Supplementary information

ROCK inhibitor combined with Ca^{2+} controls the myosin II activation and optimizes human nasal epithelial cell sheets

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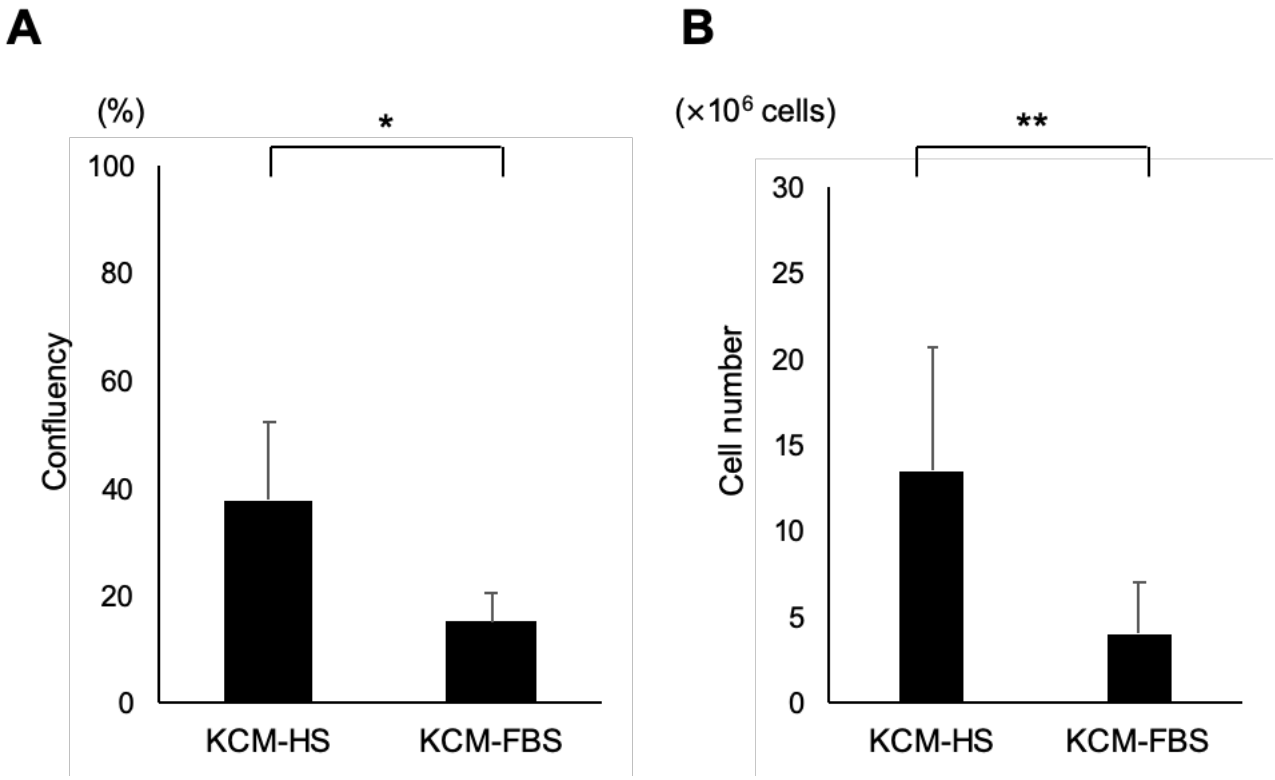

Figure S1. Cell expansion after explant culture. (A) Cell confluence in a 60-mm dish for cells cultured using normal KCM containing 5% human serum (HS; $n = 5$) or KCM containing 10% FBS (FBS; same as normal KCM $n = 4$). (B) Cell number counted from 8 dishes of cells cultured using KCM containing 5% human serum (HS) or normal KCM containing 10% FBS (FBS).

