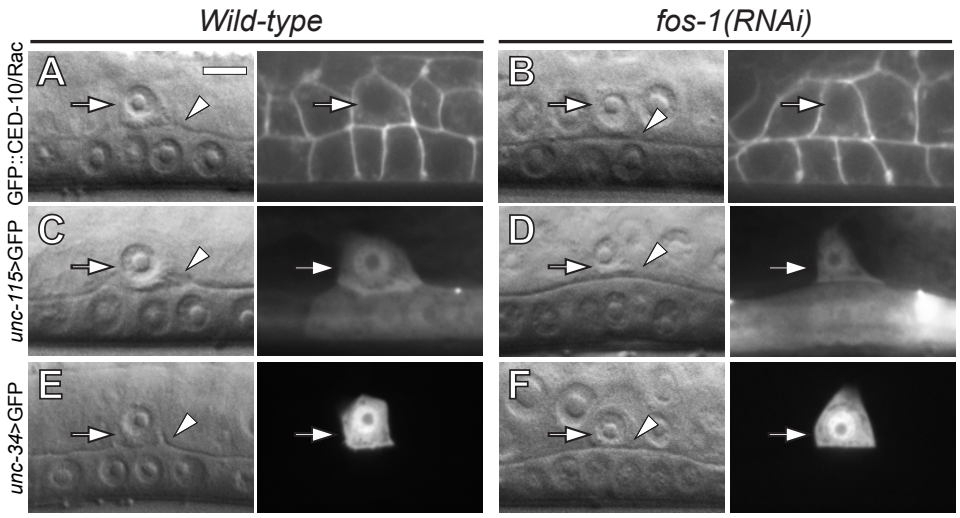

Fig. S1. FOS-1A does not regulate the expression of UNC-40 effectors.
DIC images (left) and corresponding fluorescence images (right) are shown at the P6.p four-cell stage. (A) GFP::CED-10 driven by its endogenous promoter is expressed and was localized to the membrane of the wild-type AC. (B) The expression and localization of CED-10 was unaffected (arrow) after *fos-1* RNAi treatment, which blocked AC invasion (arrowhead denotes intact phase dense line representing basement membrane). (C and E) The transcriptional reporters for *unc-115* (*unc-115 > GFP*) and *unc-34* (*unc-34 > GFP*) showed AC expression. (D and F) The expression of transcriptional reporters for *unc-115* and *unc-34* remained unchanged after *fos-1* RNAi treatment. In this and all other supplementary figures, scale bars represent 5 μm.

