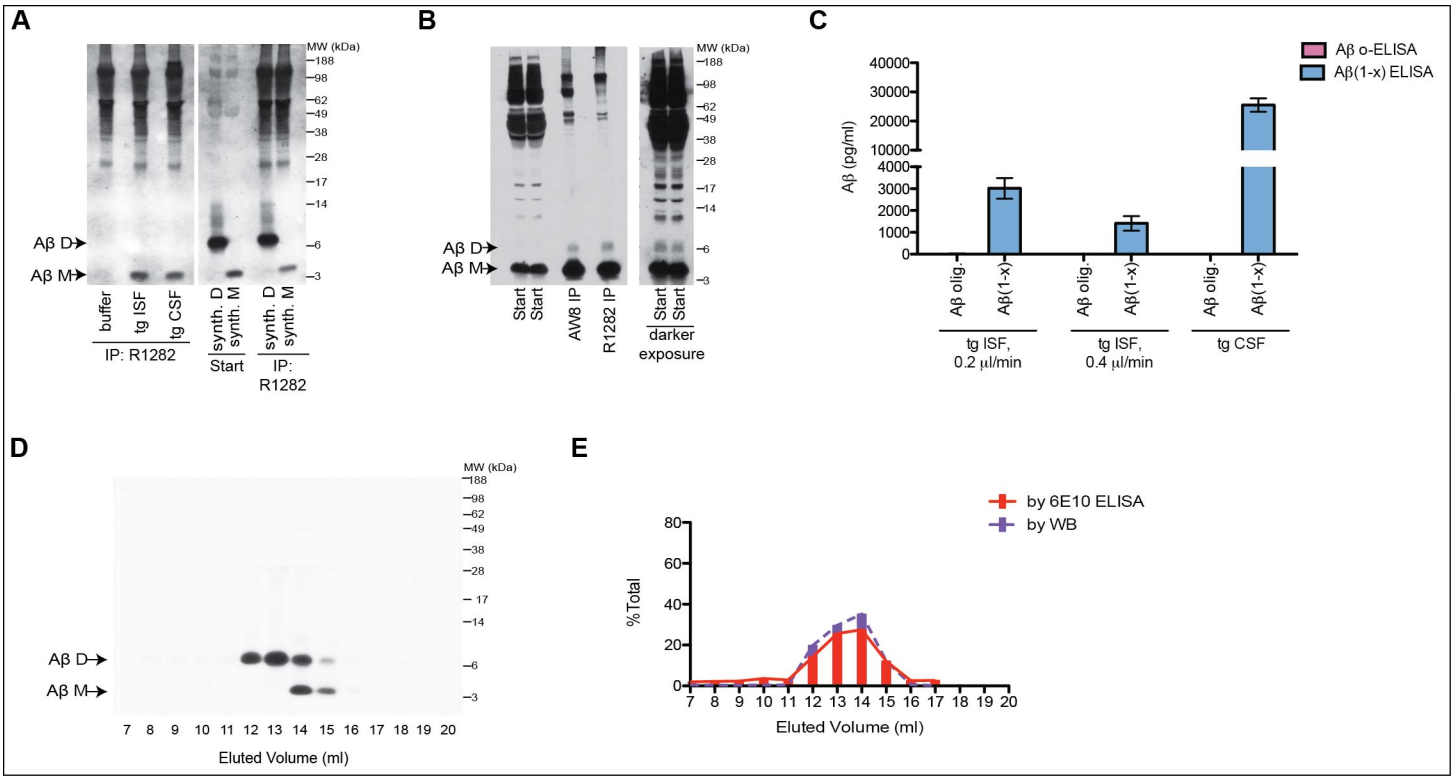

Figure S1, related to Figure 1. (A) R1282 Aβ antiserum fails to pull down Aβ dimers from tg mouse ISF or CSF 500 µl 3 mo tg hippocampal ISF and CSF, 24 mo tg TBS extract, and 2 ng synthetic Aβ S26C dimers (D) or wt monomers (M) were immunoprecipitated for Aβ using R1282 (1:75). WB: 3D6+6E10. **(B) AW8 and R1282 antibodies effectively pull down endogenous Aβ dimers and monomers from tg mouse brain** Start (20 mo tg TBS extract) was immunoprecipitated for Aβ using either AW8 (1:100) or R1282 (1:75). WB: 3D6, 6E10, 266, 2G3 and 21F12. **(C) Tg mouse ISF and CSF contain ample amounts of Aβ monomers but no detectable level of oligomers** ISF microdialyzed at 0.2 µl/min (N=4 mice) and 0.4 µl/min (N=10 mice) and CSF (N=7 mice) were tested on Aβ o-ELISA and $Aβ_{1-x}$ ELISA. **(D, E) Aβ triplex ELISA detects synthetic dimers and monomers to commensurate degrees** Non-denaturing SEC of synthetic Aβ that contained dimers and monomers was performed with Superdex 75 SEC column, followed by analysis of the SEC fractions by WB on SDS-PAGE (WB: 3D6+6E10) (**D**) or by 6E10 Aβ triplex ELISA (**E**). The

WB and ELISA elution profiles gave comparable results (**E**) (dotted line= WB, bold line= 6E10 Aβ triplex ELISA).

