Supplementary Information for:

Observations of membrane domain reorganization in mechanically compressed artificial cells

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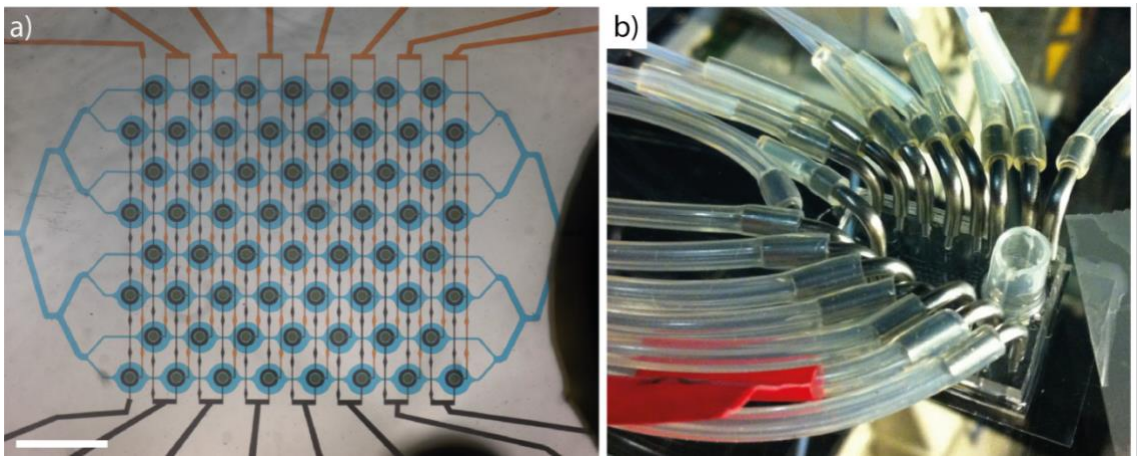

Figure S1. a) Bright-field image of entire channel network with an array of 60 chambers. Channel are filled with food dyes for visualization. Upper layer: micro-stamps (orange), and ring-valves (black). Lower layer: fluidic channels with posts for GUVs (blue). Scale bar: 2 mm. b) Photograph of final assembled device with all tubings connected: 8 for the ring-valves, 8 for the micro-stamps, and 1 to the syringe.

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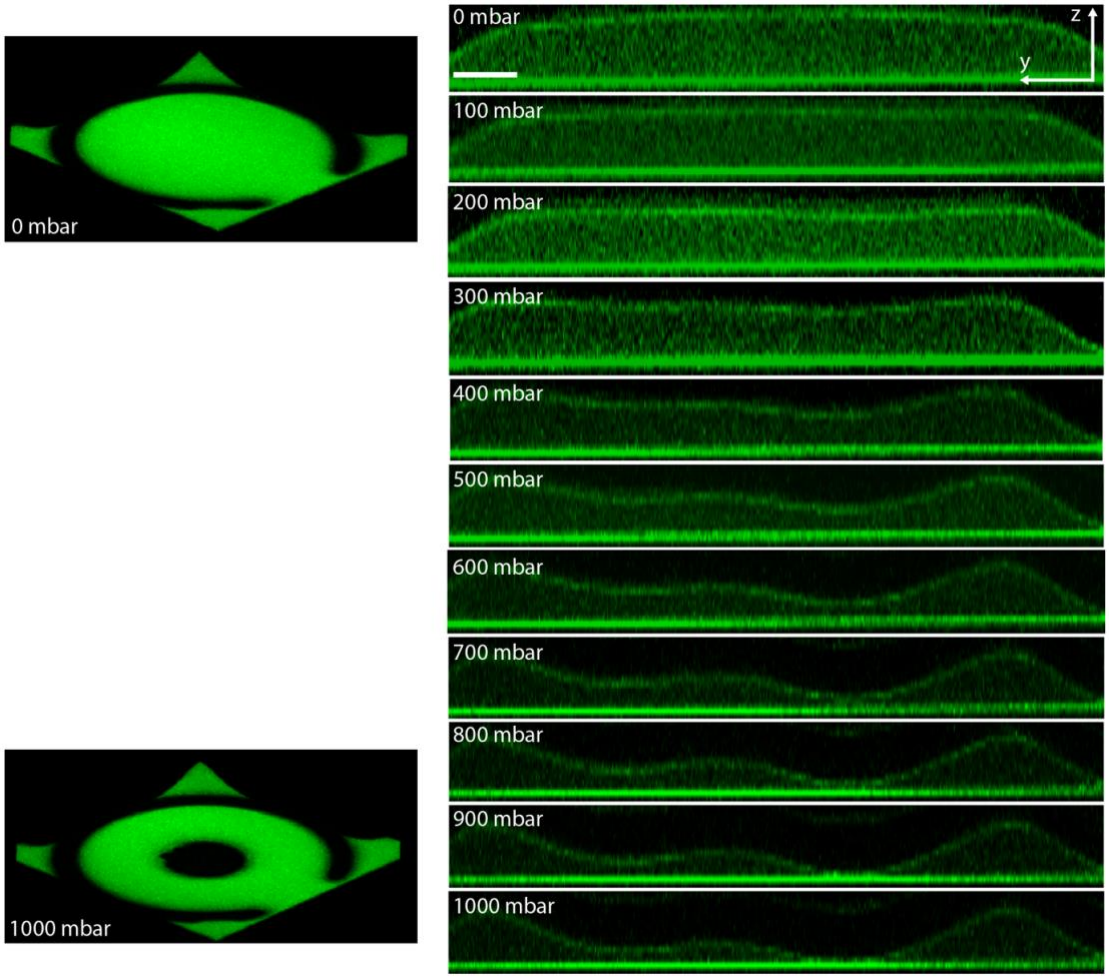

