Supplemental Figure Legends:

Figure S1: ***Genetic interactions and rescue of cyk-4 GAP alleles.*** Genetic data demonstrating that *cyk-4(or749ts)* and *cyk-4(or570ts)* (CYK-4$^{GAP(E448K)}$ and CYK-4$^{GAP(T546I)}$, respectively) are strictly recessive (orange), fail to complement each other (brown), and also fail to fully complement *cyk-4(t1689ts)* (CYK-4$^{CSA(S15L)}$, an allele that affects Centralspindlin assembly. Expression of a GFP fusion with CYK-4 from a transgene rescues the embryonic lethality of both *cyk-4(or749ts)* and *cyk-4(or570ts)* (green).

Figure S2: ***Centralspindlin mutations do not affect the accumulation of myosin II on the equatorial cortex prior to furrow ingression.*** A) Schematic summarizing the temporal progression of cytokinesis at 25ºC. During the 80s immediately following anaphase onset, myosin II becomes enriched on the equatorial cortex, forming a band that encircles the cell equator. The cortex subsequently buckles inwards to form a furrow that ingresses inwards towards the spindle center, closing the hole between the daughter cells. **B,C**) During the 80s immediately following anaphase onset, the amount and distribution of cortical myosin II in the GAP domain and Centralspindlin assembly mutants is not significantly different from that in controls. **B**) Images of the cortex from time-lapse series of embryos expressing a GFP-fusion to the *C. elegans* myosin II heavy chain (NMY-2::GFP). Scale bar, 10 μm. **C**) The mean post-anaphase accumulation of cortical NMY-2::GFP fluorescence is plotted as a function of embryo length for control and Centralspindlin mutant embryos at the indicated time points. Values were normalized by dividing by the average maximum value for controls (between 55-65% embryo width). Error bars, SEM. NOTE: Prior to anaphase onset, cortical NMY-2::GFP is present in an anterior cap that contributes to the polarity maintenance. In the early post-anaphase interval, this localization is super-imposed with its accumulation at the cell equator. In the quantification shown in **C**, the post-anaphase accumulation is measured by subtracting the distribution at anaphase onset from the distribution at subsequent time points to eliminate the contribution from the anterior cap.

Figure S3: ***Myosin II levels at the furrow tip and the rate of furrow ingression are reduced in Centralspindlin mutants.*** **A**) Schematic illustrating the quantification method used to measure the amount of myosin II heavy chain (NMY-2::GFP) at the furrow tip. **B**) Mean NMY-2::GFP fluorescence at the furrow tip is reduced in Centralspindlin mutants relative to controls. Error bars, SEM. **C**) The mean rate of furrow ingression in the CYK-4 GAP mutants is nearly identical to that in mutants disrupting Centralspindlin assembly and following RNAi-mediated depletion of CYK-4, and is ~3-fold reduced relative to controls. Error bars, SEM.

Figure S4: ***Partial depletion of ECT-2 enhances the CYK-4$^{GAP(E448K)}$ cytokinesis defect, and Rac^{CED-10} depletion rescues furrow ingression in ZEN-4$^{CSA(D520N)}$ but not in AuroraB$^{AIR-2(P265L)}$ embryos.*** **A**) Partial depletion of ECT-2 by RNAi enhances the CYK-4$^{GAP(E448K)}$ cytokinesis defect. **B**) Rac^{CED-10} depletion rescues the furrow ingression defect in the Centralspindlin assembly mutant ZEN-4$^{CSA(D520N)}$, allowing 85% of the furrows to ingress to completion. However, in 71% of these embryos cytokinesis fails to complete and the furrow ultimately regresses. **C**) Rac^{CED-10} depletion does not suppress the cytokinesis phenotype in AuroraB$^{AIR-2(P265L)}$ mutants which also fail to form a central spindle and have a partial furrow ingression defect. **D**) Double depletion of Rac^{CED-10} and Rac^{RAC-2} by RNAi did not increase the efficiency of rescue over that observed following depletion of Rac^{CED-10} alone (Fig. 3D). However, due to two (30-40 bp) stretches of identity at the nucleotide level we cannot rule out that RNAi depletion of Rac^{CED-10} also targets Rac^{RAC-2} (albeit less-efficiently) and vice versa. Scale bar, 20 μm.

