Supplementary Information

Diosgenin restores Aβ-induced axonal degeneration by reducing the expression of heat shock cognate 70 (HSC70)

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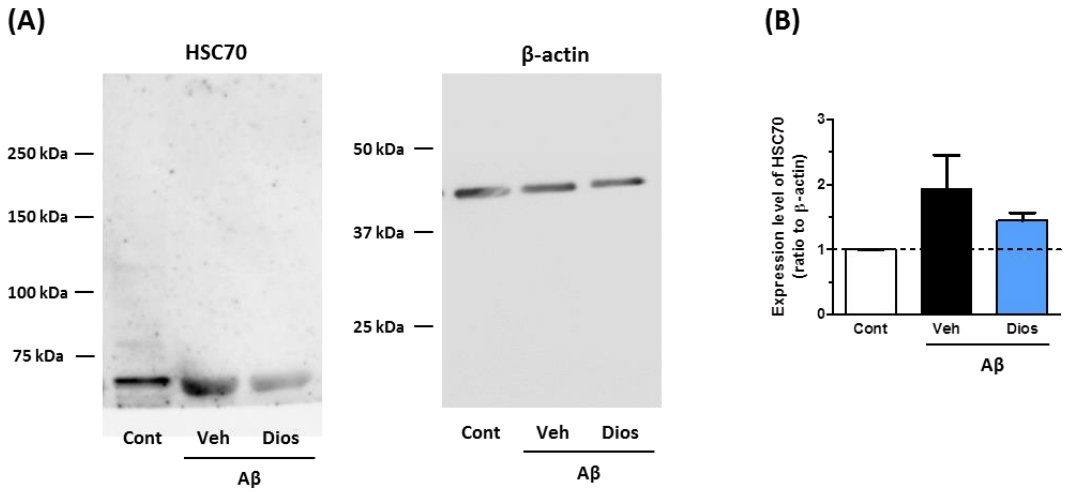
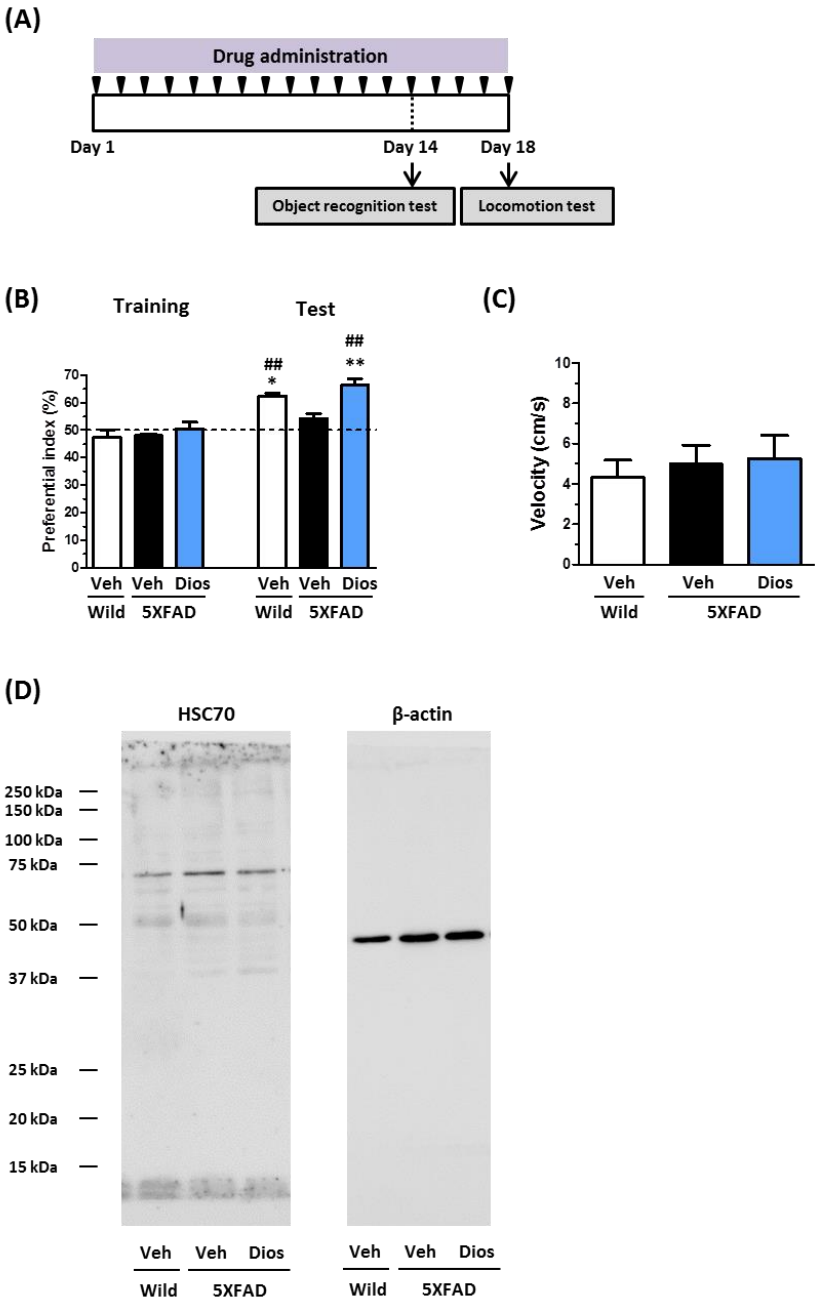
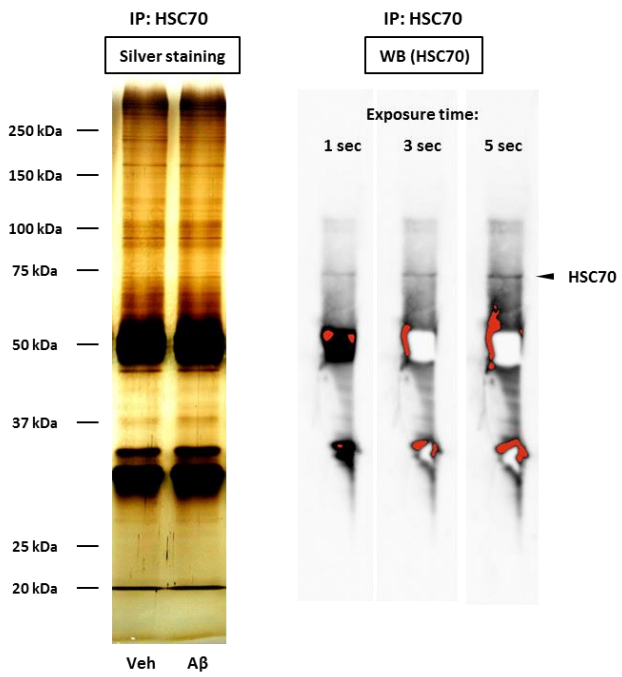


