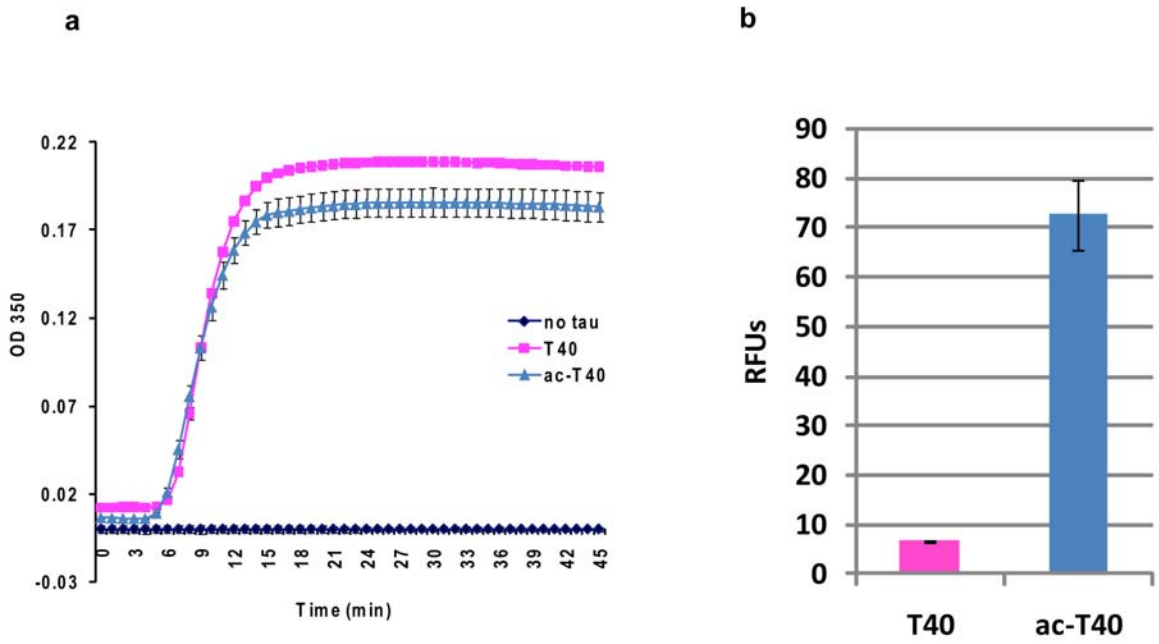
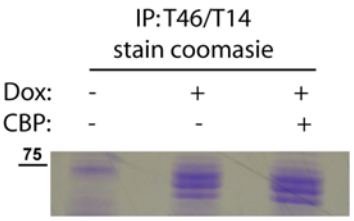


Supplementary Figure S1: Tau T40 acetylation impairs MT assembly and promotes PHF aggregation *in vitro*.

a) MT assembly activities of either unmodified or acetylated T40 proteins were evaluated in light scattering assays similar to that shown in Figure 1. 30 μM tubulin monomers were mixed with 15 μM tau proteins in MT assembly (RA) buffer supplemented with 2 mM guanosine triphosphate (GTP). MT assembly was determined by monitoring absorbance every minute at 350 nm using a SpectraMax plate reader. **b**) 15 μM tau proteins were evaluated in fibrillization reactions using 10 μM heparin to induce assembly for 3 days, at which point samples were incubated with 12.5 μM ThT. These results were confirmed from n=4 independent experiments. Error bars indicate standard error of the mean (SEM).

