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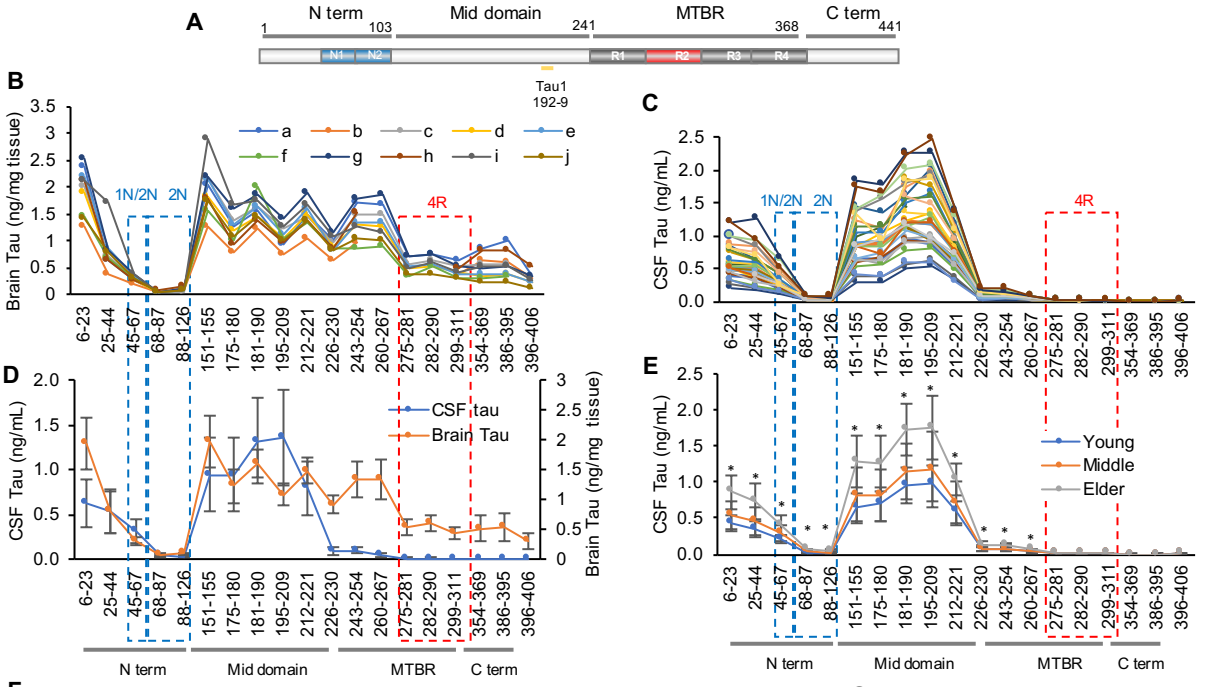
Supplemental Information

Tau Kinetics in Neurons and the Human Central Nervous System

Chihiro Sato, Nicolas R. Barthélemy, Kwasi G. Mawuenyega, Bruce W. Patterson, Brian A. Gordon, Jennifer Jockel-Balsarotti, Melissa Sullivan, Matthew J. Crisp, Tom Kasten, Kristopher M. Kirmess, Nicholas M. Kanaan, Kevin E. Yarasheski, Alaina Baker-Nigh, Tammie L.S. Benzinger, Timothy M. Miller, Celeste M. Karch, and Randall J. Bateman



F

Participant #	Brain region	Thal	Braak	CERAD	Neuropath diagnosis	PMI	RIN	Age at death	Sex
a	Frontal	1	2	0	Normal Brain	5	9	108	F
b	Frontal	3	5	3	AD	14.5	9	75	F
c	Frontal	0	4	0	Other	5	7	91	F
d	Frontal	3	1	0	Normal Brain	5	7	93	F
e	Frontal	N/A	1	4	AD	7	7	73	M
f	Frontal	2	2	0	N/A	9.1	7	96	F
g	Frontal	1	4	0	Other	9.5	7	99	F
h	Occipital	N/A	N/A	N/A	N/A	10.1	7	86	M
i	Frontal	N/A	2	4	N/A	16	7	70	F
j	Frontal	3	3	1	Probable AD	12.5	5	79	F
Mean	-	-	-	-	-	9.3	7.2	87.1	8F/
SD	-	-	-	-	-	3.9	1.1	12.4	2M

G

		Age			Sex
Group	N	Mean	SD	Range	(F/M)
Young	8	30.4	4.1	25-39	5/3
Middle	10	52.7	3.5	40-60	7/3
Elder	14	77.2	5.0	61-87	7/7
Total	32	57.8	19.8	25-87	19/13

Figure S1. Tau Profiles in 10 Human Brains and CSF from 29 controls. Related to Figure 1. (A) Schematic of the longest tau isoform (2N4R), fragments detected by MS, and epitopes of Tau1 antibody. Absolute quantitation of tau peptides in 10 human brains (B), CSF from 29 normal controls (C), and comparison of the average of each (D). (E) CSF tau profiles from Young (age 25-39), Middle (age 40-60), and Elder (age 61-87) populations suggest that CSF tau is significantly higher in Elder compared with Middle (p=0.11, F=9.28, Df=26) and Young (p=0.0012, F=9.28, Df=26). The relative abundance of tau peptides remains similar. *p<0.05 for Elder vs Young, and Elder vs Middle. Significance was determined via one-way ANOVA with

Tukey posthoc test. Data are represented as mean ± SD. (F) Thal, Braak, and CERAD staging were used to assess amyloid deposition, neurofibrillary tangle, and neuritic plaque score, respectively. PMI, post mortem interval. RIN, RNA integrity number. (G) Summary of CSF used in (C) and (E).

