Expanded View Figures

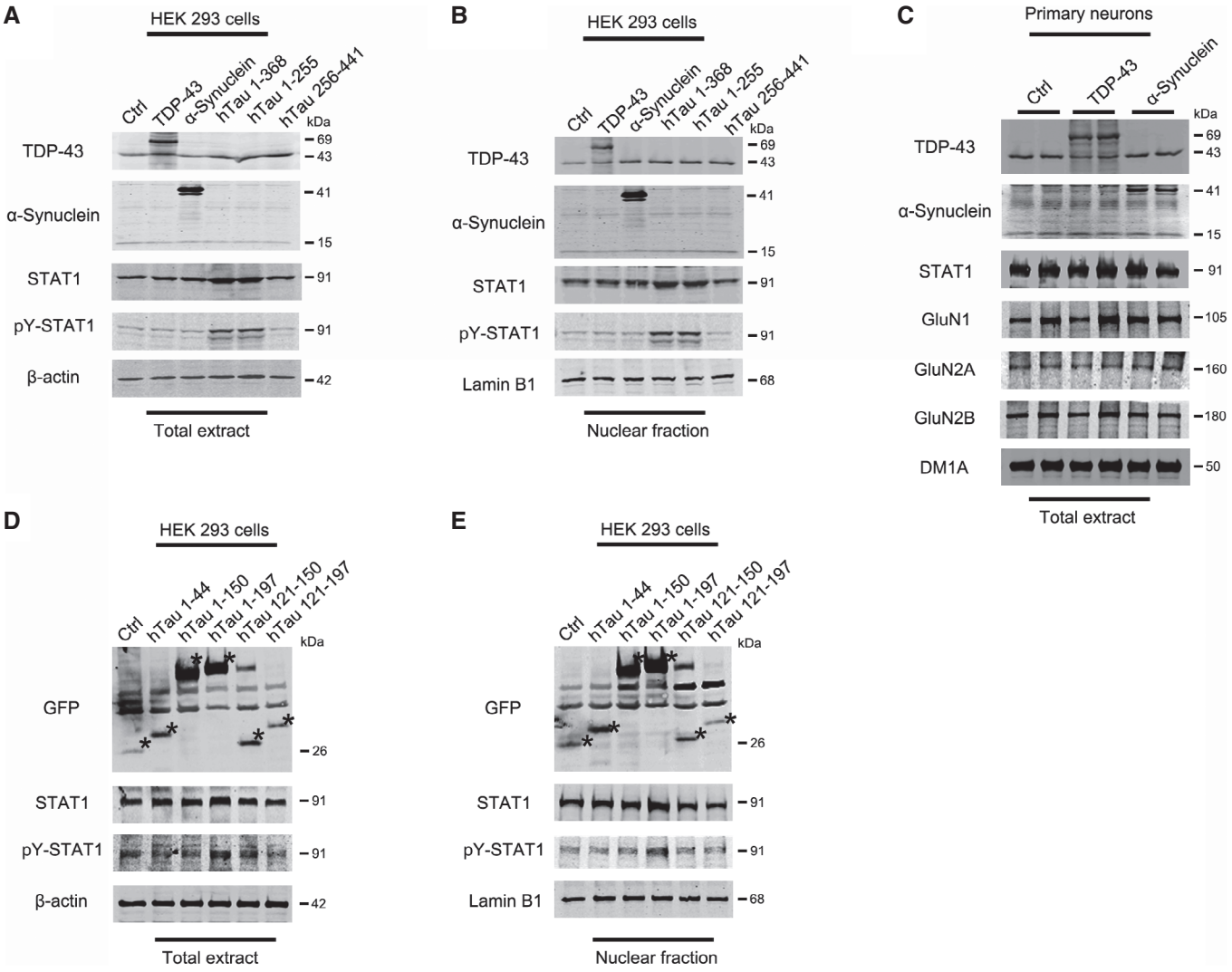


Figure EV1. Overexpressing N-terminal tau fragments not TDP-43 or α-synuclein activate STAT1.

A, B Plasmids of TDP-43, α-synuclein, or hTau fragments or the vector control were transfected into HEK293 cells for 48 h, and then, the protein levels of STAT1 and pY-STAT1 were detected by Western blotting in the total extracts (A) and the nuclear fraction (B).

C Adeno-associated virus (AAV)-eGFP-TDP43 or AAV-eGFP-α-synuclein or the vector control was transfected into the primary hippocampal neurons (6 div) and the neurons were cultured for another 48 h, and then, the protein levels of NMDARs were detected by Western blotting.

