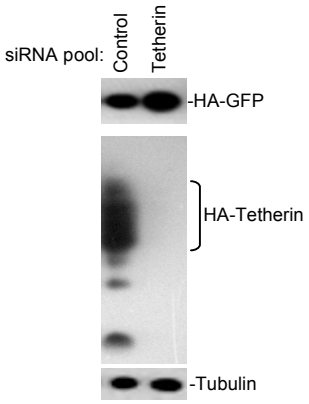
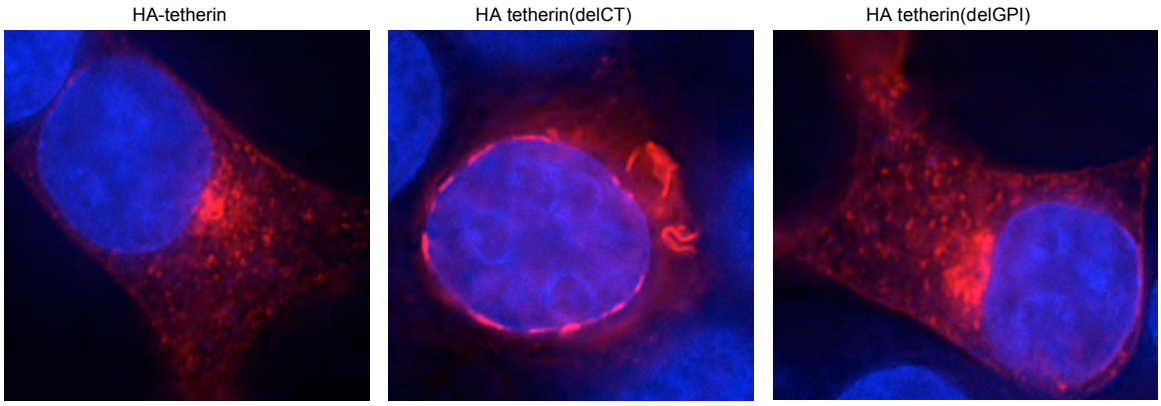
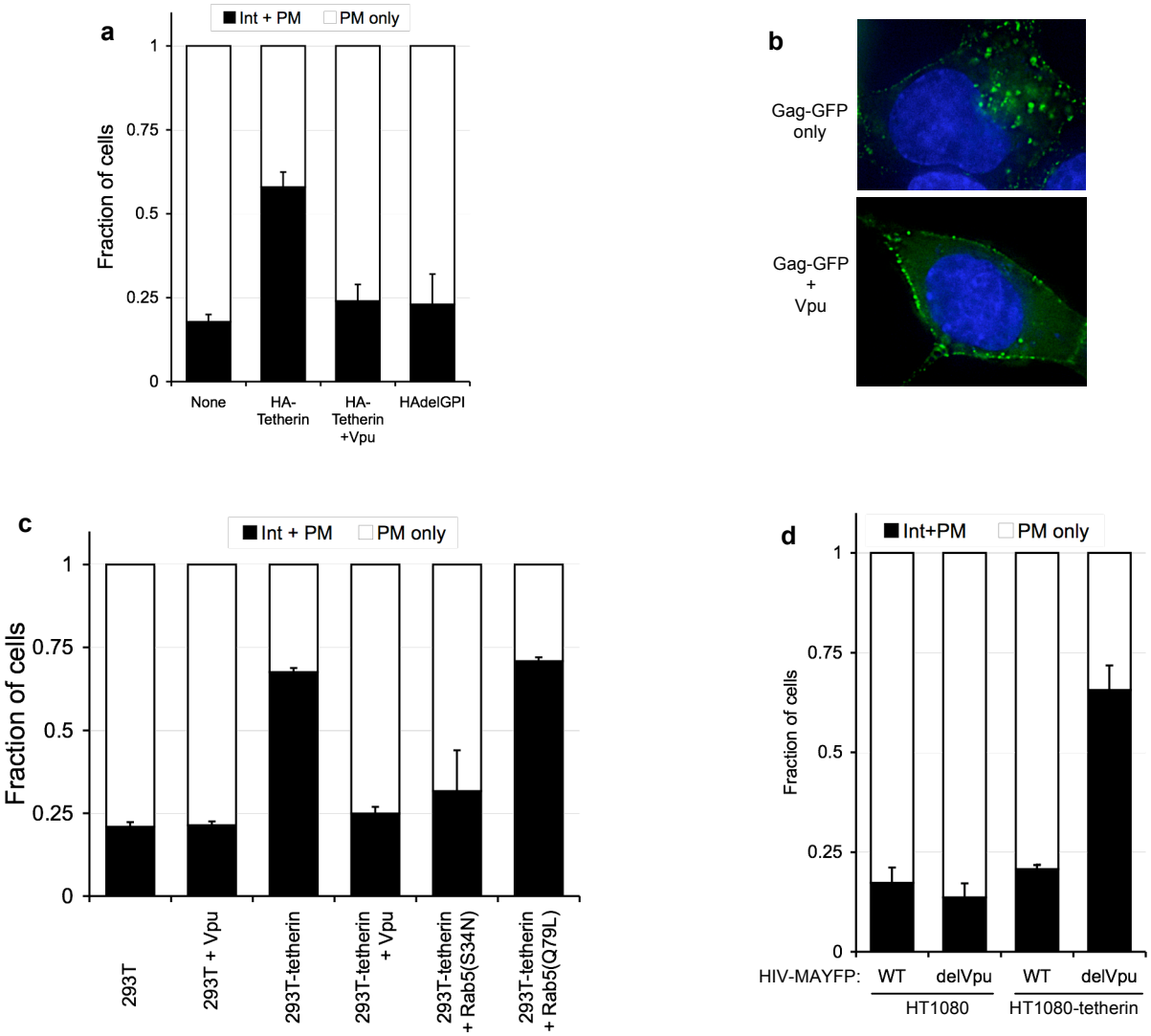