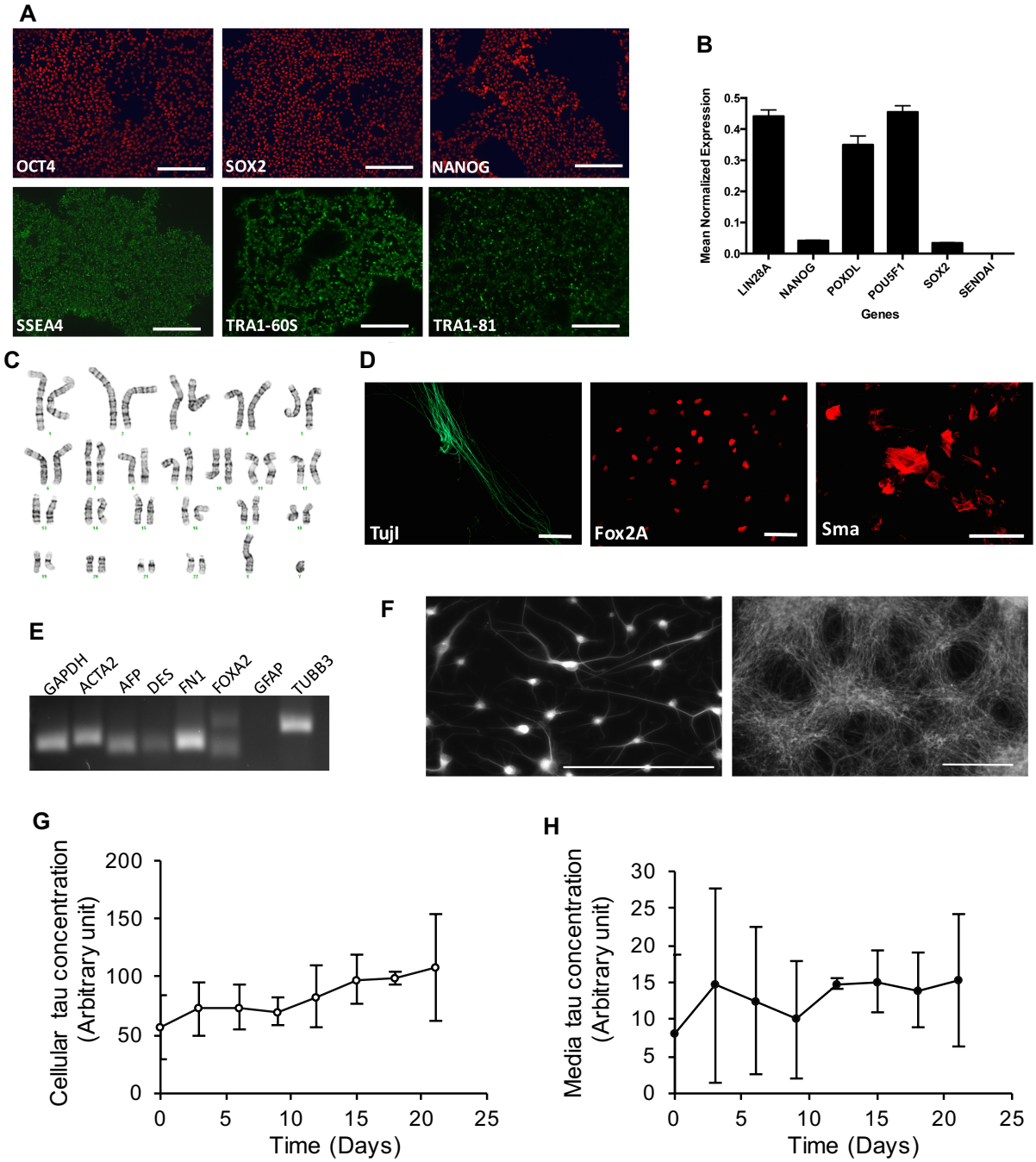

A
OCT4
SOX2
NANOG
SSEA4
TRA1-60S
TRA1-81
B
Mean Normalized Expression
0.5
0.4
0.3
0.2
0.1
0.0
LIN28A
NANOG
POXDL
POU5F1
SOX2
SENDAI
Genes
C
D
Tujl
Fox2A
Sma
E
GAPDH
ACTA2
AFP
DES
FN1
FOXA2
GFAP
TUBB3
F
G
Cellular tau concentration (Arbitrary unit)
200
150
100
50
0
0
5
10
15
20
25
Time (Days)
H
Media tau concentration (Arbitrary unit)
30
25
20
15
10
5
0
0
5
10
15
20
25
Time (Days)

