

SUPPLEMENTARY INFORMATION

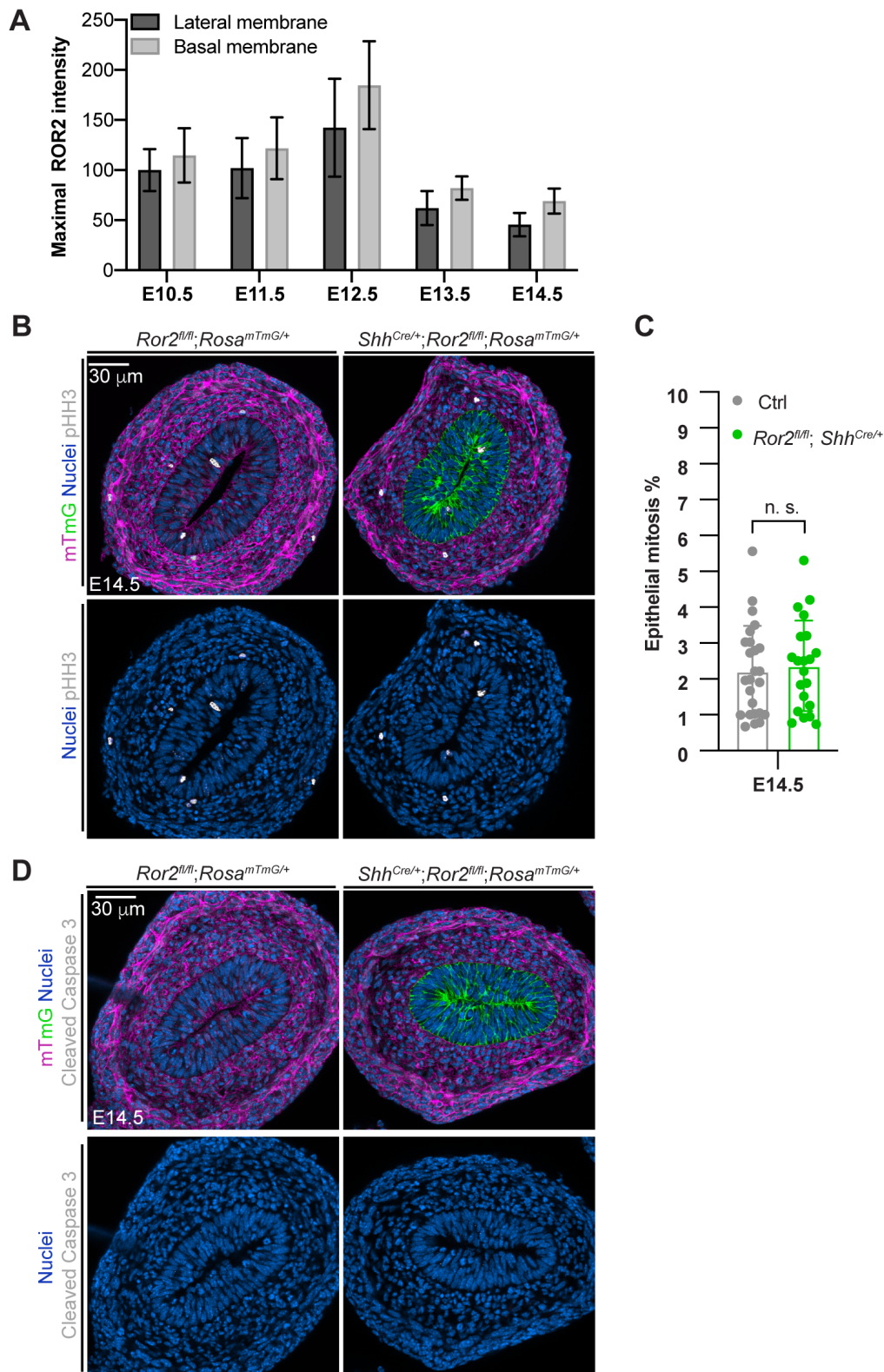


Fig. S1. Epithelial mitosis rate is unaffected after the loss of epithelial ROR2, related to Fig. 1.

(A) Quantification of the maximal ROR2 level on the lateral/basal membranes of epithelial cells at E10.5–14.5. For each stage, measurements were performed on 10 sections of 5 samples.