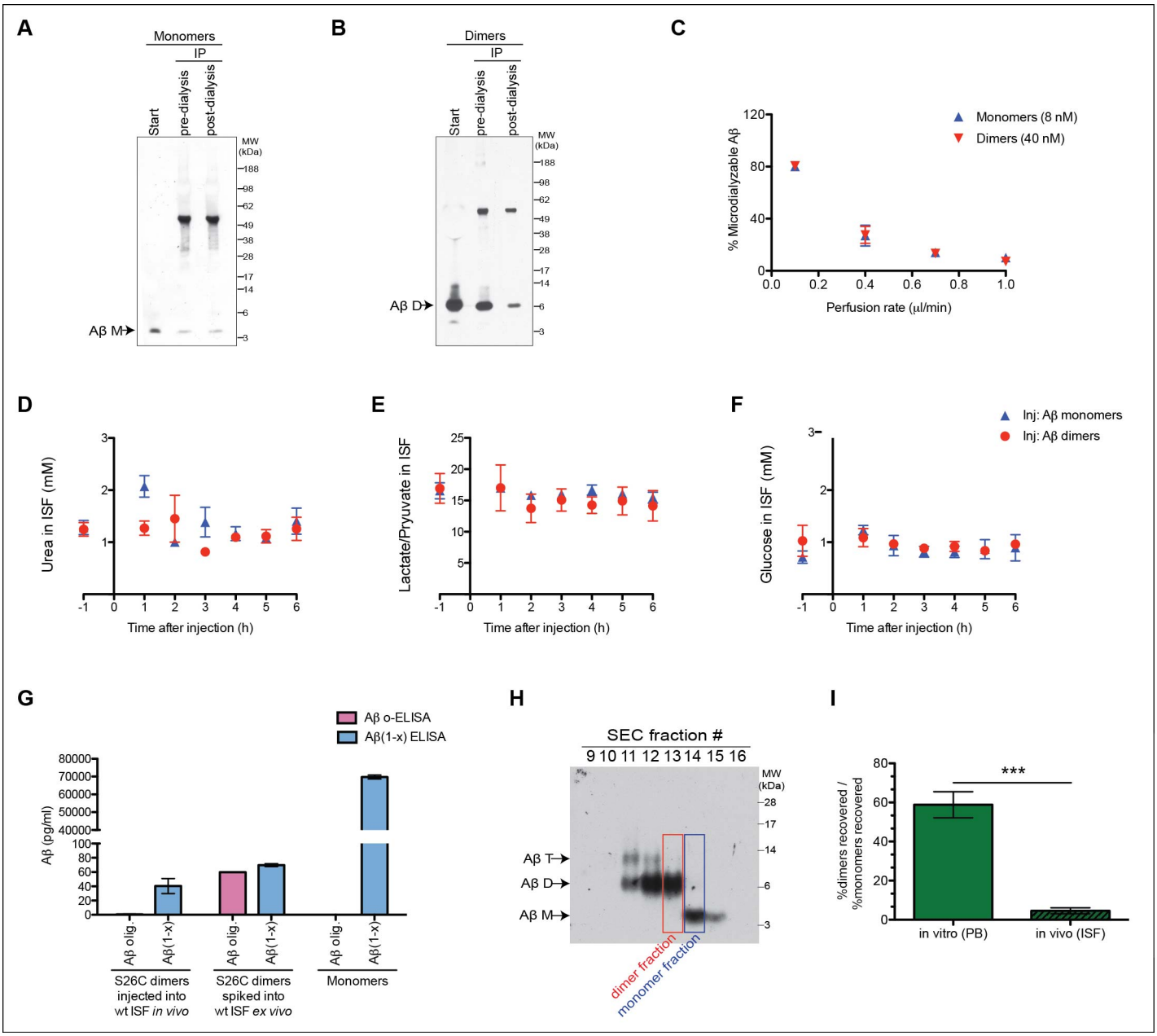

Figure S2, related to Figure 2. (A, B) The *in vitro* microdialysates reflect their respective starting materials and stay as such throughout the time of our analysis Starting materials (pre-dialysis) of Aβ monomers (**A**) or dimers (**B**) and their respective *in vitro* microdialysates (post-dialysis) were immunoprecipitated for Aβ. IP: R1282, WB: 3D6. We avoided multiple freeze-thaw cycles to

keep these Aβ species mostly in their original monomeric and dimeric forms (O'Nuallain et al., 2010). **(C) Comparable % microdialyzable Aβ is captured for both monomers (8 nM) and dimers (40 nM)** The wt Aβ monomers and S26C Aβ dimers were microdialyzed in pairs side by side *in vitro* and analyzed using the extrapolated zero flow microdialysis method (N=3 sets). **(D-F) Non-Aβ analytes in the ISF of wt mice are not altered with injection of Aβ dimers or monomers** Hourly monitoring of urea (**D**), ratio of lactate over pyruvate (**E**) and glucose (**F**) in the ISF of wt mice pre- and post-injection of the Aβ monomers (blue triangle) or Aβ dimers (red circle). N=4-8 mice. Urea in the ISF, frequently used as an endogenous reference compound in microdialysis experiments (Brody et al., 2008; Ronne-Engström et al., 2001), did