SUPPLEMENTARY INFORMATION

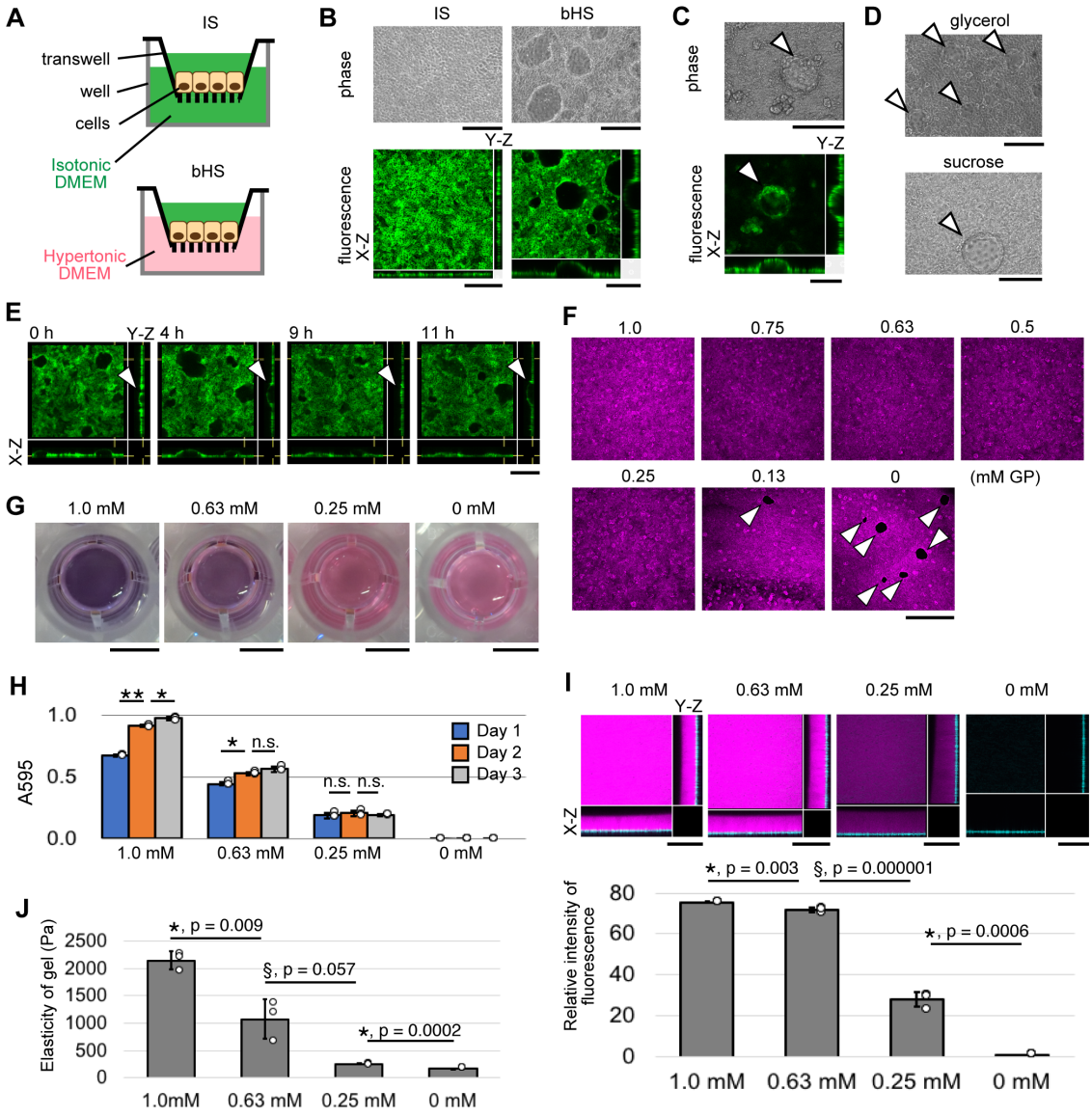


Fig. S1. Fluid-filled domes (F-domes) occurred and collapsed on non-coat permeable membrane in basal hypertonic stress (bHS); Genipin could crosslink and strengthen the Matrigel.

(A) The experimental system of bHS treatment and control isotonic stress (IS). Green and pink indicate isotonic and hypertonic DMEM, respectively.

(B) Phase-contrast and fluorescent images of confluent MDCK cells with fluorescent-labeled membrane (MDCK-CAAX cells) on non-coat 0.4 μm pore membrane in IS and bHS.

(C) The same area is taken by both a phase-contrast microscopy and a fluorescent confocal microscopy. Arrowheads indicate the same dome.

(D) Phase-contrast images of F-domes (arrowheads) on 0.4 μm pore membrane in bHS generated by glycerol or sucrose.

(E) Time development of F-domes in bHS. Arrowheads indicate collapse and rebirth of a dome. Green, MDCK-CAAX.

(F) Maximum intensity projection image of MDCK-wild type (WT) sheet on genipin treated-Matrigel (GP-Matrigel) with different concentration of genipin (GP). The cell sheets were cultured in IS. Arrowheads indicate the pore of the monolayer. Magenta, F-actin.

(G) Images of GP-Matrigel with different concentrations of GP.

