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Supplementary Information for

Delta-secretase-cleaved Tau Antagonizes TrkB Neurotrophic Signalings, Mediating Alzheimer's Disease Pathologies

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Supplementary Information Text

Fig. S1.

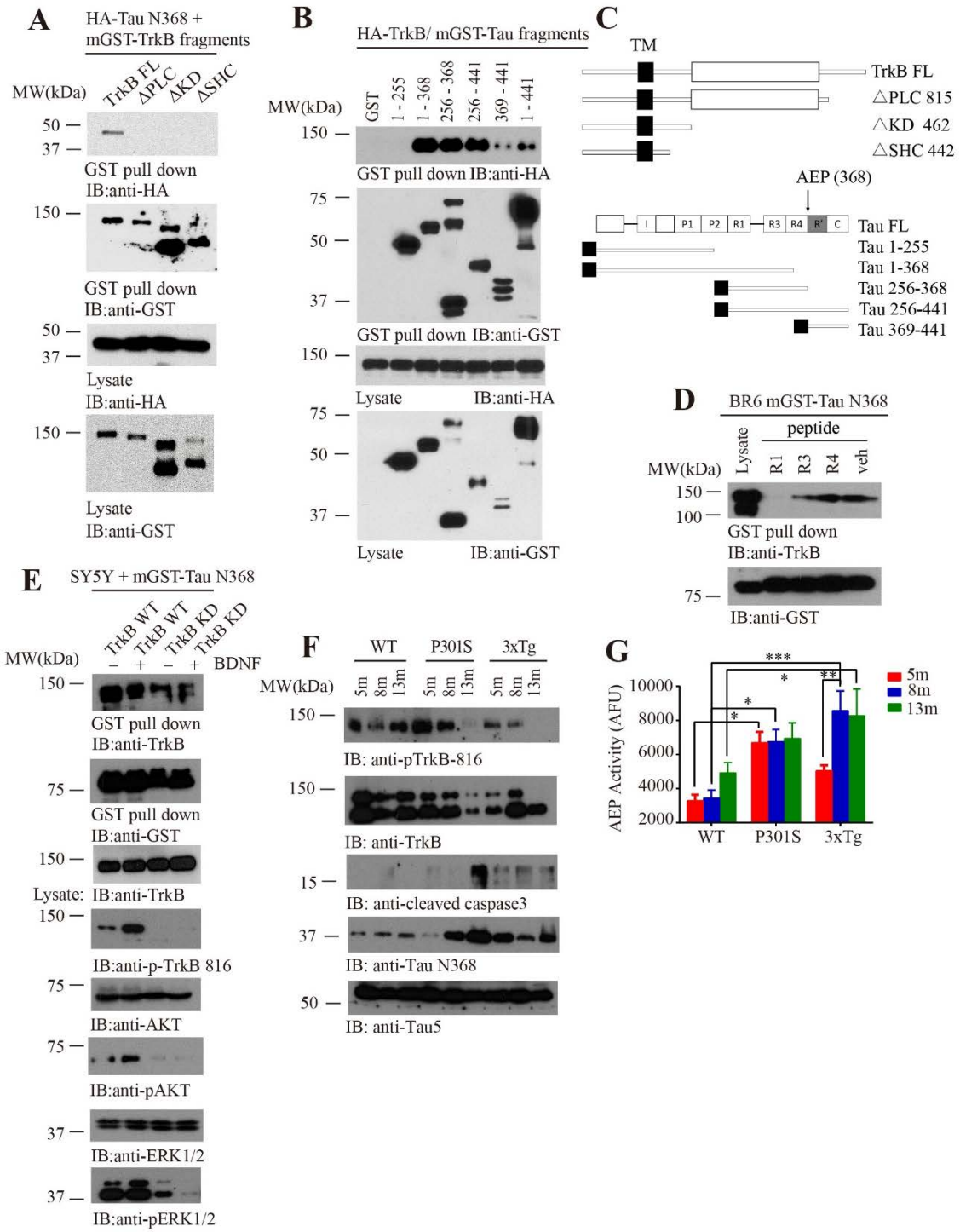

Fig. S1: TrkB C-terminus and Tau Repeat-domain 1 are implicated in association. (A) TrkB tail domain is indispensable for Tau to interact with TrkB. Mapping assay for TrkB PLC binding domain required for associating with Tau with GST pulldown assay from co-transfected HEK293 cells. (B) Tau RD region is implicated in binding to TrkB. Different mGST-tagged Tau truncates were co-transfected with HA-TrkB into HEK293 cells. GST-pull down assay was performed, and co-precipitated proteins were analyzed by immunoblotting with anti-HA. (C) Schematic diagrams of TrkB and Tau with various domains. (D) in vitro competition assay. Tau R1 peptide disrupted the association between Tau N368 and the TrkB. BR6 cell transfected with mGST-Tau N368, and cell lysates were incubated with vehicle or 10 μM R1, R3 or R4 peptide for 2 h and GST pull-down assay was performed. (E) TrkB kinase activity mediates the association between TrkB and Tau N368. SH-SY5Y cells transfected with mGST-Tau N368 and hTrkB WT/hTrkB KD plasmids for 48 h, then treated with BDNF 50 ng/ml for 15 min, and GST pull-down assay was performed, and coprecipitated proteins were analyzed by immunoblotting with anti-TrkB (Top), Cell lysates were probed with various indicated antibodies (2^{nd}-bottom panels). (F) Western blot analysis of AEP and TrkB signaling in WT, P301S and 3xTg mice brain during the aging process. P-TrkB signals were reduced and AEP and caspase-3 were activated in Tauopathies mice in an age-dependent way. (G) AEP activity increases in WT, P301S and 3xTg mice brain in an age-dependent manner. Validation of AEP enzymatic activities by fluorescent substrate cleavage assay. (Data represent mean ± s.e.m. of 3 mice per age; *P<0.05, **P<0.01, two-way ANOVA).

Fig. S2.

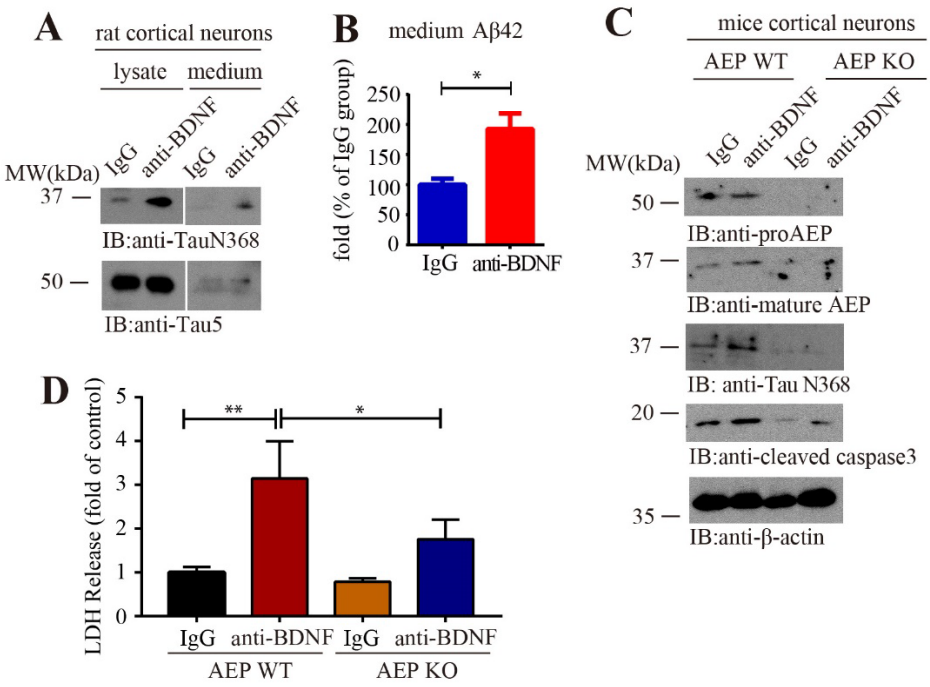

Fig. S2: Delta-secretase-induced Tau N368 exerts neurotoxicity in primary neurons.

(A) Anti-BDNF elicits Tau N368 secretion into neuronal medium. Secreted Tau N368 fragment was detected with western blot in anti-BDNF-treated primary neuron concentrated medium. (B) Secreted Aβ42 was detected with ELISA in anti-BDNF-treated primary neuron concentrated medium (Data represent mean ± s.e.m. of 3 independent experiments; *P<0.05, two-way ANOVA). (C and D) AEP is required for BDNF deprivation-induced neuronal death. Primary AEP WT or AEP KO neurons were treated with anti-BDNF 15 μg/ml for 48 h, and immunoblotting analysis with various antibodies including AEP, Tau N368 and active caspase-3 (C). LDH assay shown AEP WT and AEP KO neuronal cell death treated with anti-BDNF or anti-IgG. (Data represent mean ± s.e.m. of 3 independent experiments; *P<0.05, **P<0.01, two-way ANOVA) (D).

Fig. S3.

