SUPPLEMENTARY MATERIAL

Supplemental Figure 1. RNAi constructs selectively target individual PSD-95 family members. (A) Vectors expressing RNAi constructs targeting unique sequences in individual PSD-95 family members were co-expressed in HEK293 cells transfected with constructs expressing a single type of GFP-fusion protein: SAP97, SAP102, PSD-93, or neuroligin (Target Protein). Western blots probed for GFP expression indicated that the RNAi constructs selectively suppressed their targeted protein, without altering the expression of other members of the family. Transfection of vector alone did not alter expression of any of the family members. **(B)** CG neurons grown in culture and transfected with a PSD-95-RNAi (red) showed reduced expression of PSD-95 (green) compared to adjacent, untransfected neurons. Left: Merge of PSD-94-RNAi and PSD-95 staining. Right: PSD-95 staining alone. Scale Bar: 10 μm.

Supplemental Figure 2. PSD-95/SAP102-RNAi expression reduces PDZ-protein levels. CG neurons were transfected with RNAi constructs targeting individual PSD-95 family members. **(A)** CG neurons expressing RFP (top), PSD-95-RNAi (middle), or PSD-95/SAP102-RNAi (bottom, red) were immunostained with an antibody that recognizes all PSD-95 family members (PDZ-Protein; green). **(B)** Knockdown of PSD-95 and SAP102 together significantly reduced PDZ-protein levels in CG neurons (**$p \leq 0.01$ by ANOVA with Dunnet's post-hoc test; 4-7 cultures/condition). Scale bar: 5 μm.

Supplemental Figure 3. Knockdown of PSD-95/SAP102 has little effect on the amount or distribution of the presynaptic marker synaptotagmin 1. CG neurons were transfected with

PSD-95/SAP102-RNAi (P95/S102-RNAi), or vector expressing RFP as a control (Con), and immunostained for the presynaptic marker synaptotagmin 1 (Syntag). Knockdown of PSD-95/SAP102 did not alter total synaptotagmin levels, synaptotagmin puncta size, or the number of synaptotagmin puncta aligned with postsynaptic nAChR clusters on the soma **(A)** or on the proximal neurites **(B)**. Results have been normalized to values found for untransfected neurons in the same field of view (n = 10-11 cultures/condition).

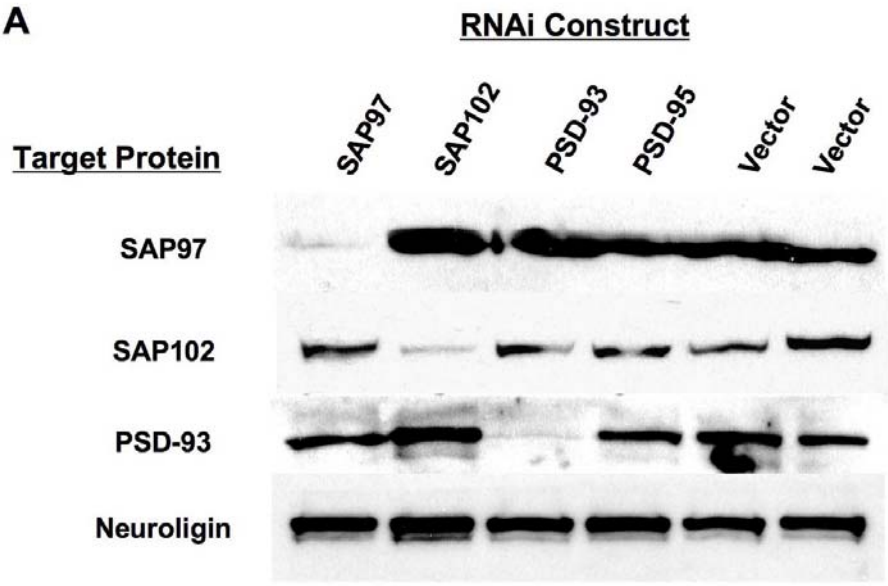
Supplemental Figure 4. Paired-pulse stimulation reveals PPD at CG synapses under control conditions. Paired EPSCs were evoked in patch-clamped CG neurons at intervals of 20-200 ms (50–5 Hz, respectively). Top: Responses to paired stimuli (arrows; S1, S2) at 5 (left) and 50 Hz (right). Bottom: Mean paired-pulse responses ± SEM. $^{**}p < 0.01$, $^{***}p < 0.001$ using a paired Student's t-test (28-31 cultures/frequency).