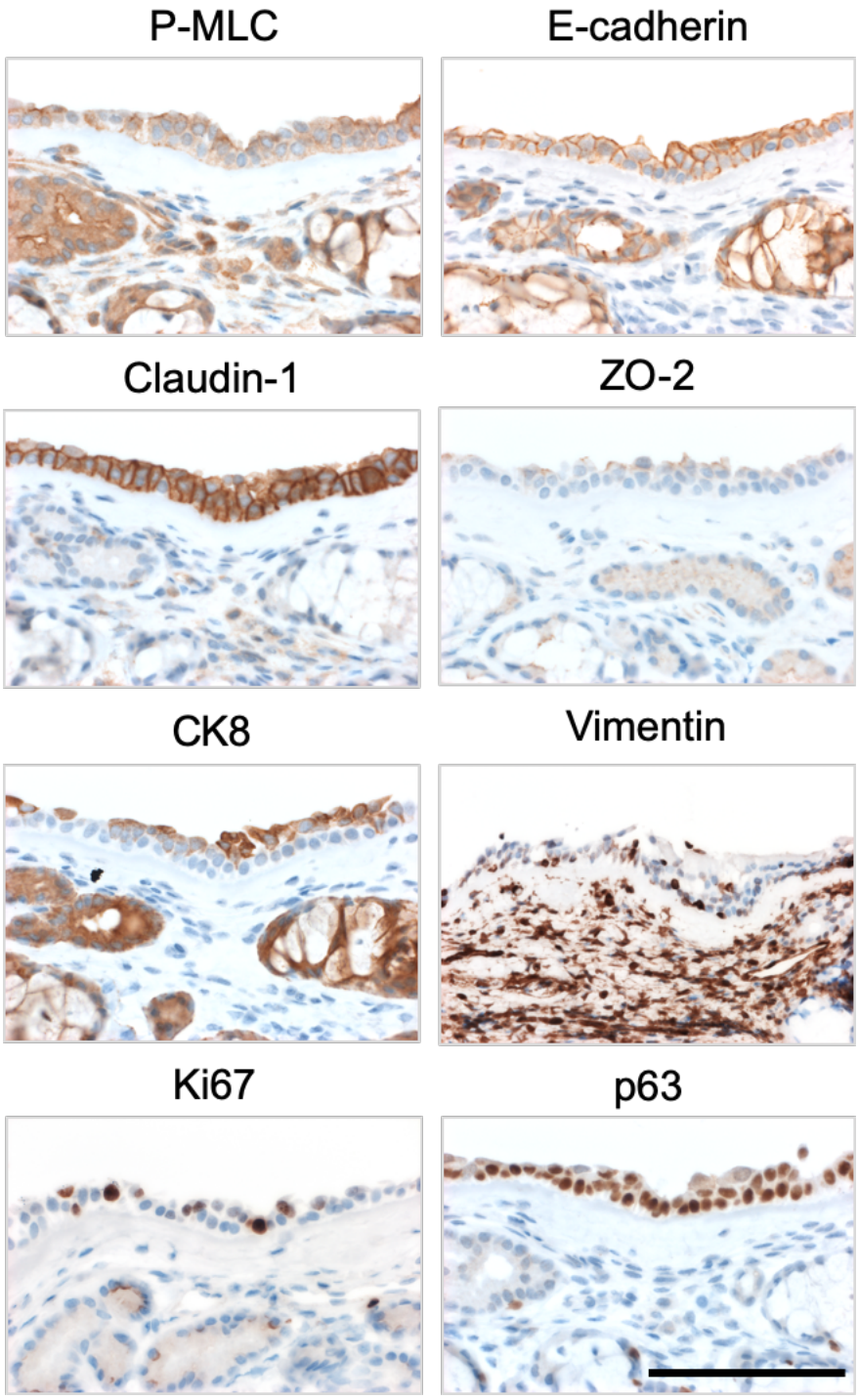

Figure S2. Immunohistological evaluation of human nasal mucosal tissue. Scale bar = 100 μm.

