Supporting Information

Rosenberger et al. 10.1073/pnas.1002620107

SI Text

DNA Staining and Flow Cytometry Analysis. Cells were harvested by trypsination, washed twice with PBS, and fixed with 70% ethanol. After fixation and centrifugation, the cell pellet was resuspended in the DNA staining solution containing DAPI as the DNA dye and SR101 as a protein counterstain following a protocol published by Stöhr et al. (1).

Immunofluorescence. Keratinocytes were grown on coverslips in K-SFM, with and without EGF depletion for 24 h, or in DMEM. After rinsing with PBS, cells were fixed in 4% paraformaldehyde for 15 min at room temperature and permeabilized with 0.1% Nonidet P-40 for 5 min. Primary antibodies (anti-keratin GP-CK10 and GP-CK18; both from PROGEN) were applied overnight. After rinsing in PBS, incubation with secondary antibody (anti-guinea pig coupled to Alexa 488) was done for 2 h at 4 °C. Nuclei were stained with DAPI. Visualization and documentation were performed with a Leica photomicroscope (2).

1. Stöhr M, Vogt-Schaden M, Knobloch M, Vogel R, Futterman G (1978) Evaluation of eight fluorochrome combinations for simultaneous DNA-protein flow analyses. *Stain Technol* 53: 205–215.
2. Langbein L, Spring H, Rogers MA, Praetzel S, Schweizer J (2004) Hair keratins and hair follicle-specific epithelial keratins. *Methods Cell Biol* 78:413–451.
3. Doorbar J (2006) Molecular biology of human papillomavirus infection and cervical cancer. *Clin Sci* 110:525–541.

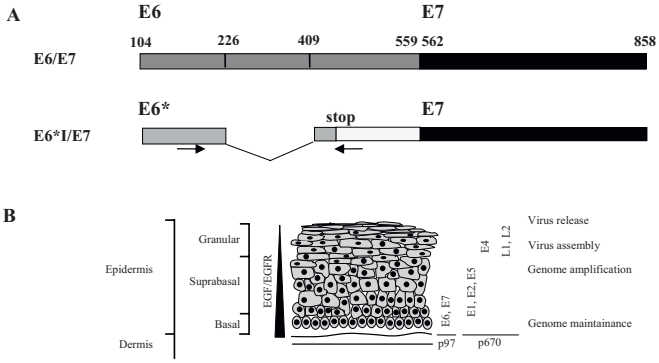

Fig. S1. (*A*) Organization of HPV16 E6/E7 and E6*/E7 early mRNA; base pair numbers are given relative to HPV genome localization. Exclusion of the exon 226–409 results in formation of the E6* ORF, which harbors a premature stop-codon. Arrows indicate primer localization for semiquantitative RT-PCR. (*B*) Illustration of replicative life cycle and gene expression of mucosal HPV within the epidermis. Modified figure according to ref. 3.

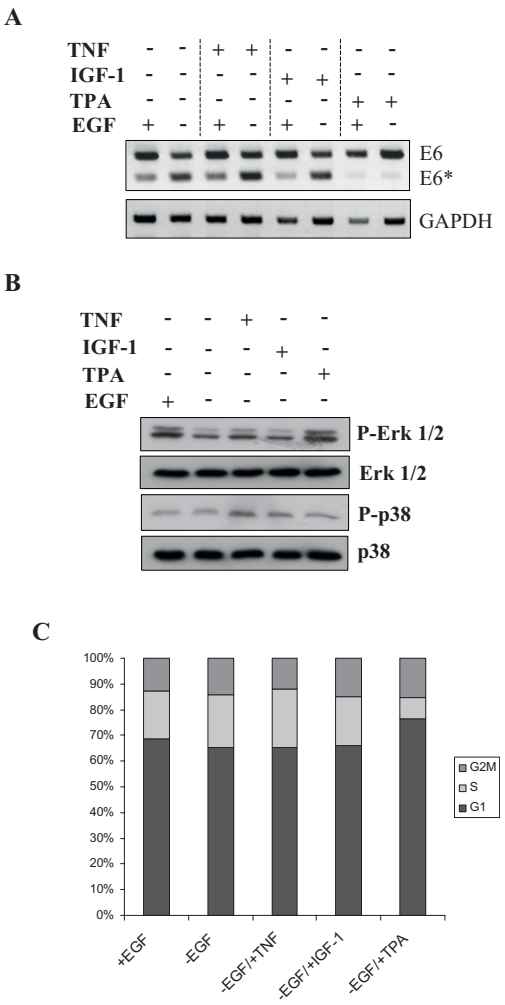

Fig. S2. TPA overcomes EGF depletion: Erk phosphorylation and E6 exon exclusion. "1321" cells were treated for 24 h as indicated. (*A*) Total RNA was harvested and E6 splicing pattern was analyzed by RT-PCR. (*B*) Total cellular extracts were analyzed by Western blot. (*C*) Cell-cycle profile analysis of the cells under the different growth factor stimuli. The percentage of cells present in different stages of the cell cycle is indicated.

