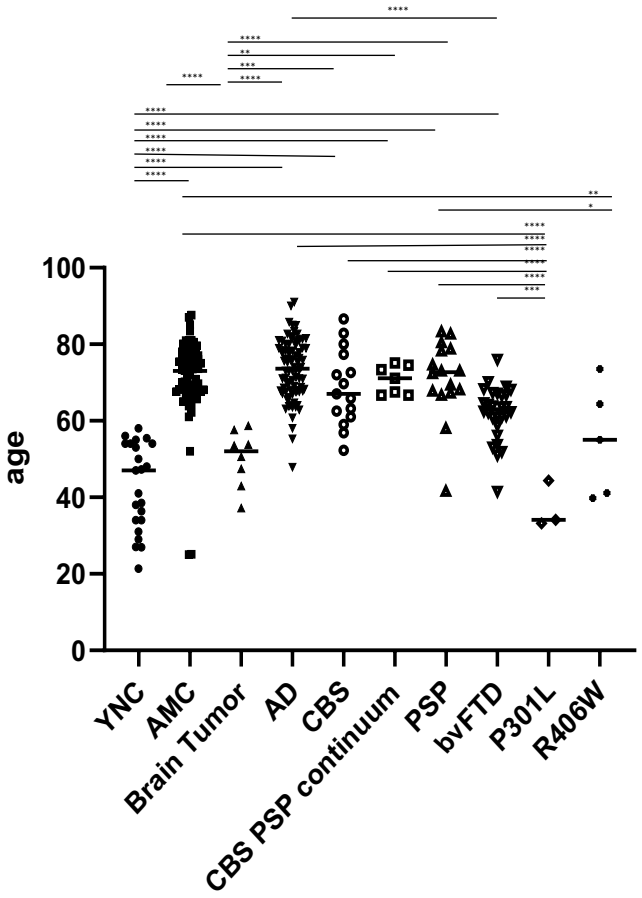
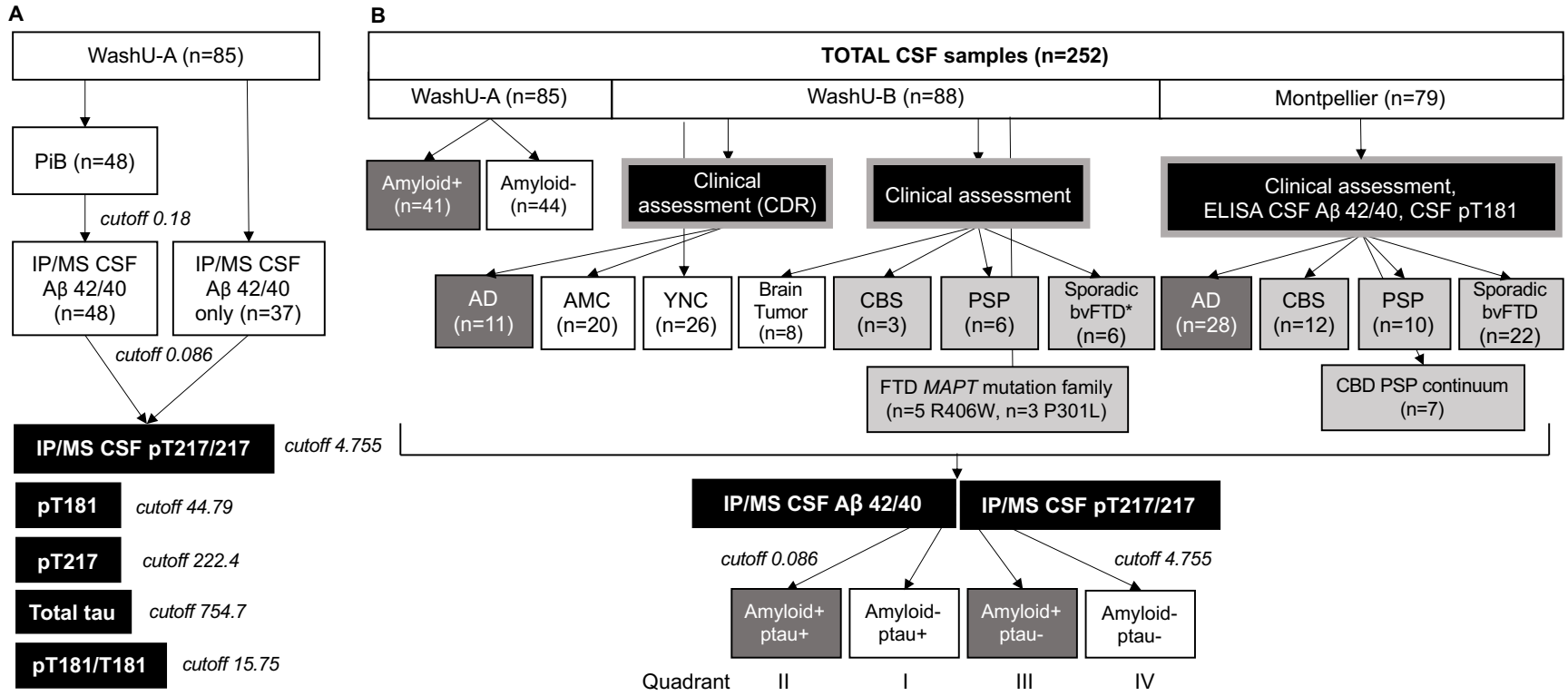
Supplementary Figures and Tables

