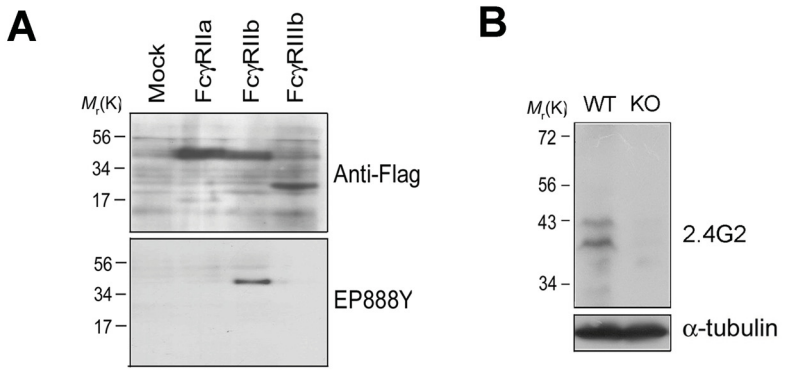
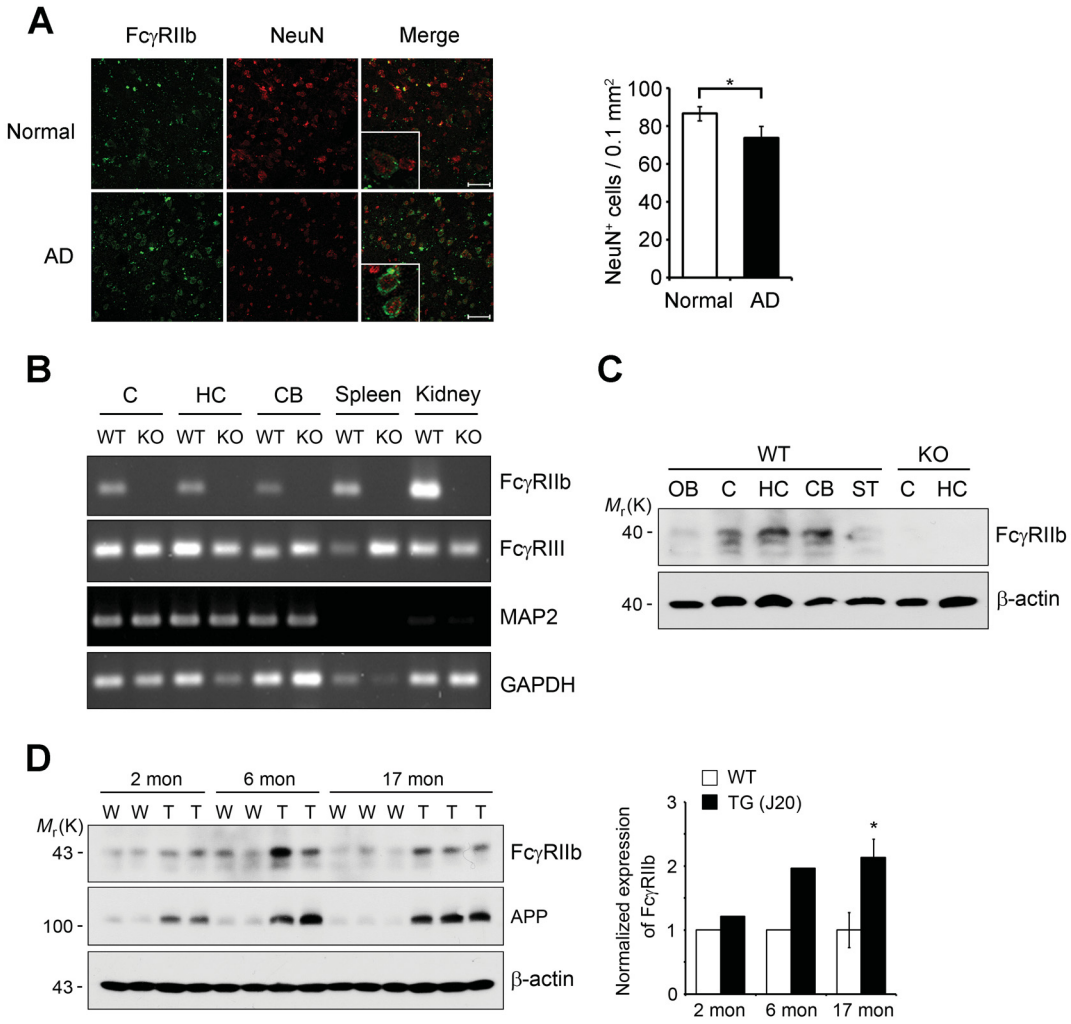


