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Supplementary Information for

A switch from non-canonical to canonical Wnt signaling stops neuroblast migration through a Slt - Robo and RGA-9b/ARHGAP dependent mechanism

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Other supplementary materials for this manuscript include the following:
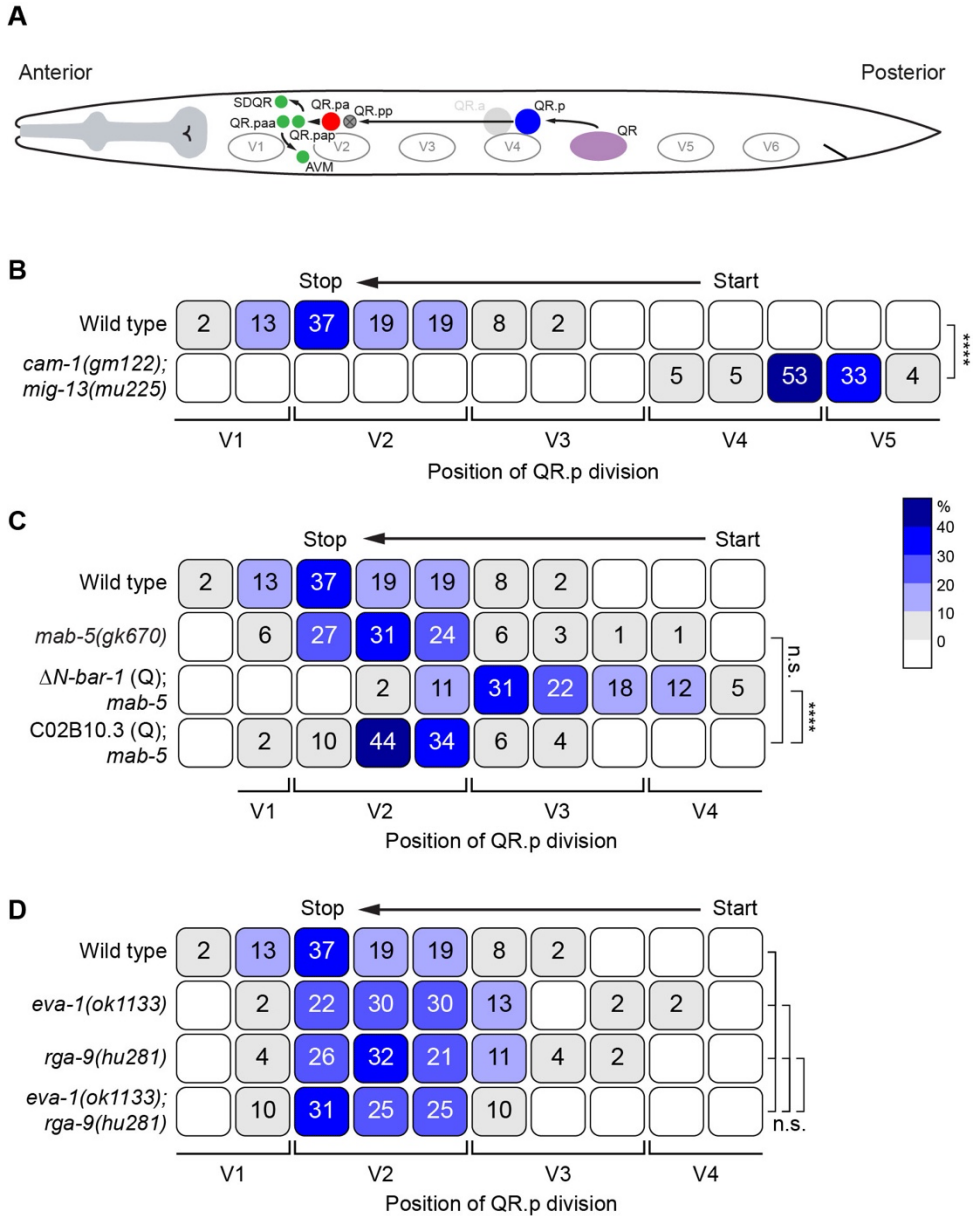
Datasets S1 to S2

