The Role of KAP1 Posttranslational Modifications during Infection with Human Adenovirus Type 5

Dissertation

zur Erlangung des akademischen Grades
eines Doktors der
Naturwissenschaften (Dr. rer. nat.)

am Fachbereich Biologie
der Fakultät für Mathematik, Informatik und Naturwissenschaften
an der Universität Hamburg

vorgelegt von
Carolin Bürck
aus Pforzheim

Mai 2015

Tag der Disputation:	10.07.2015
Gutachter:	Prof. Dr. T. Dobner
	Prof. Dr. N. Fischer
Prüfungsvorsitzende:	Prof. Dr. J. Kehr

HPI Heinrich-Pette-Institut
Leibniz-Institut für Experimentelle Virologie

HPI · Martinistraße 52 · 20251 Hamburg

Carol Stocking, Ph.D.
Retroviral Pathogenesis, Head
Phone: +49-40-48051 273
Fax: +49-40-48051 187
Email: stocking@hpi.uni-hamburg.de

Studienbüro Biologie
z.H. Frau Sült-Wüpping
MIN Fakultät
Universität Hamburg
Biozentrum Klein Flottbek
Ohnhorststraße 18
22609 Hamburg

21. April 2015

Sehr geehrte Damen und Herren,

hiermit bestätige ich, dass die von Frau Carolin Bürck mit dem Titel "The Role of KAP1 Posttranslational Modifications during Infection with Human Adenovirus Type 5 " vorgelegte Doktorarbeit in korrektem Englisch geschrieben ist.

Mit freundlichen Grüßen,

Carol Stocking

Dr. Carol Stocking
Leiterin der FG Retrovirale Pathogenese
Heinrich-Pette-Institut
(Amerikanerin)

Declaration on oath

I hereby declare, on oath, that I have written the present dissertation by my own and have not used other than the acknowledged resources and aids.

Hamburg, 20.04.2015 Signature C. Bürck

Table of contents

Abbreviations

aa	amino acid
AD	adenoid degradation
APC	adenoid-pharyngeal-conjunctival
APS	Ammonium persulfate
ARD	acute respiratory disease
ATM	Ataxia telangiectasia mutated
ATP	Adenosine triphosphate
ATR	Ataxia telangiectasia and Rad3-related protein
BRK	baby rat kidney cells
BSA	bovine serum albumin
CHD3	Chromodomain helicase DNA-binding protein 3
CHK1/2	Checkpoint Kinase 1/2
DAPI	4', 6 Diamidine-2-phenylindole dihydrochloride
Daxx	Death-domain associated protein 6
dd	double-distilled
DDR	DNA damage response
DSB	double-strand break
DEPC	Diethylpyrocarbonate
DMSO	Dimethylsulfoxide
DNA-PK	DNA-dependent protein kinase
dNTP	Desoxyribonucleoside-5'-Triphosphate
DTT	Dithiotreithol
E. coli	*Escherichia coli*
EDTA	Ethylenediaminetetraacetic acid
FCS	fetal calf serum
FITC	Fluorescein isothiocyanate
ffu	Fluorescence forming units
HA	Hemagglutinin epitope of Influenza Virus

HAd	Human Adenovirus
HAT	Histone acetyltransferase
HCV	Hepatitis C Virus
HDAC	Histone deacetylase
HEK	Human embryonic kidney
HIV	Human Immunodeficiency Virus
HMT	Histone methyltransferase
HP1	Heterochromatin protein 1
HR	homologous recombination
HRP	horse-radish peroxidase
HSV	Herpes Simplex Virus
IFN	Interferone
Ig	Immunoglobulin
IgH	Immunoglobulin heavy chain
IP	Immunoprecipitation
KAKA	KRAB- and KAP1-associated
KAP1	KRAB-associated protein 1
KRAB	Krüppel-associated box
KMT	Lysine methyltransferase
KSHV	Kaposi's sarcoma-associated herpesvirus
mAb	mouse antibody
NES	nuclear export signal
NHEJ	non-homologous end joining
NLS	nuclear localization signal
NuRD	Nucleosome Remodeling Deacetylase
ORF	open reading frame
PHD	Plant homeodomain
PML	Promyelocytic leukemia protein
PML-NB	PML nuclear body
PP	Protein phosphatase

PTM	Post-translational modification
rAb	rat antibody
Rb	Retinoblastoma tumorsuppressor protein
rbAb	rabbit antibody
RBCC	RING, B-Box, coiled-coil domain
RI	respiratory illness
RING	Really interesting new gene
RLU	Relative luminescence units
SCM	SUMO conjugation motif
SDS	Sodium dodecyl sulfate
SENP	Sentrin specific protease
SETDB1	SET domain, bifurcated 1
SFK	Srk family kinase
SIM	SUMO interaction motif
SPOC1	Survival time-associated PHD finger protein in Ovarian Cancer 1
SUMO	Small ubiquitin related modifier
TIF1β	Transcriptional Intermediary Factor 1 beta
TRIM	tripartite motif
Tris	Tris-(hydroxymethyl)-aminomethane
TSS	Tif1 signature sequence
Ubi	ubiquitin
v/v	volume per volume
w/v	weight per volume
wt	wildtype

1 Abstract

Cell survival requires mechanisms to recognize and repair DNA damage. The mechanisms involved in DNA damage response (DDR) have been intensively studied and comprise ATM-, ATR- and DNA-PK-dependent pathways, which can in severe cases initiate apoptosis. Modulation of chromatin structure is a critical step for DNA repair proteins gaining access to the DNA. A recently identified modulator of chromatin structure is the Survival-time associated PHD protein in Ovarian Cancer 1 (SPOC1). SPOC1 complexes with the cellular co-repressor KRAB-associated Protein 1 (KAP1) as well as with Histone Methyltransferases (HMTs) and the Nucleosome Remodeling and Deacetylase Complex (NuRD), resulting in chromatin condensation and heterochromatin formation after the repair of DNA double strand breaks (DSBs). KAP1 function is known to be regulated via several posttranslational modifications (PTMs), such as phosphorylation and SUMOylation. In this context, KAP1 is phosphorylated and deSUMOylated upon DSBs, leading to the dissociation of the repressive components from the damaged sites, facilitating efficient DNA repair, thereby underlining the importance of these PTMs in enlarging protein functions.

Human adenovirus type 5 (HAd5) contains a linear double-stranded genome, which is internalized into the host cell nucleus for virus replication. The free viral linear genome activates host-cellular DDR mechanisms after entering the host cell, including ATM, ATR and DNA-PK pathways. For efficient virus replication HAd5 gene products of the early regions 1 and 4 (E1 and E4) counteract these effects by inactivation of DDR components. While some of the components are targeted for degradation by the E1B-55K/E4orf6-dependent E3 ubiquitin ligase complex, other DDR factors are relocalized and/or inactivated to ensure proper virus replication. Increasing evidence suggests that modulation of chromatin remodeling factors is a crucial step in virus replication. Recently, we reported that the cellular Daxx/ATRX chromatin remodeling complex negatively regulates HAd5 replication and that this host-cellular antiviral defense is counteracted by virus-mediated proteasomal degradation during HAd5 infection. Furthermore, we recently identified SPOC1 as a restriction factor and binding partner of the viral core protein pVII, which is

relocalized to viral replication centers (VRCs) and subsequently targeted for proteasomal degradation.

This work demonstrates that the cellular co-repressor KAP1 interacts with E1B-55K via its C-terminus as well as with the viral DNA binding factor E2A/DBP. This study provides the first molecular evidence that KAP1 negatively regulates HAd5 productive infection. Although KAP1 is not degraded during HAd5 infection, its antiviral response is counteracted by induction of PTMs of the cellular factor. In this context, it was observed that KAP1 is phosphorylated in a dose-dependent manner and that its phosphorylation is counteracted by the viral phosphoprotein E1B-55K early in infection, suggesting the repression of KAP1-responsive pro-apoptotic genes. Additionally, KAP1 is deSUMOylated, known to result in dissociation of the repressive complex from the DNA, followed by DNA relaxation and transcriptional activation. Interestingly, reduction of KAP1 SUMO modification requires the presence of SUMOylated E1B-55K, indicating a tight interplay between PTMs of KAP1 and the viral factor. The fact that our group recently showed an interaction of SPOC1 with pVII suggests that the SPOC1/KAP1 complex functions to maintain the chromatinized state of the viral genome early in infection, whereas KAP1 phosphorylation and deSUMOylation of KAP1 results in the onset of viral DNA synthesis.

Consistent with the hypothesis of a flexible regulation of KAP1 co-repressor function by PTMs, this work reveals that viral proteins differentially regulate KAP1 SUMOylation status in transfection. Thereby, proteins associated with the incoming virus particle as well as immediate early proteins induce an increase of KAP1 SUMOylation, while in the presence of early and late proteins, KAP1 SUMO modification is reduced. Furthermore, this study provides evidence that KAP1 facilitates SUMOylation of several HAd5 proteins, indicating its involvement in the regulation of functional and/or localization changes of the respective viral factors.

2 Introduction

2.1 Adenoviruses

2.1.1 Classification

During the last 60 years, adenoviruses (Ads) have been intensively studied as a model system to gain a better understanding of the virus/host interplay but have also been used as vectors in gene therapy. Ads were first discovered and isolated in the early 1950s from adenoid tissues and secretions of patients suffering respiratory tract infections (Hilleman and Werner, 1954; Rowe *et al.*, 1953). These isolates were named according to their symptoms, such as acute respiratory disease (ARD), adenoid-pharyngeal-conjunctival (APC), respiratory illness (RI) or adenoid degradation (AD). Due to their shared characteristics and properties, they were finally grouped and named adenoviruses in 1956 (Enders *et al.*, 1956; Huebner *et al.*, 1954).

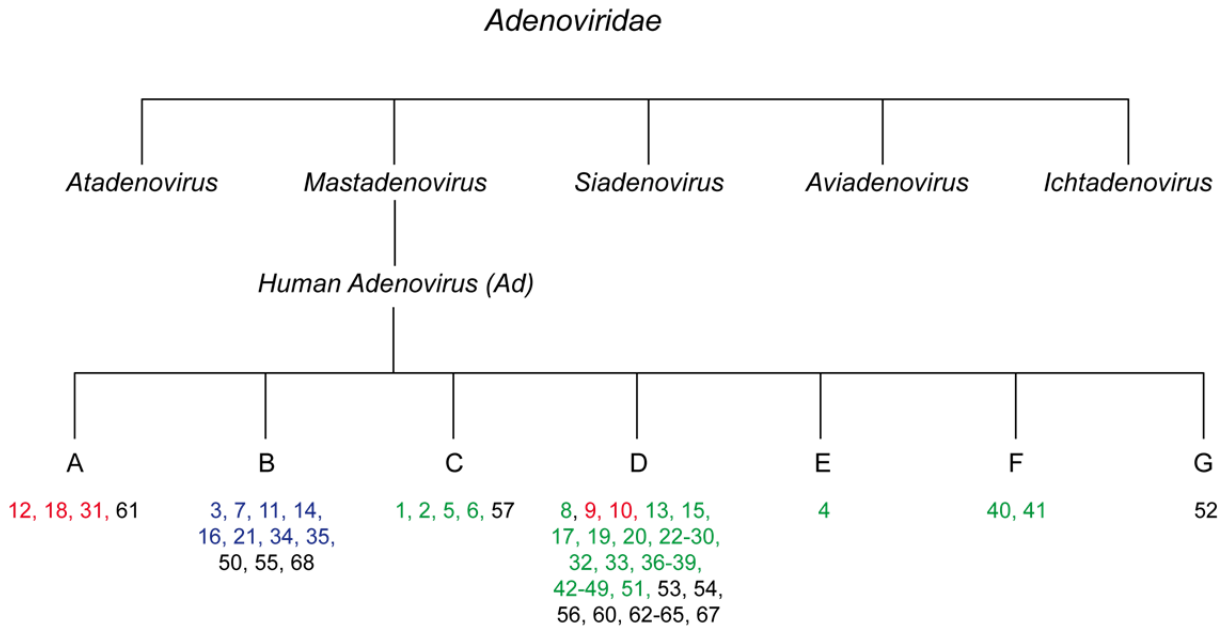


Figure 1: Classification of human Adenoviruses. Schematic representation of the family of *Adenoviridae.* The 68 human Ad types are subgrouped into the seven species A-G (Davison *et al.*, 2003; ICTV/International committee on taxonomy of viruses). Oncogenic potential in rodents is indicated by colour (red: highly oncogenic, blue: weakly oncogenic, green: non-oncogenic, black: not reported).

The family of *Adenoviridae* is able to infect a variety of hosts, including mammalian and other vertebrates and comprises more than 100 different serotypes (Benkö *et al.*, 1999). Ads can be divided into five genera depending on their host range: the *Mastadenoviruses* isolated from mammals, *Aviadenoviruses* isolated from birds, *Atadenoviruses* isolated from birds, reptiles and ruminants, *Siadenoviruses* isolated from amphibians, and *Ichtadenoviruses* isolated from fish (Benkó *et al.*, 2002; Benkö and Harrach, 1998; Davison *et al.*, 1993). Human Ads (HAds) can be distinguished dependent on their hemagglutination properties with specific human sera, sequence homology and oncogenicity in immunosuppressed rodents (Bailey and Mautner, 1994; Berk, 2007; Buckwalter *et al.*, 2012; Davison *et al.*, 2003; de Jong *et al.*, 1999; Wadell, 1984). To date, 68 human types can be clustered into the seven species A-G (Figure 1).

Infections with HAds cause lytic as well as persistent infections and are highly prevalent, as around 80% of all children are infected by the age of five. HAd infections can be associated with a variaty of ocular, respiratory and gastrointestinal diseases like appendicitis (species A, E), gastroenteritis (species G), keratoconjunctivitis (species A, D, E), pharyngitis (species A, E), pneumonia (species A, E), persisting urinary tract infections (species B), cystitis (species A, B, E), hepatitis and tick-borne encephalitis (species B). In immunocompetent individuals, HAd infections are usually mild and self-limiting, whereas in immunocompromised hosts like AIDS patients, organ transplant recipients or tumor patients receiving radiation and chemotherapy, HAd infection frequently results in a fatal outcome (Abe *et al.*, 2003; Carrigan, 1997; Horwitz, 1996).

2.1.2 Structure and genome organization of Human Adenoviruses

HAds comprise an 80-110 nm large non-enveloped icosahedral capsid containing a linear double-stranded DNA genome, tightly associated with the core proteins V, VII and μ (Shenk, 2001; Figure 2). The genome is flanked by two inverted terminal repeats (ITR) and associates with the viral 55 kDa terminal protein (TP) at both 5′ ends, responsible for the initiation of the viral DNA synthesis (Davison *et al.*, 2003).

The viral capsid consists of 252 structural units (capsomers), comprising 240 trimeric hexon and 12 penton proteins (Figure 2). The fiber proteins (spikes) are associated with the penton proteins, facilitating the adsorption and internalization of the virus via receptor-mediated uptake mainly through the Coxsackie/Adenovirus Receptor (CAR) or CD46 (Bergelson *et al.*, 1997; Gaggar *et al.*, 2003). Additionally, the penton base proteins interact with integrins on the cell surface, thereby facilitating receptor-mediated endocytosis (Mathias *et al.*, 1994; Wickham *et al.*, 1994; Wickham *et al.*, 1993). In general, the adenoviral core proteins can be classified as the minor capsid proteins pIIIa, pVI, pVIII and pIX and the viral genome-associated minor components pV, pVII, μ, IVa2 and TP (Russell, 2009; Russell and Matthews, 2003).

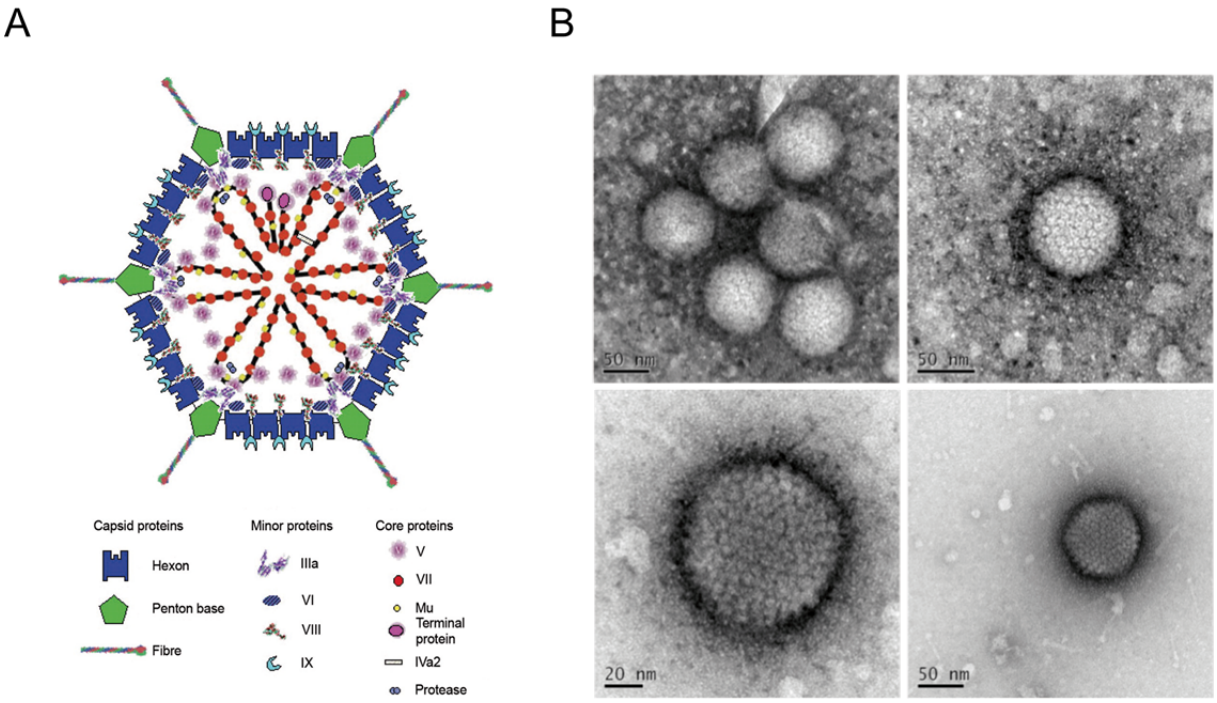


Figure 2: Schematic representation of HAds. (A). Schematic cross section of an HAd particle based on cryomicroscopic analyzation (Russell, 2009). (B) Electron microscopy picture of HAd5 particles, showing the icosahedral capsids with hexon proteins (Department of Electron Microscopy, Heinrich Pette Institute, Leibniz Institute for Experimental Virology, Hamburg).

Sequence analysis could show that all HAds contain a similar genomic organization and express a conserved set of gene products (Davison *et al.*, 2003; Shenk, 2001). Until today, most of the studies on HAds have been conducted on species C HAd2 and HAd5. Besides approximately 40 regulatory and structural proteins, the nine transcriptional units additionally encode two non-coding virus-associated RNAs

(VA-RNAs; Figure 3). The transcription units comprise five early (E1A, E1B, E2, E3 and E4), two delayed (IX and IVa2) and the major late transcription unit (MLTU), generating five late mRNA families (L1-L5). All mentioned transcription units are synthesized by RNA polymerase II, whereas the VA-RNAs are transcribed by RNA polymerase III (Shenk, 2001).

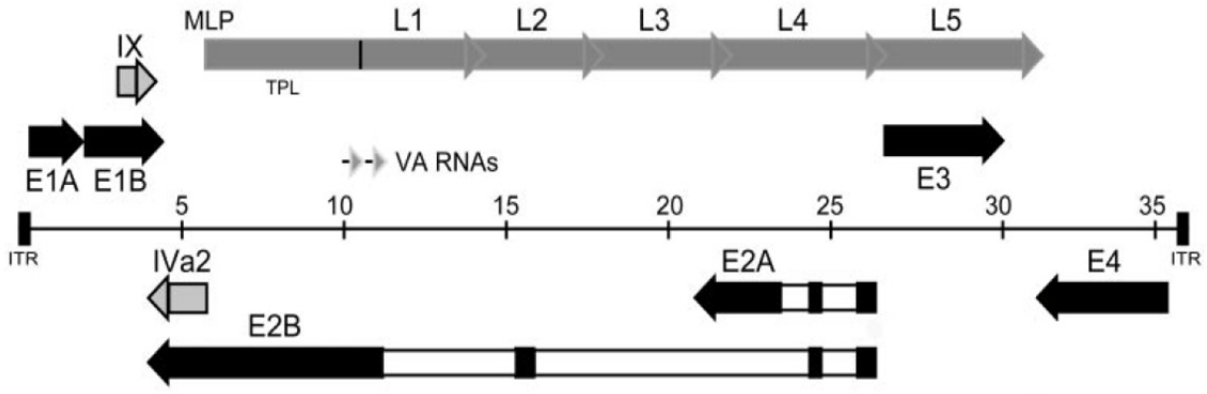


Figure 3: Genomic organization of HAd5. Organization of early (E1A, E1B, E2A, E2B, E3, E4), delayed (pIX, IVa2) and late transcription units (L1-L5). Reading direction is indicated by arrows. Early viral transcription units are already functional before viral DNA replication takes place and mainly have regulatory functions, such as DNA replication (E2), modulation of the immune system (E3) as well as transcription, RNA processing and cell cycle control (E1A, E1B and E4). Late genes (L1-L5) are transcribed after the onset of viral DNA replication by a shared promoter (MLP) and encode mainly for structural proteins. E: early; L: late; MLP: major late promoter; TPL: tripartite leader; VA RNAs: virus-associated RNA; ITR: inverted terminal repeat (from Täuber and Dobner, 2001a).

2.1.3 Productive infection cycle of Human Adenoviruses

HAds infect mainly post-mitotic resting differentiated epithelial cells of the respiratory and gastrointestinal tract. Futhermore, HAds can infect several established tumor and primary cell lines in tissue culture. In human cells HAds cause a productive lytic infection cycle, whereas infection of animal cells, especially rodent cells, results in an abortive infection (Liebermann *et al.*, 1996; Shenk, 2001). Additionally, recent work suggests the possibility of latent adenovirus infections (Garnett *et al.*, 2009; Gustafsson *et al.*, 2007; Kosulin *et al.*, 2007).

The adenoviral infection cycle can be divided into an early and a late phase, which are separated by the onset of viral DNA replication. After receptor-mediated uptake, endocytosis of the viral particle and nuclear import of the DNA/core complex, the immediate early gene E1A is expressed. This step leads to the transcription of more than 20 early regulatory proteins from the E1, E2, E3 and E4 transcription units via

the cellular RNA polymerase II, followed by alternative splicing (Avvakumov *et al.*, 2002a; Avvakumov *et al.*, 2002b; Moran *et al.*, 1986; Schaeper *et al.*, 1998). These proteins establish optimal conditions for virus replication in the late phase of infection. E1A and E1B promote cell cycle progression and inhibit apoptosis. Proteins of the E2 region, the viral DNA binding protein (E2A/DBP), the viral DNA polymerase (E2B) and the precursor of the terminal protein (TP) play roles in viral DNA replication (Shenk, 2001). Proteins of the E3 region modulate the immune response and maintain cell viability (Burgert and Blusch, 2000; Gooding and Wold, 1990). The E4 region encodes at least six different polypeptides, namely E4orf1, E4orf2, E4orf3, E4orf4, E4orf6 and E4orf6/7, according to their open reading frames. These polypeptides result from alternative splicing and are responsible for several functions during virus replication and oncogenic transformation (Täuber and Dobner, 2001a).

In the late phase of infection the transcription of the major late transcription unit (MLTU) is initiated by the activation of the major late promoter (MLP). The late viral mRNAs L1, L2, L3, L4 and L5 are produced by differential splicing of the 29 kbp precursor mRNA MLTU (Figure 3), mainly encoding structural proteins like hexon and fiber. These mRNAs contain a common 5‘-non-coding sequence (tripartite leader, TPL). The accepted model is that during the late phase of infection, host cell mRNA transport and translation are shut-off (host cell shut-off), while viral late mRNAs are efficiently transported to the cytoplasm and preferentially translated (Babich *et al.*, 1983; Beltz and Flint, 1979). However, increasing evidence occurs, suggesting a more complex regulation of the cellular and viral mRNA export. Additionally, the massive accumulation of viral DNA and the reorganization of the host cell cytosceleton result in a restructuring of the nucleus (Defer *et al.*, 1990; Puvion-Dutilleul and Puvion, 1995). Finally, packaging of viral DNA is arranged by late (L4-100K, L4-33K and L4-22K) and early regulatory proteins (E1B-55K, E4orf6 and E2A). In tissue culture, the viral life cycle is completed after approximately 24 h by host cell lysis and the release of up to 10,000 progeny viral particles per cell (Shenk, 2001).

2.1.4 Role of early HAd5 proteins during the onset of the replication cycle

In the capsid, HAd5 core protein V was shown to be associated with the viral genome. Early work on pV suggested that it is involved in core condensation (Chatterjee *et al.*, 1986; Vayda *et al.*, 1983). Recently, Ugai and co-workers revealed that pV plays a role in virus assembly and the formation of infectious virions (Ugai *et al.*, 2007). During the infection process pV is suggested to play a role in the delivery of the viral DNA (Matthews and Russell, 1998a; Matthews and Russell, 1998b) and to contain multiple DNA binding sites, known to bridge the inner viral DNA core with the outer capsid (Brown *et al.*, 1975; Chatterjee *et al.*, 1986; Matthews and Russell, 1998b). It is suggested that this function is mediated by the interaction with the minor protein pVI (Pérez-Vargas *et al.*, 2014).

Upon HAd5 infection, pVI can be found in the nucleus as well as in the cytoplasm, thereby acting as a transport molecule for importin-dependent nuclear transport of the viral hexon protein. As a pH independent lytic factor, pVI releases the viral genome from the endosome, followed by the internalization of the linear dsDNA into the nucleus of the host cell (Wiethoff *et al.*, 2005). Upon proteolytic cleavage of pVI, the viral protein loses its Nuclear Localization Sequence (NLS) and its Nuclear Export Sequence (NES). Due to this cleavage pVI function as a nucleocytoplasmic transporter is abrogated and replaced by its function as a structural protein (Wodrich *et al.*, 2003). In the virion, pVI connects genome-associated core with the capsid, resulting in a high stability of the virus particle. Recently, our group showed that upon HAd5 entry pVI activates the Ad E1A promoter independently of HAd5 gene expression, thereby connecting virus entry with the transcriptional activation of the virus genome. Daxx localizes to intranuclear dot-like structures, which are mainly formed by the Promyelocytic leukemia protein (PML). Interaction of pVI with the host-cellular antiviral factor Daxx results in the displacement from those PML nuclear bodies (PML-NBs) and inactivation of the cellular factor by pVI (Schreiner *et al.*, 2012).

During nuclear import of the viral genome, the highly conserved DNA-associated core protein pVII plays a major role. pVII harbors lysine-rich amino acid sequences,

mediating the interaction with the sugar-phosphate backbone of the viral DNA. Interestingly, pVII shows strong homology to the N-terminus of human histone 3. During the importin-dependent import, pVII stays associated with the viral DNA. Later in infection, pVII dissociates from the Ad genome and initiates viral DNA synthesis. Similar to pVI, pVII undergoes proteolytic cleavage, resulting in an N-terminally cleaved 150 amino acid protein (Lee *et al.*, 2003). Our group recently reported that SPOC1, an important cellular component of the DNA damage response (DDR) and chromatin condensation, interacts with pVII at the viral genome and negatively regulates HAd5 productive infection (Kinkley *et al.*, 2009; Mund *et al.*, 2012; Schreiner *et al.*, 2013b). Taken together, these observations suggest the model of nucleosome-like structures proposed for Ad core and encapsidated DNA.

E1A is the first protein expressed during HAd5 infection and plays an important role in cell cycle progression as well as in transcriptional activation (Flint and Shenk, 1989), thereby establishing optimal conditions for efficient progeny virus production. In primary rodent cells, E1A was shown to induce immortalization by the modulation of key regulator functions involved in cell cycle progression and programmed cell death (Gallimore *et al.*, 1984a; Gallimore *et al.*, 1984b). Alternative splicing of mRNA transcripts of the E1A gene result in two major E1A proteins: E1A-12S and E1A-13S (Chow *et al.*, 1979; Perricaudet *et al.*, 1979). E1A-13S is suggested to be responsible for transactivating viral gene expression by the interaction with a variety of transcription factors (Hiebert *et al.*, 1991; Kovesdi *et al.*, 1986; Liu and Green, 1990; Nevins, 1990; Stevens *et al.*, 2002). In this context, E1A was found to interact with the Retinoblastoma Tumor Protein (pRB), mediating its dissociation from E2F transcription factors. Thereby, E2F responsive genes are activated and cell cycle progression is induced (Buchkovich *et al.*, 1990; Cress and Nevins, 1996; Dyson *et al.*, 1992; Giordano *et al.*, 1991). Furthermore, E1A-13S was shown to interact with the cellular transcription factor p300/CBP, thereby recruiting the cellular factor to HAd promoters during infection (Pelka *et al.*, 2009). Recently, our group showed that HAd5 E1A-13S enhances p300 transcriptional activity, resulting in a positive stimulation of HAd5 promoters. Thereby, cooperation with PML-II increases E1A-mediated transcriptional activation (Berscheminski *et al.*, 2013).

Hateboerg and co-workers also revealed that E1A co-operates with the cellular murine SUMO E2 enzyme mUbc9 (Hateboer *et al.*, 1996). So far, no further work was conducted clarrifiying the role and relevance of this interaction and PML-NB association.

E1B-55K represents another HAd5 protein, which was recently identified to interact with the cellular SUMO E2 enzyme Ubc9 (Wimmer *et al.*, 2013). E1B-55K is a 496 amino acid phosphoprotein with a molecular weight of 55 kDa. E1B-55K can shuttle between cytoplasm and nucleus, due to its Nuclear Export Signal (NES) and a SUMO Conjugation Motif (SCM) at lysine 104. Thereby, E1B-55K itself is a substrate for the host-cellular SUMO modification system (Endter *et al.*, 2005; Endter *et al.*, 2001; Kindsmuller *et al.*, 2007). E1B-55K contributes to complete cell transformation of primary rodent cells and is known to antagonize apoptosis and growth arrest (Debbas and White, 1993). In this context, the E1B-55K SCM was shown to be responsible for the transforming potential in rodents in combination with E1A (Endter *et al.*, 2001). E1B-55K plays an important role by inhibiting p53-activated genes (Kao *et al.*, 1990; Sarnow *et al.*, 1982; Shen *et al.*, 2001; Yew and Berk, 1992; Yew *et al.*, 1990). In the early phase of infection, E1B-55K interacts with p53, resulting in nuclear to cytoplasmic relocalization in order to block p53 transcription-activating properties (Endter *et al.*, 2005; Endter *et al.*, 2001; Farmer *et al.*, 1992; Martin and Berk, 1998). Early *in vitro* studies revealed that E1B-55K itself shows SUMO E3 ligase activity (Martin and Berk, 1998). E1B-55K modulates p53 transcription activity by inhibiting its acetylation and simultaneously promoting its SUMO modification (Liu *et al.*, 2000; Müller and Dobner, 2008; Pennella *et al.*, 2010). Thereby, SUMO modification of E1B-55K itself is known to play a role in the functional inactivation of the cellular tumor suppressor (Endter *et al.*, 2005; Endter *et al.*, 2001). For a long time it has been assumed that there might be an interaction with a cellular co-repressor involved in order to block p53 function (Martin and Berk, 1999). In this context, our group recently showed that E1B-55K-mediated SUMOylation of p53 requires its localization at PML-NBs. Thereby, PML isoforms IV and V were identified as a prerequisite for HAd-mediated oncogenic transformation of primary rodent cells (Wimmer *et al.*, 2015). Furthermore, E1B-55K harbors a RING finger motif (Härtl *et*

al., 2008), which was shown to play a role in the ubiquitination pathway (Borden and Freemont, 1996; Deshaies and Joazeiro, 2009).

For E1B-55K nuclear localization the presence of E4orf6 was shown to be essential (Dobbelstein *et al.*, 1997; Goodrum *et al.*, 1996; Ornelles and Shenk, 1991). Marshall and co-workers suggested an involvement of the cellular factor Runt Related Transcription Factor (RUNX) in E4orf6-mediated relocalization of E1B-55K (Marshall *et al.*, 2008). E1B-55K and E4orf6 were shown to form a complex with the cellular proteins Elongin B and C, Cullin-5, Rbx/RCO1/Hrt1 to an E3 ubiquitin ligase. Thereby, E4orf6 connects E1B-55K to the ligase complex, whereas E1B-55K is believed to bind and recruit the substrates (Blanchette *et al.*, 2004; Harada *et al.*, 2002; Querido *et al.*, 2001a). In the late phase of infection, this E3 ubiquitin ligase complex leads to the ubiquitin-dependent proteolytic degradation of cellular proteins, mainly involved in the DNA damage response, apoptosis and cell surface remodeling, such as p53, Mre11, DNA ligase IV, BLM, Integrin α3, ATRX, SPOC1 by the 26S proteasome (Baker *et al.*, 2007; Carson *et al.*, 2003; Cheng *et al.*, 2011; Dallaire *et al.*, 2009; Orazio *et al.*, 2011; Querido *et al.*, 2001b; Roth *et al.*, 1998; Schreiner *et al.*, 2013a; Schreiner *et al.*, 2013b; Steegenga *et al.*, 1998; Stracker *et al.*, 2002). In addition, work of our group showed an E4orf6-independent proteasomal degradation of the host-cellular antiviral factor Daxx (Schreiner *et al.*, 2010). Furthermore, it is assumed that this viral E3 ubiquitin ligase complex might target cellular RNA export factors, thereby inhibiting cellular mRNA export (Ornelles and Shenk, 1991).

E2A/DBP is a 529 amino acid nuclear phosphoprotein with 72 kDa in size, showing high affinity for single-stranded DNA and RNA (Klein *et al.*, 1979; Linne *et al.*, 1977). This viral protein is involved in various steps during HAd infection, such as DNA replication, early and late gene expression as well as virion assembly (Cleghon and Klessig, 1986; Van der Vliet, 1995). Recently, Ahi and co-workers showed that E2A/DBP interacts with the adenoviral protein IVa2 (Ahi *et al.*, 2013).

In the course of infection, the replication centers emerge with regions of actively replicating genomes associated with E2A/DBP. The outer rim of these replication centers have been shown to be sites of viral transcription (Aspegren *et al.*, 1998;

Pombo *et al.*, 1994). In this context, E2A was shown to be required for the strand elongation reaction of viral DNA synthesis (Challberg *et al.*, 1982; Friefeld *et al.*, 1983). Recently, our group showed that *de novo*-synthesized viral RNA aggregates at the outer rims of Ad replication centers adjacent to PML tracks containing the activating factor Sp100A (Berscheminski *et al.*, 2014).

Taken together, these observations suggest that for efficient replication HAds express proteins that have evolved multiple mechanisms to interfere with the host-cellular antiviral defense, such as modulation of DDR components.

2.2 The cellular DNA damage response

2.2.1 Cellular DNA damage pathways

Cell survival requires mechanisms to recognize and repair DNA damage. The cellular DNA damage response (DDR) includes the detection of DNA damage, the initiation of cell cycle arrest and finally the repair of the lesion. In cases of severe DNA damage, apoptosis can be initiated (Olive, 1998). The mechanisms involved in DDR have been intensively studied. There exist two major pathways for DNA repair: Homologous Recombination (HR) and Non-Homologous End Joining (NHEJ).

HR occurs at homologous sequences mainly in S- and G2-phase. Autophosphorylation of ATM at Ser1981 leads to dimer dissociation, thereby releasing active ATM monomers, which phosphorylate downstream effector molecules such as the H2A variant H2AX (Redon *et al.*, 2010; Rogakou *et al.*, 1998; Thiriet and Hayes, 2005). Thereby, activation of ATM is one of the earliest characterized events in response to DSBs. γH2AX leads to the accumulation of the Mre11/Rad50/NBS1 (MRN) complex at the site of DNA damage (Lee and Paull, 2007). Additionally, more ATM is recruited to the damaged area and activated through phosphorylation, resulting in a continuous increase of γH2AX and the activation of CHK2 (Derheimer and Kastan, 2010). ATR plays a role in the response to ssDNA and is recruited to the site of DNA damage and activated by a number of

proteins, such as TOPBP1, promoting cell cycle regulation through CHK1 activation (Cimprich and Cortez, 2008).

In contrast to ATM and ATR, DNA-PK regulates the process of NHEJ, which dominates in G1 and G2 phase (Metzger and Iliakis, 1991). DNA-PK consists of a large catalytic subunit (cs) and two regulatory subunits Ku70 and Ku86. These subunits mediate the interaction of DNA-PK with DNA DSBs, resulting in the recruitment of DNA ligase IV to rejoin the broken ends of the DNA (Burma and Chen, 2004).

2.2.2 Adenoviruses interfere with the cellular DNA damage response

Early in infection, the host cell activates antiviral measures and responds to the virus. This might be due to the sensing of free viral linear DNA in the cell (Weitzman and Ornelles, 2005). Furthermore, HAd5 replication is facilitated by the viral DNA polymerase, which is thought to additionally trigger DDR pathways (Challberg and Kelly, 1989; Van der Vliet, 1995). Transcription-mediated remodeling of the HAd genome induces ATM phosphorylation/activation, suggesting that the condensed chromatin structure of the incoming viral genome may prevent DDR activation (Chen *et al.*, 2007; Karen and Hearing, 2002). Prakash and co-workers suggested that some aspects of the cellular DDR are stimulated by the incoming viral genome, but other DDR pathways are activated by the process of viral DNA replication (Prakash *et al.*, 2012). However, it was shown that activation of cellular DDR, involving ATM-, ATR- and DNA-PK-dependent pathways, is counteracted by early HAd transcripts (Blackford *et al.*, 2008; Carson *et al.*, 2003; Stracker *et al.*, 2002; Weiden and Ginsberg, 1994; Figure 4). To inactivate HR and NHEJ pathways, cellular proteins involved in the DDR are degraded upon HAd5 infection, including p53, Mre11, DNA ligase IV, BLM, Tip60 and SPOC1 (Blackford *et al.*, 2010; Carson *et al.*, 2003; Cheng *et al.*, 2011; Forrester *et al.*, 2011b; Gupta *et al.*, 2013; Orazio *et al.*, 2011; Querido *et al.*, 2001a; Schreiner *et al.*, 2013b; Stracker *et al.*, 2002). The MRN complex was found to be important for ATM activation and phosphorylation of a number of proteins involved in DNA repair and checkpoint signaling (Lee and Paull, 2007). ATM autophosphorylation and downstream signaling was shown to be impaired upon

HAd5 infection due to the degradation of MRN complex components (Carson *et al.*, 2003). This degradation is facilitated by the formation of the E1B-55K/E4orf6-dependent E3 ubiquitin ligase complex, subsequent ubiquitination of the substrate, followed by proteasomal degradation via the 26S proteasome (Blanchette *et al.*, 2004; Luo *et al.*, 2007). In cells infected with group A adenoviruses, TOPBP1 was additionally shown to be targeted for proteasomal degradation by the viral E4orf6 protein (Blackford *et al.*, 2010). Furthermore, HAd5 E4orf6 has been found to inhibit protein phosphatase PP2A, resulting in prolonged H2AX phosphorylation (Hart *et al.*, 2007). In addition, HAd5 E4orf3 and E4orf6 were shown to bind directly to DNA-PK, leading to reduced autophosphorylation during DSB repair (Boyer *et al.*, 1999; Hart *et al.*, 2005). In this context, HAd5 mutants lacking the E4 transcription unit were shown to heavily activate the cellular DDR in infected cells (Carson *et al.*, 2003).

Besides the degradation of cellular proteins involved in the DDR, HAds have been found to alter the localization of the cellular factors, such as RPA32, TOPBP1, Rad9 or Sp100 (Berscheminski *et al.*, 2014; Blackford *et al.*, 2008; Carson *et al.*, 2009; Carson *et al.*, 2003). Upon HAd infection PML-II is targeted by E4orf3 resulting in the reorganization of PML-NBs in track-like structures releasing repressive factors into viral replication centers (VRCs), suggesting the inhibition of their functions, whereas positive factors stay associated with the PML-tracks adjacent to VRCs. Interestingly, components of the ATR pathway, namely BLM and the MRN complex, have been shown to be recruited to VRCs in order to promote replication (Blackford *et al.*, 2008; Polo *et al.*, 2012). In addition, several DDR proteins have been found to localize to PML-NBs upon HAd5 infection (Lombard and Guarente, 2000). This suggests host protein specific processes mediated by HAd determinants to counteract antiviral host-cellular response and simultaneously exploiting the cellular transcription machinery. In this context, our group recently showed that Sp100A activates E1A-dependent transcription, whereas the other Sp100 isoforms Sp100B, Sp100C and Sp100HMG showed no effect on early HAd transcriptional activation. Consistent with this, Sp100B, Sp100C and Sp100HMG isoforms were recruited into VRCs (Berscheminski *et al.*, 2014). Analogous localization during HAd infection was also shown for the viral restriction factor SPOC1. As mentioned above, SPOC1 co-

operates with the viral genome via interaction with the adenoviral DNA-associated core protein pVII, resulting in transcriptional repression. Later during infection, SPOC1 is proteasomally degraded as a novel target of the E1B-55K/E4orf6-dependent E3 ubiquitin ligase complex (Schreiner *et al.*, 2013b). SPOC1 is dynamically regulated during cell cycle and important for mitotic chromatin condensation (Kinkley *et al.*, 2009). Interestingly, SPOC1 was reported to orchestrate essential functions in a chromatin remodeling complex with the cellular co-repressor KAP1, playing an important role in DDR upon DNA DSBs (Mund *et al.*, 2012). Thereby, SPOC1 is recruited in an ATM dependent manner, localizing to endogenous repair foci characteristic for delayed repair at heterochromatic sites. In this context, SPOC1 was shown to mediate dose-dependent changes in chromatin association of DNA compaction factors, thereby acting as a modulator of repair kinetics and choice of DDR pathways in combination with KAP1.

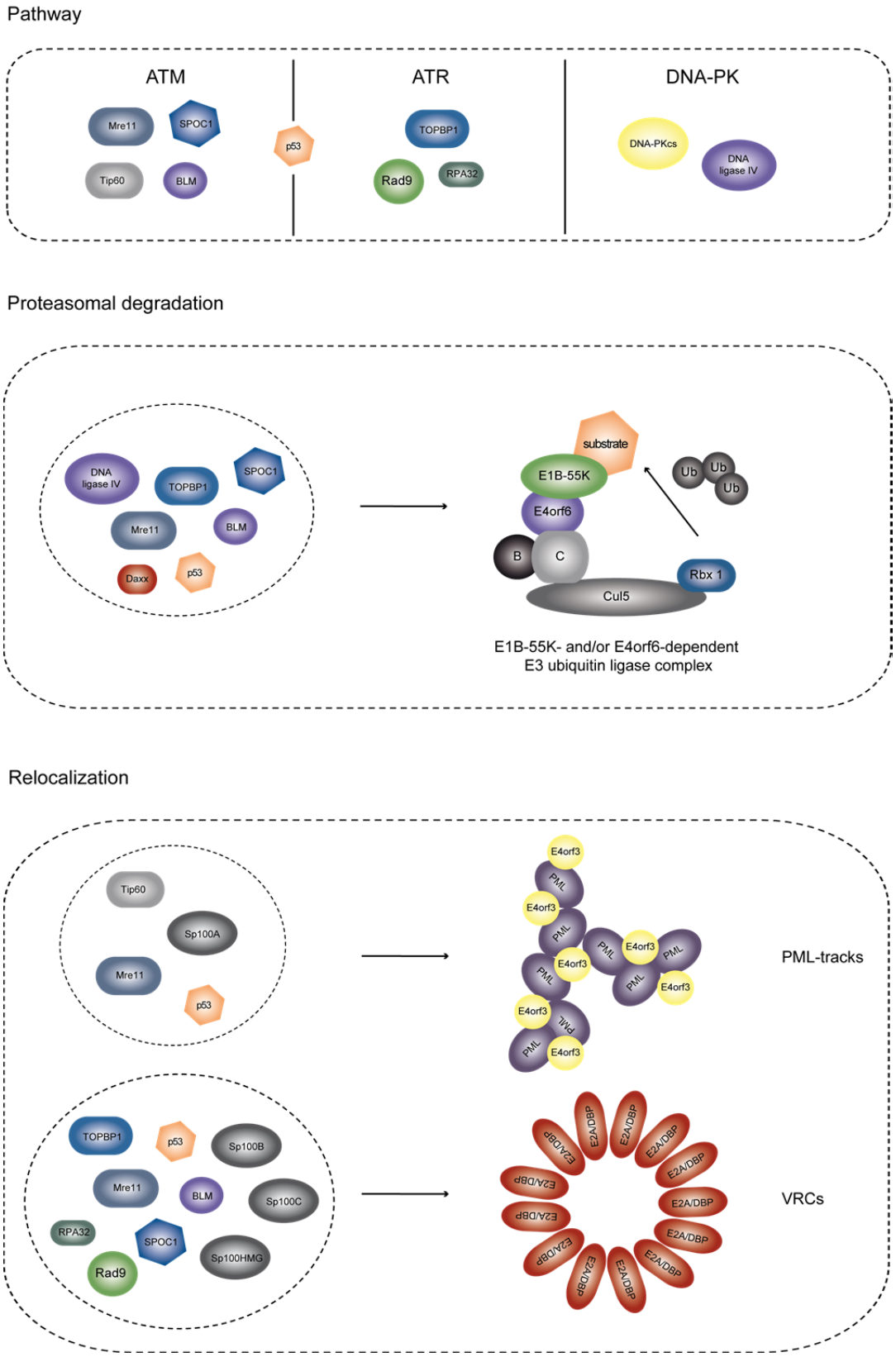


Figure 4: Adenoviruses interfere with the cellular DNA damage response. HAds interfere with ATM, ATR and DNA-PK pathways by proteasomal degradation or relocalization of their components. HAd5 E1B-55K/E4of6 complexes recruit cellular ubiquitin ligases to facilitate the degradation of p53, BLM, Mre11, DNA ligase IV and SPOC1, whereas HAd5 E1B-55K mediates degradation of Daxx and Ad12 E4orf6 facilitates TOPBP1 degradation. Components of the ATM/ATR and DNA-PK pathways are recruited to PML-tracks or VRCs in order to promote replication. Further details see chapter 2.2.2.

2.3 Cellular co-repressor KRAB domain-associated Protein 1 (KAP1)

2.3.1 Domain structure of KAP1

Several additional names exist for the KRAB domain-associated protein 1 (KAP1) in reference to its domain structure and functions. As Transcriptional Intermediary Factor 1 beta (TIF1β), KAP1 belongs to the TIF1 family, which also includes TIF1α, TIF1β, TIF1γ, and TIF1δ. KAP1 harbors an N-terminal tripartite motif, giving it the additional name Tripartite motif-containing 28 (TRIM28). The TRIM domain, also known as RBCC domain, contains a RING finger, two B-box zinc fingers and a coiled-coil region. Similar to the other members of the TIF1 family, KAP1 harbors a TIF1 signature sequence (TSS), an HP1 binding domain (HP1BD) as well as a C-terminal plant homeodomain (PHD) and a bromodomain (Figure 5). However, in contrast to the other TIF1 proteins, KAP1 does not contain a nuclear receptor (NR) box (Iyengar *et al.*, 2011).

Figure 5: Domain structure of KAP1 protein. N-terminal RBCC domain, including a RING finger, two B-box domains (B1, B2) and a coiled-coil region, a central TIF1 signature sequence (TSS) and an HP1 binding domain (HP1 Box), as well as a C-terminal plant homeodomain (PHD) and a bromodomain (Bromo). Numbers indicate the amino acid positions in the KAP1 protein.

2.3.2 Functions of KAP1

Over the past 19 years, KAP1 function has been extensively studied and could be shown to play a role in several cellular pathways, including cell differentiation, tumorigenesis, immune response, DNA damage response (DDR), and virus replication. Additionally, KAP1 is important for embryonic development, for maintaining pluripotency of embryonic stem cells (ESCs) and their differentiation (Cammas *et al.*, 2004; Cammas *et al.*, 2007; Cammas *et al.*, 2002; Herzog *et al.*, 2011). Furthermore, KAP1 plays a role in spermatogenesis, erythropoisis and the development of T- and B-cells (Barde *et al.*, 2013; Maruyama *et al.*, 2011; Santoni de Sio *et al.*, 2012a; Santoni de Sio *et al.*, 2012b; Weber *et al.*, 2002).

KAP1 was shown to inhibit apoptosis, since it represses its responsive genes, such as *p21, gadd45a, bax, puma* and *noxa*, which encode gene products causing cell cycle arrest and apoptosis (Lee *et al.*, 2007; Li *et al.*, 2007). Via its N-terminal RBCC domain KAP1 was shown to interact with several Krüppel-associated box zinc finger proteins (KRAB-ZFPs) via their KRAB repression domains (Friedman *et al.*, 1996; Kim *et al.*, 1996; Moosmann *et al.*, 1996; Peng *et al.*, 2007; Urrutia, 2003). Besides its interaction with KRAB-ZFPs, KAP1 is able to co-operate and excert its co-repressor functions with the transcription factor Pax3, which is associated with pediatric alveolar rhabdomyosarcoma (Hsieh *et al.*, 2006). Additionally, Mund and co-workers showed that KAP1 interacts with Survival time-associated PHD finger protein in Ovarian Cancer 1 (SPOC1; Mund *et al.*, 2012). SPOC1 is a recently identified modulator of chromatin structure and is involved in the DDR and DNA repair by changing the chromatin association of the DNA compaction factors KAP1, the Heterochromatin-associated Protein 1 (HP1) and Lysine Methyltransferases (KMTs; Figure 6). By recognition and binding of the active histone mark H3K4me3 through SPOC1, KAP1 is recruited to the DNA. Additionally, the central part of KAP1 contains a hydrophobic PxVxL pentapeptide region, mediating the interaction with HP1, critical for KAP1-mediated gene silencing (Lechner *et al.*, 2000; Nielsen *et al.*, 1999; Ryan *et al.*, 1999; Sripathy *et al.*, 2006). HP1 was shown to be required for the recruitment of KAP1 to DNA damage sites (Baldeyron *et al.*, 2011; White *et al.*, 2012). The C-terminal PB domain is thought to recognize histone tails and to recruit components of the Nucleosome Remodeling Deacetylase (NuRD) / Histone Deacetylase (HDAC) complex as well as the Histone Methyltransferase (HMT) SET Domain, Bifurcated 1 (SETDB1), leading to histone methylation, deacetylation and heterochromatin formation (Iyengar and Farnham, 2011; Ryan *et al.*, 1999; Schultz *et al.*, 2002; Schultz *et al.*, 2001; Underhill *et al.*, 2000; Zuo *et al.*, 2012). Thereby, SPOC1 inhibits KAP1 phosphorylation and induces H3K9 trimethylation, resulting in chromatin compaction and transcriptional repression.

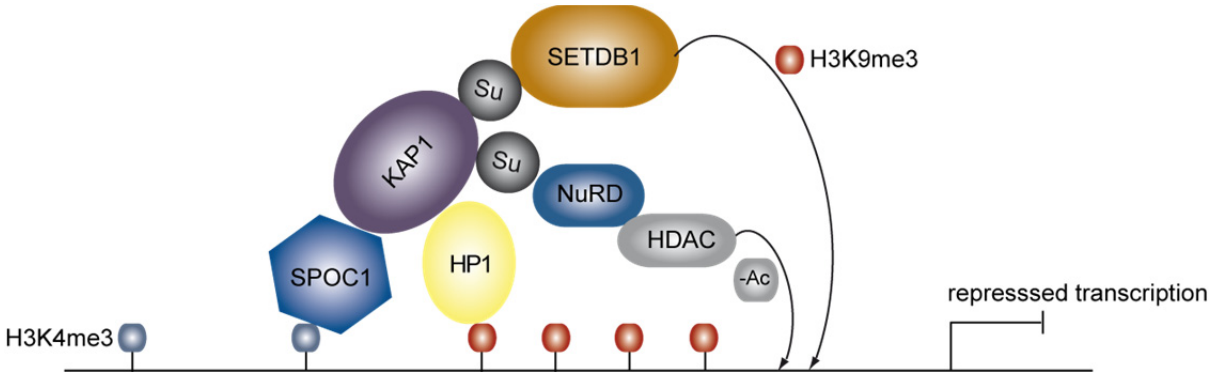


Figure 6: Schematic representation of the SPOC1/KAP1-mediated transcriptional repression. H3K4me3-mediated interaction of SPOC1 with the chromatin results in the recruitment of KAP1 and its associated enzymes (SETDB1, NuRD complex). HDAC and HMT activity results in an increase of trimethylated H3K9, thereby generating new HP1 binding sites, followed by transcriptional repression.

Given these interaction partners, KAP1 is thought to epigenetically regulate gene expression through multiple transcriptional co-repressor complexes. Thereby, KAP1 plays an important role in maintaining genome stability by facilitating DNA repair in response to DNA damage through chromatin remodeling (Liu *et al.*, 2013; Ziv *et al.*, 2006). Interestingly, the role of KAP1 in DNA DSB repair was found to be cell cycle-dependent (Beucher *et al.*, 2009; Goodarzi *et al.*, 2008; Goodarzi *et al.*, 2009; Noon *et al.*, 2010; Shibata *et al.*, 2011).

Recent studies indicate that KAP1 PB domain possesses enzymatic activity. Via its PHD domain, KAP1 possesses a SUMO E3 ligase activity to recruit the SUMO-conjugating enzyme Ubc9. Thereby, KAP1 is able to auto-SUMOylate its own bromodomain to generate a repressive form of KAP1 (Ivanov *et al.*, 2007). Besides its role in transcriptional regulation and DDR, the novel finding that KAP1 is a SUMO E3 ligase itself is intruiging and reveals additional functions and mechanisms, by which KAP1 influences gene expression and/or other cellular processes.

2.3.3 Posttranslational modifications of KAP1

The gene repressive function of KAP1 can be modulated by its posttranslational modifications (PTMs), mainly phophorylation and SUMOylation (Ivanov *et al.*, 2007; Lee *et al.*, 2007; Li *et al.*, 2007; Mascle *et al.*, 2007). KAP1 harbors several residues known to be targets for PTMs (Figure 7). Increasing evidence suggests a tight crosstalk between phosphorylation and SUMOylation in regulating KAP1 function.