(H) The absorbance values at 595 nm wavelength for 0 mM, 0.25 mM, 0.63 mM, and 1.0 mM GP-Matrigel were measured at daily intervals following GP treatment. n = 3

independent experiments. Means ± S.D. n.s., no significance. *, p<0.05. **, p<0.0001 (student *t*-test with Bonferroni correction).

(I) Fluorescence images of Matrigel treated with different concentrations of GP obtained at a constant laser power. Graph of fluorescence intensity of GP-Matrigel (excitation/emission 561/603 nm). Magenta, GP-Matrigel. Cyan, the membrane of transwell. Means ± S.D. *n* = at least 3 independent experiments.

(J) Surface stiffness of GP-Matrigel was measured by atomic force microscopy (AFM). *N* = 3 area from 1 experiment. Means ± S.D. *, student *t*-test. §, welch *t*-test.

(B-E) Scale bar, 200 μm. (F) Scale bar, 5 mm. (H and J) Scale bar, 500 μm. Images are representatives of *n* = at least 3 independent experiments. These experiments used Matrigel incubated with GP for 48 h (F) or 72 h (G, I, J).

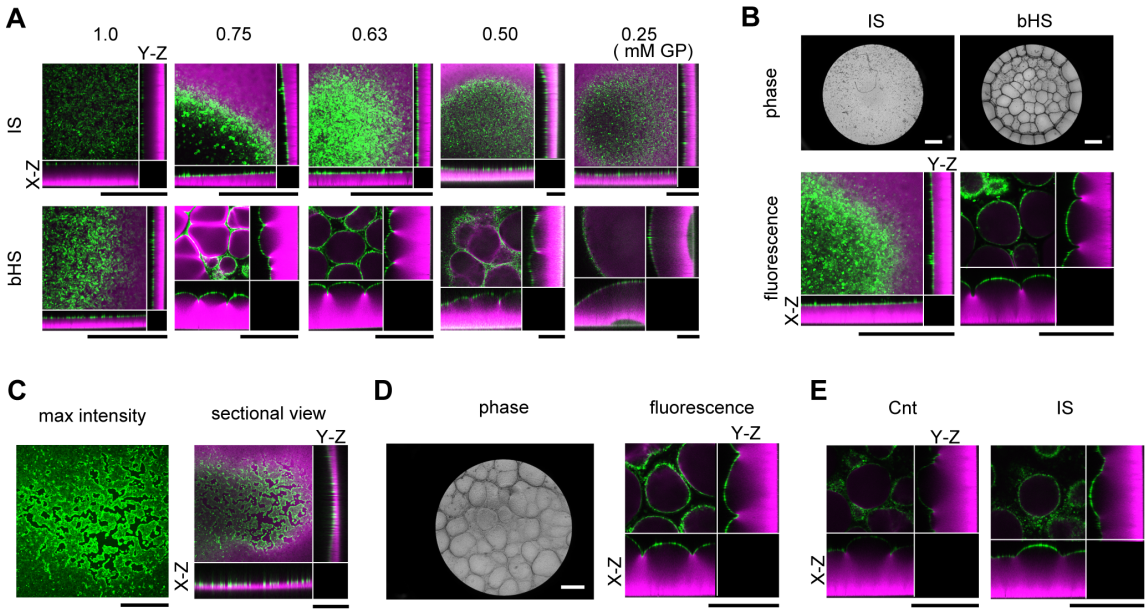


Fig. S2. bHS was essential for the gel-filled dome (G-dome) formation of MDCK nonporous sheets.

(A) Fluorescent images of Fig. 1B. Green, MDCK-CAAX. Magenta, GP-Matrigel.

(B) Images of MDCK-WT sheets on 0.63 mM GP-Matrigel after overnight incubation in IS and bHS. Green, calcein-AM. Magenta, GP-Matrigel.

(C) Images of the subconfluent MDCK-WT sheet on 0.63 mM GP-Matrigel after overnight incubation in bHS. Maximum intensity projection is only in green. Green, calcein-AM. Magenta, GP-Matrigel.

(D) Images of G-domes on 0.63 mM GP-Matrigel in a 12 well transwell insert. Green, MDCK-CAAX. Magenta, GP-Matrigel.

(E) Images of G-domes before (Cnt) and after (IS) overnight incubation with IS. Green, MDCK-CAAX. Magenta, GP-Matrigel. Scale bar, 1mm. (A-C, E) Images are the representatives of the *n* = at least 3 independent experiments. (D) Images form the *n* = 1 experiment. These experiments used Matrigel incubated with GP for 48 h.