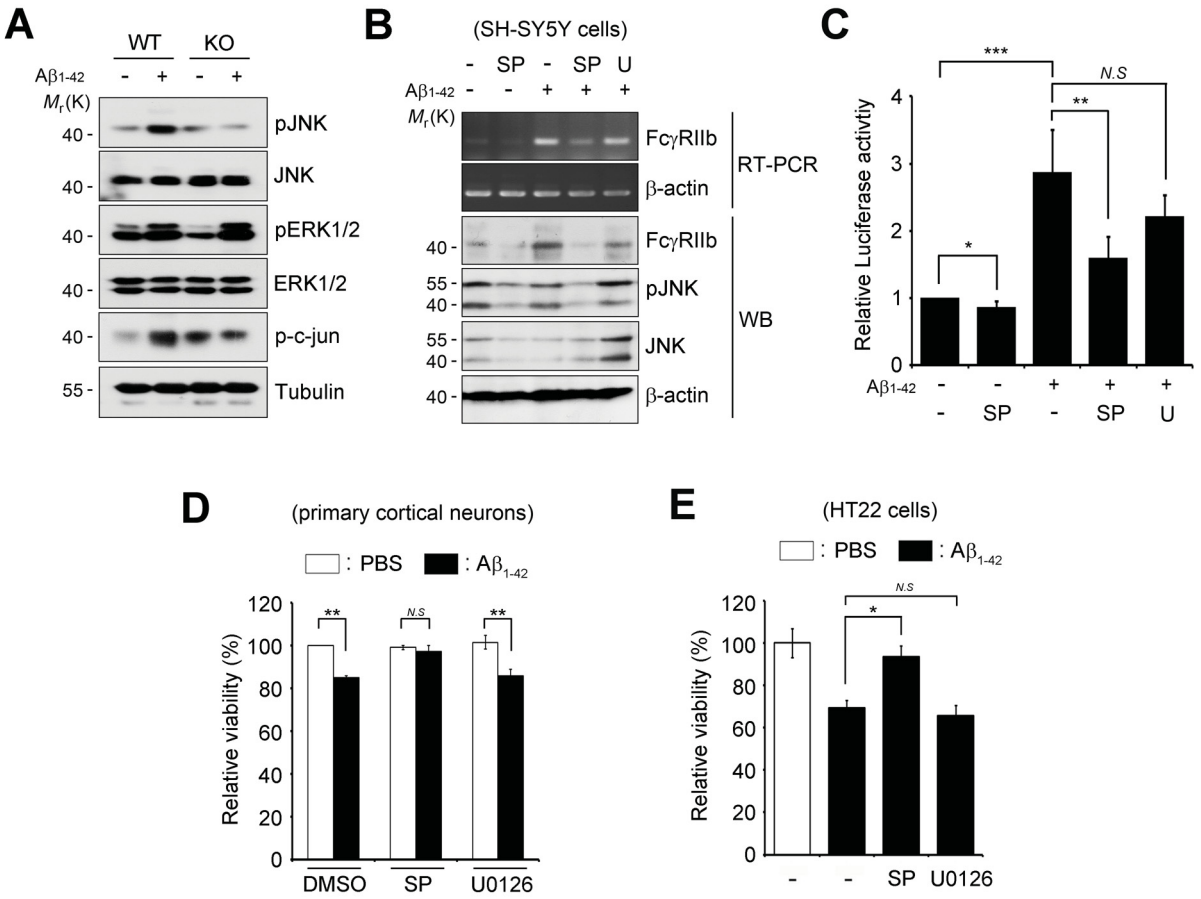
Supplemental Figure 1. Detection of human FcγRIIb in cells and mouse FcγRIIb in the brain tissues.

(A) Specificity of anti-human FcγRIIb antibody. HEK293T cells were transfected with flag-tagged human FcγRs and analyzed by Western blotting using anti-FcγRIIb (EP888Y) and anti-flag antibodies. **(B)** Detection of FcγRIIb in the mouse brains using anti-FcγRIIb antibody. Adult brains from wild type (WT) and FcγRIIb knockout (KO) mice were analyzed by Western blotting using anti-FcγRIIb (2.4G2) and anti-α-tubulin antibodies. FcγRIIb is detected in the brain of WT mice, but not in FcγRIIb KO mice.



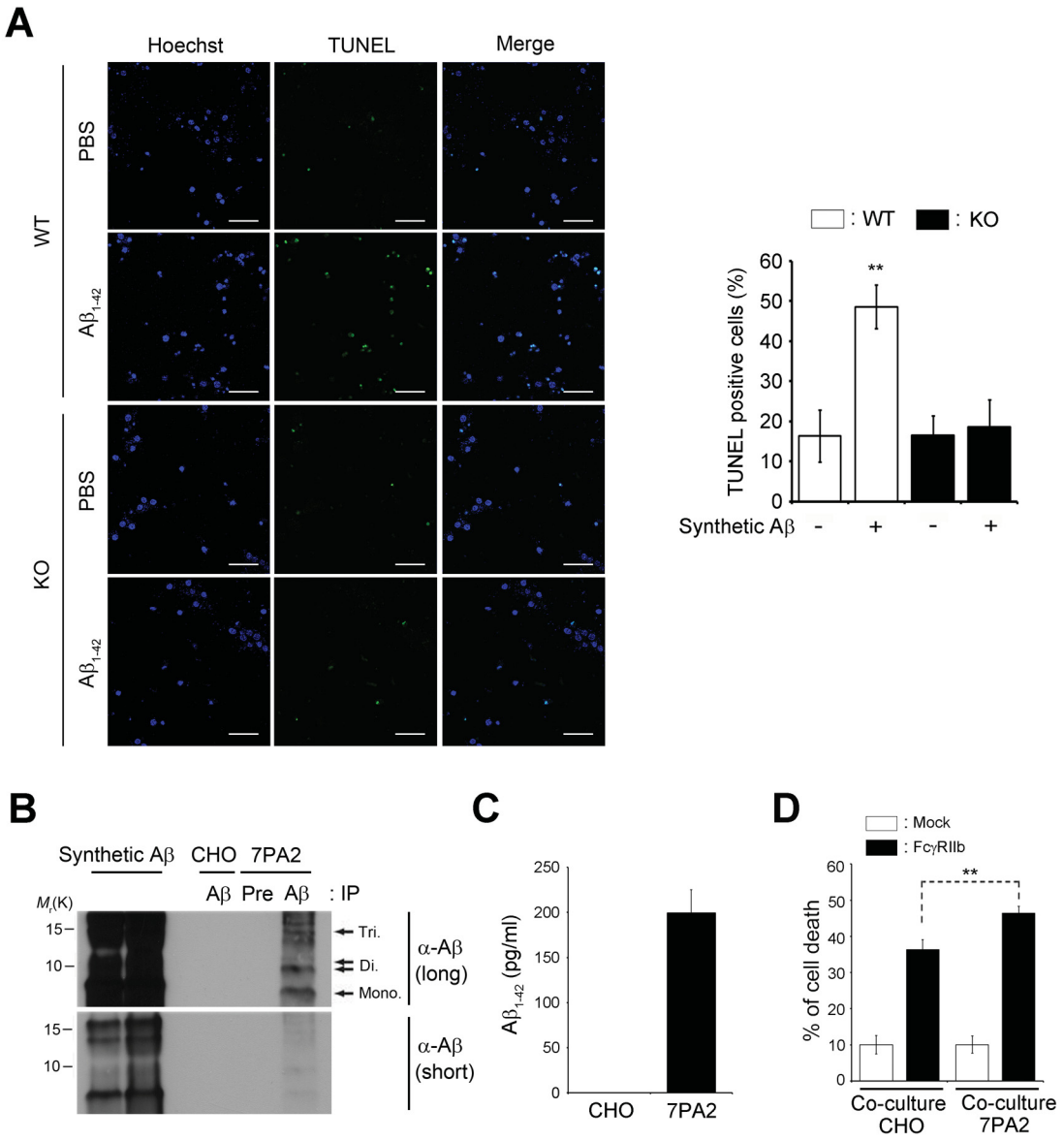
Supplemental Figure 2. Neuronal distribution of FcγRIIb and increased FcγRIIb in the hippocampus of AD brains and cortex of J20 mice.