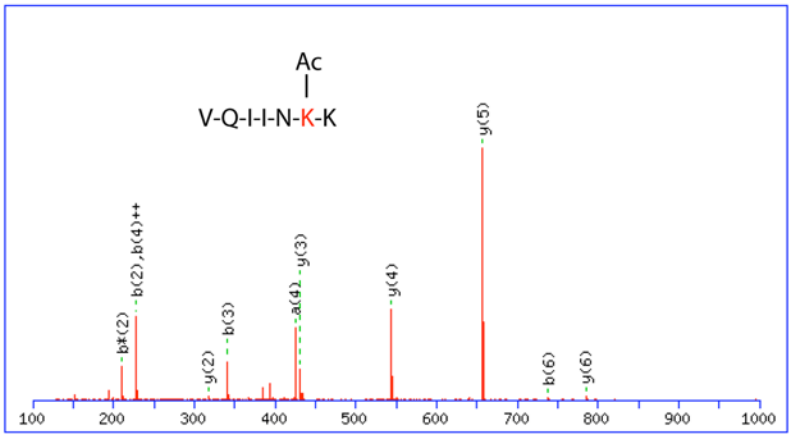
a



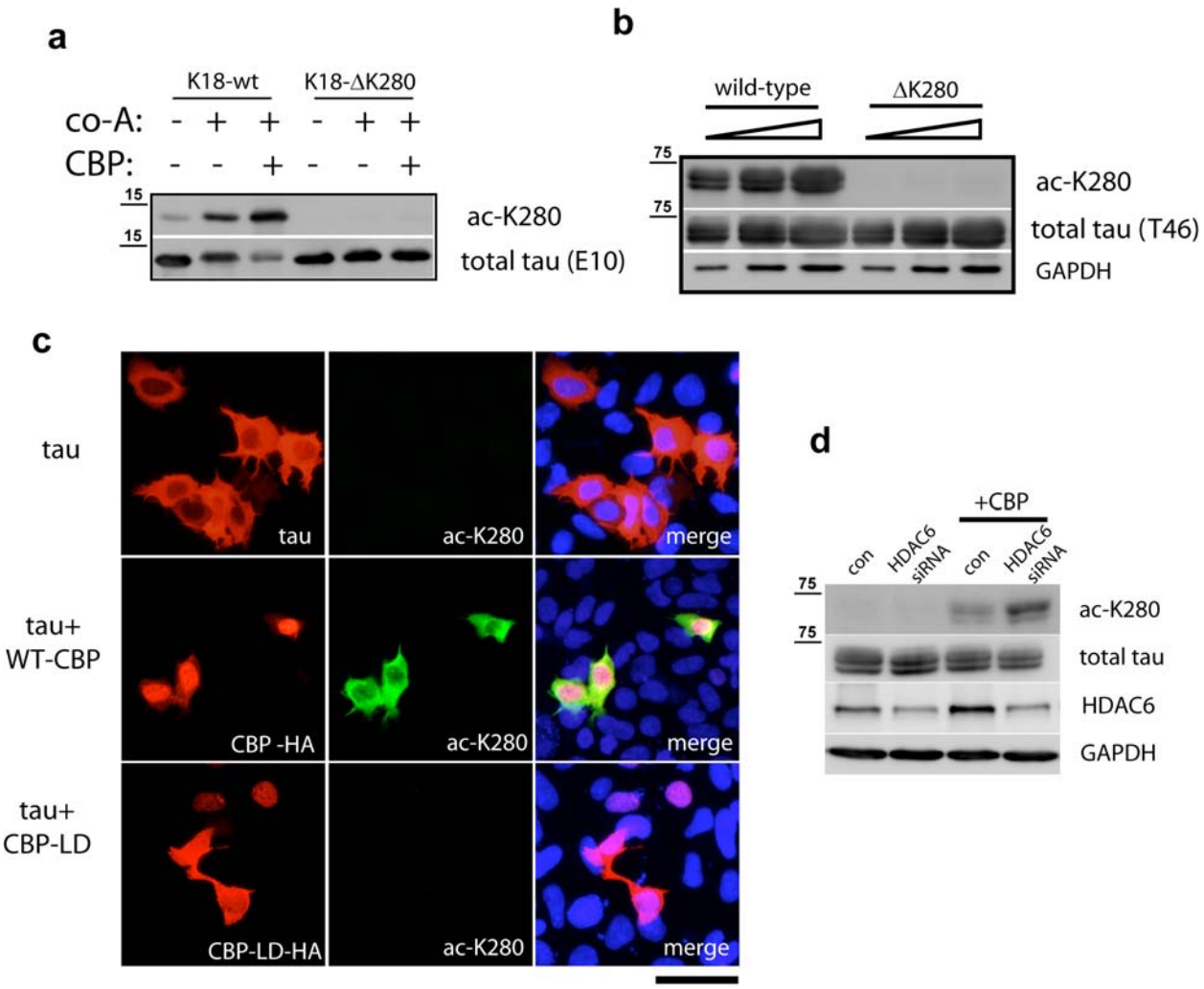
b

peptide sequence	peptide lysine	tau residue #	Ions score (>40 sig.)	Expect (p-value)
GAAPPGQKGQANATR	K8	K163	93	4.50E-07
VQIINKK	K6	K280	44	0.024
VQIINKK	K7	K281	40	0.05
IGSLDNITHVPGGGNKK	K16	K369	45	0.033