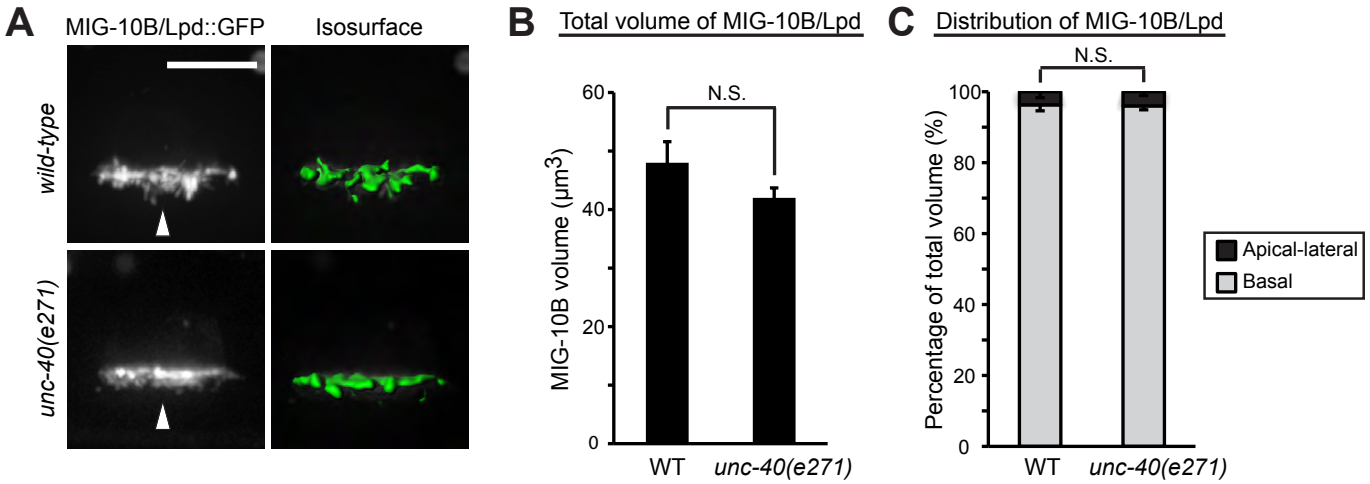

Fig. S2. MIG-10B localization remains polarized in *unc-40(e271)* mutant ACs.
(A) Images show 3D reconstructions generated from confocal z-stacks taken in animals at the P6.p four-cell stage. Fluorescence (left) and corresponding isosurface rendering of MIG-10B::GFP localization (right). MIG-10B polarizes to the invasive cell membrane (white arrowheads) in wild-type ACs and *unc-40* mutant ACs at the P6.p four-cell stage. (B) Quantification of the total MIG-10B volume in wild-type and *unc-40(e271)* mutant ACs at the P6.p four-cell stage (n ≥ 10 per genotype). (C) The basal (gray) and apical-lateral (black) distribution of MIG-10B within the AC in wild-type animals and *unc-40(e271)* mutants at the P6.p four-cell stage (n ≥ 10 per genotype). In this and all other supplementary figures, one asterisk (*), two asterisks (**) and three asterisks (***) indicate statistically-significant differences of $P < 0.05$, $P < 0.01$ and $P < 0.001$, respectively, and N.S. indicates no significant difference (Student's *t*-test). Error bars represent the standard error of the mean. Significant differences relative to wild-type animals are indicated.

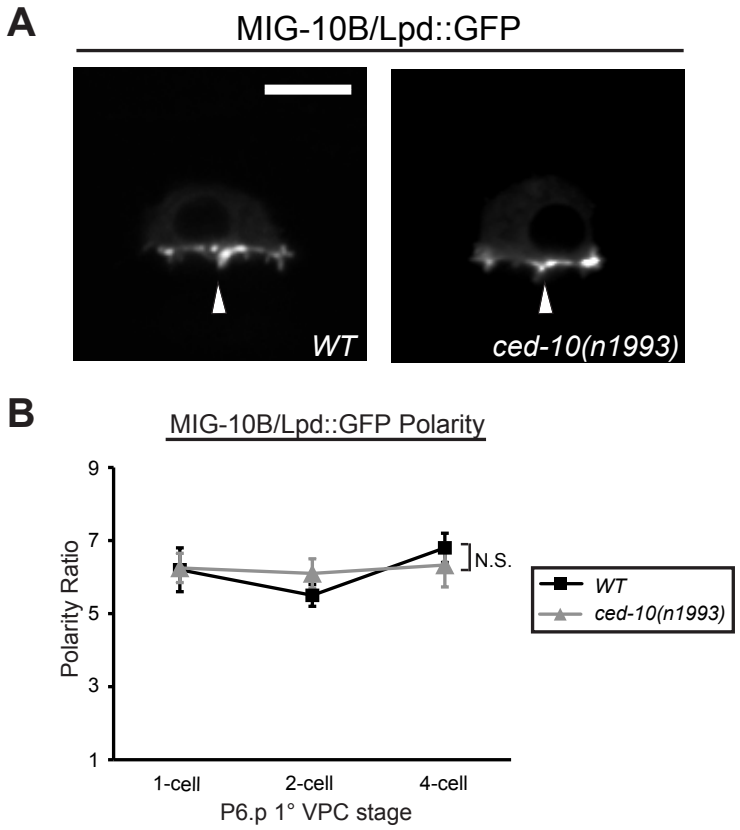

Fig. S3. MIG-10B localization remains polarized in *ced-10(n1993)* mutant ACs.
(A) MIG-10B polarizes to the invasive cell membrane (white arrowheads) in wild-type ACs and *ced-10* mutant ACs at the P6.p four-cell stage. (B) Quantification of MIG-10B polarization to the invasive cell membrane in wild-type animals (black squares), *unc-40* mutants (gray triangles) at the P6.p one-, two-, and four-cell stages (n ≥ 12 for each stage per genotype). Significant differences relative to wild-type animals are indicated.

