Supporting Information

Effects of cholesterol on nano-mechanical properties of the living cell plasma membrane

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Example of two plasma membrane tethers pulled from a cell

We used fluorescein isothiocyanate (FITC) labeled phalloidin (Phalloidin-FITC, Sigma-Aldrich) as cytochemical marker of polymerized actin. Tethers were pulled according to the single-speed pulling protocol. Once the tether force reached its equilibrium value, the bead was immobilized by lowering the axial position of the optical trap using the PZT until it came in contact with the glass surface at the bottom of Petri dish. The cells were then immediately fixed with PBS-paraformaldehyde 4% for 15 minutes, treated with PBS-triton X100 0.2% for five minutes, blocked with PBS-5% BSA for 30 minutes, and incubated for one hour with PBS-phalloidin-FITC 1 µM to stain F-actin. Fluorescent emission from stained F-actin was filtered using FITC filter set (Nikon, FITC). The filter set included an excitation filter (525-560 nm), a dichroic mirror which reflected the excitation light into the objective to illuminate the sample, and an emission filter (570-620 nm). Images were subsequently acquired by a CCD camera with 100X optical microscope, and analyzed with ImageJ software.

To investigate the presence of F-actin in plasma membrane tethers, tethers were pulled from non-cholesterol manipulated human embryonic kidney (HEK) cells. Tethers were formed and elongated at 1 µm/s to reach a length of 20 µm, and subsequently held therein for ~2 minutes to allow for tether force relaxation and then labeled and fixed as described above. In Fig.S1 we show the respective bright-field and fluorescent images of the same bead-tether-cell assembly. In these particular experiments, two tethers were formed from the same cell. The fluorescent image did not indicate the presence of F-actin within the tether under these pulling conditions.

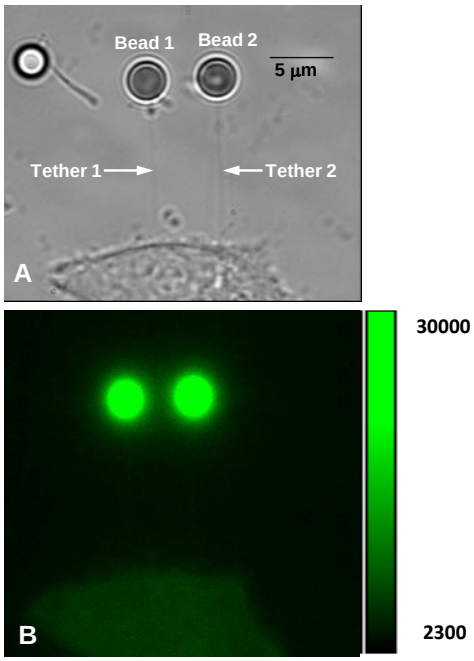

Figure S1- Images of cell-tether-bead assembly- (A) Bright-field photomicrograph of two trapped beads - two membrane tethers pulled from a control HEK cell. **(B)** Corresponding fluorescent image of the same beads-tethers-cell assembly shown in (A) after F-actin staining with FITC-labeled phalloidin.