

Role of p300 in human papillomavirus type 8 (HPV8)-mediated skin tumorigenesis

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1. Introduction

1.1 Viral Contributions to Human Carcinogenesis

Carcinogenesis involves the accumulation of DNA-damaging events, commonly referred to as oncogenic "hits". These hits occur spontaneously or result from environmental cues (e.g., UV radiation) and cause dysregulation of signalling pathways. The hallmarks of cancer summarize the key tumorigenic contributors including sustained proliferation, resistance to apoptosis, angiogenesis, invasion and metastasis, replicative immortality and immune evasion (reviewed in Hanahan, 2026).

Oncogenic viruses exploit these pathways with a specialized repertoire of viral oncoproteins occasionally resulting in malignant transformation of infected target cells (Gaglia and Munger, 2018). Viral oncogenesis commonly represents an accidental event during long-term infection which does not promote viral fitness or replication but rather a "dead-end" for the infecting virus (Chang et al., 2017). Among sexually active individuals, human papillomavirus (HPV) infection is the most prevalent sexually transmitted infection worldwide. Based on their carcinogenic potential, HPVs are categorized as high- or low-risk types. Persistent infections with high-risk HPV of genus alphapapillomavirus (HR-HPV) pose a global health threat, since these viruses can promote malignant transformation of mucosal epithelia (Jensen et al., 2024). In particular, HR-HPV16 and HPV18 have been identified as the major contributors to the development of cervical cancer, other anogenital carcinomas, as well as a subset of head and neck squamous cell carcinomas (HNSCC) (Galati et al., 2024).

Prophylactic vaccination against the most prevalent HR-HPV types has been available since 2006, which generates neutralizing antibodies against the viral capsid L1 protein. To date, six prophylactic HPV vaccines can be administered to provide long-term protection against infection with the most common HR-HPV types: three bivalent (Cervarix®, Cecolin®, Walrinvax™), two quadrivalent (GARDASIL®, Cervavac®) and one nonavalent (GARDASIL9®) vaccine (reviewed in Williamson, 2023). Currently, GARDASIL9® represents the most commonly administered vaccine, which provides protection against infection with the nine alpha-HPV types contained in the vaccine, but there is no cross-protection against other alpha-HPV or beta-HPV types (Meyer and Stockfleth, 2024). The high global prevalence of HPV-associated anogenital cancers underscores the importance of further research into the molecular mechanisms of carcinogenesis in order to develop further vaccination strategies and small-molecule inhibitors that will enable the efficient treatment of HPV-associated cancers (Nelson and Mirabello, 2023).

1.2.2 Genomic organization

Papillomaviruses have a non-enveloped, icosahedral capsid of 50 – 60 nm in size composed of the major and minor structural proteins L1 and L2. The capsid encloses the circular, approximately 8,000 base pair DNA genome which can be divided into an upstream regulatory region (URR), and an early and a late region both harboring protein-encoding open reading frames (ORFs) (Fig. 2). All ORFs are located on the sense DNA strand, and are expressed from polycistronic mRNAs, which are generated through alternative mRNA splicing. The early proteins E1, E2, E5, E6, and E7 have regulatory functions in viral replication, transcription and cellular transformation. The URR harbors the origin of replication (*ori*), binding sites for the viral E2 protein and several cellular transcription factors (TF), including AP-1 and SP1, to initiate transcription (Nelson and Mirabello, 2023, Aggarwal et al., 2025). The presence of tissue-specific TF binding sites within the URR may confer the tissue tropism of HPV types (Longworth and Laimins, 2004).

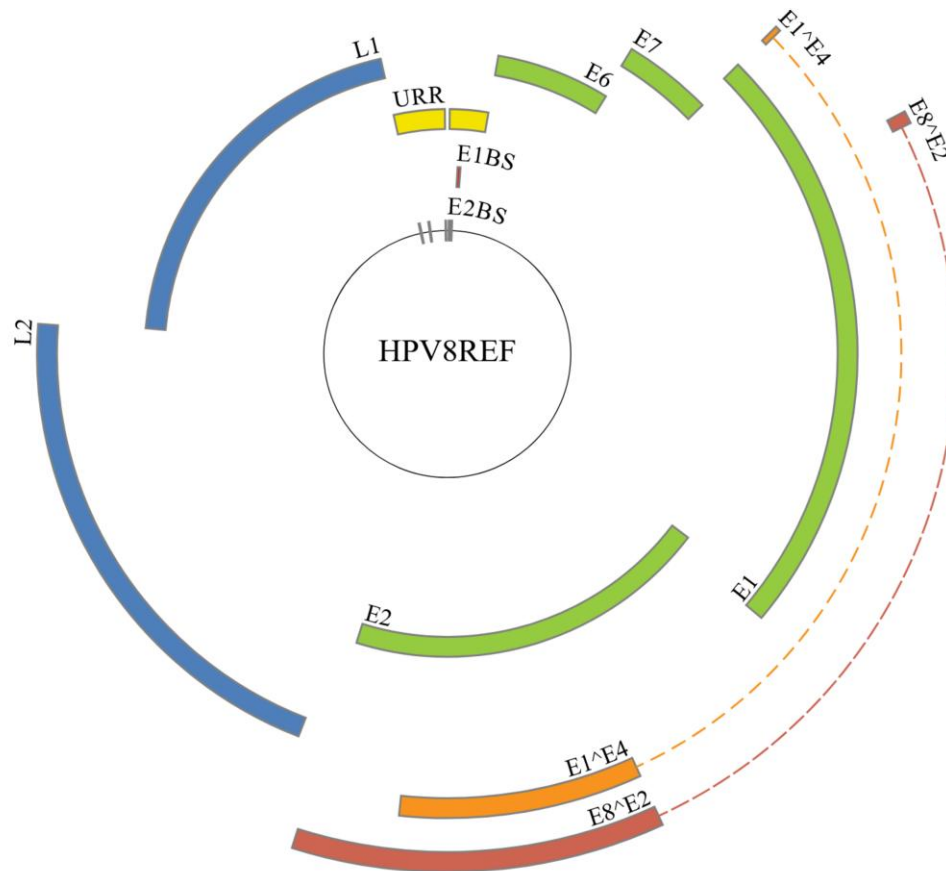


Figure 2: Genomic organization of HPV8. Early protein encoding region (green), late protein encoding region (blue) and the upstream regulatory region (URR) (yellow). Alternative splicing transcripts are indicated in orange (E1^{E4}) and red (E8^{E2}). Image taken from PaVE: <http://pave.niaid.nih.gov>. BS = binding site, URR = upstream regulatory region.

1.3 HPV Life Cycle

Most studies on the life cycle of HPVs have been conducted on HR-HPV types. However, fundamental mechanisms can be applied to other HPV types but with some genus- or even type-specific modifications. Infection of basal keratinocytes occurs through epithelial microlesions or via hair follicles (Fig. 3) (Doorbar, 2005, Becerril et al., 2021). The HPV life cycle is tightly linked to keratinocyte differentiation and comprises three sequential phases: establishment, genome maintenance and vegetative amplification (McBride, 2017).

Virus attachment to the basal cell layer is initiated by binding of the major L1 capsid protein to heparan sulfate chains of proteoglycans (HSPGs) including syndecan-1, which accumulate at epithelial wound edges. This interaction induces conformational changes within the viral capsid, ultimately allowing cleavage of L2, which is crucial for endosomal transport of the virus to the trans-Golgi network (TGN). It has been shown that L2 is required for the interaction of HPV with a secondary, yet elusive receptor to trigger virus internalization (Becker et al., 2018). Following internalization, L2 remains associated with the HPV genome and tethers it to condensed chromatin during transient mitotic breakdown of the nuclear envelope (Aydin et al., 2014).

After completion of mitosis and nuclear envelope reformation, viral DNA is released and established as low-copy episomes (DiGiuseppe et al., 2016, DiGiuseppe et al., 2017). During the maintenance phase, E2 stabilizes the viral episome at host chromatin to ensure equal partitioning to daughter cells. This tethering is mediated via binding to the cellular epigenetic reader BRD4, which recognizes acetylated histones. BRD4 acts as a scaffold protein for E2, the viral E1 helicase as well as the host DNA replication machinery, allowing the HPV genome to be replicated in synchrony with the host genome during S phase in proliferating keratinocytes. In this phase, transcription from the early promoter drives expression of the early viral genes, including E1, E2, E6 and E7 (Rani et al., 2023).

Upon division of infected basal cells, one daughter cell migrates towards the epithelial surface and differentiates, whereas the other remains in the basal layer. Keratinocyte differentiation enables vegetative genome amplification and late promoter activation. Late promoter activation results in high expression levels of E1^{E4}, L1 and L2, allowing virion assembly in the terminally differentiated, cornified epithelial layer. During vegetative amplification, HPV genomes preferentially replicate near chromatin areas characterized by high replication stress. These areas provide access to DNA damage repair (DDR) factors during recombination-directed repair, supporting viral persistence but also increasing the likelihood of accidental integration of viral DNA

into the host genome, contributing to genomic instability and malignant transformation of infected keratinocytes (Bordignon et al., 2017, Della Fera et al., 2021). Ultimately, mature virions are released through the natural shedding of terminally differentiated squamous cells from the epithelial surface (Graham, 2017, Della Fera et al., 2021).

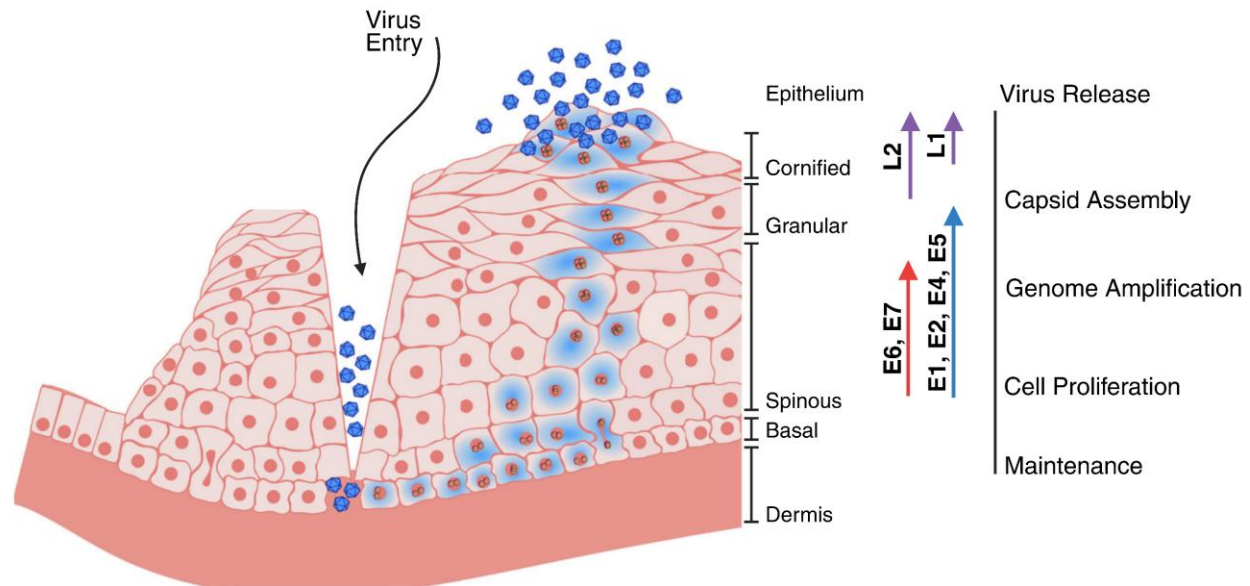


Figure 3: HPV genome organization and the HPV life cycle. The illustration shows the different epidermal layers as well as the maturation process of HPV coupled to the keratinocyte differentiation program. Image taken from Zayats et al. (2022) and modified using BioRender. E = Early gene, L = Late gene, LCR = Long control region.

1.4 Functions of the viral proteins

1.4.1 E1 and E2 proteins

Both E1 and E2 are required to induce papillomavirus replication, whereas E2 also functions as the major regulator of viral transcription. E1 is composed of a carboxy-terminal ATPase/helicase domain of the AAA+ family, a central DNA binding domain, and an amino-terminal regulatory region required for interactions with E2 and host proteins (Bergvall et al., 2013, Morgan, 2025). The E2 protein comprises a carboxy-terminal DNA binding/dimerization and an amino-terminal transactivation domain, connected by a flexible hinge region (McBride, 2013). The URR contains four DNA E2-binding sites: two sites proximal to the TATA box of the early promoter, one site near the origin of replication (*ori*), and a fourth site in the distal enhancer within the URR.

E2 tightly regulates expression of the E6/E7 oncogenes to maintain controlled viral transcription and prevent host cell transformation (Della Fera et al., 2021, Wu et al., 2024). In HPV18, this regulation has been shown to depend on E2 concentration and distinct occupancy of E2-binding

sites which balance E6/E7 expression during differentiation of infected keratinocytes in a dose-dependent manner (Steger and Corbach, 1997). Moreover, HPV8-E2 directly interacts with the acetyltransferase p300, which enhances E2-dependent activation of viral gene expression (Müller et al., 2002). A similar cooperation between E2 and p300 was observed at the HPV16 early promoter (Kruppel et al., 2008).

In addition, E2 binds to the histone acetylation reader BRD4, which contributes to low-copy episomal maintenance by repressing transcription from the early promoter (Rani et al., 2023). HR-HPV DNA is often integrated into the host genome in cervical cancer. This viral integration frequently disrupts the E2 ORF, thereby abolishing E2-mediated transcriptional control and leading to deregulated E6/E7 expression. This mechanism is considered a key event in malignant progression of HR-HPV-associated lesions (Albert and Laimins, 2020). Viral DNA replication is initiated by E2-dependent recruitment of E1 helicase to the origin of replication to facilitate genome unwinding (McBride, 2022). During mitosis, p300 acetylates E2 at a conserved lysine residue (K111), promoting recruitment of topoisomerase I (Top1) and DNA-Topoisomerase-2-binding Protein 1 (TOPBP1). While Top1 binds acetylated K111 and leads to enhanced E2 stability, TOPBP1 ensures stable tethering of E2 at mitotic chromosomes and activates the DDR response (Morgan, 2025). E1, in turn, binds replication protein A (RPA), and Topo I to facilitate DNA unwinding and promote replication (Bergvall et al., 2013).

1.4.2 E4

The E4 protein is translated from an alternatively spliced E1^{E4} mRNA. The E1^{E4} mRNA encodes an E1^{E4} fusion protein containing the first five amino acids derived from the E1 ORF followed by the E4 protein sequence. E4 expression is highest in the mid and upper epithelial layers during productive replication and serves as a marker of active HPV infection. Furthermore, E4 has been shown to contribute to G2 cell cycle arrest, which is thought to facilitate productive viral genome amplification (Doorbar, 2013). Moreover, E4 stabilizes E2, thereby supporting E2-mediated regulation of viral transcription (Davy et al., 2009).

1.4.3 E5

The transmembrane protein E5 of HR-HPVs enhances EGFR signaling (Wasson et al., 2017) which induces hyperproliferation of keratinocytes and delays differentiation (Ilahi and Bhatti, 2020). Enhanced EGFR activity also increases PD-L1 expression through a positive feedback loop, thereby contributing to immune evasion by inducing T-cell apoptosis (Allouch et al., 2020). Furthermore, E5 promotes immune evasion by retaining MHC class I molecules in the Golgi

apparatus (Ashrafi et al., 2006, Pereira Santos et al., 2025). Unlike HR-HPVs, beta-papillomaviruses such as HPV5 and HPV8 do not encode an E5 ORF (Gelbard and Munger, 2023).

1.4.4 HR-HPV E6 and E7 proteins

The HPV E6 protein contains two zinc-binding domains, which are connected by a short linker helix, and each domain comprises two zinc-stabilized CXXC motifs (Liu et al., 2009, Zanier et al., 2013). Together, they form hydrophobic pockets which recognize cellular proteins carrying LXXLL-motifs (Zanier et al., 2013). Both CXXC motifs are highly conserved across HPV genera (Liu et al., 2009) and are essential for protein integrity and function (Kanda et al., 1991).

An important function of HR-HPV E6 proteins is their ability to inhibit p53-mediated apoptosis of infected keratinocytes. HR-HPV E6 binds to p53 and the cellular ubiquitin ligase E6AP-LXXLL motif to induce proteasomal degradation of p53 (Vande Pol and Klingelhutz, 2013). In addition, it has been suggested that HPV16-E6 inhibits p53-dependent transcription by directly binding to p300 (Thomas and Chiang, 2005, White et al., 2012). Survival of infected target cells is further ensured through proteasomal degradation of the pro-apoptotic protein Bcl-2 homologous antagonist/killer (Bak) in an E6AP-dependent manner (Thomas and Banks, 1999). In parallel, interaction of HPV16-E6 with the Myc oncoprotein activates the human Telomerase Reverse Transcriptase (hTERT) promoter, thereby extending the life span of infected keratinocytes (Chen et al., 2025). A carboxy-terminal PDZ-binding motif (PBM), which is unique to HR-HPV E6 proteins, mediates the interaction with cellular PDZ-domain containing tumor suppressors to induce their proteasomal degradation (Skelin and Tomaic, 2023). Loss of these proteins facilitates proliferation, reduces cell-cell contacts, and delays keratinocyte differentiation (Nguyen et al., 2003). Through these different mechanisms, E6 prevents apoptosis and supports the continued survival and proliferation of infected keratinocytes.

The approximately 100 amino acid E7 protein is organized into three conserved regions (CR). CR1 and CR2 are located at the N-terminus, with CR2 harboring the highly conserved LXCXE motif. The C-terminal CR3 contains two CXXC motifs that form a zinc-binding domain (Roman and Munger, 2013, Mittal and Banks, 2017). HR-HPV E7 proteins bind the retinoblastoma tumor suppressor protein (pRb) via their LXCXE motif, targeting it for proteasomal degradation (Ohlenschlager et al., 2006). This results in release of activating E2F-transcription factors, which promote the expression of genes required for S-phase entry. E7 also interacts with the cyclin-dependent kinase inhibitor p21^{CIP1}, thereby inhibiting its ability to suppress cyclin-dependent

kinase activity, which further supports cell-cycle progression (Yeo-Teh et al., 2018, Pal and Kundu, 2019). In addition, HR-HPV E7 proteins interact with class I histone deacetylases (HDAC1) through their carboxy-terminal zinc-binding domain. HPV31 E7 reduces HDAC recruitment to E2F-responsive promoters, thereby promoting E2F-dependent transcription (Longworth et al., 2005). E7 also disrupts the DREAM (Dimerization partner, RB-like, E2F and MuvB) transcriptional repressor complex, by binding p130 and promoting its degradation. This results in de-repression of genes involved in cell-cycle progression (Rashid et al., 2015).

Together, E6 and E7 support the viral life cycle by preventing cell-cycle exit and maintaining infected keratinocytes in a replication-competent state during differentiation. Their activities also induce replication stress and activate DNA damage response pathways that recruit repair factors required for vegetative viral genome amplification (Albert and Laimins, 2020, Morgan, 2025). Persistent interference by HR-HPV E6 and E7 proteins with apoptosis, cell-cycle control and DNA damage responses can promote genomic instability and malignant transformation (Doorbar et al., 2015).

1.5 Role of beta-HPV in non-melanoma skin cancer

Multiple cutaneous beta-HPV types are frequently detected on healthy skin of adults and infants without causing symptoms, suggesting that they are part of the normal skin microbiome (Weissenborn et al., 2009, McBride, 2022). However, patients with the rare genetic disorder Epidermodysplasia verruciformis are particularly susceptible to symptomatic beta-HPV infections (Orth, 2006). These patients carry loss-of-function mutations in the transmembrane channel-like (TMC) genes TMC6 (EVER1) and TMC8 (EVER2), as well as the calcium- and integrin-binding protein-1 (CIB1) protein. It has been shown that these three proteins form a complex that interferes with beta-HPV infection of keratinocytes. Disruption of the CIB1-EVER1-EVER2 complex leads to chronic infection (de Jong et al., 2018a), accompanied by the appearance of multiple, life-long persisting warts (Lewandowsky and Lutz, 1922). These warts may contain many different beta-HPV types and their transcripts (Saeidian et al., 2023) and are known to progress into cutaneous squamous cell carcinoma (cSCC) upon frequent UV-exposure (Orth, 2006, de Jong et al., 2018b). HPV5 and HPV8 have been regarded as the predominant beta-HPV types found in Epidermodysplasia verruciformis-associated cSCCs (Pfister, 2003, Howley and Pfister, 2015). Moreover, it has been shown that both HPV5 and HPV8 cooperate with UV irradiation during skin tumorigenesis (Akgül et al., 2005b, Smola, 2020). Accordingly, the International Agency for Research on Cancer (IARC) has classified HPV5 and HPV8 as possibly carcinogenic in patients with Epidermodysplasia verruciformis (Dervenis, 2025).

Recently, it has been reported that iatrogenic immunosuppression or HIV infection can lead to Epidermodysplasia verruciformis-like skin lesions without the genetic defects associated with inherited Epidermodysplasia verruciformis. This is described as acquired Epidermodysplasia verruciformis, which is associated with an increased susceptibility to beta-HPV infection, most commonly involving HPV5 and HPV8. Patients with acquired Epidermodysplasia verruciformis also have an increased risk of developing cSCCs, showing that beta-HPV-associated skin cancer is not limited to inherited Epidermodysplasia verruciformis (Momin et al., 2026).

In contrast to patients with Epidermodysplasia verruciformis, an association of beta-HPV with cSCCs of the general population is less clear, since viral genomes are detectable only at very low levels (less than one genome per cell). Consistent with this, next generation sequencing transcriptome analyses failed to detect HPV expression in any of the tested skin tumors derived from immunocompetent and immunocompromised patients (Arron et al., 2011, Ganzenmueller et al., 2012, Arroyo Muhr et al., 2021). It was shown that viral loads in actinic keratoses of immunosuppressed organ transplant recipients (OTRs) exceed those in cSCCs. However, beta-HPV transcription in actinic keratoses is not common (Hultin et al., 2019). Together, these observations suggest that beta-HPVs facilitate early cellular transformation by enhancing the carcinogenic effects of UV radiation rather than maintaining tumor growth (Hasche et al., 2017, Galati et al., 2020, Ferreira et al., 2021).

In 2019, Strickley and colleagues presented a previously unrecognized role of immunity against cutaneous PV. The colonization of immunocompetent mice with *Mus musculus* Papillomavirus 1 (MmuPV1) protected against UV-induced skin cancer in a CD8⁺ T-cell-dependent manner (Strickley et al., 2019). The protective role of PV-specific immunity was further supported by recent studies showing that PV colonization maintains homeostasis of UV-damaged skin by increasing PV antigen presentation to CD8⁺ T-cell infiltrates in p53-mutated keratinocytes (Son et al., 2025). Consistent with this, it has been recently proposed that the early proteins E1, E2, E6, and E7 of commensal beta-HPVs are more immunogenic than those of HR-HPVs. This may promote a stronger T cell-mediated immune response and contribute to the mostly asymptomatic infections within the general population (Guennoun et al., 2024).

1.6 Beta-HPV8-E6

As described above, HPV5 and HPV8 (beta 1 species) are associated with cSCC development in patients with Epidermodysplasia verruciformis (Sandoval-Clavijo et al., 2025). High copy numbers of transcriptionally active HPV5 and HPV8 genomes are found in approximately 90 %

of Epidermodysplasia verruciformis-associated cSCCs. These observations indicate that high expression of the viral E6/E7 oncoproteins is associated with an increased risk for oncogenic transformation (Dell'Oste et al., 2009, Tommasino, 2017). Beta-HPV oncoproteins alter cellular signaling pathways similar to those of HR-HPV types, but through different molecular mechanisms.

Unlike HR-HPVs, HPV8-E6 does not bind to p53 and does therefore not induce its proteasomal degradation (Steger and Pfister, 1992). Instead, it affects several cellular pathways involved in keratinocyte differentiation, proliferation, and DNA damage repair (Akgül et al., 2007, Hufbauer et al., 2015, Taute et al., 2017). By binding to the transcriptional co-activator factor Mastermind-like 1 (MAML1), HPV8-E6 inhibits NOTCH-dependent transcription, which is crucial for keratinocyte differentiation (reviewed in Howley and Pfister, 2015). Through its interaction with the transcriptional co-activator p300, HPV8-E6 regulates late promoter activity, but also cellular pathways involved in differentiation, as well as the DNA damage response (Müller-Schiffmann et al., 2006, Hufbauer et al., 2015).

Furthermore, HPV8-E6 activates TEAD-dependent transcription and attenuates BCL9L-dependent canonical Wnt transcription, thereby interfering with cell density-dependent growth control and keratinocyte differentiation (Wu et al., 2023). Previous studies have shown that HPV8 can transcriptionally reprogram infected keratinocytes by altering Sp1/3-dependent transcription (Kirschberg et al., 2019). More recent transcriptome analyses showed that HPV8-E6 strongly affects gene expression and epigenetic regulation in keratinocytes (Hufbauer et al., 2026).

Besides affecting cellular transcription similar to HR-HPVs, several beta-HPV E6 proteins induce proteasomal degradation of the pro-apoptotic factor Bak to facilitate the survival of HPV-infected keratinocytes after UV-exposure (Jackson et al., 2000, Underbrink et al., 2008, Holloway et al., 2015). In addition, HPV5-E6 and HPV8-E6 interfere with the UV-induced DNA damage response, which contributes to the progression of skin carcinogenesis (Wallace et al., 2012, Hufbauer et al., 2015, Wallace et al., 2015, Wendel and Wallace, 2023).

1.6.1 Oncogenic potential of HPV8 in transgenic mouse models

The oncogenic potential of HPV8 was further investigated using transgenic mouse models. K14-HPV8-CER and K14-HPV8-E6 transgenic mice express either the complete early region (CER) of HPV8 or the E6 protein alone under the control of the human keratin 14 (K14) promoter, which directs gene expression to basal epithelial cells. Both mouse models developed spontaneous papillomas within one year, of which 6 % progressed to SCCs, revealing HPV8-E6 as the major

oncogene in the murine epidermis (Schaper et al., 2005, Marcuzzi et al., 2009). UVA/B irradiation or skin wounding by tattooing or punch biopsy led to increased E6 expression and accelerated papilloma formation to three weeks (Marcuzzi et al., 2009, Hufbauer et al., 2010). Furthermore, previous studies have shown that HPV8-E6 delays the removal of UV-induced DNA lesions in a p300-dependent manner, thus facilitating cellular transformation and skin tumorigenesis *in vivo* (Hufbauer et al., 2015). These findings suggest that UV exposure and skin injury can promote HPV8-E6-induced papilloma formation.

Analysis of K14-HPV8-CER transgenic mice revealed a selective proliferation and expansion of Lrig1-positive keratinocyte stem cells within the hair follicle junctional zone (Lanfredini et al., 2017). More recently, HPV8-E6 was shown to be sufficient for the expansion of this stem cell population into the overlying epidermis by binding to p300, which promoted STAT3 activation, thereby maintaining stem cell properties and delaying keratinocyte differentiation (Morgan et al., 2024).

The importance of p300 binding for HPV8-E6-mediated skin tumorigenesis was further investigated using a transgenic mouse model expressing the p300-binding-deficient HPV8-E6 K136N mutant. The K136N substitution is located within amino acids 132–136, which are required for the interaction of HPV8-E6 with p300 (Müller-Schiffmann et al., 2006). In contrast to mice expressing wild-type HPV8-E6, K14-HPV8-E6-K136N transgenic mice showed reduced accumulation of UV-induced DNA damage and did not develop papillomas following UV exposure or mechanical wounding (Hufbauer et al., 2015). These findings indicate that the interaction with p300 is required for the tumorigenic activity of HPV8-E6.

HPV8-E6 was previously shown to interact with three distinct regions within p300, designated as p300-1 (aa 2-439), p300-4 (aa 1453-1888), and p300-5 (aa 1889-2378) (Müller-Schiffmann et al., 2006) (Fig. 4A). Tattooing of expression plasmids encoding p300-1 and p300-4 restored wound healing and reduced papilloma formation in K14-HPV8-E6 mice, whereas p300-5 had no comparable effect (M. Hufbauer, B. Akgül, personal communication) (Fig. 4B). Together, these results support a central role of p300 for HPV8-E6-mediated skin tumorigenesis.

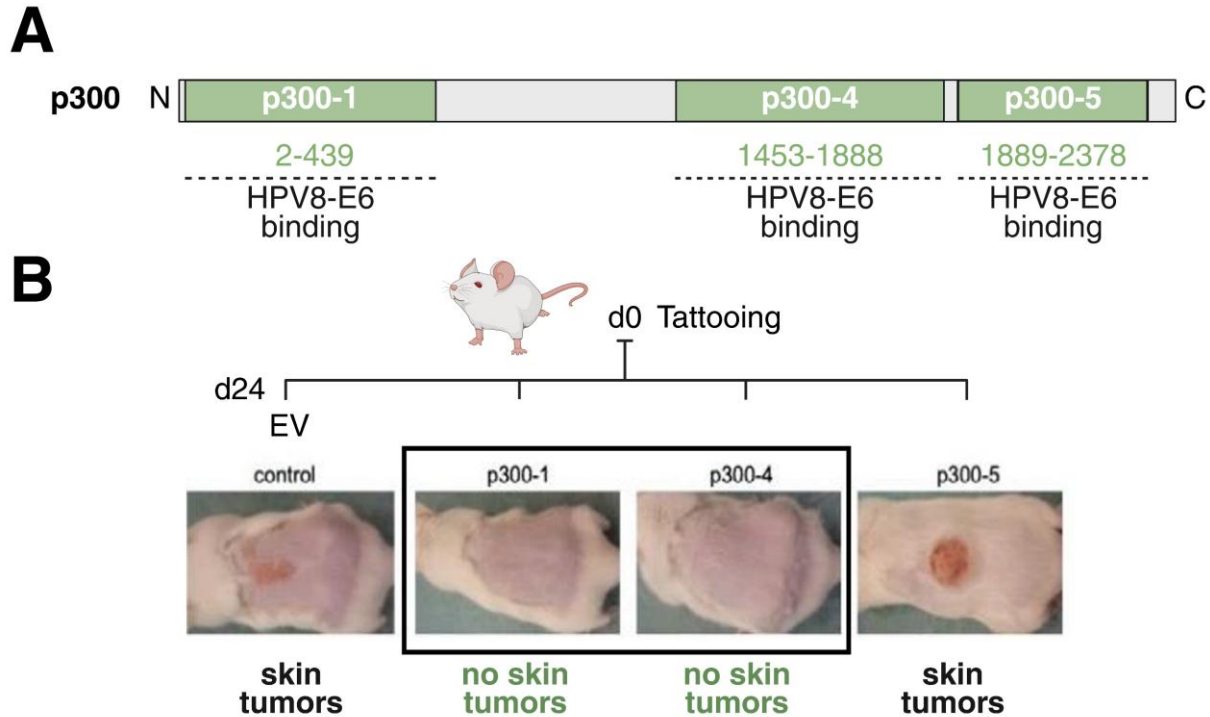


Figure 4: p300 fragments containing HPV8-E6 binding regions inhibit skin tumorigenesis. (A) Shown is the structure of p300 and three distinct regions which are recognized by HPV8-E6, designated as p300-1, p300-4, and p300-5 (colored in green). Numbers indicate the corresponding residues within p300. **(B)** K14-HPV8-E6 transgenic mice were tattooed with expression vectors encoding the fragments p300-1, p300-4, and p300-5. Three weeks post-tattooing mice expressing p300-1 and p300-4 displayed wound healing without skin tumorigenesis (d24). P300-5 did not inhibit skin tumorigenesis. Images in panel (B) were provided by M. Hufbauer and B. Akgül (personal communication). Created with BioRender. d = day, EV = empty vector.