(B-C) Immunostaining of pHH3 on cross-sections of the control and epithelial *Ror2* depleted midguts. Quantitation of the epithelial mitosis rate was performed on 25 cross-sections of 5 control samples and 20 cross-sections of 4 *Ror2^{flox/flox}; Shh^{Cre/+}* samples.

(D) Immunostaining of cleaved caspase 3 (white) on cross-sections of midguts. No clear apoptosis was detected in either control or epithelial *Ror2* depleted midguts. Data are represented as mean \pm s.e.m. Analyses were performed using unpaired nonparametric tests (Mann-Whitney test). n. s. = not significant.

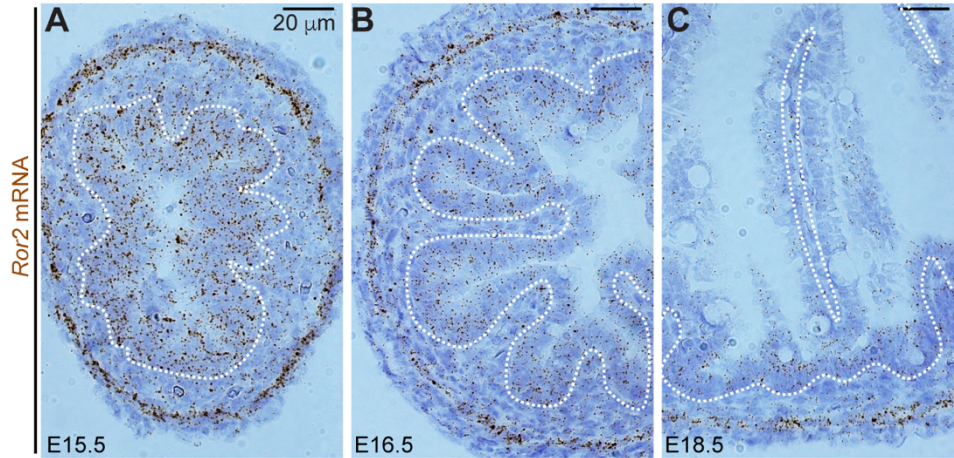


Fig. S2. *Ror2* is present in the midgut throughout Phase II, related to Fig. 2.

(A-C) RNAscope of *Ror2* on cross-sections of wildtype midguts at E15.5, E16.5, and E18.5. The epithelial-mesenchymal interface is outlined by white dashed lines. Scale bar, 20 μm.

