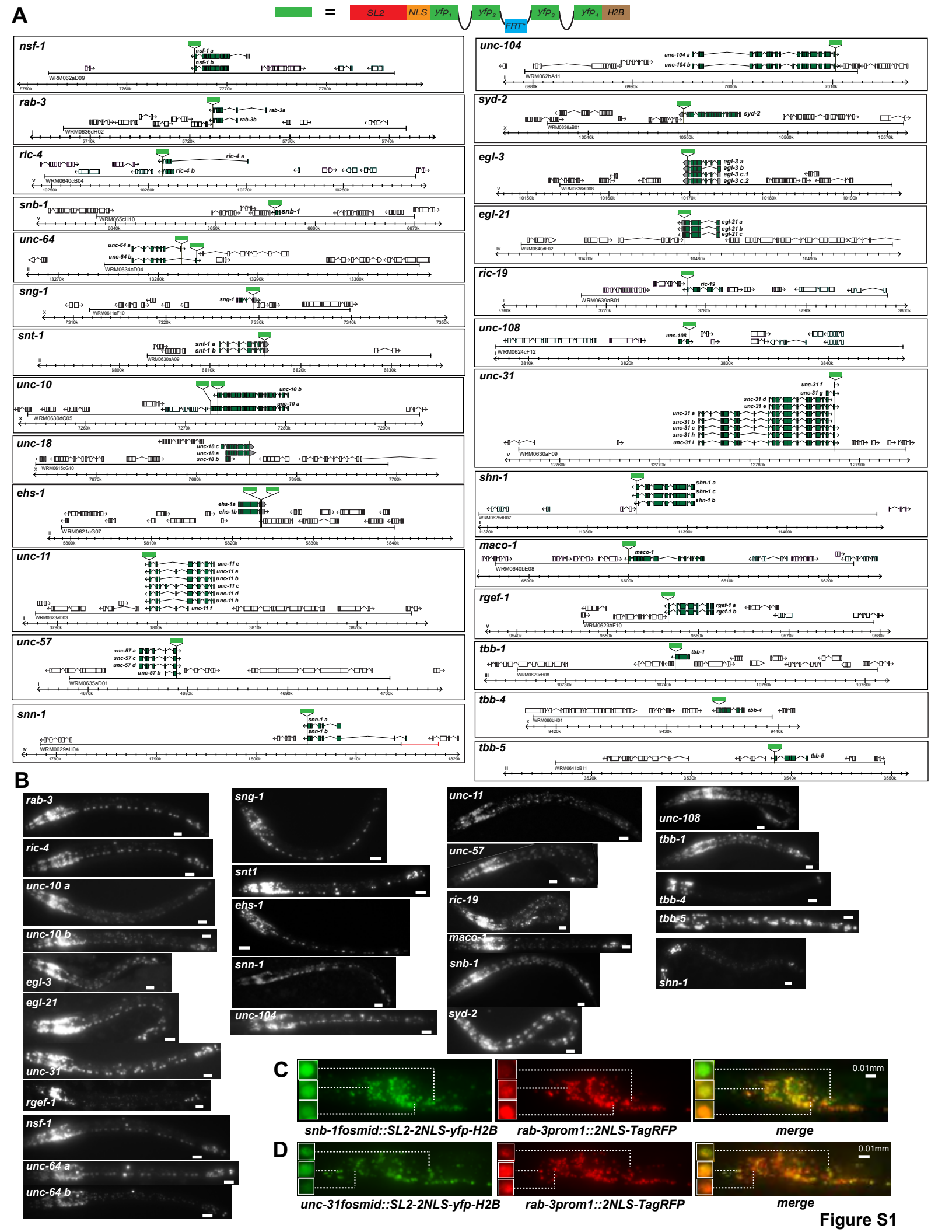

A
=
SL2
NLS
yfp1
yfp2
FRT
yfp3
yfp4
H2B
nsf-1
rab-3
ric-4
snb-1
unc-64
sng-1
snt-1
unc-10
unc-18
ehs-1
unc-11
unc-57
snn-1
unc-104
syd-2
egl-3
egl-21
ric-19
unc-108
unc-31
shn-1
maco-1
rgef-1
tbb-1
tbb-4
tbb-5
B
rab-3
ric-4
unc-10 a
unc-10 b
egl-3
egl-21
unc-31
rgef-1
nsf-1
unc-64 a
unc-64 b
sng-1
snt1
ehs-1
snn-1
unc-104
unc-11
unc-57
ric-19
maco-1
snb-1
syd-2
unc-108
tbb-1
tbb-4
tbb-5
shn-1
C
snb-1fosmid::SL2-2NLS-yfp-H2B
rab-3prom1::2NLS-TagRFP
merge
0.01mm
D
unc-31fosmid::SL2-2NLS-yfp-H2B
rab-3prom1::2NLS-TagRFP
merge
0.01mm

Figure S1

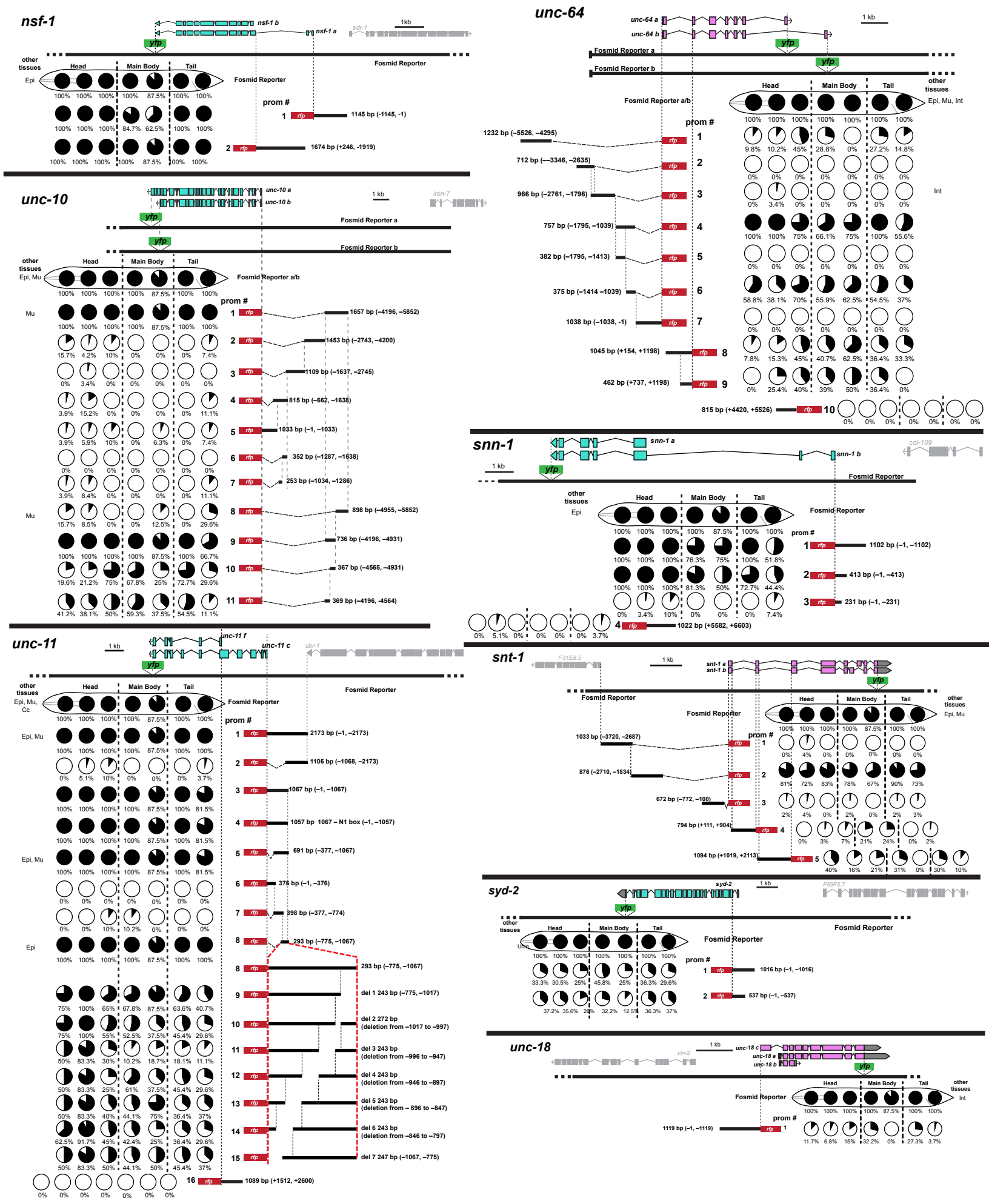

nsf-1
nsf-1 b
nsf-1 a
a:dr-1
1kb
yfp
other tissues
Head
Main Body
Tail
Epi
Fosmid Reporter
prom #
1 rfp 1145 bp (-1145, -1)
2 rfp 1674 bp (+246, -1919)
unc-10
unc-10 a
unc-10 b
1 kb
Iron-7
Fosmid Reporter a
Fosmid Reporter b
Fosmid Reporter a/b
Epi, Mu
Mu
1 rfp 1657 bp (–4196, –5852)
2 rfp 1453 bp (–2743, –4200)
3 rfp 1109 bp (–1637, –2745)
4 rfp 815 bp (–662, –1638)
5 rfp 1033 bp (–1, –1033)
6 rfp 352 bp (–1287, –1638)
7 rfp 253 bp (–1034, –1286)
8 rfp 898 bp (–4955, –5852)
9 rfp 736 bp (–4196, –4931)
10 rfp 367 bp (–4565, –4931)
11 rfp 369 bp (–4196, -4564)
unc-11
unc-11 f
unc-11 c
ubr-1
Epi, Mu, Cc
1 rfp 2173 bp (–1, –2173)
2 rfp 1106 bp (–1068, –2173)
3 rfp 1067 bp (–1, –1067)
4 rfp 1057 bp 1067 – N1 box (–1, –1057)
5 rfp 691 bp (–377, –1067)
6 rfp 376 bp (–1, –376)
7 rfp 398 bp (–377, –774)
8 rfp 293 bp (–775, –1067)
8 rfp 293 bp (–775, –1067)
9 rfp del 1 243 bp (–775, –1017)
10 rfp del 2 272 bp (deletion from –1017 to –997)
11 rfp del 3 243 bp (deletion from –996 to –947)
12 rfp del 4 243 bp (deletion from –946 to –897)
13 rfp del 5 243 bp (deletion from – 896 to –847)
14 rfp del 6 243 bp (deletion from –846 to –797)
15 rfp del 7 247 bp (–1067, –775)
16 rfp 1089 bp (+1512, +2600)
unc-64
unc-64 a
unc-64 b
Fosmid Reporter a
Fosmid Reporter b
Fosmid Reporter a/b
Epi, Mu, Int
Int
1232 bp (–5526, –4295)
712 bp (—3346, –2635)
966 bp (–2761, –1796)
757 bp (–1795, –1039)
382 bp (–1795, –1413)
375 bp (–1414 –1039)
1038 bp (–1038, -1)
1045 bp (+154, +1198)
462 bp (+737, +1198)
815 bp (+4420, +5526)
snn-1
snn-1 a
snn-1 b
col-109
Epi
1 rfp 1102 bp (–1, –1102)
2 rfp 413 bp (–1, –413)
3 rfp 231 bp (–1, –231)
4 rfp 1022 bp (+5582, +6603)
snt-1
F31E8.5
snt-1 a
snt-1 b
Epi, Mu
1033 bp (–3720, –2687)
876 (–2710, –1834)
672 bp (–772, –100)
794 bp (+111, +904)
1094 bp (+1019, +2113)
syd-2
F59F5.7
Ubq
1 rfp 1016 bp (–1, –1016)
2 rfp 537 bp (–1, –537)
unc-18
stn-2
unc-18 c
unc-18 a
unc-18 b
1119 bp (–1, –1119)