1.7 The transcriptional co-activator p300

P300 and CREB-binding protein (CBP) are multidomain proteins belonging to the superfamily of lysine acetyltransferase 3 (KAT3), sharing 61 % full-length sequence identity and 86 % identity within the catalytic core. Due to the high degree of conservation, CBP and p300 exhibit similar structural organization and overlapping but also distinct cellular functions (Chan and La Thangue, 2001). P300 is crucial for transcriptional regulation by promoting histone acetylation, which facilitates chromatin accessibility and enhances binding of transcription factors to active enhancer regions (Rubio et al., 2023).

Since p300 does not contain a sequence-specific DNA-binding domain, it is recruited to active promoters and enhancers by interacting with multiple DNA-bound transcription factors. At these genomic regions it acts as a scaffold for several co-regulators, chromatin-modifying proteins and components of the basal transcription machinery (Ferrie et al., 2024). The multidomain structure

of p300 allows it to interact with more than 400 cellular proteins, including DNA-binding transcription factors such as p53, HIF-1 α , NF- κ B, STAT proteins, and TCF7L2, as well as non-DNA-binding transcriptional co-regulators such as CITED2 and β -catenin (Bedford et al., 2010).

Several conserved domains which are connected by long, intrinsically disordered regions contribute to p300-mediated transcriptional regulation (Dyson and Wright, 2005). The N- and C-terminal regions contain the transcriptional adapter zinc finger domains TAZ1 and TAZ2, overlapping with the cysteine/histidine-rich regions CH1 and CH3, respectively. Other important interaction domains include the nuclear receptor interaction domain (NRID), kinase-inducible domain (KIX), and interferon-binding domain (IBID). The catalytic region contains a bromodomain (BD), the plant homeodomain (PHD), a RING domain, and the histone acetyltransferase (HAT) domain. The bromodomain recognizes acetylated lysine residues, whereas the HAT domain acetylates histone and various non-histone proteins. The surrounding RING, PHD and ZZ domains contribute to substrate recognition and regulation of the catalytic activity of p300 (Dyson and Wright, 2016). A simplified overview of the p300 domains is illustrated in Figure 5.

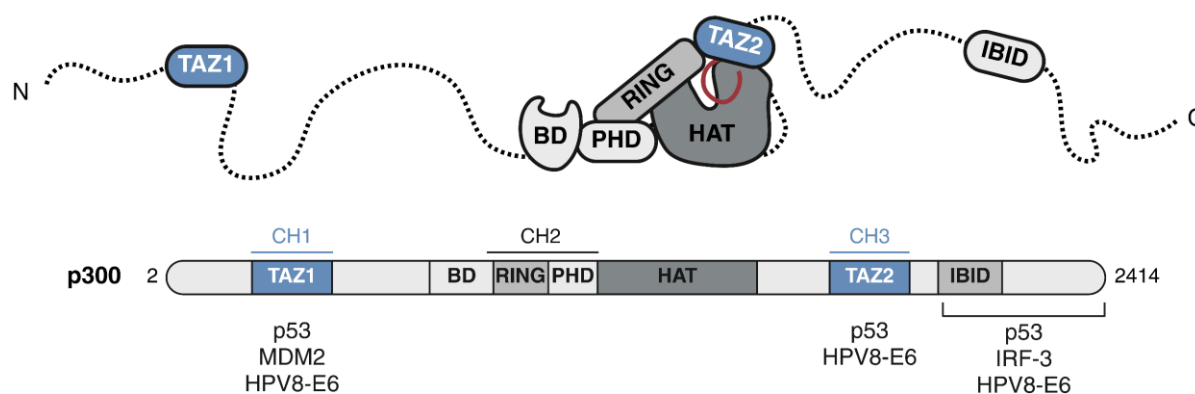


Figure 5: p300 domain architecture. Transcriptional adaptor zinc-finger 1 (TAZ1) domain (also known as cysteine-histidine-rich domain 1 (CH1); Bromodomain (BD); Really interesting new gene (RING); Plant homeodomain (PHD); RING-PHD segment is also known as cysteine-histidine-rich domain 2 (CH2); Histone acetyltransferase (HAT); Transcriptional adaptor zinc-finger 2 (TAZ2) domain (also known as cysteine-histidine rich domain 3 (CH3); Interferon-binding domain (IBID); Histone deacetylase (HDAC); Murine double minute 2 (MDM2); Interferon regulatory factor 3 (IRF-3). The autoinhibitory loop (AIL) is colored in red. Representative TAZ domain interaction partners are indicated below. Numbers indicate the amino acid positions within p300. Schematic representation of p300 was adapted from Delvecchio et al. (2013) and created with BioRender.

Among these domains, TAZ1 and TAZ2 are important for mediating protein-protein interactions that contribute to the recruitment of p300 to active genomic regions. TAZ1 and TAZ2 fold into α -helical domains that are stabilized by three zinc ions, coordinated by conserved cysteine and

histidine residues. They provide a scaffold for intrinsically disordered regions (IDR) of multiple cellular and viral proteins (De Guzman et al., 2000). Promoter-bound transcription factors recruit p300 through highly selective interaction of their IDRs with TAZ1 or TAZ2 (Wojciak et al., 2009). Upon binding to TAZ1 or TAZ2, IDRs most commonly adopt α -helical structures, thereby stabilizing the complex with p300 and promoting target gene transcription. Since several transcription factors interact with the same p300 domains, they compete or cooperate for p300 binding (Dyson and Wright, 2016). For example, HIF-1 α and CITED2 bind to overlapping surfaces within TAZ1, allowing CITED2 to displace HIF-1 α and attenuate hypoxia-dependent transcription (Freedman et al., 2003, De Guzman et al., 2004). However, p300 requires simultaneous interactions with multiple different transcription factors for stable recruitment to chromatin (Ferrie et al., 2024). Together, these mechanisms allow transcription factors to recruit and activate p300, which facilitates transcription through histone acetylation. By altering the global p300-interacting network HPV8-E6 might affect its recruitment to specific target genes to induce transcriptional reprogramming in keratinocytes.

Binding of transcription factors not only recruits p300 to chromatin but also regulates its catalytic activity. A lysine-rich autoinhibitory loop (AIL) within the HAT domain, together with the RING and TAZ2 domain limits the substrate access, thus keeping p300 in an inactive state (Thompson et al., 2004, Delvecchio et al., 2013, Xu et al., 2023). Activated and dimeric transcription factors can promote trans-autoacetylation of the AIL, thereby increasing p300 HAT activity (Ortega et al., 2018). Increased HAT activity contributes to enhanced target gene expression by acetylating particularly histone H3 at lysine residues K18 (H3K18ac) and K27 (H3K27ac). H3K18ac and H3K27ac represent the major histone modifications at active enhancers and promoters (Jiang et al., 2026).

In addition to histone acetylation, p300 contributes to transcriptional regulation of many key signaling pathways by acetylating lysine residues of transcription factors including p53 (Reed and Quelle, 2014), NF- κ B (Buerki et al., 2008), and STAT3 (Tesoriere et al., 2021). However, the establishment and maintenance of accessible chromatin involve both p300-dependent H3K27 acetylation and mammalian SWI/SNF-like nucleosome remodeling complexes (Alver et al., 2017, Ren et al., 2024).

1.8 BAF chromatin remodeling complexes

Mammalian SWI/SNF is a highly conserved multi-subunit complex that is required for ATP-dependent nucleosome remodeling. Mammalian cells express three mSWI/SNF complexes: canonical BRG1/BRM-associated factor (CBAF), polybromo-associated BAF (PBAF) and non-canonical BAF (ncBAF or GBAF). These complexes share a conserved core module but assemble with unique accessory subunits which target distinct genomic regions (Maassen et al., 2025). Their ATPase activity is provided by BRG1/BRM (SMARCA2/4). Pioneer transcription factors such as OCT4, SOX2, and AP-1 can recruit BAF complexes to closed genomic regions and thereby induce nucleosome remodeling and transcriptional regulation (Weiss et al., 2026). Depending on the cellular context, BAF complexes can contribute to both transcriptional activation and repression (Morse et al., 2024). SWI/SNF subunit mutations are found in approximately 25 % of human cancers, indicating an important role of these complexes in preventing malignant transformation (Kadoch et al., 2013, Weiss et al., 2026).

1.9 Aim of the study

Previous studies from our group have demonstrated that the interaction between HPV8-E6 and the transcriptional co-activator p300 represents a key step during HPV8-E6-mediated skin tumorigenesis. Moreover, disruption of this interaction significantly reduces skin tumorigenesis *in vivo*. However, the underlying molecular mechanisms remain largely unknown. A detailed characterization of the HPV8-E6/p300 interaction is therefore expected to provide mechanistic insights into how HPV8-E6 alters p300-dependent signaling pathways and transcriptional programs that contribute to keratinocyte transformation and skin tumorigenesis.

To address this question, the HPV8-E6/p300 interaction was comprehensively analyzed to identify the p300 domains required for HPV8-E6 binding. In addition, TurboID-based proximity labeling was employed to investigate the impact of HPV8-E6 on the global p300 interactome under basal conditions or following UV irradiation. Cellular proteins whose association with p300 was altered in the presence of HPV8-E6 were subsequently identified, and the functional consequences of these altered interactions were investigated with particular focus on HPV8-E6-mediated reprogramming of keratinocyte fate and molecular processes underlying skin tumorigenesis.

2. Material

2.1 Bacterial strains

Table 1: Bacterial strains.

Bacterial strain	Genotype	Company
<i>Epiculture coli</i> ® XL2-Blue	recA1 endA1 gyrA96 thi-1 hsdR17 supE44 relA1 lac [F'proAB lacIqZΔM15 Tn10 (TetR) Amy Camr]	Stratagene
XL10-Gold ultracompetent cells	TetR Δ(mcrA)183 Δ(mcrCB-hsdSMR-mrr)173 endA1 supE44 thi-1 recA1 gyrA96 relA1 lac Hte [F'proAB lacIqZΔM15 Tn10 (TetR) Amy CamR]	Agilent Technologies

2.2 Eukaryotic cell lines

Table 2: Eukaryotic cell lines.

Cell line	Reference	Characteristics
C33A	(Yee et al., 1985)	HPV-negative cervical carcinoma cell line
HEK293	(Graham et al., 1977)	Human embryonic kidney cells transformed with Adenovirus 5 DNA

2.2.1 Human keratinocytes

Table 3: Human keratinocyte cell lines.

Cell line	Reference	Characteristics
HaCaT	(Boukamp et al., 1988)	Spontaneously immortalized human epidermal keratinocytes
N/Tert	(Dickson et al., 2000)	telomerase reverse transcriptase (TERT) immortalized human foreskin keratinocytes
PHEK	Lonza, Cat. No. 00192906	Pooled normal human primary epithelial keratinocytes

2.3 Media

2.3.1 Media for eukaryotic cells

Table 4: Composition of cell culture media.

Medium	Cell line	Composition	Company
Gibco™ DMEM GlutaMAX™ (1x)	C33A		Thermo Fisher Scientific
Keratinocyte Growth Medium 2 (Ready-to-use)	HEK293		PromoCell GmbH
RM+ Medium	N/Tert		Prepared in-house
	SFM		
	SHM	▪ 500 ml DMEM (1x) GlutaMAX™	
	HaCaT	▪ 500 ml DMEM/F-12 (1:1) (1x) + GlutaMAX™	
		▪ RM+ (10x) additive	

2.3.2 Generic cell culture reagents

Table 5: Cell culture reagents.

Medium	Company
Fetal Bovine Serum (FBS)	Thermo Fisher Scientific
Freezing Medium Cryo-SFM Plus	PromoCell GmbH
RM+ (10x) additive	<ul style="list-style-type: none"> ▪ 40 µg/ml Hydrocortisone ▪ 500 µg/ml Insulin ▪ 1 µg/ml EGF ▪ Cholera toxin 0,84 µg/ml ▪ 500 µg/ml Transferrin ▪ 2x10⁻¹¹ M Lythyronine
Trypsin-EDTA (0,05 %)	Thermo Fisher Scientific

2.4 Antibiotics

2.4.1 For cell culture

Table 6: Antibiotics used for cell culture with stock and final concentrations.

Antibiotic	Stock concentration	End concentration	Company
Geneticin sulfate (G418)	250 µg/ml	250 µg/ml	Carl Roth GmbH + Co. KG
Gibco™ Penicillin-Streptomycin	10,000 U/ml	100 U/ml	Thermo Fisher Scientific
Puromycin dihydrochloride	1 mg/ml	1.5 µg/ml	Sigma-Aldrich

2.4.2 For cultivation of bacteria

Table 7: Antibiotics used for bacterial culture with stock and final concentrations.

Antibiotic	Stock Concentration	End concentration	Company
Ampicillin sodium salt	100 mg/ml	150 µg/ml	Carl Roth GmbH + Co. KG

2.5 Protease and phosphatase inhibitors

Table 8: Protease and phosphatase inhibitors with stock and final concentrations.

Inhibitor	Stock Concentration	End concentration	Company
cOmplete™, EDTA-free Protease Inhibitor Cocktail	25 x	1x	Roche
Phenylmethyl sulfonyl fluoride	100 mM	1 mM	Carl Roth GmbH + Co. KG
Sodium fluoride	1 M	50 mM	Carl Roth GmbH + Co. KG
Sodium orthovanadate	200 mM	1 mM	Sigma-Aldrich

2.6 Expression plasmids

2.6.1 For cloning

Table 9: Expression plasmids used for cloning.

Expression plasmid	Reference
3xHA-TurboID-NLS_pCDNA3	Addgene #107171 (Branon et al., 2018)
pCMV2-FLAG	Kodak
pGEX2T	Amersham Biosciences
PX459-SpCas9-2A-Puro	Addgene #62988 (Ran et al., 2013)
pXJ41-HA	Ulla Krüppel; based on pXJ41 (Yan et al., 2003)

2.6.2 Recombinant expression plasmids

Table 10: Recombinant mammalian expression plasmids.

Plasmid construct	Backbone	Insert	Generated
3xHA-TurboID-p300-1	3xHA-TurboID-NLS_pCDNA3	P300-1	This study
3xHA-TurboID-p300-4	3xHA-TurboID-NLS_pCDNA3	P300-4	This study
pcDNA3.1-FLAG-DPF2	pcDNA3.1-FLAG	DPF2	André Hoelz (Huber et al., 2017)
pcDNA3.1-FLAG-EV	pcDNA3.1	FLAG	Andreas Müller
pcDNA3.1-FLAG-HPV8-E6	pcDNA3.1-FLAG	HPV8-E6	Andreas Müller
pCMV2-FLAG-p300-4(1789-1814) C1789A/C1790A	pCMV2-FLAG	P300-4(1789-1814) C1789A/C1790A	This study
pCMV2-FLAG-p300-4(1789-1814)-WT	pCMV2-FLAG	P300-4	Martin Hufbauer
pXJ41-FLAG-HPV8-E6	pXJ41-FLAG	HPV8-E6	Sichtig, N. 2006, (Thesis)
pXJ41-GST-HA-p300-1.9 to p300-1.12	pXJ41-HA-p300-1.9 – p300-1.12	GST	This study
pXJ41-HA-p300-1.1 to p300-1.8	pXJ41-HA	P300-1.1 – p300-1.8	This study
pXJ41-MYC-HPV8-E6	pXJ41	MYC-HPV8-E6	This study

2.7 Oligonucleotides

All oligonucleotides used in this work were purchased from Integrated DNA technologies.

2.7.1 Cloning of p300-1 fragments

Table 11: Primers and restriction sites used for cloning of p300-1 fragments.

Fragment	Enzyme(s)	Forward (5'-3')	Reverse (5'-3')
181-303	BamHI/ KpnI	CAGTCGGGATCCAATGGAC AAGGGATAATGCCTAATCAA	CCCTCGAGAAGCTTCCCGGG GGATCAACCCATGTTGGGCA TTCTCCAC
182-303	BamHI	CAGTCGGGATCCAATGGAC AAGGGATAATGCCTAATCAA	TTAAACGGATCCACCCATGTT GGGCATTCTCCACCAGG
182-439	BamHI	CAGTCGGGATCCAATGGAC AAGGGATAATGCCTAATCAA	ATCCTAGGATCCATTTCCAAG TCCAACGGGTGCTCCAG
2-181	BamHI/ KpnI	ATGGATCCGCCGAGAATGT GGTGAACCGGGGCC	CCCTCGGTACCTTAGCCTGC AGCCAACATTCCAGGATTCAT GCCCGC
2-303	BamHI	ATGGATCCGCCGAGAATGT GGTGAACCGGGGCC	TTAAACGGATCCACCCATGTT GGGCATTCTCCACCAGG
2-331	BamHI	ATGGATCCGCCGAGAATGT GGTGAACCGGGGCC	AAGGATCCTTAATCAGCTGTA TGTGCTCCAGAACCCATCC
2-439	NheI	GAAAATGGATCCGCCGAGA ATGTGGTGAACCGGGGCC G	AGAGCTAGCATTTCCAAGTC CAACGGGTGCTCCAGTCAA
2-439	BamHI	ATGGATCCGCCGAGAATGT GGTGAACCGGGGCC	ATCCTAGGATCCATTTCCAAG TCCAACGGGTGCTCCAG
2-439	BamHI	GAAAATGGATCCGCCGAGA ATGTGGTGAACCGGGGCC G	ATCCTAGGATCCATTTCCAAG TCCAACGGGTGCTCCAG
332-390	BamHI/ KpnI	GGGGATCCCCAGAGAAGCG CAAGCTCATCCAGC	GGAGGTACCTTACACTTGGC AAGACTTGCTGACTGGCAG TG
332-439	BamHI	GGGGATCCCCAGAGAAGCG CAAGCTCATCCAGC	ATCCTAGGATCCATTTCCAAG TCCAACGGGTGCTCCAG
366-406	BamHI/ KpnI	AACCGGGATCCCTTCCCCA CTGTCGCACAATGAAGAAT GTC	GGTACCTTAACAATTCTTCCA GTGTGAAATGATTGTGCG
391-420	BamHI/ KpnI	GGACGGGATCCGCACACTG TGCATCTTCTCGACAAATCA TT	GGACGGGTACCTTAAGCATT TTTGAGGGGGAGACACACAG G

2.7.2 Cloning of p300-4 fragments

Table 12: Primers and restriction enzymes used for cloning of p300-4 fragments.

Fragment	Enzyme	Forward (5'-3')	Reverse (5'-3')
1453-1888	NheI	GAGGCTAGCCCTGACCAGAA	GGGGCTAGCGGGCAAGTAGG
		GATACCCAAGCCCAAGCGAC	GTGGCATGCTATTGGGAGGGG
		TGCAGG	TAGG

2.7.3 Cloning of GST-tag

Table 13: Primers and restriction enzymes used for cloning of GST-tag.

Name	Enzyme	Forward (5'-3')	Reverse (5'-3')
GST-tag	EcoRI	CCGTGAATTCATGTCCCCTAT	CATAGAATTCTTTTGGAGGATG
		ACTAGGTTATTGGAAAATTAA	GTCGCCACCAC
		GGGCC	

2.7.4 For cloning of p300-4 fragments

Table 14: Annealed oligonucleotides for cloning of the p300-4 fragment.

Fragment	Enzyme	Top (5'-3')	Bottom (5'-3')
1789-1814	BamHI	Phos/GATCCTGCTGCTACCAT	Phos/GATCCCCGGAGCTTCTG
		GCCAAGCACTGCCAGGAGAA	CTTGATGTTTAGGCAGAACGG
		CAAATGCCCGGTGCCGTTCT	CACCGGGCATTGTTCCTG
		GCCTAAACATCAAGCAGAAG	GCAGTGCTTGGCATGGTAGCA
		CTCCGGG	GCAG
1789-1814	NheI	Phos/CTAGCTGCTGCTACCAT	Phos/CTAGCCCCGGAGCTTCTG
		GCCAAGCACTGCCAGGAGAA	CTTGATGTTTAGGCAGAACGG
		CAAATGCCCGGTGCCGTTCT	CACCGGGCATTGTTCCTG
		GCCTAAACATCAAGCAGAAG	GCAGTGCTTGGCATGGTAGCA
		CTCCGGG	GCAG
1789-1814 CC/AA	BamHI	Phos/GATCCGCTGCTTACCAT	Phos/GATCCCCGGAGCTTCTG
		GCCAAGCACTGCCAGGAGAA	CTTGATGTTTAGGCAGAACGG
		CAAATGCCCGGTGCCGTTCT	CACCGGGCATTGTTCCTG
		GCCTAAACATCAAGCAGAAG	GCAGTGCTTGGCATGGTAGCA
		CTCCGGG	GCAG

2.7.5 For DPF2 knockout

Table 15: Annealed oligonucleotides used for DPF2 knockout.

guide RNA	Target Sequence	Top (5'-3')	Bottom (5'-3')
gRNA1	GAAGATACTCCCAAG	CACCGAAGATACTCC	AAACCGACGCTTGGGA
	CGTCG	CAAGCGTCG	GTATCTTC
gRNA2	GACCAACAGTCGAG	CACCGACCAACAGCT	AAACTTCGCGCTCGACT
	CGCGAAAGG	GAGCGCGAA	GTTGGTC

2.7.6 For sequencing of knockout clones

Table 16: Primers used for validation of DPF2 knockout.

Primer	Forward (5'-3')	Reverse (5'-3')
gRNA1 left	CGGATCCTAGAACCAGATGACT	CCAAGAAGAGCAAAGAGACACC
gRNA2 left	TGATCTCTCAGGATGGCAGTAG	GGACTCCAGCTTCTGTTTCAGT

2.7.7 For qRT-PCR

Table 17: Primers used for qRT-PCR.

Gene	Primer (5'-3')	Sequence	Primer Tm (° C)	Amplicon (bp)
CDH4	Forward	GACCGAGAGAAAGTTCAGCAG	61	138
	Reverse	GGTAAATTCTGGCGGGTTGTC	61	
GABRP	Forward	GAGTAACATGGACTACACAGCC	62	187
	Reverse	GAGCCTGTTTCCCACAGTGAC	63	
IL33	Forward	GAGTTACTATGAGTCTCAACACC	61	139
	Reverse	CTTATGGAGCTCCACAGAGTG	61	
KRT13	Forward	GGGACAAGATCCTGACCGC	62	195
	Reverse	GCATCTCCAGGTCAGTCTTAG	61	
MMP7	Forward	GGGAACAGGCTCAGGACTATC	63	174
	Reverse	GCACTCCACATCTGGGCTTC	63	
MUC4	Forward	CCTCGGGAGCAACACCTAC	62	185
	Reverse	CACACTGGCTGGGACATCAG	63	

2.7.8 DNA markers and loading dyes

Table 18: DNA markers and loading dyes used in this study.

Product	Company
6X Orange DNA Loading Dye	Thermo Fisher Scientific
GeneRuler™ 1 kb DNA Ladder	Thermo Fisher Scientific
GeneRuler™ 100 bp DNA Ladder	Thermo Fisher Scientific
peqGREEN DNA/RNA dye	VWR International, LLC.

2.8 Antibodies

2.8.1 Primary antibodies

Table 19: List of primary antibodies with working dilutions.

Primary Antibody (Clone)	Host/Clonality	Application/ Dilution	Company
Anti-DPF2 (12111-1-AP)	Rabbit polyclonal	WB 1:1,000 IHC 1:150	Proteintech Group Inc.
Anti-E-cadherin (Clone 36)	Mouse monoclonal	IF 1:200	BD Transduction Laboratories™
Anti-FLAG® (M2)	Mouse monoclonal	WB 1:1,000	Sigma-Aldrich
Anti-GAPDH (G-9)	Mouse monoclonal	WB 1:1,000	Santa Cruz Biotechnology, Inc.
Anti-HA-TAG (C29F)	Rabbit monoclonal	WB 1:3,000	Cell Signaling Technology, Inc.
Anti-MMP7 (10374-2-AP)	Rabbit polyclonal	IF 1:500 IHC 1:300	Proteintech Group Inc
Anti-Myc-TAG (71D10)	Rabbit monoclonal	WB 1:1,000	Cell Signaling Technology, Inc.
Anti-p300 (NM11)	Mouse monoclonal	WB 1:500; IF 1:50	Santa Cruz Biotechnology, Inc.
Anti-Tubulin (YL1/2)	Rat monoclonal	WB 1:500	Santa Cruz Biotechnology, Inc.
Anti-Vinculin XP® (E1E9V)	Rabbit monoclonal	WB 1:1,000	Cell Signaling Technology, Inc.
Normal mouse IgG			Santa Cruz Biotechnology, Inc.

2.8.2 Primary antibody conjugate

Table 20: List of primary antibody conjugates.

Antibody (Clone)	Source/Isotype	Conjugate	Company
Anti-FLAG (L5)	Rat monoclonal IgG2a	Pierce™ Anti-DYKDDDDK Magnetic Agarose	Thermo Fisher Scientific
Anti-HA (2-2.2.14)	Mouse monoclonal IgG1	Pierce™ Anti-HA Magnetic Beads	Thermo Fisher Scientific
Anti-Myc-Tag (9B11)	Mouse monoclonal IgG2ak	Magnetic bead conjugate	Cell Signaling Technology, Inc,
Anti-p300 (NM11)	Mouse monoclonal IgG2bk	Agarose conjugate	Santa Cruz Biotechnology, Inc.

2.8.3 Secondary antibodies

Table 21: Secondary antibodies and detection reagents used in this study.

Reagent	Dilution
Goat anti-Mouse IgG (H+L), Alexa Fluor™ 594	1:500
Goat anti-Rabbit IgG (H+L), Alexa Fluor™ 488	1:500
Goat anti-Rabbit IgG (H+L), Alexa Fluor™ 594	1:500
IRDye® 680RD Goat anti-Mouse	1:5,000
IRDye® 680RD Goat anti-Rabbit	1:5,000
IRDye® 800CW Goat anti-Mouse	1:5,000
IRDye® 800CW Goat anti-Mouse IgG1-Specific	1:5,000
IRDye® 800CW Goat anti-Rabbit	1:5,000
IRDye® 800CW Streptavidin	1:3,000

All IRDye® secondary antibodies were purchased from LI-COR Bioscience and all Alexa Fluor dye conjugated secondary antibodies were purchased from Thermo Fisher Scientific.

2.9 Enzymes

Table 22: Enzymes used in this study.

Enzyme	Company
Proteinase K	AppliChem GmbH
Taq DNA Polymerase, recombinant	Thermo Fisher Scientific
T4 DNA Ligase	Thermo Fisher Scientific
Q5® High-Fidelity DNA Polymerase	New England Biolabs GmbH

Restriction enzymes were purchased from New England Biolabs and Thermo Fisher Scientific.

2.10 Tissues

2.10.1 Epidermodysplasia verruciformis-associated skin lesions

Six paraffin-embedded skin lesions from patients with Epidermodysplasia verruciformis were kindly provided by Sławomir Majewski (Department of Dermatology and Venereology, Medical University of Warsaw, Warsaw, Poland) and have been characterized in previous studies (Heuser et al., 2016).

Table 23: Epidermodysplasia verruciformis-associated skin tissue samples.

Block number	Beta-HPV types
19529	HPV 5,8,20,23,36,50
25734	HPV 5,8,20,23,36,50
32522	HPV5, 36
32520	HPV5, 36
30089	HPV 5,8,20,23,36,50
19530	HPV 5,8,20,23,36,50

2.10.2 Human cSCC tissue samples

Five paraffin-embedded cSCC lesions of patients without Epidermodysplasia verruciformis were used as controls. These sections had been obtained by excision surgery as part of a previously approved study and provided by the Department of Dermatology, University Hospital Cologne (Ethics Committee of the University of Cologne; reference no. 06/090).

Table 24: Human cSCC tissue samples and E4 status.

Block number	E4 status
216_02F	E4 negative
6340_02L	E4 negative
4633_02E	E4 positive
4429_08C	E4 positive
746_13B	E4 positive

2.10.3 Mouse tissue samples

Paraffin-embedded skin tumor sections from K14-HPV8-E6 transgenic mice obtained 24 days after UV irradiation were kindly provided by Prof. Dr. Baki Akgül.

2.11 Buffers and solutions prepared in-house

Table 25: In-house prepared buffers and solutions.

Buffer/Solution	Composition
10 x SDS running buffer	<ul style="list-style-type: none"> 25 mM TRIS, 192 mM Glycine 0.1 % SDS
10 x TBST	<ul style="list-style-type: none"> 10 mM TRIS 150 mM NaCl 0.05 % Tween-20 adjust pH 7.5 with HCl
5 % skimmed milk/BSA	<ul style="list-style-type: none"> 5 % (w/v) non-fat dry milk/BSA, in 1 x TBST
50 x TAE buffer	<ul style="list-style-type: none"> 40 mM TRIS 1 mM EDTA adjust pH 8.0 with HCl
Blocking solution for Western blotting	<ul style="list-style-type: none"> 5 % (w/v) non-fat dry milk 5 % (w/v) BSA in 1 x TBST
Low Salt Dilution Buffer	<ul style="list-style-type: none"> 20 % glycerol 50 mM TRIS-HCl pH 7.9 0.1 % NP-40 0-1000 mM KCl
Lysis Buffer	<ul style="list-style-type: none"> 10 mM Tris-HCl pH 8.0 10 mM EDTA, 0.5 % SDS in dH₂O
RIPA Buffer	<ul style="list-style-type: none"> 50 mM Tris 150 mM NaCl 0.1 % (wt/vol) SDS 0.5 % (wt/vol) sodium deoxycholate 1 % (vol/vol) Triton X-100 adjust to pH 7.5 with HCl
Transformation and Storage Buffer	LB-broth containing <ul style="list-style-type: none"> 10 mM MgCl₂ 10 mM MgSO₄ 5 % DMSO
TRIS-EDTA pH 7.5	<ul style="list-style-type: none"> 100 mM TRIS-HCl pH 7.5 10 mM EDTA

2.12 Commercial chemicals and reagents

Table 26: Commercial chemicals and reagents.