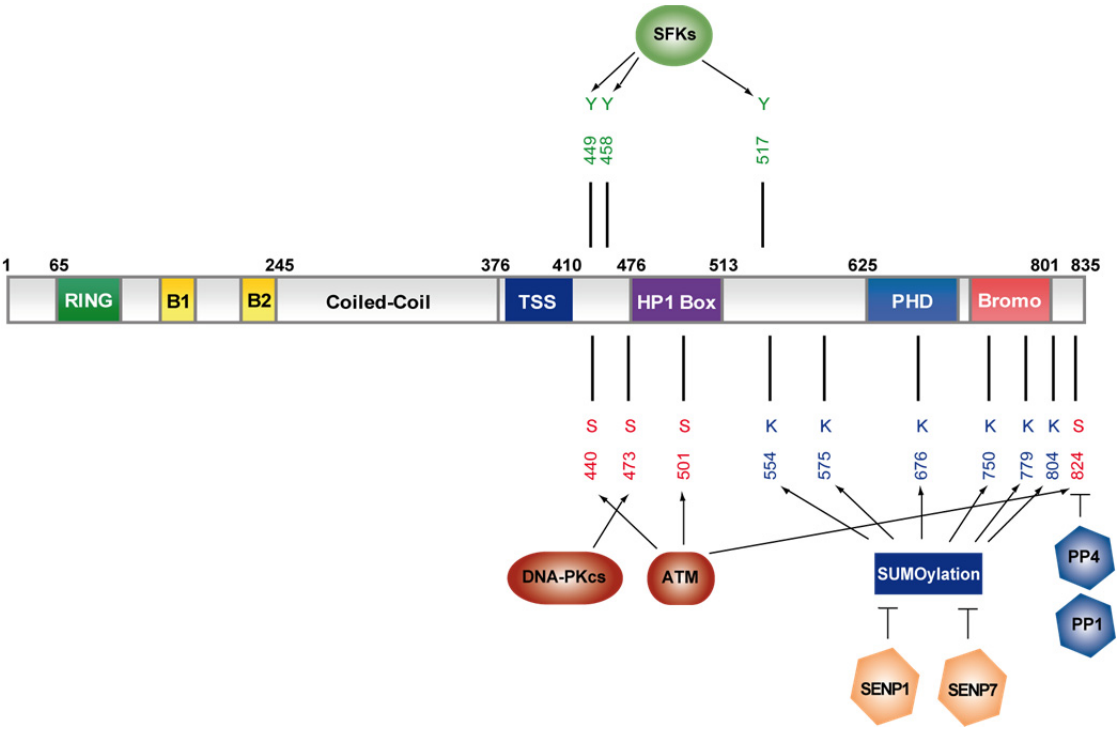


Figure 7: KAP1 posttranslational modifications. Blue: SUMOylation sites (deSUMOylases indicated in light red); Red: Serine phosphorylation sites (responsible kinase indicated in red) or antagonized by phosphatases (indicated in light blue); green: tyrosine phosphorylation sites (responsible kinase indicated in green). Numbers represent the sequence of amino acids. SFKs: Srk family kinases; ATM: Ataxia-Telangiectasia Mutated; PP1/4; Protein Phosphatase 1/4; DNA-PKcs: DNA-dependent Protein Kinase catalytic subunit, SENP1/7: SUMO/Sentrin/Smt3-specific Peptidase 1/7.

2.3.3.1 KAP1 phosphorylation

Ser473 and Ser824 are so far the most conducted phosphorylation sites of the cellular KAP1 protein. Recently, phosphorylation of KAP1 Ser473 (pKAP1S473) by ATM and ATR was shown to be a novel marker for DNA damage (Bolderson *et al.*, 2012). Protein phosphatase 4 is known to dephosphorylate pKAP1S473, impacting the DDR (Lee *et al.*, 2012; Liu *et al.*, 2012). pKAP1S473 was shown to be involved in efficient DNA repair and cell survival upon DNA damage (Bolderson *et al.*, 2012; Hu *et al.*, 2012; White *et al.*, 2012). Chang *et al.* observed that phosphorylation of KAP1S473 regulates the binding to HP1 and thereby co-repressor function of KAP1 (Chang *et al.*, 2008).

More studies dealing with KAP1 phosphorylation were conducted on phosphorylation of KAP1 Ser824 (pKAP1S824). After DNA DSBs occur, KAP1S824 is mainly phosphorylated by ATM and DNA-PKcs (White *et al.*, 2006; Ziv *et al.*, 1997), resulting in co-localization with numerous DDR factors at DNA lesions (Tomimatsu

et al., 2009; Yajima *et al.*, 2009). pKAP1S824 is known to be crucial for DDR, since it is responsible for activating DNA damage checkpoints and chromatin relaxation (Li *et al.*, 2007; Ziv *et al.*, 2006). pKAP1S824 was shown to be a prerequisite for HR repair (Geuting *et al.*, 2013) and to promote NHEJ repair (Liu *et al.*, 2012). ATM-mediated phosphorylation of KAP1S824 results in de-repression of KAP1-responsive genes involved in cell cycle arrest and apoptosis (Lee and Paull, 2007; Li *et al.*, 2007). Protein phosphatase 1 and 4 (PP1 and PP4) were found to interact with and dephosphorylate KAP1S824 (Lee *et al.*, 2012; Li *et al.*, 2010; Liu *et al.*, 2012).

Recently, Kubota and co-workers identified three other phosphorylation sites in KAP1, Tyr449, Tyr458 and Tyr517 (Kubota *et al.*, 2013), showing that phosphorylation of these sites by Src Family Kinases (SFKs) impaired HP1 binding. However, so far no further studies were conducted on possible functional consequences arising through KAP1 tyrosine phosphorylation.

2.3.3.2 KAP1 SUMOylation

The small ubiquitin-like modifier (SUMO) comprises four different isoforms, SUMO1 to SUMO4. Due to sequence and structure similarities they are grouped into the family of ubiquitin-like proteins. SUMO2 and SUMO3 show 95% sequence identity, whereas with SUMO1 they only share 50% sequence homology (Saitoh and Hinchey, 2000). Due to an internal SUMO consesus motif, SUMO2 and SUMO3 are able to form polymeric chains (Tatham *et al.*, 2001). The SUMO conjugation pathway is mechanistically identical to the ubiquitination pathway. Upon the exposure of a C-terminal di-Gly (double Glycine) motif in the SUMO protein, the activating enzyme E1 transfers SUMO to the SUMO conjugating E2 enzyme Ubc9 (Desterro *et al.*, 1999; Johnson and Blobel, 1997; Johnson *et al.*, 1997; Okuma *et al.*, 1999; Ulrich, 2009). Subsequently, Ubc9 catalyzes the covalent attachment of SUMO to a lysine within the SUMO consensus motif of the target protein (Rodriguez *et al.*, 2001).

SUMO modification of substrates is known to extensively modulate protein function. So far, SUMOylation was shown to affect nucleocytoplasmic transport, DNA repair, transcription and apoptosis (Geiss-Friedlander and Melchior, 2007; Hay, 2006;

Kerscher *et al.*, 2006; Ulrich, 2009; Verger *et al.*, 2003). As a SUMO E3 ligase, KAP1 PHD domain is known to target and auto-SUMOylate its adjacent bromodomain, thereby altering KAP1 function (Ivanov *et al.*, 2007). Recent studies showed that SUMOylation of KAP1 is a prerequisite for its repressive function. Lys554, 575, 676, 779 and 804 could be validated as putative SUMOylation sites, thereby affecting the interaction between KAP1 bromodomain and the repressive components SETDB1 and CHD3 (Lee and Paull, 2007; Mascle *et al.*, 2007; Peng *et al.*, 2007).

SUMO modification of KAP1 is balanced by the deSUMOylases, sentrin specific peptidase 1 and 7 (SENP1 and SENP7) and phosphorylation at Ser824 (Garvin *et al.*, 2013; Ivanov *et al.*, 2007; Li *et al.*, 2010). In this context, ATM-mediated KAP1S824 phosphorylation was shown to perturb SUMO-dependent interaction of KAP1 and CHD3, thereby reducing H3K9di- and trimethylation, resulting in decondensation of heterochromatin and activating transcription of KAP1-responsive pro-arrest and pro-apoptotic genes (Garvin *et al.*, 2013; Lee *et al.*, 2007; Li *et al.*, 2007).

2.3.3.3 KAP1 acetylation

So far, only little is known about KAP1 acetylation. Lai and co-workers showed that KAP1 is acetylated and that the level of KAP1 acetlyation is downregulated by HDAC10, suggesting a regulation of KAP1 transcriptional co-repressor activity (Lai *et al.*, 2010). However, further investigation is required to reveal the exact mechanism by which this possible change in function is achieved.

2.3.4 KAP1 in the context of viruses

Since KAP1 was shown to play a role in various cellular processes, including cell differentiation, tumorigenesis, immune response and DNA damage response (DDR), it is not surprising that several human viruses interfere with or take advantage of this cellular factor.

KAP1 was originally identified as a silencer of Moloney Murine Leukemia Virus (MLV; Wolf and Goff, 2007). In the case of MLV but also other retroviruses like the Human T-cell Lymphotropic Virus-1 (HTLV-1), KAP1 restricts pro-viral gene

activation (Wolf *et al.*, 2008; Wolf and Goff, 2009). Additionally, in embryonic stem cells (ESCs) retroelements from Endogenous Retroviruses (ERVs) were also found to be silenced by KAP1 in order to protect genome integrity (Matsui *et al.*, 2010; Rowe *et al.*, 2010; Schlesinger and Goff, 2013; Tan *et al.*, 2013). Furthermore, Allouch *et al.* revealed that KAP1 plays a role in the inhibition of Human Immunodeficiency Virus 1 (HIV-1) infection. Thereby, KAP1 complex formation with HDAC1 is leading to the deacetylation and inhibition of HIV-1 integrase activity, resulting in reduced infectivity and integration in the host genome (Allouch *et al.*, 2011).

Karposi's Sarcoma-Associated Herpesvirus (KSHV) was shown to exploit KAP1 chromatin remodeling function. Phosphorylation of KAP1 at Ser 824 by the viral protein kinase activates lytic genes to support KSHV lytic replication (Chang *et al.*, 2009). Furthermore, KSHV infection-induced phosphorylation of KAP1 Ser 473 was observed in endothelial cells (Chang *et al.*, 2008). Since KAP1 represses the proto-oncogene STAT3, their data on KAP1 phosphorylation suggest a contribution to the chronic inflammatory environment, which is a hallmark of Karposi's Sarcoma (KS) (King, 2013). Recent work by Sun *et al.* revealed that KSHV latency-associated nuclear antigen (LANA) interacts with KAP1, thereby repressing lytic gene expression during the early stage of KSHV primary infection to facilitate the establishment of KSHV latency (Sun *et al.*, 2014).

So far, not much is known about the role of KAP1 during adenovirus infection. However, since HAd5 was shown to take advantage of the cellular SUMOylation system, a connection between the cellular E3 SUMO ligase KAP1 and productive HAd5 infection could be suggested. This study aims to investigate the involvement of KAP1 in HAd5 infection and the alteration of posttranslational modifications on virus and host cell factors. This work should provide a better understanding of the mechanisms underlying the HAd-mediated alteration of chromatin structure and the cellular SUMOylation pathway.

3 Material

3.1 Cells

3.1.1 Bacteria

STRAIN	CHARACTERISTICS
DH5α	supE44, ΔlacU169, (ϕ80d/lacZΔM15), hsdR17, recA1, endA1, gyrA96, thi-1, relA1 (Hanahan and Meselson, 1983).

3.1.2 Cell lines

CELL LINE	CHARACTERISTICS
A549	Human lung carcinoma cell line expressing wild-type p53 (Giard *et al.*, 1973).
A549 shscrambled	A549 cell line with shRNA scrambled (this work).
A549 shKAP1	A549 cell line with shRNA against KAP1; shRNA 5‘-CCTGGCTCTGTTCTCTGTCCT-3‘ (this work).
H1299	Human lung carcinoma cell line, p53 negative (Mitsudomi *et al.*, 1992).
H1299 shscrambled	H1299 cell line with shRNA scrambled (this work).
H1299 shKAP1	H1299 cell line with shRNA against KAP1; shRNA 5‘-CCTGGCTCTGTTCTCTGTCCT-3‘ (this work).
H1299 shPML	H1299 cell line with shRNA against PML; shRNA 5‘-GGAGTTGGATCTCTCAGAA-3‘ (group database).
H1299 shPML/shscrambled	H1299 shPML cell line with shRNAscrambled (this work)
H1299 shPML/shKAP1	H1299 shPML cell line with shRNA against KAP1; shRNA 5‘-CCTGGCTCTGTTCTCTGTCCT-3‘ (this work)
HEK-293	Established HAd5-transformed, human embryonic

	kidney cell line stably expressing the adenoviral E1A and E1B gene products (Graham *et al.*, 1977).
HEK-293T	HEK-293 derived cell line expressing the SV40 large tag (DuBridge *et al.*, 1987).
HeLa	Human cervix carcinoma cell line (Grey *et al.*, 1952).
HeLa-Su1	HeLa cell line stably expressing His-SUMO1 (Tatham *et al.*, 2009).
HeLa-Su2	HeLa cell line stably expressing His-SUMO2 (Tatham *et al.*, 2009).

3.2 Adenoviruses

ADENOVIRUS	CHARACTERISTICS
H5*pg*4100	Wild-type HAd5 carrying a 1863 bp deletion (nt 28602-30465) in the E3 reading frame (Kindsmuller *et al.*, 2007).
H5*pm*4149	HAd5 E1B-55K null mutant carrying four stop codons at aa positions 3, 8, 86 and 88 of the E1B-55K sequence (Kindsmuller *et al.*, 2009).
H5*pm*4102	HAd5 E1B-55K mutant carrying one aa exchange (K104R) within the SCM of the E1B-55K sequence (Kindsmuller *et al.*, 2007).
H5*pm*4154	HAd5 E4orf6 null mutant carrying a stop codon at aa 66 within the E4orf6 sequence (Blanchette *et al.*, 2004).

3.3 Nucleic Acids

3.3.1 Oligonucleotides

The following oligonucleotides were used as primers for sequencing, PCR amplification and site-directed mutagenesis. All oligonucleotides were ordered from Metabion and numbered according to the internal *Filemaker Pro* database.

#	NAME	SEQUENCE	PURPOSE

635	pcDNA3 fwd	5‘-ATGTCGTAACAACTCCGC-3‘	sequencing
636	pcDNA3 rev	5‘-GGCACCTTCCAGGGTCAA G-3‘	sequencing
781	Seq E1-Box fwd 1582 bp	5‘-GATTGCGTGTGTGGTTAACGC-3‘	sequencing
782	Seq E1-Box fwd 2454 bp	5‘-CAAGGATAATTGCGCTAATGAGC-3‘	sequencing
1318	Seq E1B bp978-999fwd	5‘-GGCCTCCGACTGTGGTTGCTT-3‘	sequencing
2542	KAP-1 phospho S824 fw	5‘-CTGGCCTGAGTGCCCAGGAGCTG-3‘	mutagenesis
2543	KAP-1 phospho S824 rev	5‘-CAGCTCCTGGGCACTCAGGCCAG-3‘	mutagenesis
2544	pKAP-1pS824 mutant seq	5‘-GACTCCACCTTCTCCCTGG-3‘	sequencing
2545	KAP1 nt1152 seq	5‘-GATTGTGGATCCCGTGGAG-3‘	sequencing
2548	sKAP1K554A fwd	5‘-GTCTCCTCCTCCGCGACAATGG-3‘	mutagenesis
2549	sKAP1K554A rev	5‘-CCATTGTCGCGGAGGAGGAGAC-3‘	mutagenesis
2550	sKAP1K779A fwd	5‘-TGCACGTCTGCCGCGTCCTCAG-3‘	mutagenesis
2551	sKAP1K779A rev	5‘-CTGAGGACGCGGCAGACGTGCA-3‘	mutagenesis
2552	sKAP1K804A fwd	5‘-ACAGCAGAGAACGCGGTGTCACC-3‘	mutagenesis
2553	sKAP1K804A rev	5‘-GGTGACACCGCGTTCTCTGCTGT-3‘	mutagenesis
2643	KAP1 nt 317 seq	5‘-CAACAGCTCGGGGGACGG-3‘	sequencing
2644	KAP1 nt 317 rev seq	5‘-CCGTCCCCCGAGCTGTTG-3‘	sequencing
2645	KAP1 K676A fwd	5‘-CATCCTCCTCCGCCAGGTCAGG-3‘	mutagenesis
2646	KAP1 K676A rev	5‘-CCTGACCTGGCGGAGGAGGATG-3‘	mutagenesis
2651	KAP1 nt 471 seq rev	5‘-CTCACAGCTAGTGCAGCACTGG-3‘	sequencing

3.3.2 Vectors

The following vectors were used for transfection experiments. All vectors are numbered according to the internal *Filemaker Pro* database.

#	NAME	PURPOSE	REFERENCE
36	pcDNA3	Expression vector for mammalian cells, CMV promoter	Invitrogen
138	pGL3	*Firefly*-Luciferase-Assay	Promega
180	pRL-TK	*Renilla*-Luciferase-Assay	Promega
203	pcDNA3-flag	Expression vector for mammalian cells, CMV promoter	B. Schneider

3.3.3 Recombinant Plasmids

The following recombinant plasmids were used for cloning and transfection experiments. All vectors are numbered according tot he internal *Filemaker Pro* database.

#	NAME	Properties	REFERENCE
375	pGL GAL-TK-LUC	expression vector for GAL-fusions; Luciferase assay	(Sadowski and Ptashne, 1989)
1022	E1B-55K-K104R-pcDNA3	HAd5 E1B-55K (SCM) mutant, K104R	group database
1023	E1B-55kDa-(Nes-Mut)-pcDNA3	HAd5 E1B-55K (Nes) mutant, LLL83/87/91AAA	group database
1213	pG4-p300	Human p300 fused to a GAL binding domain	group database
1319	pcDNA3-E1B-55k	HAd5 E1B-55K	group database
1667	72K DBP	HAd2 E2A-72K	group database

1730	pcDNA3 E1B RF6	HAd5 E1B-55K (RF6) mutant, CC454/456SS	group database
1968	pCMV-VSV-G	Envelope protein G of Vesicular Stomatitis Virus	(Beyer *et al.*, 2002)
1969	pRSV Rev	HIV-1 *Rev*	(Dull *et al.*, 1998)
1970	pMDLg/pRRE	HIV-1 Gag Pol	(Dull *et al.*, 1998)
2076	E4orf6-HA	HAd5 HA-E4orf6	group database
2141	E1B-55K R443A	HAd5 E1B-55K (R443A) mutant, R443A	group database
2157	E1B-55K-KK185/187AA	HAd5 E1B-55K (KK) mutant, KK185/187AA	group database
2193	E1B-55K RTR448/449/540AAA	HAd5 E1B-55K (RTR) mutant, RTR448/449/540AAA	group database
2194	E1B-55K E472A	HAd5 E1B-55K (E2) mutant, E472A	group database
2421	pGL3-Basic Prom E1B	HAd5 E1B promoter; Luciferase assay	group database
2422	pGL3-Basic Prom pIX	HAd5 pIX promoter; Luciferase assay	group database
2424	pGL3-Basic Prom MLP	HAd5 major late promoter; luciferase assay	group database
2475	pcDNA3-HA-E1A-13S	HAd5 E1A 13S	group database
2628	pV Ad5	HAd5 HA-pV	group database
2889	Promoter Ad5 E4	HAd5 E4 promoter; luciferase assay	group database
2915	pcDNA3.1-flag-WT-	full length KAP1	(Iyengar *et al.*, 2011)

	KAP1		
2916	pcDNA3.1-flag -M2-KAP1	2aa mutation in HP1-binding domain	(Iyengar *et al.*, 2011)
2917	pcDNA3.1-flag -deltaPB-KAP1	deletion of C-terminal PHD and Bromo domain	(Iyengar *et al.*, 2011)
2918	pcDNA3.1-flag -deltaRBCC-KAP1	deletion of N-terminal RBCC domain	(Iyengar *et al.*, 2011)
2919	pT-Rex-3X-flag-delta(RBCC+PB)-KAP1	Deletion of N-terminal RBCC and C-terminal PHD and Bromo domain	(Iyengar *et al.*, 2011)
2922	pcDNA3.1-flag-KAP1S824A	KAP1 phosphomutant	this work
2926	pcDNA3.1-flag-KAP1-K554A	KAP1 SUMO mutant	this work
2927	pcDNA3.1-flag-KAP1-K676A	KAP1 SUMO mutant	this work
2928	pcDNA3.1-flag-KAP1-K779A	KAP1 SUMO mutant	this work
2929	pcDNA3.1-flag-KAP1-K804A	KAP1 SUMO mutant	this work
2930	pcDNA3.1-flag-KAP1-K554/676A	KAP1 SUMO mutant	this work
2931	pcDNA3.1-flag-KAP1-K779/804A	KAP1 SUMO mutant	this work
2932	pcDNA3.1-flag-KAP1-K554/676/779A	KAP1 SUMO mutant	this work
2933	pcDNA3.1-flag-KAP1-K554/676/779/804A	KAP1 SUMO mutant	this work
-	pBS L30 mRFP1-pVI	HAd5 RFP-pVI	Harald Wodrich

3.4 Antibodies

The following antibodies were used for *Western Blot* or immunofluorescence analysis. All antibodies are numbered according tot he internal *Filemaker Pro* database.

3.4.1 Primary antibodies

NAME	PROPERTIES	REFERENCE
2A6	Monoclonal mouse Ab; against N-terminus of HAd5 E1B-55K	(Sarnow *et al.*, 1982)
3F10	Monoclonal rat Ab; against the HA-tag	Roche
4E8	Monoclonal rat Ab; against the central region of HAd5 E1B-55K	(Kindsmuller *et al.*, 2009)
6B10	Monoclonal rat Ab; against HAd5 L4-100K	Group database
6His	Monoclonal mouse Ab; against 6xHis-tag	Clontech
7C11	Monoclonal rat Ab; against C-terminus of HAd5 E1B-55K	(Kindsmuller *et al.*, 2009)
AC-15	Monoclonal mouse Ab; against β-actin	Sigma Aldrich
B6-8	Monoclonal mouse Ab; against HAd5 E2A	(Reich *et al.*, 1983)
DO-I	Monoclonal mouse Ab; against N-terminus of human p53	Santa Cruz
flag-M2	Monoclonal mouse Ab; against the flag-tag	Sigma Aldrich
M73	Monoclonal mouse Ab; against HAd5 E1A-12S and 13S	(Harlow *et al.*, 1985)
RSA3	Monoclonal mouse Ab; against N-terminus of HAd5 E4orf6 and E4orf6/7	(Marton *et al.*, 1990)
Tif1ß (H-300)	Polyclonal Rabbit Ab; against C-terminus of Tif1ß	Bethyl Laboratories
pKAP1S473	Polyclonal Rabbit Ab; against phosphorylated Ser473 in Tif1ß	Abcam

pKAP1S824	Polyclonal Rabbit Ab; against phosphorylated Ser824 in Tif1ß	Bethyl Laboratories
GH3	Polyclonal rabbit Ab; against Sp100 isoforms	H. Will
PML	Polyclonal rabbit Ab; against PML isoforms	Novus Biologicals
Mre11	Polyclonal rabbit Ab; against human Mre11	Abcam/Novus
6C5	Monoclonal mouse Ab; against GAPDH	Santa Cruz
pVI	Polyclonal rabbit Ab; against HAd5 pVI	(Wodrich *et al.*, 2010)
H3	Monoclonal rabbit Ab; against Histone H3	Epitomics
CR56	Polyclonal rabbit Ab; against SPOC1	(Mund *et al.*, 2012)
Histone H3	Polyclonal rabbit Ab; against H3K9me3	Millipore (Upstate)

3.4.2 Secondary antibodies

NAME	PROPERTIES	REFERENCE
HRP-Anti-Mouse IgG	HRP (*horseradish peroxidase*)-coupled Ab; raised in sheep	Jackson
HRP-Anti-Rat IgG	HRP (*horseradish peroxidase*)-coupled Ab; raised in sheep	Jackson
HRP-Anti-Rabbit IgG	HRP (*horseradish peroxidase*)-coupled Ab; raised in sheep	Jackson
HRP-Anti-Mouse IgG; light chain specific	HRP (*horseradish peroxidase*)-coupled Ab; raised in sheep	Jackson
Cy™3-Anti-Rabbit IgG	Affinity purified, Cy™3-coupled Ab; raised in donkey (H + L)	Dianova
FITC-Anti-Mouse IgG	Affinity purified, fluorescein-isothiocyanat (FITC)-coupled Ab; raised in donkey (H + L)	Dianova

Alexa™ 488 Anti-Mouse IgG	Alexa™ 488 Ab; raised in goat (H + L; $F(ab')_2$ fragment)	Invitrogen

3.5 Standards and markers

Size determination of DNA fragments was performed with a 1 kbp and 100 bp DNA ladder (New England Biolabs). The molecular weight of proteins was determined by *PageRuler™ Prestained Protein Ladder Plus* (Fermentas).

3.6 Commercial Systems

PRODUCT	COMPANY
Dual-Luciferase® Reporter Assay System	Promega
ProFection® Mammalian Transfection System	Promega
Protein Assay	BioRad
Plasmid Mini, Midi and Maxi Kit	Qiagen
QuikChange™ Site-Directed Mutagenesis Kit	Agilent
SuperSignal® West Pico Chemiluminescent Substrate	Pierce

3.7 Chemicals, enzymes, reagents and equipment

Chemicals, enzymes and reagents used in this study were supplied by Agilent, Applichem, Biomol, Merck, New England Biolabs, Roche and Sigma Aldrich. Cell culture materials, generals plastic material and equipment were purchased from BioRad, Biozym, Brand, Engelbrecht, Eppendorf GmbH, Falcon, Gibco BRL, Greiner, Hartenstein, Hellma, Nunc, PAA, Pan, Sarstedt, Protean, Schleicher & Schuell, VWR and Whatman.

3.8 Software and Database

SOFTWARE	PURPOSE	SOURCE

Acrobat 9.0 Pro	PDF data processing	Adobe
BioEdit 7.0.5.2	sequence data processing	Open Software (*Ibis Therapeutics Carlsbad*)
CLC Main Workbench 5.0	sequence data processing	CLC bio
Endnote 9.0	reference management	Thomson
Filemaker Pro 11	database management	FileMaker, Inc.
Gene Tools	quantification of DNA/protein bands	SynGene
Illustrator CS4	layout processing	Adobe
ImageJ 1.45s	image intensity quantification	Open Software
pDRAW32	vector image processing	Open Software *(Acaclone)*
Photoshop CS4	image processing	Adobe
PubMed	literature database, open software for sequence analysis	Open Software (NCBI)

4 Methods

4.1 Bacteria

4.1.1 Propagation and storage

For bacterial liquid cultures, sterile LB-Medium containing the respective antibiotic (100 µg/mL ampicilin or 50 µg/mL kanamycin) was inoculated with a single colony and cultivated over night in an incubator shaker (New Brunswick) at 200 rpm at 30 or 37°C, respectively. For selection of single colonies, bacteria were spread on solid LB medium containing 15 g/l agar and respective antibiotics (100 µg/ml ampicillin or 50 µg/ml kanamycin) and incubated over night at 30 or 37°C. If necessary, solid plate cultures were sealed with *Parafilm* (Pechiney Plastic Packaging) and stored up to several weeks at 4°C.

For bacteria storage, liquid cultures were centrifugated (4000 rpm, 5 min, *Multifuge* 3 S-R; Heraeus) at RT, resuspended in 0.5 ml LB medium, transferred into *CryoTubes* (Sarstedt) and mixed with 0.5 ml sterile glycerol. The glycerol stocks can be longterm-stored at -80°C.

LB Media	Trypton	10 g/l
	Yeast Extract	5 g/l
	NaCl	5g/l
	(autoclaved)	
Antibiotic solutions	Ampicillin	50 mg/ml
	Kanamycin	10 mg/ml
	(filter sterilization, storage at -20°C)	

4.1.2 Transformation of E. *coli*

100 µl of chemically competent DH5α bacteria were transferred into a 10 ml *Falcon 2059* tube, mixed with 1 µl β-Mercaptoethanol (1.2M) and 1-10 µl diluted plasmid

DNA and incubated on ice for 30 min. After a heat shock at 42°C for 45 s, the bacteria were chilled on ice for 2 min. Subsequently, 1 ml LB medium without antibiotics was added and the bacteria were incubated for 1 hour at 37°C and 200 rpm in an incubation shaker (New Brunswick). After centrifugation (4000 rpm, 3 min; *Cryo centrifuge 5417R*, Eppendorf) the bacteria were resuspended in 100 µL LB, plated on LB agar containing appropriate antibiotics and incubated over night at 30 or 37°C.

4.2 Cell lines

4.2.1 Propagation of established mammalian cell lines

In this work adhesive mammalian cells growing in monolayers on polystyrene cell culture dishes (12-well, 6-well, 100 mm or 150 mm diameter dishes) were cultured with *Dulbecco's Modified Eagle Medium* (DMEM; Sigma) containing 0.11 g/l sodium pyruvate, 10% fetal calf serum (FCS; PAA) and 1% of penicillin/streptomycin solution (1,000 U/ml penicillin and 10 mg/ml streptomycin in 0.9% NaCl; PAA). For hepatoma cell lines (HepaRG, HepaRG shscrambled and HepaRG shKAP1) 5 µg/ml bovine insulin (Sigma) and 0.5 µM hydrocortisone (Sigma) were added (Gripon et al., 2002). Cultured cells were incubated at 37°C in an incubator (Heraeus) with 5% CO_2 atmosphere. To split confluent cells the media was removed, cells were washed once with sterile phosphate buffered saline (PBS) solution and incubated with trypsin/EDTA solution (PAA) for 5 min at 37°C. Trypsin activity was inhibited by adding standard culture media and the cells were transferred into 15 or 50 ml tubes and centrifuged at 2000 rpm (*Multifuge 3S-R*; Heraeus) for 3 min. The supernatant was removed, cells were resuspended in an appropriate amount of standard media and split in a constant ratio of 1:2 to 1:20. Cells, further used for transfection or infection experiments were counted by mixing 50 µl cell suspension with 50 µl trypanblue solution and subsequent transfer in the *Neubauer counter* (C. Roth). The exact cell number in cells per ml was determined by multiplication of the mean value of two manual counts (16 squares) using the light microscope (Leica DMIL) by the dilution factor and the factor 10^4. Dead cells were excluded during counting.

PBS	NaCl	140 mM
	KCl	3 mM
	Na_2HPO_4	4 mM
	KH_2PO_4 (pH7.0-7.7, autoclaved)	1.5 mM
Trypan Blue Solution	Trypan Blue	0.15%
	NaCl	0.85%

4.2.2 Storage of mammalian cell lines

For longterm storage of mammalian cell lines, subconfluent cells were detached from the cell culture dishes as described in chapter 4.2.1. After centrifugation at 2000 rpm for 3 min (*Multifuge 3S-R*, Thermo), the supernatant was removed, the cell pellet was resuspended in 1 ml FCS (PAA) containing 10% DMSO (Sigma) and transferred in *CryoTubes*™ (Sarstedt). The cells were frozen slowly using „Mr. Frosty“ (Zefa Laborservice) before stored in liquid nitrogen. For recultivation, frozen cells were thawed fastly at 37°C and resuspended in 5 ml of culture media. After centrifugation at 2000 rpm for 3 min (*Multifuge* 3S-R, Thermo) to remove the DMSO containing media, the cells were resuspended in 1 ml media, transferred in a cell culture dish containing pre-warmed media and incubated at standard conditions (see chapter 4.2.1).

4.2.3 Transfection of mammalian cell lines

4.2.3.1 Polyethylenimine (PEI) method

Polyethylenimine (PEI, 25 kDa, Polysciences) is a polycationic polymere, which is able to form a complex with the negatively charged DNA. This complex containing an anionic core (DNA) and a cationic surface (PEI) can then attach to the negatively charged cell surface and subsequently be taken up by the cell via endocytosis.

Prior to transfection, PEI was dissolved in ddH_2O at a concentration of 1 mg/ml, the pH was adjusted to pH 7.2 with 0.1 M HCl, the suspension was filter sterilized (0.45

µm), aliquoted and stored at -80°C. For efficient transfection of mammalian cell lines, cell culture media was replaced by fresh DMEM media without supplements, a DNA-media-PEI mixture was prepared (ratio 1:100:10), mixed and incubated for 20 min at RT. After dropwise application of the transfection solution, cells were incubated at standard conditions for 8 h (see chapter 4.2.1) before the media was replaced by standard culture media. Transfected cells were harvested 24 to 72 h post transfection (h p.t.) as described in chapter 4.2.4.

4.2.3.2 Calcium phosphate method

Calcium phosphate-mediated transfection procedure facilitates DNA binding to cell membranes and entry of the DNA into the cell via endocytosis. Additionally, calcium phosphate is known to protect the DNA against nucleases (Loyter *et al.*, 1982). For efficient transfection of mammalian cell lines, the *ProFection® Mammalian Transfection System* (Promega) was used. Three hours prior to transcfection cell culture media was replaced by fresh standard culture media. For each transfection DNA was mixed with 2 M $CaCl_2$ and added to a new tube containing 2XHBS solution according to the manufacturers' protocol. Transfected cells were harvested 48 h p.t. as described in chapter 4.2.4.

4.2.4 Harvest of mammalian cell lines

Adherent mammalian cells were harvested using cell scrapers (Sarstedt), subsequent transfer into 15 ml tubes and centrifugation at 2000 rpm for 3 min at RT (*Multifuge* 3 S-R; Heraeus). After discarding the supernatant, the cell pellet was washed once with PBS and stored at -20°C or directly used for subsequent experiments.

4.2.5 Generation of stable knock-down cell lines

4.2.5.1 Generation of recombinant lentiviral particles

For the generation of recombinant lentiviral particles, HEK-293T cells were co-transfected with a plasmid containing either scrambled shRNA or shRNA specific for KAP1, as well as the envelope and packaging plasmids pCMV-VSV-G,

pMDLg/pRRE and pRSV-Rev. Therefore, 1 ml of OptiMEM (GIBCO) and 1:10 PEI was added to the DNA, mixed and incubated for 30 min at RT. The cell culture media was replaced by fresh OptiMEM without supplements and the DNA-media-PEI mixture was added dropwise to the cells. After 8 h of incubation at standard conditions (see chapter 4.2.1) media was replaced by standard culture media. 3 days p.t. supernatant containing the lenitiviral particles was collected and filter sterilized (0.45 µm), aliquoted, frozen in liquid nitrogen and stored at – 80°C.

4.2.5.2 Infection of mammalian cell lines with lentiviral particles

For infection with lentiviruses, cells were grown to a confluence of 50 to 70% in 6-well culture plates. Prior to infection, media was replaced by fresh DMEM without supplements and lentiviral particles were applied dropwise. After 2 h post infection (h p.i.) fresh standard culture media was added. 48 h p.i. the media was supplemented with puromycin (1 µg/ml) in order to select transduced cells. Under these conditions cells were cultured and propagated. Knock-down efficiency was determined via Western Blot analysis.

4.3 Adenoviruses

4.3.1 Generation and storage of high titer virus stocks

For the generation of high titer virus stocks several 150 mm cell culture plates containing subconfluent HEK-293 cells were infected with a multiplicitiy of infection (moi) of 5. After 3 days the cells showed a cytopathic effect and dissociated from the culture dish. Since it could be shown that 90% of newly produced viruses associate with the cells (Jakoby and Pastan, 1979), the cells were harvested and pelleted for 5 min at 2000 rpm (*Multifuge* 3S-R, Thermo). After washing the pellet with PBS, the cells were resuspended in 4 ml DMEM without supplements and viral particles were released into the media via repeated freeze and thaw cycles. To remove cell debris the suspension was centrifuged 15 min at 4500 rmp (*Multifuge 3S-R*, Thermo). For long-time storage, sterile glycerole (87%, end concentration 10%, AppliChem) was added to the supernatant and the virus stocks were stored at -80°C.

4.3.2 Determination of virus titers

For the determination of virus titers fluorescence forming units (ffu) were assigned. Therefore, the early HAd5 protein E2A (DBP) was stained via immunohistochemistry. $4x10^5$ HEK-293 cells were seeded in each well of a 6-well plate. 24 hours after seeding media was removed, the cells were washed with PBS and infected with different dilutions of the virus stock (10^{-1} to 10^{-6} in DMEM without supplements). After 2 hours of incubation in the incubator the media was replaced with fresh standard culture media and incubated over night (o.n.) at standard conditions. After 24 h p.i. the media was removed, the cells were washed once gently with PBS and fixed with ice cold methanole for 15 min at -20°C. Afterwards, the methanole was removed and the cells were air dryed at room temperature (RT). In order to block unspecific antibody binding sites the cells were incubated with 2 ml TBS-BG per well for 1 h at RT followed by 1 ml of a 1:10 dilution of the primary antibody B6-8 in TBS-BG for 2 h at RT or o.n. at 4°C. After washing the cells three times 5 min with TBS-BG (2 ml per well) 1 ml of the secondary antibody dilution (Alexa™ 488 anti-mouse; 1:1000 in TBS-BG) was added per well and incubated for 2 h at RT or o.n. at 4°C. Finally, the cells were washed three times 5 min with TBS-BG and covered with 1 ml TBS-BG per well. Infected cells could be counted using the immunofluorescence microscope (Leica DMIL) and infectious particles per ml could be calculated by respecting the dilution factor.

TBS-BG	Tris/HCl, pH 7.6	20 mM
	NaCl	137 mM
	HCl	3 mM
	$MgCl_2$	1,5 mM
	Tween 20	0.05%
	Natriumazid	0.05%
	Glycine	5 mg/ml
	BSA	5 mg/ml

4.3.3 Infection of mammalian cell lines with adenoviruses

Prior to infection culture media was replaced by DMEM without supplements and 1 ml fresh DMEM with the respective amount of virus (moi between 1 and 100) was applied. The total volume for a well of a 6-well plate was 1 ml, for a 100 mm dish 6 ml and for a 150 mm dish 10 ml. The cells were incubated for 2 h under standard conditions, media was removed and fresh standard culture media was added. According to the experimental settings the cells were harvested between 2 and 96 h p.i. (see chapter 4.2.4).

4.3.4 Determination of virus progeny production

In order to determine the virus progeny production cells were seeded (see chapter 4.2.1), infected (see chapter 4.3.3) and harvested after different time points (see chapter 4.2.4). After pelleting and washing, cells were respuspended in 300-500 µl DMEM without supplements and lysed via three freeze and thaw circles with liquid nitrogen and subsequent 37°C waterbath incubation. Cell debris was removed by a centrifugation step (4500 rpm, 10 min; Multifuge 3S-R, Thermo). The supernatant was pipetted into a new 1.5 ml tube and stored at 4°C. The number of infectious particles was determined as described in chapter 4.3.2 and progeny production was calculated according to cell number and dilution factor.

4.3.5 Inhibition of the 26S proteasome

The 26S proteasome contains two subunits. The central 20S core unit consists of 4 rings and has proteolytic activity. At its ends two 19S units are located which have mainly regulatory functions. The rings of the core unit can be divided into a central β-unit and two α-units, which can be subdivided into seven subunits (α1-α7, β1- β7) and have different enzyme-like functions. β2 has trypsine-like, β5 chymotrypsine-like and β1 glutamylpeptidylhydrolytic (PGPH) activity (Hershko and Ciechanover, 1998). MG-132, a tripeptidaldehyde (SIGMA), known to reversibly block the β1- and β2-subunits of the proteasome additionally inhibits other proteases of the cell, like calpain or cathepsine B. In order to inhibit the proteasome after transfection (see chapter 4.2.3) or infection (see chapter 4.3.3) the culture media was supplemented

with MG-132 (final concentration 20 mM in DMSO) 4 hours before harvesting the cells (see chapter 4.2.4). Control cells were treated with DMEM containing DMSO respectively. Total-cell extracts were generated and further analyzed via SDS-PAGE (see chapter 4.5.6) and Western Blot (see chapter 4.5.7).

4.4 DNA techniques

4.4.1 Preparation of plasmid DNA from *E. coli*

For the large-scale isolation of plasmid DNA from *E. coli* 500 ml LB media was inoculated with 200-500 µl pre-cultured liquid culture derived from a single bacteria clone. After incubation for 16-20 hours at 30 or 37°C in an *Inova 4000 Incubator* (New Brunswick), bacteria were pelleted by centrifugation at 6000 rpm for 10 min (*Avanti J-E*; Beckman & Coulter) and plasmid DNA was purified according to the manufacturer's instructions using a *MaxiKit* (Qiagen). For analytical purposes, 1 ml of liquid culture was harvested, bacteria was pelleted by centrifugation (6000 rpm, 10 min; *Eppendorf 5417R*) and resuspended in 300 µl *resuspension buffer P1* (Qiagen). By adding 300 µl *lysis buffer P2* (Qiagen) the bacteria were lysed followed by 5 min incubation at RT and addition of 300 µl *neutralization buffer P3* (Qiagen). After incubation for 5 min at RT, salts and cellular debris were removed by centrifugation (14000 rpm, 10 min; *Eppendorf 5417R*) and the supernatant was transferred into a new reaction tube containing 0.9 volume isopropanol for precipitating by centrifugation (14000 rpm, 30 min; *Eppendorf 5417R*). The DNA pellet was washed once with 500 µl 75% (v/v) ethanol, air-dried and rehydrated in a volume of 50 µl ddH_2O.

4.4.2 Determination of DNA concentrations

DNA concentrations were quantitatively determined with a *NanoDrop* spectrophotometer (PEQLAB) at a wavelength of 260 nm. DNA purity was controlled by calculating the OD_{260}/OD_{280} ratio with the optimal value of 1.8.

4.4.3 DNA agarose gel electrophoresis

Analytical agarose gels were generated by dissolving *Seakem® LE agarose* (Biozym) in TBE buffer with a final concentration of 0.6-1.0% (w/v) and boiling in a microwave oven (Moulinex). After cooling down, ethidium bromide (stock concentration 10 mg/ml) was added to a final concentration of 0.05 µg/ml and the solution was poured in a respective gel tray. *6x loading buffer* was added to the DNA samples and the solutions were subjected to agarose gel electrophoresis at a voltage of 5-10 V/cm gel length for 1-2 hours. DNA visualization was done using a UV transilluminator system at 312 nm (*G-Box*; SynGene).

5xTBE	Tris	450 mM
	Boric Acid	450 mM
	EDTA	10 mM
	(pH 7.8)	
6x loading buffer	Bromphenol Blue	0.25% (w/v)
	Xylen Cyanol	0.25% (w/v)
	Glycerol	50% (v/v)
	EDTA	10 mM

4.4.4 Polymerase-Chain-Reaction (PCR) for site-directed mutagenesis

For the insertion of mutations into plasmids the *in vitro QuikChange™ Site-directed Mutagenesis* (Agilent) method was used. The respective mutation forward and reverse primers were designed and subsequently ordered from Metabion. Depending on the introduced mutation and primer length the PCR program was as follows:

1 min	95°C	DNA denaturation
45 sec	55°C	primer annealing
45 sec/kb	68-72°C	extension

4.4.5 DNA sequencing

For DNA sequencing 0.8-1.2 µg of DNA and 30 pmol of sequencing primer were mixed with ddH_2O to reach a total volume of 15 µl. Sequencing was performed by Seqlab (Göttingen).

4.5 Protein techniques

4.5.1 Preparation of total-cell lysates

All steps of cell lysis were carried out on ice or at 4°C. Harvested cell pellets (see chapter 4.2.4) were either directly used or, if frozen, carefully thawed on ice and resuspended in an appropriate volume of highly stringent RIPA lysis buffer supplemented with 0.2 mM PMSF, 1 mg/ml pepstatin A, 5 mg/ml aprotinin and 20 mg/ml leupeptin and incubated for 30 min on ice, with vortexing steps every 10 min. In order to completely disrupt the cells as well as to shear genomic DNA the cells were sonificated (30s, output 0.6, 0.8 impulses/s; *Branson Sonifier 450*) and cellular debris was pelleted by centrifugation (14,000 rpm, 5 min, 4°C; *Eppendorf 5417R*). Protein concentration was determined by spectrophotometry (see chapter 4.5.3).

RIPA	Tris/HCl, pH8.0	50 mM
	NaCl	150 mM
	EDTA	5 mM
	Nonident P-40	1% (v/v)
	Sodium Dodecyl Sulfate (SDS)	0.1% (w/v)
	Sodium Desoxycholate	0.5% (w/v)

4.5.2 Preparation of fractionated cell lysates

4.5.3 Quantitative determination of protein concentrations

Soluble protein concentration was determined by applying *Protein-Assays* (BioRad) according to Bradford (Bradford, 1976), measuring the 595 nm absorption of proteins

bound to chromogenic substrates. For determination of absolute protein concentrations a BSA standard curve was used (concentrations of 1-16 µg/µl; New England Biolabs). 1 µl of protein solution was mixed with 799 µl ddH_2O and 200 µl *Bradford Reagent* (BioRad) and measured in a SmartSpec™ Plus spectrophotometer (BioRad) against a blank of 800 µl ddH_2O and 200 µl *Bradford Reagent*.

4.5.4 Investigation of protein-protein interactions via immunoprecipitation

For the immunoprecipitation of target proteins equal amounts (1-2 mg) of total-cell lysates (see chapters 4.5.1 and 4.5.3) were precleared by adding equal amounts of protein A-sepharose (Sigma-Aldrich) for 1 hour at 4°C in a rotator (GFL) and 1 µg of purified antibody or 100 µl of hybridoma supernatant per reaction was coupled to 3 mg of protein A-sepharose/IP. After three times washing the antibody-coupled sepharose beads with 5 ml of RIPA lysis buffer (supplemented with 0.2 mM PMSF, 1 mg/ml pepstatin A, 5 mg/ml aprotinin, 20 mg/ml leupeptin, 25 mM iocetamide and 25 mM N-ethylmaleimide) the precleared protein lysates were centrifuged (600xg, 5 min, 4°C; *Eppendorf* 5417R) and the antibody-coupled beads were added to the supernatant. Immunoprecipitation was performed at 4°C in a rotator (GFL) for 2 hours. Afterwards, protein A complexes were pelleted by centrifugation (600xg, 5 min, 4°C; *Eppendorf* 5417R) followed by three steps of washing with 1.5 ml RIPA lysis buffer. 15 µl of *5xSDS sample buffer* were added to the pellet, boiled for 5 min at 95°C for protein elution and stored at -20°C until further analysis via SDS-PAGE (see chapter 4.5.6) and Western Blot (see chapter 4.5.7).

4.5.5 Purification and analysis of SUMO conjugates

To investigate the SUMO modifications of several proteins HeLa cells stably expressing 6His-SUMO1 or 6His-SUMO2 were transfected and/or infected with appropriate amounts of expression vectors and/or virus. After 48 hours cells were harvested, washed once and resuspended in 5 ml PBS. 1 ml was removed for total protein analysis, centrifuged (2000 rmp, 3 min; *Eppendorf* 5417R) and lysed with RIPA buffer (see chapter 4.5.1). Cells in the remaining 4 ml were pelleted (2000 rpm, 3 min; *Multifuge* 3S-R, Thermo) and dissolved in 5 ml Guanidinium containing lysis buffer.

After sonification (30s, output 0.6, 0.8 impulses/s; *Branson Sonifier 450*) and incubation for at least 6 hours at 4°C with prewashed 25 µl Ni-NTA agarose (Qiagen), Ni-NTA beads were pelleted (4000rpm, 10 min, 4°C; *Heraeus Megafuge 1.0*), washed once with wash buffer pH8.0 and two times with wash buffer pH6.3 (2000 rpm, 3 min, 4°C; *Eppendorf* 5417R). To elute the 6His-SUMO conjugates, 30 µl elution buffer was added and the samples were boiled at 95°C for 5 min. Afterwards, the eluates were stored at -20°C until further analysis via SDS-PAGE (see chapter 4.5.6) and Western Blot (see chapter 4.5.7).

Guanidinium lysis buffer	Guanidinium-HCl	6 M
	Na_2HPO_4	0.1 M
	NaH_2PO_4	0.1 M
	Tris/HCl pH8.0	10 mM
	Imidazole	20 mM
	β-Mercaptoethanol	5 mM
Wash buffe pH8.0	Urea	8 M
	Na_2HPO_4	0.1 M
	NaH_2PO_4	0.1 M
	Tris/HCl pH8.0	10 mM
	Imidazole	20 mM
	β-Mercaptoethanol	5 mM
Wash buffer pH6.3	Urea	8 M
	Na_2HPO_4	0.1 M
	NaH_2PO_4	0.1 M
	Tris/HCl pH6.3	10 mM
	Imidazole	20 mM
	β-Mercaptoethanol	5 mM
Elution buffer	Imidazole	200 mM
	SDS	0.1% (w/v)
	TrisHCl pH6.8	150 mM
	Glycerol	30% (v/v)
	β-Mercaptoethanol	720 mM

	Bromphenol blue	0.01% (w/v)

4.5.6 SDS Polyacrylamid gel electrophoresis (SDS-PAGE)

For analysis of protein samples cell lysates were separated by SDS-PAGE. According to their molecular weights different amounts of negatively charged SDS bind to the proteins and compensate their positive charge. Thereby, speed is exclusively dependent on the size of the protein. 8-15% Polyacrylamide gels were made using a 30% acrylamide/bisacrylamide solution (37.5:1 *Protogel*; National Diagnostics). Proteins were concentrated between the lower percentage stacking and the higher percentage separating gel via pH discrepancy (Weber et al., 2008). Polymerization of acrylamide was initiated by the addition of APS (0.1%) and N, N, N′, N′-Tetra-methylethylendiamine (TEMED; 0.01%). Gels were prepared using the SDS-PAGE system of Biometra according to the manufacturer's instructions and run at 15-20 mA/gel in TGS buffer. Before loaded on the gel cell lysates were supplemented with *5xSDS sample buffer* (Sambrook et al., 1989) and boiled at 95°C for 3 min in a thermoblock (*Thermomixer Comfort*; Eppendorf). To determine the molecular weights of the separated proteins *PageRuler™ Prestained Protein Ladder Plus* (Fermentas) was used. Subsequently, gels were subjected to Western blotting (see chapter 4.5.7).

5xSDS sample buffer	Tris/HCl pH6.8	100 mM
	SDS	10% (w/v)
	DTT	200 mM
	Bromphenol blue	0.2% (w/v)
30% Acrylamide	Acrylamide	29% (w/v)
Stock solution	N,N'Methylenbisacrylamide	1% (w/v)
Stacking Gel 5%	Acrylamide Stock Solution	17% (v/v)
	Tris/HCl, pH6.8	120 mM
	SDS	0.1% (w/v)
	APS	0.1% (w/v)
	TEMED	0.1% (v/v)
Separating Gel 8%	Acrylamide Stock Solution	27% (v/v)

	Tris/HCl, pH8.8	250 mM
	SDS	0.1% (w/v)
	APS	0.1% (w/v)
	TEMED	0.06% (v/v)
Separating Gel 10%	Acrylamide Stock Solution	34% (v/v)
	Tris/HCl, pH8.8	250 mM
	SDS	0.1% (w/v)
	APS	0.1% (w/v)
	TEMED	0.04% (v/v)
Separating Gel 12%	Acrylamide Stock Solution	40% (v/v)
	Tris/HCl, pH8.8	250 mM
	SDS	0.1% (w/v)
	APS	0.1% (w/v)
	TEMED	0.04% (v/v)
Separating Gel 15%	Acrylamide Stock Solution	50% (v/v)
	Tris/HCl, pH8.8	250 mM
	SDS	0.1% (w/v)
	APS	0.1% (w/v)
	TEMED	0.04% (v/v)
TGS Buffer	Tris	25 mM
	Glycine	200 mM
	SDS	0.1% (w/v)

4.5.7 Western Blot

For analysis of protein samples polypeptides were transferred and immobilized on nitrocellulose membranes (Protran®; Whatman) via a *Western Blot.* With the help of a *Trans-Blot® Electrophoretic Transfer Cell* (BioRad) the SDS-Gels (see chapter 4.5.6) were installed according to the manufacturer's instructions and the proteins were transferred in *Towbin* Buffer at 400 mA for 60-90 min with respect to the molecular weight of the proteins of interest. To test transfer efficiency the membranes could be reversibly stained with Ponceau S (Sigma). The de-staining could be reached by

washing with ddH_2O. To block unspecific antibody binding the nitrocellulose membranes were incubated for 1 hour at RT or overnight at 4°C in PBS containing 5% non-fat dry milk (Frema) on an *orbital shaker* (GFL). After discarding the blocking solution membranes were washed with PBS-Tween to remove residual blocking solution and incubated with dilutions of specific primary antibodies in PBS-Tween for 1-2 hours at 4°C, followed by three steps of 10 min washing with PBS-Tween and 1 hour incubation with a 1:10,000 dilution of the HRP-coupled secondary antibodies on an *orbital shaker* (GFL). After three more steps of 10 min washing with PBS-Tween protein bands were visualized by enhanced chemiluminescence using the *SuperSignal® West Pico Chemiluminescent Substrate* (Pierce) according to the manufacturer's instructions and detected with X-ray films (*RP New Medical X-Ray Film*; CEA), which were subsequently scanned, cropped using *Photoshop CS4* (Adobe) and figures were generated using *Illustrator CS4* (Adobe).

***Towbin* Buffer**	Tris/HCl, pH8.3	25 mM
	Glycine	200 mM
	SDS	0.05% (w/v)
	Methanol	20% (v/v)
Ponceau S	Ponceau S	0.2% (w/v)
	Trichloroacetic acid	3% (w/v)
	Sulfosalicyclic acid	3% (w/v)
PBS-Tween	Tween 20 (in PBS)	0.1% (v/v)

4.5.8 Immunofluorescence

Subconfluent, adherent cells were cultivated in 6-well plates on top of sterile cover slips, transfected (see chapter 4.2.3) or infected (see chapter 4.3.3) followed by fixation with methanol. Therefore, the culture media was discarded, the cells were washed once with PBS and incubated for 15 min with icecold methanol at -20°C. Afterwards, the methanol was removed, the fixed cells were air-dryed and could either be stored at -20°C or directly further analyzed. Unspecific antibody binding

areas were blocked by incubating the cells with TBS-BG buffer, followed by incubation with primary antibodies diluted in PBS for 1 hour. After three steps of washing with PBS, the appropriate secondary antibody dilution was added and the coverslips were incubated for 1 hour. Cover slips were washed three times with PBS, mounted in *Glow Mounting Media* (EnerGene) and digital images were acquired using the DM6000 *fluorescence microscope* (Leica) with a charge-coupled device camera (Leica). Images were cropped in *Photoshop CS4* (Adobe) and figures were generated with *Illustrator CS4* (Adobe).