D, E The subdivided hTau fragments were transfected into the HEK293 cells for 48 h, and then, the protein levels of STAT1 and pY-STAT1 were detected by Western blotting in total extracts (D) and the nuclear fraction (E). Asterisks indicated the bands which matched with fragments' predicted molecular weight.

Source data are available online for this figure.

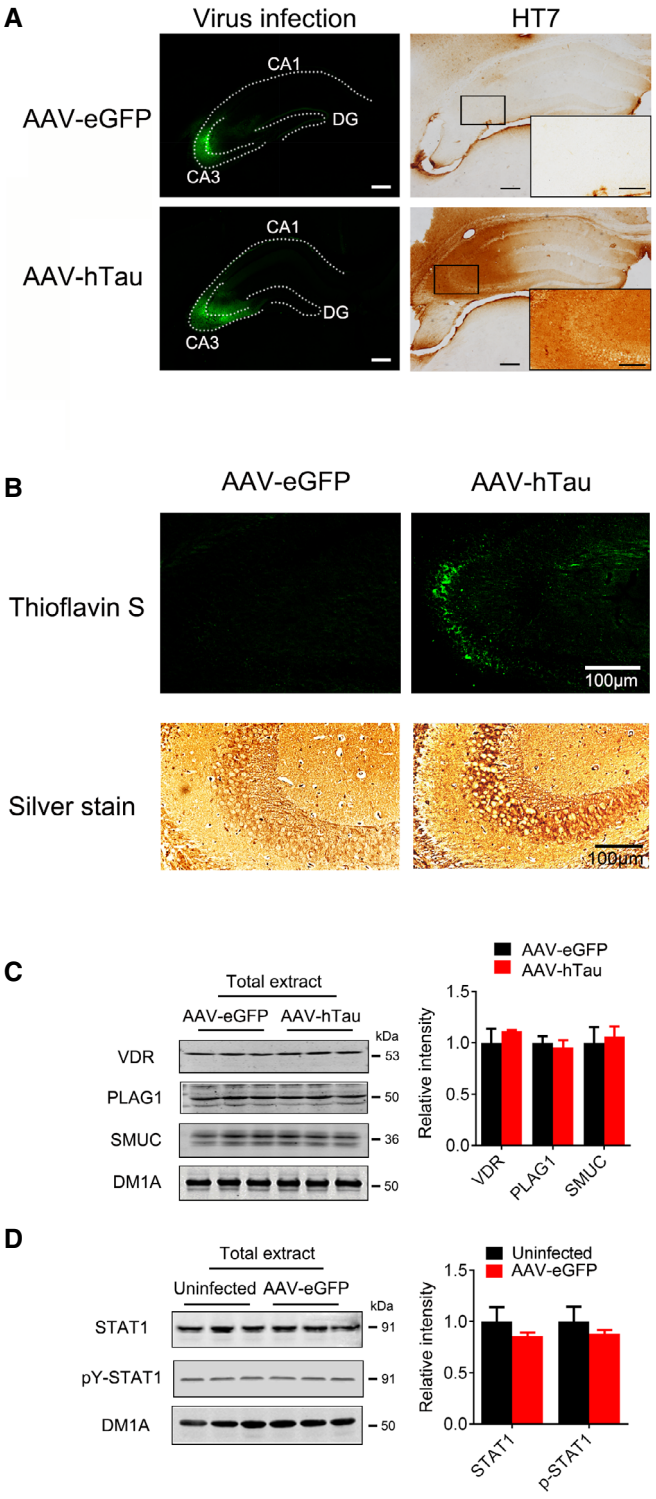


Figure EV2. Overexpressing hTau induces tau aggregation and does not affect protein level of VDR, PLAG1, and SMUC.

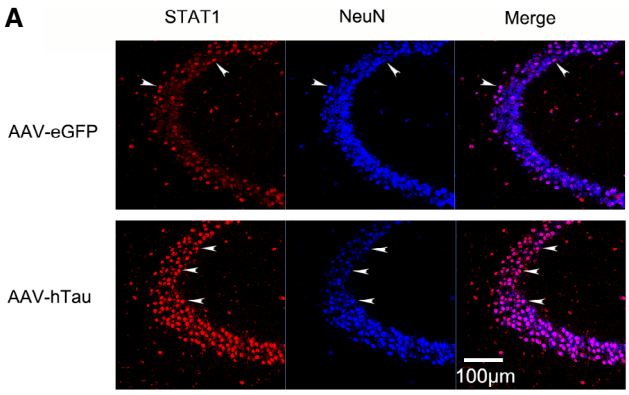
A Adeno-associated virus (AAV)-eGFP expressing wild-type full-length human tau (hTau) or the empty AAV vector (eGFP) (1.13×10^{13} v.g./ml) was stereotaxically injected into the hippocampal CA3 of 3-month-old C57 mice. One month later, the infected virus was shown by direct fluorescence (left panels) and immunohistochemical staining of HT7 (specifically reacts with human tau, right panel), respectively. Scale bar, 200 μm; 100 μm (enlarged picture).

