

1 YAP1 Activation by Human Papillomavirus E7 Promotes Basal Cell Identity in Squamous Epithelia

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16

17 **Abstract**

18 Persistent human papillomavirus (HPV) infection of stratified squamous epithelial cells causes
19 nearly five percent of cancer cases worldwide. HPV-positive oropharyngeal cancers harbor few
20 mutations in the Hippo signaling pathway compared to HPV-negative cancers at the same
21 anatomical site, prompting the hypothesis that an HPV-encoded protein inactivates the Hippo
22 pathway and activates the Hippo effector YAP1. The HPV E7 oncoprotein is required for HPV
23 infection and for HPV-mediated oncogenic transformation. We investigated the effects of HPV
24 oncoproteins on YAP1 and found that E7 activates YAP1, promoting YAP1 nuclear localization in
25 basal epithelial cells. YAP1 activation by HPV E7 required that E7 bind and degrade the tumor
26 suppressor PTPN14. E7 required YAP1 transcriptional activity to extend the lifespan of primary
27 keratinocytes, indicating that YAP1 activation contributes to E7 carcinogenic activity. Maintaining
28 infection in basal cells is critical for HPV persistence, and here we demonstrate that YAP1
29 activation causes HPV E7 expressing cells to be retained in the basal compartment of stratified
30 epithelia. We propose that YAP1 activation resulting from PTPN14 inactivation is an essential,
31 targetable activity of the HPV E7 oncoprotein relevant to HPV infection and carcinogenesis.

32 **Introduction**

33 Human papillomaviruses (HPV) are non-enveloped viruses with circular double-stranded DNA
34 genomes that infect keratinocytes in stratified squamous epithelia (Doorbar et al., 2015; Graham,
35 2017; McBride, 2017). Although most HPV infections are cleared by the immune system, some
36 infections persist and form higher grade lesions that can lead to cancer (Koshiol et al., 2008;
37 McBride, 2021; Radley et al., 2016; Rositch et al., 2013). HPV infection at mucosal epithelial sites
38 causes cancers including oropharyngeal, cervical, vaginal, penile, and anal malignancies (de
39 Martel et al., 2017; Gillison et al., 2015). Nearly 5% of human cancer cases are caused by
40 persistent infection with one of the high-risk (oncogenic) human papillomavirus genotypes (de
41 Martel et al., 2020).

42 Inactivation of host cell tumor suppressors by the high-risk HPV E6 and E7 oncoproteins
43 modulates cellular processes that enable HPV persistence. Two well-characterized instances of
44 tumor suppressor inactivation by HPV are high-risk HPV E6 proteins targeting p53 for
45 proteasome-mediated degradation and high-risk HPV E7 proteins binding and degrading the
46 retinoblastoma protein (RB1) (Heck et al., 1992; Münger et al., 1989; Scheffner et al., 1990;
47 Seavey et al., 1999; Werness et al., 1990). Both p53 degradation and RB1 inactivation are
48 required for productive HPV infection (Collins et al., 2005; Flores et al., 2000; Kho et al., 2013;
49 McLaughlin-Drubin et al., 2005; Wang et al., 2009). In addition to supporting productive infection,
50 E7 is essential for HPV-mediated carcinogenesis (Mirabello et al., 2017). The impact of the HPV
51 oncoproteins on cell growth control pathways is reflected in human cancer genomic data: genes
52 in the p53 pathway and in the RB1-related cell cycle pathway are frequently mutated in HPV-
53 negative head and neck squamous cell carcinoma (HNSCC) but infrequently mutated in HPV-
54 positive HNSCC (Sanchez-Vega et al., 2018).

55 Although some of the growth-promoting activities of high-risk HPV E6 and E7 are well
56 established, open questions remain. RB1 binding/degradation by high-risk HPV E7 is necessary
57 but insufficient for E7 transforming activity (Balsitis et al., 2006, 2005; Banks et al., 1990; Ciccolini

58 et al., 1994; Helt and Galloway, 2002; Huh et al., 2005; Ibaraki et al., 1993; Jewers et al., 1992;
59 Phelps et al., 1992; Strati and Lambert, 2007; White et al., 2015). Papillomavirus researchers
60 have sought to identify one or more activities of HPV E7 that cooperate with RB1 inactivation to
61 promote carcinogenesis and to identify the cellular pathway affected by such an activity. Human
62 cancer genomic data indicates that like the p53 and cell cycle pathways, the Hippo signaling
63 pathway is more frequently mutated in HPV-negative than in HPV-positive HNSCC. The core
64 Hippo pathway consists of a kinase cascade upstream of the effector proteins Yes-Associated
65 Protein (YAP1) and its paralogue TAZ. When the Hippo kinases are inactive, YAP1 and TAZ are
66 activated and translocate to the nucleus. In stratified squamous epithelia YAP1 is primarily
67 expressed in the basal layer, where YAP1 activation is regulated by contextual cues including cell
68 density, tension in the extracellular matrix, and contact with the basement membrane (Elbediwy
69 et al., 2016; Totaro et al., 2017; Zhang et al., 2011). In normal stratified squamous epithelia,
70 activation of YAP1 and TAZ promotes expansion of the basal cell compartment, and inhibition of
71 YAP1 and TAZ allows keratinocytes to differentiate (Beverdam et al., 2013; Elbediwy and
72 Thompson, 2018; Schlegelmilch et al., 2011; Totaro et al., 2017; Yuan et al., 2020; Zhang et al.,
73 2011). Mutations in many of the tumor suppressors upstream of YAP1/TAZ are common in a
74 variety of cancer types (Moroishi et al., 2015).

75 Non-receptor protein tyrosine phosphatase 14 (PTPN14) has been implicated as a tumor
76 suppressor and negative regulator of YAP1 (Knight et al., 2018; Mello et al., 2017; Poernbacher
77 et al., 2012; Wang et al., 2012). Diverse HPV E7 bind directly to PTPN14 and recruit the E3 ligase
78 UBR4 to direct PTPN14 for proteasome-mediated degradation (Szalmás et al., 2017; White et al.,
79 2016, 2012b; Yun et al., 2019). We have shown that PTPN14 degradation and RB1
80 binding/degradation are separable activities of HPV E7 that each contribute to E7 carcinogenic
81 activity (Hatterschide et al., 2020, 2019; White et al., 2016). However, the downstream
82 consequences of PTPN14 degradation are poorly understood, and so far we have not observed

83 that PTPN14 inactivation in human keratinocytes causes an increase in canonical YAP1 target
84 genes *CTGF* and *CYR61*.

85 These observations regarding an additional transforming activity of HPV E7, the ability of
86 E7 to inactivate PTPN14, and the relative paucity of mutations in the Hippo pathway in HPV-
87 positive HNSCC led us to hypothesize that HPV E7-mediated activation of YAP1 is required for
88 the transforming activity of high-risk HPV E7. Here we show that expression of high-risk HPV E7
89 is sufficient to activate YAP1 and that HPV E7 requires YAP1/TAZ-TEAD transcriptional activity
90 to promote cell growth. We demonstrate that HPV E7 must bind PTPN14 to activate YAP1 and
91 that PTPN14 inactivation alone is sufficient to activate YAP1. YAP1 activation by HPV E7 is
92 restricted to the basal layer of the epithelium where we found *PTPN14* expression to be enriched.

93 Our finding that either HPV E7 or PTPN14 loss activate YAP1 specifically in basal
94 epithelial cells led us to investigate the role of YAP1 activation during normal HPV infection. HPV
95 infection begins in basal epithelial keratinocytes (Day and Schelhaas, 2014; Pyeon et al., 2009;
96 Roberts et al., 2007) and infected basal cells are the site of persistent HPV infection. The basal
97 cell compartment contains the only long-lived cells in the epithelium and the HPV genome can be
98 maintained in dividing basal cells without productive replication (Egawa et al., 2012; Parish et al.,
99 2006; You et al., 2004). Activation of YAP1 and TAZ has been proposed to maintain the progenitor
100 cell state in several different epithelia (Beverdam et al., 2013; Heng et al., 2020; Hicks-Berthet et
101 al., 2021; Szymaniak et al., 2015; Yimlamai et al., 2014; Zhao et al., 2014). If YAP1 activation by
102 E7 promotes the maintenance of a basal cell state in stratified squamous epithelia, YAP1
103 activation could facilitate the persistence of HPV-positive cells. Testing this hypothesis, we found
104 that YAP1 activation and PTPN14 degradation by E7 both promote the maintenance of cells in
105 the basal compartment of stratified epithelia. We propose that YAP1 activation facilitates HPV
106 persistence and contributes to the carcinogenic activity of high-risk HPV E7.

107

108 **Results**

109 **HPV E7 activates YAP1 in basal keratinocytes**

110 A comprehensive analysis of somatic mutations and copy number variations in human tumor
111 samples revealed that the cell cycle, p53, and Hippo pathways are the three pathways that exhibit
112 the greatest difference in alteration frequency in HPV-negative vs HPV-positive HNSCC
113 (Sanchez-Vega et al., 2018). We used data made available by The Cancer Genome Atlas (TCGA)
114 through cBioPortal (Lawrence et al., 2015) to recapitulate the finding that genes in these pathways
115 are altered at a lower frequency in HPV-positive than in HPV-negative HNSCC (Figure 1A and
116 Figure 1—figure supplement 1). However, most HPV-positive HNSCC arise in the oropharynx.
117 We repeated the analysis of pathway alteration rates using data only from HPV-positive and HPV-
118 negative oropharyngeal squamous cell carcinomas (OPSCC) (Figure 1A and Figure 1—figure
119 supplement 1). Consistent with previous findings, HPV-negative OPSCC were more frequently
120 altered in the p53, cell cycle, and Hippo pathways than HPV-positive OPSCC. Many of the Hippo
121 pathway alterations in HPV-negative HNSCC or OPSCC are amplification of the YAP1/TAZ
122 oncogenes or inactivating mutation in an upstream inhibitor of YAP1/TAZ. Either alteration type
123 is consistent with a carcinogenic role for YAP1 activation in HNSCC.

124 To test whether an HPV-encoded protein activates YAP1, we grew three dimensional (3D)
125 organotypic epithelial cultures to model the differentiation of keratinocytes into basal and
126 suprabasal compartments. Organotypic cultures of primary human foreskin keratinocytes (HFK)
127 harboring an HPV18 genome exhibited increased YAP1 staining and increased YAP1 nuclear
128 localization, indicative of YAP1 activation, particularly in the basal layer of the epithelium,
129 compared to HFK cultures (Figure 1B and Figure 1—figure supplement 2A,B). Proliferating cell
130 nuclear antigen (PCNA) transcription increases upon RB1 inactivation and is a marker of HPV E7
131 expression. In contrast to the basal layer-specific compartmentalization of YAP1 activation in the
132 HPV18 genome containing cells, PCNA levels were increased in these cultures in both the basal
133 and suprabasal layers of the epithelium.

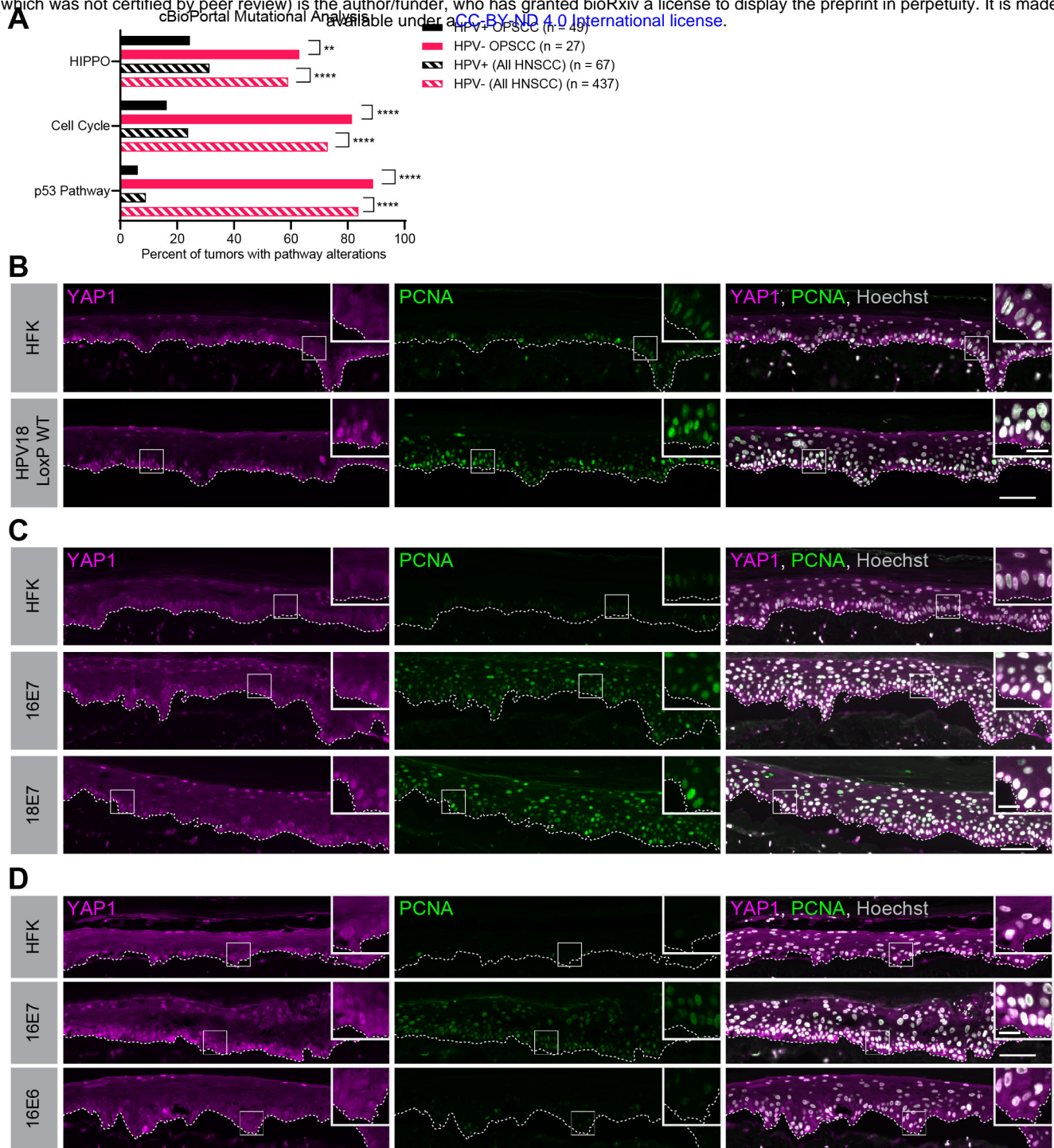


Figure 1 | HPV E7 activates YAP1 in basal epithelial keratinocytes. (A) cBioPortal analysis for total genomic mutations and copy number alterations in HPV+/- OPSCC and HNSCC. Graph displays the percent of tumors with alterations in each pathway. Statistical significance was determined by Fisher's exact test. (B-D) Organotypic cultures were grown from primary HFK, HFK harboring the HPV18 genome, or HFK transduced with retroviral expression encoding HPV E6 or E7 proteins. FFPE sections of cultures grown from (C) HFK or HFK harboring the HPV18 genome, (D) HFK or HFK expressing HPV16 E7 or HPV18 E7, or (E) HFK or HFK expressing HPV16 E6 or HPV16 E7 were stained for YAP1 (magenta), PCNA (green), and Hoechst (gray). White dashed lines indicate the basement membrane. White boxes indicate the location of insets in main images. Main image scale bars = 100 μ m. Inset scale bars = 25 μ m.

134 We next tested whether high-risk HPV E6 or E7 alone was sufficient to activate YAP1.
135 HFK transduced with retroviral expression vectors encoding HPV16 E6, HPV16 E7, or HPV18 E7
136 were used to grow organotypic cultures. YAP1 expression and nuclear localization were
137 increased in the HPV16 E7 and HPV18 E7 expressing cells relative to parental HFK cells ([Figure](#)
138 [1C](#) and [Figure 1—figure supplement 3A-C](#)). As in the HPV18 genome-containing cells, YAP1
139 activation was restricted to the basal epithelial layer. YAP1 expression or nuclear localization did
140 not increase in organotypic cultures of HPV16 E6 expressing cells ([Figure 1D](#) and [Figure 1—](#)
141 [figure supplement 4](#)). Constitutive expression of either HPV16 E7 or HPV18 E7 induced PCNA
142 expression in basal and suprabasal cells. We conclude that HPV promotes increased YAP1
143 expression and nuclear localization in basal keratinocytes and that E7 is sufficient for YAP1
144 activation.

145

146 **HPV E7 activates YAP1 in keratinocytes through PTPN14 degradation**

147 We previously discovered that HPV E7 targets the YAP1 inhibitor PTPN14 for proteasome-
148 mediated degradation (White et al., 2016, 2012b). We tested whether loss of PTPN14 expression
149 in keratinocytes was sufficient to activate YAP1 in stratified epithelia by growing 3D organotypic
150 cultures from previously described control and PTPN14 knockout (KO) N/Tert-Cas9 keratinocytes
151 (Hatterschide et al., 2019). We found that YAP1 levels and YAP1 nuclear localization were
152 increased in PTPN14 KO cultures compared to controls ([Figure 2A](#) and [Figure 2—figure](#)
153 [supplement 1A-C](#)). YAP1 activation in basal epithelial cells lacking PTPN14 was comparable to
154 YAP1 activation in HPV E7 cells. We conclude that loss of PTPN14 expression activates YAP1
155 in basal keratinocytes.

