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Biochemical and molecular characterisation of the transcription factor WUSCHEL in *Arabidopsis thaliana*

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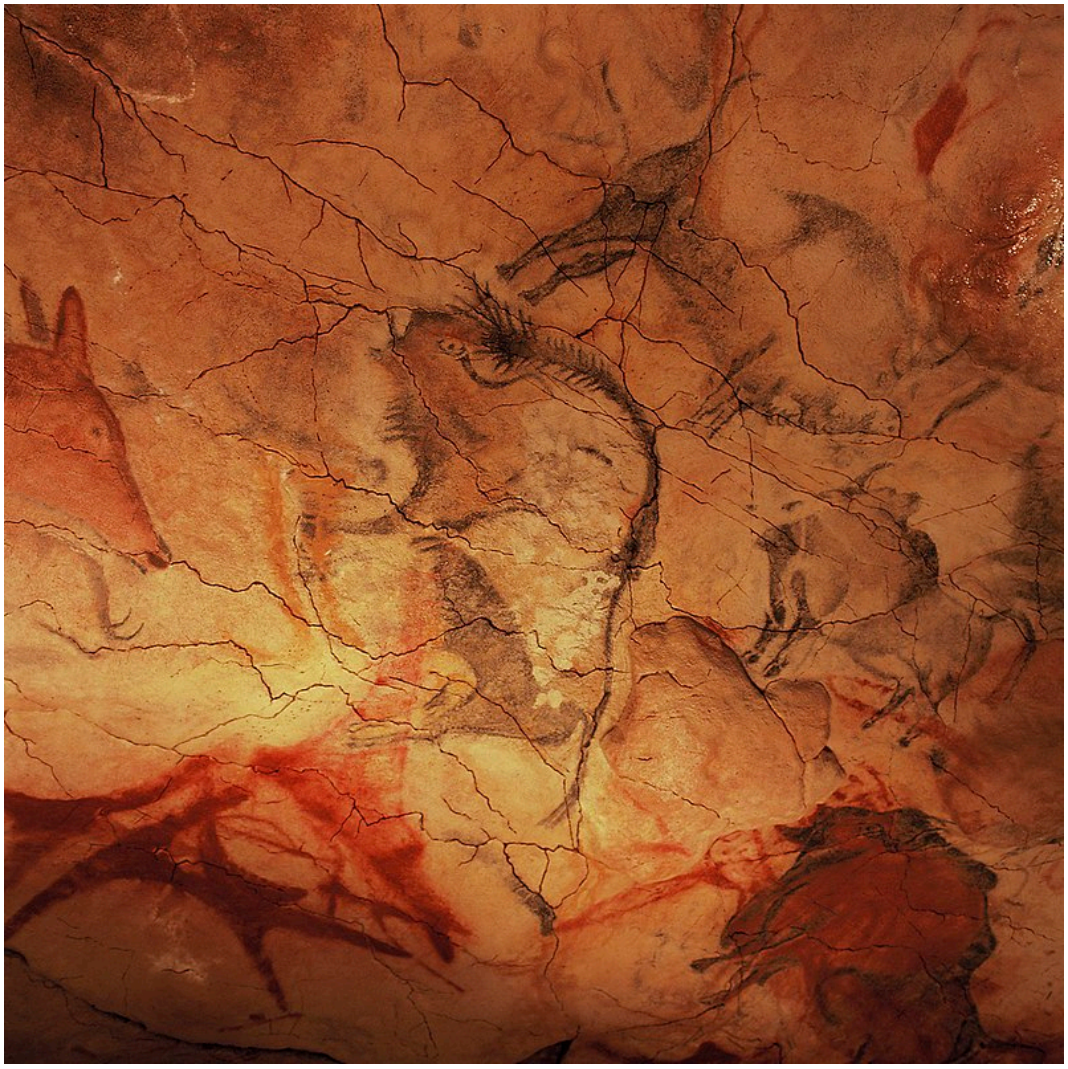
Στους γονείς μου.



Paleolithic art from the Altamira cave in northern Spain.

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Contents

Summary

Plants are autotroph sessile organisms that are characterised by extensive postembryonic development. This continuous generation of new tissues and organs, which follows the germination of the plant embryo, is driven by multipotent stem cells that reside in specialized tissues called meristems. The Shoot Apical Meristem (SAM) is the tissue where almost all aerial plant organs are generated. In the eudicot reference plant *Arabidopsis thaliana*, the stem cell pool of the SAM is located in the Central Zone (CZ) and is maintained by the cells of the adjacent Organising Centre (OC). The latter express the homeodomain transcription factor WUSCHEL (WUS), which is the key regulator of stem cell function in the SAM. WUS moves from the OC into the CZ through the plasmodesmata where it upregulates or represses its target genes. *CLAVATA3* (*CLV3*), a gene activated by WUS in the CZ, encodes an oligopeptide that is secreted in the apoplastic space and subsequently moves back to the OC where it represses *WUS*, thereby establishing a negative feedback loop that controls stem cell fate.

The biological functions of WUS cannot be fully explained yet. However, it is known that WUS exerts its role in the SAM via physical interaction with other proteins. WUS acts as negative regulator of gene expression for a multitude of genes and this function is enhanced by its interaction with the transcriptional repressor TOPLESS (TPL). In addition, the interaction of WUS with transcription factor HAIRY MERISTEM 1 (HAM1) is a prerequisite for the spatially correct activation of *CLV3* by WUS in the SAM. Therefore, I decided to ascertain the *in vivo* WUS complex with the goal of identifying novel WUS interactors in an attempt to elucidate WUS function.

To this end, I performed a yeast two-hybrid (Y2H) screening against an extensive library of transcription factors from *A. thaliana*. The interaction of a subset of these transcription factors with WUS was further validated with Acceptor Photobleaching Förster Resonance Energy Transfer (AP-FRET) *in planta*. Among the candidates that I identified were WOX9 and ESR1, whose genetic interaction with *WUS* I dissected by means of generating and studying CRISPR mutants for the respective genes. Additionally, I performed co-immunoprecipitation reactions (co-IPs) of WUS fusion proteins that were expressed under the native *WUS* promoter in *A. thaliana* meristematic tissue or were overexpressed in *Nicotiana benthamiana*. The WUS complexes that I pulled down were further analysed with Mass Spectrometry. I also studied the effect of WUS phospho-PTMs by expressing the respective phospho-mutants and phospho-mimics in *A. thaliana*.

Zusammenfassung

Pflanzen sind sessile autotrophe Organismen, die sich durch eine extensive postembryonale Entwicklung auszeichnen. Die damit verbundene ständige Bildung von neuem Gewebe und neuen Organen, die direkt nach Keimung des Embryos einsetzt, wird dabei von multipotenten Stammzellen getrieben, die in speziellen Geweben, den Meristemen, zu finden sind. Das apikale Sprossmeristem ist das Gewebe, in dem fast alle überirdischen Pflanzenorgane ihren Ursprung haben. In *Arabidopsis thaliana* befindet sich die Population der Stammzellen des Sprossmeristems in der sogenannten zentralen Zone (ZZ), wo sie durch die Aktivität der Zellen des darunter liegenden organisierenden Zentrums (OZ) aufrechterhalten wird. Letztere exprimieren den Transkriptionsfaktor WUSCHEL (WUS), bei dem es sich um den entscheidenden Regulator der Stammzellfunktion im Sprossmeristem handelt. WUS wandert durch die Plasmodesmen von den Zellen des OZ in die Zellen der ZZ, wo es die Expression seiner Zielgene hoch- oder runterreguliert. *CLAVATA 3 CLV3*, ein Gen dessen Expression durch WUS in der zentralen Zone induziert wird, codiert für ein Oligopeptid, das in den Apoplast sekretiert wird und von dort in die Zellen des OZ wandert, wo es die Expression von WUS unterdrückt und somit zur Etablierung eines negativen Feedback-Mechanismus beiträgt, der das Stammzellschicksal im Meristem kontrolliert.

Die biologische Funktion von WUS ist noch nicht vollständig aufgeklärt. Es ist jedoch bekannt, dass WUS seine Rolle im Sprossmeristem über die physikalische Interaktion mit anderen Proteinen ausübt. WUS wirkt als negativer Regulator der Genexpression für eine Reihe von Genen und diese Aktivität wird über die Wechselwirkung mit dem transkriptionalen Repressor TOPLESS (TPL) vermittelt. Darüber hinaus ist die Interaktion von WUS mit dem Transkriptionsfaktor HAIRY MERISTEM 1 (HAM1) Voraussetzung für die korrekte räumliche Expression von *CLV3* durch WUS im Sprossmeristem. Um neue potentielle WUS Bindepartner zu identifizieren, sollten in dieser Arbeit *in vivo* WUS Proteinkomplex untersucht werden, die möglicherweise zum besseren Verständnis der Funktion von WUS beitragen können.

Dazu wurde zunächst ein Yeast two-hybrid Screen mit einer umfangreichen Bibliothek von Transkriptionsfaktoren aus *A. thaliana* durchgeführt. Interaktionen zwischen WUS und einem Teil der dabei identifizierten Transkriptionsfaktoren konnten anschließend durch Förster Resonanz Energie Transfer *in planta* bestätigt werden. Unter den so identifizierten Kandidaten waren *WOX9* und *ESR1*. Um genetische Interaktionen zwischen diesen beiden Genen und *WUS* zu untersuchen, wurden entsprechende CRISPR Mutanten generiert und analysiert. Zusätzlich wurden co-Immunpräzipitationen mit WUS Fusionsproteinen, die entweder unter Kontrolle des nativen *WUS* Promotors aus *A.thaliana* Meristemgewebe oder nach Überexpression aus *Nicotiana benthamiana* Blättern isoliert wurden, durchgeführt. Die dabei gewonnenen WUS Proteinkomplexe wurden mit Hilfe von Massenspektrometrie weiter analysiert. Zudem wurde der Einfluss von posttranslationalen Phosphor-Modifikationen auf die Funktion von WUS untersucht. Hierzu wurden entsprechende Mutanten generiert und in *A. thaliana* exprimiert.

Chapter 1

Introduction

1.1 Preamble

Plants are autotroph eukaryotic organisms that harvest solar energy via photosynthesis. This energy is stored into chemical bonds and is later used for the physiological and developmental functions that plants exhibit. Securing the fundamentals for survival in a way that is independent of other life forms has made plants the entry point of energy and the site of *de novo* organic material composition in almost all ecosystems during the unfolding of biotic evolution's time spiral. Most plants are multicellular organisms that are also sessile and cannot readily avoid stressful environmental conditions and predators, seek actively favourable niches or search for a mating partner. These characteristics of their *modus vivendi* has led to the emergence of multiple adaptations, one of which is the extensive post-embryonic development. Plants generate new organs continuously during their lifespan, something that is facilitated by the presence and function of stem cells, which are pluripotent undifferentiated cells. These stem cells reside in meristems, specialized tissues that consist of the stem cells themselves and a niche, i.e. an adjacent group of cells that is responsible for maintaining the stem cell population. All these unique features of plant biology naturally raise fundamental questions, such as how do stem cells remain undifferentiated or how are they recruited into new developing organs. The answer to these questions not only has an ”abstract” value in terms of interpreting biological phenomena but can also lead to numerous and reverberating effects in the every-day life of humanity since plants, or more specifically the products of plant meristems, are widely used in numerous ways by human societies, e.g. as staple food, heat source, raw material for clothing or paper, etc **[1]**. In addition, the importance of research in the field of plant biology in general has broadened through the advances in the molecular elucidation of plant structure and function during the previous decades, since the latter have permitted additional novel uses of plants, e.g. ectopic expression of plant proteins in mammalian cells that elicit anti-tumour effects **[2]**.

This thesis focuses on the meristem organisation of flowering plants and uses the annual eudicot plant *Arabidopsis thaliana* (ecotype Col-0), an established model in the research field of plant genetics and physiology, as basis for the dissection of plant stem cell biology **[3, 4]**. Furthermore, this thesis concentrates on the shoot apical meristem, the primary meristem

that serves as the generation site of all aerial plant structures, and analyses in depth the function of the transcription factor WUSCHEL.

1.2 Meristems of *A. thaliana*

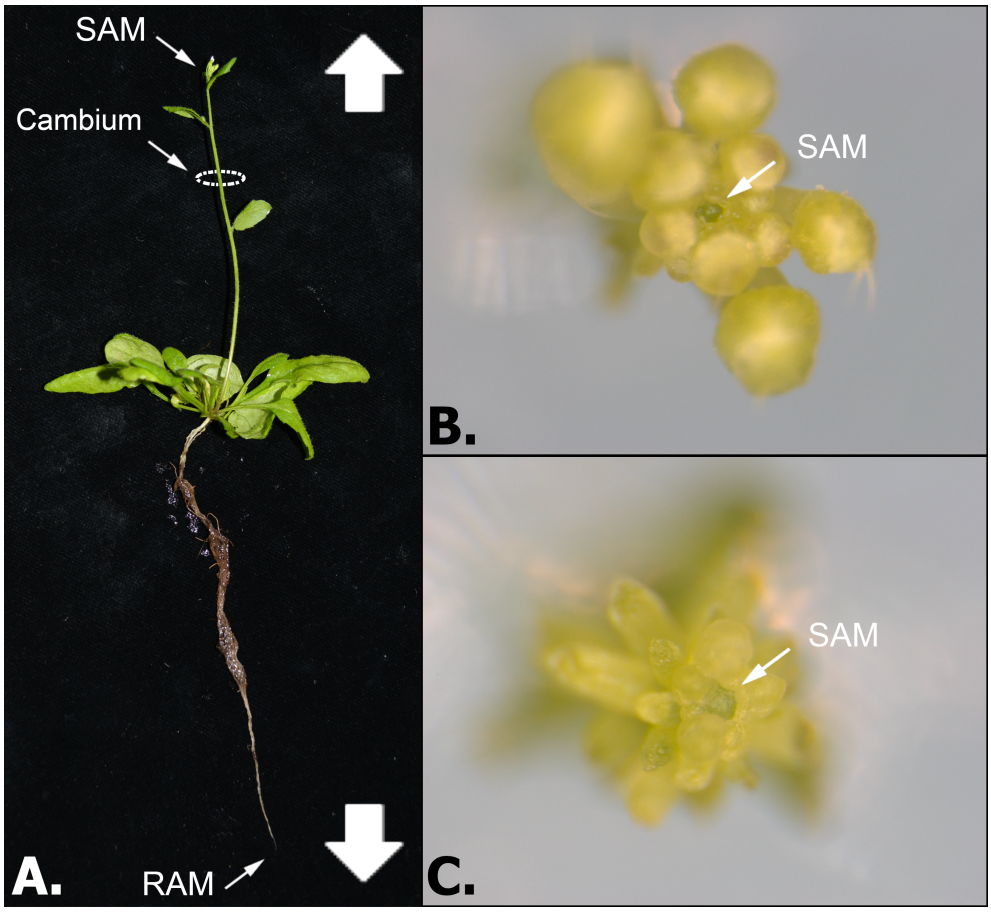

Figure 1.1: A) Overview of an adult *A. thaliana* Col-0 plant (Wus -/-, pWUS::WUS-linker-GFP). The plant is ca. 30 days old and the length of the shoot is approximately 10 cm. The thin arrows point to the location of meristems. The SAM and RAM are situated at the tip of the shoot and root respectively. In contrast, the cambium consists of several cell clusters that form a continuous stretch of rings along the stem that resemble a hollow cylinder. The fat arrows illustrate the direction of apical growth driven by the SAM and RAM, while the ring around the shoot depicts the radial growth driven by the cambium. B) Top view of the SAM surrounded by developing flowers (Col-0, 60x). C) Top view of the SAM shown in B) after removal of all emerging flowers that had developed past flower stage IV (Col-0, 100x). This dissected SAM is typical of the samples imaged with confocal laser microscopy for this study. In B) and C) the SAM is visible at the centre of the image as a dark green structure resembling a dome and is pinpointed by white arrows.

It was mentioned already that plant stem cells are located in specialised tissues, called meristems. Plants have two primary meristems that are responsible for the longitudinal growth of a plant. These are located at the tip of the shoot and the root and generate all above- and below-ground plant tissues and organs respectively (**Fig. 1.1, A**). They are known in the scientific literature as the shoot (SAM) and the root apical meristem (RAM) **[5, 6]**. The SAM and the RAM are established during embryogenesis and are characterised by

similar cellular architecture, i.e. they comprise a stem cell pool and an adjoining niche whose cells regulate stem cell function and fate **[7]**, as well as the daughter cells that result from the division of stem cells. Many plants possess also secondary meristems that are established *de novo* during post-embryonic development and drive their secondary growth. The latter is characterised by different modes that result in growth along axes that are oblique to the primary longitudinal one or in an increased circumference of the organism **[8, 9]**. Prominent examples include the cambium (**Fig. 1.1, A**), which derives from the vascular bundles and facilitates the radial growth of a plant **[10]**, as well as the axillary and lateral root meristems that enable shoot and root branching respectively **[11–13]**.

1.3 Structure of the shoot apical meristem (SAM)

The SAM (**Fig. 1.1, B and C**) has been studied extensively during the previous decades and many molecular details of its function have been discovered **[14, 15]**. The borders of the SAM as a biological structure *per se* are delineated by the expression of *SHOOTMERISTEMLESS* (*STM*). Mutant seedlings for the *STM* locus lack a SAM, since the latter is not initiated during embryonic development **[16]**. Furthermore, *stm* plants do not generate a SAM post-embryonically. *STM* itself encodes a homeodomain transcription factor (TF) and is responsible for keeping SAM cells at an undifferentiated state **[17, 18]**.

Anatomically, the outermost area of the SAM is divided in 3 clonally distinct cell monolayers, i.e. layers L1, L2 and L3 (**Fig. 1.2**) **[19]**. The stem cells reside at the very centre of these 3 layers and form the Central Zone (CZ) of the SAM. The orientation of cell division is not uniform among stem cells however. In layers L1 and L2, stem cells divide predominantly in an anticlinal orientation and the daughter cells either remain in the CZ or move towards SAM periphery while displacing adjacent cells. The daughter cells that exit the CZ lose stem cell identity, a fact manifested by the synchronous loss of the stem cell marker *CLAVATA3* (*CLV3*) **[18, 20]**, but do not differentiate yet. Instead, they form the Peripheral Zone (PZ) of the SAM, which encloses the CZ on all sides apart from the epidermis. In contrast, stem cells in L3 exhibit both anticlinal and periclinal cell divisions with their progeny either remaining in the CZ, entering the PZ or moving towards the interior of the shoot. The rate of cell division is also not uniform in the SAM, with the stem cells of the CZ dividing rarely and the daughter cells of the PZ dividing more frequently **[21]**. The cells that eventually reach the boundaries of the SAM at the end of the PZ differentiate and get incorporated in the lateral organ primordia (LOP) while the cells that move towards the interior of the shoot participate in the formation of vasculature and other mesenchymal tissues.

Directly below the stem cells lies a group of cells that is called the Organizing Centre (OC) and is characterised by expression of the gene *WUSCHEL* (*WUS*), which encodes a homeodomain transcription factor **[22]**. The discovery of *WUS*, whose identification occurred during a mutagenesis screen in *A. thaliana* **[23]**, was a turning point in our understanding of SAM biology. The respective *wus-1* mutation, like almost all deleterious *wus* mutations isolated thereafter, results in embryos failing to develop a SAM (**Fig. 1.3**). In contrast to

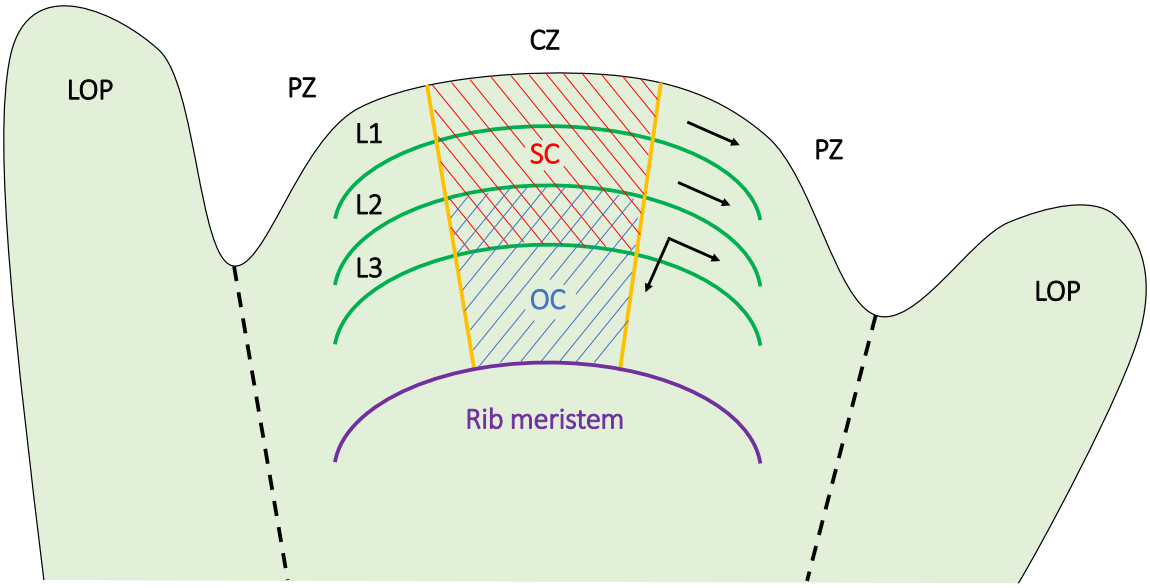

Figure 1.2: Schematic virtual longitudinal section of an inflorescence SAM from *A. thaliana*. The boundaries of the SAM are delineated by dashed lines. The outermost part of the SAM consists of three cell monolayers that are called L1, L2 and L3. The stem cells are located at the central part of these three layers and form the Central Zone (CZ). Anticlinal cell divisions in the CZ result in daughter cells moving outside of the CZ towards the SAM periphery while losing their stem cell identity (black arrows pointing sidewards of the CZ). These undifferentiated cells occupy transiently the Peripheral Zone (PZ), but will eventually differentiate upon reaching the SAM boundaries and will be recruited into the developing Lateral Organ Primordia (LOP). Cell layer L3 is the only one that also exhibits periclinal cell divisions (black arrow pointing below the CZ). Directly underneath the CZ exists a group of cells called the Organising Centre (OC), which is responsible for maintaining the pluripotency and the size of the stem cell pool. In the inflorescence SAM, the OC and the CZ overlap at L3 (as shown in this figure), while in the vegetative SAM they remain distinct with the OC lying entirely outside of the CZ. Beneath the OC is the Rib meristem, which is characterised by changes in cell shape.

the similar *stm* phenotype, however, *wus* seedlings generate *de novo* stem cells repeatedly during post-embryonic development but the resulting stem cell pool is non-sustainable and is depleted fast. Subsequently, adult *wus* plants either form an abnormal rosette with enlarged leaves and do not grow further, or develop indeed a shoot and form multiple aerial rosettes at irregular intervals giving rise to a very characteristic stop-and-go growth phenotype (**Fig. 1.4**).

The area of the SAM beneath the OC is called the Rib meristem (RM) and is characterised by alteration of cell morphology. The cells of the RM adopt a flatter shape that most likely constitutes a sign of their imminent differentiation **[14]**. Below the RM, the differentiated tissue forms vascular elements. The SAM itself is devoid of vasculature, a fact that may be responsible for its recalcitrance to viral infection **[24]**. Interestingly, a large area of the SAM that overlaps with the OC and the RM constitutes a hypoxic micro-environment, which is essential for the efficient generation of new leaves **[25]**.

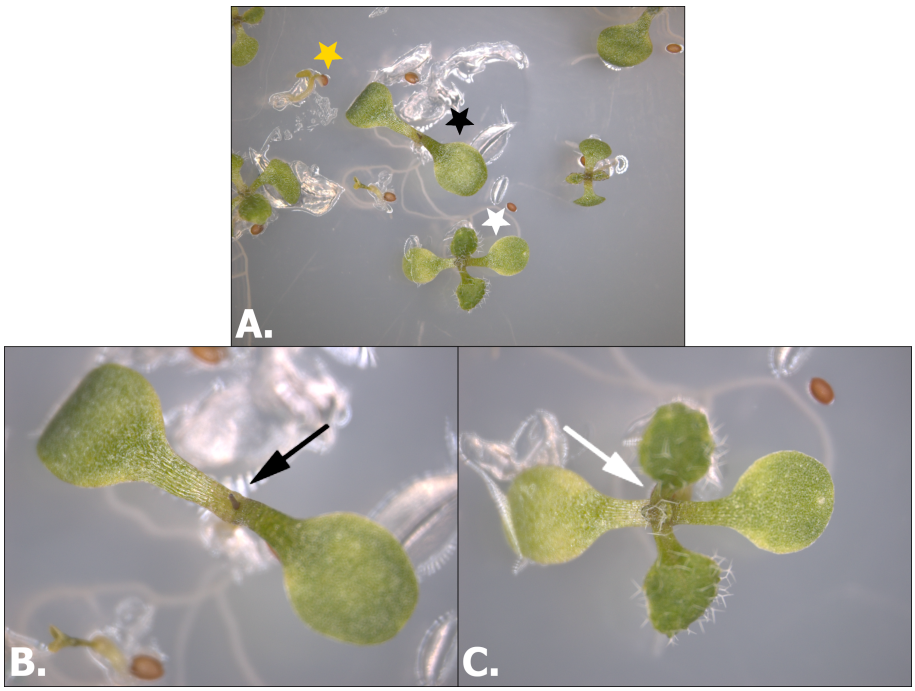

Figure 1.3: Overview of the *wus* phenotype in *A. thaliana* seedlings. *wus* lines are propagated in a heterozygous state. A) Selection for Wus -/- plants (black star). Wild-type (yellow star) and heterozygous (white star) plants are also visible (7.5x). B) A Wus -/- plant. The arrow points to the aborted SAM (20x). C) A Wus +/- plant. The arrow points to the leaf primordia. The first pair of true leaves has also developed (20x). All seedlings are 8 days old.

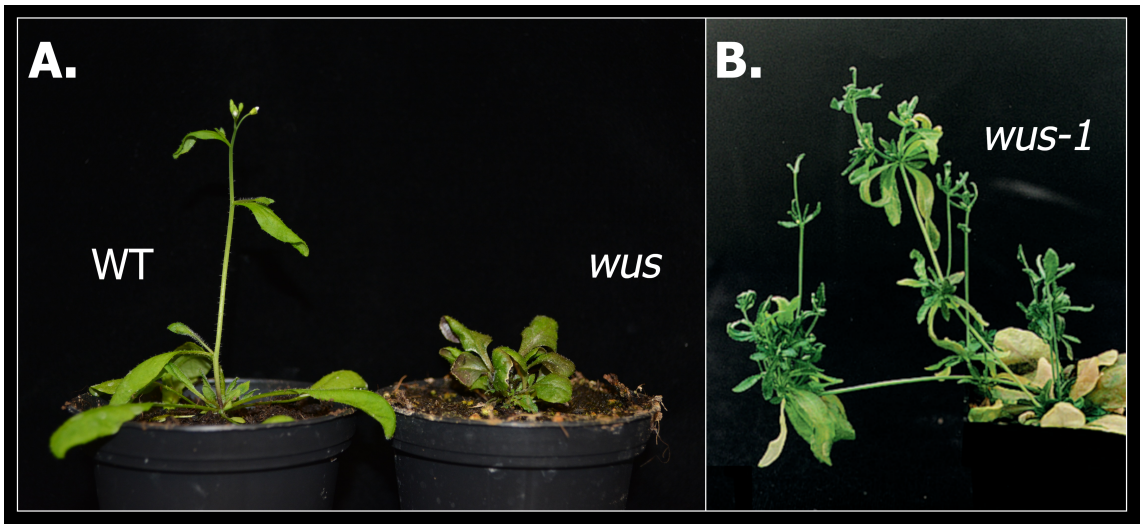

Figure 1.4: Overview of the *wus* phenotype in adult *A. thaliana* plants. A) Comparison between a WT plant and a *wus* mutant. The *wus* plant, which harbours in homozygosity the *wus-AM* mutation that was used for the experiments of this thesis, is characterised by the absence of a shoot. This *wus* mutant is 50 days old while the WT plant is 27 days old. B) The stop-and-go growth phenotype of a *wus* plant. This mutant plant is homozygous for the *wus-1* mutation. Original picture published in [23]. Shoots in both A) and B) have a length of ca. 10 cm.

1.4 *WUSCHEL* in the context of the transcriptional networks regulating SAM development and function

SAM biology is governed by an elaborate network of signalling molecules and genes, the products of the latter being involved in a series of complex interactions (**Fig. 1.5**). In

Section 1.3, the two genes (*STM* and *WUS*) responsible for the main attributes of the SAM, i.e. it being an undifferentiated tissue that hosts a stem cell pool, were already mentioned. However, as it has been manifested by the isolation of the corresponding mutants, several other genes also participate in SAM development and in the regulation of stem cell fate.

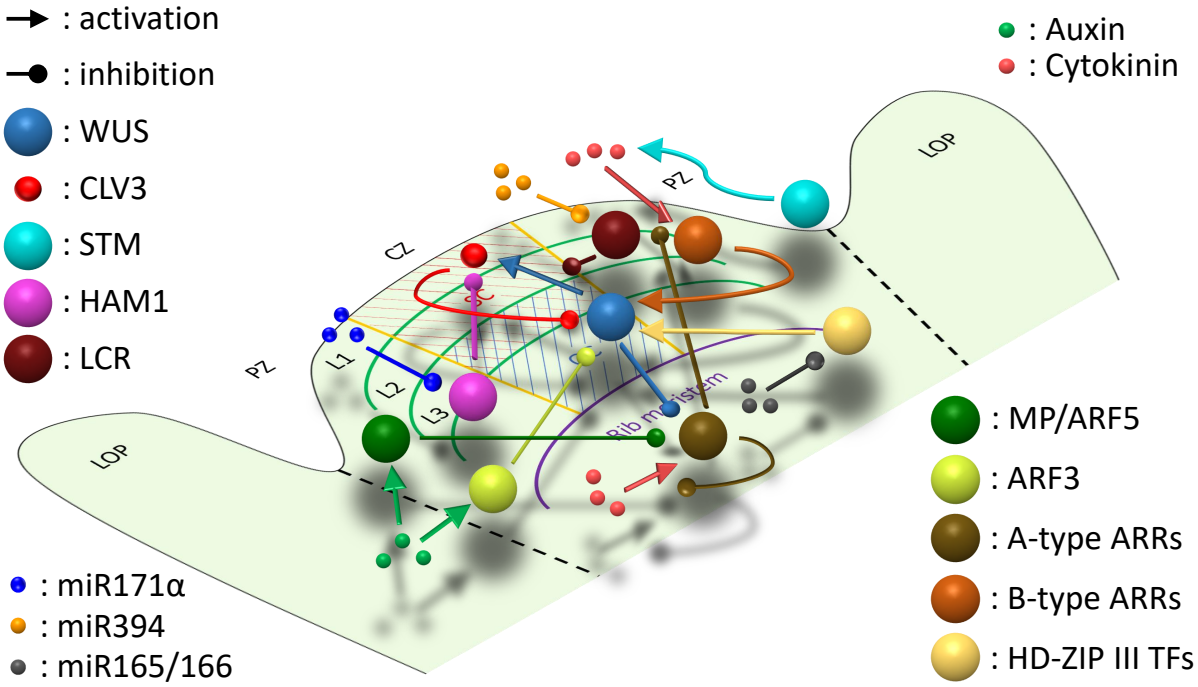

Figure 1.5: Pseudo-3D transformation of the schematic virtual longitudinal section of an inflorescence SAM from *A. thaliana* that was presented in Fig. 1.2. The interactions among various genes and signalling pathways regulating SAM development and function are depicted. WUS is expressed in the OC and subsequently moves into the CZ through the plasmodesmata, where it activates *CLV3*. The CLV3 peptide, in turn, is secreted in the apoplastic space and moves into the OC where it represses *WUS* via signalling cascades initiated by its interaction with CLV1 and CLV2-CRN receptor kinases. The miRNA miR394, which is expressed in L1, facilitates the WUS-mediated activation of *CLV3* by inhibiting *LCR*, the latter blocking by default WUS function. Additionally, HAM1 interacts with and obstructs WUS from activating *CLV3* outside of the CZ. *HAM1*, however, is repressed by miR171α, which is also expressed in L1. Cytokinin promotes *WUS* expression by activating B-type *ARRs*, which subsequently activate *WUS*. WUS boosts cytokinin signalling by inhibiting A-type *ARRs* that otherwise exert negative feedback control on cytokinin signalling. HD-ZIP III TFs, whose levels are negatively regulated by miR165 and miR166, participate in the *de novo* activation of *WUS* along with the B-type ARRs. *WUS* activation is also promoted by STM, which enhances cytokinin biosynthesis. Auxin has opposing effects on stem cell fate. *MP*/*ARF5* is upregulated by auxin and promotes *WUS* expression by repressing B-type *ARRs*. ARF3, however, which is also positively regulated by auxin, moves into the OC and represses *WUS*. The position of the various biomolecules in this figure tends to be representative of their *in vivo* localisation in the SAM (e.g. *WUS*, *CLV3*, *HAM1*, miR171α, miR394, etc), but this was not technically possible to accomplish for all cases at this scale (e.g. cytokinin, auxin, etc). More details as well as the non-abbreviated gene names are provided in the text.

Maintenance of stem cell fate in the SAM

WUS, which is already present in the apical inner cells of the 16-cell *A. thaliana* embryo **[22]**, is expressed in the OC of the vegetative and inflorescence SAMs. WUS acts in a non-

cell-autonomous manner, in the sense that it moves from the OC into the SC through the plasmodesmata (**Fig. 1.6**) **[26, 27]**, where it exerts its role as a transcriptional regulator by activating or repressing its target genes **[28]**. Among the genes that are activated by WUS is *CLV3*, the latter encoding a protein that is processed into a glycosylated 13-aa oligopeptide **[29]**. The CLV3 peptide is secreted into the apoplast and moves back to the OC, where it heralds a signalling cascade mediated by the receptor kinases CLV1 and CLV2-CRN that results in the repression of *WUS* **[30, 31]**. Thus, *WUS* and *CLV3* form a negative feedback loop that functions as the key regulatory step of stem cell fate (**Fig. 1.7**).

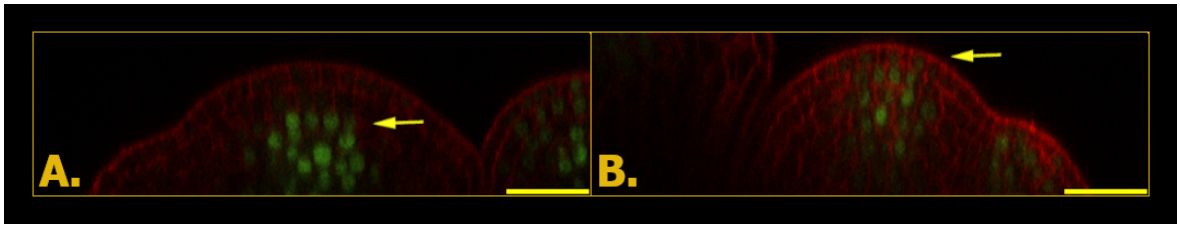

Figure 1.6: Comparison of pWUS activity and WUS distribution in the inflorescence SAM. A) pWUS is active in the OC and in layer L3 (yellow arrow). No fluorescence is detected in L1 or L2. Plant line genotype: Wus +/+, pWUS::3xVenus-NLS. Red: DAPI, Green: pWUS::3xVenus-NLS. B) WUS is present not only in the OC and in L3, but also in L2 and L1 (yellow arrow). Plant line genotype: Wus -/-, pWUS::WUS-linker-GFP. Red: DAPI, Green: pWUS::WUS-linker-GFP. Scale bars correspond to 25 µm.

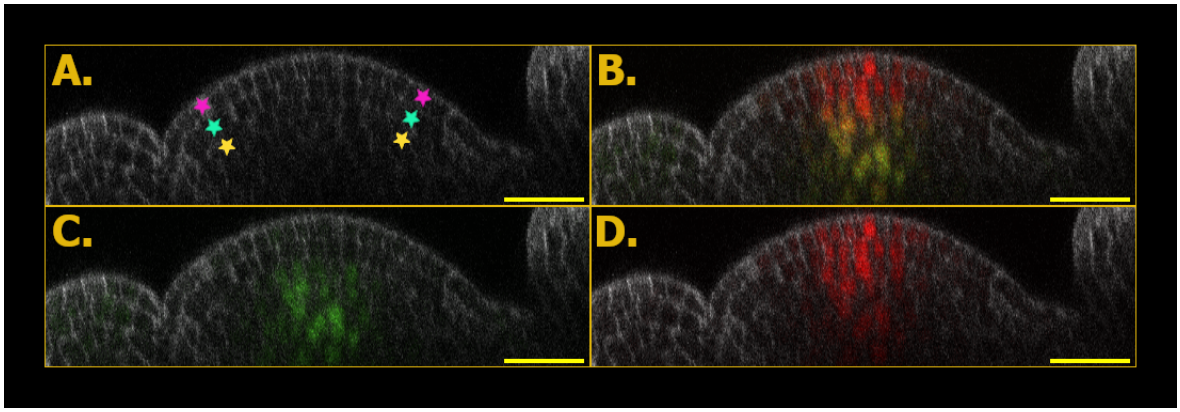

Figure 1.7: Transcriptional reporters for *WUS* and *CLV3* in the Col-0 background. All pictures depict the same longitudinal section of an inflorescence SAM. A) The L1 layer is marked with pink, the L2 layer with cyan and the L3 layer with yellow stars. B) *WUS* and *CLV3* expression overlap in L3. C) *WUS* transcriptional reporter profile. D) *CLV3* transcriptional reporter profile. Plant line genotype: pCLV3::mCherry-NLS, pWUS::3xVenus-NLS. Grey: DAPI, Red: pCLV3::mCherry-NLS, Green: pWUS::3xVenus-NLS. Scale bars correspond to 25 µm.

Activation of *WUS*

The activation of *WUS* is a biological process where the developmental gene transcriptional programme and the effects elicited by the phytohormones cytokinin and auxin converge. *WUS* is activated *de novo* by transcriptional complexes formed between B-type Arabidopsis Response Regulators (B-type ARRs) ARR1, ARR2, ARR10, ARR12 and homeodomain-leucine zipper (HD-ZIP) III TFs PHABULOSA (PHB), PHAVOLUTA (PHV), REVO-

LUTA (REV) that bind to the *WUS* promoter **[32]**. SPLAYED (SYD), a chromatin-remodelling SNF2 ATPase, is also recruited to the *WUS* promoter and participates in *WUS* activation **[33]**.

B-type ARRs are activated by cytokinin, function as transcriptional activators and relay further-on cytokinin-mediated signalling **[34]**. In turn, WUS represses A-type ARRs, which are also activated by cytokinin and constitute the auto-inhibitory pathway of cytokinin signalling, thereby promoting its own expression **[34, 35]**. The HD-ZIP III TFs PHB, PHV and REV are under negative control by miRNAs miR165 and miR166, an interaction that imposes an additional regulatory layer on *WUS* activity **[32]**. Furthermore, miR165 and miR166 are also negatively regulated by *ARGONAUTE 10*/*ZWILLE* (*AGO10*/*ZLL*), which sequesters these miRNA molecules in the vasculature below the OC, thus facilitating the activation of *WUS* by the aforementioned HD-ZIP III TFs **[14, 36]**. Cytokinin-mediated *WUS* expression is also enhanced by *STM* since the latter promotes cytokinin biosynthesis by activating the gene *ISOPENTENYLTRANSFERASE 7* (*IPT7*), which encodes the enzyme that catalyses the respective rate-limiting step **[37]**.

Auxin-mediated signalling also affects *WUS* activation, however in opposing ways. Perception of auxin by the cell transcriptional machinery results in the expression of genes that are under transcriptional control of the Auxin Response Factor (ARF) class of TFs **[38]**. At low auxin concentrations, the function of ARF TFs is suppressed by the Auxin/INDOLE-3-ACETIC ACID (Aux/IAA) repressor proteins. At higher concentrations, however, auxin mediates the degradation of Aux/IAA proteins by the proteasome, thus allowing ARF TFs to enable the transcription of auxin-responsive genes **[39]**. ARF5, a TF known also as MONOPTEROS (MP), which is expressed in the PZ and moves into the CZ, represses A-type ARRs and as a consequence promotes *WUS* expression **[40]**. On the contrary, the TF encoded by *ARF3*, which is also expressed in SAM periphery, moves into the OC where it represses *WUS* as well as the effects of cytokinin signalling **[41]**. Interestingly, WUS also exerts equivocal effects on auxin signalling. Even though WUS suppresses the latter to prevent the auxin-mediated differentiation and exhaustion of the stem cell pool, WUS simultaneously secures the rudimentary activity of auxin signalling that is required for stem cell maintenance **[42]**. Thus, the WUS domain is characterised by the coinciding presence of stem cells and of minimum auxin signalling output.

Interestingly, the hypoxic conditions that exist in a large area of the SAM (see section 1.3) seem also to contribute, albeit negatively, to the regulation of *WUS* expression. Hypoxia is necessary for the stability of LITTLE ZIPPER 2 (ZPR2), a protein that is otherwise degraded by the proteasome in the presence of O_2 **[25]**. ZPR2 is expressed in the OC, where it evades degradation by virtue of the local hypoxic conditions and subsequently represses the HD-ZIP III TFs PHB and REV by physically interacting with them. It was mentioned already that PHB and REV are directly involved in the activation of *WUS*.

Furthermore, *WUS* activation is regulated spatially by BRCA-associated RING domain 1 (BARD1), a protein known also as REPRESSOR OF WUSCHEL1 (ROW1), which is involved in DNA repair. BARD1 binds to the *WUS* promoter and confines *WUS* transcription within

the OC, a function that might be mediated by an interaction between BARD1 and SYD **[43]**. BARD1 has a similar role in the RAM, where it represses *WUSCHEL-related homeobox 5* (*WOX5*), a *WUS* paralog[§] **[44]**. Additionally, the lateral boundaries of the *WUS* expression domain, as well as that of *CLV3*, are restricted to the centre of the SAM by components of the ERECTA signalling pathway **[45]**.

Activation of *CLV3*

The activation of *CLV3* by WUS is also regulated on several layers, the main components of the loop notwithstanding. *HAIRY MERISTEM 1* (*HAM1*), which encodes a GRAS TF (GRAS being a portmanteau of the genes *GIBBERELLIC ACID INSENSITIVE*, *REPRESSOR OF GA* and *SCARECROW*), represses *CLV3* and confines its transcriptional potentiality within the CZ **[46]**. More specifically, HAM1 obstructs WUS from activating *CLV3* in the OC, where WUS is expressed. However, once WUS moves into the CZ, where HAM1 is absent, then WUS can activate *CLV3*. The repression of *CLV3* by HAM1 depends on the physical interaction between HAM1 and WUS **[47]**, which will be analysed further in Section 1.6.

Interestingly, L1 provides several cues that enable *CLV3* expression, a fact that could explain the invariant apical positioning of the CZ within the SAM. The activation of *CLV3* by WUS is inhibited by *LEAF CURLING RESPONSIVENESS* (*LCR*), which codes for an F-box protein. LCR is presumably involved in the ubiquitination of proteins that leads to the degradation of the latter by the proteasome **[48]**, but its inhibition of WUS function does not entail a decrease in WUS stability **[49]**. Nevertheless, the protodermal* miRNA miR394, which moves into L2 and L3 after being transcribed in L1, represses *LCR*, thereby permitting the efficient activation of *CLV3* by WUS **[49]**. In addition, *HAM1*, which suppresses *CLV3* expression, is repressed by miR171α, a miRNA that is also expressed in the protoderm* of the embryo and in the L1 later in development **[50]**.

The role of *CLV3* in constraining the size of the stem cell pool seems to be a novelty in angiosperms that has evolved from ancient signalling pathways present in land plants, which are mediated by *CLV3/ESR-related* (*CLE*) genes and originally promoted stem cell fate **[51]**. Interestingly, such a function seems to be also present in the *A. thaliana* SAM. *CLE40* encodes a peptide that is similar to CLV3 and actually forms along with WUS a second negative feedback loop, which also contributes to the regulation of stem cell fate **[52]**. CLE40 is expressed in the PZ and, in contrast to CLV3, activates WUS. This activation is elicited by the interaction of CLE40 with the receptor kinase BARELY ANY MERISTEM 1 (BAM1) and possibly coordinates the size of the stem cell pool with the rate of lateral organ formation **[52]**. WUS, in turn, is involved in the suppression of *CLE40*. Intriguingly, there exists interplay between the WUS-CLV3 and WUS-CLE40 loops since CLV1 represses BAM1.

[§]WUS is the founding member of the WOX TF family (see section 1.5).
*The protoderm is the embryonic tissue that eventually forms the epidermis.

Initiation of stem cell fate in the embryo

The role of B-type ARRs and HD-ZIP III TFs in *WUS* activation described above pertains to the established SAM, the axillary meristems that drive shoot branching as well as to shoot regeneration **[32]**. In embryogenesis, however, although the HD-ZIP III TFs are still essential for SAM establishment, the B-type ARRs are not. Transcriptional output of cytokinin signalling is absent in the apical region of the 16-cell embryo, the time point when *WUS* gets initially expressed, and appears only later in development, namely in heart stage embryos[‡] **[53]**. Furthermore, it seems that WUS itself is also not required for the activation of *CLV3* and the establishment of the SAM during embryogenesis **[54]**. This fact is in line with the *de novo* SAM generation in adult *wus* mutants. Instead, *WOX2* is the actual TF necessary for stem cell specification in the embryo. *WOX2* function is mediated by the HD-ZIP III TFs PHB, PHV, REV, which also participate in *WUS* activation later in development, as mentioned above, and results in the enhancement of cytokinin biosynthesis **[54]**. Once *CLV3* has been activated in transition embryos[‡] **[54]**, then WUS is needed to maintain *CLV3* expression along with stem cell fate.

1.5 Structural insights into the function of WUSCHEL

WUS is a homeodomain TF, whose discovery and identification established the WOX TF family **[57]**. The WOX proteins are a plant-specific group of homeodomain TFs and play a role in several key developmental events, in a similar fashion to animal homeodomain TFs **[58]**. WOX proteins are divided phylogenetically in three clades: the WUS clade, the intermediate clade and the ancient clade **[59]**. The WUS clade comprises WOX proteins that exist exclusively in spermatophytes, many of which can substitute to some extent each other *in vivo* **[59, 60]**. WOX5, which is expressed in the Quiescent Centre of the RAM and is responsible for maintaining the stem cells of the latter, can rescue *wus* mutants when expressed under the native *WUS* promoter **[61]**. Similarly, WUS rescues *wox5* mutants when expressed under the control of the *WOX5* promoter. This functional interchangeability of WUS and WOX5 pertains only to stem cell maintenance however **[61]**. Furthermore, the single *WUS/WOX5* ortholog from the gymnosperm *Gnetum gnemon* not only is functional in the *A. thaliana* SAM when expressed under the *WUS* promoter but also enhances stem cell function since its expression results in a phenotype similar to the one manifested by *clv* mutants **[62]**. The functional diversification that WOX proteins exhibit apparently results from different spatial and temporal patterns of expression as well as from association with different protein interactors.

The comparison of sequence homology among WOX proteins led to the identification of certain domains and motifs, some of which have been found to mediate protein-protein interactions or are implicated in specific biological functions. WOX proteins share these structural elements, although the latter are not necessarily present in each WOX protein, while

[‡]The plant embryo is named during the progressive steps of embryogenesis as follows: zygote, 1-cell, 2-cell, 4-cell, 8-cell (octant), 16-cell (dermatogen), globular, transition, heart, torpedo, walking-stick, mature **[55, 56]**.

some elements and motifs are restricted in certain clades. Naturally, the conserved WOX homeodomain is the defining feature of this protein family. Additionally, many WOX proteins belonging to the WUS clade contain a WUS-box (Thr-Leu-X-Leu-Phe-Pro-X-X) and/or an ERF-associated amphiphilic repression (EAR) motif (Leu-[Glu/Asp]-Leu-[Arg/Ser/Thr]-Leu) **[59]**. The WUS-box is a repression domain that is a prerequisite for all known functions of WUS while the EAR motif enhances the transcriptional repression induced by WUS but is otherwise redundant **[60, 63]**. Some WOX proteins also possess PEST sequences, which are rich in Pro-Glu-Ser-Thr, and apparently lead to a fast degradation of the protein by the proteasome **[60, 64]**.

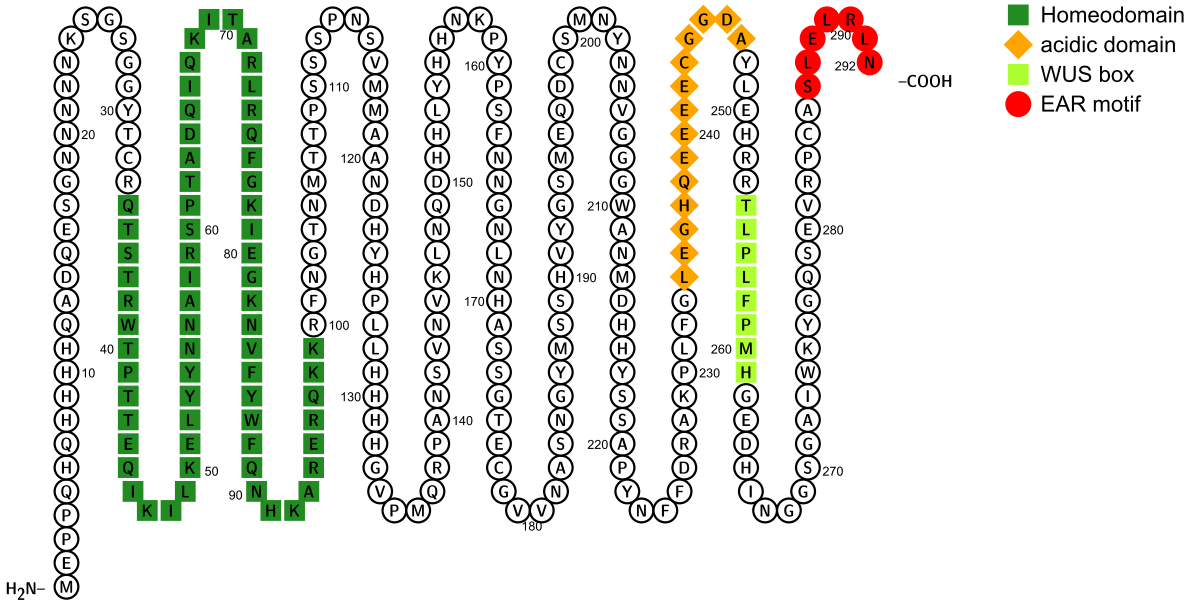

Figure 1.8: Schematic representation of WUS. The various known structural elements and motifs are coloured according to the legend.

The *WUS* gene itself encodes a 292 aa TF and the analysis of its sequence revealed that WUS possesses four regions with distinct structural characteristics: a) the WOX homeodomain (aa 34-99), which enables DNA binding, b) an acidic stretch (aa 234-247), which functions as an activation domain but is apparently only needed for the development of the gynoecium **[59, 60]**, c) the WUS-box (aa 254-261) and d) the EAR motif (286-292, **Fig. 1.8**). The three-dimensional structure of the folded full-length WUS protein remains unknown. Up to the present, only the structure of the WUS homeodomain has been solved by X-ray crystallography studies of truncated WUS protein molecules **[65]**. Intriguingly, recent advances in the computational prediction of protein structure led to the development of the AlphaFold artificial intelligence software, which reportedly accomplishes highly accurate predictions of protein structure, even in the absence of structural data from related proteins **[66]**. The structure that AlphaFold predicts for WUS can be seen in **Fig. 1.9**. This predicted 3D structure of WUS seems to confirm previous hypotheses that the folding of WUS into its tertiary structure results in a mostly disordered molecule. This fact is in line with bioinformatics analyses that propose plant TFs to be highly unstructured and to function as scaffolds where the transcrip-

tional machinery of the cell assembles **[67]**.

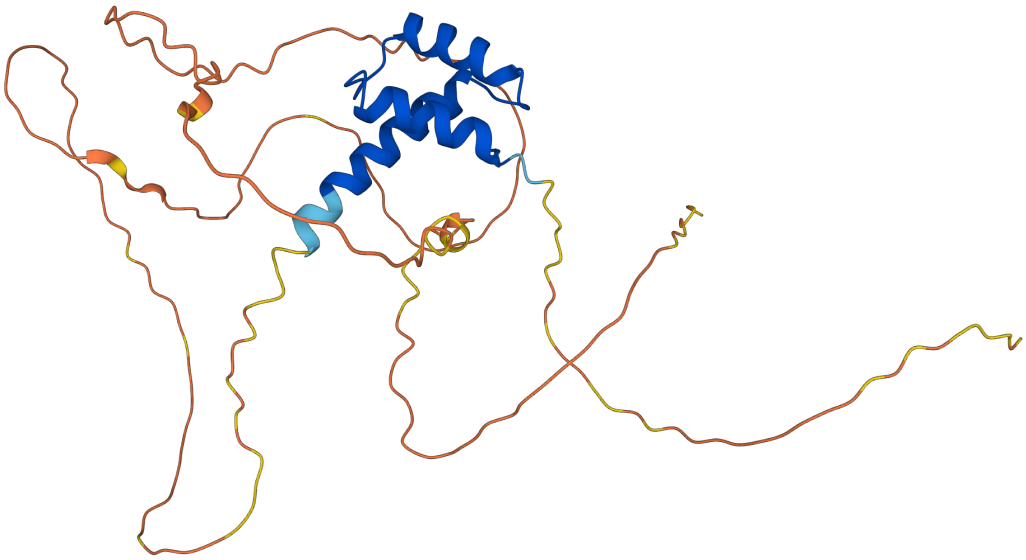

Figure 1.9: Predicted three-dimensional structure of full-length WUS by AlphaFold. The largest part of the WUS primary sequence remains unstructured after protein folding. Model confidence: very high, confident, low, very low. The predicted structure was retrieved in September 2022.

1.6 Protein interactors of WUSCHEL

After the discovery of *WUS*, several proteins have been identified as WUS interactors, although the respective experimental evidence is not always extensive. The well-studied transcriptional repressor TOPLESS (TPL) **[68, 69]** was the first protein that was verified to interact with WUS **[70]**. TPL was identified during a yeast two-hybrid (Y2H) screening and its interaction with WUS is mediated by the WUS-box and the EAR domain **[60, 70]**. Additional TPL family proteins have been shown to interact with WUS in Y2H screenings: TOPLESS-RELATED 1 (TPR1), TOPLESS-RELATED 2 (TPR2) and TOPLESS-RELATED 4 (TPR4) **[60, 70]**.

The TF HAM1, as well as the HAM family members HAM2, HAM3 and HAM4, have also been found to interact with WUS, a discovery that was once more accomplished with a Y2H screening **[47]**. Nevertheless, *ham1* mutants do not show any perturbations of stem cell function, apparently due to redundancy among the *HAM* genes. A relevant phenotype that is caused by depletion of the SAM stem cell pool becomes evident only in *ham1;ham2;ham3* triple mutants created in the sensitised *wus*-7‡ background. These results point out that HAM1 contributes along WUS to SAM stem cell maintenance. Supposedly, though, the WUS-HAM1 interaction is mediated by binding of HAM1 to a region of WUS (aa 203-236) that overlaps with the WUS acidic domain. However, Y2H screenings that were carried out for this thesis with WUS substitution mutants and will be presented in Section 4.1 contradict this assumption and suggest that it was an artefact resulting from the use of a WUS dele-

tion mutant in the original Y2H screening, which apparently undergoes misfolding **[47]**. The dissection of the WUS-HAM1 genetic interplay, however, revealed that HAM1 is involved in the main function of WUS, i.e. the activation of *CLV3* **[46]**. More specifically, it seems that HAM1 limits the ability of WUS to activate *CLV3* within the very apical layers of the SAM. Under native conditions, HAM1 is present all over the SAM except from layers L1 and L2 **[47]**. Expression of HAM1 under a L1-specific promoter does push the site of maximum *CLV3* expression deeper in the SAM **[46]**, which otherwise occurs in L1 of WT SAMs. However, the proposed model according to which HAM1 obstructs WUS from activating *CLV3* fails to explain why WUS activates *CLV3* in L3 (**Fig. 1.7**) where HAM1 is also present, why the maximum of *CLV3* expression does not correspond to the sites of higher WUS concentration, either in wt plants or in *ham1,2,3* mutants, or why WUS activates *CVL3* in developing axillary meristems, in which *HAM1* is ubiquitously, albeit non-homogeneously, expressed **[46]**.

Another protein-protein interaction involving WUS that was identified in the recent years is the one with STM **[72]**. The biological role of this interaction was interpreted in the context of a theory according to which WUS activates *CLV3* by directly binding to its promoter **[27]**. Thus, it has been hypothesised that STM binds independently to the *CLV3* promoter and subsequently interacts with WUS, thereby enhancing WUS binding on the promoter of *CLV3* **[72]**. This hypothesis, however, also does not explain the reverse relationship between the maxima of WUS concentration and *CLV3* expression, nor the fact that none of the many ChIP-seq and ChIP-chip experiments, which were done so far in an attempt to detect genome-wide WUS binding sites, have ever produced any relevant findings **[28, 42]**.

There is also some evidence that the basic leucine zipper domain TF bZIP30, known also as DRINK ME, which is expressed in the SAM as well as in other plant tissues, interacts with WUS **[73]**. However, the experimental evidence lies mostly at the biochemical level and there are no clear biological implications of this protein-protein interaction yet. Nevertheless, it seems that the WUS-bZIP30 complex is excluded from the nucleus and remains in the cytosol **[73]**.

Finally, an additional WUS protein interactor was identified in *Cucumis sativus*, but its biological role does not affect stem cell fate. The gene *SPOROCYTELESS* (*SPL*) is under positive transcriptional control by HD-ZIP III TFs in the ovule where its protein product interacts directly with WUS in a manner that enables the successful formation of female reproductive organs in the developing flower **[74]**.

1.7 Experimental approaches for the elucidation of protein-protein interactions

Interactions between proteins can be established by a large variety of experimental techniques **[75, 76]**. The ad hoc approach chosen depends on the biological question that is to be elucidated. Certain *in vitro* techniques require the prior isolation and purification of the proteins that will be tested, which makes them suitable for the validation of already identified

[‡] *wus-7* is a weak *wus* mutant that can still form a SAM and eventually many fertile flowers **[71]**.

or suspected protein-protein interactions (e.g. *in vitro* pulldown, co-immunoprecipitation reactions (co-IPs), Förster Resonance Energy Transfer (FRET), surface plasmon resonance, microscale thermophoresis, etc). Moreover, such techniques can readily be combined with overexpression of the proteins of interest in a heterologous biological system, a fact that can circumvent possible low protein concentrations in the native organism. Although following such an approach requires initially additional effort so as to set up the experimental setting (e.g. creation of expression vectors), it nevertheless makes it easier to establish which protein domains are responsible for these protein-protein interactions since it is relatively straightforward to examine the effect of protein sequence perturbations.

Other techniques are, however, more suitable for the high-throughput discovery of novel protein-protein interactions (e.g. Y2H screening, phage display, etc). Y2H screening is a very common approach which entails the expression of the protein of interest (bait molecule), fused to the binding domain of a TF, in one of the two mating types of *Saccharomyces cerevisiae*. In parallel, a virtually unrestricted number of compatible *S. cerevisiae* clones expressing any possible candidate protein interactors (prey molecules), which are fused to the activation domain of the same TF, are also generated. Following multiple pairwise matings of the yeast clone expressing the bait protein to the clones expressing pray proteins, any successful interaction between a certain couple of paired proteins results in transcriptional output that can be monitored easily, while the whole procedure can also be efficiently automated **[77]**.

In the previous decades, the direct isolation of a protein from its native organism under conditions that do not disrupt the binding of any protein interactors was of minimal use for the high-throughput discovery of novel protein-protein interactions. The resulting sample could not be efficiently analysed and its content be identified with the then current experimental arsenal, without having *a priori* knowledge of the possible proteins present in it. Yet, the coupling of protein isolation workflows to Mass Spectrometry (Mass-Spec) instruments that are capable of identifying thousands of proteins from complex biological samples lead to the formation of the field of Proteomics **[78, 79]**. For the organisms whose genome sequence is known, a typical experimental scenario starts with a co-IP, which aims to pull down the protein of interest out of a lysate that contains all proteins expressed in a tissue, along with any interacting partners. After the necessary washing steps in order to remove the background of unspecifically bound proteins and the proteolytic digestion that is needed for the generation of Mass-Spec compatible peptides, the protein content of the eluate is identified and quantified by injecting it to a tandem Mass-Spec analyser and comparing the generated MS/MS spectra to the respective proteome database. Such an experimental approach can also lead to the identification of novel post-translational modifications (PTMs) of any identified proteins.

Finally, it is worth mentioning that Mass-Spec Proteomics also enables efficient detection of transient protein-protein interactions. Such weak interplay between protein molecules cannot be easily maintained upon cell lysis. However, certain enzymes can be fused to the protein of interest that will subsequently label *in vivo* any proteins that come into proximity of the former **[80, 81]**. This chemical labelling can then be identified by Mass-Spec.

1.8 Aim

As it was described earlier in this chapter, the TF WUS is the basic regulator of stem cell fate in the SAM of *A. thaliana* and is involved in several pathways that control SAM development and function. Furthermore, it was also presented that WUS interacts physically with other proteins in the SAM and that these interactions are a prerequisite for WUS to operate as activator or repressor of gene expression. Nevertheless, WUS biology still cannot be fully explained. For example, how exactly WUS exerts transcriptional control on its gene targets or what are the exact details of the directed WUS movement from the OC into the CZ are still questions that remain unresolved. Therefore, I attempted to analyse the *in vivo* WUS complex and identify potential interacting proteins in order to elucidate such open issues. To this end, I performed:

- Y2H screenings, during which WUS was paired with an extensive library of TFs from *A. thaliana*,
- co-immunoprecipitation reactions, for which the bait were WUS fusion proteins expressed in *A. thaliana* meristematic tissue or *Nicotiana benthamiana* leaves.

Identified candidates were initially scrutinised by reproducing their interaction with WUS *in planta* and detecting it with Acceptor Photobleaching FRET (AP-FRET). Selected candidates were further validated by creating *A. thaliana* CRISPR mutants for the respective loci and examining them for perturbations of stem cell function. Moreover, the *in vivo* expression of these candidates was studied by performing *in situ* hybridization experiments using WT plants and by creating translational reporters in the WT as well as in the CRISPR mutant background. Additionally, the effect of WUS phosphorylation PTMs that were identified by Mass-Spec was investigated by mutating *WUS* and expressing the resulting phospho-mutants and phospho-mimics in *A. thaliana*.

Chapter 2

Materials

2.1 Instruments

The instruments that were used for conducting the experiments that are presented in this thesis are listed below:

Name	Company
Adaptis A1000 Incubator	Conviron, Winnipeg, Canada
Binder BD Series Incubator	Binder, Tuttlingen, Germany
CFI Apo LWD 25XW 1.10 Water Dipping Objective Lens	Nikon, Tokyo, Japan
Dyad DNA Engine Peltier Thermal Cycler	Bio-Rad, Hercules, USA
Electrophoresis Power Supply-EPS 301	GE Healthcare Bio-sciences, Fairfield, USA
Eppendorf Centrifuge 5424	Eppendorf, Hamburg, Germany
Eppendorf Centrifuge 5424R	Eppendorf, Hamburg, Germany
Eppendorf Centrifuge 5702R	Eppendorf, Hamburg, Germany
Eppendorf Centrifuge 5810R	Eppendorf, Hamburg, Germany
Eppendorf Thermomixer 5436	Eppendorf, Hamburg, Germany
gentleMACS Dissociator	Miltenyi Biotec, Bergish Gladbach, Germany
Innova 44 Shaking Incubator	New Brunswick Scientific, Enfield, USA
INTAS Imager	INTAS Science Imaging Instruments, Göttingen, Germany
Mic qPCR Cycler	Bio Molecular Systems, Upper Coomera, Australia
MicroPulser electroporator	Bio-Rad, Hercules, USA
Milli-Q Water purification system	Millipore, Billerica, USA
neoBlock-HeizerDuo Heating Block	neoLab, Heidelberg, Germany
NanoDrop 2000c spectrophotometer	ThermoScientific, Waltham, US

Name	Company
Nikon A1 Confocal Microscope	Nikon, Tokyo, Japan
Nikon D60 Digital Camera	Nikon, Tokyo, Japan
Nikon SMZ 745 stereo microscope	Nikon, Tokyo, Japan
poly klima Incubator	poly klima, Freising, Germany
Sonicator Misonix S-4000	Misonix, Farmingdale, USA
TissueLyser II	Qiagen, Hilden, Germany
SORVALL Evolution RC Centrifuge	ThermoScientific, Waltham, USA
Vortex Genie 2	Scientific Industries, Bohemia, USA
Waterbath 1083	GFL, Burgwedel, Germany
Waterbath WNB 7	Memmert, Schwabach, Germany
Zeiss Axio Imager.M1 epifluorescence microscope	Carl Zeiss Microscopy, Oberkochen, Germany
Zeiss SteREO Discovery.V20 stereo microscope	Carl Zeiss Microscopy, Oberkochen, Germany
List of instruments	

2.2 Chemicals

All chemicals were purchased from the following companies: AppliChem (Darmstadt, Germany), Merck (Darmstadt, Germany), Roth (Karlsruhe, Germany) and Sigma-Aldrich (St. Louis, USA). The chemicals used for preparing yeast media were purchased from MP Biomedicals (Irvine, USA). The only other exceptions are listed below:

Name	Company
Bromophenol Blue	Waldeck, Münster, Germany
Colloidal Coomassie Stain	Protein Ark, Sheffield, United Kingdom
Hygromycin B	Roche, Basel, Switzerland
MS medium	Duchefa, Harlem, Germany
Phyto Agar	Duchefa, Harlem, Germany
Silwet L-77	Lehle Seeds, Texas, USA

2.3 Enzymes

- Superscript III, Invitrogen, Thermo Fischer Scientific, Waltham, USA
- RevertAid RT, Thermo Fischer Scientific, Waltham, USA
- Q5 High-Fidelity DNA Polymerase, New England Biolabs, Ipswich, USA
- PHUSION High-Fidelity DNA Polymerase, Thermo Fischer Scientific, Waltham, USA
- JumpStart REDTaq ReadyMix, Sigma-Aldrich, St. Louis, USA

- JumpStart Taq ReadyMix, Sigma-Aldrich, St. Louis, USA
- Benzonase endonuclease, Merck Millipore, Darmstadt, Germany
- Proteinase K, Roche, Basel, Switzerland
- Lysozyme (powder), neoLab, Heidelberg, Germany
- T4 DNA Ligase (1 u/µl), Thermo Fischer Scientific, Waltham, USA
- T4 DNA Ligase Highly Concentrated (30 u/µl), Thermo Fischer Scientific, Waltham, USA
- Restriction endonucleases, Thermo Fischer Scientific, Waltham, USA and New England Biolabs, Ipswich, USA
- Fast Digest restriction endonucleases, Thermo Fischer Scientific, Waltham, USA
- Antarctic Phosphatase, New England Biolabs, Ipswich, USA
- Thermolabile Exonuclease I, New England Biolabs, Ipswich, USA
- DNase I, Roche, Basel, Switzerland

2.4 Molecular biology and biochemistry reagents

- 6x DNA Loading Dye, Thermo Fischer Scientific, Waltham, USA
- dNTPs, Thermo Fischer Scientific, Waltham, USA
- Generuler DNA ladder, Thermo Fischer Scientific, Waltham, USA
- Pageruler Prestained Protein ladder, Thermo Fischer Scientific, Waltham, USA
- 10x T4 DNA Ligase buffer, Thermo Fischer Scientific, Waltham, USA
- 5x High Fidelity PHUSION DNA polymerase buffer, Thermo Fischer Scientific, Waltham, USA
- 5x Q5 High Fidelity DNA polymerase buffer, New England Biolabs, Ipswich, USA
- 10x Restriction endonuclease buffers, Thermo Fischer Scientific, Waltham, USA
- 10x Fast Digest Restriction endonuclease buffers, Thermo Fischer Scientific, Waltham, USA
- 10x Restriction endonuclease buffers, New England Biolabs, Ipswich, USA
- EDTA-free Complete Protease Inhibitor, Roche, Basel, Switzerland
- Protease Inhibitor Cocktail for plant cell and tissue extracts, Sigma-Aldrich, St. Louis, USA

- Amersham ECL Prime Western Blotting Detection Reagent, Cytiva, Marlborough, USA
- Protein G PLUS-Agarose beads, Santa Cruz Biotechnology, Dallas, USA
- Strep-Tactin Superflow Agarose beads, IBA Lifesciences, Goettingen, Germany

2.5 Kits

- innuPREP Plasmid Mini Kit, analytikjena, Jena, Germany
- innuPREP DOUBLEpure Kit, analytikjena, Jena, Germany
- DNeasy Plant Mini Kit, Qiagen, Hilden, Germany
- RNeasy Plant Mini Kit, Qiagen, Hilden, Germany
- RNeasy Mini Kit, Qiagen, Hilden, Germany

2.6 Antibodies

Name	Organism	Company	Catalog Number
anti-WUSCHEL	Rabbit	Agrisera, Vännäs, Sweden	AS111759
anti-GFP	Rabbit	abcam, Cambridge, Great Britain	ab290
anti-GFP	Mouse (9F9.F9)	abcam, Cambridge, Great Britain	ab1218
anti-GFP	Rabbit	K. Schumacher group, Heidelberg University	in-house generation
anti-FLAG	Mouse (M2)	Sigma-Aldrich, St. Louis, USA	F3165
anti-diglycyl_Lysine	Mouse (GX41)	Merck Millipore, Darmstadt, Germany	MABS27
anti-histone_H2B	Rabbit	abcam, Cambridge, Great Britain	ab1790
anti-histone_H3	Rabbit	abcam, Cambridge, Great Britain	ab1791

Name	Organism	Company	Catalog Number
anti-Rabbit_IgG-HRP	Donkey	Santa Cruz Biotechnology, Dallas, USA	sc-2313
anti-Rabbit_IgG-HRP	Mouse	Santa Cruz Biotechnology, Dallas, USA	sc-2357
List of antibodies			

2.7 Antibodies coupled to beads

Name	Organism	Company	Catalog Number
GFP-Trap_A	Alpaca	ChromoTek GmbH, Planegg-Martinsried, Germany	gta-20

2.8 Antibiotics for bacterial selection

Name	Working concentration
Ampicillin	100 µg/ml
Chloramphenicol	25 µg/ml
Gentamicin	20 µg/ml
Kanamycin	50 µg/ml
Rifampicin	?? µg/ml
Spectinomycin	100 µg/ml
Streptomycin	30 µg/ml
Tetracycline	5 µg/ml

2.9 Antibiotics and herbicides for plant selection

Name	Working concentration
BASTA (Glufosinate ammonium in soil)	20 µg/ml
D-Alanine	12 mM
Hygromycin B	30 µg/ml
Kanamycin	70 µg/ml

2.10 Solutions and buffers

This section includes only solutions and buffers that were used in routine experimental procedures. The buffers and solutions that pertain to specific protocols are listed in the respective entry in the ”Methods” chapter along with the experimental details for each protocol.