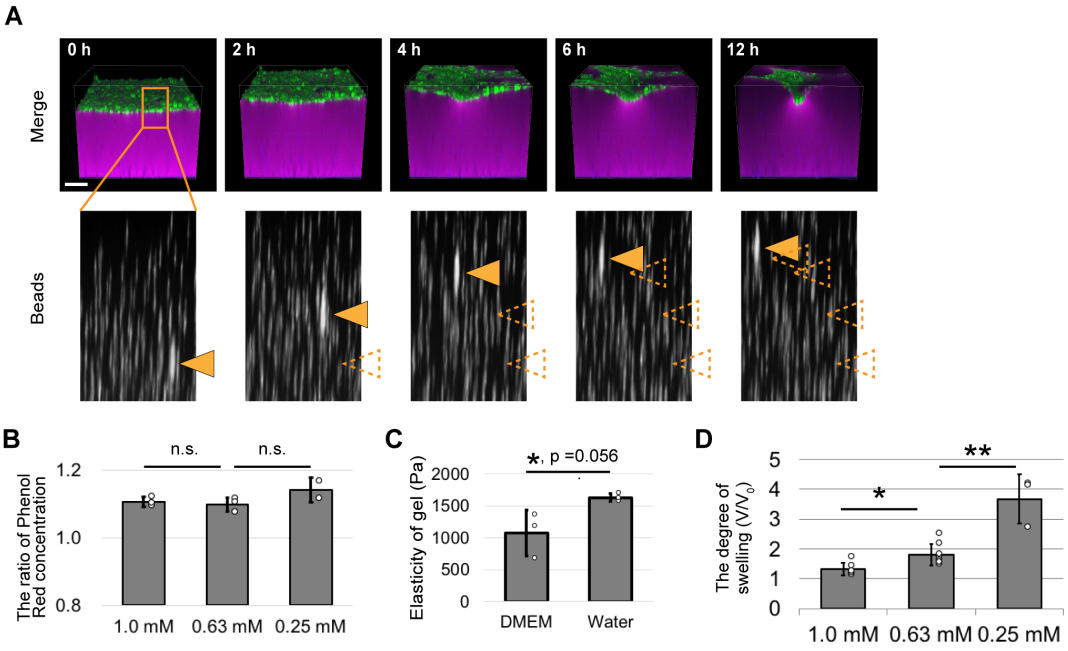


Fig. S3. The beads in GP-Matrigel moved up at the site of dome formation; Gel stiffness affected its swelling but not water transport.

(A) Time course 3D images of the G-domes on 0.63 mM GP-Matrigel containing fluorescent beads. Time development of beads is magnified view of orange box in the top panel. The orange arrowhead tracks the same bead. The arrowheads with orange dashed line indicate the beads places at earlier time points. Blue, beads. Green, MDCK-CAAX. Magenta, GP-Matrigel. Scale bar, 200 μm. Images are the representatives of at least *n* = 3 independent experiments.

(B) Water transport analysis of MDCK-WT sheet on GP-Matrigel with different gel stiffness values. *n* = at least 2 independent experiments. Means ± S.D. n.s., no significance. (student *t*-test with Bonferroni correction).

(C) Surface stiffness of 0.63 mM GP-Matrigel was measured by AFM. *n* = 3 area from 1 experiment. Means ± S.D. *, student *t*-test.

(D) Gel swelling analysis of GP-Matrigel with different stiffness values in pure water. Cells were not seeded. *n* = at least 3 independent experiments. Means ± S.D. *, $p<0.05$. **, $p<0.01$ (student *t*-test with Bonferroni correction). These experiments used Matrigel incubated with GP for 48 h (A) or 72 h (B-D).

