Supporting Information

Hyperacetylation mimetics within the tau filament core inhibits prion-like propagation of misfolded tau

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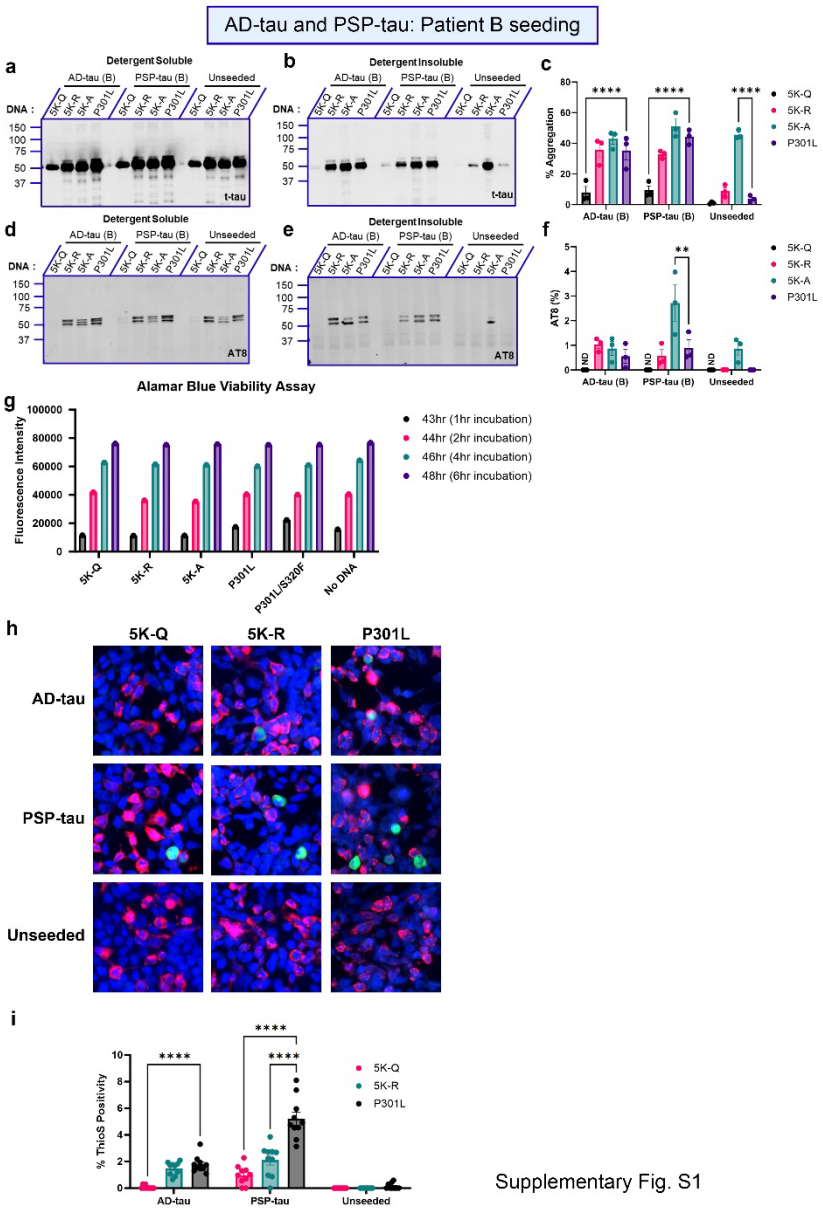
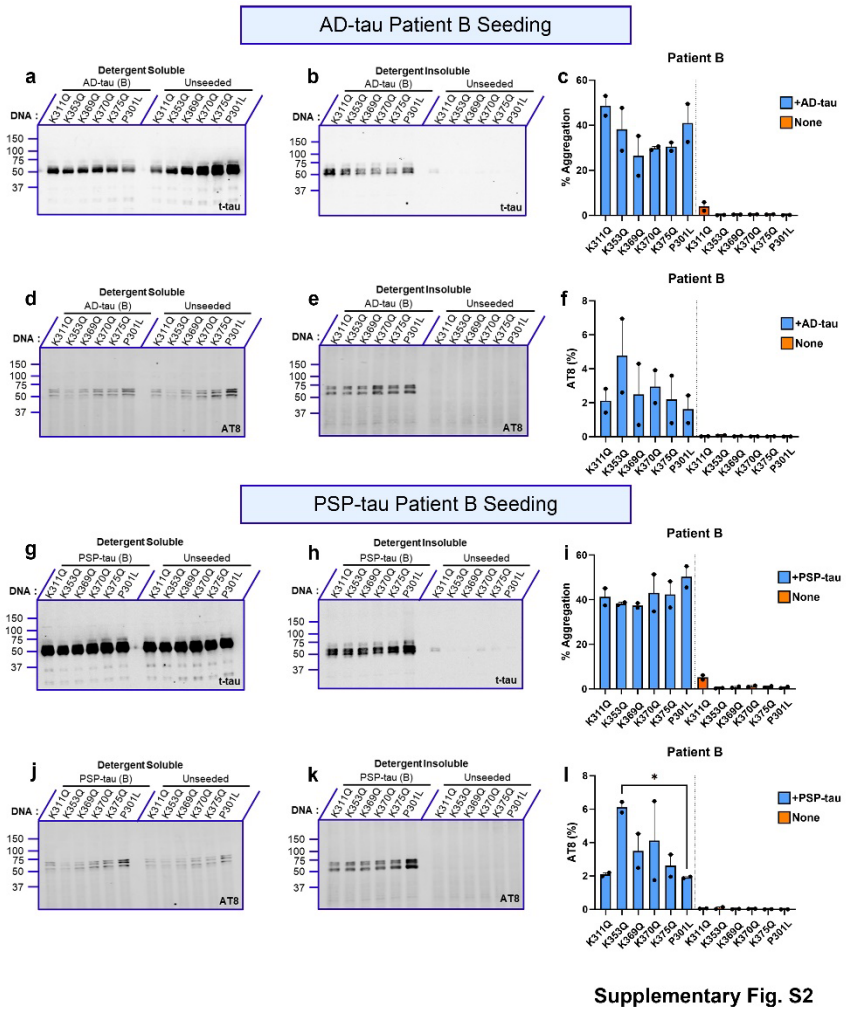