- **Tris-buffered saline (TBS)**: 50 mM Tris pH 7.5, 150 mM NaCl.
- **Tris-buffered saline Tween-20 (TBS-T)**: 50 mM Tris pH 7.5, 150 mM NaCl, 0.1% Tween-20.
- **Towbin buffer**: 25 mM Tris, 192 mM Glycine.
- **Laemmli buffer 5x**: 10% SDS, 3.5% β-mercaptoethanol, 310 mM Tris-HCl pH 6.8, 50% glycerol, 0.0025% Bromophenol blue.
- **10x Phosphate-buffered saline (PBS)**: 1.3 M NaCl, 30 mM NaH_2PO_4, 70 mM Na_2HPO_4.
- **20x SSC**: 3 M NaCl, 0.3 M Na-citrate.
- **Coomassie dye**:
- **Coomassie Destain buffer**:
- **TE buffer**: 10 mM Tris-HCl pH 8.0, 1 mM EDTA pH 8.0.

2.11 Cultivation media

- **1/2 Murashige and Skoog (1/2 MS) plates**: 0.215% w/v MS, 0.05% w/v MES, 0.7% w/v Phytoagar, pH 5.7.
- **Lysogeny Broth medium (LB)**: 1% w/v tryptone, 0.5% w/v yeast extract, 0.5% w/v NaCl, pH 7.0, 1.5% w/v agar (optional).
- **YPAD medium**: 1% w/v Bacto yeast extract, 2% w/v Bacto peptone, 80 mg/L adenine hemisulfate, 2% w/v glucose, 20 g/L Bacto agar (optional)
- **Synthetic Defined Drop-Out -LEU medium**: 6.7 g/L Yeast Nitrogen base without amino acids and with ammonium sulfate, 0.64 g/L Complete Supplement Mixture -LEU -TRP, 50 mg/L L-Tryptophan, 80 mg/L adenine hemisulfate, 20 g/L glucose, 20 g/L Bacto agar (optional)
- **Synthetic Defined Drop-Out -TRP medium**: 6.7 g/L Yeast Nitrogen base without amino acids and with ammonium sulfate, 0.64 g/L Complete Supplement Mixture -LEU -TRP, 100 mg/L L-Leucine, 80 mg/L adenine hemisulfate, 20 g/L glucose, 20 g/L Bacto agar (optional)
- **Synthetic Defined Drop-Out -LEU -TRP medium**: 6.7 g/L Yeast Nitrogen base without amino acids and with ammonium sulfate, 0.64 g/L Complete Supplement Mixture -LEU -TRP, 80 mg/L adenine hemisulfate, 20 g/L glucose, 20 g/L Bacto agar (optional)
- **Synthetic Defined Drop-Out -HIS -LEU -TRP medium**: 6.7 g/L Yeast Nitrogen base without amino acids and with ammonium sulfate, 0.61 g/L Complete Supplement Mixture -ADE -HIS -LEU -TRP, 90 mg/L adenine hemisulfate, 20 g/L glucose, 20 g/L Bacto agar (optional)

2.12 Organisms

2.12.1 Plants

All plants used in this study belong to the species *Arabidopsis thaliana*, ecotype Columbia and the species *Nicotiana benthamiana*.

2.12.2 Bacteria

All cloning was mediated by use of the *Escherichia coli* strain XL1-Blue MR. Experiments involving expression of *A. thaliana* proteins in a heterologous bacterial system were done by utilising the *E. coli* strain Rosetta pLysS. All plant transformation experiments were conducted by using the *Agrobacterium tumefaciens* strains ASE and GV3101.

XL1-Blue MR: Δ(*mcrA*)*183* Δ(*mcrCB-hsdSMR-mrr*)*173 endA1 supE44 thi-1 recA1 gyrA96 relA1 lac*.

Rosetta pLysS: F^- *ompT gal dcm lon? hsdS*$_B$*(*$r_B^- m_B^-$*)* λ(DE3 [*lacI lacUV5-T7p07 ind1 sam7 nin5*]) [*malB*$^+$]$_{K\text{-}12}$(λ^S) pLysSRARE[*T7p20 ileX argU thrU tyrU glyT thrT argW metT leuW proL ori*$_{p15A}$](Cm^R).

ASE: chromosome Chl^R, disarmed resident Ti plasmid Kan^R, pSoup helper plasmid Tet^R, cloning destination vector $Spect^R$.

GV3101: chromosome Rif^R, disarmed resident Ti plasmid $Gent^R$, cloning destination vector Kan^R.

2.12.3 Yeast

The Y2H screenings presented in this thesis were done with the yeast strains PJ69-4alpha and PJ69-4A. Both strains are identical except for the mating type.

PJ69-4alpha: *MATalpha, trp1-901, leu2-3,112, ura3-52, his3-200, gal4Δ, gal80Δ, GAL2-ADE2, LYS2::GAL1-ADE2, LYS2::GAL1-HIS3, met2::GAL7-lacZ.*

PJ69-4A: *MATa, trp1-901, leu2-3,112, ura3-52, his3-200, gal4Δ, gal80Δ, GAL2-ADE2, LYS-2::GAL1-ADE2, LYS2::GAL1-HIS3, met2::GAL7-lacZ.*

2.13 Plasmid vectors and constructs

The full list of vectors, which were constructed during the course of this thesis, is presented in the Supplement due to its size.

2.14 Oligonucleotides

The full list of primers, which were used for carrying out the experiments presented in this thesis, is presented in the Appendix due to its size.

2.15 Software

The following list includes the software that was used for carrying out this thesis.

- FIJI: analysis of microscopy images.
- Geneious v9.1: *in silico* cloning.
- GIMP v2.10.32: image annotation and figure generation.
- KNIME v4.5.2: analysis of microscopy images.
- LibreOffice v7.2: office application.
- MaxQuant v1.16.17: analysis of Mass-Spec data from *N. benthamiana*.
- MaxQuant v2.1.3: analysis of Mass-Spec data from *A. thaliana*.
- MacTex: LaTeX distribution.
- Mendeley Studio: bibliography management.
- Microsoft Office 365: office application.
- Microsoft Onenote: note-taking program.
- MikTeX: LaTeX distribution.
- NIS Elements v3: analysis of microscopy images.
- R v4.0.4: statistical analysis.
- RStudio v1.4.1103: figure generation using R packages.
- TeXStudio v4.3.1: TeX editor.

Chapter 3

Methods

3.1 Plant growth

The *A. thaliana* adult plants and many freshly sown out seedlings were grown on soil at 22 °C, on a long day cycle (16 hours light and 8 hours darkness) and at 60% relative humidity. The *A. thaliana* seedlings that were initially sown out on 1/2 MS plates were also grown at 22 °C, on a long day cycle (16 hours light and 8 hours darkness) before transplantation. In all cases, the *A. thaliana* seeds were stratified for at least 48 hours by storing them at 4 °C and preventing any exposure to light. Seeds were sterilized before stratification only if they were to be sown out on 1/2 MS plates.

The *N. benthamiana* adult plants and seedlings were grown on soil at 25 °C, on a long day cycle (16 hours light and 8 hours darkness) and at 60% relative humidity.

3.2 *A. thaliana* seed sterilization

Seed sterilisation was carried out with ethanol. The seeds were resuspended thoroughly in 70% ethanol and placed on a rotator for 20 minutes. Subsequently, the seeds were collected by gravity and the supernatant was discarded. The seeds were then resuspended in 100% ethanol and were poured immediately onto a pre-sterilized filter paper, placed in a laminar flow hood. The seeds were left to dry completely and were finally dispersed on 1/2 MS plates.

3.3 Dexamethasone treatment of inducible transgenic *A. thaliana* lines

Proteins of interest from *A. thaliana* were subcloned under the chimeric 6xpOp promoter that gets activated by the chimeric transcription activator GR-LhG4 upon induction with dexamethasone (DEX). These transgenic *A. thaliana* lines were induced by spraying adult plants with DEX solutions, which were created by diluting a stock solution (30 mM DEX in 100% ethanol) to a working concentration of either 20 µM or 30 µM DEX and adding Silwet L-77

(C_f = 0.02%). The mock solutions were identical to the DEX solutions, the only difference being the substitution of DEX with 100% ethanol.

3.4 Crosslinking plant tissue with formaldehyde

Tissue samples from *A. thaliana* and *N. benthamiana* were fixed by incubation in 1.5% formaldehyde for 15 minutes under vacuum at R/T. Subsequently, the fixation reaction was stopped by adding glycine to a C_f = 131 mM and incubating further for 5 minutes under vacuum at R/T.

The 1.5% formaldehyde working solution was created by diluting the 16% formaldehyde stock solution with H_2O.

3.5 Processing plant tissue with the gentleMACS dissociator for nuclei isolation

Each sample was submerged in 10 mL of Nuclei Extraction Buffer 1 and processed with a gentleMACS Dissociator at 4oC (tubes M, program ”Protein_01”, executed thrice). After the dissociation step had concluded, the rest of Nuclei Extraction Buffer 1 was used to rinse the tube M up to a final volume of 30 mL.

3.6 Crude protein extraction from plants with Laemmli buffer

Ground plant tissue or isolated plant nuclei were resuspended in 2x Laemmli buffer at a 1:5 ratio, i.e. 100 mg tissue were mixed with 500 µl 2x Laemmli buffer, and then incubated for 5 minutes at 95 °C.

3.7 Crude protein extraction from plant tissue and IP for GFP-tagged proteins

1-2 g of plant material were frozen in liquid nitrogen and ground into powder with a mortar and pestle. For every 100 mg of plant material 150 µl of cold Extraction buffer were added and the samples were mixed thoroughly. Next, each sample was incubated with rotation for 10 minutes at 4 °C. In the meantime, 50 µl of GFP-Trap bead slurry per sample were equilibrated with 1000 µl of Washing buffer, followed by centrifugation at 2500 g, for 2 minutes, at 4 °C. The equilibration step was repeated twice. The cell debris was removed by centrifugation at 16000 g, for 15 minutes, at 4 °C. The supernatant was transferred to a fresh tube and diluted by adding Washing buffer, until Triton X-100 reached a C_f of 0.2%. The pre-equilibrated beads were added in the samples and the latter were incubated with rotation for 1 hour at 4 °C. Subsequently, the beads were collected by centrifugation at 500 g, for 2 minutes, at 4 °C. The flow-through was removed and the beads were washed by

adding 1000 µl Washing buffer (without Protease Inhibitor). The samples were incubated for 5 minutes on ice and were then centrifuged at 500 g, for 2 minutes, at 4 °C. The supernatant was removed and the washing step was repeated twice. Finally, elution was performed by adding 100 µl 0.2 M glycine pH 2.5 to each pellet of beads and incubating under constant mixing for 60 seconds at R/T. The beads were collected by centrifugation at 500 g, for 2 minutes, at 4 °C. The supernatant was transferred into a new tube and 10 µl 1M Tris pH 10.4 were added to it.

Extraction buffer: 50 mM Tris-HCl pH 7.5, 5 mM EGTA, 150 mM NaCl, 0.5% Triton X-100, 25 mM DTT, 1x Protease Inhibitor (Sigma), 1 µl/ml benzonase.

Washing buffer: 50 mM Tris-HCl pH 7.5, 5 mM EGTA, 150 mM NaCl, 25 mM DTT, 1x Protease Inhibitor (Sigma).

Washing buffer (without Protease Inhibitor): 50 mM Tris-HCl pH 7.5, 5 mM EGTA, 150 mM NaCl, 25 mM DTT.

3.8 Crude protein extraction from fixed plant tissue and IP for GFP-tagged proteins

1-2 g of plant material, previously cross-linked with formaldehyde, were frozen in liquid nitrogen and ground into powder with a mortar and pestle. For every 100 mg of plant material 150 µl of cold Extraction buffer were added and the samples were mixed thoroughly. Next, each sample was incubated with rotation for 10 minutes at 4 °C. Subsequently, the sample was sonicated using a micro-tip with following settings: 20% - 40%, 5 x 15 seconds, 60 seconds gap. In the meantime, 50 µl of GFP-Trap bead slurry per sample were equilibrated with 1000 µl of Washing buffer, followed by centrifugation at 2500 g, for 2 minutes, at 4 °C. The equilibration step was repeated twice. The cell debris was removed by centrifugation at 16000 g, for 15 minutes, at 4 °C. The supernatant was transferred to a fresh tube and diluted by adding Washing buffer, until Triton X-100 reached a C_f of 0.2%. The pre-equilibrated beads were added in the samples and the latter were incubated with rotation for 1 hour at 4 °C. Subsequently, the beads were collected by centrifugation at 500 g, for 2 minutes, at 4 °C. The flow-through was removed and the beads were washed by adding 1000 µl Washing buffer (without Protease Inhibitor). The samples were incubated for 5 minutes on ice and were then centrifuged at 500 g, for 2 minutes, at 4 °C. The supernatant was removed and the washing step was repeated twice. Finally, elution was performed by adding 100 µl 0.2 M glycine pH 2.5 to each pellet of beads and incubating under constant mixing for 60 seconds at R/T. The beads were collected by centrifugation at 500 g, for 2 minutes, at 4 °C. The supernatant was transferred into a new tube and 10 µl 1M Tris pH 10.4 were added to it.

Extraction buffer: 50 mM Tris-HCl pH 7.5, 5 mM EGTA, 150 mM NaCl, 0.5% Triton X-100, 25 mM DTT, 1x Protease Inhibitor (Sigma), 1 µl/ml benzonase.

Washing buffer: 50 mM Tris-HCl pH 7.5, 5 mM EGTA, 150 mM NaCl, 25 mM DTT, 1x Protease Inhibitor (Sigma).

Washing buffer (without Protease Inhibitor): 50 mM Tris-HCl pH 7.5, 5 mM EGTA, 150 mM NaCl, 25 mM DTT.

3.9 Crude protein extraction from fixed plant tissue with RIPA buffer and IP for GFP-tagged proteins

This protocol is similar to the one of Section 3.8, but with different buffer compositions.

RIPA buffer: 50 mM Tris-HCl pH 7.5, 5 mM EGTA, 1 mM EDTA, 150 mM NaCl, 1.0% Triton X-100, 0.25% Na-Deoxycholate, 0.1% SDS, 25 mM DTT, 1x Protease Inhibitor (Sigma).

Washing buffer: 50 mM Tris-HCl pH 7.5, 5 mM EGTA, 1 mM EDTA, 150 mM NaCl, 25 mM DTT, 1x Protease Inhibitor (Sigma).

Washing buffer (without Protease Inhibitor): 50 mM Tris-HCl pH 7.5, 5 mM EGTA, 1 mM EDTA, 150 mM NaCl, 25 mM DTT.

3.10 Crude protein extraction from plant tissue and affinity purification for STREPII-tagged proteins

Before processing the plant material, 150 µl of Strep-Tactin Superflow bead slurry were blocked by resuspension in 2 ml solution B and incubation with rotation for 1 hour at R/T. The beads were collected by centrifugation at 1000 g, for 5 minutes, at 4 °C. Then, the beads were transferred into a gravity flow column (Micro Bio-Spin, Bio-Rad) and were equilibrated by applying 2000 µl of W-strep buffer. The equilibration step was repeated twice.

1 g of plant material was frozen in liquid nitrogen and ground into powder with a mortar and pestle. Next, 1.5 ml of cold Ex-strep buffer was added and the sample was mixed thoroughly, followed by incubation with rotation for 10 minutes at 4 °C. The cell debris was removed by centrifugation at 21000 g, for 10 minutes, at 4 °C and the supernatant was applied onto the Strep-Tactin Superflow beads. The flow-through was collected and was re-applied on the Strep-Tactin resin. Subsequently, the beads were washed as follows: 2 x 1 mL W-strep buffer, 4 x 0.5 mL W-strep buffer (without Triton X-100 and Protease Inhibitor). Finally, the elution was performed in the following pattern: 1 x 80 µl E-buffer (void volume), 4 x 100 µl E-buffer (collected as two sequential 200 µl pools).

Solution B: 0.5 mg/mL BSA, 0.5 mg/mL salmon-sperm DNA, 1x TBS-T.

Ex-strep buffer: 100 mM Tris-HCl pH 8.0, 5 mM EGTA, 150 mM NaCl, 0.5% Triton X-100, 25 mM DTT, 1x Protease Inhibitor (Sigma), 1 µl/ml benzonase, 100 µg/mL avidin.

Washing buffer: 50 mM Tris-HCl pH 8.0, 5 mM EGTA, 2.5 mM EDTA, 150 mM NaCl, 0.05% Triton X-100, 25 mM DTT, 1x Protease Inhibitor (Sigma).

Washing buffer (without Triton X-100 and Protease Inhibitor): 50 mM Tris-HCl pH 8.0, 5 mM EGTA, 2.5 mM EDTA, 150 mM NaCl, 25 mM DTT.

E-buffer: 10 mM Tris-HCl pH 8.0, 25 mM DTT, 10 mM desthiobiotin.

3.11 Nuclei isolation from plant tissue

The following protocol was used in order to isolate nuclei from *N. benthamiana* and *A.thaliana* leaves as well as from ap1/cal meristematic tissue. When this protocol was used for the subsequent detection of phospho-PTMs, the buffers listed here contained also 1x Phosphatase Inhibitor no2 (Sigma) and 1x Phosphatase Inhibitor no3 (Sigma).

2 g of plant tissue were frozen in liquid nitrogen and ground into powder using a mortar and pestle. The fine powder was resuspended in 30 mL of ice-cold Nuclei Extraction buffer 1 and the solution was filtered sequentially through a 100 µM and a 50 µM mesh. The filtrate was then centrifuged for 20 min at 1,900 g at 4 °C. The supernatant was gently removed and the pellet was resuspended in 1 mL Nuclei Extraction buffer 2a and then centrifuged for 20 min at 1,900 g at 4 °C. Next, the supernatant was collected and referred to as the “nucleus-depleted fraction”. The pellet was resuspended in 0.3 mL Nuclei Extraction buffer 2b and was slowly overlayed on 0.5 mL Nuclei Extraction buffer 3, followed by centrifugation for 45 min at 16000 g at 4 °C. This step was repeated twice. Finally, the pellet was resuspended either in lysis buffer and then used as input for an IP or in 2x Laemmli buffer and then boiled for crude protein extraction.

Nuclei Extraction buffer 1: 0.4 M sucrose, 10 mM Tris-HCl pH 8.0, 10 mM $MgCl_2$, 25 mM DTT, 1x protease inhibitor (Sigma).

Nuclei Extraction buffer 2a: 0.25 M sucrose, 10 mM Tris-HCl pH 8.0, 10 mM $MgCl_2$, 0.5% Triton X-100, 25 mM DTT, 1x protease inhibitor (Sigma).

Nuclei Extraction buffer 2b: 0.25 M sucrose, 10 mM Tris-HCl pH 8.0, 10 mM $MgCl_2$, 0.15% Triton X-100, 25 mM DTT, 1x protease inhibitor (Sigma).

Nuclei Extraction buffer 3: 1.7 M sucrose, 10 mM Tris-HCl pH 8.0, 2 mM $MgCl_2$, 0.15% Triton X-100, 25 mM DTT, 1x protease inhibitor (Sigma).

3.12 Protein extraction from isolated plant nuclei and IP for GFP-tagged proteins

A fresh plant nuclei prep or one that had been previously snap-frozen in liquid N_2 was resuspended in 0.3 ml of Extraction buffer by pipetting. Next, the sample was incubated

with rotation for 10 minutes at 4 °C. Subsequently, the sample was sonicated using a micro-tip with following settings: 10%, 5 x 15 seconds, 60 seconds gap. In the meantime, 50 µl of GFP-Trap bead slurry were equilibrated with 1000 µl of Washing buffer, followed by centrifugation at 2500 g, for 2 minutes, at 4 °C. The equilibration step was repeated twice. The cell debris was removed by centrifugation at 16000 g, for 15 minutes, at 4 °C. The supernatant was transferred to a fresh tube and diluted by adding Washing buffer, until Triton X-100 reached a C_f of 0.2%. The pre-equilibrated beads were added in the samples and the latter were incubated with rotation for 1 hour at 4 °C. Subsequently, the beads were collected by centrifugation at 500 g, for 2 minutes, at 4 °C. The flow-through was removed and the beads were washed by adding 1000 µl Washing buffer (without Protease Inhibitor). The samples were incubated for 5 minutes on ice and were then centrifuged at 500 g, for 2 minutes, at 4 °C. The supernatant was removed and the washing step was repeated twice. Finally, elution was performed by adding 100 µl 0.2 M glycine pH 2.5 to each pellet of beads and incubating under constant mixing for 60 seconds at R/T. The beads were collected by centrifugation at 500 g, for 2 minutes, at 4 °C. The supernatant was transferred into a new tube and 10 µl 1M Tris pH 10.4 were added to it.

Extraction buffer: 50 mM Tris-HCl pH 7.5, 5 mM EGTA, 150 mM NaCl, 1.0% Triton X-100, 25 mM DTT, 1x Protease Inhibitor (Sigma), 1 µl/ml benzonase.

Washing buffer: 50 mM Tris-HCl pH 7.5, 5 mM EGTA, 150 mM NaCl, 25 mM DTT, 1x Protease Inhibitor (Sigma).

Washing buffer (without Protease Inhibitor): 50 mM Tris-HCl pH 7.5, 5 mM EGTA, 150 mM NaCl, 25 mM DTT.

3.13 Protein extraction from isolated plant nuclei with RIPA buffer and IP for GFP-tagged proteins

This protocol is similar to the one presented in section 3.12, but involves different buffer compositions.

RIPA buffer: 50 mM Tris-HCl pH 7.5, 5 mM EGTA, 1 mM EDTA, 150 mM NaCl, 1.0% Triton X-100, 0.25% Na-Deoxycholate, 0.1% SDS, 25 mM DTT, 1x Protease Inhibitor (Sigma).

Washing buffer: 50 mM Tris-HCl pH 7.5, 5 mM EGTA, 1 mM EDTA, 150 mM NaCl, 25 mM DTT, 1x Protease Inhibitor (Sigma).

Washing buffer (without Protease Inhibitor): 50 mM Tris-HCl pH 7.5, 5 mM EGTA, 1 mM EDTA, 150 mM NaCl, 25 mM DTT.

3.14 Protein extraction from isolated plant nuclei and IP for GFP-tagged proteins under optimal conditions for the detection of phospho-PTMs

This protocol is similar to the one presented in section 3.12, but involves different buffer compositions.

Extraction buffer: 50 mM Tris-HCl pH 7.5, 5 mM EGTA, 150 mM NaCl, 1.0% Triton X-100, 25 mM DTT, 1x Protease Inhibitor (Sigma), 1x Phosphatase Inhibitor no2 (Sigma), 1x Phosphatase Inhibitor no3 (Sigma), 1 µl/ml benzonase.

Washing buffer: 50 mM Tris-HCl pH 7.5, 5 mM EGTA, 150 mM NaCl, 25 mM DTT, 1x Protease Inhibitor (Sigma), 1x Phosphatase Inhibitor no2 (Sigma), 1x Phosphatase Inhibitor no3 (Sigma).

Washing buffer (without Protease Inhibitor): 50 mM Tris-HCl pH 7.5, 5 mM EGTA, 150 mM NaCl, 25 mM DTT, 1x Phosphatase Inhibitor no2 (Sigma), 1x Phosphatase Inhibitor no3 (Sigma).

3.15 Protein extraction from isolated plant nuclei and IP for FLAG-tagged proteins

Before processing the plant material, 20 µl of protein G bead slurry were blocked by resuspension in 2 ml solution B and incubation with rotation for 1 hour at R/T. The beads were collected by centrifugation at 1000 g, for 5 minutes, at 4 °C. Then, the beads were equilibrated with 1000 µl of Washing buffer, followed by centrifugation at 1000 g, for 5 minutes, at 4 °C. The equilibration step was repeated twice.

A fresh plant nuclei prep or one that had been previously snap-frozen in liquid N_2 was resuspended in 0.3 ml of Extraction buffer by pipetting. Next, the sample was incubated with rotation for 10 minutes at 4 °C. Subsequently, the sample was sonicated using a micro-tip with following settings: 10%, 5 x 15 seconds, 60 seconds gap. The cell debris was removed by centrifugation at 16000 g, for 15 minutes, at 4 °C. The supernatant was transferred to a fresh tube and diluted by adding Washing buffer, until Triton X-100 reached a C_f of 0.2%. At that point, 2 µg of monoclonal anti-FLAG antibody were added to the sample, followed by incubation with rotation for 2 hours at 4 °C. Then, the pre-equilibrated beads were also added in the sample and the latter was incubated with rotation for 4 hours at 4 °C. Subsequently, the beads were collected by centrifugation at 500 g, for 5 minutes, at 4 °C. The flow-through was removed and the beads were washed by adding 1000 µl Washing buffer (without Protease Inhibitor). The sample was incubated for 5 minutes on ice and was then centrifuged at 500 g, for 5 minutes, at 4 °C. The supernatant was removed and the washing step was repeated twice. Finally, elution was performed by adding 50 µl 0.2 M glycine pH 2.5 to the pellet of beads and incubating under constant mixing for 60 seconds at R/T. The beads were collected

by centrifugation at 1000 g, for 5 minutes, at 4 °C. The supernatant was transferred into a new tube and 10 µl 1M Tris pH 10.4 were added to it.

Solution B: 0.5 mg/mL BSA, 0.5 mg/mL salmon-sperm DNA, 1x TBS-T.

Extraction buffer: 50 mM Tris-HCl pH 7.5, 5 mM EGTA, 150 mM NaCl, 1.0% Triton X-100, 25 mM DTT, 1x Protease Inhibitor (Sigma), 1 µl/ml benzonase.

Washing buffer: 50 mM Tris-HCl pH 7.5, 5 mM EGTA, 150 mM NaCl, 25 mM DTT, 1x Protease Inhibitor (Sigma).

Washing buffer (without Protease Inhibitor): 50 mM Tris-HCl pH 7.5, 5 mM EGTA, 150 mM NaCl, 25 mM DTT.

3.16 Protein extraction from isolated plant nuclei with RIPA buffer, IP, tryptic digestion and anti-diGLY IP for the enrichment of ubiquitinated tryptic peptides

WUSCHEL-GFP was extracted from plant nuclei preps according to the protocol presented in Section 3.13. However, the bound proteins were not eluted from the beads but were subjected to on-beads digestion with trypsin and the tryptic peptides were then purified with the SP3 clean-up protocol **[82]**. Next, the tryptic peptides were used as input for an anti-diGLY IP, during which an anti-diglycyl_Lysine monoclonal antibody was used to bind the tryptic peptides that had been ubiquitinated *in vivo* **[83]**. The antibody-peptide complexes were pulled out of solution with protein G beads and, finally, the purified peptides were eluted with formic acid and submitted for Mass Spectrometry.

3.17 In-gel digestion of protein samples for Mass Spectrometry (LC-MS/MS)

Protein samples were loaded in 10% polyacrylamide gels. After a brief SDS-PAGE run, enough to allow the samples to resolve for 1-1.5 cm in the separating gel, the gel lanes were cut into three slices containing the entire area of the resolved proteins. Each gel slice was fragmented into ca. 1 mm^3 pieces using a scalpel and was then destained followed by subsequent reduction, alkylation and overnight trypsin-LysC digestion.

More specifically, the gel pieces were destained by incubation in 1:1 (vol/vol) of 100 mM ammonium bicarbonate / acetonitrile for 30 minutes. Subsequently, the supernatant was discarded and the gel slices were fully covered in neat acetonitrile for 15 minutes. The proteins were reduced by incubating the gel slices in reduction solution (10 mM DTT in 100 mM ammonium bicarbonate) at 56 °C for 30 minutes. The reduction solution was removed and the gel slices were incubated in neat acetonitrile for 10 minutes. The acetonitrile was removed and the gel pieces were incubated in reduction solution (55 mM chloroacetamide in

100 mM ammonium bicarbonate) for 20 minutes at R/T. The reduction solution was discarded and neat acetonitrile was added to the gel pieces. Proteins were digested with the addition of trypsin-LysC solution (13 ng/µl in 50 mM ammonium bicarbonate) and the gel pieces were incubated for 30 minutes on ice. Subsequently, the gel slices were transferred to a thermostat shaker and incubated overnight at 37 °C. Peptides were extracted with the addition of 100 µl extraction buffer (5% formic acid / acetonitrile, 1:2 vol/vol) and incubation for 15 minutes on a shaker at 37 °C. The supernatant was removed and stored in a new tube. Neat acetonitrile was added to the gel slices and then were incubated for another 15 minutes at 37 °C. The supernatant was removed and combined with the previous one. All samples were air-dried in a speedvac for 1 hour. The peptides were finally dissolved in 0.1% formic acid (vol/vol) and stored at -20 °C until LC-MS/MS analysis.

3.18 Protein identification in co-IP samples with Mass Spectrometry (LC-MS/MS)

Peptides were resolved using the Easy NanoLC1200 liquid chromatography system fitted with a trapping (Acclaim Pepmap C18, 5 µm, 100 Å, 100 µm x 2 cm) and an analytical column (Acclaim PepMap RSLC C18, 2 µm, 100 Å, 75 µm x 50 cm). The outline of the analytical column was coupled directly to a Fusion Orbitrap (Thermo Fisher Scientific) mass spectrometer. Solvent A was 0.1% formic acid (vol/vol) and solvent B 80% acetonitrile (vol/vol), 0.1% formic acid (vol/vol). The peptides were loaded on the trap column with a constant flow of solvent A at a maximum pressure of 800 bar. Peptides were eluted from the analytical column at a constant flow rate of 300 nl/min and a temperature of 55 °C. During the elution, the percentage of solvent B was increased in a linear gradient from 3% to 8% in 4 minutes, then from 8% to 10% in 2 minutes, then from 10% to 32% for 68 minutes and then from 32% to 50% for another 12 minutes. At the end of the gradient, solvent B was kept at 100% for 7 minutes followed by re-equilibration of the analytical column for 10 minutes at 97% solvent A. The peptides were introduced to the mass spectrometer via a Pico-Tip Emitter 360 µm OD x 20 µm ID; 10 µm tip (New Objective) and a nano-source spray voltage of 2 kV. The ion transfer tube temperature was set to 275 °C. Full scan MS1 spectra were acquired within the range (m/z) of 375-1500 in the Orbitrap detector with a resolution of 120,000. The maximum injection time was set to 50 ms and automatic gain control target (AGC) to 1 x 106 ions. The most abundant ions within a 3 sec cycle time window were selected for fragmentation. Ions with unassigned charges and charges of 1 or >5 were excluded. Dynamic exclusion was set to 40 sec with a mass tolerance of ±10 ppm. For the MS2 scans, the quadrupole was used with an isolation window of 1.6 m/z. For peptide fragmentation higher-energy collisional dissociation (HCD) was used at 33%. MS2 scans were acquired in the linear ion trap that was operated in the rapid ion scan rate with an AGC target of 1 x 104 ions or a maximum injection of 50 ms. MS2 scans were acquired as centroid data type.

3.19 Analysis of Mass Spectrometry (LC-MS/MS) data

Raw files were processed using the MaxQuant software package. The peptide searches were performed either against the *Arabidopsis thaliana* Uniprot canonical database, which contained both reviewed and TrEMBL entries, or against the *Nicotiana benthamiana* database **[84]**. Enzyme digestion in MaxQuant settings was set to Trypsin-LysC while allowing for a maximum of up to 3 missed cleavages. Protein N-term acetylation, methionine oxidation and asparagine-glutamine deamidation were set as variable modifications. Carbamidomethylation of cysteine was set as a fixed modification. The minimum unique peptides option was set to 1. Both intensity-based absolute quantification and label-free quantification values were calculated. Peptide and protein hits were filtered at a false discovery rate (FDR) of 1% with a minimum peptide length of 7 or 6 amino acids. The reversed sequences of the target database were used as the respective decoy database. A second peptide search for the identification of chimeric MS2 spectra was always performed. All other MaxQuant options were left to their default settings. Subsequent analysis of the output tables was performed in Microsoft Excel and in the RStudio statistical software environment.

3.20 Yeast transformation

Liquid YPAD medium was inoculated from the appropriate yeast -80 °C stock and the culture was grown till saturation in a shaking incubator (220 rpm) at 30 °C. The cells were centrifuged at top speed for 5 seconds. The pellet volume was approximately measured. About 25 µl of yeast cells are required for a single transformation. The cells were then resuspended in 100 mM lithium acetate (LiAc), diluting the resuspension with more 100 mM LiAc if needed so as to keep a ratio of cells to LiAc at 25 µl cells / 1 ml LiAc. The appropriate volume of salmon sperm DNA (10 µl from a 10 mg/ml solution for one transformation) was denatured in boiling water for 5 minutes and then transferred immediately and stored till needed on ice. The resuspended cells were incubated for 5 minutes at 30 °C. The resuspension volume needed for one transformation, i.e. 1 ml, was transferred in a new Eppendorf tube, centrifuged at top speed for 5 seconds and the supernatant was discarded. Subsequently, the following components were pipetted into each tube: 240 µl (50 % w/v) PEG 3350, 36 µl 1 M LiAc, 10 µl salmon sperm DNA (10 mg/ml), 64 µl H2O, 5 µl plasmid DNA (between 100 ng to 5 µg). The cell pellet was then vortexed for at least 1 minute so as to resuspend it in this transformation mix, followed by incubation for 20 minutes at 42 °C. Finally, the cells were centrifuged at top speed for 10 seconds, the supernatant was removed and the pellet was resuspended in 200 µl H2O by gentle pipetting. 100 µl aliquots of the resuspended cells were plated on plates with the appropriate selective medium and incubated for 2-3 days at 30 °C.

3.21 Yeast mating

Fresh and large (2-3 mm) colonies of both PJ69-4 strains were picked from plates with the corresponding selective growth medium. Each colony was placed into a 1.5 ml Eppendorf tube containing 0.5 ml YPAD medium. The tubes were vortexed to completely resuspend the cells and then incubated for ca. 20-24 hours in a shaking incubator (200 rpm) at 30 °C. 100µl aliquots of the mating culture were spread on plates with Synthetic Defined Drop-Out -LEU -TRP selective growth medium and incubated for 2-3 days at 30 °C.

3.22 Yeast two-hybrid (Y2H) screening

The bait vectors for the Y2H screening were constructed by amplifying the coding sequence (CDS) of WUS and WUS mutants with PCR, using primers with flanking SalI and XhoI restriction sites. The PCR products were digested with SalI/XhoI, isolated and then ligated into vector pENTR1A. These intermediate pENTR1A-based vectors were then used to ligate the CDSs of interest into the destination vector pDEST32 via Gateway reactions. The resulting bait vectors had the WUS or WUS mutant CDS inserted in-frame with the GAL4 DNA binding domain. All bait vectors were transformed into yeast strain PJ69-4alpha followed by an auto-activation test. The latter was conducted by mating the aforementioned strain to yeast strain PJ69-4A containing an empty pDEST22 destination vector, i.e. no prey protein was present to facilitate the reconstitution of the GAL4 transcription factor, selecting diploid clones on Synthetic Defined Drop-Out -LEU -TRP selective growth medium and finally plating these diploid clones on Synthetic Defined Drop-Out -HIS -LEU -TRP selective growth medium and observing for possible background growth. The stringency conditions for the auto-activation test were controlled by the addition of 3-aminotriazole (3-AT, a competitive inhibitor of the HIS3 product) in the Synthetic Defined Drop-Out -HIS -LEU -TRP selective growth medium (0 mM, 5 mM, 10 mM, 15 mM and 20 mM). Some weak auto-activation was observed in some of the experiments, but 5 mM 3-AT were enough to suppress it.

The library screening per se was performed in the lab of Prof. R. Immink in Wageningen, the Netherlands. The strain PJ69-4alpha containing the bait vector was used for matings with different clones of the strain PJ69-4A containing the prey vectors, i.e. in total ca. 2000 *A. thaliana* transcription factors inserted into the destination vector pDEST22 in-frame with the GAL4 activation domain. The screening stringency was controlled by adding different concentration of 3-AT in order to differentiate between weak and strong protein-protein interactions.

3.23 Preparing *A. thaliana* tissue sections for RNA *in situ* hybridization

Shoot apical meristems (SAMs) from several *A. thaliana* plants were dissected, harvested and transferred into Eppendorf tubes with fixative solution placed on ice. The SAMs

are then placed into cassettes, which are transferred into a cassette holder and placed in the retort of the automated vacuum tissue processor Leica ASP200/ASP300. The cassettes are then incubated successively in the following solutions (at R/T, unless otherwise noted): fixative for 4 hours, 70% denatured ethanol for 1 hour, 90% denatured ethanol for 1 hour, 90% denatured ethanol for 1 hour, 100% denatured ethanol plus Eosin Y for 1 hour, 100% denatured ethanol for 1 hour, 100% absolute ethanol for 1 hour, xylol for 1 hour, xylol for 1 hour, xylol for 1 hour 15 minutes, paraplast for 1 hour at 62 °C, paraplast for 1 hour at 62 °C, paraplast for 3 hours at 62 °C. The SAMs are then moved to the Leica EG1160 embedding station and mounted onto molds. The embedded SAMs are then divided into 8 µm sections using a microtome.

Fixative: 50% ethanol, 5% glacial acetic acid, 3.7% formaldehyde.

3.24 RNA probe synthesis for RNA *in situ* hybridization

The coding sequences of interest were subcloned so as to be flanked by RNA polymerase promoters (T7 and SP6). Subsequently, PCR was carried out to generate a linear fragment. 200 ng of the latter were used for each one of two separate *in vitro* transcription reactions, one leading to the synthesis of the anti-sense probe and the other to the synthesis of the sense probe. Each transcription reaction contained 2 µl 10x transcription buffer, 1 µl RNase inhibitor, 2 µl 10x DIG NTP mix, 2 µl T7 or SP6 RNA polymerase, 200 ng purified PCR product, H_2O up to a final volume of 20 µl. Transcription reactions were incubated for 2 hours at 37 °C. Then 2 µl RNase-free DNase I were added and the samples were incubated for 15 min at 37 °C. The reactions were then stopped by adding 2 µl 0.2 M EDTA pH 8.0. The synthesized RNA probes were purified using the Qiagen RNA Isolation kit in order to remove any unincorporated DIG nucleotides. If the RNA probe was longer than 500 bases, then it was hydrolyzed by adding 100 µl 2x Hydrolysis buffer and incubating at 60 °C until the probe was reduced to ca. 200 bases. The incubation time was calculated using the formula $time = \frac{L_i - L_f}{K \times L_i \times L_f}$, where L_i is the initial probe length in kb, L_f is the final probe length in kb and K is a constant corresponding to 0.11 kb per minute. The hydrolysis was stopped by adding 20 µl 10% acetic acid and the probes were precipitated by adding 1 µl 1 M $MgCl_2$, 1 µl 20 mg/ml glycogen, 600 µl 100% ethanol and incubating at -20 °C overnight. The next day the samples were centrifuged at maximum speed, for 30 minutes, at 4 °C and washed with 750 µl ice-cold 80% ethanol. The samples were again centrifuged at maximum speed, for 30 minutes, at 4 °C and the supernatant was decanted. The samples were air-dried for 10 minutes at R/T and subsequently resuspended in 50 µl H_2O. The probes were finally diluted by adding 50 µl deionized formamide and stored at -80 °C.

2x Hydrolysis byffer: 120 mM Na_2CO_3, 80 mM $NaHCO_3$, pH 10.2.

3.25 RNA *in situ* hybridization using *A. thaliana* tissue sections

The Proteinase-K buffer was preheated to 37 °C. The slides with the sections were dewaxed and rehydrated by placing them in a rack and incubating them successively at R/T in glass jars containing the following solutions: 100% Histoclear for 10 minutes with gentle shaking, 100% Histoclear for 10 minutes with gentle shaking, 100% ethanol for 5 minutes, 100% ethanol for 5 minutes, 95% ethanol/H_2O for 1 minute, 90% ethanol/H_2O for 1 minute, 80% ethanol/H_2O for 1 minute, 60% ethanol/0.75% NaCl for 1 minute, 30% ethanol/0.75% NaCl for 1 minute, 0.75% NaCl for 2 minutes, 1x PBS for 2 minutes. Next, 10.4 µl Proteinase K were added to 200 ml of Proteinase K buffer. The slides were transferred into this solution and were incubated for 30 minutes at 37 °C. Subsequently, the slides were incubated successively at R/T in the following solutions: glycin/PBS for 2 minutes, fixative for 2 minutes, 1x PBS for 5 minutes, 1x PBS for 5 minutes. The sections were then dehydrated by incubating the slides successively at R/T in the following solutions: 0.75% NaCl for 2 minutes, 30% ethanol/0.75% NaCl for 1 minute, 60% ethanol/0.75% NaCl for 1 minute, 80% ethanol/H_2O for 1 minute, 90% ethanol/H_2O for 1 minute, 95% ethanol/H_2O for 1 minute, 100% ethanol for 2 minutes, 100% ethanol until application of the probe.

The humid chamber for the hybridization was assembled by putting 2 sheets of kitchen paper on the bottom part of a plastic tray, fixing on top of these sheets metal holders in order to keep the slides elevated and soaking the kitchen paper with soaking solution. The probe volume needed for the experiment was denatured for 5 minutes at 80 °C and placed immediately afterwards on ice. Next, the Hybridization mix was prepared fresh and kept on a heating block at 50 °C until use. The cold probe was mixed with the Hybridization mix and the resulting mix was placed on the heating block. The slides were taken out of the ethanol, one by one, and placed on the heating block in order to dry (the sections turn from transparent to white). When dry, each slide was removed from the block and 100 µl of the Hybridization/probe mix were applied on it. Each slide was then covered with a parafilm piece of similar size and placed into the humid chamber. The latter was then sealed with plastic foil and placed into an incubator set at 55 °C for overnight incubation (> 14 hours). The washing solutions needed for the subsequent washes were also preheated at 55 °C overnight.

The following day, the slides were immersed in a preheated glass jar with 2x SSC at 55 °C for 2 minutes so as to enable the buffer to infiltrate the space between the slide and the parafilm. The later was then removed with a pair of forceps and the slide was placed back in the glass jar. Once all slides had been processed, they were transferred in a fresh preheated glass jar with 0.2x SSC and incubated for 30 minutes at 55 °C. This washing step was repeated with new 0.2x SSC solution three times for a total incubation of 2 hours. The slides were then washed successively with the following solutions: 0.2x SSC for 5 minutes at 37 °C, 0.2x SSC for 5 minutes at R/T, 1x PBS for 5 minutes at R/T, blocking solution for 30 minutes at R/T with gentle shaking, antibody buffer for 15 minutes at R/T with gentle shaking. During these last blocking steps, the anti-DIG antibody was diluted from stock into the antibody buffer at a ratio of 1:1250. Also, a new humid chamber was assembled, but the

sheets were soaked with water this time. 100 µl anti-DIG antibody solution were applied to each slide. The slides were then covered with parafilm and placed inside the humid chamber for 90 minutes. Afterwards, the slides were immersed in a glass jar with antibody buffer and the parafilm was removed. The rack with the slides was moved into another jar with fresh antibody buffer and incubated for 30 minutes with gentle shaking. The antibody buffer was exchanged with fresh one and the incubation continued for 30 minutes with gentle shaking.

The slides were finally transferred into the detection buffer and incubated for 5 minutes without shaking. In the meantime, the NBT-BCIP solution was prepared by adding NBT-BCIP in the detection buffer at a ratio of 1:50. 100 µl NBT-BCIP solution were applied to each slide. The slides were then covered with coverslips and placed again inside the humid chamber. The latter was covered with aluminum foil to allow the signal to develop, something that usually required overnight incubation. After checking briefly under the microscope, the development reaction was stopped by immersing the slides in a glass jar with H_2O for 2 minutes so as to enable the H_2O to infiltrate the space between the slide and the coverslip, which was then removed by lifting the slide vertically. The slides were incubated in H_2O for a few minutes. Then, 80 µl 50% glycerol were applied to each slide, which was finally covered with a coverslip and inspected under the microscope. After pictures were taken, the slides were either discarded or sealed with nail polish and stored at 4 °C.

Proteinase K: Proteinase K, recombinant, PCR Grade, 19.2 mg/ml, Roche.

Proteinase-K buffer: 100 mM Tris pH 7.5, 50 mM EDTA pH 8.0.

Fixative: 50% ethanol, 5% glacial acetic acid, 3.7% formaldehyde.

Glycin in PBS: 2 mg/ml Glycin in 1x PBS.

Soaking solution: 50% Formamide, 2x SSC.

10x *in situ* salts: 3 M NaCl, 100 mM Tris pH 8.0, 50 mM EDTA, 53.7 mM NaH_2PO_4, 46.3 mM Na_2HPO_4.

50x Denhardt's solution: 1% Ficoll, 1% Polividon 25, 1% BSA.

Hybridization mix: 50% deionized formamide, 10% dextrane sulfate, 1x *in situ* salts, 1x Denhardt's solution, 0.5 mg/ml tRNA.

Blocking solution: 1% Blocking reagent (Roche), 0.3% Triton-X 100, 1x TBS.

Antibody buffer: 1% BSA, 0.3% Triton-X 100, 1x TBS.

Detection buffer: 100 mM Tris pH 9.5, 50 mM $MgCl_2$, 100 mM NaCl.

3.26 Preparation of chemically competent *E. coli* cells for transformation

The appropriate *E. coli* strain was streaked from the -80 °C stock on an LB agar plate and was incubated overnight at 37 °C. The next day, 10-12 large colonies were picked and used to inoculate 2 x 250 ml LB cultures in 1 L flasks. The cultures were incubated at 22 °C with vigorous shaking until an OD_{600} of 0.5 was attained (24-32 hours usually needed). The cultures were then placed for 10 minutes on ice. The cells were pelleted by centrifugation at 3000 g, for 10 minutes, at 4 °C. Each pellet was gently resuspended in 40 ml ice-cold TB solution and stored for 10 minutes on ice. The resuspended cells were pooled and then pelleted by centrifugation at 3000 g, for 10 minutes, at 4 °C. The final pellet was resuspended in a solution consisting of 18.6 ml ice-cold TB solution and 1.4 ml DMSO (R/T). The cells were incubated for 10 minutes on ice, aliquoted in 100 µl aliquots snd stored at -80 °C.

TB solution: 10 mM PIPES, 250 mM KCl, 15 mM $CaCl_2$, 55 mM $MnCl_2$, pH 6.7.

3.27 Preparation of electro-competent agrobacteria for transformation

A preculture of agrobacteria is started by inoculating 20 ml LB medium plus the appropriate antibiotics directly from the glycerol stock. The preculture is grown at 28 °C, 180 rpm for 36 hours. The final OD_{600} should be around 4.0. Then, two 2 litre flasks are inoculated, each with 500 ml LB medium plus the appropriate antibiotics and 9.4 ml of the preculture. The two flasks are incubated at 28 °C, 180 rpm until the OD_{600} reaches a value of 0.6-0.8. This takes ca. 7 hours. Then, both cultures are divided into a total number of 4 parts, each being 250 ml, and transferred in pre-chilled centrifugation bottles. The latter are placed on ice for 15 minutes, followed by centrifugation at 3000 g, for 15 minutes, at 4 °C. The supernatants are discarded and each pellet is gently resuspended in 200 ml H20 (4 °C), followed by centrifugation at 3000 g, for 15 minutes, at 4 °C. The supernatants are discarded and each pellet is gently resuspended in 100 ml H20 (4 °C). The number of the centrifugation bottles is reduced from 4 to 2 by pooling the cells and centrifuging at 3000 g, for 15 minutes, at 4 °C. The supernatants are discarded and each pellet is gently resuspended in 4.8 ml pre-chilled 10% glycerol, i.e. in total 9.6 ml 10% glycerol is used. Finally, 50 µl aliquots of the resuspended cells are distributed in pre-chilled Eppendorf tubes, which are snap-frozen in liquid nitrogen and stored at -80 °C.

3.28 Agro-infiltration of *N. benthamiana*

A. tumefaciens cultures containing the construct of interest were grown in LB medium plus the respective antibiotics in a shaking incubator for ca. 36 hours at 28 °C. The cells were then centrifuged at 3000 g, for 30 minutes, at R/T. The supernatant was discarded and

the pellet was resuspended in an equal volume of infiltration solution. The resuspended cells were then injected without any incubation into the leaves of 2-4 weeks old *N. benthamiana* plants. The infiltration was performed with the syringe only, i.e. without a needle, by pressing on the lower side of the leaf. The expression of our constructs was verified after ca. 48 hours either by checking for fluorescence or by harvesting leaf tissue that was subsequently used for protein extraction and Western blotting.

Infiltration solution: 10 mM $MgCl_2$, 10 mM MES, 0,15 mM acetosyringone, pH 5.7.

3.29 Agro-infiltration of *A. thaliana*

A. tumefaciens cultures containing the construct of interest were grown in LB medium plus the respective antibiotics in a shaking incubator for ca. 36 hours at 28 °C. The cells were then centrifuged at 3000 g, for 30 minutes, at R/T. The supernatant was discarded and the pellet was resuspended in an equal volume of infiltration solution. The resuspended cells were then applied directly on the floral buds by dipping the whole aerial part of a plant into the infiltration solution. The transformed plants were covered with a dome, left for a night at a place with reduce light and transferred the following day into the growth room.

Infiltration solution: 5% sucrose, 0.05% Silwet L-77.

3.30 Expression and purification of His-tagged proteins from *E. coli*

The appropriate expression vector containing the CDS of interest under the control of the *lac* promoter was transformed in the *E. coli* strain Rosetta pLysS. The transformed Rosetta cells were used to inoculate a 2 ml culture that was grown in a shaking incubator (200 rpm) overnight at 37 °C. The following day 150 µl of the overnight culture were used to inoculate a fresh 15 ml culture, which was grown in a shaking incubator (200 rpm) at 37 °C, until an $O.D_{600}$ of 0.5 had been reached. At that point, IPTG was added to the culture at a C_f of 1 mM. Afterwards, the culture was grown for an additional 4 hours in a shaking incubator (200 rpm) at 37 °C (or at 30 °C in the case of GFP-fusion proteins). The cells were harvested by centrifugation and were processed immediately or were frozen at -80 °C.

All the steps of the purification process were performed on ice. The cell pellet was resuspended in 1.5 ml of Lysis buffer and sonicated (10%, 6 x 10 seconds, 30 seconds gap). The sample was centrifuged at maximum speed, for 5 minutes, 4 °C. In the meantime, 400 µl of Ni-NTA bead slurry were loaded onto a microspin column. The beads were washed with 2 ml H_2O and equilibrated with 2 ml buffer W2. The supernatant from the centrifuged sample was decanted on the beads and the flowthrough was collected and loaded once more on the column. The beads were then washed successively with 2 ml of each of the W2, W3 and W4 buffers. Finally, the bound protein was eluted from the beads by applying on them 1 ml of Elution buffer.

Lysis buffer: 20 mM Tris pH 8.0, 10 mM imidazole pH 8.0, 150 mM NaCl, 2 mM DTT, 1 mg/ml lysozyme, 0.3 µl/ml benzonase, 1x protease inhibitor (Roche).

W2 buffer: 20 mM Tris pH 8.0, 10 mM imidazole pH 8.0, 150 mM NaCl, 2 mM DTT.

W3 buffer: 20 mM Tris pH 8.0, 10 mM imidazole pH 8.0, 1000 mM NaCl, 2 mM DTT.

W4 buffer: 20 mM Tris pH 8.0, 20 mM imidazole pH 8.0, 150 mM NaCl, 2 mM DTT.

Elution buffer: 20 mM Tris pH 8.0, 330 mM imidazole pH 8.0, 150 mM NaCl, 2 mM DTT.

3.31 Extraction of genomic DNA from plants using the Edwards buffer

One young cauline leaf per plant is harvested. The tissue is ground for 10-15 seconds with a pestle. Alternatively, the leaf is placed in an Eppendorf tube along with glass beads, snap-frozen in liquid nitrogen and ground using a TissueLyser II. 400 µl of Edwards buffer are added. If using a pestle, grinding is repeated briefly to remove any remaining tissue from it. Afterwards, the samples are vortexed for 5 seconds. The preps can be kept at R/T for >1 hour. The samples are centrifuged at 13000 rpm, for 1 minute, at R/T. Then, 300 µl of the supernatant are transferred to a fresh tube, 300 µl isopropanol are added and the tubes are inverted several times. The samples are left for 2 minutes at R/T and, then, centrifuged at 13000 rpm, for 5 minutes, at R/T. The supernatant gets discarded and the pellet is washed with 700 µl 70% Ethanol. The samples are centrifuged at 13000 rpm, for 2 minutes, at R/T and the DNA pellet is air-dried for 10-15 minutes. Alternatively, the pellet can be dried for 5 min at 50 °C. Finally, the pellet is resuspended in 50 µl sterile water.

Edwards buffer: 200 mM Tris-HCl pH 7.5, 25 mM EDTA, 250 mM NaCl, 0.5% SDS.

3.32 DNA minipreparation from yeast

A saturated yeast culture was spun down and the supernatant discarded. The following ingredients were added into the Eppendorf tube: 200 µl Breaking buffer, 200 µl phenol/chloroform/isoamyl alcohol (25:25:1) solution, 100-200 µl glass beads. The tube was vortexed vigorously for 2 minutes and then centrifuged at 16000 g, for 10 minutes, at R/T. The aqueous phase (ca. 200 µl) was transferred to a new tube and the rest was discarded. Subsequently, 1 ml of cold 100% ethanol (stored at -20 °C) was added to the aqueous phase and the sample was centrifuged at 16000 g, for 20 minutes, at R/T. The supernatant was discarded, 1 ml of 70% ethanol was pipetted into the tube and the sample was again centrifuged at 16000 g, for 10 minutes, at R/T. Finally, the pellet was air-dried for 10-15 minutes and then resuspended in 50 µl TE + RNase.

Breaking buffer: 2% Triton X-100, 1% SDS, 100 mM NaCl, 100 mM Tris-HCl pH 8.0, 1 mM EDTA.

TE buffer + RNase: 100 µg/ml RNase A in TE buffer.

3.33 Polymerase chain reaction (PCR)

The specific details of PCR amplification depended on the instructions supplied by the polymerase's manufacturer. A representative protocol is listed below.

PCR reaction composition: 0.5 µM forward primer, 0.5 µM reverse primer, 200 µM dNTPs, 1x High Fidelity Buffer (supplied), 1.0 unit / 50 µl reaction of thermostable DNA polymerase, 1 ng - 1 µg genomic DNA or 1 pg - 10 ng plasmid DNA.

Thermal profile: Initial denaturation for 30 seconds at 98 °C, denaturation for 8 seconds at 98 °C, annealing for 20 seconds at T_m, extension for 30 seconds per kb at 72 °C, 35 cycles, final extension for 5 minutes at 72 °C.

3.34 DNA extraction from agarose gels (Freeze and Squeeze)

The band from the agarose gel is excised and transferred to a Biorad Micro Bio-Spin chromatography column (No 7326204). The column is placed inside an Eppendorf tube and then stored for 10 min at -20 °C. The column is centrifuged at 12000 rpm, for 3 minutes, at R/T. Then, the column is discarded and the flowthrough is transferred to a new 1.5 ml Eppendorf tube. The volume of the flowthrough is approximately measured and an equal volume of isopropanol is added. Then, 1 µl glycogen is also added and the tube is inverted several times, followed by centrifugation at 13000 rpm, for 10 minutes, at R/T. The supernatant is decanted and the pellet gets washed with 500 µl 70% ethanol. The sample is centrifuged at 13000 rpm, for 5 minutes, at R/T and the ethanol is discarded. A short spin is performed and the residual ethanol is also discarded. The pellet is air-dried for 10-15 minutes or, alternatively, dried for 5 minutes at 50 °C. Finally, the pellet is resuspended with 20 µl Elution Buffer P (AnalytikJena innuPREP DOUBLEpure Kit).

3.35 SDS-PAGE

Proteins from plant or bacterial extracts were separated in polyacrylamide gels of varying acrylamide concentration. Each gel run was conducted by applying electric current at 300 V, 30 mA per gel, for approximately 1 hour (details pertain to 1.5 mm thick polyacrylamide gels). After the run, the gels were either incubated in Coomassie solution so as to visualise the protein separation or used for protein detection via Western blotting. Gels that were submitted for Mass-Spec analysis were incubated in commercial colloidal Coomassie solution.

3.36 Western blot

Resolved proteins from polyacrylamide gels were further analysed with semi-dry Western blot. The gel was incubated in blotting buffer for 10 minutes with gentle shaking. In the meantime, PVDF membrane and Whatman blotting paper were cut in parts of size equivalent to that of the gel. The membrane piece was incubated in methanol for 15 seconds, followed by incubation in blotting buffer for 10 minutes with gentle shaking. The Whatman paper pieces were soaked briefly with blotting buffer. Subsequently, the Western apparatus was assembled by placing on top of each other three pieces of Whatman paper, the PVDF membrane piece, the polyacrylamide gel and finally three additional pieces of Whatman paper. The transfer was carried out at 30 V, 150 mA per gel, for 1 hour 15 minutes (details pertain to 1.5 mm thick polyacrylamide gels). After the transfer had completed, the gel was discarded and the membrane was blocked in 5% w/v milk/TBS-T solution for 1 hour with gentle shaking at R/T. The membrane was then successively incubated in the primary antibody solution (dilution was antibody-specific, e.g. 1:1000 for the anti-WUS primary antibody) for 2 hours with gentle shaking at R/T or overnight with gentle shaking at 4 °C (overnight incubation was always performed for the anti-WUS primary antibody), followed by three washing steps in TBS-T, each lasting 10 minutes with gentle shaking. The membrane was then incubated in the secondary antibody solution (dilution was antibody-specific, e.g. 1:5000 for the anti-rabbit secondary antibody) for 2 hours with gentle shaking at R/T, followed again by three washing steps in TBS-T, each lasting 10 minutes. The membrane was finally incubated in ECL solution for 2 minutes and imaged for the detection of chemiluminescence signal with an appropriate imager.

Blotting buffer (Towbin): Recipe missing!.

3.37 Generation of CRISPR *A. thaliana* mutants

The CRISPR mutants were created by using the Cas9 endonuclease. The gene of interest was analyzed with the web tool CHOPCHOP (`http://chopchop.cbu.uib.no/`) in order to find suitable gRNAs. The chosen gRNAs were created by annealing complementary oligos, digesting the duplexes with Eco31I and finally subcloning the fragments into vector pHEE-401E **[85]**. The resulting constructs were transformed into GV3101 and finally into *A. thaliana*. The resistant transgenic T1 plants were genotyped and the putative mutated loci sequenced.

3.38 High resolution melting curve analysis (HRM)

The HRM analysis was performed for the initial scanning of possible CRISPR mutants among selected T1 plants. Short amplicons <150 bp spanning the potential mutation site were amplified by PCR in a Mic qPCR Cycler for 40 cycles in the presence of the EvaGreen

intercalating fluorescent dye. After the final extension step at 72 °C the samples were denatured at 95 °C in order to also bring the last synthesized molecules from heterozygous plants in the pool of mismatched molecules. The subsequent annealing step at 50 °C was followed by a conditioning step at 72 °C. Finally, the data for the melting curves was collected by heating up the samples from 72 °C to 95 °C in steps of 0.1 °C.

3.39 Acceptor Photobleaching FRET (AP-FRET)

AP-FRET was applied in order to elucidate the possible *in vivo* interaction of chimeric fusion proteins, both in *N. benthamiana* as well as in *A. thaliana*. The donor-acceptor fluorophore pair consisted always of GFP and mCherry respectively. mCherry fusion proteins were bleached with a strong 561 nm laser pulse (90%-95% laser power for 1-2 seconds) and any possible flunctuations in GFP fluorescence intensity were measured with the in-house Nikon A1 laser confocal microscope. FRET efficiency (E) was calculated according to the following equation: $E(\%) = \frac{I_{DONOR(post-bleach)} - I_{DONOR(pre-bleach)}}{I_{DONOR(post-bleach)}} \times 100$. An E value of >3% was the threshold for identifying protein-protein interactions. At least 10 cells were studied for each pair of fusion proteins.

Chapter 4

Results

4.1 Y2H screening for WUS interactors

As mentioned in the Introduction of this Thesis, one of the approaches that was applied for the identification of WUS interacting proteins was a Y2H screening of an *A. thaliana* transcription factor library. The latter has been generated in the lab of Prof. Dr. R. Immink in Wageningen, the Netherlands and encompasses 1982 transcription factors. The full list with the members of this library is presented in the Appendix, along with the corresponding interaction data for the first out of the total four screenings for the WUS wt protein. In this Y2H setup, WUS or WUS mutant coding sequences were fused in-frame with the transcription activation domain of the GAL4 protein and thus served as the prey protein of each mating. On the other hand, each TF of the library was subcloned in-frame with the DNA binding domain of the GAL4 protein and served as the bait protein of each mating. The stringency of the screenings was controlled by the addition of the HIS3 inhibitor 3-AT (Section 3.22).

The mutant sequences were named WUS-D3 and WUS-D7 and were chosen after evaluating the initial results of another project in our lab that focuses on the mobility of the WUS protein. They were created by substituting the stretch of amino acids to be mutated with a Ser-Gly linker (**Fig. 4.1**). WUS-D7 exhibits restricted mobility while WUS-D3 moves significantly farther along the apical-basal axis compared to the pattern of the wild-type WUS protein (results not shown). The rationale behind this choice was to correlate this functional difference between WUS and the WUS mutants with a putative interaction partner.

The initial screening was done by pairing WUS against the whole TF library and resulted in the identification of 211 interactions. These were detected and initially evaluated by a robot. 117 of these were classified as strong interactions while the rest 94 were considered to be background noise. The 117 strong interactions were also visually inspected and 55 of them where chosen as definite strong interactions, since the respective clones had fully grown on the selective medium. The genes that were further studied in my experiments were chosen out of this pool of 55 genes.

Nevertheless, the Y2H screening for the 211 strong and weak interactions was repeated in the meantime thrice for wild-type WUS. Moreover, this subset of the yeast library was also used for the screenings that involved the WUS-D3 and WUS-D7 mutant proteins as prey. It is

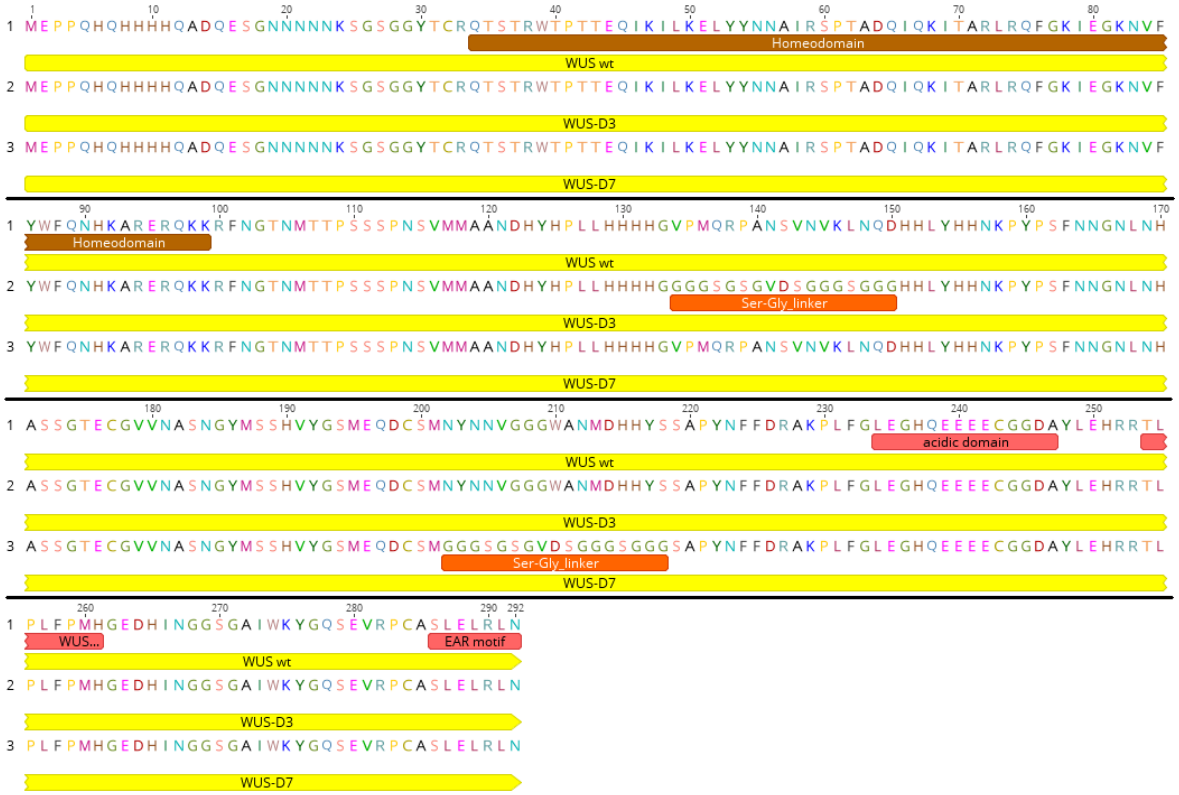

Figure 4.1: The WUS-D3 and WUS-D7 mutants used in the Y2H screenings. The Ser-Gly stretches are depicted in orange on the WUS mutant sequences (no2 and no3). The motifs known for WUS are depicted on the wt sequence (no1).

worth noting that these additional screenings were performed manually, in contrast to the first screening that was done by a robot. The summary of the interaction data for these additional screenings is presented in the following diagram (**Fig. 4.2, A**). Overall, it is obvious that the follow-up screenings for WUS were more stringent compared to the initial one, since the three repetitions resulted in a decrease of the detected strong interactions by 41%, 38% and 24-54% for the second, third and fourth screening respectively. These repetitions, however, allowed the verification of the detected interactions. Out of the 149 strong interactions, 57 were observed only once, 24 twice, 29 thrice and 30 four times, i.e. in all of the screenings (**Fig. 4.2, B**). This comparison for the wt WUS protein was done by comparing screenings that were done with 5 mM 3-AT. It is worth noting that although the 5 mM of 3-AT were the more stringent condition for the first three screenings, the fourth screening was performed not only with 5 mM but also with 10 mM 3-AT. The reason for this deviation was that the fourth screening was done in parallel to the screenings for the WUS mutants, which had to be carried out with 10 mM 3-AT due to higher auto-activation. Thus, the fourth screening was done redundantly using both 5 mM and 10 mM 3-AT in order to compare its findings to the results from the previous screenings for wt WUS and to the results from the screenings for the WUS mutants respectively.

