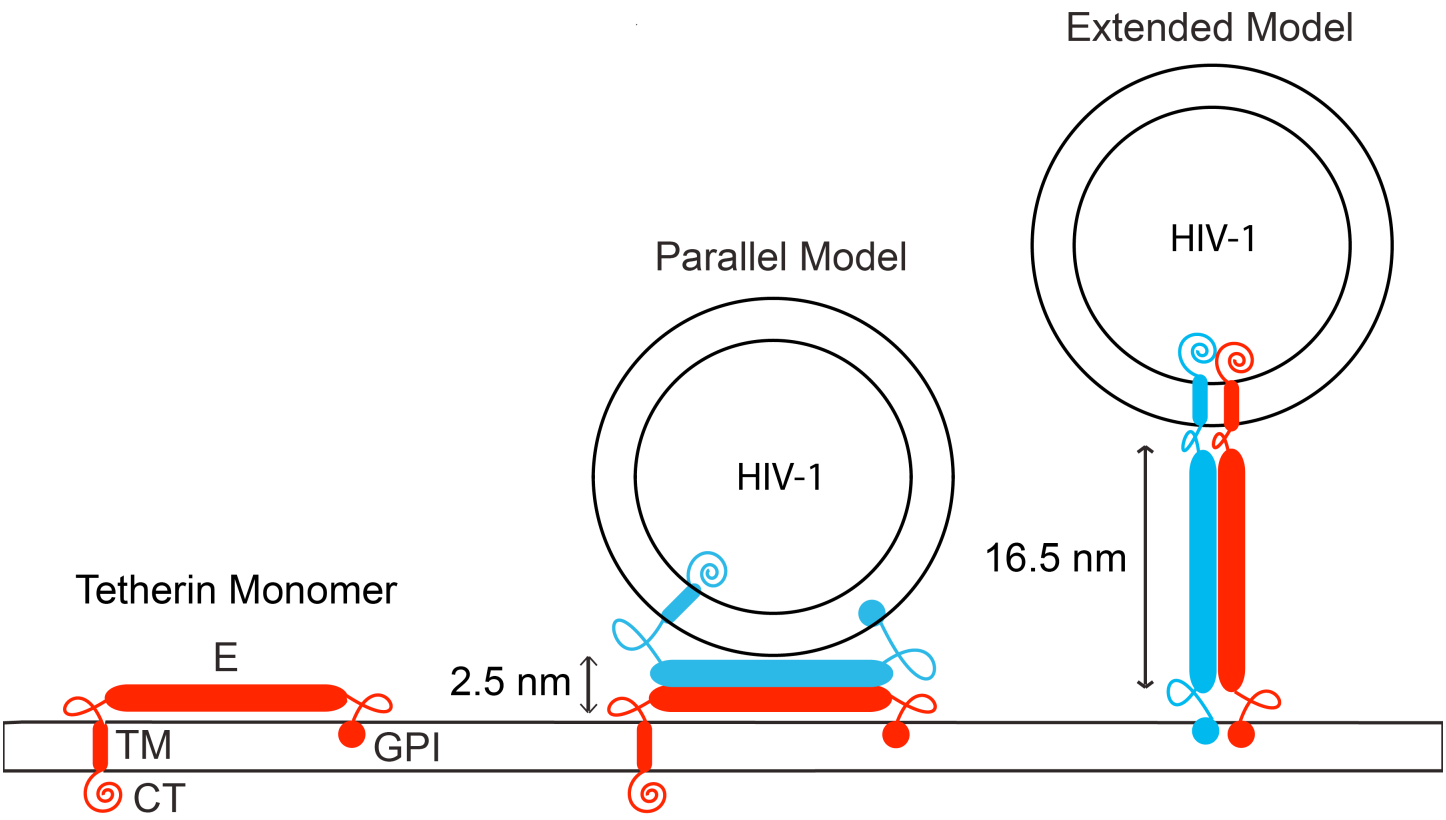
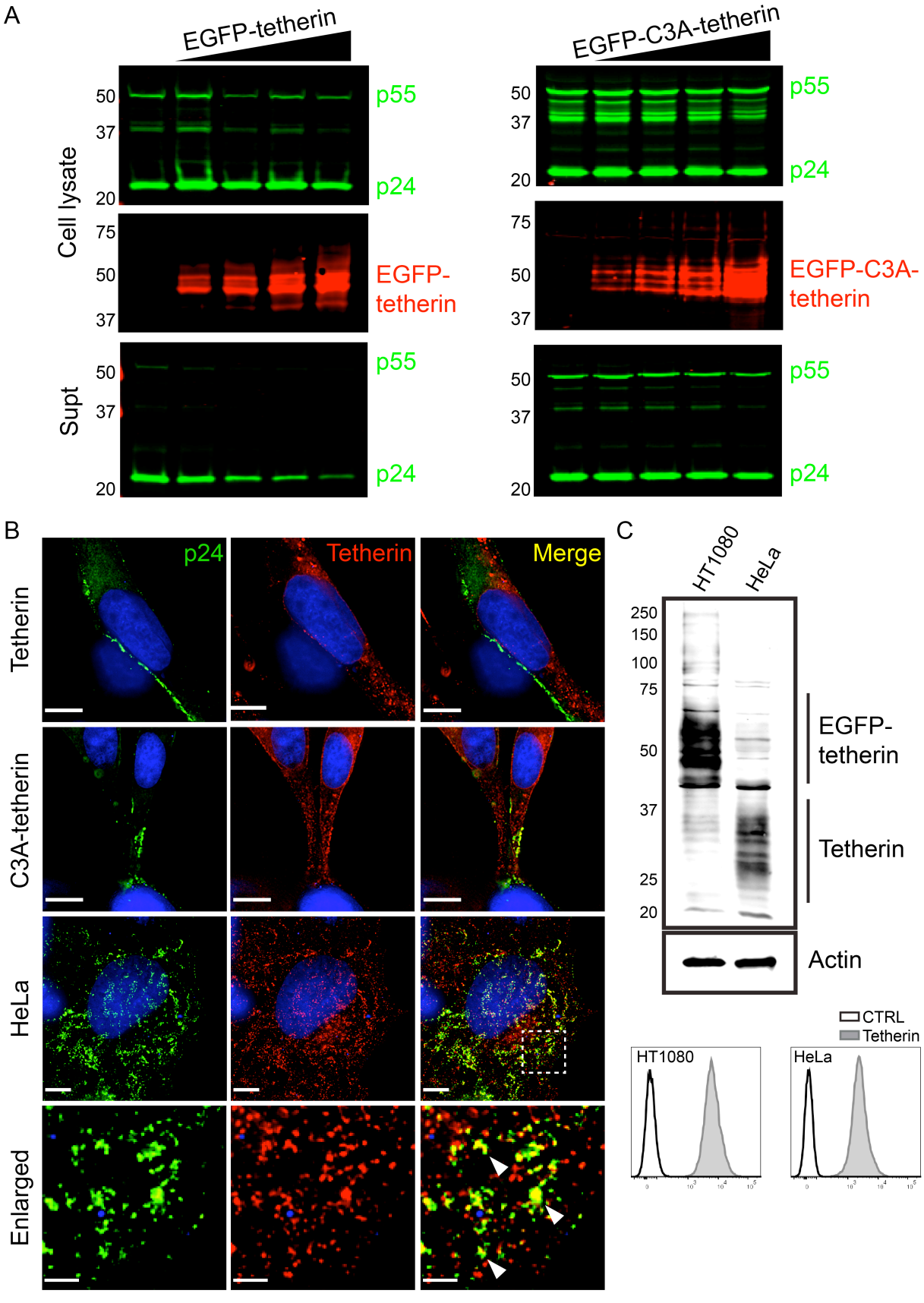
