Supplemental material

Cheng et al., http://www.jcb.org/cgi/content/full/jcb.201504042/DC1



Figure S1. Mechanical stretch causes loss of morphologically defined caveolae at the plasma membrane. (A) Quantification of caveolae per micrometer of membrane after different times in hypo-osmotic medium. Each point represents one complete perimeter of a different bEnd5 cell. (B) Quantification of caveolae per micron of membrane after different times of mechanical stretching. The deformable substrate on which the cells were grown was stretched by 20%, repeated at 1 Hz. Each point represents one complete perimeter of a different cell.



Figure S2. **Supporting data for experiments with dobutamine-treated mice.** (A) Pulse plethysmography of dobutamine-treated mice. Pulse sensor was placed over the carotid artery; mice were shaved in this region. Each dot is one animal, and ages were 20–25 wk. (B) Biochemical assay of the disassembly of caveolae in vivo after increases in cardiac output. Western blots with anti–cavin-1 antibodies are shown, analyzing either membrane fractions or total tissue lysate.



Figure S3. An assay for acute loss of plasma membrane integrity reveals that caveolae protect cells from rupture under mechanical stress. (A) Staining of control and *caveolin* $1^{-/-}$ mouse embryonic fibroblasts with wheat germ agglutinin–Alexa 488, to allow visualization of cell outlines, and SYTOX Green, to label nuclei in cells where the integrity of the plasma membrane has been compromised. (B) Quantification by flow cytometry of the intensity of SYTOX Green labeling in control and *caveolin* $1^{-/-}$ cells. (C) Quantification of the proportion of cells with SYTOX Green–positive nuclei after 10 min of 20% stretch at 1 Hz. Each point represents an image containing 5–15 cells.