Supporting Information

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SI Methods

Immunofluorescence Microscopy. HeLa cells plated on coverslips were transfected with pCAGGS-CFP-VP40 and Vpu or Ebola GP plasmids. HT1080 cells were transfected with pCAGGS-GFP-VP40 alone or in the presence of tetherin and Ebola GP DNA. At 24 h after transfection, HeLa cells were stained with anti-Ebola GP mAb 42 (a gift from Y. Kawaoka) or anti-Vpu mAb 969 (National Institutes of Health AIDS Research and Reference Reagent Program). HT1080s were stained with anti-AU1 (Bethyl Labs) and anti-Ebola GP mAb. The *z*-section images were collected on a Leica DMRE fluorescence microscope using Open Lab software (Improvision). Thirty *z*-sections per image were collected at 0.2-μm intervals. The *z*-section data were deconvoluted by iterative restoration using Velocity software (Improvision) to a 98% confidence level or 15 iterations. Images shown are reassembled *z*-stacks.

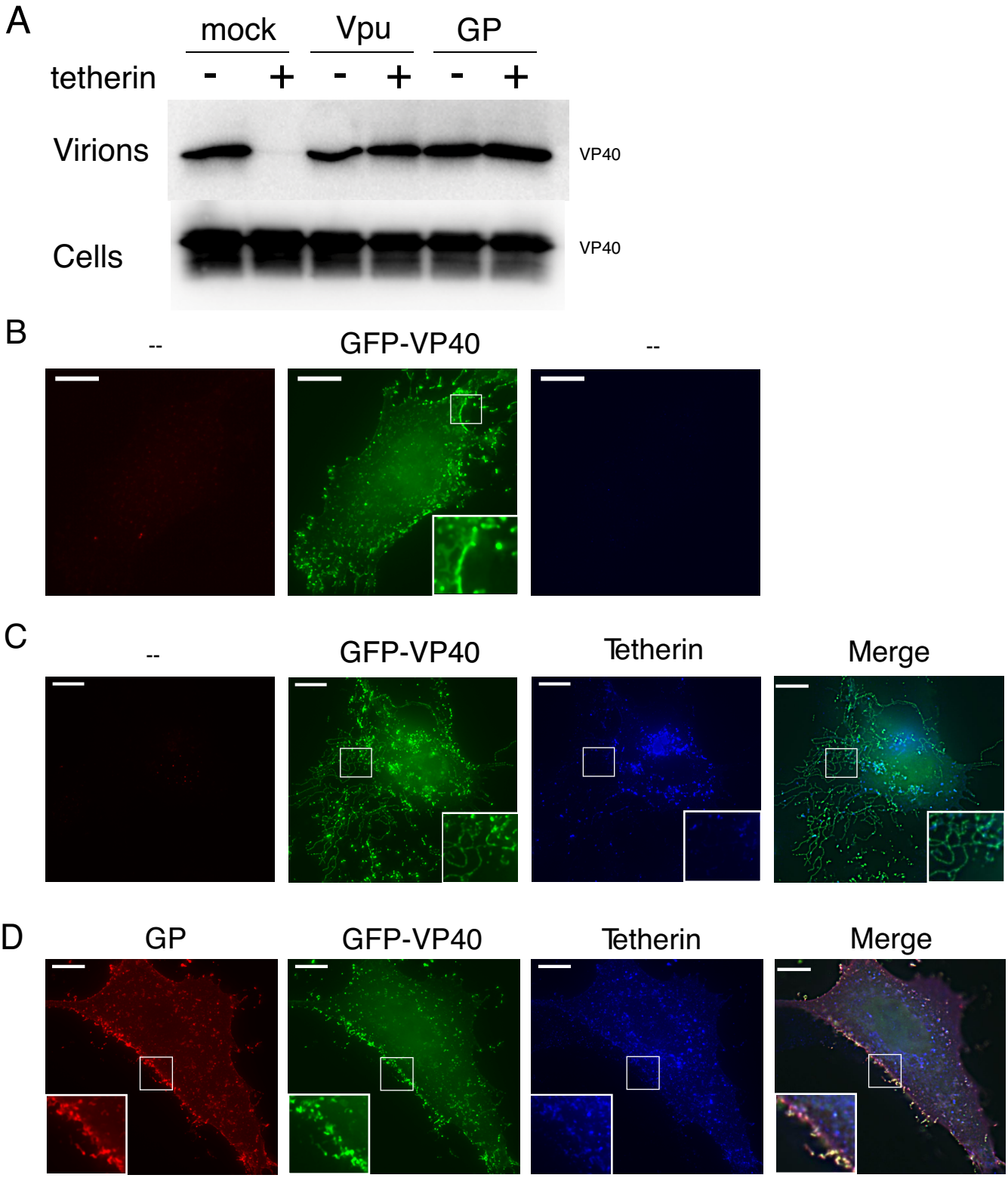