Figure S2

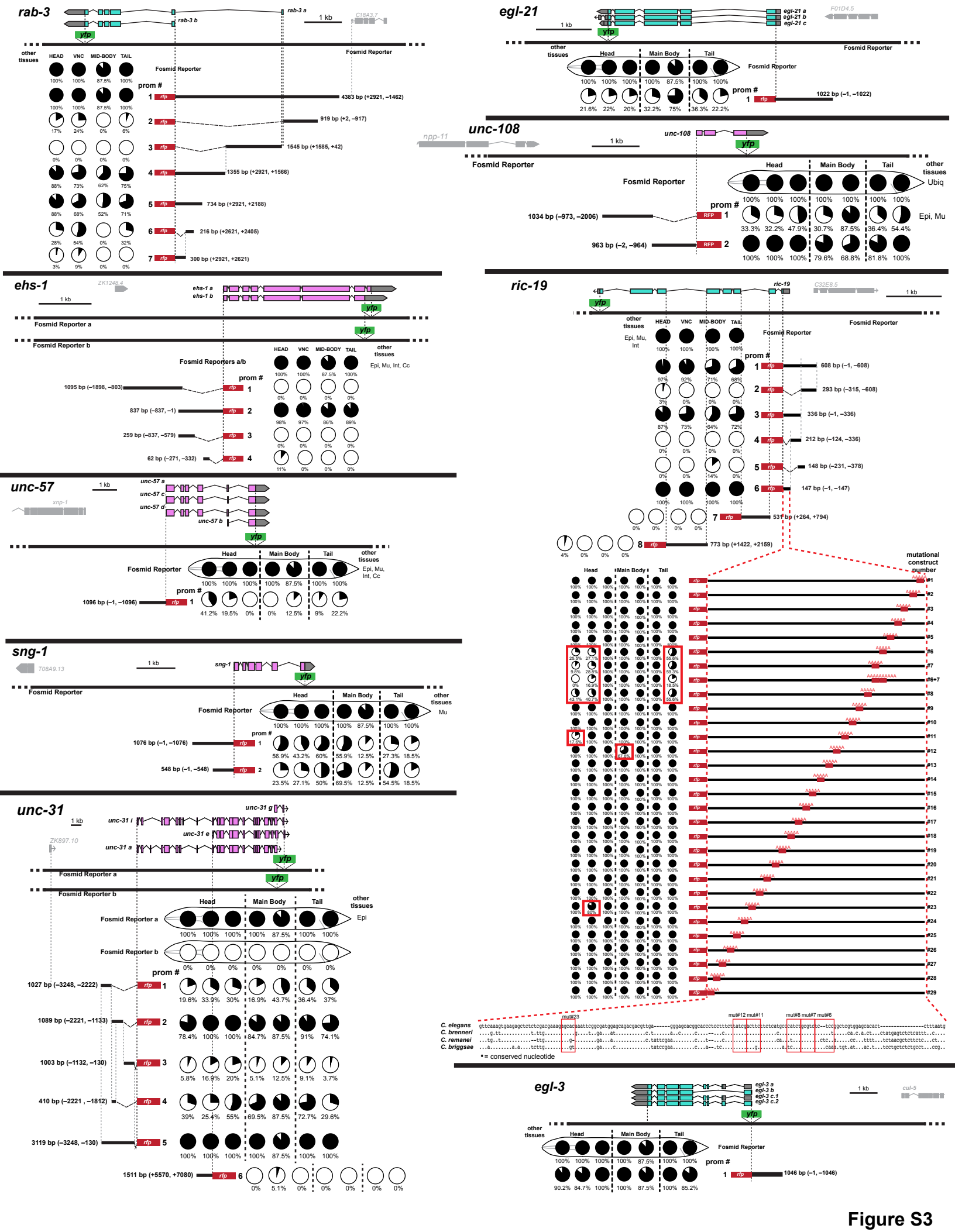
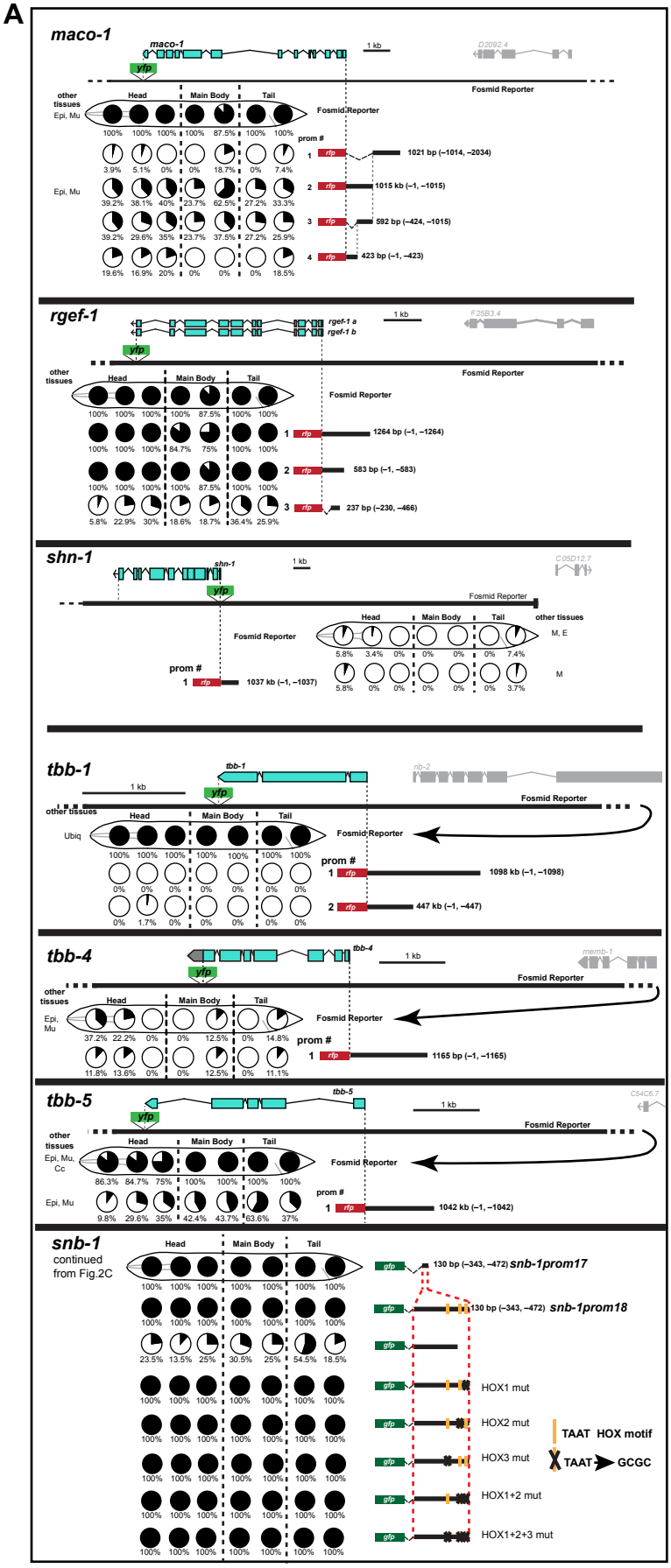
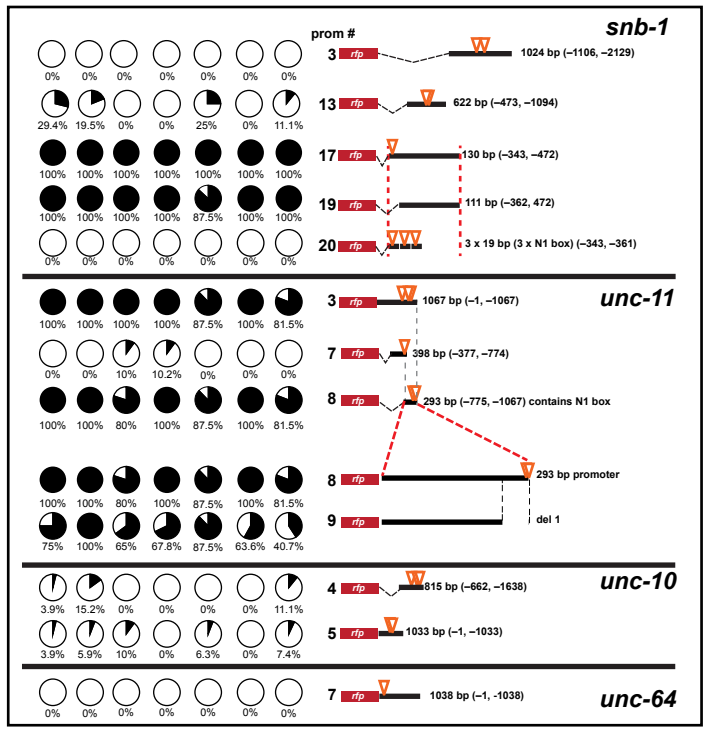

rab-3
egl-21
unc-108
ehs-1
ric-19
unc-57
sng-1
unc-31
egl-3
Fosmid Reporter
Head
Main Body
Tail
other tissues
prom #
mutational construct number
C. elegans
C. brenneri
C. remanei
C. briggsae
• = conserved nucleotide

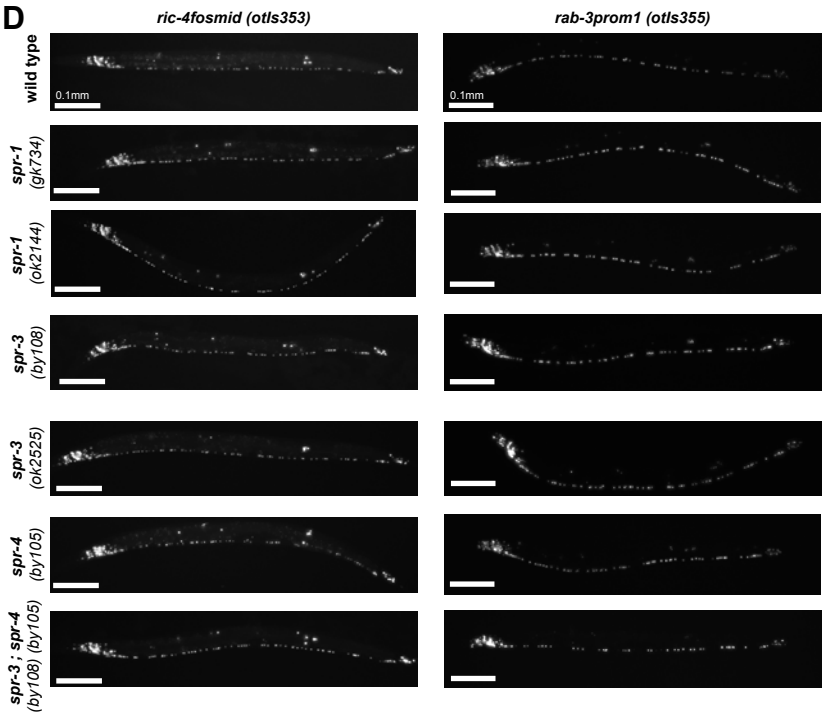
Figure S3





C

Rank	*C.elegans* Hit	E value	reciprocal best homolog
1	BLMP-1	7e-26	human PRDM1/BLIMP1
2	EOR-1	2e-25	human PLZF
3	Y55F3AM.14	7e-20	human ZNF658
4	F47E1.3	2e-19	human PRDM14
5	FEZF-1	2e-19	human FEZF-1
6	CHE-1	2e-18	Drosophila Glass



Supplemental Figure S4

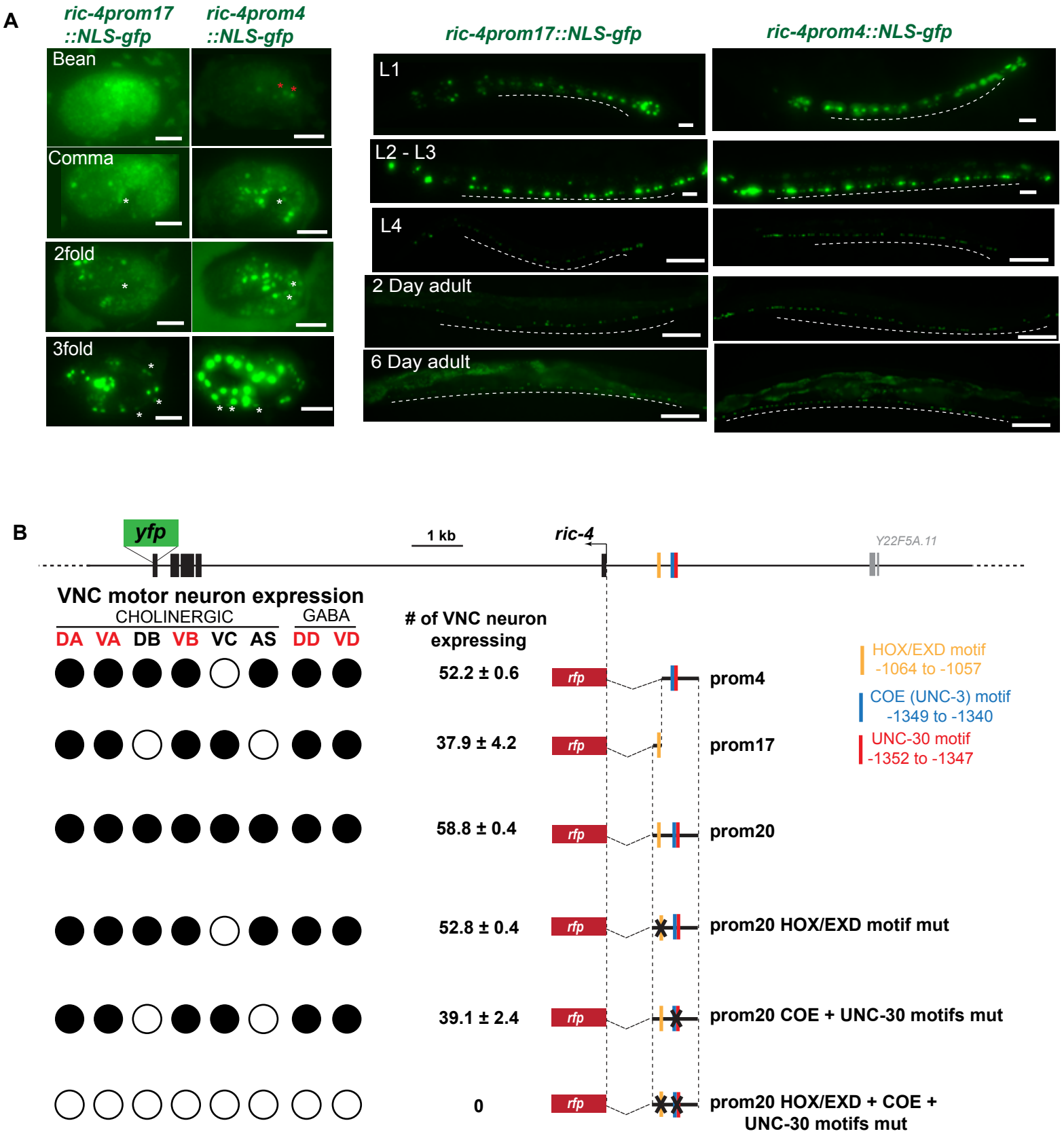

A
ric-4prom17 ::NLS-gfp
ric-4prom4 ::NLS-gfp
Bean
Comma
2fold
3fold
ric-4prom17::NLS-gfp
ric-4prom4::NLS-gfp
L1
L2 - L3
L4
2 Day adult
6 Day adult
B
yfp
1 kb
ric-4
Y22F5A.11
VNC motor neuron expression
CHOLINERGIC
GABA
DA VA DB VB VC AS DD VD
# of VNC neuron expressing
52.2 ± 0.6
37.9 ± 4.2
58.8 ± 0.4
52.8 ± 0.4
39.1 ± 2.4
0
rfp
prom4
prom17
prom20
prom20 HOX/EXD motif mut
prom20 COE + UNC-30 motifs mut
prom20 HOX/EXD + COE + UNC-30 motifs mut
HOX/EXD motif -1064 to -1057
COE (UNC-3) motif -1349 to -1340
UNC-30 motif -1352 to -1347

