Supplemental Figure Legends

Supplemental Figure S1. Neuroligin-1 clusters glutamate and GABA synaptic vesicles. **(A, B)** Fibroblasts expressing HA-neuroligin-1 co-cultured with hippocampal neurons induced clusters of VGlut1**(A)** and GAD **(B)** in contacting glutamatergic and GABAergic axons. The induced clusters of VGlut1 **(A)** lacked postsynaptic PSD-95 immunoreactivity (arrowhead) in contrast to endogenous synaptic clusters (arrow). The induced clusters of GAD **(B)** lacked postsynaptic gephyrin immunoreactivity (arrowhead), in contrast to endogenous synaptic clusters (arrow). **(C, D)** Fibroblasts expressing YFP co-cultured with hippocampal neurons had no effect on VGlut1 **(C)** or GAD **(D)** in contacting axons. Only endogenous clusters of VGlut1 associated with postsynaptic PSD-95 immunoreactivity **(C),** and endogenous clusters of GAD associated with postsynaptic gephyrin immunoreactivity **(D)** (arrows) were observed. Scale bar 10 μm.

Supplemental Figure S2. Recombinant CFP-neurexin-1β localizes to glutamate and GABA presynaptic terminals. **(A)** GABAergic neurons expressing CFP-neurexin-1β co-clustered the neurexin with co-expressed synaptophysin-YFP and endogenous GAD at GABAergic terminals. The synaptophysin-YFP was co-expressed to mark transfected neuron terminals; two GABAergic axons are seen in the field of view, only one of which was transfected. A similar clustered distribution of CFP-neurexin was observed when it was expressed alone. **(B)** Glutamatergic neurons expressing CFP-neurexin-1β co-clustered the neurexin with co-expressed synaptophysin-YFP and endogenous VGlut1 at glutamatergic terminals. Some untransfected VGlut1-positive terminals are also seen in the field of view. Scale bar 10 μm.

Supplemental Figure S3. N-cadherin and NgCAM do not induce postsynaptic protein clustering. Coculture of fibroblasts expressing N-cadherin-YFP **(A, B)** or NgCAM-YFP **(C, D)** with hippocampal neurons did not induce clustering of gephyrin or PSD-95 at dendrite contacts with expressing fibroblasts. Only endogenous synaptic clusters of gephyrin and PSD-95

colocalized with synapsin were observed. This is in contrast to the ability of neurexin-CFP to induce clustering of gephyrin and PSD-95 as shown in Figure 1. Scale bar 10 μm.

Supplemental Figure S4. Neurexin induces postsynaptic protein clustering in immature or mature neurons. **(A, B)** Fibroblasts expressing neurexin-1β-CFP were cocultured with hippocampal neurons at only 2-3 days in culture, before neurons form endogenous synapses. Even in these very young neurons, small but distinct non-synaptic clusters of gephyrin and PSD-95 were consistently observed in association with fibroblasts expressing neurexin-CFP, often following the edges or filopodia of the expressing fibroblasts. Insets show enlarged regions containing neurexin-induced gephyrin and PSD-95 clusters. **(C)** Fibroblasts expressing neurexin-1β-CFP also induced non-synaptic clusters of gephyrin and PSD-95 in mature 14-16 day cultured neurons, at neighboring but largely distinct sites in contacting dendrites. These results for hippocampal neurons at different ages are similar to the results at 1 week in culture shown in Figure 1. Scale bar 10 μm.

Supplemental Figure S5. Neurexin clusters multiple glutamatergic postsynaptic scaffolding and signaling proteins. **(A, C)** Fibroblasts expressing neurexin-1β-CFP co-cultured with hippocampal neurons induced clusters of the excitatory scaffolding protein GKAP/SAPAP **(A)** and excitatory signaling protein SynGAP **(C)** in contacting dendrites. Induced clusters (arrowhead) lacked immunoreactivity for VGlut1, unlike endogenous synaptic clusters (arrow). Induced clusters were often associated with the edges of transfected fibroblasts. **(B, D)** Fibroblasts expressing mCFP co-cultured with hippocampal neurons did not affect the distribution of GKAP **(B)** or SynGAP **(D)** in contacting dendrites. Only endogenous clusters associated with VGlut1-positive terminals (arrow) were observed. Scale bar 10 μm.

Supplemental Figure S6. Neurexin but not mCFP clusters GABA and NMDA glutamate receptors. **(A, B)** Fibroblasts expressing mCFP co-cultured with hippocampal neurons did not

affect the distribution of $GABA_AR\gamma2$ **(A)** or NR1 **(B)** , the essential subunit of NMDA receptors, in contacting dendrites. Only endogenous clusters associated with synapsin-positive terminals (arrow) were observed. This is in contrast to the ability of neurexin-CFP to induce clusters of $GABA_AR\gamma2$ and NR1 in a similar assay (Figure 3A, B). **(C)** Fibroblasts expressing mCFP co-cultured with hippocampal neurons did not affect the distribution of GluR1 AMPA receptor subunit in contacting dendrites. Only endogenous clusters associated with synapsin-positive terminals (arrow) were observed. Scale bar 10 μm.

