Supporting Information

McLaughlin-Drubin et al. 10.1073/pnas.1310432110

SI Materials and Methods

Cells. U2OS-tet on were maintained in DMEM supplemented with 10% (vol/vol) tet system-approved FBS, 50 U/mL penicillin, 50 mg/mL streptomycin, and 250 mg/mL G418. CaSki, SiHa, and HeLa cells (ATCC) were maintained in DMEM with 10% newborn calf serum, 50 U/mL penicillin, and 50 mg/mL streptomycin. Me-180 cells (ATCC) were maintained in McCoy's 5a Medium modified with 10% (vol/vol) FBS, 50 U/mL penicillin, and 50 mg/mL streptomycin. GSK J4 was obtained from Xcess Biosciences.

RNAi. The cells were transfected with the following shRNA constructs for depletion of histone lysine demethylases (KDM)6B-shKDM6B: TRCN0000095268 (Open Biosystems) and MISSION Non-Target shRNA control vector (Sigma). The cells were transfected with the following shRNA constructs for depletion of tumor suppressor $p16^{INK4A}$ – pbabeshp16AB, pbabep16CD, and pbabe-p16EF or pbabeU6 as a control (a generous gift from James Rocco, Massachusetts General Hospital, Boston, MA).

The cells were transfected with 40 nM of the following ON-TARGET*plus* SMARTpools: KDM6B-specific ON-TARGET*plus* SMARTpool (L-023013–01; Thermo Scientific Dharmacon), cyclin-dependent kinases 4 (CDK4)-specific ON-TARGET*plus* SMARTpool (L-003238-00; Thermo Scientific Dharmacon), CDK6-specific ON-TARGET*plus* SMARTpool (L-003240-00; Thermo Scientific Dharmacon), $p16^{INK4A}$-specific ON-TARGET*plus* SMARTpool (sequence AACGCACCGAAUAGUUACGGUUU or AAUAGUUACGGUCGGAGGCCGUU; Thermo Scientific Dharmacon), or ON-TARGET*plus* Non-Targeting Pool (D-001810-10; Thermo Scientific Dharmacon) using Lipofectamine 2000 (Invitrogen).

Wild-type $p16^{INK4A}$ (missing the first 24 nucleotides; a gift of Robert Weinberg, Whitehead Institute, Massachusetts Institute of Technology, Cambridge MA) (1) cloned into LXSN (2) was used in rescue experiments with shp16AB. Rescue forms of $p16^{INK4A}$ (resp16CD and resp16EF, resistant to their respective shRNA), were generated by introducing silent mutations in the $p16^{INK4A}$ sequence using the following primers: shp16CD sense 5′-GGGTCGGGTAGAAGATGTGCGGGCGCTG-3′, shp16CD antisense 5′-CAGCGCCCGCACATCTTCTACCCGACCC-3′, shp16EF sense 5′-GGGGCGCTGCCCAAAGCACCGAATAGT-3′, and shp16EF antisense 5′-ACTATTCGGTGCATTGGGCAGCGCCCC-3′. The mutations were constructed by PCR using the QuikChange site-directed mutagenesis kit (Stratagene) according to the manufacturer's instructions.

1. Medema RH, Herrera RE, Lam F, Weinberg RA (1995) Growth suppression by p16ink4 requires functional retinoblastoma protein. *Proc Natl Acad Sci USA* 92(14):6289–6293.

2. Piboonniyom SO, Timmermann S, Hinds P, Münger K (2002) Aberrations in the MTS1 tumor suppressor locus in oral squamous cell carcinoma lines preferentially affect the INK4A gene and result in increased cdk6 activity. *Oral Oncol* 38(2):179–186.

