Supplementary Information for manuscript # MOLPHARM-AR-2021-000269

Tacrine induces endoplasmic reticulum-stressed apoptosis via disrupting the proper assembly of oligomeric acetylcholinesterase in cultured neuronal cells

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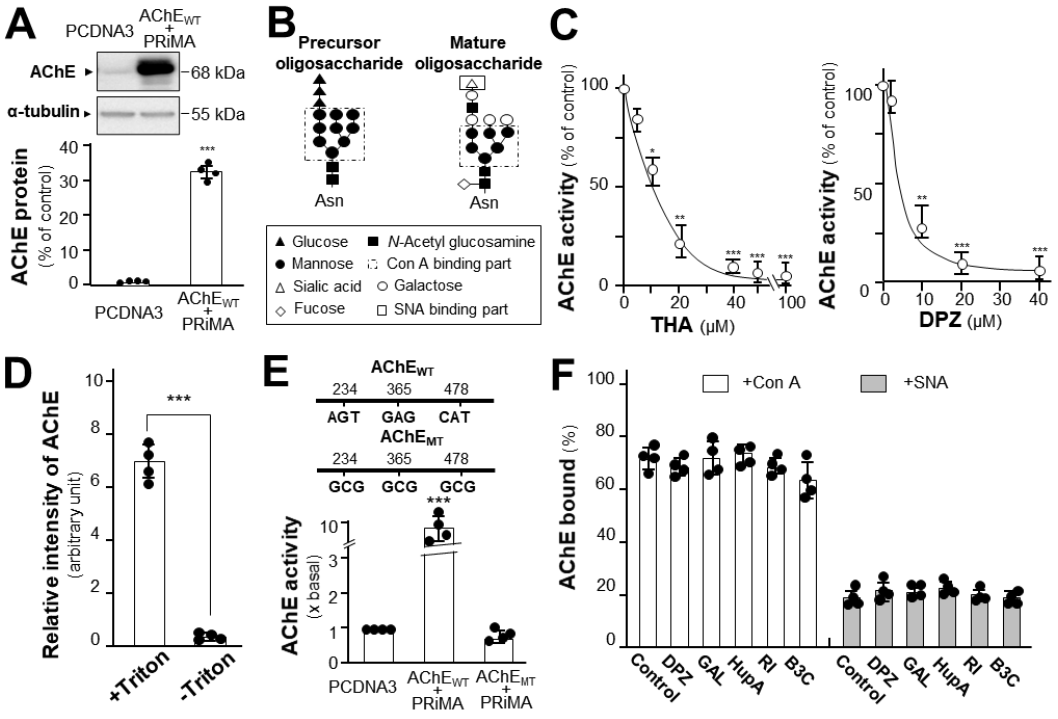
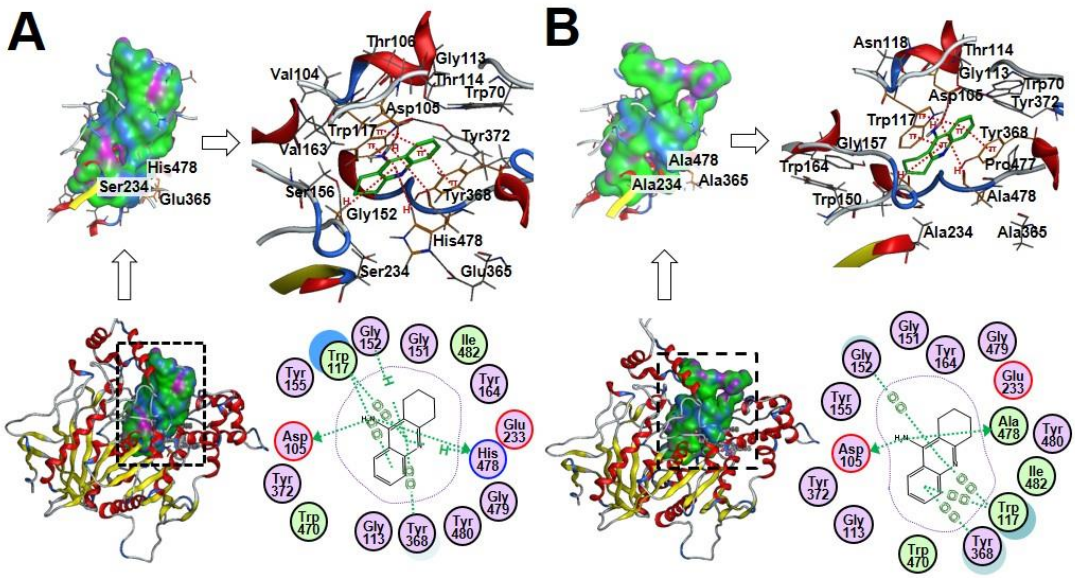


