Preliminary data (Cryo-EM analysis of native AMPA receptors in native post-synaptic membrane)

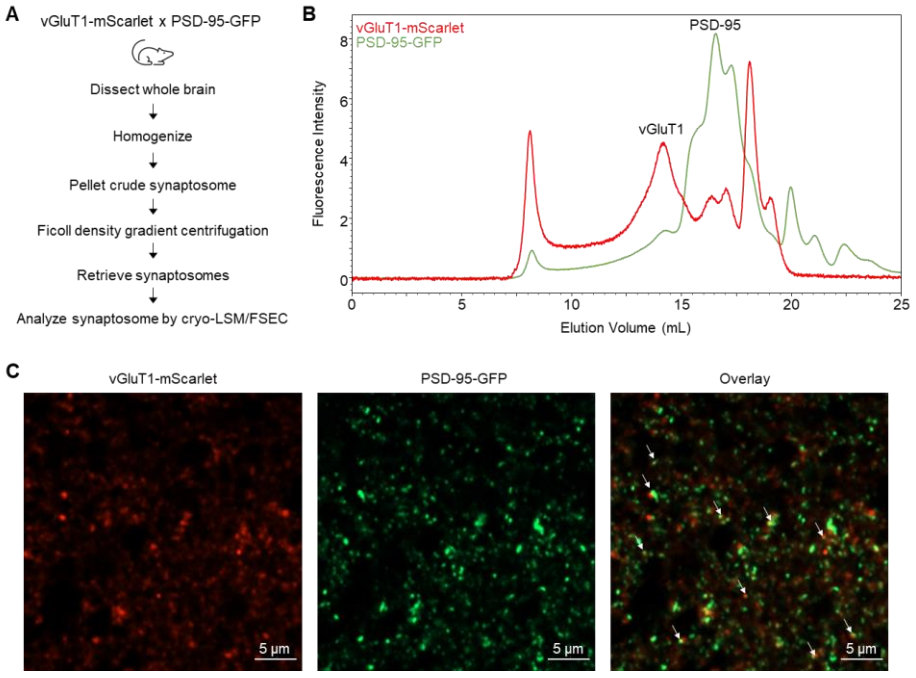

Figure 1. Isolation of intact glutamatergic synaptosomes from vGluT1-mScarlet x PSD-95-GFP mouse. (A) Schematic for the isolation of synaptosomes from mouse brain using a Ficoll density gradient. (B) FSEC traces of PSD-95-GFP and vGluT1-mScarlet in fractionated synaptosomes. (C) Cryo-LSM images of glutamatergic synaptosomes on cryo-EM grids. Fluorescence signals of vGluT1-mScarlet and PSD-95-GFP at the pre- and post-synaptic compartments of synaptosomes are shown in the red and green channels, respectively. Representative glutamatergic synaptosomes are highlighted with white arrows.

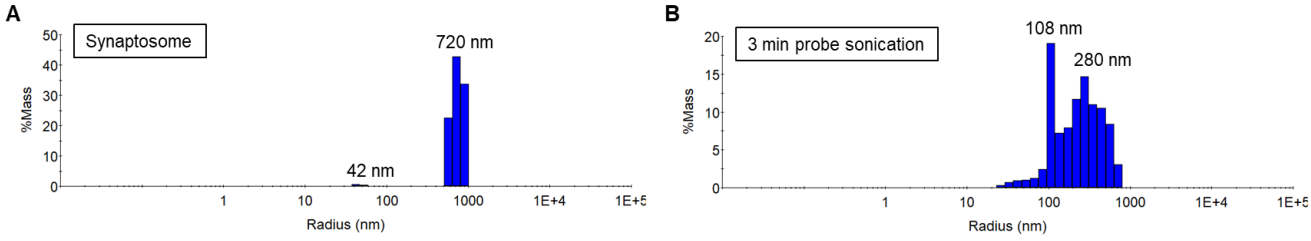

Figure 2. Analysis of the diameter of synaptosomes and sonicated synaptosome vesicles. Dynamic light scattering analysis showed the distribution of the diameter of synaptosomes (A) and smaller vesicles prepared by probe sonication (B).

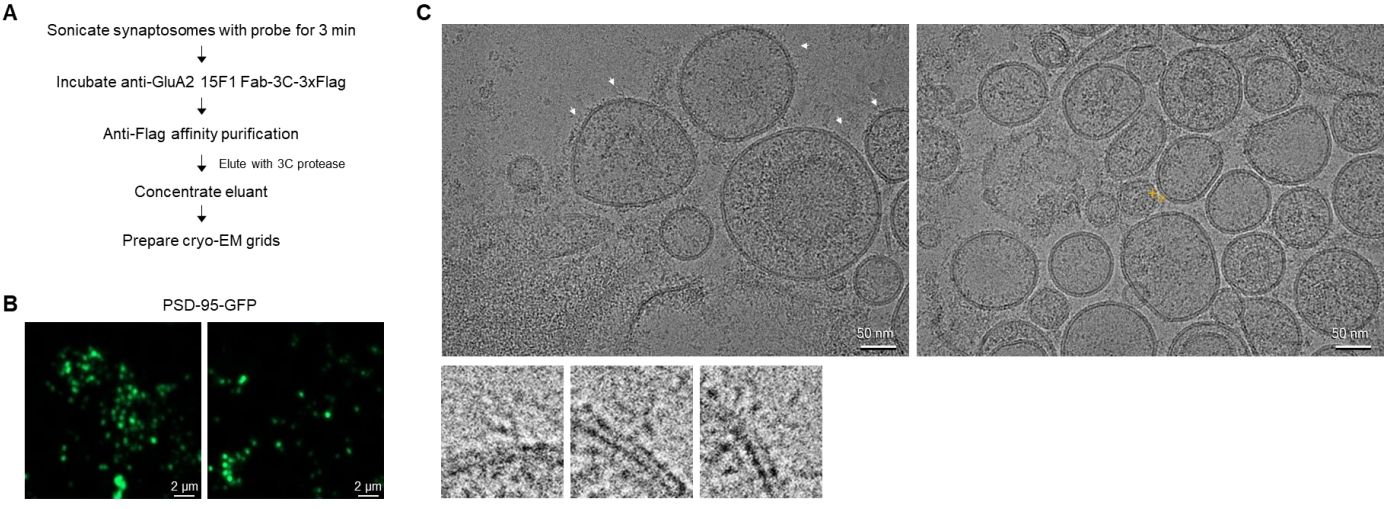

Figure 3. Purification of AMPAR-containing synaptosome vesicles. (A) Schematic for the purification of AMPAR-containing synaptosome vesicles using anti-GluA2 15F1 Fab-3C-3xFlag. (B) Cryo-LSM images of purified AMPAR-containing vesicles on cryo-EM grids. Fluorescence signals of PSD-95-GFP are shown in the green channel. (C) Representative cryo-TEM micrograph of purified AMPAR-containing vesicles on cryo-EM grids. Receptor-like particles are highlighted with white arrows.

References

- Tao, Xiao, Chen Zhao, and Roderick MacKinnon. "Membrane protein isolation and structure determination in cell-derived membrane vesicles." Proceedings of the National Academy of Sciences 120.18 (2023): e2302325120.
- Zhao, Yan, et al. "Architecture and subunit arrangement of native AMPA receptors elucidated by cryo-EM." Science 364.6438 (2019): 355-362.
- Yu, Jie, et al. "Hippocampal AMPA receptor assemblies and mechanism of allosteric inhibition." Nature 594.7863 (2021): 448-453.