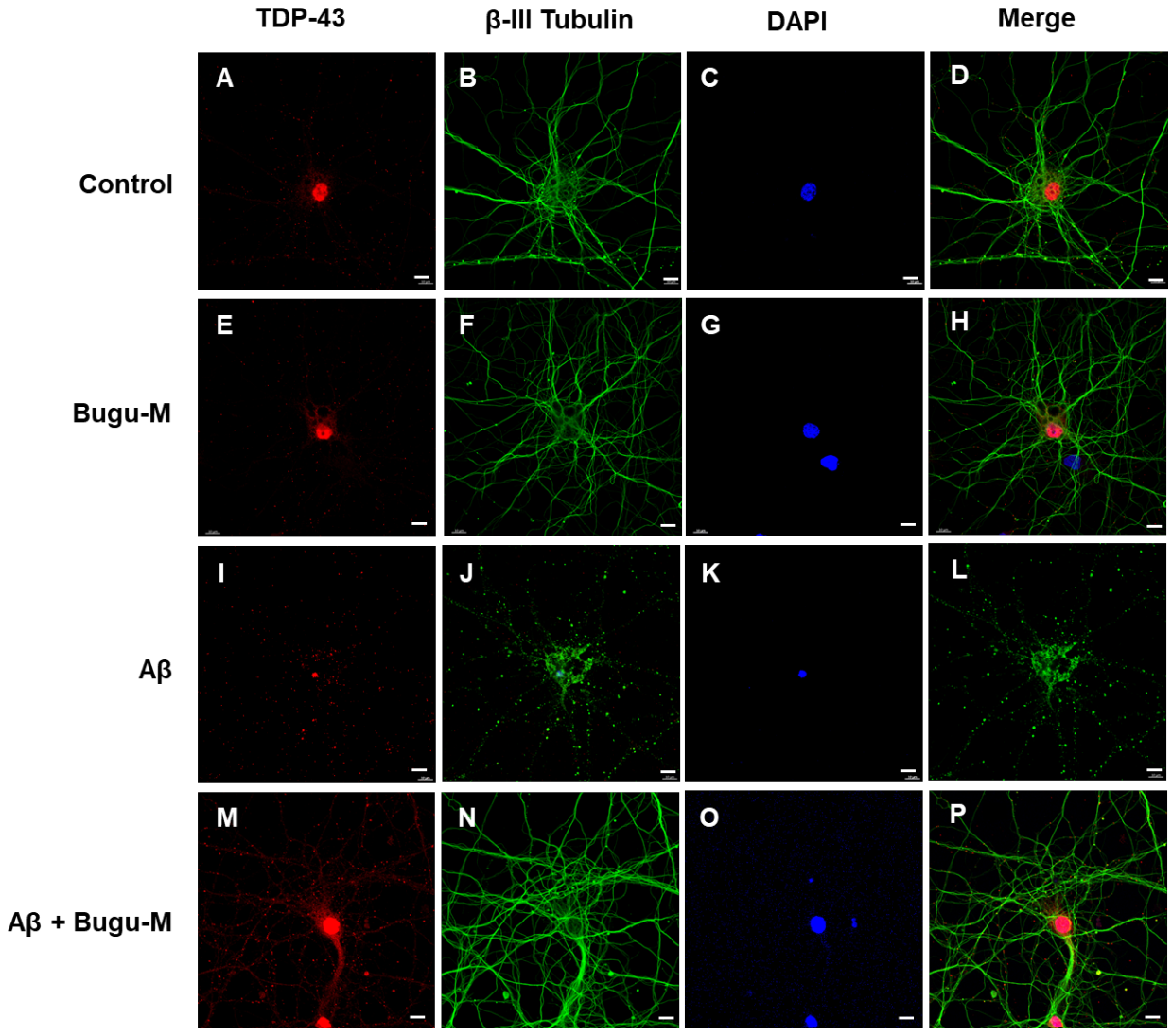
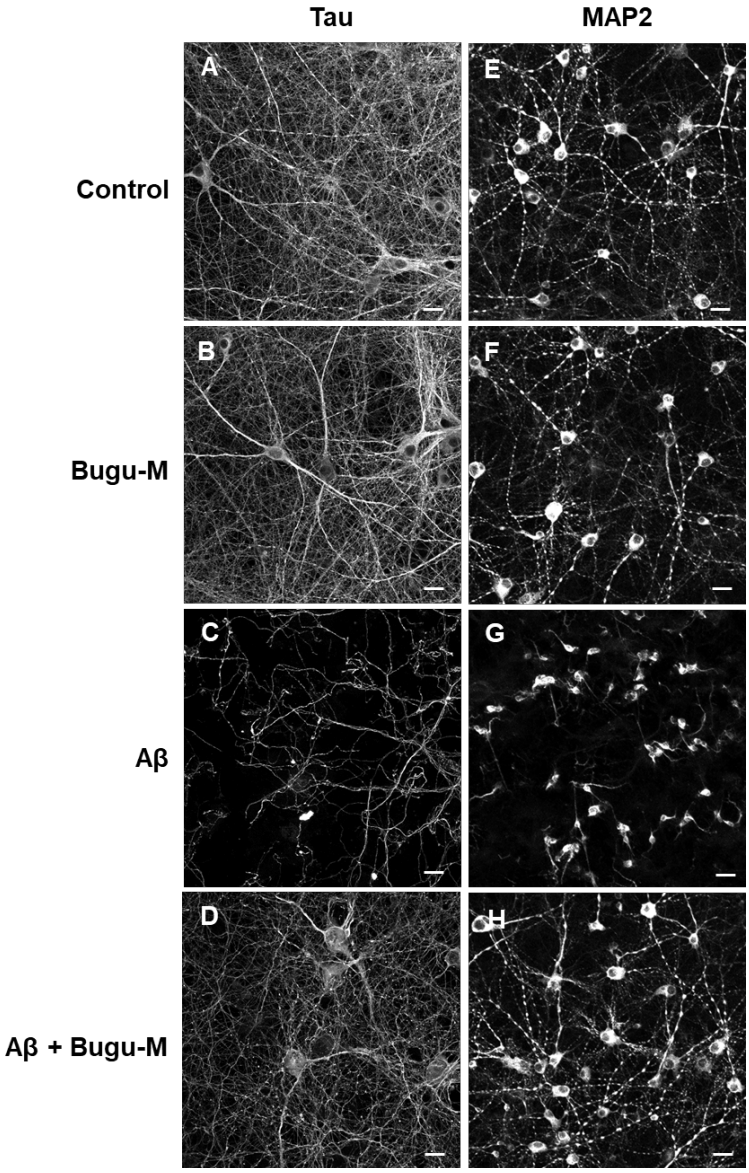
Supplementary Material

Neuroprotective Effects of a Multi-Herbal Extract on Axonal and Synaptic Disruption in Vitro and Cognitive Impairment in Vivo

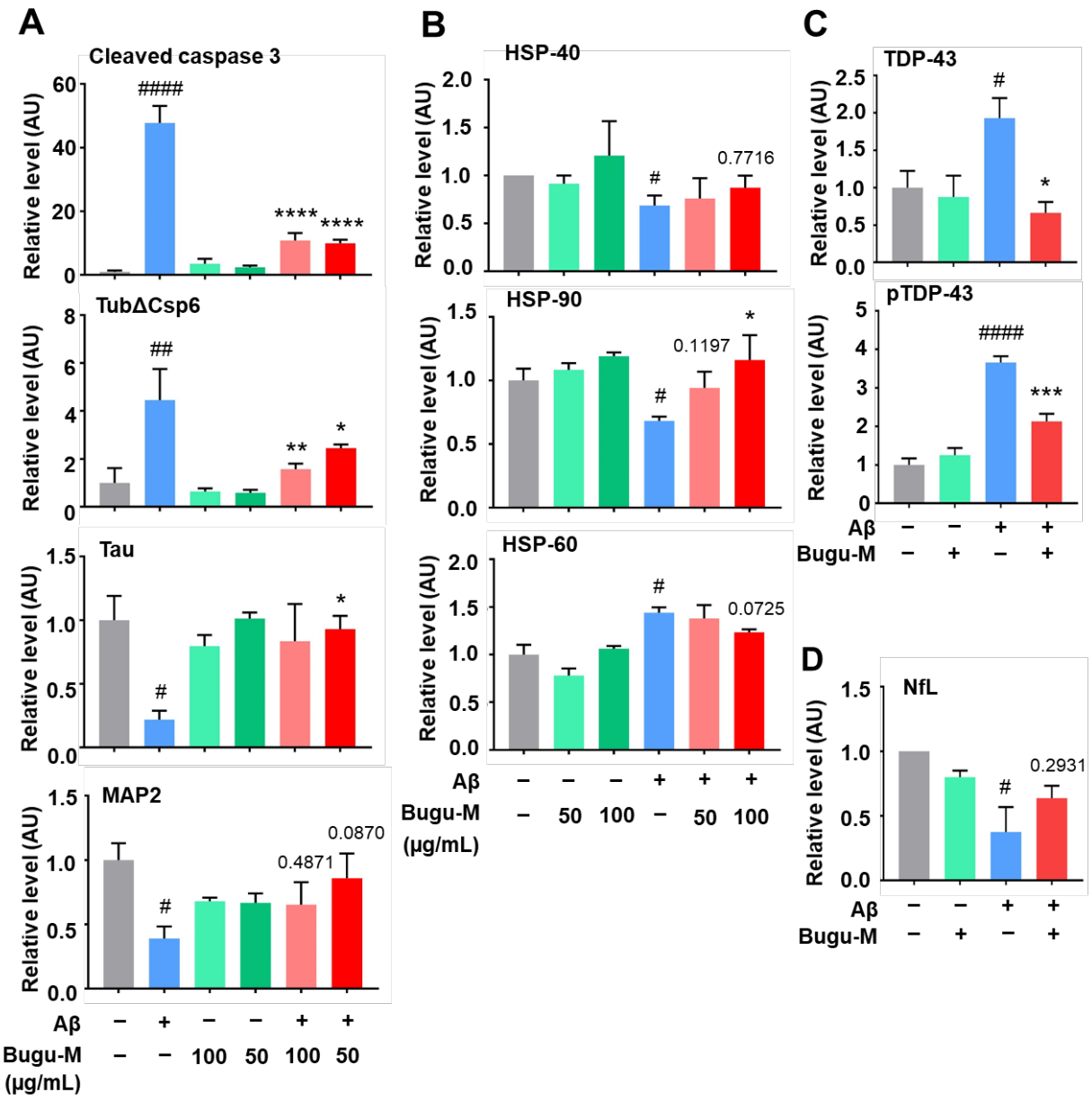
Supplementary Figure 1. Bugu-M remains TDP-43 in the nucleus. Rat cortical neurons were pretreated with Bugu (100 μg/mL) for 1 h prior to $A\beta_{25-35}$ incubation for 72 h. Immunofluorescent labeling of TDP-43 (A, E, I, M) and β-III tubulin (B, F, J, N) was performed. Nucleus was stained by DAPI. Confocal images are single optical slices, and camera and microscope setting were equivalent for comparisons between groups. Shown are representative images. Scale bar, 10 μm.



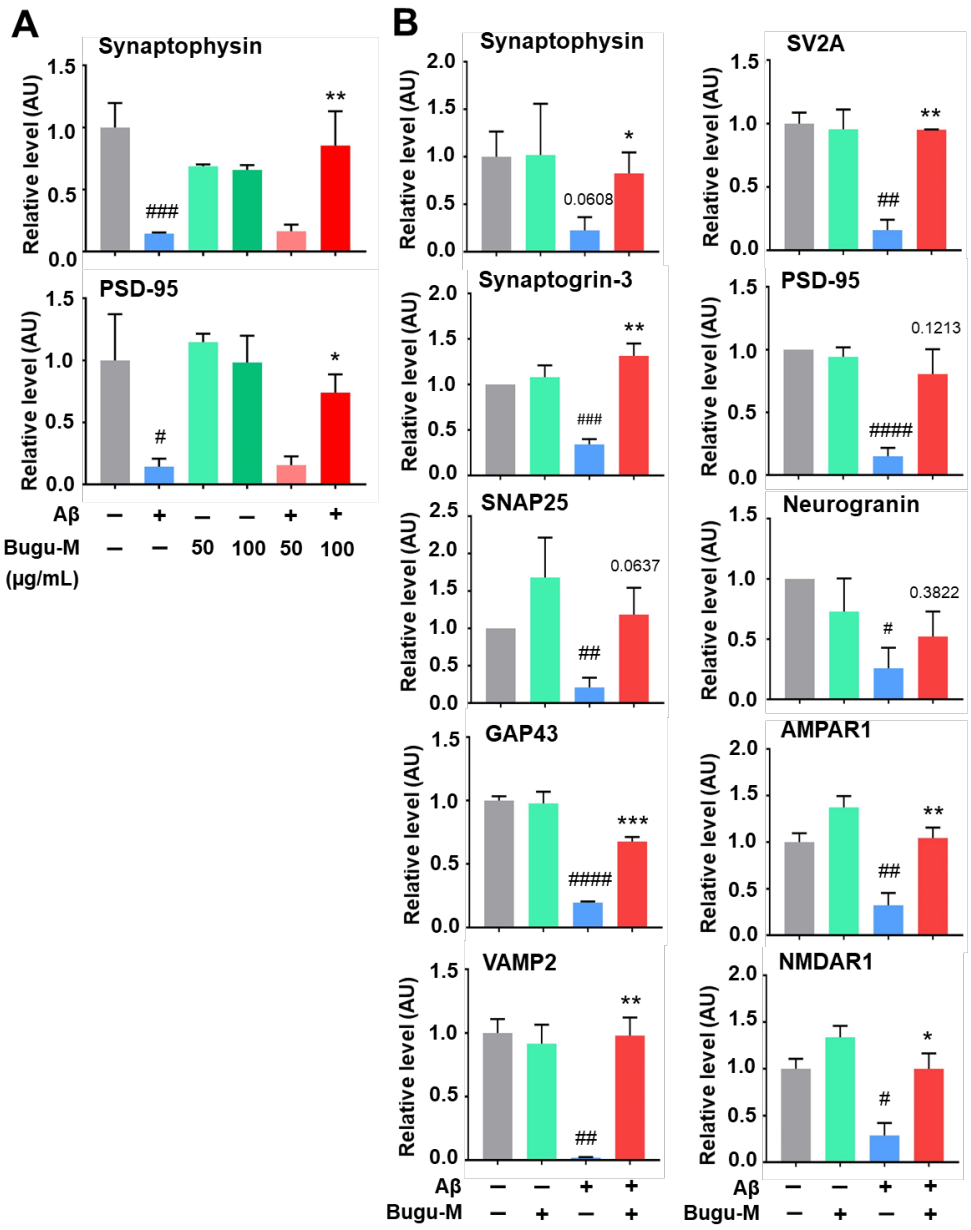
Supplementary Figure 2. Bugu-M reserves integrity of microtubule-associated proteins. Rat cortical neurons were pretreated with Bugu-M (100 μg/mL) for 1 h prior to $A\beta_{25-35}$ incubation for 72 h. Immunofluorescent labeling of Tau (A to D) and MAP2 (E to H) was performed. Confocal images are maximum projections, and camera and microscope setting