Supplemental Material for

**Force-induced transcellular tunnel formation in endothelial cells**

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Supplemental Figure S1.

(A) Histograms of penetration step sizes for control, EDIN-treated and ROCK-inhibited cells.

(B) Force-induced tunnels in EDIN-treated and ROCK-inhibited cells. Time-lapse TIRF images of HUVECs expressing EDIN or treated with 50 µM Y-27632 forming a tunnel when indented with an AFM cantilever tip. Displacement of some actin fibers by the tip can be observed in the EDIN-expressing cell. Arrow heads indicate location of the tip. The time points of each image relative to tip contact (t = 0 s) are displayed above the image. Scale bar: 10 µm.
Supplemental Figure S2. Bacterial toxin EDIN decreases barrier against tunnel formation in HUVECs

(A) Histograms of force required to form tunnels in cells for the three conditions. (B) Histograms of the total work required to indent and push away cytoplasmic materials in the cell to induce tunnel opening for the three conditions.
Supplemental Figure S3. Bacterial toxin EDIN decreases the level of active actin response to small deformations in HUVECs

Histograms of the maximum normalized actin intensity change measured when cells were indented with a 2–5 nN force clamp with an AFM cantilever tip for 3–5 minutes. Histograms shown for (A) control cells, (B) EDIN-expressing, and (C) ROCK-inhibited cells. The darker shades correspond to counts where the normalized actin intensity change was less than 1.25x the initial mean actin intensity.