Supporting Information:

Materials and Methods:

Neonatal foreskin keratinocytes, transfection, raft cultures and analysis: Primary human keratinocytes were isolated from neonatal foreskins that were collected per approved IRB protocol from the Newborn Nursery of the University of Alabama at Birmingham. Early passage (P0 or P1) PHKs were transfected with pNeo-Lox-HPV18 and pCAGGS-nlsCre, a plasmid that expresses nls-Cre recombinase, using Fugene 6.0 (Promega) and selected with G418 (110 μg/ml for 3 days) as described (1). Multiple raft cultures from each batch of transfected PHKs were prepared. Independent experiments used different batches of PHKs harvested on different days. Vorinostat was dissolved in DMSO as a concentrated stock (10 mM). A subset of cultures were treated with Vorinostat (Cat. V8477, LC Labs, Wuburn, MA, USA) at different concentrations. Unless otherwise specified, cultures were exposed to Vorinostat from day 6 through day 13 and harvested on day 13. Culture media with or without Vorinostat were refreshed every other day until harvest. The control cultures were untreated or exposed to DMSO (0.1 %) for the same duration. This vehicle did not affect cell proliferation or differentiation, nor HPV DNA amplification. 5-Bromo-2'-deoxyuridine (BrdU, cat. B5002, Millipore Sigma, St. Louis, MO, USA), an analogue of thymidine, was added to the culture medium at 100 μg/ml for the final 6 h prior to harvesting. One of the cultures at each condition was harvested by fixation in 10% buffered formalin, embedded in paraffin (FFPE) and used for in situ analyses. Another set of cultures was harvested for total DNA to determine the relative viral DNA amplification. The remaining unfixed cultures were used to isolate total proteins in MCLB buffer for immunoblot analyses (2). Belinostat (Cat. S1085) and Panobinostat

(Cat. S1030) were procured from Selleckchem (Houston, TX, USA). Raft cultures were treated at a range of concentrations from day 6 to day 14 and harvested on day 14.

W12-E cells were grown as described (3) and used to prepare raft cultures. Cultures were harvested on day 14 with or without exposure to Vorinostat at 1 and 5 μM from day 6 to day 14. The Moloney murine leukemia retroviral vector expressing HPV-11 E7 G22D mutant form and the preparation of raft cultures of PHKs infected with amphotropic recombinant retrovirus have been described (4). Cultures were harvested on day 14, with or without an exposure to Vorinostat as described above. BrdU was added to these two raft cultures as described above. Cervical cancer cell line, CaSki, (5) were grown in DMEM supplemented with 10% fetal bovine serum. Raft cultures were similarly prepared with or with exposure to Vorinostat from day 5 to day 14 and harvested on Day 14.

Histology and indirect immunofluorescence (IF) assays

Freshly cut 4 μm tissue sections of FFPE raft cultures were deparaffinized and rehydrated and stained with hematoxylin and eosin (H&E) (Fisher Scientific). For additional in situ assays, rehydrated sections were heat treated for antigen retrieval in 10 mM citrate buffer (pH 6.0 in microwave) as per standard protocols. Tissues were then treated with 3% hydrogen peroxide for 10 minutes and then blocked with 10% goat serum for 1 h. The sections were probed with primary antibodies (*SI Appendix,* Table 1). Indirect immunofluorescence (IF) signals were detected via HRP-conjugated or fluorescently labeled secondary antibodies. HRP-conjugated secondary antibodies were labeled with fluorescein or cy3 by the tyramide-based signal amplification and detection system (Perkin-Elmer, MA, USA) as described (6). Tissue sections were mounted with 4',6-

diamidino-2-phenylindole (DAPI) containing mounting media (H1200, Vector Laboratories, Burlingame, CA). Images were captured with a 20x objective in an Olympus BX63 microscope fitted with appropriate filters for immunofluorescence using an Olympus DP73 camera and cellSens software. Numbers of total nuclei and those positive for specific antigens as well as signal intensity were determined from four non-overlapping areas of each slide using the Count and Measure application of the cellSens software. The same threshold level of signal intensity was set to count objects in each set of experiments. Images were processed using Adobe Photoshop software.