Figure S2. Characterization of iPSCs and iPSC-derived neurons. Related to Figure 1, 2. Human dermal fibroblasts were reprogrammed into iPSCs using non-integrating Sendai virus. (A) iPSCs express markers of pluripotency. (B) Quantitative PCR. Probes specific to Sendai virus-driven pluripotency genes demonstrate that Sendai virus has been diluted out of the clones. (C) Normal karyotype for iPSCs. iPSCs were cultured in DMEM/F12 supplemented with FBS to form embryoid bodies. Embryoid bodies were then plated and analyzed by immunocytochemistry (D) and qPCR (E) to demonstrate that the iPSC can spontaneously form cells from any of the three germ layers. iPSC-derived neurons express markers of neuronal cell fate and produce steady-state levels of tau (F-H). iPSC-derived neurons were stained with TujI. (F, left) 20x magnification. (F, right) 10x magnification. Absolute quantitation of tau using mass spectrometry in the cells (G) and media (H) from iPSC-derived neurons during 21 days of labeling starting at 4 weeks in culture. Data are represented as mean ± SD.

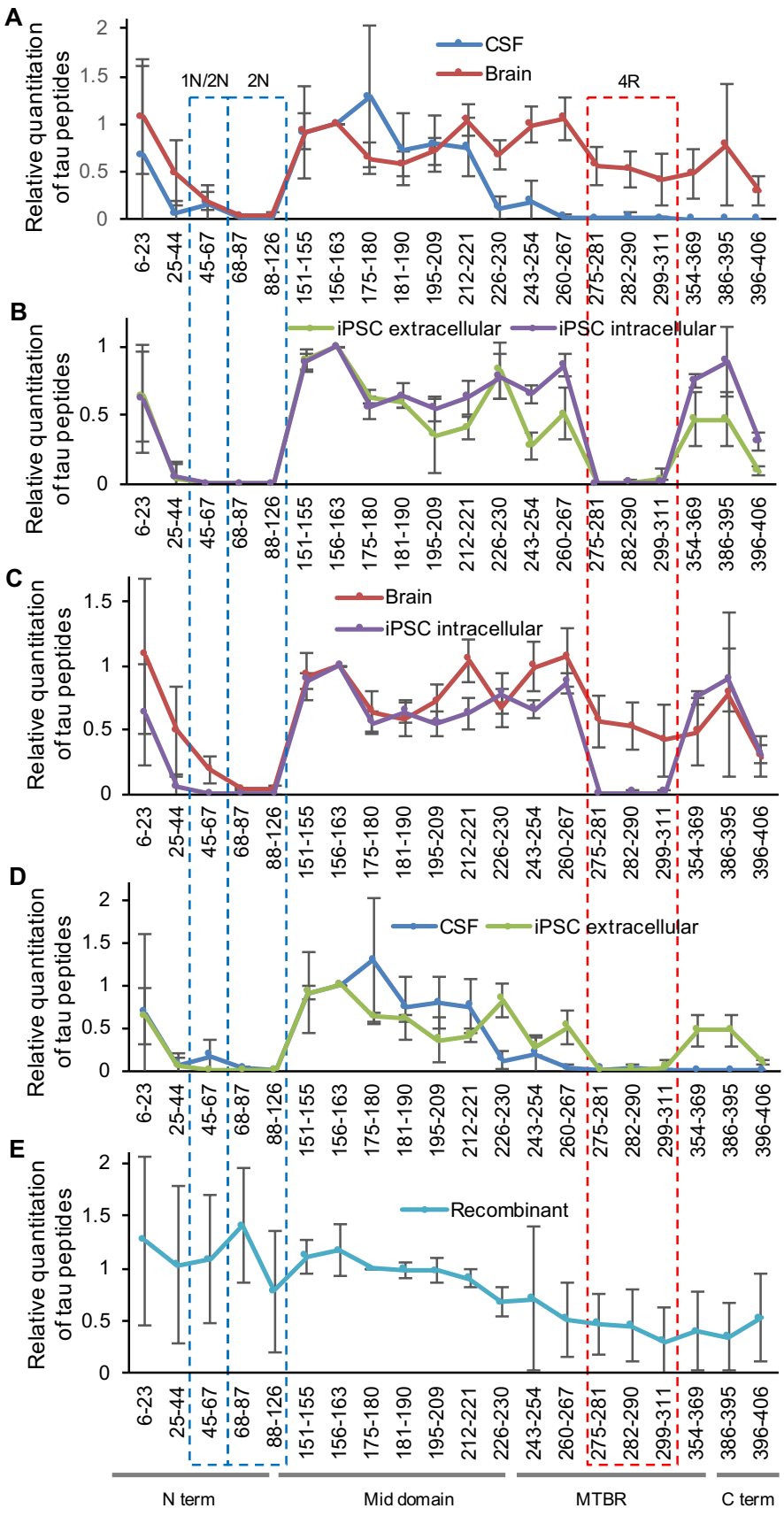

A
Relative quantitation of tau peptides
CSF
Brain
1N/2N
2N
4R
B
iPSC extracellular
iPSC intracellular
C
Brain
iPSC intracellular
D
CSF
iPSC extracellular
E
Recombinant
6-23
25-44
45-67
68-87
88-126
151-155
156-163
175-180
181-190
195-209
212-221
226-230
243-254
260-267
275-281
282-290
299-311
354-369
386-395
396-406
N term
Mid domain
MTBR
C term

