

Expanded View Figures

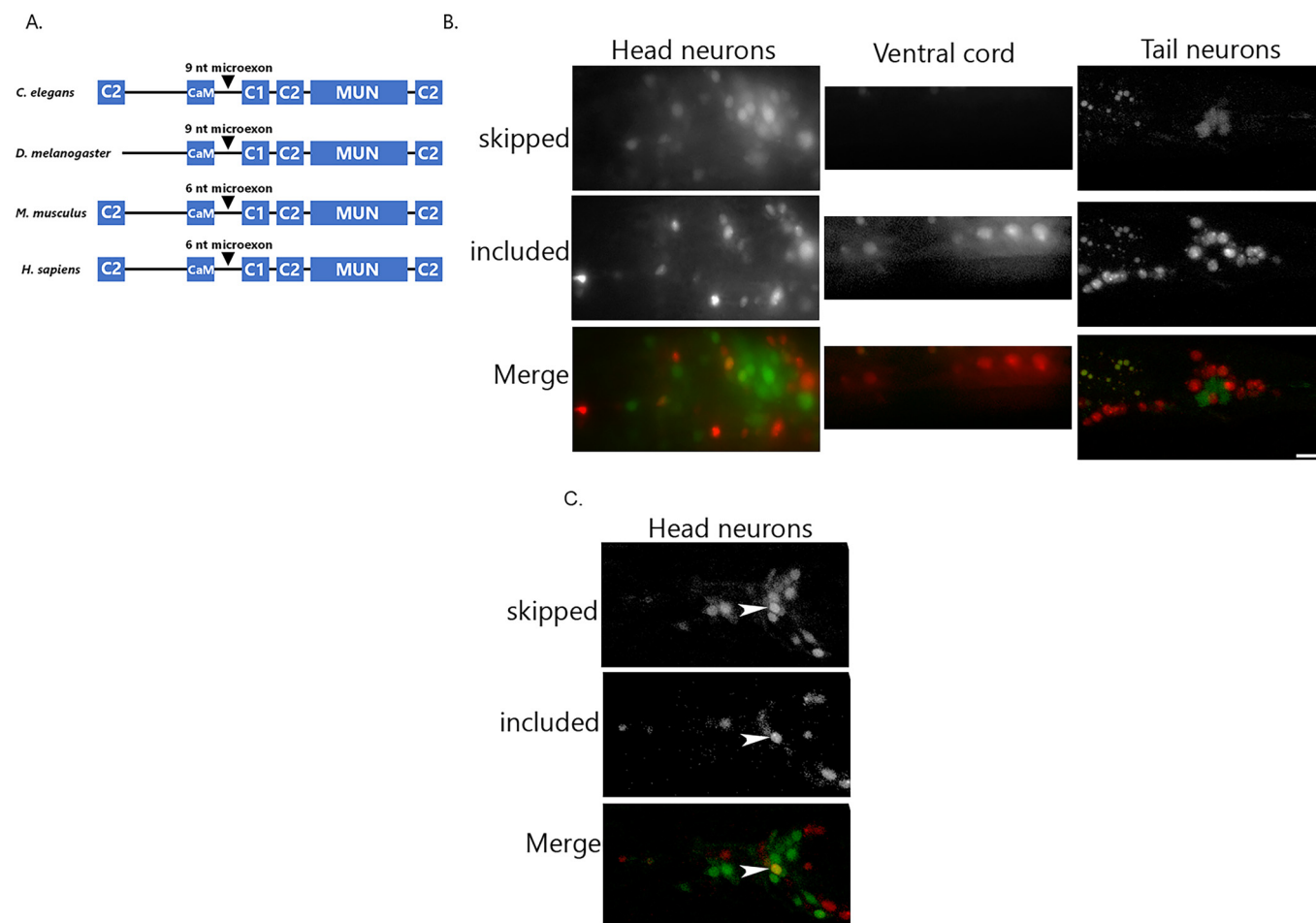


Figure EV1. The presence of an alternative microexon is conserved across species and its splicing pattern is invariant across multiple isolated independent integrated transgenic lines in *C. elegans*.

(A) Schematic of the position of the alternatively spliced microexon in the *unc-13* transcript across various species. In mice and humans, it's 6 nt microexon, whereas in *C. elegans* and *Drosophila*, it's 9 nt. Black arrowheads mark the position of the microexon in different species. (B) *unc-13* microexon-splicing pattern in various regions of the nervous system of an independently isolated integrated transgenic line. (C) A neuron marked by a white arrowhead expressing both skipped and included versions in the nerve ring neurons of the *unc-13* microexon splicing reporter expressing animal. Scale bar 20 μ m.

