# Genetic deletion of $GABA_A$ receptors reveals distinct requirements of neurotransmitter receptors for GABAergic and glutamatergic synapse development

Jingjing Duan, Saurabh Pandey, Tianming Li, David Castellano, Xinglong Gu, Jun Li, Qingjun Tian, Wei Lu

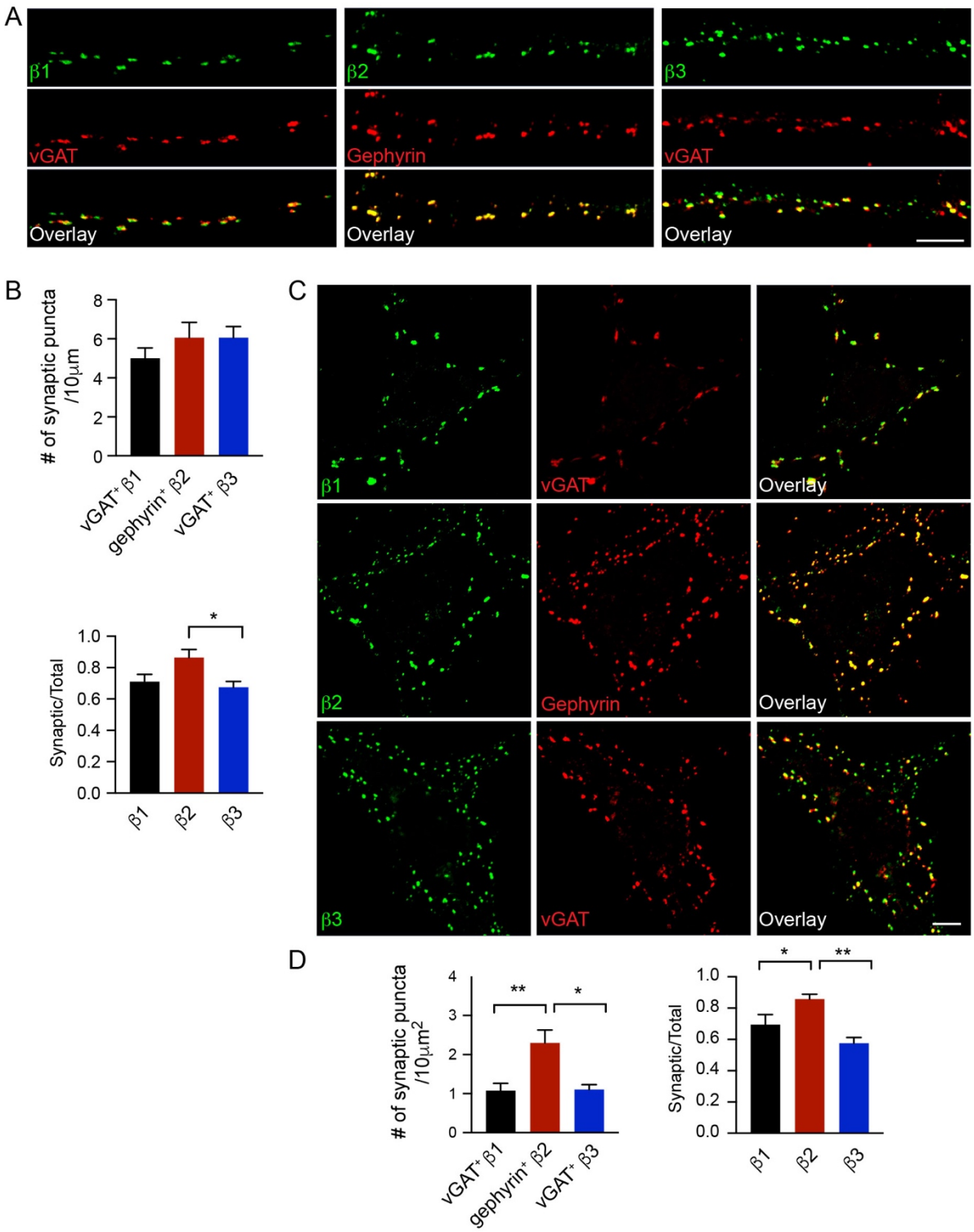


**Figure S1. Subcellular distribution of the GABA$_A$R β1-3 subunits in hippocampal neurons.**

(A-B) Representative images and summary graphs showed dendrites of neurons at DIV 18 immunolabelled with $GABA_A$ receptor β1, 2, or 3 subunits (green) and synaptic marker gephyrin/vGAT (green). Synaptic $GABA_A$ receptor β subunits (indicated by co-localization with gephyrin or vGAT) was calculated (n = 15; N = 3, *p < 0.05, one-way ANOVA followed by Bonferroni's post-hoc test). Choice of gephyrin or vGAT depends on antibody compatibility. Scale bar, 5 µm.