156 A highly conserved C-terminal arginine in E7 makes a direct interaction with the C-
157 terminus of PTPN14, and the HPV18 E7 R84S variant is unable to bind or degrade PTPN14
158 (Hatterschide et al., 2020; Yun et al., 2019). To test whether PTPN14 degradation by HPV E7 is
159 required for activation of YAP1, we grew 3D organotypic cultures using primary HFK transduced

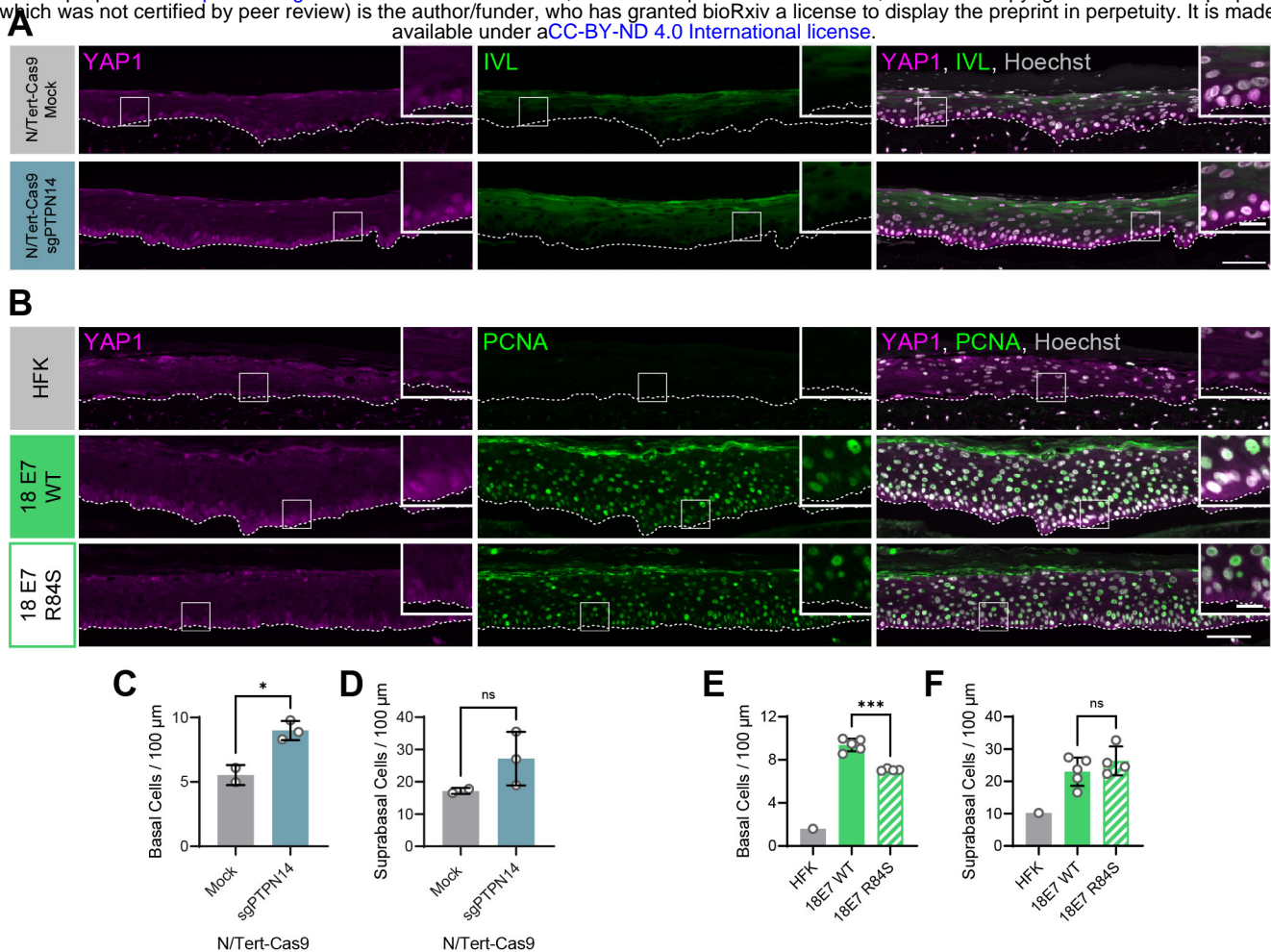


Figure 2 | HPV E7 activates YAP1 in basal keratinocytes through PTPN14 degradation. Organotypic cultures were grown from N/Tert-Cas9 keratinocytes or primary HFK transduced with retroviral expression vectors encoding HPV18 E7 WT or R84S. (A) FFPE sections of cultures grown from mock or sgPTPN14 transfected N/Tert-Cas9 keratinocytes were stained for YAP1 (magenta), IVL (green), and Hoechst (Gray). (B) FFPE sections of cultures grown from parental HFK, HPV18 E7 WT or HPV18 E7 R84S expressing HFK were stained for YAP1 (magenta), PCNA (green), and Hoechst (Gray). White dashed lines indicate the basement membrane. White boxes indicate the location of insets in main images. Main image scale bars = 100 μm. Inset scale bars = 25 μm. (C-F) Quantification of the number of (C and E) basal cells and (D and F) suprabasal cells per 100 μm of epidermis. Graphs display the mean ± SD and each individual data point (independent cultures). Statistical significance was determined by ANOVA (* $p < 0.05$, *** $p < 0.001$).

160 with retroviral expression vectors encoding HPV18 E7 wild type (WT) or HPV18 E7 R84S. Indeed,
161 YAP1 expression and nuclear localization in the basal layer of HPV18 E7 R84S cultures were
162 reduced compared to HPV18 E7 WT controls (Figure 2B and Figure 2—figure supplement 2).

163 In addition to activating YAP1, PTPN14 loss increased basal cell density from an average
164 of 5.5 cells per 100 μm in control cultures to 9.0 cells per 100 μm in PTPN14 KO cultures (Figure
165 2C). Basal cell density was higher in HPV18 E7 WT cultures (9.4 cells per 100 μm) than in HPV18
166 E7 R84S cultures (to 7.1 cells per 100 μm) (Figure 2E). No statistically significant difference in
167 suprabasal cell density was observed in either comparison (Figure 2D,F). We conclude that E7
168 expression or PTPN14 loss in stratified squamous epithelia is sufficient to activate YAP1 in the
169 basal layer of the epithelium and increase basal cell density.

170

171 **PTPN14 expression is enriched in basal keratinocytes**

172 YAP1 activation was restricted to basal epithelial cells in our organotypic cultures leading us to
173 hypothesize that PTPN14 may act as a basal layer specific inhibitor of YAP1. We therefore sought
174 to determine whether *PTPN14* expression is restricted to a specific subset of cells in the stratified
175 epithelium. In a recent single cell-RNA seq analysis of human neonatal foreskin epidermis,
176 *PTPN14* mRNA expression was enriched in the basal-III cluster, a subset of basal cells predicted
177 to differentiate directly into spinous cells (Figure 3A,B) (S. Wang et al., 2020). *PTPN14* expression
178 was higher in basal-III cells than in the spinous or granular cell clusters. To test whether *PTPN14*
179 expression is higher in basal or suprabasal cells in our cultures, we used laser capture
180 microdissection to isolate basal and suprabasal layers from 3D organotypic cultures grown from
181 unmodified primary HFK (Figure 3C). We found that there was a ~5-fold enrichment of *PTPN14*
182 mRNA in the basal epithelial layer compared to the suprabasal layers (Figure 3D). As expected,
183 the basal integrins *ITGA6* and *ITGB4* were expressed in the basal layer (Figure 3E) and the
184 differentiation markers *KRT1* and *IVL* were expressed in the suprabasal layers (Figure 3F). The
185 same pattern of *PTPN14* mRNA expression was observed in an organotypic culture grown from

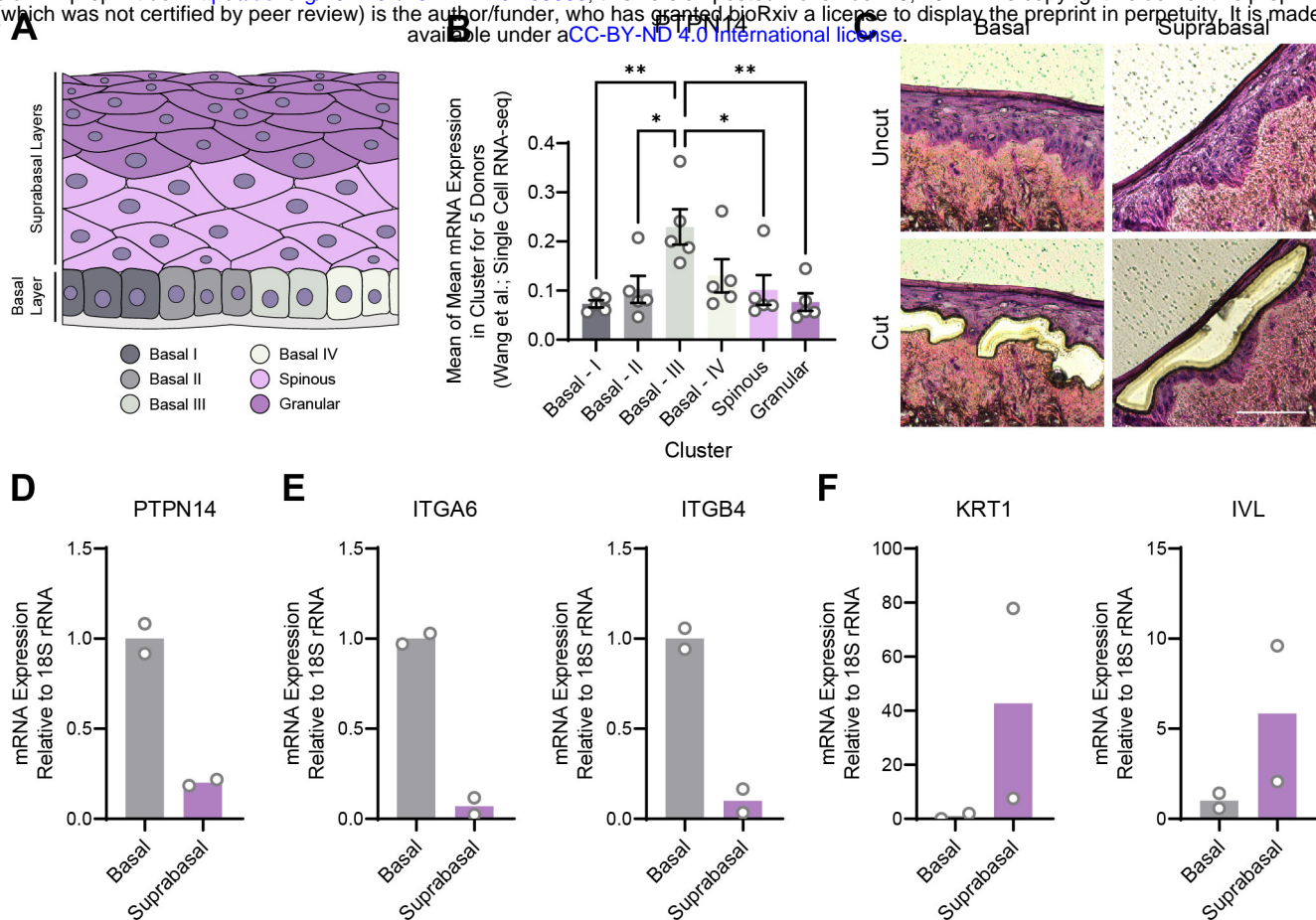


Figure 3 | PTPN14 expression is enriched in basal keratinocytes. (A-B) Single-cell RNA sequencing data and clustering analysis from Wang et al. was reanalyzed to assess PTPN14 expression in different subsets of epidermal cells. (A) Diagram of epidermis; shading depicts tissue localization of cell clusters. (B) For each donor, the mean of PTPN14 mRNA expression was calculated for each cell cluster. Graphs display the mean of PTPN14 mRNA expression for each donor (circles) as well as the mean of all five donors \pm SEM (bars and error bars). Statistical significance was determined by ANOVA ($*p < 0.05$, $**p < 0.01$). (C-F) Basal and suprabasal layers from organotypic cultures were dissected using laser capture microdissection. (C) Representative images of HFK cultures before and after individual laser dissections. Hundreds of such cuts were performed per sample. (D-F) RNA was purified from isolated layers and qRT-PCR was used to assess the expression of PTPN14 (D), basal cell markers ITGA6 and ITGB4 (E), and differentiation markers KRT1 and IVL (F). Graphs display the mean and each individual data point.

186 primary HFK expressing HPV18 E7 WT (Figure 3—figure supplement 1A-C). We conclude that
187 *PTPN14* mRNA is enriched in basal keratinocytes in the presence or absence of HPV E7. Our
188 data support that *PTPN14* acts as a YAP1 inhibitor specifically in the basal compartment of
189 stratified epithelia.

190

191 **YAP1/TAZ regulate differentiation downstream of PTPN14**

192 In previous unbiased experiments we found that the primary effect of *PTPN14* inactivation on
193 transcription is to repress epithelial differentiation gene expression (Hatterschide et al., 2020,
194 2019). However, we also observed that *PTPN14* inactivation did not increase expression of the
195 canonical YAP1/TAZ targets *CTGF* and *CYR61*. Consistent with this difference there was minimal
196 overlap between *PTPN14*-dependent differentially expressed genes and the genes listed in the
197 MSigDB conserved YAP1 signature (Figure 4A). We therefore asked whether the ability of
198 *PTPN14* to regulate differentiation gene expression requires YAP1/TAZ as intermediates.
199 Transduction of keratinocytes with a *PTPN14* lentivirus induced the expression of the
200 differentiation markers *KRT10* and *IVL* in a dose-dependent manner (Figure 4—figure supplement
201 1A-C). To test whether *PTPN14* required YAP1/TAZ to increase *KRT1* and *IVL*, we transfected
202 HFK with siRNAs targeting *YAP1* and *WWTR1* then transduced the cells with *PTPN14* lentivirus
203 (Figure 4B). HFK transfected with control siRNA exhibited the expected increase in *KRT1* and
204 *IVL* after transduction with *PTPN14* lentivirus (Figure 4C,D and Figure 4—figure supplement
205 2A,B). However, keratinocytes depleted of YAP1/TAZ did not express relatively more *KRT1* or
206 *IVL* when *PTPN14* was overexpressed than when it was not. We conclude that *PTPN14* requires
207 YAP1 and/or TAZ to regulate differentiation gene expression in keratinocytes. Both pairs of
208 YAP1/TAZ siRNA had the same effect on differentiation in response to *PTPN14* overexpression
209 yet only one pair efficiently depleted TAZ protein levels (Figure 4B), leading us to speculate that
210 YAP1 is the key intermediate connecting *PTPN14* levels to differentiation gene expression.

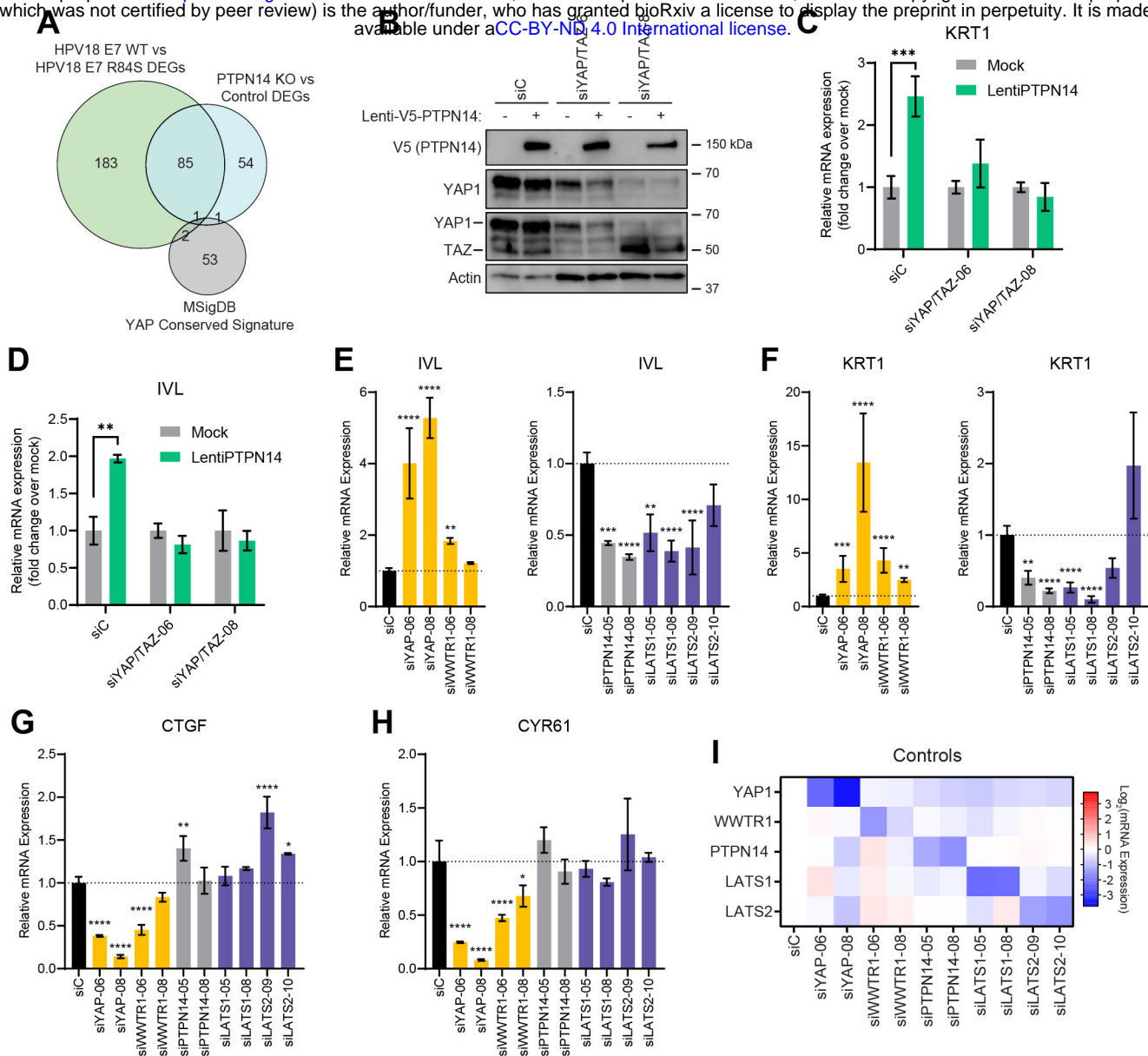


Figure 4 | YAP1/TAZ regulate differentiation downstream of PTPN14. (A) Venn diagram comparing the MSigDB YAP conserved signature to the differentially expressed genes (DEG) from our two published experiments that reflect PTPN14 loss in keratinocytes. (B-D) YAP1 and WWTR1 were simultaneously knocked down by siRNA transfection in HFK. Transfected HFK were then transduced with PTPN14 lentivirus at 24h post transfection. Cells were lysed for protein and total cellular RNA at 72h post transfection. (B) Cell lysates were subjected to SDS/PAGE/Western analysis and probed with antibodies to PTPN14, YAP1, TAZ, and Actin. (C and D) qRT-PCR was used to measure the expression of the differentiation markers KRT1 and IVL relative to G6PD. Graphs display fold change in gene expression relative to the mock transduced cells. (E-I) Primary HFK were transfected with siRNAs targeting YAP1, WWTR1 (TAZ), PTPN14, LATS1, and LATS2. Two siRNAs were used per target. qRT-PCR was used to measure gene expression for: the differentiation markers IVL (E) and KRT1 (F), and the canonical YAP1/TAZ targets CTGF (G) and CYR61 (H). Data confirming that individual siRNA transfections depleted intended transcripts is summarized in a heatmap of log₂(fold-change) levels (I). Bar graphs display the mean \pm SD of three independent replicates. Statistical significance was determined by ANOVA (*p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001).

211 Next, we tested whether repression of keratinocyte differentiation occurs upon loss of
212 LATS1 and LATS2, the core Hippo pathway kinases that phosphorylate and inhibit YAP1 and
213 TAZ. We used siRNAs to deplete *PTPN14*, *LATS1*, or *LATS2* and measured the expression of
214 the differentiation markers *KRT1* and *IVL* (Figure 4E,F). Depletion of *PTPN14*, *LATS1*, or *LATS2*
215 all decreased differentiation gene expression to a similar degree. Consistent with our previous
216 experiments, none of the three knockdowns significantly affected the levels of *CTGF* or *CYR61*
217 (Figure 4G-H). Direct depletion of *YAP1* or *WWTR1* affected both differentiation gene expression
218 and *CTGF/CYR61* levels. *YAP1* knockdown always had a stronger effect than did *WWTR1*
219 knockdown and our qRT-PCR analyses supported that *WWTR1* transcript levels were low in HFK.
220 This result shows that inactivation of three different YAP1 inhibitors dampens differentiation gene
221 expression and does not increase canonical YAP1 target gene expression in keratinocytes. Taken
222 together, these data support that PTPN14 promotes differentiation through inhibition of YAP1/TAZ
223 despite not affecting canonical YAP1/TAZ target genes.

