Supplementary Materials for

Extracellular vesicle formation mediated by local phosphatidylserine exposure promotes efficient cell extrusion

Akihito Kira[1], Machiko Murata[1†], Keisuke Saito[1†], Ichiko Tatsutomi[1†], Izumi Hattori[1], Haruna Kajita[1], Naoko Muraki[1], Yukako Oda[2], Saya Satoh[3], Yuta Tsukamoto[3], Seisuke Kimura[4,5], Hiroki Kato[3], Kohki Kawane[1*]

Correspondence to: kawane@cc.kyoto-su.ac.jp

This PDF file includes:

Other Supplementary Materials for this manuscript include the following:

Materials and Methods

Plasmid

pCAG-mGFP (palmitoylated EGFP) and pEGFP-actin were purchased from Addgene (14757) and Clontech, respectively. pCAG-MFG-E8 (D89E):GFP and pCAG-Lifeact:mRuby were constructed. Briefly, the MFG-E8 coding sequence amplified from pEF1-MFG-E8 (D89E) (gifted by Dr. S. Nagata [1]) with the primers 5-ATGCAGGTCTCCCGTGTGCTG-3′ and 5-ACAGCCCAGCAGCTCCAGGC-3′ was subcloned into EcoRI sites in pCAG-mLSR:cGFP (gifted by Dr. M. Furuse [2]). The Lifeact:mRuby coding sequence amplified from Lifeact:mRuby (Addgene, 54674) with the primers 5-GCGGCCGCGCTAGCGCCACCATGGGCGT-3′ and 5-GGTACCTTACTTGTACAGCTGCTCCA-3′ was subcloned into the NheI and KpnI sites in pCAG-mRFP.

Cell culture

MDCKI, MDCKII, and EpH4 cells (gifted from Dr. E. Reichmann) were grown in Dulbecco's modified Eagle's medium (D-MEM) (High Glucose) with L-Glutamine, Phenol Red and Sodium Pyruvate (Wako, 043-30085) including antibiotics (Penicillin-Streptomycin Mixed Solution, Nakarai Tesque, 26253-84) and Fetal Bovine Serum (FBS, HyClone) (5% for MDCKII and 10% for MDCKI and EpH4 cells) (hereinafter referred to as standard culture medium). For the transient gene knockdown experiments, siRNA for each specific gene (Santa Cruz, sc-154404 for *Ano6/TMEM16F*; sc-141186 for *Arf1*; sc-43620 for *Arf6*; sc-41629 for *PC-Pld1*; sc-155377 for *Xkr8*; and sc-37007 for the control) were transfected using Lipofectamine™ RNAiMAX Transfection Reagent (Thermo Fisher) into EpH4 cells, together with BLOCK-iT™ Alexa Fluor™ Red Fluorescent Control (Thermo Fisher, 14750100) to monitor siRNA-

incorporated cells. Briefly, the transfection reagent and siRNA prepared following the manufacturer's instructions were added to a tissue culture-treated glass-bottom dish (CELLVIEW™ CELL CULTURE DISH, Greiner Bio-One, 627870), and then cells were seeded onto them. Two days after transfection, time-lapse imaging with a laser confocal microscope (TSC SP8, Leica) was performed.

For the transient expression of MFG-E8 (D89E), MDCKII and EpH4 cells with 30–60% confluence on a tissue culture-treated glass-bottom dish were transfected with pCAG-MFG-E8 (D89E) using Lipofectamine™ LTX reagent (Thermo Fisher). Briefly, the standard culture medium was replaced with DMEM, high glucose, no glutamine, no calcium (Thermo Fisher, 21068028) without FBS 30 min before transfection. Transfection was performed according to the manufacturer's instructions, except for the use of DMEM, high glucose, no glutamine, and no calcium also in the step of the plasmid-Lipofectamine complex formation. The medium was changed to the standard culture medium 5 h after transfection. Two days post-transfection, imaging experiments were performed.

To establish a stable transformant expressing membrane-bound GFP, RFP, Actin:GFP, or Lifeact:mRuby, transfection was similarly performed as abovementioned in a 6 cm diameter cell culture dish (VIOLAMO) and 2 d after, the standard culture medium was replaced with the one containing 400 μg/mL G418 sulfate (Wako) or 200 μg/mL HygromycinB (Wako) and cells were cultivated for 10–14 days. Surviving colonies were selected and diluted to obtain single cell-derived clones.

For UV-induced cell extrusion, MDCKII and EpH4 cells were grown to a confluent state on a tissue culture-treated glass-bottom dish. The standard culture medium was replaced with a minimum volume of PBS, and the cells were irradiated using a UV Stratalinker 2400

(Stratagene), followed by time-lapse imaging analysis after further incubation in the standard culture medium for the times indicated in the figure legends.

For mosaic analysis, palmitoylated GFP-expressing MDCKII cells and RFP-expressing MDCKI cells were seeded at a 1:4 ratio 2 d before time-lapse imaging. Mouse intestinal organoids were prepared from female C57BL/6N mice (SLC) and maintained as described in [3].

Drosophila genetics and maintenance

Flies were grown at 25 °C and fed with standard fly food. Females were isolated from males 1–3 d after eclosion and used for analysis. For experiments using the GeneSwitch system [4] flies were transferred to a vial with food containing 73 μg/mL Mifepristone (Sigma Aldrich, M8046) 5 d after eclosion. Adult flies were transferred to new vials containing Mifepristone every 2 days. Myo1A-Gal4 (112-001) and Esg-GFP (114-268) were obtained from Kyoto Stock Center (DGRC). UAS-*Arf51F* RNAi (51417), UAS-*Arf79F* RNAi (66174), UAS-*Xk* (CG32579) RNAi (57784), RNAi isogenic control (36303, 36304), UAS-Arf51F:GFP (65867), Eip71CD-Gal4 (6871), hs-FLPG5 (55817), act < cd2 < Gal4, UAS-RFP (30558), and UAS-mCD8:mRFP (27398) were obtained from Bloomington Drosophila Stock Center (BDSC). UAS-*Xk* (CG32579) RNAi (110645), RNAi isogenic control (60100), and UAS-Dicer2 (60007) were obtained from Vienna Drosophila Resource Center (VDRC). Lgl:GFP was provided by Dr. Y. Hong; Pswitch CSG5966, Dr. E. Piddini; Tsh-Gal4, Dr. T. Tabata; DE-Cad:GFP, Dr. Y. Oda; Sqh:mKate2, Dr. Y. Bellaïche; UAS-AnnexinV:GFP and UAS-AnnexinV (mutant):GFP, Dr. C. Han; act < y+ < LexA, LexAop-CD8:GFP, Dr. L. LeGoff. For clonal analysis, 13–19 d after eclosion (grown at 18 °C), flies were heated at 37 °C for 15 min. After 7 d (grown at 25 °C),

their midguts were dissected and observed using laser confocal microscope. The genotypes of the *Drosophila* lines used in each experiment are listed in Table S2.

Time-lapse imaging

Cells were seeded on a tissue culture-treated glass-bottom dish (CELLVIEW™ CELL CULTURE DISH, Greiner Bio-One, 627870). After 2 or 3 d (1 d after the cells reached a confluent state), time-lapse images were acquired. The standard culture medium was replaced with FluoroBrite™ DMEM (Thermo Fisher, A1896701) containing 5% or 10% FBS, antibiotics, and GlutaMax™ Supplement (Thermo Fisher, 35050061) 1 h before imaging. For the overexpression of MFG-E8 (D89E), the medium change was done 6 h before imaging to accumulate the secreted MFG-E8 (D89E) protein in the medium. Time-lapse images were acquired using a laser confocal microscope (TSC SP8, Leica) equipped with a 20× or 63× oil-immersion objective, a time-lapse module, a multi-position module, and a hybrid detector (Leica) in a chamber (Tokai Hit) containing 5% CO_2 and at 37 °C. Las X software (Leica) was used for analysis of confocal images and movie file preparation. All images and movies used in the report were prepared from confocal images at a single z-position unless otherwise stated.

