Supplemental Experimental Procedures

Quantitative real-time PCR (qPCR). Total RNAs were extracted from cortical primary astrocytes using TRIzol® Reagent (Invitrogen). RNAs were reverse transcribed with High Capacity cDNA Reverse Transcription kit (Applied Biosystems). qPCR was performed with SYBR® Advantage® qPCR Premix (Clontech) in ABI 7500 instrument (Applied Biosystemes) using the default thermal cycling. The forward primer for ApoE was 5'- CTGACAGGATGCCTAGCCG -3', and the reverse primer was 5'- CGCAGGTAATCCCAGAAGC -3'. U6 primer sets included in the mir-X miRNA First-Strand Synthesis kit (Clontech) were used to normalize qPCR signals among samples. To confirm the specificity of qPCR reactions, dissociation curves were analyzed at the end of qPCR assays. Relative mRNA levels were calculated by comparative Ct method using the Applied Biosystems 7500 software.

Ligand blotting. Purified LDLR extracellular domain (2 μg, Sino Biological) was resolved on nonreducing SDS-PAGE (3-8% Tris-acetate, sample was not boiled and no reducing agent was added) and the protein was then transferred to a PVDF membrane. The LDLR protein was then denatured/renatured in Guan-HCl. The blot was incubated in sequential 30 min washes at room temperature of 6 M, 3 M and 1 M Guan-HCl. The blot was then washed in 0.1 M Guan-HCl for 30 min at 4°C and no Guan overnight at 4°C. For all of the steps the Guan-HCl was diluted into the denaturing/renaturing buffer (10% glycerol, 100 mM NaCl, 20 mM Tris (pH 7.6), 0.5 mM EDTA, 0.1% Tween-20, and 2% milk). The blot was then blocked in 2.5% milk in TBS-T (tris-buffered saline with 0.125% Tween-20). Either Aβ or recombinant apolipoprotein E3 (5 μg/mL, Leinco Technologies, St. Louis, MO) was then incubated with the blot for 3 hr at room temperature in TBS (50 mM Tris-HCl, 150 mM NaCl, pH=7.5), followed by three 10 minute washes in TBS-T. Immunoblotting for either Aβ, apoE, or the His tag was then performed.

Supplemental Figure Legends

Supplemental Fig. 1. Effect of LDLR levels on apoE mRNA amount in astrocytes. Primary astrocytes were cultured from the cortices of Wt, LDLR$^{-/-}$, and LDLR Tg mice. ApoE mRNA levels were then assessed by qPCR, and the values were normalized to U6 snRNA values. Mean ± SEM (n≥4) *** denotes $p<0.001$.

Supplemental Fig. 2. Effect of LDLR levels on the amount of LRP1 and RAP in astrocytes. Primary astrocytes were cultured from the cortices of Wt, LDLR$^{-/-}$, and LDLR Tg mice. **(A)** LRP1 levels and **(B)** RAP levels were measured by immunoblot and normalized to either actin or tubulin levels, respectively. Mean ± SEM (n≥4) *** denotes $p<0.001$, n.s. not significant.

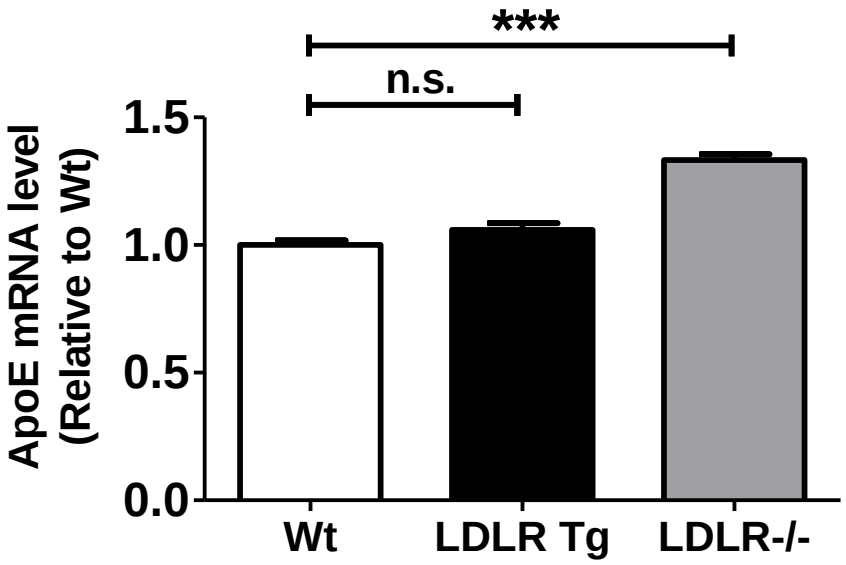
Supplemental Fig. 3. Comparison of LDLR levels in Wt and LDLR$^{-/-}$ primary astrocytes. (A) Primary astrocytes were cultured from the cortices of Wt and LDLR$^{-/-}$ mice. After reaching confluency, the cells were lysed in a 1% Triton X-100 lysis buffer. The lysates were then analyzed by SDS-PAGE and probed using an LDLR antibody. **(B)** LDLR was expressed in LDLR$^{-/-}$ astrocytes via lentiviral transduction. LDLR expression was confirmed by immunoblot for HA.