(**A**) Immunohistochemical detection of FcγRIIb in the NeuN-positive hippocampal neurons of AD patients. Tissue samples from normal and AD patients (n=3 for each group) were immunostained using anti-FcγRIIb (EP888Y) and anti-NeuN antibodies and examined under a confocal microscope. Scale bars, 50 μm (*left*). The numbers of NeuN-positive cells were quantified (*right*). (**B, C**) Neuronal expression of FcγRIIb in the mouse brain tissues. Total RNA from the indicated tissues of WT and FcγRIIb KO mice was isolated for RT-PCR analysis using synthetic primers for FcγRIIb, FcγRIII, MAP2 (neuronal marker) and GAPDH (B). The lysates from WT and FcγRIIb KO mice were subjected to Western blotting using anti-FcγRIIb (2.4G2) and anti-β-actin antibodies (C). (**D**) Increased FcγRIIb in the cortex of J20 mice. Cortical homogenates from 2, 6, 17 month-old WT (W) and J20 transgenic mice (T were subjected to Western blotting. APP antibody was used as a marker for transgenic mice (*left*). Levels of FcγRIIb were quantified by densitometric measurement (*right*). Values are mean ± s.d.; **P* < 0.02, two-tailed *t*-test (17-month-old samples).

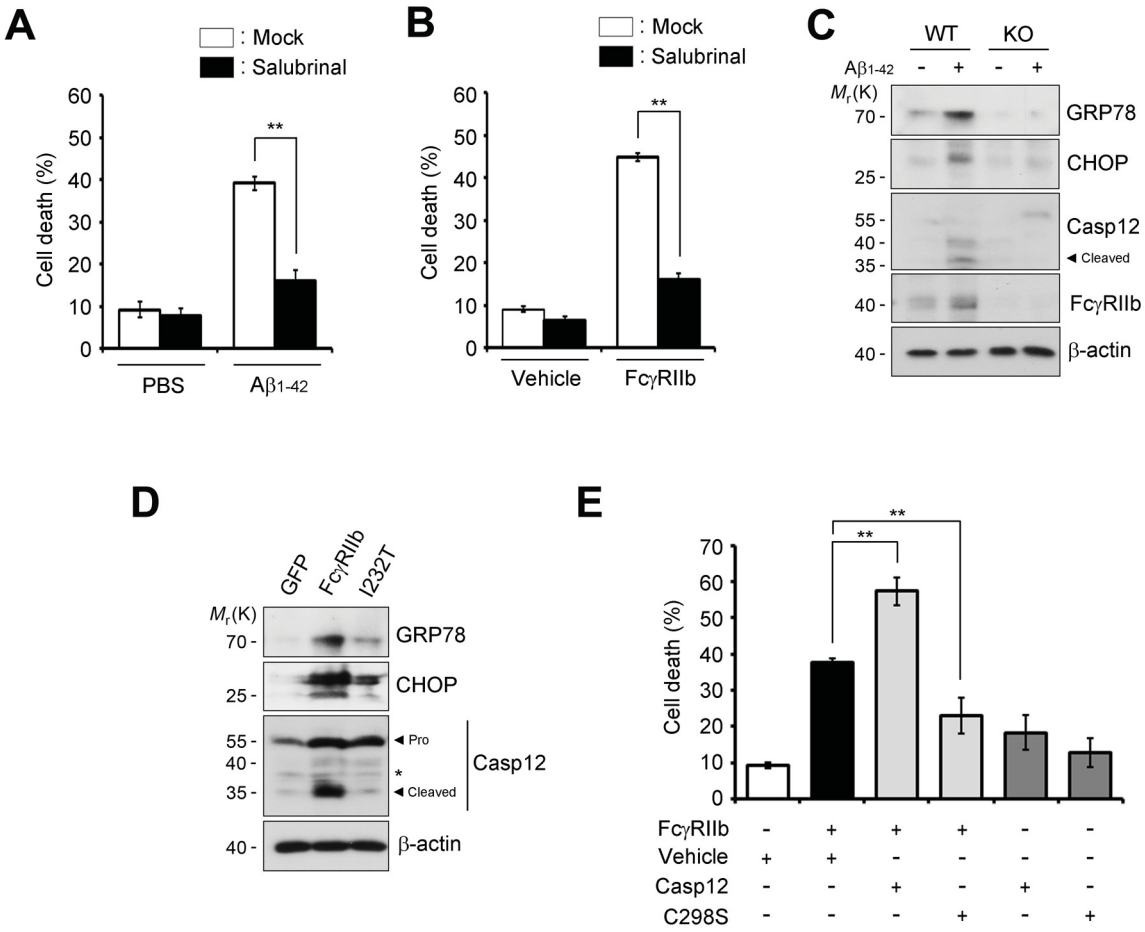


Supplemental Figure 3. Regulation of Aβ-induced FcγRIIb expression by JNK-c-Jun pathway.