RNA-seq analysis

Total RNA was isolated from EpH4 cells or adult *Drosophila* midguts using an RNAeasy micro kit (Qiagen) with DNaseI treatment according to the manufacturer's instructions. For the *Drosophila* experiment, the posterior part of the midguts of 15 adult flies were homogenized using a syringe with a 26G needle (Terumo) in the RLT solution provided with the kit. After RNA integrity was confirmed using an Agilent RNA 6000 Nano Chip (Agilent Technologies),

total RNA samples were used for RNA-seq library preparation using an Illumina TruSeq Stranded mRNA LT sample kit according to the manufacturer's instructions (Illumina). The pooled libraries were sequenced on an Illumina NextSeq500 sequencing platform, and approximately 20 million raw reads for each sample were obtained by single-end sequencing of 76 bp length. The obtained reads were mapped to the reference genome (GRCm38 for EpH4 cells and BDGP assembly release 6 for adult *Drosophila* midgut) using TopHat2 [5] and the FPKM (fragments per kilobase of exon per million reads mapped reads) values were defined using cuffdiff [6].

Analysis of *Drosophila* pupal epidermis

Pupae were collected 16–18 h after pupal formation and placed on double-sided tapes attached to glass slides. The pupal case was removed, and Halocarbon oil 700 (Sigma Aldrich, H8898) was added to the dorsal abdomen, which was then covered with cover glass. Time-lapse imaging was performed using laser confocal microscope with multi-positioning and adaptive focus control modes.

Quantitative and statistical analysis

Quantification was performed using the basic measurement tool in Fiji/ImageJ. To quantify cell protrusion from the cell layer, sequential confocal images were analyzed, and the length between the apex of extruding cells and the surface of the neighboring cell layer was calculated. To quantify the width of LEC region, maximum projection images of z-stacks of up to 200 μm were analyzed. To quantify the progress of extrusion of LECs, the length from the apical surface of cell layer to a section in which the width of the extruding cell is at the maximum was calculated.

Two-tailed Welch's *t*-tests were used to determine *P*-values. *P*-values < 0.05 were considered significant. Survival curves were analyzed using the log-rank test with BellCurve for Excel (Social Survey Research Information).

Immunostaining and other staining methods

Drosophila midguts were dissected in PBS, fixed with 4% paraformaldehyde in phosphate buffer (Wako) for 1 h at 25 °C, and post-fixed with methanol for 30 min at −30 °C. After permeabilization with 0.1% (v/v) Triton X-100 in PBS and blocking with Blocking One (Nakarai Tesque, 03953-66), the midguts were incubated with mouse anti-discs large monoclonal antibody (4F3) (1:100, Developmental Studies Hybridoma Bank) and subsequently with Alexa Fluor 546 conjugated goat anti-mouse IgG (1:1000, Life Technology, A11003). Counterstaining was performed with 1 μg/mL of DAPI (Wako, 340-07971) and Phalloidin-TRITC (1:2000 Sigma Aldrich, P1951).

Cultured cells were seeded in a chamber slide (Matsunami) 2 or 3 d before the staining experiments. Cells were fixed with 1% paraformaldehyde in PBS for 20 min at 25 °C. After permeabilization with 0.1% (v/v) Triton X-100 in PBS and blocking with Blocking One (Nakarai Tesque), samples were incubated with rabbit anti-cleaved caspase3 monoclonal antibody (Asp175) (1:300, Cell Signaling Technology, 9661) and then Goat anti-Rabbit IgG (H+L) Cross-Absorbed Secondary Antibody, Alexa Fluor 568 (1:1200, Life Technology, A11011). Counterstaining with 0.5 μg/mL of DAPI was performed.

For Annexin V staining of cultured cells, Annexin V Alexa Fluor™ 555-conjugate (1:20, Molecular Probes, A35108) was used for staining with 1 μg/mL of Hoechst33342 (Wako, 346-07951) according to the manufacturer's instruction. Before staining, the cell layer was scratched

at some points with a microtip to allow Annexin V to access the basolateral parts of cells. Images were captured within 30 min after the completion of staining.

To visualize the cell membrane, 2.5 μg/mL of CellMask™ Deep Red Plasma membrane Stain (Thermo Fisher, C10046) was added 1 hour before the acquisition of time-lapse images. For lysosome staining, 150 nM of LysoTracker™ Red DND-99 (Thermo Fisher, L7528) was added 1 h before the acquisition of time-lapse images.

Western blotting

For the preparation of cell lysates, scraped culture cells in a 6 cm diameter cell culture dish (VIOLAMO) were incubated in 20 mM Tris-HCl (pH 7.5) supplemented with 150 mM NaCl, 1 mM EDTA, and 1% (v/v) Triton-X100 containing a Protease Inhibitor Cocktail Set III DMSO Solution (EDTA free) (1:100, Wako, 163-26061) for 1 h at 4 °C. The cell lysates were then mixed with 2 × SDS loading buffer (10% glycerol, 4% SDS, and 0.004% bromophenol blue in 0.25 M Tris-HCl (pH 6.8) containing 2% 2-mercaptoethanol and incubated for 1 h at 25 °C. Proteins (10 μg) were separated by SDS–PAGE after the determination of protein concentration and transferred onto Immobilon-P PVDF membranes (Millipore). Signals were detected using an ECL chemiluminescence system (Western BLoT Quant HRP Substrate, TAKARA, T7102A) and Fuji LAS-4000 mini system (Fujifilm). For reblotting, Western BLoT Stripping Buffer was used (TAKARA, T7135A). The primary antibodies used were the following: mouse Anti-DYKDDDDK tag, Monoclonal Antibody (1:1000, Wako, 014-22383), mouse anti-Arf6 (3A-1) (1:200, Santa Cruz, sc-7971), and rabbit anti-Glyceraldehyde-3-Phosphate Dehydrogenase Polyclonal (1:1000, Trevigen, 2275-PC-100). The secondary antibodies used were Peroxidase-

AffiniPure Donkey Anti-Rabbit IgG (H+L) (1:20000, Jackson ImmunoResearch, 711-035-152) and Goat Anti-Mouse IgG (H+L)-HRP Conjugate (1:1000, Biorad, 170-6516).

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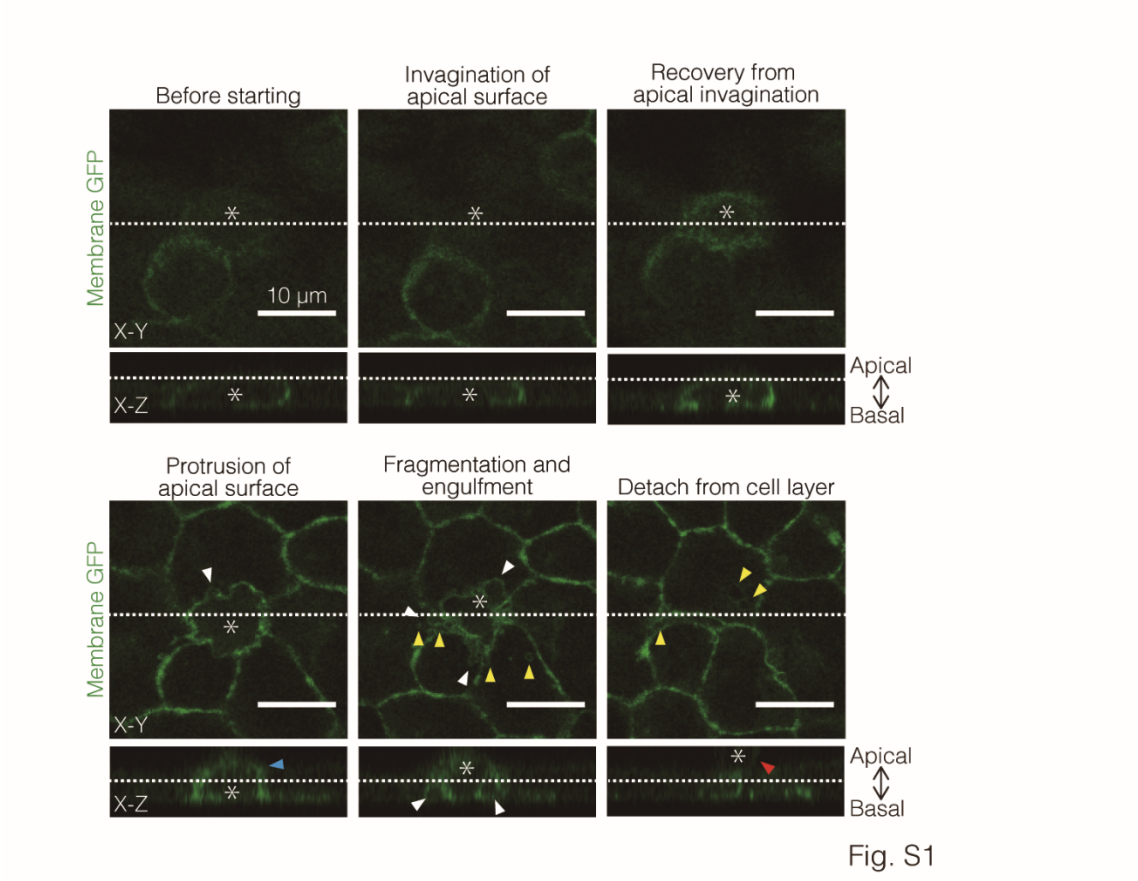

Fig. S1.

