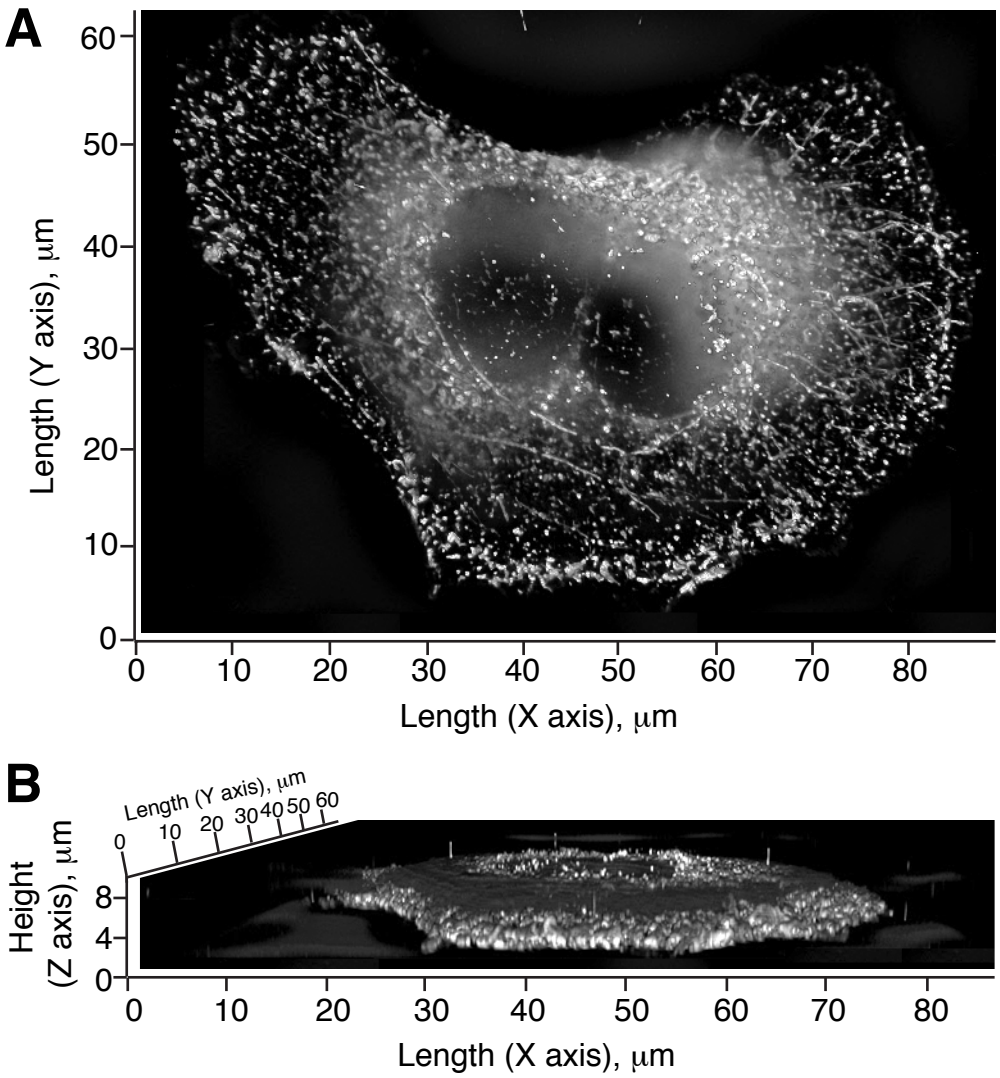

Figure S1. 3-Dimensional images of an SK-BR-3 cell expressing caveolin-1-GFP. Caveolin-1-GFP was expressed in SK-BR-3 cells grown on glass-bottomed dishes. Cells were fixed and examined by deconvolution microscopy. A 3D image was constructed from a Z-stack and rotated to obtain views from the top (A) and the side (B). Movie 1 shows a rotation of the same cell.

