SUPPLEMENTARY INFORMATION

Supplementary Materials and Methods

Nematode strains and genetics: In addition to those referenced in the main text, a strain harboring the following genetic lesion was utilized: *LG I - crml-1(n1962)*. In addition to those in the main text, the following transgenic arrays were utilized in this study: *Ex1039[P_{ced-10}::GFP::ced-10, unc-76(+)]* (Lundquist et al., 2001), *qyEx123 [P_{ephx-1}::GFP, unc-119(+)]* (Ziel et al., 2009), *sEx14969[P_{vav-1}::GFP, dpy-5(+)]* (McKay et al., 2003), *sEx13403[$P_{unc-73a::}$GFP, dpy-5(+)]* (McKay et al., 2003), *qyEx115 [P_{tiam-1}::GFP, unc-119(+)]* (Ziel et al., 2009); *muIs28[mig-2::GFP, unc-31(+)]* (Zipkin et al., 1997).

GFP Induction using NMD system: To induce the conditional expression system, we incubated mothers at 25°C for 24 hours prior to harvesting embryos. Embryos of both induced and uninduced groups were filmed at 20°C using the same settings. ImageJ was used to measure the total GFP signal per embryo. Area-adjusted background signal was subtracted from the total GFP signal prior to statistical analysis.

Expression Data: RNA sequencing data from wild-type embryos by the Waterson group in conjunction with the ModENCODE project (Celniker et al., 2009) were accessed on WormBase (Howe et al., 2012). SAGE data were obtained from the Genome BC *C. elegans* Gene Expression Consortium http://elegans.bcgsc.bc.ca/.

RNA interference: yk656a1 (a kind gift from Dr. Yuji Kohara) was used to synthesize *vav-1* double stranded RNA. The pseudocoelomic cavities of *unc-73(rh40)* worms were injected with *vav-1* RNA at 2 µg/µL. Embryos were analyzed 20-24 hours later.

Supplementary Figures

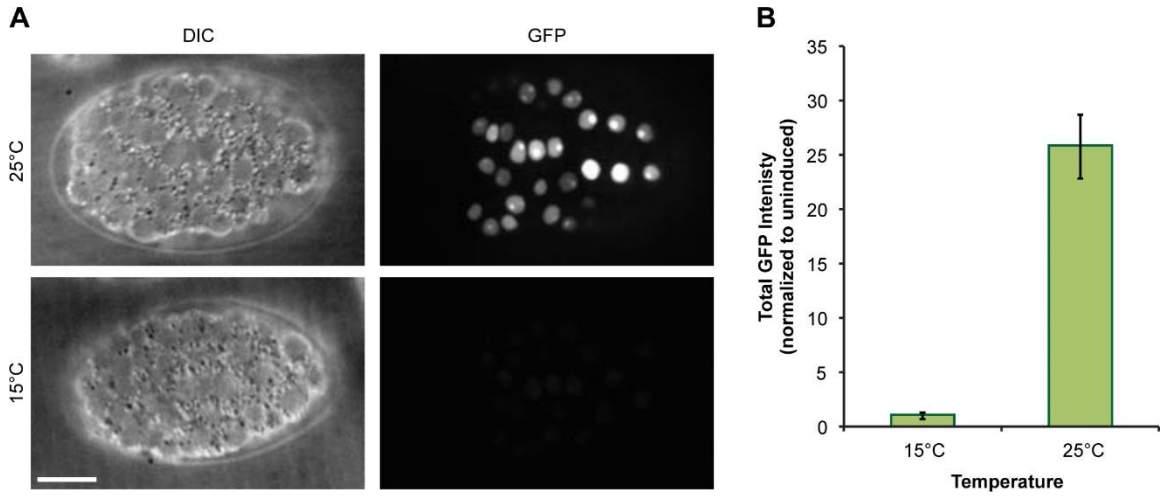


Figure S1. GFP induction using the NMD-based conditional expression system. A) Micrographs of intercalating *P_{lin-26}::gfp::smg sensi ive 3 TR; smg-1(cc546*ts*)* embryos with (25°C) or without (15°C) induction. Scale bar is 10 µm. B) Quantification of induction based on total embryo GFP intensity. Intensity values were normalized to uninduced controls. Induced embryos express significantly more GFP than uninduced control (Student's T-test, $p<0.001$).

