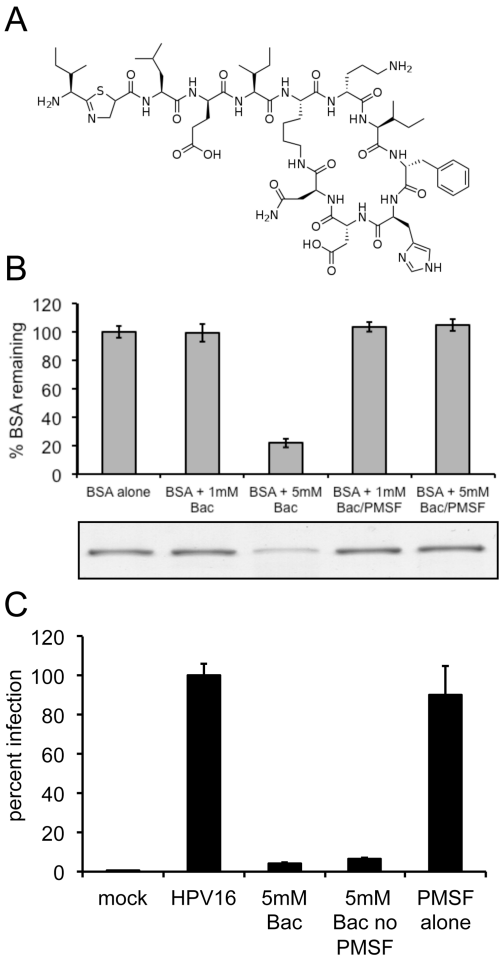
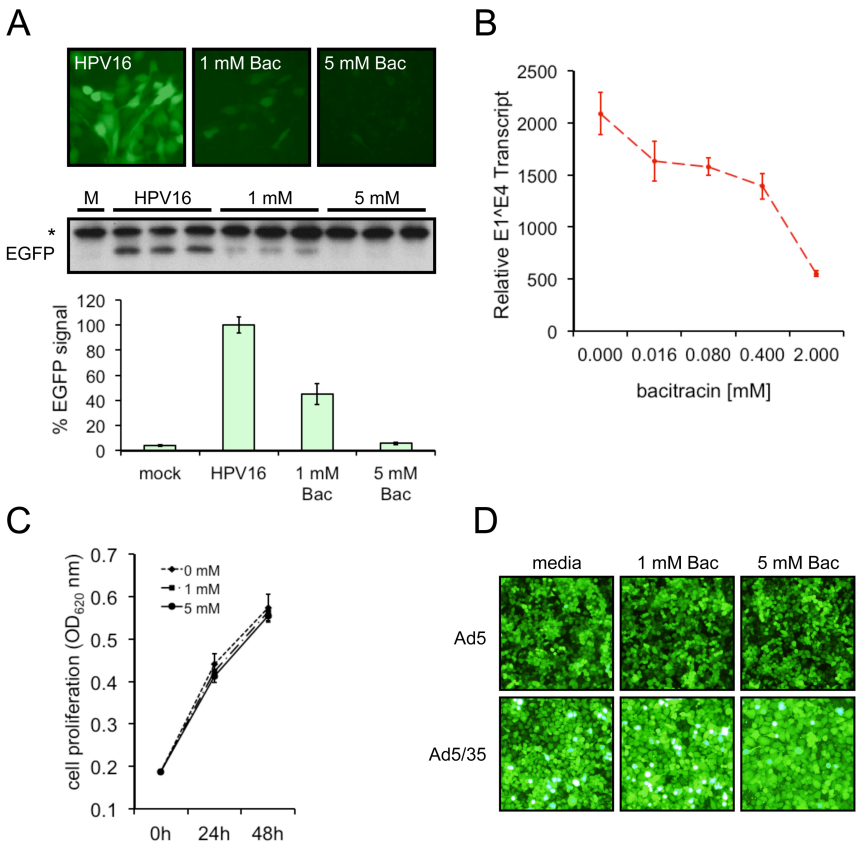
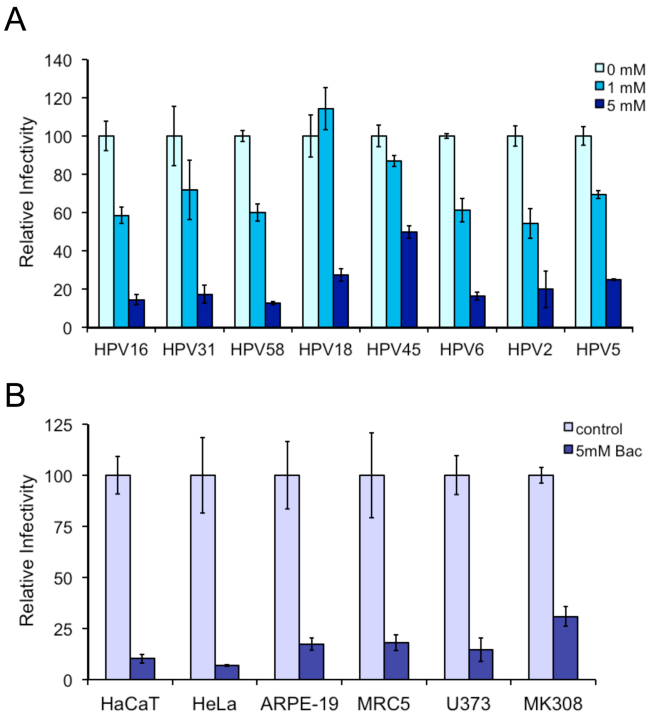
Campos et al. Supplemental Figures & Legends