224

225 **HPV-positive HNSCC are less differentiated than HPV-negative HNSCC**

226 We next asked whether the gene expression pattern observed downstream of PTPN14 loss is
227 reflected in HPV-positive cancers. HPV-positive HNSCC have a strong propensity toward poorly
228 differentiated, basaloid histology (Mendelsohn et al., 2010; Pai and Westra, 2009), which is
229 reflected in their transcriptional profile (Hatterschide et al., 2019). We confirmed the relationship
230 between HPV positivity and greater impairment of differentiation by immunohistochemical
231 analysis of the differentiation marker *KRT1* in sections of 14 HPV-negative tumors and 48 HPV-
232 positive tumors (Figure 5A). 43% of HPV-negative tumors and 12.5% of HPV-positive tumors
233 stained positive for *KRT1*. We additionally measured gene expression in patient-derived xenograft
234 (PDX) models generated from human HNSCC. We measured *KRT1*, *KRT10*, and *IVL* levels using
235 RNA extracted from 11 HPV-negative and 8 HPV-positive HNSCC PDX. Each differentiation
236 marker was expressed at a markedly lower level in HPV-positive PDX than in HPV-negative PDX

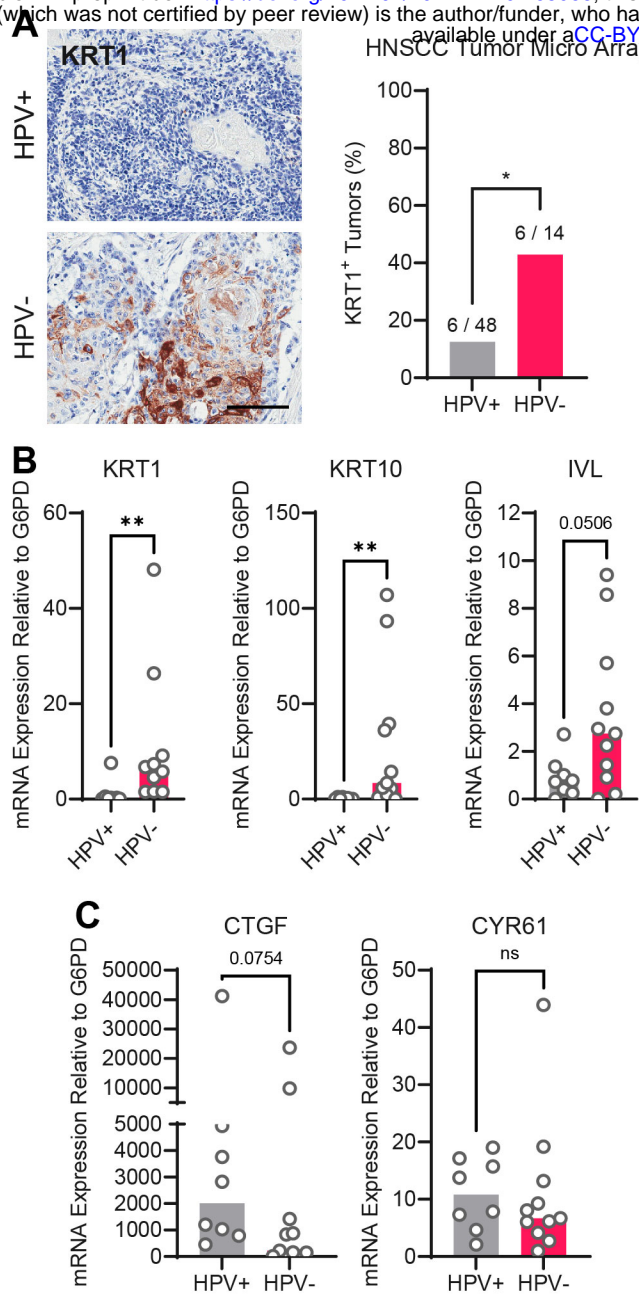


Figure 5 | HPV-positive HNSCC are less differentiated than HPV-negative HNSCC. (A) Human HNSCC tumor samples were stained for KRT1 (left). Scale bar = 100 μ m. Graph displays the percentage of tumors that were KRT1⁺ (right). Statistical significance was determined by Fisher's exact test. (B-C) Total RNA was purified from PDX samples and qRT-PCR was used to assess gene expression of (B) the differentiation markers KRT1, KRT10, and IVL and (C) the canonical YAP1/TAZ targets CTGF and CYR61. Statistical significance was determined by Mann-Whitney nonparametric test. (* $p < 0.05$, ** $p < 0.01$, **** $p < 0.0001$).

237 (Figure 5B). We observed the same pattern of differentiation marker gene expression in an
238 analysis of transcriptomic data from other cohorts (Figure 5—figure supplement 1A-C) (Lawrence
239 et al., 2015). Having confirmed that HPV-positive HNSCC exhibit reduced expression of
240 differentiation markers than do HPV-negative HNSCC, we measured *CTGF* and *CYR61* levels.
241 We found no significant difference in expression of these canonical YAP1/TAZ target genes in
242 HPV-positive vs HPV-negative PDX, although there was a trend towards higher *CTGF* in the HPV-
243 positive PDX (Figure 5C and Figure 5—figure supplement 1D,E). The pattern of low expression
244 of differentiation markers and unchanged canonical YAP1/TAZ target gene expression in HPV-
245 positive versus HPV-negative patient samples is consistent with the effects of PTPN14
246 inactivation in cultured cells.

247

248 **High-risk HPV E7 require YAP1/TAZ-TEAD transcriptional activity to extend the lifespan of**
249 **primary keratinocytes.**

250 High-risk but not low-risk HPV E7 proteins can extend the lifespan of primary keratinocytes
251 (Halbert et al., 1991). The TEADi protein is a genetically encoded competitive inhibitor that
252 prevents binding between YAP1/TAZ and TEAD transcription factors (Yuan et al., 2020). We used
253 TEADi to test whether YAP1/TAZ-TEAD transcriptional activity was required for high-risk HPV E7
254 to extend the lifespan of primary HFK. We transduced HFK with retroviral vectors encoding GFP,
255 HPV16 E7, or HPV18 E7 plus a lentiviral vector encoding doxycycline-inducible GFP-TEADi. As
256 anticipated, HPV16 E7 or HPV18 E7 extended the lifespan of primary HFK based on cumulative
257 population doublings (Figures 6A,B). TEADi induction upon doxycycline treatment decreased the
258 lifespan of primary HFK in the presence or absence of E7, but the effect of YAP1/TAZ-TEAD
259 inhibition was greater in the HPV16 E7 and HPV18 E7 cells, where E7 had minimal ability to
260 promote growth in the presence of TEADi. We conclude that high-risk HPV E7 proteins require
261 YAP1/TAZ-TEAD transcriptional activity for their lifespan extending capacity in primary
262 keratinocytes.

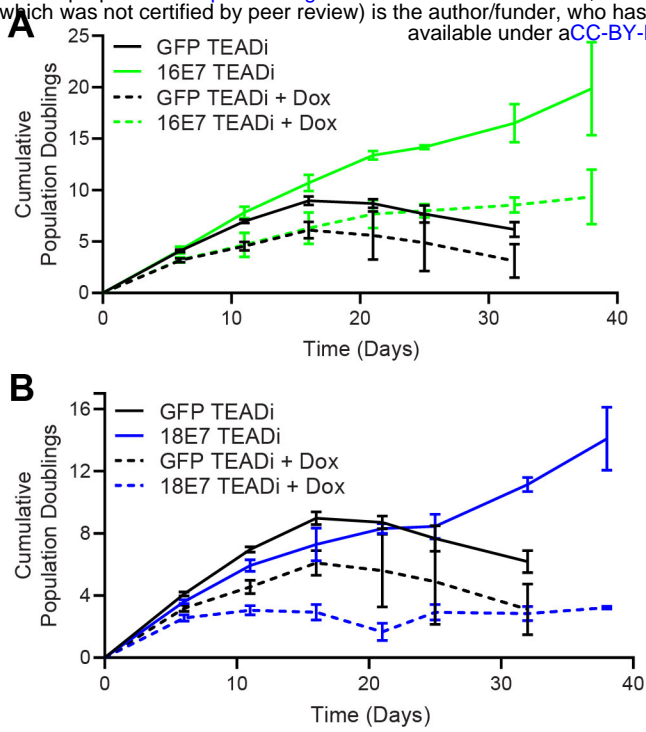


Figure 6 | High-risk HPV E7 requires YAP1/TAZ-TEAD transcriptional activity to extend the lifespan of primary keratinocytes. Primary HFK were transduced with retroviruses encoding HPV16 E7, HPV18 E7, or GFP, plus pInducer20 TEADi lentivirus. Each cell population was cultured with or without 1 μ g/mL doxycycline in the media for 38 days and population doublings were tracked with each passage. Graph displays the mean \pm SD of two independently transduced cell populations per condition.

263

264 **PTPN14 loss and YAP1 activation promote basal cell retention in organotypic cultures**

265 YAP1 overexpression impairs differentiation and promotes progenitor cell identity in squamous
266 and non-squamous epithelia. HPV infection is maintained in a reservoir of infected basal cells and
267 productive virus replication begins upon commitment to differentiation. To better understand how
268 repression of differentiation downstream of YAP1 activation affects HPV viral biology, we
269 developed an assay to measure cell retention in the basal epithelial layer. We hypothesized that
270 YAP1 activation by HPV E7 might promote the adoption of a basal cell identity in stratified
271 squamous epithelia. In our cell fate monitoring assay, a small proportion of GFP-labeled cells
272 were mixed with unmodified, parental HFK, and the pool was used to generate organotypic
273 cultures in which normal labeled cells are randomly distributed throughout the epithelium.

274 Our initial experiment tested whether YAP1 activation altered cell fate in stratified
275 squamous epithelia. We used GFP-labeled tracing cells that expressed doxycycline-inducible
276 YAP1 WT, YAP1 S127A (hyperactive), or YAP1 S94A (cannot bind TEAD transcription factors)
277 ([Figure 7—figure supplement 1A,B](#)). In organotypic cultures grown from a 1:25 mixture of GFP-
278 labeled cells and unmodified HFK, about 20% of uninduced GFP+ cells were found in the basal
279 layer. Induction of YAP1 WT or YAP1 S127A expression was sufficient to promote the retention
280 of nearly 60% of labeled cells in the basal layer of the epithelium ([Figure 7A,B](#)). Only around 40%
281 of GFP+ cells were found in the basal layer when YAP1 S94A was induced. These data indicate
282 that YAP1 activation causes cells to be retained in the basal layer of a stratified squamous
283 epithelium. The ability of YAP1 to bind TEAD transcription factors contributed to its activity in the
284 cell fate assay.

285 We next tested whether loss of PTPN14 expression was sufficient to promote basal cell
286 identity. We grew organotypic cultures from mixtures of unmodified primary HFK and GFP-labeled
287 control or PTPN14 KO HFK ([Figure 7—figure supplement 1C,D](#)). 60-70% of PTPN14 KO tracer
288 cells were found in the basal layer when either of two PTPN14 guide RNAs were used whereas

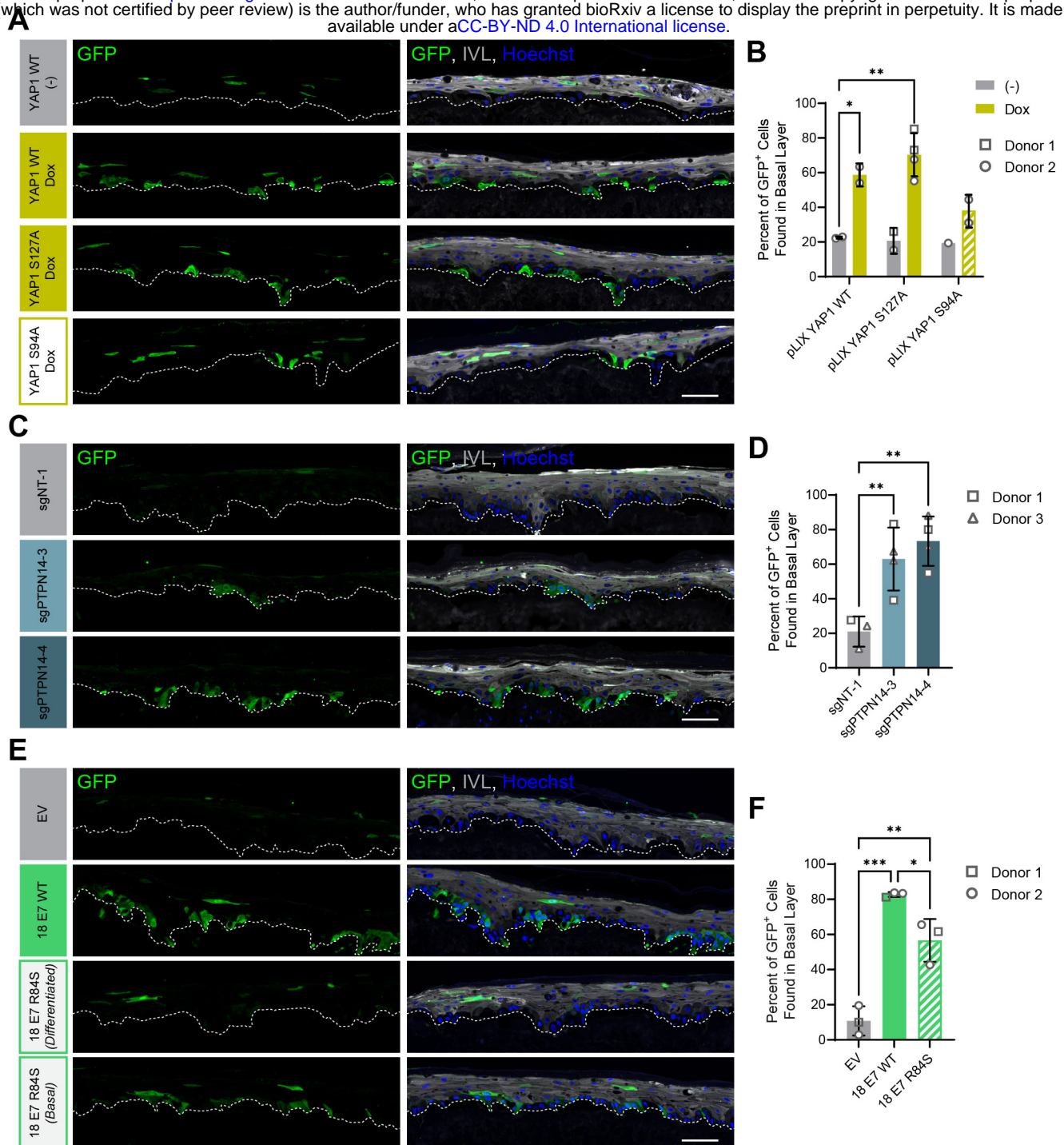


Figure 7 | PTPN14 loss and YAP1 activation by HPV E7 promote basal cell retention in organotypic cultures. Organotypic cultures were grown from GFP-labeled HFK mixed with unmodified HFK. (A-B) GFP-labeled HFK were transduced with lentiviral vectors encoding YAP1 WT, YAP1 S127A, or YAP1 S94A under the control of a doxycycline inducible promoter. GFP-labeled YAP1 cells were mixed 1:25 into unmodified HFK and organotypic cultures were grown from the mixture. Cultures were grown +/- 1 μ g/mL doxycycline. (C-D) GFP-labeled HFK were transduced with LentiCRISPR v2 vectors encoding control or PTPN14 targeting sgRNAs. GFP-labeled cells were mixed 1:25 into unmodified HFK and organotypic cultures were grown from the mixture. (E-F) GFP-labeled HFK were transduced with HPV18 E7 WT, HPV18 E7 R84S, or the empty vector (EV). GFP-labeled HPV18 E7 cells were mixed 1:50 into unmodified HFK and organotypic cultures were grown from the mixture. (A, C, E) FFPE sections of cultures were stained for GFP (green), IVL (grey), and Hoechst (blue). Scale bar = 100 μ m. (B, D, F) Quantification of the percentage of GFP+ cells found in the basal layer. Graphs display the mean \pm SD and each individual data point (independent cultures). Shapes indicate cultures grown from different HFK donors. Statistical significance was determined by ANOVA. (* p <0.05, ** p <0.01).

289 about 20% of control tracer cells were retained in the basal layer (Figure 7C,D). Thus, PTPN14
290 knockout is sufficient to promote basal cell fate determination in keratinocytes.

291 Next, we tested whether HPV E7 promoted basal cell retention and if so, whether its cell
292 retention activity required PTPN14 degradation. We grew organotypic cultures from mixtures of
293 GFP-labeled HFK expressing HPV18 E7 WT, HPV18 E7 R84S, or the empty vector control diluted
294 1:50 into unmodified primary HFK (Figure 7—figure supplement 1E,F). We found that nearly 80%
295 of GFP-labeled HPV18 E7 WT tracer cells were retained in the basal layer compared to about
296 10% of labeled control cells (Figure 7E,F). HPV18 E7 WT labeled cells were numerous and
297 grouped in clusters in the basal layer, suggesting that E7 promoted the clonal expansion of
298 labeled basal cells. Both effects were dampened in experiments using HPV18 E7 R84S tracer
299 cells (cannot degrade PTPN14). Labeled HPV18 E7 R84S cells exhibited varying degrees of
300 basal cell expansion and basal cell retention and approximately 60% of labeled cells were in the
301 basal layer. HPV18 E7 R84S retains the ability to inactivate RB1 and we interpret these data to
302 mean that the proliferation of labeled basal cells resulted from RB1 inactivation. Finally, HPV18
303 E7 Δ DLLC cannot bind RB1 but can bind and degrade PTPN14. In a cell fate experiment using
304 GFP-labeled HPV18 E7 Δ DLLC tracer cells, the labeled cells were present mainly as single cells
305 in the basal layer (Figure 7—figure supplement 2A-B). The behavior of the two mutant HPV E7
306 proteins supports that PTPN14 degradation is required for basal cell retention and RB1
307 inactivation is required for basal cell expansion. We conclude that PTPN14 degradation and YAP1
308 activation by HPV18 E7 promote basal cell retention.

309

310 Discussion

311 YAP1 and TAZ are oncogenes that promote growth and inhibit differentiation in stratified
312 squamous epithelia (Elbediwy et al., 2016; Schlegelmilch et al., 2011; Totaro et al., 2017; Yuan
313 et al., 2020; Zhang et al., 2011). Here we report that HPV E7 activates YAP1 (Figure 1).
314 YAP1/TAZ-TEAD transcriptional activity is required for the carcinogenic activity of HPV E7 (Figure

315 6) and YAP1 activation by E7 biases HPV E7-expressing cells to be retained in the basal epithelial
316 layer (Figure 7). Based on these findings we propose that YAP1 activation by HPV E7 enables
317 HPV-infected cells to persist in stratified epithelia. There is substantial evidence that RB1
318 inactivation is necessary but insufficient for the transforming activity of high-risk HPV E7 (Balsitis
319 et al., 2006, 2005; Banks et al., 1990; Ciccolini et al., 1994; Helt and Galloway, 2002; Huh et al.,
320 2005; Ibaraki et al., 1993; Jewers et al., 1992; Phelps et al., 1992; Strati and Lambert, 2007; White
321 et al., 2015). We propose that YAP1 activation cooperates with RB1 inactivation to enable the
322 transforming activity of HPV E7.

323 PTPN14 binding by HPV18 E7 was required for activation of YAP1 in the basal layer and
324 PTPN14 KO was sufficient for the same effect (Figure 2). Highly conserved amino acids in E7
325 participate in binding to PTPN14 (Hatterschide et al., 2020; Yun et al., 2019), indicating that YAP1
326 activation and maintenance of basal cell state is likely shared among diverse papillomavirus E7
327 proteins. Some minor genotype-specific differences were apparent. HPV18 E7 depletes PTPN14
328 protein levels more efficiently than HPV16 E7 (Hatterschide et al., 2020; White et al., 2016), which
329 is consistent with the observed stronger effect of HPV18 E7 on YAP1 nuclear localization in basal
330 cells (Figure 1). Genotype-specific differences could also explain the stronger effect of TEADi on
331 HPV18 E7 in lifespan extension assays (Figure 6). Although other reports have suggested that
332 HPV might activate YAP1 (He et al., 2015; Morgan et al., 2020; Olmedo-Nieva et al., 2020; Webb
333 Strickland et al., 2018), no specific activity of an HPV protein has previously been shown to enable
334 YAP1 activation. Other groups have proposed that HPV E6 activates YAP1 (He et al., 2015; Webb
335 Strickland et al., 2018), but we did not observe YAP1 activation by HPV E6. We conclude that
336 activation of YAP1 by HPV E7 is contingent upon its ability to bind and degrade PTPN14.

337 Even when HPV E7 was expressed in all layers of a stratified epithelium, YAP1 levels and
338 nuclear localization increased only in basal epithelial cells. We found that E7 required PTPN14
339 degradation to activate YAP1 and that PTPN14 was expressed predominantly in basal
340 keratinocytes (Figure 3). Basal cell-specific expression of *PTPN14* is consistent with the

341 observation that it is regulated by p63, the master regulator of basal cell identity in stratified
342 epithelia (Perez et al., 2007). We propose that PTPN14 inhibits YAP1 primarily in basal cells and
343 that unlike the effects of E7 on RB1 in both differentiated and undifferentiated cells, E7 activates
344 YAP1 primarily in basal cells.