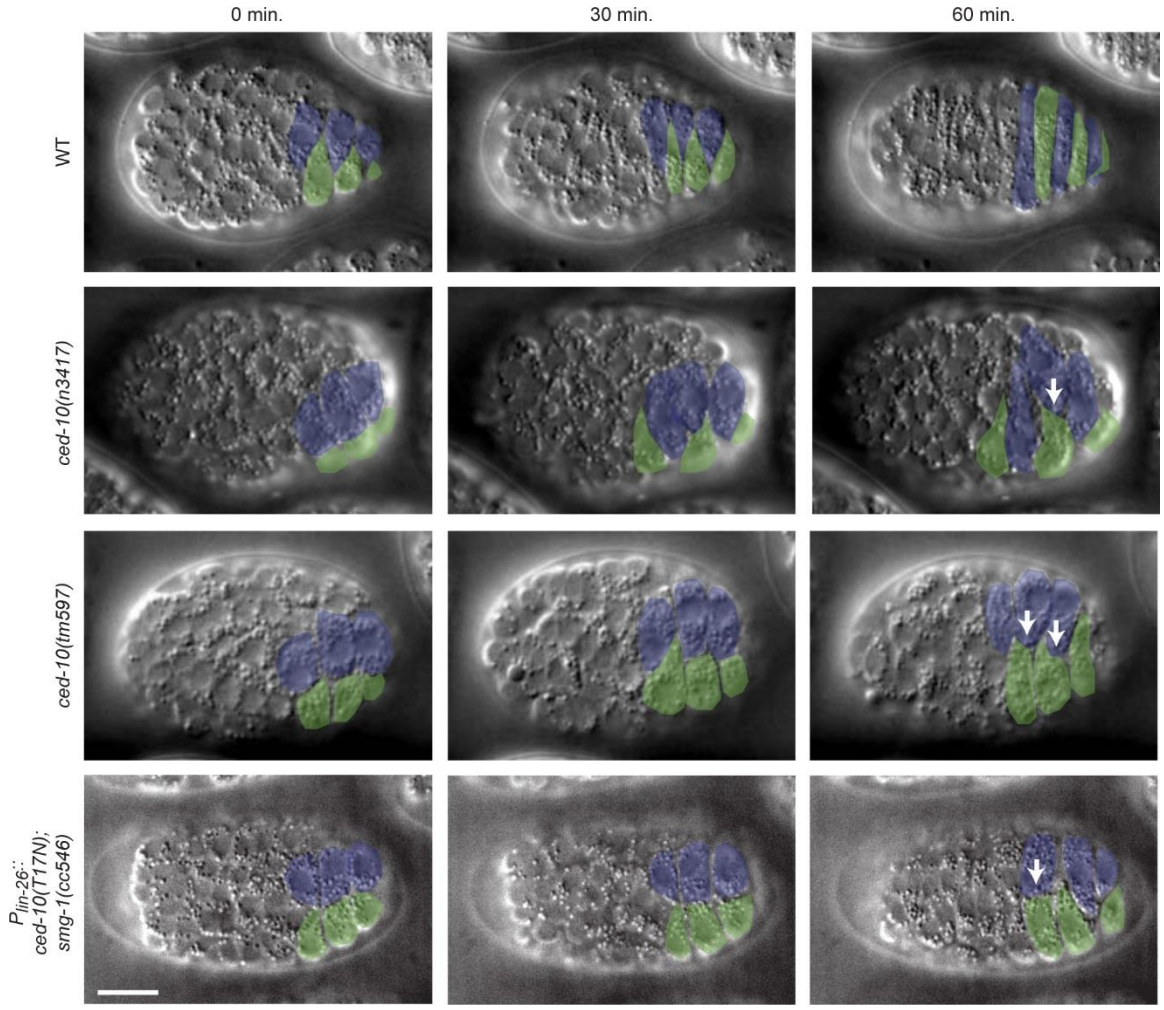


Figure S2. Epidermal-specific dominant-negative *ced-10/Rac* expression phenocopies *ced-10* null mutants. *ced-10(n3417)* and *ced-10(m597)* intercalating cells have blunt medial edges (white arrows), similar to *ced-10(T17N/ N)*. The first time point (0 min.) is one hour after terminal epidermal cell divisions. Left-hand cells are pseudocolored green, right-hand cells are pseudocolored blue. Scale bar = 10 μm.

