Supporting Information

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SI Materials and Methods

C. elegans Strains and Genetics. *C. elegans* strains were raised on NGM (Nematode Growth Medium) plate seeded with the *Escherichia coli* strain OP50 at 20 °C using standard methods. Wild-type animals were of the Bristol variety, strain N2. Strains are listed in Table S1. Integrated Pmec-4::gfp (zdIs5), Pgcy-32:: mCherry (casIs35), and Pgcy-32::gfp (casIs35) transgenes were used to visualize Q-neuroblast progenies, AVM/PVM and AQR/PQR. Ethyl methane sulfonate mutagenesis was carried out, with either a single transgenic marker or both, to identify Q-cell migration mutations. Four mutations affecting AQR and AVM migration were isolated and mapped with snip-SNP techniques and complementation testing. The candidate genes in these regions were sequenced to identify mutations. The data showed that these four alleles caused point mutations in or deletion of the *mig-13* gene.

Molecular Biology and Transgenesis. We used PCR fusion and molecular cloning techniques to generate fluorescence reporters in Q cells. All of the PCRs were performed with Phusion DNA polymerase (New England Biolabs). The PCR templates and primers are listed Table S2 and the plasmid constructs are listed in Table S3. Transgenic *C. elegans* were created by germ-line transformation. The PCR products and DNA plasmids at 10–30 ng/ μ L were injected into N2 hermaphrodites with a selection marker pRF4 or *unc-76 (e911)* with WT *unc-76* gene.

Live-Cell Imaging. *C. elegans* L1 larva were anesthetized with 0.1 mmol/L levamisole in M9 buffer, and then mounted on 2% (wt/ vol) agar pads and maintained at room temperature (20 °C). Our imaging system includes an Axio Observer Z1 microscope (Carl

Zeiss MicroImaging) equipped with a 100×, 1.45 N.A. objective, an EM CCD camera (Andor iXon+ DU-897D-C00-#BV-500), and the 405-, 488-, and 568-nm lines of a Sapphire CW CDRH USB Laser System attached to a spinning disk confocal scan head (Yokogawa CSU-X1 Spinning Disk Unit). Time-lapse images were acquired with exposure time of 300 ms at every 40–60 s with μ Manager (www.micro-manager.org) or Focus Image software (developed by Xiang Zhang at the Institute of Biophysics, Chinese Academy of Sciences, Beijing, China). ImageJ software (http://rsbweb.nih.gov/ij/) was used to process the images.

Statistical Analysis. The Student t test was used to examine significant differences in Q-cell migration between WT and migration mutants as indicated in the figure legends.

Quantification of Relative Position of Q Cells. The positions of the Qcell progenies in adult *C. elegans* reflect the outcomes of Q-cell migration. We thus quantified the final positions of Q-cell progenies in the adult stage. We chose three nonmotile cells, URX, PLM, and PVM, which can be labeled by either *Pmec-4:: gfp* or *Pgcy-32::mCherry*, as fiduciary markers (Fig. S3*A*). For example, the relative position of AQR is calculated as the distance between URX and AQR divided by the distance between URX and PLM (Fig. S3*A*).

LIN-39 and MAB-5 Binding Peaks Display. IGB (Integrated Genome Browser 6.7.3) was used to analyze LIN-39 and MAB-5 binding peaks to *mig-13* or *lin-39* promoters. The ChIP-Seq data were downloaded from the modENCODE Consortium (www.modencode.org).



Fig. 51. MIG-13 and LIN-39 expression and localization in Q-cell lineage. (A) AVM (*Left*) and AQR (*Right*) migration defects in four new alleles of *mig-13*. n = 23-47. Quantifications are described in Fig. S3A and *Materials and Methods*. (*B–D*) Still images of MIG-13::GFP in Q cells using different strains: *muls62* (*B*), *muls42* (*C*), and *casEx593* (*D*). Time on the *Right* top is in minutes. (*E*) MIG-13::GFP expression in *lin-39* (*n1760*, *Upper*) and *mab-5* (*e2088*, *Lower*) mutants. (*F*) Pegl-13::gfp expression pattern (green) in the Q-cell (mCherry, red) lineages. (Scale bar, 5 µm.) (*G* and *H*) The expression of Pegl-46 (*G*) and Pmec-7 (*H*) in *lin-39* (*n1760*) mutants. (Scale bars: in *B–G*, 5 µm; in *H*, 50 µm.) (*I*) MIG-13::GFP in *lin-39* (*n1760*) mutants. In QR.ap (AQR) without MIG-13::GFP, QR.ap stayed close to QR.pa (mother cell of AVM) (*Left*), whereas QR.ap with MIG-13::GFP was more anterior than QR.pa (*Right*).