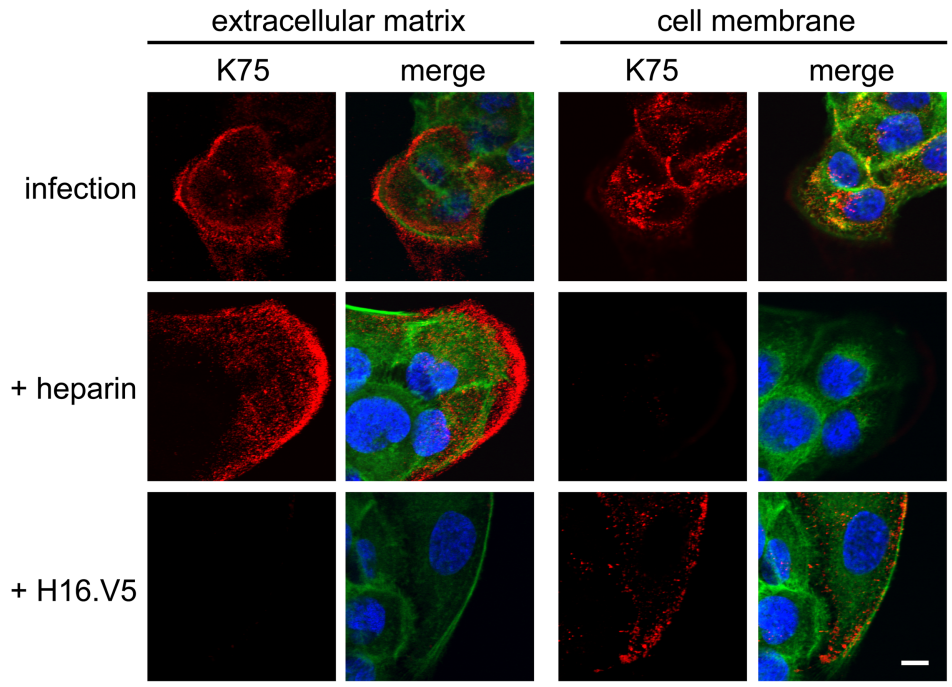
Supplemental Fig 1. Elimination of protease contaminant in commercial bacitracin. (A) Chemical structure of Bac A, the major form found in commercial Bac preparations, MW = 1423 g/mol. **(B)** BSA proteolysis assay. Bac was treated with PMSF as described in *materials and methods*, or simply dissolved at the indicated concentrations in PBS. Bac solutions were mixed with 500 ng BSA and incubated at 37°C for 8h prior to SDS-PAGE and Coomassie staining. **(C)** HaCaT cells were infected with HPV16 alone or in the presence of 5 mM PMSF-inactivated Bac, 5 mM Bac alone, or PMSF alone. Infection was measured at 48h by luciferase assay, revealing no significant inhibition by PMSF alone.