As mentioned in the previous paragraph, the screenings for WUS-D3 and WUS-D7 were performed exclusively with 10 mM 3-AT in order to eliminate a small degree of auto-activation, which was present at 5 mM 3-AT. They were, therefore, evaluated by comparing them to the

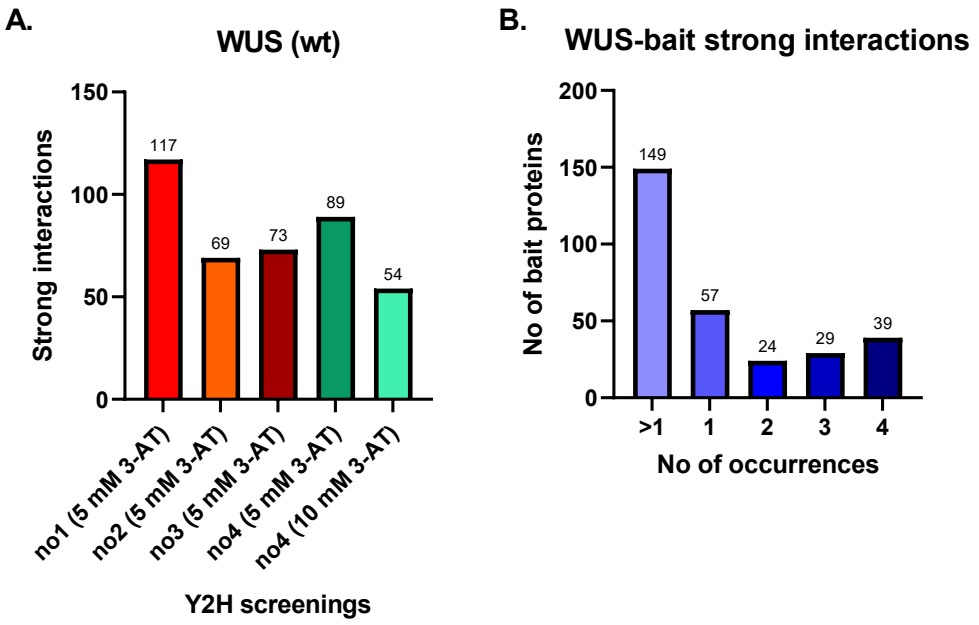

Figure 4.2: Y2H screening for wt WUS. A) Number of strong interactions in the different screenings done for WUS. B) Number of occurrences for each WUS-bait interaction.

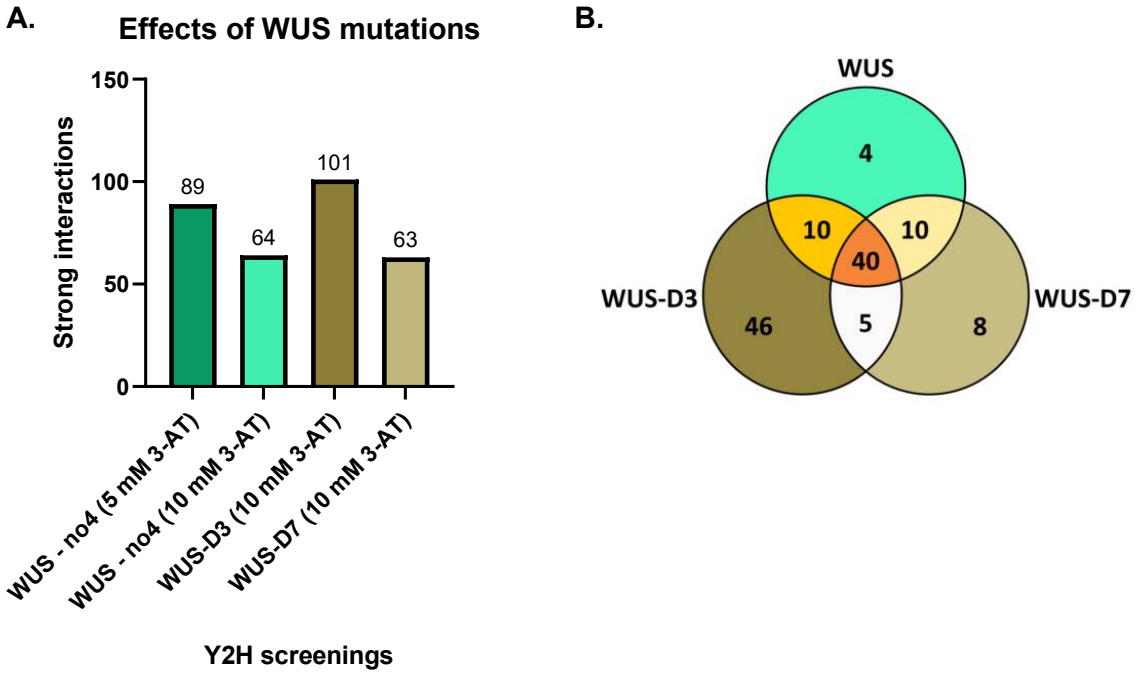

Figure 4.3: Effects of the WUS mutations on the WUS-bait interactions. A) Total interactions for each prey protein. B) Venn diagram depicting overlapping interactions among all different prey proteins.

10 mM 3-AT version of the fourth screening for WUS. WUS-D7 exhibited 63 strong interactions, a similar number to that of WUS. However, WUS-D3 showed a pronounced overactivity, since it interacted with 101 bait proteins, a 58% increase compared to WUS (**Fig. 4.3.A**). This is in-line with the increased mobility of WUS-D3, although the Y2H screenings did not shed any further light into the behaviour of this mutant. Furthermore, 40 out of the

64 observed interactions for WUS were not affected by the mutations while the remaining 24 were abolished either by one or both of the mutations (**Fig. 4.3.B**). Another fact worth mentioning is that although the Ser-Gly linker in WUS-D3 overlaps with and therefore destroys the WUS region that is known to be necessary for the WUS-HAM1 interaction **[47]**, the corresponding interaction persists in my screenings, in contrast to the published results.

As mentioned earlier in this section, out of the initial pool of 55 confirmed strong interactions observed in the first Y2H screening several genes were selected for further investigation. These genes were the following: *HAM1* (AT2G45160, as a positive control), *ESR1* (AT1G12980, also known as *DRN*), *GRF1* (AT2G22840), *RFI2* (AT2G47700), *WOX9* (AT2G33880), *PUCHI* (AT5G18560), *HAT2* (AT5G47370), *SPL9* (AT2G42200), *LEC1* (AT1G-21970) and *AIL6* (AT5G10510). This selection was based on existing evidence in the literature, according to which these proteins are functional in the SAM and on the microarray data provided by the Arabidopsis eFP browser at `http://www.bar.utoronto.ca/` **[86]**, **[87]** (**Fig. 4.4**).

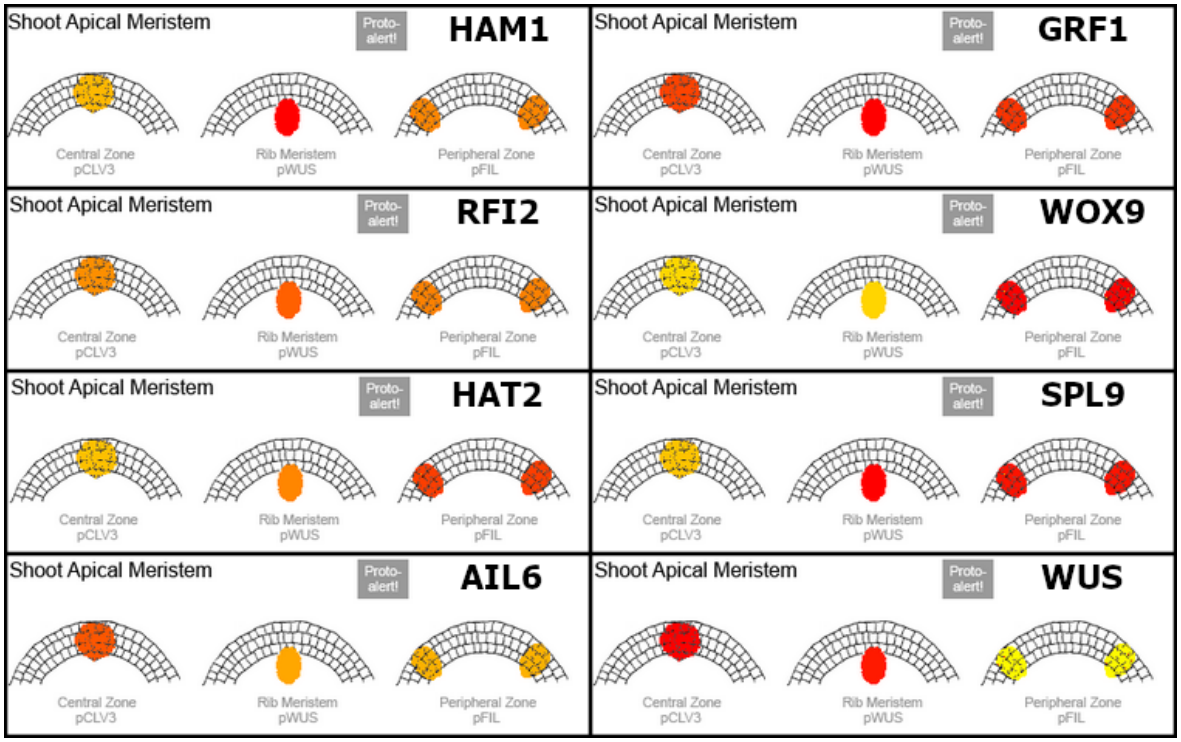

Figure 4.4: Microarray expression data for the *A. thaliana* SAM (not all selected genes had an entry in the eFP browser). The expression in the regions of interest is depicted using a colour gradient (red: strong expression, orange: intermediate expression, yellow: no or below detection threshold expression).

The additional validation experiments that will be presented in the next section led to the selection of genes *WOX9* and *ESR1* for further analysis. Therefore, a more detailed report of the Y2H results of these two genes ought to be mentioned. The interaction between WUS and WOX9 was detected and visually confirmed as strong in the initial Y2H screening, but surprisingly in none of the subsequent repetitions that were done later. Thus, I repeated the mating and the screening exclusively for the putative WUS-WOX9 interaction (**Fig. 4.5**). The results did confirm the existence of this interaction in yeast, although it does not appear to

be particularly strong. It is also worth noting that WUS exhibited extensive auto-activation in this small-scale screening that vanished upon addition of 3-AT. It was surprising, however, because in similar experiments I performed earlier there was no auto-activation for WUS (results not shown). On the other hand, the protein ESR1 was detected as a strong interacting partner for WUS in all Y2H screenings done with WUS serving as the prey protein as well as in the screening for the WUS-D3 and WUS-D7 substitution constructs (**Fig. 4.6**).

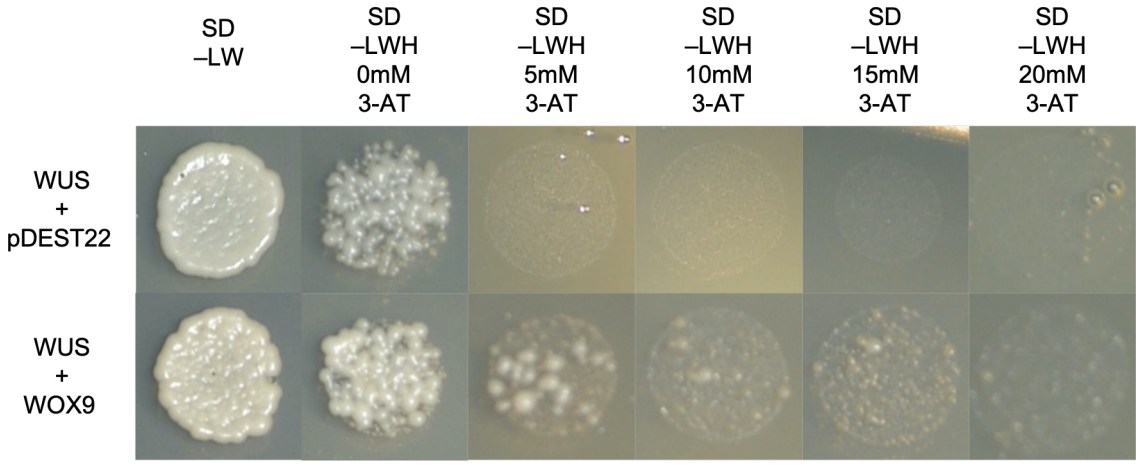

Figure 4.5: Confirmation Y2H screening for the WUS-WOX9 interaction.

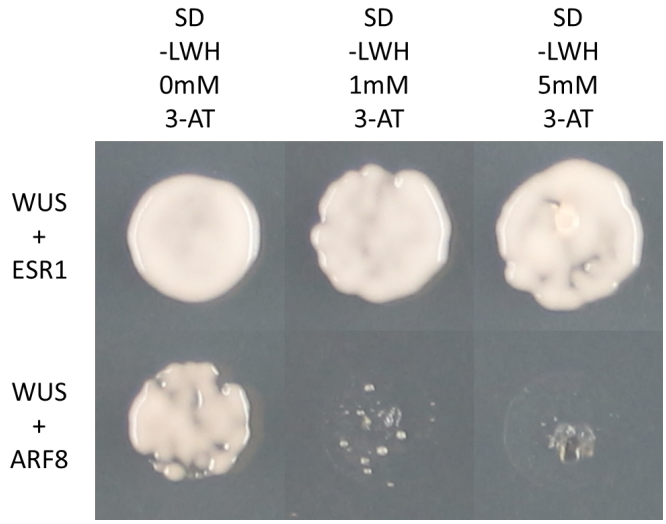

Figure 4.6: Growth comparison for the diploid WUS-ESR1 clone on selective media (3rd Y2H screening for WUS). The transcription factor ARF8 was one of the several negative controls.

4.2 *in planta* verification of WUS interactors by AP-FRET

The next step after the Y2H screenings was to evaluate the putative interactions using an assay *in planta*. This was done so as to eliminate any possible false-positive hits by adding a second layer of validation in my workflow and also in order to check the interactions in a more relevant biological system since all TFs under examination come from *A. thaliana*. To this end, I used Acceptor Photobleaching FRET (AP-FRET), a technique that is based on the

non-radiative energy transfer from an excited donor fluorophore to an acceptor fluorophore. This energy transfer takes place when both fluorophores are only <10 nm apart and the emission spectrum of the donor overlaps with the absorption spectrum of the acceptor. Thus, I fused WUS to GFP under the control of the constitutive viral 35S promoter and also fused each candidate interacting protein to mCherry under the control of the same promoter. The GFP and mCherry fluorophores are a compatible pair for AP-FRET and the 35S promoter permits the overexpression of the fusion proteins in *N. benthamiana* leaves, an established and widely used model system for heterologous protein overexpression *in planta*. In addition, WUS-GFP has been successfully used in the past in our lab to rescue *wus* mutant plants, a fact that verifies its functionality and its subsequent usefulness in assays aiming to dissect WUS function.

Fusion protein no1	**Fusion protein no2**	**Expression of fusion protein no2 in *N. benthamiana***	**FRET**	**n**
-	mCherry-linker-GFP-NLS	Yes	Yes	12
WUS-GFP	mCherry-NLS	Yes	No	10
WUS-GFP	mCherry-HAM1	No	-	-
WUS-GFP	HAM1-mCherry	No	-	-
WUS-GFP	mCherry-ESR1	Yes	Yes	21
WUS-GFP	ESR1-mCherry	No	-	-
WUS-GFP	mCherry-GRF1	No	-	-
WUS-GFP	GRF1-mCherry	No	-	-
WUS-GFP	mCherry-RFI2	No	-	-
WUS-GFP	RFI2-mCherry	No	-	-
WUS-GFP	mCherry-WOX9	Yes	Yes	25
WUS-GFP	WOX9-mCherry	Yes	Yes	30
WUS-GFP	mCherry-PUCHI	No	-	-
WUS-GFP	PUCHI-mCherry	No	-	-
WUS-GFP	mCherry-HAT2	No	-	-
WUS-GFP	HAT2-mCherry	Yes	No	14
WUS-GFP	mCherry-SPL9	Yes	No	5
WUS-GFP	SPL9-mCherry	Yes	No	8
WUS-GFP	mCherry-LEC1	No	-	-
WUS-GFP	LEC1-mCherry	No	-	-
WUS-GFP	mCherry-AIL6	No	-	-
WUS-GFP	AIL6-mCherry	No	-	-

Table 4.1: Overview of the AP-FRET validation experiments. The WUS-GFP protein was always strongly expressed in *N. benthamiana*. All fusion proteins were cloned without any linker between the TF and the fluorophore. The proteins mCherry-linker-GFP-NLS and mCherry-NLS served as the positive and negative control respectively. The ”n” values correspond to the nuclei that were bleached for each donor-acceptor combination.

Nevertheless, expression in *A. thaliana* was not successful for many of the TFs that were fused to mCherry (**Table 4.1**). Repetition of the infiltration or the use of other *A. tumefaciens* strains also did not circumvent this problem. It is worth noting, however, that also WUS-mCherry fusion proteins do not express well in *N. benthamiana* in contrast to WUS-GFP, although in this case it is a matter of erroneous subcellular localization instead of expression efficiency (results not shown).

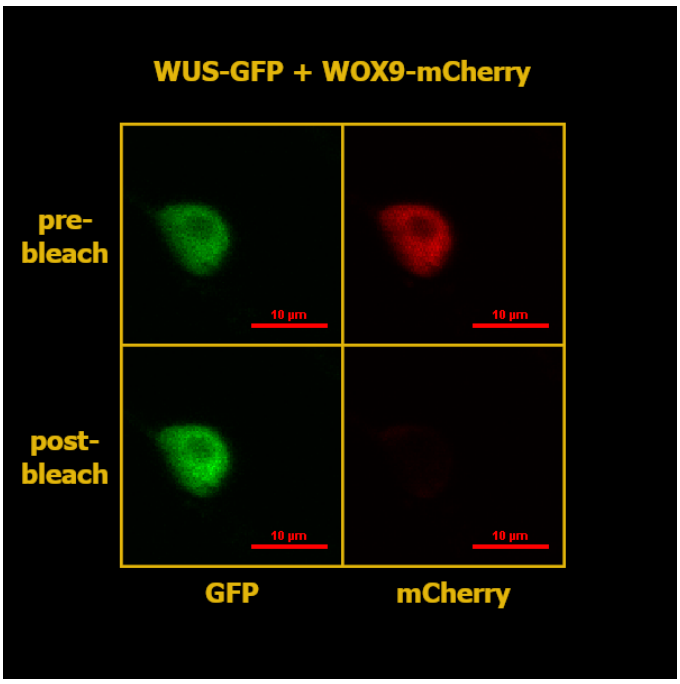

Figure 4.7: A representative successful AP-FRET experiment in *N. benthamiana*. The donor-acceptor pair comprises WUS-GFP and WOX9-mCherry. Bleaching of mCherry results in an increase of GFP fluorescence. Scale bars correspond to 10 µm.

The mCherry fusion proteins that were successfully expressed in *N. benthamiana* were then bleached in order to detect a possible increase in donor fluorescence (**Fig. 4.7**). For each pair of fusion proteins at least 10 nuclei were bleached for the AP-FRET experiments. The only exception to this were the SPL9 fusion proteins, due to the fact that they did not express so well in *N. benthamiana*. Fluorescence intensity was monitored before and after bleaching and the respective values for the donor channel (GFP) were used to calculate the FRET efficiency (E).

The detection of FRET transfer upon protein-protein interactions depends on multiple factors, e.g. the relative abundance of the acceptor and the donor, the efficient bleaching of the acceptor as well as the intra-molecular distance between the fluorophores on the protein complex. The latter is influenced by the tertiary structure of the fusion proteins and it can always be that even stable protein-protein interactions do not result in positive FRET experiments. This is the reason why I created acceptor fusion proteins tagged with mCherry either N- or C-terminally. Nevertheless, although the absence of FRET does not exclude the possibility that two proteins interact with each other, positive FRET, i.e. with E (%) values >3, is a very good indicator that such an interaction takes place indeed.

The AP-FRET experiments that I performed in the course of this study, in order to validate the interactors identified in the Y2H screening, are summarised in **Fig. 4.8**. Proteins WOX9 and ESR1 were identified again as WUS interactors and I chose, subsequently, to generate mutants for the respective genes and study their phenotype, focusing on stem cell function in the shoot. On the other hand, proteins HAT2 and SPL9 appear to not interact with WUS in *N. benthamiana* leaves.

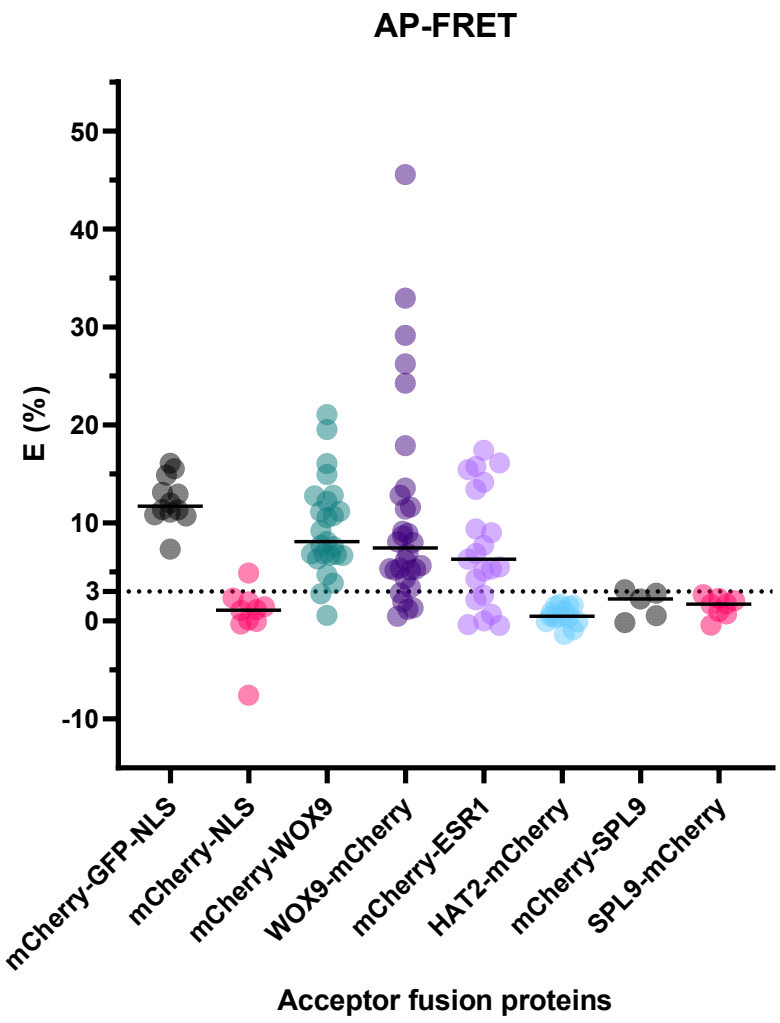

Figure 4.8: AP-FRET in *N. benthamiana* leaves. The E (%) values on the Y axis show the FRET efficiency of each experiment. The entries on the X axis correspond to the respective acceptor fusion protein. The donor protein is WUS-GFP, except for control mCherry-GFP-NLS. The black lines depict the median value for each group of measurements. E (%) values >3 indicate protein-protein interaction.

4.3 Generation and characterisation of CRISPR mutants for *WOX9* and *ESR1*

A. thaliana seed collections like NASC (`http://arabidopsis.info/`) contain mutants for the vast majority of *A. thaliana* genes, which have been generated by T-DNA insertion. However, the exact nature of such mutants can be challenging to verify experimentally and there is always the chance that additional uncharacterised mutations co-segregate. In addition, I did not locate any *wox9* or *esr1* mutants with a known striking phenotype. Therefore, I opted to generate the corresponding mutants via CRISPR.

4.3.1 *wox9*

A *wox9* CRISPR mutant existed already in the seed stock of my laboratory. This mutant was generated by addition of a single T after base no.51 of the *WOX9* CDS in the Double-

Reporter background (DR: Col-0, pCLV3::mCherry-NLS, pWUS::3xVenus-NLS). This mutation resulted in the premature termination of WOX9 and the generation of a truncated 23 aa polypeptide in contrast to the 378 aa WOX9 protein (**Fig. 4.9**). Thus, I chose to work with this mutant due to the fact that this *wox9* mutation generates a very short polypeptide lacking the homeodomain and also due to the absence of any alternative translation initiation sites downstream of the mutation that could result in a biologically active molecule.

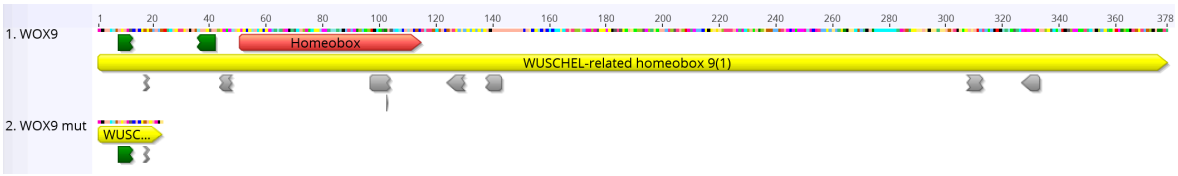

Figure 4.9: The WOX9 wild-type (1) and WOX9 CRISPR mutant (2) proteins. The homeodomain is depicted as a red ribbon on the WOX9 wild-type sequence. The jagged gray line shows the CAS9 cutting site.

In addition to the microarray expression data presented earlier (**Fig. 4.4**), WOX9 presence in the SAM had also been confirmed by published *in situ* experiments **[88]**. According to the authors, *WOX9* expression could be detected throughout the shoot apex both in a 6-day-old seedling as well as in the inflorescences of adult plants, albeit at a lower level (**Fig. 4.10**). Furthermore, the same study provided evidence for pWOX9 activity in the SAM by GUS staining (results not shown). Therefore, I continued with the evaluation of *wox9* mutants.

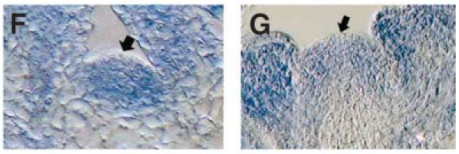

Figure 4.10: Published *in situ* of *WOX9* mRNA in *A. thaliana* wild-type plants, both in a 6-day-old seedling (F) and in the inflorescences of an adult plant (G) [88].

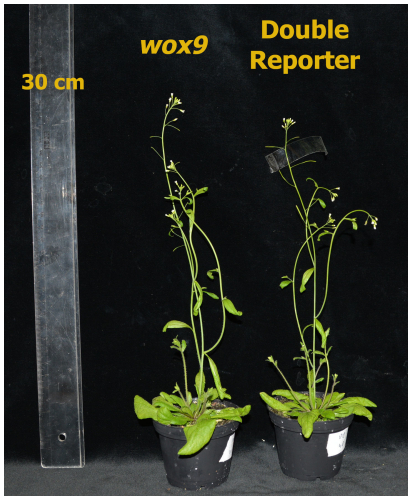

Figure 4.11: A *wox9* plant (left) and a Double-Reporter plant (right). Both plants are 34 days old.

Previous studies of *wox9* plants revealed the emergence of an embryonic lethal phenotype that can be rescued by addition of sucrose in the 1/2 MS growth medium **[88]**, although this phenotype was characterised by incomplete penetrance in my experiments. Nevertheless, I focused on *wox9* adult plants, yet my study did not reveal any striking phenotype at the macroscopic level (**Fig. 4.11**). However, I also performed a plastochron measurement, i.e. I calculated how many lateral organs had been produced by the SAM at a certain time-point and then normalized this value against the length of the shoot. This measurement revealed that the *wox9* mutants produced more flowers compared to the background Double-Reporter plants, a difference that becomes apparent only at later growth stages (**Fig. 4.12**). This pattern suggests that the stem cell pool of *WOX9* plants gets exhausted faster in comparison to *wox9* mutants. More specifically, it appeared that the *wox9* mutants either had more SAM stem cells or were able to maintain them in

an undifferentiated state for a longer time period, resulting in stem cells being present and therefore recruited for lateral organ formation at a later time-point in the life cycle of the plant.

Another interesting characteristic of the *wox9* mutant plants became apparent only during senescence. These plants required a total of about 3 months to complete their life cycle, that is a 50% increase compared to wild-type *A. thaliana*. Moreover, this extended longevity was accompanied by a severe reduction in fertility. The *wox9* plants produced only 100-200 seeds each, something that is a decrease by 1-2 orders of magnitude compared to the wild-type.

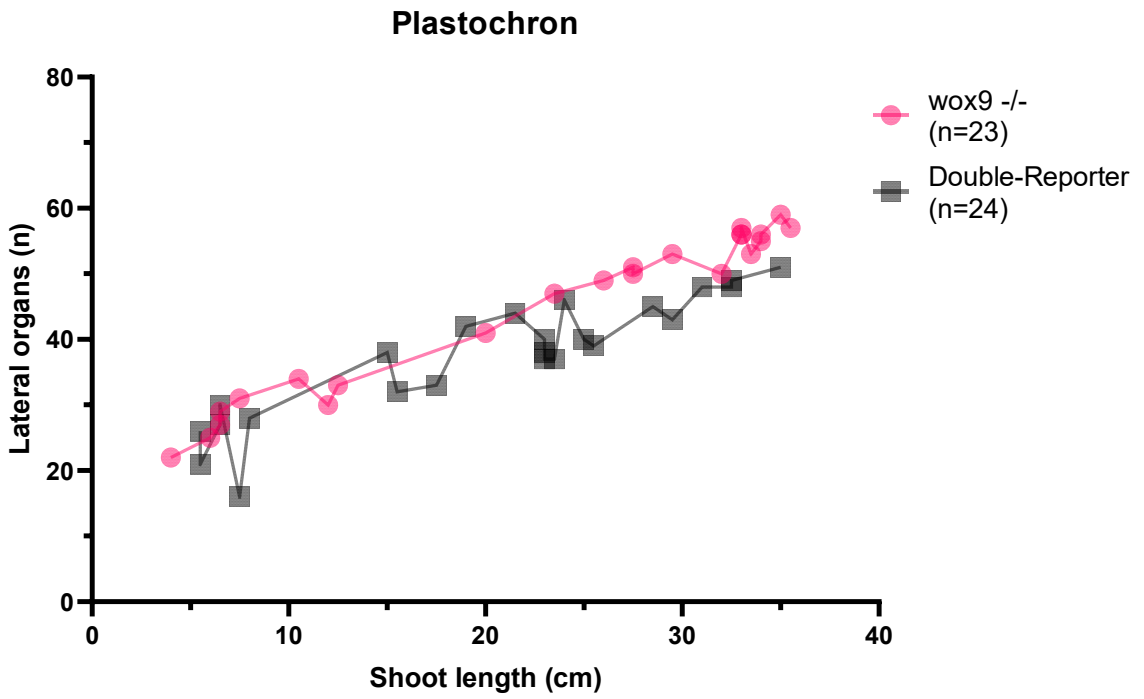

Figure 4.12: Plastochron measurement for *wox9* and Double-Reporter (*WOX9*) plants. The *wox9* mutants are able to generate more new flowers at later growth stages. Plant line genotypes: Double-Reporter (wox9 +/+, pCLV3::mCherry-NLS, pWUS::3xVenus-NLS), *wox9* (wox9 -/-, pCLV3::mCherry-NLS, pWUS::3xVenus-NLS).

Next, I examined the effect of the *wox9* mutation on the SAM size using laser confocal microscopy. Since I was trying to elucidate a possible WUS-WOX9 interaction and had already initial findings hinting towards a perturbation of the SAM stem cell niche in *wox9* plants, it was really intriguing to observe the respective SAM structure in the absence of WOX9. A broader WUS domain could be the cause of the previously observed plastochron phenotype.

Measuring the size of the SAM is not a trivial task, since this structure resembles a dome with irregular protrusions that eventually differentiate to new lateral organs. For the initial analysis, I decided to use a method that has been previously applied in the lab, that is to measure the SAM diameter thrice and calculate the respective average value. The measurement was done by drawing lines on a maximum projection image across the SAM and through its centre, while excluding any lateral primordia (**Fig. 4.13**). The latter were detected by the presence of fluorescence signal driven by pWUS, even if their structural differentiation had not already begun.

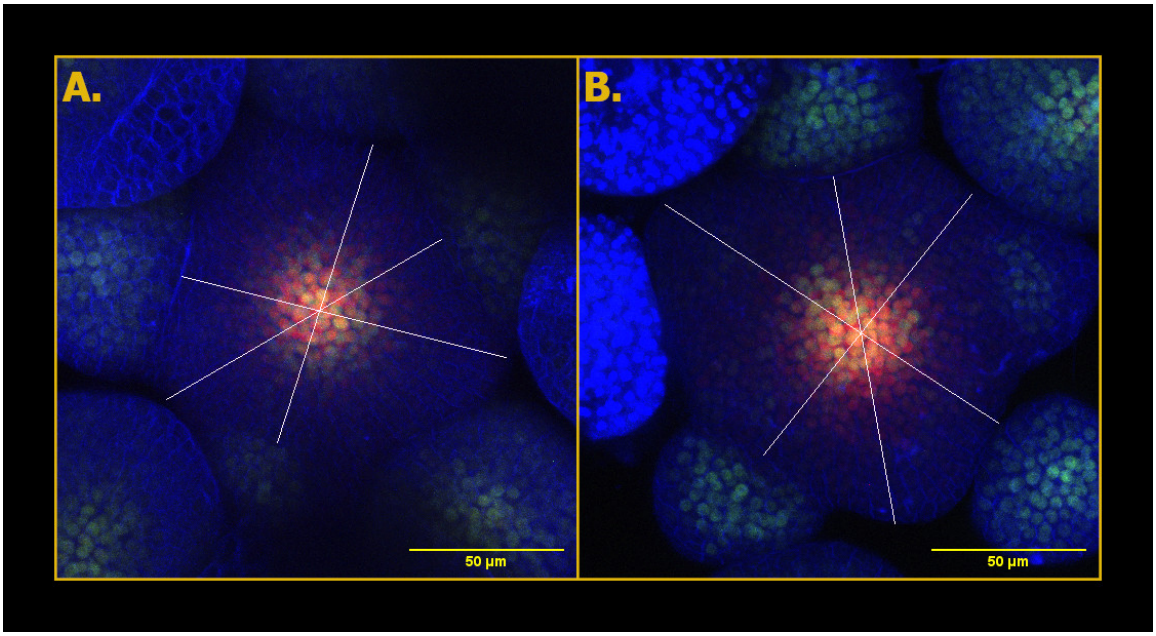

Figure 4.13: Measuring SAM diameter by drawing lines on a maximum intensity projection of a Double-Reporter (A) and a *wox9* (B) SAM. Plant line genotypes: Double-Reporter (wox9 +/+, pCLV3::mCherry-NLS, pWUS::3xVenus-NLS), *wox9* (wox9 -/-, pCLV3::mCherry-NLS, pWUS::3xVenus-NLS). Blue: DAPI, Red: pCLV3::mCherry-NLS, Green: pWUS::3xVenus-NLS. Scale bars correspond to 50 µm.

The initial SAM measurements provided very interesting findings. The *wox9* mutation results in a significantly increased meristem size compared to the *WOX9* Double-Reporter background (**Fig. 4.14**). These results suggest that WOX9 represses WUS and the *wox9* mutation cancels this repression, thereby boosting WUS function, which subsequently enlarges the SAM. Taking into account that both WUS and WOX9 contain homeodomains, the possibility of these proteins forming hetero-dimers offered a plausible mechanistic explanation of this putative repressive protein-protein interaction. The abolishment of this repression could be manifested in various ways, e.g. either by WUS moving into additional cells towards the SAM periphery in the absence of WOX9 and resulting in the spatial expansion of the stem cell niche or by WUS remaining active for a longer time during plant growth.

The mode of repression would of course depend on the exact localization of WOX9. The microarray expression data contrasted the published *WOX9 in situ*, the former showing a strong peripheral expression of *WOX9* while the latter a weaker ubiquitous expression throughout the SAM. Moreover, it could still be the case that WOX9 exhibits cell-to-cell mobility in the SAM, something that is also characteristic of WUS distribution.

I continued by testing first the hypothesis of a possibly enlarged WUS domain in the absence of WOX9, something that of course requires monitoring WUS distribution in the SAM. To this end, I had to combine the *wox9* mutant line that was generated in the Double-Reporter background (stimpy) to an already existing *wus* rescue line that is most frequently used in the lab for confocal laser microscopy studies (pGD044), while excluding the pWUS and pCLV3 reporter constructs. Specifically, I crossed stimpy to pGD044 in order to create line CTL008 and bring the *wox9* mutation in our *wus* rescue background:

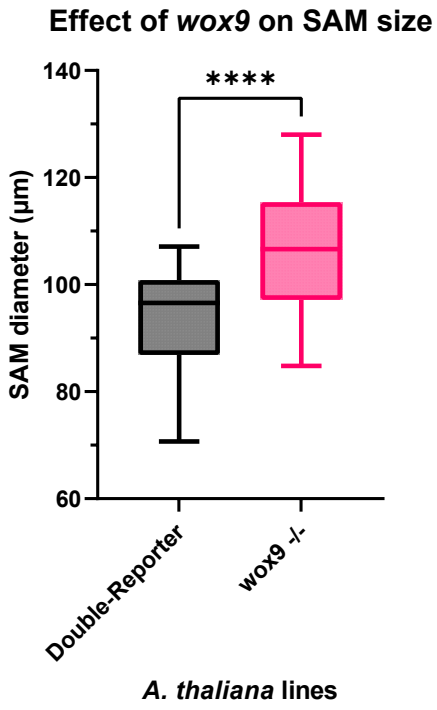

Figure 4.14: The *wox9* mutation results in SAM enlargement. Plant line genotypes: Double-Reporter (wox9 +/+, pCLV3::mCherry-NLS, pWUS::3xVenus-NLS), *wox9* (wox9 -/-, pCLV3::mCherry-NLS, pWUS::3xVenus-NLS). Sample size: Double-Reporter (n=29), *wox9* (n=39). Statistical test: unpaired two-tailed t-test with P value < 0.0001.

$$wus^{-/-},\ wox9^{+/+},\ pWUS::WUS-linker-GFP\ (pGD044)$$
$$x$$
$$wus^{+/+},\ wox9^{-/-},\ pCLV3::mCherry-NLS,\ pWUS::3xVenus-NLS\ (stimpy)$$
$$\downarrow$$
$$wus^{-/-},\ wox9^{-/-},\ pWUS::WUS-linker-GFP\ (CTL008)$$

Using line CTL008 I was able to repeat the SAM size measurements in a *wus* mutant background rescued by WUS-linker-GFP and thus also validate the possible presence of an expanded WUS domain in *wox9* mutants. The SAM size measurements were initially performed in a similar way to the previous experiments (**Fig. 4.13, Fig. 4.15**). The results confirmed that the SAM becomes enlarged in *wox9* plants (**Fig. 4.16, A - SAM columns**). Interestingly, the absolute values for SAM size differed considerably between the two experiments. The measurements done for the Double-Reporter background gave the following average SAM diameter values: 93.85 µm (Double-Reporter) and 106.1 µm (wox9 -/-). In contrast, the measurements for the wus -/- background gave considerably smaller average SAM diameter values: 68.99 µm (pGD044) and 77.97 µm (CTL008), that is a reduction of 26.49% and 26.51% for *WOX9* and *wox9* mutant plants respectively. This identical reduction in SAM size most likely resulted from the difference in the background genotype, since all plants grew in the same plant growth chambers, albeit at different time points, and therefore the environmental conditions should be similar.

The WUS-linker-GFP fusion protein rescuing the wus -/- background permitted the detection of WUS distribution in the SAM via confocal laser microscopy. The WUS domain size was calculated by drawing lines on maximum projection images (**Fig. 4.15**). The values collected did reveal a small enlargement of the WUS domain in the absence of WOX9 (**Fig. 4.16, A - WUS domain columns**), but the normalization of these measurements against

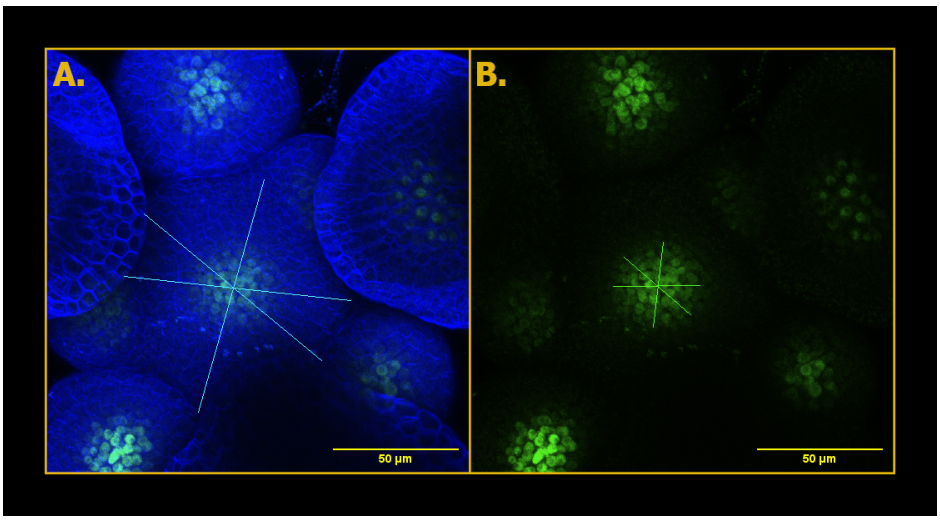

Figure 4.15: Measuring the diameter of the SAM (A) and the WUS domain (B) of a CTL008 plant by drawing lines on a maximum intensity projection of the SAM. Plant line genotype: CTL008 (wus -/-, wox9 -/-, pWUS::WUS-linker-GFP). Blue: DAPI, Green: pWUS::WUS-linker-GFP. Scale bars correspond to 50 µm.

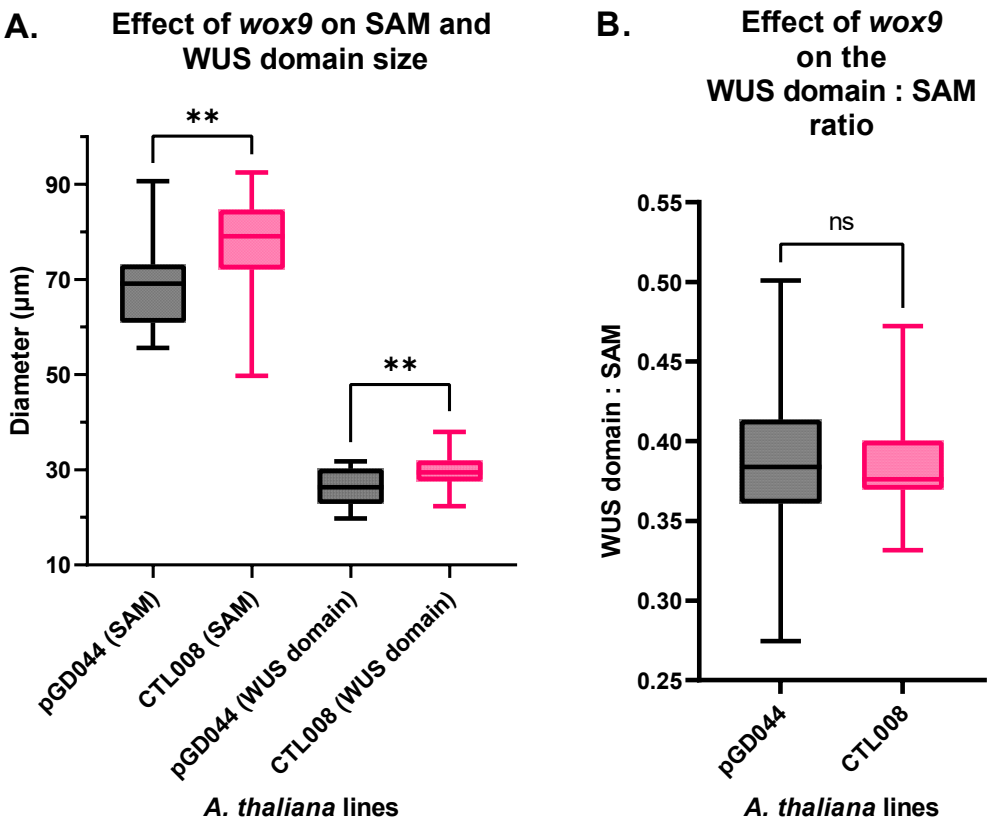

Figure 4.16: A. The *wox9* mutation results in SAM and WUS domain enlargement. Sample size: pGD044 (n=19), CTL008 (n=17). Statistical test: unpaired two-tailed t-test with P value = 0.0086 (SAM), unpaired two-tailed t-test with P value = 0.0058 (WUS domain). B. The *wox9* mutation results in a proportional increase in SAM and WUS domain sizes. Sample size: pGD044 (n=19), CTL008 (n=17). Statistical test: unpaired two-tailed t-test with P value = 0.7471. Plant line genotypes: pGD044 (wus -/-, wox9 +/+, pWUS::WUS-linker-GFP), CTL008 (wus -/-, wox9 -/-, pWUS::WUS-linker-GFP).

the SAM size showed that this change does not affect SAM architecture. The *wox9* mutation results apparently in a proportional increase in both the SAM and the WUS domain size (**Fig. 4.16, B**). Thus, a possible repression of WUS by WOX9 does not depend on spatial restriction of WUS expression or movement.

Nevertheless, I repeated the SAM size measurements for the same data that was collected for the CTL008-pGD044 comparison using a different approach (**Fig. 4.17**). I used the Fiji image processing software to pick the coordinates of the centre of ca. 20 cells in the L1 layer of each SAM. These cells were selected from top to bottom along the Z axis of each stack of confocal images. The coordinates were then inserted into a workflow developed in the KNIME environment and were used to generate a geometrical shape (hemisphere) that closely resembles the dome-shaped SAM. These hemispheres naturally permit the execution of precise geometrical calculations (**Fig. 4.17**).

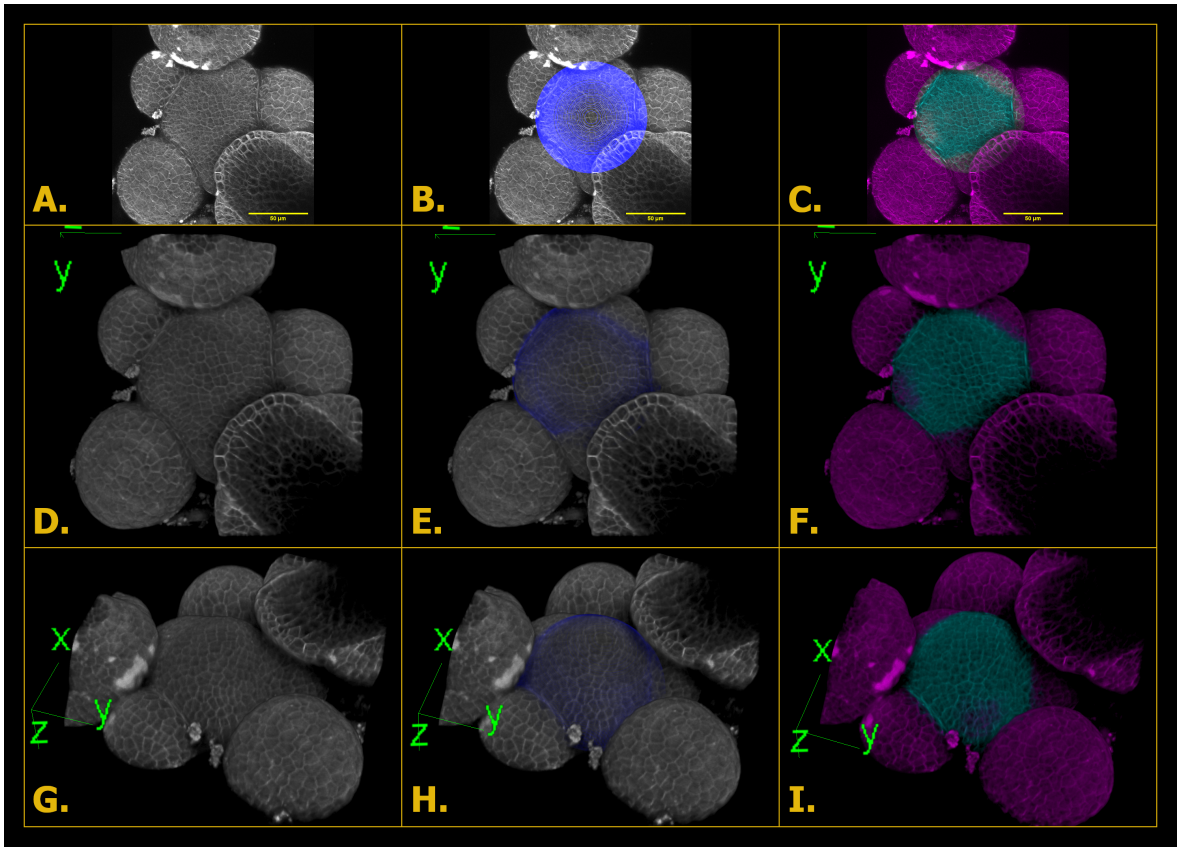

Figure 4.17: Output of the KNIME workflow. All pictures show the same CTL008 plant (only the DAPI channel was used for the analysis). Pictures A, B, C are maximum projections. Pictures D, E, F are volume reconstructions (top view). Pictures G, H, I are volume reconstructions (side view). Pictures A, D, G are the original images. Pictures B, E, H show the hemisphere generated after inserting the coordinate list for the L1 cells into KNIME. Pictures C, F, I depict the delineated SAM after the juxtaposition of the hemisphere with the original image via KNIME. Plant line genotype: CTL008 (wus -/-, wox9 -/-, pWUS::WUS-linker-GFP). Grey: DAPI. Magenta: DAPI. Blue: hemisphere. Cyan: optically segmented SAM. Scale bars in A, B, C correspond to 50 µM.

This second measurement provided additional validation to the finding, that the *wox9* plants manifest a larger SAM compared to the *WOX9* lines (**Fig. 4.18, A**). Nevertheless, since it is unavoidable that certain small differences will arise when calculating SAM sizes

with different methods, it was worth investigating whether these differences might have affected my conclusions. However, such differences were minor and within statistical tolerance (**Fig. 4.18, B**).

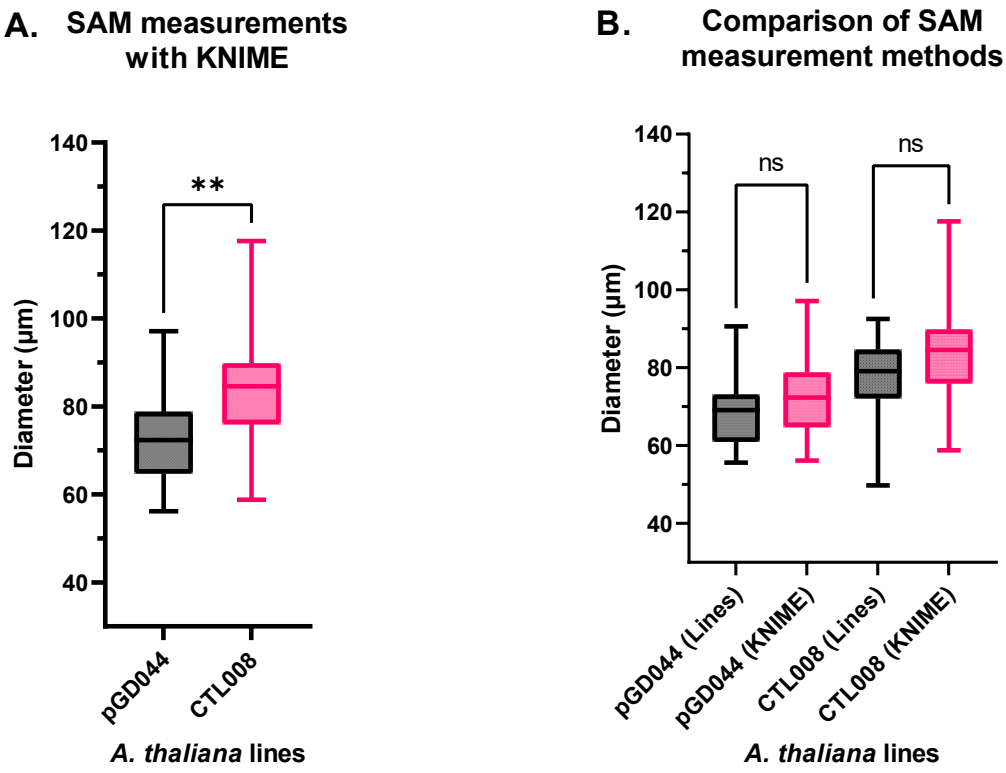

Figure 4.18: A. The *wox9* mutation results in SAM enlargement. Measurements done with KNIME. Sample size: pGD044 (n=18), CTL008 (n=19). Statistical test: unpaired two-tailed t-test with P value = 0.0066. B. The different methods of measuring SAM size did not result in a statistically significant difference between the respective measurements. Sample size: pGD044 Lines (n=19), pGD044 KNIME (n=18), CTL008 Lines (n=17), CTL008 KNIME (n=19). Statistical tests: unpaired two-tailed t-tests with P value = 0.1958 (pGD044) and P value = 0.1292 (CTL008). Plant line genotypes: pGD044 (wus -/-, wox9 +/+, pWUS::WUS-linker-GFP), CTL008 (wus -/-, wox9 -/-, pWUS::WUS-linker-GFP).

The next step was to examine where WOX9 is expressed in the SAM and if it co-localises with WUS. Therefore, I transformed wild-type Col-0 plants with a construct harbouring the pWOX9::WOX9-linker-mCherry expression cassette. The results were, however, disappointing, notwithstanding the fact that working with the native pWOX9 was difficult since it appears to be a weak promoter. Nevertheless, the detected signal revealed that the WOX9 fusion protein is present at the outer rim of the peripheral zone, at the sites where apparently the primordia will eventually develop (**Fig. 4.19**). In addition, later in development the WOX9 fusion protein gets incorporated into the developing lateral organs, thereby refuting the hypothesis that WOX9 and WUS might overlap in the SAM of *A. thaliana*.

The fact that the WOX9 protein localisation differed drastically from the published mRNA *in situ* experiments led me to repeat them utilising both sense and anti-sense WOX9 RNA probes. The experimental outcome was compatible to the WOX9 localisation in the SAM but invalidated the previously published results **[88]**. The *WOX9* mRNA was detected in the SAM periphery exclusively, coinciding with the distribution pattern of WOX9 (**Fig. 4.20**).

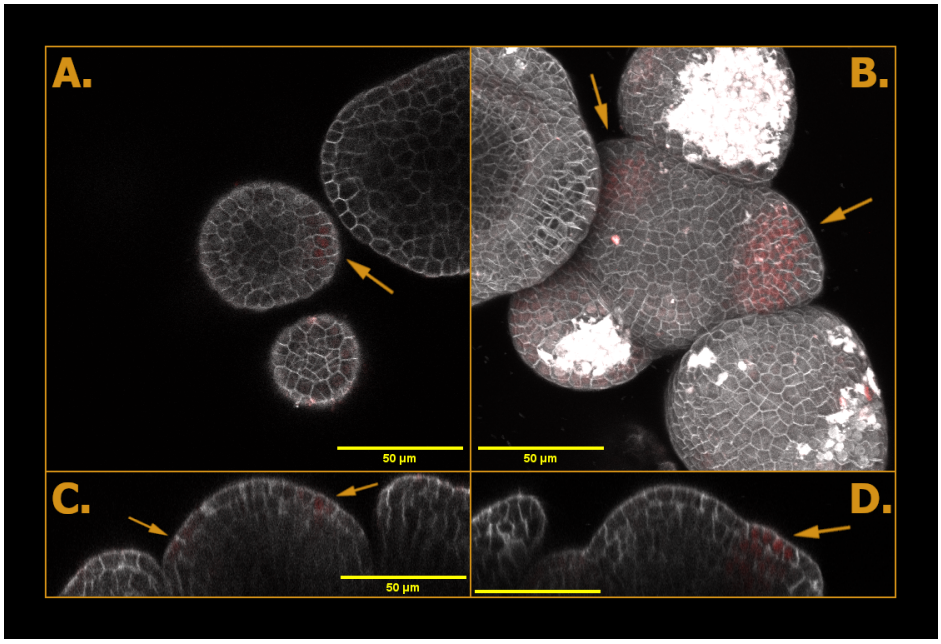

Figure 4.19: Expression of pWOX9::WOX9-linker-mCherry in *A. thaliana*. All T1 plants exhibited low transgene expression (n=26). Signal appears at the SAM periphery, away from the WUS domain (A and C). The transgene signal eventually gets incorporated in the developing lateral organs (B and D). A) Optical cross-section of a SAM at the L2 layer. B) Maximum-intensity projection of a SAM. C) Optical longitudinal section of the SAM depicted in figure part A. D) Optical longitudinal section of the SAM depicted in figure part B. Fluorescence signal is highlighted by orange arrows. Grey: DAPI. Red: WOX9-linker-mCherry. Scale bars correspond to 50 µm.

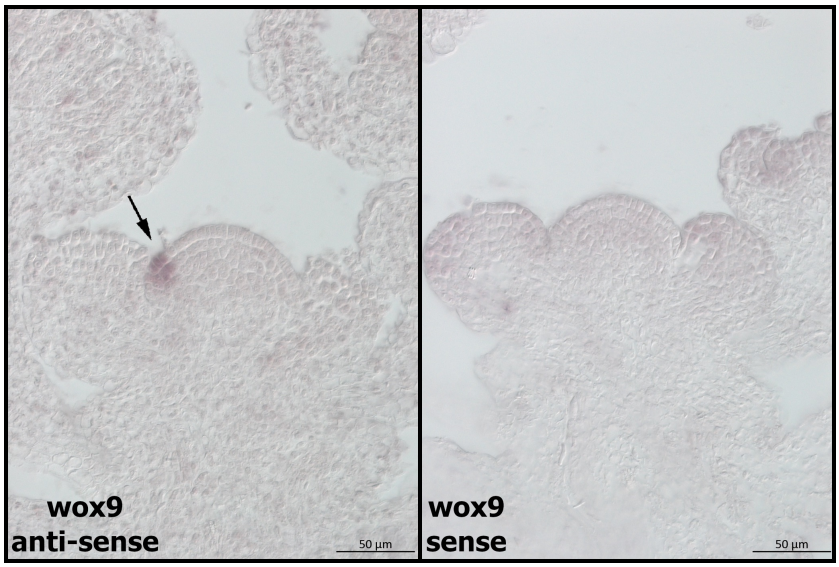

Figure 4.20: *In situ* detection of *WOX9* mRNA in Col-0 plants. Images were captured with DIC microscopy. Scale bars correspond to 50 µm.

Since it became obvious that WOX9 and WUS do not overlap in the SAM, a potential interaction between the two proteins in a wild-type context had to be excluded. The SAM enlargement that was observed in *wox9* plants is apparently the product of aberrant development that is not influenced by WUS and the stem cell niche. Nevertheless, I still examined if a WOX9-WUS interaction takes place in *A. thaliana*, when the two proteins are both present in the same SAM cell population, as well as the biological implications of such a co-expression.

In order to examine the notions stated above, I used the WUS promoter as well as the CLV3 promoter for expressing WOX9-linker-mCherry in Col-0 *A. thaliana* plants. In this way, it should become obvious if WOX9 exerts a function on WUS *in planta* since both proteins would co-localise in the cells where WUS is natively present. Both transgenes expressed strongly and in the pattern expected from pWUS and pCLV3, i.e. in the Organising Centre and in the Central Zone respectively (**Fig. 4.22**). However, the transgenic plants did not exhibit any kind of aberrant phenotype, neither in general nor with respect to WUS function. The only exception was a single T2 plant for the line expressing pWUS::WOX9-linker-mCherry (**4.21**). This plant developed many shoots that had a *clv3*-like phenotype with multiple lateral organs developing in the SAM, something that would be in line with a WOX9-induced repression of WUS, as well as phyllotaxis defects. However, the fact that it was only a single T2 plant out of dozens precluded any further investigation of the matter.

Figure 4.21: A T2 Col-0 plant expressing pWUS::WOX9-linker-mCherry and exhibiting a *clv3*-like phenotype.

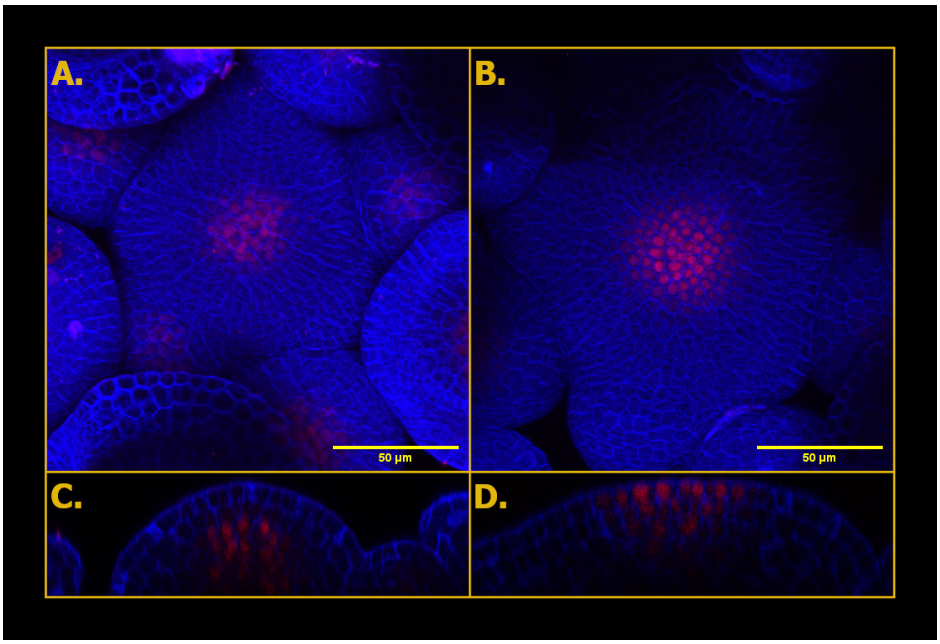

Figure 4.22: Expression of pWUS::WOX9-linker-mCherry (A, C) and pCLV3::WOX9-linker-mCherry (B, D) in *A. thaliana*. These transgenes were imaged at the T2 generation (27 T2 plants from 3 independent T1 events for pWUS::WOX9-linker-mCherry, 13 T2 plants from 2 independent T1 events for pCLV3::WOX9-linker-mCherry). The pWUS and pCLV3 exhibit the expected expression pattern. However, WOX9 ectopic expression in the SAM does not result in any aberrant phenotypes. A) Maximum-intensity projection of a plant expressing pWUS::WOX9-linker-mCherry. B) Maximum-intensity projection of a plant expressing pCLV3::WOX9-linker-mCherry. C) Optical longitudinal section of the SAM depicted in figure part A. D) Optical longitudinal section of the SAM depicted in figure part B. Blue: DAPI, Red: WOX9-linker-mCherry. Scale bars correspond to 50 µm.

Finally, even though there appeared to be no biological significance of a potential WUS-WOX9 interaction, I attempted to verify it in *A. thaliana* using AP-FRET. To this end, I crossed line CTL008 to line TTL027 in order to create line CTL010:

$$wus^{-/-},\ wox9^{-/-},\ pWUS :: WUS - linker - GFP\ (CTL008)$$
$$x$$
$$wus^{+/+},\ wox9^{+/+},\ pCLV3 :: WOX9 - linker - mCherry\ (TTL027)$$
$$\downarrow$$
$$wus^{-/-},\ wox9^{-/-},\ pWUS :: WUS - linker - GFP,$$
$$pCLV3 :: WOX9 - linker - mCherry\ (CTL010).$$

This experiment was also meant as proof-of-concept since something similar had never been performed in my lab before due to the fact that bleaching fluorophores and monitoring FRET in cells further down from the epidermis is not trivial. Nevertheless, the experiment worked technically (**Fig. 4.23**).

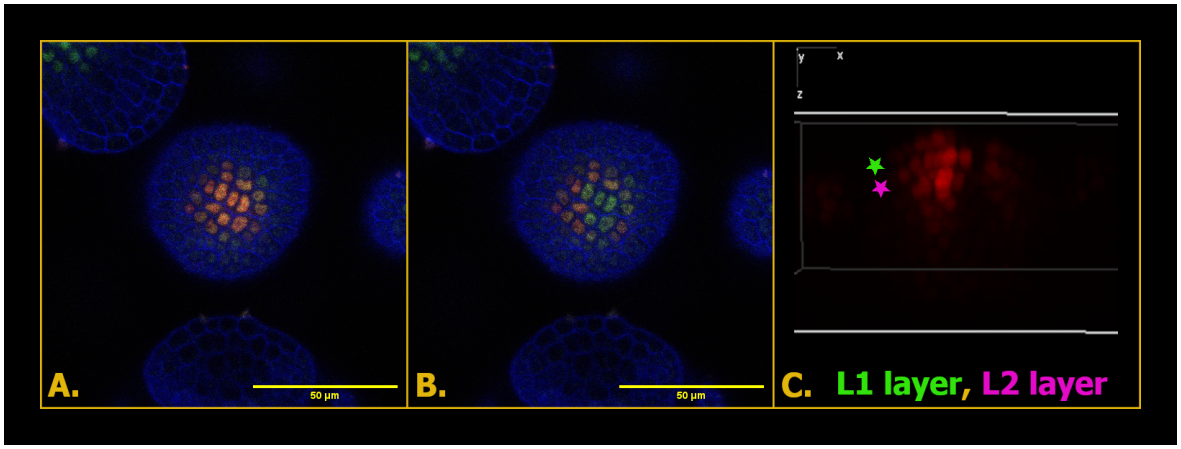

Figure 4.23: AP-FRET in *A. thaliana* SAMs. A. Pre-bleach cross-section of a CTL010 SAM at the L2 layer. B. Post-bleach cross-section of a CTL010 SAM at the L2 layer. C. Volume reconstruction (side view) of the SAM of a control plant expressing mCherry-NLS showing successful bleaching also in the rib meristem, i.e. below the L2 layer. Plant line genotypes: CTL010 (wus -/-, wox9 -/-, pWUS::WUS-linker-GFP, pCLV3::WOX9-linker-mCherry), control (wus -/-, wox9 -/-, pWUS::WUS-linker-GFP, pCLV3::mCherry-NLS). Blue: DAPI, Green: pWUS::WUS-linker-GFP, Red: pCLV3::WOX9-linker-mCherry (A, B) and pCLV3::mCherry-NLS (C). Scale bars correspond to 50 µm.

Unfortunately, however, even though there was some indication of interaction between WUS and WOX9, these initial results had to be disregarded since the mCherry-NLS negative control acceptor protein, which was included in the experiment, interacted strongly with WUS-linker-GFP (negative control line genotype: wus -/-, wox9 -/-, pWUS::WUS-linker-GFP, pCLV3::mCherry-NLS). This surprising finding cannot be explained adequately. Genotyping for the mCherry-NLS CDS confirmed the presence of this transgene though. Moreover, the deviations in the FRET measurements for the possible WUS-WOX9 interaction between the two different plant model organisms, which were used in this study, most likely arose due to the different fusion proteins used for the respective experiments. AP-FRET in *N. benthamiana* was done by utilising fusion proteins with no linker between the TF and the fluorophore

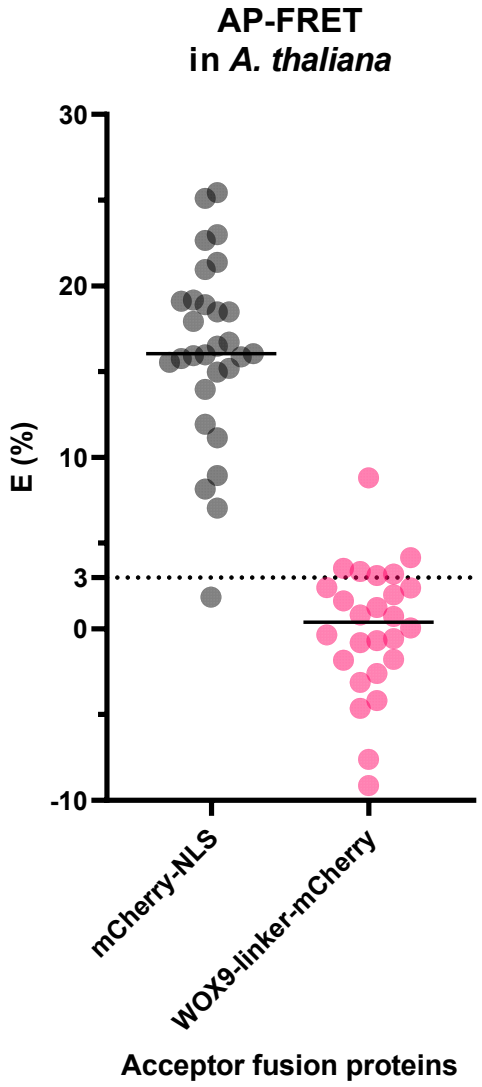

Figure 4.24: AP-FRET in *A. thaliana* SAMs. The E (%) values on the Y axis show the FRET efficiency of each experiment. The entries on the X axis correspond to the respective acceptor fusion protein. The donor protein is WUS-linker-GFP. The black lines depict the median value for each group of measurements. E (%) values >3 indicate protein-protein interaction.

coding sequences, while AP-FRET in *A. thaliana* was conducted with fusion proteins that included a Ser-Gly linker (e.g. WUS-GFP vs WUS-linker-GFP). The decision to omit the linker from the constructs used for AP-FRET in *N. benthamiana* was based on previous experience gathered in the lab with similar experiments. On the other hand, including the linker in the design of the fusion proteins expressed in *A. thaliana* was also based on prior *wus* rescue experiments done in the lab, whereby fluorophores that were directly fused downstream of WUS resulted in reduction of the fusion protein's biological activity.