Chemical/Reagent	Company
2-Propanol	Carl Roth GmbH + Co. KG
(ROTIPURAN® ≥99,7 %, p.a.)	
4',6-Diamidin-2-phenylindol (DAPI)	Merck KGaA
Agarose basic	AppliChem GmbH
BOND Epitope Retrieval Solution 1	Leica Biosystems
Chameleon® Duo Pre-Stained Protein Ladder	LI-COR Biosciences
D-Biotin	Thermo Fisher Scientific
Dimethyl sulfoxide (DMSO) for cell culture	AppliChem GmbH
Disodium ethylene diamine tetraacetate	Sigma-Aldrich
DPBS	Thermo Fisher Scientific
Ethanol	Carl Roth GmbH + Co. KG
(≥99,5 %, Ph. Eur., extra pure)	
Ethanol	Carl Roth GmbH + Co. KG
(≥99,8 %, denatured)	
Glycerol	AppliChem GmbH
Glycine	Carl Roth GmbH + Co. KG
Goat Serum	Cell Signaling Technology
HEPES, buffer grade	AppliChem GmbH
Hydrogen chloride (HCl), 37 %	Carl Roth GmbH + Co. KG
Isopropyl alcohol, >99,8 %	Carl Roth GmbH + Co. KG
LB-Agar powder	Thermo Fisher Scientific
LB-Broth powder	Carl Roth GmbH + Co. KG
Magnesium chloride hexahydrate	Merck KGaA
Magnesium sulfate heptahydrate	Sigma-Aldrich
Methanol	Carl Roth GmbH + Co. KG
(ROTIPURAN® ≥99,8 %, p.a.)	
NP-40	Sigma-Aldrich
Pierce™ 16% Formaldehyde (w/v),	Thermo Fisher Scientific
Methanol-free 10 x 1 mL	
Polyethylene glycol 3,350	Sigma-Aldrich
Potassium Chloride (KCl)	Carl Roth GmbH + Co. KG
ProLong™ Gold Antifade Mountant	Thermo Fisher Scientific
RNAse free water	Promega
ROTI®Load, 2 x	Carl Roth GmbH + Co. KG
ROTI®Load, 4 x	Carl Roth GmbH + Co. KG
Shandon™ Immu-Mount™	Epredia
Skimmed milk powder	Hügli Nahrungsmittel GmbH
Sodium chloride (NaCl)	Merck KGaA
Sodium deoxycholate (C ₂₄ H ₃₉ O ₄ Na)	AppliChem GmbH

Tris-(hydroxymethyl)-aminomethane
 Triton® X-100
 Tween® 20

Carl Roth GmbH + Co. KG
 SERVA Electrophoresis GmbH
 Carl Roth GmbH + Co. KG

2.13 Kits

Table 27: Commercial kits.

Kit	Company
Macherey-Nagel™ NucleoSpin™ Gel and PCR Clean-up Kit	MACHEREY-NAGEL GmbH & Co. KG
Monarch® Plasmid Miniprep Kit	New England Biolabs GmbH
NucleoBond® Maxi kit	MACHEREY-NAGEL GmbH & Co. KG
P3 Primary Cell 4D-Nucleofector® X Kit S	Lonza Group AG
QuikChange® XL Site-Directed Mutagenesis Kit	Agilent Technologies
RNeasy Mini Kit (250)	Qiagen
Thermo Scientific™ Pierce™ BCA Protein Assay Kit	Thermo Fisher Scientific
VECTASTAIN® Elite ABC-HRP Kit, Peroxidase (Rabbit IgG)	Vector Laboratories Inc.

2.14 Equipment

Table 28: Equipment used in this study.

Equipment	Company
4D-Nucleofector® Core Unit	Lonza Group AG
4D-Nucleofector® X Unit	Lonza Group AG
90° Ground Edge Frosted Slides	Engelbrecht GmbH
All-In-One Life Science Microscope BZ-X1000	KEYENCE Corporation
Axiovert 25	Carl Zeiss Microscopy GmbH
Bioruptor® Sonicator Plus	Diagenode, LLC.
BRAND® HandyStep® S manual repetitive pipette	BRAND GmbH + Co KG
Cell Counting Slides	Bio-Rad Laboratories,
Cell culture flask, ventilated lid, 25, 75, 150 cm²	TPP Techno Plastic Products AG
Cell culture petri dish, Ø96 mm, 60 cm²	TPP Techno Plastic Products AG
Cell culture plates, 24 wells, Ø15.4 mm	TPP Techno Plastic Products AG
CO ₂ Incubator Modell CB 260	BINDER GmbH
Countstar Mira FL Counter	Alit Biotech Co., Ltd.
CryoPure Röhre, 2 ml	SARSTEDT AG & Co. KG
Dispenser-Tips PD-Tips II	BRAND GmbH + Co KG
DynaMag-2 magnetic rack	Thermo Fisher Scientific
Eppendorf Centrifuge 5424 R	Eppendorf
Eppendorf® Thermomixer Compact	Eppendorf

GloMax® Discover Microplate Reader	Promega
IKA ROLLER 6 basic	IKA®-Werke GmbH & CO. KG
IKA Vortexer VF2	IKA®-Werke GmbH & CO. KG
Innova® S44i Inkubationsschüttler	Eppendorf
Leica DMI6000	Leica Microsystems GmbH
MagneSphere® Technology Magnetic Separation Stands	Promega
MaxQ™ 4000 Benchtop Orbital Shaker	Thermo Fisher Scientific
Mini-Centrifuge ROTILABO®	Carl Roth GmbH + Co. KG
Mixer Mill MM 301	Retsch GmbH
Multifuge X4 Pro Centrifuge	Thermo Fisher Scientific
NanoDrop™ One/OneC Mikrovolumen-UV/VIS- Spektralphotometer	Thermo Fisher Scientific
NanoZoomer® S360 Digital slide scanner	Hamamatsu Photonics K.K
Odyssey® XF	LI-COR Biosciences
Pico™ Microcentrifuge	Thermo Fisher Scientific
PIPETBOY acu 2	INTEGRA Biosciences GmbH
POWER PAC 200 and 300	Bio-Rad Laboratories
PT-9700PC	Brother Industries Ltd.
QuantStudio™ 5 Real-Time PCR System	Thermo Fisher Scientific
Scaltec SBA 52	SCALTEC Instruments GmbH
ScanLaf Mars - Biological safety cabinet class 2	LaboGene A/S
Severin MW 7838	SEVERIN Elektrogeräte GmbH
Snijders 34528 Test-Tube Rotator	Snijders Labs
Thermomixer Comfort 5355	Eppendorf
Trans-Blot® Turbo™ Transfer System	Bio-Rad Laboratories GmbH
Ultrapure water Milli-QPlusPF	Millipore
UVP ChemStudio PLUS Bioimaging System	Analytik Jena GmbH+Co. KG
UVP CL-1000M Midrange UV Crosslinker	Analytik Jena GmbH+Co. KG
Waterbath Hydro H 24	LAUDA DR. R. WOBSE GMBH & CO. KG

2.15 Software

Table 29: Software and corresponding versions.

Software	Version
© BioRender	Web-based application
Fiji/ImageJ	1.54p
Image Studio	V5.2.5
Leica Application Suite X	V3.7.1.21655
NDP.view2	2.8.24
SnapGene	V7.2

3. Methods

3.1 Bacterial culture

3.1.1 Production of competent bacteria for transformation

Production of competent *E. coli* strains was performed according to the protocol described by Chung and Miller (Chung and Miller, 1988). 5 ml LB-medium were inoculated with a pipette tip containing frozen bacteria and incubated at 37 °C overnight. 50 ml LB-medium were inoculated with the complete preculture and incubated at 37 °C on a rotator for approximately one hour. Bacteria were centrifuged at 1,000 x g for 10 min at 4 °C and the pellet was resuspended in 5 ml ice-cold TSB-buffer. After incubation on ice for 10 min, competent *E. coli* strains were aliquoted and stored at -80 °C for long-term preservation. Transformation efficiency was determined by transforming 100 µl competent bacteria with 1 ng puc19 plasmid. Colonies were counted to calculate the efficiency of bacteria when transformed with 1 µg DNA. The efficiency was at 7×10^6 colonies per microgram DNA.

3.1.2 Transformation of competent bacteria

100 µl of competent *E. coli* bacteria (DH5α and XL2 strains) were transformed by adding appropriate amounts of plasmid DNA. After 30 min of incubation on ice, bacteria were heat shocked at 42 °C for 30 s and allowed to recover on ice for five minutes. To initiate the expression of resistance genes, 300 µl SOC-medium were added and the bacterial suspensions were left to incubate at 37 °C for 1 h with gentle mixing. Transformed bacteria were then plated on LB-agar plates containing 100 µg/ml ampicillin and grown over night at 37 °C.

3.1.3 Storage of bacteria

Bacterial culture plates, sealed with parafilm or liquid bacterial cultures were short-term stored up to four weeks at 4 °C. For long-term storage at -80 °C, 750 µl of bacterial culture were mixed with 250 µl 50 % glycerol (autoclaved).

3.1.4 Plasmid preparation

For mini-preps: 6 ml LB-medium containing 150 µg/ml ampicillin were inoculated with bacterial clones and incubated at 37 °C overnight. To purify larger amounts of plasmid DNA 200-400 ml LB-medium containing 150 µg/ml ampicillin was inoculated with 0.5 ml – 1 ml of the preculture. Incubation took place at 37 °C overnight.

For maxi-preps: LB-medium containing 150 µg/ml ampicillin was inoculated with a single clone picked from LB-ampicillin-agar plates and incubated overnight at 37°C. 200-400 ml LB-medium supplemented with 150 µg/ml ampicillin were inoculated with 0.5 ml of the preculture in an Erlenmeyer flask and incubated overnight at 37 °C on a rotator. Plasmid DNA was isolated using the Monarch® Plasmid Miniprep Kit or NucleoBond® Xtra Maxi kit according to the manufacturer's protocol. DNA concentrations were determined using the NanoDrop™ spectral photometer.

3.2 DNA methods

3.2.1 Isolation of genomic DNA

Cells were detached and centrifuged as described in 3.3.1. Cell pellets were resuspended in 200 µl lysis buffer containing 20 mg/ml Proteinase K and incubated overnight at 55 °C with gentle shaking. To precipitate DNA, 1/10 volume of 3 M NaCl was added and mixed by inverting six times. Two volumes of ice-cold ethanol (100 %) were added and samples incubated overnight at -80 °C, followed by centrifugation for 10 min at 12,000 x g and 4 °C. DNA precipitates were washed once with ice-cold ethanol (70 %) and air-dried for 5-10 min. DNA was resuspended in nuclease-free water and incubated for 10 min at 55 °C to improve solubility. Concentration and purity were determined using the NanoDrop™ spectral photometer.

3.2.2 Molecular cloning

3.2.2.1 Polymerase chain reaction (PCR)

Specific DNA fragments were amplified via PCR. The reaction was performed using 5 ng template DNA in a total volume of 50 µl containing Q5® High-Fidelity DNA Polymerase, 200 mM deoxynucleotide triphosphates (dNTPs) and 0.5 µM of forward and reverse primers each. The cycling protocol was carried out in an Eppendorf® Mastercycler®.

Table 30: PCR cycling conditions.

Step		Temperature	Time
Initial Denaturation	1 x	98 °C	30 s
Amplification		1) 98 °C	1) 8 s
	30	2) 55 °C	2) 20 s
	x	3) 72 °C	3) 25 s
Final Extension	1 x	72 °C	2 min
Hold	1 x	4 °C	∞

3.2.2.2 Isolation of DNA from agarose gels

Amplified PCR products were separated on an agarose gel. DNA bands were cut out from the gel under UV-light using the UVP ChemStudio PLUS Bioimaging System. DNA fragments were purified from the gel using the Macherey-Nagel™ NucleoSpin™ Gel and PCR Clean-up Kit according to the manufacturer's protocol. The following steps were carried out for subsequent molecular cloning:

1. DNA digestion by restriction enzymes
2. Agarose gel electrophoresis
3. Purification of DNA from gel
4. Dephosphorylation of 5'-end DNA fragments by alkaline phosphatase
5. Ligation of DNA by T4-DNA-Ligase
6. Spectrophotometric determination of concentration at 260 nm and 280 nm using NanoDrop™ spectral photometer

3.2.2.3 *In vitro* site-directed mutagenesis

The QuikChange® XL Site-Directed Mutagenesis Kit was used to introduce amino acid changes in a protein sequence of interest according to the manufacturer's protocol.

3.3 Cell culture and generation of cellular models

3.3.1 General cultivation of cell lines

Adherent cell lines were cultivated at 37 °C, 6 % CO₂ with the corresponding culture medium, as listed in Table 4. All cell culture experiments were executed under biological safety class 1 laminar flow cabinet. Cell passaging was performed at 70 – 80 % density. After washing once with 1x DPBS, detachment of cells was achieved by adding 0.05 % Trypsin-EDTA at 37 °C. Detached cells were resuspended in fresh culture medium, centrifuged for 5 min at 300 x g, resuspended in fresh culture medium and seeded in a new flask in the desired density.

3.3.2 Freezing of cell lines

Cells were grown in 150 cm² tissue culture flasks until reaching approximately 80 % confluency and detached as described above. Detached cells were centrifuged at 300 x g for 5 min. and the cell pellet was resuspended in 4.5 ml FBS containing 0.5 ml dimethyl sulfoxide (DMSO). Cells were aliquoted into 1.5 ml freezing vials for long-term storage at -150 °C.

3.3.3 Cell counting

Automated cell counting was performed with the Countstar Mira FL Counter.

3.3.4 Calcium phosphate transfection of cell lines

For transfection, cells were seeded in 10 cm cell culture dishes to reach 60 – 80 % confluency after overnight incubation at 37 °C. DNA diluted in dH₂O (ad 450 µl) was mixed with 500 µl 2x HBS pH 7.05. Finally, 50 µl 2 M CaCl₂ were added to each sample. Each sample was instantly vortexed for 10 s and incubated 10 min at room temperature (RT). The DNA mixture was then added to the cells drop by drop, carefully swirled a few times and incubated 5 h at 37 °C. Transfected cells were washed twice with 1x DPBS and supplied with fresh medium. Whole-cell protein extracts were produced 48 h after transfection.

3.3.5 Transfection of human keratinocytes

N/Tert keratinocytes (serum condition) were transfected using the P3 Primary Cell 4D-Nucleofector® X Kit S with the 4D-Nucleofector® either in a Nucleocuvette™ or Nucleocuvette™ Strip. Electroporation was performed with the designated DS-138 (Keratinocytes, Human) program.

3.3.6 Generation of human keratinocytes stably expressing HPV8-E6

N/Tert keratinocytes were cultured in a T75 cm² tissue culture flask with RM+ medium to reach 80 % confluency. Cells were detached and prepared for transfection in a Nucleocuvette™. Per sample, 1 x 10⁵ cells were centrifuged 10 min at 200 x g. Cell pellets were gently resuspended in 20 µl P3 Primary Cell 4D-Nucleofector™ X Solution containing 10 µg pcDNA-FLAG-EV or pcDNA-FLAG-HPV8-E6 plasmids. The Nucleocuvettes™ were placed in the 4D-Nucleofector X Unit for electroporation (pulsing) followed by adding 1 ml fresh, pre-heated culture medium, and incubation for 10 min at RT. Finally, the cells were carefully pipetted into 24-well-plates. Transfected cells were selected by adding RM+ medium containing 250 µg/ml G418 48 h post transfection. G418-containing medium was regularly replaced until the H₂O-transfected control cells were dead.

3.3.7 CRISPR-Cas9-mediated knockout of DPF2 in human keratinocytes

For CRISPR-Cas9-mediated gene knockout, N/Tert^{EV} and N/Tert^{HPV8-E6} cells cultivated in serum-containing medium were transfected with the 5 µg pSpCas9(BB)-2A-Puro plasmid (Ran et al., 2013) as described above. Selection was started 48 h post-transfection using culture medium

containing 1.5µg/ml puromycin. Single cell clones were isolated using cloning cylinders and transferred into 96-well-plates.

3.3.8 Verification of DPF2^{KO} via PCR and sequencing

To validate positive DPF2^{KO} clones, genomic DNA was isolated from transfected keratinocytes as described in section 3.2.1. Site-specific primers listed in Table 16 were used to amplify the corresponding gRNA target region using *Taq* DNA Polymerase. PCR fragments were purified using the Macherey-Nagel™ NucleoSpin™ Gel and PCR Clean-up Kit following the manufacturer's instructions. Purified PCR fragments were analyzed by Sanger Sequencing and evaluated using SnapGene software. If sequencing indicated a heterozygous population, transfected cells were single-cell cloned using cloning cylinders.

Table 31: Reaction mixture for PCR-based verification of DPF2^{KO} clones.

Component	Amount per 50 µl reaction
10X Standard <i>Taq</i>	5 µl
Reaction Buffer	
25 mM MgCl ₂	2 µl
10 mM dNTPs	1 µl
10 µM Forward Primer	1 µl
10 µM Reverse Primer	1 µl
Template DNA	50 ng
<i>Taq</i> DNA Polymerase	0.25 µl
Nuclease-free water	ad 50 µl

Table 32: PCR cycling conditions for verification of DPF2^{KO} clones.

Step		Temperature	Time
Initial Denaturation	1x	95 °C	2 min
	30x	1) 95 °C	1) 30 s
Amplification		2) 58 °C	2) 30 s
		3) 72 °C	3) 30 s
Final Extension	1x	72 °C	5 min
Hold		4 °C	

3.3.9 UV irradiation

For UV-irradiation, cells were washed once with 1x PBS and irradiated with 15 mJ/cm² UV using the UVP CL-1000M Midrange UV Crosslinker 30 h after transfection.

3.4 RNA methods

3.4.1 RNA isolation

Total RNA from cells was isolated using the RNeasy mini kit according to the manufacturer's protocol. RNA concentration was determined using the NanoDrop™ spectral photometer. RNA from primary human epithelial keratinocytes cultured in serum-free KGM medium was provided by Dr. Martin Hufbauer.

3.4.2 cDNA synthesis

The Omniscript RT Kit was used to reverse transcribe 1 µg RNA in a total volume of 20 µl according to the manufacturer's protocol. Reverse transcription was carried out for one hour at 37 °C, followed by 10 min at 95 °C to inactivate the enzyme.

3.4.3 Quantitative real-time polymerase chain reaction (qRT-PCR)

mRNA levels of target genes were measured using the efficiency-corrected $\Delta\Delta C_t$ method (Pfaffl, 2001). For each reaction, 20 ng cDNA was used in a total volume of 20 µl. The reaction mix contained 1x GoTaq® qPCR Master Mix and 250 nM forward and reverse primers each. Samples were analyzed in duplicates. Transcript levels were normalized to the housekeeping gene HPRT1.

Table 33: qRT-PCR cycling conditions.

Step		Temperature	Time
Initial denaturation	1 x	95 °C	60 s
Amplification	40 x	1) 95 °C	1) 1 s
		2) 55 °C	2) 5 s
		3) 72 °C	3) 15 s
Melting Curve	1 x	1) 95 °C	1) 15 s
		2) 70 °C	2) 20 s
		3) 95 °C	3) 1 s
Cool down	1 x	40 °C	10 s

3.5 Protein methods

3.5.1 Production of whole-cell extracts

For the preparation of whole-cell extracts, adherent cells were scraped off cell culture dishes with a scraper in 100 mM LSDB buffer + PI (1 mM PMSF, 1 mM OV, 50 mM NaF and 1x cOmplete™ EDTA-free protease inhibitor cocktail). Lysates were incubated on ice for 45 min and then sonicated using the Bioruptor® Sonicator Plus for five cycles of 30 s pulse followed by 30 s pause at 4 °C to ensure complete cell lysis. Lysates were centrifuged for 15 min at 14,000 x g and 4 °C. The supernatant was transferred to pre-chilled, new 1.5 ml tubes and protein concentrations were measured by either Bradford or Pierce™ BCA Protein Assay Kit. Absorbance was measured using the GloMax® Discover Microplate Reader. Whole-cell extracts were stored at -80 °C.

3.5.2 Co-immunoprecipitation (Co-IP)

FLAG and HA-tagged proteins were precipitated using Pierce™ Anti-DYKDDDDK or Pierce™ Anti-HA Magnetic Agarose. Per sample, 20 µl magnetic agarose were equilibrated in 1 ml ice-cold 100 mM LSDB buffer (0.1 mM PMSF) on a rotating platform three times. The beads were collected using a magnetic rack. 200 µg - 500 µg whole-cell extracts were incubated with 20 µl equilibrated magnetic beads in 500 µl with ice-cold 100 mM LSDB (1 mM PMSF, 1x cOmplete™ EDTA-free protease inhibitor cocktail, 1 mM OV, 50 mM NaF) for 30 min at RT, 2 h at 4 °C, or overnight at 4 °C on a rotating platform. Beads containing the enriched proteins were collected using a magnetic stand and the supernatant was discarded. Each sample was washed three to five times with 500 µl ice-cold 150 mM – 250 mM LSDB buffer for 5 min on a rotating platform. After a final wash step with 500 µl ice-cold 100 mM LSDB buffer, enriched proteins were eluted by adding one bead volume of 2x ROTI®Load reducing sample buffer and incubating for 5 min at 95 °C. Residual beads were collected using a magnetic rack and the denatured eluates were loaded on an appropriate SDS-gel for separation followed by Western Blotting.

For relative quantification of Co-IP experiments, band intensities of co-precipitated proteins were quantified using the Image Studio™ acquisition software (v 5.2.5) and normalized to the corresponding precipitated protein or reference signal, depending on the experimental setup. Relative values were calculated by setting the indicated control condition to 1.

3.5.3 Western blotting

Protein extracts were separated by SDS-PAGE at a constant voltage of 100 V (Laemmli, 1970). To remove SDS remnants, gels were placed in ultrapure water for 5 min with gentle shaking.

Separated proteins were transferred to a nitrocellulose membrane at a voltage of 25 V for 30 minutes using the Trans-Blot® Turbo™ Transfer System. The membrane was blocked with 5 % BSA or low-fat milk in 1x TBST for 1 h at RT to reduce unspecific binding of antibodies. Blocked membranes were incubated overnight with appropriate dilutions of primary antibody in 5 % skimmed milk or BSA at 4 °C. After three wash steps for five minutes each with 1x TBST, the corresponding IRDye® secondary antibodies were added for one hour at RT in the dark. After three additional wash steps of five minutes each with 1x TBST, specific proteins were detected with the Odyssey® XF and associated Image Studio™ acquisition software (v 5.2.5).

3.5.4 Immunostaining and microscopy

3.5.4.1 DAB-staining of paraffin-embedded tissue

Paraffin-embedded sections from organotypic N/Tert skin cultures and human skin cSCC tissue samples were analyzed by DAB staining. Organotypic skin cultures from N/Tert keratinocytes transduced with retroviral pLZRS-EV, pLZRS-HPV8-E6, pLZRS-HPV8-E7, or pLZRS-HPV8-E6/E7 constructs were provided through the collaboration with Mariet C. W. Feltkamp (Department of Medical Microbiology, Leiden University Medical Center, Netherlands). Generation of the organotypic skin cultures was described previously (Struijk et al., 2008, Kazem et al., 2012).

DAB staining was performed using the VECTASTAIN® Elite ABC HRP Kit according to the manufacturer's instructions. Antigen retrieval was performed in Nucleobond citrate buffer by heating the slides in a microwave for 3 min followed by incubation in the hot buffer for 15 min. Images were viewed and exported using NDP.view2 software.

3.5.4.2 Immunofluorescent staining of human keratinocytes

For immunofluorescent stainings, cells were seeded on coverslips (15 mm) in a 24-well plate and cultured overnight at 37 °C. After washing each well once with 1x DPBS, cells were fixed with 4 % formaldehyde for 15 min at RT. To completely remove formaldehyde, cells were washed three times with 1x DPBS. Cells were permeabilized with 0.5 % Triton® X-100-PBS for 10 min at RT and washed three times with 1x DPBS. Unspecific binding of primary antibodies was reduced by incubating the coverslips with 0.1 % Tween-PBS/5 % BSA/3 % goat serum for one hour at RT. Primary antibodies were diluted in 0.1 % Tween-PBS/3 % BSA/1 % goat serum and incubated overnight at 4 °C with gentle shaking. Three washing steps with 0.1 % Tween-PBS followed. The coverslips were then incubated with secondary antibodies diluted in 0.1 % Tween-PBS/3 % BA/1 % goat serum for one hour at RT in the dark followed by three washing steps with 0.05 %

Tween-PBS. Nuclei were stained with 4',6-diamidino-2-phenylindole (DAPI) diluted 1:1,000 in 1x DPBS for 10 min in the dark. After three washes with 1x DPBS coverslips were mounted on microscopic slides using ProLong™ Gold Antifade mountant and left to dry in the dark overnight at RT. Images were acquired using the All-In-One Life Science Microscope BZ-X1000.

3.5.4.3 Immunofluorescence image analysis

For quantitative analysis, immunofluorescence images were acquired using identical exposure and acquisition settings within each staining experiment. To analyze the p300 and DPF2 colocalization, 30 nuclear regions of interest (ROI) per condition were defined in N/Tert^{EV} and N/Tert^{HPV8-E6} cells using Fiji/ImageJ. The analyzed nuclei were obtained from multiple randomly selected regions. Prior to analysis, background fluorescence was removed from the p300 and DPF2 channels using the “Subtract Background” function in Fiji/ImageJ (rolling ball radius = 50 pixels). To assess the correlation between p300 and DPF2 fluorescence signals, Pearson's correlation coefficient was calculated within each nuclear ROI using the Coloc2 plugin (Schindelin et al., 2012). Pearson's correlation coefficients were compared using an unpaired two-tailed Welch's t-test in GraphPad Prism 11. The distribution of p300 and DPF2 signals within the nucleus was visualized by drawing a straight line across selected ROIs. Representative fluorescence intensity profiles were generated by measuring the individual ROIs using the “Plot Profile” function.

The localization of MMP7 and E-cadherin was analyzed by drawing a straight ROI line across individual cell-cell junctions and the adjacent cells. Representative fluorescence intensities of both channels were measured along the selected line using the “Plot Profile” function in Fiji/ImageJ. Mean fluorescence intensities were determined for 20 cell-cell junctions per condition. Statistical significance was assessed using an unpaired two-tailed Welch's t-test in GraphPad Prism 11.

3.6 TurboID proximity proteomics

3.6.1 Transient TurboID transfection and UV irradiation of HEK293 cells

HEK293 cells were transiently co-transfected with appropriate amounts of either 3xHA-TurboID empty vector, 3xHA-TurboID-p300-1 or 3xHA-TurboID-p300-4 together with pcDNA-FLAG-HPV8-E6, as described in section 3.3.4. All conditions were performed in triplicate. For UV-irradiation, cells were washed once with 1x PBS and irradiated with 15 mJ/cm² UV using the UVP CL-1000M Midrange UV Crosslinker 30 h after transfection. Post irradiation, cells were cultured at 37 °C for 18 h in fresh medium.

3.6.2 Biotin labeling of proximal proteins

48 h post-transfection cells were incubated with warm culture medium containing 50 μ M D-Biotin for 10 min at 37 °C. The biotinylation reaction was stopped by placing the tissue culture dishes on ice and washing 5x with ice-cold 1x DPBS. The cells were scraped off, resuspended in 800 μ l ice-cold 1x DPBS (1 mM PMSF and 1x cOmplete™ EDTA-free protease inhibitor cocktail), and centrifuged for 15 min at 300 x g and 4 °C. The cell pellet was resuspended in 550 μ l RIPA buffer (1 mM PMSF, 1x cOmplete™ EDTA-free protease inhibitor cocktail, 1 mM Orthovanadate (OV) and 50 mM Sodium Fluoride (NaF)). Lysates were incubated on ice for 45 min, then sonicated using the Bioruptor® Sonicator Plus for five cycles of 30 s pulse followed by 30 s rest at 4 °C to ensure complete cell lysis. The lysates were centrifuged for 15 min at 14,000 x g and 4 °C. The supernatant was transferred to pre-chilled, clean 1.5 ml tubes. The Pierce™ BCA Protein Assay Kit was used to measure the protein concentration with the GloMax® Discover Microplate Reader.

3.6.3 Enrichment of biotinylated proteins

Biotinylated proteins were enriched using magnetic Sera-Mag™ streptavidin beads. The beads were equilibrated 3x with RIPA buffer containing 1 mM PMSF and 1x cOmplete™ EDTA-free protease inhibitor cocktail (RIPA + PI). Equilibrated streptavidin beads (50 μ l) were incubated with 1 mg protein in 500 μ l RIPA + PI overnight at 4 °C on a rotating platform. The beads were collected using a magnetic rack and the supernatant was discarded. The streptavidin beads were washed twice with RIPA buffer (2 min, RT, rotating), once with 1 M KCl (2 min, RT, rotating), once with 0.1 M Na₂CO₃ (10 s, inverting), once with 2 M urea in 10 mM Tris-HCl pH 8 (10 s, inverting) and finally twice with RIPA buffer (2 min, RT, rotating). The beads were finally collected using a magnetic rack and transferred to a fresh tube in RIPA buffer.

3.6.4 Characterization of construct expression and activity

The expression of TurboID-EV and the TurboID fusion constructs, as well as the efficiency of biotin labeling, was assessed by Western blotting. 10 % of the beads containing the enriched proteins were transferred to fresh tubes and boiled for 10 min at 95 °C in 20 μ l reducing 2x ROTI® Load buffer. After transfer, nitrocellulose membranes were blocked 1 h at RT in 5 % BSA. To detect biotin labeling and fusion construct expression by Western blotting, membranes were incubated with IRDye® 800CW Streptavidin and an anti-HA-Tag antibody, respectively, both diluted 1:3,000 in 5 % BSA, for 2 h at RT. Imaging was performed using the LI-COR ODYSSEY® XF imaging system.

3.6.5 On-bead digestion

Enriched proteins were pulled down from the magnetic beads to prepare proteomic samples for mass spectrometry analysis. Proteins bound to the magnetic streptavidin beads were washed once with 50 mM Tris-HCl and twice with 2M urea in 50 mM Tris-HCl pH 7.5. The beads were then incubated for one hour at 25 °C and 1,000 rpm with 2M urea in 50 mM Tris-HCl pH 7.5 containing 1 mM DTT and 0.5 µg trypsin. In total, the beads were washed three times with 2M urea in 50 mM Tris-HCl pH 7.5. A magnetic rack was used to collect and combine the eluates of the three wash steps. To reduce disulfide bonds, eluates were incubated for 30 min with 4 mM DTT at 25 °C and 1,000 rpm. Alkylation was induced by incubating samples for 45 min with 10 mM chloroacetamide at 25 °C and 1,000 rpm in the dark. An additional 0.5 µg of trypsin was added to each sample and digestion proceeded over night at 25 °C in the dark (700 rpm). Finally, samples were acidified by adding 1 % (v/v) formic acid pH 3. Acidified peptide samples were purified using C18 Stage Tips.

3.6.6 SDB-RP C18 stage tip purification

C18 Stage Tips were placed into 2 ml tubes and equilibrated sequentially with 20 µl methanol, 20 µl buffer B (0.1 % formic acid in 80 % acetonitrile), and 20 µl buffer A (0.1 % formic acid in water) applied twice. All buffers were provided by the CECAD Proteomics Core Facility (University Cologne). After each equilibration step, the Stage Tips were centrifuged for 1 min at 800 × g. Before loading the supernatant, the acidified samples were centrifuged at full speed for 5 min. After loading, Stage Tips were centrifuged for 5 min at 800 x g. Each sample was washed once with 30 µl buffer A and twice with 30 µl buffer B followed by centrifugation for 1 min at 800 x g. A syringe was used to completely dry the Stage Tips, which were then dried overnight at 4 °C. Mass spectrometric analysis was performed by the CECAD Proteomics Core Facility.

