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# Regulation of p53 target gene transcription by a TBL1-mediated epigenetic mechanism

**Inauguraldissertation**

zur Erlangung des Doctor scientiarum humanarum (Dr. sc. hum.)
an der
Medizinischen Fakultät Heidelberg
der
Ruprecht-Karls-Universität

**vorgelegt von**
**Qianchao Wu**
**aus Dezhou, P.R. China**
**2019**

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# Abbreviations

| | |
|---|---|
| 5-FU | 5-fluorouracil |
| µl | Microliter |
| µg | Microgram |
| ABC | ATP binding cassette |
| AKT | Protein kinase B |
| AML | Acute myeloid leukemia |
| AMP | Ampicillin |
| AP-1 | Activator protein 1 |
| Apaf-1 | Apoptotic Protease Activating Factor 1 |
| APTX | Aprataxin |
| ATCC | American Type Culture Collection |
| ATM | Ataxia-Telangiectasia Mutated |
| ATP | Adensosin Triphosphate |
| ATR | ATM and Rad3 Related |
| ATRIP | ATR interacting protein |
| BAX | Bcl-2 Associated X Protein |
| BBC3 | BCL2 binding component 3 |
| BCA | Bicinchoninic acid |
| Bcl-2 | B-cell lymphoma 2 |
| BER | Base Excision Repair |
| BH3 | Bcl-2 homology 3 |
| bp | Base Pairs |
| BRCA1 | Breast Cancer 1 |
| BSA | Bovin serum albumin |
| CaM | $Ca^{2+}$ binding to calmodulin |
| CBP | CREB-binding protein |
| CDKN1A | Cyclin Dependent Kinase Inhibitor 1A |
| CDKN2A | Cyclin Dependent Kinase Inhibitor 2A |
| ChIP | Chromatin immunoprecipitation |
| Chk1 | Checkpoint Kinase 1 |
| Chk2 | Checkpoint Kinase 2 |
| COUPTF | Chicken ovalbumin upstream promoter transcription factor |
| CSCs | Cancer stem cells |
| CtBP | C-terminal-binding protein |
| CTCF | CCCTC-binding factor |
| °C | Degree celcius |
| ds | Double strand |
| DAD | Deacetylase activation domain |
| DAX1 | Dosage-sensitive sex reversal, adrenal hypoplasia critical region, on chromosome X, gene 1 |
| DBD | DNA Binding Domain |
| DDR | DNA damage response |
| DISC | Death inducing signaling complex |
| DKFZ | German Cancer Research Center |
| DMSO | Dimethyl sulfoxide |
| DNA | Deoxyribonucleic acid |
| DNA-PKcs | DNA-Dependent Protein Kinase catalytic subunit |

| | |
|---|---|
| DNMT | DNA methyltransferase |
| dNTP | Deoxyribonucleotides |
| Dox | Doxorubicin |
| DR | Death receptor |
| DREAM | Dimerization partner, RB-like, E2F and multi-vulval class B |
| DSB | DNA double strand break |
| E4F1 | E4F Transcription Factor 1 |
| ECM | Extra-cellular matrix |
| E. coli | Escherichia coli |
| EGFR | Epidermal growth factor receptor |
| ER | Estrogen receptors |
| ESC | Embryonic stem cells |
| et al. | et alteri |
| FACS | Fluoresence Activated Cell Sorting |
| FADD | Fas-associated death domain |
| FBS | Fetal bovine serum |
| FDA | Food and drug administration |
| FITC | Fluorescein isothiocyanate |
| FOXO | Forkhead box O |
| g | Gram |
| GLUT1 | Glucose transporter 1 |
| GO | Gene ontology |
| GOF | Gain-of-function |
| GPS2 | G Protein Pathway Suppressor 2 |
| GSH | Glutathione |
| GST | Glutathione S-Transferase |
| h | hours |
| H2AX | H2A histone family member X |
| HAT | Histone acetyltransferase |
| HCT116 | Human colon cancer cells |
| HDAC | Histone Deacetylase |
| HID | Histone interaction domain |
| HIPK2 | Htiomeodomain-interacting kinase 2 |
| His | Polyhistidine |
| hMOF | Human males absent on the first |
| HR | Homologous recombination |
| HRP | Horseradish peroxidase |
| HSCs | Hematopoietic stem cells |
| ID | Interaction domain |
| IgG | Immunoglobulin G |
| INPP5D | Inositol Polyphosphate-5-Phosphatase D |
| IP | Immunoprecipitation |
| IPTG | Isopropyl β-D-1-thiogalactopyranoside |
| IR | Ionizing radiation |
| kb | Kilobase |
| kd | Kilodalton |
| l | Liter |
| LB | Lysogeny Broth |
| LBD | Ligand-binding domain |

| | |
|---|---|
| LisH | Lis Homology |
| Lys | Lysine |
| M | Molar |
| MCF-7 | Human breast adenocarcinoma cell line |
| MDM2 | Murine double minute 2 |
| min | Minute |
| ml | Milliliter |
| MLH1 | MutL Homolog 1 |
| MMR | Mismatch Repair |
| MRN | Mre11-Rad50-Nbs1 |
| MSCs | Mesenchymal stem cells |
| MSH2 | MutS Homolog 2 |
| MYBL2 | MYB Proto-Oncogene Like 2 |
| NAFLD | Non-alcoholic fatty liver disease |
| NCoR | Nuclear Receptor Corepressor |
| NER | Nucleotide Excision Repair |
| ng | Nanogram |
| NGS | Next-Generation Sequencing |
| NHEJ | Non-Homologous End Joining |
| nm | Nanometer |
| Noxa | BH-only member of the BCL-2 family |
| NR | Nuclear Receptors |
| ns | Not significant |
| OASD | Ocular Albinism with Sensorineural Deafness |
| ON | Over night |
| p | Significance |
| PARP | Poly ADP ribose polymerase |
| PBS | Phosphate buffered saline |
| PCAF | p300/CBP-Associated Factor |
| PCR | Polymerase Chain Reaction |
| PD-1 | Programmed cell death protein 1 |
| PD-L1 | Programmed death-ligand 1 |
| PDAC | Pancreatic ductal adenocarcinoma |
| Pen/Strep | Penicillin/Strptomycin |
| PHLDA3 | Pleckstrin Homology Like Domain Family A Member 3 |
| PI | Propidium Iodide |
| PI3K | Phosphatidylinositol-3-kinase |
| Pit-1 | POU Class 1 Homeobox 1 |
| PPAR | Peroxisome proliferator activated receptor |
| PTM | Posttranslational Modification |
| PUMA | p53 Upregulated Modulator of Apoptosis |
| PVDF | Polyvinylidene fluoride |
| RC | Restrictive combination |
| RD | Repression domain |
| RNA | Ribonucleic acid |
| ROS | Reactive oxygen species |
| RPA | Replication protein A |
| rpm | Revolutions per minute |
| RT | Reverse Transcriptase |

| | |
|---|---|
| RT | Room Temperature |
| SANT | SW13/ADA2/NCoR/TFIIB |
| SDS | Sodiumdodecylsulfate |
| SDS-PAGE | SDS polyacrylamide gel electrophoresis |
| SENP1 | SUMO specific protease I |
| Ser | Serine |
| Siah1 | seven in absentia homolog 1 |
| SLC7A11 | Solute carrier family 7 member 11 |
| SMRT | Silencing Mediator of the Retinoid and Thyroid Hormone Receptors |
| ss | Single Strand |
| SSB | Single Strand Break |
| SUMO | Small Ubiquitin-Related Modifier |
| TAD | Transactivation Domain |
| TAE | Tris-Acetate-EDTA |
| TE | Tris-EDTA |
| TBL1 | Transducin β-like protein 1 |
| TBLR1 | Transducin β-like related 1 |
| TEMED | Tetramethylethylenediamine |
| TET | Ten-eleven translocation |
| TLS | Translesion synthesis |
| TNF | Tumor Necrosis Factor |
| TNFR | Tumor Necrosis Factor Receptor |
| TNFRSF10C | TNF Receptor Superfamily Member 10c |
| TopBP1 | DNA Topoisomerase II Binding Protein 1 |
| TR | Thyroid Hormone Receptor |
| TRADD | TNFR-1-associated death domain |
| TRAIL | TNF related apoptosis inducing ligand |
| TS | Template switching |
| U2OS | Human Bone Osteosarcoma Epithelial Cells |
| U.S. | United States |
| UV | Ultraviolet |
| V | Volt |
| v/v | Volume per volume |
| Vol | Volume |
| wt | Wildtype |

# 1 Introduction

## 1.1 Cancer, cancer treatment and cancer drug resistance

Cancer is one of the leading causes of death all over the world. There are several typical hallmarks of cancer cells, like transformation, metastasis and immortality. They do not behave like normal cells, which would die when they grow old or become damaged and replaced by new cells. Cancer cells would not stop dividing and become more and more abnormal. If tumours are benign, which means they do not spread into surrounding tissues, they do not come back if they are removed. Unlike benign tumours, malignant tumours can invade nearby tissues. In addition, some cancer cells can leave their original site and travel to distant organs through the blood or the lymph system and form new tumours, which is a big challenge for clinical treatment (Carter et al., 1989, Elston and Ellis, 1991, Maheswaran and Haber, 2010).

In the past several decades, the landscape of tumour treatment has largely changed. In addition to surgery, chemotherapy and radiotherapy, targeted cancer therapy and cancer immunotherapy have come to the forefront. As the name suggested, targeted cancer therapy is to interfere with specific genes or proteins involved in tumourigenesis. Monoclonal antibodies, immunomodulators and small molecule inhibitors are three main types of targeted therapies. Even though different cancer types and the same cancer type in individual people have different driving mutations, it is also obvious that some aberrations appear in a broad range of cancers. Since 2000, the United States (U.S.) food and drug administration (FDA) has approved over 15 targeted cancer therapies (Baudino, 2015).

Cancer immunotherapy is to use immune system to eliminate tumour cells. At the moment, several strategies are used to augment the patients' immune system targeting tumour cells, including adoptive cell therapies, vaccines and antibodies against immune checkpoint inhibitors, like programmed death-1 (PD-1) receptor on T cells and its ligand PD-L1. Although they have shown promising results in only a few malignancies to date, now they are further developed in other tumour entities, which is expected to broadly benefit patients (Oiseth et al., 2017).

Despite its limitations and adverse effects, currently conventional cancer therapies are still the most common treatments for cancer. This is mainly due to its good effectiveness and comparatively low cost. There are two major ways how chemotherapeutic agents inhibit cancer growth: One is to impair mitosis and the other is to induce apoptosis by causing DNA damage (Lind, 2011, Makin and Hickman, 2000, Malhotra and Perry, 2003). Cancer cells are known to show high growth rates and uncontrolled cell division. As chemotherapy affects cell division, quickly dividing cancer cells are more sensitive to chemotherapy (Corrie, 2011).

One of the major challenges in cancer treatment is chemoresistance which often results in treatment failure and sometimes makes cancer cells even more aggressive (Margaret et al., 2014, Szakacs et al., 2006). There are two types of chemoresistance: intrinsic resistance and acquired resistance (Fig. 1). Intrinsic resistance means that cancer cells were already chemo-resistant even before they were exposed to anti-cancer drugs. Conversely, acquired resistance means that initially, the cancer cells are sensitive to anti-cancer drugs but later develop a resistance against them (Abdullah and Chow, 2013, Tapia and Diaz-Padill, 2013).

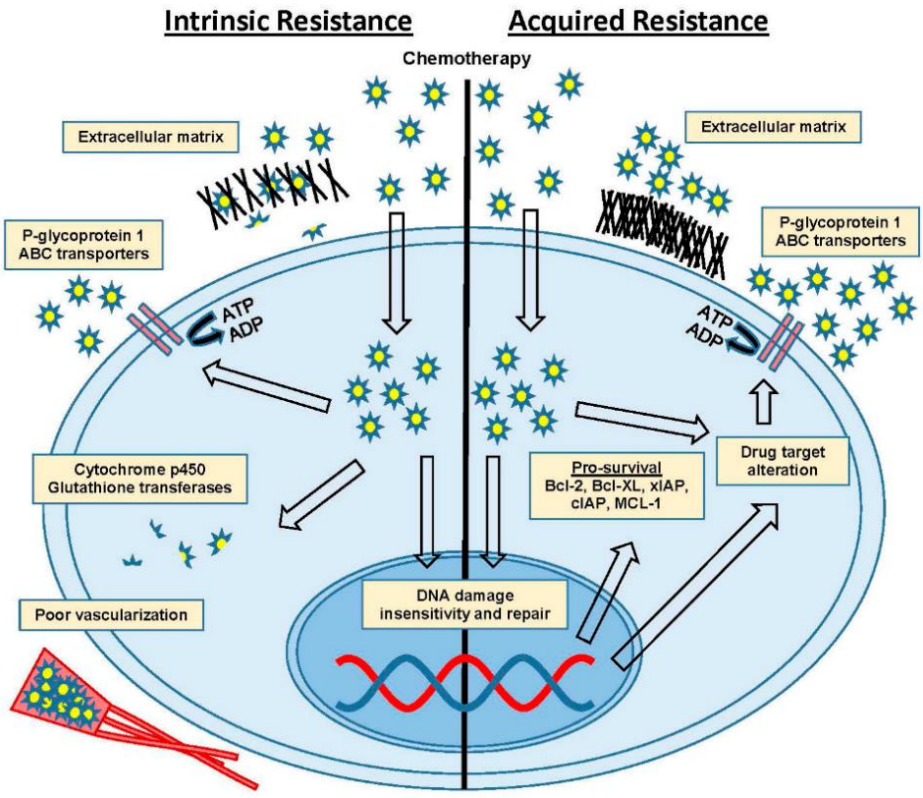


**Fig. 1: Intrinsic and acquired resistance.** The common mechanisms of intrinsic chemoresistance include adenosine triphosphate (ATP) binding cassette (ABC) transporters to lower drug concentrations within cells, cytochrome p450 and glutathione transferases to degrade drugs, poor vascularization and extracellular matrix (ECM) interactions. The common mechanisms of acquired chemoresistance include modulation of the expression of genes that drive increased anti-apoptotic signaling (e.g. xIAP/cIAP, BCL-2, BCL-XL and MCL-1), DNA repair capacity, drug target alteration and changes to ECM-collagen VI surface proteins (taken from Robert 2017)**.**

Chemoresistance is a complex network of many different endo- and exogenous mechanisms. It was recently hypothesized that a small fraction of cancer stem cells (CSCs) with the ability of self-renewal and recreating a full repertoire of cancer cells plays an important role in chemoresistance (Abdullah and Chow, 2013). Therefore, it is not surprising that tumours can never be completely eliminated upon chemotherapy, because CSCs are not targeted by chemotherapy and repopulate afterwards, leading to tumour recurrence or relapse. The mutation of oncogenes and tumour suppressor genes also contribute to drug resistance (Hanahan and Weinberg, 2011). One of the most popular examples is the tumour suppressor gene p53 which is a key executor in the DNA damage response (DDR). Many anti-cancer drugs directly activate the DDR in order to induce cell cycle arrest or apoptosis in cancer cells by regulating p53 activity. However, p53 is mutated or deleted in more than half of all human cancers increasing the drug resistance of the cancer cells (Khoo et al., 2014). Furthermore, the tumour microenvironment plays an important role in the development of chemoresistance in terms of oxygen status, the amount of extracellular matrix (ECM) proteins, and the presence of stromal cells (Tadeo et al., 2017, Mumenthaler et al., 2015, Gentric et al., 2017, Chan et al., 2016). For example, ECM proteins could either bind to drugs directly or form barriers impeding the delivery of drugs to the centre of the tumour (Gjorevski et al., 2016, Affo et al., 2017).

However, chemotherapy non-selectively targets both healthy and cancer cells (Partridge et al., 2001). Additionally, because chemotherapy mainly kills rapidly dividing cells, the slowly dividing CSCs cannot be effectively eliminated. One approach that could potentially reduce chemoresistance is to combine two or more

therapeutic agents or methods which could enhance efficiency compared to the conventional monotherapy. Combination therapy including agents which target CSCs (e.g. gamma-secretase inhibitors and Notch inhibitors) would attenuate drug resistance and reduce the risk for tumour recurrence and relapse (Takebe et al., 2015). Furthermore, since the drugs are already FDA-approved, the overall costs would be reduced. In a recent study, researchers investigated and optimized combinational administration of chemotherapy and photothermal therapy. They found that if chemotherapy is administered before photothermal treatment, therapeutic outcomes are much better compared to other administration sequences (Zhu et al., 2018).

The more advanced stage for combination therapy is the so-called restrictive combination (RC). This strategy includes a more specific dosing and drug administration with the aim to selectively kill cancer cells, but spare out the healthy cells. Using the molecular differences between healthy and cancer cells (e.g. increased number of growth factor receptors, driver gene mutations, etc.), restrictive regimens are designed to specifically target cancer cells (Blagosklonny, 2008). For example, cell cycle arrest inducing agents are applied to induce cell cycle arrest in normal cells for treating tumours with mutated or deleted p53. Then drugs that have targeted cytotoxic effect on quickly dividing cells could be used to eliminate cancer cells with high grow rates (Blagosklonny, 2008).

In order to overcome chemoresistance the development of drugs inducing chemosensitivity should be taken into consideration. Conventional cytotoxic agents could be used in combination with drugs that effectively circumvent intrinsic and adaptive drug resistance in order to improve therapy outcomes (Holohan et al., 2013). Furthermore, advanced high throughput screening methods can be helpful to predict the responses of different tumours to specific combination therapies.

## 1.2 The DNA Damage Response

### 1.2.1 DDR and DDR signaling

DNA damage is permanently happening in every cell of the human body with a frequency of up to $10^5$ DNA lesions per day per cell (Hoeijmakers, 2009). In contrast

to DNA mutations which are changes in the nucleotide sequence, DNA damage includes changes in the structure of DNA, such as DNA double strand breaks (DSBs), DNA single strand break (SSBs) and DNA lesions (Köhler et al., 2016).

The DDR is an essential system that protects genome stability and prevents cancer formation (Jackson and Bartek, 2009) (Fig. 2). In response to DNA damage, DDR signaling is activated to induce DNA repair, cell cycle arrest or apoptosis (Bartek et al., 2007). Similar to other signaling pathways, there are many sensor, transducer and effector proteins involved in DDR (Zhou and Elledge, 2000). The sensor proteins first detect the aberrant DNA structures which were induced by DNA damage and subsequently activate upstream DDR kinases. The ataxia-telangiectasia mutated (ATM), ATM- and Rad3-Related (ATR) and DNA-dependent protein kinase catalytic subunit (DNA-PKcs) kinases are the three major upstream DDR kinases (Abraham, 2001, Caporali et al., 2004, Zhou and Bartek, 2004). In contrast to ATM or ATR, DNA-PKcs seems to regulate only a small number of targets and primarily play a role in non-homologous end joining (NHEJ ) (Burma et al., 2006).

ATM is mainly activated by DSBs and recruited by the DSB-recognizing protein complex Mre11-Rad50-Nbs1 (MRN). MRN is one of the first factor recruited to DSBs and is required for rapid localization of ATM to DSBs site as well as its activation (Uziel et al., 2003). ATM phosphorylates a large number of adapter and transducer proteins which ultimately activate downstream effectors such as checkpoint kinase 2 (Chk2), breast cancer 1 (BRCA1) and p53 to mediate the relevant DNA damage response (Lavin, 2008, Shiloh, 2003). In addition, ATM also phosphorylates the histone variant H2A histone family member X (H2AX) on the chromatin resulting in γH2AX which localizes to DSBs and hence is known to be a marker for DSBs. Although phosphorylation of H2AX is not essential to activate downstream effectors, it is required to recruit DNA repair proteins and chromatin-remodelling complexes around DSBs (Lukas et al., 2011).

ATR is activated by wide range of different DNA lesions, including SSBs. The major mechanism of ATR activation is initiated by binding of the replication protein A (RPA), which is a single strand (ss) DNA-binding protein complex. ATR interacting protein (ATRIP) binds directly to RPA-coated ssDNA and enables ATR-ATRIP complex to localize to the site of damage. Ultimately, this leads to the activation of

downstream substrates like checkpoint kinase 1 (Chk1) (Tibbetts et al., 2000). Furthermore, ATR and some of its substrates are also localized to damaged DNA sites in a DDR-independent manner to enhance the local concentrations of these proteins (Chen and Sanchez, 2004). Moreover, ATR gets also activated upon the junction of ssDNA and double strand (ds) DNA (Ellison and Stillman, 2003).

Although ATM and ATR have different properties, it has become clear that their DDR signaling pathways do overlap to a certain extent. ATR has been shown to phosphorylate ATM at Ser1981 in response to DNA replication stress (Stiff et al., 2006). In contrast, ATM regulates ATR activation via phosphorylation of DNA Topoisomerase II Binding Protein 1 (TopBP1) (Cuadrado et al., 2006, Yoo et al., 2007). Additionally, ATM may also promote the recruitment of TopBP1 to DNA damage sites by γH2AX and mediator of DNA damage checkpoint protein 1 (Mdc1) (Wang et al., 2011). On the other hand, ATR does phosphorylate H2AX upon DNA replication stress which leads to the recruitment of ATM to the chromatin adjacent to stressed replication forks (Ward and Chen, 2001).

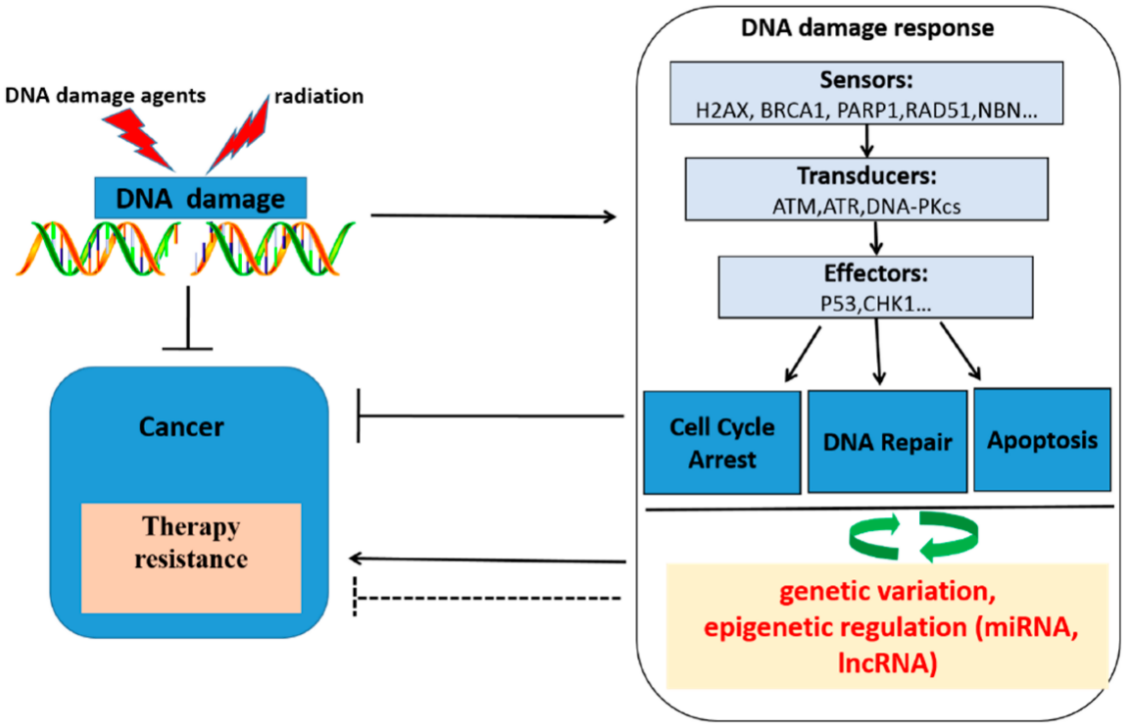


**Fig. 2: DNA Damage Response.** Schematic overview of DDR including examples of sensors, transducers and effectors. Proteins involved in this signaling pathways are promising therapeutic targets. However, cancer cells frequently develop genetic variations leading to therapy resistance (taken from Li 2016).

### 1.2.2 Apoptosis

Irreparable DNA damage induces programmed cell death. Until recently, the terms “programmed cell death” and “apoptosis” were used synonymously. However, now other types of programmed cell death that are non-apoptotic have been described, such as necroptosis, pyroptosis and the recently discovered ferroptosis (Dixon et al., 2012, Wallach et al., 2016). Apoptosis plays an important role in human physiology from embryonic development to execution of immune effector functions (Green, 2011). However, too much apoptosis can lead to neurodegenerative diseases like Parkinson's disease or Alzheimer's disease, whereas too little apoptosis might result in autoimmunity (Mattson, 2000, Nagata, 2010). Apoptosis is regulated by a set of caspases (cysteine-aspartic proteases, cysteine aspartases or cysteine-dependent aspartate-directed proteases) which are synthesized as inactive precursor proteins and get activated upon specific cleavages when apoptosis is initiated (Taylor et al., 2008). The caspases 2, 3 and 6-10 are involved in apoptosis while other caspases like caspase 1, 4 and 5 mediate pyroptosis (Taylor et al., 2008). The executioner caspases 3 and 7 are responsible for ultimately killing cells and the resulting apoptotic bodies are removed by macrophages (deCathelineau and Henson, 2003).

Apoptosis is mediated by two pathways: the intrinsic pathway (also called mitochondrial pathway) and the extrinsic pathway (also called death receptor pathway) (Riedl and Salvesen, 2007). Different intracellular stimuli like DNA damage or lack of growth factor initiate the intrinsic apoptotic pathway, which is regulated by the B-cell lymphoma 2 (Bcl-2) protein family. There are three subfamilies of Bcl-2 proteins: pro-apoptotic Bcl-2 homology 3 (BH3)-only members (Bim, Bid, PUMA, Noxa, Hrk, Bmf, and Bad), pro-apoptotic effector proteins (Bax and Bak), and anti-apoptotic Bcl-2 family proteins (Bcl-2, Bcl-xL, Mcl1, A1, and Bcl-B) (Czabotar et al., 2014). Once apoptosis is initiated, BH3-only proteins antagonize the anti-apoptotic Bcl-2 family members and activate Bax and Bak that forms pores in the

mitochondrial membrane, which permeabilize mitochondrial membrane and stimulate the release of cytochrome c from the mitochondrial. The released cytochrome c forms a cytoplasmic complex with apoptotic protease activating factor 1 (Apaf-1) that induces caspase 3 activation (Tait and Green, 2010).

The extrinsic pathway is triggered by external factors that are recognized by death receptor (DR) family members, such as Fas/CD95, tumour necrosis factor (TNF) receptor 1 (TNFR-1) or TNF related apoptosis inducing ligand (TRAIL) receptors DR-4 and DR-5 (Nowsheen and Yang, 2012). After ligand binding, DRs trimerize and transmit the apoptotic signal to their intracellular death domain. Upon binding to the TNFR-1-associated death domain (TRADD) or Fas-associated death domain protein (FADD), the death inducing signaling complex (DISC) is formed. This complex activates caspase 8 and 10 which lead to widespread cleavage of caspase substrates and rapid induction of cell death (Elmore, 2007, Roos and Kaina, 2013).

### 1.2.3 DDR and Chemosensitivity

Chemotherapeutic agents target rapidly dividing cancer cells by inducing DNA damage resulting in stalled replication and cell death. However, cancer cells show several mutations in DDR signaling pathways leading to alterations in sensitivity and resistance against a range of chemotherapeutics. For example, mutations in MutS Homolog 2 (MSH2) and MutL Homolog 1 (MLH1) genes are frequently found in relapsed acute myeloid leukaemia (AML) patients with reduced sensitivity to chemotherapeutics (Mao et al., 2008). Defects in SSB repair pathways are also described to affect chemosensitivity by determining tumour cell sensitivity to chemotherapeutic treatment (Banescu et al., 2014, Liddiard et al., 2010). In addition, mutations of the tumour suppressor p53 have a dominant-negative effect on wild type p53 and hence, reduce the chemosensitivity of cancer cells (Velculescu and El-Deiry, 1996, Vogelstein and Kinzler, 1992).

Therefore, the DDR is an important target that could be therapeutically exploited in order to increase chemosensitivity of cancer cells. One exemplary approach is to restore the p53 function by inhibiting its degradation or by demethylating silenced promoter regions of p53 target genes (Baylin and Jones, 2011, Martins et al., 2006, Ventura et al., 2007). Another possibility is to override cell cycle checkpoints by

inhibiting certain modulators of DDR which would result in mitotic catastrophe and cell death (Baylin and Jones, 2011, Squatrito et al., 2010). Furthermore, it is also possible to target proteins that are involved in DNA repair, such as poly (ADP-ribose) polymerase (PARP) and some specific histone deacetylases (HDACs) (Donawho et al., 2007, Miller et al., 2010).

## 1.3 Tumour Suppressor p53

### 1.3.1 Regulation of p53

p53 was discovered in 1979 as a target of SV40 virus which was found to induce tumour formation (Lane and Crawford, 1979, Linzer and Levine, 1979). However, in 1989 it was found that wild-type p53 represses growth as well as oncogenic transformation in cell cultures (Finlay et al., 1989). Later, p53 was found to be frequently mutated in human cancers and that p53 deletions in mice induce tumour formation at high penetrance (Baker et al., 1990, Donehower et al., 1992). Today, p53 is one of the best studied tumour suppressors and its role has been validated in numerous studies.

The p53 tumour suppressor function is primarily based on its role as a transcription factor that regulates expression of stress response genes and mediates anti-proliferative processes (Fridman and Lowe, 2003). Although this has been described more than 25 years ago, there are still a lot of unresolved questions concerning its mechanism of action. As a typical transcription factor, the p53 protein consists of a central DNA binding domain (DBD), two N-terminal transactivation domains (TADs), a conserved proline-rich domain, an oligomerization domain required for transcriptional activity and a C-terminal regulatory domain for nuclear localization signals and for regulation of p53 via posttranslational modifications (PTMs) (Fig. 3) (Joerger and Fersht, 2008). p53 functions as a tetramer to recognize and bind to p53 response elements, which consist of two copies of a 10 base pair motif with the consensus 5′-PuPuPuC(A/T)(T/A)GPyPyPy-3′ (el-Deiry et al., 1992). Its stability and transcriptional activity are tightly controlled by a variety of PTMs and various regulators (Meek and Anderson, 2009, Toledo and Wahl, 2006). Mouse double minute 2 homolog (MDM2) is known as a primary negative regulator of p53 (Fig. 3).

Early studies demonstrated that p53 is constantly degraded due to its ubiquitylation mediated mainly by MDM2 keeping the basal protein levels of p53 rather low (Haupt et al., 1997, Honda et al., 1997, Kubbutat et al., 1997). Emerging evidence shows that this is not the single mode for the repression of p53 by MDM2. MDM2 recruits HDAC1 to inhibit p53 activity via promoting p53 deacetylation (Ito et al., 2002). In addition, it has been identified that MDM2 directly represses p53 activity by conjugating ubiquitin monomer to the chromatin in the vicinity of p53 binding site and forming a transcription repressive atmosphere, which is ubiquitylation independent pathway (Minsky and Oren, 2004).

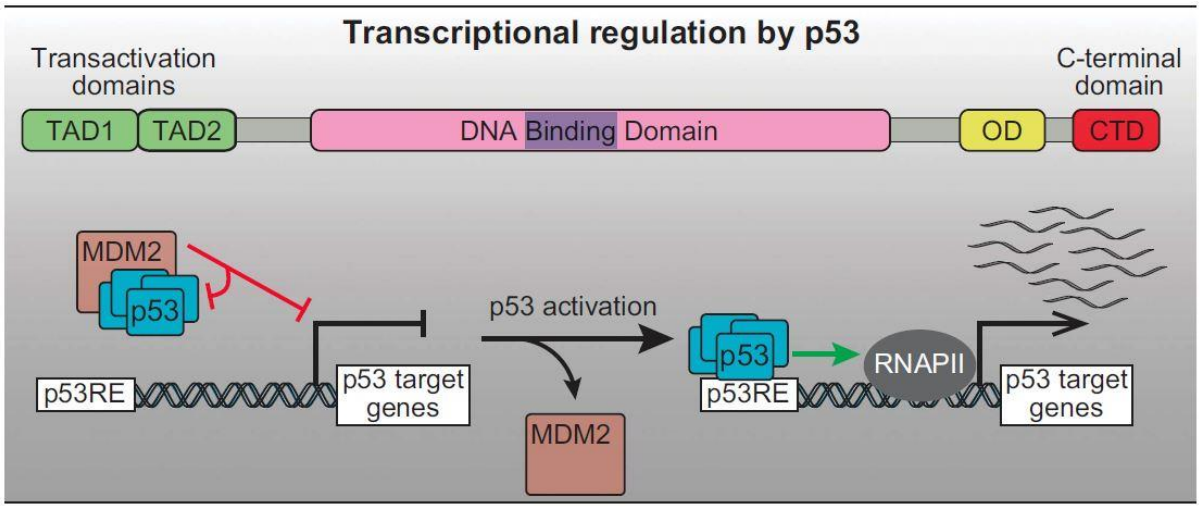



**Fig. 3: p53 regulation by MDM2.** p53 contains a N-terminal transactivation domain, a central DNA-binding domain, a tetramerization domain and a C-terminal regulatory domain. In steady state, MDM2 represses p53 activity in both ubiquitination dependent and ubiquitination independent pathways (taken from Shi 2012).

It is the fact that p53 regulates genes expression that shows both mRNA upregulation and downregulation. But more and more research revealed that its repressive effects are indirect and are driven by its target genes like p21 and E2F7. More recently, it was demonstrated that p53 indirectly represses transcription via p21 dependent RB-E2F4 complex and the dimerization partner, RB-like, E2F and multi-vulval class B (DREAM) complex (Fischer et al., 2015, Quaas et al., 2012). Genome-wide DREAM chromatin binding data showed that more than 200 genes are predicted to be regulated by the p53-p21-DREAM axis (Fischer et al., 2016).

In addition to mediating cellular responses via a transcription-dependent mechanism, p53 also mediates apoptosis in a transcription-independent way. Hallmark of this mechanism is the stress-induced accumulation of p53 in the cytosol or mitochondria which inhibits membrane protective and anti-apoptotic members of the Bcl-2 family. This leads to the activation of the pro-apoptotic protein Bax stimulating the release of cytochrome c, which results in the initiation of the caspase cascade and induction of apoptotic cell death. (Khoo et al., 2014). Furthermore, p53 contributes to death receptor trafficking and activation of procaspase 8 (Vousden and Lu, 2002).

The canonical functions of p53 include DNA damage-induced (p53-mediated) DNA repair, cell cycle arrest and apoptosis. Upon genotoxic stress, like DNA damage and DNA replication stress, p53 dissociates from its negative regulators leading to its stabilization and activation of downstream signaling (Horn and Vousden, 2007). Several mechanisms lead to p53 activation. For example, upon different extent of stresses, p53 is phosphorylated at Ser15 via ATM and ATR as well as at Ser20 by Chk1 and Chk2 (Appella and Anderson, 2001, Bode and Dong, 2004). If it is a low level of stress, the G1 phase checkpoint is induced, due to the transcriptional activation of the p21 cyclin-dependent kinase inhibitor gene which allows DNA repair prior to further cell division. Another possible outcome is cellular senescence meaning that cells do not longer replicate (el-Deiry et al., 1993). In addition, phosphorylation of Thr18 in the TAD of p53 dramatically reduces MDM2 binding that stabilizes and activates p53 (Teufel et al., 2009).

Besides the phosphorylation of p53, acetylation also plays an important role in the control of p53 activation. The acetyl transferases human males absent on the first (hMOF) and Tat-interactive protein, 60 kD (TIP60) acetylate p53 at Lys120 (Sykes et al., 2006, Tang et al., 2006), thereby enhancing pro-apoptotic gene expression (e.g. Bax, Noxa or Puma). HDACs can also remove acetyl groups from p53 such as HDAC1 and SIRT1. Inhibition of these HDACs leads to increased p53 acetylation and p53-dependent activation of apoptosis or senescence (Brooks and Gu, 2011). Moreover, p53 Lys320 is acetylated by p300/CBP-associated factor (PCAF) and ubiquitinated by E4F1. Both modifications result in increased transcription of genes involved in cell cycle arrest like CDKN1A (Le Cam et al., 2006, Liu et al., 1999). Furthermore, the acetylation of Lys98 in murine p53 (corresponding to Lys101 in

human p53) is crucial for p53-mediated ferroptosis and tumour suppression (Wang et al., 2016b).