Cell extrusion processes in MDCKII cells. Representative images for each process in palmitoylated GFP (membrane GFP, green)-expressing extruding MDCKII cells (the same extruding cell shown in Fig 2A as control). Asterisks indicate an extruding cell. White arrowheads indicate budding in fragmentation; yellow arrowheads, engulfed fragments; blue arrowhead, protrusion of extruding cell; and a red arrowhead, cell detachment from the cell layer. Dashed lines correspond to the position from another angle (x-y or x-z images).

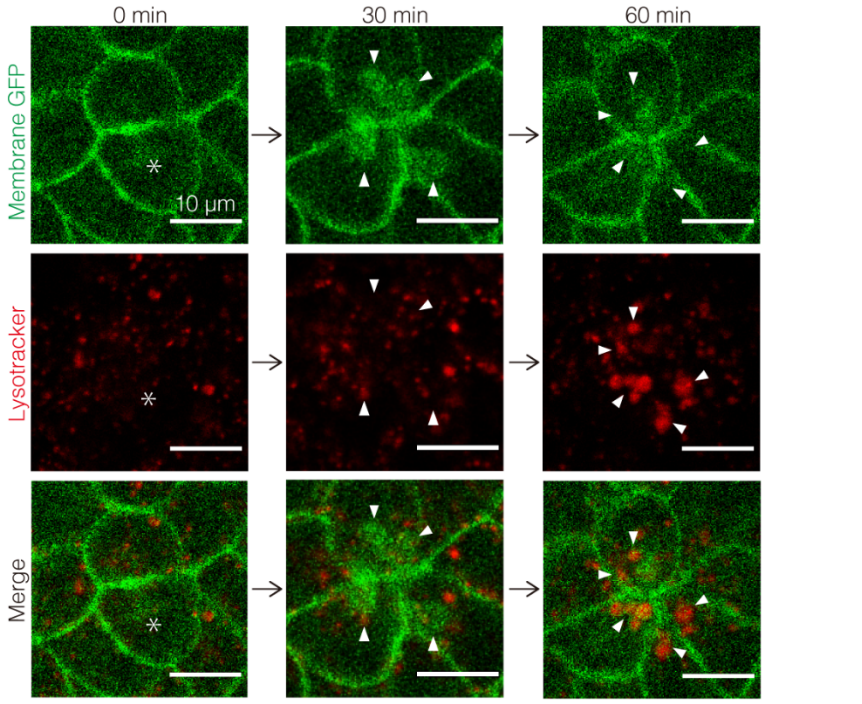

Fig. S2.

Lysosomal transport of engulfed vesicles derived from the fragmentation of extruding cells. Lysotracker staining (red) in palmitoylated GFP (membrane GFP, green)-expressing MDCKII cells. The engulfed vesicles in the neighboring cells were positive for the lysotracker signal (32/36 extrusion events) 30–60 min after incorporation. Asterisks indicate an extruding cell, whereas arrowheads indicate engulfed vesicles.

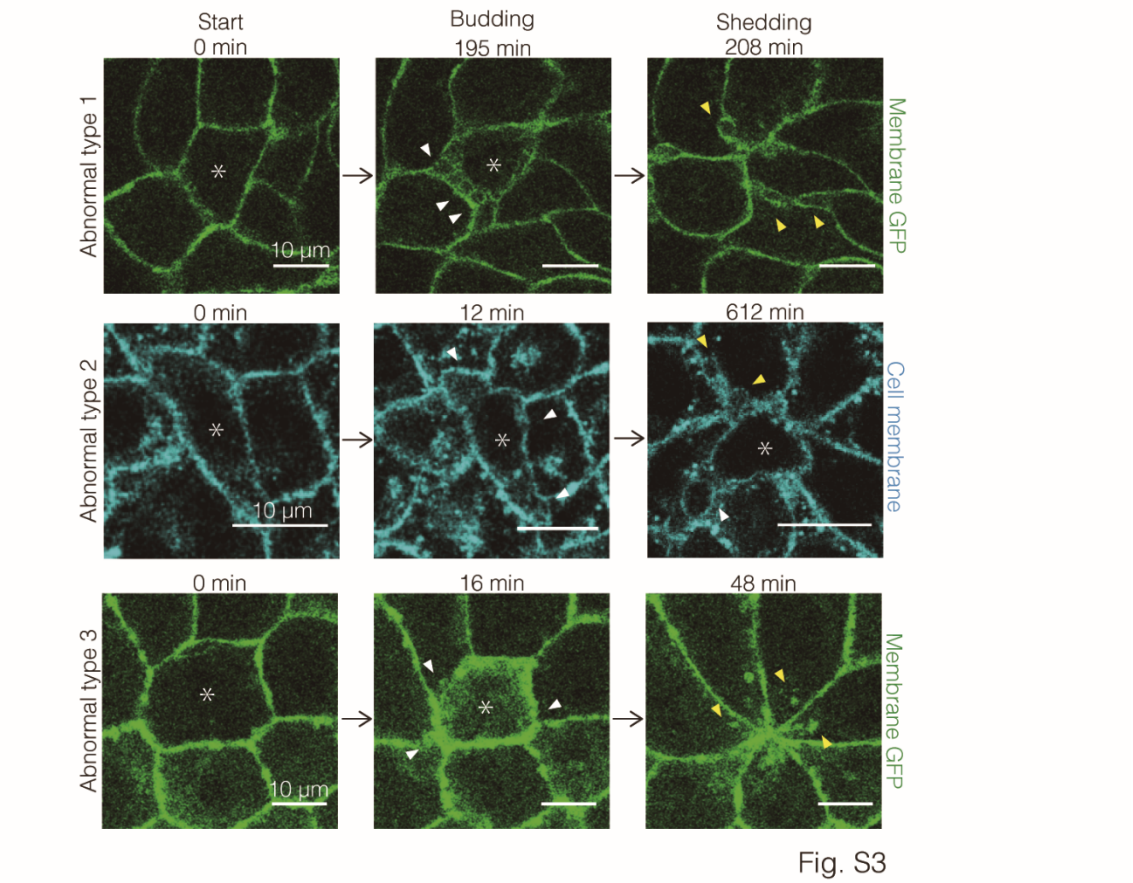

Fig. S3.

Characterization of abnormal types of fragmentation in extruding mammalian cultured cells. Representative images for each type of abnormality in fragmentation. Abnormal type 1 shows delayed budding-like movement of the cell membrane; Abnormal type 2, delayed shedding after budding; and Abnormal type 3, fewer or smaller vesicles. This typing is utilized in Fig. 2A, 2F, 2J, 4C, and S5B. (Abnormal type 1) An extruding D89E- and palmitoylated GFP (membrane GFP, green)-expressing EpH4 cell (same one used in Fig. 2F). (Abnormal type 2) An extruding *Pld1*-siRNA EpH4 cell stained with CellMask (cyan). (Abnormal type 3) An extruding D89E- and palmitoylated GFP (membrane GFP, green)-expressing MDCKII cell. Asterisks indicate extruding cells. White arrowheads indicate budding in fragmentation; yellow arrowheads, shed fragments in the intercellular space or those engulfed by neighboring cells.

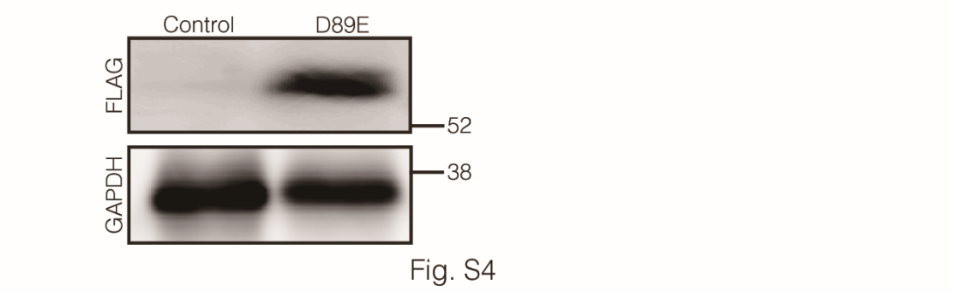

Fig. S4.