Supplementary Fig. S1

Figure S1: Comparative effect of combinatorial acetyl variants in the tau core domain on seed-induced P301L tau aggregation. a-f. HEK293T cell seeding assay using the acetyl variants seeded with AD-tau seeds or PSP-tau seeds. Samples were fractionated into detergent-soluble and detergent-insoluble lysates and probed for total tau (t-tau) and p-tau (AT8). Representative immunoblot and % Aggregation of acetyl-tau variants seeded with AD:Patient B tau seeds or PSP:Patient B tau seeds. N=2 for each experimental replicate. g. AlamarBlue cell viability was conducted on cells transfected with different plasmids. Measurements were taken at 4 different time points. h-i. Immunofluorescence of HEK293T expressing 5K-Q, 5K-R, and P301L tau, seeded with either AD-tau, PSP-tau, or unseeded were probed with ThioS (green) and total

tau (red) and DAPI (blue) for nuclei. Quantification of immunofluorescent images as a ratio of ThioS-positive cells to tau-positive cells calculated from 10 individual fields of view for each group (i). 2-way ANOVA with Dunnett's multiple comparisons test, with single pooled variance. ****$p<0.0001$.



Supplementary Fig. S2

Figure S2: Comparative profiles of individual pseudo-acetyl tau variants seeded with AD-tau and PSP-tau seeds. a-f. Representative immunoblots and quantitation depicting % tau aggregation (a-c) and AT8 insolubility index (d-f) of individual acetyl-mimetic tau seeded with AD:Patient B tau seeds. g-l. Representative immunoblots and quantitation depicting % tau aggregation (g-i) and AT8 insolubility index (j-l) of individual acetyl-mimetic tau seeded with PSP:Patient B tau seeds. Relative molecular masses (kDa) are indicated on the left of each blot. N=2 for each experimental replicate. 1-way ANOVA with Sidak's multiple comparisons test, with single pooled variance. *p<0.05.