Figure S5: ***Strains used in this study.*** Genotype and strain names for strains used in this study. Note: CYK-4$^{GAP(E448K)}$ is *cyk-4(or749ts)*, CYK-4$^{GAP(T546I)}$ is *cyk-4(or570ts),* CYK-4$^{CSA(S15L)}$ is *cyk-4(t1689ts)*, and ZEN-4$^{CSA(D520N)}$ is *zen-4(or153ts)* in this manuscript.

Figure S6: ***dsRNAs used in this study.*** Primers, template, and concentrations of the dsRNAs used in this study.

Supplemental Movie Legends:

Movie S1: ***Movie montage of cytokinesis in cyk-4 mutant and control embryos.*** The GAP domain mutants CYK-4$^{GAP(E448K)}$ and CYK-4$^{GAP(T546I)}$ show a partial ingression cytokinesis defect that resembles the cytokinesis defect in the Centralspindlin assembly mutant CYK-4$^{CSA(S15L)}$. Embryos are co-expressing a GFP fusion with a PH domain that binds to a phospholipid specifically produced on the plasma membrane, and a RFP^{mCherry} fusion with a histone H2B that labels the chromosomes. Images were collected every 15s and are played back at 150x real time.

Movie S2: ***AIR-2 dynamics during cytokinesis in cyk-4 mutant and control embryos.*** A GFP-fusion with AuroraB^{AIR-2} localizes to the central spindle normally in CYK-4$^{GAP(E448K)}$ and CYK-4$^{GAP(T546I)}$ embryos, in contrast to CYK-4$^{CSA(S15L)}$ embryos, which do not form a central spindle. Images were collected every 10s and are played back at 100x real time.

Supplemental References:

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2. S. E. Encalada et al., Dev Biol 228, 225 (Dec 15, 2000).
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4. P. Gonczy et al., J Cell Biol 144, 927 (Mar 8, 1999).
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6. K. Oegema, A. Desai, S. Rybina, M. Kirkham, A. A. Hyman, J Cell Biol 153, 1209 (Jun 11, 2001).
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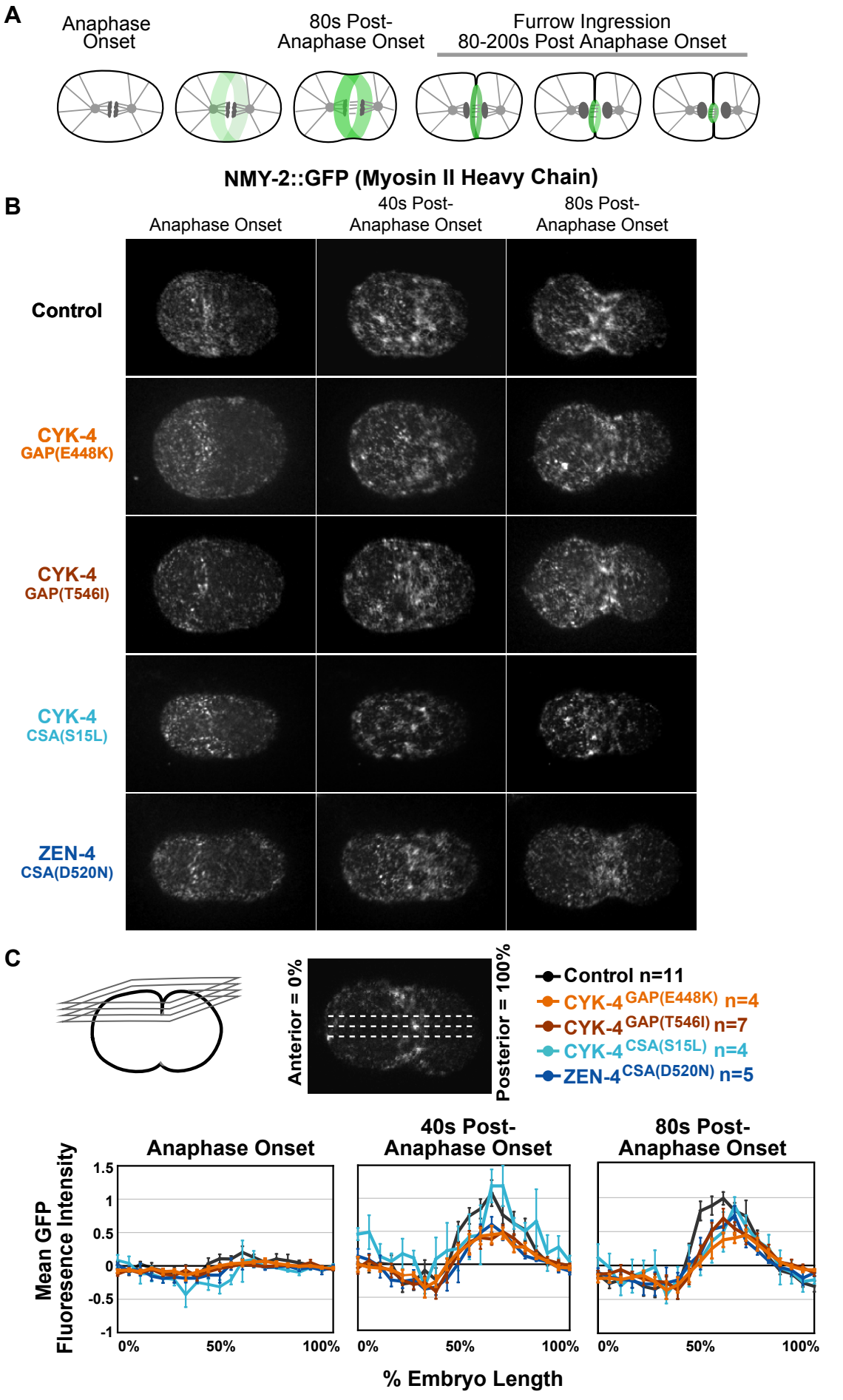
Canman *et al.* Supplemental Figure 1