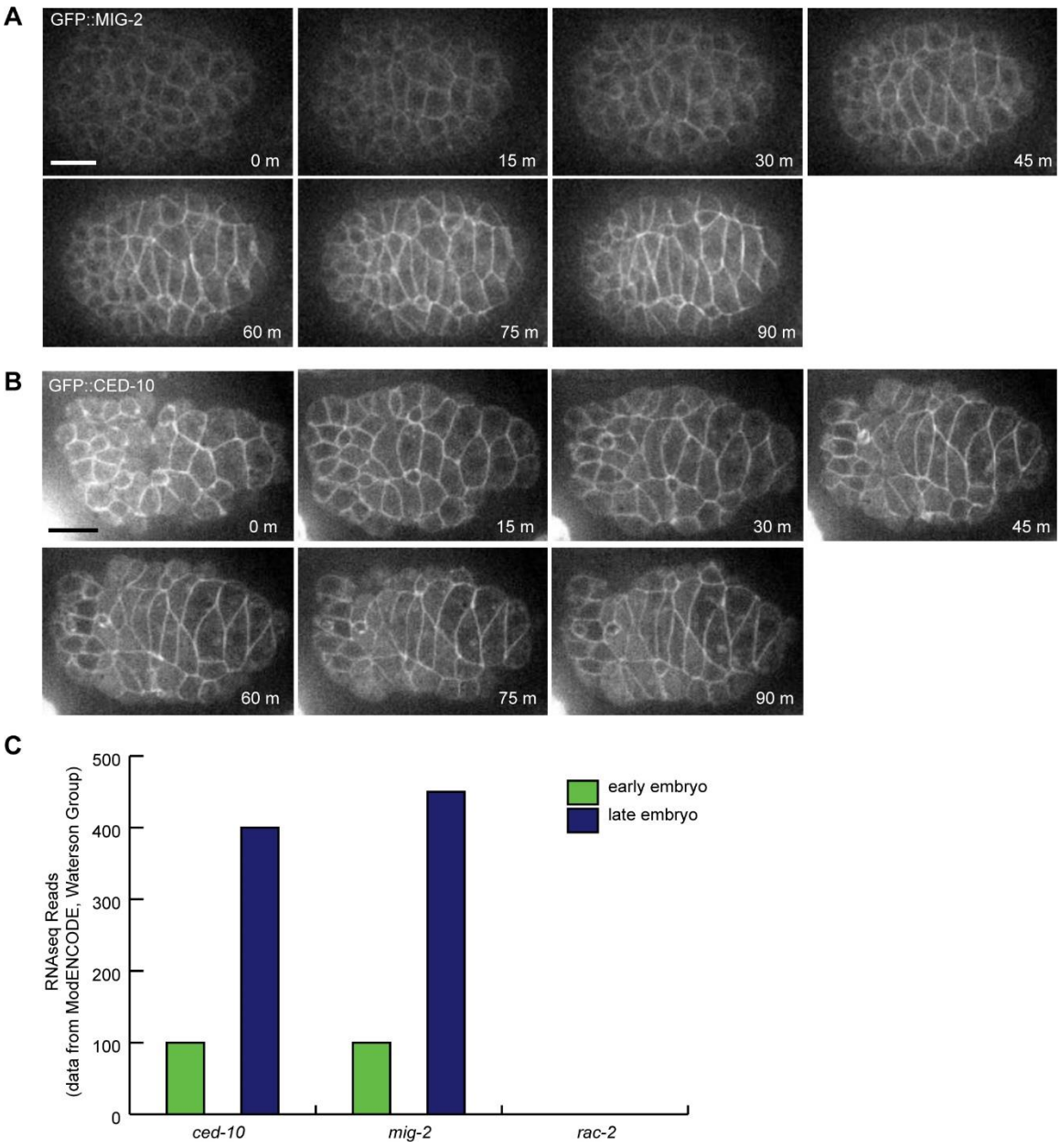


Figure S3. Expression of *C. elegans* Rac homologs during dorsal intercalation. A) GFP::MIG-2 becomes more intense as intercalation proceeds. Scale bar = 10 μm. B) GFP::CED-10 localizes to cell membranes during intercalation. Scale bar = 10 μm. C) *ced-10* and *mig-2* but not *rac-2* are expressed in embryos. RNAseq data (freely available from the ModENCODE pro ect; Celniker et al, 2009) from early and late embryos shows that mRNA for *ced-10* and *mig-2* accumulates in late embryos, consistent with GFP expression.

