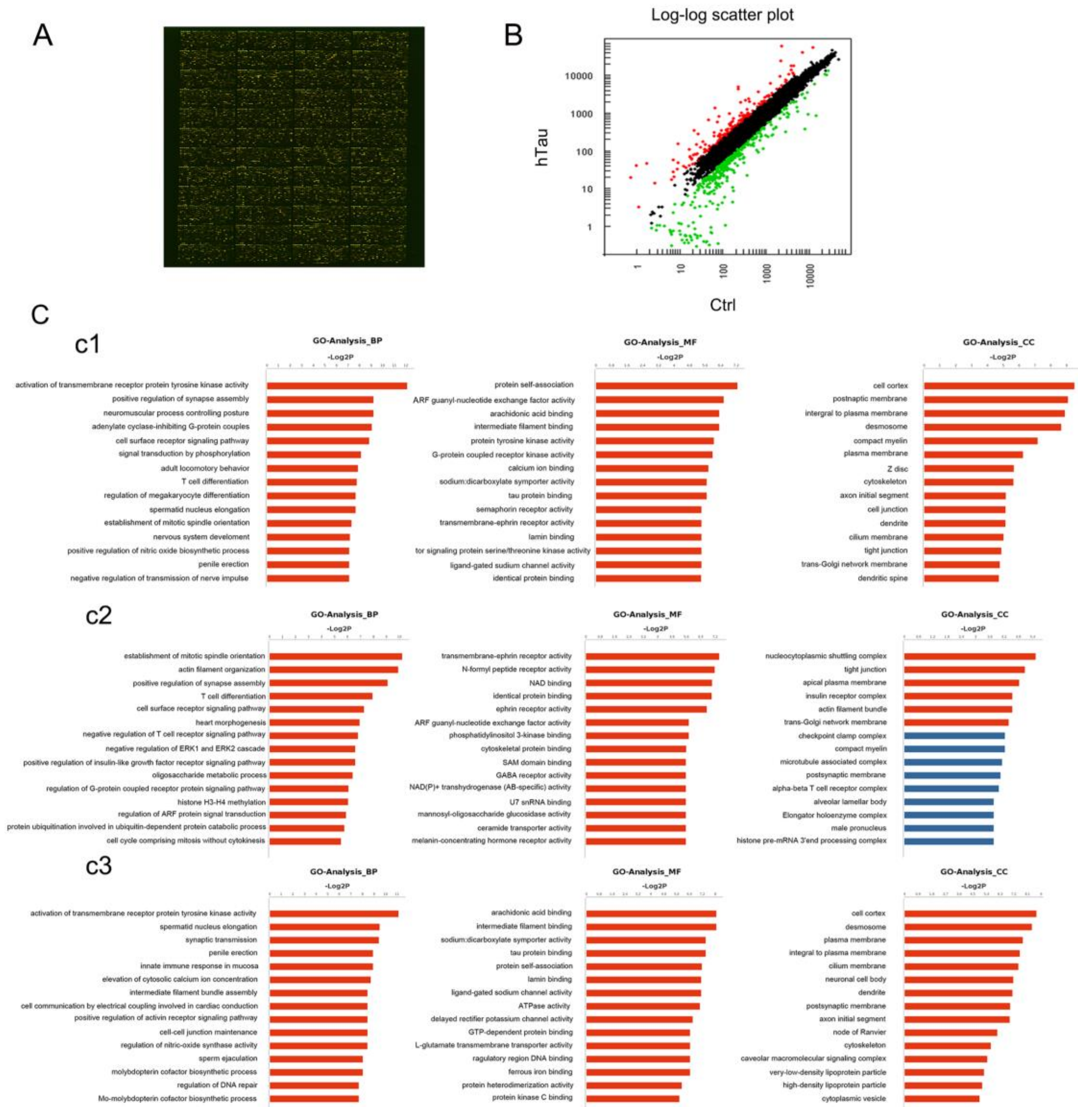
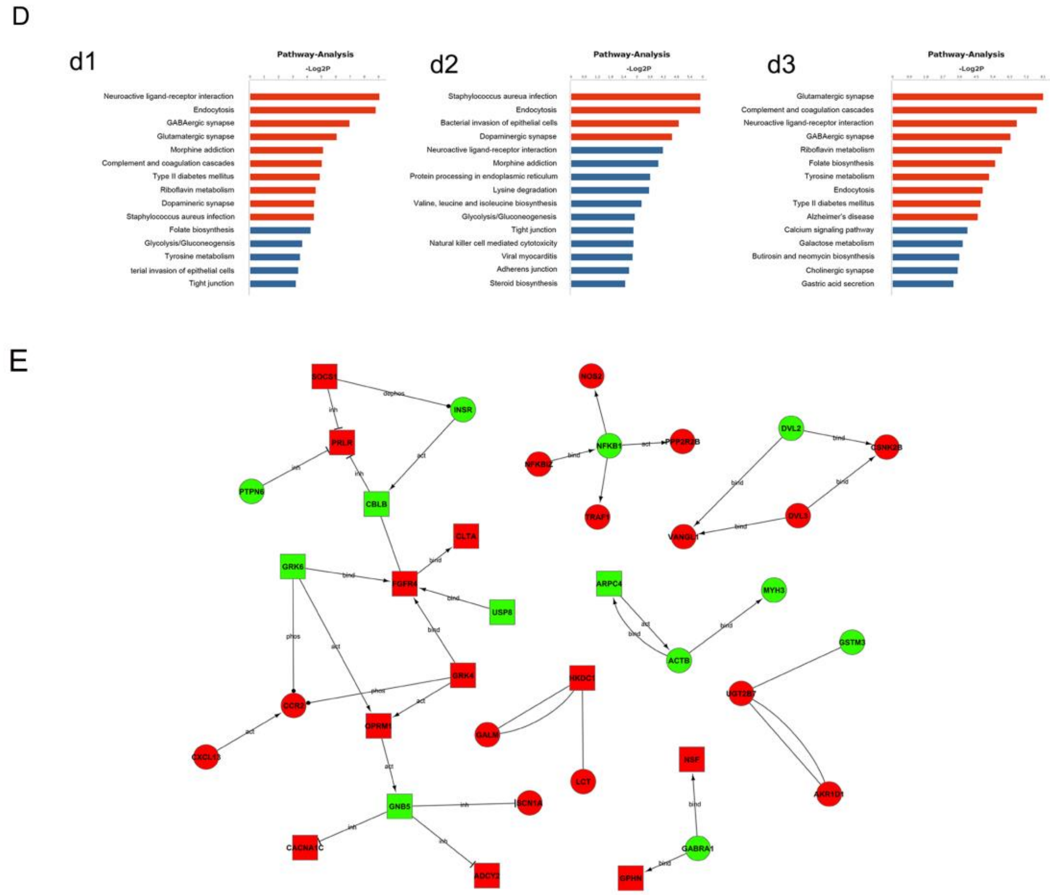
The Appendix