Supplemental Fig. 4. Ligand blotting to detect Aβ-LDLR interaction. The extracellular domain of LDLR (2μg) was resolved by non-reducing SDS-PAGE and transferred to a PVDF membrane. The LDLR protein was then denatured and renatured on the membrane using sequential treatment with decreasing concentrations of guanidine-HCl. To detect the binding of proteins with LDLR in the membrane, either apoE or Aβ40 (5 ug/mL for each) was incubated with the membrane. NA represents no

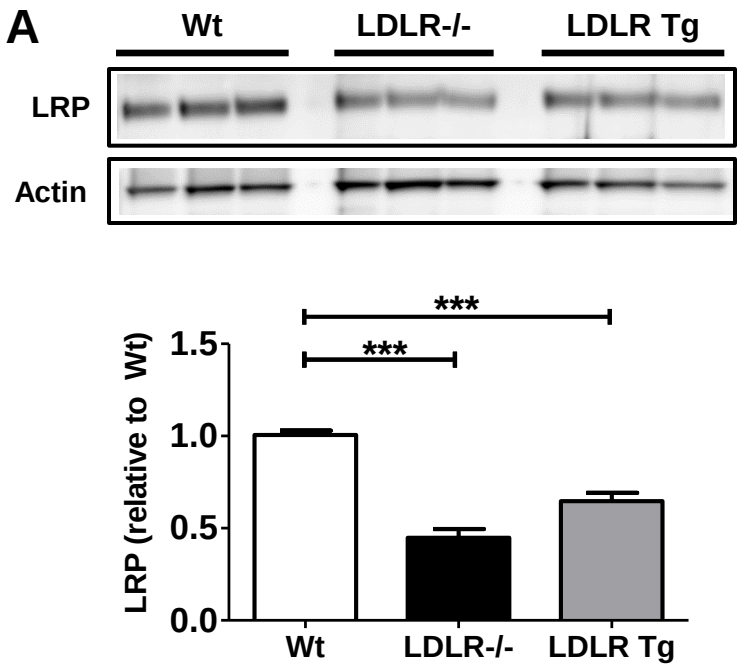
addition. Bound protein was then detected by immunoblot with the respective antibody. To analyze the size of nonreduced LDLR in the membrane, a His tag antibody was used.