3.6.6.1 LC-MS/MS data acquisition

Proteomic samples were analyzed by the CECAD Proteomics Facility using an Orbitrap Exploris 480 mass spectrometer (Thermo Scientific) coupled to a Vanquish Neo ultra-high-performance liquid chromatography (UHPLC) system in a trap-and-elute configuration.

Peptides were first loaded onto a precolumn (Acclaim 5 µm PepMap 300 µ Cartridge) at a flow rate of 60 µL/min. Following loading, samples were reverse-flushed onto an in-house packed analytical column (30 cm length, 75 µm inner diameter) filled with 2.7 µm Poroshell EC120 C18 (Agilent). Chromatographic separation was performed using a multi-step gradient of Solvent A

(0.1 % formic acid) and Solvent B (0.1 % formic acid in 80 % acetonitrile): Initial: 2 % B at 400 nL/min, 0–3 min: Linear increase to 6 % B, 3–29 min: Flow reduced to 300 nL/min; B increased to 20 %. 29–44 min: B increased to 35 %. 44–45 min: B increased to 98 % while flow returned to 400 nL/min. Wash/Re-equilibration: Column wash at 95 % B followed by re-equilibration to starting conditions. The mass spectrometer was operated in Data-Dependent Acquisition (DDA) mode with a 1 s cycle time. MS1 scans were acquired from 350 m/z to 1400 m/z at 60,000 resolution with an Automatic Gain Control (AGC) target of 300 %. MS2 scans were acquired at 15,000 resolution, a maximum injection time of 118 ms, a normalized AGC target of 50 %, a 2 Th isolation window, and a fixed first mass of 110 m/z. MS1 data were stored in profile mode; MS2 data were stored as centroids.

3.6.7 Proteomic data processing and statistical analysis

Raw data files were processed using MaxQuant (v2.4.0.0) against the human reference proteome database (UniProt UP000005640) and the sequence of Protein E6 (P06428) of Human papillomavirus type 8. Standard settings were employed, including the "match-between-runs" (MBR) option enabled across replicates (Tyanova et al., 2016a). Downstream bioinformatic analysis was performed in Perseus (v1.6.15). Protein groups were filtered to remove entries labeled as "Reverse," "Potential contaminants," or "Only identified by site." The remaining identifications were filtered for data completeness in at least one group. Missing values were imputed from a normal distribution using a width of 0.3σ and a downshift of 1.8σ . Differential expression was determined using FDR-controlled two-sided t-tests (Tyanova et al., 2016b).

3.6.8 Downstream statistical analysis and data visualization

$\log_2$ -transformed raw intensities were used as input for downstream statistical analysis. Principal component analysis (PCA) was performed to identify the principal components explaining the greatest variability between samples. Comparisons of interest were analyzed using Welch's t-test (Welch, 1947). Raw p-values were adjusted for multiple testing using the Benjamini–Hochberg procedure (Benjamini and Hochberg, 1995). Heatmaps were generated in R using the Bioconductor package epiNEM (Gentleman et al., 2004, Pirkl et al., 2017, R Core Team, 2024).

3.7 RNA-seq sequencing and data analysis

Poly(A)-selected mRNA libraries were prepared using the Illumina Stranded mRNA Prep kit according to the manufacturer's protocol and sequenced on an Illumina NovaSeq 6000 platform at the Cologne Center for Genomics (CCG), generating approximately 35 million reads per

sample. Primary bioinformatic processing was also performed by the CCG. Quality control was performed using FastQC, and quality-control reports were summarized using MultiQC. Reads were trimmed using Trimmomatic, aligned using STAR, and quantified using featureCounts. Differential expression analysis was performed using DESeq2. Genes with an adjusted p-value of < 0.005 and an absolute $\log_2$ fold change of ≥ 2 were considered differentially expressed.

3.8 DynaTag/ChIP-sequencing

3.8.1 Dynamic targets and tagmentation (DynaTag)

DynaTag was carried out as described previously (Hunold et al., 2025). DynaTag is an adaptation of Cut&Tag (Kaya-Okur et al., 2020) and uses a physiological intracellular salt concentration, thereby retaining transcription factor-DNA interactions. In brief, 5×10^5 cells were pelleted, washed once with cold PBS and nuclei were isolated by incubation with 500 μ l cold NE1 (20 mM HEPES-KOH, pH 7.9; 10 mM KCl, 0.5 mM spermidine, 0.1 % Triton X-100, 20 % glycerol), supplemented with 1x complete EDTA-free protease inhibitor (Roche) for 3 min on ice. Nuclei were pelleted by centrifugation at $1,300 \times g$ for 2 min at 4 °C and fixed with 0.1 % methanol-free formaldehyde (Thermo Fisher) in 1 ml PBS at RT. Fixation was stopped by adding 40 μ l 2 M glycine. Nuclei were pelleted by centrifugation at $450 \times g$ for 3 min at 4 °C, resuspended in AB (25 mM HEPES-KOH pH 7.5, 110 mM KCl, 10 mM NaCl, 1 mM $MgCl_2$, 1 % BSA, 0.01 % digitonin, 0.5 mM spermidine) and stored on ice until primary antibody was added. 2×10^5 nuclei in 50 μ l AB were incubated with a 1:50 dilution of primary antibody EP300 or DPF2 overnight at 4 °C on a rotating platform. Samples were centrifuged and incubated with a 1:100 dilution of the secondary antibody (guinea pig anti-Rabbit IgG, NBP1-72763, Lot: 52392) in 50 μ l AB for 1 h at RT, two times washed with 200 μ l AB and incubated with 25 nM pre-loaded pATn5 (Diagenode) in 50 μ l AB buffer for 1 hour at RT. Nuclei were washed two times with AB and incubated in 100 μ l tagmentation buffer (10 mM $MgCl_2$ in AB buffer) for 1 h at 37 °C. Nuclei were pelleted, resuspended in 100 μ l ICS buffer (25 mM HEPES-KOH pH 7.5, 110 mM KCl, 10 mM NaCl, 1 mM $MgCl_2$) and counted. 20,000 nuclei were pelleted, resuspended in 70 μ l denaturation buffer, incubated at 58 °C for 3 h and then at 18 °C overnight. Subsequently, DNA was isolated using the ChIP Clean and Concentrator kit (Zymo Research).

DynaTag libraries were amplified (14-22 cycles) adding barcoded indexes. After validation (TapeStation, Agilent Technologies) and quantification (Qubit, Thermo Fisher Scientific) individual libraries were pooled. The library pools were quantified using the Colibri Library Quantification Kit (Thermo Fisher Scientific) and the QuantStudio 5 Real-Time PCR System (Thermo Fisher

Scientific). Libraries were subsequently sequenced on an Illumina NovaSeq X Plus instrument using a 2x150 bp sequencing protocol and aiming for 30 million clusters per sample.

3.8.2 DynaTag data processing

The DynaTag sequencing reads were checked for quality with FASTQC v0.12.1 (Andrews, 2010) and MultiQC v1.22.3 (Ewels et al., 2016). The Nextera Transposase sequence (CTGTCTCTTATA) was trimmed from the 3' end with Cutadapt v4.4 (Martin, 2011) using -O5 and -m50 options. Trimmed reads were mapped to hs1 (Nurk et al., 2022) as described in (Hunold et al., 2025) using bowtie2 v2.5.4 (Langmead and Salzberg, 2012), SAMtools v1.19.2 (Li et al., 2009, Quinlan and Hall, 2010) and BEDtools v2.31.1 (Quinlan and Hall, 2010). Peaks were called on all technical replicates together with MACS v2.2.9.1 (Zhang et al., 2008) with IgG experiments as control (background) with --keep-dup all --nomodel --extsize 55 options. Differential peaks between conditions were called with MACS bgdiff function with -l 50 -g 25 options and -d1 and -d2 calculated for each condition. For the visualization in UCSC browser (Kent et al., 2002), the read coverage was normalized to signal per million reads (SPMR).

3.9 Use of generative artificial intelligence

OpenAI ChatGPT was used as a supportive tool for language editing, formulation suggestions, and consistency checks during the preparation of this thesis. All scientific content, interpretations, conclusions, and references were critically reviewed and verified by the author. Generative AI was not used to generate experimental data or perform the scientific analyses presented in this thesis.

4. Results

Experiments with K14-HPV8-E6 transgenic mice suggest that binding of HPV8-E6 to the tumor suppressor p300 represents a key step for skin tumorigenesis (Marcuzzi et al., 2009, Hufbauer et al., 2015). This work aims to elucidate the oncogenic activities of HPV8-E6 by characterizing its interaction with p300 and the molecular consequences in human keratinocytes.

4.1 Identification of HPV8-E6 binding regions within p300

4.1.1 Mapping the major HPV8-E6 recognition motif in p300-4

Previous work using p300 fragment constructs mapped HPV8-E6 binding to three regions within p300 spanning residues 2-439 (p300-1), 1453-1888 (p300-4), and 1889-2378 (p300-5) (Müller-Schiffmann et al., 2006) (Fig. 4). *In vivo* experiments with K14-HPV8-E6 transgenic mice revealed that overexpression of p300-1 and p300-4 inhibited skin tumorigenesis in transgenic animals (see section 1.6.1), indicating that targeting p300-1 and p300-4 by HPV8-E6 is mechanistically relevant for tumor induction. Our group previously followed the intention of identifying the HPV8-E6 interaction surface within p300, by focusing on p300-4. The 45-amino-acid fragment aa 1770-1814 within TAZ2 was identified to mediate HPV8-E6 binding (Fig. 6A). However, the use of various overlapping p300 fragments suggested that a 26-amino-acid fragment spanning residues 1789-1814 was sufficient (Müller-Schiffmann et al., 2006).

To confirm this, a DNA fragment encoding residues 1789-1814 of p300 was cloned into the pCMV2-FLAG expression vector and designated FLAG-p300-4⁽¹⁷⁸⁹⁻¹⁸¹⁴⁾-WT. The FLAG-tagged fragment was co-expressed with MYC-tagged HPV8-E6 in transiently transfected C33A cells. FLAG-IPs revealed co-precipitation of MYC-HPV8-E6, confirming that the 26-amino-acid fragment is sufficient for HPV8-E6 binding (Fig. 6B). It has been reported that alanine substitutions of the cysteine residues C1738, C1746, C1789, and C1790 within the p300 TAZ2 domain did not impair its biological function and allowed a more stable protein fold (De Guzman et al., 2000, Jenkins et al., 2009). Since we were interested in crystallizing p300-4⁽¹⁷⁸⁹⁻¹⁸¹⁴⁾-WT in complex with HPV8-E6 to analyze the interaction surface in more detail, we introduced C1789A and C1790A substitutions by *in vitro* mutagenesis, thereby generating the double mutant FLAG-p300-4⁽¹⁷⁸⁹⁻¹⁸¹⁴⁾-MUT. Interestingly, Western blotting revealed that FLAG-p300-4⁽¹⁷⁸⁹⁻¹⁸¹⁴⁾-MUT did not co-precipitate with MYC-HPV8-E6 (Fig. 6B). Together, these data confirm that the p300 fragment spanning residues 1789-1814 within TAZ2 is sufficient for HPV8-E6 binding and suggest that C1789 and/or C1790 contribute to this interaction.

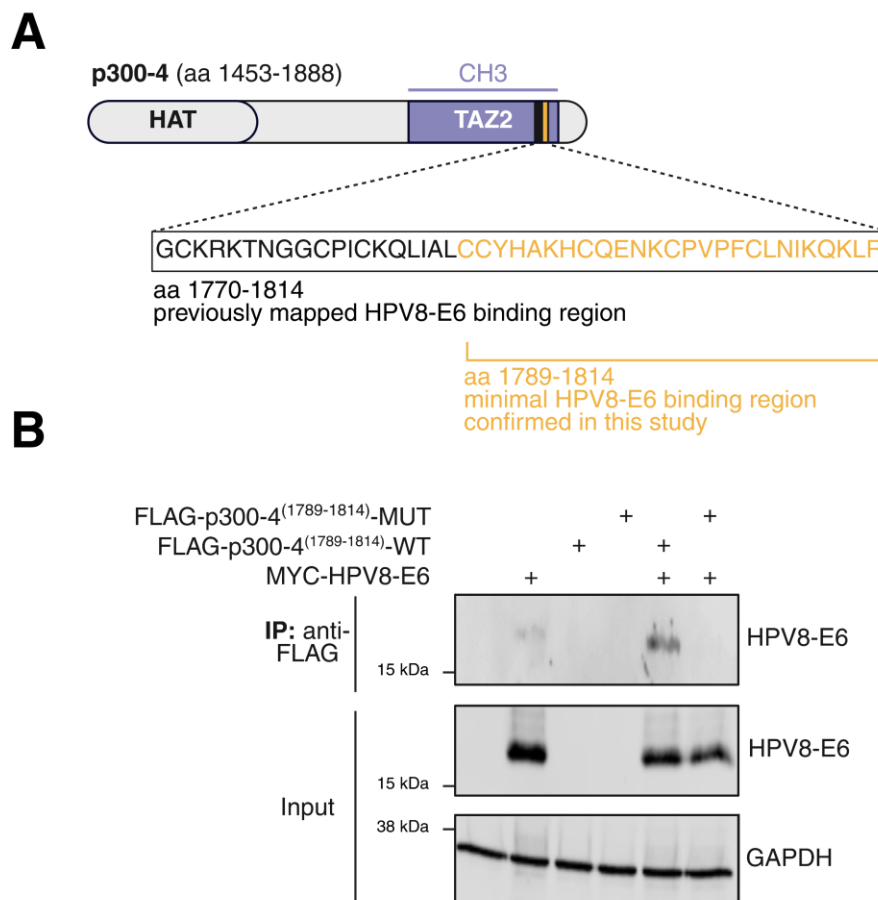


Figure 6: Identification of a minimal HPV8-E6-binding region within the p300 TAZ2 domain. (A) Shown is a schematic representation of the p300-4 fragment spanning residues 1453-1888 which contains the Transcription adaptor putative zinc-finger 2 (TAZ2), and part of the Histone acetyltransferase (HAT) domain. The previously mapped HPV8-E6 binding region spanning residues 1770-1814 (black) and the minimal binding region spanning residues 1789-1814 identified in this study (yellow) are indicated. **(B)** Following transfection of C33A cells, whole-cell lysates (500 µg) were used to perform FLAG-IPs, to precipitate FLAG-p300-4⁽¹⁷⁸⁹⁻¹⁸¹⁴⁾-WT and FLAG-p300-4⁽¹⁷⁸⁹⁻¹⁸¹⁴⁾-MUT (C1789A/C1790A). Co-precipitated MYC-HPV8-E6 was detected by Western blotting using a MYC-tag antibody. Expression of MYC-HPV8-E6 in the samples was confirmed in the input lysates. Due to their small size, the FLAG-tagged p300 fragments could not be detected by Western blotting. GAPDH served as a loading control. Created with BioRender. CH = cysteine/histidine-rich region.

4.1.2 Mapping the HPV8-E6 recognition motifs within p300-1

Although HPV8-E6 was shown to bind p300-1, the molecular basis of this interaction had not been characterized. To identify the binding region within p300-1 (aa 2-439), PCR products encoding several overlapping p300-1 fragments were cloned into the pXJ41-HA expression vector. C33A cells were transiently co-transfected with FLAG-tagged HPV8-E6 and the overlapping HA-p300-1 constructs. FLAG-HPV8-E6 was immunoprecipitated by performing FLAG-IPs, and

co-precipitated HA-p300-1 fragments were detected by Western blotting. FLAG-HPV8-E6 co-precipitated with HA-p300-1.1 (Fig. 7A, lane 6) spanning residues 2-331 and HA-p300-1.2 (Fig. 7A, lane 8) spanning residues 332-439. These findings suggest that HPV8-E6 recognizes at least two distinct binding regions within p300-1.

To further narrow down the minimal HPV8-E6 binding region, additional overlapping HA-p300-1 fragments were analyzed (Fig. 7B). Quantification of HA-p300-1 fragments co-precipitated with FLAG-HPV8-E6 showed weaker signals for HA-p300-1.5 (Fig. 7B, lane 5) and HA-p300-1.8 (Fig. 7B, lane 7) compared with HA-p300-1.6 (Fig. 7B, lane 6). Interestingly, GST-HA-p300-1.9 (aa 332-390) showed the strongest relative Co-IP signal among the analyzed fragments (Fig. 7B, lane 8). These results indicate a major binding region between residues 332-390 within p300-1, corresponding to the TAZ1 domain. To further narrow down specific residues within TAZ1 that are involved in HPV8-E6 binding, p300-1.9, -1.10, and -1.11 fragments were fused to GST to facilitate their detection by Western blotting without impairing HPV8-E6 binding.

To this end, the GST-tag was amplified from the bacterial pGEX-2T expression vector via PCR and cloned into pXJ41-HA-p300-1.9, -1.10, and -1.11 plasmids, allowing the expression of GST-fusion proteins. HA-IP revealed co-precipitation of FLAG-HPV8-E6 with GST-HA-p300-1.9, (aa 332-390; Fig. 7C, lane 5) but not with GST-HA-p300-1.10 (aa 366-406) or p300-1.11 (aa 391-420) (Fig. 7C, lane 6 and 7). However, p300-1.10 and p300-1.11 showed only weak signals in the input and were precipitated less efficiently than p300-1.9 (Fig. 7C, lane 3 and 4). Therefore, it cannot be excluded that the absence of detectable HPV8-E6 binding was partly caused by the lower expression of these fragments. Together, these findings indicate that the 59-amino acid fragment spanning residues 332-390 within p300-TAZ1 mediates HPV8-E6 binding, whereas the shorter fragment spanning residues 332-366 is sufficient for this interaction. The analyzed p300-1 fragments and their observed HPV8-E6 binding are summarized in Fig. 7D.

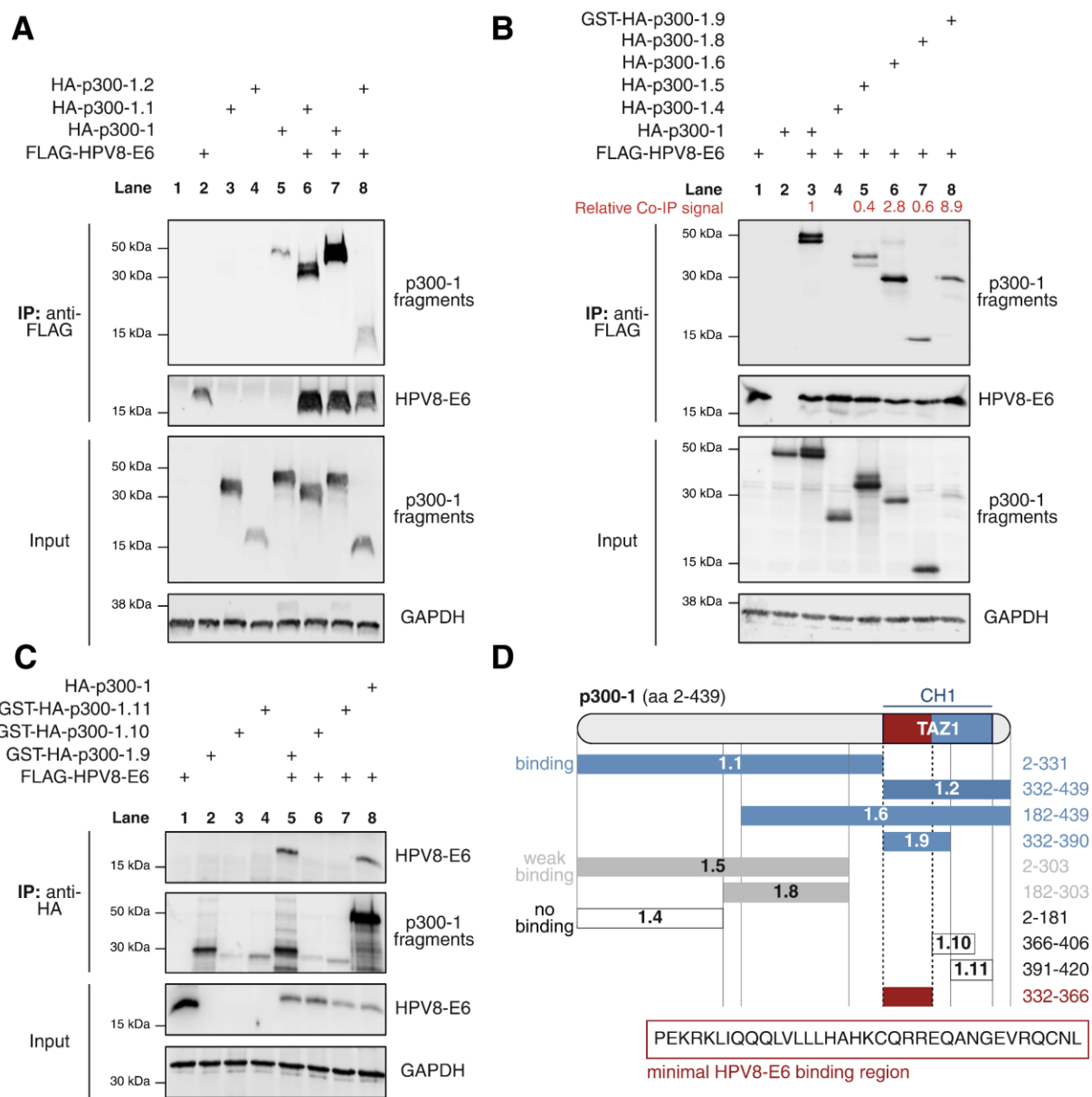


Figure 7: Identification of a minimal HPV8-E6-binding region within the p300 TAZ1 domain. (A, B, C) Whole-cell lysates of transiently transfected C33A cells were used in FLAG-IPs to precipitate FLAG-HPV8-E6. Co-precipitated HA-p300-1 fragments were detected by Western blotting using an HA-tag antibody (A, B). Relative Co-IP signals in panel (B) are shown in red and represent expression relative to HA-p300-1 in lane 3, which was set to 1. (C) HA-IPs were performed to precipitate HA-GST-p300-1 fragments. Co-precipitated FLAG-HPV8-E6 was detected using a FLAG-tag antibody. Expression of the indicated recombinant proteins was confirmed in the input lysates. GAPDH served as a loading control. (D) Shown is a schematic representation of p300-1 (aa 2-439) including the TAZ1 domain. The analyzed HA-p300-1 fragments and binding to HPV8-E6 are shown. Fragments showing no detectable binding to HPV8-E6 are labeled in white, whereas fragments showing binding or weak binding are shown in blue and grey, respectively. Numbers indicate amino acid residues within p300. Created with BioRender. CH = cysteine/histidine-rich region.

4.1.3 Relevance of HPV8-E6 binding to p300-1.9

To determine whether p300-1.9 (aa 332-390) competes with p300-1 for HPV8-E6 binding, we performed competitive binding assays. C33A cells were transiently co-transfected with constant amounts of HA-p300-1 and FLAG-HPV8-E6 and increasing amounts of GST-HA-p300-1.9 as indicated (Fig. 8). Co-expression of p300-1.9 reduced the amount of co-precipitated HA-p300-1 with FLAG-HPV8-E6 (Fig. 8A, lane 2). Increasing amounts of p300-1.9 slightly reduced p300-1 co-precipitation (Fig. 8A, lane 3). To determine whether p300-1.9 also affects the interaction of HPV8-E6 and endogenous p300, the competitive binding assay was repeated. Relative p300 Co-IP signals for endogenous p300 decreased from 1 to 0.45 and 0.36 upon co-expression of 5 μ g and 8 μ g GST-HA-p300-1.9, respectively (Fig. 8B, lane 3 and 4). In contrast, relative p300 input signals remained largely unchanged. These findings indicate that p300-1.9 interferes with HPV8-E6-binding to both p300-1 (Fig. 8A) and endogenous p300 (Fig. 8B), suggesting that the 59-amino-acid fragment spanning residues 332-390 contributes to the interaction between HPV8-E6 and p300.

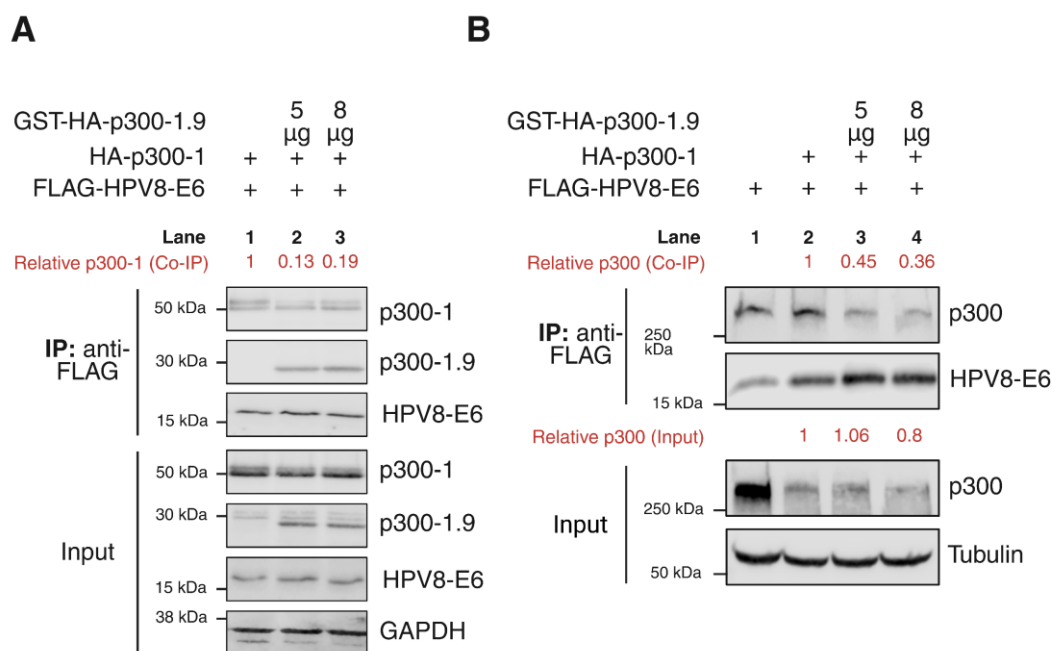


Figure 8: p300-1.9 reduces HPV8-E6 binding to p300-1 and endogenous p300. (A, B) C33A cells were transiently co-transfected with constant amounts of expression vectors encoding HA-p300-1 and FLAG-HPV8-E6 but increasing amounts of p300-1.9 as indicated. FLAG-IPs were performed to precipitate FLAG-HPV8-E6. Co-precipitated HA-p300-1 was detected by Western blotting using an HA-tag antibody (A), whereas co-precipitated endogenous p300 was detected using a p300-antibody (B). Relative Co-IP signals are shown in red. The HA-p300-1 signal in panel (A) was expressed relative to lane 1, which was set to 1. Relative Co-IP and input signals for endogenous p300 in panel (B) were each expressed relative to lane 2, which was set to 1. Expression of the indicated proteins was confirmed in the input lysates. GAPDH and Tubulin served as loading controls. Created with BioRender.

4.2 HPV8-E6-dependent modulation of the p300 interactome

4.2.1 TurboID proteomics to identify proteins modulated proximal to p300-1

The overexpression of p300-1 (aa 2-439) or p300-4 (aa 1453-1888) suppressed UV-induced skin tumorigenesis in K14-HPV8-E6 transgenic mice (M. Hufbauer, B. Akgül, personal communication). Based on these *in vivo* findings, we propose that HPV8-E6 alters the global p300 interaction network which results in a disruption of p300-dependent skin homeostasis, thereby promoting skin tumorigenesis.

To identify cellular factors whose proximity to p300-1 and p300-4 is altered by HPV8-E6, PCR fragments encoding p300-1 (aa 2-439) or p300-4 (aa 1453-1888) were cloned into the 3xHA-TurboID-NLS_pcDNA3 vector as described by Branon et al. (2018). HEK293 cells were transiently co-transfected either with the fusion constructs TurboID-p300-1 or TurboID-p300-4 and FLAG-HPV8-E6 in three independent experiments. TurboID empty vector served as control. To include the effect of UV on the p300 interactome, one set of transfected cells was irradiated once in a UV crosslinker at 15 mJ/cm². Expression of the TurboID-p300-4 fusion protein did not produce reliable mass spectrometric data due to consistently inducing severe cytotoxicity, with the majority of cells dying within 24–48 hours post-transfection. Thus, we focused only on the results obtained with TurboID-p300-1 for our following analyses.

As shown in Fig. 9A, we confirmed efficient expression of the TurboID-p300-1 fusion protein and a robust biotin labeling activity, supported by the detection of multiple biotin-labeled proteins, including the TurboID-EV (Fig. 9A, lane 1-2 and 5-6) and TurboID-p300-1 (Fig. 9A, lane 3-4 and 7-8) proteins. Enrichment of the labeled proteins was performed using Streptavidin-beads, followed by mass spectrometry (MS) in collaboration with the CECAD Proteomics Core Facility (University of Cologne), and downstream statistical analysis in collaboration with Dr. Martin Pirkl (Institute of Virology).

The quality and reproducibility of the proteomics data were assessed by performing a Principal component analysis (PCA), based on log₂-transformed peptide intensities. PCA analysis revealed that the biological replicates generally clustered within their respective experimental groups (Fig. 9B). When comparing TurboID-p300-1 samples with and without HPV8-E6 co-expression (112 vs 111), we observed a clear separation between the two groups (Fig. 9B). A similar but less pronounced separation was observed with UV treated samples (116 vs 115). In contrast, TurboID-EV samples with and without HPV8-E6 co-expression either with (113 vs. 114) or without UV

treatment (109 vs. 110) did not show clear clustering (Fig. 9B). Overall, HPV8-E6 co-expression altered TurboID-mediated proximity labeling of p300-1 more clearly than that of TurboID-EV.

