Supplemental Information

Alzheimer Amyloid-ß Oligomer Bound to Post-Synaptic Prion Protein Activates Fyn to Impair Neurons

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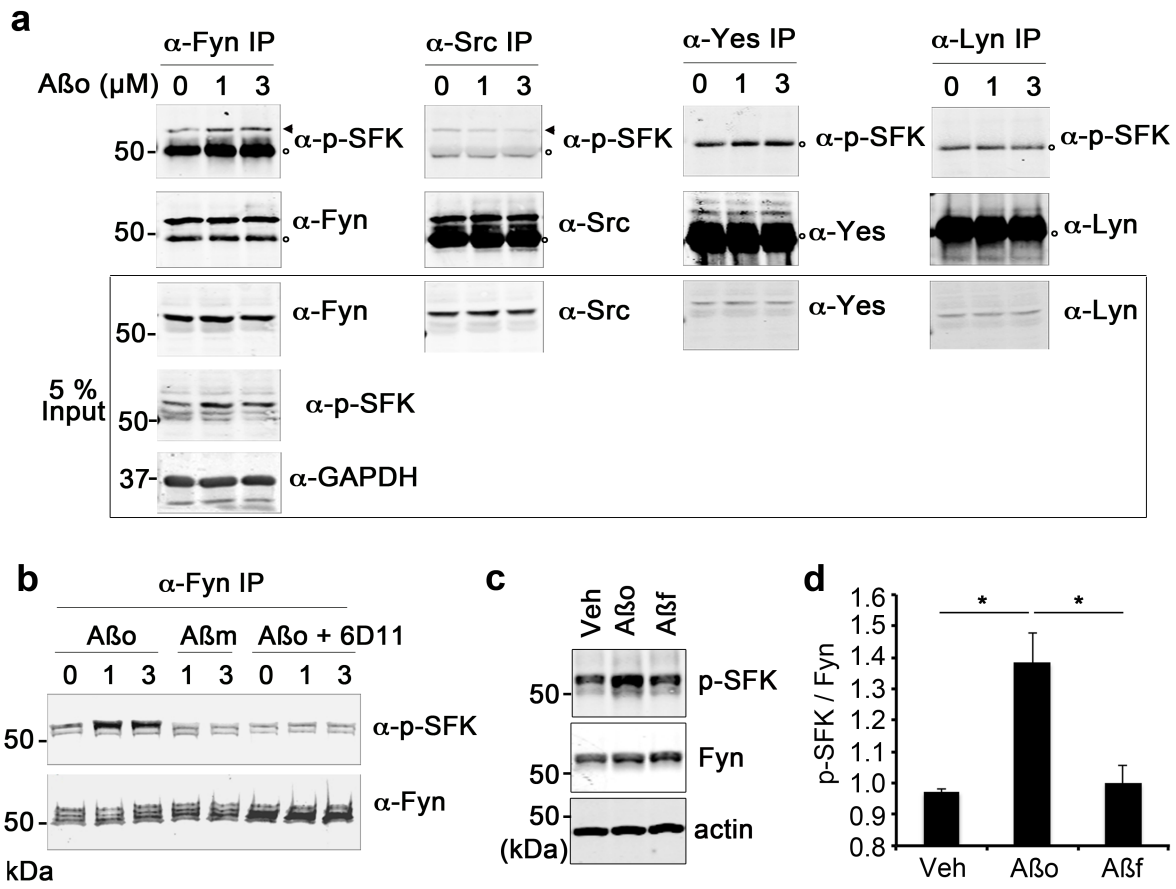


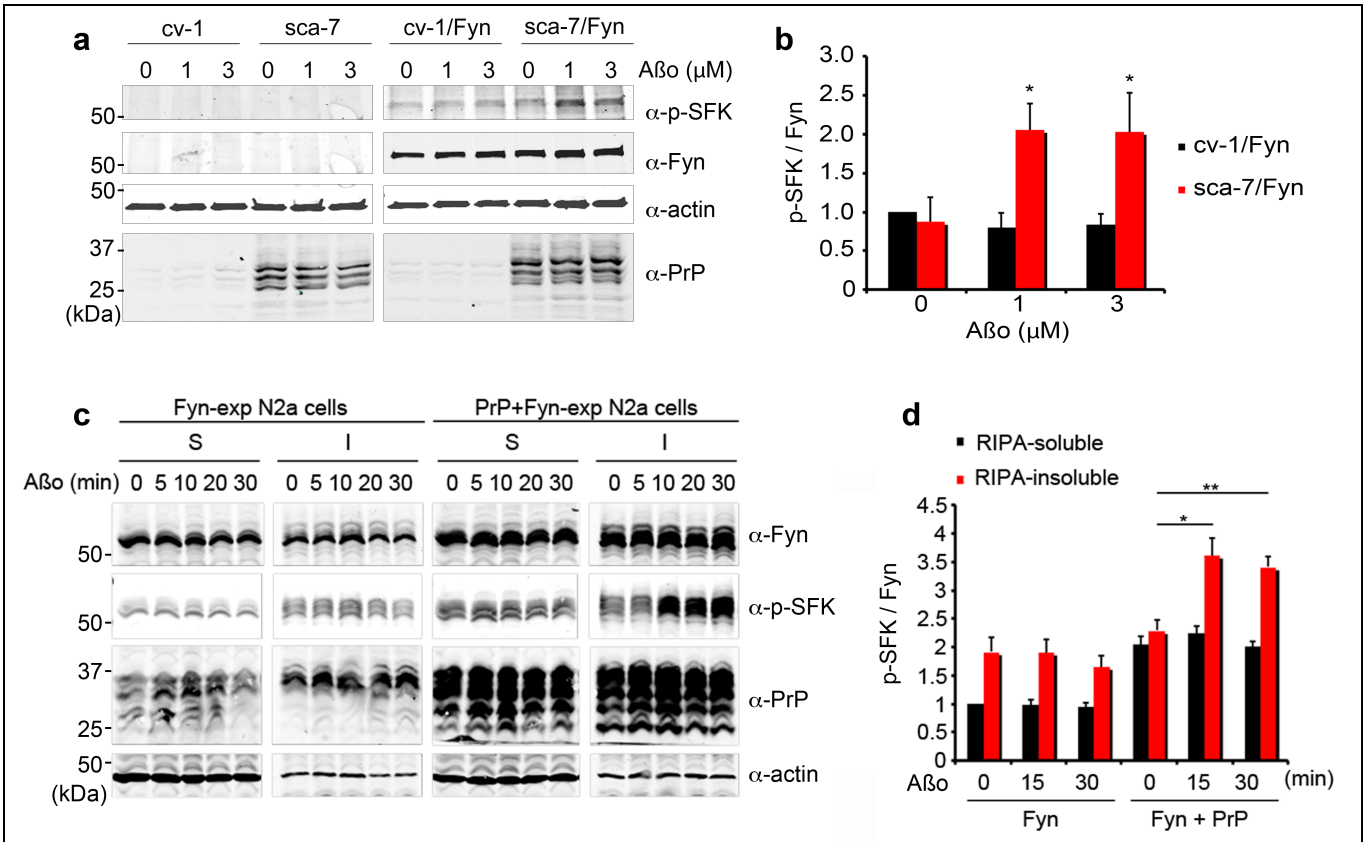
Supplemental Figure S1. Fyn kinase is activated specifically by Aβ oligomers.