Expression of MFG-E8 D89E mutant protein (D89E) in EpH4 cells. Representative images of western blotting of transiently FLAG-tagged D89E-expressing EpH4 cells 2 d after the transfection, using anti-FLAG antibody with GAPDH expression as a loading control. The control sample was not transfected, and the experiments were conducted thrice.

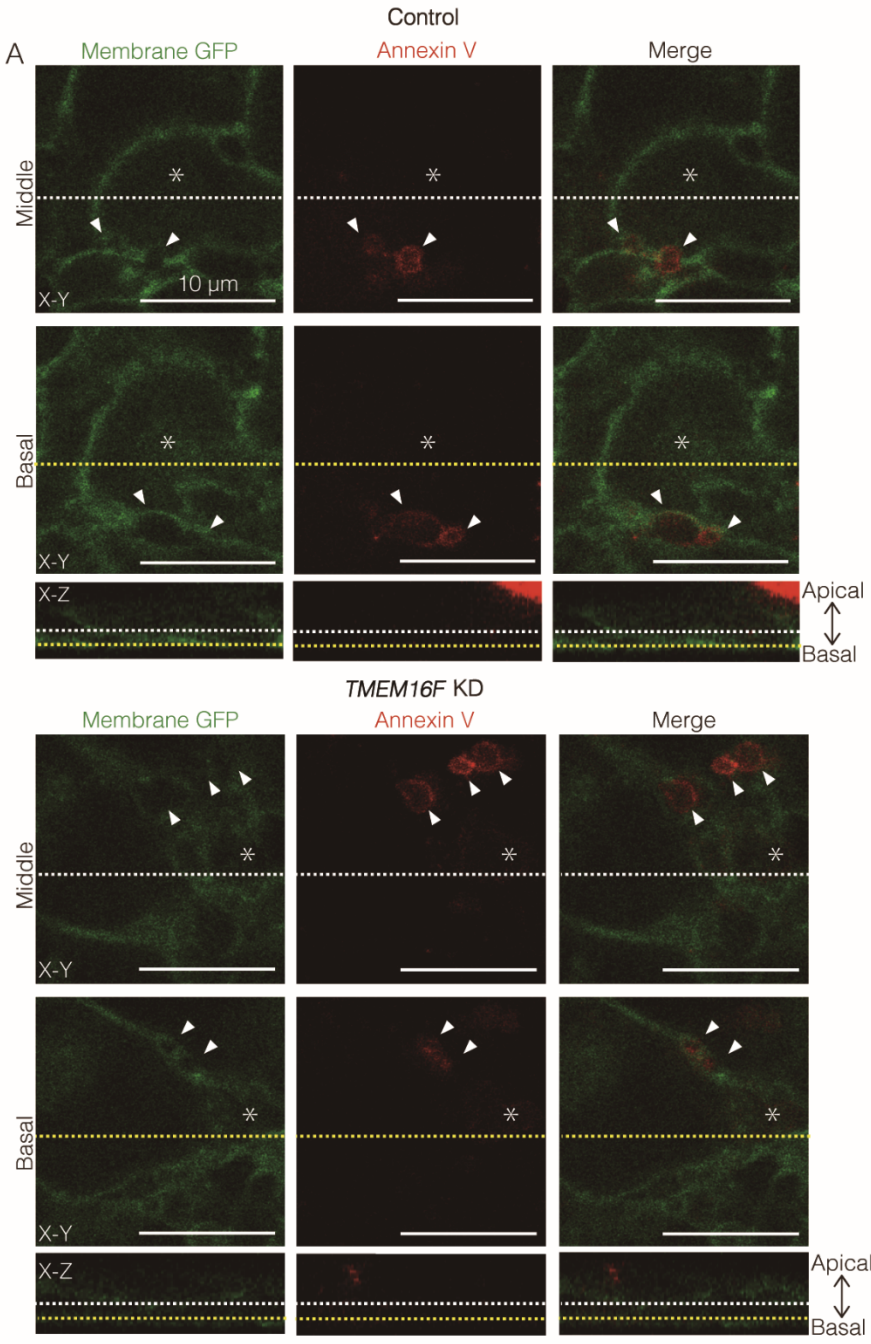
Control
A
Membrane GFP
Annexin V
Merge
Middle
X-Y
10 µm
Basal
X-Y
X-Z
Apical
Basal
TMEM16F KD
Membrane GFP
Annexin V
Merge
Middle
X-Y
Basal
X-Y
X-Z
Apical
Basal

Fig. S5A

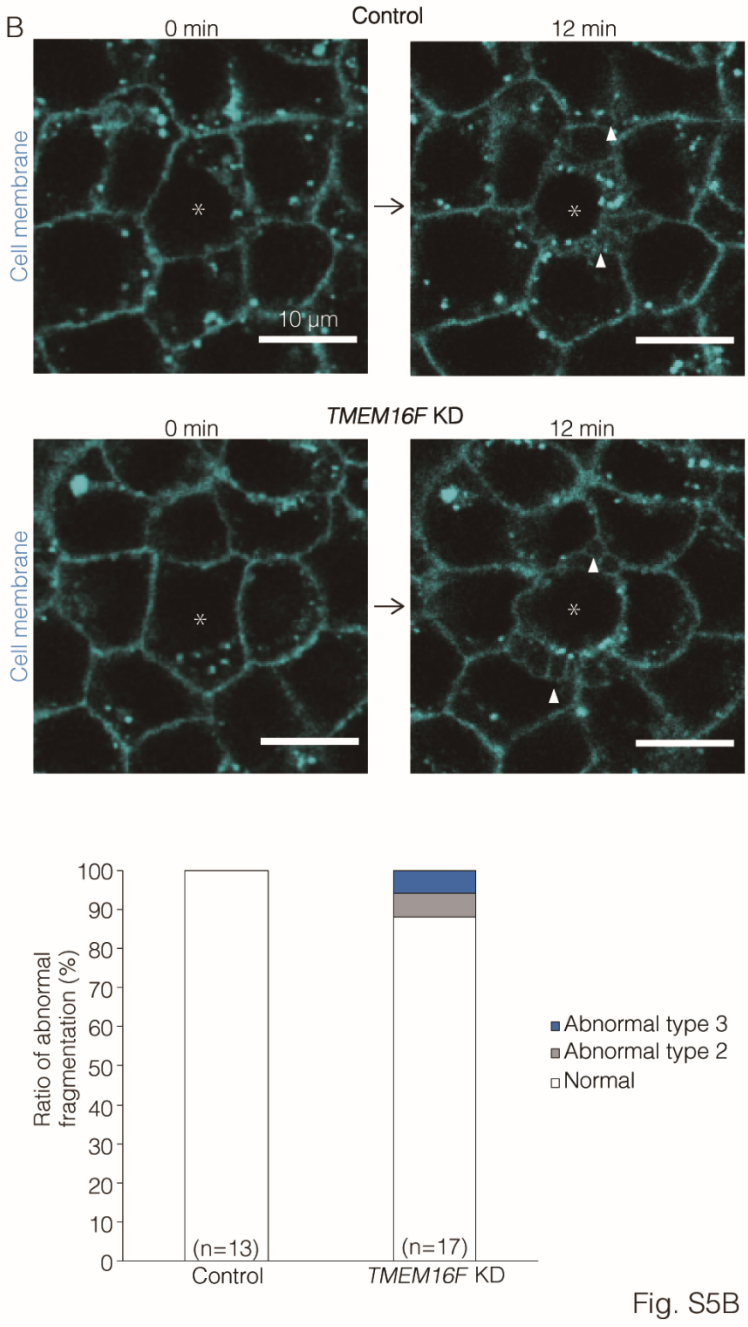
B
Control
0 min
12 min
Cell membrane
10 μm
TMEM16F KD
0 min
12 min
Cell membrane
Ratio of abnormal fragmentation (%)
100
90
80
70
60
50
40
30
20
10
0
(n=13)
(n=17)
Control
TMEM16F KD
Abnormal type 3
Abnormal type 2
Normal

