

1 **Supplementary figure legends:**

2 **Fig. S1.** *susi-5* was identified as *dis-3*. (A) Schematic of SUSI-5(ceDIS-3) (top) and
3 sequence alignments of DIS-3 in different organisms: hs, *homo sapiens*; mm, *Mus*
4 *musculus*; sp, *Schizosaccharomyces pombe*; dm, *Drosophila melanogaster*; and ce, *C.*
5 *elegans*. (B) Images of seam cells of *eri-1(mg366);dis-3(ust56);gfp::nrde-*
6 *3;mCherry::dis-3* animals at L4 stage. mCherry::DIS-3 rescued the cytoplasmic
7 localization of GFP::NRDE-3 in *eri-1(-)* animals. Scale bars, 10 μ m. (C) Brood size of
8 the *dis-3* mutant grown at 20°C and 25°C, respectively. mean \pm s.d.; n > 10 animals.

9

10 **Fig. S2.** risiRNAs were accumulated in *dis-3(ust56)* mutants. (A) Results from the deep
11 sequencing of total small RNAs from indicated animals. Green dashed lines indicate
12 risiRNAs. (B) Distribution from the deep sequencing of NRDE-3-associated small
13 RNAs in indicated animals. Sense fragments of lincRNA, protein-coding transcripts,
14 transposon transcripts, ncRNA and ribosomal RNA are not counted. (C) Results from
15 the deep sequencing of total small RNAs from the indicated animals. Green dashed
16 lines indicate risiRNAs.

17

18 **Fig. S3.** Alleles of the exosome subunits used in this study. (A) Brood sizes of indicated
19 animals grown at 20°C. Data are presented as mean \pm s.d.; n > 10 animals. Progeny
20 reached L4 or young adult stages are counted to evaluate the fertility. (B-J) The *ust*
21 alleles were generated via a dual sgRNA-directed CRISPR/Cas9 gene knockout
22 technology in *C. elegans*. The *tm* alleles and *ok* alleles were acquired from National
23 Bioresource Project and the CGC, respectively. *exos-5(ust61)*, *exos-7(ust62)*, and *exos-*
24 *10(ok2269)* alleles are in-frame mutations and the translated products are predicted to
25 lose 120, 89 and 208 amino acids respectively. All other alleles are frame-shift
26 mutations, and likely nulls. Red bars indicate deleted regions in gene loci. Green arrows
27 indicate premature stop codons.

28

29 **Fig. S4.** RPOA-2 localized to nucleoli in embryos. (A) Brood size of wild type animals
30 upon RNAi targeting of *rpoa-2*. Data are presented as mean \pm s.d.; n=16 animals. (B)

31 Images of young gravid adult animals expressing GFP::RPOA-2. Scale bars, 25 μ m.
32 (C) Images of *C. elegans* embryos expressing GFP::RPOA-2 (green) and mCherry::FIB-
33 1 (red). Scale bars, 10 μ m. (D) Images of the embryos expressing GFP::RPOA-2. Scale
34 bars, 25 μ m. White arrows indicate nucleoli. Scale bars, 10 μ m.

35

36 **Fig. S5.** Images of the animals expressing indicated transgenes in the presence of
37 actinomycin D. Shown are embryos (A), soma of L4 stage animals (B), and germline
38 (C). White arrows indicate nucleoli. Scale bars, 10 μ m.

39

40 **Fig. S6.** *dis-3* mutation did not change the expression of RPOA-2. (A) Images of seam
41 cells of L4 stage animals expressing GFP::NRDE-3 in the presence of actinomycin D
42 in the *eri-1(mg366)* background. Scale bars, 5 μ m. (B) Images show somatic cells of
43 L4 stage animals expressing GFP::EXOS-10 in the presence of actinomycin D. The
44 percentage of animals with nucleolar localized GFP::EXOS-10 is indicated (% NCL).
45 The number of scored animals is indicated in parentheses. Scale bars, 10 μ m. (C)
46 Western Blotting of GFP::RPOA-2 in indicated actinomycin D-treated animals. (D)
47 Western Blotting of GFP::RPOA-2 in indicated animals.

48

49 **Fig. S7.** (A) Feeding RNAi targeting 18S rRNAs did not significantly change the brood
50 size of animals. Animals were grown at 20°C. Data are presented as mean \pm s.d.; n > 10
51 animals; ns, nonsignificance. (B) Results of the ChIP assay of RPOA-2 occupancy at
52 rDNA loci by non-specific IgG or anti-GFP antibody. The enrichment of each sample
53 was normalized to 1% input. Statistics were performed by comparing the data from the
54 IgG or anti-GFP antibody groups. Mean \pm s.d.; n = 4, ***P<0.001. (C) Results of the
55 ChIP assay of RPOA-2 at rDNA loci after feeding dsRNA targeting a protein coding
56 gene *oma-1*. (D) Results of the ChIP assay of RPOA-2 at rDNA loci after feeding
57 dsRNA targeting a 26S rRNA segment. The enrichment of each sample was first
58 normalized to 1% input. And then fold changes were calculated by dividing the
59 enrichment of indicated mutants by the number of control animals.

60

61 **Fig. S8.** Images of the animals expressing indicated transgenes. (A) L3 animals and (B)
62 germline of young gravid adults. Scale bars, 25 μm . (C) Images of 1-to-8 cell embryos
63 from the animals expressing indicated transgenes. Scale bars, 10 μm .

64

65 **Fig. S9.** Subcellular localization of the exosome subunits of L4 stage animals. (A)
66 Images of somatic cells of the animals expressing indicated transgenes. The
67 fluorescence intensity indicated by dashed lines was measured by ImageJ, and the
68 values are shown on the right. Scale bars, 5 μm . (B) Images of somatic cells of the
69 animals expressing indicated transgenes. Scale bars, 5 μm . (C) (left) Images of somatic
70 cells of the indicated animals. Scale bars, 10 μm . (right) Quantification of the nucleolar
71 localization of GFP::EXOS-10. mean \pm s.d.; $n > 70$ animals; ns, not significant. (D)
72 (top) Images of somatic cells of the indicated animals. Scale bars, 10 μm . (bottom)
73 Quantification of the nucleolar localization of GFP::EXOS-10. mean \pm s.d.; $n > 70$
74 animals; ns, not significant.

75

76 **Fig. S10.** Schematics of the alleles that were generated via dual sgRNA-directed
77 CRISPR/Cas9 gene knockout technology. Each of the alleles contains a frameshift
78 mutation and is likely a null allele.

79

80 **Fig. S11.** The localization of nucleolar proteins. The expression and localization of
81 mCherry::RBD-1 (A), mCherry::FIB-1 (B) and GFP::RRP-8 (C) upon RNAi indicated
82 genes. Bleached embryos were placed on RNAi plates and grew up to L4 stage for
83 photographing. Scale bars, 10 μm .

84

85

86

87

88 **Table S1.** Candidate-base RNAi screening for factors affecting the subcellular
89 localization of GFP::EXOS-10. The percentage of animals with nucleolar-localized
90 GFP::EXOS-10 is indicated (% NCL).

91

92 **Table S2.** List of strains used in this study.

93

94 **Table S3.** Sequences of sgRNAs for CRISPR/Cas9-mediated gene editing.

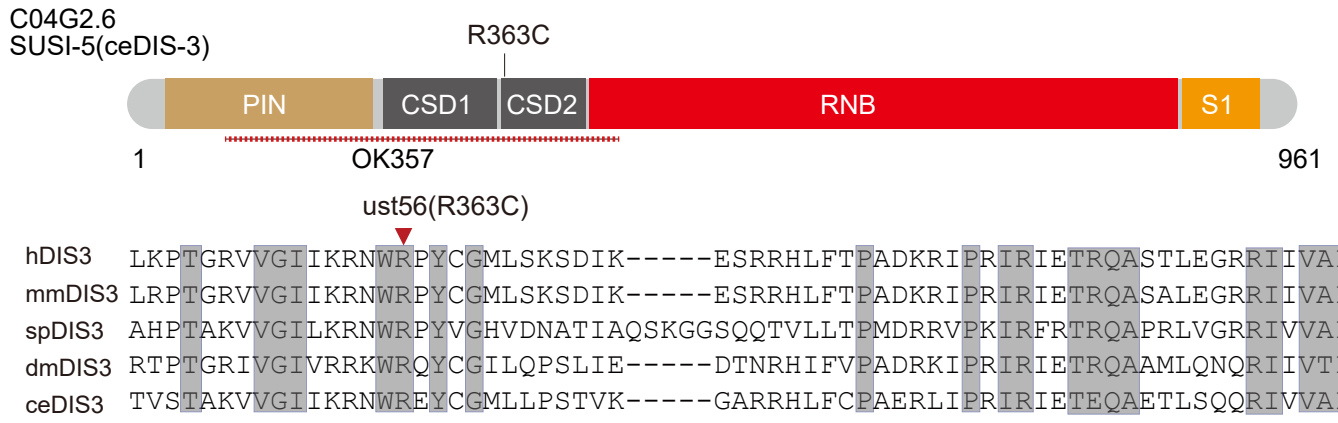
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96 **Table S4.** Sequences of quantitative real-time PCR primers for ChIP experiments.

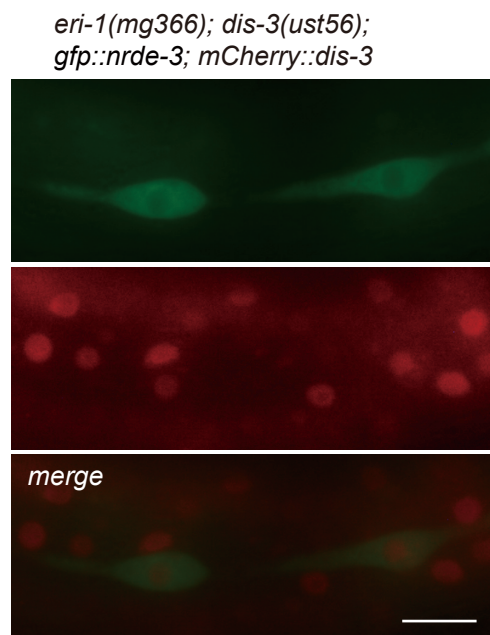
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98 **Table S5.** Sequences of quantitative real-time PCR primers for detecting the pre-rRNA
99 and rRNA levels.

