Supplemental Materials

LIPOPROTEIN LIPASE IS A NOVEL Aβ-BINDING PROTEIN THAT PROMOTES CELLULAR UPTAKE OF Aβ IN ASTROCYTES

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Methods

Aβ oligomers were prepared as previously described (Lambert et al., Journal of Neurochemistry, 2001, 79, 595-605). In brief, Aβ42 was dissolved in hexafluoro-2-propanol (HFIP) and aliquots were placed into to microcentrifuge tubes. HFIP was removed by evaporation with traces removed under vacuum and the tubes were stored at -80 ˚C. An aliquot of Aβ42 was mixed with DMSO to a final concentration of 5 mM, which was then added to ice-cold F12 medium without phenol red to 100 μM. This solution was incubated at 4 ˚C for 24 h and then centrifuged at 14,000 x g for 10 min. The supernatant was used as the Aβ oligomer preparation.

Legend

Supplemental Fig.1. Determination of assembly state of Aβ which binds to LPL. (A, Left blot). Freshly dissolved Aβ (50 ng) was separated by SDS-PAGE and transferred to a PVDF membrane. Aβ was probed with 6E10 followed by the horseradish peroxidase-labeled anti-mouse antibody and the chemiluminescent substrate ECL Plus. **(A, Right blot).** LPL (5 μg/ml) and Aβ (500 nM) were incubated in DMEM at 37 ˚C for 3 h. Protein complexes formed were immunoprecipitated with an anti-LPL antibody (α-LPL) and the immunoprecipitates were analyzed by Western blotting using 6E10, an anti-Aβ antibody. **(B, Left blot).** Aβ oligomer preparation (1 μg) was separated by SDS-PAGE and transferred to a PVDF membrane. Aβ was probed with 6E10 followed by the horseradish peroxidase-labeled anti-mouse antibody and the chemiluminescent substrate ECL Plus. **(B, Right blot).** LPL (5 μg/ml) and Aβ oligomer (500 nM) preparation were incubated in DMEM at 37 ˚C for 3 h. Protein complexes formed were immunoprecipitated with an anti-LPL antibody (α-LPL) and the immunoprecipitates were analyzed by Western blotting using 6E10, an anti-Aβ antibody.

Supplemental Fig. 1
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