A

Anterior   Posterior

SDQR QR.pa QR.pp
QR.paa   QR.pap
        AVM
QR.a   QR.p
              QR

V1 V2 V3 V4 V5 V6

B

Stop ← → Start

Position of QR.p division

Genotype													
Wild type	2	13	37	19	19	8	2					
cam-1(gm122); mig-13(mu225)									5	5	53	33	4

V1   V2   V3   V4   V5

****

C

Stop ← → Start

Position of QR.p division

Genotype									
Wild type	2	13	37	19	19	8	2		
mab-5(gk670)		6	27	31	24	6	3	1	1
ΔN-bar-1 (Q); mab-5				2	11	31	22	18	12	5
C02B10.3 (Q); mab-5		2	10	44	34	6	4		

V1   V2   V3   V4

n.s.
****

%
40
30
20
10
0

D

Stop ← → Start

Position of QR.p division

Genotype									
Wild type	2	13	37	19	19	8	2		
eva-1(ok1133)		2	22	30	30	13		2	2
rga-9(hu281)		4	26	32	21	11	4	2	
eva-1(ok1133); rga-9(hu281)		10	31	25	25	10			

V1   V2   V3   V4

n.s.

Fig. S1. QR neuroblast lineage. (*A*) QR neuroblast lineage and migration of the QR descendants. (*B*), (*C*) and (*D*) Position of QR.p division with respect to the seam cells, indicated as percentiles of the total number of