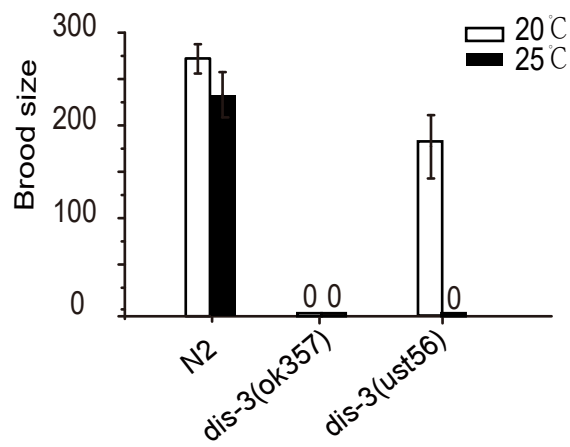
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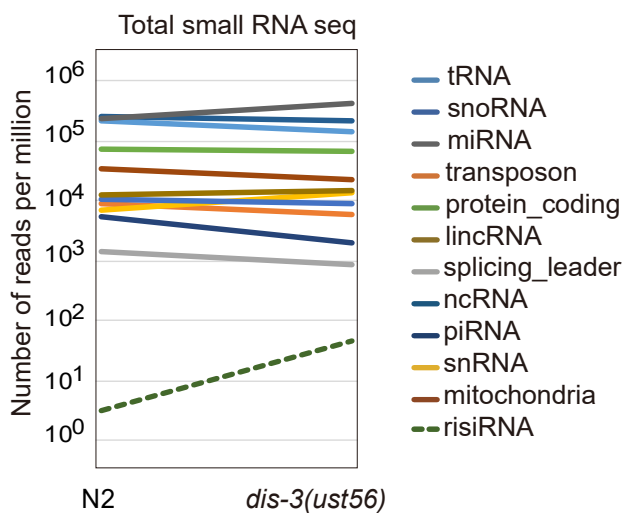
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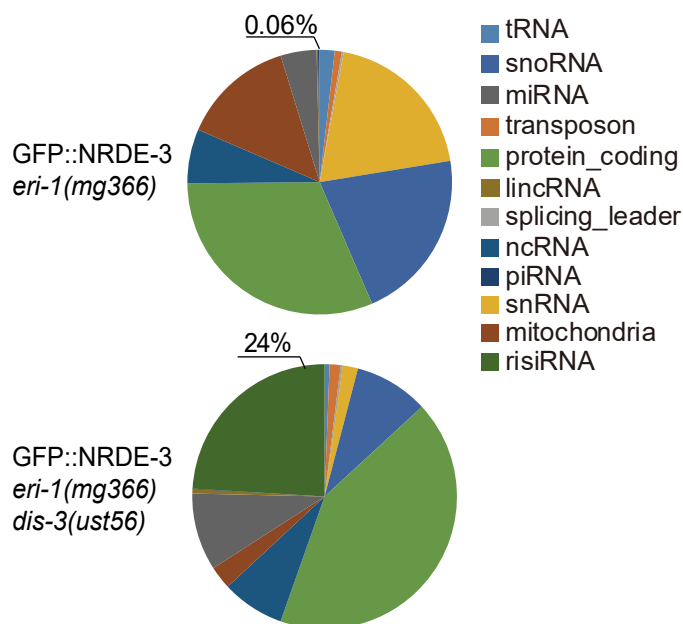
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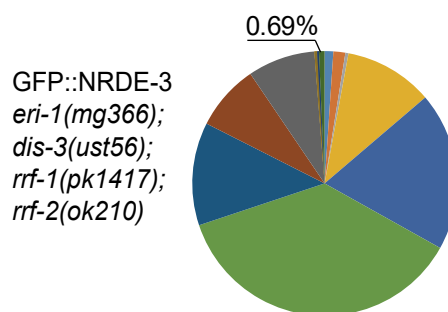
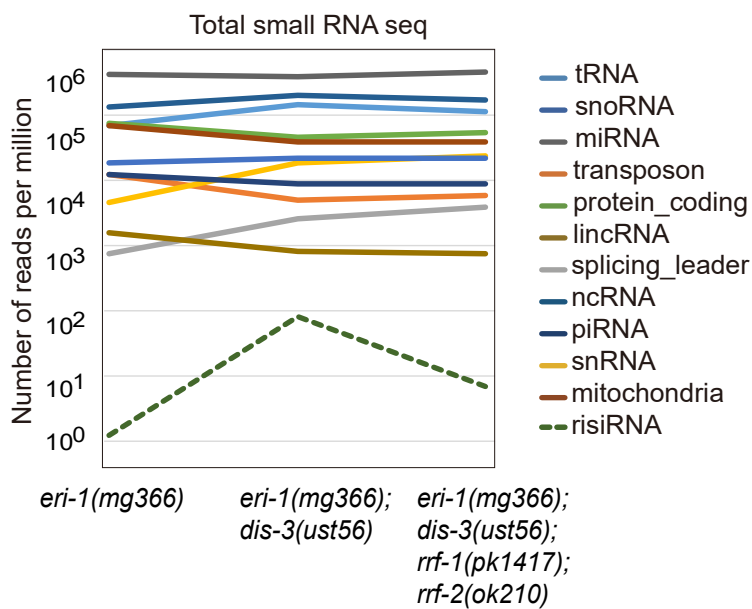
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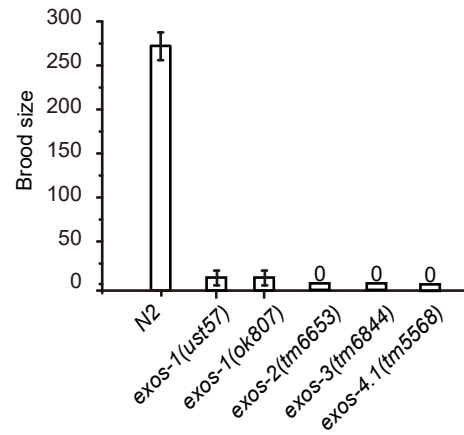
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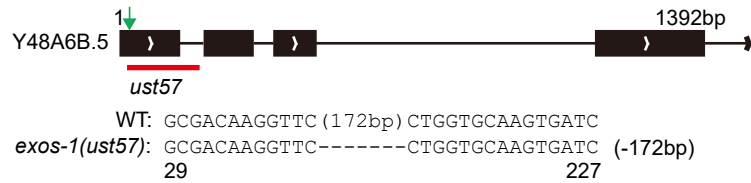
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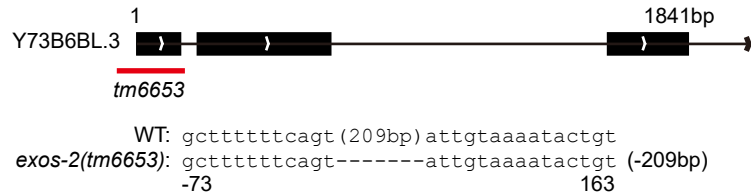
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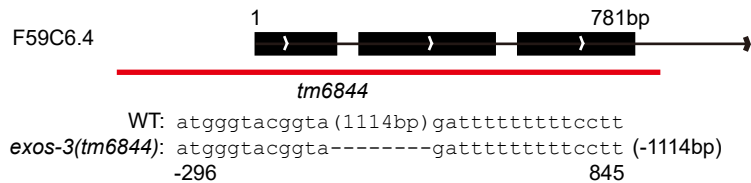
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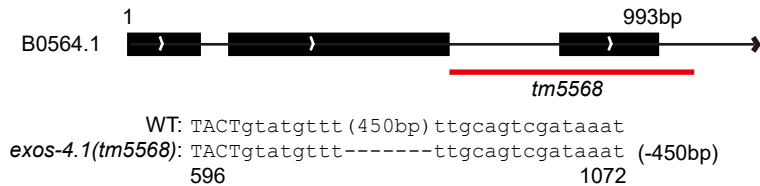
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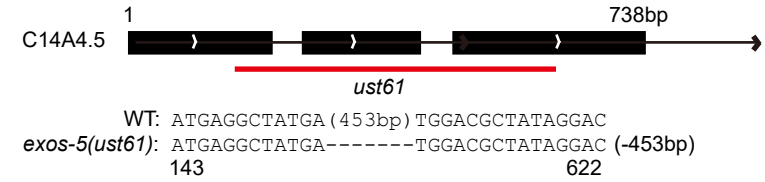