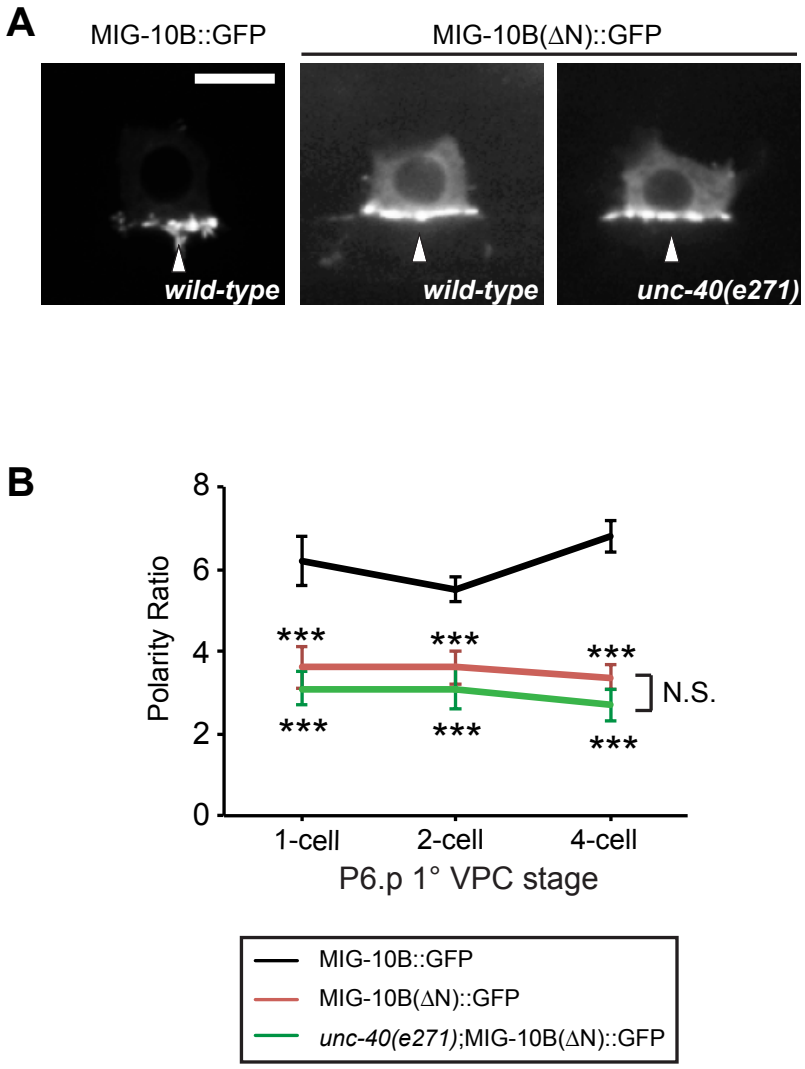

Fig. S4. The N-terminal domain of MIG-10B promotes localization to the invasive membrane.
(A) In wild-type ACs, MIG-10B was strongly polarized to the invasive cell membrane (white arrowhead). MIG-10B(ΔN) showed reduced localization to the invasive cell membrane (white arrowhead), which was not further reduced in *unc-40* mutants. (B) Quantification of polarization of MIG-10B in wild-type animals (black line) and MIG-10B(ΔN) in wild-type (red line) and *unc-40* (green line) mutant animals at the P6.p one-, two-, and four-cell stages ($n \geq 12$ for each stage per genotype). Significant differences relative to wild-type animals are indicated.