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Fig. 52. COR-1::GFP localization in *mig-2 (mu28* null allele), *mig-2 (rh17* gain of function allele), or *ina-1 (gm144)* mutants or *mig-13* ectopic expression animals (Pegl-13::mig-13). (A and B) COR-1::GFP localization in QR descendants of *mig-2 (mu28* null) (A), *mig-2 (rh17 gf)* (B), and *ina-1 (gm144)* (C) mutants. The yellow arrows in A–C show the anterior direction in which QR.ap normally polarizes. The white asters in A show the abnormal distribution of COR-1::GFP in QR.x. (D and E) Still images show the localization of *casls49* [COR-1::GFP] during QL.ap migration in WT (D) or with *mig-13* ectopic expression (Pegl-13::mig-13) (E). COR-1::GFP is labeled by GFP (*Top*). Q-cell plasma membrane and chromosomes are visualized with mCherry (*Middle*). The merged images are at *Bottom*. The white arrows in D and E show that the posterior direction that QL.ap normally migrates. The white asters in D show the asymmetric distribution of COR-1::GFP in the leading edge of migrating QL.ap. The white arrowhead in E indicates the mislocalization of COR-1::GFP opposite to the direction of QL.ap. The cell name is adjacent. The anterior of the cell is on the *Left*. Time is in minutes. (Scale bar, 5 µm.)



Fig. S3. Statistical analysis of Q-cell migration in WT and mutants. (A) Schematics of quantifications of the position of Q-cell descendants in adult *Caenorhabditis elegans* (see *Materials and Methods* for details). AQR position is illustrated as an example. (B) AVM position in WT, *mig-13(mu225)*, and *mig-13* rescue experiments with *C. elegans* or *Mus musculus mig-13*. (C) AQR (*Left*) and AVM (*Right*) and (*D*) PQR position in *mig-13* cell type-specific rescue animals. (*E*) AQR (*Left*) and AVM (*Right*) position in *lin-39* mutants and the cell type-specific expression of *mig-13* in *lin-39 (n1760)* animals. Data in *B*, *C* and *D*, and *E* are the same as the data in Figs. 1*E*, 2 *B*–D, and 4*D*. Data shown are the mean \pm SD, *n* = 20–100 per group in a single experiment. **P* < 0.01 by Student t test. (*F*) ChIP-seq data of MAB-5 binding site in the *lin-39* promoter (*Top*, blue). The input is in the *Middle*, red; the *lin-39* gene model on the *Bottom*, black.



Fig. 54. Genetic interactions between *mig-2* and *mig-13*. (A) Quantifications of AQR and AVM positions in *mig-13* and *mig-2*-related genetic backgrounds (indicated on *Top Right*). (B) Statistical analysis of AQR and AVM positions in the single or double mutants of *mig-13* or *mig-2*. (C) The expression of Pmig-2:: *mig-2::gfp* in WT and *mig-13* mutants (*Left*) or Pmig-13::mig-13::gfp in mig-2 mutants (*Right*). (Scale bar, 5 µm.)



Fig. S5. Q-cell fate in WT and *mig-13*-related mutants. (A) The expression of Pmec-7::gfp and Pgcy-32::mCherry in WT and mig-13 mutants (Left). (Scale bar, 50 μm.) (B) The expression of Posm-6::gfp in WT and mig-13 loss or gain-of function mutants (Left). (Scale bar, 5 μm.)