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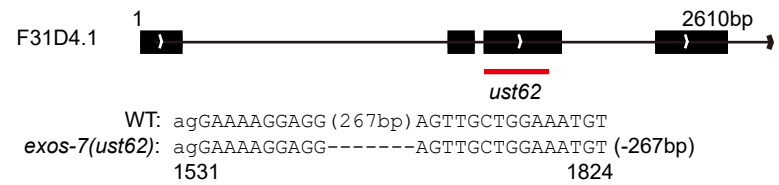
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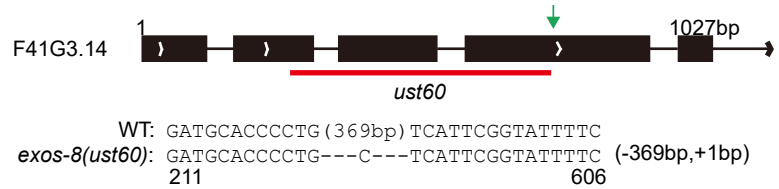
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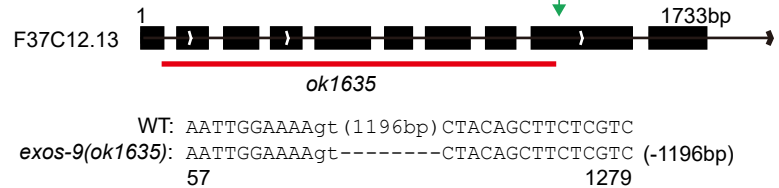
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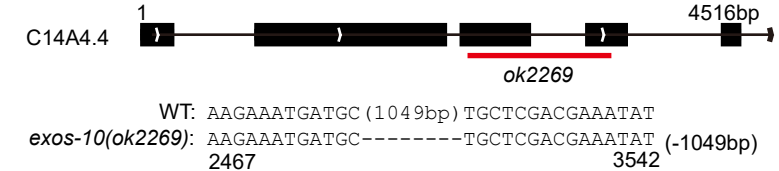
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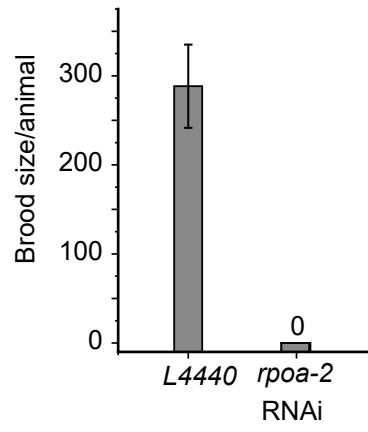
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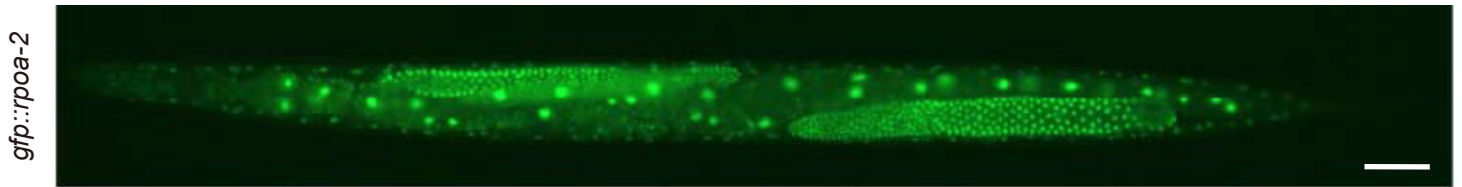
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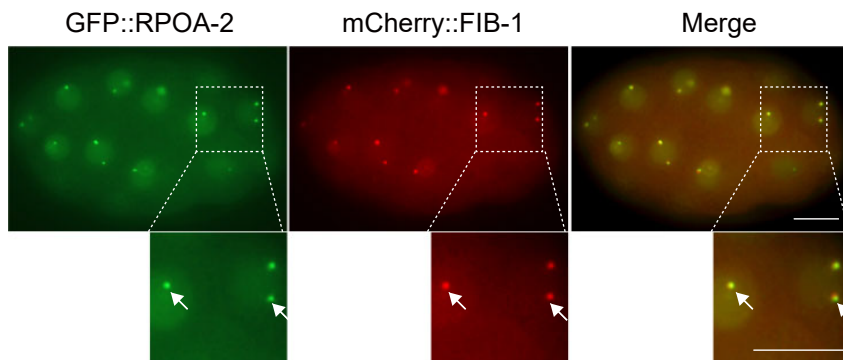
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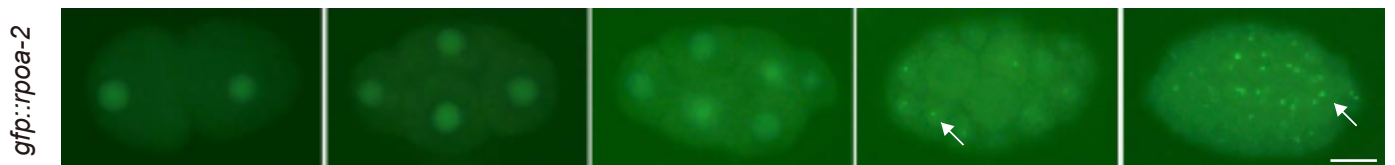
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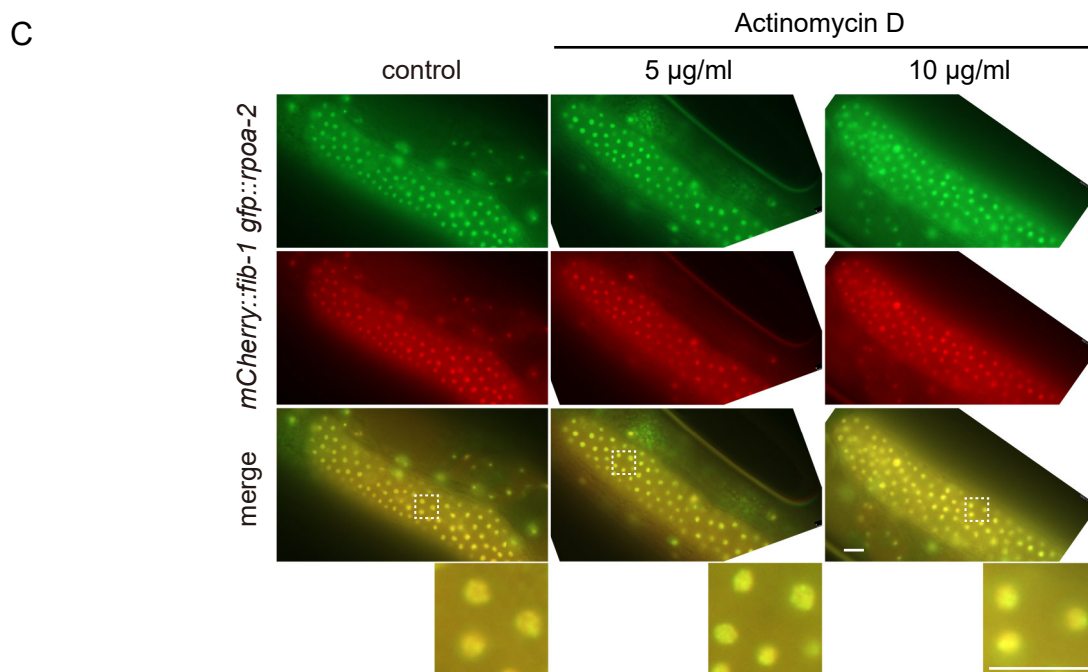
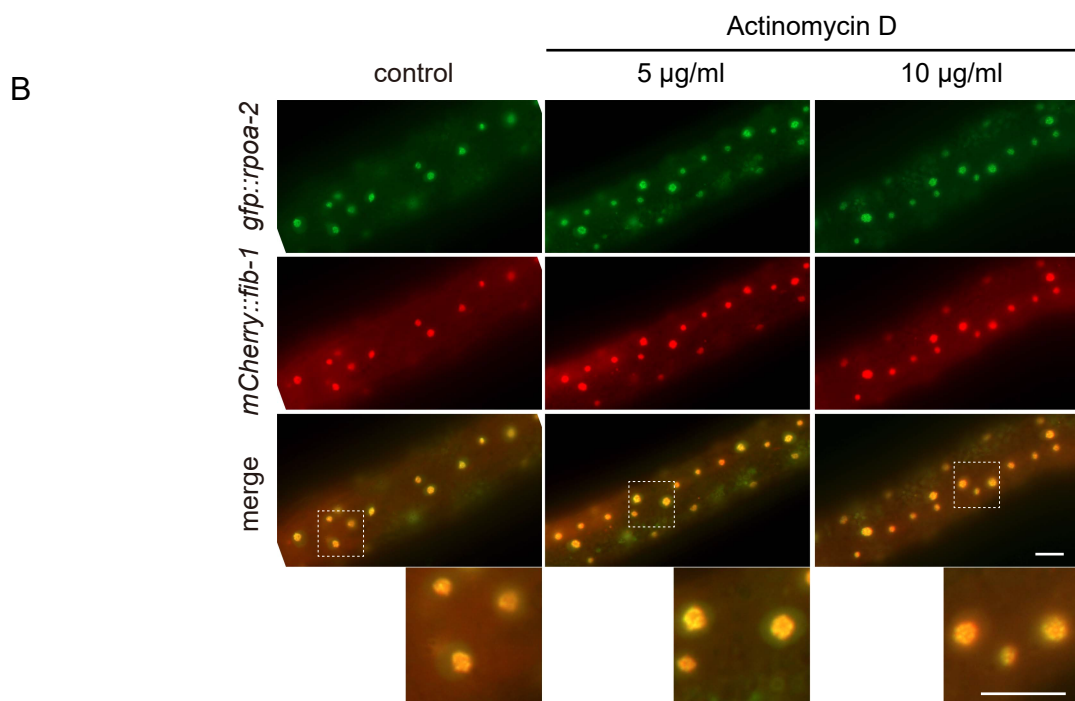
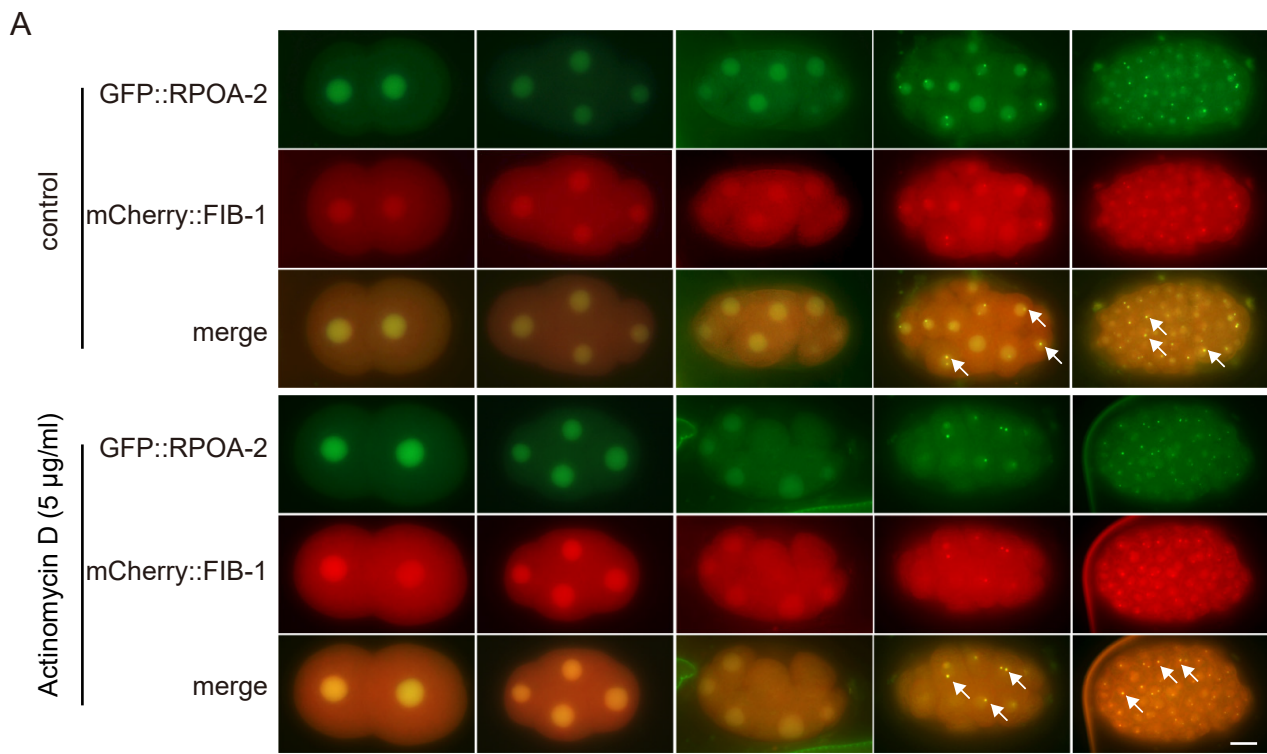