In-situ hybridization and qPCR

Fluorescence in-situ hybridization to detect HPV-18 DNA amplification in FFPE tissue sections was performed as reported (6). Real time quantitative PCR to determine the relative HPV-18 plasmid DNA copy number/cell was conducted as described (1, 7). The average values from triplicate qPCR reactions were used to compute DNA copy numbers per cell.

Immunoblot assays

Total protein was prepared from raft cultures in the presence of protease and phosphatase inhibitors as described (8). Lysates were resolved in 4-15% polyacrylamide gels (Mini-Protean TGX Stain-Free, BIO-RAD, Hercules, California) and transferred to PVDF membranes by the standard wet blot transfer method. Target proteins were probed with specific primary antibodies and detected with appropriate horseradish peroxidase

(HRP)-conjugated secondary antibodies in combination with Pierce ECL substrate (Cat. 32106) from Thermo-Scientific (Rockford, IL). Alternatively, mouse and rabbit antibodies were detected with IRDye680RD anti-mouse (green) and IRDye800CW anti-rabbit (red) secondary antibodies and scanned using an LI-COR CLx documentation system. Antibodies, suppliers, and dilutions used in immunoblots are presented in *Supplemental Information Appendix,* Table 1.

TUNEL assay

In-situ Cell Death Detection Kit Fluorescein (Cat # 11684795910 Roche, marketed by Sigma-Aldrich, Saint Louis, MO) was used for Terminal deoxynucleotidyl Transferase (TdT) dUTP Nick-End Labeling (TUNEL) assay. Raft culture sections were processed as described, equilibrated in PBS, and incubated with the detection reagents for 1 hour at 37^{o}C as per the kit protocols. The sections were then washed with 1X Phosphate buffered saline and transferred to microscope slides in DAPI-containing mounting media. Images were captured by immunofluorescence microscopy with a 20X objective.

Figure Legends:

Fig. S1. Histology of PHK or day 13 HPV-18 raft cultures exposed to DMSO or 200 nM Vorinostat from day 6 to 13, revealed by hematoxylin and eosin staining. Images were captured with 20X objective.

Fig. S2. Day 14 raft cultures of PHKs acutely transduced with a recombinant retrovirus expressing HPV-11 E7 D22G. Cultures were exposed to 1 or 5 μM Vorinostat from day

6 to day 14. BrdU was added to the medium in the last 6 hr. Top row, histology, revealed by hematoxylin and eosin staining. Middle row, BrdU incorporation. Lower row, in situ detection of γ-H2AX (red) and TUNEL (green). Nuclei were stained with DAPI. Images were captured with 20X objective.

Fig. S3. Day 14 HPV-18 infected raft cultures exposed to Vorinostat, Belinostat or Panobinostat. (*A*) Histology was revealed by hematoxylin and eosin staining. (B) L1 antibody staining (green) of Balinostat and Panobinostat treated cultures. Images were captured with 20X objective.

Fig. S4. Double-stranded DNA damage and apoptosis in Belinostat and Panobinostat treated, HPV-18 infected raft cultures. γ-H2AX (red) and TUNEL signals (green) were revealed by indirect immunofluorescence detection. Nuclei were stained with DAPI. Images were captured with 20X objective.

References:

