SUPPLEMENTAL MATERIAL

Figure S1. PAR protein and exocyst expression in polarized cell types. (A-D') Lateral views of excretory canals in L4 larvae expressing the indicated fluorescent fusion proteins; arrowheads point towards canal cell lumen. (D) and (D') are DIC and fluorescent images of the same canal to show PAR-6-mCherry localization along canal lumen; canal cell is outlined with dashed lines in D. Scale bar is 10 μm. (E-F) *sec-5* mutant expressing SEC-5-YFP in 1-cell (E) and 8-cell embryos (F); arrowheads indicate enrichment at the anterior (E) or contact-free (F) surface. (G-G') Comma-stage embryos co-expressing the indicated fluorescent fusion proteins; arrowheads point towards protein enrichment at the lumenal surface of the intestinal epithelium. (H-I''') Fluorescent images of immunostained late embryos showing PKC-3 (H'') and PAR-6 (I'') expression in the excretory canal; canal cell is marked and co-immunostained with ERM-1, shown in H' and I'.

Figure S2. Exocyst localization in *ral-1(MZ)* and *sec-5(MZ)* mutant embryos. (A-B') 8-cell stage *sec-5(MZ)* embryos expressing the indicated fluorescent fusion proteins in embryos depleted of maternal ZF1-tagged protein (A, A') (n = 29), or with depletion prevented by *zif-1* RNAi (B, B') (n = 40). (C-D') 26-cell to 28-cell stage *ral-1(MZ)* mutant embryos (C-C') (n = 30) and embryos with *zif-1* RNAi to block depletion (D, D') (n=22). Dashed areas outline mutant early embryonic cells depleted of the indicated maternally provided ZF1-tagged protein; arrowheads point to enrichment at contact-free surfaces.

Figure S3. Disorganized canals in *ral-1(MZ)* and *sec-5(MZ)* mutant larvae. (A-C) Control (n = 58/58 lumenized) (A), *ral-1(MZ)* (n = 42/45 lumenized) (B), and *sec-5(MZ)* (n

= 32/43 lumenized) (C) mutant L1 larval stage animals displaying a portion of the disorganized posterior canal immediately distal to the pharynx; B and C display distal-most region of truncated mutant canals, and cytoplasmic extensions can be seen extending beyond the lumenized portion. (A'-C') Expression of cytoplasmic mCherry (A', B') or IFB-1-GFP (C') in the larvae shown above in A-C. Pearl regions can be seen in the control larvae in A'. In all panels, boxes display higher resolution of dashed regions. Arrows direct toward canal lumen in each panel. Scale bars = 5 μm.

Figure S4. *sec-5* and *ral-1* mutant canal analysis. (A) L4 larval stage *ral-1* mutant canal. Arrow indicates posterior-most extent of lumen, dotted line underscores continued posterior extension of cytoplasm. Scale bar is 10 μm. (B) Schematic of excretory canal and table displaying quantitation of canal elongation in control and mutant L4 larvae of the indicated genotype. Top bar percentage bins (in gray) are percent body length distal to the excretory canal cell body to the posterior end, traversed by canals. Table percentages (in white) are percent of animals of indicated genotype within each bin.

Figure S5. Localization of SEC-8-mCherry and YFP-RAL-1 relative to canal cytoplasm. (A-B'') L4 larval canals co-expressing the indicated fluorescent fusion proteins. Scale bar is 10 μm, and all panels are at equivalent scales.

Figure S6. Exocyst and PAR protein asymmetry in early embryonic cells. (A-B) 8-cell embryos expressing fluorescently tagged exocyst reporter produced by adults depleted of *par-6* (*n* = 71/81 not polarized) and *par-3* (*n* = 39/39 not polarized) by RNAi.