TBS-BG	Tris/HCl pH7.6	20 M
	NaCl	137 mM
	KCl	3 mM
	$MgCl_2$	1.5 mM
	Tween 20	0.05% (v/v)
	Sodium Azide	0.05% (w/v)
	Glycine	5% (w/v)
	BSA	5% (w/v)

4.6 Luminescence based reporter gene assay

For the quantitative determination of promoter activities the *Dual-Luciferase® Reporter Assay System* (Promega) was used according to the manufacturer's instructions. The reporter constructs used in this work contain the *Firefly* luciferase gene, isolated from the firefly *Photinus pyralis*, which is under the control of a promoter of interest. As an internal control the reporter constructs were co-transfected with pRL-TK, a vector containing the *Renilla* luciferase gene isolated from *Renilla reniformis* under the control of the thymidine kinase promoter. The value of the *Renilla* luciferase was used for normalization by calculating the quotient of the *Firefly* luciferase activity to the *Renilla* luciferase activity. For the experiments $1.5x10^5$ H1299 cells were seeded in 12-well dishes (see chapter 4.2.1) and after 24 hours they were co-transfected with 0.5 µg pRL-TK, 1 µg of the respective reporter plasmid and if required 0.5 µg of a pcDNA3.1-flag based construct with the gene for human KAP1 with the PEI transfection method (see chapter 4.2.3.1). The cells were cultivated under standard

conditions (see chapter 4.2.1), washed once with PBS 48 h p.t. and incubated at RT for 10 min on an *orbital shaker* (GFL) with 100 µl *passive lysis buffer* (Promega) per well. 10 µl of lysate was subjected to measuring of *Firefly* and *Renilla* luciferase activity in a *Lumat LB 9507 luminometer* (Berthold Technologies).

5 Results

5.1 Early HAd5 proteins modulate regulators of chromatin structure and DNA damage response

For efficient infection of a cell, chromatin structure has to be modified and therefore the host-cellular DNA damage response (DDR) has to be manipulated. HAd5 interferes with the DDR in various ways, including the degradation of proteins involved in the DDR or pro-apoptotic processes like p53, integrin α3, Mre11, DNA ligase IV, Bloom Helicase (BLM) or Death domain-associated protein 6 (Daxx; Baker *et al.*, 2007; Dallaire *et al.*, 2009; Orazio *et al.*, 2011; Daxx; Salone *et al.*, 2003; Schreiner *et al.*, 2010; Steegenga *et al.*, 1998; Stracker *et al.*, 2002).

In this context, our group recently showed that early HAd5 proteins interfere with the cellular factors Daxx and X-linked α-thalassaemia retardation syndrome protein (ATRX) as well as with SPOC1, all involved in transcriptional repression and chromatin compaction, in order to ensure proper virus replication (Figure 8; Schreiner *et al.*, 2013a; Schreiner *et al.*, 2013b).

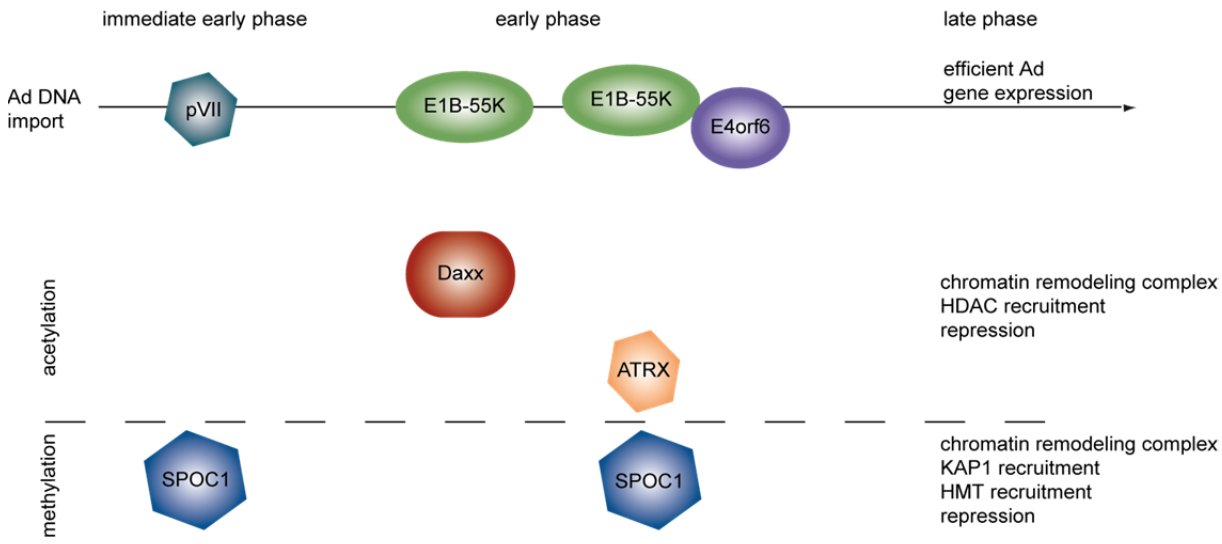


Figure 8: Early HAd5 proteins modulate regulators of chromatin structure and DNA damage response. Schematic representation of the interference of immediate early HAd5 protein pVII, E1B-55K and E4orf6 on the cellular chromatin-associated factors Daxx/ATRX and SPOC1. The chromatin remodeling complex Daxx/ATRX negatively regulates HAd5 replication via HDAC recruitment, resulting in transcriptional repression. Cullin-based E3-ubiquitin ligase complexes containing the early HAd5 proteins E1B-55K or E1B-55K in combination with E4orf6 counteract the host-cellular response by targeting the two cellular factors Daxx/ATRX for proteasomal degradation. The chromatin-associated factor and epigenetic reader protein SPOC1 also plays a role

in the antiviral host-cellular response during HAd5 infection. The SPOC1-mediated antiviral response is counteracted by the interaction with HAd5 core protein pVII, resulting in dissociation of SPOC1 from the viral genome. Subsequently, the E1B-55K/E4orf6-dependent E3-ubiquitin ligase complex targets SPOC1 for proteasomal degradation to ensure proper virus replication (Schreiner *et al.*, 2013a; Schreiner *et al.*, 2013b).

Daxx is known to repress transcription via interaction with ATRX in a functional, ATP-dependent, SWI/SNF chromatin remodeling complex, recruiting histone deacetylases (HDACs) and depositioning histone variant H3.3 (Goldberg *et al.*, 2007; Hollenbach *et al.*, 2002). Our group reported that the chromatin remodeling complex Daxx/ATRX negatively affects HAd5 infection (Schreiner *et al.*, 2013a). Furthermore, functional inactivation of Daxx/ATRX by viral E3 ubiquitin ligase which induced proteasomal degradation resulted in dissociation of histone variant H3.3 from HAd5 promoters as well as in modulation of chromatin compaction.

Recently, our group identified SPOC1, a regulator of DDR and chromatin structure (Kinkley *et al.*, 2009; Mund *et al.*, 2012) as a negative regulator of HAd5 infection (Schreiner *et al.*, 2013b). Interestingly, SPOC1 interacts with the viral core protein pVII, known to be associated with the viral genome during entry (Karen and Hearing, 2002). Our group showed that SPOC1 overexpression reduces HAd5 gene expression as well as HAd5 promoter activity. Furthermore, SPOC1 antiviral capacity is antagonized by interaction with E1B-55K and subsequent targeting for proteasomal degradation via the E1B-55K/E4orf6 E3-ubiquitin ligase complex. In contrast to Daxx/ATRX, which gain their repressive function through the recruitment of HDACs, SPOC1 recruits HMTs for transcriptional repression. Moreover, SPOC1 forms a chromatin remodeling complex via cooperation with the cellular co-repressor KAP1, thereby regulating repair kinetics in DNA damage response (Mund *et al.*, 2012).

In this context, the aim of this study was to identifiy novel KAP1 interacting factors of the virus and to unravel the role of the multifunctional host factor KAP1 during HAd5 infection.

5.2 Identification and characterization of KAP1-association with early HAd5 proteins

5.2.1 E1B-55K interacts with KAP1

The HAd5 early region 1B 55-kDa protein E1B-55K is a multifunctional protein involved in regulating cell cycle control and apoptosis, since it interacts with and inactivates cellular factors involved in DDR, apoptosis or chromatin structure. As mentioned above, SPOC1 plays a key role in restricting HAd5 gene expression and progeny production. Furthermore, SPOC1 is bound by E1B-55K and degraded via the E1B-55K/E4orf6-dependent Cullin-based E3 ubiquitin ligase complex (Schreiner *et al.*, 2013b). SPOC1 recruits KAP1 to chromatin regions containing the active histone mark H3K4me3, resulting in chromatin compaction via heterochromatin spreading (Groner *et al.*, 2010; Mund *et al.*, 2012).

To investigate, whether E1B-55K associates with KAP1, co-precipitation experiments were performed (Figure 9). Therefore, human H1299 and A549 cells were infected with wt virus (H5*pg*4100) at a multiplicity of infection (moi) of 50 ffu/cell and harvested 24 hours post infection (h p.i.). After precipitating endogenous KAP1 and subsequent staining for E1B-55K, a specific interaction between KAP1 and the viral factor could be detected in both cell lines tested (Figure 9A, lanes 2 and 4).

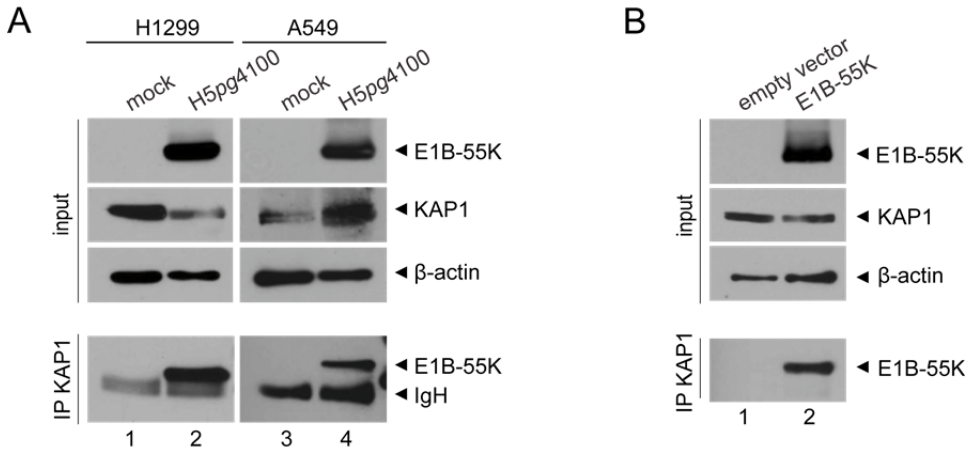


Figure 9: E1B-55K interacts with KAP1. (A) Subconfluent H1299 and A549 cells were infected with wt virus (H5*pg*4100) at a multiplicity of 50 ffu/cell, harvested after 24 hours and total-cell extracts were prepaired. Immunoprecipitation of endogenous KAP1 was performed using rbAb H-300 (α-KAP1), and proteins were visualized by immunoblotting. Input levels of total-cell lysates and co-precipitated proteins were detected using mAb 2A6 (α-E1B-55K), rbAb H-300 (α-KAP1) and mAb AC-15 (α-β-actin). Note that heavy chains (IgH) are

detected at 55 kDa. (B) Subconfluent H1299 cells were transfected with a plasmid encoding for E1B-55K, harvested after 48 hours and total-cell extracts were prepared. Immunoprecipitation of endogenous KAP1 was performed using rbAb H-300 (α-KAP1), and proteins were visualized by immunoblotting as in A.

To exclude indirect KAP1/E1B-55K binding or bridged binding due to the presence of other viral proteins, H1299 cells were transfected with a plasmid encoding for E1B-55K. Precipitation of endogenous KAP1 revealed an interaction between KAP1 and E1B-55K (Figure 9B, lane 2). Consistent with the obtained data, this interaction could recently be confirmed in HEK-293 cells by Forrester and co-workers (Forrester *et al.*, 2011a).

5.2.2 E1B-55K binds to the C-terminal PHD/bromo domain of KAP1

Various studies have identified the functions of the different domains in KAP1. Since the cellular factor does not posess a DNA binding domain itself, the protein requires DNA binding partners for efficient transcriptional regulation. At its N-terminus, KAP1 contains a RING finger, two B boxes and a leucine zipper coiled-coil region (RBCC or TRIM domain) facilitating interaction with DNA-binding factors, like KRAB ZNF transcription factors (Zeng *et al.*, 2008) or SPOC1 (Mund *et al.*, 2012). The central part of KAP1 contains a PxVxL pentapeptide region mediating the interaction with heterochromatin protein 1 (HP1). At its C-terminus, KAP1 contains a plant homeodomain (PHD) finger and a bromodomain, responsible for the recruitment of components of the NuRD histone deacetylase complex and the H3K9-specific histone methyltransferase SETDB1 (Mund *et al.*, 2012; Nielsen *et al.*, 1999; Schultz *et al.*, 2002; Schultz *et al.*, 2001).

To narrow down the binding domain required for E1B-55K interaction, co-precipitation experiments with KAP1 truncation mutants (Figure 10A) were performed. Therefore, KAP1-depleted H1299 cells (characterization see in Figure 16) were co-transfected with plasmids encoding for E1B-55K and KAP1-wt, the HP1 binding mutant M2, the deletion mutants dRBCC, dPB or the double deletion mutant d(RBCC+PB) and harvested 48 h after transfection. These experiments revealed that the deletion mutant lacking the PHD/bromo domain as well as the double mutant

additionally lacking the RBCC domain exhibit impaired E1B-55K binding properties (Figure 10B, lanes 6 and 7).

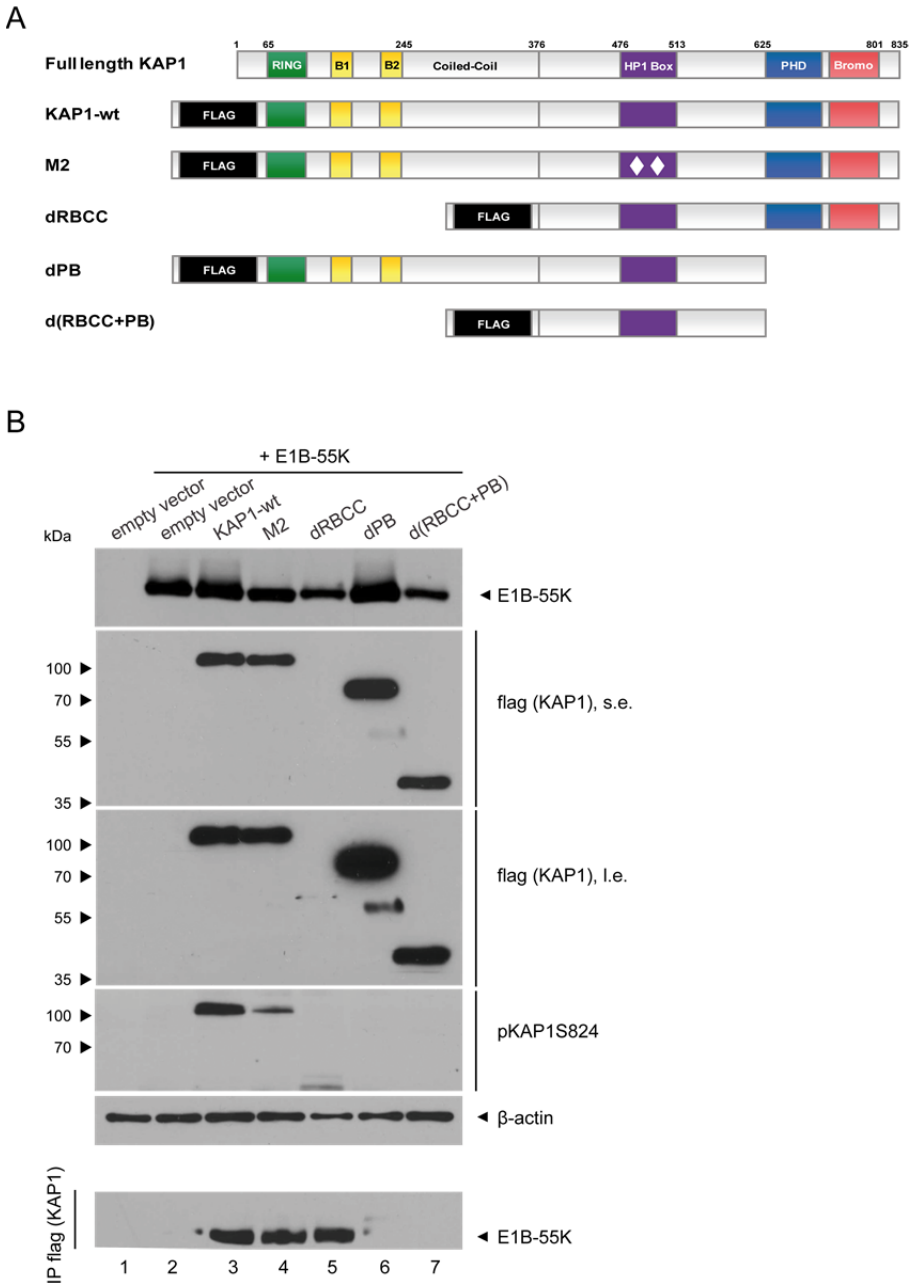


Figure 10: E1B-55K binds to the C-terminal PHD/bromo domain of KAP1. (A) Schematic representation of KAP1-wt and the truncation constructs M2 (2 aa mutation in the HP1 binding domain), dRBCC (deletion of the RBCC domain), dPB (deletion of the PHD and Bromo domain) and d(RBCC+PB) consisting of the aa region 376 – 625). (B) Subconfluent H1299 shKAP1 cells were transfected with plasmids encoding for E1B-55K and KAP1-wt, M2, dRBCC, dPB or d(RBCC+PB). Cells were harvested after 48 hours and total-cell extracts were prepared. Co-immunoprecipitation assays were performed using mAb flag-M2 (α-flag/KAP1), and proteins were visualized by immunoblotting. Input levels of total-cell lysates and co-precipitated proteins were detected using mAb 2A6 (α-E1B-55K), mAb flag-M2 (α-flag/KAP1), rbAb phospho KAP1S824 (α-pKAP1S824) and mAb AC-15 (α-β-actin). s.e.: short exposure; l.e.: long exposure. Molecular weight in kDa is indicated on the left, while the relevant proteins are labeled on the right.

To identify putative KAP1 binding domains in the E1B-55K protein, H1299 cells were transfected with plasmids encoding for E1B-55K, E1B-55K-RTR448/449/450AAA (RTR), E1B-55K-CC454/456SS (RF6), E1B-55K-K104R (K104R), E1B-55K-E472A (E2), E1B-55K-LLL83/87/91AAA (Nes), E1B-55K-KK185/187AA (KK) or E1B-55K-R443A (R443A). After precipitating endogenously expressed KAP1 and subsequent staining for E1B-55K, interactions with all tested E1B-55K mutants could be observed (Figure 11). The signals obtained for binding affinity were detected in high dependance to the respective steady-state concentrations.

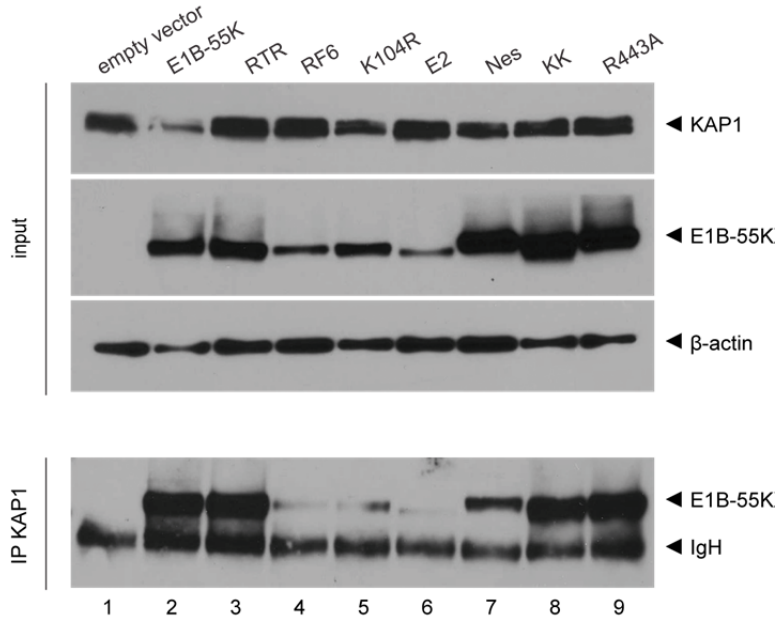


Figure 11: Identification of putative KAP1 binding domains in E1B-55K. Subconfluent H1299 cells were transfected with plasmids encoding for E1B-55K, E1B-55K-RTR448/449/450AAA (RTR), E1B-55K-454/456SS (RF6), E1B-55K-K104R (K104R), E1B-55K-E472A (E2), E1B-55K-LLL83/87/91AAA (Nes), E1B-55K-KK185/187AA (KK) or E1B-55K-R443A (R443A). Cells were harvested after 48 hours and total-cell extracts were prepared. Co-immunoprecipitation assays were performed using rbAb H-300 (α-KAP1) and proteins were visualized by immunoblotting. Input levels of total-cell lysates and co-precipitated proteins were detected using rbAb H-300 (α-KAP1), mAb 2A6 (α-E1B-55K) and mAb AC-15 (α-β-actin). Note that heavy chains (IgH) are detected at 55 kDa.

Taken together, these data indicate that E1B-55K interacts with the C-terminal PHD/bromo domain of KAP1. However, the KAP1 binding domain of E1B-55K could not yet be identified.

5.2.3 HAd5 DNA binding protein E2A/DBP interacts with KAP1

Due to the lack of a DNA-binding domain, KAP1 requires interaction partners with the ability to bind DNA. In the host cell this is mediated by KAP1 interaction with KRAB ZNF transcription factors or other DNA bridging proteins, like SPOC1 (Mund *et al.*, 2012; Peng *et al.*, 2009; Urrutia, 2003). As SPOC1 is degraded during HAd5 infection (Schreiner *et al.*, 2013b), the question was raised whether KAP1 compensates the loss of SPOC1 and interacts with a DNA-associated viral factor. The viral early regulatory protein E2A/DBP was reported to possess DNA binding properties. This viral factor was identified as a marker for HAd5 replication sites in the nucleus and is involved in the initiation of viral DNA replication and chain elongation with the help of other viral proteins, like pTP (terminal protein), AdPol (viral polymerase) and cellular transcription factors (Puvion-Dutilleul and Puvion, 1990; Shenk, 2001). To test possible cooperations between KAP1 and E2A/DBP, co-immunoprecipitation experiments were performed. Therefore, H1299 and A549 cells were infected with wt virus H5*pg*4100 and harvested after 24 hours. Precipitation of endogenously expressed KAP1 with KAP1 specific antibodies and subsequent immunostaining of E2A/DBP revealed a specific interaction of both proteins (Figure 12, lane 2 and 4).

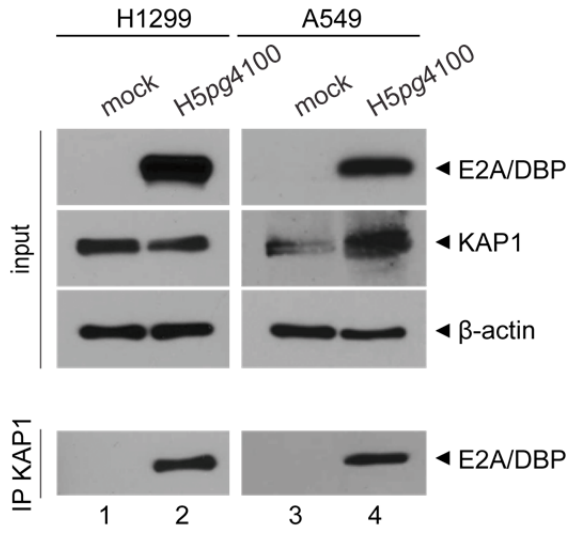


Figure 12: HAd5 E2A/DBP (DNA binding protein) interacts with KAP1 in infected cells. Subconfluent H1299 and A549 cells were infected with wt virus (H5*pg*4100) at a multiplicity of 50 ffu/cell, harvested after 24 hours and total-cell extracts were prepaired. Immunoprecipitation of endogenous KAP1 was performed using rbAb H-300 (α-KAP1), and proteins were visualized by immunoblotting. Input levels of total-cell lysates and co-precipitated proteins were detected using mAb B6-8 (E2A/DBP), rbAb H-300 (KAP1) and mAb AC-15 (α-β-actin).

To exclude an indirect KAP1-E2A/DBP cooperation mediated by the KAP1 binding to the viral protein E1B-55K, binding capacity without the virus background was monitored. Thus, H1299 cells were transfected with plasmids encoding for E1B-55K and E2A/DBP as indicated (Figure 13) and harvested after 48 hours. In the absence of E1B-55K, E2A/DBP still co-precipitated with KAP1-specific antibody (Figure 13, lane 3).

In sum, these data revealed an E1B-55K-independent cooperation between KAP1 and the viral DNA binding protein E2A/DBP.

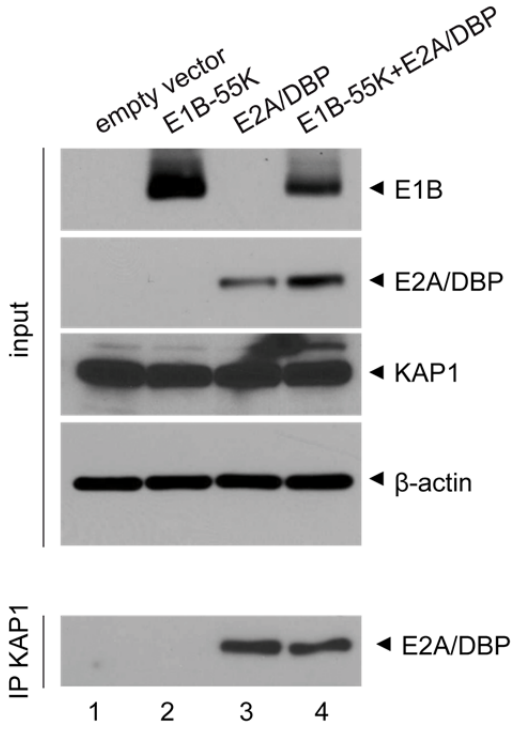


Figure 13: HAd5 E2A/DBP (DNA binding protein) interacts with KAP1 in transfected cells. Subconfluent H1299 cells were transfected with plasmids encoding for E1B-55K and E2A/DBP at indicated combinations, harvested after 48 hours and total-cell extracts were prepared. Immunoprecipitation of endogenous KAP1 was performed using rbAb H-300 (α-KAP1), and proteins were visualized by immunoblotting. Input levels of total-cell lysates and co-precipitated proteins were detected using mAb 2A6 (E1B-55K), mAb B6-8 (E2A/DBP), rbAb H-300 (KAP1) and mAb AC-15 (α-β-actin).

5.2.4 KAP1 localization is not altered upon HAd5 infection

Recent reports conclude that under normal conditions KAP1 localizes in the nucleus and interacts with KRAB ZNF transcription factors as well as with proteins not containing zinc fingers but serving as bridging proteins. These interactions facilitate

its access to DNA and allow KAP1 to function as a transcriptional co-repressor (Friedman *et al.*, 1996; Mund *et al.*, 2012; Peng *et al.*, 2009; Urrutia, 2003).

To evaluate whether the cooperation between KAP1 and E1B-55K or E2A/DBP affect its intracellular localization, H1299 and A549 cells were infected with wt virus (H5*pg*4100) and fixed after 24 hours. Analysis of KAP1 localization via indirect immunofluorescence staining confirmed the diffuse nuclear localization of endogenous KAP1 in mock infected cells (Figure 14, a-d and i-l) and showed no relocalization upon HAd5 infection (Figure 14, e-h and m-p).

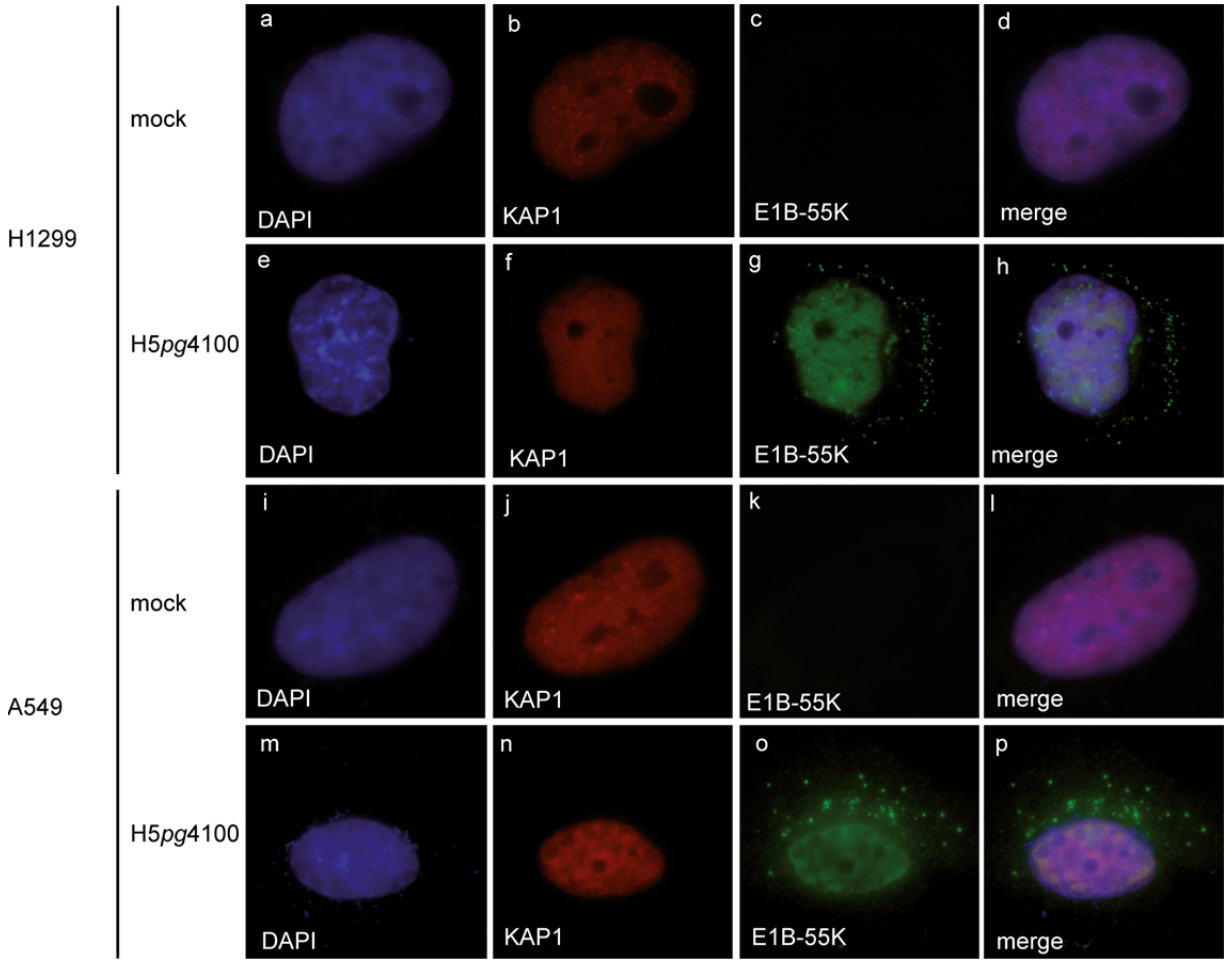


Figure 14: KAP1 localization is not altered upon HAd5 infection. H1299 and A549 cells were infected with wt virus (H5*pg*4100), fixed with methanol 24 h p.i. and double-labeled with rbAb H-300 (α-KAP1) and mAb 2A6 (α-E1B-55K). Primary antibodies were detected using Cy3 (α-KAP1; red) and FITC (α-E1B-55K; green) conjugated secondary antibodies. Nuclei are labeled with DAPI. Overlays of single images (merge) are shown in d, h, l, p (magnification x 7600).

5.3 KAP1 is a negative regulator of HAd5 productive infection

5.3.1 KAP1 overexpression counteracts HAd5 productive infection

KAP1 is involved in virus infection, executing different functions like the regulation of latency (KSHV, Karposi's Sarcoma Associated Herpesvirus; Chang *et al.*, 2009) or the inhibition of genome integration (HIV-1, Human Immunodeficiency Virus - type 1; Allouch *et al.*, 2011). In this context, the effect of KAP1 overexpression on HAd5 progeny production and protein expression was examinated.

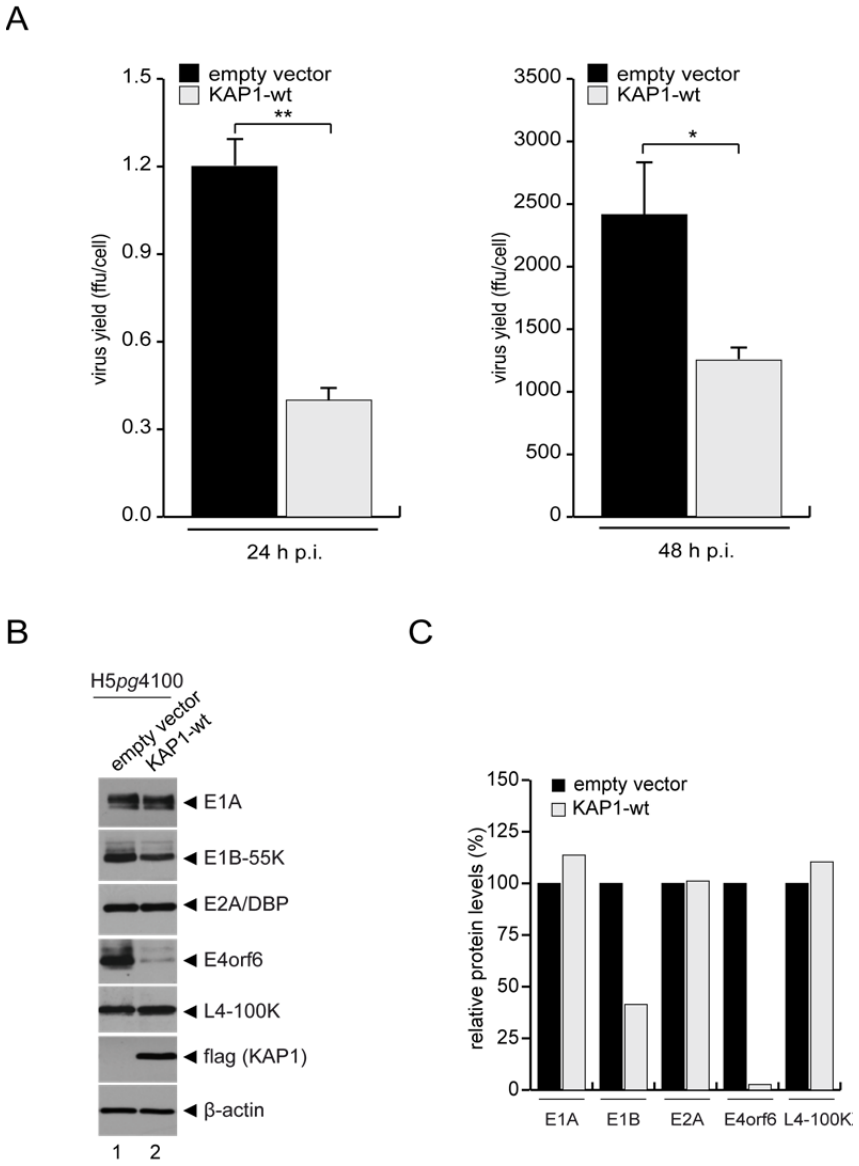


Figure 15: KAP1 overexpression counteracts HAd5 productive infection. Subconfluent H1299 cells were transfected with an empty vector control or a plasmid encoding for KAP1-wt and superinfected with wt virus (H5*pg*4100) at a multiplicity of 20 ffu/cell. (A) Viral particles were harvested 24 and 48 h p.i. and virus yield was determined by quantitative E2A/DBP immunofluorescence staining on HEK-293 cells. The mean and standard

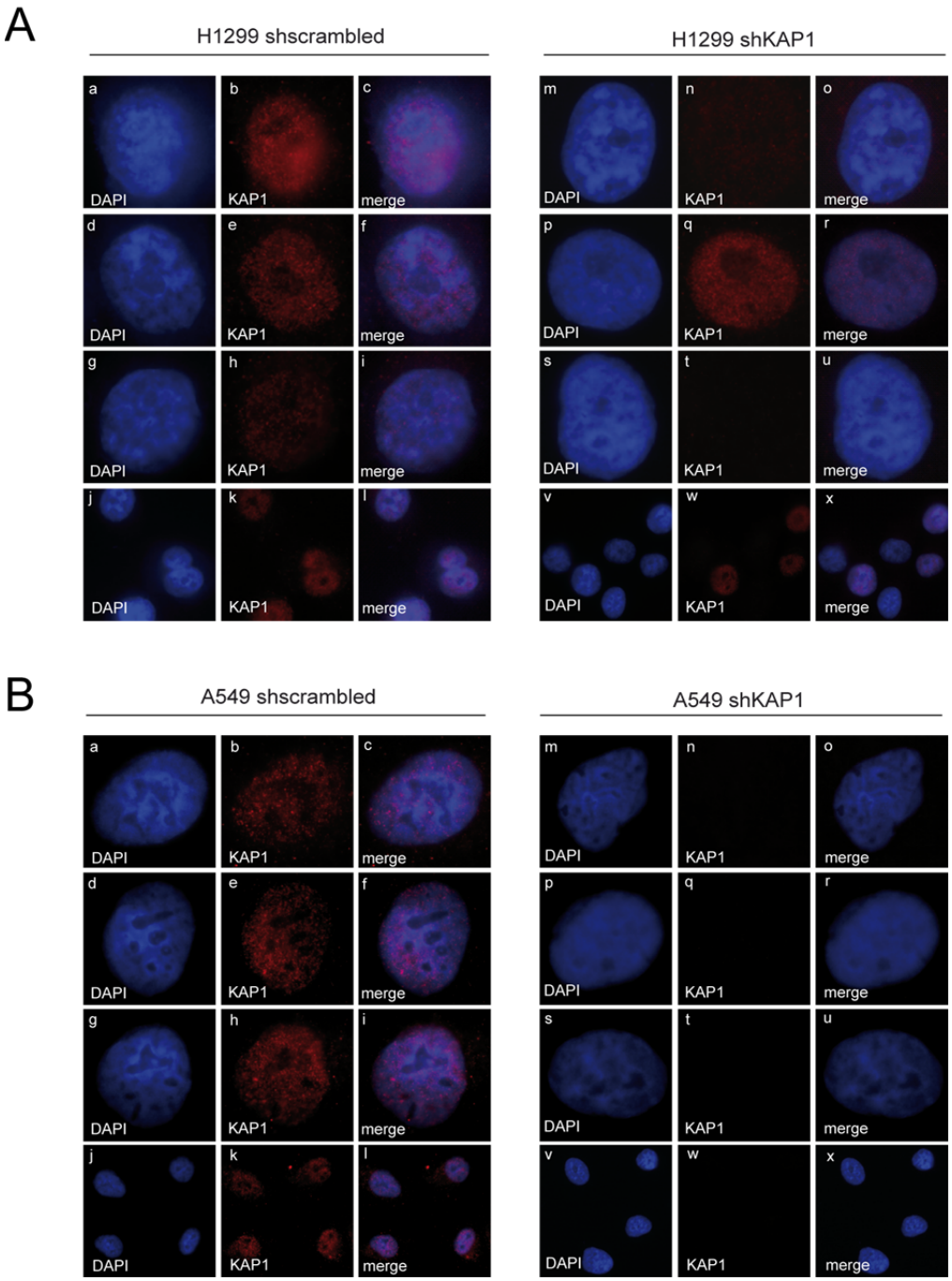
deviation are presented for three independent experiments. Two-tailed *t* tests were applied to calculate significance (* P<0.05; ** P<0.002). (B) Cells were harvested 24 h p.i., total-cell extracts were prepared and steady-state expression levels of total-cell lysates were detected using mAb M73 (α-E1A), mAb 2A6 (α-E1B), mAb B6-8 (α-E2A/DBP), mAb RSA3 (α-E4orf6), rAb 6B10 (α-L4-100K), mAb flag-M2 (α-flag/KAP1) and mAb AC-15 (α-β-actin). (C) Densiometric analysis of protein levels in B, quantified with ImageJ 1.45s, normalized with the respective β-actin levels to 100. Values are shown as relative protein levels in %.

Therefore, H1299 cells were transfected with a plasmid encoding for KAP1-wt, superinfected with wt virus (H5*pg*4100) after 24 hours, harvested at the indicated time points and virus yield was determined by quantitative E2A/DBP immunofluorescence staining on HEK-293 cells. Overexpression of KAP1 resulted in a decrease of virus progeny production compared to the empty vector control (Figure 15A). Interestingly, this effect seems to be dependent on the infection kinetics, since at 24 h p.i. virus progeny production was decreased three-fold (Figure 15A, left side) whereas at 48 h p.i. the reduction was only two-fold (Figure 15A, right side). Since efficient virus replication requires the expression of all viral proteins, it was further tested whether KAP1 overexpression affects the levels of HAd5 proteins. Therefore, H1299 cells were transfected with KAP1, superinfected with wt virus (H5*pg*4100) 24 h p.t. and harvested after additional 24 hours. Immunoblotting (Figure 15B) and densiometric analysis (Figure 15C) revealed that KAP1 had also a selective negative effect on the expression of early viral proteins. Reduced protein levels upon KAP1 overexpression could be observed for the early regulatory proteins E1B-55K and E4orf6, whereas E1A, E2A/DBP and L4-100K were not affected.

5.3.2 KAP1 depletion results in a modest increase of HAd5 productive infection

To further analyze the effect of KAP1 on HAd5 productive infection, a lentivirus expressing shRNA against KAP1 (shKAP1) was generated to deplete cellular KAP1 protein from human H1299 and A549 cells. Therefore, HEK-293T cells were co-transfected with KAP1 or scrambled shRNA as a control, an envelope and a packaging vector (see chapter 4.2.5.1). Lentiviral particles were harvested from the supernatant and sterile filtered after three days. To generate stable KAP1-depleted cells, H1299 and A549 cells were infected with the newly generated lentiviruses containing scrambled shRNA or shRNA against KAP1 and then selected based on their puromycin resistance cassette.

To test knock-down efficiency, indirect immunofluorescence staining and Western Blot analysis of KAP1 in the newly generated H1299 shscrambled, H1299 shKAP1 (Figure 16A), A549 shscrambled and A549 shKAP1 cells (Figure 16B) were performed. As shown by immunofluorescence and immunoblotting analysis KAP1 was efficiently downregulated in both cell lines (Figure 16A, B and C).



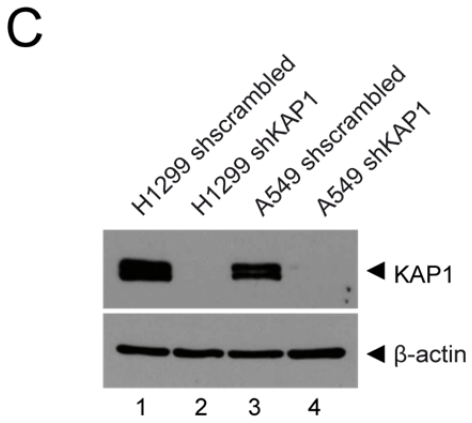


Figure 16: Characterization of KAP1-depleted human cells. (A) and (B) H1299 shscrambled, H1299 shKAP1, A549 shscrambled and A549 shKAP1 cells were fixed with methanol and cells were labelled with rbAb H-300 (α-KAP1). The primary antibody was detected with Cy3 (red) conjugated secondary antibody. DAPI was used for nuclear staining. Overlays of single images (merge) are shown in C/D c, f, i, l, o, r, u and x (magnification x 7600). (C) Endogenous KAP1 levels were determined by the preparation of total-cell extracts followed by protein separation by 10% SDS-PAGE and visualization by immunoblotting. Steady-state expression levels of total-cell lysates were detected using rbAb H-300 (α-KAP1) and mAb AC-15 (α-β-actin).

As KAP1 was shown to play a role in proliferation and cell growth (Lee *et al.*, 2007; Li *et al.*, 2007; Xiao *et al.*, 2011), it was investigated whether KAP1-depleted cells show a difference in morphology or growth behavior (Figure 17). Morphology was monitored using light microscopy of the newly generated H1299 shscrambled, H1299 shKAP1, A549 shscrambled and A549 shKAP1 cells. These data revealed that knock-down of KAP1 had no effect on the morphology of both cell lines (Figure 17A). Additionally, growth behavior was not altered in cells treated with shKAP1 compared to control shRNA (shscrambled; Figure 17B) during the time frame of the conducted experiments, except for a slight growth reduction in A549 shKAP1 cells.

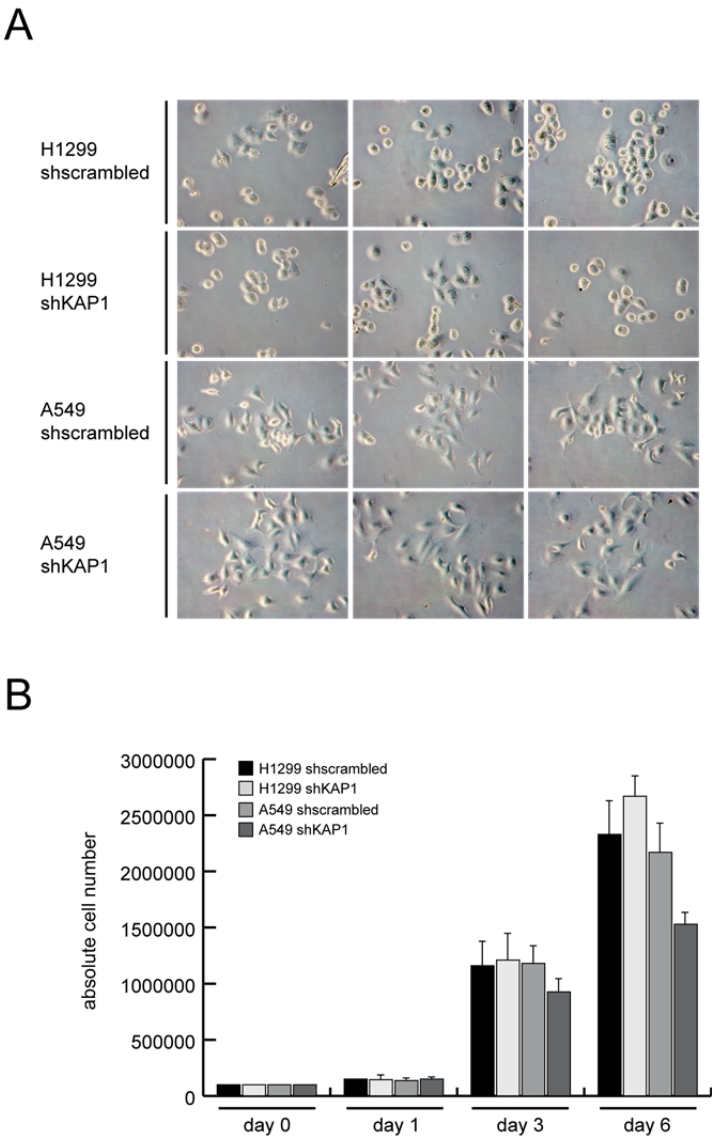


Figure 17: Morphology and growth behavior of KAP1-depleted cells. (A) Analysis of the cell morphology using bright field microscopy (magnification x 100). (B) $1x10^5$ cells (H1299 shscrambled, H1299 shKAP1, A549 shscrambled or A549 shKAP1) were cultivated and absolute cell number was determined after the indicated time points. The mean and standard deviations are presented for three independent experiments.

Compared to the control cell lines (shscrambled), knock-down of KAP1 showed no significant increase in virus progeny production (data not shown). To investigate whether the antiviral effect of KAP1 is specific to the absence of the host transcription factor, KAP1 was reintroduced in the newly generated H1299 shKAP1 cells by transient transfection. After superinfection with wt virus (H5*pg*4100) at 24 h p.t. cells were harvested after the indicated time points and virus yield was determined via quantitative E2A/DBP immunofluorescence staining on HEK-293 cells. Reintroduction of KAP1 in KAP1-depleted cells resulted in a decrease in progeny

production compared to the empty vector control 24 h p.i. (Figure 18A). However, this effect could not be detected 48 and 72 h p.i. (Figure 18B), suggesting that since KAP1 is not degraded, HAd5 counteracts KAP1 antiviral properties during infection by a so far unknown mechanism.

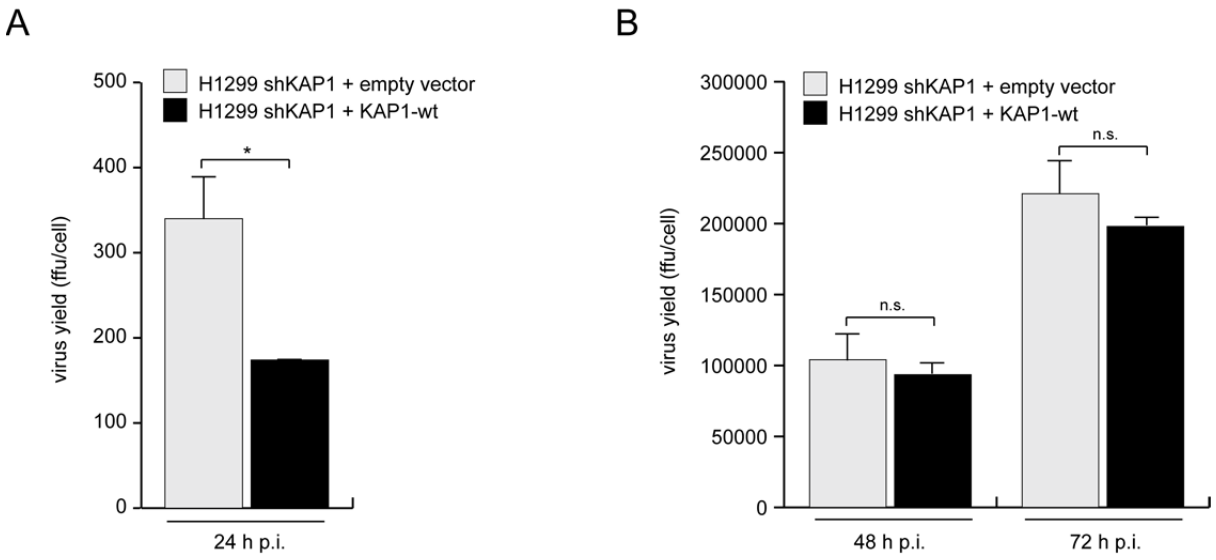


Figure 18: KAP1 reconstitution in knock-down cells negatively regulates HAd5 progeny production. Subconfluent KAP1-depleted H1299 cells (H1299 shKAP1) were transfected with a plasmid encoding for KAP1-wt and 24 hours later superinfected with wt virus (H5*pg*4100) at a multiplicity of 20 ffu/cell. Viral particles were harvested after indicated time points and virus yield was determined by quantitative E2A/DBP immunofluorescence staining on HEK-293 cells. The mean and standard deviation are presented for three independent experiments. Two-tailed *t* tests were applied to calculate significance (* $P<0.05$; n.s.: not significant).

To further analyze the role of KAP1 in HAd5 replication and protein expression, time course experiments were performed (Figure 19). Therefore, the newly generated H1299 shscrambled, H1299 shKAP1, A549 shscrambled and A549 shKAP1 cells were infected with wt virus (H5*pg*4100) and harvested after the indicated time points. Western Blot analysis revealed an only modest effect on HAd5 protein expression in both KAP1-depleted cell lines compared to their respective control. H1299 shKAP1 cells showed only a slight increase of E2A/DBP, E4orf6, E4orf6/7 and L4-100K protein levels compared to H1299 shscrambled cells (Figure 19, lanes 4-6 and 10-12). However, protein levels of E1A, E1B-55K and the capsid proteins hexon, penton and fiber were slightly decreased in KAP1-depleted H1299 cells compared to H1299 shscrambled cells. KAP1-depleted A549 cells on the other hand showed slightly increased levels of E1B-55K, E2A and the capsid proteins hexon, penton and fiber

compared to A549 shscrambled cells (Figure 19, lanes 16-18 and 21-24). However, protein expression levels of E1A, E4orf6, E4orf6/7 and L4-100K showed a modest decrease in KAP1-depleted A549 cells compared to A549 shscrambled cells. Notably, in both KAP1-depleted cell lines an increase of hexon trimers could be detected compared to their respective control cell line (Figure 19, lanes 4, 5 and 11, 12, lanes 17, 18 and 22-24).