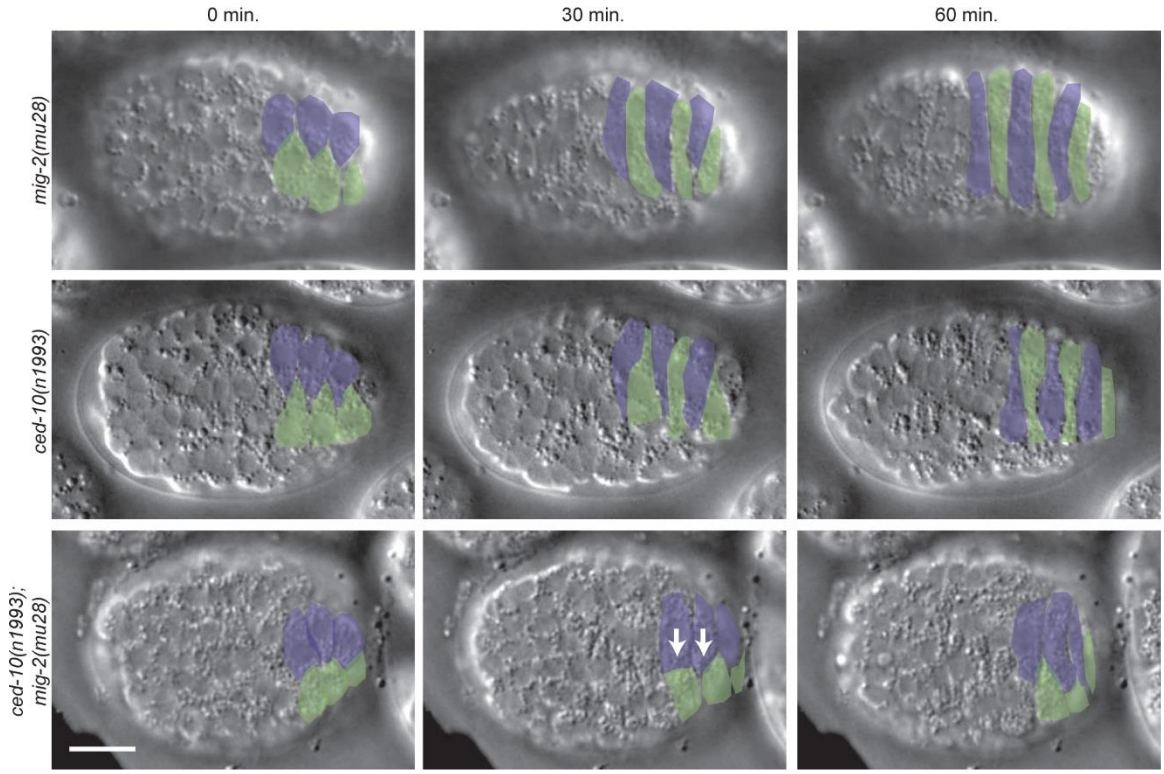


Figure S4. *mig-2(mu28); ced-10(n1993)* intercalating cells have blunt medial edges. Whereas *mig-2(mu28)* null and hypomorphic *ced-10(n1993)* embryos intercalate normally, double mutants often exhibit blunt medial edges (white arrows). Left-hand cells are pseudocolored green, right-hand cells are pseudocolored blue. The first time point (0 min.) is one hour after terminal epidermal cell divisions. Scale bar = 10 μm.

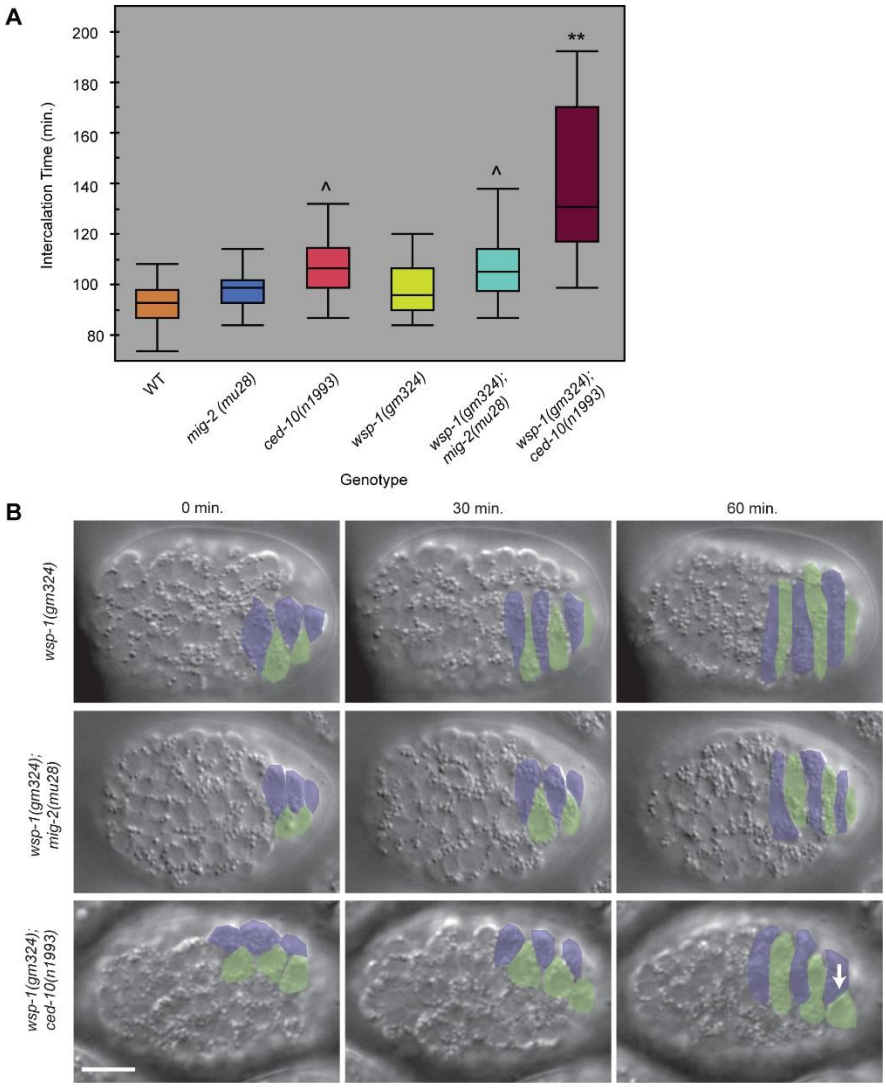


Figure S5. *wsp-1/WASP* and *ced-10/Rac* are in parallel pathways during intercalation. A) Intercalation delay in *wsp-1(gm324)* embryos is enhanced by the weak *ced-10* allele, *n1993*. *mig-2(mu28);wsp-1(gm324)* mutants are not significantly different than either single mutant. **, significantly different than all other groups, $p \leq 1x10^{-4}$ (ANOVA); ^, significantly different than wildtype (WT), $p \leq 4x10^{-3}$ (ANOVA). B) Dorsal cells in *wsp-1(gm324);ced-10(n1993)* embryos exhibit blunt medial edges (white arrow), while single mutants appear wild-type. Left-hand cells are pseudocolored green, right-hand cells are pseudocolored blue. The first time point (0 min.) is one hour after terminal epidermal cell divisions. Scale bar = 10 μm.

