Supplementary Materials for

Crosstalk Between Microtubule Attachment Complexes Ensures Accurate Chromosome Segregation

Dhanya K. Cheerambathur, Reto Gassmann, Brian Cook, Karen Oegema, Arshad Desai*

*Corresponding author. E-mail: abdesai@ucsd.edu

Published 14 November 2013 on Science Express
DOI: 10.1126/science.1246232

This PDF file includes:

Materials and Methods
Figs. S1 to S9
Tables S1 and S2
References
Materials & Methods:

C. elegans strains & methods

*C. elegans* strains used in this study are listed in Table S1. Strains carrying integrated single-copy RNAi-resistant *spdl-1* and *ndc-80* transgenes were constructed using the MosSCI method as previously described (28). Transgene integration was confirmed by PCR of regions spanning each side of the insertion.

Genetic analysis was performed using the *ndc-80(tm5271)* deletion which causes 100% larval lethality when homozygous. Strains heterozygous for *ndc-80(tm5271)* (on Chr IV) and homozygous for a specific *ndc-80* transgene (inserted on Chr II) were generated by standard genetic methods. Approximately 200 F1 progeny (L4 stage) from each strain were singled and placed for 48 hrs at 20°C. Each plate was scored for visible phenotype(s) of the F1 worm as well as for presence of embryos, and the F1 worm (which on certain plates was an arrested larvae or a highly abnormal sterile adult) was then removed and genotyped to identify those that were homozygous for the *ndc-80(tm5271)* deletion. When applicable, the fate of embryos laid by worms homozygous for a *ndc-80* transgene and *ndc-80(tm5271)* deletion was scored 2 days later; plates homozygous for *ndc-80(tm5271)* with viable F2 progeny that could be propagated through multiple generations enabled establishment of stably rescued lines (such lines were never obtained for *ndc-80* but were obtained for the other transgenes).

RNA-mediated Interference

All RNAi was performed by microinjection. Double-stranded RNAs were generated as described (10) using DNA templates prepared by PCR-amplifying regions using the oligonucleotides and templates in Table S2. L4 hermaphrodites were injected with dsRNA
and incubated at 20°C for 36–46 h before dissection and imaging of their embryos. For double depletions, dsRNAs were mixed to obtain an equal concentration of 1 mg/mL for each dsRNA. For lethality assays investigating the functionality of spdl-1 and ndc-80 transgenes, L4 worms injected with dsRNA were singled 24 hours post-injection. Adult worms were removed from the plates 48 hours post-injection and hatched larvae and unhatched embryos were counted 20 hours later at 20°C.

**Immunoblotting**

Immunoblots were performed on whole-worm extracts (15 worms per lane for 100% loading) with affinity purified antibodies to NDC-80 (11) and SPDL-1 (7) using anti-α-tubulin as a loading control (DM1-α; Sigma-Aldrich). The primary antibodies, rabbit anti-NDC-80 and rabbit anti-SPDL-1, were pre-incubated with 0.5 mL of immobilized *E. coli* lysate (Thermo Scientific) per µg of antibody at room temperature for 1hr before addition to the nitrocellulose membrane.

**Yeast Two-Hybrid Analysis**

Two-hybrid analysis was performed according to the manufacturer’s instructions (MATCH-MAKER™, Clontech).

**Live Imaging**

For embryos expressing GFP:H2b and GFP:γ-tubulin, 5 x 2 µm z-stacks were collected with 2x2 binning and a 60x 1.4 NA PlanApo objective (Olympus) every 10 s at 19°C on a DeltaVision microscope (Applied Precision) equipped with a CoolSnap camera (Roper Scientific). Spindle pole separation was quantified as described (11). Imaging for all other worms strains was performed on an inverted Zeiss Axio Observer Z1 system with a
Yokogawa spinning-disk confocal head (CSU-X1), a 63x 1.4 NA Plan Apochromat objective, and a QuantEM: 512SC EMCCD camera (Photometrics).