1. Wang HK, Duffy AA, Broker TR, & Chow LT (2009) Robust production and passaging of infectious HPV in squamous epithelium of primary human keratinocytes. *Genes Dev.* 23(2):181-194.
2. Genovese NJ, Banerjee NS, Broker TR, & Chow LT (2008) Casein kinase II motif-dependent phosphorylation of human papillomavirus E7 protein promotes p130 degradation and S-phase induction in differentiated human keratinocytes. *J. Virol.* 82(10):4862-4873.
3. Jeon S, Allen-Hoffmann BL, & Lambert PF (1995) Integration of human papillomavirus type 16 into the human genome correlates with a selective growth advantage of cells. *J. Virol.* 69(5):2989-2997.
4. Genovese NJ, Broker TR, & Chow LT (2011) Nonconserved lysine residues attenuate the biological function of the low-risk human papillomavirus E7 protein. *J. Virol.* 85(11):5546-5554.
5. Baker CC*, et al.* (1987) Structural and transcriptional analysis of human papillomavirus type 16 sequences in cervical carcinoma cell lines. *J. Virol.* 61(4):962-971.
6. Van Tine BA, Broker TR, & Chow LT (2005) Simultaneous in situ detection of RNA, DNA, and protein using tyramide-coupled immunofluorescence. *Methods Mol. Biol.* 292:215-230.
7. Banerjee NS, Wang HK, Beadle JR, Hostetler KY, & Chow LT (2017) Evaluation of ODE-Bn-PMEG, an acyclic nucleoside phosphonate prodrug, as an antiviral against productive HPV infection in 3D organotypic epithelial cultures. *Antiviral Res.* 150:164-173.

8. Banerjee NS, Wang HK, Broker TR, & Chow LT (2011) Human papillomavirus (HPV) E7 induces prolonged G2 following S phase reentry in differentiated human keratinocytes. *J. Biol. Chem.* 286(17):15473-15482.