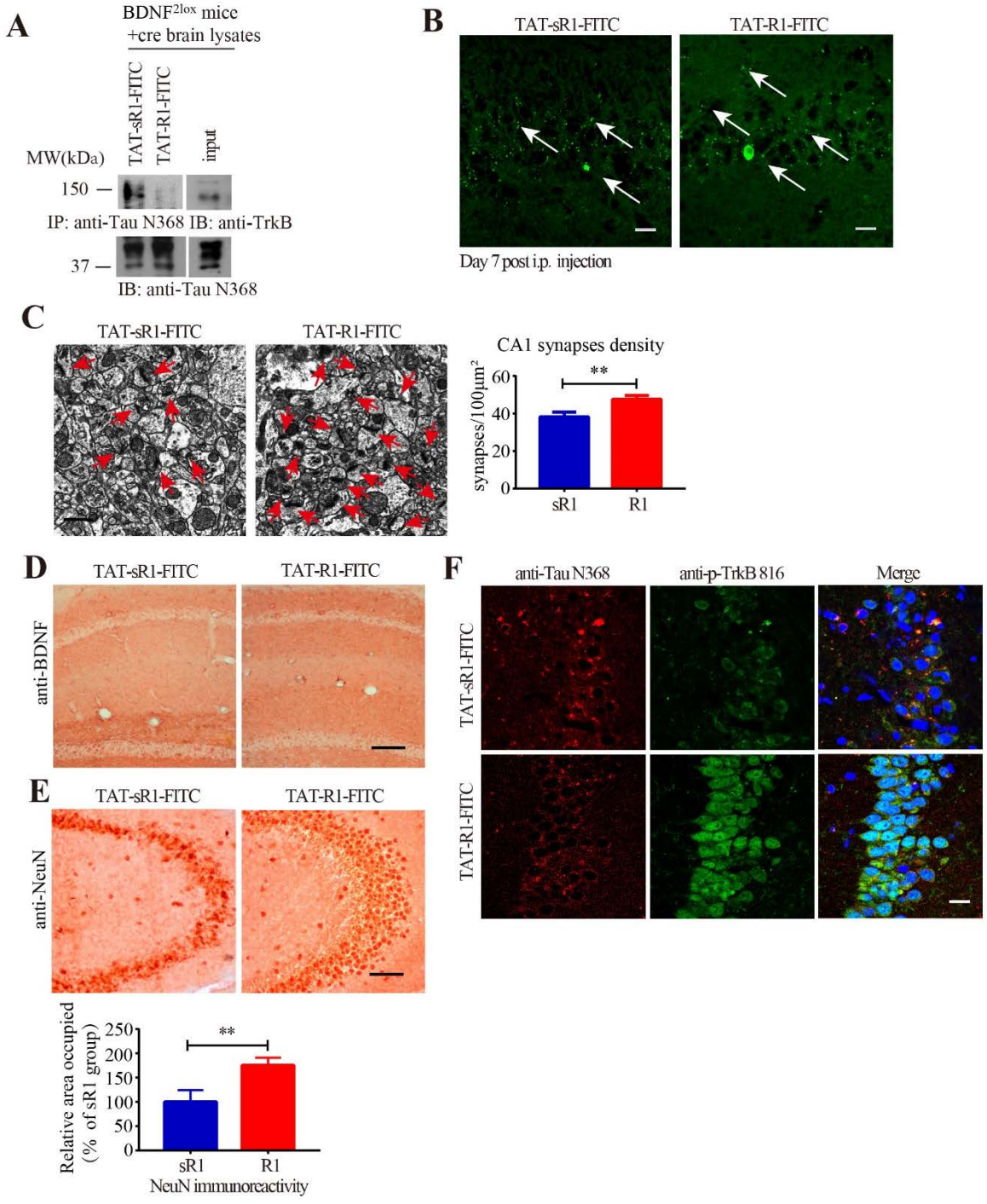
A
BDNF2lox mice
+cre brain lysates
TAT-sR1-FITC
TAT-R1-FITC
input
MW(kDa)
150
IP: anti-Tau N368 IB: anti-TrkB
37
IB: anti-Tau N368
B
TAT-sR1-FITC
TAT-R1-FITC
Day 7 post i.p. injection
C
TAT-sR1-FITC
TAT-R1-FITC
CA1 synapses density
synapses/100μm²
60
40
20
0
**
sR1
R1
D
TAT-sR1-FITC
TAT-R1-FITC
anti-BDNF
E
TAT-sR1-FITC
TAT-R1-FITC
anti-NeuN
Relative area occupied
(% of sR1 group)
250
200
150
100
50
0
**
sR1
R1
NeuN immunoreactivity
F
anti-Tau N368
anti-p-TrkB 816
Merge
TAT-sR1-FITC
TAT-R1-FITC

Fig. S3: BDNF depletion triggers Tau N368 cleavage and synaptic dysfunctions in BDNF2lox mice, rescued by the blocking peptide.