(C) 8-cell *par-3(MZ)* embryo carrying fluorescently tagged exocyst reporter (*n* = 13/13 not polarized). Arrows in A-C indicate SEC-8-mCherry mislocalized to cell contacts. (D-D') *ral-1(MZ)* embryo co-immunostained for PAR-3 (*n*=31/31 polarized) (D) and ZF1-YFP-RAL-1 (D'). (E-H') Co-immunostained control (*n*=18/18 polarized PAR-6, *n*=17/17 polarized PKC-3) (E,G) and *ral-1(MZ)* mutant embryos (*n*=20/20 polarized PAR-6, *n*=24/24 polarized PKC-3) (F-F', H-H'). PAR-6 and PKC-3 are present at contact-free surfaces (arrowheads). Cells depleted of ZF1-tagged proteins are outlined by dashed areas.

Video S1. Z-stack of tomogram through wild-type L4 excretory canal.

Video S2. Z-stack of tomogram through *ral-1* mutant L4 excretory canal.

Table S1. Phenotype of *ral-1* and exocyst mutants

Genotype	Fertile	Sterile	*n*
wild type	99%	1%	82
ral-1	0%	100%	133
ral-1; yfp-ral-1	52%	48%	216
sec-5	87%[a]	13%	104
sec-5; sec-5-yfp	97%[b]	3%	121
sec-8	3%	97%	69
sec-8; sec-8-mCherry	72%	28%	222

[a]Fertile animals ruptured after laying a few dead eggs and arrested L1 larvae

[b]Rupturing and maternal-effect lethality were rescued

Table S2. Rescue by ZF1-tagged transgenes

Genotype	Fertile	Sterile	*n*
Wild type[a]	99%	1%	82
ral-1[a]	0%	100%	133
ral-1; zf1-yfp-ral-1	100%	0%	98
sec-5[a]	87%[b]	13%	104
sec-5; sec-5-zf1-yfp	98%[c]	2%	130
sec-8[a]	3%	97%	69
sec-8; sec-8-zf1-mCherry	97%	3%	125

[a]Data from Table S1

[b]Fertile animals ruptured after laying a few dead eggs and arrested L1 larvae

[c]Rupturing and maternal-effect lethality were rescued

Table S3. Phenotype of maternal-zygotic *ral-1* and exocyst mutants

Parental genotype	Viable progeny	Dead embryos	Dead larvae	*n*
wild type	99.5%	0.5%	0%	940
ral-1; zf1-yfp-ral-1	97%	2%	1%	134
ral-1; zf1-yfp-ral-1 / +[a]	78%	1%	21%[b]	231
sec-5; sec-5-zf1-yfp	98%	0.5%	1.5%	624
sec-5; sec-5-zf1-yfp / +[a]	79%	5%	16%[b]	788
sec-8; sec-8-zf1-mCherry	99%	0.5%	0.5%	657
sec-8; sec-8-zf1-mCherry / +[a]	79%	8%	13%[b]	804

[a]25% of progeny are expect to be maternal-zygotic mutants

[b]Dead larvae examined lacked transgene and are therefore maternal-zygotic mutants

