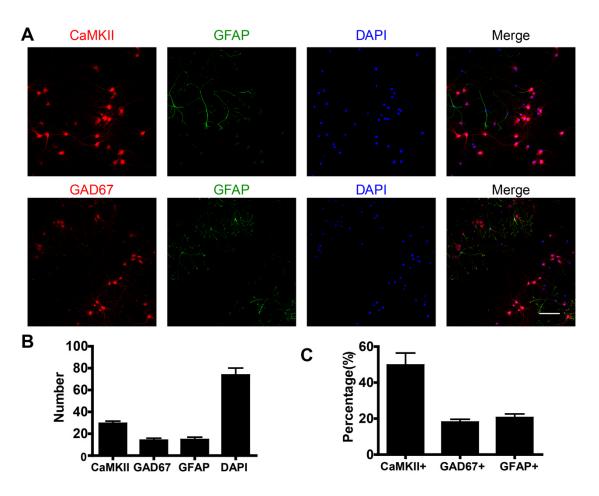
Electronic supplementary material

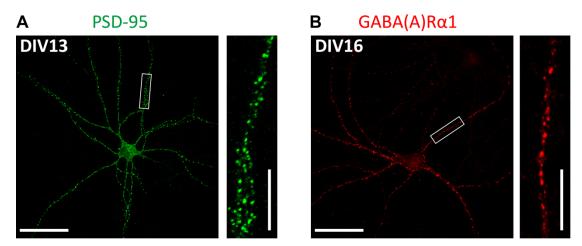
## Neuroligins Differentially Mediate Subtype-Specific Synapse Formation in Pyramidal Neurons and Interneurons

Qiang-Qiang Xia<sup>1</sup>, Jing Xu<sup>1</sup>, Tai-Lin Liao<sup>1</sup>, Jie Yu<sup>1</sup>, Lei Shi<sup>2</sup>, Jun Xia<sup>3</sup>, Jian-Hong Luo<sup>1\*</sup>, Junyu Xu<sup>1\*</sup>



**Fig. S1** Antibody testing in cultured cortical neurons as neuronal markers. **A** Representative images of neuronal markers. GFAP, astrocyte marker; DAPI, nuclear stain; CaMKII, pyramidal neuron marker; GAD67, interneuron marker, scale bar, 100 μm. **B** Numbers of pyramidal neurons, interneurons, astrocytes, and total cells in one microscope field. **C** Percentages of pyramidal neuron (CaMKII<sup>+</sup>), interneurons

(GAD67<sup>+</sup>), and astrocytes (GFAP<sup>+</sup>) in culture.



**Fig. S2** Antibody testing in cultured cortical neurons as synapse markers. **A** Representative images for excitatory synapse marker PSD-95. **B** Representative images for the GABAergic synapse marker GABA(A)R $\alpha$ 1. Scale bars, 50  $\mu$ m for the original image and 10  $\mu$ m for the enlarged image.

