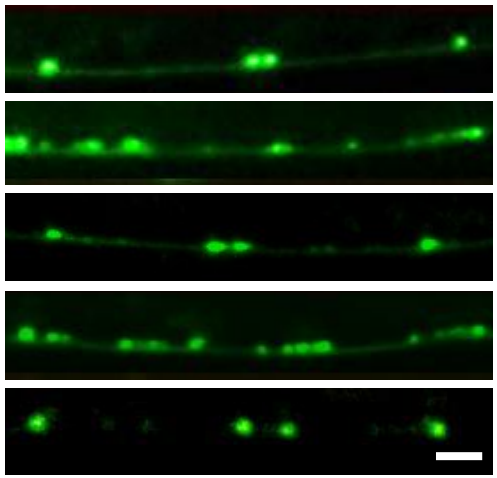
Norris *et al*. Figure S1

unc-16 exon 16 skipped isoform



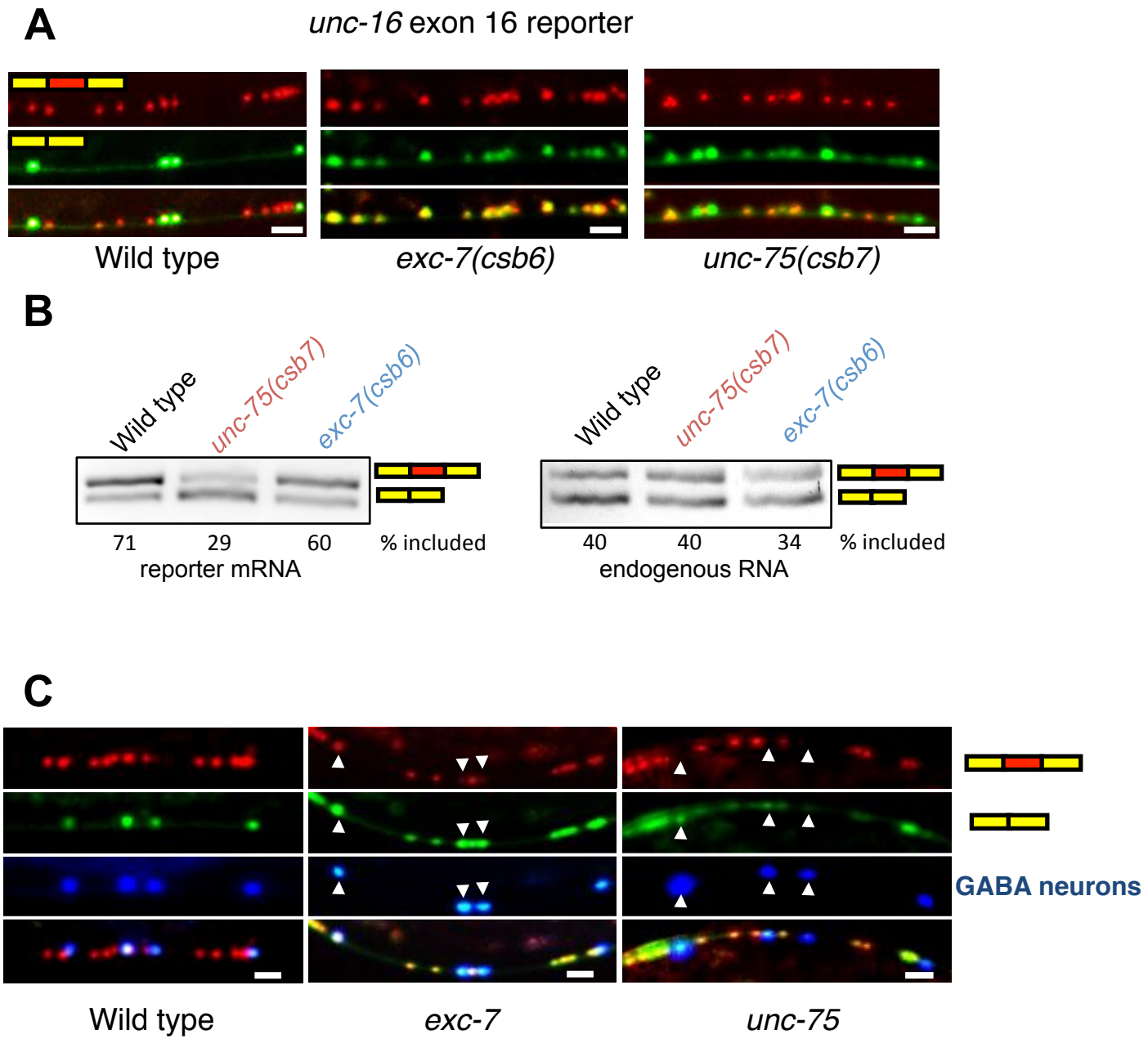
Wild type

unc-75

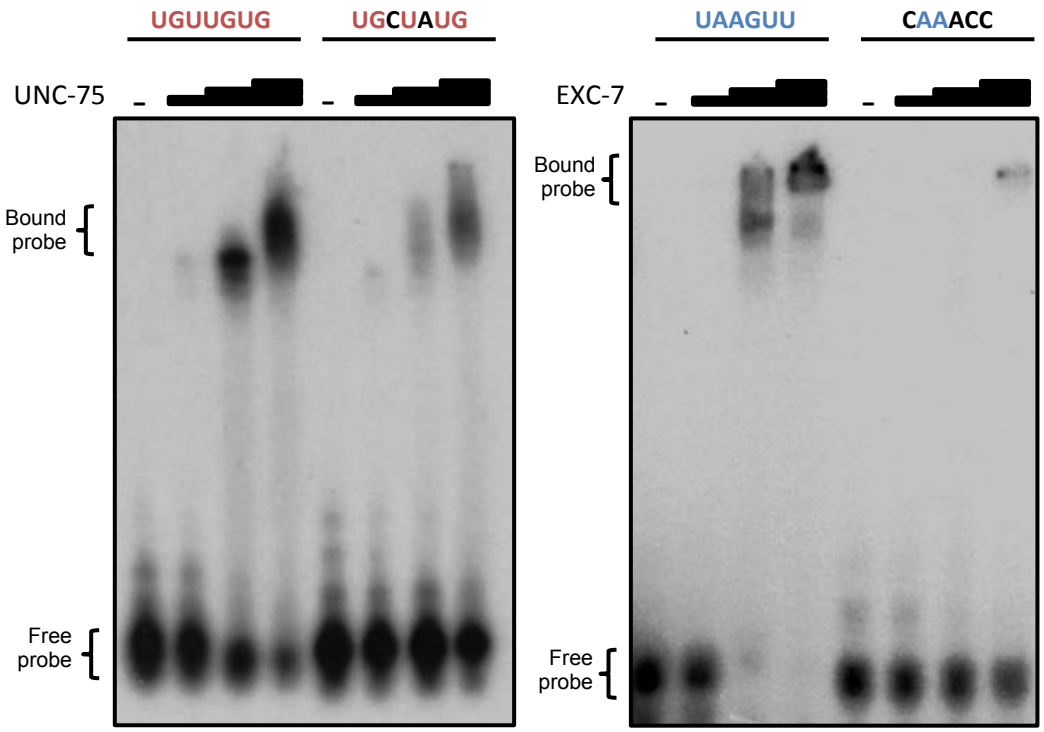