cells analyzed (n≥50 for all genotypes). Statistical significance was calculated using Fisher's exact test (n.s. p≥0.05, **** p<0.0001). The ΔN-BAR-1 (Q) and C02B10.3 (Q) expressing strains contain *mab-5(gk670)*.
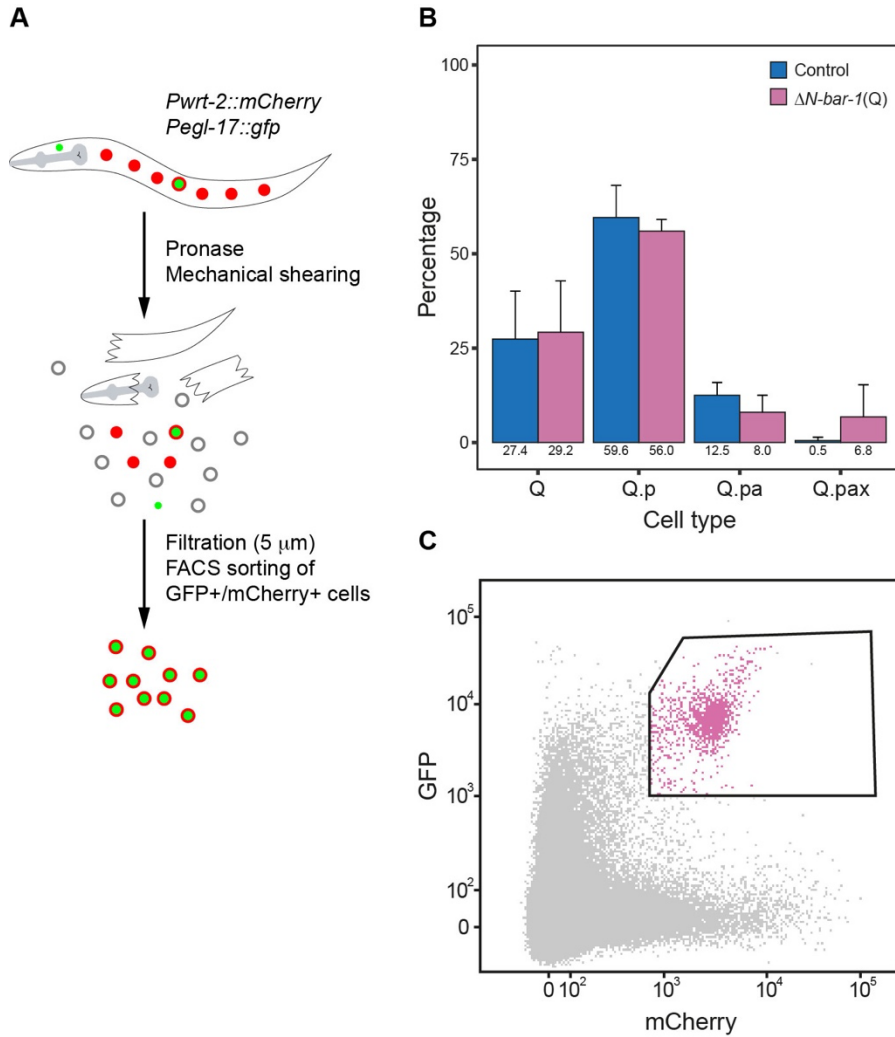
Fig. S2. Isolation of the Q neuroblast and their descendants. (*A*) Schematic representation of the cell isolation procedure. See methods for details. (*B*) Quantification of Q lineage cells present in synchronized *mab-5(gk670)* control and *mab-5(gk670);* ΔN-BAR-1 (Q) animals used for cell isolation. Error bars represent SEM, n>50. (*C*) Representative FACS profile of double labeled Q neuroblast descendants isolated for RNA-sequencing.
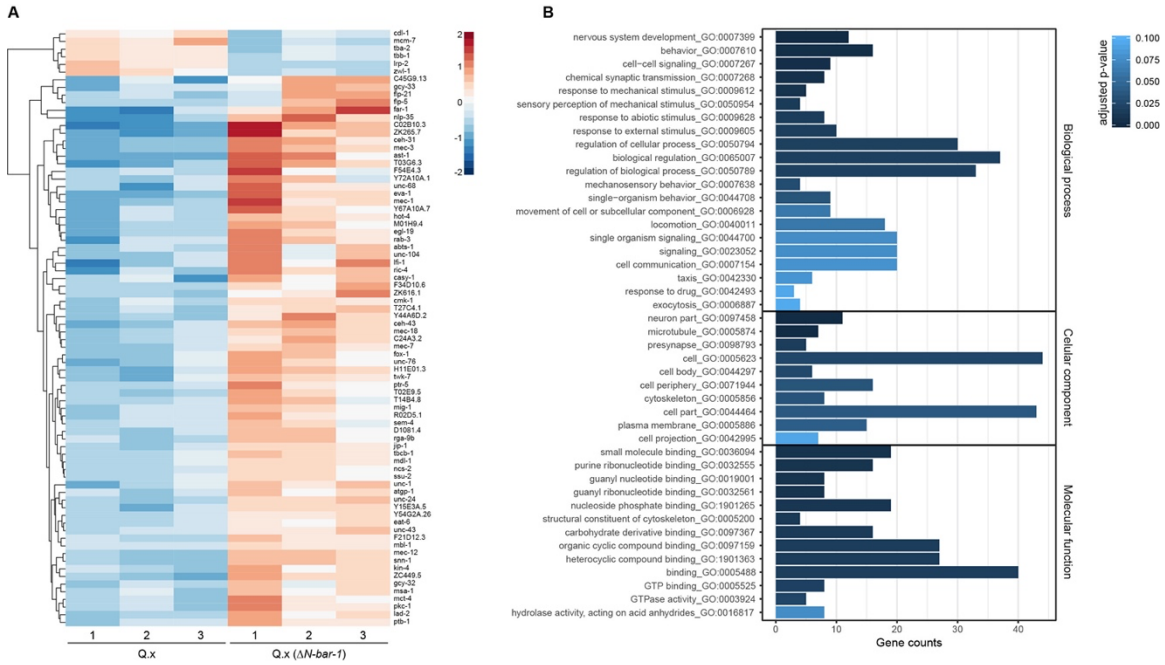
Fig. S3. Gene expression changes induced by expression of ΔN-BAR-1 in the Q cell lineage. (*A*) Heat map of genes differentially expressed between *mab-5(gk670)* control and *mab-5(gk670); ΔN-BAR-1 (Q). (*B*) Gene ontology enrichment of the same differentially expressed genes.