Fig. S5B

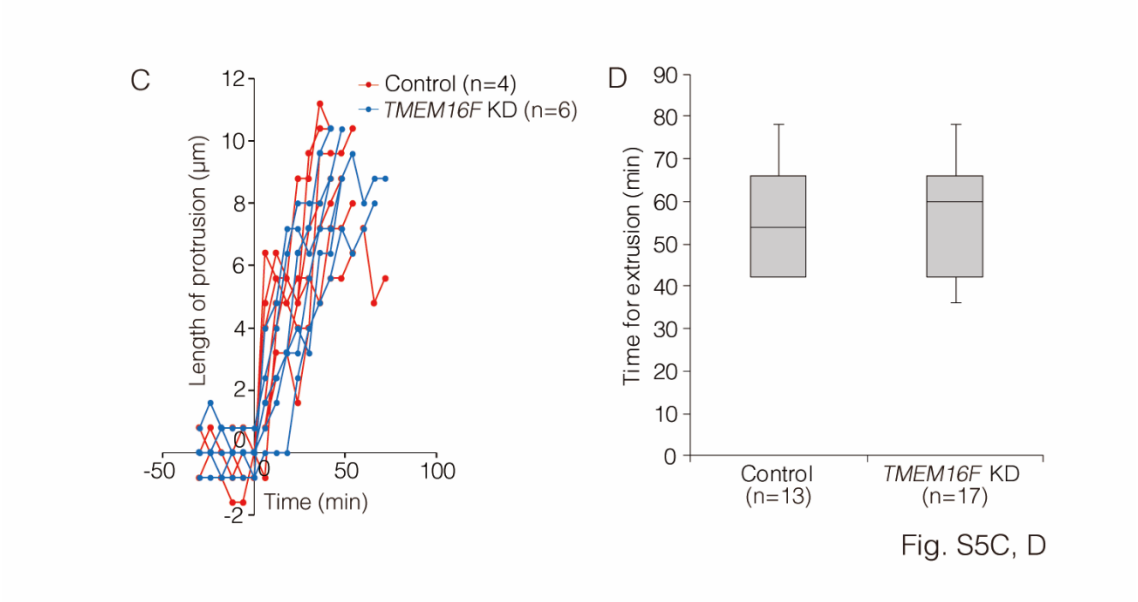

Fig. S5.

TMEM16F (Ano6) is not required for the fragmentation of extruding cells and cell extrusion in EpH4 cells. (A) Representative images of Annexin V (red)-stained control (scrambled siRNA) and *TMEM16F*-siRNA palmitoylated GFP (membrane GFP, green)-expressing EpH4 cells. Asterisks indicate extruding cells. Arrowheads indicate cell fragmentation. White and yellow dashed lines correspond to the position from another angle at the middle part and basal part in x-z axis, respectively. (B) Representative images (Normal for *TMEM16F*-siRNA) and the typing of fragmentation in the control and *TMEM16F*-siRNA extruding EpH4 cells stained with CellMask (cyan) according to the criterion of each abnormality shown in Fig. S3. Asterisks indicate extruding cells, whereas arrowheads indicate fragments in the intercellular space. (C) Quantification of the apical protrusion of extruding cells in the control (red) and *TMEM16F*-siRNA (blue) EpH4 cells. (D) Time for the completion of cell extrusion in the control and *TMEM16F*-siRNA EpH4 cells ($p = 0.28$, two-tailed Welch's *t*-tests).

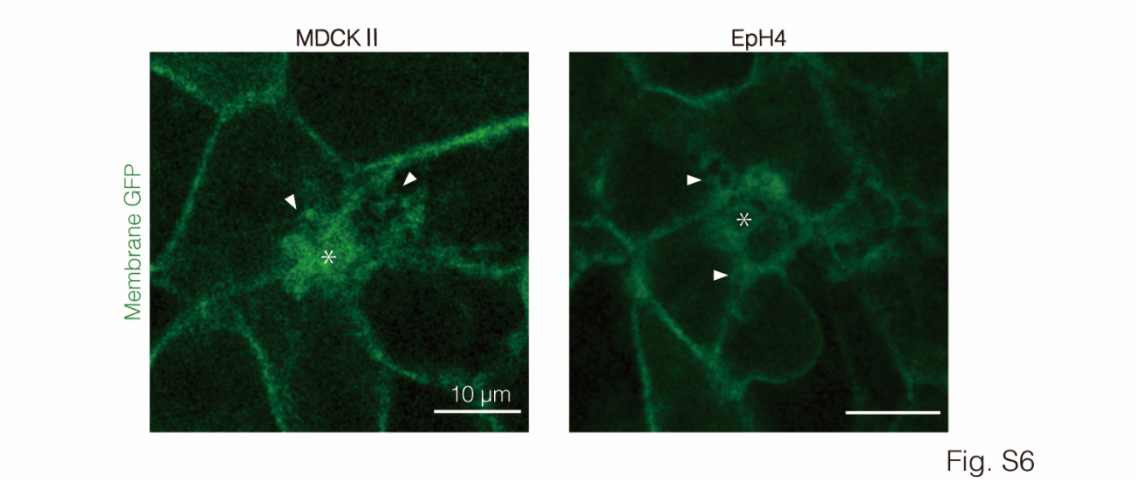

Fig. S6.

Cell fragmentation in UV irradiation-induced cell extrusion. Palmitoylated GFP (membrane GFP, green)-expressing MDCKII and EpH4 cells were irradiated by 180 and 150 mJ of UV, respectively, and images were acquired 17 and 24 h after irradiation. Asterisks indicate extruding cells, whereas arrowheads indicate fragments in the intercellular space or those engulfed by neighboring cells.

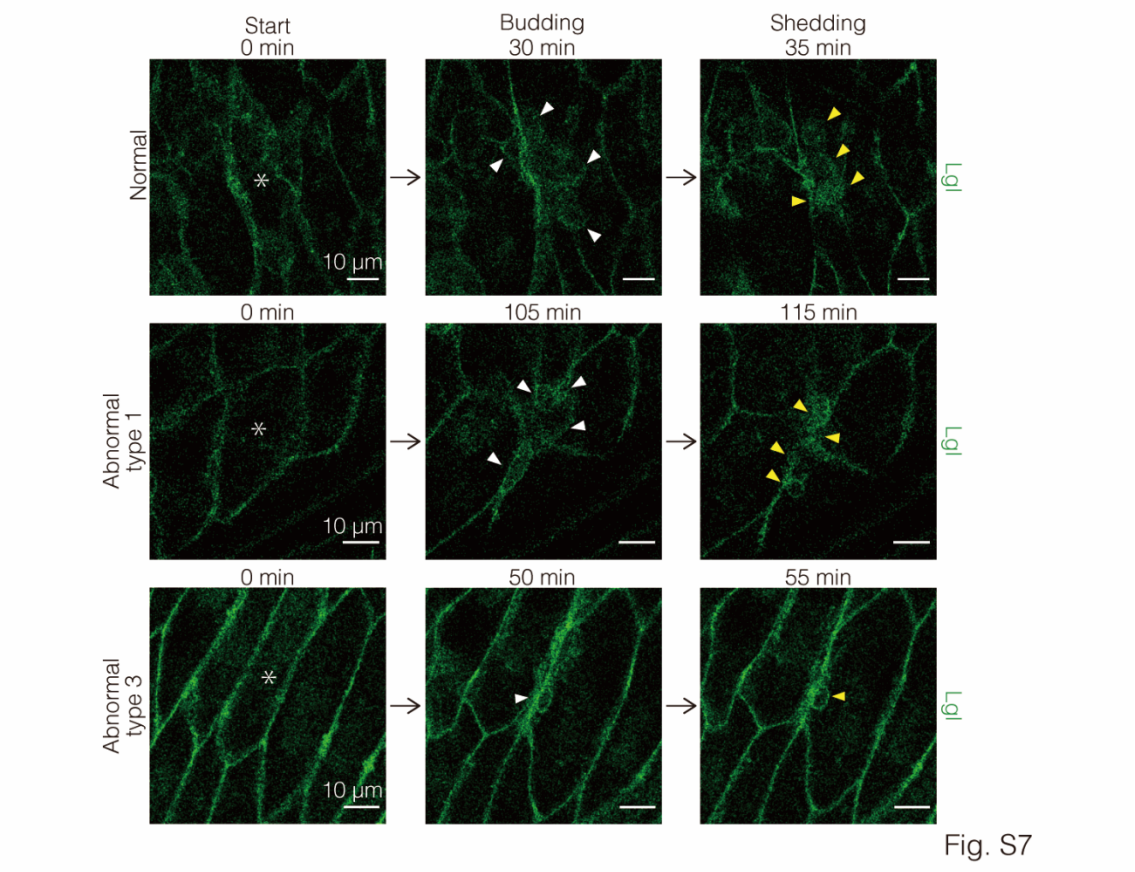

Fig. S7.