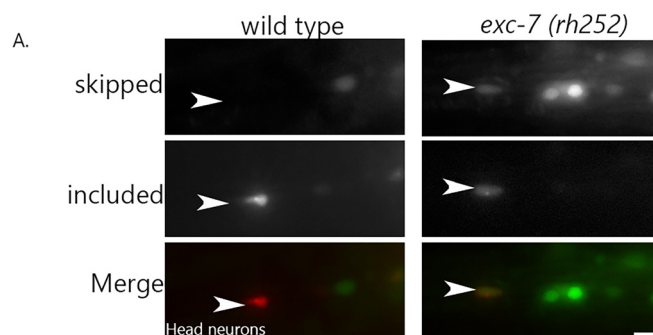


Figure EV2. Alternative splicing of the *unc-13* microexon is affected in a neuron-specific manner in *exc-7* animals.

(A) A region of the head neurons, where an identified neuron in wild type exclusively has RFP, whereas in *exc-7(rh252)* it has both, GFP and RFP signal. The white arrowhead marks that neuron. Scale bar 10 μ m.

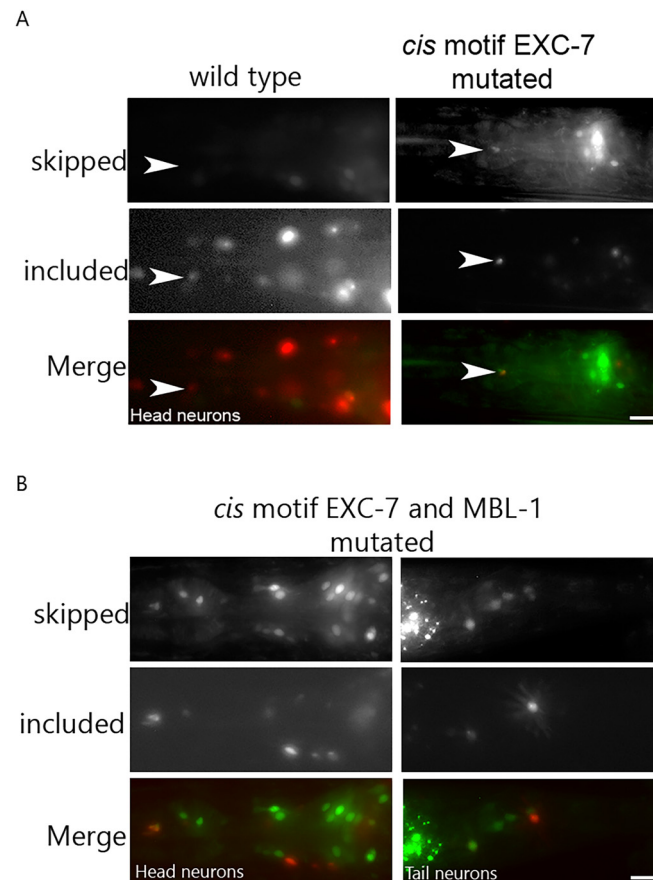


Figure EV3. Mutating *cis* motifs for EXC-7 and/or MBL-1 affect splicing in a neuronal-subtype-specific manner.

(A) A neuron in pharyngeal region shows the expression of GFP and RFP, whereas in wild type it expresses only included form. The white arrowhead marks that individual neuron. (B) Representative image showing *cis* motif mutation in both EXC-7 and MBL-1, where a set of animals (~20% of animals) has lesser RFP neuronal cell bodies in head and tail neurons compared to wild type. Scale bar 20 μ m.