(A) TAT-R1-FITC peptide blocks TrkB and Tau N368 association in vitro. BDNF2lox mice injected with AAV-Cre virus in CA1 for a month, brain lysates were incubated with 10 μM TAT-R1-FITC or TAT-control-FITC for 2 h and then immunoprecipitation was performed with control IgG or anti-Tau N368, and the co-precipitated proteins were analyzed by immunoblotting with anti-TrkB. (B) TAT-R1-FITC and TAT-sR1-FITC peptides are brain permeable and stable in the brain. Representative images of hippocampus region from 7 days after i.p. injection with TAT-R1-FITC or TAT-sR1-FITC peptide. The fluorescent peptide penetrated into the brains. Scale bar, 20 μm. (C) Electron microscopy of synapses. Arrows indicated the synapses (left). Scale bar, 1μm. Quantification of the synaptic density (right) (mean ± s.e.m.; n=6 mice per group; **P<0.01, two-tailed Student's t-test). (D) BDNF was depleted by Cre virus in the hippocampal region of BDNF2lox mice. BDNF immunostaining of hippocampal regions. Scale bar, 50 μm. (E) NeuN immunostaining of hippocampal regions, scale bar, 50μm (left). Bar graph, quantification of positive cells was analyzed. (Data represent mean ± s.e.m.; n=6 mice per group; **P<0.01, two-tailed Student's t-test). (F) Tau R1 peptide treatment rescues p-TrkB signals in BDNF-depleted mice. Tau N368 and p-TrkB co-staining were performed in BDNF2lox AAV-Cre injected mice hippocampal region treated with TAT-R1-FITC or TAT-sR1-FITC peptide, scale bar, 10 μm.

Fig. S4.

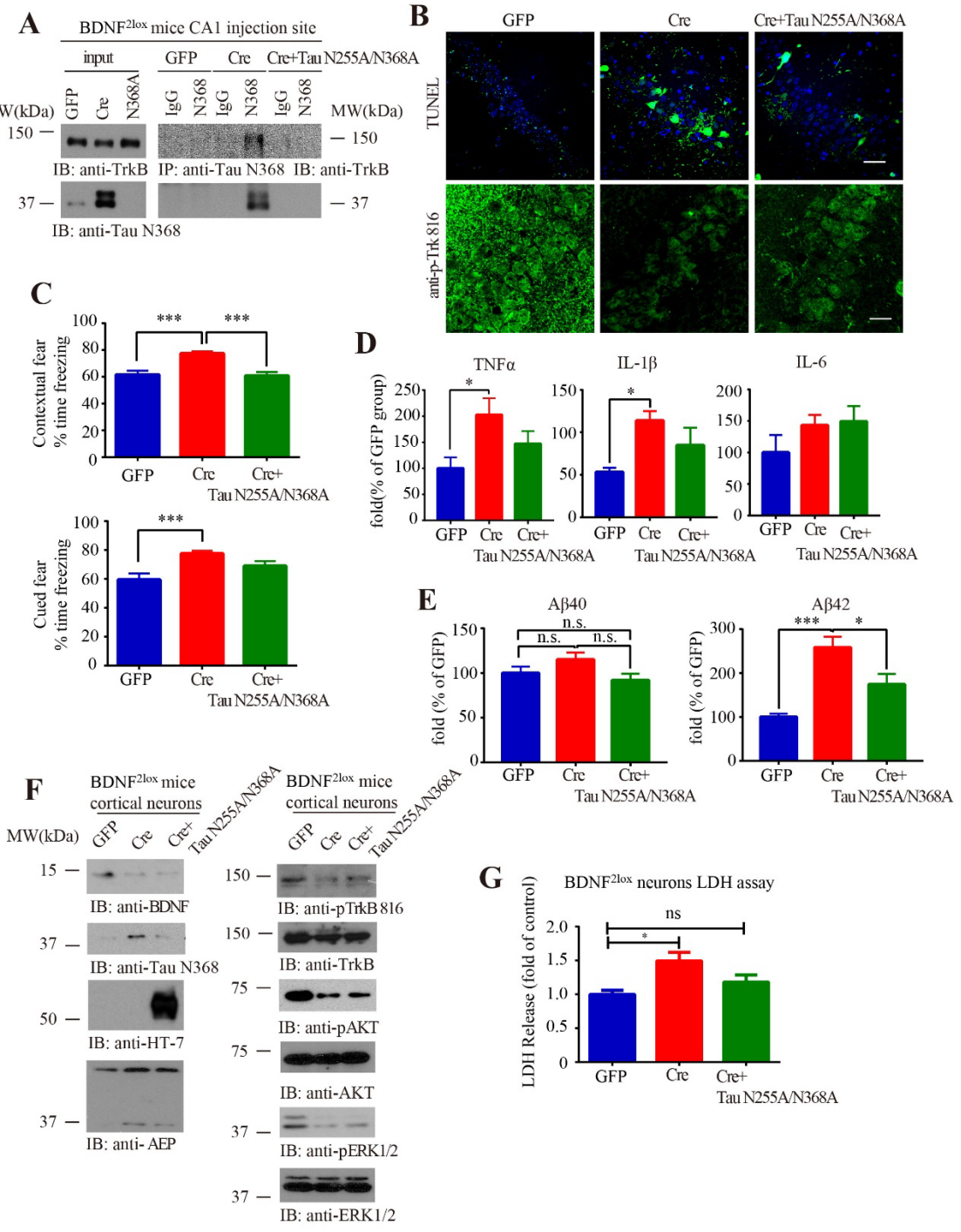
A
BDNF2lox mice CA1 injection site
input
GFP
Cre
Cre+Tau N255A/N368A
GFP
Cre
N368A
IgG
N368
MW(kDa)
150
IB: anti-TrkB
IP: anti-Tau N368
IB: anti-TrkB
37
IB: anti-Tau N368
B
GFP
Cre
Cre+Tau N255A/N368A
TUNEL
anti-p-Trk 816
C
Contextual fear
% time freezing
***
GFP
Cre
Cre+
Tau N255A/N368A
Cued fear
% time freezing
D
TNFα
IL-1β
IL-6
fold(% of GFP group)
E
Aβ40
Aβ42
n.s.
fold (% of GFP)
F
BDNF2lox mice
cortical neurons
Tau N255A/N368A
MW(kDa)
IB: anti-BDNF
IB: anti-Tau N368
IB: anti-HT-7
IB: anti- AEP
IB: anti-pTrkB 816
IB: anti-TrkB
IB: anti-pAKT
IB: anti-AKT
IB: anti-pERK1/2
IB: anti-ERK1/2
G
BDNF2lox neurons LDH assay
LDH Release (fold of control)
ns