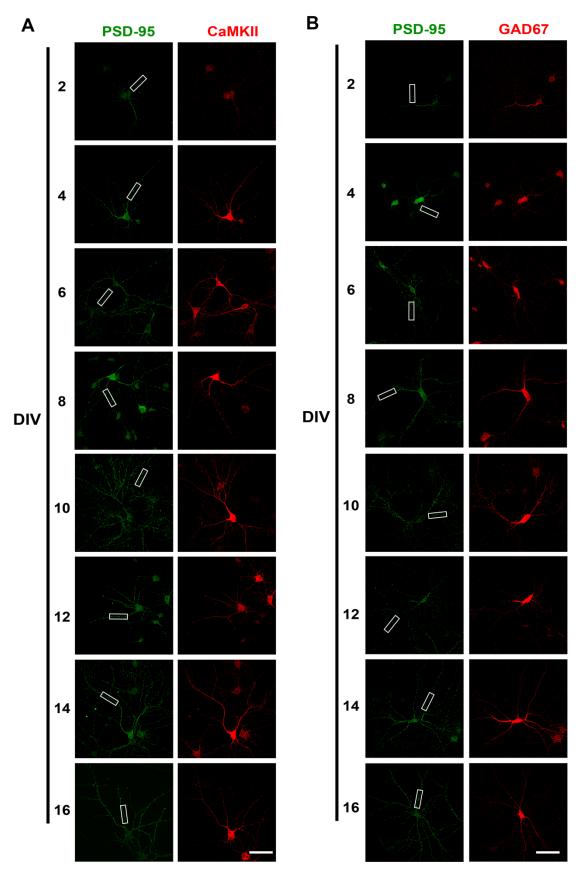
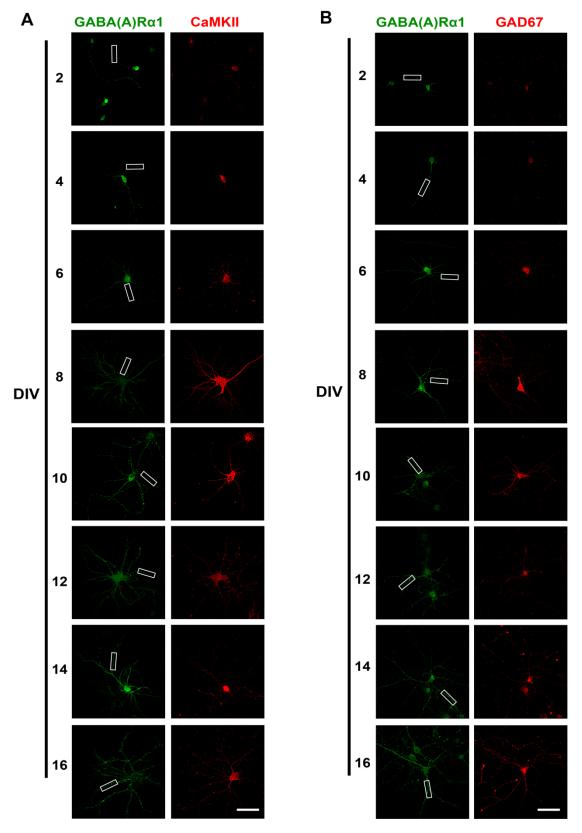


Fig. S3 Development of excitatory synapses in cultured cortical neurons. A, B

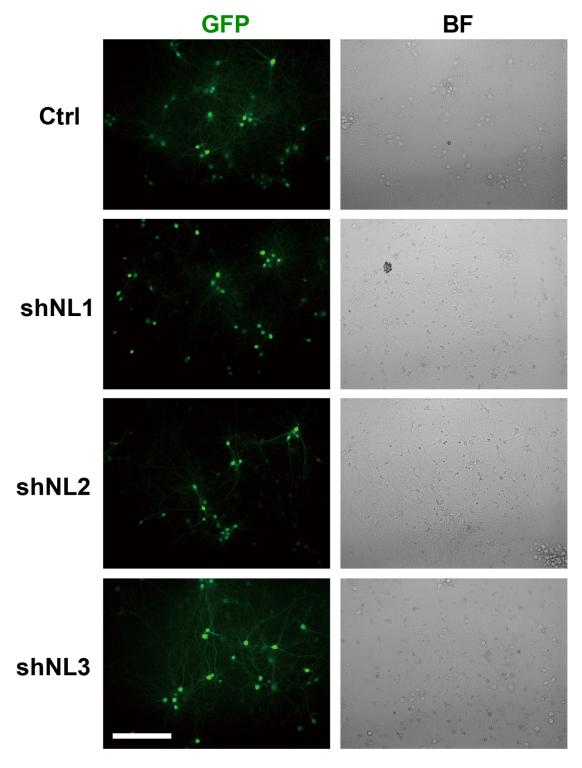
Representative images of PSD-95 puncta from DIV 2 to DIV 16 in cultured cortical

pyramidal neurons ( $\bf A$ ) and interneurons ( $\bf B$ ). The frames are selected regions of the PSD-95 signal shown in Fig. 1A. CaMKII, pyramidal neuron marker; GAD67, interneuron marker; scale bars, 50  $\mu m$ .



