Supplemental Information

MADD-4/Punctin and Neurexin Organize C. elegans GABAergic Postsynapses through Neuroligin

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Figure S1, Related to Figure 3, NLG-1 clustering is independent of its intracellular tail, and relies on extracellular cues other than NRX-1

(A) An internally-tagged NLG-1 missing the C-terminal PDZ-binding domain (NLG-1ΔPDZBD::YFP) localizes normally apposite to inhibitory presynaptic terminals. (B) The same construct lacking the entire cytoplasmic tail of NLG-1 (NLG-1ΔintraC::YFP) also localizes to inhibitory postsynapses. (C) This is not due to dimerization with the endogenous NLG-1, since its distribution is unchanged in nlg-1(ok259) null mutants. (D) In contrast deletion of the extracellular domain of NLG-1 leads to a diffuse distribution of the corresponding protein (NLG-1ΔextraC::YFP) at the cell plasma membrane. (E) A putative NRX-1 binding mutant of NLG-1, NLG-1(QED), shows a normal distribution at inhibitory postsynapses. (A—E) Inhibitory presynaptic terminals are visualized by expressing the active zone marker UNC-10::tdTomato under the control of the unc-47 promoter. (F) Quantification of UNC-49::YFP clustering in wild-type, nlg-1(ok259) mutant and nlg-1(ok259) mutant animals expressing full-length or truncated NLG-1 constructs in body-wall muscles. The number of animals analyzed is indicated. p<0.001, Fisher’s exact test. *p values are ***<0.001, ****<0.0001. Scale bar: 5 μm.
Figure S2, Related to Figure 3, Clustering of presynaptic active zones and synaptic vesicles is normal in nrx-1 mutants

(A) The active zone protein UNC-10::TdT forms clusters apposite to NLG-1::YFP in nrx-1(wy778) animals. (B) Synaptic vesicles labeled with mCherry::RAB-3 accumulate normally at inhibitory presynaptic terminals in nrx-1(wy778) mutants.

Scale bar: 5 μm.
Figure S3, Related to Figure 6, Expression of MADD-4S and NRX-1 extracellular fragments in Drosophila S2 culture for the ECIA. Double (**) and single (*) asterisks indicate no detectable expression and very low levels of expression, respectively.
Figure S4, Related to Figure 6, Multiple protein interactions cluster GABA\textsubscript{A} receptors at the postsynaptic membrane.
MADD-4S/Punctin is secreted from the presynaptic boutons and deposited locally. MADD-4S interacts with presynaptic NRX-1/neurexin and postsynaptic NLG-1/neuroligin. MADD-4S and N RX-1 act together to recruit NLG-1 to GABAergic synapses, which in turn induces GABA\textsubscript{A} receptor clustering.