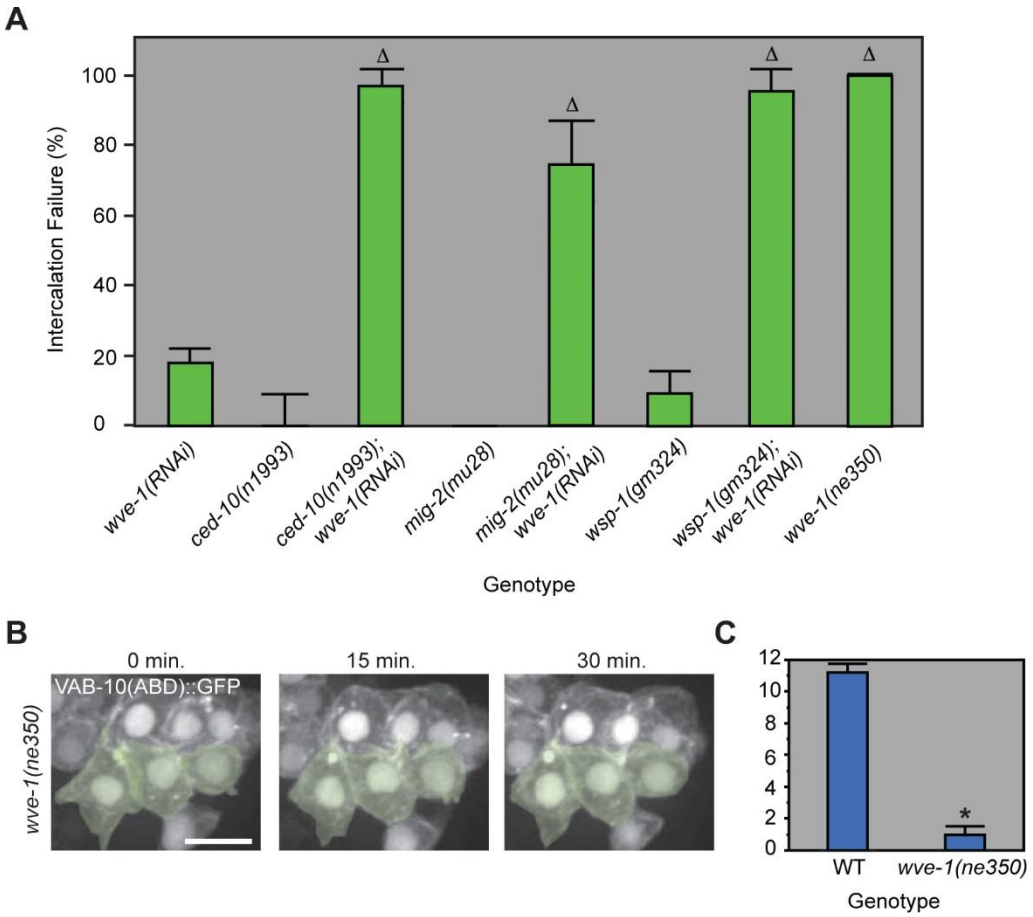


Figure S6. Weak loss of *wve-1* enhances intercalation failure in *mig-2(mu28), ced-10(n1993),* and *wsp-1(gm324)* embryos. A) Genotypes labeled Δ are significantly different from unlabeled groups, $p \leq 1x10^{-4}$, (ANOVA); error bars = s.e.m. B) F-actin in *wve-1(ne350)* homozygotes during intercalation. Scale bar = 5 μm. C) Quantification of protrusion number in *wve-1(ne350).* Both protrusion number and area (not shown) were significantly different than WT (wildtype) (Student's T-test, $p<0.0001$, denoted by *). Error bars = s.e.m.