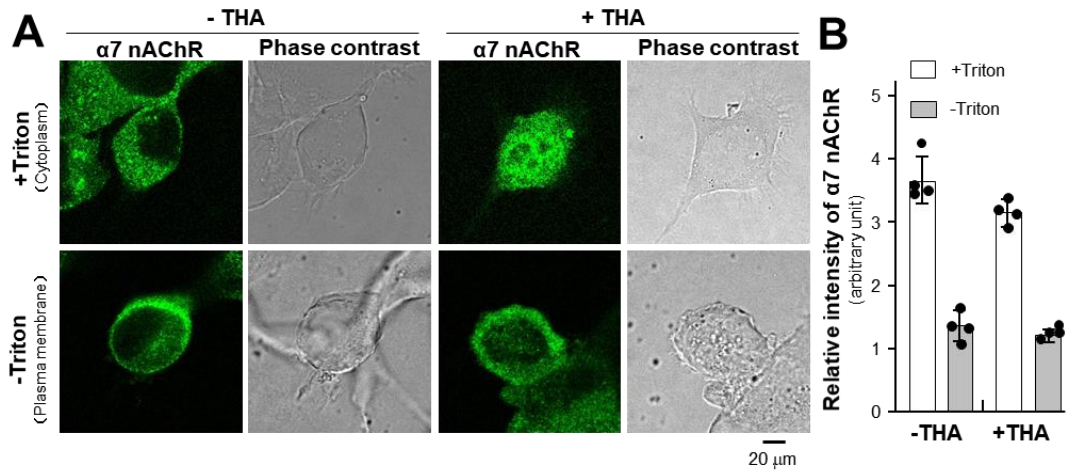
Supplementary Figure 1.

(A) NG108-15 cells were transfected with pcDNA3 and AChE$_{WT}$ with PRiMA. Protein lysates of the cultures were performed for Western blot assay. **(B)** A schematic diagram of binding parts of Con A and SNA were shown. **(C)** Cultured NG108-15 cells were treated with or without tacrine (THA), or donepezil (DPZ), at different concentrations for 24 hours. Protein lysates of the cultures were performed Ellman assay. **(D)** Cultured NG108-15 cells co-transfected with AChE and PRiMA were exposed with thapsigargin (Tg, 1 μM) for 24 hours. Cells were immuno-stained by anti-AChE antibody with or without permeabilization of Triton X-100 (0.2%). The quantification of florescence intensity of AChE was shown. The values of average florescence intensity/cell were analyzed by ImageJ software. **(E)** The cDNAs encoded wild type AChE (AChE$_{WT}$) and AChE activity-deleted mutant (AChE$_{MT}$) were shown by diagram. AChE$_{WT}$ S234 (AGT), E365 (GAG), H478 (CAT) were mutated to A (GCG) (left panel). HEK293T cells were transfected with pcDNA3, AChE$_{WT}$ and AChE$_{MT}$ with PRiMA. Protein lysates of the cultures were performed Ellman assay (right panel). **(F)** AChEI-treated NG108-15 cell lysate was incubated with Con A,

SNA or agarose. The concentrations of AChEIs were shown as in Fig. 10B. Percentage of precipitated AChE was calculated. The data are normalized to the percentage of control, or the amount of agarose-precipitated AChE. Values are expressed as the percentage of control to basal reading, or the fold of basal value, and are in means ± SD, n=4, each with triplicate samples. Statistical significance was analyzed by one-way repeated measures of ANOVA with subsequent application of Dunnett's multiple comparisons test. *P<0.5, **P<0.01, ***P<0.001 vs. control.



Supplementary Figure 4. Proposed binding modes of tacrine with wild type and activity-deleted mutant AChE. (A) Interactions of tacrine with AChE$_{WT}$ (ligand is shown as green) are shown as 3D and 2D images. **(B)** Interactions of tacrine with AChE$_{MT}$ (ligand is shown as green) are shown as 3D and 2D images. Key resides for binding of tacrine are shown. These binding modes show certain similarity to that of rivastigmine.



Supplementary Figure 5. Tacrine dose not block the trafficking of α7 nAChR to membrane.

(A) Cultured NG108-15 cells transfected with α7 nAChR were exposed with or without tacrine (THA; 20 μM) for 24 hours. Cells were immuno-stained by the anti-α7 nAChR antibody with or without permeabilization of Triton X-100 (0.2%). Florescence and phase photos were taken. **(B)** The quantification of florescence intensity of α7 nAChR was shown. The values of average florescence intensity/cell were analyzed by ImageJ software. Values are in means ± SD, *n=4*.

Supplemental Data File 1. The structure of $AChE_{WT}$.

Supplemental Data File 2. The structure of $AChE_{MT}$.