### 1.3.2 The Network of p53

Besides the transcriptional and canonical functions, p53 was also found to be involved in a variety of other “non-canonical” pathways. p53 is also involved in metabolism, autophagy, reactive oxygen species (ROS) control, cell plasticity and pluripotency (Alexandrov et al., 2016, Wang et al., 2016b). For example, p53 was found to increase glutamine catabolism, downregulate lipid synthesis and stimulate gluconeogenesis (Kruiswijk et al., 2015). Furthermore, it was recently reported that p53 is required for the tight regulation of DNA methylation by DNA methyltransferases (DNMTs) and enzymes of the ten-eleven translocation family (TET) (Tovy et al., 2017). In addition, it has been shown that inactivation of p53 rescues cultured cells from apoptosis caused by DMNT1 deficiency and subsequent genomic demethylation. Hence, p53 seems to be capable of sensing and responding to perturbations in the epigenome (Jackson-Grusby et al., 2001).

Because p53 is involved in a wide variety of cellular processes, the exact mechanisms of regulation of different responses are still not clear. However, it is widely accepted that the p53 response varies between different cell types and stress conditions. One proposed mechanism is similar to its function in DDR, which is based on different PTMs depending on different stresses leading to the induction of specific targets. However, there are also other factors which affect how the cell interprets p53 activation. For example, although p53 is induced to bind to p21 promoter in embryonic stem cells (ESCs), p21 is not effectively activated due to cell type specific repressive histone H3K27me3 marks at the according locus (Itahana et al., 2016). Other transcriptional factors like nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) and forkhead box O (FOXO) can change the p53 target spectrum by cooperation with p53 (Cooks et al., 2014, Eijkelenboom and Burgering, 2013).

Taken together, all these determining signals and factors create a complex cellular system that is essential for the biochemical aspects of p53 activity like

phosphorylation status and protein binding, as well as the biological outcomes of a p53 response.

### 1.3.3 p53 based cancer therapies

The important role of p53 in tumour suppression and the high rate of p53 mutations in cancer has encouraged many researchers to develop strategies to directly target the p53 network in cancer therapy. Indeed, *in vivo* mouse studies have shown that activation of wild type p53 expression can efficiently initiate tumour elimination (Martins et al., 2006, Ventura et al., 2007, Xue et al., 2007). Generally, there are three major strategies depending on the p53 status of the tumour: targeting wild type p53, targeting mutant p53 and targeting p53 regulators (Fig. 4).

In many tumours, the main p53 inhibitor MDM2 is aberrantly overexpressed which effectively abolishes p53 function. Consequently, one great effort for cancer therapy is to inhibit MDM2 in cancers harbouring wild type p53. Nutlin-3 was reported in 2004 to be a small molecule antagonist of MDM2 which stabilizes p53 by blocking the p53 binding site in the N-terminal domain of MDM2 (Vassilev et al., 2004). The drug has passed phase I and was about to be investigated in several phase II clinical trials. This novel treatment approach has been shown to work synergistically with adenovirus-mediated gene therapy as well as mutant p53 reactivators (Graat et al., 2007, Liu et al., 2013).

Due to the fact that MDM2 inhibitors stabilize p53 and initiate cell cycle arrest in normal cells but have no effect on mutant p53 cancer cells, these drugs can also be used in order to reduce toxic side effects which are caused by targeting also healthy cells by chemotherapy. The underlying mechanism is based on the fact that chemotherapy selectively targets actively cycling cells. Hence, the cytotoxins do not affect the non-cycling healthy cells resulting in less systemic toxicity and higher tolerable doses of the drugs (Cheok and Lane, 2017).

In order to target mutant p53 in cancer therapy, efforts have been made to identify small molecules promoting proper folding of mutant p53 and restoring wild type p53 function. These molecules stabilize the active conformation of classical structural p53 mutants in order to restore sequence-specific DNA binding and activation of p53

which finally leads to the induction of cancer cell death. Although p53-directed drug design is quite challenging due to the absence of well-defined binding pockets in the DBD, several compounds have been identified via chemical library screening, molecular modelling and rational drug design. The first small molecule targeting p53 was CP-31398 which refolds specific p53 mutants and protects wild-type p53 from thermal denaturation (Foster et al., 1999). The drug APR-246 was designed to reactivate mutant p53 and is now investigated in clinical trials. However, it has already been shown to have some off-target effects (Deneberg et al., 2016).

Another approach to target mutant p53 is to consider it as a tumour-specific neoantigen. Several decades ago it has been shown that mutant p53 is highly overexpressed in cancer cells and might act as an antigen (Crawford et al., 1982). *In vivo* experiments showed that vaccination against mutant p53 reduced cancer progression in mice with xenografted tumours (Roth et al., 1996). Recently, the viral vectors and peptide vaccines targeting mutant p53 have been investigated in phase I/II clinical trials (van der Burg et al., 2002, Zeestraten et al., 2013).

An attractive way to target mutant p53 in cancer is to combine p53 immunotherapy with so called immune checkpoint blockade in order to enhance T-cell reactivity. It has been reported that loss of p53 can protect cancer cells from $CD8^+$ T-cells via PD-L1 depression thereby accelerating cancer development. This has been shown in mouse models and in human lung cancer (Cha et al., 2016) indicating that this combinatorial approach results in the desired cytotoxicity and clinical response based on the generation of p53 mutant-specific T-cells.

Besides the p53 protein itself, its associated regulatory pathways also offer a number of potential targets for anti-cancer therapy. For example, tenovins was found to induce tumour regression in mouse models with chronic myelogenous leukaemia by selectively killing leukaemia stem cells. This function is due to the ability of tenovins to inhibit the nicotinamide adenine dinucleotide ($NAD^{+)}$-dependent sirtuin deacetylases SIRT1 and SIRT2 (Lain et al., 2008). SIRT1 is overexpressed in a variety of cancer cell lines including chronic myelogenous leukaemia stem cells with wild-type p53 and is known to downregulate the transcriptional activity of p53 (Li et al., 2012). Therefore, tenovis activates the tumour suppression ability of p53 by inhibiting SIRT1.

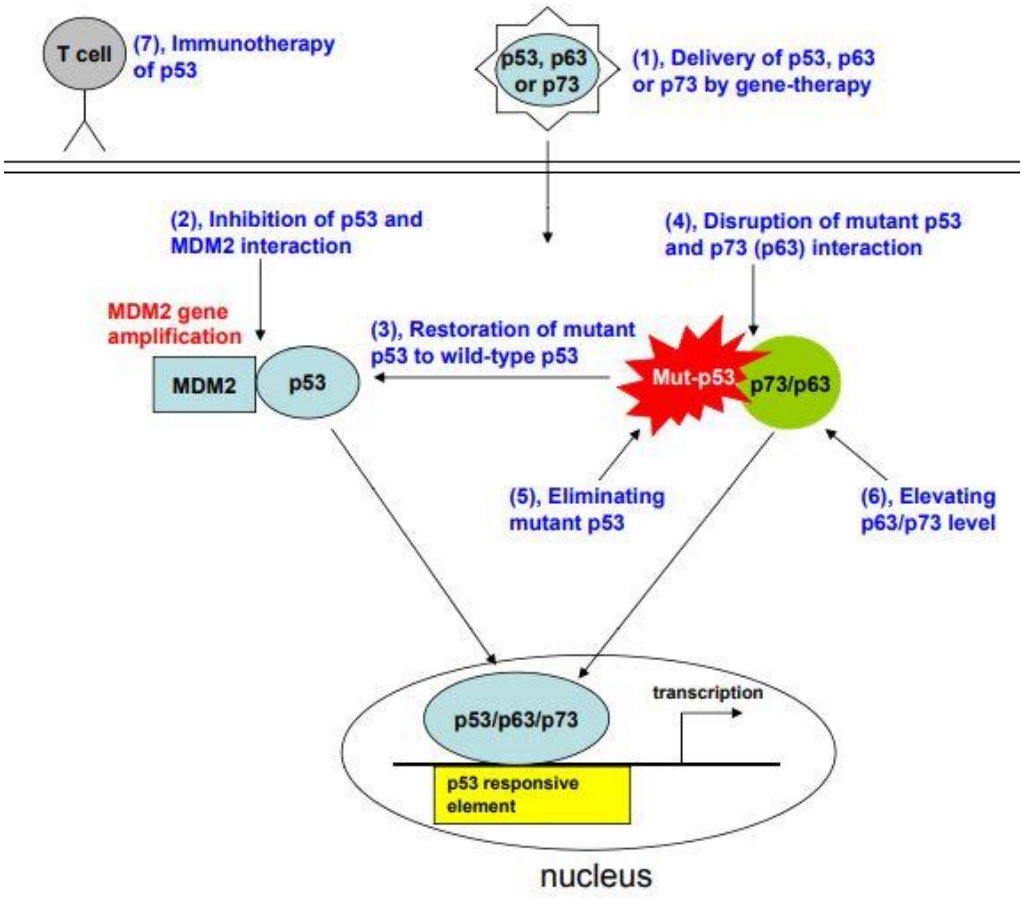



**Fig. 4: p53 based cancer therapies.** p53 inactivation in cancer is primarily based on MDM2 amplification, p53 mutation or deletion, and the inhibition of the transcriptional activity of p53 protein family members (p63 and p73). The figure shows seven strategies for restoration of p53 function (taken from Hong 2014).

## 1.4 Transcriptional Regulator Transducin β-like Protein 1

### 1.4.1 TBL1 and Nuclear Receptor Repressors

Initially, TBL1 was found to play a role in the X-linked human hearing defect Ocular Albinism with Sensorineural Deafness (OASD) which might be caused by a C-terminal microdeletion in the TBL1 gene (Bassi et al., 1999). Later TBL1 was reported and confirmed to be a subunit of the nuclear receptor co-repressors nuclear receptor co-repressor (NCoR) and silencing mediator for tetanoid and thyroid hormone receptors (SMRT) complex (Guenther et al., 2000, Zhang et al., 2002). As

the names suggest, co-repressors generally supress gene transcription by binding to non-ligand-bound nuclear receptors (NRs).

NCoR and SMRT are the two most studied co-repressors and were first identified through their interaction with nuclear receptors in absence of a ligand (Chen and Evans, 1995, Horlein et al., 1995). They share highly homologous domains with an overall sequence identity of 40 % and the molecular weight is around 270 kDa. Besides TBL1, the corepressor NCoR/SMRT also consists of several other components (Fig. 5), such as TBL related 1 (TBLR1), HDAC3, G protein pathway suppressor 2 (GPS2) and WD-repeat protein (IR-10) (Guenther et al., 2000, Li et al., 2000, Yoon et al., 2003, Zhang et al., 2002). Other complexes that include the Class II HDACs have also been shown to bind strongly to NCoR and SMRT (Grozinger and Schreiber, 2000, Huang et al., 2000, Kao et al., 2000).

The repression by NCoR/SMRT is initiated through the recruitment of multiple HDAC enzymes. It is known that HDAC3 is essential for the repression of the thyroid hormone receptor by the NCoR/SMRT complex (Ishizuka and Lazar, 2003). In addition, other HDACs such as HDAC1, HDAC4, HDAC7 and Sirt1 also contribute to this repression activity (Ariyoshi and Schwabe, 2003, Fischle et al., 2002, Kao et al., 2000, Picard et al., 2004).

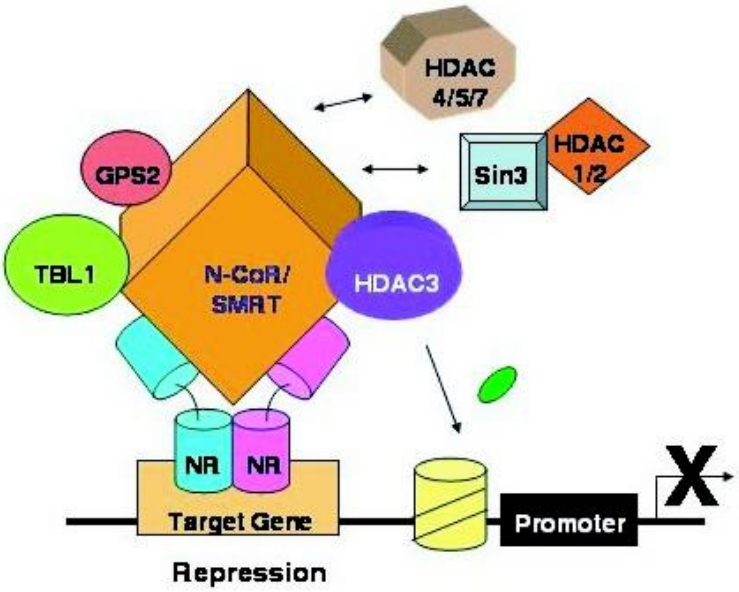



**Fig. 5: TBL1 is a subunit of the NCoR/SMRT repressor complexes.** A variety of proteins have been confirmed to interact with NCoR and SMRT. Alternative complexes like HDAC1-

Sin3 and HDAC4/5/7 have also been reported to interact with NCoR/SMRT but this has not been validated yet.

### 1.4.2 TBL1 as a Nuclear Exchange Factor

TBL1 has a highly conserved N-terminal domain that contains a Lis homology (LisH) domain which is required for homo- and hetero-dimerization as well as stable chromatin targeting (Choi et al., 2008). The C-terminal domain consists of seven WD40 repeat domains which provide a platform for protein-protein interactions and assembly (Perissi et al., 2004). The centrally located F-Box motif is essential for the recruitment of E3 ligases (Perissi et al., 2004).

At first, TBL1 was thought to be exclusively associated with transcriptional repression (Guenther et al., 2000, Yoon et al., 2003, Zhang et al., 2002). However, it has been revealed to have co-activating functions in gene expression as well. The co-activating activity of TBL1 appears to be attributed to its role as an adaptor protein and its ability to recruit the ubiquitin proteasome system which subsequently promotes the exchange of transcriptional co-repressors for co-activators (Perissi et al., 2004). For example, TBL1 and TBLR1 are required to dismiss C-terminal-binding protein (CtBP) and NCoR/SMRT corepressors by recruiting 19S proteasome particles to degrade them respectively (Fig. 6) (Perissi et al., 2008). This happens not only on NRs but also on c-Jun and NF-κB binding sites of affected genes (Perissi et al., 2004, Perissi et al., 2008).

A potential mechanism could be that TBL1 resides in the co-repressor complex and acts as a receptor co-repressor as well as a transcriptional repressor. Once it becomes phosphorylated on certain residues, TBL1 is enabled to recruit E3 ligases giving rise to the degradation of other co-repressors (Perissi et al., 2008).

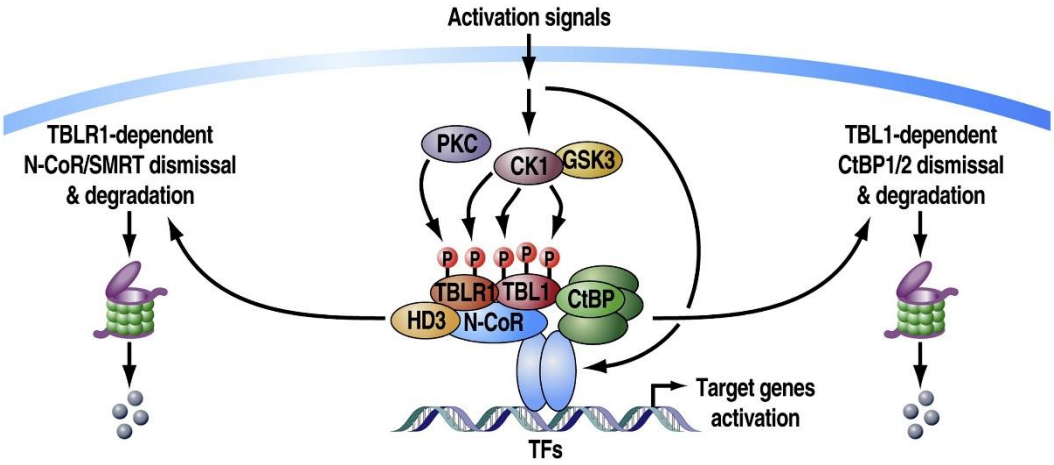



**Fig. 6: TBL1 as a nuclear exchange factor.** Upon phosphorylation, TBL1 promotes the ubiquitylation and degradation of the co-repressor CtBP. TBLR1 does also dismiss and degrade the NCoR/SMRT/HDAC3 co-repressor complex (taken from Perissi 2008).

### 1.4.3 Functions of TBL1 in different pathways

Recent studies provided evidence that TBL1 plays an important role in the development of obesity-induced non-alcoholic fatty liver disease (NAFLD) as a direct target of miR-367 (Li et al., 2017). TBL1 was also described to be overexpressed in both human and murine pancreatic ductal adenocarcinoma (PDAC) (Stoy et al., 2015). Its therapeutic inactivation could prevent and reverse pancreatic tumour growth which correlates with diminished glucose uptake, glycolytic flux, and oncogenic PI3 kinase signaling.

Upon tumour necrosis factor alpha (TNF-α) stimulation, TBL1 forms a complex with NF-κB and facilitates its recruitment to target gene promoters (Ramadoss et al., 2011). The mechanism was discovered four years later which is due to the SUMOylation of TBL1 and TBLR1 in response to TNF-α treatment. This resulted in the formation of the TBL1-TBLR1-NF-κB complex which leads to NF-κB-mediated transcriptional activation. Conversely, SUMO-specific protease I (SENP1)-mediated deSUMOylation of TBL1 and TBLR1 inhibits NF-κB target gene expression by dissociating TBL1 and TBLR1 from the NCoR/SMRT complex (Park et al., 2016).

TBL1 and TBLR1 were also identified as key players in the Wnt signaling pathway by recruiting β-catenin to Wnt target gene promoters and subsequently activating their

transcription. A deeper insight into the molecular mechanism of this regulation was given three years later: In normal cells, TBL1 plays a role in protecting β-catenin from Siah-1-mediated ubiquitination and subsequent degradation leading to Wnt target gene expression (Dimitrova et al., 2010). Upon UV irradiation, TBL1 promotes Siah1-mediated β-catenin degradation which leads to the inhibition of Wnt signaling. Similar to the NF-κB pathway, SUMOylation of TBL1 increases the recruitment of the TBL1-TBLR1-β-catenin complex to activate expression of Wnt target genes and SENP1 deSUMOylates TBL1/TBLR1 resulting in the dissociation of the β-catenin/TBL1/TBLR1 complex and inhibition of β-catenin-mediated transcription (Choi et al., 2011).

Furthermore, Ebi, the *Drosophila* homologue of TBL1, was found to be involved in the regulation of apoptosis (Lim et al., 2012). Ebi formed a complex with activator protein 1 (AP-1) and was required to suppress AP-1-mediated activation of pro-apoptotic target genes. Ebi depletion caused late-onset neuronal apoptosis and increased sensitivity to oxidative stress. Therefore, Ebi is essential to protect photoreceptor neurons from stress-induced apoptosis and age-related degeneration which contributes to long-term survival (Lim and Tsuda, 2016).

## 1.5 Aim of the Project

TBL1 is associated with metabolic control on one hand and with regulation of apoptosis and cell growth pathways on the other hand. Furthermore, the link of corepressor complex NCoR/SMRT to p53 suggested a potential connection between TBL1 and p53 (Adikesavan et al., 2014, Konduri et al., 2010).

The aim of this thesis was to identify the role for TBL1 in regulation of p53. This involved to investigate if TBL1 regulates p53 transcriptional activity and to biochemically characterize the link between these two proteins in order to gain a deeper molecular understanding of the underlying mechanism. In addition, the function of TBL1 in chemoresistance/chemosensitivity and the interplay between TBL1 and p53 in DNA damage response were also studied to provide some evidence for the potential clinical application.

# 2 Materials and Methods

## 2.1 Materials

### 2.1.1 Materials and Kits

| Material | Manufacture |
|---|---|
| 1.5 ml Eppendorf tube | Sarstedt AG |
| 2 ml Eppendorf tube | Sarstedt AG |
| 15 ml Falcon tube | Greiner |
| 50 ml Falcon tube | Greiner |
| 6 cm cell culture dish | Greiner |
| 10 cm cell culture dish | Greiner |
| 75 cm cell culture flask | Greiner |
| 6-well plate | Nunc GmbH |
| 96-well plate | Falcon |
| PCR tube | Nerbe |
| FACS tube | BD Biosciences |
| Complete protease inhibitor cocktail | Roth |
| Cryo vials | Roche |
| Glutathion-Sepharose | Amersham Biosciences |
| Lipofectamine™ 2000 | Invitrogen |
| Nickel-NTA-Agarose | Qiagen |
| Protein A/G Plus Sepharose | Santa Cruz |
| ChIP-Grade Protein G Magnetic Beads | Cell Signaling |
| PVDF-membrane | GE Healthcare |
| Pierce ECL-Kits | Thermo Fischer Scientific |
| Pierce® BCA Protein Assay Kit | Thermo Fischer Scientific |
| QIAprep® Spin Miniprep Kit | Qiagen |
| QIAquick® Gel Extraction Kit | Qiagen |
| QIAquick® PCR Purification Kit | Qiagen |
| QIAGEN® Plasmid Maxi Kit | Qiagen |
| X-ray films „Fuji Super FX“ | FUJIFILM |
| RNA Clean & Concentrator – 5 (R1015) | ZYMO RESEARCH |
| High Capacity RNA-to-cDNA Kit | Applied Biosystems |

| | |
|---|---|
| Taqman® Gene Expression Master Mix | Applied Biosystems |

### 2.1.2 Chemicals

| Reagent | Manufacture |
|---|---|
| 5-Fluorouracil | Sigma Aldrich |
| Ampicilin | Gerbu |
| Nutlin-3a | Sigma Aldrich |
| Doxycycline | Sigma Aldrich |
| BSA | Servia |
| Coomassie brilliant blue G250 | BioRad |
| Annexin V-FITC | BD Pharmingen™ |
| Glutathion | Sigma Aldrich |
| Imidazole | Sigma Aldrich |
| IPTG | Roth |
| Propidium Iodide | Sigma Aldrich |
| RNase | Invitrogen |
| Goat Serum | Sigma Aldrich |
| Lysozyme | Sigma Aldrich |
| MG-132 | US Biological |
| Sodium Vanadate ($Na_3VO_4$) | Sigma Aldrich |
| Crystal Violet | Sigma Aldrich |
| TRIzol Reagent | Invitrogen |
| PMSF | Sigma Aldrich |
| Adriamycin/Doxorubicin | Sigma Aldrich |
| Complete protease inhibitor cocktail | Roche |

Standard chemicals were purchased from Roche Diagnostics, Merck, Roth, Gerbu, Sigma Aldrich and Biozol.

### 2.1.3 Proteins and enzymes

| Protein/Enzyme | Manufacture |
|---|---|
| Restriction Enzymes | New England BioLabs |
| Antarctic phosphastase | New England BioLabs |
| T4 DNA ligase | New England BioLabs |

| | |
|---|---|
| Deoxynucleotides (dNTPs) | Invitrogen |
| Taq DNS Polymerase | Invitrogen |

### 2.1.4 Markers

| Marker | Manufacture |
|---|---|
| PageRuler$^{TM}$ Plus Prestained Protein Ladder | Fermentas |
| GeneRulerTM 1kb DNA Ladder | Fermentas |

### 2.1.5 Buffers and Solutions

| Buffer | Substance | Concentration |
|---|---|---|
| Annexin V-FITC Binding Buffer (10x) | HEPES pH 7.5 | 100 mM |
| | NaCl | 1.4 M |
| | CaCl2 | 25 mM |
| PBS | $Na_2HPO4/KH_2PO_4$ | 12 mM |
| | NaCl | 137 mM |
| | KCl | 2.7 mM |
| HB Buffer | Tris-HCl pH 7.9 | 20 mM |
| | KCl | 10 mM |
| | $MgCl_2$ | 1.5 mM |
| | PMSF | 1 mM |
| BC400 Buffer | Tris-HCl pH 7.9 | 20 mM |
| | NaCl | 400 mM |
| | Glycerol | 10 % |
| | EDTA | 0.2 mM |
| | Triton X-100 | 0.5 % |
| | PMSF | 1 mM |
| TSE I | Tris-HCl pH 8.0 | 20 mM |
| | EDTA | 2 mM |
| | NaCl | 150 mM |
| | SDS | 0.1 % |
| | Triton X-100 | 1 % |
| TSE II | Tris-HCl pH 8.0 | 1 mM |
| | EDTA | 2 mM |

| | | |
|---|---|---|
| | NaCl | 500 mM |
| | SDS | 0.1 % |
| | Triton X-100 | 1 % |
| LiCl Buffer | Tris-HCl pH 8.0 | 10 mM |
| | EDTA | 1 mM |
| | LiCl | 250 mM |
| | NP40 | 1 % |
| TE Buffer | Tris-HCl pH 8.0 | 10 mM |
| | EDTA | 1 mM |
| Blotting Buffer | Potassium Acetate | 100 mM |
| | HEPES/KOH | 30 mM |
| | Magnesium Acetate | 2 mM |
| Crystal Violet Solution | Crystal Violet | 0.1 % (w/v) |
| | Ethanol | 10 % (v/v) |
| | Acetic Acid | 10% (v/v) |
| Coomassie-Staining-Solution | Coomassie | 0.006 % (w/v) |
| | Methanol | 10 % (v/v) |
| | Acetic Acid | 10 % (v/v) |
| Coomassie-Destain-Solution | Methanol | 10% (v/v) |
| | Acetic Acid | 10% (v/v) |
| GST-Lysis Buffer | HEPES pH 7.5 | 20 mM |
| | NaCl | 300 mM |
| | EDTA | 1 mM |
| | Lysozyme | 2 mg/ml |
| | PMSF | 1mM |
| GST-Elution Buffer | GST-Lysis Buffer | 1 x |
| | Glutathione | 10 mM |
| GST dialysis buffer | HEPES pH 7.5 | 30 mM |
| | NaCl | 150 mM |
| HBS Transfection Buffer, 2 x | NaCl | 275 mM |
| | KCl | 10 mM |
| | $Na_2HPO_4$ | 1.7 mM |
| | D-Glucose | 10 mM |

| | | |
|---|---|---|
| | HEPES pH 7.5 | 45 mM |
| HEMGN Buffer | KCl | 100 mM |
| | HEPES pH 8.0 | 25 mM |
| | EDTA pH 8.0 | 0.1 mM |
| | $MgCl_2$ | 12.5 mM |
| | Glycerol | 10 % (v/v) |
| His-Elution Buffer | HEMGN Buffer | 1 x |
| | KCl | 100 mM |
| | Imidazole | 10 mM |
| TB Buffer | EDTA | 1 mM |
| | KCl | 183 mM |
| | NaCl | 47 mM |
| | Tris-HCl | 10 mM |
| | PMSF | 1 mM |
| Lysis Buffer NP40/0.1 % SDS | Tris-HCl pH 7.4 | 20 mM |
| | NaCl | 150 mM |
| | NP40 | 1 % (v/v) |
| | SDS | 0.1 % (v/v) |
| | EDTA | 5 mM |
| | NaF | 25 mM |
| | Glycerol | 10 % (v/v) |
| SDS-PAGE Buffer, 10 x | Tris-Base | 0.25 M |
| | Glycine | 1.92 M |
| | SDS | 1 % (w/v) |
| SDS Loading Dye, 5 x | Tris/HCl pH 6.8 | 312.5 mM |
| | β-Mercaptoethanol | 25 % (v/v) |
| | Glycerol | 10 % (v/v) |
| | SDS | 10 % (w/v) |
| | Bromophenol Blue | 0.01 % (w/v) |
| SDS Stacking Gel Solution | Tris/HCl pH 6.8 | 125 mM |
| | Acrylamide | 5 % (v/v) |
| | SDS | 0.1 % (w/v) |
| | APS | 0.04 % (w/v) |

| | | |
|---|---|---|
| | TEMED | 0.075 % (v/v) |
| SDS Resolving Gel Solution | Tris/HCl pH 8.8 | 35 mM |
| | Acrylamide | 5 % (v/v) |
| | SDS | 0.1 % (w/v) |
| | APS | 0.04 % (w/v) |
| | TEMED | 0.074 % (v/v) |
| TAE Buffer, 50 x | Tris/HCl pH 8.0 | 2 M |
| | Acetic Acid | 1 M |
| | EDTA | 50 mM |
| TBS | Tris pH 7.5 | 50 mM |
| | NaCl | 150 mM |
| TBS-T Buffer | TBS | 1 x |
| | Tween 20 | 0.1 % (v/v) |
| West-Blotting Buffer | Tris | 25 mM |
| | Glycine | 190 mM |
| | Methanol | 10 % (v/v) |
| Ponceau S Staining Solution | Ponceau S | 0.5 % (w/v) |
| | Acetic acid | 1 % (v/v) |

### 2.1.6 Media and Supplements for Cell Culture

| Reagent | Manufacturer |
|---|---|
| DMEM 4.5 g/l Glocose, L-Glutamine | Gibco (Invitrogen) |
| FBS 10 % | PAA Laboratories |
| Sodium Pyruvate 100 mM | Gibco (Invitrogen) |
| HEPES 1 M | Gibco (Invitrogen) |
| L-Glutamine 200 mM | Gibco (Invitrogen) |
| Penicillin/Streptomycin | Gibco (Invitrogen) |
| Opti-MEM® | Gibco (Invitrogen) |
| Trpsin/EDTA Solution 1 x | Gibco (Invitrogen) |

### 2.1.7 Mammalian Cell Lines

| Cell Line | Description | Company |
|---|---|---|
| HCT116 WT | human Colon Carcinoma | Dr. B. Vogelstein, Baltimore |

| | | |
|---|---|---|
| HCT116 p53 KO | human Colon Carcinoma | Dr. B. Vogelstein, Baltimore |
| RPE1 | human Retina | ATCC |
| U2OS | human Bone Osteosarcoma | ATCC |
| MCF7 | human Breast Cancer | ATCC |

### 2.1.8 Media for Bacteria Cultivation

| | |
|---|---|
| LB-Medium | 10 g/l Tryptone<br>5 g/l yeast extract<br>5 g/l NaCl |
| SOB-Medium | 20g/l Tryptone<br>5 g/l yeast extract<br>0.5 g/l NaCl<br>2.5 mM KCl<br>10 mM $MgCl_2$ |

### 2.1.9 Bacterial Strains

E. coli DH5α (Invitrogen)

E. coli BL21 pLysS (Novagen)

E. coli Rosetta (Novagen)

E. coli Stbl3 (Invitrogen)

### 2.1.10 Expression Vectors

| cDNA | Backbone | Tag | Source |
|---|---|---|---|
| p53 wt | pcDNA3 | HA | T.G. Hofmann |
| TBL1X wt | pGEX4T.1 | GST | A. Jones |

### 2.1.11 Antibodies

#### 2.1.11.1 Primary Antibodies

| Antibody | Species | Dilution | Company |
|---|---|---|---|

| | | | |
|---|---|---|---|
| p53 | mouse (monoclonal) | 1:1000 | Santa Cruz |
| p53 | rabbit (polyclonal) | 1:1000 | Santa Cruz |
| p53 | mouse (monoclonal) | 1:200 | Sigma Aldrich |
| p53 pSer46 | mouse (monoclonal) | 1:1000 | BD Pharmingen |
| p53 acetyl Lys373/382 | rabbit (polyclonal) | 1:1000 | Millipore (Upstate) |
| p21 | rabbit (monoclonal) | 1:1000 | Cell Signaling |
| PUMA | rabbit (monoclonal) | 1:1000 | Cell Signaling |
| cl-PARP | rabbit (polyclonal) | 1:1000 | Abcam |
| Actin | mouse (monoclonal) | 1:100000 | MP Biomedicals |
| TBL1 | rabbit (polyclonal) | 1:1000 | Abcam |
| TBL1 | Guinea pig (polyclonal) | 1:500 | Gift from Valentina Perissi |
| Acetyl-H3K9 | rabbit (monoclonal) | 1:50 | Cell Signaling |
| Methyl-H3K9 | mouse (polyclonal) | 1:100 | Cell Signaling |
| Acetyl-H3K27 | rabbit (polyclonal) | 1:100 | Cell Signaling |
| Methyl-H3K27 | rabbit (monoclonal) | 1:50 | Cell Signaling |
| Acetyl-H4K16 | rabbit (polyclonal) | 1:50 | Cell Signaling |

The TBL1 Geinea pig polyclonal antibody from Valentina Perissi was only used in Fig. 12 for the Co-IP experiment. The TBL1 antibody used in other experiments was rabbit polyclonal antibody which is from Abcam.

#### 2.1.11.2 Senondary Antibodies

Immunoblot: HRP-conjugated secondary antibodies were obtained from Dianova and diluted 1:20000 in 5% milk/TBST.

### 2.1.12 siRNA and Oligonucleotides

siTBL1: Hs-TBL1X_7 FlexiTube siRNA (Qiagen)

siNCoR1: L-003518-00-0005 (Dharmacon)

siSMRT: L-020145-01-0005 (Dharmacon)

siTBLR1: Hs_TBL1XR1_10, (Qiagen)

siHDAC3: J-003496-09-0010 (Dharmacon)

siHDAC1: L-003493-00-0005 (Dharmacon)

### 2.1.13 TaqMan Probes

| Gene | Description |
|---|---|
| CDKN1A | Hs00355782_m1 |
| HPRT1 | Hs02800695_m1 |
| TBL1X | Hs00959540_m1 |
| BBC3 | Hs00248075_m1 |
| NCoR1 | Hs01094541_m1 |
| HDAC3 | Hs00187320_m1 |
| NCoR2 | Hs00196955_m1 |

### 2.1.14 Primers

| Gene | Up | Down |
|---|---|---|
| HDAC1 | 5'-CTGGGGACCTACGGGATATT-'3 | 5'-TGTCAGGGTCTTCCTCATCC-'3 |
| GAPDH | 5'-CCAAAGTTGTCATGGATGAC-'3 | 5'-GTGAAGGTCGGTGTGAACG G-'3 |
| CDKN1A | 5'-GTGGCTCTGATTGGCTTTCT G -'3 | 5'-CTGAAAACAGGCAGCCCAA G -'3 |
| BBC3 | 5'-GCGAGACTGTGGCCTTGTGT -'3 | 5'-CGTTCCAGGGTCCACAAAG T -'3 |

### 2.1.15 Software

| Application | Software |
|---|---|
| Virtual Cloning | Serial cloner 2.6 |
| FACS Analysis | BD CellQuest™ Pro, BD Biosciences |
| Absorbance Detection | Ascent Vs. 2.6, Labsystems |

| Statistics | Prism 7.0, GraphPad |
|---|---|

### 2.1.16 Instruments

| Instrument | Name | Company |
|---|---|---|
| Absorbance Detector | Multiskan Ms | Labsystems |
| Developing Machine | Curix 60 | AGFA HealthCare |
| FACS | FACS Calibur | BD Biosciences |
| Spectrophotometer | Photometer Ultrospec 3000 pro | Amersham Biosciences |
| Luminescence Reader | Synergy 2 | BioTek |
| PCR-Cycler | T3000 Thermocycler | Biometra |
| qPCR Cycler | LightCycler® Systems | Roche |

## 2.2 Methods

### 2.2.1 Methods of Molecular Biology

#### 2.2.1.1 Preparation of chemically competent E. coli

Glycerol stock (E. coli DH5α, Invitrogen; E. coli BL21 pLysS, Novagen; E. coli Stbl3, Invitrogen) was scratched to inoculate an overnight culture in 3 ml LB medium. Overnight culture was used to inoculate 120 ml SOB medium and grow at 20 °C until $OD_{600}$ of 0.4 - 0.6. Flask was put on ice for 10 min and collected by centrifugation at 4000 rpm, 10 min at 4 °C. Cells were resuspended gently in 40 ml of ice-cold TB buffer and on ice for another 10 min. Cells were collected by centrifugation again and resuspended in 10 ml of ice-cold TB buffer. DMSO was added to a final concentration of 7 % and the buffer was mixed gently. Cells were placed on ice for 10 min, aliquoted into 100 µl and frozen in liquid nitrogen and stored in -80 °C.

#### 2.2.1.2 Transformation of chemically competent E. coli

100 µl bacteria were thawed on ice and 1 µl plasmid DNA or 1 µl ligation mix was added and gently mixed without vortexing. Bacteria were incubated on ice for 30 min and then were heated-shock for 45 sec at 42 °C. Immediately they were put on ice for 2 min and 400 µl LB medium was added. The bacteria in medium were put in the thermomixer at 37 °C for 60 min at 500 rpm. All bacteria were put on selective LB-plates and incubated at 37 °C, 180 rpm overnight. Single clone was inoculated to LB medium the next day.

#### 2.2.1.3 Plasmid preparation

For analytical preparation a single bacteria clone was inoculated to 3 ml selective LB medium and incubated at 37 °C overnight. Bacteria were collected by centrifugation for 5 min at 8000 rpm. Plasmid DNA was extracted using QIAprep Spin Miniprep Kit (Qiagen) following the manufacturer's protocol.

To get higher amount of plasmid 300 ml selective LB medium was inoculated at 37 °C, 180 rpm overnight and pelleted for 15 min at 8000 rpm, 4 °C. Extraction of plasmid DNA was performed with the QIAGEN Plasmid Maxi Kit (Qiagen) following the manufacturer's protocol.