Table S1. C. elegans strains used in this study

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Strain name	Genotype	Method	Resource	Reference
GOU246	zdls5[Pmec-4::GFP];casls35[Pgcy-32::mCherry];him-5(e1490)	Injection and cross	CGC and	Fig. 1C
GOU191	mig-13(cas14); zdls5[Pmec-4::GFP];casls35[Pgcy-32::mCherry]	EMS	This study	Fig. 1 <i>D</i> and Fig. 51A
GOU192	mig-13(cas15); zdls5[Pmec-4::GFP];casls35[Pgcy-32::mCherry]	EMS	This study	Fig. 1D and Fig. S1A
GOU655	mig-13(cas64); zdls5[Pmec-4::GFP];casls35[Pgcy-32::mCherry]	EMS	This study	Fig. 1D and Fig. S1A
GOU656	mig-13(cas65);	EMS	This study	Fig. 1D and Fig. S1A
CF726	mig-13(mu225)		CGC	Fig. 1 <i>E</i>
CF1295	[[][][][][][][][][]][][][][]][][][][][
GO0649	casex593[Pmig-13::Mig-13::1EV-5::GFP::mig-13_3'UTR+unc-76(+); Pegl-17::Myri-mcherry::pie-1 3'UTR;Pegl-17::mcherry-TEV-5::his-24]; unc-76(e911)	Injection	This study	Movie S2
GOU648	casEx790[Pmig-13::mouse_MIG-13::GFP,pRF4];mig-13(mu225); zdls5[Pmec-4::GFP]	Injection	CGC and this study	Fig. 1 <i>E</i> and Fig. S3 <i>B</i>
GOU646	casEx788[Pegl-17::mouse_MIG-13::GFP,pRF4];mig-13(mu225); zdls5[Pmec-4::GFP]	Injection	CGC and this study	Fig. 1 <i>E</i> and Fig. S3 <i>B</i>
GOU263	muls62[MIG-13::GFP + lin-15(+)]; rdvls1[Pegl-17::Myri-mcherry::pie-13'UTR, Pegl-17::mig-10::YFP::unc-543'UTR, Pegl-17::mcherry-TEV-S::his-24,pRF4]	Cross	CGC and this study	Fig. 1 F and G, Fig. S1B, and Movie S1
GOU560	muls42[MIG-13::GFP + lin-15(+)]; rdvls1[Pegl-17::Myri-mcherry::pie-13'UTR, Pegl-17::mig-10::YFP::unc-543'UTR, Pegl-17::mcherry:TFV-5::his-24.pRF4]	Cross	CGC and this study	Fig. S1C
GOU644	lin-39(n1760);zdls5[Pmec-4::GFP];casls35[Pgcy-32::mCherry]	Cross	CGC and	Fig. 4D and
GOU652	lin-39(n1760):zdls5[Pmec-4::GFP]:cas/s35[Pacv-32::mCherry]:	Injection	this study CGC and	Fig. S3 <i>E</i> Fig. 4 <i>D</i> and
	casEx600[Pegl-46::mig-13]	and cross	this study	Fig. S3E
GOU653	lin-39(n1760);zdls5[Pmec-4::GFP];casls35[Pgcy-32::mCherry]; casEv1129[Pmec-7::mig-13::GEP]	Injection and cross	CGC and	Fig. 4D and
6011536	lin_39(n1760); rdyk1[Peal_17::Myri_mcherry::nie_1_3/LITR	Cross	CGC and	Fig. 1G and
000550	Pegl-17::mig-10::YFP::unc-54 3'UTR,Pegl-17::mcherry-TEV-S::his-24, pRF4]; muls62[MIG-13::GFP + lin-15(+)]	Closs	this study	Fig. S1 <i>E</i>
GOU680	mab-5(e2088);	Injection and cross	CGC and	Fig. 1G and
GOU745	mig-14(mu71);	Injection and cross	CGC and this study	Fig. 1G
GOU650	casEx779[Pegl-13::mig-13];mig-13(cas15);zdls5[Pmec-4::GFP];	Injection	This study	Fig. 2 <i>B–D</i> and
GOU551	casts59[Pgcy-32::mig-13];mig-13(cas15);zdls5[Pmec-4::GFP];	Injection	This study	Fig. 2 <i>B</i> – <i>D</i> and
GOU544	casts50[Pegl-46::mig-13];mig-13(cas15);zdls5[Pmec-4::GFP];	Injection	This study	Fig. 2 <i>B</i> – <i>D</i> and
GOU651	casiss5[Pgcy-32::mCherry] casEx1129[Pmec-7::MIG-13::GFP];mig-13(cas15);zdls5[Pmec-4::GFP];	Injection	This study	Fig. 2 <i>B–D</i> and
	casls35[Pgcy-32::mCherry]			Fig. S3 C and D
GOU347	casis49[PegI-17::Myri-mcherry::pie-1 3'UTR, PegI-17:: COR-1::GFP::unc-54 3'UTR, PegI-17::mcherry-TEV-S:: his-24]	integration	This study	Fig. 3 A, D, and E, and Movies S3 and S8
GOU422	mig-13(cas15);casls49[Pegl-17::Myri-mcherry::pie-1 3'UTR, Peal-17::COR-1::GFP::unc-54 3'UTR.Peal-17::mcherry-TEV-S:: his-24]	Cross	This study	Fig. 3 <i>B</i> , <i>D</i> , and <i>E</i> , and Movie S7
GOU482	casIs126[PegI-13::mig-13]; casIs49[PegI-17::Myri-mcherry::pie-1 3'UTR, PegI-17::COR-1::GFP::unc-54 3'UTR,PegI-17::mcherry-TEV-S::his-24]	Cross	This study	Fig. 3 C–E, Fig. S2E, and Movie S9
GOU785	casls131[Pegl-17::mcherry::zen-4];	Injection and cross	CGC and this study	Fig. 5 A and B, Movies S11 and S12
GOU855	casEx831[Pmec-7::lin-39::GFP]; zdls5[Pmec-4::GFP];casls35 [Pgcy-32::mCherry]	Injection and cross	This study	Fig. 5 C and D
GOU856	casEx832[Pgcy-32::lin-39::GFP]; zdls5[Pmec-4::GFP];casls35 [Pgcy-32::mCherry]	Injection and cross	This study	Fig. 5 C and D
GOU857	casEx833[Pegl-13::/in-39::GFP] zdls5[Pmec-4::GFP];casIs35	Injection	This study	Fig. 5 C and D
GOU802	casEx816[dePmig-13A::mig-13::GFP]; mig-13(mu225); zdls5[Pmec-4::GFP];casls35[Pgcy-32::mCherry]	Injection and cross	This study	Fig. 4C

Table S1. Cont.