~~Tau Phosphorylation at T217 Increases in *MAPT* R406W Mutation Carriers without Amyloid Pathology~~

Chihiro Sato**, Nipun Mallipeddi, Nupur Ghoshal, Brenton A. Wright, Gregory S. Day, Albert A. Davis, Albert H. Kim, Gregory J Zipfel, Randall J. Bateman, Audrey Gabelle**, and Nicolas R. Barthélemy**

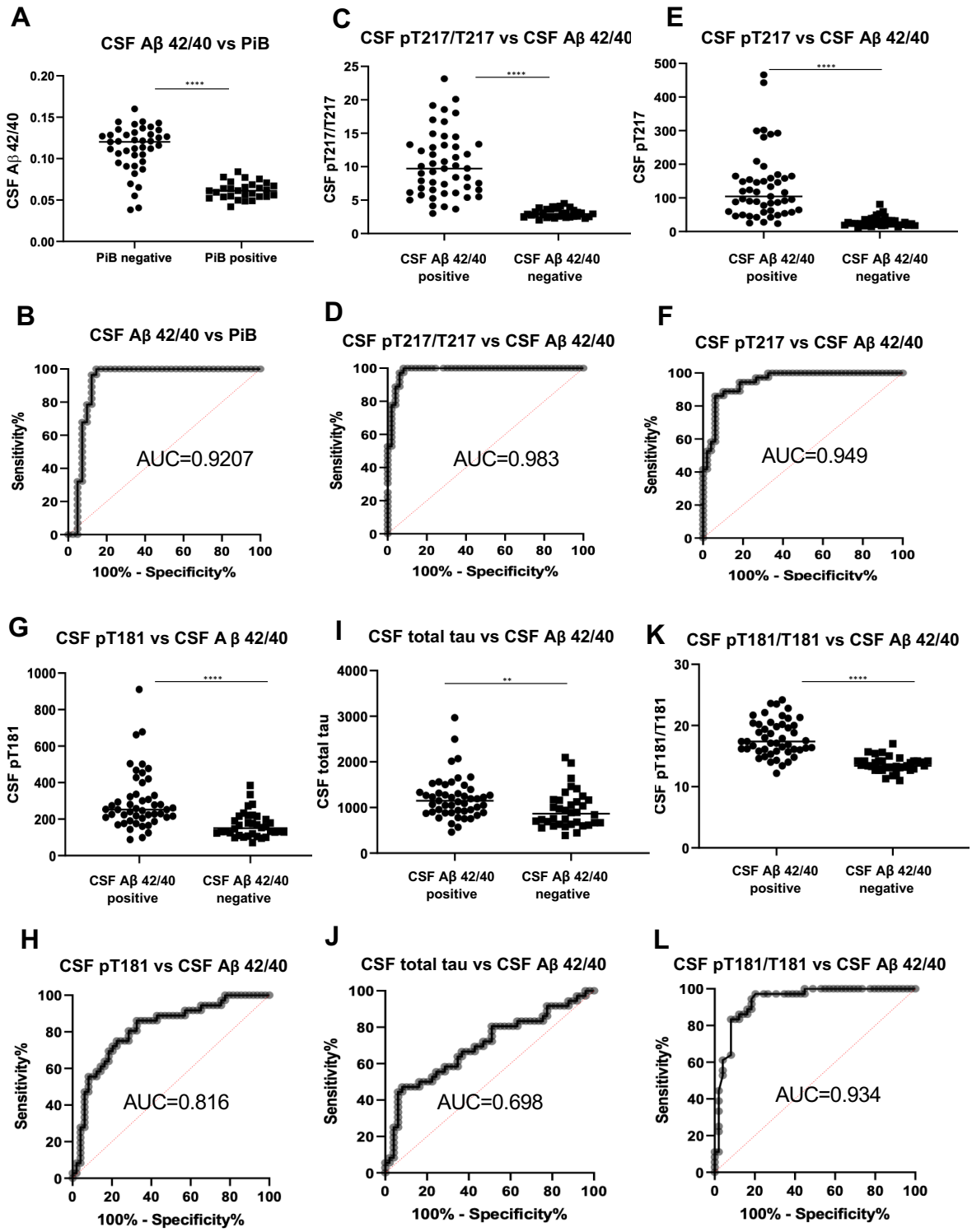


Supplementary Fig. 1. Age of the participants in each subgroup. YNC, Brain tumor, P301L groups are significantly younger than AMC and participants with neurodegenerative diseases.

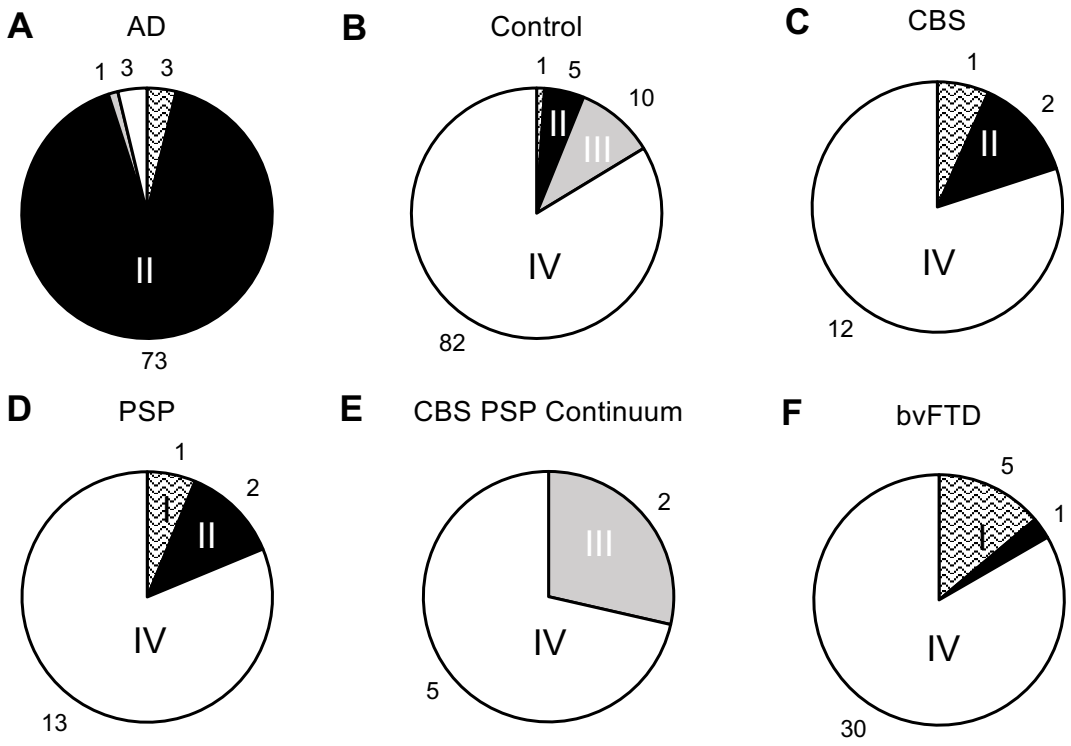


Supplementary Fig. 2. Flowchart of cohort analyzed in this study. (A) WashU-A cohort includes AD and age matched controls with a subset (n=48) having PiB PET imaging data. This subset and a PiB PET cutoff of 0.18 were used to determine amyloid positivity of immunoprecipitation and Mass spectrometry (IP/MS) measures of CSF Aβ 42/40 (cutoff 0.086). Next, all CSF from WashU-A was used to determine IP/MS CSF pT217/T217 cutoff of 4.76. Cutoff of CSF concentrations of pT181, pT217, total tau, and ratio of pT181/T181 were 44.79, 222.4, 754.7, and 15.75, respectively. (B) WashU-A (n=85), WashU-B (n=88), and Montpellier cohort (n=79) were analyzed for IP/MS CSF Aβ 42/40 and pT217/T217 in quadrant analyses (total n=252). Samples were categorized into quadrant