c

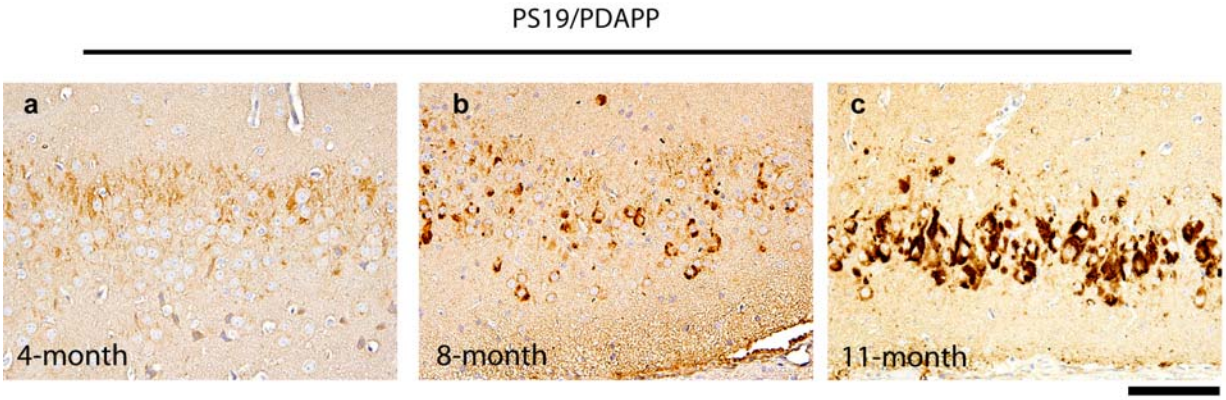


Supplementary Figure S2: Mass spectrometry analysis identified K280 as a tau acetylation site in HEK-T40 cells. **a**) HEK-T40 cells treated with Dox were transfected with vector alone or CBP and T40 was immunoprecipitated with T14/T46 antibodies and was separated by SDS-PAGE followed by gel excision and mass spectrometry analysis. In the absence of CBP, no acetylation was detected. In the presence of CBP, acetylation was detected on the four indicated lysine residues with significant ion scores and p-values shown in (**b**). Acetylated K280 was detected on the peptide sequence VQIINKK. The corresponding m/z spectrum is shown in (**c**) and ion scores are represented in Supplementary table S2.



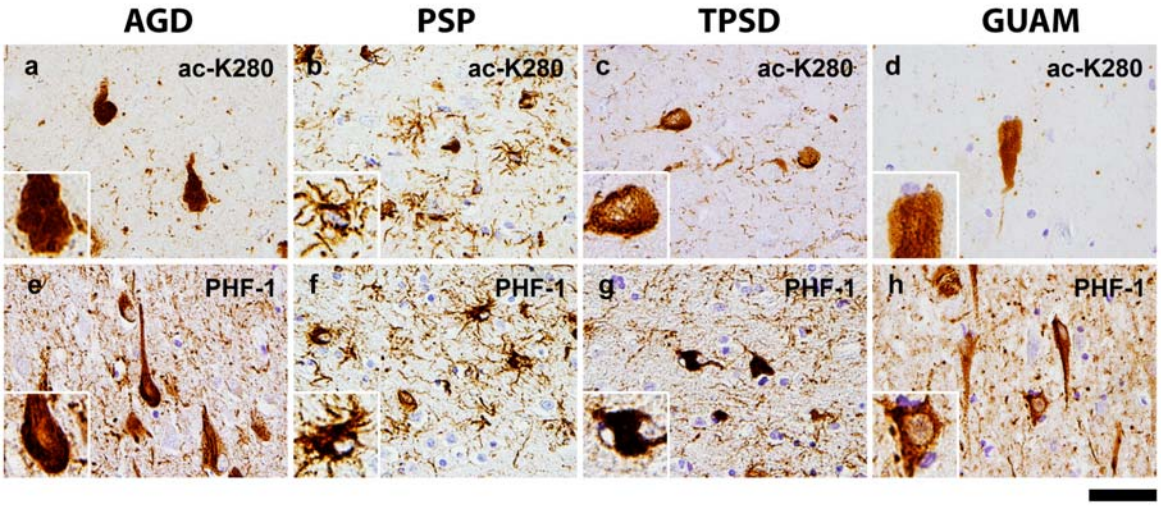
Supplementary Figure S3: Charaterization of the ac-K280 site-specific acetylated tau antibody.

a) Recombinant WT-K18 or ΔK280-K18 proteins (1 µg) were incubated in the presence or absence of acetyl-CoA or CBP as indicated and K280 acetylation was determined by immunoblotting using anti-ac-K280 and a polyclonal antibody raised to exon 10 of tau protein (E10). **b**) QBI-293 cells were co-transfected with WT-T40 tau or ΔK280-T40 mutant tau expression plasmids along with CBP to promote tau acetylation. 1, 2, and 5 µg total cell lysates were analyzed by western blotting using anti-ac-K280, total tau (T46), and GAPDH antibodies. **c**) QBI-293 cells were transfected with WT-40, WT-CBP-HA, or enyzyme-inactive CBP-LD-HA expression plasmids and analyzed by immunofluroescence using anti-tau (T46), monoclonal HA (Santa Cruz), or anti-ac-K280 antibodies. Note, ac-K280 reactivity is specifically detected in the presence of WT-CBP, but not the inactive mutant CBP-LD. Scale bar represents 50µm. **d**) Control or HDAC6 siRNA expressing HEK-T40 cells were analyzed by immunoblotting using ac-K280, total tau, HDAC6, and GAPDH antibodies.