**Protein Expression & Purification**

Open reading frames encoding all 4 subunits of the *C. elegans* NDC-80 complex were PCR-amplified from cDNA and cloned into polycistronic expression construct pST39 (29). Mutant versions were generated with the QuikChange Lightning Kit (Agilent Technologies). For expression of RNZ, ROD-1^[1,4] and Zwl-1-6XHis were PCR amplified and cloned into pET21a and pACYC Duet-1 (Novagen), respectively. Proteins were expressed in BL21(DE3) cells at 20 °C for 3-4hrs. Induced BL21(DE3) cells were lysed in Lysis Buffer (50mM HEPES [pH 7.4], 300mM NaCl, 10mM imidazole, 5mM β-mercaptoethanol [BME]) and clarified at 42,000g for 30 min at 4°C. For NDC-80 complex purifications, the clarified extract was fractionated by 30% ammonium sulfate precipitation and the pellet resuspended in Lysis Buffer with 100mM NaCl. Ni-NTA agarose (Qiagen) was incubated with clarified lysates or resuspended ammonium sulfate pellets for 45min, washed with Wash Buffer (25mM HEPES [pH 7.4], 400mM NaCl, 10mM imidazole, 5mM BME), and eluted with 25mM HEPES [pH 7.4], 250mM NaCl, 250mM imidazole, 5mM BME. The eluted protein was desalted into 25mM HEPES [pH 7.4], 50mM NaCl, 1mM EDTA, 1mM BME with a EconoPac PD10 desalting column (Bio-Rad), loaded on an ion exchange column (NDC-80 WT & CH^* on HiTrap SP HP, GE Healthcare & RNZ & NDC-80 ΔTail on Mono Q, GE Healthcare), and eluted with a linear salt gradient. The peak fractions from ion exchange were fractionated on a Superose 6 gel filtration column. Protein concentrations were determined using densitometry of Coomassie-stained gels relative to a BSA standard.
**Microtubule Cosedimentation Analysis**

Increasing amounts of taxol-stabilized microtubules were incubated with 100nM NDC-80 complex variants or 100 nM NDC-80 complex preincubated with R^{NZ} (10 min at room temperature) in 12.5mM HEPES [pH7.4], 5µM BSA and 50 mM (2 µM R^{NZ}) or 100mM (14 µM R^{NZ}) NaCl. Reactions were pelleted through a BRB80 + 40% glycerol cushion at 80,000 rpm in a TLA100 rotor at 25°C. An aliquot from the top was removed as the supernatant sample; the rest of the supernatant was discarded. The pellet was resuspended in BRB80 + 10mM CaCl$_2$ for 10 min on ice. Equivalent amounts of supernatant (S) and pellet (P) were analyzed by anti-NDC-80 immunoblotting.

**In vitro Translation**

$[^{35}]$S labeled fragments of NDC-80 were prepared using TnT® Quick Coupled Transcription/Translation System (Promega). 10 µl of the in vitro translation lysate was incubated for 1hr at 4°C with 10 µg R^{NZ} complex (in 25mM HEPES [pH7.4], 100mM NaCl, 0.1% NP40, 10mM Imidazole) in a final volume of 50 µl, mixed with 30 µl of a 1:1 nickel agarose slurry equilibrated with the binding buffer for an additional hour at 4°C. Beads were washed three times with binding buffer, eluted using sample buffer and the elution analyzed by SDS-PAGE and autoradiography.
Cheerambathur et al. Fig. S1

Fig. S1. An RNAi resistance-based replacement system for NDC-80 in the *C. elegans* embryo.

(A) Schematic of the RNAi-resistant *ndc-80* and *ndc-80::mCherry* transgene integrations (the single Mos transposon used for both integrations was located on Chromosome II). The *ndc-80::mCherry* transgene did not rescue depletion of endogenous *NDC-80* as robustly as the untagged *ndc-80* transgene. A dsRNA corresponding to this region was used to deplete endogenous *NDC-80* in all RNAi-based replacement experiments.

(B) Re-encoded nucleotide sequence of exons 3-5 of *ndc-80* that was used to generate the RNAi-resistant *ndc-80* transgene. A dsRNA corresponding to this genomic locus perfectly rescues the premature pole separation defect observed following *NDC-80* depletion (black squares). The control and *ndc-80(RNAi)* curves are the same as in Fig. 1C. The plot in (F) demonstrates that *NDC-80WT* (green diamonds) and *NDC-804A* (blue squares) are equally sensitive to inhibition by RZZ (induced by depletion of SPDL-1). The control curve (*ndc-80(RNAi) + NDC-80WT*) is the same as in Fig. S1F.