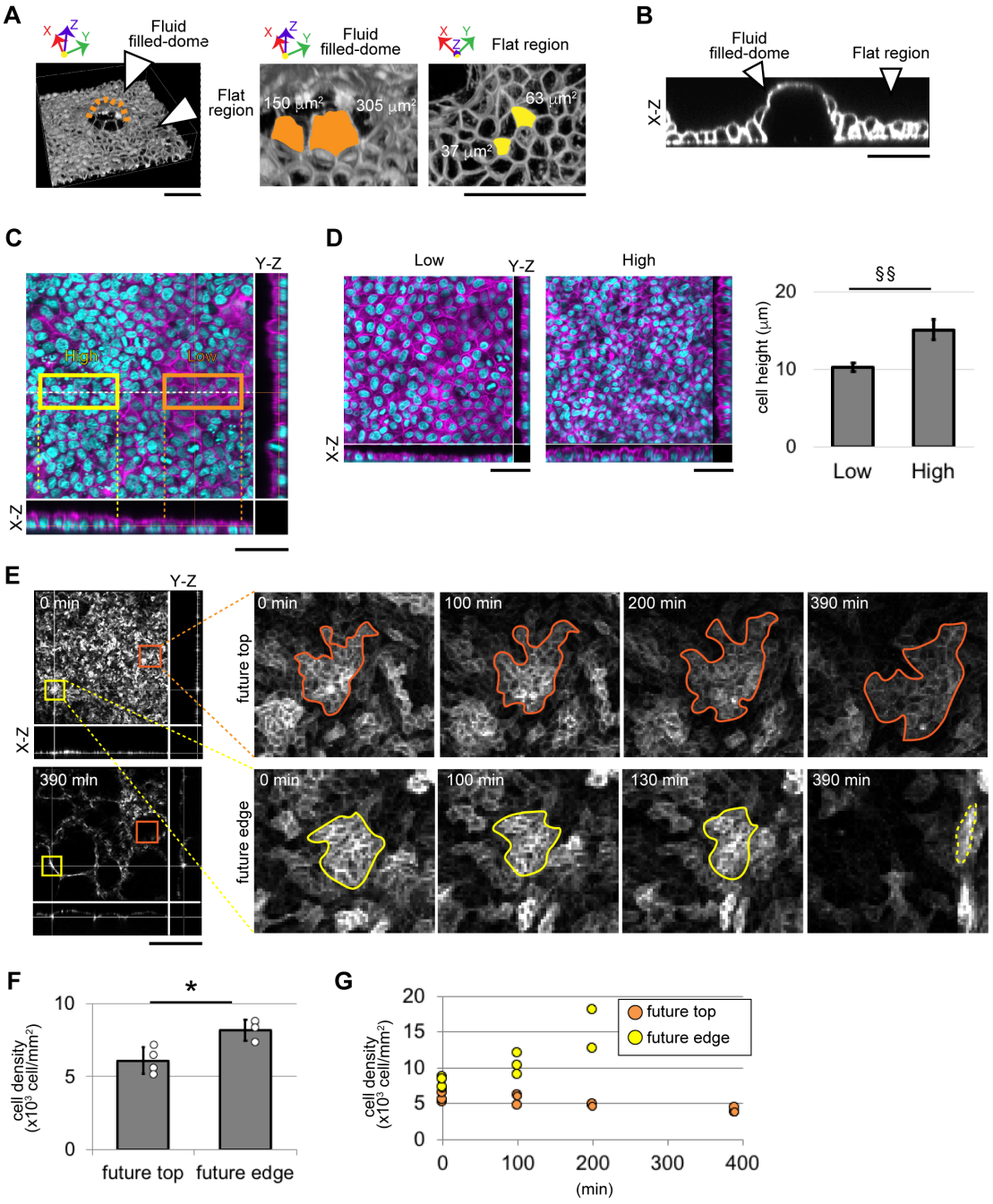


Fig. S4. Cell height was thinner at F-domes and in low cell density; cell density was lower at future G-dome top than at future G-dome edge.

(A) 3D view of fluorescent images of a F-dome of MDCK-WT cells induced by bHS on non-coat 0.4 μm pore transwells. Orange dashed line indicates the surface of the F-dome. Orange and yellow areas show the cell surface of the F-dome and the flat region, respectively. Each apical area is shown in the picture.

(B) Sectional view of (A) (A, B) White, F-actin. Scale bar, 50 μm. Images are from *n* = 1 experiment.

(C) Fluorescent images of MDCK sheet on GP-Matrigel before bHS treatment. White dashed line indicates the Z-section. Yellow and orange boxes showed the area with high and low cell density, respectively. Yellow and orange dot lines indicate the XZ-sectional view corresponded to each color boxes in XY section. Images are from *n* = 1 experiment.

(D) (Left) Fluorescent images of MDCK-WT sheets on non-coat 1.0 μm pore transwells. Cells were seeded with low or high cell density (seeded with 1.0 x 10^5 or 4.0 x 10^5 cells/24 well insert, respectively) and incubated in bHS for 9 h. (Right) The quantitative analysis of cell height. *n* = 9 area from three independent experiments. Images are representatives. Means ± S.D. §§, $P < 0.0001$ (Welch's *t*-test). (C, D) Cyan, nucleus. Magenta, F-actin. Scale bar, 50 μm.

(E) 3D live imaging of the G-dome formation with MDCK-CAAX on 0.63 mM GP-Matrigel. Orange and yellow lines indicate the area of future G-dome top and

future G-dome edge, respectively. Yellow dashed line indicates the unmeasurable state after aggregation of another area with strong fluorescence intensity. White, MDCK-CAAX. Scale bar, 500 μm. Images are representatives from *n* = 1 experiment.

(F) Cell density analysis of the area between the future top and future edge of G-domes at 0 h. We used 0.63 mM GP-Matrigel. *n* = at least 3 area from 1 experiment. Means ± S.D. *, $P < 0.05$ (Student's *t*-test).

(G) Cell density tracking of the area in the future top and future edge of G-domes on 0.63 mM GP-Matrigel. Orange and yellow dots represent future top and future edge, respectively. *n* = at least 2 area from 1 experiment. (E-G) These experiments used Matrigel incubated with GP for 48 h.