Characterization of abnormal types of fragmentation in extruding LECs. Representative images for each type of abnormality in fragmentation. Abnormal type 1 shows delayed budding-like movement of the cell membrane; and Abnormal type 3, fewer or smaller vesicles. Abnormal type 2 corresponding to Fig. S3 was not observed in LECs. This typing is utilized in Fig. 3G and 4G. (Normal and Abnormal type 3) Extruding *Xk*-RNAi or without RNAi Lgl:GFP (green)-expressing LECs (same images used in Fig. 3G). (Abnormal type 1) *Arf51F*-RNAi Lgl:GFP-expressing LECs (same image used in Fig. 4G). Asterisks indicate extruding cells. White arrowheads indicate budding in fragmentation; yellow arrowheads, shed fragments engulfed by neighboring cells.

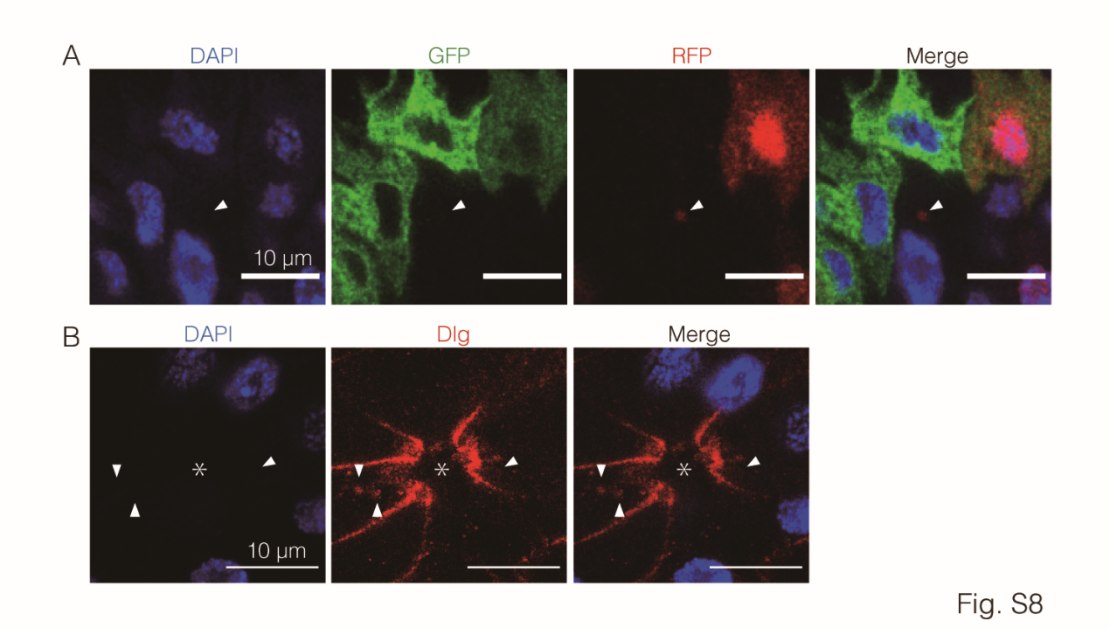

Fig. S8.

Fragmentation and engulfment in adult *Drosophila* midgut epithelium. (A) Representative images of cell mosaic in the midgut epithelium, wherein each epithelial cell randomly expresses GFP (green) and/or RFP (red) or neither, stained with DAPI (blue). RFP-positive vesicles (arrowheads) were observed in the fluorescent non-expressing cell. (B) Immunostaining for Dlg (red) with DAPI (blue) in the midgut epithelium. Engulfed vesicles (arrowheads) in the neighboring cells adjacent to an extruding cell (asterisks) were observed.

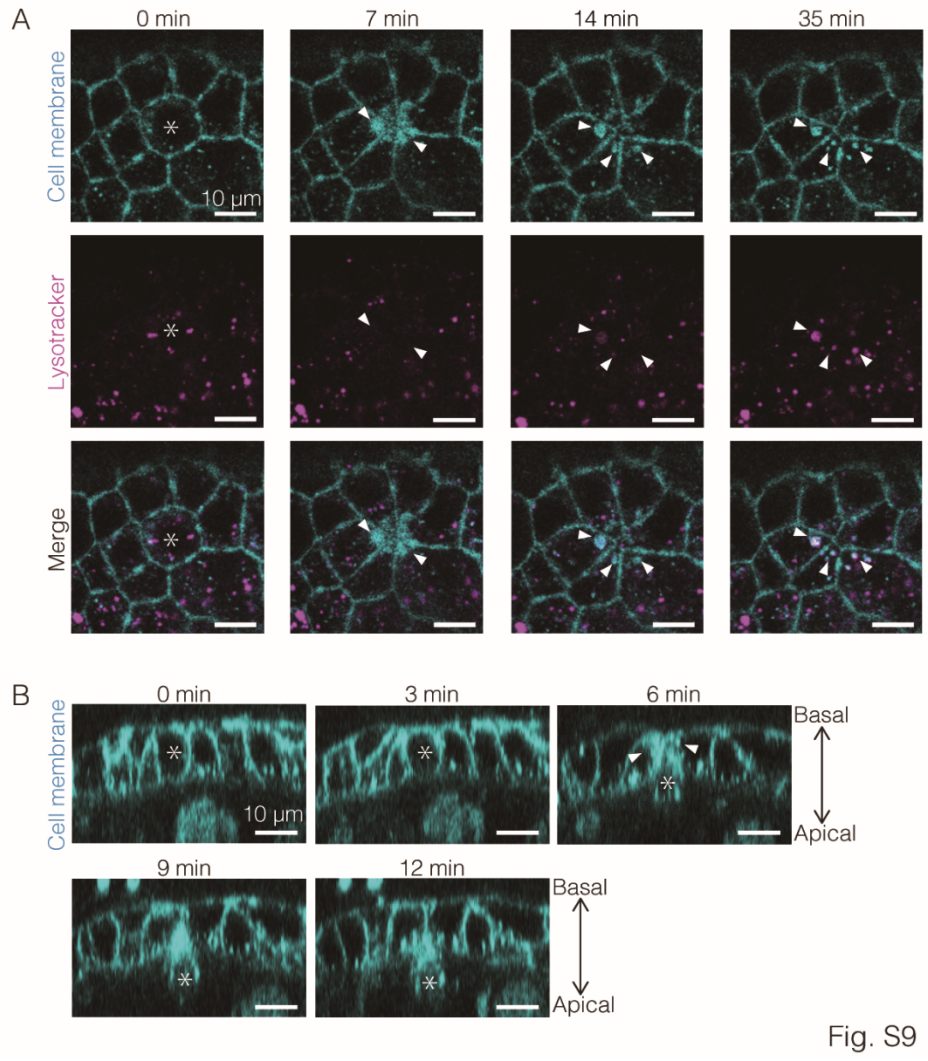

Fig. S9.

Fragmentation at the basal half of the extruding cells in mouse intestinal organoid. (A) Representative x-y images of an extruding cell in mouse intestinal organoid stained with CellMask (cyan) and lysotracker (magenta). Asterisks indicate extruding cells, whereas arrowheads indicate fragments engulfed by neighboring cells. (B) Representative transverse optical section of a cell, which extrude to the apical direction, in the organoid stained with CellMask (cyan). Asterisks indicate extruding cells, whereas arrowheads indicate fragmentation at the basal part of extruding cell.

