Figure S1

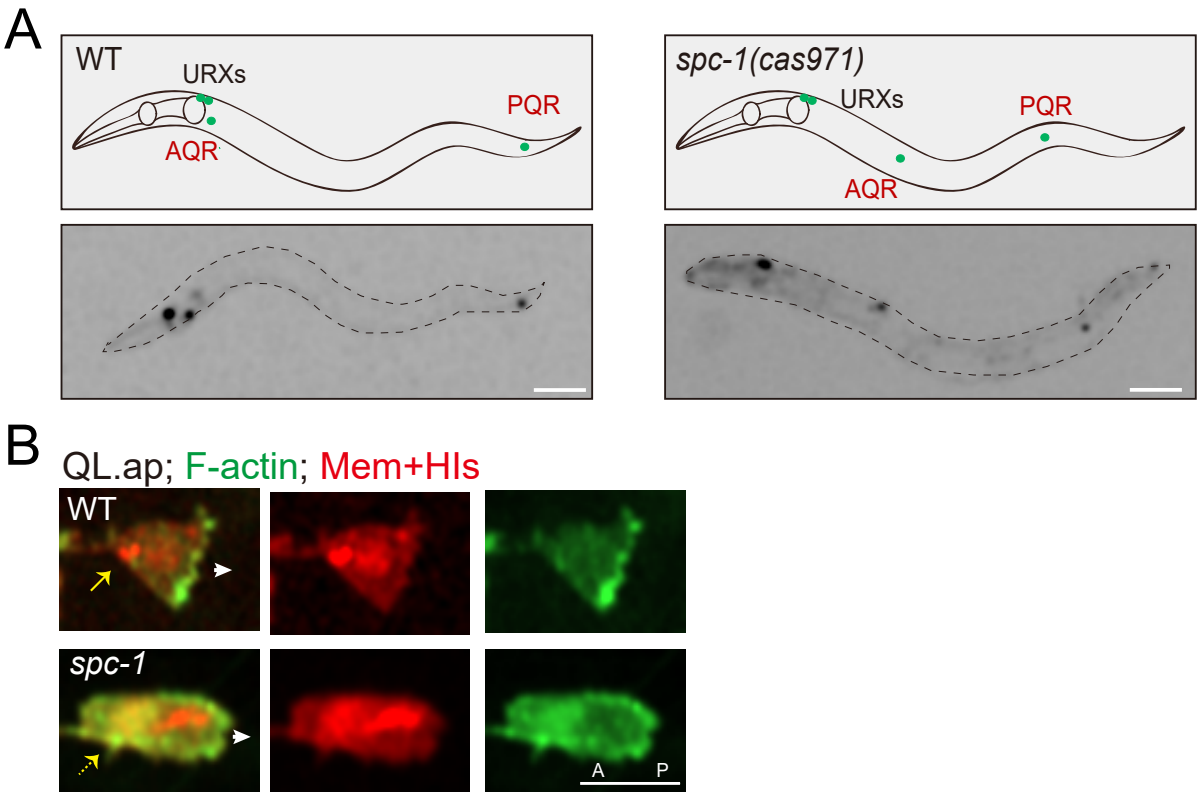
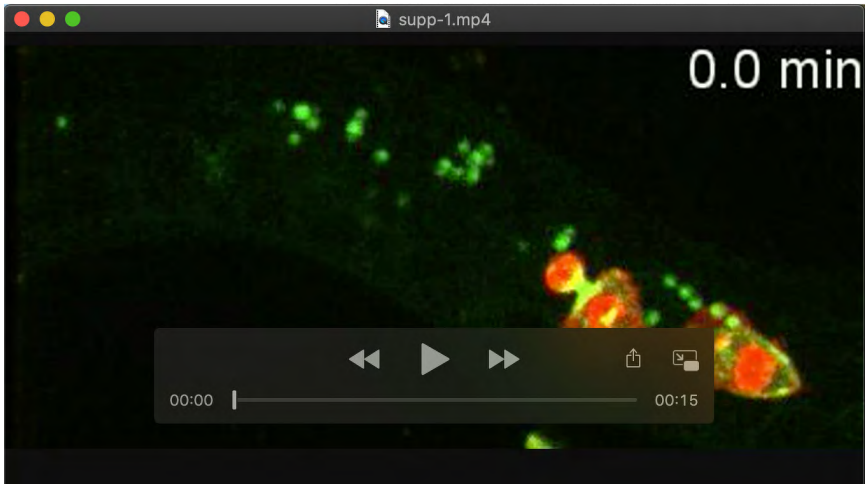
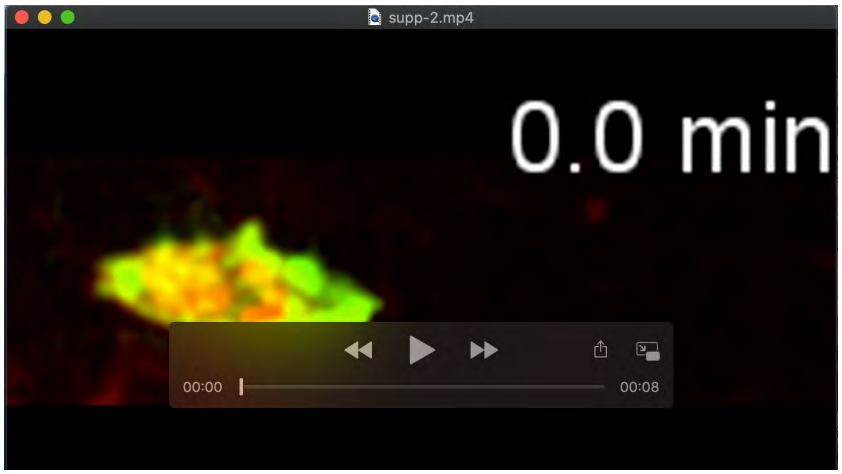