Table S1. Primers and oligonucleotides.

sgRNA oligos	
dpy-10	gtaatacgactcactatagggctaccataggcaccacgaggtttttagagctagaaatagc
Peva-1_TCFm #1	gtaatacgactcactataggtacttttcaaaaaaggcgtagtttttagagctagaaatagc
Peva-1_TCFm #2	gtaatacgactcactataggctctgtactacatatttcaagtttttagagctagaaatagc
Prga9_TCFm	gtaatacgactcactataggacattcgtgaattgtcggctgtttttagagctagaaatagc
rga-9 #1	gtaatacgactcactataggaaaggagtcgggcgtcgagggtttttagagctagaaatagc
rga-9 #2	gtaatacgactcactatagggcttgctcgtcaacgaccgcgtttttagagctagaaatagc
rga-9 #3	gtaatacgactcactataggttagagcagttacaaaataagtttttagagctagaaatagc
rga-9 #4	gtaatacgactcactataggtcgtctttttctatttctaggtttttagagctagaaatagc
srgp-1 #1	gtaatacgactcactataggcaagtttgctctaacgaaatgtttttagagctagaaatagc
srgp-1 #2	gtaatacgactcactatagggaagatcgacttgatgcaatgtttttagagctagaaatagc
ssODN repair templates	
dpy-10	acttgaacttcaatacggcaagatgagaatgactggaaaccgtaccgcatgcggtgcctatggtagcggagcttcacatggcttcagaccaacagcctat
Peva-1_TCFm	cggaaattccagaacttcaatttcaatgggaaaaattgtacgcatcctatgaatgtccctacgcctaagggcccaagtaagcgaattctatgaaaatatctaaagaaaacggaaaaaatttcaaaaaggtacagttttaagtgtttcccaattataaaaaaaatccctctaaacatttccggcaaacggcattttgccgga
Prga9_TCFm	tttgaacgcggatttggagactgtaaaaagtgaaatatccaatagaaaagtttggtgaactttggcaaatttttactaacattcgataactaaaggatcccattcgtgaattgtcggctaggatgtataaagcttttggcaatttgtttagctttctctaatgatttaccaactttcaccaatttgttttttcaactttc
srgp-1	ttttattactttgttttatatttcaaaaacatcaagtcgatcttcaaatcgatcaaaagc
Gateway compatible primers	
ΔN-bar-1(Q)_F	ggggacaagtttgtacaaaaaagcaggctttatggccgactatgagccg
ΔN-bar-1(Q)_R	ggggaccactttgtacaagaaagctgggttttaaaatcgactattcctag
C02B10.3(Q)_F	ggggacaagtttgtacaaaaaagcaggctttatgaccggagcactatgc
C02B10.3 (Q)_R	ggggaccactttgtacaagaaagctgggttctaatagttgaacttgttg
eva-1(Q)_F	ggggacaagtttgtacaaaaaagcaggctcaatgaatatgcatatagtttcccc
eva-1(Q)_R	ggggaccactttgtacaagaaagctgggtcttaaaaatcatagtgactataatgtggatt
rga-9b(Q)_F	ggggacaagtttgtacaaaaaagcaggctttatgacagtggcgagttac
rga-9b(Q)_R	ggggaccactttgtacaagaaagctgggtttcagaactgagtcgatgt
sax-3(Q)_F	ggggacaagtttgtacaaaaaagcaggctatgttcaatcgaaaaacgctg
sax-3(Q)_R	ggggaccactttgtacaagaaagctgggttcaagtttgttcttgtgtgacg

Table S2. Reagents and resources.

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Antibodies		
Bacterial and Virus Strains		
OP50	CGC	N/A
One Shot® Top10 chemically competent *E. coli*	Invitrogen™	C404003
Biological Samples		
Chemicals, Peptides, and Recombinant Proteins		
Ultra-pure RNase free water	Invitrogen™	10977049
RNase OUT	Invitrogen™	10777-019
Second strand buffer	Invitrogen™	10812-014
DNA polymerase I (*E. coli*)	Invitrogen™	18010-025
E. coli DNA ligase	Invitrogen™	18052-019
T4 RNA ligase 2, truncated	NEB Inc.	M0242
RnaseH (*E. coli*)	Invitrogen™	18021-071
TRIzol™ Reagent	Ambion®	15596018
GlycoBlue™ Coprecipitant	Ambion®	AM9515
Chloroform stabilized with ethanol	Boom	76025322.2500
Ethanol 100% absolut	Boom	84028185.2500
2-propanol, HPCL	Boom	76025379.2500
Acetic acetate pH 8.1	Sigma-Aldrich®	93337
KOAc	Sigma-Aldrich®	95843
MgOAc	Sigma-Aldrich®	63052
Ethylenediaminetetraacetic acid solution pH 8	Sigma-Aldrich®	03690
Ampicillin	Sigma-Aldrich®	A9518
Kanamycin	Sigma-Aldrich®	K1377
Luria Agar Base	Difco™	241320
Luria Bertani Broth	OXOID	101417
NGM Agar	OXOID	CM1110
Sodium azide	Sigma-Aldrich®	S2002
Paraffin, liquid, GPR Rectapur®	VWR™	24679.291
UltraPure™ Agarose	Invitrogen™	16500-500
Critical Commercial Assays		
Qubit dsDNA HS Assay Kit	Invitrogen™	Q32854
RNA pico kit	Agilent Technologies	5067-1513
High sensitivity DNA kit	Agilent Technologies	5067-4626
ERCC RNA spike-ins mix	Ambion®	4456740
MessageAmpII kit	Ambion®	AM1751
SuperScript II Reverse Transcriptase	Invitrogen™	18064-014
Deoxynucleotide (dNTP) Solution Mix	NEB Inc.	N0447S
MEGAscript T7 transcription kit	Ambion®	AM1334
AMPure XP beads	Beckman Coulter	A63880
High-fidelity PCR Master Mix with HF buffer	NEB Inc.	M0531