Figure S5

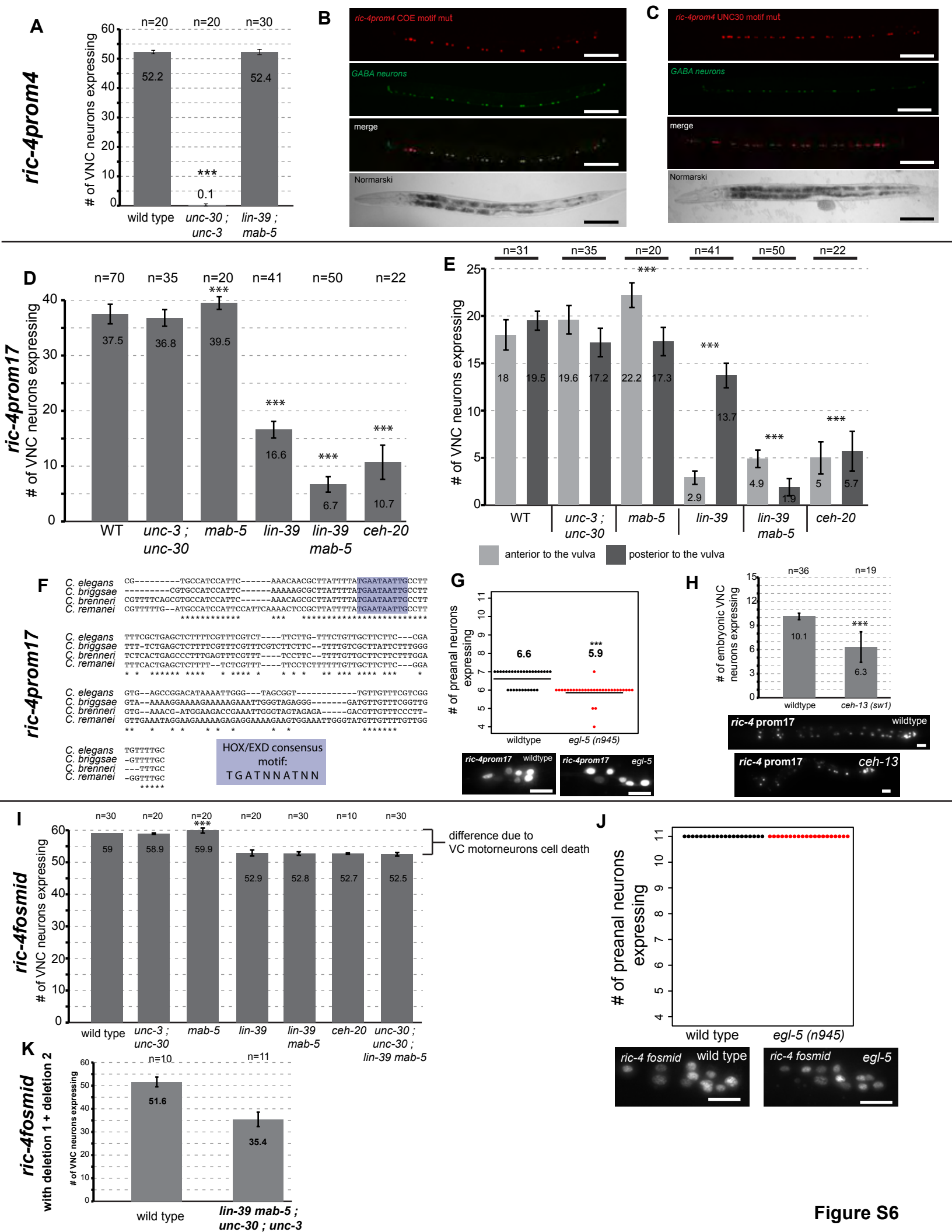

A
ric-4prom4
n=20
n=20
n=30
# of VNC neurons expressing
52.2
0.1
52.4
***
wild type
unc-30 ; unc-3
lin-39 ; mab-5
B
ric-4prom4 COE motif mut
GABA neurons
merge
Normarski
C
ric-4prom4 UNC30 motif mut
GABA neurons
merge
Normarski
D
ric-4prom17
n=70
n=35
n=20
n=41
n=50
n=22
# of VNC neurons expressing
37.5
36.8
39.5
16.6
6.7
10.7
***
WT
unc-3 ; unc-30
mab-5
lin-39
lin-39 mab-5
ceh-20
E
n=31
n=35
n=20
n=41
n=50
n=22
# of VNC neurons expressing
18
19.5
19.6
17.2
22.2
17.3
2.9
13.7
4.9
1.9
5
5.7
***
WT
unc-3 ; unc-30
mab-5
lin-39
lin-39 mab-5
ceh-20
anterior to the vulva
posterior to the vulva
F
ric-4prom17
C. elegans CG----------TGCCATCCATTC------AAACAACGCTTATTTTATGAATAATTGCCTT
C. briggsae ---------CGTGCCATCCATTC------AAAAAGCGCTTATTTTATGAATAATTGCCTT
C. brenneri CGTTTTCAGCGTGCCATCCATTC------AAACAGCGCTTATTTTATGAATAATTGCCTT
C. remanei CGTTTTTG--ATGCCATCCATTCCATTCAAAACTCCGCTTATTTTATGAATAATTGCCTT
C. elegans TTTCGCTGAGCTCTTTTCGTTTCGTCT----TTCTTG-TTTCTGTTGCTTCTTC---CGA
C. briggsae TTT-TCTGAGCTCTTTTCGTTTCGTTTCGTCTTCTTC--TTTTGTCGCTTATTCTTTGGG
C. brenneri TCTCACTGAGCCCTTTGAGTTTCGTTT----TCCTTC--TTTTGTTGCTTCTTCTTTGGA
C. remanei TTTCACTGAGCTCTTTT--TCTCGTTT----TTCCTCTTTTTTTGTTGCTTCTTC---GGA
C. elegans GTG--AGCCGGACATAAAATTGGG---TAGCGGT------------TGTTGTTTCGTCGG
C. briggsae GTA--AAAAGGAAAAGAAAAAGAAATTGGGTAGAGGG-------GATGTTGTTTCGGTTG
C. brenneri GTG--AAACG-ATGGAAGACCGAAATTGGGTAGTAGAGA-----GACGTTGTTTCCCTT-
C. remanei GTTGAAAATAGGAAGAAAAAGAGAGGAAAAGAAGTGGAAATTGGGTATGTTGTTTTGTTGG
C. elegans TGTTTTGC
C. briggsae -GTTTTGC
C. brenneri ---TTTGC
C. remanei -GGTTTGC
HOX/EXD consensus motif:
TGATNNATNN
G
# of preanal neurons expressing
6.6
5.9
***
wildtype
egl-5 (n945)
ric-4prom17 wildtype
ric-4prom17 egl-5
H
# of embryonic VNC neurons expressing
n=36
n=19
10.1
6.3
***
wildtype
ceh-13 (sw1)
ric-4 prom17 wildtype
ric-4 prom17 ceh-13
I
ric-4fosmid
# of VNC neurons expressing
n=30
n=20
n=20
n=20
n=30
n=10
n=30
59
58.9
59.9
52.9
52.8
52.7
52.5
***
difference due to VC motorneurons cell death
wild type
unc-3 ; unc-30
mab-5
lin-39
lin-39 mab-5
ceh-20
unc-30 ; unc-30 ; lin-39 mab-5
J
# of preanal neurons expressing
wild type
egl-5 (n945)
ric-4 fosmid wild type
ric-4 fosmid egl-5
K
ric-4fosmid
with deletion 1 + deletion 2
# of VNC neurons expressing
n=10
n=11
51.6
35.4
wild type
lin-39 mab-5 ; unc-30 ; unc-3

Figure S6

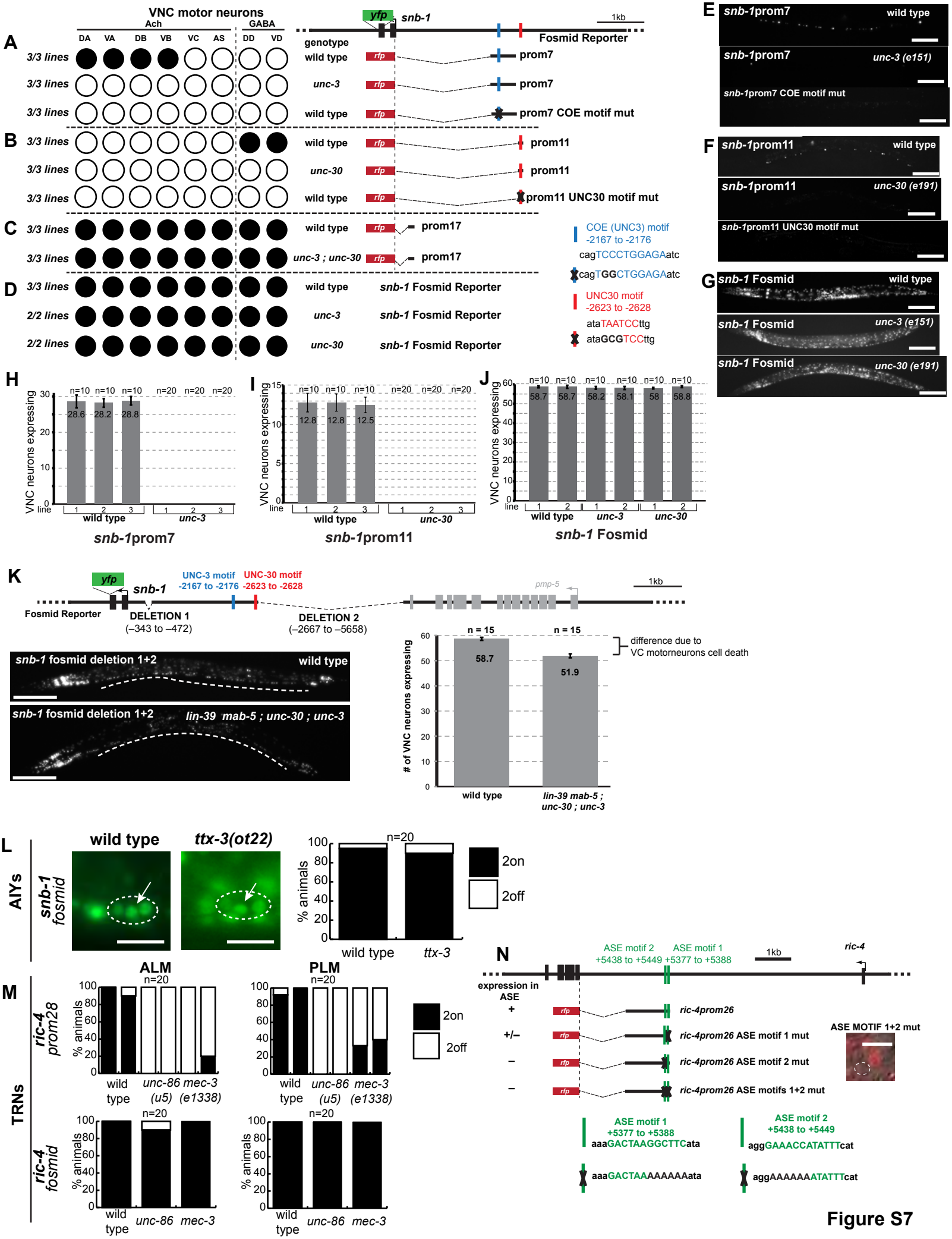

VNC motor neurons
Ach
GABA
DA VA DB VB VC AS DD VD
genotype
yfp
snb-1
1kb
Fosmid Reporter
A
3/3 lines
wild type
rfp
prom7
unc-3
prom7
prom7 COE motif mut
B
prom11
unc-30
prom11
prom11 UNC30 motif mut
C
prom17
unc-3 ; unc-30
D
2/2 lines
snb-1 Fosmid Reporter
COE (UNC3) motif
-2167 to -2176
cagTCCCTGGAGAatc
cagTGGCTGGAGAatc
UNC30 motif
-2623 to -2628
ataTAATCCttg
ataGCGTCCttg
E
snb-1prom7
wild type
unc-3 (e151)
snb-1prom7 COE motif mut
F
snb-1prom11
unc-30 (e191)
snb-1prom11 UNC30 motif mut
G
snb-1 Fosmid
H
VNC neurons expressing
n=10 n=10 n=10 n=20 n=20 n=20
28.6 28.2 28.8
line
wild type
unc-3
snb-1prom7
I
12.8 12.8 12.5
unc-30
snb-1prom11
J
58.7 58.7 58.2 58.1 58 58.8
snb-1 Fosmid
K
UNC-3 motif
-2167 to -2176
UNC-30 motif
-2623 to -2628
pmp-5
Fosmid Reporter
DELETION 1
(–343 to –472)
DELETION 2
(–2667 to –5658)
snb-1 fosmid deletion 1+2
wild type
lin-39 mab-5 ; unc-30 ; unc-3
n = 15
58.7
51.9
difference due to
VC motorneurons cell death
# of VNC neurons expressing
wild type
lin-39 mab-5 ;
unc-30 ; unc-3
L
AIYs
snb-1
fosmid
wild type
ttx-3(ot22)
% animals
n=20
2on
2off
ttx-3
M
TRNs
ric-4
prom28
ALM
PLM
wild type
unc-86 (u5)
mec-3 (e1338)
ric-4
fosmid
unc-86
mec-3
N
ASE motif 2
+5438 to +5449
ASE motif 1
+5377 to +5388
ric-4
expression in ASE
+
+/–
–
ric-4prom26
ric-4prom26 ASE motif 1 mut
ric-4prom26 ASE motif 2 mut
ric-4prom26 ASE motifs 1+2 mut
ASE MOTIF 1+2 mut
ASE motif 1
+5377 to +5388
aaaGACTAAGGCTTCata
ASE motif 2
+5438 to +5449
aggGAAACCATATTTcat
aaaGACTAAAAAAAAata
aggAAAAAAATATTTcat