(**A**) FcγRIIb deficiency prevents the activation of JNK-c-Jun pathway by $A\beta_{1\text{-}42}$. Mouse primary cortical neurons from WT or FcγRIIb KO embryos (DIV 8) were incubated with 5 μM $A\beta_{1\text{-}42}$ for 12 h. Cell extracts were subjected to Western blotting using anti-pJNK, total anti-JNK, anti-pERK1/2, total anti-ERK1/2, anti-p-c-Jun and anti-α-tubulin antibodies. (**B**) The increased expression of FcγRIIb by $A\beta_{1\text{-}42}$ is prevented by JNK inhibitor. SH-SY5Y cells were pre-treated with 5 μM SP600125 or 10 μM U0126 for 2 h, and then further incubated with 5 μM $A\beta_{1\text{-}42}$ for 12 h. Total RNA was isolated for RT-PCR analysis using synthetic primers for FcγRIIb and β-actin (*upper*). Cell lysates were subjected to Western blotting (*lower*) using anti-FcγRIIb (EP888Y), anti-pJNK, total anti-JNK and anti-β-actin antibodies. (**C**) Suppression of Aβ-induced luciferase activity of FcγRIIb promoter-luciferase construct by JNK inhibitor. SH-SY5Y cells were cotransfected with FcγRIIb promoter-luciferase construct and pLacZ for 24 h. Cells were pre-treated with 5 μM SP600125 or 10 μM U0126 for 2 h, and then further incubated with 5 μM $A\beta_{1\text{-}42}$ for 12 h in the absence or presence of the inhibitors. The luciferase activity in cell lysates was determined using a luminometer and then normalized by that of β-galactosidase. (**D, E**) Aβ-induced cell death is blocked by JNK inhibitor. Primary cortical neurons (DIV 8) (D) or HT22 cells (E) were pre-treated with 5 μM SP600125 or 10 μM U0126 for 2 h, and then incubated with 5 μM $A\beta_{1\text{-}42}$ for 48 h. The cell death was determined d by using Calcein-AM (D) or MTT assay (E). Data are mean ± s.d. (n=4). *$p < 0.01$, **$p < 0.005$, unpaired *t*-test.