Figure S5

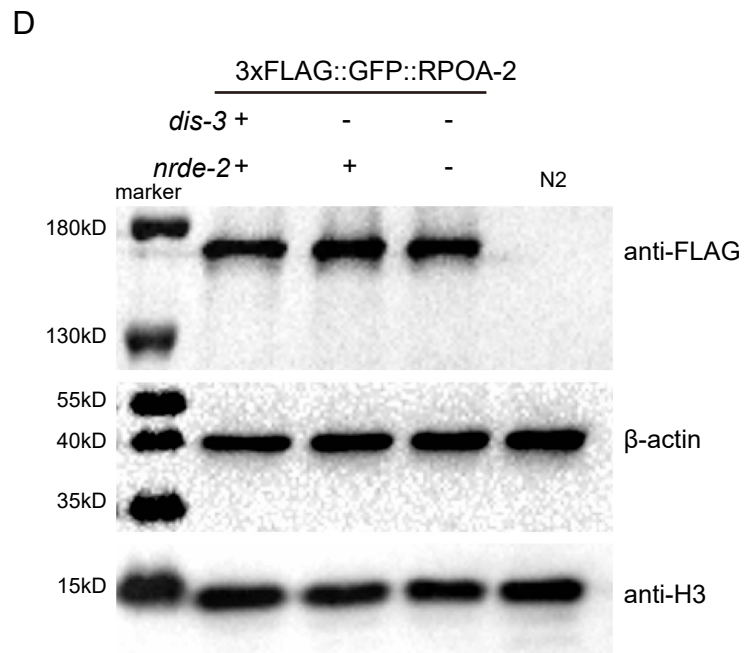
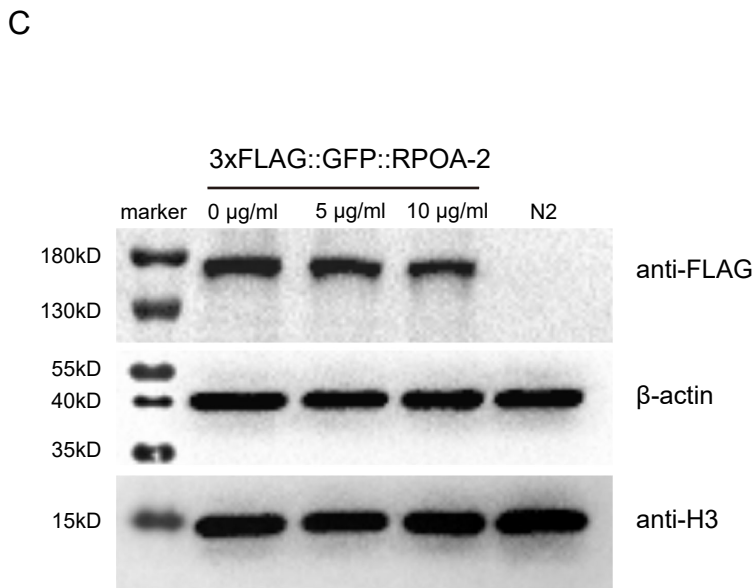
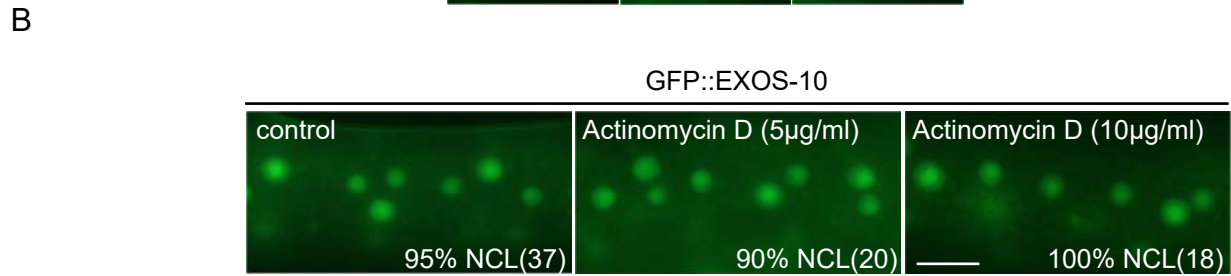
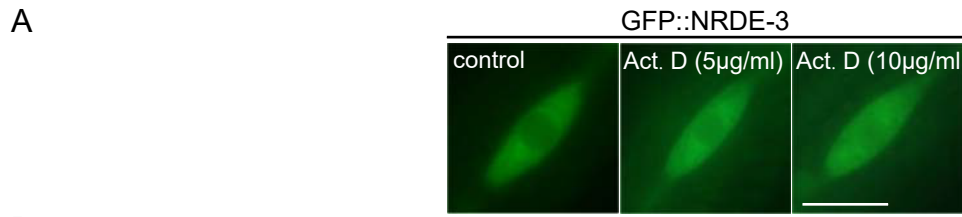
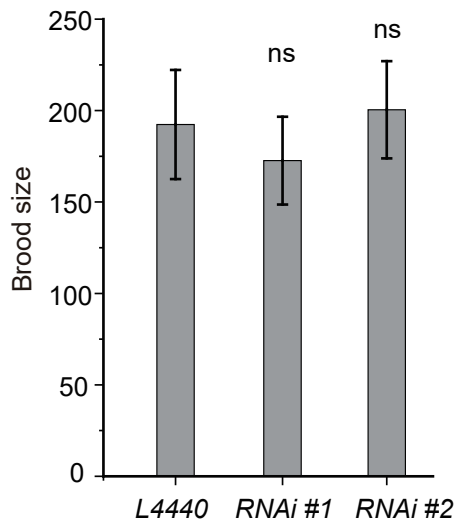
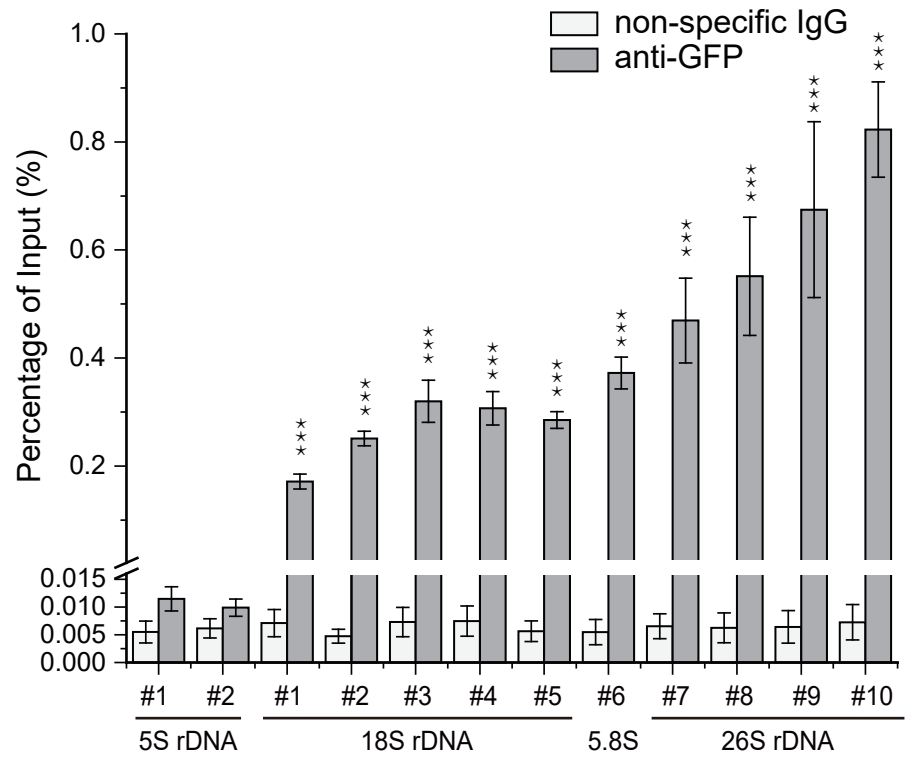