Figure S1. (A) Schematics (upper) and fluorescence inverted images of the A/PQR position in WT and *spc-1(cas971)* mutant animals. A/PQR neurons were visualized using P*gcy-32::mCherry*. The image is inverted so that high mCherry fluorescence intensity is black. The cell identities are denoted adjacent to the cells. Dotted blue lines show the periphery of *C. elegans*. Scale bar, 50 μm. **(B)** Fluorescence images of GFP-tagged F-actin (green) with mCherry (red) labeled plasma membrane and histone in QL.ap cells in WT or *spc-1(cas971)* animals. Yellow arrows show the rear of migrating cells; White arrows indicate the direction of migration. AP, anterior, and posterior. Scale bar, 5 μm. **(C)** Quantification of the GFP (green) of SPC-1 (left) or UNC-70 (right), and mCherry (red) fluorescence intensity ratio between the posterior and the anterior plasma membrane portions in the QL cell lineages (N = 10-20 animals). Anterior and posterior were divided by a dashed line in Fig. 2A. **(D)** Quantification of the fluorescence intensity ratio of split-7xGFP-tagged SPC-1 (green), and mCherry-membrane (red) between the posterior and the anterior of the migrating QL.ap cells (N = 15 animals). The error bars indicate the standard error of the mean (SEM). Statistical significance is based on Student's *t*-test, *** $P < 0.001$.