not change with injection of either Aβ monomers or dimers (**D**). Also, at the low nanomolar concentrations of synthetic Aβ we injected (3 µl injections, corresponding to 96-960 pg Aβ), neither the ratio of lactate/pyruvate nor the level of glucose in the ISF was altered (**E**, **F**). **(G) S26C dimers injected at pg levels into *in vivo* wt ISF are promptly reduced to monomers** Hippocampal ISF was collected from wt mice for 1 h immediately following administration of 960 pg S26C dimers and was analyzed for Aβ levels using $Aβ_{1-x}$ ELISA or Aβ o-ELISA. N=3 mice. A low amount (<80 pg/ml) of Aβ dimers and a high amount (>70 ng/ml) of Aβ monomers were spiked to wt ISF *ex vivo* to test the sensitivity and specificity of the two ELISAs. **(H-I) Non-crosslinked Aβ dimers are also rapidly sequestered away from brain ISF** wt $Aβ_{1-40}$ dimers and monomers were separated using non-denaturing SEC (**H**) and the resulting dimer-enriched and monomer-enriched fractions were used for *in vitro* and *in vivo* microdialysis. The *in vivo* recovery of the Aβ dimers was 4.54 ± 1.53% of that of the Aβ monomers *in vivo* (N=3 pairs), in contrast to that of the *in vitro* recovery, 58.8 ± 6.65% (N=3 pairs) (**I**). ***: P=0.0014 by two-tailed Student's t test.