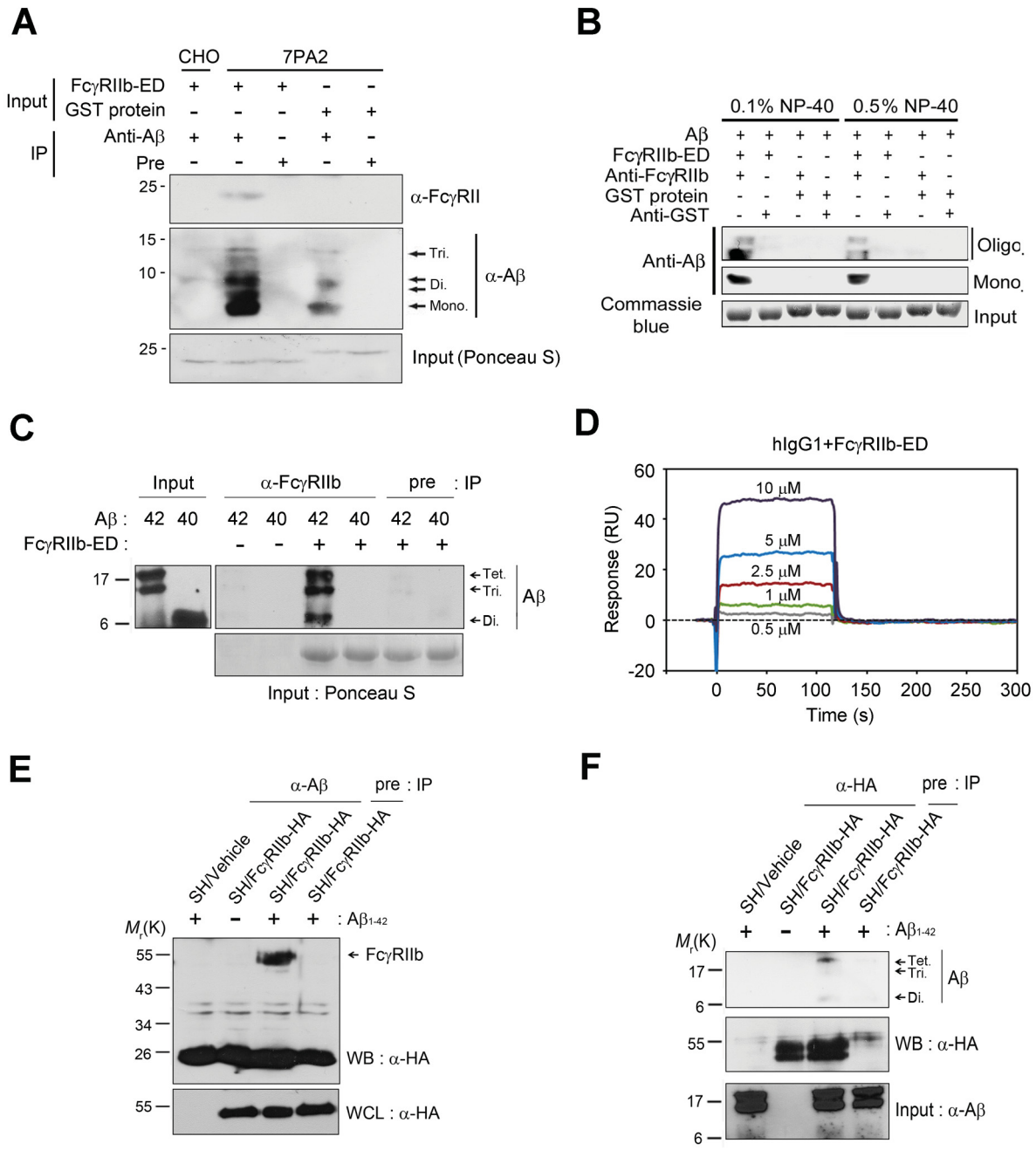


Supplemental Figure 4. FcγRIIb KO neurons are resistant to Aβ toxicity.
(**A**) FcγRIIb KO neurons are resistant to synthetic Aβ toxicity. Primary hippocampal neurons were cultured from WT and FcγRIIb KO embryos and then incubated with 5 μM synthetic $A\beta_{1\text{-}42}$ oligomers for 2 days (from 5 DIV), after which neuronal apoptosis was determined using TUNEL assay. Data are mean ± s.d. (n=3-5 per groups). $^{**}P < 0.001$, one-way ANOVA. (**B, C**) Detection and quantification of cell-derived Aβ. CHO- and 7PA2-conditioned medium (CM) were immunoprecipitated with pre-immune (Pre) or 6E10 (Aβ) antibodies, and the immunoprecipitates were western blotted with anti-Aβ antibody (4G8). Synthetic Aβ was used as a loading control (50 ng, *lane 1*; 200 ng, *lane 2*). Mono, monomer; Di, dimer; Tri, trimer (B). Levels of total $A\beta_{1\text{-}42}$ in CHO- and 7PA2-CM determined by ELISA (C). **(D)** Induction of cell death by 7PA2-CM in FcγRIIb-expressing HT22 cells. HT22 cells were grown on glass cover slip and transfected with GFP (Mock) or FcγRIIb-GFP (FcγRIIb) for 12 h. Then, cover slips were transferred into the plates containing a ~80 % confluent cell layer of CHO or 7PA2 cells. After 36 h of co-cultivation, cell death was determined as described previously. Values are mean ± s.d. (n=3). $^{**}P < 0.005$, two-tailed *t*-test.



Supplemental Figure 5. FcγRIIb mediates $A\beta_{1\text{-}42}$ neurotoxicity through ER stress and caspase-12 activation.

(**A, B**) Inhibition of Aβ- and FcγRIIb-induced cell death by Salubrinal. HT22 cells were pre-treated with 75 μM Salubrinal for 2 h, and then incubated with 5 μM $A\beta_{1\text{-}42}$ oligomers for 48 h (A) or transfected with either pEGFP-N1 (Vehicle) or pFcγRIIb-GFP for 36 h in the absence or presence of 75 μM Salubrinal (B). Bars depict the incidence of cell death. Values are mean ± s.d.; n=3. $^{**}P < 0.005$, unpaired *t*-test. (**C**) FcγRIIb deficiency prevents ER stress response and caspase-12 activation by $A\beta_{1\text{-}42}$. Mouse primary cortical neurons from WT or FcγRIIb KO embryos (DIV 8) were incubated with 5 μM $A\beta_{1\text{-}42}$ for 48 h. Cell extracts were subjected to Western blotting using anti-GRP78, anti-CHOP, anti-caspase-12, anti-FcγRIIb and anti-β-actin antibodies. (**D**) FcγRIIb, but not I232T mutant, induces ER stress and caspase-12 activation. HT22 cells were transfected with pEGFP-N1, pFcγRIIb-GFP or pFcγRIIb I232T-GFP for 36 h and cell lysates were subjected to Western blotting using anti-GRP78, anti-CHOP, anti-caspase-12, anti-GFP and anti-β-actin antibodies. (**E**) Suppression of FcγRIIb-induced cell death by caspase-12 activity-dead mutant. HT22 cells were cotransfected with pFcγRIIb and pEGFP-N1 (Vehicle), pCaspase-12-GFP (Casp12) or pCaspase-12 C298S-GFP (C298S) for 36 h. The cell death was determined based on the morphology of GFP-positive cells under a fluorescence microscope. Values are mean ± s.d.; n=3. $^{**}P < 0.001$, unpaired *t*-test.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7PA2
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GST protein
IP
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Pre
25
α-FcγRII
15
10
Tri.
Di.
Mono.
α-Aβ
25
Input (Ponceau S)
B
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0.5% NP-40
Aβ
FcγRIIb-ED
Anti-FcγRIIb
GST protein
Anti-GST
Anti-Aβ
Oligo.
Mono.
Commassie blue
Input
C
Input
α-FcγRIIb
pre
: IP
Aβ : 42 40 42 40 42 40 42 40
FcγRIIb-ED :
17
6
Tet.
Tri.
Di.
Aβ
Input : Ponceau S
D
hIgG1+FcγRIIb-ED
Response (RU)
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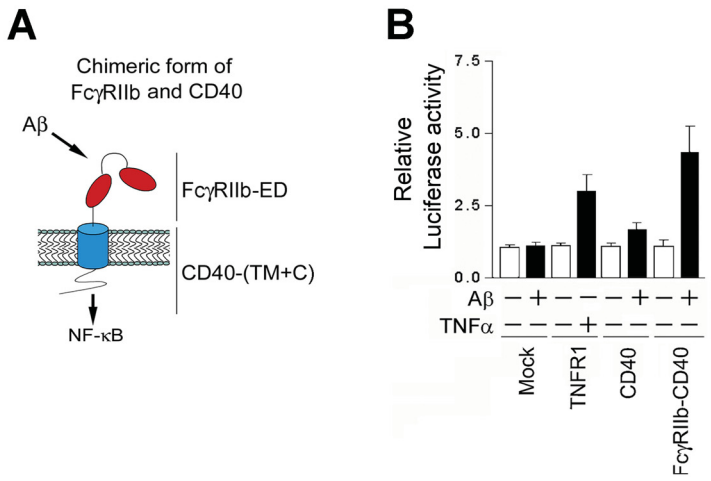
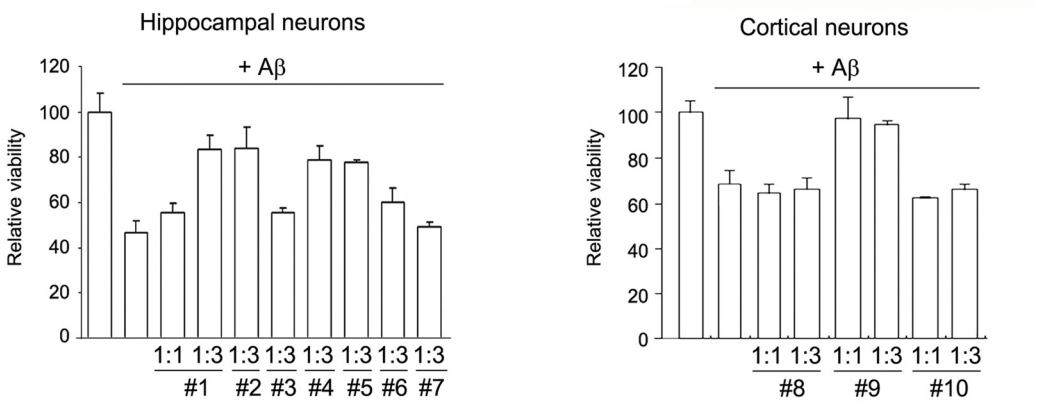
40
20
0
-20
10 μM
5 μM
2.5 μM
1 μM
0.5 μM
0
50
100
150
200
250
300
Time (s)
E
α-Aβ
pre : IP
SH/Vehicle
SH/FcγRIIb-HA
SH/FcγRIIb-HA
SH/FcγRIIb-HA
Mr(K)
: Aβ1-42
55
43
34
26
← FcγRIIb
WB : α-HA
55
WCL : α-HA
F
α-HA
pre : IP
SH/Vehicle
SH/FcγRIIb-HA
SH/FcγRIIb-HA
SH/FcγRIIb-HA
Mr(K)
: Aβ1-42
17
6
Tet.
Tri.
Di.
Aβ
55
WB : α-HA
17
6
Input : α-Aβ

