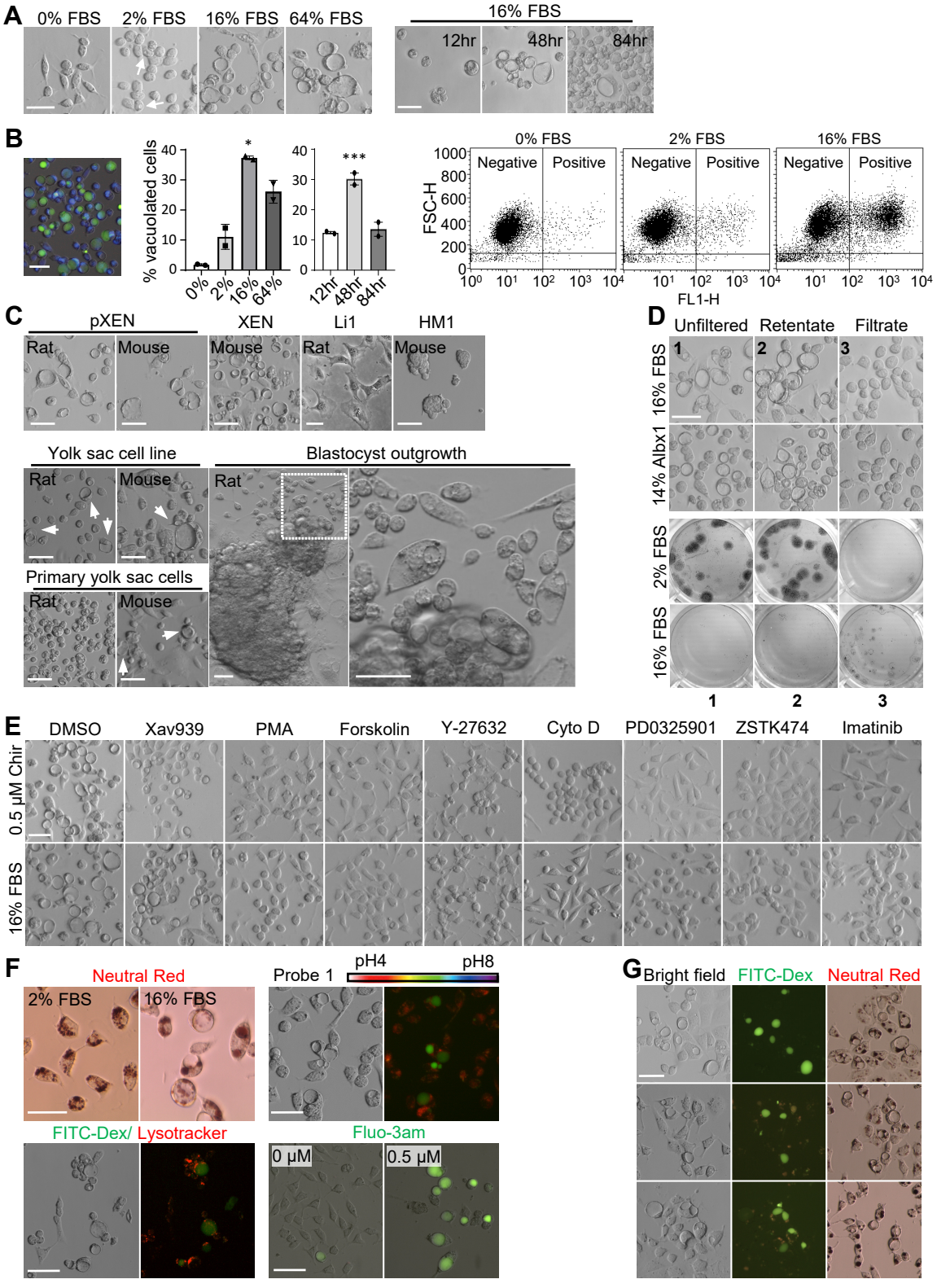

Fig. S1. Additional characterization of vacuoles and vacuolization factors. **(A)** Effects of serum concentration and culture time/cell density. Left row: Rat pXEN cells (line CX1) were cultured for 2 days in different concentrations of FBS (final concentrations: 2%, 16%, 64%); arrows indicate smaller vacuoles. Right row: Cells were cultured in 16% FBS for the indicated time points. **(B)** The proportion of vacuolated cells was quantified by flow cytometry after AP staining on Day 2. The photo illustrates the specificity of the AP dye (green: AP dye; blue: DAPI). Columns show mean ± s.e.m. from two independent experiments (n=2), each performed in duplicate. *, $p < 0.05$; ***, $p < 0.001$ (left columns: versus 2% FBS ("ground state"); right columns: versus 12 hours) by two-tailed Student's t-test. The plots show representative flow cytometry patterns. **(C)** Comparison of cell types for the propensity to vacuolate. Vacuolization tendency appeared to decrease in the order: blastocyst outgrowths > pXEN cells > XEN cells > primary yolk sac cells > yolk sac cell line; no vacuolization was observed in rat fibroblast cells (line Li1) and mouse ESCs (line HM1). E4.5 blastocysts, E12.5 dissociated yolk sacs and ExEn lineage cells, except XEN cells, were cultured in condition A with additional 14% FBS for 7 days (blastocyst), 4 days (yolk sac), or 2 days (cell lines), respectively. XEN cells, Li1, and HM1 cells were cultured for 2 days in their respective routine culture media but with additional 20% FBS. Arrow head indicates vacuole. **(D)** Vacuole-inducing capacity of serum and Albumax1 filtrate. 2% or 16% of unfiltered FBS or Albumax1, of >50 KDa retentate, and of the <50KDa filtrate were compared. Upper row: 5,000 cells were cultured for 2 days at standard cell density (for vacuole formation). Lower row: 100 cells were seeded in plating assays and analyzed after 5 days. The numbers represent each condition. **(E)** Preventive or inhibitory effects of signaling drugs on vacuolization. The drugs were present for 1 day in addition to vacuole-inducing factors (0.5 µM Chir or 16% FBS). Drug concentrations: 6 µM Xav939; 40 µM PMA; 20 µM Forskolin; 20 µM Y-27632; 65 µM Cytochalasin D (Cyto D); 50 µM PD0325901; 1 µM ZSTK474; 50 nM Imatinib. **(F)** Assessment of the intravacuolar milieu. For the pH assessments, cells were cultured with and without additional 14% FBS (final concentrations: 2 vs. 16% FBS) and then stained with Neutral Red, Probe 1, or FITC-Dextran/Lysotracker. The pH color scale for Probe 1 is taken from Lee MH et al., 2013. For the Ca assessment, cells were cultured without or with 0.5 µM of Chir, and then stained with Fluo-3am. **(G)** Assessment of vacuolar pH in vacuoles that were induced with three different drugs: 0.5 µM Chir, 1 µM Bim1, or 2 µM Gö6983. pH was assessed by incubation with FITC-Dextran or Neutral Red on Day 2. Scale bars, 50 µm (A-G). All photos represent at least two independent experiments. Except for (D), the experiments were based on condition A. FITC-Dex, FITC-Dextran. Albx1, Albumax1.