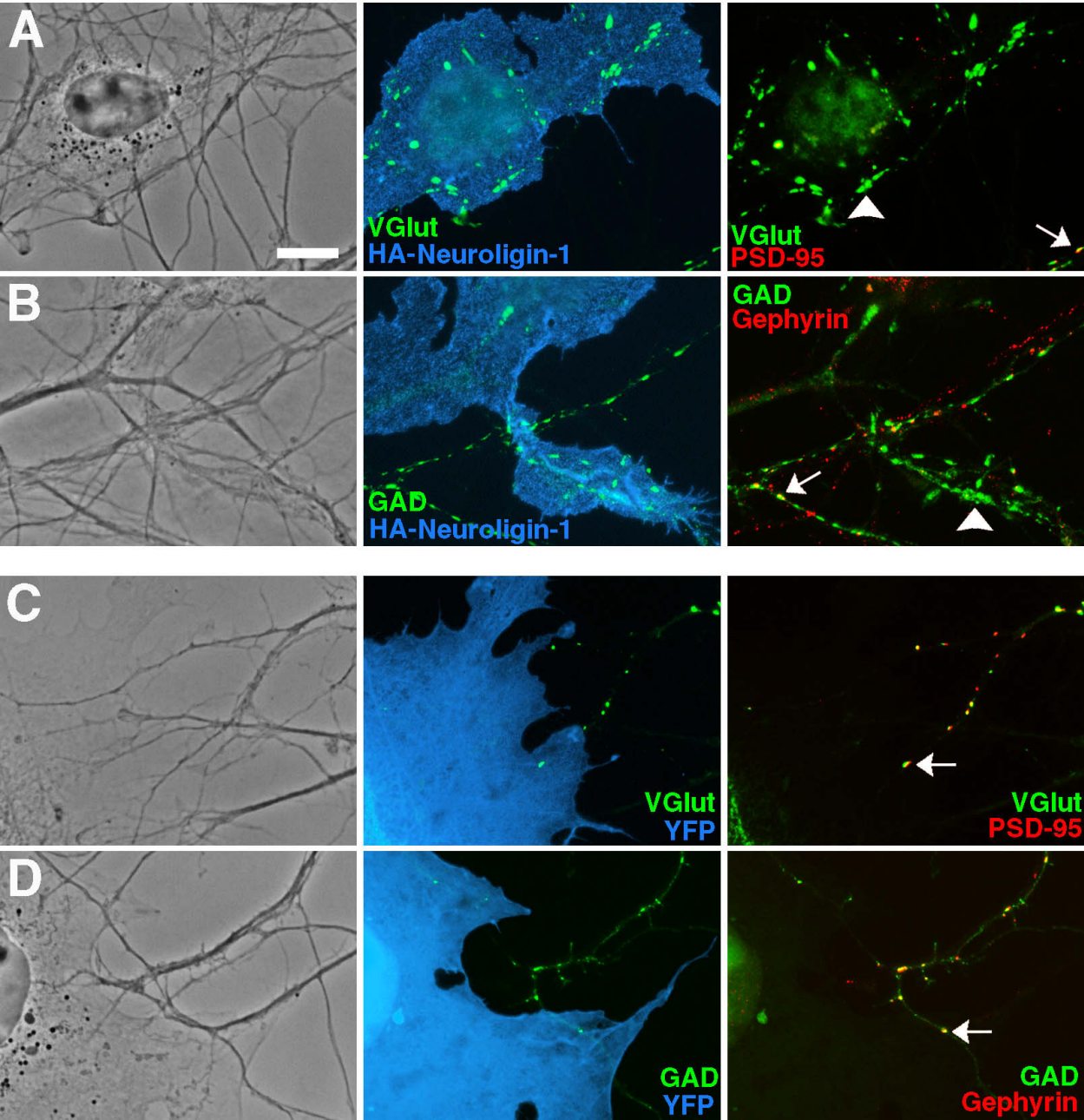
Supplemental Figure S7. Neurexin-induced receptor clusters are on the dendrite surface. **(A, B)** Apparent induced clusters (arrowhead) of $GABA_AR\gamma2$ **(A)** and NR1 **(B)** associated with the edges of fibroblasts expressing neurexin-1β-CFP were present on neuronal dendrites identified as MAP2-immunoreactive. **(C)** Neurexin-1β-CFP did not induce clustering of GluR1 in contacting MAP2-immunoreactive dendrites; only apparent endogenous clusters were observed (arrow). **(D, E)** Live cell incubation with an antibody against an extracellular epitope of the GABA receptor showed that the nonsynaptic receptor clusters induced by contact with fibroblasts expressing neurexin-1β-CFP are on the cell surface. This example also illustrates that concentrations of neurexin-CFP are sometimes seen in the contacting fibroblasts at concentrations of induced receptors and scaffolding proteins (E). Scale bars A-C 10 μm; D 20 μm; E 5 μm.

Supplemental Figure S8. The agrin LNS domain cannot functionally substitute for the neurexin LNS. **(A-C)** Fibroblasts expressing either full-length agrin **(A, B)** or the neurexin construct with the agrin LNS swap **(C)** co-cultured with hippocampal neurons did not affect the distribution of PSD-95 or gephyrin in contacting dendrites. Only endogenous synaptic clusters of PSD-95 and gephyrin colocalizing with synapsin were observed (arrows). **(D-I)** Inactive neurexin deletion constructs and agrin LNS swap reach the surface of transfected HEK cells as indicated by confocal optical sections. **(D)** Control CFP filled the HEK cells. **(E)** mCFP appeared associated

with the HEK cell plasma membrane. **(F)** Full length neurexin-1β-CFP appeared on the surface of HEK cells, indicated by association with the periphery and labeling of filopodia. **(G)** The neurexin-1β-CFP glycosylation domain deletion showed some intracellular pools but appeared largely cell surface. **(H)** The neurexin-1β-CFP LNS domain deletion showed some intracellular pools but appeared largely cell surface. **(I)** The neurexin construct with the agrin LNS swap also appeared to reach the cell surface efficiently, indicated by association with the periphery and labeling of filopodia. Scale bars 10 μm.