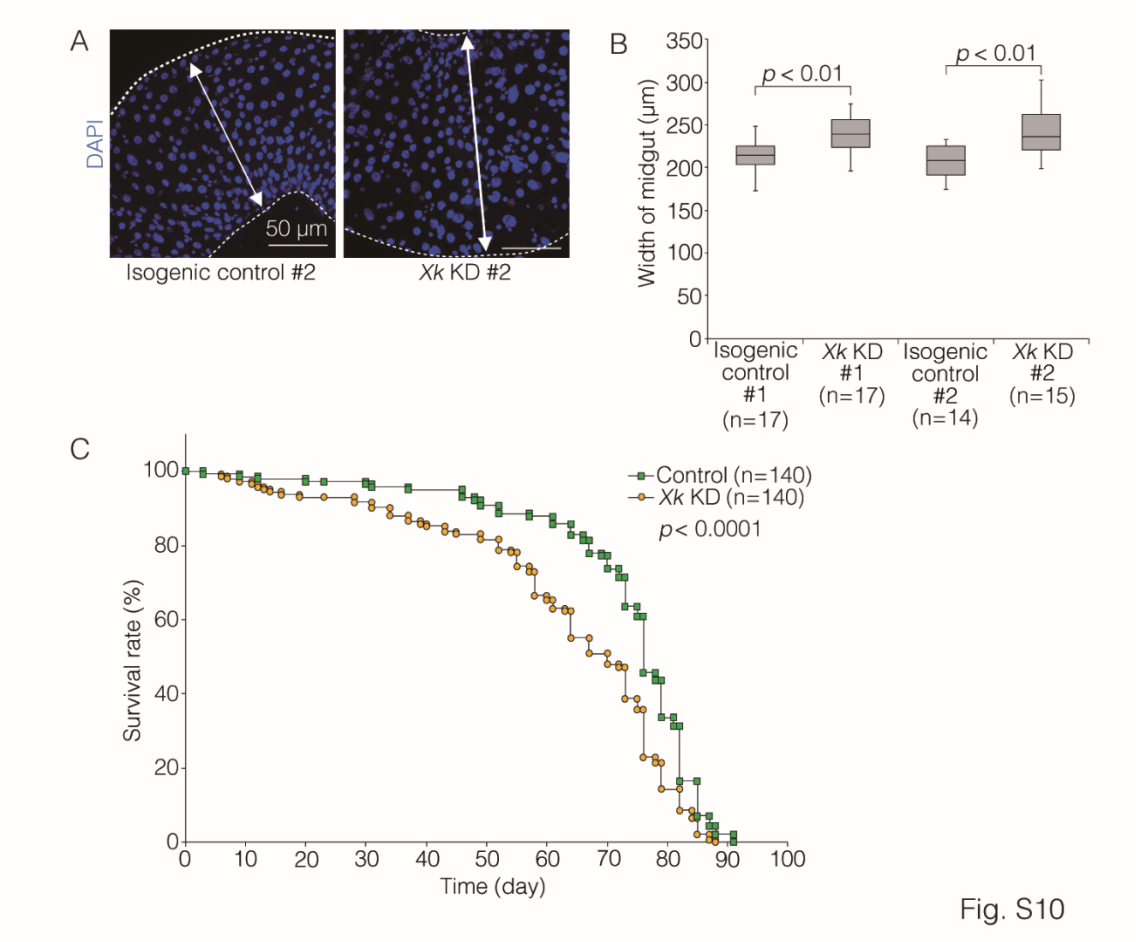

Fig. S10.

***Xk*-knockdown in adult *Drosophila* midgut.** *Xk* gene knockdown was induced in the adult stage in a midgut enterocyte-specific manner using the GeneSwitch system. (A) Representative maximum projection images of the isogenic control and the enterocyte-specific *Xk*-RNAi midgut (region 4 [7]) stained with DAPI (blue). Dashed lines indicate each midgut. (B) Width of region 4 in the isogenic control and enterocyte-specific *Xk*-RNAi midguts measured by the length as indicated by double-sided arrows in (A). (C) Survival curves of the isogenic control (green) and the enterocyte-specific *Xk*-RNAi (yellow) *Drosophila*. *P*-value is determined using the log-rank test.

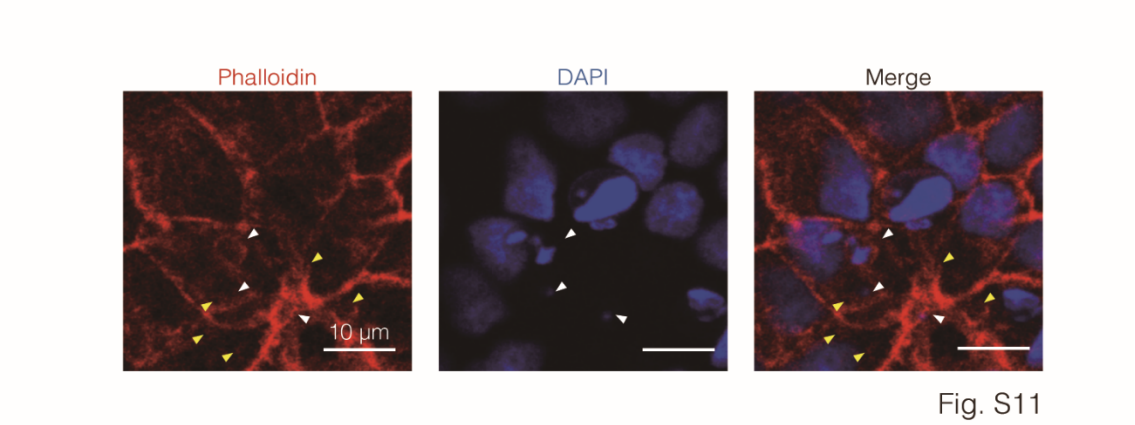

Fig. S11.

DNA in some vesicles derived from extruding cells. Representative images of an extruding MDCKII cell stained with phalloidin (red) and DAPI (blue). White and yellow arrowheads indicate the engulfed vesicles in the neighboring cells with and without DNA, respectively.

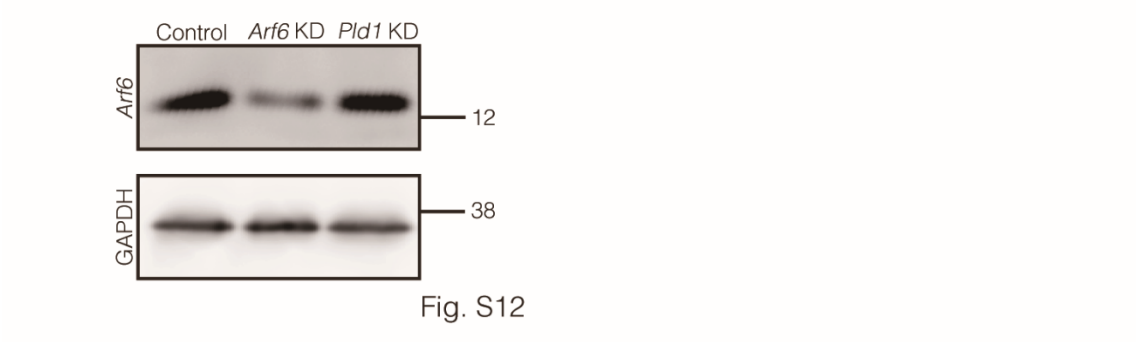

Fig. S12.

Decreased expression of Arf6 protein in *Arf6*-siRNA EpH4 cells. Representative images of western blotting of transient *Arf6*- or *Pld1*-siRNA EpH4 cells 2 d after the transfection, using anti-Arf6 antibody with GAPDH expression as a loading control. The control represents scrambled siRNA transfection, and the experiments were conducted thrice.