a Cultured neurons from E18 wild type rats after 21 DIV were untreated or treated with 1 or 3 μM Aβo for 15 minutes. Immunoprecipitation (IP) was performed with anti-Fyn, anti-SFK, anti-Yes, or anti-Lyn antibody, followed by immunoblotting with anti-phospho-SFK (Tyr 416) antibody. Total cell lysates were immunoblotted with anti-Fyn, anti-Src, anti-Yes, anti-Lyn or anti-phospho-SFK (Tyr416) antibody. GAPDH served as a loading control. Arrowhead indicates phospho-SFK bands. Arrowheads indicate the kinase, and the open circles indicate IgG heavy chains.

b Cultured neurons at DIV21 from E18 wild type rats were treated with 0, 1 or 3 μM Aßo or Aßm for 15 minutes. Prior to Aβo exposure, the indicated cultures were pre-incubated with 10 μg/ml of 6D11 antibody for 1 hour. Immunoprecipitation (IP) was performed with anti-Fyn, followed by immunoblotting with anti-phospho-SFK (Tyr416) antibody.

c Cortical neurons from E17 wild type after 21 DIV were treated with 1 μM Aßo (Aßo) or Aß fibril (Aßf) for 20 min. Whole cell lysates were analyzed by anti-phospho-SFK (Tyr 416) or anti-Fyn immunoblot. Actin served as a loading control.

d Quantification of phospho-SFK level in the lysates (from c) normalized to Fyn immunoreactivity from three independent experiments. Mean ± s.e.m. *, $P < 0.05$; one-way ANOVA, with Tukey post-hoc pairwise comparisons.



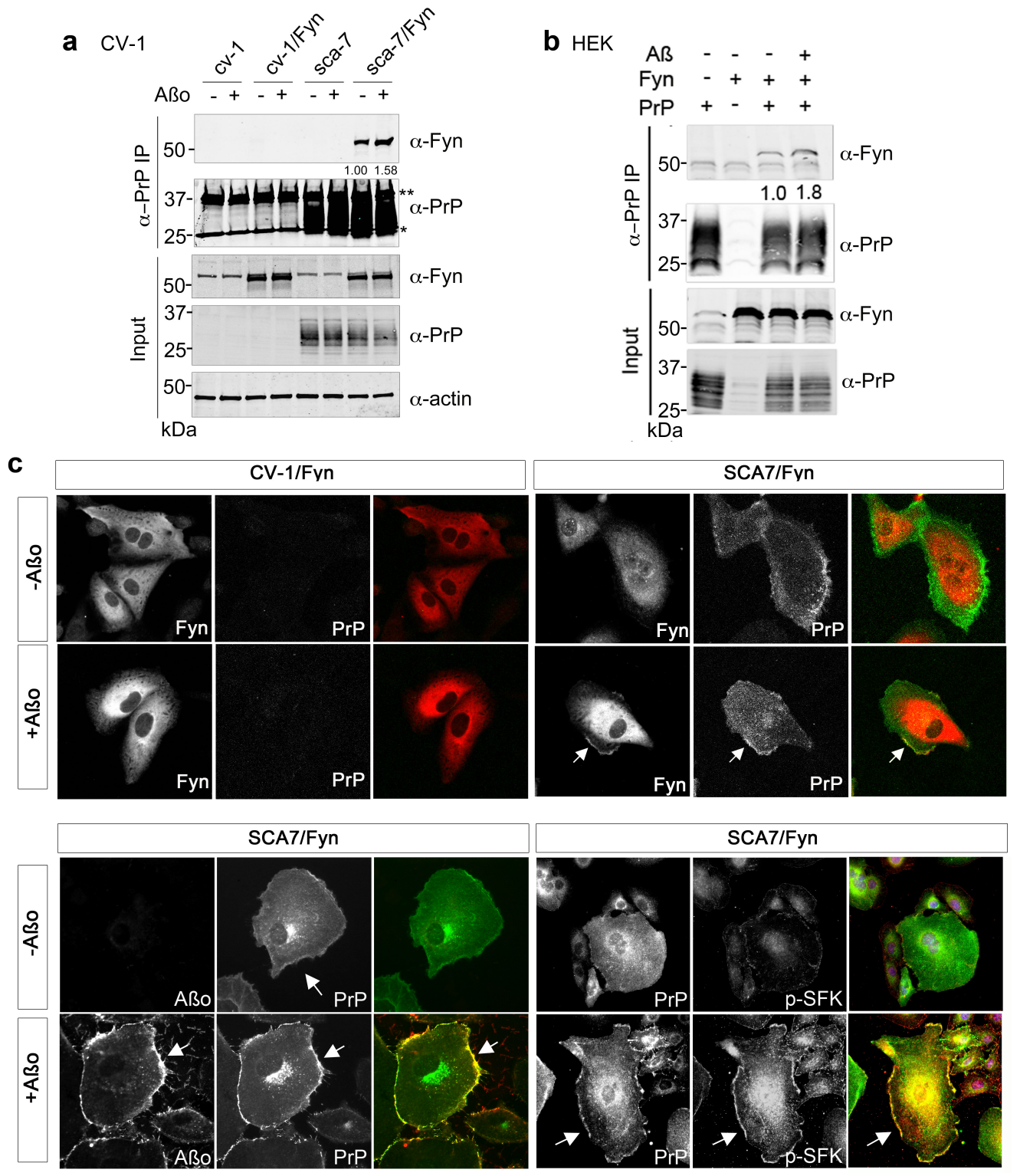
Supplemental Figure S2. Fyn kinase is activated by Aβ binding to PrP^C in cell lines.