Table S4. Strains list

Strain	Genotype
N2	wild type
EG1322	*unc-119(ed3)*
WM186	*avr-14(ad1302); tTTi5605; unc-119; glc-1(pk54::Tc1) avr-15(ad1051)*
FT1310	*avr-14; xnSi31(Psec-8::sec-8-mCherry); unc-119; glc-1 avr-15*
FT1379	*avr-14; xnSi34(Psec-15::sec-15-yfp); unc-119; glc-1 avr-15*
VC2648	*sec-8(ok2187)/hT2*
DV2689	*sec-5(pk2358)/mIn1*
FT1411	*ral-1(tm5205)/qC1*
BC06288	*dpy-5(e907); sIs10089(Ppgp-12::gfp)*
FT1370	*xnIs484(Psec-10::mCherry-sec-10); unc-119*
FT1221	*unc-119; xnIs459(Pral-1::yfp-ral-1a)*
FT1371	*unc-119; xnIs485(Psec-10::mCherry-sec-10)*
FT1243	*unc-119; xnIs465(Psec-8::sec-8-mCherry)*
FT1246	*unc-119; xnIs468(Psec-8::sec-8::mCherry)*
FT828	*unc-119; xnIs312(Ppar-6::par-6-mCherry)*
FT1265	*sec-5; xnIs461(Psec-5::sec-5-yfp)*
FT1278	*sec-5; xnIs471(Psec-5::sec-5-zf1-yfp)*
FT1412	*sec-8; xnIs465*
FT1199	*sec-8; xnIs403(Psec-8::sec-8-zf1-mCherry)*
FT1250	*ral-1 xnIs459(Pral-1a::yfp-ral-1a)*
FT1279	*ral-1; xnIs472(Pral-1a::zf1-yfp-ral-1a)*
FT1290	*ral-1; xnIs472; xnIs468*
FT1417	*ral-1; xnIs472; xnIs484*
FT1576	*sec-5; xnIs471; xnIs485*
FT1266	*xnIs3(Ppar-6::par-6-gfp); xnIs465*
FT1414	*sIs10089; ral-1/qC1*
FT1425	*pk2358 sIs10089/mIn1*
FT1413	*xnSi31; juIs176(ifb-1a-gfp)*
FT1294	*unc-119(ed3); xnIs465; xnEx285(Phsp-16.2::gfp-ral-1CA/pTG96*, 20 ng/μL*)*
FT1415	*xnSi31; xnEx322 (Ppgp-12::vha-5-gfp*, 20 ng/μL*)*
FT1419	*unc-119(ed3) xnIs459(yfp-ral-1); xnEx329(Ppgp-12::vha-5-mCherry/pRF4*, 20 ng/μL*)*
FT484	*unc-119; xnIs200(gfp-par-3)*
FT1428	*ral-1; xnIs472; sIs10089*

FT1432	*ral-1/qC1; xnEx335(Ppgp-12::yfp-ral-1; rol-6,* 10 ng/μL*)*
VC277	*pkc-3(ok544)/mIn1*
FT1430	*pkc-3(ne4250); sIs10089*
FT1387	*xnIs459; xnEx313(Ppgp-12::mCherry,* 10 ng/μL*)*
FT1391	*xnEx316(Ppgp-12::yfp-ral-1; rol-6,* 50 ng/μL*)*
FT1281	*par-3(tm2716) unc-32(e189); zuIs20(Ppar-3::par-3-zf1-gfp); xnIs468*
FT1431	*xnSi31; pac-1(xn6) unc-32(e189)*
FT1427	*xnIs468; sIs10089*
FT1587	*tm5205; xnIs472; xnEx487(Ppgp-12::mCherry/pRF4*, 10 ng/μL)
FT1595	*pk2358/mIn1; juIs176*