4.3.2 *esr1*

In parallel to the generation of CRISPR mutants for *ESR1*, I also performed an *in situ* experiment for this gene in order to verify its expression pattern in the SAM. At that point there were no relevant data in the eFP browser and earlier publications either focused on the *ESR1* function in the embryo **[89], [90]** or presented *in situs* with very low resolution **[91]**. My experiments showed that *ESR1* is transcribed in the L1 layer of the SAM as well as at the distal end of the developing flowers (**Fig. 4.25**).

Since I confirmed the expression of *ESR1* in an area of the SAM that overlaps with the

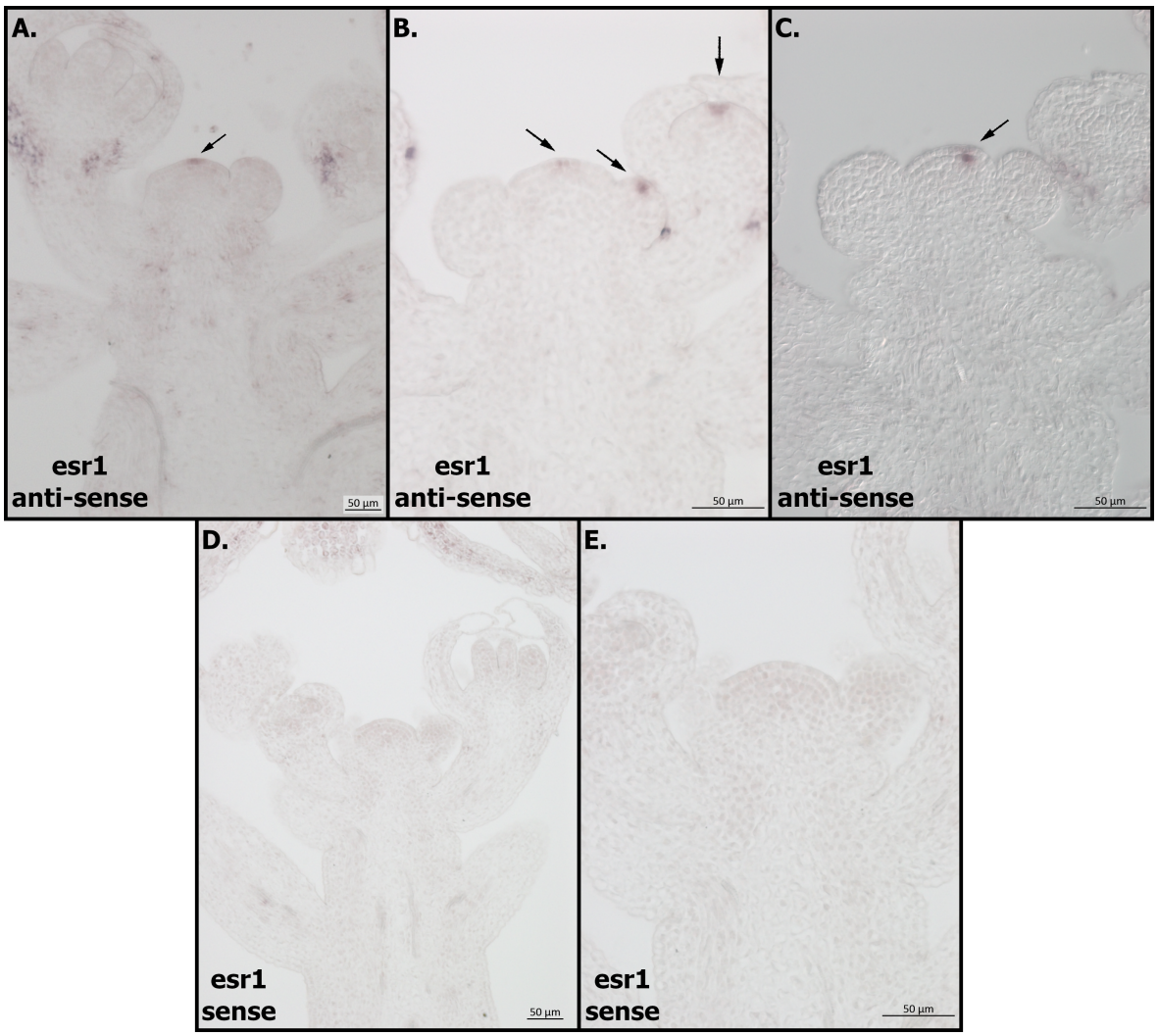

Figure 4.25: *In situ* detection of *ESR1* mRNA in Col-0 plants. Images A, B, C show sections from three different plants at varying magnifications. The plant in image C is tilted but gave the strongest signal for the anti-sense probe and was selected for DIC microscopy. Images D and E show the same plant at different magnifications. Images A, B, D, E were captured with bright-field while image C with DIC microscopy. Scale bars correspond to 50 µm.

WUS domain, I chose as a next step to examine the ESR1 protein distribution. For this purpose, I transformed Col-0 plants with a construct coding for the pESR1::mCherry-linker-ESR1 expression cassette. Using laser confocal microscopy I was able to detect that mRNA and protein distribution for *ESR1* overlap (**Fig. 4.26**). Unfortunately, the native pESR1 seems to be a weak promoter, at least when it comes to expression in the SAM. Nevertheless, it cannot be excluded that expressing transgenes under pESR1 would be stronger in a *esr1* mutant background. The technical difficulties notwithstanding, it seems that the ESR1 protein, when compared to the *ESR1* transcription domain, expands laterally towards the organ primordia and possibly also into the L2 layer. However, no signal was detected in the OC. These results seem also to confirm that the *ESR1* expression in the SAM of the developing embryo remains unaltered in adult plants **[92]**.

I created a CRISPR mutant collection for *ESR1* by transforming Double-Reporter plants with a construct expressing the gRNA by an egg-specific promoter (**Section 3.37**). These

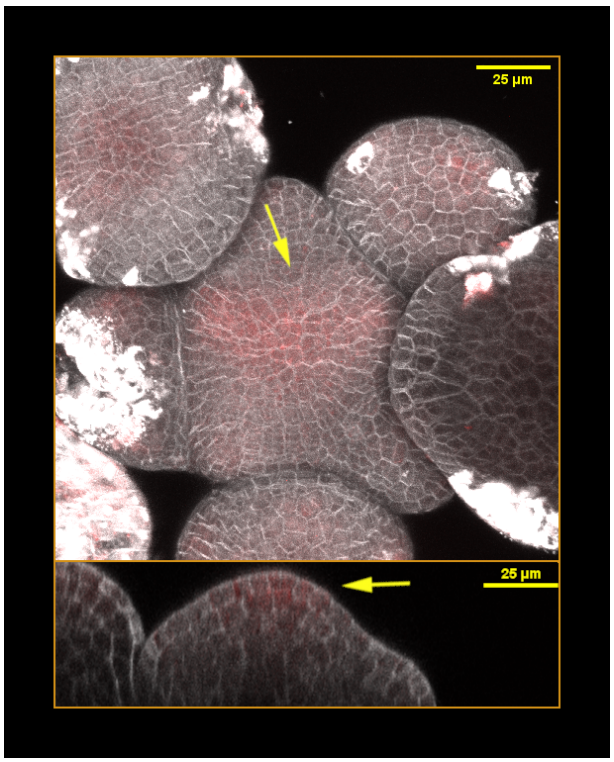

Figure 4.26: Expression of pESR1::mCherry-linker-ESR1 in *A. thaliana*. All T1 plants exhibited low transgene expression (n=40). Signal appears at the L1 and L2 within the CZ. Up) Maximum-intensity projection of a SAM from a plant expressing pESR1::mCherry-linker-ESR1. Down) Optical longitudinal section of the SAM depicted in the upper part of the figure. Fluorescence signal is highlighted by yellow arrows. Grey: DAPI. Red: pESR1::mCherry-linker-ESR1. Scale bars correspond to 25 µm.

mutants were the result of single base insertions or deletions (A, T or G) as well as a 4-base and a 47-base deletion. The first mutant that was used in this study was generated by the addition of a single G after base no.162 of the *ESR1* CDS. This mutation resulted in the premature termination of ESR1 and the generation of a truncated 73 aa protein in contrast to the 328 aa full-length ESR1 protein (**Fig. 4.27**). Moreover, this mutation terminates translation at the beginning of the AP2/ERF DNA-binding domain, thereby practically abolishing it. In addition to the absent AP2/ERF domain, this mutation could not otherwise result in a biologically active molecule due to the absence of any alternative downstream translation initiation sites. The second mutant used in these experiments was generated by the deletion of a single G (base no.162 of the *ESR1* CDS) and resulted in a frame-shift that creates a truncated 144 aa protein (**Fig. 4.27**). Although this mutant protein is longer compared to the one resulting from the single base addition and still contains the region originally coding for the AP2/ERF domain, the frame-shift alters severely the amino acid composition of the latter thereby once again abolishing it. This mutant protein also lacks any alternative downstream translation initiation sites.

Both types of *ESR1* CRISPR mutants were used to study the effects of *esr* on the SAM stem cell pool. It is worth noting, however, that there appears to be functional redundancy between *ESR1* and its paralog *ESR2*, which is also present in *A. thaliana*. Mutants for both loci that were generated in the framework of another project running in our lab exhibit a severe lethal phenotype at the seedling stage (results not shown). Yet, *esr1* mutants show

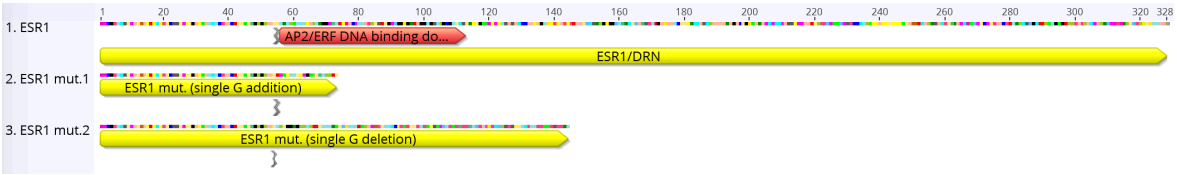

Figure 4.27: The ESR1 wild-type (1) and two of the ESR1 CRISPR mutant (2, 3) proteins. The AP2/ERF DNA-binding domain is depicted as a red ribbon on the ESR1/DRN wild-type sequence. The shorter ESR1 mutant depicted in line 2 results from a single G addition. The longer mutant shown in line 3 is the product of a single base deletion and although it encompasses the region originally coding for the AP2 DNA binding domain, the mutation nevertheless abolishes it due to frame-shift. The jagged gray line shows the CAS9 cutting site.

macroscopically no visible effects, although an embryo-specific phenotype has been reported **[89]**. They do, however, exhibit prolonged longevity and reduced fertility, similar to *wox9* plants.

The SAM measurements for the *esr1* plant were conducted in the manner described earlier for *wox9* (**Fig. 4.28**). A initial comparison between the different CRISPR *esr1* mutants showed that there was no statistically significant deviation among them with respect to the size of the SAM (results not shown). Thus, I grouped all *esr1* SAM measurements and compared them against the control Double-Reporter plants. The results of this comparison proved that the *esr1* mutants are characterised by an enlarged SAM (**Fig. 4.29**). Such a phenotype could be a direct effect of a larger stem cell pool, which could in turn be caused by altered WUS function or distribution.

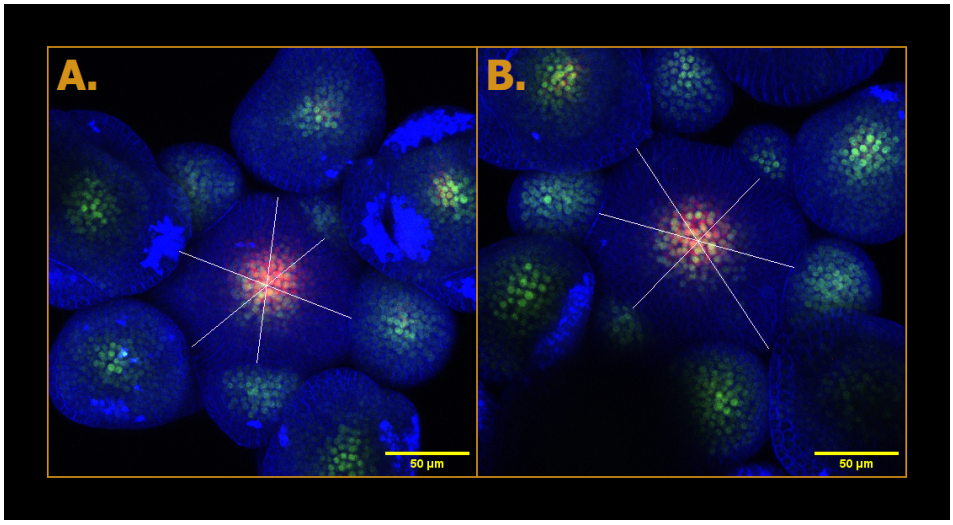

Figure 4.28: Measuring SAM diameter by drawing lines on a maximum intensity projection of a Double-Reporter (A) and an *esr1* (B) SAM. Plant line genotypes: Double-Reporter (esr1 +/+, pCLV3::mCherry-NLS, pWUS::3xVenus-NLS), *esr1* (esr1 -/-, pCLV3::mCherry-NLS, pWUS::3xVenus-NLS). Blue: DAPI, Red: pCLV3::mCherry-NLS, Green: pWUS::3xVenus-NLS. Scale bars correspond to 50 µm.

As a first step in the direction of dissecting WUS activity in the esr -/- background, I planned to examine the distribution of WUS in *esr* mutants.Therefore, I attempted to create a line that would harbour the WUS-linker-GFP fusion protein in the wus -/-, esr1 -/- double-

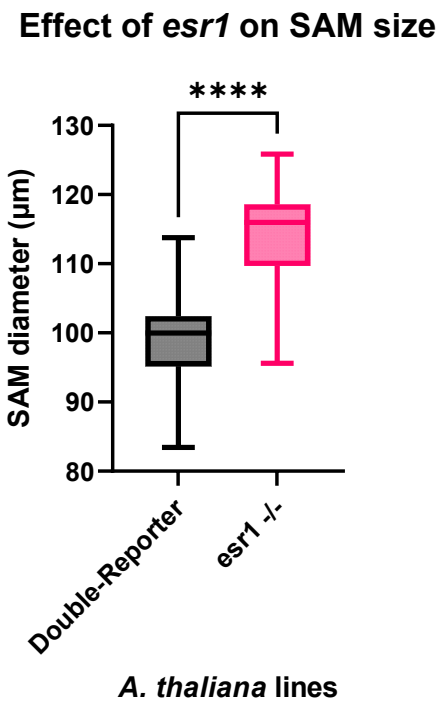

Figure 4.29: The *esr1* mutation also results in SAM enlargement. Plant line genotypes: Double-Reporter (esr1 +/+, pCLV3::mCherry-NLS, pWUS::3xVenus-NLS), *esr1* (esr1 -/-, pCLV3::mCherry-NLS, pWUS::3xVenus-NLS). Sample size: Double-Reporter (n=18), *esr1* (n=31). Statistical test: unpaired two-tailed t-test with P value < 0.0001.

mutant background (CTL013). Since the *esr1* mutation had been generated in the Double-Reporter background, I would also need to exclude the pWUS and pCLV3 reporter constructs during segregation. To this end, I crossed an *esr1* mutant into line pGD044, our lab's *wus* rescue line::

$$wus^{-/-},\ esr1^{+/+},\ pWUS::WUS-linker-GFP\ (pGD044)$$
$$x$$
$$wus^{+/+},\ esr1^{-/-},\ pCLV3::mCherry-NLS,\ pWUS::3xVenus-NLS\ (esr1)$$
$$\downarrow$$
$$wus^{-/-},\ esr1^{-/-},\ pWUS::WUS-linker-GFP\ (CTL013)$$

However, it was proven that this line was impossible to generate. I discovered that during the generation of the *wus* rescule line pGD044, the pWUS::WUS-linker-GFP contruct had been inserted in the proximity of the wild-type *ESR1* locus. Thus, the segregation of pWUS::WUS-linker-GFP was always associated with an esr +/+ or +/- background due to genetic linkage. Time restrictions did not allow me to pursue the generation of a new *wus*-rescue line.

Nevertheless, I tried to gain more insight into the putative WUS-ESR1 interaction by ectopically expressing ESR1 under the regulatory control of pWUS or pCLV3. In order to accomplish this, I transformed Col-0 plants with constructs harbouring the pWUS::mCherry-linker-ESR1 or pCLV3::mCherry-linker-ESR1 expression cassettes. To my surprise, the T1 plants manifested strong termination phenotypes (**Fig. 4.30** and **4.31**). Intriguingly, plants that terminated early after germination possessed an apical bulge instead of a SAM. This bulge remained dormant and its formation was frequently followed by death, but in many cases aberrant leaves and shoots would emerge out of its base after several weeks **Fig. 4.32**.

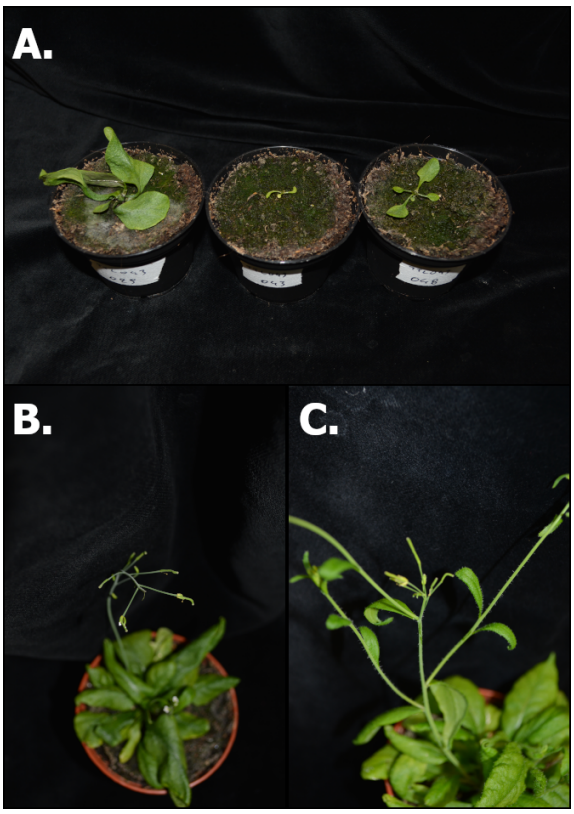

Figure 4.30: SAM termination upon ESR1 ectopic expression driven by pWUS. All T1 plants depicted have been genotyped.

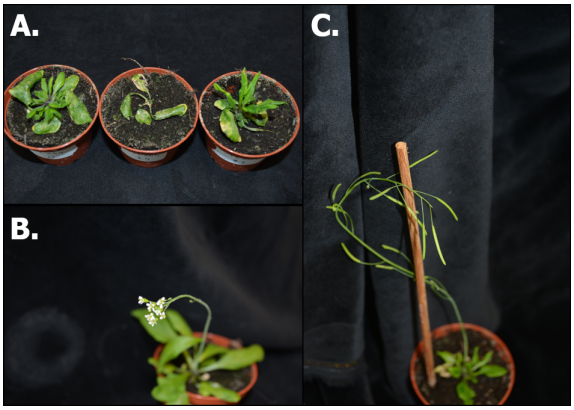

Figure 4.31: SAM termination upon ESR1 ectopic expression driven by pCLV3. All T1 plants depicted have been genotyped.

I imaged such shoots with confocal microscopy but the experiments did not yield conclusive results since any fluorescence detected was extremely weak (**Fig. 4.33**). Overall, it seems that ESR1 exerts strong repression on *WUS* function, but only when it is expressed in the L3 and farther below.

Figure 4.32: T1 plant expressing pWUS::mCherry-linker-ESR1. The SAM has terminated and resembles a bulge (pinpointed by white arrows). Picture A was taken 15 days before picture B.

It appears that ESR1 expression driven by pWUS or pCLV3 arrests stem cell function in the respective plant lines, but growth might restart if the corresponding transgenes are silenced. This assumption is supported by my attempts to visualize ESR1 ectopic expression in the SAM. The pWUS-driven expression of ESR1 was practically absent from all T1 plants that I imaged (**Fig. 4.33**). On the other hand, approximately 25% of the T1 plants expressing pCLV3::mCherry-linker-ESR1 showed weak fluorescence, but the signal was detected only in the rib meristem, outside of the *CLV3* domain (**Fig. 4.33**).

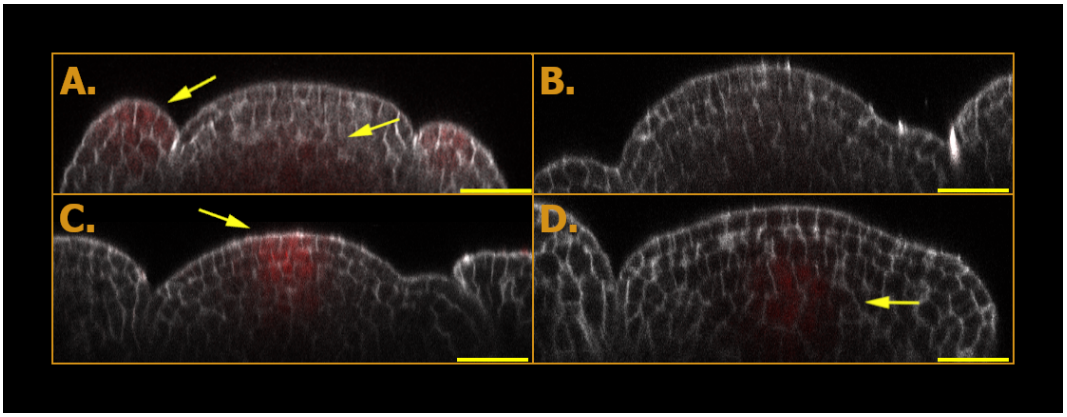

Figure 4.33: Ectopic expression of ESR1 under pWUS (A, B) or pCLV3 (C, D). The expression of ESR1 under pWUS was very weak. Only 2 out of 31 T1 plants showed faint fluorescence, which was detected across the rib meristem below L3 (A), while the rest did not show any signal (B). The expression of ESR1 under pCLV3 was also very weak, with only 10 out of 39 T1 plants exhibiting fluorescence. This signal though was incompatible with pCLV3-driven expression, since it was detected only in the rib meristem below the L3 layer (D). Only a single T1 plant exhibited fluorescence in the *CLV3* domain (C). Fluorescence signal is highlighted by yellow arrows. Plant line genotypes: pWUS::mCherry-linker-ESR1 (A, B), pCLV3::mCherry-linker-ESR1 (C, D). Grey: DAPI, Red: pWUS::mCherry-linker-ESR1 (A, B) or pCLV3::mCherry-linker-ESR1 (C, D). Scale bars correspond to 25 μm.

4.4 Co-IP of WUS protein complexes from *A. thaliana* and *N. benthamiana*

4.4.1 Initial attempts

I mentioned already in the Introduction that a major goal of this thesis was to identify the protein complexes that WUS forms in the SAM of *A. thaliana*. Thus, I performed a series of co-IP experiments with the aim of isolating such WUS complexes. Although I used fusion proteins with different types of tags for my co-IPs in an attempt to exploit different affinity purification approaches, all the co-IP experiments that will be reported in this chapter were done with the fusion protein WUS-linker-GFP, which has been successfully used in the past to rescue the *wus* mutation in *A. thaliana*.

Initially, I used as input material for my co-IPs *A. thaliana* seedlings in an attempt to enrich my input with meristematic tissue. The usual 1-2 g of input material that I used contained hundreds of seedlings, i.e. SAMs. However, I was never able to detect the presence of

WUS-linker-GFP in either the input or the more concentrated elution fraction (results not shown). Since WUS is expressed in roughly 50 cells in the SAM, it became obvious that this enrichment approach was not sufficient.

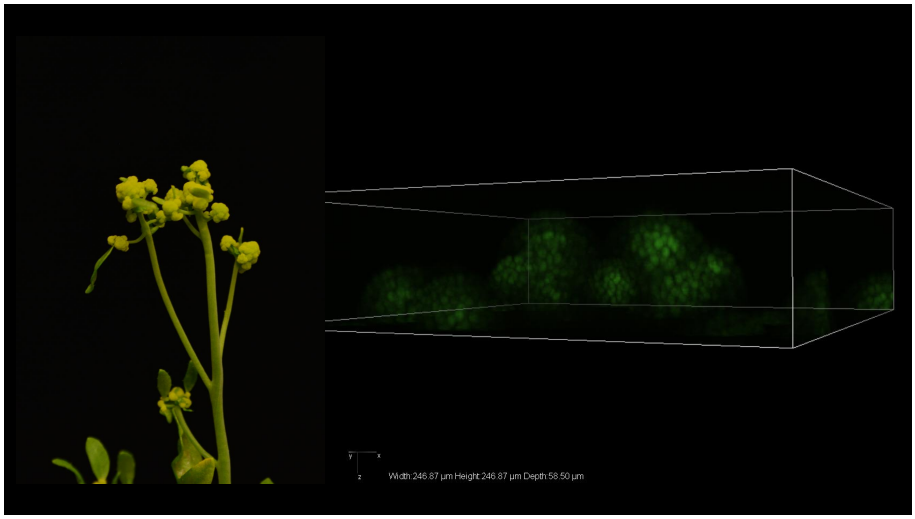

Figure 4.34: Transgenic *ap1/cal* double mutant transformed with pWUS::WUS-linker-GFP (left). Even a tiny part of inflorescence tissue contains multiple meristems (right).

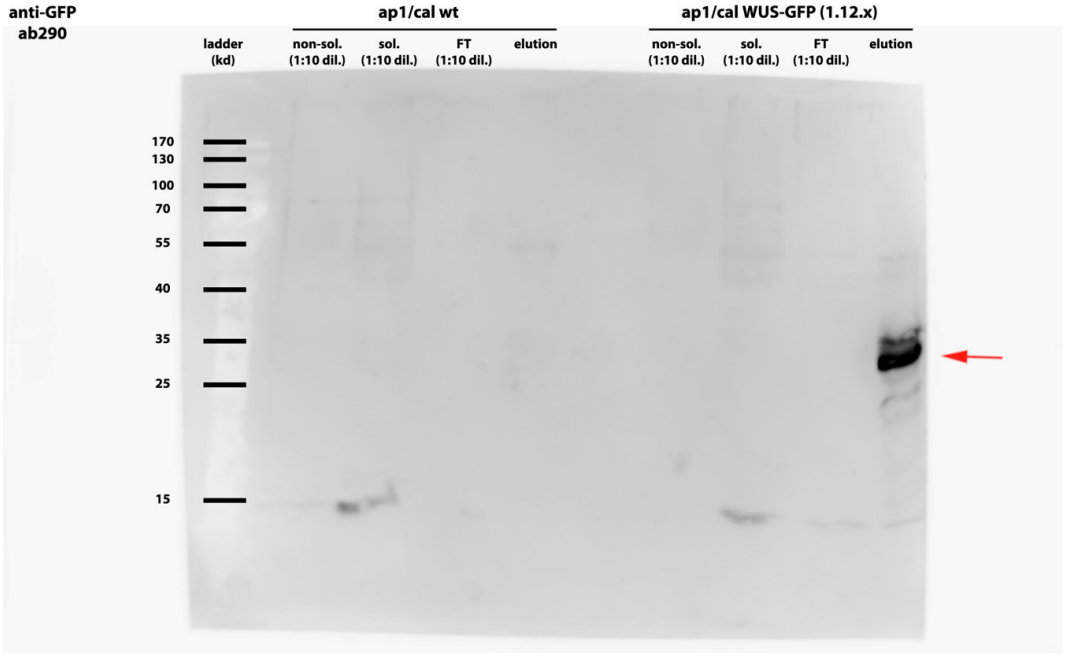

Figure 4.35: Western blot for an IP against WUS-linker-GFP. Only the GFP part of the fusion protein could be isolated (red arrow). Input: *ap1/cal*. Antibody: anti-GFP.

As a next step, I decided to use the *apetala/cauliflower* (*ap1/cal*) double mutant **[93]**. The *ap1/cal* plants are characterised by a massive overproliferation of meristematic tissue **Fig. 4.34**, which is achieved by the protracted retention of inflorescence meristems and the delayed generation of flowers. Thus, the use of *ap1/cal* plants as input material would provide me with significantly higher quantity of WUS fusion proteins while maintaining the genetic background of the input tissue close to the wild-type. Unfortunately, though, the respective co-IPs were only partially successful as I could only pull down a molecule that

had an apparent molecular weight (MW) corresponding to half of that of the WUS-linker-GFP protein (**Fig. 4.35**). Also, this molecule was detected in a western blot only when using an anti-GFP antibody. On the contrary, the western blots developed with an anti-WUS antibody did not generate any signal. Therefore, I got the first indications that the WUS-linker-GFP protein is not stable during my co-IP workflow and as a result I could only manage to isolate the GFP part of the fusion protein.

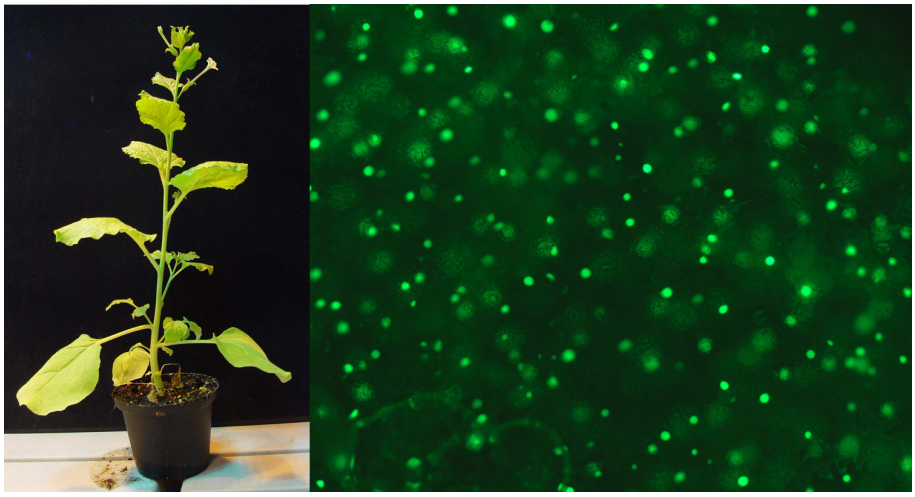

Figure 4.36: The tobacco plant *N. benthamiana* (left). Overexpression of proteins under viral promoters is very efficient (right).

Since the use of *ap1/cal* inflorescences was not sufficient to detect the WUS fusion protein in the input fraction, but only in the more concentrated eluate, I decided to overexpress WUS-linker-GFP in the leaves of the tobacco plant *Nicotiana benthamiana* (**Fig. 4.36**). The overexpression of proteins under the regulatory control of viral promoters in *N. benthamiana* is of great advantage for the study of a lowly abundant protein such as WUS. *N. benthamiana* can be readily and easily transformed in a transient manner with agrobacteria. Moreover, the overexpressed proteins attain high *in vivo* concentrations. This last attribute of this plant heterologous system proved to be invaluable for the troubleshooting of my IP workflow, since it allowed me to detect WUS in every step of my co-IPs. By using *N. benthamiana* as input for an IP I could establish that the WUS-linker-GFP did indeed get degraded during isolation (**Fig. 4.37** and **4.38**). Thus, I had to tackle the instability that my WUS fusion proteins exhibited before I could isolate any WUS complexes.

When I processed *N . benthamiana* tissue as input for my IPs, I was able to detect the WUS-linker-GFP in western blots both with an anti-GFP as well as with an anti-WUS antibody. Incidentally, I briefly considered the possibility of the truncated molecule, which I detected in the IP eluate, being the one and the same, but also being detected by two different antibodies due to cross-reactivity. However, I excised the respective bands from the SDS-PAGE gels and analysed them with Mass-Spec. Subsequently, I could verify the simultaneous presence in the IP eluate of both the WUS and the GFP truncated parts of the WUS-linker-GFP fusion protein (results not shown).

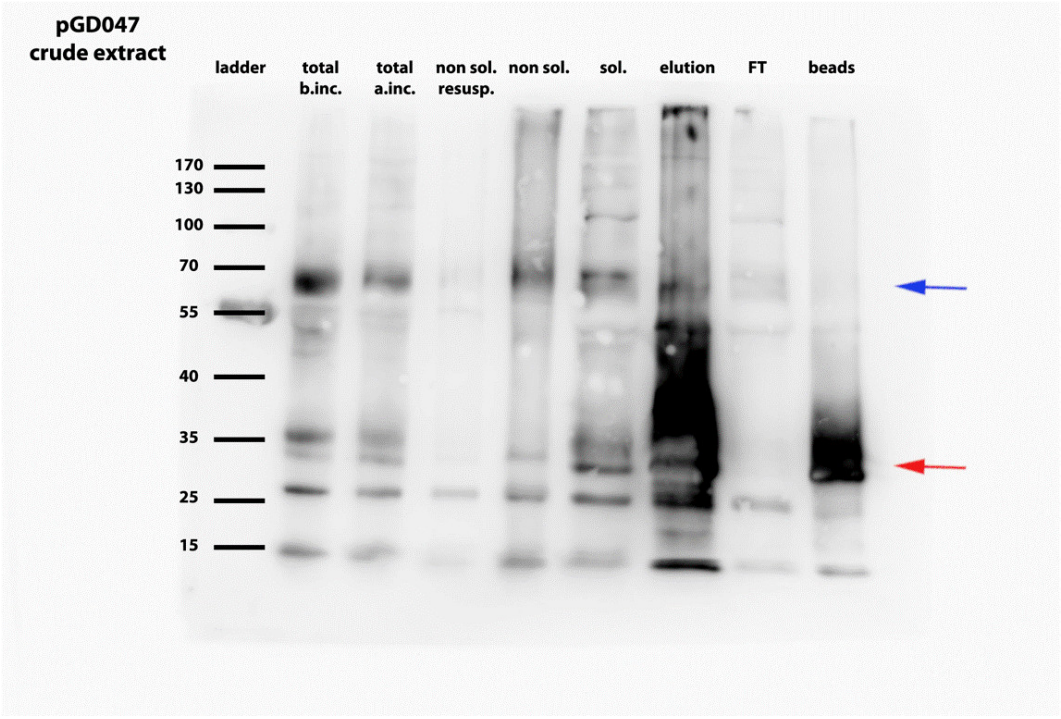

Figure 4.37: Western blot for an IP against WUS-linker-GFP. The complete fusion protein (blue arrow) can be detected in the input fraction but gets degraded during isolation, resulting in a truncated molecule (red arrow). Input: *N. benthamiana*. Antibody: anti-GFP.

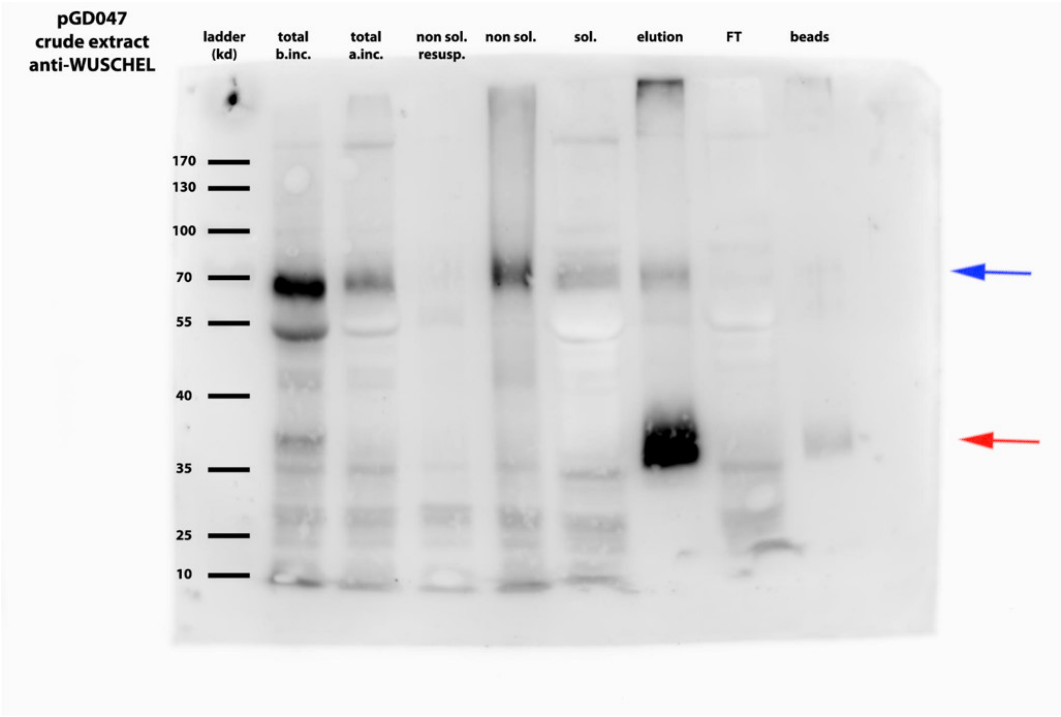

Figure 4.38: Western blot for an IP against WUS-linker-GFP. The complete fusion protein (blue arrow) can be detected in the input fraction but gets degraded during isolation, resulting in a truncated molecule (red arrow). Input: *N. benthamiana*. Antibody: anti-WUS.

4.4.2 Troubleshooting WUS degradation during immunoprecipitation experiments

I exploited the advantages of *N. benthamiana* as a protein overexpression system to establish a simple protein stability assay in order to expedite the troubleshooting of my IP

workflow (**Fig. 4.39** and **4.40**). This way, I was able to detect that the degradation of my WUS fusion proteins began fast, while still in the Extraction buffer, and was therefore not caused by any erroneous experimental handling during the downstream purification steps.

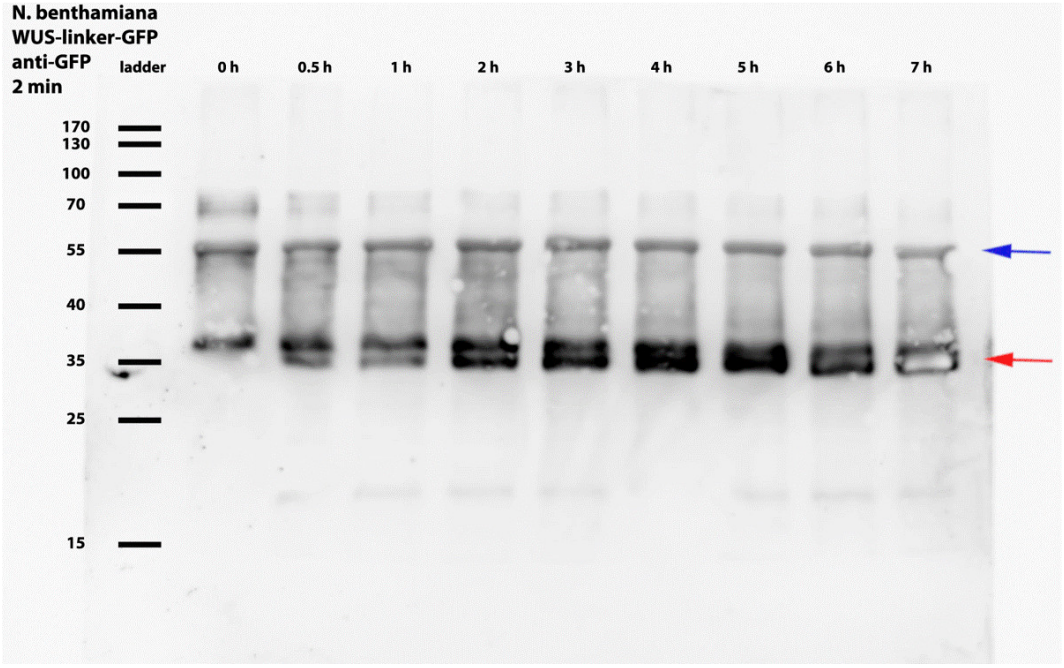

Figure 4.39: Western blot for a time-course stability assay for WUS-linker-GFP. The complete fusion protein (blue arrow) is not stable and gets degraded fast resulting in a truncated molecule (red arrow). Input: *N. benthamiana*. Antibody: anti-GFP.

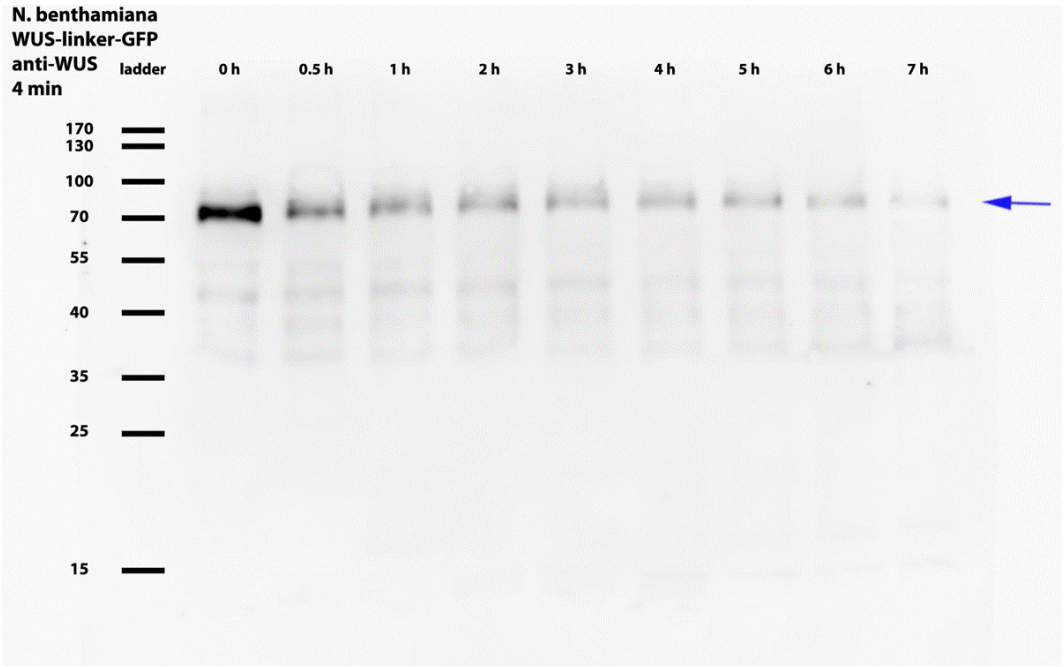

Figure 4.40: Western blot for a time-course stability assay for WUS-linker-GFP. The complete fusion protein (blue arrow) is not stable and gets degraded fast. The WUS truncated part of the fusion protein is apparently further degraded and is never detected. Input: *N. benthamiana*. Antibody: anti-WUS.

These assays also revealed that while the GFP truncated segment remains stable after the initial degradation of the fusion protein and constantly accumulates, the WUS part

does not. Instead, the WUS segment undergoes apparently further degradation and never reaches a concentration that is high enough to overcome the western blotting detection threshold. This result hints that WUS itself is responsible for the instability of the WUS fusion proteins that I used for my co-IPs.

In the following table, I summarize all the modifications I did to my protein extraction protocol, as well as their outcome:

Modification of the crude extraction protocol	Outcome
IP elution with acidic glycine solution (instead of Laemmli buffer)	Failure
Heating up eluates to 42 °C instead of 95 °C before SDS-PAGE	Failure
Omitting benzonase from the extraction buffer	Failure
Addition of 5x more protease inhibitor in the extraction buffer	Failure
Increase of EDTA concentration to 20 mM in the extraction buffer	Failure
Omitting the Gly-Ser polypeptide linker between WUS and GFP	Failure
N-terminally tagged WUS fusion proteins instead of C-terminally	Failure
Nuclei isolation instead of crude extract	**Success**
Cross-linking with formaldehyde	**Success**

After extensive and lengthy troubleshooting, I was able to identify workarounds for the instability that WUS fusion proteins exhibit in solution. The first successful approach was to abandon the crude extract protocol and perform a nuclei isolation (**Fig. 4.41**). The subsequent co-IP was successful (**Fig. 4.42**). The respective IP eluates were analysed with Mass-Spec and the results will be presented later in this chapter.

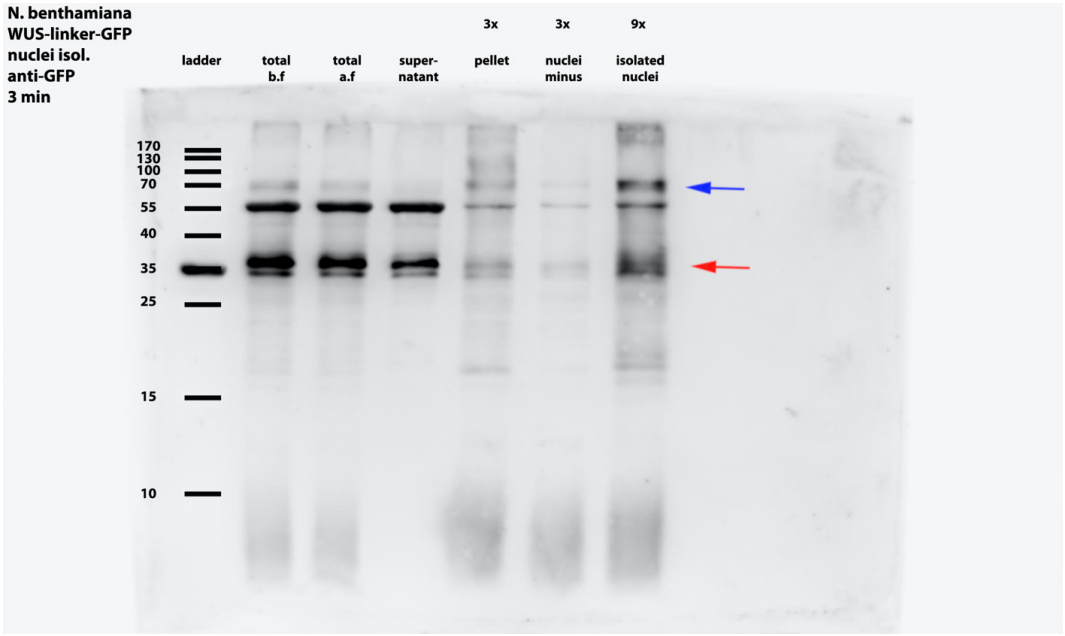

Figure 4.41: Western blot for nuclei isolation from *N. benthamiana* expressing WUS-linker-GFP. The fusion protein (blue arrow) remains intact for the complete duration of the protocol (ca. 4 hours). Truncated GFP is still detected (red arrow), which most likely originates from *in vivo* protein turnover, but does not accumulate over time. Input: *N. benthamiana*. Antibody: anti-GFP.

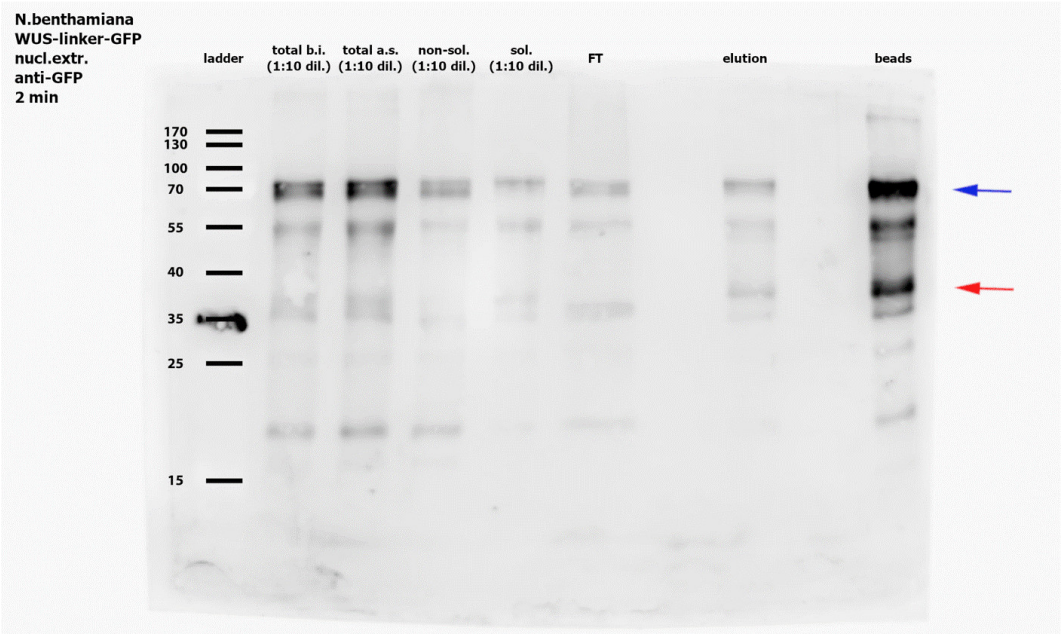

Figure 4.42: Western blot for nuclear protein extraction and IP against WUS-linker-GFP. The fusion protein (blue arrow) remains intact for the complete duration of the protocol (ca. 3 hours). Truncated GFP is still detected (red arrow), which most likely originates from *in vivo* protein turnover, but does not accumulate over time. Input: *N. benthamiana* nuclei. Antibody: anti-GFP.

Nevertheless, all attempts to perform nuclei isolation from *ap1/cal* tissue failed (results not shown). Thus, I continued the troubleshooting in order to find a protocol that would be applicable to *A. thaliana*. The only other protocol modification that enabled the successful isolation of intact WUS fusion proteins consisted of cross-linking the input tissue with formaldehyde prior to protein extraction (**Fig. 4.43**).

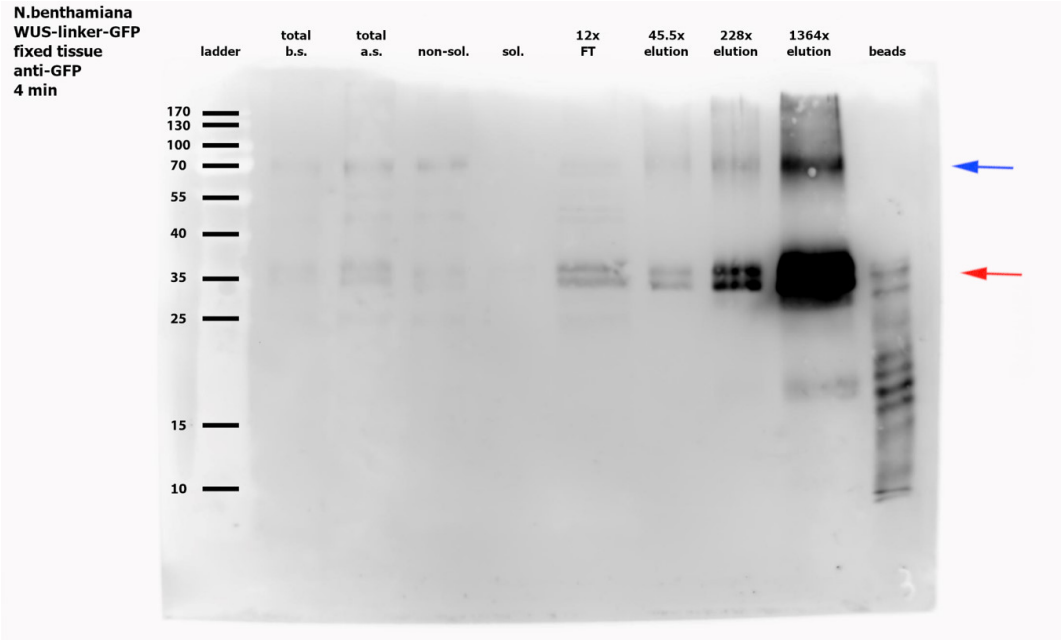

Figure 4.43: Western blot for an IP against WUS-linker-GFP. Intact fusion protein (blue arrow) was successfully isolated. Truncated GFP is still detected (red arrow), indicating protein degradation. Input: *N. benthamiana* tissue, cross-linked with formaldehyde. Antibody: anti-GFP.

Although the cross-linking of plant tissue with formaldehyde resulted in the isolation of intact WUS fusion protein, the latter was still partially degraded during the experiment. This is evident by the increased ratio of ”truncated protein”:”intact protein” in the eluate when compared to the input fraction (**Fig. 4.43**). This, however, is not an unequivocal sign of protein instability during purification. The WUS-linker-GFP fusion protein has always been degraded in a consistent manner during my IPs, a fact suggesting that this degradation is mediated by a biomolecule. It is plausible that a protease is digesting WUS-linker-GFP. Such a protease could become inert due to the cross-linking but could regain its activity when the formaldehyde cross-linking is reversed by applying heat. In such a case, the degradation of WUS fusion proteins happens only after they have been purified. The stability of WUS fusion proteins that are isolated from fixed tissue could also be studied with the time-course experiment I presented earlier (**Fig. 4.39** and **4.40**), but unfortunately I did not perform such an assay due to time restrictions.

4.4.3 Mass-Spec analysis of WUS protein complexes isolated from *N. benthamiana* tissue

I mentioned earlier that I was able to purify WUS complexes from *N. benthamiana* nuclei. The respective co-IP was performed in biological triplicates and the eluates were analysed with Mass-Spec. The controls for this experiment were *N. benthamiana* tissue, which expressed N7_NLS-3xGFP, and wild-type uninfiltrated *N. benthamiana* tissue. One of my WUS-linker-GFP samples, however, had to be discarded due to technical problems with the Mass-Spec instrument during the run.

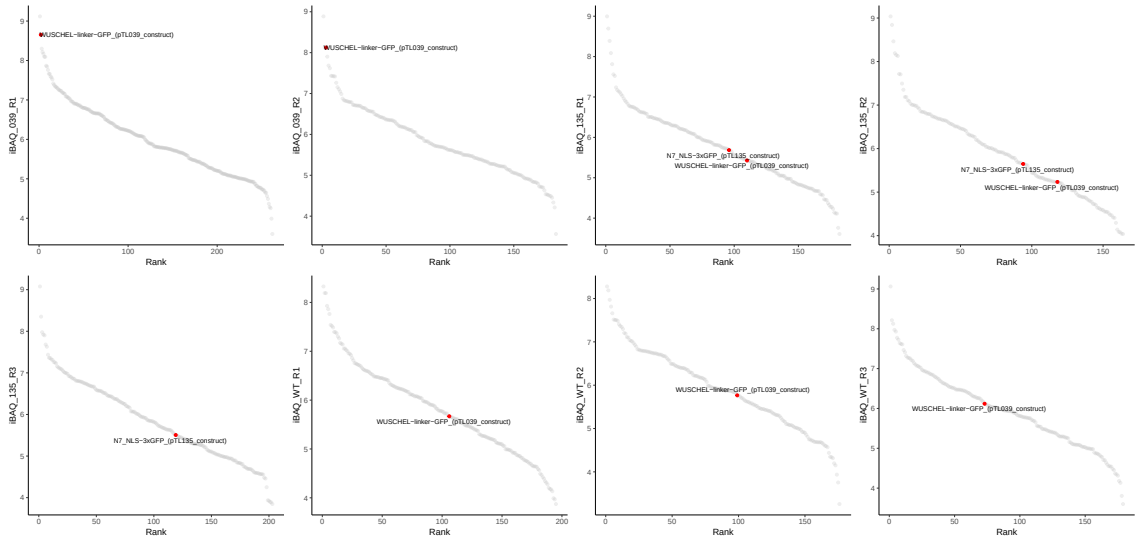

Figure 4.44: IBAQ values for the proteins isolated from the IPs done for *N. benthamiana*. 039: WUS-linker-GFP samples, 135: N7_NLS-3xGFP samples, WT: wild-type samples.

The IBAQ values, which reflect the relative quantitation of proteins within a certain sample, revealed that the IP was highly successful, since WUS-linker-GFP ranked very high in the respective samples (**Fig. 4.44**). The LFQ values, which permit the comparison of the quantitation values for a given protein across all samples, also confirmed the success of the

IPs, since WUS-linker-GFP was significantly enriched in the WUS-linker-GFP samples **Fig. 4.45**. The rest of the proteins that were significantly enriched (fold-change > 4) in the WUS-linker-GFP are presented in the table below. Overall, 57 proteins were significantly enriched in the WUS-linker-GFP samples in all pairwise comparisons against both controls. It is worth noting that among the enriched proteins is TPLR 3, a fact that adds additional confidence to these results. TPL and TPLR proteins are established co-factors of WUS.

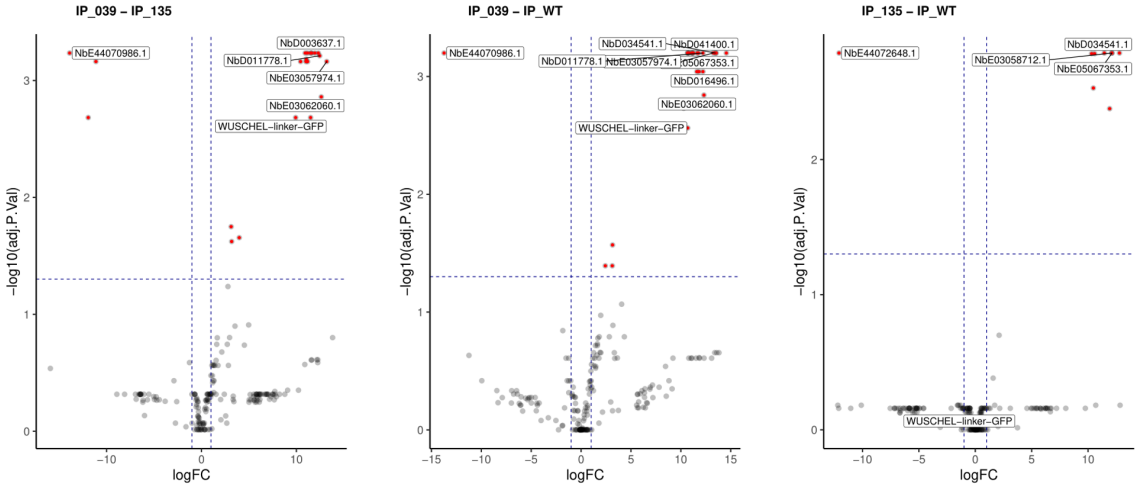

Figure 4.45: LFQ values and pairwise comparisons for the proteins isolated from the IPs done for *N. benthamiana*. 039: WUS-linker-GFP samples, 135: N7_NLS-3xGFP samples, WT: wild-type samples.

Mass-Spec results from *N. benthamiana*		
log2(FC 039-135)	**log2(FC 039-wt)**	**Enriched proteins in 039 against both controls**
14.65	10.48	**WUSCHEL-linker-GFP_(pTL039_construct)**
13.96	8.64	NbE05064048.1uncharacterizedproteinLOC108947531(XP_018631163.1)
13.23	9.81	NbE03057974.1eukaryotictranslationinitiationfactor3subunitAisoformX1(XP_019250286.1)
12.78	12.68	NbE05068848.1Partial,photosystemIP700chlorophyllaapoproteinA2(plastid)(NP_054496.1)
12.23	12.25	NbE03062060.1uncharacterizedproteinLOC104118817(XP_009628473.1)
11.84	12.02	NbE03059012.1glutaminesynthetase,chloroplastic(XP_019243456.1)
11.73	11.82	NbD011778.1proteinTOC75-3,chloroplastic(XP_019247175.1)
11.55	11.41	**NbD003637.1topless-relatedprotein3-likeisoformX1(XP_019252621.1)**
11.43	11.48	NbE44071943.1lignin-forminganionicperoxidase-like(XP_019240945.1)
11.05	11.22	NbE03056644.1ADP,ATPcarrierprotein,mitochondrial(XP_016436941.1)
11.02	10.85	NbD046002.1ruBisCOlargesubunit-bindingproteinsubunitalpha(XP_009799931.1)
10.89	10.94	NbE05068545.1calmodulin-bindingprotein60D-likeisoformX1(XP_009621876.1)
10.89	10.79	NbE44072886.1putativetranscriptionelongationfactorSPT5homolog1(XP_019225136.1)
10.27	10.34	NbD028931.1DNA-damage-repair/tolerationproteinDRT100-like(XP_009617306.1)
10.04	13.57	NbD024468.1eukaryotictranslationinitiationfactor3subunitI-likeisoformX1(XP_016515077.1)
9.12	9.43	NbD033030.1histoneH4-like(XP_019250598.1)
8.79	12.37	NbD016496.1endochitinaseEP3-like(XP_016454693.1)
8.75	8.61	NbE03059850.1tubulinbeta-8chain-like(XP_016463870.1)
8.34	4.63	NbD039848.1chlorophylla-bbindingprotein13,chloroplastic-like(XP_016513166.1)
8.25	8.51	NbD039872.1ATPsynthasesubunitb',chloroplastic-like(XP_016481186.1)
8.07	11.98	NbE05066820.1MIP1.4a(AGY48886.1)
8.04	4.61	NbD009795.1photosystemIIproteinH(plastid)(NP_054529.1)
7.95	8.07	NbD006438.1alpha-xylosidase1-like(XP_009597805.1)
7.85	8.05	NbD049285.1malatedehydrogenase2,mitochondrial(XP_016456818.1)
7.67	3.69	NbE03055645.1rhodanese-likedomain-containingprotein4,chloroplastic(XP_019243697.1)
7.51	3.97	NbE44072991.1coatomersubunitalpha-1-like(XP_019258380.1)
7.00	3.01	NbD051708.1elongationfactor1-delta1-likeisoformX1(XP_016469408.1)
6.95	3.91	NbD007120.1probablehistoneH2A.1(XP_019240828.1)
6.09	6.49	NbE44071763.1eukaryotictranslationinitiationfactor3subunitC(XP_019247301.1)
6.00	13.42	NbD015090.1serineproteaseinhibitor1-like(XP_019233492.1)
5.55	13.50	NbD008120.1glucanendo-1,3-beta-glucosidase,acidicisoformGI9-like(XP_009780913.1)
5.40	2.42	NbD012851.1ribulosebisphosphatecarboxylase/oxygenaseactivase1,chloroplasticisoformX2(XP_016501446.1)
5.10	8.90	NbE03061757.1phosphoglyceratekinase(chloroplast)(ADR71054.1)
5.02	13.46	NbD034541.1N-alpha-acetyltransferase35,NatCauxiliarysubunit(XP_019230114.1)
4.62	9.28	NbD032055.1GDSLesterase/lipaseAPG-like(XP_016444095.1)
4.59	8.63	NbD000124.1Partial,actin-like(XP_019240219.1)

log2(FC 039-135)	log2(FC 039-wt)	Enriched proteins in 039 against both controls
4.46	2.54	NbD043897.1Partial,uncharacterizedproteinLOC109208384isoformX1(XP_019227040.1)
4.45	11.75	NbD038578.1nucleosomeassemblyprotein1(XP_019235670.1)
4.44	9.23	NbE03053520.1Partial,probablephosphoinositidephosphataseSAC9isoformX2(XP_019238026.1)
4.21	4.18	NbD015649.1photosystemII10kDapolypeptide,chloroplastic(XP_016498221.1)
4.15	11.00	NbD041400.1LOWQUALITYPROTEIN:eukaryotictranslationinitiationfactor3subunitB-like(XP_019240596.1)
4.13	2.97	NbD047408.1AMSH-likeubiquitinthioesterase1isoformX1(XP_009777130.1)
4.12	4.22	NbD052327.1uncharacterizedproteinLOC107807147(XP_016486961.1)
4.09	11.66	NbD042545.1luminal-bindingprotein5(XP_019237640.1)
3.74	7.80	NbD004745.1uncharacterizedproteinLOC107773647,partial(XP_016448537.1)
3.73	8.36	NbD052698.1putativeglucose-6-phosphate1-epimerase(XP_016484535.1)
3.56	3.99	NbD014257.1MIP1.1a(AFR66645.1)
3.51	2.52	NbE05063957.1aluminum-activatedmalatetransporter8-likeisoformX1(XP_016444055.1)
3.32	10.46	NbE44069843.1ribosomalproteinL23(plastid)(NP_054576.1)
3.02	2.70	NbD031389.1Partial,alpha-tubulin6(ALH22047.1)
2.74	3.10	NbD048805.1heatshockcognate70kDaprotein2-like(XP_019236347.1)
2.67	3.22	NbD038830.1histoneH2B-like(XP_019245582.1)
2.65	2.51	NbE03056217.1ribulosebisphosphatecarboxylase/oxygenaseactivase2,chloroplastic(XP_019254757.1)
2.61	3.23	NbE03058344.1ribulosebisphosphatecarboxylasesmallchainS41,chloroplastic-like(XP_016461746.1)
2.57	3.82	NbD028813.1histoneH1-like(XP_019229955.1)
2.40	2.55	NbE05064989.1ribulose-1,5-bisphosphatecarboxylase/oxygenaselargesubunit(plastid)(NP_054507.1)
2.37	10.55	NbD048983.1fructose-bisphosphatealdolase1,chloroplastic(XP_016505617.1)

4.4.4 Mass-Spec analysis of WUS protein complexes isolated from *ap1/cal* meristematic tissue

I also used meristematic *ap1/cal* tissue that expressed WUS-linker-GFP and had been previously cross-linked with formaldehyde as input for a co-IP experiment. The respective western blot did not show any signal for the WUS-linker-GFP protein, a fact most likely caused by the low concentration of WUS in *A. thaliana*. Even the *ap1/cal* tissue cannot reach the protein levels of overexpression in *N. benthamiana*. Nevertheless, since the cross-linking approach had been repeatedly successful for *N. benthamiana* tissue, I decided to analyse the IP eluates with Mass-Spec, since the Mass-Spec instruments are more sensitive that the detection methods used for western blotting. Fortunately, the analysis worked and I was able to identify WUS-linker-GFP in the transgenic *ap1/cal* tissue. This experiment was performed in singlets and the control was wild-type *ap1/cal* tissue.

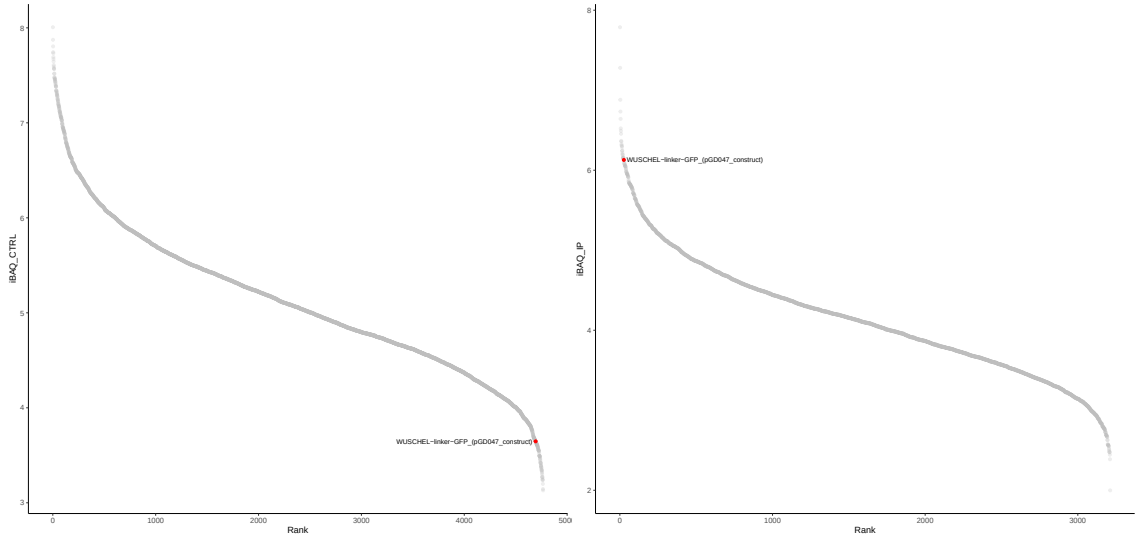

Figure 4.46: IBAQ values for the proteins isolated from the IPs done for *A. thaliana*. IP: WUS-linker-GFP sample, CTRL: wild-type control.

The IBAQ values for the experiment showed that the IP worked efficiently, since WUS-linker-GFP was among the most abundant proteins in the IP sample (**Fig. 4.46, right**). The LFQ values provided additional confirmation that the IP was successful, since WUS-linker-GFP was the most enriched protein in the IP sample upon comparison to the control (**Fig. 4.47**). Incidentally, TPL was identified in my samples, but it was actually enriched in the control. This finding cannot be interpreted, however, e.g. as a direct sign of too stringent pull-down conditions. TPL is a generic transcriptional repressor and interacts with many TFs, therefore its exact function in *ap1/cal* tissue needs to be experimentally ascertained. Overall, 34 proteins were significantly enriched in the WUS-linker-GFP *ap1/cal* samples, i.e. these proteins exhibited fold-change > 4. These enriched proteins are presented in the following table.