Appendix Figure S1

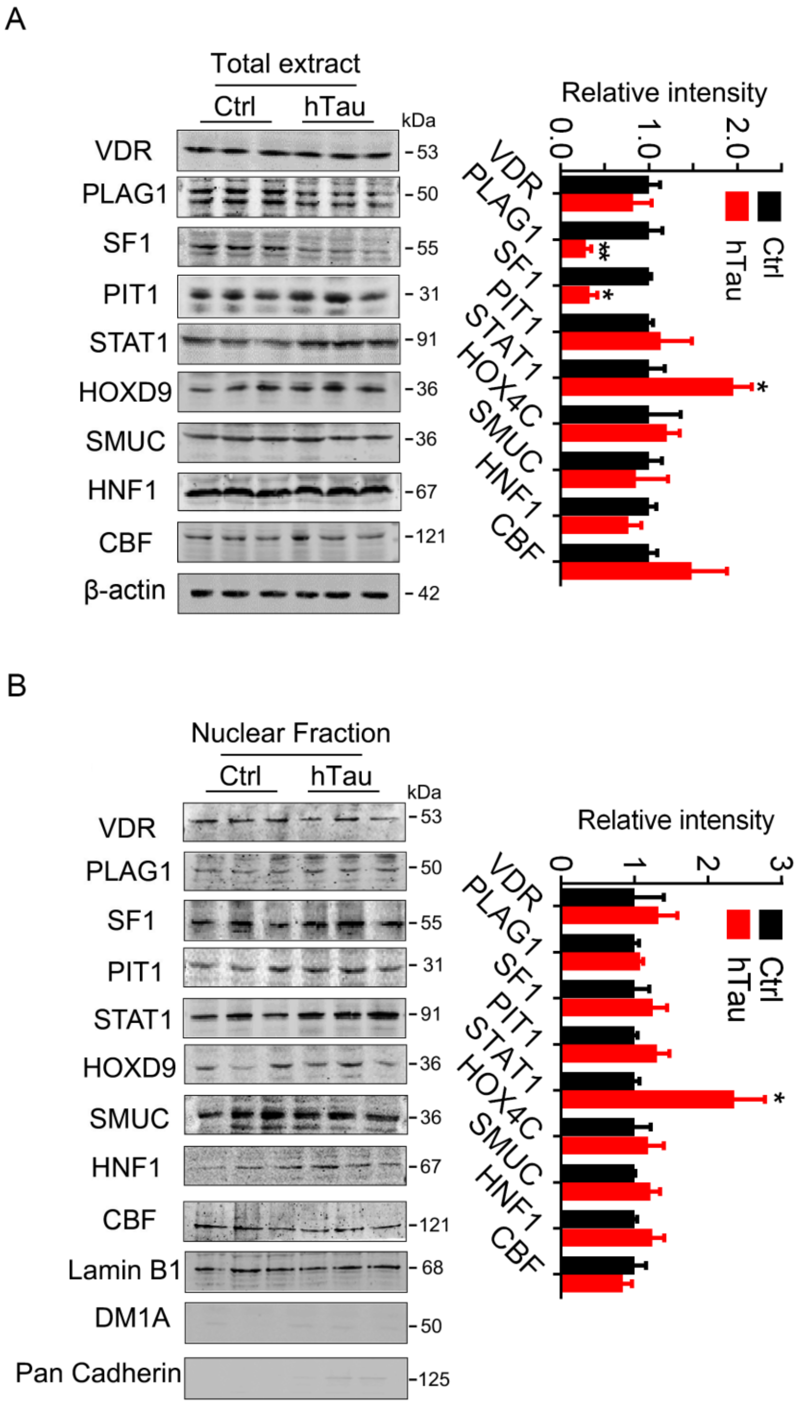




Appendix Figure S1 Tau accumulation changes mRNA levels.