a CV-1, SCA-7, CV-1/Fyn and SCA-7/Fyn cells were treated with 0-3 μM oligomeric Aβ (Aβo, monomer equivalents) for 15 minutes, and cell lysates were analyzed by immunoblot with anti-phospho-SFK (Tyr 416), anti-Fyn, or anti-PrP^C antibody. Actin served as a loading control.

b Quantification of phospho-SFK level in the lysate normalized to Fyn immunoreactivity from three independent experiments. Data are mean ± s.e.m. *, $P < 0.05$, Student's two-tailed *t* test.

c After N2a cells were transfected with Fyn alone or together with PrP^C, cells were untreated or treated with 1 μM Aβo for 5, 10, 20 or 30 minutes. Cell lysates were fractionated into RIPA-soluble fraction (S) and RIPA-insoluble fraction (I). Each fraction was analyzed by immunoblotting with anti-Fyn, anti-phospho-SFK (Tyr 416) or anti-PrP^C antibody. Actin served as a loading control.

d Quantification of phosphor-SFK level in the lysate normalized to Fyn immunoreactivity from 4 independent same experiments as c.



Supplemental Figure S3. Aßo induce PrP^C/Fyn Association and Co-Localization.

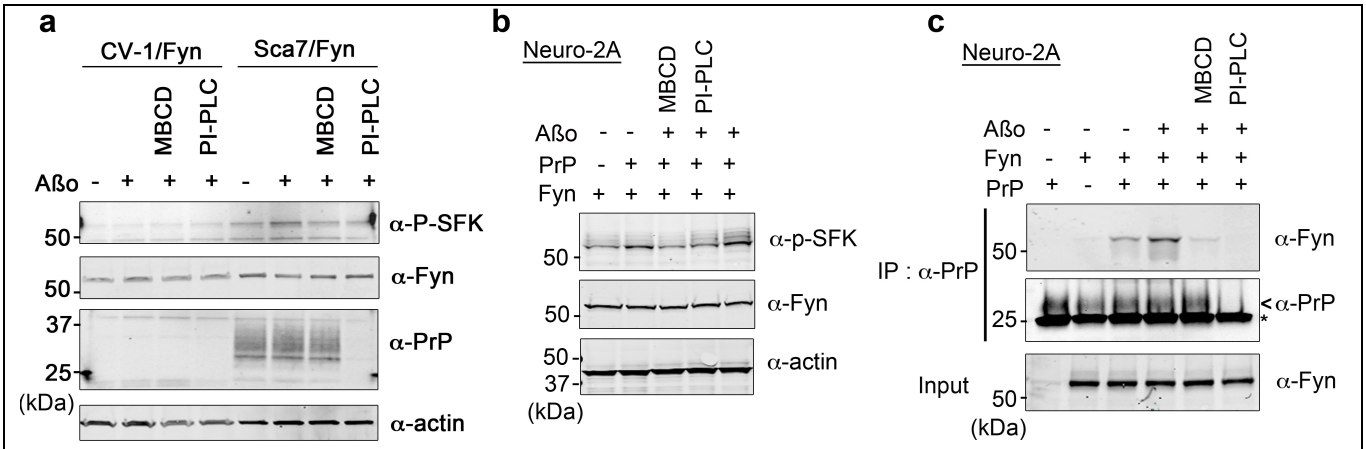
a CV-1, CV-1/Fyn, SCA-7 and SCA-7/Fyn cells were treated with 0 or 1 μM Aβo for 1.5 hours, and immunoprecipitation (IP) was performed with anti-PrP^C antibody. Whole lysates and anti-PrP^C immunoprecipitates were immunoblotted with anti-Fyn or anti-PrP^C antibody. The values at the bottom of the top panel indicate the relative quantities of Fyn bands. Actin served as a loading control. The asterix are non-specific bands from the immunoprecipitation.

b After HEK293 cells were transfected with PrP^C and Fyn, cells were treated with 0 or 1 μM Aßo for 1.5

hours. Immunoprecipitation (IP) was performed with anti-PrP antibody, and then whole lysates and anti-PrP^C immunoprecipitates were immunoblotted with anti-Fyn or anti- PrP^C antibody. The values at the bottom of the top panel indicate the relative quantities of Fyn bands.

c CV-1/Fyn (upper left set) and SCA-7/Fyn cells (upper right set) were treated with 0-1 μM Aβo for 15 minutes, and labeled with mouse anti-PrP^C on ice and then fixed, permeablilized and labeled with rabbit anti-Fyn antibodies. Immunostained preparations were examined using laser scanning confocal microscope. Note colocalization in ruffles indicated by arrowheads.

In the lower set of panels, SCA-7/Fyn cells were treated with 0-1 μM biotinylated Aβo (lower left set) or non-biotinylated Aßo (lower right set) and then fixed, permeablilized and labeled with mouse anti-PrP^C and streptavidin covalently attached with Alexa Fluor 568 (lower left) or with mouse anti-PrP^C (3F4 clone) and rabbit anti-phospho-SFK (Tyr 416) antibodies (lower right). Immunostained preparations were examined using epifluorescent microscopy.

