Pettem et al., http://www.jcb.org/cgi/content/full/jcb.201206028/DC1

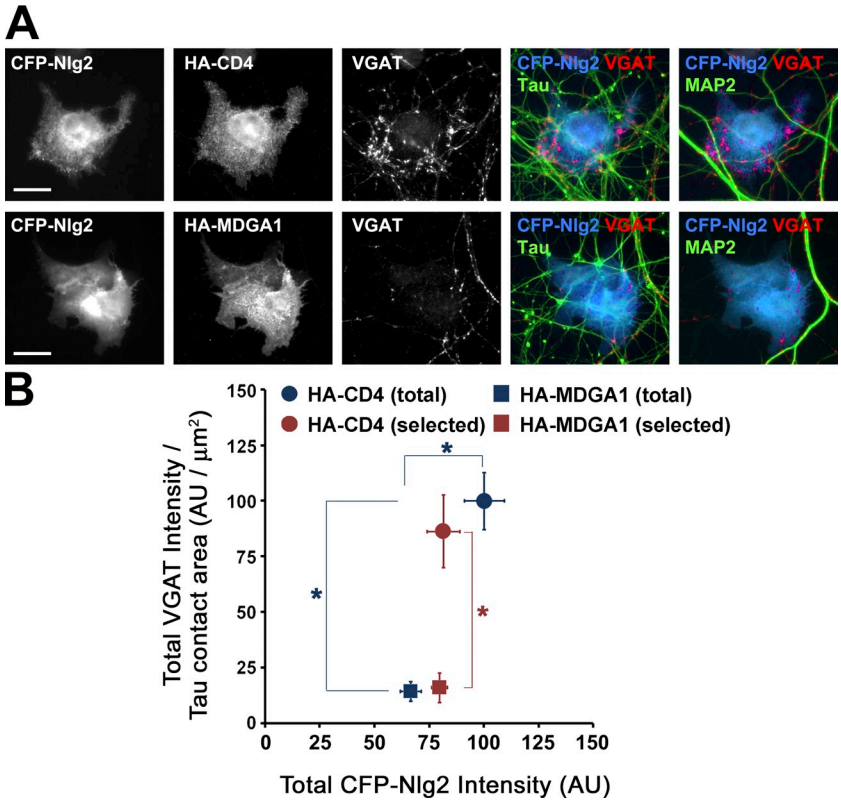

Figure S1. **MDGA1 suppresses the inhibitory presynaptic differentiation induced by neuroligin-2.** (A) COS7 cells were cotransfected with CFP–neuroligin-2 (CFP-Nlg2) and HA-CD4 (top panels) or HA-MDGA1 (bottom panels), then co-cultured with hippocampal neurons. COS7 cells expressing HA-CD4 with CFP-Nlg2 (top) induced robust clustering of vesicular GABA transporter (VGAT) in contacting axons, whereas COS7 cells coexpressing HA-MDGA1 with CFP-Nlg2 (bottom) showed diminished VGAT clustering on tau-positive axon contact sites. (B) Quantification of total integrated intensity of VGAT immunofluorescence not associated with MAP2 and associated with COS7 cells coexpressing HA-CD4 or HA-MDGA1 with CFP-Nlg2, divided by tau-positive axon contact area (shown in Y-axis) and quantification of total fluorescent intensity of CFP-Nlg2 on the same COS7 cells (shown in X-axis). Blue symbols represent the average of the data based on all analyzed COS7 cells (n = 20 cells each). Red symbols represent the average of the data based on the selected COS7 cells with similar total CFP-Nlg2 expression level (n = 13 cells each). t test; *, $P < 0.0001$. Data are mean ± SEM. Bars, 20 µm.

