# Supporting Information

## Remodeling of Adhesion Network within Cancer Spheroids via Cell-Polymer Interaction

Youngbin Cho†, Seung Jung Yu‡, Jiwon Kim†, Ung Hyun Ko†, Eun Young Park†, Jinseung Cheong†, Goro Choi‡, Daehyun Kim†, Eunjung Lee‡, Sung Gap Im‡*, and Jennifer H. Shin†*

†Department of Mechanical Engineering, Korea Advanced Institute of Science and Technology,

‡Department of Chemical and Biomolecular Engineering, KAIST Institute for the NanoCentury, 291, Daehak-ro, Daejeon 34141, Republic of Korea

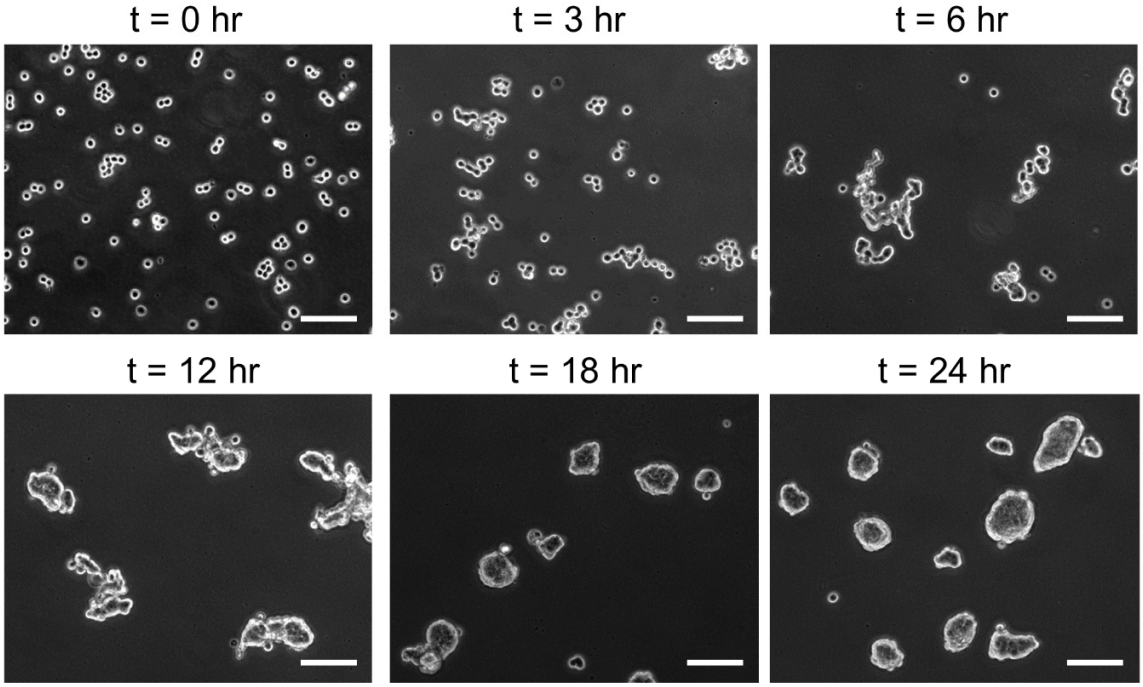



**Figure S1**. 3D aggregation of MCF7 cells on pV4D4 surface during early time-points (0-24 hr). All scale bars represent 100 μm.

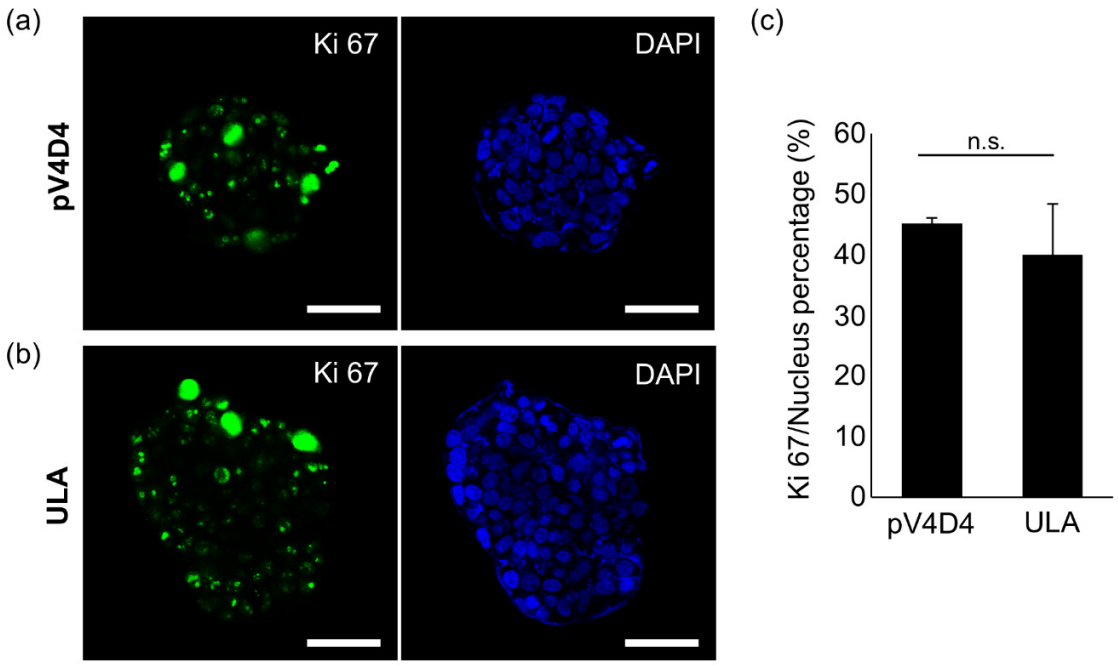



**Figure S2**. Confocal images of Ki67 (green) and DAPI (blue) within (a) pV4D4-cultured spheroid and (b) ULA-cultured spheroid. (c) Quantification of the Ki67/nucleus ratio of pV4D4 and ULA-cultured spheroids. Scale bars, 50 μm.