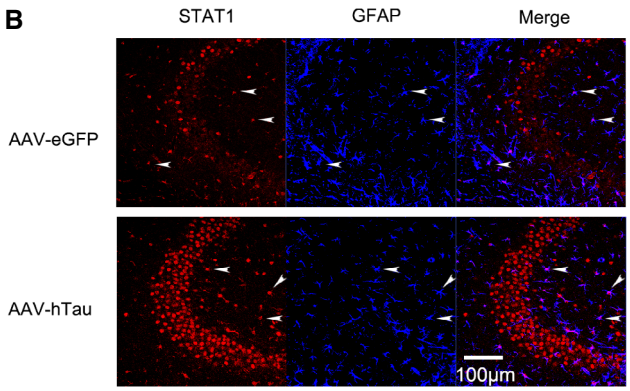
B The representative images of Thioflavin S and Bielschowsky silver staining showed tau aggregation in the hippocampus of the virus-injected mice.

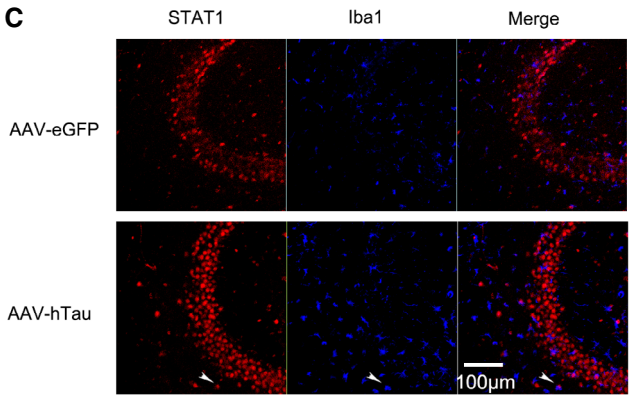
C AAV-hTau or the empty vector (eGFP) (1.13×10^{13} v.g./ml) was stereotaxically injected into the hippocampal CA3 of 3-month-old C57 mice. One month later, the protein levels of VDR (vitamin D receptor), PLAG1 (pleiomorphic adenoma gene 1), and SMUC (snail-related transcription factor) were detected by Western blotting (n = 4).

D AAV-eGFP (1.13×10^{13} v.g./ml) or PBS was stereotaxically injected into the hippocampal CA3 of 3-month-old C57 mice. PBS-injected tissue was used as uninfected control. One month later, the protein levels of STAT1 and pY-STAT1 were detected by Western blotting (n = 4).

Data information: Data were presented as mean ± SD (Mann–Whitney test).
Source data are available online for this figure.







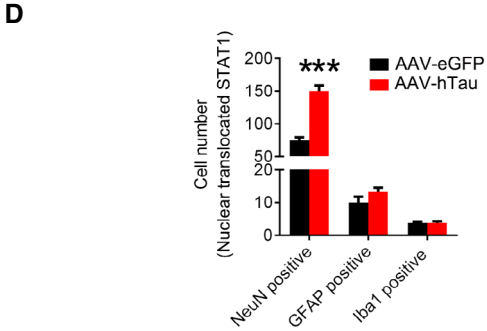


Figure EV3. STAT1 is predominantly expressed in neurons and elevation of STAT1 by overexpressing hTau is mostly significant in neurons.

A–D AAV-hTau-eGFP (tau) or the empty vector AAV-eGFP (eGFP) (1.13×10^{13} v.g./ml) was stereotaxically injected into hippocampal CA3 of 3-month-old C57 mice. After 1 month, the cell type of STAT1 staining was detected by co-staining of STAT1 with NeuN (A), GFAP (B), or IBA1 (C) and cell number quantitative analysis (D). Arrowheads indicated typical co-localization of nuclear-translocated STAT1 with NeuN (A), GFAP (B) or IBA1 (C). ***$P < 0.001$ vs. eGFP. $N = 4$–5 each group.