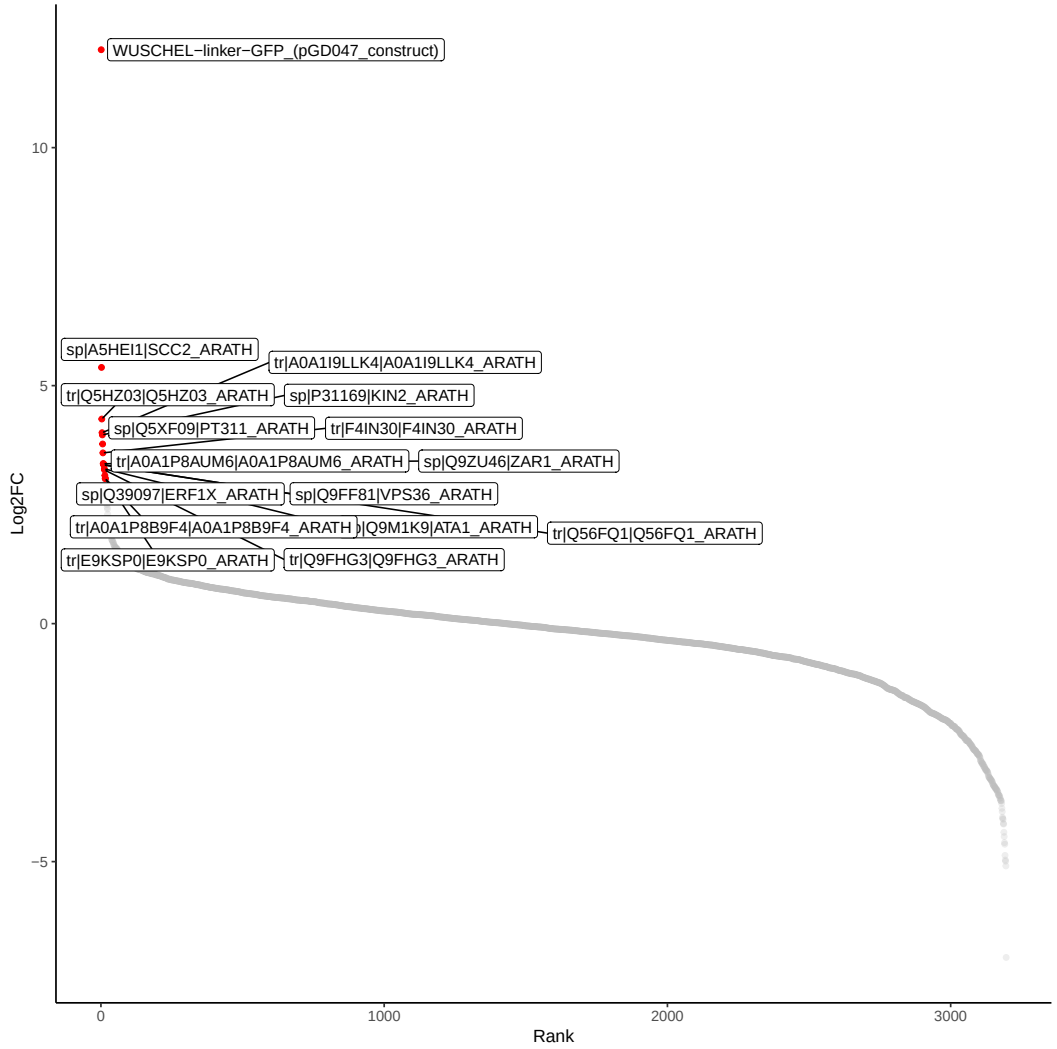

Figure 4.47: LFQ values and pairwise comparison for the proteins isolated from the IPs done for *A. thaliana*. IP: WUS-linker-GFP sample, CTRL: wild-type control.

Mass-Spec results from *A. thaliana*	
Proteins enriched in WUS-linker-GFP samples	**log2(FC)**
WUSCHEL-linker-GFP	12.06
sp|A5HEI1|SCC2_ARATH Sister chromatid cohesion protein SCC2 OS=Arabidopsis thaliana OX=3702 GN=SCC2 PE=1 SV=1	5.38
tr|Q5HZ03|Q5HZ03_ARATH At5g55530 OS=Arabidopsis thaliana OX=3702 GN=MTE17.25 PE=1 SV=1	4.30
sp|Q9M9L7|LONM4_ARATH Lon protease homolog 4, chloroplastic/mitochondrial OS=Arabidopsis thaliana OX=3702 GN=LON4 PE=3 SV=1	4.01

Proteins enriched in the WUS-linker-GFP samples	log2(FC)
sp|P31169|KIN2_ARATH Stress-induced protein KIN2 OS=Arabidopsis thaliana OX=3702 GN=KIN2 PE=1 SV=1	3.96
sp|Q5XF09|PT31_ARATH Probable sugar phosphate/phosphate translocator At3g11320 OS=Arabidopsis thaliana OX=3702 GN=At3g11320 PE=2 SV=1	3.77
tr|F4IN30|F4IN30_ARATH Alpha/beta-Hydrolases superfamily protein OS=Arabidopsis thaliana OX=3702 GN=MHK10.17 PE=1 SV=1	3.59
tr|A0A1P8AUM6|A0A1P8AUM6_ARATH Binding protein OS=Arabidopsis thaliana OX=3702 GN=At1g58230 PE=1 SV=1	3.36
sp|Q9ZU46|ZAR1_ARATH Receptor protein kinase-like protein ZAR1 OS=Arabidopsis thaliana OX=3702 GN=ZAR1 PE=1 SV=1	3.36
sp|Q9FF81|VPS36_ARATH Vacuolar protein sorting-associated protein 36 OS=Arabidopsis thaliana OX=3702 GN=VPS36 PE=1 SV=1	3.34
tr|Q56FQ1|Q56FQ1_ARATH Activator of spomin LUC3 OS=Arabidopsis thaliana OX=3702 GN=ASML3 PE=2 SV=1	3.33
sp|Q9M1K9|ATA1_ARATH Short-chain dehydrogenase reductase ATA1 OS=Arabidopsis thaliana OX=3702 GN=TA1 PE=1 SV=1	3.25
tr|Q9FHG3|Q9FHG3_ARATH 16S rRNA processing protein RimM family OS=Arabidopsis thaliana OX=3702 GN=At5g46420 PE=1 SV=1	3.23
sp|Q39097|ERF1X_ARATH Eukaryotic peptide chain release factor subunit 1-1 OS=Arabidopsis thaliana OX=3702 GN=ERF1-1 PE=1 SV=2	3.11
sp|Q9SHJ3|CALS7_ARATH Callose synthase 7 OS=Arabidopsis thaliana OX=3702 GN=CALS7 PE=3 SV=3	3.05
tr|A0A1P8B9F4|A0A1P8B9F4_ARATH Uncharacterized protein OS=Arabidopsis thaliana OX=3702 GN=At5g37470 PE=4 SV=1	3.05
sp|O80990|CIA1_ARATH Protein CIA1 OS=Arabidopsis thaliana OX=3702 GN=CIA1 PE=1 SV=2	2.98
sp|Q8VYU6|GOGC4_ARATH Golgin candidate 4 OS=Arabidopsis thaliana OX=3702 GN=GC4 PE=2 SV=1	2.97
sp|Q9SCX9|GPDA1_ARATH Glycerol-3-phosphate dehydrogenase [NAD(+)] 1, chloroplastic OS=Arabidopsis thaliana OX=3702 GN=DHAPRD PE=1 SV=1	2.84
sp|O64948|LONP2_ARATH Lon protease homolog 2, peroxisomal OS=Arabidopsis thaliana OX=3702 GN=LON2 PE=2 SV=1	2.59
sp|F4K5T4|STKLU_ARATH Probable transcription factor At5g28040 OS=Arabidopsis thaliana OX=3702 GN=At5g28040 PE=1 SV=1	2.50
tr|Q8L9X1|Q8L9X1_ARATH Alpha/beta-Hydrolases superfamily protein OS=Arabidopsis thaliana OX=3702 GN=At5g20060 PE=1 SV=1	2.44
sp|O24496|GLO2C_ARATH Hydroxyacylglutathione hydrolase cytoplasmic OS=Arabidopsis thaliana OX=3702 GN=GLX2-2 PE=1 SV=2	2.41
sp|Q9ZTP3|EIN4_ARATH Protein EIN4 OS=Arabidopsis thaliana OX=3702 GN=EIN4 PE=1 SV=1	2.30
sp|Q9SYK0|HEXO2_ARATH Beta-hexosaminidase 2 OS=Arabidopsis thaliana OX=3702 GN=HEXO2 PE=1 SV=1	2.19
sp|Q9FNG3|RICE2_ARATH Protein RISC-INTERACTING CLEARING 3-5 EXORIBONUCLEASE 2 OS=Arabidopsis thaliana OX=3702 GN=RICE2 PE=1 SV=1	2.15
sp|P57751|UGPA1_ARATH UTP–glucose-1-phosphate uridylyltransferase 1 OS=Arabidopsis thaliana OX=3702 GN=UGP1 PE=2 SV=1	2.08
tr|Q8GWB3|Q8GWB3_ARATH Protein YIP OS=Arabidopsis thaliana OX=3702 GN=At3g05280 PE=1 SV=1	2.06
sp|Q9LVJ1|SBT14_ARATH Subtilisin-like protease SBT1.4 OS=Arabidopsis thaliana OX=3702 GN=SBT1.4 PE=2 SV=1	2.03
sp|Q9SII8|DSK2B_ARATH Ubiquitin domain-containing protein DSK2b OS=Arabidopsis thaliana OX=3702 GN=DSK2B PE=1 SV=1	2.03
sp|P62126|RR12_ARATH 30S ribosomal protein S12, chloroplastic OS=Arabidopsis thaliana OX=3702 GN=rps12-A PE=2 SV=2	2.02
tr|Q9SFV3|Q9SFV3_ARATH At3g07230 OS=Arabidopsis thaliana OX=3702 GN=T1B9.10 PE=1 SV=1	1.98
sp|Q8GWE1|UCH3_ARATH Ubiquitin carboxyl-terminal hydrolase 3 OS=Arabidopsis thaliana OX=3702 GN=UCH3 PE=2 SV=1	1.97
sp|F4JRP8|STIL2_ARATH Protein STICHEL-like 2 OS=Arabidopsis thaliana OX=3702 GN=At4g24790 PE=3 SV=1	1.94

4.5 Investigation of WUS PTMs

In the Mass-Spec analyses that I performed, I detected several post-translational modifications (PTMs) of WUS. The following residues of WUS were found to be phosphorylated: Y53, S60, T70 and S286. In addition, the following WUS residues were found to be ubiquitinated: K47, K50 and K99. PTMs of WUS are a very interesting finding because they may unveil previously unknown aspects of WUSCHEL function. For example, S286 lies within the EAR domain, which is involved in transcriptional repression. Therefore, it is credible to envisage a possible effect of this phosphorylation site on the role of WUS as transcriptional repressor. Recently, a draft version of the *A. thaliana* proteome was published **[94]**. These data provided evidence for the phosphorylation of the following WUS residues: S17 and S286.

Following up on these findings, I created WUS-linker-GFP fusion proteins harbouring the mutations S17A, S286A as well as the double mutation S17A, S286A and subsequently transformed them in the *wus* mutant background. In parallel, I also created phospho-mimic versions of WUS-linker-GFP by generating the following WUS mutations: S17D, S286D as well as the double mutation S17D, S286D. These phospho-mimic WUS mutant proteins were also transformed into the *wus* mutant background.

Due to time restrictions, I has able to screen only T1 plants for any significant phenotypes. However, the *wus* mutation still segregates in the T1 generation and it is difficult to obtain in this step a wus -/- plant that harbours the construct of interest. Nevertheless, I did observe a few phospho-mutant plants with terminated SAMs (**Fig. 4.48**). Interestingly, I did not observe any plants with the double phosphomutation exhibiting terminated meristems. Concerning the phospho-mimic mutants, the transformations of the respective constructs into *A. thaliana* were very inefficient and I did not manage to get a sufficient number of T1 plants. Overall, I was not able to generate enough data to gain insights in the role of the WUS phospho-PTMs.

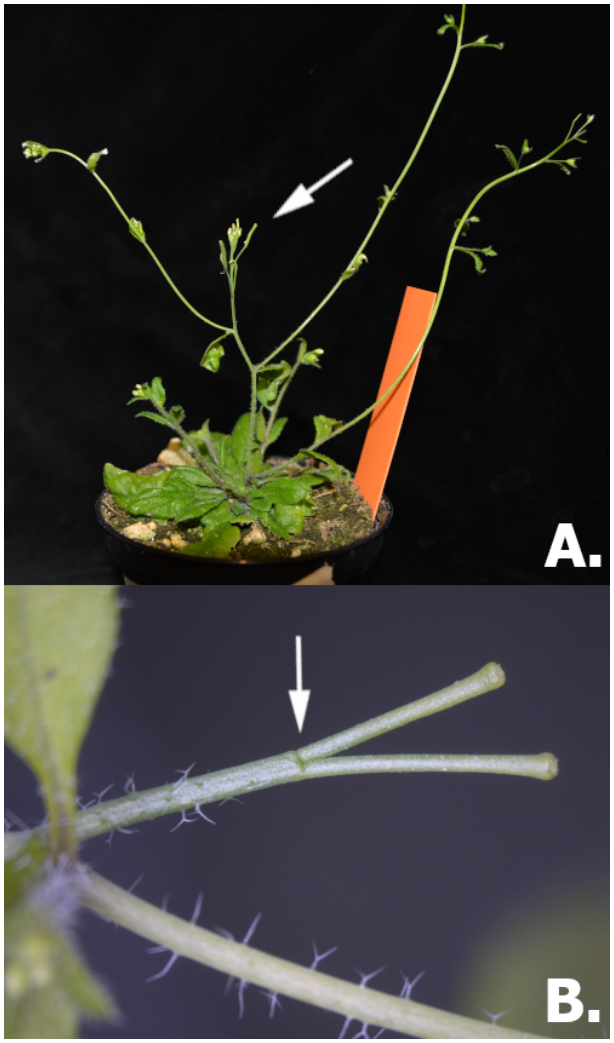

Figure 4.48: Segregating *wus* mutants harbouring phospho-mutant versions of WUS. A) A plant expressing pWUS::WUS_(S17A)-linker-GFP. The SAM has terminated. B) Close-up view of a terminated SAM (14x). This plant expressed pWUS::WUS_(S286A)-linker-GFP. The white arrows point to the terminated SAMs.

Chapter 5

Discussion

5.1 Investigating a possible interaction between WUS and WOX9

The decision to investigate a possible interaction between WUS and WOX9 was based on the very first Y2H screening I performed for this thesis. The positive result, along with encouraging data that had been published earlier regarding the localisation of WOX9 in the SAM **[88]**, convinced me to validate the WUS-WOX9 interaction *in planta*. The positive AP-FRET experiment in combination with the enlarged SAM that *wox9* mutants display was a consistent sign that a possible WUS-WOX9 interaction affects the SAM stem cell pool. However, ectopic expression of WOX9 under the control of pWUS and pCLV3 did not lead to any relevant phenotypes. Moreover, I visualised the localisation of WOX9 as well as of *WOX9* mRNA in the SAM and found out that WUS and WOX9 do not overlap *in vivo*. Thus, the hypothesis that WUS and WOX9 interact in the *A. thaliana* SAM, which was based on findings in heterologous systems, had to be disregarded.

Nevertheless, *wox9* mutants still manifest the interesting phenotype of an enlarged SAM, a phenotype initiated without any apparent aberrations with respect to stem cell function. As I described in the Introduction of this thesis, the cells that exit the CZ remain initially undifferentiated until they are finally incorporated in the developing lateral organs. It is intriguing to examine a possible contribution of WOX9 to cell differentiation. I saw during my experiments that WOX9 is expressed in the SAM periphery and finally gets incorporated into the developing flowers. STM is known to suppress differentiation genes throughout the SAM and it would be interesting to examine the effect of WOX9 on STM expression (and vice versa) by using inducible overexpression lines. Furthermore, it would be interesting to examine the auxin transcriptional output in *wox9* mutants and ascertain if it participates in the increased production of lateral organs that the *wox9* mutation provokes **[42, 95]**.

5.2 Investigating a possible interaction between WUS and ESR1

ESR1 was a candidate for interaction with WUS that was consistently identified in my Y2H screenings. The additional positive AP-FRET experiments convinced me to examine further the biological implications of a possible WUS-ESR1 interaction. The CRISPR *esr1*

mutants that I generated exhibited an enlarged SAM, a phenotype that can be caused by increased WUS activity (e.g. like in the *clv* mutants). However, my attempt to visualise WUS distribution in the SAM of *esr1* mutants had to be aborted since the necessary transgenic line (esr1 -/-, wus -/-, pWUS::WUS-linker-GFP) could not be created in a timely manner with the available resources that were at my disposal at that moment. Nevertheless, I performed ectopic expression studies of ESR1, which produced very interesting results. When ESR1 is expressed under the regulatory control of pWUS or pCLV3, it causes the apparent depletion of the stem cell pool of the SAM and the termination of the latter. This phenotype is, however, very surprising given the fact that ESR1 is present natively in the L1 (and possibly also in the L2[‡]) of the CZ. It seems plausible that *ESR1* expression is subject to very tight spatial regulation, since the presence of its protein product in L3 or farther below causes an arrest of stem cell function. Given the deleterious effects of the *esr1* mutation, it is very difficult to examine *in vivo* in *A. thaliana* the possibility of a direct physical interaction between WUS and ESR1. However, the expertise I acquired by performing successful AP-FRET experiments in the SAM could be combined with inducible lines that express ESR1 under pWUS and pCLV3. Performing an AP-FRET experiment shortly after ESR1 induction in the SAM should avoid the negative effects that ESR1 has on stem cell and possibly also on WUS function.

While I was performing the experiments for my thesis, a very interesting study concerning the role of ESR1 in SAM function was published **[96]**. According to this study, ESR1 indirectly activates *CLV3* via an unknown mechanism. This assumption would be compatible with the repression that ESR1 seems to exert on *WUS* function, but does not take into consideration the role of HAM1 in the WUS-mediated activation of *CLV3* **[46]**. Furthermore, a significant portion of *clv3* mutants that express pCLV3::ESR1 develop meristems that are even larger compared to the *clv3* phenotype, while these enlarged SAMs seem to undergo termination (Fig. 6 in **[96]**). Thus, the contribution of *ESR1* to the regulation of stem cell fate in the SAM seems to be more complex. Finally, it is also interesting that the publication on the ESR1-CLV3 interplay reports the successful propagation of esr1 -/-, esr2 -/- double-mutants **[96]**, although work that was done in my lab for a different project clearly shows that this combination of mutations always provokes embryonic lethality.

5.3 Mass-Spec results from *N. benthamiana*

The co-IP experiments that I performed using samples from *N. benthamiana* and the subsequent Mass-Spec results that I generated were crucial for the troubleshooting of my IP workflow. However, extracting biological information about WUS from such results is not trivial. Many protein families, e.g. WOX TFs, have multiple members expressed in different tissues and organs. A possible interaction between WUS and a certain TF in the leaf of a heterologous protein expression system might be misleading, since WUS and the ortholog *A. thaliana* protein might never come in contact in *A. thaliana*. Furthermore, such interactions

[‡]Ascertaining the possibility of *ESR1* being expressed in the L2 would need the generation of an *ESR1* transcriptional reporter, which I did not create due to time restrictions.

might be ”evolutionary inheritance” that proteins related to WUS, e.g. WOX5 might, actually exhibit. In addition, WUS overexpression has a dramatic effect on the transcriptional programme of a tissue. Such an effect in the case of *N. benthamiana* takes place out of the native biological context of WUS and could therefore be distorted by artefacts.

5.4 Mass-Spec results from *A. thaliana*

Most of the Mass-Spec analyses that were conducted for this thesis used *N .benthamiana* tissue as input. Some of these analyses were done for crude protein extraction experiments while others were performed for nuclear protein extractions. When comparing these two types of experiments, a major conclusion that can be drawn is that the crude protein extractions result in an under-representation of nuclear proteins in the IP eluate. However, the crude protein extraction for the cross-linked *ap1/cal* tissue resulted in the enrichment of several nuclear proteins including two TFs (Q56FQ1_ARATH and STKLU_ARATH), which definitely warrant further investigation. Apparently, tissue fixation can maintain protein-protein interactions that most likely do not survive the harsher initial cell lysis that characterises crude protein extraction protocols.

Furthermore, three proteases were enriched in the WUS-linker-GFP samples. The first two belong to the Lon protease family, i.e. LONM4_ARATH (Lon protease homolog 4) and LONP2_ARATH (Lon protease homolog 2), while the third one is a subtilase, i.e. SBT14_ARATH (Subtilisin-like protease SBT1.4). The presence of these proteases in my IP samples could offer a plausible explanation for the degradation of WUS fusion proteins that have undermined my IPs. However, the Lon proteases are located in the mitochondria, in the chloroplasts and in the peroxisome and, thus, their enrichment is most likely an experimental artefact resulting from the lysis of the respective organelles during protein extraction. The subtilisin-like protease, however, is a secreted protease that according to the Shoot Apex Translatome eFP browser is present in the L1 of the SAM. The daughter cells that exit the CZ upon stem cell division would still contain WUS protein. However, no WUS has ever been detected laterally outside of the CZ in wild-type *A. thaliana* plants. The possible digestion of WUS by a protease outside of the CZ could be a possible mechanism, which mediates the transition of the former stem cells to differentiation.

Finally, the protein CALS7_ARATH (Callose synthase 7) was also enriched in the IP samples. I mentioned earlier in the Introduction that WUS moves from the OC into the CZ through the plasmodesmata. Callose synthases are an inherent structural component of the plasmodesmata and it would be interesting to examine the possibility of an interaction between a callose synthase and WUS, which might influence the cell-to-cell movement that WUS exhibits.

5.5 Perturbation of WUS phospho-PTM sites

The Mass-Spec data that I generated contained evidence of phosphorylated and ubiquitinated WUS peptides. These findings, along with published Proteomics data for *A. thaliana*, led me to create WUS fusion proteins that harboured phospho- (S17A, S286A, [S17A,S286A]) and phospho-mimic mutations (S17D, S286D, [S17D,S286D]). The data I acquired on the function of these WUS phospho-PTM sites were limited since I worked only with segregating *wus* mutant T1 plants. Nevertheless, I observed that a fraction of the plants, which had been transformed with either the construct pWUS::WUS_(S17A)-linker-GFP or the construct pWUS::WUS_(S286A)-linker-GFP, manifested terminated SAMs. Since the respective plants also expressed wild-type WUS, there exists the interesting possibility that these phospho-mutations are dominant-negative. Further experiments are required, however, to investigate the issue. A starting point would be to ascertain whether these constructs can rescue the *wus* mutation or not.

References

[1]F. Food and Agriculture Organization of the United Nations, *Dimensions of Need: An Atlas of Food and Agriculture* (Rome, Italy, 1995).

[2]Y. Qi, L. Ding, S. Zhang, S. Yao, J. Ong, Y. Li, H. Wu and P. Du, “A plant immune protein enables broad antitumor response by rescuing microRNA deficiency.”, Cell **185**, 1888–1904.e24 (2022).

[3]M. Somssich, “The Dawn of Plant Molecular Biology: How Three Key Methodologies Paved the Way”, Current Protocols **2**, 1–19 (2022).

[4]F. M. Ausubel, “Tracing my roots: How i became a plant biologist”, Annual Review of Genetics **52**, 1–20 (2018).

[5]R. S. Poethig, “Phase change and the regulation of shoot morphogenesis in plants”, Science **250**, 923–930 (1990).

[6]C. V. D. Berg, V. Willemsen, W. Haget, P. Weisbeek and B. Scheres, “Cell fate in the Arabidopsis root meristem determined by directional signalling”, Nature **378**, 3–6 (1995).

[7]T. Greb and J. U. Lohmann, “Plant Stem Cells”, Current Biology **26**, R816–R821 (2016).

[8]T. Greb, O. Clarenz, E. Schafer, R. Herrero, G. Schmitz and K. Theres, “Molecular analysis of the LATERAL SUPPRESSOR gene in Arabidopsis”, Genes & Development **17**, 1175–1187 (2003).

[9]Z. H. Ye, “Vascular tissue differentiation and pattern formation in plants”, Annual Review of Plant Biology **53**, 183–202 (2002).

[10]J. Agusti and T. Greb, “Going with the wind - Adaptive dynamics of plant secondary meristems”, Mechanisms of Development **130**, 34–44 (2013).

[11]Y. Wang and Y. Jiao, “Axillary meristem initiation — a way to branch out”, Current Opinion in Plant Biology **41**, 61–66 (2018).

[12]V. Grbić and A. B. Bleecker, “Axillary meristem development in Arabidopsis thaliana”, Plant Journal **21**, 215–223 (2000).

[13]J. E. Malamy and P. N. Benfey, “Organization and cell differentiation in lateral roots of Arabidopsis thaliana”, Development **124**, 33–44 (1997).

[14]E. Aichinger, N. Kornet, T. Friedrich and T. Laux, “Plant Stem Cell Niches”, Annual Review of Plant Biology **63**, 615–636 (2012).

[15]L. Eshed Williams, “Genetics of Shoot Meristem and Shoot Regeneration”, Annual Review of Genetics **55**, 661–681 (2021).

[16]M. K. Barton and R. S. Poethig, “Formation of the shoot apical meristem in Arabidopsis thaliana: an analysis of development in the wild type and in the shoot meristemless mutant”, Development **119**, 823–831 (1993).

[17]J. A. Long, E. I. Moan, J. I. Medford and M. K. Barton, *A member of the KNOTTED class of homeodomain proteins encoded by the STM gene of Arabidopsis*, 1996.

[18]M. Lenhard, G. Jürgens and T. Laux, “The WUSCHEL and SHOOTMERISTEMLESS genes fulfil complementary roles in Arabidopsis shoot meristem regulation”, Development **129**, 3195–3206 (2002).

[19]S. Satina, A. F. Blakeslee and A. G. Avery, “Demonstration of the Three Germ Layers in the Shoot Apex of Datura By Means of Induced Polyploidy in Periclinal Chimeras”, American Journal of Botany **27**, 895–905 (1940).

[20]J. C. Fletcher, “Signaling of cell fate decisions by CLAVATA3 in Arabidopsis shoot meristems”, Science **283**, 1911–1914 (1999).

[21]P. Laufs, O. Grandjean, C. Jonak, K. Kiêu and J. Traas, “Cellular parameters of the shoot apical meristem in Arabidopsis”, Plant Cell **10**, 1375–1389 (1998).

[22]K. F. X. Mayer, H. Schoof, A. Haecker, M. Lenhard, G. Jürgens and T. Laux, “Role of WUSCHEL in regulating stem cell fate in the Arabidopsis shoot meristem”, Cell **95**, 805–815 (1998).

[23]T. Laux, K. F. Mayer, J. Berger and G. Jürgens, “The WUSCHEL gene is required for shoot and floral meristem integrity in Arabidopsis.”, Development (Cambridge, England) **122**, 87–96 (1996).

[24]R. L. M. Pierik, “Production of disease-free plants”, in *In vitro culture of higher plants* (Springer Science+Business Media Dordrecht, 1997), pp. 169–181.

[25]D. A. Weits, A. B. Kunkowska, N. C. W. Kamps, K. M. S. Portz, N. K. Packbier, Z. N. Venza, C. Gaillochet, J. U. Lohmann, O. Pedersen, J. T. van Dongen and F. Licausi, “An apical hypoxic niche sets the pace of shoot meristem activity”, Nature **569**, 714–717 (2019).

[26]G. Daum, A. Medzihradszky, T. Suzaki and J. U. Lohmann, “A mechanistic framework for noncell autonomous stem cell induction in Arabidopsis.”, Proceedings of the National Academy of Sciences of the United States of America **111**, 14619–24 (2014).

[27]R. K. Yadav, M. Perales, J. Gruel, T. Girke, H. Jönsson and G. Venugopala Reddy, “WUSCHEL protein movement mediates stem cell homeostasis in the Arabidopsis shoot apex”, Genes and Development **25**, 2025–2030 (2011).

[28]W. Busch, A. Miotk, F. D. Ariel, Z. Zhao, J. Forner, G. Daum, T. Suzaki, C. Schuster, S. J. Schultheiss, A. Leibfried, S. Haubei??, N. Ha, R. L. Chan and J. U. Lohmann, “Transcriptional control of a plant stem cell niche”, Developmental Cell **18**, 849–861 (2010).

[29]K. Ohyama, H. Shinohara, M. Ogawa-Ohnishi and Y. Matsubayashi, “A glycopeptide regulating stem cell fate in Arabidopsis thaliana”, Nature Chemical Biology **5**, 578–580 (2009).

[30]E. Rojo, V. K. Sharma, V. Kovaleva, N. V. Raikhel and J. C. Fletcher, “CLV3 is localized to the extracellular space, where it activates the Arabidopsis CLAVATA stem cell signaling pathway”, Plant Cell **14**, 969–977 (2002).

[31]H. Schoof, M. Lenhard, A. Haecker, K. F. X. Mayer, G. Ju, T. Laux, M. D. Pflanzen, A. D. Morgenstelle, G. Jürgens and T. Laux, “The stem cell population of Arabidopsis shoot meristems in maintained by a regulatory loop between the CLAVATA and WUSCHEL genes”, Cell **100**, 635–44 (2000).

[32]T.-Q. Zhang, H. Lian, C.-M. Zhou, L. Xu, Y. Jiao and J.-W. Wang, “A Two-Step Model for de Novo Activation of WUSCHEL during Plant Shoot Regeneration”, The Plant Cell **29**, 1073–1087 (2017).

[33]C. S. Kwon, C. Chen and D. Wagner, “WUSCHEL is a primary target for transcriptional regulation by SPLAYED in dynamic control of stem cell fate in Arabidopsis”, Genes and Development **19**, 992–1003 (2005).

[34]J. P. To, J. Deruère, B. B. Maxwell, V. F. Morris, C. E. Hutchison, F. J. Ferreira, G. E. Schaller and J. J. Kieber, “Cytokinin regulates type-A Arabidopsis response regulator activity and protein stability via two-component phosphorelay”, Plant Cell **19**, 3901–3914 (2007).

[35]A. Leibfried, J. P. C. To, W. Busch, S. Stehling, A. Kehle, M. Demar, J. J. Kieber and J. U. Lohmann, “WUSCHEL controls meristem function by direct regulation of cytokinin-inducible response regulators.”, Nature **438**, 1172–1175 (2005).

[36]Y. Zhou, M. Honda, H. Zhu, Z. Zhang, X. Guo, T. Li, Z. Li, X. Peng, K. Nakajima, L. Duan and X. Zhang, “Spatiotemporal sequestration of miR165/166 by arabidopsis argonaute10 promotes shoot apical meristem maintenance”, Cell Reports **10**, 1819–1827 (2015).

[37]O. Yanai, E. Shani, K. Dolezal, P. Tarkowski, R. Sablowski, G. Sandberg, A. Samach and N. Ori, “Arabidopsis KNOXI proteins activate cytokinin biosynthesis”, Current Biology **15**, 1566–1571 (2005).

[38]A. Israeli, J. W. Reed and N. Ori, “Genetic dissection of the auxin response network”, Nature Plants **6**, `10.1038/s41477-020-0739-7` (2020).

[39]M. R. A. de Figueiredo and L. C. Strader, “Intrinsic and extrinsic regulators of Aux/IAA protein degradation dynamics”, Trends in Biochemical Sciences **47**, 865–874 (2022).

[40]Z. Zhao, S. U. Andersen, K. Ljung, K. Dolezal, A. Miotk, S. J. Schultheiss and J. U. Lohmann, “Hormonal control of the shoot stem-cell niche”, Nature **465**, 1089–1092 (2010).

[41]K. Zhang, H. Zhang, Y. Pan, Y. Niu, L. Guo, Y. Ma, S. Tian, J. Wei, C. Wang, X. Yang, Y. Fu, P. Qu, L. Liu, Y. Zhang, H. Sun, Z. Bai, J. Dong, C. Li and X. Liu, “Cell- and noncell-autonomous AUXIN RESPONSE FACTOR3 controls meristem proliferation and phyllotactic patterns”, Plant Physiology, 1–15 (2022).

[42]Y. Ma, A. Miotk, Z. Šutiković, O. Ermakova, C. Wenzl, A. Medzihradszky, C. Gaillochet, J. Forner, G. Utan, K. Brackmann, C. S. Galván-Ampudia, T. Vernoux, T. Greb and J. U. Lohmann, “WUSCHEL acts as an auxin response rheostat to maintain apical stem cells in Arabidopsis”, Nature communications **10**, 5093 (2019).

[43]P. Han, Q. Li and Y. X. Zhu, “Mutation of Arabidopsis BARD1 causes meristem defects by failing to confine WUSCHEL expression to the organizing center”, Plant Cell **20**, 1482–1493 (2008).

[44]Y. Zhang, Y. Jiao, Z. Liu and Y. X. Zhu, “ROW1 maintains quiescent centre identity by confining WOX5 expression to specific cells”, Nature Communications **6**, 10.1038/ncomms7003 (2015).

[45]L. Zhang, D. D. Gennaro, G. Lin, J. Chai and E. D. Shpak, “ERECTA family signaling constrains CLAVATA3 and WUSCHEL to the center of the shoot apical meristem”, Development (Cambridge) **148**, 1–10 (2021).

[46]Y. Zhou, A. Yan, H. Han, T. Li, Y. Geng, X. Liu and E. M. Meyerowitz, “HAIRY MERISTEM with WUSCHEL confines CLAVATA3 expression to the outer apical meristem layers”, Science **506**, 502–506 (2018).

[47]Y. Zhou, X. Liu, E. M. Engstrom, Z. L. Nimchuk, J. L. Pruneda-Paz, P. T. Tarr, A. Yan, S. A. Kay and E. M. Meyerowitz, “Control of plant stem cell function by conserved interacting transcriptional regulators.”, Nature **517**, 377–80 (2015).

[48]C. Bai, P. Sen, K. Hofmann, L. Ma, M. Goebl, J. W. Harper and S. J. Elledge, “SKP1 connects cell cycle regulators to the ubiquitin proteolysis machinery through a novel motif, the F-box”, Cell **86**, 263–274 (1996).

[49]S. Knauer, A. L. Holt, I. Rubio-Somoza, E. J. Tucker, A. Hinze, M. Pisch, M. Javelle, M. C. Timmermans, M. R. Tucker and T. Laux, “A Protodermal miR394 Signal Defines a Region of Stem Cell Competence in the Arabidopsis Shoot Meristem”, Developmental Cell **24**, 125–132 (2013).

[50]H. Takanashi, H. Sumiyoshi, M. Mogi, Y. Hayashi, T. Ohnishi and N. Tsutsumi, “miRNAs control HAM1 functions at the single-cell-layer level and are essential for normal embryogenesis in Arabidopsis”, Plant Molecular Biology **96**, 627–640 (2018).

[51]Y. Hirakawa, “Evolution of meristem zonation by CLE gene duplication in land plants”, Nature Plants **8**, 735–740 (2022).

[52]J. Schlegel, G. Denay, R. Wink, K. G. Pinto, Y. Stahl, J. Schmid, P. Blümke and R. Simon, “Control of Arabidopsis shoot stem cell homeostasis by two antagonistic CLE peptide signalling pathways”, eLife **10**, 1–30 (2021).

[53]E. Zürcher, D. Tavor-Deslex, D. Lituiev, K. Enkerli, P. T. Tarr and B. Müller, “A robust and sensitive synthetic sensor to monitor the transcriptional output of the cytokinin signaling network in planta”, Plant Physiology **161**, 1066–1075 (2013).

[54]Z. Zhang, E. Tucker, M. Hermann and T. Laux, “A Molecular Framework for the Embryonic Initiation of Shoot Meristem Stem Cells”, Developmental Cell **40**, 264–277.e4 (2017).

[55]R. B. Goldberg, G. de Paiva and R. Yadegari, “Plant Embryogenesis: Zygote to Seed”, Science **266**, 605–614 (1994).

[56]J. Palovaara, T. De Zeeuw and D. Weijers, “Tissue and Organ Initiation in the Plant Embryo: A First Time for Everything”, Annual Review of Cell and Developmental Biology **32**, 47–75 (2016).

[57]A. Haecker, R. Groß-Hardt, B. Geiges, A. Sarkar, H. Breuninger, M. Herrmann and T. Laux, “Expression dynamics of WOX genes mark cell fate decisions during early embryonic patterning in Arabidopsis thaliana”, Development **131**, 657–668 (2004).

[58]W. J. Gehring, Y. Q. Qian, M. Billeter, K. Furukubo-Tokunaga, A. F. Schier, D. Resendez-Perez, M. Affolter, G. Otting and K. Wüthrich, “Homeodomain-DNA recognition”, Cell **78**, 211–223 (1994).

[59]E. van der Graaff, T. Laux and S. A. Rensing, “The WUS homeobox-containing (WOX) protein family.”, Genome biology **10**, 248 (2009).

[60]A. Dolzblasz, J. Nardmann, E. Clerici, B. Causier, E. van der Graaff, J. Chen, B. Davies, W. Werr and T. Laux, “Stem Cell Regulation by Arabidopsis WOX Genes”, Molecular Plant **9**, 1028–1039 (2016).

[61]A. K. Sarkar, M. Luijten, S. Miyashima, M. Lenhard, T. Hashimoto, K. Nakajima, B. Scheres, R. Heidstra and T. Laux, “Conserved factors regulate signalling in Arabidopsis thaliana shoot and root stem cell organizers.”, Nature **446**, 811–814 (2007).

[62]J. Nardmann, P. Reisewitz and W. Werr, “Discrete shoot and root stem cell-promoting WUS/WOX5 functions are an evolutionary innovation of angiosperms”, Molecular Biology and Evolution **26**, 1745–1755 (2009).

[63]M. Ikeda, N. Mitsuda and M. Ohme-Takagi, “Arabidopsis wuschel is a bifunctional transcription factor that acts as a repressor in stem cell regulation and as an activator in floral patterning”, Plant Cell **21**, 3493–3505 (2009).

[64]M. Rechsteiner and S. W. Rogers, “PEST sequences and regulation by proteolysis”, Trends in Biochemical Sciences **21**, 267–271 (1996).

[65]J. Sloan, J. P. Hakenjos, M. Gebert, O. Ermakova, A. Gumiero, G. Stier, K. Wild, I. Sinning and J. U. Lohmann, “Structural basis for the complex DNA binding behavior of the plant stem cell regulator WUSCHEL”, Nature Communications **11**, 2223 (2020).

[66]J. Jumper, R. Evans, A. Pritzel, T. Green, M. Figurnov, O. Ronneberger, K. Tunyasuvunakool, R. Bates, A. Žídek, A. Potapenko, A. Bridgland, C. Meyer, S. A. Kohl, A. J. Ballard, A. Cowie, B. Romera-Paredes, S. Nikolov, R. Jain, J. Adler, T. Back, S. Petersen, D. Reiman, E. Clancy, M. Zielinski, M. Steinegger, M. Pacholska, T. Berghammer, S. Bodenstein, D. Silver, O. Vinyals, A. W. Senior, K. Kavukcuoglu, P. Kohli and D. Hassabis, “Highly accurate protein structure prediction with AlphaFold”, Nature **596**, 583–589 (2021).

[67]L. Strader, D. Weijers and D. Wagner, “Plant transcription factors — being in the right place with the right company”, Current Opinion in Plant Biology **65**, 102136 (2022).

[68]J. A. Long, C. Ohno, Z. R. Smith and E. M. Meyerowitz, “TOPLESS Regulates Apical Embryonic Fate in Arabidopsis”, Science **312**, 1520–1522 (2006).

[69]A. R. Plant, A. Larrieu and B. Causier, “Repressor for hire! The vital roles of TOPLESS-mediated transcriptional repression in plants”, New Phytologist **231**, 963–973 (2021).

[70]M. Kieffer, Y. Stern, H. Cook, E. Clerici, C. Maulbetsch, T. Laux and B. Davies, “Analysis of the transcription factor WUSCHEL and its functional homologue in Antirrhinum reveals a potential mechanism for their roles in meristem maintenance”, Plant Cell **18**, 560–573 (2006).

[71]P. Graf, A. Dolzblasz, T. Würschum, M. Lenhard, U. Pfreundt and T. Laux, “MGOUN1 encodes an Arabidopsis type IB DNA topoisomerase required in stem cell regulation and to maintain developmentally regulated gene silencing”, Plant Cell **22**, 716–728 (2010).

[72]Y. H. Su, C. Zhou, Y. J. Li, Y. Yu, L. P. Tang, W. J. Zhang, W. J. Yao, R. Huang, T. Laux and X. S. Zhang, “Integration of pluripotency pathways regulates stem cell maintenance in the arabidopsis shoot meristem”, Proceedings of the National Academy of Sciences of the United States of America **117**, 22561–22571 (2020).

[73]P. Lozano-Sotomayor, R. A. Chávez Montes, M. Silvestre-Vañó, H. Herrera-Ubaldo, R. Greco, J. Pablo-Villa, B. M. Galliani, D. Diaz-Ramirez, M. Weemen, K. Boutilier, A. Pereira, L. Colombo, F. Madueño, N. Marsch-Martínez and S. de Folter, “Altered expression of the bZIP transcription factor DRINK ME affects growth and reproductive development in Arabidopsis thaliana”, Plant Journal **88**, 437–451 (2016).

[74]X. Liu, K. Ning, G. Che, S. Yan, L. Han, R. Gu, Z. Li, Y. Weng and X. Zhang, “CsSPL functions as an adaptor between HD-ZIP III and CsWUS transcription factors regulating anther and ovule development in Cucumis sativus (cucumber)”, Plant Journal, 1–13 (2018).

[75]E. M. Phizicky and S. Fields, “Protein-Protein Interactions: Methods for Detection and Analysis”, Microbiological Reviews **59**, 94–123 (1995).

[76]T. Berggård, S. Linse and P. James, “Methods for the detection and analysis of protein-protein interactions”, Proteomics **7**, 2833–2842 (2007).

[77]A. Paiano, A. Margiotta, M. De Luca and C. Bucci, “Yeast Two-Hybrid Assay to Identify Interacting Proteins”, Current Protocols in Protein Science **95**, 1–33 (2019).

[78]R. Aebersold and M. Mann, “Mass-spectrometric exploration of proteome structure and function”, Nature **537**, 347–355 (2016).

[79]X. Han, A. Aslanian and J. R. Yates, “Mass spectrometry for proteomics”, Current Opinion in Chemical Biology **12**, 483–490 (2008).

[80]K. J. Roux, D. I. Kim and B. Burke, “BioID: A screen for protein-protein interactions”, Current Protocols in Protein Science, 1–14 (2013).

[81]J. S. Rees, X.-W. Li, S. Perrett, K. S. Lilley and A. P. Jackson, “Protein Neighbors and Proximity Proteomics.”, Molecular & cellular proteomics : MCP **14**, 2848–2856 (2015).

[82]C. S. Hughes, S. Moggridge, T. Müller, P. H. Sorensen, G. B. Morin and J. Krijgsveld, “Single-pot, solid-phase-enhanced sample preparation for proteomics experiments”, Nature Protocols **14**, 68–85 (2019).

[83]A. Fulzele and E. J. Bennett, “Ubiquitin diGLY proteomics as an approach to identify and quantify the ubiquitin-modified proteome”, Methods in Molecular Biology **1844**, 363–384 (2018).

[84]J. Kourelis, F. Kaschani, F. M. Grosse-Holz, F. Homma, M. Kaiser and R. A. Van Der Hoorn, “A homology-guided, genome-based proteome for improved proteomics in the alloploid Nicotiana benthamiana”, BMC Genomics **20**, 1–15 (2019).

[85]Z. P. Wang, H. L. Xing, L. Dong, H. Y. Zhang, C. Y. Han, X. C. Wang and Q. J. Chen, “Egg cell-specific promoter-controlled CRISPR/Cas9 efficiently generates homozygous mutants for multiple target genes in Arabidopsis in a single generation”, Genome Biology **16**, 1–12 (2015).

[86]D. Winter, B. Vinegar, H. Nahal, R. Ammar, G. V. Wilson and N. J. Provart, “An ”electronic fluorescent pictograph” Browser for exploring and analyzing large-scale biological data sets”, PLoS ONE **2**, 1–12 (2007).

[87]R. K. Yadav, T. Girke, S. Pasala, M. Xie and G. V. Reddy, “Gene expression map of the Arabidopsis shoot apical meristem stem cell niche”, Proceedings of the National Academy of Sciences **106**, 1–6 (2009).

[88]X. Wu, T. Dabi and D. Weigel, “Requirement of Homeobox Gene STIMPY/WOX9 for Arabidopsis Meristem Growth and Maintenance”, Current Biology **15**, 436–440 (2005).

[89]J. W. Chandler, M. Cole, A. Flier, B. Grewe and W. Werr, “The AP2 transcription factors DORNRÖSCHEN and DORNRÖSCHEN-LIKE redundantly control Arabidopsis embryo patterning via interaction with PHAVOLUTA”, Development **134**, 1653–1662 (2007).

[90]J. W. Chandler, M. Cole, B. Jacobs, P. Comelli and W. Werr, “Genetic integration of DORNRÖSCHEN and DORNRÖSCHEN-LIKE reveals hierarchical interactions in auxin signalling and patterning of the Arabidopsis apical embryo”, Plant Molecular Biology **75**, 223–236 (2011).

[91]T. Kirch, R. Simon, M. Grünewald and W. Werr, “The Dornröschen/Enhancer of Shoot Regeneration1 Gene of Arabidopsis acts in the control of meristem cell fate and lateral organ development”, Plant Cell **15**, 694–705 (2003).

[92]M. Cole, J. Chandler, D. Weijers, B. Jacobs, P. Comelli and W. Werr, “DORNRÖSCHEN is a direct target of the auxin response factor MONOPTEROS in the Arabidopsis embryo”, Development **136**, 1643–1651 (2009).

[93]C. Ferrándiz, Q. Gu, R. Martienssen and M. F. Yanofsky, “Redundant regulation of meristem identity and plant architecture by FRUITFULL, APETALA1 and CAULIFLOWER.”, Development (Cambridge, England) **127**, 725–734 (2000).

[94]J. Mergner, M. Frejno, M. List, M. Papacek, X. Chen, A. Chaudhary, P. Samaras, S. Richter, H. Shikata, M. Messerer, D. Lang, S. Altmann, P. Cyprys, D. P. Zolg, T. Mathieson, M. Bantscheff, R. R. Hazarika, T. Schmidt, C. Dawid, A. Dunkel, T. Hofmann, S. Sprunck, P. Falter-Braun, F. Johannes, K. F. Mayer, G. Jürgens, M. Wilhelm, J. Baumbach, E. Grill, K. Schneitz, C. Schwechheimer and B. Kuster, “Mass-spectrometry-based draft of the Arabidopsis proteome”, Nature **579**, 409–414 (2020).

[95]B. Shi and T. Vernoux, “Hormonal control of cell identity and growth in the shoot apical meristem”, Current Opinion in Plant Biology **65**, 102111 (2022).

[96]L. Luo, J. Zeng, H. Wu, Z. Tian and Z. Zhao, “A Molecular Framework for Auxin-Controlled Homeostasis of Shoot Stem Cells in Arabidopsis”, Molecular Plant **11**, 899–913 (2018).

Appendix

Genotypes of background *Arabidopsis thaliana* lines

Double-Reporter: pCLV3::mCherry-NLS, pWUS::3xVenus-NLS

Genotypes of transgenic *Arabidopsis thaliana* lines generated for this thesis

Transgenic *A. thaliana* lines generated for this thesis		
Name)	**Background**	**Construct**
TTL001	ap1/cal	5.6 kb WUS prom::WUS_linker-STREPII
TTL002	ap1/cal	8.8 kb WUS prom::WUS_linker-STREPII
TTL003	ap1/cal	pTL020 (5.6 kb WUS prom::STREPII-WUS)
TTL004	ap1/cal	8.8 kb WUS prom::STREPII-WUS
TTL005	WUS-AM	pTL020 (5.6 kb WUS prom::STREPII-WUS)
TTL006	ap1/cal	pGD044 (WUS 4.4 kb::WUS-GFP)
TTL007	WUS-AM	pTL021 (WUS 4.4 kb::3xHA-WUS_linker-BirA*)
TTL008	WUS-AM	pTL022 (WUS 4.4 kb::3xHA-WUS_short_linker-BirA*)
TTL009	WUS-AM	pTL023 (WUS 4.4 kb::BirA*-linker_WUS-3xHA)
TTL010	WUS-AM	pTL024 (WUS 4.4 kb::BirA*-short_linker_WUS-3xHA)
TTL011	WUS-AM	pTL025 (N7_NLS_linker-3xHA-BirA*)
TTL012	Col-0	pTL026 (DEX inducible 6xOP::STREPII-WUSCHEL)
TTL013	Col-0	pTL027 (DEX inducible 6xOP::WUSCHEL_linker-STREPII)
TTL014	WUS-AM	5.6 kb WUS prom::WUS_linker-STREPII
TTL015	WUS-AM	8.8 kb WUS prom::STREPII-WUS
TTL016	WUS-AM	8.8 kb WUS prom::WUS_linker-STREPII
TTL017	ap1/cal	pTL040 (5.6 kb WUS prom::1xHA-WUS)
TTL018	ap1/cal	pTL041 (5.6 kb WUS prom::WUS_linker-1xHA)
TTL019	WUS-AM	pTL040 (5.6 kb WUS prom::1xHA-WUS)
TTL020	WUS-AM	pTL041 (5.6 kb WUS prom::WUS_linker-1xHA)
TTL021	double-reporter (Col-0)	pTL057 (sgRNA 2 for ESR1 in pHEE401E)
TTL022	ap1/cal	pTL090 (pCLV3::NLS-GFP, pWUS::NLS-GFP)
TTL023	ap1/cal (Col-0 background, N556708 x N624971)	pTL090 (pCLV3::NLS-GFP, pWUS::NLS-GFP)
TTL024	Col-0	pTL123 (pWUS 4.4 kb::WOX9)
TTL025	Col-0	pTL124 (pCLV3::WOX9)
TTL026	Col-0	pTL125 (pWUS 4.4 kb::WOX9_linker-mCherry)
TTL027	Col-0	pTL126 (pCLV3::WOX9_linker-mCherry)
TTL028	Col-0	pTL129 (pWOX9::WOX9_linker-mCherry)
TTL029	Col-0	pTL130 (pWOX9::WOX9-3xFLAG)
TTL030	WUS-AM	pTL131 (5.6 kb WUS prom::WUS_linker-TurboID-1xHA)
TTL031	WUS-AM	pTL132 (5.6 kb WUS prom::WUS_short_linker-TurboID-1xHA)
TTL032	WUS-AM	pTL133 (5.6 kb WUS prom::N7_NLS-TurboID-1xHA)
TTL033	double-reporter (Col-0)	pMG315 (pCLV3::WUSCHEL)
TTL034	drn-1 (T-DNA insertion mutant)	pMG315 (pCLV3::WUSCHEL)
TTL035	Col-0	pYC050 (sgRNA for AIL6 and AIL7 in pHEE401E)
TTL036	double-reporter (Col-0)	pYC050 (sgRNA for AIL6 and AIL7 in pHEE401E)

Name)	Background	Construct
TTL037	drn-1 (T-DNA insertion mutant)	pYC050 (sgRNA for AIL6 and AIL7 in pHEE401E)
TTL038	WUS-AM pTL137	(pWUS::WUS_(S17A)-linker-GFP)
TTL039	WUS-AM pTL138	(pWUS::WUS_(S286A)-linker-GFP)
TTL040	WUS-AM pTL139	(pWUS::WUS_(S17A, S286A)-linker-GFP)
TTL041	Col-0 pTL140	(pCmYLCV_(CmpC)::N7_NLS-GFP)
TTL042	Col-0 pTL141	(pCmYLCV_(CmpS)::N7_NLS-GFP)
TTL043	Col-0 pTL154	(pWUS 4.4 kb::mCherry-linker-ESR1)
TTL044	Col-0 pTL155	(pCLV3::mCherry-linker-ESR1)
TTL045	Col-0 pTL156	(pESR1::mCherry-linker-ESR1)
TTL046	WUS-AM pTL158	(pWUS::WUS_(S17D)-linker-GFP)
TTL047	WUS-AM pTL159	(pWUS::WUS_(S286D)-linker-GFP)
TTL048	WUS-AM pTL160	(pWUS::WUS_(S17D, S286D)-linker-GFP)
CTL001	ap1/cal (Col-0)	male –> ap1/cal (in Col-0 background): N556 x N624, #1.2.x, siblings No.1 and No.3
	Col-0	female –> pGD044 (WUS::WUS-GFP)
CTL002	ap1/cal (Col-0)	male –> ap1/cal (in Col-0 background): N664 x N624, #1.31.x, sibling No.4
	Col-0	female –> pGD044 (WUS::WUS-GFP)
CTL003	double-reporter	male –> stimpy 157.12.1.12.1 (stimpy CrispR mutant (Anne P.))
	double-reporter	female –> double-reporter
CTL004	double-reporter	male –> stimpy 157.12.1.13.1 (stimpy CrispR mutant (Anne P.))
	double-reporter	female –> double-reporter
CTL005	double-reporter	male –> TTL021 #28
	double-reporter	female –> double-reporter
CTL006	double-reporter	male –> TTL021 #49
	double-reporter	female –> double-reporter
CTL007	double-reporter	male –> TTL021 #53
	double-reporter	female –> double-reporter
CTL008	double-reporter	male –> stimpy 157.12.1.13.2.11 (stimpy CrispR mutant (Anne P.))
	Col-0	female –> pGD044 (WUS::WUS-GFP)
CTL009	Col-0 ???	male –> CTL008 #1.240.3.11
	Col-0	female –> TTL026 #12.16.1
CTL010	Col-0 ???	male –> CTL008 #1.240.3.2
	Col-0	female –> TTL027 #1.2.6
CTL011	Col-0 ???	male –> CTL008 #1.240.3.3
	Col-0	female –> TTL028 #6.1
CTL012	Col-0	male –> clv3-10
	Double-reporter	female –> stimpy 157.12.1.13.2.11.x (stimpy CrispR mutant (Anne P.))
CTL013	double-reporter	male –> CTL005 10.5.x
	Col-0	female –> pGD044 (WUS::WUS-GFP)
CTL014	Col-0	male –> clv3-10
	double-reporter	female –> CTL005 10.5.x
CTL015	Col-0	male –> clv3-10
	Col-0	female –> drn-1 (T-DNA insertion mutant)
CTL016	double-reporter	male –> TTL036 #39.3
	double-reporter	female –> CTL005 10.5.x
CTL017	double-reporter	male –> TTL036 #39.3
	Col-0	female –> drn-1 (T-DNA insertion mutant)
CTL018	double-reporter	male –> TTL036 #39.3
	double-reporter	female –> double-reporter

List of the *A. thaliana* transcription factors used in the Y2H screenings

The following list presents the full range of proteins that served as baits in the first Y2H screening. This first screening was done against the whole library set. The following attempts were done by screening against only that subset of the library that contained the strong as well as the weak interactions from the first screening, coloured below in pink and blue respectively. The three last columns of this list indicate the growth status for the respective yeast clone under the conditions mentioned on the title of each column: 0 signifies no growth,

1 signifies good growth.

Gene identifier (AGI)	Primary Gene Symbol	TF family	1 mM 3AT	5 mM 3AT	visual in-spec-tion
AT5G45980	WUSCHEL RELATED HOMEOBOX 8 (WOX8)	HB a,b,c	1	1	1
AT1G67260	(TCP1)	TCP a,c	1	1	1
AT1G35560	TCP23	TCP a,b,c	1	1	1
AT5G08070	TCP17	TCP a,b,c	1	1	1
AT4G35280	DUO1-ACTIVATED ZINC FINGER 2 (DAZ2)	C2H2 a,b,c	1	1	1
AT1G60250	BBX26	Orphans a	1	1	1
AT3G30530	BZIP42	bZIP a,b,c	1	1	1
AT1G72010	TCP22	TCP a,b,c	1	1	1
AT1G69690	TCP15	TCP a,b,c	1	1	1
AT4G16780	HOMEOBOX PROTEIN 2 (HB-2)	HB a,b	1	1	1
AT5G47370	(HAT2)	HB a,b	1	1	1
AT3G51960	BASIC LEUCINE ZIPPER 24 (BZIP24)	bZIP a,b,c	1	1	1
AT3G47620	TCP14	TCP a,b,c	1	1	1
AT3G17860	JASMONATE-ZIM-DOMAIN PROTEIN 3 (JAZ3)	ZIM b,c	1	1	1
AT2G31370	BZIP59	bZIP a,b,c	1	1	1
AT5G65790	MYB DOMAIN PROTEIN 68 (MYB68)	MYB a,b	1	1	1
AT2G42200	SQUAMOSA PROMOTER BINDING PROTEIN-LIKE 9 (SPL9)	SBP a,b,c	1	1	1
AT4G18390	TCP2	TCP a,b,c	1	1	1
AT5G60970	TCP5	TCP a,b,c	1	1	1
AT5G16560	KANADI (KAN)	G2-like a,b	1	1	1
AT3G21330	bHLH transcription factor	bHLH a,b	1	1	1
AT2G20350	ERF/AP2 transcription factor	AP2-EREBP a,b,c	1	1	1
AT2G23760	BEL1-LIKE HOMEODOMAIN 4 (BLH4)	HB a,b	1	1	1
AT3G60530	GATA TRANSCRIPTION FACTOR 4 (GATA4)	C2C2-GATA a,b	1	1	1
AT2G28610	PRESSED FLOWER (PRS)	HB a,b,c	1	1	1
AT1G28160	ERF/AP2 transcription factor	AP2-EREBP a,b,c	1	1	1
AT4G15248	BBX30	Orphans a	1	1	1
AT2G31310	LOB DOMAIN-CONTAINING PROTEIN 14	LOB a / AS2 b	1	1	1
AT1G21970	LEAFY COTYLEDON 1 (LEC1)	CCAAT a / CCAAT-HAP3 b	1	1	1
AT3G02150	PLASTID TRANSCRIPTION FACTOR 1 (PTF1)	TCP a,b,c	1	1	1
AT5G18560	(PUCHI)	AP2-EREBP a,b,c	1	1	1
AT1G68510	LOB DOMAIN-CONTAINING PROTEIN 42	LOB a / AS2 b	1	1	1
AT2G36400	GROWTH-REGULATING FACTOR 3 (GRF3)	GRF a,b	1	1	1
AT4G37780	MYB DOMAIN PROTEIN 87 (MYB87)	MYB a,b	1	1	1
AT2G42280	ABA-RESPONSIVE KINASE SUBSTRATE 3	bHLH a,b	1	1	1
AT5G09750	HECATE 3	bHLH a,b	1	1	1
AT1G27660	PERICYCLE FACTOR TYPE-A 5	ND	1	1	1
AT2G45160	HAIRY MERISTEM 1 (HAM1)	GRAS a,b	1	1	1
AT3G13960	GROWTH-REGULATING FACTOR 5 (GRF5)	GRF a,b	1	1	1
AT3G55370	OBF-BINDING PROTEIN 3 (OBP3)	C2C2-DOF a,b,c	1	1	1
AT3G18550	BRANCHED 1 (BRC1)	TCP a,b,c	1	1	1
AT2G33880	HOMEOBOX-3 (HB-3)	HB a,b,c	1	1	1
AT1G24590	ENHANCER OF SHOOT REGENERATION 2	AP2-EREBP a,b,c	1	1	1
AT2G20570	GBF'S PRO-RICH REGION-INTERACTING FACTOR 1 (GPRI1)	G2-like a,b	1	1	1
AT1G68640	PERIANTHIA (PAN)	bZIP a,b,c	1	1	1
AT1G53230	TCP3	TCP a,b,c	1	1	1
AT1G32240	KANADI 2 (KAN2)	G2-like a,b	1	1	1
AT2G28550	TARGET OF EARLY ACTIVATION TAGGED (EAT) 1	AP2-EREBP a,b,c	1	1	1
AT5G10510	AINTEGUMENTA-LIKE 6 (AIL6)	AP2-EREBP a,b,c	1	1	1
AT2G47700	RED AND FAR-RED INSENSITIVE 2	ND	1	1	1
AT5G44190	GOLDEN2-LIKE 2 (GLK2)	G2-like a,b	1	1	1
AT3G49690	MYB DOMAIN PROTEIN 84 (MYB84)	MYB a,b	1	1	1
AT3G50510	LOB DOMAIN-CONTAINING PROTEIN 28	LOB a / AS2 b	1	1	1
AT4G32570	TIFY8	ZIM b,c	1	1	1
AT4G04885	PCF11P-SIMILAR PROTEIN 4 (PCFS4)	C2H2 d	1	1	1
AT3G62100	INDOLE-3-ACETIC ACID INDUCIBLE 30 (IAA30)	AUX-IAA a,b	1	1	
AT3G14020	NUCLEAR FACTOR Y, SUBUNIT A6	CCAAT a / CCAAT-HAP2 b / CBFB_NFYA c	1	1	
AT2G36890	REGULATOR OF AXILLARY MERISTEMS 2 (RAX2)	MYB a,b	1	1	
AT3G58070	GLABROUS INFLORESCENCE STEMS (GIS)	C2H2 a,b,c	1	1	
AT1G16530	ASYMMETRIC LEAVES 2-LIKE 9	LOB a / AS2 b	1	1	
AT1G07900	LOB DOMAIN-CONTAINING PROTEIN 1	LOB a / AS2 b	1	1	
AT1G06280	LOB DOMAIN-CONTAINING PROTEIN 2	LOB a / AS2 b	1	1	
AT5G03790	HOMEOBOX 51 (HB51)	HB a,b,c	1	1	
AT4G01720	WRKY47	WRKY a,b,c	1	1	
AT5G15130	WRKY DNA-BINDING PROTEIN 72	WRKY a,b,c	1	1	
AT4G30080	AUXIN RESPONSE FACTOR 16 (ARF16)	ARF a,b,c	1	1	
AT3G25710	BASIC HELIX-LOOP-HELIX 32 (BHLH32)	bHLH a,b	1	1	

Gene identifier (AGI)	Primary Gene Symbol	TF family	1 mM 3AT	5 mM 3AT	visual in-spec-tion
AT1G69120	APETALA1 (AP1)	MADS a,b	1	1	
AT2G41940	ZINC FINGER PROTEIN 8 (ZFP8)	C2H2 a,b,c	1	1	
AT3G15030	TCP FAMILY TRANSCRIPTION FACTOR 4 (TCP4)	TCP a,b,c	1	1	
AT2G47460	MYB DOMAIN PROTEIN 12 (MYB12)	MYB a,b	1	1	
AT5G44160	INDETERMINATE DOMAIN 8	C2H2 a,b,c	1	1	
AT4G02590	PERICYCLE FACTOR TYPE-B 1	bHLH a,b	1	1	
AT4G18020	(APRR2)	ARR-B a / G2-like b	1	1	
AT3G01470	HOMEOBOX 1 (HB-1)	HB a,b	1	1	
AT1G69170	SPL6	SBP a,b,c	1	1	
AT5G15840	CONSTANS (CO)	C2C2-CO-like a,b	1	1	
AT5G07310	ETHYLENE RESPONSE FACTOR 115	AP2-EREBP a,b,c	1	1	
AT3G08500	MYB DOMAIN PROTEIN 83 (MYB83)	MYB a,b	1	1	
AT2G20880	ERF DOMAIN 53 (ERF53)	AP2-EREBP a,b,c	1	1	
AT1G31630	AGAMOUS-LIKE 86	MADS a,b	1	1	
AT2G02540	HOMEOBOX PROTEIN 21 (HB21)	zf-HD a,b	1	1	
AT1G48500	JASMONATE-ZIM-DOMAIN PROTEIN 4	ZIM b,c	1	1	
AT4G27330	SPOROCYTELESS (SPL)	NOZZLE a / NZZ b	1	1	
AT1G66550	WRKY DNA-BINDING PROTEIN 67	WRKY a,b,c	1	1	
AT1G79430	ALTERED PHLOEM DEVELOPMENT (APL)	G2-like a,b	1	1	
AT5G60120	TARGET OF EARLY ACTIVATION TAGGED (EAT) 2	AP2-EREBP a,b,c	1	1	
AT2G41370	BLADE ON PETIOLE2 (BOP2)	TRAF a	1	1	
AT5G25220	KNOTTED1-LIKE HOMEOBOX GENE 3 (KNAT3)	HB a / KNOX2 c	1	1	
AT2G22800	(HAT9)	HB a,b	1	1	
AT2G43220	Cysteine/Histidine-rich C1 domain family protein	ND	1	1	
AT4G37540	LOB DOMAIN-CONTAINING PROTEIN 39	LOB a / AS2 b	1	1	
AT4G37740	GROWTH-REGULATING FACTOR 2 (GRF2)	GRF a,b	1	1	
AT5G06080	LOB DOMAIN-CONTAINING PROTEIN 33	LOB a / AS2 b	1	1	
AT4G08150	KNOTTED-LIKE FROM ARABIDOPSIS THALIANA (KNAT1)	HB a,b	1	1	
AT3G51180	Zinc finger C-x8-C-x5-C-x3-H type family protein	ND	1	1	
AT3G21890	B-BOX DOMAIN PROTEIN 31	Orphans a	1	1	
AT3G25790	HRS1 HOMOLOGUE 1	G2-like a,b	1	1	
AT5G66350	SHORT INTERNODES (SHI)	SRS a,b	1	1	
AT3G62610	MYB DOMAIN PROTEIN 11 (MYB11)	MYB a,b	1	1	
AT5G17430	BABY BOOM (BBM)	AP2-EREBP a,b,c	1	1	
AT2G22840	GROWTH-REGULATING FACTOR 1 (GRF1)	GRF a,b	1	1	
AT4G32010	HSI2-LIKE 1	ABI3-VP1 a,b	1	1	
AT3G16857	RESPONSE REGULATOR 1 (RR1)	ARR-B a,b	1	1	
AT1G73360	HOMEODOMAIN GLABROUS 11 (HDG11)	HB a,b	1	1	
AT1G19220	AUXIN RESPONSE FACTOR 19 (ARF19)	ARF a,b,c	1	1	
AT5G41410	BELL 1 (BEL1)	HB a,b	1	1	
AT5G46880	HOMEOBOX-7	HB a,b	1	1	
AT1G30460	CLEAVAGE AND POLYADENYLATION SPECIFICITY FACTOR 30 (CPSF30)	C3H a,b	1	1	
AT5G23280	TCP7	TCP a,b,c	1	1	
AT1G12980	ENHANCER OF SHOOT REGENERATION 1 (ESR1)	AP2-EREBP a,b,c	1	1	
AT5G08330	TCP21	TCP a,b,c	1	1	
AT2G30130	ASL5	LOB a / AS2 b	1	1	
AT4G03250	Homeodomain-like superfamily protein	HB a,b	1	1	
AT2G27110	FAR1-RELATED SEQUENCE 3	FAR1 a	1	1	
AT1G21700	SWITCH/SUCROSE NONFERMENTING 3C (SWI3C)	MYB-related a	1	1	
AT2G31280	LONESOME HIGHWAY LIKE 2	bHLH d	1	1	
AT5G23260	TRANSPARENT TESTA16 (TT16)	MADS a,b	1	0	
AT5G40220	AGAMOUS-LIKE 43	MADS a,b	1	0	
AT5G67060	HECATE 1	bHLH a,b	1	0	
AT5G59340	WUSCHEL RELATED HOMEOBOX 2 (WOX2)	HB a,b,c	1	0	
AT5G38800	BZIP43	bZIP a,b,c	1	0	
AT5G62320	MYB DOMAIN PROTEIN 99 (MYB99)	MYB a,b	1	0	
AT1G71450	FUF1	AP2-EREBP a,b,c	1	0	
AT3G10590	homeodomain-like superfamily protein	MYB a,b	1	0	
AT3G61250	MYB DOMAIN PROTEIN 17 (MYB17)	MYB a,b	1	0	
AT3G10580	Homeodomain-like superfamily protein	MYB-related a / MYB b	1	0	
AT1G68320	MYB DOMAIN PROTEIN 62 (MYB62)	MYB a,b	1	0	
AT3G57600	member of the DREB subfamily A-2 of ERF/AP2 TF family	AP2-EREBP a,b,c	1	0	
AT3G50410	OBF BINDING PROTEIN 1 (OBP1)	C2C2-DOF a,b,c	1	0	
AT1G35490	bZIP family transcription factor	bZIP b	1	0	
AT4G26150	CYTOKININ-RESPONSIVE GATA FACTOR 1 (CGA1)	C2C2-GATA a,b	1	0	
AT5G57660	CONSTANS-LIKE 5	C2C2-CO-like a,b	1	0	
AT3G11440	MYB DOMAIN PROTEIN 65 (MYB65)	MYB a,b	1	0	
AT2G42400	VOZ2	VOZ a,b	1	0	
AT5G25190	ETHYLENE AND SALT INDUCIBLE 3 (ESE3)	AP2-EREBP a,b,c	1	0	
AT1G27370	SQUAMOSA PROMOTER BINDING PROTEIN-LIKE 10 (SPL10)	SBP a,b,c	1	0	
AT3G20310	ETHYLENE RESPONSE FACTOR 7 (ERF7)	AP2-EREBP a,b,c	1	0	