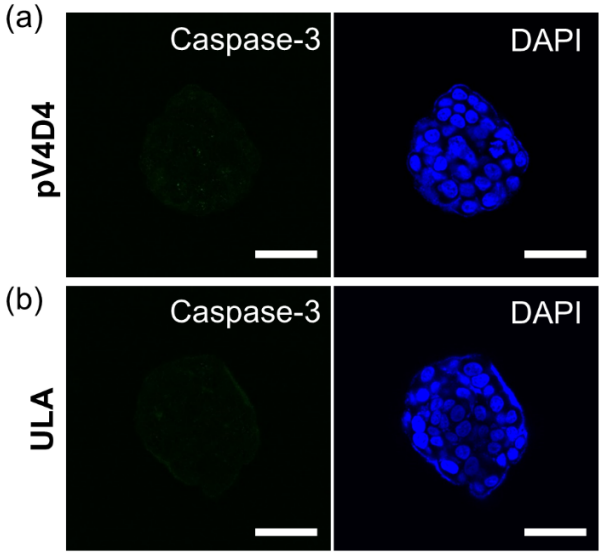



**Figure S3**. Confocal images of cleaved caspase-3 (green) and DAPI (blue) within (a) pV4D4-cultured spheroid and (b) ULA-cultured spheroid. Scale bars, 50 μm.

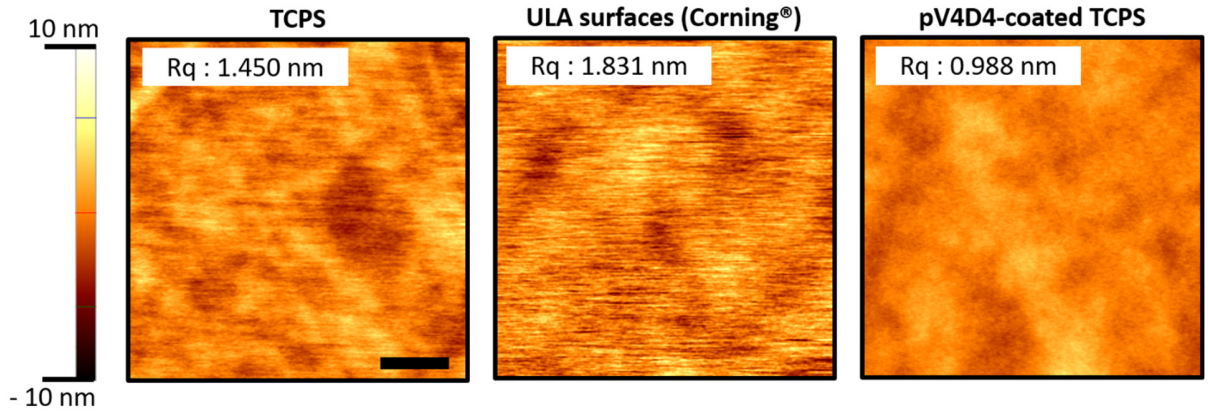



**Figure S4**. Roughness analysis of cell seeding surfaces by atomic force microscopy. (Left : tissue culture plate, middle : ultra-low attachment plate, right : pV4D4-caoted tissue culture plate). All scale bars represent 1 μm.