Figure S6

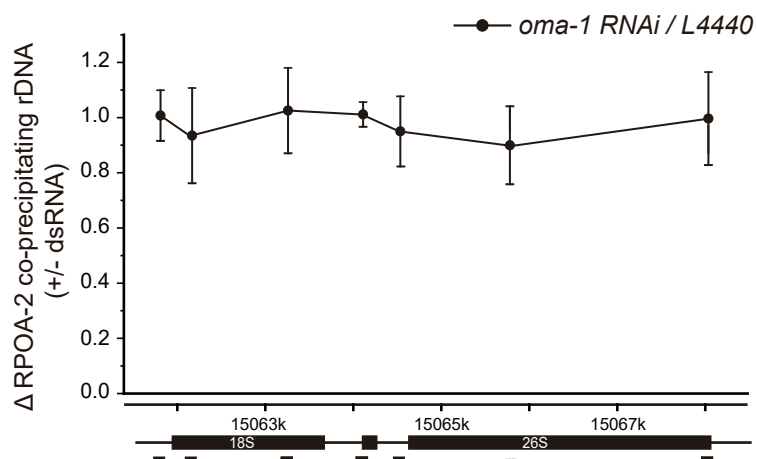
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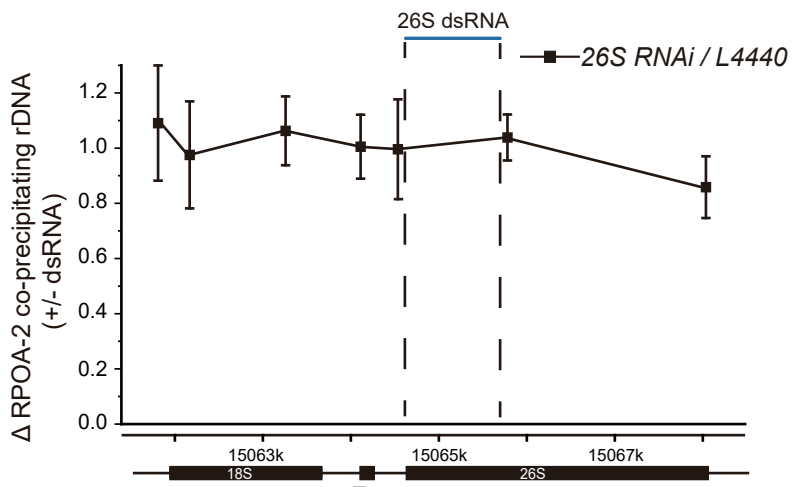
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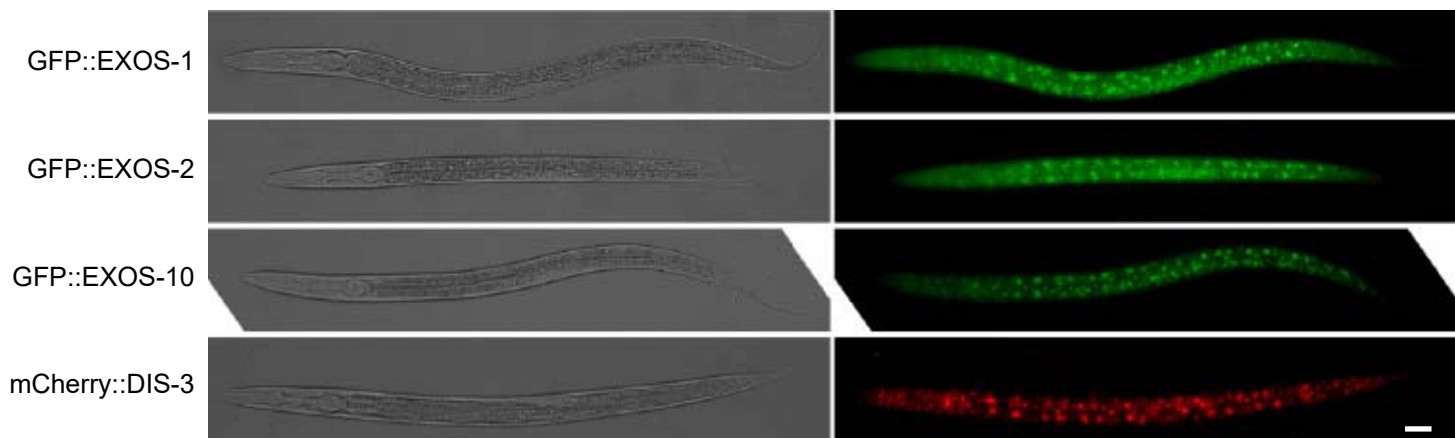
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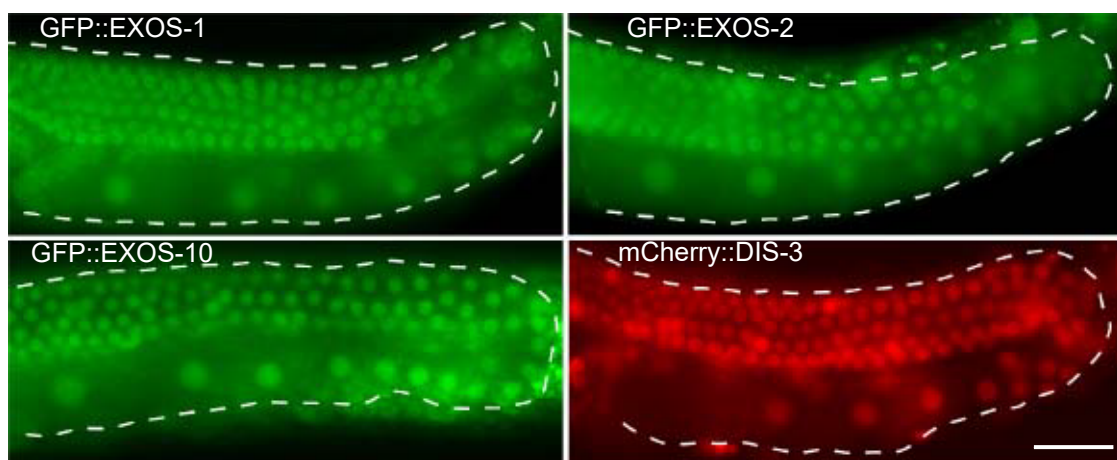
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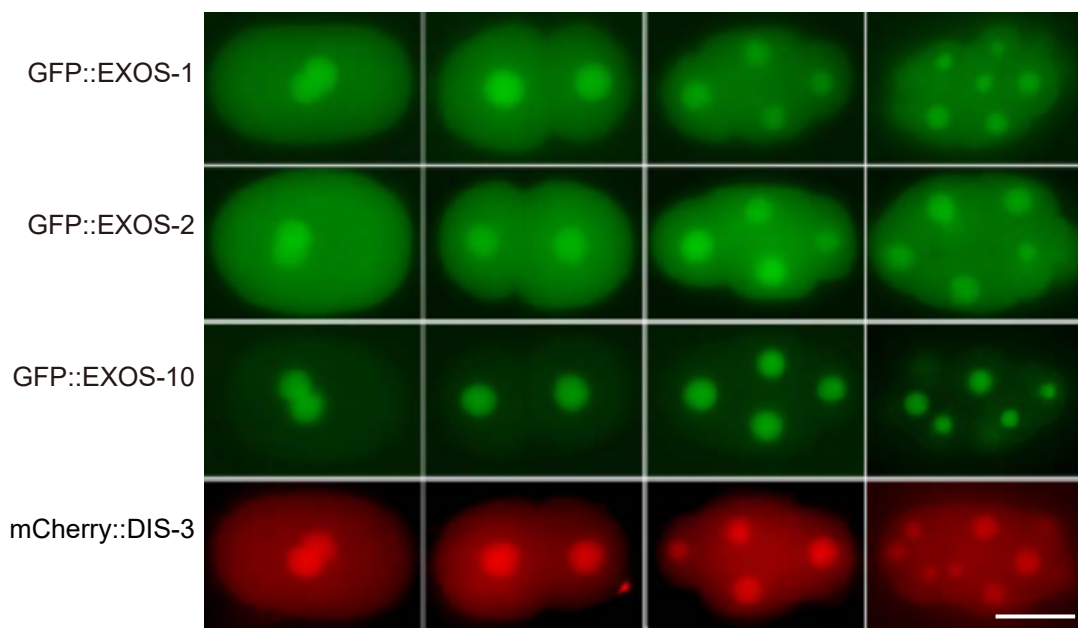
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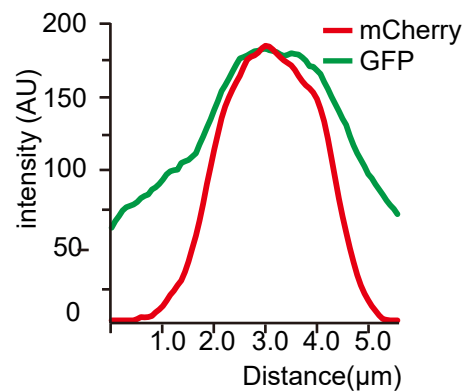
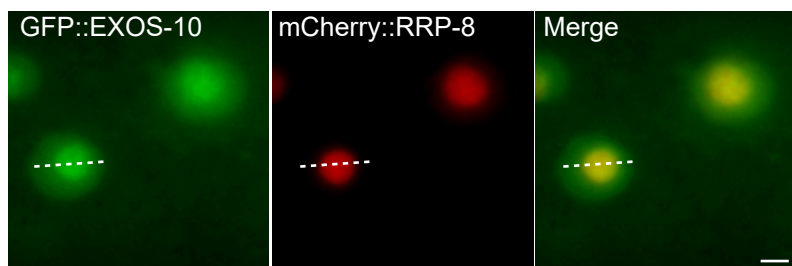
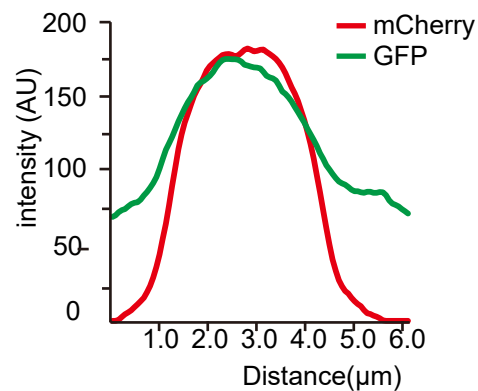
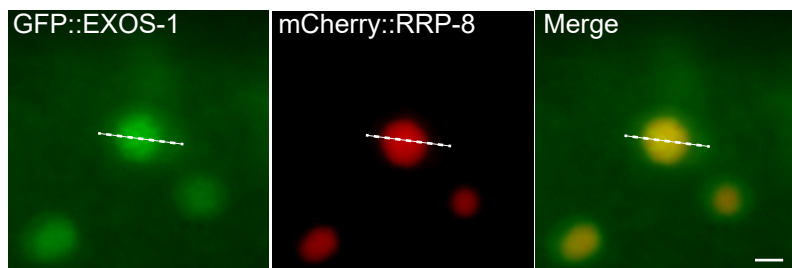
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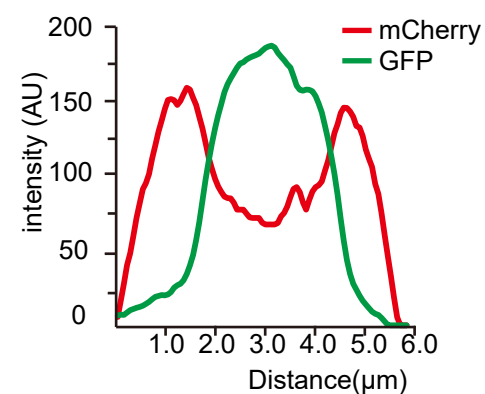