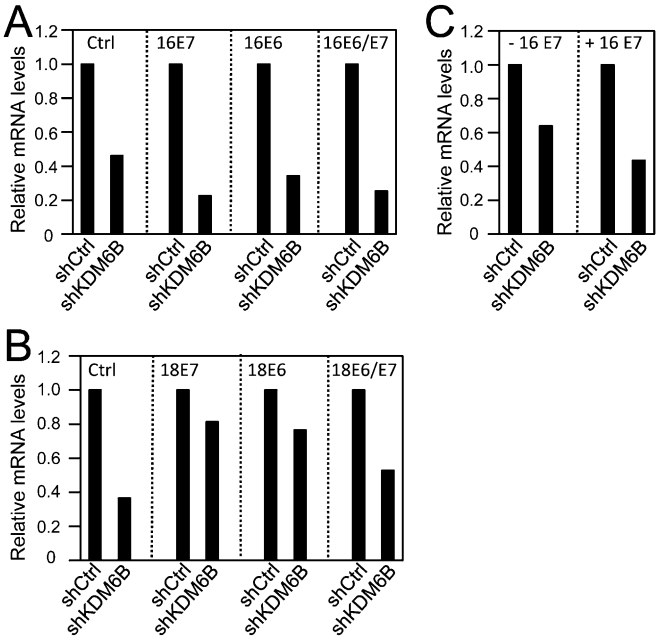

Fig. S1. KDM6B depletion in human foreskin keratinocyte (HFK) populations and U2OS osteosarcoma cells. KDM6B was depleted in: (*A*) HFKs expressing control vector, human papillomavirus (HPV)16 E7, E6, or E6 and E7; (*B*) HFKs expressing control vector, HPV18 E7, E6, or E6 and E7; and (*C*) U2OS-tet on cells with doxycycline-inducible expression of HPV16 E7. mRNA levels were determined by quantitative RT-PCR (qRT-PCR). The graphs depict representative experiments.

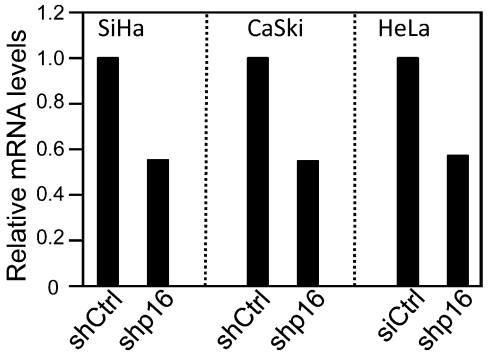

Fig. S2. p16^{INK4A} depletion in cervical carcinoma lines. p16^{INK4A} was depleted in the HPV16^{+} SiHa and CaSki cervical carcinoma lines, and the HPV18^{+} HeLa cervical cancer line. mRNA levels were determined by qRT-PCR. The graph depicts representative experiments.

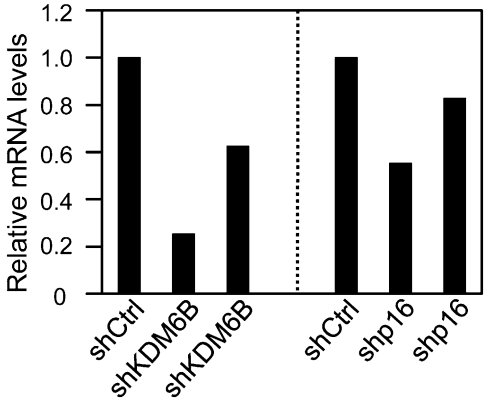

Fig. S3. KDM6B and p16^{INK4A} depletion in SiHa cervical carcinoma cells. KDM6B and p16^{INK4A} were depleted in the HPV16^{+} SiHa cervical carcinoma line. mRNA levels were determined by qRT-PCR.

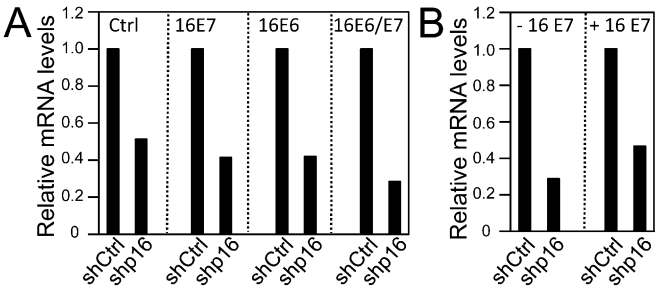

Fig. S4. p16^{INK4A} depletion in HFK populations. p16^{INK4A} was depleted in (*A*) HFKs expressing control vector, HPV16 E7, E6, or E6 and E7; and (*B*) in U2OS-tet on cells with doxycycline-inducible HPV16 E7 expression. mRNA levels were determined by qRT-PCR. The graphs depict representative experiments.