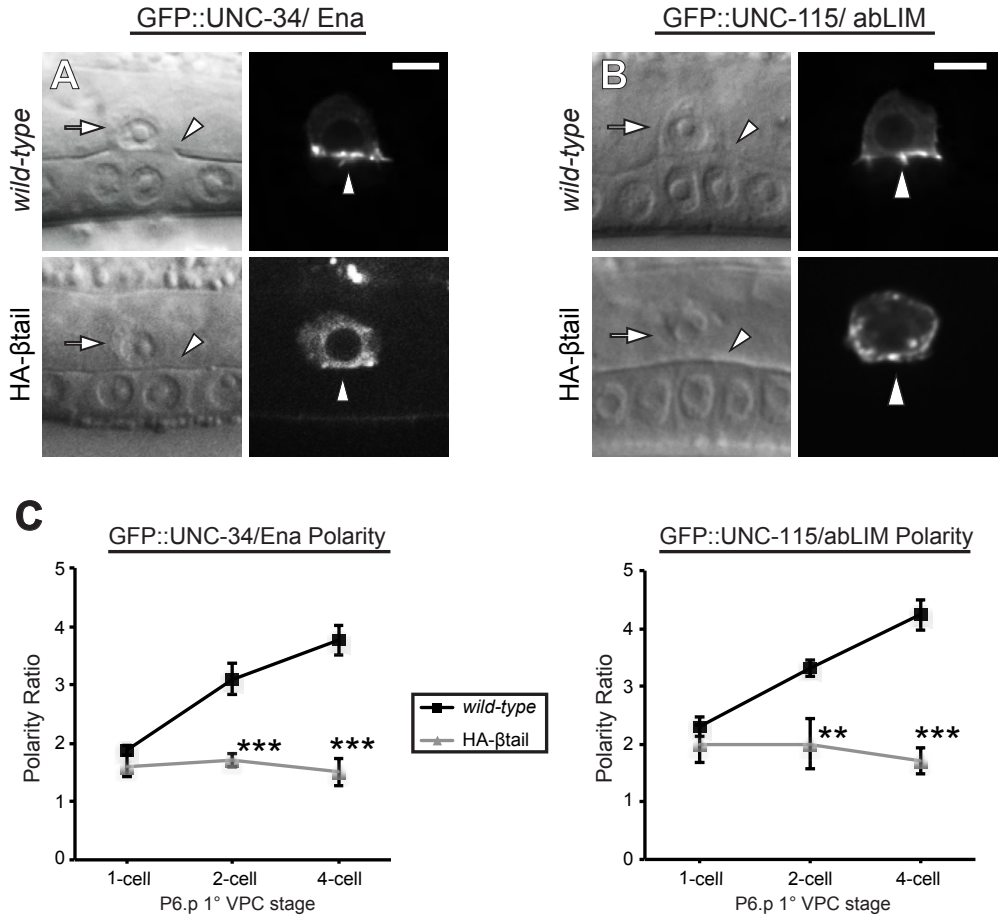

Fig. S5. Integrin localizes UNC-34 and UNC-115 to the invasive cell membrane.
(A and B) DIC images (left) and corresponding fluorescence (right). In wild-type ACs, UNC-34 and UNC-115 polarized to the invasive cell membrane (white arrowheads). In contrast, expression of a dominant negative integrin PAT-3 β subunit in the AC (*zmp-1 > HA- βtail*) reduced the polarization of UNC-34 and UNC-115 to the invasive membrane. ACs in *HA- βtail* animals still adhered to the underlying basement membrane (arrowhead, DIC image). (C) Quantification of UNC-34 and UNC-115 polarization to the invasive cell membrane in wild-type animals (black squares) and HA- βtail (gray triangles) at the P6.p one-, two-, and four-cell stages ($n \geq 12$ for each stage per genotype). Significant differences relative to wild-type animals are indicated.