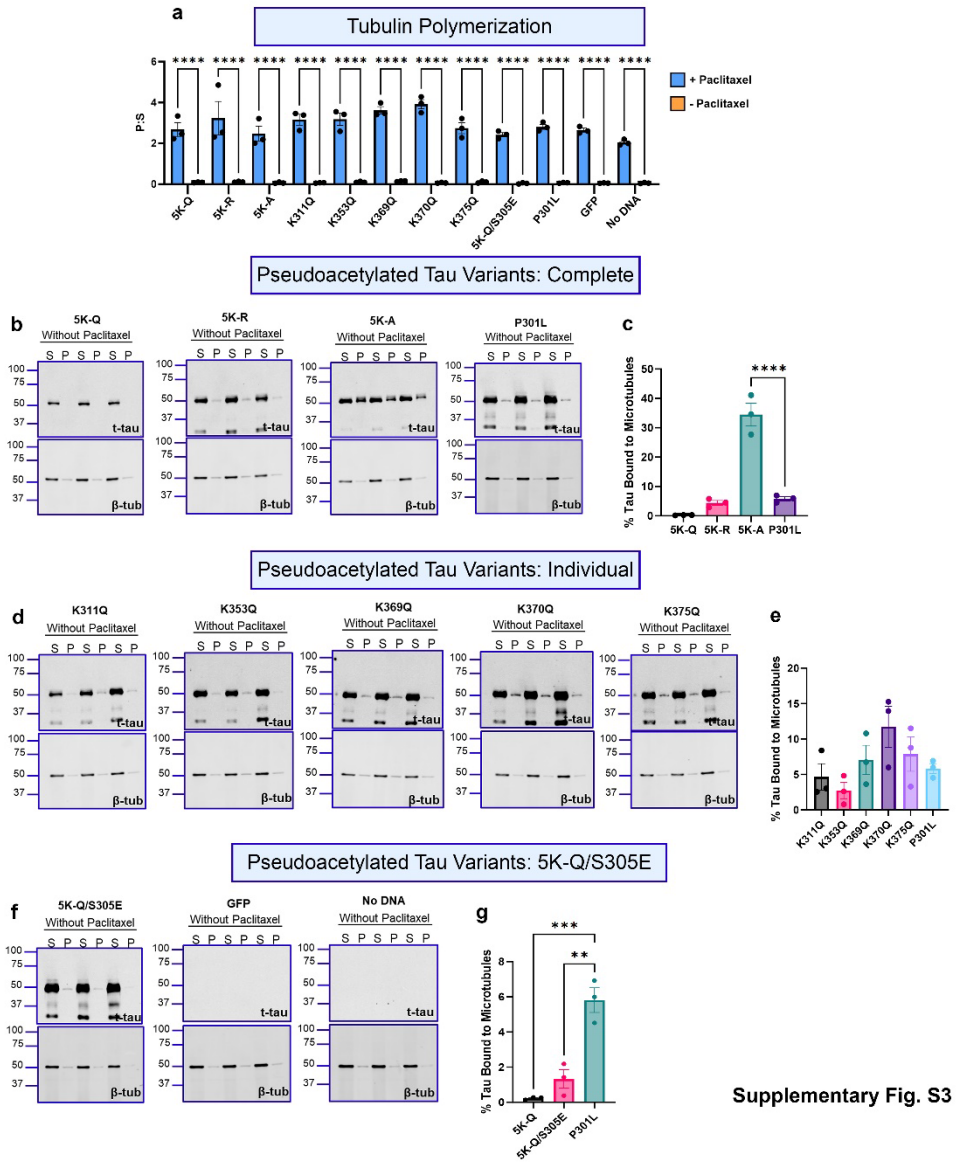
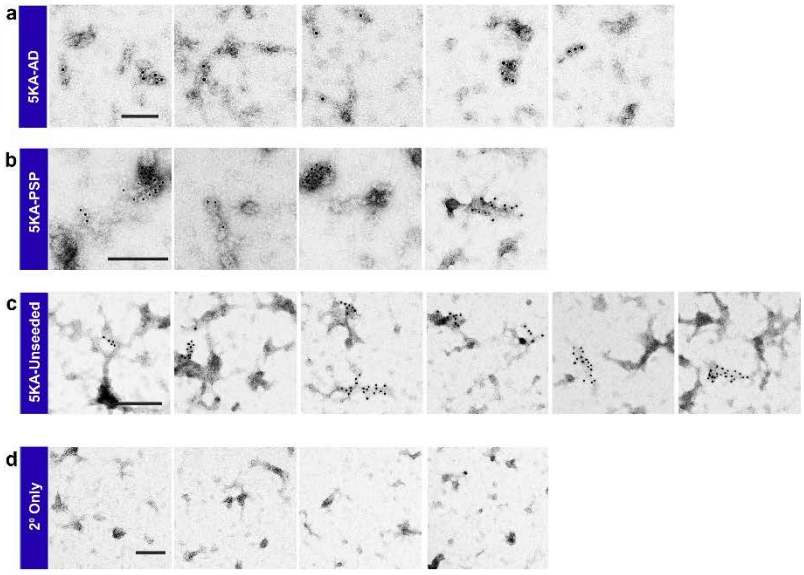


Figure S3: Microtubule binding of acetyl-substituted tau variants in the absence of paclitaxel. HEK293T cells were transfected with acetyl-substituted tau variants as indicated and cell lysates used for microtubule binding assay. a. Quantification of the tubulin polymerization efficiency for conditions treated with and without Paclitaxel. b-c. Quantification of microtubule-binding of 5K-Q, 5K-R, 5K-A compared to parent P301L tau. d-e. Quantification of microtubule-binding of K311Q, K353Q, K369Q, K370Q, K375Q compared to parent P301L tau. f-g. Quantification of microtubule-binding for 5K-Q/S305E, 5K-Q and P301L tau. 1-way ANOVA with Dunnett's multiple comparisons test, with single pooled variance. **p<0.01, ***p<0.001, ****p<0.0001. S= supernatant fraction; P= pellet fraction.