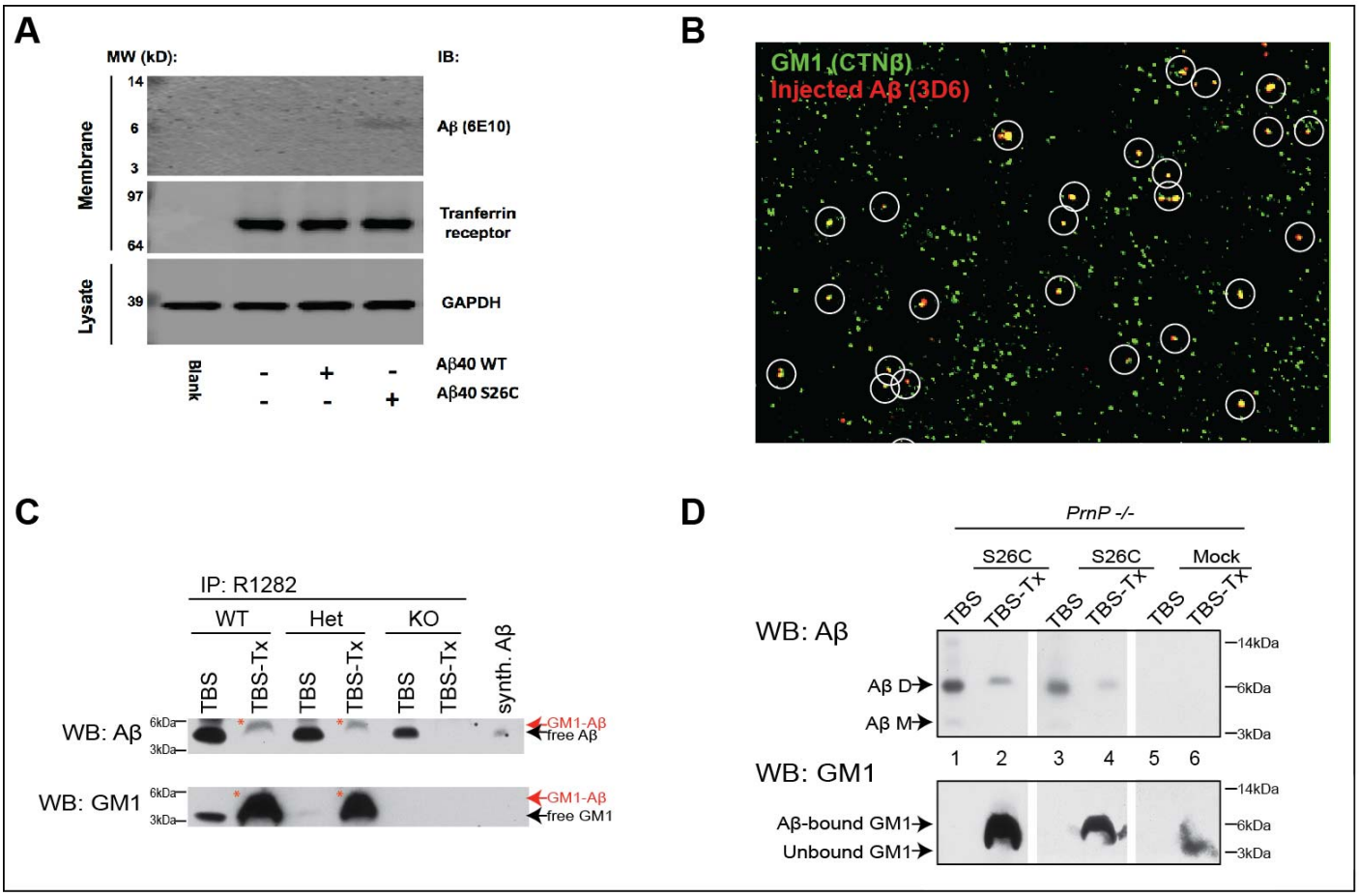

Figure S3, related to Figure 3. (A) Aβ dimers are pulled down from plasma membranes of hippocampal neurons Healthy rat hippocampal neurons were treated for 24 hours with either wt $Aβ_{40}$ monomers or S26C oligomers (500 nM), then subjected to surface biotinylation prior to lysing of cells. The biotinylated plasma membrane proteins were isolated using Streptavidin and separated by SDS-PAGE. WB: Aβ (6E10), Transferrin receptor or GAPDH. **(B) Most of the injected Aβ that remains in the injected brain tissue is associated with endogenous GM1 ganglioside** Mice were perfused 72 hours post ICV injection of 5 ng S26C and their brains fixed and embedded for cryostat sectioning. Immunohistochemistry on such wt mouse brain that received *in vivo* Aβ injection demonstrated that most of the remaining injected Aβ (3D6; red) in CA1 tissue was co-localized with endogenous GM1 (cholera toxin β-subunit; green). **(C) 5-kDa Aβ can no longer be recovered in**