Table S5. Oligonucleotide List

Transgene	Region amplified (source)	Oligo Sequences
Several	*pgp-12* promotor (genomic)	for = 5'-tgtgcttgcagtgaaccaga-3' rev = 5'-gtttaacctatttcagaagaatatctgttg-3'
Psec-8::sec-8::mCherry *Psec-8-sec-8-zf1-mCherry*	Promoter & coding sequence (genomic)	for = 5'-ggcaagatatgtacggccgc-3' rev = 5'-tttcttctgattgaggatagaatttagaaccatttgtc-3'
	3' UTR (genomic)	for = 5'-ccatgatctttttttattaattaaatttattttaaatatttatgattttaagtgc-3' rev = 5'-ccaagaaggttaatttttcatccaaatttgtg-3'
Psec-5::sec-5-yfp *Psec-5::sec-5-zf1-yfp*	Promoter & coding sequence (genomic)	for = 5'-gcgtaaatggaagaaaacgctcaagc-3' rev = 5'-gatgttggaggtgttgtgagagtc-3'
	3' UTR (genomic)	for = 5'-atcaatttattaaaaacgctttcaatttttctgtg-3' rev = 5'-agactcctttaaaatccccctctt-3'
Pral-1::yfp-ral-1 *Pral-1::zf1-yfp-ral-1*	Promoter (genomic)	for = 5'-ccgattagcaatagagcgc-3' rev = 5'-ggctgaaaattcgagttttag-3'
	Coding sequence & 3' UTR (genomic)	for = 5'-gcatcgaaaaaagcaagcgga-3' rev = 5'-ttcattttgagccgaagaattgtgc-3'
Ppgp-12::vha-5-gfp	Coding sequence & GFP (*mcEx337* from CGC)	for = 5'-atggggtcgttgtcgcgc-3' rev = 5'-ctatttgtatagttcatccatgccatgtgtaatcc-3'
Ppgp-12::vha-5-mCherry	Coding sequence (genomic)	for = 5'-atggggtcgttgtcgcgc-3' rev = 5'-cggtaccaaagctgcttc-3'
Phsp-16.2::GFP-ral-1CA	*ral-1* cDNA (N2 cDNA)	for = 5'-atggcatcgaaaaaagcaagcggaac-3' rev = 5'-ttaaagaattgtgcaatgcttcttgattccgg-3'
par-3 RNAi	*par-3* exon (genomic)	for = 5'-ggatttggcttcactgtgacc-3' rev = 5'-ctgtggatcagcagctgctcc-3'