were equivalent for comparisons between groups. Shown are representative images. Scale bar, 10 μm.



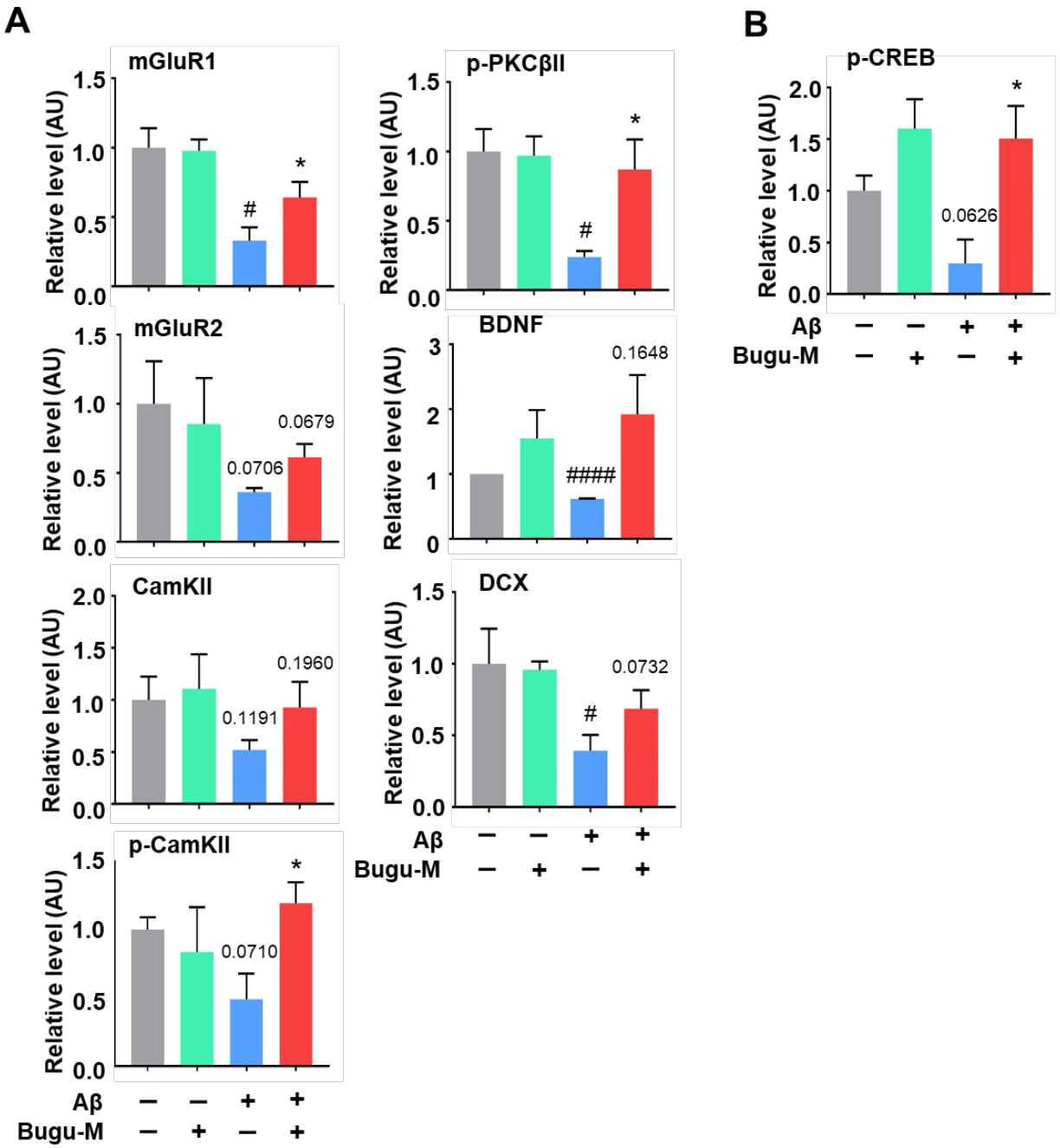
Supplementary Figure 3. Quantification of western blots in Figure 2. A) Blots in Fig. 2A. B) Blots in Fig. 2B. C) Blots in Fig. 2C. Data are expressed as mean ± SEM (n = 3). Differences among groups were analyzed by unpaired t-test and one-way ANOVA followed by Dunnett's multiple comparisons test. The p values shown in Aβ and Aβ + Bugu-M groups are compared to the untreated control and Aβ groups, respectively. $^{\#}p < 0.05$, $^{\#\#}p < 0.01$, and $^{\#\#\#\#}p < 0.0001$ versus the untreated control group; $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$, and $^{****}p < 0.0001$ versus the Aβ group.



