



Figure S4. Analysis of protein spectrum data on proteins that may interact with GPR158 in WT and GPR158^{Tag} mice.

A Co-immunoprecipitation (co-IP) silver staining results of Flag-tagged GPR158 in the whole brain of WT and GPR158^{Tag} mice (WT mice: n=3; GPR158^{Tag} mice: n=3). The bands were cut into 8 samples and sent together for protein spectrum.

B Schematic diagram of co-IP for mass spectrometry identification of candidate GPR158 interacting proteins (Method 1).

C Waterfall diagram made according to protein spectrum data of Sample 7 (WT^{comb}) and Sample 8 (Tag^{comb}) was used to screen the candidate GPR158 interacting protein (Method 1).

D Schematic diagram of co-IP for mass spectrometry identification of candidate GPR158 interacting proteins (Method 2).

E Volcano plots of GPR158 candidate interacting proteins were quantified by label-free quantification. A two-sided Student's t-test was used in the volcano plot. Wild-type mouse samples (Samples 1-3, n=3, male) were used as negative controls. GPR158^{Tag} mouse samples (Samples 4-6, n=3, male) were used as the experimental group (Method 2). Significantly changed proteins were highlighted in red ($p < 0.05$ and >2 -fold difference in intensity). GPR158 candidate interacting proteins that were present only in GPR158^{Tag} mice but not in WT mice were labeled in green.

F The final list of GPR158 candidate interacting proteins from analytical Methods 1 and 2.