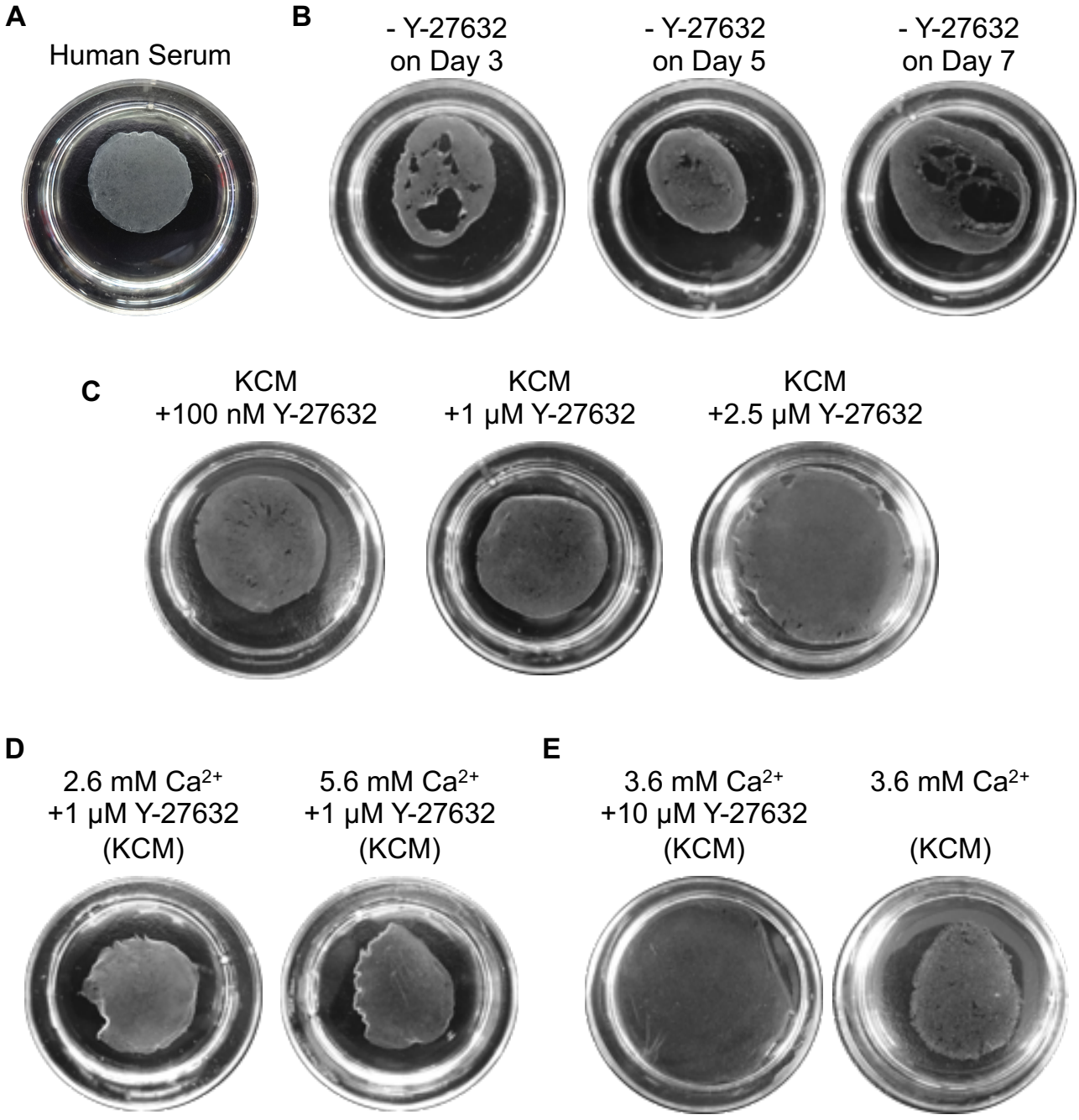

Figure S3. Representative images showing cell sheets cultured under various conditions. (A) Conventional method using autologous human serum. (B) Changing medium method that from KCM containing 1.6 mM Ca^{2+} and 10 μM Y-27632 to KCM containing 1.6 mM Ca^{2+} on day 0, 3, or 5. (C) Cell sheet cultured by different concentration of Y-27632. (D) Cell sheet cultured by KCM with 1 μM Y-27632 and different concentration of Ca^{2+}. (E) Cell sheet cultured by KCM containing 3.6 mM Ca^{2+} + 10 μM Y-27632. (F) Cell sheet cultured by KCM containing 3.6 mM Ca^{2+}.