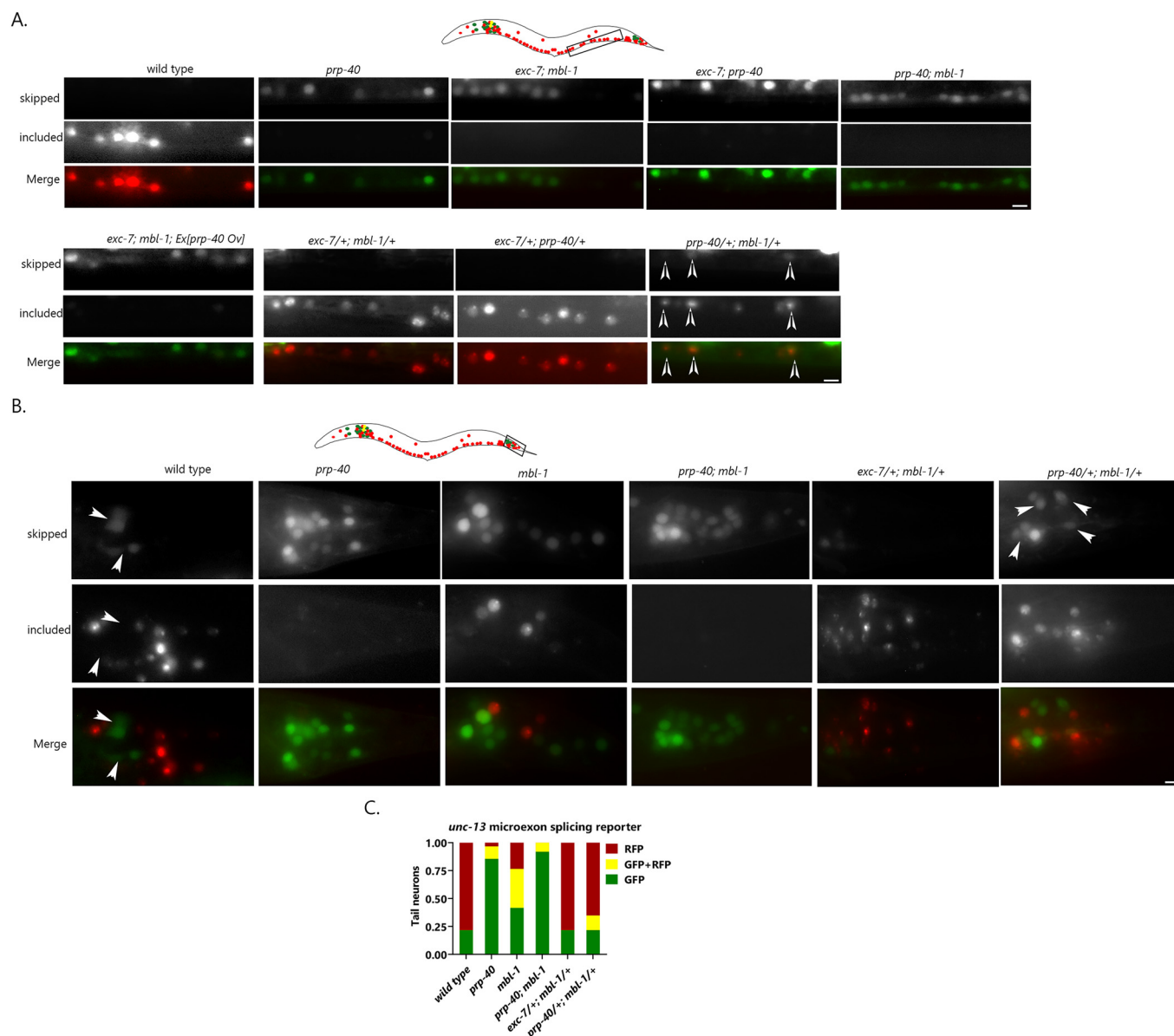


Figure EV4. Genetic interaction between splicing regulators for the alternative splicing of *unc-13* microexon in different neuronal subtypes (also see Fig. 3G, H in the main section for quantification).

(A) Upper panel: Worm schematic showing the splicing reporter pattern and the below images are from the area marked by dotted rectangle in the ventral cord region. Note that the wild-type control panel is the same worm as in Figs. 2D and 1F. Bottom panel: Representative images of the ventral cord region of the for various genotypes. White arrowheads mark the GFP appearance in the double heterozygous animals. (B) Upper panel: A worm schematic showing splicing reporter with the area marked by a dotted rectangle in the tail region is represented in the below mentioned images for various genotypes. Bottom panel: Representative images of the tail neurons in the mentioned genotypes. White arrowheads in the wild type panel marks a set of tail neurons expressing the skipped isoform (GFP) (C) Quantification of the splicing analysis of the tail neurons in the indicated genotypes. $n = 15-20$ animals. Scale bar 10 μ m.