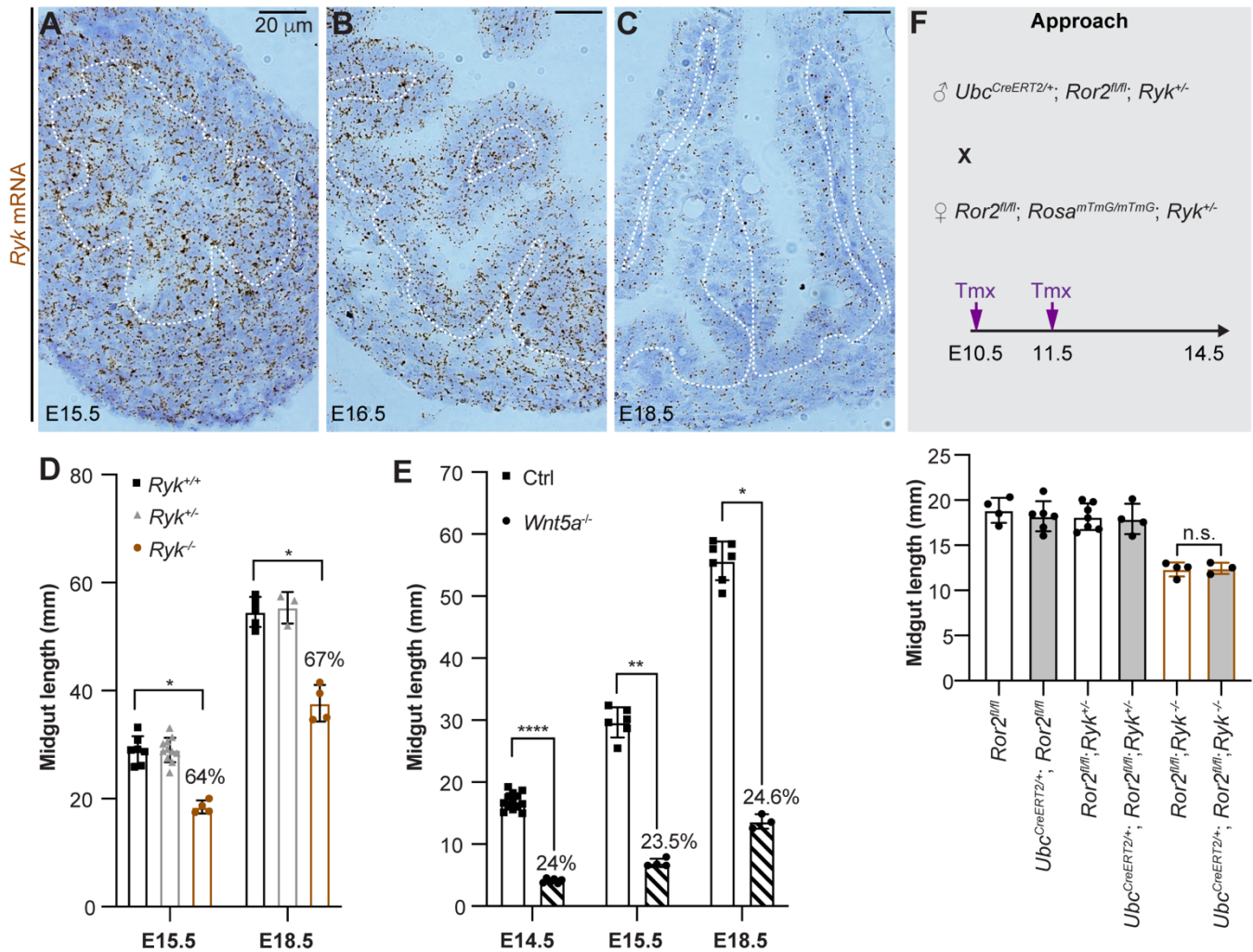


Fig. S3. *Ryk* is present during Phase II but is not required for Phase II midgut elongation, related to Fig. 3.

(A-C) RNAscope of *Ryk* on cross-sections of wildtype midguts at E15.5, E16.5, and E18.5. The epithelial-mesenchymal interface is outlined by white dotted lines. Scale bar, 20 μ m.

(D) Quantitation of the midgut length in $Ryk^{+/+}$, $Ryk^{+/-}$, and $Ryk^{-/-}$ embryos at E15.5 and E18.5. $Ryk^{+/+}$, n=7 (E15.5), n=4 (E18.5); $Ryk^{+/-}$, n=11 (E15.5), n=3 (E18.5); $Ryk^{-/-}$, n=4 (E15.5), n=4 (E18.5).

(E) Quantitation of the midgut length in control ($Wnt5a^{+/+}$ and $Wnt5a^{+/-}$) and $Wnt5a^{-/-}$ embryos at E14.5, E15.5, and E18.5. Ctrl: n=13 (E14.5), n=6 (E15.5), n=7 (E18.5); $Wnt5a^{-/-}$, n=6 (E14.5), n=4 (E15.5), n=3 (E18.5).

(F) Approach for *Ror2/Ryk* double depletion (tamoxifen treatment beginning at E10.5) and quantitation of the midgut length of indicated genotypes. $Ror2^{fl/fl}$, n=4; $Ror2^{fl/fl}; Ubc^{CreERT2/+}$, n=6; $Ror2^{fl/fl}; Ryk^{+/-}$, n=7; $Ror2^{fl/fl}; Ubc^{CreERT2/+}; Ryk^{+/-}$, n=4; $Ror2^{fl/fl}; Ryk^{-/-}$, n=4; $Ror2^{fl/fl}; Ubc^{CreERT2/+}; Ryk^{-/-}$, n=3. Data are represented as mean \pm s.e.m. Analyses were performed using unpaired nonparametric tests (Mann-Whitney test). * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$, and n. s. = not significant.