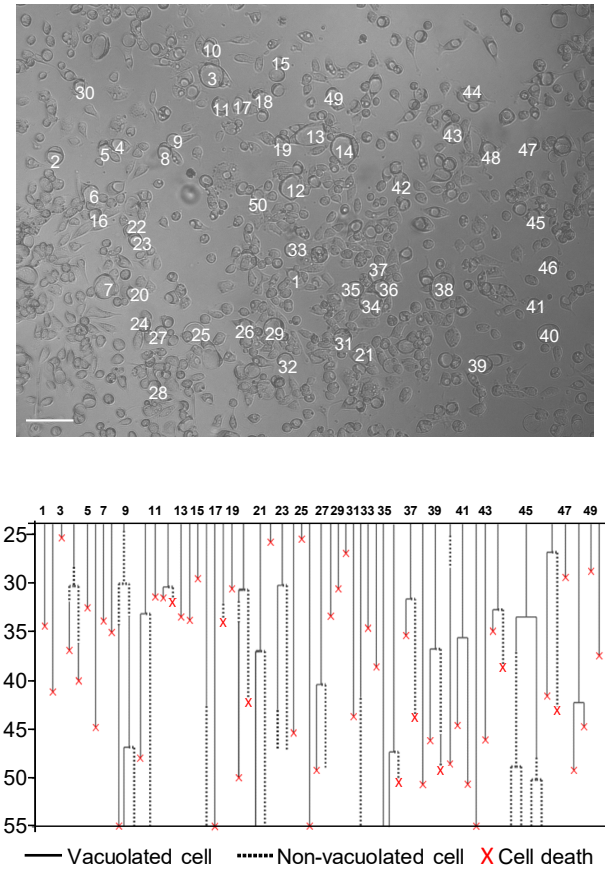

Fig. S2. Fate of vacuolated cells. 50 cells were tracked in condition A with additional 14% FBS on live cell imager from 48 to 80 hours (Video SV5). Scale bar, 100 µm.

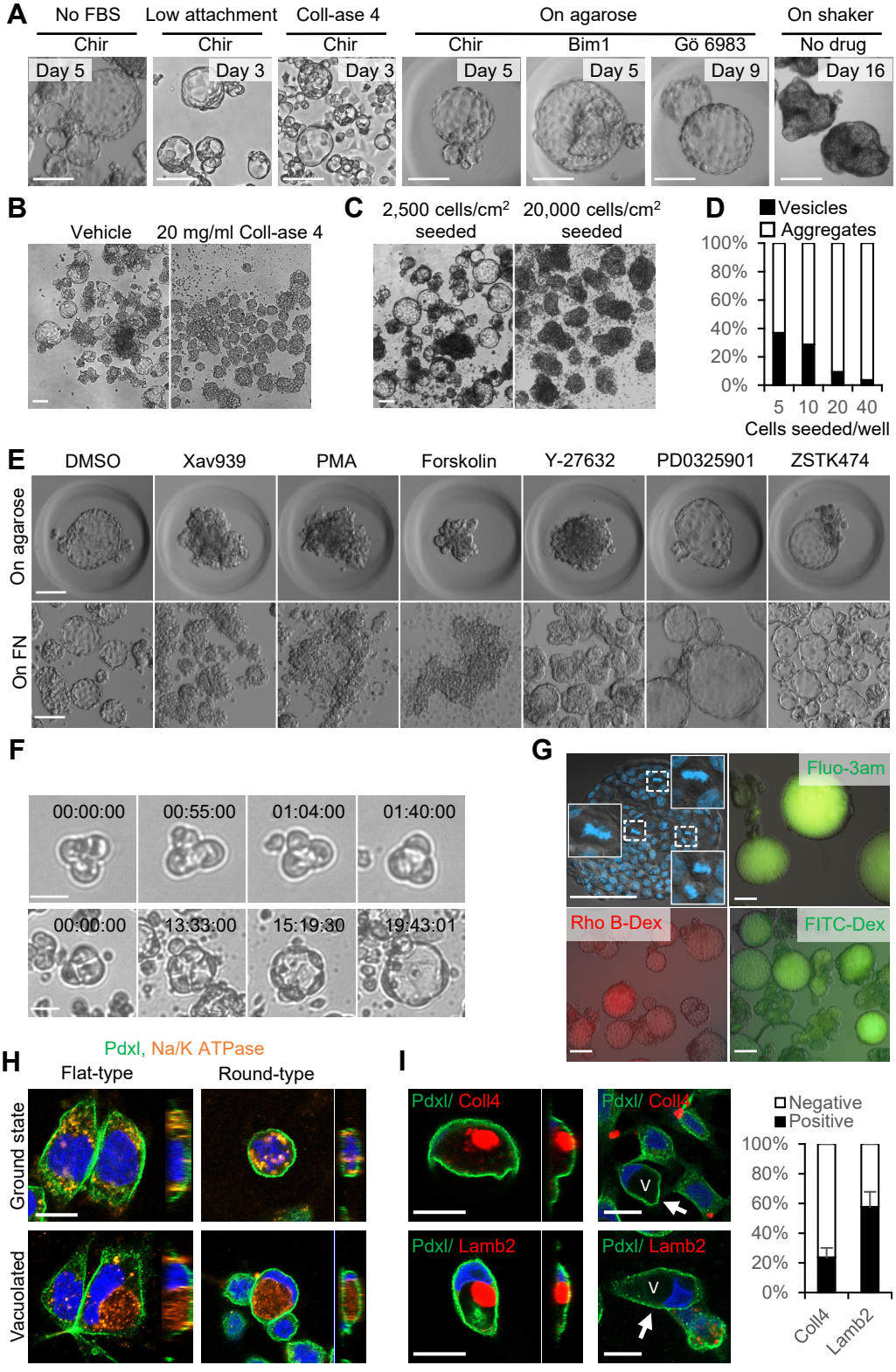

Fig. S3. Additional characterization of vesicle-producing cultures. **(A)** Comparison of vesicle-inducing conditions. pXEN cells were cultured for the indicated times under condition A, but with the indicated modifications (including no FBS instead of 2% FBS; low attachment plastic plates or agarose microwells instead of fibronectin-coated plastic plates; on shaker instead of non-moving plates) and in the additional presence of the indicated drug (3 µM Chir, 3 µM Bim1, 5 µM Gö 6983) or of collagenase 4 (0.1 mg/ml). Scale bars, 100 µm (except on shaker), 300 µm (on shaker). **(B)** Treatment with a high collagenase 4 concentration prevents vesiculation and instead causes aggregation. Cells were cultured in 4-well plates under condition B. Scale bar, 100 µm. The photos are representative of 3 independent experiments. **(C)** High cell density prevents vesiculation and instead causes aggregation. Cells were cultured in 4-well plates under condition B. Scale bar, 100 µm. The photos are representative of 3 independent experiments. **(D)** High cell density prevents vesiculation and instead causes aggregation. Cells were cultured in agarose microwells under condition A + 3 µM Chir. Numbers are based on 2 independent experiments. **(E)** Inhibitory effects of some signaling drugs on vesicle formation. Vesicles were formed over 5 days in condition A plus 3 µM Chir or condition B (which already includes 3 µM Chir). Cells were cultured in 3% agarose microwells for condition A and on fibronectin-coated wells for condition B. 3 µM Xav939; 20 µM PMA; 20 µM Forskolin; 40 µM Y-27632; 50 µM PD0325901; 1 µM ZSTK474. Scale bars, 100 µm. **(F)** Movie images showing one example of transition from vacuolar to vesicular lumen (see Videos SV9 and 10). 3 vacuolated cells formed one lumen (top) and kept growing (bottom). hr:min:sec. Scale bar, 25 µm (top), 50 µm (bottom). **(G)** Expansion of vesicles. Shown are vesicles stained with DAPI (boxes: mitotic chromosomes) or photographed after incubation with Fluo-3am, Rho B-Dextran, or FITC-Dextran. Scale bars, 100 µm. **(H)** Top and side views of “ground state” and vacuolated pXEN cells stained for Pdxl and Na/K ATPase. Note that in rounded but not flat cells, Pdxl is also present in the bottom part of the plasma membrane. The photos represent three independent experiments. Blue, nuclei (DAPI stain). **(I)** Photos: Top and side views of vacuolated pXEN cells immunostained for Pdxl, collagen 4, and laminin B2. The photos show examples of vacuoles that are filled with laminin or collagen (left) or free of these proteins (right). Columns: Percentages of collagen 4-positive or laminin B2-positive vacuoles at the vacuolated stage. V and arrows indicate vacuoles. Scale bars, 20 µm (H, I). Mean±s.e.m. from 5 independent experiments.