Fig. S4: AEP-uncleavable Tau N255A/N368A antagonizes BDNF depletion-induced neuronal cell death and cognitive impairments.

(A) Tau N368/TrkB association is decreased in the presence of Tau N255A/N368A mutant. $BDNF^{2lox}$ mice injected AAV-Cre, AAV-Cre plus AAV-Tau N255A/N368A or AAV-GFP control in CA1 region. Co-immunoprecipitation was conducted from the injected site brain lysates and performed with anti-Tau N368 antibody, the coprecipitated proteins were analyzed by immunoblotting with anti-TrkB. (B) Uncleavable Tau mutant inhibits neuronal apoptosis and increases p-TrkB. p-TrkB 816 immunofluorescent and TUNEL co-staining in hippocampal region were shown. Uncleavable Tau mutant rescued TrkB signal and decreased cell apoptosis in Cre+Tau N255A/N368A group mice compared with Cre mice group (Scale bar, 20μm). (C) Fear conditioning test (mean ± s.e.m.; n=9 mice per group; *P<0.05, **P<0.01, one-way ANOVA). (D) Uncleavable Tau mutant decreases BDNF knockout-induced neuro-inflammation. ELISA analysis of injected brain lysates TNFα, IL-1βand IL-6 concentration (Data represent mean ± s.e.m. of 3 independent experiments; *P<0.05, one-way ANOVA). (E) Aβ42 is repressed by uncleavable Tau mutant. ELISA results showing the concentration of Aβ1-40 and Aβ1-42 in injected region brain lysates from Cre, Cre + Tau N255A/N368A and GFP control group (Data represent mean ± s.e.m. of 3 independent experiments; *P<0.05, ***P<0.001, one-way ANOVA). (F) Uncleavable Tau mutant rescues p-TrkB signaling. The western blot conducted with $BDNF^{2lox}$ mice primary neuron infected AAV-Cre plus AAV-Tau N255A/N368A virus showing TrkB signaling restoration. (G) Uncleavable Tau mutant represses BDNF depletion-induced neuronal cell death. LDH assay conducted with cell medium infected with AAV-Cre, AAV-Cre plus AAV-Tau

N255A/N368A or AAV-GFP control. (Data represent mean ± s.e.m. of 3 independent experiments; *P<0.05, one-way ANOVA).