Supplemental Figure 5. PSD-95/SAP102-RNAi expression in the postsynaptic cell does not change either the readily releasable pool of transmitter or the vesicle re-filling rate at release sites contacting the cell. CG neurons were patch-clamped in the presence of TTX and given a 4-second focal application of hyperosmotic aCSF (containing 0.5 M sucrose) twice (App1 and App2) separated by 1 minute. Top: Responses in a control neuron (upper) and a neuron expressing PSD-95/SAP102-RNAi (lower). Bottom: Mean ± SEM total charge flux (pC) during a 5 second period starting with the initiation of the sucrose challenge.

Supplemental Figure 6. Distributions of SAP97-GFP and SAP102-GFP differ substantially at the cell surface. CG neurons transfected with SAP97-GFP (left) or SAP102-GFP (right)

were immunostained for GFP 7 days later. SAP97-GFP was distributed primarily along the cell membrane, comprising numerous puncta together with intervening diffuse distributions. SAP102-GFP was localized at the cell membrane in discreet clusters. Scale bar: 5 μm.

Supp. Fig. 1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PSD-95
Vector
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SAP97
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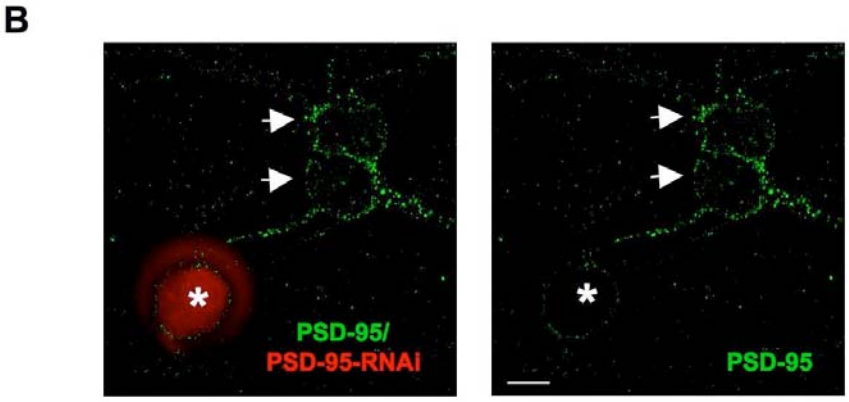
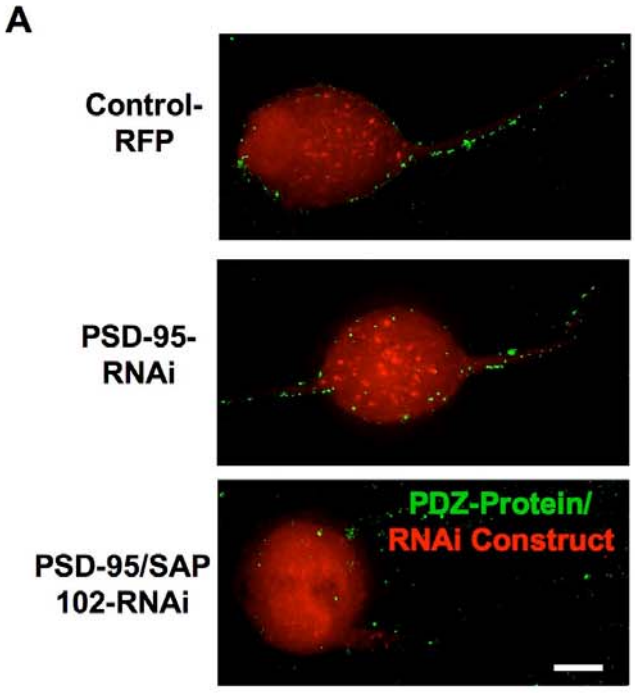
PSD-93
Neuroligin