345 Degradation of PTPN14 by HPV E7 represses keratinocyte differentiation but does not
346 induce canonical Hippo pathway target genes (Hatterschide et al., 2020, 2019). Nonetheless, we
347 found that PTPN14 overexpression promoted differentiation only in the presence of YAP1/TAZ
348 (Figure 4C,D). Few studies have tested how YAP1 inhibitor inactivation alters gene expression
349 downstream of YAP1. Here we demonstrate that inactivation of LATS1 or LATS2, two well-
350 characterized inhibitors of YAP1/TAZ, also repressed differentiation genes but did not induce
351 canonical YAP1/TAZ targets (Figure 4E-I). Taken together, these experiments indicate that
352 PTPN14 acts through YAP1/TAZ to regulate differentiation in keratinocytes. It is so far unclear
353 why *CTGF* and *CYR61* expression is sensitive to large changes in total levels of YAP1 or TAZ
354 yet is unaffected by alterations in regulators upstream of YAP1/TAZ. Nonetheless, the pattern of
355 low differentiation gene expression and unchanged expression of canonical YAP1/TAZ target
356 genes caused by PTPN14 loss is consistent with gene expression differences between HPV-
357 positive and HPV-negative HNSCC.

358 PTPN14 knockout and knockdown reduced differentiation gene expression in monolayer
359 culture. Even so, we did not observe reduced differentiation in suprabasal layers of organotypic
360 cultures grown from PTPN14 knockout cells (Figure 2A and Figure 2—figure supplement 1A-C).
361 Using our cell fate monitoring assay, we determined that instead, HPV18 E7 promotes basal cell
362 retention and that either YAP1 overexpression or PTPN14 KO are sufficient for this activity (Figure
363 7). The effect of YAP1 activation on cell fate in our assay resembles several experiments in which
364 YAP1 promotes progenitor cell identity in airway and liver epithelia (Yimlamai et al., 2014; Zhao
365 et al., 2014). Our findings demonstrate that YAP1 activation enables basal cell fate determination
366 in stratified squamous epithelia and show that loss of an inhibitor of YAP1 has the same effect.

367 We conclude that one consequence of YAP1 activation by HPV E7 is that E7-expressing cells are
368 retained in the basal layer of stratified squamous epithelia.

369 Although persistent infection is a prerequisite for HPV-mediated carcinogenesis, the
370 mechanisms used by papillomaviruses to establish persistent infections remain incompletely
371 understood. Maintaining infection in the basal cell compartment is critical for papillomavirus
372 persistence. Substantial effort has been devoted to the mechanistic understanding of how the
373 papillomavirus genome is stably maintained in the basal layer upon cell division. However, much
374 less is known about how papillomaviruses manipulate epithelial cell fate to establish and expand
375 the pool of infected basal cells. Previously, HPV E7 was believed to be primarily required to
376 establish a cellular environment conducive to HPV DNA replication in suprabasal cells. We
377 propose that a so far unappreciated role of E7 is that it activates YAP1 to facilitate HPV
378 persistence by biasing infected cells to remain in the basal layer of the epithelium. Not every HPV
379 E7-expressing cell was retained in the basal layer, so we do not anticipate that YAP1 activation
380 would block differentiation-dependent HPV replication. HPV E6 also represses differentiation
381 gene expression in keratinocytes and has been proposed to promote basal cell retention (Kranjec
382 et al., 2017). Further research is needed to determine the extent to which different HPV genotypes
383 depend on the activities of E6 or E7 for basal cell retention activity.

384 To the best of our knowledge, no other viruses are recognized to modulate cell fate
385 decisions in solid tissues in a way that facilitates persistence. Some herpesviruses impact the
386 choice between progenitor/differentiated cell fates in infected immune cells, for example Epstein-
387 Barr Virus (EBV) restricts B-cell differentiation to facilitate viral latency (Knox and Carrigan, 1992;
388 Niiya et al., 2006; Onnis et al., 2012; Romeo et al., 2019; Styles et al., 2017). Herpesviruses,
389 polyomaviruses, and hepadnaviruses encode proteins proposed to activate YAP1/TAZ or alter
390 Hippo signaling (Hwang et al., 2014; Liu et al., 2014, 2015; Nguyen et al., 2014; Shanzer et al.,
391 2015; Tian et al., 2004; Z. Wang et al., 2020). Not all of the mechanisms used by these viruses
392 to activate YAP1 nor the downstream consequences of YAP1 activation have been well defined.

393 Our finding that HPV E7 activates YAP1 to manipulate cell fate opens up an exciting new line of
394 inquiry into how YAP1, TAZ, and the Hippo signaling pathway could impact viral infections by
395 regulating tissue developmental processes.

396 YAP1 activation and PTPN14 are relevant to both viral and non-viral cancers. We found
397 that a genetically encoded inhibitor of YAP1/TAZ-TEAD transcription inhibited the growth of high-
398 risk HPV E7 expressing cells (Figure 6), indicating that high-risk HPV E7 proteins require YAP1
399 or TAZ for carcinogenesis. YAP1/TAZ activation is sufficient to drive carcinogenesis in mouse
400 models of cervical and oral cancer (He et al., 2019; Nishio et al., 2020; Omori et al., 2020), and
401 the YAP1 inhibitor verteporfin reduced the growth of HPV-positive tumors in a xenograft model
402 (Liu et al., 2019). YAP1 activation correlates with the clinical stage of HPV infection (Nishio et al.,
403 2020), and YAP1 localizes to the nucleus in HPV-positive cancers (Alzahrani et al., 2017). Basal
404 cell carcinoma (BCC) is the non-viral cancer that is most clearly linked to PTPN14. Germline
405 inactivating mutations in *PTPN14* are associated with a 4- to 8-fold increase in risk of BCC by age
406 70 (Olafsdottir et al., 2021) and somatic mutations in *PTPN14* are frequent in BCC (Bonilla et al.,
407 2016). YAP1/TAZ-TEAD transcriptional activity also restricts differentiation in BCC cells (Yuan et
408 al., 2021). We propose that the specific association of PTPN14 with BCC is related to our
409 observation that PTPN14 loss activates YAP1 in basal epithelial cells. YAP1 inhibition is of major
410 clinical interest for several cancer types, and it is appealing to speculate that targeting YAP1 could
411 treat persistent HPV infection and/or HPV-positive cancers.

412

413

414 **Materials and Methods**

415 **Plasmids and cloning.** pInducer20 EGFP-TEADi was a gift from Ramiro Iglesias-Bartolome
416 (Addgene plasmid # 140145) (Yuan et al., 2020). pQCXIH-Myc-YAP (Addgene plasmid # 33091),
417 pQCXIH-Flag-YAP-S127A (Addgene plasmid # 33092), and pQCXIH-Myc-YAP-S94A (Addgene
418 plasmid # 33094) were gifts from Kun-Liang Guan (Zhao et al., 2007). Each YAP1 ORF was
419 amplified by PCR from pQCXIH, cloned into pDONR223, and transferred into pLIX_402 lentiviral
420 backbone using Gateway recombination. pLIX_402 was a gift from David Root (Addgene plasmid
421 # 41394). pLenti CMV GFP Hygro (656-4) was a gift from Eric Campeau & Paul Kaufman
422 (Addgene plasmid # 17446) (Campeau et al., 2009). PHAGE-P-CMVt N-HA GFP was previously
423 described (Galligan et al., 2014). pNeo-loxP-HPV18 was the kind gift of Thomas Broker and
424 Louise Chow (Wang et al., 2009). The Δ DLLC mutation was introduced into the pDONR HPV18
425 E7 vector using site-directed mutagenesis. HPV18 E7 Δ DLLC and GFP ORFs were cloned into
426 MSCV-P C-FlagHA GAW or MSCV-Neo C-HA GAW destination vectors using Gateway
427 recombination. The remaining MSCV-P C-FlagHA and MSCV-Neo C-HA HPV E6 and HPV E7
428 retroviral plasmids and pHAGE lentiviral plasmids have been previously described (Hatterschide
429 et al., 2020; White et al., 2016, 2012a, 2012b). A complete list of all plasmids used in this study
430 is in [Supplemental File 1](#).

431

432 **Cell culture, retrovirus production, and lentivirus production.** Deidentified primary human
433 foreskin keratinocytes (HFK) and human foreskin fibroblasts (HFF) were provided by the
434 University of Pennsylvania Skin Biology and Disease Resource-Based Center (SBDRRC). N/Tert-
435 1 cells are hTert-immortalized HFK (Dickson et al., 2000), and N/Tert-Cas9 mock and sgPTPN14-
436 1 are N/Tert-1 cells further engineered to constitutively express Cas9 (Hatterschide et al., 2019).
437 Keratinocytes for cell fate experiments were cultured in keratinocyte serum-free media (KSFM)
438 (Life Technologies, Carlsbad, California) mixed 1:1 with Medium 154 (Thermo Fisher Scientific,
439 Waltham, Massachusetts) with the human keratinocyte growth supplement (HKGS) (Thermo

440 Fisher Scientific) (Duperret et al., 2015; Egolf et al., 2019). Keratinocytes for all other experiments
441 were cultured as previously described (White et al., 2012a). HFF were cultured in Dulbecco's
442 Modified Eagle Medium (DMEM) (Thermo Fisher Scientific) supplemented with antibiotic and
443 antimycotic. HFK harboring the HPV18 genome were previously described (Hatterschide et al.,
444 2020), and were generated by transfecting cells with the pNeo-loxP-HPV18 vector (Wang et al.,
445 2009) along with NLS-Cre and selecting with G418 to generate a stable population. Lentiviruses
446 and retroviruses were produced in 293T or 293 Phoenix cells respectively as previously described
447 (White et al., 2016). Stable keratinocyte populations were generated following transduction by
448 selection with puromycin, G418, or hygromycin alone or in combination.

449

450 **Lifespan extension assay.** Primary HFK were engineered and cultured as described in cell
451 culture, retrovirus production, and lentivirus production. The growth of engineered HFK was
452 monitored in culture for 38 days. Population doublings were calculated using the number of cells
453 at the beginning and end of each passage.

454

455 **Organotypic epithelial culture.** Devitalized human dermis was provided as deidentified material
456 from the University of Pennsylvania SBDRRC. Stands for organotypic epithelial cultures were
457 printed using high temperature, autoclavable resin at the University of Pennsylvania Biotech
458 Commons 3D-printing facility. Organotypic cultures were generated as previously described
459 (Duperret et al., 2015; Egolf et al., 2019). Devitalized dermis was seeded with primary HFF on
460 the dermal side at a density of 3×10^4 cells per cm^2 of culturing area and cultured for four days.
461 Dermis and fibroblasts were then stretched across 3D-printed stands. The epidermal side of the
462 dermis was seeded with unmodified or engineered keratinocytes at a density of 1×10^6 cells per
463 cm^2 . Organotypic cultures were cultured in E media (Fehrmann and Laimins, 2005) with the
464 dermal layer maintained at the air-liquid interface starting on the day of seeding keratinocytes.
465 Cultures were allowed to stratify for 12-14 days, then trimmed and fixed in 10% neutral buffer

466 formalin for 24 hours. Tissues were embedded in paraffin and sectioned by the SBDR Core A.

467 A complete list of all organotypic cultures used in this study is in [Supplemental File 2](#).

468

469 **siRNA transfection.** Primary HFK were transfected with siRNAs using the Dharmafect 1
470 transfection reagent. All siRNA experiments were collected 72 h post transfection. Two siRNAs
471 were used to target each gene in an experiment. The siRNAs used in this study were all
472 purchased from Dharmacon (Lafayette, Colorado): nontargeting siRNA, siYAP1-06, siYAP1-08,
473 siWWTR1-06, siWWTR1-08, siPTPN14-05, siPTPN14-08, siLATS1-05, siLATS1-08, siLATS2-
474 09, siLATS2-10.

475

476 **Laser capture microdissection.** Formalin-fixed paraffin-embedded (FFPE) organotypic cultures
477 were sectioned onto polyethylene naphthalate (PEN) membrane glass slides by the SBDR Core
478 A. Laser capture microdissection was performed on a Leica LMD 7000 microscope. Hundreds of
479 microdissections were made per sample amounting to ~1.5 mm² of total dissected area per
480 sample. RNA was isolated using the RNeasy FFPE kit (Qiagen, Germantown, Maryland). RNA
481 concentration was determined using Qubit RNA HS assay kit (Life Technologies).

482

483 **Patient derived xenografts.** The PDXs were previously established from surgical resections of
484 treatment-naive HPV-positive OPSCC as described (Facompre et al., 2020). Human tumors were
485 engrafted subcutaneously in NSG mice and passaged at least twice before cryopreservation
486 when they reached a volume of 0.5-1.0 cm³. Total tumor RNA was isolated using the QIAamp
487 RNA Blood Mini Kit (Qiagen).

488

489 **Western blotting.** Western blots were performed using Mini-PROTEAN (Bio-Rad Laboratories,
490 Hercules, California) or Criterion (Bio-Rad) Tris/Glycine SDS-PAGE gels and transfers were
491 performed onto polyvinylidene difluoride (PVDF). Membranes were blocked with 5% nonfat dried

492 milk in Tris-buffered saline with 0.05% Tween 20 (TBST). Membranes were incubated with
493 primary antibodies as specified in [Supplemental File 1](#). Following TBST washes, membranes
494 were incubated with horseradish peroxidase-coupled secondary antibodies and imaged using
495 chemiluminescent substrate on an Amersham Imager 600 (GE Healthcare, Chicago, Illinois).

496

497 **qRT-PCR.** Unless otherwise specified, total cellular RNA was isolated using the NucleoSpin RNA
498 extraction kit (Macherey-Nagel/Takara, San Jose, California). cDNA was generated from bulk
499 RNA with the high-capacity cDNA reverse transcription kit (Applied Biosystems, Waltham,
500 Massachusetts). cDNAs were used as a template for qPCR using Fast SYBR green master mix
501 (Applied Biosystems) and a QuantStudio 3 system (Thermo Fisher Scientific). 18S rRNA qRT-
502 PCR primers were ordered from Integrated DNA Technologies (Integrated DNA Technologies,
503 Inc., Coralville, Iowa): FWD, 5- CGCCGCTAGAGGTGAAATTCT; REV, 5-
504 CGAACCTCCGACTTTCGTTCT (Roh et al., 2005). KiCqStart SYBR green primers for qRT-PCR
505 (MilliporeSigma, St. Louis, Missouri) were used for the remaining genes assayed in this study:
506 KRT1, KRT10, IVL, ITGB4, ITGA6, CYR61, CTGF, PTPN14, YAP1, WWTR1, LATS1, LATS2,
507 G6PD, and GAPDH.

508

509 **Immunofluorescence, immunohistochemistry, and microscopy.** FFPE sections were
510 prepared for immunofluorescence by deparaffinization with xylene washes, rehydration through
511 an ethanol gradient, and heat induced epitope retrieval (HIER). Tissue sections were blocked with
512 PBS containing 1% bovine serum albumin, 10% normal goat serum, and 0.3% Triton X-100.
513 Tissue sections were incubated with primary antibodies at 4°C overnight, washed with PBS with
514 0.05% Tween 20, and incubated with fluorescently labeled secondary antibodies and Hoechst
515 33342 at room temperature. Antibody dilutions and HIER conditions are specified in [Supplemental](#)
516 [File 1](#). Fluorescent micrographs were captured using an Olympus IX81 microscope. All

517 fluorescent micrograph images within the same figure panels were captured using the same
518 exposure time and batch processed using the same contrast settings.

519 The TMA was constructed from surgical resection specimens of 120 HNSCC that vary by
520 TNM stage and HPV status ([Supplemental File 3](#)). Archival FFPE tumors of the oral cavity and
521 oropharynx were identified retrospectively and oropharyngeal tumors were evaluated for HPV
522 status as per College of American Pathologists criteria (Lewis et al., 2018) using IHC for p16.
523 When present, lymph node metastases were included in association with the primary tumor of
524 origin. All FFPE specimens were represented in the TMA by at least three tissue cores that
525 incorporate both non-necrotic central tumor regions and invasive margins. Tumor materials and
526 clinical data were accessed under University of Pennsylvania IRB protocol 417200. Staining for
527 KRT1 was performed by the Clinical Services Laboratory in the University of Pennsylvania
528 Department of Pathology and Laboratory Medicine. Antibody information can be found in
529 [Supplemental File 1](#). The KRT1 stained slides were reviewed with a standard light microscope,
530 and evaluation was based on the presence or absence of staining in the cytoplasm of tumor cells.

531

532 **Bioinformatic analysis.** Genomic mutation and copy number variation data as well as tumor
533 RNA-seq gene expression data from TCGA (Lawrence et al., 2015) were analyzed using the
534 cBioPortal.org graphical interface (Cerami et al., 2012; Gao et al., 2013). RNA-seq V2 RSEM
535 (RNA-Seq by Expectation Maximization) normalized expression values for individual genes were
536 downloaded directly from cBioPortal.org. OPSCC were distinguished from HNSCC by clinical
537 annotation of primary tumor site and HPV-positive and HPV-negative status was assigned based
538 on previously reported HPV transcript status (Chakravarthy et al., 2016). Genes included as a
539 part of each pathway analysis are listed in [Supplemental File 4](#). Missense, truncating, and splice
540 mutations of unknown significance as well as amplifications of tumor suppressor genes and
541 deletion of oncogenes were excluded from total alteration tallies.

542 Single cell-RNA sequencing dataset derived from the human neonatal foreskin epidermis
543 and subsequent clustering analysis were retrieved from GitHub (S. Wang et al., 2020) and
544 reanalyzed with MATLAB. PTPN14 expression was calculated by averaging mRNA expression
545 for all cells by cluster and donor.
546

547 **Acknowledgments**

548 We thank the members of our laboratories, particularly Pavithra Rajagopalan, for helpful
549 discussions. We thank Stephen M. Prouty, Ph.D. from the SBDRC for help with tissue processing
550 and sectioning. Stands for organotypic cultures were printed courtesy of the University of
551 Pennsylvania Libraries' Biotech Commons. This work was supported by American Cancer Society
552 grant 131661-RSG-18-048-01-MPC and NIH/NIAID R01 AI148431 to EAW. DB is supported by
553 NIH/NIDCR R01 DE027185. JH was supported by NIH/NIAID T32 AI007324 and NIH/NIDCR F31
554 DE030365. SBDRC was funded by NIH grant P30 AR068589.

555

556 **Author Contributions**

557 Conception and design: JH, EAW. Acquisition of data: JH, PC, HWK, KTM, EAW. Analysis and
558 interpretation of data: JH, PC, KTM, DB, EAW. Drafting or revising the article: JH, PC, DB, EAW.
559 Contributing unpublished essential data or reagents: SMS, KTM, DB.

560 **Figure Legends**

561 **Figure 1 | HPV E7 activates YAP1 in basal epithelial keratinocytes.** (A) cBioPortal analysis
562 for total genomic mutations and copy number alterations in HPV+/- OPSCC and HNSCC. Graph
563 displays the percent of tumors with alterations in each pathway. Statistical significance was
564 determined by Fisher's exact test. (B-D) Organotypic cultures were grown from primary HFK, HFK
565 harboring the HPV18 genome, or HFK transduced with retroviral expression encoding HPV E6 or
566 E7 proteins. FFPE sections of cultures grown from (C) HFK or HFK harboring the HPV18 genome,
567 (D) HFK or HFK expressing HPV16 E7 or HPV18 E7, or (E) HFK or HFK expressing HPV16 E6
568 or HPV16 E7 were stained for YAP1 (magenta), PCNA (green), and Hoechst (gray). White dashed
569 lines indicate the basement membrane. White boxes indicate the location of insets in main
570 images. Main image scale bars = 100 μ m. Inset scale bars = 25 μ m.