Supplemental Figure S9. Neurexin but not mCFP clusters neuroligins. **(A, B)** Fibroblasts expressing neurexin-1β-CFP co-cultured with hippocampal neurons did not affect the distribution of α-dystroglycan **(A)** or β-dystroglycan **(B)** in contacting dendrites. Clusters of α-dystroglycan were relatively uniformly distributed along MAP2-positive processes, with no particular association with neurexin contact sites **(A),** and only endogenous clusters of β-dystroglycan associated with synapsin-positive terminals (arrow) were observed **(B)**. **(C, D)** Fibroblasts expressing mCFP co-cultured with hippocampal neurons did not affect the distribution of YFP-neuroligin-1 **(C)** or YFP-neuroligin-2 **(D)** in contacting dendrites of transfected neurons. Only YFP-neuroligin-1 and YFP-neuroligin-2 clusters associated with synapsin-positive terminals (arrow) were observed. This is in contrast to the ability of neurexin-CFP to induce non-synaptic clusters of YFP-neuroligin-1 and -2 in a similar assay (Figure 3B, C). **(E)** Fibroblasts expressing neurexin-1β-CFP co-cultured with hippocampal neurons induced clusters of endogenous neuroligin-2 in contacting dendrites. Induced clusters (arrowhead) lacked immunoreactivity for GAD or VGlut, in contrast to endogenous synaptic clusters (arrow). **(F)** Fibroblasts expressing mCFP co-cultured with hippocampal neurons did not affect the distribution of endogenous neuroligin-2 in contacting dendrites. Only neuroligin-2 clusters associated with GAD-positive terminals (arrow) were observed. Scale bar 10 μm.