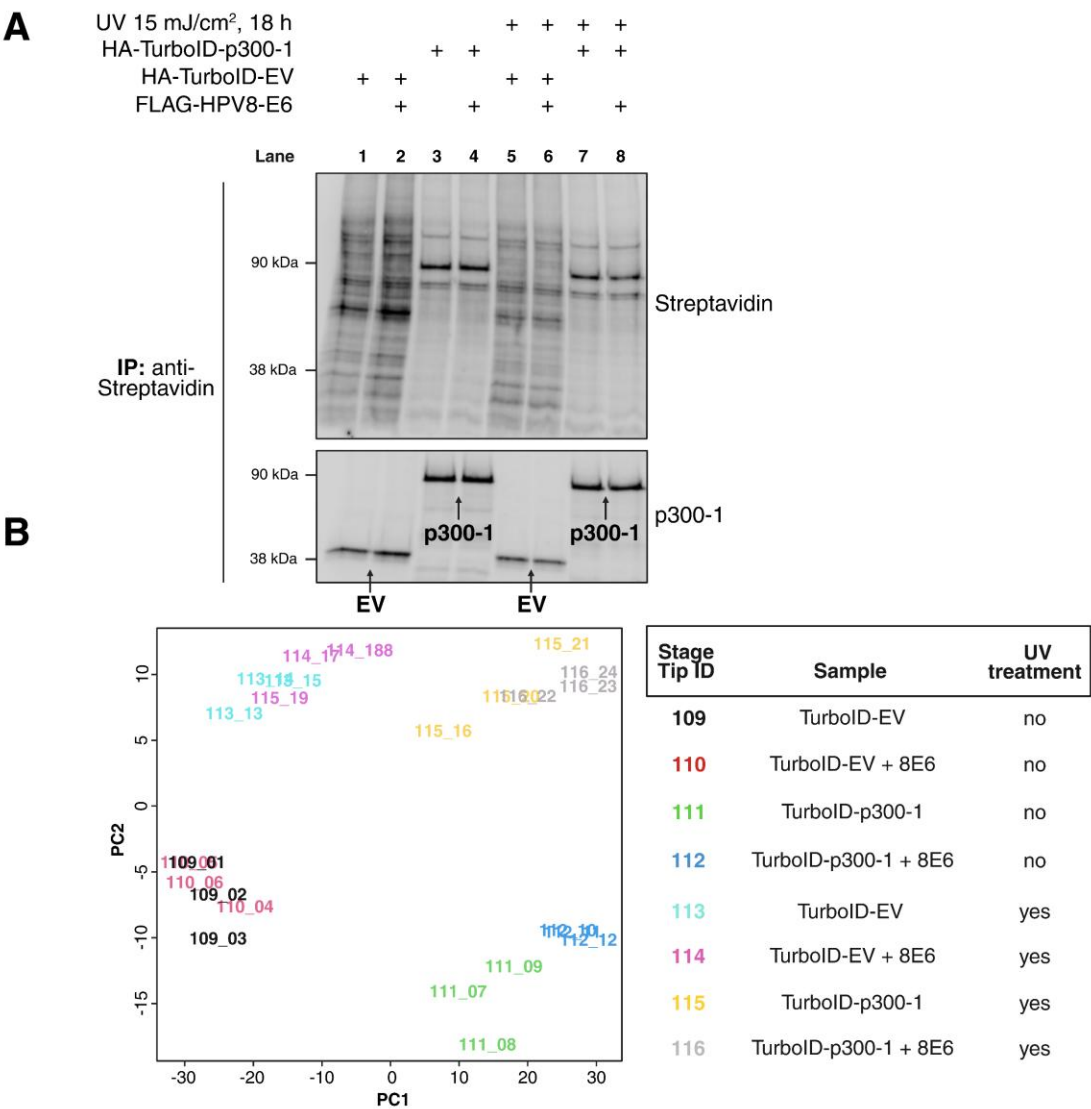


Figure 9: Quality control of TurboID-p300-1 proximity-labeling samples. (A) HEK293 cells were transiently co-transfected with HA-TurboID-p300-1 and FLAG-HPV8-E6 and exposed to UV as indicated. Biotinylated proteins were enriched from whole-cell lysates (1 mg) using magnetic Streptavidin beads and detected by Western blotting using the IRDye® 800CW anti-Streptavidin antibody. Expression of the TurboID constructs was detected using an HA-tag antibody. **(B)** PCA matrix showing clustering of proteomics samples. The table on the right summarizes the sample IDs and corresponding experimental conditions. PCA analysis and the original plot were generated by M. Pirkl and adapted for presentation using BioRender. EV = empty vector, PC = principal component.

To characterize the p300-1 interactome, log₂ peptide intensities of samples expressing TurboID-p300-1 with and without HPV8-E6 co-expression were compared with those of the corresponding empty vector controls. Proteins displaying only one peptide were removed from the list. In total

we identified 1,806 protein entries as significantly altered across all comparisons (FDR < 0.1, > 2-fold difference). Of these, the top 20 proteins from each comparison were sorted by their fold change and false discovery rate (FDR), and plotted on a heatmap to illustrate enrichment or reduction proximal to p300-1 (Fig. 10).

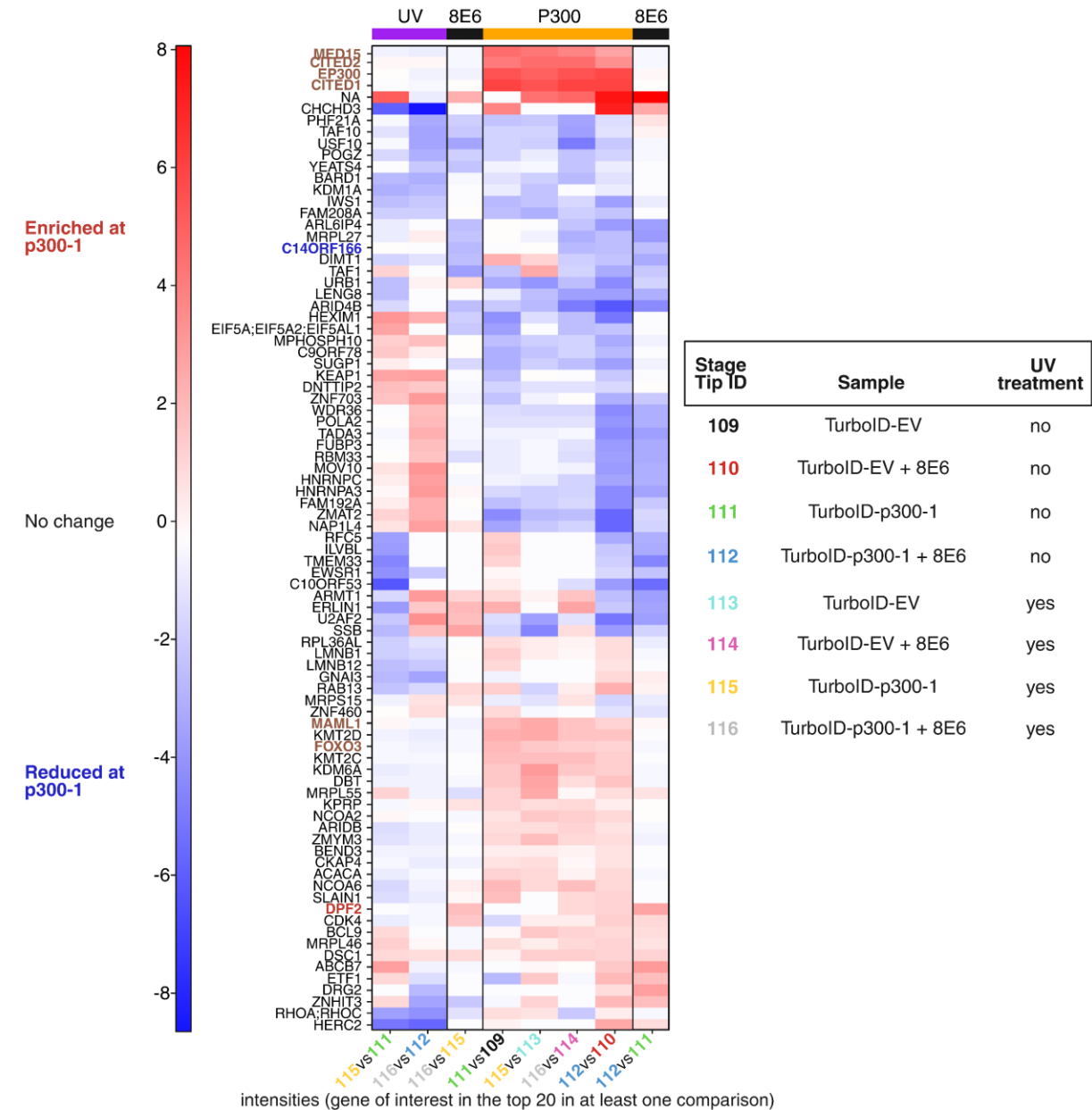


Figure 10: Comparison of protein enrichment in TurboID-p300-1 samples. Shown are the peptide LFQ intensities of proteins ranked among the top 20 hits in all comparisons. Each column represents one comparison. The experimental conditions and the corresponding sample IDs are summarized in the table on the right. Black boxes indicate the comparisons between TurboID-p300-1 samples with and without HPV8-E6 under untreated (112 vs. 111) and UV-treated (116 vs. 115) conditions. Statistical analysis and heatmap generation were performed by M. Pirkl and adapted for presentation using BioRender. EV = empty vector, ID = identifier, LFQ = label-free quantification.

Comparison of protein lists obtained from p300-1-expressing cells and empty vector controls (111 vs. 109) revealed already well-characterized transcriptional co-activators which interact with p300 independent of HPV8-E6, including CITED1/2, MAML1, and FOXO3 (Yazici and McIntyre, 2025). This confirmed that our experimental approach generated biologically plausible proteomics data. In the presence of HPV8-E6, we identified 56 proteins in untreated (112 vs. 111) and 11 proteins in UV-treated samples (116 vs. 115). The comparison 112 vs. 111 is summarized in a volcano plot (Fig. 11A), whereas a Venn diagram was used to show the 67 protein hits obtained when comparing 112 vs. 111 and 116 vs. 115 (Fig. 11B).

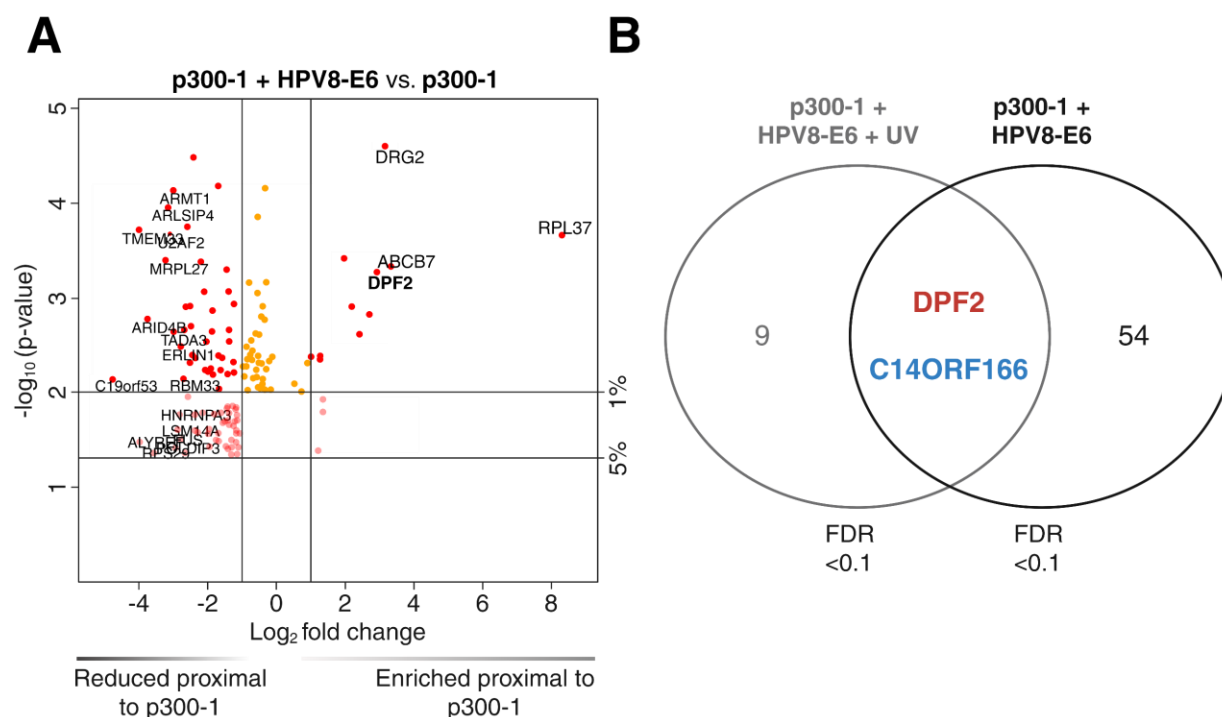


Figure 11: HPV8-E6 affects protein enrichment in TurboID-p300-1 samples. (A) Volcano plot showing significantly altered proteins proximal to p300-1 in the presence of HPV8-E6. The log₂ fold change on the x-axis is plotted against the -log₁₀(p-value) on the y-axis. Each point represents one protein. Selected significant proteins are highlighted according to the defined threshold ($|\log_2 \text{fold change}| \geq 1$, FDR < 0.1). Vertical lines indicate the fold change threshold. Horizontal lines indicate unadjusted p-value thresholds of 0.05 and 0.01. **(B)** Venn diagram showing significantly modulated proteins proximal to p300-1 upon HPV8-E6 co-expression with and without UV treatment. C14ORF166 was reduced (blue), while DPF2 was enriched (red) under both conditions. Statistical analysis and the original volcano plot were generated by M. Pirkl and adapted for presentation using BioRender. FDR = false discovery rate.

Under both experimental conditions, HPV8-E6 consistently reduced C14ORF166 and enriched DPF2 proximal to p300-1 (Fig. 11B). To obtain a functional overview of these 65 distinct proteins, we submitted their UniProt IDs to ShinyGO v0.85 and performed Gene ontology and KEGG enrichment analyses. KEGG pathway analysis revealed an enrichment of proteins involved in

DNA mismatch-repair pathways as well as DNA replication (Fig. 12A). As shown in Fig. 12B, when applying the term “molecular function” we identified the strongest enrichment for proteins participating in (lysine) acetylated histone binding. Together, these data suggest that HPV8-E6 modulates p300-specific functions related to these pathways.

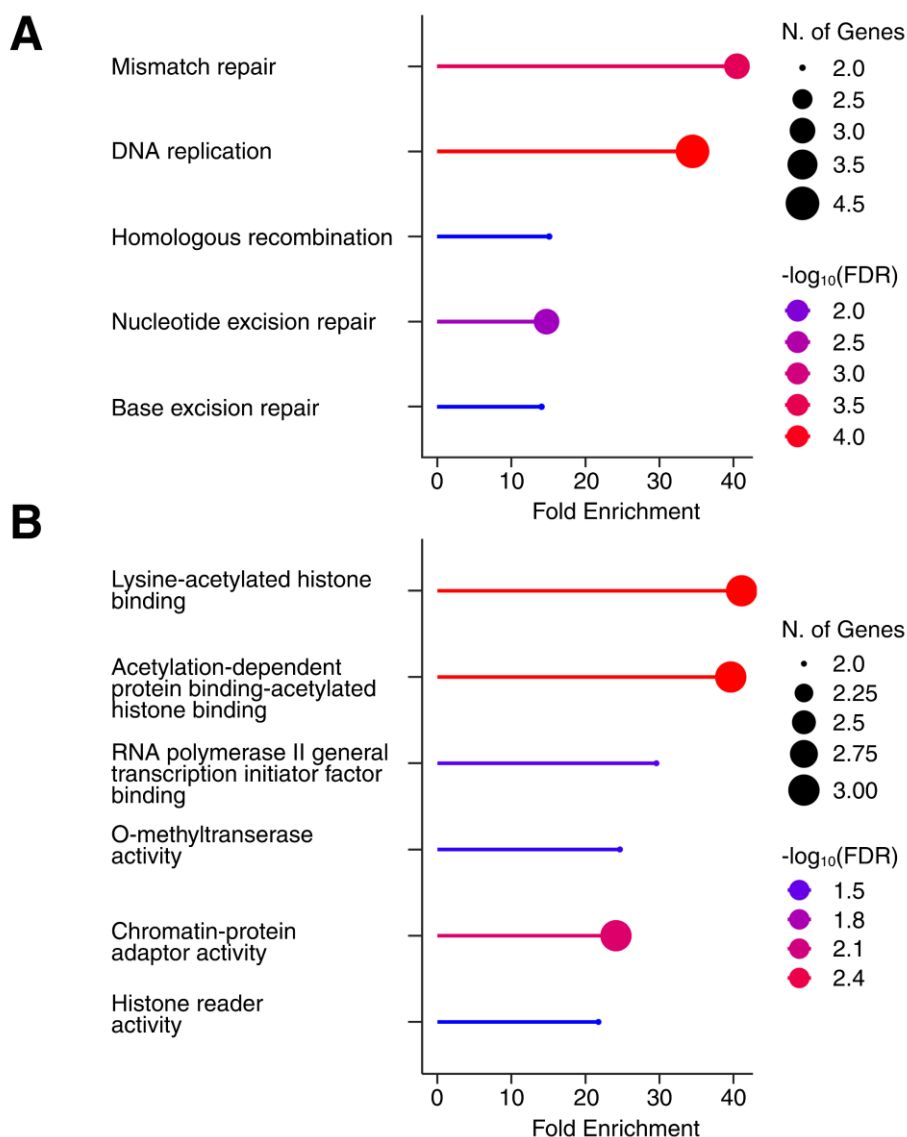


Figure 12: Functional enrichment analysis of proteins enriched proximal to p300-1 upon HPV8-E6 expression. Shown are the top enriched KEGG (A) and Gene Ontology (GO) molecular function terms (B). Of the 67 significantly altered proteins across both comparisons, 65 represented distinct proteins, whose UniProt IDs were mapped to 63 human genes and used for enrichment analysis with ShinyGO v0.85. An FDR below 0.05 and pathway size thresholds of 5–100 for KEGG (A), and 8–50 genes for GO (B) were used. Fold enrichment is shown on the x-axis. Dot size indicates the number of associated proteins, and dot color represents the significance level ($-\log_{10}(\text{FDR})$). Created with BioRender.

4.2.2 DPF2 interacts with endogenous p300

GO analysis revealed an enrichment of proteins binding to lysine-acetylated histones, whose association proximal to p300-1 was altered by HPV8-E6 (Fig. 12). Since the epigenetic reader DPF2 was consistently identified as enriched proximal to p300-1 in the presence of HPV8-E6, we selected it for further analysis.

DPF2 is a ubiquitously expressed histone reader subunit of the CBAF complex (Mashtalir et al., 2018). Its tandem PHD finger recognizes H3 and H4 acetylation and thus contributes to regulation of lineage-specific promoter regions (Huber et al., 2017) as well as p53-dependent transcription (Hyun et al., 2024). BAF complexes contribute to various biological processes, including cell proliferation, differentiation, DNA damage repair, and the immune response, by regulating chromatin accessibility and gene expression (Malone and Roberts, 2024). Several studies have demonstrated that p300 and DPF2 colocalize at active enhancer regions through physical interaction (Alver et al., 2017, Zhang et al., 2019).

Since TurboID-based proximity proteomics identified DPF2 as enriched proximal to p300-1, we wanted to assess a potential physical interaction by performing Co-IP followed by Western blotting. Human N/Tert^{EV} and N/Tert^{HPV8-E6} cells (generated as described in 3.3.6) were used to investigate the DPF2/p300 interaction. The N/Tert cell line has been widely used as a model for studying HPV functions in keratinocytes (White et al., 2012).

Endogenous p300 was precipitated from whole-cell lysates of N/Tert^{EV} and N/Tert^{HPV8-E6} cells, treated with UV or left untreated. Western blotting revealed co-precipitation of DPF2 in both cell lines independent of UV irradiation (Fig. 13A, B). Quantification of DPF2 relative to precipitated p300 was comparable in untreated N/Tert^{EV} and N/Tert^{HPV8-E6} cells. However, normalized DPF2/p300 Co-IP signals were significantly increased in HPV8-E6-expressing cells after UV treatment (Fig. 13B).

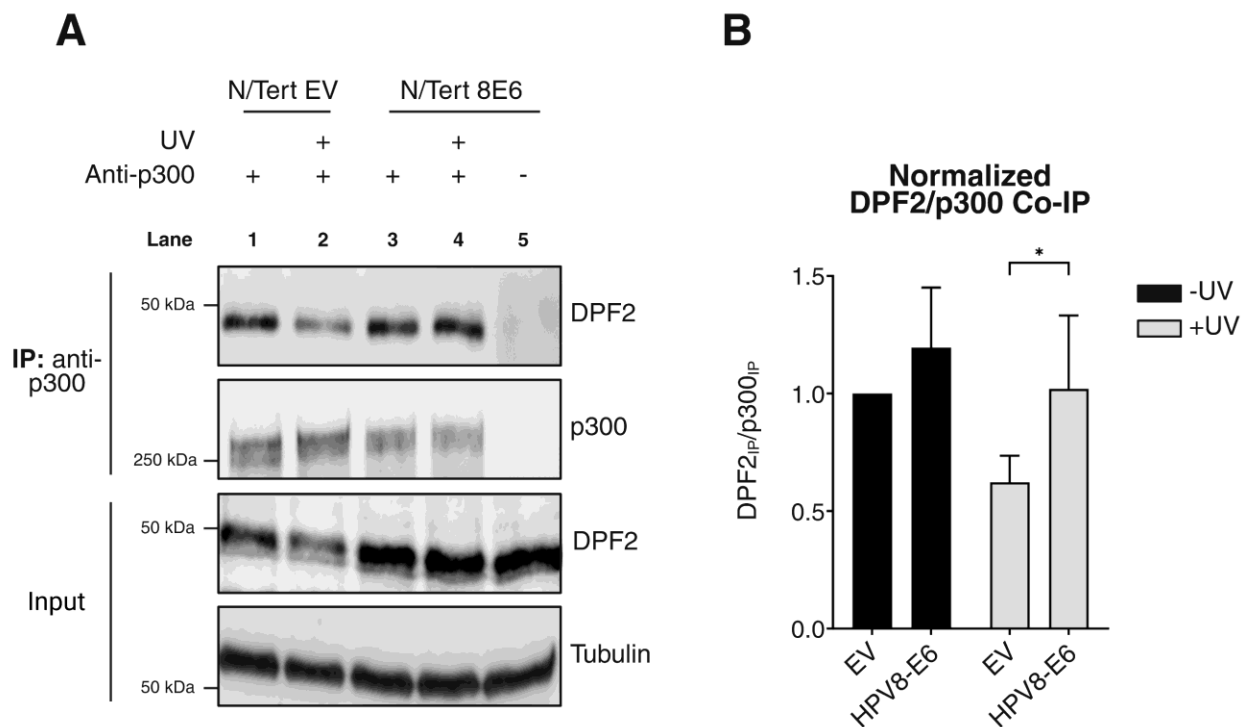


Figure 13: HPV8-E6 stabilizes the interaction between DPF2 and p300. (A) Whole-cell lysates of N/Tert cells stably expressing pcDNA-FLAG-EV or pcDNA-FLAG-HPV8-E6 with or without UV treatment were used to precipitate p300. Co-precipitated DPF2 was detected by WB using a DPF2 antibody. DPF2 expression was confirmed in the input lysates. Tubulin served as a loading control. Lane 5 served as a negative control without p300 antibody. **(B)** DPF2 IP signals were normalized to DPF2 input and subsequently to precipitated p300. Error bars represent the sample standard deviation (mean $\pm$ SD, $n = 2$ biological replicates). Statistical significance was determined using an unpaired t-test. $p < 0.05$ (*). Created with BioRender.

To characterize the physical interaction between DPF2 and p300-1 in more detail, we co-transfected pcDNA3.1-FLAG-DPF2 (Huber et al., 2017), HA-p300-1, and MYC-HPV8-E6 into C33A cells, followed by Co-IP and Western blotting. FLAG-IPs showed a strong co-precipitation of HA-p300-1 with FLAG-DPF2 in the presence of HPV8-E6 (Fig. 14A, lane 4). FLAG-DPF2 also co-precipitated with MYC-HPV8-E6 in the absence of p300-1 (Fig. 14A, lane 5). MYC-IPs confirmed a physical interaction between HPV8-E6 and DPF2, even in the absence of p300-1 (Fig. 14B, lane 6).

HA-IPs further confirmed co-precipitation of FLAG-DPF2 with HA-p300-1 also in the absence of HPV8-E6 (Fig. 14C, lane 9). In addition, HA-IPs revealed that FLAG-DPF2 is co-precipitated with HA-p300-1.9 (aa 332-390; Fig. 14C, lane 8). These data suggest that DPF2, p300-1 and HPV8-E6 form a ternary complex, which is stabilized by HPV8-E6. Interestingly, it appears that DPF2 and HPV8-E6 both interact with the p300 residues 332-390.

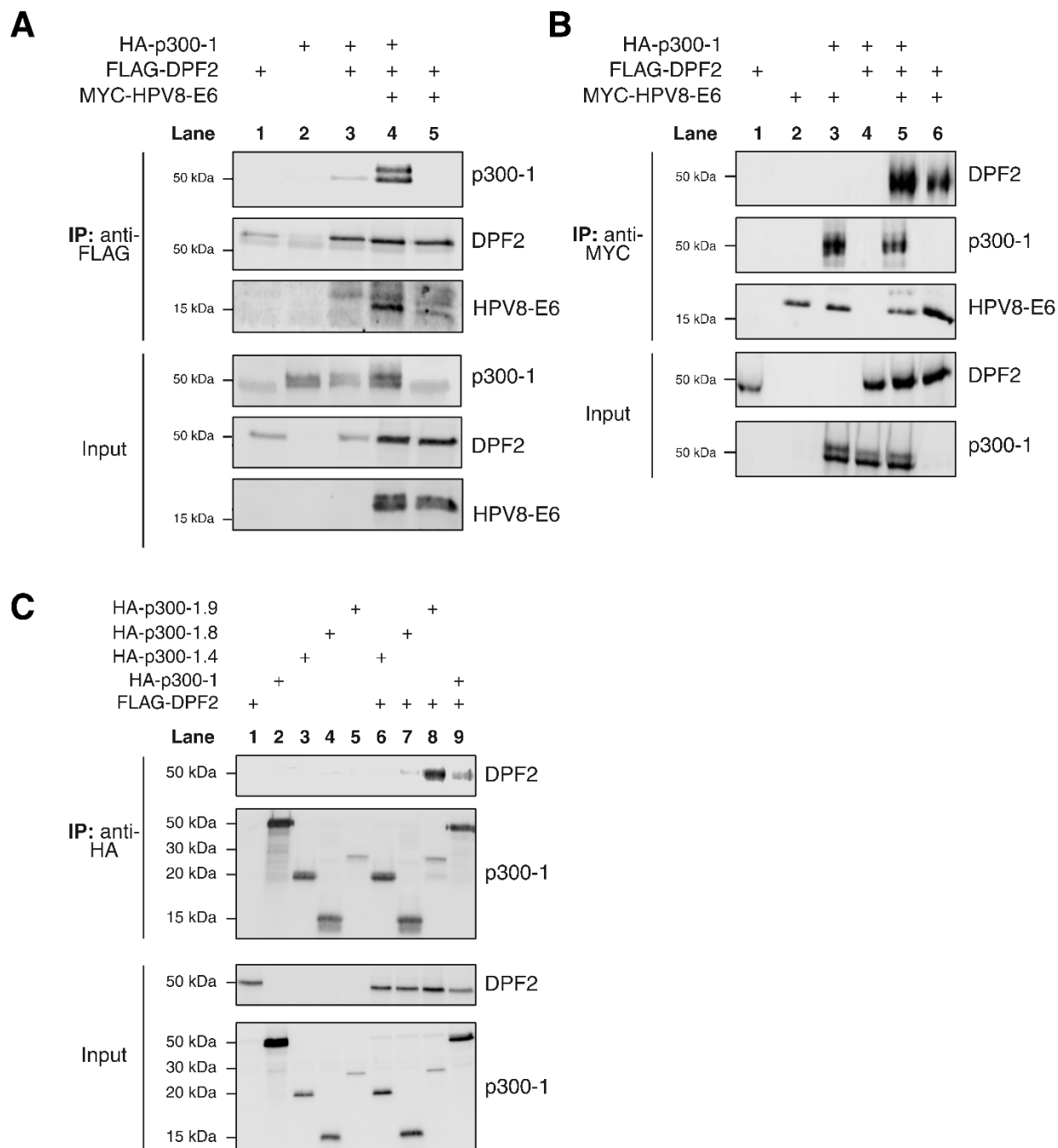


Figure 14: DPF2 interacts with p300-1 and HPV8-E6 in a ternary complex. C33A cells were co-transfected with FLAG-DPF2, HA-p300-1 and MYC-HPV8-E6 as indicated. Whole-cell extracts were used for **(A)** FLAG-IPs to precipitate FLAG-DPF2, **(B)** MYC-IPs to precipitate MYC-HPV8-E6, and **(C)** HA-IPs to precipitate HA-p300-1 and the indicated p300-1 fragments. Co-precipitated DPF2, p300-1, and HPV8-E6 were analyzed as indicated. Expression of the recombinant proteins was confirmed in the input lysates. Created with BioRender.

To investigate colocalization of endogenous p300 and DPF2, we performed immunofluorescence (IF) in N/Tert^{EV} and N/Tert^{HPV8-E6} keratinocytes as indicated (Fig. 15A). As reported previously, p300 was mainly distributed within the nucleus. Some nuclei, however, displayed heterogeneous regions with increased p300 intensity, which is in agreement with previous observations. It has been suggested that these focal accumulations of p300 represent discrete compartments with elevated protein concentrations (Zhang et al., 2021).

As expected, DPF2 was also mainly localized in the nucleus. However, some cells showed distinct spots within the nucleoplasm and cytosol. Similar observations have been reported in U2OS cells (Human Protein Atlas). As shown in Fig. 15A, colocalization of DPF2 and p300, indicated by the yellow fluorescence signal, was more pronounced in N/Tert^{HPV8-E6} cells than in N/Tert^{EV} cells.

To quantify the degree of colocalization between DPF2 and p300, Pearson's *r* coefficient was determined for 30 individual nuclear regions of interest (ROI) in both cell lines (Adler and Parmryd, 2010, Mukaka, 2012, McDonald and Dunn, 2013). HPV8-E6-expressing cells showed significantly higher Pearson's *r* (0.43) compared with cells expressing empty vector (0.35) (Fig. 15B). Line scan analysis of a representative nuclear ROI showed more overlapping DPF2 and p300 intensity peaks in N/Tert^{HPV8-E6} cells (Fig. 15C). These results suggest an enhanced nuclear colocalization of p300/DPF2 in the presence of HPV8-E6.