RNA RT primer (RP1)	Illumina®	N/A
RNA PCR primers	Illumina®	N/A
RNeasy MinElute kit	Qiagen®	74204
MultiSite Gateway® Three- Fragment Vector Construction Kit	Invitrogen™	12537-023
QIAprep Spin Miniprep Kit	Qiagen®	27106
Deoxynucleotide Triphosphates mix	Promega	U1420
Experimental Models: Organisms/Strains		
C. elegans: *sax-3(ky123)*	CGC	CX3198
C. elegans: *slt-1(eh15)*	CGC	CX5000
C. elegans: *bar-1(ga80)*	CGC	EW15
C. elegans: heIs63[P*wrt-2*::GFP::H2B; P*wrt-2*::GFP::PH; P*lin-48*::tdTomato] V; *unc-73(e936)*	This study	KN2195
C. elegans: heIs63[P*wrt-2*::GFP::H2B; P*wrt-2*::GFP::PH; P*lin-48*::tdTomato] V ; *mab-5(gk670)*	This study	KN2591
C. elegans: heIs63[P*wrt-2*::GFP::H2B; P*wrt-2*::GFP::PH; P*lin-48*::tdTomato] V ; *pix-1(ok982)*	This study	KN2592
C. elegans: casIs328[P*egl-17*::myri-mCherry; P*egl-17*::mCherry-TEV-*his-24*, P*egl-17*::GFP::moesinABD]	Gift from G. Ou	
C. elegans: heIs63[P*wrt-2*::GFP::H2B; P*wrt-2*::GFP::PH; P*lin-48*::tdTomato] V; huIs179[P*egl-17*::∆N-*bar-1*::*unc-54* 3' UTR; P*myo-2*::mCherry] IV ; *mab-5(gk670)*	This study	KN2651
C. elegans: ayIs9[P*egl-17*::GFP] V ; huIs166[P*wrt-2*::mCherry::H2B; P*wrt-2*::mCherry::PH] X ; *mab-5(gk670)*	This study	KN2664
C. elegans: heIs63[P*wrt-2*::GFP::H2B; P*wrt-2*::GFP::PH; P*lin-48*::tdTomato] V ; *cam1(gm122) ; mig-13(mu225)*	This study	KN2680
C. elegans: ayIs9[P*egl-17*::GFP] V ; huIs166[P*wrt-2*::mCherry::H2B; P*wrt-2*::mCherry::PH] X ; huIs179[P*egl-17*::∆N-*bar-1*::*unc-54* 3' UTR; P*myo-2*::mCherry] IV ; *mab-5(gk670)*	This study	KN2683
C. elegans: heIs63[P*wrt-2*::GFP::H2B; P*wrt-2*::GFP::PH; P*lin-48*::tdTomato] V; huIs179[P*egl-17*::∆N-*bar-1*::*unc-54* 3' UTR; P*myo-2*::mCherry] IV ; *mab-5(gk670); pop-1(hu9)*	This study	KN2684
C. elegans: heIs63[P*wrt-2*::GFP::H2B; P*wrt-2*::GFP::PH; P*lin-48*::tdTomato] V; huIs179[P*egl-17*::∆N-*bar-1*::*unc-54* 3' UTR; P*myo-2*::mCherry] IV ; *cam-1(gm122) ; mab-5(gk670)*	This study	KN2707
C. elegans: heIs63[P*wrt-2*::GFP::H2B; P*wrt-2*::GFP::PH; P*lin-48*::tdTomato] V; huIs179[P*egl-17*::∆N-*bar-1*::*unc-54* 3' UTR; P*myo-2*::mCherry] IV ; *cwn-1(ok546) ; mab-5(gk670)*	This study	KN2717
C. elegans: heIs63[P*wrt-2*::GFP::H2B; P*wrt-2*::GFP::PH; P*lin-48*::tdTomato] V; huIs179[P*egl-17*::∆N-*bar-1*::*unc-54* 3' UTR; P*myo-2*::mCherry] IV ; *mab-5(gk670) ; mig-13(mu225)*	This study	KN2754
C. elegans: heIs63[P*wrt-2*::GFP::H2B; P*wrt-2*::GFP::PH; P*lin-48*::tdTomato] V; cam-1(gm122); *mab-5(gk670)*	This study	KN2808
C. elegans: heIs63[P*wrt-2*::GFP::H2B; P*wrt-2*::GFP::PH; P*lin-48*::tdTomato] V ; huEx731[P*egl-17*::*eva-1* cds::*unc-54* 3' UTR; pRF4; P*lin-32*::tdTomato] ; *mab-5(gk670)*	This study	KN2817