Supplemental Figure S10. Model for the role of neurexins and neuroligins in glutamate and GABA synapse assembly. Lines indicate reported protein-protein interactions; dashed lines indicate proposed, most likely indirect, interactions. The model is based on the results presented here and previously reported protein localization and interaction data.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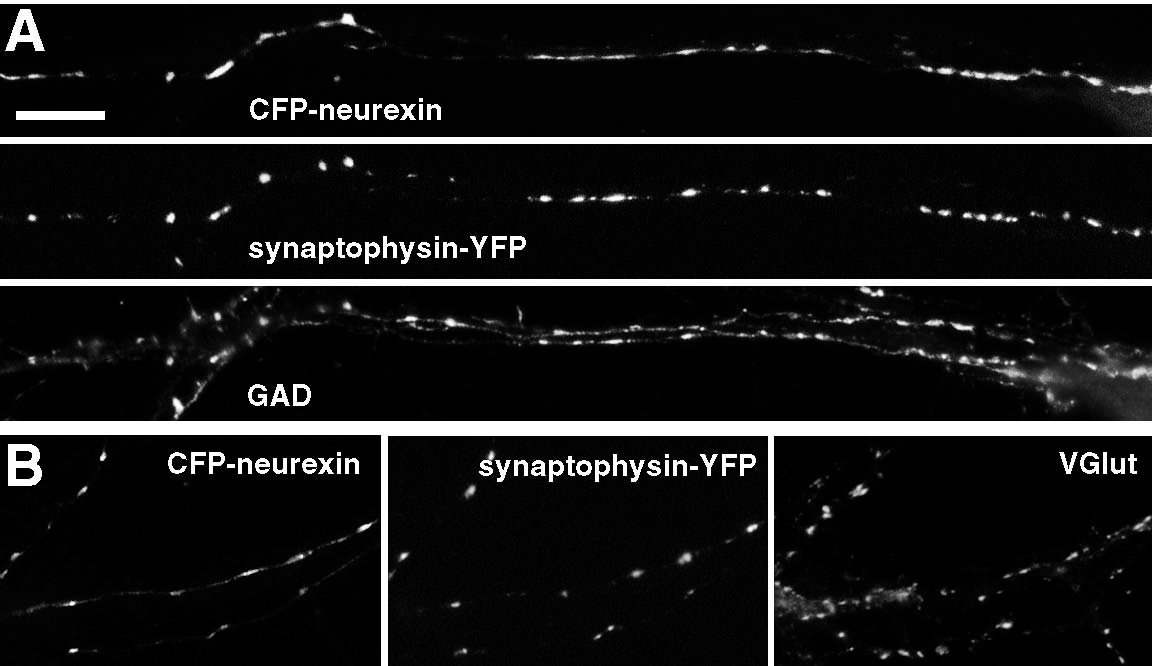