Fig. S1. Ebola GP antagonizes tetherin in HT1080 cells. (*A*) Cell lysates and purified virions from HT1080 cells transfected with FLAG-VP40 with or without tetherin and the indicated plasmids were analyzed by Western blot analysis using anti-FLAG antibodies. (*B–D*) HT1080 cells were transfected with GFP-VP40 (*B*) alone or in combination with AU1-tetherin (*C* and *D*) and Ebola GP (*D*). Immunofluorescence of GFP, anti-AU1, and anti-Ebola GP is shown as indicated. In *C* and *D*, the righthand image represents the merge of a single focal plane overlaying tetherin and VP40 (*C*) or tetherin, VP40, and GP (*D*) signals. GFP panels in *B–D* are identical to those shown in Fig. 2 *K–M*. (Scale bar: 10.6 μM.)

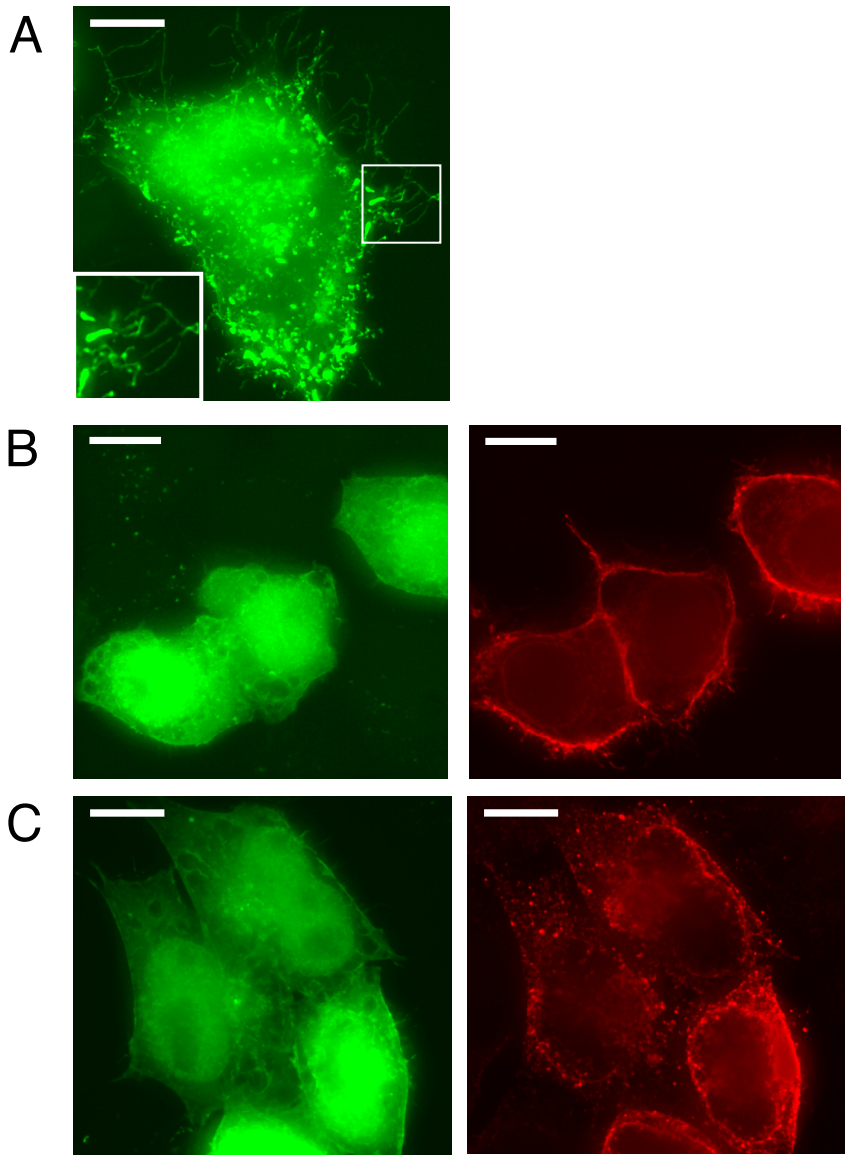

Fig. S2. Tethering of Ebola VLPs on HeLa cells is overcome by Vpu and Ebola GP. (*A*) Immunofluorescence of HeLa cells transfected with CFP-VP40. Cells cotransfected with CFP-VP40 and Ebola GP (*B*) or Vpu (*C*) are shown. (*Left*) CFP expression in transfected cells is shown in green. (*Right*) Cells stained for Ebola GP (*B*) or Vpu (*C*) are shown in red. (Scale bar: 10.6 μM.)