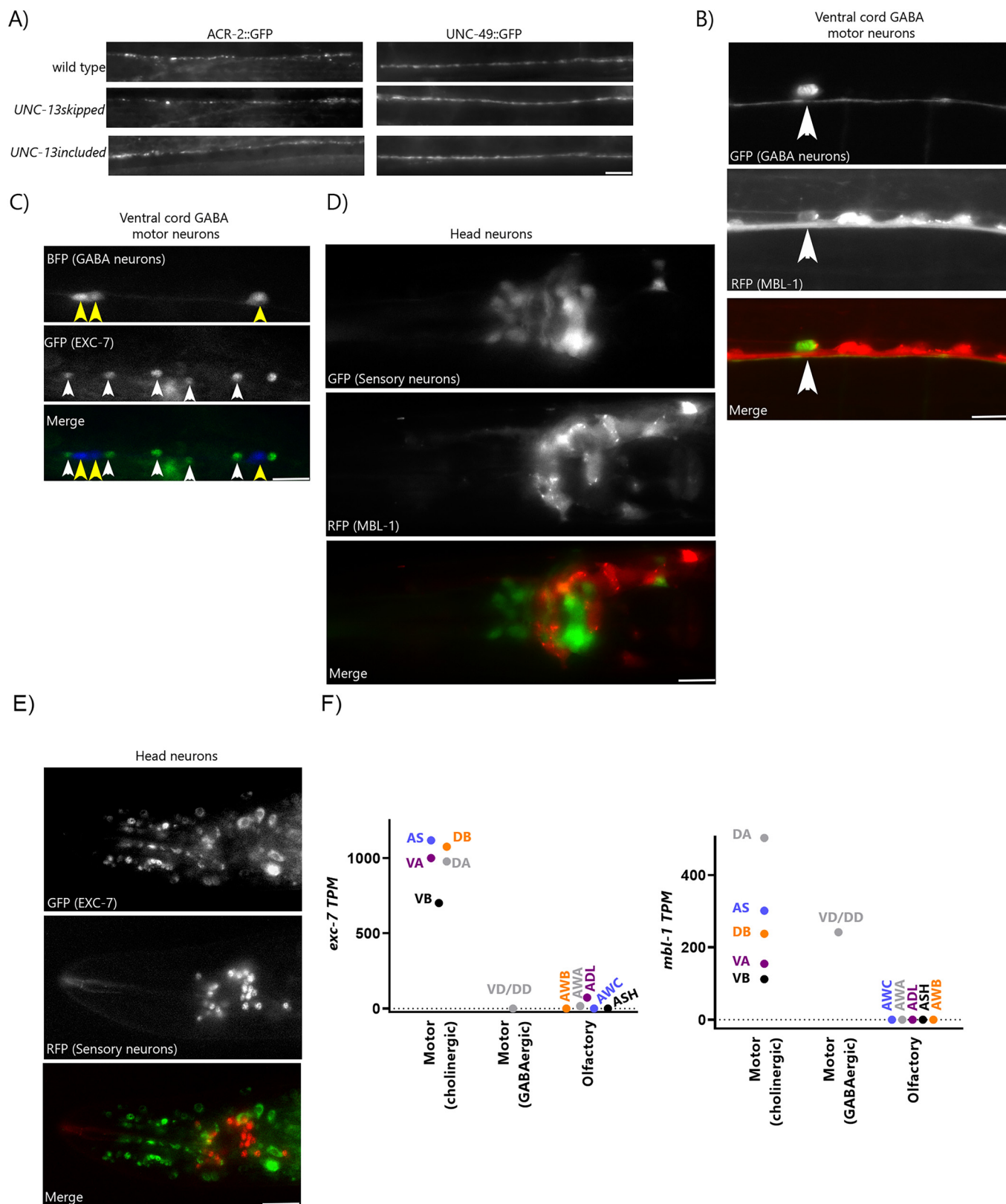




Figure EV5. Localization of SNB-1::GFP in dorsal cords and expression analysis of RBPs EXC-7 and MBL-1 by using reporter strains in addition to the CeNGEN database.

(A) Representative images of localization of post-synaptic markers such as acetylcholine receptors (left panel) and GABA receptors (right panel) in the muscle compartment. (B) The upper panel shows GABA motor neurons (white arrowhead) in the ventral cord, which colocalize with MBL-1::RFP driven by a fosmid. (C) GABA motor neurons in the ventral cord marked by BFP (yellow arrowheads) driven under *unc-25* promoter does not colocalize with EXC-7::GFP (marked by white arrowheads) in ventral cord neurons. (D) Sensory neurons marked by GFP driven under *osm-6* promoter and MBL-1::RFP expression by the fosmid, indicates many of the sensory neurons does not have detectable MBL-1 expression. (E) The upper panel indicates EXC-7::GFP expression in the head region, and the sensory neurons marked by HIS-11::mcherry driven by *osm-6* promoter (middle panel). Sensory neurons have little to no detectable expression of EXC-7 as scored by the reporter analysis. (F) Single-cell RNA seq analysis for *exc-7* and *mb1-1* transcripts in multiple sensory and motor neurons (GABA and cholinergic neurons). Analysis indicates significant transcript level in motor neurons for *mb1-1*, whereas a set of sensory neurons lack the same, whereas *exc-7* transcripts are lower or undetectable in both sensory and GABA motor neurons, but enriched in cholinergic motor neurons. Scale bar 20 μ m.