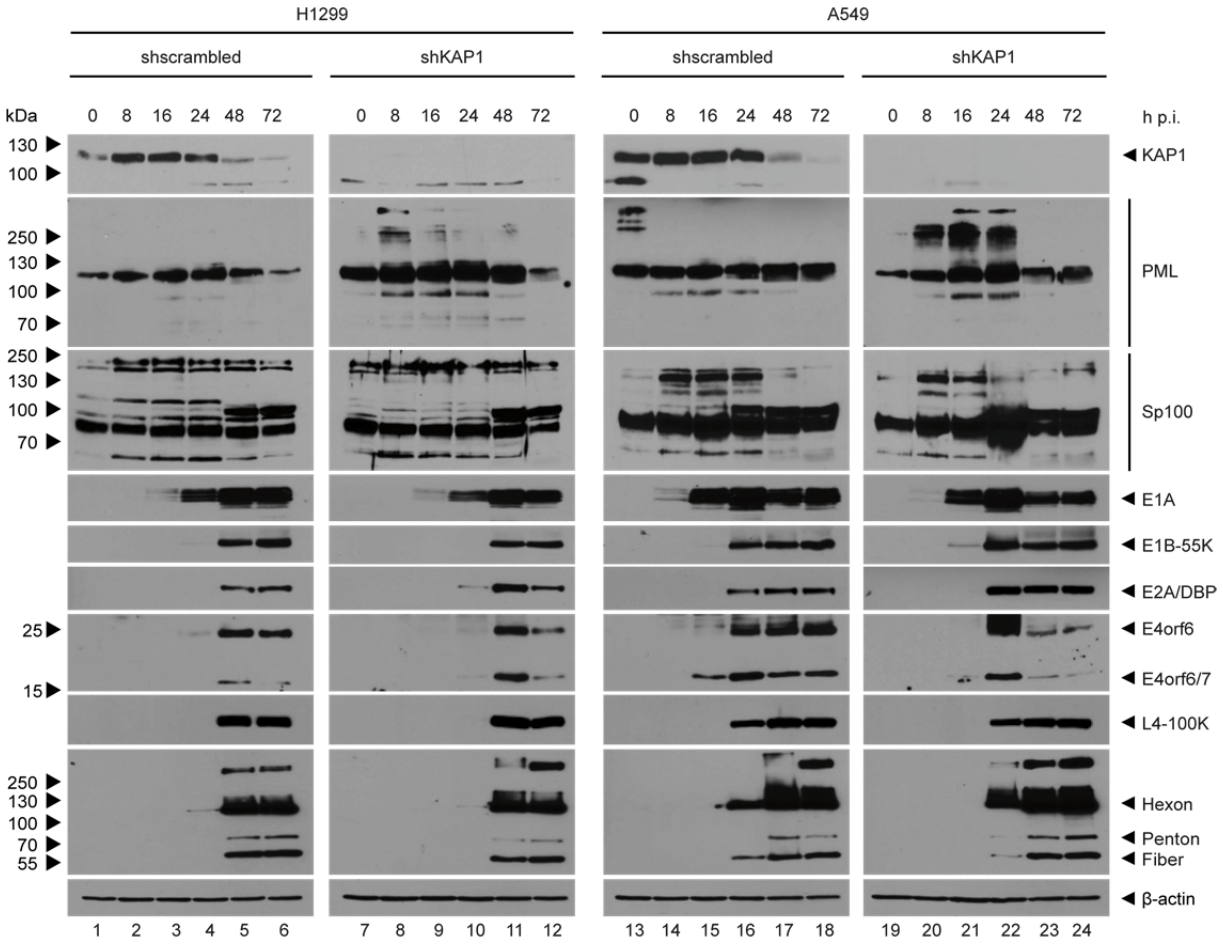


Figure 19: KAP1 depletion affects viral protein synthesis. H1299 shscrambled, H1299 shKAP1, A549 shscrambled and A549 shKAP1 cells were infected with wt virus (H5*pg*4100) at a multiplicity of 50 ffu/cell, harvested at indicated time points and proteins from total-cell extracts were separated by SDS-PAGE and subjected to immunoblotting using rbAb H-300 (KAP1), pAb NB 100-59787 (α-PML), pAb GH3 (α-Sp100), mAb M-73 (α-E1A), mAb 2A6 (α-E1B-55K), mAb B6-8 (α-E2A/DBP), mAb RSA3 (α-E4orf6), rAb 6B10 (α-L4-100K), rabbit antiserum L133 specific for HAd5 capsid and mAb AC-15 (α-β-actin). (B) A549 shscrambled and A549 shKAP1 cells were infected as in A and proteins from total-cell extracts were separated by SDS-PAGE and subjected to immunoblotting using the same antibodies as in A. Molecular weight in kDa is indicated on the left, while the relevant proteins are labeled on the right.

Recently, Kepkay and co-workers showed that KAP1 depletion results in an increase of intranuclear PML-NBs (Kepkay *et al.*, 2011). Consistent with this, the levels of PML were increased after KAP1 depletion in both cell lines tested, whereas the levels of

the other prominent PML-NB component Sp100 showed no difference in expression levels (Figure 19). PML is involved in cellular defense mechanisms against incoming viruses (Everett and Chelbi-Alix, 2007; Tavalai and Stamminger, 2008). Glass & Everett could show that upon PML depletion the expression levels of the cellular Daxx protein were reduced (Glass and Everett, 2012). Interestingly, our group identified Daxx as a novel restriction factor of HAd5 productive infection (Schreiner *et al.*, 2010).

In this context, our data strongly suggest that upon KAP1 depletion upregulated PML exhibits antiviral function and mimicks KAP1-dependent effects, resulting in similar levels of HAd5 protein expression and progeny production.

5.3.3 Generation of a PML/KAP1-depleted cell line

PML-NBs are dynamic intranuclear structures, which harbor numerous transiently or permanently localized proteins, playing various roles in host antiviral defences (Everett and Chelbi-Alix, 2007). Since our results on increasing PML protein levels upon KAP1 depletion (Figure 19) were consistent with the observation that PML-NB numbers were increased after knock-down of KAP1 (Kepkay *et al.*, 2011), we hypothesized that increased PML protein levels overtake KAP1 antiviral function.

To avoid the antiviral effects of increased PML protein levels after KAP1 depletion, PML-depleted H1299 cells (H1299 shPML) were subsequently treated with scrambled shRNA as a control or shRNA against KAP1 (shKAP1). Additional knock-down of KAP1 had no effect on morphology or growth behaviour of the cells treated with shKAP compared to control shRNA (shscrambled) or parental H1299 cells (Figure 20A and B) during the time frame of the conducted experiments.

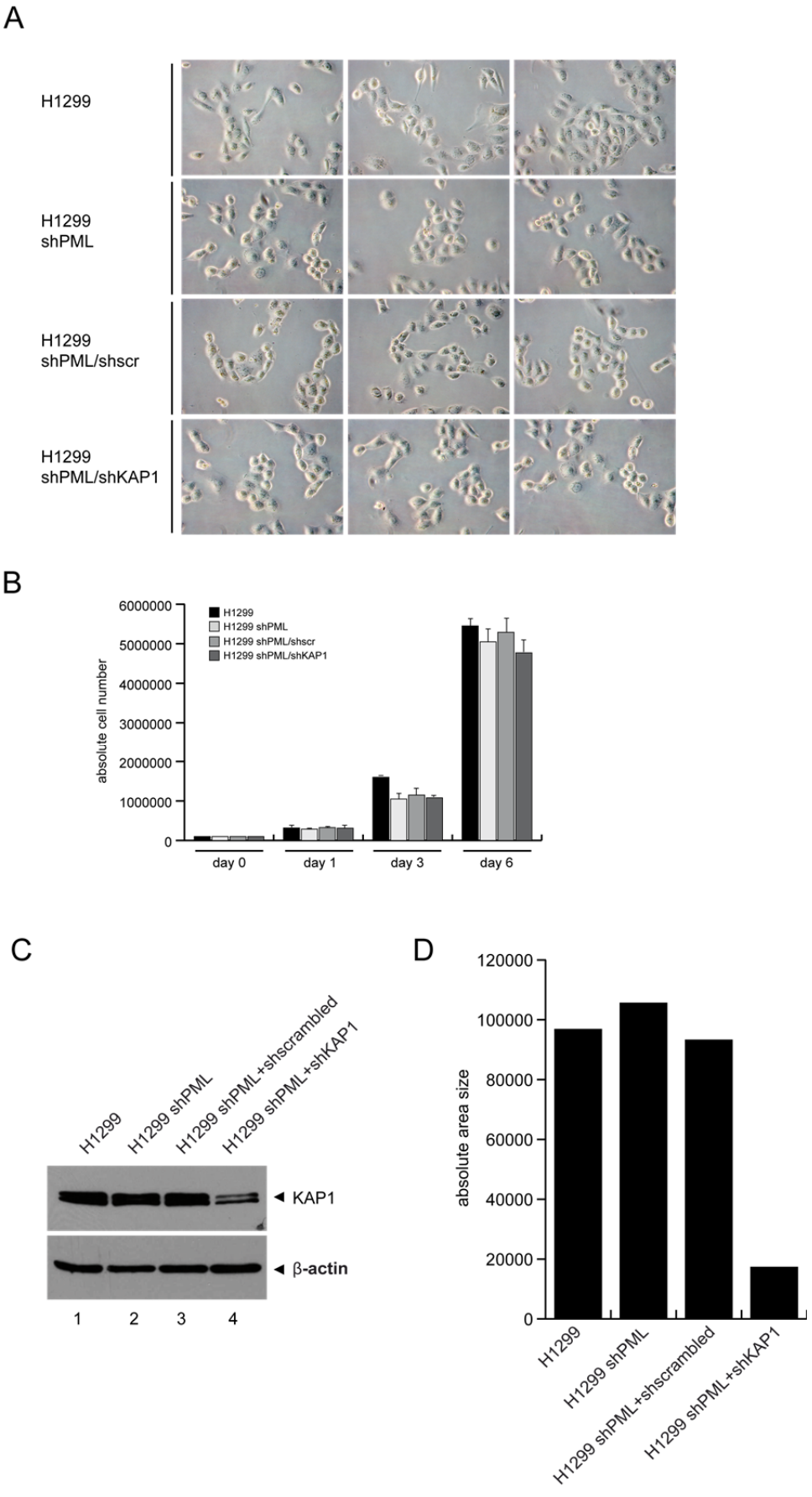

A
H1299
H1299 shPML
H1299 shPML/shscr
H1299 shPML/shKAP1
B
absolute cell number
6000000
5000000
4000000
3000000
2000000
1000000
0
H1299
H1299 shPML
H1299 shPML/shscr
H1299 shPML/shKAP1
day 0
day 1
day 3
day 6
C
H1299
H1299 shPML
H1299 shPML+shscrambled
H1299 shPML+shKAP1
KAP1
β-actin
1
2
3
4
D
absolute area size
120000
100000
80000
60000
40000
20000
0
H1299
H1299 shPML
H1299 shPML+shscrambled
H1299 shPML+shKAP1

Figure 20: Generation of a PML/KAP1-depleted cell line. Comparison of KAP1-depleted H1299 shPML cells to parental H1299 cells and cells treated with scrambled shRNA. (A) Analysis of the cell morphology using bright field microscopy (magnification x 100). (B) $1x10^5$ cells (H1299, H1299 shPML, H1299 shPML/shscrambled or H1299 shPML/shKAP1) were cultivated and absolute cell number was determined after the indicated time points. The mean and standard deviations are presented for three independent experiments. (C) Endogenous KAP1 levels were determined by the preparation of total-cell extracts followed by protein separation in 10% SDS-PAGE and visualization by immunoblotting. Steady-state expression levels of total-cell lysates were detected using rbAb H-300 (α-KAP1) and mAb AC-15 (α-β-actin). (D) Densiometric analysis of protein levels in C, quantified with ImageJ 1.45s, normalized with the respective β-actin levels. Values are shown as absolute area sizes.

To investigate whether KAP1 depletion affects virus replication, H1299, H1299 shPML, H1299 shPML/shscrambled and H1299 shPML/shKAP1 cells were infected with wt virus (H5*pg*4100) and virus yield was determined via quantitative E2A/DBP immunofluorescence staining on HEK-293 cells. Compared to the control cell lines (H1299, H1299 shPML and H1299shPML/shscrambled), knock-down of KAP1 showed no significant increase in virus progeny production (data not shown). To further investigate the role of KAP1 depletion on HAd5 protein synthesis, time course experiments were performed. Therefore, H1299, H1299 shPML, H1299 shPML/shscrambled and H1299 shPML/shKAP1 cells were infected with wt virus (H5*pg*4100), harvested after the indicated time points and subjected to Western Blot analysis. As expected, H1299 shPML and H1299 shPML/shscrambled cells showed higher expression levels of the early HAd5 proteins E1A, E1B, E2A/DBP and E4orf6 24 and 48 h p.i. (Figure 21, lanes 3-5, 9-11 and 15-17) as well as the late L4-100K and capsid proteins hexon, penton and fiber at 48 and 72 h p.i. compared to parental H1299 cells (Figure 21, lanes 5, 6, 11, 12, 17 and 18). Additional knock-down of KAP1 in H1299 shPML cells revealed an only slight increase of E2A/DBP after 48 and 72 hours compared to H1299 shPML cells, whereas the late capsid proteins hexon, penton and fiber showed decreased protein levels (Figure 21, lanes 17, 18 and 23, 24). Hence, the data obtained from these experiments suggest that the modest reduction of KAP1 is not sufficient to confirm KAP1 antiviral function in this experimental setup.

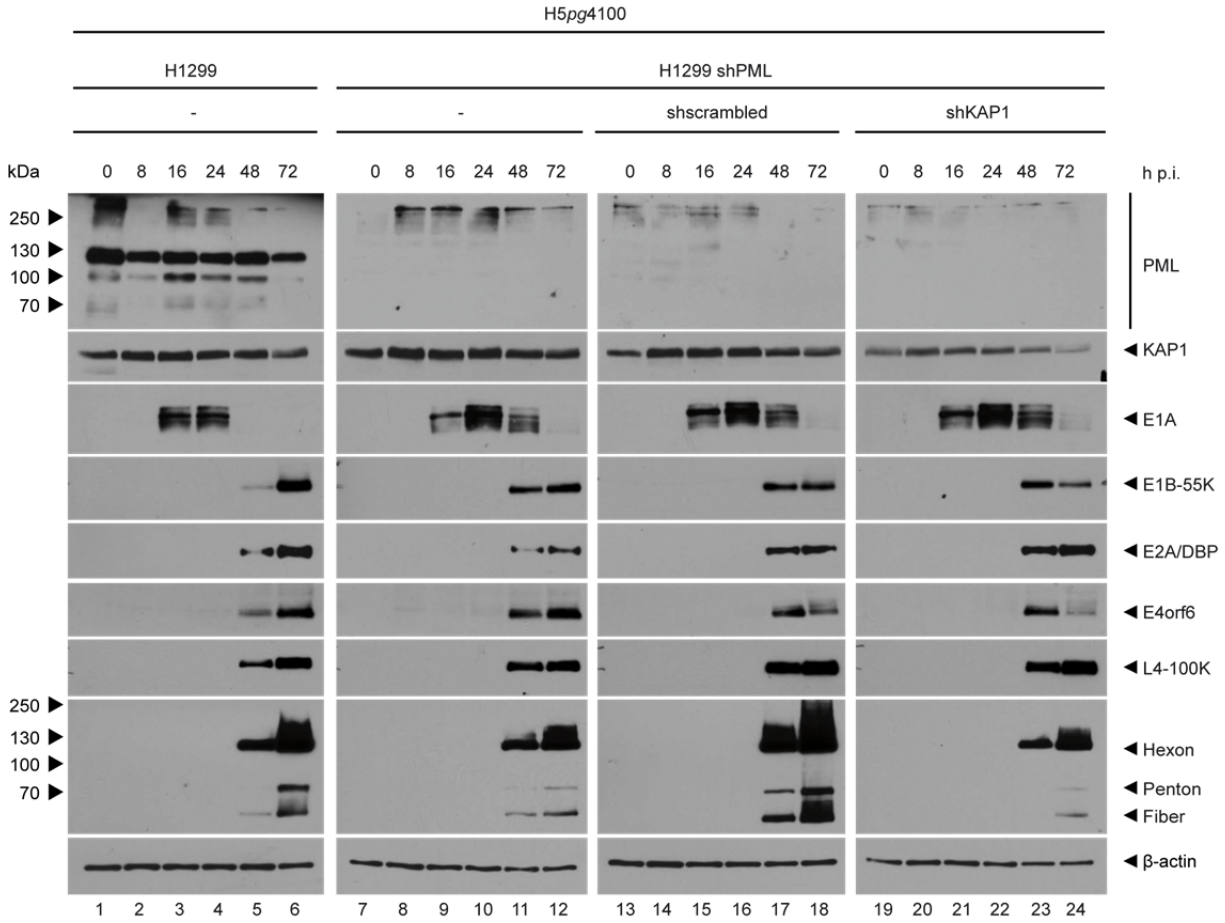


Figure 21: Effect of PML/KAP1 double knock-down on HAd5 protein expression. H1299, H1299 shPML, H1299 shPML/shscrambled and H1299 shPML/shKAP1 cells were infected with wt virus (H5*pg*4100) at a multiplicity of 50 ffu/cell, harvested after indicated time points and proteins from total-cell extracts were separated by SDS-PAGE and subjected to immunoblotting using pAb NB 100-59787 (α-PML), rbAb H-300 (KAP1), mAb M-73 (α-E1A), mAb 2A6 (α-E1B-55K), mAb B6-8 (α-E2A/DBP), mAb RSA3 (α-E4orf6), rAb 6B10 (α-L4-100K), rabbit antiserum L133 to HAd5 capsid and mAb AC-15 (α-β-actin). Molecular weight in kDa is indicated on the left, while the relevant proteins are labeled on the right.

5.4 KAP1 is a negative regulator of HAd5 promoter activity

KAP1 is able to modulate chromatin structure by the recruitment of silencing factors to distinct genomic regions, resulting in silencing of euchromatic genes. Schultz and co-workers identified the histone H3 lysine 9-specific methyltransferase SETDB1 and the Mi-2α subunit of NuRD as novel interaction partners of KAP1 (Schultz *et al.*, 2002; Schultz *et al.*, 2001), resulting in HP1-mediated transcriptional repression of genes such as *p21, bax, puma* and *noxa*, whose products are involved in cell cycle arrest and pro-apoptotic processes during DDR (Lee *et al.*, 2007; Li *et al.*, 2007).

To investigate whether the KAP1 negative function in HAd5 productive infection affects HAd5 promoter expression, luciferase reporter assays with different HAd5

promoters were performed. Therefore, HAd5 E1B, pIX, E4 and MLP promoter sequences were introduced into the reporterplasmid pGL3 basic (Invitrogen) upstream of a *luciferase* gene (Schreiner *et al.*, 2013a; Figure 22).

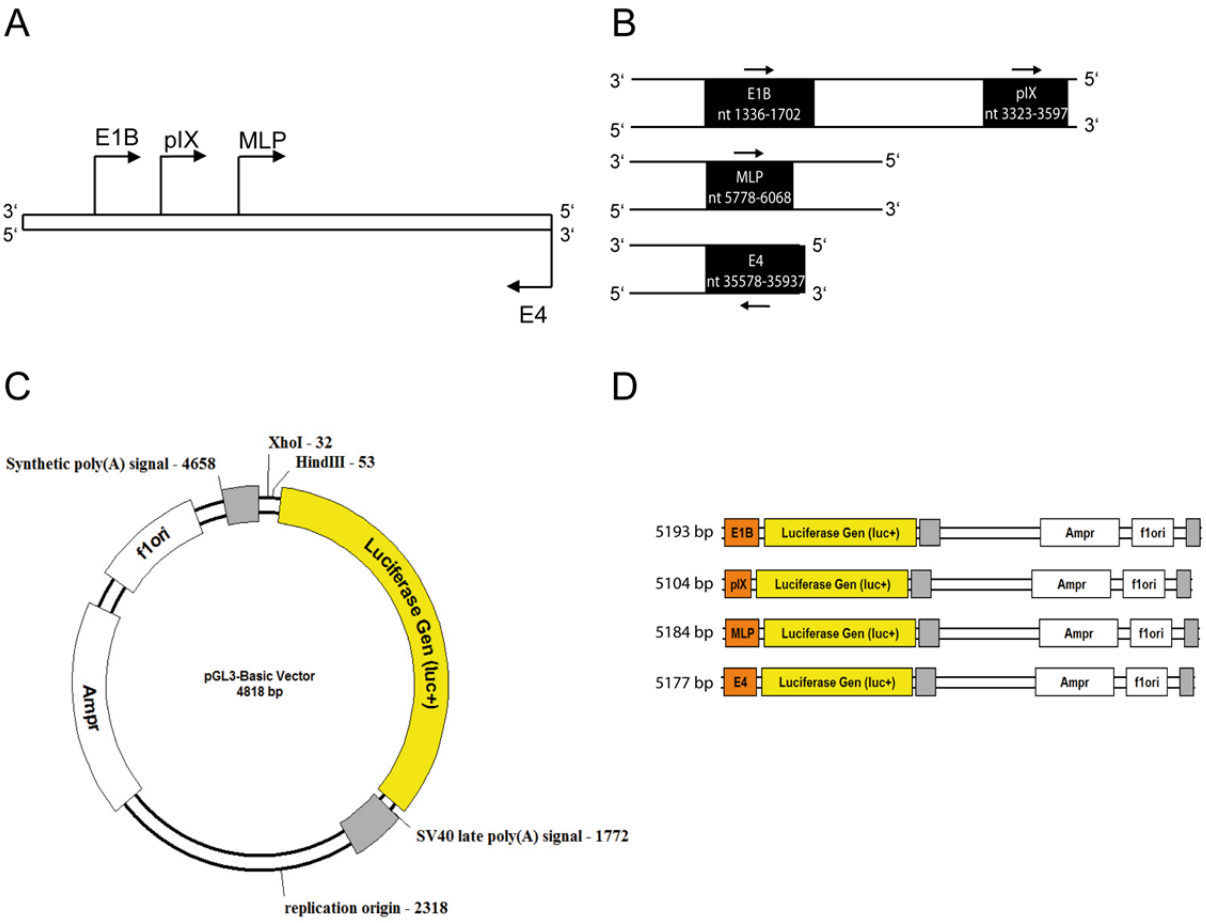


Figure 22: Overview of generated reporter constructs with HAd5 promoter regions. (A) Distribution of the examined HAd5 promoters in the HAd5 genome. (B) Exact nucleotide position (nt) of the examined HAd5 promoters. (C) Vector map of the pGL3 basic plasmid with XhoI/HindIII restriction site upstream of a luciferase gene. (D) Linearized representation of the pGL3 promoter constructs of the examined HAd5 promoters.

To investigate whether KAP1 affects HAd5 promoters, H1299 cells were co-transfected with the luciferase constructs for the E1B, pIX, E4 or MLP promoters and a plasmid encoding for KAP1-wt and luciferase assays were performed (Figure 23). Consistent with the data obtained from the virus yield and protein expression experiments, co-transfection of KAP1 together with the HAd5 promoters E1B (Figure 23A), pIX (Figure 23B), E4 (Figure 23C) and MLP (Figure 23D) showed a decrease of luciferase activity compared to the empty vector control.

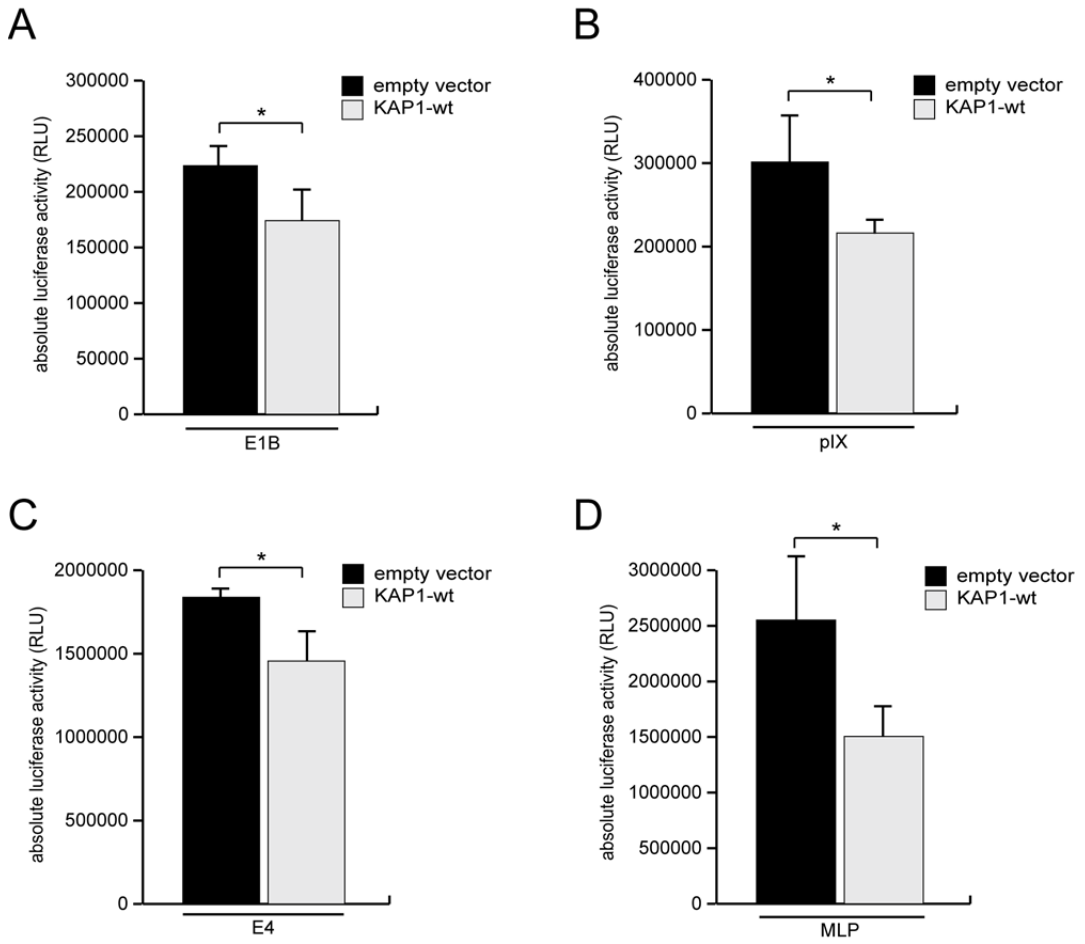


Figure 23: KAP1 is a negative regulator of HAd5 promoter activity. H1299 cells were transfected with 0.5 µg of pRL-TK (Renilla-Luc), 0.5 µg pGL3Basic Prom (E1B, pIX, E4 or MLP) and 1 µg of a plasmid encoding for KAP1-wt or an empty vector control. 24 h p.t. total-cell extracts were prepared and luciferase activity was determined. The mean and standard deviation are presented for three independent experiments. Two-tailed *t* tests were applied to calculate significance (* P<0.05).

Taken together, the data on virus progeny production, protein expression and transcriptional regulation suggest that KAP1 is a negative regulator of HAd5 promoter activity, resulting in transcriptional repression and reduced HAd5 progeny production.

5.5 KAP1 represses E1A-dependent transcriptional activity

HAd5 E1A-13S interacts with the cellular transcription factor p300, resulting in its recruitment to HAd5 promoters during infection (Pelka *et al.*, 2009). Recently, our group could show that HAd5 E1A-13S enhances p300 transcriptional activity, resulting in a positive stimulation of HAd5 promoters (Berscheminski *et al.*, 2013). Since decreased levels of HAd5 promoter activity upon KAP1 overexpression was

detected (Figure 23), the hypothesis that KAP1 could also affect E1A transcriptional activation emerged. To address this idea, luciferase assays with a GAL4-responsive promoter and p300 fused to GAL4 DNA binding domain in combination with E1A-13S and KAP1 were performed (Figure 24). KAP1 alone did not affect p300 transcriptional activity (Figure 24A). Consistent with data shown by Berscheminski *et al.*, E1A strongly stimulated the luciferase activity from the promoter. Co-transfection of increasing KAP1 amounts resulted in a decrease of luciferase activity. These results show that KAP1 negatively affects E1A-dependent transactivation, illustrating a possible mechanism by which KAP1 negatively regulates HAd5 gene expression (Figure 24B and C).

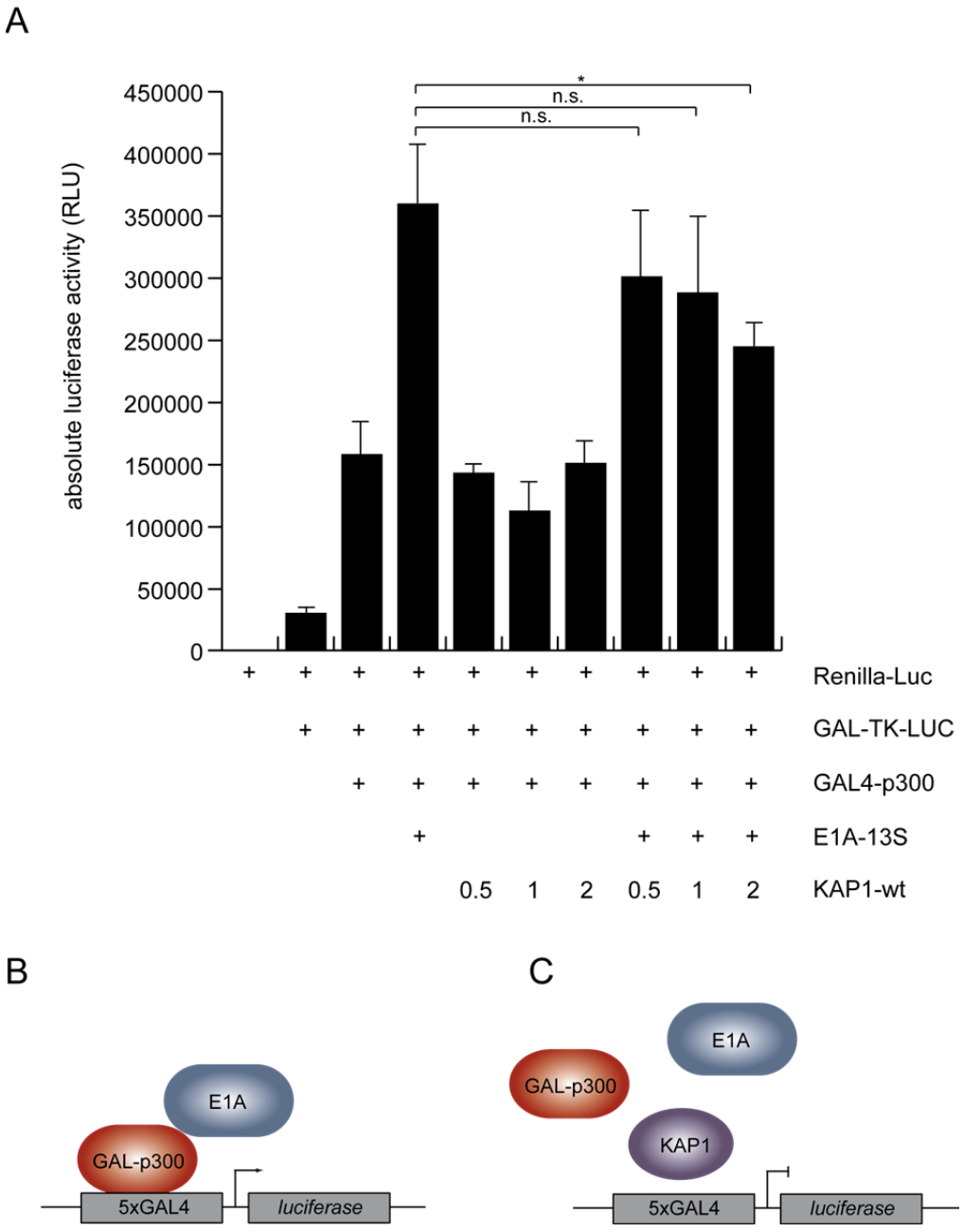


Figure 24: KAP1 represses E1A-dependent transcriptional activity. (A) H1299 cells were transfected with 0.5 µg Renilla-Luc, 0.5µg pGL-C3G5-luc, 0.5 µg pG4-p300, 1µg pE1A-13S and increasing amounts of a plasmid encoding for KAP1-wt in the combinations indicated (+). At 48 h p.t. total-cell extracts were prepared and luciferase activity was determined. The mean and standard deviations are presented for three independent experiments. Two-tailed *t* tests were applied to calculate significance (* P<0.05, n.s.: not significant). (B) and (C) Schematic presentation of the luciferase reporter assay. The luciferase gene is under the control of a promoter containing five GAL4 sequences, which is bound and activated by a complex of GAL-p300 and E1A, counteracted by KAP1.

5.6 KAP1 acts as a co-repressor of E1B-55K

E1B-55K binds to the p53 tumor suppressor protein (Sarnow *et al.*, 1982), converting it from a transcriptional activator regulated in response to DNA damage (Ko and Prives, 1996) to an upregulated repressor of genes with p53-binding sites (Martin and

Berk, 1998; Yew and Berk, 1992; Yew *et al.*, 1994). Since HAd5 E1A promotes apoptosis (White, 1995), E1B-55K thereby defends the virus against p53-induced antiviral host cell responses. HAd2/5 or HAd12 E1B-55K is known to repress transcription of promoters with GAL4-DNA bindingsites when fused to the GAL4-DNA binding domain isolated from *S. cerevisiae* (Yew *et al.*, 1994). This activity is independent of p53 but requires cellular co-repressors interacting with the C-terminus of E1B-55K. So far, the co-repressor of E1B-55K could not be identified by testing general transcription factors, but *in vitro* studies suggested that it interacts with the RNA polymerase II (Martin and Berk, 1999). The fact that KAP1 was identified as a novel interaction partner of E1B-55K (Figure 9; Forrester *et al.*, 2011a) and the report that it represses transcription of pro-apoptotic genes (Li *et al.*, 2007) resulting in decreased RNA Pol II recruitment (Groner *et al.*, 2010), raised the question whether KAP1 acts as a co-repressor in combination with E1B-55K to inactivate p53-responsive genes.

To investigate this, luciferase assays were conducted with a GAL4-responsive promoter and E1B-wt-GAL and KAP1-wt in combinations indicated (Figure 25). As expected, co-transfection of the GAL4-responsive promoter with E1B-55K alone decreased luciferase activity three-fold, while co-transfection of the promoter with KAP1-wt did not affect its activity. However, co-transfection of the promoter with both E1B-55K and KAP1-wt resulted in a dramatic reduction of luciferase activity.

Taken together, these data strongly suggest that KAP1 acts as a co-repressor of E1B-55K-dependent trancriptional repression.

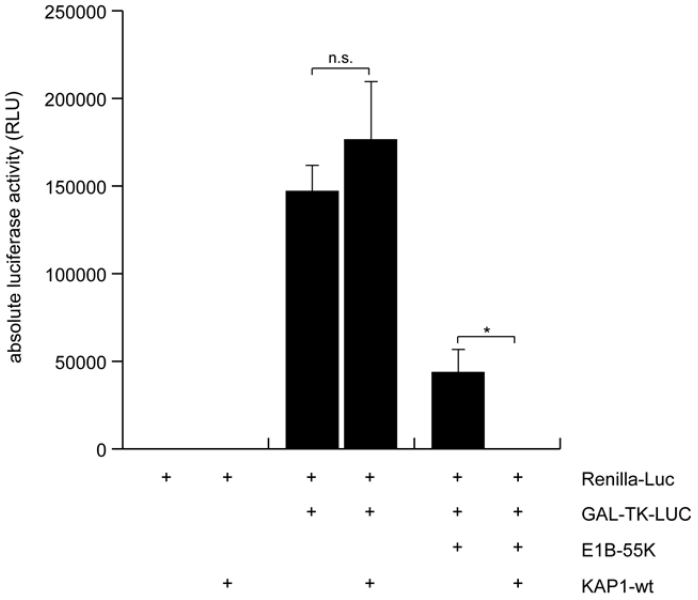


Figure 25: KAP1 acts as a co-repressor of E1B-55K. H1299 cells were transfected with 0.5 µg Renilla-Luc, 0.5 µg pGALTK-Luc and pGAL4-E1B-55K with 1 µg KAP1-wt in the combinations indicated (+). At 48 h p.t. total-cell extracts were prepared and luciferase activity was determined. The mean and standard deviations are presented for three independent experiments. Two-tailed *t* tests were applied to calculate significance (* P<0.05, n.s.: not significant).

5.7 KAP1 is not degraded during HAd5 infection

Various studies have shown that HAd5 targets several repressive cellular factors to ensure proper virus replication. A cooperation of the early viral proteins E1B-55K and E4orf6 with cellular E3 ubiquitin ligase components ensures the proteasomal degradation of several cellular factors, thereby regulating apoptosis, modulation of cell surface receptors, DNA double strand break repair/nonhomologous end joining (NHEJ) and cell cycle checkpoint signaling (Gupta *et al.*, 2013; Querido *et al.*, 2001a; Salone *et al.*, 2003; Schreiner *et al.*, 2013a; Schreiner *et al.*, 2013b; Steegenga *et al.*, 1998; Stracker *et al.*, 2002). Additionally, our group previously revealed that the cooperation of E1B-55K alone with cellular E3 ubiquitin ligase components is able to target cellular Daxx for proteasomal degradation, thereby interfering with chromatin structure and transcriptional repression (Schreiner *et al.*, 2010).

Since KAP1 interacts with HAd5 E1B-55K and negatively regulates HAd5 productive infection, it was investigated whether HAd5 also targets this cellular factor for proteasomal degradation. In this context, time course experiments were performed. Therefore, H1299 cells were infected with wt virus (H5*pg*4100) and harvested after

the indicated time points (Figure 26). Immunoblotting revealed no reduction of KAP1 protein expression during HAd5 infection while Mre11 showed decreased protein levels already 16 h p.i. (Figure 26, lane 3). Consistent with the data obtained, Forrester and co-workers recently reported that KAP1 is not degraded during HAd5 infection (Forrester *et al.*, 2011a).

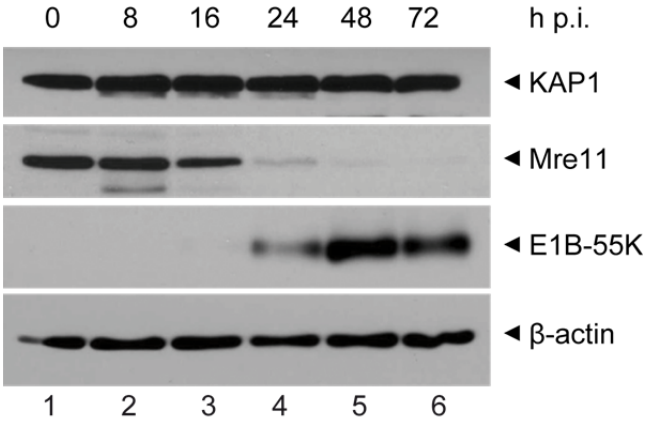


Figure 26: KAP1 is not degraded during HAd5 infection. Subconfluent H1299 cells were infected with wt virus (H5*pg*4100) at a multiplicity of 20 ffu/cell and harvested after indicated time points. Total-cell extracts were prepared and steady-state expression levels of total-cell lysates were detected using rbAb H-300 (α-KAP1), rbAb Mre11 (α-Mre11), mAb 2A6 (E1B-55K) and mAb AC-15 (α-β-actin).

Notably, a slight increase of KAP1 protein expression was detected early in infection (Figure 26, lane 2). To further investigate this, time course experiments spanning the early time points of infection were performed. Therefore, H1299 and A549 cells were infected with wt virus (H5*pg*4100) and harvested after the indicated time points (Figure 27). Immunoblotting revealed an increase of KAP1 protein levels in both cell lines already 2 h p.i (Figure 27, lanes 2 and 11) followed by a decrease at 24 h p.i. to levels comparable to the mock infected cells (Figure 27, lanes 7 and 16).

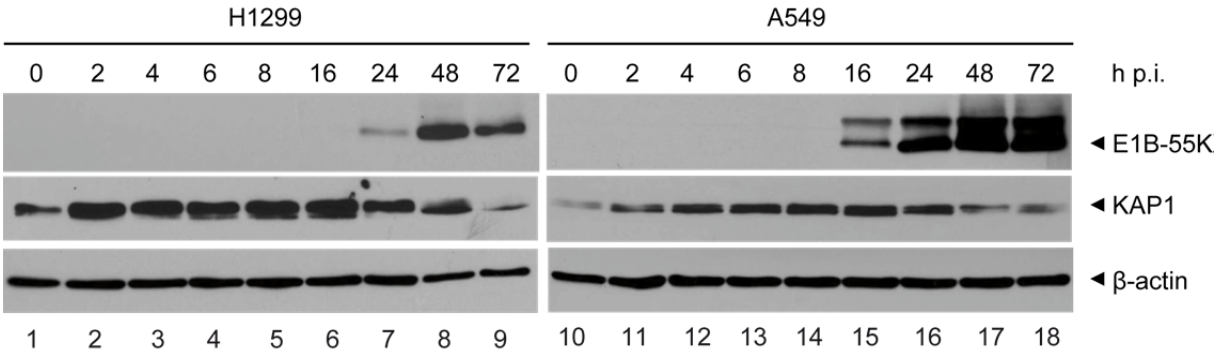


Figure 27: KAP1 is upregulated early in infection. Subconfluent H1299 and A549 cells were infected with wt virus (H5*pg*4100) at a multiplicity of 50 ffu/cell and harvested after indicated time points. Total-cell extracts were prepared and steady-state expression levels of total-cell lysates were detected using mAb 2A6 (α-E1B-55K), rbAb H-300 (α-KAP1) and mAb AC-15 (α-β-actin).

5.8 HAd5 infection induces PTMs of KAP1

5.8.1 KAP1 is phosphorylated upon HAd5 infection

Early during infection, the host cell responds to the virus by activating damage response pathways. Extensive studies showed that various adenovirus early region mutants resulted in pronounced cellular responses with increased phosphorylation of DDR substrates and concatenation of viral DNA (Blackford *et al.*, 2008; Carson *et al.*, 2003; Stracker *et al.*, 2002; Weiden and Ginsberg, 1994). Additionally, E4orf6 was shown to impair DDR by inhibiting protein phosphatase 2A (PP2A), resulting in a prolonged phosphorylation of H2AX in order to protect the incoming linear viral genome (Hart *et al.*, 2007). E4orf3 and E4orf6 bind to DNA-PK, although no inhibition of its kinase activity could be observed (Boyer *et al.*, 1999). Furthermore, E4orf3 represses downstream DDR responses by promoting epigenetic silencing of p53 transcription and relocalization of Mre11 to the cytoplasm (Carson *et al.*, 2009; Soria *et al.*, 2010).

The repressive function of KAP1 is regulated by modulating KAP1 posttranslational modifications (PTMs), including phosphorylation at the two major phosphosites Ser473 and Ser824. Recent reports showed that KAP1 S473 is phosphorylated by ATM and ATR in response to DNA damage (Bolderson *et al.*, 2012) and that this phosphorylation site is required for efficient HP1 binding (Chang *et al.*, 2008). Various studies indicated that KAP1 is phosphorylated at Ser824 by ATM after the

occurance of DNA double strand breaks resulting in co-localization with numerous DDR factors at DNA lesions (Tomimatsu *et al.*, 2009; White *et al.*, 2006; Yajima *et al.*, 2009; Ziv *et al.*, 2006). Recently, KAP1S824 phosphorylation was reported to cause transcriptional de-repression of KAP1-dependent genes (Li *et al.*, 2007).

To investigate whether KAP1 function is altered during HAd5 infection time course experiments were performed. Therefore, H1299 and A549 cells were infected with wt virus (H5*pg*4100), harvested after the indicated time points and subjected to immunoblotting (Figure 28). Interestingly, these experiments revealed an increase of KAP1 phosphorylation at Ser824 upon HAd5 infection in both cell lines tested. In H1299 cells, KAP1 was phosphorylated after 24 hours, whereas in A549 cells KAP1 phosphorylation occured already 16 h p.i. (Figure 28, lanes 4 and 9). Moreover, this correlated with the expression pattern of the early HAd5 proteins E1A, E1B-55K and E4orf6. Notably, KAP1 protein bands showed reduced intensities after the onset of KAP1 phosphorylation (Figure 28, lanes 5 and 11). Consistent with these data, Forrester and co-workers recently reported that KAP1 is phosphorylated during HAd5 infection in an ATM-dependent manner (Forrester *et al.*, 2011b).

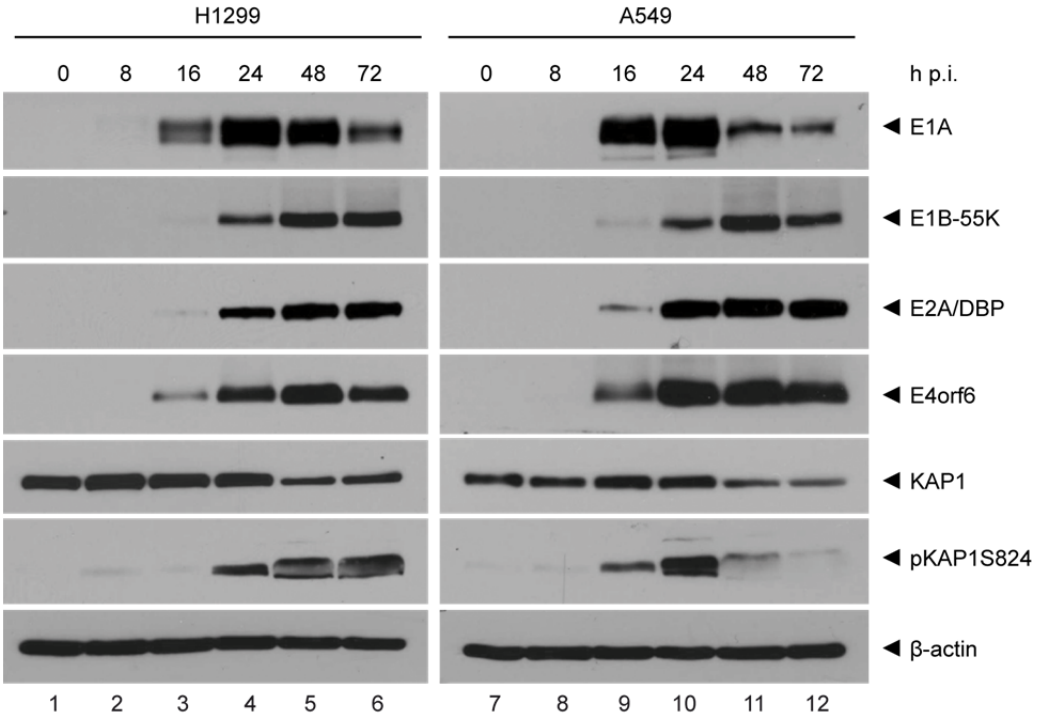


Figure 28: KAP1 is phosphorylated upon HAd5 infection. Subconfluent H1299 and A549 cells were infected with wt virus (H5*pg*4100) at a multiplicity of 50 ffu/cell, harvested after indicated time points and proteins from

total-cell extracts were separated by SDS-PAGE and subjected to immunoblotting using mAb M-73 (α-E1A), mAb 2A6 (αE1B-55K), mAb B6-8 (α-E2A/DBP), mAb RSA3 (α-E4orf6), rbAb H-300 (α-KAP1), rbAb phospho KAP1S824 (α-pKAP1S824) and mAb AC-15 (α-β-actin).

To substantiate these results, infection experiments with increasing numbers of viral particles were performed. Therefore, subconfluent H1299 cells were infected with wt virus (H5*pg*4100) at different multiplicities of infection, harvested after 24 hours and subjected to immunoblotting (Figure 29A). In line with the results obtained from the time course experiments (Figure 28), KAP1 was phosphorylated upon HAd5 infection while no effect on endogenous KAP1 protein levels could be observed (Figure 29A, lanes 2-4). Additionally, cells infected with more viral particles, reflected by increasing E2A/DBP levels, showed respectively higher amounts of phosphorylated KAP1.

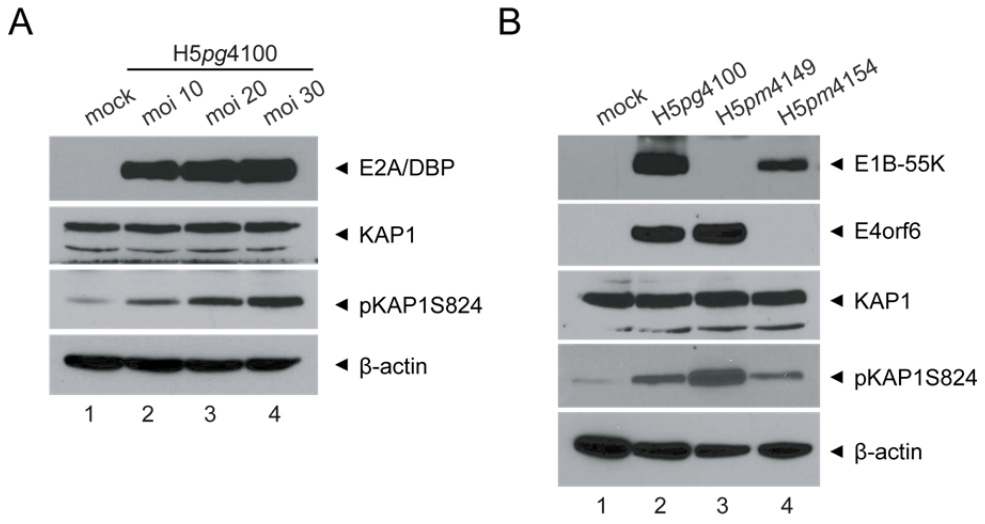


Figure 29: KAP1 phosphorylation is HAd5 concentration-dependent and affected by E1B-55K. (A) Subconfluent H1299 cells were infected with wt virus (H5*pg*4100) at the indicated multiplicities. Cells were harvested 24 h p.i., total-cell extracts were separated by SDS-PAGE and subjected to immunoblotting. Steady-state expression levels of total-cell lysates were detected using mAb B6-8 (α-E2A/DBP), rbAb H-300 (α-KAP1), rbAb phospho KAP1S824 (α-pKAP1S824) and mAb AC-15 (α-β-actin). (B) Subconfluent H1299 cells were infected with wt virus (H5*pg*4100), E1B-minus virus (H5*pm*4149) or E4orf6-minus virus (H5*pm*4154) at a multiplicity of 50 ffu/cell. Cells were harvested 24 h p.i. and total-cell extracts were prepared. Steady-state expression levels of total-cell lysates were detected as described in A using mAb 2A6 (α-E1B-55K), mAb RSA3 (α-E4orf6), rbAb H-300 (α-KAP1), rbAb phospho KAP1S824 (α-pKAP1S824) and mAb AC-15 (α-β-actin).

Various reports revealed that HAd5, especially the early viral proteins E1B-55K and E4orf6, interfere with the cellular phosphorylation pathways to ensure proper virus replication. On the one hand, the two viral proteins can initiate the degradation of cellular proteins involved in phosphorylation processes, like BLM, p53, Mdm2 or

Daxx (Baker *et al.*, 2007; Carson *et al.*, 2003; Cheng *et al.*, 2011; Forrester *et al.*, 2011b; Orazio *et al.*, 2011; Querido *et al.*, 2001a; Schreiner *et al.*, 2010; Stracker *et al.*, 2002). On the other hand, HAd5 inhibits the DDR independently of protein degradation. E4orf6 and E4orf3 have been identified as interaction partners of DNA-PK (Boyer *et al.*, 1999). Additionally, E4orf6 was shown to impair DDR by inhibiting protein phosphatase 2A (PP2A), preventing dephosphorylation of H2AX leading to apoptosis (Hart *et al.*, 2007). Furthermore, HAd5 infection promotes the ATR-dependent phosphorylation of RPA32 (Blackford *et al.*, 2008).

Next, it was analyzed which viral factor is involved in KAP1 phosphorylation. Since KAP1 interacts with E1B-55K (see chapter 5.2.1), infection experiments with wt virus (H5*pg*4100), E1B-minus virus (H5*pm*4149) and E4orf6-minus virus (H5*pm*4154) were performed (Figure 29B). Compared to wt or E4orf6-minus virus infected cells (Figure 29B, lane 2 and 4), cells infected with the E1B-minus virus showed higher levels of phosphorylated KAP1 (Figure 29B, lane 3), suggesting that DNA damage induced KAP1 phosphorylation after the entry of the viral DNA genome is counteracted by the viral phosphoprotein E1B-55K in order to ensure proper virus replication.

To further identify the viral factor which is responsible for KAP1 phosphorylation, transfection experiments were performed in H1299 cells with the early viral proteins E1A-13S, E1B-55K, the phosphorylation-deficient E1B-55K-delP, E2A/DBP and E4orf6 (Figure 30). Since KAP1 phosphorylation occurs upon genotoxic stress (Li *et al.*, 2007), stress conditions were minimized by transfecting the cells with calcium phosphate (see chapter 4.2.3.2), thereby minimizing the basal KAP1 phosphorylation levels. Subsequent immunoblotting revealed that all tested viral proteins were able to increase KAP1 phosphorylation levels (Figure 30, lanes 2 to 6).

Notably, cells transfected with a plasmid encoding for E2A/DBP showed reduced levels of KAP1 phosphorylation in this particular experiment (Figure 30, lane 5). Additional experiments showed no effect on phosphorylated KAP1 after E2A/DBP expression (data not shown). Although equal amounts of plasmids were transfected, viral proteins showed different expression levels, such as E1B-55K and E1B-55K-delP

(Figure 30, lanes 3 and 4). Taken together, no exclusive viral factor could be identified being responsible for KAP1 phosphorylation upon HAd5 infection.

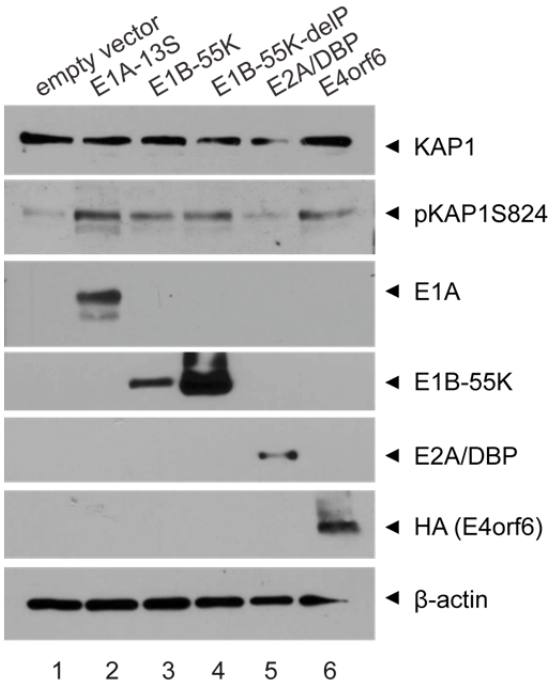


Figure 30: KAP1 phosphorylation is induced by early HAd5 proteins. Subconfluent H1299 cells were transfected with plasmids encoding for E1A-13S, E1B-55K, E1B-55K-delP, E2A/DBP or E4orf6 with Ca-Phosphate (see chapter 4.2.3.2). Cells were harvested 48 h p.t., total-cell extracts were separated by SDS-PAGE and subjected to immunoblotting. Steady-state expression levels of total-cell lysates were detected using rbAb H-300 (α-KAP1), rbAb phospho KAP1S824 (α-pKAP1S824), mAb M73 (E1A), mAb 2A6 (α-E1B-55K), mAb B6-8 (α-E2A/DBP), mAB RSA3 (α-E4orf6) and mAb AC-15 (α-β-actin).

5.8.2 Chromatin-associated factors are altered during HAd5 infection

KAP1 phosphorylation plays a role in chromatin decondensation and enhanced expression of KAP1-dependent genes involved in cell cycle progression and pro-apoptotic processes (el-Deiry *et al.*, 1993; Taylor and Stark, 2001; Vousden and Lu, 2002). Increasing evidence suggests an important role for KAP1 PTM in the highly flexible transcriptional regulation and DNA double-strand break response due to dissociation from chromatin and the alteration of chromatin structure (Goodarzi *et al.*, 2011; Li *et al.*, 2007; White *et al.*, 2011).