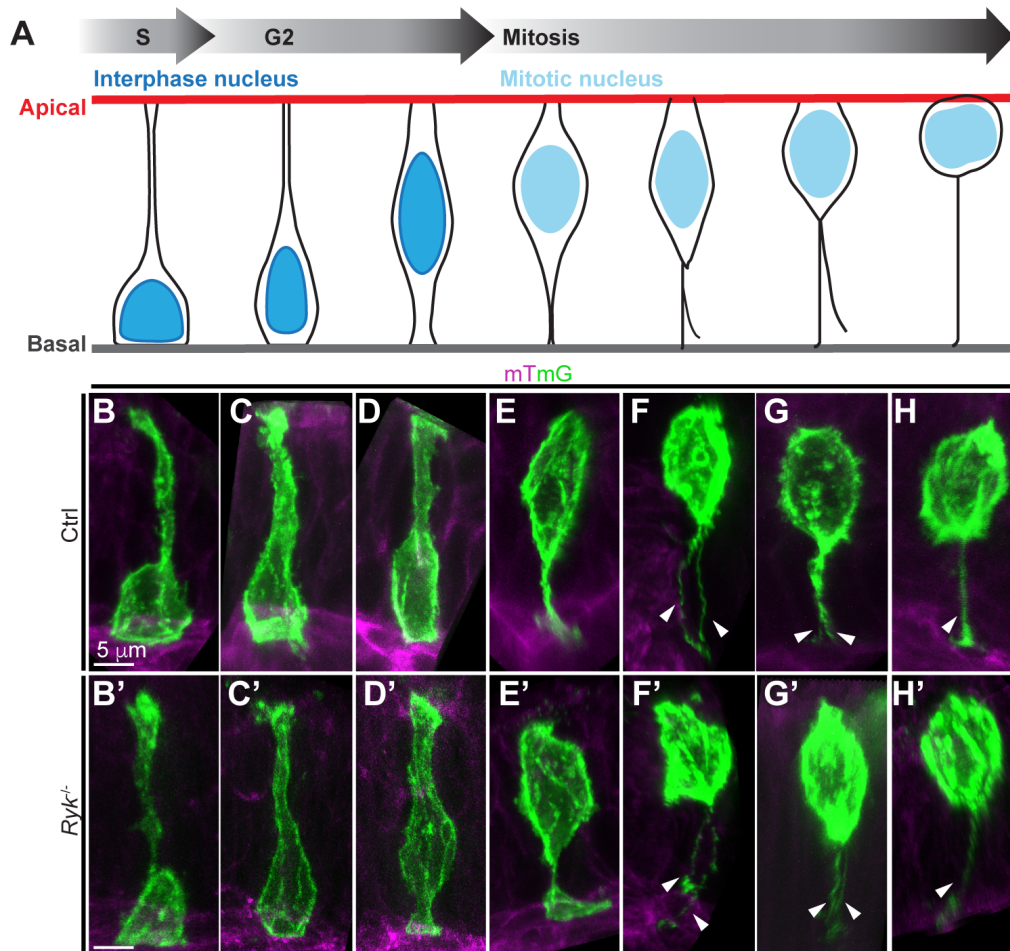


Fig. S4. Epithelial cell behaviors before cytokinesis were unaffected in *Ryk*^{-/-} midguts, related to Fig. 5.

(A) Schematic illustration of cell behaviors before cytokinesis in Phase I midgut epithelium.

(B-H') 3D reconstructions of individual cells before cytokinesis in control and *Ryk*^{-/-} midgut epithelium at E13.5, demonstrating that the apical nuclear migration (B-E, B'-E') and basal process splitting (F, F', G, G') were not affected in the absence of RYK; at the end of mitosis, usually, only one basal process is retained for both control and *Ryk*^{-/-} cells (H, H'). Scale bar, 5 μm.