571

572 **Figure 2 | HPV E7 activates YAP1 in basal keratinocytes through PTPN14 degradation.**

573 Organotypic cultures were grown from N/Tert-Cas9 keratinocytes or primary HFK transduced with
574 retroviral expression vectors encoding HPV18 E7 WT or R84S. (A) FFPE sections of cultures
575 grown from mock or sgPTPN14 transfected N/Tert-Cas9 keratinocytes were stained for YAP1
576 (magenta), IVL (green), and Hoechst (Gray). (B) FFPE sections of cultures grown from parental
577 HFK, HPV18 E7 WT or HPV18 E7 R84S expressing HFK were stained for YAP1 (magenta),
578 PCNA (green), and Hoechst (Gray). White dashed lines indicate the basement membrane. White
579 boxes indicate the location of insets in main images. Main image scale bars = 100 μ m. Inset scale
580 bars = 25 μ m. (C-F) Quantification of the number of (C and E) basal cells and (D and F) suprabasal
581 cells per 100 μ m of epidermis. Graphs display the mean \pm SD and each individual data point
582 (independent cultures). Statistical significance was determined by ANOVA (* p <0.05, *** p <0.001).

583

584 **Figure 3 | PTPN14 expression is enriched in basal keratinocytes.** (A-B) Single-cell RNA
585 sequencing data and clustering analysis from Wang et al. was reanalyzed to assess PTPN14

586 expression in different subsets of epidermal cells. (A) Diagram of epidermis; shading depicts
587 tissue localization of cell clusters. (B) For each donor, the mean of PTPN14 mRNA expression
588 was calculated for each cell cluster. Graphs display the mean of PTPN14 mRNA expression for
589 each donor (circles) as well as the mean of all five donors \pm SEM (bars and error bars). Statistical
590 significance was determined by ANOVA (* $p < 0.05$, ** $p < 0.01$). (C-F) Basal and suprabasal layers
591 from organotypic cultures were dissected using laser capture microdissection. (C) Representative
592 images of HFK cultures before and after individual laser dissections. Hundreds of such cuts were
593 performed per sample. (D-F) RNA was purified from isolated layers and qRT-PCR was used to
594 assess the expression of PTPN14 (D), basal cell markers ITGA6 and ITGB4 (E), and
595 differentiation markers KRT1 and IVL (F). Graphs display the mean and each individual data point.
596

597 **Figure 4 | YAP1/TAZ regulate differentiation downstream of PTPN14.** (A) Venn diagram
598 comparing the MSigDB YAP conserved signature to the differentially expressed genes (DEG)
599 from our two published experiments that reflect PTPN14 loss in keratinocytes. (B-D) *YAP1* and
600 *WWTR1* were simultaneously knocked down by siRNA transfection in HFK. Transfected HFK
601 were then transduced with PTPN14 lentivirus at 24h post transfection. Cells were lysed for protein
602 and total cellular RNA at 72h post transfection. (B) Cell lysates were subjected to
603 SDS/PAGE/Western analysis and probed with antibodies to PTPN14, YAP1, TAZ, and Actin. (C
604 and D) qRT-PCR was used to measure the expression of the differentiation markers *KRT1* and
605 *IVL* relative to *G6PD*. Graphs display fold change in gene expression relative to the mock
606 transduced cells. (E-I) Primary HFK were transfected with siRNAs targeting *YAP1*, *WWTR1*
607 (*TAZ*), *PTPN14*, *LATS1*, and *LATS2*. Two siRNAs were used per target. qRT-PCR was used to
608 measure gene expression for: the differentiation markers *IVL* (E) and *KRT1* (F), and the canonical
609 YAP1/TAZ targets *CTGF* (G) and *CYR61* (H). Data confirming that individual siRNA transfections
610 depleted intended transcripts is summarized in a heatmap of $\log_2(\text{fold-change})$ levels (I). Bar

611 graphs display the mean \pm SD of three independent replicates. Statistical significance was
612 determined by ANOVA (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$).

613

614 **Figure 5 | HPV-positive HNSCC are less differentiated than HPV-negative HNSCC.** (A)

615 Human HNSCC tumor samples were stained for KRT1 (left). Scale bar = 100 μ m. Graph displays
616 the percentage of tumors that were KRT1⁺ (right). Statistical significance was determined by
617 Fisher's exact test. (B-C) Total RNA was purified from PDX samples and qRT-PCR was used to
618 assess gene expression of (B) the differentiation markers KRT1, KRT10, and IVL and (C) the
619 canonical YAP1/TAZ targets CTGF and CYR61. Statistical significance was determined by Mann-
620 Whitney nonparametric test. (* $p < 0.05$, ** $p < 0.01$, **** $p < 0.0001$).

621

622 **Figure 6 | High-risk HPV E7 requires YAP1/TAZ-TEAD transcriptional activity to extend the**

623 **lifespan of primary keratinocytes.** Primary HFK were transduced with retroviruses encoding
624 HPV16 E7, HPV18 E7, or GFP, plus pInducer20 TEADi lentivirus. Each cell population was
625 cultured with or without 1 μ g/mL doxycycline in the media for 38 days and population doublings
626 were tracked with each passage. Graph displays the mean \pm SD of two independently transduced
627 cell populations per condition.

628

629 **Figure 7 | PTPN14 loss and YAP1 activation by HPV E7 promote basal cell retention in**

630 **organotypic cultures.** Organotypic cultures were grown from GFP-labeled HFK mixed with
631 unmodified HFK. (A-B) GFP-labeled HFK were transduced with lentiviral vectors encoding YAP1
632 WT, YAP1 S127A, or YAP1 S94A under the control of a doxycycline inducible promoter. GFP-
633 labeled YAP1 cells were mixed 1:25 into unmodified HFK and organotypic cultures were grown
634 from the mixture. Cultures were grown +/- 1 μ g/mL doxycycline. (C-D) GFP-labeled HFK were
635 transduced with LentiCRISPR v2 vectors encoding control or PTPN14 targeting sgRNAs. GFP-

636 labeled cells were mixed 1:25 into unmodified HFK and organotypic cultures were grown from the
637 mixture. (E-F) GFP-labeled HFK were transduced with HPV18 E7 WT, HPV18 E7 R84S, or the
638 empty vector (EV). GFP-labeled HPV18 E7 cells were mixed 1:50 into unmodified HFK and
639 organotypic cultures were grown from the mixture. (A, C, E) FFPE sections of cultures were
640 stained for GFP (green), IVL (grey), and Hoechst (blue). Scale bar = 100 μ m. (B, D, F)
641 Quantification of the percentage of GFP+ cells found in the basal layer. Graphs display the mean
642 \pm SD and each individual data point (independent cultures). Shapes indicate cultures grown from
643 different HFK donors. Statistical significance was determined by ANOVA. (* p <0.05, ** p <0.01).

644

645 **Figure 1—figure supplement 1 | HPV-positive HNSCC have fewer Hippo pathway**
646 **alterations and lower expression of differentiation genes.** cBioPortal analysis for genomic
647 mutations and copy number alterations in HPV+/- HNSCC and OPSCC. Oncoprint displays
648 specific genomic alterations in individual tumor samples.

649

650 **Figure 1—figure supplement 2 | HPV18 E7 activates YAP1 in basal keratinocytes.** (A-B)
651 Additional replicates of organotypic cultures grown from primary HFK or HFK harboring the
652 HPV18 genome. FFPE sections were stained for YAP1 (magenta), PCNA (green), and Hoechst
653 (gray). White dashed lines indicate the basement membrane. White boxes indicate the location
654 of insets in main images. Main image scale bars = 100 μ m. Inset scale bars = 25 μ m.

655

656 **Figure 1—figure supplement 3 | HPV E7 activates YAP1 in basal keratinocytes.** Additional
657 replicates of organotypic cultures grown from primary HFK or HFK transduced with retroviral
658 expression encoding HPV E7 proteins. FFPE sections of cultures grown from (A) HFK or HFK
659 expressing HPV16 E7 or HPV18 E7, (B) HFK or HFK transduced with HPV16 E7, or (E) HFK and
660 HFK expressing HPV18 E7 were stained for YAP1 (magenta), PCNA (green), and Hoechst (gray).

661 White dashed lines indicate the basement membrane. White boxes indicate the location of insets
662 in main images. Main image scale bars = 100 μm . Inset scale bars = 25 μm .

663

664 **Figure 1—figure supplement 4 | HPV E6 does not activate YAP1 in basal keratinocytes.**

665 Additional replicates of organotypic cultures grown from primary HFK or HFK transduced with
666 retroviral expression encoding HPV E6 or E7 proteins. FFPE sections were stained for YAP1
667 (magenta), PCNA (green), and Hoechst (gray). White dashed lines indicate the basement
668 membrane. White boxes indicate the location of insets in main images. Main image scale bars =
669 100 μm . Inset scale bars = 25 μm .

670

671 **Figure 2—figure supplement 1 | PTPN14 knockout activates YAP1 in basal keratinocytes.**

672 Additional replicates of organotypic cultures grown from N/Tert-Cas9 keratinocytes (A-C) FFPE
673 sections from mock or sgPTPN14 transfected N/Tert-Cas9 keratinocytes were stained for YAP1
674 (magenta), IVL (green), and Hoechst (Gray). White dashed lines indicate the basement
675 membrane. White boxes indicate the location of insets in main images. Main image scale bars =
676 100 μm . Inset scale bars = 25 μm .

677

678 **Figure 2—figure supplement 2 | HPV E7 activates YAP1 in basal keratinocytes through**

679 **PTPN14 degradation.** Additional replicates of organotypic cultures grown from primary HFK
680 transduced with retroviral expression vectors encoding HPV18 E7 WT or R84S. FFPE sections
681 from parental HFK, HPV18 E7 WT or HPV18 E7 R84S expressing HFK were stained for YAP1
682 (magenta), PCNA (green), and Hoechst (Gray). White dashed lines indicate the basement
683 membrane. White boxes indicate the location of insets in main images. Main image scale bars =
684 100 μm . Inset scale bars = 25 μm .

685

686 **Figure 3—figure supplement 1 | PTPN14 expression is enriched in basal keratinocytes in**
687 **HPV 18 E7 expressing organotypic cultures.** Basal and suprabasal layers from a 3D
688 organotypic culture grown from HFK transduced with a retroviral expression vector encoding
689 HPV18 E7 were dissected using laser capture microdissection. RNA was purified from isolated
690 layers and qRT-PCR was used to assess the expression of PTPN14 (A), the basal cell markers
691 ITGA6 and ITGB4 (B), and the differentiation marker IVL (C). Graphs display individual data
692 points.

693

694 **Figure 4—figure supplement 1 | PTPN14 overexpression promotes differentiation in**
695 **keratinocytes.** NTert-Cas9 Mock and sgPTPN14-1 keratinocytes were transduced with
696 lentiviruses encoding GFP or PTPN14 or the empty vector control. (A) Cell lysates were subjected
697 to SDS/PAGE/Western analysis and probed with antibodies to PTPN14, V5-tag, Involucrin, and
698 Actin. (B) qRT-PCR was used to measure the expression of the differentiation markers IVL and
699 KRT10 relative to G6PD. Graphs display the mean \pm SD of two independent replicates.

700

701 **Figure 4—figure supplement 2 | YAP1 and TAZ are required for PTPN14 to promote**
702 **keratinocyte differentiation.** Primary HFK were transfected with control or YAP1 and WWTR1
703 targeting siRNAs then transduced with PTPN14 encoding lentivirus. qRT-PCR was used to
704 measure the expression of the differentiation markers (A) KRT1 and (B) IVL relative to G6PD.
705 Graphs portray the change in gene expression relative to siC. Graphs display the mean \pm SD of
706 three independent replicates. Statistical significance was determined by ANOVA (**p<0.01,
707 ***p<0.001).

708

709 **Figure 5—figure supplement 1 | HPV-positive HNSCC express lower levels of**
710 **differentiation genes.** RNA-seq data from TCGA were accessed through cBioPortal. Violin plots
711 display the distribution in log₂ mRNA expression of differentiation markers (A) KRT1, (B) KRT10,

712 and (C) IVL, and the canonical YAP1/TAZ targets (D) CTGF and (E) CYR61. Statistical
713 significance was determined by Mann-Whitney nonparametric test. (**p<0.01, ***p<0.001,
714 ****p<0.0001).

715

716 **Figure 7—figure supplement 1 | PTPN14 degradation by HPV E7 promotes basal cell**
717 **retention.** (A-B) GFP-labeled HFK were transduced with YAP1 WT, YAP1 S127A, or YAP1 S94A
718 under the control of a doxycycline inducible promoter. (A) GFP expression was confirmed by
719 fluorescence microscopy. Scale bar = 100 μ m. (B) Total RNA was purified from monolayer cells
720 +/- treatment with 1 μ g/mL doxycycline for 72h. qRT-PCR was used to assess gene expression
721 of YAP1 and CTGF. (C-D) GFP-labeled HFK were transduced with retroviral vectors encoding
722 HPV18 WT, HPV18 Δ DLLC, HPV18 E7 R84S, or the empty vector control (EV). (C) GFP
723 expression was confirmed by fluorescence microscopy. Scale bar = 100 μ m. (D) Cell lysates were
724 subjected to SDS/PAGE/Western analysis and probed with antibodies to PTPN14, RB1, and
725 Actin. (E-F) GFP-labeled HFK were transduced with LentiCRISPR v2 sgNT-1, sgPTPN14-3, or
726 sgPTPN14-4 vectors. (E) GFP expression was confirmed by fluorescence microscopy. Scale bar
727 = 100 μ m (F) Cell lysates were subjected to SDS/PAGE/Western analysis and probed with
728 antibodies to PTPN14 and Actin.

729

730 **Figure 7—figure supplement 2 | HPV18 E7 can promote basal cell retention in the absence**
731 **of RB1 binding.** Organotypic cultures were grown from GFP-labeled cells mixed with unmodified
732 HFK. GFP-labeled HFK were transduced with HPV18 E7 Δ DLLC or the empty vector (EV). GFP-
733 labeled cells were mixed 1:50 into unmodified HFK. (A) FFPE sections were stained for GFP
734 (green), IVL (grey), and Hoechst (blue). Scale bar = 100 μ m (B) Quantification of the percentage
735 of GFP+ cells found in the basal layer. Graphs display the mean \pm SD and each individual data
736 point (independent cultures). Statistical significance was determined by t-test. (**p<0.01).

737

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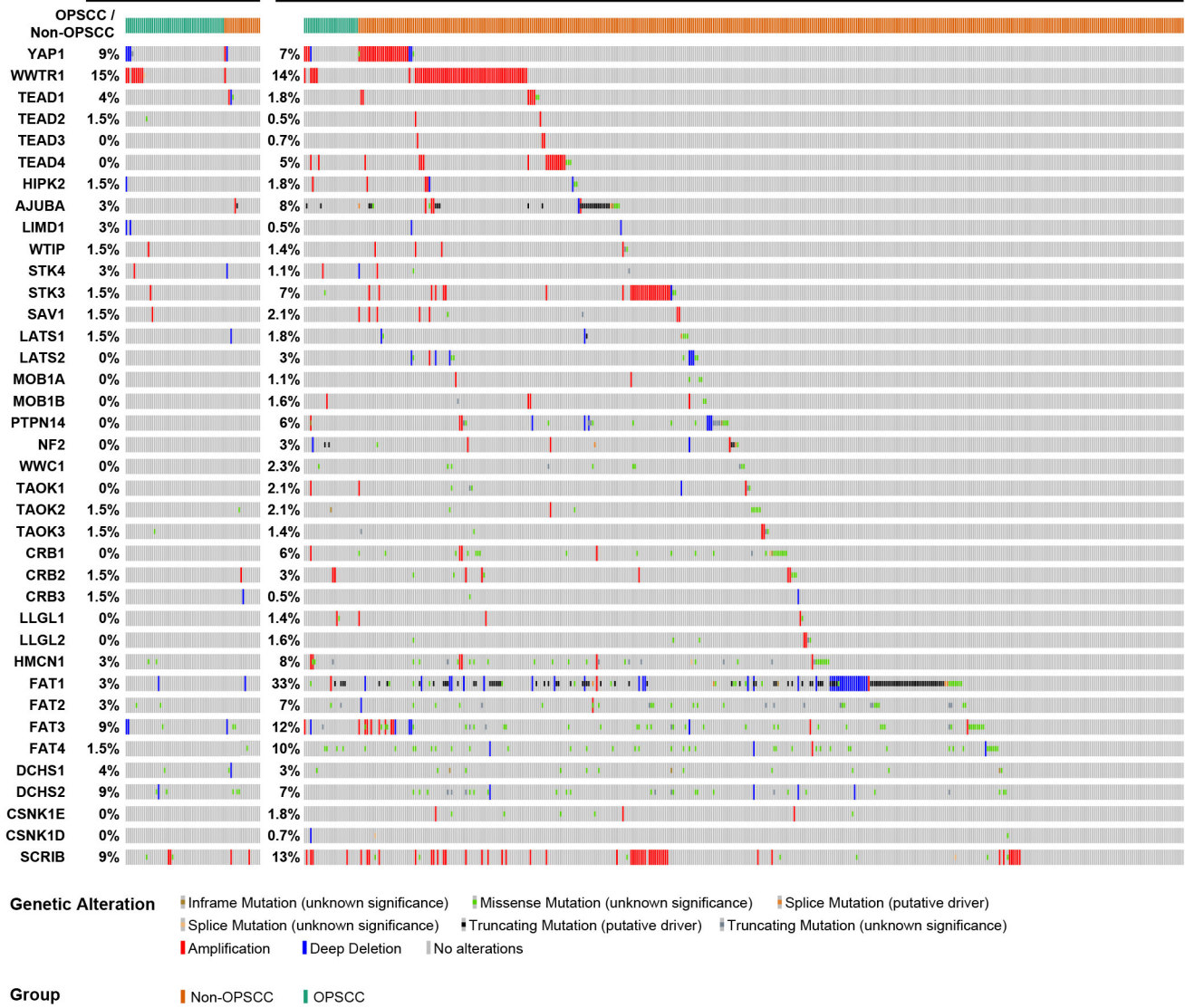


Figure 1—figure supplement 1 | HPV-positive HNSCC have fewer Hippo pathway alterations and lower expression of differentiation genes. cBioPortal analysis for genomic mutations and copy number alterations in HPV+/- HNSCC and OPSCC. Oncoprint displays specific genomic alterations in individual tumor samples.

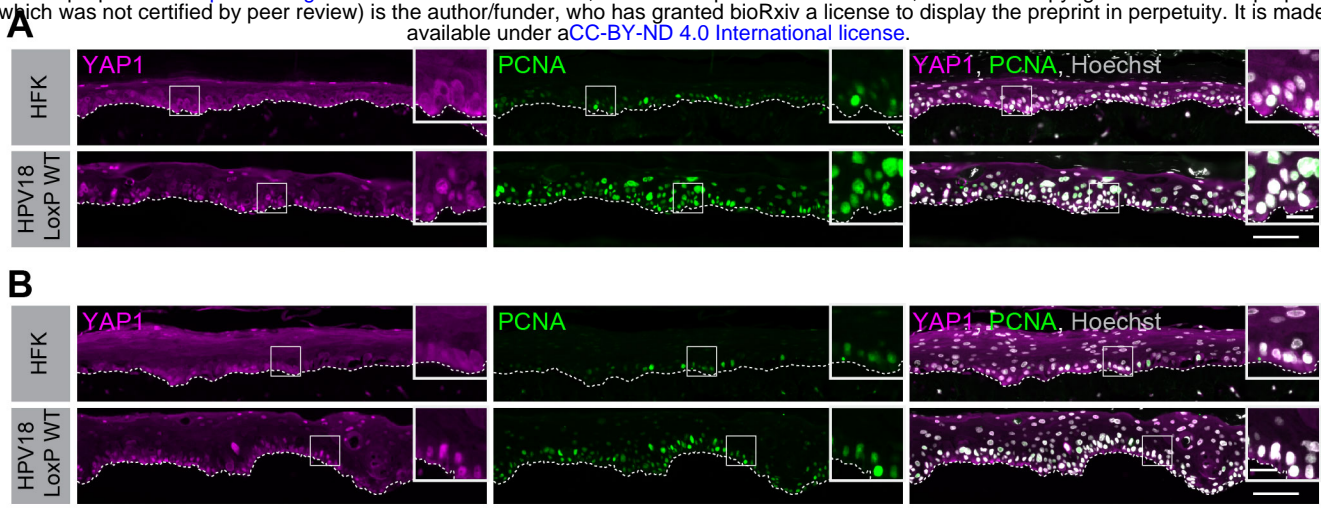


Figure 1—figure supplement 2 | HPV18 E7 activates YAP1 in basal keratinocytes. (A-B) Additional replicates of organotypic cultures grown from primary HFK or HFK harboring the HPV18 genome. FFPE sections were stained for YAP1 (magenta), PCNA (green), and Hoechst (gray). White dashed lines indicate the basement membrane. White boxes indicate the location of insets in main images. Main image scale bars = 100 μm . Inset scale bars = 25 μm .