mice lacking GM1 Mice with gene disruption of GM2/GD2 synthase received acute hippocampal Aβ injections and their TBS and TBS-Tx brain extracts were immunoprecipitated for Aβ using R1282. In contrast to their WT and Het controls, R1282 immunoprecipitates of TBS-Tx extracts of KO mice failed to yield 5-kDa species that was both Aβ- (upper panel; 3D6+6E10+266+2G3+21F12) and GM1- (lower panel; CTβ) positive. **(D) PrP^{c} does not seem to be required for Aβ-GM1 interaction** S26C 320 ng or DPBS (mock) were injected to hippocampi of *Prnp* -/- mice. Mice were immediately sacrificed for brain fractionation and the brain extracts were immunoprecipitated for Aβ using R1282. Aβ S26C dimers were recovered as bound to endogenous GM1 in *PrnP* null mice in a manner indistinguishable from the injection results in wt mice (Figure 3), as evidenced by Aβ (Upper panel: 3D6 and 6E10) and GM1 (CTβ) Western blotting.

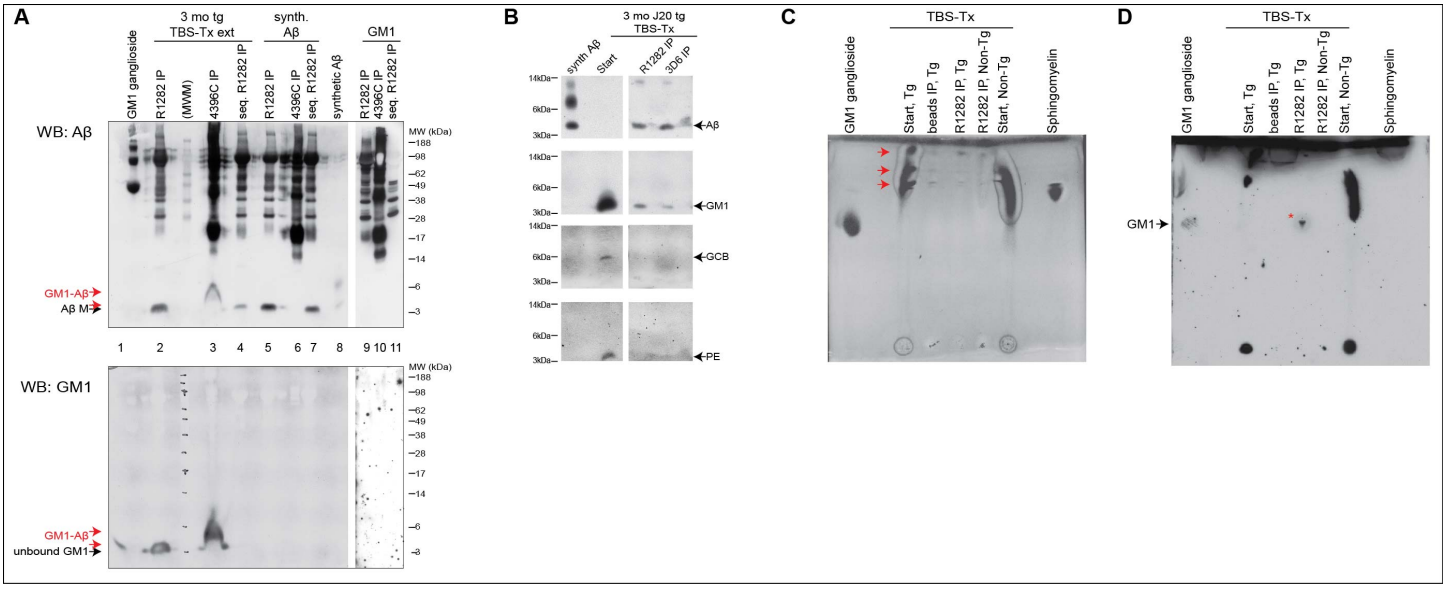

Figure S4, related to Figure 6. (A) 4396C antibody efficiently immunoprecipitates GM1-bound Aβ from J20 tg brain membrane extract TBS-Tx extract of 3 mo tg (lanes 2-4), synthetic Aβ (lanes 5-7, 8) and pure GM1 ganglioside (lanes 1, 9-11) were immunoprecipitated using either R1282 or 4396C. 4396C-immunodepleted supernatants were subsequently subjected to R1282 IP. WB:

3D6+6E10+266+2G3+21F12 for Aβ (upper panel) or HRP-conjugated CTβ for GM1 (lower panel). **(B) Aβ recovered from J20 tg brain membrane extract is preferentially bound to GM1 over other brain lipids** 3 mo tg TBS-Tx was immunoprecipitated for Aβ using R1282 polyclonal and 3D6 monoclonal anti-Aβ antibodies and run on SDS-PAGE. Both Aβ immunoprecipitates yield a band co-immunoreactive for Aβ (3D6+6E10+266+2G3+21F12) and GM1 (CTβ) but not for galactocerebroside (GCB) or phosphatidylethanolamine (PE). (**C-D**) **HP-TLC failed to show enrichment in the major brain lipids**. R1282 Aβ IP of 3 mo tg or non-tg control TBS-Tx extracts were applied onto a high-performance thin-layer chromatography (HP-TLC) plate and lipids were separated using the ganglioside developer. The resulting HP-TLC plate failed to show enrichment in the major brain lipids detectable by primulin (**C**, red arrows) in the Aβ IP of tg and non-tg TBS-Tx. Subsequent CTβ blotting detected GM1-positive smear only in the Aβ IP of the tg but not that of the non-tg (**D**, red asterisk).