(C-D) Representative images and summary graphs showed somata of neurons at DIV 18 immunolabelled with $GABA_A$ receptor β1, 2, or 3 subunits (green) and synaptic marker gephyrin/vGAT (green). Synaptic $GABA_A$ receptor β subunits (indicated by co-localization with gephyrin or vGAT) was calculated (n = 10 - 15; N = 3, *p < 0.05, ** p < 0.01, one-way ANOVA followed by Bonferroni's post-hoc test). Choice of gephyrin or vGAT depends on antibody compatibility. Scale bar, 5 µm.

n represents the number of cells analyzed and N represents the number of independent experiments.

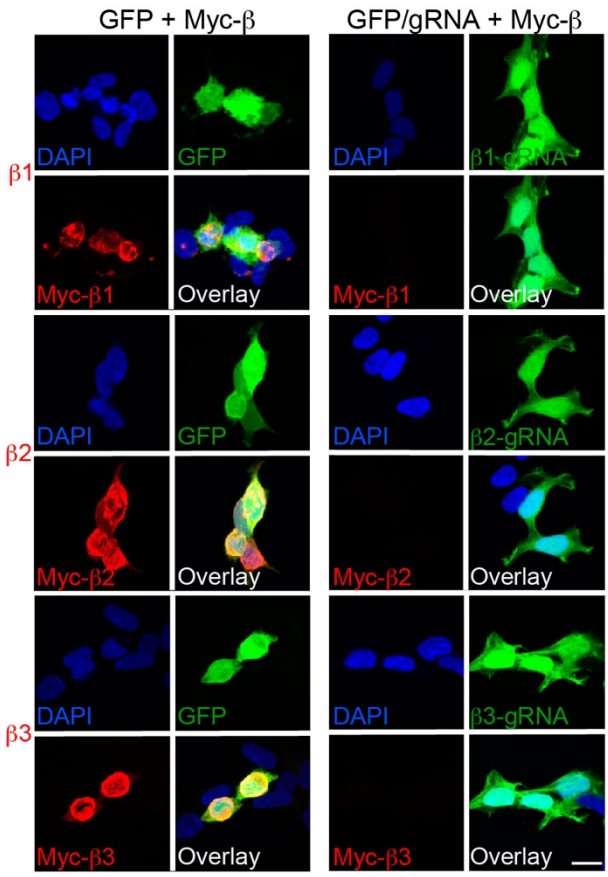


**Figure S2. Validation of gRNAs in HEK293T cells**

Validation of gRNA-mediated KO of the β1, 2, 3 subunits in HEK293T cells using the CRISPR/Cas9 technique. HEK293T cells were transfected with single β1, 2, 3 gRNAs candidate (also expressing GFP) or GFP alone together with β1, 2 or 3 subunits, respectively. Confocal images showed the loss of β1, 2 or 3 subunits in cells expressing both Myc-tagged β subunits and the respective gRNA candidate (N = 3). Scale bar, 5 μm.

N represents the number of independent experiments.