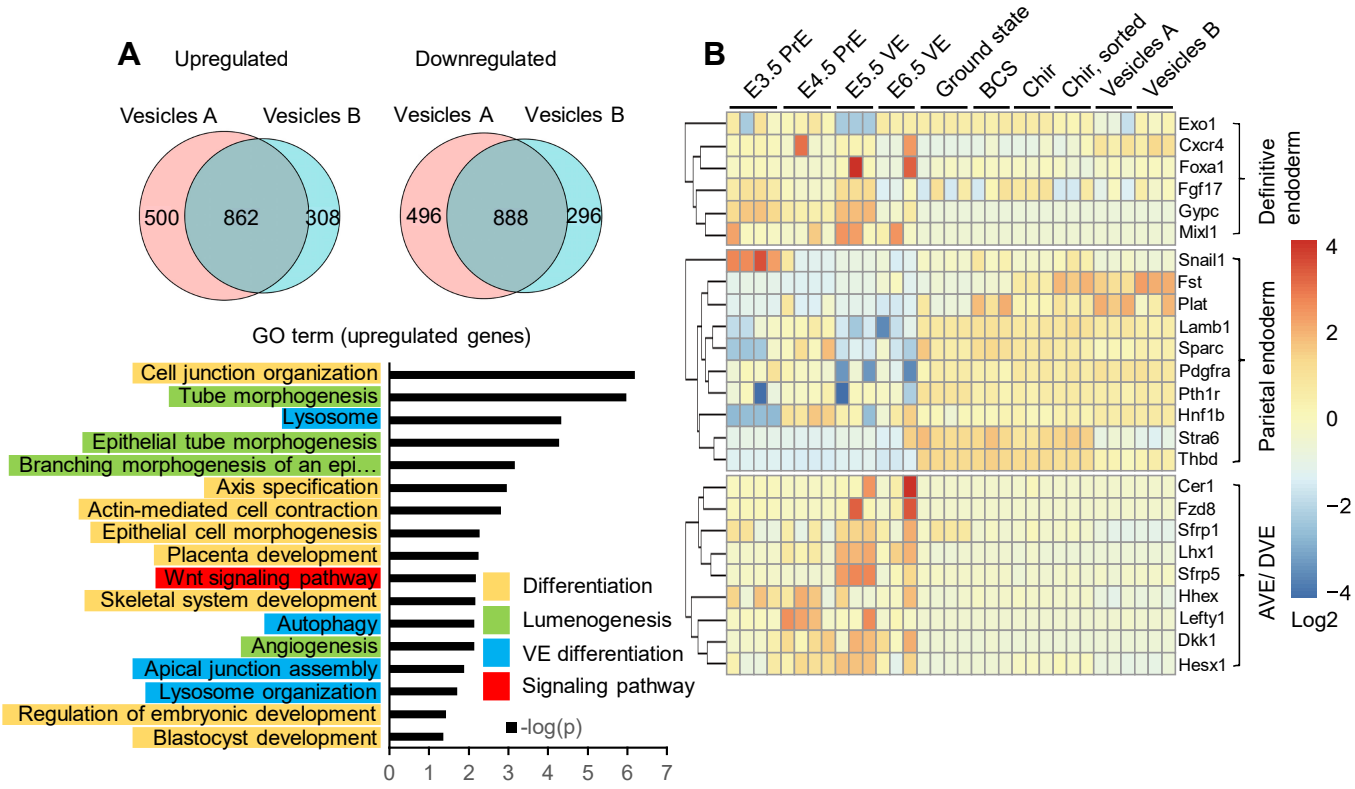

Fig. S4. Transcriptome analysis of vesiculation. **(A)** Comparison of normal pXEN cells with "Vesicles A" (condition A plus 3 µM Chir) and "Vesicles B" (condition B, which already includes 3 µM Chir). Top, Venn diagrams. Bottom, Gene ontology of biological annotation; same colors indicate related meanings (see color legend). See also Tables S10 and S11. **(B)** Heatmap comparison of definitive endoderm, parietal endoderm, anterior visceral endoderm (AVE)/ distal visceral endoderm (DVE) marker gene expression in "ground state" (condition A= maintenance condition) pXEN cells, vacuolated pXEN cells, pXEN cell-derived vesicles, and related in vivo samples. **(C)** Immunostainings showing disappearance of the pluripotency marker Oct4, but maintenance of the PrE marker Gata4, during vacuolization and vesicular morphogenesis of pXEN cells. **(D)** Epithelial morphologies of immature (Vesicles B; with Chir) and more mature (Mature vesicles B; Chir withdrawn) vesicles. Images in (C) and (D) were obtained with a confocal microscope. Scale bars, 20 µm. All photos represent at least two independent experiments.