Supplemental Figure 6. Interaction of FcγRIIb with $A\beta_{1-42}$.
(A, B) *In vitro* binding of FcγRIIb-ED protein to Aβ. Purified GST and hFcγRIIb-ED proteins were incubated with CHO- or 7PA2-CM for 6 h (A) or synthetic $A\beta_{1-42}$ for 2 h (B), and then immunoprecipitated with anti-Aβ (A) or anti-FcγRIIb antibody (B). The precipitates were separated with SDS-PAGE and analyzed by Western blotting using anti-FcγRII (AT10) or Nu-1 antibody. Monomeric (Mono.), dimeric (Di.) and trimeric (Tri.) forms of Aβ are indicated. **(C)** Selective interaction of oligomeric $A\beta_{1-42}$ with FcγRIIb *in vitro*. Purified hFcγRIIb-ED protein was incubated with oligomeric $A\beta_{1-42}$ or $A\beta_{1-40}$ for 2 h and then immunoprecipitated with anti-FcγRIIb antibody or pre-immune (pre). Precipitates were analyzed by Western blotting using anti-Aβ (Nu-1) antibody. Oligomeric forms [dimer (Di.), trimer (Tri.) and tetramer (Tet.)] of Aβ are indicated. (**D**) Interaction of FcγRIIb-ED with IgG1 with low affinity in SPR analysis. BSA or human IgG1 were immobilized on a CM5 chip® and their interactions with hFcγRIIb-ED protein (0.5-10 μM) were analyzed using Biacore3000®. **(E, F)** Cellular interaction between FcγRIIb and Aβ oligomers. SH-SY5Y cells that stably express hFcγRIIb-HA were left untreated or incubated with 1 μM $A\beta_{1-42}$ for 1 h. Cell lysates were immunoprecipitated with anti-Aβ (Nu-1) antibody (D) or anti-HA antibody (E). Murine pre-immune (pre) served as a negative control. Immunoprecipitated proteins were then analyzed with Western blotting using anti-HA antibody (D) or anti-Aβ antibody (E). WCL, whole cell lysates.