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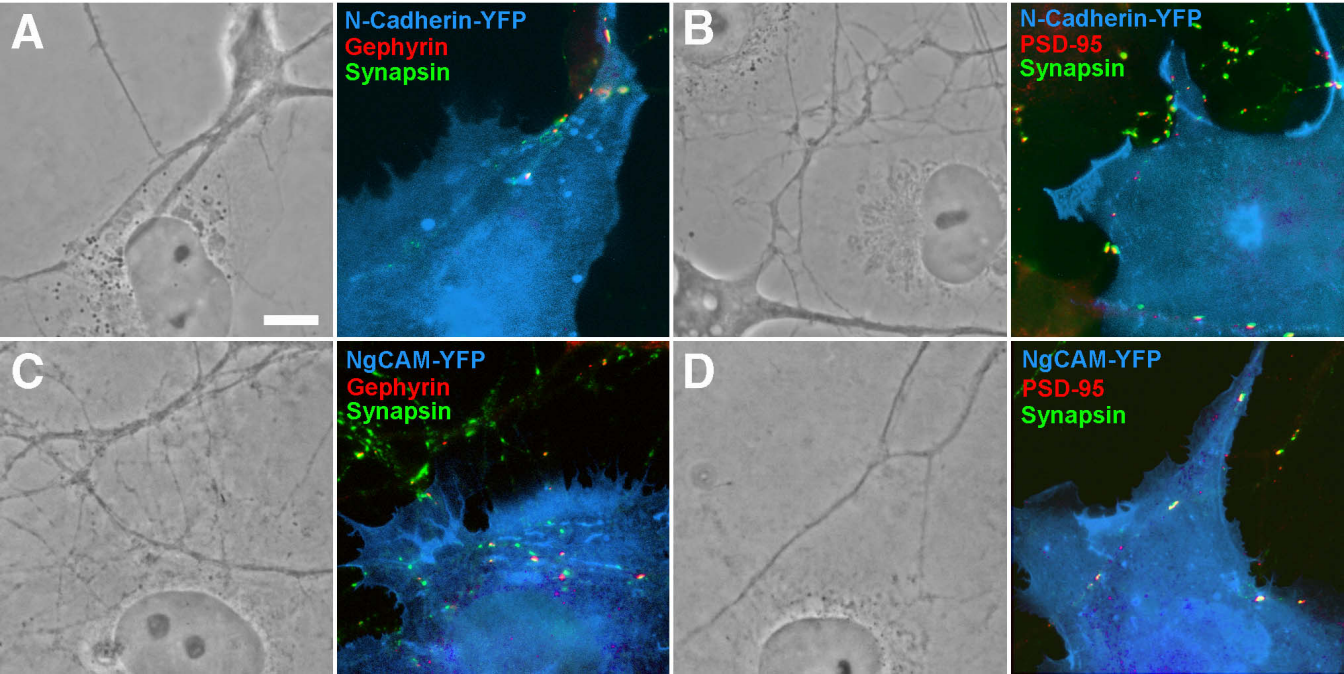
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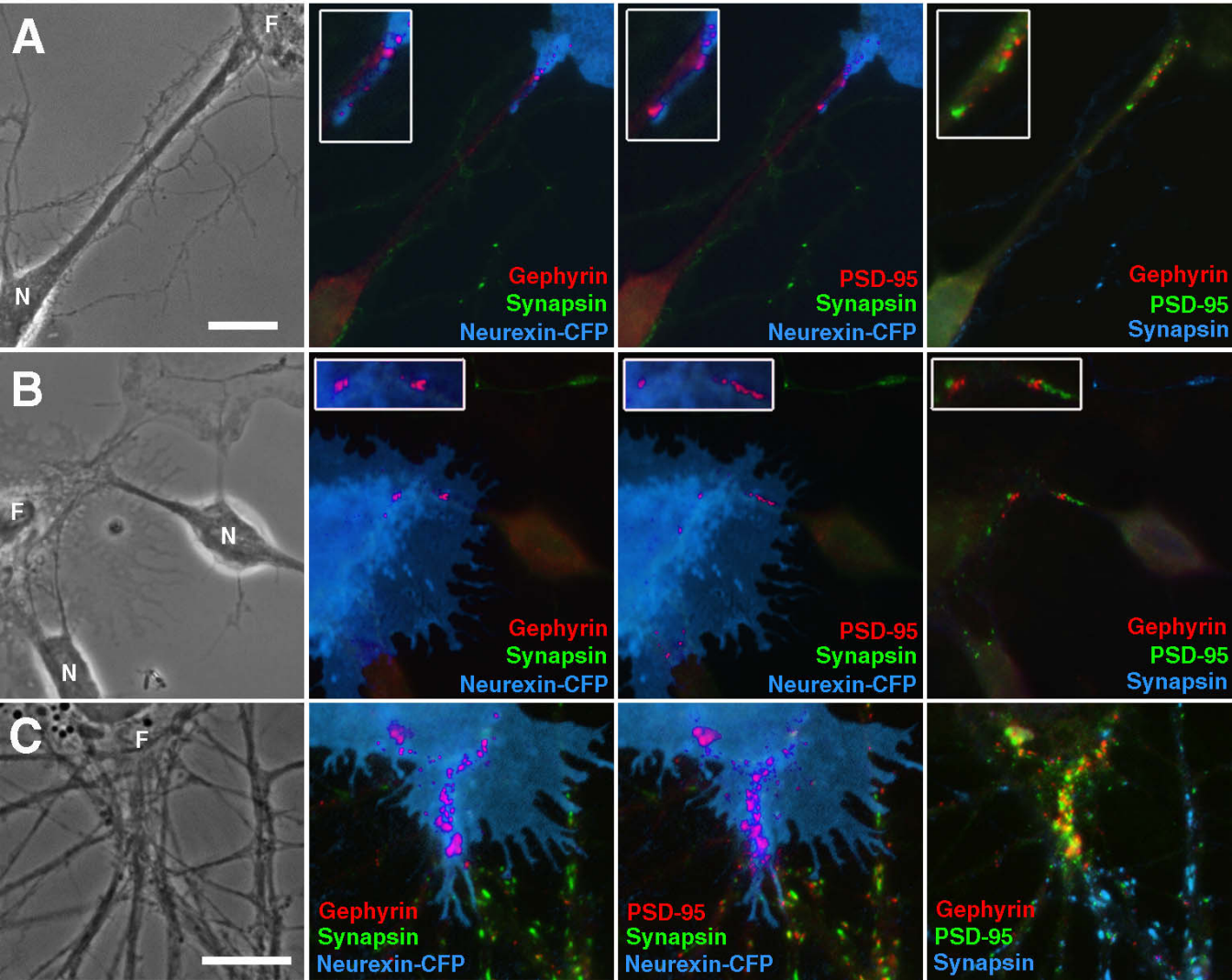
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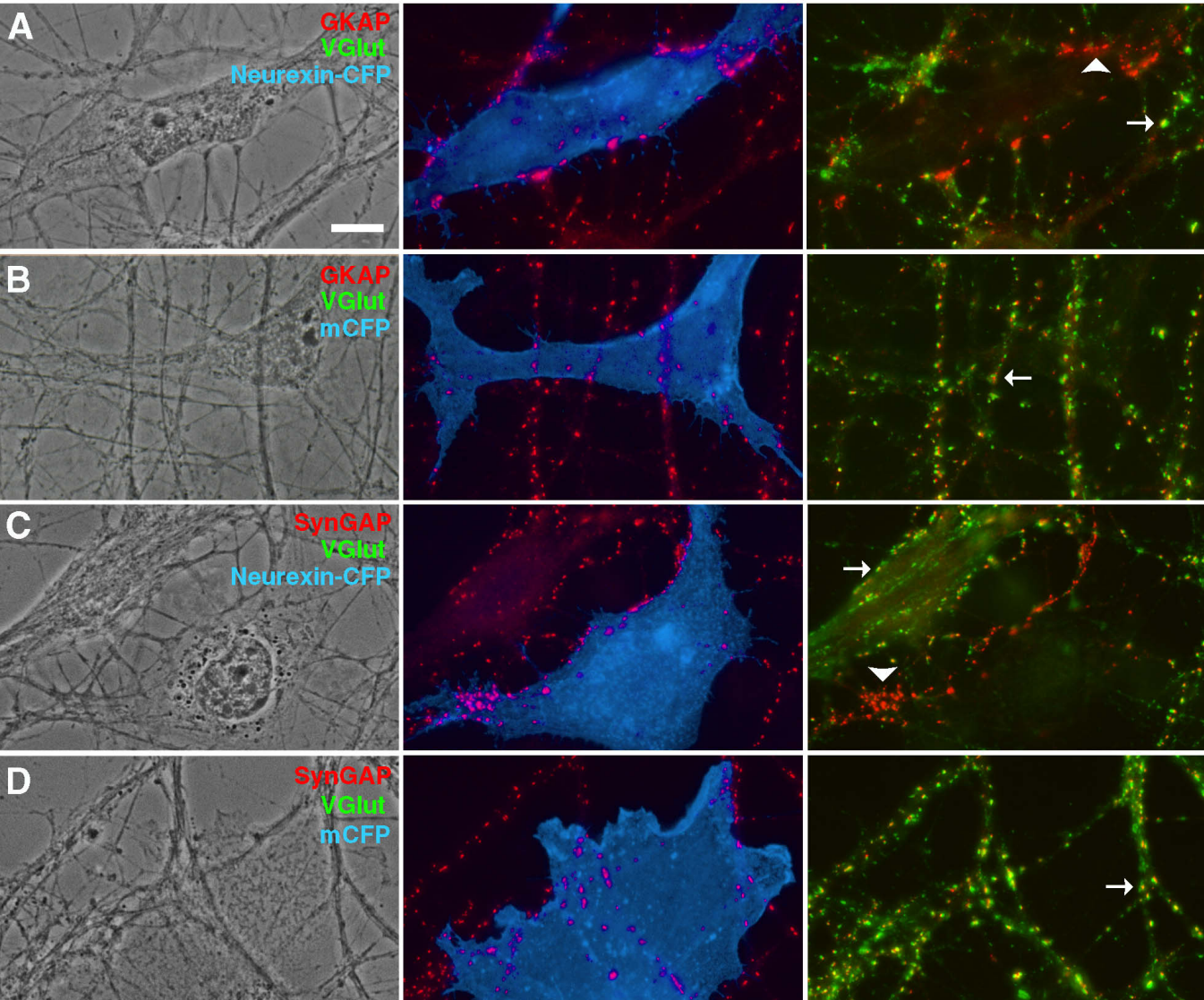
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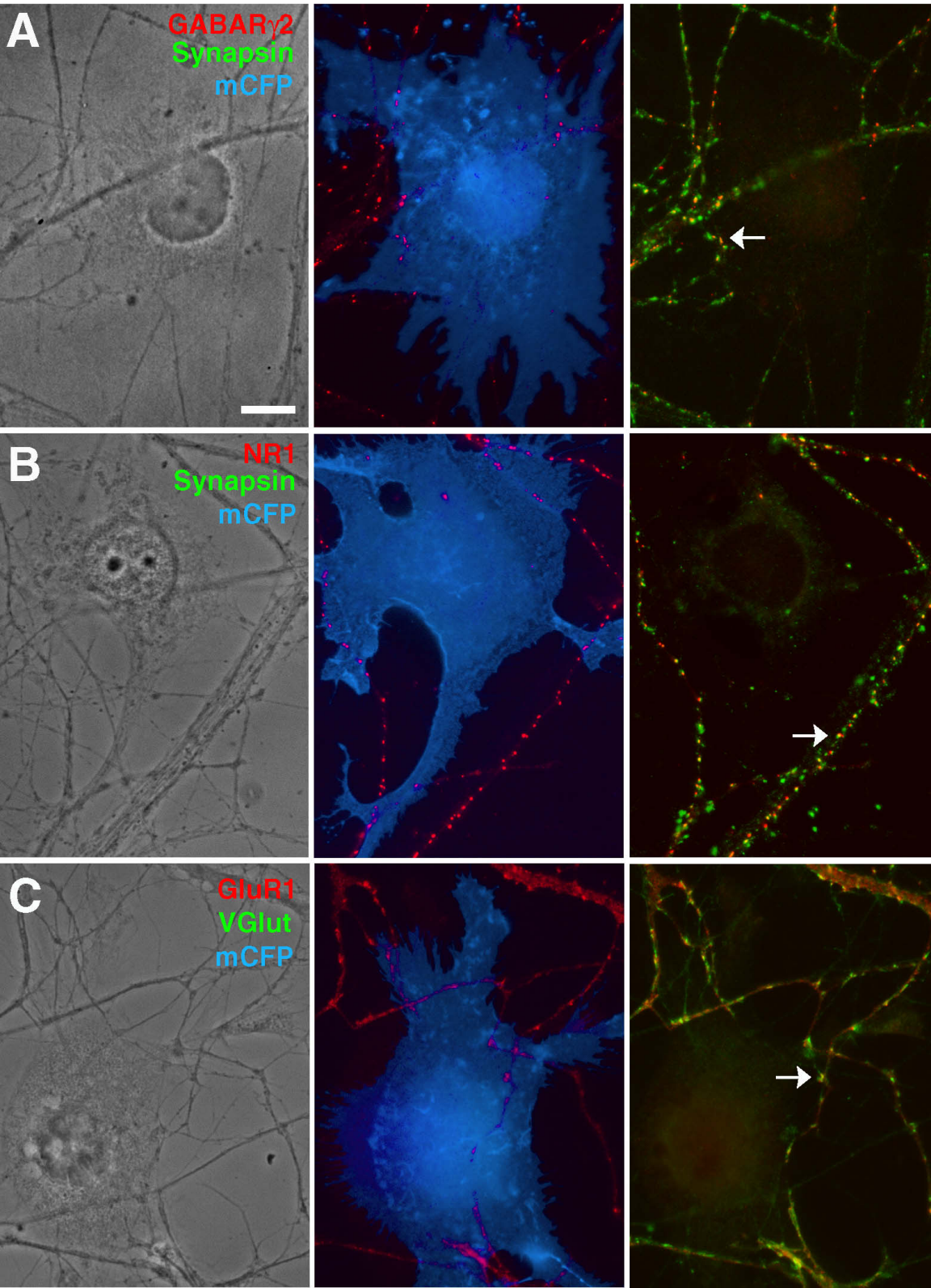
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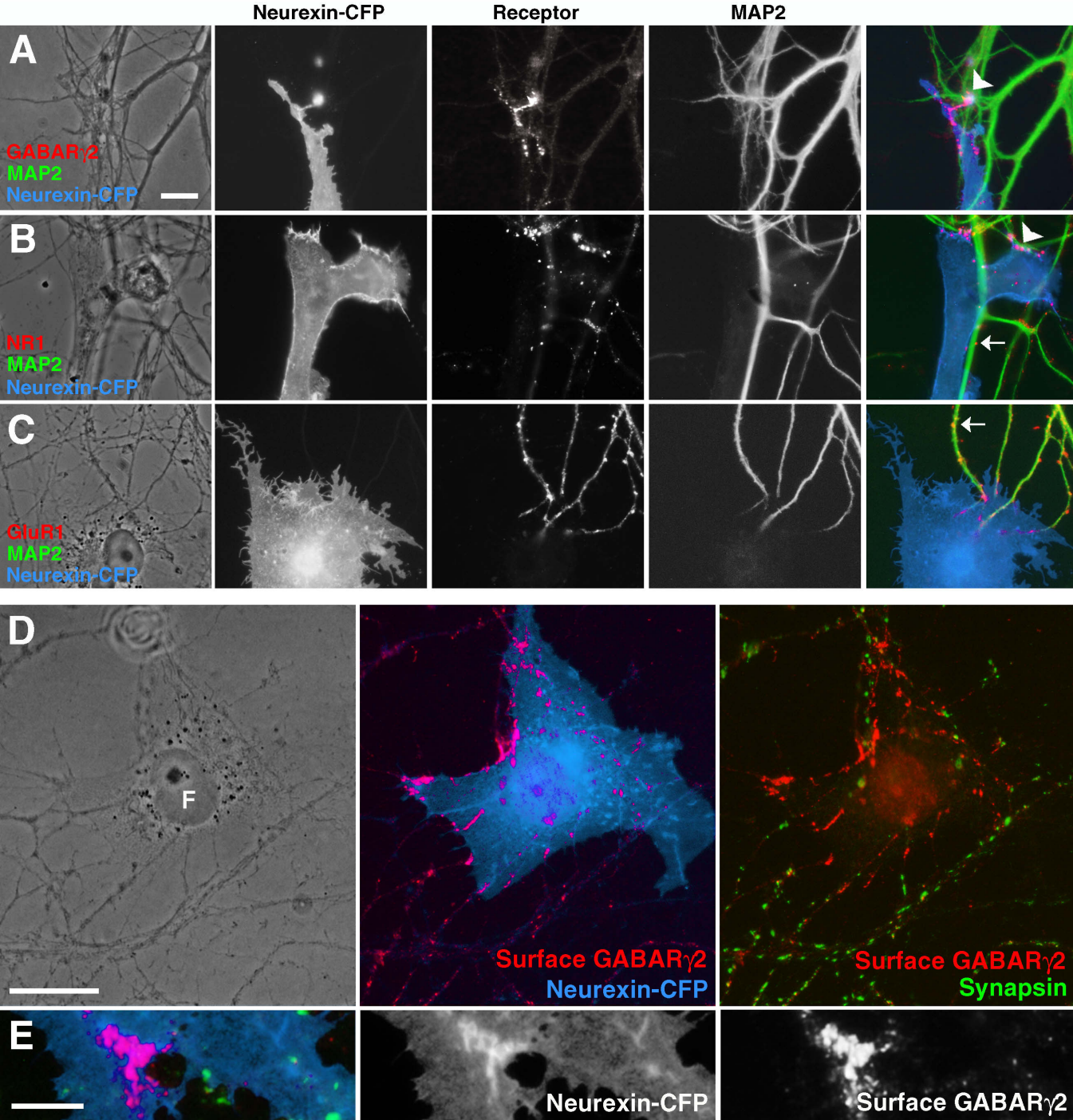