Gene identifier (AGI)	Primary Gene Symbol	TF family	1 mM 3AT	5 mM 3AT	visual in-spec-tion
AT4G00180	YABBY3 (YAB3)	C2C2-YABBY a,b,c	1	0	
AT1G14920	GIBBERELLIC ACID INSENSITIVE (GAI)	GRAS a,b	1	0	
AT5G15800	SEPALLATA1 (SEP1)	MADS a,b	1	0	
AT1G03840	MAGPIE (MGP)	C2H2 a,b,c	1	0	
AT3G60390	HOMEOBOX-LEUCINE ZIPPER PROTEIN 3 (HAT3)	HB a,b	1	0	
AT2G01570	REPRESSOR OF GA1-3 1 (RGA1)	GRAS a,b	1	0	
AT1G51600	ZIM-LIKE 2 (ZML2)	C2C2-GATA a / ZIM b / CCT c	1	0	
AT4G36540	BR ENHANCED EXPRESSION 2	bHLH a,b	1	0	
AT2G45190	ABNORMAL FLORAL ORGANS (AFO)	C2C2-YABBY a,b,c	1	0	
AT1G58110	bZIP transcription factor	bZIP b,c	1	0	
AT1G23420	INNER NO OUTER (INO)	C2C2-YABBY a,b,c	1	0	
AT1G10120	CRY2-INTERACTING BHLH 4	bHLH a,b	1	0	
AT1G69810	WRKY DNA-BINDING PROTEIN 36	WRKY a,b,c	1	0	
AT5G53950	CUP-SHAPED COTYLEDON 2 (CUC2)	NAC a,b	1	0	
AT1G50640	ETHYLENE RESPONSIVE ELEMENT BINDING FACTOR 3 (ERF3)	AP2-EREBP a,b,c	1	0	
AT4G36930	SPATULA (SPT)	bHLH a,b	1	0	
AT5G25160	ZINC FINGER PROTEIN 3 (ZFP3)	C2H2 a,b	1	0	
AT1G27650	U2 auxiliary factor small subunit	C3H a,b	1	0	
AT1G61660	PFA4	bHLH a	1	0	
AT4G13480	MYB DOMAIN PROTEIN 79 (MYB79)	MYB a,b	1	0	
AT5G47230	ETHYLENE RESPONSIVE ELEMENT BINDING FACTOR 5 (ERF5)	AP2-EREBP a,b,c	1	0	
AT1G03040	PERICYCLE FACTOR TYPE-B 2	bHLH a,b	1	0	
AT5G23000	MYB DOMAIN PROTEIN 37 (MYB37)	MYB a,b	1	0	
AT4G04450	WRKY42	WRKY a,b,c	1	0	
AT4G21080	DOF4.5	C2C2-DOF a,b,c	1	0	
AT4G29100	BASIC HELIX LOOP HELIX 68	ND	1	0	
AT4G22360	SWIB complex BAF60b domain-containing protein	SWI/SNF-BAF60b a	1	0	
AT1G06170	BASIC HELIX LOOP HELIX PROTEIN 89	bHLH a,b	1	0	
AT1G15050	INDOLE-3-ACETIC ACID INDUCIBLE 34 (IAA34)	AUX-IAA a,b	1	0	
AT3G24120	PHR1-LIKE 2	G2-like a,b	1	0	
AT5G13330	RELATED TO AP2 6L (Rap2.6L)	AP2-EREBP a,b,c	1	0	
AT3G51910	HEAT SHOCK TRANSCRIPTION FACTOR A7A (HSFA7A)	HSF a,b,c	1	0	
AT5G66980	AP2/B3-like transcriptional factor family protein	ABI3-VP1 a,b	1	0	
AT1G80590	WRKY DNA-BINDING PROTEIN 66	WRKY a,b,c	1	0	
AT5G57390	AINTEGUMENTA-LIKE 5 (AIL5)	AP2-EREBP a,b,c	1	0	
AT1G12890	member of the ERF subfamily B-1 of ERF/AP2 TF family	AP2-EREBP a,b,c	1	0	
AT4G37750	AINTEGUMENTA (ANT)	AP2-EREBP a,b,c	1	0	
AT5G48890	LATE FLOWERING (LATE)	C2H2 b	1	0	
AT4G04890	PROTODERMAL FACTOR 2 (PDF2)	HB a,b	1	0	
AT3G03660	WUSCHEL RELATED HOMEOBOX 11 (WOX11)	HB a,b,c	1	0	
AT5G39820	NAC DOMAIN CONTAINING PROTEIN 94	NAC a,b	1	0	
AT1G26780	MYB DOMAIN PROTEIN 117 (MYB117)	MYB a,b	1	0	
AT5G66870	ASYMMETRIC LEAVES 2-LIKE 1 (ASL1)	LOB a / AS2 b	1	0	
AT5G09460	bHLH143	ND	1	0	
AT1G72210	BHLH96	bHLH a,b	1	0	
AT4G36710	HAM4	GRAS a,b	1	0	
AT3G45150	TCP16	TCP a,b,c	1	0	
AT3G12910	NAC (No Apical Meristem) domain transcriptional regulator superfamily protein	NAC a / NAM c	1	0	
AT2G43010	PHYTOCHROME INTERACTING FACTOR 4 (PIF4)	bHLH a,b	1	0	
AT1G65620	ASYMMETRIC LEAVES 2	LOB a / AS2 b	1	0	
AT3G01220	HOMEOBOX PROTEIN 20 (HB20)	HB a,b	1	0	
AT1G68810	ABNORMAL SHOOT 5	bHLH a,b	1	0	
AT5G57620	MYB DOMAIN PROTEIN 36 (MYB36)	MYB a,b	1	0	
AT2G23660	LOB DOMAIN-CONTAINING PROTEIN 10	LOB a / AS2 b	1	0	
AT3G12890	ACTIVATOR OF SPOMIN::LUC2 (ASML2)	Orphans a / C2C2-CO-like b	1	0	
AT5G01160	HAKAI	C2H2 d	1	0	
AT1G43160	ERF113	AP2-EREBP a,b,c	1	0	
AT5G51910	TCP19	TCP a,b,c	1	0	
AT1G66810	Encodes a tandem CCCH zinc finger (TZF) protein	C3H a,b	1	0	
AT3G24650	ABA INSENSITIVE 3 (ABI3)	ABI3-VP1 a,b	1	0	
AT4G34000	ABSCISIC ACID RESPONSIVE ELEMENTS-BINDING FACTOR 3 (ABF3)	bZIP a,b,c	1	0	
AT4G17695	KANADI 3	G2-like a,b	1	0	
AT1G75540	B-BOX DOMAIN PROTEIN 21 (BBX21)	Orphans a	1	0	
AT4G38180	FAR1-RELATED SEQUENCE 5	FAR1 a	1	0	
AT1G52150	ATHB15	HB a,b	1	0	
AT2G35940	BEL1-LIKE HOMEODOMAIN 1 (BLH1)	HB a,b	1	0	
AT5G45300	BETA-AMYLASE 2 (BMY2)	BES1 a,b	1	0	
AT5G52170	HOMEODOMAIN GLABROUS 7 (HDG7)	HB a,b	1	0	
AT4G00150	HAIRY MERISTEM 3 (HAM3)	GRAS a,b	1	0	
AT5G64750	ABA REPRESSOR1 (ABR1)	AP2-EREBP a,b,c	1	0	

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AT1G79000	HISTONE ACETYLTRANSFERASE OF THE CBP FAMILY 1 (HAC1)	TAZ a,b	1	0	
AT5G61380	TIMING OF CAB EXPRESSION 1 (TOC1)	Pseudo ARR-B a / C2C2-CO-like b	1	0	
At5g65330	AGAMOUS-LIKE 78	MADS	1	0	
AT1G68150	protein_coding	WRKY a,b,c	0	0	
AT1G55600	protein_coding	WRKY a,b,c	0	0	
AT4G18470	SUPPRESSOR OF NPR1-1, INDUCIBLE 1 (SNI1)	ND	0	0	
AT5G06500	INVOLVED IN: regulation of transcription, DNA-dependent	MADS a,b	0	0	
AT2G24430	INVOLVED IN: multicellular organismal development, regulation of transcription	NAC a,b	0	0	
AT5G26650	INVOLVED IN: regulation of transcription, DNA-dependent	MADS a,b	0	0	
AT1G60920	INVOLVED IN: N-terminal protein myristoylation, regulation of transcription, DNA-dependent	MADS a,b	0	0	
AT1G60880	AGAMOUS-LIKE-56 (AGL56)	MADS a,b	0	0	
AT3G24050	GATA TRANSCRIPTION FACTOR 1 (GATA1)	C2C2-GATA a,b	0	0	
AT2G37430	INVOLVED IN: response to chitin, regulation of transcription	C2H2 a,b,c	0	0	
AT5G04340	ZINC FINGER OF ARABIDOPSIS THALIANA 6 (ZAT6)	C2H2 a,b	0	0	
AT4G25470	C-REPEAT/DRE BINDING FACTOR 2 (CBF2)	AP2-EREBP a,b,c	0	0	
AT5G42910	BEST Arabidopsis thaliana protein match is: abscisic acid responsive elements-binding factor 2 (TAIR:AT1G45249.1)	bZIP a,b,c	0	0	
AT4G14550	INDOLE-3-ACETIC ACID INDUCIBLE 14 (IAA14)	AUX-IAA a,b	0	0	
AT2G46990	INDOLE-3-ACETIC ACID INDUCIBLE 20 (IAA20)	AUX-IAA a,b	0	0	
AT4G11880	AGAMOUS-LIKE 14 (AGL14)	MADS a,b	0	0	
AT3G57230	AGAMOUS-LIKE 16 (AGL16)	MADS a,b	0	0	
AT2G22630	AGAMOUS-LIKE 17 (AGL17)	MADS a,b	0	0	
AT5G10140	FLOWERING LOCUS C (FLC)	MADS a,b	0	0	
AT5G65050	AGAMOUS-LIKE 31 (AGL31)	MADS a,b	0	0	
AT5G62165	AGAMOUS-LIKE 42 (AGL42)	MADS a,b	0	0	
AT5G39750	INVOLVED IN: regulation of transcription, DNA-dependent, embryo development ending in seed dormancy	MADS a,b	0	0	
AT1G09530	PHYTOCHROME INTERACTING FACTOR 3 (PIF3)	bHLH a,b	0	0	
AT3G05200	ARABIDOPSIS TOXICOS EN LEVADURA 6 (ATL6)	ND	0	0	
AT2G42360	BEST Arabidopsis thaliana protein match is: RING/U-box superfamily protein (TAIR:AT2G42350.1)	ND	0	0	
AT4G11680	LOCATED IN: plasma membrane	ND	0	0	
AT1G63840	INVOLVED IN: response to abscisic acid stimulus	ND	0	0	
AT2G20180	PHYTOCHROME INTERACTING FACTOR 3-LIKE 5 (PIL5)	bHLH a,b	0	0	
AT2G22750	INVOLVED IN: regulation of transcription	bHLH a,b	0	0	
AT4G37730	INVOLVED IN: regulation of transcription, DNA-dependent	bZIP a,b,c	0	0	
AT5G24800	BASIC LEUCINE ZIPPER 9 (BZIP9)	bZIP a,b,c	0	0	
AT2G22760	INVOLVED IN: regulation of transcription	bHLH a,b	0	0	
AT5G10030	TGACG MOTIF-BINDING FACTOR 4 (TGA4)	bZIP a,b,c	0	0	
AT4G36900	RELATED TO AP2 10 (RAP2.10)	AP2-EREBP a,b,c	0	0	
AT1G80580	0	AP2-EREBP a,b,c	0	0	
AT3G56970	(BHLH038)	bHLH a,b	0	0	
AT4G30980	LJRHL1-LIKE 2 (LRL2)	bHLH a,b	0	0	
AT1G01720	(ATAF1)	NAC a,b	0	0	
AT3G18400	INVOLVED IN: multicellular organismal development, regulation of transcription	NAC a,b	0	0	
AT5G22380	INVOLVED IN: multicellular organismal development, regulation of transcription	NAC a,b	0	0	
AT2G41240	BASIC HELIX-LOOP-HELIX PROTEIN 100 (BHLH100)	bHLH a,b	0	0	
AT5G41030	BEST Arabidopsis thaliana protein match is: TEOSINTE BRANCHED 1, cycloidea, PCF (TCP)-domain family protein 20 (TAIR:AT3G27010.1)	TCP a,b,c	0	0	
AT1G69180	CRABS CLAW (CRC)	C2C2-YABBY a,b,c	0	0	
AT1G08465	YABBY2 (YAB2)	C2C2-YABBY a,c	0	0	
AT5G64060	INVOLVED IN: multicellular organismal development, regulation of transcription	NAC a,b	0	0	
AT3G50650	BEST Arabidopsis thaliana protein match is: GRAS family transcription factor (TAIR:AT5G66770.1)	GRAS a,b	0	0	
AT1G66600	protein_coding	WRKY a,b,c	0	0	
AT4G35610	INVOLVED IN: regulation of transcription	C2H2 b	0	0	
AT5G05410	DRE-BINDING PROTEIN 2A (DREB2A)	AP2-EREBP a,b,c	0	0	
AT3G16280	0	AP2-EREBP a,b,c	0	0	
AT4G32980	HOMEOBOX GENE 1 (ATH1)	HB a,b	0	0	
AT4G34610	INVOLVED IN: regulation of transcription, DNA-dependent, regulation of transcription	HB a,b	0	0	
AT3G12820	MYB DOMAIN PROTEIN 10 (MYB10)	MYB a,b	0	0	
AT1G79180	MYB DOMAIN PROTEIN 63 (MYB63)	MYB a,b	0	0	
AT3G21880	INVOLVED IN: regulation of transcription	C2C2-CO-like a,b	0	0	
AT2G47890	INVOLVED IN: regulation of transcription	C2C2-CO-like a,b	0	0	
AT5G61420	MYB DOMAIN PROTEIN 28 (MYB28)	MYB a,b	0	0	

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AT5G06800	BEST Arabidopsis thaliana protein match is: Homeodomain-like superfamily protein (TAIR:AT3G04450.1)	G2-like a,b	0	0	
AT5G45580	BEST Arabidopsis thaliana protein match is: Homeodomain-like superfamily protein (TAIR:AT3G24120.1)	G2-like a,b	0	0	
AT1G17950	MYB DOMAIN PROTEIN 52 (MYB52)	MYB a,b	0	0	
AT5G21120	ETHYLENE-INSENSITIVE3-LIKE 2 (EIL2)	EIL a,b	0	0	
AT1G73410	MYB DOMAIN PROTEIN 54 (MYB54)	MYB a,b	0	0	
AT3G04450	BEST Arabidopsis thaliana protein match is: Homeodomain-like superfamily protein (TAIR:AT5G29000.2)	G2-like a,b	0	0	
AT2G20400	BEST Arabidopsis thaliana protein match is: phosphate starvation response 1 (TAIR:AT4G28610.1)	G2-like a,b	0	0	
AT4G39070	INVOLVED IN: response to karrikin, response to chitin, regulation of transcription	Orphans a	0	0	
AT1G01060	LATE ELONGATED HYPOCOTYL (LHY)	MYB-related a,b	0	0	
AT2G28920	CONTAINS InterPro DOMAIN/s: Zinc finger, RING-type (InterPro:IPR001841), Zinc finger, C3HC4 RING-type (InterPro:IPR018957)	ND	0	0	
AT4G31580	RS-CONTAINING ZINC FINGER PROTEIN 22 (RSZ22)	ND	0	0	
AT4G21040	BEST Arabidopsis thaliana protein match is: Dof-type zinc finger domain-containing protein (TAIR:AT4G21080.1)	C2C2-DOF a,b,c	0	0	
AT3G52440	BEST Arabidopsis thaliana protein match is: Dof-type zinc finger DNA-binding family protein (TAIR:AT1G21340.1)	C2C2-DOF a,b,c	0	0	
AT1G21340	BEST Arabidopsis thaliana protein match is: Dof-type zinc finger DNA-binding family protein (TAIR:AT3G52440.1)	C2C2-DOF a,b,c	0	0	
AT2G17900	SET DOMAIN GROUP 37 (SDG37)	ND	0	0	
AT4G31680	INVOLVED IN: regulation of transcription, DNA-dependent	ABI3-VP1 a,b	0	0	
AT3G11580	INVOLVED IN: regulation of transcription, DNA-dependent	ABI3-VP1 a,b	0	0	
AT2G44910	HOMEOBOX-LEUCINE ZIPPER PROTEIN 4 (HB4)	HB a,b	0	0	
AT1G20700	WUSCHEL RELATED HOMEOBOX 14 (WOX14)	HB a,b,c	0	0	
AT5G52020	0	AP2-EREBP a,b,c	0	0	
AT4G06746	RELATED TO AP2 9 (RAP2.9)	AP2-EREBP a,b,c	0	0	
AT1G64380	0	AP2-EREBP a,b,c	0	0	
AT3G25890	CYTOKININ RESPONSE FACTOR 11 (CRF11)	AP2-EREBP a,b	0	0	
AT3G61910	NAC DOMAIN PROTEIN 66 (NAC066)	NAC a,b	0	0	
AT3G44350	BEST Arabidopsis thaliana protein match is: NAC domain containing protein 90 (TAIR:AT5G22380.1)	NAC a,b	0	0	
AT1G35515	HIGH RESPONSE TO OSMOTIC STRESS 10 (HOS10)	MYB a,b	0	0	
AT1G17310	INVOLVED IN: regulation of transcription, DNA-dependent	MADS a,b	0	0	
AT3G53340	INVOLVED IN: regulation of transcription, DNA-dependent	CCAAT a / CCAAT-HAP3 b	0	0	
AT1G47760	INVOLVED IN: regulation of transcription, DNA-dependent	MADS a,b	0	0	
AT5G60890	protein_coding	MYB a,b	0	0	
AT3G02310	SEPALLATA 2 (SEP2)	MADS a,b	0	0	
AT2G42830	SHATTERPROOF 2 (SHP2)	MADS a,b	0	0	
AT1G65360	AGAMOUS-LIKE 23 (AGL23)	MADS a,b	0	0	
AT3G30260	INVOLVED IN: regulation of transcription, DNA-dependent	MADS a,b	0	0	
AT2G34440	INVOLVED IN: regulation of transcription, DNA-dependent	MADS a,b	0	0	
AT5G27910	INVOLVED IN: regulation of transcription, DNA-dependent	CCAAT a / CCAAT-HAP5 b	0	0	
AT3G12250	TGACG MOTIF-BINDING FACTOR 6 (TGA6)	bZIP a,b,c	0	0	
AT2G04038	INVOLVED IN: regulation of transcription, DNA-dependent	bZIP a,b,c	0	0	
AT3G17609	HY5-HOMOLOG (HYH)	bZIP a,b,c	0	0	
AT5G06839	INVOLVED IN: regulation of transcription, DNA-dependent	bZIP a,b,c	0	0	
AT1G48000	MYB DOMAIN PROTEIN 112 (MYB112)	MYB a,b	0	0	
AT1G25340	MYB DOMAIN PROTEIN 116 (MYB116)	MYB a,b	0	0	
AT3G27785	MYB DOMAIN PROTEIN 118 (MYB118)	MYB a,b	0	0	
AT3G30210	MYB DOMAIN PROTEIN 121 (MYB121)	MYB a,b	0	0	
AT5G35550	TRANSPARENT TESTA 2 (TT2)	MYB a,b	0	0	
AT5G02320	MYB DOMAIN PROTEIN 3R-5 (MYB3R-5)	MYB a,b	0	0	
AT4G25560	LONG AFTER FAR-RED LIGHT 1 (LAF1)	MYB a,b	0	0	
AT5G01200	BEST Arabidopsis thaliana protein match is: Duplicated homeodomain-like superfamily protein (TAIR:AT2G38090.1)	MYB a,b	0	0	
AT1G71030	MYB-LIKE 2 (MYBL2)	MYB-related a,b	0	0	
AT5G26660	BEST Arabidopsis thaliana protein match is: myb domain protein 50 (TAIR:AT1G57560.1)	MYB a,b	0	0	
AT4G11250	INVOLVED IN: regulation of transcription, DNA-dependent	MADS a,b	0	0	
AT5G41200	INVOLVED IN: regulation of transcription, DNA-dependent	MADS a,b	0	0	
AT1G65330	PHERES1 (PHE1)	MADS a,b	0	0	
AT1G13450	GT-1 (GT-1)	TRIHELIX a,b	0	0	
AT1G49560	BEST Arabidopsis thaliana protein match is: myb-like transcription factor family protein (TAIR:AT1G68670.1)	G2-like a,b	0	0	
AT2G40260	BEST Arabidopsis thaliana protein match is: myb-like HTH transcriptional regulator family protein (TAIR:AT2G38300.1)	G2-like a,b	0	0	
AT3G27920	MYB DOMAIN PROTEIN 0 (MYB0)	MYB a,b	0	0	

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AT5G56110	(MYB80)	MYB a,b	0	0	
AT5G56840	BEST Arabidopsis thaliana protein match is: myb-like transcription factor family protein (TAIR:AT5G47390.1)	MYB-related a,b	0	0	
AT1G15580	INDOLE-3-ACETIC ACID INDUCIBLE 5 (IAA5)	AUX-IAA a,b	0	0	
AT3G23140	UPRIGHT ROSETTE (URO)	C2H2 b	0	0	
AT5G53040	RWP-RK DOMAIN-CONTAINING 4 (RKD4)	RWP-RK a / NIN-like b	0	0	
AT2G41130	INVOLVED IN: regulation of transcription	bHLH a,b	0	0	
AT3G56770	INVOLVED IN: response to chitin, regulation of transcription	bHLH a,b	0	0	
AT3G56980	(BHLH039)	bHLH a,b	0	0	
AT1G22490	INVOLVED IN: regulation of transcription	bHLH a,b	0	0	
AT5G53210	SPEECHLESS (SPCH)	bHLH a,b	0	0	
AT3G57920	SQUAMOSA PROMOTER BINDING PROTEIN-LIKE 15 (SPL15)	SBP a,b,c	0	0	
AT1G49120	CYTOKININ RESPONSE FACTOR 9 (CRF9)	AP2-EREBP a,b	0	0	
AT3G60490	0	AP2-EREBP a,b,c	0	0	
AT4G27950	CYTOKININ RESPONSE FACTOR 4 (CRF4)	AP2-EREBP a,b,c	0	0	
AT5G11190	SHINE2 (SHN2)	AP2-EREBP a,b,c	0	0	
AT2G47270	UPBEAT1 (UPB1)	bHLH d	0	0	
AT5G25830	GATA TRANSCRIPTION FACTOR 12 (GATA12)	C2C2-GATA a,b	0	0	
AT2G28500	LOCATED IN: chloroplast	LOB a / AS2 b	0	0	
AT2G42430	LATERAL ORGAN BOUNDARIES-DOMAIN 16 (LBD16)	LOB a / AS2 b	0	0	
AT2G45410	BEST Arabidopsis thaliana protein match is: LOB domain-containing protein 31 (TAIR:AT4G00210.1)	LOB a / AS2 b	0	0	
AT2G45420	BEST Arabidopsis thaliana protein match is: Lateral organ boundaries (LOB) domain family protein (TAIR:AT4G00220.1)	LOB a / AS2 b	0	0	
AT3G47870	LOB DOMAIN-CONTAINING PROTEIN 27 (LBD27)	LOB a / AS2 b	0	0	
AT5G15830	INVOLVED IN: regulation of transcription, DNA-dependent	bZIP a,b,c	0	0	
AT1G06070	INVOLVED IN: regulation of transcription, DNA-dependent	bZIP a,b,c	0	0	
AT4G30180	BEST Arabidopsis thaliana protein match is: unknown protein (TAIR:AT2G18969.1)	bHLH d	0	0	
AT1G26590	INVOLVED IN: regulation of transcription	C2H2 a,b	0	0	
AT1G26610	INVOLVED IN: regulation of transcription	C2H2 a,b,c	0	0	
AT1G51220	WIP DOMAIN PROTEIN 5 (WIP5)	C2H2 a,b,c	0	0	
AT2G26940	INVOLVED IN: regulation of transcription	C2H2 a,b,c	0	0	
AT3G10470	INVOLVED IN: regulation of transcription	C2H2 a,b,c	0	0	
AT5G22990	INVOLVED IN: regulation of transcription	C2H2 a,b,c	0	0	
AT3G27650	BEST Arabidopsis thaliana protein match is: Lateral organ boundaries (LOB) domain family protein (TAIR:AT5G63090.4)	LOB a / AS2 b	0	0	
AT1G05710	INVOLVED IN: response to ethylene stimulus, regulation of transcription	ND	0	0	
AT3G13445	TATA BINDING PROTEIN 1 (TBP1)	ND	0	0	
AT1G06040	SALT TOLERANCE (STO)	Orphans a	0	0	
AT2G36930	INVOLVED IN: biological_process unknown	C2H2 b	0	0	
AT3G19290	ABRE BINDING FACTOR 4 (ABF4)	bZIP a,b,c	0	0	
AT2G40620	INVOLVED IN: regulation of transcription, DNA-dependent	bZIP a,b,c	0	0	
AT2G33550	BEST Arabidopsis thaliana protein match is: sequence-specific DNA binding transcription factors (TAIR:AT4G31270.1)	TRIHELIX a,b	0	0	
AT3G13040	BEST Arabidopsis thaliana protein match is: phosphate starvation response 1 (TAIR:AT4G28610.1)	G2-like a,b	0	0	
AT3G51080	GATA TRANSCRIPTION FACTOR 6 (GATA6)	C2C2-GATA a,b	0	0	
AT1G08290	BEST Arabidopsis thaliana protein match is: WIP domain protein 5 (TAIR:AT1G51220.1)	C2H2 a,b	0	0	
AT2G31380	SALT TOLERANCE HOMOLOGUE (STH)	Orphans a	0	0	
AT1G21200	BEST Arabidopsis thaliana protein match is: unknown protein (TAIR:AT1G76870.1)	TRIHELIX b	0	0	
AT1G13260	RELATED TO ABI3/VP1 1 (RAV1)	AP2-EREBP a,b	0	0	
AT1G74840	INVOLVED IN: in 9 processes	MYB-related a,b	0	0	
AT5G48250	LOCATED IN: plasma membrane	C2C2-CO-like a,b	0	0	
AT4G09450	BEST Arabidopsis thaliana protein match is: Homeodomain-like superfamily protein (TAIR:AT3G10580.2)	MYB-related a / MYB b	0	0	
AT1G72450	JASMONATE-ZIM-DOMAIN PROTEIN 6 (JAZ6)	ZIM b,c	0	0	
AT2G42680	MULTIPROTEIN BRIDGING FACTOR 1A (MBF1A)	MBF1 a,b	0	0	
AT4G01250	protein_coding	WRKY a,b,c	0	0	
AT2G36990	RNAPOLYMERASE SIGMA-SUBUNIT F (SIGF)	SIGMA70-like a	0	0	
AT2G47210	INVOLVED IN: N-terminal protein myristoylation, negative regulation of transcription, regulation of transcription, DNA-dependent	MYB-related a,b,c	0	0	
AT2G25650	INVOLVED IN: biological_process unknown	GeBP a,b	0	0	
AT1G25560	TEMPRANILLO 1 (TEM1)	AP2-EREBP a,b	0	0	
AT3G07670	INVOLVED IN: biological_process unknown	SET a / PcG b	0	0	
AT1G04950	TATA BOX ASSOCIATED FACTOR II 59 (TAFII59)	ND	0	0	
AT5G67300	MYB DOMAIN PROTEIN R1 (MYBR1)	MYB a,b	0	0	
AT5G08520	BEST Arabidopsis thaliana protein match is: Homeodomain-like transcriptional regulator (TAIR:AT5G23650.1)	MYB a,b	0	0	
AT3G14180	Has 515 Blast hits to 439 proteins in 35 species: Archae - 0	TRIHELIX a,b	0	0	

Gene identifier (AGI)	Primary Gene Symbol	TF family	1 mM 3AT	5 mM 3AT	visual in-spec-tion
AT1G42990	BASIC REGION/LEUCINE ZIPPER MOTIF 60 (BZIP60)	bZIP a,b,c	0	0	
AT4G10240	INVOLVED IN: regulation of transcription	Orphans a	0	0	
AT2G46270	G-BOX BINDING FACTOR 3 (GBF3)	bZIP a,b	0	0	
AT4G29190	BEST Arabidopsis thaliana protein match is: CCCH-type zinc finger family protein (TAIR:AT2G19810.1)	C3H a,b	0	0	
AT4G27410	RESPONSIVE TO DESICCATION 26 (RD26)	NAC a,b	0	0	
AT3G10480	INVOLVED IN: multicellular organismal development, regulation of transcription	NAC a,b	0	0	
AT1G68550	CYTOKININ RESPONSE FACTOR 10 (CRF10)	AP2-EREBP a,b,c	0	0	
AT2G41835	INVOLVED IN: biological_process unknown	C2H2 a,b	0	0	
AT5G39760	INVOLVED IN: regulation of transcription	zf-HD a,b	0	0	
AT2G44150	HISTONE-LYSINE N-METHYLTRANSFERASE ASHH3 (ASHH3)	SET a / PcG b	0	0	
AT1G50420	protein_coding	GRAS a,b	0	0	
AT5G44260	BEST Arabidopsis thaliana protein match is: Zinc finger C-x8-C-x5-C-x3-H type family protein (TAIR:AT1G03790.1)	C3H a,b	0	0	
AT1G51140	FLOWERING BHLH 3 (FBH3)	bHLH a,b	0	0	
AT4G39100	SHORT LIFE (SHL1)	PHD a,b	0	0	
AT5G39610	NAC DOMAIN CONTAINING PROTEIN 6 (NAC6)	NAC a,b	0	0	
AT1G27360	SQUAMOSA PROMOTER-LIKE 11 (SPL11)	SBP a,b,c	0	0	
AT2G31230	ETHYLENE-RESPONSIVE ELEMENT BINDING FACTOR 15 (ERF15)	AP2-EREBP a,b,c	0	0	
AT5G65320	INVOLVED IN: regulation of transcription	bHLH a,b	0	0	
AT1G53160	SQUAMOSA PROMOTER BINDING PROTEIN-LIKE 4 (SPL4)	SBP a,b,c	0	0	
AT5G22570	protein_coding	WRKY a,b,c	0	0	
AT1G24625	ZINC FINGER PROTEIN 7 (ZFP7)	C2H2 a,b,c	0	0	
AT5G47640	INVOLVED IN: regulation of transcription, DNA-dependent	CCAAT a / CCAAT-HAP3 b	0	0	
AT3G44460	protein_coding	bZIP a,b,c	0	0	
AT4G09460	MYB DOMAIN PROTEIN 6 (MYB6)	MYB a,b	0	0	
AT2G18300	INVOLVED IN: response to cytokinin stimulus, regulation of transcription	bHLH a,b	0	0	
AT5G28640	ANGUSTIFOLIA 3 (AN3)	GIF b	0	0	
AT3G15510	NAC DOMAIN CONTAINING PROTEIN 2 (NAC2)	NAC a,b	0	0	
AT3G48160	DP-E2F-LIKE 1 (DEL1)	E2F-DP a,b,c	0	0	
AT2G28510	INVOLVED IN: regulation of transcription	C2C2-DOF a,b,c	0	0	
AT2G47810	INVOLVED IN: regulation of transcription, DNA-dependent	CCAAT a / CCAAT-HAP3 b	0	0	
AT4G16610	INVOLVED IN: regulation of transcription	C2H2 a,b	0	0	
AT4G01060	CAPRICE-LIKE MYB3 (CPL3)	MYB-related a,b,c	0	0	
AT2G03470	LOCATED IN: nucleus	MYB-related a	0	0	
AT1G08810	MYB DOMAIN PROTEIN 60 (MYB60)	MYB a,b	0	0	
AT4G17785	MYB DOMAIN PROTEIN 39 (MYB39)	MYB a,b	0	0	
AT5G67580	(TRB2)	MYB-related a,b	0	0	
AT1G23380	KNOTTED1-LIKE HOMEOBOX GENE 6 (KNAT6)	HB a,b	0	0	
AT4G31610	REPRODUCTIVE MERISTEM 1 (REM1)	ABI3-VP1 a,b	0	0	
AT3G58630	Has 367 Blast hits to 356 proteins in 28 species: Archae - 0	TRIHELIX a,b	0	0	
AT2G38880	NUCLEAR FACTOR Y, SUBUNIT B1 (NF-YB1)	CCAAT a / CCAAT-HAP3 b	0	0	
AT5G45420	MEMBRANE ANCHORED MYB (maMYB)	MYB a,b	0	0	
AT5G42780	INVOLVED IN: regulation of transcription	zf-HD a,b	0	0	
AT5G04390	INVOLVED IN: biological_process unknown	C2H2 a,b,c	0	0	
AT4G16141	INVOLVED IN: regulation of transcription, DNA-dependent	C2C2-GATA d	0	0	
AT3G02790	INVOLVED IN: regulation of transcription	C2H2 b	0	0	
AT5G28770	(BZO2H3)	bZIP a,b,c	0	0	
AT5G60850	OBF BINDING PROTEIN 4 (OBP4)	C2C2-DOF a,b,c	0	0	
AT3G49530	NAC DOMAIN CONTAINING PROTEIN 62 (NAC062)	NAC a,b	0	0	
AT4G30935	protein_coding	WRKY a,b,c	0	0	
AT2G45800	PLIM2A (PLIM2a)	LIM a,b	0	0	
AT1G30500	INVOLVED IN: negative regulation of gene-specific transcription, regulation of transcription, DNA-dependent	CCAAT a / CCAAT-HAP2 b / CBFB_NFYA c	0	0	
AT1G66140	ZINC FINGER PROTEIN 4 (ZFP4)	C2H2 a,b,c	0	0	
AT1G78700	INVOLVED IN: biological_process unknown	BES1 a,b	0	0	
AT3G50260	COOPERATIVELY REGULATED BY ETHYLENE AND JASMONATE 1 (CEJ1)	AP2-EREBP a,b,c	0	0	
AT3G51880	HIGH MOBILITY GROUP B1 (HMGB1)	HMG a,b	0	0	
AT5G13180	NAC DOMAIN CONTAINING PROTEIN 83 (NAC083)	NAC a,b	0	0	
AT2G30580	DREB2A-INTERACTING PROTEIN 2 (DRIP2)	ND	0	0	
AT2G18670	EXPRESSED IN: 21 plant structures	ND	0	0	
AT3G61550	EXPRESSED IN: 20 plant structures	ND	0	0	
AT2G06200	GROWTH-REGULATING FACTOR 6 (GRF6)	GRF a,b	0	0	
AT1G69780	(ATHB13)	HB a,b	0	0	
AT1G08970	NUCLEAR FACTOR Y, SUBUNIT C9 (NF-YC9)	CCAAT a / CCAAT-HAP5 b	0	0	
AT3G10030	INVOLVED IN: cellular amino acid biosynthetic process	TRIHELIX a,b	0	0	

Gene identifier (AGI)	Primary Gene Symbol	TF family	1 mM 3AT	5 mM 3AT	visual in-spec-tion
AT1G13300	HYPERSENSITIVITY TO LOW PI-ELICITED PRIMARY ROOT SHORTENING 1 (HRS1)	G2-like a,b	0	0	
AT3G11200	ALFIN-LIKE 2 (AL2)	ALFIN-like a,b	0	0	
AT3G04030	INVOLVED IN: regulation of transcription	G2-like a,b	0	0	
AT3G12730	INVOLVED IN: regulation of transcription	G2-like a,b	0	0	
AT2G33480	INVOLVED IN: multicellular organismal development, regulation of transcription	NAC a,b	0	0	
AT3G12720	MYB DOMAIN PROTEIN 67 (MYB67)	MYB a,b	0	0	
AT4G31800	WRKY DNA-BINDING PROTEIN 18 (WRKY18)	WRKY a,b,c	0	0	
AT2G25900	(ATCTH)	C3H a,b	0	0	
AT3G23240	ETHYLENE RESPONSE FACTOR 1 (ERF1)	AP2-EREBP a,b,c	0	0	
AT3G04420	INVOLVED IN: multicellular organismal development, regulation of transcription	NAC a,b	0	0	
AT3G13810	INVOLVED IN: regulation of transcription	C2H2 a,b,c	0	0	
AT5G09790	ARABIDOPSIS TRITHORAX-RELATED PROTEIN 5 (ATXR5)	PHD a,b	0	0	
AT5G06960	OCS-ELEMENT BINDING FACTOR 5 (OBF5)	bZIP a,b,c	0	0	
AT4G13980	(AT-HSFA5)	HSF a,b,c	0	0	
AT4G28140	0	AP2-EREBP a,b,c	0	0	
AT3G50060	MYB DOMAIN PROTEIN 77 (MYB77)	MYB a,b	0	0	
AT2G39900	WLIM2A (WLIM2a)	LIM a,b	0	0	
AT4G14410	INVOLVED IN: regulation of transcription	bHLH a,b	0	0	
AT1G04990	INVOLVED IN: biological_process unknown	C3H a,b	0	0	
AT3G13350	LOCATED IN: nucleus, intracellular	ARID a,b	0	0	
AT5G52010	INVOLVED IN: regulation of transcription	C2H2 a,b,c	0	0	
AT3G12480	INVOLVED IN: regulation of transcription, DNA-dependent	CCAAT a / CCAAT-HAP5 b	0	0	
AT5G03740	HISTONE DEACETYLASE 2C (HD2C)	C2H2 a,b,c	0	0	
AT2G29580	MOS4-ASSOCIATED COMPLEX SUBUNIT 5B (MAC5B)	C3H a		0	
AT1G21000	BEST Arabidopsis thaliana protein match is: PLATZ transcription factor family protein (TAIR:AT1G76590.1)	PLATZ a,b	0	0	
AT5G58620	INVOLVED IN: regulation of transcription	C3H a,b	0	0	
AT4G35550	WUSCHEL RELATED HOMEOBOX 13 (WOX13)	HB a,b,c	0	0	
AT3G45260	INVOLVED IN: regulation of transcription	C2H2 a,b,c	0	0	
AT4G18880	HEAT SHOCK TRANSCRIPTION FACTOR A4A (HSF A4A)	HSF a,b,c	0	0	
AT1G01010	INVOLVED IN: multicellular organismal development, regulation of transcription	NAC a,b	0	0	
AT2G46310	CYTOKININ RESPONSE FACTOR 5 (CRF5)	AP2-EREBP a,b,c	0	0	
AT1G77080	MADS AFFECTING FLOWERING 1 (MAF1)	MADS a,b	0	0	
AT4G30410	Has 35333 Blast hits to 34131 proteins in 2444 species: Archae - 798	bHLH d	0	0	
AT2G27990	BEL1-LIKE HOMEODOMAIN 8 (BLH8)	HB a,b	0	0	
AT1G55520	TATA BINDING PROTEIN 2 (TBP2)	ND	0	0	
AT3G29035	NAC DOMAIN CONTAINING PROTEIN 3 (NAC3)	NAC a,b	0	0	
AT3G58120	(BZIP61)	bZIP a,b,c	0	0	
AT2G46130	protein_coding	WRKY a,b,c	0	0	
AT1G08780	INVOLVED IN: protein folding	ND	0	0	
AT1G15360	SHINE 1 (SHN1)	AP2-EREBP a,b,c	0	0	
AT5G14000	INVOLVED IN: multicellular organismal development, regulation of transcription	NAC a,b	0	0	
AT4G32890	GATA TRANSCRIPTION FACTOR 9 (GATA9)	C2C2-GATA a,b	0	0	
AT4G36570	BEST Arabidopsis thaliana protein match is: RAD-like 6 (TAIR:AT1G75250.1)	MYB-related d	0	0	
AT2G25000	WRKY DNA-BINDING PROTEIN 60 (WRKY60)	WRKY a,b,c	0	0	
AT5G05090	BEST Arabidopsis thaliana protein match is: Homeodomain-like superfamily protein (TAIR:AT3G10760.1)	G2-like a,b	0	0	
AT5G02470	(DPA)	E2F-DP a,b,c	0	0	
AT3G15270	SQUAMOSA PROMOTER BINDING PROTEIN-LIKE 5 (SPL5)	SBP a,b,c	0	0	
AT3G57480	INVOLVED IN: biological_process unknown	C2H2 a,b	0	0	
AT1G25470	CYTOKININ RESPONSE FACTOR 12 (CRF12)	AP2-EREBP a,b	0	0	
AT3G55530	SALT- AND DROUGHT-INDUCED RING FINGER1 (SDIR1)	ND	0	0	
AT2G47260	protein_coding	WRKY a,b,c	0	0	
AT1G75390	INVOLVED IN: regulation of transcription, DNA-dependent	bZIP a,b,c	0	0	
AT5G62920	RESPONSE REGULATOR 6 (ARR6)	Orphans a	0	0	
AT3G54390	INVOLVED IN: regulation of transcription	TRIHELIX a,b	0	0	
AT1G30650	protein_coding	WRKY a,b,c	0	0	
AT5G60200	TARGET OF MONOPTEROS 6 (TMO6)	C2C2-DOF a,b,c	0	0	
AT5G65410	HOMEOBOX PROTEIN 25 (HB25)	zf-HD a,b	0	0	
AT3G50750	INVOLVED IN: biological_process unknown	BES1 a,b	0	0	
AT3G46640	PHYTOCLOCK 1 (PCL1)	G2-like a,b	0	0	
AT1G72830	NUCLEAR FACTOR Y, SUBUNIT A3 (NF-YA3)	CCAAT a / CCAAT-HAP2 b / CBFB_NFYA c	0	0	
AT5G41570	protein_coding	WRKY a,b,c	0	0	
AT5G43700	AUXIN INDUCIBLE 2-11 (ATAUX2-11)	AUX-IAA a,b	0	0	

Gene identifier (AGI)	Primary Gene Symbol	TF family	1 mM 3AT	5 mM 3AT	visual in-spec-tion
AT5G24110	protein_coding	WRKY a,b,c	0	0	
AT3G45610	BEST Arabidopsis thaliana protein match is: TARGET OF MONOPTEROS 6 (TAIR:AT5G60200.1)	C2C2-DOF a,b,c	0	0	
AT2G38340	DEHYDRATION RESPONSE ELEMENT-BINDING PROTEIN 19 (DREB19)	AP2-EREBP a,b,c	0	0	
AT4G11660	(AT-HSFB2B)	HSF a,b,c	0	0	
AT1G14900	HIGH MOBILITY GROUP A (HMGA)	ND	0	0	
AT1G26260	CRYPTOCHROME-INTERACTING BASIC-HELIX-LOOP-HELIX 5 (CIB5)	bHLH a,b	0	0	
AT3G49940	BEST Arabidopsis thaliana protein match is: LOB domain-containing protein 37 (TAIR:AT5G67420.1)	LOB a / AS2 b	0	0	
AT1G66470	BEST Arabidopsis thaliana protein match is: RHD SIX-LIKE 1 (TAIR:AT5G37800.1)	bHLH a,b	0	0	
AT5G07680	INVOLVED IN: multicellular organismal development, regulation of transcription	NAC a,b	0	0	
AT5G24590	TCV-INTERACTING PROTEIN (TIP)	NAC a,b	0	0	
AT5G63790	NAC DOMAIN CONTAINING PROTEIN 102 (NAC102)	NAC a,b	0	0	
AT2G30250	protein_coding	WRKY a,b,c	0	0	
AT4G01580	INVOLVED IN: regulation of transcription, DNA-dependent	ABI3-VP1 a,b	0	0	
AT4G00850	GRF1-INTERACTING FACTOR 3 (GIF3)	GIF b	0	0	
AT5G19280	KINASE ASSOCIATED PROTEIN PHOSPHATASE (KAPP)	FHA a,b	0	0	
AT3G62690	ATL5 (ATL5)	ND	0	0	
AT3G09370	MYB DOMAIN PROTEIN 3R-3 (MYB3R-3)	MYB a,b	0	0	
AT2G45050	GATA TRANSCRIPTION FACTOR 2 (GATA2)	C2C2-GATA a,b	0	0	
AT1G76710	INVOLVED IN: vegetative to reproductive phase transition of meristem, histone methylation	SET a / PcG b	0	0	
AT2G17560	HIGH MOBILITY GROUP B4 (HMGB4)	HMG a,b	0	0	
AT2G01650	PLANT UBX DOMAIN-CONTAINING PROTEIN 2 (PUX2)	C2H2 b	0	0	
AT3G47640	POPEYE (PYE)	bHLH a	0	0	
AT3G03750	INVOLVED IN: chromatin modification	SET a / PcG b	0	0	
AT4G11070	protein_coding	WRKY a,b,c	0	0	
AT5G29000	INVOLVED IN: regulation of transcription	G2-like a,b	0	0	
AT4G38960	INVOLVED IN: regulation of transcription	Orphans a	0	0	
AT3G20670	HISTONE H2A 13 (HTA13)	CCAAT a	0	0	
AT4G34410	REDOX RESPONSIVE TRANSCRIPTION FACTOR 1 (RRTF1)	AP2-EREBP a,b,c	0	0	
AT2G02060	BEST Arabidopsis thaliana protein match is: Homeodomain-like superfamily protein (TAIR:AT1G14600.1)	G2-like a,b	0	0	
AT4G09180	INVOLVED IN: regulation of transcription	bHLH a,b	0	0	
AT4G25400	INVOLVED IN: regulation of transcription	bHLH a,b	0	0	
AT4G28110	MYB DOMAIN PROTEIN 41 (MYB41)	MYB a,b	0	0	
AT2G22850	INVOLVED IN: regulation of transcription, DNA-dependent	bZIP a,b,c	0	0	
AT1G69490	NAC-LIKE, ACTIVATED BY AP3/PI (NAP)	NAC a,b	0	0	
AT3G53200	MYB DOMAIN PROTEIN 27 (MYB27)	MYB a,b	0	0	
AT5G59820	RESPONSIVE TO HIGH LIGHT 41 (RHL41)	C2H2 a,b	0	0	
AT3G47120	INVOLVED IN: biological_process unknown	C3H a,b	0	0	
AT4G31420	INVOLVED IN: regulation of transcription	C2H2 b	0	0	
AT1G22190	0	AP2-EREBP a,b,c	0	0	
AT1G80840	WRKY DNA-BINDING PROTEIN 40 (WRKY40)	WRKY a,b,c	0	0	
AT1G46480	WUSCHEL RELATED HOMEOBOX 4 (WOX4)	HB a,b,c	0	0	
AT1G67100	BEST Arabidopsis thaliana protein match is: LOB domain-containing protein 41 (TAIR:AT3G02550.1)	LOB a / AS2 b	0	0	
AT2G46870	BEST Arabidopsis thaliana protein match is: AP2/B3-like transcriptional factor family protein (TAIR:AT3G61970.1)	ABI3-VP1 a,b	0	0	
AT5G66320	GATA TRANSCRIPTION FACTOR 5 (GATA5)	C2C2-GATA a,b	0	0	
AT4G11080	3XHIGH MOBILITY GROUP-BOX1 (3xHMG-box1)	HMG a,b	0	0	
AT1G78080	RELATED TO AP2 4 (RAP2.4)	AP2-EREBP a,b,c	0	0	
AT2G37000	INVOLVED IN: regulation of transcription	TCP a,b,c	0	0	
AT3G21150	B-BOX DOMAIN PROTEIN 32 (BBX32)	Orphans a	0	0	
AT3G16770	ETHYLENE-RESPONSIVE ELEMENT BINDING PROTEIN (EBP)	AP2-EREBP a,b,c	0	0	
AT1G54330	INVOLVED IN: multicellular organismal development, regulation of transcription	NAC a,b	0	0	
AT1G08000	GATA TRANSCRIPTION FACTOR 10 (GATA10)	C2C2-GATA a,b	0	0	
AT1G53170	ETHYLENE RESPONSE FACTOR 8 (ERF8)	AP2-EREBP a,b,c	0	0	
AT1G26960	HOMEOBOX PROTEIN 23 (AtHB23)	HB a,b	0	0	
AT4G37790	(HAT22)	HB a,b	0	0	
AT2G34600	BEST Arabidopsis thaliana protein match is: jasmonate-zim-domain protein 8 (TAIR:AT1G30135.1)	ZIM b,c	0	0	
AT1G51060	HISTONE H2A 10 (HTA10)	CCAAT a	0	0	
AT5G13080	WRKY DNA-BINDING PROTEIN 75 (WRKY75)	WRKY a,b,c	0	0	
AT1G52830	INDOLE-3-ACETIC ACID 6 (IAA6)	AUX-IAA a,b	0	0	
AT3G10595	INVOLVED IN: regulation of transcription, DNA-dependent	MYB a,b	0	0	
AT3G60670	BEST Arabidopsis thaliana protein match is: PLATZ transcription factor family protein (TAIR:AT2G12646.1)	PLATZ a,b	0	0	

Gene identifier (AGI)	Primary Gene Symbol	TF family	1 mM 3AT	5 mM 3AT	visual in-spec-tion
AT2G40470	BEST Arabidopsis thaliana protein match is: LOB domain-containing protein 13 (TAIR:AT2G30340.1)	LOB a / AS2 b	0	0	
AT5G55690	INVOLVED IN: regulation of transcription, DNA-dependent	MADS a,b	0	0	
AT3G17600	INDOLE-3-ACETIC ACID INDUCIBLE 31 (IAA31)	AUX-IAA a,b	0	0	
AT1G71692	AGAMOUS-LIKE 12 (AGL12)	MADS a,b	0	0	
AT5G21960	0	AP2-EREBP a,b,c	0	0	
AT1G51190	PLETHORA 2 (PLT2)	AP2-EREBP a,b,c	0	0	
AT5G45710	ROOT HANDEDNESS 1 (RHA1)	HSF a,b,c	0	0	
AT3G53310	INVOLVED IN: regulation of transcription, DNA-dependent	ABI3-VP1 a,b	0	0	
AT5G46710	BEST Arabidopsis thaliana protein match is: PLATZ transcription factor family protein (TAIR:AT4G17900.1)	PLATZ a,b	0	0	
AT5G65130	0	AP2-EREBP a,b,c	0	0	
AT4G00220	JAGGED LATERAL ORGANS (JLO)	LOB a / AS2 b	0	0	
AT1G22640	MYB DOMAIN PROTEIN 3 (MYB3)	MYB a,b	0	0	
AT3G04070	INVOLVED IN: multicellular organismal development, regulation of transcription	NAC a,b	0	0	
AT1G31320	BEST Arabidopsis thaliana protein match is: ASYMMETRIC LEAVES 2-like 9 (TAIR:AT1G16530.1)	LOB a / AS2 b	0	0	
AT5G46590	INVOLVED IN: multicellular organismal development, regulation of transcription	NAC a,b	0	0	
AT4G39780	0	AP2-EREBP a,b,c	0	0	
AT2G13570	INVOLVED IN: regulation of transcription, DNA-dependent	CCAAT a / CCAAT-HAP3 b	0	0	
AT2G30340	INVOLVED IN: biological_process unknown	LOB a / AS2 b	0	0	
AT1G54690	-H2AX foci throughout the chromatin. However, their accumulation is not contemporaneous with that of AtSPO11-1. At 3 h post-S, no #947	CCAAT a	0	0	
AT4G17810	INVOLVED IN: regulation of transcription	C2H2 a,b,c	0	0	
AT3G49950	BEST Arabidopsis thaliana protein match is: GRAS family transcription factor (TAIR:AT5G67411.1)	GRAS a,b	0	0	
AT5G44210	ERF DOMAIN PROTEIN 9 (ERF9)	AP2-EREBP a,b,c	0	0	
AT1G71130	CYTOKININ RESPONSE FACTOR 8 (CRF8)	AP2-EREBP a,b,c	0	0	
AT4G24540	protein_coding	MADS a,b	0	0	
AT2G33310	AUXIN-INDUCED PROTEIN 13 (IAA13)	AUX-IAA a,b	0	0	
AT3G23030	INDOLE-3-ACETIC ACID INDUCIBLE 2 (IAA2)	AUX-IAA a,b	0	0	
AT4G35570	HIGH MOBILITY GROUP B5 (HMGB5)	HMG a,b	0	0	
AT1G62990	KNOTTED-LIKE HOMEOBOX OF ARABIDOPSIS THALIANA 7 (KNAT7)	HB a / KNOX2 c	0	0	
AT4G25440	INVOLVED IN: biological_process unknown	C3H a,b	0	0	
AT1G74430	MYB DOMAIN PROTEIN 95 (MYB95)	MYB a,b	0	0	
AT1G04240	SHORT HYPOCOTYL 2 (SHY2)	AUX-IAA a,b	0	0	
AT4G29080	PHYTOCHROME-ASSOCIATED PROTEIN 2 (PAP2)	AUX-IAA a,b	0	0	
AT1G49480	RELATED TO VERNALIZATION1 1 (RTV1)	ABI3-VP1 a,b	0	0	
AT5G23405	INVOLVED IN: regulation of transcription, DNA-dependent	HMG a,b	0	0	
AT5G59570	BROTHER OF LUX ARRHYTHMO (BOA)	G2-like a,b	0	0	
AT5G46690	INVOLVED IN: regulation of transcription	bHLH a,b	0	0	
AT1G03800	ERF DOMAIN PROTEIN 10 (ERF10)	AP2-EREBP a,b,c	0	0	
AT2G35000	ARABIDOPSIS TOXICOS EN LEVADURA 9 (ATL9)	ND	0	0	
AT4G16750	0	AP2-EREBP a,b,c	0	0	
AT3G15540	INDOLE-3-ACETIC ACID INDUCIBLE 19 (IAA19)	AUX-IAA a,b	0	0	
AT1G29280	protein_coding	WRKY a,b,c	0	0	
AT4G28190	ULTRAPETALA1 (ULT1)	ULT a,b	0	0	
AT3G09230	MYB DOMAIN PROTEIN 1 (MYB1)	MYB a,b	0	0	
AT5G15210	HOMEOBOX PROTEIN 30 (HB30)	zf-HD a,b	0	0	
AT2G35310	INVOLVED IN: regulation of transcription, DNA-dependent	ABI3-VP1 a,b	0	0	
AT1G01520	BEST Arabidopsis thaliana protein match is: Homeodomain-like superfamily protein (TAIR:AT4G01280.1)	MYB-related a,b	0	0	
AT5G26950	INVOLVED IN: regulation of transcription, DNA-dependent	MADS a,b	0	0	
AT4G13620	0	AP2-EREBP a,b,c	0	0	
AT3G53600	INVOLVED IN: response to chitin, regulation of transcription	C2H2 a,b,c	0	0	
AT5G51780	INVOLVED IN: regulation of transcription	bHLH a,b	0	0	
AT1G14580	LOCATED IN: intracellular	C2H2 a,b,c	0	0	
AT4G38000	BEST Arabidopsis thaliana protein match is: DOF zinc finger protein 1 (TAIR:AT1G51700.1)	C2C2-DOF a,b,c	0	0	
AT2G31220	INVOLVED IN: regulation of transcription	bHLH a,b	0	0	
AT1G19210	0	AP2-EREBP a,b,c	0	0	
AT1G57560	MYB DOMAIN PROTEIN 50 (MYB50)	MYB a,b	0	0	
AT1G29160	BEST Arabidopsis thaliana protein match is: Dof-type zinc finger DNA-binding family protein (TAIR:AT2G34140.1)	C2C2-DOF a,b,c	0	0	
AT5G06950	(AHBP-1B)	bZIP a,b,c	0	0	
AT1G17460	TRF-LIKE 3 (TRFL3)	MYB-related a,b	0	0	
AT2G44940	0	AP2-EREBP a,b,c	0	0	
AT3G50700	INDETERMINATE(ID)-DOMAIN 2 (IDD2)	C2H2 a,b,c	0	0	
AT3G23250	MYB DOMAIN PROTEIN 15 (MYB15)	MYB a,b	0	0	
AT1G13600	INVOLVED IN: regulation of transcription, DNA-dependent	bZIP a,b,c	0	0	

Gene identifier (AGI)	Primary Gene Symbol	TF family	1 mM 3AT	5 mM 3AT	visual in-spec-tion
AT5G50480	INVOLVED IN: regulation of transcription, DNA-dependent	CCAAT a / CCAAT-HAP5 b	0	0	
AT1G45249	ABSCISIC ACID RESPONSIVE ELEMENTS-BINDING FACTOR 2 (ABF2)	bZIP a,b,c	0	0	
AT2G36270	ABA INSENSITIVE 5 (ABI5)	bZIP a,b,c	0	0	
AT5G61600	ETHYLENE RESPONSE FACTOR 104 (ERF104)	AP2-EREBP a,b,c	0	0	
AT4G14560	INDOLE-3-ACETIC ACID INDUCIBLE (IAA1)	AUX-IAA a,b	0	0	
AT1G49720	ABSCISIC ACID RESPONSIVE ELEMENT-BINDING FACTOR 1 (ABF1)	bZIP a,b,c	0	0	
AT2G46770	NAC SECONDARY WALL THICKENING PROMOTING FACTOR1 (NST1)	NAC a,b	0	0	
AT2G35530	BASIC REGION/LEUCINE ZIPPER TRANSCRIPTION FACTOR 16 (bZIP16)	bZIP a,b	0	0	
AT5G43290	protein_coding	WRKY a,b,c	0	0	
AT1G73830	INVOLVED IN: regulation of transcription	bHLH a,b	0	0	
AT1G08010	GATA TRANSCRIPTION FACTOR 11 (GATA11)	C2C2-GATA a,b	0	0	
AT5G67110	ALCATRAZ (ALC)	bHLH a,b	0	0	
AT1G66230	MYB DOMAIN PROTEIN 20 (MYB20)	MYB a,b	0	0	
AT1G02210	INVOLVED IN: regulation of transcription	NAC b	0	0	
AT1G62975	INVOLVED IN: regulation of transcription	bHLH a,b	0	0	
AT1G71260	WHIRLY 2 (ATWHY2)	PBF-2-like a	0	0	
AT2G35910	CONTAINS InterPro DOMAIN/s: Zinc finger, RING-type (InterPro:IPR001841), Zinc finger, C3HC4 RING-type (InterPro:IPR018957)	ND	0	0	
AT5G18270	INVOLVED IN: multicellular organismal development, regulation of transcription	NAC a,b	0	0	
AT1G16640	INVOLVED IN: regulation of transcription, DNA-dependent	ABI3-VP1 a,b	0	0	
AT3G26744	INDUCER OF CBF EXPRESSION 1 (ICE1)	bHLH a,b	0	0	
AT3G02990	HEAT SHOCK TRANSCRIPTION FACTOR A1E (HSFA1E)	HSF a,b,c	0	0	
AT4G35700	DUO1-ACTIVATED ZINC FINGER 3 (DAZ3)	C2H2 b	0	0	
AT3G43440	BEST Arabidopsis thaliana protein match is: jasmonate-zim-domain protein 12 (TAIR:AT5G20900.1)	ZIM b,c	0	0	
AT5G18450	0	AP2-EREBP a,b,c	0	0	
AT3G62260	INVOLVED IN: protein amino acid dephosphorylation	DBP a	0	0	
AT3G23050	INDOLE-3-ACETIC ACID 7 (IAA7)	AUX-IAA a,b	0	0	
AT5G54640	RESISTANT TO AGROBACTERIUM TRANSFORMATION 5 (RAT5)	CCAAT a	0	0	
AT4G17460	(HAT1)	HB a,b	0	0	
AT3G03760	BEST Arabidopsis thaliana protein match is: lateral organ boundaries-domain 16 (TAIR:AT2G42430.1)	LOB a / AS2 b	0	0	
AT2G21530	LOCATED IN: chloroplast thylakoid membrane, chloroplast stroma, chloroplast	FHA a,b	0	0	
AT1G24580	LOCATED IN: endomembrane system	ND	0	0	
AT2G31180	MYB DOMAIN PROTEIN 14 (MYB14)	MYB a,b	0	0	
AT5G12840	NUCLEAR FACTOR Y, SUBUNIT A1 (NF-YA1)	CCAAT a / CCAAT-HAP2 b / CBFB_NFYA c	0	0	
AT5G06070	RABBIT EARS (RBE)	C2H2 a,b,c	0	0	
AT1G04370	ETHYLENE-RESPONSIVE ELEMENT BINDING FACTOR 14 (ERF14)	AP2-EREBP a,b,c	0	0	
AT1G75490	0	AP2-EREBP a,b,c	0	0	
AT5G05610	ALFIN-LIKE 1 (AL1)	ALFIN-like a,b	0	0	
AT5G15160	BANQUO 2 (BNQ2)	bHLH d	0	0	
AT1G54140	TATA BINDING PROTEIN ASSOCIATED FACTOR 21KDA SUBUNIT (TAFII21)	ND	0	0	
AT2G41070	ENHANCED EM LEVEL (EEL)	bZIP a,b,c	0	0	
AT1G72350	INVOLVED IN: regulation of transcription, DNA-dependent	MADS a,b	0	0	
AT4G23550	(WRKY29)	WRKY a,b,c	0	0	
AT3G06380	TUBBY-LIKE PROTEIN 9 (TLP9)	TUB a,c	0	0	
AT1G14350	FOUR LIPS (FLP)	MYB a,b	0	0	
AT2G27300	BEST Arabidopsis thaliana protein match is: NAC domain containing protein 60 (TAIR:AT3G44290.1)	NAC a,b	0	0	
AT4G18890	BEST Arabidopsis thaliana protein match is: BES1/BZR1 homolog 4 (TAIR:AT1G78700.1)	BES1 a,b	0	0	
AT4G01280	BEST Arabidopsis thaliana protein match is: Homeodomain-like superfamily protein (TAIR:AT1G01520.1)	MYB-related a,b	0	0	
AT3G55080	BEST Arabidopsis thaliana protein match is: Rubisco methyltransferase family protein (TAIR:AT3G07670.1)	SET a / PcG b	0	0	
AT5G53200	BEST Arabidopsis thaliana protein match is: Homeodomain-like superfamily protein (TAIR:AT2G30420.1)	MYB-related a,b	0	0	
AT3G02940	MYB DOMAIN PROTEIN 107 (MYB107)	MYB a,b	0	0	
AT5G14750	MYB DOMAIN PROTEIN 66 (MYB66)	MYB a,b	0	0	
AT3G15170	CUP-SHAPED COTYLEDON1 (CUC1)	NAC a,b	0	0	
AT5G38140	INVOLVED IN: regulation of transcription, DNA-dependent	CCAAT a / CCAAT-HAP5 b	0	0	
AT5G53290	CYTOKININ RESPONSE FACTOR 3 (CRF3)	AP2-EREBP a,b,c	0	0	
AT1G75250	BEST Arabidopsis thaliana protein match is: RAD-like 5 (TAIR:AT1G19510.1)	MYB-related a	0	0	
AT5G62380	NAC-DOMAIN PROTEIN 101 (NAC101)	NAC a,b	0	0	

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AT1G12260	NAC 007 (NAC007)	NAC a,b	0	0	
AT4G02640	(BZO2H1)	bZIP a,b,c	0	0	
AT5G26210	ALFIN-LIKE 4 (AL4)	ALFIN-like a,b	0	0	
AT1G10480	ZINC FINGER PROTEIN 5 (ZFP5)	C2H2 a,b,c	0	0	
AT5G57520	ZINC FINGER PROTEIN 2 (ZFP2)	C2H2 a,b,c	0	0	
AT1G19860	INVOLVED IN: biological_process unknown	C3H a	0	0	
AT3G46090	INVOLVED IN: response to chitin, regulation of transcription	C2H2 a,b	0	0	
AT4G32040	KNOTTED1-LIKE HOMEOBOX GENE 5 (KNAT5)	HB a / KNOX2 c	0	0	
AT5G42630	ABERRANT TESTA SHAPE (ATS)	G2-like a,b	0	0	
AT2G31720	LOCATED IN: cellular_component unknown	ND	0	0	
AT5G43270	SQUAMOSA PROMOTER BINDING PROTEIN-LIKE 2 (SPL2)	SBP a,b,c	0	0	
AT5G24520	TRANSPARENT TESTA GLABRA 1 (TTG1)	ND	0	0	
AT3G01970	protein_coding	WRKY a,b,c	0	0	
AT1G06180	MYB DOMAIN PROTEIN 13 (MYB13)	MYB a,b	0	0	
AT2G41710	INVOLVED IN: regulation of transcription, DNA-dependent	AP2-EREBP a,b,c	0	0	
AT1G09030	INVOLVED IN: regulation of transcription	CCAAT a / CCAAT-HAP3 b	0	0	
AT5G43170	ZINC-FINGER PROTEIN 3 (ZF3)	C2H2 a,b	0	0	
AT1G59940	RESPONSE REGULATOR 3 (ARR3)	Orphans a	0	0	
AT4G11140	CYTOKININ RESPONSE FACTOR 1 (CRF1)	AP2-EREBP a,b,c	0	0	
AT4G00050	INVOLVED IN: double fertilization forming a zygote and endosperm, regulation of transcription	bHLH a,b	0	0	
AT2G04890	SCARECROW-LIKE 21 (SCL21)	GRAS a,b	0	0	
AT5G13910	LEAFY PETIOLE (LEP)	AP2-EREBP a,b,c	0	0	
AT1G01780	PLIM2B (PLIM2b)	LIM a,b	0	0	
AT1G77250	INVOLVED IN: regulation of transcription, DNA-dependent	PHD a,b	0	0	
AT2G24260	LJRHL1-LIKE 1 (LRL1)	bHLH a,b	0	0	
AT2G25620	DNA-BINDING PROTEIN PHOSPHATASE 1 (DBP1)	DBP a	0	0	
AT5G61590	0	AP2-EREBP a,b,c	0	0	
AT5G54070	HEAT SHOCK TRANSCRIPTION FACTOR A9 (HSFA9)	HSF a,b,c	0	0	
AT3G49850	TELOMERE REPEAT BINDING FACTOR 3 (TRB3)	MYB-related a,b	0	0	
AT4G16430	INVOLVED IN: regulation of transcription	bHLH a,b	0	0	
AT1G25580	SUPPRESSOR OF GAMMA RADIATION 1 (SOG1)	NAC a,b	0	0	
AT1G21450	SCARECROW-LIKE 1 (SCL1)	GRAS a,b	0	0	
AT3G02830	protein_coding	C3H a,b	0	0	
AT3G07740	HOMOLOG OF YEAST ADA2 2A (ADA2A)	MYB-related a / SWIRM c	0	0	
AT3G61830	INVOLVED IN: response to hormone stimulus, regulation of transcription, DNA-dependent, regulation of transcription	ARF a,b,c	0	0	
AT5G44080	INVOLVED IN: regulation of transcription, DNA-dependent	bZIP a,b,c	0	0	
AT3G61460	BRASSINOSTEROID-RESPONSIVE RING-H2 (BRH1)	ND	0	0	
AT1G47870	protein_coding	E2F-DP a,b,c	0	0	
AT5G16470	INVOLVED IN: regulation of transcription	C2H2 b	0	0	
AT1G58100	TCP DOMAIN PROTEIN 8 (TCP8)	TCP a,b,c	0	0	
AT4G10350	NAC DOMAIN CONTAINING PROTEIN 70 (NAC070)	NAC a,b	0	0	
AT3G20910	INVOLVED IN: negative regulation of gene-specific transcription, regulation of transcription, DNA-dependent	CCAAT a / CCAAT-HAP2 b / CBFB_NFYA c	0	0	
AT5G56860	GATA, NITRATE-INDUCIBLE, CARBON METABOLISM-INVOLVED (GNC)	C2C2-GATA a,b	0	0	
AT5G66160	RECEPTOR HOMOLOGY REGION TRANSMEMBRANE DOMAIN RING H2 MOTIF PROTEIN 1 (RMR1)	ND	0	0	
AT5G20900	INVOLVED IN: biological_process unknown	ZIM b,c	0	0	
AT3G23690	INVOLVED IN: regulation of transcription	bHLH a,b	0	0	
AT1G74410	INVOLVED IN: response to chitin	ND	0	0	
AT5G23090	INVOLVED IN: regulation of transcription	CCAAT a / CCAAT-Dr1 b	0	0	
AT3G27940	LOCATED IN: cellular_component unknown	LOB a / AS2 b	0	0	
AT4G22950	AGAMOUS-LIKE 19 (AGL19)	MADS a,b	0	0	
AT5G40880	INVOLVED IN: N-terminal protein myristoylation	C3H a,b	0	0	
AT5G07690	MYB DOMAIN PROTEIN 29 (MYB29)	MYB a,b	0	0	
AT2G01930	BASIC PENTACYSTEINE1 (BPC1)	BBR-BPC a,b	0	0	
AT5G08190	INVOLVED IN: regulation of transcription	CCAAT a / CCAAT-Dr1 b	0	0	
AT2G37120	INVOLVED IN: regulation of transcription	S1Fa-like a,b	0	0	
AT5G17240	BEST Arabidopsis thaliana protein match is: SET domain-containing protein (TAIR:AT3G55080.1)	SET a / PcG b	0	0	
AT4G33450	MYB DOMAIN PROTEIN 69 (MYB69)	MYB a,b	0	0	
AT1G05805	INVOLVED IN: regulation of transcription	bHLH a,b	0	0	
AT3G07650	CONSTANS-LIKE 9 (COL9)	C2C2-CO-like a,b	0	0	
AT1G14410	WHIRLY 1 (WHY1)	PBF-2-like a / WHIRLY b	0	0	
AT4G00270	INVOLVED IN: biological_process unknown	GeBP a,b	0	0	
AT2G31210	INVOLVED IN: regulation of transcription	bHLH a,b	0	0	