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Strain name	Genotype	Method	Resource	Reference
GOU800	casEx813[dePmig-13B::MIG-13::GFP];	Injection	This study	Fig. 4C
	zdls5[Pmec-4::GFP];casls35[Pgcy-32::mCherry]	and cross		
GOU801	casEx814[dePmig-13C::mig-13::GFP];	Injection	This study	Fig. 4C
	zdls5[Pmec-4::GFP];casls35[Pgcy-32::mCherry]	and cross		
GOU854	casEx828[Del_motif::MIG-13::GFP];	Injection	This study	Fig. 4C
	zdls5[Pmec-4::GFP];casls35[Pgcy-32::mCherry]	and cross		-
GOU783	mab-5(e2088); casis131[Pegl-17::mcherry::zen-4];	Injection	CGC and	Fig. 5 <i>B</i>
	wqls18[LIN-39::GFP]	and cross	this study	5
GOU786	miq-14(mu71);	Injection	CGC and	Fig. 5 <i>B</i>
	wqls18[LIN-39::GFP]	and cross	this study	5
GOU350	miq-2(rh17);casls49[Pegl-17::Myri-mcherry::pie-1 3'UTR,	Injection and	CGC and	Fig. S2 <i>B</i> and
	Pegl-17::COR-1::GFP::unc-54 3'UTR, Pegl-17::mcherry-TEV-S::his-24]	integration	this study	Movie S5
GOU420	ina-1(gm144),casIs48[Pegl-17::Myri-mcherry::pie-1_3'UTR,	Injection and	CGC and	Fig. S2C and
	Pegl-17::COR-1::GFP::unc-54 3'UTR, Pegl-17::mcherry-TEV-S::his-24]	integration	this study	Movie S6
GOU746	casEx788[Pegl-17::mig-13::GFP];mig-13(mu225);	Injection	This study	Fig. S3D
	zdls5[Pmec-4::GFP];casls35[Pgcy-32::mCherry]	and cross		5
GOU944	casEx5002[Punc-47::MIG-13::GFP]; zdls5[Pmec-4::GFP];	Injection	CGC and	Fig. 2 B and C,
	miq-13(mu225) casls35[Pgcy-32::mCherry]	and cross	this study	and Fig. S3C
GOU945	casEx5003[Punc-47::MIG-13::GFP]; zdls5[Pmec-4::GFP];	Injection	CGC and	Fig. 2 B and C,
	miq-13(mu225) casls35[Pgcy-32::mCherry]	and cross	this study	and Fig. S3C
GOU890	miq-2(mu28);casls49[Peql-17::Myri-mcherry::pie-1 3'UTR,	Injection	CGC and	Fig. S2A and
	Peql-17:: COR-1::GFP::unc-54 3'UTR, Peql-17::mcherry-TEV-S:: his-24]	and cross	this study	Movie S4
GOU971	miq-2(mu28) casIs35[Pqcy-32::mCherry]; zdIs5[Pmec-4::GFP]	Cross	CGC and	Fig. 3 <i>F</i> and
			this study	Fig. S4 A
				and B
GOU976	miq-2(mu28) miq-13(mu225) casIs35[Pgcy-32::mCherry];	Cross	CGC and	Fig. 3 <i>F</i> and
	zdls5[Pmec-4::GFP]		this study	Fig. S4 <i>B</i>
GOU1004	mig-2(rh17) casls35[Pgcy-32::mCherry]; zdls5[Pmec-4::GFP]	Cross	CGC and	Fig. S4 A
			this study	and B
GOU1005	miq-2(rh17) miq-13(mu225) casIs35[Pgcy-32::mCherry];	Cross	CGC and	Fig. S4 <i>A</i>
	zdls5[Pmec-4::GFP]		this study	and B
GOU1006	mig-2(mu28) casls35[Pgcy-32::mCherry]; casls222[Pmig-13::	Injection,	CGC and	Fig. S4C
	MIG-13::MYC::GFP Pegl-17 > Myri-mcherry > pie-1 3'UTR,	integration,	this study	5
	Peql-17 > mcherry-TEV-S::his-24]	and cross		
GOU963	miq-13(mu225) casls35[Pgcy-32::mCherry]; muls28[MIG-2::GFP];	Cross	CGC and	Fig. S4C
			this study	5
GOU988	lin-39(n1760); casls48[Pegl-17::Myri-mcherry::pie-1 3'UTR, Pegl-17::	Cross	CGC and	Fig. 4A and
	COR-1::GFP::unc-54 3'UTR, Pegl-17::mcherry-TEV-S:: his-24];		this study	Movie S10
	rdvls1[Pegl-17::Myri-mcherry::pie-13'UTR,Pegl-17::mig-10::			
	YFP::unc-54 3'UTR, Pegl-17::mcherry-TEV-S::his-24,pRF4]			
GOU953	lin-39(n1760); muls32[Pmec-7::GFP];	Cross	CGC and	Fig. S1 <i>H</i>
			this study	-
GOU954	lin-39(n1760);	Cross	CGC and	Fig. S1G
			this study	-
GOU949	mig-13(mu225)	Cross	CGC and	Fig. S5A
			this study	-
GOU955	mig-13(mu225)	Cross	CGC and	Fig. S5 <i>B</i>
			this study	-
GOU978	casls179[Pegl-46::MIG-13, Pegl-17::Myri-mcherry::pie-1 3'UTR,	Injection	CGC and	Fig. S5 <i>B</i>
	Pegl-17::mcherry-TEV-S:: his-24]; mnls17[osm-6::GFP unc-36(+)]	and cross	this study	