(C) Metaphase and Anaphase chromosomes stained with *γ*-tubulin and *Dendra*::H2b. *NDC-80WT* is perfectly rescued by the *NDC-80WT* transgene; hence, *ndc-80* integrations (the single Mos transposon used for both integrations was located on *ndc-80* genomic locus) demonstrate that *NDC-80WT* (green diamonds) and *NDC-804A* (*NDC-80WT* and the *NDC-804A* mutant rescue the embryonic lethality associated with endogenous *NDC-80* depletion.

(D) (top) Schematic of wild-type (WT) and Aurora B phosphosite mutant (4A) *NDC-80*. (bottom) Anti- *NDC-80* immunoblot showing replacement of endogenous *ndc-80* by transgene-encoded *NDC-80* variants. α-tubulin serves as a loading control.

(E) Embryo viability analysis. Error bars are SD of embryo lethality per worm. >10 worms and >1000 embryos were scored per condition. Both the *NDC-80WT* and the *NDC-804A* mutant rescue the embryonic lethality associated with endogenous *NDC-80* depletion.

(F) & (G) Plot of spindle pole separation kinetics for the indicated conditions. Error bars represent the SEM with a 95% confidence interval. The plot in (F) demonstrates that *NDC-80WT* (gray circles) perfectly rescues the premature pole separation defect observed following *NDC-80* depletion (black squares). The control and *ndc-80(RNAi)* curves are the same as in Fig. 1C. The plot in (G) demonstrates that *NDC-80WT* (green diamonds) and *NDC-804A* (blue squares) are equally sensitive to inhibition by RZZ (induced by depletion of SPDL-1). The control curve (*ndc-80(RNAi) + NDC-80WT*) is the same as in Fig. S1F.
Fig. S2. Yeast two-hybrid and microtubule co-sedimentation analysis.

(A) Schematic of NDC-80, Nuf2HIM-10 and RZZ complex subunits and summary of yeast two-hybrid analysis. Strong interaction of ZwilchZW-1 with the N-terminus of ROD-1, and weak interaction of Zw10CZW-1 with internal fragments of ROD-1 indicate that the ZwilchZW-1 and Zw10CZW-1 fusions were expressed in yeast, suggesting that the absence of any interaction between them and the tested NDC-80 and Nuf2HIM-10 fragments are not false negatives.

(B) Coomassie-stained gel lanes of recombinant *C. elegans* NDC-80 complexes (WT, ΔTail, 4D or CH*) expressed and purified from bacteria for use in microtubule-binding assays.

(C) Microtubule cosedimentation analysis conducted with indicated concentration of taxol-stabilized microtubules, 100 nM NDC-80 complex, and 14 µM RNZ complex. 5 µM BSA was included in all binding assays conducted in BRB80 buffer supplemented with 100 mM NaCl. Supernatant (S) and Pellet (P) fractions were immunoblotted using anti-NDC-80 antibody. Quantification of the binding analysis is plotted on the right.

(D) Microtubule cosedimentation analysis demonstrating that the RNZ complex does not bind to microtubules. Binding assays were performed with indicated concentrations of taxol-stabilized microtubules, 2 µM RNZ complex, and 5 µM BSA; the binding buffer was BRB80 + 50 mM NaCl. Supernatant (S) and Pellet (P) fractions were immunoblotted using anti-ZWL-1 antibody.
Fig. S3. Location of CH domain mutations, pole separation kinetics of NDC-80\(^{\Delta \text{Tail}}\), and sensitivity of NDC-80\(^{4D}\) to RZZ inhibition.

(A) Location in the structure of the human Ndc80 complex (PDB 2ve7,(15)) of the 3 residues mutated in NDC-80\(^{CH^*}\). The red lettering marks the human residues; in parentheses below in black are the corresponding *C. elegans* residues that were mutated to alanine to generate the CH\(^*\) mutant NDC-80.