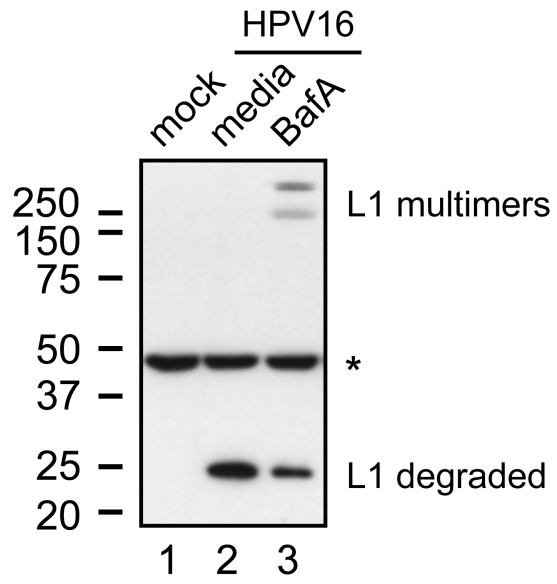
Supplemental Fig 2. Bacitracin inhibits HPV16 but not adenovirus and is not cytotoxic. HaCaT cells were infected with HPV16 virions containing a GFP reporter plasmid **(A)** or recircularized viral genome **(B)** in the presence of various amounts of Bac. **(A)** Fluorescence micrograph of infected cells and western blot of whole cell lysates showing GFP expression levels. Asterisk denotes a non-specific band. GFP bands were quantified by densitometry and plotted in the graph. **(B)** Viral E1^E4 mRNA transcript levels were measured 48h post infection by RT-qPCR. **(C)** Cytotoxicity assay, HaCaT cells were grown in media ± Bac and cell proliferation was quantified by MTT assay. **(D)** HeLa cells were infected with either Ad5-GFP or Ad5/35-GFP at 500 VP/cell ± Bac. Fluorescence micrographs were taken 48h post-infection.



Supplemental Fig 3. Bacitracin inhibition of multiple HPV and cell types. (A) HaCaT cells were infected with the indicated HPV type (luciferase-expressing reporter virions) in media ± Bac. **(B)** Indicated cell types were infected with HPV16 in media ± Bac. Infection was measured at 48h by luciferase assay for both panels.



Supplemental Fig 4. Neutralization in the presence of bacitracin. HaCaT cells were infected with HPV16 alone or with neutralizing amounts of heparin or H16.V5 monoclonal antibody, in media plus 5 mM Bac. Infection proceeded for 4h prior to fixation and IF and as described in *materials and methods*. L1 was stained with the rabbit polyclonal K75 and AF555 anti-rabbit (red). Actin was stained with AF488-phalloidin (green). Nuclei were stained with DAPI. Two different optical planes (extracellular matrix and cell membrane) of the same cell clusters are shown for each infection. Scale bar = 10 μm.



Supplemental Fig 5. L1 degradation is reduced but not eliminated in the presence of BafA. HaCaT cells were mock infected (lane 1) or pre-bound with virus and infected for 7h in media alone (lane 2) or media plus 32 nM BafA (lane 3) prior to trypsinization and processing for non-reducing SDS-PAGE and western blot for L1. Molecular masses (kDa) are shown to the left and positions of the L1 forms are denoted on the right. The asterisk again denotes the non-specific band seen with anti-L1 ab30908.