The QIAquick Gel Extraction Kit (Qiagen) was used for extraction of plasmid from agarose following manufacturer's instruction.

#### 2.2.1.4 RNA isolation and purification

Total RNA was isolated with TRIzol Reagent (Invitrogen) according to the manufacturer's protocol. RNA was transcribed to cDNA with the High Capacity RNA-to-cDNA Kit (Applied Biosystems) following the manual.

To purify RNA isolated from TRIzol, RNA Clean & Concentrator - 5 (R1015) (ZYMO RESEARCH) was used according to the manufacturer's protocol.

#### 2.2.1.5 Quality control for DNA and RNA

Concentration and purity were determined with Nano Drop spectrophotometer.

Only for the DNA precipitated for ChIP, Qubit 4 Fluorometer (Thermo Fisher) was used to determine the very accurate concentration.

4200 TapeStation System (Integrated Sciences) was used to analyze the quality of RNA for RNA-seq.

#### 2.2.1.6 Enzymatic Modification of DNA

**DNA digestion and dephosphorylation**

Restriction enzymes from New England Biolabs were used to perform digestion according to the manufacturer's protocol. For analysis 400 ng plasmid DNA was incubated with 0.5 U enzyme in a total volume of 20 µl for 60 min at 37 °C. Preparative digestions were performed with 2 µg DNA and 1 U enzyme in a total volume of 50 µl at 37 °C for 3 - 4 h. To avoid relegation Antarctic phosphatase (NEB) was used for dephosphorylation.

**Ligation**

Ligation of DNA with compatible ends was done with T4 DNA Ligase (NEB) following the manual. Basically, 50 ng of the digested and dephosphorylated vector was incubated with a 3-fold molar amount of insert DNA fragment and 1 U T4 ligase in appropriate buffer containing ATP. The total volume was 20 µl and incubated overnight at 16 °C.

#### 2.2.1.7 Agarose gel electrophoresis

Agarose gel electrophoresis was performed to separate DNA fragments of analytic and preparative digestions. Ethidium bromide (Roth) was added for visualization with a final concentration of 1 µg/ml. 1 kb DNA Ladder GeneRuler (Fermentas) and 100 bp DNA ladder (Invitrogen) were used to determine the length of DNA fragment.

#### 2.2.1.8 Quantitative Real-time Polymerase Chain Reaction (qRT-PCR)

qRT-PCR was performed for expression analysis (e.g. analysis of knockdown efficiency) and binding level analysis (e.g. amount of transcription factor on gene promoter). Therefore, the TaqMan® Gene Expression Assays from Applied Biosystems and iTaq™ Universal SYBR® Green Supermix from BIO-RAD were both used following respective instruction. For relative gene expression, analysis was done by applying the ΔΔCt method, which is based on the relative quantification through compared amplification of an endogenous control gene (HPRT1). For exact binding level of proteins to gene promoters, the Percent Input Method and the Fold Enrichment Method were used, which are based on the comparison between Ct values.

qRT-PCR Reaction Setup (TaqMan):

| Component | Volume for one reaction |
|---|---|
| TaqMan® Universal PCR Master Mix (2X) | 5 µL |
| TaqMan® probe | 0.5 µL |
| DNA template | 1 µL |
| Nuclease-free Water | 3.5 µL |
| Total Volume | 10 µL |

qRT-PCR protocol (TaqMan):

| Parameter | UNG incubation | Polymerase activation | PCR (40 cycles) | |
|---|---|---|---|---|
| | Hold | Hold | Denature | Anneal/extend |
| Temperature | 50 °C | 95 °C | 95 °C | 60 °C |
| Time (mm:ss) | 02:00 | 10:00 | 00:15 | 01:00 |

qRT-PCR Reaction Setup (SYBR® Green):

| Component | Volume for one reaction |
|---|---|
| iTaq™ Universal SYBR® Green supermix (2X) | 5 µL |
| Forward and reverse primers | 1 µL |
| DNA template | 1 µL |
| Nuclease-free Water | 3 µL |
| Total Volume | 10 µL |

qRT-PCR protocol (SYBR® Green):

| Parameter | Polymerase activation | PCR (35 cycles) | |
|---|---|---|---|
| | Hold | Denature | Anneal/extend |
| Temperature | 95 °C | 95 °C | 60 °C |
| Time (mm:ss) | 00:30 | 00:15 | 01:00 |

#### 2.2.1.9 Recombinant Protein Expression in E. coli

Polyhistidine (His-) and glutathione S-transferase (GST-) fusion proteins were recombinantly expressed in E. coli BL21 pLysS or E. coli Rosetta and purified via affinity chromatography. In general, 3 ml LB medium were inoculated first and incubated overnight at 37 °C. Then 100 ml LB medium were inoculated with pre-cultured bacterial and incubated at 37 °C until $OD_{600}$ = 0.3 - 0.6. Protein expression was induced by adding IPTG (final concentration 1 mM) and incubated for 6 h at 30 °C. Pellets were collected by centrifugation for 15 min at 8000 rpm and washed once with ice cold PBS. Pellets were centrifuged again and resuspended in 10 ml lysis buffer with protease inhibitor. Bacteria were sonicated and then added 1 % Triton X to incubate on ice for 15 min. Then bacteria were centrifuged to collect supernatants and added respective washed beads (Ni-NTA or GSH) to incubate for 3 h under constant mixing at 4 °C. Beads were washed three times with lysis buffer and the protein amount was determined by SDS PAGE and Coomassie staining. Elution from beads was performed by 1 ml elution buffer with PMSF and 1 % Triton X for 1 h

under constant mixing at 4 °C. Buffer exchange was done by dialysis or by using centricon filters.

## 2.2.2 Methods of Cell Biology

### 2.2.2.1 Cultivation of Mammalian Cells

Cells were cultivated in T75 flasks at 37 °C, 5 % $CO_2$ and 95 % humidity. All the work concerning cell cultivation and treatment was done under sterile condition. DMEM medium and additives (see **2.1.6**) were prewarmed to 37 °C prior to use. Cells were passaged every 2 - 3 days based on the individual proliferation rate. Cells were washed with PBS, detached with Trypsin-EDTA, resuspended in fresh culture media and transferred into a new flask with respective dilution.

### 2.2.2.2 Cryoconservation

To stock cells in liquid nitrogen or –80 °C freezer, cells were centrifuged at 1800 rpm, 4 °C for 5 min to get cell pellet and dissolved with freezing media (5 ml FCS, 1 ml DMSO and 4 ml DMEM). To thaw cells, cells were put quickly into 37 °C water bath until they become liquid. Then cells were pipetted into prewarmed culture medium and put into the incubator.

### 2.2.2.3 Transient Transfection

HCT116, RPE-1, MCF7 and U2OS cells were transfected with Lipofectamine RNAiMax following the manufacturer's instructions. For siRNA transfection 6 well plates were seeded with 2 x $10^5$ cells/well. 24 h later 100 nM siRNA (for TBL1, NCoR, SMRT, TBLR1 knockdown) or 50 nM siRNA (for HDAC1, HDAC2 and HDAC3 knockdown) and 5 µl or 2.5 µl Lipofectamine were prepared in Opti-MEM medium, distributed evenly in each well. Opti-MEM medium was changed to culture medium after 4 - 5 h and cells were harvested 24 - 48 h post transfection.

### 2.2.2.4 RNA-seq

HCT116 (wt or p53-deficient) cells were transfected with control siRNA or TBL1 specific siRNA (Pool of 7 different siRNAs) for 24 h. Each sample group had three biological replicates. Total RNA was prepared as said in **2.2.1.4**. The RNA quality was evaluated as written in **2.2.1.5** and the RNA integrity number (RIN) is more than

8. Before performing RNA-seq analysis, a small aliquot of each sample was analyzed by qRT-PCR to confirm TBL1 knockdown efficiency. RNA-seq analysis was performed at the Genomics and Proteomics Core Facility in German Cancer Research Center. Transcriptome-sequencing libraries were prepared from total RNA extractions using the TruSEQ RNA sample preparation kit (Illumina). Single-read 50 bp sequencing was performed on a HiSeq-2000 sequencing machine (Illumina). Reads were trimmed by removing stretches of bases at the end of the reads, which had a phred quality score of less than 30. Reads were mapped using Tophat 2.0.6 (Kim et al., 2013) against the hg19 assembly of the human genome. Differential expression was quantified using DESeq2 (Love et al., 2014) and subjected to multiple testing corrections. Genes with a q-value smaller than 0.05 were considered to be differentially expressed. FPKM values were computed using Cuffdiff 2.0 (Trapnell et al., 2012). Principal Component Analysis (PCA) plots and heatmaps were done in R using the FactoMineR or gplot packages, respectively. DAVID Functional Annotation Bioinformatics Microarray Analysis was used to predict the pathways and biological processes most likely to be affected by the observed gene expression changes.

#### 2.2.2.5 Treatment of Eukaryotic cells

**5-Fluorouracil**

5-Fluorouracil acts principally as a thymidylate synthase (TS) inhibitor. Interrupting the action of this enzyme blocks synthesis of the pyrimidine thymidine, which is a nucleoside required for DNA replication. In this work it was mainly used to induce DNA damage. It was dissolved in DMSO (30 mM 5-FU stock) and diluted with medium to 50 μM for indicated time points.

**Nutlin-3a**

Nutlin-3a is a compound that can inhibit the interaction between MDM2 and p53, in this way stabilizing p53. In this work it was used to stabilize p53 and activate its activity without inducing DNA damage. It was dissolved in DMSO (10 mM stock) and diluted with medium to 10 μM for indicated time points.

**Adriamycin/Doxorubicin**

Adriamycin intercalates into the DNA and inhibits biosynthesis by inhibiting the topoisomerase II complex. In this work it was also used to induce DNA damage, in which the role of TBL1 was studied. It was dissolved in distilled water (10 mg/ml) and diluted with medium to 0.5 µg/ml for indicated time points.

### 2.2.3 Cell based Assays

#### 2.2.3.1 Colony Formation Assay

Colony formation assay was used to analyze cellular proliferation rate and cytotoxicity of treatments. Cells were seeded in a certain density (around 1000 cells/well in 6 well plate) and transfected with siTBL1 to knock down TBL1. Then the respective drug in defined concentration was added for the indicated time points. For staining, the medium was removed and cells were rinsed carefully with PBS. Then PBS was removed, stained for at least 30 min with 2 ml crystal violet staining solution. The crystal blue was removed and cells were washed with tap water carefully. The plate was dried in normal air at room temperature and the number of colonies were calculated.

#### 2.2.3.2 FITC Annexin V Apoptosis Detection

Apoptotic and necrotic cells were detected by flow cytometry following Annexin V/PI staining (FITC Annexin V Apoptosis Detection Kit I, BD Pharmingen™). Cells were seeded and treated in 6 well plate. Then medium was removed and cells were detached with Trypsin/EDTA. Cells were collected by centrifugation at 1000 rpm, 4 °C for 5 min and washed once with ice cold PBS. 50 µl Binding buffer and 2.5 µl Annexin V were added to cells. The mixer was incubated 15 min on ice in the dark. 430 µl Binding buffer was added to the cell mixture and transferred to FACS tube. At the end 10 µl PI (50 µg/ml) was added and subsequently measured by FACS (BD Biosciences). In general, Annexin V positive cells were combined to a total cell death rate and the percentage cell death of an untreated cell fraction was subtracted from the one of a treated cell fraction to obtain the specific cell death rate.

## 2.2.4 Methods of Protein Biochemistry

### 2.2.4.1 Preparation of Cell Lysates

For lysis cells were scraped from plates with culture media and centrifuged at 1800 rpm, 4 °C for 5 min. Cell pellets were washed once with ice cold PBS and spinned down again. Cell pellets used for immunoblotting were resuspended in NP40/0.1% SDS lysis buffer including protease inhibitors (Complete protease inhibitor cocktail, Roche) and incubated on ice for 60 min. Co-immunoprecipitation samples were first prepared in HB buffer (see **2.1.5**) for the remove of cytosolic fraction and BC400 buffer (see **2.1.5**) for nuclear fraction. Lysates were then cleared by centrifugation at 13000 rpm, 4 °C for 30 min. For immunoblotting 5 x Loading buffer was added and samples were heated at 95 °C for 5 min.

### 2.2.4.2 Determination of Protein Concentration

To make an equal loading of samples on SDS-PAGE gel, lysates concentration was determined by using the BCA Protein Assay Kit (Thermo Fisher Scientific) following the manual and absorbance at 570 nm was measured in a plate reader (Labsystems). Reference curves were regenerated by measuring absorbance of BSA protein standard solutions.

### 2.2.4.3 SDS-PAGE

Sodium dodecyl sulfate (SDS) polyacrylamide gel electrophoresis under denaturing conditions was used to separate proteins according to their electrophoretic mobility. The prepared lysates (see **2.2.4.1**) and desired protein amount were loaded on 8 - 12.5 % gel which were self-made. Electrophoresis procedure was accomplished in apparatuses purchased from Bio Rad Laboratories (Hercules, USA).

### 2.2.4.4 Western blot (Immunoblotting)

Immunoblotting was performed using PVDF membranes which were activated in methanol for 10 sec prior to using. SDS-gel was placed on the membrane and both parts were covered by Whatman paper and fiber pads. Wet electrophoretic transfer method was used and the condition was 80 V at 4 °C for 1 h and 30 min. After that transfer efficiency was checked by Ponceau S staining. Then membranes were incubated in 5 % fat-free milk in TBST buffer for around 1 h to block unspecific binding sites. Primary antibody (see **2.1.11.1**) was added and membranes were

incubated on a rotator at 4 °C overnight. The next day membranes were washed 3 x with TBST to remove excess primary antibody and Horseradish peroxidase (HRP) coupled second antibody was added for 60 min at room temperature on a rotator. Membranes were washed again 3 times with TBST and developed by incubating with HRP substrate.

#### 2.2.4.5 Co-immunoprecipitation

Lysates were precleared with 10 µl protein A/G Plus Sepharose beads (Santa Cruz, USA) on a rotator at 4 °C for 1 h. Solid phase was discarded by centrifugation for 5 min, 4 °C 1800 rpm. 2 µl TBL1 antibody (Abcam) and 25 µl p53 antibody (Santa Cruz) against the target protein were added to the lysate and incubated at 4 °C overnight. Next day 10 µl washed protein A/G Plus Sepharose beads were added to lysates and incubated for 4 h at 4 °C. Beads were washed 3 times with 500 µl lysis buffer and finally, resuspended in 1 x SDS sample buffer and heated at 95°C for 5 min before analyzed on SDS-PAGE gel and western blot.

#### 2.2.4.6 GST pulldown assay

To examine protein *in vitro* interaction, GST pulldown assay was performed. The GST-TBL1 protein was expressed in E. coli BL21pLysS-Rosetta, purified, and kept bound to glutathione beads. His-p53 protein was purified and eluted (unattached to beads). The lysates were precleared 2 x in 500 µl IVB buffer containing 0.2 % NP40 and 50 µl Glutathione-Sepharose 4 Fast Flow (GSH beads) for 1 h at 4 °C on a rotating wheel. Then a constant volume of p53 protein was added to the lysates which was incubated at 4 °C for 2 h on rotating wheel. After that beads were washed 3 times with buffer and samples were separated by denaturing SDS-PAGE. GST loading was analyzed by Coomassie staining and Western blot (primary antibody used see **2.1.11.1)**.

#### 2.2.4.7 Chromatin immunoprecipitation

10 million cells were fixed with 1% formaldehyde for 10 min at room temperature and the fixation was stopped by glycine with a final concentration of 125 mM. Harvested cells were lysed first with cell lysis buffer for 10 min at 4 °C. The nuclei were spinned down and the pellet was resuspended with ice-cold nuclear lysis buffer for 10 min on ice. The sample was sonicated 20x 30 sec with maximal output. After sonication, the

lysates were centrifuged, and the supernatants were collected and pre-cleaned by 30 µl ChIP-Grade Protein G Magnetic Beads (Cell Signaling Technology, 9006S) in dilution buffer for 1 h at 4 °C. The pre-cleaned lysates were aliquoted equally and incubated with antibody TBL1 (Abcam, 1:500) or antibody p53 (CST, 1:250) overnight at 4 °C. 30 µl Saturated protein G magnetic beads were added to each sample and incubated for 2 h at 4 °C. The beads were washed with TSE I, TSE II, buffer LiCl, and buffer TE (see **2.1.5)**, sequentially. The binding components were eluted in 1 % SDS and 0.1 M $NaHCO_3$ and reverse cross-linkage was performed at 65 °C overnight. DNA was extracted using the PCR purification Kit (Qiagen, 28106). Real-time PCR was performed to detect relative enrichment of the protein on indicated genes.

### 2.2.5 Statistical analysis

qRT-PCR, ChIP qRT-PCR and apoptosis detection results are shown as means ± Standard Deviation (s.d.). Statistical significance was determined by using two-tailed, unpaired Student *t*-test in all figures except those described below. In Fig. 11, significance was determined by 2way Anova with Bonferroni-adjusted posttest. In Fig. 17 and Fig. 30, significance was determined by 1way Anova with Dunnetts-adjusted posttest. All statistical analysis was performed using GraphPad Prism software. $P < 0.05$ and $P < 0.01$ were denoted as statistically significant.

# 3 Results

## 3.1 TBL1 regulates p53 in the absence of cellular stress

### 3.1.1 Altered gene expression upon knockdown of TBL1 in HCT116 cells

Previous findings from our laboratory indicated that there might be a potential link between the co-transcriptional factor TBL1 and the tumour suppressor p53. In order to find out if there is a connection between TBL1 and p53, the influence of TBL1 on p53 target gene expression was first investigated via RNA-seq. Therefore, TBL1 was depleted by a pool of seven siRNAs in HCT116 wt and p53-deficient cells. After 24 h, RNA was isolated and sent to the Genomics and Proteomics Core Facility of the German Cancer Research Centre (DKFZ) for sequencing (Fig. 7 A). Fig 7 B showed that a two-dimensional principle component analysis (PCA) was generated to visualize the relationship between the samples. It could be seen that the clustering of the replicates and clear separation between siCtr and siTBL1 treated cells (PC2) as well as between HCT116 wt and p53 deficient cells (PC1). As shown in Fig. 7 C, there were 1055 genes upregulated and 1108 genes downregulated upon TBL1 depletion in HCT116 wt cells, whereas in the HCT116 p53-deficient cells, 1351 genes were upregulated and 1364 genes were downregulated (Fig. 7 D). By rejecting the overlapping genes affected by TBL1 depletion in both HCT116 wt and p53-deficient cells, the remaining genes affected in HCT116 wt cells have been considered to be p53 dependent and TBL1 regulated genes. As a result, 294 upregulated and 196 downregulated genes were found to be p53-dependent genes upon TBL1 knockdown (Fig. 7 E). As it has been revealed that gene downregulation by p53 is indirect, so here upregulated genes are more focused (Allen et al., 2014, Brady et al., 2011, Fischer, 2017, Verfaillie et al., 2016).

Recently, Fisher evaluated over 3000 p53 target genes by performing a meta-analysis of data sets from the literature and identified 116 genes as high-confident p53 targets in at least 6 out of 16 genome-wide data sets. These p53 target genes are involved in a variety of cellular responses, including cell cycle arrest, DNA repair, apoptosis, autophagy, metabolism and mRNA translation (Fischer, 2017). Hence, I compared the 294 upregulated genes with these 116 genes and found 51 overlapping genes (Fig. 7 F and supplementary data) which strengthened my finding that expression of certain p53 targets were suppressed by TBL1.

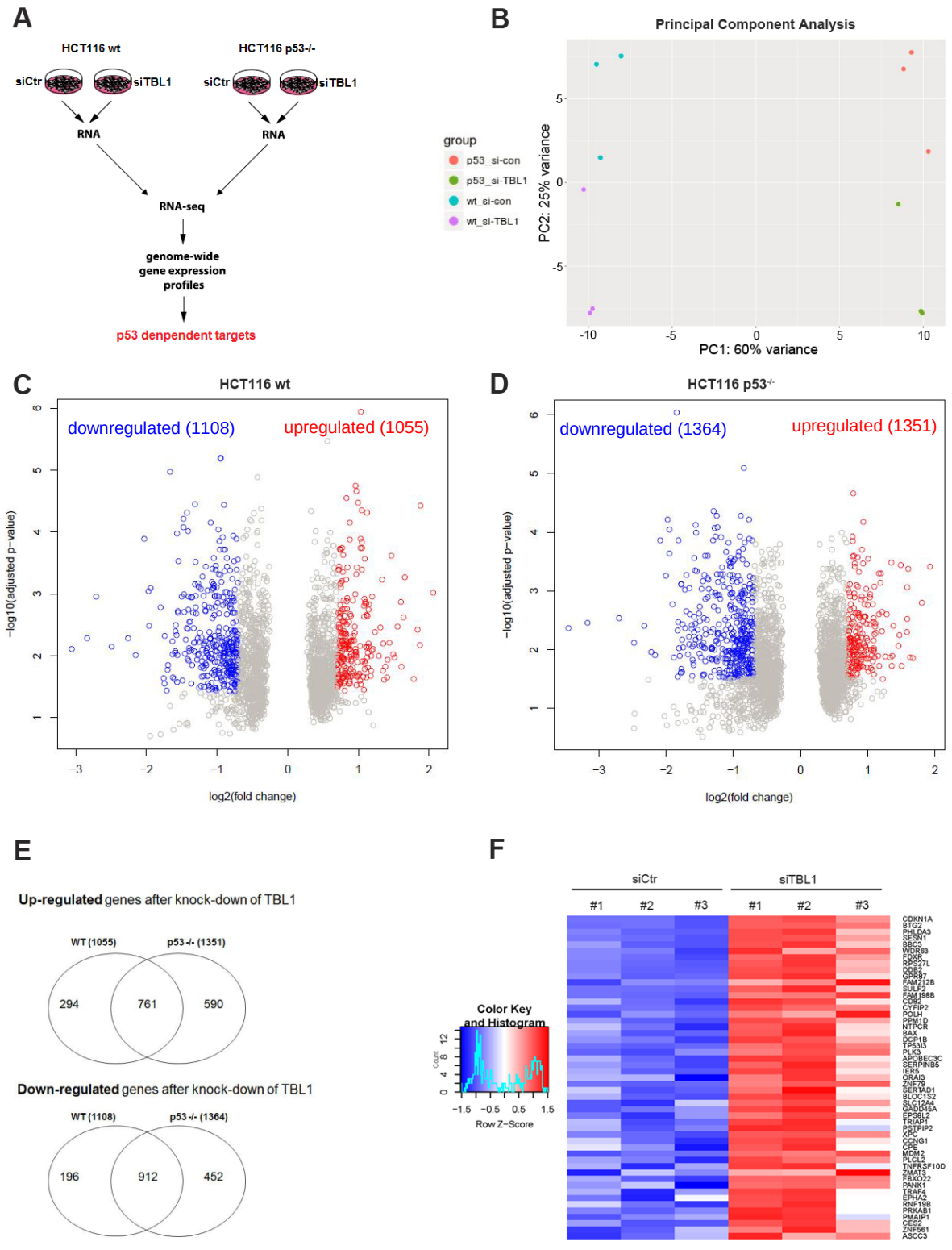
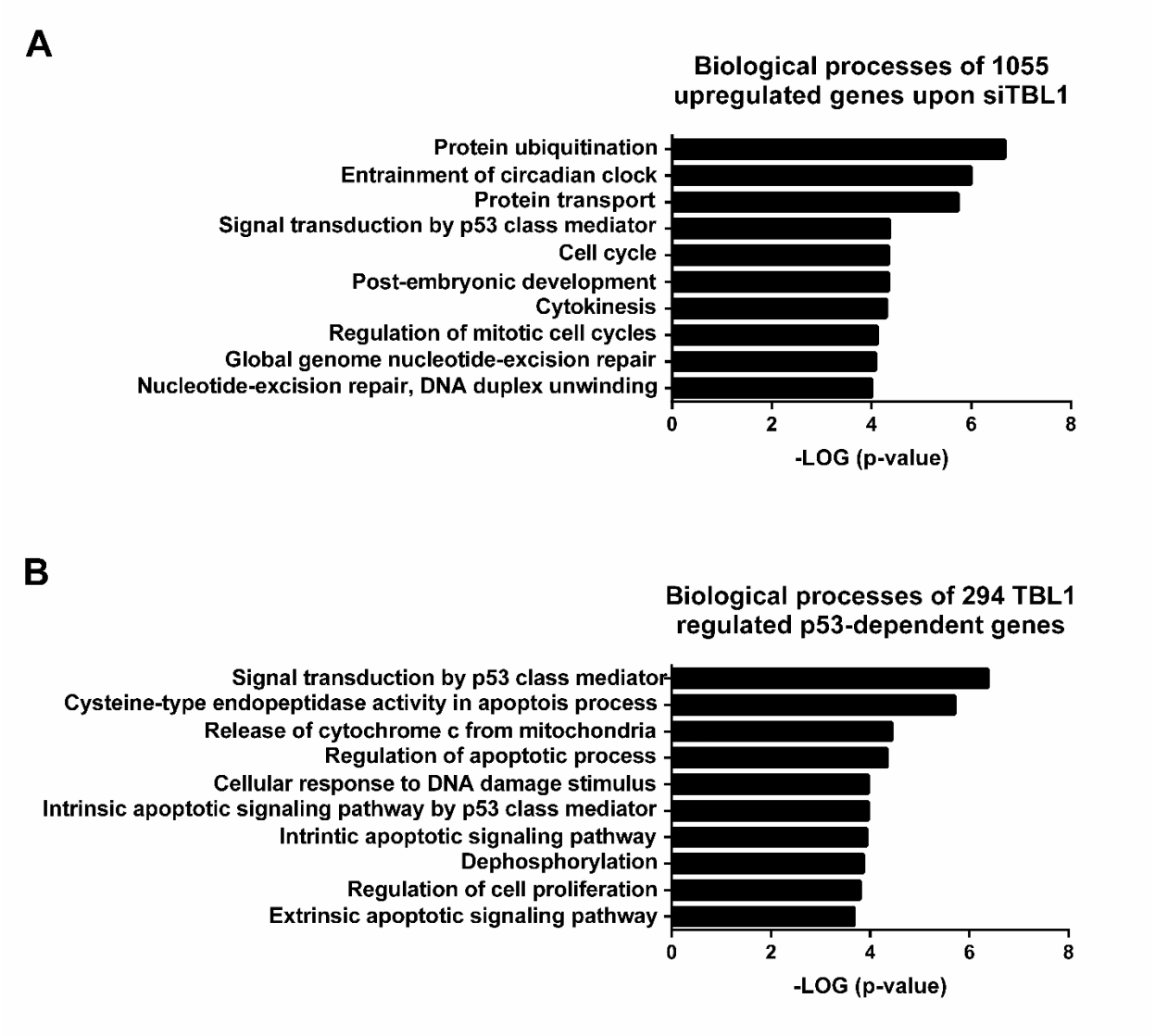



**Fig. 7: RNA-seq analysis to identify genes regulated by p53-TBL1 interplay. A** Experimental outline. **B** PCA analysis. **C** Genes regulated upon TBL1 depletion in HCT116 p53 wt cells. **D** Genes regulated upon TBL1 depletion in HCT116 p53$^{-/-}$ cells. **E** 294

upregulated and 196 downregulated p53 target genes are dependent on TBL1 depletion. **F** Heat map of high-confident p53 targets upon TBL1 depletion.

Gene Ontology (GO)-term analysis of biological process was performed on the 1055 upregulated genes upon TBL1 knockdown (Fig. 8 A) and specified the analysis on the 294 p53-dependently upregulated genes (Fig. 8 B). It showed that these TBL1-regulated p53-dependent genes were indeed involved in p53 signaling pathway.



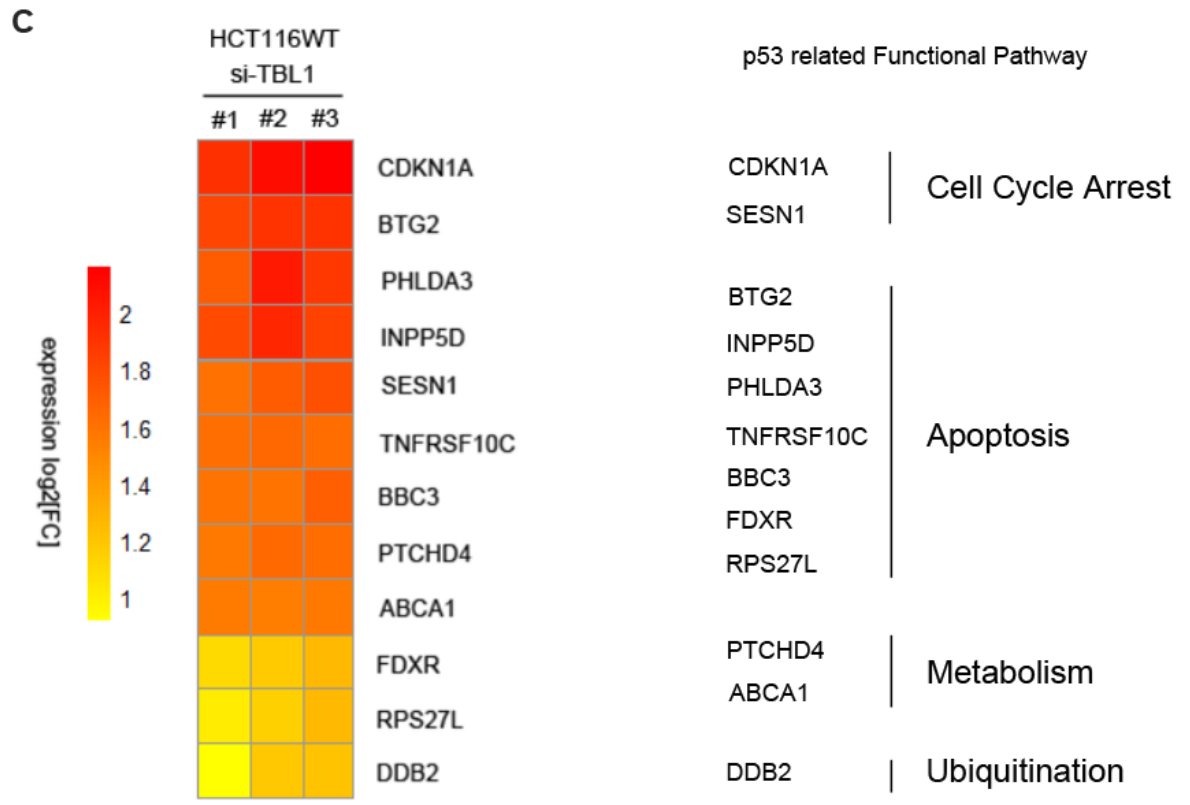



**Fig. 8: Functional analysis of TBL1 regulated genes**. **A** GO-term functional analysis of 1055 upregulated genes upon siTBL1 in HCT116 wt cells. **B** GO-term functional analysis of 294 p53-dependently upregulated genes upon siTBL1 in HCT116 wt cells. **C** Heat map of top-12-upregulated p53 target genes by depletion of TBL1 and functional analysis of them.

Fig. 8 C illustrates fold changes in expression of the top 12 TBL1-upregulated p53 target genes. It turned out that seven of these genes code for pro-apoptotic factors and two others are involved in the control of cell cycle arrest. For subsequent experiments, two canonical p53 target genes, namely CDKN1A (codes for p21) involved in cell cycle arrest and BBC3 (also called PUMA) involved in cell apoptosis, were selected as representatives of these cellular processes.

### 3.1.2 Validation of RNA-seq data by qRT-PCR

Several genes from top 12 TBL1-regulated genes (Fig. 8 C) were selected to confirm the RNA-seq result by qRT-PCR. Fig. 9 showed that these genes expression level upon TBL1 knockdown was consistent to the RNA-seq result.

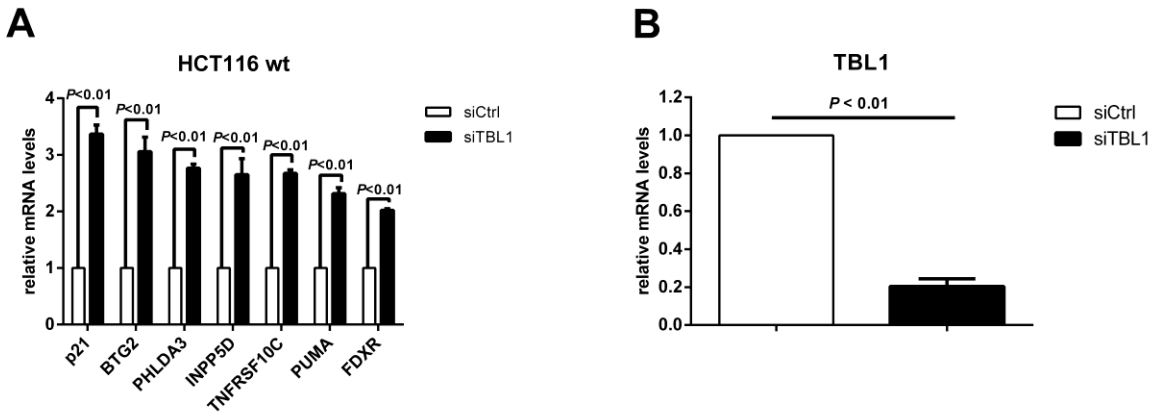



**Fig. 9: Validation of differentially expressed genes by qRT-PCR.** HCT116 cells were transfected with 100 nM siCtrl or siTBL1 and expression of the p53 target genes p21, BTG2, PHLDA3, INPP5D, TNFRSF10C, PUMA and FDXR (**A**) was analyzed 24 h after transfection by qRT-PCR. **B** Knockdown efficiency of TBL1 was verified by qRT-PCR; mRNA levels were normalized to HPRT1 mRNA expression and mRNA levels of siCtrl cells were set to 1.0. Error bars indicate mean ± s.d., n = 3 for biological replicates.

### 3.1.3 TBL1 depletion has a mild effect on cell growth arrest

The gene with the highest upregulation upon TBL1 knockdown is the p53 target gene CDKN1A which encodes for the cyclin-dependent kinase inhibitor 1 and functions as a regulator of cell cycle progression during G1 phase. In order to investigate an effect of TBL1 depletion on the cell cycle, propidium iodide (PI) staining was performed at different time points after TBL1 knockdown. 24 h later, there was almost no change in cell cycle distribution (Fig. 10 A). After 48 h a statistically significant increase in the G1 fraction has been detected in HCT116 wt cells (Fig. 10 B). There was no further increase in cell cycle status at 72 h after TBL1 depletion compared with that after 48 h (Fig. 10 C). In HCT116 p53-deficient cells there was almost no change in cell cycle distribution upon TBL1 depletion (Fig. 10 D, E, F).