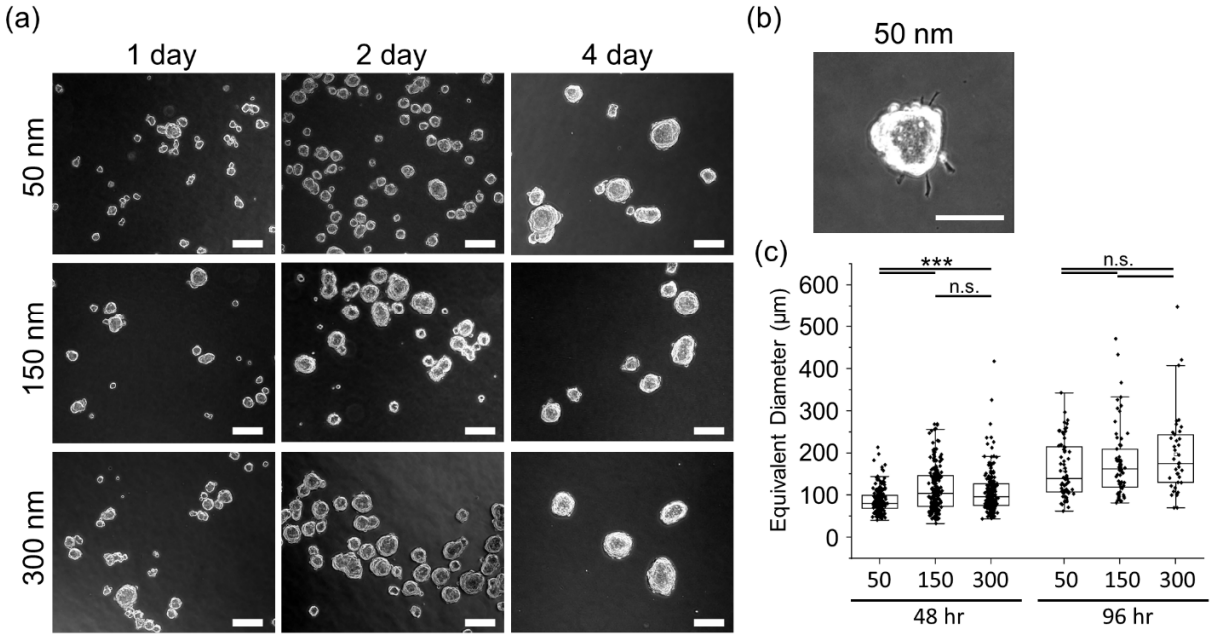
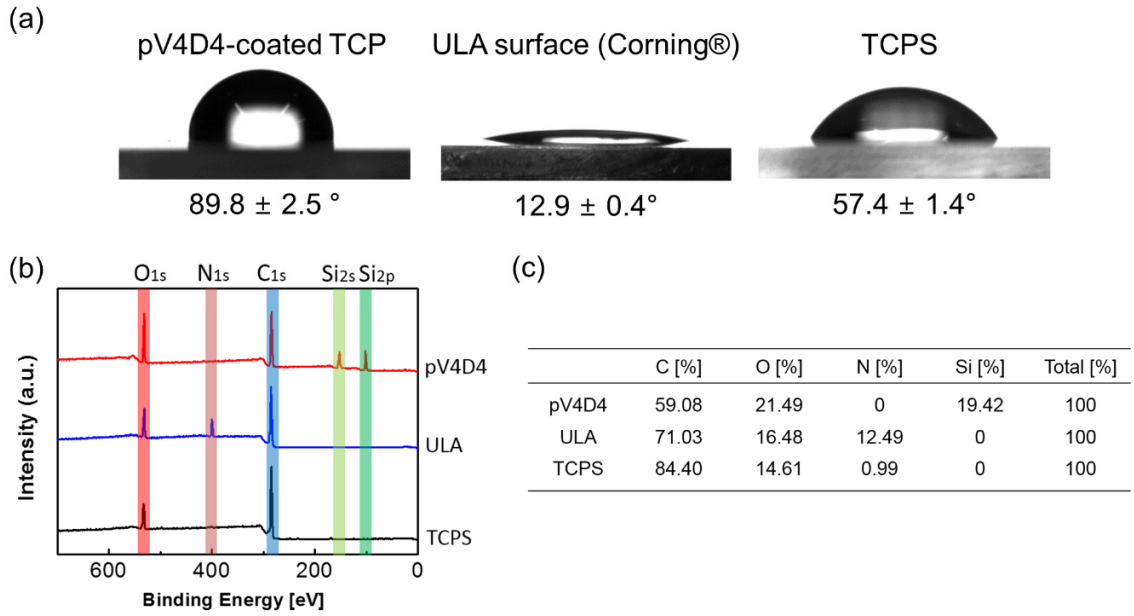



**Figure S5.** MCF7 cell aggregation on the different thickness of the pV4D4 coating surface. (a) Phase-contrast images of spheroids on 50, 150, 300 nm height of pV4D4 coating surface. Scale bars, 200 μm. (b) Formation of sharp lamellipodia between MCF7 spheroid and 50 nm pV4D4 coating surface. Scale bar, 100 μm. (c) Box plots of the equivalent diameter of spheroids on 50, 150, 300 nm height of pV4D4 coating surface at 48, 96 hr after seeding (***$p < 0.0005$).




| | C [%] | O [%] | N [%] | Si [%] | Total [%] |
|---|---|---|---|---|---|
| pV4D4 | 59.08 | 21.49 | 0 | 19.42 | 100 |
| ULA | 71.03 | 16.48 | 12.49 | 0 | 100 |
| TCPS | 84.40 | 14.61 | 0.99 | 0 | 100 |

**Figure S6.** Chemical properties of the pV4D4 surface. (a) Contact angle of water on pV4D4, ULA and TCPS surface. (b) XPS spectrum analysis for pV4D4, ULA, and TCPS surface. (c) Surface composition of the pV4D4, ULA and TCPS surface.