unc-75; unc-75 rescuing construct

exc-7

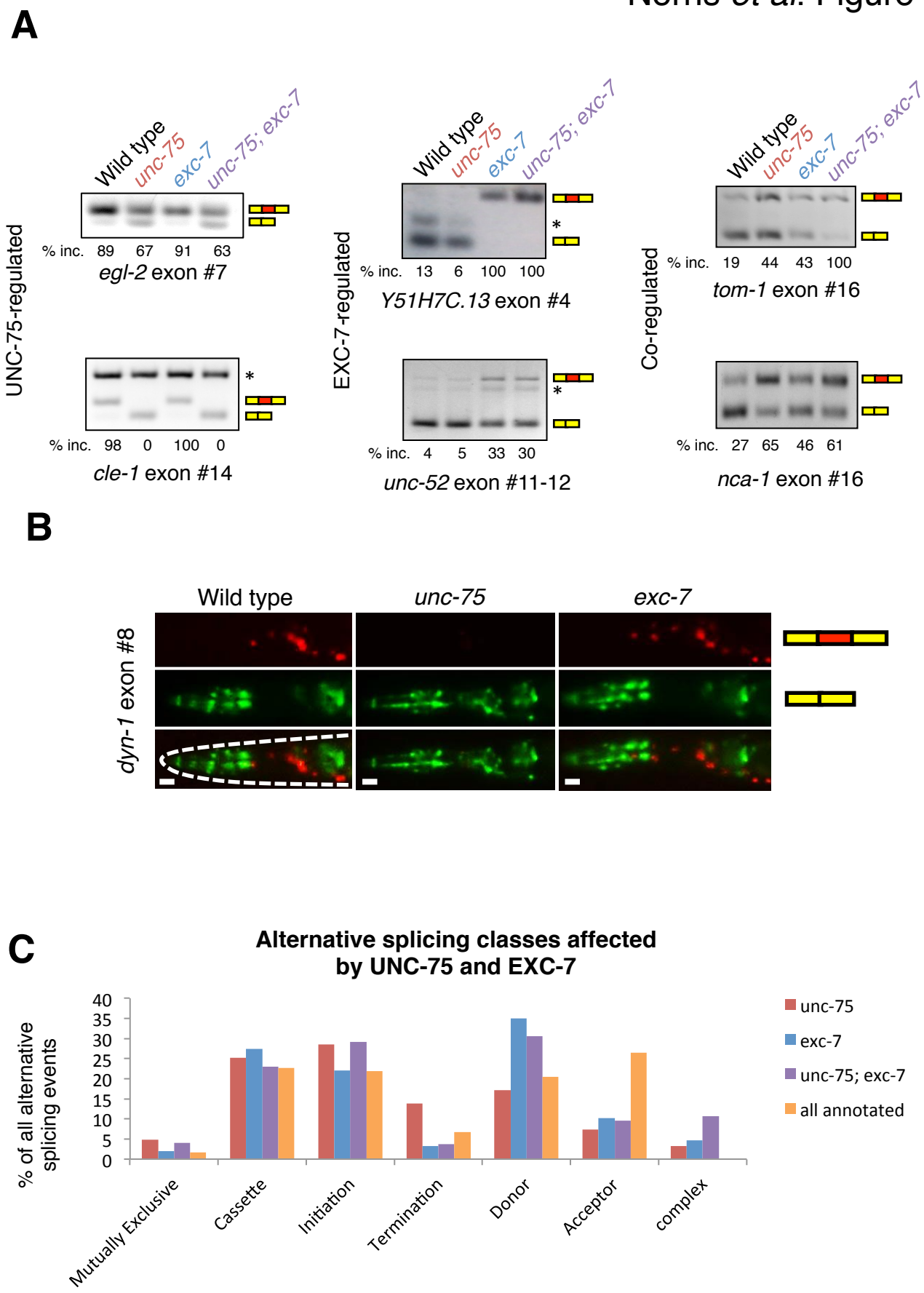
exc-7; exc-7 rescuing construct


A
unc-16 exon 16 reporter
Wild type
exc-7(csb6)
unc-75(csb7)
B
Wild type
unc-75(csb7)
exc-7(csb6)
71
29
60
% included
reporter mRNA
Wild type
unc-75(csb7)
exc-7(csb6)
40
40
34
% included
endogenous RNA
C
GABA neurons
Wild type
exc-7
unc-75