(B) Plot of spindle pole separation kinetics, as in Fig. 1C. The *ndc-80 (RNAi)* curve is the same as in Fig. 1C and control curve (*ndc-80(RNAi) + NDC-80\(^{WT}\)*) is the same as in Fig. S1F.

(C) Plot of spindle pole separation kinetics for the indicated conditions. Error bars represent the SEM with a 95% confidence interval. The plot demonstrates that NDC-80\(^{WT}\) (*green diamonds*) and NDC-80\(^{4D}\) (*pink squares*) are equally sensitive to inhibition by RZZ (induced by depletion of SPDL-1). The control curve (*ndc-80(RNAi) + NDC-80\(^{WT}\)*) is the same as in Fig. S1F.
**Fig. S4.** The Ska complex does not compensate for the absence of the NDC-80 tail.

(A) Evidence that the ska-3 dsRNA is effective. A strain expressing SKA-1::GFP was imaged at metaphase. In the control metaphase embryo, SKA-1::GFP labels the spindles (white arrow) and kinetochores (yellow arrowhead) of metaphase embryos. Following SKA-3 depletion, SKA-1::GFP signal is no longer detected (the residual punctate signal is embryo autofluorescence). Bar, 5 µm.

(B) Embryo viability analysis for the indicated conditions. Error bars represent the SD of embryo lethality per worm. >1000 embryos from ≥10 worms were scored per condition. The results show that, in contrast to NDC-80 complex depletion, Ska complex depletion does not lead to significant embryonic lethality.

(C) Chromosome segregation phenotypes for the indicated conditions. Bar, 5 µm.

(D) Plot of spindle pole separation kinetics for the indicated conditions. The ndc-80(RNAi) + NDC-80WT curve is the same as in Fig. S1F. Error bars represent the SEM with a 95% confidence interval.

(E) Embryo viability analysis for the indicated conditions. Error bars represent the SD of embryo lethality per worm. >1000 embryos from >10 worms were scored for each condition. Depletion of SKA-3 in the presence of ΔTail mutant NDC-80 does not result in embryonic lethality approaching that observed with the CH* mutant NDC-80.
Fig. S5. The ndc-80(tm5271) mutant and example phenotypes observed in the presence of ndc-80^ΔTail transgene.

(A) Schematic of the ndc-80 genomic locus and the tm5271 deletion mutant allele. The tm5271 allele is lethal and harbors an in-frame internal deletion, predicted to encode a truncated form of NDC-80 (NDC-80^Δ41-118), in which part of the basic tail and the initial segment of the NDC-80 CH domain are lacking. In a balanced heterozygous mutant (panel on the right), the predicted truncated NDC-80^Δ41-118 protein is detectable by immunoblotting. Based on structural and biochemical analysis of the NDC-80 complex (15, 20), the in-frame deletion likely abolishes NDC-80 microtubule-binding activity. Homozygous ndc-80(tm5271) embryos derived from a heterozygous mother hatch but arrest and die as larvae. As ndc-80(RNAi), which depletes maternal load, causes penetrant embryonic lethality (Fig. 2E), the hatching of the mutant embryos is likely supported by maternally loaded wildtype NDC-80 protein. Image panels on the bottom show the larval arrest phenotype compared to a wildtype L4 larva. Bar, 50 µm.

(B) Images of homozygous ndc-80(tm5271) worms in the presence of the ndc-80^WT or ndc-80^ΔTail transgenes. A quantitative description of the phenotypes observed with the ndc80^ΔTail transgene is shown in Fig. 3D. Bar, 50 µm.
Fig. S6. The spindle checkpoint functions normally in the NDC-80\(^{\Delta\text{Tail}}\) mutant.

(A) (left) Schematic of assay used to generate monopolar versus bipolar second division embryos by depletion of the centriole duplication kinase \(\text{zyg-1}\). Cell cycle timing is measured in the AB cell in the two types of embryos to assess whether the spindle checkpoint is active (30). (right) Time from NEBD–Chromosome Decondensation measured in the indicated conditions. Both NDC-80\(^{\text{WT}}\) and NDC-80\(^{\Delta\text{Tail}}\) support a monopolar spindle-induced cell cycle delay. By contrast, no delay is observed following NDC-80 depletion (30). Error bars represent the SEM with a 95% confidence interval.

