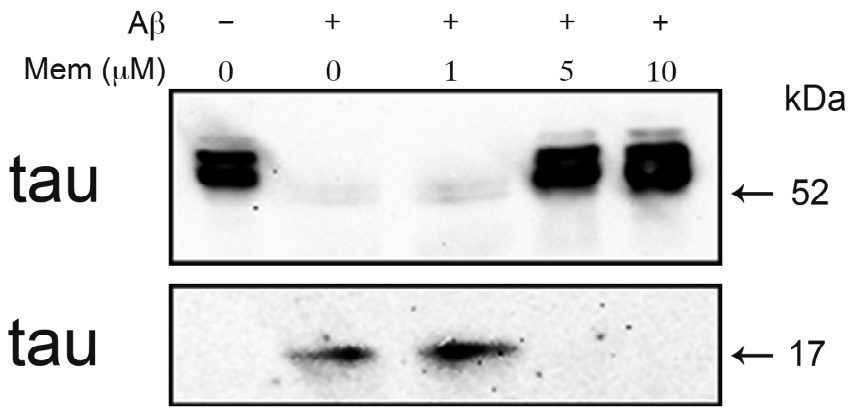
Supplemental Data

Supplemental Figure 1. **Memantine prevented Aβ-induced 17 kDa tau generation in a dose-dependent manner in mature hippocampal neurons**. Immunoblot analysis of 17 kDa tau production in whole cell lysates prepared from mature hippocampal neurons cultured in the presence (+) or absence (-) of Aβ peptide (20 mM) after their preincubation with memantine (1-10 mM) using a tau antibody (clone tau5). Decreased in full-length tau immunoreactivity and a strong 17 kDa tau fragment immunoreactive band were detected in Aβ-treated lysates obtained from neurons preincubated with lower doses of memantine. Aβ-induced tau cleavage was prevented when neurons were pretreated with 10 μM memantine.