Strains carrying extrachromosome arrays were exposed to gamma-ray irradiation for integration and were backcrossed with wild-type N2 animals at least three times. *rdvIs1* (Q cell membrane and nuclei marked with mCherry) was constructed previously (1). CGC, *Caenorhabditis* Genetics Center; EMS, ethyl methanesulfonate.

1. Ou G, Stuurman N, D'Ambrosio M, Vale RD (2010) Polarized myosin produces unequal-size daughters during asymmetric cell division. Science 330(6004):677-680.

Table S2. PCR products for *C. elegans* transgenesis

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PCR product	Primer 5′	Primer 3′	Template	Reference
egl-17 promoter	cagatggatg tttactgcca actgg	agctcacatt tcgggcacct gaa	N2 genomic DNA	
myr-mCherry	caggtgcccgaaatgtgagct atgggttcct gtattggaaa agtctc	ctaaagggaa caaaagctgg agc	pJWZ50.3	
P _{egl-17} myr-mCherry	cttccgttct atggaacact c	gaatcatcgt tcacttttca cgg	egl-17 promoter and MYR-mCherry	Fig. 3 A and D, and Figs. S1D and S2C
P _{egl-17} mCherry::his-24	cttccgttct atggaacact c	gaagacgttg aacgtcaaat tatc	<i>egl-17</i> promoter and mCherry + his-24	Fig. 3 A and D, and Figs. S1D and S2C
Р _{дсу-32}	cacattatatacgatcgaggcatg	caccctttgagaccattctat aatacaatcgtgatcttcg	N2 genomic DNA	
mCherry	atggtctcaaagggtgaagaagataac	AAGGGCCCGTACGGCCGACTAGTAGG	pPD49.26-mCherry	
mig-13+3'UTR	CCAGCATGAGCCGTAGACGAA tgactaaactactcatagc	ataaacgaattcaatgcgac	N2 genomic DNA	
P _{eal-13}	TCACCTGCCCCGACATTA	TCGTCTACGGCTCATGCTGG	N2 genomic DNA	
P _{egl-13} ::mig-13	GAGTGTGTGACACCTTGAG	ataaacgaattcaatgcgac	mig-13+3'UTR and P _{egl-13}	Fig. 2 <i>B–D</i> and Fig. S3 C and D
cor-1+3'UTR	ACATGGACAGCGGAGGTGGAGGTACTAT GGCGCAAATCGTCCGGCAGAG	CCATTTATTAGGTTGCAATAC	N2 genomic DNA	5
P _{egl-17} ::cor-1	CTTCCGTTCTATGGAACACTC	GGTTGCAATACAATACAACACAC	cor-1+3'UTR and P _{egl-17}	Fig. 3 A–E and Fig. S2 A–D and Movies S3 and S7
P _{mec-7}	ggtggagaacgtcatgaaattacg	gttgcttgaaatttggaccc	N2 genomic DNA	
P _{mig-13}	tctctgcagtgttacacg	ttcctactgtccaaaccg	N2 genomic DNA	
P _{unc-47}	CGCGGTTTTGGCGCG	gagtagtttagtcatctgt	N2 genomic DNA	
	gtttcccggagtctacgc	aatgaaataaatgtgacgc		