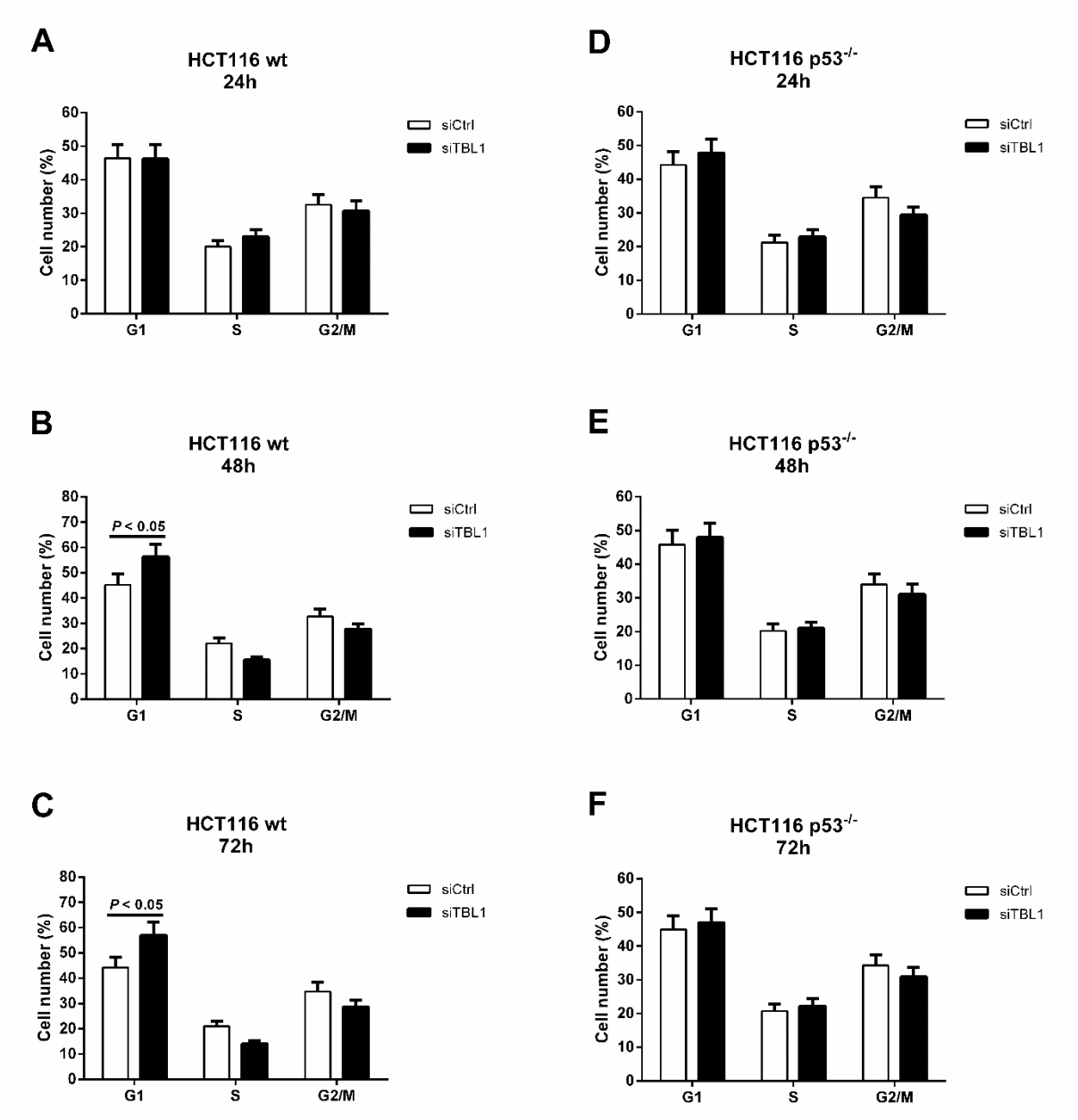



**Fig. 10: Effects of TBL1 depletion on cell cycle progression in HCT116 cells.** Cell cycle analysis was performed by flow cytometry. **A** Knockdown of TBL1 by siRNA in HCT116 wt cells did not induce cell cycle arrest in G0/G1 phase at 24 h after transfection. Knockdown of TBL1 by siRNA in HCT116 wt cells induced cell cycle arrest in G0/G1 phase at 48 h after transfection **B** and 72 h after transfection **C**. **D, E, F** Knockdown of TBL1 by siRNA in HCT116 p53 $^{-/-}$ cells did not induce cell cycle arrest in G0/G1 phase at 24 h, 48 h and 72 h after transfection (knockdown efficiency of TBL1 not shown). Error bars indicate mean ± s.d., n = 3 for biological replicates.

### 3.1.4 TBL1 regulates p53 target genes in RPE-1, U2OS and MCF7 cell lines

In order to determine whether p53 target genes induction in response to TBL1 depletion is also evident in other tumour cell lines and in non-cancerous cells, different cell lines were chosen for further gene expression experiments. U2OS is a well-established osteosarcoma cell line which is used in many studies (Lauvrak et al., 2013). MCF7 is a breast cancer cell line which is the origin of a wide spectrum of current knowledge in breast cancer (Levenson and Jordan, 1997). RPE-1 (Retinal pigmented epithelial cells) has been used to represent non-cancerous cells.

In MCF7 and U2OS, the gene expression of p21 and PUMA was highly increased upon TBL1 knockdown (Fig. 11 A, B). However, the slight increase in p21 and PUMA expression was not statistically significant in RPE-1 (Fig. 11 A, B). This indicated that TBL1 might be exploited by tumour cells in order to suppress p53 activity.

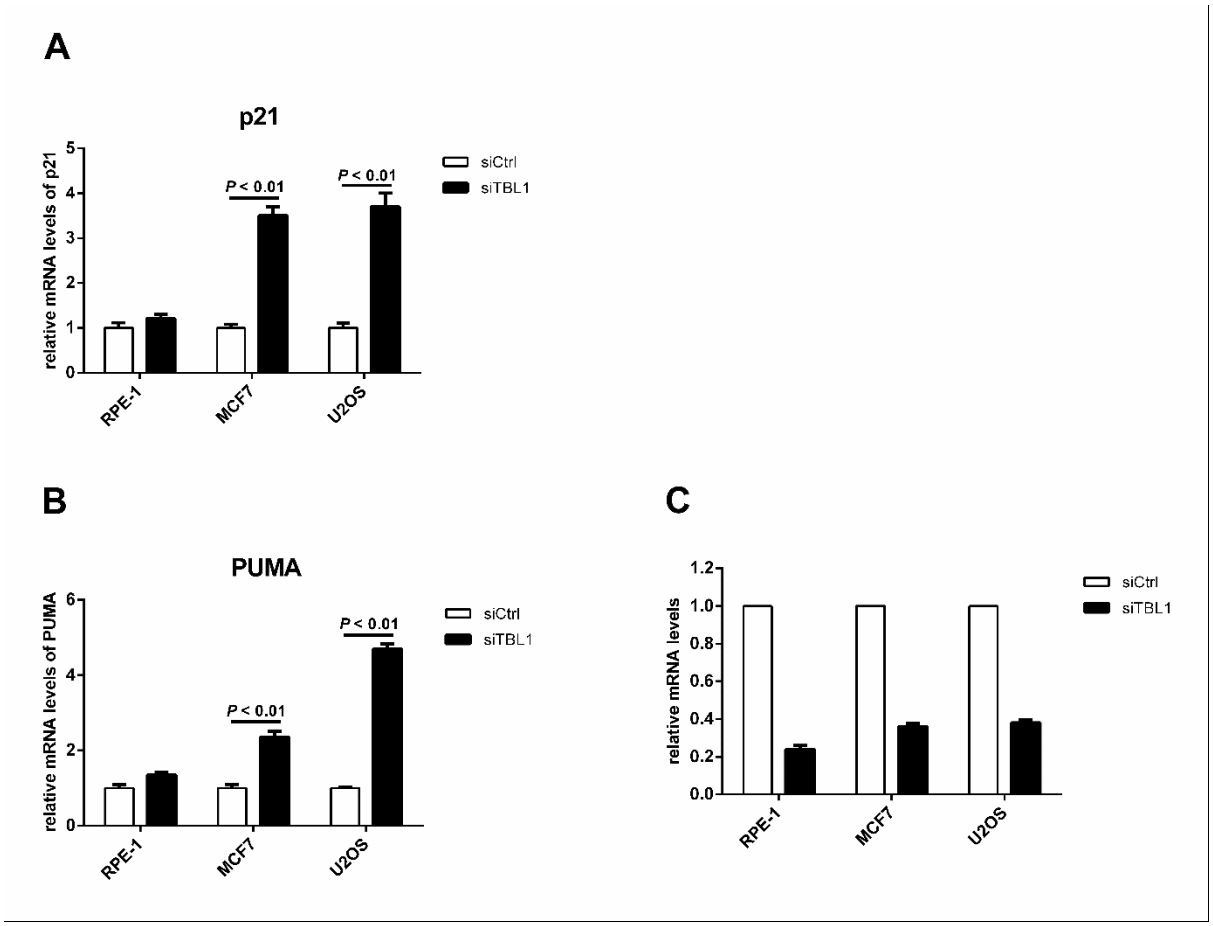


**Fig. 11: Knockdown of TBL1 enhances p21 and PUMA expression in MCF7 and U2OS, but not in RPE-1.** RPE-1, MCF7 and U2OS cells were transfected with 100 nM siCtrl or siTBL1 and expression of the p53 target genes p21 (**A**) and PUMA (**B**) was analyzed 24 h after transfection by qRT-PCR. **C** Knockdown efficiency of TBL1 was verified by qRT-PCR; mRNA levels were normalized to HPRT1 mRNA expression and mRNA levels of siCtrl cells were set to 1.0. Error bars indicate mean ± s.d., n = 3 for biological replicates.

### 3.1.5 Interaction of TBL1 with p53 *in vitro* and *in vivo*

Investigation of gene regulation revealed that TBL1 regulates a subset of p53 target genes. Because of this finding, it was then interesting to explore whether these two proteins bind to each other. In order to determine whether these two proteins endogenously interact with each other, co-immunoprecipitation (Co-IP) has been performed. Fig. 12 A showed that p53 protein was successfully precipitated by the corresponding p53 antibody in HCT116 wt cells and that TBL1 could be co-precipitated with p53. Although TBL1 was found to be co-precipitated in HCT116 wt cells, it was not detected in p53-deficient cells. Vice versa, p53 was detected in co-precipitated binding partners of pulled TBL1 and there was no p53 found from pulled control IgG antibody which served as a negative control (Fig. 12 B).

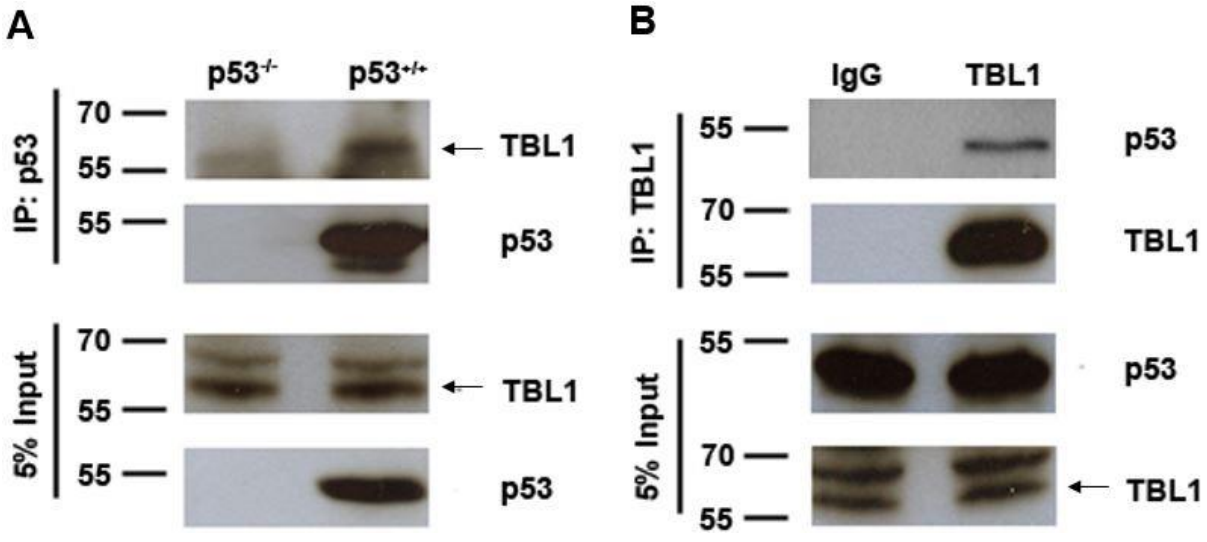



**Fig. 12: p53 and TBL1 endogenously interact in HCT116 cells.** Co-IP of endogenous TBL1 and p53 **A** HCT116 wt cells and HCT116 p53 $^{-/-}$ cells (as control) were used, anti-p53 antibody was added and then pulled from total lysate using protein A/G plus-agarose beads, TBL1 was found to be co-precipitated. **B** HCT116 wt cells were used, anti-IgG antibody (as negative control) and anti-TBL1 antibody were added and then pulled, p53 was co-

precipitated; proteins were detected by immunoblotting with anti-TBL1 and anti-p53 antibodies and HRP-coupled secondary antibodies. TBL1 antibody used here is the Guinea pig polyclonal antibody which is from Valentina Perissi. Data are shown as representative of three experiments.

To determine whether the interaction is direct or indirect, GST-Pulldown assay was performed *in vitro*. Bacterially expressed GST-TBL1 or GST was incubated with bacterially expressed His-p53 respectively and co-precipitation was analyzed by western blot. The results shown in Fig. 13 clearly revealed a specific interaction between TBL1 and p53, since His-p53 could be only detected with GST-TBL1 instead of GST.

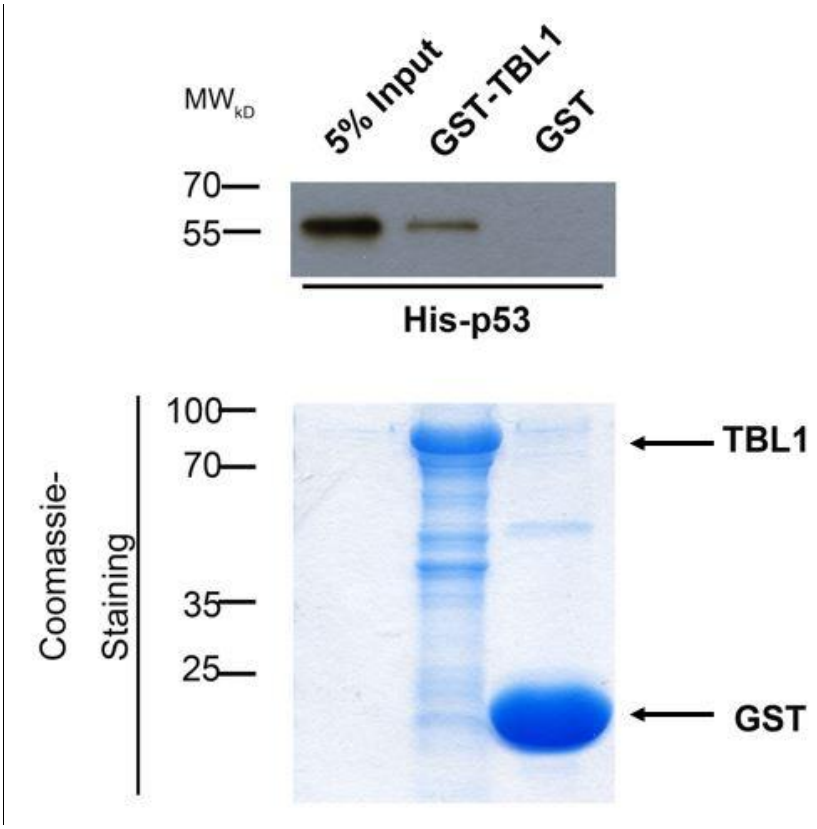



**Fig. 13: p53 and TBL1 interact *in vitro*.** *In vitro* GST-Pulldown with GST-TBL1 and His-p53: GST and GST-TBL1 were bacterially expressed, purified and incubated with bacterially expressed His-p53, co-precipitation was analyzed by western blot. GST-proteins were Coomassie stained as loading control, GST served as negative binding control, 5% of the His-protein was loaded as input control. Data are shown as representative of three experiments.

### 3.1.6 p53 and TBL1 are recruited to p53 target gene promoters in unstressed cells

Based on the findings that TBL1 depletion could induce the expression of a set of p53 target genes without any external stress and that TBL1 binds to p53, it was hypothesized that p53 and TBL1 were already associated to some of its target promoters under unstressed conditions. In order to prove this, chromatin immunoprecipitation (ChIP) qRT-PCR was performed analyzing p21 and PUMA as representative p53 targets. As expected, p53 and its binding partner TBL1 were both found to be bound to the promoter of p21 and PUMA in HCT116 wt cells. But in HCT116 p53-deficient cells TBL1 was not bound to the p21 and PUMA promoter anymore, which indicated this action was p53 dependent (Fig. 14 A-D).

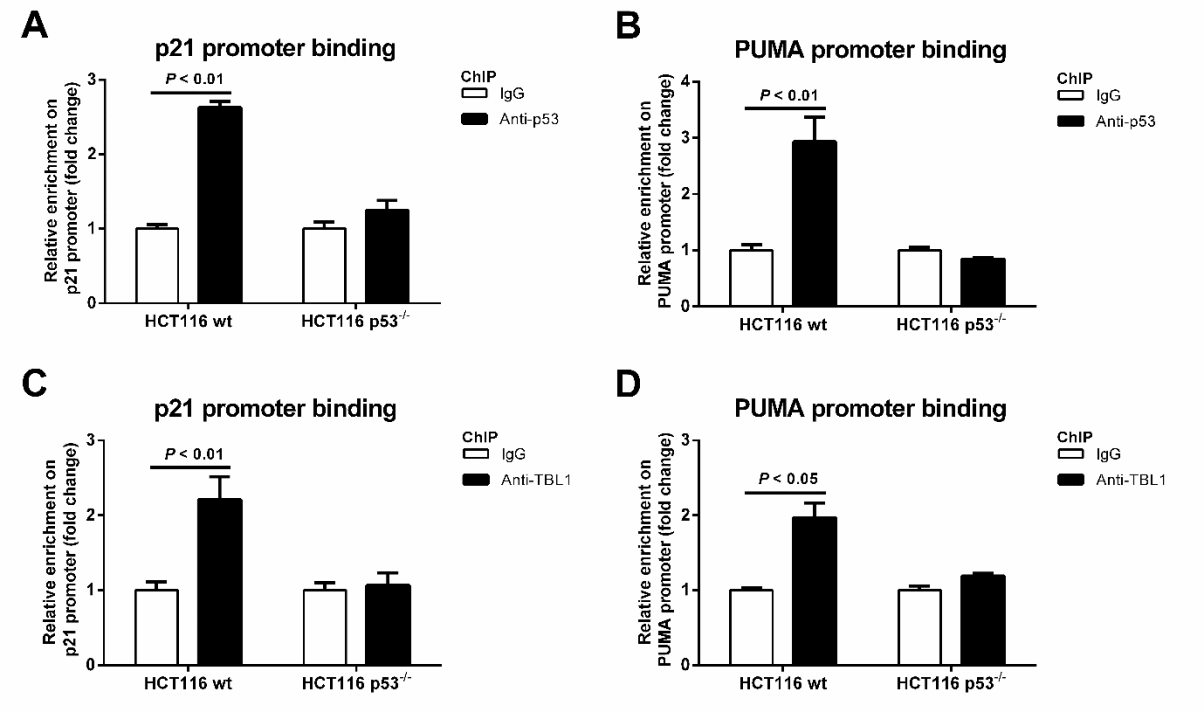



**Fig. 14: Binding of p53 and TBL1 on p53 target promoters by ChIP analysis.** DNA was isolated following chromatin immunoprecipitation by **A**, **B** anti-p53 antibody and **C**, **D** anti-TBL1 antibody. p21 and PUMA promoter DNA was determined by qRT-PCR. The outcome is presented in relation to the extracts precipitated with IgG antibody and set to 1.0. HCT116 $p53^{-/-}$ cells were used as control. Error bars indicate mean ± s.d., n = 3 for biological replicates.

### 3.1.7 TBL1 depletion has virtually no effect on p53 protein level

Consequently, I wanted to know how TBL1 represses p53 target gene transcription and unravel the mechanism behind it. It is known that rapid accumulation of large amounts p53 is a marker for p53 activation. At first, whether p53 protein level change was investigated upon TBL1 depletion. In four replicates it was shown that there were no significant changes in p53 protein level upon TBL1 knockdown (Fig. 15). Consistent with the RNA-seq result, protein levels of two p53 targets p21 and PUMA were enhanced compared to the control (Fig. 15). Hence, the suppression of p53 activity is not due to the inhibition of p53 protein accumulation.

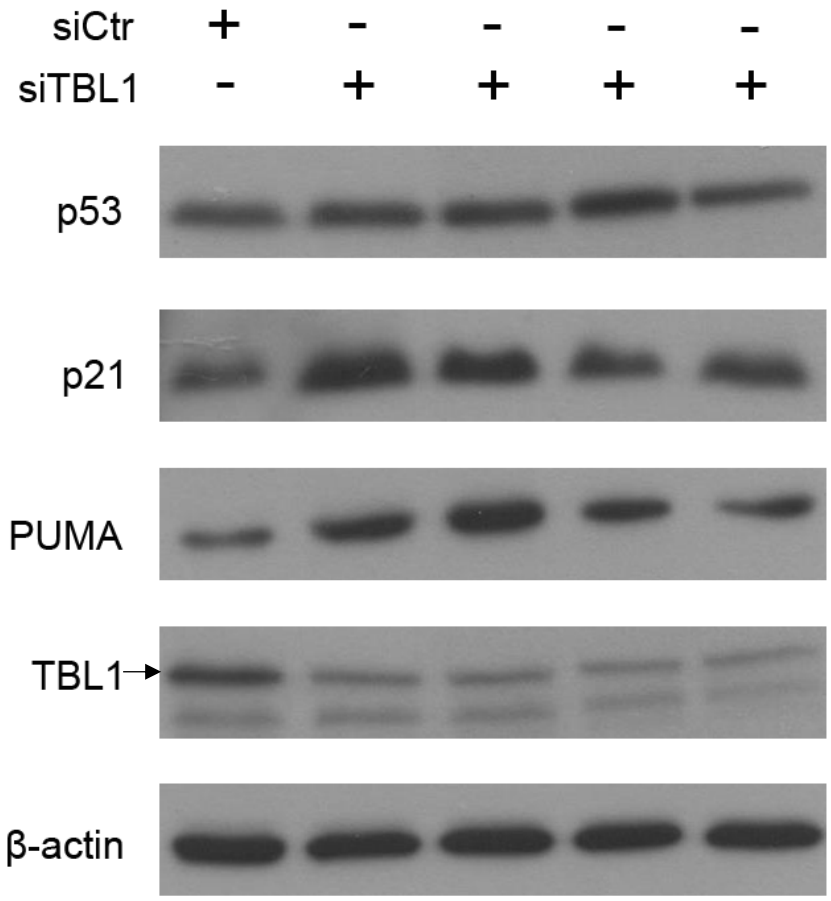



**Fig. 15: TBL1 knockdown does not increase p53 protein levels.** HCT116 wt cells were transfected with siCtr and siTBL1 for 48 h. Lysates were analyzed by immunoblotting with anti-p53, anti-TBL1, anti-p21, anti-PUMA and anti-β-actin antibodies and specific HRP-coupled secondary antibodies. The TBL1 antibody from here were all used rabbit polyclonal antibody which was from Abcam. Data are shown as representative of three experiments.

### 3.1.8 TBL1 depletion has virtually no effect on p53 modifications

Post-translational modifications like phosphorylation and acetylation are quite essential for p53 activation. It has been shown that acetylation at lysine 373 and 382 as well as phosphorylation at serine 15 of p53 play an important role in the induction of p53 target genes transcription (Zhao et al., 2006, Appella and Anderson, 2001, Bode and Dong, 2004). Therefore, these modifications have been detected upon TBL1 knockdown. The topoisomerase II inhibitor doxorubicin was used as a positive control for p53 activation. However, there were again no significant differences between the acetylation and phosphorylation of cells treated with siCtr or siTBL1 (Fig. 16).

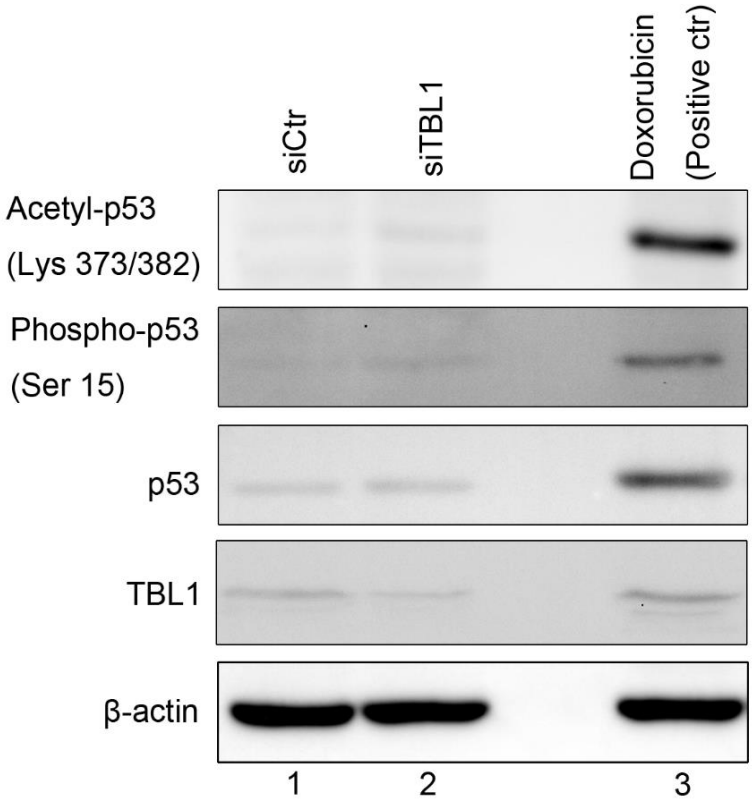



**Fig. 16: TBL1 knockdown does not affect p53 modifications.** HCT116 wt cells were transfected with siCtr or siTBL1or treated with 0.5 µg/ml Doxorubicin for 24 h as positive control. Lysates were analyzed by immunoblotting with anti-p53AcLys373/382, anti-p53pSer15, anti-p53, anti-TBL1 and anti-β-actin antibodies and specific HRP-coupled secondary antibodies. Data are shown as representative of three experiments.

### 3.1.9 TBL1 depletion has virtually no effect on promoter recruitment of p53

As stated in 3.1.6, p53 binds to several promoters in unstressed cells. Another potential mechanism how TBL1 regulates p53 target gene expression could be that TBL1 depletion influences p53 binding activity to its target promoters. Hence, ChIP was performed using a p53 antibody upon TBL1 knockdown. However, there was almost no change for the amount of p53 binding to p21 and PUMA promoters between the mock transfected and the TBL1 knockdown cells (Fig. 17 A, B).

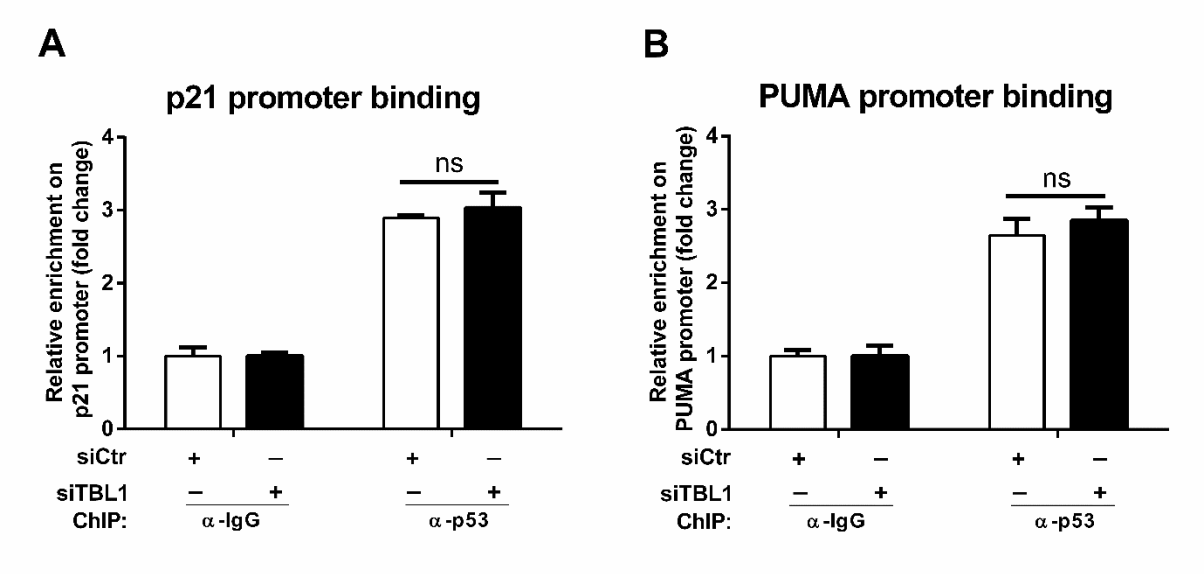



**Fig. 17: TBL1 knockdown does not influence p53 binding to target promoters.** HCT116 wt cells were transfected with siCtr and siTBL1 for 48 h. Then DNA was extracted following chromatin immunoprecipitation by anti-p53 antibody. **A** p21 and **B** PUMA promoter DNA was determined by qRT-PCR. The outcome is expressed in relation to extract precipitated with IgG antibody, which was set to 1.0. ns: not significant. Error bars indicate mean ± s.d., n = 3 for biological replicates.

### 3.1.10 TBL1 depletion increases H3K9/27 acetylation at p53 target gene promoters

It becomes more and more clear that epigenetic modifications on the chromatin play an important role in the regulation of gene expression. It has been reported that H3K9 and H3K27 acetylation at p53 target promoters regulate p53 target genes expression at the transcriptional level. Therefore, they were detected to investigate if

TBL1 knockdown does increase their binding to p53 target promoters. Fig. 18 A, C showed that there was an increase in acetylated-H3K9 and acetylated-H3K27 levels on p21 and PUMA promoters upon TBL1 knockdown in HCT116 wt cells. However, it has not been found for acetylated-H4K16. In p53-deficient cells, none of the three histones showed increased acetylation, indicating that this effect is p53 dependent (Fig. 18 B, D).

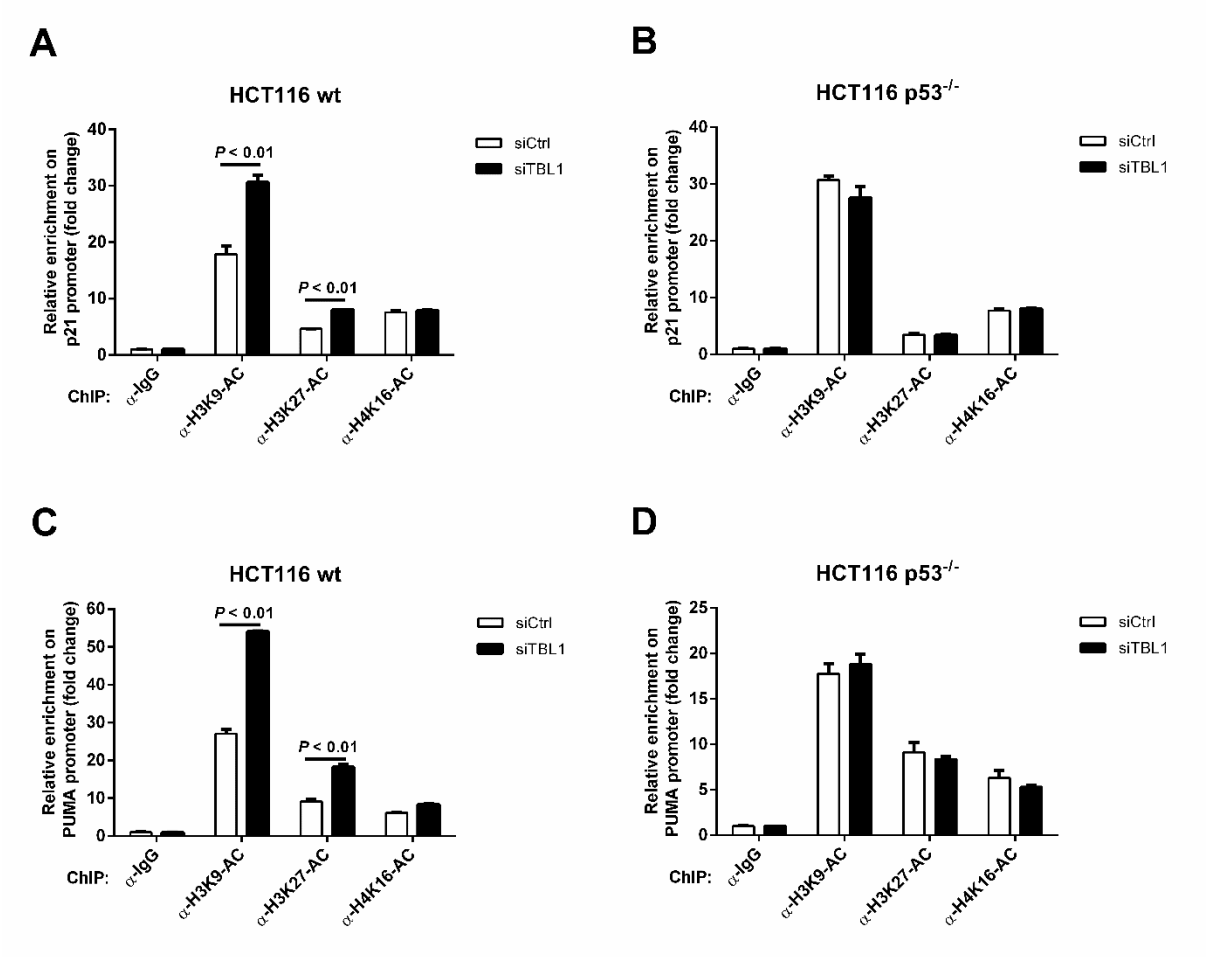



**Fig. 18: TBL1 knockdown increases histone acetylation at p21 and PUMA promoters in HCT116 wt cells. A**, **C** HCT116 wt cells and **B**, **D** HCT116 p53 $^{-/-}$ cells were transfected with siCtr and siTBL1 for 48 h. The chromatin was immunoprecipitated with anti-H3K9-AC, anti- H3K27-AC, anti-H4K16-AC and anti-IgG and then was extracted and determined by qRT-PCR on p21 and PUMA promoters. The outcome is expressed in relation to extract precipitated with IgG antibody which was set to 1.0. Error bars indicate mean ± s.d., n = 3 for biological replicates.

### 3.1.11 TBL1 depletion decreases H3K9/27 methylation at p53 target gene promoters

Histone acetylation partly unravels the chromatin to promote gene transcription. Conversely, most histone methylations lead to much denser chromatin structures which inhibits gene transcription. Based on the above findings of increased histone acetylation at p53 target promoters upon TBL1 depletion, correspondingly, this should be connected with the decrease of methylation at the same sites. Tri-methylated H3K9 and H3K27 are two markers for the repression of genes transcription. Therefore, these two histone methylation markers were detected via ChIP assay. Fig. 19 A, C revealed that methylated H3K9 and H3K27 were attenuated on p21 and PUMA promoter in HCT 116 wt cells in response to TBL1 knockdown, but not in HCT116 p53-deficient cells (Fig. 19 B, D).

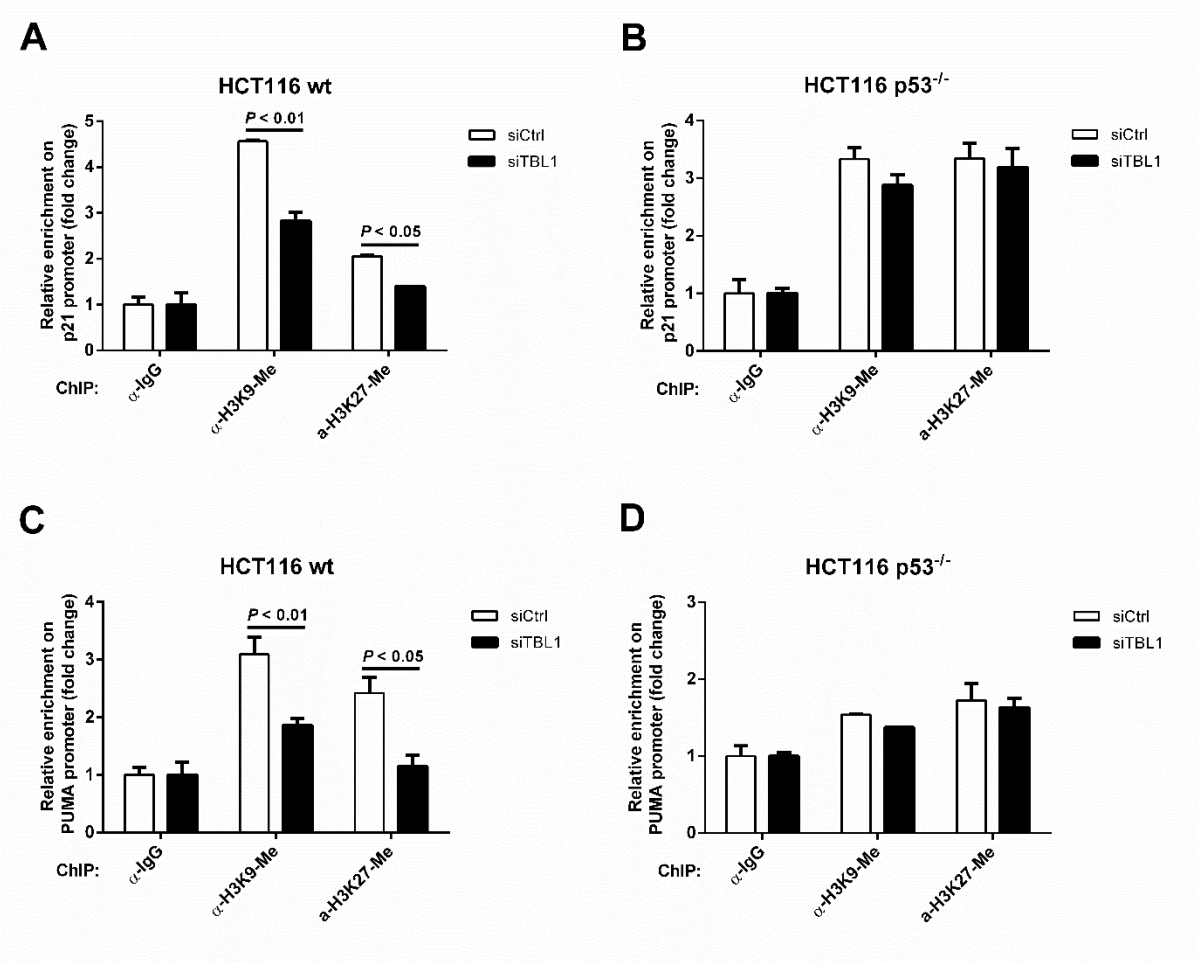



**Fig. 19: TBL1 knockdown decreases histone methylation at p21 and PUMA promoter in HCT116 wt cells. A**, **C** HCT116 wt cells and **B**, **D** HCT116 p53$^{-/-}$ cells were transfected with siCtr and siTBL1 for 48 h. The chromatin was immunoprecipitated with anti-H3K9-Me,

anti-H3K27-Me and anti-IgG antibodies. Upon chromatin extraction, relative enrichment was determined by qRT-PCR on p21 and PUMA promoters. The outcome is expressed in relation to extract precipitated with IgG antibody which was set to 1.0. Error bars indicate mean ± s.d., n = 3 for biological replicates.