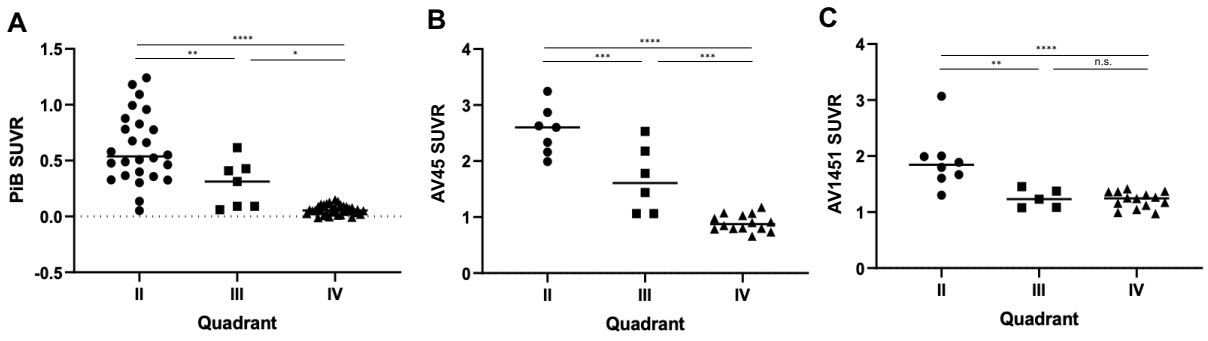
I-IV using the cutoffs for amyloid and ptau positivity determined in (A). AMC: age matched control, YNC: Young Normal Control, CBS: Corticobasal Syndrome, PSP: Progressive Supranuclear Palsy, bvFTD: behavioral variant Frontotemporal Dementia.


A
CSF Aβ 42/40 vs PiB
CSF Aβ 42/40
PiB negative
PiB positive
B
CSF Aβ 42/40 vs PiB
Sensitivity%
100% - Specificity%
AUC=0.9207
C
CSF pT217/T217 vs CSF Aβ 42/40
CSF pT217/T217
CSF Aβ 42/40 positive
CSF Aβ 42/40 negative
D
CSF pT217/T217 vs CSF Aβ 42/40
AUC=0.983
E
CSF pT217 vs CSF Aβ 42/40
CSF pT217
F
CSF pT217 vs CSF Aβ 42/40
AUC=0.949
G
CSF pT181 vs CSF A β 42/40
CSF pT181
H
CSF pT181 vs CSF Aβ 42/40
AUC=0.816
I
CSF total tau vs CSF Aβ 42/40
CSF total tau
J
CSF total tau vs CSF Aβ 42/40
AUC=0.698
K
CSF pT181/T181 vs CSF Aβ 42/40
CSF pT181/T181
L
CSF pT181/T181 vs CSF Aβ 42/40
AUC=0.934

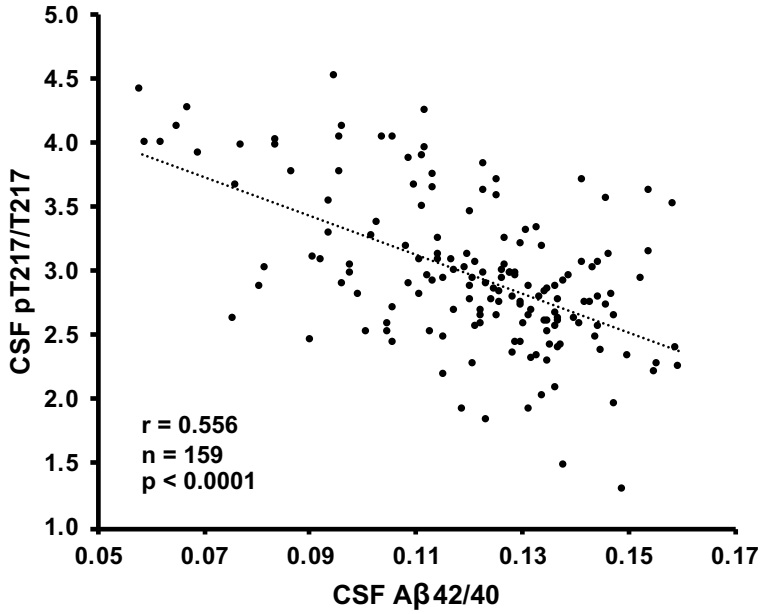
Supplementary Fig. 3. ROC analyses of IP/MS CSF Aβ 42/40, CSF tau and ptau. (A) CSF Aβ 42/40 ratio is significantly increased in amyloid PET-PiB+ participants in Wash-A cohort (n=48). (B) CSF Aβ 42/40 can differentiate amyloid PET-PiB+ from PiB- individuals (AUC = 0.9207, $p<0.0001$). (C, E, G, I, K) CSF pT217/T217 ratio, CSF pT217, CSF T181, CSF total tau concentrations, CSF pT181/T181 ratio are significantly increased in amyloid+ participants defined by CSF Aβ 42/40 ratio in Wash-A cohort (n=85). (D, F, H, J, L) CSF pT217/T217 ratio, CSF pT217, CSF T181, CSF total tau concentrations, CSF pT181/T181 ratio can differentiate CSF Aβ 42/40 positive (amyloid +) from CSF Aβ 42/40 negative (amyloid –) individuals with AUC = 0.983, 0.949, 0.816, 0.693, 0.934, respectively. *$p<0.05$, **$p<0.01$, ***$p<0.001$, ****$p<0.0001$.