HEK293 cells were transfected with wildtype full-length human tau plasmid (hTau) or its empty vector (Ctrl) for 48 h, and then the mRNA was extracted and the gene expression was detected by the Jingxin® 35K oligo gene array (CapitalBio Inc., Beijing, China). (A) Heatmap presentation of hTau-induced gene expression. (B) Scatter plots show variations in mRNA expression in hTau and the control cells: 'Red' indicates high relative expression and 'Blue' indicates low relative expression. (C) Gene ontology-(GO) analysis, (c1) up-regulated genes GO-analysis, (c2) down-regulated genes GO-analysis, (c3) up- & down-regulated genes GO-analysis, BF (Biological Processes), MF (Molecular Function), CC (Cellular Component). 'Red' or 'Blue' indicates significant or non-significant GO Term. (D) Pathway Analysis, (d1) up-regulated genes Pathway Analysis, (d2) down-regulated genes Pathway Analysis, (d3) up- & down-regulated genes Pathway Analysis. ‘Red’ or ‘Blue’ indicates significant or non-significant Pathway Term. (E) Network model analysis, ‘Red’ or ‘Blue’ indicates high or low relative expression.

Appendix Figure 2

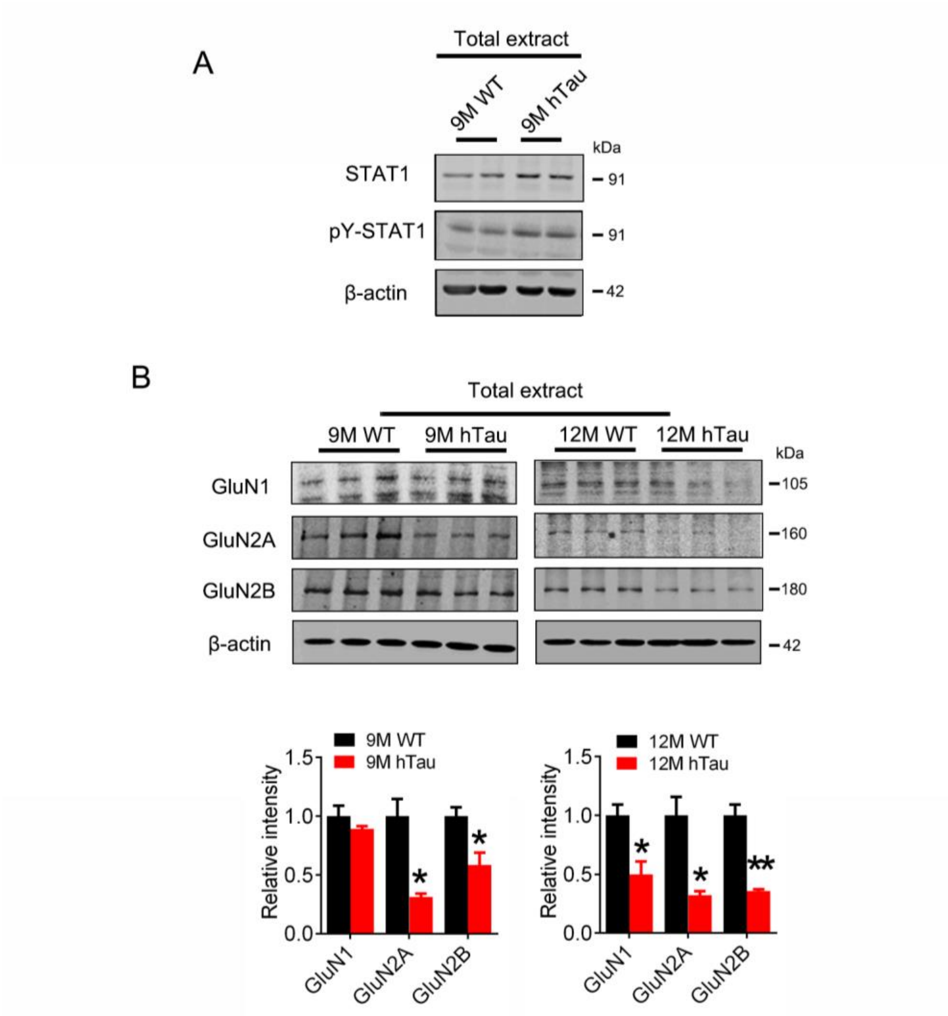


Appendix Figure S2 Overexpression of hTau increases protein level of STAT1 in HEK293 cells.

(A, B) The human tau plasmid (hTau) or its empty vector (Ctrl) was transfected in HEK293 cells for 48 h. Then the protein levels of the transcription factors (TFs) in total extracts and the nuclear fraction, screened out from Fig. 1A by using TF Activation Profiling Plate Array II, were measured by Western blotting and quantitative analysis. Find the full name of transcription factors in Table S2.

Data information: *, $p<0.05$, **, $p<0.01$ vs Ctrl. Data were presented as mean ± SD (n=4, Mann-Whitney test).