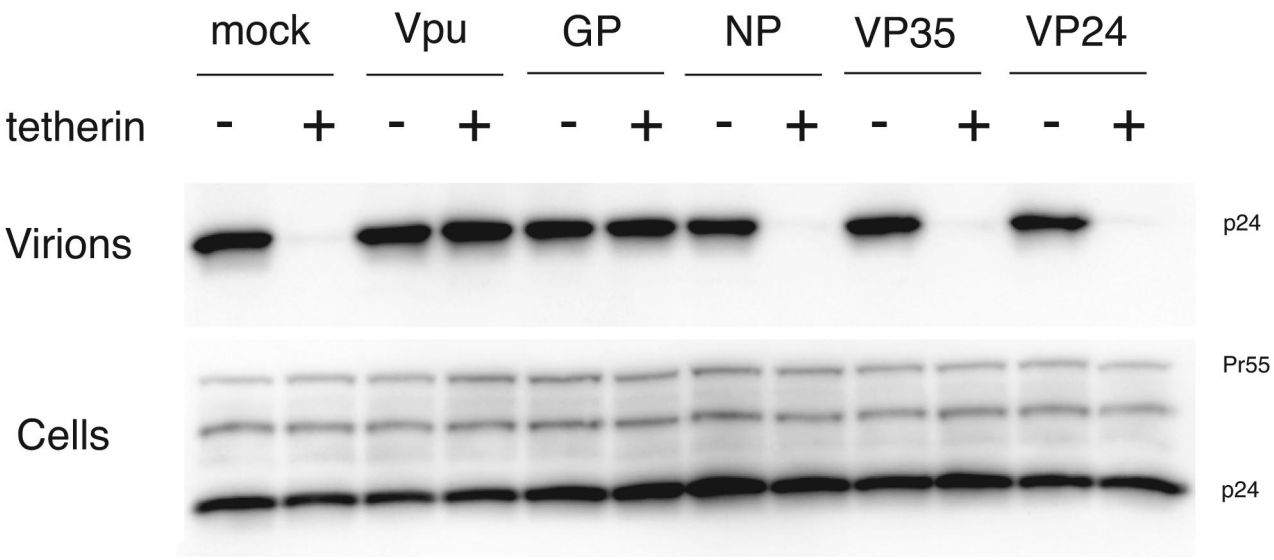

Fig. S3. Ebola GP rescues HIV budding in the presence of tetherin. 293T cells were transfected with a plasmid encoding HIV gag-pol, with or without 25 ng of tetherin, and empty vector (mock) or plasmids encoding viral proteins. Supernatants and cell lysates were harvested 48 h after transfection. Virions were pelleted through sucrose and were analyzed with cell lysates by Western blot analysis using an anti-p24 monoclonal antibody.