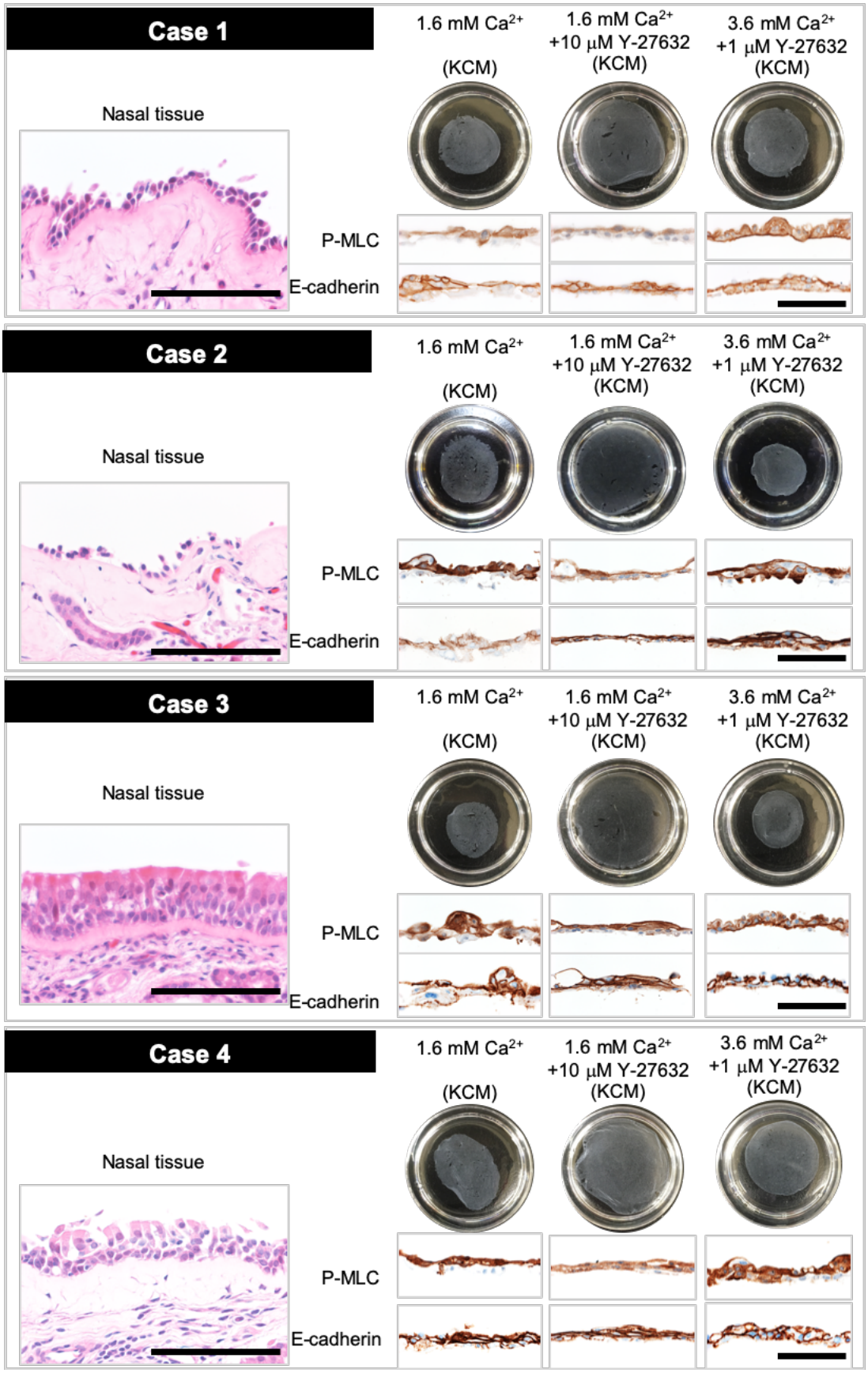

Figure S4. Reproducibility for shrinkage behavior and P-MLC and E-cadherin expression of a cell sheet independent on original tissue condition. Scale bar = 100 μm.

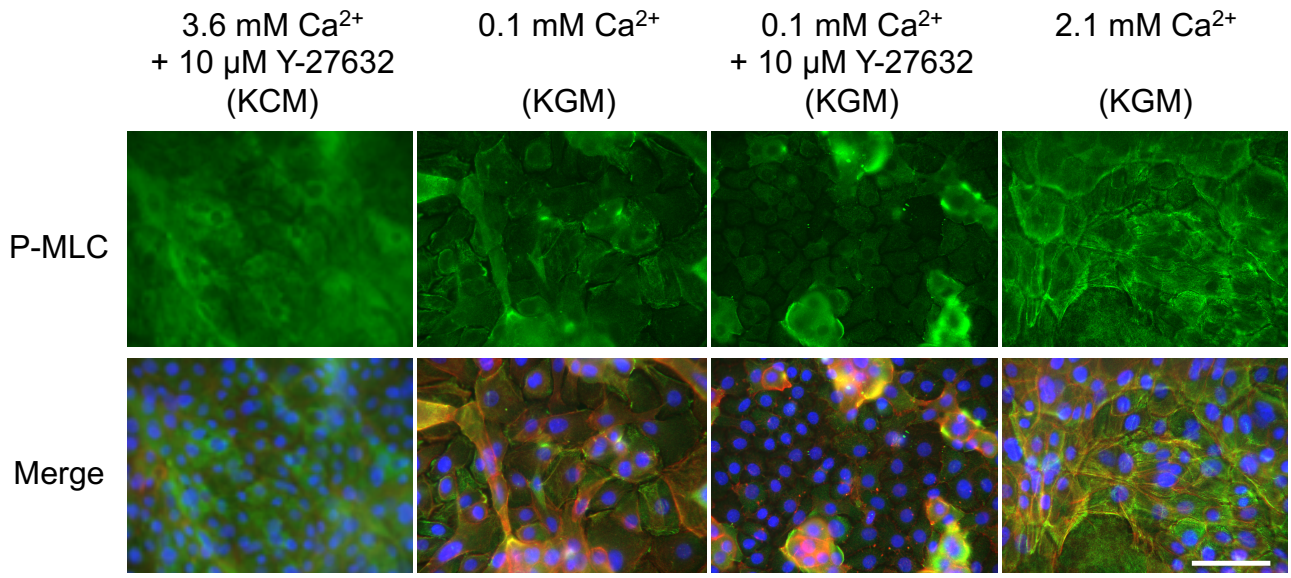

Figure S5. Immunofluorescence analysis of P-MLC expression. P-MLC (green), phalloidin (red), and DAPI (blue). Scale bar = 100 μm.

