Supporting Information

Kho et al. 10.1073/pnas.1304855110

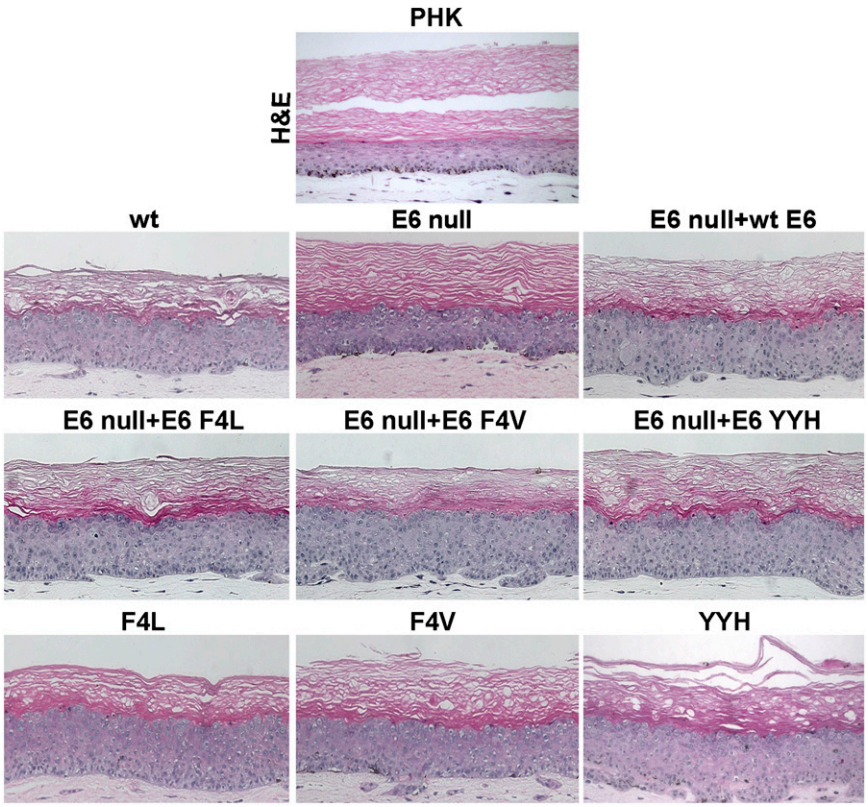

Fig. S1. Histology of organotypic cultures. H&E-stained thin sections of raft cultures contained human papillomavirus (HPV) -18 WT, *E6* mutant genomes, or E6 null mutant *trans* complemented by pBabe Puro HPV-18 upstream regulatory region-*E6* or *E6* mutation as indicated.

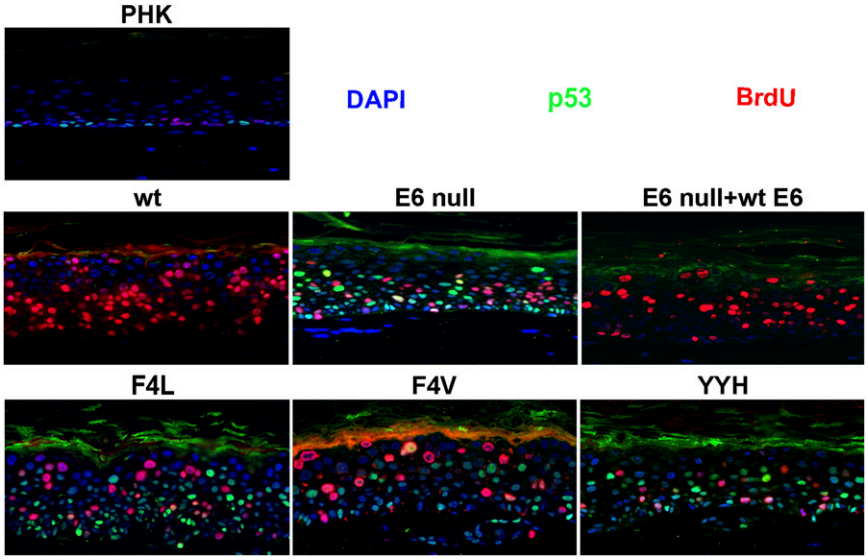

Fig. S2. p53 stabilization and BrdU incorporation in organotypic cultures. Thin sections of raft cultures containing HPV-18 WT, *E6* mutants, or E6 null mutant *trans* complemented by pBabe Puro HPV-18 upstream regulatory region-*E6* were probed for p53 (green, Alexa Fluor 488) and BrdU (red, Alexa Fluor 555) by immunofluorescence. Nuclei were stained with DAPI (blue). The experiments were conducted on multiple independent experiments, and the results were qualitatively similar.