To investigate whether HAd5 infection results in changes of dynamic chromatin-associated protein complexes, such as SPOC1 and KAP1, time course experiments

were performed upon wt virus (H5*pg*4100) infection (Figure 31). SPOC1, a DNA-bridging factor of KAP1, is known to inhibit KAP1 phosphorylation and to recruit H3K9 Methyltransferases (KMTs), enhancing H3K9 trimethylation and heterochromatin formation (Mund *et al.*, 2012; Figure 31A). As published recently, SPOC1 shows decreased protein levels during HAd5 infection (Schreiner *et al.*, 2013b; Figure 31B, lanes 4-6). In line with the data obtained so far, KAP1 is phosphorylated upon HAd5 infection (Figure 31B, lanes 2-6). Recently, our group reported that after HAd5 infection, association of the histone variant H3.3 at viral promoters was significantly lost (Schreiner *et al.*, 2013a). Here, decreasing protein levels of histone 3 (H3) were detected (Figure 31B, lanes 5 and 6) due to a reduction of the protein or upcoming modifications, which mask the antibody epitope. Interestingly, the repressive histone mark H3K9me3 showed increasing protein levels early in infection, followed by a decrease late in infection (Figure 31B, lanes 2 and 6).

Taken together, these data suggest a HAd5-mediated alteration of chromatin-associated factors, including SPOC1 proteasomal degradation, KAP1 phosphorylation and H3 modulation to ensure proper HAd5 replication.

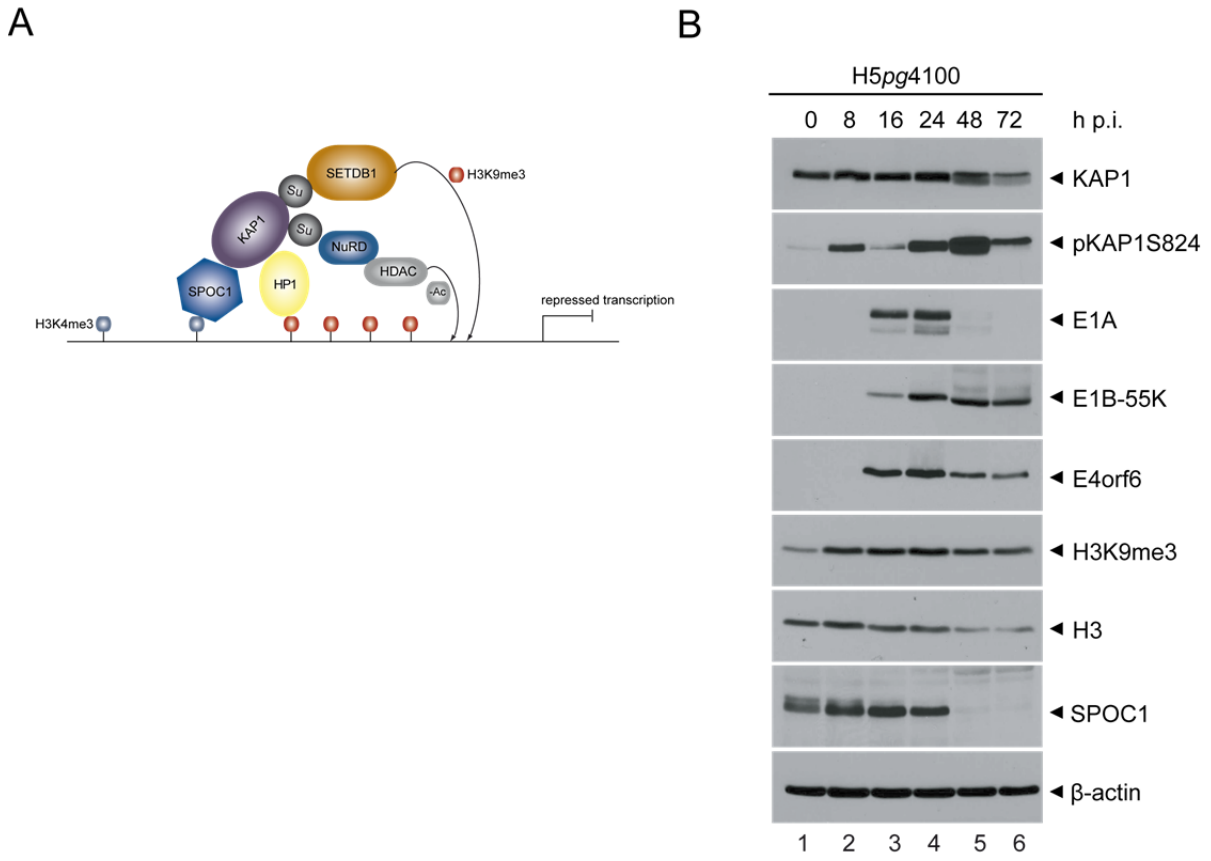


Figure 31: Chromatin-associated factors are altered during HAd5 infection. (A) Schematic representation of SPOC1/KAP1-mediated transcriptional repression via the recruitment of the histone methyl transferase (HMT) SETDB1 and subsequent H3K9me3 modifications. (B) Subconfluent H1299 cells were infected with wt virus (H5*pg*4100) at a multiplicity of 50 ffu/cell and harvested after indicated time points. Total-cell extracts were separated by SDS-PAGE and subjected to immunoblotting. Steady-state expression levels of total-cell lysates were detected using rbAb H-300 (α-KAP1), rbAb phospho KAP1S824 (α-pKAP1S824), mAb M73 (α-E1A), mAb 2A6 (α-E1B-55K), mAb RSA3 (α-E4orf6), rbAb Histone H3 (α-H3K9me3), rbAb H3 (α-Histone 3), rbAb CR56 (α-SPOC1) and mAb AC-15 (α-β-actin).

5.8.3 KAP1 is phosphorylated and relocalized into the soluble fraction

To further investigate the effect of HAd5-mediated KAP1 phosphorylation on its localization, fractionation experiments were performed upon infection with wt virus (H5*pg*4100), E1B-55K-minus virus (H5*pm*4149) and E4orf6-minus virus (H5*pm*4154; Figure 32). Consistent with the data obtained so far, KAP1 was phosphorylated upon HAd5 infection (Figure 32, lanes 2-4). Intriguingly, phosphorylated KAP1 could almost exclusively be found in the soluble fraction (Figure 32, lanes 1-4) and not associated to chromatin. To control each fraction, protein levels of GAPDH (soluble) and histone variant H3 (chromatin fraction) were included. These results provide evidence that upon HAd5 infection KAP1 is efficiently phosphorylated and therefore

recruited into the soluble fraction of the host cell to liberate the chromatin from repressive transcription factors to support efficient gene expression.

In sum, the data strongly indicate a HAd5-mediated alteration of chromatin composition for efficient viral replication.

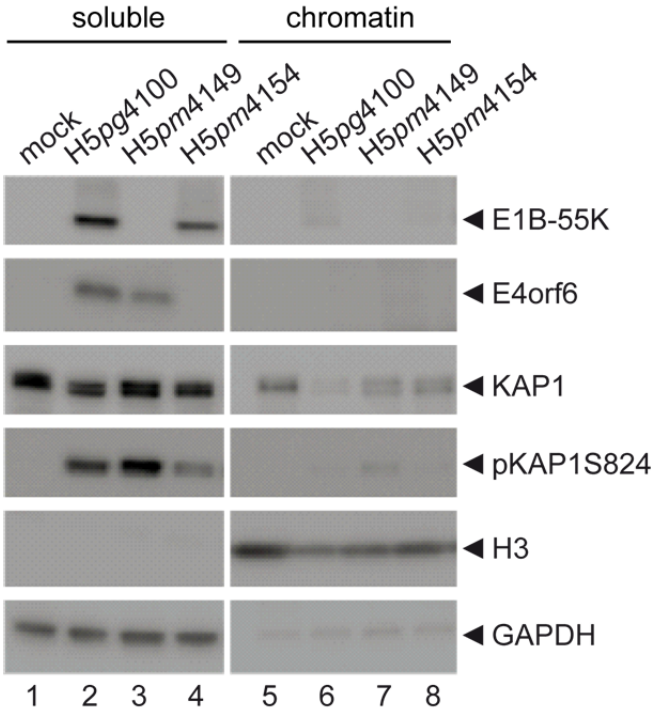


Figure 32: KAP1 is phosphorylated and relocalized into the soluble fraction. Subconfluent H1299 cells were infected with wt virus (H5*pg*4100), E1B-minus virus (H5*pm*4149) or E4orf6-minus virus (H5*pm*4154) at a multiplicity of 50 ffu. Cells were harvested 24 h p.i. and a fractionation was performed as described in chapter 4.5.2. Proteins from the soluble and chromatin fraction were separated by SDS-PAGE and subjected to immunoblotting using mAb 2A6 (α-E1B-55K), mAb RSA3 (α-E4orf6), rbAb H-300 (α-KAP1), rbAb phospho KAP1S824 (α-pKAP1S824), rbAb H3 (α-Histone 3) and mAb 6C5 (α-GAPDH).

5.8.4 KAP1 phosphorylation is necessary for productive infection

Next, it was investigated whether KAP1 phosphorylation affects HAd5 productive infection. Therefore, the phosphorylation-deficient mutant KAP1S824A was generated (Figure 33A) via exchange of the serine at position 824 by an alanine (Figure 32B). First, this mutant version of KAP1 was tested for efficient loss of phosphorylation (Figure 32C) and for its intracellular localization pattern (Figure 32D). Therefore, subconfluent H1299 cells were transfected with KAP1-wt or KAP1S824A, harvested after 48 hours and subjected to immunoblotting. Both constructs showed similar expression levels, however, the antibody specific for

KAP1S824 phosphorylation only detected KAP1 phosphorylation in cells transfected with KAP1-wt (Figure 33C), confirming the generation of a phosphorylation-deficient KAP1 mutant.

To investigate, whether loss of phosphorylation at Ser824 affects intracellular localization of KAP1, immunofluorescence analysis was performed. Therefore, H1299 cells were transfected with flag-tagged KAP1-wt or with the phosphorylation-deficient mutant KAP1S824A, fixed after 48 hours and KAP1 was stained with flag-M2 antibody (Figure 33D). KAP1-wt diffusely localized in the nucleus (Figure 33D, b), which confirms the localization studies with endogenous KAP1 (Figure 14). The newly generated phosphorylation-deficient mutant KAP1S824A showed a similar localization pattern compared to KAP1-wt (Figure 33D, b and e).

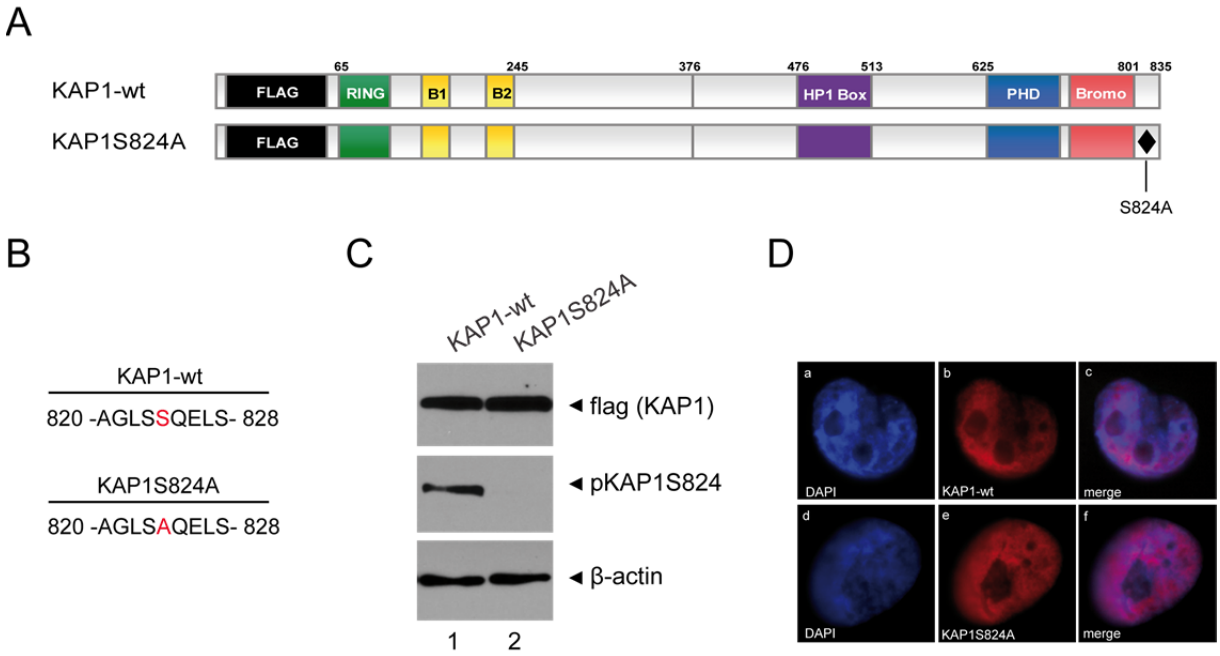


Figure 33: Generation of the phosphorylation-deficient KAP1S824A mutant. (A) Schematic representation of KAP1-wt construct and the phosphomutant KAP1S824A. (B) Amino acid exchange within the KAP1S824A mutant. Mutated amino acid is indicated in red. (C) Subconfluent H1299 cells were transfected with plasmids encoding for KAP1-wt or the phosphomutant KAP1S824A. Total-cell extracts were separated by SDS-PAGE and subjected to immunoblotting. Steady-state expression levels of total-cell lysates were detected using mAb flag-M2 (α-flag/KAP1), rbAb phospho KAP1S824 (α-pKAP1S824) and mAb AC-15 (α-β-actin). (D) H1299 cells were transfected with plasmids encoding for KAP1-wt or the phosphomutant KAPS824A, fixed with methanol 48 h p.t. and labeled with mAb flag-M2 (α-flag/KAP1). The primary antibody was detected using Cy3 (α-flag; red) conjugated secondary antibody. Nuclei are labeled with DAPI. Overlays of single images (merge) are shown in b and d (magnification x 7600).

Next, the phosphorylation-deficient mutant KAP1S824A was included in the infection analysis to investigate the role of KAP1 phosphorylation on virus growth. To eliminate the endogenous KAP1 background, KAP1-depleted H1299 cells were used which were transiently transfected with various KAP1 constructs. Here KAP1-wt, M2, dRBCC, dPB, the double mutant d(RBCC+PB) as internal controls (Figure 34A) and the phosphorylation-deficient mutant KAP1S824A were compared (Figure 34B). After subsequent superinfection with wt virus (H5*pg*4100) reduced levels of E4orf6 and hexon could be observed in cells transfected with KAP1-wt or the HP1 binding-deficient M2 mutant (Figure 34C, lane 2), whereas transfection of the truncation mutants dRBCC, dPB as well as the double mutant d(RBCC+PB) did not impair HAd5 protein expression (Figure 34C, lanes 4-6). Interestingly, cells transfected with the KAP1 phosphorylation-deficient mutant showed an even stronger impact on the reduction of E4orf6 protein levels compared to phosphorylated KAP1-wt (Figure 34C, lane 7).

Taken together, these data strongly indicate a positive effect of KAP1 phosphorylation on HAd5 protein expression later in infection. Moreover, interaction of KAP1 with HP1 seems to be dispensable for efficient repression of HAd5 protein expression, whereas the incapability of KAP1 to get efficiently recruited to DNA (dRBCC) or/and to recruit repressive components (dPB, d(RBCC+PB)) results in a loss of KAP1 repressive function.

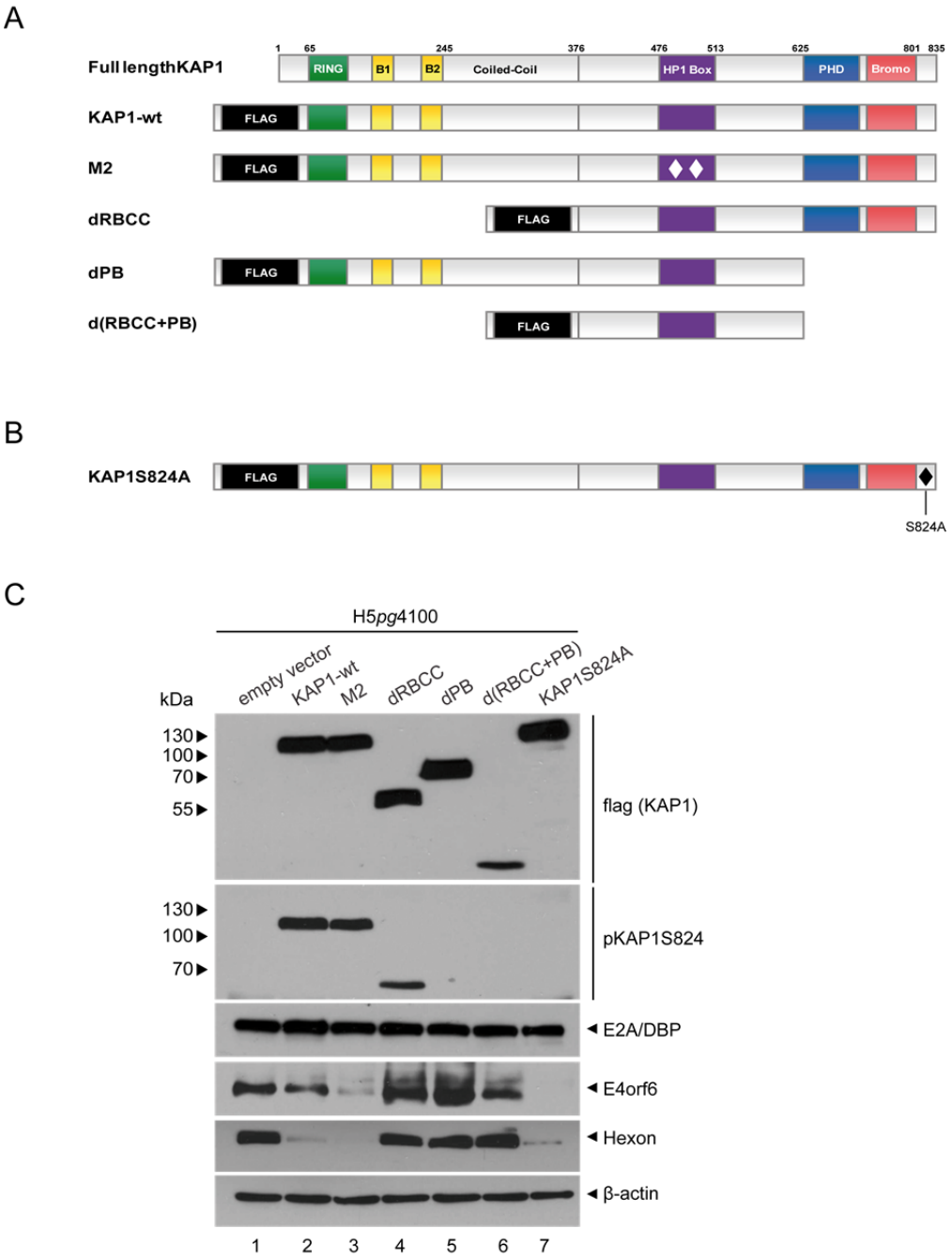


Figure 34: KAP1 phosphorylation is necessary for productive infection. (A) Schematic representation of KAP1-wt and the truncation constructs M2 (2 aa mutation in the HP1 binding domain), dRBCC (deletion of the RBCC domain), dPB (deletion of the PHD and Bromo domain) and d(RBCC+PB) (consisting of the aa region 376 – 625). (B) Schematic representation of the phosphorylation-deficient mutant KAP1S824A. (C) Subconfluent H1299 shKAP1 cells were transfected with plasmids encoding for KAP1-wt or the truncation constructs M2 (two aa mutation in the HP1 binding domain), dRBCC (deletion of the RBCC domain), dPB (deletion of the PHD and Bromo domain) and d(RBCC+PB) (consisting of the aa region 376 – 625). 24 h p.t. the cells were superinfected with wt virus (H5*pg*4100) at a multiplicity of 50 ffu/cell and harvested 24 h p.i.. Total-cell extracts were separated by SDS-PAGE and subjected to immunoblotting. Steady-state expression levels of total-cell lysates were detected using mAb flag-M2 (α-flag/KAP1), rbAb phospho KAP1S824 (α-pKAP1S824), mAb B6-8 (α-E2A/DBP), mAb RSA3 (α-E4orf6), rabbit antiserum L133 to Ad capsid and mAb AC-15 (α-β-actin).

5.8.5 E1B-55K interacts with unphosphorylated and phosphorylated KAP1

Since a HAd5-dependent phosphorylation of KAP1 and strong interaction with E1B-55K was observed, the question was raised whether the viral factor interacts with phosphorylated and unphosphorylated KAP1. To test this, co-immunoprecipitation assays were performed. Therefore, H1299 cells were infected with wt virus (H5*pg*4100). After precipitating endogenous pKAP1S824 with a phosphorylation-specific antibody and subsequent staining for E1B-55K, a specific interaction of both proteins could be detected (Figure 35A, lane 2).

To exclude an involvement of one or more other viral proteins facilitating this interaction, H1299 cells were transfected with a plasmid encoding for E1B-55K and after precipitation of endogenous KAP1 an interaction with E1B-55K could be verified (Figure 35B, lane 2).

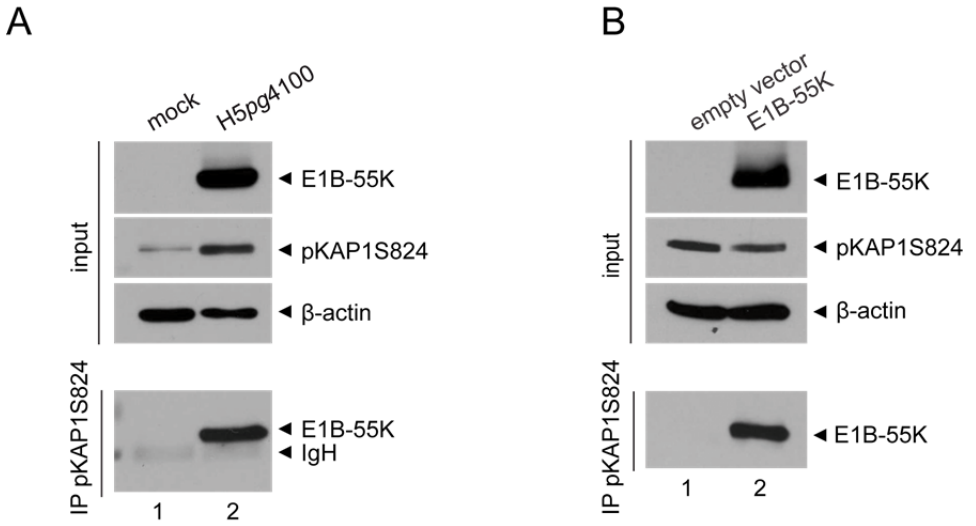


Figure 35: E1B-55K interacts with phosphorylated KAP1. (A) Subconfluent H1299 cells were infected with wt virus (H5*pg*4100), harvested after 24 hours and total-cell extracts were prepaired. Immunoprecipitation of endogenous pKAP1S824 was performed using rbAb phospho KAP1S824 (α-pKAP1S824), and proteins were visualized by immunoblotting. Input levels in total-cell lysates and co-precipitated proteins were detected using mAb 2A6 (α-E1B-55K), rbAb phospho KAP1S824 (α-pKAP1S824) and mAb AC-15 (α-β-actin). (B) Subconfluent H1299 cells were transfected with a plasmid encoding for E1B-55K, harvested after 48 hours and total-cell extracts were prepared. Immunoprecipitation of endogenous pKAP1S824 was performed using rbAb phospho KAP1S824 (α-pKAP1S824), and proteins were visualized by immunoblotting. Input levels in total-cell lysates and co-precipitated proteins were detected using mAb 2A6 (α-E1B-55K), rbAb phospho KAP1S824 (α-pKAP1S824) and mAb AC-15 (α-β-actin).

To confirm the results that KAP1 phosphorylation is no prerequisite for binding to E1B-55K, co-immunoprecipitation experiments with E1B-55K and either KAP1

wildtype or the phosphorylation-deficient mutant KAP1S824A were performed. Therefore, H1299 cells were co-transfected with plasmids encoding for E1B-55K and KAP1 wildtype or KAP1S824A as indicated (Figure 36) and harvested after 48 hours. Co-immunoprecipitation and subsequent immunoblotting revealed that E1B-55K still interacted with the phosphomutant KAP1S824A (Figure 36, lane 3), supporting the hypothesis that E1B-55K interacts not only with phosphorylated but also with unphosphorylated KAP1.

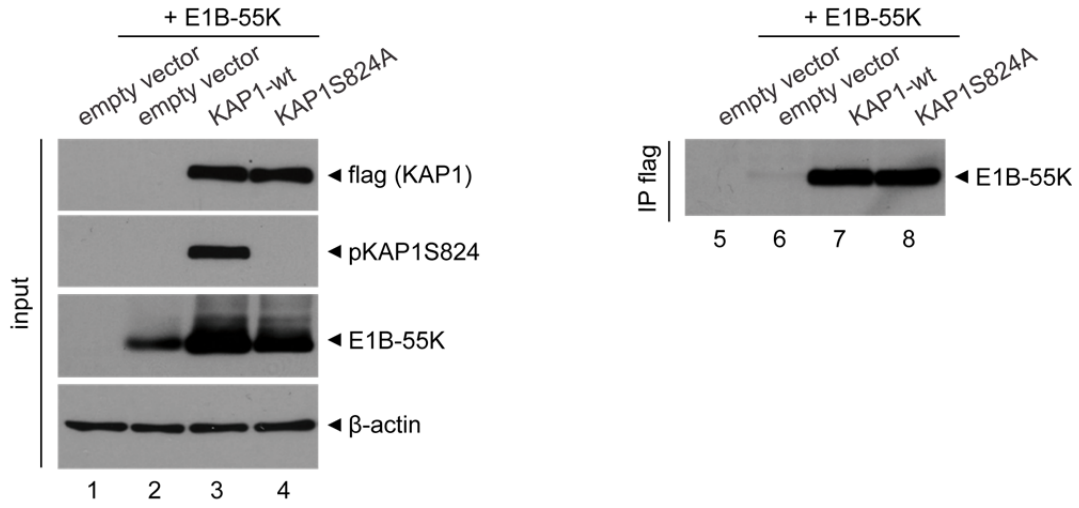


Figure 36: E1B-55K interacts with unphosphorylated and phosphorylated KAP1. Subconfluent H1299 cells were co-transfected with plasmids encoding for E1B-55K and KAP1-wt or the phosphorylation-deficient mutant KAP1S824A, harvested after 48 hours and total-cell extracts were prepared. Immunoprecipitation of KAP1 was performed using M2-flag beads (Sigma) and proteins were visualized by immunoblotting. Input levels in total-cell lysates and co-precipitated proteins were detected using mAb flag-M2 (α-flag/KAP1), rbAb phospho KAP1S824 (α-pKAP1S824) and mAb AC-15 (α-β-actin).

To substantiate the results obtained from the co-immunoprecipitation studies, localization of endogenous KAP1 phosphorylated at Ser 824 was monitored (Figure 37). Therefore, H1299 and A549 cells were infected with wt virus (H5*pg*4100), fixed after 24 hours and stained for phosphorylated KAP1S824 and E1B-55K. In line with the results obtained so far, immunofluorescence studies showed an increase of KAP1S824 phosphorylation in HAd5 infected cells (Figure 37, f, g, and n, o), whereas no phosphorylation could be detected in uninfected cells (Figure 37, b, c and j, k). Additionally, intranuclear localization of the cellular factor was not affected by HAd5 infection. However, compared to unphosphorylated KAP1, phosphorylated

KAP1 showed more distinct loci, which partially co-localized with the nuclear fraction of E1B-55K (Figure 37, f, g, and n, o).

In sum, the co-immunoprecipitation and immunofluorescence data suggest a cooperation of E1B-55K with unphosphorylated and phosphorylated KAP1.

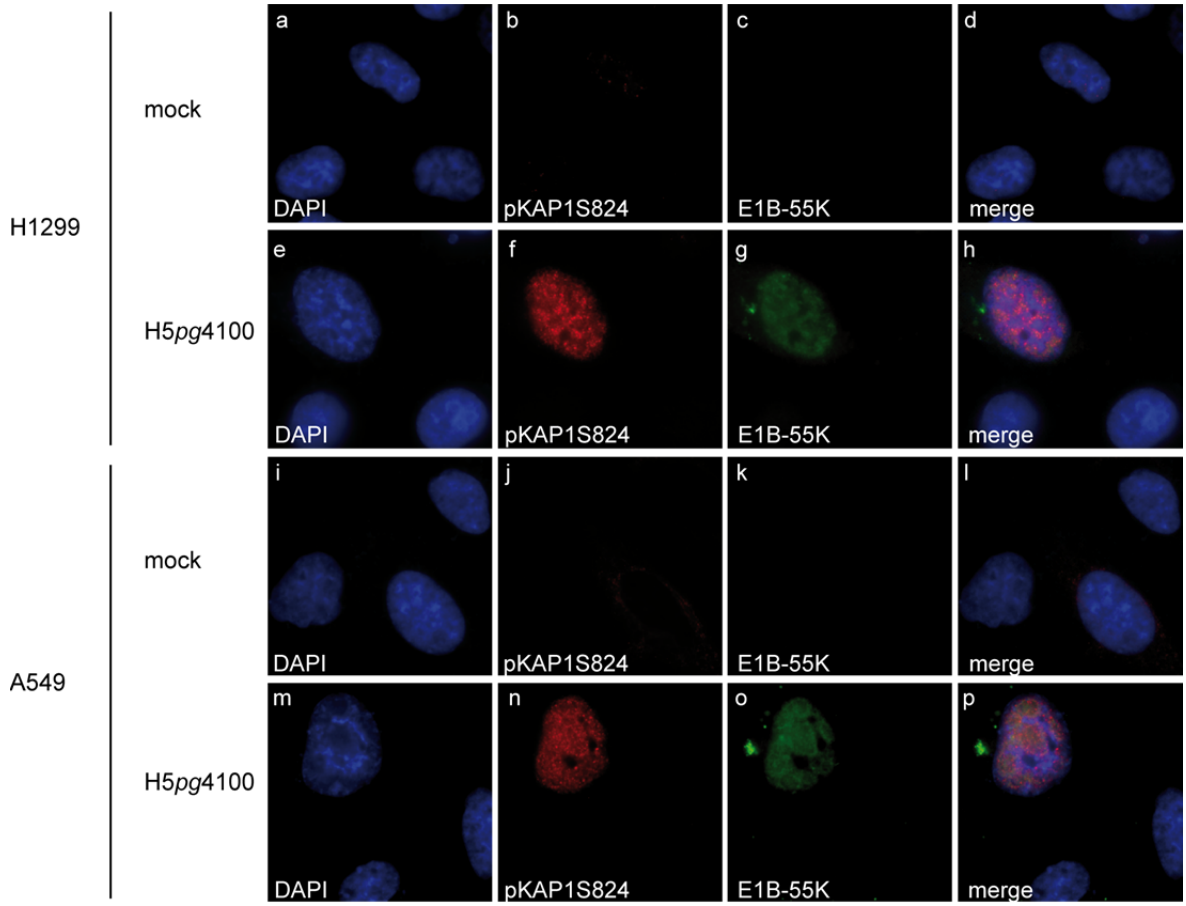


Figure 37: KAP1 is phosphorylated upon HAd5 infection. H1299 and A549 cells were infected with wt virus (H5*pg*4100), fixed with methanol 24 h p.i. and double-labeled with rbAb phospho KAP1S824 (α-pKAP1S824) and mAb 2A6 (α-E1B-55K). Primary antibodies were detected using Cy3 (α-pKAP1S824; red) and FITC (α-E1B-55K; green) conjugated secondary antibodies. Nuclei are labeled with DAPI. Overlays of single images (merge) are shown in d, h, l, p (magnification x 7600).

5.8.6 KAP1 is deSUMOylated upon HAd5 infection

Several studies have shown that besides phosphorylation KAP1 also undergoes SUMOylation as a prerequisite for its repressive function (Ivanov *et al.*, 2007; Mascle *et al.*, 2007). The small ubiquitin-like modifiers (SUMOs) share sequence and structural similarity with ubiquitin (Geiss-Friedlander and Melchior, 2007; Johnson, 2004) and are required for cell viability (Hayashi *et al.*, 2002; Johnson *et al.*, 1997; Nacerddine *et al.*, 2005; Seufert *et al.*, 1995). Extensive work in this field led to the

identification of a large set of SUMO substrates involved in gene transcription (Gill, 2005; Girdwood *et al.*, 2004; Vertegaal *et al.*, 2006).

Recently, Li and co-workers reported that KAP1 SUMOylation decreases KAP1-dependent gene expression, suggesting a regulation of its co-repressive functions (Li *et al.*, 2007). DeSUMOylation of KAP1 is required to relieve its transcriptional repression at KAP1-responsive genes, such as *p21, bax, puma* and *noxa* (Lee *et al.*, 2007; Li *et al.*, 2007). Although extensive literature exists regarding KAP1 functions, little is known about the coordinated loss of KAP1-mediated repression which is orchestrated and regulated by multiple PTMs. Lee and co-workers identified Lys554, Lys676, Lys779 and Lys804 as highly active SUMOylation sites in KAP1, responsible for the repressive transcriptional potential (Lee *et al.*, 2007).

Several early HAd5 proteins interact with enzymes or resemble substrates of the SUMOylation pathway. In this context, E1B-55K is the most extensively studied HAd5 protein and was shown to interact with the SUMO E2 enzyme Ubc9 (Wimmer *et al.*, 2013). Futhermore, E1B-55K harbors a SUMO conjugation motive (SCM) and represents a substrate for the host cell SUMO modification system (Endter *et al.*, 2005; Endter *et al.*, 2001). Recently, evidence was generated that PML-NB-associated E1B-55K bears also E3 SUMO ligase activity towards p53 (Müller and Dobner, 2008; Pennella *et al.*, 2010; Wimmer *et al.*, 2015).

To investigate the effect of HAd5 infection on KAP1 SUMOylation, H1299 cells were co-transfected with KAP1-wt and SUMO1 encoding constructs in the combinations indicated. 24 hours later, the cells were infected with wt virus (H5*pg*4100) and harvested after additional 24 hours (Figure 38). Staining of endogenous KAP1 revealed a reduction of unSUMOylated KAP1 when co-expressed with SUMO1 in uninfected (Figure 38, lanes 2 and 3) and infected cells (Figure 38, lanes 4 and 6). Additionally, in uninfected cells co-transfected with KAP1 and SUMO1 a slower migrating band at approximately 160 to 200 kDa could be observed (Figure 38, lane 3) which could not be detected in cells where SUMO1 was not overexpressed (Figure 38, lane 2). Interestingly, this higher migrating band was dramatically reduced upon HAd5 infection (Figure 38, lane 6). Furthermore, slower migrating bands already

occured in cells transfected with the empty vector control (Figure 38, lane 1), which also were reduced in HAd5 infected cells (Figure 38, lane 4). Interestingly, staining of the early HAd5 protein E2A/DBP revealed increasing protein levels upon co-transfection of KAP1 alone and even more in combination with SUMO1 (Figure 38, lane 6), suggesting a KAP1-dependent stabilization of E2A/DBP.

This data strongly suggests a HAd5-mediated regulation of KAP1 function by reducing SUMO-KAP1 moieties.

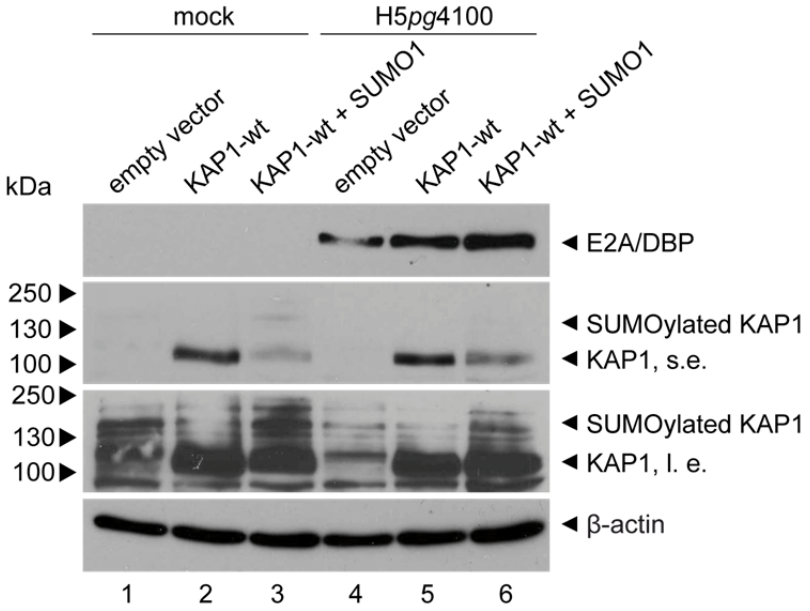


Figure 38: KAP1 is deSUMOylated upon HAd5 infection. Subconfluent H1299 cells were transfected with plasmids encoding for KAP1-wt and SUMO1 as indicated and 24 h p.t. superinfected with wt virus (H5*pg*4100) at a multiplicity of 50 ffu/cell. 24 h p.i. cells were harvested and total-cell extracts were separated by SDS-PAGE and subjected to immunoblotting. Steady-state expression levels of total-cell lysates were detected using mAb B6-8 (α-E2A/DBP), rbAb H-300 (α-KAP1) and mAb AC-15 (α-β-actin). s.e.: short exposure; l.e.: long exposure. Molecular weight in kDa is indicated on the left, while the relevant proteins are labeled on the right.

To substantiate the observation that KAP1 SUMO modification is reduced upon HAd5 infection, a model system developed by Tatham *et al.* was used, allowing the detection of protein SUMOylation *in vivo* (Tatham *et al.*, 2009). In their system, the 6His-SUMO1, SUMO2 or SUMO3 constructs stably expressed in HeLa cells can be purified from extracts using nickel affinity chromatography under denaturing conditions. Proteins covalently modified by 6His-SUMO can be purified and analyzed by Western Blot for the protein of interest (Figure 39A).

In this context, parental and HeLa cells expressing 6His-SUMO1/2 were transfected with a plasmid encoding for KAP1-wt and superinfected with wt virus (H5*pg*4100) 8 h p.t. (Figure 39B). Immunoblotting of Ni-NTA-purified His-SUMO conjugates (Figure 39B, left side) and crude lysates (Figure 39B, right side) revealed that KAP1 SUMO2 modification is reduced during HAd5 infection (Figure 39B, lanes 5 and 6). However, SUMO1 conjugation on KAP1 could not be detected at this time point of infection (Figure 39B, lanes 3 and 4). Interestingly, after staining the Ni-NTA-purified conjugates with an antibody specific for 6His an increase of SUMO1 and SUMO2 modified proteins in HAd5 infected cells could be observed compared to the mock controls (Figure 39B, lanes 3, 4 and 5, 6).

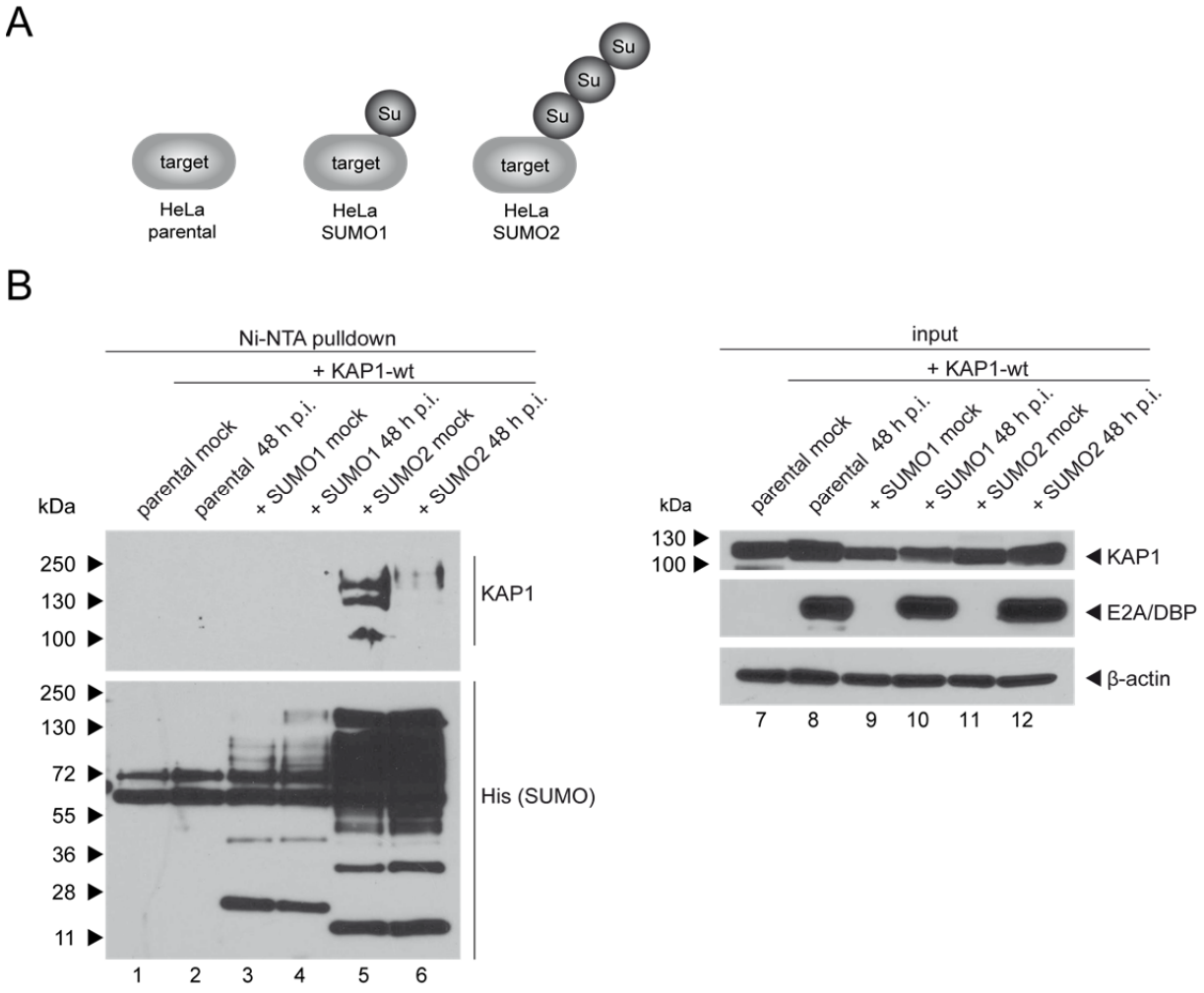


Figure 39: KAP1 SUMO2 modification is reduced upon HAd5 infection. (A) Schematic representation of the modelsystem consisting of parental and HeLa cells expressing 6His-SUMO1/2 (developed by Tatham *et al.*, 2009). (B) Parental HeLa cells and HeLa cells stably expressing 6His-SUMO1 or 6His-SUMO2 were transfected with a plasmid encoding for KAP1-wt and superinfected with wt virus (H5*pg*4100) at a multiplicity of 50 ffu/cell. 48 h p.i. total-cell lysates were prepared with guanidinium chloride buffer, subjected to Ni-NTA purification of 6His-SUMO conjugates and analyzed via SDS-PAGE and immunoblotting. Input levels of total-cell lysates and

Ni-NTA-purified proteins were detected using rbAb H-300 (α-KAP1), mAb 6xHis, mAb B6-8 (α-E2A/DBP) and mAb AC-15 (α-β-actin). Molecular weight in kDa is indicated on the left, while the relevant proteins are labeled on the right.

5.8.7 E1B-55K is responsible for KAP1 deSUMOylation in infected cells

Several early HAd5 proteins interact with enzymes or substrates of the SUMOylation pathway. E1B-55K itself is a substrate for the host cell SUMO modification system and bears a SUMO conjugation motif (SCM; Endter *et al.*, 2005; Endter *et al.*, 2001). PTM of E1B-55K is critical for its localization and function regarding the inactivation of the tumor suppressor p53 and the degradation of Daxx (Schreiner *et al.*, 2011; Schreiner *et al.*, 2010). Additionally, early HAd5 E4orf6 was identified as a negative factor for E1B-55K SUMOylation (Lethbridge *et al.*, 2003). Recently, our group reported that E1B-55K interacts with the cellular SUMO E2 enzyme Ubc9 (Wimmer *et al.*, 2013). Interestingly, the PHD domain of cellular KAP1 functionally also interacts with Ubc9 to facilitate the SUMOylation of the adjacent bromodomain (Ivanov *et al.*, 2007).

To investigate whether the reduction of high molecular weight forms of SUMO2-modified KAP1 depends on the viral early proteins E1B-55K or E4orf6, parental and HeLa cells expressing His-SUMO2 were transfected with a plasmid encoding for KAP1-wt and superinfected with wt virus (H5*pg*4100) or mutant HAd5 viruses lacking either E1B-55K (H5*pm*4149) or E4orf6 (H5*pm*4154; Figure 40). After immunoblotting of Ni-NTA-purified His-SUMO conjugates (Figure 40, left side) and crude lysates (Figure 40, right side), significantly less high molecular weight SUMO2 modified forms of KAP1 could be observed upon infection with wt virus or the E4orf6-minus virus (Figure 40, lanes 6 and 8). However, infection with the E1B-55K-minus virus did not affect KAP1 SUMOylation (Figure 40, lane 7), indicating that KAP1 deSUMOylation highly depends on the expression of early viral E1B-55K. Notably, viral protein levels of E1B-55K, E4orf6 and E2A/DBP were increased in His-SUMO2 expressing HeLa cells (Figure 40, right side, lanes 10-12 and 14-16).

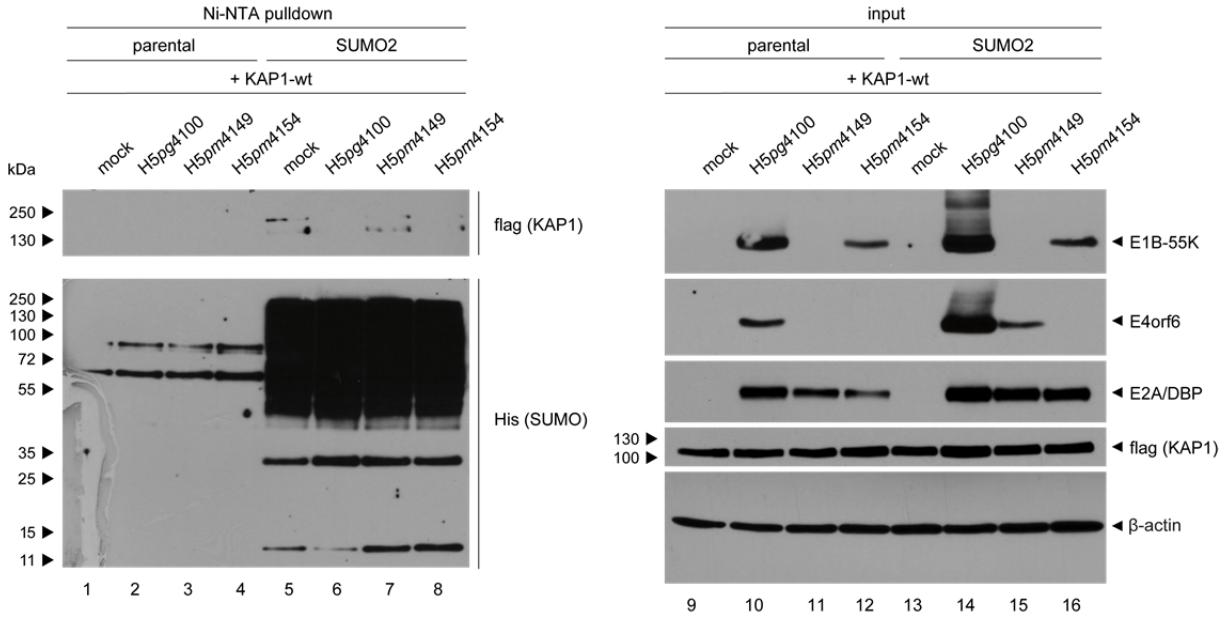


Figure 40: E1B-55K is responsible for KAP1 deSUMOylation in infected cells. Parental HeLa cells and HeLa cells stably expressing 6His –SUMO2 were transfected with a plasmid encoding for KAP1-wt and superinfected with wt virus (H5*pg*4100), E1B-minus virus (H5*pm*4149) or E4orf6-minus virus (H5*pm*4154) at a multiplicity of 50 ffu/cell. 48 h p.i. total-cell lysates were prepared with guanidinium chloride buffer, subjected to Ni-NTA purification on 6His-SUMO conjugates and analyzed via SDS-PAGE and immunoblotting. Input levels of total-cell lysates and Ni-NTA purified proteins were detected using mAb flag-M2 (α-flag/KAP1), mAb 6xHis, mAb 2A6 (α-E1B-55K), mAb RSA3 (α-E4orf6), mAb B6-8 (α-E2A/DBP) and mAb AC-15 (α-β-actin). Molecular weight in kDa is indicated on the left, while the relevant proteins are labeled on the right.

Taken together, these data indicate that KAP1 is deSUMOylated upon HAd5 infection in an E1B-55K-dependent manner.

5.8.8 E1B-55K SUMOylation at lysine 104 promotes KAP1 deSUMOylation

SUMOylation of E1B-55K is a prerequisite for the inhibition of p53 by its nuclear export to cytoplasmic E1B-55K aggresomes as well as for E1B-55K association with PML-NBs. Additionally, E1B-55K was shown to function as an E3 SUMO1 ligase for p53 (Müller and Dobner, 2008; Pennella *et al.*, 2010). Furthermore, unpublished data from our group also suggest an E1B-55K-dependent increase of Sp100A SUMOylation. However, E1B-55K could so far never been shown to be involved in deSUMOylation processes.

Based on the novel findings that KAP1 deSUMOylation is dependent on E1B-55K (Figure 40), it was tested whether E1B-55K SUMOylation itself affects KAP1 deSUMOylation. Our group identified a SUMO1-conjugation motif (SCM) at Lys104 in the E1B-55K protein, which dramatically affects its function and localization

(Endter *et al.*, 2001; Kindsmuller *et al.*, 2007). This raised the question whether the SCM of E1B-55K might affect KAP1 deSUMOylation. Therefore, parental and HeLa cells expressing His-SUMO2 were transfected with KAP1-wt and superinfected with wt virus (H5*pg*4100) or E1B-55K-K104R mutant virus (H5*pm*4102; Figure 41). Immunoblotting of Ni-NTA-purified His-SUMO conjugates and crude lysates revealed that the reduction of high molecular weight forms of SUMO2 modified KAP1 depends on SUMOylation of E1B-55K at lysine residue 104 (Figure 41, lane 6).

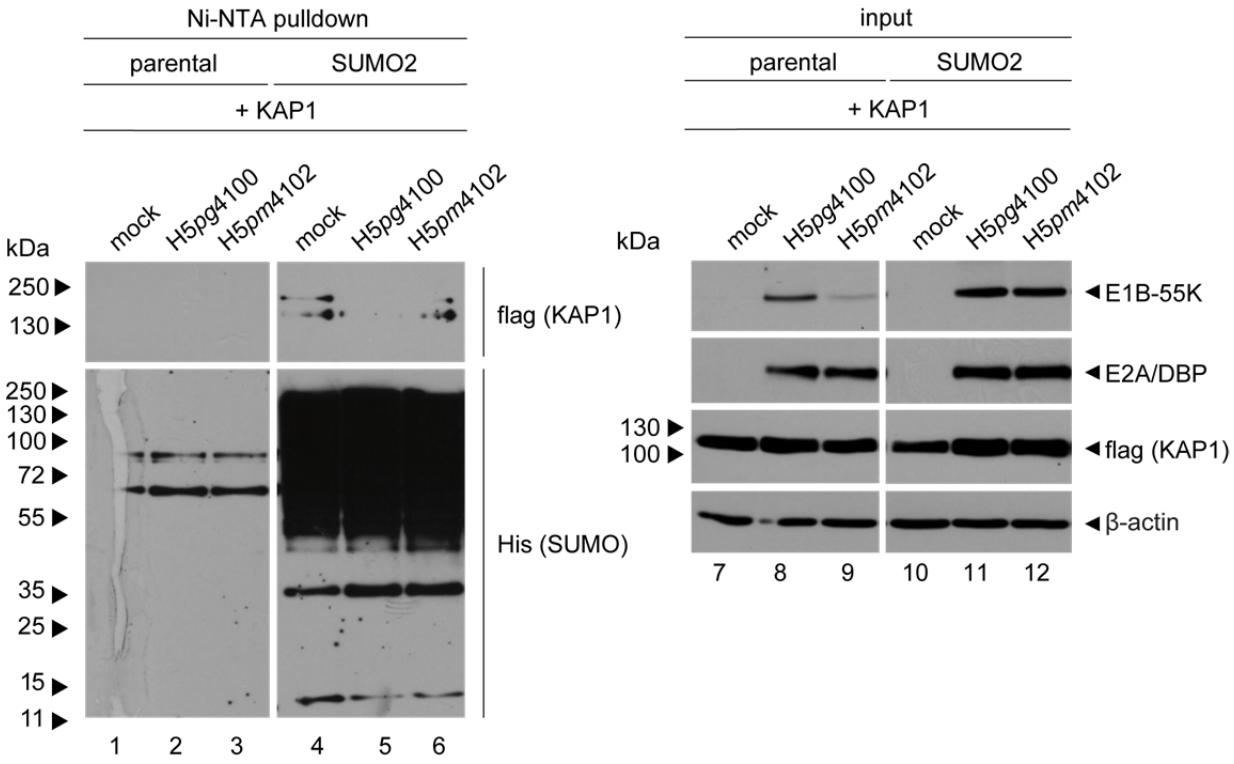


Figure 41: E1B-55K K104 SUMOylation is necessary for KAP1 deSUMOylation in infected cells. Parental HeLa cells and HeLa cells stably expressing 6His-SUMO2 were transfected with a plasmid encoding for KAP1-wt and after 24 hours superinfected with wt virus (H5*pg*4100) or the E1B-55K-K104R mutant (H5*pm*4102) at a multiplicity of 50 ffu/cell. 48 h p.i. total-cell lysates were prepared with guanidinium chloride buffer, subjected to Ni-NTA purification on 6His-SUMO conjugates and analyzed via SDS-PAGE and immunoblotting. Input levels of total-cell lysates and Ni-NTA purified proteins were detected using mAb flag-M2 (α-flag/KAP1), mAb 6xHis, mAb 2A6 (α-E1B-55K), mAb B6-8 (α-E2A/DBP) and mAb AC-15 (α-β-actin). Molecular weight in kDa is indicated on the left, while the relevant proteins are labeled on the right.