Supplementary Fig. 4. Quadrant analyses by diagnosis. Pie charts showing the quadrants (I, II, III, IV) where participants from each disease group are located.



Supplementary Fig. 5. Amyloid and tau PET Imaging by quadrant. (A) Amyloid PET imaging measured by PiB SUVR is significantly and gradually increased in quadrant II > III > IV (ANOVA, $p<0.0001$). (B) Amyloid PET imaging measured by AV45 SUVR is significantly and gradually increased in quadrant II > III > IV (ANOVA, $p<0.0001$). (C) Tau PET imaging measured by AV1451 SUVR is only increased in quadrant II (ANOVA, $p<0.0001$). There was no participant with imaging data in quadrant I. ANOVA *$p<0.05$, **$p<0.01$, ***$p<0.001$, ****$p<0.0001$.

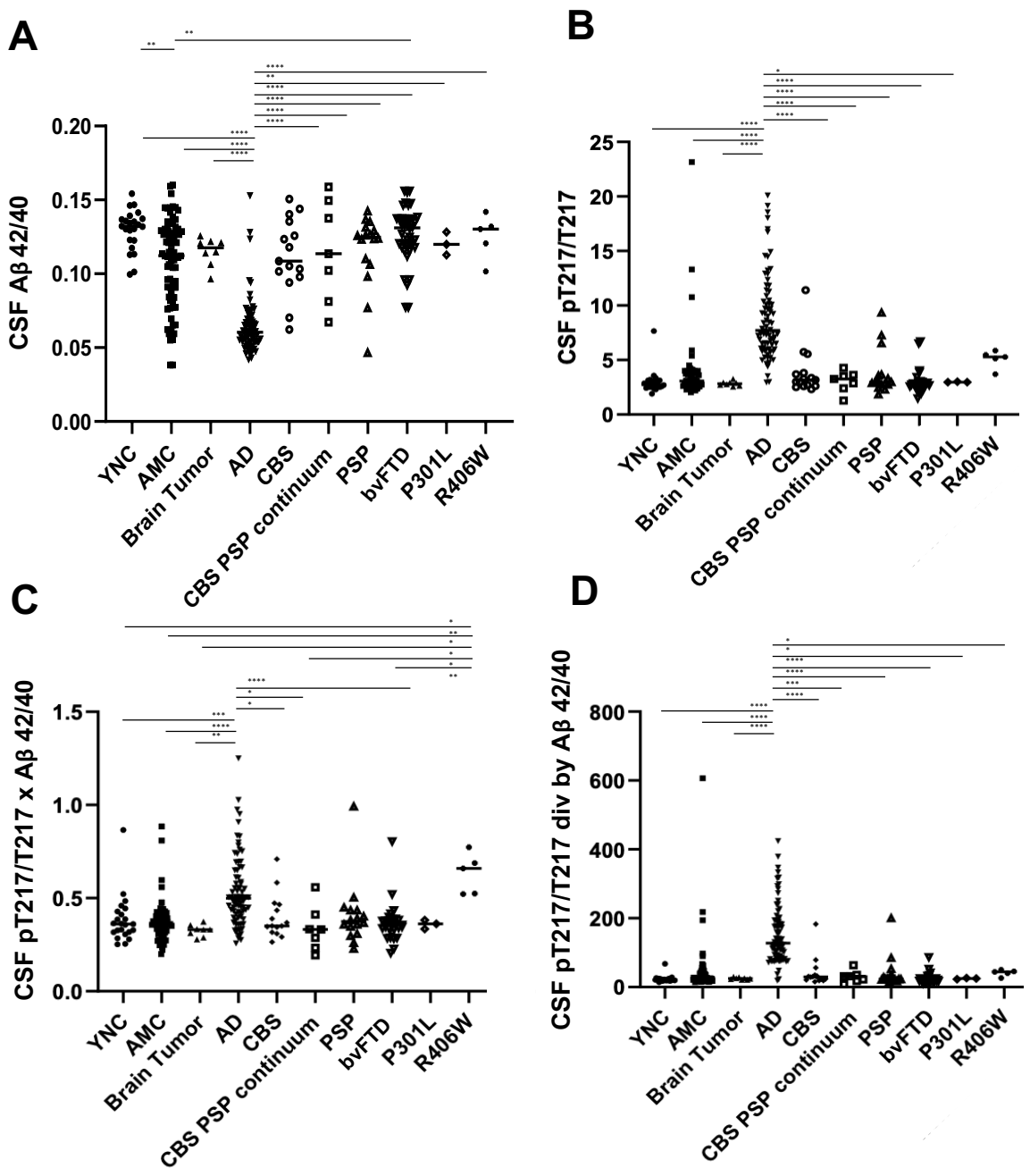


Supplementary Fig. 6. CSF Aβ 42/40 and CSF pT217/T217 correlate in quadrant III and IV.

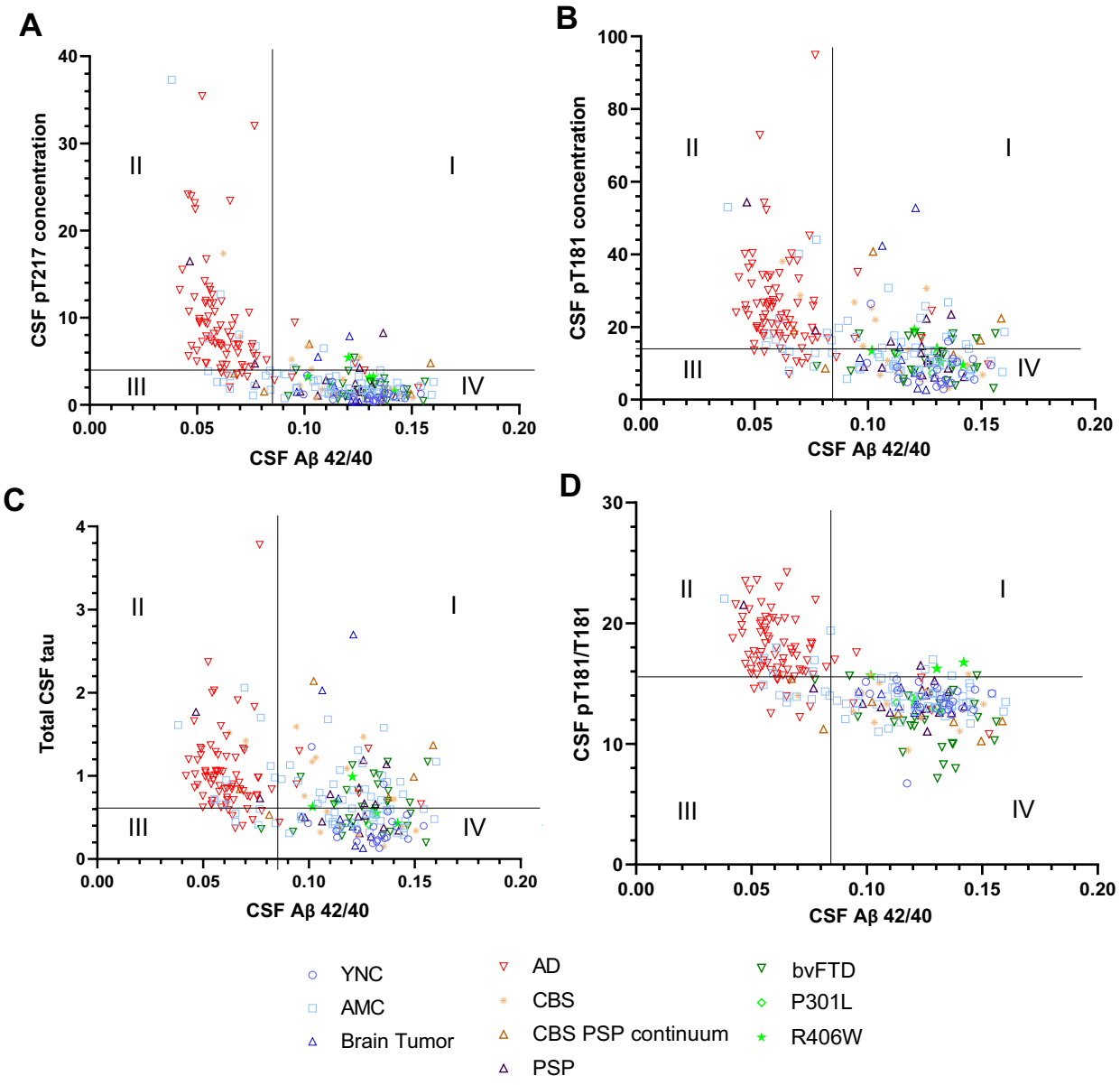
CSF pT217/T217 negatively correlates with CSF Aβ 42/40 in quadrant III and IV below cutoff for ptau positivity (Pearson correlation r=0.556, p<0.0001).