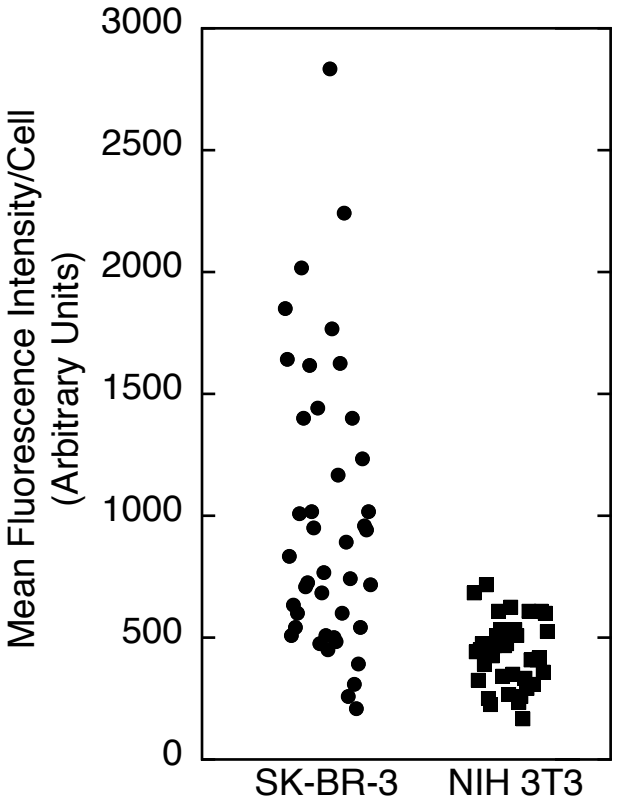

Figure S2. Comparison of endogenous caveolin-1 levels in NIH 3T3 cells with caveolin-1 levels in transfected SK-BR-3 cells containing tubules. NIH 3T3 cells and SK-BR-3 cells transfected with caveolin-1 were grown on coverslips and processed in parallel for confocal microscopy, detecting caveolin-1 with anti-caveolin-1 antibodies and AF-594-conjugated secondary antibodies. Mean fluorescence intensity in 36 randomly-chosen NIH 3T3 cells, and in 36 transfected SK-BR-3 cells that contained caveolin-1-positive tubules was measured and is shown. The same acquisition parameters were used for all cells, and images were not processed.

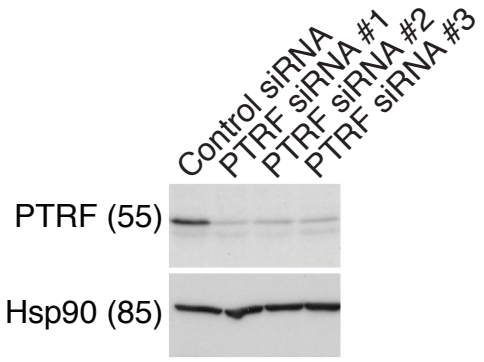

Figure S3. siRNA-mediated suppression of PTRF/cavin-1 synthesis. MDA-MB-231 cells were transfected with control or one of three PTRF/cavin-1 siRNA duplexes (as indicated). Levels of PTRF/cavin-1 and Hsp90 (loading control) were determined by SDS-PAGE and Western blotting. Molecular masses (in parentheses) are in kilodaltons.

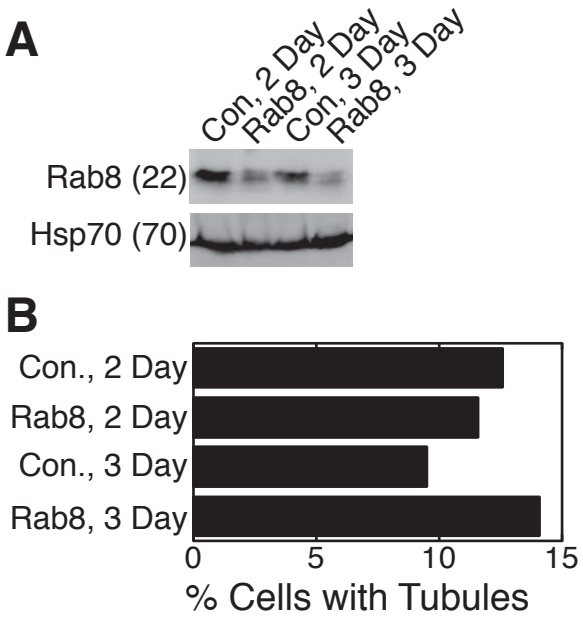

Figure S4. siRNA-mediated suppression of Rab8a synthesis does not affect caveolin-1-GFP tubule formation. HeLa cells were transfected with control (Con.) or Rab8a siRNA duplexes and caveolin-1-GFP as in Methods, and grown for two or three days post-siRNA transfection (as indicated) before harvest. (A) Levels of Rab8 and Hsp70 (loading control) were determined by SDS-PAGE and Western blotting. Molecular masses (in parentheses) are in kilodaltons. (B) The percent of transfected, coverslip-grown cells containing long tubules positive for caveolin-1-GFP was determined. The mean of two experiments (counting at least 150 cells on each slide) is shown.