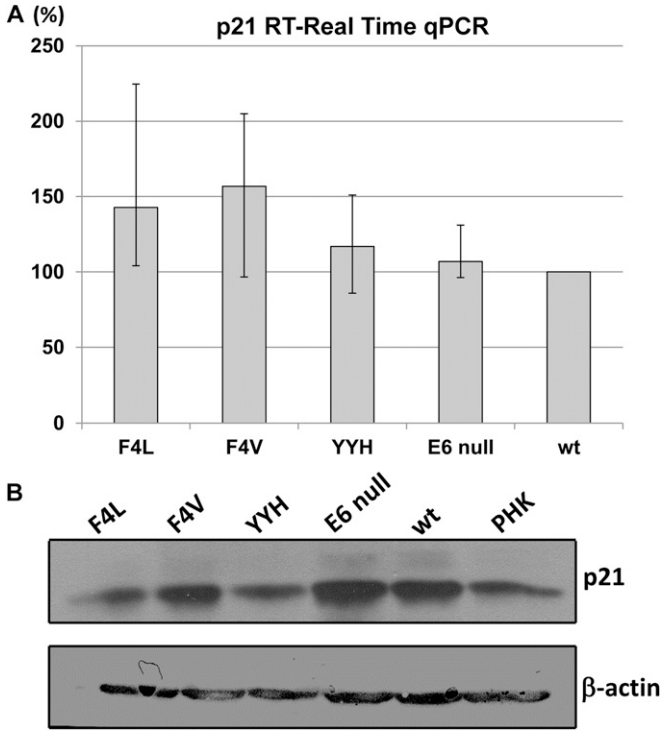

Fig. S3. p21cip1 expression in organotypic cultures. Lysates from raft cultures containing HPV-18 WT and *E6* mutants were assayed. (*A*) p21cip1 RNA was assayed by real-time quantitative RT-PCR (RT-qPCR) and normalized to β-actin as also determined by real-time RT-qPCR. The graph is the average of four independent experiments. (*B*) p21cip1 protein by Western blots from one of four experiments; β-actin was used as an internal loading reference.