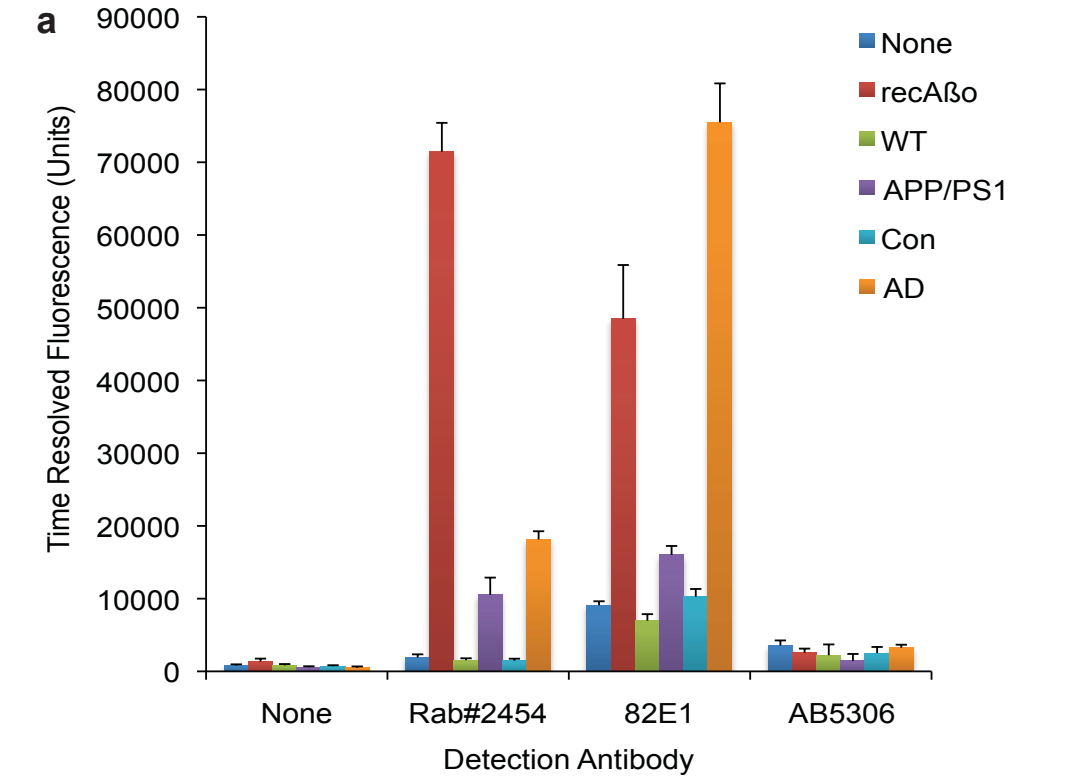


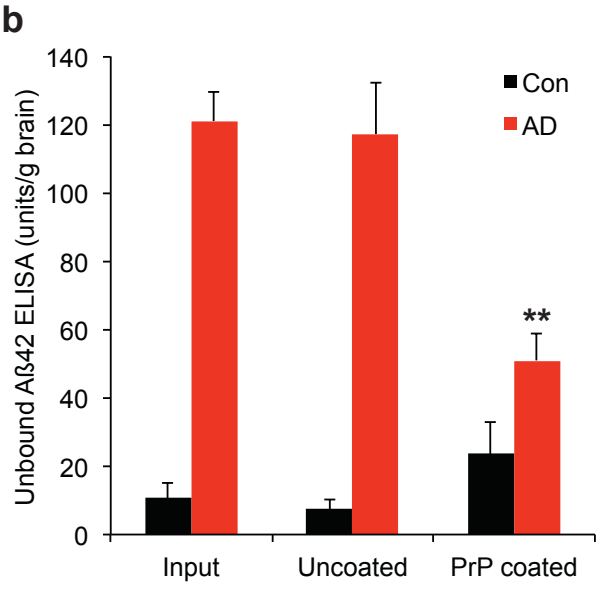
Supplemental Figure S4. Aßo Activation of PrP/Fyn Requires Lipid Rafts and GPI Anchorage in Cell Lines.

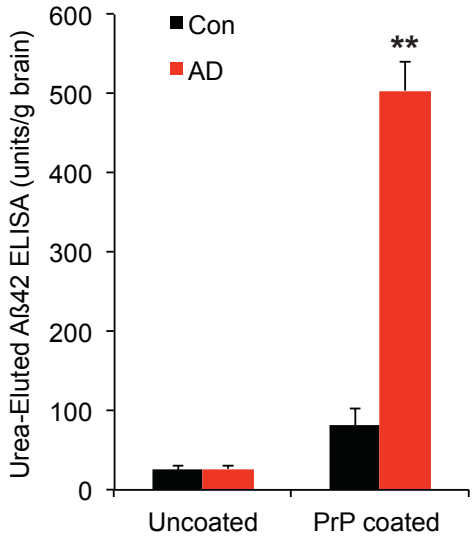
a CV-1/Fyn or SCA7/Fyn cells were treated with 0 or 1 μM Aßo for 20 min. Prior to Aßo exposure, the indicated cultures were pre-treated with 5 mg/ml MBCD for 1 hour or 0.1 unit of PI-PLC for 10 min. Cell lysates were analyzed by immunoblot with anti-phospho-SFK (Tyr 416), anti-Fyn, or anti-PrP^C antibody. Actin served as a loading control.

b After N2a cells were transfected with PrP^C and Fyn, cells were treated with 0 or 1 μM Aßo for 20 min. Prior to Aßo exposure, the indicated cultures were pre-treated with 5 mg/ml MBCD for 1 hour or 0.1 unit of PI-PLC for 10 min. Cell lysates were analyzed by immunoblotting with anti-phospho-SFK (Tyr 416) or anti-Fyn antibodies. Actin served as a loading control.

c After N2a cells were transfected with PrP^C and Fyn, cells were treated with 0 or 1 μM Aßo for 1.5 hours. Prior to Aßo exposure, the indicated subcultures were pre-treated with 5 mg/ml methyl-ß-cyclodextrin (MBCD) for 1 hour or 0.1 unit of PI-PLC for 10 min. IP was performed with anti-PrP antibody, and then anti- PrP^C immunoprecipitates were immunoblotted with anti-Fyn or anti- PrP^C antibody. The asterick indicates IgG light chain and the arrowhead, PrP^C.







d

	Control	AD
Number	25	29
Age (mean + sem)	74+3 years	77+2 years
Gender (female, %)	52	54
Post-Mortem Interval	12+2 hours	13+1 hours