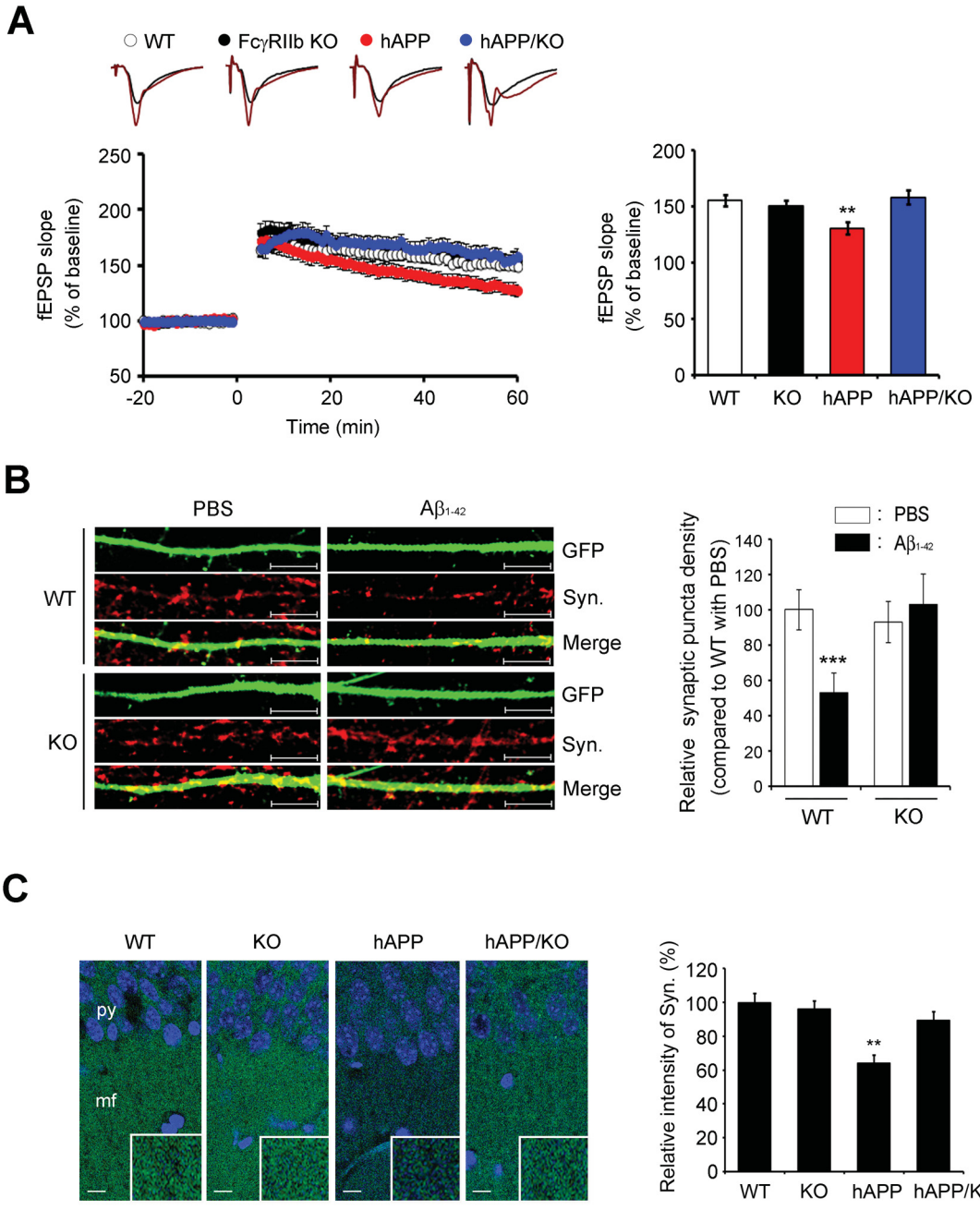


Supplemental Figure 7. A rFcγRIIb-CD40 chimera reporter assay showing the interaction of FcγRIIb with $A\beta_{1\text{-}42}$.
(A) Schematic diagram of a reporter assay using rFcγRIIb-CD40 chimera. Rat FcγIIb-ED (rFcγIIb) was fused to the transmembrane and cytosolic regions (TM+C) of CD40. **(B)** Reporter activity showing the binding of $A\beta_{1\text{-}42}$ to FcγRIIb-ED in cells. NIH3T3 cells were cotransfected with pNF-κB-luciferase, pβact-lacZ and either pEGFP, pTNF receptor1 (TNFR1), pCD40 or pFcγRIIb-CD40, and then incubated for 24 h with 5 μM $A\beta_{1\text{-}42}$ or 10 ng/ml murine TNF-α (mean ± s.d.; n=3).



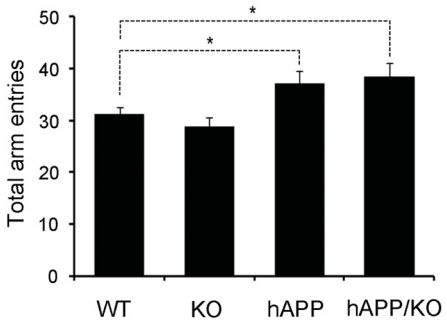
Supplemental Figure 8. Inhibition of Aβ neurotoxicity by FcγRIIb- or Aβ-derived synthetic peptides.

Effects of FcγRIIb- or Aβ-derived synthetic peptides on Aβ-induced cell death. Beginning at 7 days *in vitro* (DIV), primary hippocampal neurons were incubated for 2 days with 5 μM $A\beta_{1\text{-}42}$ w/wo the indicated ratios (to $A\beta_{1\text{-}42}$) of $A\beta_{1\text{-}9}$ WT or mutant synthetic peptide (#1 to #7 shown in Fig. 3 D) (*left*). Beginning at 5 DIV, primary cortical neurons were incubated for 2 days with 5 μM $A\beta_{1\text{-}42}$ w/wo FcγRIIb-derived WT and mutant synthetic peptides (#8 to #10 shown in Fig. 3 D) (*right*). Relative viability was determined by counting neurons for dead cells after staining with Calcein-AM and neurons showing intact cell bodies and neurites for live cells. Bars represent mean ± s.d. (n=3).