Figure S7

SUPPLEMENTAL FIGURE LEGENDS

Figure S1, Related to Figure 1. Fosmid reporter constructs for the 26 genes of this study.

A: Schematic representations of the tagging cassette and the fosmid reporter constructs for all 26 genes. Our tagging cassette contains a NLS signal and a histone tag (H2B) for nuclear localization, a SL2 sequence for trans-splicing (Tursun et al., 2009), and intron containing YFP. After successful recombineering, a "FRT*" scar remains in one of the introns of the YFP. **B**: Fluorescent images of L1 stage worms and fosmid reporter schematics are shown for each gene. For *snb-1fosmid reporter* (**C**) and *unc-31fosmid* reporter (**D**) images of head neuron expression and intensity level variability are shown the same way as in **Fig.1D**. Scale bars are 0.01 mm.

Figure S2, Related to Figure 3. Modular Architecture of *Cis*-Regulatory Regions of Pan-neuronal Genes. *Cis*-regulatory analysis for *nsf-1*, *unc-10*, *unc-11*, *unc-18*, *unc-64*, *snn-1*, *snt-1*, *syd-2* is shown**. Fig.3** legend explains in detail how the scoring data are presented.

Figure S3, Related to Figure 3. Modular Architecture of *Cis*-Regulatory Regions of Pan-neuronal Genes. *Cis*-regulatory analysis for *rab-3, ehs-1, unc-57, sng-1, unc-31, egl-21, unc-108, ric-19, egl-3* is shown. **Fig.3** legend explains in detail how the scoring data are presented. 29 scanning substitution constructs were made to reveal the modularity of the *ric-19prom6* 147bp element that still drove pan-neuronal expression. Alignment of *ric-19prom6* sequence within four nematode species is also shown.

Figure S4, Related to Figure 3. Modular Architecture of *Cis*-Regulatory Regions of pan-neuronal genes and evidence against REST (model#1) and N1 box (model #2) as players in the regulation of pan-neuronal genes.

A: *Cis*-regulatory analysis for *maco-1, rgef-1, shn-1, tbb-1, tbb-4, tbb-5* and continued *snb-1* from Fig.3C. **B**: N1 box is not a master *cis*-regulatory element for pan-neuronal gene expression. Examples of the *cis*-regulatory analysis showing that the N1 box (Ruvinsky et al., 2007) is neither sufficient (***snb-1***: *prom3, prom13, prom17, prom20*, ***unc-11***: *prom3, prom7, prom8*, ***unc-10***: *prom4, prom5,* and ***unc-64****prom7*) nor required (***snb-1****prom19*, ***unc-11****prom9*) for broad neuronal expression, excluding its role as a *cis*-regulatory element that

binds a master regulator transcription factor for pan-neuronal gene expression. **C – D**: The *C. elegans* REST/NRSF functional homologues have no effect on pan-neuronal gene expression in *C. elegans*. **C**: *C.elegans* genes with highest similarity to vertebrate NRSF/REST. Shown are the results of a BLASTP with human NRSF/REST against the *C.elegans* genome. All the top hits are genes that are the orthologs of other genes; in addition, SPR-3, SPR-4 Zn finger proteins claimed to be REST homolog (Lakowski et al., 2003; Lu et al., 2014) do not pick up NRSF/REST as top hit. This indicates that in spite of previous claims (Lakowski et al., 2003; Lu et al., 2014), *C. elegans* may not contain a true NRSF/REST ortholog. However, SPR-3/4 do act similarly as SPR-1, the sole clear ortholog of vertebrate CoREST (Jarriault and Greenwald, 2002). The existence of CoREST in *C.elegans* is not indicative of the presence of an as yet unidentified NRSF/REST factor because CoREST is part of a broadly employed chromatin modifying complex (Laugesen and Helin, 2014). **D**: The *ric-4fosmid reporter* and *rab-3prom1* reporter show no derepression of expression in non-neuronal cell types when crossed into *spr-1*, *spr-3*, *spr-4* single mutant and *spr-3 ; spr-4* double mutant backgrounds. *ric-4*/SNAP25 is a confirmed target of REST/NRSF in vertebrates (Bruce et al., 2004). Fluorescent images of young adult *C. elegans* hermaphrodites are shown. Scale bars are 0.1 mm.

Figure S5, Related to Figure 5. *ric-4prom4* and *ric-prom17* act redundantly and produce coincident temporal expression in VNC MNs

A. Expression patterns of *ric-4prom4::2xNLS::GFP (otIs490)* and *ric-4prom17::2xNLS::GFP (otIs414)* transgenes in different stages from embryo through adulthood. For both transgenes VNC MN expression starts being detected at the comma - twofold stage (white asterisks) and stays on until adulthood. Red asterisks in the bean stage of *ric-4prom4* indicate early expression of the intestinal co-injection marker. Scale bars are 0.01 mm from bean stage to L2 – L3 stage, and 0.1 mm from L4 stage to 6 day adults.

B. *ric-4prom4* and *ric-4prom7* act redundantly in the context of a larger promoter (*ric-4prom20*) that contains both *cis*-regulatory elements. Expression of these two non-overlapping *cis*-regulatory elements overlaps in the DA, VA, VB cholinergic and DD, VD GABAergic VNC MNs (shown in red). Mutagenesis of the terminal selector motifs or HOX motif separately in the context of *ric-4prom20* a larger promoter (resulting from “stitching back together” *ric-4prom4* and *ric-4prom17)*, does not result in loss of expression in the VNC MNs expressing redundantly from both elements. Only when both terminal selector and HOX

motifs are both mutated expression is completely lost. Quantification is also shown as (average number of VNC MNs with expression) ± standard deviation.

Figure S6, Related to Figure 5. Terminal selectors act in parallel to HOX Genes to regulate *ric-4* expression in VNC MNs.

A – C: *ric-4prom4* expression in cholinergic and GABAergic VNC MNs depends on *unc-3* and *unc-30* but not on the *lin-39* and *mab-5* HOX genes. **A**: Quantification of data shown in (**Fig.5A, D, E)**. **B**: Upon mutation of the COE (UNC3) motif, *ric-4prom4* loses expression specifically in cholinergic VNC MNs but not in the GABAergic MNs. **C**: Upon mutation of the UNC-30 motif, *ric-4prom4* loses expression specifically in the GABAergic MNs, but not in the cholinergic MNs.

D – F: *ric-4prom17* expression in VNC MNs depends on HOX genes but not the *unc-3* and *unc-30* terminal selectors. **D**: Quantification of data shown in (**Fig.5B, G, H, I)**. **E**: same as in panel **D** but VNC MNs are separated into neurons anterior and posterior to the vulva to show the HOX positional specificity.

F: *ric-4prom17* sequence alignment among four nematode species and conservation of the HOX/EXD motif (blue box). **G – H**: The HOX genes *egl-5* and *ceh-13* also affect expression of *ric-4prom17*. **G**: *ric-4 prom17* expression in the preanal ganglion neurons is affected in the mutant background of the posteriorly expressed *egl-5* HOX gene. Data are presented in a dot-plot. Black line represents average number of neurons expressing in each genotype (6.6 in the wildtype and 5.9 in the *egl-5* mutant). **H**: *ric-4 prom17* expression in the VNC MNs neurons is affected in the mutant background of the *che-13* HOX gene. *ceh-13* null mutants are L1 larva lethal.

I – J: *ric-4 fosmid* reporter expression in VNC MNs is not affected in the *unc-3, unc-30,* HOX and quadruple mutant backgrounds. **I**: Quantification of data shown in (**Fig.5C, K – O)**. In *lin-39* and *ceh-20* mutants the VC neurons of the VNC are not generated. Therefore for the statistical analysis the average number of VNC MNs expressing the *ric-4fosmid* reporter in these mutants is compared to the total number of VNC neurons expressed in the wild type (59) minus the VC neurons (6), which is 53 VNC MNs. **J**: *ric-4fosmid* reporter expression in the preanal ganglion neurons is unaffected in the *egl-5* mutants.

K: Quantification of VNC MN expression of *ric-4fosmid* reporter carrying deletions of redundant VNC *cis*-regulatory elements shown in (**Fig.5T**). After crossing this deleted fosmid

reporter construct into a *lin-39 mab-5 ; unc-30 ; unc-3* quadruple mutant background, the number of VNC MNs still expressing the reporter is significantly reduced, but expression is still observed in ~60% of VNC MNs. This suggest the existence of at least three regulatory elements, other than the terminal selectors (*unc-3, unc-30*) and the *lin-39* and *mab-5* HOX genes, so at least 5 elements total, demonstrating the extreme number of parallel-acting *cis*-regulatory inputs for *ric-4* expression in the VNC MNs.
Error bars show standard deviation. Two-tailed student's t-test was used for statistical analysis. * = p<0.05, **=p<0.01, ***=p<0.001. Scale bars are 0.1 mm in **B** and **C** and 0.01 mm in **G**, **H**, and **J**.

Figure S7, Related to Figure 7. Terminal selectors affect pan-neuronal gene expression only in the context of isolated *cis*-regulatory elements but not in the context of the fosmid reporters.

A – J: Expression in VNC MNs of isolated *snb-1 cis*-regulatory regions depends on the terminal selectors *unc-3* and *unc-30*.

A: *snb-1prom7* expression in the A-type and B-type motor neurons is abolished in an *unc-3* mutant background or by mutagenesis of the conserved COE (UNC3) motif present in *prom7* in all 3 different transgenic lines tested. **B**: Similarly, *snb-1prom11* expression in the D-type motor neurons is abolished in an *unc-30* mutant background or by mutagenesis of the conserved UNC-30 motif present in *prom11*. **C**: In contrast *snb-1prom17* does not contain UNC3 and UNC-30 motifs and its expression in the VNC MNs does not depend on *unc-3* and *unc-30*. **D**: Expression of the *snb-1* fosmid reporter remains unaffected in the terminal selector mutant backgrounds. Fluorescent worm images (L4 / young adult stage) and quantification of this analysis is shown in **E** and **H** for *snb-1prom7*, **F** and **I** for *snb-1prom11* and in **G** and **J** for *snb-1fosmid* reporter.

K: Deletion of *snb-1prom17* (deletion 1) and *snb-1prom1* and *snb-1prom9* (deletion 2) (see **Fig.3C** for *snb-1prom* constructs), which in isolation produce VNC MN expression, does not affect *snb-1 fosmid* reporter in VNC MNs in both wildtype and quadruple *unc-3; unc-30; lin-39 mab-5* mutants. This argues for the existence of at least three more regulatory elements, other than the terminal selectors (*unc-3, unc-30*), so at least 4 elements total, demonstrating the extreme number of parallel-acting *cis*-regulatory inputs for *snb-1* expression in the VNC MNs.

L: *snb-1 fosmid* reporter expression in AIY is not affected in *ttx-3* (AIY terminal selector) mutants. **M**: *ric-4prom28*, but not *ric-4 fosmid*, expression in Touch Receptor Neurons (TRN) ALM and PLM depends on TRN terminal selector *unc-86*. Coordinates of *ric-4prom28*, (+6396, +6427), have been described before (Hwang and Lee, 2003). **N**: Expression of *ric-4prom26* in ASE depends on the terminal selector *che-1* (see **Fig.6D**). Mutational analysis of the two ASE motifs of this promoter recapitulate that result, suggesting that the *che-1* effect on the ASE expression of this reporter construct is direct.