B
PSD-95/
PSD-95-RNAi
PSD-95


A
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RNAi
PSD-95/SAP
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PDZ-Protein/
RNAi Construct
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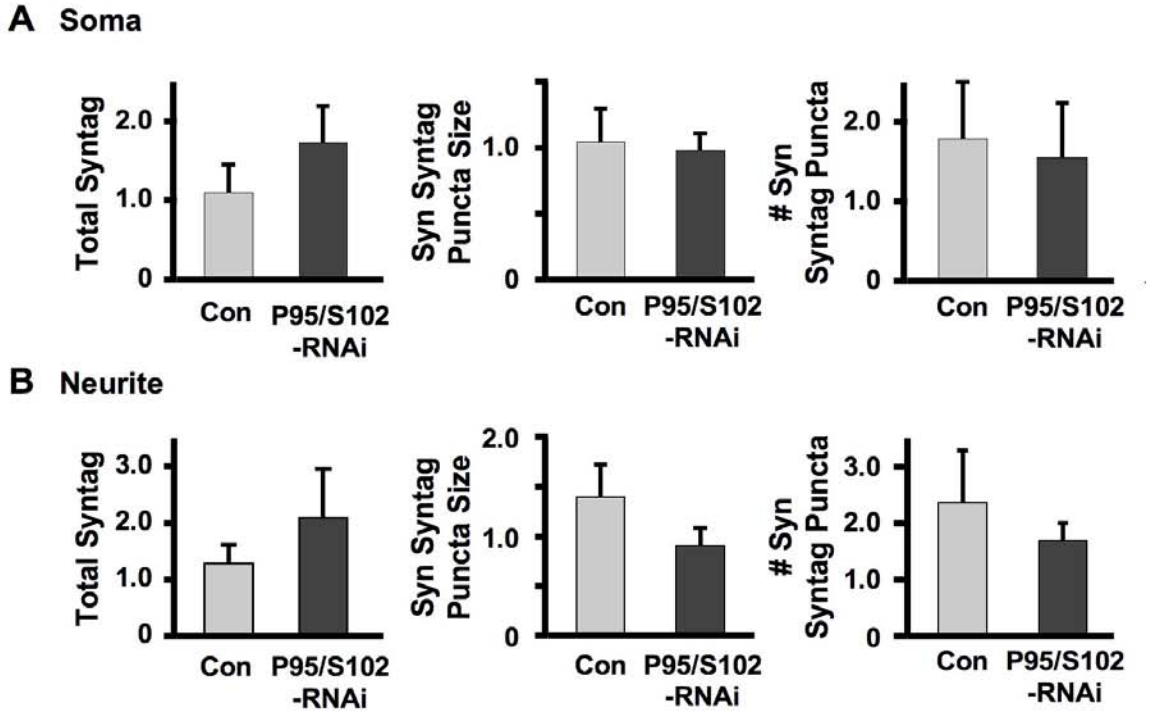
100
50
0
**
Con-
RFP
P-95-
RNAi
P-95/S102
-RNAi