Movie 1 is related to Figure 2. Dynamic distribution of SPC-1::-7xGFP during QR.ap cell migration

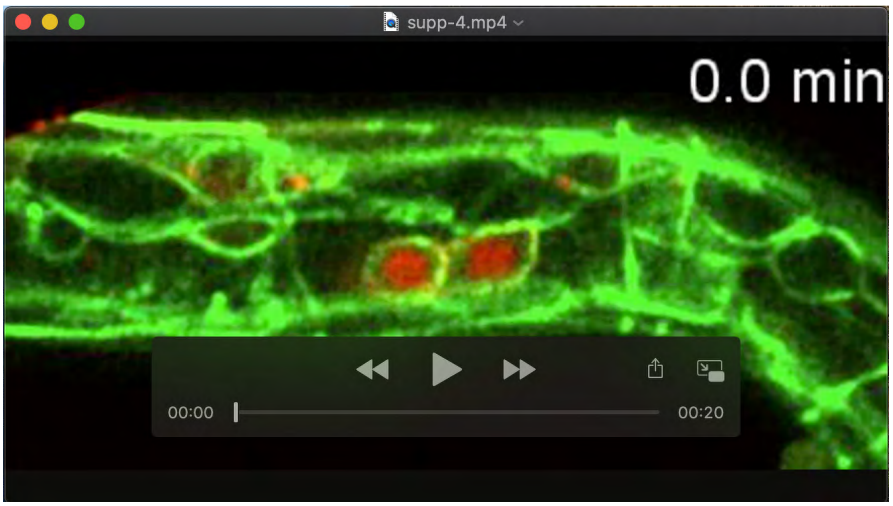
Fluorescence time-lapse movies of QR.ap cell migration with 7xGFP-tagged SPC-1 and mCherry-tagged plasma membrane in a WT animal. Frames were taken every 60 seconds. The display rate is 7 frames per second.



Movie 2 is related to Figure 3. The neuritogenesis of QL.ap in WT Fluorescence time-lapse movies of GFP-tagged F-actin and mCherry-tagged plasma membrane during QL.ap neuritogenesis, showing the transformation of the leading edge into the growth cone. Frames were taken every 60 seconds. The display rate is 7 frames per second.

Movie 3 is related to Figure 3. The neuritogenesis of QL.ap in *spc-1(cas971)* mutant animal

Fluorescence time-lapse movies of GFP-tagged F-actin and mCherry-tagged plasma membrane during QL.ap neuritogenesis in *spc-1(cas971)* mutants, showing the transformation of the leading edge into the branched growth cone. Frames were taken every 60 seconds. The display rate is 7 frames per second.



Movie 4 is related to Figure 4. Dynamic distribution of GFP::UNC-44 during QR.a and QR.ap cell migration in the knock-in animals

Fluorescence time-lapse movies of QR.ap cell migration with GFP-tagged UNC-44 and mCherry-tagged plasma membrane in a knock-in animal. Frames were taken every 60 seconds. The display rate is 7 frames per second.

Supplemental Tables

Table S1 Targets of CRISPR and primers for molecular analysis

Gene	CRISPR-Cas9 targets (PAM)	Primers (For: forward; Rev: reverse)
spc-1 knock in	sg1: GCGAGATTAGCTCTGGAACA **AGG**	For: ATTAGCTCTGGAACAGTTTTAGAGCTAGAAAT AGCAAG
		Rev: TGTTCCAGAGCTAATCTCGCCAAGACATCTCG CAATAGG
	sg2:ACAATTGGCGAGATTAGCT C **TGG**	For: TGGCGAGATTAGCTCGTTTTAGAGCTAGAAAT AGCAAG
		Rev: GAGCTAATCTCGCCAATTGTCAAGACATCTCG CAATAGG
unc-70 knock in	*sg1:*CGTCGTCGGCAATATGG CTA **CGG**	For: TAGCCATATTGCCGACGACGCAAGACATCTCG CAATAGG
		Rev: TCGGCAATATGGCTAGTTTTAGAGCTAGAAAT AGCAAG
	sg2: GCGAAACGTCGTCGGCAATA **TGG**	For: TATTGCCGACGACGTTTCGCCAAGACATCTCG CAATAGG
		Rev: ACGTCGTCGGCAATAGTTTTAGAGCTAGAAAT AGCAAG
spc-1-L268P knock in	*sg1:* ACTCGTAAGGAAGGGCTCTT **TGG**	For: TAAGGAAGGGCTCTTGTTTTAGAGCTAGAAAT AGCAAG
		Rev: AAGAGCCCTTCCTTACGAGTCAAGACATCTCG CAATAGG
	sg2: AAAGAGCCCTTCCTTACGAG **TGG**	For: CTCGTAAGGAAGGGCTCTTTCAAGACATCTCG CAATAGG
		Rev:

		GCCCTTCCTTACGAGGTTTTAGAGCTAGAAATAGCAAG
unc-70-ΔH590-L598 knock in	*sg1:* CGAGAGTAAGAAGCATATCG **TGG**	For: CGATATGCTTCTTACTCTCGCAAGACATCTCGCAATAGG
		Rev: GTAAGAAGCATATCGGTTTTAGAGCTAGAAATAGCAAG
	sg2: GCTTCTTACTCTCGATCTCA **TGG**	For: TGAGATCGAGAGTAAGAAGCCAAGACATCTCGCAATAG
		Rev: TTACTCTCGATCTCAGTTTTAGAGCTAGAAATAGCAAG
unc-44 knock in	*sg1:* CGCCTTCGTTCGACATGGT **CGG**	For: CCTTCGTTCGACATGGTGTTTTAGAGCTAGAAATAGCAAG
		Rev: ATGTCGAACGAAGGCGCAAGACATCTCGCAATAGGAGGTG
	sg2: CAACAACAGCCGGAGTCAC **AGG**	For: ACAACAGCCGGAGTCACGTTTTAGAGCTAGAAATAGCAAG
		Rev: GACTCCGGCTGTTGTTGCAAGACATCTCGCAATAGGAGGTG

Table S2 Primers and plasmids used for plasmid cloning in this study

Plasmid Name	**Primer 5'**	**Primer 3'**	**Notes**
pDD162-P*eft-3*::Cas9 + P*U6:: spc-1 knock in sg1*	TGTTCCAGAGCTAATCTCGCCAAGACATCTCGCAATAG	ATTAGCTCTGGAACAGTTTTAGAGCTAGAAATAGCAA	PCR from pDD162-P*eft-3*::Cas9+P*U6::Empty sgRNA*
pDD162-P*eft-3*::Cas9 + P*U6::spc-1 knock in sg2*	GAGCTAATCTCGCCAATTGTCAAGACATCTCGCAATAG	TGGCGAGATTAGCTCGTTTTAGAGCTAGAAATAGCAA	PCR from pDD162-P*eft-3*::Cas9+P*U6::Empty sgRNA*
pDD162-P*eft-3*::Cas9 + P*U6:: unc-70 knock in sg1*	TAGCCATATTGCCGACGACGCAAGACATCTCGCAATA	TCGGCAATATGGCTAGTTTTAGAGCTAGAAATAGCAA	PCR from pDD162-P*eft-3*::Cas9+P*U6::Empty sgRNA*
pDD162-P*eft-3*::Cas9 + P*U6:: unc-70 knock in sg2*	TATTGCCGACGACGTTTCGCCAAGACATCTCGCAATAG	ACGTCGTCGGCAATAGTTTTAGAGCTAGAAATAGCAA	PCR from pDD162-P*eft-3*::Cas9+P*U6::Empty sgRNA*
pDD162-P*eft-3*::Cas9 + P*U6:: spc-1-L268 knock in sg1*	AAGAGCCCTTCCTTACGAGTCAAGACATCTCGCAATAG	TAAGGAAGGGCTCTTGTTTTAGAGCTAGAAATAGCAA	PCR from pDD162-P*eft-3*::Cas9+P*U6::Empty sgRNA*
pDD162-P*eft-3*::Cas9 + P*U6:: spc-1-L268 knock in sg2*	TTCCTTACGAGTGGCCGCCACAAGACATCTCGCAATA	GCCACTCGTAAGGAAGTTTTAGAGCTAGAAATAGCAA	PCR from pDD162-P*eft-3*::Cas9+P*U6::Empty sgRNA*
pDD162-P*eft-3*::Cas9 + P*U6:: unc-70-ΔH590-L598 knock in sg1*	CGATATGCTTCTTACTCTCGCAAGACATCTCGCAATA	GTAAGAAGCATATCGGTTTTAGAGCTAGAAATAGCAA	PCR from pDD162-P*eft-3*::Cas9+P*U6::Empty sgRNA*