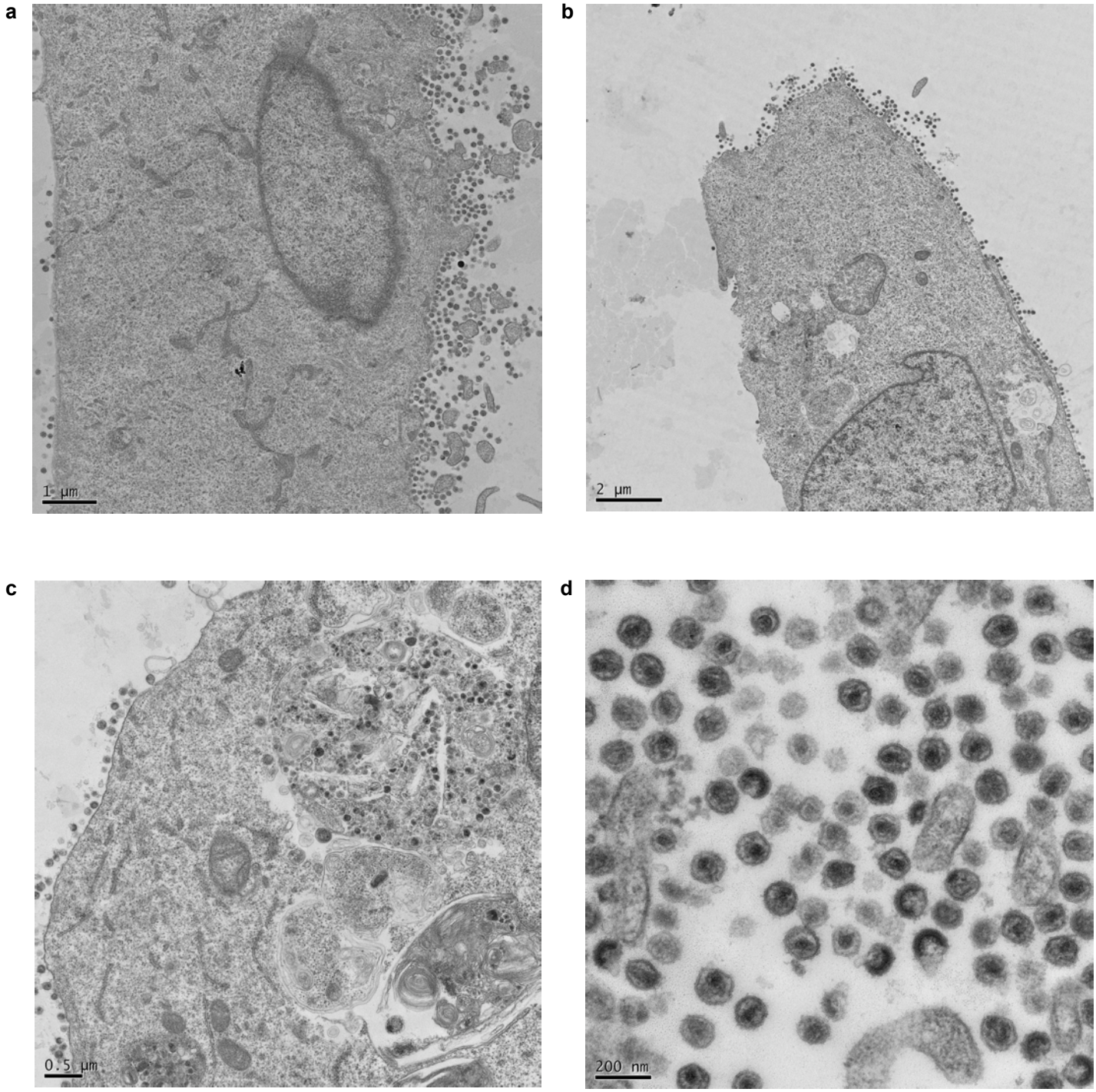
Supplementary Figure S1. Knockdown of HA-tagged tetherin expression by tetherin specific siRNAs. HeLa cells were cotransfected with plasmids expressing HA-tagged GFP (upper panel) or HA tagged tetherin (center and lower panels) along with a control siRNA pool, or an siRNA pool targeting tetherin, as indicated. At 24h after transfection, cell lysates were separated by SDS PAGE and western blots probed with anti-HA antibodies (upper and center panels) or an anti-tubulin antibody (lower panel) Note that tetherin migrates as several species by SDS-PAGE, as a result of post translational modifications.