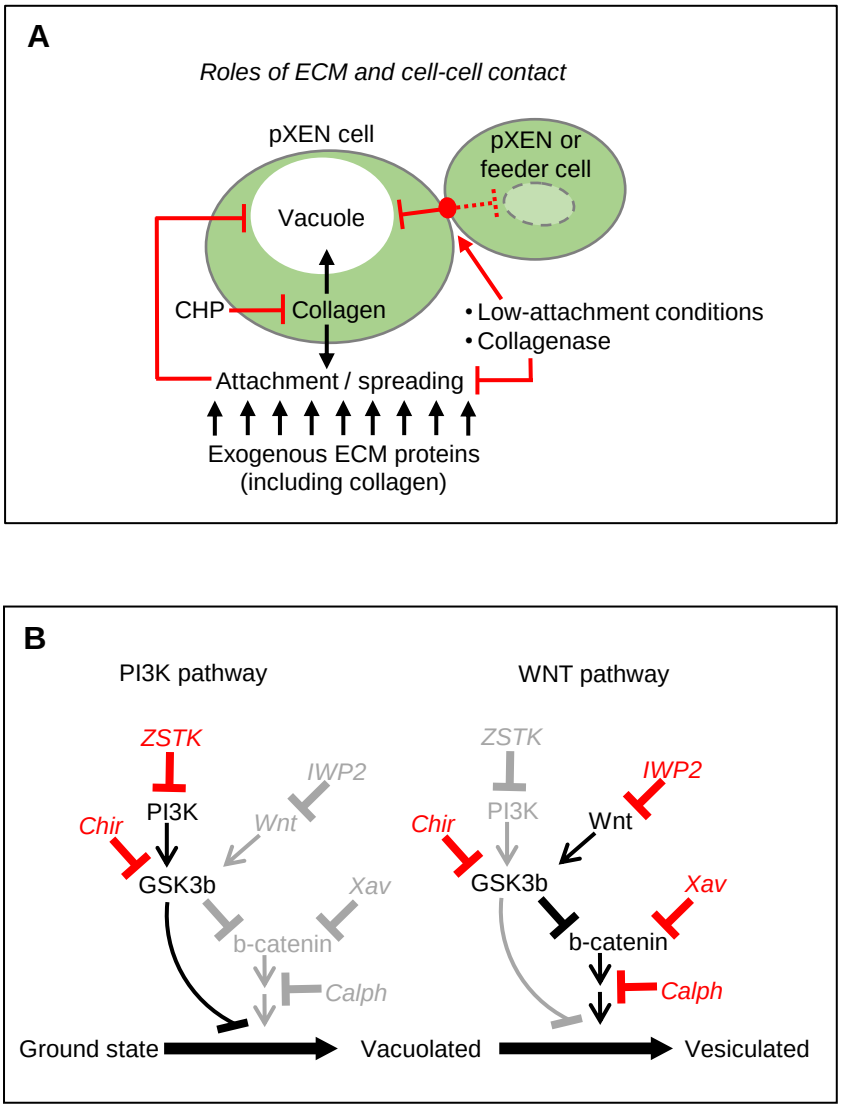

Fig. S5. Control mechanisms of vesicular morphogenesis. **(A)** Schematic summarizing control of vacuolization. pXEN cells have an inherent tendency to create vacuoles, which is held in check by ECM proteins (notably including self-synthesized collagen) and by cell-cell contact. Note that endogenously synthesized collagen can be secreted both out of the cell and into vacuoles, although not necessarily at the same time. **(B)** Potential roles of PI3K and Wnt pathways. Only the results obtained with drugs that are directly relevant to those pathways are included. Each drug was tested both in the vacuolization and in the vesiculation stage. Drugs that showed effects are depicted in red color. When a drug showed no effect, it is depicted in grey color. Full drug names, see Materials and Methods.

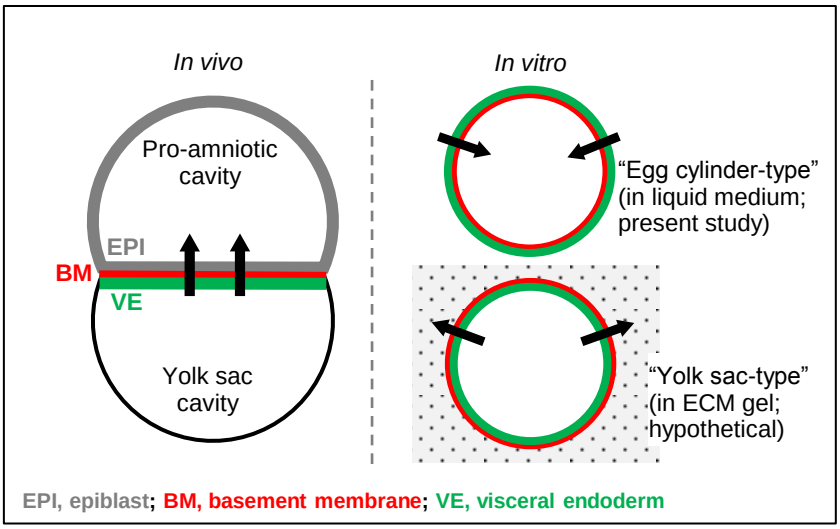

Fig. S6. Schematic explaining relationship of VE-like vesicles with egg cylinder. It depends on the function studied whether a vesicle is "in vivo-like". **Left**, Schematic of the egg cylinder-stage embryo with distorted proportions to emphasize topology (for a schematic of the egg cylinder with realistic proportions, see Fig. 6B). Also shown is the transport function of the early VE (arrow), which is executed in tandem with the epiblast. The schematic ignores that later the pro-amniotic cavity also extends into the extraembryonic ectoderm, but this does not alter the notions discussed. **Right**, Depending on their orientation, VE vesicles represent different functions of the VE in vitro. For transport studies, "egg cylinder-type" VE vesicles (present study) are more in vivo-like, as the yolk sac cavity is the "outside". Such vesicles are also more practical, as they do not need pre-loading. "Yolk sac-type" VE vesicles were not produced in the present study but probably are feasible when using an ECM gel.

Table S1. Chemicals, culture additives, growth factors

Product	Company	Catalog No.
0.05% trypsin/ EDTA	Welgene, Gyeongsan, Korea	LS015-09
A83-01	Peprotech, RI, U.S.	9094360
Advanced DMEM/F12	ThermoFisher, Waltham, MA, U.S.	12634-010
Albumax1	ThermoFisher	11020-021
Ascorbic acid	Sigma, St. Louis, MO. U.S.	A8960
B27 supplement without vitamin A	ThermoFisher	12587-010
β-mercaptoethanol	Sigma	M7522
BCS	Welgene	S003-05
Bovine fibronectin	ThermoFisher	33010018
Crytal violet	Sigma	C.I. 42555
DMEM (high glucose)	Sigma	D6429
DMEM (low glucose)	Sigma	D6046
DMEM/F12	ThermoFisher	21331-020
Donkey serum	Genetex, Irvine, CA U.S.	GTX73205
EGF	Miltenyi Biotec, Bergisch Gladbach, Germany	130-093-825
Fatty acid-free BSA	GenDEPOT, Katy, TX, U.S.	A0100-010
FBS	MP biomedicals, Santa Ana, CA, U.S.	2916754 (Lot# M6164)
Fraction V BSA	Roche, Basel, Switzerland	10774111103
Glutamax	ThermoFisher	35050061
Hybrid-R (RNA extraction)	GeneAll, Seoul, Korea	305-101
ITS	ThermoFisher	41400
Knockout serum replacement	ThermoFisher	10828-010
LA-BSA	Sigma	L9530
LIF	ORF Genetics, Kopavogur, Iceland	01-A1140
MCDB-201	Sigma	M6770
Mitomycin C	Tocris, Bristol, UK	3258
N2 supplement	ThermoFisher	17502-048
N-acetylcysteine	Sigma	A7250
Neurobasal	ThermoFisher	21103-049
Paraformaldehyde	JUNSEI, Tokyo, Japan	58295-1201
PDGF	ThermoFisher	14-8501-80
Penicillin/ streptomycin	ThermoFisher	10378016
Polyethylenimine (PEI) system	Polyscience, Warrington, PA, U.S.	23966
Propidium iodide	Sigma	P4170
Puromycin	Invivogen, Pak Shek Kok, Hong Kong	ant-pr-1
Random hexamer	Enzynomics, Daejeon, Korea	N101M
Reverse transcriptase kit	Solgent, Daejeon, Korea	DR23-R10k
RNAse A	m.biotech, Hanam, Korea	43014
SYBR green	Enzynomics	RT500M

Triton X-100	Fisher Scientific, Hampton, NH, U.S.	T/3751/08
Trizol	TAKARA, Kusatsu, Japan	9109
Vectashield	Vector Laboratories, Burlingame, CA, U.S.	H-1000

Table S2. Molecules tested for effects on vacuolization and vesiculation

Drug	Catalog No.	Company
Bisindolylmaleimide 1 (Bim1)	B-6191	LC laboratories, Woburn, MA, U.S.
Calphostin C	AG-CN2-0430-C100	Adipogen, San Diego, CA, U.S.
Chir99021	4423	Tocris
Cytochalasin D	1233	Tocris
Forskolin	1099	Tocris
Gö6983	G-7700	LC laboratories
Imatinib	I-5508	LC laboratories
IWP2	6866167	Peprotech
PD0325901	4192	Tocris
PMA (Phorbol 12-myristate 13-acetate)	P-1680	LC laboratories
XAV939	13596	Cayman, Ann Arbor, MI, U.S.
Y-27632	A3008	Apexbio, Boston, MA, U.S.
ZSTK474	S1072	Selleck, Houston, TX, U.S.
cis-L-hydroxy-proline (CHP)	H1169	Tokyo chemical, Nihonbashi-honcho, Tokyo, Japan
Collagenase 4 (Coll-ase 4)	LS004186	Worthington, Lakewood, NJ, U.S.