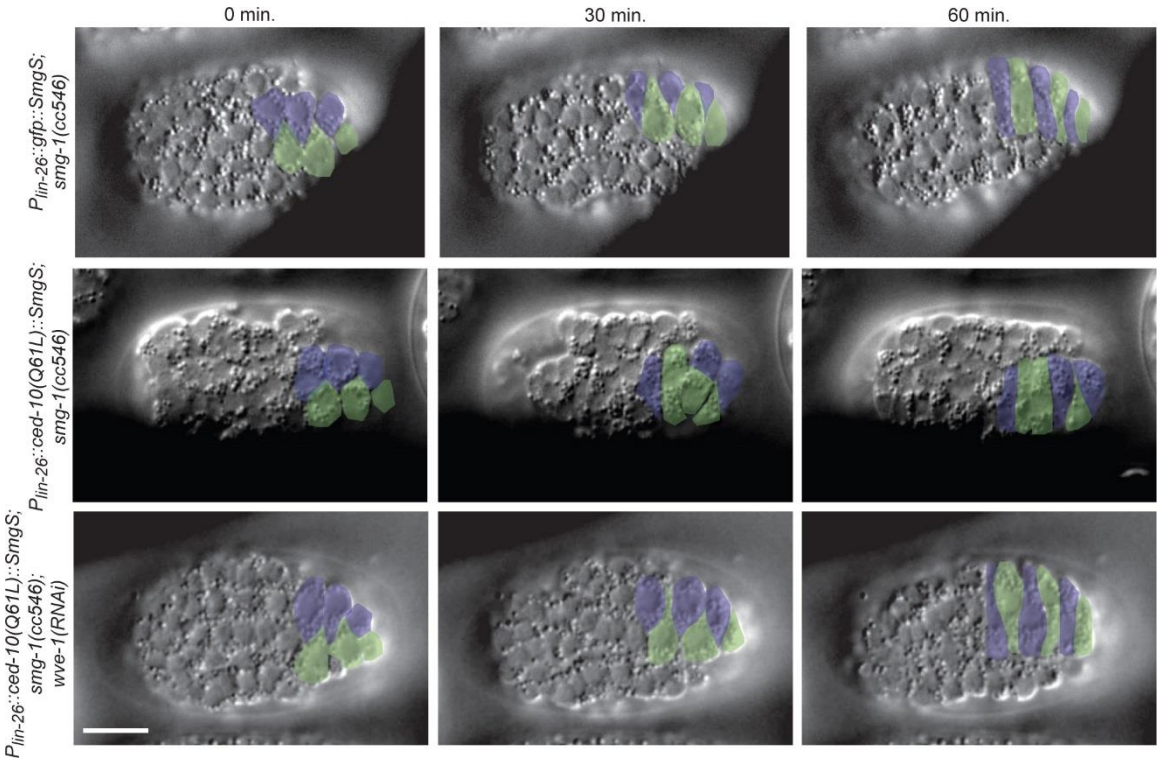
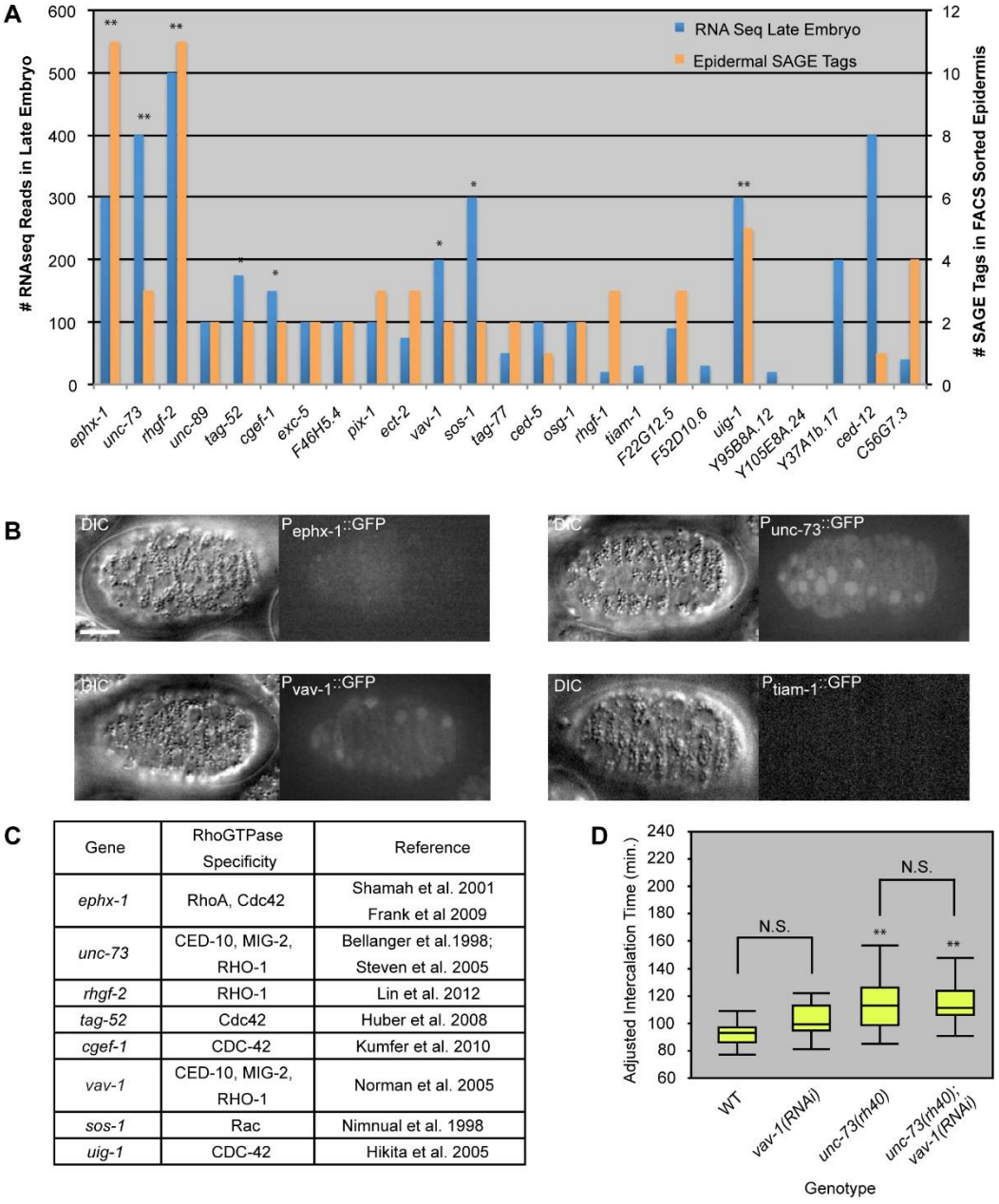