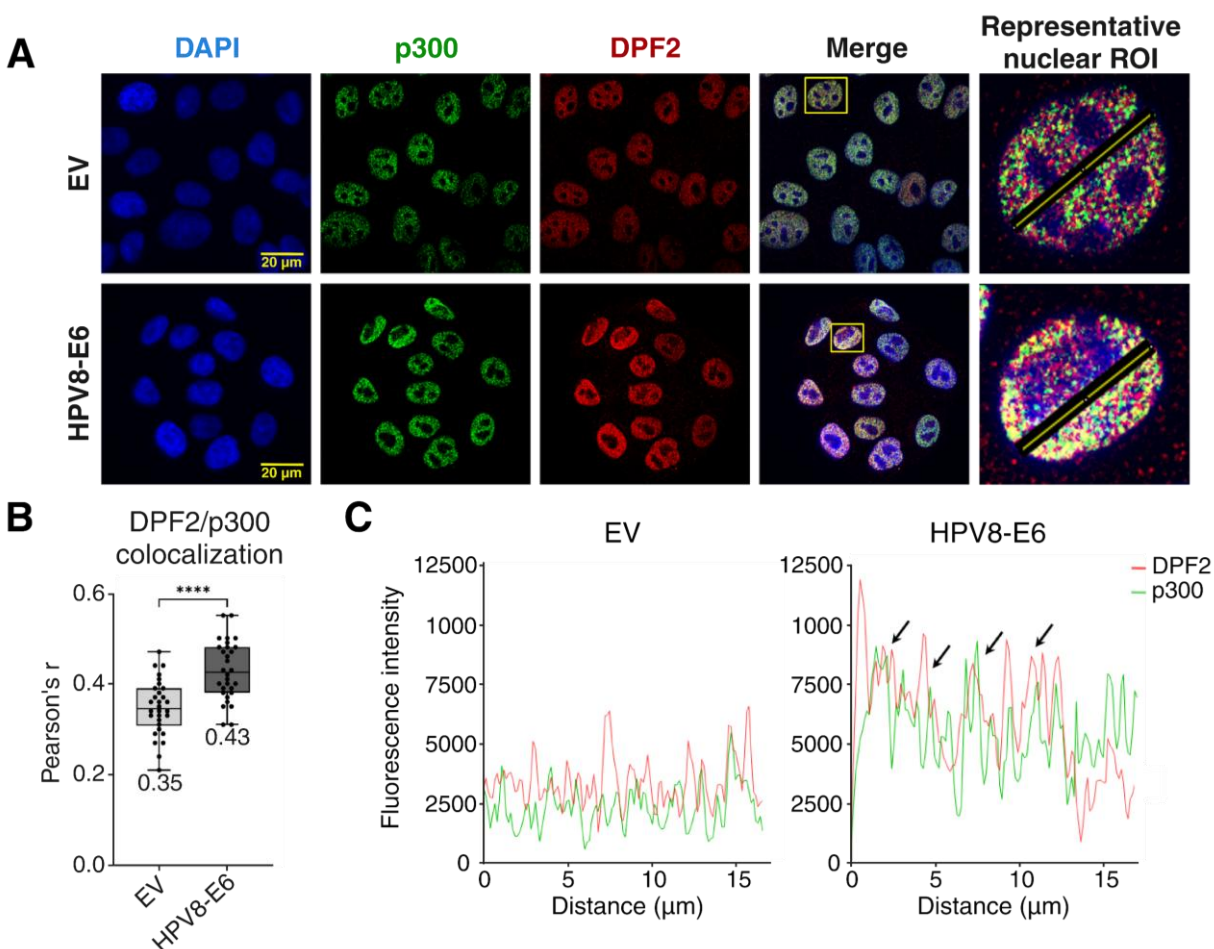


Figure 15: HPV8-E6 increases nuclear colocalization of DPF2 and p300. (A) Representative IF images of N/Tert^{EV} and N/Tert^{HPV8-E6} keratinocytes stained for p300 (green) and DPF2 (red). Nuclei were counterstained with DAPI (blue). Overlapping DPF2 and p300 signals appear yellow. Scale bars: 20 μm. (B) Pearson's r was determined for 30 independent nuclear ROIs. Each dot represents one nuclear ROI. Horizontal lines represent the median, and whiskers indicate the minimum and maximum values. Statistical significance was assessed using an unpaired two-tailed Welch's t-test. $p < 0.0001$ (****). (C) Representative fluorescence intensity line scans of p300 and DPF2 along the lines as indicated in (A). Arrows indicate regions with overlapping p300 and DPF2 intensity peaks in HPV8-E6-expressing cells. Graphs were generated using GraphPad Prism, and the figure was assembled for presentation using BioRender. ROI = region of interest.

4.2.3 Analysis of DPF2 expression in HPV8-E6-driven skin tumors

To assess DPF2 expression in HPV8-E6-driven skin tumors, paraffin-embedded tumor sections from K14-HPV8-E6 transgenic mice obtained 24 days post UV irradiation were analyzed for DPF2 expression by immunohistochemical staining using 3,3'-diaminobenzidine (DAB). DPF2 was detected throughout the tumor tissue both in basal cells and suprabasal layers with a predominantly nuclear localization within keratinocytes (Fig. 16). This is consistent with previous studies, reporting DPF2 as a ubiquitously expressed protein (Huber et al., 2017).

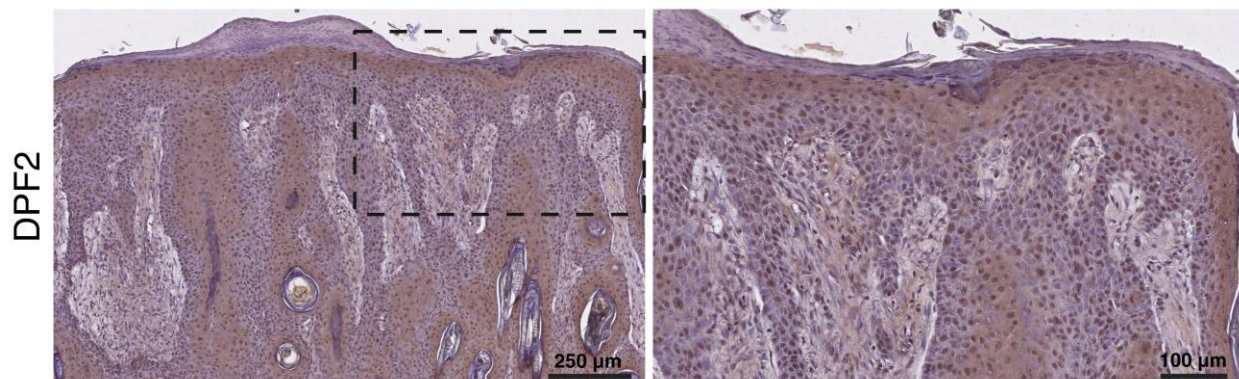


Figure 16: DPF2 expression in skin tumors of K14-HPV8-E6 transgenic mice. Representative images of tumor biopsies obtained from K14-HPV8-E6 transgenic mice 24 days post UV irradiation stained for DPF2. DAB (brown) indicates DPF2-positive staining, nuclei were counterstained with hematoxylin (purple). Magnification: 50x (left), 100x (right). Created with BioRender.

4.3 Investigating the downstream effects of the DPF2/p300 interaction

4.3.1 CRISPR-Cas9-mediated DPF2 knockout in N/Tert^{HPV8-E6} cells

Recently, our group demonstrated that HPV8-E6 modulates cellular gene expression (Hufbauer et al., 2026). Since our previous experiments identified DPF2 as a p300-1-interacting protein whose association is enhanced by HPV8-E6, we next investigated whether DPF2 contributes to HPV8-E6-dependent transcription. To this end, DPF2 was knocked out in human N/Tert^{EV} and N/Tert^{HPV8-E6} keratinocytes using the CRISPR-Cas9 system.

Two different guide RNAs (gRNA1 and gRNA2) were cloned into the pSpCas9(BB)-2A-Puro V2.0 expression plasmid (Ran et al., 2013). In addition to the expression cassette encoding the corresponding gRNA, the plasmid also encodes the Cas9 nuclease as well as a puromycin-resistance gene for selection. Cas9-mediated cleavage introduces double-strand breaks at the target locus, which are repaired by error-prone non-homologous end joining, ultimately resulting in gene knockout (KO).

Cas9-Puro-gRNA constructs were delivered into the cells by electroporation. After puromycin selection single clones were isolated and the DPF2^{KO} was validated via Sanger sequencing, using primers flanking the corresponding target region (Sequencing data is not shown). While DPF2^{KO} clones were generated with both gRNAs in N/Tert^{HPV8-E6} cells, only gRNA2-derived clones were obtained in N/Tert^{EV} cells. Therefore, two independent N/Tert^{EV} DPF2^{KO} clones (#2-1 and #2-2) and one N/Tert^{HPV8-E6} DPF2^{KO} clone for each gRNA (#1 and #2) were used for further analysis. Western blotting confirmed DPF2^{KO} for both cell lines at the protein level (Fig. 17).

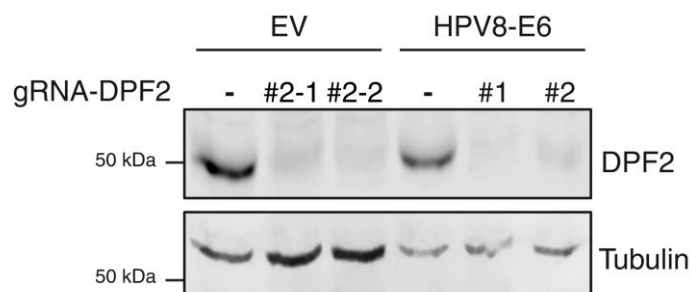


Figure 17: Validation of DPF2^{KO} in N/Tert^{EV} and N/Tert^{HPV8-E6} cells. Two independent EV DPF2^{KO} clones obtained with gRNA2 (#2-1, #2-2), as well as HPV8-E6 DPF2^{KO} clones obtained with gRNA1 (#1) and gRNA2 (#2) were detected by Western blotting (40 µg) using a DPF2 antibody. Tubulin served as a loading control. Created with BioRender. EV = empty vector, gRNA = guide RNA.

4.3.2 Loss of DPF2 alters HPV8-E6-dependent transcription

To evaluate the effect of DPF2^{KO} on HPV8-E6-dependent transcription, RNA was isolated in triplicate from untreated or UV-irradiated N/Tert^{HPV8-E6} keratinocytes expressing DPF2^{WT} or DPF2^{KO} (gRNA2, clone #2). RNA-seq analysis was performed in collaboration with the Cologne Center for Genomics (CCG). Since we had not yet succeeded in establishing DPF2^{KO} in N/Tert^{EV} cells at that time, these cells were not included in the RNA-seq analysis. Instead, we focused on the effect of DPF2 loss in HPV8-E6-expressing keratinocytes. PCA analysis showed clear clustering of the three biological replicates within their respective experimental groups according to DPF2 status and UV treatment (Fig. 18).

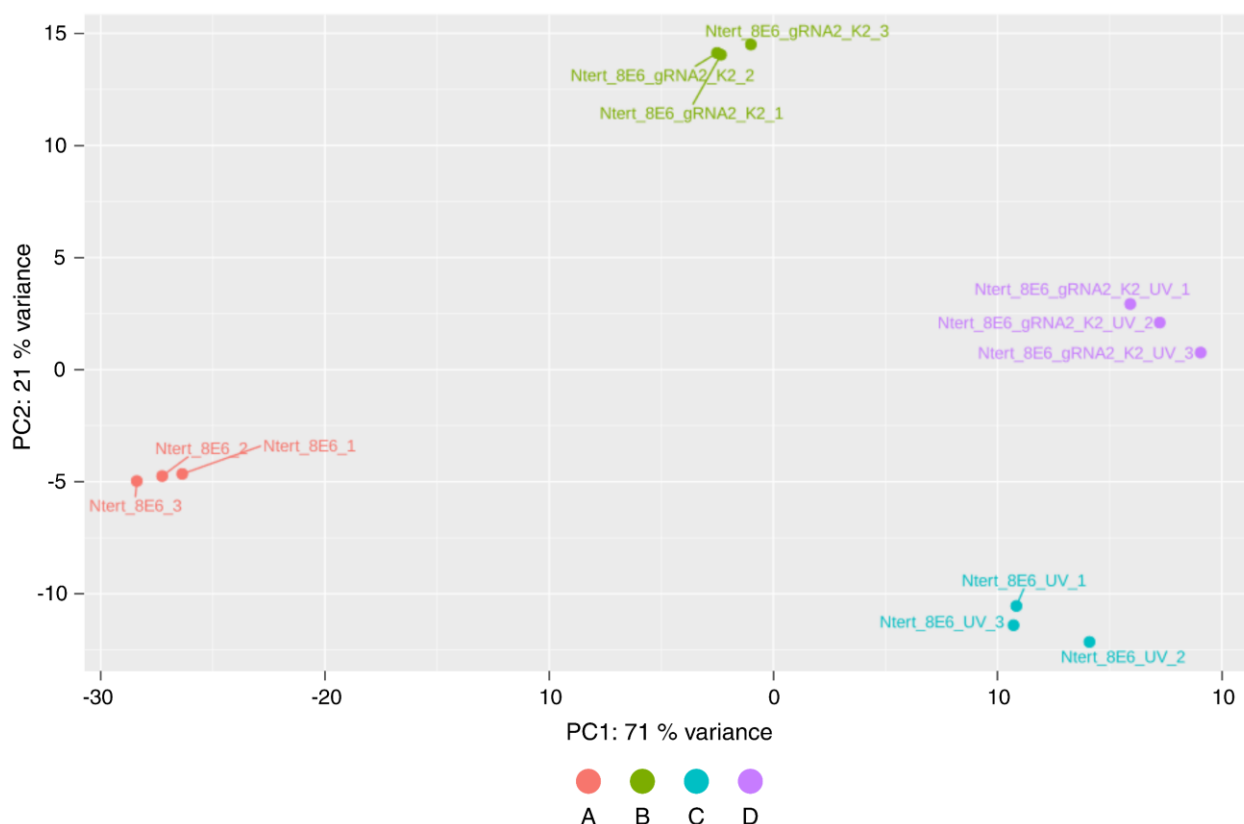


Figure 18: PCA matrix of RNA-seq results. Original sample identifiers assigned during RNA-seq processing are retained in the PCA plot. Group A (orange) N/Tert^{HPV8-E6}; Group B (green) N/Tert^{HPV8-E6} DPF2^{KO} clone #2; Group C (turquoise) N/Tert^{HPV8-E6} + UV; Group D (lilac) N/Tert^{HPV8-E6} DPF2^{KO} clone #2 + UV. RNA-seq data analysis and PCA generation were performed by R. Remy. The PCA plot was adapted for presentation using BioRender. K = clone, PC = principal component, UV = ultraviolet irradiation.

In total, 1,613 genes showed an FDR < 0.005, of which 437 additionally met the $|\log_2\text{FC}| \geq 2$ cutoff and were therefore considered differentially expressed. To investigate DPF2-dependent regulation, genes displaying the strongest transcriptional changes upon DPF2 loss were ranked according to their $\log_2$ fold change. Among the most strongly DPF2-dependent genes, MMP7, IL33, GABRP, and MUC4 were significantly reduced upon DPF2 loss, whereas CDH4 expression was significantly increased (Fig. 19A).

For KEGG enrichment analysis, all 1,613 genes with an FDR < 0.005 were analyzed using ShinyGO v0.85. Enriched pathways included extracellular matrix (ECM)-receptor interaction, transcriptional misregulation in cancer, and cell cycle (Fig. 19B).

To identify HPV8-E6-regulated genes that also depend on DPF2, we compared our RNA-seq data from N/Tert^{HPV8-E6} cells with a newly generated dataset from N/Tert^{EV} cells (G. Steger, personal communication). In total, 179 genes were identified as regulated by both HPV8-E6 and DPF2

($|\log_2FC| \geq 2$). HPV8-E6 induced MMP7, MUC4, and GABRP expression, while reducing CDH4. Consistently, DPF2 loss reduced MMP7, MUC4, and GABRP, whereas CDH4 expression was increased (Fig. 19C). Together, these findings indicate that DPF2 contributes to the transcriptional regulation of selected HPV8-E6 target genes.

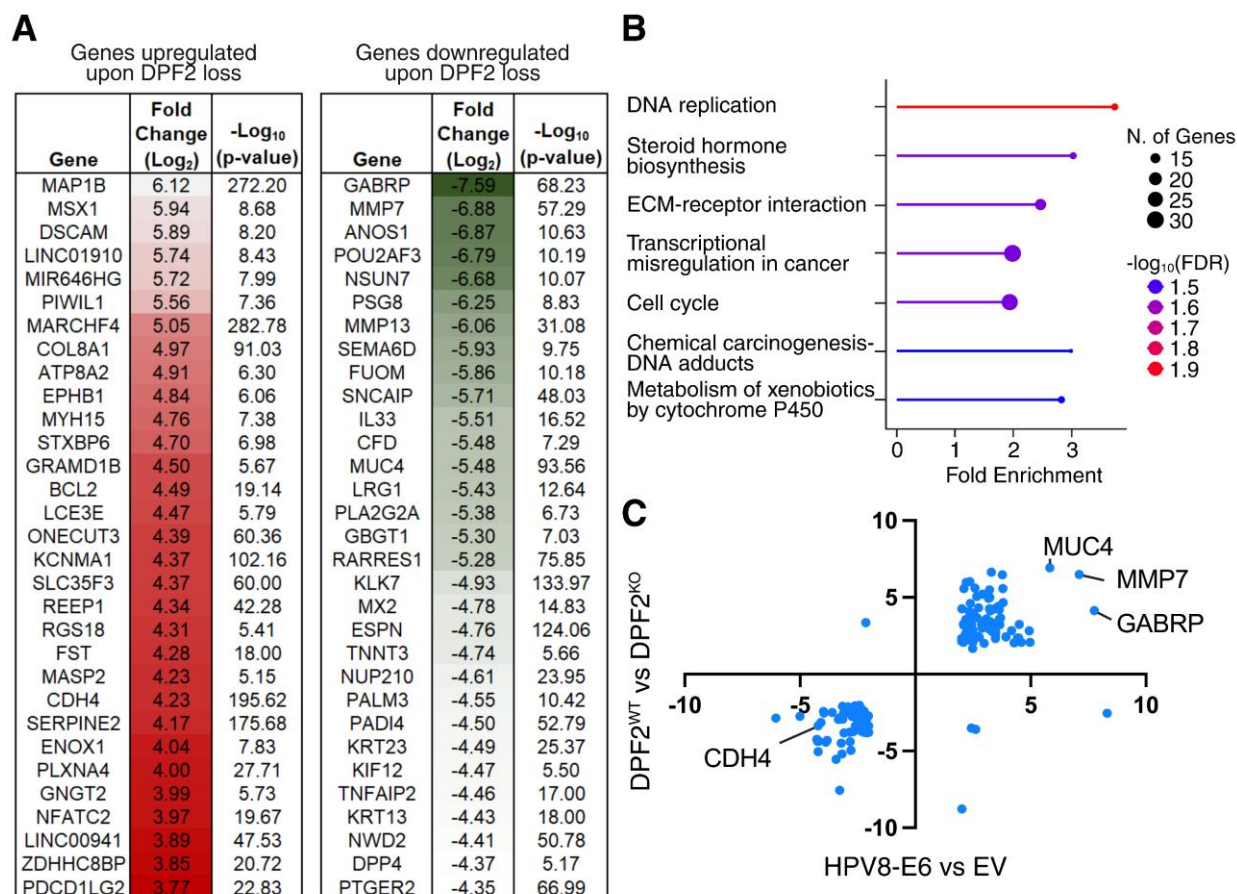


Figure 19: DPF2 loss alters gene expression in HPV8-E6-expressing N/Tert cells. (A) Top DPF2-regulated genes in HPV8-E6-expressing cells are indicated. Downregulation upon DPF2 loss is shown in green, whereas upregulation is shown in red. (B) All genes with an FDR < 0.005 from the DPF2^{WT} versus DPF2^{KO} comparison were used as input, while all genes detected in the RNA-seq dataset were used as background. 1,612 Ensembl gene IDs were mapped to 1,612 human genes and used for KEGG enrichment analysis with ShinyGO v0.85. Pathway analysis was performed using an FDR cutoff of 0.05 and pathway size thresholds of 5–300 genes. Fold enrichment is shown on the x-axis, dot size represents the number of genes assigned to each pathway. Color represents significance ($-\log_{10}(\text{FDR})$). (C) Log₂ fold changes in N/Tert^{HPV8-E6} versus N/Tert^{EV} cells (x-axis) were plotted against log₂ fold changes in N/Tert^{HPV8-E6} DPF2^{WT} versus N/Tert^{HPV8-E6} DPF2^{KO} cells (y-axis). RNA-seq data processing and differential expression analysis were performed by R. Remy. Visualizations were generated based on these analyses and adapted for presentation using BioRender. EV = empty vector, FDR = false discovery rate. KO = knockout, WT = wild-type.

To validate the DPF2-dependent transcriptional regulation of selected genes, we compared gene expression in N/Tert^{EV} and N/Tert^{HPV8-E6} cells expressing either DPF2^{WT} or DPF2^{KO}. Two independent DPF2^{KO} clones were analyzed in each cell line. Exon-junction spanning qRT-PCR primers were used as listed in section 2.7.7, Table 17 to assess gene expression of MMP7, CDH4, GABRP, MUC4, KRT13, and IL33.

Consistent with the RNA-seq data obtained in N/Tert^{HPV8-E6} cells, loss of DPF2 strongly reduced MMP7 and KRT13 expression (Fig. 20A). In contrast, CDH4 expression was increased upon DPF2^{KO} in both cell lines, although this effect was weaker in HPV8-E6-expressing keratinocytes. For MUC4 and GABRP, the situation was more complex. While DPF2 loss abolished expression of GABRP and MUC4 in N/Tert^{HPV8-E6} cells, both genes were increased upon DPF2^{KO} in N/Tert^{EV} cells. Due to high Ct values and variability among the biological replicates, DPF2-dependent regulation of IL33 could not be reliably evaluated (Fig. 20A).

To analyze which of these DPF2-regulated genes were also modulated by HPV8-E6, we additionally compared their expression in N/Tert^{EV} and N/Tert^{HPV8-E6} cells. In DPF2^{WT} cells, HPV8-E6 strongly induced MMP7, KRT13, MUC4, and GABRP, while reducing CDH4 expression. Together, these data indicate that DPF2 supports HPV8-E6-mediated transcriptional activation of MMP7 and KRT13, and contributes to transcriptional repression of CDH4. HPV8-E6 expression was validated in DPF2^{WT} and DPF2^{KO} cells.

Both DPF2^{KO} clones showed approximately 50-fold lower HPV8-E6 transcripts than DPF2^{WT} cells (Fig. 20B), suggesting that DPF2 contributes to efficient expression of the pcDNA-FLAG-HPV8-E6 construct. Despite the lower HPV8-E6 expression, we still observed differences in the expression of most analyzed genes when comparing N/Tert^{EV} and N/Tert^{HPV8-E6} DPF2^{KO} cells. However, it cannot be completely ruled out that the lower HPV8-E6 expression may have contributed to the reduced MMP7 expression.

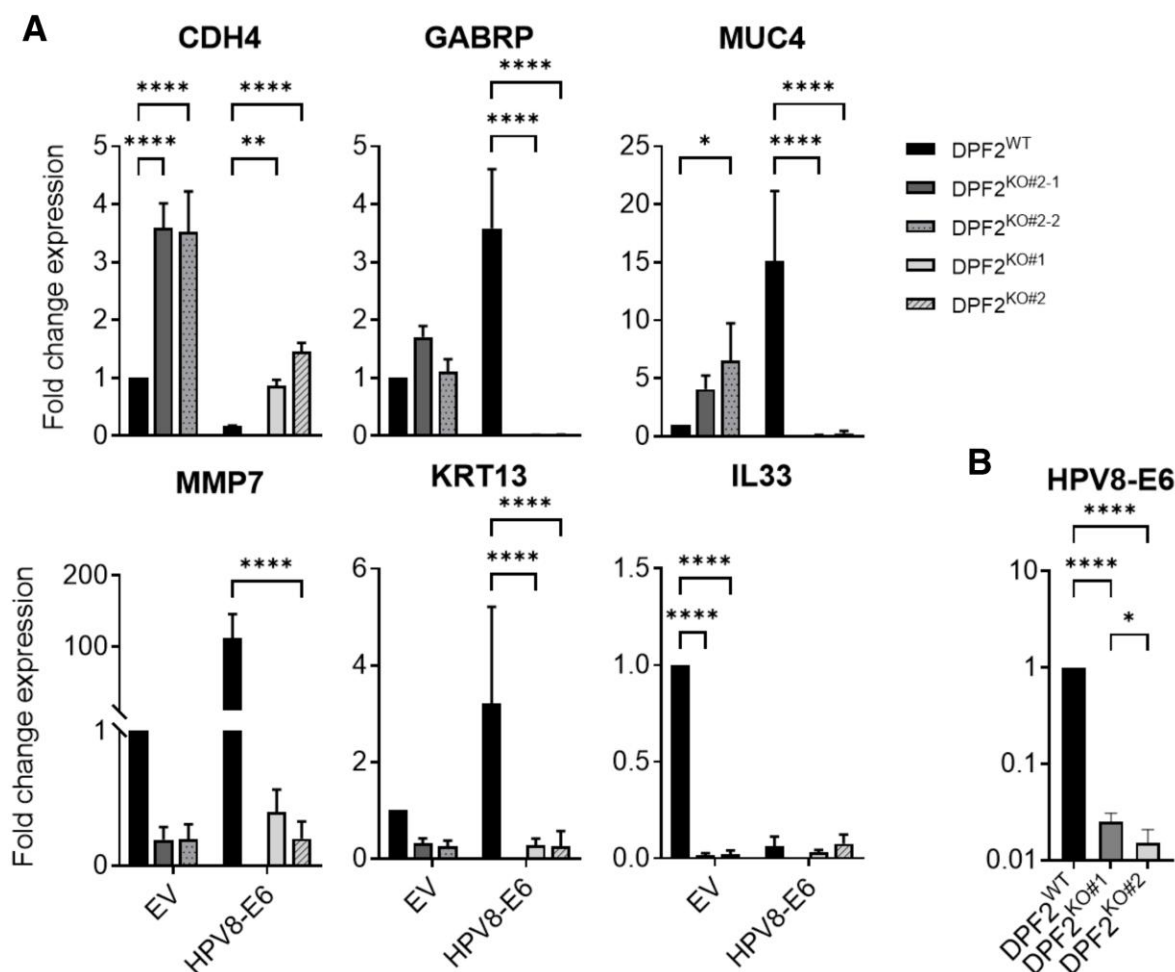


Figure 20: HPV8-E6 alters expression of DPF2-regulated genes. (A) RNA from N/Tert^{EV} and N/Tert^{HPV8-E6} cells expressing DPF2^{WT} or DPF2^{KO} was used to quantify transcription of target genes as indicated. Data are shown as mean $\pm$ SD of $n = 2$ biological replicates, each analyzed in four qRT-PCRs. Statistical significance was determined using a two-way ANOVA followed by Tukey's multiple comparisons test. **(B)** HPV8-E6 transcript levels were analyzed in DPF2^{WT} cells and DPF2^{KO} clones. Gene expression is shown relative to DPF2^{WT} on a logarithmic y-axis. Statistical significance was determined using an ordinary one-way ANOVA followed by Tukey's multiple comparison test. $p < 0.05$ (*), $p < 0.01$ (**), $p < 0.001$ (***), $p < 0.0001$ (****). Graphs were generated using GraphPad Prism and adapted for presentation using BioRender. EV = empty vector, KO#1 = gRNA1, clone #1, KO#2 = gRNA2, clone #2, KO#2-1 = gRNA2, clone #2-1, KO#2-2 = gRNA2, clone #2-2, WT = wild-type.

To address whether the loss of DPF2 affected gene expression in general, FPKM values of selected genes were compared using the RNA-seq data. While DPF2 and MMP7 expression was strongly reduced upon DPF2 loss, EP300 and the housekeeping gene HPRT1 showed only moderate changes (Fig. 21). This indicates that despite the lower HPV8-E6 expression, DPF2 loss selectively affected gene expression instead of causing a general transcriptional repression.

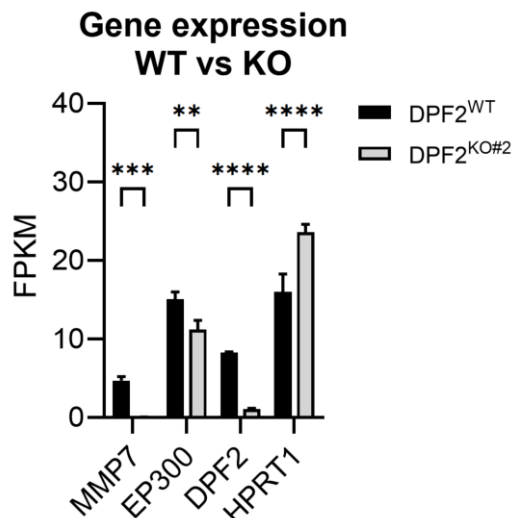


Figure 21: Expression of selected genes in DPF2^{WT} and DPF2^{KO} cells. FPKM values of MMP7, EP300, DPF2 and HPRT1 were taken from the RNA-seq count tables of N/Tert^{HPV8-E6} cells expressing DPF2^{WT} or DPF2^{KO}. Data are shown as mean $\pm$ SD of $n = 3$ biological replicates. Statistical significance was assessed using an ordinary two-way ANOVA followed by Šídák's multiple comparisons test. $p < 0.05$ (*), $p < 0.01$ (**), $p < 0.001$ (***), $p < 0.0001$ (****). The graph was generated using GraphPad Prism and adapted for presentation using BioRender. FPKM = Fragments Per Kilobase of transcript per Million mapped reads, KO#2 = gRNA2, clone #2, WT = wild-type.

Since MMP7 showed the strongest HPV8-E6-mediated increase, we further validated its expression under different culture conditions. CDH4 was included because it showed the most consistent reduction among the remaining target genes. It has been reported previously that culture components (Aldehlawi et al., 2020) and calcium concentrations can influence keratinocyte gene expression (Borowiec et al., 2013, Wilson, 2014). To analyze whether culture conditions used in the previous experiments affected HPV8-E6-mediated regulation of MMP7 and CDH4, we compared N/Tert cells cultivated in medium containing 10 % fetal bovine serum (SHM) or serum-free medium (SFM).

While SHM contains higher calcium concentrations and promotes a more differentiated phenotype, the low calcium concentration in SFM keeps keratinocytes in a basal state (Borowiec et al., 2013, Wilson, 2014). HPV8-E6-mediated repression of CDH4 remained largely unaffected by the different culture conditions. In contrast, MMP7 expression was increased approximately 100-fold in N/Tert^{HPV8-E6} cells cultured under SHM, but only 6-fold under SFM conditions (Fig. 22A). These findings indicate that different culture conditions can strongly influence HPV8-E6-dependent activation of MMP7.

