Supporting Information

Part I. Example microscope images of adherent cells for 4 and 8 h cholesterol depletion

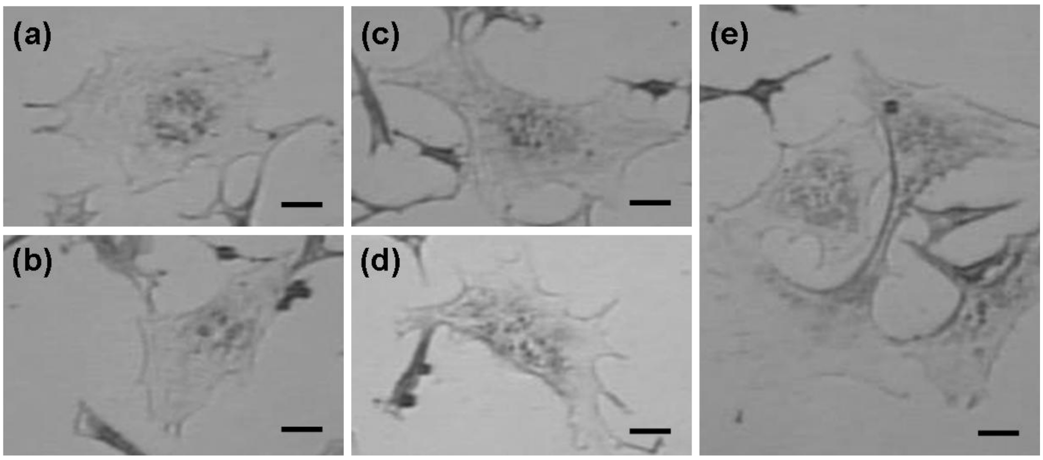

Figure S1. Optical microscope images of cholesterol-depleted cell samples for nano-indentation tests, demonstrating analogous cell morphology with cholesterol depletion for 4 (a, b) and 8 h (c, d, e). Scale bar, 10 μm.

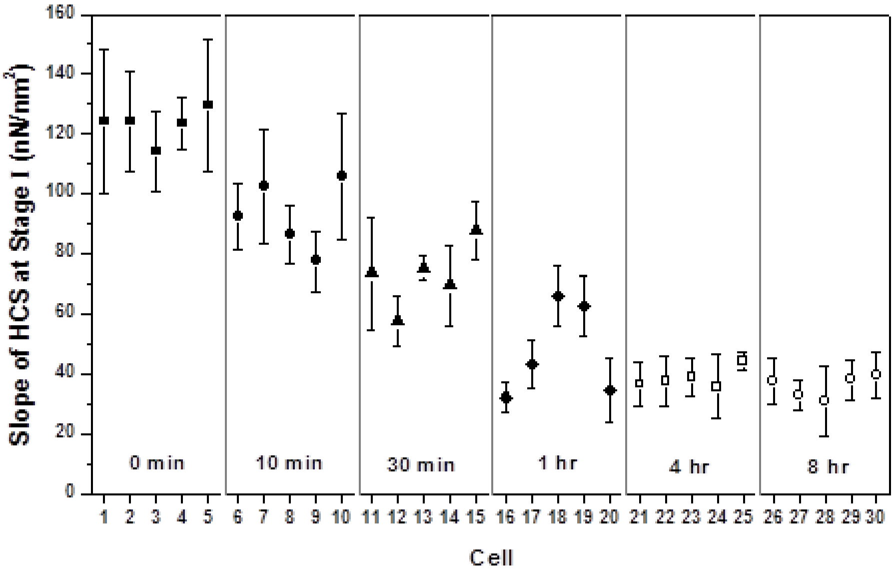

Figure S2: Statistic diagram of the slope of HCS at Stage I of every sampled cell in each group of cholesterol depletion.

Part II. AFM nano-indentation for the cholesterol depletion experiment of living cells

The inset in Figure S3 illustrates the way AFM nano-indentation works. During the indentation process, through monitoring the cantilever deflection, which is proportional to the applied force as a function of the indentation depth, a force-displacement curve can be obtained. The initial 100 nm of the measured force-displacement curve is then fitted with Bilodeau model to calculate the apparent Young's modulus of the indented cells by minimizing square errors between the model and experimental data [Ref. S1]. Figure S3 shows the apparent Young's modulus data calculated from force-displacement curves of living 3T3 fibroblast cells incubated with 5 mM MβCD in serum-free medium from 0 min to 8 h. The Young's modulus of cells treated with MβCD for 8 h (0.62 ± 0.08 kPa) decreased drastically as compared to that of untreated cells (6.37 ± 1.08 kPa). The results of apparent Young's modulus measured by AFM exhibit an analogous exponential decay tendency to the results of HCS measurement with instrumented nano-indenter.

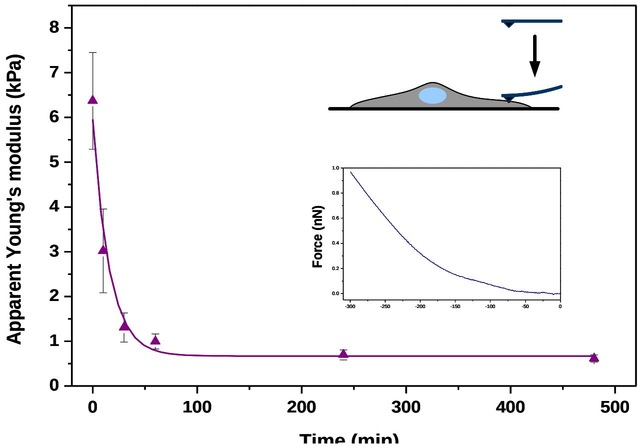

Figure S3. The apparent Young's modulus data of living 3T3 fibroblast cells incubated with 5 mM MβCD prepared in serum-free medium for 8 h. (n = 16~18) The inset shows the AFM nano-indentation process and an example of the obtained force-displacement curve for the calculation of Young's modulus data.

The experimental of AFM nano-indentation

AFM force—distance curves were obtained at ambient temperature using a commercial atomic force microscope (Agilent 5500 AFM System, *Agilent Technologies*, USA) combined with an inverted optical microscope (TE2000-U, *Nikon*, Japan). Commercial silicon nitride cantilever probe (MSCT, *Veeco Instrument*, USA) with a nominal tip radius of curvature of ≈10 nm and a nominal spring constant of 0.01 N/m was used in the experiment. The spring constant of the cantilever was calibrated using Nanoscope 4.43 software calibration tool. The AFM tip was cleaned in 70% ethanol and rinsed in Milli-Q water prior to each measurement. The AFM was operated at a force distance curve acquisition frequency of 0.5 Hz. Chosen cells in each group were well spread in around the same size, and nano-indentation tests were also performed in the lamellipodium region. The measured force-distance curves were fitted with Bilodeau model to calculate the apparent Young's modulus of the indented cells by minimizing square errors between the model and experimental data [Ref. S1].

Reference

S1. Bilodeau, G. G. Regular Pyramid Punch Problem *J. Appl. Mech.* **1992,** 59, 519-523.