Table S3. Composition of media

Culture condition	Media components	Application
A	Low glucose DMEM, MCDB, 1% (v/v) penicillin/streptomycin, 1x (v/v) LA-BSA, 1x (v/v) ITS, 0.1 nM L-ascorbic acid, 2% (v/v) FBS, 0.5 µM Dexamethasone, 10 ng/ml PDGF, 10 ng/ml EGF, 10 ng/ml LIF, 55 µM β-mercaptoethanol, 100 ng/ml fibronectin	Routine culture condition for rat and mouse pXEN and yolk sac stem cell culture
B	DMEM/F12, Neurobasal, 1x (v/v) N2, 1x (v/v) B27, 1% (v/v) penicillin/streptomycin, 1x (v/v) Glutamax, 10 ng/ml LIF, 100 µM β-mercaptoethanol, 3 µM Chir99021	Vesicle and duct formation
C	Advanced DMEM/F12, 1x (v/v) N2, 1x (v/v) B27, 1% (v/v) penicillin/streptomycin, 1x (v/v) Glutamax, 1.25 mM N-acetylcysteine, 50 ng/ml EGF, 500 nM A83-01	Differentiation of vesicles
XEN cells	70% (v/v) conditioned medium (three days on Mitomycin C treated MEF) with 30% (v/v) fresh medium (RPMI 1640 (Sigma, R0883), 20% (v/v) FBS, 1% (v/v) penicillin/streptomycin, 1 mM sodium pyruvate (Sigma, P5280), 2 mM L-glutamine (Sigma, G8540))	Observation of vacuoles
Li1 cells	High glucose DMEM, 1% (v/v) penicillin/streptomycin, 7.5% (v/v) FBS	Feeder cells or control cells
HM1 cells	High glucose DMEM, 1% (v/v) penicillin/streptomycin, 15% (v/v) FBS, 10 ng/ml LIF, 100 µM β-mercaptoethanol, 0.1% gelatin	Control cells

Table S4. Organisms/strains

Species (organ)	Sex	Strain	Age	Company
Mouse (Yolk sac)	Female	ICR	12 weeks	Koatech, Pyeongtaek, Korea
Rat (Blastocyst)	Female	SD	11 weeks	Koatech
Rat (Yolk sac)	Female	SD	10 weeks	Koatech

Table S5. Cell lines

Cell line	Species	Strain	Sex	Reference
CX1 (RX1)	rat	WKY	M	Debeb et al., 2009, Zhong et al., 2018
HM1	mouse	129	M	Magin et al., 1992
Li1	rat	SD	F	Debeb et al., 2009
MAX3	rat	F344	M	Zhong et al., 2018, Lo Nigro et al., 2012
pXEN6	mouse	ICR	F	Zhong et al., 2018
XEN5	mouse	ICR	M	Zhong et al., 2018

Table S6. Organelle markers

Plasmid	Target	Company	Catalog No.
B4GALT1-pmTurquoise2	Golgi	Addgene, Watertown, MA, U.S.	36205, RRID: Addgene_36205
COX8A-pmTurquoise2	Mitochondria		36208, RRID: Addgene_36208
Lamp1-RFP	Lysosome		1817, RRID: Addgene_1817
Sec61-mCh	ER		49155, RRID: Addgene_49155
Rab5-mRFP	Eary endosome		14437, RRID: Addgene_14437
Rab7-GFP	Late endosome		12605, RRID: Addgene_12605

Table S7. Antibodies, phalloidin, DBA

	Target	Company	Catalog No.	Dilution	Host
1st	Afp (Alpha-fetoprotein)	Cusabio, Houston, TX, U.S.	CSB-PA001421LA01MO, RRID: Not found	1:200	Rabbit
1st	Cdh1 (E-cadherin)	Bioss, Woburn, MA, U.S.	bs-10009R, RRID: Not found	1:200	Rabbit
1st	Cer1 (Cerberus 1)	Santa Cruz, Dallas, TX, U.S.	sc-515324, RRID: Not found	1:200	Mouse
1st	Collagen4 (type 4 collagen)	Hybridoma bank	M3F7, RRID: AB_528167	1:200	Mouse
1st	DBA (Dolichos biflorus agglutinin)	Vector lab	RL-1032, RRID: AB_2336396	1:100	N/A
1st	Gata4 (GATA Binding Protein 4)	Santa Cruz	sc-9053, RRID: AB_2247396	1:100	Rabbit
1st	Lamb2 (Laminin, beta 2)	Hybridoma bank, IA, U.S.	D18, RRID: AB_2281095	1:200	Mouse
1st	Lrp2 (Low density lipoprotein-related protein 2)	Hybridoma bank	20B megalin, RRID: AB_2753214	1:200	Mouse

1st	Oct4 (octamer-binding transcription factor 4)	Santa Cruz	sc-5279, RRID: AB_628051	1:100	Mouse
1st	Fluorescent Dye 594-I Phalloidin	Abnova, Taipei, Taiwan	A10200, RRID: Not found	1:10000	N/A
1st	Pdxl (Podocalyxin)	Abclonal, Woburn, MA, U.S.	bs-1345R, RRID: AB_2757722	1:200	Rabbit
1st	Na/K ATPase	Hybridoma bank	A6F, RRID: AB_528092	1:100	Mouse
1st	Na/K ATPase	Abclonal	A7878, RRID: AB_2768499	1:100	Rabbit
1st	Zo-1	Biorbyt, Cambs, UK	orb340146 RRID: Not found	1:200	Rabbit
2nd	anti-mouse Alexa 488	Jackson Immunoresearch, West Grove, PA, U.S.	715-545-150, RRID: AB_2340846	1:200	Donkey
2nd	anti-rabbit DyLight 488	Bethyl, Montgomery, TX, U.S.	A120-108D2, RRID: AB_10634082	1:200	Donkey
2nd	anti-mouse DyLight 594	Bethyl	A90-337D4, RRID: AB_10630877	1:200	Donkey