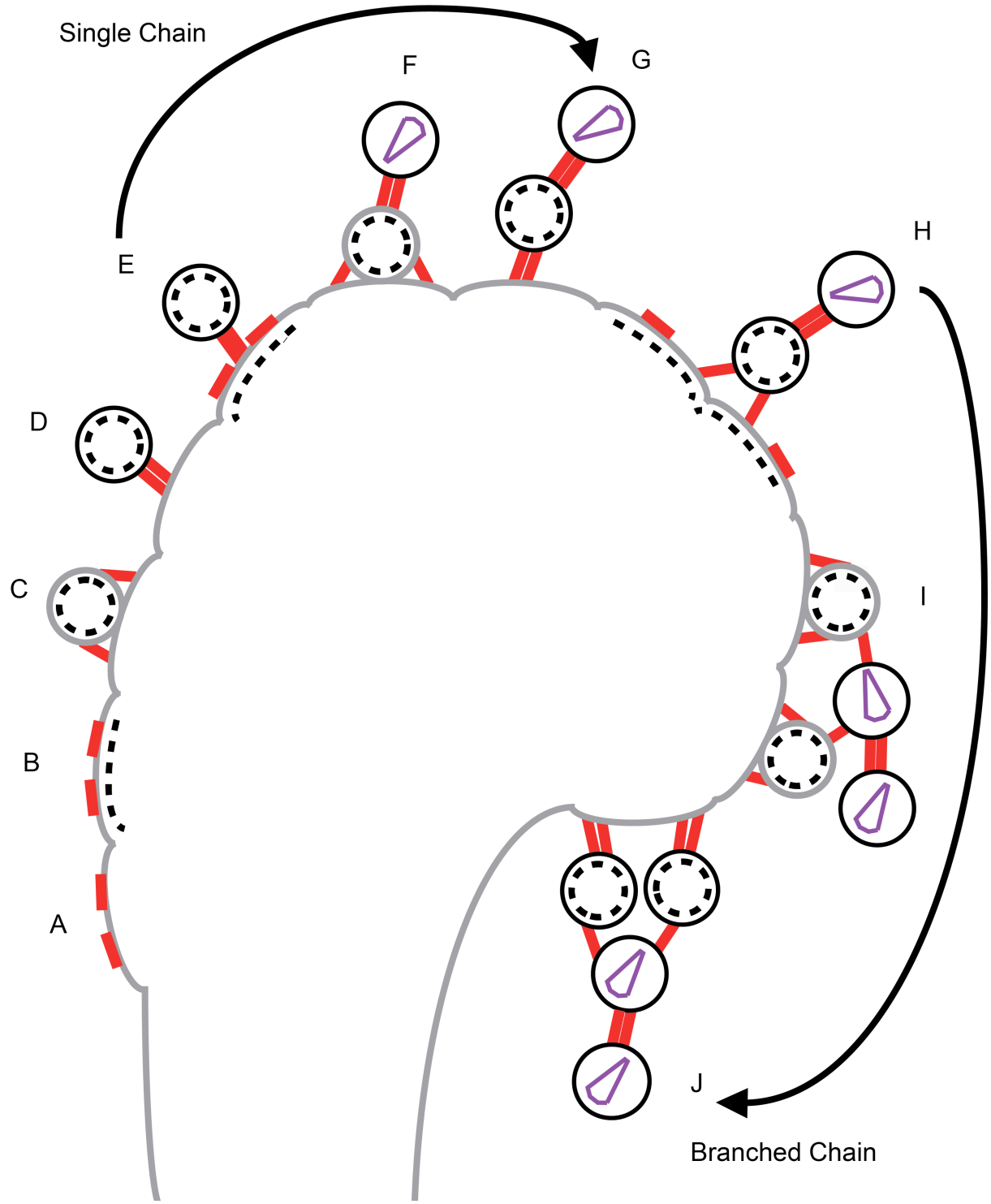
SUPPLEMENTARY FIGURES, FIGURE LEGENDS, AND MOVIE LEGENDS