### 3.1.12 Effect of co-repressor depletion on p53 target genes expression

So far it has been found that induction of p53 target gene expression upon TBL1 depletion is due to increased histone acetylation at p53 target promoters which means the suppression of p53 transcriptional activity by TBL1 in unstressed status is regulated via deacetylation on p53 target gene promoters. However, TBL1 has no enzymatic histone-deacetylating activity which means that there must be other factors recruited by TBL1 to the target promoters which ultimately initiate deacetylation. In order to determine these downstream mediators, several co-repressors that are components of NCoR/SMRT complex were knocked down by corresponding siRNAs and the gene expression of the representative p53 targets p21 and PUMA was detected. Knockdown of NCoR, HDAC3, and TBL1 all led to a significant induction of p21 and PUMA to a similar extent (Fig. 20 A, B).

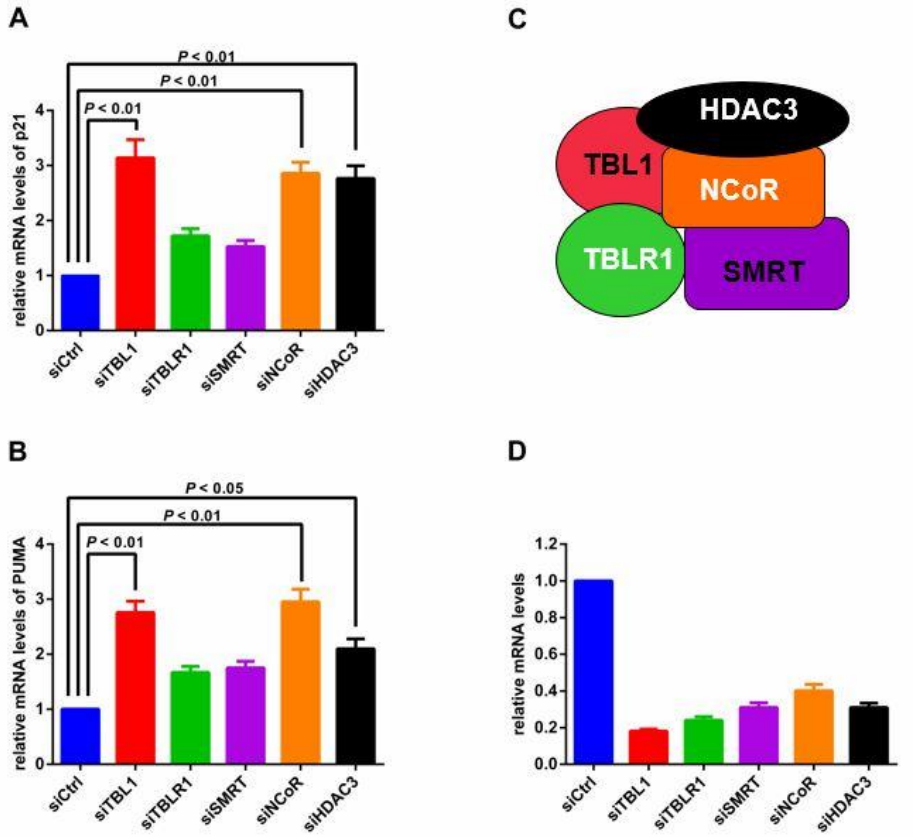


**Fig. 20: Knockdown of HDAC3 and NCoR enhances p21 and PUMA expression in HCT116 wt cells.** HCT116 wt cells were transfected with siCtrl, siTBL1, siTBLR1, siSMRT, siNCoR, and siHDAC3. After 24 h, expression of p53 target genes p21 (**A**) and PUMA (**B**) was analyzed by qRT-PCR. **D** Knockdown efficiency of TBL1, TBLR1, SMRT, NCoR and HDAC3 was verified by qRT-PCR; mRNA levels were normalized to HPRT1 mRNA expression and mRNA levels of siCtrl cells were set to 1.0. Error bars indicate mean ± s.d., n = 3 for biological replicates.

### 3.1.13 HDAC3 and NCoR knockdown phenocopies TBL1 depletion regarding the increased acetylation of H3K9/K27 at p53 target gene promoters

Another ChIP assay has been performed to analyze the histone acetylation status upon co-repressor depletion in comparison to TBL1 knockdown. It turned out that siHDAC3 significantly enhanced H3K9 and H3K27 acetylation compared to siCtr on p21 (Fig. 21 A) and PUMA promoters (Fig. 21 C). However, this enhancement was abolished in HCT116 p53-deficient cells (Fig. 21 B, D).

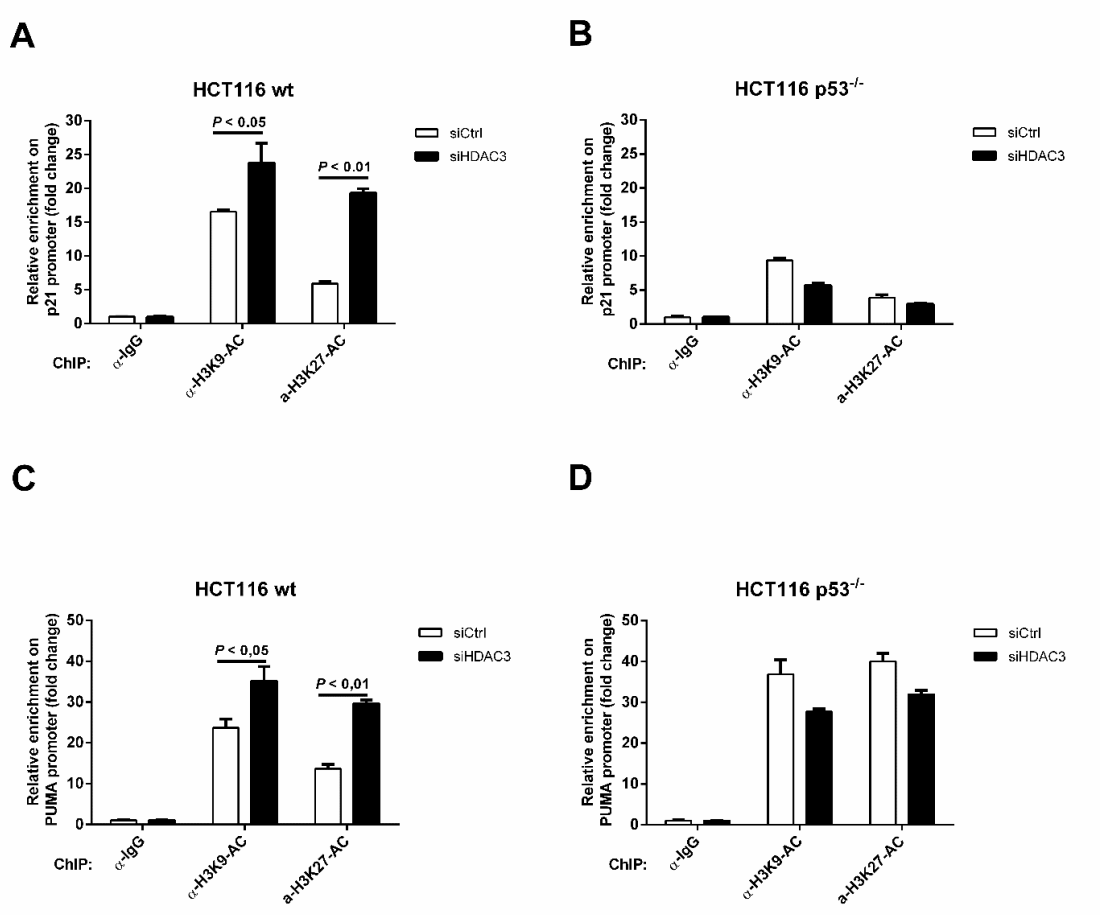


**Fig. 21: HDAC3 knockdown increases histone acetylation at p21 and PUMA promoters in HCT116 wt cells, but no p53-deficient cells. A**, **C** HCT116 wt cells and **B**, **D** HCT116 p53$^{-/-}$ cells were transfected with siCtr and siHDAC3 for 48 h. The chromatin was immunoprecipitated with anti-H3K9-AC, anti- H3K27-AC, or anti-IgG antibodies and then extracted and determined by qRT-PCR on p21 and PUMA promoters. The results are presented relatively to extract precipitated with IgG antibody which was set to 1.0. Error bars indicate mean ± s.d., n = 3 for biological replicates.

Knockdown of NCoR showed a similar result which also significantly enhanced H3K9 and H3K27 acetylation compared to siCtr on p21 (Fig. 22 A) and PUMA promoters (Fig. 22 C). This enhancement was abolished in HCT116 p53-deficient cells as well (Fig. 22 B, D).

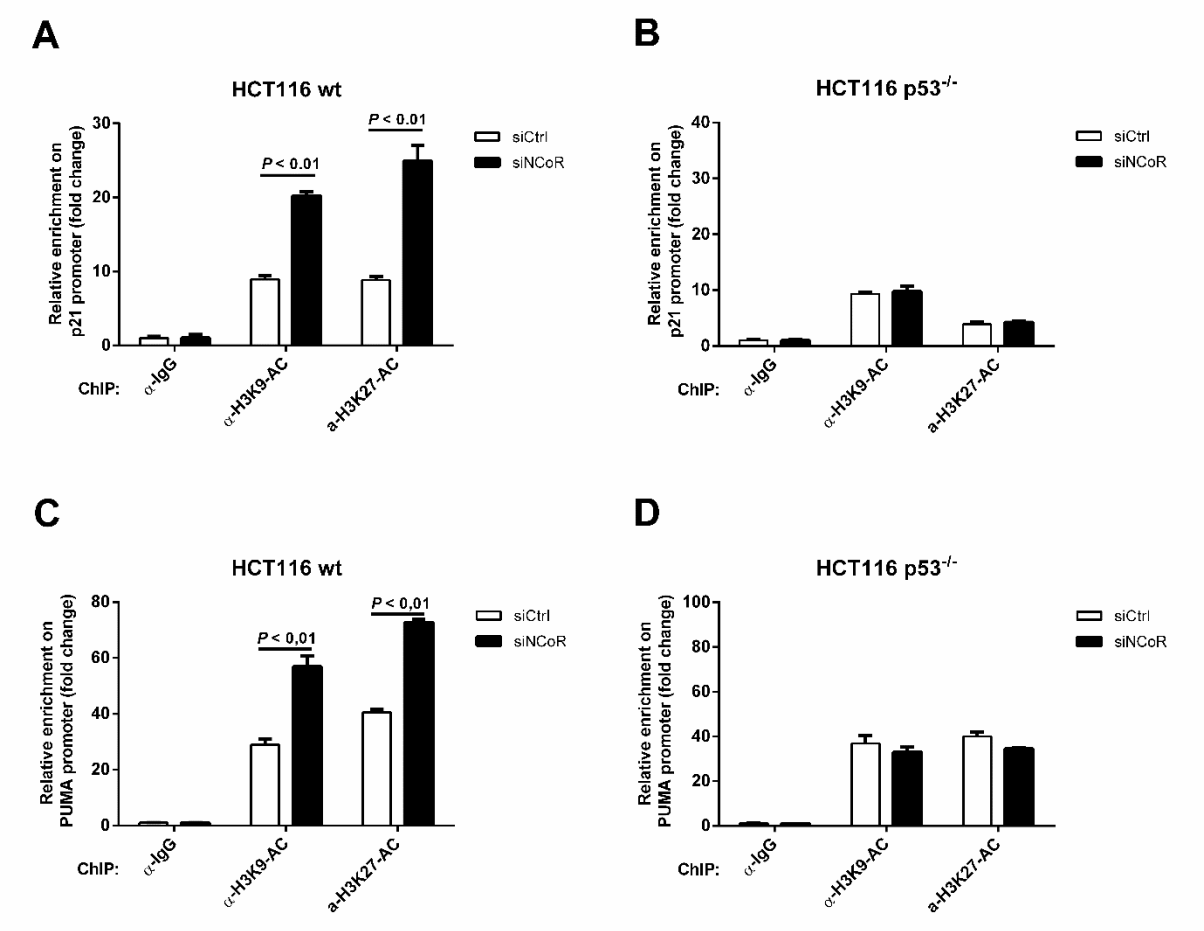



**Fig. 22: NCoR knockdown increases histone acetylation at p21 and PUMA promoters in HCT116 wt cells, but no p53-deficient cells. A**, **C** HCT116 wt cells and **B**, **D** HCT116 p53$^{-/-}$ cells were transfected with siCtr and siNCoR for 48 h. The chromatin was

immunoprecipitated with anti-H3K9-AC, anti- H3K27-AC, or anti-IgG and then extracted and determined by qRT-PCR on p21 and PUMA promoters. The results are presented relatively to extract precipitated with IgG antibody which was set to 1.0. Error bars indicate mean ± s.d., n = 3 for biological replicates.

## 3.2 Functional interplay of TBL1 and p53 in response to chemotherapeutic drug treatment

### 3.2.1 TBL1 depletion chemosensitizes HCT116 cells to 5-fluoroucil treatment in a p53-dependent manner

Based on the fact that TBL1 depletion induced a subset of p53 targets involved in cell cycle arrest or cell apoptosis, it was of huge interest to test whether it might also increase tumour cell sensitivity to chemotherapeutic agents. Therefore, FITC Annexin V Apoptosis assay was performed upon TBL1 knockdown and 5-FU treatment in HCT116 cells. As shown in Fig. 23 A, TBL1 depletion significantly increased cell apoptosis in comparison to siCtrl. Moreover, TBL1 depletion led to an increase of 15 % cell apoptosis compared to control cells in response to 5-FU treatment. This indicated that depletion of TBL1 sensitizes cells to chemotherapeutic drug treatment. Furthermore, since p53 is an important player in the induction of apoptosis upon genotoxic stress, it was essential to determine if the above findings are dependent on p53 activity. Fig. 23 C showed that cell apoptosis in HCT116 p53-deficient cells after knockdown of TBL1 and 5-FU treatment was not significantly altered, indicating an important role of p53 in this process.

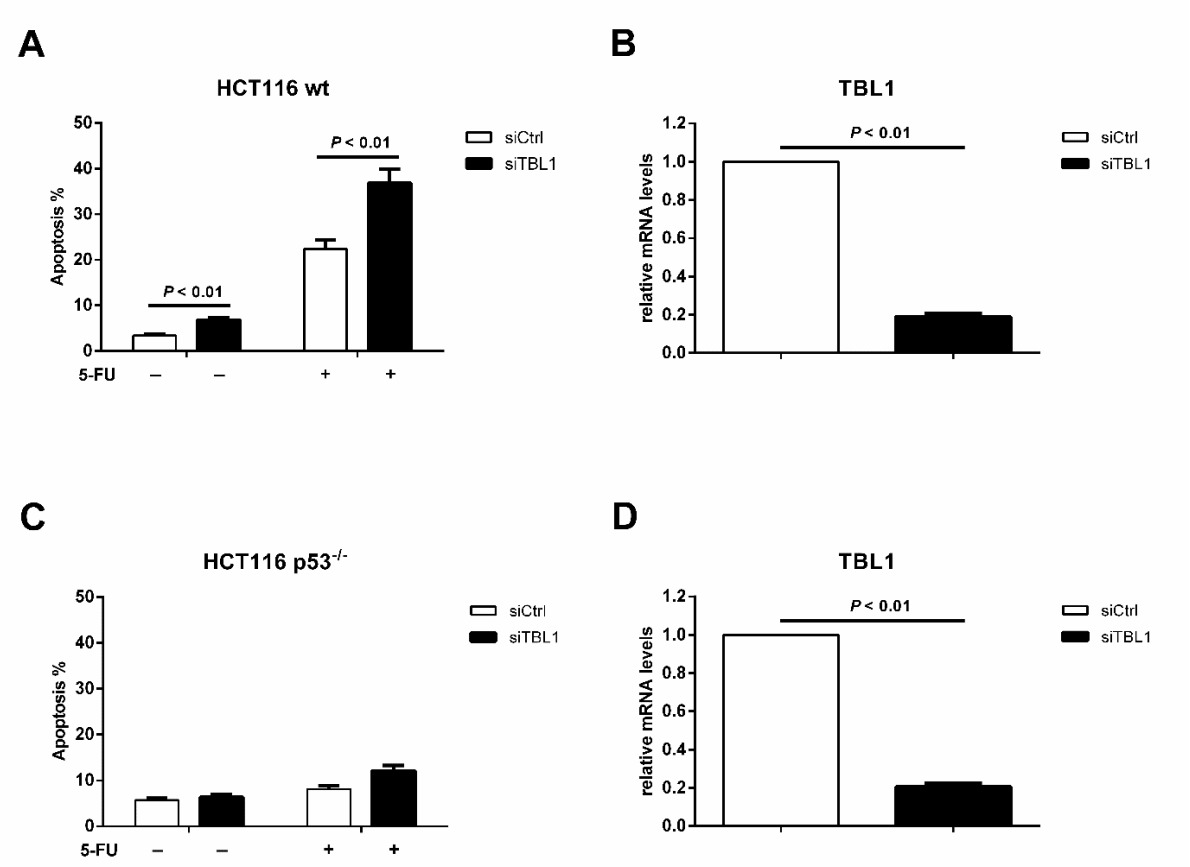



**Fig. 23: 5-FU treatment upon TBL1 depletion induces p53-dependent apoptosis. A, C** HCT116 wt cells and HCT116 $p53^{-/-}$ cells were transfected with siCtrl and siTBL1 and subsequently exposed to 50 µM 5-FU for 48 h. Cells were then harvested and stained with Annexin V / PI and analyzed via flow cytometry. Apoptosis is the annexin V positive but PI negative fraction, % of total population. **B, D** qRT-PCR confirmed knockdown efficiency; mRNA levels were normalized to HPRT1 mRNA expression, mRNA levels of siCtrl cells were normalized to 1.0. Error bars indicate mean ± s.d., n = 3 for biological replicates.

During apoptosis, Poly (ADP-ribose) Polymerase (PARP) is known to be activated at an intermediate stage of apoptosis and to be cleaved and inactivated at a later stage by caspases. Cleaved PARP facilitates cellular disassembly and serves as a marker of cells undergoing apoptosis (Oliver et al., 1998). Therefore, protein levels of cleaved PARP were detected upon TBL1 knockdown and 5-FU treatment (Fig. 24). The amount of cleaved PARP was significantly increased upon TBL1 depletion and 5-FU treatment in HCT116 wt cells. In addition, protein levels of p21 and PUMA were also found to be enhanced by knockdown of TBL1 (Fig. 24).

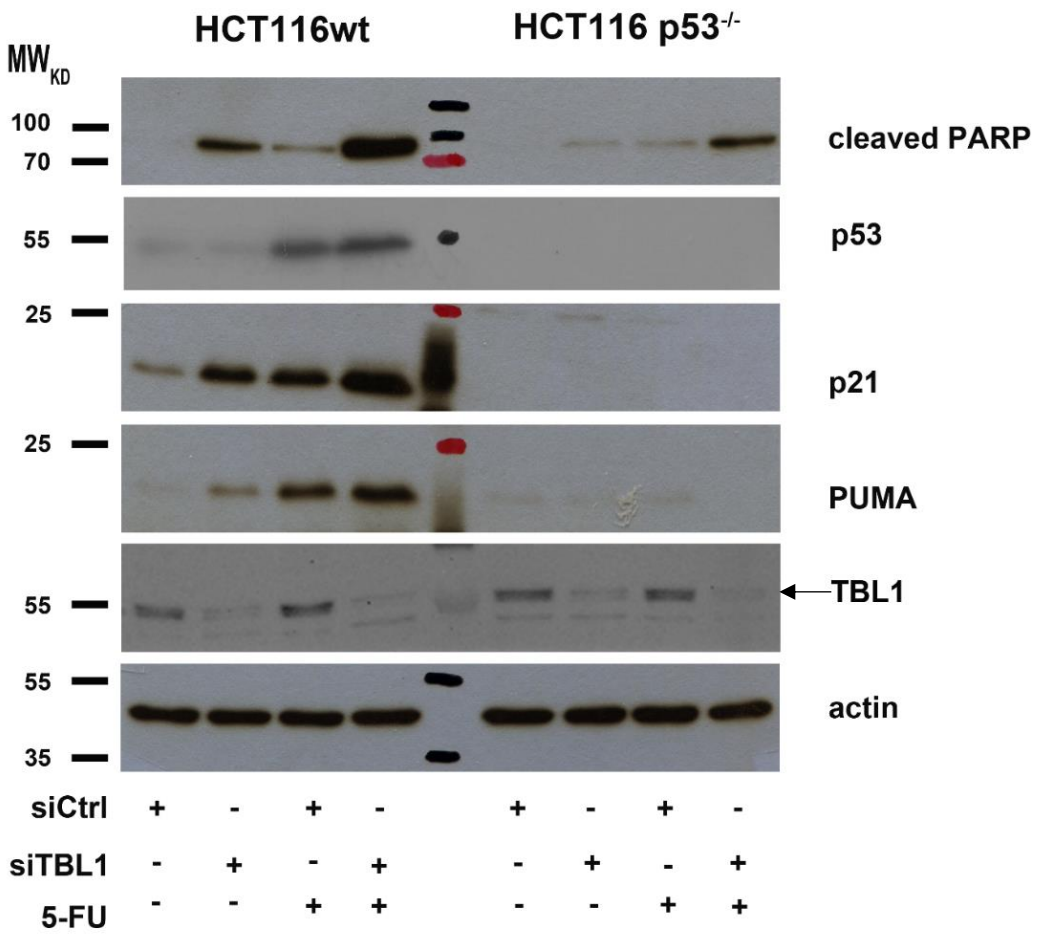



**Fig. 24: TBL1 depletion increases levels of cleaved PARP in response to 5-FU.** HCT116 wt cells and HCT116 p53$^{-/-}$ cells were transfected with siCtrl and siTBL1 and subsequently exposed to 50 µM 5-FU for 48 h. Cell lysates were analyzed by immunoblotting with anti-cleaved PARP, anti-p53, anti-p21, anti-PUMA, and anti-actin antibodies as well as specific HRP-coupled secondary antibodies. Data are shown as representative of three experiments.

### 3.2.2 TBL1 depletion chemosensitizes HCT116 cells to Nutlin-3a in a p53-dependent manner

Nutlin-3a is a novel anti-cancer drug currently investigated in clinical trials which activates p53 via inhibition of the interaction between p53 and the E3 ubiquitin ligase MDM2. This leads to a stabilization of p53 without inducing a DNA damage response. Hence, the same experiment as described in **3.2.1** has been performed using Nutlin-3a instead of 5-FU. The results presented in Fig. 25 A showed that TBL1 knockdown and Nutlin-3a treatment together significantly enhanced cell death

to around 25%. Of note, this phenomenon was almost completely diminished in p53-deficient cells (Fig. 25 C).

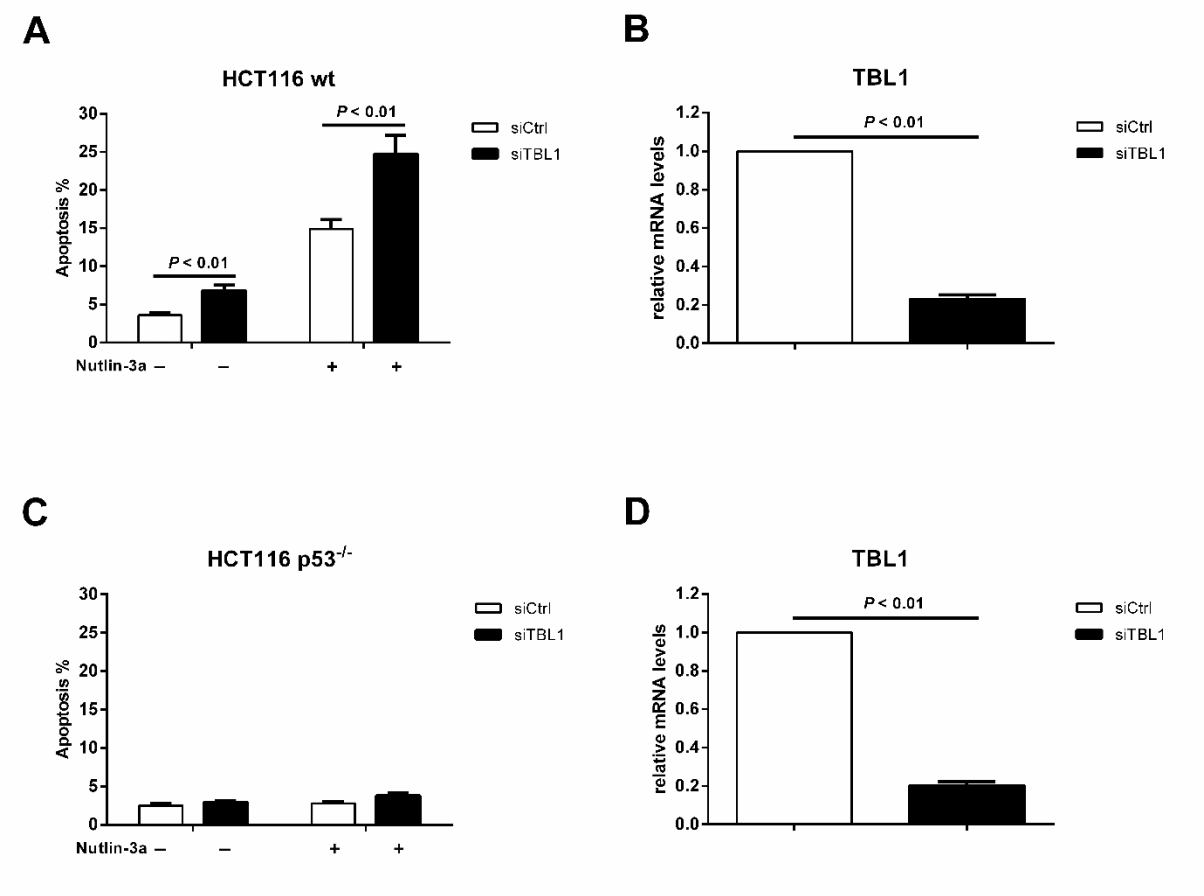



**Fig. 25: Nutlin-3a treatment upon TBL1 depletion induces p53-dependent cell apoptosis. A, C** HCT116 wt cells and HCT116 $p53^{-/-}$ cells were transfected with siCtrl and siTBL1 and subsequently exposed to 10 µM Nutlin-3a for 48 h. Cells were harvested and stained with Annexin V / PI and analyzed by flow cytometry. Apoptosis is the annexin V positive but PI negative fraction, % of total population. **B, D** qRT-PCR to confirm knockdown efficiency; mRNA levels were normalized to HPRT1 mRNA expression, mRNA levels of siCtrl cells were set to 1.0. Error bars indicate mean ± s.d., n = 3 for biological replicates.

Since Nutlin-3a could only activate wild-type p53 and has been proved that there is no effect in p53 mutant or p53 deficient cells (Vassilev et al., 2004), the detection on the protein level was done in HCT116 wt cells. The elevated level of cleaved PARP was clear to be obserbed upon TBL1 knockdown and Nutlin-3a treatment (Fig. 26).

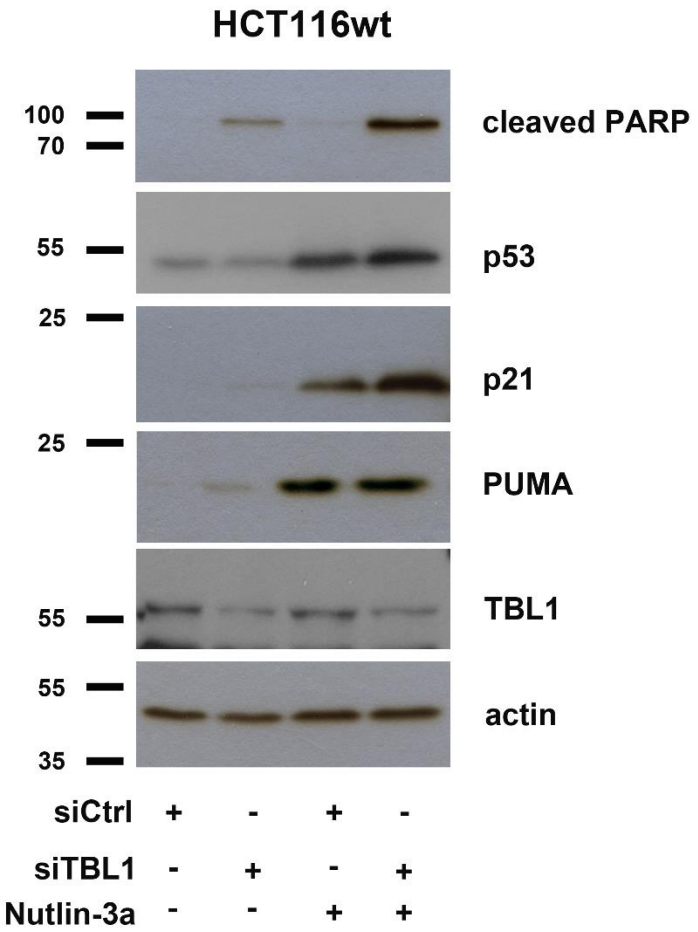



**Fig. 26: Nutlin-3a treatment upon TBL1 depletion increases levels of cleaved PARP.** HCT116 wt cells and HCT116 p53$^{-/-}$ cells were transfected with siCtrl and siTBL1 and subsequently exposed to 10 µM Nutlin-3a for 48 h. Cell lysates were analyzed by immunoblotting with anti-cleaved-PARP, anti-p53, anti-p21, anti-PUMA and anti-actin antibodies as well as specific HRP-coupled secondary antibodies. Data are shown as representative of three experiments.

### 3.2.3 TBL1 depletion inhibits cell growth and induces cell death

In order to test whether TBL1 knockdown affects overall cell survival particularly under conditions of genotoxic stress, colony formation assays were performed. Therefore, TBL1 was depleted by siRNA transfection and cells were subsequently exposed to 5-FU or Nutlin-3a. After two weeks, cells were washed, fixed, and stained with crystal violet. Upon TBL1 knockdown HCT116 wt cell number was already attenuated in unstressed conditions (Fig. 27 A). This might have been due to the inhibition of cell proliferation as well as induction of cell death. With 5-FU or Nutlin-3a

treatment, cell numbers were drastically reduced which suggested a huge induction of cell death. Upon TBL1 knockdown with additional drug treatment almost no colony formation was detected (Fig. 27 A). In HCT116 p53-deficient cells there was a similar tendency but with slightly weaker effects (Fig. 27 B). Knockdown efficiency was confirmed by western blot (Fig. 27 C).

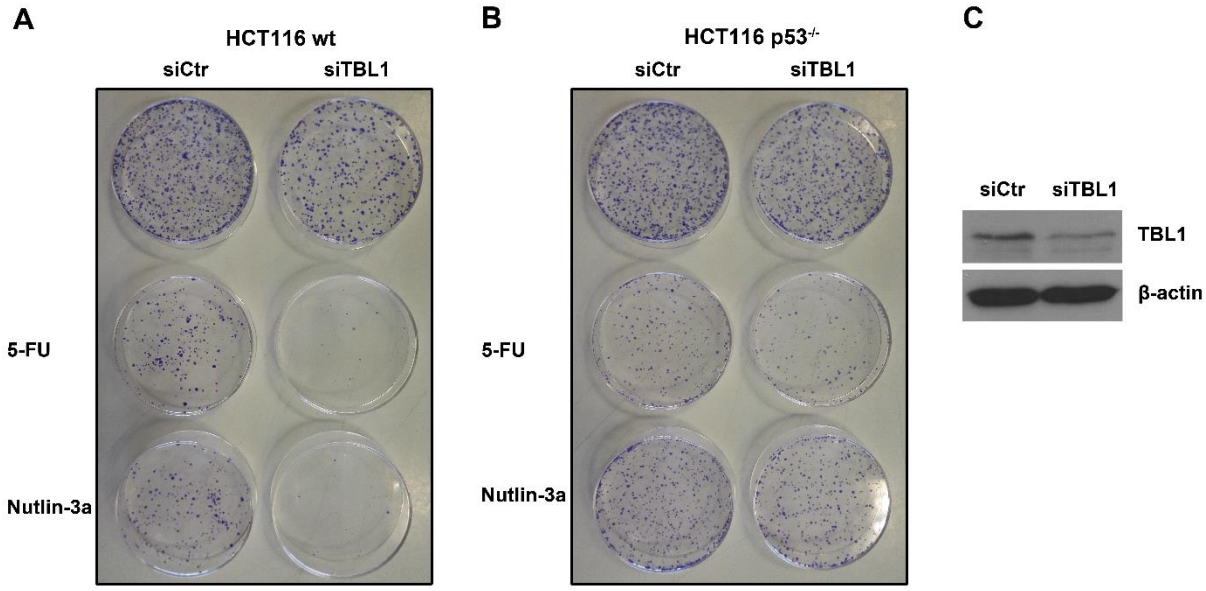



**Fig. 27: Knockdown of TBL1 inhibits colony formation and enhances cell death in response to 5-FU and Nutlin-3a respectively.** Colony formation assay: 1 x $10^3$ HCT116 wt cells **A** and HCT116 $p53^{-/-}$ cells **B** were seeded in 6 cm dishes and transfected with 100 nM siCtrl and siTBL1. Cells were then treated with 50 µM 5-FU or 10 µM Nutlin-3a for 24 h. After another 14 days, cells were fixed and stained with crystal violet. **C** Knockdown efficiency was analyzed by western blot. Data are shown as representative of three experiments.

### 3.2.4 TBL1 interacts with p53 in DDR

Under unstressed conditions, TBL1 binds to p53 in order to suppress its transcription activity via HDAC3-mediated histone deacetylation on p53 target promoters. Consequently, it was of very big interest to investigate the status of TBL1 in stressed cells. As it is known that p53 is activated in response to DNA damage, I expected that TBL1-p53 binding as well as the TBL1-mediated p53 repression would be disrupted to allow p53 to transactivate its target genes. Surprisingly, as shown in Fig.