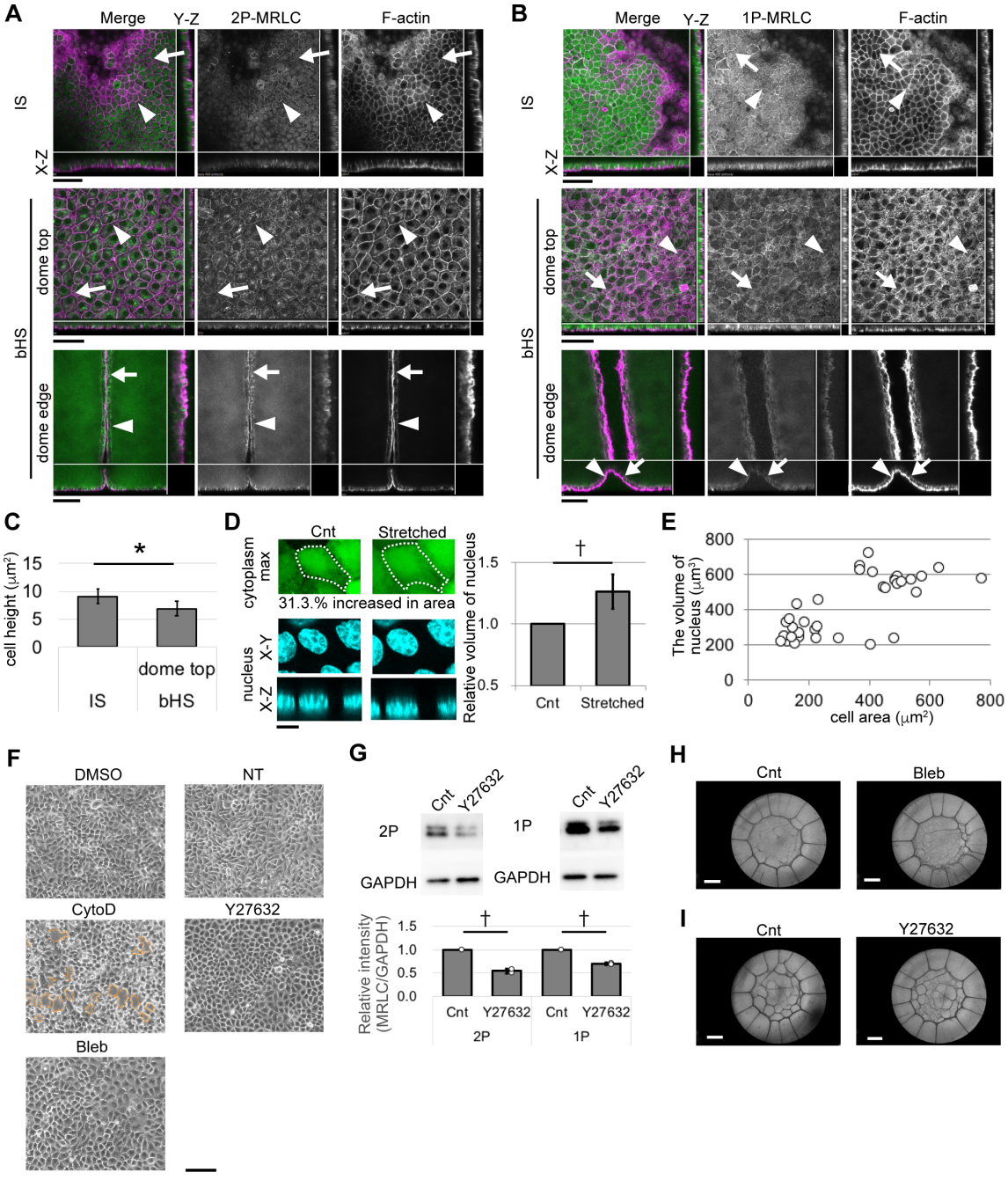


Fig. S5. MRLC contractility did not contribute to G-dome formation; cell stretching increased nucleus volume in MDCK cells.

(A-B) 2P-MRLC (A) and 1P-MRLC (B) images of MDCK sheet on 0.63 mM GP-Matrigel before and after bHS treatment. The samples were inverted to observe under a high magnification objective lens. Arrowheads and arrows indicate colocalization and non-colocalization of P-MRLC and F-actin, respectively. Green, F-actin. Magenta, P-MRLCs. Scale bar, 50 μm. Images are from *n* = at least 1 experiment.

(C) Cell height analysis of MDCK sheet in IS and bHS (dome top). *n* = 15 cells from at least 1 experiment. Means ± S.D. *, $P < 0.001$ (Student's *t*-test).

(D) Images for nuclear volume analysis of MDCK cells before (Cnt) and after (Stretched) cell stretching using a silicone rubber chamber. Cytoplasm and nucleus were shown in max intensity and sectional image, respectively. White dot line indicates the single cell area. Cyan, nucleus. Green, Calcein-AM. Scale bar, 10 μm. Images are representative. *n* = 26 cells from 1 experiment. Means ± S.D. †, significance by 99% confidence interval.

(E) Nucleus volume analysis of MDCK cells seeded at different densities on glass. *n* = 35 cells from 1 experiment.

(F) Phase-contrast images of MDCK sheet after 4 h incubation with actomyosin inhibitors. DMSO is control for cytochalasin D (CytoD) and blebbistatin (Bleb). NT is

control for Y27632. Orange dashed lines indicate the hole in MDCK monolayer. Scale bar, 100 μm. Images are representatives from *n* = 1 experiment.

(G) Western blot analysis for 2P-MRLC, 1P-MRLC, and GAPDH in G-dome on 0.63 mM GP-Matrigel treated with Y27632. *N* = 2 independent experiments. Means ± S.D. †, significance by 99% confidence interval.

(H-I) Phase-contrast images of G-domes on 0.63 mM GP-Matrigel after 9 h incubation in bHS with Bleb (H) and Y27632 (I). Scale bar, 1 mm. Images are representatives form *n* = 3 independent experiments. These experiments used Matrigel incubated with GP for 72 h.