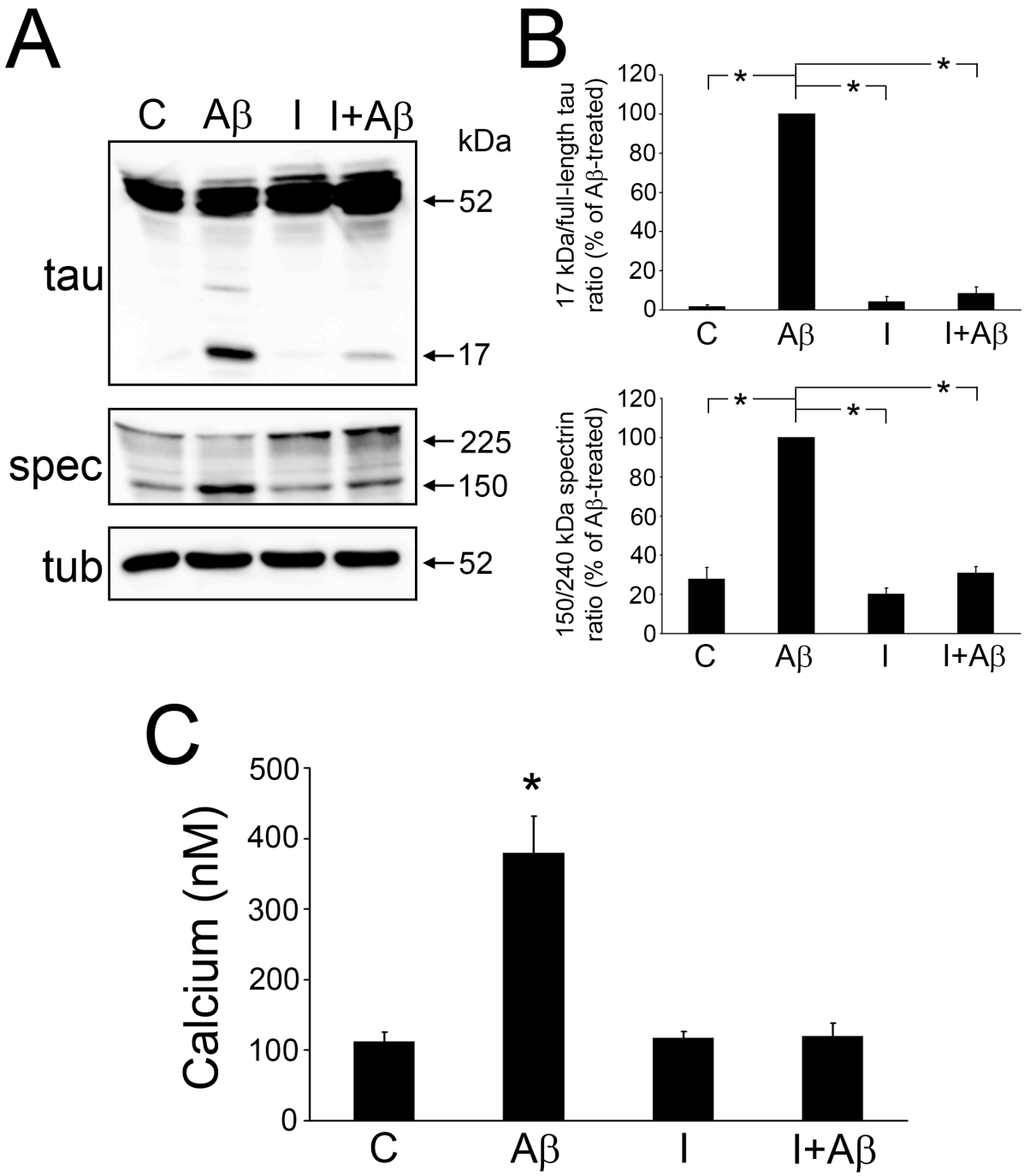
Supplemental Figure 2. **Ifenprodil significantly attenuated Aβ-induced 17 kDa tau generation, calpain activation, and Ca^{2+} influx in hippocampal mature neurons.** (A) Immunoblot analysis of 17 kDa tau production and calpain activity, by means of spectrin degradation, in whole cell lysates prepared from mature hippocampal cultures incubated with or without Aβ (20 μM), Ifenprodil (5 μM), or both. The 17 kDa tau fragment and calpain activation were greatly reduced in Aβ-treated lysates that were preincubated with Ifenprodil as compared to those treated with Aβ alone. (B) Quantification of the 17 kDa/full-length tau ratio as well as the 150/240 kDa spectrin ratio in whole cell lysates treated as described in (A). Tubulin was used as an internal control and the values were expressed as a percent of Aβ-treated neurons. (C) Using fura-2 imaging, intracellular Ca^{2+} levels were calculated in live mature neurons treated as mentioned in (A) after their baseline levels had been established. Ca^{2+} influx induced by Aβ treatment was significantly decreased in cells that were pretreated with Ifenprodil. Values represent the mean ± S.E.M from 5 independent experiments per condition. *Differs from Aβ-treated cells (B) or untreated controls (C), $p < 0.01$. C: control, Aβ: beta-amyloid, I: Ifenprodil.

Supplemental Figure 3. Synapses number was significantly increased in cholesterol-enriched young hippocampal neurons. (A-H) Young (A, B, E, F) and mature (C, D, G, H) neurons were incubated with (B, F, D, H) or without (A, C, E, G) cholesterol-modifying drugs. These cells were then immunolabeled with a tubulin (A-D) or synaptophysin (E-H) antibody. (I-J) Quantification of the number of synaptophysin-positive puncta per cell in young (I) and mature (J) cells treated as described in (A-H). Values represent the mean ± S.E.M. for 5 independent experiments per condition (n=90 cells/condition). Note the increase in synaptophysin labeling of cholesterol-enriched young neurons as compared to young neuron controls. *Differs from age-matched control, $p < 0.05$. Scale bar: 50 μm. C: control, CH: cholesterol-treated, MBCD: MBCD-treated.

Supplemental Figure 1



Supplemental Figure 2



Supplemental Figure 3