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

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### Methods

A $\beta$  oligomers were prepared as previously described (Lambert et al., Journal of Neurochemistry, 2001, 79, 595-605). In brief, A $\beta$ 42 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 A $\beta$ 42 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  $\mu$ M. 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 A $\beta$  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 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 immunoprecipitates were analyzed by Western blotting using 6E10, an anti-Aβ antibody.

Oligomer A<sub>β</sub> Fresh А В Oligomer Aβ + - + LPL(5 μg/ml) Fresh Αβ Αβ IP:α-LPL kDa α-LPL α-LPL IgG IP kDa -220--220 ... - 120 -- 80 -— 60 — - 60 -- Heavy chain — Heavy chain - 50 ---- 40 ----Aβ oligomer Light Chain — 30 — — Light Chain - 20 — 15 —  $\begin{array}{c} A\beta \ trimer \longrightarrow \\ A\beta \ dimer \longrightarrow \end{array}$ 15 - Aβ monomer 6 2 Aβ monomer — Aβ monomer IB:6E10 2

### Supplemental Fig. 1 Nishitsuji et al

IB:6E10