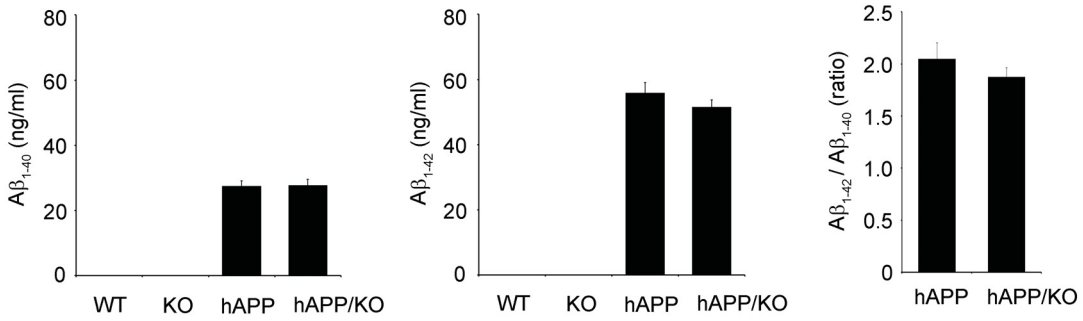

A
WT
FcγRIIb KO
hAPP
hAPP/KO
fEPSP slope (% of baseline)
Time (min)
WT
KO
hAPP
hAPP/KO
**
B
PBS
Aβ1-42
WT
KO
GFP
Syn.
Merge
Relative synaptic puncta density (compared to WT with PBS)
: PBS
: Aβ1-42
***
C
py
mf
Relative intensity of Syn. (%)

Supplemental Figure 9. Requirement of FcγRIIb in the inhibition of hippocampal LTP and synaptic loss in hAPP mice.

(**A**) Lack of hippocampal LTP inhibition by FcγRIIb deficiency in hAPP mice. Field potentials were recorded from the hippocampal slices from 9 to 13 month-old WT (n=23 slices from 5 mice), FcγRIIb KO (n=17 slices from 5 mice), hAPP (n=14 slices from 4 mice) and hAPP/FcγRIIb KO (n=10 slices from 5 mice) (*left*). Summary bar graphs represent the magnitude of LTP between 50 and 60 min (*right*). All data shown are mean ± s.e.m.; $^{**}P < 0.005$, one-way ANOVA. (**B**) Images represent synaptic clusters in WT and FcγRIIb KO primary hippocampal neurons. Cultured neurons at 13 DIV were transfected with pEGFP-N1 for 3 days and then exposed to 2 μM $A\beta_{1\text{-}42}$. After 2 days, neurons were immunostained for synaptophysin (Syn.). Synaptic sites were identified as both synaptophysin- and GFP-positive puncta (*left*). Both synaptophysin^{+} and GFP^{+} puncta were counted from 10 randomly chosen dendrites with 100 μm in length (*right*). Bars depict mean ± s.d. $^{***}P < 0.0005$, one-way ANOVA. (**C**) Prevention of synaptic loss in hAPP/FcγRIIb KO mice. Images represent the synaptophysin immunoreactivity on CA1 in WT, FcγRIIb KO, hAPP and hAPP/FcγRIIb KO mice. py, pyramidal neuron layer; mf, mossy fiber. Scale bar, 10 μm (*left*). The synaptophysin^{+} intensity was measured by densitometric analysis (*right*). Bars depict mean ± s.e.m. (n=10). $^{**}P < 0.01$, one-way ANOVA.



Supplemental Figure 10. FcγRIIb deficiency does not affect the hyperactivity in hAPP mice. Total arm entries in Y-maze test of WT, FcγRIIb KO, hAPP and hAPP/KO mice were analyzed (n=7-18 mice per group). Asterisk indicates significant difference between WT mice and either hAPP or hAPP/KO mice. No significant difference in total arm entries was observed between WT and KO mice or between hAPP and hAPP/KO mice. Data are mean ± s.e.m.; *$P < 0.05$ versus WT, *t*-test.



Supplemental Figure 11. Aβ levels in the hippocampus of hAPP and hAPP/FcγRIIb KO mice.

Levels of $A\beta_{1-40}$ (left), $A\beta_{1-42}$ (middle) and $A\beta_{1-42}/A\beta_{1-40}$ ratio (right) in the hippocampus of 8-month-old WT, FcγRIIb KO, hAPP or hAPP/KO mice were determined by ELISA (n=3). Human APP and hAPP/KO groups did not differ significantly by Student's *t*-test. Values are means ± s.e.m.

Supplemental Table 1. Relevant clinical information of control and AD subjects

Brain	Diagnosis	Age	Sex	PMI[A]	Neuronal loss[B]	Senile plaques[B]
4625	Normal	53	F	24	-	-
4725	Normal	58	F	17.8	-	-
5081	Normal	60	F	17.58	-	-
5083	Normal	38	M	28.83	-	-
0704	Normal	82	F	15.70	-	-
2921	Normal	89	F	14.12	+	-
8396	Normal	76	M	12.25	-	-
10180	Normal	73	M	24	-	-
10329	Normal	81	F	26.18	-	-
7494	Braak III	84	F	8.58	-	-
7510	Braak III	82	M	22.08	-	+
7525	Braak III	89	M	30.58	-	+
7547	Braak III	82	F	17.37	-	+
7614	Braak III	84	F	21.67	-	+
5258	Braak V	92	F	5	+++	++
5272	Braak V	83	F	19.25	+	++
5273	Braak V	83	M	18	++	+++
5275	Braak V	77	M	11.05	-	++
5290	Braak V	95	F	7.58	+++	++
5842	Braak V	89	F	27	+	++
5279	Braak VI	83	M	25.41	++	++

5283	Braak VI	82	F	20.75	+++	+++
5285	Braak VI	68	M	8.62	++	++
6785	Braak VI	59	F	22.42	-	++
6803	Braak VI	82	F	19.17	++	+
7427	Braak VI	76	F	12.08	++	++
7604	Braak VI	64	F	17.42	++	+++
7633	Braak VI	82	F	27	+++	+++

Diagnosis	**Mean Age (y) ± s.d.**	**Sex (F/M)**	**Mean PMI (h) ± s.d.**
Normal	67.8 ± 16.5	6/3	20.1 ± 5.8
Braak III	84.2 ± 2.9	3/2	20.1 ± 8.0
Braak V/VI	80.2 ± 10.6	10/4	16.3 ± 6.7

[A] PMI: Post-mortem interval (h)

[B] - : None, +: Mild, ++: Moderate, +++: Abundant/Severe