C. elegans: heIs63[P*wrt-2*::GFP::H2B; P*wrt-2*::GFP::PH; P*lin-48*::tdTomato] V ; huEx737[P*egl-17*::*rga-9* cds::*unc-54* 3' UTR; pRF4; P*lin-32*::tdTomato] ; *mab-5(gk670)*	This study	KN2825
C. elegans: heIs63[P*wrt-2*::GFP::H2B; P*wrt-2*::GFP::PH; P*lin-48*::tdTomato] V ; huEx740[P*egl-17*::C02B10.3 cds::*unc-54* 3' UTR; pRF4; P*lin-32*::tdTomato] ; *mab-5(gk670)*	This study	KN2828
C. elegans: heIs63[P*wrt-2*::GFP::H2B; P*wrt-2*::GFP::PH; P*lin-48*::tdTomato] V ; huEx743[pRF4; P*lin-32*::tdTomato] ; *mab-5(gk670)*	This study	KN2831
C. elegans: heIs63[P*wrt-2*::GFP::H2B; P*wrt-2*::GFP::PH; P*lin-48*::tdTomato] V; *cwn-1(ok546)*; *mab-5(gk670)*	This study	KN2841
C. elegans: heIs63[P*wrt-2*::GFP::H2B; P*wrt-2*::GFP::PH; P*lin-48*::tdTomato] V ; *cwn-1(ok546)*; *pix-1(ok982)*	This study	KN2845
C. elegans: heIs63[P*wrt-2*::GFP::H2B; P*wrt-2*::GFP::PH; P*lin-48*::tdTomato] V ; huIs214[P*egl-17*::*eva-1* cds::*unc-54* 3' UTR; pRF4; P*lin-32*::tdTomato] ; *mab-5(gk670)*	This study	KN2866
C. elegans: heIs63[P*wrt-2*::GFP::H2B; P*wrt-2*::GFP::PH; P*lin-48*::tdTomato] V; huIs179[P*egl-17*::ΔN-*bar-1*::*unc-54* 3' UTR; P*myo-2*::mCherry] IV ; *mab-5(gk670); slt-1(eh15)*	This study	KN2879
C. elegans: heIs63[P*wrt-2*::GFP::H2B; P*wrt-2*::GFP::PH; P*lin-48*::tdTomato] V; huIs179[P*egl-17*::ΔN-*bar-1*::*unc-54* 3' UTR; P*myo-2*::mCherry] IV ; *eva-1(ok1133); mab-5(gk670)*	This study	KN2880
C. elegans: heIs63[P*wrt-2*::GFP::H2B; P*wrt-2*::GFP::PH; P*lin-48*::tdTomato] V; huIs179[P*egl-17*::ΔN-*bar-1*::*unc-54* 3' UTR; P*myo-2*::mCherry] IV ; *eva-1(hu266[Peva-1TCFm]); mab-5(gk670)*	This study	KN2891
C. elegans: heIs63[P*wrt-2*::GFP::H2B; P*wrt-2*::GFP::PH; P*lin-48*::tdTomato] V huIs221[P*egl-17*::*rga-9b* cds::*unc-54* 3' UTR; pRF4; P*lin-32*::tdTomato] ; *mab-5(gk670)*	This study	KN2900
C. elegans: *rga-9(hu281)*	This study	KN2931
C. elegans: casIs328[P*egl-17*::myri-mCherry; P*egl-17*::mCherry-TEV-*his-24*, P*egl-17*::GFP::moesinABD] ; huIs179[P*egl-17*::ΔN-*bar-1*::*unc-54* 3' UTR; P*myo-2*::mCherry] IV ; *mab-5(gk670)*	This study	KN2939
C. elegans: heIs63[P*wrt-2*::GFP::H2B; P*wrt-2*::GFP::PH; P*lin-48*::tdTomato] V; huIs179[P*egl-17*::ΔN-*bar-1*::*unc-54* 3' UTR; P*myo-2*::mCherry] IV ; *eva-1(ok1133); mab-5(gk670); rga-9(hu281)*	This study	KN2962
C. elegans: heIs63[P*wrt-2*::GFP::H2B; P*wrt-2*::GFP::PH; P*lin-48*::tdTomato] V; *eva-1(ok1133); mab-5(gk670); rga-9(hu281)*	This study	KN2963
eva-1(ok1133); rga-9(hu281)	This study	KN2964
C. elegans: heIs63[P*wrt-2*::GFP::H2B; P*wrt-2*::GFP::PH; P*lin-48*::tdTomato] V; huIs179[P*egl-17*::ΔN-*bar-1*::*unc-54* 3' UTR; P*myo-2*::mCherry] IV ; *mab-5(gk670); rga-9(hu281)*	This study	KN2976
C. elegans: heIs63[P*wrt-2*::GFP::H2B; P*wrt-2*::GFP::PH; P*lin-48*::tdTomato] V; huIs179[P*egl-17*::ΔN-*bar-1*::*unc-54* 3' UTR; P*myo-2*::mCherry] IV ; *mab-5(gk670) ; sax-3(ky123)*	This study	KN3034

