



**Figure S2. GPR158 knockout mice showed significant differences in the co-localization of individual neurons in the cerebral cortex in comparison to Gpr158<sup>Tag</sup> mice in HA with PSD95 or SYN at P60.**

A It displayed the raw immunofluorescence staining, 3D modeling and 3D co-localization of HA with SYN in the cerebral cortex of individual mCherry-positive neurons in adult Gpr158<sup>Tag</sup> mice.

B It showed the scatter plot of SYN binding to HA in individual mCherry-positive cells in the cerebral cortex of Gpr158<sup>Tag</sup> mice (heter) and GPR158 knockout mice (heter). mCherry<sup>+</sup> cells in the cerebral cortex of Gpr158<sup>Tag</sup> mice, n=10;  $\beta$ -gal<sup>+</sup> cells in the cerebral cortex of GPR158 knockout mice, n=10; male Gpr158<sup>Tag</sup> mice (heter) and GPR158 knockout mice (heter) at P60; \*\*: P<0.01.

C It presented the original immunofluorescence staining, 3D modelling and 3D co-localization results of  $\beta$ -gal with SYN in individual mCherry-positive neurons in the cerebral cortex of adult GPR158 knockout mice.

D Scatter plot of the number of SYN punctate signals around single mCherry-positive cells in the cerebral cortex of Gpr158<sup>Tag</sup> mice (heter) and GPR158 knockout mice (heter). mCherry<sup>+</sup> cells in the cerebral cortex of Gpr158<sup>Tag</sup> mice, n=10;  $\beta$ -gal<sup>+</sup> cells in the cerebral cortex of GPR158 knockout mice, n=10; male Gpr158<sup>Tag</sup> mice (heter) and GPR158 knockout mice (heter) at P60; \*\*: P<0.01.

E It illustrated the original immunofluorescence staining, 3D modelling and 3D co-localization of HA with PSD95 in the cerebral cortex of adult Gpr158<sup>Tag</sup> mice.

F It displayed the scatter plot of PSD95 binding to HA in individual mCherry-positive cells in the cerebral cortex of Gpr158<sup>Tag</sup> mice (heter) and GPR158 knockout mice (heter). mCherry<sup>+</sup> cells in the cerebral cortex of Gpr158<sup>Tag</sup> mice, n=8;  $\beta$ -gal<sup>+</sup> cells in the cerebral cortex of GPR158 knockout mice, n=10; male Gpr158<sup>Tag</sup> mice (heter) and GPR158 knockout mice (heter) at P60; \*\*: P<0.01.

G It presented the original immunofluorescence staining, 3D modeling and 3D co-localization results of  $\beta$ -gal with PSD95 in individual mCherry-positive neurons in the cerebral cortex of adult GPR158 knockout mice.

H It showed the scatter plot of the number of PSD95 punctate signals around single mCherry-positive cells in the cerebral cortex of Gpr158<sup>Tag</sup> mice (heter) and GPR158 knockout mice (heter). mCherry<sup>+</sup> cells in the cerebral cortex of Gpr158<sup>Tag</sup> mice, n=9;  $\beta$ -gal<sup>+</sup> cells in the cerebral cortex of GPR158 knockout mice, n=10; male Gpr158<sup>Tag</sup> mice (heter) and GPR158 knockout mice (heter) at P60; \*\*: P<0.01. Scale bars, 4  $\mu$ m.