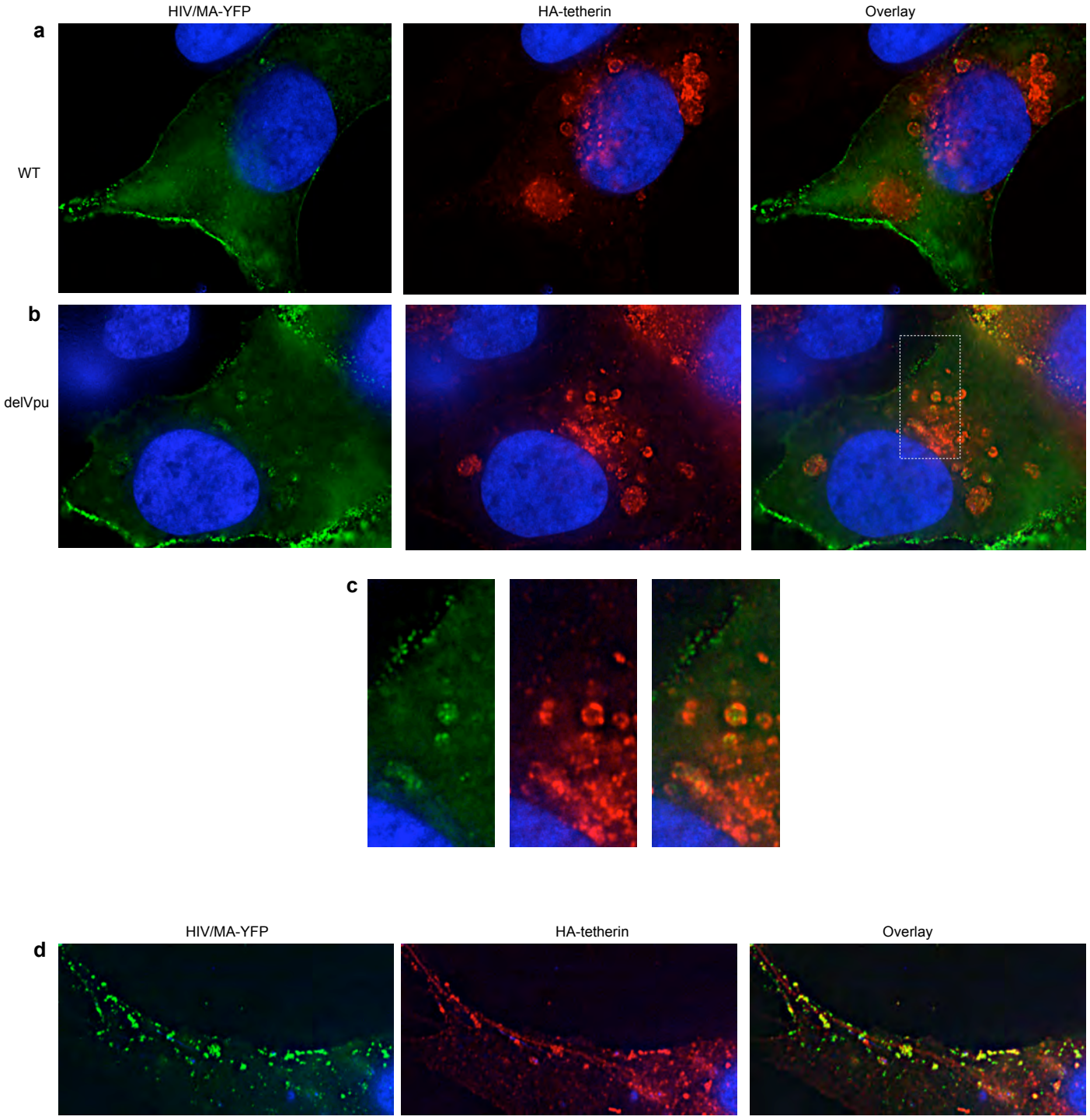
Supplementary Figure S2. Subcellular localization of HA tetherin and deletion mutants. 293T cells were transiently transfected with plasmids expressing the indicated HA-tetherin proteins (see Fig 3a), fixed and stained with anti-HA antibodies