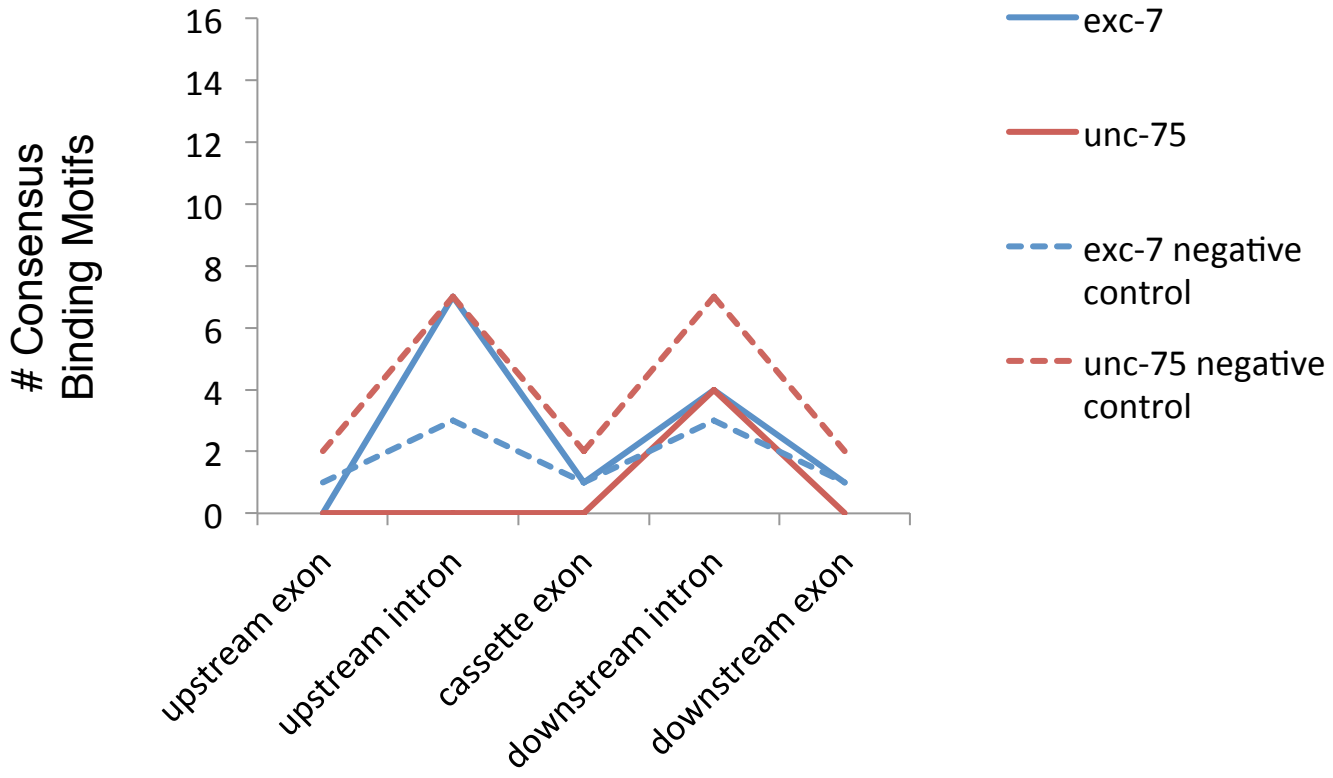
Norris *et al*. Figure S3



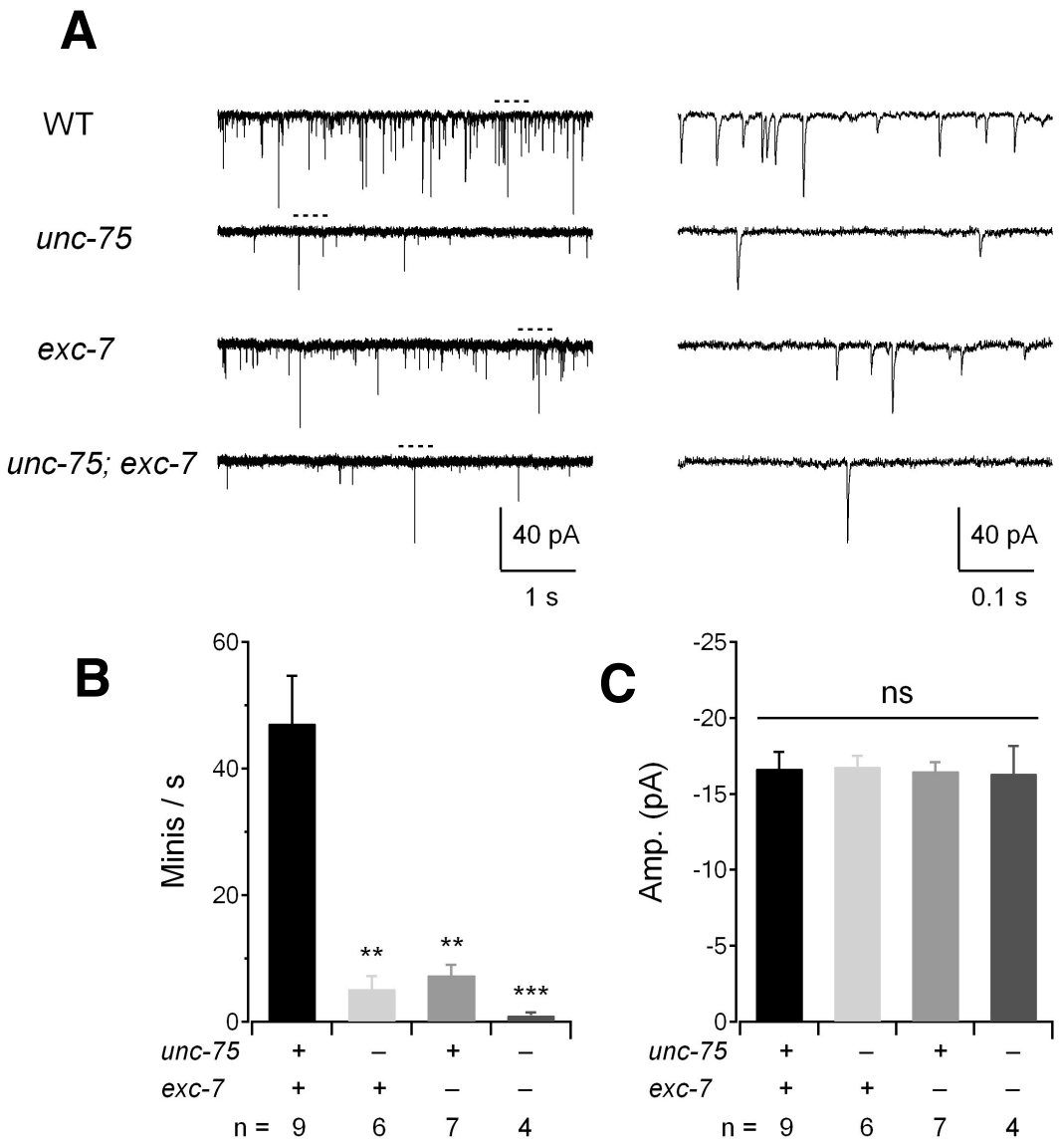
Norris *et al*. Figure S4



Alternative exons with increased inclusion in *unc-75; exc-7* mutants

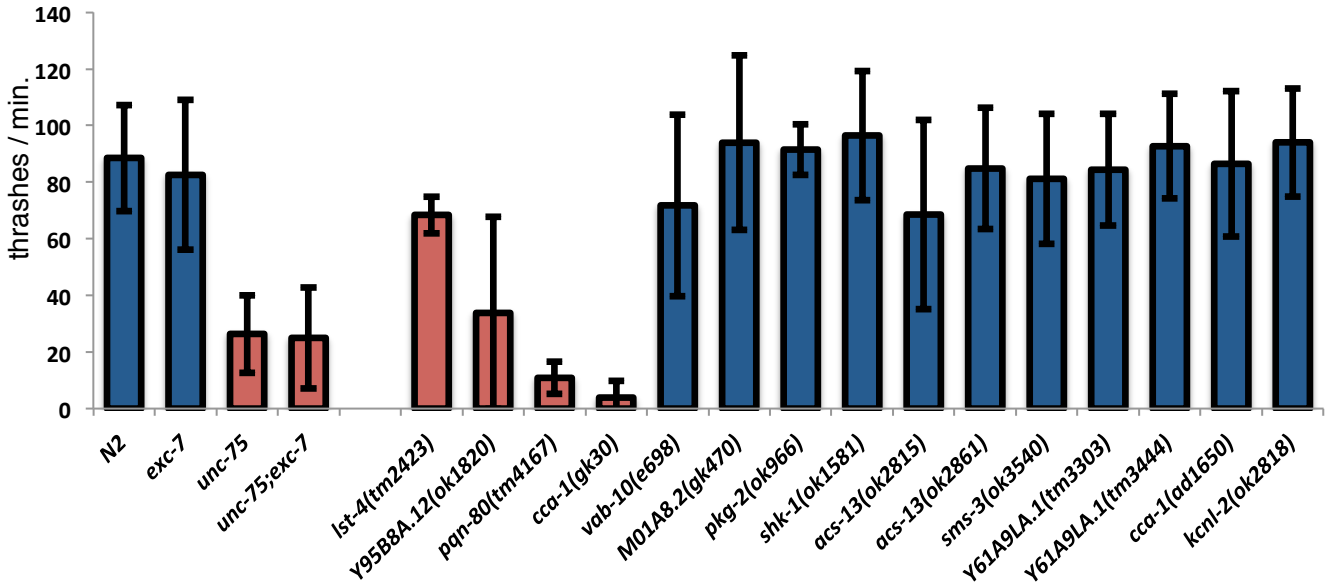