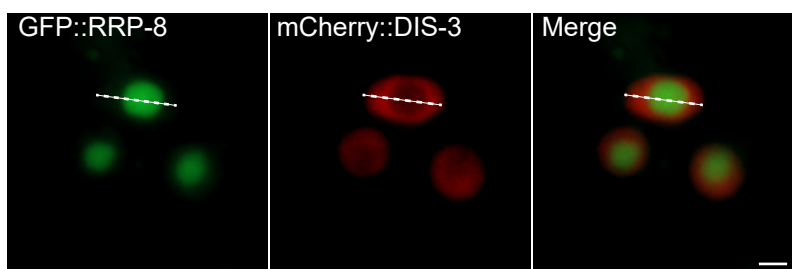
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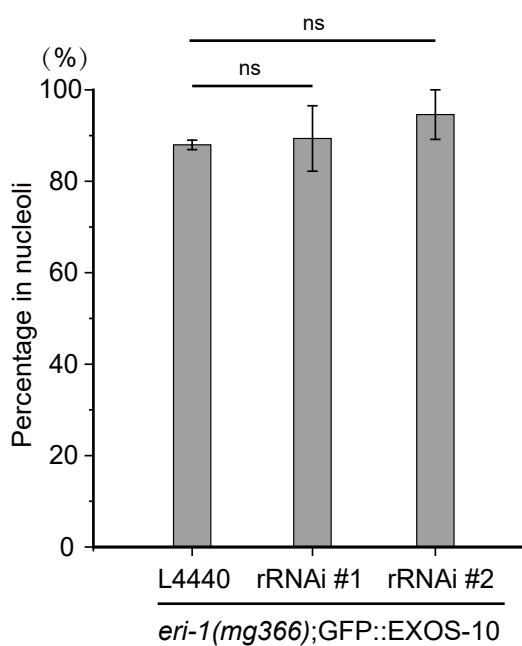
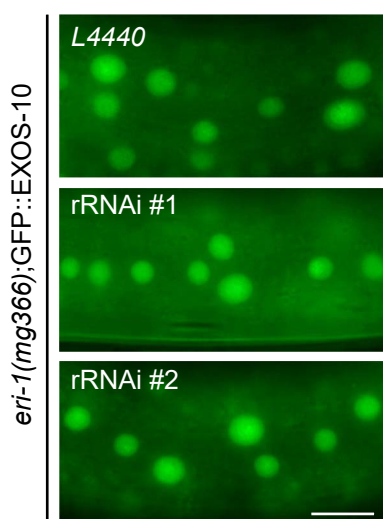
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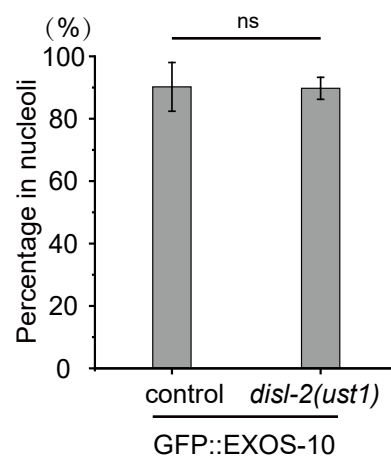
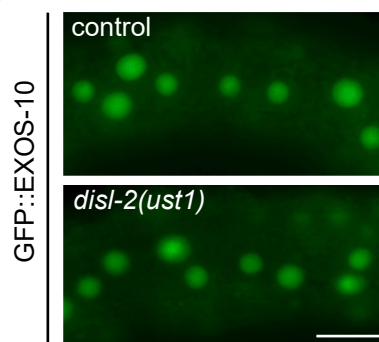
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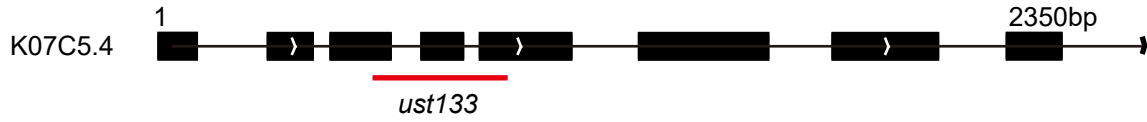
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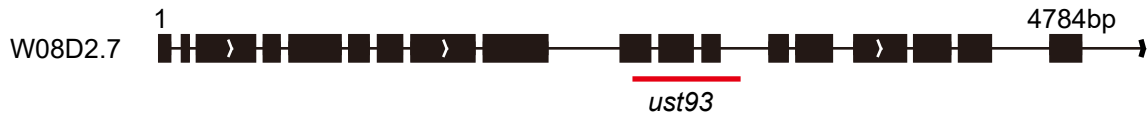
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WT: CGGTGGTCGTGG (24bp) CAGTTTCG (134bp) GTGGTTTT (573bp) atatttatattacag
fib-1(ust132): CGGTGGTCGTGG-----CAGTTTCG (134bp) GTGGTTTT--TAT--atatttatattacag (-597bp, +3bp)
 144 917