Figure S7. Intercalation defects in epidermal-specific *ced-10(Q61L/CA)* are suppressed by weak *wve-1(RNAi).* DIC images corresponding to data presented in Fig. 4B. All images were gathered at 20°C after a 24 hour incubation at 18°C, except *smg-1(cc546),* which was incubated at 25°C for 24 hours. *wve-1(RNAi)* proceeded for 6 hours prior to mounting. Left-hand cells are pseudocolored green, right-hand cells are pseudocolored blue. Scale bar = 10 µm.



Gene	RhoGTPase Specificity	Reference
ephx-1	RhoA, Cdc42	Shamah et al. 2001 Frank et al 2009
unc-73	CED-10, MIG-2, RHO-1	Bellanger et al.1998; Steven et al. 2005
rhgf-2	RHO-1	Lin et al. 2012
tag-52	Cdc42	Huber et al. 2008
cgef-1	CDC-42	Kumfer et al. 2010
vav-1	CED-10, MIG-2, RHO-1	Norman et al. 2005
sos-1	Rac	Nimnual et al. 1998
uig-1	CDC-42	Hikita et al. 2005

Figure S8. RhoGEF expression during *C. elegans* embryogenesis. A) Graph of high-throughput expression analyses of all RhoGEFs in the *C. elegans* genome. RNAseq reads from late embryos based on publicly available data (Celniker et al, 2009) are plotted on the left axis and epidermal SAGE tag reads are plotted on the right axis (McKay et al., 2003). ** denotes GEFs that are highly expressed both in the embryo (≥300 reads) and

epidermis (≥3 SAGE tags); * denotes GEFs that are moderately expressed in both the embryo (≥100 reads) and epidermis (≥2 SAGE tags). B) Expression of transcriptional reporters corresponding to a subset of GEFs during dorsal intercalation. Both *vav-1/Vav* and *unc-73/Trio* are expressed during intercalation. Scale bar = 10 μm. C) Table summarizing specificities of RhoGEFs that are expressed in embryos and in the epidermis in *C. elegans*. GEFs whose specificity has been demonstrated directly in *C. elegans* are listed using the relevant worm protein name (CED-10, MIG-2, RHO-1, CDC- 42), while likely specificities of *C. elegans* GEF homologs based on the specificities of the corresponding vertebrate proteins are indicated using the generic names of the relevant GTPases. D) *vav-1* does not function during intercalation. Intercalation times of *vav-1(RNAi)* embryos are not significantly different from wildtype, nor are *vav-1(RNAi); unc-73(rh40)* times different from *unc-73(rh40)* alone. **, significantly different than wildtype, $p \leq 0.0004$ (ANOVA).