28 A, B, TBL1 and p53 still bind to each other even upon treatment with Doxorubicin to induce the DDR.

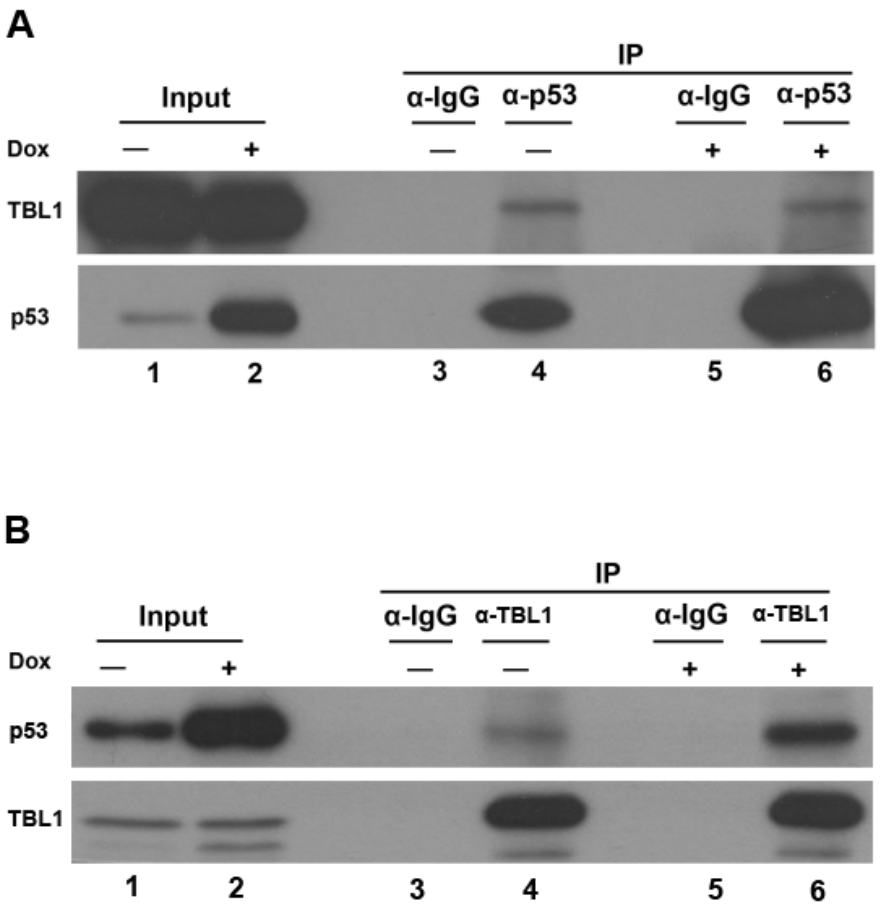



**Fig. 28: p53 and TBL1 interact with each other in absence or presence of DNA damage.** Co-IP of endogenous TBL1 and p53. **A** HCT116 wt cells treated with 0.5 µg/ml Doxorubicin for 24 h to induce the DDR and untreated HCT116 wt cells were analyzed. IgG antibody (negative control) and p53 antibody were added and then pulled from total lysate using protein A/G plus-agarose beads. **B** HCT116 wt cells treated with 0.5 µg/ml Doxorubicin for 24 h to induce the DDR and untreated HCT116 wt cells were analyzed. IgG antibody (negative control) and TBL1 antibody were added and then pulled. As a result, p53 was co-precipitated. Proteins were detected by immunoblotting with anti-TBL1 and anti-p53 antibodies as well as HRP-coupled secondary antibodies. Data are shown as representative of three experiments.

### 3.2.5 Doxorubicin treatment has virtually no effect on TBL1 protein level

These results tempted me to investigate if there are any changes in TBL1 protein levels during the DDR. Therefore, HCT116 wt cells were treated with Doxorubicin for different time periods between 2 h and 24 h. It can be clearly seen that p53 activation increases with the time after adding the genotoxic stress. However, there was no significant change in TBL1 protein levels over time (Fig. 29).

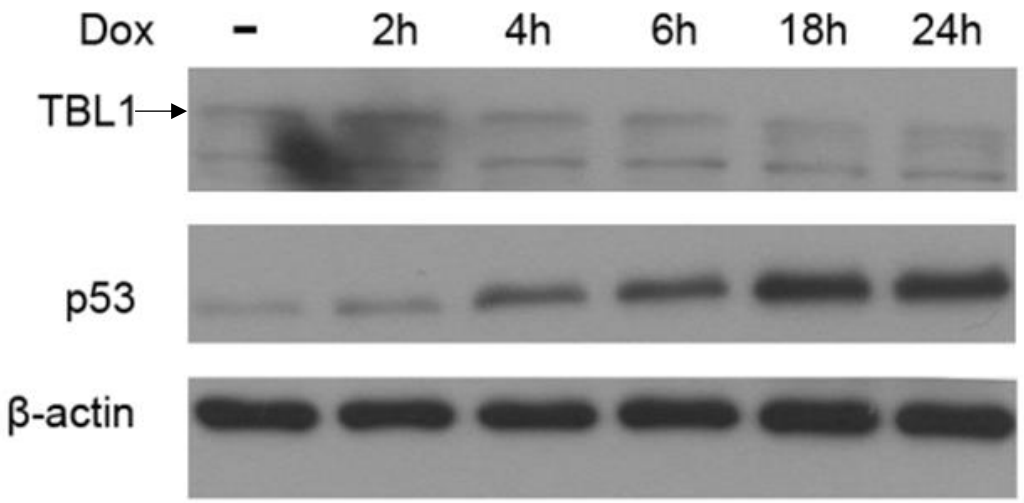



**Fig. 29: DDR does not alter TBL1 protein level.** HCT116 wt cells were treated with 0.5 µg/ml Doxorubicin for 2 h, 4 h, 6 h, 18 h and 24 h respectively. Lysates were analyzed by immunoblotting with anti-p53, anti-TBL1 and anti-β-actin antibodies as well as specific HRP-coupled secondary antibodies. Data are shown as representative of three experiments.

### 3.2.6 Doxorubicin treatment has virtually no effect on the recruitment of TBL1 to p53 target gene promoters

Based on the results presented above, it seemed that that there would be no change in TBL1 recruitment to p53 target promoters upon treatment of Doxorubicin. As a positive control recruitment of p53 was analyzed by ChIP. This analysis showed a dramatic increase in the p53 promoter binding during Doxorubicin-induced DDR (Fig. 30 A, B). However, there is no change for TBL1 binding to promoters with or without genotoxic stress (Fig. 30 C, D).

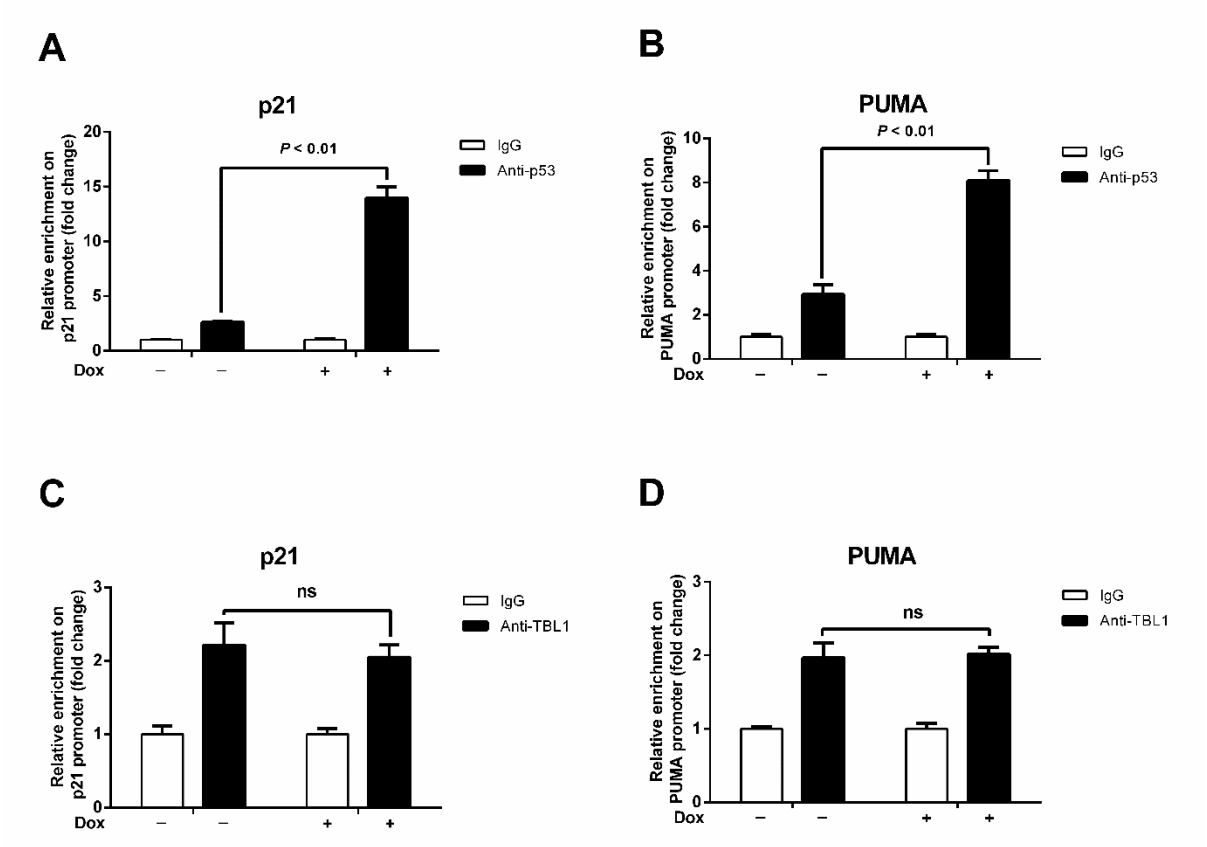



**Fig. 30: Doxorubicin treatment does not influence TBL1 binding to p53 target promoters.** HCT116 wt cells treated with 0.5 µg/ml Doxorubicin for 24 h were analyzed compared to untreated cells as a control. Then DNA was isolated via chromatin immunoprecipitation by anti-p53 antibody **A**, **B** or anti-TBL1 antibody **C**, **D** and p21 and PUMA promoter DNA was determined by qRT-PCR. The outcome is presented in relation to extract precipitated with IgG antibody which was set to 1.0. ns: not significant. Error bars indicate mean ± s.d., n = 3 for biological replicates.

### 3.2.7 TBL1 depletion increases p53 target gene expression upon Doxorubicin treatment

It is now clear that TBL1 binds to p53 upon genotoxic stress. Hence, if TBL1 was depleted after the DDR has already been initiated, it would be able to give rise to a higher extent of p53 targets expression. As expected, Fig. 31 A showed that 24 h treatment with Doxorubicin upon TBL1 knockdown significantly enhanced p21 expression. Although the induction for PUMA is not statistically significant, the trend shows an increase of PUMA expression (Fig. 31 A).

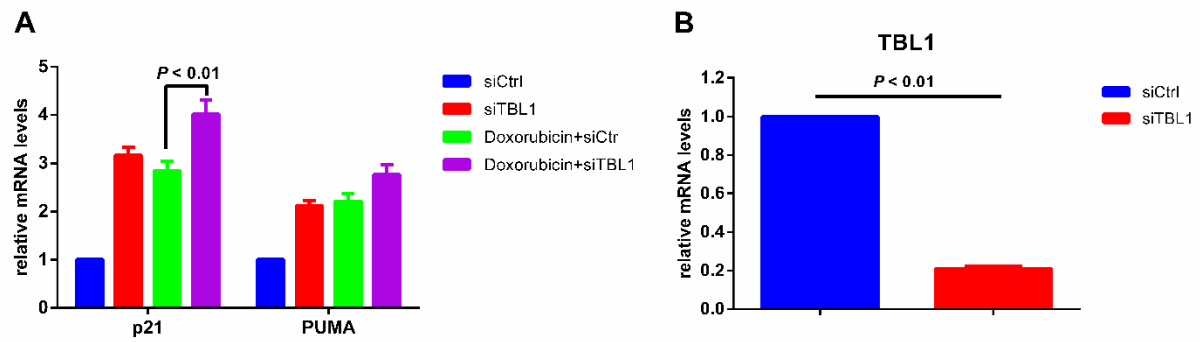



**Fig. 31: TBL1 depletion enhances p53 target gene expression upon Doxorubicin-induced DDR. A** HCT116 wt cells were treated with 0.5 µg/ml Doxorubicin for 24 h, then transfected with 100 nM siCtrl or siTBL1, and expression of p53 target genes p21 and PUMA was analyzed by qRT-PCR 24 h after transfection. **B** Knockdown efficiency of TBL1 was verified by qRT-PCR. mRNA levels were normalized to HPRT1 mRNA expression and mRNA levels of siCtrl cells were set to 1.0. Error bars indicate mean ± s.d., n = 3 for biological replicates.

# 4 Discussion

As a key regulator of a number of cellular functions, p53 has been found to interact with a variety of co-factors which regulate p53 activity in different signaling networks. As a factor regulating transcription, TBL1 is involved in different cellular pathways, such as cell growth control (Dimitrova et al., 2010, Li and Wang, 2008, Ramadoss et al., 2011), metabolic signaling (Kulozik et al., 2011, Rohm et al., 2013) and cell death (Lim et al., 2012). Previous finding from our laboratory suggested that TBL1 might also play a role in the regulation of the transcriptional activity of p53 (Adikesavan et al., 2014, Dimitrova et al., 2010, Lim et al., 2012, Perissi et al., 2008), which tempted me to investigate a possible relation of these two proteins.

RNA-seq analysis data showed that a subset of p53 target genes was regulated by TBL1. However, there were still a lot of questions waiting to be answered. The first part of this thesis showed that TBL1 interacts with p53 and suppresses p53 transcriptional activity by HDAC3-mediated histone deacetylation on p53 target gene promoters. In the second part, TBL1 is associated with chemosensitivity of cancer cells. But the question remains whether it is involved in DDR directly.

Taken together, regulating p53 transcriptional activity was identified as a new function of TBL1. Furthermore, TBL1 appears to be an attractive molecular target to be exploited in the future to chemosensitize cancer cells.

## 4.1 TBL1 regulates the expression of a subset of p53 target genes in the absence of cellular stress

RNA-seq analysis was used to find out if there are p53 target genes regulated by TBL1. Among 1055 genes upregulated upon TBL1 depletion in HCT116 wt cells, 294 genes were identified as p53 targets (Fig. 7 E). Although a huge number of p53 target genes have been identified, some of them are poorly investigated. Moreover, recent studies support the idea that p53 functions as a transcription activator. The downregulation of genes mediated by p53 is indirect and requires p21 (Allen et al., 2014, Brady et al., 2011, Fischer, 2017, Verfaillie et al., 2016). That is the reason why here upregulated p53 target genes were focused on. Fischer summarized most of the proposed p53 target genes from literature and defined 116 genes, which were

found in at least 6 out of 16 genome-wide data sets, as high-confident p53 target genes (Fischer, 2017). Among 294 TBL1 regulated p53 targets there were 51 genes that were found among these 116 genes (Fig. 7 F and supplementary data), which supports our finding that around one sixth of TBL1 regulated p53 dependent genes were “high-confident p53 targets”. However, there are many more p53 target genes besides the 116 genes postulated by Fischer. For example, among the top 12 TBL1 upregulated p53 targets (Fig. 8 C), TNFRSF10C and INPP5D were not among the “Fischer genes” but were identified in multiple data sets and have been proven to be p53 targets (Leszczynska et al., 2015, Sheikh et al., 1999).

Functional analysis showed that 9 out of the 12 most TBL1 upregulated p53 targets were involved in the inhibition of cell proliferation or induction of cell apoptosis (Fig. 8 C). Interestingly, Leszczynska *et al.* found that PHLDA3 and INPP5D (2 of the 12 top genes) mediate apoptosis through AKT pathway inhibition. Furthermore, inhibition of AKT led to apoptosis in p53-deficient tumours and increased radio-sensitivity (Leszczynska et al., 2015). PI3K is known to be the major mode of AKT activation and it has been reported that TBL1 was involved in PI3K signaling pathway (Stoy et al., 2015). This explains why these two TBL1 regulated genes exert their function via AKT signaling pathway. In addition, GO-term analysis was done to 1055 upregulated genes in HCT116 wt cells. It was found that TBL1 participated in a number of different functions including p53 mediated signal transduction (Fig. 8 A). Moreover, GO-term analysis of 294 upregulated p53 targets showed that most of these genes were involved in intrinsic or extrinsic apoptotic signaling pathways (Fig. 8 B).

One of the genes from our RNA-seq list upon knockdown of TBL1, namely SLC7A11 (solute carrier family 7 member 11) caught my attention. SLC7A11 is a key component of the cystine/glutamate antiporter, which imports cystine to support elimination of lipid peroxides in the cell (Lewerenz et al., 2013). In case SLC7A11 is repressed, the reluctant lipid peroxides cannot be removed which subsequently leads to the induction of ferroptosis. Ferroptosis is a specific type of programmed cell death which is characterized by iron-dependent accumulation of lipid hydroperoxides leading to death (Stockwell et al., 2017). Recently, Jiang *et al.* found that p53 inhibits cystine uptake and sensitizes cells to ferroptosis by repressing the expression of SLC7A11, which leads to glutathione reduction and ROS increase which is an important component of ferroptosis (Jiang et al., 2015). In my study, however, the

mRNA expression of SLC7A11 was decreased up to 65 % upon TBL1 depletion in HCT116 wt cell and to 55 % in HCT116 p53-deficient cells (data not shown). This indicated that SLC7A11 is not completely dependent on p53. A study by Ou *et al.* on p53 and ferroptosis showed that p53 alone seems not to be able to induce ferroptosis but rather to regulate the threshold of sensitivity to ferroptosis inducers (Ou et al., 2016). Nevertheless, the search was ongoing for an elucidation of p53 regulated ferroptosis and it could be seen the potential of TBL1 depletion to contribute to ferroptosis.

It is worth mentioning that the induction of these p53 target genes is completely dependent on the knockdown of TBL1, as it has been observed in the absence of additional cellular stresses. This is a very rare phenomenon, because most studies focus on the function of p53 after its activation upon genotoxic stress. This finding gives rise to a question that what the basal status or basal function of p53 is. For a very long time, the activity of p53 under basal conditions (unstressed cells) has received relatively little attention. Initially, the basal level of p53 was simply determined by averaging over cell populations prior to different treatments and the expression of p53 was considered to be dispensable for normal cell survival. However, recent studies revealed more and more functions of p53 in the absence of cellular stress. For instance, Hafsi *et al.* showed that an essential role of basal p53 in physiological processes such as stem cell maintenance, development, aging and senescence, as well as the regulation of basal oxidative cell metabolism (Hafsi and Hainaut, 2011). In detail, they found that p53 is required to maintain hematopoietic stem cells (HSCs) in a quiescent state via modulating intracellular levels of ROS and the expression of growth suppressors. This remarkably impacts stem and progenitor cell functions under both normal and pathologic conditions (Asai et al., 2011, Liu et al., 2009, Urao and Ushio-Fukai, 2013). Moreover, basal p53 expression is also critical in maintaining mesenchymal stem cell (MSC) integrity. Alterations in MSC function resulting from p53 inactivation may contribute to the pathophysiology of skeletal-related disease more significantly than currently appreciated (Boregowda et al., 2018). In the absence of genotoxic stress, cells also suffer from intrinsic transient damage from cell replication and metabolic process. Loewer *et al.* pointed out that transient low damage is insufficient to convert inactive p53 to its active form, which is mainly because of post translational modifications keeping p53 inactive. When the

deacetylase inhibitor JW152 was used to induce the accumulation of acetylated p53, p21 expression was activated in the absence of extrinsic damage (Loewer et al., 2010). This indicated that in non-stressed cells acetylation patterns of p53 could increase its transcriptional activity. However, it was not known whether the induction of p53 targets upon TBL1 depletion was due to p53 modifications, accumulation or other reasons.

Taken together, TBL1 was identified as a new negative regulator of p53 activity and its depletion induced transcription of p53 target genes. This reinforces my hypothesis that TBL1 is a promising target for the repression of cancer growth and development.

## 4.2 TBL1 interacts with p53 *in vitro* and *in vivo*

As the main negative regulator of p53, the E3 ubiquitin ligase MDM2 interacts with p53 and constantly degrades it. This prompted me to investigate if there is a similar correlation between TBL1 and p53.

By ectopically expressing TBL1 and p53, a former PhD student in our laboratory found that p53 and TBL1 interact with each other in cells (Greiner, 2014). However, overexpression experiments are not necessarily representative for the actual situation in the cell. These synthetic conditions might lead to specific defects in the cell, like promiscuous interactions or pathway modulation associated with the degree of overexpression (Moriya, 2015). Therefore, these findings needed to be validated on the endogenous level. Although there is comparatively less p53 protein in unstressed cells, the endogenous interaction between p53 and TBL1 was confirmed in colorectal cancer cells via Co-IP experiments by adapting and optimizing cell numbers and techniques (Fig. 12).

As the Co-IP result could not distinguish between direct or indirect interactions, another strategy deployed to investigate this was GST-Pulldown assay. The recombinantly expressed proteins GST-TBL1 and His-p53 in E. coli were isolated, purified and incubated together and the result showed that TBL1 and p53 directly bind to each other (Fig. 13).

Moreover, it has been shown that p53 is one of the transcriptional factors that is associated with the NCoR/SMRT complex, in which TBL1 is a subunit of this

complex. For instance, Adikesavan *et al.* showed that the deacetylase activation domain (DAD) of SMRT can directly bind to p53 (Adikesavan et al., 2014). This binding blocks HDAC3 interaction with DAD leading to a net increase in HAT activity, which contributes to the activation of p53 target genes in response to DNA damage. In addition, it was reported that ERα represses p53 mediated transcriptional activation in human breast cancer cells by recruiting NCoR/SMRT and HDAC1 (Konduri et al., 2010). However, SMRT (but not NCoR) has also been shown to act as a co-activator for ERα in MCF7 breast cancer cells (Peterson et al., 2007). In addition to the fact that there are at least two corepressors in one complex directly binding to p53, the single component like SMRT has different functions on the same receptor depending on the cellular context. Concerning the role of TBL1 as a nuclear factor exchanger, I hypothesized that that TBL1 dissociates from p53 upon DNA damage. However, upon Doxorubicin treatment p53 still interacts with TBL1 (Fig. 28). TBL1 knockdown upon DNA damage led to an additional increase in the expression of p53 target genes (Fig. 31). This finding showed that TBL1 is a negative regulator of p53 activity during the DNA damage response and thereby counteracts the activity of SMRT. Depending on the different conditions and different time points, it is expected to see the varying roles of the subunits. Furthermore, it is very interesting that during the instant activation of p53 upon DNA damage, TBL1 still represses p53 activity, presumably in order to prevent it from over-activation. It remains to be investigated whether TBL1 permanently inhibits p53 or it dissociates upon another trigger, which is not DNA damage.

There are two possibilities for the interaction between p53 and TBL1 which differ in the localization of p53: One is that a small fraction of p53 has already bound to its target promoters in an inactive condition which is stabilized by TBL1, the other theory is that TBL1 inhibits binding of p53 to the target promoters. In both cases TBL1 inhibits the expression of p53 target genes. ChIP-p53 analysis proved that p53 already binds to its target gene promoters even in unstressed cells (Fig. 14), reinforcing the first hypothesis.

In this study, I postulated that a fraction of p53 already binds to its target gene promoters in unstressed cells waiting to be instantly initiated upon specific triggers. It is known that upon DNA damage, p53 is activated to preserve genome integrity to prevent abnormal cell behaviour, otherwise DNA damage may accumulate and result

in the cancer formation. Regarding its critical role as a tumour suppressor and “guardian of the genome”, a wide range of sensing mechanisms are needed to detect cellular damage with extremely high sensitivity which activate the corresponding responses, such as cell cycle arrest or apoptosis (Bakkenist and Kastan, 2004). Therefore, it is very efficient that a fraction of p53 already binds to the promoters of its target genes, as it saves the time for p53 to be recruited to the DNA. However, upon constant damage, the accumulation of p53 continues leading to increased binding of p53 to the appropriate target promoters and thereby enhancing the DDR.

As TBL1 was found to be a direct binding partner of p53, TBL1 also binds indirectly to the p53 target promotes (Fig. 14). The result of ChIP-p53 upon DNA damage is as expected since more p53 protein binds to its target gene promoters, which leads to a much higher increase compared with that under normal conditions (Fig. 30). However, there is no difference for the result of ChIP-TBL1 under unstressed and stressed conditions (Fig. 30). Since there is no change of protein level of TBL1 upon DNA damage response (Fig. 29), it may interact with a fixed amount of p53. Another possibility is that there are no more TBL1 molecules free to interact with the increased molecules of p53 upon genotoxic stress. Therefore, even when more p53 binds to promoters this would not affect the interaction of TBL1 with the original p53 there. That could be why there was no increased binding of TBL1 to p53 target gene promotors observed upon genotoxic stress.

## 4.3 TBL1 depletion promotes active histone markers on p53 target gene promoters

It is known that p53 is stabilized in response to various cellular stresses, but mechanisms leading to p53 activation are stimulus dependent. For instance, DNA damage leads to p53 phosphorylation, which blocks MDM2 mediated p53 degradation (Shieh et al., 1997). Oncogenic signaling activates p53 by inducing the ARF tumour suppressor (also known as CDKN2A) to inhibit MDM2 (Pomerantz et al., 1998, Zhang et al., 1998). In all cases, p53 degradation mediated by MDM2 is inhibited which results in an accumulation of p53 protein. However, what I found is a novel MDM2-independent mechanism of p53 regulation via TBL1.

Due to the fact that no increase of p53 protein levels could be detected upon TBL1 knockdown, it seems like this mechanism does not result in p53 stabilization. However, the exact mechanism still remains to be investigated. Different PTMs occur on the p53 protein, like phosphorylation, acetylation, SUMOylation, glycosylation, and prolyl isomerization which all affect protein stability and activity (Kumari et al., 2014). Among them, phosphorylation and acetylation are the two most prominent ways of p53 activation. It has been revealed that Ser15 is phosphorylated by various kinases such as ATM, ATR and AMPK. Ser 15 phosphorylation represents an early cellular response to a variety of genotoxic stresses (Shieh et al., 1997, Siliciano et al., 1997). For example, UV-irradiation and γ-radiation could trigger Ser 15 phosphorylation (Canman et al., 1994, Lees-Miller et al., 1992, Siliciano et al., 1997). In addition, p300 and CBP (CREB-binding protein) mediated C-terminal acetylation of K373 and K382 are very common in response to various stresses (Ivanov et al., 2007). In the present study, these p53 modifications were detected and indeed, there was no change upon TBL1 knockdown (Fig. 16).

Although p53 pre-bound to its target promoters, TBL1 knockdown did not recruit more p53 to the promoters (Fig. 17). It is known that not only transcription factors, but also chromatin modifications contribute to development and homeostasis by initiating and maintaining stable patterns of gene expression (Jaenisch and Bird, 2003). Among different histone modifications, histone acetylation and methylation are two main modifications that responds to various cellular signals as transcription regulating marks. A recent study showed that cell-type and state-specific chromatin modifications may alter the accessibility of particular genes to p53 transactivation (Su et al., 2015). Gomes *et al.* found that PUMA expression could be determined by repressive histone modifications under certain conditions, in which the PUMA locus is insulated by CCCTC-binding factor (CTCF) (Gomes and Espinosa, 2010). In ESCs, it was revealed that p53 can be induced to bind to the p21 promoter, but that efficient p21 activation depends on the loss of cell type specific repressive histone H3K27me3 that marks at the locus (Itahana et al., 2016).

In this study, ChIP analysis showed that TBL1 depletion significantly increased the acetylation levels of H3K9 and H3K27 at the p21 and PUMA promoter without significantly affecting H4K16 acetylation (Fig. 18). Since coactivators p300/CBP mediate H3K27 acetylation and p300/CBP-associated factor (PCAF) mediates H3K9

acetylation (Jin et al., 2011). I postulated that they are the potential histone acetyltransferases which are recruited after TBL1 depletion and increase histone acetylation on p53 target promoters. Histone acetylation is connected with the activation of gene transcription. It has been pointed out that H3K9 acetylation leads to chromatin decondensation as well as formation of chromatin loops, which makes genes from compact chromosome territories more accessible for transcription (Chambeyron and Bickmore, 2004). Besides that, H3K27 acetylation also plays an important role in the regulation of key developmental genes in stem cells (Creyghton et al., 2010). In contrast to histone acetylation, histone methylation can result in repression or activation of gene transcription depending on the histone residue that is modified. Trimethylation of H3K9 and H3K27 frequently occurs in condensed heterochromatin, which is linked to transcriptional repression. Indeed, the results showed that TBL1 depletion decreased the trimethylation of H3K9 and H3K27 at p21 and PUMA promoters in HCT116 wt cells but not in HCT116 p53-deficient cells, indicating that this action is p53 dependent (Fig. 19).

It is clear that the way TBL1 represses p53 activity is via histone deacetylation on p53 target gene promoters. Once TBL1 is depleted, the deacetylation on the p53 target promoters would be attenuated and the corresponding repression on p53 activity is released. In this way, the pre-bound p53 is free to induce the transcription of its target genes.

Then I inferred that should not be only TBL1, but there are also other factors which have similar functions to inhibit p53 activity in unstressed state. Indeed, Wang et al. found that the oncoprotein SET interacts with p53 and profoundly represses p53 transcriptional activity in steady state by inhibiting H3K18 and H3K27 acetylation on the p53 target promoters (Wang et al., 2016a). Furthermore, calcineurin binding protein 1 (Cabin1) was also shown to impede p53 transcriptional activity via regulating H3K9 modification on p53 target promoters in the absence of genotoxic stress (Jang et al., 2009). In addition, the transcription factor Bach1 (BTB and CNC homology 1) recruits to a subset of p53 target promoters to repress p53 action by forming a complex containing HDAC1 and NCoR to promote histone deacetylation (Dohi et al., 2008).

My findings and these above published data indicated that this mechanism of deacetylation-dependent repression of p53 activity in unstressed state is widespread in nature. The modulation of p53 activity by these negative regulators is essential for cell survival in steady state.

## 4.4 Corepressors recruited by TBL1

Since TBL1 is a component of NCoR/SMRT complex, it would be interesting to know if other subunits are involved in this process. Indeed, knockdown of NCoR or HDAC3 phenocopies TBL1 depletion in terms of p53 target genes induction and enrichment of histone acetylation on p53 target gene promoters (Fig. 21, 22).

Based on the fact that TBL1 and NCoR have no histone-deacetylating enzymatic activity, this result suggested that HDAC3 is the critical key player which suppresses p53 target gene transcription via histone deacetylation on p53 target gene promoters. According to the literature, the regulation and mechanisms underlying HDAC3 function are linked to the association with the NCoR/SMRT complex. Some transcription factors, such as COUP-TF, MAD, Rev-Erb, Pit-1 and DAX1 have been reported to be suppressed by NCoR/SMRT recruited HDAC3 (Jepsen and Rosenfeld, 2002, Urnov et al., 2000). Now my data showed that there is also a direct functional connection between HDAC3 and the transcription factor p53 as I found that p53 activity underlies the suppression by HDAC3.

The role of NCoR and SMRT is not only to be a platform to recruit these corepressors, but they also stimulate the enzymatic activity of HDAC3 via its deacetylase activation domain. In case the domain is mutated, the deacetylation activity of HDAC3 decreases dramatically (Guenther et al., 2001, Wen et al., 2000, Zhang et al., 2002). This explains the observation that knockdown of NCoR results in a similar phenotype like that upon HDAC3 or TBL1 knockdown. However, SMRT knockdown had a much weaker effect on the expression of p53 targets p21 and PUMA. In this context, NCoR is more essential than SMRT to repress p53 activity with HDAC3 and TBL1 together.

Combining all of findings from this study, Fig. 32 showed my proposed mechanism of how TBL1 regulates p53 activity: Nuclear co-repressor complexes including NCoR

and HDAC3 are recruited by TBL1, leading to inhibition of p53 target gene transcription through HDAC3-mediated histone deacetylation of H3K9 and H3K27 located on p53 target gene promoters. Once TBL1 is depleted, this co-repressor complex dissociates from p53. Subsequently, the histone deacetylation on p53 target promoters does not occur anymore which leads to an increase in histone acetylation of H3K9/27. In parallel, the repressive histone marks, trimethylation of H3K9/27, decreases on the p21 and the PUMA promoter. These changes of histone modification on p53 target promoters result in chromatin decondensation and initiation of p53 target genes transcription.

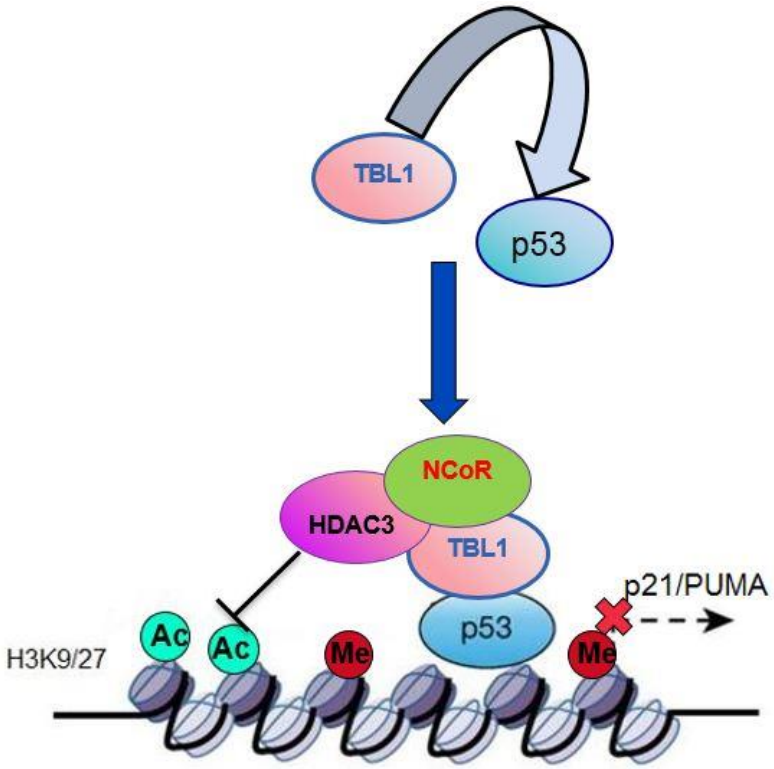



**Fig. 32: Mechanism of TBL1-dependent p53 regulation.** TBL1 indirectly represses p53 transcriptional activity through the recruitment of nuclear co-repressor complexes like NCoR and HDAC3. HDAC3 is the direct mediator which inhibits p53 target gene transcription via histone deacetylation on p53 target promoters.

## 4.5 TBL1 depletion increases chemosensitivity of cancer cell lines in a p53-dependent manner

Based on these findings, the apoptosis studies showed that TBL1 knockdown increased chemosensitivity in colorectal cancer cells and other cell lines. 5-FU is an antimetabolite used for anti-cancer therapy which is known to prolong survival of patients with various cancers and it has the largest impact in colorectal cancer (Longley et al., 2003). Chemoresistance of colorectal cancer to 5-FU has been reported and co-treatment with irinotecan and oxaliplatin was applied to improve the overall survival of colorectal cancer patients. However, this combination therapy is associated with increased toxicity and side effects (Boige et al., 2010, de Gramont et al., 2000). In contrast to cytotoxic treatments, TBL1 inhibition would not have this kind of side effects. It is quite clear that a strong induction of cell apoptosis was seen upon 5-FU and TBL1 knockdown treatment in HCT116 wt cells (Fig. 23). In HCT116 p53-deficient cells, there is a very mild induction after the combination treatment compared with 5-FU treatment alone, although it is not statistically significant. Consistent to this, a difference in the expression of cleaved-PARP was found via western blot analysis (Fig. 24), which means p53 plays an important role in the induction of cell apoptosis under this circumstance, but it is not completely p53-dependent. p53 is a known target of PARP (Alvarez-Gonzalez, 2007, Malanga and Althaus, 2005). But up until now, only one study proposed a reciprocal regulation of PARP-mediated cell death by p53. They showed that loss of p53 enhances resistance to PARP-mediated cell death and concluded that p53 regulates PARP activity (Montero et al., 2013). Besides p53, other factors such as APTX, MYBL2 and DNA modifications were found to be involved in the regulation of PARP activity as well (Cervellera and Sala, 2000, Gueven et al., 2004). My results indicated an additional unknown regulation mechanism of PARP, which implies p53 and TBL1.

As mentioned earlier, Nutlin-3a is a non-cytotoxic p53 activator which is now investigated in phase I/II clinical trials. My results showed that TBL1 knockdown could also enhance colorectal cancer cell sensitivity to Nutlin-3a treatment (Fig. 25) which was similar to 5-FU treatment. However, the difference is that in HCT116 p53-deficient cells the combination treatment has no additional cell death induction anymore. This is due to the different mechanisms of 5-FU which is not completely

dependent on p53 signaling and Nutlin-3a which exclusively functions via p53 activation.

Colony formation assays showed that TBL1 knockdown sensitized colorectal cancer cells as the overall cell survival upon 5-FU or Nutlin-3a treatment was significantly reduced (Fig. 27). This may be an explanation why TBL1 is upregulated in some cancer types like breast cancer (Ramadoss et al., 2011) and pancreatic cancer (Stoy et al., 2015). Upregulation of TBL1 expression would have a pro-survival effect in cancer cells leading to protection of cells against genotoxic stress.

The data here is consistent with the function of the *Drosophila* TBL1 homolog Ebi, which was linked to anti-apoptotic regulation. Lim *et al.* described that Ebi depletion induced neuronal cell death and sensitized retina cells to oxidative stress (Lim and Tsuda, 2016). Accordingly, Ebi protects these cells from damage induced apoptosis and thereby promotes long term survival.

Conclusively, this study identified the role TBL1 in chemosensitivity/chemoresistance of colorectal cancer cells. This was a highly interesting and promising discovery.