Supplementary Figure S4: Ac-K280 pathology accumulates with age in PS19/PDAPP mice.

a-c) Cortical brain sections from PS19/PDAPP mice at 4-month (panel **a**), 8-month (panel **b**), or 11-months (panel **c**) were analyzed by IHC using anti-ac-K280 antibody. Shown are representative images highlighting increased ac-K280 immuno-reactivity in older mice. Scale bar represents 100 μM.



Supplementary Figure S5: Ac-K280-positive tau inclusions are detected in a diverse set of human tauopathies

a-h) Cortical brain sections from the indicated tauopathies were analyzed by IHC using anti-ac-K280 (panels **a-d**) and PHF-1 (panels **e-h**) antibodies. Shown are representative images highlighting NFTs and tau inclusions in each tauopathy. Scale bar represents 50 μM, insets depict 60X magnification.

peptide sequence	peptide lysine	tau residue #	Ions score (>40 sig.)	Expect (p-value)
TKIATPR	K2	K150	34	0.12
GAAPPGQKGQANATR	K8	K163	106	5.30E-09
IPAKTPPAPK	K4	K174	47	0.0049
TPPKSPSSAK	K4	K234	46	0.0066
TPPKSPSSAKSR	K10	K240	47	0.0047
SKIGSTENLK	K2	K259	44	0.011
HQPGGGKVQIINK	K7	K274	76	6.20E-06
VQIINKK	K6	K280	46	0.0059
VQIINKK	K7	K281	44	0.0099
KLDLSNVQSK	K10	K290	61	0.00021
HVPGGGSVQIVYKPVDLSK	K13	K311	58	0.00029
IGSLDNITHVPGGGNKK	K16	K369	73	1.00E-05
TDHGAEIVYKSPVVSGDTSPR	K10	K395	56	0.00038

Supplementary Table S1: Mass spectrometry analysis identified tau acetylation sites from *in vitro* acetylated tau-T40.

Recombinant CBP protein was incubated with 1µg T40 and 0.4 mM acetyl-CoA in 30 µl of reaction buffer (50 mM Tris-HCl pH 8.0, 10% glycerol, 1 mM DTT, 100 mM EDTA, 1 mM phenylmethylsulfonyl fluoride) for 1 hr at 37°C. Acetylation was analyzed by SDS-PAGE and Coomassie staining followed by gel excision for mass spectrometry using LTQ XL* Linear Ion Trap Mass Spectrometer (Thermo Scientific). Data was acquired using Xcalibur software (Thermo Scientific) and analyzed using Mascot software (Matrix Science).

Monoisotopic mass of neutral peptide Mr(calc): 883.5491

Variable modifications:

K6 : Acetyl (K)

Ions Score: 44 Expect: 0.024

Matches (Bold Red): 11/68 fragment ions using 14 most intense peaks

#	a	a^{++}	a*	a^{*++}	b	b^{++}	b*	b^{*++}	Seq.	y	y^{++}	y*	y^{*++}	#
1	72.0808	36.544			100.0757	50.5415			V					7
2	200.1394	100.5733	183.1128	92.06	**228.1343**	114.5708	**211.1077**	106.0575	Q	**785.488**	393.2476	768.4614	384.7343	6
3	313.2234	157.1153	296.1969	148.6021	**341.2183**	171.1128	324.1918	162.5995	I	**657.4294**	329.2183	640.4028	320.7051	5
4	**426.3075**	213.6574	409.2809	205.1441	454.3024	**227.6548**	437.2758	219.1416	I	**544.3453**	272.6763	527.3188	264.163	4
5	540.3504	270.6788	523.3239	262.1656	568.3453	284.6763	551.3188	276.163	N	**431.2613**	216.1343	414.2347	207.621	3
6	710.4559	355.7316	693.4294	347.2183	**738.4508**	369.7291	721.4243	361.2158	K	**317.2183**	159.1128	300.1918	150.5995	2
7									K	147.1128	74.06	130.0863	65.5468	1

Supplementary Table S2: Ion scores from acetylated VQIINNK peptide identified by mass spectrometry analysis of tau in cells

Tau-T40 was immunoprecipitated with T14/T46 antibodies and separated by SDS-PAGE followed by gel excision and mass spectrometry analysis. Listed in the table above are the ion scores that correspond to the m/z spectrum depicted in Supplementary Figure 2. The numbers in red bold are statistically significant and confirm the presence of acetylation on Lys-280 (K280). These data were generated using Mascot software (Matrix Science).