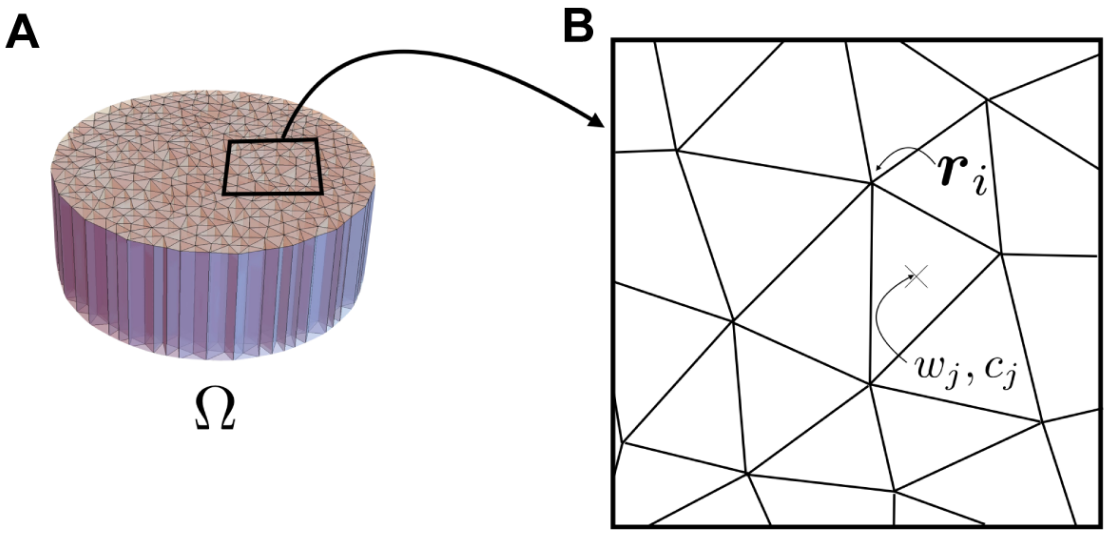


Fig. S6. Details of the symbols appearing in the model.

(A, B) Position of variable and its conceptual diagram.

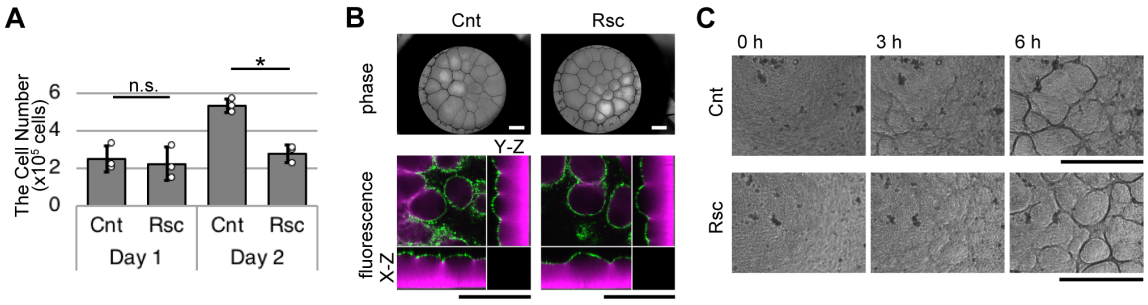


Fig. S7. Cell proliferation was not necessary for the G-dome formation

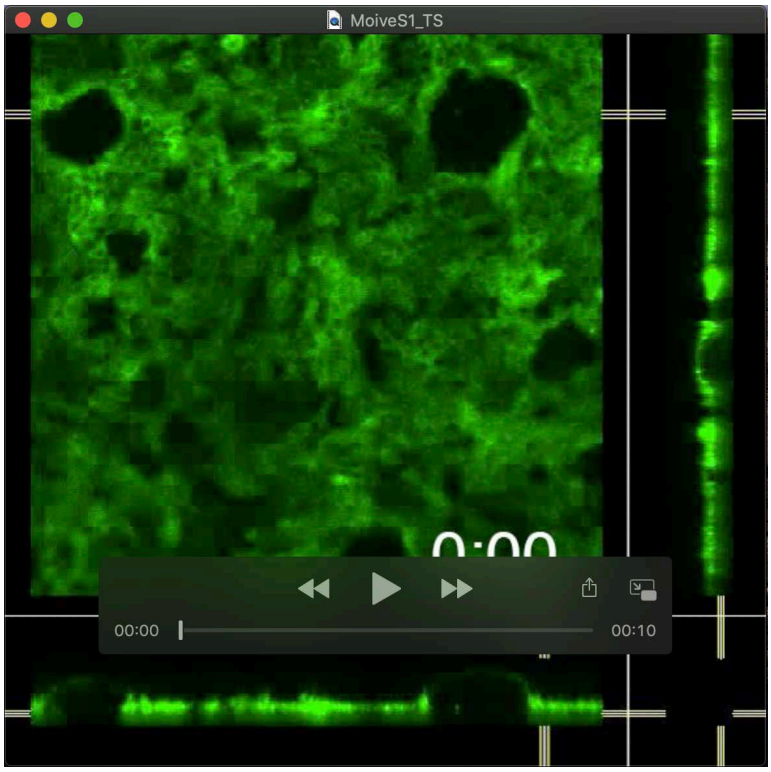
(A) Cell growth assay of MDCK-CAAX cells with treatment of 10 μM Roscovitine (Rsc), a proliferation inhibitor, and DMSO (control; Cnt). *n* = 3 experiments. Means ± S.D. n.s., no significance. *, $P < 0.05$ (Student's *t*-test).

(B) Images of G-domes on 0.63 mM GP-Matrigel after overnight incubation in bHS with Rsc and Cnt. Green, MDCK-CAAX. Magenta, GP-Matrigel.

(C) Time-lapse phase contrast images of MDCK-CAAX sheets on GP-Matrigel in bHS with treatment of Rsc or Cnt. Scale bar, 1 mm. Images are the representatives of *n* = 3 independent experiments. These experiments used Matrigel incubated with GP for 48 h.