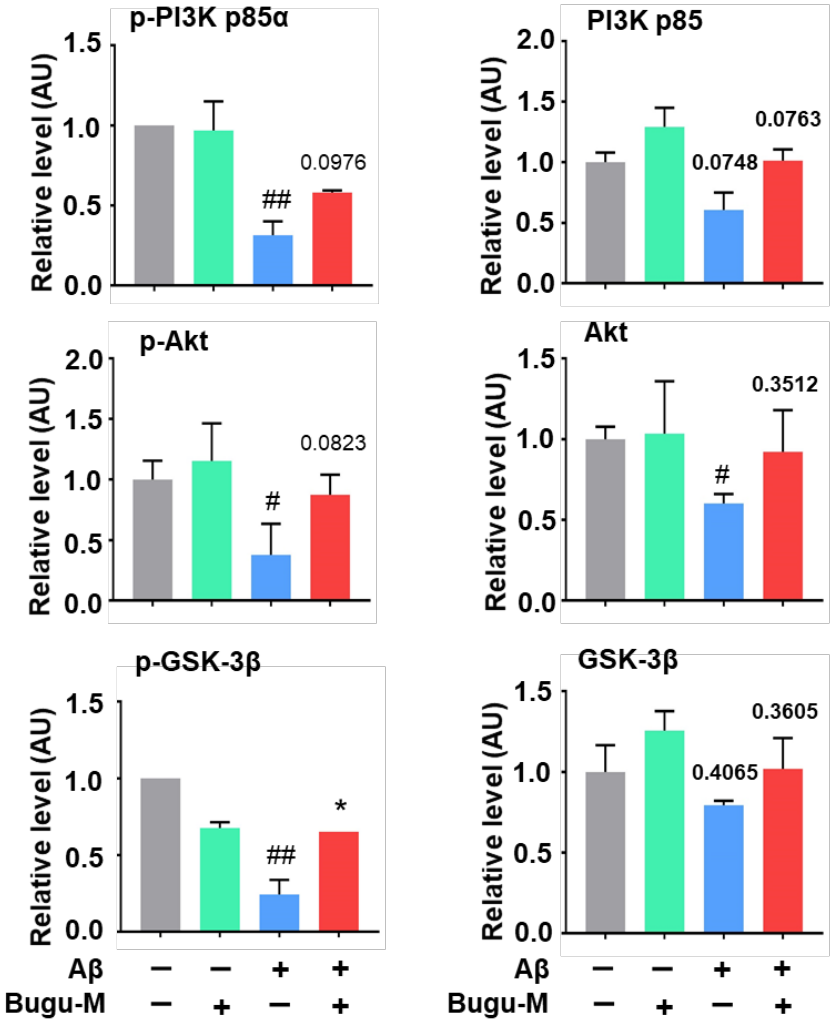
Supplementary Figure 4. Quantification of western blots in Fig. 3. A) Blots in Fig. 3A. B) Blots in Fig. 3D. Data are expressed as mean ± SEM (n = 3). Differences among groups were analyzed by unpaired t-test and one-way ANOVA followed by Dunnett's multiple comparisons test. The p values shown in Aβ and Aβ + Bugu-M groups are compared to the untreated control and Aβ groups, respectively. $^{\#}p < 0.05$, $^{\#\#}p < 0.01$, $^{\#\#\#}p < 0.001$, and $^{\#\#\#\#}p < 0.0001$ versus the untreated control group; $^{*}p < 0.05$, $^{**}p < 0.01$, and $^{***}p < 0.001$ versus the Aβ group.



Supplementary Figure 5. Quantification of western blots in Fig. 4. A) Blots in Fig. 4A. B) Blots in Fig. 4B. Data are expressed as mean ± SEM (n = 3). Differences among groups were analyzed by unpaired t-test and one-way ANOVA followed by Dunnett's multiple comparisons test. The p values shown in Aβ and Aβ + Bugu-M groups are compared to the untreated control and Aβ groups, respectively. $^{\#}p < 0.05$ and $^{\#\#\#\#}p < 0.0001$ versus the untreated control group; $^{*}p < 0.05$ versus the Aβ group.



Supplementary Figure 6. Quantification of western blots in Figure 5. Data are expressed as mean ± SEM (n = 3). Differences among groups were analyzed by unpaired t-test and one-way ANOVA followed by Dunnett's multiple comparisons test. The p values shown in Aβ and Aβ + Bugu-M groups are compared to the untreated control and Aβ groups, respectively. $^{\#}p < 0.05$ and $^{\#\#}p < 0.01$ versus the untreated control group; $^{*}p < 0.05$ versus the Aβ group.



Supplementary Figure 7. Activity in the open field and the elevated plus maze. The open-field test (OFT) (A-C) was conducted in a white acrylic box (50 × 50 × 30 cm). Mice were placed in the corner of the box and locomotor activities were recorded for 5 min. The elevated plus maze (EPM) (D-F) was a plus-shaped maze with a center square (5 × 5 cm) connected to 2 open arms (30 × 5 cm) with 0.5 cm-high guardrail and 2 closed arms (30 × 5 cm) with 15 cm high walls. The EPM was made of white acrylic and 50 cm-high from the floor. Mice were placed in the center square and allowed to explore for 5 min. Movements were tracked with EthoVision XT ver 13. Data are expressed as mean ± SEM (n = 9-10). Differences among groups were analyzed by one-way ANOVA and Dunnett's multiple comparisons test. $^{\#\#}p < 0.01$ and $^{\#\#\#\#}p < 0.0001$ versus NonTg control.