

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-1 Δ PDZBD::YFP) localizes normally apposite to inhibitory presynaptic terminals. (B) The same construct lacking the entire cytoplasmic tail of NLG-1 (NLG-1 Δ intraC::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-1 Δ extraC::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 μ m.

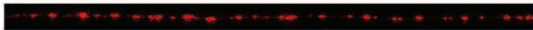
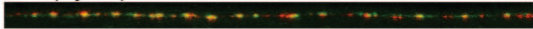
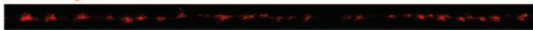
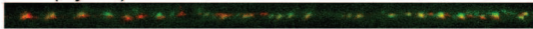
A*NLG-1::YFP**UNC-10::TdT**nrx-1(wy778)***B***NLG-1::YFP**mCherry::RAB-3**nrx-1(wy778)*

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 μ m.

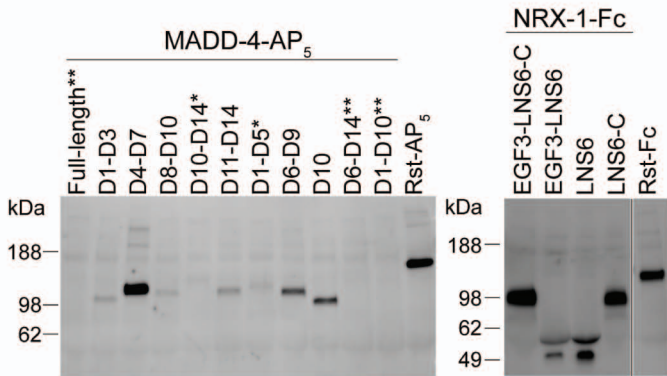


Figure S3, Related to Figure 6, Expression of MADD-4S and NRX-1 extracellular fragments in *Drosophila* S2 culture for the ECIA. Double (**) and single (*) asterisks indicate no detectable expression and very low levels of expression, respectively.

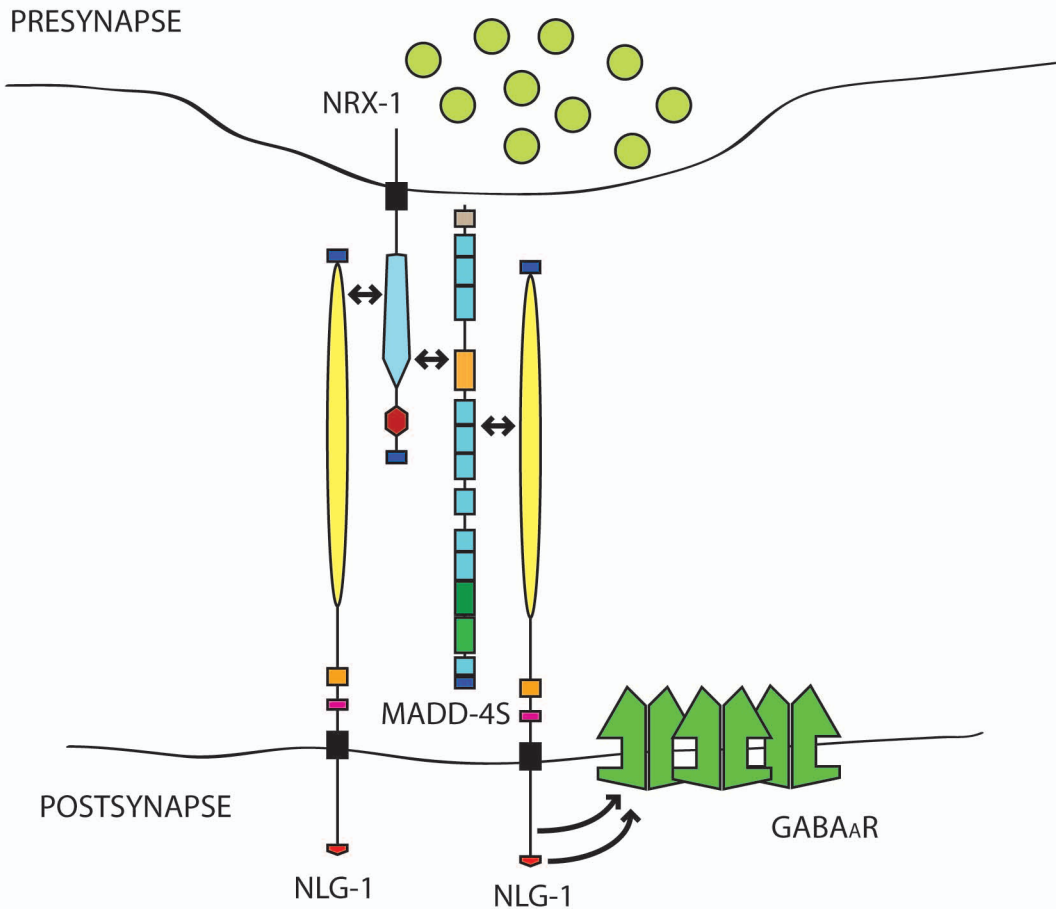


Figure S4, Related to Figure 6, Multiple protein interactions cluster GABA_A receptors at the postsynaptic membrane. MADD-4S/Punctin is secreted from the presynaptic boutons and deposited locally. MADD-4s interacts with presynaptic NRX-1/Neurexin and postsynaptic NLG-1/Neuroigin. MADD-4S and NRX-1 act together to recruit NLG-1 to GABAergic synapses, which in turn induces GABA_A receptor clustering.