Figure S3. Relative quantification of tau in the human brain, CSF, and iPSC-derived neurons. Related to Figure 1. Analyses of tau using the merged results from the eight different immunopurification averaged and normalized by the signal from peptide 156-163. Comparison of tau profiles between (A) human brain vs CSF, (B) intracellular vs extracellular tau from iPSC-derived neurons, (C) human brain vs intracellular tau from iPSC-derived neurons, and (D) CSF tau vs extracellular tau from iPSC-derived neurons are shown. (E) Relative amount of recombinant tau recovered from tau antibodies reflects immunoprecipitation efficiencies and mass spectrometry quality within the domains defined. Data are represented as mean ± SD.

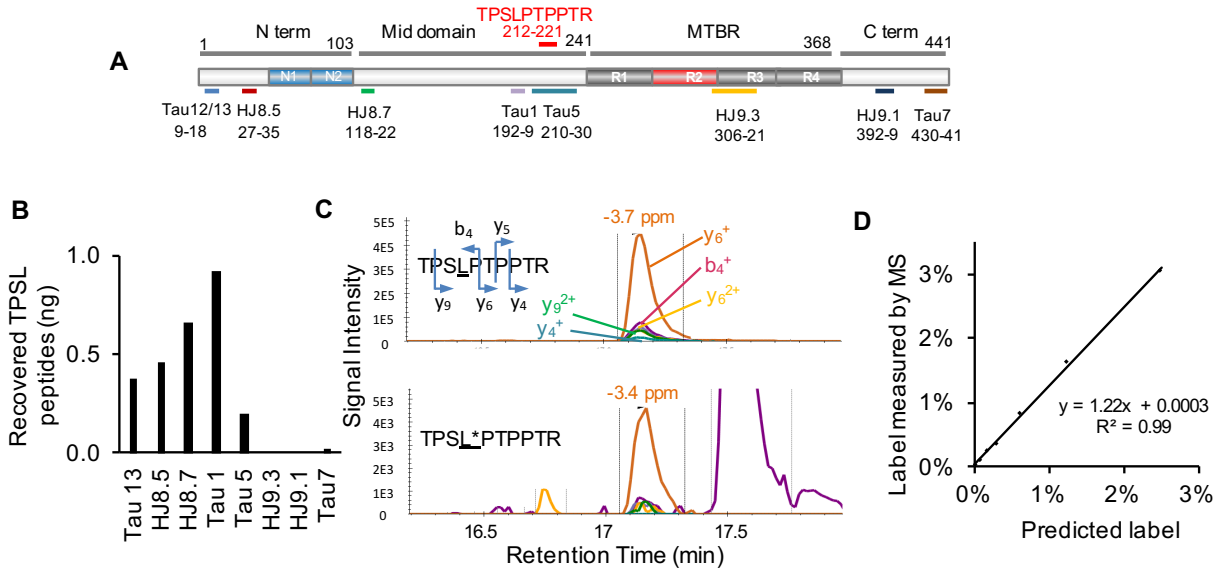


Figure S4. Tau SILK method development. Related to Figure 2, 3. (A) Schematic of 2N4R full length tau, epitopes of Tau antibodies, and TPSLPTTPR peptide. (B) IP/MS efficiencies of Tau antibodies in the normal control CSF assessed by mass spectrometry. (C) Examples of LC-MS/MS signals from TPSLPTPPTR (unlabeled, top) and TPSL*PTPPTR (labeled, bottom) peptide obtained from $^{13}C_6$-leucine labeled samples. Approximately 1% of total tau is labeled. (D) Standard curve of TPSLPTPPTR peptides showing the limit of quantitation of 0.1%.