## 4.6 The potential role of other HDACs in TBL1-dependent p53 regulation

The suppression of p53 target gene transcription through TBL1 is mediated by histone deacetylation via HDAC3. This tempted me to investigate if other HDACs might also participate in the process. In most cases, HDACs are within complexes composed of other proteins which are believed to modulate the activity of their catalytic subunits. Similar to the NCoR/SMRT complex which associates with HDAC3 (Hartman et al., 2005), two other complexes, namely uRD/Mi2/NRD and Sin3/HDAC contain both HDAC1 and HDAC2 (Knoepfler and Eisenman, 1999). Furthermore, although HDACs usually influence distinct cellular process, they also appear to have overlapping functions (Khochbin et al., 2001). It has been shown that HDAC1/2 as well as HDAC4/5/7 are all somehow related to NCoR/SMRT (Kao et al., 2000, Ariyoshi and Schwabe, 2003, Fischle et al., 2002), although it is still not known if they also contribute to the repression of p53 target genes expression. Lastly, several other HDACs are also known to deacetylate p53 protein. Juan *et al.* showed

that HDAC1 interacts with p53 *in vitro* and *in vivo* and this interaction results in the downregulation of p53 dependent gene activation (Juan et al., 2000). Similarly, Luo *et al.* showed that HDAC1 containing complex PID/MTA2 associated NuRD modulate the deacetylation of p53 (Luo et al., 2000). In addition, it has been reported that HDAC2 regulates p53 transcriptional activity by altering p53-DNA binding activity (Harms and Chen, 2007). HDAC4 was shown to repress p21 expression in a p53-independent mechanism. However, upon DDR, HDAC4 promotes repression of G(2)/M genes transcription via deacetylation of C-terminal lysines on p53 which is in a p53-dependent way (Basile et al., 2006, Mottet et al., 2009). Considering the report that histone-deacetylating enzymatic activity associated with HDAC4 is dependent on NCoR/SMRT complex (Fischle et al., 2002), it is very likely that HDAC4 is also involved in the repression of p53 target gene transcription.

Taken together, HDAC activity and its regulation are very complex and diffuse. The same is true for the regulation of p53 as many different factors contribute directly or indirectly to the transcriptional activity of p53. Hence, further studies are required to get a much deeper molecular understanding of the role of other HDACs in TBL1-dependent p53 regulation.

## 4.7 Summary and Outlook

In this thesis, TBL1 was found to suppress p53 transcriptional activity via HDAC3-mediated deacetylation at p53 target gene promoters in unstressed cells. As an indirect transcriptional regulator, TBL1 is also recruited to p53 target gene promoters via interaction with p53. Knockdown of TBL1 was able to induce the expression of a subset of p53 target genes, most of which were involved in intrinsic or extrinsic apoptosis pathways. This might be the reason why TBL1 depletion chemosensitizes cancer cells to 5-FU or Nutlin-3a.

Next step in the project would primarily focus on the role of other HDACs in the regulation of p53 activity by TBL1. This would also contribute to figure out how HDACs work in different contexts. Meanwhile, the role of NCoR in DDR would be investigated to find out if it is like TBL1 which still represses p53 target genes transcription in the presence of genotoxic stress. It is also intriguing to find out the

potential histone acetyltransferases and histone demethyltransferases recruited after TBL1 depletion to increase histone acetylation and decrease histone methylation on p53 target promoters. Furthermore, TBL1 expression is upregulated in some cancer types such as breast cancer and pancreatic cancer (Ramadoss et al., 2011, Stoy et al., 2015). Now the investigation of TBL1 expression in other tumour types especially in colorectal cancer is performed in collaboration. Meanwhile, it would be interesting to investigate genome-wide binding of p53 to its target gene promotors in unstressed cells. Several studies have investigated p53-bound genes in response to different stresses (Wei et al., 2006, Riley et al., 2008, Allen et al., 2014, Kenzelmann Broz et al., 2013), but the work has not been done in normal conditions without any stress. Finally, a potential clinical relevance of these findings should be analyzed *in vivo*. *In vitro* it has been shown that the chemosensitivity of cancer cells upon TBL1 depletion with the combination of chemotherapeutic drugs. It would be of particular interest to investigate an impact of TBL1 depletion on tumour growth in *in vivo* models.

In summary, the findings of this project helped to know more about the mechanism of p53 regulation. Additionally, it might offer a new strategy to overcome chemoresistance in some cancer types and to enhance chemotherapeutic treatment efficiency.

# 5 Abstract

Despite undisputed achievements of cancer research and numerous breakthroughs, benefits for patients in terms of prolonged survival time have not been as high as expected. Despite the rapid development of new ways to treat cancers, most patients are still treated with conventional approaches like radiation therapy or chemotherapy. Radio- and chemotherapy induce DNA damage thereby activating the DNA damage response and DNA damage response outcomes like apoptosis to eliminate cancer cells. However, many tumours become resistant to therapy creating a need for new innovative therapeutic strategies. The tumour suppressor p53 is a key effector of the DNA damage response and thus plays a pivotal role in cell fate-decision making upon genotoxic stress. Thus, enhancing p53 activity would be an intriguing approach to increase cancer cell chemosensitivity.

In this study, TBL1 was identified as a novel regulator of p53. In unstressed cells, RNA-Sequencing analysis showed that knockdown of TBL1 induced the expression of a subset of p53 target genes. Mechanistically, I found that TBL1 and p53 bind to each other *in vitro* and *in vivo* and that both bind to the promotors of the p53 target genes CDKN1A and BBC3 in the absence of p53 activation. Moreover, chromatin immunoprecipitation analysis showed that TBL1 depletion increases the presence of activating histone marks and in parallel, decreases repressive histone marks on the p21 and the PUMA promoter. These findings suggest that (1.) in absence of stress, a subset of p53 promoters are pre-occupied by p53 and (2.) the activity of promoter-bound p53 is suppressed by TBL1 through an epigenetic mechanism. TBL1 is a subunit of the NCoR/SMRT repressor complexes. Knockdown of the co-repressor NCoR and histone deacetylase HDAC3, which is a part of the complex, phenocopies the knockdown of TBL1 and induces p53 target gene expression and increases activating histone acetylation at the p21 and the PUMA promoter indicating that TBL1 represses p53 target gene expression by recruiting co-repressors. Functionally, I found that TBL1 depletion sensitizes colorectal cancer cells to 5-Fluorouracil or Nutlin-3a treatment.

Taken together, my work identified TBL1 as a repressor of p53 activity, suggesting a novel strategy to be exploited in the future to chemosensitize cancer cells.

# Zusammenfassung

Trotz unbestrittener Erfolge in der Krebsforschung und zahlreicher Durchbrüche in der Tumortherapie haben sich die Überlebenszeiten von Patienten nicht wie erwartet verlängert. Trotz der rasanten Entwicklung neuer Krebsmedikamente werden die meisten Patienten noch immer mit konventionellen Methoden wie Strahlen- oder Chemotherapie behandelt. Radio- und Chemotherapie induzieren DNA-Schäden, welche die DNA-Schadensantwort und Apoptose aktivieren, was zur Eliminierung entarteter Zellen führt. Viele Tumore entwickeln jedoch Therapieresistenzen, weshalb die Entwicklung neuer innovativer Therapiestrategien erforderlich ist. Der Tumorsuppressor p53 ist ein zentraler Regulator der DNA-Schadensantwort und spielt eine entscheidende Rolle bei Zellschicksalsentscheidungen nach genotoxischem Stress. Eine Steigerung der p53-Aktivität könnte daher ein faszinierender Ansatz zur Erhöhung der Chemosensitivität von Tumorzellen sein.

In dieser Studie wurde TBL1 als ein neuer Regulator von p53 identifiziert. In ungestressten Zellen konnte mittels RNA-Sequenzierungsanalyze gezeigt werden, dass die Depletion von TBL1 die Expression bestimmter p53-Zielgene wie p21 und PUMA induziert. Mechanistisch fand ich heraus, dass TBL1 und p53 *in vitro* und *in vivo* miteinander interagieren und dass beide an die Promotorsequenzen der p53-Zielgene p21 und PUMA in Abwesenheit von p53-aktivierenden Stimuli binden. Darüber hinaus zeigten Chromatin-Immunopräzipitationsexperimente, dass die Reduktion der TBL1-Expression die Histon-Modifikationen in den Promotoren von p21 und PUMA beeinflusst: die Depletion von TBL1 führt zu einer Zunahme aktivierender Histon-Modifikationen (z. B. H3K9/27-Acetylierung) und zeitlich zu einer Abnahme reprimierender Histon-Modifikationen (z. B. H3K9/27-Trimethylierung). Diese Ergebnisse legen nahe, dass (1.) p53 in Abwesenheit von zellulärem Stress an einen Teil seiner Zielgen-Promotoren bindet und (2.) die Aktivität von Promotor-gebundenem p53 von TBL1 durch einen epigenetischen Mechanismus unterdrückt wird. TBL1 ist eine Untereinheit der NCoR/SMRT-Repressorkomplexe. Die Depletion des Co-Repressors NCoR oder der Histon-Deacetylase HDAC3, die Teil des Komplexes sind, zeigen den gleichen Phänotyp wie die TBL1-Reduktion: eine Induktion der p53-Zielgenexpression und eine Zunahme der Histon-Acetylierung in den Promotoren von p21 und PUMA. Dies weist

darauf hin, dass TBL1 durch die Rekrutierung von Co-Repressoren die p53-Zielgenexpression unterdrückt. Funktionell konnte ich zeigen, dass die Depletion von TBL1 Darmkrebszellen für die Behandlung mit 5-Fluorouracil oder Nutlin-3a sensitiviert.

In dieser Arbeit konnte TBL1 als Repressor der p53-Aktivität identifiziert werde. Die in dieser Studie gewonnen Ergebnisse zeigen eine neue Strategie auf, die in Zukunft zur Chemosensitivierung von Krebszellen genutzt werden könnte.

# 6 References


Abdullah, L. N. & Chow, E. K. 2013. **Mechanisms of chemoresistance in cancer stem cells**. Clin Transl Med*, 2*, 3.

Abraham, R. T. 2001. **Cell cycle checkpoint signaling through the atm and atr kinases**. Genes Dev*, 15*, 2177-96.

Adikesavan, A. K., Karmakar, S., Pardo, P., Wang, L., Liu, S., Li, W. & Smith, C. L. 2014. **Activation of p53 transcriptional activity by smrt: A histone deacetylase 3-independent function of a transcriptional corepressor**. Mol Cell Biol*, 34*, 1246-61.

Affo, S., Yu, L. X. & Schwabe, R. F. 2017. **The role of cancer-associated fibroblasts and fibrosis in liver cancer**. Annu Rev Pathol*, 12*, 153-186.

Alexandrov, L. B., Ju, Y. S., Haase, K., Van Loo, P., Martincorena, I., Nik-Zainal, S., Totoki, Y., Fujimoto, A., Nakagawa, H., Shibata, T., Campbell, P. J., Vineis, P., Phillips, D. H. & Stratton, M. R. 2016. **Mutational signatures associated with tobacco smoking in human cancer**. Science*, 354*, 618-622.

Allen, M. A., Andrysik, Z., Dengler, V. L., Mellert, H. S., Guarnieri, A., Freeman, J. A., Sullivan, K. D., Galbraith, M. D., Luo, X., Kraus, W. L., Dowell, R. D. & Espinosa, J. M. 2014. **Global analysis of p53-regulated transcription identifies its direct targets and unexpected regulatory mechanisms**. Elife*, 3*, e02200.

Alvarez-Gonzalez, R. 2007. **Genomic maintenance: The p53 poly(adp-ribosyl)ation connection**. Sci STKE*, 2007*, pe68.

Appella, E. & Anderson, C. W. 2001. **Post-translational modifications and activation of p53 by genotoxic stresses**. Eur J Biochem*, 268*, 2764-72.

Ariyoshi, M. & Schwabe, J. W. 2003. **A conserved structural motif reveals the essential transcriptional repression function of spen proteins and their role in developmental signaling**. Genes Dev*, 17*, 1909-20.

Asai, T., Liu, Y., Bae, N. & Nimer, S. D. 2011. **The p53 tumor suppressor protein regulates hematopoietic stem cell fate**. J Cell Physiol*, 226*, 2215-21.

Baker, S. J., Preisinger, A. C., Jessup, J. M., Paraskeva, C., Markowitz, S., Willson, J. K., Hamilton, S. & Vogelstein, B. 1990. **P53 gene mutations occur in combination with 17p allelic deletions as late events in colorectal tumorigenesis**. Cancer Res*, 50*, 7717-22.

Bakkenist, C. J. & Kastan, M. B. 2004. **Initiating cellular stress responses**. Cell*, 118*, 9-17.

Banescu, C., Duicu, C., Trifa, A. P. & Dobreanu, M. 2014. **Xrcc1 arg194trp and arg399gln polymorphisms are significantly associated with shorter survival in acute myeloid leukemia**. Leuk Lymphoma*, 55*, 365-70.

Bartek, J., Bartkova, J. & Lukas, J. 2007. **DNA damage signalling guards against activated oncogenes and tumour progression**. Oncogene*, 26*, 7773-9.

Basile, V., Mantovani, R. & Imbriano, C. 2006. **DNA damage promotes histone deacetylase 4 nuclear localization and repression of g2/m promoters, via p53 c-terminal lysines**. J Biol Chem*, 281*, 2347-57.

Bassi, M. T., Ramesar, R. S., Caciotti, B., Winship, I. M., De Grandi, A., Riboni, M., Townes, P. L., Beighton, P., Ballabio, A. & Borsani, G. 1999. **X-linked late-onset sensorineural deafness caused by a deletion involving oa1 and a novel gene containing wd-40 repeats**. Am J Hum Genet*, 64*, 1604-16.

Baudino, T. A. 2015. **Targeted cancer therapy: The next generation of cancer treatment**. Curr Drug Discov Technol*, 12*, 3-20.

Baylin, S. B. & Jones, P. A. 2011. **A decade of exploring the cancer epigenome - biological and translational implications**. Nat Rev Cancer*, 11*, 726-34.

Blagosklonny, M. V. 2008. **"Targeting the absence" and therapeutic engineering for cancer therapy**. Cell Cycle*, 7*, 1307-12.

Bode, A. M. & Dong, Z. 2004. **Post-translational modification of p53 in tumorigenesis**. Nat Rev Cancer*, 4*, 793-805.

Boige, V., Mendiboure, J., Pignon, J. P., Loriot, M. A., Castaing, M., Barrois, M., Malka, D., Tregouet, D. A., Bouche, O., Le Corre, D., Miran, I., Mulot, C., Ducreux, M., Beaune, P. & Laurent-Puig, P. 2010. **Pharmacogenetic assessment of toxicity and outcome in patients with metastatic colorectal cancer treated with lv5fu2, folfox, and folfiri: Ffcd 2000-05**. J Clin Oncol*, 28*, 2556-64.

Boregowda, S. V., Krishnappa, V., Strivelli, J., Haga, C. L., Booker, C. N. & Phinney, D. G. 2018. **Basal p53 expression is indispensable for mesenchymal stem cell integrity**. Cell Death Differ*, 25*, 677-690.

Brady, C. A., Jiang, D., Mello, S. S., Johnson, T. M., Jarvis, L. A., Kozak, M. M., Kenzelmann Broz, D., Basak, S., Park, E. J., McLaughlin, M. E., Karnezis, A. N. & Attardi, L. D. 2011. **Distinct p53 transcriptional programs dictate acute DNA-damage responses and tumor suppression**. Cell*, 145*, 571-83.

Brooks, C. L. & Gu, W. 2011. **The impact of acetylation and deacetylation on the p53 pathway**. Protein Cell*, 2*, 456-62.

Burma, S., Chen, B. P. & Chen, D. J. 2006. **Role of non-homologous end joining (nhej) in maintaining genomic integrity**. DNA Repair (Amst)*, 5*, 1042-8.

Canman, C. E., Wolff, A. C., Chen, C. Y., Fornace, A. J., Jr. & Kastan, M. B. 1994. **The p53-dependent g1 cell cycle checkpoint pathway and ataxia-telangiectasia**. Cancer Res*, 54*, 5054-8.

Caporali, S., Falcinelli, S., Starace, G., Russo, M. T., Bonmassar, E., Jiricny, J. & D'Atri, S. 2004. **DNA damage induced by temozolomide signals to both atm and atr: Role of the mismatch repair system**. Mol Pharmacol*, 66*, 478-91.

Carter, C. L., Allen, C. & Henson, D. E. 1989. **Relation of tumor size, lymph node status, and survival in 24,740 breast cancer cases**. Cancer*, 63*, 181-7.

Cervellera, M. N. & Sala, A. 2000. **Poly(adp-ribose) polymerase is a b-myb coactivator**. J Biol Chem*, 275*, 10692-6.

Cha, Y. J., Kim, H. R., Lee, C. Y., Cho, B. C. & Shim, H. S. 2016. **Clinicopathological and prognostic significance of programmed cell death ligand-1 expression in lung adenocarcinoma and its relationship with p53 status**. Lung Cancer*, 97*, 73-80.

Chambeyron, S. & Bickmore, W. A. 2004. **Chromatin decondensation and nuclear reorganization of the hoxb locus upon induction of transcription**. Genes Dev*, 18*, 1119-30.

Chan, R., Sethi, P., Jyoti, A., McGarry, R. & Upreti, M. 2016. **Investigating the radioresistant properties of lung cancer stem cells in the context of the tumor microenvironment**. Radiat Res*, 185*, 169-81.

Chen, J. D. & Evans, R. M. 1995. **A transcriptional co-repressor that interacts with nuclear hormone receptors**. Nature*, 377*, 454-7.

Chen, Y. & Sanchez, Y. 2004. **Chk1 in the DNA damage response: Conserved roles from yeasts to mammals**. DNA Repair (Amst)*, 3*, 1025-32.

Cheok, C. F. & Lane, D. P. 2017. **Exploiting the p53 pathway for therapy**. Cold Spring Harb Perspect Med*, 7*.

Choi, H. K., Choi, K. C., Kang, H. B., Kim, H. C., Lee, Y. H., Haam, S., Park, H. G. & Yoon, H. G. 2008. **Function of multiple lis-homology domain/wd-40 repeat-containing proteins in feed-forward transcriptional repression by**

**silencing mediator for retinoic and thyroid receptor/nuclear receptor corepressor complexes**. Mol Endocrinol*, 22*, 1093-104.

Choi, H. K., Choi, K. C., Yoo, J. Y., Song, M., Ko, S. J., Kim, C. H., Ahn, J. H., Chun, K. H., Yook, J. I. & Yoon, H. G. 2011. **Reversible sumoylation of tbl1-tblr1 regulates beta-catenin-mediated wnt signaling**. Mol Cell*, 43*, 203-16.

Cooks, T., Harris, C. C. & Oren, M. 2014. **Caught in the cross fire: P53 in inflammation**. Carcinogenesis*, 35*, 1680-90.

Corrie, P. G. 2011. **Cytotoxic chemotherapy: Clinical aspects**. Medicine*, 39*, 717-722.

Crawford, L. V., Pim, D. C. & Bulbrook, R. D. 1982. **Detection of antibodies against the cellular protein p53 in sera from patients with breast cancer**. Int J Cancer*, 30*, 403-8.

Creyghton, M. P., Cheng, A. W., Welstead, G. G., Kooistra, T., Carey, B. W., Steine, E. J., Hanna, J., Lodato, M. A., Frampton, G. M., Sharp, P. A., Boyer, L. A., Young, R. A. & Jaenisch, R. 2010. **Histone h3k27ac separates active from poised enhancers and predicts developmental state**. Proc Natl Acad Sci U S A*, 107*, 21931-6.

Cuadrado, M., Martinez-Pastor, B., Murga, M., Toledo, L. I., Gutierrez-Martinez, P., Lopez, E. & Fernandez-Capetillo, O. 2006. **Atm regulates atr chromatin loading in response to DNA double-strand breaks**. J Exp Med*, 203*, 297-303.

Czabotar, P. E., Lessene, G., Strasser, A. & Adams, J. M. 2014. **Control of apoptosis by the bcl-2 protein family: Implications for physiology and therapy**. Nat Rev Mol Cell Biol*, 15*, 49-63.

de Gramont, A., Figer, A., Seymour, M., Homerin, M., Hmissi, A., Cassidy, J., Boni, C., Cortes-Funes, H., Cervantes, A., Freyer, G., Papamichael, D., Le Bail, N., Louvet, C., Hendler, D., de Braud, F., Wilson, C., Morvan, F. & Bonetti, A. 2000. **Leucovorin and fluorouracil with or without oxaliplatin as first-line treatment in advanced colorectal cancer**. J Clin Oncol*, 18*, 2938-47.

deCathelineau, A. M. & Henson, P. M. 2003. **The final step in programmed cell death: Phagocytes carry apoptotic cells to the grave**. Essays Biochem*, 39*, 105-17.

Deneberg, S., Cherif, H., Lazarevic, V., Andersson, P. O., von Euler, M., Juliusson, G. & Lehmann, S. 2016. **An open-label phase i dose-finding study of apr-246 in hematological malignancies**. Blood Cancer J*, 6*, e447.

Dimitrova, Y. N., Li, J., Lee, Y. T., Rios-Esteves, J., Friedman, D. B., Choi, H. J., Weis, W. I., Wang, C. Y. & Chazin, W. J. 2010. **Direct ubiquitination of beta-catenin by siah-1 and regulation by the exchange factor tbl1**. J Biol Chem*, 285*, 13507-16.

Dixon, S. J., Lemberg, K. M., Lamprecht, M. R., Skouta, R., Zaitsev, E. M., Gleason, C. E., Patel, D. N., Bauer, A. J., Cantley, A. M., Yang, W. S., Morrison, B., 3rd & Stockwell, B. R. 2012. **Ferroptosis: An iron-dependent form of nonapoptotic cell death**. Cell*, 149*, 1060-72.

Dohi, Y., Ikura, T., Hoshikawa, Y., Katoh, Y., Ota, K., Nakanome, A., Muto, A., Omura, S., Ohta, T., Ito, A., Yoshida, M., Noda, T. & Igarashi, K. 2008. **Bach1 inhibits oxidative stress-induced cellular senescence by impeding p53 function on chromatin**. Nat Struct Mol Biol*, 15*, 1246-54.

Donawho, C. K., Luo, Y., Luo, Y., Penning, T. D., Bauch, J. L., Bouska, J. J., Bontcheva-Diaz, V. D., Cox, B. F., DeWeese, T. L., Dillehay, L. E., Ferguson, D. C., Ghoreishi-Haack, N. S., Grimm, D. R., Guan, R., Han, E. K., Holley-Shanks, R. R., Hristov, B., Idler, K. B., Jarvis, K., Johnson, E. F., Kleinberg, L. R., Klinghofer, V., Lasko, L. M., Liu, X., Marsh, K. C., McGonigal, T. P., Meulbroek, J. A., Olson, A. M., Palma, J. P., Rodriguez, L. E., Shi, Y., Stavropoulos, J. A., Tsurutani, A. C., Zhu, G. D., Rosenberg, S. H., Giranda, V. L. & Frost, D. J. 2007. **Abt-888, an orally active poly(adp-ribose) polymerase inhibitor that potentiates DNA-damaging agents in preclinical tumor models**. Clin Cancer Res*, 13*, 2728-37.

Donehower, L. A., Harvey, M., Slagle, B. L., McArthur, M. J., Montgomery, C. A., Jr., Butel, J. S. & Bradley, A. 1992. **Mice deficient for p53 are developmentally normal but susceptible to spontaneous tumours**. Nature*, 356*, 215-21.

Eijkelenboom, A. & Burgering, B. M. 2013. **Foxos: Signalling integrators for homeostasis maintenance**. Nat Rev Mol Cell Biol*, 14*, 83-97.

el-Deiry, W. S., Kern, S. E., Pietenpol, J. A., Kinzler, K. W. & Vogelstein, B. 1992. **Definition of a consensus binding site for p53**. Nat Genet*, 1*, 45-9.

el-Deiry, W. S., Tokino, T., Velculescu, V. E., Levy, D. B., Parsons, R., Trent, J. M., Lin, D., Mercer, W. E., Kinzler, K. W. & Vogelstein, B. 1993. **Waf1, a potential mediator of p53 tumor suppression**. Cell*, 75*, 817-25.

Ellison, V. & Stillman, B. 2003. **Biochemical characterization of DNA damage checkpoint complexes: Clamp loader and clamp complexes with specificity for 5' recessed DNA**. PLoS Biol*, 1*, E33.

Elmore, S. 2007. **Apoptosis: A review of programmed cell death**. Toxicol Pathol*, 35*, 495-516.

Elston, C. W. & Ellis, I. O. 1991. **Pathological prognostic factors in breast cancer. I. The value of histological grade in breast cancer: Experience from a large study with long-term follow-up**. Histopathology*, 19*, 403-10.

Finlay, C. A., Hinds, P. W. & Levine, A. J. 1989. **The p53 proto-oncogene can act as a suppressor of transformation**. Cell*, 57*, 1083-93.

Fischer, M. 2017. **Census and evaluation of p53 target genes**. Oncogene*, 36*, 3943-3956.

Fischer, M., Quaas, M., Nickel, A. & Engeland, K. 2015. **Indirect p53-dependent transcriptional repression of survivin, cdc25c, and plk1 genes requires the cyclin-dependent kinase inhibitor p21/cdkn1a and cde/chr promoter sites binding the dream complex**. Oncotarget*, 6*, 41402-17.

Fischer, M., Quaas, M., Steiner, L. & Engeland, K. 2016. **The p53-p21-dream-cde/chr pathway regulates g2/m cell cycle genes**. Nucleic Acids Res*, 44*, 164-74.

Fischle, W., Dequiedt, F., Hendzel, M. J., Guenther, M. G., Lazar, M. A., Voelter, W. & Verdin, E. 2002. **Enzymatic activity associated with class ii hdacs is dependent on a multiprotein complex containing hdac3 and smrt/n-cor**. Mol Cell*, 9*, 45-57.

Foster, B. A., Coffey, H. A., Morin, M. J. & Rastinejad, F. 1999. **Pharmacological rescue of mutant p53 conformation and function**. Science*, 286*, 2507-10.

Fridman, J. S. & Lowe, S. W. 2003. **Control of apoptosis by p53**. Oncogene*, 22*, 9030-40.

Gentric, G., Mieulet, V. & Mechta-Grigoriou, F. 2017. **Heterogeneity in cancer metabolism: New concepts in an old field**. Antioxid Redox Signal*, 26*, 462-485.

Gjorevski, N., Sachs, N., Manfrin, A., Giger, S., Bragina, M. E., Ordonez-Moran, P., Clevers, H. & Lutolf, M. P. 2016. **Designer matrices for intestinal stem cell and organoid culture**. Nature*, 539*, 560-564.

Gomes, N. P. & Espinosa, J. M. 2010. **Gene-specific repression of the p53 target gene puma via intragenic ctcf-cohesin binding**. Genes Dev*, 24*, 1022-34.

Graat, H. C., Carette, J. E., Schagen, F. H., Vassilev, L. T., Gerritsen, W. R., Kaspers, G. J., Wuisman, P. I. & van Beusechem, V. W. 2007. **Enhanced tumor cell kill by combined treatment with a small-molecule antagonist of mouse double minute 2 and adenoviruses encoding p53**. Mol Cancer Ther*, 6*, 1552-61.

Green, D. R. 2011. **Means to an end: Apoptosis and other cell death mechanisms**.

Greiner, V. 2014. **Tbl1 as a novel modulator in DNA damage response and chemosensitivity**. Biological Dissertation, Universität Heidelberg.

Grozinger, C. M. & Schreiber, S. L. 2000. **Regulation of histone deacetylase 4 and 5 and transcriptional activity by 14-3-3-dependent cellular localization**. Proc Natl Acad Sci U S A*, 97*, 7835-40.

Guenther, M. G., Barak, O. & Lazar, M. A. 2001. **The smrt and n-cor corepressors are activating cofactors for histone deacetylase 3**. Mol Cell Biol*, 21*, 6091-101.

Guenther, M. G., Lane, W. S., Fischle, W., Verdin, E., Lazar, M. A. & Shiekhattar, R. 2000. **A core smrt corepressor complex containing hdac3 and tbl1, a wd40-repeat protein linked to deafness**. Genes Dev*, 14*, 1048-57.

Gueven, N., Becherel, O. J., Kijas, A. W., Chen, P., Howe, O., Rudolph, J. H., Gatti, R., Date, H., Onodera, O., Taucher-Scholz, G. & Lavin, M. F. 2004. **Aprataxin, a novel protein that protects against genotoxic stress**. Hum Mol Genet*, 13*, 1081-93.

Hafsi, H. & Hainaut, P. 2011. **Redox control and interplay between p53 isoforms: Roles in the regulation of basal p53 levels, cell fate, and senescence**. Antioxid Redox Signal*, 15*, 1655-67.

Hanahan, D. & Weinberg, R. A. 2011. **Hallmarks of cancer: The next generation**. Cell*, 144*, 646-74.

Harms, K. L. & Chen, X. 2007. **Histone deacetylase 2 modulates p53 transcriptional activities through regulation of p53-DNA binding activity**. Cancer Res*, 67*, 3145-52.

Hartman, H. B., Yu, J., Alenghat, T., Ishizuka, T. & Lazar, M. A. 2005. **The histone-binding code of nuclear receptor co-repressors matches the substrate specificity of histone deacetylase 3**. EMBO Rep*, 6*, 445-51.

Haupt, Y., Maya, R., Kazaz, A. & Oren, M. 1997. **Mdm2 promotes the rapid degradation of p53**. Nature*, 387*, 296-9.

Hoeijmakers, J. H. 2009. **DNA damage, aging, and cancer**. N Engl J Med*, 361*, 1475-85.

Holohan, C., Van Schaeybroeck, S., Longley, D. B. & Johnston, P. G. 2013. **Cancer drug resistance: An evolving paradigm**. Nat Rev Cancer*, 13*, 714-26.

Honda, R., Tanaka, H. & Yasuda, H. 1997. **Oncoprotein mdm2 is a ubiquitin ligase e3 for tumor suppressor p53**. FEBS Lett*, 420*, 25-7.

Horlein, A. J., Naar, A. M., Heinzel, T., Torchia, J., Gloss, B., Kurokawa, R., Ryan, A., Kamei, Y., Soderstrom, M., Glass, C. K. & et al. 1995. **Ligand-independent repression by the thyroid hormone receptor mediated by a nuclear receptor co-repressor**. Nature*, 377*, 397-404.

Horn, H. F. & Vousden, K. H. 2007. **Coping with stress: Multiple ways to activate p53**. Oncogene*, 26*, 1306-16.

Huang, E. Y., Zhang, J., Miska, E. A., Guenther, M. G., Kouzarides, T. & Lazar, M. A. 2000. **Nuclear receptor corepressors partner with class ii histone deacetylases in a sin3-independent repression pathway**. Genes Dev*, 14*, 45-54.

Ishizuka, T. & Lazar, M. A. 2003. **The n-cor/histone deacetylase 3 complex is required for repression by thyroid hormone receptor**. Mol Cell Biol*, 23*, 5122-31.

Itahana, Y., Zhang, J., Goke, J., Vardy, L. A., Han, R., Iwamoto, K., Cukuroglu, E., Robson, P., Pouladi, M. A., Colman, A. & Itahana, K. 2016. **Histone modifications and p53 binding poise the p21 promoter for activation in human embryonic stem cells**. Sci Rep*, 6*, 28112.

Ito, A., Kawaguchi, Y., Lai, C. H., Kovacs, J. J., Higashimoto, Y., Appella, E. & Yao, T. P. 2002. **Mdm2-hdac1-mediated deacetylation of p53 is required for its degradation**. Embo j*, 21*, 6236-45.

Ivanov, G. S., Ivanova, T., Kurash, J., Ivanov, A., Chuikov, S., Gizatullin, F., Herrera-Medina, E. M., Rauscher, F., 3rd, Reinberg, D. & Barlev, N. A. 2007. **Methylation-acetylation interplay activates p53 in response to DNA damage**. Mol Cell Biol*, 27*, 6756-69.

Jackson-Grusby, L., Beard, C., Possemato, R., Tudor, M., Fambrough, D., Csankovszki, G., Dausman, J., Lee, P., Wilson, C., Lander, E. & Jaenisch, R. 2001. **Loss of genomic methylation causes p53-dependent apoptosis and epigenetic deregulation**. Nat Genet*, 27*, 31-9.

Jackson, S. P. & Bartek, J. 2009. **The DNA-damage response in human biology and disease**. Nature*, 461*, 1071-8.

Jaenisch, R. & Bird, A. 2003. **Epigenetic regulation of gene expression: How the genome integrates intrinsic and environmental signals**. Nat Genet*, 33 Suppl*, 245-54.

Jang, H., Choi, S. Y., Cho, E. J. & Youn, H. D. 2009. **Cabin1 restrains p53 activity on chromatin**. Nat Struct Mol Biol*, 16*, 910-5.

Jepsen, K. & Rosenfeld, M. G. 2002. **Biological roles and mechanistic actions of co-repressor complexes**. J Cell Sci*, 115*, 689-98.

Jiang, L., Kon, N., Li, T., Wang, S. J., Su, T., Hibshoosh, H., Baer, R. & Gu, W. 2015. **Ferroptosis as a p53-mediated activity during tumour suppression**. Nature*, 520*, 57-62.

Jin, Q., Yu, L. R., Wang, L., Zhang, Z., Kasper, L. H., Lee, J. E., Wang, C., Brindle, P. K., Dent, S. Y. & Ge, K. 2011. **Distinct roles of gcn5/pcaf-mediated h3k9ac and cbp/p300-mediated h3k18/27ac in nuclear receptor transactivation**. Embo j*, 30*, 249-62.

Joerger, A. C. & Fersht, A. R. 2008. **Structural biology of the tumor suppressor p53**. Annu Rev Biochem*, 77*, 557-82.

Juan, L. J., Shia, W. J., Chen, M. H., Yang, W. M., Seto, E., Lin, Y. S. & Wu, C. W. 2000. **Histone deacetylases specifically down-regulate p53-dependent gene activation**. J Biol Chem*, 275*, 20436-43.

Kao, H. Y., Downes, M., Ordentlich, P. & Evans, R. M. 2000. **Isolation of a novel histone deacetylase reveals that class i and class ii deacetylases promote smrt-mediated repression**. Genes Dev*, 14*, 55-66.

Kenzelmann Broz, D., Spano Mello, S., Bieging, K. T., Jiang, D., Dusek, R. L., Brady, C. A., Sidow, A. & Attardi, L. D. 2013. **Global genomic profiling reveals an extensive p53-regulated autophagy program contributing to key p53 responses**. Genes Dev*, 27*, 1016-31.

Khochbin, S., Verdel, A., Lemercier, C. & Seigneurin-Berny, D. 2001. **Functional significance of histone deacetylase diversity**. Curr Opin Genet Dev*, 11*, 162-6.

Khoo, K. H., Verma, C. S. & Lane, D. P. 2014. **Drugging the p53 pathway: Understanding the route to clinical efficacy**. Nat Rev Drug Discov*, 13*, 217-36.

Kim, D., Pertea, G., Trapnell, C., Pimentel, H., Kelley, R. & Salzberg, S. L. 2013. **Tophat2: Accurate alignment of transcriptomes in the presence of insertions, deletions and gene fusions**. Genome Biol*, 14*, R36.

Knoepfler, P. S. & Eisenman, R. N. 1999. **Sin meets nurd and other tails of repression**. Cell*, 99*, 447-50.

Köhler, K., Ferreira, P., Pfander, B. & Boos, D. 2016. Regulation of the initiation of DNA replication upon DNA damage in eukaryotes. *In:* KAPLAN, D. L. (ed.) *The initiation of DNA replication in eukaryotes.* Cham: Springer International Publishing.

Konduri, S. D., Medisetty, R., Liu, W., Kaipparettu, B. A., Srivastava, P., Brauch, H., Fritz, P., Swetzig, W. M., Gardner, A. E., Khan, S. A. & Das, G. M. 2010. **Mechanisms of estrogen receptor antagonism toward p53 and its implications in breast cancer therapeutic response and stem cell regulation**. Proc Natl Acad Sci U S A*, 107*, 15081-6.

Kruiswijk, F., Labuschagne, C. F. & Vousden, K. H. 2015. **P53 in survival, death and metabolic health: A lifeguard with a licence to kill**. Nat Rev Mol Cell Biol*, 16*, 393-405.

Kubbutat, M. H., Jones, S. N. & Vousden, K. H. 1997. **Regulation of p53 stability by mdm2**. Nature*, 387*, 299-303.

Kulozik, P., Jones, A., Mattijssen, F., Rose, A. J., Reimann, A., Strzoda, D., Kleinsorg, S., Raupp, C., Kleinschmidt, J., Muller-Decker, K., Wahli, W., Sticht, C., Gretz, N., von Loeffelholz, C., Stockmann, M., Pfeiffer, A., Stohr, S., Dallinga-Thie, G. M., Nawroth, P. P., Diaz, M. B. & Herzig, S. 2011. **Hepatic deficiency in transcriptional cofactor tbl1 promotes liver steatosis and hypertriglyceridemia**. Cell Metab*, 13*, 389-400.

Kumari, R., Kohli, S. & Das, S. 2014. **P53 regulation upon genotoxic stress: Intricacies and complexities**. Mol Cell Oncol*, 1*, e969653.