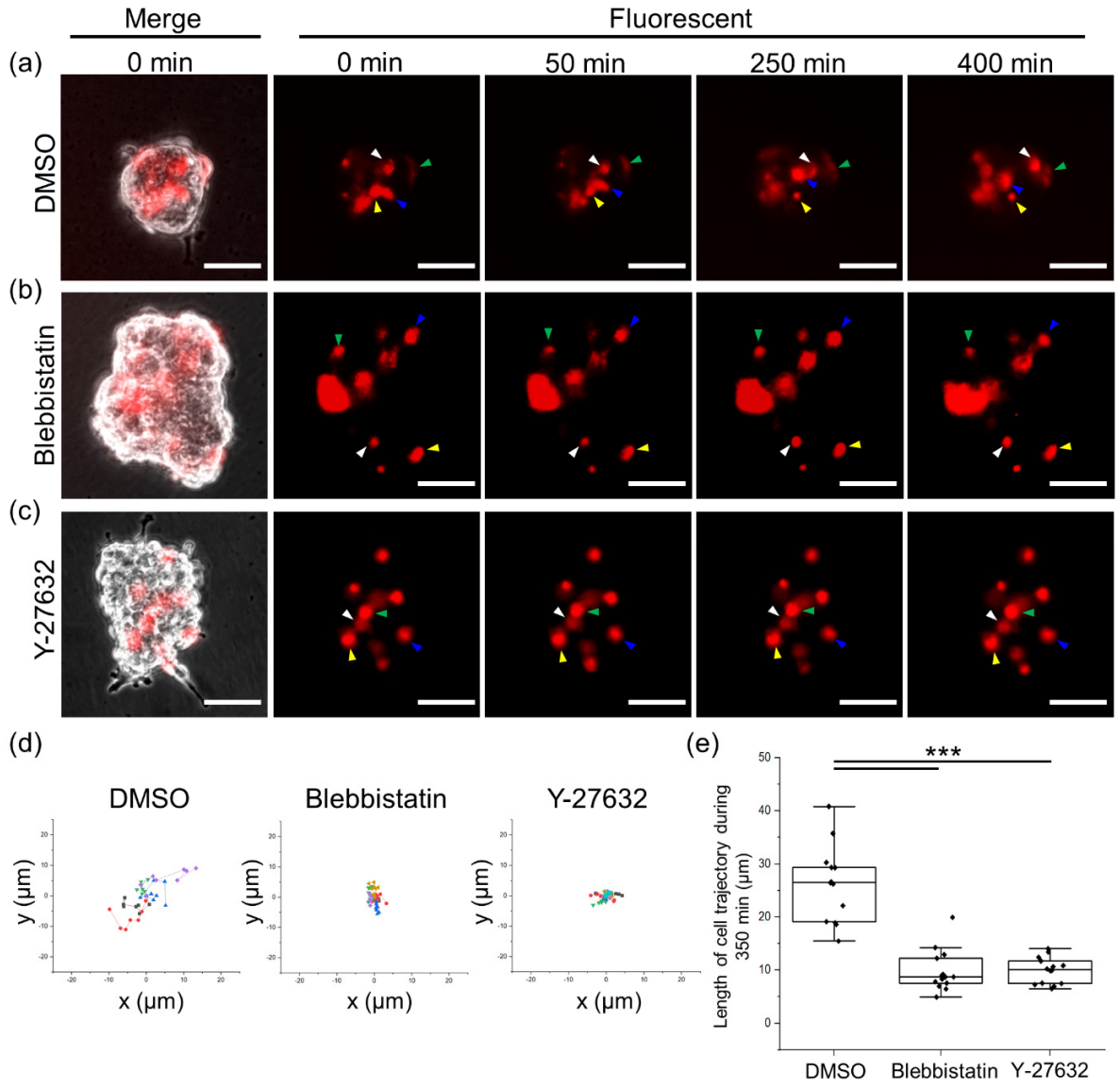



**Figure S7.** Time-dependent dynamics of constituent cells within DMSO control, blebbistatin, Y-27632 treated spheroid on the pV4D4 surface. Merge of phase and fluorescent images at 0 min and time-development of fluorescent images of the labeled constituent cells within the (a) DMSO, (b) blebbistatin, (c) Y-27632 treated spheroid. 0 min indicates the initiation of time-lapse imaging of the 1 day-cultured spheroid. The color of each arrow marks the same cell. Trajectory of constituent cells within the (d) DMSO, (e) blebbistatin, (f) Y-27632 treated spheroids for 350 min. (g) Box plots of the total length of constituent cell trajectories during 350 min within DMSO, blebbistatin, Y-27632 treated spheroids. **$p < 0.005$, ***$p < 0.0005$. Scale bars, 100 μm.

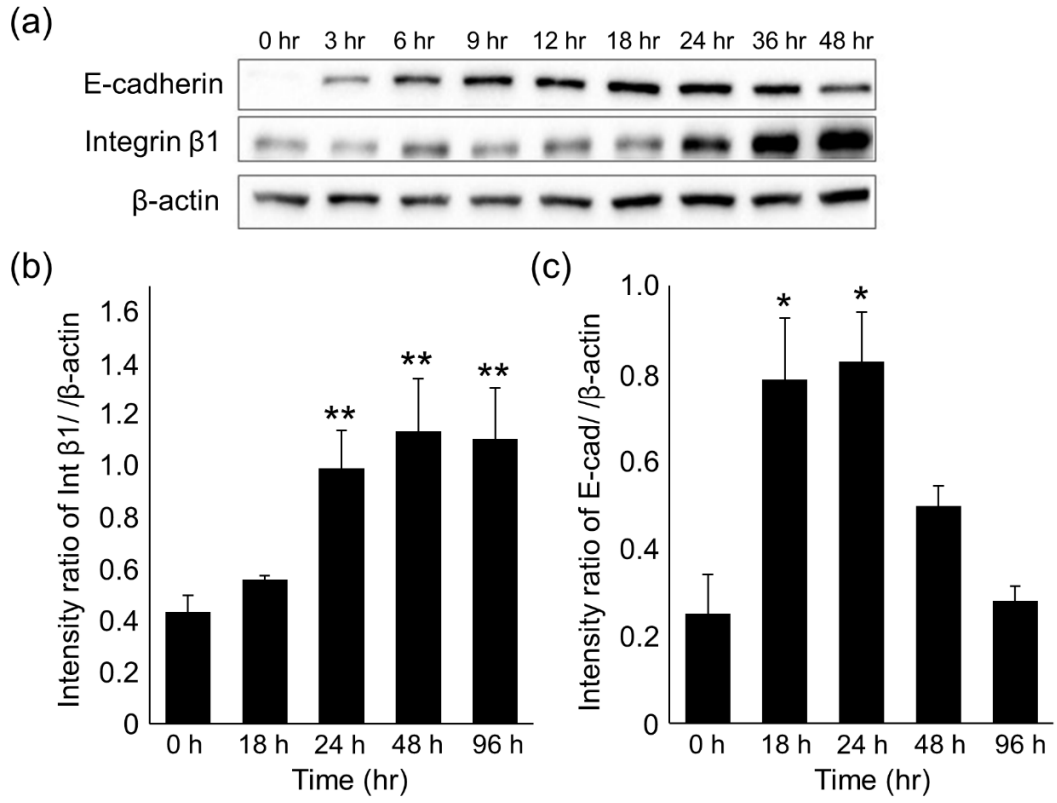



**Figure S8**. (a) Western assays during 3D spheroid formation on pV4D4 at early timepoints (0-48 hr). (b,c) To account for the difference in protein loading per each sample, intensity of each (b) integrin β1 and (c) E-cadherin band was normalized by the respective β-actin band intensity. (n=4, *$p<0.05$, **$p<0.01$)