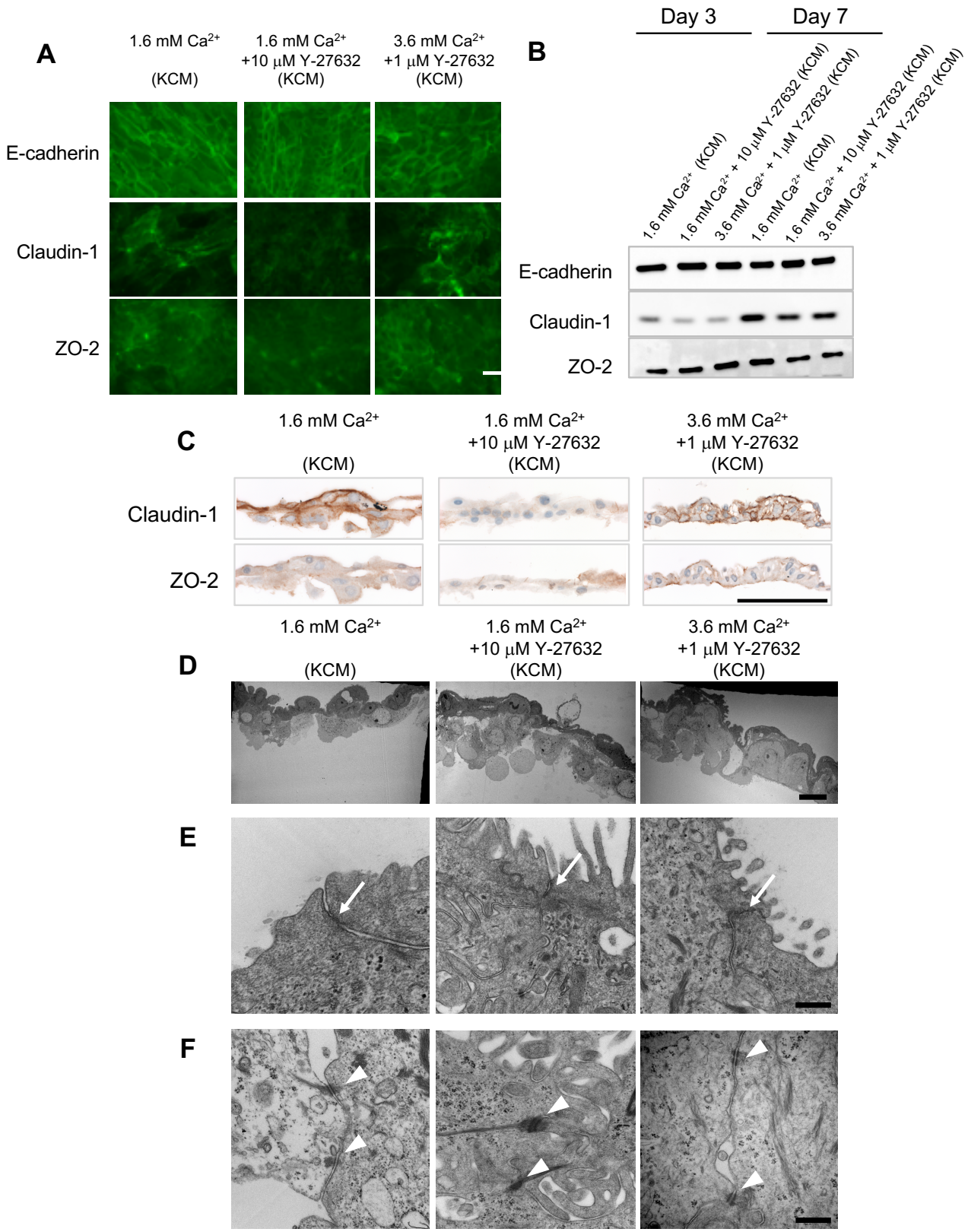

Figure S6. Analysis of cell-cell adhesion. (A) Immunofluorescence images showing the expressions of E-cadherin, claudin-1 and ZO-2 at day 7 of cell sheet culture. (B) Western blot analysis of the expressions of E-cadherin, claudin-1 and ZO-2 at day 3 and day 7 of cell sheet culture. The samples were the same as those used to obtain the data in Fig. 4C. (C) Immunohistological evaluation of the harvested cell sheet cultured in KCM containing 1.6 mM Ca^{2+}, KCM containing 1.6 mM Ca^{2+} + 10 μM Y-27632, or KCM containing 3.6 mM Ca^{2+} + 1 μM Y-27632. (D) TEM images of cell sheets