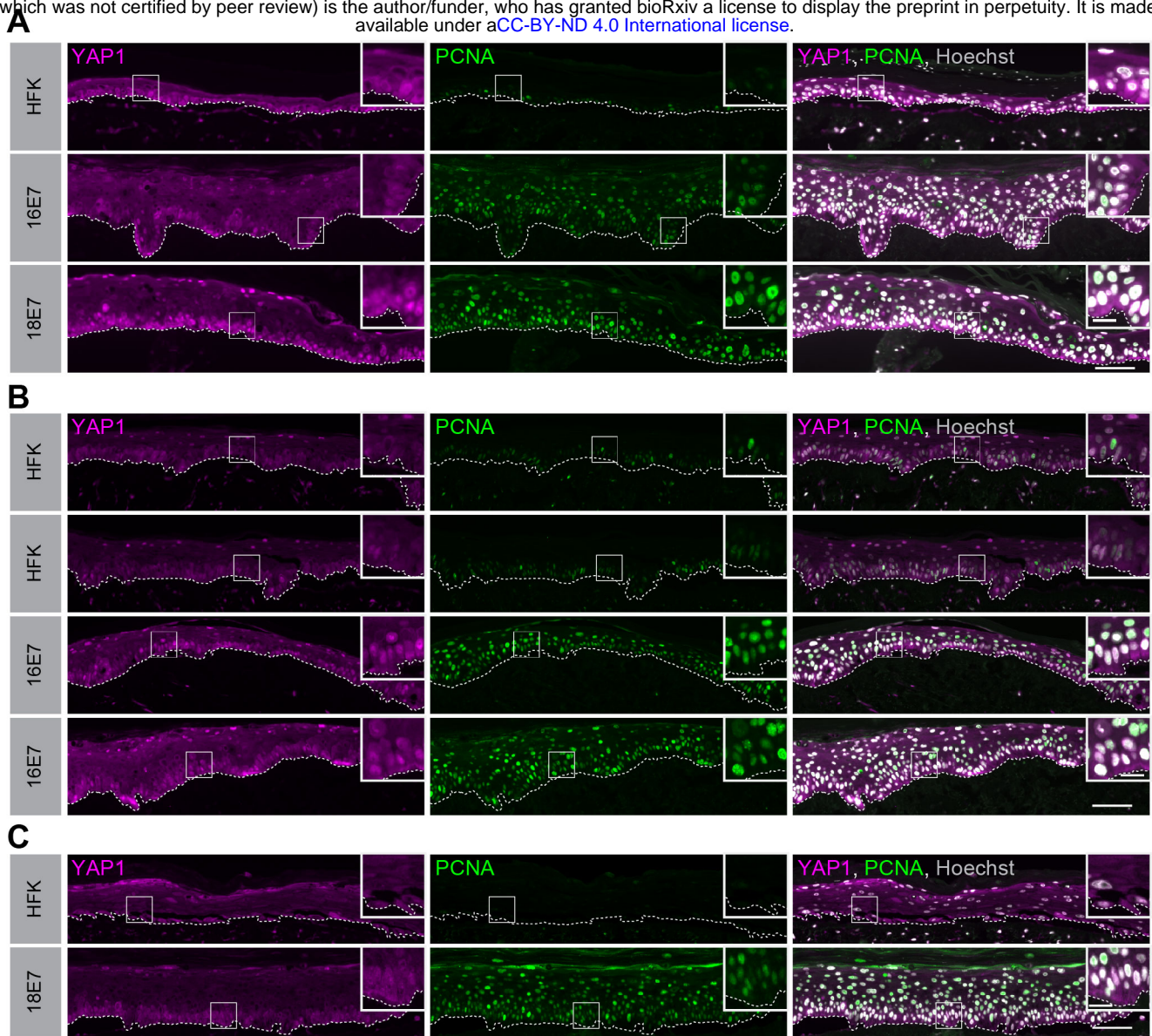


Figure 1—figure supplement 3 | HPV E7 activates YAP1 in basal keratinocytes. Additional replicates of organotypic cultures grown from primary HFK or HFK transduced with retroviral expression encoding HPV E7 proteins. FFPE sections of cultures grown from (A) HFK or HFK expressing HPV16 E7 or HPV18 E7, (B) HFK or HFK transduced with HPV16 E7, or (E) HFK and HFK expressing HPV18 E7 were stained for YAP1 (magenta), PCNA (green), and Hoechst (gray). White dashed lines indicate the basement membrane. White boxes indicate the location of insets in main images. Main image scale bars = 100 μm . Inset scale bars = 25 μm .

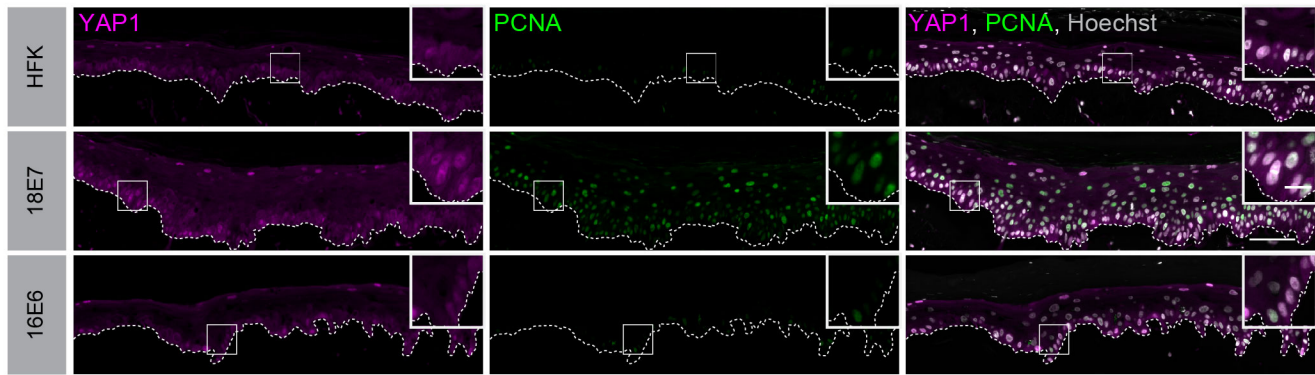


Figure 1—figure supplement 4 | HPV E6 does not activate YAP1 in basal keratinocytes. Additional replicates of organotypic cultures grown from primary HFK or HFK transduced with retroviral expression encoding HPV E6 or E7 proteins. FFPE sections were stained for YAP1 (magenta), PCNA (green), and Hoechst (gray). White dashed lines indicate the basement membrane. White boxes indicate the location of insets in main images. Main image scale bars = 100 μ m. Inset scale bars = 25 μ m.

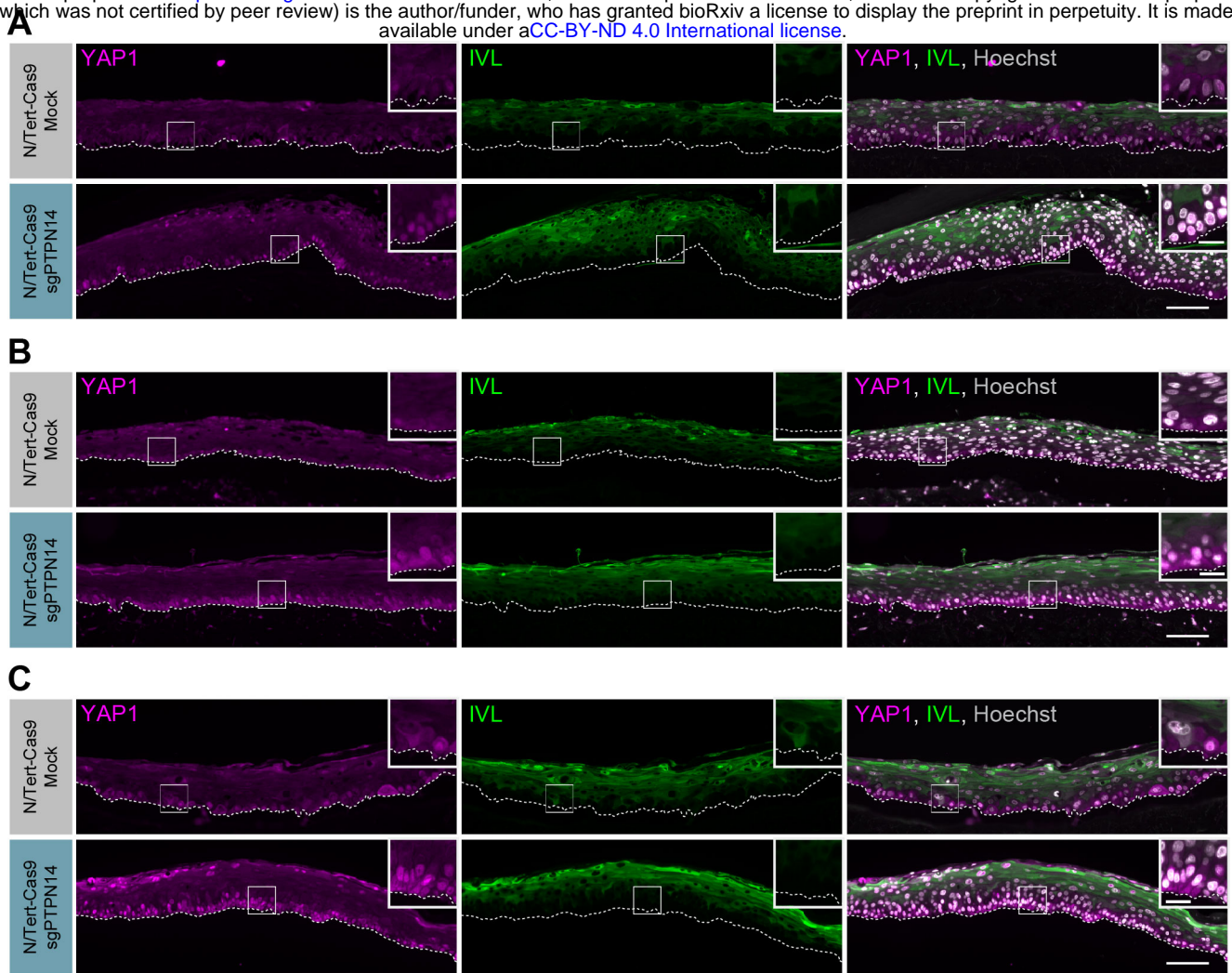


Figure 2—figure supplement 1 | PTPN14 knockout activates YAP1 in basal keratinocytes. Additional replicates of organotypic cultures grown from N/Tert-Cas9 keratinocytes (A-C) FFPE sections from mock or sgPTPN14 transfected N/Tert-Cas9 keratinocytes were stained for YAP1 (magenta), IVL (green), and Hoechst (Gray). White dashed lines indicate the basement membrane. White boxes indicate the location of insets in main images. Main image scale bars = 100 μ m. Inset scale bars = 25 μ m.

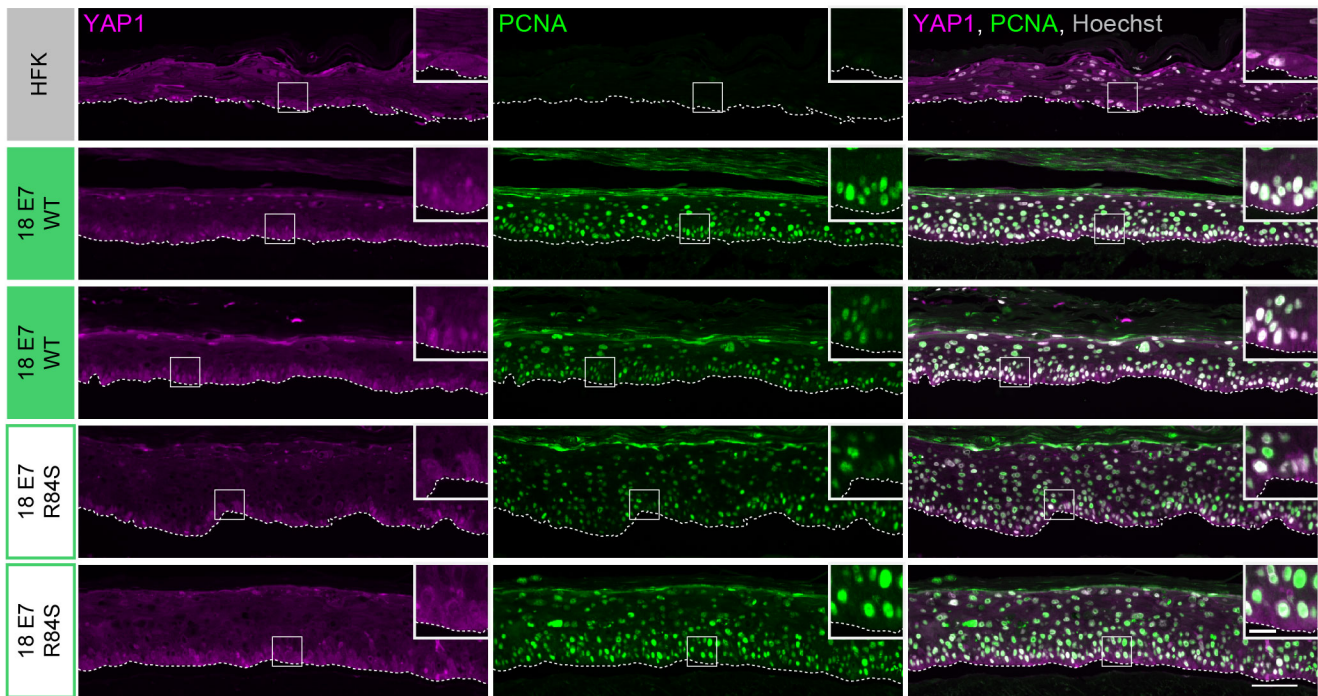


Figure 2—figure supplement 2 | HPV E7 activates YAP1 in basal keratinocytes through PTPN14 degradation. Additional replicates of organotypic cultures grown from primary HFK transduced with retroviral expression vectors encoding HPV18 E7 WT or R84S. FFPE sections from parental HFK, HPV18 E7 WT or HPV18 E7 R84S expressing HFK were stained for YAP1 (magenta), PCNA (green), and Hoechst (Gray). White dashed lines indicate the basement membrane. White boxes indicate the location of insets in main images. Main image scale bars = 100 μm. Inset scale bars = 25 μm.

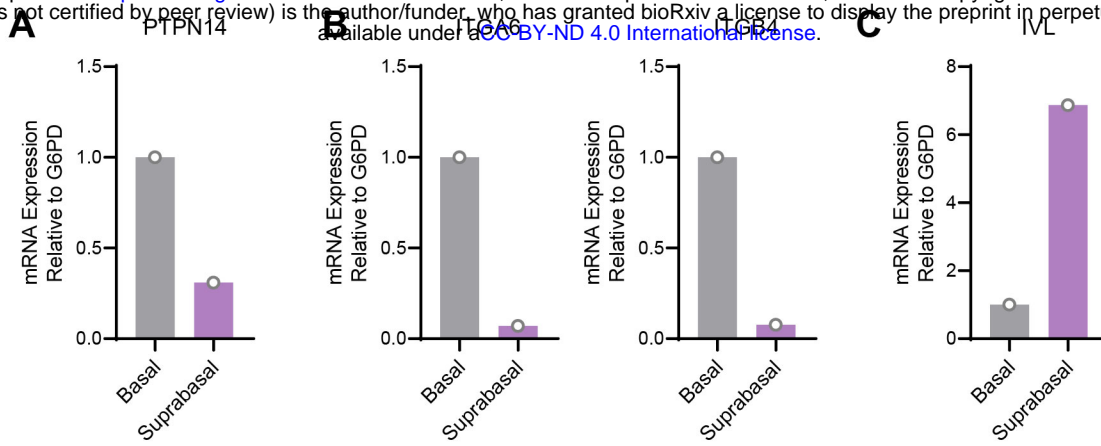


Figure 3—figure supplement 1 | PTPN14 expression is enriched in basal keratinocytes in HPV 18 E7 expressing organotypic cultures. Basal and suprabasal layers from a 3D organotypic culture grown from HFK transduced with a retroviral expression vector encoding HPV18 E7 were dissected using laser capture microdissection. RNA was purified from isolated layers and qRT-PCR was used to assess the expression of PTPN14 (A), the basal cell markers ITGA6 and ITGB4 (B), and the differentiation marker IVL (C). Graphs display individual data points.

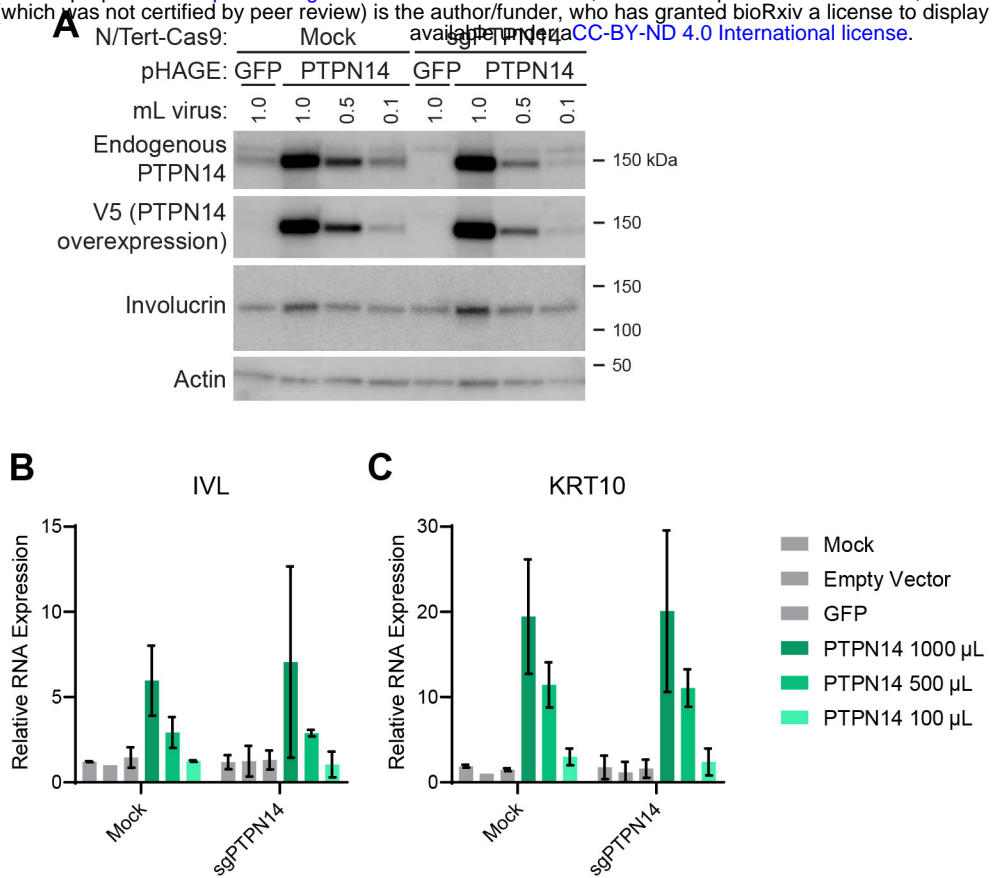


Figure 4—figure supplement 1 | PTPN14 overexpression promotes differentiation in keratinocytes.