(B) Embryo viability analysis for the indicated conditions. Error bars are the SD of embryo lethality per worm; >10 worms and >1000 embryos analyzed per condition.
Fig. S7. A replacement system for SPDL-1 in the *C. elegans* embryo.

(A) Schematic of the RNAi-resistant spdl-1 and spdl-1::mCherry transgene integrations (the single Mos transposon used for the integrations was located on Chromosome I for untagged spdl-1 transgenes and on Chromosome IV for spdl-1::mCherry transgenes). The wildtype spdl-1::mCherry transgene did not rescue removal of endogenous SPDL-1 as robustly as the untagged spdl-1 transgene; hence, untagged spdl-1 transgenes were used for all functional analysis.

(B) Re-encoded nucleotide sequence of exon 4 of spdl-1 that was used to engineer RNAi-resistance into the spdl-1 transgene. A dsRNA corresponding to this region was used to deplete endogenous SPDL-1 in all RNAi-based replacement experiments.

(C) Panels showing localization of SPDL-1::mCherry and SPDL-1F199A::mCherry to unattached kinetochores of monopolar spindles generated by depletion of the centriole duplication kinase ZYG-1. Bar, 2.5 µm.

(D) Plot of spindle pole separation kinetics, demonstrating rescue of the defect in formation of kinetochore-microtubule attachments in spdl-1(RNAi) by the spdl-1WT transgene. Error bars represent the SEM with a 95% confidence interval. The control and spdl-1(RNAi) curve is the same as in Fig. 1C.

(E) Plot of spindle pole separation kinetics, demonstrating the defect in kinetochore-microtubule attachment formation in the presence of SPDL-1F199A (red diamonds). Note the perfect overlap between SPDL-1WT (green outlined squares) and SPDL-1F199A (purple triangles) in the presence of rod-1(RNAi). Error bars represent the SEM with a 95% confidence interval.
Fig. S8. SPDL-1F199A triggers a spindle checkpoint-dependent cell cycle delay.

In *C. elegans* embryos, SPDL-1 is required for dynein localization to kinetochores (7) as well as for spindle checkpoint activation by promoting kinetochore localization of the Mad1/Mad2 complex (7,31). This figure shows that SPDL-1F199A, while inhibiting dynein recruitment (see Fig. 4B), does not prevent checkpoint activation and in fact triggers a checkpoint-dependent delay. Combining the SPDL-1F199A mutant with checkpoint inhibition (via depletion of the conserved checkpoint protein Mad2MDF-2) recapitulates the SPDL-1 depletion phenotype.

(A) Time-aligned image panels of GFP::H2b and GFP::γ-tubulin (relative to NEBD) for the indicated conditions. The images depict delayed anaphase onset in SPDL-1F199A mutant embryos and the abrogation of this delay following spindle checkpoint inhibition by depletion of Mad2MDF-2. Bar, 5 µm.

(B) Quantification of the NEBD-anaphase onset interval for the indicated number of embryos per condition. Anaphase onset was defined as the first visible separation of sister chromatids in images of the type shown in panel A. Error bars represent the SD.

(C) Plot of spindle pole separation kinetics, demonstrating that inhibition of the spindle checkpoint in the SPDL-1F199A mutant (green circles) results in a profile similar to depletion of SPDL-1 (gray diamonds). The *spdl-1(RNAi)* and *spdl-1(RNAi) + SPDL-1F199A* curves are the same as in Fig.1C & S7E, respectively. Error bars represent the SEM with a 95% confidence interval.

(D) Panels showing recruitment of the spindle checkpoint component Mad2MDF-2 to unattached kinetochores of monopolar spindles in *SPDL-1::mCherry* and *SPDL-1F199A::mCherry* embryos. Bar, 2.5 µm.
Fig. S9. Model for the transition from dynein-mediated capture to Ndc80-mediated end-coupled attachment.

(A) (left) Cartoon summarizing the control of Ndc80 complex microtubule binding by RZZ. As the Ndc80 tail deletion does not exhibit a defect in end-coupled attachment formation in C. elegans embryos, we propose that RZZ inhibits Ndc80 via control of the CH domain. (right) Dynein recruitment by Spindly is required to turn off RZZ inhibition.