Error bars show standard deviation. Two-tailed student's t-test was used for statistical analysis. * = $p<0.05$, **=$p<0.01$, ***=$p<0.001$. Scale bars are 0.1 mm in **E**, **F**, **G** and **K** and 0.01 mm in **L** and **N**.

SUPPLEMENTAL EXPERIMENTAL PROCEDURES

Strains and Transgenes

Wild type is strain N2, *C. elegans* variety Bristol. Strains were maintained by standard methods. Mutant alleles [strain name] used in this study: *unc-3 (e151)* [CB151], *unc-30 (e191)* [CB845], *lin-39 (n1760)* [MT4009], *mab-5 (e1239)* [CB3531], *lin-39 (n1760) mab-5 (e1239)* [MT7419], *ceh-13 (sw1)* [FR431], *egl-5 (n945)* [MT1975], *unc-10 (md1117)* [NM1657], *ehs-1 (ok146)* [NM1568], *mec-3 (e1338)* [DR1367], *unc-86 (n846)* [MT1859], *unc-86 (u5)*, *pha-1 (e2123)* [GE24], *ttx-3 (ot22)* [OH161], *lim-4 (ky403)* [CX3937], *ceh-36 (ky640)* [CX5922], *pag-3 (ls20)* [EA81], *ceh-14 (ch3)* [TB528], *che-1 (ot75)* [OH13098], *ceh-20 (ok541)* [VC447], *unc-42 (e270)* [CB270], *fax-1 (gm83)* [NG83], *spr-1 (gk734)* [VC1608], *spr-1 (ok2144)* [VC1815], *spr-3 (by108)* [LA59], *spr-3 (ok2525)* [RB1930], *spr-4 (by105)* [LA95].

The following transgenes were used for neuronal ID: *otIs388 [eat-4^{FOSMID}::SL2::NLS-YFP-H2B], otIs534 [cho-1^{FOSMID}::SL2::NLS-YFP-H2B (this study)], oxIs12 [unc-47::gfp], oyIs44 [odr-1::rfp], evIs82b [unc-129::gfp], wdIs3 [del-1::gfp], syIs80 [lin-11::gfp*].

Transgenes generated in this study

EXTRACHROMOSOMAL ARRAYS			
PROMOTER CONSTRUCTS			
Strain name	**Transgene name**	**Construct**	**Coordinates (in relation to the ATG)**
OH12070	*otEx5450*	*unc-64prom1*	-4295 -5526
OH12300	*otEx5565*	*unc-64prom2*	-3346 -2635
OH10500	*otEx4648*	*unc-64prom3*	-1796 -2761
OH10500	*otEx4647*	*unc-64prom4*	1039 -1795
OH10807	*otEx4851*	*unc-64prom5*	-1795 -1414
OH10806	*otEx4850*	*unc-64prom6*	-1413 -1039
OH10499	*otEx4646*	*unc-64prom7*	-1 -1038
OH10502	*otEx4649*	*unc-64prom8*	+154 +1198
OH12072	*otEx5452*	*unc-64prom9*	+737 +1198
OH12071	*otEx5451*	*unc-64prom10*	+4420 +5234
OH12080	*otEx5460*	*nsf-1prom1*	-1 -1145
OH12081	*otEx5461*	*nsf-1prom2*	+246 +1919
OH11557	*otEx5246*	*ric-4prom1*	–3774 –4958
OH11556	*otEx5244*	*ric-4prom2*	–2768 –3773
OH11557	*otEx5246*	*ric-4prom3*	–1724 –2740
OH11555	*otEx5244*	*ric-4prom4*	–1075 –1727
OH10505	*otEx4652*	*ric-4prom5*	–1 –1095

OH11427	*otEx5178*	*ric-4prom6*	–1 –1727
OH12074	*otEx5454*	*ric-4prom7*	–3449 –3773
OH12075	*otEx5455*	*ric-4prom8*	–3009 –3448
OH12076	*otEx5456*	*ric-4prom9*	–2748 –3008
OH12077	*otEx5457*	*ric-4prom10*	–4558 –4958
OH12078	*otEx5458*	*ric-4prom11*	–4187 –4535
OH12079	*otEx5459*	*ric-4prom12*	–3774 –4186
OH13189	*otEx6082*	*ric-4prom13*	–1075 –1538
OH13190	*otEx6083*	*ric-4prom14*	–1 –601
OH11424	*otEx5177*	*ric-4prom15*	–634 –1095
OH13191	*otEx6084*	*ric-4prom16*	–634 –939
OH11418	*otEx5176*	*ric-4prom17*	–948 –1095
OH12073	*otEx5453*	*ric-4prom18*	–996 –1095
OH13193	*otEx6086*	*ric-4prom19*	–948 –1063
OH13192	*otEx6085*	*ric-4prom20*	–948 –1727
OH11558	*otEx5247*	*ric-4prom21*	+73 +1083
OH12363	*otEx5585*	*ric-4prom22*	+690 +2278
OH11559	*otEx5248*	*ric-4prom23*	+2259 +3263
OH11560	*otEx5249*	*ric-4prom24*	+3267 +4309
OH11561	*otEx5250*	*ric-4prom25*	+4310 +5317
OH11562	*otEx5251*	*ric-4prom26*	+5362 +6592
OH10506	*otEx4653*	*ric-4prom27*	+6594 +7621
OH11433	*otEx5182*	*snb-1prom1*	–2995 –5658
OH10518	*otEx4665*	*snb-1prom2*	–2129 –3007
OH10517	*otEx4664*	*snb-1prom3*	–1106 –2129
OH10515	*otEx4662*	*snb-1prom4*	–1 –1094
OH10519	*otEx4666*	*snb-1prom5*	–2131 –2444
OH13194	*otEx6087*	*snb-1prom6*	–2013 –2444
OH11434	*otEx5183*	*snb-1prom7*	–1906 –2444
OH10809	*otEx4853*	*snb-1prom8*	–2444 –3007
OH10813	*otEx4857*	*snb-1prom9*	–2667 –3007
OH10812	*otEx4856*	*snb-1prom10*	–2444 –2666
OH12086	*otEx5466*	*snb-1prom11*	–2557 –2666
OH10488	*otEx4635*	*snb-1prom12*	–1 –472
OH10521	*otEx4668*	*snb-1prom13*	–473 –1094
OH10522	*otEx4669*	*snb-1prom14*	–1 –173
OH10523	*otEx4670*	*snb-1prom15*	–174 –472
OH10525	*otEx4672*	*snb-1prom16*	–174 –342
OH10524	*otEx4671*	*snb-1prom17*	–343 –472
OH10811	*otEx4855*	*snb-1prom18*	–343 –446
OH10526	*otEx4673*	*snb-1prom19*	–362 –472
OH10810	*otEx4854*	*snb-1prom20*	3x (–343 –361)
OH11563	*otEx5252*	*snb-1prom21*	29bp deletion (–343 –472)
OH11567	*otEx5256*	*snb-1prom22*	–343 –472 see Fig 2C
OH11568	*otEx5257*	*snb-1prom23*	–343 –472 see Fig 2C
OH11569	*otEx5258*	*snb-1prom24*	–343 –472 see Fig 2C
OH11570	*otEx5259*	*snb-1prom25*	–343 –472 see Fig 2C
OH11571	*otEx5260*	*snb-1prom26*	–343 –472 see Fig 2C
OH11572	*otEx5261*	*snb-1prom27*	–343 –472 see Fig 2C
OH12088	*otEx5468*	*snb-1prom28*	–378 –406
OH12089	*otEx5469*	*snb-1prom29*	3x(–378, –406)
OH11564	*otEx5253*	*snb-1prom30*	–174 –472
OH11565	*otEx5254*	*snb-1prom31*	–1 –1094
OH10482	*otEx4629*	*unc-10prom1*	–4196 –5852
OH10483	*otEx4630*	*unc-10prom2*	–2743 –4200
OH13195	*otEx6088*	*unc-10prom3*	–1637 –2745

OH13196	*otEx6089*	*unc-10prom4*	–662 –1638
OH10480	*otEx4627*	*unc-10prom5*	–1 –1033
OH12299	*otEx5564*	*unc-10prom6*	–1287 –1638
OH10481	*otEx4628*	*unc-10prom7*	–1034 –1286
OH10801	*otEx4845*	*unc-10prom8*	–4955 –5852
OH10802	*otEx4846*	*unc-10prom9*	–4196 –4931
OH10803	*otEx4847*	*unc-10prom10*	–4565 –4931
OH10804	*otEx4848*	*unc-10prom11*	–4196 -4564
OH10484	*otEx4631*	*unc-11prom1*	–1 –2173
OH10485	*otEx4632*	*unc-11prom2*	–1068, –2173
OH10487	*otEx4634*	*unc-11prom3*	–1, –1067
OH10486	*otEx4633*	*unc-11prom4*	–1, –1057
OH10489	*otEx4636*	*unc-11prom5*	–377 –1067
OH10488	*otEx4635*	*unc-11prom6*	–1 –376
OH10491	*otEx4638*	*unc-11prom7*	–377 –774
OH10490	*otEx4637*	*unc-11prom8*	–775 –1067
OH11442	*otEx5190*	*unc-11prom9*	del 1 –775 –1017
OH11443	*otEx5191*	*unc-11prom10*	del 2 deletion from –1017 to –997
OH11444	*otEx5192*	*unc-11prom11*	del 3 deletion from –996 to –947
OH11445	*otEx5193*	*unc-11prom12*	del 4 deletion from –946 to –897
OH11446	*otEx5194*	*unc-11prom13*	del 5 deletion from – 896 to –847
OH11447	*otEx5195*	*unc-11prom14*	del 6 deletion from –846 to –797
OH11448	*otEx5196*	*unc-11prom15*	del 7 –1067 –775
OH10534	*otEx4680*	*unc-11prom16*	+1512 +2600
OH10507	*otEx4654*	*snn-1prom1*	–1 –1102
OH13197	*otEx6090*	*snn-1prom2*	–1 –413
OH13198	*otEx6091*	*snn-1prom3*	–1 –231
OH10508	*otEx4655*	*snn-1prom4*	+5582 +6603
OH13199	*otEx6092*	*unc-104prom1*	–4206 –2809
OH13200	*otEx6093*	*unc-104prom2*	–2807 –1902
OH10808	*otEx4852*	*unc-104prom3*	–1984 –884
OH10503	*otEx4650*	*unc-104prom4*	–1084 –1
OH12360	*otEx5582*	*unc-104prom5*	+1260 +2860
OH12362	*otEx5584*	*unc-104prom6*	+4627 –5766
OH10498	*otEx4645*	*unc-31prom1*	–3248 –2222
OH10497	*otEx4644*	*unc-31prom2*	–2221 –1133
OH10495	*otEx4642*	*unc-31prom3*	–1132 –130
OH10805	*otEx4849*	*unc-31prom4*	–2221 –1812
OH10496	*otEx4643*	*unc-31prom5*	–3248 –130
OH12364	*otEx5586*	*unc-31prom6*	+5570 +7080
OH13201	*otEx6094*	*egl-3prom1*	–1 –1046
OH13202	*otEx6095*	*egl-21prom1*	–1 –1022
OH13203	*otEx6096*	*maco-1prom1*	–1014 –2034
OH10817	*otEx4858*	*maco-1prom2*	–1 –1015
OH12301	*otEx5566*	*maco-1prom3*	–424 –1015
OH12302	*otEx5567*	*maco-1prom4*	–1 –423
OH10509	*otEx4656*	*shn-1prom1*	–1 –1037
OH10510	*otEx4657*	*tbb-1prom1*	–1 –1098
OH13204	*otEx6097*	*tbb-1prom2*	–1 –447
OH13205	*otEx6098*	*tbb-2prom1*	–1179 –2513
OH10511	*otEx4658*	*tbb-2prom2*	–1 –1110