To substantiate the novel finding that K104 SUMOylated E1B-55K plays a role in the reduction of KAP1 SUMO2 modification, co-transfection experiments without the viral background were performed. Therefore, His-SUMO2 conjugates were purified (Ni-NTA) after co-transfection of plasmids encoding for KAP1-wt and wt E1B-55K or the SUMO mutant E1B-55K-K104R (Figure 42). Subsequent immunoblotting of Ni-

NTA-purified His-SUMO conjugates and crude lysates showed significantly less high molecular weight SUMO2 modified forms of KAP1 in cells co-transfected with wt E1B-55K (Figure 42, lane 2). However, decrease of KAP1 SUMO2 modification was not observed when co-transfected with E1B-55K-K104R (Figure 42, lane 3).

Taken together, these data strongly suggest that SUMOylation of E1B-55K-K104 is a prerequisite for KAP1 deSUMOylation.

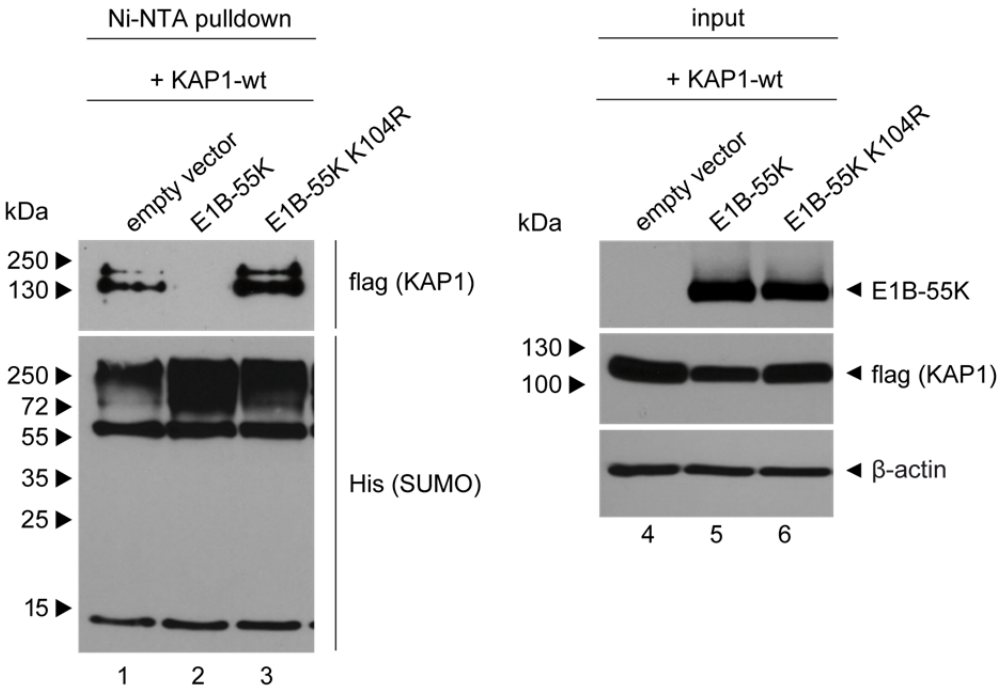


Figure 42: E1B-55K K104 SUMOylation is necessary for KAP1 deSUMOylation in transfected cells. HeLa cells stably expressing 6His-SUMO2 were co-transfected with plasmids encoding for KAP1-wt and E1B-55K or the SUMO mutant E1B-55K K104R. 48 h p.t. total-cell lysates were prepared with guanidinium chloride buffer, subjected to Ni-NTA purification on 6His-SUMO conjugates and analyzed via SDS-PAGE and immunoblotting. Input levels of total-cell lysates and Ni-NTA purified proteins were detected using mAb flag-M2 (α-flag/KAP1), mAb 6xHis, mAb 2A6 (α-E1B-55K), mAb 2A6 (α-E1B-55K), mAb B6-8 (α-E2A) and mAb AC-15 (α-β-actin). Molecular weight in kDa is indicated on the left, while the relevant proteins are labeled on the right.

5.8.9 HAd5 proteins differentially regulate KAP1 SUMOylation

Since SUMO proteins regulate different processes within the cell (Hay, 2006; Seeler and Dejean, 2003), it is not surprising that viral pathogens take advantage of this PTM to manipulate cellular pathways and maintain the integrity of viral proteins (Wimmer *et al.*, 2013). KAP1 SUMOylation enhances its co-repressive function and condensation of chromatin in order to silence KAP1-dependent genes (Ivanov *et al.*, 2007; Iyengar and Farnham, 2011; Iyengar *et al.*, 2011; Lee *et al.*, 2007; Schultz *et al.*,

2002). For efficient gene expression the regulation of its repressive function by SUMOylation and deSUMOylation processes has to be highly dynamic. Since KAP1 deSUMOylation is E1B-55K-dependent during infection (Figure 40), the question is raised whether other HAd5 proteins could also affect KAP1 SUMOylation status.

5.8.9.1 HAd5 virus particle-associated and immediate early proteins induce KAP1 SUMOylation

Our group recently reported that Daxx forms a chromatin remodeling complex with ATRX, resulting in the repression of HAd5 replication (Schreiner *et al.*, 2013a). As one of the first HAd5 proteins occuring in the cell, the internal capsid protein pVI is released by the partial disassembly of the endocytosed capsid (Wiethoff *et al.*, 2005). Besides its role in lysis of endosomal membranes and capsid transport to the nucleus (Wiethoff *et al.*, 2005; Wodrich *et al.*, 2010), our group showed that it is targeted to PML-NBs, where it interacts with and counteracts Daxx, thereby activating the HAd5 E1A promoter (Schreiner *et al.*, 2013a). The immediate early protein E1A is the first viral protein to be expressed upon HAd5 infection, thereby activating downstream viral expression units and promoting cellular gene expression (Berk, 2005). Previously, E1A was reported to repress SUMO modification of the retinoblastoma protein and interacts with the SUMO-conjugating enzyme Ubc9 (Ledl *et al.*, 2005; Yousef *et al.*, 2010).

To investigate the effect of the HAd5 virus particle-associated protein pVI and the immediate early protein E1A on the SUMO modification of KAP1, HeLa cells expressing His-SUMO2 were co-transfected with plasmids encoding for KAP1-wt and increasing amounts of the respective HAd5 protein. His-SUMO2 conjugates were purified (Ni-NTA) and immunoblotting revealed that the expression of the HAd5 proteins pVI (Figure 43A) and E1A-13S (Figure 43B) increased the levels of SUMO2 modified KAP1 in a dose-dependent manner (Figure 43A and B, lanes 2 and 3).

Taken together, these data indicate that the virus particle-associated protein pVI and the immediate early protein E1A-13S induce KAP1 SUMO modification.

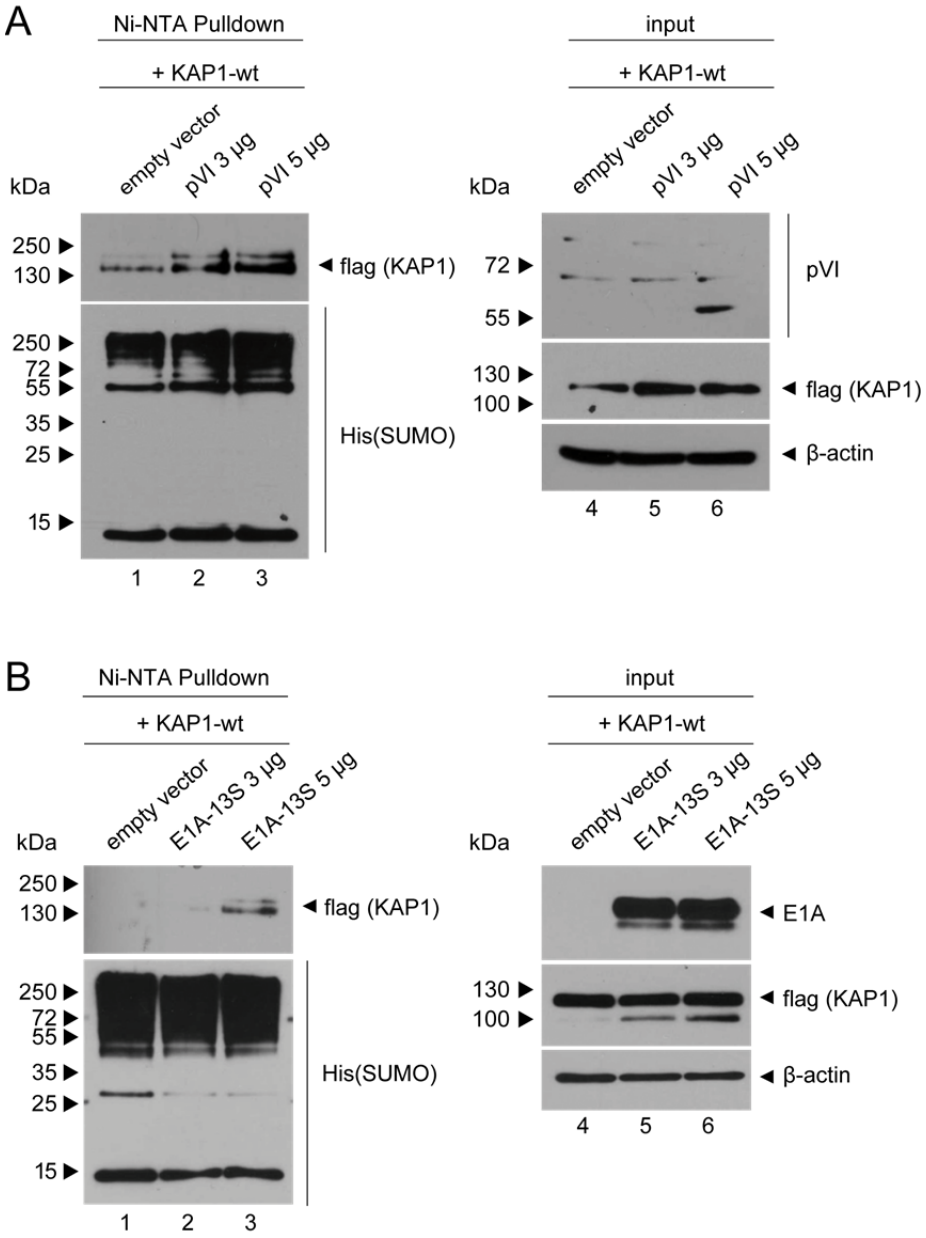
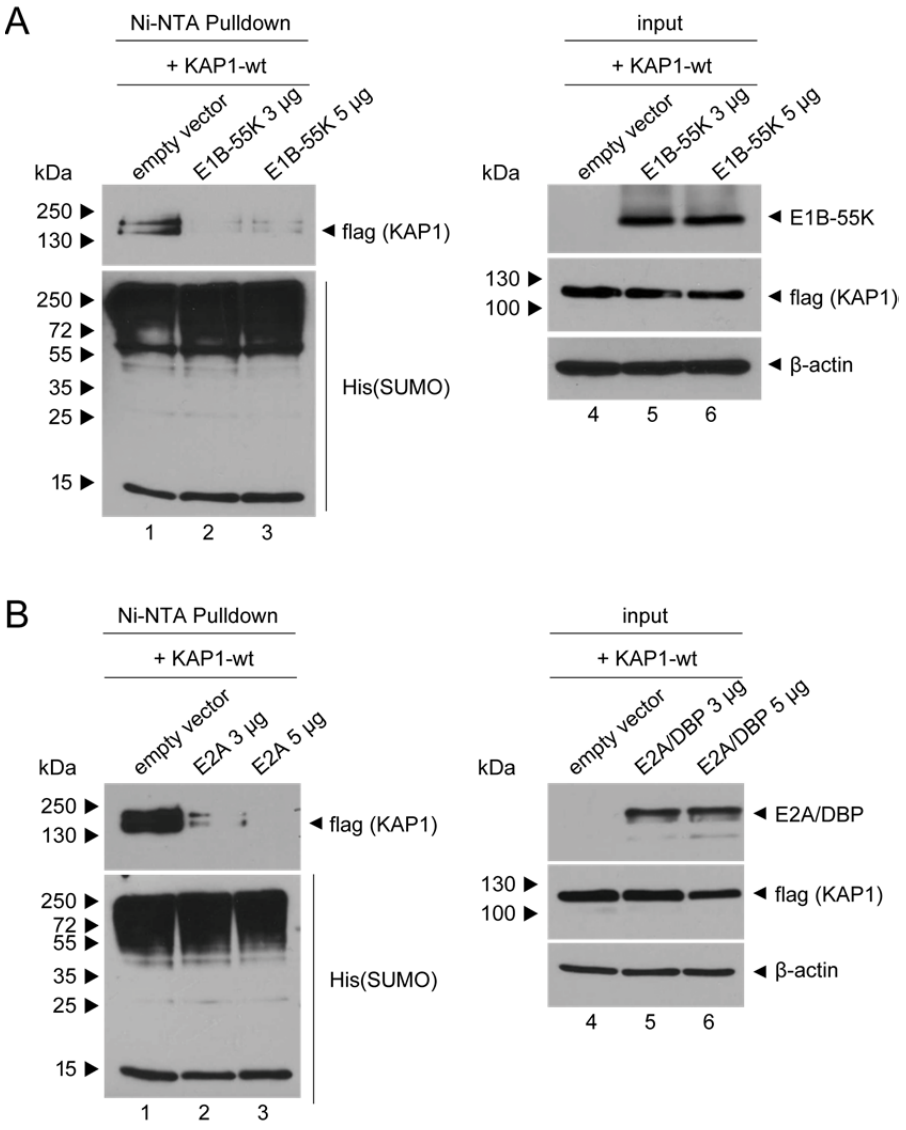


Figure 43: HAd5 virus particle-associated and immediate early proteins induce KAP1 SUMOylation. HeLa cells stably expressing 6His-SUMO2 were transfected with plasmids encoding for KAP1-wt and increasing amounts of (A) pVI or (B) E1A-13S. 48 h p.t. total-cell lysates were prepared with guanidinium chloride buffer, subjected to Ni-NTA purification on 6His-SUMO conjugates and analyzed via SDS-PAGE and immunoblotting. Input levels of total-cell lysates and Ni-NTA purified proteins were detected using mAb flag-M2 (α-flag/KAP1), mAb 6xHis, rbAb pVI (α-pVI), mAb M73 (α-E1A) and mAb AC-15 (α-β-actin). Molecular weight in kDa is indicated on the left, while the relevant proteins are labeled on the right.

5.8.9.2 HAd5 early and late proteins induce KAP1 deSUMOylation

The expression of E1A activates the downstream viral expression units E1B, E2, E3 and E4 (Berk, 2005). Since co-transfection of KAP1 together with the early viral

protein E1B-55K led to a decrease of SUMO2-modified KAP1 levels (Figure 42, lanes 1 and 2), it was further investigated whether other HAd5 proteins affect KAP1 SUMOylation as well. Therefore, HeLa cells expressing His-SUMO2 were co-transfected with plasmids encoding for KAP1-wt and increasing amounts of the respective HAd5 protein. His-SUMO2 conjugates were purified (Ni-NTA) and subjected to immunoblotting. Consistent with the data obtained, co-transfection of KAP1 together with increasing amounts of E1B-55K resulted in a decrease of KAP1 SUMO2 modification (Figure 44A, lanes 2 and 3). Interestingly, this effect could also be observed after co-transfection with the early viral proteins E2A/DBP (Figure 44B, lanes 2 and 3), E4orf6 (Figure 44C, lane 3) and the late core protein pV (Figure 44D, lanes 2 and 3).



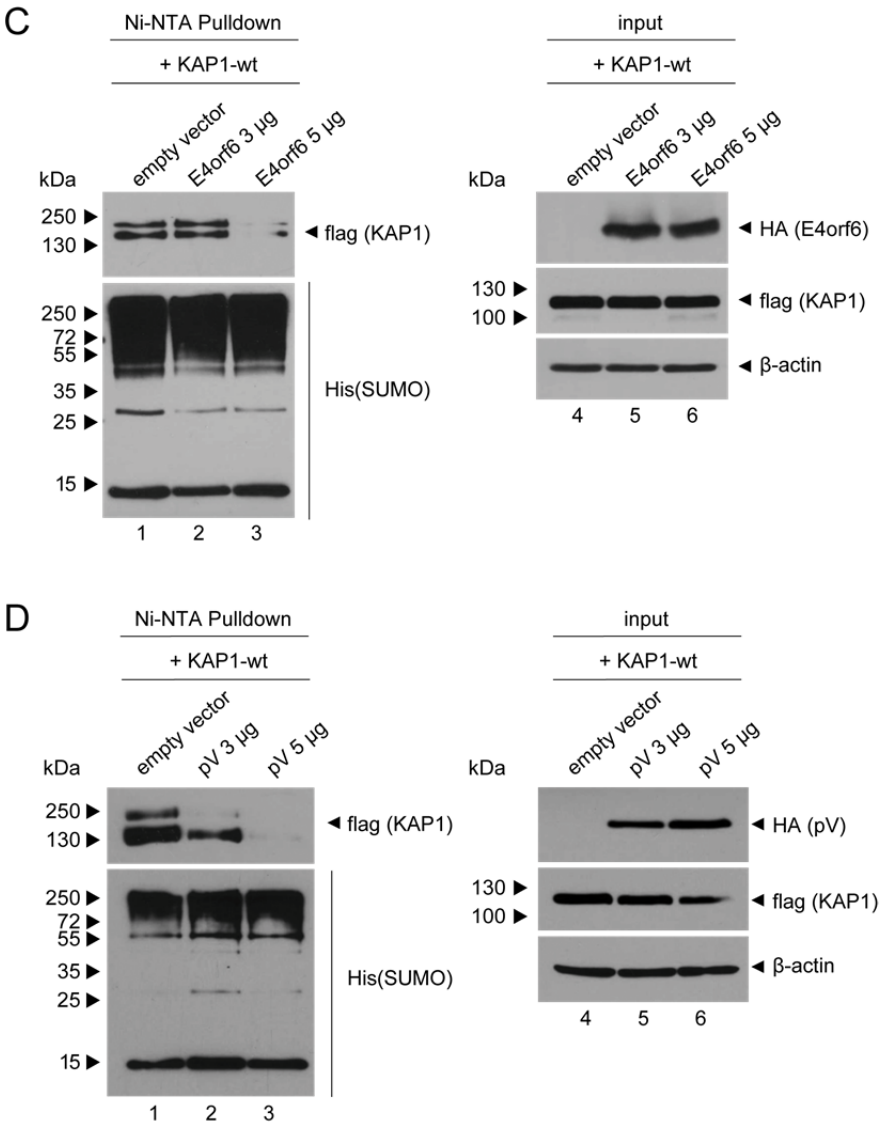


Figure 44: Later HAd5 proteins induce KAP1 deSUMOylation. HeLa cells stably expressing 6His-SUMO2 were transfected with plasmids encoding for KAP1-wt and increasing amounts of (A) E1B-55K, (B) E2A/DBP, (C) E4orf6 or (D) pV. 48 h p.t. total-cell lysates were prepared with guanidinium chloride buffer, subjected to Ni-NTA purification on 6His-SUMO conjugates and analyzed via SDS-PAGE and immunoblotting. Input levels of total-cell lysates and Ni-NTA purified proteins were detected using mAb flag-M2 (α-flag/KAP1), mAb 6xHis, mAb 2A6 (α-E1B-55K), mAb B6-8 (α-E2A/DBP), rAb 3F10 (α-HA/E4orf6 and pV) and mAb AC-15 (α-β-actin). Molecular weight in kDa is indicated on the left, while the relevant proteins are labeled on the right.

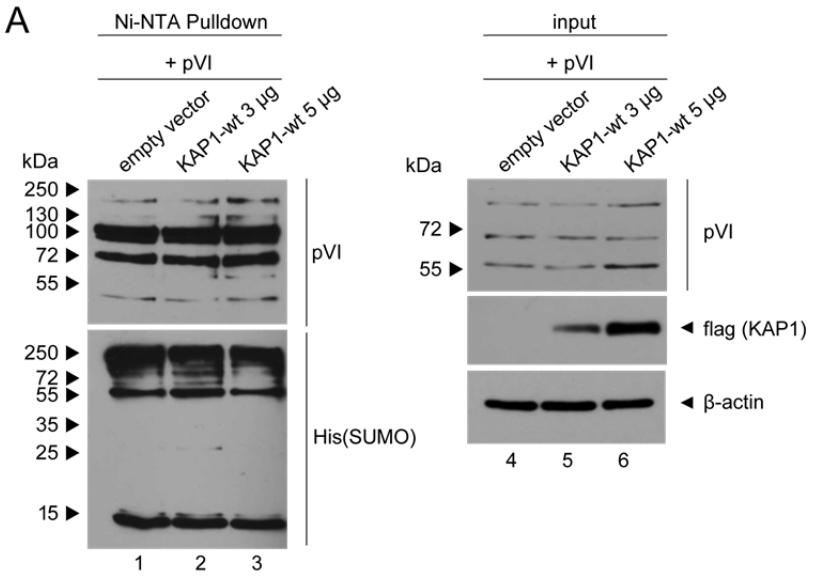
In sum, dependent on their kinetics and onset of expression, a differential regulation of KAP1 SUMOylation could be observed for the different HAd5 proteins. Whereas the HAd5 virus particle-associated protein pVI and the immediate early protein E1A-13S lead to increased amounts of KAP1 SUMOylation, later HAd5 protein expression induced a decrease of KAP1 SUMO modification. These data suggest a highly flexible and dynamic KAP1 SUMOylation switch to modulate KAP1 function during HAd5 infection.

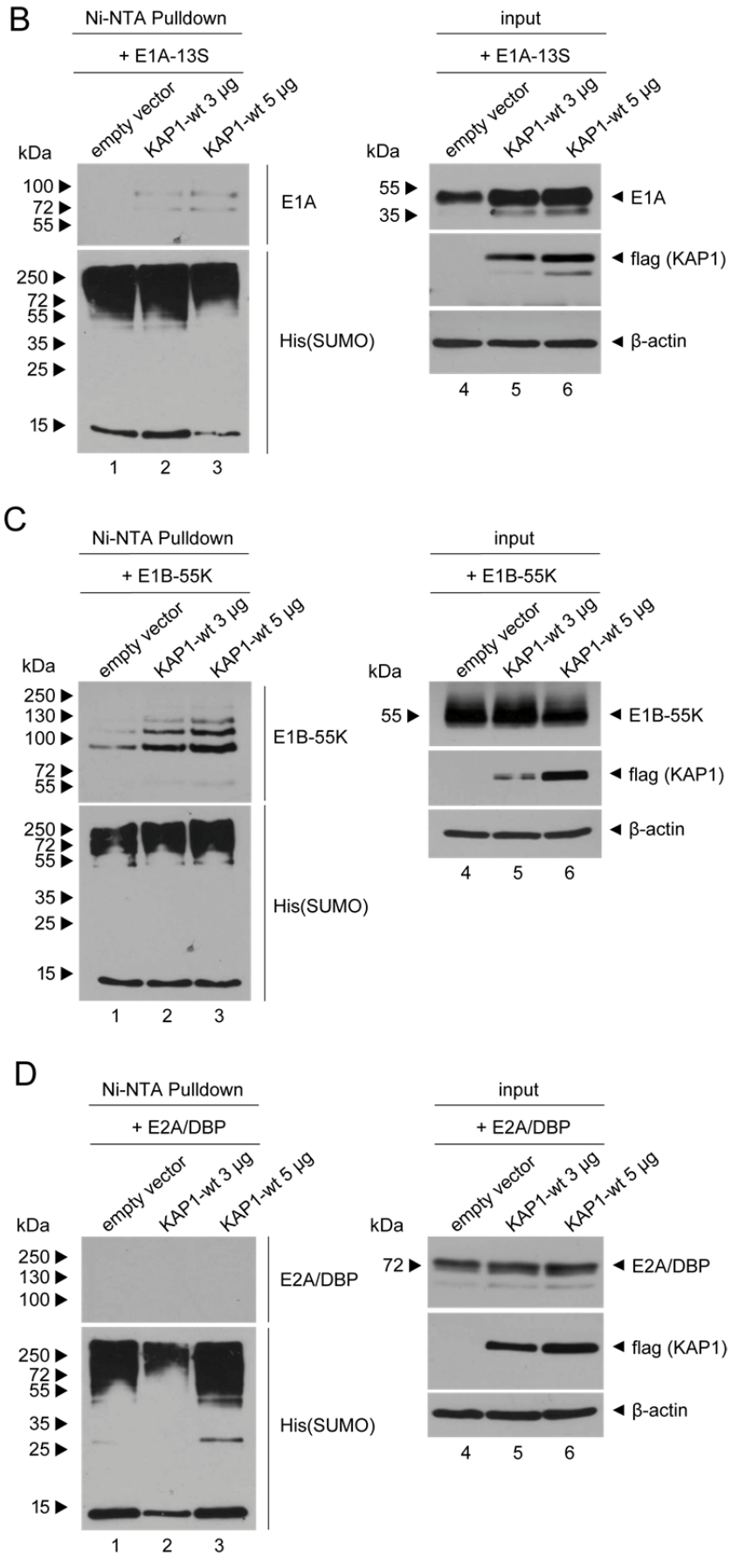
5.9 KAP1 induces PTMs of HAd5 proteins

5.9.1 KAP1 affects SUMOylation status of HAd5 proteins

Recent studies have shown, that KAP1 can recruit the SUMO-conjugating enzyme Ubc9 to act as a SUMO E3 ligase either to auto-SUMOylate its own bromodomain to generate a repressive form or to SUMOylate other cellular substrates, like IRF7 or Vps34 (Ivanov *et al.*, 2007; Liang *et al.*, 2011; Yang *et al.*, 2013).

Since reduced levels of KAP1 SUMO2 modification during HAd5 infection could be observed (Figure 39), the question was raised whether SUMO2 modification of HAd5 proteins might in turn also be affected by the cellular factor. To investigate this, HeLa cells expressing His-SUMO2 were co-transfected with plasmids encoding for different HAd5 proteins (Figure 45), including the virus particle-associated protein pVI (A), the immediate early protein E1A-13S (B), the early proteins E1B-55K (C), E2A/DBP (D), E4orf6 (E) or the late core protein pV (F) with increasing amounts of KAP1-wt. His-SUMO2 conjugates were purified (Ni-NTA) and immunoblotting revealed that all viral proteins tested, except E2A/DBP, showed SUMO2 modification in this experimental set-up. Interestingly, KAP1 overexpression resulted in an increased SUMO2 modification of E1A-13S, E1B-55K, E4orf6 and pV (Figure 45B, C, E and F, lanes 2 and 3), suggesting an interplay of SUMOylation processes between KAP1 and the viral factors.




B
Ni-NTA Pulldown
+ E1A-13S
empty vector
KAP1-wt 3 µg
KAP1-wt 5 µg
kDa
100
72
55
250
72
55
35
25
15
E1A
His(SUMO)
1 2 3
input
+ E1A-13S
empty vector
KAP1-wt 3 µg
KAP1-wt 5 µg
kDa
55
35
E1A
flag (KAP1)
β-actin
4 5 6
C
Ni-NTA Pulldown
+ E1B-55K
empty vector
KAP1-wt 3 µg
KAP1-wt 5 µg
kDa
250
130
100
72
55
250
72
55
35
25
15
E1B-55K
His(SUMO)
1 2 3
input
+ E1B-55K
empty vector
KAP1-wt 3 µg
KAP1-wt 5 µg
kDa
55
E1B-55K
flag (KAP1)
β-actin
4 5 6
D
Ni-NTA Pulldown
+ E2A/DBP
empty vector
KAP1-wt 3 µg
KAP1-wt 5 µg
kDa
250
130
100
250
72
55
35
25
15
E2A/DBP
His(SUMO)
1 2 3
input
+ E2A/DBP
empty vector
KAP1-wt 3 µg
KAP1-wt 5 µg
kDa
72
E2A/DBP
flag (KAP1)
β-actin
4 5 6

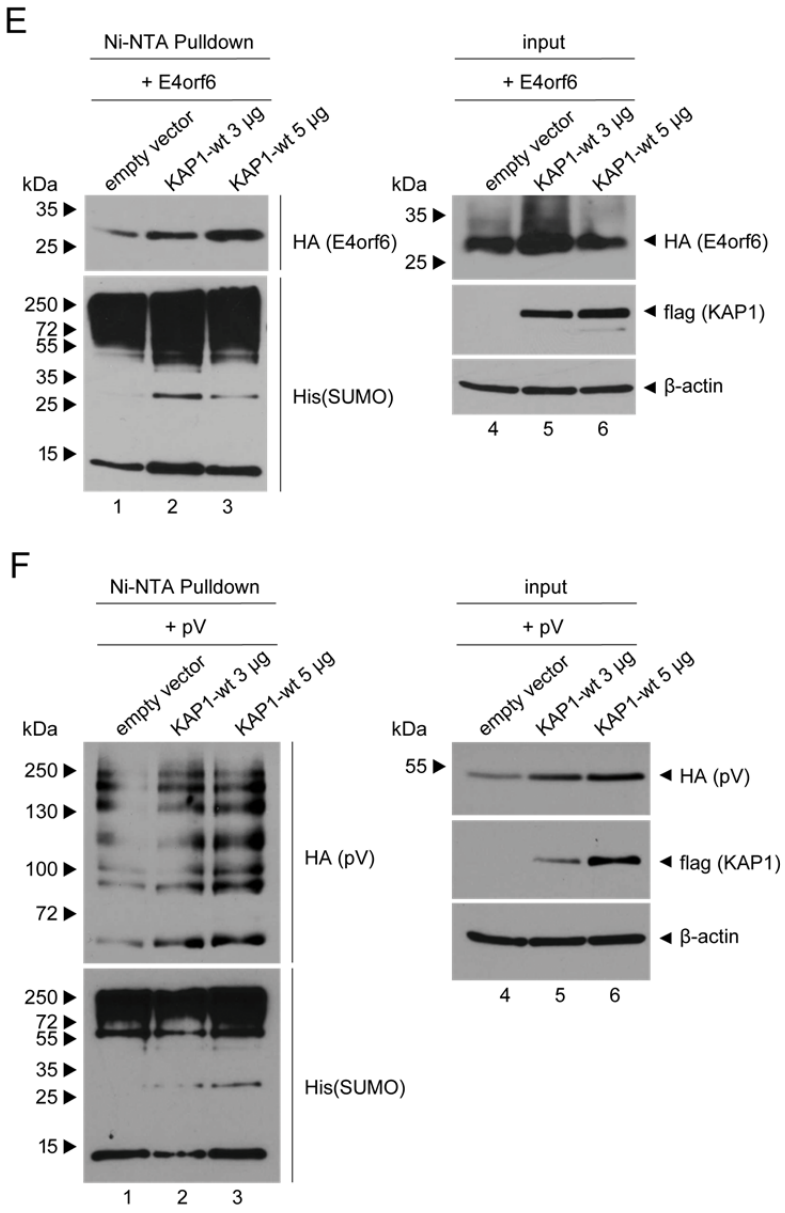


Figure 45: KAP1 affects SUMOylation status of HAd5 proteins. HeLa cells stably expressing 6His-SUMO2 were transfected with plasmids encoding for (A) pVI, (B) E1A-13S, (C) E1B-55K, (D) E2A/DBP, (E) E4orf6 or (F) pV and increasing amounts of KAP1-wt.48 h p.t. total-cell lysates were prepared with guanidinium chloride buffer, subjected to Ni-NTA purification on 6His-SUMO conjugates and analyzed via SDS-PAGE and immunoblotting. Input levels of total-cell lysates and Ni-NTA purified proteins were detected using rbAb pVI (α-pVI), mAb M73 (α-E1A), mAb 2A6 (α-E1B-55K), mAb B6-8 (α-E2A/DBP), rAb 3F10 (α-HA/E4orf6 and pV), mAb 6xHis, mAb flag-M2 (α-flag/KAP1) and mAb AC-15 (α-β-actin). Molecular weight in kDa is indicated on the left, while the relevant proteins are labeled on the right.

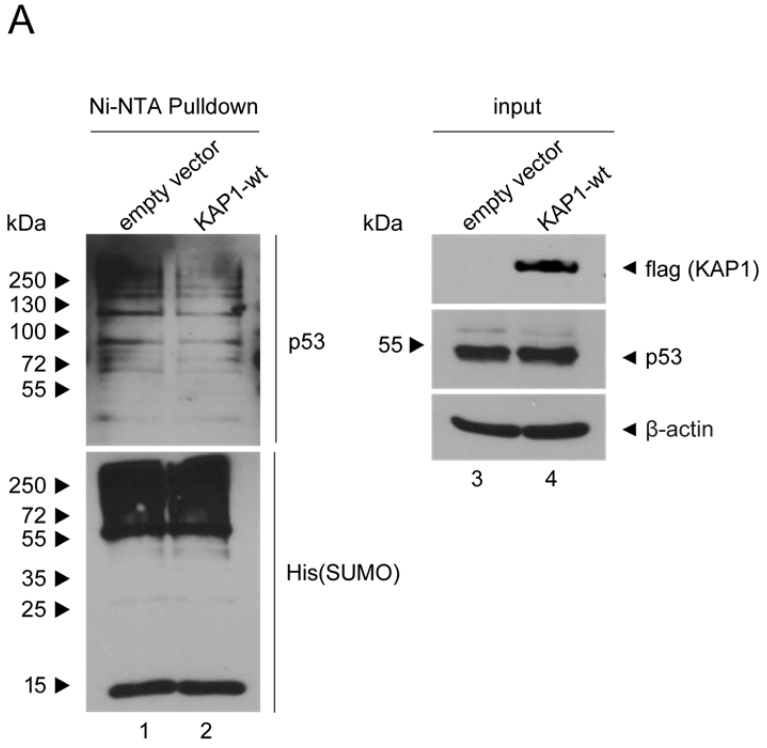
5.9.2 KAP1 does not affect p53 SUMOylation status

As a functional E3 SUMO-ligase, E1B-55K was shown to induce SUMOylation of the tumor suppressor p53. This process is dependent on E1B-55K SUMOylation itself, resulting in p53 spatial restriction to PML-NBs and transcriptional repression. The SUMOylation process of this HAd5 SUMO target is highly flexible and linked to

E1B-55K induced cellular transformation (Müller and Dobner, 2008; Pennella *et al.*, 2010; Wimmer *et al.*, 2015).

To substantiate the findings that KAP1 specifically and not randomly alters SUMO2 modification of HAd5 proteins, it was tested whether KAP1 also affects SUMOylation of p53. Therefore, HeLa cells expressing His-SUMO2 were transfected with a plasmid only encoding for KAP1-wt (Figure 46A) or co-transfected with p53 and increasing amounts of KAP1-wt (Figure 46B). His-SUMO2 conjugates were purified (Ni-NTA) and subjected to immunoblotting. KAP1 overexpression showed no effect on neither endogenous (Figure 46A, lanes 1 and 2) nor on co-transfected p53 SUMOylation (Figure 46B, lanes 1-3). However, after co-transfection of p53 with KAP1 a slight increase of SUMOylated proteins at around 300 kDa after staining the purified SUMO2 conjugates with 6His antibody was observed (Figure 46B, lanes 2 and 3).

Taken together, KAP1 overexpression had no effect on SUMO modification of endogenous or overexpressed p53, suggesting a specific regulation of HAd5 protein SUMOylation by the cellular E3 SUMO ligase.



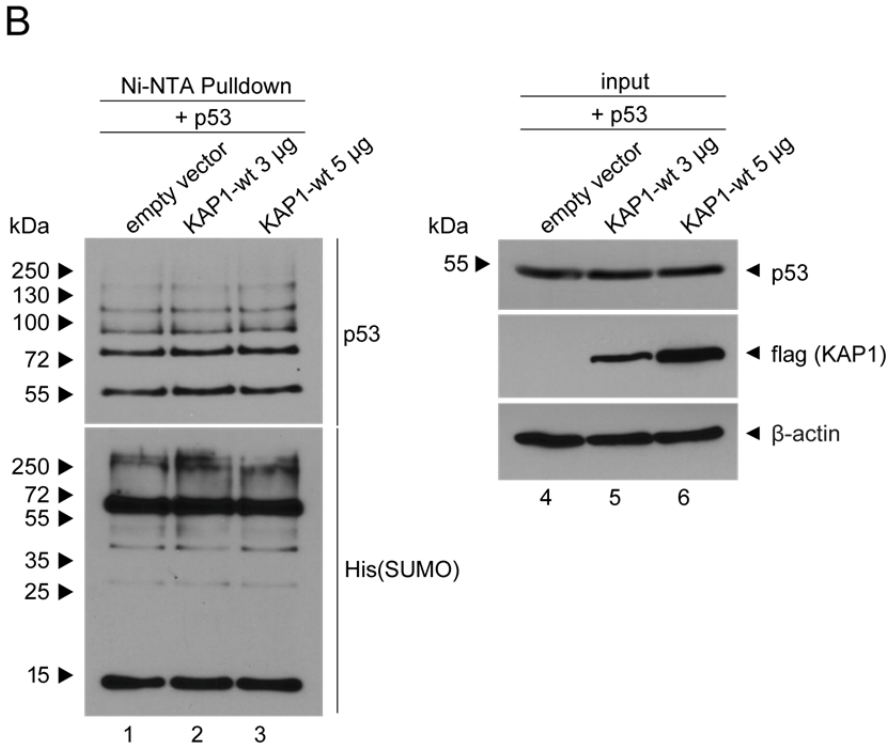


Figure 46: KAP1 does not affect SUMOylation status of the classical cellular SUMO-target p53. (A) HeLa cells stably expressing 6His-SUMO2 were transfected with a plasmid encoding for KAP1-wt. 48 h p.t. total-cell lysates were prepared with guanidinium chloride buffer, subjected to Ni-NTA purification on 6His-SUMO conjugates and analyzed via SDS-PAGE and immunoblotting. Input levels of total-cell lysates and Ni-NTA purified proteins were detected using mAb D-01 (α-p53), mAb 6xHis, mAb flag-M2 (α-flag) and mAb AC-15 (α-β-actin). (B) HeLa cells stably expressing 6His-SUMO2 were co-transfected with p53 and increasing amounts of KAP1-wt as indicated. 48 h p.t. total-cell lysates were prepared and analyzed as described in A. Molecular weight in kDa is indicated on the left, while the relevant proteins are labeled on the right.

5.9.3 KAP1 SUMOylation is no prerequisite for E1B-55K SUMOylation

Since E1B-55K-dependent deSUMOylation of KAP1 during HAd5 infection and in turn KAP1-dependent SUMOylation of E1B-55K was observed (Figure 39 and Figure 45), the question was raised whether SUMOylation status of KAP1 affects its ability to alter E1B-55K SUMO modification. KAP1 harbors several SUMOylation sites including the major SUMOylation sites Lys554, Lys575, Lys779 and Lys804, as well as the minor SUMOylation site Lys676 (Lee and Paull, 2007; Lee *et al.*, 2007; Zeng *et al.*, 2008). Lee and co-workers showed that SUMOylation of KAP1 at Lys554, Lys779 and Lys804 is no prerequisite for binding to KRAB Zink finger proteins as their DNA bridging factors but that SUMOylation at these sites is necessary for transcriptional co-repressor activity (Lee *et al.*, 2007).

To investigate the effect of KAP1 SUMOylation on the KAP1-mediated E1B-55K SUMOylation, KAP1 SUMO mutants were generated by mutating the minor SUMOylation site Lys676 as well as three major SUMOylation sites Lys554, Lys779 and Lys804 (Figure 47A and B). Thereby, the two double mutants K554/676A and K779/804A as well as one mutant with all four amino acid changes K554/676/779/804A were created. Next, HeLa cells expressing His-SUMO2 were co-transfected with plasmids encoding for E1B-55K and KAP1-wt or the respective KAP1 SUMO mutants (Figure 47C, left side). Immunoblotting of Ni-NTA purified His-SUMO2 conjugates and crude lysates showed that mutation of KAP1 SUMOylation sites resulted in decreased levels of SUMO2 modified protein levels (Figure 47C, lanes 3-6). In line with the results obtained so far, co-expression of KAP1 together with E1B-55K resulted in a decrease of KAP1 SUMO2 modification (Figure 47C, lanes 1 and 3). Additionally, all KAP1 mutants showed similar SUMOylation patterns of E1B-55K (Figure 47C, lanes 3-6), indicating that KAP1 SUMO modification does not affect its ability to enhance SUMOylation of the viral protein. Notably, although all KAP1 SUMO mutants showed similar expession levels, in cells co-transfected with the KAP1 mutant harboring all four amino acid changes E1B-55K showed the highest quantities of SUMO2 modification (Figure 47C, lane 6).

Since the SUMO mutant E1B-55K-K104R was not able to reduce KAP1 SUMO2 modification, co-transfection experiments with E1B-55K-K104R and KAP1-wt or the respective KAP1 SUMO mutants were performed (Figure 47C, right side). Consistent with the results obtained so far, co-transfection with the E1B-55K-K104R mutant showed no reduction of KAP1-SUMO2 modification (Figure 47, lanes 7 and 9), allowing the assumption that there might be a direct interplay between KAP1deSUMOylation and the increase in E1B-55K SUMOylation. Notably, co-transfection of the KAP1 SUMO mutants resulted in an enhanced stabilization of E1B-55K protein (Figure 47C, lanes 10-12).

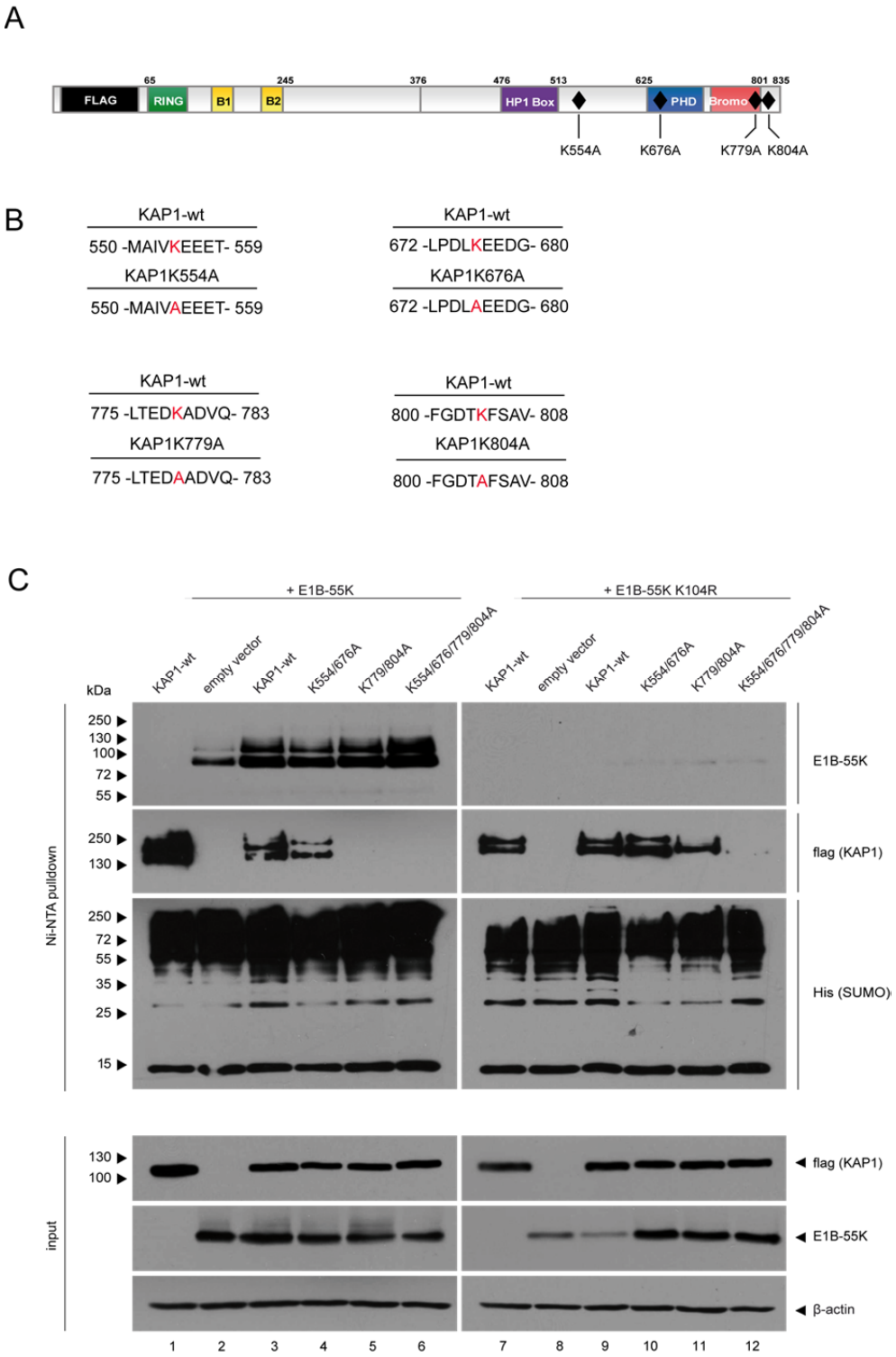


Figure 47: SUMO modification of KAP1 is no prerequisite for E1B-55K SUMOylation. (A) Schematic representation of the newly generated KAP1 SUMO mutants and the position of the mutations in KAP1. (B) Amino acid exchange within the KAP1 protein. Mutated amino acid is indicated in red. (C) HeLa cells stably expressing 6His-SUMO2 were co-transfected with plasmids encoding for KAP1-wt, the SUMO double mutants K554/676A, K779/804A or the construct with all four SUMO sites mutated and E1B-55K or the E1B-55K K104R SUMO mutant as indicated. 48 h p.t. total-cell lysates were prepared with guanidinium chloride buffer, subjected to Ni-NTA purification on 6His-SUMO conjugates and analyzed via SDS-PAGE and immunoblotting. Input levels of total-cell lysates and Ni-NTA purified proteins were detected using mAb 2A6 (α-E1B-55K), mAb flag-M2 (α-flag/KAP1), mAb 6xHis and mAb AC-15 (α-β-actin). Molecular weight in kDa is indicated on the left, while the relevant proteins are labeled on the right.

Taken together, these observations suggest a functional interplay between HAd5 infection and the PTMs of KAP1 as well as of viral proteins. Although many questions remain to be answered, the correlation between KAP1 PTM by phosphorylation and deSUMOylation may provide the first insights into the molecular mechanisms of HAd5-mediated regulation of KAP1 transcriptional repressor function.

6 Discussion

6.1 Components of chromatin remodeling and DDR pathways restrict HAd5 productive infection

HAd genomes are highly condensed by viral core proteins within the capsid (Chatterjee *et al.*, 1986; Matsumoto *et al.*, 1993; Okuwaki and Nagata, 1998). As for HSV-1, the HAd genome was shown to associate with H3.3 (Placek *et al.*, 2009; Ross *et al.*, 2011), suggesting that Ad DNA is chromatinized. The core/DNA complex enters the nucleus and is decondensed prior to the onset of early viral gene transcription (Komatsu *et al.*, 2011). In this context, we recently identified the cellular chromatin remodeling complex Daxx/ATRX as a negative regulator of HAd5 infection. This Daxx/ATRX chromatin remodeling complex recruits HDACs to deacetylate histone tails, resulting in chromatin compaction and transcriptional repression (Hollenbach *et al.*, 2002). We demonstrated that this complex associates with the HAd genome, thereby repressing active viral gene transcription (Schreiner *et al.*, 2013a; Schreiner *et al.*, 2010).

Besides the Daxx/ATRX chromatin remodeling complex we recently identified SPOC1 as a negative regulator of HAd5 infection (Schreiner *et al.*, 2013b). SPOC1 is also involved in regulating chromatin structure and DDR by modulating chromatin association of DNA compaction factors (Mund *et al.*, 2012). We showed that SPOC1 complexes with the HAd5 pVII protein and negatively regulates HAd5 gene expression (Schreiner *et al.*, 2013b). Interestingly, viral pVII shows strong homology to various domains in the human histone 3. In addition, pVII was reported to remain associated with the viral DNA during nuclear import, followed by dissociation and the onset of viral DNA synthesis (Matsumoto *et al.*, 1993). SPOC1 was shown to form a chromatin remodeling complex with KAP1, resulting in the recruitment of repressive components, such as HMT and NuRD (Mund *et al.*, 2012). Although no interaction of the SPOC1-binding protein KAP1 was observed, it is tempting to speculate that KAP1 is recruited to the linear double-stranded HAd5 genome via interaction of SPOC1 with pVII. As a transcriptional co-repressor and chromatin

modifier KAP1 might maintain the chromatinized state of the HAd genome after nuclear entry. This might be facilitated via deacetylation of histone-like pVII via HDAC recruitment as well as the deposition of repressive histone mark H3K9me3 via HMTs.

This study shows that KAP1 overexpression negatively regulates HAd5 progeny production (Figure 15A and Figure 18). However, this negative effect is attenuated later during infection, suggesting that HAd5 counteracts KAP1 negative function by exploiting so far unknown cellular pathways to ensure proper virus replication. Additionally, HAd5 protein levels were reduced upon KAP1 overexpression (Figure 15B and C). In this context, the most severe reduction on protein level was observed for E1B-55K and E4orf6. Interestingly, luciferase assays (Figure 23) showed a KAP1-mediated negative effect on the E1B and E4 promoter. In addition, ChIP analysis recently revealed an interaction of SPOC1 with HAd5 promoters in transfected cells (Schreiner *et al.*, 2013b), suggesting that KAP1-mediated transcriptional repression might be facilitated via its recruitment to HAd5 promoters by the SPOC1 protein. Although KAP1 overexpression did not affect the E1A promoter activity (data not shown), it negatively affected E1A-dependent transcriptional activity (Figure 24). Kamitani and co-workers observed that KAP1 suppresses TNF-α-induced IL-6 production and transcriptional activation of NF-κB via the modulation of the interactions among NF-κB, p300 and STAT3 (Kamitani *et al.*, 2008; Kamitani *et al.*, 2011), although the exact mechanism by which KAP1 modulates these interactions still needs to be clarified. Although no interaction of KAP1 with E1A could be observed, it is likely that KAP1 interferes with the association of E1A and p300, leading to a reduction of E1A-dependent transcriptional activity.

To substantiate the results concerning the KAP1-mediated negative regulation of HAd5 productive infection, extensive but so far unsuccessful approaches were conducted during this work to generate KAP1-depleted cell lines. Since KAP1 is involved in cell cycle progression and apoptosis, it is not suprising that KAP1 depletion has several downstream effects. Li and co-workers observed that KAP1 depletion increases apoptotic phenotypes and showed severe negative effects on cell

proliferation rates (Li *et al.*, 2010). Additionally, Kepkay and co-workers reported an increase of PML-NBs upon KAP1 depletion (Kepkay *et al.*, 2011). Although the KAP1-depleted cell lines generated in this work showed comparable proliferation rates to those of the control cell lines (Figure 17), in line with Kepkay *et al.* increased PML protein levels upon KAP1 depletion could be observed (Figure 19). As PML plays a role in host-cellular antiviral defense (Everett and Chelbi-Alix, 2007; Tavalai and Stamminger, 2008), its upregulation might compensate the loss of KAP1, resulting in similar levels of HAd5 progeny production. In line with this, Glass and Everett observed an increase of the cellular Daxx protein upon PML depletion (Glass and Everett, 2012), indicating a complex regulation of these important cellular compartments. As Daxx is a negative regulator of HAd5 replication (Schreiner *et al.*, 2010), this might highlight an additional reason, why KAP1 depletion in that context does not induce HAd5 gene expression. To further clarify the role of KAP1 depletion on HAd5 productive infection, its correlation with altered PML-NB composition has to be fully understood. This global effect remains to be investigated by double and triple knock-down of all constitutive PML-NB components to monitor a combined effect on HAd5 productive infection.

6.2 HAd5 counteracts host-cellular antiviral defense and DDR mechanisms

6.2.1 HAd5 impacts functions of KAP1 by modulating posttranslational modifications of this cellular co-repressor

HAds have evolved several ways to circumvent host-cellular antiviral mechanisms mainly by proteasomal degradation of DDR components or proteins involved in chromatin remodeling. Recently, we showed that the HAd5 restriction factor SPOC1 is degraded upon HAd5 infection via the E1B-55K/E4orf6-dependent E3 ubiquitin ligase complex (Schreiner *et al.*, 2013b). Interestingly, this work revealed that SPOC1-associated cellular factor KAP1 is not degraded upon HAd5 infection (Figure 26). Since KAP1 exerts antiviral functions against HAd5 infection, it was tempting to speculate about inhibition of KAP1 function via mechanisms besides degradation.

HAd5 early regulatory proteins promote cell cycle progression while simultaneously blocking apoptosis and growth arrest (Kosulin *et al.*, 2007; Shenk, 2001; Täuber and Dobner, 2001a; Täuber and Dobner, 2001b). E1A-mediated E2F activation via inactivation of Rb is known to initiate the transcription of genes required for the passage from G1- to S-phase (Ben-Israel and Kleinberger, 2002; Weinberg, 1995). Studies in this field revealed that E2F-mediated transcriptional repression requires the recruitment of a variety of transcriptional co-repressors and chromatin remodeling proteins, such as HDACs and HMTs (David *et al.*, 2008; Grandinetti and David, 2008; Grandinetti *et al.*, 2009). In this context, the cellular co-repressor KAP1 was shown to stimulate E2F1-HDAC1 complex formation, resulting in deacetylation of E2F1, thereby suppressing E2F1-mediated apoptotic gene expression in response to DNA damage (Wang *et al.*, 2007). This work shows that KAP1 is phosphorylated upon HAd5 infection (Figure 28). Thus, it is tempting to speculate that besides Rb inactivation, E1A takes advantage of KAP1 PTMs to promote cell cycle progression from G1- to S-phase, thereby establishing optimal conditions for virus replication.

However, KAP1 phosphorylation is counteracted by E1B-55K early in infection (Figure 29B). E1B-55K was reported to block the E1A-induced apoptosis to ensure proper virus replication (Schaeper *et al.*, 1998; White, 1995). Since unphosphorylated KAP1 is known to repress pro-apoptotic genes (Lee *et al.*, 2007; Li *et al.*, 2007) this leads to the hypothesis that after E1A-mediated cell cycle progression to S-phase E1B-55K antagonizes HAd5-mediated KAP1 phosphorylation to prevent cell death. This hypothesis is supported by the findings that KAP1 Ser824 phosphorylation is facilitated by ATM or the DNA-PKcs, which were shown to be functionally inactivated during HAd5 infection (Boyer *et al.*, 1999; Gautam and Bridge, 2013; Huang and Hearing, 1989; White *et al.*, 2006; Ziv *et al.*, 1997). Additionally, this work revealed that KAP1 phosphorylation is a prerequisite for productive HAd5 infection (Figure 34), indicating that flexible regulation of KAP1-mediated gene silencing is required for virus replication.

