Legends for Supplementary Data, Hansen et al.

Supplementary Figure 1
Alignment of the amino acid sequences of human PTRF, SDPR, SRBC and MURC. Generated using Jalview [1].

Supplementary Figure 2
Localization of PTRF-mCh, SDPR-mCh, SRBC-mCh and MURC-GFP to caveolae. Total internal reflection images of HeLa cells fixed and stained using indirect immunofluorescence with antibodies against caveolin 1. Cells were expressing the constructs shown. Bars 10 µm.

Supplementary Figure 3
A. Rescue of loss of PTRF and caveolin 1 expression in SDPR shRNA cell line upon re-expression of non-targeted SDPR. SDPR-CFP with silent mutations to avoid targeting by the shRNA was expressed in cell line SDPR shRNA 2 by transient transfection. As a control, a separate population of cells was transfected with plasmid expressing GFP. FACS was used to select and separate equal numbers of transfected from untransfected cells, extracts of which were then analyzed by Western blotting with antibodies against the proteins indicated in the figure. Antibodies against flotillin 1 were used to provide a control for equal loading.
B. Estimation of the extent of over-expression of SDPR-mCh in different cells. HeLa cells expressing SDPR-mCh, and neighboring untransfected cells, were fixed and stained using indirect immunofluorescence with antibodies against SDPR. Comparison of the mean fluorescence intensity from SDPR antibody staining between transfected and untransfected cells allow estimation of minimum fold over-expression of SDPR-mCh. In the cell labeled 1, where SDPR-mCh is punctate, over-expression is approximately 2x, in the cell labeled 2, where SDPR is in tubes, over-expression is approximately 10x. These are minimum estimates because in un-transfected cells a significant fraction of total fluorescence signal may come from non-specific labeling. Bar 20 µm.

Supplementary Figure 4
Quantification of the number and morphology of caveolae in reconstructed cell sections by electron microscopy. Images at 36,000 x magnification were acquired using a CCD camera, so as to completely track the perimeter of selected cells. Typically this required 20-30 images per cell. The number of caveolae clearly connected to the cell surface in each cell was counted, and their outline traced by hand and shown in the enlarged panels. Potential caveolae not open at the plasma membrane, such as the vesicular structures marked * in the left hand inset were not counted. Clathrin coated structures could be readily identified. The bars are 500 nm.

Supplementary Figure 5
A. Recruitment of endogenous PTRF to cytosolic puncta composed of SDPR49-290, K272E K273E. The SDPR mutant was GFP-tagged at the C-terminus. PTRF was detected by indirect immunofluorescence. The lower panels show a zoomed view of the region outlined with a white box. Bar 20 µm.
B. **SDPR over-expression induces membrane tubulation.** Cells over-expressing non-tagged SDPR, fixed and labeled with anti-SDPR antibody. Bar 20µm.

C. **Comparison of membrane tubulation in cells over-expressing the same amount of SDPR-GFP, with and without STB.** Confocal sections of live cells. Bars 20µm.

D. **Co-localization between STB and caveolin 1 without energy depletion.** Confocal image. Bar 10µm. White arrows highlight localization of caveolin 1 at the end of STB-positive tubes, yellow arrows highlight co-localization in membrane puncta. The lower panels show a magnified view of the region delineated by the dashed line. Note that both the abundance of STB tubes and the extent of co-localization between STB and caveolin 1 in puncta are less than in energy depleted cells (Figure 6B).

E. **Western blots showing down-regulation of caveolin 1 in caveolin 1 shRNA cell lines.** Blots of whole cell lysates were probed with the antibodies shown. The anti-caveolin 1 blot was Ponceau stained to provide an indication of total protein levels in each sample.

**Supplementary Figure 6**

**Full scans of all blots and gels from main figures.** Red text indicates which main figure each panel relates to, which antibodies were used in blots, and positions of relevant size markers. Red boxes indicate the regions shown in the main figure. Black text is the labeling of the relevant lanes used in the main figure.

**Supplementary Movies 1 and 2.**

Both movies show time-lapse images of an energy-depleted cell expressing caveolin 1-GFP during addition of STB conjugated to Cy3. Movie 1 provides a lower resolution view of the whole cell, Movie 2 a zoomed in view of a region in the upper left hand corner of the cell. In both images the lefthand monochrome panel is STB-Cy3, right hand is caveolin1-GFP, and in the color overlay GFP is green and Cy3 red. Images were acquired at 4 images per minute, and play back at 15 images per second (i.e. 225x real time).

1. Clamp, M., Cuff, J., Searle, S.M., and Barton, G.J. (2004). The Jalview Java alignment editor. Bioinformatics 20, 426-427.