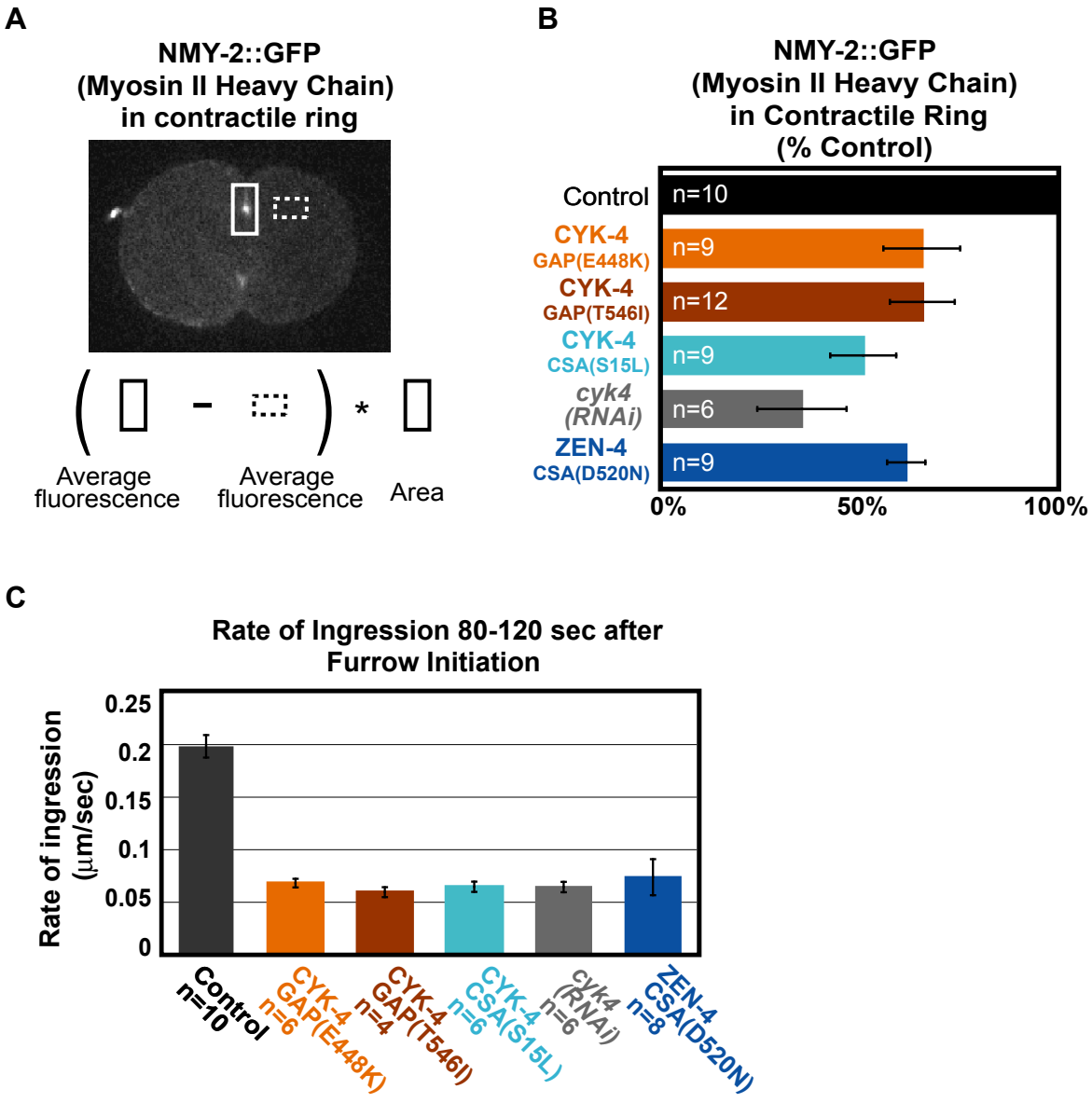
Genetic interactions and rescue of cyk-4 alleles at 25∫C