Taken together, the observations on KAP1 phosphorylation suggest that HAd5-mediated KAP1 Ser824 phosphorylation is exploited early in infection by E1A,

introducing the infected cells into S phase. Afterwards, this step is followed by E1B-55K-induced counteraction of KAP1 phosphorylation to block cell death. Later during infection, KAP1-mediated transcriptional repression of HAd5 gene expression is antagonized by KAP1 phosphorylation facilitated through ATM- and DNA-PK-independent mechanisms due to their inactivation. However, so far it is unclear which viral factor is responsible for KAP1 phosphorylation. These observations lead to the assumption that KAP1 phosphorylation might be no exclusive cellular antiviral response to the free linear viral genome but an active viral mechanism to alter KAP1 functions to generate a positive environment for viral replication.

KAP1 co-repressor function is flexibly regulated by Ser824 phosphorylation and SUMO modification. KAP1 phosphorylation results in chromatin decondensation, whereas KAP1 SUMOylation increased its repressive function by the recruitment of HMTs and NuRD (Lee and Paull, 2007; Li *et al.*, 2010). Here it is shown that HAd5 infection reduces levels of SUMO-modified KAP1 (Figure 38 and 39), substantiating the hypothesis that thereby KAP1 might dissociate from SPOC1 to release the repressive HMTs and HDACs, facilitating H3K9 acetylation and demethylation for relaxation of chromatin, transcriptional activation and enhanced gene expression.

Additionally, this work reveals that HAd5-mediated KAP1 deSUMOylation is dependent on the presence of E1B-55K (Figure 40), which was reported to contain a SCM and represents a substrate for the host cell SUMO modification system (Endter *et al.*, 2005; Endter *et al.*, 2001). Interestingly, E1B-55K interacts with the cellular SUMO E2 enzyme Ubc9 (Wimmer *et al.*, 2012). Studies from Penella and co-workers suggest that E1B-55K represents a p53-SUMO1 E3 ligase (Pennella *et al.*, 2010). In line with this, our group recently showed that E1B-55K SUMOylation and hence PML-NB localization is a prerequisite for SUMO ligase activity of the viral protein (Wimmer *et al.*, 2015). However, the detailed mechanism by which E1B-55K mediates SUMOylation of other proteins is still not fully understood.

For the first time, this work provides evidence that SUMO modification of E1B-55K is a prerequisite for efficient KAP1 deSUMOylation upon HAd5 infection. Since KAP1

deSUMOylation does not occur in cells expressing the E1B-55K SUMOylation-deficient mutant K104R (Figure 41 and 42) it is tempting to speculate that E1B-55K facilitates KAP1 deSUMOylation by taking over KAP1-associated SUMO moieties.

The transfection experiments testing several HAd5 immediate early, early and late proteins for their capacity to induce KAP1 SUMOylation changes revealed that levels of KAP1 SUMO modification were differentially regulated in the presence of the respective viral protein (Figure 43 and 44). Thereby, overexpression of the minor capsid protein pVI as well as the immediate early protein E1A, two of the first proteins present during infection, increased levels of KAP1 SUMO modification, whereas early and late protein expression reduced KAP1 SUMOylation.

In accordance with the role of KAP1 during apoptosis these data lead to the hypothesis that early in infection, SUMOylated KAP1 associates with DNA-bridging factors on KAP1-responsive pro-apoptotic genes. As infection progresses, E1B-55K is expressed and continues to block E1A-induced apoptosis. Furthermore, we recently showed that the KAP1-associated protein SPOC1 is able to bind to the viral genome and pVII (Schreiner *et al.*, 2013b). Given these findings, one can hypothesize that HAd5-mediated KAP1 phosphorylation, deSUMOylation and SPOC1 degradation leads to the dechromatinization of the viral DNA, thereby mediating the onset of HAd5 gene expression.

6.2.2 HAd5 exploits KAP1-mediated SUMOylation to affect localization and functions of viral and cellular proteins

SUMOylation of a substrate is known to alter its inter- and/or intramolecular interactions, thereby modulating its stability, localization or activity. In this context, it is suggested that SUMO modification of a target protein is associated with the recruitment of SIM-containing effector proteins (Kerscher *et al.*, 2006; Song *et al.*, 2004). KAP1 represents a cellular SUMO E3 ligase, which was shown to not only regulate its own SUMOylation status but is also able to SUMOylate other cellular proteins, such as the cellular factor IFN regulatory factor 7 (IRF7), thereby reducing its transcriptional activity, resulting in suppressed IFN-based antiviral response

(Liang *et al.*, 2011). In addition, the Class III PI 3-kinase Vps34 was recently reported to be targeted by KAP1 for SUMOylation. SUMOylation of Vps34 enhances its binding to Beclin1, which triggers autophagosome formation (Yang *et al.*, 2013). As a subset of RNA viruses was shown to exploit this autophagic pathway to promote viral replication (Jackson *et al.*, 2005), it is not clear if and how HAd5 might exploit this pathway.

This work revealed that KAP1 induces SUMO2 modification of several regulatory and structural HAd5 proteins, including E1A-13S, E1B-55K, E4orf6 and pV in a dose dependent manner (Figure 45). As a classical target for E1B-55K-mediated SUMOylation, p53 showed no increase of SUMO modification when co-transfected with KAP1 (Figure 46), eliminating the assumption of unspecific KAP1-mediated SUMOylation of SIM containing proteins. However, KAP1 SUMOylation itself is not required for this process (Figure 47).

Based on this work, one can hypothesize that KAP1 mediated SUMOylation of adenoviral proteins might alter localization of the viral factors and therefore modulate their functions. In line with this assumption, it could be shown that E1B-55K SUMOylation is critical for localization and function of the viral protein, especially for its ability to inactivate p53 and degrade Daxx (Pennella *et al.*, 2010; Schreiner *et al.*, 2011; Schreiner *et al.*, 2010; Wimmer *et al.*, 2012; Wimmer *et al.*, 2010). Although so far no functional connection could be observed in this context, E1B-55K might exploit KAP1 SUMO E3 ligase activity to regulate its own localization. Furthermore, if HAd5 exploits KAP1 function in this context it could also be used for the SUMOylation of cellular proteins and their relocalization to PML-NBs.

As described in the introduction, studies of our group suggested the model that PML-tracks localize to the outer rim of the VRCs, harboring positive factors for viral replication, whereas negative factors are captured in the VRCs or degraded via the E1B-55K/E4orf6-dependent E3 ubiquitin ligase. Since KAP1 forms nucleoplasmic foci with KRAB-ZNPs to so-called KAKA foci adjacent to PML-NBs (Briers *et al.*, 2009) one might speculate about its possible role to SUMOylate viral as well as cellular factors, e.g. transcription factors required for viral mRNA synthesis. As

mentioned before, KAP1 was shown to SUMOylate IRF7 to reduce its transcription activity, leading to the suppression of the IFN response (Liang *et al.*, 2011). Since this work indicates that KAP1 SUMOylation is not required for its function as an E3 SUMO ligase, this might be one mechanism by which HAd5 can interfere with the IFN response. Clearly, the IFN response is a complex pathway and HAd5 most likely interferes with several of its components. However, the exact mechanism by which HAd5 blocks this pathway are subjects for future studies.

Additionally, KAP1 possesses ubiquitin E3 ligase activity via its RING domain. So far, p53 could be identified as a KAP1 ubiquitination substrate, resulting in p53 degradation (Doyle *et al.*, 2010; Xiao *et al.*, 2011; Yang *et al.*, 2007). These observations raise the question whether HAd5 might also exploit KAP1 function concerning ubiquitination of cellular substrates to induce their degradation. Further work on this has to be conducted to clarify the mechanism by which HAd5 uses the emerging functional repertoire of the cellular co-repressor KAP1 to support its replication.

6.3 Roles for KAP1 during HAd5-mediated transformation and latency

High KAP1 expression levels have been linked to various cancer types, including pro-metastatic cervical cancer, colorectal cancer, gastric gancer and thyroid carcinoma (Hector *et al.*, 2012; Lin *et al.*, 2013; Martins *et al.*, 2013; Wang *et al.*, 2013; Yokoe *et al.*, 2009). Thus, KAP1 has been proposed to be a target for anti-cancer therapy (Okamoto *et al.*, 2006; Wang *et al.*, 2005). However, the role of KAP1 in tumorigenesis is still inconclusive and seems to be tissue specific, since it showed tumor suppressive functions in early-stage lung cancer by inactivating the oncogenic transcription factors STAT3 and HIF-1α (Chen *et al.*, 2012; Huang *et al.*, 2013; Tsuruma *et al.*, 2008). Furthermore, KAP1 was shown to play a role in the MDM2/p53/HDAC1 complex, thereby promoting deacetylation and MDM2-mediated degradation of p53 (Wang *et al.*, 2005). This effect is enhanced by the presence of melanoma antigen (MAGE) family proteins (Yang *et al.*, 2007).

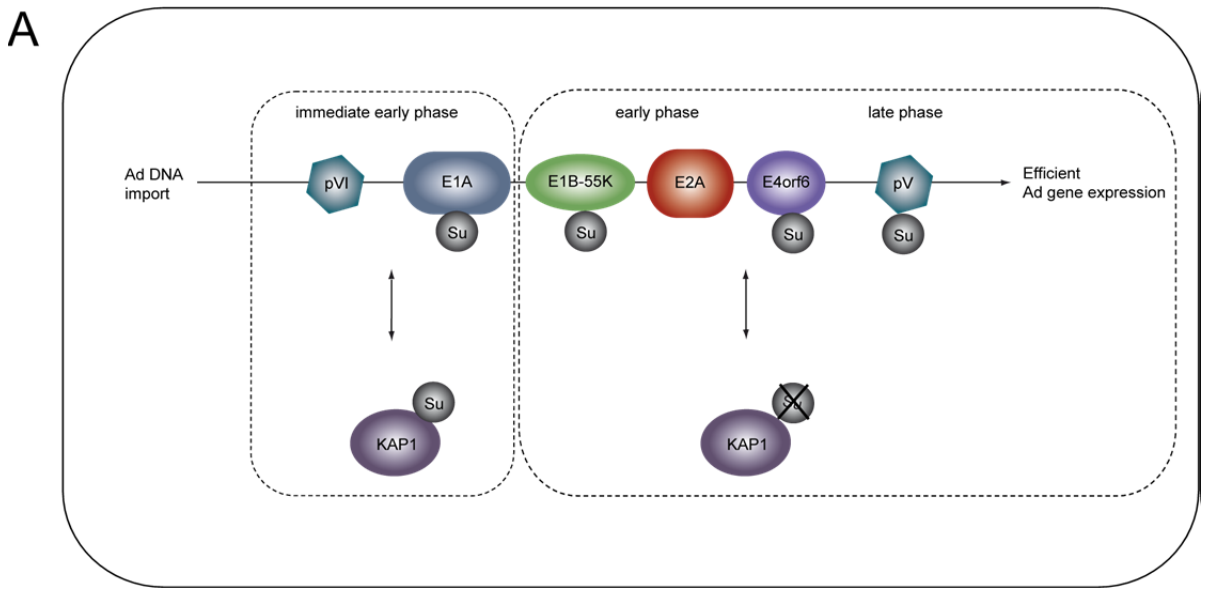
HAds are able to transform primary rodent cells. HAd5-mediated transformation requires the early viral proteins E1A and E1B and is substantially increased by the additional expression of E4 proteins (Branton *et al.*, 1985; Gaggar *et al.*, 2003; Graham *et al.*, 1984; Nevins and Vogt, 1996; Ricciardi, 1995; Täuber and Dobner, 2001a). For efficient transformation, E1A supports cell cycle progression, thereby establishing optimal conditions for progeny virus production (Endter and Dobner, 2004; Ferrari *et al.*, 2009; Ferrari *et al.*, 2008; Frisch and Mymryk, 2002). As described above, E1A is able to induce immortalization of primary rodent cells by the modulation of key regulators controlling cell cycle progression in the course of an abortive infection (Gallimore *et al.*, 1984a; Gallimore *et al.*, 1984b). E1A additionally induces p53 stabilization and atypical apoptosis, which is counteracted by E1B proteins. Thereby, E1B contributes to a completely transformed phenotype (Debbas and White, 1993; Grand *et al.*, 1994; Lowe and Ruley, 1993; Mymryk *et al.*, 1994; Ruley, 1983; Sabbatini *et al.*, 1995; Samuelson and Lowe, 1997; Turnell *et al.*, 2000).

The tumorigenic functions of E1B-55K are mainly mediated by the modulation of the tumor suppressor p53, including interaction, transcriptional repression and nuclear-cytoplasmic relocalization (Endter *et al.*, 2005; Endter *et al.*, 2001; Kao *et al.*, 1990; Martin and Berk, 1998; Martin and Berk, 1999; Sarnow *et al.*, 1982; Yew *et al.*, 1994). In this context, we identified KAP1 as a co-repressor of E1B-55K (Figure 25). Recent work from our group showed that PTMs of E1B-55K regulate its transforming activities (Endter *et al.*, 2001). Thereby, phosphorylation and SUMOylation of the oncoprotein E1B-55K is required for the modulation of p53 and the degradation of Daxx (Schreiner *et al.*, 2010; Wimmer *et al.*, 2012). Additionally, SUMO modification of E1B-55K is indispensable for the interaction with PML-IV and V. In this context, this interaction was shown to be essential for HAd5-mediated transformation (Wimmer *et al.*, 2015). Since this work indicated that SUMOylation of E1B-55K is a prerequisite for its ability to deSUMOylate KAP1, it is interesting whether the SUMO status of KAP1 plays an important role during transformation of primary rodent cells.

Besides abortive infections in non-permissive cells, different observations suggest that HAds can establish long-term low-level persistent or even latent infections (Garnett *et al.*, 2009; Gustafsson *et al.*, 2007; Kosulin *et al.*, 2007). In this context, it is proposed that the HAd genome is maintained in the cell in an unintegrated episomal state. This model might be the explanation of the high prevalence of HAd infections in immunocompromised individuals. As mentioned in the introduction, KSHV was recently shown to exploit KAP1 chromatin remodeling function via phosphorylation of KAP1S824 by the viral protein kinase, leading to the activation of its lytic genes resulting in lytic KSHV replication (Chang *et al.*, 2009). Furthermore, KAP1 phosphorylation is thought to support the chronic inflammatory environment of Karposi's Sarcoma by activating STAT3 (King, 2013). Additionally, the latency-associated nuclear antigen (LANA) functionally interacts with KAP1 in order to repress lytic gene expression during the early stage of KSHV infection, which was suggested to facilitate the establishment of KSHV latency after primary infections (Sun *et al.*, 2014).

Whether KAP1 contributes to HAd5 latency still remains under investigation. However, KAP1 is involved in the regulation of episomal gene expression through KRAB/KAP1-mediated histone modifications (Barde *et al.*, 2009). Thereby, KAP1 might be involved in maintaining HAd5 latent state by repressing HAd5 episomal gene expression. This hypothesis is supported by the observation that KAP1 reduces HAd5 promoter activity (Figure 23). Since KAP1 is phosphorylated upon DNA DSBs it is likely that in chemotherapy-treated patients, irradiation-mediated KAP1 phosphorylation results in the activation of HAd5 lytic gene expression. Thereby, KAP1 could play an important role in HAd5 reactivation in immunocompromised patients, underlining the importance of this factor to be considered in cancer therapy.

In sum, the data of this thesis reveales a complex HAd5-mediated regulation of KAP1 co-repressor and chromatin remodeler function as illustrated in Figure 48. Based on these results, one can suggest the model that early in infection HAd5 exploits KAP1 phosphorylation to generate a positive environment for virus replication, thereby circumventing host-cellular pro-apoptotic pathways. Immediately after this, KAP1 constant phosphorylation and deSUMOylation leads to a dechromatinized state of the viral and cellular genome, resulting in transcriptional activation and enhanced viral and cellular gene expression. Simultaneously, HAd5 benefits from KAP1 as an E3 SUMO ligase, SUMOylating cellular factors as well as HAd5 proteins. These PTMs might alter protein localization and/or functions during HAd5 infection. Constantly growing knowledge about KAP1 places great importance on this cellular factor, since it is involved in several important cellular pathways, including cell differentiation, tumorigenesis, immune response, DDR as well as virus replication. However, to reveal the dimension of the interplay between HAd5, KAP1 and the emerging relevance of PTMs will clearly be subject of further studies.



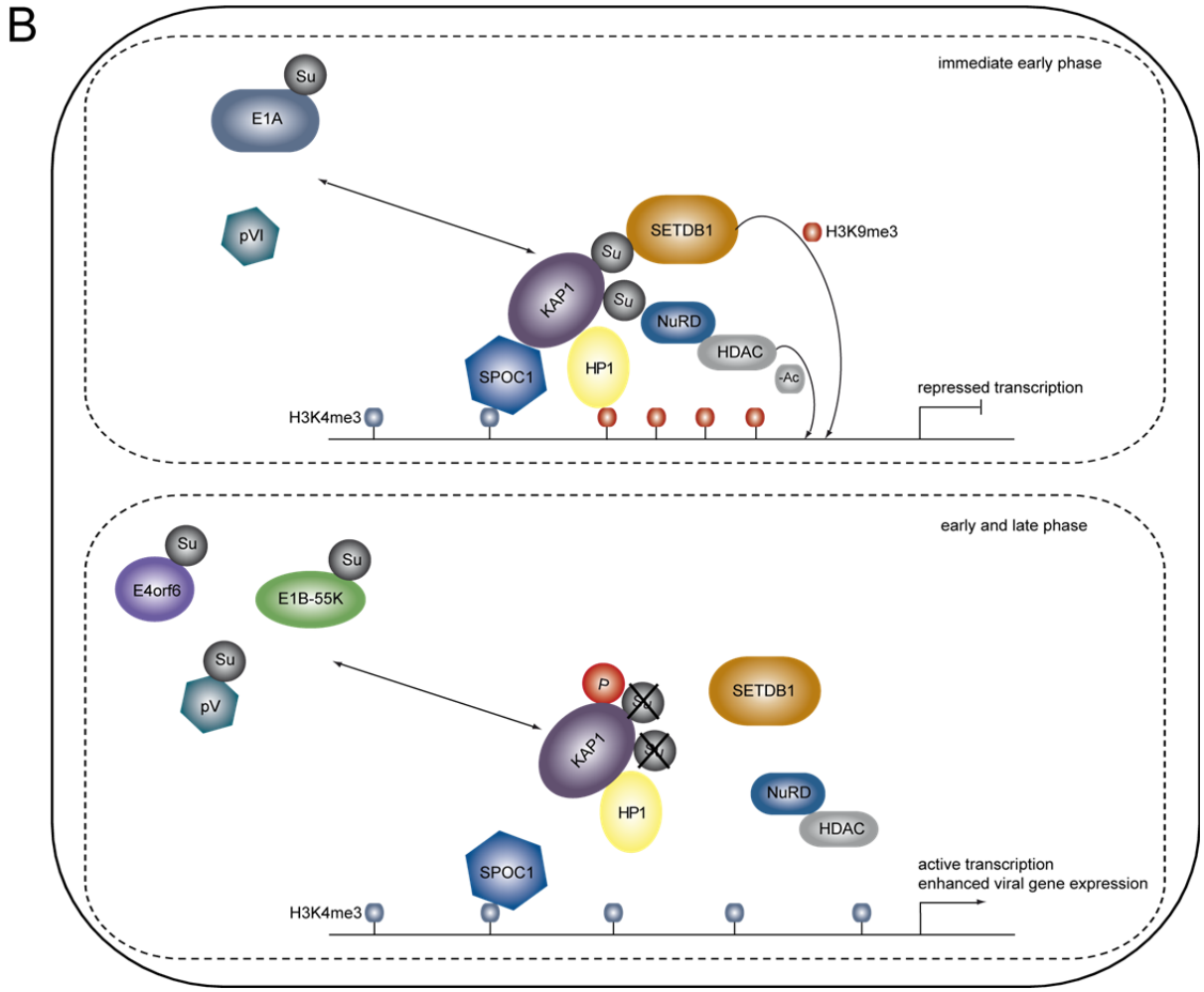


Figure 48: Model of KAP1 PTM by HAd5 proteins. A schematic representation illustrating the proposed model of modulation of KAP1 posttranslational modifications by viral factors, simultaneous alteration of viral factors by KAP1 and the resulting consequences on HAd5 infection. (A) After import of viral DNA the incoming viral proteins pVI and E1A induce KAP1 SUMOylation, whereas the viral factors of the early and late phase of infection induce KAP1 deSUMOylation. Simultaneously, KAP1 induces SUMOylation of the viral factors E1A, E1B-55K, E4orf6 and pV. (B) Early in infection pVI and E1A induce/maintain KAP1 SUMOylation status in order to strengthen the repressive complex and avoid the expression of pro-apoptotic genes. Later in infection E1B-55K, E4orf6 and pV induce KAP1 deSUMOylation and phosphorylation resulting in chromatin decondensation and enhanced viral gene expression.

References

Abe, S., Miyamura, K., Oba, T., Terakura, S., Kasai, M., Kitaori, K., Sasaki, T., and Kodera, Y. (2003). Oral ribavirin for severe adenovirus infection after allogeneic marrow transplantation. *Bone Marrow Transplant* **32**(11)**,** 1107-8.

Ahi, Y. S., Vemula, S. V., and Mittal, S. K. (2013). Adenoviral E2 IVa2 protein interacts with L4 33K protein and E2 DNA-binding protein. *J Gen Virol* **94**(Pt 6)**,** 1325-34.

Allouch, A., Di Primio, C., Alpi, E., Lusic, M., Arosio, D., Giacca, M., and Cereseto, A. (2011). The TRIM family protein KAP1 inhibits HIV-1 integration. *Cell Host Microbe* **9**(6)**,** 484-95.

Aspegren, A., Rabino, C., and Bridge, E. (1998). Organization of splicing factors in adenovirus-infected cells reflects changes in gene expression during the early to late phase transition. *Exp Cell Res* **245**(1)**,** 203-13.

Avvakumov, N., Sahbegovic, M., Zhang, Z., Shuen, M., and Mymryk, J. S. (2002a). Analysis of DNA binding by the adenovirus type 5 E1A oncoprotein. *J Gen Virol* **83**(Pt 3)**,** 517-24.

Avvakumov, N., Wheeler, R., D'Halluin, J. C., and Mymryk, J. S. (2002b). Comparative sequence analysis of the largest E1A proteins of human and simian adenoviruses. *J Virol* **76**(16)**,** 7968-75.

Babich, A., Feldman, L. T., Nevins, J. R., Darnell, J. E., Jr., and Weinberger, C. (1983). Effect of adenovirus on metabolism of specific host mRNAs: transport control and specific translational discrimination. *Mol Cell Biol* **3**(7)**,** 1212-21.

Bailey, A., and Mautner, V. (1994). Phylogenetic relationships among adenovirus serotypes. *Virology* **205**(2)**,** 438-52.

Baker, A., Rohleder, K. J., Hanakahi, L. A., and Ketner, G. (2007). Adenovirus E4 34k and E1b 55k oncoproteins target host DNA ligase IV for proteasomal degradation. *J Virol* **81**(13)**,** 7034-40.

Baldeyron, C., Soria, G., Roche, D., Cook, A. J., and Almouzni, G. (2011). HP1alpha recruitment to DNA damage by p150CAF-1 promotes homologous recombination repair. *J Cell Biol* **193**(1)**,** 81-95.

Barde, I., Laurenti, E., Verp, S., Groner, A. C., Towne, C., Padrun, V., Aebischer, P., Trumpp, A., and Trono, D. (2009). Regulation of episomal gene expression by KRAB/KAP1-mediated histone modifications. *J Virol* **83**(11)**,** 5574-80.

Barde, I., Rauwel, B., Marin-Florez, R. M., Corsinotti, A., Laurenti, E., Verp, S., Offner, S., Marquis, J., Kapopoulou, A., Vanicek, J., and Trono, D. (2013). A KRAB/KAP1-miRNA cascade regulates erythropoiesis through stage-specific control of mitophagy. *Science* **340**(6130)**,** 350-3.

Beltz, G. A., and Flint, S. J. (1979). Inhibition of HeLa cell protein synthesis during adenovirus infection. Restriction of cellular messenger RNA sequences to the nucleus. *J Mol Biol* **131**(2)**,** 353-73.

Ben-Israel, H., and Kleinberger, T. (2002). Adenovirus and cell cycle control. *Front Biosci* **7,** d1369-95.

Benkó, M., Elo, P., Ursu, K., Ahne, W., LaPatra, S. E., Thomson, D., and Harrach, B. (2002). First molecular evidence for the existence of distinct fish and snake adenoviruses. *J Virol* **76**(19)**,** 10056-9.

Benkö, M., and Harrach, B. (1998). A proposal for a new (third) genus within the family Adenoviridae. *Arch Virol* **143**(4)**,** 829-37.

Benkö, M., Harrach, B., and Russel, W. C. (1999). Adenoviridae. In Virus Taxonomy. Seventh Report of the International Comittee on Taxonomy of Viruses. M. H. V. van Regenmortel, C. M. Fauquet, and D. H. L. Bishop, editors. Academic Press, San Diego, 227-238.

Bergelson, J. M., Cunningham, J. A., Droguett, G., Kurt-Jones, E. A., Krithivas, A., Hong, J. S., Horwitz, M. S., Crowell, R. L., and Finberg, R. W. (1997). Isolation of a common receptor for Coxsackie B viruses and adenoviruses 2 and 5. *Science* **275**(5304)**,** 1320-3.

Berk, A. J. (2005). Recent lessons in gene expression, cell cycle control, and cell biology from adenovirus. *Oncogene* **24**(52)**,** 7673-85.

Berk, A. J. (2007). Adenoviridae: The Viruses and Their Replication. *In Fields Virology***,** 5th edn, pp. 2355-2394.

Berscheminski, J., Groitl, P., Dobner, T., Wimmer, P., and Schreiner, S. (2013). The adenoviral oncogene E1A-13S interacts with a specific isoform of the tumor suppressor PML to enhance viral transcription. *J Virol* **87**(2)**,** 965-77.

Berscheminski, J., Wimmer, P., Brun, J., Ip, W. H., Groitl, P., Horlacher, T., Jaffray, E., Hay, R. T., Dobner, T., and Schreiner, S. (2014). Sp100 isoform-specific regulation of human adenovirus 5 gene expression. *J Virol* **88**(11)**,** 6076-92.

Beucher, A., Birraux, J., Tchouandong, L., Barton, O., Shibata, A., Conrad, S., Goodarzi, A. A., Krempler, A., Jeggo, P. A., and Lobrich, M. (2009). ATM and Artemis promote homologous recombination of radiation-induced DNA double-strand breaks in G2. *Embo J* **28**(21)**,** 3413-27.

Beyer, W. R., Westphal, M., Ostertag, W., and von Laer, D. (2002). Oncoretrovirus and lentivirus vectors pseudotyped with lymphocytic choriomeningitis virus glycoprotein: generation, concentration, and broad host range. *J Virol* **76**(3)**,** 1488-95.

Blackford, A. N., Bruton, R. K., Dirlik, O., Stewart, G. S., Taylor, A. M., Dobner, T., Grand, R. J., and Turnell, A. S. (2008). A role for E1B-AP5 in ATR signaling pathways during adenovirus infection. *J Virol* **82**(15)**,** 7640-52.

Blackford, A. N., Patel, R. N., Forrester, N. A., Theil, K., Groitl, P., Stewart, G. S., Taylor, A. M., Morgan, I. M., Dobner, T., Grand, R. J., and Turnell, A. S. (2010). Adenovirus 12 E4orf6 inhibits ATR activation by promoting TOPBP1 degradation. *Proc Natl Acad Sci U S A* **107**(27)**,** 12251-6.

Blanchette, P., Cheng, C. Y., Yan, Q., Ketner, G., Ornelles, D. A., Dobner, T., Conaway, R. C., Conaway, J. W., and Branton, P. E. (2004). Both BC-box motifs of adenovirus protein E4orf6 are required to efficiently assemble an E3 ligase complex that degrades p53. *Mol Cell Biol* **24**(21)**,** 9619-29.

Bolderson, E., Savage, K. I., Mahen, R., Pisupati, V., Graham, M. E., Richard, D. J., Robinson, P. J., Venkitaraman, A. R., and Khanna, K. K. (2012). Kruppel-associated Box (KRAB)-associated co-repressor (KAP-1) Ser-473 phosphorylation regulates heterochromatin protein 1beta (HP1-beta) mobilization and DNA repair in heterochromatin. *J Biol Chem* **287**(33)**,** 28122-31.

Borden, K. L., and Freemont, P. S. (1996). The RING finger domain: a recent example of a sequence-structure family. *Curr Opin Struct Biol* **6**(3)**,** 395-401.

Boyer, J., Rohleder, K., and Ketner, G. (1999). Adenovirus E4 34k and E4 11k inhibit double strand break repair and are physically associated with the cellular DNA-dependent protein kinase. *Virology* **263**(2)**,** 307-12.

Branton, P. E., Bayley, S. T., and Graham, F. L. (1985). Transformation by human adenoviruses. *Biochim Biophys Acta* **780**(1)**,** 67-94.

Briers, S., Crawford, C., Bickmore, W. A., and Sutherland, H. G. (2009). KRAB zinc-finger proteins localise to novel KAP1-containing foci that are adjacent to PML nuclear bodies. *J Cell Sci* **122**(Pt 7)**,** 937-46.

Brown, D. T., Westphal, M., Burlingham, B. T., Winterhoff, U., and Doerfler, W. (1975). Structure and composition of the adenovirus type 2 core. *J Virol* **16**(2)**,** 366-87.

Buchkovich, K., Dyson, N., Whyte, P., and Harlow, E. (1990). Cellular proteins that are targets for transformation by DNA tumour viruses. *Ciba Found Symp* **150,** 262-71; discussion 271-8.

Buckwalter, S. P., Teo, R., Espy, M. J., Sloan, L. M., Smith, T. F., and Pritt, B. S. (2012). Real-time qualitative PCR for 57 human adenovirus types from multiple specimen sources. *J Clin Microbiol* **50**(3)**,** 766-71.

Burgert, H. G., and Blusch, J. H. (2000). Immunomodulatory functions encoded by the E3 transcription unit of adenoviruses. *Virus Genes* **21**(1-2)**,** 13-25.

Burma, S., and Chen, D. J. (2004). Role of DNA-PK in the cellular response to DNA double-strand breaks. *DNA Repair (Amst)* **3**(8-9)**,** 909-18.

Cammas, F., Herzog, M., Lerouge, T., Chambon, P., and Losson, R. (2004). Association of the transcriptional corepressor TIF1beta with heterochromatin protein 1 (HP1): an essential role for progression through differentiation. *Genes Dev* **18**(17)**,** 2147-60.

Cammas, F., Janoshazi, A., Lerouge, T., and Losson, R. (2007). Dynamic and selective interactions of the transcriptional corepressor TIF1 beta with the heterochromatin protein HP1 isotypes during cell differentiation. *Differentiation* **75**(7)**,** 627-37.

Cammas, F., Oulad-Abdelghani, M., Vonesch, J. L., Huss-Garcia, Y., Chambon, P., and Losson, R. (2002). Cell differentiation induces TIF1beta association with centromeric heterochromatin via an HP1 interaction. *J Cell Sci* **115**(Pt 17)**,** 3439-48.

Carrigan, D. R. (1997). Adenovirus infections in immunocompromised patients. *Am J Med* **102**(3A)**,** 71-4.

Carson, C. T., Orazio, N. I., Lee, D. V., Suh, J., Bekker-Jensen, S., Araujo, F. D., Lakdawala, S. S., Lilley, C. E., Bartek, J., Lukas, J., and Weitzman, M. D. (2009). Mislocalization of the MRN complex prevents ATR signaling during adenovirus infection. *Embo J* **28**(6)**,** 652-62.

Carson, C. T., Schwartz, R. A., Stracker, T. H., Lilley, C. E., Lee, D. V., and Weitzman, M. D. (2003). The Mre11 complex is required for ATM activation and the G2/M checkpoint. *Embo J* **22**(24)**,** 6610-20.

Challberg, M. D., and Kelly, T. J. (1989). Animal virus DNA replication. *Annu Rev Biochem* **58,** 671-717.

Challberg, M. D., Ostrove, J. M., and Kelly, T. J., Jr. (1982). Initiation of adenovirus DNA replication: detection of covalent complexes between nucleotide and the 80-kilodalton terminal protein. *J Virol* **41**(1)**,** 265-70.

Chang, C. W., Chou, H. Y., Lin, Y. S., Huang, K. H., Chang, C. J., Hsu, T. C., and Lee, S. C. (2008). Phosphorylation at Ser473 regulates heterochromatin protein 1 binding and corepressor function of TIF1beta/KAP1. *BMC Mol Biol* **9,** 61.

Chang, P. C., Fitzgerald, L. D., Van Geelen, A., Izumiya, Y., Ellison, T. J., Wang, D. H., Ann, D. K., Luciw, P. A., and Kung, H. J. (2009). Kruppel-associated box domain-associated protein-1 as a latency regulator for Kaposi's sarcoma-associated herpesvirus and its modulation by the viral protein kinase. *Cancer Res* **69**(14)**,** 5681-9.

Chatterjee, P. K., Vayda, M. E., and Flint, S. J. (1986). Identification of proteins and protein domains that contact DNA within adenovirus nucleoprotein cores by ultraviolet light crosslinking of oligonucleotides 32P-labelled in vivo. *J Mol Biol* **188**(1)**,** 23-37.

Chen, B. P., Uematsu, N., Kobayashi, J., Lerenthal, Y., Krempler, A., Yajima, H., Lobrich, M., Shiloh, Y., and Chen, D. J. (2007). Ataxia telangiectasia mutated (ATM) is essential for DNA-PKcs phosphorylations at the Thr-2609 cluster upon DNA double strand break. *J Biol Chem* **282**(9)**,** 6582-7.

Chen, L., Chen, D. T., Kurtyka, C., Rawal, B., Fulp, W. J., Haura, E. B., and Cress, W. D. (2012). Tripartite motif containing 28 (Trim28) can regulate cell proliferation by bridging HDAC1/E2F interactions. *J Biol Chem* **287**(48)**,** 40106-18.

Cheng, C. Y., Gilson, T., Dallaire, F., Ketner, G., Branton, P. E., and Blanchette, P. (2011). The E4orf6/E1B55K E3 ubiquitin ligase complexes of human adenoviruses exhibit heterogeneity in composition and substrate specificity. *J Virol* **85**(2)**,** 765-75.

Chow, L. T., Broker, T. R., and Lewis, J. B. (1979). Complex splicing patterns of RNAs from the early regions of adenovirus-2. *J Mol Biol* **134**(2), 265-303.

Cimprich, K. A., and Cortez, D. (2008). ATR: an essential regulator of genome integrity. *Nat Rev Mol Cell Biol* **9**(8), 616-27.

Cleghon, V. G., and Klessig, D. F. (1986). Association of the adenovirus DNA-binding protein with RNA both in vitro and in vivo. *Proc Natl Acad Sci U S A* **83**(23), 8947-51.

Cress, W. D., and Nevins, J. R. (1996). Use of the E2F transcription factor by DNA tumor virus regulatory proteins. *Curr Top Microbiol Immunol* **208**, 63-78.

Dallaire, F., Blanchette, P., Groitl, P., Dobner, T., and Branton, P. E. (2009). Identification of integrin alpha3 as a new substrate of the adenovirus E4orf6/E1B 55-kilodalton E3 ubiquitin ligase complex. *J Virol* **83**(11), 5329-38.

David, G., Grandinetti, K. B., Finnerty, P. M., Simpson, N., Chu, G. C., and Depinho, R. A. (2008). Specific requirement of the chromatin modifier mSin3B in cell cycle exit and cellular differentiation. *Proc Natl Acad Sci U S A* **105**(11), 4168-72.

Davison, A. J., Benko, M., and Harrach, B. (2003). Genetic content and evolution of adenoviruses. *J Gen Virol* **84**(Pt 11), 2895-908.

Davison, A. J., Telford, E. A., Watson, M. S., McBride, K., and Mautner, V. (1993). The DNA sequence of adenovirus type 40. *J Mol Biol* **234**(4), 1308-16.

de Jong, D., Aleman, B. M., Taal, B. G., and Boot, H. (1999). Controversies and consensus in the diagnosis, work-up and treatment of gastric lymphoma: an international survey. *Ann Oncol* **10**(3), 275-80.

Debbas, M., and White, E. (1993). Wild-type p53 mediates apoptosis by E1A, which is inhibited by E1B. *Genes Dev* **7**(4), 546-54.

Defer, C., Belin, M. T., Caillet-Boudin, M. L., and Boulanger, P. (1990). Human adenovirus-host cell interactions: comparative study with members of subgroups B and C. *J Virol* **64**(8), 3661-73.

Derheimer, F. A., and Kastan, M. B. (2010). Multiple roles of ATM in monitoring and maintaining DNA integrity. *FEBS Lett* **584**(17), 3675-81.

Deshaies, R. J., and Joazeiro, C. A. (2009). RING domain E3 ubiquitin ligases. *Annu Rev Biochem* **78**, 399-434.

Desterro, J. M., Rodriguez, M. S., Kemp, G. D., and Hay, R. T. (1999). Identification of the enzyme required for activation of the small ubiquitin-like protein SUMO-1. *J Biol Chem* **274**(15), 10618-24.

Dobbelstein, M., Roth, J., Kimberly, W. T., Levine, A. J., and Shenk, T. (1997). Nuclear export of the E1B 55-kDa and E4 34-kDa adenoviral oncoproteins mediated by a rev-like signal sequence. *Embo J* **16**(14), 4276-84.

Doyle, J. M., Gao, J., Wang, J., Yang, M., and Potts, P. R. (2010). MAGE-RING protein complexes comprise a family of E3 ubiquitin ligases. *Mol Cell* **39**(6), 963-74.

DuBridge, R. B., Tang, P., Hsia, H. C., Leong, P. M., Miller, J. H., and Calos, M. P. (1987). Analysis of mutation in human cells by using an Epstein-Barr virus shuttle system. *Mol Cell Biol* **7**(1)**,** 379-87.

Dull, T., Zufferey, R., Kelly, M., Mandel, R. J., Nguyen, M., Trono, D., and Naldini, L. (1998). A third-generation lentivirus vector with a conditional packaging system. *J Virol* **72**(11)**,** 8463-71.

Dyson, N., Guida, P., McCall, C., and Harlow, E. (1992). Adenovirus E1A makes two distinct contacts with the retinoblastoma protein. *J Virol* **66**(7)**,** 4606-11.

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., and Vogelstein, B. (1993). WAF1, a potential mediator of p53 tumor suppression. *Cell* **75**(4)**,** 817-25.

Enders, J. F., Bell, J. A., Dingle, J. H., Francis, T., Jr., Hilleman, M. R., Huebner, R. J., and Payne, A. M. (1956). Adenoviruses: group name proposed for new respiratory-tract viruses. *Science* **124**(3212)**,** 119-20.

Endter, C., and Dobner, T. (2004). Cell transformation by human adenoviruses. *Curr Top Microbiol Immunol* **273,** 163-214.

Endter, C., Hartl, B., Spruss, T., Hauber, J., and Dobner, T. (2005). Blockage of CRM1-dependent nuclear export of the adenovirus type 5 early region 1B 55-kDa protein augments oncogenic transformation of primary rat cells. *Oncogene* **24**(1)**,** 55-64.

Endter, C., Kzhyshkowska, J., Stauber, R., and Dobner, T. (2001). SUMO-1 modification required for transformation by adenovirus type 5 early region 1B 55-kDa oncoprotein. *Proc Natl Acad Sci U S A* **98**(20)**,** 11312-7.

Everett, R. D., and Chelbi-Alix, M. K. (2007). PML and PML nuclear bodies: implications in antiviral defence. *Biochimie* **89**(6-7)**,** 819-30.

Farmer, G., Bargonetti, J., Zhu, H., Friedman, P., Prywes, R., and Prives, C. (1992). Wild-type p53 activates transcription in vitro. *Nature* **358**(6381)**,** 83-6.

Ferrari, R., Berk, A. J., and Kurdistani, S. K. (2009). Viral manipulation of the host epigenome for oncogenic transformation. *Nat Rev Genet* **10**(5)**,** 290-4.

Ferrari, R., Pellegrini, M., Horwitz, G. A., Xie, W., Berk, A. J., and Kurdistani, S. K. (2008). Epigenetic reprogramming by adenovirus e1a. *Science* **321**(5892)**,** 1086-8.

Flint, J., and Shenk, T. (1989). Adenovirus E1A protein paradigm viral transactivator. *Annu Rev Genet* **23,** 141-61.

Forrester, N. A., Patel, R. N., Speiseder, T., Groitl, P., Sedgwick, G. G., Shimwell, N. J., Seed, R. I., Catnaigh, P. O., McCabe, C. J., Stewart, G. S., Dobner, T., Grand, R. J., Martin, A., and Turnell, A. S. (2011a). Adenovirus E4orf3 targets transcriptional intermediary factor 1gamma for proteasome-dependent degradation during infection. *J Virol* **86**(6)**,** 3167-79.

Forrester, N. A., Sedgwick, G. G., Thomas, A., Blackford, A. N., Speiseder, T., Dobner, T., Byrd, P. J., Stewart, G. S., Turnell, A. S., and Grand, R. J. (2011b). Serotype-specific inactivation of the cellular DNA damage response during adenovirus infection. *J Virol* **85**(5)**,** 2201-11.

Friedman, J. R., Fredericks, W. J., Jensen, D. E., Speicher, D. W., Huang, X. P., Neilson, E. G., and Rauscher, F. J., 3rd (1996). KAP-1, a novel corepressor for the highly conserved KRAB repression domain. *Genes Dev* **10**(16)**,** 2067-78.

Friefeld, B. R., Lichy, J. H., Hurwitz, J., and Horwitz, M. S. (1983). Evidence for an altered adenovirus DNA polymerase in cells infected with the mutant H5ts149. *Proc Natl Acad Sci U S A* **80**(6)**,** 1589-93.

Frisch, S. M., and Mymryk, J. S. (2002). Adenovirus-5 E1A: paradox and paradigm. *Nat Rev Mol Cell Biol* **3**(6)**,** 441-52.

Gaggar, A., Shayakhmetov, D. M., and Lieber, A. (2003). CD46 is a cellular receptor for group B adenoviruses. *Nat Med* **9**(11)**,** 1408-12.

Gallimore, P. H., Byrd, P. J., and Grand, R. J. (1984a). Adenovirus genes involved in transformation. What determines the oncogenic phenotype? *In Viruses and Cancer. Symposium of the Society for General Microbiology***,** edited by P. W. J. Rigby. Cambridge: Cambridge University Press. pp. 125-172.

Gallimore, P. H., Byrd, P. J., Grand, R. J., Whittaker, J. L., Breiding, D., and Williams, J. (1984b). An examination of the transforming and tumor-inducing capacity of a number of adenovirus type 12 early region 1, host-range mutants and cells transformed by subgenomic fragments of Ad12 E1 region. *Cancer Cells***,** 2, 519-526.

Garnett, C. T., Talekar, G., Mahr, J. A., Huang, W., Zhang, Y., Ornelles, D. A., and Gooding, L. R. (2009). Latent species C adenoviruses in human tonsil tissues. *J Virol* **83**(6)**,** 2417-28.

Garvin, A. J., Densham, R. M., Blair-Reid, S. A., Pratt, K. M., Stone, H. R., Weekes, D., Lawrence, K. J., and Morris, J. R. (2013). The deSUMOylase SENP7 promotes chromatin relaxation for homologous recombination DNA repair. *EMBO Rep* **14**(11)**,** 975-83.

Gautam, D., and Bridge, E. (2013). The kinase activity of ataxia-telangiectasia mutated interferes with adenovirus E4 mutant DNA replication. *J Virol* **87**(15)**,** 8687-96.

Geiss-Friedlander, R., and Melchior, F. (2007). Concepts in sumoylation: a decade on. *Nat Rev Mol Cell Biol* **8**(12)**,** 947-56.

Geuting, V., Reul, C., and Lobrich, M. (2013). ATM release at resected double-strand breaks provides heterochromatin reconstitution to facilitate homologous recombination. *PLoS Genet* **9**(8)**,** e1003667.

Giard, D. J., Aaronson, S. A., Todaro, G. J., Arnstein, P., Kersey, J. H., Dosik, H., and Parks, W. P. (1973). In vitro cultivation of human tumors: establishment of cell lines derived from a series of solid tumors. *J Natl Cancer Inst* **51**(5)**,** 1417-23.

Gill, G. (2005). Something about SUMO inhibits transcription. *Curr Opin Genet Dev* **15**(5), 536-41.

Giordano, A., Lee, J. H., Scheppler, J. A., Herrmann, C., Harlow, E., Deuschle, U., Beach, D., and Franza, B. R., Jr. (1991). Cell cycle regulation of histone H1 kinase activity associated with the adenoviral protein E1A. *Science* **253**(5025), 1271-5.

Girdwood, D. W., Tatham, M. H., and Hay, R. T. (2004). SUMO and transcriptional regulation. *Semin Cell Dev Biol* **15**(2), 201-10.

Glass, M., and Everett, R. D. (2012). Components of promyelocytic leukemia nuclear bodies (ND10) act cooperatively to repress herpesvirus infection. *J Virol* **87**(4), 2174-85.

Goldberg, A. D., Allis, C. D., and Bernstein, E. (2007). Epigenetics: a landscape takes shape. *Cell* **128**(4), 635-8.

Goodarzi, A. A., Kurka, T., and Jeggo, P. A. (2011). KAP-1 phosphorylation regulates CHD3 nucleosome remodeling during the DNA double-strand break response. *Nat Struct Mol Biol* **18**(7), 831-9.

Goodarzi, A. A., Noon, A. T., Deckbar, D., Ziv, Y., Shiloh, Y., Lobrich, M., and Jeggo, P. A. (2008). ATM signaling facilitates repair of DNA double-strand breaks associated with heterochromatin. *Mol Cell* **31**(2), 167-77.

Goodarzi, A. A., Noon, A. T., and Jeggo, P. A. (2009). The impact of heterochromatin on DSB repair. *Biochem Soc Trans* **37**(Pt 3), 569-76.

Gooding, L. R., and Wold, W. S. (1990). Molecular mechanisms by which adenoviruses counteract antiviral immune defenses. *Crit Rev Immunol* **10**(1), 53-71.

Goodrum, F. D., Shenk, T., and Ornelles, D. A. (1996). Adenovirus early region 4 34-kilodalton protein directs the nuclear localization of the early region 1B 55-kilodalton protein in primate cells. *J Virol* **70**(9), 6323-35.

Graham, F. L., Rowe, D. T., McKinnon, R., Bacchetti, S., Ruben, M., and Branton, P. E. (1984). Transformation by human adenoviruses. *J Cell Physiol Suppl* **3**, 151-63.

Graham, F. L., Smiley, J., Russell, W. C., and Nairn, R. (1977). Characteristics of a human cell line transformed by DNA from human adenovirus type 5. *J Gen Virol* **36**(1), 59-74.

Grand, R. J., Grant, M. L., and Gallimore, P. H. (1994). Enhanced expression of p53 in human cells infected with mutant adenoviruses. *Virology* **203**(2), 229-40.

Grandinetti, K. B., and David, G. (2008). Sin3B: an essential regulator of chromatin modifications at E2F target promoters during cell cycle withdrawal. *Cell Cycle* **7**(11), 1550-4.

Grandinetti, K. B., Jelinic, P., DiMauro, T., Pellegrino, J., Fernandez Rodriguez, R., Finnerty, P. M., Ruoff, R., Bardeesy, N., Logan, S. K., and David, G. (2009).

Sin3B expression is required for cellular senescence and is up-regulated upon oncogenic stress. *Cancer Res* **69**(16)**,** 6430-7.

Groner, A. C., Meylan, S., Ciuffi, A., Zangger, N., Ambrosini, G., Denervaud, N., Bucher, P., and Trono, D. (2010). KRAB-zinc finger proteins and KAP1 can mediate long-range transcriptional repression through heterochromatin spreading. *PLoS Genet* **6**(3)**,** e1000869.

Gupta, A., Jha, S., Engel, D. A., Ornelles, D. A., and Dutta, A. (2013). Tip60 degradation by adenovirus relieves transcriptional repression of viral transcriptional activator EIA. *Oncogene* **32**(42)**,** 5017-25.

Gustafsson, B., Huang, W., Bogdanovic, G., Gauffin, F., Nordgren, A., Talekar, G., Ornelles, D. A., and Gooding, L. R. (2007). Adenovirus DNA is detected at increased frequency in Guthrie cards from children who develop acute lymphoblastic leukaemia. *Br J Cancer* **97**(7)**,** 992-4.

Hanahan, D., and Meselson, M. (1983). Plasmid screening at high colony density. *Methods Enzymol* **100,** 333-42.

Harada, J. N., Shevchenko, A., Shevchenko, A., Pallas, D. C., and Berk, A. J. (2002). Analysis of the adenovirus E1B-55K-anchored proteome reveals its link to ubiquitination machinery. *J Virol* **76**(18)**,** 9194-206.

Harlow, E., Franza, B. R., Jr., and Schley, C. (1985). Monoclonal antibodies specific for adenovirus early region 1A proteins: extensive heterogeneity in early region 1A products. *J Virol* **55**(3)**,** 533-46.

Hart, L. S., Ornelles, D., and Koumenis, C. (2007). The adenoviral E4orf6 protein induces atypical apoptosis in response to DNA damage. *J Biol Chem* **282**(9)**,** 6061-7.

Hart, L. S., Yannone, S. M., Naczki, C., Orlando, J. S., Waters, S. B., Akman, S. A., Chen, D. J., Ornelles, D., and Koumenis, C. (2005). The adenovirus E4orf6 protein inhibits DNA double strand break repair and radiosensitizes human tumor cells in an E1B-55K-independent manner. *J Biol Chem* **280**(2)**,** 1474-81.

Härtl, B., Zeller, T., Blanchette, P., Kremmer, E., and Dobner, T. (2008). Adenovirus type 5 early region 1B 55-kDa oncoprotein can promote cell transformation by a mechanism independent from blocking p53-activated transcription. *Oncogene* **27**(26)**,** 3673-84.

Hateboer, G., Hijmans, E. M., Nooij, J. B., Schlenker, S., Jentsch, S., and Bernards, R. (1996). mUBC9, a novel adenovirus E1A-interacting protein that complements a yeast cell cycle defect. *J Biol Chem* **271**(42)**,** 25906-11.

Hay, R. T. (2006). Role of ubiquitin-like proteins in transcriptional regulation. *Ernst Schering Res Found Workshop*(57)**,** 173-92.

Hayashi, T., Seki, M., Maeda, D., Wang, W., Kawabe, Y., Seki, T., Saitoh, H., Fukagawa, T., Yagi, H., and Enomoto, T. (2002). Ubc9 is essential for viability of higher eukaryotic cells. *Exp Cell Res* **280**(2)**,** 212-21.

Hector, S., Chen, H., Kijanka, G., Murray, F., and Prehn, J. H. (2012). A reverse-ELISA for the detection of TRIM28/KAP1 serum autoantibodies in colorectal cancer patients. *Acta Oncol* **51**(3)**,** 394-6.

Hershko, A., and Ciechanover, A. (1998). The ubiquitin system. *Annu Rev Biochem* **67,** 425-79.

Herzog, M., Wendling, O., Guillou, F., Chambon, P., Mark, M., Losson, R., and Cammas, F. (2011). TIF1beta association with HP1 is essential for post-gastrulation development, but not for Sertoli cell functions during spermatogenesis. *Dev Biol* **350**(2)**,** 548-58.

Hiebert, S. W., Blake, M., Azizkhan, J., and Nevins, J. R. (1991). Role of E2F transcription factor in E1A-mediated trans activation of cellular genes. *J Virol* **65**(7)**,** 3547-52.

Hilleman, M. R., and Werner, J. H. (1954). Recovery of new agent from patients with acute respiratory illness. *Proc Soc Exp Biol Med* **85**(1)**,** 183-8.

Hollenbach, A. D., McPherson, C. J., Mientjes, E. J., Iyengar, R., and Grosveld, G. (2002). Daxx and histone deacetylase II associate with chromatin through an interaction with core histones and the chromatin-associated protein Dek. *J Cell Sci* **115**(Pt 16)**,** 3319-30.

Horwitz, M. S. (1996). Adenoviruses. *In Virology***,** Vol. 2. B. N. Fields, D. M. Knipe, and P. M. Howley, editors. Lippincott-Raven, New York. 2149-2171.

Hsieh, M. J., Yao, Y. L., Lai, I. L., and Yang, W. M. (2006). Transcriptional repression activity of PAX3 is modulated by competition between corepressor KAP1 and heterochromatin protein 1. *Biochem Biophys Res Commun* **349**(2)**,** 573-81.

Hu, C., Zhang, S., Gao, X., Gao, X., Xu, X., Lv, Y., Zhang, Y., Zhu, Z., Zhang, C., Li, Q., Wong, J., Cui, Y., Zhang, W., Ma, L., and Wang, C. (2012). Roles of Kruppel-associated Box (KRAB)-associated Co-repressor KAP1 Ser-473 Phosphorylation in DNA Damage Response. *J Biol Chem* **287**(23)**,** 18937-52.

Huang, C., Martin, S., Pfleger, C., Du, J., Buckner, J. H., Bluestone, J. A., Riley, J. L., and Ziegler, S. F. (2013). Cutting Edge: a novel, human-specific interacting protein couples FOXP3 to a chromatin-remodeling complex that contains KAP1/TRIM28. *J Immunol* **190**(9)**,** 4470-3.

Huang, M. M., and Hearing, P. (1989). Adenovirus early region 4 encodes two gene products with redundant effects in lytic infection. *J Virol* **63**(6)**,** 2605-15.

Huebner, R. J., Rowe, W. P., Ward, T. G., Parrott, R. H., and Bell, J. A. (1954). Adenoidal-pharyngeal-conjunctival agents: a newly recognized group of common viruses of the respiratory system. *N Engl J Med* **251**(27)**,** 1077-86.

Ivanov, A. V., Peng, H., Yurchenko, V., Yap, K. L., Negorev, D. G., Schultz, D. C., Psulkowski, E., Fredericks, W. J., White, D. E., Maul, G. G., Sadofsky, M. J., Zhou, M. M., and Rauscher, F. J., 3rd (2007). PHD domain-mediated E3 ligase activity directs intramolecular sumoylation of an adjacent bromodomain required for gene silencing. *Mol Cell* **28**(5)**,** 823-37.

Iyengar, S., and Farnham, P. J. (2011). KAP1 protein: an enigmatic master regulator of the genome. *J Biol Chem* **286**(30)**,** 26267-76.

Iyengar, S., Ivanov, A. V., Jin, V. X., Rauscher, F. J., 3rd, and Farnham, P. J. (2011). Functional analysis of KAP1 genomic recruitment. *Mol Cell Biol* **31**(9)**,** 1833-47.