Graf S7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GluR1
MAP2
Neurexin-CFP
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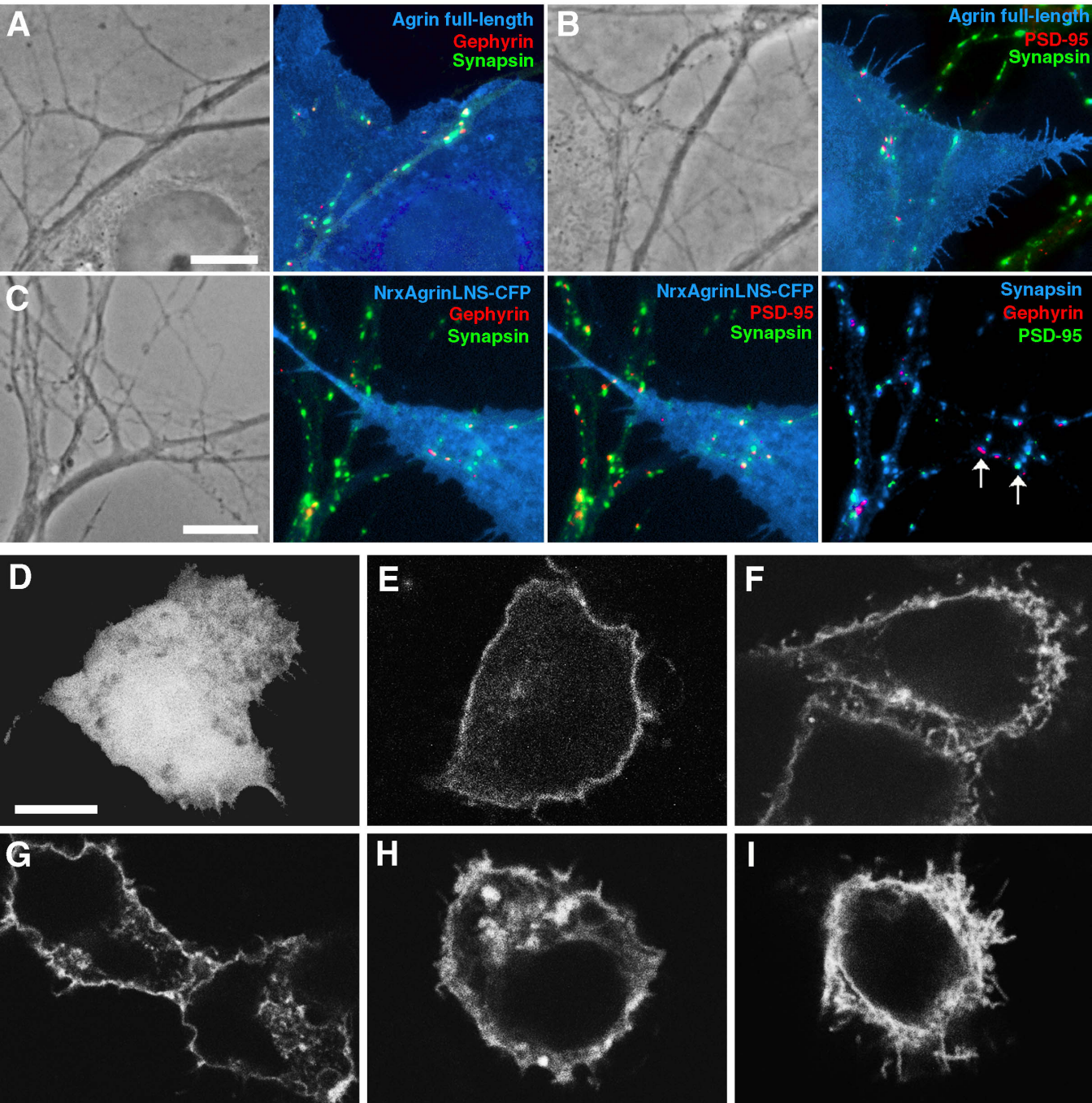
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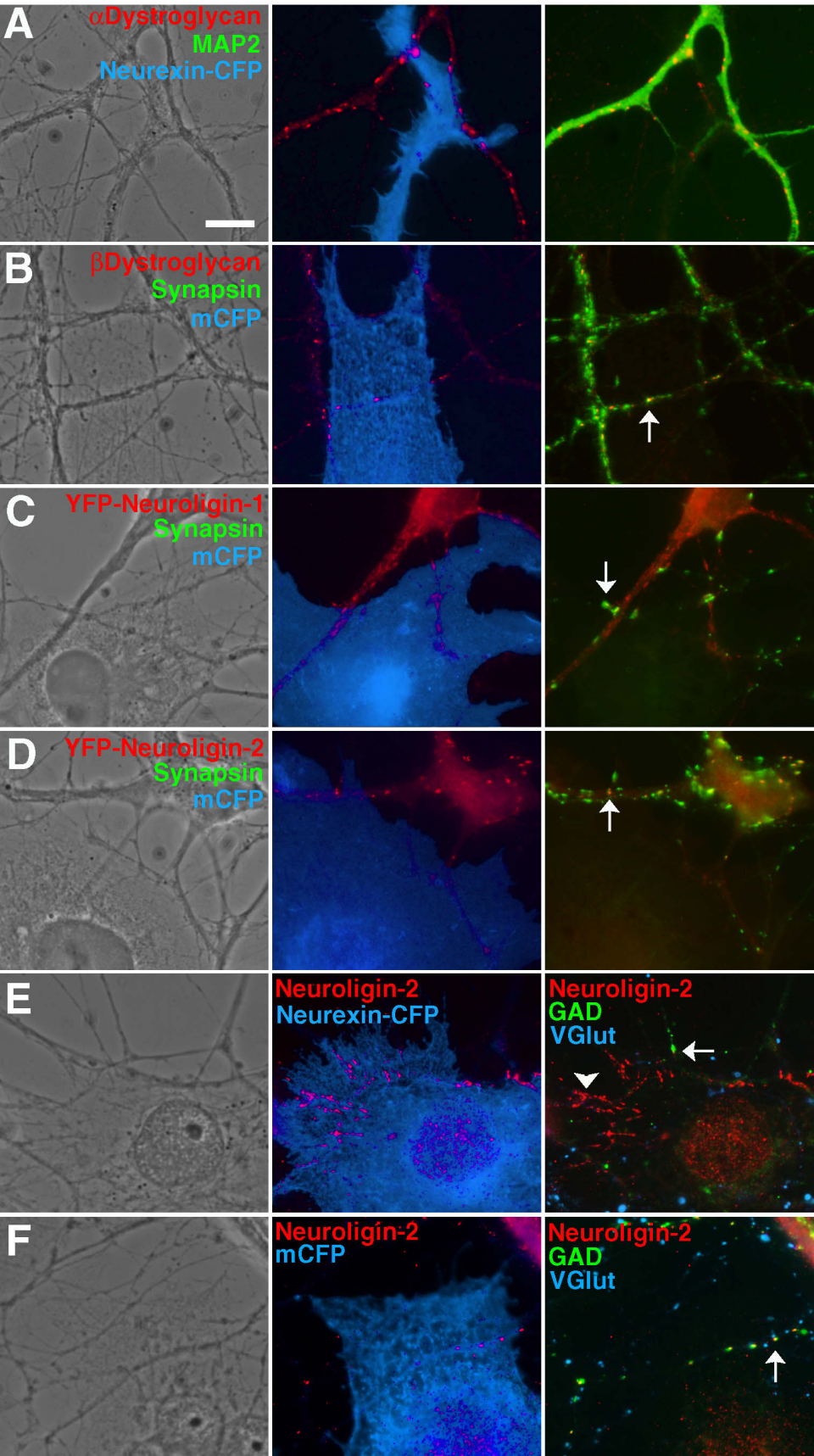
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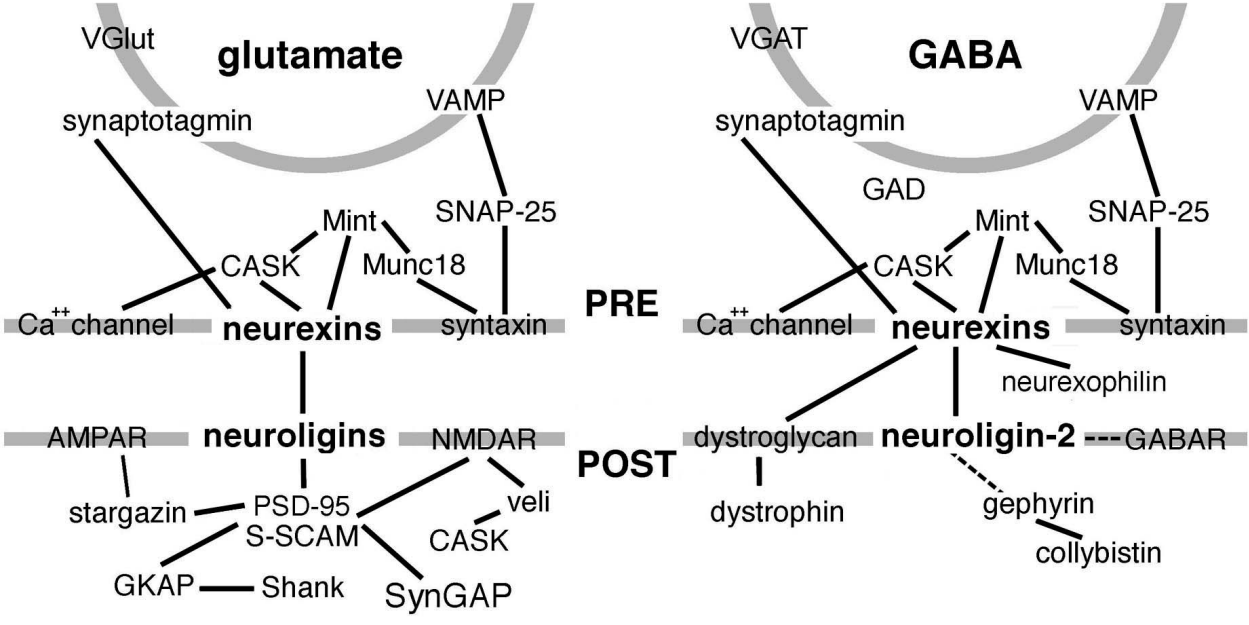
Graf S10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NMDAR
POST
stargazin
PSD-95
S-SCAM
veli
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