Norris *et al*. Figure S6

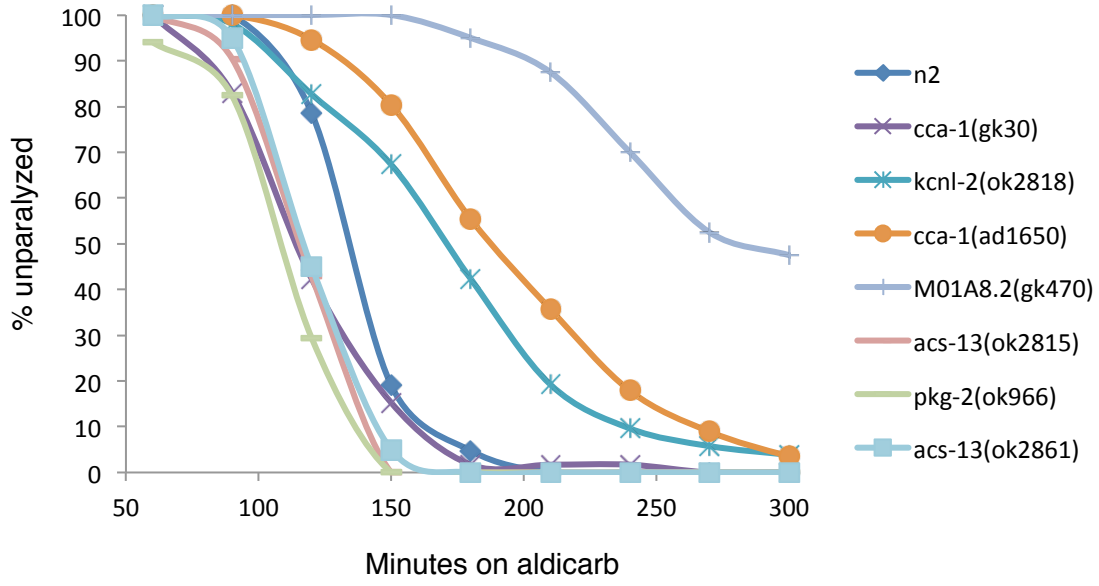


Norris *et al*. Figure S7

A



B



Supplementary Figure legends

Figure S1: UNC-75 and EXC-7 expressing transgenes rescue neuron-specific alternative splicing defects. Related to Figure 2.
UNC-75 and EXC-7 expressing transgenes driven by their endogenous promoters rescue the *unc-16* exon 16 alternative splicing defects observed in the respective *unc-75* or *exc-7* mutants.