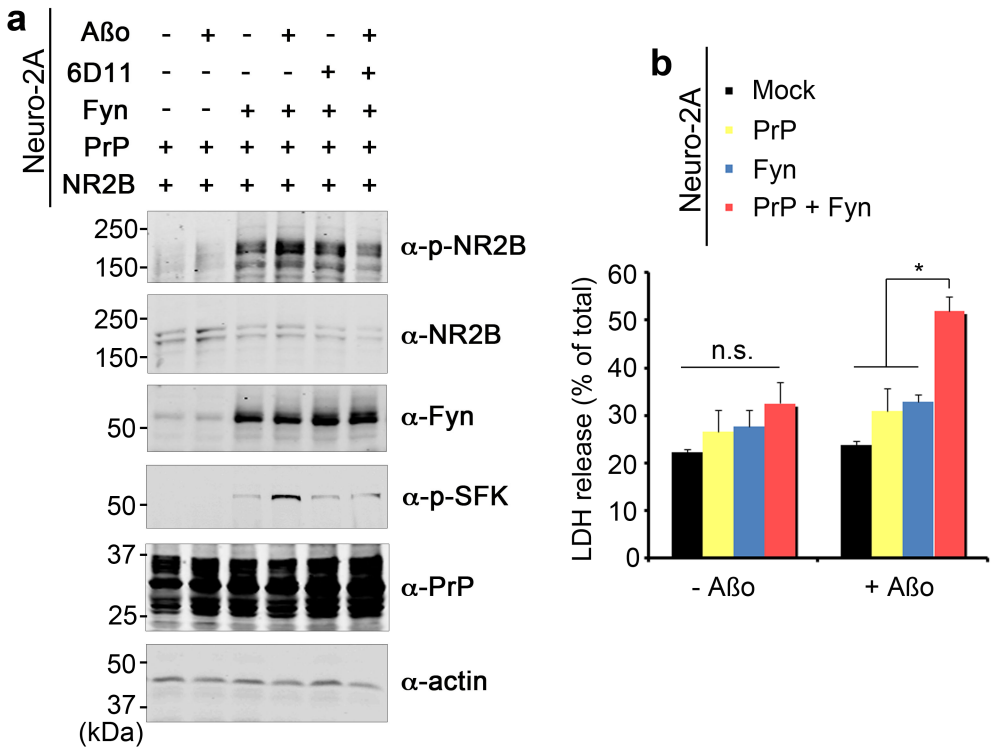
Supplemental Figure S5. Detection of Aßo Bound to PrP (23-111).
a Different samples containing Aß species were incubated with immobilized PrP(23-111). Samples: pure Aßo (recAßo, 1 ng), wild type mouse brain (WT, 10 μg total protein), APPswe/PS1ΔE9 mouse brain (APP/PS1, 10 μg protein), control human TBS brain extract (Con, 50 μg protein) or AD human TBS brain extract (AD, 50 μg protein). Bound Aß was detected by time-resolved fluorescence derived

from anti-Aß antibody and europium-tagged secondary reagents, using the indicated primary antibodies. Mean ± s.e.m. for 3 replicates.

b Total Aß42 immunoreactivity in TBS-Soluble extracts pooled from 4 Control or 4 AD Brain specimens was assessed by standard sandwich Aß42 ELISA assay. The brain extract was assayed before (Input) and after incubation with microtiter wells coated with or without PrP(23-111), as indicated. The level of Aß42 immunoreactivity after incubation with PrP-coated wells is decreased (P<0.01, ANOVA) relative to the Input or incubation with control non-PrP wells. Mean ± sem from n=4 replicates.

c Uncoated or PrP-coated wells previously exposed to AD or Control brain TBS-soluble extracts were eluted with 10 M Urea, and the eluted material was assayed for Aß42 immunoreactivity. Greater immunoreactivity is eluted from PrP-coated wells exposed to AD extract (P<0.01, ANOVA) relative to other conditions. Mean ± sem from n=4 replicates.

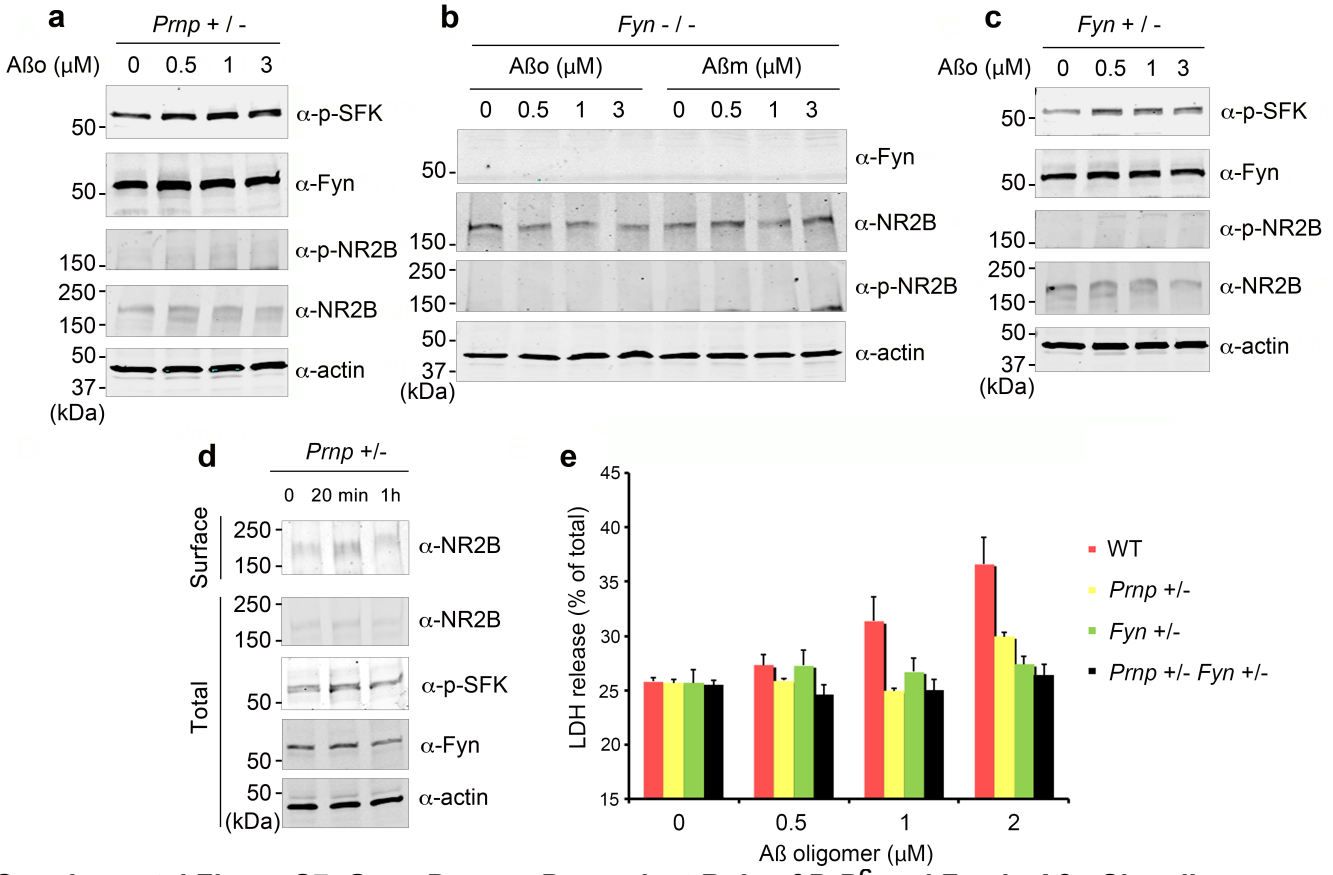
d Characteristics of human brain samples used for assay of PrP-interacting Aß psecies.