OH13206	*otEx6099*	*tbb-4prom1*	–1 –1165
OH10512	*otEx4659*	*tbb-5prom1*	–1 –1042
OH10513	*otEx4660*	*tbb-6prom1*	–1060 –1
OH13603	*otEx6315*	*unc-108prom1*	–973 –2006
OH12908	*otEx5944*	*unc-108prom2*	–2 –964
OH13225	*otEx6118*	*unc-18prom1*	–1 –1119
OH13224	*otEx6117*	*unc-57prom1*	–1 –1096
OH13226	*otEx6119*	*syd-2prom1*	–1 –1016
OH13227	*otEx6120*	*syd-2prom2*	–1 –537
OH13259	*otEx6145*	*rab-3prom2*	+2 –917
OH12919	*otEx5955*	*rab-3prom3*	+1585 +42
OH12916	*otEx5952*	*rab-3prom4*	+2921 +1566
OH13262	*otEx6148*	*rab-3prom5*	+2921 +2188
OH13265	*otEx6151*	*rab-3prom6*	+2380 +2167
OH13268	*otEx6154*	*rab-3prom7*	+2921 +2559
OH13271	*otEx6157*	*ehs-1prom2*	–837 –1
OH13274	*otEx6160*	*ehs-1prom4*	–271 –332
OH13277	*otEx6163*	*ric-19prom1*	–1 –608
OH13283	*otEx6169*	*ric-19prom2*	–315 –608
OH13280	*otEx6166*	*ric-19prom3*	–1 –336
OH13284	*otEx6170*	*ric-19prom5*	–231 –378
OH13287	*otEx6173*	*ric-19prom6*	–1 –147
OH13331	*otEx6216*	*ric-19prom7*	+264 +794
OH13332	*otEx6217*	*ric-19prom8*	+1422 +2159
OH13294	*otEx6180*	*ric-19prom6del1*	-147 to -143 AAAAA
OH13295	*otEx6181*	*ric-19prom6del2*	-142 to -138 AAAAA
OH13296	*otEx6182*	*ric-19prom6del3*	-137 to -133 AAAAA
OH13297	*otEx6183*	*ric-19prom6del4*	-132 to -128 AAAAA
OH13298	*otEx6184*	*ric-19prom6del5*	-127 to -123 AAAAA
OH13299	*otEx6185*	*ric-19prom6del6*	-122 to -118 AAAAA
OH13300	*otEx6186*	*ric-19prom6del7*	-117 to -113 AAAAA
OH13301	*otEx6187*	*ric-19prom6del8*	-112 to -108 AAAAA
OH13302	*otEx6188*	*ric-19prom6del9*	-107 to -103 AAAAA
OH13303	*otEx6189*	*ric-19prom6del10*	-102 to -98 AAAAA
OH13304	*otEx6190*	*ric-19prom6del11*	-97 to -93 AAAAA
OH13305	*otEx6191*	*ric-19prom6del12*	-92 to -88 AAAAA
OH13306	*otEx6192*	*ric-19prom6del13*	-87 to -83 AAAAA
OH13307	*otEx6193*	*ric-19prom6del14*	-82 to -78 AAAAA
OH13308	*otEx6194*	*ric-19prom6del15*	-77 to -73 AAAAA
OH13309	*otEx6195*	*ric-19prom6del16*	-72 to -68 AAAAA
OH13310	*otEx6196*	*ric-19prom6del17*	-67 to -63 AAAAA
OH13311	*otEx6197*	*ric-19prom6del18*	-62 to -58 AAAAA
OH13312	*otEx6198*	*ric-19prom6del19*	-57 to -53 AAAAA
OH13313	*otEx6199*	*ric-19prom6del20*	-52 to -48 AAAAA
OH13314	*otEx6200*	*ric-19prom6del21*	-47 to -43 AAAAA
OH13315	*otEx6201*	*ric-19prom6del22*	-42 to -38 AAAAA
OH13316	*otEx6202*	*ric-19prom6del23*	-37 to -33 AAAAA
OH13317	*otEx6203*	*ric-19prom6del24*	-32 to -28 AAAAA
OH13318	*otEx6204*	*ric-19prom6del25*	-27 to -23 AAAAA
OH13319	*otEx6205*	*ric-19prom6del26*	-22 to -18 AAAAA
OH13320	*otEx6206*	*ric-19prom6del27*	-17 to -13 AAAAA
OH13321	*otEx6207*	*ric-19prom6del28*	-12 to -8 AAAAA
OH13322	*otEx6208*	*ric-19prom6del29*	-7 to -3 AAAAA
OH13325	*otEx6211*	*ric-19prom6del6+del7*	
OH12909	*otEx5945*	*snt-1prom1*	–3720 –2687
OH12901	*otEx5937*	*snt-1prom2*	–2710 –1834

OH12896	*otEx5932*	*snt-1prom3*	–772 –100
OH12911	*otEx5947*	*snt-1prom4*	+111 +904
OH12922	*otEx5958*	*snt-1prom5*	+1019 +2113
OH13247	*otEx6133*	*rgef-1prom1*	–1 –1264
OH13250	*otEx6136*	*rgef-1prom2*	–1 –583
OH13253	*otEx6139*	*rgef-1prom3*	–230 –466
OH13256	*otEx6142*	*sng-1prom1*	–1 –1076
OH12939	*otEx5974*	*sng-1prom2*	–1 –548
PROMOTER CONSTRUCTS CARRYING MUTAGENIZED BINDING MOTIFS			
Strain name	**Transgene name**	**Construct**	
OH12082	*otEx5462*	*ric-4 prom4 COEmut*	
OH12083	*otEx5463*	*ric-4prom4 UNC30mut*	
OH11429	*otEx5180*	*ric-4prom4 COE+UNC30mut*	
OH13208	*otEx6101*	*ric-4prom26 ASE1mut*	
OH13209	*otEx6102*	*ric-4prom26 ASE2mut*	
OH13210	*otEx6103*	*ric-4prom26 ASE1+2mut*	
OH13212	*otEx6105*	*ric-4prom17 HOXmutA*	
OH13214	*otEx6107*	*ric-4prom17 HOXmutB*	
OH13215	*otEx6108*	*ric-4prom17 HOXmutC*	
OH11441	*otEx5189*	*snb-1prom7 COEmut*	
OH13211	*otEx6104*	*snb-1prom11 UNC30mut*	
OH13216	*otEx6109*	*snb-1prom17 HOX1mut*	
OH13217	*otEx6110*	*snb-1prom17 HOX2mut*	
OH13218	*otEx6111*	*snb-1prom17 HOX3mut*	
OH13219	*otEx6112*	*snb-1prom17 HOX1+2mut*	
OH13220	*otEx6113*	*snb-1prom17 HOX1+2+3mut*	
FOSMID REPORTERS			
Strain name	**Transgene name**	**Construct**	
OH10251	*otEx4556*	*ric-4fosmid A*	
OH11575	*otEx5264*	*ric-4fosmid B*	
OH11576	*otEx5265*	*ric-4fosmid C*	
OH10246	*otEx4551*	*unc-10fosmid isoform a*	
OH10245	*otEx4550*	*unc-10fosmid isoform b*	
OH10247	*otEx4552*	*unc-11fosmid*	
OH11411	*otEx5174*	*unc-31fosmid*	
OH10249	*otEx4553*	*unc-64fosmid isoform a*	
OH10248	*otEx4553*	*unc-64fosmid isoform b*	
OH10250	*otEx4555*	*unc-104fosmid*	
OH10253	*otEx4558*	*snb-1fosmid*	
OH10535	*otEx4681*	*snn-1fosmid*	
OH10243	*otEx4548*	*tbb-1fosmid*	
OH10244	*otEx4549*	*tbb-5fosmid*	
OH11410	*otEx5173*	*nsf-1fosmid*	
OH11412	*otEx5175*	*maco-1fosmid*	
OH10252	*otEx4557*	*shn-1fosmid (co-injected with linearized pRF4 (rol-6) marker)*	
OH12883	*otEx5920*	*rab-3fosmid*	
OH13237	*otEx6123*	*sng-1fosmid*	
OH12888	*otEx5924*	*snt-1fosmid*	
OH13239	*otEx6125*	*ehs-1fosmid isoform a*	
OH13238	*otEx6124*	*ehs-1fosmid isoform b*	
OH12849	*otEx5886*	*unc-57fosmid*	
OH12889	*otEx5925*	*unc-18fosmid isoform a*	
OH12884	*otEx5921*	*syd-2fosmid*	

OH12857	*otEx5894*	*egl-3fosmid*	
OH12860	*otEx5897*	*egl-21fosmid*	
OH13290	*otEx6176*	*ric-19fosmid*	
OH13207	*otEx6100*	*unc-108fosmid*	
OH12867	*otEx5904*	*rgef-1fosmid*	
OH12863	*otEx5900*	*tbb-4fosmid*	
FOSMID REPORTERS CARRYING MUTAGENIZED SEQUENCES			
Strain name	**Transgene name**	**Construct**	
OH13172	*otEx6066*	*cho-1fosmid COEmut*	
OH13221	*otEx6114*	*cho-1fosmid COEmut control*	
OH13222	*otEx6115*	*cho-1fosmid TTX3mut*	
OH13241	*otEx6127*	*eat-4fosmid UNC86mut*	
OH12872	*otEx5909*	*gcy-5fosmid ASEmut*	
OH13223	*otEx6116*	*ric-4fosmid del1+2*	
INTEGRATED TRANSGENES			
Strain name	**Transgene name**	**Construct**	**Co-injection marker (chromosome if known)**
OH10684	*otIs350*	*ric-4fosmid A – pBALU23*	*pha-1*
OH10686	*otIs352*	*ric-4fosmid A – pBALU23*	*pha-1*
OH10687	*otIs353*	*ric-4fosmid A – pBALU23*	*pha-1*
OH10688	*otIs354*	*ric-4fosmid A – pBALU23*	*pha-1*
OH12541	*otIs532*	*cho-1fosmid – pBALU23*	*–*
OH12542	*otIs533*	*cho-1fosmid – pBALU23*	*–*
OH12543	*otIs534*	*cho-1fosmid – pBALU23*	*–*
OH12544	*otIs535*	*cho-1fosmid – pBALU23*	*– (X)*
OH12545	*otIs536*	*cho-1fosmid – pBALU23*	*– (X)*
OH12546	*otIs537*	*cho-1fosmid – pBALU23*	*–*
OH12547	*otIs538*	*cho-1fosmid – pBALU23*	*–*
OH11344	*otIs414*	*ric-4prom17 – NLS::GFP*	*pha-1*, *elt-2::DsRed2*
OH11417	*otIs420*	*ric-4prom17 – NLS::GFP*	*pha-1*, *elt-2::DsRed2*
OH11419	*otIs421*	*ric-4prom17 – NLS::GFP*	*pha-1*, *elt-2::DsRed2*
OH11420	*otIs422*	*ric-4prom17 – NLS::GFP*	*pha-1*, *ttx-3::mCherry*
OH11421	*otIs423*	*ric-4prom17 – NLS::GFP*	*pha-1*, *ttx-3::mCherry (X)*
OH11422	*otIs424*	*ric-4prom17 – NLS::GFP*	*pha-1*, *ttx-3::mCherry*
OH11416	*otIs419*	*snb-1prom17 – NLS::GFP*	*pha-1*, *elt-2::DsRed2*
OH12313	*otIs489*	*ric-4prom4 – NLS::GFP*	*elt-2::DsRed2 (X)*
OH12314	*otIs490*	*ric-4prom4 – NLS::GFP*	*elt-2::DsRed2 (IV)*
OH9545	*otIs287*	*rab-3prom1 – NLS::YFP*	*rol-6 (IV)*
OH9609	*otIs291*	*rab-3prom1 – NLS::YFP*	*rol-6 (V)*
OH10689	*otIs355*	*rab-3prom1 – NLS::TagRFP*	*– (IV)*
OH10690	*otIs356*	*rab-3prom1 – NLS::TagRFP*	*– (V)*
OH11061	*otIs380*	*ric-19prom6 – NLS::GFP*	*elt-2::DsRed2*
OH11062	*otIs381*	*ric-19prom6 – NLS::GFP*	*elt-2::DsRed2 (V)*
OH12410	*otIs501*	*ehs-1prom4 – NLS::GFP*	*rps-5::Dsred2*

Generation of fosmid reporters

All fosmid reporter constructs were generated using λ-Red-mediated recombineering in bacteria as previously described (Tursun et al., 2009).

To generate a *snn-1* fosmid reporter, a fosmid extension protocol was used (as previously described (Tursun et al., 2009))**.** Fosmid WRM0629aH04 containing pan-neuronal gene *snn-1* was extended by 3306 bp to include the first exon of *snn-1* and 2748 bp of genomic sequence just upstream of the ATG of *snn-1* (covering the entire upstream intergenic region, shown as a red line in **Fig.S1**).

Deletions of the terminal selector binding sites for the *cho-1*, *gcy-5* and *eat-4* fosmids were done by replacement of the binding site by FRT or FRT* depending on which pBALU was used for reporter tagging. For the mutagenesis of COE motif in the *cho-1* fosmid a FRT scar was placed 23 base pairs upstream of the mutated COE motif. A control *cho-1* fosmid containing the FRT in the same place but the wild type COE motif was also made. Deletions 1 and 2 in the *ric-4 fosmid* reporter (**Fig.5T**) were done by replacement of the corresponding fosmid sequence by Lox2272 and FRT respectively. Deletions of prom28, prom17 and prom1+prom9 in the *snb-1* fosmid reporter (**Fig.2C and Fig.S7K**) were done by replacement of the corresponding fosmid sequence by FRT, Lox2272 and FRT respectively. The fosmid clones, primer sequences for tagging and mutagenesis and the sequences of the recombineering cassettes made and used in this study are provided in the Supplementary Methods. All fosmid constructs were linearized using SdaI or NotI and injected as complex extrachromosomal arrays in a *pha-1 (e2123)* mutant background strain (Granato et al., 1994) in the following concentrations: linearized fosmid construct 15 ng/µL, linearized *pha-1* rescuing plasmid pBX 2.5 ng/µL, sonicated OP50 bacterial genomic DNA 100 ng/µL (apart from *shn-1* fosmid that was injected in N2 background using as co-injection marker linearized pRF4 (*rol-6*) at 2.5ng/µL instead of *pha-1* rescuing plasmid pBX). Integrated fosmid reporters *ric-4^{Fosmid}::SL2::NLS-YFP-H2B* transgenes (*otIs350, otIs352, otIs353, otIs354*), and integrated *cho-1^{Fosmid}::SL2::NLS-YFP-H2B* transgenes (*otIs532 - otIs538*), were generated by gamma irradiation and outcrossed four to six times.

Fosmid reporters were generated using the recombineering cassettes pBALU23 and pBALUNI that we created for the purposes of this study. The following table contains information of the fosmid clones and primers used to create each fosmid reporter. Sequences

of the recombineering cassettes are provided below. pBALU23 was used for all fosmid reporters except for fosmid reporters *ric-4* B and *ric-4* C marked with (*), where pBALUNI was used instead to tag the different *ric-4* isoforms at the 5' end of the transcript.