Appendix Figure S3



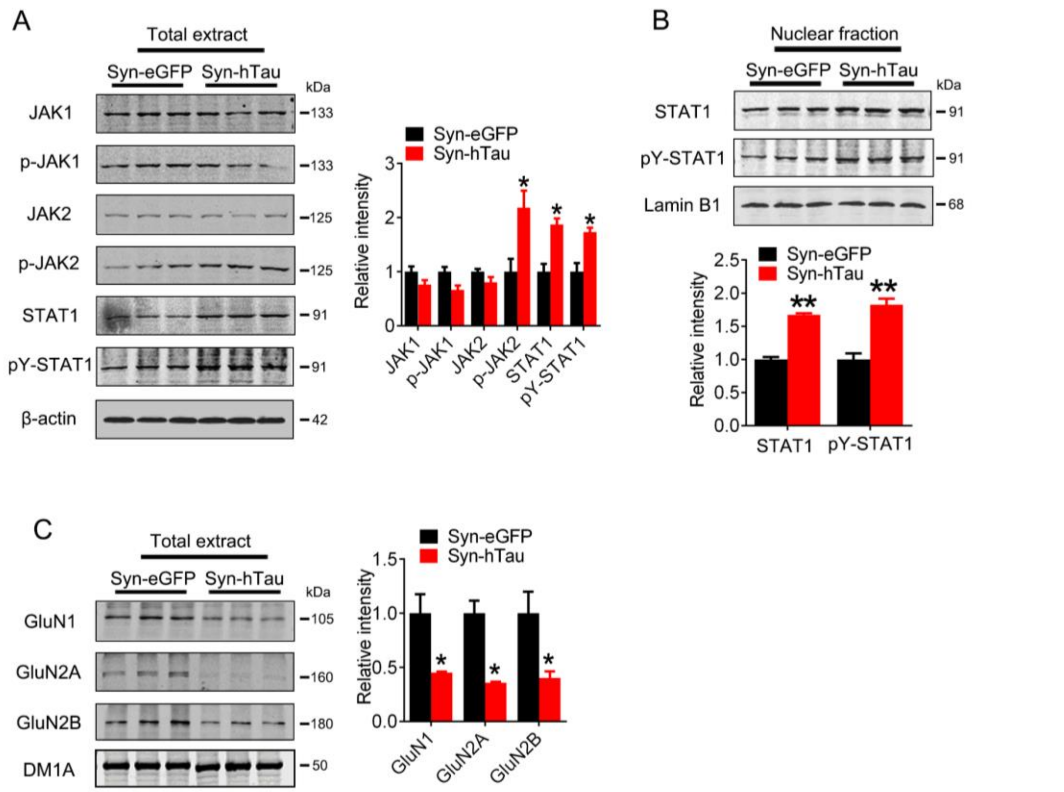
Appendix Figure S3 Phosphorylated STAT1 level increases and NMDAR levels decrease in the 9-month- and 12-month-old hTau mice.

(A) The increased STAT1 and pY-STAT1 in hippocampal total extracts of 9-month-old hTau transgenic mice measured by Western blotting.

(B) The decreased NMDAR levels in hippocampal total extracts of 9-month- and 12-month-old hTau transgenic mice measured by Western blotting and quantitative analysis (n=4, Mann-Whitney test).

Data information: *, $p<0.05$； **, $p<0.01$ vs WT. Data were presented as mean ± SD.

Appendix Figure S4



Appendix Figure S4 Specifically overexpressing hTau in neurons activated JAK2-STAT1 axis and decrease NMDAR levels.

(A-C) Syn-hTau-AAV (Syn-hTau) or the empty vector AAV-Syn (Syn-eGFP) (1.99×10^{13} v.g./ml) was injected stereotaxically into hippocampal CA3 of 3-month-old C57 mice. After one month, the total hippocampal extracts and the nuclear fraction were prepared for Western blotting analyses.

(A) The increased levels of STAT1, pY-STAT1 and p-JAK2 in hippocampal total extracts were detected in hTau group.

(B) The increased levels of STAT1 and pY-STAT1 in the nuclear fraction of the hippocampus were detected in hTau group.

(C) The reduced protein levels of GluN1, GluN2A and GluN2B in total hippocampal extracts in hTau group.

Data information: *, $p<0.05$, **, $p<0.01$ vs Syn-eGFP. Data were presented as mean ± SD (n=4, Mann-Whitney test).