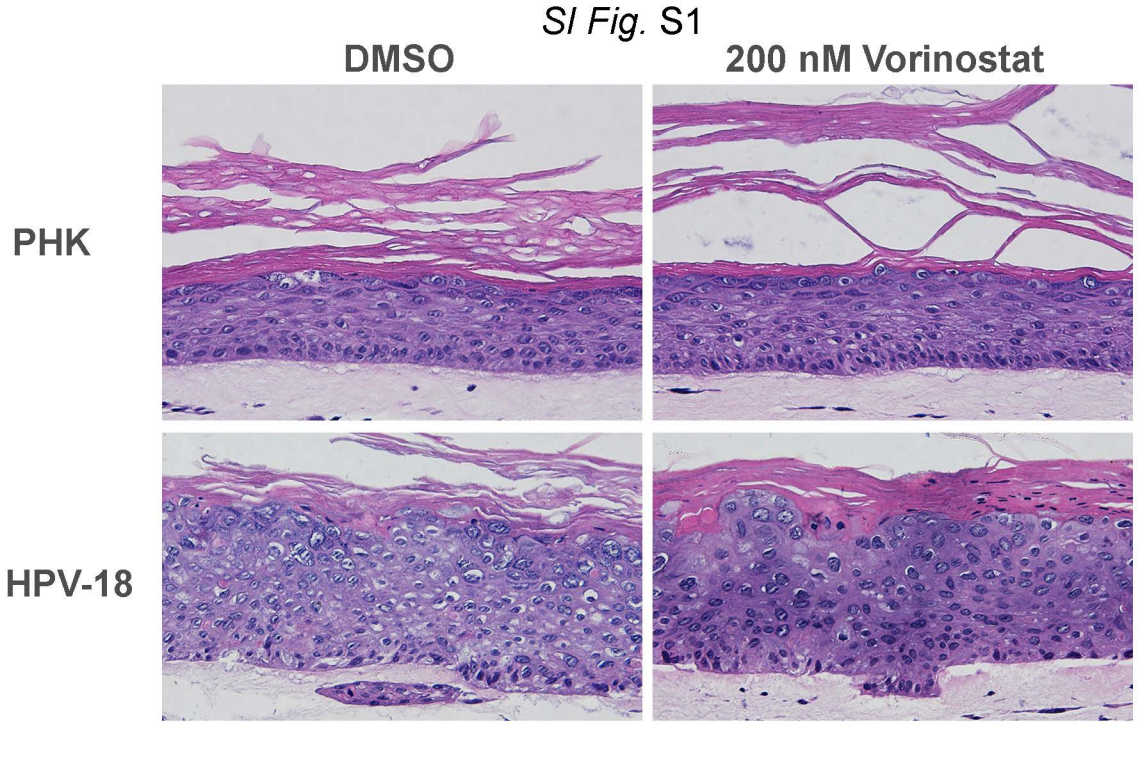
SI Appendix Table 1

Sl#	Antibody	Clone	Species	Vendor/Source	Catalogue #	Application
1	anti-ATM	D2E2	Rabbit	Cell Signaling	2873	WB
2	anti-Phospho-ATM (S1981)	EP1890Y	Rabbit	AbCam	ab81292	WB
3	Apaf1	Clone 24	Mouse, IgG1	BD Biosciences	611364	WB
4	Bim	C34C5	Rabbit	Cell Signaling	2933	WB
5	Phospho-Histone g-H2AX (s139)	Polyclonal	Rabbit	Cell Signaling	2577	WB
6	Phospho-Histone g-H2AX	3F2	Mouse, IgG1	Abcam	ab22551	IHC-P
7	BAX	D2E11	Rabbit	Cell Signaling	5023	WB
8	BrdU	ZBU30	Mouse, IgG1	Thermo Fisher Scientific	03 3900	IHC-P
9	Cleaved caspase-3 (Asp175)	5A1E	Rabbit	Cell Signaling	9664	WB
10	EZH2	Polyclonal	Rabbit	Millipore Sigma	07-689	WB
11	pRB	G3-245	Mouse, IgG1	BD Biosciences	554136	WB
12	RB-2/p130	Clone 10	Mouse, IgG2a	BD Biosciences	610261	WB
13	p53	DO-7	Mouse, IgG2b	Leica Biosystems	p53-DO7-L-CE	WB, IHC
14	PCNA	PC-10	Mouse, IgG2a	Invitrogen	180110	IHC-P
15	CycB1	Y106	Rabbit	AbCam	ab32053	WB, IHC
16	NOXA	Polyclonal	Goat	Santa Cruz Biotech	sc-26917	WB, IHC
17	PUMA	EP512Y	Rabbit	AbCam	ab33906	WB
18	HDAC1	Polyclonal	Rabbit	Bethyl Laboratories	A300-713A	WB
19	HDAC2	Polyclonal	Rabbit	Bethyl Laboratories	A300-705A	WB
20	HDAC3	7G6C5	Mouse, IgG2a	Cell Signaling	3949	WB
21	HDAC4	D15C3	Rabbit	Cell Signaling	7628	WB
22	HDAC5	Polyclonal	Rabbit	Bethyl Laboratories	A-303-463A	WB
23	HDAC6	D2E5	Rabbit	Cell Signaling	7558	WB
24	HDAC7	Polyclonal	Rabbit	Bethyl Laboratories	A-301-384A	WB
25	SirT1	D1D7	Rabbit	Cell Signaling	9475	WB
26	Total DNA-PKcs	3H6	Mouse, IgG1	Cell Signaling	12311	WB
27	Phosphorylated DNA-PKcs (S2056)	Polyclonal	Rabbit	Abcam	ab18192	WB
28	HPV-18 E6	G-7	Mouse, IgG1	Santa Cruz Biotech	sc-365089	WB
29	HPV-18 E7	F-7	Mouuse, IgG1	Santa Cruz Biotech	sc-365035	WB
30	E6AP	E-4	Mouse, IgG2b	Santa Cruz Biotech	sc-166689	WB
31	H4K12Ac	Polyclonal	Rabbit	Epigentek	A-4029-050	WB
32	H4K16 Ac	Polyclonal	Goat	Epigentek	A-4030-050	WB