Figure S1

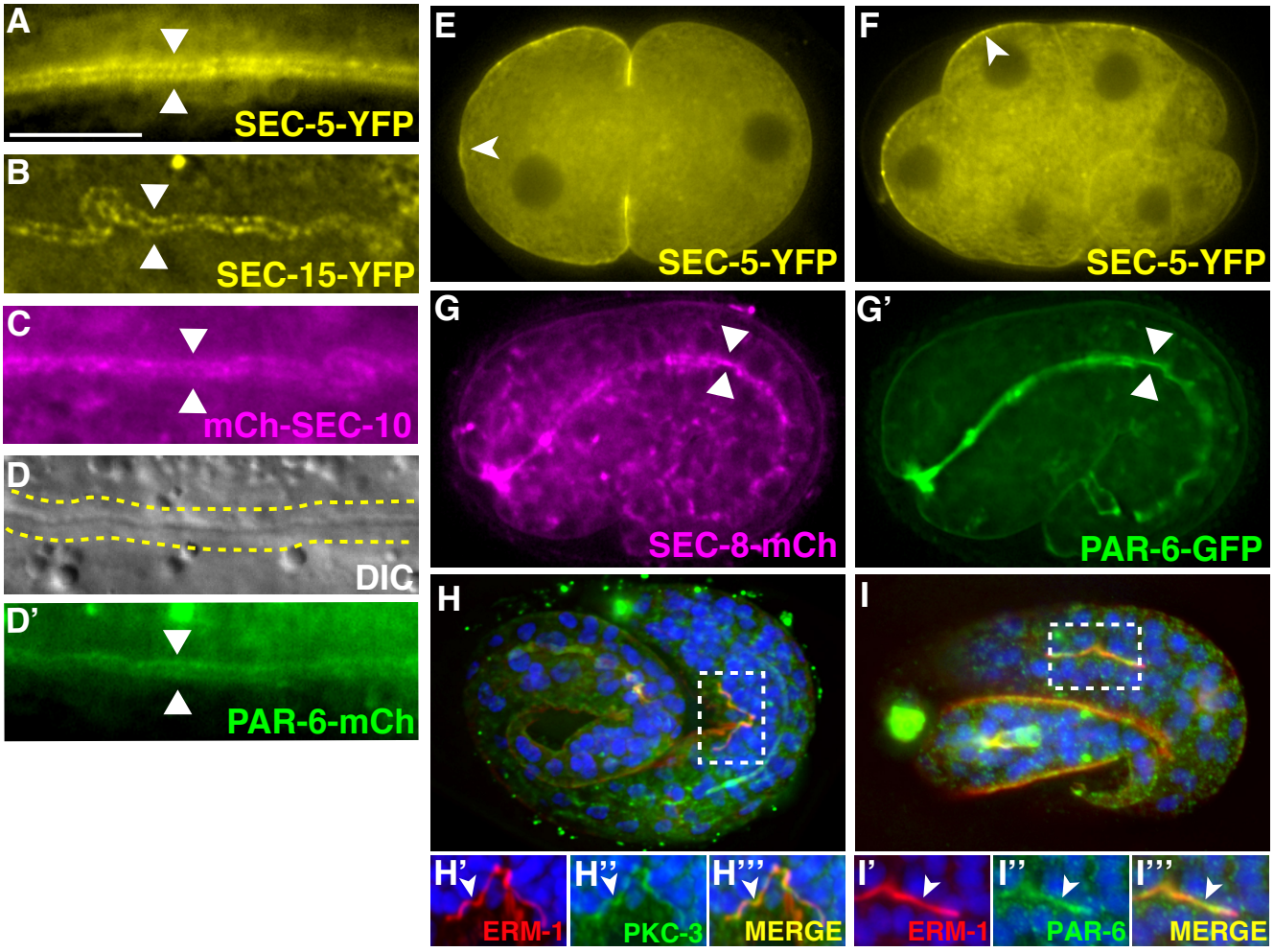

A
SEC-5-YFP
B
SEC-15-YFP
C
mCh-SEC-10
D
DIC
D'
PAR-6-mCh
E
SEC-5-YFP
F
SEC-5-YFP
G
SEC-8-mCh
G'
PAR-6-GFP
H
I
H'
ERM-1
H''
PKC-3
H'''
MERGE
I'
ERM-1
I''
PAR-6
I'''
MERGE