Suppl. Fig. S6. Aßo Phopshorylates NR2B and Produces Cell Toxicity in N2A Neuroblastoma Cells via PrP and Fyn.

a N2A cells were transfected with expression vectors for PrP^C plus NR2B with or without Fyn, and some cultures were then pre-incubated for 1 h with 10 μg/ml of 6D11 antibody prior to Aß exposure. After treatment of cells with 0 or 1 μM of Aβo (monomer equivalent, 10 nM estimated oligomer) for 20 min, lysates were subjected to immunoblot with anti-phospho-NR2B (Tyr 1472), anti-NR2B, anti-Fyn, anti-phospho-SFK (Tyr 416) or anti-PrP antibody. Actin served as a loading control.

b N2A cells were transfected with expression vectors for PrP^C, or Fyn, or both, and then treated 48 hours later with 0 or 1 μM Aβo for 1.5 h. Cell toxicity was determined by LDH release. Data are mean ± s.e.m. *, $P < 0.05$; one-way ANOVA, with Tukey post-hoc pairwise comparisons.



Supplemental Figure S7. Gene Dosage Dependent Role of PrP^C and Fyn in Aßo Signaling.

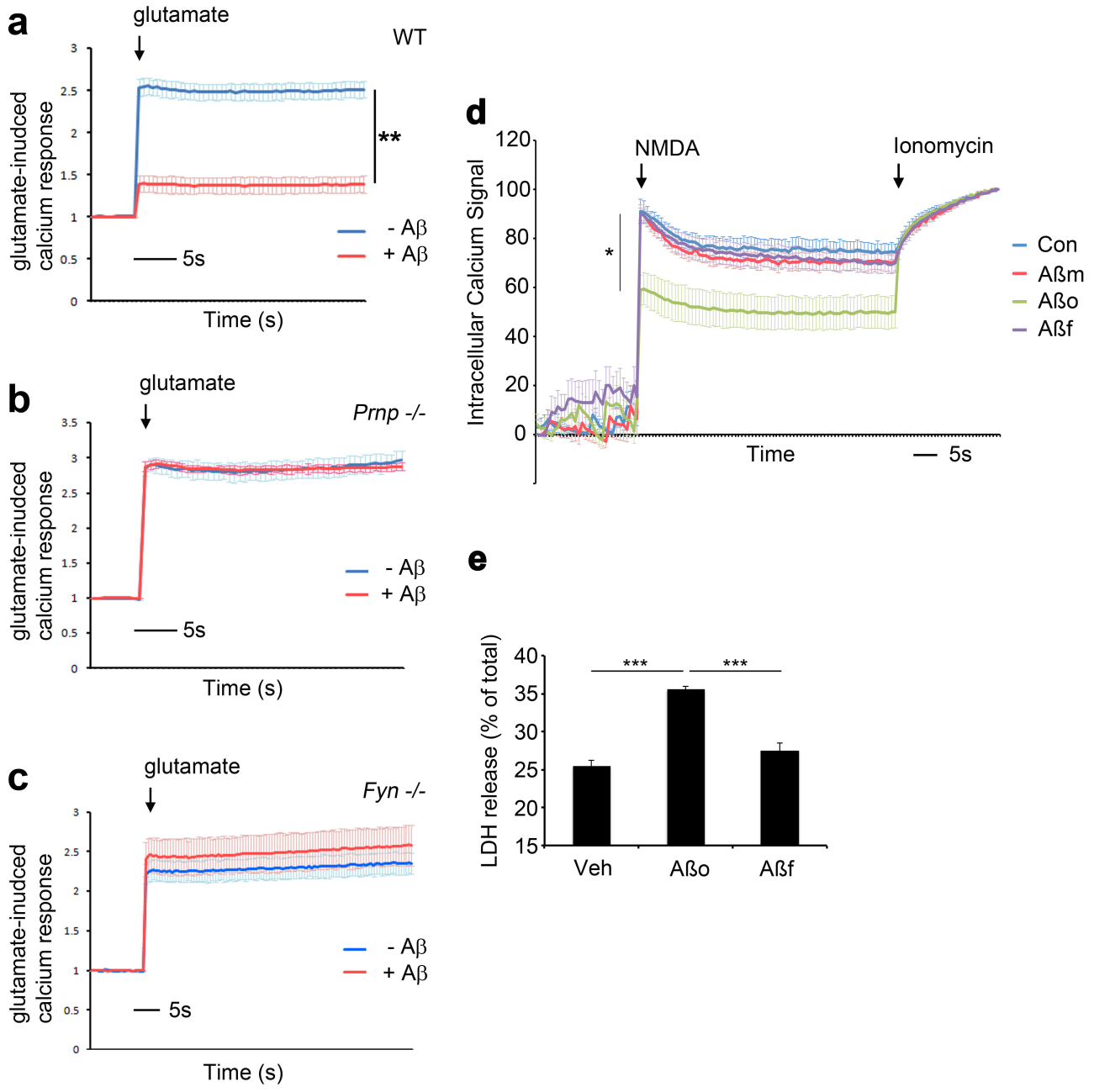
a Cortical neurons from E17 *Prnp*$^{+/-}$ after 21 DIV were treated with 0-3 µM Aβo for 20 min. Cell lysates were analyzed by immunoblotting with anti-phospho-SFK (Tyr 416), anti-Fyn, anti-phospho-NR2B (Tyr 1472) or anti-NR2B antibody. Actin served as a loading control. One of three experiments with similar results in panels A-D.