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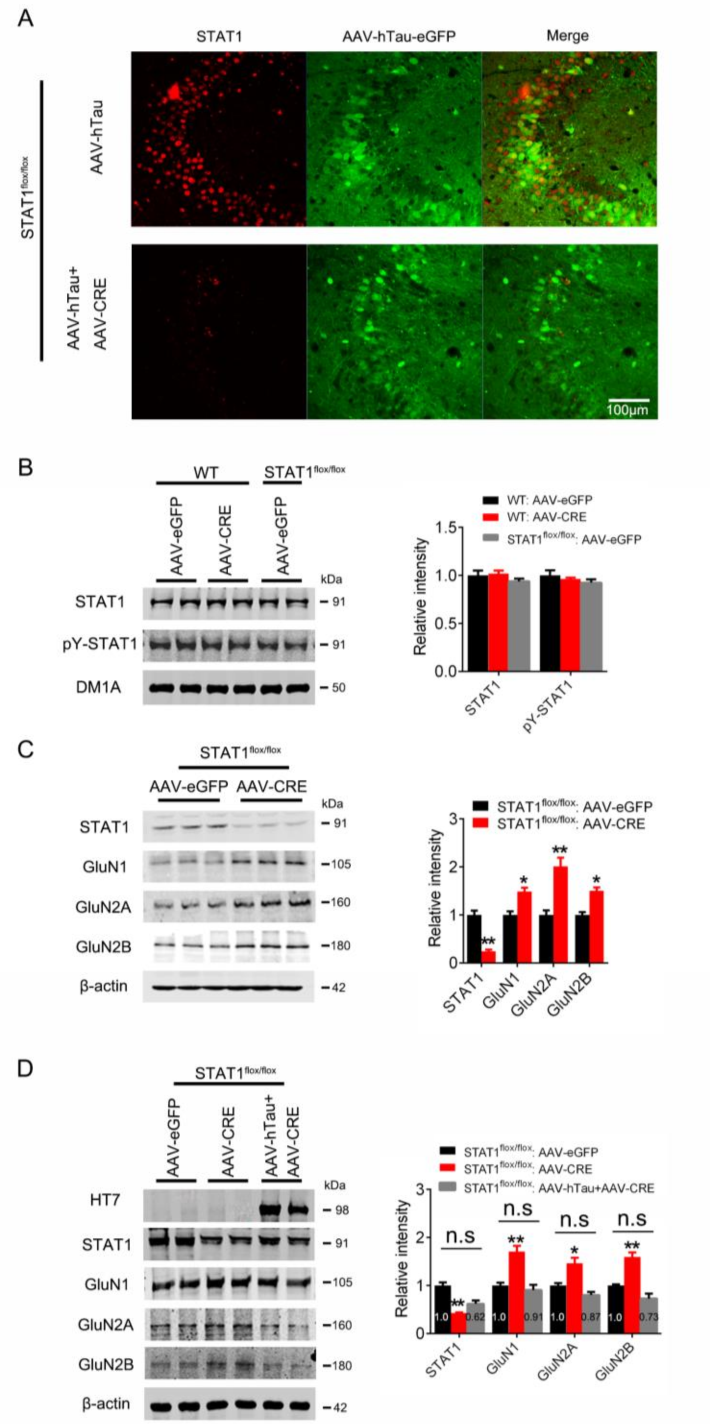

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Appendix Figure S5 Knockdown STAT1 negatively regulates the expression of NMDARs.

(A) AAV-Cre (5×10^{12} v.g./ml) mixed with AAV-hTau were stereotaxically injected into the hippocampal CA3 of 3-month-old $STAT1^{flox/flox}$ mice. One month later, downregulation of STAT1 was confirmed by immunofluorescence staining.

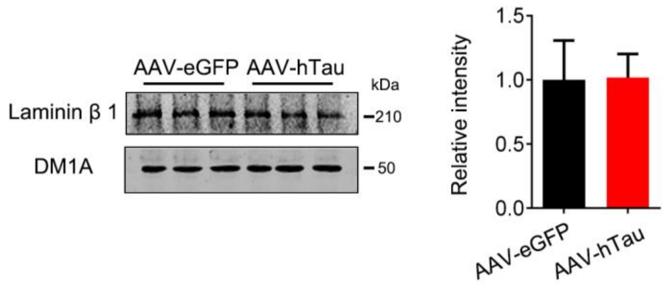
(B) AAV-Cre or AAV-eGFP (1.13×10^{13} v.g./ml) was stereotaxically injected into the hippocampal CA3 of 3-month-old C57 WT or $STAT1^{flox/flox}$ mice. One month later, the protein levels of STAT1 and pY-STAT1 were detected by Western blotting.

(C) AAV-Cre or AAV-eGFP (1.13×10^{13} v.g./ml) was stereotaxically injected into the hippocampal CA3 of 3-month-old $STAT1^{flox/flox}$ mice. One month later, the protein levels of STAT1 and NMDARs were detected by Western blotting.

(D) AAV-Cre, AAV-eGFP (1.13×10^{13} v.g./ml), or AAV-Cre mix with AAV-hTau was stereotaxically injected into the hippocampal CA3 of 3-month-old $STAT1^{flox/flox}$ mice. One month later, the protein levels of STAT1 and NMDARs were detected by Western blotting.

Data information: Data were presented as mean ± SD (n=3). (one-way ANOVA, Bonferroni's post hoc test). *, p<0.05, **, p<0.01, ***, p<0.001 vs AAV-eGFP group.

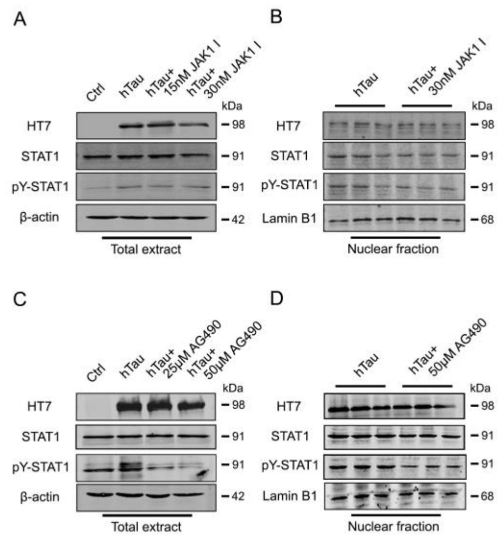
Appendix Figure S6



Appendix Figure S6 Overexpressing hTau does not affect the protein level of laminin β1.