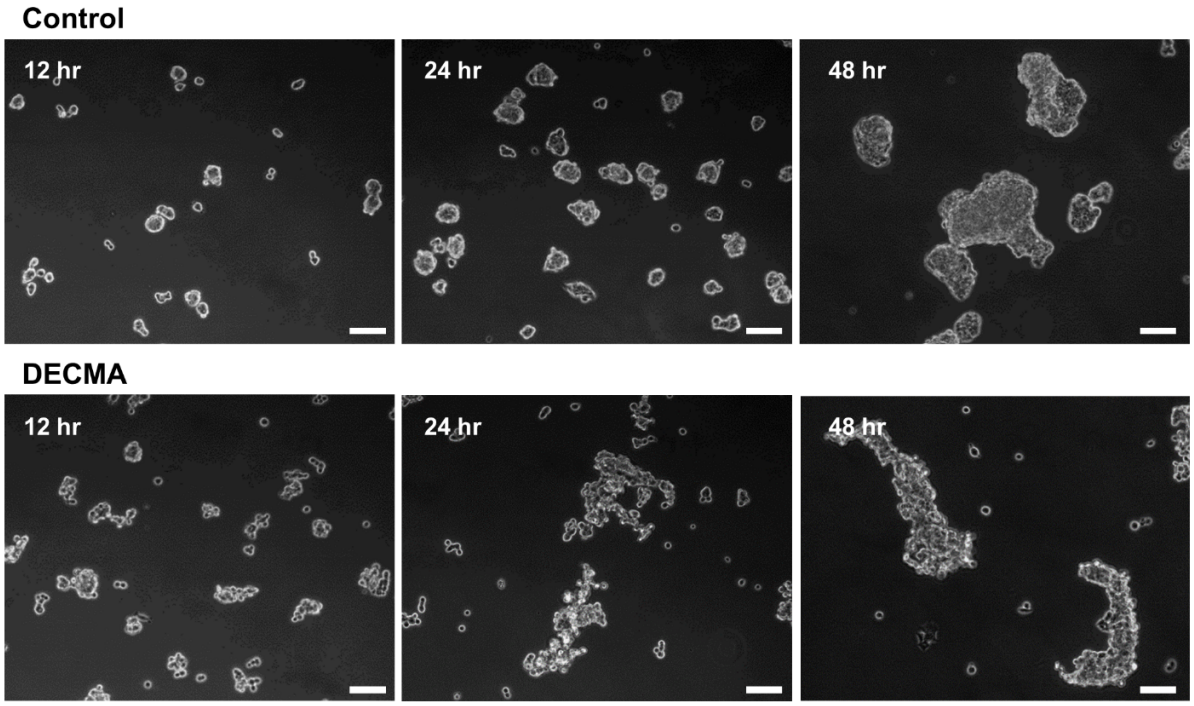



**Figure S9**. 3D aggregation of control (up) and E-cadherin antibody-treated (down) MCF7 cells at 12,24,48 hr. All scale bars represent 100 μm.

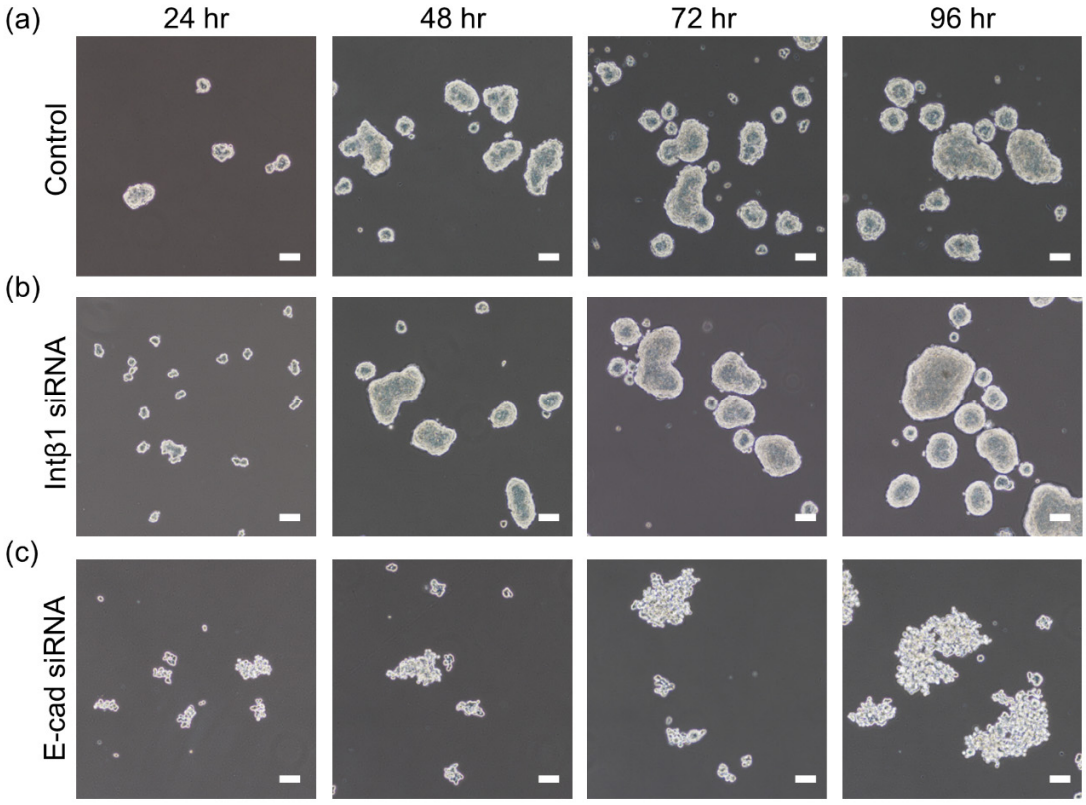



**Figure S10.** Phase images of the spheroid formation of (a) control, (b) integrin β1 siRNA, (c) E-cadherin siRNA cells on the ULA surface. Scale bars, 100 μm.