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AT5G56900	INVOLVED IN: biological_process unknown	C3H a,b	0	0	
AT3G61970	BEST Arabidopsis thaliana protein match is: AP2/B3-like transcriptional factor family protein (TAIR:AT2G46870.1)	ABI3-VP1 a,b	0	0	
AT2G33860	ETTIN (ETT)	ARF a,b,c	0	0	
AT4G20280	TBP-ASSOCIATED FACTOR 11 (TAF11)	ND	0	0	
AT2G42040	Bacteria - 0	Orphans a	0	0	
AT1G19510	INVOLVED IN: regulation of transcription, DNA-dependent	MYB-related a	0	0	
AT1G67030	ZINC FINGER PROTEIN 6 (ZFP6)	C2H2 b	0	0	
AT2G34140	INVOLVED IN: regulation of transcription	C2C2-DOF a,b,c	0	0	
AT3G11280	protein_coding	MYB a,b	0	0	
AT1G43700	VIRE2-INTERACTING PROTEIN 1 (VIP1)	bZIP a,b,c	0	0	
AT3G54430	SHI-RELATED SEQUENCE 6 (SRS6)	SRS a,b	0	0	
AT5G17490	RGA-LIKE PROTEIN 3 (RGL3)	GRAS a,b	0	0	
AT1G28370	ERF DOMAIN PROTEIN 11 (ERF11)	AP2-EREBP a,b,c	0	0	
AT2G22670	INDOLEACETIC ACID-INDUCED PROTEIN 8 (IAA8)	AUX-IAA a,b	0	0	
AT2G03710	SEPALLATA 4 (SEP4)	MADS a,b	0	0	
AT3G57800	INVOLVED IN: regulation of transcription	bHLH a,b	0	0	
AT1G14030	LYSINE METHYLTRANSFERASE (LSMT)-LIKE (LSMT-L)	SET a / PcG b	0	0	
AT2G45680	BEST Arabidopsis thaliana protein match is: TCP family transcription factor (TAIR:AT5G51910.2)	TCP a,b,c	0	0	
AT3G01330	DP-E2F-LIKE PROTEIN 3 (DEL3)	E2F-DP a,b,c	0	0	
AT5G25890	INDOLE-3-ACETIC ACID INDUCIBLE 28 (IAA28)	AUX-IAA a,b	0	0	
AT2G19810	OXIDATION-RELATED ZINC FINGER 1 (OZF1)	C3H a,b	0	0	
AT4G17500	ETHYLENE RESPONSIVE ELEMENT BINDING FACTOR 1 (ERF-1)	AP2-EREBP a,b,c	0	0	
AT3G28920	INVOLVED IN: regulation of transcription	zf-HD a,b	0	0	
AT2G24500	(FZF)	C2H2 b	0	0	
AT1G06850	INVOLVED IN: regulation of transcription, DNA-dependent	bZIP a,b,c	0	0	
AT2G31070	TCP DOMAIN PROTEIN 10 (tcp10)	TCP a,b,c	0	0	
AT1G28360	ERF DOMAIN PROTEIN 12 (ERF12)	AP2-EREBP a,b,c	0	0	
AT4G36990	HEAT SHOCK FACTOR 4 (HSF4)	HSF a,b,c	0	0	
AT4G40060	HOMEOBOX PROTEIN 16 (HB16)	HB a,b	0	0	
AT4G27900	INVOLVED IN: biological_process unknown	Orphans a / C2C2-CO-like b	0	0	
AT2G40140	BEST Arabidopsis thaliana protein match is: salt-inducible zinc finger 1 (TAIR:AT3G55980.1)	C3H a,b	0	0	
AT4G05100	MYB DOMAIN PROTEIN 74 (MYB74)	MYB a,b	0	0	
AT4G28610	PHOSPHATE STARVATION RESPONSE 1 (PHR1)	G2-like a,b	0	0	
AT5G66750	CHROMATIN REMODELING 1 (CHR1)	SNF2 a	0	0	
AT3G61890	HOMEOBOX 12 (HB-12)	HB a,b	0	0	
AT5G04410	NAC DOMAIN CONTAINING PROTEIN 2 (NAC2)	NAC a,b	0	0	
AT1G54830	INVOLVED IN: regulation of transcription, DNA-dependent	CCAAT a / CCAAT-HAP5 b	0	0	
AT1G18710	MYB DOMAIN PROTEIN 47 (MYB47)	MYB a,b	0	0	
AT5G12870	MYB DOMAIN PROTEIN 46 (MYB46)	MYB a,b	0	0	
AT2G33710	0	AP2-EREBP a,b,c	0	0	
AT4G01460	INVOLVED IN: regulation of transcription	bHLH a,b	0	0	
AT1G68520	INVOLVED IN: regulation of transcription	C2C2-CO-like a,b	0	0	
AT2G34720	BEST Arabidopsis thaliana protein match is: nuclear factor Y, subunit A7 (TAIR:AT1G30500.2)	CCAAT a / CCAAT-HAP2 b / CBFB_NFYA c	0	0	
AT1G74930	(ORA47)	AP2-EREBP a,b,c	0	0	
AT3G55730	MYB DOMAIN PROTEIN 109 (MYB109)	MYB a,b	0	0	
AT5G66730	INVOLVED IN: regulation of transcription	C2H2 a,b	0	0	
AT1G56170	NUCLEAR FACTOR Y, SUBUNIT C2 (NF-YC2)	CCAAT a / CCAAT-HAP5 b	0	0	
AT3G16500	PHYTOCHROME-ASSOCIATED PROTEIN 1 (PAP1)	AUX-IAA a,b	0	0	
AT2G24790	CONSTANS-LIKE 3 (COL3)	C2C2-CO-like a,b	0	0	
AT5G53420	INVOLVED IN: biological_process unknown	Orphans a / C2C2-CO-like b	0	0	
AT5G49450	BASIC LEUCINE-ZIPPER 1 (bZIP1)	bZIP a,b,c	0	0	
AT4G01680	MYB DOMAIN PROTEIN 55 (MYB55)	MYB a,b	0	0	
AT5G63470	BEST Arabidopsis thaliana protein match is: nuclear factor Y, subunit C1 (TAIR:AT3G48590.1)	CCAAT a / CCAAT-HAP5 b	0	0	
AT3G28910	MYB DOMAIN PROTEIN 30 (MYB30)	MYB a,b	0	0	
AT2G21230	INVOLVED IN: regulation of transcription, DNA-dependent	bZIP a,b,c	0	0	
AT4G36240	GATA TRANSCRIPTION FACTOR 7 (GATA7)	C2C2-GATA a,b	0	0	
AT5G65210	(TGA1)	bZIP a,b,c	0	0	
AT3G48590	NUCLEAR FACTOR Y, SUBUNIT C1 (NF-YC1)	CCAAT a / CCAAT-HAP5 b	0	0	
AT5G54230	MYB DOMAIN PROTEIN 49 (MYB49)	MYB a,b	0	0	
AT3G47500	CYCLING DOF FACTOR 3 (CDF3)	C2C2-DOF a,b,c	0	0	
AT5G58850	MYB DOMAIN PROTEIN 119 (MYB119)	MYB a,b	0	0	
AT2G20825	INVOLVED IN: biological_process unknown	ULT a,b	0	0	

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AT5G39860	PACLOBUTRAZOL RESISTANCE1 (PRE1)	bHLH d	0	0	
AT2G16210	INVOLVED IN: regulation of transcription, DNA-dependent	ABI3-VP1 a,b	0	0	
AT1G75520	SHI-RELATED SEQUENCE 5 (SRS5)	SRS a,b	0	0	
AT3G16160	BEST Arabidopsis thaliana protein match is: Tesmin/TSO1-like CXC domain-containing protein (TAIR:AT5G25790.1)	CPP a,b	0	0	
AT2G01370	BEST Arabidopsis thaliana protein match is: DNA-binding storekeeper protein-related transcriptional regulator (TAIR:AT1G55950.1)	GeBP a,b	0	0	
AT1G68240	INVOLVED IN: regulation of transcription	bHLH a,b	0	0	
AT1G19790	SHI-RELATED SEQUENCE 7 (SRS7)	SRS a,b	0	0	
AT4G18770	MYB DOMAIN PROTEIN 98 (MYB98)	MYB a,b	0	0	
AT4G00130	LOCATED IN: mitochondrion	GeBP a,b	0	0	
AT3G06120	MUTE (MUTE)	bHLH a,b	0	0	
AT4G01260	BEST Arabidopsis thaliana protein match is: DNA-binding storekeeper protein-related transcriptional regulator (TAIR:AT4G25210.1)	GeBP a,b	0	0	
AT4G09820	TRANSPARENT TESTA 8 (TT8)	bHLH a,b	0	0	
AT4G00232	BEST Arabidopsis thaliana protein match is: DNA-binding storekeeper protein-related transcriptional regulator (TAIR:AT5G41765.1)	GeBP a,b	0	0	
AT1G68200	BEST Arabidopsis thaliana protein match is: Zinc finger C-x8-C-x5-C-x3-H type family protein (TAIR:AT1G66810.1)	C3H a,b	0	0	
AT1G72570	BEST Arabidopsis thaliana protein match is: AINTEGUMENTA-like 5 (TAIR:AT5G57390.1)	AP2-EREBP a,b,c	0	0	
AT1G77980	AGAMOUS-LIKE 66 (AGL66)	MADS a,b	0	0	
AT1G74480	BEST Arabidopsis thaliana protein match is: RWP-RK domain-containing protein (TAIR:AT1G18790.1)	RWP-RK a / NIN-like b	0	0	
AT1G59530	INVOLVED IN: regulation of transcription, DNA-dependent	bZIP a,b,c	0	0	
AT2G27220	INVOLVED IN: regulation of transcription, DNA-dependent, regulation of transcription	HB a,b	0	0	
AT1G77200	0	AP2-EREBP a,b,c	0	0	
AT4G28500	BEST Arabidopsis thaliana protein match is: NAC domain containing protein 10 (TAIR:AT1G28470.1)	NAC a,b	0	0	
AT5G09780	INVOLVED IN: regulation of transcription, DNA-dependent	ABI3-VP1 a,b	0	0	
AT1G25330	CESTA (CES)	bHLH a,b	0	0	
AT5G60130	INVOLVED IN: regulation of transcription, DNA-dependent	ABI3-VP1 a,b	0	0	
AT1G12630	0	AP2-EREBP a,b,c	0	0	
AT3G19184	INVOLVED IN: regulation of transcription, DNA-dependent	ABI3-VP1 a,b	0	0	
AT4G36590	INVOLVED IN: regulation of transcription, DNA-dependent	MADS a,b	0	0	
AT2G18328	EXPRESSED IN: rosette leaf	MYB-related a	0	0	
AT5G07700	MYB DOMAIN PROTEIN 76 (MYB76)	MYB a,b	0	0	
AT5G67411	BEST Arabidopsis thaliana protein match is: GRAS family transcription factor (TAIR:AT3G49950.1)	GRAS a,b	0	0	
AT2G12646	BEST Arabidopsis thaliana protein match is: PLATZ transcription factor family protein (TAIR:AT3G60670.1)	PLATZ a,b	0	0	
AT5G04150	INVOLVED IN: regulation of transcription	bHLH a,b	0	0	
AT5G43840	HEAT SHOCK TRANSCRIPTION FACTOR A6A (HSFA6A)	HSF a,b,c	0	0	
AT5G51990	C-REPEAT-BINDING FACTOR 4 (CBF4)	AP2-EREBP a,b,c	0	0	
AT5G25470	INVOLVED IN: regulation of transcription, DNA-dependent	ABI3-VP1 b / B3 c	0	0	
AT5G35900	LOCATED IN: endomembrane system	LOB a / AS2 b	0	0	
AT2G46970	PHYTOCHROME INTERACTING FACTOR 3-LIKE 1 (PIL1)	bHLH a,b	0	0	
AT5G58340	INVOLVED IN: regulation of transcription	MYB-related a,b	0	0	
AT4G22700	BEST Arabidopsis thaliana protein match is: LOB domain-containing protein 35 (TAIR:AT5G35900.1)	LOB a / AS2 b	0	0	
AT2G36340	BEST Arabidopsis thaliana protein match is: DNA-binding storekeeper protein-related transcriptional regulator (TAIR:AT2G25650.1)	GeBP a,b	0	0	
AT3G56520	INVOLVED IN: multicellular organismal development, regulation of transcription	NAC b	0	0	
AT3G26620	BEST Arabidopsis thaliana protein match is: LOB domain-containing protein 24 (TAIR:AT3G26660.1)	LOB a / AS2 b	0	0	
AT5G23650	BEST Arabidopsis thaliana protein match is: Duplicated homeodomain-like superfamily protein (TAIR:AT5G08520.1)	MYB a,b	0	0	
AT5G15480	INVOLVED IN: regulation of transcription	C2H2 a,b,c	0	0	
AT5G50470	INVOLVED IN: regulation of transcription, DNA-dependent	CCAAT a / CCAAT-HAP5 b	0	0	
AT1G10200	WLIM1 (WLIM1)	LIM a,b	0	0	
AT3G44750	HISTONE DEACETYLASE 3 (HDA3)	C2H2 b	0	0	
AT2G18350	INVOLVED IN: regulation of transcription	zf-HD a,b	0	0	
AT3G44785	INVOLVED IN: biological_process unknown	C3H a	0	0	
AT5G18300	INVOLVED IN: multicellular organismal development, regulation of transcription	NAC a,b	0	0	
AT5G43540	INVOLVED IN: regulation of transcription	C2H2 a,b,c	0	0	
AT1G07050	BEST Arabidopsis thaliana protein match is: CCT motif family protein (TAIR:AT4G25990.1)	Orphans a / C2C2-CO-like b	0	0	
AT1G47655	BEST Arabidopsis thaliana protein match is: Dof-type zinc finger DNA-binding family protein (TAIR:AT5G66940.1)	C2C2-DOF a,b,c	0	0	

Gene identifier (AGI)	Primary Gene Symbol	TF family	1 mM 3AT	5 mM 3AT	visual in-spec-tion
AT1G49475	INVOLVED IN: regulation of transcription, DNA-dependent	ABI3-VP1 a,b	0	0	
AT1G04445	INVOLVED IN: biological_process unknown	C2H2 b	0	0	
AT1G10470	RESPONSE REGULATOR 4 (ARR4)	Orphans a	0	0	
AT2G40670	RESPONSE REGULATOR 16 (RR16)	Orphans a	0	0	
AT4G17900	BEST Arabidopsis thaliana protein match is: PLATZ transcription factor family protein (TAIR:AT1G32700.1)	PLATZ a,b	0	0	
AT5G09240	INVOLVED IN: regulation of transcription, DNA-dependent	Coactivator p15 a	0	0	
AT1G03150	INVOLVED IN: metabolic process	GNAT a	0	0	
AT1G03650	INVOLVED IN: metabolic process	GNAT a	0	0	
AT1G24040	INVOLVED IN: metabolic process	GNAT a	0	0	
AT1G26220	INVOLVED IN: metabolic process	GNAT a	0	0	
AT2G32030	INVOLVED IN: metabolic process	GNAT a	0	0	
AT5G11340	INVOLVED IN: metabolic process	GNAT a	0	0	
AT5G15770	GLUCOSE-6-PHOSPHATE ACETYLTRANSFERASE 1 (GNA1)	GNAT a	0	0	
AT3G22560	INVOLVED IN: metabolic process	GNAT a	0	0	
AT1G54760	INVOLVED IN: regulation of transcription	ND	0	0	
AT5G03500	INVOLVED IN: regulation of transcription from RNA polymerase II promoter	MED7 a	0	0	
AT2G30424	TRICHOMELESS 2 (TCL2)	MYB-related a,c	0	0	
AT3G17730	INVOLVED IN: multicellular organismal development, regulation of transcription	NAC a,b	0	0	
AT5G64530	BEST Arabidopsis thaliana protein match is: NAC domain containing protein 25 (TAIR:AT1G61110.1)	NAC a,b	0	0	
AT5G19910	INVOLVED IN: regulation of transcription	SOH1 a	0	0	
AT2G14880	INVOLVED IN: biological_process unknown	SWI/SNF-BAF60b a	0	0	
AT2G35605	BEST Arabidopsis thaliana protein match is: SWIB/MDM2 domain superfamily protein (TAIR:AT1G31760.1)	SWI/SNF-BAF60b a	0	0	
AT3G48600	INVOLVED IN: biological_process unknown	SWI/SNF-BAF60b a	0	0	
AT1G12440	INVOLVED IN: biological_process unknown	zf-AN1 c	0	0	
AT3G28917	BEST Arabidopsis thaliana protein match is: mini zinc finger (TAIR:AT1G18835.1)	zf-HD a,b	0	0	
AT1G74950	BEST Arabidopsis thaliana protein match is: jasmonate-zim-domain protein 1 (TAIR:AT1G19180.1)	ZIM b,c	0	0	
AT1G26945	KIDARI (KDR)	ND	0	0	
AT1G51200	INVOLVED IN: biological_process unknown	zf-AN1 c	0	0	
AT1G60240	BEST Arabidopsis thaliana protein match is: NAC (No Apical Meristem) domain transcriptional regulator superfamily protein (TAIR:AT1G60340.1)	ND	0	0	
AT1G32540	LSD ONE LIKE 1 (LOL1)	zf-LSD1 c	0	0	
AT3G14700	BEST Arabidopsis thaliana protein match is: SART-1 family (TAIR:AT5G16780.1)	SART-1 c	0	0	
AT3G28857	PACLOBUTRAZOL RESISTANCE 5 (PRE5)	HLH c	0	0	
AT3G46580	METHYL-CPG-BINDING DOMAIN PROTEIN 5 (MBD5)	MBD c	0	0	
AT4G19630	INVOLVED IN: regulation of transcription, DNA-dependent	ND	0	0	
AT4G20380	LESION SIMULATING DISEASE (LSD1)	zf-LSD1 c	0	0	
AT4G22745	METHYL-CPG-BINDING DOMAIN 1 (MBD1)	MBD c	0	0	
AT4G22820	INVOLVED IN: biological_process unknown	zf-AN1 c	0	0	
AT4G00416	METHYL-CPG-BINDING DOMAIN 3 (MBD3)	ND	0	0	
AT4G12040	INVOLVED IN: biological_process unknown	zf-AN1 c	0	0	
AT5G18037	INVOLVED IN: regulation of transcription	ND	0	0	
AT5G27810	INVOLVED IN: regulation of transcription, DNA-dependent	ND	0	0	
AT5G27944	INVOLVED IN: regulation of transcription, DNA-dependent	MADS a / SRF-TF c	0	0	
AT5G59800	METHYL-CPG-BINDING DOMAIN 7 (MBD7)	ND	0	0	
AT2G44745	BEST Arabidopsis thaliana protein match is: WRKY DNA-binding protein 13 (TAIR:AT4G39410.1)	WRKY a,b,c	0	0	
AT5G52830	WRKY DNA-BINDING PROTEIN 27 (WRKY27)	WRKY a,b,c	0	0	
AT2G37260	protein_coding	WRKY a,b,c	0	0	
AT1G48150	INVOLVED IN: regulation of transcription, DNA-dependent	MADS a,b	0	0	
AT1G65300	AGAMOUS-LIKE 38 (AGL38)	MADS a,b	0	0	
AT2G14210	AGAMOUS-LIKE 44 (AGL44)	MADS a,b	0	0	
AT1G28450	INVOLVED IN: regulation of transcription, DNA-dependent	MADS a,b	0	0	
AT5G48670	AGAMOUS-LIKE 80 (AGL80)	MADS a,b	0	0	
AT1G22590	INVOLVED IN: regulation of transcription	MADS a,b	0	0	
AT3G45170	GATA TRANSCRIPTION FACTOR 14 (GATA14)	C2C2-GATA a,b	0	0	
AT4G24470	ZINC-FINGER PROTEIN EXPRESSED IN INFLORESCENCE MERISTEM (ZIM)	C2C2-GATA a / ZIM b / CCT c	0	0	
AT2G18380	GATA TRANSCRIPTION FACTOR 20 (GATA20)	C2C2-GATA a,b	0	0	
AT1G80730	ZINC-FINGER PROTEIN 1 (ZFP1)	C2H2 a,b,c	0	0	
AT3G23130	SUPERMAN (SUP)	C2H2 a,b,c	0	0	
AT1G02030	INVOLVED IN: regulation of transcription	C2H2 a,b,c	0	0	
AT4G25490	C-REPEAT/DRE BINDING FACTOR 1 (CBF1)	AP2-EREBP a,b,c	0	0	
AT1G03970	G-BOX BINDING FACTOR 4 (GBF4)	bZIP a,b,c	0	0	
AT5G42820	(U2AF35B)	C3H a,b	0	0	
AT1G17380	BEST Arabidopsis thaliana protein match is: jasmonate-zim-domain protein 6 (TAIR:AT1G72450.1)	ZIM b,c	0	0	

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AT3G21175	ZIM-LIKE 1 (ZML1)	C2C2-GATA a / ZIM b / CCT c	0	0	
AT4G36730	G-BOX BINDING FACTOR 1 (GBF1)	bZIP a,b	0	0	
AT1G24610	INVOLVED IN: biological_process unknown	SET a / PcG b	0	0	
AT5G60910	AGAMOUS-LIKE 8 (AGL8)	MADS a,b	0	0	
AT5G61430	INVOLVED IN: multicellular organismal development, regulation of transcription	NAC a,b	0	0	
AT2G19520	(FVE)	ND	0	0	
AT4G23860	INVOLVED IN: biological_process unknown	PHD b	0	0	
AT1G70700	(TIFY7)	ZIM b,c	0	0	
AT1G64860	SIGMA FACTOR A (SIGA)	SIGMA70-like a	0	0	
AT4G01120	G-BOX BINDING FACTOR 2 (GBF2)	bZIP a,b	0	0	
AT4G36860	LOCATED IN: plasma membrane	LIM b	0	0	
AT1G07360	MOS4-ASSOCIATED COMPLEX SUBUNIT 5A (MAC5A)	C3H a	0	0	
AT3G48100	RESPONSE REGULATOR 5 (RR5)	Orphans a	0	0	
AT3G46590	TRF-LIKE 1 (TRFL1)	MYB-related d	0	0	
AT2G45650	AGAMOUS-LIKE 6 (AGL6)	MADS a,b	0	0	
AT5G27580	INVOLVED IN: regulation of transcription, DNA-dependent	MADS a,b	0	0	
AT5G59990	BEST Arabidopsis thaliana protein match is: CCT motif family protein (TAIR:AT5G41380.1)	Orphans a / C2C2-CO-like b	0	0	
AT1G63170	EXPRESSED IN: 24 plant structures	ND	0	0	
AT2G46160	CONTAINS InterPro DOMAIN/s: Zinc finger, RING-type (InterPro:IPR001841), Zinc finger, C3HC4 RING-type (InterPro:IPR018957)	ND	0	0	
AT2G32600	INVOLVED IN: biological_process unknown	C2H2 d	0	0	
AT3G18010	WUSCHEL RELATED HOMEOBOX 1 (WOX1)	HB a,b,c	0	0	
AT1G66380	MYB DOMAIN PROTEIN 114 (MYB114)	MYB a,b	0	0	
AT5G65510	BEST Arabidopsis thaliana protein match is: AINTEGUMENTA-like 6 (TAIR:AT5G10510.2)	AP2-EREBP a,b,c	0	0	
AT2G28810	BEST Arabidopsis thaliana protein match is: Dof-type zinc finger DNA-binding family protein (TAIR:AT1G07640.3)	C2C2-DOF a,b,c	0	0	
AT3G10760	BEST Arabidopsis thaliana protein match is: Homeodomain-like superfamily protein (TAIR:AT5G05090.1)	G2-like a,b	0	0	
AT1G69580	BEST Arabidopsis thaliana protein match is: Homeodomain-like superfamily protein (TAIR:AT3G04030.3)	G2-like a,b	0	0	
AT2G18550	HOMEOBOX PROTEIN 21 (HB21)	HB a,b,c	0	0	
AT5G19790	RELATED TO AP2 11 (RAP2.11)	AP2-EREBP a,b,c	0	0	
AT1G66390	MYB DOMAIN PROTEIN 90 (MYB90)	MYB a,b	0	0	
AT4G23800	3XHIGH MOBILITY GROUP-BOX2 (3xHMG-box2)	HMG a,b	0	0	
AT3G20810	JUMONJI DOMAIN CONTAINING 5 (JMJD5)	JUMONJI d	0	0	
AT1G09540	MYB DOMAIN PROTEIN 61 (MYB61)	MYB a,b	0	0	
AT5G52260	MYB DOMAIN PROTEIN 19 (MYB19)	MYB a,b	0	0	
AT2G13960	INVOLVED IN: regulation of transcription, DNA-dependent, regulation of transcription	MYB-related a,b	0	0	
AT3G25990	BEST Arabidopsis thaliana protein match is: Homeodomain-like superfamily protein (TAIR:AT1G13450.1)	TRIHELIX a,b	0	0	
AT5G47660	BEST Arabidopsis thaliana protein match is: Duplicated homeodomain-like superfamily protein (TAIR:AT5G28300.1)	TRIHELIX a,b	0	0	
AT5G37800	INVOLVED IN: regulation of transcription	bHLH a,b	0	0	
AT5G61270	PHYTOCHROME-INTERACTING FACTOR7 (PIF7)	bHLH a,b	0	0	
AT2G41690	HEAT SHOCK TRANSCRIPTION FACTOR B3 (HSFB3)	HSF a,b,c	0	0	
AT1G01250	0	AP2-EREBP a,b,c	0	0	
AT1G63030	DWARF AND DELAYED FLOWERING 2 (ddf2)	AP2-EREBP a,b,c	0	0	
AT3G25730	INVOLVED IN: regulation of transcription, DNA-dependent	AP2-EREBP a,b	0	0	
AT2G20100	INVOLVED IN: regulation of transcription	ND	0	0	
AT3G50890	INVOLVED IN: regulation of transcription, DNA-dependent, regulation of transcription	zf-HD a,b	0	0	
AT5G23420	HIGH-MOBILITY GROUP BOX 6 (HMGB6)	HMG a,b	0	0	
AT2G40350	0	AP2-EREBP a,b,c	0	0	
AT3G05800	Has 150 Blast hits to 150 proteins in 12 species: Archae - 0	bHLH d	0	0	
AT3G17100	INVOLVED IN: regulation of transcription	bHLH d	0	0	
AT3G12130	BEST Arabidopsis thaliana protein match is: KH domain-containing protein / zinc finger (CCCH type) family protein (TAIR:AT5G06770.1)	C3H a,b	0	0	
AT1G76510	INVOLVED IN: regulation of transcription	ARID a,b	0	0	
AT5G57420	INDOLE-3-ACETIC ACID INDUCIBLE 33 (IAA33)	AUX-IAA b	0	0	
AT1G11490	LOCATED IN: intracellular	C2H2 b	0	0	
AT1G68360	INVOLVED IN: regulation of transcription	C2H2 b	0	0	
AT2G28710	INVOLVED IN: regulation of transcription	C2H2 a,b,c	0	0	
AT3G09290	TELOMERASE ACTIVATOR1 (TAC1)	C2H2 b	0	0	
AT3G49930	INVOLVED IN: regulation of transcription	C2H2 a,b	0	0	
AT1G64000	protein_coding	WRKY a,b,c	0	0	
AT3G58710	protein_coding	WRKY a,b,c	0	0	
AT4G23810	protein_coding	WRKY a,b,c	0	0	
AT5G01900	protein_coding	WRKY a,b,c	0	0	

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AT2G22770	(NAI1)	bHLH a,b	0	0	
AT1G14685	BASIC PENTACYSTEINE 2 (BPC2)	BBR-BPC a,b	0	0	
AT1G29950	INVOLVED IN: regulation of transcription	ND	0	0	
AT3G22100	INVOLVED IN: regulation of transcription	bHLH a	0	0	
AT1G03350	INVOLVED IN: biological_process unknown	BSD a	0	0	
AT1G10720	BEST Arabidopsis thaliana protein match is: BSD domain-containing protein (TAIR:AT3G49800.1)	BSD a	0	0	
AT2G23320	WRKY DNA-BINDING PROTEIN 15 (WRKY15)	WRKY a,b,c	0	0	
AT3G58680	MULTIPROTEIN BRIDGING FACTOR 1B (MBF1B)	MBF1 a,b	0	0	
AT2G47900	TUBBY LIKE PROTEIN 3 (TLP3)	TUB a,c	0	0	
AT5G18090	INVOLVED IN: regulation of transcription, DNA-dependent	ABI3-VP1 a,b	0	0	
AT1G64620	BEST Arabidopsis thaliana protein match is: Dof-type zinc finger DNA-binding family protein (TAIR:AT4G24060.1)	C2C2-DOF a,b,c	0	0	
AT4G12240	BEST Arabidopsis thaliana protein match is: C2H2-like zinc finger protein (TAIR:AT5G52010.1)	C2H2 a,b,c	0	0	
AT1G75080	BRASSINAZOLE-RESISTANT 1 (BZR1)	BES1 a,b	0	0	
AT1G53910	RELATED TO AP2 12 (RAP2.12)	AP2-EREBP a,b,c	0	0	
AT1G15720	TRF-LIKE 5 (TRFL5)	MYB-related a,b	0	0	
AT3G60580	INVOLVED IN: regulation of transcription	C2H2 a,b,c	0	0	
AT5G25475	INVOLVED IN: regulation of transcription, DNA-dependent	ABI3-VP1 b / B3 c	0	0	
AT1G78600	BEST Arabidopsis thaliana protein match is: B-box zinc finger family protein (TAIR:AT1G06040.1).	Orphans a	0	0	
AT2G24570	negative regulator of basal resistance to Pseudomonas syringae.	WRKY a,b,c	0	0	
AT4G38170	INVOLVED IN: response to red or far red light	ND	0	0	
AT2G46680	HOMEOBOX 7 (HB-7)	HB a,b,c	0	0	
AT1G18400	INVOLVED IN: regulation of transcription	bHLH a,b	0	0	
AT3G21270	DOF ZINC FINGER PROTEIN 2 (DOF2)	C2C2-DOF a,b,c	0	0	
AT3G15210	ETHYLENE RESPONSIVE ELEMENT BINDING FACTOR 4 (ERF4)	AP2-EREBP a,b,c	0	0	
AT1G54060	6B-INTERACTING PROTEIN 1-LIKE 1 (ASIL1)	TRIHELIX a,b	0	0	
AT1G77450	INVOLVED IN: multicellular organismal development, regulation of transcription	NAC a,b	0	0	
AT2G22200	0	AP2-EREBP a,b,c	0	0	
AT3G04730	INDOLEACETIC ACID-INDUCED PROTEIN 16 (IAA16)	AUX-IAA a,b	0	0	
AT1G73870	INVOLVED IN: regulation of transcription	C2C2-CO-like a,b	0	0	
AT5G06550	Has 1762 Blast hits to 1747 proteins in 292 species: Archae - 0	JUMONJI a,b	0	0	
AT5G39660	CYCLING DOF FACTOR 2 (CDF2)	C2C2-DOF a,b,c	0	0	
AT1G77640	0	AP2-EREBP a,b,c	0	0	
AT1G34190	INVOLVED IN: multicellular organismal development, regulation of transcription	NAC a,b	0	0	
AT2G26580	BEST Arabidopsis thaliana protein match is: Plant-specific transcription factor YABBY family protein (TAIR:AT1G08465.1)	C2C2-YABBY a,b,c	0	0	
AT4G33880	ROOT HAIR DEFECTIVE 6-LIKE 2 (RSL2)	bHLH a,b	0	0	
AT1G04100	INDOLEACETIC ACID-INDUCED PROTEIN 10 (IAA10)	AUX-IAA a,b	0	0	
AT5G05790	BEST Arabidopsis thaliana protein match is: Duplicated homeodomain-like superfamily protein (TAIR:AT3G11280.2)	MYB a,b	0	0	
AT1G30135	BEST Arabidopsis thaliana protein match is: jasmonate-zim-domain protein 7 (TAIR:AT2G34600.1)	ZIM b,c	0	0	
AT1G48040	INVOLVED IN: protein amino acid dephosphorylation	DBP a	0	0	
AT2G38090	BEST Arabidopsis thaliana protein match is: Homeodomain-like transcriptional regulator (TAIR:AT5G58900.1)	MYB a,b	0	0	
AT3G42860	INVOLVED IN: biological_process unknown	zf-GRF c	0	0	
AT2G18850	BEST Arabidopsis thaliana protein match is: Rubisco methyltransferase family protein (TAIR:AT3G07670.1)	SET a / PcG b	0	0	
AT1G21910	DEHYDRATION RESPONSE ELEMENT-BINDING PROTEIN 26 (DREB26)	AP2-EREBP a,b,c	0	0	
AT1G71930	VASCULAR RELATED NAC-DOMAIN PROTEIN 7 (VND7)	NAC a,b	0	0	
AT2G40340	(DREB2C)	AP2-EREBP a,b,c	0	0	
AT1G68130	INDETERMINATE(ID)-DOMAIN 14 (IDD14)	C2H2 a,b,c	0	0	
AT5G41920	BEST Arabidopsis thaliana protein match is: GRAS family transcription factor (TAIR:AT3G54220.1)	GRAS a,b	0	0	
AT5G47220	ETHYLENE RESPONSIVE ELEMENT BINDING FACTOR 2 (ERF2)	AP2-EREBP a,b,c	0	0	
AT5G42520	BASIC PENTACYSTEINE 6 (BPC6)	BBR-BPC a,b	0	0	
AT1G79700	BEST Arabidopsis thaliana protein match is: ARIA-interacting double AP2 domain protein (TAIR:AT1G16060.1)	AP2-EREBP a,b,c	0	0	
AT1G51070	INVOLVED IN: regulation of transcription	bHLH a,b	0	0	
AT5G63160	BTB AND TAZ DOMAIN PROTEIN 1 (bt1)	TRAF a / TAZ b	0	0	
AT4G27240	INVOLVED IN: regulation of transcription	C2H2 b	0	0	
AT5G18240	MYB-RELATED PROTEIN 1 (MYR1)	G2-like a,b	0	0	
AT5G02840	LHY/CCA1-LIKE 1 (LCL1)	MYB-related a,b	0	0	
AT4G13640	BEST Arabidopsis thaliana protein match is: Homeodomain-like superfamily protein (TAIR:AT3G24120.2)	G2-like a,b	0	0	
AT2G01760	RESPONSE REGULATOR 14 (RR14)	ARR-B a,b	0	0	
AT1G19490	INVOLVED IN: regulation of transcription, DNA-dependent	bZIP a,b,c	0	0	

Gene identifier (AGI)	Primary Gene Symbol	TF family	1 mM 3AT	5 mM 3AT	visual in-spec-tion
AT4G34680	GATA TRANSCRIPTION FACTOR 3 (GATA3)	C2C2-GATA a,b	0	0	
AT4G35040	(bZIP19)	bZIP a,b,c	0	0	
AT2G01060	BEST Arabidopsis thaliana protein match is: Homeodomain-like superfamily protein (TAIR:AT3G04450.1)	G2-like a,b	0	0	
AT2G16720	MYB DOMAIN PROTEIN 7 (MYB7)	MYB a,b	0	0	
AT5G51190	0	AP2-EREBP a,b,c	0	0	
AT3G42790	ALFIN-LIKE 3 (AL3)	ALFIN-like a,b	0	0	
AT1G60380	BEST Arabidopsis thaliana protein match is: NAC domain containing protein 24 (TAIR:AT1G60350.1)	NAC b	0	0	
AT3G06220	INVOLVED IN: regulation of transcription, DNA-dependent	ABI3-VP1 a,b	0	0	
AT4G00390	LOCATED IN: nucleolus	GeBP a,b	0	0	
AT5G64340	SUPPRESSOR OF ACAULIS 51 (SAC51)	bHLH d	0	0	
AT1G43770	INVOLVED IN: regulation of transcription, DNA-dependent	PHD a,b	0	0	
AT1G70000	BEST Arabidopsis thaliana protein match is: myb-like transcription factor family protein (TAIR:AT5G47390.1)	MYB-related a,b	0	0	
AT4G00250	INVOLVED IN: biological_process unknown	GeBP a,b	0	0	
AT4G24660	BEST Arabidopsis thaliana protein match is: homeobox protein 25 (TAIR:AT5G65410.1)	zf-HD a,b	0	0	
AT5G43250	INVOLVED IN: regulation of transcription, DNA-dependent	CCAAT a / CCAAT-HAP5 b	0	0	
AT5G03720	HEAT SHOCK TRANSCRIPTION FACTOR A3 (HSFA3)	HSF a,b,c	0	0	
AT5G09330	INVOLVED IN: multicellular organismal development, regulation of transcription	NAC a,b	0	0	
AT5G15150	HOMEOBOX 3 (HB-3)	HB a,b,c	0	0	
AT1G01030	BEST Arabidopsis thaliana protein match is: AP2/B3-like transcriptional factor family protein (TAIR:AT4G01500.1)	ABI3-VP1 a,b	0	0	
AT1G02065	SQUAMOSA PROMOTER BINDING PROTEIN-LIKE 8 (SPL8)	SBP a,b,c	0	0	
AT1G05690	BTB AND TAZ DOMAIN PROTEIN 3 (bt3)	TRAF a / TAZ b	0	0	
AT2G24700	INVOLVED IN: regulation of transcription, DNA-dependent	ABI3-VP1 a,b	0	0	
AT3G04380	(SUVR4)	SET a / PcG b	0	0	
AT3G07220	BEST Arabidopsis thaliana protein match is: SMAD/FHA domain-containing protein (TAIR:AT3G07260.1)	FHA a,b	0	0	
AT3G09735	INVOLVED IN: regulation of transcription	S1Fa-like a,b	0	0	
AT3G50870	MONOPOLE (MNP)	C2C2-GATA a,b	0	0	
AT3G51470	INVOLVED IN: protein amino acid dephosphorylation	DBP a	0	0	
AT3G59470	BEST Arabidopsis thaliana protein match is: Far-red impaired responsive (FAR1) family protein (TAIR:AT3G07500.1)	FAR1 a	0	0	
AT3G54610	HISTONE ACETYLTRANSFERASE OF THE GNAT FAMILY 1 (HAG1)	GNAT a	0	0	
AT4G19985	INVOLVED IN: N-terminal protein myristoylation, metabolic process	GNAT a	0	0	
AT3G18870	INVOLVED IN: biological_process unknown	mTERF a	0	0	
AT2G03050	EMBRYO DEFECTIVE 93 (EMB93)	mTERF a	0	0	
AT5G23930	INVOLVED IN: biological_process unknown	mTERF a	0	0	
AT5G07900	INVOLVED IN: biological_process unknown	mTERF a	0	0	
AT5G08790	(ATAF2)	NAC a,b	0	0	
AT2G18500	LOCATED IN: plasma membrane	OFP a	0	0	
AT3G20800	BEST Arabidopsis thaliana protein match is: Cell differentiation, Rcd1-like protein (TAIR:AT5G12980.1)	RCD1-like a	0	0	
AT1G80400	EXPRESSED IN: 22 plant structures	ND	0	0	
AT4G34290	INVOLVED IN: biological_process unknown	SWI/SNF-BAF60b a	0	0	
AT3G01890	BEST Arabidopsis thaliana protein match is: SWIB/MDM2 domain superfamily protein (TAIR:AT5G14170.1)	SWI/SNF-BAF60b a	0	0	
AT2G40450	BEST Arabidopsis thaliana protein match is: BTB/POZ domain-containing protein (TAIR:AT5G48510.1)	TRAF a	0	0	
AT1G01640	0	TRAF a	0	0	
AT4G08455	BEST Arabidopsis thaliana protein match is: BTB/POZ domain-containing protein (TAIR:AT1G01640.2)	TRAF a	0	0	
AT3G61600	POZ/BTB CONTAININ G-PROTEIN 1 (POB1)	TRAF a	0	0	
AT1G22310	METHYL-CPG-BINDING DOMAIN 8 (MBD8)	ND	0	0	
AT1G24250	INVOLVED IN: regulation of transcription, DNA-dependent	Orphans a	0	0	
AT2G27580	INVOLVED IN: biological_process unknown	zf-AN1 c	0	0	
AT3G15790	METHYL-CPG-BINDING DOMAIN 11 (MBD11)	ND	0	0	
AT4G21610	LSD ONE LIKE 2 (LOL2)	zf-LSD1 c	0	0	
AT4G25380	INVOLVED IN: biological_process unknown	zf-AN1 c	0	0	
AT3G19580	ZINC-FINGER PROTEIN 2 (ZF2)	C2H2 a,b	0	0	
AT5G60100	PSEUDO-RESPONSE REGULATOR 3 (PRR3)	Pseudo ARR-B a / C2C2-CO-like b	0	0	
AT2G32905	BEST Arabidopsis thaliana protein match is: Domain of unknown function (DUF313) (TAIR:AT2G27410.1)	REM(B3) d	0	0	
AT3G05760	INVOLVED IN: biological_process unknown	C2H2 d	0	0	
AT3G61850	DOF AFFECTING GERMINATION 1 (DAG1)	C2C2-DOF a,b,c	0	0	
AT1G55760	BEST Arabidopsis thaliana protein match is: BTB/POZ domain-containing protein (TAIR:AT1G21780.2)	TRAF a	0	0	
AT4G01550	NAC DOMAIN CONTAINING PROTEIN 69 (NAC069)	NAC a,b	0	0	

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AT1G02220	INVOLVED IN: multicellular organismal development, regulation of transcription	NAC a,b	0	0	
AT1G33280	NAC DOMAIN CONTAINING PROTEIN 15 (NAC015)	NAC a,b	0	0	
AT1G63910	MYB DOMAIN PROTEIN 103 (AtMYB103)	MYB a,b	0	0	
AT2G39880	MYB DOMAIN PROTEIN 25 (MYB25)	MYB a,b	0	0	
AT4G22680	MYB DOMAIN PROTEIN 85 (MYB85)	MYB a,b	0	0	
AT1G66370	MYB DOMAIN PROTEIN 113 (MYB113)	MYB a,b	0	0	
AT2G42660	BEST Arabidopsis thaliana protein match is: myb-like HTH transcriptional regulator family protein (TAIR:AT2G38300.1)	G2-like a,b	0	0	
AT3G24310	MYB DOMAIN PROTEIN 305 (MYB305)	MYB a,b	0	0	
AT5G62470	MYB DOMAIN PROTEIN 96 (MYB96)	MYB a,b	0	0	
AT1G75710	LOCATED IN: intracellular	C2H2 a,b	0	0	
AT5G03150	JACKDAW (JKD)	C2H2 a,b,c	0	0	
AT5G46830	NACL-INDUCIBLE GENE 1 (NIG1)	bHLH a,b	0	0	
AT1G02340	LONG HYPOCOTYL IN FAR-RED (HFR1)	bHLH a,b	0	0	
AT1G06160	OCTADECANOID-RESPONSIVE ARABIDOPSIS AP2/ERF 59 (ORA59)	AP2-EREBP a,b,c	0	0	
AT3G23230	TRANSCRIPTIONAL REGULATOR OF DEFENSE RESPONSE 1 (TDR1)	AP2-EREBP a,b,c	0	0	
AT5G61890	0	AP2-EREBP a,b,c	0	0	
AT5G63260	INVOLVED IN: biological_process unknown	C3H a,b	0	0	
AT4G26030	INVOLVED IN: regulation of transcription	C2H2 d	0	0	
AT2G03500	BEST Arabidopsis thaliana protein match is: myb-like transcription factor family protein (TAIR:AT1G68670.1)	G2-like a,b	0	0	
AT5G62020	HEAT SHOCK TRANSCRIPTION FACTOR B2A (HSFB2A)	HSF a,b,c	0	0	
AT1G19040	BEST Arabidopsis thaliana protein match is: NAC domain containing protein 6 (TAIR:AT1G03490.1)	NAC b	0	0	
AT3G54990	SCHLAFMUTZE (SMZ)	AP2-EREBP a,b,c	0	0	
AT1G64105	INVOLVED IN: multicellular organismal development, regulation of transcription	NAC a,b	0	0	
AT1G77570	INVOLVED IN: regulation of transcription, DNA-dependent	HSF a,b	0	0	
AT5G50570	INVOLVED IN: regulation of transcription	SBP a,b,c	0	0	
AT2G18490	INVOLVED IN: regulation of transcription	C2H2 a,b,c	0	0	
AT5G54360	INVOLVED IN: regulation of transcription	C2H2 d	0	0	
AT1G63820	BEST Arabidopsis thaliana protein match is: CCT motif family protein (TAIR:AT5G41380.1)	Orphans a / C2C2-CO-like b	0	0	
AT2G40210	INVOLVED IN: regulation of transcription, DNA-dependent	MADS a,b	0	0	
AT3G11100	INVOLVED IN: regulation of transcription	TRIHELIX a,b	0	0	
AT1G66420	BEST Arabidopsis thaliana protein match is: DNA-binding storekeeper protein-related transcriptional regulator (TAIR:AT2G01370.1)	GeBP a,b	0	0	
AT1G76870	Bacteria - 2	TRIHELIX b	0	0	
AT2G15740	INVOLVED IN: regulation of transcription	C2H2 a,b,c	0	0	
AT2G37740	INVOLVED IN: regulation of transcription	C2H2 a,b,c	0	0	
AT1G18960	INVOLVED IN: regulation of transcription, DNA-dependent, regulation of transcription	MYB-related a,b	0	0	
AT1G27740	ROOT HAIR DEFECTIVE 6-LIKE 4 (RSL4)	bHLH a,b	0	0	
AT1G32770	NAC DOMAIN CONTAINING PROTEIN 12 (NAC012)	NAC a,b	0	0	
AT1G43000	BEST Arabidopsis thaliana protein match is: PLATZ transcription factor family protein (TAIR:AT1G21000.1)	PLATZ a,b	0	0	
AT1G55650	LOCATED IN: chloroplast	ARID a,b	0	0	
AT2G01818	INVOLVED IN: biological_process unknown	PLATZ a	0	0	
AT2G27930	BEST Arabidopsis thaliana protein match is: PLATZ transcription factor family protein (TAIR:AT1G32700.1)	PLATZ a,b	0	0	
AT2G30432	TRICHOMELESS1 (TCL1)	MYB-related a,b	0	0	
AT3G04280	RESPONSE REGULATOR 22 (RR22)	Orphans a	0	0	
AT3G17010	REPRODUCTIVE MERISTEM 22 (REM22)	ABI3-VP1 a,b	0	0	
AT5G14960	INVOLVED IN: regulation of transcription, DNA-dependent	E2F-DP a,b,c	0	0	
AT5G51790	INVOLVED IN: regulation of transcription	bHLH a,b	0	0	
AT5G60440	AGAMOUS-LIKE 62 (AGL62)	MADS a,b	0	0	
AT2G17770	BASIC REGION/LEUCINE ZIPPER MOTIF 27 (BZIP27)	bZIP c	0	0	
AT2G35430	BEST Arabidopsis thaliana protein match is: Zinc finger (CCCH-type) family protein (TAIR:AT1G32360.1)	C3H a,b	0	0	
AT5G13780	INVOLVED IN: metabolic process	GNAT a	0	0	
AT5G16800	INVOLVED IN: metabolic process	GNAT a	0	0	
AT1G62085	BEST Arabidopsis thaliana protein match is: Mitochondrial transcription termination factor family protein (TAIR:AT1G62110.1)	mTERF a	0	0	
AT1G62110	INVOLVED IN: biological_process unknown	mTERF a	0	0	
AT1G79580	SOMBRERO (SMB)	NAC a,b	0	0	
AT1G06920	OVATE FAMILY PROTEIN 4 (OFP4)	OFP a	0	0	
AT3G52525	BEST Arabidopsis thaliana protein match is: Ovate family protein (TAIR:AT2G36026.1)	OFP a	0	0	
AT2G32550	BEST Arabidopsis thaliana protein match is: Cell differentiation, Rcd1-like protein (TAIR:AT5G12980.1)	RCD1-like a	0	0	
AT3G11090	BEST Arabidopsis thaliana protein match is: LOB domain-containing protein 25 (TAIR:AT3G27650.1)	LOB a / AS2 b	0	0	

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AT3G11260	WUSCHEL RELATED HOMEOBOX 5 (WOX5)	HB a,b,c	0	0	
AT2G01940	SHOOT GRAVITROPISM 5 (SGR5)	C2H2 a,b,c	0	0	
AT5G01860	INVOLVED IN: regulation of transcription	C2H2 a,b,c	0	0	
AT5G14010	KNUCKLES (KNU)	C2H2 a,b,c	0	0	
AT3G58190	LATERAL ORGAN BOUNDARIES-DOMAIN 29 (LBD29)	LOB a / AS2 b	0	0	
AT5G67420	LOB DOMAIN-CONTAINING PROTEIN 37 (LBD37)	LOB a / AS2 b	0	0	
AT1G04880	INVOLVED IN: regulation of transcription	ARID a,b	0	0	
AT2G31730	INVOLVED IN: response to ethylene stimulus, response to gibberellin stimulus	ND	0	0	
AT2G40200	INVOLVED IN: regulation of transcription	bHLH a,b	0	0	
AT4G00870	INVOLVED IN: regulation of transcription	bHLH a,b	0	0	
AT1G30970	SUPPRESSOR OF FRIGIDA4 (SUF4)	C2H2 a,b	0	0	
AT5G49520	WRKY DNA-BINDING PROTEIN 48 (WRKY48)	WRKY a,b,c	0	0	
AT3G02380	CONSTANS-LIKE 2 (COL2)	C2C2-CO-like a,b	0	0	
AT5G57180	CHLOROPLAST IMPORT APPARATUS 2 (CIA2)	Orphans a / C2C2-CO-like b	0	0	
AT3G24860	INVOLVED IN: biological_process unknown	TRIHELIX a,b	0	0	
AT5G24930	INVOLVED IN: regulation of transcription	C2C2-CO-like a,b	0	0	
AT4G39410	protein_coding	WRKY a,b,c	0	0	
AT5G07100	WRKY DNA-BINDING PROTEIN 26 (WRKY26)	WRKY a,b,c	0	0	
AT5G51860	AGAMOUS-LIKE 72 (AGL72)	MADS a,b	0	0	
AT1G70510	KNOTTED-LIKE FROM ARABIDOPSIS THALIANA 2 (KNAT2)	HB a,b	0	0	
AT1G74650	MYB DOMAIN PROTEIN 31 (MYB31)	MYB a,b	0	0	
AT2G02820	MYB DOMAIN PROTEIN 88 (MYB88)	MYB a,b	0	0	
AT5G10120	INVOLVED IN: response to karrikin, regulation of transcription	EIL a,b	0	0	
AT2G37590	BEST Arabidopsis thaliana protein match is: OBF-binding protein 3 (TAIR:AT3G55370.2)	C2C2-DOF a,b,c	0	0	
AT1G19270	BEST Arabidopsis thaliana protein match is: LIM domain-containing protein (TAIR:AT4G36860.1)	Orphans a / LIM b	0	0	
AT1G56160	MYB DOMAIN PROTEIN 72 (MYB72)	MYB a,b	0	0	
AT1G01160	GRF1-INTERACTING FACTOR 2 (GIF2)	GIF b	0	0	
AT4G27310	INVOLVED IN: regulation of transcription	Orphans a	0	0	
AT1G25440	INVOLVED IN: regulation of transcription	C2C2-CO-like a,b	0	0	
AT2G24590	RS-CONTAINING ZINC FINGER PROTEIN 22A (RSZ22a)	ND	0	0	
AT5G14260	INVOLVED IN: biological_process unknown	SET a / PcG b	0	0	
AT4G31550	negative regulator of basal resistance to Pseudomonas syringae.	WRKY a,b,c	0	0	
AT1G76110	INVOLVED IN: regulation of transcription	ARID a,b	0	0	
AT5G18550	INVOLVED IN: biological_process unknown	C3H a,b	0	0	
AT5G22890	INVOLVED IN: response to chitin, regulation of transcription	C2H2 a,b,c	0	0	
AT1G66350	RGA-LIKE 1 (RGL1)	GRAS a,b	0	0	
AT4G17570	GATA TRANSCRIPTION FACTOR 26 (GATA26)	C2C2-GATA a,b	0	0	
AT1G51700	DOF ZINC FINGER PROTEIN 1 (DOF1)	C2C2-DOF a,b,c	0	0	
AT5G48150	PHYTOCHROME A SIGNAL TRANSDUCTION 1 (PAT1)	GRAS a,b	0	0	
AT2G28160	FER-LIKE REGULATOR OF IRON UPTAKE (FRU)	bHLH a,b	0	0	
AT1G22070	TGA1A-RELATED GENE 3 (TGA3)	bZIP b,c	0	0	
AT5G26805	Has 35333 Blast hits to 34131 proteins in 2444 species: Archae - 798	REM(B3) d	0	0	
AT3G13540	MYB DOMAIN PROTEIN 5 (MYB5)	MYB a,b	0	0	
AT1G54160	NUCLEAR FACTOR Y, SUBUNIT A5 (NF-YA5)	CCAAT a / CCAAT-HAP2 b / CBFB_NFYA c	0	0	
AT4G25210	INVOLVED IN: biological_process unknown	GeBP a,b	0	0	
AT3G20550	DAWDLE (DDL)	FHA a,b	0	0	
AT2G04845	INVOLVED IN: metabolic process	GNAT a	0	0	
AT5G67180	BEST Arabidopsis thaliana protein match is: Integrase-type DNA-binding superfamily protein (TAIR:AT4G36920.2)	AP2-EREBP a,b,c	0	0	
AT1G04250	AUXIN RESISTANT 3 (AXR3)	AUX-IAA a,b	0	0	
AT5G49330	MYB DOMAIN PROTEIN 111 (MYB111)	MYB a,b	0	0	
AT5G01380	BEST Arabidopsis thaliana protein match is: Homeodomain-like superfamily protein (TAIR:AT2G38250.1)	TRIHELIX a,b	0	0	
AT2G40970	BEST Arabidopsis thaliana protein match is: Homeodomain-like superfamily protein (TAIR:AT3G10760.1)	G2-like a,b	0	0	
AT4G03170	INVOLVED IN: regulation of transcription, DNA-dependent	ABI3-VP1 a,b	0	0	
AT1G02250	INVOLVED IN: multicellular organismal development, regulation of transcription	NAC a,b	0	0	
AT4G34990	MYB DOMAIN PROTEIN 32 (MYB32)	MYB a,b	0	0	
AT1G63040	0	ND	0	0	
AT1G34670	MYB DOMAIN PROTEIN 93 (MYB93)	MYB a,b	0	0	
AT3G57670	NO TRANSMITTING TRACT (NTT)	C2H2 a,b,c	0	0	
AT1G64625	Has 35333 Blast hits to 34131 proteins in 2444 species: Archae - 798	bHLH d	0	0	
AT5G10570	INVOLVED IN: regulation of transcription	bHLH a,b	0	0	
AT1G26300	INVOLVED IN: biological_process unknown	BSD a	0	0	
AT3G49800	INVOLVED IN: biological_process unknown	BSD a	0	0	
AT3G55770	WLIM2B (WLIM2b)	LIM a,b	0	0	

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AT3G54340	APETALA 3 (AP3)	MADS a,b	0	0	
AT4G16845	REDUCED VERNALIZATION RESPONSE 2 (VRN2)	ND	0	0	
AT3G01530	MYB DOMAIN PROTEIN 57 (MYB57)	MYB a,b	0	0	
AT5G17300	REVEILLE 1 (RVE1)	MYB-related a,b	0	0	
AT3G54810	BLUE MICROPYLAR END 3 (BME3)	C2C2-GATA a,b	0	0	
AT2G33610	SWITCH SUBUNIT 3 (SWI3B)	MYB-related a / SWIRM c	0	0	
AT4G12670	INVOLVED IN: response to jasmonic acid stimulus, response to salicylic acid stimulus	MYB-related d	0	0	
AT5G66270	INVOLVED IN: biological_process unknown	C3H a	0	0	
AT5G67480	BTB AND TAZ DOMAIN PROTEIN 4 (BT4)	TAZ a,b	0	0	
AT1G19700	BEL1-LIKE HOMEODOMAIN 10 (BEL10)	HB a,b	0	0	
AT5G22290	NAC DOMAIN CONTAINING PROTEIN 89 (NAC089)	NAC a,b	0	0	
AT2G18160	BASIC LEUCINE-ZIPPER 2 (bZIP2)	bZIP a,b,c	0	0	
AT5G54630	INVOLVED IN: regulation of transcription	C2H2 b	0	0	
AT2G22430	HOMEOBOX PROTEIN 6 (HB6)	HB a,b	0	0	
AT1G75340	INVOLVED IN: biological_process unknown	C3H a,b	0	0	
AT4G17880	(MYC4)	bHLH a,b	0	0	
AT5G59430	TELOMERIC REPEAT BINDING PROTEIN 1 (TRP1)	MYB-related d	0	0	
AT5G46760	(MYC3)	bHLH a,b	0	0	
AT3G60630	HAIRY MERISTEM 2 (HAM2)	GRAS a,b	0	0	
AT1G73730	ETHYLENE-INSENSITIVE3-LIKE 3 (EIL3)	EIL a,b	0	0	
AT2G46040	FUNCTIONS IN: DNA binding	ARID a,b	0	0	
AT4G15420	INVOLVED IN: proteolysis, ubiquitin-dependent protein catabolic process	C2H2 b	0	0	
AT4G29930	INVOLVED IN: regulation of transcription	bHLH a,b	0	0	
AT5G65640	INVOLVED IN: regulation of transcription	bHLH a,b	0	0	
AT1G55580	LATERAL SUPPRESSOR (LAS)	GRAS a,b	0	0	
AT1G76420	protein_coding	NAC a,b	0	0	
AT3G01600	INVOLVED IN: multicellular organismal development, regulation of transcription	NAC a,b	0	0	
AT4G02670	INVOLVED IN: regulation of transcription	C2H2 a,b,c	0	0	
AT4G14720	(PPD2)	ZIM b,c	0	0	
AT4G36160	NAC DOMAIN CONTAINING PROTEIN 76 (NAC076)	NAC a,b	0	0	
AT5G19490	INVOLVED IN: biological_process unknown	CCAAT a	0	0	
AT3G21350	INVOLVED IN: regulation of transcription from RNA polymerase II promoter	MED6 a	0	0	
AT1G62120	INVOLVED IN: biological_process unknown	mTERF a	0	0	
AT1G78930	INVOLVED IN: biological_process unknown	mTERF a	0	0	
AT2G44020	BEST Arabidopsis thaliana protein match is: Mitochondrial transcription termination factor family protein (TAIR:AT4G02990.1)	mTERF a	0	0	
AT4G14605	BEST Arabidopsis thaliana protein match is: Mitochondrial transcription termination factor family protein (TAIR:AT2G21710.1)	mTERF a	0	0	
AT2G18060	VASCULAR RELATED NAC-DOMAIN PROTEIN 1 (VND1)	NAC a,b	0	0	
AT1G05420	BEST Arabidopsis thaliana protein match is: ovate family protein 16 (TAIR:AT2G32100.1)	OFP a	0	0	
AT2G36050	EXPRESSED IN: sepal, carpel, stamen	OFP a	0	0	
AT2G30400	EXPRESSED IN: 13 plant structures	OFP a	0	0	
AT5G19650	BEST Arabidopsis thaliana protein match is: ovate family protein 7 (TAIR:AT2G18500.1)	OFP a	0	0	
AT1G49520	BEST Arabidopsis thaliana protein match is: SWIB complex BAF60b domain-containing protein (TAIR:AT3G19080.1)	SWI/SNF-BAF60b a	0	0	
AT5G13730	SIGMA FACTOR 4 (SIG4)	SIGMA70-like a	0	0	
AT5G45110	NPR1-LIKE PROTEIN 3 (NPR3)	TRAF a	0	0	
AT5G48510	BEST Arabidopsis thaliana protein match is: BTB/POZ domain-containing protein (TAIR:AT2G40450.1)	TRAF a	0	0	
AT3G04670	protein_coding	WRKY a,b,c	0	0	
AT1G23810	INVOLVED IN: regulation of transcription, DNA-dependent	Orphans a	0	0	
AT1G24230	INVOLVED IN: regulation of transcription, DNA-dependent	Orphans a	0	0	
AT1G27280	INVOLVED IN: regulation of transcription, DNA-dependent	Orphans a	0	0	
AT1G49830	INVOLVED IN: regulation of transcription	ND	0	0	
AT3G19070	INVOLVED IN: regulation of transcription	ND	0	0	
AT2G36740	INVOLVED IN: regulation of transcription	YL1 c	0	0	
AT4G25110	METACASPASE 2 (MC2)	PEPTIDASE_C14 c	0	0	
AT5G15040	INVOLVED IN: regulation of transcription, DNA-dependent	Orphans a	0	0	
AT5G60142	INVOLVED IN: regulation of transcription, DNA-dependent	ABI3-VP1 a / B3 c	0	0	
AT4G01540	NAC WITH TRANSMEMBRANE MOTIF1 (NTM1)	NAC a,b	0	0	
AT3G06490	MYB DOMAIN PROTEIN 108 (MYB108)	MYB a,b	0	0	
AT5G38860	INVOLVED IN: regulation of transcription	bHLH a,b	0	0	
AT1G32330	HEAT SHOCK TRANSCRIPTION FACTOR A1D (HSFA1D)	HSF a,b,c	0	0	
AT5G60480	INVOLVED IN: regulation of transcription	zf-HD a,b	0	0	
AT1G55950	BEST Arabidopsis thaliana protein match is: DNA-binding storekeeper protein-related transcriptional regulator (TAIR:AT2G01370.1)	GeBP a,b	0	0	
AT3G12977	INVOLVED IN: regulation of transcription	NAC a / NAM c	0	0	
AT4G06634	YIN YANG 1 (YY1)	C2H2 a,b	0	0	

Gene identifier (AGI)	Primary Gene Symbol	TF family	1 mM 3AT	5 mM 3AT	visual in-spec-tion
AT4G20970	INVOLVED IN: defense response to fungus, regulation of transcription	bHLH a,b	0	0	
AT1G32510	BEST Arabidopsis thaliana protein match is: NAC domain containing protein 71 (TAIR:AT4G17980.1)	NAC a,b	0	0	
AT1G18335	INVOLVED IN: metabolic process	GNAT a	0	0	
AT5G04760	BEST Arabidopsis thaliana protein match is: Duplicated homeodomain-like superfamily protein (TAIR:AT5G08520.1)	MYB a,b	0	0	
AT1G68670	BEST Arabidopsis thaliana protein match is: myb-like transcription factor family protein (TAIR:AT1G25550.1)	G2-like a,b	0	0	
AT2G02470	ALFIN-LIKE 6 (AL6)	ALFIN-like a,b	0	0	
AT2G34450	LOCATED IN: nucleus, chloroplast	HMG a,b	0	0	
AT1G59640	BIG PETAL P (BPEP)	bHLH a,b	0	0	
AT5G07580	0	AP2-EREBP a,b,c	0	0	
AT5G10280	MYB DOMAIN PROTEIN 92 (MYB92)	MYB a,b	0	0	
AT5G17260	INVOLVED IN: multicellular organismal development, regulation of transcription	NAC a,b	0	0	
AT3G02550	BEST Arabidopsis thaliana protein match is: LOB domain-containing protein 40 (TAIR:AT1G67100.1)	LOB a / AS2 b	0	0	
AT4G14540	INVOLVED IN: regulation of transcription, DNA-dependent	CCAAT a / CCAAT-HAP3 b	0	0	
AT3G24140	FAMA (FMA)	bHLH a,b	0	0	
AT5G52660	BEST Arabidopsis thaliana protein match is: Homeodomain-like superfamily protein (TAIR:AT4G01280.1)	MYB-related a,b	0	0	
AT2G33350	BEST Arabidopsis thaliana protein match is: CCT motif family protein (TAIR:AT1G04500.1)	Orphans a / C2C2-CO-like b	0	0	
AT2G46670	BEST Arabidopsis thaliana protein match is: pseudo-response regulator 9 (TAIR:AT2G46790.1)	Orphans a / C2C2-CO-like b	0	0	
AT3G03200	INVOLVED IN: multicellular organismal development, regulation of transcription	NAC a,b	0	0	
AT4G05170	INVOLVED IN: regulation of transcription	bHLH a	0	0	
AT2G32020	INVOLVED IN: response to abscisic acid stimulus, metabolic process	GNAT a	0	0	
AT2G38130	(ATMAK3)	GNAT a	0	0	
AT3G46600	BEST Arabidopsis thaliana protein match is: GRAS family transcription factor (TAIR:AT5G59450.1)	GRAS a,b	0	0	
AT1G50600	SCARECROW-LIKE 5 (SCL5)	GRAS a,b	0	0	
AT4G38900	INVOLVED IN: regulation of transcription, DNA-dependent	bZIP a,b,c	0	0	
AT1G76890	(GT2)	TRIHELIX a,b	0	0	
AT2G47190	MYB DOMAIN PROTEIN 2 (MYB2)	MYB a,b	0	0	
AT2G42380	(BZIP34)	bZIP a,b,c	0	0	
AT5G66940	BEST Arabidopsis thaliana protein match is: OBF binding protein 1 (TAIR:AT3G50410.1)	C2C2-DOF a,b,c	0	0	
AT1G10610	INVOLVED IN: regulation of transcription	bHLH a,b	0	0	
AT1G29860	protein_coding	WRKY a,b,c	0	0	
AT5G49490	INVOLVED IN: regulation of transcription, DNA-dependent	MADS a,b	0	0	
AT1G14687	INVOLVED IN: regulation of transcription	zf-HD a,b	0	0	
AT2G17040	NAC DOMAIN CONTAINING PROTEIN 36 (NAC036)	NAC a,b	0	0	
AT1G68840	RELATED TO ABI3/VP1 2 (RAV2)	AP2-EREBP a,b	0	0	
AT2G35700	ERF FAMILY PROTEIN 38 (ERF38)	AP2-EREBP a,b,c	0	0	
AT1G62150	BEST Arabidopsis thaliana protein match is: Mitochondrial transcription termination factor family protein (TAIR:AT1G62085.1)	mTERF a	0	0	
AT3G26790	FUSCA 3 (FUS3)	ABI3-VP1 a,b	0	0	
AT2G38470	WRKY DNA-BINDING PROTEIN 33 (WRKY33)	WRKY a,b,c	0	0	
AT1G59810	INVOLVED IN: regulation of transcription, DNA-dependent	MADS a,b	0	0	
AT1G31140	GORDITA (GOA)	MADS a,b	0	0	
AT5G49300	GATA TRANSCRIPTION FACTOR 16 (GATA16)	C2C2-GATA a,b	0	0	
AT2G28200	INVOLVED IN: regulation of transcription	C2H2 a,b,c	0	0	
AT5G16540	ZINC FINGER NUCLEASE 3 (ZFN3)	C3H a,b	0	0	
AT2G47620	SWITCH/SUCROSE NONFERMENTING 3A (SWI3A)	MYB-related a / SWIRM c	0	0	
AT1G22985	CYTOKININ RESPONSE FACTOR 7 (CRF7)	AP2-EREBP a,b,c	0	0	
AT3G45880	LOCATED IN: cellular_component unknown	JUMONJI d	0	0	
AT2G22540	SHORT VEGETATIVE PHASE (SVP)	MADS a,b	0	0	
AT5G41315	GLABROUS 3 (GL3)	bHLH a,b	0	0	
AT2G43000	NAC DOMAIN CONTAINING PROTEIN 42 (NAC042)	NAC a,b	0	0	
AT3G13840	BEST Arabidopsis thaliana protein match is: GRAS family transcription factor (TAIR:AT3G49950.1)	GRAS a,b	0	0	
AT5G65590	INVOLVED IN: regulation of transcription	C2C2-DOF a,b,c	0	0	
AT4G12210	CONTAINS InterPro DOMAIN/s: Zinc finger, RING-type (InterPro:IPR001841), Zinc finger, C3HC4 RING-type (InterPro:IPR018957)	ND	0	0	
AT3G46070	INVOLVED IN: regulation of transcription	C2H2 a,b,c	0	0	
AT2G36080	0	ABI3-VP1 a,b	0	0	
AT4G23750	CYTOKININ RESPONSE FACTOR 2 (CRF2)	AP2-EREBP a,b,c	0	0	
AT5G16600	MYB DOMAIN PROTEIN 43 (MYB43)	MYB a,b	0	0	
AT5G14340	MYB DOMAIN PROTEIN 40 (MYB40)	MYB a,b	0	0	