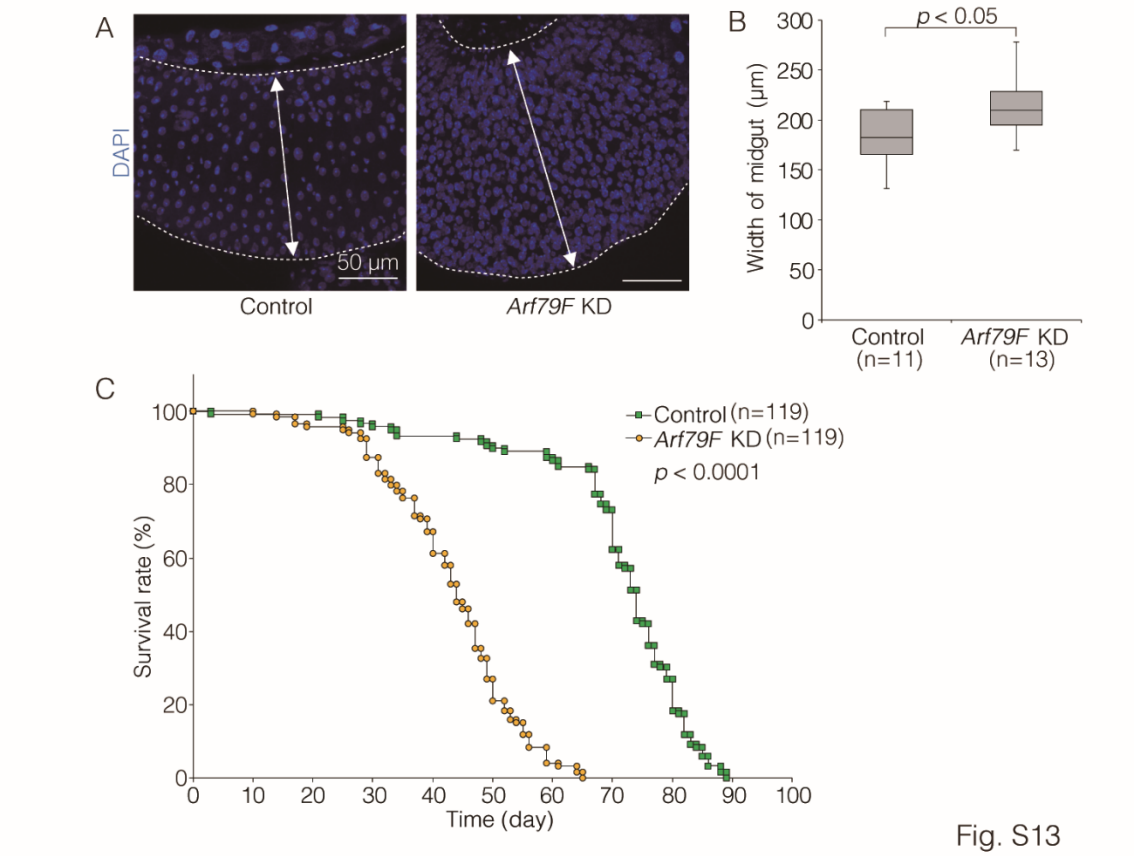

Fig. S13.

***Arf79F*-knockdown in adult *Drosophila* midgut.** *Arf79F* gene knockdown was induced in the adult stage in a midgut enterocyte-specific manner using the GeneSwitch system. (A) Representative maximum projection images of the midgut region 4 stained with DAPI (blue) and (B) width of the region 4 in the measured by the length as indicate by the double-sided arrows in (A) in control and enterocyte-specific *Arf79F*-RNAi midguts. (C) Survival curve of the isogenic control (green) and the enterocyte-specific *Arf79F*-RNAi (yellow) *Drosophila*. *P*-value is determined using the log-rank test.

Table S1.

Gene expression from RNAseq analysis in EpH4 cell and *Drosophila* adult midgut.

Fragments per kilobase of exon per million reads mapped (FKPM) of some Arf family genes, TMEM16 family genes, and Xkr family genes in RNAseq analysis of EpH4 cell and *Drosophila* midgut is shown. The number of biological replicates was three.

EpH4 cell

		FPKM				
gene name	gene ID	Replicate 1	Replicate 2	Replicate 3	Average	SD
Arf1	ENSMUSG00000048076	291.043	394.692	327.569	337.768	52.571794
Arf6	ENSMUSG00000044147	36.2654	35.495	33.4041	35.0548333	1.4805639
Ano3	ENSMUSG00000074968	0.0159882	0	0	0.0053294	0.0092308
Ano4	ENSMUSG00000035189	0	0.015948	0.955046	0.32366467	0.5468504
Ano6	ENSMUSG00000064210	25.2188	34.5515	30.4293	30.0665333	4.6769137
Ano7	ENSMUSG00000034107	0	0	0	0	0
Ano9	ENSMUSG00000054662	2.68279	1.96306	2.8719	2.50591667	0.4795421
Xkr4	ENSMUSG00000051951	0	0	0	0	0
Xkr8	ENSMUSG00000037752	0.897262	2.19177	0.900079	1.32970367	0.7465727
Xkr9	ENSMUSG00000067813	0	0	0	0	0

Drosophila midgut

		FPKM				
gene name	gene ID	Replicate 1	Replicate 2	Replicate 3	Average	SD
arf51f (arf6)	FBgn0013750	51.3448	42.221	48.1021	47.2226333	4.6250435
arf79f (arf1)	FBgn0010348	498.962	418.416	455.613	457.663667	40.312138
xk (CG32579)	FBgn0052579	9.36984	10.4461	9.0385	9.61814667	0.7359189

Table S2.

The genotype details of the *Drosophila* lines used in Figures and Supplemental Figures.

Figure	Genetype	Stage or tissue
3B, C	Eip71CD-Gal4, Lgl:GFP/UAS-mCD8:mRFP	Pupa
3D	Lgl:GFP/+	Pupa
3E	Esg-Gal4/UAS-AnnexinV: GFP or UAS-AnnexinV (mutant):GFP	Pupa
3F	Eip71CD-Gal4/UAS-AnnexinV: GFP or UAS-AnnexinV (mutant):GFP	Adult (abdomen)
3G, H	Eip71CD-Gal4, Lgl:GFP/UAS-Xk (CG32579) RNAi or +	Pupa
3I	Tsh-Gal4, DE-Cad:GFP/UAS-Xk (CG32579) RNAi or +	Pupa
4B	Sqh:mKate2, Lgl:GFP	Pupa
4F	Tsh-Gal4, Sqh:mKate2/UAS-Arf51F:GFP	Pupa
4G, H	Tsh-Gal4, Lgl:GFP/UAS-*Arf51F* RNAi (VDRC) or +	Pupa
S8A	hs-FLPG5; act < cd2 < Gal4, UAS-RFP/act < y+ < LexA, LexAop-CD8:GFP	Adult (midgut)
S8B	UAS-Dicer2; Pswitch CSG5966/+	Adult (midgut)
S10A	UAS-Dicer2; Pswitch CSG5966/UAS-Xk (CG32579) RNAi (BDSC) or +	Adult (midgut)
S10B	UAS-Dicer2; Pswitch CSG5966/UAS-Xk (CG32579) RNAi (VDRC) or UAS-Xk (CG32579) RNAi (BDSC) or +	Adult (midgut)
S10C	UAS-Dicer2; Pswitch CSG5966/UAS-Xk (CG32579) RNAi (VDRC) or +	Adult
S13A, B	UAS-Dicer2; Pswitch CSG5966/UAS-*Arf79F* RNAi or +	Adult (midgut)
S13C	UAS-Dicer2; Pswitch CSG5966/UAS-*Arf79F* RNAi or +	Adult

Movie S1.

Corresponds to Fig. 1A. Cell extrusion of a palmitoylated GFP (green)-expressing MDCKII cell. A time-lapse image with a single z-position at 63× magnification. Scale bar, 10 μm

Movie S2.

Corresponds to Fig. 1D. Co-culture of palmitoylated GFP- and RFP-expressing MDCK cells. Cell extrusion of a RFP (red)-expressing cell adjacent to palmitoylated GFP (green)-expressing cells. A time-lapse image with a single z-position at 63× magnification. Scale bar, 10 μm

Movie S3.

Corresponds to Fig. 1E. Co-culture of palmitoylated GFP- and RFP-expressing MDCK cells. Cell extrusion of a RFP (red)-expressing cells adjacent to palmitoylated GFP (green)-expressing cells. Transverse optical section (x-z) time-lapse image at 63× magnification. Scale bar, 5 μm

Movie S4

Corresponds to Fig. 2A. Cell extrusion of a MFG-E8 D89E mutant protein- and palmitoylated GFP (green)-expressing MDCKII cells. A time-lapse image with a single z-position at 63X. Scale bar, 10 μm

Movie S5

Corresponds to Fig. 2E. Cell extrusion of a palmitoylated GFP (green)-expressing EpH4 cells. A time-lapse image with a single z-position at 63X. Scale bar, 10 μm

Movie S6

Corresponds to Fig. 2F. Cell extrusion of a MFG-E8 D89E mutant protein- and palmitoylated GFP (green)-expressing EpH4 cell. A projected (3 confocal images, 0.75 μm interval each) time-lapse image at 63X. Scale bar, 10 μm

Movie S7

Corresponds to Fig. 3A. Cell extrusion of a Lgl:GFP (green)-expressing LEC in the basal direction. A time-lapse image at 63X. Scale bar, 10 μm.

Movie S8

Corresponds to Fig. 3D. Cell extrusion of a Lgl:GFP (green)-expressing LEC in the basal direction. Fragmentation (arrow) occurred immediately after the apical surface closure with neighboring cells. Transverse optical section (x-z) time-lapse image at 63X. Scale bar, 10 μm.

Movie S9

Corresponds to Fig. 3E. Cell extrusion of an AnnexinV:GFP (green)-expressing LEC (dotted line). A projected (3 confocal images in apical site, 1 μm interval each) time-lapse image at 63X. Scale bar, 10 μm.

Movie S10

Corresponds to Fig. S9A. Cell extrusion of a cell in mouse intestinal organoid stained with CellMask (cyan) and lysotracker (magenta). A time-lapse image with a single z-position at 63× magnification. Scale bar, 10 μm.

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