NTert-Cas9 Mock and sgPTPN14-1 keratinocytes were transduced with lentiviruses encoding GFP or PTPN14 or the empty vector control. (A) Cell lysates were subjected to SDS/PAGE/Western analysis and probed with antibodies to PTPN14, V5-tag, Involucrin, and Actin. (B) qRT-PCR was used to measure the expression of the differentiation markers IVL and KRT10 relative to G6PD. Graphs display the mean \pm SD of two independent replicates.

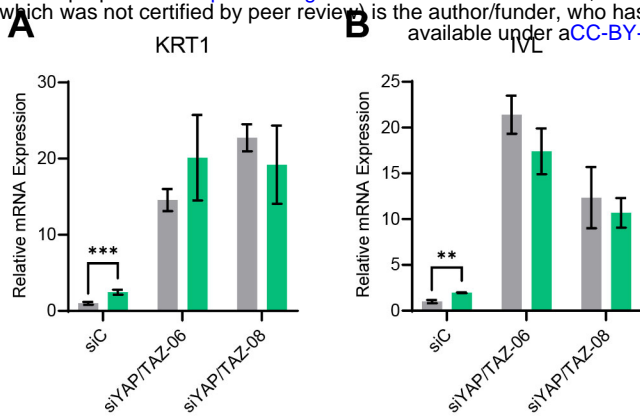


Figure 4—figure supplement 2 | YAP1 and TAZ are required for PTPN14 to promote keratinocyte differentiation. Primary HFK were transfected with control or YAP1 and WWTR1 targeting siRNAs then transduced with PTPN14 encoding lentivirus. qRT-PCR was used to measure the expression of the differentiation markers (A) KRT1 and (B) IVL relative to G6PD. Graphs portray the change in gene expression relative to siC. Graphs display the mean \pm SD of three independent replicates. Statistical significance was determined by ANOVA (** $p < 0.01$, *** $p < 0.001$).

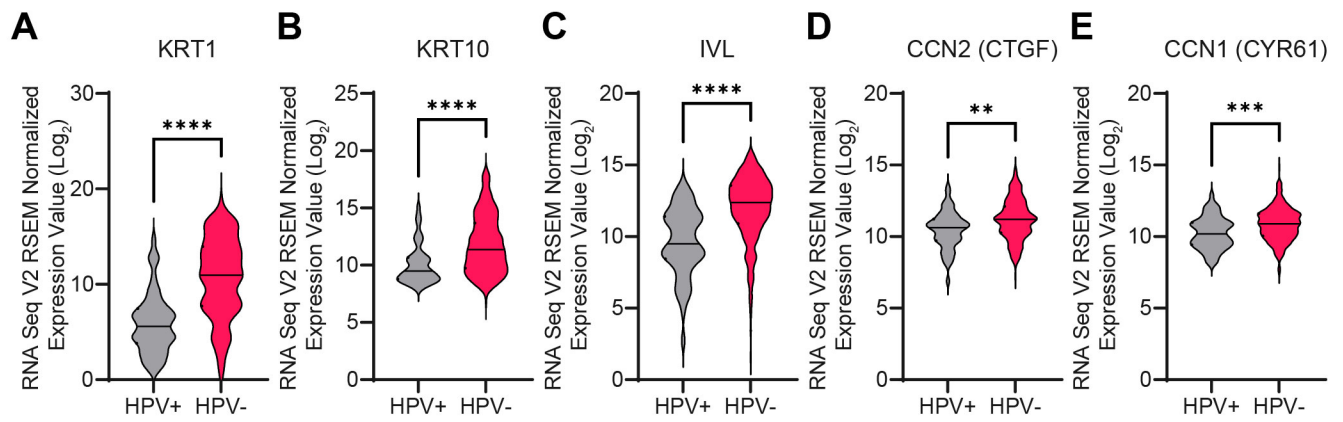


Figure 5—figure supplement 1 | HPV-positive HNSCC express lower levels of differentiation genes. RNA-seq data from TCGA were accessed through cBioPortal. Violin plots display the distribution in log₂ mRNA expression of differentiation markers (A) KRT1, (B) KRT10, and (C) IVL, and the canonical YAP1/TAZ targets (D) CTGF and (E) CYR61. Statistical significance was determined by Mann-Whitney nonparametric test. (**p<0.01, ***p<0.001, ****p<0.0001).

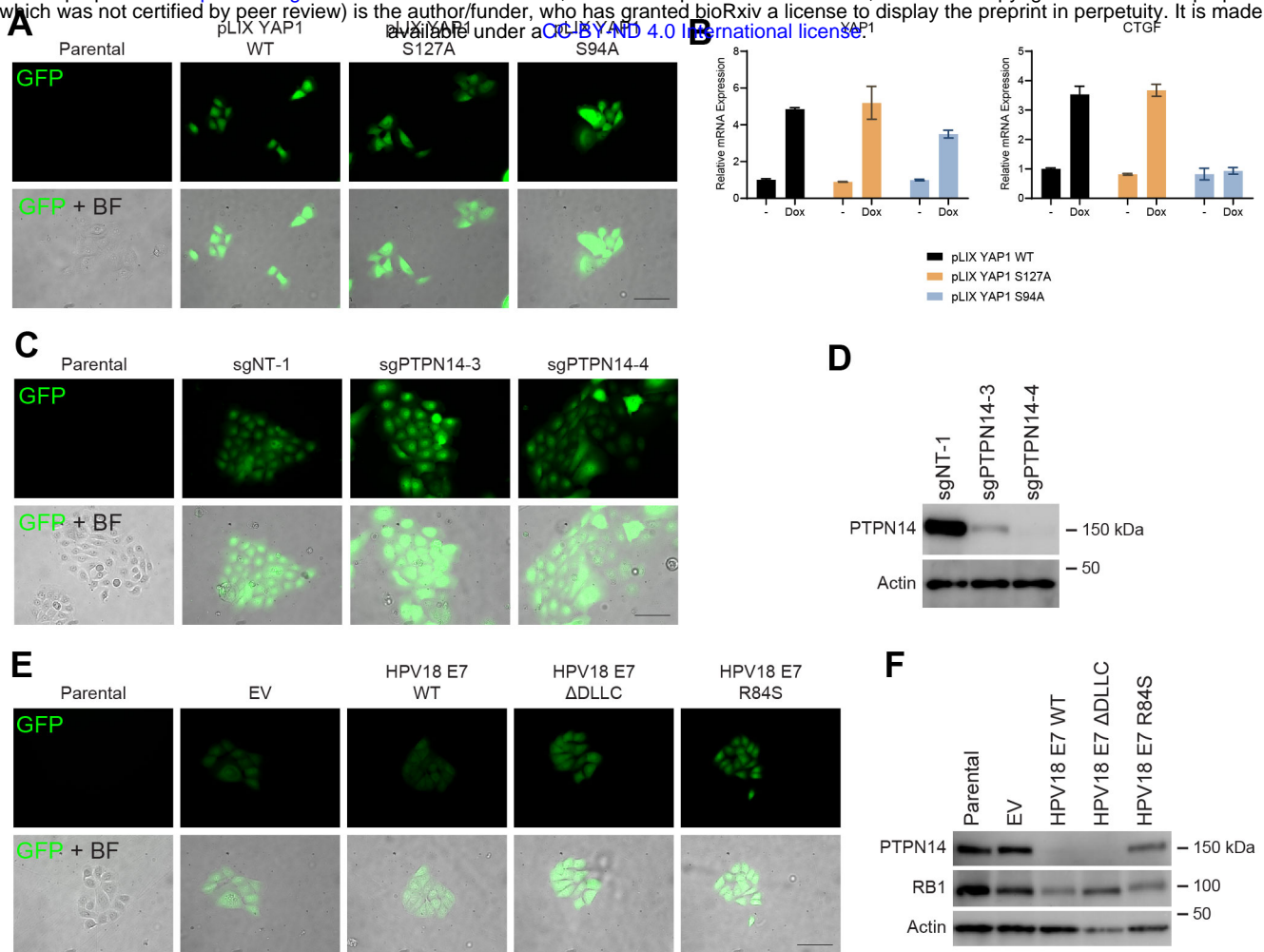


Figure 7—figure supplement 1 | PTPN14 degradation by HPV E7 promotes basal cell retention. (A-B) GFP-labeled HFK were transduced with YAP1 WT, YAP1 S127A, or YAP1 S94A under the control of a doxycycline inducible promoter. (A) GFP expression was confirmed by fluorescence microscopy. Scale bar = 100 μ m. (B) Total RNA was purified from monolayer cells +/- treatment with 1 μ g/mL doxycycline for 72h. qRT-PCR was used to assess gene expression of YAP1 and CTGF. (C-D) GFP-labeled HFK were transduced with retroviral vectors encoding HPV18 WT, HPV18 Δ DLCC, HPV18 E7 R84S, or the empty vector control (EV). (C) GFP expression was confirmed by fluorescence microscopy. Scale bar = 100 μ m. (D) Cell lysates were subjected to SDS/PAGE/Western analysis and probed with antibodies to PTPN14, RB1, and Actin. (E-F) GFP-labeled HFK were transduced with LentiCRISPR v2 sgNT-1, sgPTPN14-3, or sgPTPN14-4 vectors. (E) GFP expression was confirmed by fluorescence microscopy. Scale bar = 100 μ m (F) Cell lysates were subjected to SDS/PAGE/Western analysis and probed with antibodies to PTPN14 and Actin.

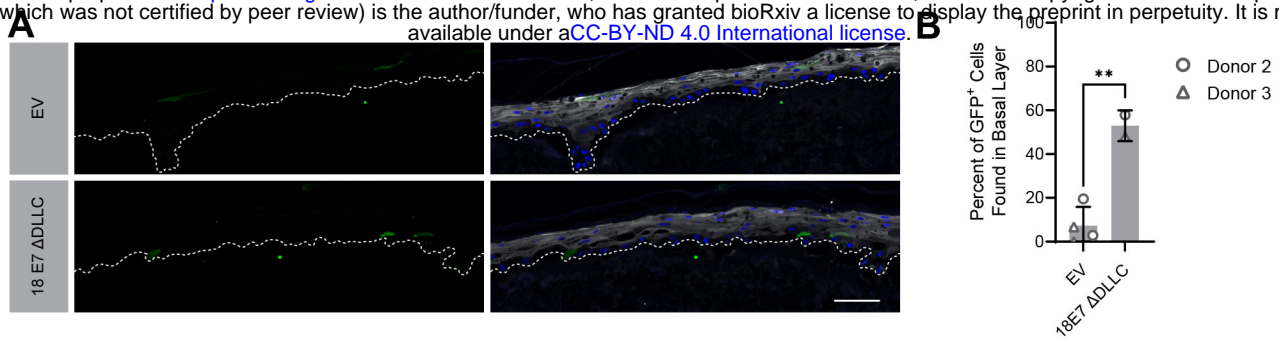


Figure 7—figure supplement 2 | HPV18 E7 can promote basal cell retention in the absence of RB1 binding. Organotypic cultures were grown from GFP-labeled cells mixed with unmodified HFK. GFP-labeled HFK were transduced with HPV18 E7 Δ DLLC or the empty vector (EV). GFP-labeled cells were mixed 1:50 into unmodified HFK. (A) FFPE sections were stained for GFP (green), IVL (grey), and Hoechst (blue). Scale bar = 100 μ m (B) Quantification of the percentage of GFP+ cells found in the basal layer. Graphs display the mean \pm SD and each individual data point (independent cultures). Statistical significance was determined by t-test. (** $p < 0.01$).

Key Resources Table				
Reagent type (species) or resource	Designation	Source or reference	Identifiers	Additional information
antibody	anti-Actin (Mouse monoclonal)	Sigma-Aldrich	Cat#: MAB1501	WB (1:20,000)
antibody	anti-GFP (Rabbit polyclonal)	Invitrogen	Cat#: A6455	WB (1:1,000); IHC-P (1:2000)
antibody	anti-Mouse IgG Alexa Fluor 488 (Goat polyclonal)	Invitrogen	Cat#: A11001	IHC-P (1:250)
antibody	anti-Mouse IgG HRP (Horse monoclonal)	Cell Signaling Technologies	Cat#: 7076	WB (1:2000)
antibody	anti-Rabbit IgG Alexa Fluor 594 (Goat polyclonal)	Invitrogen	Cat#: A11012	IHC-P (1:250)
antibody	anti-Rabbit IgG HRP (Goat monoclonal)	Cell Signaling Technologies	Cat#: 7074	WB (1:2000)
antibody	anti-HA-Peroxidase (Rat monoclonal)	Roche	Cat#: 12013819001	WB (1:500)
antibody	anti-ITGB4 (Rabbit polyclonal)	Sigma-Aldrich	Cat#: HPA036348	IHC-P (1:100)
antibody	anti-IVL (Mouse monoclonal)	Santa Cruz Biotechnology	Cat#: sc-398952	IHC-P (1:100)
antibody	anti-KRT1 (Mouse monoclonal)	Enzo Life Sciences	Cat#: C34904	
antibody	anti-PCNA	Santa Cruz Biotechnology	Cat#: sc-56	IHC-P (1:100)

antibody	Anti-PTPN14 (Rabbit monoclonal)	Cell Signaling Technology	D5T6Y; Cat#: 13808	WB (1:500)
antibody	anti-TAZ (Rabbit monoclonal)	Cell Signaling Technology	D3I6D; Cat#: 70148	WB (1:1000)
antibody	anti-V5 (Mouse monoclonal)	Invitrogen	Cat#: 46-0705	WB (1:1000)
antibody	anti-YAP1 (Rabbit monoclonal)	Cell Signaling Technology	D8H1X; Cat#: 14074	WB (1:1000); IHC-P (1:50)
transfected construct (human)	nontargeting siRNA	Dharmacon	Cat#: D-001810-01	
transfected construct (human)	siRNA to YAP1 (OnTarget Plus)	Dharmacon	Cat#: J-012200-06	
transfected construct (human)	siRNA to YAP1 (OnTarget Plus)	Dharmacon	Cat#: J-012200-08	
transfected construct (human)	siRNA to WWTR1 (OnTarget Plus)	Dharmacon	Cat#: J-016083-06	
transfected construct (human)	siRNA to WWTR1 (OnTarget Plus)	Dharmacon	Cat#: J-016083-08	
transfected construct (human)	siRNA to PTPN14 (OnTarget Plus)	Dharmacon	Cat#: J-008509-05	
transfected construct (human)	siRNA to PTPN14 (OnTarget Plus)	Dharmacon	Cat#: J-008509-08	
transfected construct (human)	siRNA to LATS1 (OnTarget Plus)	Dharmacon	Cat#: J-004632-05	
transfected construct (human)	siRNA to LATS1 (OnTarget Plus)	Dharmacon	Cat#: J-004632-08	

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transfected construct (human)	siRNA to LATS2 (OnTarget Plus)	Dharmacon	Cat#: J-003865-09	
transfected construct (human)	siRNA to LATS2 (OnTarget Plus)	Dharmacon	Cat#: J-003865-10	

White lab plasmid #	Plasmid name	Gene	Promoter	Bacterial Resistance	Tag	Tag location	Selectable Marker	Addgene Number	Original Source
8130	MSCV/GFP Pure C-FlagHA	GFP	MSCV LTR	Ampicillin	Flag, HA	C terminus	Puromycin	n/a	This Study
6640	MSCV/P-C-FlagHA 1BE7	HPV18 E7	MSCV LTR	Ampicillin	Flag, HA	C terminus	Puromycin	35018	White et al. (2012) PNAS: 109(5):E280–E287
6641	MSCV/P-C-FlagHA 1BE7	HPV18 E7	MSCV LTR	Ampicillin	Flag, HA	C terminus	Puromycin	35019	White et al. (2012) PNAS: 109(5):E280–E287
8193	MSCV/P-C-FlagHA 1BE7 R84S	HPV18 E7	MSCV LTR	Ampicillin	Flag, HA	C terminus	Puromycin	163307	Hatterschilde et al. (2020) J. Virol. 94:e1024-20
6659	MSCV/P-N-FlagHA 16E6	HPV16 E6	MSCV LTR	Ampicillin	Flag, HA	N terminus	Puromycin	44152	White et al. (2012) J. Virol. 86(24):13174-86
8208	MSCV/Neo C-HA Empty	Emply	MSCV LTR	Ampicillin	HA	C terminus	Neomycin	163310	Hatterschilde et al. (2020) J. Virol. 94:e1024-20
8133	MSCV/GFP Neo C-HA	HPV18 E7	MSCV LTR	Ampicillin	HA	C terminus	Neomycin	n/a	This Study
6993	MSCV/Neo C-HA 1BE7 R84S	HPV18 E7	MSCV LTR	Ampicillin	HA	C terminus	Neomycin	163311	Hatterschilde et al. (2020) J. Virol. 94:e1024-20
8220	MSCV/Neo C-HA 1BE7 R84S	HPV18 E7	MSCV LTR	Ampicillin	HA	C terminus	Neomycin	163312	Hatterschilde et al. (2020) J. Virol. 94:e1024-20
8340	plenti CMV GFP Hygro (656-4)	GFP	CMV	Ampicillin	HA	C terminus	Neomycin	n/a	This Study
6571	PHAGE-P-CMV NHA GFP	PTPN14	CMV	Ampicillin	HA	N terminus	Puromycin	17246	Campau et al. (2009) PLoS One. 4(8):e6529
7522	PHAGE-P-NV5 PTPN14	CMV	CMV	Ampicillin	V5	N terminus	Puromycin	n/a	Galligan et al. (2015) J. Proteome Res. 14(2): 953-966.
8251	POCK/HiMVC-YAP	YAP1 isoform 3	CMV	Ampicillin	Myc	C terminus	Puromycin	33091	White et al. (2016) mBio. 7(5):e01530-16
8252	POCK/HiMVC-YAP S12ZA	YAP1 isoform 3	CMV	Ampicillin	Flag	C terminus	Hygromycin	33092	Zhao et al. (2007) Genes Dev. 21(21):2747-61
8254	POCK/HiMVC-YAP S94A	YAP1 isoform 3	CMV	Ampicillin	Myc	C terminus	Hygromycin	33094	Zhao et al. (2007) Genes Dev. 21(21):2747-61
8319	PDONR AT-G-YAP1 WT-Stop	YAP1 isoform 3	n/a	Spectinomycin	n/a	n/a	n/a	n/a	This Study
8321	PDONR AT-G-YAP1 S12ZA-Stop	YAP1 isoform 3	n/a	Spectinomycin	n/a	n/a	n/a	n/a	This Study
8322	PDONR AT-G-YAP1 S94A-Stop	YAP1 isoform 3	TRE promoter, Tet ON	Ampicillin	n/a	n/a	Puromycin	n/a	This Study
8325	PLX YAP1 WT	YAP1 isoform 3	TRE promoter, Tet ON	Ampicillin	n/a	n/a	Puromycin	n/a	This Study
8327	PLX YAP1 S12ZA	YAP1 isoform 3	TRE promoter, Tet ON	Ampicillin	n/a	n/a	Puromycin	n/a	This Study
8328	PLX YAP1 S94A	YAP1 isoform 3	TRE promoter, Tet ON	Ampicillin	n/a	n/a	Puromycin	n/a	This Study
8278	plinduce20 EGFP-TEAD1	EGFP-TEAD1	TRE promoter, Tet ON	Ampicillin	GFP	C terminus	Neomycin	140145	Yuan et al. (2020) Nat Commun. 11, 1472

White lab plasmid #	Plasmid name	HPV genome	Bacterial Resistance	Selectable Marker	Original source
8216	pNeo-loP-HPV18	HPV18	Kanamycin	Neomycin	Wang et al. (2009) Genes Dev. 23:181-94.