(B) Model, invoking RZZ-mediated inhibition, for the transition from dynein-mediated capture to Ndc80-mediated end-coupled attachment. We propose that it is not solely Spindly-dependent dynein recruitment but rather a subsequent dynein activity-dependent mechanical force that alleviates RZZ inhibition and that this alleviation only occurs when a state that minimizes the potential for merotely is achieved. We postulate that during an initial capture event by kinetochore dynein, RZZ inhibits the Ndc80 complex (“Lateral” state in model). Given the short half-life of mitotic microtubules (32), the kinetochore dynein-bound microtubule will catastroph e leading its depolymerizing plus end to reach the kinetochore, where it is stabilized by dynein and exerts a force on the kinetochore dynein module (“Intermediate” state in model)—such a scenario is plausible based on in vitro biophysical experiments analyzing dynein interaction with depolymerizing microtubule ends (33, 34). We suggest that this end-coupled force orients the chromosome and alleviates inhibition of Ndc80, either via a change in RZZ conformation or dissociation of RZZ from the kinetochore (“End-coupled” state in model). In this way, RZZ inhibition would help ensure that a kinetochore is oriented toward one spindle pole before end-coupled attachments are formed. Additional mechanisms, including chromokinesins (35, 36), likely also contribute to this orientation function.
### Supplementary Tables:

**Table S1: *C. elegans* strains used in this study**

<table>
<thead>
<tr>
<th>Strain number</th>
<th>Genotype</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>N2</strong> (ancestral)</td>
<td></td>
</tr>
<tr>
<td>OD927</td>
<td>unc-119(ed3)III; ltSi421[pDC204;Pspdl-1:spdl-1 reencoded; cb-unc-119(+)]I #1</td>
</tr>
<tr>
<td>OD928</td>
<td>unc-119(ed3)III; ltSi422[pDC205;Pspdl-1:spdl-1(F199A) reencoded; cb-unc-119(+)]I #1</td>
</tr>
<tr>
<td>OD941</td>
<td>unc-119(ed3)III; orls17[~dhc-1::dhc-1::GFp;cb-unc-119(+);ltSi424 spdl-1[pDC206;Pspdl-1:reencoded; cb-unc-119(+)]II</td>
</tr>
<tr>
<td>OD942</td>
<td>unc-119(ed3)III; orls17[~dhc-1::dhc-1::GFp;cb-unc-119(+); ltSi425 [pDC205;Pspdl-1:spdl-1(F199A) reencoded; cb-unc-119(+)]I #1</td>
</tr>
<tr>
<td>OD943</td>
<td>unc-119(ed3)III; ltSi421[pDC207;Pspdl-1:spdl-1reencoded; cb-unc-119(+)]II; ruls32[pAZ132; pie-1/GFP::histone H2B] III; ddls6 [GFP::tbg-1; unc-119(+)] V</td>
</tr>
<tr>
<td>OD945</td>
<td>unc-119(ed3)III; ltSi422[pDC205;Pspdl-1:spdl-1(F199A) reencoded; cb-unc-119(+)]II #1; ruls32[pAZ132; pie-1/GFP::histone H2B] III; ddls6 [GFP::tbg-1; unc-119(+)] V</td>
</tr>
<tr>
<td>OD464</td>
<td>cb-unc-119(+)]IV; ruls32[pAZ132; pie-1/GFP::histone H2B] III; ddls6 [GFP::tbg-1; unc-119(+)] V</td>
</tr>
<tr>
<td>OD465</td>
<td>unc-119(ed3)III; ltSi36[pOD1035/pRG48; Pspdl-1:spdl-1(F199A) reencoded::mCherry; cb-unc-119(+)]IV; lts32[pOD379; pie-1/GFP::histone H2B] III; ddls6 [GFP::tbg-1; unc-119(+)] V</td>
</tr>
<tr>
<td>OD601</td>
<td>unc-119(ed3)III; ltSi34[pOD1034/pRG47; Pspdl-1:spdl-1::mCherry; cb-unc-119(+)]IV; lts32[pOD379; pie-1/GFP::histone H2B] III; ddls6 [GFP::tbg-1; unc-119(+)] V</td>
</tr>
<tr>
<td>OD602</td>
<td>unc-119(ed3)III; ltSi36[pOD1035/pRG48; Pspdl-1:spdl-1(F199A) reencoded::mCherry; cb-unc-119(+)]IV; lts32[pOD379; pie-1/GFP::histone H2B] III; ddls6 [GFP::tbg-1; unc-119(+)] V</td>
</tr>
<tr>
<td>OD611</td>
<td>unc-119(ed3)III; ltSi425[pDC207;Pspdl-1:SPDL-1(F199A) reencoded; cb-unc-119(+)]II</td>
</tr>
<tr>
<td>OD612</td>
<td>unc-119(ed3)III; ltSi121[pDC175;Pndc-80:ndc-80 (Mutant D1-59) reencoded; cb-unc-119(+)]II #1</td>
</tr>
<tr>
<td>OD634</td>
<td>unc-119(ed3)III; ltSi122[pDC178;Pndc-80:ndc-80 (8,18,44,51AAAA) reencoded; cb-unc-119(+)]II #1</td>
</tr>
<tr>
<td>OD642</td>
<td>unc-119(ed3)III; ltSi129[pDC181;Pndc-80:ndcC-80 (100,144,155AAAA) reencoded; cb-unc-119(+)]II #2</td>
</tr>
<tr>
<td>OD613</td>
<td>unc-119(ed3) III; ltSi120[pDC170;Pndc-80:ndc-80 reencoded; cb-unc-119(+)]II #3; ruls32[pAZ132; pie-1/GFP::histone H2B] III; ddls6 [GFP::tbg-1; unc-119(+)] V</td>
</tr>
<tr>
<td>OD614</td>
<td>unc-119(ed3) III; ltSi121[pDC175;Pndc-80:ndc-80 (Mutant D1-59) reencoded; cb-unc-119(+)]II #1; ruls32[pAZ132; pie-1/GFP::histone H2B] III; ddls6 [GFP::tbg-1; unc-119(+)] V</td>
</tr>
<tr>
<td>OD644</td>
<td>unc-119(ed3) III; ltSi122[pDC178;Pndc-80:ndc-80 (8,18,44,51AAAA) reencoded; cb-unc-119(+)]II #1; ruls32[pAZ132; pie-1/GFP::histone H2B] III; ddls6 [GFP::tbg-1; unc-119(+)] V</td>
</tr>
<tr>
<td>OD688</td>
<td>unc-119(ed3) III; ltSi122[pDC178;Pndc-80:ndc-80 (8,18,44,51AAAA) reencoded; cb-unc-119(+)]II #1; ruIs32[pAZ132; pie-1/GFP::histone H2B] III; ddls6 [GFP::tbg-1; unc-119(+)] V</td>
</tr>
<tr>
<td>OD647</td>
<td>unc-119(ed3)III; ltSi131[pDC122;Pndc-80:ndc-80:mCherry reencoded; cb-unc-119(+)]II #2; ruIs32[pAZ132; pie-1/GFP::histone H2B] III; ddls6 [GFP::tbg-1; unc-119(+)] V</td>
</tr>
<tr>
<td>OD1135</td>
<td>ndc-80(tm5271) IV/nT1<a href="IV;V">qls51</a></td>
</tr>
<tr>
<td>OD1136</td>
<td>unc-119(ed3)III; ndc-80(tm5271) IV/nT1<a href="IV;V">qls51</a>; ltSi120[[pDC170;Pndc-80:ndc-80 reencoded; cb-unc-119(+)]II #3ndc-80(tm5271) IV/nT1<a href="IV;V">qls51</a></td>
</tr>
<tr>
<td>OD1137</td>
<td>unc-119(ed3)III; ndc-80(tm5271) IV/nT1<a href="IV;V">qls51</a>; ltSi121[pDC175;Pndc-80:ndc-80(D1-59) reencoded; cb-unc-119(+)]II #1ndc-80(tm5271) IV/nT1<a href="IV;V">qls51</a></td>
</tr>
<tr>
<td>OD650</td>
<td>unc-119(ed3)III; ltSi428[pDC159;Pska-1:ska-1:gfp reencoded; cb-unc-119(+)]III#3</td>
</tr>
<tr>
<td>TH32</td>
<td>unc-119(ed3) III; ruIs32[pAZ132; pie-1/GFP::histone H2B] III; ddls6 [GFP::tbg-1; unc-119(+)] V</td>
</tr>
<tr>
<td>OD935</td>
<td>unc-119(ed3)III; ltSi590[pDC178;Pndc-80:ndc-80 (8,18,44,51DDDD) reencoded; cb-unc-119(+)]II #1</td>
</tr>
<tr>
<td>OD1255</td>
<td>unc-119(ed3)III; ltSi590[pDC178;Pndc-80:ndc-80 (8,18,44,51DDDD) reencoded; cb-unc-119(+)]II #1; ruIs32[pAZ132; pie-1/GFP::histone H2B] III; ddls6 [GFP::tbg-1; unc-119(+)] V</td>
</tr>
</tbody>
</table>
### Table S2: Oligos & Templates Used for dsRNA Production