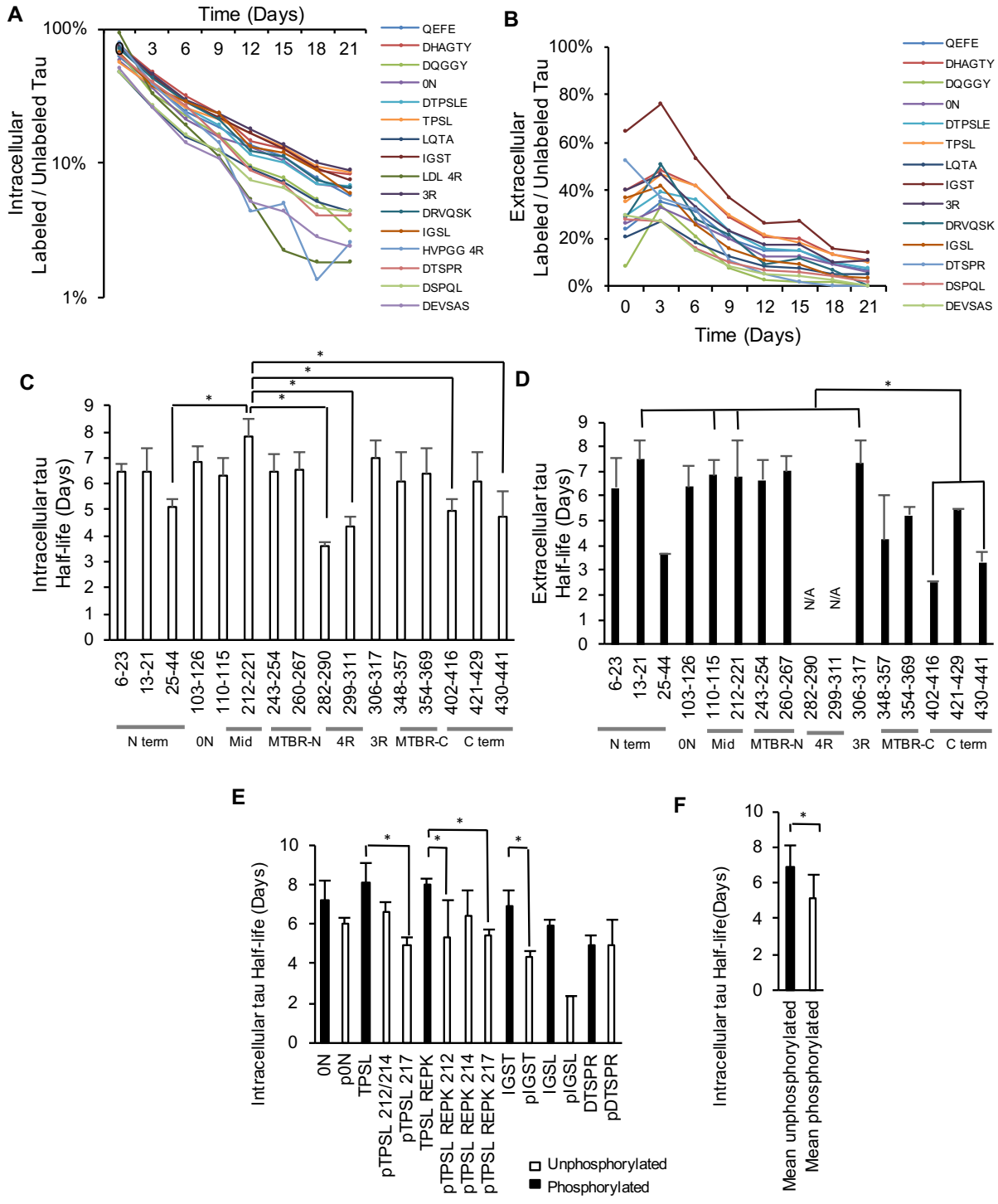
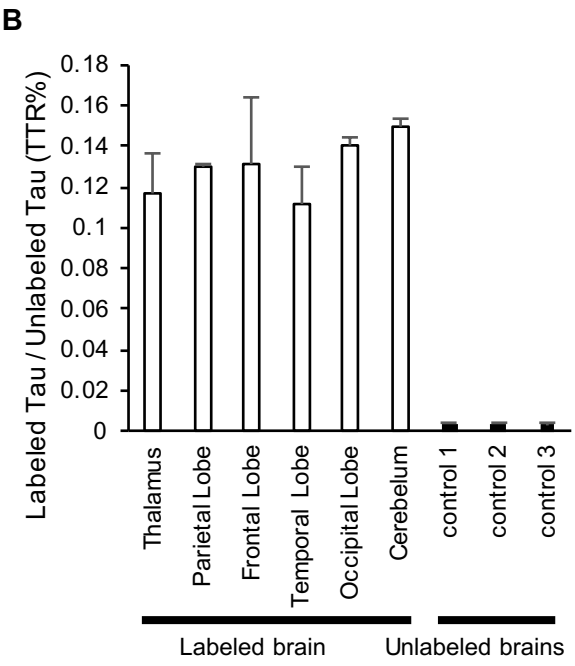
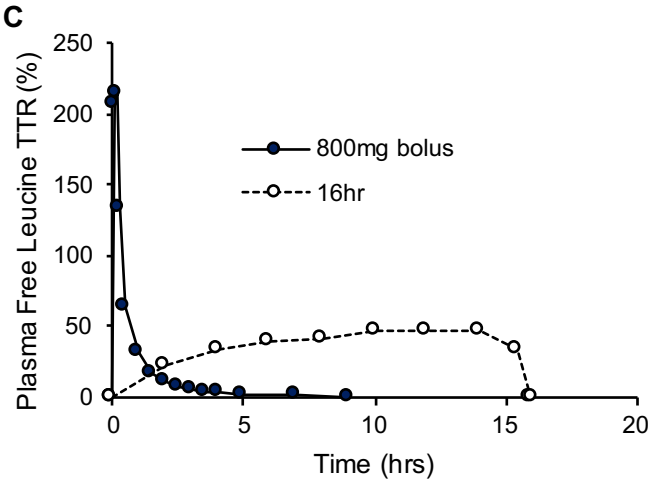