Figure S2

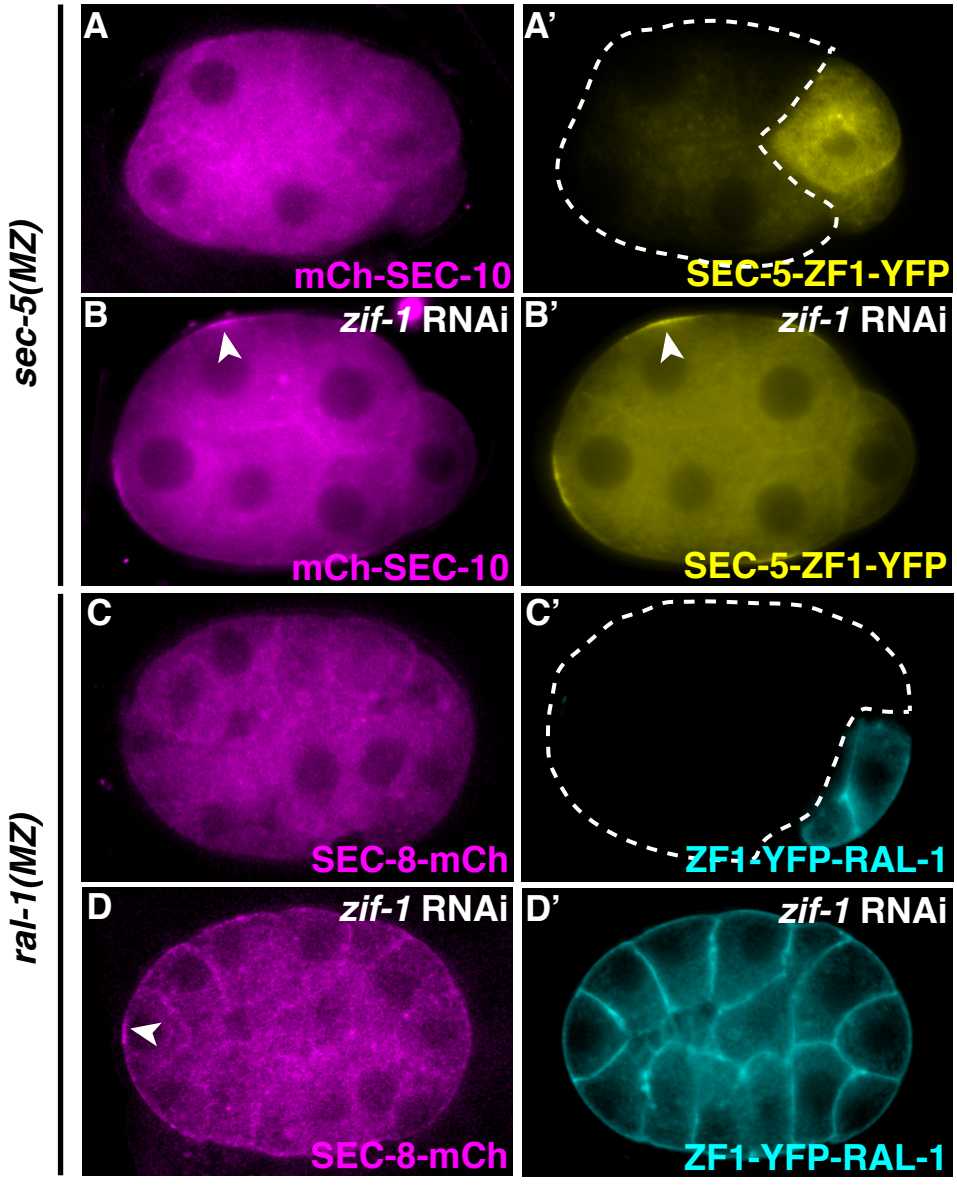

sec-5(MZ)
ral-1(MZ)
A
mCh-SEC-10
A'
SEC-5-ZF1-YFP
B
zif-1 RNAi
mCh-SEC-10
B'
zif-1 RNAi
SEC-5-ZF1-YFP
C
SEC-8-mCh