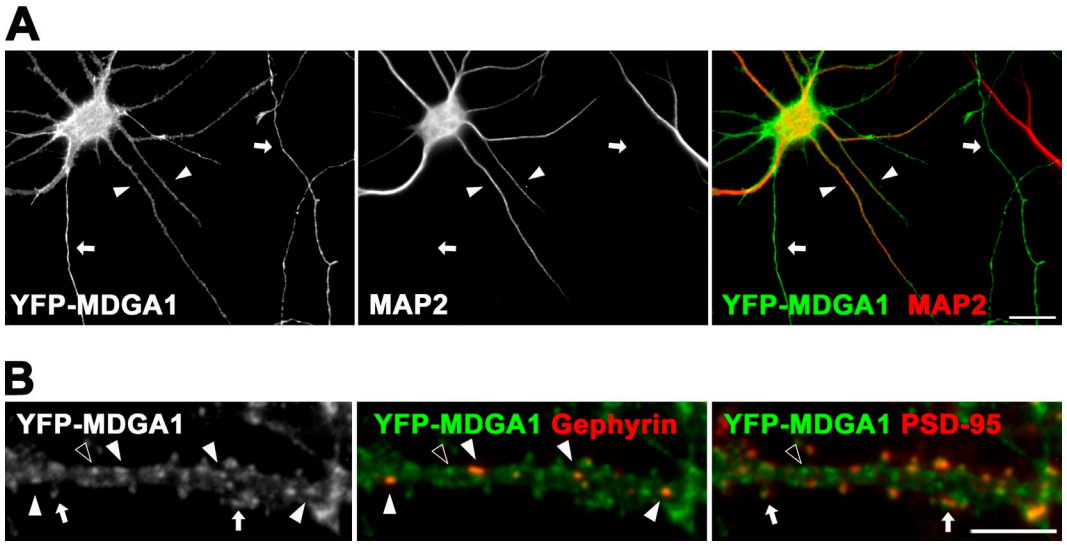

Figure S2. **Subcellular localization of recombinant MDGA1 in cultured hippocampal neurons.** (A) Recombinant extracellularly YFP-tagged MDGA1 (YFP-MDGA1) expressed at low level in cultured hippocampal neurons at 16 DIV and labeled for surface YFP was detected on dendrites, MAP2-positive neurites (arrowheads), and on axons, MAP2-negative neurites (arrows). (B) YFP-MDGA1 was sometimes observed in a punctate pattern in dendrites. Some YFP-MDGA1 clusters in dendrites were colocalized with gephyrin clusters (red in middle, filled arrows), some with PSD-95 clusters (red at right, arrows), and a few YFP-MDGA1 clusters did not overlap with either gephyrin or PSD-95 (open arrowhead). Bars: (A) 20 µm; (B) 5 µm.