Jackson, W. T., Giddings, T. H., Jr., Taylor, M. P., Mulinyawe, S., Rabinovitch, M., Kopito, R. R., and Kirkegaard, K. (2005). Subversion of cellular autophagosomal machinery by RNA viruses. *PLoS Biol* **3**(5)**,** e156.

Jakoby, W. B., and Pastan, I. H. (1979). Cell culture. Methods in enzymology**,** Academic press, Inc., San Diego, California.

Johnson, E. S. (2004). Protein modification by SUMO. *Annu Rev Biochem* **73,** 355-82.

Johnson, E. S., and Blobel, G. (1997). Ubc9p is the conjugating enzyme for the ubiquitin-like protein Smt3p. *J Biol Chem* **272**(43)**,** 26799-802.

Johnson, E. S., Schwienhorst, I., Dohmen, R. J., and Blobel, G. (1997). The ubiquitin-like protein Smt3p is activated for conjugation to other proteins by an Aos1p/Uba2p heterodimer. *Embo J* **16**(18)**,** 5509-19.

Kamitani, S., Ohbayashi, N., Ikeda, O., Togi, S., Muromoto, R., Sekine, Y., Ohta, K., Ishiyama, H., and Matsuda, T. (2008). KAP1 regulates type I interferon/STAT1-mediated IRF-1 gene expression. *Biochem Biophys Res Commun* **370**(2)**,** 366-70.

Kamitani, S., Togi, S., Ikeda, O., Nakasuji, M., Sakauchi, A., Sekine, Y., Muromoto, R., Oritani, K., and Matsuda, T. (2011). Kruppel-associated box-associated protein 1 negatively regulates TNF-alpha-induced NF-kappaB transcriptional activity by influencing the interactions among STAT3, p300, and NF-kappaB/p65. *J Immunol* **187**(5)**,** 2476-83.

Kao, C. C., Yew, P. R., and Berk, A. J. (1990). Domains required for in vitro association between the cellular p53 and the adenovirus 2 E1B 55K proteins. *Virology* **179**(2)**,** 806-14.

Karen, K. A., and Hearing, P. (2002). Adenovirus core protein VII protects the viral genome from a DNA damage response at early times after infection. *J Virol* **85**(9)**,** 4135-42.

Kepkay, R., Attwood, K. M., Ziv, Y., Shiloh, Y., and Dellaire, G. (2011). KAP1 depletion increases PML nuclear body number in concert with ultrastructural changes in chromatin. *Cell Cycle* **10**(2)**,** 308-22.

Kerscher, O., Felberbaum, R., and Hochstrasser, M. (2006). Modification of proteins by ubiquitin and ubiquitin-like proteins. *Annu Rev Cell Dev Biol* **22,** 159-80.

Kim, S. S., Chen, Y. M., O'Leary, E., Witzgall, R., Vidal, M., and Bonventre, J. V. (1996). A novel member of the RING finger family, KRIP-1, associates with the KRAB-A transcriptional repressor domain of zinc finger proteins. *Proc Natl Acad Sci U S A* **93**(26)**,** 15299-304.

Kindsmuller, K., Groitl, P., Hartl, B., Blanchette, P., Hauber, J., and Dobner, T. (2007). Intranuclear targeting and nuclear export of the adenovirus E1B-55K protein are regulated by SUMO1 conjugation. *Proc Natl Acad Sci U S A* **104**(16)**,** 6684-9.

Kindsmuller, K., Schreiner, S., Leinenkugel, F., Groitl, P., Kremmer, E., and Dobner, T. (2009). A 49-kilodalton isoform of the adenovirus type 5 early region 1B 55-kilodalton protein is sufficient to support virus replication. *J Virol* **83**(18)**,** 9045-56.

King, C. A. (2013). Kaposi's sarcoma-associated herpesvirus kaposin B induces unique monophosphorylation of STAT3 at serine 727 and MK2-mediated inactivation of the STAT3 transcriptional repressor TRIM28. *J Virol* **87**(15)**,** 8779-91.

Kinkley, S., Staege, H., Mohrmann, G., Rohaly, G., Schaub, T., Kremmer, E., Winterpacht, A., and Will, H. (2009). SPOC1: a novel PHD-containing protein modulating chromatin structure and mitotic chromosome condensation. *J Cell Sci* **122**(Pt 16)**,** 2946-56.

Klein, H., Maltzman, W., and Levine, A. J. (1979). Structure-function relationships of the adenovirus DNA-binding protein. *J Biol Chem* **254**(21)**,** 11051-60.

Ko, L. J., and Prives, C. (1996). p53: puzzle and paradigm. *Genes Dev* **10**(9)**,** 1054-72.

Komatsu, T., Haruki, H., and Nagata, K. (2011). Cellular and viral chromatin proteins are positive factors in the regulation of adenovirus gene expression. *Nucleic Acids Res* **39**(3)**,** 889-901.

Kosulin, K., Haberler, C., Hainfellner, J. A., Amann, G., Lang, S., and Lion, T. (2007). Investigation of adenovirus occurrence in pediatric tumor entities. *J Virol* **81**(14)**,** 7629-35.

Kovesdi, I., Reichel, R., and Nevins, J. R. (1986). Identification of a cellular transcription factor involved in E1A trans-activation. *Cell* **45**(2)**,** 219-28.

Kubota, S., Fukumoto, Y., Aoyama, K., Ishibashi, K., Yuki, R., Morinaga, T., Honda, T., Yamaguchi, N., Kuga, T., Tomonaga, T., and Yamaguchi, N. (2013). Phosphorylation of KRAB-associated protein 1 (KAP1) at Tyr-449, Tyr-458, and Tyr-517 by nuclear tyrosine kinases inhibits the association of KAP1 and heterochromatin protein 1alpha (HP1alpha) with heterochromatin. *J Biol Chem* **288**(24)**,** 17871-83.

Lai, I. L., Lin, T. P., Yao, Y. L., Lin, C. Y., Hsieh, M. J., and Yang, W. M. (2010). Histone deacetylase 10 relieves repression on the melanogenic program by maintaining the deacetylation status of repressors. *J Biol Chem* **285**(10)**,** 7187-96.

Lechner, M. S., Begg, G. E., Speicher, D. W., and Rauscher, F. J., 3rd (2000). Molecular determinants for targeting heterochromatin protein 1-mediated gene silencing: direct chromoshadow domain-KAP-1 corepressor interaction is essential. *Mol Cell Biol* **20**(17)**,** 6449-65.

Ledl, A., Schmidt, D., and Muller, S. (2005). Viral oncoproteins E1A and E7 and cellular LxCxE proteins repress SUMO modification of the retinoblastoma tumor suppressor. *Oncogene* **24**(23), 3810-8.

Lee, D. H., Goodarzi, A. A., Adelmant, G. O., Pan, Y., Jeggo, P. A., Marto, J. A., and Chowdhury, D. (2012). Phosphoproteomic analysis reveals that PP4 dephosphorylates KAP-1 impacting the DNA damage response. *Embo J* **31**(10), 2403-15.

Lee, J. H., and Paull, T. T. (2007). Activation and regulation of ATM kinase activity in response to DNA double-strand breaks. *Oncogene* **26**(56), 7741-8.

Lee, T. W., Blair, G. E., and Matthews, D. A. (2003). Adenovirus core protein VII contains distinct sequences that mediate targeting to the nucleus and nucleolus, and colocalization with human chromosomes. *J Gen Virol* **84**(Pt 12), 3423-8.

Lee, Y. K., Thomas, S. N., Yang, A. J., and Ann, D. K. (2007). Doxorubicin down-regulates Kruppel-associated box domain-associated protein 1 sumoylation that relieves its transcription repression on p21WAF1/CIP1 in breast cancer MCF-7 cells. *J Biol Chem* **282**(3), 1595-606.

Lethbridge, K. J., Scott, G. E., and Leppard, K. N. (2003). Nuclear matrix localization and SUMO-1 modification of adenovirus type 5 E1b 55K protein are controlled by E4 Orf6 protein. *J Gen Virol* **84**(Pt 2), 259-68.

Li, X., Lee, Y. K., Jeng, J. C., Yen, Y., Schultz, D. C., Shih, H. M., and Ann, D. K. (2007). Role for KAP1 serine 824 phosphorylation and sumoylation/desumoylation switch in regulating KAP1-mediated transcriptional repression. *J Biol Chem* **282**(50), 36177-89.

Li, X., Lin, H. H., Chen, H., Xu, X., Shih, H. M., and Ann, D. K. (2010). SUMOylation of the transcriptional co-repressor KAP1 is regulated by the serine and threonine phosphatase PP1. *Sci Signal* **3**(119), ra32.

Liang, Q., Deng, H., Li, X., Wu, X., Tang, Q., Chang, T. H., Peng, H., Rauscher, F. J., 3rd, Ozato, K., and Zhu, F. (2011). Tripartite motif-containing protein 28 is a small ubiquitin-related modifier E3 ligase and negative regulator of IFN regulatory factor 7. *J Immunol* **187**(9), 4754-63.

Liebermann, H., Mentel, R., Dohner, L., Modrow, S., and Seidel, W. (1996). Inhibition of cell adhesion to the virus by synthetic peptides of fiber knob of human adenovirus serotypes 2 and 3 and virus neutralisation by anti-peptide antibodies. *Virus Res* **45**(2), 111-22.

Lin, L. F., Li, C. F., Wang, W. J., Yang, W. M., Wang, D. D., Chang, W. C., Lee, W. H., and Wang, J. M. (2013). Loss of ZBRK1 contributes to the increase of KAP1 and promotes KAP1-mediated metastasis and invasion in cervical cancer. *PLoS One* **8**(8), e73033.

Linne, T., Jornvall, H., and Philipson, L. (1977). Purification and characterization of the phosphorylated DNA-binding protein from adenovirus-type-2-infected cells. *Eur J Biochem* **76**(2), 481-90.

Liu, B., Wang, Z., Ghosh, S., and Zhou, Z. (2012). Defective ATM-Kap-1-mediated chromatin remodeling impairs DNA repair and accelerates senescence in progeria mouse model. *Aging Cell* **12**(2), 316-8.

Liu, B., Wang, Z., Ghosh, S., and Zhou, Z. (2013). Defective ATM-Kap-1-mediated chromatin remodeling impairs DNA repair and accelerates senescence in progeria mouse model. *Aging Cell* **12**(2), 316-8.

Liu, F., and Green, M. R. (1990). A specific member of the ATF transcription factor family can mediate transcription activation by the adenovirus E1a protein. *Cell* **61**(7), 1217-24.

Liu, Y., Colosimo, A. L., Yang, X. J., and Liao, D. (2000). Adenovirus E1B 55-kilodalton oncoprotein inhibits p53 acetylation by PCAF. *Mol Cell Biol* **20**(15), 5540-53.

Lombard, D. B., and Guarente, L. (2000). Nijmegen breakage syndrome disease protein and MRE11 at PML nuclear bodies and meiotic telomeres. *Cancer Res* **60**(9), 2331-4.

Lowe, S. W., and Ruley, H. E. (1993). Stabilization of the p53 tumor suppressor is induced by adenovirus 5 E1A and accompanies apoptosis. *Genes Dev* **7**(4), 535-45.

Loyter, A., Scangos, G., Juricek, D., Keene, D., and Ruddle, F. H. (1982). Mechanisms of DNA entry into mammalian cells. II. Phagocytosis of calcium phosphate DNA co-precipitate visualized by electron microscopy. *Exp Cell Res* **139**(1), 223-34.

Luo, K., Ehrlich, E., Xiao, Z., Zhang, W., Ketner, G., and Yu, X. F. (2007). Adenovirus E4orf6 assembles with Cullin5-ElonginB-ElonginC E3 ubiquitin ligase through an HIV/SIV Vif-like BC-box to regulate p53. *Faseb J* **21**(8), 1742-50.

Marshall, L. J., Moore, A. C., Ohki, M., Kitabayashi, I., Patterson, D., and Ornelles, D. A. (2008). RUNX1 permits E4orf6-directed nuclear localization of the adenovirus E1B-55K protein and associates with centers of viral DNA and RNA synthesis. *J Virol* **82**(13), 6395-408.

Martin, M. E., and Berk, A. J. (1998). Adenovirus E1B 55K represses p53 activation in vitro. *J Virol* **72**(4), 3146-54.

Martin, M. E., and Berk, A. J. (1999). Corepressor required for adenovirus E1B 55,000-molecular-weight protein repression of basal transcription. *Mol Cell Biol* **19**(5), 3403-14.

Martins, M. B., Marcello, M. A., Morari, E. C., Cunha, L. L., Soares, F. A., Vassallo, J., and Ward, L. S. (2013). Clinical utility of KAP-1 expression in thyroid lesions. *Endocr Pathol* **24**(2), 77-82.

Marton, M. J., Baim, S. B., Ornelles, D. A., and Shenk, T. (1990). The adenovirus E4 17-kilodalton protein complexes with the cellular transcription factor E2F, altering its DNA-binding properties and stimulating E1A-independent accumulation of E2 mRNA. *J Virol* **64**(5), 2345-59.

Maruyama, A., Nishikawa, K., Kawatani, Y., Mimura, J., Hosoya, T., Harada, N., Yamamato, M., and Itoh, K. (2011). The novel Nrf2-interacting factor KAP1 regulates susceptibility to oxidative stress by promoting the Nrf2-mediated cytoprotective response. *Biochem J* **436**(2)**,** 387-97.

Mascle, X. H., Germain-Desprez, D., Huynh, P., Estephan, P., and Aubry, M. (2007). Sumoylation of the transcriptional intermediary factor 1beta (TIF1beta), the Co-repressor of the KRAB Multifinger proteins, is required for its transcriptional activity and is modulated by the KRAB domain. *J Biol Chem* **282**(14)**,** 10190-202.

Mathias, P., Wickham, T., Moore, M., and Nemerow, G. (1994). Multiple adenovirus serotypes use alpha v integrins for infection. *J Virol* **68**(10)**,** 6811-4.

Matsui, T., Leung, D., Miyashita, H., Maksakova, I. A., Miyachi, H., Kimura, H., Tachibana, M., Lorincz, M. C., and Shinkai, Y. (2010). Proviral silencing in embryonic stem cells requires the histone methyltransferase ESET. *Nature* **464**(7290)**,** 927-31.

Matsumoto, K., Nagata, K., Ui, M., and Hanaoka, F. (1993). Template activating factor I, a novel host factor required to stimulate the adenovirus core DNA replication. *J Biol Chem* **268**(14)**,** 10582-7.

Matthews, D. A., and Russell, W. C. (1998a). Adenovirus core protein V interacts with p32--a protein which is associated with both the mitochondria and the nucleus. *J Gen Virol* **79 (Pt 7),** 1677-85.

Matthews, D. A., and Russell, W. C. (1998b). Adenovirus core protein V is delivered by the invading virus to the nucleus of the infected cell and later in infection is associated with nucleoli. *J Gen Virol* **79 (Pt 7),** 1671-5.

Metzger, L., and Iliakis, G. (1991). Kinetics of DNA double-strand break repair throughout the cell cycle as assayed by pulsed field gel electrophoresis in CHO cells. *Int J Radiat Biol* **59**(6)**,** 1325-39.

Mitsudomi, T., Steinberg, S. M., Nau, M. M., Carbone, D., D'Amico, D., Bodner, S., Oie, H. K., Linnoila, R. I., Mulshine, J. L., Minna, J. D., and et al. (1992). p53 gene mutations in non-small-cell lung cancer cell lines and their correlation with the presence of ras mutations and clinical features. *Oncogene* **7**(1)**,** 171-80.

Moosmann, P., Georgiev, O., Le Douarin, B., Bourquin, J. P., and Schaffner, W. (1996). Transcriptional repression by RING finger protein TIF1 beta that interacts with the KRAB repressor domain of KOX1. *Nucleic Acids Res* **24**(24)**,** 4859-67.

Moran, E., Grodzicker, T., Roberts, R. J., Mathews, M. B., and Zerler, B. (1986). Lytic and transforming functions of individual products of the adenovirus E1A gene. *J Virol* **57**(3)**,** 765-75.

Müller, S., and Dobner, T. (2008). The adenovirus E1B-55K oncoprotein induces SUMO modification of p53. *Cell Cycle* **7**(6)**,** 754-8.

Mund, A., Schubert, T., Staege, H., Kinkley, S., Reumann, K., Kriegs, M., Fritsch, L., Battisti, V., Ait-Si-Ali, S., Hoffbeck, A. S., Soutoglou, E., and Will, H. (2012).

SPOC1 modulates DNA repair by regulating key determinants of chromatin compaction and DNA damage response. *Nucleic Acids Res* **40**(22), 11363-79.

Mymryk, J. S., Shire, K., and Bayley, S. T. (1994). Induction of apoptosis by adenovirus type 5 E1A in rat cells requires a proliferation block. *Oncogene* **9**(4), 1187-93.

Nacerddine, K., Lehembre, F., Bhaumik, M., Artus, J., Cohen-Tannoudji, M., Babinet, C., Pandolfi, P. P., and Dejean, A. (2005). The SUMO pathway is essential for nuclear integrity and chromosome segregation in mice. *Dev Cell* **9**(6), 769-79.

Nevins, J. R. (1990). Adenovirus E1A-dependent trans-activation of transcription. *Semin Cancer Biol* **1**(1), 59-68.

Nevins, J. R., and Vogt, P. K. (1996). Cell transformation by viruses. In Virology, third edn, edited by B. N. Fields, D. M. Knipe & P. M. Howley. New York: Lippicott-Raven, pp. 301-343.

Nielsen, A. L., Ortiz, J. A., You, J., Oulad-Abdelghani, M., Khechumian, R., Gansmuller, A., Chambon, P., and Losson, R. (1999). Interaction with members of the heterochromatin protein 1 (HP1) family and histone deacetylation are differentially involved in transcriptional silencing by members of the TIF1 family. *Embo J* **18**(22), 6385-95.

Noon, A. T., Shibata, A., Rief, N., Lobrich, M., Stewart, G. S., Jeggo, P. A., and Goodarzi, A. A. (2010). 53BP1-dependent robust localized KAP-1 phosphorylation is essential for heterochromatic DNA double-strand break repair. *Nat Cell Biol* **12**(2), 177-84.

Okamoto, K., Kitabayashi, I., and Taya, Y. (2006). KAP1 dictates p53 response induced by chemotherapeutic agents via Mdm2 interaction. *Biochem Biophys Res Commun* **351**(1), 216-22.

Okuma, T., Honda, R., Ichikawa, G., Tsumagari, N., and Yasuda, H. (1999). In vitro SUMO-1 modification requires two enzymatic steps, E1 and E2. *Biochem Biophys Res Commun* **254**(3), 693-8.

Okuwaki, M., and Nagata, K. (1998). Template activating factor-I remodels the chromatin structure and stimulates transcription from the chromatin template. *J Biol Chem* **273**(51), 34511-8.

Olive, P. L. (1998). The role of DNA single- and double-strand breaks in cell killing by ionizing radiation. *Radiat Res* **150**(5 Suppl), S42-51.

Orazio, N. I., Naeger, C. M., Karlseder, J., and Weitzman, M. D. (2011). The adenovirus E1b55K/E4orf6 complex induces degradation of the Bloom helicase during infection. *J Virol* **85**(4), 1887-92.

Ornelles, D. A., and Shenk, T. (1991). Localization of the adenovirus early region 1B 55-kilodalton protein during lytic infection: association with nuclear viral inclusions requires the early region 4 34-kilodalton protein. *J Virol* **65**(1), 424-9.

Pelka, P., Ablack, J. N., Torchia, J., Turnell, A. S., Grand, R. J., and Mymryk, J. S. (2009). Transcriptional control by adenovirus E1A conserved region 3 via p300/CBP. *Nucleic Acids Res* **37**(4)**,** 1095-106.

Peng, H., Gibson, L. C., Capili, A. D., Borden, K. L., Osborne, M. J., Harper, S. L., Speicher, D. W., Zhao, K., Marmorstein, R., Rock, T. A., and Rauscher, F. J., 3rd (2007). The structurally disordered KRAB repression domain is incorporated into a protease resistant core upon binding to KAP-1-RBCC domain. *J Mol Biol* **370**(2)**,** 269-89.

Peng, H., Ivanov, A. V., Oh, H. J., Lau, Y. F., and Rauscher, F. J., 3rd (2009). Epigenetic gene silencing by the SRY protein is mediated by a KRAB-O protein that recruits the KAP1 co-repressor machinery. *J Biol Chem* **284**(51)**,** 35670-80.

Pennella, M. A., Liu, Y., Woo, J. L., Kim, C. A., and Berk, A. J. (2010). Adenovirus E1B 55-kilodalton protein is a p53-SUMO1 E3 ligase that represses p53 and stimulates its nuclear export through interactions with promyelocytic leukemia nuclear bodies. *J Virol* **84**(23)**,** 12210-25.

Pérez-Vargas, J., Vaughan, R. C., Houser, C., Hastie, K. M., Kao, C. C., and Nemerow, G. R. (2014). Isolation and characterization of the DNA and protein binding activities of adenovirus core protein V. *J Virol* **88**(16)**,** 9287-96.

Perricaudet, M., Akusjarvi, G., Virtanen, A., and Pettersson, U. (1979). Structure of two spliced mRNAs from the transforming region of human subgroup C adenoviruses. *Nature* **281**(5733)**,** 694-6.

Placek, B. J., Huang, J., Kent, J. R., Dorsey, J., Rice, L., Fraser, N. W., and Berger, S. L. (2009). The histone variant H3.3 regulates gene expression during lytic infection with herpes simplex virus type 1. *J Virol* **83**(3)**,** 1416-21.

Polo, S. E., Blackford, A. N., Chapman, J. R., Baskcomb, L., Gravel, S., Rusch, A., Thomas, A., Blundred, R., Smith, P., Kzhyshkowska, J., Dobner, T., Taylor, A. M., Turnell, A. S., Stewart, G. S., Grand, R. J., and Jackson, S. P. (2012). Regulation of DNA-end resection by hnRNPU-like proteins promotes DNA double-strand break signaling and repair. *Mol Cell* **45**(4)**,** 505-16.

Pombo, A., Ferreira, J., Bridge, E., and Carmo-Fonseca, M. (1994). Adenovirus replication and transcription sites are spatially separated in the nucleus of infected cells. *Embo J* **13**(21)**,** 5075-85.

Prakash, A., Jayaram, S., and Bridge, E. (2012). Differential activation of cellular DNA damage responses by replication-defective and replication-competent adenovirus mutants. *J Virol* **86**(24)**,** 13324-33.

Puvion-Dutilleul, F., and Puvion, E. (1990). Replicating single-stranded adenovirus type 5 DNA molecules accumulate within well-delimited intranuclear areas of lytically infected HeLa cells. *Eur J Cell Biol* **52**(2)**,** 379-88.

Puvion-Dutilleul, F., and Puvion, E. (1995). Immunocytochemistry, autoradiography, in situ hybridization, selective stains: complementary tools for ultrastructural

study of structure-function relationships in the nucleus. Applications to adenovirus-infected cells. *Microsc Res Tech* **31**(1)**,** 22-43.

Querido, E., Blanchette, P., Yan, Q., Kamura, T., Morrison, M., Boivin, D., Kaelin, W. G., Conaway, R. C., Conaway, J. W., and Branton, P. E. (2001a). Degradation of p53 by adenovirus E4orf6 and E1B55K proteins occurs via a novel mechanism involving a Cullin-containing complex. *Genes Dev* **15**(23)**,** 3104-17.

Querido, E., Morrison, M. R., Chu-Pham-Dang, H., Thirlwell, S. W., Boivin, D., and Branton, P. E. (2001b). Identification of three functions of the adenovirus e4orf6 protein that mediate p53 degradation by the E4orf6-E1B55K complex. *J Virol* **75**(2)**,** 699-709.

Redon, C. E., Nakamura, A. J., Zhang, Y. W., Ji, J. J., Bonner, W. M., Kinders, R. J., Parchment, R. E., Doroshow, J. H., and Pommier, Y. (2010). Histone gammaH2AX and poly(ADP-ribose) as clinical pharmacodynamic biomarkers. *Clin Cancer Res* **16**(18)**,** 4532-42.

Reich, N. C., Sarnow, P., Duprey, E., and Levine, A. J. (1983). Monoclonal antibodies which recognize native and denatured forms of the adenovirus DNA-binding protein. *Virology* **128**(2)**,** 480-4.

Ricciardi, R. P. (1995). Transformation and tumorigenesis mediated by the adenovirus E1A and E1B oncogenes. In DNA Tumor Viruses: Oncogenic Mechanisms**,** edited by G. Barbanti-Brodano. New York: Plenum Press, pp. 195-210.

Rodriguez, M. S., Dargemont, C., and Hay, R. T. (2001). SUMO-1 conjugation in vivo requires both a consensus modification motif and nuclear targeting. *J Biol Chem* **276**(16)**,** 12654-9.

Rogakou, E. P., Pilch, D. R., Orr, A. H., Ivanova, V. S., and Bonner, W. M. (1998). DNA double-stranded breaks induce histone H2AX phosphorylation on serine 139. *J Biol Chem* **273**(10)**,** 5858-68.

Ross, P. J., Kennedy, M. A., Christou, C., Risco Quiroz, M., Poulin, K. L., and Parks, R. J. (2011). Assembly of helper-dependent adenovirus DNA into chromatin promotes efficient gene expression. *J Virol* **85**(8)**,** 3950-8.

Roth, J., Konig, C., Wienzek, S., Weigel, S., Ristea, S., and Dobbelstein, M. (1998). Inactivation of p53 but not p73 by adenovirus type 5 E1B 55-kilodalton and E4 34-kilodalton oncoproteins. *J Virol* **72**(11)**,** 8510-6.

Rowe, H. M., Jakobsson, J., Mesnard, D., Rougemont, J., Reynard, S., Aktas, T., Maillard, P. V., Layard-Liesching, H., Verp, S., Marquis, J., Spitz, F., Constam, D. B., and Trono, D. (2010). KAP1 controls endogenous retroviruses in embryonic stem cells. *Nature* **463**(7278)**,** 237-40.

Rowe, W. P., Huebner, R. J., Gilmore, L. K., Parrott, R. H., and Ward, T. G. (1953). Isolation of a cytopathogenic agent from human adenoids undergoing spontaneous degeneration in tissue culture. *Proc Soc Exp Biol Med* **84**(3)**,** 570-3.

Ruley, H. E. (1983). Adenovirus early region 1A enables viral and cellular transforming genes to transform primary cells in culture. *Nature* **304**(5927), 602-6.

Russell, W. C. (2009). Adenoviruses: update on structure and function. *J Gen Virol* **90**(Pt 1), 1-20.

Russell, W. C., and Matthews, D. A. (2003). Nuclear perturbations following adenovirus infection. *Curr Top Microbiol Immunol* **272**, 399-413.

Ryan, R. F., Schultz, D. C., Ayyanathan, K., Singh, P. B., Friedman, J. R., Fredericks, W. J., and Rauscher, F. J., 3rd (1999). KAP-1 corepressor protein interacts and colocalizes with heterochromatic and euchromatic HP1 proteins: a potential role for Kruppel-associated box-zinc finger proteins in heterochromatin-mediated gene silencing. *Mol Cell Biol* **19**(6), 4366-78.

Sabbatini, P., Chiou, S. K., Rao, L., and White, E. (1995). Modulation of p53-mediated transcriptional repression and apoptosis by the adenovirus E1B 19K protein. *Mol Cell Biol* **15**(2), 1060-70.

Sadowski, I., and Ptashne, M. (1989). A vector for expressing GAL4(1-147) fusions in mammalian cells. *Nucleic Acids Res* **17**(18), 7539.

Saitoh, H., and Hinchey, J. (2000). Functional heterogeneity of small ubiquitin-related protein modifiers SUMO-1 versus SUMO-2/3. *J Biol Chem* **275**(9), 6252-8.

Salone, B., Martina, Y., Piersanti, S., Cundari, E., Cherubini, G., Franqueville, L., Failla, C. M., Boulanger, P., and Saggio, I. (2003). Integrin alpha3beta1 is an alternative cellular receptor for adenovirus serotype 5. *J Virol* **77**(24), 13448-54.

Samuelson, A. V., and Lowe, S. W. (1997). Selective induction of p53 and chemosensitivity in RB-deficient cells by E1A mutants unable to bind the RB-related proteins. *Proc Natl Acad Sci U S A* **94**(22), 12094-9.

Santoni de Sio, F. R., Barde, I., Offner, S., Kapopoulou, A., Corsinotti, A., Bojkowska, K., Genolet, R., Thomas, J. H., Luescher, I. F., Pinschewer, D., Harris, N., and Trono, D. (2012a). KAP1 regulates gene networks controlling T-cell development and responsiveness. *Faseb J* **26**(11), 4561-75.

Santoni de Sio, F. R., Massacand, J., Barde, I., Offner, S., Corsinotti, A., Kapopoulou, A., Bojkowska, K., Dagklis, A., Fernandez, M., Ghia, P., Thomas, J. H., Pinschewer, D., Harris, N., and Trono, D. (2012b). KAP1 regulates gene networks controlling mouse B-lymphoid cell differentiation and function. *Blood* **119**(20), 4675-85.

Sarnow, P., Ho, Y. S., Williams, J., and Levine, A. J. (1982). Adenovirus E1b-58kd tumor antigen and SV40 large tumor antigen are physically associated with the same 54 kd cellular protein in transformed cells. *Cell* **28**(2), 387-94.

Schaeper, U., Subramanian, T., Lim, L., Boyd, J. M., and Chinnadurai, G. (1998). Interaction between a cellular protein that binds to the C-terminal region of adenovirus E1A (CtBP) and a novel cellular protein is disrupted by E1A through a conserved PLDLS motif. *J Biol Chem* **273**(15), 8549-52.

Schlesinger, S., and Goff, S. P. (2013). Silencing of proviruses in embryonic cells: efficiency, stability and chromatin modifications. *EMBO Rep* **14**(1)**,** 73-9.

Schreiner, S., Burck, C., Glass, M., Groitl, P., Wimmer, P., Kinkley, S., Mund, A., Everett, R. D., and Dobner, T. (2013a). Control of human adenovirus type 5 gene expression by cellular Daxx/ATRX chromatin-associated complexes. *Nucleic Acids Res* **41**(6)**,** 3532-50.

Schreiner, S., Kinkley, S., Burck, C., Mund, A., Wimmer, P., Schubert, T., Groitl, P., Will, H., and Dobner, T. (2013b). SPOC1-mediated antiviral host cell response is antagonized early in human adenovirus type 5 infection. *PLoS Pathog* **9**(11)**,** e1003775.

Schreiner, S., Martinez, R., Groitl, P., Rayne, F., Vaillant, R., Wimmer, P., Bossis, G., Sternsdorf, T., Marcinowski, L., Ruzsics, Z., Dobner, T., and Wodrich, H. (2012). Transcriptional activation of the adenoviral genome is mediated by capsid protein VI. *PLoS Pathog* **8**(2)**,** e1002549.

Schreiner, S., Wimmer, P., Groitl, P., Chen, S. Y., Blanchette, P., Branton, P. E., and Dobner, T. (2011). Adenovirus type 5 early region 1B 55K oncoprotein-dependent degradation of cellular factor Daxx is required for efficient transformation of primary rodent cells. *J Virol* **85**(17)**,** 8752-65.

Schreiner, S., Wimmer, P., Sirma, H., Everett, R. D., Blanchette, P., Groitl, P., and Dobner, T. (2010). Proteasome-dependent degradation of Daxx by the viral E1B-55K protein in human adenovirus-infected cells. *J Virol* **84**(14)**,** 7029-38.

Schultz, D. C., Ayyanathan, K., Negorev, D., Maul, G. G., and Rauscher, F. J., 3rd (2002). SETDB1: a novel KAP-1-associated histone H3, lysine 9-specific methyltransferase that contributes to HP1-mediated silencing of euchromatic genes by KRAB zinc-finger proteins. *Genes Dev* **16**(8)**,** 919-32.

Schultz, D. C., Friedman, J. R., and Rauscher, F. J., 3rd (2001). Targeting histone deacetylase complexes via KRAB-zinc finger proteins: the PHD and bromodomains of KAP-1 form a cooperative unit that recruits a novel isoform of the Mi-2alpha subunit of NuRD. *Genes Dev* **15**(4)**,** 428-43.

Seeler, J. S., and Dejean, A. (2003). Nuclear and unclear functions of SUMO. *Nat Rev Mol Cell Biol* **4**(9)**,** 690-9.

Seufert, W., Futcher, B., and Jentsch, S. (1995). Role of a ubiquitin-conjugating enzyme in degradation of S- and M-phase cyclins. *Nature* **373**(6509)**,** 78-81.

Shen, Y., Kitzes, G., Nye, J. A., Fattaey, A., and Hermiston, T. (2001). Analyses of single-amino-acid substitution mutants of adenovirus type 5 E1B-55K protein. *J Virol* **75**(9)**,** 4297-307.

Shenk, T. (2001). Adenoviridae: the viruses and their replication. *In Fields Virology***,** 4th edn, pp. 2265-2300.

Shibata, A., Conrad, S., Birraux, J., Geuting, V., Barton, O., Ismail, A., Kakarougkas, A., Meek, K., Taucher-Scholz, G., Lobrich, M., and Jeggo, P. A. (2011). Factors

determining DNA double-strand break repair pathway choice in G2 phase. *Embo J* **30**(6)**,** 1079-92.

Song, J., Durrin, L. K., Wilkinson, T. A., Krontiris, T. G., and Chen, Y. (2004). Identification of a SUMO-binding motif that recognizes SUMO-modified proteins. *Proc Natl Acad Sci U S A* **101**(40)**,** 14373-8.

Soria, C., Estermann, F. E., Espantman, K. C., and O'Shea, C. C. (2010). Heterochromatin silencing of p53 target genes by a small viral protein. *Nature* **466**(7310)**,** 1076-81.

Sripathy, S. P., Stevens, J., and Schultz, D. C. (2006). The KAP1 corepressor functions to coordinate the assembly of de novo HP1-demarcated microenvironments of heterochromatin required for KRAB zinc finger protein-mediated transcriptional repression. *Mol Cell Biol* **26**(22)**,** 8623-38.

Steegenga, W. T., Riteco, N., Jochemsen, A. G., Fallaux, F. J., and Bos, J. L. (1998). The large E1B protein together with the E4orf6 protein target p53 for active degradation in adenovirus infected cells. *Oncogene* **16**(3)**,** 349-57.

Stevens, J. L., Cantin, G. T., Wang, G., Shevchenko, A., Shevchenko, A., and Berk, A. J. (2002). Transcription control by E1A and MAP kinase pathway via Sur2 mediator subunit. *Science* **296**(5568)**,** 755-8.

Stracker, T. H., Carson, C. T., and Weitzman, M. D. (2002). Adenovirus oncoproteins inactivate the Mre11-Rad50-NBS1 DNA repair complex. *Nature* **418**(6895)**,** 348-52.

Sun, R., Liang, D., Gao, Y., and Lan, K. (2014). Kaposi's sarcoma-associated herpesvirus-encoded LANA interacts with host KAP1 to facilitate establishment of viral latency. *J Virol* **88**(13)**,** 7331-44.

Tan, X., Xu, X., Elkenani, M., Smorag, L., Zechner, U., Nolte, J., Engel, W., and Pantakani, D. V. (2013). Zfp819, a novel KRAB-zinc finger protein, interacts with KAP1 and functions in genomic integrity maintenance of mouse embryonic stem cells. *Stem Cell Res* **11**(3)**,** 1045-59.

Tatham, M. H., Jaffray, E., Vaughan, O. A., Desterro, J. M., Botting, C. H., Naismith, J. H., and Hay, R. T. (2001). Polymeric chains of SUMO-2 and SUMO-3 are conjugated to protein substrates by SAE1/SAE2 and Ubc9. *J Biol Chem* **276**(38)**,** 35368-74.

Tatham, M. H., Rodriguez, M. S., Xirodimas, D. P., and Hay, R. T. (2009). Detection of protein SUMOylation in vivo. *Nat Protoc* **4**(9)**,** 1363-71.

Täuber, B., and Dobner, T. (2001a). Adenovirus early E4 genes in viral oncogenesis. *Oncogene* **20**(54)**,** 7847-54.

Täuber, B., and Dobner, T. (2001b). Molecular regulation and biological function of adenovirus early genes: the E4 ORFs. *Gene* **278**(1-2)**,** 1-23.

Tavalai, N., and Stamminger, T. (2008). New insights into the role of the subnuclear structure ND10 for viral infection. *Biochim Biophys Acta* **1783**(11)**,** 2207-21.

Taylor, W. R., and Stark, G. R. (2001). Regulation of the G2/M transition by p53. *Oncogene* **20**(15)**,** 1803-15.

Thiriet, C., and Hayes, J. J. (2005). Chromatin in need of a fix: phosphorylation of H2AX connects chromatin to DNA repair. *Mol Cell* **18**(6)**,** 617-22.

Tomimatsu, N., Mukherjee, B., and Burma, S. (2009). Distinct roles of ATR and DNA-PKcs in triggering DNA damage responses in ATM-deficient cells. *EMBO Rep* **10**(6)**,** 629-35.

Tsuruma, R., Ohbayashi, N., Kamitani, S., Ikeda, O., Sato, N., Muromoto, R., Sekine, Y., Oritani, K., and Matsuda, T. (2008). Physical and functional interactions between STAT3 and KAP1. *Oncogene* **27**(21)**,** 3054-9.

Turnell, A. S., Grand, R. J., Gorbea, C., Zhang, X., Wang, W., Mymryk, J. S., and Gallimore, P. H. (2000). Regulation of the 26S proteasome by adenovirus E1A. *Embo J* **19**(17)**,** 4759-73.

Ugai, H., Borovjagin, A. V., Le, L. P., Wang, M., and Curiel, D. T. (2007). Thermostability/infectivity defect caused by deletion of the core protein V gene in human adenovirus type 5 is rescued by thermo-selectable mutations in the core protein X precursor. *J Mol Biol* **366**(4)**,** 1142-60.

Ulrich, H. D. (2009). The SUMO system: an overview. *Methods Mol Biol* **497,** 3-16.

Underhill, C., Qutob, M. S., Yee, S. P., and Torchia, J. (2000). A novel nuclear receptor corepressor complex, N-CoR, contains components of the mammalian SWI/SNF complex and the corepressor KAP-1. *J Biol Chem* **275**(51)**,** 40463-70.

Urrutia, R. (2003). KRAB-containing zinc-finger repressor proteins. *Genome Biol* **4**(10)**,** 231.

Van der Vliet, P. C. (1995). Adenovirus DNA replication. *Curr Top Microbiol Immunol* **199 (Pt 2),** 1-30.

Vayda, M. E., Rogers, A. E., and Flint, S. J. (1983). The structure of nucleoprotein cores released from adenovirions. *Nucleic Acids Res* **11**(2)**,** 441-60.

Verger, A., Perdomo, J., and Crossley, M. (2003). Modification with SUMO. A role in transcriptional regulation. *EMBO Rep* **4**(2)**,** 137-42.

Vertegaal, A. C., Andersen, J. S., Ogg, S. C., Hay, R. T., Mann, M., and Lamond, A. I. (2006). Distinct and overlapping sets of SUMO-1 and SUMO-2 target proteins revealed by quantitative proteomics. *Mol Cell Proteomics* **5**(12)**,** 2298-310.

Vousden, K. H., and Lu, X. (2002). Live or let die: the cell's response to p53. *Nat Rev Cancer* **2**(8)**,** 594-604.

Wadell, G. (1984). Molecular epidemiology of human adenoviruses. *Curr Top Microbiol Immunol* **110,** 191-220.

Wang, C., Ivanov, A., Chen, L., Fredericks, W. J., Seto, E., Rauscher, F. J., 3rd, and Chen, J. (2005). MDM2 interaction with nuclear corepressor KAP1 contributes to p53 inactivation. *Embo J* **24**(18)**,** 3279-90.

Wang, C., Rauscher, F. J., 3rd, Cress, W. D., and Chen, J. (2007). Regulation of E2F1 function by the nuclear corepressor KAP1. *J Biol Chem* **282**(41)**,** 29902-9.

Wang, Y. Y., Li, L., Zhao, Z. S., and Wang, H. J. (2013). Clinical utility of measuring expression levels of KAP1, TIMP1 and STC2 in peripheral blood of patients with gastric cancer. *World J Surg Oncol* **11,** 81.

Weber, P., Cammas, F., Gerard, C., Metzger, D., Chambon, P., Losson, R., and Mark, M. (2002). Germ cell expression of the transcriptional co-repressor TIF1beta is required for the maintenance of spermatogenesis in the mouse. *Development* **129**(10)**,** 2329-37.

Weiden, M. D., and Ginsberg, H. S. (1994). Deletion of the E4 region of the genome produces adenovirus DNA concatemers. *Proc Natl Acad Sci U S A* **91**(1)**,** 153-7.

Weinberg, R. A. (1995). The retinoblastoma protein and cell cycle control. *Cell* **81**(3)**,** 323-30.

Weitzman, M. D., and Ornelles, D. A. (2005). Inactivating intracellular antiviral responses during adenovirus infection. *Oncogene* **24**(52)**,** 7686-96.

White, D., Rafalska-Metcalf, I. U., Ivanov, A. V., Corsinotti, A., Peng, H., Lee, S. C., Trono, D., Janicki, S. M., and Rauscher, F. J., 3rd (2012). The ATM substrate KAP1 controls DNA repair in heterochromatin: regulation by HP1 proteins and serine 473/824 phosphorylation. *Mol Cancer Res* **10**(3)**,** 401-14.

White, D. E., Negorev, D., Peng, H., Ivanov, A. V., Maul, G. G., and Rauscher, F. J., 3rd (2006). KAP1, a novel substrate for PIKK family members, colocalizes with numerous damage response factors at DNA lesions. *Cancer Res* **66**(24)**,** 11594-9.

White, E. (1995). Regulation of p53-dependent apoptosis by E1A and E1B. *Curr Top Microbiol Immunol* **199 (Pt 3),** 34-58.

White, J. S., Yue, N., Hu, J., and Bakkenist, C. J. (2011). The ATM kinase signaling induced by the low-energy beta-particles emitted by (33)P is essential for the suppression of chromosome aberrations and is greater than that induced by the energetic beta-particles emitted by (32)P. *Mutat Res* **708**(1-2)**,** 28-36.

Wickham, T. J., Filardo, E. J., Cheresh, D. A., and Nemerow, G. R. (1994). Integrin alpha v beta 5 selectively promotes adenovirus mediated cell membrane permeabilization. *J Cell Biol* **127**(1)**,** 257-64.

Wickham, T. J., Mathias, P., Cheresh, D. A., and Nemerow, G. R. (1993). Integrins alpha v beta 3 and alpha v beta 5 promote adenovirus internalization but not virus attachment. *Cell* **73**(2)**,** 309-19.

Wiethoff, C. M., Wodrich, H., Gerace, L., and Nemerow, G. R. (2005). Adenovirus protein VI mediates membrane disruption following capsid disassembly. *J Virol* **79**(4)**,** 1992-2000.

Wimmer, P., Berscheminski, J., Blanchette, P., Groitl, P., Branton, P. E., Hay, R. T., Dobner, T., and Schreiner, S. (2015). PML isoforms IV and V contribute to adenovirus-mediated oncogenic transformation by functionally inhibiting the tumor-suppressor p53. *Oncogene*.

Wimmer, P., Blanchette, P., Schreiner, S., Ching, W., Groitl, P., Berscheminski, J., Branton, P. E., Will, H., and Dobner, T. (2012). Cross-talk between phosphorylation and SUMOylation regulates transforming activities of an adenoviral oncoprotein. *Oncogene* **32**(13)**,** 1626-37.

Wimmer, P., Blanchette, P., Schreiner, S., Ching, W., Groitl, P., Berscheminski, J., Branton, P. E., Will, H., and Dobner, T. (2013). Cross-talk between phosphorylation and SUMOylation regulates transforming activities of an adenoviral oncoprotein. *Oncogene* **32**(13)**,** 1626-37.

Wimmer, P., Schreiner, S., Everett, R. D., Sirma, H., Groitl, P., and Dobner, T. (2010). SUMO modification of E1B-55K oncoprotein regulates isoform-specific binding to the tumour suppressor protein PML. *Oncogene* **29**(40)**,** 5511-22.

Wodrich, H., Guan, T., Cingolani, G., Von Seggern, D., Nemerow, G., and Gerace, L. (2003). Switch from capsid protein import to adenovirus assembly by cleavage of nuclear transport signals. *Embo J* **22**(23)**,** 6245-55.

Wodrich, H., Henaff, D., Jammart, B., Segura-Morales, C., Seelmeir, S., Coux, O., Ruzsics, Z., Wiethoff, C. M., and Kremer, E. J. (2010). A capsid-encoded PPxY-motif facilitates adenovirus entry. *PLoS Pathog* **6**(3)**,** e1000808.

Wolf, D., Cammas, F., Losson, R., and Goff, S. P. (2008). Primer binding site-dependent restriction of murine leukemia virus requires HP1 binding by TRIM28. *J Virol* **82**(9)**,** 4675-9.

Wolf, D., and Goff, S. P. (2007). TRIM28 mediates primer binding site-targeted silencing of murine leukemia virus in embryonic cells. *Cell* **131**(1)**,** 46-57.

Wolf, D., and Goff, S. P. (2009). Embryonic stem cells use ZFP809 to silence retroviral DNAs. *Nature* **458**(7242)**,** 1201-4.

Xiao, T. Z., Bhatia, N., Urrutia, R., Lomberk, G. A., Simpson, A., and Longley, B. J. (2011). MAGE I transcription factors regulate KAP1 and KRAB domain zinc finger transcription factor mediated gene repression. *PLoS One* **6**(8)**,** e23747.

Yajima, H., Lee, K. J., Zhang, S., Kobayashi, J., and Chen, B. P. (2009). DNA double-strand break formation upon UV-induced replication stress activates ATM and DNA-PKcs kinases. *J Mol Biol* **385**(3)**,** 800-10.

Yang, B., O'Herrin, S. M., Wu, J., Reagan-Shaw, S., Ma, Y., Bhat, K. M., Gravekamp, C., Setaluri, V., Peters, N., Hoffmann, F. M., Peng, H., Ivanov, A. V., Simpson, A. J., and Longley, B. J. (2007). MAGE-A, mMage-b, and MAGE-C proteins form complexes with KAP1 and suppress p53-dependent apoptosis in MAGE-positive cell lines. *Cancer Res* **67**(20)**,** 9954-62.

Yang, Y., Fiskus, W., Yong, B., Atadja, P., Takahashi, Y., Pandita, T. K., Wang, H. G., and Bhalla, K. N. (2013). Acetylated hsp70 and KAP1-mediated Vps34 SUMOylation is required for autophagosome creation in autophagy. *Proc Natl Acad Sci U S A* **110**(17)**,** 6841-6.

Yew, P. R., and Berk, A. J. (1992). Inhibition of p53 transactivation required for transformation by adenovirus early 1B protein. *Nature* **357**(6373)**,** 82-5.

Yew, P. R., Kao, C. C., and Berk, A. J. (1990). Dissection of functional domains in the adenovirus 2 early 1B 55K polypeptide by suppressor-linker insertional mutagenesis. *Virology* **179**(2)**,** 795-805.

Yew, P. R., Liu, X., and Berk, A. J. (1994). Adenovirus E1B oncoprotein tethers a transcriptional repression domain to p53. *Genes Dev* **8**(2)**,** 190-202.

Yokoe, T., Toiyama, Y., Okugawa, Y., Tanaka, K., Ohi, M., Inoue, Y., Mohri, Y., Miki, C., and Kusunoki, M. (2009). KAP1 is associated with peritoneal carcinomatosis in gastric cancer. *Ann Surg Oncol* **17**(3)**,** 821-8.

Yousef, A. F., Fonseca, G. J., Pelka, P., Ablack, J. N., Walsh, C., Dick, F. A., Bazett-Jones, D. P., Shaw, G. S., and Mymryk, J. S. (2010). Identification of a molecular recognition feature in the E1A oncoprotein that binds the SUMO conjugase UBC9 and likely interferes with polySUMOylation. *Oncogene* **29**(33)**,** 4693-704.

Zeng, L., Yap, K. L., Ivanov, A. V., Wang, X., Mujtaba, S., Plotnikova, O., Rauscher, F. J., 3rd, and Zhou, M. M. (2008). Structural insights into human KAP1 PHD finger-bromodomain and its role in gene silencing. *Nat Struct Mol Biol* **15**(6)**,** 626-33.

Ziv, Y., Bar-Shira, A., Pecker, I., Russell, P., Jorgensen, T. J., Tsarfati, I., and Shiloh, Y. (1997). Recombinant ATM protein complements the cellular A-T phenotype. *Oncogene* **15**(2)**,** 159-67.

Ziv, Y., Bielopolski, D., Galanty, Y., Lukas, C., Taya, Y., Schultz, D. C., Lukas, J., Bekker-Jensen, S., Bartek, J., and Shiloh, Y. (2006). Chromatin relaxation in response to DNA double-strand breaks is modulated by a novel ATM- and KAP-1 dependent pathway. *Nat Cell Biol* **8**(8)**,** 870-6.

Zuo, X., Sheng, J., Lau, H. T., McDonald, C. M., Andrade, M., Cullen, D. E., Bell, F. T., Iacovino, M., Kyba, M., Xu, G., and Li, X. (2012). Zinc finger protein ZFP57 requires its co-factor to recruit DNA methyltransferases and maintains DNA methylation imprint in embryonic stem cells via its transcriptional repression domain. *J Biol Chem* **287**(3)**,** 2107-18.

Publications

I. Publications in scientific journals

- Meyer, RP, Gehlhaus M, Schwab R, **Bürck C**, Knoth R, Hagemeyer CE.
  Concordant up-regulation of cytochrome P450 Cyp3a11, testosterone oxidation and androgen receptor expression in mouse brain after xenobiotic treatment. J Neurochem. 2009 Apri, 109(2):670-81.

- Hagemeyer CE, **Bürck C**, Schwab R, Knoth R, Meyer RP.
  7-Benzyloxyresorufin-O-dealkylase activity as a marker for measuring cytochrome P450 CYP3A induction in mouse liver. Anal Biochem. 2010 Mar 1; 398(1):104-11.

- Meyer RP, Pantazis G, Killer N, **Bürck C**, Schwab R, Brandt M, Knoth R, Gehlhaus M.
  Xenobiotics in the limbic system – affecting brain's network function. Vitam Horm. 2010; 82:87-106.

- Schreiner S, **Bürck C**, Glass M, Groitl P, Wimmer P, Kinkley S, Mund A, Everett RD, Dobner T.
  Control of human adenovirus type 5 gene expression by cellular Daxx/ATRX chromatin-associated complexes. Nucleic Acids Res. 2013 Apr 1; 41(6):3532-50.

- Schreiner S, Kinkley S, **Bürck C**, Mund A, Wimmer P, Schubert T, Groitl P, Will H, Dobner T.
  SPOC1-mediated antiviral host cell response is antagonized early in human adenovirus type 5 infection. PLoS Pathog. 2013;9(11):E1003775.

- **Bürck C**, Mund A, Berscheminski J, Lisa Kieweg, Dobner T, Schreiner S.
  Efficient replication of Human Adenovirus Type 5 (HAdV5) requires KAP1 posttranslational modification. (manuscript in preparation)

II. Oral presentations at scientific meetings

- Schreiner S, **Bürck C**, Wimmer P, Groitl P, Chen SY, Blanchette P, Branton PE, Dobner T.
  Functional interaction between the Ad5 E1B-55K oncoprotein and the PML-associated protein Daxx.
  10th GfV-Workshop on the Cell Biology of Viral Infections, Deidesheim, Germany (2011)

- Schreiner S, **Bürck C**, Glass M, Groitl P, Wimmer P, Kinkley S, Mund A, Everett RD, Dobner T.
  Control of Adenovirus type 5 gene expression by cellular Daxx/ATRX chromatin remodeling complex.
  11th GfV-Workshop on the Cell Biology of Viral Infections, Deidesheim, Germany (2012)

- Schreiner S, **Bürck C**, Glass M, Groitl P, Wimmer P, Kinkley S, Mund A, Everett RD, Dobner T.
  Control of Adenovirus type 5 gene expression by cellular Daxx/ATRX chromatin remodeling complexes.
  23rd Annual Meeting of the Society for Virology, Kiel, Germany (2013)

- **Bürck C**, Berscheminski J, Mund A, Dobner T, Schreiner S.
  Efficient replication of human Adenovirus type 5 (Ad5) requires phosphorylation of the cellular co-repressor KAP1 at Ser824.
  DNA Tumor Virus Meeting Birmingham, UK (2013)

- **Bürck C**, Berscheminski J, Mund A, Dobner T, Schreiner S.
  Efficient replication of human Adenovirus type 5 (Ad5) requires phosphorylation of the cellular co-repressor KAP1 at Ser824.
  12th GfV-Workshop on the Cell Biology of Viral Infections, Deidesheim, Germany (2013)

- **Bürck C**, Berscheminski J, Mund A, Dobner T, Schreiner S.
  Productive Ad infection requires PTM of the cellular co-repressor KAP1.
  DNA Tumor Virus Meeting Madison, Wisconsin, USA (2014)

III. Poster presentations at scientific meetings

- Schreiner S, **Bürck C**, Wimmer P, Groitl P, Chen SY, Blanchette P, Branton PE, Dobner T.
  Functional interaction between the Ad5 E1B-55K oncoprotein and the PML-associated protein Daxx.
  21st Annual Meeting of the Society for Virology Freiburg, Germany (2011)

- **Bürck C**, Berscheminski J, Mund A, Dobner T, Schreiner S.
  Efficient replication of human Adenovirus type 5 (Ad5) requires phosphorylation of the cellular co-repressor KAP1 at Ser824.
  24th Annual Meeting of the Society for Virology Alpbach, Austria (2014)

- **Bürck C**, Berscheminski J, Mund A, Dobner T, Schreiner S.
  Efficient replication of human Adenovirus type 5 (Ad5) requires PTM of the cellular co-repressor KAP1.
  11th International Adenovirus Meeting La Jolla/CA, USA (2014)

- **Bürck C**, Berscheminski J, Mund A, Dobner T, Schreiner S.
  Efficient replication of human Adenovirus type 5 (Ad5) requires PTM of the cellular co-repressor KAP1.
  Meeting of the Department Biology, University of Hamburg, Germany (2015)

IV. Participation in scientific meetings/workshops

- Soft skill course: Communication & presentation in the academic context, Hamburg, Germany (2012)
- Soft skill course: Effective Scientific Writing, Hamburg, Germany (2012)
- 4th Adenovirus workshop Hannover, Germany (2013)