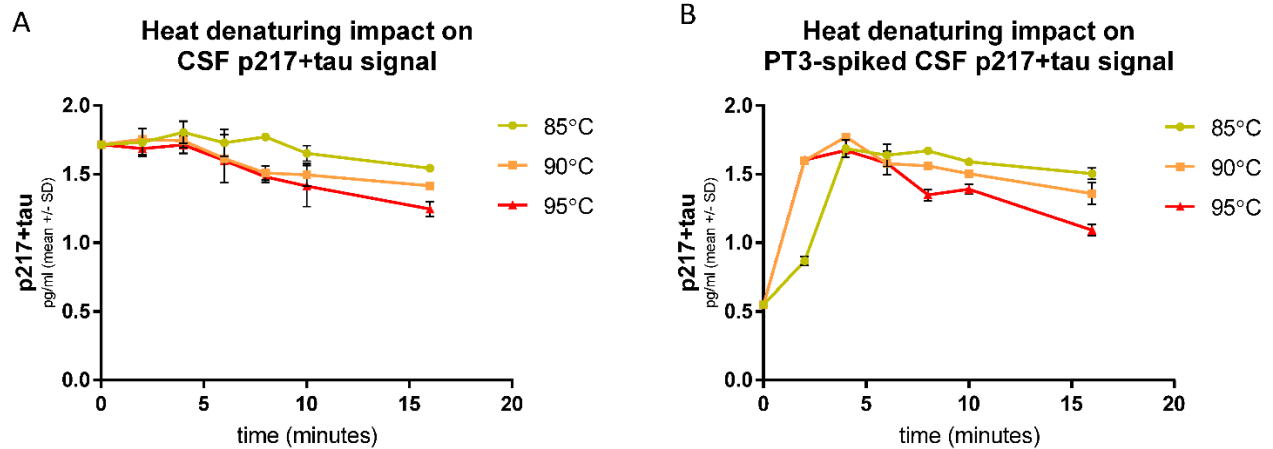# Supplementary Material

Development and Validation of a High Sensitivity Assay for Measuring p217+tau in Cerebrospinal Fluid

Supplementary Figure 1. Temperature dependency of heat denaturing method. A) CSF was heated at the indicated temperature and times, then measured with p217+tau assay (PT3xHT43) revealing stability of the p217+tau signal for 4-10 min, depending on temperature. B) CSF was spiked with 1 µg/ml PT3 then heated for the temperature and times, then measured with p217+tau assay, revealing that heat rescues the PT3-competed signal within 2-4 min. Ideal conditions for denaturing were selected as 85°C x 7 min but should be evaluated at each testing site.
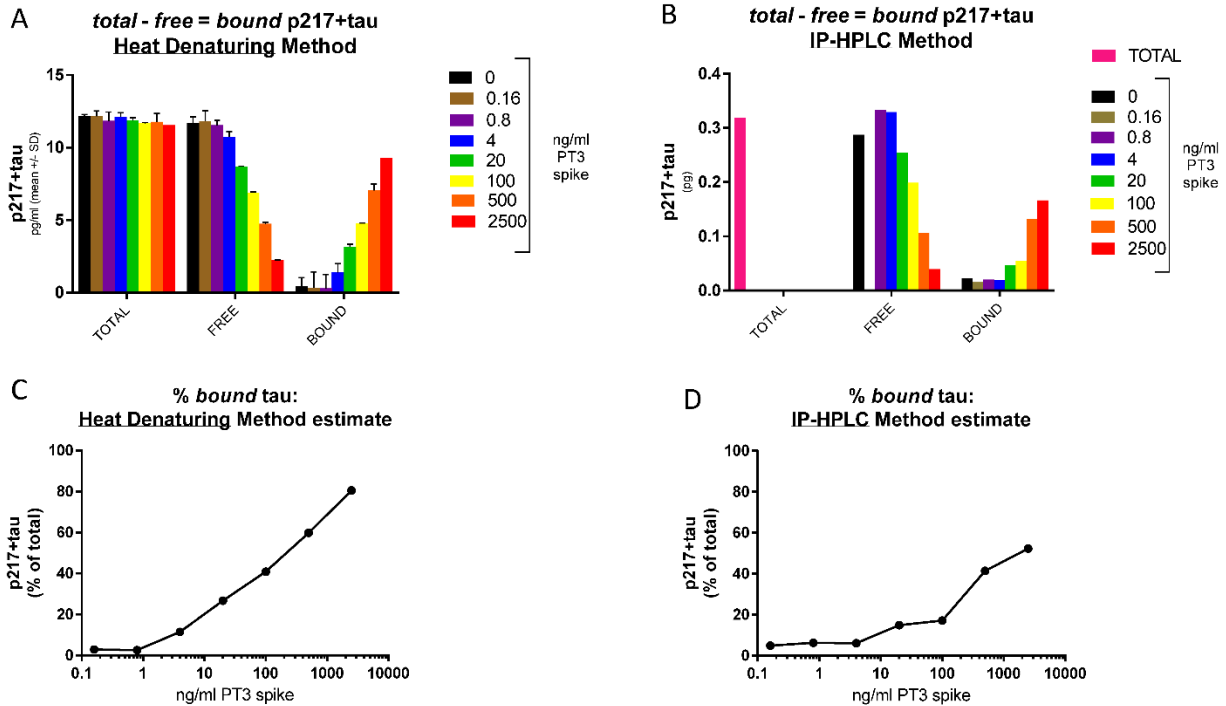
Supplementary Figure 2. Comparable quantification of antibody-bound CSF p217+tau using heat denaturing or immunoprecipitation methods. CSF spiked with PT3 at indicated concentrations, incubated at 22°C for 2 h to allow PT3-tau complexes to form, then measured in the following two methods to calculate % of p217+tau bound by PT3. A) Samples were measured directly (*free* p217+tau) or after heat denaturing (*total* p217+tau) with the p217+tau assay (PT3xHT43), then *bound* p217 was calculated as *total-free=bound*. B) Samples were immunoprecipitated to capture all IgG, then eluted off the beads, denatured, and fractionated by HPLC before measuring with the p217+tau assay (aka *bound* p217+tau). Depleted supernatants were also treated with denaturant/HPLC and measured with the same p217+tau assay (aka *free* p217+tau). A non-spiked non-immunoprecipitated sample was also treated with denaturant/HPLC and measured with the p217+tau assay (aka *total* p217+tau). C, D) Alignment of % *bound* p217+tau reveal that the heat denaturing and IP/HPLC methods result in similar dose-response curves.