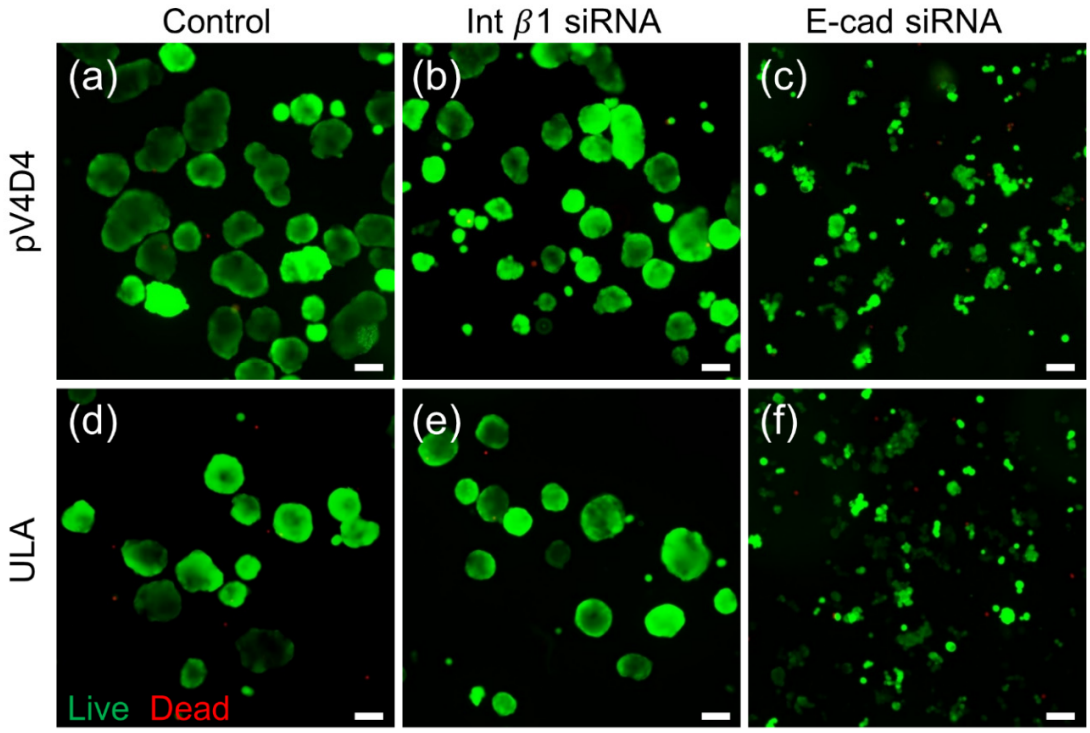



**Figure S11.** Live-dead assay of 4-day cultured control, integrin β1, E-cadherin downregulated cells on (a-c) pV4D4 and (d-f) ULA surfaces (Green: live, Red: dead). Scale bars, 100 μm

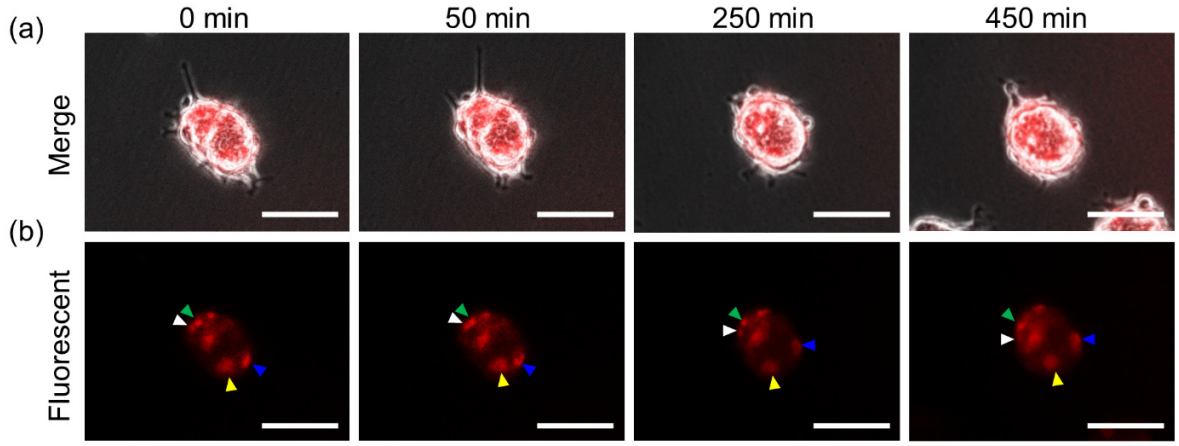



**Figure S12.** Time-dependent dynamics of integrin β1 siRNA spheroid on the pV4D4 surface. (a) Merge of phase and fluorescent images, (b) fluorescent images of the labeled constituent cells within the integrin β1 siRNA spheroid. Non-labeled cells and fluorescent-labeled cells were simultaneously seeded on the pV4D4 surface at the ratio of 10:1 and cultured for 1 day. 0 min indicates the initiation of time-lapse imaging of the 1 day-cultured spheroid. Same color arrowheads mark the same cells in each time sequence. Scale bars, 100 μm.