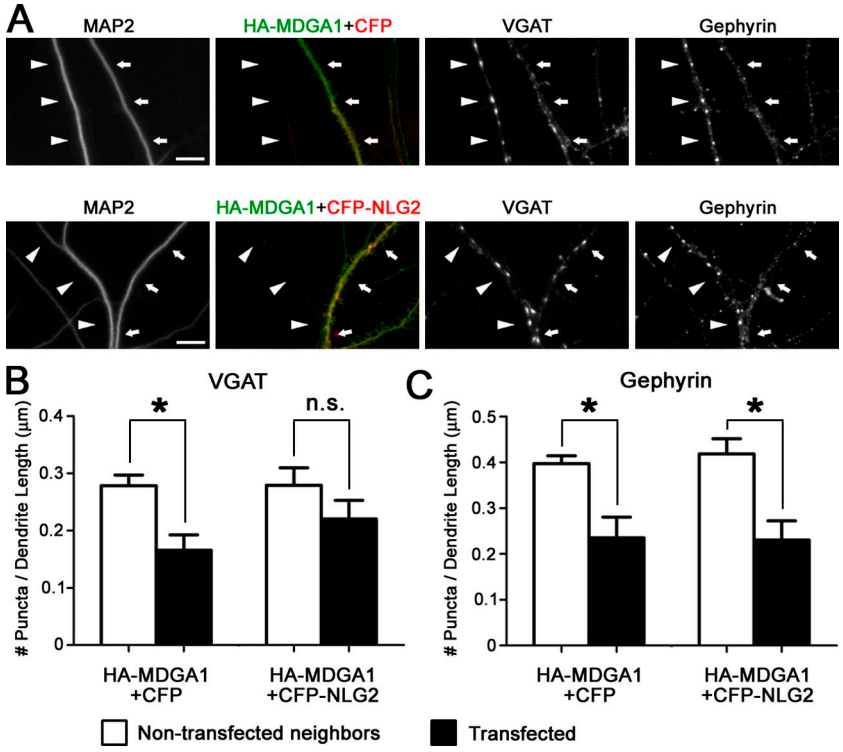

Figure S3. **Neuroligin-2 overexpression partially rescues the effects of MDGA1 overexpression.** Cultured hippocampal neurons were transfected at 9 DIV with HA-MDGA1 and either CFP–neuroligin-2 (CFP-NLG2) or CFP as a control, then fixed at 15 DIV and immunostained for the indicated synaptic markers plus the HA tag and MAP2 as a dendrite marker. (A) Clusters of VGAT and gephyrin were hardly detected along dendrites expressing HA-MDGA1 and CFP (top, arrows), whereas such clusters were more readily detected along nontransfected neighboring dendrites (top, arrowheads). In contrast, VGAT clusters along dendrites expressing HA-MDGA1 and CFP-NLG2 (bottom, arrows) appeared nearly comparable to those along nontransfected neighboring dendrites (bottom, arrowheads), although gephyrin clusters appeared reduced (bottom, arrows). (B and C) Quantitation of cluster number per dendrite length for VGAT (B) and gephyrin (C) in neurons overexpressing HA-MDGA1 with CFP or CFP-NLG2. ANOVA, $P < 0.05$ for B and $P < 0.01$ for C; $n = 11$ cells each; *, $P < 0.05$ in post-hoc Bonferroni test. n.s. indicates no significant difference in post-hoc Bonferroni test. Data are mean ± SEM. Bar, 5 µm.