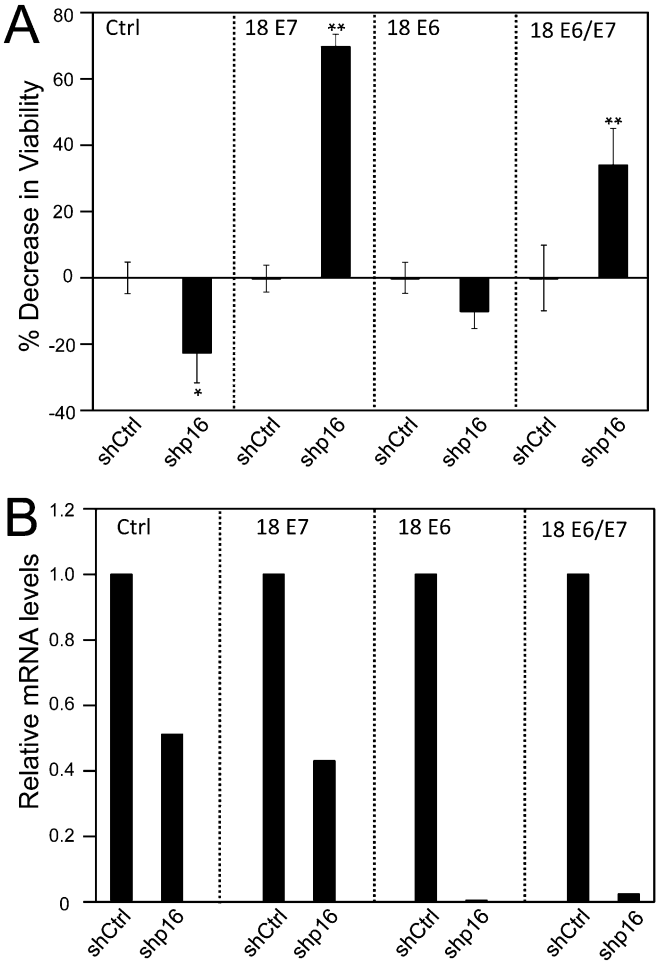

Fig. S5. Loss of viability upon p16^{INK4A} depletion in HPV18-expressing HFK populations. p16^{INK4A} was depleted in (*A*) HFKs expressing control vector, HPV18 E7, HPV18 E6, or HPV18 E6 and E7. Cell viability was measured by AlamarBlue assay. Averages and SDs for three independent experiments are shown. Statistically significant changes are indicated: $^{**}P < 0.01$. (*B*) p16 mRNA levels were determined by qRT-PCR. The graphs depict representative experiments.

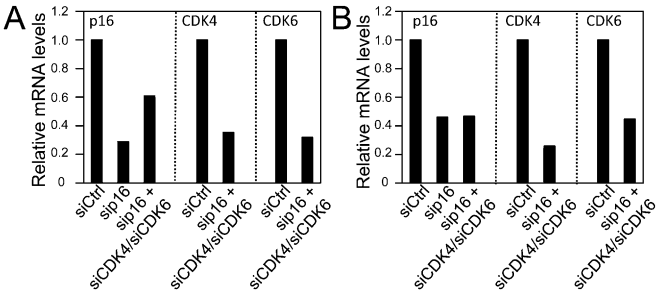

Fig. S6. Analysis of p16^{INK4A}, CDK4, and CDK6 mRNAs. (*A*) p16^{INK4A} was depleted in U2OS-tet on cells (– dox) by transfection with p16^{INK4A}-specific siRNA duplexes or control siRNA (siCtrl), and CDK4 and CDK6 were depleted by transfection with CDK4- and CDK6-specific siRNA duplexes. (*B*) p16^{INK4A} was depleted in U2OS-tet on cells with doxycycline-inducible expression of HPV16 E7 (+ dox) by transfection with p16^{INK4A}-specific siRNA duplexes or control siRNA (siCtrl), and CDK4 and CDK6 were depleted by transfection with CDK4- and CDK6-specific siRNA duplexes. mRNA levels were determined by qRT-PCR. The graphs depict representative experiments.