Supplementary Fig. S4

Figure S4: **Additional immuno-EM images for 5K-A tau filaments**. a-c. Representative immuno-EM images of detergent-insoluble 5K-A filaments from HEK293T cells that were seeded with AD-tau (a), PSP-tau (b) or left unseeded (c). d. Representative Immuno-EM images of 5K-A filaments exposed to secondary antibody only. All filaments were stained with 10nm gold conjugated secondary antibody followed by negative staining with 1% uranyl acetate.

Supplementary Table Legends.

Supplementary Table S1. Demographics and diagnoses of the AD and PSP brain donors.

Individually characterized brains in the University of Florida Neuromedicine Human Brain and Tissue Bank were used in the study. Experimental Group denotes the designation for this study. NP Dx1, primary diagnosis based on neuropathology; NP Dx2 and NP Dx3, secondary diagnoses based on neuropathology; Thal phase, burden of immunostained amyloid deposits in cortical and subcortical area; Braak stage, CERAD score, neuritic plaque frequency; AD, Alzheimer's disease; ARTAG, Aging-related tau astrogliopathy; CAA, cerebral amyloid angiopathy; CVD, cardiovascular disease; HC, healthy control with no dementia; LATE, limbic-predominant age-related TDP-43 encephalopathy; PSP, Progressive supranuclear Palsy. N/A: Not applicable; ND, not done.

Supplementary Table S2. Antibodies used in this study.

Table S1. Demographics and diagnoses of the patient cohorts used in this study. Case IDs correspond to individually characterized brains in the University of Florida Neuromedicine Human Brain and Tissue Bank. Experimental Group denotes the designation for this study. NP Dx1, primary diagnosis based on neuropathology; NP Dx2 and NP Dx3, secondary diagnoses based on neuropathology; Thal phase, burden of immunostained amyloid deposits in cortical and subcortical area; Braak stage, CERAD score, neuritic plaque frequency; AD, Alzheimer's disease; ARTAG, Aging-related tau astrogliopathy; CAA, cerebral amyloid angiopathy; CVD, cardiovascular disease; HC, healthy control with no dementia; LATE, limbic-predominant age-related TDP-43 encephalopathy. N/A: Not applicable; ND, not done.

Experimental Group	Post-mortem Diagnosis	Case ID	NP Dx1	NP Dx2	NP Dx3	Thal stage	Braak stage	CERAD score	APOE genotype	Sex	Age	PMI
AD-A	AD	A19-015	AD high	CAA widespread, mild		4	V	frequent	3/4	m	77	5
AD-B	AD	A19-027	AD high	CAA widespread, moderate		5	VI	frequent	3/4	m	63	2
PSP-A	PSP	A22-040	PSP	AD low	CAA focal, mild to moderate	3	I	none		m	70	19.5
PSP-B	PSP	A21-041	PSP	AD intermediate	CAA widespread, severe	5	III	moderate	4/4	m	72	27

Table S2. Antibodies used in this study

Antibody name	Specificity	Host Species	Dilutions	Source
Recombinant anti-tau	Mouse and human tau	Rabbit monoclonal	1:10000 (WB)	Abcam Cat# ab254256, RRID:AB_2894402
CP27	human tau (130-150)	Mouse monoclonal	1:500 (EM)	Gift from Dr. Peter Davies (Duff et al., 2000; RRID:AB_2716722)
CP13	pSer202	Mouse monoclonal	1:1000 (WB)	Gift from Dr. Peter Davies (Weaver et al., 2000; RRID:AB_2314223)
PHF1	pSer396/pSer404	Mouse monoclonal	1:1000 (WB)	Gift from Dr. Peter Davies (Greenberg et al., 1992; RRID:AB_2313687)
AT8	pSer202/pT205	Mouse monoclonal	1:1000 (WB)	Thermo Fisher Scientific Cat#MN1020, RRID:AB_223647
AT180	pThr231	Mouse monoclonal	1:1000 (WB)	Thermo Fisher Scientific Cat# MN1040, RRID:AB_223649
AT270	pThr181	Mouse monoclonal	1:1000 (WB)	Thermo Fisher Scientific Cat# MN1060, RRID:AB_223652
AC-15	Actin	Mouse monoclonal	1:1000 (WB)	(Abcam Cat# ab6276, RRID:AB_2223210)
TUB 2.1	β-tubulin	Mouse monoclonal	1:1000 (WB)	(Sigma-Aldrich Cat# T4026, RRID:AB_477577)