Supplementary Figure 1. Illustration of the extended and parallel tetherin models. For the parallel model, tetherin monomers are in *cis*-orientation, with the single pass transmembrane (TM) domain and C-terminal glycosylphosphitidylinositol (GPI) of each monomer embedded in the viral membrane (blue tetherin monomer) or in the cell plasma membrane (red tetherin monomer). The tetherin ectodomains (E) are oriented parallel to the plasma membrane. In the extended model, the tetherin monomers are in the *trans*-configuration, with the TM and GPI of each monomer embedded in both the viral membrane and in the plasma membrane (blue and red tetherin monomers). The ectodomains are oriented perpendicular to the cell plasma membrane. Based on X-ray crystal structures of the mammalian ectodomain homodimer the predicted distance separating viral membrane from the host cell plasma membrane is 2.5 nm for the parallel model and 16.5 nm for the extended model.


A
EGFP-tetherin
EGFP-C3A-tetherin
Cell lysate
Supt
50
37
20
75
p55
p24
EGFP-tetherin
EGFP-C3A-tetherin
B
p24
Tetherin
Merge
Tetherin
C3A-tetherin
HeLa
Enlarged
C
HT1080
HeLa
250
150
100
75
50
37
25
20
EGFP-tetherin
Tetherin
Actin
CTRL
Tetherin
HT1080
HeLa