Table S1. Primer sequences and templates used for PCR fusions and cloning

Primer sequence (5'->3')	Primer type	Amplicon	Template
TAATGTGAGTTAGCTCACT CATTAGG	Forward	*cdh-3 promoter*	pPD107.94/mk62 -63
AACGATGGATACGCTAACA ACTTGG	Forward nested	*cdh-3 promoter*	pPD107.94/mk62 -63
TTTCTGAGCTCGGTACCCTC CAAG	Reverse	*cdh-3 promoter*	pPD107.94/mk62 -63
ATGAGTAAAGGAGAAGAA CTTTTCAC	Forward	*GFP*	pPD95.81 (GFP)
GGAAACAGTTATGTTTGGT ATATTGGG	Reverse nested	*GFP*	pPD95.81 (GFP); Plasmid *unc-86 > mig-10::GFP*
AAGGGCCCGTACGGCCGAC TA	Reverse	*GFP*	pPD95.81 (GFP); Plasmid *unc-86 > mig-10::GFP*
TTTGTATAGTTCATCCATGC CATGTG	Reverse for GFP extension to N-terminus of protein of interest	*GFP*	Plasmid *cdh-3 > GFP*
GTGCCCGTAAATCAATACC TAGTC	Forward	*unc-34 promoter*	N2 genomic DNA
GCACTTTTACGGCAGATTTT GTGT	Reverse	*unc-34 promoter*	N2 genomic DNA
GCTCATCCCTGATTACAAG TTT	Forward	*unc-115 promoter for unc-115 > GFP*	N2 genomic DNA
CGAAGCACGGAATAAATCA T	Forward nested	*unc-115 promoter for unc-115 > GFP*	N2 genomic DNA
GGTATAGAATAGCGGAGAG AGGTCT	Reverse	*unc-115 promoter for unc-115 > GFP*	N2 genomic DNA
GACCTCTCTCCGCTATTCTA TACCATGAGTAAAGGAGAA GAACTTTT	Forward GFP extension	*GFP for unc-115 > GFP*	pPD95.81 (GFP)
ATGGGCAAAAAATGCGACG TATGT	Forward	*unc-115 cDNA for cdh-3 > GFP::unc-115*	N2 cDNA
GACTTGGAGACAAATAACG GGGAT	Reverse	*unc-115 cDNA for cdh-3 > GFP::unc-115*	N2 cDNA
CGAGATTCCGCGTAGAAGA CAAA	Reverse nested	*unc-115 cDNA for cdh-3 > GFP::unc-115*	N2 cDNA
CATACGTCGCATTTTTTGCC CATTTTGTATAGTTCATCCA TGCCA	Reverse for GFP with *unc-115* extension	*GFP for cdh-3 > GFP::unc-115*	Plasmid *cdh-3 > GFP*
CTTGGAGGGTACCGAGCTC AGAAAATGTATCACGATCG ACGG	Forward *cdh-3* promoter extension	*mig-10b::GFP for cdh-3 > mig-10b::GFP*	Plasmid *unc-86 > mig-10::GFP*
CTTGGAGGGTACCGAGCTC AGAAAATGTCCGCAGATTG GCAGTTG	Forward *cdh-3* promoter extension	*mig-10b(ΔN)::GFP for cdh-3 > mig-10b(ΔN)::GFP*	Plasmid *unc-86 > mig-10::GFP*

Table S2. Extrachromosomal array and integrated strain generation

Strain Designation	PCR fusion or plamids	Injection concentration (ng/µl)	Co-injection marker
qyEx196	*unc-115 > GFP* [a]	50	*unc-119+*
qyEx258	*unc-34 > GFP* [b]	50	*unc-119+*
qyEx259 (overexpression)	*cdh-3 > unc-40::GFP* [a]	50	*unc-119+, myo-2 > GFP*
qyEx412	*cdh-3 > mig-10b(ΔN)::GFP* [a]	10	*unc-119+, myo-2 > GFP*
qyIs182	*cdh-3 > GFP::unc-115* [a]	50	*unc-119+*
qyIs183	*cdh-3 > mig-10b::GFP* [a]	10	*unc-119+*

[a] PCR fusion product; [b] plasmid