C'
ZF1-YFP-RAL-1
D
zif-1 RNAi
SEC-8-mCh
D'
zif-1 RNAi
ZF1-YFP-RAL-1

Figure S3

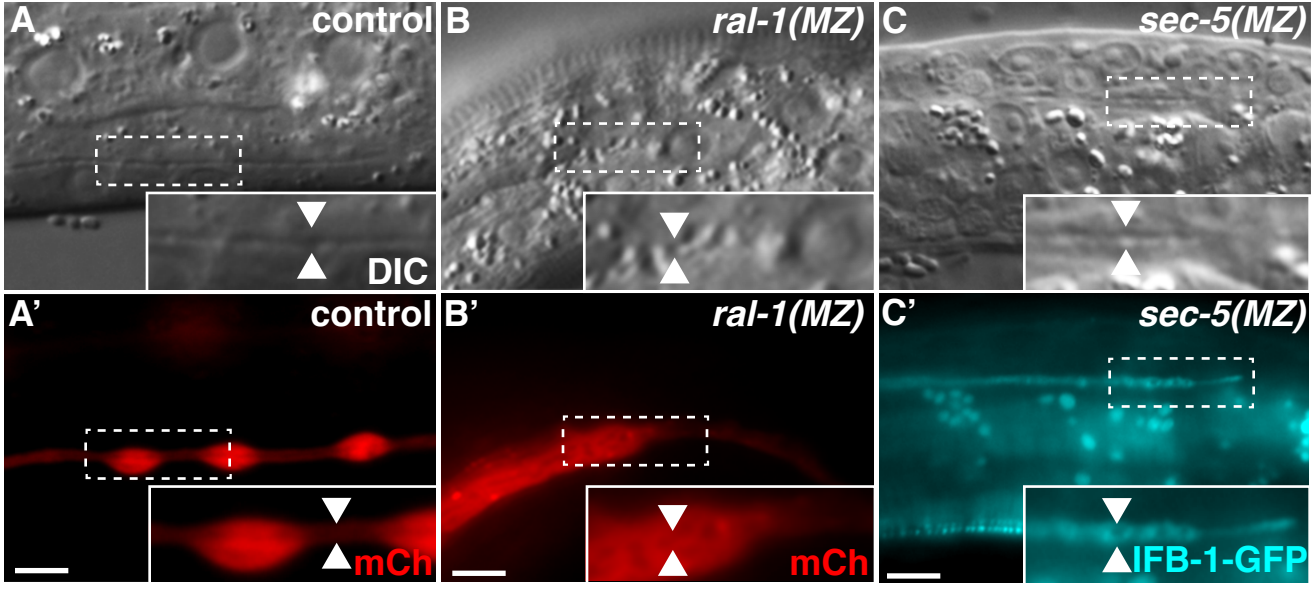
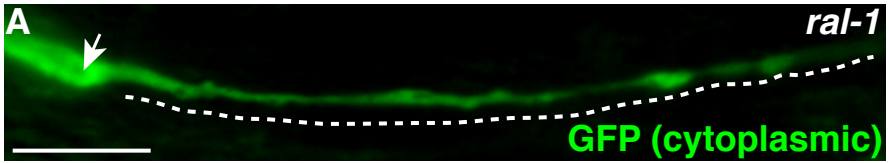