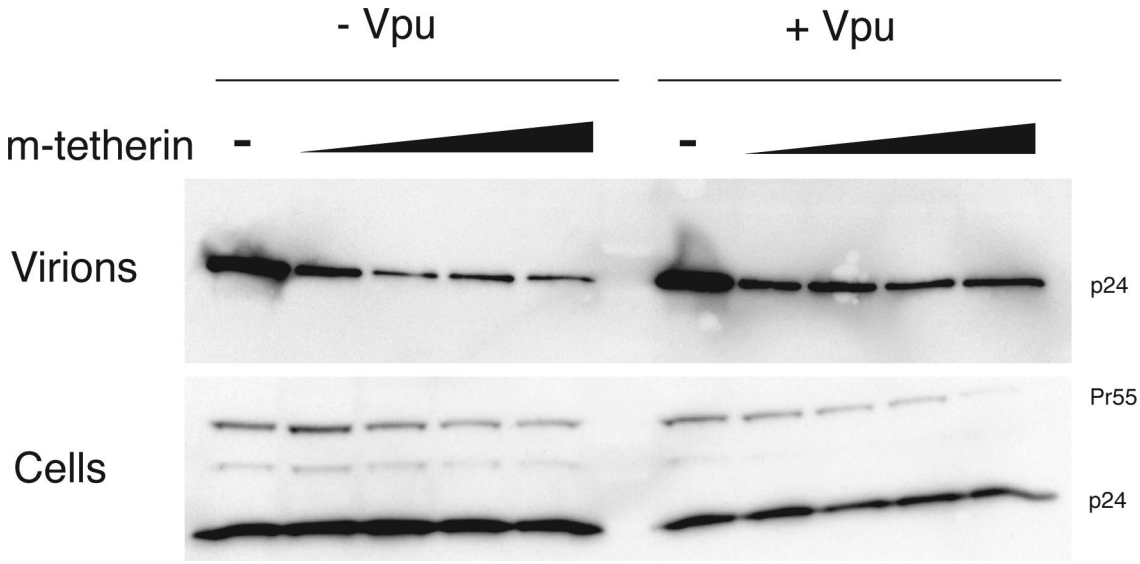

Fig. S4. Effect of murine tetherin on HIV budding. 293T cells were cotransfected with HIV Gag-Pol, with or without HIV vpu, and 0 to 100 ng of murine tetherin DNA. Cell lysates and supernatants were harvested 48 h after transfection. Virions in clarified supernatants were pelleted through sucrose. HIV p24 in cell lysates and pelleted virion samples was analyzed by Western blot analysis.

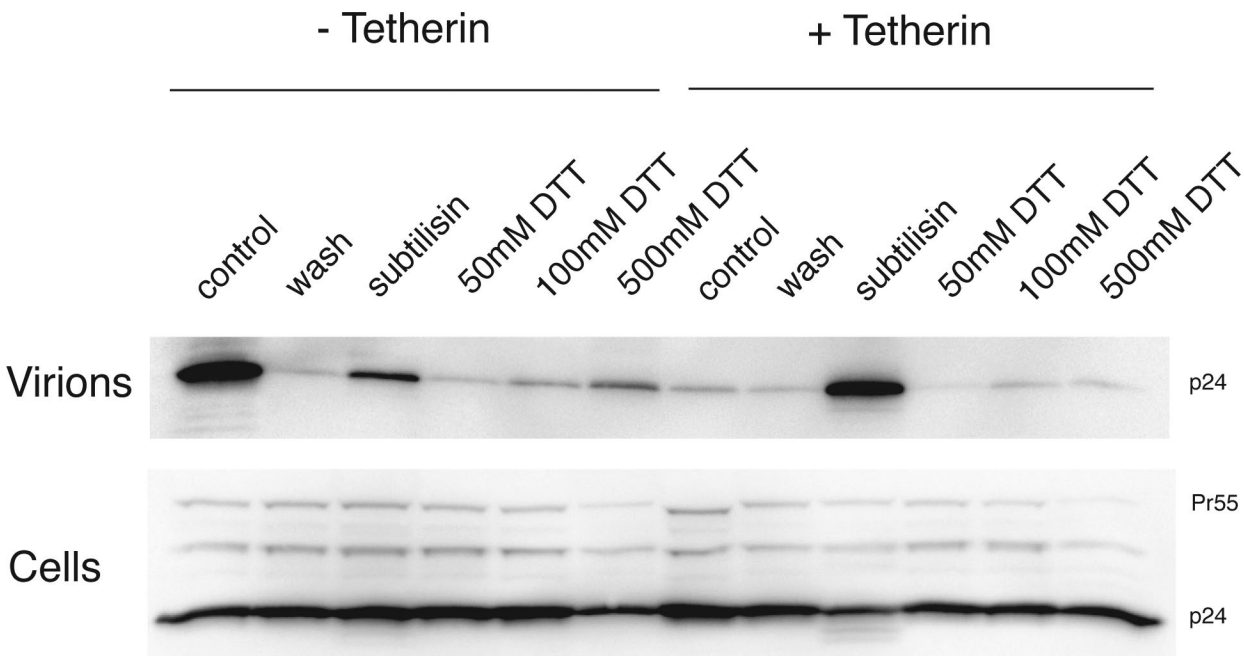

Fig. S5. DTT is not sufficient to release tethered HIV from the cell surface. 293T cells were transfected with an HIV Gag-Pol-expressing plasmid with or without tetherin. At 48 h after transfection, supernatants were harvested, and cells were treated with 1 mg/mL subtilisin, DTT, or PBS alone for 10 min at 37 °C. Virions in supernatants were pelleted through sucrose, resuspended, and analyzed with cell lysates by Western blot analysis for expression of HIV p24.