White lab plasmid #	Plasmid name	sgRNA sequence from Broad Brnucleic library	Promoter	Bacterial Resistance	Selectable Marker	Addgene Number	Original Source
8092	LentiCRISPR v2 sgNT-1	AGCTCGCAGTGTCCGTTCTC	U6	Ampicillin	Puromycin	163315	Hatterschilde et al. (2020) J. Virol. 94:e1024-20
8115	LentiCRISPR v2 sgPTPN14-3	CGACACTGGACGTGAAACGGG	U6	Ampicillin	Puromycin	163314	Hatterschilde et al. (2019) PNAS: 116:7033–7042
8116	LentiCRISPR v2 sgPTPN14-4	TGTGCTTACCCTGTGAAAGA	U6	Ampicillin	Puromycin	163316	Hatterschilde et al. (2020) J. Virol. 94:e1024-20

Supplemental File 1
Antibodies used in the study

Target	Antibody Name	Company	Product Number	Use	Dilution	Notes
Actin	Anti-Actin Antibody, clone C4	Sigma-Aldrich	MAB1501	Western Blot	1:20000	
GFP	GFP Polyclonal Antibody	Invitrogen	A6455	IHC-P	1:2000	HIER: 10 mM Sodium Citrate pH 6
GFP	GFP Polyclonal Antibody	Invitrogen	A6455	Western Blot	1:1000	
Goat anti-Mouse IgG 488	Goat anti-Mouse IgG (H+L) Alexa Fluor 488	Invitrogen	A11001	IHC-P	1:250	
Goat anti-mouse IgG HRP	Anti-mouse IgG, HRP-linked Antibody	Cell Signaling Technology	7076	Western Blot	1:2000	
Goat anti-Rabbit IgG 594	Goat anti-Rabbit IgG (H+L) Alexa Fluor 594	Invitrogen	A11012	IHC-P	1:250	
Goat anti-Rabbit IgG HRP	Anti-Rabbit IgG, HRP-linked Antibody	Cell Signaling Technology	7074	Western Blot	1:2000	
HA	Anti-HA-Peroxidase	Roche	12013819001	Western Blot	1:500	
ITGB4	Anti-ITGB4 antibody	Sigma-Aldrich	HPA036348	IHC-P	1:100	HIER: Tris-EDTA pH 10
IVL	Anti-Involucrin Antibody (A-5)	Santa Cruz Biotechnology	sc-398952	IHC-P	1:100	HIER: either 10 mM Sodium Citrate pH 6 or Tris-EDTA pH 10
KRT1	Cytokeratin 1 (human) monoclonal antibody (349B4)	Enzo Life Sciences	C34904	IHC-P (TMA)	-	
PCNA	Anti-PCNA Antibody (PC-10)	Santa Cruz Biotechnology	sc-56	IHC-P	1:100	HIER: Tris-EDTA pH 10
PTPN14	PTPN14 (D5T6Y)	Cell Signaling Technology	13808	Western Blot	1:500	
TAZ	TAZ (D3I6D)	Cell Signaling Technology	70148	Western Blot	1:1000	
V5	Anti-V5 Tag Antibody	Invitrogen	46-0705	Western Blot	1:1000	
YAP1	YAP (D8H1X) XP	Cell Signaling Technology	14074	IHC-P	1:50	HIER: Tris-EDTA pH 10
YAP1	YAP (D8H1X) XP	Cell Signaling Technology	14074	Western Blot	1:1000	

Homogeneous HFk Organotypic Cultures		White Lab ID		Vector	HFk Donor	List of Figures *Inclusion of a single culture in >1 figure indicates that separate sections of the culture were processed independently	
HFk-1	08072021032 HFk	None	None	None	4659	Figure 2B	Figure 1-figure supplement 3C
HFk-2	06902211301 HFk-1	None	None	None	4659	Figure 1B	Figure 1-figure supplement 2A
HFk-3	06902211301 HFk-2	None	None	None	4659	Figure 1B	Figure 1-figure supplement 2A
HFV18 LoP-1	012720201431 WT1	pNeo-LoP-HFV18	pNeo-LoP-HFV18	4659	4659	Figure 1-figure supplement 2A	Figure 1-figure supplement 2B
HFV18 LoP-2	040520211003 1B-E7-1	MSCV-P-C-FlagHA 1B-E7	MSCV-P-C-FlagHA 1B-E7	4659	4659	Figure 1-figure supplement 2A	Figure 1-figure supplement 2B
HFV18 ET-WT-1	072220201051 1B-E7-WT1	MSCV-P-C-FlagHA 1B-E7	MSCV-P-C-FlagHA 1B-E7	4659	4659	Figure 1-figure supplement 2A	Figure 1-figure supplement 2B
HFV18 ET-WT-2	072220201051 1B-E7-WT2	MSCV-P-C-FlagHA 1B-E7	MSCV-P-C-FlagHA 1B-E7	4659	4659	Figure 1-figure supplement 2A	Figure 1-figure supplement 2B
HFV18 ET-WT-3	080720201032 1B-E7-WT3	MSCV-P-C-FlagHA 1B-E7	MSCV-P-C-FlagHA 1B-E7	4659	4659	Figure 1-figure supplement 2A	Figure 1-figure supplement 2B
HFV18 ET-WT-4	080720201032 1B-E7-WT4	MSCV-P-C-FlagHA 1B-E7	MSCV-P-C-FlagHA 1B-E7	4659	4659	Figure 1-figure supplement 2A	Figure 1-figure supplement 2B
HFV18 ET-R84S-1	072220201051 1B-E7-R84S1	MSCV-P-C-FlagHA 1B-E7 R84S	MSCV-P-C-FlagHA 1B-E7 R84S	4659	4659	Figure 2-figure supplement 2A	Figure 2-figure supplement 2A
HFV18 ET-R84S-2	072220201051 1B-E7-R84S2	MSCV-P-C-FlagHA 1B-E7 R84S	MSCV-P-C-FlagHA 1B-E7 R84S	4659	4659	Figure 2-figure supplement 2A	Figure 2-figure supplement 2A
HFV18 ET-R84S-3	080720201032 1B-E7-R84S1	MSCV-P-C-FlagHA 1B-E7 R84S	MSCV-P-C-FlagHA 1B-E7 R84S	4659	4659	Figure 2-figure supplement 2A	Figure 2-figure supplement 2A
HFV18 ET-R84S-4	080720201032 1B-E7-R84S2	MSCV-P-C-FlagHA 1B-E7 R84S	MSCV-P-C-FlagHA 1B-E7 R84S	4659	4659	Figure 2-figure supplement 2A	Figure 2-figure supplement 2A
HFV16 EB-1	040520211003 1B-E6-2	MSCV-P-N-FlagHA 1B-E6	MSCV-P-N-FlagHA 1B-E6	4659	4659	Figure 1-figure supplement 4A	Figure 1-figure supplement 4A
HFV16 EB-2	040520211003 1B-E6-3	MSCV-P-N-FlagHA 1B-E6	MSCV-P-N-FlagHA 1B-E6	4659	4659	Figure 1-figure supplement 4A	Figure 1-figure supplement 4A

Homogeneous N/Tert-Cas9 Organotypic Cultures		White Lab ID		sgRNA	Cell Line	List of Figures *Inclusion of a single culture in >1 figure indicates that separate sections of the culture were processed independently	
N/Tert-Cas9 Mock-1	072220201051 Mock-1 N-Tert	None	None	None	N/Tert-Cas9	Figure 2A	Figure 2-figure supplement 1B
N/Tert-Cas9 Mock-2	072220201051 Mock-2 N-Tert	None	None	None	N/Tert-Cas9	Figure 2-figure supplement 1A	Figure 2-figure supplement 1C
N/Tert-Cas9 sgPTPN14-1	072220201051 sgPTPN14-1 N-Tert	sgPTPN14-1	sgPTPN14-1	N/Tert-Cas9	N/Tert-Cas9	Figure 2-figure supplement 1A	Figure 2-figure supplement 1C
N/Tert-Cas9 sgPTPN14-2	072220201051 sgPTPN14-2 N-Tert	sgPTPN14-2	sgPTPN14-2	N/Tert-Cas9	N/Tert-Cas9	Figure 2-figure supplement 1B	Figure 2-figure supplement 1C
N/Tert-Cas9 sgPTPN14-3	072220201051 sgPTPN14-3 N-Tert	sgPTPN14-3	sgPTPN14-3	N/Tert-Cas9	N/Tert-Cas9	Figure 2-figure supplement 1B	Figure 2-figure supplement 1C

Cell Fate Organotypic Cultures		White Lab ID		Vectors in Tracer Cells	HFk Donor	Dilution (Tracer:Unmodified)	
Tracer Cell Condition	White Lab ID	Tracer Cell Condition	White Lab ID	Vectors in Tracer Cells	HFk Donor	Dilution (Tracer:Unmodified)	List of Figures
PLX YAP1 WT (-)-1	082020211055 WT(-)1	PLX YAP1 WT	plenti CMV/GFP Hygro (656-4)	PLX YAP1 WT	4948	1:25	Figure 7A
PLX YAP1 WT (-)-2	082020211055 WT(-)2	PLX YAP1 WT	plenti CMV/GFP Hygro (656-4)	PLX YAP1 WT	4948	1:25	Figure 7A
PLX YAP1 WT (Dox)-1	082020211055 WT(Dox)1	PLX YAP1 WT	plenti CMV/GFP Hygro (656-4)	PLX YAP1 WT	4948	1:25	Figure 7A
PLX YAP1 WT (Dox)-2	082020211055 WT(Dox)2	PLX YAP1 WT	plenti CMV/GFP Hygro (656-4)	PLX YAP1 WT	4948	1:25	Figure 7A
PLX YAP1 S127A (-)-1	043020211055 S127A(-)1	PLX YAP1 S127A	plenti CMV/GFP Hygro (656-4)	PLX YAP1 S127A	4659	1:25	Figure 7A
PLX YAP1 S127A (-)-2	043020211055 S127A(-)2	PLX YAP1 S127A	plenti CMV/GFP Hygro (656-4)	PLX YAP1 S127A	4659	1:25	Figure 7A
PLX YAP1 S127A (Dox)-1	082020211055 S127A(Dox)1	PLX YAP1 S127A	plenti CMV/GFP Hygro (656-4)	PLX YAP1 S127A	4948	1:25	Figure 7A
PLX YAP1 S127A (Dox)-2	043020211055 S127A(Dox)2	PLX YAP1 S127A	plenti CMV/GFP Hygro (656-4)	PLX YAP1 S127A	4948	1:25	Figure 7A
PLX YAP1 S127A (Dox)-3	082020211055 S127A(Dox)3	PLX YAP1 S127A	plenti CMV/GFP Hygro (656-4)	PLX YAP1 S127A	4948	1:25	Figure 7A
PLX YAP1 S127A (Dox)-4	082020211055 S127A(Dox)4	PLX YAP1 S127A	plenti CMV/GFP Hygro (656-4)	PLX YAP1 S127A	4948	1:25	Figure 7A
PLX YAP1 S94A (-)-1	082020211055 S94A(-)1	PLX YAP1 S94A	plenti CMV/GFP Hygro (656-4)	PLX YAP1 S94A	4948	1:25	Figure 7A
PLX YAP1 S94A (Dox)-1	082020211055 S94A(Dox)1	PLX YAP1 S94A	plenti CMV/GFP Hygro (656-4)	PLX YAP1 S94A	4948	1:25	Figure 7A
PLX YAP1 S94A (Dox)-2	082020211055 S94A(Dox)2	PLX YAP1 S94A	plenti CMV/GFP Hygro (656-4)	PLX YAP1 S94A	4948	1:25	Figure 7A
PLX YAP1 S94A (Dox)-3	082020211055 S94A(Dox)3	PLX YAP1 S94A	plenti CMV/GFP Hygro (656-4)	PLX YAP1 S94A	4948	1:25	Figure 7A
PLX YAP1 S94A (Dox)-4	082020211055 S94A(Dox)4	PLX YAP1 S94A	plenti CMV/GFP Hygro (656-4)	PLX YAP1 S94A	4948	1:25	Figure 7A
LentCRISPR sgNT-1-1	043020211353 CF sgNT-1-1	LentCRISPR v2 sgNT-1	plenti CMV/GFP Hygro (656-4)	LentCRISPR v2 sgNT-1	4952	1:25	Figure 7C
LentCRISPR sgNT-1-2	100620211327 sgNT-1-2	LentCRISPR v2 sgNT-1	plenti CMV/GFP Hygro (656-4)	LentCRISPR v2 sgNT-1	4952	1:25	Figure 7C
LentCRISPR sgNT-1-3	100620211327 sgNT-1-3	LentCRISPR v2 sgNT-1	plenti CMV/GFP Hygro (656-4)	LentCRISPR v2 sgNT-1	4952	1:25	Figure 7C
LentCRISPR sgPTPN14-3-1	043020211353 CF sgPTPN14-3-1	LentCRISPR v2 sgPTPN14-3	plenti CMV/GFP Hygro (656-4)	LentCRISPR v2 sgPTPN14-3	4659	1:25	Figure 7C
LentCRISPR sgPTPN14-3-2	043020211353 CF sgPTPN14-3-2	LentCRISPR v2 sgPTPN14-3	plenti CMV/GFP Hygro (656-4)	LentCRISPR v2 sgPTPN14-3	4659	1:25	Figure 7C
LentCRISPR sgPTPN14-3-3	100620211327 sgPTPN14-3-1	LentCRISPR v2 sgPTPN14-3	plenti CMV/GFP Hygro (656-4)	LentCRISPR v2 sgPTPN14-3	4952	1:25	Figure 7C
LentCRISPR sgPTPN14-3-4	100620211327 sgPTPN14-3-2	LentCRISPR v2 sgPTPN14-3	plenti CMV/GFP Hygro (656-4)	LentCRISPR v2 sgPTPN14-3	4952	1:25	Figure 7C
LentCRISPR sgPTPN14-4-1	043020211353 CF sgPTPN14-4-1	LentCRISPR v2 sgPTPN14-4	plenti CMV/GFP Hygro (656-4)	LentCRISPR v2 sgPTPN14-4	4659	1:25	Figure 7C
LentCRISPR sgPTPN14-4-2	043020211353 CF sgPTPN14-4-2	LentCRISPR v2 sgPTPN14-4	plenti CMV/GFP Hygro (656-4)	LentCRISPR v2 sgPTPN14-4	4659	1:25	Figure 7C
LentCRISPR sgPTPN14-4-3	100620211327 sgPTPN14-4-1	LentCRISPR v2 sgPTPN14-4	plenti CMV/GFP Hygro (656-4)	LentCRISPR v2 sgPTPN14-4	4952	1:25	Figure 7C
LentCRISPR sgPTPN14-4-4	100620211327 sgPTPN14-4-2	LentCRISPR v2 sgPTPN14-4	plenti CMV/GFP Hygro (656-4)	LentCRISPR v2 sgPTPN14-4	4952	1:25	Figure 7C
Empty Vector-1	100620201141 Empty	MSCV-Neo CHA Empty	PHAGE-P-CMV/NHA GFP	MSCV-Neo CHA Empty	4659	1:50	Figure 7-figure supplement 2A
Empty Vector-2	90222021137 48 EV1	MSCV-Neo CHA Empty	PHAGE-P-CMV/NHA GFP	MSCV-Neo CHA Empty	4948	1:50	Figure 7-figure supplement 2A
Empty Vector-3	90222021137 48 EV2	MSCV-Neo CHA Empty	PHAGE-P-CMV/NHA GFP	MSCV-Neo CHA Empty	4948	1:50	Figure 7-figure supplement 2A
Empty Vector-4	90222021137 52 EV1	MSCV-Neo CHA Empty	PHAGE-P-CMV/NHA GFP	MSCV-Neo CHA Empty	4948	1:50	Figure 7-figure supplement 2A
Empty Vector-5	90222021137 52 EV2	MSCV-Neo CHA Empty	PHAGE-P-CMV/NHA GFP	MSCV-Neo CHA Empty	4948	1:50	Figure 7-figure supplement 2A
HFV18 ET-WT-1	10262020114 1B-E7	MSCV-Neo CHA 1B-E7	PHAGE-P-CMV/NHA GFP	MSCV-Neo CHA 1B-E7	4659	1:50	Figure 7C
HFV18 ET-WT-2	10262020114 1B-E7	MSCV-Neo CHA 1B-E7	PHAGE-P-CMV/NHA GFP	MSCV-Neo CHA 1B-E7	4659	1:50	Figure 7C
HFV18 ET-WT-3	90222021137 48 WT 2	MSCV-Neo CHA 1B-E7	PHAGE-P-CMV/NHA GFP	MSCV-Neo CHA 1B-E7	4948	1:50	Figure 7C
HFV18 ET-R84S-1	10262020114 R84S	MSCV-Neo CHA 1B-E7 R84S	PHAGE-P-CMV/NHA GFP	MSCV-Neo CHA 1B-E7 R84S	4659	1:50	Figure 7C
HFV18 ET-R84S-2	90222021137 48 R84S 1	MSCV-Neo CHA 1B-E7 R84S	PHAGE-P-CMV/NHA GFP	MSCV-Neo CHA 1B-E7 R84S	4948	1:50	Figure 7C
HFV18 ET-R84S-3	90222021137 48 R84S 2	MSCV-Neo CHA 1B-E7 R84S	PHAGE-P-CMV/NHA GFP	MSCV-Neo CHA 1B-E7 R84S	4948	1:50	Figure 7C
HFV18 ET-R84S-4	90222021137 48 R84S 1	MSCV-Neo CHA 1B-E7 R84S	PHAGE-P-CMV/NHA GFP	MSCV-Neo CHA 1B-E7 R84S	4948	1:50	Figure 7C
HFV18 ET-ADLLC-1	90222021137 48 ADLLC 1	MSCV-Neo CHA 1B-E7 ADLLC	PHAGE-P-CMV/NHA GFP	MSCV-Neo CHA 1B-E7 ADLLC	4952	1:50	Figure 7-figure supplement 2A
HFV18 ET-ADLLC-2	90222021137 52 ADLLC 1	MSCV-Neo CHA 1B-E7 ADLLC	PHAGE-P-CMV/NHA GFP	MSCV-Neo CHA 1B-E7 ADLLC	4952	1:50	Figure 7-figure supplement 2A

Supplemental File 3

Tumor microarray specimen information

	Oral Cavity	Oropharynx	Total	HPV-positive	HPV-negative*
# Patients	72	48	120	33	87
Primary tumor (T-stage) †					
Early (T1 or T2)	60	40	100	27	73
Advanced (T3 or T4)	12	8	20	6	14
Nodal metastasis					
Positive	24	39	63	29	34
Negative	48	9	57	4	53
Overall pathologic stage †					
Early (I or II)	43	7	50	3	47
Advanced (III or IV)	29	41	70	30	40

*HPV status was defined by IHC for p16 for oropharyngeal tumors during routine clinical and was inferred as negative for oral cavity tumors per standards of the College of American Pathologists (Lewis et al. (2018) Archives of Pathology & Laboratory Medicine 142:559–597).

† 7th edition AJCC staging manual

Supplemental File 4

Gene Lists for Pathway Mutational Analyses

HIPPO Pathway

STK4
STK3
SAV1
LATS1
LATS2
MOB1A
MOB1B
YAP1
WWTR1
TEAD1
TEAD2
TEAD3
TEAD4
PTPN14
NF2
WWC1
TAOK1
TAOK2
TAOK3
CRB1
CRB2
CRB3
LLGL1
LLGL2
HMCN1
SCRIB
HIPK2
FAT1
FAT2
FAT3
FAT4
DCHS1
DCHS2
CSNK1E
CSNK1D
AJUBA
LIMD1
WTIP

Cell Cycle

CDKN1A
CDKN1B
CDKN2A
CDKN2B
CDKN2C
CCND1
CCND2
CCND3
CCNE1
CDK2
CDK4
CDK6
RB1
E2F1
E2F3

p53

TP53
MDM2
MDM4
ATM
CHEK2
RPS6KA3