Genotype	Average/Median % Embryonic Lethal	Viable/Total # Embryos
cyk-4(or749ts) / *cyk-4(or749ts)*	100/100%	0/495
cyk-4(or570ts) / *cyk-4(or570ts)*	100/100%	0/276
cyk-4(or570ts) / *cyk-4(or749s)*	99/100%	2/311
cyk-4(or749ts) / +	1/0%	530/533
cyk-4(or570ts) / +	1/0%	343/348
unc-32(e189) cyk-4(t1689ts) / *unc-32(e189) cyk-4(t1689ts)*	100/100%	0/503
unc-32(e189) cyk-4(t1689ts) / *cyk-4(or749ts)*	95/99%	30/698
unc-32(e189) cyk-4(t1689ts) / *cyk-4(or570ts)*	85/87%	140/888
unc-32(e189) cyk-4(t1689ts) / +	41/26%	362/540
unc-119(ed3) cyk-4(or749ts) / *unc-119(ed3) cyk-4(or749ts)* ; *unc-119+ gfp::cyk-4+* / +	39/36%	213/601
unc-119(ed3) cyk-4(or570ts) / *unc-119(ed3) cyk-4(or570ts)* ; *unc-119+ gfp::cyk-4+* / +	29/23%	222/911
unc-119(ed3) / *unc-119(ed3)* ; *unc-119+ gfp::cyk-4+* / +	1/0%	368/373