Table S1. Key resources

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Antibodies		
mouse anti-ROR2	DSHB	Cat# N/A RRID: AB_10804796
rabbit anti-Ki67	Thermo Fisher	Cat# RM-9106-S RRID: AB_149707
mouse anti-Phospho-histone H3	Millipore	Cat# 05-806 RRID: AB_310016
rabbit Cleaved Caspase-3 (Asp175)	Cell signaling	Cat# 9661 RRID: AB_2341188
rabbit anti-GFP Alexa Fluor 488 conjugated	Thermo Fisher	Cat# A-21311 RRID: AB_221477
Alexa Fluor® 488 Goat Anti-Mouse IgG (H+L)	Thermo Fisher	Cat# A-11001 RRID: AB_2534069
Alexa Fluor® 488 Goat Anti-Rabbit IgG (H+L) Antibody	Thermo Fisher	Cat# A-11008 RRID: AB_143165
Alexa Fluor® 546 Goat Anti-Rabbit IgG (H+L)	Thermo Fisher	Cat# A-11010 RRID: AB_2534077
Alexa Fluor® 555 Donkey Anti-Mouse IgG (H+L)	Thermo Fisher	Cat# A-31570 RRID: AB_2536180
Alexa Fluor® 647 Goat Anti-Rabbit IgG (H+L)	Thermo Fisher	Cat# A-21244 RRID: AB_141663
Bacterial and Virus Strains		
Biological Samples		
Chemicals, Peptides, and Recombinant Proteins		
Hoechst	Life Technologies	Cat# 33258
Alexa Fluor™ 647 Phalloidin	Thermo Fisher	Cat# A22287
ProLong™ Gold Antifade Mountant	Life Technologies	Cat# P36930
Tamoxifen	Sigma	Cat# T5648
Focus Clear Kit	Cedarlane Labs	Cat# F101-KIT
1% Penicillin-Streptomycin-Glutamine	Gibco	Cat# 10378016
DMEM	Gibco	Cat# 21063029
Glass-bottom culture dish 35-mm	MatTek Corporation	Cat# P35G-0-20-C
Richard-Allan Scientific™ HistoGel™ Specimen Processing Gel	Thermo Fisher	Cat# HG-4000-012
RNAscope® 2.5 HD Assay- BROWN	ACD	322300
RNAscope® Probe-Mm-Ror2	ACD	430041
RNAscope® Probe-Mm-Ryk	ACD	549981
RNAscope® Negative Control Probe-DapB	ACD	310043
RNAscope® Positive Control Probe-Mm-Ppib	ACD	313911
Critical Commercial Assays		
Deposited Data		
Experimental Models: Cell Lines		
Experimental Models: Organisms/Strains		
Mouse: C57BL6/J	Charles River	Strain Code: 027
Mouse: Ryk ^{+/-} : B6j.129S1/Sv-Ryk ^{tm1STAC}	Stacker lab (Halford et al., 2000)	N/A
Mouse: Ror2 ^{fl/fl} : B6;129S4-Ror2 ^{tm1.1Meg/J}	The Jackson Laboratory	RRID:IMSR_JAX:018354
Mouse: Rosa ^{mTmG/+} : Gt(ROSA)26Sor ^{tm4(ACTB-tdTomato,-EGFP)Luo/J}	The Jackson Laboratory	RRID:IMSR_JAX:007576

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Mouse: <i>Shh</i> ^{CreERT2/+} ; B6.129S6- <i>Shh</i> ^{tm2(cre/ERT2)Cjt/J}	The Jackson Laboratory	RRID:IMSR_JAX:005623
Mouse: <i>Shh</i> ^{Cre/+} ; B6.Cg- <i>Shh</i> ^{tm1(EGFP/cre)Cjt/J}	The Jackson Laboratory	RRID:IMSR_JAX:005622
Mouse: <i>Twist2</i> ^{Cre/+} ; B6.129X1- <i>Twist2</i> ^{tm1.1(cre)Dor/J}	The Jackson Laboratory	RRID:IMSR_JAX:008712
Mouse: <i>Shh</i> ^{CreERT2/+} ; B6.Cg-Ndor1 ^{Tg(UBC-cre/ERT2)1Ejb/1J}	The Jackson Laboratory	RRID:IMSR_JAX:007001
Oligonucleotides		
Primers: <i>Ryk</i>	Stacker lab	
<i>Ryk</i> -GTR: CAAGTAACATGCTCCCCAAAAC	(Halford et al., 2000)	
<i>Ryk</i> -GT WT: CAGGGCAGTGCATTCCATCT		
<i>Ryk</i> -GTKO3: GCGTTGGCTACCCGTGATA		
Primers: <i>Ror2</i> ^{fllox}	The Jackson Laboratory	14698; 14699
Primers: <i>mTmG</i>	The Jackson Laboratory	22163; oIMR9020; oIMR9021
Primers: <i>Shh</i> ^{Cre}	The Jackson Laboratory	oIMR1857; oIMR5960; oIMR5961
Primers: <i>Shh</i> ^{CreERT2}	The Jackson Laboratory	oIMR3798; oIMR7338; oIMR7339; oIMR8346;
Primers: <i>Twist2</i> ^{Cre}	The Jackson Laboratory	15199; 15200; oIMR9174; oIMR9176
Primers: <i>Ubc</i> ^{CreERT2/+}	The Jackson Laboratory	25285; oIMR7338; oIMR7339; oIMR9074
Recombinant DNA		
Software and Algorithms		
Imaris	Bitplane	http://www.bitplane.com/imaris
Fiji	Schindelin et al., 2012	https://imagej.net/Fiji
Other		