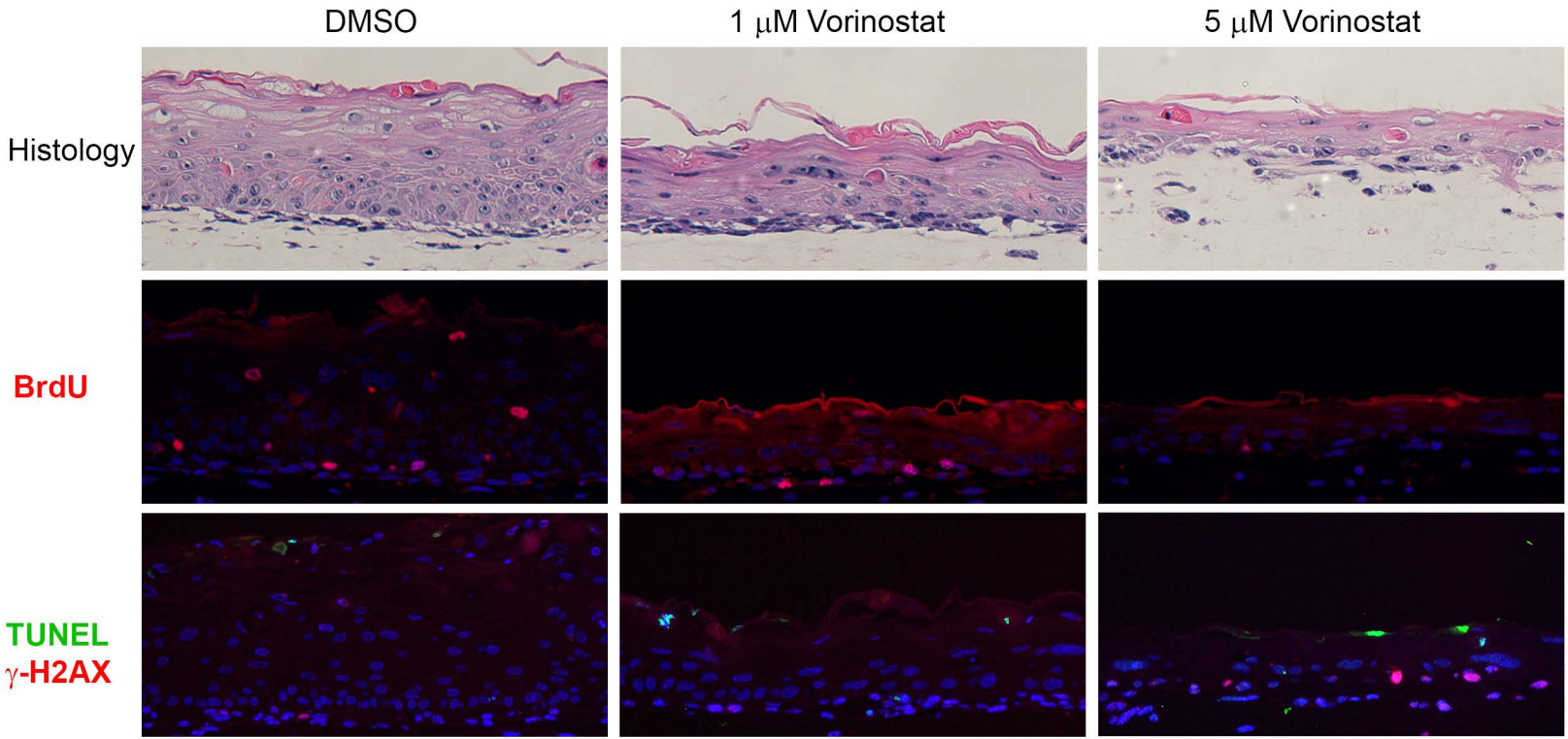
SI Appendix , Table 1 (Continued)

33	Mre11	EPR3471	Rabbit	AbCam	109623	WB
34	Nbs1	Y112	Rabbit	AbCam	32074	WB
35	actin	AC-15	Mouse, IgG1	Millipore Sigma	A1978	WB
36	CK10	DE-K10	Mouse, IgG1	Abcam	ab9026	WB, IHC
37	Loricrin	Polyclonal	Rabbit	AbCam	ab85679	IHC
38	H4 (acetyl K5 + K8 + K12 + K16)	EPR16606	Rabbit	AbCam	ab177790	WB, IHC