Table S3. Plasmids constructed for C. elegans transgenesis

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Plasmid name	Primer 5'	Primer 3'	Notes	Reference
pPD95.77-P <i>egl-17</i> ::GFP	TTTTTCTACCGGTACC CTCAAGGG	AGACCCAAGCTTGGTACCAT GAGTAAAGGAGAAGAACTTTTCAC	Pegl-17 sequence was amplified from N2 genomic DNA and inserted into pPD95.77 plasmid via In-fusion advantage PCB cloping kit	
pPD95.77-Pe <i>gl-17</i> :: mouse_ <i>mig-13</i> _cDNA1::GFP	ccagcgacgggccatagctca catttcgggcacctgaa	taagaacaaccgctgATGA GTAAAGGAGAAGAAC	Mouse_mig-13 cDNA1 was amplified from mouse cDNA library and inserted into pPD95.77-Pegl-17::GFP via In-fusion advantage PCR cloning kit	
pPD95.77-Pe <i>gl-17</i> :: mouse_ <i>mig-13</i> _cDNA::GFP	ccactgtaagaacaaccgctg	gctttgctgctgtgtATG AGTAAAGGAGAAGAAC	Mouse_mig-13 cDNA2 was amplified from mouse cDNA library and inserted into pPD95.77-Pegl-17:: mouse_mig-13_cDNA1:: GFP via In-fusion advantage PCP cloping kit	Fig. 1 <i>E</i> and Fig. S3 <i>B</i>
pCF95-P <i>mig-13</i> :: mouse_ <i>mig-13</i> _cDNA::GFP	ccagcgacgggccattacct gaaattctgaattaaatg	GTACCGGTAGAAAAA ccctgacactaagtt	Mouse_mig-13 cDNA was amplified from pPD95.77-Pegl-17:: mouse_mig-13_cDNA::GFP and inserted into pCF95 via In-fusion advantage PCR cloning kit	Fig. 1 <i>E</i> and Fig. S3 <i>B</i>
pPD49.26-P <i>mig-13</i> ::mig-13	Cgtgtaacactgcagaga TGCAAGCTTGGCGTAATC	gtcgcattgaattcgtttat GACGGTACCATGGTATTG	Pmig-13::mig-13 sequence was amplified from N2 genomic DNA and inserted into pPD49.26 plasmid via In-fusion advantage PCR cloning kit	
pPD49.26-P <i>gcy-32</i> ::mig-13	cgattgtattatagaat gactaaactactcatagc	atcgtatataatgtg TGCAAGCTTGGCGTAATC	Pgcy-32 sequence was amplified from N2 genomic DNA and inserted into pPD49.26-Pmig-13:: mig-13 plasmid via In-fusion advantage PCR cloning kit	Fig. 2 <i>B–D</i> and Fig. S3 C and <i>L</i>
pPD49.26-Pe <i>gl-46</i> ::mig-13	ggaaaacatctggaa TGCAAGCTTGGCGTAATC	tgatttcagaaggccA tgactaaactactcatagc	Pegl-46 sequence was amplified from N2 genomic DNA and inserted into pPD49.26-Pmig-13::mig-13 plasmid via In-fusion advantage PCR cloping kit	Figs. 2 <i>B–D</i> and 4 <i>D</i> , and Fig. S3 C and <i>D</i>
pPD49.26-P <i>mig-13</i> ::mig-13:: TEV-S::GFP_mig-13_3'UTR	gagtagattacgatgtacatc	GATGAACTATACAAAtaaaat ctgttctttaattttc	TEV-S::GFP was amplified by soeing PCR and inserted into pPD49.26-P <i>mig-13</i> :: mig-13 via In-fusion advantage PCR cloning kit	Fig. S1 <i>D</i>
pPD49.26-P <i>mec-7</i> ::mig-13:: TEV-S::GFP_mig-13_3′UTR	atgacgttctccacc TGCAAGCTTGGCGTAATC	caaatttcaagcaacAt gactaaactactcatagc	Pmec-7 sequence was amplified from N2 genomic DNA and inserted into pPD49.26- Pmig-13::mig-13::TEV-S:: GFP_mig-13_3'UTR	Fig. 2 <i>B–D</i> and 4 <i>D</i> , and Fig. S3 C and <i>L</i>
pPD95.77 <i>-DePmig-13A</i> :: mig-13::GFP	AGGTGACCACCACAAACACC GTTTCTTTTTGTTCAAC	TTGTGGTGGTCACCTGTCGA	pCF95(MIG-13::GFP) plasmid was used to amplify sequence deleting Pmig-13A. then it was circled by In-fusion advantage PCR cloning kit	Fig. 4C

Table S3. Cont.