C. elegans: heIs63[P*wrt-2*::GFP::H2B; P*wrt-2*::GFP::PH; P*lin-48*::tdTomato] V; huIs179[P*egl-17*::ΔN-*bar-1*::*unc-54* 3' UTR; P*myo-2*::mCherry] IV ; *rga-9(hu293[Prga-9TCFm]); mab-5(gk670)*	This study	KN3055
C. elegans: heIs63[P*wrt-2*::GFP::H2B; P*wrt-2*::GFP::PH; P*lin-48*::tdTomato] V; huIs179[P*egl-17*::ΔN-*bar-1*::*unc-54* 3' UTR; P*myo-2*::mCherry] IV ; *mab-5(gk670) ; srgp-1(hu294)*	This study	KN3066
C. elegans: heIs63[P*wrt-2*::GFP::H2B; P*wrt-2*::GFP::PH; P*lin-48*::tdTomato] V ; *rga-9(hu281)*	This study	KN3079
C. elegans: heIs63[P*wrt-2*::GFP::H2B; P*wrt-2*::GFP::PH; P*lin-48*::tdTomato] V ; *mab-5(gk670) ; pix-1(ok982)*	This study	KN3080
C. elegans: heIs63[P*wrt-2*::GFP::H2B; P*wrt-2*::GFP::PH; P*lin-48*::tdTomato] V ; *mab-5(gk670) ; mig-13(mu225)*	This study	KN3081
C. elegans: heIs63[P*wrt-2*::GFP::H2B; P*wrt-2*::GFP::PH; P*lin-48*::tdTomato] V huIs221[P*egl-17*::*rga-9b* cds::*unc-54* 3' UTR; pRF4; P*lin-32*::tdTomato] ; *mab-5(gk670); pix-1(ok982)*	This study	KN3082
C. elegans: heIs63[P*wrt-2*::GFP::H2B; P*wrt-2*::GFP::PH; P*lin-48*::tdTomato] V huIs221[P*egl-17*::*rga-9b* cds::*unc-54* 3' UTR; pRF4; P*lin-32*::tdTomato] ; *pix-1(ok982)*	This study	KN3083
C. elegans: *eva-1(ok1133); sax-3(ky123)*	This study	KN3120
C. elegans: heIs63[P*wrt-2*::GFP::H2B; P*wrt-2*::GFP::PH; P*lin-48*::tdTomato] V; huIs221[P*egl-17*::*rga-9b* cds::*unc-54* 3' UTR; pRF4; P*lin-32*::tdTomato] ; *mab-5(gk670); unc-73(e936)*	This study	KN3123
C. elegans: huEx829 [*Pegl-17::sax-3, Ptoe-2*::GFP, *Plin-48*::tdTomato]; *sax-3(ky123)*	This study	KN3150
C. elegans: huEx830 [*Pegl-17::sax-3, Ptoe-2*::GFP, *Plin-48*::tdTomato]; *sax-3(ky123)*	This study	KN3151
C. elegans: heIs63[P*wrt-2*::GFP::H2B; P*wrt-2*::GFP::PH; P*lin-48*::tdTomato] V; *mab-5(gk670); pop-1(hu9)*	This study	KN3161
C. elegans: Bristol (N2) strain as wild-type (WT)	CGC	N2
C. elegans: *srgp-1(ok300)*	CGC	RB570
C. elegans: heIs63[P*wrt-2*::GFP::H2B; P*lin-48*::tdTomato] V	Gift from S. van den Heuvel	SV1009
C. elegans: *eva-1(ok1133)*	CGC	VC868