cultured cultured in KCM containing 1.6 mM Ca^{2+}, KCM containing 1.6 mM Ca^{2+} + 10 μM Y-27632, or KCM containing 3.6 mM Ca^{2+} + 1 μM Y-27632. (E) TEM images of the upper layer of each cell sheet. White arrows indicate tight junctions. (F) TEM images of the basal layer of each cell sheet. White arrowheads show adherens junctions. Scale bar = 100 μm (A, C), 20 μm (D), 500 nM (E, F).

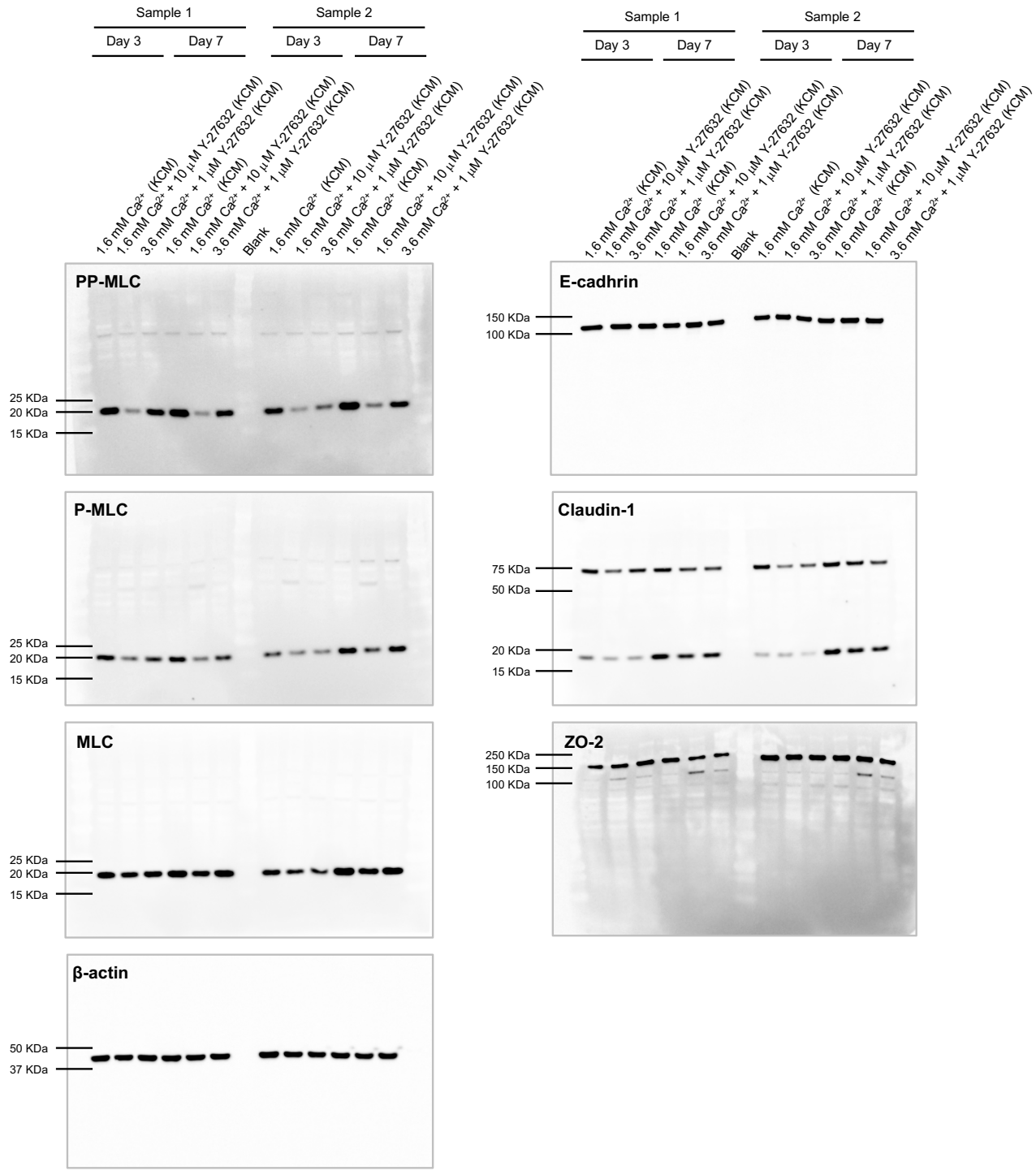

Figure S7. Full-length photograph of western blot shown in Figure 3D and Figure S4 (n = 2).