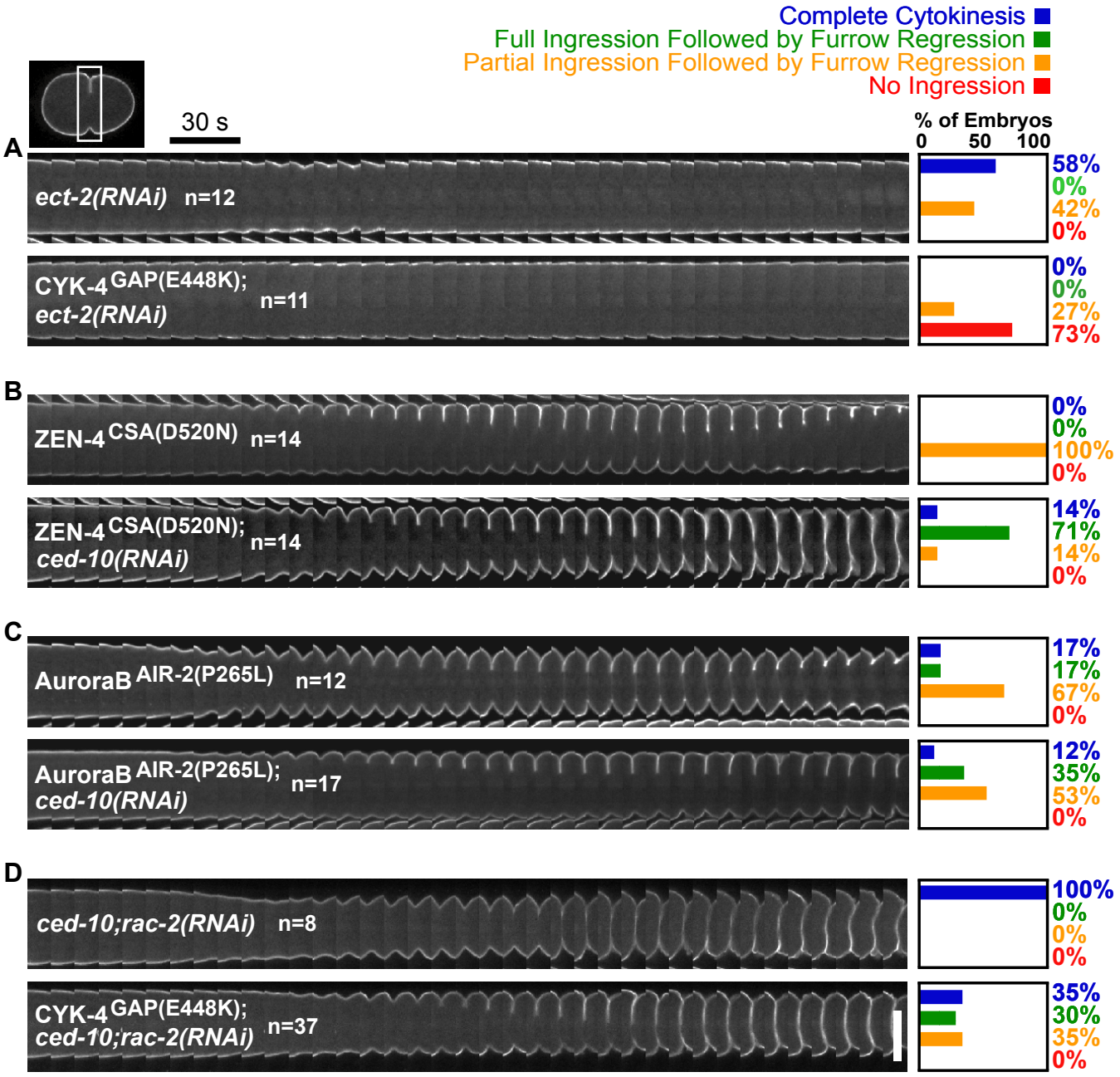
Canman *et al.* Supplemental Figure 2



Canman *et al.* Supplemental Figure 3



Canman *et al.* Supplemental Figure 4



Canman *et al.* Supplemental Figure 5

Worm strains used in the study

Strain #	Genotype
N2	*wild-type (ancesteral)*
CB1309	*lin-2(e1309)X.*
CB1489	*him-8(e1489)IV.*
MT3751	*dpy-5(e61)I; rol-6(e187)II; unc-32(e189)III.*
MT464	*unc-5(e53)IV; dpy-11(e224)V; lon-2(e678)X.*
DR104	*dpy-18(e364) unc-25(e156)III.*
CB2053	*dpy-18(e364) unc-64(e246)III.*
EU1303	*cyk-4(or570ts)III.*
EU1302	*cyk-4(or570ts)III; him-8(e1489)IV.*
OD227	*cyk-4(or749ts)III.*
OD228	*unc-32(e189) cyk-4(t1689ts)III.*
WH0279	*unc-119(ed3)III; ojIs12[cyk-4::GFP unc-119(+)]/+*
OD229	*cyk-4(or749ts) unc-119(ed3)III; ojIs12[cyk-4::GFP unc-119(+)]/+*
OD230	*cyk-4(or570ts) unc-119(ed3)III; ojIs12[cyk-4::GFP unc-119(+)]/+*
EU716	*zen-4(or153ts)IV.*
OD27	*unc-119(ed3)III; ltIs14 [pASM05; pie-1/GFP-TEV-STag::air-2; unc-119 (+)]IV.*
OD231	*cyk-4(or570ts)III; ltIs14 [pASM05; pie-1/GFP-TEV-STag::air-2; unc-119 (+)]IV.*
OD232	*cyk-4(or749ts)III; ltIs14 [pASM05; pie-1/GFP-TEV-STag::air-2; unc-119 (+)]IV.*
OD233	*unc-32(e189) cyk-4(t1689ts)III; ltIs14 [pASM05; pie-1/GFP-TEV-STag::air-2; unc-119 (+)]IV.*
JJ1473	*unc-119(ed3)III; zuIs45[nmy-2::NMY-2::GFP + unc-119(+)]V.*
OD234	*cyk-4(or570ts)III; zuIs45[nmy-2::NMY-2::GFP + unc-119(+)]V.*
OD235	*cyk-4(or749ts)III; zuIs45[nmy-2::NMY-2::GFP + unc-119(+)]V.*
OD236	*zen-4(or153ts)IV; zuIs45[nmy-2::NMY-2::GFP + unc-119(+)]V.*
OD237	*unc-32(e189) cyk-4(t1689ts)III; zuIs45[nmy-2::NMY-2::GFP + unc-119(+)]V.*
OD95	*unc-119(ed3) ltIs38 [pAA1; pie-1/GFP::PH(PLC1delta1)III; unc-119 (+)]; ltIs37 [pAA64; pie-1/mCHERRY::his-58; unc-119 (+)]IV*
OD238	*zen-4(or153ts)IV; ltIs38 [pAA1; pie-1/GFP::PH(PLC1delta1)III.*
OD105	*air-2(or207ts)I; ltIs38 [pAA1; pie-1/GFP::PH(PLC1delta1)III.*
OD239	*cyk-4(or749ts) ltIs38 [pAA1; pie-1/GFP::PH(PLC1delta1) unc-119 (+)]III; ltIs37 [pAA64; pie-1/mCHERRY::his-58; unc-119 (+)]IV*
OD240	*cyk-4(or570ts) ltIs38 [pAA1; pie-1/GFP::PH(PLC1delta1) unc-119 (+)]III; ltIs37 [pAA64; pie-1/mCHERRY::his-58; unc-119 (+)]IV*
OD241	*cyk-4(t1689ts) ltIs38 [pAA1; pie-1/GFP::PH(PLC1delta1) unc-119 (+)]III; tIs37 [pAA64; pie-1/mCHERRY::his-58; unc-119 (+)]IV*