Data information: Data were presented as mean ± SD (two-way ANOVA, Bonferroni's *post hoc* test).

Figure EV4. STAT1 regulates the expression of NMDARs.

A AAV-STAT1-eGFP (WT-STAT1) or the empty vector (AAV-eGFP) (1×10^{12} v.g./ml) was transfected into the primary hippocampal neurons (6 div) for 6 days, and expression of the virus was shown by direct fluorescence. Scale bar, 20 μm.

B, C Overexpression of WT-STAT1 selectively decreased protein and mRNA levels of GluN1, GluN2A, and GluN2B detected by Western blotting and qRT–PCR. *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$ vs eGFP. Data were presented as mean ± SD ($n = 4$ from four independent experiments. Mann–Whitney test).

D AAV-hTau-eGFP (tau) or the empty vector AAV-eGFP (eGFP) (1.13×10^{13} v.g./ml) was stereotaxically injected into hippocampal CA3 of 3-month-old C57 mice. After 1 month, the decreased GluN1, GluN2A, and GluN2B expression was detected by immunohistochemical staining. Scale bar, 100 μm.

E The mixture of AAV-hTau (1.13×10^{13} v.g./ml) and AAV-Cre (5×10^{12} v.g./ml) (1 μl AAV-hTau plus 2 μl AAV-Cre) was stereotaxically infused into the hippocampal CA3 of 3-month-old STAT1flox/flox mice. One month later, the restored GluN1, GluN2A, and GluN2B expression was detected by immunohistochemical staining. Scale bar, 100 μm.

Source data are available online for this figure.