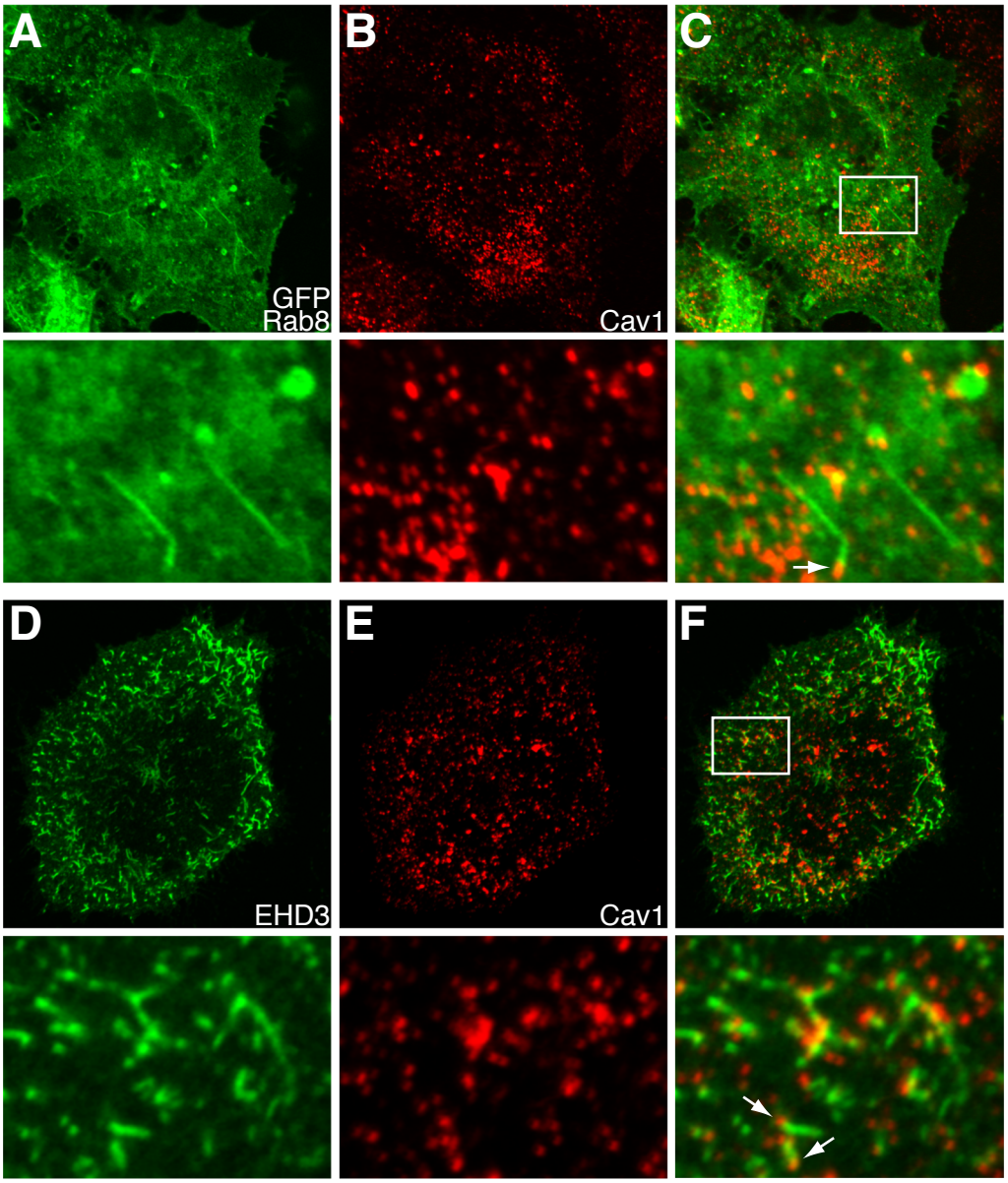

Figure S5. Endogenous caveolin-1 is not recruited to tubules formed by myc-EHD3 or GFP-Rab8-Q67L. HeLa cells were transfected with GFP-Rab8-Q67L (A-C) or myc-EHD3 (D-F), fixed, and processed for confocal microscopy. (C, F); Merged images with regsions shown at high magnification below each panel indicated. Arrows show caveolin-1 puncta at the ends of GFP-Rab8-Q67L or myc-EHD3 tubules.