Canman *et al.* Supplemental Figure 6

dsRNAs used in the study

Gene	Oligo 1	Oligo 2	Template	Conc. (mg/mL)
zen-4 (M03D4.1)	AATTAACCCTCACTAA AGGAATTGGTTATGG CTCCGAG	TAATACGACTCACTAT AGGATTGGAGCTGTT GGATGAGC	Kohara cDNA yk35d10	1.3
cyk-4 (K08E3.6)	TAATACGACTCACTAT AGGCGCAAGCTGTGG AAAGATTC	AATTAACCCTCACTAA AGGTTGCGATGTCAC GAGTTGTT	N2 genomic	2.0
rho-1 (Y51H4A.3)	TAATACGACTCACTAT AGGTGGCTGCGATTA GAAAGAAG	AATTAACCCTCACTAA AGGCCTCACGAATTC CGTCCTTA	Kohara cDNA yk435f7	2.0
ect-2 (T19E10.1)	TAATACGACTCACTAT AGGTGGATCCGATTC TCGAACTT	AATTAACCCTCACTAA AGGACATTTGGCTTTG TGCTTCC	N2 genomic	1.7
ced-10 (C09G12.8)	TAATACGACTCACTAT AGGAAATGTGTCGTC GTTGGTGA	AATTAACCCTCACTAA AGGCCGTACACTTGC TCTTTTTGG	N2 cDNA	1.0
rac-2 (K03D3.10)	TAATACGACTCACTAT AGGGCAAGCAATCAA ATGTGTCG	AATTAACCCTCACTAA AGGACCGTGCAATTG CTCTTTTT	N2 cDNA	1.2
cdc-42 (R07G3.1)	TAATACGACTCACTAT AGGGATCAAGTGCGT CGTCGTT	AATTAACCCTCACTAA AGGGAGAATATTGCA CTTCTTCTTCTTCTC	N2 cDNA	0.7
mig-2 (C35C5.4)	TAATACGACTCACTAT AGGCTTCACCGTCGA GGCAGAT	AATTAACCCTCACTAA AGGATTGCAAGACTTC TTCTTTTTCTG	N2 cDNA	1.6
arx-2 (K07C5.1)	TAATACGACTCACTAT AGGTCAGCTTCGTCAA ATGCTTG	AATTAACCCTCACTAA AGGTGCAATACGCGA TCCAAATA	N2 genomic	1.7
wsp-1 (C07G1.4)	AATTAACCCTCACTAAA GGTCTTCAGGAATCGG ATCCAC	TAATACGACTCACTATA GGCGGCTCCAGAAGT CGTACTC	N2 cDNA	4.1
wve-1 (R06C1.3)	AATTAACCCTCACTAA AGGCTCTAACAAAACG GGCGGTA	TAATACGACTCACTAT AGGGGAGGTGGTGGAG GGATATT	N2 cDNA	4.8