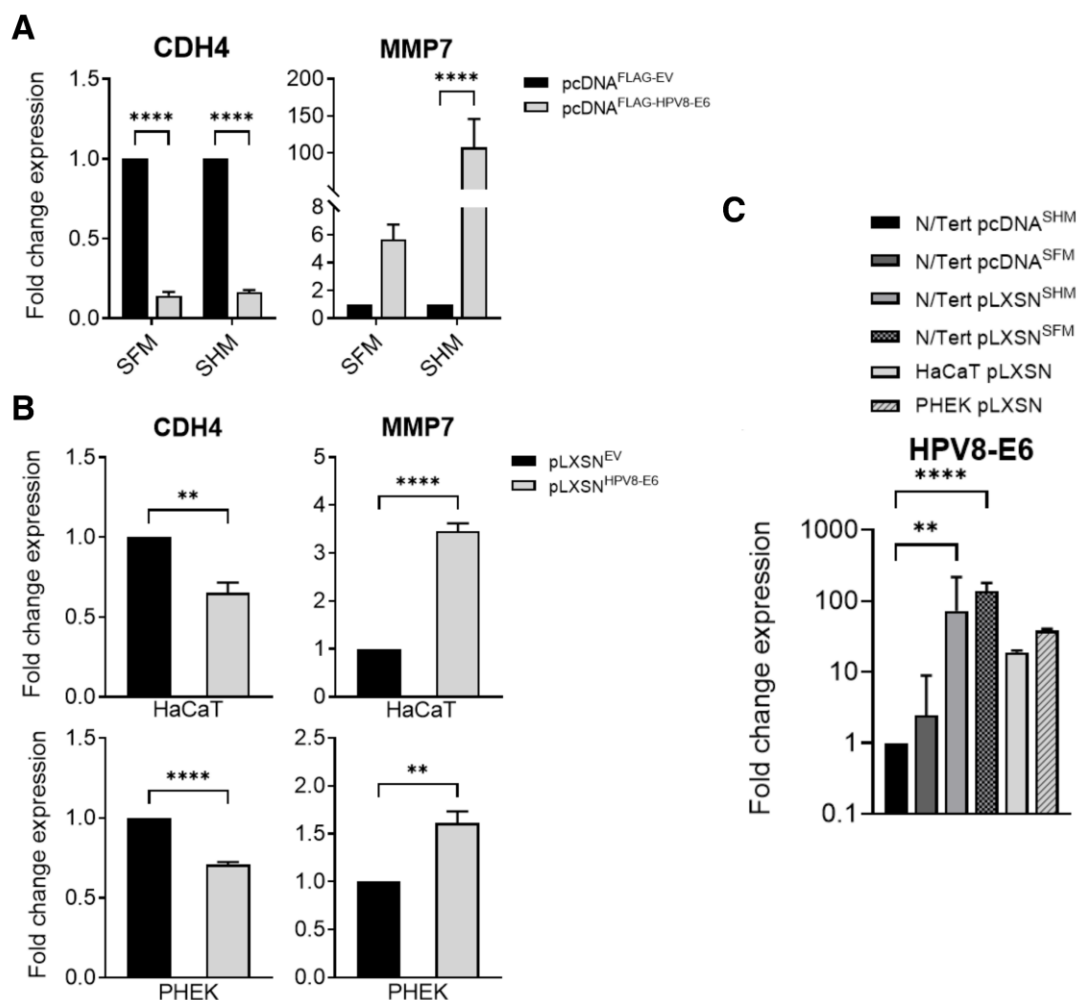


Figure 22: HPV8-E6 alters MMP7 and CDH4 expression in different keratinocyte models. (A) N/Tert keratinocytes expressing pcDNA-FLAG-EV or pcDNA-FLAG-HPV8-E6 were analyzed under serum-containing medium (SHM) or serum-free (SFM) culture conditions. (B) HaCaT cells were cultured under SHM conditions, while PHEKs were cultured under SFM conditions. (C) HPV8-E6 transcript levels in the indicated cell lines were measured by qRT-PCR and are shown relative to N/Tert cells expressing pcDNA-FLAG-HPV8-E6 under SHM conditions, which was set to 1. Data are shown as mean $\pm$ SD of $n = 3$ biological replicates on a logarithmic y-axis. Statistical significance was determined using a two-way ANOVA followed by Šídák's multiple comparisons test (A), an unpaired two-tailed Welch's t-test (B), or an ordinary one-way ANOVA followed by Dunnett's multiple comparisons test. $p < 0.05$ (*), $p < 0.01$ (**), $p < 0.0001$ (****). Graphs were generated using GraphPad Prism and adapted for presentation using BioRender. EV = empty vector, PHEK = primary human epithelial keratinocytes.

Expression of MMP7 and CDH4 was also analyzed in immortalized HaCaT (Taute et al., 2017), and primary human epithelial keratinocytes (PHEK) expressing either EV or HPV8-E6 from a retroviral pLXSN-based plasmid construct. HPV8-E6 slightly reduced CDH4 transcripts in both models (Fig. 22B). In contrast, MMP7 expression was increased approximately 3.6-fold in HaCaT and 1.6-fold in PHEKs, which were cultured in serum-free keratinocyte growth medium (Fig. 22B).

These results were consistent with HPV8-E6-mediated MMP7 induction observed in N/Tert cells, although the effects were weaker. As shown in Fig. 22C, HPV8-E6 expression was detected in all cell lines. However, pLXSN-HPV8-E6 constructs resulted in much higher HPV8-E6 transcription than the pcDNA-FLAG-HPV8-E6 (Fig. 22C). Overall, HPV8-E6 increased MMP7 and reduced CDH4 expression across different keratinocyte models.

4.3.3 Analysis of DPF2 and p300 binding at selected regulatory regions

Since MMP7 was identified as an HPV8-E6-regulated target gene, we investigated whether HPV8-E6 might alter the recruitment of DPF2 and p300 at MMP7 regulatory regions. To this end, we performed ChIP-seq analysis using N/Tert^{EV} and N/Tert^{HPV8-E6} cells in collaboration with the Institute for Translational Epigenetics Cologne. Chromatin was prepared from four biological replicates and precipitated using anti-DPF2 and anti-p300 antibodies. Rabbit IgG was used as a negative control. The precipitated chromatin was analyzed by next generation sequencing as described in section 3.8. The MMP7 promoter is located on chromosome 11 at genomic positions 102,522,678 and 102,532,920 on the minus strand. Three STAT3-binding sites (Yuan et al., 2008) and two TCF7L2-binding sites (Brabletz et al., 1999) have been previously described close to the transcription start site (TSS) (Fig. 23A). DPF2 ChIP-seq peaks in both N/Tert^{EV} and N/Tert^{HPV8-E6} cells remained largely unchanged around the TSS, whereas p300 peaks were increased in three out of four replicates among HPV8-E6-expressing cells (-286/+751 bp).

This region overlapped with H3K27ac-enrichment detected in an epithelial prostate cancer cell line (Gershman et al., 2022). We detected an additional region downstream of the TSS (+1.3/+2.4 kb). DPF2 ChIP peaks were again largely unaffected by HPV8-E6-expression, whereas p300 peaks were increased in all HPV8-E6 replicates compared with EV samples. Although the H3K27ac-enrichment detected in the prostate cancer cells was weaker than around the TSS, it still overlapped with the p300 peaks, suggesting that the identified region was transcriptionally active (Fig. 23B). In addition, GeneHancer annotations identified two adjacent regions approximately 21.4 kb and 23.6 kb upstream of the TSS (Fishilevich et al., 2017), which displayed altered DPF2 and p300 ChIP peaks. At the region -22.7/-23.6 kb, DPF2 and p300 peaks were similar in both N/Tert^{EV} and N/Tert^{HPV8-E6} cells across all replicates. In contrast, DPF2 and p300 peaks at the region -21.4/-22.4 kb were simultaneously increased in N/Tert^{HPV8-E6} cells in three out of four replicates. This region also colocalized with H3K27ac-enrichment detected in the prostate cancer cells (Fig. 23C).

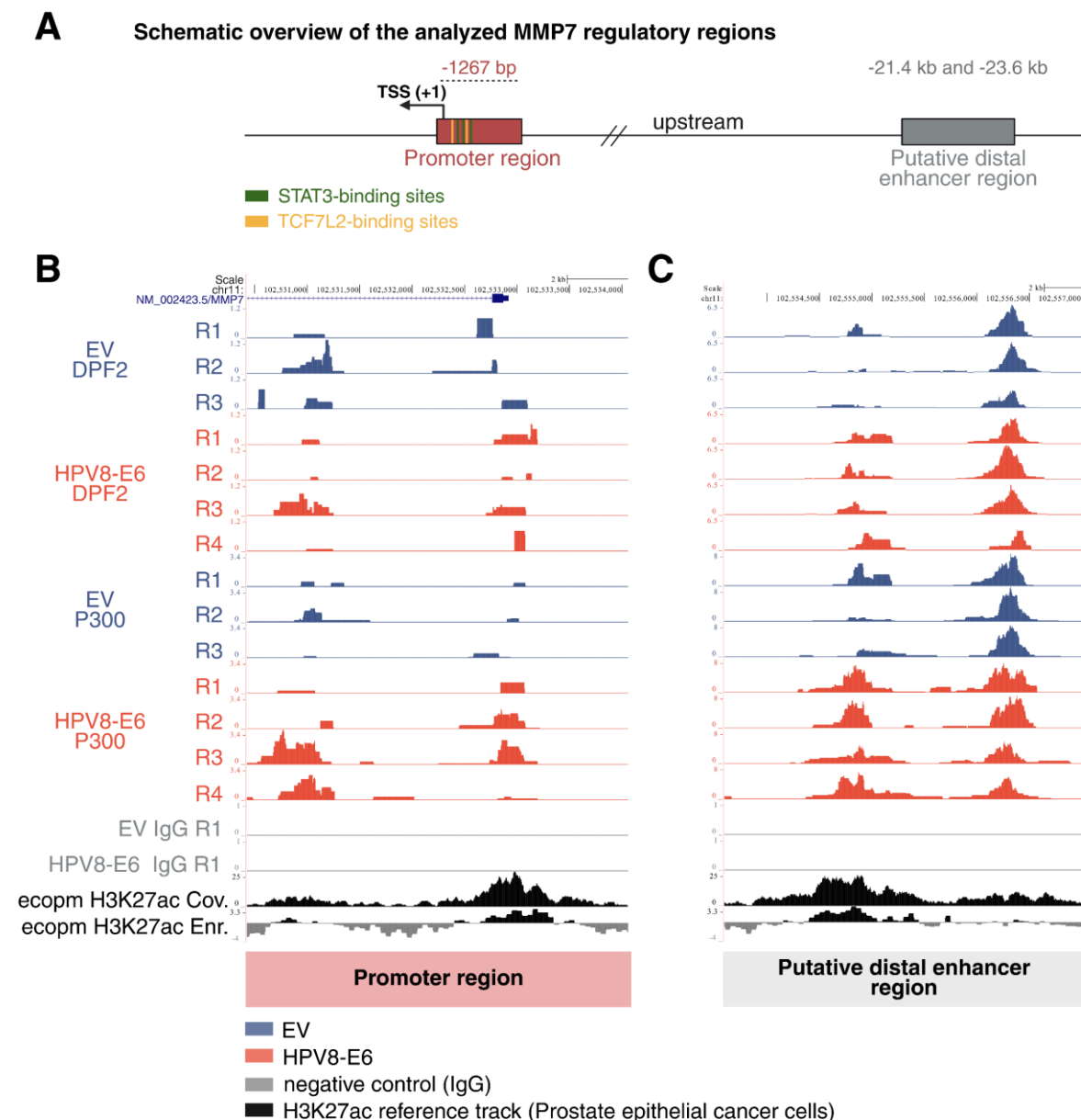


Figure 23: HPV8-E6 alters chromatin association of DPF2 and p300 at MMP7 regulatory regions. (A) Schematic representation of the MMP7 promoter (-1267 to +44 bp relative to the TSS) and a putative promoter-distal enhancer region located approximately 21.4 and 23.6 kb upstream of the TSS. Published STAT3- and TCF7L2-binding sites are indicated in green (Brabletz et al., 1999, Yuan et al., 2008). (B, C) Genome browser views of the MMP7 promoter region (B) and the putative distal enhancer region (C) showing DPF2 and p300 ChIP-seq tracks in N/Tert^{EV} (blue) and N/Tert^{HPV8-E6} cells (red), with IgG controls in grey. Published H3K27ac coverage and enrichment tracks derived from prostate cancer cells are included as a reference for active chromatin (Gershman et al., 2022). ChIP-seq tracks are shown as SPMR using the human T2T-CHM13v2.0 reference assembly (UCSC hs1). Bioinformatic analysis was performed in collaboration with the Institute for Translational Epigenetics Cologne. Created with BioRender. bp = base pairs, ChIP-seq = chromatin immunoprecipitation sequencing, Cov. = coverage, Enr. = enrichment, EV = empty vector, H3K27ac = histone H3 lysine 27 acetylation, IgG = immunoglobulin G, R = replicate, SPMR = signal per million reads, TSS = transcription start site.

Since CDH4 was also identified as an HPV8-E6-regulated target gene by qRT-PCR, DPF2 and p300 ChIP peaks at the CDH4 regulatory region were also analyzed. In contrast to the MMP7 regulatory regions, no clear HPV8-E6-dependent differences were observed, indicating that not all expression changes detected by qRT-PCR were accompanied by detectable changes in DPF2 or p300 chromatin association. Taken together, these data suggest that HPV8-E6 affects p300 and DPF2 binding around the MMP7 promoter-proximal region and a promoter-distal enhancer region. These changes correlate with enhanced transcriptional activation.

4.3.4 MMP7 expression in human N/Tert keratinocytes

Since HPV8-E6 strongly induced MMP7 expression in N/Tert keratinocytes, we subsequently analyzed the relationship between MMP7 and E-cadherin in N/Tert^{EV} and N/Tert^{HPV8-E6} cells by performing immunofluorescence (IF) stainings.

Epidermal keratinocytes express the transmembrane protein E-cadherin to maintain cell-cell contacts. It is known that E-cadherin decreases during wound healing to facilitate cellular migration (Jiang et al., 2020). In addition, E-cadherin is needed for regulating keratinocyte differentiation (Charest et al., 2009). MMP7 reportedly cleaves the extracellular domain of E-cadherin and thereby contributes to weakened cell-cell contacts and increased cellular migration (Noë et al., 2001, McGuire et al., 2003).

Compared with EV cells, HPV8-E6-expressing keratinocytes showed a less defined membrane-associated E-cadherin pattern (Fig. 24A). In contrast, MMP7 remained detectable at cell-cell junctions but was more broadly distributed within the cytoplasm (Fig. 24A). Mean fluorescence intensities along representative cell-cell-junctions revealed a clear E-cadherin peak, which was reduced in HPV8-E6-expressing cells (Fig. 24B). MMP7 fluorescence intensity appeared more broadly distributed along the measured area in cells expressing HPV8-E6 and did not show an individual peak as observed in EV cells (Fig. 24B).

Quantification of the mean fluorescence intensity confirmed a significantly reduced E-cadherin signal in N/Tert^{HPV8-E6} cells compared with N/Tert^{EV} cells. In contrast, the mean MMP7 fluorescence intensity was only slightly but significantly reduced in the presence of HPV8-E6 (Fig. 24C). These observations suggest that HPV8-E6 expression is associated with reduced membrane-associated E-cadherin and altered MMP7 localization.

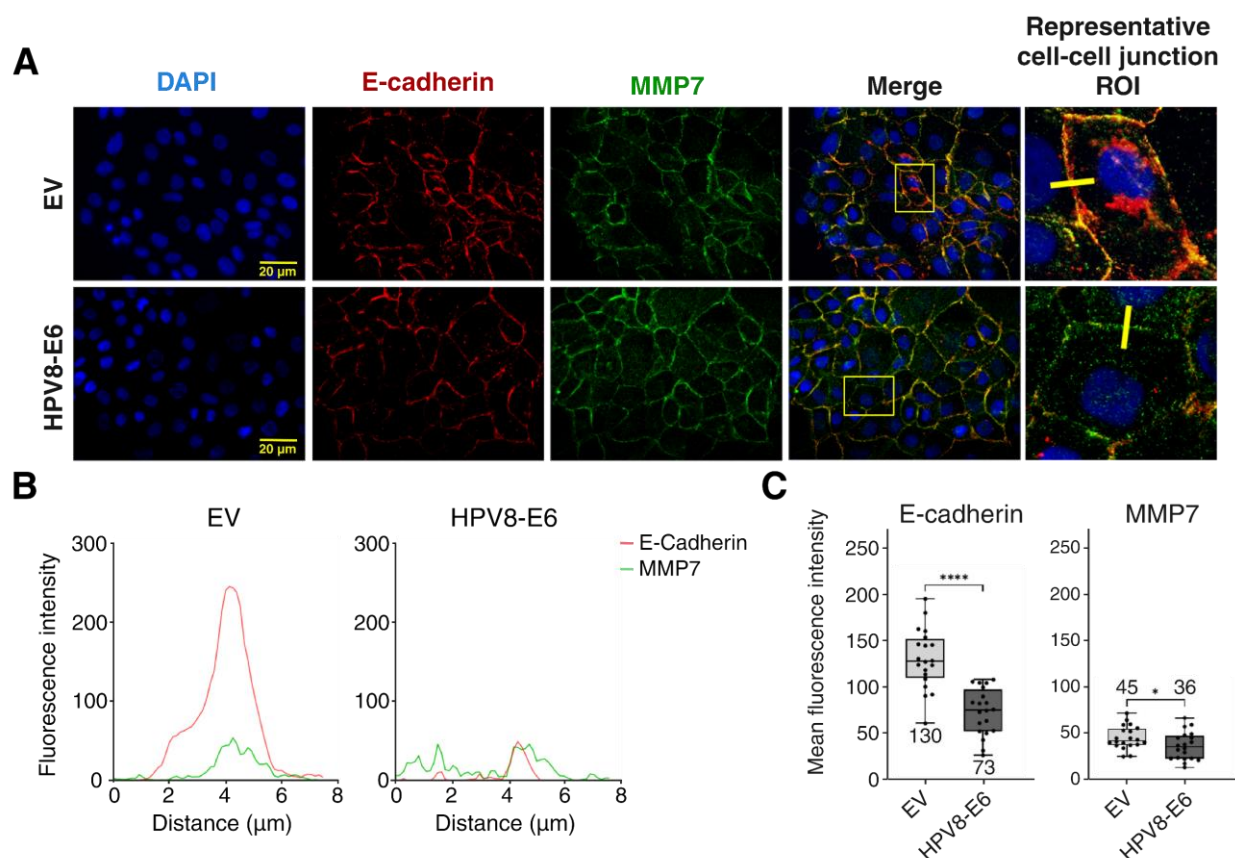


Figure 24: HPV8-E6 alters E-cadherin and MMP7 staining at cell-cell junctions. (A) Representative IF images of N/Tert^{EV} and N/Tert^{HPV8-E6} cells stained for MMP7 (green) and E-cadherin (red). Nuclei were counterstained with DAPI (blue). Scale bars: 20 μm . (B) Representative fluorescence intensity line profiles of E-cadherin and MMP7 were determined along the indicated line in (A). (C) Mean fluorescence intensities were calculated for 20 individual cell-cell-junctions. Each dot represents one analyzed cell-cell junction. Horizontal lines represent the median, and whiskers indicate the minimum and maximum values. Statistical significance was assessed using an unpaired two-tailed Welch's t-test. $p < 0.05$ (*); $p < 0.0001$ (****). Graphs were generated using GraphPad Prism, and the figure was assembled for presentation using BioRender. EV = empty vector, IF = immunofluorescence

4.4 Analysis of MMP7 expression in organotypic cultures and human skin tissue

4.4.1 MMP7 expression in HPV8-E6-positive organotypic skin cultures

Our qRT-PCR results have shown that MMP7 was consistently upregulated by HPV8-E6 in primary keratinocytes as well as immortalized N/Tert and HaCaT cells. This effect was observed in two different expression systems driven by either the CMV promoter or a retroviral long terminal repeat (Miller and Rosman, 1989). Since MMP7 has not been reported as a target of HPV E6 oncogenes, we subsequently characterized its distribution in N/Tert^{HPV8-E6} keratinocytes. MMP7, also known as matrilysin, is a zinc-dependent, matrix metalloproteinase. In its secreted form it

can degrade several ECM components as well as non-matrix substrates, including E-cadherin, membrane receptors, cytokines, and growth factors (Visse and Nagase, 2003). MMP7 has been associated with tissue remodeling, cell proliferation, migration, differentiation, and invasion (Laronha and Caldeira, 2020), which is consistent with the enrichment of ECM-receptor interaction and cell-cycle pathways observed in our RNA-seq analysis. Increased matrilysin expression has also been reported in metastatic cutaneous SCCs, particularly at the invasive front (Ahmed Haji Omar et al., 2015). We therefore analyzed MMP7 expression at the protein level in HPV8-E6-positive organotypic skin cultures.

MMP7 could not be reliably detected by Western blotting in N/Tert^{HPV8-E6} cells (data not shown). We therefore analyzed MMP7 protein expression by IHC in organotypic N/Tert skin culture models. Sections from these 3D-organotypic cultures were obtained in collaboration with S. Kazem and M. Feltkamp (Kazem et al., 2012). N/Tert^{pLZRS-EV} skin cultures displayed a well-organized, differentiated stratified epithelium with flat cornified cells at the top. Compared with organotypic N/Tert^{pLZRS-EV} skin cultures, those expressing N/Tert^{HPV8-E6} showed an altered morphology with enlarged suprabasal cells and no clear separation of distinct epithelial layers. A similar histology was observed in HPV8-E6/E7-positive skin cultures (Fig. 25).

These observations are consistent with previous studies, showing that HPV8-E6 delays terminal keratinocyte differentiation in organotypic raft cultures (O'Shaughnessy et al., 2007, Lazic et al., 2012). MMP7 staining was weak in organotypic skin cultures expressing empty vector, and was mostly localized at cell-cell contacts, while the undifferentiated basal cell layers were largely MMP7-negative. In contrast, HPV8-E6-expressing organotypic skin cultures showed much stronger MMP7 staining throughout the epidermis, with additional staining within the cytoplasm. A similar pattern was observed with HPV8-E6/E7, although MMP7 staining was slightly weaker (Fig. 25). Together, these findings suggest that the presence of HPV8-E6 in organotypic N/Tert skin cultures is associated with increased MMP7 expression.

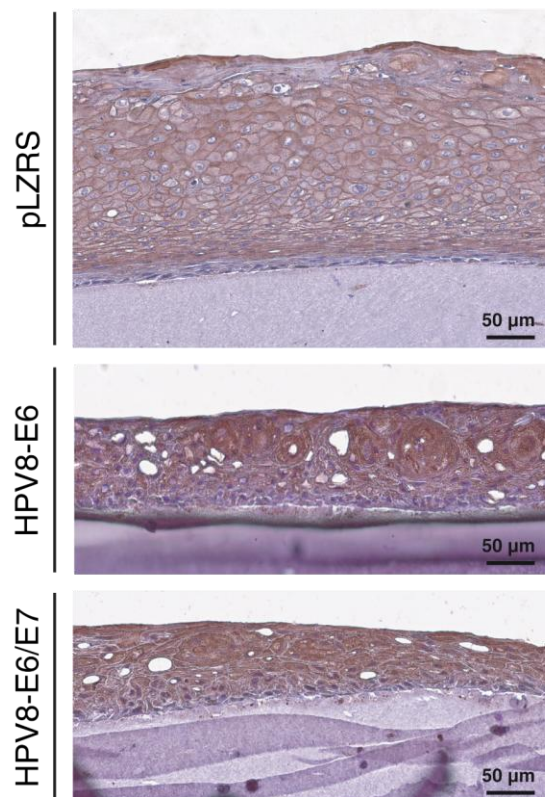


Figure 25: Increased MMP7 expression in HPV8-E6-expressing organotypic skin cultures. Two independent sections per condition were stained for MMP7. Representative IHC images of sections from organotypic N/Tert skin cultures expressing pLZRS empty vector, HPV8-E6, or HPV8-E6/E7 are shown. DAB (brown) indicates MMP7 staining. Nuclei were counterstained with hematoxylin (purple). Scale bars: 50 µm. Created with BioRender. IHC = immunohistochemistry.

4.4.2 MMP7 expression in human skin tissue

To assess the relevance of HPV8-E6-induced MMP7 expression in cutaneous SCCs obtained from patients with Epidermodysplasia verruciformis, we performed IHC staining of paraffin-embedded SCC tissue sections as described in section 3.5.4.1. Epidermodysplasia verruciformis-associated lesions displayed heterogeneous MMP7 expression throughout the stratified cell layers of the tumorous tissue. In areas with “normal” epidermal differentiation features, basal cells were largely MMP7-negative, while the more differentiated cells of the stratified layer showed increased MMP7 staining. Notably, koilocytes in Epidermodysplasia verruciformis-associated SCCs showed increased MMP7 staining (Fig. 26). Koilocytes are squamous epithelial cells that are histologically characterized by an enlarged nucleus and perinuclear cytoplasmic vacuolization, and are regarded as a surrogate marker for a cytopathic effect caused by HPV infection (Krawczyk et al., 2008). These findings suggest an association between increased MMP7 expression and HPV-infected differentiating keratinocytes in Epidermodysplasia

verruciformis-associated cSCCs. MMP7 expression in cSCC tissue sections obtained from a patient without Epidermodysplasia verruciformis (block no. 216-02F) was included as a control. Similar to the Epidermodysplasia verruciformis-associated lesions, basal cells were largely MMP7-negative, while more differentiated cells showed increased MMP7 staining. These findings are consistent with previous reports associating MMP7 expression with cSCC progression (Hei et al., 2025).

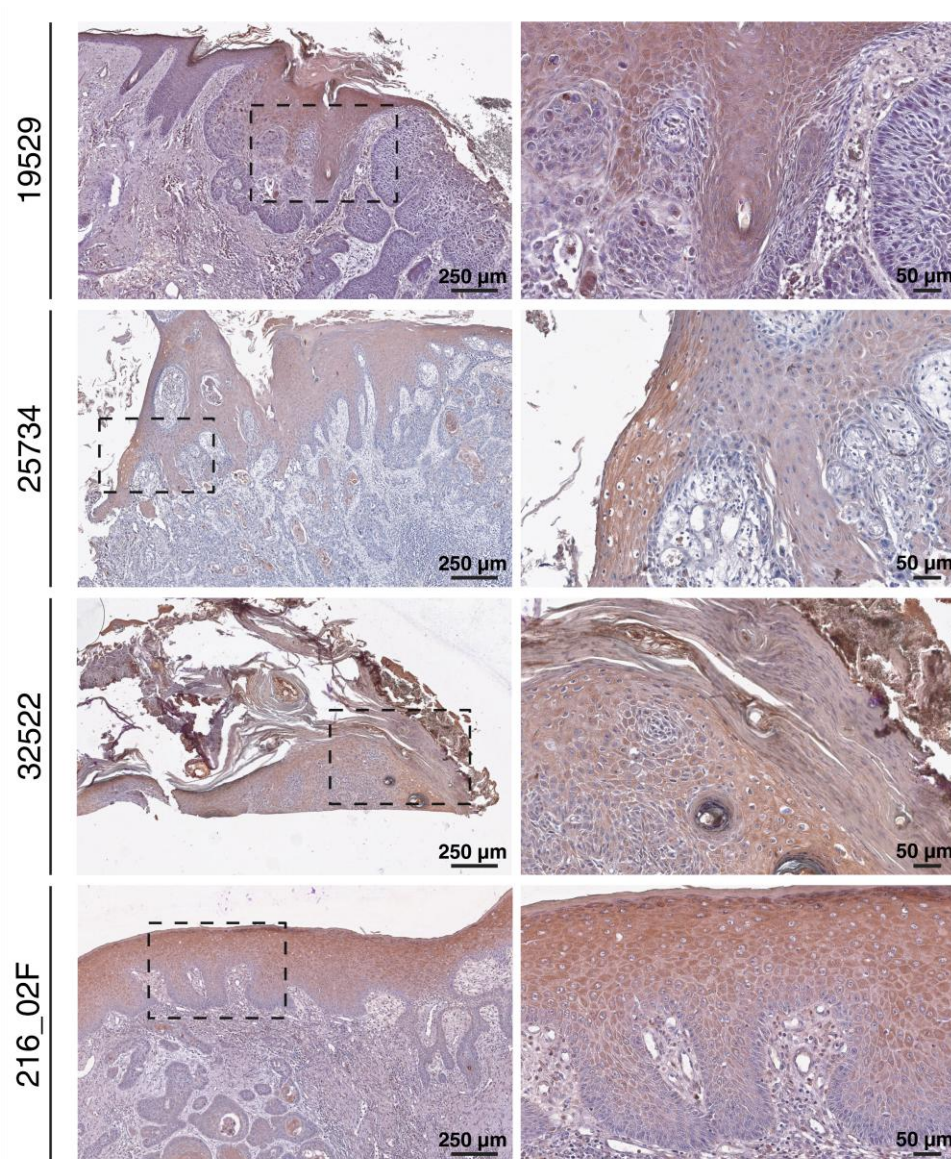


Figure 26: MMP7 expression in human cSCC tissue. MMP7 IHC was performed on six Epidermodysplasia verruciformis-associated cSCC tissue blocks and five control tissue blocks. Representative IHC images of sections from Epidermodysplasia verruciformis-associated tissue blocks (19529, 25734, 32522) and from one control block (216_02F) are shown. DAB (brown) indicates MMP7 staining. Nuclei were counterstained with hematoxylin (purple). Scale bars: 250 μm (left) and 50 μm (right). Created with BioRender. cSCC = cutaneous squamous cell carcinoma, IHC = immunohistochemistry.

5. Discussion

HPV8 has been classified as possibly carcinogenic in EV-patients (Bouvard et al., 2009). In immunosuppressed OTRs, higher beta-HPV DNA loads are frequently detected in actinic keratoses. Due to the low viral copy numbers in cSCC lesions, their contribution to tumor development remains unclear (reviewed in Smola, 2020). These observations led to the development of a “hit-and-run” model, which suggests that beta-HPVs act at early steps in skin tumor development (Hufbauer and Akgül, 2017). Previous *in vivo* and *in vitro* studies indicated that an early tumor-promoting role of HPV8-E6 depends on its interaction with p300 (Müller-Schiffmann et al., 2006, Howie et al., 2011, Hufbauer et al., 2015). However, the molecular basis of this interaction and its contribution to tumor growth are not fully understood and were addressed in this thesis.

First, we characterized binding of HPV8-E6 to the p300 fragments p300-1 and p300-4, which were both shown to suppress skin tumorigenesis in K14-HPV8-E6 transgenic mice upon overexpression (M. Hufbauer, personal communication). We successfully identified two minimal regions within p300, ranging from aa 332–390 within TAZ1 and aa 1789–1814 within TAZ2 as sufficient for HPV8-E6 binding. To further characterize the minimal TAZ2 binding region, we mutated the cysteine residues C1789 and C1790 within the p300-4 fragment, which are not involved in zinc coordination of the domain (De Guzman et al., 2000). It was previously shown that alanine substitutions of the non-zinc-coordinating cysteine residues C1738, C1746, C1789, and C1790 improved TAZ2 stability without altering its ability to bind p53 (Jenkins et al., 2009). Interestingly, we observed that HPV8-E6 failed to bind the mutated TAZ2 peptide, suggesting that C1789 and C1790 within TAZ2 might be required for this interaction. Introducing the same mutations into full-length p300 may help to determine if this minimal TAZ2 region is also required for the association of HPV8-E6 with the whole protein. Structural analyses of HPV8-E6 in complex with the minimal binding regions of TAZ1 and TAZ2 are currently ongoing and may contribute to identifying key residues of the interaction surface. Tattooing of these two minimal p300 peptides into the skin of K14-HPV8-E6 transgenic mice would provide additional insight into whether these regions contribute to HPV8-E6-mediated skin tumorigenesis *in vivo*.