AAV-hTau or the empty vector (eGFP) (1.13×10^{13} v.g./ml) (1μl) was stereotaxically infused into the hippocampal CA3 of 3-month-old C57 mice. One month later, laminin β1 protein level in the extract of hippocampal CA3 was detected by Western blotting. Data were presented as mean ± SD (n=4, Mann-Whitney test).

Appendix Figure S7

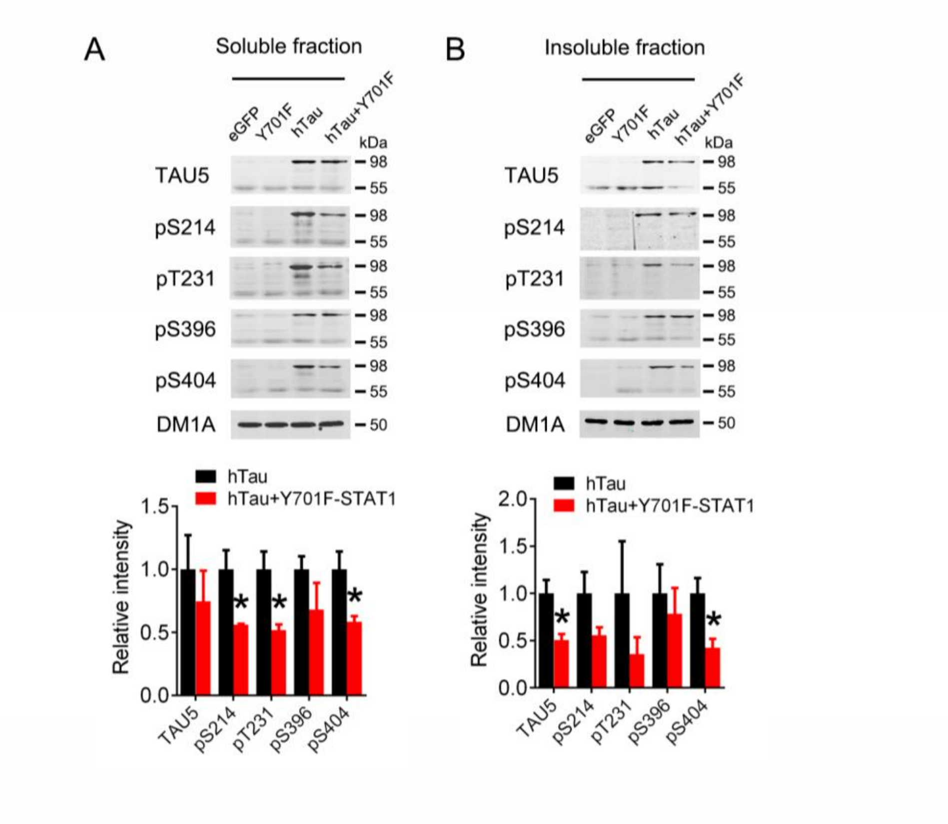


Appendix Figure S7 JAK2 non-specific inhibitor AG490 abolishes hTau-induced STAT1 phosphorylation at Tyr701 in total extracts and the nuclear fraction.

(A, B) Overexpression of hTau in HEK293 cells for 48 h, pharmacological inhibition of JAK1 (A, B) for 24 h did not significantly affect hTau-induced STAT1 phosphorylation at pY-STAT1 (Tyr701) in total extracts (A) and the nuclear fraction (B) measured by Western blotting.

(C, D) Overexpression of hTau in HEK293 cells for 48 h, AG490 (non-specific inhibitor of JAK2) abolishes the hTau-induced STAT1 phosphorylation at Tyr701 in total extracts (C) and the nuclear fraction (D).

Appendix Figure S8



Appendix Figure S8 STAT1 inhibition reduces hyperphosphorylation and aggregation of tau in vivo.

(A, B) AAV-hTau or the empty vector (eGFP) (1.13×10^{13} v.g./ml) with or without AAV-Y701F-STAT1 (5×10^{12} v.g./ml) was stereotaxically injected into the hippocampal CA3 of 3-month-old C57 mice. One month later, the hippocampal CA3 tissues were separated into soluble and insoluble fraction. Tau protein level and tau hyperphosphorylation at pS214, pT231, pS396 and pS404 epitopes was detected by Western blotting.

Data information: *, $p<0.05$ vs hTau. Data were presented as mean ± SD (n=4, Mann-Whitney test).