Parameter	Value	Descriptions
μ	10.0	Parameter of the function f
c_0	1.0	Parameter of the function f
ρ	0.001	Parameter of convert ratio
κ_1	3.564	Coefficient of the potential energy U^{Bend}
κ_2	0.05	Coefficient of the potential energy U^{Volume}
N	6459	Number of vertices
M	12695	Number of vertices
$Atol$	1.0e-8	Absolute tolerance value of embedded R.K. method
$Rtol$	1.0e-8	Absolute tolerance value of embedded R.K. method

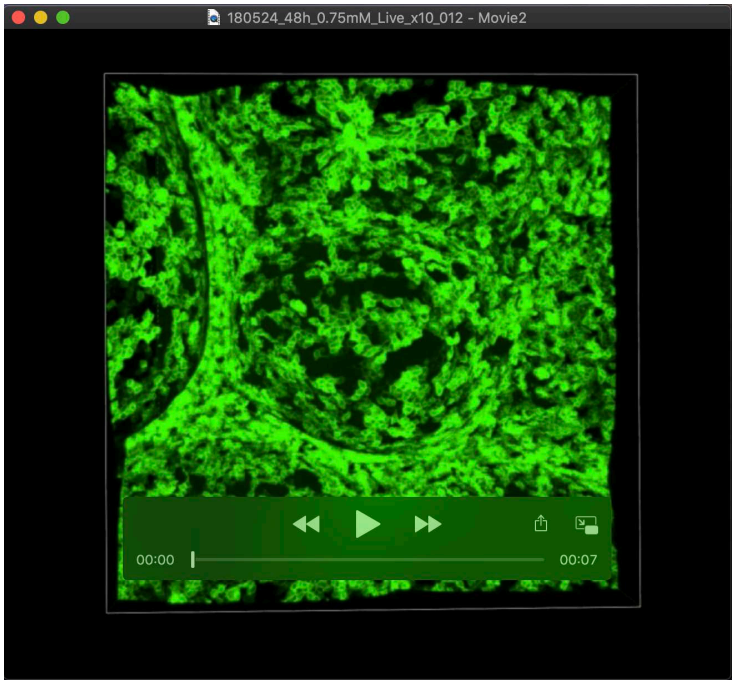
Table S1. Parameters of the simulation.



Movie 1.

3D live cell imaging of the basal hypertonic stress (bHS) induced fluid-filled dome (F-dome) collapse and reform on a permeable membrane.

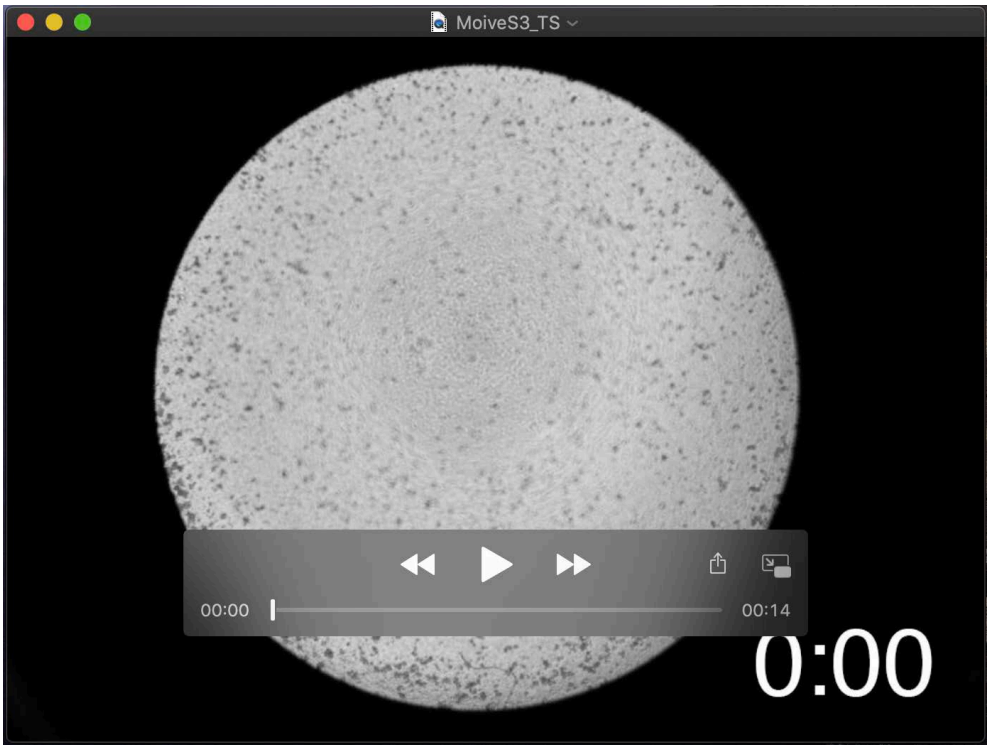
3D live cell imaging video that F-domes collapsed and reformed on permeable membrane. Video started immediately after the bHS was applied. Green, MDCK cells with fluorescent-labeled membrane (MDCK-CAAX cells). 75 min/frame



Movie 2

3D view of the bHS induced domes on 0.75 mM GP-Matrigel.