Our findings indicate that HPV8-E6 recognizes distinct interaction surfaces within TAZ1 and TAZ2. The identified HPV8-E6 binding region within TAZ1 corresponds to the zinc-binding motif Zn1 between helices $\alpha 1$ and $\alpha 2$, and to the Zn3 motif between helices $\alpha 3$ and $\alpha 4$ within TAZ2, based on the structural models described by De Guzman et al. (2005). Although the zinc-coordinating histidine and cysteine residues are conserved in both TAZ domains, differences in

α -helical orientation create highly selective scaffolds for cellular binding partners (De Guzman et al., 2000, De Guzman et al., 2005). Although TAZ1 and TAZ2 share several binding partners, they may differ in their functions (Teufel et al., 2007, Wojciak et al., 2009). For instance, HIF-1 α only interacts with TAZ1 (Freedman et al., 2002), whereas p53 binds to both domains but recognizes distinct interaction sites within TAZ1 and TAZ2 (Grossman, 2001). STAT transcription factors and NF- κ B subunits are also among the sequence-specific transcriptional regulators targeting TAZ1 and TAZ2 (Wojciak et al., 2009, Bedford et al., 2010). These interactions with multiple transcription factors are required for the efficient recruitment of p300 to promoters and for its co-activator function including p300-dependent histone acetylation and transcriptional activation (Wang et al., 2013, Ferrie et al., 2024). More recently, p53 binding to TAZ2 was shown to induce conformational changes that are associated with increased p300 activity (Xu et al., 2023).

Based on these observations, HPV8-E6 binding to TAZ1 and TAZ2 might also have different consequences on the co-activator function of p300. The results presented in this work suggest that targeting TAZ1 alters the global p300 interaction network. We used TurboID-based proximity proteomics to systematically identify cellular proteins whose proximity to the p300-1 (TAZ1) was altered in the presence of HPV8-E6.

To determine specific interactions, we compared the protein list obtained with TurboID empty vector with those obtained from samples expressing TurboID-p300-1. The enrichment of several well-characterized p300-associated proteins, including MAML1 (Hansson et al., 2009), CITED1 (Yahata et al., 2001), CITED2 (De Guzman et al., 2004), and FOXO3 (Wang et al., 2012) in samples expressing p300-1, supported the biological relevance of the identified data set. In total, we identified 56 proteins in untreated and 11 proteins in UV-irradiated cells as significantly altered by HPV8-E6 in its proximity to p300-1. GO enrichment analysis showed that HPV8-E6 mostly affected proteins associated with acetylated protein binding and chromatin remodeling. This is consistent with known functions of p300 linked to global histone acetylation (Weinert et al., 2018) and enhancer regulation (Van et al., 2026), suggesting that HPV8-E6 modulates the proximity of p300-1 to chromatin regulators. Although gene lists obtained from UV irradiated samples did not reveal any plausible results, DPF2 and C14orf166 were consistently altered by HPV8-E6 under both untreated and UV-irradiated conditions, further supporting their biological relevance. C14orf166 has been linked to influenza virus replication (Rodriguez-Frandsen et al., 2016), but to date neither an association with p300 nor a role in HPV biology have been reported. Both DPF2 and C14orf166 show nuclear localization, as does p300. In addition, DPF2 and C14orf166 have

also been detected in the cytoplasm (Rodriguez-Frandsen et al., 2016, Shin et al., 2017, Ononye et al., 2025).

Since DPF2 is an epigenetic reader subunit of the CBAF complex that links p300-dependent acetylation to chromatin remodeling (Black and Kutateladze, 2023), it emerged as a relevant candidate for detailed analysis. Our TurboID proteomics data showed that DPF2 was enriched in proximity to p300-1 in the presence of HPV8-E6. Consistent with this, Co-IPs revealed a stronger interaction between DPF2 and p300-1 upon co-expression of HPV8-E6 accompanied by increased nuclear colocalization of both proteins in HPV8-E6-expressing keratinocytes. In addition, our results suggest that DPF2 is a new interaction partner of HPV8-E6, as HPV8-E6 co-precipitated with DPF2 also in the absence of co-expressed p300-1. Together, these findings imply that HPV8-E6 acts as an adaptor protein to stabilize the interaction between p300-TAZ1 and DPF2.

To address the molecular consequences of this enhanced interaction between DPF2 and p300 in complex with HPV8-E6, we combined a DPF2 knockout with RNA-seq analysis. KEGG pathway analysis of all significantly altered genes revealed an association with pathways including ECM-receptor interaction, cell cycle, and transcriptional mis-regulation in cancer. In agreement with these findings, high DPF2 expression has previously been associated with poor prognosis in hepatocellular carcinoma and with gene signatures related to cell-cycle regulation, DNA replication, and Wnt signaling (Yang et al., 2024). A recent study further demonstrated that β -catenin can serve as a molecular adapter and contribute to CBAF-dependent transcriptional regulation by mediating the association of CBAF with TCF/LEF transcription factors, YAP1, FOXO3, and p300 (Chan et al., 2025). These observations suggest that DPF2-containing CBAF complexes cooperate with p300 to regulate Wnt and Hippo-dependent transcription.

We confirmed that DPF2 is required for efficient expression of MMP7, MUC4, and GABRP, while contributing to transcriptional repression of CDH4. Our analyses further revealed reduced HPV8-E6 transcript levels in DPF2^{KO} cells, indicating that DPF2 supports transcription of HPV8-E6 from the CMV immediate-early promoter present in pcDNA-FLAG-HPV8-E6 construct used in the N/Tert cells. This reduction may have contributed to some of the detected gene expression changes upon DPF2 loss.

The induction of MMP7 transcripts and repression of CDH4 in HaCaT cells and PHEKs expressing pLXSN-HPV8-E6 allowed us to exclude cell type- and vector-specific effects. However, HPV8-E6-mediated transcriptional activation of MMP7 in PHEKs and HaCaT cells was

weaker than observed in N/Tert cells. This might be explained by the different culture conditions of the two keratinocyte models: primary keratinocytes were cultivated in serum-free medium, whereas N/Tert cells were cultivated in medium containing FBS. By cultivating N/Tert^{HPV8-E6} and N/Tert^{EV} cells under both conditions, we confirmed that HPV8-E6-mediated MMP7 activation was strongly enhanced in the presence of serum. In contrast, MMP7 expression in EV cells was not affected by the different culture conditions.

To further characterize HPV8-E6-dependent MMP7 regulation, we performed ChIP-seq analysis of DPF2 and p300. DPF2 was shown to co-occupy enhancer regions with p300 and the CBAF subunit BRG1, thereby supporting a functional link between DPF2, p300-dependent acetylation and BAF-induced chromatin remodeling (Zhang et al., 2019). Our ChIP-seq data showed that genome-wide DPF2 and p300 binding sites largely overlapped and colocalized with previously described H3K27ac-enriched regions (Gershman et al., 2022), suggesting the presence of biologically relevant, transcriptionally active elements. The largely comparable DPF2 and p300 peak patterns between N/Tert^{EV} and N/Tert^{HPV8-E6} cells indicate that HPV8-E6 does not globally alter the recruitment of DPF2 and p300. Consistent with this, binding of DPF2 and p300 at regulatory regions of the CDH4 promoter remained largely unaffected in the presence of HPV8-E6, indicating that the observed transcriptional repression is not associated with altered binding of these factors. However, ChIP-seq analysis revealed that HPV8-E6 induces region-specific alterations in DPF2 and p300 chromatin binding.

DPF2 peaks at the MMP7 promoter and around the TSS were comparable in both N/Tert^{EV} and N/Tert^{HPV8-E6} cells. In contrast, p300 peaks were higher around the TSS and at a downstream region in the presence of HPV8-E6. These genomic sequences present within the promoter and proximal to the TSS displaying altered DPF2 and p300 binding partially overlapped with published binding sequences for known transcriptional regulators of MMP7, including STAT3 (Yuan et al., 2008), c-Jun (Guan et al., 2015), and TCF7L2 (Brabletz et al., 1999, Katoh et al., 2016). A functional interaction of these transcriptional regulators with the CBAF complex has been shown previously (Tando et al., 2010, Ho et al., 2011, Vierbuchen et al., 2017), suggesting that they contribute to CBAF recruitment at MMP7 regulatory regions. In N/Tert^{HPV8-E6} cells, detected DPF2 and p300 peaks simultaneously increased at a promoter-distal regulatory region located approximately -22 kb upstream of the TSS, further supporting an HPV8-E6-dependent, region-specific alteration of DPF2 and p300 chromatin binding. This region also colocalized with H3K27ac marks detected in epithelial prostate cancer cells (Gershman et al., 2022), suggesting transcriptionally active chromatin.

Based on our current findings, it cannot be determined whether MMP7 transcriptional activation results from the HPV8-E6-mediated stabilization of DPF2 and p300, or involves a third factor that is also regulated by HPV8-E6. Since high affinity ChIP-grade antibodies against HPV8-E6 are currently not available, a direct association of HPV8-E6 with the analyzed MMP7 regulatory regions could not be analyzed. Overall, these findings suggest that HPV8-E6 modulates DPF2 and p300 chromatin association at selected MMP7 regulatory regions. The HPV8-E6-dependent modulation of chromatin-associated DPF2 and p300 observed in this study may contribute to the transcriptional and epigenetic reprogramming of keratinocytes recently reported by Hufbauer et al. (2026).

Previous studies have reported that HPV8-E6 promotes proteasomal degradation of p300 (Howie et al., 2011). We observed that p300 ChIP-seq peaks were largely similar between N/Tert^{EV} and N/Tert^{HPV8-E6} cells, which argues against an HPV8-E6-induced reduction of chromatin-associated p300. Moreover, we did not observe reduced p300 protein levels in the presence of HPV8-E6 in any of our Western blot experiments. Given that EDTA can interfere with zinc-dependent folding, its presence in the buffers may disturb the detection of specific protein interactions (Matt et al., 2004, Nyborg and Peersen, 2004, De Guzman et al., 2005). EDTA-free buffers were used for the detection of specific HPV8-E6/p300 interactions in this work. Of note, Howie et al. used an EDTA-containing lysis buffer for the preparation of whole-cell lysates. To evaluate whether differences in lysis conditions may result in the distinct observations regarding p300 protein levels, a direct comparison of EDTA-containing and EDTA-free lysis conditions would be required.

We were unable to detect specific MMP7 signals by Western blotting to confirm the HPV8-E6-mediated MMP7 induction at the protein level. A possible explanation might be a low protein abundance in N/Tert cell lysates. However, IHC stainings of N/Tert^{HPV8-E6} cells grown in a monolayer as well as 3D-organotypic skin cultures, revealed higher MMP7 staining. This indicates that HPV8-E6 expression correlates with MMP7 activation. Most importantly, SCC tissue samples from patients with Epidermodysplasia verruciformis showed enhanced MMP7 signals in regions containing koilocytes. These findings are consistent with our observations in HPV8-E6-positive organotypic skin cultures and further support an association between MMP7 expression and keratinocyte differentiation, which is required for productive HPV infection (Moody, 2017). HPV8-E6-mediated MMP7 induction may therefore also favor the productive HPV8 life cycle. However, whether MMP7 directly contributes to viral replication remains unclear.

The HPV8-E6-mediated activation of MMP7 may have broader molecular consequences. Since MMP7 reportedly targets E-cadherin for proteolytic cleavage, increased MMP7 expression could

contribute to reduced E-cadherin levels (McGuire et al., 2003, Lynch et al., 2010) and enhanced proliferation and migration of epithelial cells (Lynch et al., 2010). Although high MMP7 expression is associated with invasion in several cancers (Sizemore and Keri, 2012, Zhang et al., 2017, Du et al., 2021), HPV8-E6 has not been shown to promote an invasive phenotype (Akgül et al., 2005a, Akgül et al., 2007). Instead, HPV8-E6 may contribute to reduced cell-cell adhesion by promoting MMP7-mediated E-cadherin cleavage, which is in line with the well-documented role of E-cadherin in maintaining adherens junctions and supporting epithelial differentiation (McGuire et al., 2003, Young et al., 2003, Lynch et al., 2010).

Previous studies have reported that HR-HPV E6 can weaken cell adhesion by reducing E-cadherin expression at the cell surface (Matthews et al., 2003, D'Costa et al., 2012). HPV8-E6-induced reduction of E-cadherin may therefore also affect adhesion-dependent signaling in infected keratinocytes. At the plasma membrane, E-cadherin associates with β -catenin and thereby links adherens junctions to the actin cytoskeleton (Niessen and Gottardi, 2008, Bruner and Derksen, 2018). It is well established that MMP7-mediated E-cadherin cleavage results in nuclear accumulation of β -catenin and activation of canonical Wnt signaling (McGuire et al., 2003, Wang et al., 2017). Nuclear β -catenin may subsequently facilitate TCF/LEF-dependent transcription, thus promoting proliferation, migration, and ultimately epithelial-to-mesenchymal transition (Wang et al., 2017, Chong and Doorbar, 2024).

Moreover, E-cadherin-stabilized cell-cell contacts also support active Hippo signaling by limiting YAP (Yes-associated protein) and TAZ (transcriptional co-activator with PDZ-binding motif) accumulation within the nucleus, which reduces TEAD-dependent transcription (Kim et al., 2011, Kehrberg and DeMali, 2025). Thus, enhanced MMP7 expression and subsequent proteolytic cleavage of E-cadherin might contribute to YAP/TAZ/TEAD-dependent transcription, thereby promoting cell proliferation and survival. Recently, HPV8-E6 was shown to activate TEAD-dependent transcription while simultaneously inhibiting Wnt signaling through binding to BCL9L, thereby interfering with keratinocyte differentiation (Wu et al., 2023). Dysregulation of Hippo signaling has also been reported for mucosal HR-HPV oncoproteins (Olmedo-Nieva et al., 2020) as well as for beta-HPV types, although to a reduced level (Sominsky et al., 2017). Together, our findings suggest that MMP7 upregulation represents an additional mechanism by which HPV8-E6 modulates Hippo and Wnt signaling to maintain a basal-like phenotype of infected keratinocytes.

Selective inhibition of MMP7 was shown to reduce tumor invasion in a prostate cancer mouse model (Zhang et al., 2017). Recent studies have demonstrated that an antibody targeting the

MMP7/syndecan-2 interaction reduced tumor growth and metastasis in colon cancer models (Min et al., 2025). Since STAT3 strongly induces MMP7 expression in several cancer cell lines (Udayakumar et al., 2002, Yuan et al., 2008, Fukuda et al., 2011), STAT3-inhibition may represent an additional approach to reduce MMP7 expression. Inhibiting STAT3-dependent MMP7 expression may therefore represent a potential therapeutic strategy in the treatment of beta-HPV-associated SCCs.

Figure 27A summarizes the main findings of this study. Based on our findings, we propose that HPV8-E6 stabilizes the interaction between DPF2 and the p300 TAZ1 region in a ternary complex. This correlates with altered chromatin association of both factors at MMP7 regulatory regions, and elevated MMP7 expression in N/Tert^{HPV8-E6} keratinocytes (Fig. 27A, B). In line with this model, we observed enhanced MMP7 staining in Epidermodysplasia verruciformis-associated SCCs, particularly in regions with koilocytes, suggesting that MMP7 expression may be relevant to cSCC development. Together, these findings suggest that modulation of DPF2 and p300 represents a novel mechanism contributing to HPV8-E6-driven skin tumorigenesis.

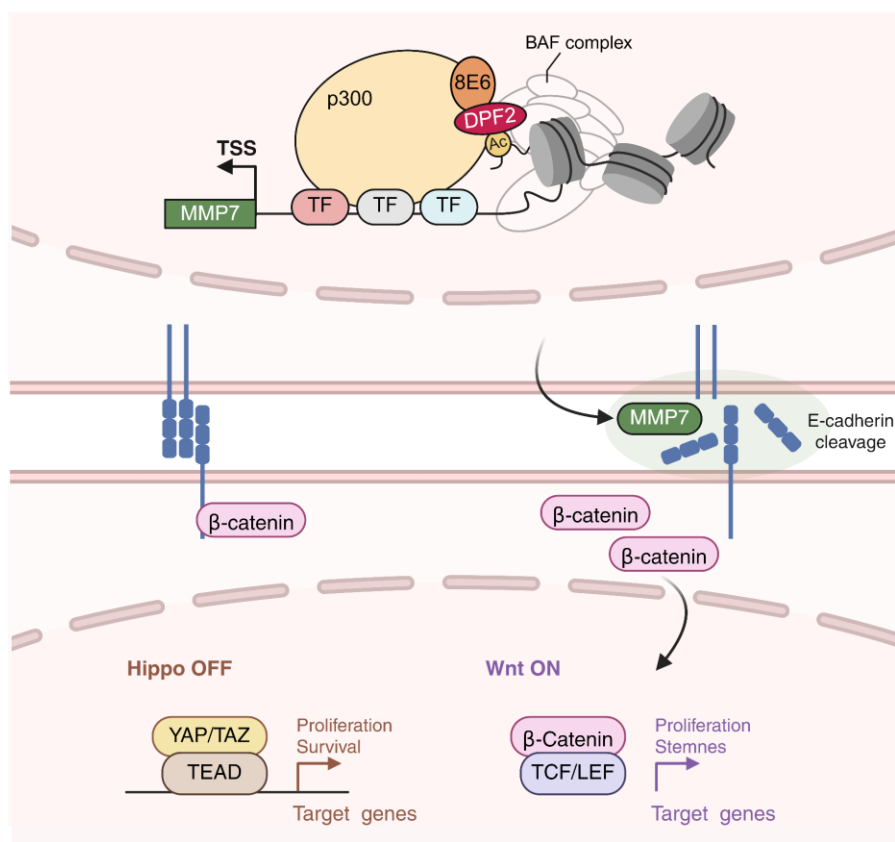
A Findings of this study**B Proposed model**

Figure 27: Summary of findings and proposed model. (A) Shown is a schematic overview of the main findings presented in this study. Experimental approaches are indicated below. The dashed arrow shows a potential link between increased MMP7 expression and reduced E-cadherin at cell-cell junctions. **(B)** Proposed model of HPV8-E6-mediated MMP7 regulation and its downstream effects. HPV8-E6 stabilizes the interaction between p300 and DPF2-containing CBAF complexes. This alters their chromatin association at MMP7 regulatory regions and may contribute to increased MMP7 expression. MMP7-mediated E-cadherin cleavage weakens cell-cell adhesion, promotes β-catenin accumulation and Wnt signaling, and reduces Hippo signaling. These HPV8-E6-mediated changes may contribute to proliferation, survival, and stemness of infected keratinocytes. Created with BioRender. Ac = acetylation, ChIP-seq = chromatin immunoprecipitation sequencing, IF = immunofluorescence, IHC = immunohistochemistry, qRT-PCR = quantitative real-time PCR, TF = transcription factor, TSS = transcription start site.

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Abstract

Viral oncogenes can reshape the host-cell transcriptome to promote transformation, yet the mechanisms by which HPV E6 oncogenes exert such control remain poorly understood. Beta-HPV infection is associated with cutaneous squamous cell carcinomas (cSCCs) in immunosuppressed organ-transplant recipients and patients with the rare hereditary disease Epidermodysplasia verruciformis. Previous work from our group showed that beta-HPV8-E6 physically interacts with the transcriptional co-activator p300 and that this interaction is crucial for skin tumorigenesis. This study aimed to elucidate how HPV8-E6 modulates p300 and the consequences for HPV8-E6-driven tumorigenesis.

In this work, the HPV8-E6-binding domain within p300 was mapped to a 59-amino-acid fragment spanning residues 332–390 and to a 26-amino-acid fragment spanning residues 1789–1814. These minimal binding regions may provide a basis for inhibitors targeting the HPV8-E6/p300 interaction. To investigate how HPV8-E6 modulates the p300 interactome, p300-1 (aa 2–439) was used as bait in TurboID-based proximity proteomics. GO analysis revealed an enrichment of proteins involved in lysine-acetylated histone binding, DNA damage repair, and DNA replication. We focused on DPF2, the epigenetic reader subunit of the chromatin-remodeling CBAF complex, whose proximity to p300-1 increased in the presence of HPV8-E6. Co-IPs suggested that HPV8-E6 stabilizes a ternary complex with DPF2 and p300. CRISPR-Cas9-mediated DPF2 knockout and RNA-seq analysis were used to investigate DPF2-dependent transcription in human N/Tert keratinocytes. Matrix metalloproteinase 7 (MMP7) was among the most strongly affected genes and was induced by HPV8-E6, with increased expression also detected in Epidermodysplasia verruciformis-associated cSCCs. ChIP-seq analysis revealed increased p300 signals close to the MMP7 transcription start site in HPV8-E6-expressing cells compared with empty-vector keratinocytes. In addition, DPF2 and p300 signals were simultaneously increased at a putative promoter-distal MMP7 regulatory region. This region overlapped with chromatin enriched for histone H3 lysine 27 acetylation, suggesting that HPV8-E6-mediated transcriptional activation of MMP7 is associated with altered p300 and DPF2 binding.

In summary, our findings suggest that the small non-DNA-binding HPV8-E6 oncogene modulates the p300 interactome and DPF2-dependent transcriptional regulation. This involves altered chromatin association of p300 and DPF2 at MMP7 regulatory regions and increased MMP7 expression. MMP7 induction may contribute to cellular transformation and HPV8-E6-driven skin tumorigenesis.

Zusammenfassung

Virale Onkogene können das Transkriptom der Wirtszelle verändern und dadurch die zelluläre Transformation fördern. Die zugrunde liegenden Mechanismen sind jedoch noch nicht vollständig verstanden. Beta-HPV-Infektionen sind mit kutanen Plattenepithelkarzinomen (cSCCs) bei immunsupprimierten Organtransplantierten und Patienten mit Epidermodysplasia verruciformis assoziiert. Frühere Arbeiten unserer Gruppe zeigten, dass HPV8-E6 mit dem transkriptionellen Koaktivator p300 interagiert und diese Interaktion für die Hauttumorentstehung entscheidend ist. Ziel dieser Arbeit war es, die zugrunde liegenden molekularen Mechanismen und deren funktionelle Konsequenzen zu untersuchen.

Die HPV8-E6-Bindedomäne innerhalb von p300 wurde auf zwei Fragmente zwischen den Aminosäuren 332–390 und 1789–1814 eingegrenzt. Um zu untersuchen, wie HPV8-E6 das p300-Interaktom beeinflusst, wurde p300-1 als Köderprotein in einer TurboID-basierten Proximity-Proteomik eingesetzt. Die GO-Analyse zeigte eine Anreicherung von Proteinen, die an der Bindung lysinacetylierter Histone, der DNA-Schadensreparatur und der DNA-Replikation beteiligt sind. Dabei wurde DPF2, eine Untereinheit des chromatinremodellierenden CBAF-Komplexes, in Anwesenheit von HPV8-E6 vermehrt in der Nähe von p300-1 nachgewiesen. Co-Immunpräzipitationen deuteten darauf hin, dass HPV8-E6 einen ternären Komplex aus DPF2 und p300 stabilisiert.

Ein CRISPR-Cas9-vermittelter DPF2-Knockout mit anschließender RNA-Sequenzierung zeigte, dass DPF2 zur HPV8-E6-abhängigen Transkriptionsregulation in humanen N/Tert-Keratinocyten beiträgt. Matrix-Metalloproteinase 7 (MMP7) gehörte zu den am stärksten beeinflussten Genen und wurde durch HPV8-E6 induziert. Eine erhöhte MMP7-Expression wurde außerdem in Epidermodysplasia-verruciformis-assoziierten cSCCs nachgewiesen. ChIP-seq-Analysen zeigten erhöhte p300-Signale nahe der MMP7-Transkriptionsstartstelle sowie gleichzeitig erhöhte DPF2- und p300-Signale an einer potenziellen promotordistalen regulatorischen Region. Diese Region überlappte mit Chromatin, das mit Histon-H3-Lysin-27-Acetylierung angereichert war, und weist damit auf eine veränderte Chromatinassoziation von p300 und DPF2 im Zusammenhang mit der MMP7-Aktivierung hin.

Zusammenfassend zeigen unsere Ergebnisse, dass HPV8-E6 das p300-Interaktom und die DPF2-abhängige Transkriptionsregulation beeinflusst. Die HPV8-E6-vermittelte Induktion von MMP7 könnte zur zellulären Transformation und Hauttumorentstehung beitragen.

Abbreviations

Abbreviation	Definition
AIL	autoinhibitory loop
ANOVA	analysis of variance
AP-1	activator protein 1
AP-1	activator protein 1
BAF	BRG1/BRM-associated factor
Bak	Bcl-2 homologous antagonist/killer
BCL9L	B-cell CLL/lymphoma 9-like protein
BD	bromodomain
BRD4	bromodomain-containing protein 4
BRG1	brahma-related gene 1
BRM	brahma homolog
BSA	bovine serum albumin
CBAF	canonical BAF chromatin remodeling complex
CBP	CREB-binding protein
CCG	Cologne Center for Genomics
cDNA	complementary DNA
CECAD	cluster of excellence for aging research
CER	complete early region
CH1	cysteine/histidine-rich region 1
CH2	cysteine/histidine-rich region 2
CH3	cysteine/histidine-rich region 3
ChIP	chromatin immunoprecipitation
ChIP-seq	chromatin immunoprecipitation sequencing
CIB1	calcium- and integrin-binding protein 1
CITED1	CBP/p300-interacting transactivator with Glu/Asp-rich carboxy-terminal domain 1
CITED2	CBP/p300-interacting transactivator with Glu/Asp-rich carboxy-terminal domain 1
CMV	cytomegalovirus
Co-IP	co-immunoprecipitation
CR	conserved region
CRISPR	clustered regularly interspaced short palindromic repeats
cSCC	cutaneous squamous cell carcinoma
DAB	3,3'-diaminobenzidine
DAPI	4',6-diamidino-2-phenylindole
DDR	DNA damage response
DMSO	dimethyl sulfoxide
DNA	deoxyribonucleic acid
DPBS	Dulbecco's phosphate-buffered saline
DPF2	double plant homeodomain fingers 2
DREAM	dimerization partner, RB-like, E2F and MuvB

DTT	dithiothreitol
E1	HPV early protein 1
E1 [^] E4	HPV E1 [^] E4 protein
E2	HPV early protein 2
E2F	E2 promoter-binding factor
E4	HPV early protein 4
E5	HPV early protein 5
E6	HPV early protein 6
E6AP	E6-associated protein
E7	HPV early protein 7
ECM	extracellular matrix
EDTA	ethylenediaminetetraacetic acid
EGFR	epidermal growth factor receptor
EV	empty vector
EVER1	Epidermodysplasia verruciformis protein 1
EVER2	Epidermodysplasia verruciformis protein 2
FBS	fetal bovine serum
FDR	false discovery rate
FLAG	FLAG octapeptide epitope tag
FPKM	fragments per kilobase of transcript per million mapped reads
G418	Geneticin sulfate
GAPDH	glyceraldehyde-3-phosphate dehydrogenase
GBAF	GLTSCR1/1L-containing BAF chromatin remodeling complex
GO	Gene Ontology
gRNA	guide RNA
GST	glutathione S-transferase
H3K18ac	histone H3 lysine 18 acetylation
H3K27ac	histone H3 lysine 27 acetylation
HA	hemagglutinin
HAT	histone acetyltransferase
HBS	HEPES-buffered saline
HDAC	histone deacetylase
HEPES	4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid
HIF-1 α	hypoxia-inducible factor 1 alpha
HIV	human immunodeficiency virus
HNSCC	head and neck squamous cell carcinomas
HPRT1	hypoxanthine-guanine phosphoribosyltransferase 1
HPV	human papillomavirus
HR-HPV	high-risk human papillomavirus
HSPG	heparan sulfate proteoglycan
hTERT	human telomerase reverse transcriptase
hTERT	human Telomerase Reverse Transcriptase
IARC	International Agency for Research on Cancer
IBID	interferon-binding domain

IDR	intrinsically disordered region
IF	immunofluorescence
IgG	immunoglobulin G
IHC	immunohistochemistry
IP	immunoprecipitation
K111	Lysine 111
K14	keratin 14
KAT3	lysine acetyltransferase type 3 family
KEGG	Kyoto Encyclopedia of Genes and Genomes
KIX	kinase-inducible domain
KO	knockout
L1	late protein 1
L2	late protein 2
LB	lysogeny broth
LFQ	label-free quantification
Lrg1	leucine-rich repeats and immunoglobulin-like domains protein 1
LSDB	low-salt dilution buffer
MAML1	Mastermind-like 1
MHC	major histocompatibility complex
MMP7	matrix metalloproteinase 7
MmuPV1	<i>Mus musculus</i> papillomavirus 1
mRNA	messenger RNA
MS	mass spectrometry
mSWI/SNF	mammalian SWI/SNF
Myc	myelocytomatosis oncogene cellular homolog
MYC	MYC epitope tag
ncBAF	non-canonical BAF
NF- κ B	nuclear factor kappa B
NRID	nuclear receptor interaction domain
OCT4	octamer-binding transcription factor 4
ORF	open reading frame
ori	origin of replication
OTR	organ transplant recipient
p130	retinoblastoma-like protein 2
PaVE	Papillomavirus Episteme
PBAF	polybromo-associated BAF chromatin remodeling complex
PBM	PDZ-binding motif
PBS	phosphate-buffered saline
PCA	principal component analysis
PCR	polymerase chain reaction
PD-L1	programmed death-ligand 1
PHD	plant homeodomain
PHEK	primary human epithelial keratinocyte
PMSF	phenylmethylsulfonyl fluoride

pRb	retinoblastoma protein
PV	papillomavirus
qRT-PCR	quantitative real-time polymerase chain reaction
RING	Really Interesting New Gene
RIPA	radioimmunoprecipitation assay buffer
RNA	ribonucleic acid
RNA-seq	RNA sequencing
ROI	region of interest
RPA	replication protein A
RT	room temperature
SCC	squamous cell carcinoma
SD	standard deviation
SDS	sodium dodecyl sulfate
SDS-PAGE	sodium dodecyl sulfate-polyacrylamide gel electrophoresis
SFM	serum-free medium
SHM	serum-containing medium
SOX2	SRY-box transcription factor 2
SP1/3	specificity protein 1 and 3 transcription factors
SPMR	signal per million reads
STAT1	signal transducer and activator of transcription 1
STAT3	signal transducer and activator of transcription 3
SWI/SNF	switching defective/sucrose non-fermenting chromatin remodeling complex
TAZ	transcriptional co-activator with PDZ-binding motif
TAZ1	transcriptional adaptor zinc-finger 1
TAZ2	transcriptional adaptor zinc-finger 2
TBST	Tris-buffered saline containing Tween-20
TCF/LEF	T-cell factor/lymphoid enhancer factor
TCF7L2	transcription factor 7-like 2
TEAD	TEA domain transcription factor
TF	transcription factor
TGN	trans-Golgi network
TMC6	transmembrane channel-like 6
TMC8	transmembrane channel-like 8
Top1	Topoisomerase I
TOPBP1	DNA-topoisomerase-2-binding protein 1
TSS	transcription start site
UCSC	University of California, Santa Cruz
UHPLC	ultra-high-performance liquid chromatography
URR	upstream regulatory region
UV	ultraviolet
WB	Western blotting
WT	wild type
YAP	Yes-associated protein

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