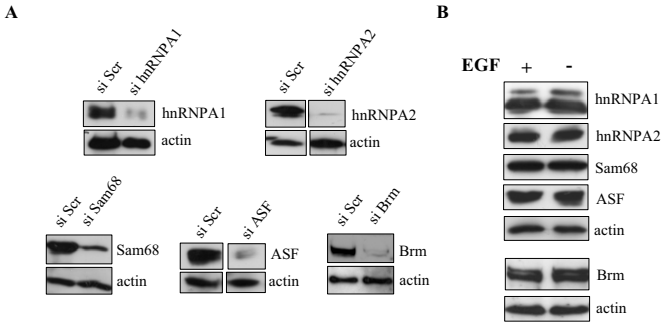

Fig. S3. Expression and siRNA knockdown of splicing factors. (*A*) "1321" keratinocytes were left untreated or transfected with 100 nM siRNAs directed against the respective splicing factor as indicated. Nuclear proteins were harvested and analyzed by Western blot. (*B*) Nuclear protein extracts of "1321" keratinocytes, grown in the presence or absence of EGF for 24 h, were analyzed by Western blot. The filters were incubated with antibodies as indicated.

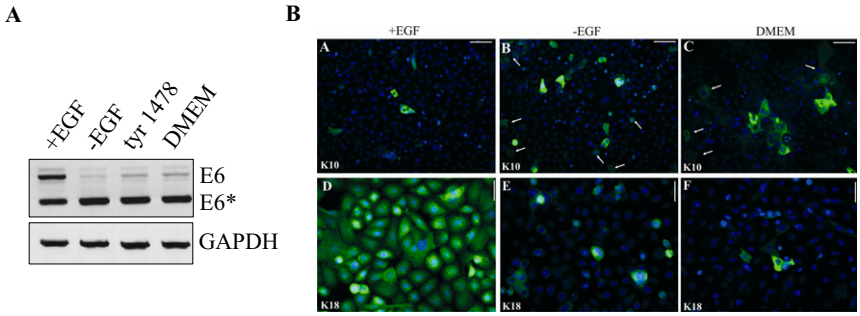

Fig. S4. DMEM-induced differentiation is correlated with enhanced E6 exon exclusion. (*A*) RT-PCR showing cytoplasmic E6 and E6* mRNA levels obtained from "1321" keratinocytes cultivation in the presence of EGF, tyrphostin 1478, and DMEM/10% serum for 24 h. (–EGF): EGF withdrawal for 24 h. (*B*) Immunofluorescence of cytokeratin K10 (*A–C*) and K18 (*D–F*) in keratinocytes grown in K-SFM medium with EGF (*A* and *D*) (+EGF), without EGF (*B* and *E*) (–EGF), or in DMEM/10% FCS (*C* and *F*). The arrows in *B* and *D* indicate cells weaker positive for K10 cytokeratin expression. (Scale bars: 50 μm.)

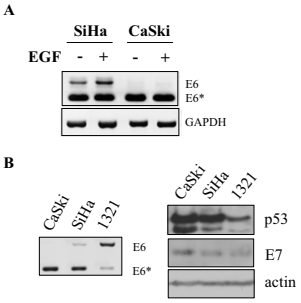

Fig. S5. Analysis of E6 splicing pattern in cervical carcinoma cell lines SiHa and CaSki. (*A*) SiHa and CaSki cells were treated with 5 ng/mL EGF for 24 h where indicated. Cytoplasmic RNA was harvested for RT-PCR analysis. (*B Left*) Cytoplasmic RNA from SiHa, CaSki, and "1321" keratinocytes were analyzed by RT-PCR. (*Right*) Western Blot showing the correlating nuclear protein levels of p53 and E7. Protein and RNA was extracted from the same samples.

Table S1. Primers and experimental conditions used for RT-PCR

Primer	Sequence	Product length	Annealing temperature	Cycle number
E6/E6*	ss 5′ actgcaatgtttcaggaccca 3′ as 5′ tcaggacacagtggctttt 3′	343 bp 161 bp	60°C	35
p53	ss 5′ ctgaggttggctctgactgtaccaccatcc 3′ as 5′ ctcattcagctctcggaacatctcgaagcg 3′	370 bp	53°C	30
c-Jun	ss 5′ gcatgaggaaccgcatcgctgcctccaagt 3′ as 5′ gcgaccaagtccttcccactcgtgcacact 3′	409 bp	55°C	35
GAPDH	ss 5′ tggatattgttgccatcaatgacc 3′ as 5′ gatggcatggactgtggtcatg 3′	460 bp	65°C	28