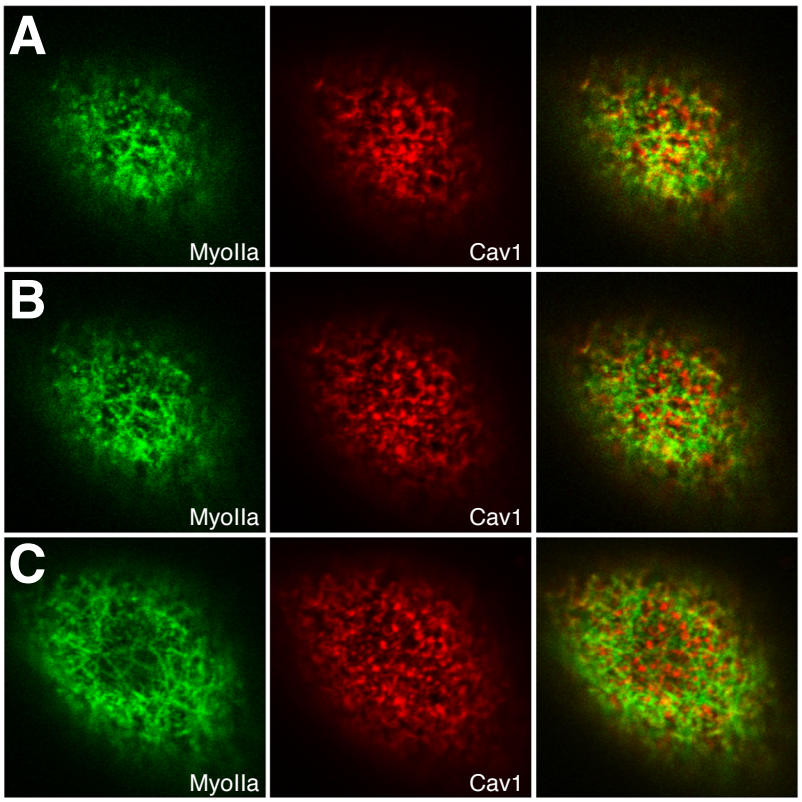

Figure S6. Caveolin-1-RFP short tubules associate most closely with GFP-myosin IIa closer to the cell surface than the zone of dorsal stress fibers. SK-BR-3 cells co-expressing GFP-myosin IIa and caveolin-1-RFP were examined by confocal microscopy. (A; also shown in Figure 8F) shows a focal plane close to the top of the cell. (B) and (C) show sequentially lower focal planes. Caveolin-1-RFP short tubules, often close to myosin IIa-positive puncta and short filaments, were seen close to the top of the cell (A), but not in lower focal planes (B, C). Well-developed dorsal stress fibers, positive for GFP-myosin IIa (C), were present below the region containing caveolin-1-RFP short tubules. Although caveolin-1-RFP-positive puncta were abundant in this region, they did not colocalize with stress fibers.

Movie legends

Movie 1. 3-Dimensional view of an SK-BR-3 cell expressing caveolin-1-GFP. A Z-stack was created from images of a fixed cell taken by deconvolution microscopy, and rotated through 60°. Top and side views of the same cell, taken from the Z-stack, are shown in Supplemental Figure 1.

Movie 2. A vacuole fuses with the plasma membrane in an MBCD-treated, caveolin-1-GFP-expressing SK-BR-3 cell. Time between frames: 15 seconds. Elapsed time: 16 min, 15 sec.

Movie 3. Dynamic long tubules and vacuoles in a LatA-treated, caveolin-1-GFP expressing SK-BR-3 cells. Time between frames: 10 sec. Elapsed time: 8 min, 20 sec.

Movie 4. Unstable short tubules in a nocodazole-treated, caveolin-1-GFP-expressing SK-BR-3 cell. Time between frames: 15 sec. Elapsed time: 16 min, 15 sec.

Movie 5. Unstable long tubules in a caveolin-1-GFP-expressing SK-BR-3 cell. Time between frames: 10 sec. Elapsed time: 11 min, 40 sec.

Movie 6. Stable long tubules in a caveolin-1-GFP-expressing, LatA-treated SK-BR-3 cell. Time between frames: 10 sec. Elapsed time: 15 min, 40 sec.

Movie 7. Unstable long tubules in a caveolin-1-RFP-expressing SK-BR-3 cell. Time between frames: 10 sec. Elapsed time: 8 min, 10 sec.

Movie 8. Stable long tubules in a caveolin-1-RFP-expressing, blebbistatin-treated SK-BR-3 cell. Time between frames: 10 sec. Elapsed time: 10 min, 40 sec.