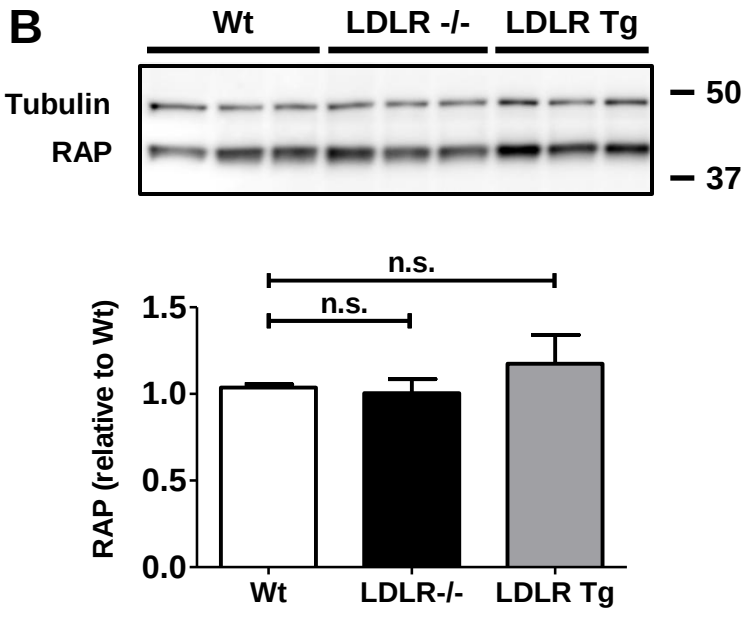
Supplemental Fig. 5. Assessment of Aβ-LDLR interaction via surface plasmon resonance. (A) The interaction between the extracellular domain of LDLR and Aβ was measured by SPR. Aβ40, Aβ42, or DIAβ40 were immobilized on the SPR chip and various concentrations of LDLR were flown over the surface. In order to calculate the dissociation constant for the interaction (K_D), we plotted the resonance units as a function of LDLR concentration. **(B)** Representive sensorgrams show the response over time in resonance units (RU) for the binding of both Aβ40 and Aβ42 at a pH of 7.4 to LDLR.

Supplemental Figure 1

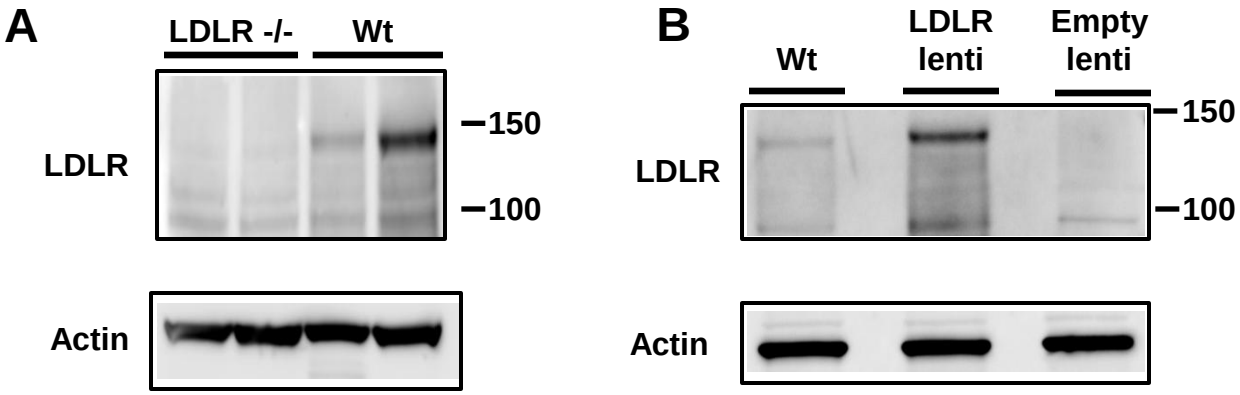


Supplemental Figure 2

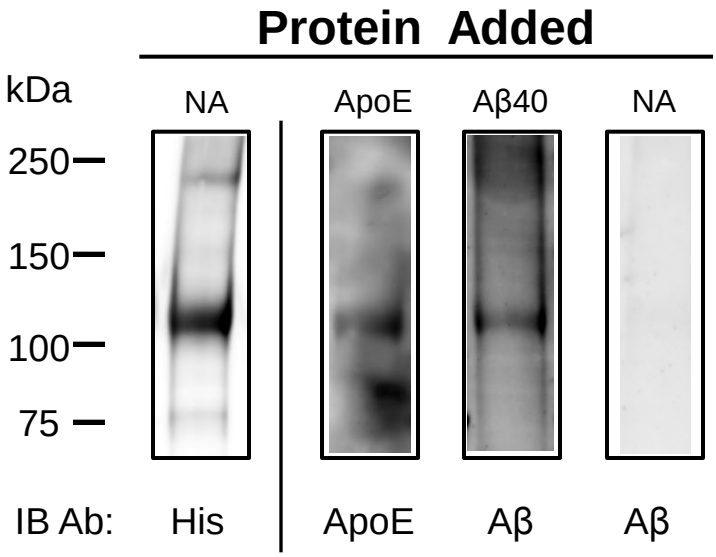




Supplemental Figure 3



Supplemental Figure 4



Supplemental Figure 5