WT: TACTGAAGCGTT (339bp) GGACAAGGATATCAA
nol-56(ust133): TACTGAAGCGTT-----GGACAAGGATATCAA (-339bp)
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WT: AGAGTTGAAGGA (339bp) tcaaaatgtaaaca
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 2460 3020

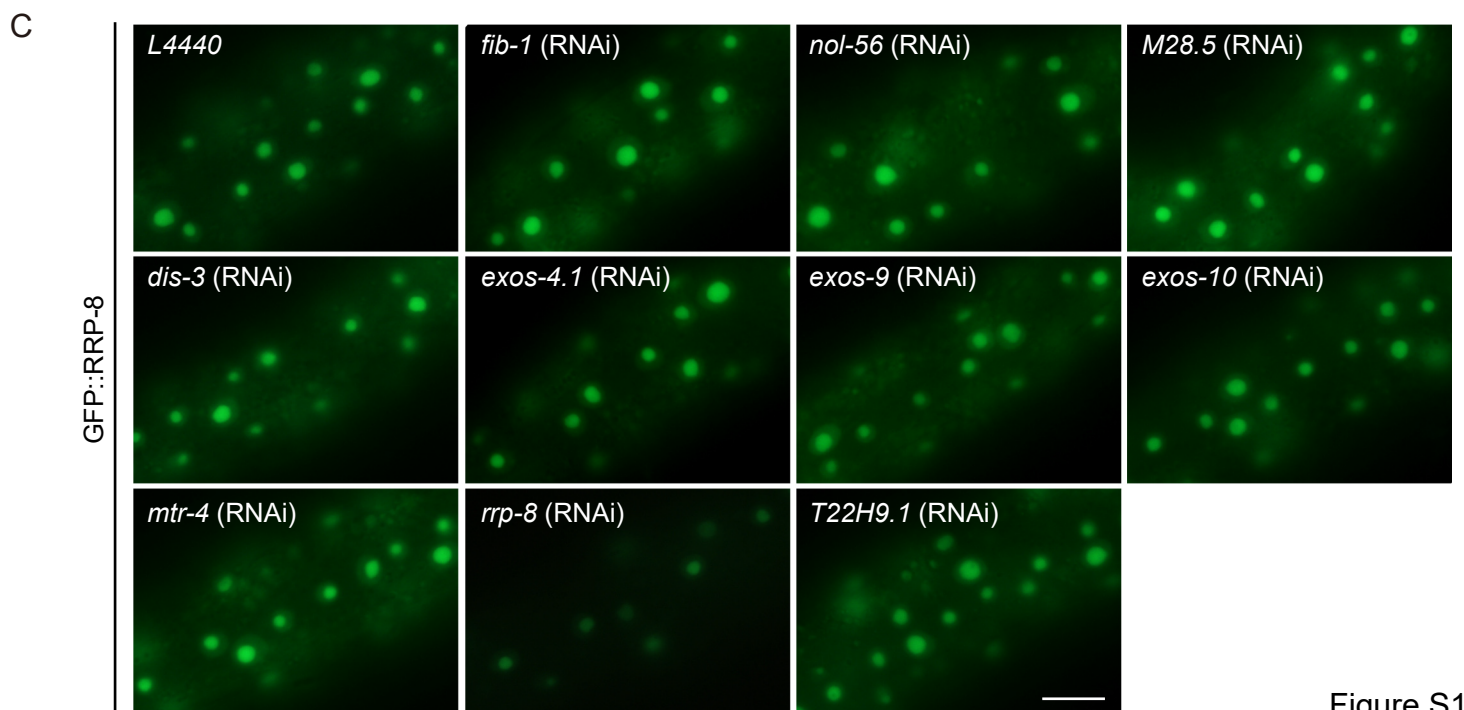
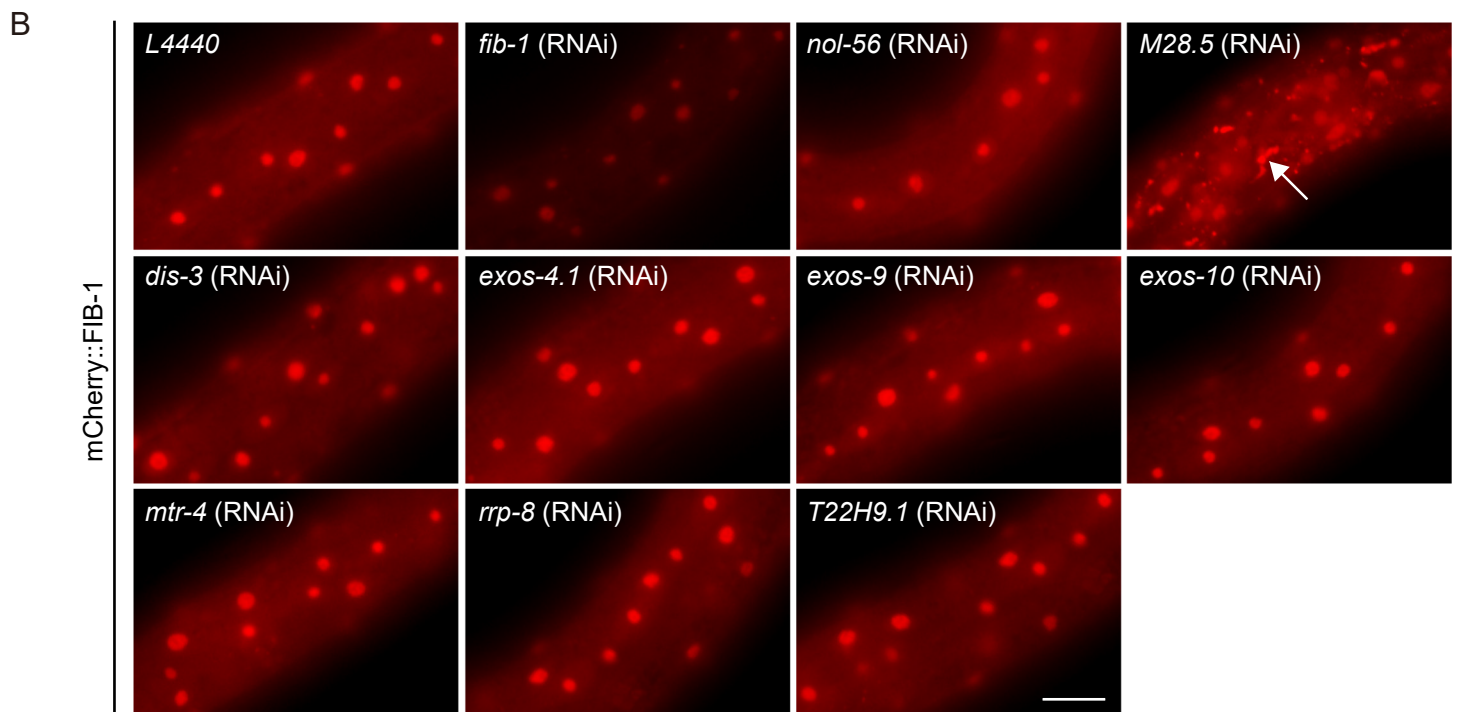
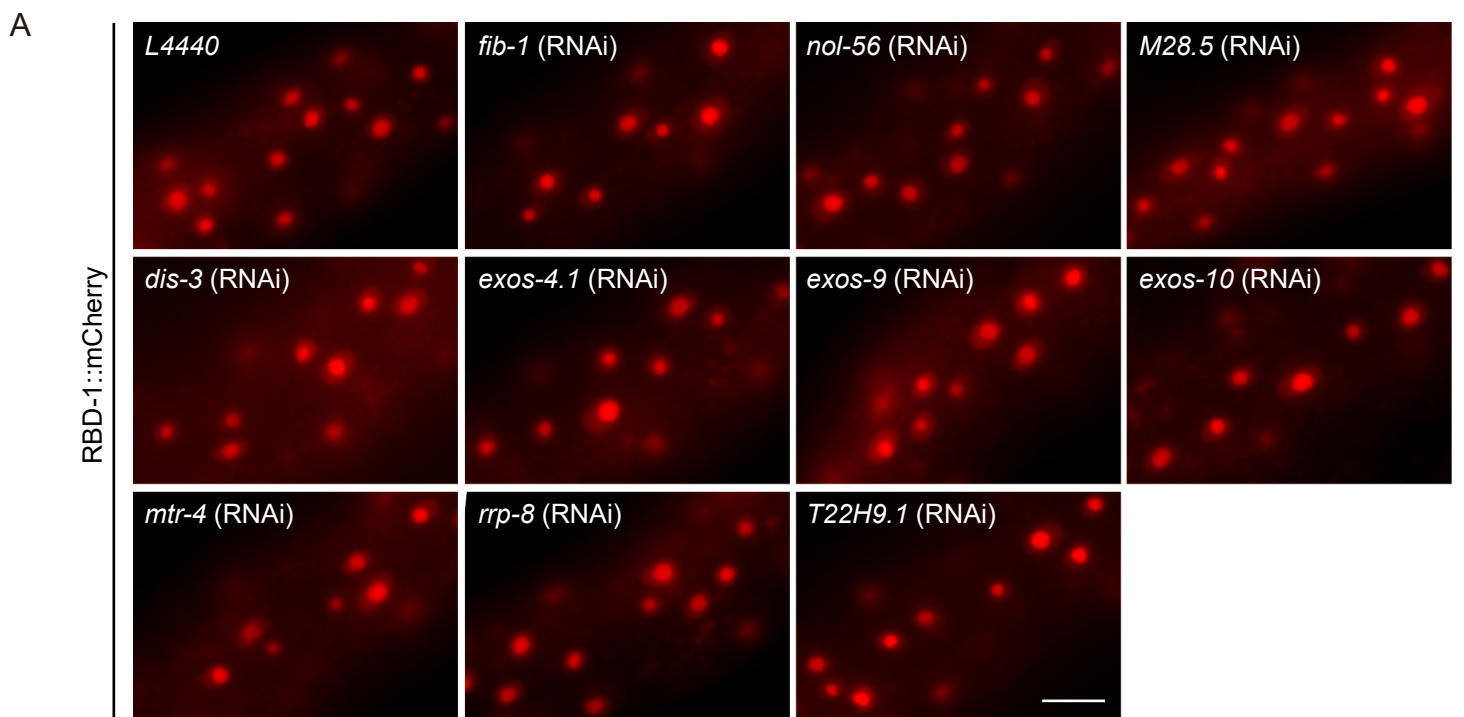


Figure S11

Table S1: Candidate-base RNAi screening for factors affecting the subcellular localization of GFP::EXOS-10.

gene ID	gene name	NCL%	yeast	human	predicted functions
L4440(control)	/	95.5%			
K07C5.4	<i>nol-56</i>	0%	NOP56	NOP56	histone methyltransferase binding activity and snoRNA binding activity
T01C3.7	<i>fib-1</i>	4.6%	FIB1	FBL	have RNA binding activity and methyltransferase activity
M28.5	<i>phi-9</i>	11.9%	SNU13	SNU13	box C/D snoRNP complex
W08D2.7	<i>mtr-4</i>	22.7%	MTR4	MTR4	have ATP binding activity, RNA binding activity, and RNA helicase activity
F10B5.1	<i>rpl-10</i>	31.8%	RPL10	RPL10	ribosomal large subunit assembly
F37C12.13	<i>exos-9</i>	60.0%	RRP45	EXOSC9	exonucleolytic trimming to generate mature 3'-end of 5.8S rRNA from tricistronic rRNA transcript
C04G2.6	<i>dis-3</i>	68.2%	RRP44	DIS3	exosome endoribonuclease and 3'-5' exoribonuclease
ZK265.6	<i>nol-16</i>	75.0%	NOP16	NOP16	ribosomal large subunit biogenesis
K09B11.2	<i>nol-9</i>	78.8%	GRC3	NOL9	Polynucleotide 5'-kinase involved in rRNA processing
F25H8.2	/	85.0%	NAF1	NAF1	box H/ACA snoRNP assembly
K01G5.5	/	90.9%	CBF5	DKC1	box H/ACA snoRNP complex
F32E10.1	<i>nol-10</i>	91.7%	NOL10	NOL10	an ortholog of human NOL10 (nucleolar protein 10); exhibits RNA binding activity
C27H6.2	<i>ruvb-1</i>	95.0%	RUVB1	RUVBL1	involved in TOR signaling, box C/D snoRNP assembly
Y48A6B.3	/	95.0%	NHP2	NHP2	box H/ACA snoRNP complex
Y66H1A.4	/	95.5%	GAR1	GAR1	box H/ACA snoRNP complex

Table S2: Strains used in the work.