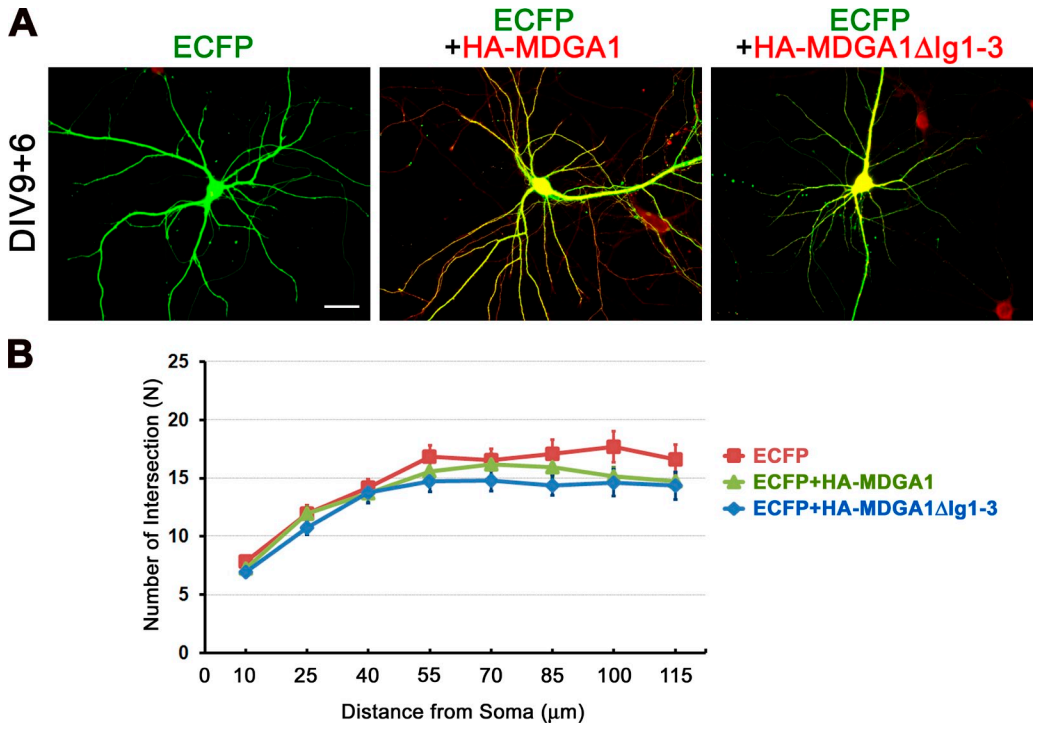

Figure S4. **MDGA1 overexpression has no significant effect on dendritic arborization.** (A) Cultured hippocampal neurons were transfected at 9 DIV with a vector expressing ECFP (left) or cotransfected with an ECFP-expressing vector and either a vector expressing extracellularly HA-tagged MDGA1 (HA-MDGA1, middle) or HA-MDGA1 lacking first three Ig domains (HA-MDGA1ΔIg1-3, right), as in Fig. 8. Neurons were labeled with anti-GFP antibody (recognizes ECFP) and analyzed at 15 DIV. (B) Sholl analysis of the transfected neurons. n = 28 cells each. ANOVA, $P > 0.11$ at each distance from soma. Data are mean ± SEM. Bar, 20 μm.

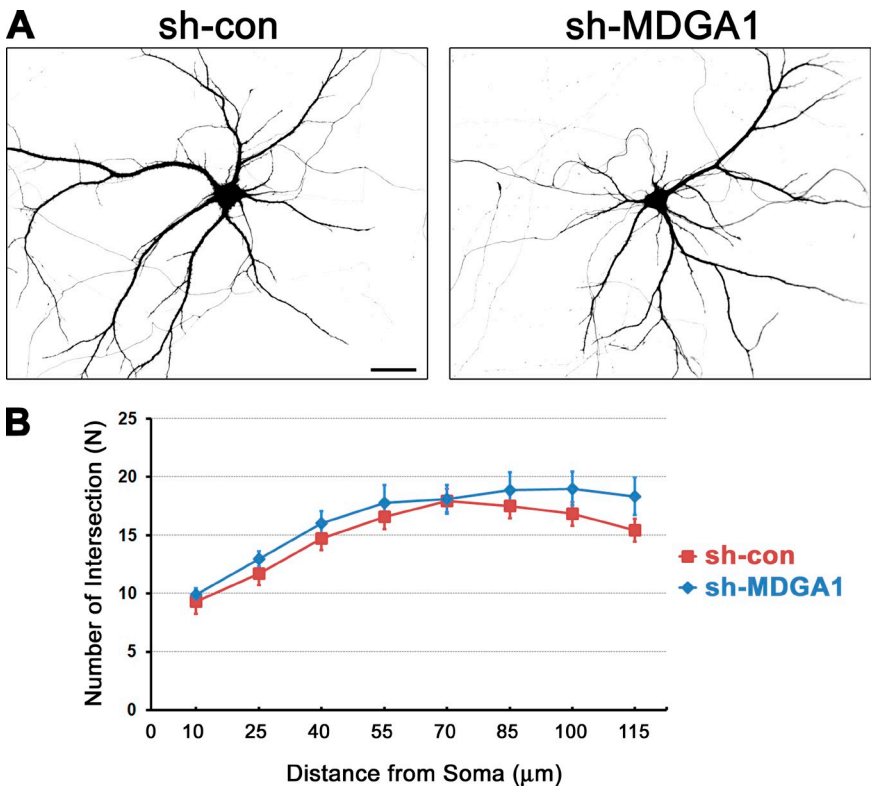

Figure S5. **MDGA1 knockdown has no significant effect on dendritic arborization.** (A) Cultured hippocampal neurons were transfected at 9 DIV with a vector coexpressing ECFP and either control shRNA (sh-con) or shRNA sequence that was able to knock down MDGA1 (sh-MDGA1), as in Fig. 9. Neurons were labeled with anti-GFP antibody (recognizes ECFP) and analyzed at 15 DIV. (B) Sholl analysis of neurons expressing sh-con or sh-MDGA1. $n = 29$ and 25 cells for sh-con and sh-MDGA1, respectively. *t* test, $P > 0.16$ at each distance from soma. Data are mean ± SEM. Bar, 20 µm.