Table S8. Primers used for quantitative RT-PCR

Primer name	**Forward primer (5'→3')**	**Reverse primer (5'→3')**	**Product size (bp)**	**Species**
beta-actin	CCTGGGTATGGAATCCTGTG	AGCAATGATCTTGATCTTCATGG	196	Rat and Mouse
Oct4	GAGGGATGGCATACTGTGGAC	GGTGTACCCCAAGGTGATCC	272	
Gata4	GTGCCAACTGCCAGACTACC	CCTCCTTCCGCATTGCAAGA	282	
Gata6	CATCATCACCACCCGACCTA	GCTGAGGCCGTTCATCTTACT	282	
Sox17	GAACCCGGATCTGCACAACG	CTTCTCCGCCAAGGTCAACG	76	
Sox7	GAGAGGAAACGTCTGGCAGT	CTGCCTCATCCACATAGGGC	121	
Occludin	GGCCTTTTGAGAGTCCACCT	AAAGAGTATGCCGGCTGAGA	127	
Cdh1	AAGGGCTTGGATTTTGAGG	AAATGGGGGCTTCATTCAC	139	
Ihh	CCTGTCAGCTGTAAAGCCAGG	GGAGCATAGGACCCAAGGG	336	
Epcam	GAGTCCTTGTTCCATTCAT	TCTCCTTTATCTCAGCCTTC	250	
Cited1	CCACTAGCCCCTCTGGATCA	AGCCCCTTGGTACTGGCTAT	146	
Lrp2	GCAGAGATGGACAGTGAGGT	GCTGGCGAGGCTATACG	175	
Cer1	TTGCTTTGGGAAATGCGGTT	GTGCGTGGTGGTGAATTTGG	100	
Lefty1	CTTGATCGACCGCCAGTCTTG	CTGCCACCTCTCGAAAGTTCT	151	
Otx2	TCTTCATGCGGGAGGAGGTA	TCTGACCTCCGTTCTGTTGC	123	
Afp	GCCCAGCATATGAAGAAAACA	TCTCTTTGTCTGGAAGCATTCCT	176	
Ttr	TGGACACCAAATCGTACTGG	GATGGTGTAGTGGCGATGA	104	
Apoa4	CCCAGCTAAGCAACAATGCC	CATCATGTTGGCCCGTAGGT	236	
Krt19	CTAATGGCGAGCTGGAGGTGAAG	GGCGGGCATTGTCGATCTGTAGGA	168	Rat only
Hnf4a	GAGTTTGAAAATGTGCAGGTGTTGAC	GCTGTTGGATGAGTTGAGGTTG	81	
Zo1	GCGGGCTACCTTATTGAATG	GTCATGGGAGCGAACTGAAT	124	
Apoa1	AGGGTGAAGGATTTCGCCAC	CTGTTCCTGTAGGCGACCAA	162	
ApoB	TGGAGAACTGACTGCACACG	CCCTCCAAGTCCCAAAGTCC	276	

Table S9. Published gene expression profiling data downloaded from GEO database

Accession No.	Source	Cell type	Plat-form	Species
SRX2963215	https://www.ncbi.nlm.nih.gov/sra/SRX2963215	E3.5 Epiblast	RNA-seq	Mouse
SRX2963247	https://www.ncbi.nlm.nih.gov/sra/SRX2963247		RNA-seq	Mouse
SRX2963257	https://www.ncbi.nlm.nih.gov/sra/SRX2963257		RNA-seq	Mouse
SRX2963266	https://www.ncbi.nlm.nih.gov/sra/SRX2963266		RNA-seq	Mouse
SRX2963325	https://www.ncbi.nlm.nih.gov/sra/SRX2963325	E4.5 Epiblast	RNA-seq	Mouse
SRX2963353	https://www.ncbi.nlm.nih.gov/sra/SRX2963353		RNA-seq	Mouse
SRX2963356	https://www.ncbi.nlm.nih.gov/sra/SRX2963356		RNA-seq	Mouse
SRX2963362	https://www.ncbi.nlm.nih.gov/sra/SRX2963362		RNA-seq	Mouse
SRX2963469	https://www.ncbi.nlm.nih.gov/sra/SRX2963469	E5.5 Epiblast	RNA-seq	Mouse
SRX2963478	https://www.ncbi.nlm.nih.gov/sra/SRX2963478		RNA-seq	Mouse
SRX2963490	https://www.ncbi.nlm.nih.gov/sra/SRX2963490		RNA-seq	Mouse
SRX2963498	https://www.ncbi.nlm.nih.gov/sra/SRX2963498		RNA-seq	Mouse
SRX2963686	https://www.ncbi.nlm.nih.gov/sra/SRX2963686	E6.5 Epiblast	RNA-seq	Mouse
SRX2963796	https://www.ncbi.nlm.nih.gov/sra/SRX2963796		RNA-seq	Mouse
SRX2963808	https://www.ncbi.nlm.nih.gov/sra/SRX2963808		RNA-seq	Mouse
SRX2963827	https://www.ncbi.nlm.nih.gov/sra/SRX2963827		RNA-seq	Mouse
SRX2963225	https://www.ncbi.nlm.nih.gov/sra/SRX2963225	E3.5 Primitive endoderm	RNA-seq	Mouse
SRX2963238	https://www.ncbi.nlm.nih.gov/sra/SRX2963238		RNA-seq	Mouse
SRX2963241	https://www.ncbi.nlm.nih.gov/sra/SRX2963241		RNA-seq	Mouse
SRX2963267	https://www.ncbi.nlm.nih.gov/sra/SRX2963267		RNA-seq	Mouse
SRX2963315	https://www.ncbi.nlm.nih.gov/sra/SRX2963315	E4.5 Primitive endoderm	RNA-seq	Mouse
SRX2963319	https://www.ncbi.nlm.nih.gov/sra/SRX2963319		RNA-seq	Mouse
SRX2963326	https://www.ncbi.nlm.nih.gov/sra/SRX2963326		RNA-seq	Mouse
SRX2963346	https://www.ncbi.nlm.nih.gov/sra/SRX2963346		RNA-seq	Mouse
SRX2963467	https://www.ncbi.nlm.nih.gov/sra/SRX2963467	E5.5 Visceral endoderm	RNA-seq	Mouse
SRX2963481	https://www.ncbi.nlm.nih.gov/sra/SRX2963481		RNA-seq	Mouse
SRX2963506	https://www.ncbi.nlm.nih.gov/sra/SRX2963506		RNA-seq	Mouse
SRX2963699	https://www.ncbi.nlm.nih.gov/sra/SRX2963699	E6.5 Visceral endoderm	RNA-seq	Mouse
SRX2963705	https://www.ncbi.nlm.nih.gov/sra/SRX2963705		RNA-seq	Mouse
SRX2963787	https://www.ncbi.nlm.nih.gov/sra/SRX2963787		RNA-seq	Mouse
SRX1479560	https://www.ncbi.nlm.nih.gov/sra/SRX1479560	E9.5 Visceral yolk sac	RNA-seq	Mouse
SRX1479561	https://www.ncbi.nlm.nih.gov/sra/SRX1479561		RNA-seq	Mouse
SRX1479562	https://www.ncbi.nlm.nih.gov/sra/SRX1479562		RNA-seq	Mouse
SRX1479563	https://www.ncbi.nlm.nih.gov/sra/SRX1479563		RNA-seq	Mouse
SRX1479564	https://www.ncbi.nlm.nih.gov/sra/SRX1479564		RNA-seq	Mouse
SRX1479565	https://www.ncbi.nlm.nih.gov/sra/SRX1479565		RNA-seq	Mouse

SRX1479566	https://www.ncbi.nlm.nih.gov/sra/SRX1479566	E12.5 Visceral yolk sac	RNA-seq	Mouse
SRX1479567	https://www.ncbi.nlm.nih.gov/sra/SRX1479567		RNA-seq	Mouse
SRX1479568	https://www.ncbi.nlm.nih.gov/sra/SRX1479568	E16.5 Visceral yolk sac	RNA-seq	Mouse
SRX1479569	https://www.ncbi.nlm.nih.gov/sra/SRX1479569		RNA-seq	Mouse
SRX1479570	https://www.ncbi.nlm.nih.gov/sra/SRX1479570		RNA-seq	Mouse
SRX1479571	https://www.ncbi.nlm.nih.gov/sra/SRX1479571		RNA-seq	Mouse
SRX3325623	https://www.ncbi.nlm.nih.gov/sra/SRX3325623	pXEN cells (ground state)	RNA-seq	Rat
SRX3325624	https://www.ncbi.nlm.nih.gov/sra/SRX3325624		RNA-seq	Rat
SRX3325625	https://www.ncbi.nlm.nih.gov/sra/SRX3325625		RNA-seq	Rat
SRX3325626	https://www.ncbi.nlm.nih.gov/sra/SRX3325626		RNA-seq	Rat