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Plasmid name	Primer 5'	Primer 3'	Notes	Reference
pPD95.77 <i>-DePmig-13B</i> :: mig-13::GFP	TAGTGTGGTGATAACGAAG AAGGGGGGGGGGGGTCTA	GTTATCACCACACTAGAAG	pCF95(MIG-13::GFP) plasmid was used to amplify sequence deleting Pmig-13B. then it was circled by In-fusion advantage PCR cloning kit	Fig. 4C
pPD95.77 <i>-DePmig-13C</i> :: mig-13::GFP	CCTCTCCCTTGTTAGTCATA ATTCCTAGGCCAGGG	CTAACAAGGGAGAGGTCTATG	pCF95(MIG-13::GFP) plasmid was used to amplify sequence deleting Pmig-13C. then it was circled by In-fusion advantage PCR cloning kit	Fig. 4C
pPD95.77 <i>-DePmig-13 motif</i> :: mig-13::GFP	ATCGTATAGTCAATTAATT AGGCCGACTGTCG	AATTGACTATACGATTGA GTGTTAATGTGCCG	pCF95(MIG-13::GFP) plasmid was used to amplify sequence deleting Pmig-13 motif. then it was circled by In-fusion advantage PCR cloning kit	Fig. 4C
pPD95.77- <i>lin-39</i> ::GFP	ATGACCACATCAACATCACCG	TTCTCCTTTACTCATGAATTGA TTGAAAAGTGGGAACC	lin-39 cDNA was amplified and inserted into pPD95.77	
pPD95.77-P <i>mec-7</i> :: <i>lin-39</i> ::GFP	GTACCGGTAGAAAAA ttccagatgttttccttccg	TGTTGATGTGGTCATggcct tctgaaatcaaaacg	P <i>mec-7</i> was amplified and inserted into pPD95.77- <i>lin-39</i> ::GFP	Fig. 5 C and D
pPD95.77-Pgcy-32:: lin-39::GFP	GTACCGGTAGAAAAAcacat tatatacgatcgaggc	TGTTGATGTGGTCAtctata atacaatcgtgatcttcgc	Pgcy-32 was amplified and inserted into pPD95.77- <i>lin-39</i> ::GFP	Fig. 5 C and D
pPD95.77-Pegl-13:: lin-39::GFP	GTACCGGTAGAAAAATCA CCTGCCCCGACATTA	TGTTGATGTGGTCATTCGTC TACGGCTCATGCTGG	Pegl-13 was amplified and inserted into pPD95.77-lin-39::GFP	Fig. 5 C and D
pCF95-Punc-47:: <i>MIG-13</i> ::GFP	CGCGCCAAAACCGCG GTCTAGAG	atgactaaactactc atagctctcatcc	Punc-47 was amplified from N2 genomic DNA and inserted into pCF95	Fig. 2 <i>B</i> and C, and Fig. S3C



Movie S1. MIG-13::GFP in QR.x migration. Transgenic *C. elegans* strain (GOU263) expressing GFP-tagged MIG-13 (green) and mCherry in Q cells (red). Images were taken by time-lapse fluorescence microscope (Axio Observer Z1 microscope; Carl Zeiss) attached to a spinning disk confocal scan head (Yokogawa CSU-X1 Spinning Disk Unit). The arrow in the first frame shows the WT migration direction. Cell names are adjacent. The anterior of the animal is toward the *Left*. Frames were taken every 40 s for 194 min. The display rate is 7 frames per second. The time stamp on the movie indicates hours:minutes:seconds.



Movie S2. MIG-13::GFP in QR.x migration. Transgenic *C. elegans* strain (GOU649) expressing GFP-tagged MIG-13 (green) and mCherry in Q cells (red). Images were taken by time-lapse fluorescence microscope (Axio Observer Z1 microscope; Carl Zeiss) attached to a spinning disk confocal scan head (Yokogawa CSU-X1 Spinning Disk Unit). The arrow in the first frame shows the WT migration direction. Cell names are adjacent. The anterior of the animal is toward the *Left*. Frames were taken every 40 s for 72 min. The display rate is 7 frames per second. The time stamp on the movie indicates hours:minutes:seconds.

Movie S2



Movie S3. COR-1::GFP in QR.x migration. Transgenic *C. elegans* strain (GOU347) expressing GFP-tagged COR-1 (green) and mCherry in Q cells (red). Images were taken by time-lapse fluorescence microscope (Axio Observer Z1 microscope; Carl Zeiss) attached to a spinning disk confocal scan head (Yokogawa CSU-X1 Spinning Disk Unit). The arrow in the first frame shows the WT migration direction. Cell names are adjacent. The anterior of the animal is toward the *Left*. Frames were taken every 60 s for 65 min. The display rate is 7 frames per second. The time stamp on the movie indicates hours:minutes:seconds.

Movie S3



Movie S4. COR-1::GFP in QR.x migration of *mig-2(mu28)* mutant. Transgenic *C. elegans* strain (GOU890) expressing GFP-tagged COR-1 (green) and mCherry in Q cells (red). Images were taken by time-lapse fluorescence microscope (Axio Observer Z1 microscope; Carl Zeiss) attached to a spinning disk confocal scan head (Yokogawa CSU-X1 Spinning Disk Unit). The arrow in the first frame shows the WT migration direction. Cell names are adjacent. The anterior of the animal is toward the *Left*. Frames were taken every 60 s for 111 min. The display rate is 7 frames per second. The time stamp on the movie indicates hours:minutes: seconds.



Movie S5. COR-1::GFP in QR.x migration of *mig-2(rh17)* mutant. Transgenic *C. elegans* strain (GOU350) expressing GFP-tagged COR-1 (green) and mCherry in Q cells (red). Images were taken by time-lapse fluorescence microscope (Axio Observer Z1 microscope; Carl Zeiss) attached to a spinning disk confocal scan head (Yokogawa CSU-X1 Spinning Disk Unit). The arrow in the first frame shows the WT migration direction. Cell names are adjacent. The anterior of the animal is toward the *Left*. Frames were taken every 40 s for 186 min. The display rate is 7 frames per second. The time stamp on the movie indicates hours:minutes: seconds.