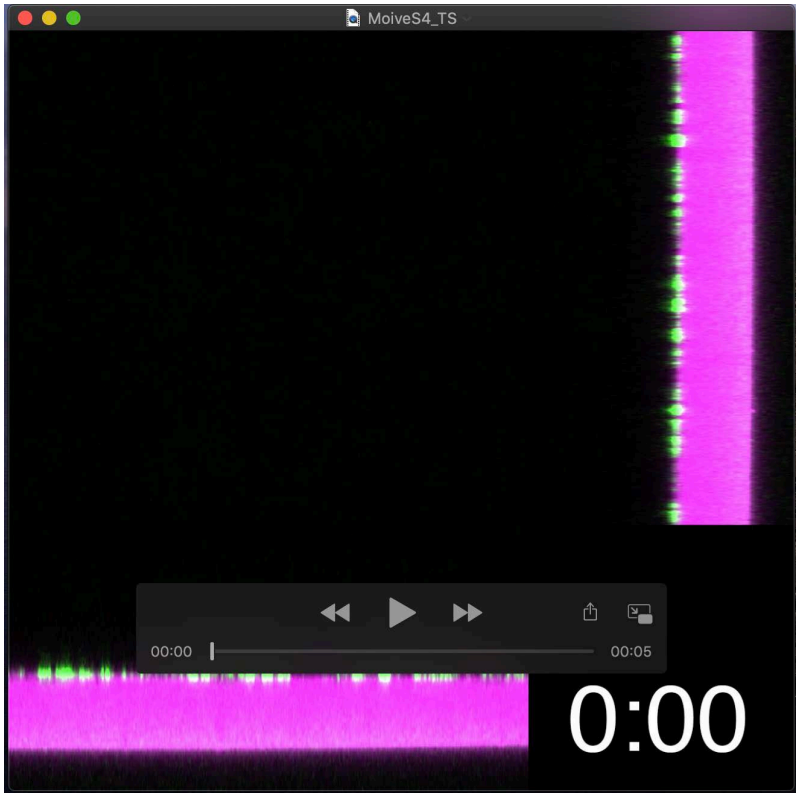
3D view of the domes on 0.75 mM genipin-treated Matrigel (GP-Matrigel) in bHS. Green, MDCK-CAAX



Movie 3

Phase contrast time-lapse video of the bHS induced gel-filled domes (G-domes).

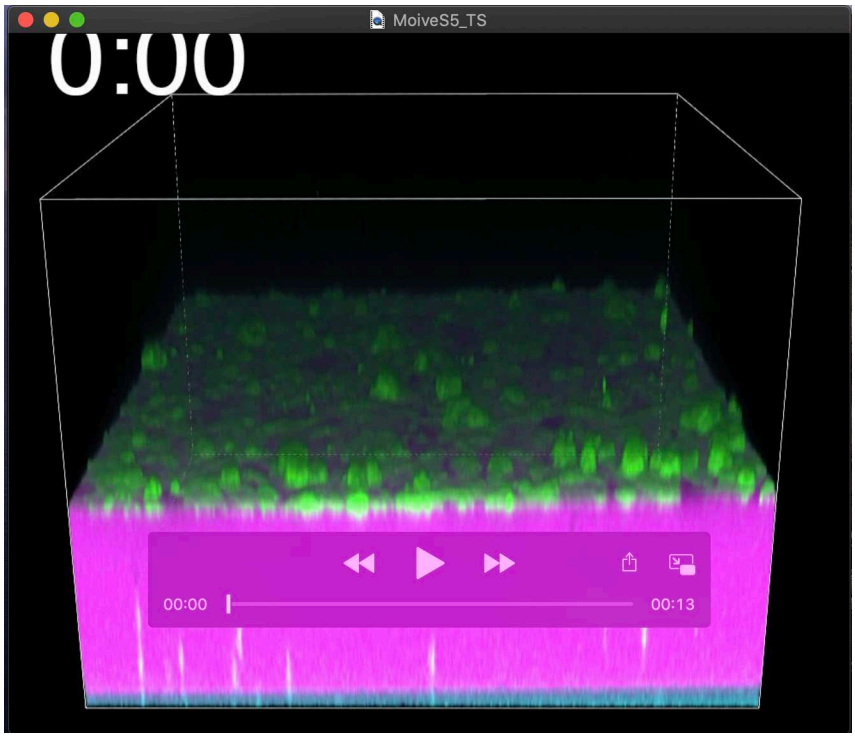
Time-lapse video that MDCK sheets formed and maintained domes on GP-Matrigel. Video started immediately after the bHS was applied. 75 min/frame



Movie 4

3D live cell imaging of the bHS induced G-domes.

3D live cell imaging video that MDCK sheets formed domes on 0.63 mM GP-Matrigel. Video started immediately after the bHS was applied. Green, MDCK-CAAX. Magenta, GP-Matrigel. 180 min/frame



Movie 5

3D live cell imaging of G-dome formation with fluorescent beads.

3D live cell imaging video that MDCK sheets formed domes on 0.63 mM GP-Matrigel containing fluorescent beads. The first half of the video was merge and the second half was beads image that was extracted from the first half. Video started immediately after the bHS was applied. Green, MDCK-CAAX. Magenta, GP-Matrigel. Cyan and white, beads. 180 min/frame.