Lain, S., Hollick, J. J., Campbell, J., Staples, O. D., Higgins, M., Aoubala, M., McCarthy, A., Appleyard, V., Murray, K. E., Baker, L., Thompson, A., Mathers, J., Holland, S. J., Stark, M. J., Pass, G., Woods, J., Lane, D. P. & Westwood, N. J. 2008. **Discovery, in vivo activity, and mechanism of action of a small-molecule p53 activator**. Cancer Cell*, 13*, 454-63.

Lane, D. P. & Crawford, L. V. 1979. **T antigen is bound to a host protein in sv40-transformed cells**. Nature*, 278*, 261-3.

Lauvrak, S. U., Munthe, E., Kresse, S. H., Stratford, E. W., Namlos, H. M., Meza-Zepeda, L. A. & Myklebost, O. 2013. **Functional characterisation of osteosarcoma cell lines and identification of mrnas and mirnas associated with aggressive cancer phenotypes**. Br J Cancer*, 109*, 2228-36.

Lavin, M. F. 2008. **Ataxia-telangiectasia: From a rare disorder to a paradigm for cell signalling and cancer**. Nat Rev Mol Cell Biol*, 9*, 759-69.

Le Cam, L., Linares, L. K., Paul, C., Julien, E., Lacroix, M., Hatchi, E., Triboulet, R., Bossis, G., Shmueli, A., Rodriguez, M. S., Coux, O. & Sardet, C. 2006. **E4f1 is an atypical ubiquitin ligase that modulates p53 effector functions independently of degradation**. Cell*, 127*, 775-88.

Lees-Miller, S. P., Sakaguchi, K., Ullrich, S. J., Appella, E. & Anderson, C. W. 1992. **Human DNA-activated protein kinase phosphorylates serines 15 and 37 in the amino-terminal transactivation domain of human p53**. Mol Cell Biol*, 12*, 5041-9.

Leszczynska, K. B., Foskolou, I. P., Abraham, A. G., Anbalagan, S., Tellier, C., Haider, S., Span, P. N., O'Neill, E. E., Buffa, F. M. & Hammond, E. M. 2015. **Hypoxia-induced p53 modulates both apoptosis and radiosensitivity via akt**. J Clin Invest*, 125*, 2385-98.

Levenson, A. S. & Jordan, V. C. 1997. **Mcf-7: The first hormone-responsive breast cancer cell line**. Cancer Res*, 57*, 3071-8.

Lewerenz, J., Hewett, S. J., Huang, Y., Lambros, M., Gout, P. W., Kalivas, P. W., Massie, A., Smolders, I., Methner, A., Pergande, M., Smith, S. B., Ganapathy, V. & Maher, P. 2013. **The cystine/glutamate antiporter system x(c)(-) in health and disease: From molecular mechanisms to novel therapeutic opportunities**. Antioxid Redox Signal*, 18*, 522-55.

Li, D. D., Liu, Y., Xue, L., Su, D. Y. & Pang, W. Y. 2017. **Up-regulation of microrna-367 promotes liver steatosis through repressing tbl1 in obese mice**. Eur Rev Med Pharmacol Sci*, 21*, 1598-1603.

Li, J. & Wang, C. Y. 2008. **Tbl1-tblr1 and beta-catenin recruit each other to wnt target-gene promoter for transcription activation and oncogenesis**. Nat Cell Biol*, 10*, 160-9.

Li, J., Wang, J., Wang, J., Nawaz, Z., Liu, J. M., Qin, J. & Wong, J. 2000. **Both corepressor proteins smrt and n-cor exist in large protein complexes containing hdac3**. Embo j*, 19*, 4342-50.

Li, L., Wang, L., Li, L., Wang, Z., Ho, Y., McDonald, T., Holyoake, T. L., Chen, W. & Bhatia, R. 2012. **Activation of p53 by sirt1 inhibition enhances elimination of cml leukemia stem cells in combination with imatinib**. Cancer Cell*, 21*, 266-81.

Liddiard, K., Hills, R., Burnett, A. K., Darley, R. L. & Tonks, A. 2010. **Ogg1 is a novel prognostic indicator in acute myeloid leukaemia**. Oncogene*, 29*, 2005-12.

Lim, Y. M., Hayashi, S. & Tsuda, L. 2012. **Ebi/ap-1 suppresses pro-apoptotic genes expression and permits long-term survival of drosophila sensory neurons**. PLoS One*, 7*, e37028.

Lim, Y. M. & Tsuda, L. 2016. **Ebi, a drosophila homologue of tbl1, regulates the balance between cellular defense responses and neuronal survival**. Am J Neurodegener Dis*, 5*, 62-8.

Lind, M. J. 2011. **Principles of cytotoxic chemotherapy**. Medicine*, 39*, 711-716.

Linzer, D. I. & Levine, A. J. 1979. **Characterization of a 54k dalton cellular sv40 tumor antigen present in sv40-transformed cells and uninfected embryonal carcinoma cells**. Cell*, 17*, 43-52.

Liu, L., Scolnick, D. M., Trievel, R. C., Zhang, H. B., Marmorstein, R., Halazonetis, T. D. & Berger, S. L. 1999. **P53 sites acetylated in vitro by pcaf and p300 are acetylated in vivo in response to DNA damage**. Mol Cell Biol*, 19*, 1202-9.

Liu, X., Wilcken, R., Joerger, A. C., Chuckowree, I. S., Amin, J., Spencer, J. & Fersht, A. R. 2013. **Small molecule induced reactivation of mutant p53 in cancer cells**. Nucleic Acids Res*, 41*, 6034-44.

Liu, Y., Elf, S. E., Miyata, Y., Sashida, G., Liu, Y., Huang, G., Di Giandomenico, S., Lee, J. M., Deblasio, A., Menendez, S., Antipin, J., Reva, B., Koff, A. & Nimer, S. D. 2009. **P53 regulates hematopoietic stem cell quiescence**. Cell Stem Cell*, 4*, 37-48.

Loewer, A., Batchelor, E., Gaglia, G. & Lahav, G. 2010. **Basal dynamics of p53 reveal transcriptionally attenuated pulses in cycling cells**. Cell*, 142*, 89-100.

Longley, D. B., Harkin, D. P. & Johnston, P. G. 2003. **5-fluorouracil: Mechanisms of action and clinical strategies**. Nat Rev Cancer*, 3*, 330-8.

Love, M. I., Huber, W. & Anders, S. 2014. **Moderated estimation of fold change and dispersion for rna-seq data with deseq2**. Genome Biol*, 15*, 550.

Lukas, J., Lukas, C. & Bartek, J. 2011. **More than just a focus: The chromatin response to DNA damage and its role in genome integrity maintenance**. Nat Cell Biol*, 13*, 1161-9.

Luo, J., Su, F., Chen, D., Shiloh, A. & Gu, W. 2000. **Deacetylation of p53 modulates its effect on cell growth and apoptosis**. Nature*, 408*, 377-81.

Maheswaran, S. & Haber, D. A. 2010. **Circulating tumor cells: A window into cancer biology and metastasis**. Curr Opin Genet Dev*, 20*, 96-9.

Makin, G. & Hickman, J. A. 2000. **Apoptosis and cancer chemotherapy**. Cell Tissue Res*, 301*, 143-52.

Malanga, M. & Althaus, F. R. 2005. **The role of poly(adp-ribose) in the DNA damage signaling network**. Biochem Cell Biol*, 83*, 354-64.

Malhotra, V. & Perry, M. C. 2003. **Classical chemotherapy: Mechanisms, toxicities and the therapeutic window**. Cancer Biol Ther*, 2*, S2-4.

Mao, G., Yuan, F., Absher, K., Jennings, C. D., Howard, D. S., Jordan, C. T. & Gu, L. 2008. **Preferential loss of mismatch repair function in refractory and relapsed acute myeloid leukemia: Potential contribution to aml progression**. Cell Res*, 18*, 281-9.

Margaret, L. T., Krysta, M. C., Mohammad, S., Ahmad, V. & Paola, M. 2014. **Chemoresistance in cancer stem cells and strategies to overcome resistance**. Chemotherapy: Open Access*, 03*.

Martins, C. P., Brown-Swigart, L. & Evan, G. I. 2006. **Modeling the therapeutic efficacy of p53 restoration in tumors**. Cell*, 127*, 1323-34.

Mattson, M. P. 2000. **Apoptosis in neurodegenerative disorders**. Nat Rev Mol Cell Biol*, 1*, 120-9.

Meek, D. W. & Anderson, C. W. 2009. **Posttranslational modification of p53: Cooperative integrators of function**. Cold Spring Harb Perspect Biol*, 1*, a000950.

Miller, K. M., Tjeertes, J. V., Coates, J., Legube, G., Polo, S. E., Britton, S. & Jackson, S. P. 2010. **Human hdac1 and hdac2 function in the DNA-damage response to promote DNA nonhomologous end-joining**. Nat Struct Mol Biol*, 17*, 1144-51.

Minsky, N. & Oren, M. 2004. **The ring domain of mdm2 mediates histone ubiquitylation and transcriptional repression**. Mol Cell*, 16*, 631-9.

Montero, J., Dutta, C., van Bodegom, D., Weinstock, D. & Letai, A. 2013. **P53 regulates a non-apoptotic death induced by ros**. Cell Death Differ*, 20*, 1465-74.

Moriya, H. 2015. **Quantitative nature of overexpression experiments**. Mol Biol Cell*, 26*, 3932-9.

Mottet, D., Pirotte, S., Lamour, V., Hagedorn, M., Javerzat, S., Bikfalvi, A., Bellahcene, A., Verdin, E. & Castronovo, V. 2009. **Hdac4 represses p21(waf1/cip1) expression in human cancer cells through a sp1-dependent, p53-independent mechanism**. Oncogene*, 28*, 243-56.

Mumenthaler, S. M., Foo, J., Choi, N. C., Heise, N., Leder, K., Agus, D. B., Pao, W., Michor, F. & Mallick, P. 2015. **The impact of microenvironmental heterogeneity on the evolution of drug resistance in cancer cells**. Cancer Inform*, 14*, 19-31.

Nagata, S. 2010. **Apoptosis and autoimmune diseases**. Ann N Y Acad Sci*, 1209*, 10-6.

Nowsheen, S. & Yang, E. S. 2012. **The intersection between DNA damage response and cell death pathways**. Exp Oncol*, 34*, 243-54.

Oiseth, S. J., Aziz, M. S. J. J. o. C. M. & Treatment 2017. **Cancer immunotherapy: A brief review of the history, possibilities, and challenges ahead**. 2017*, 3*, 250-61.

Oliver, F. J., de la Rubia, G., Rolli, V., Ruiz-Ruiz, M. C., de Murcia, G. & Murcia, J. M. 1998. **Importance of poly(adp-ribose) polymerase and its cleavage in apoptosis. Lesson from an uncleavable mutant**. J Biol Chem*, 273*, 33533-9.

Ou, Y., Wang, S. J., Li, D., Chu, B. & Gu, W. 2016. **Activation of sat1 engages polyamine metabolism with p53-mediated ferroptotic responses**. Proc Natl Acad Sci U S A*, 113*, E6806-e6812.

Park, S. Y., Na, Y., Lee, M. H., Seo, J. S., Lee, Y. H., Choi, K. C., Choi, H. K. & Yoon, H. G. 2016. **Sumoylation of tbl1 and tblr1 promotes androgen-independent prostate cancer cell growth**. Oncotarget*, 7*, 41110-41122.

Partridge, A. H., Burstein, H. J. & Winer, E. P. 2001. **Side effects of chemotherapy and combined chemohormonal therapy in women with early-stage breast cancer**. J Natl Cancer Inst Monogr, 135-42.

Perissi, V., Aggarwal, A., Glass, C. K., Rose, D. W. & Rosenfeld, M. G. 2004. **A corepressor/coactivator exchange complex required for transcriptional activation by nuclear receptors and other regulated transcription factors**. Cell*, 116*, 511-26.

Perissi, V., Scafoglio, C., Zhang, J., Ohgi, K. A., Rose, D. W., Glass, C. K. & Rosenfeld, M. G. 2008. **Tbl1 and tblr1 phosphorylation on regulated gene promoters overcomes dual ctbp and ncor/smrt transcriptional repression checkpoints**. Mol Cell*, 29*, 755-66.

Peterson, T. J., Karmakar, S., Pace, M. C., Gao, T. & Smith, C. L. 2007. **The silencing mediator of retinoic acid and thyroid hormone receptor (smrt) corepressor is required for full estrogen receptor alpha transcriptional activity**. Mol Cell Biol*, 27*, 5933-48.

Picard, F., Kurtev, M., Chung, N., Topark-Ngarm, A., Senawong, T., Machado De Oliveira, R., Leid, M., McBurney, M. W. & Guarente, L. 2004. **Sirt1 promotes fat mobilization in white adipocytes by repressing ppar-gamma**. Nature*, 429*, 771-6.

Pomerantz, J., Schreiber-Agus, N., Liegeois, N. J., Silverman, A., Alland, L., Chin, L., Potes, J., Chen, K., Orlow, I., Lee, H. W., Cordon-Cardo, C. & DePinho, R. A. 1998. **The ink4a tumor suppressor gene product, p19arf, interacts with mdm2 and neutralizes mdm2's inhibition of p53**. Cell*, 92*, 713-23.

Quaas, M., Muller, G. A. & Engeland, K. 2012. **P53 can repress transcription of cell cycle genes through a p21(waf1/cip1)-dependent switch from mmb to dream protein complex binding at chr promoter elements**. Cell Cycle*, 11*, 4661-72.

Ramadoss, S., Li, J., Ding, X., Al Hezaimi, K. & Wang, C. Y. 2011. **Transducin beta-like protein 1 recruits nuclear factor kappab to the target gene promoter for transcriptional activation**. Mol Cell Biol*, 31*, 924-34.

Riedl, S. J. & Salvesen, G. S. 2007. **The apoptosome: Signalling platform of cell death**. Nat Rev Mol Cell Biol*, 8*, 405-13.

Riley, T., Sontag, E., Chen, P. & Levine, A. 2008. **Transcriptional control of human p53-regulated genes**. Nat Rev Mol Cell Biol*, 9*, 402-12.

Rohm, M., Sommerfeld, A., Strzoda, D., Jones, A., Sijmonsma, T. P., Rudofsky, G., Wolfrum, C., Sticht, C., Gretz, N., Zeyda, M., Leitner, L., Nawroth, P. P., Stulnig, T. M., Berriel Diaz, M., Vegiopoulos, A. & Herzig, S. 2013. **Transcriptional cofactor tblr1 controls lipid mobilization in white adipose tissue**. Cell Metab*, 17*, 575-85.

Roos, W. P. & Kaina, B. 2013. **DNA damage-induced cell death: From specific DNA lesions to the DNA damage response and apoptosis**. Cancer Lett*, 332*, 237-48.

Roth, J., Dittmer, D., Rea, D., Tartaglia, J., Paoletti, E. & Levine, A. J. 1996. **P53 as a target for cancer vaccines: Recombinant canarypox virus vectors expressing p53 protect mice against lethal tumor cell challenge**. Proc Natl Acad Sci U S A*, 93*, 4781-6.

Sheikh, M. S., Huang, Y., Fernandez-Salas, E. A., El-Deiry, W. S., Friess, H., Amundson, S., Yin, J., Meltzer, S. J., Holbrook, N. J. & Fornace, A. J., Jr. 1999. **The antiapoptotic decoy receptor trid/trail-r3 is a p53-regulated DNA damage-inducible gene that is overexpressed in primary tumors of the gastrointestinal tract**. Oncogene*, 18*, 4153-9.

Shieh, S. Y., Ikeda, M., Taya, Y. & Prives, C. 1997. **DNA damage-induced phosphorylation of p53 alleviates inhibition by mdm2**. Cell*, 91*, 325-34.

Shiloh, Y. 2003. **Atm and related protein kinases: Safeguarding genome integrity**. Nat Rev Cancer*, 3*, 155-68.

Siliciano, J. D., Canman, C. E., Taya, Y., Sakaguchi, K., Appella, E. & Kastan, M. B. 1997. **DNA damage induces phosphorylation of the amino terminus of p53**. Genes Dev*, 11*, 3471-81.

Squatrito, M., Brennan, C. W., Helmy, K., Huse, J. T., Petrini, J. H. & Holland, E. C. 2010. **Loss of atm/chk2/p53 pathway components accelerates tumor development and contributes to radiation resistance in gliomas**. Cancer Cell*, 18*, 619-29.

Stiff, T., Walker, S. A., Cerosaletti, K., Goodarzi, A. A., Petermann, E., Concannon, P., O'Driscoll, M. & Jeggo, P. A. 2006. **Atr-dependent phosphorylation and activation of atm in response to uv treatment or replication fork stalling**. Embo j*, 25*, 5775-82.

Stockwell, B. R., Friedmann Angeli, J. P., Bayir, H., Bush, A. I., Conrad, M., Dixon, S. J., Fulda, S., Gascon, S., Hatzios, S. K., Kagan, V. E., Noel, K., Jiang, X., Linkermann, A., Murphy, M. E., Overholtzer, M., Oyagi, A., Pagnussat, G. C., Park, J., Ran, Q., Rosenfeld, C. S., Salnikow, K., Tang, D., Torti, F. M., Torti, S. V., Toyokuni, S., Woerpel, K. A. & Zhang, D. D. 2017. **Ferroptosis: A regulated cell death nexus linking metabolism, redox biology, and disease**. Cell*, 171*, 273-285.

Stoy, C., Sundaram, A., Rios Garcia, M., Wang, X., Seibert, O., Zota, A., Wendler, S., Mannle, D., Hinz, U., Sticht, C., Muciek, M., Gretz, N., Rose, A. J., Greiner, V., Hofmann, T. G., Bauer, A., Hoheisel, J., Berriel Diaz, M., Gaida, M. M., Werner, J., Schafmeier, T., Strobel, O. & Herzig, S. 2015. **Transcriptional co-factor transducin beta-like (tbl) 1 acts as a checkpoint in pancreatic cancer malignancy**. EMBO Mol Med*, 7*, 1048-62.

Su, D., Wang, X., Campbell, M. R., Song, L., Safi, A., Crawford, G. E. & Bell, D. A. 2015. **Interactions of chromatin context, binding site sequence content, and sequence evolution in stress-induced p53 occupancy and transactivation**. PLoS Genet*, 11*, e1004885.

Sykes, S. M., Mellert, H. S., Holbert, M. A., Li, K., Marmorstein, R., Lane, W. S. & McMahon, S. B. 2006. **Acetylation of the p53 DNA-binding domain regulates apoptosis induction**. Mol Cell*, 24*, 841-51.

Szakacs, G., Paterson, J. K., Ludwig, J. A., Booth-Genthe, C. & Gottesman, M. M. 2006. **Targeting multidrug resistance in cancer**. Nat Rev Drug Discov*, 5*, 219-34.

Tadeo, I., Berbegall, A. P., Navarro, S., Castel, V. & Noguera, R. 2017. **A stiff extracellular matrix is associated with malignancy in peripheral neuroblastic tumors**. Pediatr Blood Cancer*, 64*.

Tait, S. W. & Green, D. R. 2010. **Mitochondria and cell death: Outer membrane permeabilization and beyond**. Nat Rev Mol Cell Biol*, 11*, 621-32.

Takebe, N., Miele, L., Harris, P. J., Jeong, W., Bando, H., Kahn, M., Yang, S. X. & Ivy, S. P. 2015. **Targeting notch, hedgehog, and wnt pathways in cancer stem cells: Clinical update**. Nat Rev Clin Oncol*, 12*, 445-64.

Tang, Y., Luo, J., Zhang, W. & Gu, W. 2006. **Tip60-dependent acetylation of p53 modulates the decision between cell-cycle arrest and apoptosis**. Mol Cell*, 24*, 827-39.

Tapia, G. & Diaz-Padill, I. 2013. Molecular mechanisms of platinum resistance in ovarian cancer. *Ovarian cancer - a clinical and translational update.*

Taylor, R. C., Cullen, S. P. & Martin, S. J. 2008. **Apoptosis: Controlled demolition at the cellular level**. Nat Rev Mol Cell Biol*, 9*, 231-41.

Teufel, D. P., Bycroft, M. & Fersht, A. R. 2009. **Regulation by phosphorylation of the relative affinities of the n-terminal transactivation domains of p53 for p300 domains and mdm2**. Oncogene*, 28*, 2112-8.

Tibbetts, R. S., Cortez, D., Brumbaugh, K. M., Scully, R., Livingston, D., Elledge, S. J. & Abraham, R. T. 2000. **Functional interactions between brca1 and the checkpoint kinase atr during genotoxic stress**. Genes Dev*, 14*, 2989-3002.

Toledo, F. & Wahl, G. M. 2006. **Regulating the p53 pathway: In vitro hypotheses, in vivo veritas**. Nat Rev Cancer*, 6*, 909-23.

Tovy, A., Spiro, A., McCarthy, R., Shipony, Z., Aylon, Y., Allton, K., Ainbinder, E., Furth, N., Tanay, A., Barton, M. & Oren, M. 2017. **P53 is essential for DNA methylation homeostasis in naive embryonic stem cells, and its loss promotes clonal heterogeneity**. Genes Dev*, 31*, 959-972.

Trapnell, C., Roberts, A., Goff, L., Pertea, G., Kim, D., Kelley, D. R., Pimentel, H., Salzberg, S. L., Rinn, J. L. & Pachter, L. 2012. **Differential gene and transcript expression analysis of rna-seq experiments with tophat and cufflinks**. Nat Protoc*, 7*, 562-78.

Urao, N. & Ushio-Fukai, M. 2013. **Redox regulation of stem/progenitor cells and bone marrow niche**. Free Radic Biol Med*, 54*, 26-39.

Urnov, F. D., Yee, J., Sachs, L., Collingwood, T. N., Bauer, A., Beug, H., Shi, Y. B. & Wolffe, A. P. 2000. **Targeting of n-cor and histone deacetylase 3 by the oncoprotein v-erba yields a chromatin infrastructure-dependent transcriptional repression pathway**. Embo j*, 19*, 4074-90.

Uziel, T., Lerenthal, Y., Moyal, L., Andegeko, Y., Mittelman, L. & Shiloh, Y. 2003. **Requirement of the mrn complex for atm activation by DNA damage**. Embo j*, 22*, 5612-21.

van der Burg, S. H., Menon, A. G., Redeker, A., Bonnet, M. C., Drijfhout, J. W., Tollenaar, R. A., van de Velde, C. J., Moingeon, P., Kuppen, P. J., Offringa, R. & Melief, C. J. 2002. **Induction of p53-specific immune responses in colorectal cancer patients receiving a recombinant alvac-p53 candidate vaccine**. Clin Cancer Res*, 8*, 1019-27.

Vassilev, L. T., Vu, B. T., Graves, B., Carvajal, D., Podlaski, F., Filipovic, Z., Kong, N., Kammlott, U., Lukacs, C., Klein, C., Fotouhi, N. & Liu, E. A. 2004. **In vivo activation of the p53 pathway by small-molecule antagonists of mdm2**. Science*, 303*, 844-8.

Velculescu, V. E. & El-Deiry, W. S. 1996. **Biological and clinical importance of the p53 tumor suppressor gene**. Clin Chem*, 42*, 858-68.

Ventura, A., Kirsch, D. G., McLaughlin, M. E., Tuveson, D. A., Grimm, J., Lintault, L., Newman, J., Reczek, E. E., Weissleder, R. & Jacks, T. 2007. **Restoration of p53 function leads to tumour regression in vivo**. Nature*, 445*, 661-5.

Verfaillie, A., Svetlichnyy, D., Imrichova, H., Davie, K., Fiers, M., Kalender Atak, Z., Hulselmans, G., Christiaens, V. & Aerts, S. 2016. **Multiplex enhancer-reporter assays uncover unsophisticated tp53 enhancer logic**. Genome Res*, 26*, 882-95.

Vogelstein, B. & Kinzler, K. W. 1992. **P53 function and dysfunction**. Cell*, 70*, 523-6.

Vousden, K. H. & Lu, X. 2002. **Live or let die: The cell's response to p53**. Nat Rev Cancer*, 2*, 594-604.

Wallach, D., Kang, T. B., Dillon, C. P. & Green, D. R. 2016. **Programmed necrosis in inflammation: Toward identification of the effector molecules**. Science*, 352*, aaf2154.

Wang, D., Kon, N., Lasso, G., Jiang, L., Leng, W., Zhu, W. G., Qin, J., Honig, B. & Gu, W. 2016a. **Acetylation-regulated interaction between p53 and set reveals a widespread regulatory mode**. Nature*, 538*, 118-122.

Wang, J., Gong, Z. & Chen, J. 2011. **Mdc1 collaborates with topbp1 in DNA replication checkpoint control**. J Cell Biol*, 193*, 267-73.

Wang, S. J., Li, D., Ou, Y., Jiang, L., Chen, Y., Zhao, Y. & Gu, W. 2016b. **Acetylation is crucial for p53-mediated ferroptosis and tumor suppression**. Cell Rep*, 17*, 366-373.

Ward, I. M. & Chen, J. 2001. **Histone h2ax is phosphorylated in an atr-dependent manner in response to replicational stress**. J Biol Chem*, 276*, 47759-62.

Wei, C. L., Wu, Q., Vega, V. B., Chiu, K. P., Ng, P., Zhang, T., Shahab, A., Yong, H. C., Fu, Y., Weng, Z., Liu, J., Zhao, X. D., Chew, J. L., Lee, Y. L., Kuznetsov, V. A., Sung, W. K., Miller, L. D., Lim, B., Liu, E. T., Yu, Q., Ng, H. H. & Ruan, Y. 2006. **A global map of p53 transcription-factor binding sites in the human genome**. Cell*, 124*, 207-19.

Wen, Y. D., Perissi, V., Staszewski, L. M., Yang, W. M., Krones, A., Glass, C. K., Rosenfeld, M. G. & Seto, E. 2000. **The histone deacetylase-3 complex contains nuclear receptor corepressors**. Proc Natl Acad Sci U S A*, 97*, 7202-7.

Xue, W., Zender, L., Miething, C., Dickins, R. A., Hernando, E., Krizhanovsky, V., Cordon-Cardo, C. & Lowe, S. W. 2007. **Senescence and tumour clearance is triggered by p53 restoration in murine liver carcinomas**. Nature*, 445*, 656-60.

Yoo, H. Y., Kumagai, A., Shevchenko, A., Shevchenko, A. & Dunphy, W. G. 2007. **Ataxia-telangiectasia mutated (atm)-dependent activation of atr occurs through phosphorylation of topbp1 by atm**. J Biol Chem*, 282*, 17501-6.

Yoon, H. G., Chan, D. W., Huang, Z. Q., Li, J., Fondell, J. D., Qin, J. & Wong, J. 2003. **Purification and functional characterization of the human n-cor complex: The roles of hdac3, tbl1 and tblr1**. Embo j*, 22*, 1336-46.

Zeestraten, E. C., Speetjens, F. M., Welters, M. J., Saadatmand, S., Stynenbosch, L. F., Jongen, R., Kapiteijn, E., Gelderblom, H., Nijman, H. W., Valentijn, A. R., Oostendorp, J., Fathers, L. M., Drijfhout, J. W., van de Velde, C. J., Kuppen, P. J., van der Burg, S. H. & Melief, C. J. 2013. **Addition of interferon-alpha to the p53-slp(r) vaccine results in increased production of interferon-gamma in vaccinated colorectal cancer patients: A phase i/ii clinical trial**. Int J Cancer*, 132*, 1581-91.

Zhang, J., Kalkum, M., Chait, B. T. & Roeder, R. G. 2002. **The n-cor-hdac3 nuclear receptor corepressor complex inhibits the jnk pathway through the integral subunit gps2**. Mol Cell*, 9*, 611-23.

Zhang, Y., Xiong, Y. & Yarbrough, W. G. 1998. **Arf promotes mdm2 degradation and stabilizes p53: Arf-ink4a locus deletion impairs both the rb and p53 tumor suppression pathways**. Cell*, 92*, 725-34.

Zhao, Y., Lu, S., Wu, L., Chai, G., Wang, H., Chen, Y., Sun, J., Yu, Y., Zhou, W., Zheng, Q., Wu, M., Otterson, G. A. & Zhu, W. G. 2006. **Acetylation of p53 at lysine 373/382 by the histone deacetylase inhibitor depsipeptide induces expression of p21(waf1/cip1)**. Mol Cell Biol*, 26*, 2782-90.

Zhou, B. B. & Bartek, J. 2004. **Targeting the checkpoint kinases: Chemosensitization versus chemoprotection**. Nat Rev Cancer*, 4*, 216-25.

Zhou, B. B. & Elledge, S. J. 2000. **The DNA damage response: Putting checkpoints in perspective**. Nature*, 408*, 433-9.

Zhu, X., Li, J., Qiu, X., Liu, Y., Feng, W. & Li, F. 2018. **Upconversion nanocomposite for programming combination cancer therapy by precise control of microscopic temperature**. Nat Commun*, 9*, 2176.

# Supplementary Data

**Table 1.** Top 116 genes identified as activated p53 targets in at least 6 out of 16 genome-wide data sets

| Gene symbol | Literature | No. of genome-wide data sets | Gene symbol | Literature | No. of genome-wide data sets | Gene symbol | Literature | No. of genome-wide data sets |
|---|---|---|---|---|---|---|---|---|
| CDKN1A | 11,12 | 16 | HSPA4L | | 9 | PLCL2 | | 7 |
| RRM2B | 31 | 16 | ISCU | 54 | 9 | PRKAB1 | 35,201 | 7 |
| MDM2 | 14,15 | 15 | PHLDA3 | 233 | 9 | PTP4A1 | 234 | 7 |
| GDF15 | 32 | 14 | SERPINB5 | 235 | 9 | SPATA18 | 236 | 7 |
| SUSD6 | 33,34 | 14 | SLC12A4 | | 9 | TGFA | 161 | 7 |
| BTG2 | 36 | 13 | TRAF4 | 179 | 9 | TLR3 | 237 | 7 |
| DDB2 | 165 | 13 | TRIM22 | 223 | 9 | ZNF219 | | 7 |
| GADD45A | 13 | 13 | CCDC90B | | 8 | ZNF337 | | 7 |
| PLK3 | 35 | 13 | CES2 | 238 | 8 | ZNF79 | 160 | 7 |
| TIGAR | 35,37 | 13 | DYRK3 | | 8 | ARHGEF3 | | 6 |
| RPS27L | 239,240 | 12 | FAM13C | | 8 | CD82 | 241 | 6 |
| TNFRSF10B | 38,39 | 12 | FAM198B | | 8 | CDIP1 | 242 | 6 |
| TRIAP1 | 189 | 12 | FAM212B | | 8 | CERS5 | | 6 |
| ZMAT3 | 243 | 12 | KITLG | 50 | 8 | CSF1 | 244 | 6 |
| BAX | 41–43 | 11 | NADSYN1 | | 8 | DUSP14 | | 6 |
| BLOC1S2 | | 11 | NTPCR | | 8 | EPS8L2 | | 6 |
| PGF | 160 | 11 | ORAI3 | | 8 | FAM210B | | 6 |
| POLH | 170 | 11 | SESN2 | 52 | 8 | FUCA1 | 160,196 | 6 |
| PPM1D | 40 | 11 | SLC30A1 | | 8 | GRHL3 | | 6 |
| PSTPIP2 | | 11 | TM7SF3 | | 8 | HHAT | | 6 |
| SULF2 | 245 | 11 | TMEM68 | | 8 | IER5 | 246 | 6 |
| XPC | 166 | 11 | WDR63 | | 8 | IGDCC4 | | 6 |
| AEN | 44 | 10 | ZNF561 | | 8 | IKBIP | | 6 |
| ANKRA2 | | 10 | ACER2 | | 7 | LAPTM5 | | 6 |
| FAS | 47–49 | 10 | ANXA4 | | 7 | MAST4 | | 6 |
| GPR87 | 247 | 10 | APOBEC3C | | 7 | MICALL1 | | 6 |
| NINJ1 | 160 | 10 | ASCC3 | | 7 | PADI4 | 248 | 6 |
| PLK2 | 45 | 10 | ASTN2 | | 7 | PANK1 | 199,200 | 6 |
| SERTAD1 | | 10 | ATF3 | 249 | 7 | PMAIP1 | 182 | 6 |
| SESN1 | 46 | 10 | BBC3 | 181 | 7 | PRDM1 | 250 | 6 |
| TP53I3 | 251,252 | 10 | CPE | | 7 | RAP2B | 253 | 6 |
| TP53INP1 | 254 | 10 | DCP1B | | 7 | RNF19B | | 6 |
| ABCA12 | 255 | 9 | EDA2R | 256 | 7 | RRAD | 257 | 6 |
| CCNG1 | 219 | 9 | ENC1 | | 7 | SAC3D1 | | 6 |
| CMBL | | 9 | EPHA2 | 258 | 7 | SYTL1 | | 6 |
| CYFIP2 | 259 | 9 | FDXR | 202 | 7 | TNFRSF10D | 39 | 6 |
| DRAM1 | 209 | 9 | FOSL1 | | 7 | TSPAN11 | | 6 |
| FBXO22 | 240 | 9 | LIF | 260 | 7 | VWCE | | 6 |
| FBXW7 | 159 | 9 | PGPEP1 | | 7 | | | |

Taken from Fischer 2017, in the table the yellow marked are the 51 overlapped genes with TBL1 regulated p53 target genes, related to Fig. 7F.

# Curriculum Vitae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## Experience

10/2015 – 04/2018 PhD in German Cancer Research Center (DKFZ)
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05/2018 – 2019 PhD in Institute of Toxicology, Mainz University
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## Education

2015 – 2019 PhD in Molecular and Cellular Biology
Heidelberg University, Germany

2012 – 2015 Master in Basic Veterinary Medicine
Jilin University, China

2007 – 2012 Bachelor in Veterinary Medicine
Jilin University, China

## Conference Contributions

06/2018 The 12th International PhD Student Cancer Conference, London, Britain

- **Selected speaker**

10/2018 The 2nd international conference on "Networks of Cellular Surveillance Mechanisms", Heidelberg, Germany

- **Poster presentation**

## Honors & Awards

- 10/2015 Scholarship from China Scholarship Council for PhD study
- 07/2015 Excellent Master Dissertation in Jilin Province
- 10/2014 Winner of National Scholarship from China's Ministry of Education

## Languages

- English – Full professional proficiency
- German – Professional working proficiency
- Mandarin – Native or bilingual proficiency

## Publications

- He, Y., Roos, W., **Wu, Q.**, Hofmann, T., Kaina, B. (2019). The SIAH1-HIPK2-p53ser46 damage response pathway is involved in temozolomide induced glioblastoma cell death. Mol Cancer Res. Accepted.
- **Wu, Q.**, Yu, L., Qiu, J., *et al*. Linalool attenuates lung Inflammation induced by Pasteurella multocida via activating Nrf-2 signaling pathway. Int Immunopharmacol. 2014 Aug; 21(2): 456 - 63.

# Acknowledgement

I would like to express my sincere gratitude to all the people who have contributed to this thesis. With you all, living and studying here is a wonderful experience!

At first, I would like to thank **Prof. Dr. Thomas Hofmann** for giving me opportunity to work in his laboratory and for supervision of this project.

I appreciated the suggestions from the members of my Thesis Advisory Committee: **Prof. Dr. Thomas Hofmann**, **Prof. Dr. Stefan Wölfl** and **Prof. Dr. Frank Rösl**. They have promoted my work a lot by scientific discussions and inspirations.

I also would like to thank **Prof. Dr. Frank Lyko**, **Prof. Dr. Alwin Krämer** and **Prof. Dr. Frank Rösl** for evaluating my thesis and my final examination.

Further, I want to say thank you to my colleagues for the daily help in the laboratory. Many thanks to people in **Prof. Dr. Frank Lyko** laboratory for cooperativeness. A special thanks to **Günter**, who helped analysis of RNA-seq data.

I also thank my friends, who helped me solve problems and made it easier for me to live well in Germany.

I could never forget the **China Scholarship Council (CSC)**, which financially supports my PhD study.

Most of all, a big thank you to my wife, **Ying**. Thank you for being my side all the time and for incomparable and affectionate encouragement. It is her credit if I have made any progress.

In the end, I would like to thank my **parents** and my **sisters**. Thank you for their care and support. Nothing would be possible without the inspiration from them.

最后，我要感谢我最爱的家人。感谢我的父母，你们对我的关心和支持就是我前进路上的最大动力。感谢我的姐姐，你们对我的鼓励和以及对父母的照顾使我能够专心求学。感谢我的老婆，如果我能做出什么成就，那都是你的功劳。千言万语汇成一句话，我爱你们!

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1. Bei der eingereichten Dissertation zu dem Thema

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Ort und Datum: Heidelberg, 26. February. 2019 Unterschrift: Wu, Qianchao