A
Time (Days)
Intracellular Labeled / Unlabeled Tau
100%
10%
1%
0 3 6 9 12 15 18 21
QEFE
DHAGTY
DQGGY
0N
DTPSLE
TPSL
LQTA
IGST
LDL 4R
3R
DRVQSK
IGSL
HVPGG 4R
DTSPR
DSPQL
DEVSAS
B
Extracellular Labeled / Unlabeled Tau
100%
80%
60%
40%
20%
0%
0 3 6 9 12 15 18 21
Time (Days)
QEFE
DHAGTY
DQGGY
0N
DTPSLE
TPSL
LQTA
IGST
3R
DRVQSK
IGSL
DTSPR
DSPQL
DEVSAS
C
Intracellular tau Half-life (Days)
0 1 2 3 4 5 6 7 8 9
6-23 13-21 25-44 103-126 110-115 212-221 243-254 260-267 282-290 299-311 306-317 348-357 354-369 402-416 421-429 430-441
N term 0N Mid MTBR-N 4R 3R MTBR-C C term
D
Extracellular tau Half-life (Days)
0 1 2 3 4 5 6 7 8 9
N/A
N/A
6-23 13-21 25-44 103-126 110-115 212-221 243-254 260-267 282-290 299-311 306-317 348-357 354-369 402-416 421-429 430-441
N term 0N Mid MTBR-N 4R 3R MTBR-C C term
E
Intracellular tau Half-life (Days)
0 2 4 6 8 10
0N p0N TPSL pTPSL 212/214 pTPSL 217 TPSL REPK pTPSL REPK 212 pTPSL REPK 214 pTPSL REPK 217 IGST pIGST IGSL pIGSL DTSPR pDTSPR
Unphosphorylated
Phosphorylated
F
Intracellular tau Half-life(Days)
0 2 4 6 8 10
Mean unphosphorylated
Mean phosphorylated

Figure S5. Tau Kinetics in iPSC-derived neurons. Related to Figure 2, Table 1, 2. SILK curves and half-lives of tau peptides of iPSC-derived neurons in the cells (A, C; intracellular) and in the media (B, D; extracellular), respectively. See **Tables 1 and 2** for peptide abbreviations. (C) In intracellular fraction, DQGGYT peptides (residue 25-44), 4R specific peptides (residues 282-290 and 299-304), and C term peptides (residues 402-407 and 430-441) were significantly shorter than TPSLPTPPTR peptides (residue 212-221) (*p≤0.05, F=5.455, Df=29). (D) In extracellular tau, C term peptides (residues 402-416 and 430-441) were significantly shorter than N term to MTBR-C peptides (residues 13-21, 110-115, 212-221, and 306-317 (*p ≤0.05, F=4.596, Df=22)). (E-F) Graph summarizing the half-lives of phosphorylated and unphosphorylated tau peptides. Phosphorylated tau peptides have significantly shorter half-lives than their unphosphorylated pair. TPSL p217 (p=0.0057, F=8.5, Df=12), TPSL REPK p212 (p=0.029, F=4.6, Df=15), TPSL REPK p217 (p=0.036, F=4.6, Df=15), and pIGST (p=0.018, F=14.7, Df=5). Significance was determined via one-way ANOVA with Tukey posthoc test. Data are represented as mean ± SD.

A

$_{13}C_6$-leucine	Brain region	TTR (%)
Labeled	Thalamus	0.117
Labeled	Parietal Lobe	0.130
Labeled	Frontal Lobe	0.132
Labeled	Temporal Lobe	0.112
Labeled	Occipital Lobe	0.141
Labeled	Cerebellum	0.150
Unlabeled 1	N/A	0.004
Unlabeled 2	N/A	0.004
Unlabeled 3	N/A	0.004
Mean labeled		0.130
SD labeled		0.014





D

Infusion method	N	Mean AUC	SD
800mg bolus	20	118.6	24.6
16hr, 4mg/kg/hr	2	537.2	64.8

Figure S6. Labeling of Tau in the Human Brain Related to Figure 3. (A) Summary table describing the labeling (TTR%) of three control brains and a brain donated by a participant who had 800mg bolus infusion eight days before passing away. (B) Quantitation of (A). Data are represented as mean ± SD. (C) Examples of plasma free leucine curves obtained from 800mg bolus infusion and 16hr 4mg/kg/hr infusion. (D) Summary of Area Under the Curve (AUC) obtained from plasma free leucine measurements from 20 participants who received 800mg bolus infusion and 2 participants who received 16hr infusion. Plasma leucine from the participant who passed away 8 days from 800mg bolus infusion was not available. Thus, average AUC from 20 participants were used for normalization in **Figure 3G**.