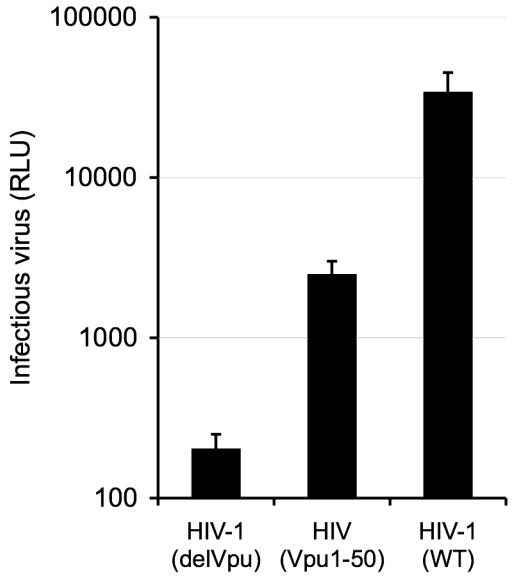
Supplementary Figure S3. Influence of Tetherin and Vpu on subcellular distribution of HIV-1 Gag in Gag-GFP transfected or HIV/MA-YFP infected cells. a, 293T cells were transiently transfected with plasmids expressing Gag-GFP alone (none), or Gag-GFP and the indicated proteins. At 18h after transfection, cells were enumerated according to whether Gag-GFP was localized in both prominent intracellular accumulations as well as at the plasma membrane (Int + PM) or primarily at the plasma membrane (PM only, see Fig. 4a for examples) **b**, 293T cells stably expressing untagged tetherin and transfected with plasmids expressing Gag-GFP either alone of with Vpu. **c**, Unmodified 293T cells, or 293T cells stably expressing untagged tetherin were transiently transfected with plasmids expressing Gag-GFP alone or Gag-GFP and the indicated Vpu or Rab5 proteins. Cells were enumerated as in **a**. **d**, HT1080 cells that were either unmodified or stably expressed untagged tetherin were infected with HIV-MAYFP(WT) or HIV-MAYFP(delVpu). At 48h after infection cells were enumerated according to whether YFP signal was accumulated at the plasma membrane only, or additionally in intracellular compartments. For panels **a**, **c**, and **d** a total of 70 to 80 Gag-GFP expressing cells were evalutated, and the mean fraction of cells (± SD) exhibiting each phenotype is plotted