A
control
DIC
B
ral-1(MZ)
C
sec-5(MZ)
A'
control
mCh
B'
ral-1(MZ)
mCh
C'
sec-5(MZ)
IFB-1-GFP

Figure S4



B

	0-25%	25-50%	50-75%	75-100%	Total
control	0%	0%	0%	100%	*n* = 189
sec-5	2%	9%	26%	63%	*n* = 144
ral-1	31%	55%	13%	1%	*n* = 129

Figure S5

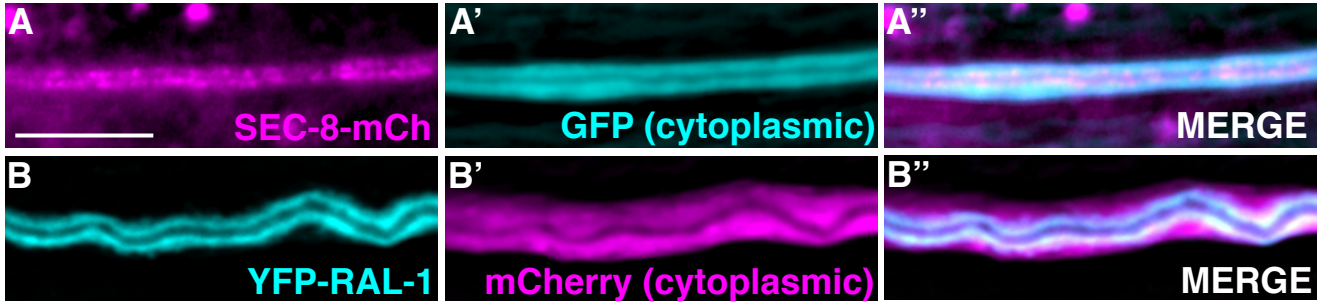
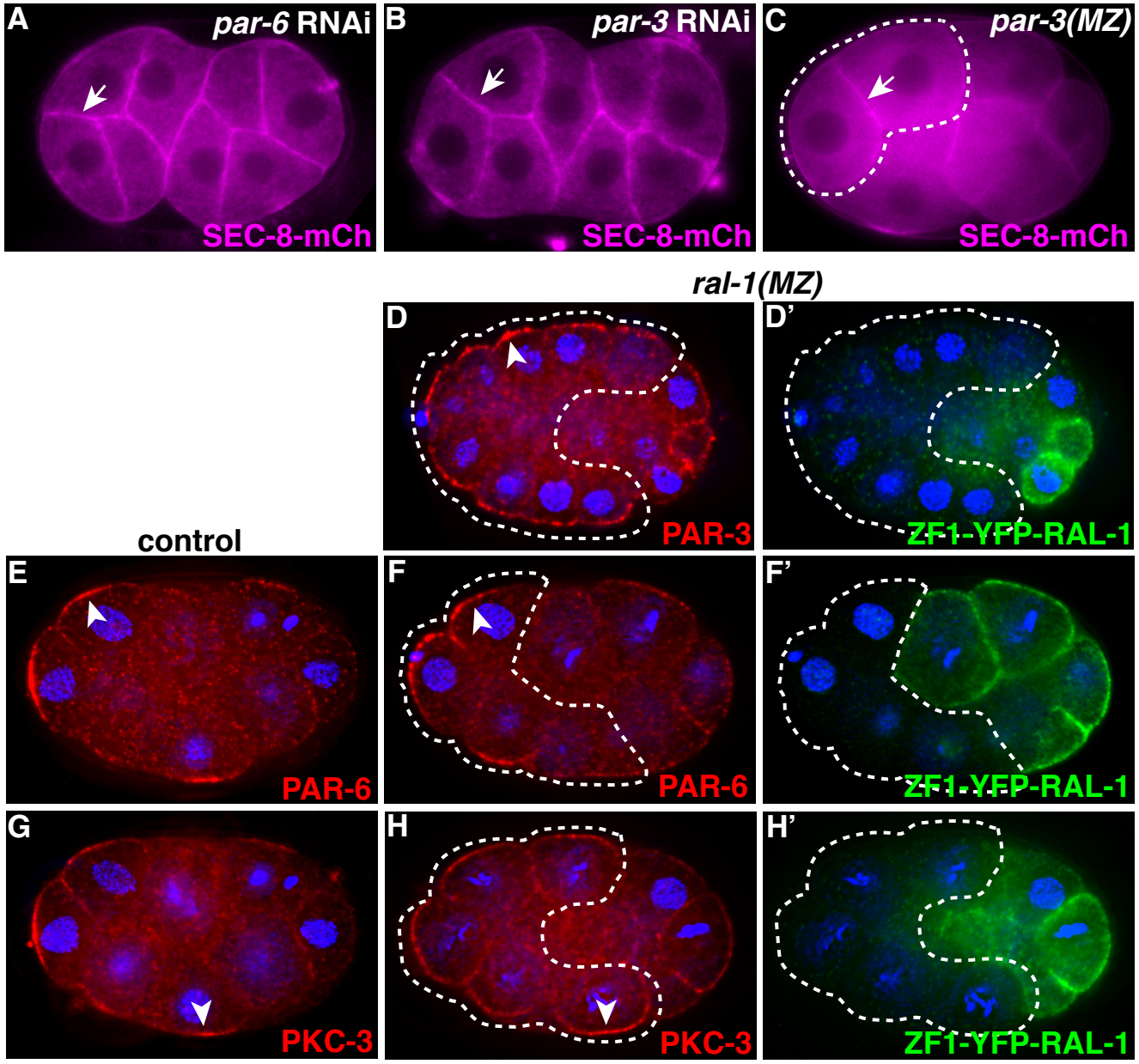

A
SEC-8-mCh
A'
GFP (cytoplasmic)
A''
MERGE
B
YFP-RAL-1
B'
mCherry (cytoplasmic)
B''
MERGE

Figure S6


A
par-6 RNAi
SEC-8-mCh
B
par-3 RNAi
SEC-8-mCh
C
par-3(MZ)
SEC-8-mCh
ral-1(MZ)
D
PAR-3
D'
ZF1-YFP-RAL-1
control
E
PAR-6
F
PAR-6
F'
ZF1-YFP-RAL-1
G
PKC-3
H
PKC-3
H'
ZF1-YFP-RAL-1