SUPPLEMENTAL EXPERIMENTAL PROCEDURES

Animals

J20 line carrying hAPP minigene with FAD mutations KM670/671NL and V717F was a kind gift of L. Mucke (UCSF) and was maintained on a C57BL6xDBA2 background. Wild-type mice used in this study were C57BL/6xDBA2, except for C57BL/6x129 for LTP studies and C57BL/6 for use as appropriate controls for the KO mice. Mice that were heterozygous or KOs for gangliotetraose gangliosides were offsprings of mice with disrupted gene for GM2/GD2 synthase (C57BL/6 background) and a very generous gift of R. Ledeen (New Jersey Medical School). *PrnP* null mice were a kind gift of D. Harris (Boston University). All animal procedures were approved by the Harvard Medical School Institutional Animal Care and Use Committee.

Immunoprecipitation and Western blot for Aβ, GM1 ganglioside or other lipids

Samples were pre-cleared using Protein A (Sigma), then immunoprecipitated using either AW8 (1:100, gift of D. Walsh, BWH) or R1282 (1:75) Aβ antisera and Protein A. Alternatively, samples were immunoprecipitated using 4396C monoclonal antibody and Protein G (Santa Cruz). For IP/WB, a previously described protocol (Walsh et al., 2000) was modified; briefly, samples were electrophoresed using 12% Bis-Tris gel and MES SDS running buffer (Invitrogen), transferred onto 0.2-μm nitrocellulose, boiled, then blotted to human Aβ using 3D6 (against $Aβ_{1-5}$, gift of Elan) and 6E10 (against $Aβ_{5-9}$, Covance), to total Aβ using 3D6, 6E10 plus 266, 2G3 and 21F12 (against $Aβ_{16-23}$, $Aβ_{31-40}$ and $Aβ_{33-42}$, respectively, gifts of Elan) antibodies, to GM1 ganglioside using HRP-conjugated cholera-toxin β-subunit (Sigma), to GCB (Millipore), or to PE (Echelon Biosciences), and detected using either ECL Prime WB Detection Reagent (GE Healthcare) or LiCor Odyssey Infrared Imaging System.