pDD162-P*eft-3*::Cas9 + P*U6:: unc-70-ΔH590-L598 knock in sg2*	TGAGATCGAGAGTAAGAAGCCAAGACATCTCGCAATA	TTACTCTCGATCTCAGTTTTAGAGCTAGAAATAGCAAG	PCR from pDD162-P*eft-3*::Cas9+P*U6::Empty sgRNA*
pPD95.77-*spc-1-5' arm::gfp knock in*	GAAGAGTAATTGGACCACAATGCGAGTGGCGTTTC	GTACCGGTAGAAAAAGGAAGAGATCCGTGCCTT	The 5' arm sequences were amplified from N2 and cloned into pPD95.77 via In-Fusion Advantage PCR Cloning Kit.
pPD95.77-*spc-1-5' arm::gfp-3' arm knock in*	AGACCCAAGCTTGGTACCATGAGT	CTATTTGTATAGTTCATCCATGCC	The 3' arm sequences were amplified from N2 and cloned into pPD95.77-*spc-1-5' arm::gfp* knock in via In-Fusion Advantage PCR Cloning Kit.
pPD*95.77-unc-70-5' arm::gfp knock in*	GAAGAGTAATTGGACCAGACGTTCACCGGAAAGAACTGCG	GTACCGGTAGAAAAACCACCCATCACTCTCTCGTAACCTC	The 5' arm sequences were amplified from N2 and cloned into pPD95.77 via In-Fusion Advantage PCR Cloning Kit.
pPD95.77-*unc-70-5' arm::gfp-3' arm knock in*	GGAAGTGGTAGCGGTATGGCTACGGTGAGTTTTTT	ATGAGTAAAGGAGAAGAACTTTTC	The 3' arm sequences were amplified from N2 and cloned into pPD95.77-*unc-70-1-5' arm::gfp knock in* via In-Fusion Advantage PCR Cloning Kit.
pPD95.77-*spc-1-5' arm::7×gfp11 knock in*	GAAGAGTAATTGGACCACAATGCGAGTGGCGTTTC	5 GTACCGGTAGAAAAAGGAAGAGATCCGTGCCTTGC	The 5' arm sequences were amplified from N2 and cloned into pPD95.77 via In-Fusion Advantage PCR Cloning Kit.
pPD95.77-*spc-1-5' arm::7×gfp11-3' arm knock in*	GGTGATACCGGCAGCATTGACATATTCG	ATGCGTGACCACATGGTCCTTCATGA	The 3' arm sequences were amplified from N2 and cloned into pPD95.77-*spc-1-5' arm::7×gfp11* knock in via In-Fusion Advantage PCR Cloning Kit.
pPD95.77-*spc-1-L268P arm knock in*	GAACGAGTCAGCACGAGCATCAAT	GCCCTCTGGGATAAGCTCTTCTTCAAACTG	The *spc-1* sequences were amplified from N2 and cloned into pPD95.77 via In-Fusion Advantage PCR Cloning Kit.
pPD95.77-*spc-1-L268P knock in repair template*	CCAAATGGCCCTTCCTTACGAGTGGCCGCCAAAGT	GGAAGGGCCATTTGGAGCTCATCAAGTTCAACGCT	PCR on pPD95.77-*spc-1-L268P* knock in repair template
pPD95.77-*unc-70-ΔH590-L598 arm knock in*	GCATGGCAATCCCTTGAGAAGGCAGAACACGAAC	GAAGAGTAATTGGACTGGCTCTTCCTCTGAGGCAAC	The *unc-70* sequences were amplified from N2 and cloned into pPD95.77 via In-Fusion Advantage PCR Cloning Kit.