Appendix Table S1 The transcription factors detected by TF activation plate array II

1	2	3	4	5	6	7	8
AP1	AP2	AR	ATF2	Brn-3	C\EBP	CAR	CBF
CDP	CREB	E2F-1	EGR	ER	Ets	FAST-1	GAS/ISR
GATA	GR/PR	HIF	HNF4	IRF	MEF2	Myb	Myc-Ma
NF-1	NFAT	NF-E2	NFkB	4-Oct	p53	Pax-5	Pbx1
Pit	PPAR	PXR	SMAD	Sp1	SRF	SATB1	Stat1
Stat3	Stat4	Stat5	Stat6	TCF/LEF	YY1	TR	TFIID
XBP	AP3	AP4	COUP-TF	ELK	FOXA1	FoxC1	FOXD3
FOXG1	FOXO1(FKHR)	FREAC2(FOXF2)	Gli-1	Gli-1	HEN(NSL-1)	HNF-1	HOX4C
HoxA-5	HSF	KLF4	MyoD	MZF	Nkx2-5	Nkx3-2	NRF1
NRF2(A)	1-Oct	Pax2	Pax3	Pax8	PITI	PLAG1	MEF1
Prox1	RB	RNUX	ROR(RZR)	RXR	SF-1	SMUC	Snail
SOX2	SOX9	SOX18	SRY	TFE3	USF-1	VDR	WT1

Appendix Table S2 The description of TF gene detected in TF activation plate array II

TF names	Gene Description
AP1	Activator protein 1(JUN/FOS)
AP2	Activator protein2
AR	Androgen receptor
ATF2	activating transcription factor 2
Brn-3	POU domain,class 4,transcription factor1
C/EBP	CCAAT/enhancer binding protein(C/EBP),alpha
CAR	nuclear receptor subfamily 1,groupI,member3
CBF	CCAAT/enhancer binding protein(C/EBP),zeta
CDP	cut-like homeobox1;CCAAT displacement protein
CREB	cAMP responsive element binding protein1
E2F1	E2F transcription factor1
EGR	Early growth response
ER	Estrogen receptor
Ets	v-ets erythroblastosisi virus E26 oncogene homolog 1
FAST1(FOXH1)	Forkhead boxH1
GAS/ISRE	IFN-stimulated response element
GATA	GATA transcription factor
GR/PR	Glucocorticoid receptor/Progesterone receptor
HIF	Hypoxia inducible factor

HNF4	Hepatocyte nuclear factor4
IRF	Interferon regulatory factor
MEF2	Myocyte enhancer factor 2
Myb	v-myb myeloblastosis viral oncogene homolog
Myc-Max	v-myc myelocytomatosis biral oncogene homolog
NF-1	Nuclear factor1
NFAT	Nuclear factor of activeated T-cells
NF-E2	Neclear factor(erythroid derived 2)
NFkb	neclear factor of kappa light polypeptide gene
OCT4	POU class 5 homeobox1
p53	Tumor protein p53
Pax-5	Paired box5
Pbx1	Pre-B cell leukemia transcription factor1
Pit	Pituitary specific transcription factor1
PPAR	Peroxisome proliferator-activated receptor
PXR	Pregnane X Receptor
SMAD(MADH)	SMAD family
Sp1	SP1 transcription factor
SRF	Serum response factor
SATB1	Special AT-rich sequence binding protein 1
Stat1	Signal transducer and activator of transcription1
Stat3	Signal transducer and activator of transcription3

Stat4	Signal transducer and activator of transcription4
Stat5	Signal transducer and activator of transcription5
Stat6	Signal transducer and activator of transcription6
TCF/LEF	Runt-related transcription factor 2
YY1	YY1 transcription factor
TR	Thyroid hormone receptor
TFIID	TATA box binding protein
TF names	Gene Description
XBP-1	X-Box binding protein 1
AP3	AP3 protein
AP4	AP4 protein
COUP-TF	nuclear receptor subfamily 2,group F,
ELK	ETS domain-containing protein Elk-1
FOXA1	homeoboxA1
FoxC1	homeoboxC1
FOXD3	forkhead box D3
FOXG1	FOXbox G1
FOXO1(FKHR)	FOXbox O1
FREAC-2	Forkhead-related activator 2
Gfi-1	growth factor independent 1 transcription
Gli-1	GLI zinc finger transcription factor
HEN(NSCL1)	helix-lool-helix protein

HNF-1	Hepatocyte Nuclear Factor1
HOX4C	HOX4C homobox
HOXA-5	homobox A5
HSF	Heat shock transcription factor 1
KLF4	Kruppel-like factor4
MyoD	muogenic differentiation i protein
MZF	zinc finger type transcription factor MZF
Nkx2-5	Homeobox protein Nkx-2.5
Nkx3-2	Homeobox protein Nkx-3.2
NRF1	nuclear respiratory factor
NRF2(ARE)	NRF2-related antioxidant responsive
Oct-1	POU domain,class 2,transcription factor
Pax2	Pair box-2 protein
Pax3	Pair box-3 protein
Pax8	Pair box-8 protein
PIT1	POU class 1 homeobox1
PLAG1	pleiomorphic adenoma gene 1
MEF1	Myocyte enhancer factor 1
Prox1	Prospero homeobox protein1
RB	Retinoblastoma control element
RNUX	SL3-3 enhancer factor1
ROR(RZR)	retinoic acid receptor related orphan

RXR	retinoid Xreceptor
SF1	Steroidogenic factor1
SMUC	snail-related transcription factor Smuc
Snail	Snail 1 zinc finger protein
SOX2	SOX protein 18
SOX9	SOX protein 2
SOX-18	SOX protein 9
SRY	sex determining region Y
TFE3	transcription factor binding to IGHM
USF-1	upstream transcription factor 1
VDR	vitamin D(1,25dihydroxy vitamin D3)

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Appendix Table S3 The antibodies used in the study