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AT5G15310	MYB DOMAIN PROTEIN 16 (MYB16)	MYB a,b	0	0	
AT2G38250	INVOLVED IN: regulation of transcription	TRIHELIX a,b	0	0	
AT1G44830	0	AP2-EREBP a,b,c	0	0	
AT5G12330	LATERAL ROOT PRIMORDIUM 1 (LRP1)	SRS a,b	0	0	
AT2G21320	INVOLVED IN: regulation of transcription	Orphans a	0	0	
AT5G03510	INVOLVED IN: regulation of transcription	C2H2 a,b,c	0	0	
AT5G05120	INVOLVED IN: regulation of transcription	C2H2 a,b,c	0	0	
AT5G06650	INVOLVED IN: trichome differentiation, response to gibberellin stimulus, response to cytokinin stimulus, regulation of transcription	C2H2 a,b,c	0	0	
AT5G10970	INVOLVED IN: regulation of transcription	C2H2 a,b	0	0	
AT4G36260	STYLISH 2 (STY2)	SRS a,b	0	0	
AT3G06590	INVOLVED IN: regulation of transcription	ND	0	0	
AT3G47710	BANQUO 3 (BNQ3)	ND	0	0	
AT3G22830	HEAT SHOCK TRANSCRIPTION FACTOR A6B (HSFA6B)	HSF a,b,c	0	0	
AT1G30210	TEOSINTE BRANCHED 1, CYCLOIDEA, AND PCF FAMILY 24 (TCP24)	TCP a,b,c	0	0	
AT3G24520	HEAT SHOCK TRANSCRIPTION FACTOR C1 (HSFC1)	HSF a,b,c	0	0	
AT4G32800	0	AP2-EREBP a,b,c	0	0	
AT5G58900	INVOLVED IN: regulation of transcription	MYB a,b	0	0	
AT2G38300	BEST Arabidopsis thaliana protein match is: Homeodomain-like superfamily protein (TAIR:AT2G40260.1)	G2-like a,b	0	0	
AT1G68920	INVOLVED IN: regulation of transcription	bHLH a,b	0	0	
AT4G34530	CRYPTOCHROME-INTERACTING BASIC-HELIX-LOOP-HELIX 1 (CIB1)	bHLH a,b	0	0	
AT3G61950	INVOLVED IN: regulation of transcription	bHLH a,b	0	0	
AT1G19000	INVOLVED IN: regulation of transcription, DNA-dependent	MYB-related a,b	0	0	
AT1G19180	JASMONATE-ZIM-DOMAIN PROTEIN 1 (JAZ1)	ZIM b,c	0	0	
AT5G40330	MYB DOMAIN PROTEIN 23 (MYB23)	MYB a,b	0	0	
AT4G27230	HISTONE H2A 2 (HTA2)	CCAAT a	0	0	
AT1G32700	BEST Arabidopsis thaliana protein match is: PLATZ transcription factor family protein (TAIR:AT4G17900.1)	PLATZ a,b	0	0	
AT2G17950	WUSCHEL (WUS)	HB a,b,c	0	0	
AT4G37610	BTB AND TAZ DOMAIN PROTEIN 5 (bt5)	TAZ a,b	0	0	
AT5G67190	DREB AND EAR MOTIF PROTEIN 2 (DEAR2)	AP2-EREBP a,b,c	0	0	
AT1G46768	RELATED TO AP2 1 (RAP2.1)	AP2-EREBP a,b,c	0	0	
AT5G40710	INVOLVED IN: biological_process unknown	C2H2 b	0	0	
AT3G56850	ABA-RESPONSIVE ELEMENT BINDING PROTEIN 3 (AREB3)	bZIP a,b,c	0	0	
AT5G63090	LATERAL ORGAN BOUNDARIES (LOB)	LOB a / AS2 b	0	0	
AT3G54620	BASIC LEUCINE ZIPPER 25 (BZIP25)	bZIP a,b,c	0	0	
AT2G30590	WRKY DNA-BINDING PROTEIN 21 (WRKY21)	WRKY a,b	0	0	
AT3G14230	RELATED TO AP2 2 (RAP2.2)	AP2-EREBP a,b,c	0	0	
AT1G44810	BEST Arabidopsis thaliana protein match is: DNA-binding storekeeper protein-related transcriptional regulator (TAIR:AT4G00250.1)	GeBP a,b	0	0	
AT2G25820	ETHYLENE AND SALT INDUCIBLE 2 (ESE2)	AP2-EREBP a,b,c	0	0	
AT3G58780	SHATTERPROOF 1 (SHP1)	MADS a,b	0	0	
AT5G27050	INVOLVED IN: regulation of transcription, DNA-dependent	MADS a,b	0	0	
AT2G27470	INVOLVED IN: regulation of transcription	CCAAT a / CCAAT-HAP3 b	0	0	
AT4G10600	INVOLVED IN: regulation of transcription, DNA-dependent	PHD b	0	0	
AT5G03415	protein_coding	E2F-DP a,b,c	0	0	
AT1G68030	INVOLVED IN: biological_process unknown	PHD a,b	0	0	
AT1G74890	RESPONSE REGULATOR 15 (ARR15)	Orphans a	0	0	
AT2G41310	RESPONSE REGULATOR 3 (RR3)	Orphans a	0	0	
AT3G14740	EXPRESSED IN: 21 plant structures	PHD a,b	0	0	
AT2G39000	INVOLVED IN: metabolic process	GNAT a	0	0	
AT1G61990	BEST Arabidopsis thaliana protein match is: Mitochondrial transcription termination factor family protein (TAIR:AT1G61960.1)	mTERF a	0	0	
AT2G34620	BEST Arabidopsis thaliana protein match is: Mitochondrial transcription termination factor family protein (TAIR:AT2G03050.1)	mTERF a	0	0	
AT1G31760	BEST Arabidopsis thaliana protein match is: SWIB/MDM2 domain superfamily protein (TAIR:AT2G35605.1)	SWI/SNF-BAF60b a	0	0	
AT5G50670	INVOLVED IN: biological_process unknown	SBP a,b,c	0	0	
AT5G14170	(CHC1)	SWI/SNF-BAF60b a	0	0	
AT4G14713	PEAPOD 1 (PPD1)	ZIM b,c	0	0	
AT1G70030	INVOLVED IN: regulation of transcription, DNA-dependent	Orphans a	0	0	
AT3G02860	INVOLVED IN: biological_process unknown	C2H2 d	0	0	
AT1G69030	EXPRESSED IN: 24 plant structures	BSD a	0	0	
AT2G16770	(bZIP23)	bZIP a,b,c	0	0	
AT1G36060	0	AP2-EREBP a,b,c	0	0	
AT1G62300	(WRKY6)	WRKY a,b,c	0	0	
AT4G25610	INVOLVED IN: biological_process unknown	C2H2 a,b,c	0	0	
AT5G50820	INVOLVED IN: multicellular organismal development, regulation of transcription	NAC a,b	0	0	
AT5G66770	BEST Arabidopsis thaliana protein match is: GRAS family transcription factor (TAIR:AT3G50650.1)	GRAS a,b	0	0	

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AT5G50010	BEST Arabidopsis thaliana protein match is: sequence-specific DNA binding transcription factors	bHLH d	0	0	
AT1G01260	INVOLVED IN: regulation of transcription	bHLH a,b	0	0	
AT1G08540	RNAPOLYMERASE SIGMA SUBUNIT 2 (SIG2)	SIGMA70-like a	0	0	
AT2G33835	FRIGIDA-ESSENTIAL 1 (FES1)	C3H a,b	0	0	
AT1G24260	SEPALLATA3 (SEP3)	MADS a,b	0	0	
AT1G61970	BEST Arabidopsis thaliana protein match is: Mitochondrial transcription termination factor family protein (TAIR:AT1G61980.1)	mTERF a	0	0	
AT2G33720	INVOLVED IN: regulation of transcription, DNA-dependent	REM(B3) d	0	0	
AT2G40740	protein_coding	WRKY a,b,c	0	0	
AT2G40750	protein_coding	WRKY a,b,c	0	0	
AT5G02030	REPLUMLESS (RPL)	HB a,b	0	0	
AT2G26960	MYB DOMAIN PROTEIN 81 (MYB81)	MYB a,b	0	0	
AT1G68190	INVOLVED IN: regulation of transcription	Orphans a	0	0	
AT5G65310	HOMEOBOX PROTEIN 5 (HB5)	HB a,b	0	0	
AT4G31660	INVOLVED IN: regulation of transcription, DNA-dependent	ABI3-VP1 a,b	0	0	
AT2G01200	INDOLE-3-ACETIC ACID INDUCIBLE 32 (IAA32)	AUX-IAA a,b	0	0	
AT5G47670	NUCLEAR FACTOR Y, SUBUNIT B6 (NF-YB6)	CCAAT a / CCAAT-HAP3 b	0	0	
AT3G27810	MYB DOMAIN PROTEIN 21 (MYB21)	MYB a,b	0	0	
AT3G66656	INVOLVED IN: regulation of transcription, DNA-dependent	MADS a,b	0	0	
AT5G58890	INVOLVED IN: regulation of transcription, DNA-dependent	MADS a,b	0	0	
AT1G17590	INVOLVED IN: regulation of transcription, DNA-dependent	CCAAT a / CCAAT-HAP2 b / CBFB_NFYA c	0	0	
AT1G74080	MYB DOMAIN PROTEIN 122 (MYB122)	MYB a,b	0	0	
AT1G60040	INVOLVED IN: regulation of transcription, DNA-dependent	MADS a,b	0	0	
AT1G69540	AGAMOUS-LIKE 94 (AGL94)	MADS a,b	0	0	
AT1G80390	INDOLE-3-ACETIC ACID INDUCIBLE 15 (IAA15)	AUX-IAA a,b	0	0	
AT3G07340	INVOLVED IN: regulation of transcription	bHLH a,b	0	0	
AT1G12610	DWARF AND DELAYED FLOWERING 1 (DDF1)	AP2-EREBP a,b,c	0	0	
AT1G51120	INVOLVED IN: regulation of transcription, DNA-dependent	AP2-EREBP a,b	0	0	
AT5G11590	TINY2 (TINY2)	AP2-EREBP a,b,c	0	0	
AT3G12680	ENHANCER OF AG-4 1 (HUA1)	C3H a,b	0	0	
AT3G48440	INVOLVED IN: biological_process unknown	C3H a,b	0	0	
AT3G55980	BEST Arabidopsis thaliana protein match is: zinc finger (CCCH-type) family protein (TAIR:AT2G40140.2)	C3H a,b	0	0	
AT2G43060	INVOLVED IN: regulation of transcription	bHLH d	0	0	
AT4G18960	AGAMOUS (AG)	MADS a,b	0	0	
AT1G28300	LEAFY COTYLEDON 2 (LEC2)	ABI3-VP1 a,b	0	0	
AT4G14490	BEST Arabidopsis thaliana protein match is: SMAD/FHA domain-containing protein (TAIR:AT3G02400.1)	FHA a,b	0	0	
AT2G48100	INVOLVED IN: biological_process unknown	C2H2 a,b	0	0	
AT4G37940	AGAMOUS-LIKE 21 (AGL21)	MADS a,b	0	0	
AT5G65080	MADS AFFECTING FLOWERING 5 (MAF5)	MADS a,b	0	0	
AT4G09100	LOCATED IN: endomembrane system	ND	0	0	
AT3G43430	EXPRESSED IN: 11 plant structures	ND	0	0	
AT1G18780	LOCATED IN: endomembrane system	ND	0	0	
AT1G18760	CONTAINS InterPro DOMAIN/s: Zinc finger, RING-type (InterPro:IPR001841), Zinc finger, C3HC4 RING-type (InterPro:IPR018957), Ubiquitin interacting motif (InterPro:IPR003903)	ND	0	0	
AT3G61630	CYTOKININ RESPONSE FACTOR 6 (CRF6)	AP2-EREBP a,b,c	0	0	
AT1G62360	SHOOT MERISTEMLESS (STM)	HB a,b	0	0	
AT4G08250	BEST Arabidopsis thaliana protein match is: RGA-like protein 3 (TAIR:AT5G17490.1)	GRAS a,b	0	0	
AT5G26170	protein_coding	WRKY a,b,c	0	0	
AT4G00210	BEST Arabidopsis thaliana protein match is: LOB domain-containing protein 19 (TAIR:AT2G45410.1)	LOB a / AS2 b	0	0	
AT1G09250	INVOLVED IN: regulation of transcription	ND	0	0	
AT3G56400	protein_coding	WRKY a,b,c	0	0	
AT2G04240	(XERICO)	ND	0	0	
AT3G47600	MYB DOMAIN PROTEIN 94 (MYB94)	MYB a,b	0	0	
AT1G72360	ETHYLENE RESPONSE FACTOR 73 (ERF73)	AP2-EREBP a,b,c	0	0	
AT5G17810	WUSCHEL RELATED HOMEOBOX 12 (WOX12)	HB a,b,c	0	0	
AT3G11020	DRE/CRT-BINDING PROTEIN 2B (DREB2B)	AP2-EREBP a,b,c	0	0	
AT3G03550	INVOLVED IN: response to karrikin	ND	0	0	
AT1G03790	SOMNUS (SOM)	C3H a,b	0	0	
AT1G25280	TUBBY LIKE PROTEIN 10 (TLP10)	TUB a,c	0	0	
AT4G22140	INVOLVED IN: positive regulation of flower development, regulation of transcription, DNA-dependent, seed germination	PHD a,b	0	0	
AT1G69010	INVOLVED IN: dTDP-rhamnose biosynthetic process, regulation of transcription	bHLH a,b	0	0	
AT4G13040	INVOLVED IN: regulation of transcription, DNA-dependent	AP2-EREBP a,b	0	0	

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AT4G39250	BEST Arabidopsis thaliana protein match is: Homeodomain-like superfamily protein (TAIR:AT2G21650.1)	MYB-related a,b	0	0	
AT3G05690	NUCLEAR FACTOR Y, SUBUNIT A2 (NF-YA2)	CCAAT a / CCAAT-HAP2 b / CBFB_NFYA c	0	0	
AT1G18570	MYB DOMAIN PROTEIN 51 (MYB51)	MYB a,b	0	0	
AT5G03780	TRF-LIKE 10 (TRFL10)	MYB-related d	0	0	
AT3G27010	protein_coding	TCP a,b,c	0	0	
AT1G68880	INVOLVED IN: regulation of transcription, DNA-dependent	bZIP a,b,c	0	0	
AT5G26930	GATA TRANSCRIPTION FACTOR 23 (GATA23)	C2C2-GATA a,b	0	0	
AT2G21060	GLYCINE-RICH PROTEIN 2B (GRP2B)	CSD a,c	0	0	
AT2G32460	MYB DOMAIN PROTEIN 101 (MYB101)	MYB a,b	0	0	
AT2G18280	TUBBY LIKE PROTEIN 2 (TLP2)	TUB a,c	0	0	
AT3G49760	INVOLVED IN: regulation of transcription, DNA-dependent	bZIP a,b,c	0	0	
AT5G08130	(BIM1)	bHLH a,b	0	0	
AT1G70920	INVOLVED IN: regulation of transcription, DNA-dependent, regulation of transcription	HB a,b	0	0	
AT5G24050	LOCATED IN: cellular_component unknown	REM(B3) d	0	0	
AT5G43650	INVOLVED IN: regulation of transcription	bHLH a,b	0	0	
AT5G26749	INVOLVED IN: biological_process unknown	C3H d	0	0	
AT1G77920	BEST Arabidopsis thaliana protein match is: TGA1A-related gene 3 (TAIR:AT1G22070.1)	bZIP a,b,c	0	0	
AT1G07640	(OBP2)	C2C2-DOF a,b,c	0	0	
AT3G23210	INVOLVED IN: regulation of transcription	bHLH a,b	0	0	
AT5G06420	LOCATED IN: membrane	C3H a,b	0	0	
AT3G19860	INVOLVED IN: regulation of transcription	bHLH a,b	0	0	
AT5G64810	protein_coding	WRKY a,b,c	0	0	
AT4G21440	MYB-LIKE 102 (MYB102)	MYB a,b	0	0	
AT2G14760	INVOLVED IN: regulation of transcription	bHLH a,b	0	0	
AT5G43620	INVOLVED IN: biological_process unknown	C2H2 d	0	0	
AT1G04500	BEST Arabidopsis thaliana protein match is: CCT motif family protein (TAIR:AT2G33350.2)	Orphans a / C2C2-CO-like b	0	0	
AT1G02170	METACASPASE 1 (MC1)	PEPTIDASE_C14 c	0	0	
AT1G13960	WRKY DNA-BINDING PROTEIN 4 (WRKY4)	WRKY a,b,c	0	0	
AT1G28470	BEST Arabidopsis thaliana protein match is: NAC domain containing protein 73 (TAIR:AT4G28500.1)	NAC a,b	0	0	
AT1G47270	TUBBY LIKE PROTEIN 6 (TLP6)	TUB a,c	0	0	
AT1G56010	NAC DOMAIN CONTAINING PROTEIN 1 (NAC1)	NAC a,b	0	0	
AT2G47850	INVOLVED IN: biological_process unknown	C3H a,b	0	0	
AT1G61730	INVOLVED IN: biological_process unknown	GeBP a,b	0	0	
AT4G00610	INVOLVED IN: biological_process unknown	GeBP a,b	0	0	
AT1G13400	NUBBIN (NUB)	C2H2 b	0	0	
AT1G46264	HEAT SHOCK TRANSCRIPTION FACTOR B4 (HSFB4)	HSF a,b,c	0	0	
AT1G48195	INVOLVED IN: biological_process unknown	C3H a,b	0	0	
AT5G11260	ELONGATED HYPOCOTYL 5 (HY5)	bZIP a,b,c	0	0	
AT1G01350	CONTAINS InterPro DOMAIN/s: Zinc finger, CCCH-type (InterPro:IPR000571), Zinc finger, RING-type, conserved site (InterPro:IPR017907), Zinc finger, RING-type (InterPro:IPR001841)	C3H a,b	0	0	
AT1G01920	Has 611 Blast hits to 608 proteins in 165 species: Archae - 0	SET a / PcG b	0	0	
AT1G60700	BEST Arabidopsis thaliana protein match is: Forkhead-associated (FHA) domain-containing protein (TAIR:AT3G54350.3)	FHA a,b	0	0	
AT1G75530	INVOLVED IN: biological_process unknown	FHA a,b	0	0	
AT2G21240	BASIC PENTACYSTEINE 4 (BPC4)	BBR-BPC a,b	0	0	
AT2G36010	E2F TRANSCRIPTION FACTOR 3 (E2F3)	E2F-DP a,b,c	0	0	
AT2G45480	GROWTH-REGULATING FACTOR 9 (GRF9)	GRF a,b	0	0	
AT4G10920	(KELP)	Coactivator p15 a	0	0	
AT4G36920	APETALA 2 (AP2)	AP2-EREBP a,b,c	0	0	
AT5G49420	INVOLVED IN: regulation of transcription, DNA-dependent	MADS a,b	0	0	
AT1G34370	SENSITIVE TO PROTON RHIZOTOXICITY 1 (STOP1)	C2H2 a,b,c	0	0	
AT3G06410	INVOLVED IN: biological_process unknown	C3H a,b	0	0	
AT3G24490	BEST Arabidopsis thaliana protein match is: sequence-specific DNA binding transcription factors (TAIR:AT3G14180.1)	TRIHELIX a,b	0	0	
AT5G65100	INVOLVED IN: regulation of transcription	EIL a,b	0	0	
AT1G19050	RESPONSE REGULATOR 7 (ARR7)	Orphans a	0	0	
AT2G23060	INVOLVED IN: metabolic process	GNAT a	0	0	
AT5G38740	INVOLVED IN: regulation of transcription, DNA-dependent	MADS a,b	0	0	
AT2G03060	AGAMOUS-LIKE 30 (AGL30)	MADS a,b	0	0	
AT1G60350	BEST Arabidopsis thaliana protein match is: NAC (No Apical Meristem) domain transcriptional regulator superfamily protein (TAIR:AT1G60300.1)	NAC a,b	0	0	
AT2G42410	ZINC FINGER PROTEIN 11 (ZFP11)	C2H2 a,b,c	0	0	
AT3G51060	STYLISH 1 (STY1)	SRS a,b	0	0	
AT5G54067	Has 30201 Blast hits to 17322 proteins in 780 species: Archae - 12	REM(B3) d	0	0	
AT1G75240	HOMEOBOX PROTEIN 33 (HB33)	zf-HD a,b	0	0	

Gene identifier (AGI)	Primary Gene Symbol	TF family	1 mM 3AT	5 mM 3AT	visual in-spec-tion
AT5G15850	CONSTANS-LIKE 1 (COL1)	C2C2-CO-like a,b	0	0	
AT1G34180	BEST Arabidopsis thaliana protein match is: NAC domain containing protein 17 (TAIR:AT1G34190.1)	NAC a,b	0	0	
AT1G69560	MYB DOMAIN PROTEIN 105 (MYB105)	MYB a,b	0	0	
AT3G10000	BEST Arabidopsis thaliana protein match is: Duplicated homeodomain-like superfamily protein (TAIR:AT5G03680.1)	TRIHELIX a,b	0	0	
AT5G56200	0	C2H2 a,b,c	0	0	
AT1G69570	BEST Arabidopsis thaliana protein match is: Dof-type zinc finger DNA-binding family protein (TAIR:AT1G26790.1)	C2C2-DOF a,b,c	0	0	
AT1G68120	BEST Arabidopsis thaliana protein match is: basic pentacysteine1 (TAIR:AT2G01930.2)	BBR-BPC a,b	0	0	
AT1G68480	JAGGED (JAG)	C2H2 b	0	0	
AT2G46735	Bacteria - 0	ND	0	0	
AT4G35580	NAC TRANSCRIPTION FACTOR-LIKE 9 (NTL9)	NAC a,b	0	0	
AT5G50915	INVOLVED IN: response to gibberellin stimulus	bHLH a,b	0	0	
AT5G65910	INVOLVED IN: biological_process unknown	BSD a	0	0	
AT5G25390	SHINE3 (SHN3)	AP2-EREBP a,b,c	0	0	
AT3G04930	BEST Arabidopsis thaliana protein match is: DNA-binding storekeeper protein-related transcriptional regulator (TAIR:AT5G28040.1)	GeBP a,b	0	0	
AT2G02450	INVOLVED IN: multicellular organismal development, regulation of transcription	NAC a,b	0	0	
AT1G10585	INVOLVED IN: regulation of transcription	bHLH a,b	0	0	
AT1G61980	INVOLVED IN: response to cold	mTERF a	0	0	
AT1G15340	METHYL-CPG-BINDING DOMAIN 10 (MBD10)	MBD c	0	0	
AT1G72740	INVOLVED IN: nucleosome assembly, regulation of transcription	MYB-related a,b	0	0	
AT4G33280	INVOLVED IN: regulation of transcription, DNA-dependent	ABI3-VP1 a,b	0	0	
AT2G29660	BEST Arabidopsis thaliana protein match is: zinc finger protein-related (TAIR:AT5G54630.1)	C2H2 a,b,c	0	0	
AT5G47790	BEST Arabidopsis thaliana protein match is: SMAD/FHA domain-containing protein (TAIR:AT5G38840.1)	FHA a,b	0	0	
AT1G67710	RESPONSE REGULATOR 11 (ARR11)	ARR-B a,b	0	0	
AT5G05330	0	HMG d	0	0	
AT3G56570	BEST Arabidopsis thaliana protein match is: Rubisco methyltransferase family protein (TAIR:AT1G14030.1)	SET a / PcG b	0	0	
AT4G38910	BASIC PENTACYSTEINE 5 (BPC5)	BBR-BPC a,b	0	0	
AT5G24330	ARABIDOPSIS TRITHORAX-RELATED PROTEIN 6 (ATXR6)	PHD a,b	0	0	
AT4G18830	OVATE FAMILY PROTEIN 5 (OFP5)	OFP a	0	0	
AT5G58360	BEST Arabidopsis thaliana protein match is: ovate family protein 4 (TAIR:AT1G06920.1)	OFP a	0	0	
AT5G22240	BEST Arabidopsis thaliana protein match is: ovate family protein 6 (TAIR:AT3G52525.1)	OFP a	0	0	
AT5G12980	BEST Arabidopsis thaliana protein match is: Cell differentiation, Rcd1-like protein (TAIR:AT3G20800.1)	RCD1-like a	0	0	
AT3G56230	BEST Arabidopsis thaliana protein match is: BTB/POZ domain-containing protein (TAIR:AT1G01640.2)	TRAF a	0	0	
AT3G53440	INVOLVED IN: biological_process unknown	ND	0	0	
AT1G08320	INVOLVED IN: regulation of transcription, DNA-dependent	bZIP a,b,c	0	0	
AT5G49200	INVOLVED IN: biological_process unknown	C3H a,b	0	0	
AT1G18750	AGAMOUS-LIKE 65 (AGL65)	MADS a,b	0	0	
AT5G05550	Has 359 Blast hits to 349 proteins in 25 species: Archae - 0	TRIHELIX a,b	0	0	
AT1G62700	ARABIDOPSIS NAC DOMAIN CONTAINING PROTEIN 26 (ANAC026)	NAC a,b	0	0	
AT4G34400	INVOLVED IN: regulation of transcription, DNA-dependent	ABI3-VP1 a,b	0	0	
AT5G53660	GROWTH-REGULATING FACTOR 7 (GRF7)	GRF a,b	0	0	
AT2G21650	MATERNAL EFFECT EMBRYO ARREST 3 (MEE3)	MYB-related d	0	0	
AT4G12850	BEST Arabidopsis thaliana protein match is: Far-red impaired responsive (FAR1) family protein (TAIR:AT2G43280.1)	FAR1 a	0	0	
AT2G36026	INVOLVED IN: biological_process unknown	OFP a	0	0	
AT4G34590	G-BOX BINDING FACTOR 6 (GBF6)	bZIP a,b,c	0	0	
AT1G18330	EARLY-PHYTOCHROME-RESPONSIVE1 (EPR1)	MYB-related a,b	0	0	
AT4G12350	MYB DOMAIN PROTEIN 42 (MYB42)	ND	0	0	
AT1G19350	BRI1-EMS-SUPPRESSOR 1 (BES1)	BES1 a,b	0	0	
AT5G25810	TINY (tny)	AP2-EREBP a,b,c	0	0	
AT5G06160	ATROPOS (ATO)	C2H2 d	0	0	
AT5G26610	INVOLVED IN: biological_process unknown	C2H2 b	0	0	
AT3G56380	RESPONSE REGULATOR 17 (RR17)	Orphans a	0	0	
AT5G25790	BEST Arabidopsis thaliana protein match is: Tesmin/TSO1-like CXC domain-containing protein (TAIR:AT4G29000.1)	CPP a,b	0	0	
AT5G41090	INVOLVED IN: multicellular organismal development, regulation of transcription	NAC a,b	0	0	
AT5G09250	(KIWI)	Coactivator p15 a	0	0	
AT3G07500	BEST Arabidopsis thaliana protein match is: Far-red impaired responsive (FAR1) family protein (TAIR:AT2G43280.1)	FAR1 a	0	0	
AT1G72030	INVOLVED IN: metabolic process	GNAT a	0	0	

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AT2G39030	N-ACETYLTRANSFERASE ACTIVITY 1 (NATA1)	GNAT a	0	0	
AT5G20240	PISTILLATA (PI)	MADS a,b	0	0	
AT3G15500	NAC DOMAIN CONTAINING PROTEIN 3 (NAC3)	NAC a,b	0	0	
AT3G02400	INVOLVED IN: regulation of transcription, DNA-dependent	FHA a,b	0	0	
AT3G53920	RNAPOLYMERASE SIGMA-SUBUNIT C (SIGC)	SIGMA70-like a	0	0	
AT2G27050	ETHYLENE-INSENSITIVE3-LIKE 1 (EIL1)	EIL a,b	0	0	
AT4G29230	INVOLVED IN: multicellular organismal development, regulation of transcription	NAC a,b	0	0	
AT4G37580	HOOKLESS 1 (HLS1)	GNAT a	0	0	
AT4G02990	BELAYA SMERT (BSM)	mTERF a	0	0	
AT4G31060	0	AP2-EREBP a,b,c	0	0	
AT5G60142	INVOLVED IN: regulation of transcription, DNA-dependent	ABI3-VP1 a / B3 c	0	0	
AT5G18000	VERDANDI (VDD)	ABI3-VP1 a,b	0	0	
AT1G55750	BEST Arabidopsis thaliana protein match is: BSD domain (BTF2-like transcription factors, Synapse-associated proteins and DOS2-like proteins) (TAIR:AT3G61420.1)	BSD a	0	0	
AT3G52910	GROWTH-REGULATING FACTOR 4 (GRF4)	GRF a,b	0	0	
AT4G37850	INVOLVED IN: regulation of transcription	bHLH a,b	0	0	
AT5G06770	BEST Arabidopsis thaliana protein match is: KH domain-containing protein / zinc finger (CCCH type) family protein (TAIR:AT3G12130.1)	C3H a,b	0	0	
AT2G39250	SCHNARCHZAPFEN (SNZ)	AP2-EREBP a,b,c	0	0	
AT5G03220	INVOLVED IN: regulation of transcription from RNA polymerase II promoter	MED7 a	0	0	
AT2G04740	Has 35333 Blast hits to 34131 proteins in 2444 species: Archae - 798	TRAF a	0	0	
AT1G76900	TUBBY LIKE PROTEIN 1 (TLP1)	TUB a,c	0	0	
AT3G28730	HIGH MOBILITY GROUP (HMG)	HMG a,b	0	0	
AT4G28030	INVOLVED IN: metabolic process	GNAT a	0	0	
AT1G62010	BEST Arabidopsis thaliana protein match is: Mitochondrial transcription termination factor family protein (TAIR:AT1G62120.1)	mTERF a	0	0	
AT1G74120	BEST Arabidopsis thaliana protein match is: plastid transcriptionally active 15 (TAIR:AT5G54180.1)	mTERF a	0	0	
AT2G36000	INVOLVED IN: biological_process unknown	mTERF a	0	0	
AT1G79220	BEST Arabidopsis thaliana protein match is: Mitochondrial transcription termination factor family protein (TAIR:AT5G64950.1)	mTERF a	0	0	
AT2G23290	MYB DOMAIN PROTEIN 70 (MYB70)	MYB a,b	0	0	
AT2G32100	BEST Arabidopsis thaliana protein match is: ovate family protein 12 (TAIR:AT1G05420.1)	OFP a	0	0	
AT1G76590	BEST Arabidopsis thaliana protein match is: PLATZ transcription factor family protein (TAIR:AT1G21000.1)	PLATZ a,b	0	0	
AT2G46260	LIGHT-RESPONSE BTB 1 (LRB1)	TRAF a	0	0	
AT1G24210	INVOLVED IN: regulation of transcription, DNA-dependent	Orphans a	0	0	
AT3G63030	METHYL-CPG-BINDING DOMAIN 4 (MBD4)	zf-CW c	0	0	
AT4G17490	ETHYLENE RESPONSIVE ELEMENT BINDING FACTOR 6 (ERF6)	AP2-EREBP a,b,c	0	0	
AT5G07500	(PEI1)	C3H a,b	0	0	
AT5G41380	BEST Arabidopsis thaliana protein match is: CCT motif family protein (TAIR:AT1G63820.1)	Orphans a / C2C2-CO-like b	0	0	
AT3G53820	INVOLVED IN: regulation of transcription	C2H2 a,b	0	0	
AT4G18450	0	AP2-EREBP a,b,c	0	0	
AT1G16490	MYB DOMAIN PROTEIN 58 (MYB58)	MYB a,b	0	0	
AT5G54470	INVOLVED IN: response to cold, regulation of transcription	Orphans a	0	0	
AT2G34830	protein_coding	WRKY a,b,c	0	0	
AT5G62610	INVOLVED IN: regulation of transcription	bHLH a,b	0	0	
AT3G24010	INHIBITOR OF GROWTH 1 (ING1)	PHD a,b	0	0	
AT4G25990	(CIL)	Orphans a / C2C2-CO-like b	0	0	
AT1G32070	NUCLEAR SHUTTLE INTERACTING (NSI)	GNAT a	0	0	
AT2G03340	WRKY DNA-BINDING PROTEIN 3 (WRKY3)	WRKY a,b,c	0	0	
AT1G22130	AGAMOUS-LIKE 104 (AGL104)	MADS a,b	0	0	
AT1G54360	TBP-ASSOCIATED FACTOR 6B (TAF6B)	ND	0	0	
AT5G27090	INVOLVED IN: regulation of transcription, DNA-dependent	MADS a,b	0	0	
AT1G68800	TCP DOMAIN PROTEIN 12 (TCP12)	TCP a,b,c	0	0	
AT5G17800	MYB DOMAIN PROTEIN 56 (MYB56)	MYB a,b	0	0	
AT1G56650	PRODUCTION OF ANTHOCYANIN PIGMENT 1 (PAP1)	MYB a,b	0	0	
AT3G61120	INVOLVED IN: regulation of transcription, DNA-dependent	MADS a,b	0	0	
AT1G20693	HIGH MOBILITY GROUP B2 (HMGB2)	HMG a,b	0	0	
AT4G36780	BEST Arabidopsis thaliana protein match is: BES1/BZR1 homolog 1 (TAIR:AT3G50750.1)	BES1 a,b	0	0	
AT2G02080	LOCATED IN: intracellular, chloroplast	C2H2 a,b,c	0	0	
AT4G28530	INVOLVED IN: multicellular organismal development, regulation of transcription	NAC a,b	0	0	
AT4G38680	GLYCINE RICH PROTEIN 2 (GRP2)	CSD a,c	0	0	
AT1G72310	(ATL3)	ND	0	0	
AT5G67000	0	AP2-EREBP a,b,c	0	0	

Gene identifier (AGI)	Primary Gene Symbol	TF family	1 mM 3AT	5 mM 3AT	visual in-spec-tion
AT1G07980	INVOLVED IN: regulation of transcription	CCAAT a / CCAAT-HAP5 b	0	0	
AT3G23220	ETHYLENE AND SALT INDUCIBLE 1 (ESE1)	AP2-EREBP a,b,c	0	0	
AT2G02740	WHIRLY 3 (WHY3)	PBF-2-like a / WHIRLY b	0	0	
AT3G16870	GATA TRANSCRIPTION FACTOR 17 (GATA17)	C2C2-GATA a,b	0	0	
AT4G31620	INVOLVED IN: regulation of transcription, DNA-dependent	ABI3-VP1 a,b	0	0	
AT5G47140	GATA TRANSCRIPTION FACTOR 27 (GATA27)	C2C2-GATA a,b	0	0	
AT5G58010	LJRHL1-LIKE 3 (LRL3)	bHLH a,b	0	0	
AT5G18680	TUBBY LIKE PROTEIN 11 (TLP11)	TUB a,c	0	0	
AT1G50680	INVOLVED IN: regulation of transcription, DNA-dependent	AP2-EREBP a,b	0	0	
AT1G52890	NAC DOMAIN CONTAINING PROTEIN 19 (NAC019)	NAC a,b	0	0	
AT4G37260	MYB DOMAIN PROTEIN 73 (MYB73)	MYB a,b	0	0	
AT1G20910	INVOLVED IN: regulation of transcription	ARID a,b	0	0	
AT3G01030	LOCATED IN: intracellular	C2H2 b	0	0	
AT3G19500	INVOLVED IN: regulation of transcription	ND	0	0	
AT3G24820	BEST Arabidopsis thaliana protein match is: BSD domain-containing protein (TAIR:AT1G69030.1)	BSD a	0	0	
AT5G35330	METHYL-CPG-BINDING DOMAIN PROTEIN 02 (MBD02)	ND	0	0	
AT4G24060	BEST Arabidopsis thaliana protein match is: Dof-type zinc finger DNA-binding family protein (TAIR:AT1G64620.1)	C2C2-DOF a,b,c	0	0	
AT2G05900	SET DOMAIN PROTEIN 11 (SDG11)	SET a / PcG b	0	0	
AT1G11510	BEST Arabidopsis thaliana protein match is: DNA-binding storekeeper protein-related transcriptional regulator (TAIR:AT1G61730.1)	GeBP a,b	0	0	
AT5G51980	INVOLVED IN: biological_process unknown	C3H a,b	0	0	
AT2G17180	DUO1-ACTIVATED ZINC FINGER 1 (DAZ1)	C2H2 a,b,c	0	0	
AT3G55210	BEST Arabidopsis thaliana protein match is: NAC domain containing protein 93 (TAIR:AT5G39690.1)	NAC a,b	0	0	
AT5G61470	INVOLVED IN: regulation of transcription	C2H2 a,b,c	0	0	
AT4G05630	Has 22 Blast hits to 22 proteins in 3 species: Archae - 0	ND	0	0	
AT4G31615	INVOLVED IN: regulation of transcription, DNA-dependent	ABI3-VP1 a,b	0	0	
AT5G24120	SIGMA FACTOR E (SIGE)	SIGMA70-like a	0	0	
AT3G52540	LOCATED IN: chloroplast	OFP a	0	0	
AT5G67450	ZINC-FINGER PROTEIN 1 (ZF1)	C2H2 a,b	0	0	
AT1G32360	BEST Arabidopsis thaliana protein match is: Zinc finger C-x8-C-x5-C-x3-H type family protein (TAIR:AT2G35430.1)	C3H a,b	0	0	
AT4G04030	Has 1 Blast hits to 1 proteins in 1 species: Archae - 0	OFP a	0	0	
AT5G59380	METHYL-CPG-BINDING DOMAIN 6 (MBD6)	MBD c	0	0	
AT5G37260	REVEILLE 2 (RVE2)	MYB-related a,b,c	0	0	
AT1G31040	BEST Arabidopsis thaliana protein match is: PLATZ transcription factor family protein (TAIR:AT2G12646.1)	PLATZ a,b	0	0	
AT5G35770	STERILE APETALA (SAP)	SAP a,b	0	0	
AT2G45120	INVOLVED IN: regulation of transcription	C2H2 a,b,c	0	0	
AT2G39020	0	GNAT a	0	0	
AT3G20640	INVOLVED IN: regulation of transcription	ND	0	0	
AT1G28520	Has 77 Blast hits to 70 proteins in 13 species: Archae - 0	VOZ a,b	0	0	
AT2G35550	BEST Arabidopsis thaliana protein match is: basic pentacysteine1 (TAIR:AT2G01930.2)	BBR-BPC a,b	0	0	
AT4G36020	COLD SHOCK DOMAIN PROTEIN 1 (CSDP1)	CSD a,c	0	0	
AT2G18120	SHI-RELATED SEQUENCE 4 (SRS4)	SRS a,b	0	0	
AT3G04100	INVOLVED IN: regulation of transcription, DNA-dependent	MADS a,b	0	0	
AT2G28910	CAX INTERACTING PROTEIN 4 (CXIP4)	ND	0	0	
AT5G10380	(RING1)	ND	0	0	
AT4G37180	protein_coding	G2-like a,b	0	0	
AT5G04110	INVOLVED IN: DNA topological change, DNA metabolic process	MYB-related a	0	0	
AT2G37060	INVOLVED IN: regulation of transcription, DNA-dependent	CCAAT a / CCAAT-HAP3 b	0	0	
AT4G00238	LOCATED IN: nucleolus	GeBP a,b	0	0	
AT4G32280	INDOLE-3-ACETIC ACID INDUCIBLE 29 (IAA29)	AUX-IAA a,b	0	0	
AT1G22810	0	AP2-EREBP a,b,c	0	0	
AT5G26630	INVOLVED IN: regulation of transcription, DNA-dependent	MADS a,b	0	0	
AT5G52600	MYB DOMAIN PROTEIN 82 (MYB82)	MYB a,b	0	0	
AT5G15060	BEST Arabidopsis thaliana protein match is: LOB domain-containing protein 22 (TAIR:AT3G13850.1)	LOB a	0	0	
AT1G53320	TUBBY LIKE PROTEIN 7 (TLP7)	TUB a,c	0	0	
AT1G20696	HIGH MOBILITY GROUP B3 (HMGB3)	HMG a,b	0	0	
AT1G04550	INDOLE-3-ACETIC ACID INDUCIBLE 12 (IAA12)	AUX-IAA a,b	0	0	
AT1G54390	INHIBITOR OF GROWTH 2 (ING2)	PHD a,b	0	0	
AT5G51870	AGAMOUS-LIKE 71 (AGL71)	MADS a,b	0	0	
AT5G62430	CYCLING DOF FACTOR 1 (CDF1)	C2C2-DOF a,b,c	0	0	
AT1G28050	INVOLVED IN: regulation of transcription	C2C2-CO-like a,b	0	0	
AT2G34710	PHABULOSA (PHB)	HB a,b	0	0	

Gene identifier (AGI)	Primary Gene Symbol	TF family	1 mM 3AT	5 mM 3AT	visual in-spec-tion
AT3G60400	BEST Arabidopsis thaliana protein match is: Mitochondrial transcription termination factor family protein (TAIR:AT5G06810.1)	mTERF a	0	0	
AT1G32870	BEST Arabidopsis thaliana protein match is: NAC domain containing protein 16 (TAIR:AT1G34180.1)	NAC a,b	0	0	
AT3G20840	PLETHORA 1 (PLT1)	AP2-EREBP a,b,c	0	0	
AT2G20110	LOCATED IN: cellular_component unknown	CPP a,b	0	0	
AT1G25550	BEST Arabidopsis thaliana protein match is: myb-like transcription factor family protein (TAIR:AT1G68670.1)	G2-like a,b	0	0	
AT4G32880	HOMEOBOX GENE 8 (HB-8)	HB a,b	0	0	
AT3G16350	BEST Arabidopsis thaliana protein match is: myb-like transcription factor family protein (TAIR:AT5G47390.1)	MYB-related a,b	0	0	
AT1G73100	SU(VAR)3-9 HOMOLOG 3 (SUVH3)	SET a / PcG b	0	0	
AT2G16910	ABORTED MICROSPORES (AMS)	bHLH a,b	0	0	
AT1G32130	(IWS1)	IWS1 a	0	0	
AT3G10500	NAC DOMAIN CONTAINING PROTEIN 53 (NAC053)	NAC a,b	0	0	
AT4G22070	protein_coding	WRKY a,b,c	0	0	
AT3G54320	WRINKLED 1 (WRI1)	AP2-EREBP a,b,c	0	0	
AT1G49010	BEST Arabidopsis thaliana protein match is: Duplicated homeodomain-like superfamily protein (TAIR:AT5G08520.1)	MYB a,b	0	0	
AT2G10950	BEST Arabidopsis thaliana protein match is: BSD domain-containing protein (TAIR:AT3G49800.1)	BSD a	0	0	
AT2G44840	ETHYLENE-RESPONSIVE ELEMENT BINDING FACTOR 13 (ERF13)	AP2-EREBP a,b,c	0	0	
AT5G02460	BEST Arabidopsis thaliana protein match is: DNA binding with one finger 2.4 (TAIR:AT2G37590.1)	C2C2-DOF a,b,c	0	0	
AT4G36620	GATA TRANSCRIPTION FACTOR 19 (GATA19)	C2C2-GATA a,b	0	0	
AT4G18170	protein_coding	WRKY a,b,c	0	0	
AT3G57390	AGAMOUS-LIKE 18 (AGL18)	MADS a,b	0	0	
AT5G13790	AGAMOUS-LIKE 15 (AGL15)	MADS a,b	0	0	
AT3G57040	RESPONSE REGULATOR 9 (ARR9)	Orphans a	0	0	
AT5G38490	LOCATED IN: cellular_component unknown	REM(B3) d	0	0	
AT1G10586	INVOLVED IN: regulation of transcription	bHLH a	0	0	
AT1G21780	protein_coding	TRAF a	0	0	
AT2G46400	protein_coding	WRKY a,b,c	0	0	
AT3G18650	INVOLVED IN: regulation of transcription, DNA-dependent	MADS a,b	0	0	
AT1G32150	BASIC REGION/LEUCINE ZIPPER TRANSCRIPTION FACTOR 68 (bZIP68)	bZIP a,b	0	0	
AT3G21810	INVOLVED IN: biological_process unknown	C3H a,b	0	0	
AT4G21030	BEST Arabidopsis thaliana protein match is: Dof-type zinc finger domain-containing protein (TAIR:AT4G21050.1)	C2C2-DOF a,b,c	0	0	
AT3G01140	MYB DOMAIN PROTEIN 106 (MYB106)	MYB a,b	0	0	
AT3G24500	MULTIPROTEIN BRIDGING FACTOR 1C (MBF1C)	MBF1 a,b	0	0	
AT4G01500	BEST Arabidopsis thaliana protein match is: AP2/B3-like transcriptional factor family protein (TAIR:AT1G01030.1)	ABI3-VP1 a,b	0	0	
AT2G17870	COLD SHOCK DOMAIN PROTEIN 3 (CSP3)	CSD a,c	0	0	
AT2G21400	SHI-RELATED SEQUENCE3 (SRS3)	SRS a,b	0	0	
AT2G21900	protein_coding	WRKY a,b,c	0	0	
AT5G05770	WUSCHEL RELATED HOMEOBOX 7 (WOX7)	HB a,b,c	0	0	
AT5G64610	HISTONE ACETYLTRANSFERASE OF THE MYST FAMILY 1 (HAM1)	C2H2 b	0	0	
AT4G36740	HOMEOBOX PROTEIN 40 (HB40)	HB a,b,c	0	0	
AT5G50320	ELONGATA 3 (ELO3)	GNAT a	0	0	
AT4G11400	FUNCTIONS IN: DNA binding	ARID a,b	0	0	
AT2G46790	PSEUDO-RESPONSE REGULATOR 9 (PRR9)	Pseudo ARR-B a / C2C2-CO-like b	0	0	
AT5G06100	MYB DOMAIN PROTEIN 33 (MYB33)	MYB a,b	0	0	
AT1G02230	INVOLVED IN: multicellular organismal development, regulation of transcription	NAC a,b	0	0	
AT4G14225	INVOLVED IN: biological_process unknown	zf-AN1 c	0	0	
AT5G39810	INVOLVED IN: regulation of transcription, DNA-dependent	MADS a,b	0	0	
AT4G17980	INVOLVED IN: multicellular organismal development, regulation of transcription	NAC a,b	0	0	
AT1G25310	Has 109 Blast hits to 109 proteins in 9 species: Archae - 0	bHLH a / HLH c	0	0	
AT2G33500	INVOLVED IN: regulation of transcription	C2C2-CO-like a,b	0	0	
AT3G10113	BEST Arabidopsis thaliana protein match is: Homeodomain-like superfamily protein (TAIR:AT1G18330.2)	MYB-related a,b,c	0	0	
AT4G25410	INVOLVED IN: regulation of transcription	bHLH a,b	0	0	
AT3G61740	INVOLVED IN: regulation of transcription, DNA-dependent	PHD a,b	0	0	
AT4G39160	EXPRESSED IN: 15 plant structures	MYB-related a,b	0	0	
AT5G20730	NON-PHOTOTROPHIC HYPOCOTYL (NPH4)	ARF a,b,c	0	0	
AT2G47070	SQUAMOSA PROMOTER BINDING PROTEIN-LIKE 1 (SPL1)	SBP a,b,c	0	0	
AT2G27230	LONESOME HIGHWAY (LHW)	bHLH d	0	0	
AT4G23980	AUXIN RESPONSE FACTOR 9 (ARF9)	ARF a,b,c	0	0	
AT5G37020	AUXIN RESPONSE FACTOR 8 (ARF8)	ARF a,b,c	0	0	
AT3G54220	protein_coding	GRAS a,b	0	0	

Gene identifier (AGI)	Primary Gene Symbol	TF family	1 mM 3AT	5 mM 3AT	visual in-spec-tion
AT2G36960	TSL-KINASE INTERACTING PROTEIN 1 (TKI1)	MYB-related a,b	0	0	
AT1G32640	(MYC2)	bHLH a,b	0	0	
AT2G32700	LEUNIG_HOMOLOG (LUH)	LUG a,b	0	0	
AT5G62000	AUXIN RESPONSE FACTOR 2 (ARF2)	ARF a,b,c	0	0	
AT3G19510	(HAT3.1)	HB a,b	0	0	
AT1G58220	INVOLVED IN: response to jasmonic acid stimulus, response to abscisic acid stimulus, response to salicylic acid stimulus	MYB-related a,b	0	0	
AT5G64220	INVOLVED IN: regulation of transcription	CAMTA a,b	0	0	
AT3G43240	LOCATED IN: intracellular	ARID a,b	0	0	
AT2G36720	INVOLVED IN: regulation of transcription, DNA-dependent	PHD a,b	0	0	
AT3G12280	RETINOBLASTOMA-RELATED 1 (RBR1)	RB a	0	0	
AT1G05230	HOMEODOMAIN GLABROUS 2 (HDG2)	HB a,b	0	0	
AT5G56270	WRKY DNA-BINDING PROTEIN 2 (WRKY2)	WRKY a,b,c	0	0	
AT3G20770	ETHYLENE-INSENSITIVE3 (EIN3)	EIL a,b	0	0	
AT4G14770	INVOLVED IN: regulation of transcription	CPP a,b	0	0	
AT2G17410	INVOLVED IN: regulation of transcription	ARID a,b	0	0	
AT1G79350	INVOLVED IN: regulation of transcription, DNA-dependent, embryo development ending in seed dormancy	PHD a,b	0	0	
AT3G08020	EXPRESSED IN: 23 plant structures	PHD b	0	0	
AT3G10800	protein_coding	bZIP a,b,c	0	0	
AT5G43990	(SUVR2)	SET a / PcG b	0	0	
AT1G62310	EXPRESSED IN: 22 plant structures	JUMONJI a,b	0	0	
AT2G22300	SIGNAL RESPONSIVE 1 (SR1)	CAMTA a,b	0	0	
AT3G19210	HOMOLOG OF RAD54 (RAD54)	SNF2 a	0	0	
AT3G53680	INVOLVED IN: regulation of transcription, DNA-dependent	PHD a,b	0	0	
AT3G54350	BEST Arabidopsis thaliana protein match is: Forkhead-associated (FHA) domain-containing protein (TAIR:AT1G75530.1)	FHA a,b	0	0	
AT1G10240	INVOLVED IN: response to red or far red light	FAR1 a	0	0	
AT4G37670	INVOLVED IN: cellular amino acid biosynthetic process, arginine biosynthetic process, metabolic process	GNAT a	0	0	
AT1G62830	LSD1-LIKE 1 (LDL1)	SWI/SNF-SWI3 a	0	0	
AT3G10390	FLOWERING LOCUS D (FLD)	SWI/SNF-SWI3 a	0	0	
AT5G19330	ARM REPEAT PROTEIN INTERACTING WITH ABF2 (ARIA)	TRAF a	0	0	
AT1G10170	NF-X-LIKE 1 (NFXL1)	zf-NF-X1 c	0	0	
AT1G59890	INVOLVED IN: regulation of transcription, DNA-dependent	Orphans a	0	0	
AT5G52230	METHYL-CPG-BINDING DOMAIN PROTEIN 13 (MBD13)	ND	0	0	
AT4G32730	(PC-MYB1)	MYB a,b	0	0	
AT5G05130	EXPRESSED IN: 24 plant structures	SNF2 a	0	0	
AT5G27610	INVOLVED IN: biological_process unknown	MYB-related a,b	0	0	
AT4G00730	ANTHOCYANINLESS 2 (ANL2)	HB a,b	0	0	
AT1G50410	EXPRESSED IN: 24 plant structures	SNF2 a	0	0	
AT4G00990	EXPRESSED IN: 20 plant structures	JUMONJI a,b	0	0	
AT2G17150	BEST Arabidopsis thaliana protein match is: Plant regulator RWP-RK family protein (TAIR:AT4G35270.1)	RWP-RK a / NIN-like b	0	0	
AT3G60030	INVOLVED IN: regulation of transcription	SBP a,b,c	0	0	
AT2G02160	INVOLVED IN: biological_process unknown	C3H a,b	0	0	
AT5G28300	INVOLVED IN: regulation of transcription	TRIHELIX a,b	0	0	
AT2G02090	LOCATED IN: chloroplast	SNF2 a	0	0	
AT2G02070	INVOLVED IN: regulation of transcription	C2H2 a,b,c	0	0	
AT1G09060	FUNCTIONS IN: sequence-specific DNA binding transcription factor activity, zinc ion binding	JUMONJI a,b	0	0	
AT3G52250	0	MYB a,b	0	0	
AT4G24020	NIN LIKE PROTEIN 7 (NLP7)	RWP-RK a / NIN-like b	0	0	
AT5G53430	protein_coding	PHD a,b	0	0	
AT5G45260	RESISTANT TO RALSTONIA SOLANACEARUM 1 (RRS1)	WRKY a	0	0	
AT3G18640	EXPRESSED IN: 18 plant structures	C3H a	0	0	
AT4G21430	INVOLVED IN: biological_process unknown	Orphans a	0	0	
AT2G32250	INVOLVED IN: response to red or far red light	FAR1 a	0	0	
AT4G15090	FAR-RED IMPAIRED RESPONSE 1 (FAR1)	FAR1 a	0	0	
AT3G01320	SIN3-LIKE 1 (SNL1)	Orphans a	0	0	
AT4G35270	BEST Arabidopsis thaliana protein match is: Plant regulator RWP-RK family protein (TAIR:AT2G17150.1)	RWP-RK a / NIN-like b	0	0	
AT5G19310	INVOLVED IN: biological_process unknown	SNF2 a	0	0	
AT5G22750	(RAD5)	SNF2 a	0	0	
AT1G07530	SCARECROW-LIKE 14 (SCL14)	GRAS a,b	0	0	
AT1G30490	PHAVOLUTA (PHV)	HB a,b	0	0	
AT1G59750	AUXIN RESPONSE FACTOR 1 (ARF1)	ARF a,b,c	0	0	
AT3G05380	CONTAINS InterPro DOMAIN/s: SANT, DNA-binding (InterPro:IPR001005), DIRP (InterPro:IPR010561)	MYB-related a,b	0	0	
AT1G30810	JUMONJI DOMAIN-CONTAINING PROTEIN 18 (JMJ18)	JUMONJI a,b	0	0	
AT4G34430	(CHB3)	MYB-related a / SWIRM c	0	0	

Gene identifier (AGI)	Primary Gene Symbol	TF family	1 mM 3AT	5 mM 3AT	visual in-spec-tion
AT1G79840	GLABRA 2 (GL2)	HB a,b	0	0	
AT5G18830	SQUAMOSA PROMOTER BINDING PROTEIN-LIKE 7 (SPL7)	SBP a,b,c	0	0	
AT5G66630	INVOLVED IN: apoptosis	Orphans a / LIM b	0	0	
AT4G20400	JUMONJI 14 (JMJ14)	JUMONJI a,b,c	0	0	
AT2G44430	EXPRESSED IN: stem, male gametophyte, flower, carpel	MYB-related a,b	0	0	
AT2G28450	INVOLVED IN: acetate biosynthetic process from carbon monoxide, methanol oxidation, RNA processing	C3H a,b	0	0	
AT5G51230	EMBRYONIC FLOWER 2 (EMF2)	C2H2 d	0	0	
AT2G18090	INVOLVED IN: regulation of transcription, DNA-dependent	SWI/SNF-BAF60b a / PHD b	FALSE	0	
AT1G32810	EXPRESSED IN: 22 plant structures	PHD a	FALSE	0	
AT5G65490	LOCATED IN: chloroplast	SGT1 c	FALSE	0	
AT5G04940	SU(VAR)3-9 HOMOLOG 1 (SUVH1)	SET a / PcG b	FALSE	0	
AT1G04050	HOMOLOG OF SU(VAR)3-9 1 (SUVR1)	SET a / PcG b	FALSE	0	
AT5G42700	INVOLVED IN: regulation of transcription, DNA-dependent	ABI3-VP1 a,b	0	0	
AT4G28640	INDOLE-3-ACETIC ACID INDUCIBLE 11 (IAA11)	AUX-IAA a,b	0	0	
AT5G47390	BEST Arabidopsis thaliana protein match is: Homeodomain-like superfamily protein (TAIR:AT3G16350.1)	MYB-related a,b	0	0	
AT4G24240	WRKY DNA-BINDING PROTEIN 7 (WRKY7)	WRKY a,b,c	0	0	
AT2G31460	BEST Arabidopsis thaliana protein match is: Domain of unknown function (DUF313) (TAIR:AT2G27410.1)	REM(B3) d	0	0	
AT5G27130	INVOLVED IN: regulation of transcription, DNA-dependent	MADS a,b	0	0	
AT2G16400	INVOLVED IN: regulation of transcription, DNA-dependent, regulation of transcription	HB a,b	0	0	
AT5G66700	HOMEOBOX 53 (HB53)	HB a,b,c	0	0	
AT1G14510	ALFIN-LIKE 7 (AL7)	ALFIN-like a,b	0	0	
AT3G18960	INVOLVED IN: regulation of transcription, DNA-dependent	ABI3-VP1 a,b	0	0	
AT2G30420	ENHANCER OF TRY AND CPC 2 (ETC2)	MYB-related a,b	0	0	
AT1G02040	LOCATED IN: intracellular	C2H2 a,b,c	0	0	
AT1G51970	Has 29 Blast hits to 29 proteins in 4 species: Archae - 0	REM(B3) d	0	0	
AT1G04850	INVOLVED IN: biological_process unknown	C2H2 d	0	0	
AT5G04820	LOCATED IN: endomembrane system	OFP a	0	0	
AT1G49130	INVOLVED IN: regulation of transcription	C2C2-CO-like a,b	0	0	
AT5G06250	BEST Arabidopsis thaliana protein match is: AP2/B3-like transcriptional factor family protein (TAIR:AT3G11580.1)	ABI3-VP1 a,b	0	0	
AT5G67430	INVOLVED IN: metabolic process	GNAT a	0	0	
AT1G16060	ARIA-INTERACTING DOUBLE AP2 DOMAIN PROTEIN (ADAP)	AP2-EREBP a,b,c	0	0	
AT1G33760	0	AP2-EREBP a,b,c	0	0	
AT3G18990	REDUCED VERNALIZATION RESPONSE 1 (VRN1)	ABI3-VP1 a,b	0	0	
AT3G61230	PLIM2C (PLIM2c)	LIM a,b	0	0	
AT3G05860	INVOLVED IN: regulation of transcription, DNA-dependent	MADS a,b	0	0	
AT5G42640	INVOLVED IN: regulation of transcription	C2H2 a,b,c	0	0	
AT2G15660	INVOLVED IN: regulation of transcription, DNA-dependent	ND	0	0	
AT4G15250	LOCATED IN: intracellular	C2C2-CO-like a,b	0	0	
AT1G08880	-H2AX foci throughout the chromatin. However, their accumulation is not contemporaneous with that of AtSPO11-1. At 3 h post-S, no #947	CCAAT a	0	0	
AT5G27880	INVOLVED IN: regulation of transcription	C2H2 a,b,c	0	0	
AT5G61620	BEST Arabidopsis thaliana protein match is: myb-like transcription factor family protein (TAIR:AT5G47390.1)	MYB-related a,b	0	0	
AT3G07260	BEST Arabidopsis thaliana protein match is: SMAD/FHA domain-containing protein (TAIR:AT3G07220.1)	FHA a,b	0	0	
AT3G48360	BTB AND TAZ DOMAIN PROTEIN 2 (bt2)	TRAF a	0	0	
AT3G08505	Zinc finger, C3HC4	C3H a,b	0	0	
AT5G28040	EXPRESSED IN: 25 plant structures	GeBP a,b	0	0	
AT1G35240	INVOLVED IN: regulation of transcription	ARF a,b,c	0	0	
AT3G50330	INVOLVED IN: ovary septum development, transmitting tissue development, carpel formation, regulation of transcription	bHLH a,b	0	0	
AT5G16820	HEAT SHOCK FACTOR 3 (HSF3)	HSF a,b,c	0	0	
AT4G38160	INVOLVED IN: biological_process unknown	mTERF a	0	0	
AT5G46350	protein_coding	WRKY a,b,c	0	0	
AT4G36060	INVOLVED IN: regulation of transcription	bHLH a,b	0	0	
AT1G72050	TRANSCRIPTION FACTOR IIIA (TFIIIA)	C2H2 a,b,c	0	0	
AT1G63650	ENHANCER OF GLABRA 3 (EGL3)	bHLH a,b	0	0	
AT5G60140	INVOLVED IN: regulation of transcription, DNA-dependent	ABI3-VP1 a,b	0	0	
AT4G31920	RESPONSE REGULATOR 10 (RR10)	ARR-B a,b	0	0	
AT2G17600	BEST Arabidopsis thaliana protein match is: Cysteine/Histidine-rich C1 domain family protein (TAIR:AT2G17590.1)	ND	0	0	
AT5G63080	BEST Arabidopsis thaliana protein match is: transferases, transferring glycosyl groups (TAIR:AT1G78280.1)	JUMONJI a,b	0	0	
AT3G59060	PHYTOCHROME INTERACTING FACTOR 3-LIKE 6 (PIL6)	bHLH a,b	0	0	
AT1G67370	ASYNAPTIC 1 (ASY1)	SWI/SNF-SWI3 a	0	0	
AT2G39830	DA1-RELATED PROTEIN 2 (DAR2)	Orphans a / LIM b	0	0	
AT4G21340	INVOLVED IN: regulation of transcription	ND	0	0	

Gene identifier (AGI)	Primary Gene Symbol	TF family	1 mM 3AT	5 mM 3AT	visual in-spec-tion
AT2G27760	TRNAISOPENTENYLTRANSFERASE 2 (IPT2)	C2H2 d	0	0	
AT3G51950	INVOLVED IN: biological_process unknown	C3H a,b	0	0	
AT2G05330	BEST Arabidopsis thaliana protein match is: BTB/POZ domain-containing protein (TAIR:AT5G48510.1)	TRAF a	0	0	
AT5G12850	BEST Arabidopsis thaliana protein match is: CCCH-type zinc finger protein with ARM repeat domain (TAIR:AT2G41900.1)	C3H a,b	0	0	
AT5G62940	HIGH CAMBIAL ACTIVITY2 (HCA2)	C2C2-DOF a,b,c	0	0	
AT5G66300	NAC DOMAIN CONTAINING PROTEIN 105 (NAC105)	NAC a,b	0	0	
AT4G26640	protein_coding	WRKY a,b,c	0	0	
AT4G17230	SCARECROW-LIKE 13 (SCL13)	GRAS a,b	0	0	
AT1G14440	INVOLVED IN: regulation of transcription	zf-HD a,b	0	0	
AT1G75410	BEL1-LIKE HOMEODOMAIN 3 (BLH3)	HB a,b	0	0	
AT5G56780	Has 86 Blast hits to 52 proteins in 16 species: Archae - 0	HRT a / HRT-like b	0	0	
AT2G05160	INVOLVED IN: biological_process unknown	C3H a,b	0	0	
AT3G53370	INVOLVED IN: regulation of transcription	S1Fa-like a,b	0	0	
AT2G32930	ZINC FINGER NUCLEASE 2 (ZFN2)	C3H a,b	0	0	
AT4G31690	INVOLVED IN: regulation of transcription, DNA-dependent	ABI3-VP1 a,b	0	0	
AT5G46915	Has 30201 Blast hits to 17322 proteins in 780 species: Archae - 12	ABI3-VP1 b	0	0	
AT4G12620	ORIGIN OF REPLICATION COMPLEX 1B (ORC1B)	PHD a,b	0	0	
AT2G33290	SU(VAR)3-9 HOMOLOG 2 (SUVH2)	SET a / PcG b	0	0	
AT1G33240	GT-2-LIKE 1 (GTL1)	TRIHELIX a,b	0	0	
AT2G45880	BETA-AMYLASE 7 (BAM7)	BES1 a,b	0	0	
AT3G13682	LSD1-LIKE2 (LDL2)	SWI/SNF-SWI3 a	0	0	
AT1G78280	BEST Arabidopsis thaliana protein match is: unknown protein (TAIR:AT5G06550.1)	JUMONJI a,b	0	0	
AT1G09710	INVOLVED IN: regulation of transcription	MYB-related a,b	0	0	
AT1G09770	CELL DIVISION CYCLE 5 (CDC5)	MYB a,b	0	0	
AT2G46530	INVOLVED IN: response to hormone stimulus, regulation of transcription, DNA-dependent, regulation of transcription	ARF a,b,c	0	0	
AT3G23150	ETHYLENE RESPONSE 2 (ETR2)	Orphans a	0	0	
AT2G41450	FUNCTIONS IN: N-acetyltransferase activity	GNAT a	0	0	
AT1G65910	INVOLVED IN: multicellular organismal development, regulation of transcription	NAC a,b	0	0	
AT2G23380	CURLY LEAF (CLF)	SET a / PcG b	0	0	
AT1G24190	SIN3-LIKE 3 (SNL3)	Orphans a	0	0	
AT5G15020	SIN3-LIKE 2 (SNL2)	Orphans a	0	0	
AT5G41580	EXPRESSED IN: 23 plant structures	zf-MIZ c	0	0	
AT5G16780	DEFECTIVELY ORGANIZED TRIBUTARIES 2 (DOT2)	SART-1 c	0	0	
AT2G38950	INVOLVED IN: regulation of transcription	JUMONJI a,b,c	0	0	
AT2G19260	INVOLVED IN: regulation of transcription, DNA-dependent	PHD a,b	0	0	
AT5G08630	Has 7496 Blast hits to 4698 proteins in 418 species: Archae - 4	DDT a,c	0	0	
AT3G05670	INVOLVED IN: regulation of transcription, DNA-dependent	PHD a,b	0	0	
AT2G40950	(BZIP17)	bZIP a,b,c	0	0	
AT2G30470	HIGH-LEVEL EXPRESSION OF SUGAR-INDUCIBLE GENE 2 (HSI2)	ABI3-VP1 a,b	0	0	
AT1G20980	SQUAMOSA PROMOTER BINDING PROTEIN-LIKE 14 (SPL14)	SBP a,b,c	0	0	
AT1G48310	INVOLVED IN: biological_process unknown	SNF2 a	0	0	
AT1G03750	LOCATED IN: membrane	SNF2 a	0	0	
AT1G11950	BEST Arabidopsis thaliana protein match is: transcription factor jumonji (jmjC) domain-containing protein (TAIR:AT1G62310.1)	JUMONJI a,b	0	0	
AT1G66340	ETHYLENE RESPONSE 1 (ETR1)	Orphans a	0	0	
AT1G77300	EARLY FLOWERING IN SHORT DAYS (EFS)	SET a / PcG b	0	0	
AT2G13370	INVOLVED IN: chromatin assembly or disassembly	SNF2 a	0	0	
AT2G31650	HOMOLOGUE OF TRITHORAX (ATX1)	PHD a,b	0	0	
AT3G22170	FAR-RED ELONGATED HYPOCOTYLS 3 (fhy3)	FAR1 a	0	0	
AT5G45050	TOLERANT TO TOBACCO RINGSPOT NEPOVIRUS 1 (TTR1)	WRKY a,b,c	0	0	
AT5G56930	INVOLVED IN: embryo development ending in seed dormancy	C3H a,b	0	0	
AT5G63420	INVOLVED IN: metabolic process, embryo development ending in seed dormancy	TRIHELIX a,b	0	0	
AT3G12270	INVOLVED IN: protein amino acid methylation	C2H2 d	0	0	
AT1G76880	BEST Arabidopsis thaliana protein match is: Duplicated homeodomain-like superfamily protein (TAIR:AT1G76890.2)	TRIHELIX a,b	0	0	
AT2G41900	BEST Arabidopsis thaliana protein match is: CCCH-type zinc finger protein with ARM repeat domain (TAIR:AT5G12850.1)	C3H a,b	0	0	
AT5G07400	INVOLVED IN: DNA repair	FHA a,b	0	0	
AT3G21430	INVOLVED IN: biological_process unknown	MYB-related a / DIRP c	0	0	
AT5G18960	INVOLVED IN: response to red or far red light	FAR1 a	0	0	
AT3G06250	INVOLVED IN: response to red or far red light	FAR1 a	0	0	
AT1G76320	INVOLVED IN: response to red or far red light	FAR1 a	0	0	
AT5G11510	MYB DOMAIN PROTEIN 3R-4 (MYB3R-4)	MYB a,b	0	0	
AT2G22740	SU(VAR)3-9 HOMOLOG 6 (SUVH6)	SET a / PcG b	0	0	
AT3G62240	LOCATED IN: intracellular	C2H2 b	0	0	
AT2G37520	INVOLVED IN: regulation of transcription, DNA-dependent	PHD a,b	0	0	

Gene identifier (AGI)	Primary Gene Symbol	TF family	1 mM 3AT	5 mM 3AT	visual in-spec-tion
AT4G13460	SU(VAR)3-9 HOMOLOG 9 (SUVH9)	SET a / PcG b	0	0	
AT4G16150	FUNCTIONS IN: calmodulin binding, transcription regulator activity	CAMTA a,b	0	0	
AT2G21710	LOCATED IN: cellular_component unknown	mTERF a	0	0	
AT5G09410	ETHYLENE INDUCED CALMODULIN BINDING PROTEIN (EICBP.B)	CAMTA a,b	0	0	
AT2G35160	SU(VAR)3-9 HOMOLOG 5 (SUVH5)	SET a / PcG b	0	0	
AT5G24470	PSEUDO-RESPONSE REGULATOR 5 (PRR5)	Pseudo ARR-B a / C2C2-CO-like b	0	0	
AT5G02810	PSEUDO-RESPONSE REGULATOR 7 (PRR7)	Pseudo ARR-B a / C2C2-CO-like b	0	0	
AT2G46830	CIRCADIAN CLOCK ASSOCIATED 1 (CCA1)	MYB-related a,b	0	0	
AT1G35460	INVOLVED IN: regulation of transcription	bHLH a,b	0	0	
At2g03710			0	0	
At2g03710			0	0	
At1g26310			0	0	
At4g09960			0	0	
At2g45660			0	0	
At1g01530			0	0	
At5g23260			0	0	
At5g23260			0	0	
At5g26580			0	0	
At2g26880			0	0	
At2g28700			0	0	
AT4G02235			0	0	
At1g28460			0	0	
At2g24840			0	0	
AT1G29962			0	0	
At1g77950			0	0	
At5g65080			0	0	
At5g38620			0	0	
At1g54760			0	0	
At5g27960			0	0	
At1g31640			0	0	
At1g46408			0	0	
At5g04640			0	0	
AT5G37415			0	0	

The full list of bait proteins used in the Y2H screenings.