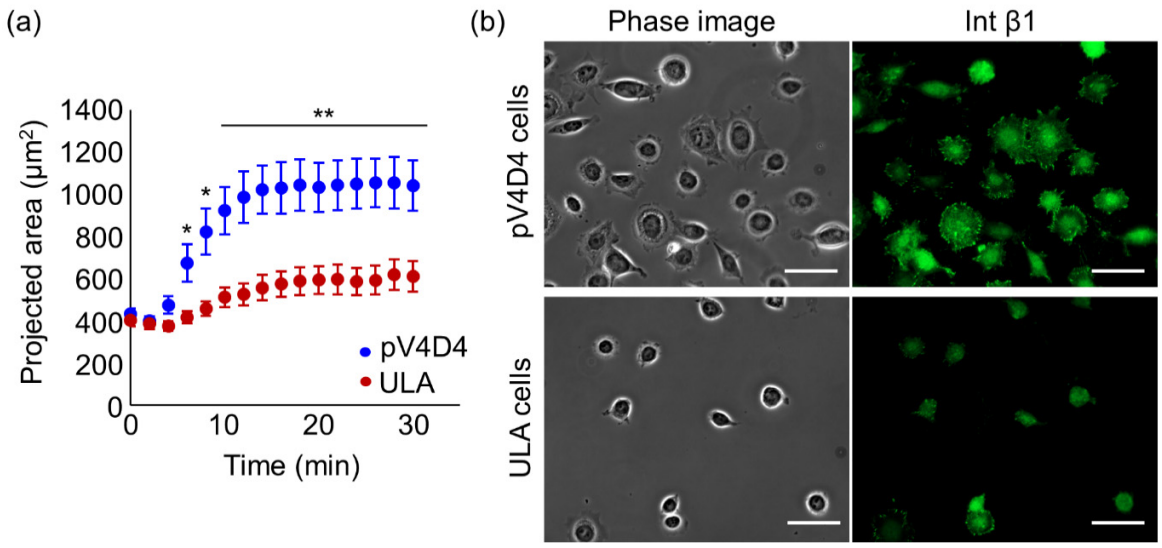



**Figure S13.** Initial attachment of dissociated single cells on the collagen-coated 2D surface. (a) Quantification of the projected area of single cells for 30 min after seeding. Data are presented as mean ± SEM (n = 17 for pV4D4, n = 18 for ULA). (*p < 0.05 versus ULA, **p < 0.005 versus ULA). (b) Phase and immunofluorescent images of integrin β1 (green) of single cells at 2 hr after seeding. Scale bars, 50 μm.

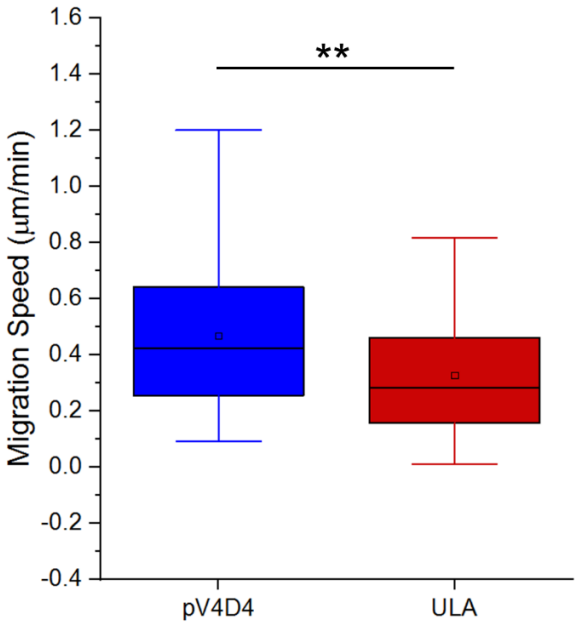



**Figure S14.** Box plot of cell migration speed of single cells dissociated from pV4D4 and ULA spheroids. Black lines indicate median and black squares indicate mean values. (n = 62 for pV4D4, n = 63 for ULA) (**p < 0.005 versus ULA).