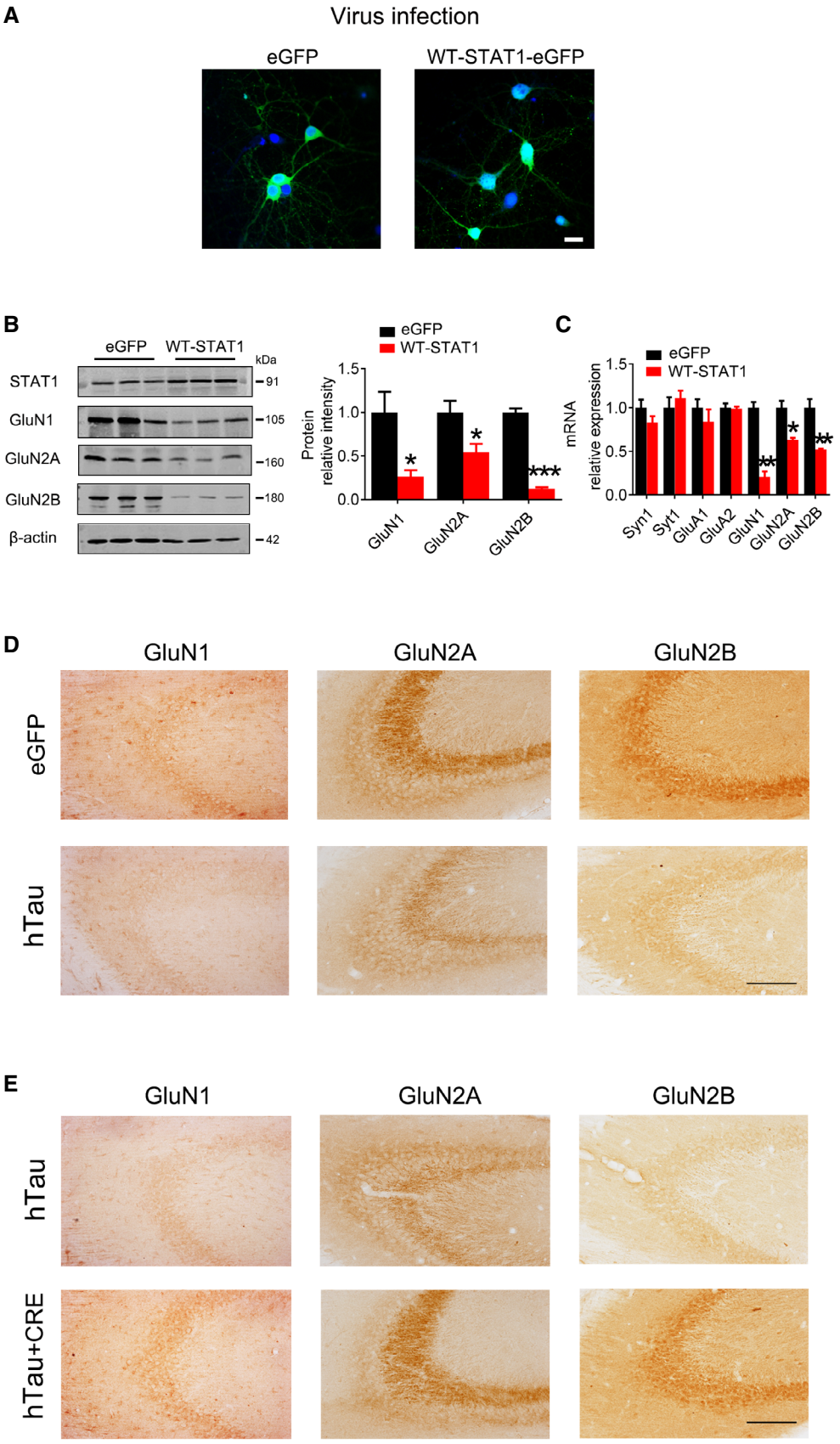

A
Virus infection
eGFP
WT-STAT1-eGFP
B
eGFP
WT-STAT1
kDa
STAT1
GluN1
GluN2A
GluN2B
β-actin
91
105
160
180
42
Protein relative intensity
1.5
1.0
0.5
0.0
*
*
***
GluN1
GluN2A
GluN2B
C
eGFP
WT-STAT1
mRNA relative expression
1.5
1.0
0.5
0.0
**
*
**
Syn1
Syt1
GluA1
GluA2
GluN1
GluN2A
GluN2B
D
GluN1
GluN2A
GluN2B
eGFP
hTau
E
GluN1
GluN2A
GluN2B
hTau
hTau+CRE

Figure EV4.

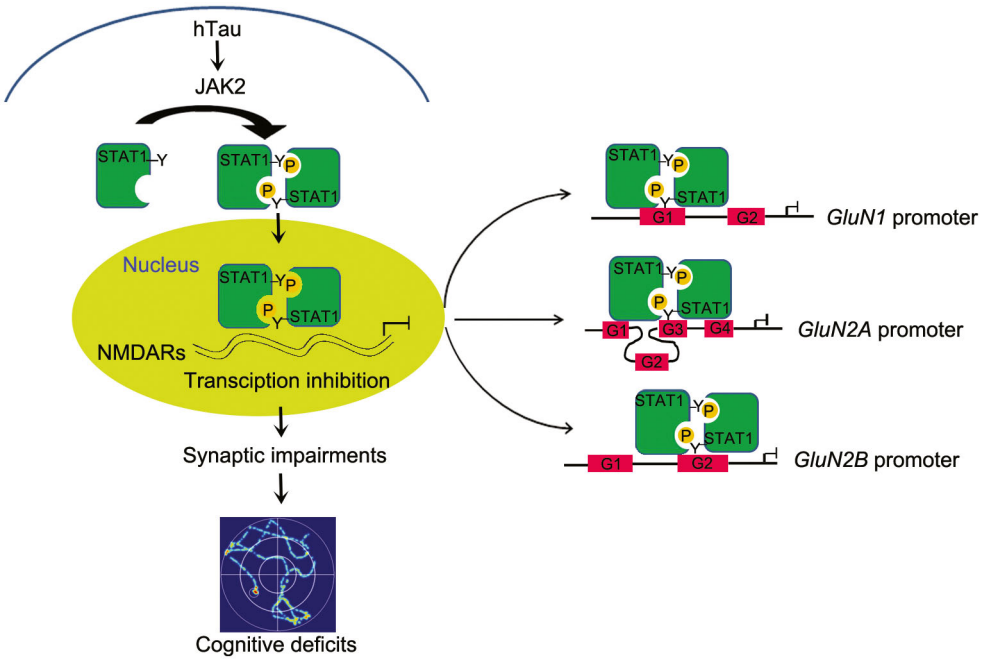


Figure EV5. The proposed working model by which STAT1 mediates hTau-induced toxicities.
Intracellular accumulation of human tau (hTau) activates JAK2 to phosphorylate STAT1 at Tyr701 leading to STAT1 dimerization and its transcriptional activation in the nuclei. The hTau-induced STAT1 activation inhibits expression of GluN1, GluN2A, and GluN2B by binding to the specific domain of the promoters, i.e., the GAS1 of GluN1 promoter, GAS2 of GluN2B promoter, or GAS3 combined with GAS1 or GAS4 of GluN2A promoter, which consequently leads to synaptic dysfunctions and memory deficits. "G" means GAS promoter element (GAS).