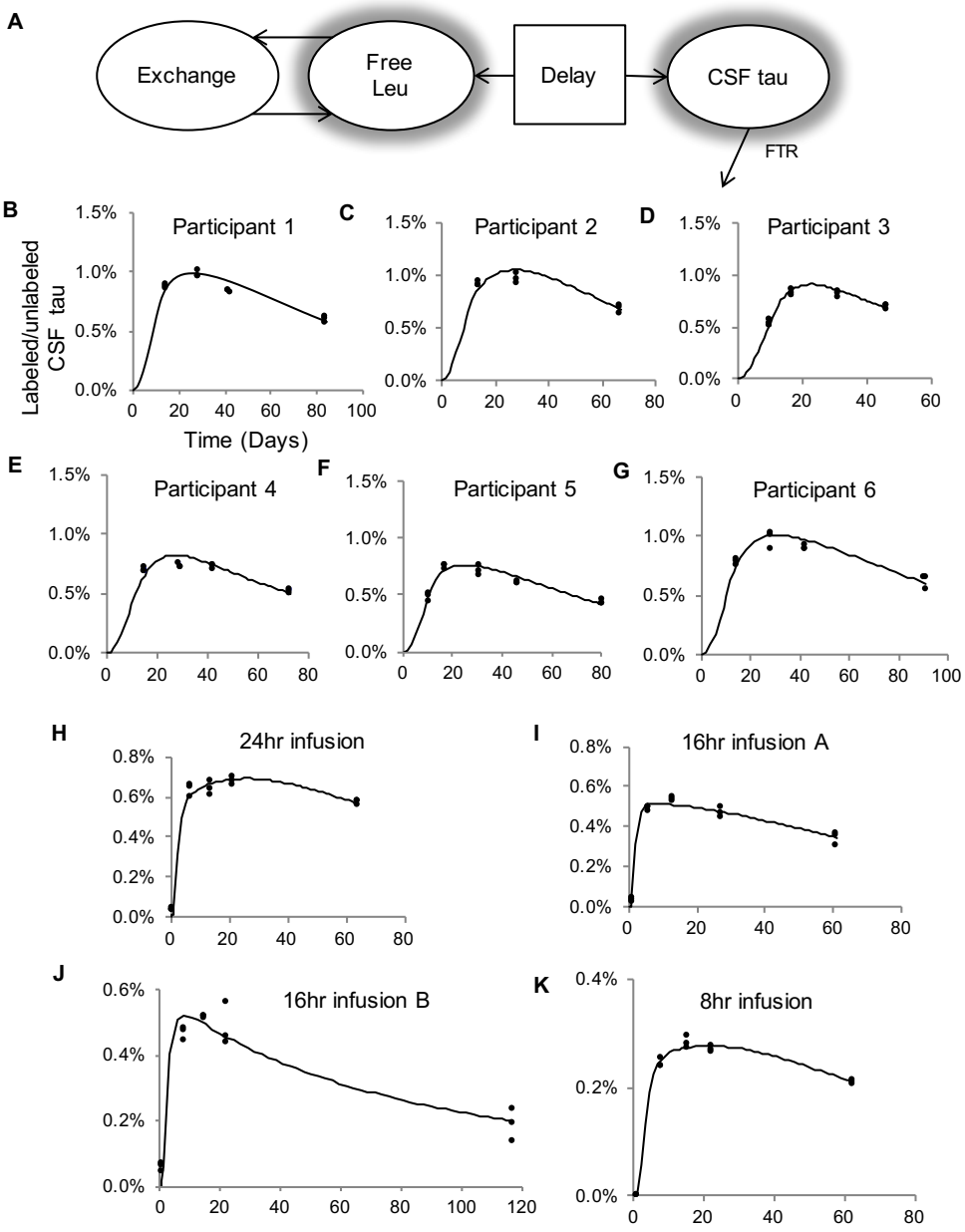


Figure S7. Compartmental modeling of $^{13}C_6$-leucine labeling in healthy participants. Related to Figure 3. (A) The model consists of a 2-compartment system to represent the free $^{13}C_6$-leucine enrichment time course, a delay element, and CSF tau. The fractional turnover rate (FTR) of tau is optimized to fit the shape of the CSF tau enrichment time course. The model fits to the labeling of CSF tau of the six orally labeled participants (B-G) and four infusion participants (H-K).