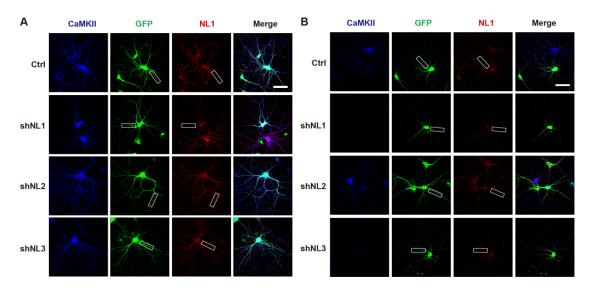
**Fig. S4** Development of GABAergic synapses in cultured cortical neurons. **A**, **B** Representative images of GABA(A)Rα1 puncta from DIV 2 to DIV 16 in cultured cortical pyramidal neurons and interneurons. The frames are selected regions of the

GABA(A)R $\alpha$ 1 signal shown in **Fig. 1A**. CaMKII, pyramidal neuron marker; GAD67, interneuron marker; scale bars, 50  $\mu$ m.

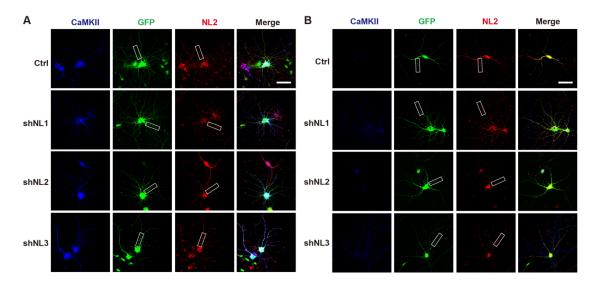


**Fig. S5** Viral infection efficiency test in cultured cortical neurons. Representative images of lentivirus infection in cultured cortical neurons. GFP signal indicating the

successful infection of the neuron by lentivirus. BF, bright-field view. DIV 13. Scale bar,  $50 \mu m$ .

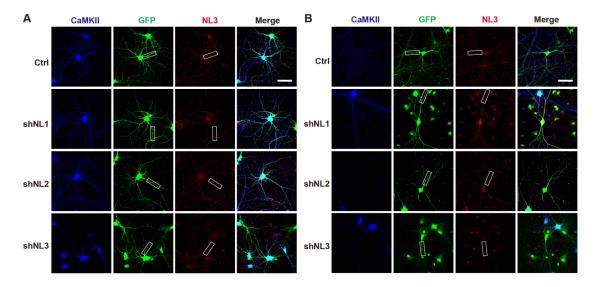


**Fig. S6** NL1 puncta in cultured cortical neurons after shNL lentiviral infection. **A**, **B** Representative images of NL1 puncta in cultured cortical pyramidal neurons (**A**) and interneurons (**B**). CaMKII, pyramidal neuron marker; scale bars, 50 μm. The frames are the selected regions whose enlarged images are shown in **Fig. 3**.



**Fig. S7** NL2 puncta in cultured cortical neurons after shNL lentiviral infection. **A**, **B** Representative images of NL2 puncta in cultured cortical pyramidal neurons (**A**) and interneurons (**B**). CaMKII, pyramidal neuron marker; scale bars, 50 μm. The frames

are the selected regions whose enlarged images are shown in Fig. 3.



**Fig. S8** NL3 puncta in cultured cortical neurons after shNL lentiviral infection. **A**, **B** Representative images of NL3 puncta in cultured cortical pyramidal neurons (**A**) and interneurons (**B**). CaMKII, pyramidal neuron marker; scale bars, 50 μm. The frames are the selected regions whose enlarged images are shown in **Fig. 3**.