pPD95.77-*unc-70 ΔH590-L598 knock in repair template*	ATGAGATCCATTGACAACTCCAATCGAACTCTGCG	GTCAATGGATCTCATGGATGATATTAAGAGCAGAC	PCR on pPD95.77-*unc-70-ΔH590-L598* arm knock in
pPD95.77-P*egl-17::gfp*$_{1-10}$	CCCGAAATGTGAGCTATGTCCAAAGGAGAAGAACTG	GAAGAGTAATTGGACCTAACTTCCGCCGCCACCTGTTCC	The *gfp*$_{1-10}$ sequences were cloned into pPD95.77-P*egl-17* via In-Fusion Advantage PCR Cloning Kit.
pPD95.77-*unc-44-HR arm knock in*	GTACCGGTAGAAAAACCACCTAGTCCTCTACATCTCATC	GAAACGCGCGAGACGCATGTCCATAATGGGCTGC	The HR arm sequences were amplified from N2 and cloned into pPD95.77 via In-Fusion Advantage PCR Cloning Kit.
pPD95.77-*unc-44-5' arm::gfp-3' arm knock in*	GGAGCTAGTGGTAGCTCGAACGAAGGCGATCCA	TTCTCCTTTACTCATGTTAGATGTTAGTCCTGC	The gfp sequences were cloned into pPD95.77-*unc-44-HR arm knock in* via In-Fusion Advantage PCR Cloning Kit.
pDD162-P*hsp-16.2*::Cas9+P*U6*::*spc-1-T1 sgRNA*	TCATGCTCCACCAGAGCGTTTTAGAGCTAGAAATAGC	TCTGGTGGAGCATGAGTGCAAGACATCTCGCAATAGGAGG	PCR from pDD162-P*hsp-16.2*::Cas9+P*U6::Empty sgRNA*
pDD162-P*hsp-16.2*::Cas9+P*U6*::*spc-1-T2 sgRNA*	AAAACGAGATCGTCTCGGTTTTAGAGCTAGAAATAGC	AGACGATCTCGTTTTGTCCAAGACATCTCGCAATAGGAGG	PCR from pDD162-P*hsp-16.2*::Cas9+P*U6::Empty sgRNA*
pDD162-P*hsp-16.2*::Cas9+P*U6*::*unc-44-T1 sgRNA*	TCCAGCTGCTCCGGAACCGTTTTAGAGCTAGAA ATA GC	TTCCGGAGCAGCTGGAGCCAAGACATCTCGCAATAGGA	PCR from pDD162-P*hsp-16.2*::Cas9+P*U6::Empty sgRNA*
pDD162-P*egl-17*::Cas9+P*U6*::*spc-1-T1 sgRNA*	TCATGCTCCACCAGAGCGTTTTAGAGCTAGAAATAGC	TCTGGTGGAGCATGAGTGCAAGACATCTCGCAATAGGAGG	PCR from pDD162-P*egl-17*::Cas9+P*U6::Empty sgRNA*
pDD162-P*egl-17*::Cas9+P*U6*::*spc-1-T2 sgRNA*	AAAACGAGATCGTCTCGGTTTTAGAGCTAGAAATAGC	AGACGATCTCGTTTTGTCCAAGACATCTCGCAATAGGAGG	PCR from pDD162-P*egl-17*::Cas9+P*U6::Empty sgRNA*