Oligonucleotides		
See Table S1 for primer sequences		

Recombinant DNA		
P*egl-17*::ΔN-*bar-1*::unc-54 3' UTR in pDESTR4-R3	This study	pKN525
P*egl-17*::C02B10.3 cds::unc-54 3' UTR in pDESTR4-R3	This study	pKN576
P*egl-17*::*rga-9*b cds::unc-54 3' UTR in pDESTR4-R3	This study	pKN585
P*egl-17*::*eva-1* cds::unc-54 3' UTR in pDESTR4-R3	This study	pKN597
P*lin-32*::tdTomato::unc-54 3' UTR in pDESTR4-R3	This study	pKN611
Plin-48::tdTomato::unc-54 3'UTR	This study	pKN641
P*egl-17*::*sax-3* cds::unc-54 3' UTR in pDESTR4-R3	This study	pKN708
P*toe-2*::GFP::unc-54 3'UTR	Gift from B. Conradt	pBC1565
pBlueScript II empty vector	Agilent Technologies	212205-8

pRF4 *rol-6(su1006)*	(Mello et al., 1991)	N/A
Software and Algorithms		
Excel 2016	Microsoft	https://products.office.com/en-us/excel
ImageJ2	ImageJ developers	https://imagej.net
ZEN2 core imaging software	Zeiss	https://www.zeiss.com/microscopy/int/products/microscope-software/zen-2-core.html
BWA v0.7.10	Li H. and Durbin R. (2010). PMID: 20080505	http://bio-bwa.sourceforge.net/
MapAndGo v2	This study	http://bit.ly/MapAndGo_v2
Samtools v1.4.1	Li H.*, Handsaker B.*, et al. (2009). PMID: 19505943	http://samtools.sourceforge.net/
R (v 3.3.2), using the packages: DEseq2, pheatmap, MarkdownReports, ggplot2, corrr, and DNAcopy.		https://cran.r-project.org/, https://bioconductor.org/, https://github.com
Other		
Borosilicated thin wall capillary	Harvard apparatus	30-0039
LoBind tubes 1,5ml	Eppendorf Tubes®	Z666548
LoBind tubes 0,5ml	Eppendorf Tubes®	Z666521

Dataset S1 (separate file). Genes differentially expressed between *mab-5(gk670)* control and *mab-5(gk670);* ΔN-BAR-1 (Q).

Dataset S2 (separate file). TCF binding sites (HMG motif) found in the 1.5 kb sequence upstream of genes differentially expressed between ΔN-BAR-1 and control.