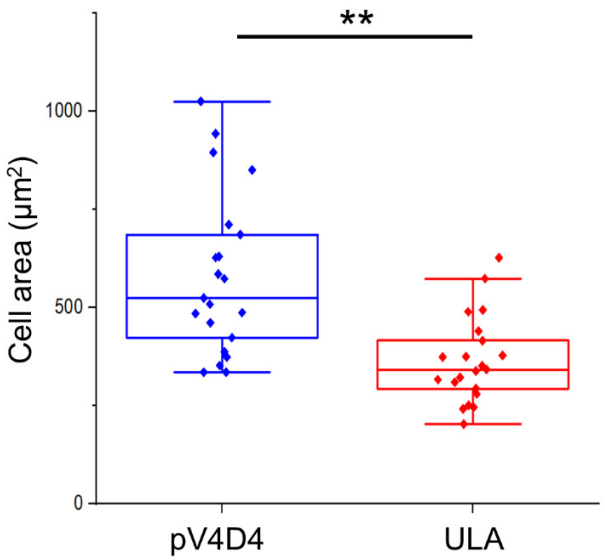



**Figure S15.** Area of cells at the leading edge of 12 hr spread pV4D4 and ULA spheroids (**p < 0.005).

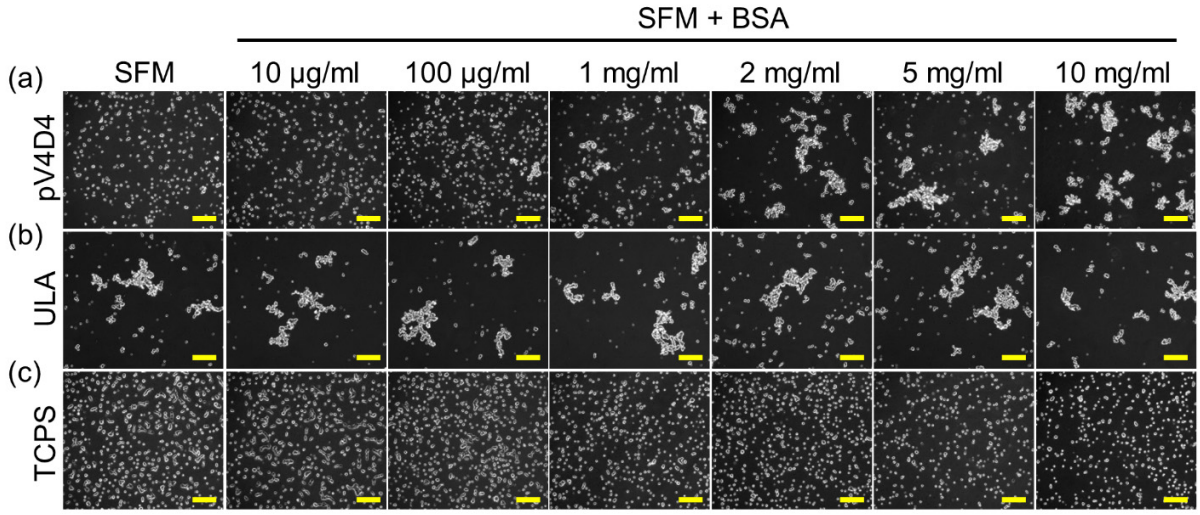



**Figure S16.** Effect of BSA concentration in serum free medium (SFM) on formation of MCF7 cell aggregates. Phase contrast images for MCF7 cell aggregates after culturing 24 hr in various BSA conditions on (a) pV4D4, (b) ULA, and (c) TCPS surfaces. Scale bars, 200 μm.

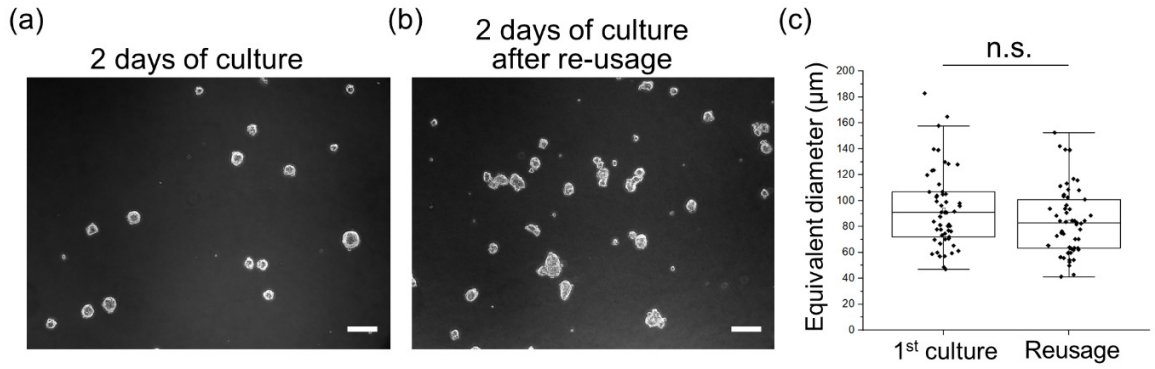



**Figure S17.** MCF7 cells cultured for 2days on (a) fresh pV4D4 surface and (b) re-used pV4D4-coated plates. For the re-usage, the pV4D4 surface was rinsed with PBS 3 times, and MCF7 cells were re-seeded. Scale bars, 200 μm. (c) Box plots for the equivalent diameter of spheroids cultured on fresh pV4D4 surface and re-used pV4D4 surface.

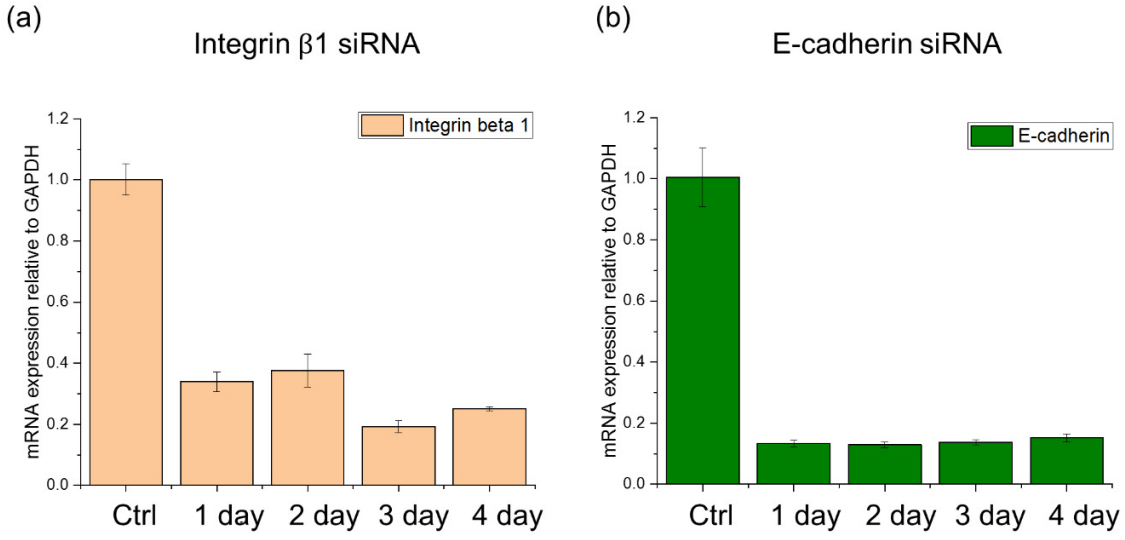



**Figure S18.** qPCR data for (a) integrin β1 siRNA and (b) E-cadherin siRNA cells.

# Supplementary movies

**Supplementary movie 1.** Time-lapse movie of 3D cell aggregation on pV4D4 surface.

**Supplementary movie 2.** Time-lapse movie of 3D cell aggregation on ULA surface.

**Supplementary movie 3.** Stretching and relaxation behavior of MCF7 spheroids on pV4D4 surface.

**Supplementary movie 4.** Behavior of MCF7 spheroids treated with Y-27632 on pV4D4 surface.

**Supplementary movie 5.** Time-lapse movie of 2D spreading experiment of spheroids cultured for 4 days on pV4D4 or ULA surfaces.

**Supplementary movie 6.** Time-lapse movie of 3D invasion experiment of spheroids cultured for 4 days on pV4D4 or ULA surfaces.