Table S3. *C. elegans* strains used in this study

Strain name	Genotype	Method	Resource
N2	Wild type	-	CGC
GOU2936	*cas815[spc-1::gfp knock in]*	Microinjection	This study
GOU3238	*cas971[spc-1-L260P knock in]*	Microinjection	This study
GOU3519	*cas1047[spc-1::7×gfp11 knock in]*	Microinjection	This study
GOU3617	*cas1047[spc-1::7×gfp11 knock in]; casEx5751[Pegl-17::gfp1-10,pRF4(+)]*	Genetic cross	This study
GOU3103	*cas962[gfp::unc-70 knock in]*	Microinjection	This study
GOU3237	*cas983[unc-70-ΔH590-L598 knock in]*	Microinjection	This study
GOU2039	*cas963[gfp::unc-44 knock in]*	Microinjection	This study
GOU3659	*cas963[gfp::unc-44 knock in]; cas961[spc-1::rfp knock in]*	Genetic cross	This study
GOU174	*casIs35[Pgcy-32::mCherry, unc-76(+)] ; zdIs5[Pmec-4::gfp, lin-15(+)]*	Genetic cross	This study
GOU3648	*casEx5752[Pegl-17::Cas9+PU6::spc-1-sg2,pRF4]; casIs35[Pgcy-32::mCherry, unc-76(+)]; zdIs5[Pmec-4::gfp, lin-15(+)]*	Microinjection	This study
GOU3649	*casEx5753[Phsp16.2::Cas9+PU6::spc-1-sg1,pRF4]; casIs35[Pgcy-32::mCherry, unc-76(+)]; zdIs5[Pmec-4::gfp, lin-15(+)]*	Microinjection	This study
GOU3651	*casEx5754[Phsp16.2::Cas9+PU6::spc-1-sg2,pRF4]; casIs35[Pgcy-32::mCherry, unc-76(+)]; zdIs5[Pmec-4::gfp, lin-15(+)]*	Microinjection	This study
GOU3072	*cas971[spc-1-L260P knock in]; casIs35[Pgcy-32::mCherry, unc-76(+)]; zdIs5[Pmec-4::gfp, lin-15(+)]*	Genetic cross	This study

GOU3079	*unc-44(e362); casIs35[Pgcy-32::mCherry, unc-76(+)]; zdIs5[Pmec-4::gfp, lin-15(+)]*	Genetic cross	This study
GOU4190	*unc-44(e362); casEx5752[Pegl-17::Cas9+PU6::spc-1-sg2,pRF4]; casIs35[Pgcy-32::mCherry, unc-76(+)]; zdIs5[Pmec-4::gfp, lin-15(+)]*	Genetic cross	This study
GOU2937	*cas815[spc-1::gfp knock in]; casIs165[Pegl-17:: myri-mCherry, Pegl-17::mCherry-TEV-S::his-24, unc-76(+)]*	Genetic cross	This study
GOU4191	*cas962[gfp::unc-70 knock in]; casIs165[Pegl-17:: myri-mCherry, Pegl-17::mCherry-TEV-S::his-24, unc-76(+)]*	Genetic cross	This study
GOU3065	*cas963[gfp::unc-44 knock in]; casIs165[Pegl-17:: myri-mCherry, Pegl-17::mCherry-TEV-S::his-24, unc-76(+)]*	Genetic cross	This study
GOU1544	*casIs165[Pegl-17:: myri-mCherry, Pegl-17::mCherry-TEV-S::his-24, unc-76(+)]; casIs555[Pegl-17::gfp::moesinABD]*	Genetic cross	This study
GOU3676	*cas971[spc-1-L260P knock in]; casIs165[Pegl-17:: myri-mCherry, Pegl-17::mCherry-TEV-S::his-24, unc-76(+)] II; casIs555[Pegl-17::gfp::moesinABD]*	Genetic cross	This study
GOU1409	*unc-44(e362); mnIs17[osm-6::gfp]*	Genetic cross	This study
GOU2035	*casEx2000[Phsp-16.2::Cas9+PU6::unc-44-T3 sgRNA; Podr-1::dsRed;]; mnIs17[osm-6::gfp]*	Genetic cross	This study

Table S4

Protein identified from SPC-1::GFP affinity purification and mass spectrometry analysis

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