Movie S5



Movie S6. COR-1::GFP in QR.x migration of *ina-1(gm144)* mutant. Transgenic *C. elegans* strain (GOU420) expressing GFP-tagged COR-1 (green) and mCherry in Q cells (red). Images were taken by time-lapse fluorescence microscope (Axio Observer Z1 microscope; Carl Zeiss) attached to a spinning disk confocal scan head (Yokogawa CSU-X1 Spinning Disk Unit). The arrow in the first frame shows the WT migration direction. Cell names are adjacent. The anterior of the animal is toward the *Left*. Frames were taken every 60 s for 102 min. The display rate is 7 frames per second. The time stamp on the movie indicates hours:minutes: seconds.

Movie S6



Movie S7. COR-1::GFP in QR.x migration of *mig-13(cas15)* mutant. Transgenic *C. elegans* strain (GOU422) expressing GFP-tagged COR-1 (green) and mCherry in Q cells (red). Images were taken by time-lapse fluorescence microscope (Axio Observer Z1 microscope; Carl Zeiss) attached to a spinning disk confocal scan head (Yokogawa CSU-X1 Spinning Disk Unit). The arrow in the first frame shows the WT migration direction. Cell names are adjacent. The anterior of the animal is toward the *Left*. Frames were taken every 60 s for 151 min. The display rate is 7 frames per second. The time stamp on the movie indicates hours: minutes:seconds.



Movie S8. COR-1::GFP in QL.ap migration. Transgenic C. *elegans* strain (GOU347) expressing GFP-tagged COR-1 (green) and mCherry in Q cells (red). Images were taken by time-lapse fluorescence microscope (Axio Observer Z1 microscope; Carl Zeiss) attached to a spinning disk confocal scan head (Yokogawa CSU-X1 Spinning Disk Unit). The arrow in the first frame shows the WT migration direction. Cell names are adjacent. The anterior of the animal is toward the *Left*. Frames were taken every 60 s for 90 min. The display rate is 7 frames per second. The time stamp on the movie indicates hours:minutes:seconds.

Movie S8



Movie S9. COR-1::GFP in QL.ap migration with *mig-13* ectopic expression. Transgenic *C. elegans* strain (GOU482) expressing GFP-tagged COR-1 (green) and mCherry in Q cells (red). Images were taken by time-lapse fluorescence microscope (Axio Observer Z1 microscope; Carl Zeiss) attached to a spinning disk confocal scan head (Yokogawa CSU-X1 Spinning Disk Unit). The arrow in the first frame shows the WT migration direction. Cell names are adjacent. The anterior of the animal is toward the *Left*. Frames were taken every 60 s for 121 min. The display rate is 7 frames per second. The time stamp on the movie indicates hours:minutes:seconds.

Movie S9



Movie S10. COR-1::GFP during QR.x migration of *lin-39(n1760)* mutant. Transgenic *C. elegans* strain (GOU988) expressing mCherry in Q cells (red). Images were taken by time-lapse fluorescence microscope (Axio Observer Z1 microscope; Carl Zeiss) attached to a spinning disk confocal scan head (Yokogawa CSU-X1 Spinning Disk Unit). The arrow in the first frame shows the WT migration direction. Cell names are adjacent. The anterior of the animal is toward the *Left*. Frames were taken every 60 s for 61 min. The display rate is 7 frames per second. The time stamp on the movie indicates hours:minutes:seconds.



Movie S11. LIN-39::GFP in QR.x migration. Transgenic *C. elegans* strain (GOU785) expressing GFP-tagged LIN-39 (green) and mCherry in Q cells (red). Images were taken by time-lapse fluorescence microscope (Axio Observer Z1 microscope; Carl Zeiss) attached to a spinning disk confocal scan head (Yokogawa CSU-X1 Spinning Disk Unit). The arrow in the first frame shows the WT migration direction. Cell names are adjacent. The anterior of the animal is toward the *Left*. Frames were taken every 60 s for 99 min. The display rate is 7 frames per second. The time stamp on the movie indicates hours:minutes:seconds.

Movie S11



Movie S12. LIN-39::GFP in QL.x migration. Transgenic *C. elegans* strain (GOU785) expressing GFP-tagged LIN-39 (green) and mCherry in Q cells (red). Images were taken by time-lapse fluorescence microscope (Axio Observer Z1 microscope; Carl Zeiss) attached to a spinning disk confocal scan head (Yokogawa CSU-X1 Spinning Disk Unit). The arrow in the first frame shows the WT migration direction. Cell names are adjacent. The anterior of the animal is toward the *Left*. Frames were taken every 60 s for 135 min. The display rate is 7 frames per second. The time stamp on the movie indicates hours:minutes:seconds.