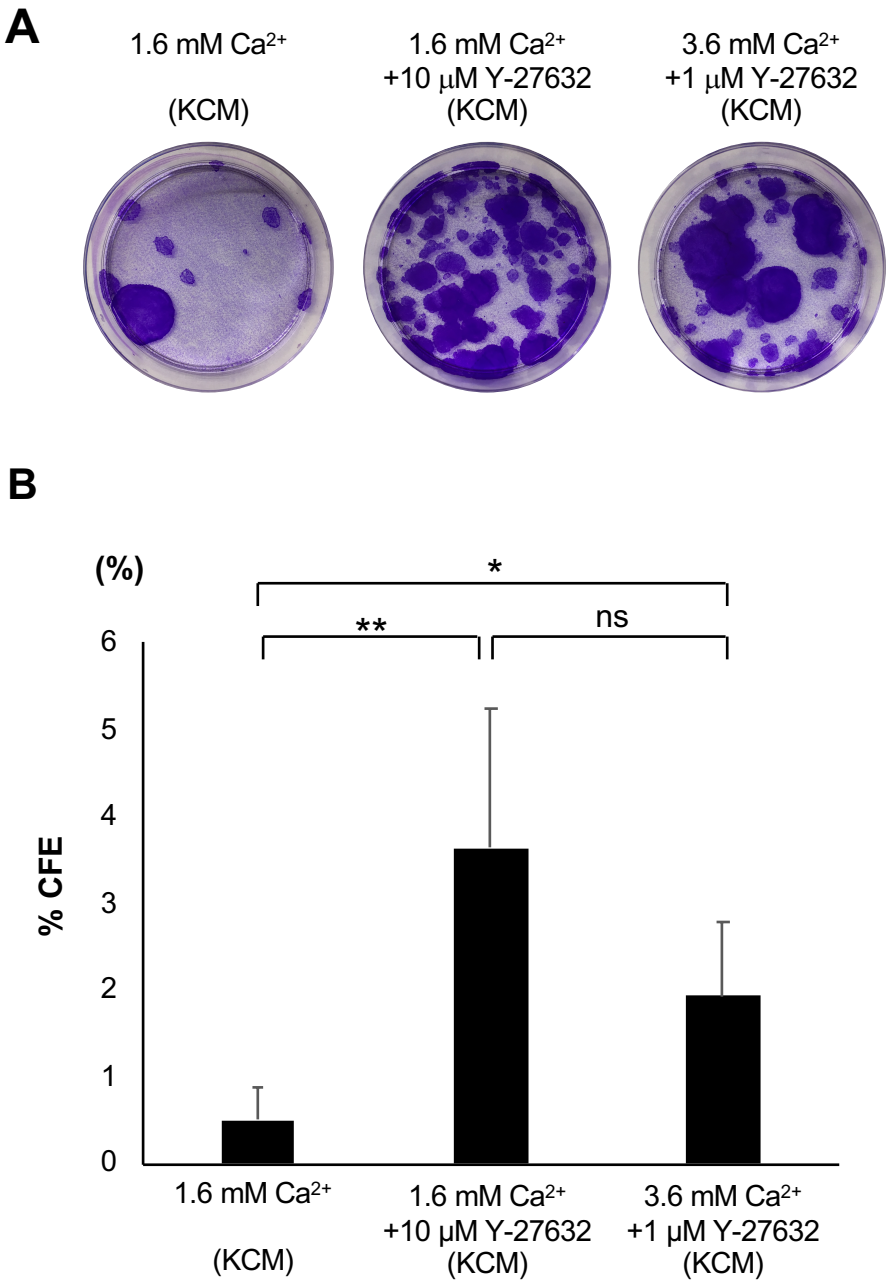

Figure S8. Evaluation of the colony forming efficacy (CFE) of the cell sheet cultured in KCM containing 1.6 mM Ca^{2+}, KCM containing 1.6 mM Ca^{2+} + 10 μM Y-27632, or KCM containing 3.6 mM Ca^{2+} + 1 μM Y-27632. (A) Representative images showing colony-forming assays for cell sheets. (B) Colony forming efficiency. Values are expressed as the mean ± SEM (n = 6). *P < 0.05, **P < 0.01; ns, not significant.