Participant	Quadrant	Diagnosis / groups	Age	Sex	CSF Aβ 42/40 (Cutoff = 0.086)	CSF pT217/T217 (Cutoff = 4.76)	CSF pT217/T217 x CSF Aβ 42/40 (Cutoff for R406W vs control = 0.50)	CSF pT217/T217 div CSF Aβ 42/40 (Cutoff for R406W vs control = 39.9)
A	I	PSP	57	M	0.127	6.107	0.776	48.1
	I	PSP	58		0.137	7.259	0.992	53.1
B	IV	CBS	58	M	0.123	3.116	0.385	25.2
	IV	CBS	59		0.105	2.515	0.265	23.9
C	IV	CBS	66	F	0.127	2.977	0.378	23.5
	IV	CBS	67		0.123	3.832	0.473	31.1
D	IV	AMC	81	F	0.111	2.818	0.314	25.3
	IV	AMC	82		0.121	3.190	0.387	26.3
E	IV	AMC	87	M	0.087	3.762	0.328	43.2
	IV	AMC	88		0.089	4.312	0.385	48.3

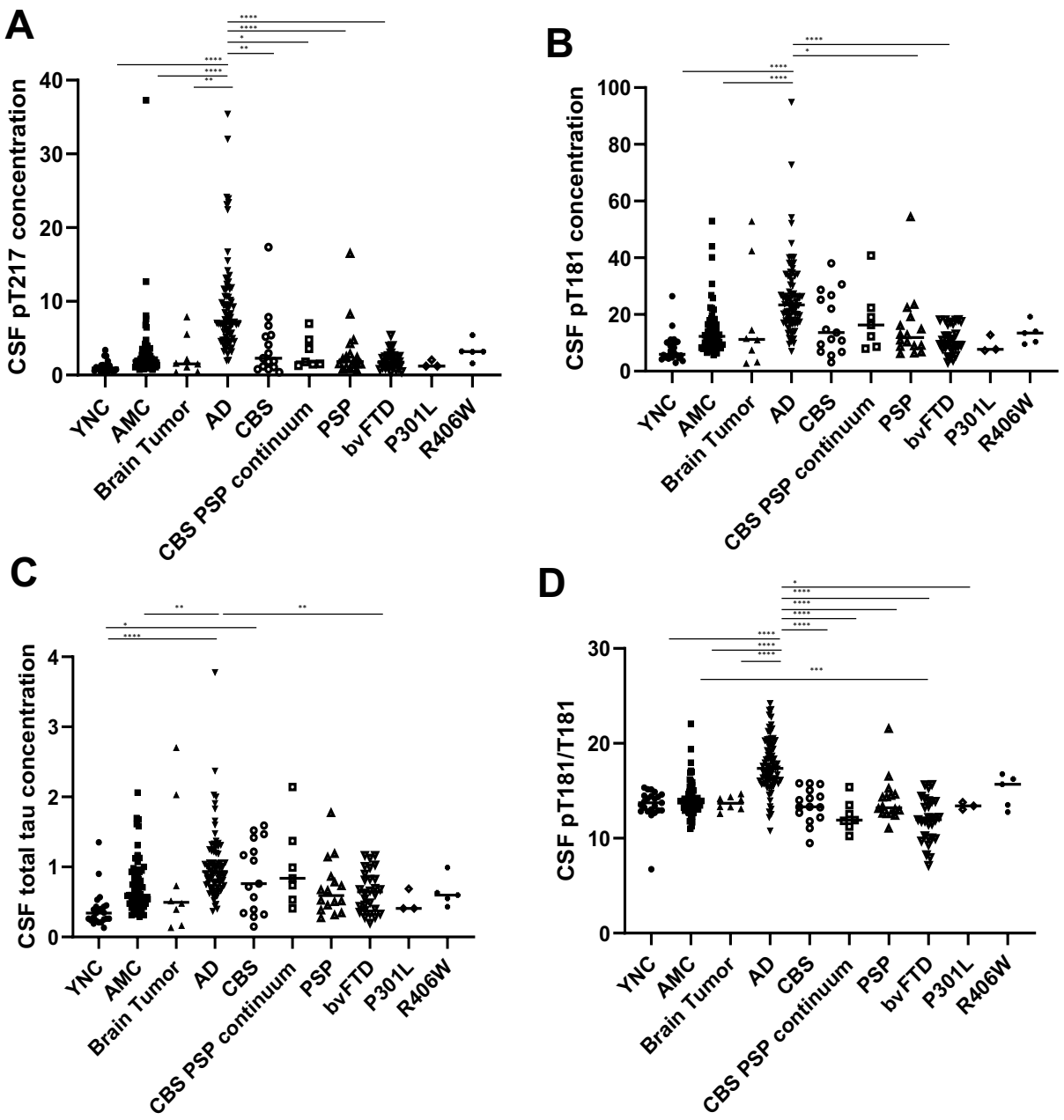
Supplementary Table 1. Demographics and summary of biomarker values for participants with follow up visits. Participant A-E had baseline and follow up visit 1 year apart. Participant A (PSP) was in quadrant I (amyloid-, ptau+) at baseline and follow up. Participant B-E were in quadrant IV (amyloid-, ptau-) at baseline and follow up.