Figure S2: UNC-75 and EXC-7 combinatorially control neuron-specific alternative splicing. Related to Figure 2.
(A) Fluorescence images of wild type, and our recovered *unc-75(csb7)* and *exc-7(csb6)* mutants expressing the *unc-16* exon 16 two color alternative splicing reporter.
(B) RT-PCR assays monitoring *unc-16* exon 16 alternative splicing in wild type and *unc-75(csb7)* and *exc-7(csb6)* mutant animals. Left panel shows two color reporter mRNA splicing patterns, and right panel shows endogenous mRNA splicing patterns.
(C) GABAergic neurons lose *unc-16* exon 16 inclusion in *unc-75* mutants (right panels, arrow-heads). However, GABAergic neurons in *exc-7* mutants still display exon 16 inclusion (middle panels, arrowheads). Scale bar represents 10 μm.

Figure S3: mutations in UNC-75 and EXC-7 consensus motifs reduce binding affinities. Related to Figure 3.
Electrophoretic mobility shift assay demonstrating that recombinant UNC-75 and EXC-7 bind their cognate binding motifs with high affinity, but not a mutated version of the motif.

Figure S4: Validations of UNC-75 and EXC-7-regulated alternative splicing events. Related to Figure 4.
(A) RT-PCR validations of alternative splicing events subject to different regulatory modes identified by our mRNA-Seq analysis. Asterisks mark additional potential splice variants not annotated in gene models.
(B) *dyn-1* exon 8 fluorescent alternative splicing reporter confirms mRNA-Seq and RT-PCR analysis showing that UNC-75, but not EXC-7, mediates exon inclusion, and furthermore shows that this regulation is neuronal cell-specific.
(C) Classes of alternative splicing events affected by the three mutant conditions, compared to all such alternative splicing events previously reported by *C. elegans* genome-wide analysis (Ramani et al 2011).

Figure S5: No enrichment of UNC-75 and EXC-7 binding motifs in the introns flanking alternative exons undergoing increased inclusion in the absence of these RNA binding proteins. Related to Figure 4.
Among the 19 exons that undergo increased exon inclusion in an *unc-75; exc-7* double mutant, no local binding-site signature is found for either UNC-75 or EXC-7 ($p>0.05$, Chi-squared test).

Figure S6: mPSCs are defective in *unc-75 and exc-7* mutants. Related to Figure 5.

(A) Representative traces with different time scales of spontaneous mPSCs in wild-type (WT), *unc-75(e950)*, *exc-7(rh252)* and *unc-75(e950); exc-7(rh252)* mutants. Muscles were holding at -60 mV, all recordings were performed under *zxIs6* background. The right panels show enlarged view of the indicated regions (dashed lines) in the left panel.
(B) The frequency of mPSCs was significantly decreased in *unc-75(e950)*, *exc-7(rh252)* and *unc-75(e950); exc-7(rh252)* mutants.
(C) The amplitude of mPSCs in all mutants, however, was comparable to that of wild-type animals. ** $P < 0.01$, *** $P < 0.001$, ns: not significant by Mann-Whitney U test.

Figure S7: UNC-75 alternative splicing targets have locomotion and aldicarb sensitivity defects. Related to Figure 6.
(A) Data showing mobility in liquid media of wild type, *unc-75, exc-7,* double mutant, and UNC-75 target gene mutants (measured in number of thrashes per minute). A total of at least 20 worms were assayed for each genotype. Error bars represent standard deviations, and red highlighted columns represent mutants that have significantly reduced locomotion relative to wild type worms ($p < 0.05$, t-test).
(B) Aldicarb sensitivity defects in wild type and UNC-75 target gene mutants, measured as a time course of the percentage of worms not paralyzed at each given time point.