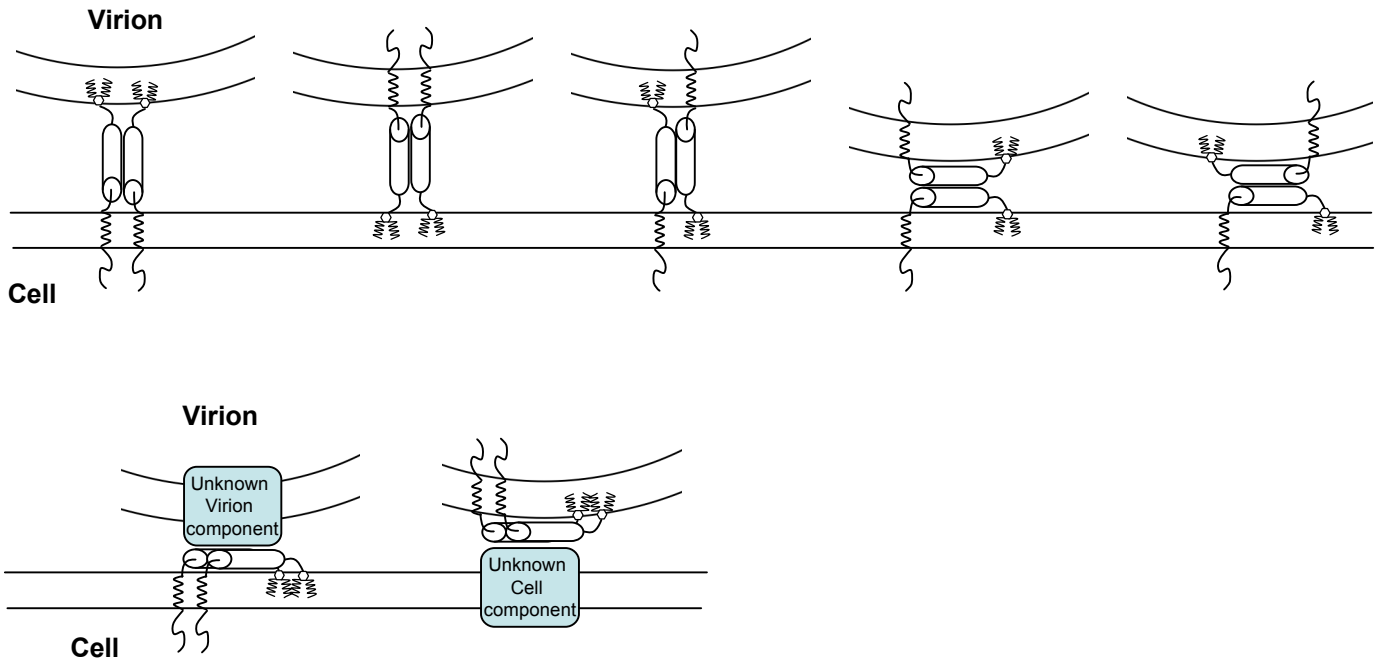
Supplementary Figure S4. Examples of tethered and endocytosed virions in HT1080 cells stably expressing tetherin and infected with HIV-1(delVpu). a,**b**, Overview of two infected cells showing virions accumulated at the cells surface. **c**, Example of a cell with virions accumulated in an intracellular compartment as well as at the cell's surface. **d**, Higher magification view of virions accumulated at an infected cell surface, showing the predominantly mature morphology of tethered virions.