<table>
<thead>
<tr>
<th>Gene</th>
<th>Oligonucleotide 1</th>
<th>Oligonucleotide 2</th>
<th>Template</th>
<th>mg/mL</th>
</tr>
</thead>
<tbody>
<tr>
<td>W01B6.9</td>
<td>5’- aattaacccctactaagg GTGACAGATTGCTATTTGAGACATTACAAATGATC-3’</td>
<td>5’- taatacgactcataagg GTGGTTCAAGATTCATTGGAATATTAGGATTCCA CGATC-3’</td>
<td>N2 cDNA</td>
<td>2.5</td>
</tr>
<tr>
<td></td>
<td>C06A8.5 (spdl-1)</td>
<td>5’- acctctactaagg</td>
<td>N2 cDNA</td>
<td>3.7</td>
</tr>
<tr>
<td></td>
<td>5’- aattaacccctactaagg ACGTTACCCGAATG ACCAC-3’</td>
<td>5’- acctcgactcataagg GCTAATTGAGGCATG GGTTC-3’</td>
<td>N2 genomic DNA</td>
<td>2.5</td>
</tr>
<tr>
<td>F55G1.4</td>
<td>5’- aattaacccctactaagg AATGCAAATCTTTTTG GATGGGAGAAAC-3’</td>
<td>5’- taatacgactcataagg CATCGACGAATTTGAT TCAGATCAATC-3’</td>
<td>N2 genomic DNA</td>
<td>3.2</td>
</tr>
<tr>
<td></td>
<td>Y106G6H.15 (ska-1)</td>
<td>5’- aattaacccctactaagg TTATGGGATAATAGAAA GTCAAGC-3’</td>
<td>N2 genomic DNA</td>
<td>3.0</td>
</tr>
<tr>
<td></td>
<td>5’- aattaacccctactaagg ATGGCTAACGAAACG CTGGGAACATG-3’</td>
<td>5’- taatacgactcataagg ATGGGAATTCTGGTTAATGTG GCTG-3’</td>
<td>N2 genomic DNA</td>
<td>4.4</td>
</tr>
<tr>
<td></td>
<td>F54E7.8 (ska-3)</td>
<td>5’- aattaacccctactaagg ATGGCTAACGAAACG CTGGGAACATG-3’</td>
<td>N2 genomic DNA</td>
<td>4.0</td>
</tr>
<tr>
<td></td>
<td>Y69A2AR.30 (mdf-2)</td>
<td>5’- aattaacccctactaagg GTGAATCGACGTCGA GAATGAG-3’</td>
<td>N2 genomic DNA</td>
<td>4.4</td>
</tr>
<tr>
<td></td>
<td>F59E12.2 (zyg-1)</td>
<td>5’- aattaacccctactaagg GGACCGAAAATTCCCGAT-3’</td>
<td>N2 genomic DNA</td>
<td>4.0</td>
</tr>
</tbody>
</table>

* Lowercase letters denote T3 and T7 sequences included for RNA synthesis.
References and Notes