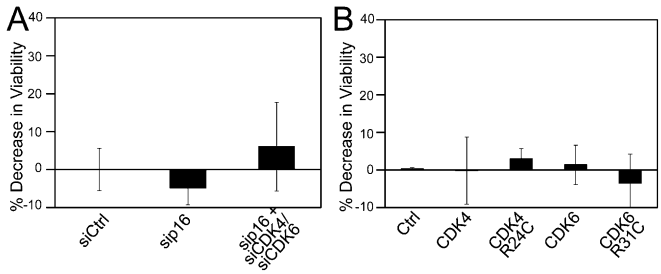

Fig. S7. Cell death caused by p16^{INK4A} depletion in p16^{INK4A}-addicted cells is dependent on CDK4 and CDK6. (*A*) p16^{INK4A} was depleted in vehicle-treated U2OS-tet on cells by transfection with p16^{INK4A}-specific siRNA duplexes or control siRNA (siCtrl), and CDK4 and CDK6 were depleted by transfection with CDK4- and CDK6-specific siRNA duplexes. (*B*) Vehicle-treated U2OS-tet on cells expression were transfected with CDK4, CDK6, and oncogenic CDK4 or CDK6 mutants that cannot be inhibited by p16^{INK4A} (R24C and R31C, respectively).

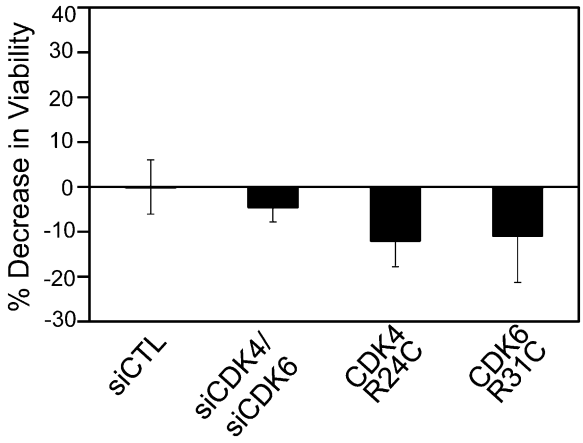

Fig. S8. HeLa cell viability is not significantly affected by CDK4/6 depletion or ectopic expression of p16^{INK4A}-insensitive CDK4 or CDK6 mutants. CDK4 and CDK6 were depleted by transfection with CDK4 and CDK6-specific siRNA duplexes in HeLa cells. HeLa cells were transfected with oncogenic mutants of CDK4 or CDK6 that cannot be inhibited by p16^{INK4A} (R24C and R31C, respectively).

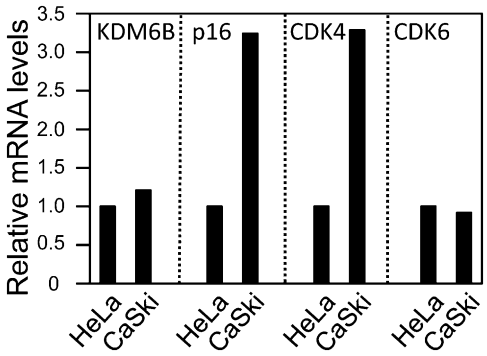

Fig. S9. Comparative analysis of KDM6B, p16^{INK4A}, CDK4, and CDK6 mRNA levels in HeLa and CaSki cervical carcinoma lines. KDM6B, p16^{INK4A}, CDK4, and CDK6 mRNA levels were determined by qRT-PCR in the HPV16^{+} cervical carcinoma cell line SiHa and the HPV18^{+} cervical cancer cell line HeLa.