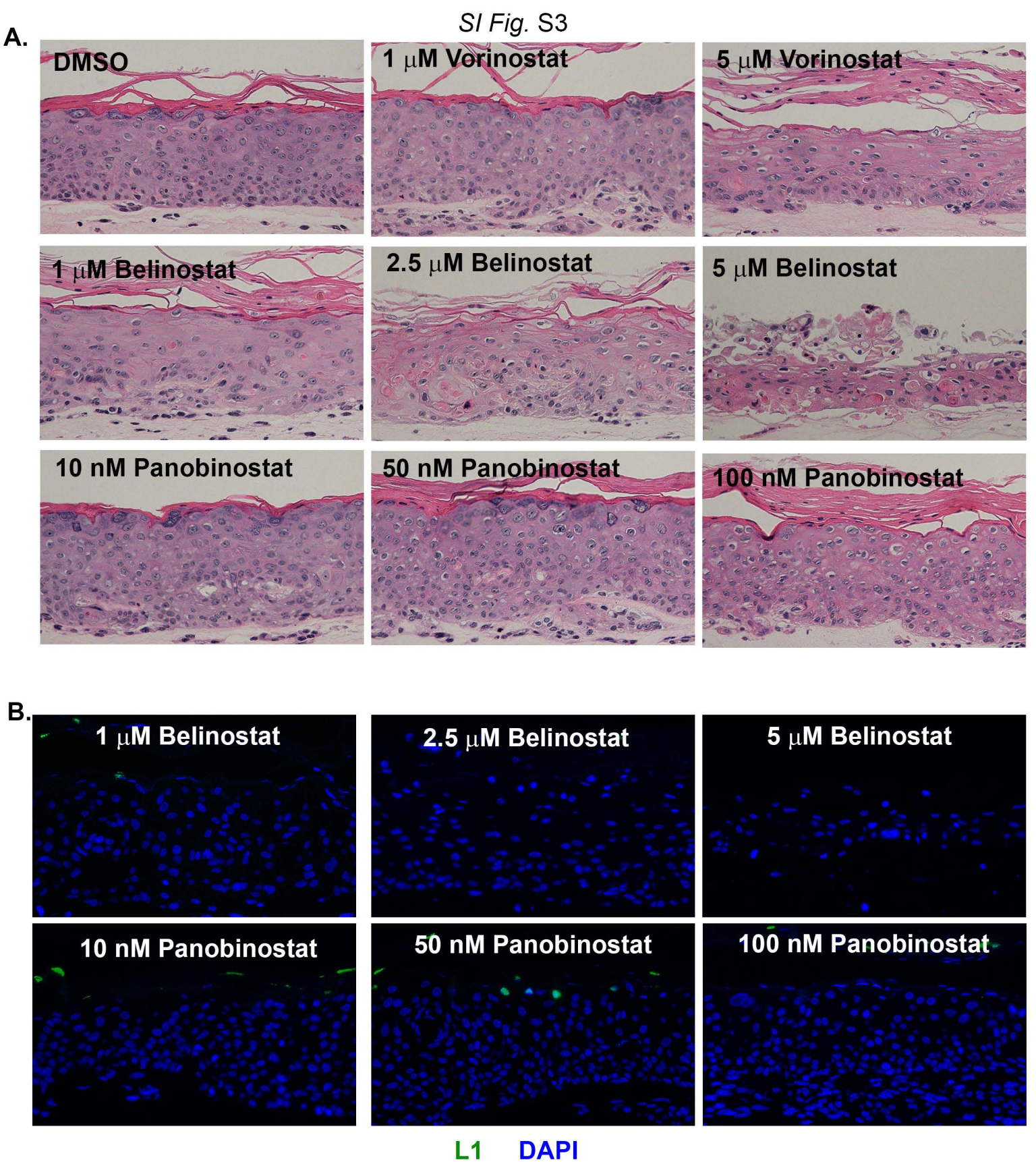
SI Fig. S1



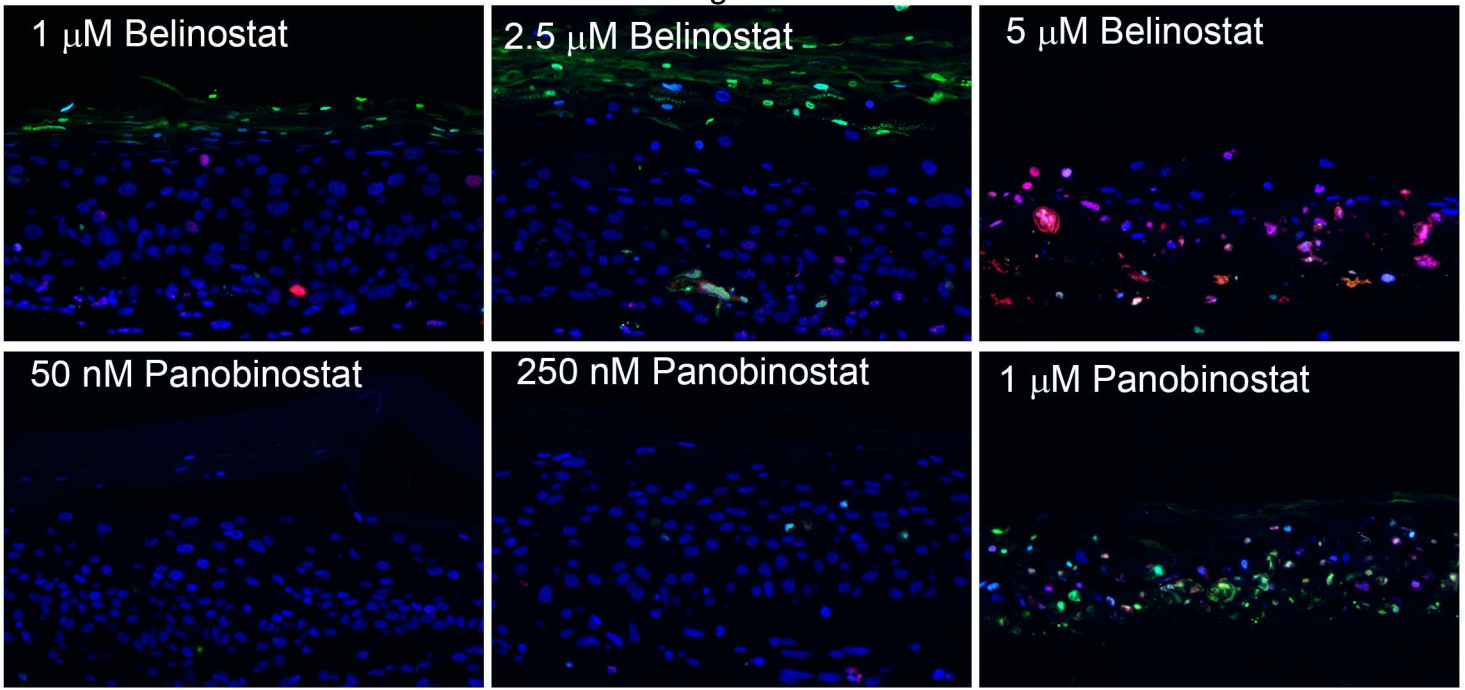
SI Fig. S2


DMSO
1 μM Vorinostat
5 μM Vorinostat
Histology
BrdU
TUNEL
γ-H2AX
HPV-11 E7(G22D) Exposed to Agents From Days 6 to 14

SI Fig. S3


A.
DMSO
1 μM Vorinostat
5 μM Vorinostat
1 μM Belinostat
2.5 μM Belinostat
5 μM Belinostat
10 nM Panobinostat
50 nM Panobinostat
100 nM Panobinostat
B.
1 μM Belinostat
2.5 μM Belinostat
5 μM Belinostat
10 nM Panobinostat
50 nM Panobinostat
100 nM Panobinostat
L1
DAPI

SI Fig. S4


1 μM Belinostat
2.5 μM Belinostat
5 μM Belinostat
50 nM Panobinostat
250 nM Panobinostat
1 μM Panobinostat
γ-H2AX TUNEL DAPI