Table S1: Antibodies used in Immunofluorescence analysis

Antibodies	Cat.No.	Source	Dilution
Rabbit anti-human double-phospho-MLC polyclonal antibody (PP-MLC)	3674	Cell Signaling Technology, Danvers, MA, USA	1:100
Rabbit anti-human phospho-MLC polyclonal antibody (P-MLC)	3671	Cell Signaling Technology, Danvers, MA, USA	1:100
Rabbit anti-human MLC antibody (MLC)[D18E2]	8505	Cell Signaling Technology, Danvers, MA, USA	1:100
Rabbit anti-human claudin-1 monoclonal antibody [D5H1D]	13255	Cell Signaling Technology, Danvers, MA, USA	1:100
Rabbit anti-human zonula occludens-2 polyclonal antibody (ZO-2)	2847	Cell Signaling Technology, Danvers, MA, USA	1:100
Mouse anti-human E-cadherin monoclonal antibody [NCH-38]	M3612	Dako, Carpinteria, CA, USA	1:100
Alexa Fluor Plus 488-conjugated secondary antibody: goat anti-rabbit IgG antibody	A32731	Thermo Fisher Scientific, Waltham, MA, USA	1:1000
Alexa Fluor Plus 488-conjugated secondary antibody: goat anti-mouse IgG antibody	A32723	Thermo Fisher Scientific, Waltham, MA, USA	1:1000

Table S2: Antibodies used in Immunohistological analysis

Antibodies	Cat.No.	Source	Dilution
Rabbit anti-human phospho-MLC polyclonal antibody (P-MLC)	3671	Cell Signaling Technology, Danvers, MA, USA	1:100
Rabbit anti-human claudin-1 monoclonal antibody [D5H1D]	13255	Cell Signaling Technology, Danvers, MA, USA	1:100
Rabbit anti-human zonula occludens-2 polyclonal antibody (ZO-2)	2847	Cell Signaling Technology, Danvers, MA, USA	1:100
Mouse anti-human E-cadherin monoclonal antibody [NCH-38]	M3612	Dako, Carpinteria, CA, USA	1:100
Mouse anti-human Ki67 monoclonal antibody [MIB-1]	M7240	Dako, Carpinteria, CA, USA	1:100
Mouse anti-human p63 monoclonal antibody [4A4]	ab735	Abcam, Cambridge, UK	1:100
Mouse anti-human vimentin monoclonal antibody [V9]	MA5-11883	Thermo Fisher Scientific, Waltham, MA, USA	1:100
Mouse anti-human cytokeratin-8 monoclonal antibody [M20]	sc-52324	Santa Cruz Biotechnology, Dallas, CA, USA	1:100

Table S3: Antibodies used in western blot analysis

Antibodies	Cat.No.	Source	Molecular weight (kDa)	Dilution
Rabbit anti-human double-phospho-MLC polyclonal antibody (PP-MLC)	3674	Cell Signaling Technology, Danvers, MA, USA	18	1:1000
Rabbit anti-human phospho-MLC polyclonal antibody (P-MLC)	3671	Cell Signaling Technology, Danvers, MA, USA	18	1:1000
Rabbit anti-human MLC antibody (MLC)[D18E2]	8505	Cell Signaling Technology, Danvers, MA, USA	18	1:1000
Rabbit anti-human claudin-1 monoclonal antibody [D5H1D]	13255	Cell Signaling Technology, Danvers, MA, USA	20	1:1000
Rabbit anti-human zonula occludens-2 polyclonal antibody (ZO-2)	2847	Cell Signaling Technology, Danvers, MA, USA	150	1:1000
Rabbit anti-human β-actin monoclonal antibody	4970	Cell Signaling Technology, Danvers, MA, USA	45	1:1000
Mouse anti-human E-cadherin monoclonal antibody [NCH-38]	M3612	Dako, Carpinteria, CA, USA	120	1:1000
HRP-conjugated goat anti-rabbit IgG antibody	7074	Cell Signaling Technology, Danvers, MA, USA	-	1:10000
HRP-conjugated goat anti-mouse IgG antibody	7076	Cell Signaling Technology, Danvers, MA, USA	-	1:10000

Movie 1. Video image showing time-lapse differential interference contrast microscopy of the leading-edge cells of the explant culture in normal KCM. Partial backward movements were observed in the lower-left region of the migrating edge from 2 h to 4 h.

Movie 2. Video image showing time-lapse differential interference contrast microscopy of the leading-edge cells of the explant culture in KCM-Ri.