Supplementary Table 1. Demographic and clinical characteristics in 54861911ALZ1005/2002 cohort (Fig. 4)

	CDR 0, A-	CDR 0.5, A-	CDR 0, A+	CDR 0.5, A+	p
# subjects	10	10	10	10	
Age (y)	70.6 (6.7)	66.5 (5.0)	67.8 (7.0)	70.7 (6.4)	0.3333
Sex (F/M)	4/6	4/6	1/9	5/5	0.2662
CSF Aβ_{42}; (pg/ml)	847 (206)	856 (184)	404 (96)	385 (99)	<0.0001
CSF t-tau; (pg/ml)	326 (110)	257 (94)	396 (183)	656 (341)	0.0042
CSF p181tau; (pg/ml)	51 (15)	42 (15)	62 (24)	80 (46)	0.0182
CSF p217+tau (pMolar)	0.32 (0.41)	0.18 (0.20)	0.69 (0.73)	1.38 (1.04)	0.0163
Data are shown as mean (SD) unless otherwise specified. Differences between the groups were tested using Kruskal-Wallis or chi-square (sex) tests.					

Supplementary Table 2. Demographic and clinical characteristics in ELN115727301/302 cohort (Figs. 5, 6F, 7B-D)

	A +	A-	p
# subjects	204	31	
Age (y)	71.7 (9.2)	72.6 (8.5)	0.6765
Sex (F/M)	107/97	14/17	0.4206
CSF Aβ$_{42}$; (pg/ml)	406 (99)	947 (226)	<0.0001
CSF Aβ$_{42/40}$; (ratio)	0.045 (0.010)	0.101 (0.007)	<0.0001
CSF t-tau; (pg/ml)	747 (399)	340 (173)	<0.0001
CSF p181tau; (pg/ml)	93.3 (38.2)	53.8 (15.7)	<0.0001
CSF p217+tau (pg/ml)	33.6 (22.0)	6.6 (8.6)	<0.0001
Data are shown as mean (SD) unless otherwise specified. Differences between the groups were tested using Mann-Whitney or chi-square (sex) tests.			

Supplementary Table 3. Demographic and clinical characteristics in 54861911ALZ2003 cohort (Fig. 6A-E)

	A +	A-	p
# subjects	146	38	
Age (y)	69.7 (5.4)	69.6 (5.4)	0.7515
Sex (F/M)	85/61	25/13	0.3966
CSF Aβ$_{42}$; (pg/ml)	509 (98)	1062 (195)	<0.0001
CSF Aβ$_{42/40}$; (ratio)	0.055 (0.012)	0.108 (0.014)	<0.0001
CSF t-tau; (pg/ml)	438 (234)	295 (92)	<0.0001
CSF p181tau; (pg/ml)	70 (29)	53 (16)	0.0005
CSF p217+tau (pMolar)	13.2 (14.0)	3.8 (2.6)	<0.0001
Data are shown as mean (SD) unless otherwise specified. Differences between the groups were tested using Mann-Whitney or chi-square (sex) tests.			

Supplementary Table 4. Demographic and clinical characteristics in AAB-001-201 cohort (Fig. 7a data)

	A +	A-	p
# subjects	19	2	
Age (y)	64.9 (6.0)	65.0 (2.8)	0.6784
Sex (F/M)	7/12	0/2	0.2931
CSF Aβ_{42}; (pg/ml)	383 (96)	870 (369)	0.0095
CSF A$\beta_{42/40}$; (ratio)	0.046 (0.009)	0.101 (0.013)	0.0095
CSF p217+tau (pg/ml)	52.1 (44.5)	6.0 (2.0)	0.0095
MMSE	20.9 (3.5)	23.0 (4.2)	0.5619
Data are shown as mean (SD) unless otherwise specified. Differences between the groups were tested using Mann-Whitney or chi-square (sex) tests.			