b Cortical neurons from E17 *Fyn*$^{-/-}$ after 21 DIV were treated with 0-3 µM Aβo or Aβm for 20 min. Cell lysates were analyzed by immunoblotting anti-Fyn, anti-phospho-NR2B (Tyr 1472), or anti-NR2B antibody. Actin served as a loading control.

c Cortical neurons from E17 *Fyn*$^{+/-}$ after 21 DIV were treated with 0-3 µM Aβo or Aβm for 20 min. Cell lysates were analyzed by immunoblotting with anti-phospho-SFK (Tyr 416), anti-Fyn, anti-phospho-NR2B (Tyr 1472) or anti-NR2B antibody. Actin served as a loading control.

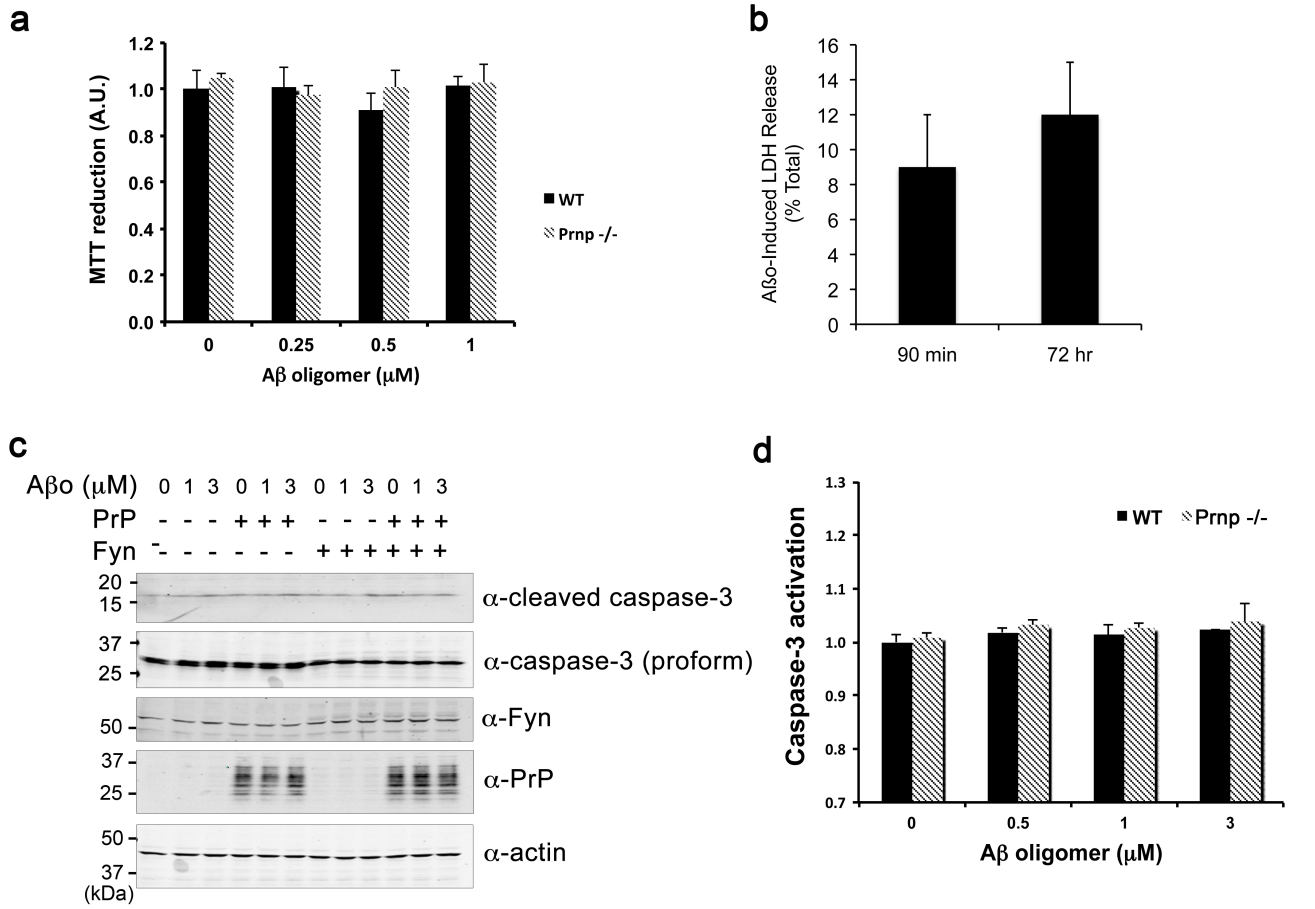
d Cortical neurons from E17 *Prnp*$^{+/-}$ after 21 DIV were treated with 0 or 1 µM Aβo for 20 min or 1 h. Cell surface proteins were biotinylated and purified by using NeutrAvidin-conjugated beads. Purified cell surface proteins were immunoblotted for NR2B, and total cell lysates were immunoblotted for NR2B, phospho-SFK, Fyn or Actin.

e After cortical neurons at 21 DIV from wild type, *Prnp*$^{+/-}$, *Fyn*$^{+/-}$, or *Prnp*$^{+/-}$*Fyn*$^{+/-}$ mice were treated with 0-2 µM Aβo for 1.5 h, cell death was determined by LDH release. Data are mean and s.e.m. for n = 15-25 wells from 3 separate embryos for each genotype.