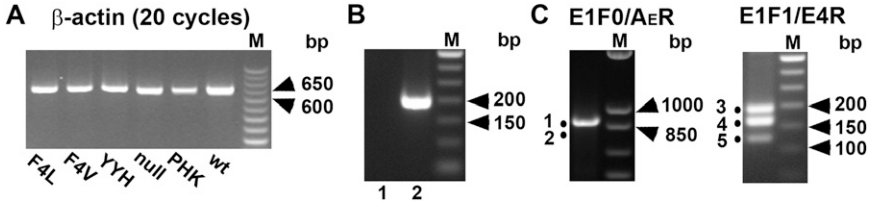

Fig. S4. RT-PCR to detect host or viral RNA. (*A*) PCR of β-actin cDNA in raft cultures containing HPV-18 WT or *E6* mutants as an internal loading control for viral sequences presented in Fig. 5. A product of 642 bp was obtained after 20 cycles of PCR amplification using published primers (1). (*B*) PCR amplification to show the specificity of junction primer E1F2. The reaction used E1F2/E4R (lane 1) or E1F1/E4R (lane 2) primer set on 5 ng pET16b-HPV-18 E1^E4 cDNA (914–929^3434–3682) as a template. PCR was performed for 25 cycles, and annealing temperature was 58 °C, identical to the temperature used to generate the data in Fig. 5*F*. Only the E1F1/E4R generated the anticipated product, whereas the junction primer yielded no product. (*C*) RT-PCR to detect three alternatively spliced early RNAs in WT HPV-18. cDNA was first conducted with a primer set of E1F0/A_ER (Fig. 5*A* and Table S3). The A_ER primer can only anneal to cDNA of RNA polyadenylated at the early polyA site (30). A major band of 899 bp was obtained (band 1), corresponding to species a in Table 1. DNA recovered from the agarose gel just below the major band (spanning dot 2 in *C*) was used in PCR amplification with the E1F1/E4R primer set. A major band of 190 bp and a minor band of 159 bp were obtained. DNA recovered from gel around the 159-bp band was subject to reamplification using the same primer set. The three bands (denoted by numbered dots) were obtained, sequenced, and identified to be species a (dot 3, 190 bp), b (dot 4, 159 bp), and C (dot 5, 118 bp), respectively (Table 1). Thus, all three transcripts were polyadenylated at the A_E site, with species a being the most abundant, whereas species C was extremely rare.

1. Meyers C, et al. (2002) Infectious virions produced from a human papillomavirus type 18/16 genomic DNA chimera. *J Virol* 76(10):4723–4733.

Table S1. Quantification of suprabasal BrdU-positive nuclei in raft cultures harboring HPV-18 WT or *E6* mutants

HPV-18	Average of suprabasal BrdU-positive cells (range)	Ratio
WT	1,391 (1,283 ~ 1,553)	1
F4L	1,129 (840 ~ 1,310)	0.81
F4V	604 (395 ~ 810)	0.43
YYH	227 (165 ~ 349)	0.16
E6 null	117 (82 ~ 165)	0.08

Day 13 raft cultures were analyzed. The average numbers (and the range) of suprabasal BrdU-positive cells were obtained from two sections per raft culture from three independent experiments. The cultures were a subset used for copy number determination presented in Fig. 3.

Table S2. Real-time qPCR of WT HPV-18 copy numbers in the presence or absence of ectopic WT *E6*

Raft cultures	Ratio of HPV-18 copy numbers/cell* (%)
PHKs	<0.004
pBabe Puro + HPV-18	81
pBabe Puro 18E6 + HPV-18	53
HPV-18 (PHK1)	100
HPV-18 (PHK2)†	86

*Transduction of retroviruses was conducted in passage 1 primary human keratinocyte (PHK1). HPV-18 WT plasmid was then generated in transduced cells by Cre-loxP recombination. After sequential selection for drug resistance, raft cultures were developed from cells equivalent to passage 2 PHKs (PHK2). Except for pBabe Puro + HPV-18, which was assayed one time, the copy numbers per cell were determined from two independent experiments (harvested on day 13 or 16) and compared with WT HPV-18 recovered from raft cultures developed from PHK1.

†WT HPV-18 was recovered from raft cultures developed from PHK2.

Table S3. Primers used in HPV-18 real-time qPCR or RT-PCR

Primer	Sequences*
qPCR (DpnI) F†	5′ nt 6896-CTGTCAAAAGGATGCTGCAC-nt 6915 3′
qPCR (DpnI) R†	7040-ACGCAATCCAGCCTGAAC-7023
E1F0	888-GGTGTGCATCCCAGCAGTAAG-908
E1F1	909-CAACAATGGCTGATCCAGAAG-929
E1F2‡	911-ACAATGGCTGATCCAGAAG ^ CT-3466
E1F3‡	911-ACAATGGCTGATCCAGAAG ^ CA-3507
E4R	3602-AGGTCCACAATGCTGCTTCT-3583
E5R	3996-GGACATGGCAGCACACATAC-3977
A_ER	TTTTTTTTTTTTTTTTTTTGCG-4268
L2R	4417-GCCAAGTCCACCCAAAAATA-4398
L1R	5778-CACCTGCAGGAACCCTAAAA-5759

*Primer sequence and the 5′ and 3′ nucleotide positions in HPV-18 genome are given.

†Primers for real-time qPCR to quantify viral DNA were designed to bracket DpnI sites.

‡Primers spanning splice junctions, nucleotides 929^3465 or 929^3506, are indicated by a caret.