Table S10 A. Overlapped upregulated genes of three versions of vacuolated rat pXEN cells (BCS-treated; Chir-treated; Chir-treated and AP-sorted) (related to Fig.2A, upper left)
B. Overlapped downregulated genes of three versions of vacuolated rat pXEN cells (BCS-treated; Chir-treated; Chir-treated and AP-sorted) (related to Fig.2A, upper right)
C. Overlapped upregulated genes of two versions of rat pXEN vesicles (serum-treated; serum free) (related to Fig.S4A, upper left)
D. Overlapped downregulated genes of two versions of rat pXEN vesicles (serum-treated; serum free) (related to Fig.S4A, upper right)

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Table S11 A. Functional annotation of vacuolization-associated rat pXEN cell genes, identified by comparison with the ground state ($p < 0.002$) (related to Fig.2A, bottom)
B. Functional annotation of vesiculation-associated rat pXEN cell genes, identified by comparison with the ground state ($p < 0.004$) (related to Fig.S4A, bottom)

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Table S12. Normalized gene expression profiling data.

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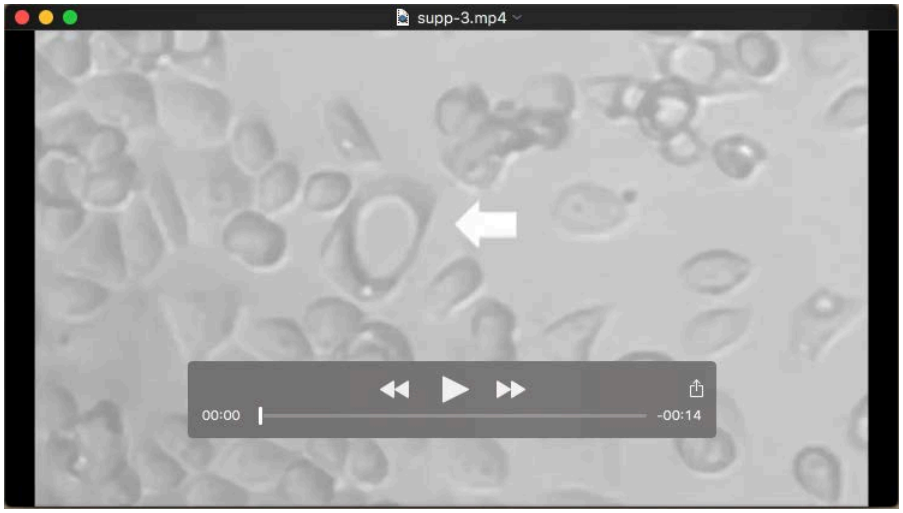
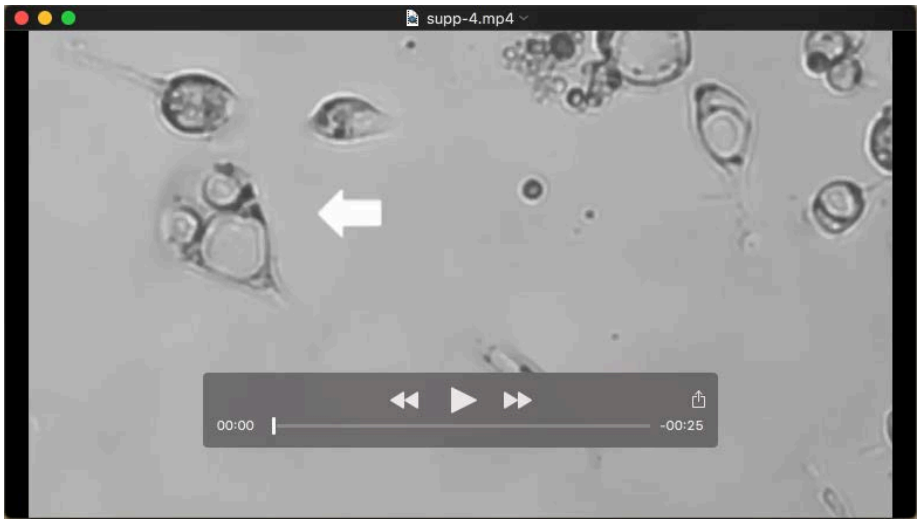
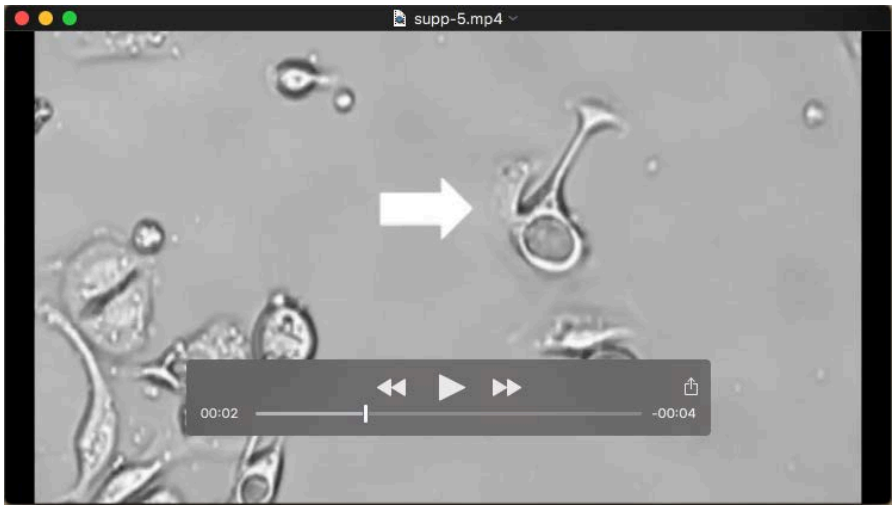


Movie 1. Reversal of vacuolization by cell-cell contact. It is shown how a single vacuolated cell (arrow) loses its vacuole when coming into contact with several non-vacuolated cells. The movie was taken 48 hours after adding 16% FBS.

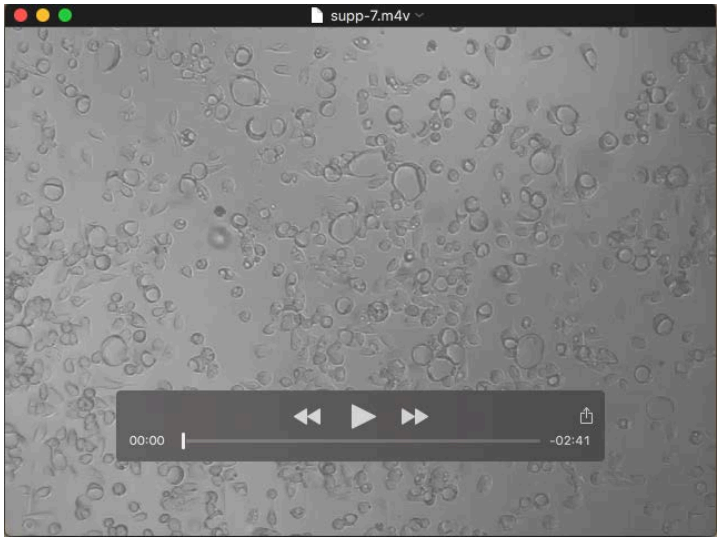


Movie 2. Intracellular fusion of vacuoles. Movie shows that in a single cell (arrow), 3 vacuoles merged into one. This movie relates to Fig. 1J.