Supplementary Figure 2. Tetherin restriction phenotyping, subcellular localization, and comparative expression levels in HT1080 and HeLa cells. (A) Comparative HIV-1 restriction profile for EGFP-tetherin and EGFP-tetherin-C3A. HT1080 cells were transfected with pNLenv1ΔU and a titration of either pEGFP-tetherin or pEGFP-C3A-tetherin. EGFP-tetherin-C3A is a non-restrictive mutant form of tetherin that lacks three conserved ectodomain cysteine residues. Supernatants and cell lysates were harvested 36 hours post-transfection and analyzed by Western blotting. Supernatant and cell lysate detection using anti-p24 (green) and anti-tetherin (red) specific antibodies. (B) Tetherin is located along the plasma membrane and colocalizes with HIV-1 Gag. HT1080 cells were cotransfected with pEGFP-tetherin or pEGFP-C3A-tetherin and pNLenv1ΔU. HeLa cells were transfected with pNLenv1ΔU only. Sixteen hours post-transfection cells were fixed and immunolabeled for HIV-1 p24 (green) and tetherin (red). White arrows in enlargement denote plasma membrane puncta of colocalized HIV-1 p24 and tetherin. Samples were imaged using widefield fluorescence microscopy. Representative maximum intensity deconvolved images are shown. Scale bars, HT1080 10 μm, HeLa 6 μm, and enlargements 2 μm. (C) Comparison of tetherin expression levels between HT1080 transfected with pEGFP-tetherin and endogenous tetherin in HeLa cells. Western blot of 15 μg/lane of pEGFP-tetherin transfected HT1080 and HeLa cell lysates using rabbit anti-tetherin antisera. Tetherin cell surface expression level of pEGFP-tetherin transfected HT1080 and endogenous HeLa using rabbit anti-tetherin antisera by flow cytometry.


Single Chain
F
G
E
H
D
C
I
B
A
J
Branched Chain

Supplementary Figure 3. Dynamic tetherin model illustrating arrangement and orientation of tetherin homodimers at sites of HIV-1 assembly and budding. (A) Tetherin homodimers (red) are located on the cell surface (i.e. tetherin microdomains) prior to HIV-1 assembly. The TM and GPI anchors are embedded into the plasma membrane (grey) and the tetherin homodimer ectodomains are oriented tangentially to the plasma membrane. (B) Gag molecules (dashed black lines) polymerize on the plasma membrane at the tetherin microdomain. The HIV-1 virion assembles and the tetherin homodimers are incorporated into the viral membrane. (C) Flexibility of the tetherin ectodomains enables tetherin homodimers to remain attached to the viral and plasma membrane during budding and scission. (D) Tetherin homodimers in the extended conformation directly cross-link the HIV-1 virion to the plasma membrane. (E) A new HIV-1 virion begins to assemble at the plasma membrane directly below the tethered virion. A single chain of HIV-1 virions tethered to the cell is generated by tetherin homodimers cross-linking the virion to the cell plasma membrane and incorporating into a single HIV-1 assembly site. (F and G) During budding and membrane scission (mature HIV-1 cores are represented as purple cones, indicating the passing of time), the tetherin homodimers remain attached to the viral membrane. This process can repeat, thus adding more HIV-1 virions to this chain. (H) Formation of a branched chain occurs as tetherin homodimers bound to the tethered HIV-1 virion (in this case a single chain) are incorporated into two different HIV-1 assembly sites. (I and J) Tetherin homodimers remain attached to the virions during assembly, budding, and membrane scission leading to formation of a branched chain of HIV-1 virions tethered to the cell.

Supplementary Movie S1. Cryo-ET of HIV-1 VLPs tethered to a HT1080 cell, corresponding to Fig 1E. Tomographic slices are displayed in the beginning of the movie, followed by the segmented HIV-1 VLPs (yellow), tethers (red), and the plasma membrane (cyan). At the end of the movie the segmented volume is rotated around the y-axis and then rotated around the x-axis.

Supplementary Movie S2. Cryo-ET of HIV-1 virions tethered to a HT1080 cell, corresponding to Fig. 3C. Tomographic slices are displayed in the beginning of the movie, followed by segmented HIV-1 virions (yellow), immature Gag lattices (green), mature conical-capsid cores (purple), tethers (red), and the plasma membrane (cyan). At the end of the movie the segmented volume is rotated around the y-axis and then rotated around the x-axis.

Supplementary Movie S3. Cryo-ET of released HIV-1. Movie shows tomographic slices (7.64 nm) of the released HIV-1 virions corresponding to Fig. 3G. In the movie, the tomogram was rotated 90° counter-clockwise relative to the montages and tomographic slice shown in Fig. 3G. Scale bar 100 nm.