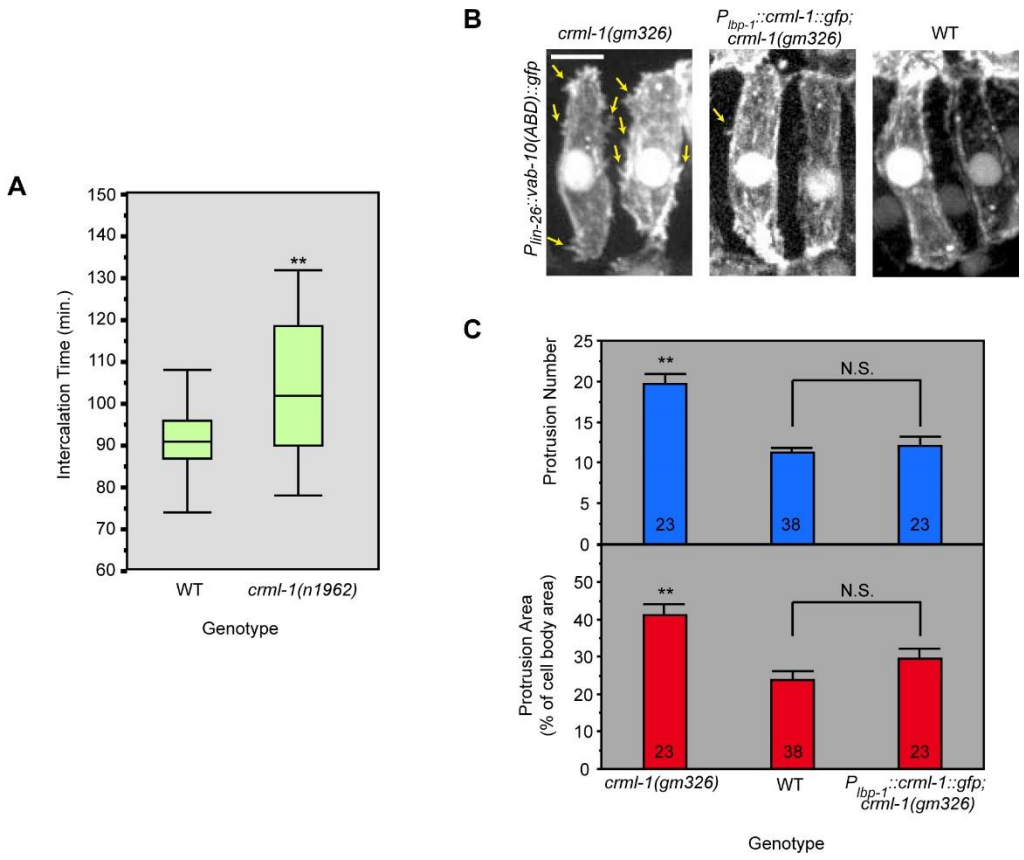


Figure S9: CRML-1 regulates intercalate time and is required in epidermal cells. A) Intercalation time in wildtype and *crml-1(n1962)* based on DIC images. **, $p < 0.001$, Student's T-test. B) Extra protrusions seen in *crml-1(gm326)* mutants are rescued when CRML-1 is expressed from the *lbp-1* promoter. *crml-1(gm326)* has significantly more numerous (top) and significantly larger (bottom) protrusions than $P_{lbp\text{-}1}$*::crml-1::gfp; crml-1(gm326)* or wildtype (WT). **, $p<0.007$ (ANOVA). However, protrusion number and area were not significantly different (N.S.) when comparing $P_{lbp\text{-}1}$*::crml-1::gfp; crml-1(gm326)* and WT (ANOVA). Error bars = s.e.m. Sample size (number of cells) is at the bottom of each bar. C) Representative micrographs of mosaic F-actin reporter expression in *crml-1(gm326)*, $P_{lbp\text{-}1}$*::crml-1::gfp; crml-1(gm326),* and WT. Yellow arrows point to extra protrusions. Scale bar = 5 μm.

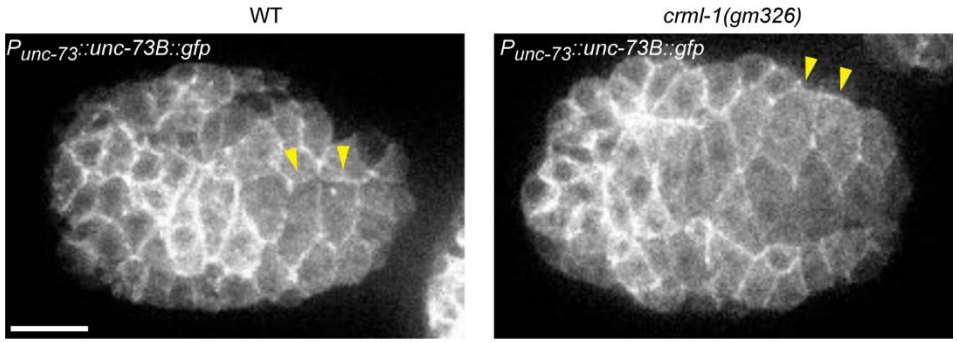


Figure S10: UNC-73B::GFP localization is unchanged in *crml-1(gm326)* mutants.

UNC-73B::GFP localizes laterally (yellow arrowheads) in both wild type and *crml-1(gm326)* backgrobackgrounds. Scale bar = 10 μm.

Supplementary Movies

Supplemental Movie 1: Protrusions in wildtype (WT) and epidermally-expressed CED-10(DN) viewed with an F-actin reporter. Minutes denoted in top left.

Supplemental Movie 2: Protrusions in wildtype (WT) and epidermally-expressed CED-10(CA) viewed with an F-actin reporter. Minutes denoted in top left. As noted on the first frame, the *ced-10(CA)* embryo has an extra contralateral cell labeled with the F-actin reporter relative to the wild-type movie.

Supplemental Movie 3: Protrusions in wildtype (WT) and *unc-73(gm40/GEF1)* viewed with an F-actin reporter. Minutes denoted in top left.

Supplemental Movie 4: Protrusions in wildtype (WT) and *crml-1(RNAi)* viewed with an F-actin reporter. Minutes denoted in top left.