Supplementary Figure S5. Localization of HIV-1/MA-YFP and tetherin in infected cells a, HA-tetherin expressing HT1080 cells infected with HIV/MA-YFP(WT) and stained with anti-HA (red). Note the absence of tetherin-associated YFP puncta in HIV/MA-YFP(WT) infected cells. **b-d**, HA-tetherin expressing HT1080 cells infected with HIV/MA-YFP(delVpu) (green) and stained with anti-HA (red). Note that intracellular tetherin-positive compartments frequently exhibit associated YFP puncta in HIV/MA-YFP(delVpu) infected cells.**c**, Expanded view of a portion of **b**, as indicated by the white box.**d**, Expanded view of of a portion of an HIV/MA-YFP(delVpu) infected cell, showing apparent association of HA-tetherin with YFP puncta at the cell surface.



Supplementary Figure S6. Single cycle replication assay in HeLa cells infected with HIV-1(delVpu), HIV-1(WT) or HIV-1 carrying a Vpu protein truncated at residue 50 in the cytoplasmic tail. HeLa cells were infected with equivalent titers of the indicated VSV-G pseudotyped viruses, and subsequent virus yield was determined using TZMbl indicator cells (see methods).



Supplementary Figure S7. Models depicting potential configurations in which tetherin might inhibit enveloped virus particle release form the surface of cells.

Supplementary Table 1. Effects if Vpu and IFNα on HIV-1 release in various cell lines.

Cell line	Infectious virus release[a]	
	HIV-1(WT)	HIV-1(del Vpu)
HeLa	100	2
HOS	100	120
293T	100	79
293T + IFNα[b]	79	7
HT1080	100	116
HT1080 + IFNα[b]	30	1.4

[a]Infectious virus release, measured using TZMbl indicator cells following a single cycle of replication (see methods), and expressed as a percentage of infectious virus release measured following a single cycle of HIV-1 (WT) replication in each cell line in the absence of IFNα.

[b]IFNα (5000U/ml) was added to 293T and HT1080 cells 24h after infection i.e. 24h before measurement of infectious virus production

Supplementary Table 2. Expression of tetherin candidates in various cell lines.

Gene	HT1080[a] (No IFN)	HT1080[a] (+ IFN)	**HT1080 (IFN induction)[b]**	293T[a] (No IFN)	293T[a] (+ IFN)	**293T (IFN induction)[b]**	Jurkat[a] (No IFN)	Jurkat[a] (+ IFN)	**Jurkat (IFN induction)[b]**	HOS[a]	HeLa[a]	**Ratio HeLa:HOS**
CD317/BST2 /HM1.24	98.15526	2041.68	**20.80051543**	76.83003	1084.639	**14.11738353**	2041.438	3887.298	**1.904195964**	80.62917	1733.295	**21.49712071**
IFITM1	273.3351	8843.597	**32.35441405**	94.25755	5148.819	**54.62500351**	1637.325	8626.978	**5.268946605**	84.27998	332.8661	**3.949527515**
IFITM3	3395.984	17170.56	**5.056136896**	57.63969	2740.139	**47.53910023**	52.10429	63.01514	**1.209404063**	1264	3655.756	**2.892212025**
IFITM2	5125.539	17680.78	**3.449545501**	255.8283	3371.004	**13.17682211**	2774.601	3944.022	**1.421473574**	2650.925	5644.129	**2.129116818**

[a] mRNA levels as measured by microarray analysis.

[b] ratio of mRNA levels in the presence versus absence of IFNα.