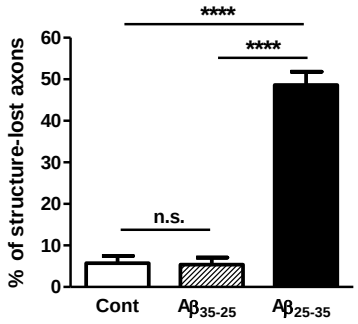
Supplementary Figure 1. Diosgenin reduced the expression level of HSC70 in neuron lysates. Mouse cortical neurons (ddY, E14) were cultured for three days and then treated with or without $A\beta_{25\text{-}35}$ (10 μM) for three days. After the three days of $A\beta_{25\text{-}35}$ treatment, neurons were treated with diosgenin (1 μM) or vehicle solution (0.1% EtOH) for four days. After the cells were washed with PBS, they were lysed and loaded on SDS-PAGE for WB analysis. **(A)** Proteins were detected by anti-HSC70 antibody or β-actin antibody, respectively. A whole membrane was cropped around 60 kDa into two pieces, upper one (for anti-HSC70) and lower one (for anti-β-actin) before WB. **(B)** Quantitative value for the expression levels of HSC70 (ratio to β-actin), n = 3 samples (2–4 lanes per a sample were quantified for the analysis).



Supplementary Figure 2. Diosgenin administration recovered memory deficits in the 5XFAD mice. Wild-type and 5XFAD mice (Female, 7–8 months old) were treated with diosgenin (0.1 μmol/kg/day, p.o.) or vehicle solution (sesame oil) for 18 days. **(A)** The time schedule of this study. On administration day 14, an object recognition memory test was performed. On the last day of administration, locomotion test was performed. **(B)** The preferential indexes of the training and test session are shown. *p < 0.05, **p < 0.01 vs 5XFAD/Veh, one-way ANOVA *post hoc* Dunnett's test, ##p < 0.01 vs 50%, one sample *t*-test, n = 4 mice. **(C)** The velocity (cm/s) within 10 min is shown. One-way ANOVA *post hoc* Dunnett's test, n = 4 mice. **(D)** The full-length WB images for the Figure 2C in the main text.



Supplementary Figure 3. The full-lengths gels for the Figure 5 in the main text.



Supplementary Figure 4. The effects of $A\beta_{35-25}$ (a negative control peptide) and $A\beta_{25-35}$ on axonal structures. Mouse cortical neurons were cultured for two days and treated with $A\beta_{35-25}$ (10 μM) or $A\beta_{25-35}$ (10 μM) for one day. After the medium was removed, the neurons were treated with fresh medium for four days. Then, neurons were fixed and double-immunostained for α-tubulin and pNF-H or observed with DIC. The percentage of structure-lost (low expression of α-tubulin) axons were quantified for each treatment. ****$p < 0.0001$, two-tailed one-way ANOVA *post hoc* Bonferroni's multiple comparison test. n = 3 photos were quantified for the analysis.