Aβ ELISA

Sandwich ELISAs for Aβ were performed using the MULTI-ARRAY® 96-well Plate platform (MesoScale Discovery) following the manufacturer's protocol and as described previously (Hong et al., 2011; Yang et al., 2013). For Aβ o-ELISA, Aβ N-terminal 3D6 was used as both capture at 3 μg/ml and biotinylated detection at 100 ng/ml with SULFO-TAG™ Streptavidin (MesoScale Discovery). For $Aβ_{1-x}$, $Aβ_{1-40}$ and $Aβ_{1-42}$ ELISAs, 266, 2G3 and 21F12 antibodies were used as capture antibodies, respectively, and biotinylated 3D6 was used as detection. For 6E10 Aβ Triplex ELISA, we used the MSD® 96-well MULTI-SPOT® Human (6E10) Abeta Triplex Assay, which uses MULTI-SPOT®

microplates pre-coated with antibodies specific to the C-termini of $A\beta_{38}$, $A\beta_{40}$ and $A\beta_{42}$, and detected with SULFO-TAG^{TM}-labeled 6E10 antibody.

Test tube microdialysis for crossover efficiency

To determine concentrations where equal percentages of monomers and dimers cross over the microdialysis membrane, we performed *in vitro* microdialysis at 0.4 µl/min with differing concentrations of wt $A\beta_{1\text{-}40}$ monomers (MesoScale Discovery) in parallel with the $(A\beta_{1\text{-}40}$ S26C$)_2$ dimers (gift of D. Walsh, BWH), and assessed amount of Aβ recovered in the microdialysates using $A\beta_{1\text{-}x}$ ELISA. % Starting material (SM) that crossed over the microdialysis membrane increased in a non-linear fashion with increasing concentrations of SM. The crossover efficiency of dimers was calculated to be comparable to that of monomers when enhanced 5-fold, i.e., dimers at 40 nM and monomers at 8 nM. To verify this, test tubes containing S26C dimers at 40 nM or wt monomers at 8 nM were microdialyzed side by side at 0.1, 0.4 and 1.0 µl/min, and the resulting microdialysates were assessed for levels of Aβ using the $A\beta_{1\text{-}x}$ ELISA. Oligomerization states of SMs and their corresponding microdialysates were checked by IP/WB, $A\beta_{1\text{-}x}$ ELISA and o-ELISA.

Interpolated zero-flow method

In vitro % recovery was assessed as previously described (Hong et al., 2011). Microdialysis was performed from SM containing 8 nM Aβ monomers or 40 nM dimers, while varying the perfusion rates (PR): 1.0, 0.7, 0.4, and 0.1 µl/min. Aβ levels were measured using $A\beta_{1\text{-}x}$ ELISA and values obtained at each PR were plotted versus the PR. 100% recovery (i.e., the theoretical maximal amount of exchangeable ISF Aβ occurring at zero PR) was calculated by extrapolating back the curve to a zero-

flow rate. Then, for each PR, % recovery was determined by calculating how much % Aβ was captured as compared to the theoretical [Aβ] at zero PR.

Surface biotinylation assay

Intact, healthy rat E18 hippocampal neurons at 21 days in vitro were treated with wt Aβ40 monomer or Aβ40 S26C oligomers (500 nM) for 24 h, placed the neurons on ice and rinsed in cold PBS. The intact neurons were then incubated in PBS containing 1 mg/ml Sulfo-NHS-SS-Biotin (Thermo Scientific) for 30 min at RT and the reaction quenched by adding 1M Tris pH 8.0 to a final concentration of 25-50 mM and rocked for 5 min. Neurons were then rinsed in PBS to remove unbound biotin and extracted in lysis buffer (1% Triton X-100, 0.1% SDS, complete protease inhibitor cocktail (Roche), phosphatase inhibitor (Roche)). The biotinylated plasma membrane proteins were isolated by using Streptavidin agarose (Sigma), separated by SDS-PAGE, and analyzed by Western blotting.