Genotype

N2

CB4856

dis-3(ust56)

exos-1(ust57)

eri-1(mg366);3XFLAG::GFP::NRDE-3(ggIS1)

eri-1(mg366);dis-3(ust56);3XFLAG::GFP::NRDE-3(ggIS1)

eri-1(mg366);dis-3(ok357);3XFLAG::GFP::NRDE-3(ggIS1)

eri-1(mg366);dis-3(ust56);mCherry::DIS-3(ustIS115);3XFLAG::GFP::NRDE-3(ggIS1)

eri-1(mg366);dis-3(ust56);rrf-1(pk1417);3XFLAG::GFP::NRDE-3(ggIS1)

eri-1(mg366);dis-3(ust56);rrf-2(ok210);3XFLAG::GFP::NRDE-3(ggIS1)

eri-1(mg366);dis-3(ust56);rrf-3(pk1426);3XFLAG::GFP::NRDE-3(ggIS1)

eri-1(mg366);dis-3(ust56);rrf-1(pk1417);rrf-2(ok210);3XFLAG::GFP::NRDE-3(ggIS1)

eri-1(mg366);exos-1(ust57);3XFLAG::GFP::NRDE-3(ggIS1)

eri-1(mg366);exos-2(tm6653);3XFLAG::GFP::NRDE-3(ggIS1)

eri-1(mg366);exos-3(tm6844);3XFLAG::GFP::NRDE-3(ggIS1)

eri-1(mg366);exos-4.1(tm5568);3XFLAG::GFP::NRDE-3(ggIS1)

eri-1(mg366);exos-5(ust61);3XFLAG::GFP::NRDE-3(ggIS1)

eri-1(mg366);exos-7(ust62);3XFLAG::GFP::NRDE-3(ggIS1)

eri-1(mg366);exos-8(ust60);3XFLAG::GFP::NRDE-3(ggIS1)

eri-1(mg366);exos-9(ok1635);3XFLAG::GFP::NRDE-3(ggIS1)

eri-1(mg366);exos-10(ok2269);3XFLAG::GFP::NRDE-3(ggIS1)

3XFLAG::GFP::EXOS-1(ustIS112)

3XFLAG::GFP::EXOS-2(ustIS113)

3XFLAG::GFP::EXOS-10(ustIS114)

mCherry::DIS-3(ustIS115)

mCherry::RRP-8(ustIS75)

GFP::RRP-8(ustIS76)

RBD-1::mCherry(ustIS207)

mCherry::FIB-1(ustIS36)
mCherry::DIS-3(ustIS115);GFP::RRP-8(ustIS76)
dis-3(ust56);3XFLAG::GFP::EXOS-1(ustIS112)
dis-3(ust56);3XFLAG::GFP::EXOS-10(ustIS114)
3XFLAG::GFP::EXOS-1(ustIS112);mCherry::RRP-8(ustIS75)
3XFLAG::GFP::EXOS-10(ustIS114);mCherry::RRP-8(ustIS75)
mCherry::DIS-3(ustIS115);GFP::RRP-8(ustIS76)
eri-1(mg366);fib-1(ust132);3XFLAG::GFP::NRDE-3(ggIS1)
eri-1(mg366);nol-56(ust133);3XFLAG::GFP::NRDE-3(ggIS1)
eri-1(mg366);mtr-4(ust93);3XFLAG::GFP::NRDE-3(ggIS1)
3XFLAG::GFP::RPOA-2(ustIS116)
3XFLAG::GFP::RPOA-2(ustIS116);mCherry::FIB-1(ustIS36)
GFP::NRDE-2(ustIS117);mCherry::RPOA-2(ustIS116)
eri-1(mg366);3XFLAG::GFP::RPOA-2(ustIS116)
eri-1(mg366);nrde-2(gg91);3XFLAG::GFP::RPOA-2(ustIS116)

Table S3. Sequences of sgRNAs for CRISPR/Cas9-mediated gene editing.

rpoa-2_sg#1	TTCAGTTCGGCCACAATTCG
rpoa-2_sg#2	ATTGTGGCCGAACACTGAACAG
rpoa-2_sg#3	GCGACAGCCACTGTTTCAGTT
exos-1_sg#1	ACAAGGTTCTCGACGCGAT
exos-1_sg#2	ATCACTTGCACCAGGTTGT
exos-1_sg#3	ATACTGATGATGTCACATT
exos-1_sg#4	CCGACAGCCATCACTTTGG
exos-5_sgRNA #1	GAGTGATGAGGCTATGACTC
exos-5_sgRNA #2	GTACATGGAATTCAGGATGA
exos-5_sgRNA #3	GCATCCAGAAGTGTGTGCGA
exos-7_sgRNA #1	GGCGAATTGACTGCTCGCGT
exos-7_sgRNA #2	GGTGACGTTGCACCAGATGA
exos-7_sgRNA #3	GTTGAGATTGTCAGCAGCAG
exos-8_sg#1	GAGTCTATCCTGACGGACG
exos-8_sg#2	GTTAGTGATGCACCCCTGG
exos-8_sg#3	TGTGGCAAACCTTTGCCTC
exos-8_sg#4	CGTCAATCGATGCATTGTT
nrde-2_sgRNA#1	GGAACAATGTTTTCGAGCGTATGG
nrde-2_sgRNA#2	GAAACATTGTTTCATTAAGTTTGG
fib-1_sgRNA#1	GCGGTGGTCGTGGAGGATA
fib-1_sgRNA#2	TGTCCGTCGATGACGGAGC
fib-1_sgRNA#3	CCACTTGAGCAGGTAACCC
nol-56_sgRNA#1	TCGATGCCGCTCATGCTGA
nol-56_sgRNA#2	ATCTGAAGCTTGTCTTCGG
nol-56_sgRNA#3	ATTGCCCTTCTCGATCAGT
mtr-4_sgRNA#1	TGAAGGAATGGCTGTTTCA
mtr-4_sgRNA#2	GTACAAAGTACATTGCATT
mtr-4_sgRNA#3	TCGTATATCAATGGGTAA
mtr-4_sgRNA#4	GCCAAGGCTTTAGCGAATA

Table S4. Sequences of quantitative real-time PCR primers for ChIP experiments.

<i>eft-3</i> qRT F	CAAGGATATTCGTCGTGGATCC
<i>eft-3</i> qRT R	AATCGAGAACTGGAGTGTATCCG
<i>ama-1</i> qRT F	CGAACCTGCCGATTGATA
<i>ama-1</i> qRT R	ACCACGATTGACCAACTC
<i>lin-15b</i> qRT #1F	ACCGAGACCAGCCAATGT
<i>lin-15b</i> qRT #1R	TCTTCATCCAGTGGTTCATCCT
<i>lin-15b</i> qRT #2F	CACTGAACTCACAAGACCACAC
<i>lin-15b</i> qRT #2R	TCCAATTTGAAGTCATCCCTCTG
5ETS-1F	CCTACACTCATGTCTTTGCAGA
5ETS-1R	GCCGTACTIONATGCAGCAAGG
5ETS-2F	CCACATTCAGAGGCTGGTGA
5ETS-2R	CCTCTCACCAGCCTATCATTCG
5ETS-3F	CGTCTCTCAAATTGCACACTGC
5ETS-3R	CCTGAGACATCACGTCTCAGAC
rDNA#1 F	CAAGACCAATACCGCAACATCA
rDNA#1 R	TCATTGCGCCGATCCATAGAT
rDNA#2 F	GGAGCTAATACATGCAACTATACCC
rDNA#2 R	CAGTCGAAACTGACAGTAAACTGC
rDNA#3 F	GGATGAGTTATTTCAATGAGTTGAATAC
rDNA#3 R	CGCAGCAATAACGAGATACTAG
rDNA#4 F	CTTCGAGTAGCAAGGAGAGG
rDNA#4 R	GCACGTAACCTAGATCCAACACTAC
rDNA#5 F	GGACTGTCGCTTCGAGGTTTAA
rDNA#5 R	GCGATGATCCAGCTGCAG
rDNA#6 F	GAATCAGTACACTGATTGCCAAAAG
rDNA#6 R	GTTCTTCATCGATACTCGATGC
rDNA#7 F	CGATAGCGAACAAGTACC
rDNA#7 R	TCTCCAAGCAACATCAAC
rDNA#8 F	TAATGTCTCAACCTATTCTCAA
rDNA#8 R	GCCAGTTCTGCTTACCAA
rDNA#9 F	GGAATCCGACTGTCTAAT
rDNA#9 R	CGCTTACTCGAATTACTAC
rDNA#10 F	CATACGACTTGGTCTCTTGG
rDNA#10 R	CTGCAAAGACATGAGTGTAGG
rDNA#11 F	GTCTGGTTTATTCGATAACGAG
rDNA#11 R	GACCTGTTATCGCTCAATCTC
rDNA#12 F	CTCGGCTGATCATCAAGACG
rDNA#12R	CGATATCAACACACACACGAGAC
rDNA#13F	GTGAACTGTCAACGTGAATGC
rDNA#13R	GGTCTCATGAGCGAGAAGTTAG

Table S5. Sequences of quantitative real-time PCR primers for detecting the pre-rRNA and rRNA levels.

<i>eft-3</i> E2L F	GTAAGGGATCTTTCAAGTACGC
<i>eft-3</i> E2L R	CATCGATGATGGTGATGTAGTAC
5S 1F	CTCTCCACACAACACACAGC
5S 1R	ATATGGTCGTAAGCGTCTATGG
<i>lin-15b</i> pre#1 F	CAATTCCTAACAATCATAAACTCTCAA
<i>lin-15b</i> pre#1 R	GGAGGTATATAAGGTGGTTGAATGTTTC
<i>lin-15b</i> pre#2 F	CAATTCCTAACAATCATAAACTCTCAA
<i>lin-15b</i> pre#2 R	CATTGTCAATGTAGTGGAGGATG
pre#1 F	TCATTGCGCCGATCCATAGAT
pre#1 R	CAAGACCAATACCGCAACATCA
pre#2 F	CAAGACCAATACCGCAACATCA
pre#2 R	CGCAGACATATAGTCTAGCGAG
pre#3 F	CCCTTGCTGCATAGTACGGC
pre#3 R	TCAGCAGCATGTCAATGTGGT
pre#4 F	GGACACACCACCAAAGTCTCAA
pre#4 R	TTGAGAGACGGCAGACAACG
pre#5 F	CTGTATGAGTGTCCCATCTCACG
pre#5 R	GCATGGCTTAATCTTTTACTTGAGC
pre#6 F	CGCATTGGTTTGAACCGG
pre#6 R	CAACTGACCGTGAAGCCAG
pre#7 F	CGTAACAAGGTAGCTGTAGGTG
pre#7 R	CTGGAACGTTGACATTTTCGAC
pre#8 F	CATACGACTTGGTCTCTTGG
pre#8 R	CTGCAAAGACATGAGTGTAGG
18S#1 F	CGATAACAGGTCTGTGATGCCC
18S#1 R	TACCCTATCCCGGACATGGAAG
26S#1 F	TAATGTCCTCAACCTATTCTCAA
26S#1 R	GCCAGTTCTGCTTACCAA
26S#2 F	GGAATCCGACTGTCTAAT
26S#2 R	CGCTTACTCGAATTACTAC