Supp. Fig. 3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B Neurite
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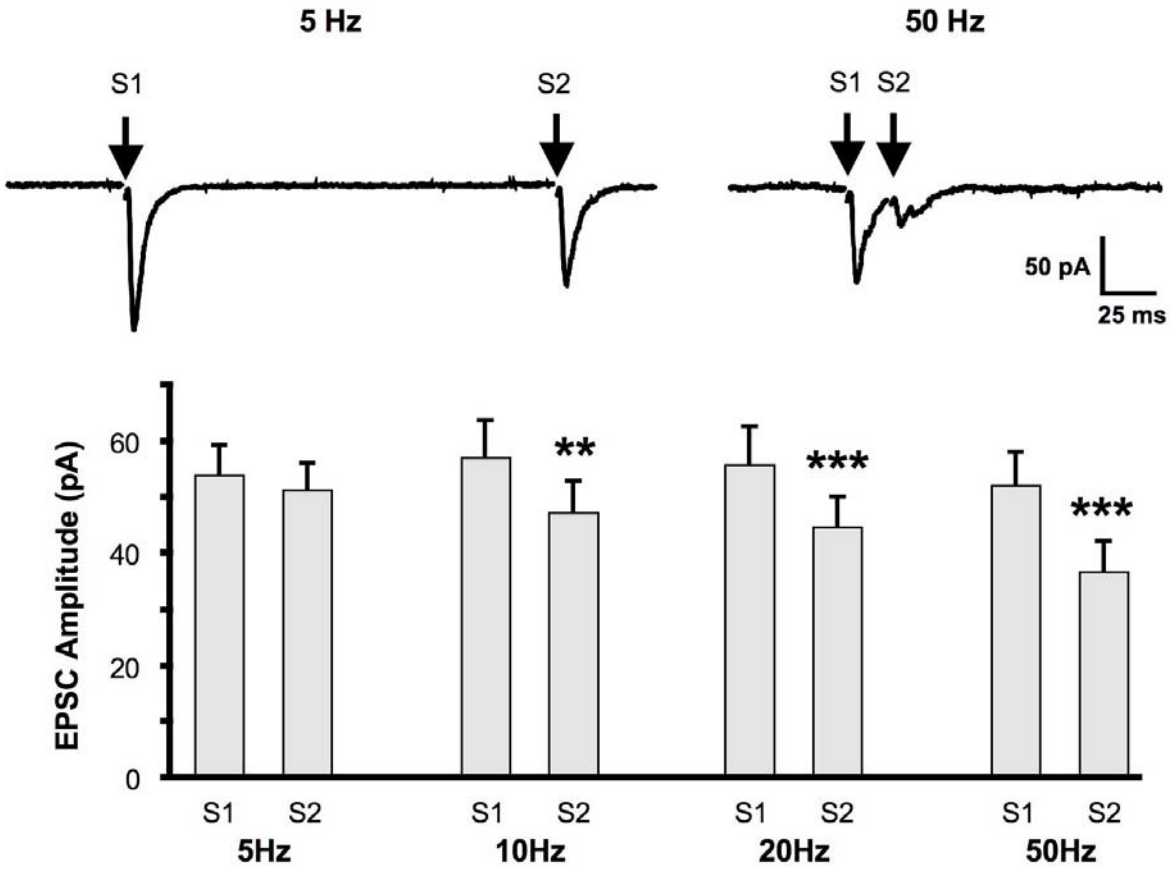
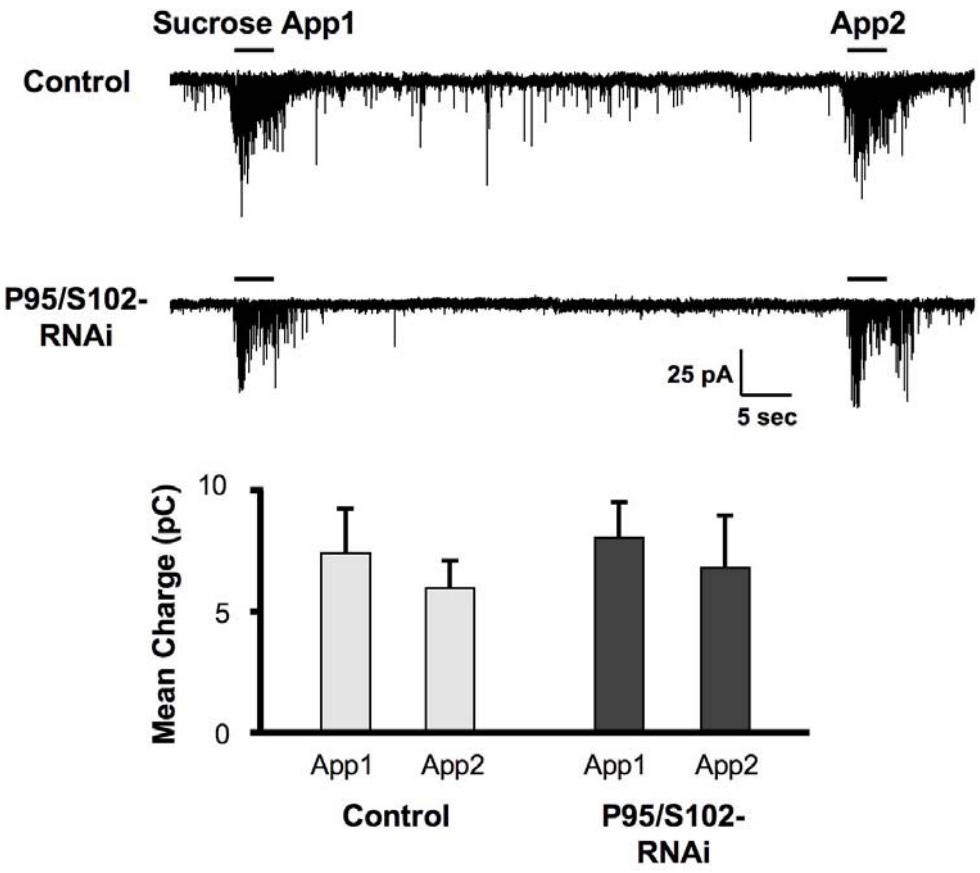
Supp. Fig. 4


5 Hz
50 Hz
S1
S2
S1 S2
50 pA
25 ms
EPSC Amplitude (pA)
60
40
20
0
**
***
***
S1 S2
5Hz
S1 S2
10Hz
S1 S2
20Hz
S1 S2
50Hz

Supp. Fig. 5


Sucrose App1
App2
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P95/S102-RNAi
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5 sec
Mean Charge (pC)
10
5
0
App1
App2
App1
App2
Control
P95/S102-RNAi

Supp. Fig. 6