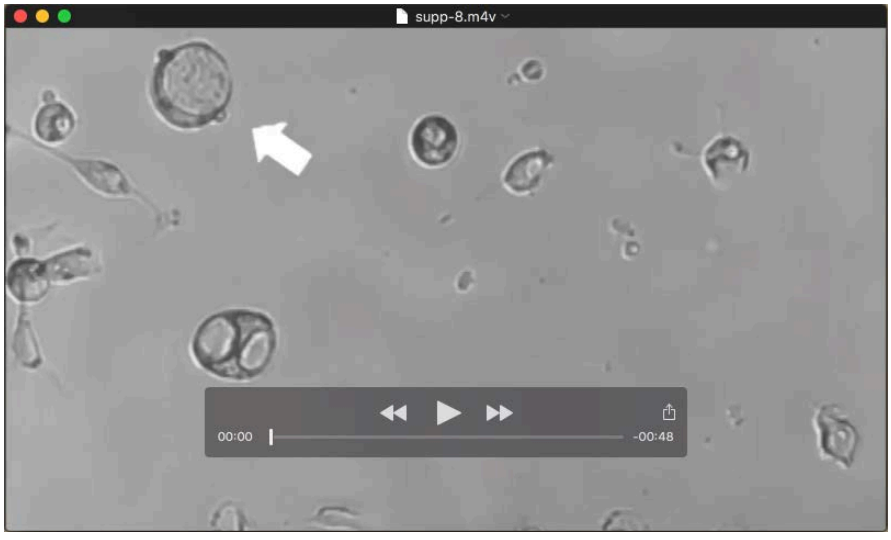


Movie 3. Reversibility of vacuolization by transfer into low-serum condition. Arrow indicates affected cell. This movie is related to Fig. 3B.

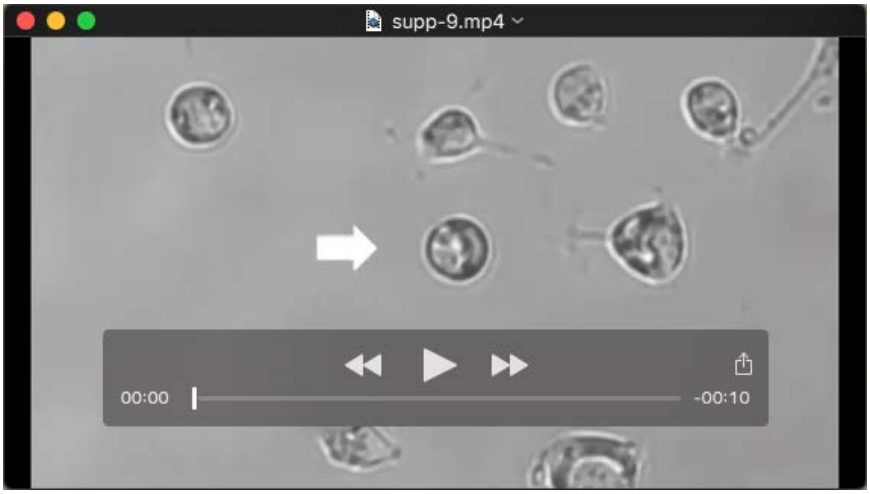
Movie 4. Aberrant cytokinesis and death of a vacuolated cell. Arrow indicates cell of interest. This movie is related to Fig. 3D.



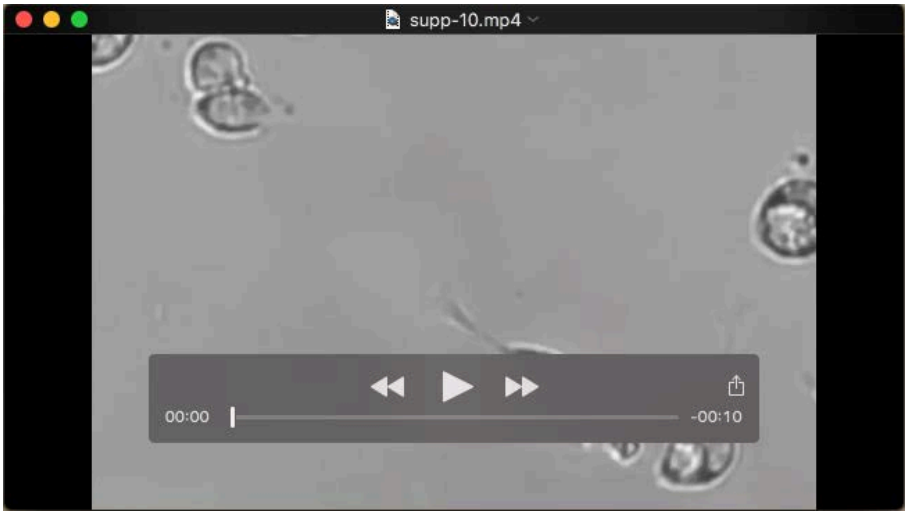
Movie 5. Population analysis (50 cells) of vacuolated cell fates. This movie is related to Fig. S2.



Movie 6. Patterns of cell division in surviving vacuolated cells: Type 1. This movie is related to Fig. 3E (top). Arrow indicates mother cell and arrow head indicates daughter cell.

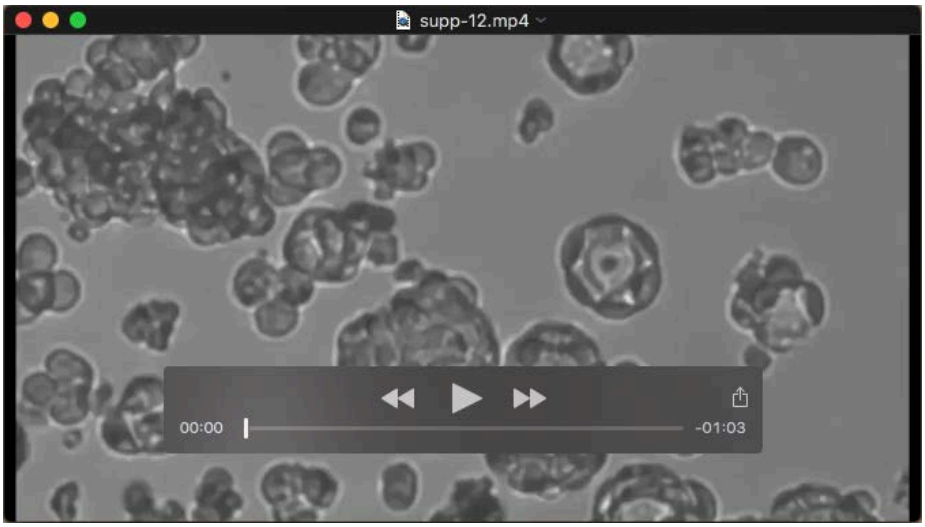


Movie 7. Patterns of cell division in surviving vacuolated cells: Type 2. This movie is related to Fig. 3E (middle). Arrow indicates cell of interest.



Movie 8. Patterns of cell division in surviving vacuolated cells: Type 3. This movie is related to Fig. 3E (bottom). Arrow indicates cell of interest.

Movie 9. Formation of nascent vesicle. It is shown how 3 vacuolated cells that are in contact produce a single lumen from the vacuoles. This movie is related to Fig. S3F (top). The arrow indicates the microaggregate of interest.



Movie 10. Expansion of vesicle. The movie shows how, starting from the 3-cell stage, the vesicle is expanding in size and cell number. This movie is related to Fig. S3F (bottom). The arrow indicates the vesicle of interest.