Supplemental Figure S8. Specificity of Aß effects on neuronal calcium signals and toxicity.
a-c Cortical neurons from wild type (**a**), *Prnp*$^{-/-}$ (**b**) or *Fyn*$^{-/-}$ (**c**) were treated with 0 or 1 μM Aβo for 60 min. The change of intracellular calcium concentration in response to glutamate (100 μM) was monitored by using FLIPR Calcium 4 assay kit. Mean ± s.e.m. For each genotype, n = 15 separate wells derived from 3 mouse embryos. **, $P < 0.01$ by Repeated Measures ANOVA for 1-20 seconds after glutamate addition. Data are normalized to pre-glutamate fluorescence; the pre-glutamate values without normalization for WT Aßo 60 min, *Prnp*$^{-/-}$ Aßo 60 min and *Fyn*$^{-/-}$ Aßo 60 min are 1.33±0.17, 1.05±0.04, and 1.11±0.08, respectively.
d Cortical neurons from wild type were treated with 1 μM Aß oligomer (Aßo), Aß monomer (Aßm) or Aß fibril (Aßf) for 60 min. The change of intracellular calcium concentration in response to NMDA (50 μM) or ionomycin (500 nM) was monitored by using FLIPR Calcium 4 assay kit. Mean ± s.e.m. n = 24 separate wells derived from 3 mouse embryos. **, $P < 0.01$ by Repeated Measures ANOVA for 1-20 seconds after NMDA addition.

e Cortical neurons at 21 DIV from wild type were treated with 1 μM Aβo or Aßf for 2 h, prior to measurement of LDH release. Data are mean ± s.e.m. ***, $P < 0.001$; one-way ANOVA, with Tukey post-hoc pairwise comparisons.



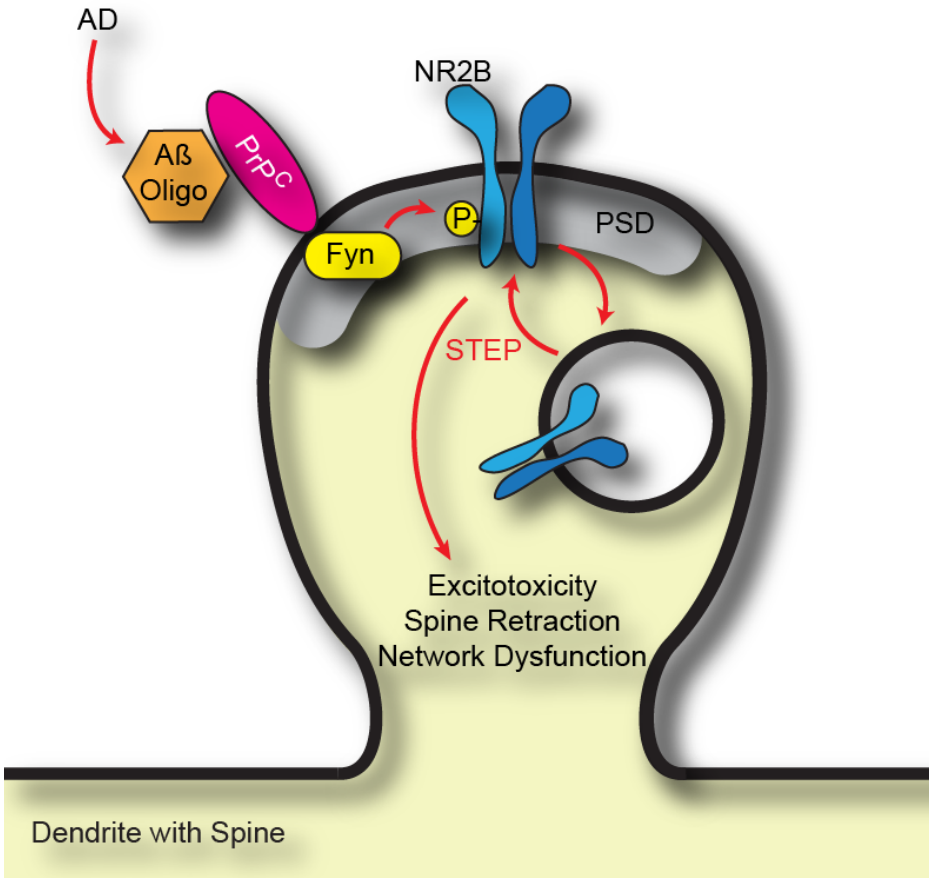
Supplemental Figure S9. Assays of Aßo cell toxicity.

a, Cortical neurons from wild type or *Prnp*$^{-/-}$ mouse embryos were treated with 0-1 μM Aβo for 90 min. Cell viability was determined by the MTT dye reduction assay as described in Experimental Procedures. Data are mean ± s.e.m. for n = 3 separate experiments.

b Wild type cortical neurons were treated with vehicle or 1-3 μM Aβo for 90 min or 72 h. LDH release was measured as in Fig. 6H-J. Mean ± s.e.m. for n = 3 separate experiments.

c N2A cells were transfected with expression vectors for Fyn or PrP. After treatment of cells with 0 -3 μM Aβo for 90 min, cell lysates were analyzed by anti-PrP, anti-Fyn, anti-caspase 3, anti-cleaved caspase 3. Actin served as a loading control.

d Cortical neurons from wild type or *Prnp*$^{-/-}$ mouse embryos were treated with 0-3 μM Aβo for 90 min. Caspase-3 activation was measured by hydrolysis of the fluorogenic substrate, DEVD-AMC. Data are mean and s.e.m. for n = 3 separate experiments.



Supplemental Figure S10. Aß Oligomer Actions via PrP^C at the Synapse.
A model illustrates the dendritic spine and the post-synaptic density. Aß oligomers bind to PrP^C and cause Fyn activation and NMDA-R redistribution. The biphasic activation effect initially increases surface NMDA-R and calcium influx, but is followed by loss of spines and surface NMDA-R and calcium signals.