Figure S2. Micro-stamp actuation. The chamber was filled with 1 μM calcein in water, and the ring-valve was closed. Confocal stacks were acquired as the micro-stamp was pressurized from 0 mbar to 1000 mbar. Left: 3-D renderings. Right: the y-z plane in order to visualize the stamp as it is lowered to the glass surface. The PDMS posts at the center of the chamber prevent the stamp from fully reaching the bottom. Scale bar arrows: 20 μm.

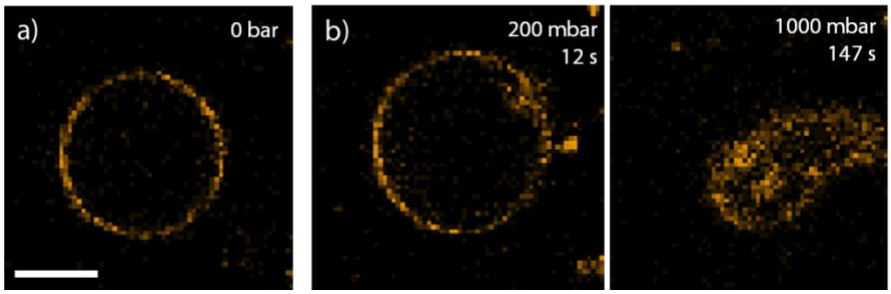

Figure S3. Confocal time series of an osmotically balanced GUV bursting under compression. a) A pure DOPC with 0.1 mol% DiI and grown in 900 mM sucrose before the application of pressure to the micro-stamp. b) After 12 s of compression with 200 mbar the GUV is larger and already appears to have a defect. After increasing to 1000 mbar at 147 s the GUV bursts (n=4). Note that the pixel resolution is low due large field of view imaging and higher frame rate to track the bursting live. Scale bar: 10 μm.

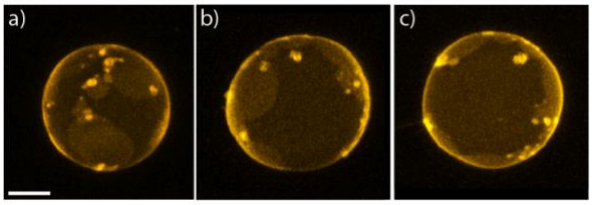

Figure S4. Example of l_d domains (DiI, orange) of a phase separated SM/DOPC/Chol 4:4:2 GUV migrating to the equatorial region with increasing compression. Scale bar: 5 µm. In this example, the l_o phase was not stained.

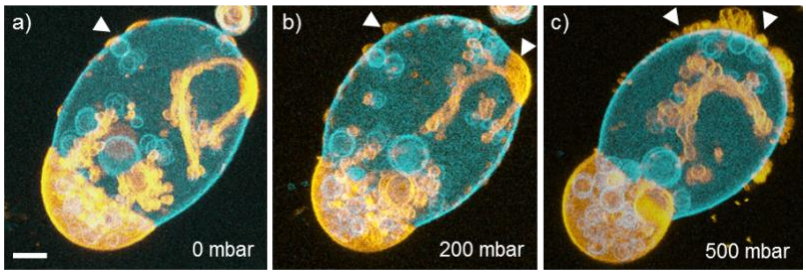

Figure S5. Compression dependent budding and detachment of domains. 3-D renderings of a GUV, SM/DOPC/Chol 4:4:2, with applied pressures of a) 0 mbar, b) 200 mbar, and c) 500 mbar. Budding of a large domain can be seen in the bottom left but also smaller domains bud and detach on the opposite side as indicated by the arrows. Scale bar: 5 µm. DiI (orange) and NAP (cyan) partition into the l_d and l_o phases respectively. Note that the intraluminal vesicles (often found via electroformation [1]) and budded outer vesicles were not attached to the main vesicle membrane and can be seen moving in between the acquisition of the confocal optical slices.

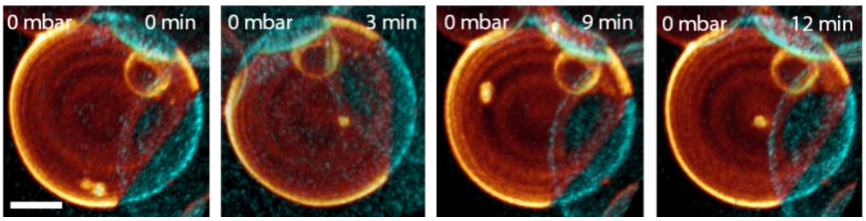

Figure S6. Control confocal 3-D renderings of a phase-separated GUV, SM/DOPC/Chol 4:4:2, without compression. Here the exemplary GUV had two large l_d domains which remained separate and did not merge (n=3). Scale bar: 5 µm. DiI (orange) and NAP (cyan) partition into the l_d and l_o phases respectively.

[1] T. Baumgart, S. T. Hess, W. W. Webb, *Nature* **2003**, *425*, 821–824.