GENE	FOSMID	PRIMERS FOR RECOMBINEERING SL2 H2B (*NLS)
nsf-1	WRM062aD09	5'CCGACACTTCTTAATATGATGGAAGGTCTTGCTCTAAACTTGTA CCGT**TAA**GCTGTCTCATCCTACTTTCAC3' 5'GAATAAATTTCTTAGCTTTTAGAGTTCTATGTTCAACTGAAGAG CTACTTCTACTTGCTGGAAGTGTACTTGG3'
rab-3	WRM0636dH02	5'CTCGAAGCGAATCCGACCCAAAAGCCTGCTCAACAGCAATGC AATTGC**TAA**GCTGTCTCATCCTACTTTCAC3' 5'GAATTTGGGAAAATTTTGAGTTTTTATAGATAGTATAATAGAAC GTAGAATTTCTACTTGCTGGAAGTGTACTTGG**3'**
ric-4 (reporter A Fig 2B)	WRM0640cB04	5'GTCCGTGTGGAATCTGCTAACAAGCGTGCGAAGAATCTCATCA CAAAA**TAA**GCTGTCTCATCCTACTTTCAC3' 5'GGAAAATGGAATGGTAAGATGAGACACAGAGAGTTTTGAAAAC ATTAAAATACTACTTGCTGGAAGTGTACTTGG3'
*ric-4 (reporter B Fig 2B)**	WRM0640cB04	5'CATAAAATTTATTTTTCAGGTTCTGTGAACGGTCAGACAACAAG CAATAATGACCGCTCCAAAGAAGAAACGCA3' 5'TTGAGGTTGATAGCCTCAAGACCCTCTGGAATATCATCATCTC CTGACATTGAATTAAAATTAGAAGTTGAAAATAC 3'
*ric-4 (reporter C Fig 2B)**	WRM0640cB04	5'ATCGTTTCCCCGTGTAGCCGGGGCGGTTTTGAAAGTTTGAAAA AACGGGGATGACCGCTCCAAAGAAGAAACGCA3' 3'TACGGACGGGGATGCCGTTGGCCGCCGGGAGCGCCGCGCCG AGCCGACATTGAATTAAAATTAGAAGTTGAAAA3'
snb-1	WRM065cH10	5'ATCGTCGTCATTCTTATTATCATCATCGTTTTATGGGCTGGAGG AAAA**TAA**GCTGTCTCATCCTACTTTCAC3' 5'GAAATTACAGCGTTTCGGGGGATTTTTTTATCCGGGACAAAGG TCGTGTACCTACTTGCTGGAAGTGTACTTGG**3'**
unc-64 (a)	WRM0634cD04	5'CTAATCGGCTTCGTTTCTCTGTGGCTCATTCAGTATATTCCTGG CATT**TAA**GCTGTCTCATCCTACTTTCAC3' 5'GAAATTTTGAAATTTTTTTAGCGTGGGCGTTTAGACGGGGAAA CAAATGGGCCTACTTGCTGGAAGTGTACTTGG3'
unc-64 (b)	WRM0634cD04	5'CTCATCACTGGCCTAATTATTTTTATTTTGTTTTATGCGAAAGTA TTA**TAA**GCTGTCTCATCCTACTTTCAC3' 5'CAATTTTTTCATTTTGGTTAAGTTTTTCTTTTCGTCGAGATTTGA AAAAATCTACTTGCTGGAAGTGTACTTGG3'
sng-1	WRM0611aF10	5'CAACAACCACCATCAAACCCATACACTCAGTCGGAAGGATATG GTTAT**TAG**GCTGTCTCATCCTACTTTCAC3' 5'TACAAGTGCTCAGGTCCATTTGTATGTGTTTTTTTTTGTTTGGC TAAAAAACTACTTGCTGGAAGTGTACTTGG3'
snt-1	WRM0630cA09	5'ACACTTGGACCAGTTGAAGAAGAAGGTGATAAGAAAGATGATA AGAAA**TAA**GCTGTCTCATCCTACTTTCAC3' 5'TTTAGATTGAATAAGGCATTTAAATTTAAAAAAAATGATAAAGTC

		ATACATCTACTTGCTGGAAGTGTACTTGG3’
unc-10 (a)	WRM0630dC05	5’GTCCAGTTCGAAAAGATTCCGATGTATCAGTTGGAGGTGCTCA GCAG**TAA**GCTGTCTCATCCTACTTTCAC3’ 5’ATGACCTGTGGTAATTAAGACAAAAAACAAACAAAAACATATGA AATTTGCTACTTGCTGGAAGTGTACTTGG3’
unc-10 (b)	WRM0630dC05	5’CTTCGTGTTTTTGAGTGACGTAATTTTCAGTTGGCTTCCAGTGC TCGC**TGA**GCTGTCTCATCCTACTTTCAC3’ 5’CGACAACCTGACCCGGACCAAGATTGTCGACAAATGTACCAAG AGGACCACTACTTGCTGGAAGTGTACTTGG3’
unc-18 (a/c)	WRM0615cG10	5’GATAAGTTCCTGACCAACTTGCGTGACCTGAACAAACCGCGTG ACATA**TGA**GCTGTCTCATCCTACTTTCAC3’ 5’GTATCTTGTTGTTTCGTCAATTGTCGATTCTTTTCGGTACCCCG CACTCTGCTACTTGCTGGAAGTGTACTTGG3’
ehs-1 (a)	WRM0621aG07	5’CAACCGGCTGGATTCGCTGATTTTGCGGACTTTGGATCGGCTT TCAAT**TGA**GCTGTCTCATCCTACTTTCAC3’ 5’CAATTTGACTGGCGTTGGGGCTTATAGTGGGTAAGGTATAATT ATTAAAAACTACTTGCTGGAAGTGTACTTGG3’
ehs-1 (b)	WRM0621aG07	5’GCTCCTCCAAAATCTGCTCGCGAAACACCTGTCAATGATCCTT TTGCG**TAA**GCTGTCTCATCCTACTTTCAC3’ 5’GTGATGGGCTGAAATTATTTAGACTTTAAATAAATTGATACTCA CTAAGACCTACTTGCTGGAAGTGTACTTGG3’
unc-11	WRM0623aD03	5’CACAAGCTCAACAGGCCCAGGCCGCCTCAGCCGATCCATTTG GATTA**TAG**GCTGTCTCATCCTACTTTCAC3’ 5’ATAAATCAGGGAATGTTACGAGAGAGATAGAGAGAAATAGACG ACTAGACTACTTGCTGGAAGTGTACTTGG3’
unc-57	WRM0635aD01	5’ACTGGATTATTCCCTGTTACCTATGTACAGGTTCTAGTGCCTCT TAAA**TAA**GCTGTCTCATCCTACTTTCAC3’ 5’CAAAAAATGGAAAGAATTGACTGGGTTTTGGAGGGAGAAATAC ATTTTTTTCTACTTGCTGGAAGTGTACTTGG3’
snn-1	WRM0629aH04 (extended by 3306 bp)	5’GACACGATGGGACAGTTGAAGCGCACTTTTGCCGGATTTTTTG GAGAA**TAA**GCTGTCTCATCCTACTTTCAC3’ 5’GAAATTGATGCGAGTTCAGTATTATGCAGATTGGAGCAGGCGG CACGAGTCTACTTGCTGGAAGTGTACTTGG3’
unc-104	WRM062bA11	5’GGAACAACATTGAAATCTCCAACATCATCATCTTCAATTGCTGC TTCA**TAA**GCTGTCTCATCCTACTTTCAC3’ 5’GTGAATAATCACAGAAAGAAAAACAGATAGGAAAGCAAAGAAA ATTAGATACTCTACTTGCTGGAAGTGTACTTGG3’
syd-2	WRM0636aB01	5’CGTAAGAATGATTCCATAGCAAAATCCTACGAGTTTCATTTATA TACC**TAG**GCTGTCTCATCCTACTTTCAC3’ 5’AGACACGAACGAGTGAGAGGGAGCGGAAAAAATTTTAATTTAA CTAACTAACTACTTGCTGGAAGTGTACTTGG3’
egl-3	WRM0636dD08	5’ACCAACAAGAAGCTCGACACCGTTCAAAAAGCCCACAAACGCA GCCAC**TAA**GCTGTCTCATCCTACTTTCAC3’ 5’AATTCTTAGAAATTTTTTGAAGGAAGGAAGAAATTGGGGAATTT TGTACATCTACTTGCTGGAAGTGTACTTGG3’
egl-21	WRM0640dE02	5’GAGCAAGAGCAAATCGCCGAGCTCGTCAACGAGATTGCCCGT CGTCGT**TAA**GCTGTCTCATCCTACTTTCAC3’

		5’ATGCAATGTGAATTGGTGTGTGGGAGTGATGACGGCGAATGA CTCGTGGGC CTACTTGCTGGAAGTGTACTTGG3’
ric-19	WRM0639aB01	5’CAATCTCTTATCGATGGATTCGACAGAGAGAATGAGGATAACT TGTTG**TGA**GCTGTCTCATCCTACTTTCAC3’ 5’AGAAAAATATAGTTTGAAAACATTTATTTTTCGATTTTACACATA GAACATCCTACTTGCTGGAAGTGTACTTGG3’
unc-31	WRM0630aF09	5’CAAGTATGGTCGAAGGAGCCGGTGCAAAGATGTTCTCGCTTTT TAAA**TAG**GCTGTCTCATCCTACTTTCAC3’ 5’CAAATAAATCCCGTGAGAAAACACCGCTTAAAGAAAAATATTAG AAAAAATCTACTTGCTGGAAGTGTACTTGG3’
unc-108	WRM0624cF12	5’CCAGGTGGAAATGCGACGGGAGGATTGGGCGGTGGATCTGG ATGCTGT**TAA**GCTGTCTCATCCTACTTTCAC3’ 5’AACGATTTAGTTAAAACATTCAAAGCTCATTCAAAGAAACATCA TCAAAAACTACTTGCTGGAAGTGTACTTGG3’
shn-1	WRM0625dB07	5’GAATTGCTCATCGCCAGATAATTGAATCAGCTCTCCGTGGCCT CCTCCAG**TGA**GCTGTCTCATCCTACTTTCAC3’ 5’GCTACGCGGAGAAAATCGAGAAAGTAGAAATGAGGGGGGAAA ATCATAAACTACTTGCTGGAAGTGTACTTGG3’
maco-1	WRM0640bE08	5’GAACAAGGGAAAATTTGGAGCTCCATCTCAACCAGCCGCTCGT CTTGCT**TGA**GCTGTCTCATCCTACTTTCAC3’ 5’TAAGAGAAAGAGTTTTTAAAGTTGGAAATTCACGTGGATCAGAA ACGAGTCTACTTGCTGGAAGTGTACTTGG3’
rgef-1	WRM0623bF10	5’GAAGATGATGATCTAGCAGATATTTCATCTGCGTCATACCGTA CCGCC**TGA**GCTGTCTCATCCTACTTTCAC3’ 5’ATTCTTGCTAAAAATAAAGAGAGTGCAGAGAAAGAGATAGATG GAGACAAA CTACTTGCTGGAAGTGTACTTGG3’
tbb-1	WRM0629cH08	5’CACTCGACGAGTTCGCCGGAGAAGGGGAGACATACGAGTCTG AGCAA**TAA**GCTGTCTCATCCTACTTTCAC3’ 5’GTACAGGAAATTACGAAACGGGGCAGGATAATTTAGTGATTGA TACACAGA CTACTTGCTGGAAGTGTACTTGG 3’
tbb-4	WRM066bH01	5’ACCGCCGACGACGAAGGCGAGTTTGATGAGCACGATCAAGAT GTGGAA**TAA**GCTGTCTCATCCTACTTTCAC3’ 5’TAATATGACAACTTTGGAAATTAGAAAAAAAACGTAGAAAATGA ATTAAGC CTACTTGCTGGAAGTGTACTTGG3’
tbb-5	WRM0641bB11	5’CTGCAGAGGAAGATGGAGAACTTGATGGAACTGATGGAGATG CTGAA**TAG**GCTGTCTCATCCTACTTTCAC3’ 5’ATTTCAAGTTTTTTTTTCATAAATAATTGTTCAAATCATAGTTTTC TATTCTACTTGCTGGAAGTGTACTTGG3’
cho-1	WRM0613dC12	5’TATTACATCCATATTCGGACCAAAGTTATTATTCCACAAATAGC AAT**TAA**GCTGTCTCATCCTACTTTCAC3’ 5’AAAAAATTTGGGAGGAAATTAAGTGAACACGTGGAACAAGTTC GTCTCTTCTACTTGCTGGAAGTGTACT3’