Antibody	Specific	Type	WB	IHC	Source
STAT1	Total STAT1	Poly-	1:500	1:100	abcam
pY-STAT1	p-STAT1 at Tyr701	Mono-	1:500	1:200	abcam
pY-STAT1	p-STAT1 at Tyr701	Poly-	1:300		Thermo Fisher Scientific
pS-STAT1	p-STAT1 at Ser727	Poly-	1:300		Thermo Fisher Scientific
GluN2A	GluN2A C-term	Poly-	1:1000	1:200	abcam
GluN2B	GluN2B C-term	Poly-	1:1000	1:200	abcam
GluN1	GluN1 C-term	Poly-	1:1000	1:200	Merck Millipore
GluA1	Total GluA1	Poly-	1:1000		abcam
GluA2	Total GluA2	Mono-	1:1000		Merck Millipore

SYN1	Total Synaptophysin	Poly-	1:1000		abcam
SYT1	Total Synaptotagmin	Mono-	1:1000		abcam
β-actin	Toal β-actin	Mono-	1:1000		abcam
DM1A	Toal α -tubulin	Mono-	1:1000		abcam
Lamin B1	Total Lamin B1	Poly-	1:1000		abcam
GFP	GFP-tag	Mono-	1:1000		abcam
FLAG	FLAG-tag	Mono-	1:1000		Merck Millipore
TAU5	Total tau	Mono-	1:1000		Merck Millipore
HT7	Total human tau	Mono-	1:1000		Thermo Fisher Scientific
AT8	PHF-tau (Ser202/Thr205)	Mono-	1:1000	1:200	Thermo Fisher Scientific

TDP-43	Total TDP-43	Poly-	1:1000		proteintech
α-synuclein	Total α-synuclein	Poly-	1:1000		proteintech
IBA1	Total IBA1	Poly-		1:200	Wako
GFAP	Total GFAP	Mono-		1:200	Cell Signaling Technology
CBF	Total C/EBP ζ	Goat-	1:300		Santa Cruz Biotechnology
PIT1	Total PIT1	Poly-	1:300		Santa Cruz Biotechnology
SF1	Total SF1	Poly-	1:300		Santa Cruz Biotechnology
PLAG1	Total PLAG1	Poly-	1:1000		abcam
HNF1	Total HNF1	Poly-	1:300		Santa Cruz Biotechnology

HOXD9	total HOXD9	Poly-	1:300		Santa Cruz Biotechnology
VDR	Total Vitamin D Receptor	Poly-	1:500		abcam
SMUC	Total SMUC	Poly-	1:200		Santa Cruz Biotechnology
JAK1	Total JAK1	Poly-	1:1000		Cell Signaling Technology
p-JAK1	p-JAK1 at Tyr1022/1023	Poly-	1:300		abcam
JAK2	Total JAK2	Poly-	1:1000		Cell Signaling Technology
p-JAK2	p-JAK2 at Tyr1007/1008	Poly-	1:500		Cell Signaling Technology
JNK	Total JNK	Poly-	1:300		Santa Cruz Biotechnology
p-JNK	p-JNK at Tyr185	Poly-	1:500		Cell Signaling

					Technology
ERK	Total ERK	Poly-	1:100 0		Cell Signaling Technology
p-ERK	p-ERK at Thr202/Tyr204	Poly-	1:500		Cell Signaling Technology
AKT	Total AKT	Poly-	1:100 0		Cell Signaling Technology
p-AKT	p-AKT at Ser473	Poly-	1:500		Cell Signaling Technology
SGK1	Total SGK1	Poly-	1:100 0		abcam
p-SGK1	p-SGK1 at Ser78	Poly-	1:100 0		Cell Signaling Technology
P38	Total P38	Poly-	1:100 0		Cell Signaling Technology
p-P38	p-P38 at Thr180/Tyr182	Poly-	1:500		Cell Signaling

					Technology
Lamininβ1	Total Laminin β1	Poly-	1:100		Santa Cruz Biotechnology

Appendix Table S4 Human brain tissues used in the study

Case Number	Frozen Tissues Provided	Paraffin Sections Provided	Primary Neuropathologic Diagnosis	Secondary Neuropathologic Diagnosis	Age At Death	ApoE	Race/ Sex
E07-34	✓		AD-Braak VI	Microinfarct-Hp	72	E3/4	w/m
E11-97	✓		AD-Braak VI		74	E3/3	w/m
E12-06	✓		AD-Braak VI		68	E3/3	w/m
E16-66		✓	AD-Braak VI		69	unknow	w/m
E05-67		✓	AD-Braak VI		62	E3/4	w/m
E05-194		✓	AD-Braak VI	Infarct	71	E3/4	w/m
E06-18		✓	AD-Braak VI	TDP-43 Inclusions	81	E3/4	-/f
E06-61		✓	AD-Braak VI		86	E3/4	w/m
E08-112		✓	AD-Braak V-VI		77	E4/4	w/m
A87-50	✓		Control		66	E3/3	w/m
E04-34	✓		Control	Infarct	69	E3/3	b/f
E16-45	✓		Control	Braak I	70	unknow	b/m
E06-137		✓	Control	Infarct	92	E3/3	w/f
E09-170		✓	Control	Infarct	88	E2/3	w/f
E10-142		✓	Control	Microinfarct-Hp	94	E3/3	w/m
E13-27		✓	Control	NFT-Braak III	91	E3/3	w/f
E14-06		✓	Control		56	unknow	w/m