Thin-Layer Chromatography

R1282 Aβ immunoprecipitates of 3 mo tg or WT control TBS-Tx extracts were solubilized using 2:1 $CHCl_3$:MeOH and applied onto a 10 cm x 10 cm silica gel (0.15-mm thick) high-performance thin-layer chromatography (HPTLC) plate (Sigma) along with lipid standards (Avanti). Lipids were then separated using ganglioside developer $CHCl_3$:MeOH:0.2% $CaCl_2$ (60:40:9, by vol.) as previously described (Taki et al., 2009). HPTLC plate was then dried and visualized using primulin (Sigma) and Imager at UV 315 nm. Following primulin detection, plate was then treated with 0.4%

polyisobutylmethacrylate and developed for GM1 detection using HRP-conjugated CTβ and ECL Prime.

Immunohistochemistry and microscopy

For immunohistochemical analyses, a similar protocol was modified (Schafer et al., 2012). Briefly, mice were perfused with PBS followed by 4% PFA and brains were postfixed for 2 h at 4°C, transferred to 30% sucrose for 24-48 h then embedded for cryostat sectioning. 14-µm thick cryosections were permeabilized for 1 h in 5% BSA and 0.2% Triton-X 100 (Sigma) at RT followed by primary antibody (for Aβ: 3D6; for GM1: cholera toxin β-subunit) overnight at 4°C. Sections were then treated with the appropriate Alexa-fluorophore-conjugated secondary antibodies and mounted on slides with vectashield containing DAPI (Vector labs). All images were acquired using LSM700 confocal microscope and Zen 2009 image acquisition software (Carl Zeiss).

SUPPLEMENTAL REFERENCES Brody, D.L., Magnoni, S., Schwetye, K.E., Spinner, M.L., Esparza, T.J., Stocchetti, N., Zipfel, G.J., and Holtzman, D.M. (2008). Amyloid-beta dynamics correlate with neurological status in the injured human brain. Science *321*, 1221–1224.

Hong, S., Quintero-Monzon, O., Ostaszewski, B.L., Podlisny, D.R., Cavanaugh, W.T., Yang, T., Holtzman, D.M., Cirrito, J.R., and Selkoe, D.J. (2011). Dynamic analysis of amyloid β-protein in behaving mice reveals opposing changes in ISF versus parenchymal Aβ during age-related plaque formation. Journal of Neuroscience *31*, 15861–15869.

O'Nuallain, B., Freir, D.B., Nicoll, A.J., Risse, E., Ferguson, N., Herron, C.E., Collinge, J., and Walsh, D.M. (2010). Amyloid beta-protein dimers rapidly form stable synaptotoxic protofibrils. J Neurosci *30*, 14411–14419.

Ronne-Engström, E., Cesarini, K.G., Enblad, P., Hesselager, G., Marklund, N., Nilsson, P., Salci, K., Persson, L., and Hillered, L. (2001). Intracerebral microdialysis in neurointensive care: the use of urea as an endogenous reference compound. J Neurosurg *94*, 397–402.

Schafer, D.P., Lehrman, E.K., Kautzman, A.G., Koyama, R., Mardinly, A.R., Yamasaki, R., Ransohoff, R.M., Greenberg, M.E., Barres, B.A., and Stevens, B. (2012). Microglia Sculpt Postnatal Neural Circuits in an Activity and Complement-Dependent Manner. Neuron *74*, 691–705.

Taki, T., Gonzalez, T.V., Goto-Inoue, N., Hayasaka, T., and Setou, M. (2009). TLC blot (far-eastern blot) and its applications. Methods Mol Biol *536*, 545–556.

Walsh, D.M., Tseng, B.P., Rydel, R.E., Podlisny, M.B., and Selkoe, D.J. (2000). The oligomerization of amyloid beta-protein begins intracellularly in cells derived from human brain. Biochemistry *39*, 10831–10839.

Yang, T., Hong, S., O'Maley, T., Sperling, R.A., Walsh, D.M., and Selkoe, D.J. (2013). New ELISAs with high specificity for soluble oligomers of amyloid β-protein detect natural Aβ oligomers in human brain but not CSF. Alzheimer's and Dementia.