Fosmid deletion analysis

GENE	FOSMID/	PRIMERS FOR RECOMBINEERING

	pBALU tagged with	(target sequence) FRT GalK Lox2272 GalK
gcy-5 ASE motif deletion	WRM0630dC06/pBALU9 (a gift from Tulsi Patel)	F1 5'TTAAAACTTCAAACATTAAGCAAGAAGTTCCTATACTATTTGAAGAATAGGAACTTCCCTGTTGACAATTAATC3' R1 5'CTTTTTCTTTGAAACCATACGCAGAAGTTCCTATTCTTCAAATAGTATAGGAACTTCGCGGCCGCTCTAGAAC3' F2 5'TTGATGCAGATACCAACAAGATTAAAACTTCAAACATTAAGCAAG3' R2 5'GATTATCTTCCCAATTTTGCACCTTTTTCTTTGAAACCATACGCA3' F2 5'GCTGGGTCCCACCCGTCTTTTACTAGAAGTTCCTATTCTCTAGAAAGTATAGGAACTTCGCGGCCGCTCTAGAACTAG3'
eat-4 POU Homeodomain motif deletion	WRM0623aF12 / pBalu23	F1 5'AACCAAGCTTGTCAGAAGACAAGTGAAGTTCCTATACTTTCTAGAGAATAGGAACTTCCCTGTTGACAATTAATC3' R1 5'GCTGGGTCCCACCCGTCTTTTACTAGAAGTTCCTATTCTCTAGAAAGTATAGGAACTTCGCGGCCGCTCTAGAACTAG3' F2 5'TATCAAAAACCAGGCAGTGAGTCCTAGAACCAAGCTTGTCAGAAGAC3' R2 5'CTTTTTTATGATCTACTACTCACCGCGCTGGGTCCCACCCGTC3'
cho-1 AIY motif deletion	WRM0613dC12 / pBalu23	F1 5'CAAATCCTTTCTTAAAACTTGTTTGAGAAGTTCCTATACTTTCTAGAGAATAGGAACTTCCCTGTTGACAATTAATC3' R1 5'GAGGAGGATGAGCAAAAGAGCAACGGAAGTTCCTATTCTCTAGAAAGTATAGGAACTTCGCGGCCGCTCTAGAACTAG3' F2 5'CTTGACAAAACATTTCCGCAGTTGCAAATCCTTTCTTAAAACTTGTTTTGA3' R2 5'TTTCGATGTGTGTGTATGGGAAAGAGAGGAGGATGAGCAAAAGAGCAACG3'
cho-1 COE motif mutation (mutated nucleotides shown in bold)	WRM0613dC12 / pBalu23	F1 5'TCTTCCTGCTCCTCTTCTACCATCACGAAGTTCCTATACTTTCTAGAGAATAGGAACTTCCCTGTTGACAATTAATC3' R1 5'**GG**CTGGAGACCGTTTTTGTGTCGTTGAAGTTCCTATTCTCTAGAAAGTATAGGAACTTCGCGGCCGCTCTAGAACTAG3' F2 5'GAATGATGTATACACGAGAAGCTGCTCTTCCTGCTCCTCTTCTACCATCA3' R2 5'CTATCCTCCCTCTTCTCATTTCTCT**GG**CTGGAGACCGTTTTTGTGTCGTT3'
ric-4 deletion 1	WRM0640cB04 / pBalu23	F1 5'CCATTTGAAAGTTGTAAATTTTATATAACTTCGTATAGGATACTTTATACGAAGTTATCCTGTTGACAATTAATC3'

		R1 5'GGGAGCGCCGCGCCGAGCCGACATATAACTTCGTATAAAGTATCCTATACGAAGTTATGCGGCCGCTCTAGAACT3' F2 5'TTGGGAGCTAGTTTATTTTTAAATCAACCATTTGAAAGTTGTAAATTTTATATAACTTCG3' R2 5'CGGCATACGGACGGGGATGCCGTTGGCCGCCGGGAGCGCCGCGCCGAGCCGACATATAACTTCG3'
ric-4 deletion 2	WRM0640cB04 / pBalu23	F1 5'ATAAGTTCATTAGCTAAAAAAAATGAAGTTCCTATACTTTCTAGAGAATAGGAACTTCCCTGTTGACAATTAATC3' R1 5'GCAAACAAAATCTCTTCCAAAAATAGAAGTTCCTATTCTCTAGAAAGTATAGGAACTTCGCGGCCGCTCTAGAAC3' F2 5'AATAAAGTTGTCTTATCCCACTATGTATAAGTTCATTAGCTAAAAAAAATGAAG3' R2 5'CTAGAACATTTTTATTGGAAAACAAAACTTTTGCAAACAAAATCTCTTCCAAAAATAGAAG3'
snb-1 deletion of prom28 (29bp)	WRM065cH10 / pBalu23	F1 5'TCATAATGCCCAGTACGCAAAATGTGAAGTTCCTATACTTTCTAGAGAATAGGAACTTCCCTGTTGACAATTAATC3' R1 5'CGATGAATGGAAATTCAATGAGAGAGAAGTTCCTATTCTCTAGAAAGTATAGGAACTTCGCGGCCGCTCTAGAACTAG3' F2 5'ATCCCGAAATAGAGATGCGCGTAGGTCATAATGCCCAGTACGCAAAATGT3' R2 5'TAAGAAATGGGGTCAGATGACGAAGCGATGAATGGAAATTCAATGAGAGA3'
snb-1 deletion of prom17	WRM065cH10 / pBalu23	F1 5'TGAAAGTGAATGGATGACGGTCATATAACTTCGTATAGGATACTTTATACGAAGTTATCCTGTTGACAATTAATC3' R1 5'ATGAAAAAATAAGAAATGGGGTCAATAACTTCGTATAAAGTATCCTATACGAAGTTATGCGGCCGCTCTAGAACT3' F2 5'CATTTCAATTTATTTATTAATCGATGATTGAAAGTGAATGGATGACGGTCATATAACTTCGTATAGGATAC3' R2 5'GTAAGGAACGATGAGAACAGGGAAAAGGATGAAAAAATAAGAAATGGGGTCAATAACTTCGTATAAAG3'
snb-1 deletion of prom1+prom9		F1 5'GAAGTATGCATTTTGCTGTCAGAAGTTCCTATACTTTCTAGAGAATAGGAACTTCCCTGTTGACAATTAATC3' R1 5'GTCGGCGAGTATGTGTGTGTGCGAAGTTCCTATTCTCTAGAAAGTATAGGAACTTCGCGGCCGCTCTAGAACTAG3' F2 5'CTGAAATGATGCTTCGTTTGTGGACACTGGAGCCGTTTGAAGTATGCATTTTGCTGTCAGAAGTTCCTATACTTTC3' R2 5'GCAGTAGCACAAGGATTATATGGGCAAAGAAGACGTCGGCGAGTATGTGTGTGTGCGAAGTTCCTATTCTCTAGA3'

pBALU23: TagRFP-SL2-NLS-YFP-FGF*-YFP-H2B

ATGGTGTCTAAGGGCGAAGAGCTGATTAAGGAGAACATGCACATGAAGCTGTACATGGA
GGGCACCGTGAACAACCACCACTTCAAGTGCACATCCGAGGGCGAAGGCAAGCCCTAC
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CCTCAAGCAAGTTCATCCAGACACTGGAGTTTCCTCCAAAGCCATGTCTATCATGAACTC
TTTTGTCAACGATGTCTTCGAGCGTATTGCTGCTGAAGCATCCCGTCTTGCTCACTACAA
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GAGAGCTTGCCAAGCACGCCGTGTCTGAGGGAACCAAGGCCGTTACCAAGTACACTTC
CAGCAAGTAG

.....	TagRFP
.....	SL2 (*gpd-2* intergenic region)
.....	1XNLS
.....	YFP
.....	intron
.....	FRT*
.....	GALK
.....	H2B

pBALUNI: NLS-YFP-FGF*-YFP-H2B-SL2

ATGACCGCTCCAAAGAAGAAACGCAAAGTACCGGTAGAAAAAATGAGTAAAGGAGAAGA
ACTTTTCACTGGAGTTGTCCCAATTCTTGTTGAATTAGATGGTGATGTTAATGGGCACAA
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TATTTGCACTACTGGAAAACTACCTGTTCCATGGGTAAGTTTAAACATATATATACTAACT
AACCCTGATTATTTAAATTTTCAGCCAACACTTGTCACTACTTTCGGTTATGGTCTTCAAT
GCTTCGCCAGATACCCAGATCATATGAAACAGCATGACTTTTTCAAGAGTGCCATGCCC
GAAGGTTATGTACAGGAAAGAACTATATTTTTCAAAGATGACGGGAACTACAAGACACGT
AAGTTTAAACAGTTCGGTACGAAGTTCCTATACTATTTGAAGAATAGGAACTTCCCTGTT
GACAATTAATCATCGGCATAGTATATCGGCATAGTATAATACGACAAGGTGAGGAACTAA
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AACTACGTTCGTGGCGTGGTGAAACATCTGCAACTGCGTAACAACAGCTTCGGCGGCGT
GGACATGGTGATCAGCGGCAATGTGCCGCAGGGTGCCGGGTTAAGTTCTTCCGCTTCA
CTGGAAGTCGCGGTCGGAACCGTATTGCAGCAGCTTTATCATCTGCCGCTGGACGGCG
CACAAATCGCGCTTAACGGTCAGGAAGCAGAAAACCAGTTTGTAGGCTGTAACTGCGGG
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GAGACTTTTTACGTTTGTAAACCATCACAAGGAGCAGGACAGTGCTGAGGATCCACTAG
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ACATATTTAAATTTTCAGGTGCTGAAGTCAAGTTTGAAGGTGATACCCTTGTTAATAGAAT
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CAACTATAACTCACACAATGTATACATTATGGCAGACAAACAAAAGAATGGAATCAAAGT
TGTAAGTTTAAACATGATTTTACTAACTAACTAATCTGATTTAAATTTTCAGAACTTCAAAA
TTAGACACAACATTGAAGATGGAAGCGTTCAACTAGCAGACCATTATCAACAAAATACTC
CAATTGGCGATGGCCCTGTCCTTTTACCAGACAACCATTACCTGTCCTACCAATCTGCCC
TTTCGAAAGATCCCAACGAAAAGAGAGACCACATGGTCCTTCTTGAGTTTGTAACAGCTG
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GCCAAGAAGGCCGCCAAGACCGTTACGAAGCCAAAGGACGGAAAGAAGAGACGTCATG
CCCGTAAGGAATCATACTCCGTCTACATCTACCGTGTCCTCAAGCAAGTTCATCCAGACA
CTGGAGTTTCCTCCAAAGCCATGTCTATCATGAACTCTTTTGTCAACGATGTCTTCGAGC
GTATTGCTGCTGAAGCATCCCGTCTTGCTCACTACAACAAGCGTTCCACAATCTCATCCC
GCGAAATTCAGACCGCTGTCCGTCTGATCCTTCCAGGAGAGCTTGCCAAGCACGCCGT
GTCTGAGGGAACCAAGGCCGTTACCAAGTACACTTCCAGCAAGTAGGCTGTCTCATCCT
ACTTTCACCTAGTTAACTGCTTGTCTTAAAATCTATGCTTCTCTTTAGTATCTAAAATTTTC
CTAGAAGCTTACAAGTATATAAATGGTCTCTTCTCAATAAAGGTTGTATATTTATTCATCTT
ATTGAATCTGCCATTTCCTCGTTTTTGCGAGTTTATATACCTTCCAATTTTCTTTCTATTGT
ATTTTCAACTTCTAATTTTAATTCA

…… 1x NLS
…… YFP
…… introns
…… FRT*
…… GalK
…… H2B
…… SL2 (*gpd-2* intergenic region)

Generation of other reporter constructs

All reporter gene fusions for *cis*-regulatory analysis (except *rab-3prom1* transcriptional reporter) were generated using a PCR fusion approach (Hobert, 2002). Genomic fragments were fused to a nuclearly localized 2xNLS-TagRFP coding sequence, which was followed by the *unc-54* 3′ untranslated region. PCR fusion DNA fragments were injected as simple extrachromosomal arrays in a *pha-1(e2123)* mutant background strain that harbors an integrated array of the *rab-3prom1*::2xNLS-YFP(*otIs287* or *otIs291*) in the following concentrations: *cis*- regulatory element ("prom") construct 50ng/µL, *pha-1* rescuing plasmid pBX 50ng/µL.

For those *cis*-element where changes in the sequence were introduced, the PCR-fusion consisting of the *cis*-element and the fluorescent reporter where first cloned into TA cloning vectors (INVITROGEN) and sequenced. Mutagenesis was performed using the QuikChange II XL Site-Directed Mutagenesis Kit (Stratagene). The new "PCR-fusion" was amplified from the mutagenized plasmid and the amplicon injected in worms as described above for all other fusions.

The *rab-3prom1* reporter (**Fig.1E; Fig.S3**) was cloned adding PstI and BamHI restriction sites to primers F 5'GCGAGTTTTGACTGGCTTTC 3' and R 5'CTGAAAATAGGGCTACTG 3' and cloned into pPD95.67 vector containing 2xNLS-*yfp* and the *unc-54* 3'UTR.

Integrated constructs *rab-3prom1::2xNLS-YFP* (*otIs287* [IV], *otIs291* [V]), *rab-3prom1::2xNLS::TagRFP* (*otIs355* [IV], *otIs356* [V]), *ric-4prom4::2xNLS::GFP* (*otIs489* [X], *otIs490* [IV]), *ric-4prom17::2xNLS::GFP* (*otIs414, otIs420-otIs424*), *snb-1prom17::2xNLS::GFP (otIs419)*, *ric-19prom6* (*otIs380, otIs381* [V]), and *ehs-1prom4::2xNLS::GFP* (*otIs501*) were generated by gamma irradiation and outcrossed four to eight times.

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