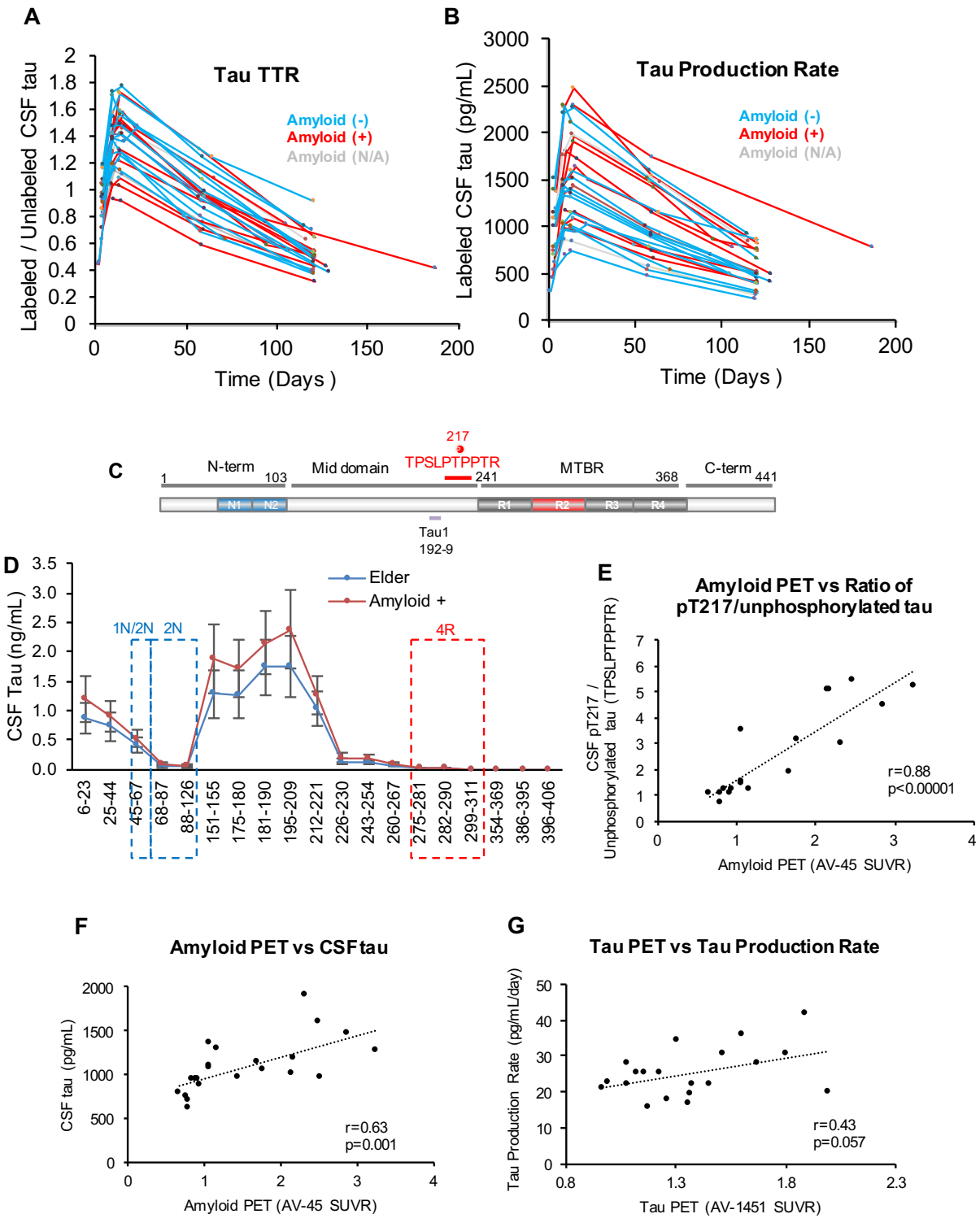

A
Tau TTR
Amyloid (-)
Amyloid (+)
Amyloid (N/A)
Labeled / Unlabeled CSF tau
Time (Days)
B
Tau Production Rate
Amyloid (-)
Amyloid (+)
Amyloid (N/A)
Labeled CSF tau (pg/mL)
Time (Days)
C
217
N-term
1
103
Mid domain
TPSLPTPPTR
241
MTBR
368
C-term
441
N1
N2
R1
R2
R3
R4
Tau1
192-9
D
Elder
Amyloid +
1N/2N
2N
4R
CSF Tau (ng/mL)
6-23
25-44
45-67
68-87
88-126
151-155
175-180
181-190
195-209
212-221
226-230
243-254
260-267
275-281
282-290
299-311
354-369
386-395
396-406
E
Amyloid PET vs Ratio of pT217/unphosphorylated tau
CSF pT217 / Unphosphorylated tau (TPSLPTPPTR)
r=0.88
p<0.00001
Amyloid PET (AV-45 SUVR)
F
Amyloid PET vs CSF tau
CSF tau (pg/mL)
r=0.63
p=0.001
Amyloid PET (AV-45 SUVR)
G
Tau PET vs Tau Production Rate
Tau Production Rate (pg/mL/day)
r=0.43
p=0.057
Tau PET (AV-1451 SUVR)

Figure S8. Tau kinetics curves from 24 participants with and without Alzheimer's pathology. Related to Figure 4. (A) Tau kinetics curves shown by the ratio of labeled/unlabeled tau over time, normalized by the amount of leucine received (plasma free leucine). (B) Absolute concentration of labeled CSF tau over time. Cut-off of amyloid PET (AV-45 SUVR) = 1.22 was used to determine amyloid positivity. (C) Schematic of the longest tau isoform (2N4R), the epitope of Tau1 antibody, TPSLPTTPR peptide and phosphorylation at T217. (D) CSF tau profile is similar across the protein in amyloid positive (+) and negative Elder group (Age 61-87). Data are represented as mean ± SD. (E) The ratio of phosphorylated tau at T217 (pT217) and unphosphorylated tau (TPSLPTPPTR peptide) positively correlates with amyloid PET. (F) The correlation between pT217/tau ratio and amyloid PET (r=0.88) is greater than that of CSF tau absolute concentration and amyloid PET (r=0.63). (G) Tau PET positively correlates with Tau production rate. Spearman correlation (r and p value) was calculated to determine each association.