Supplementary Fig. 7. Subcategory of diagnosis are shown from Fig. 2. (A) CSF Aβ 42/40 is significantly decreased in AD and AD focal. (B) CSF pT217/T217 is significantly increased in AD and AD focal. (C) CSF pT217/T217 x CSF Aβ 42/40, is significantly increased in AD and MAPT R406W mutation carriers. (D) CSF pT217/T217 divided by CSF Aβ 42/40, is significantly increased in AD and AD focal. ANOVA *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001.



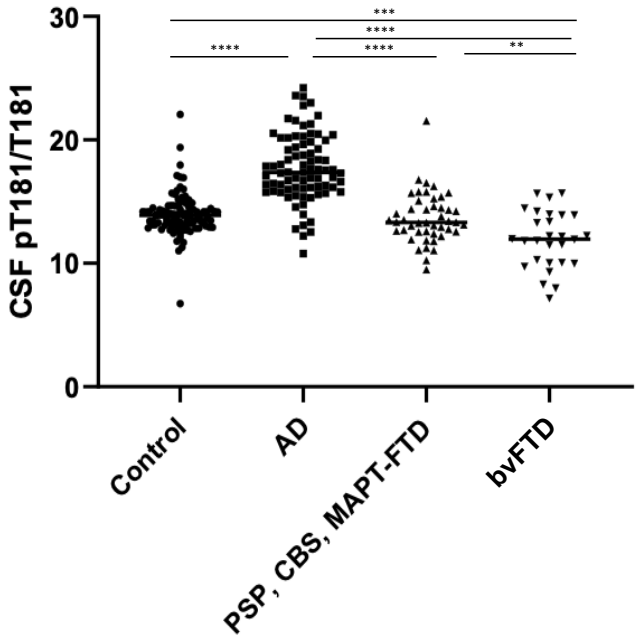
Supplementary Fig. 8. Quadrant Analyses using CSF Aβ 42/40 and CSF total tau and ptau. CSF concentrations of pT217 (A), pT181 (B), total tau (C) do not separate *MAPT* R406W mutation carriers or other groups. (D) CSF pT181/T181 ratio does not separate *MAPT* R406W mutation carriers. However, sporadic bvFTD may have lower CSF pT181/T181 in quadrant IV.



Supplementary Fig. 9. IP/MS CSF total tau and ptau in subgroups of tauopathies.

(A-B) AD has significantly increased CSF pT217 and pT181 concentrations. (C) Total CSF tau is significantly increased in AMC, AD, AD focal, CBS PSP continuum than YNC. (D) Sporadic bvFTD containing FTLD-tau, FTLD-TDP, FTLD-FUS has significantly lower CSF pT181/T181 than R406W (*p<0.05), AMC (***p<0.001), and AD/AD focal (****p<0.0001).

A



B

Test	Diagnostic groups	n per group	AUC	95% CI	P value
CSF pT181/T181	bvFTD vs AD	28 vs 80	0.959	0.9265 to 0.9913	<0.0001
	PSP, CBS, *MAPT*-FTD vs AD	46 vs 80	0.894	0.8340 to 0.9540	<0.0001
	AD vs Control	80 vs 98	0.889	0.8325 to 0.9450	<0.0001
	bvFTD vs Control	28 vs 98	0.752	0.6295 to 0.8742	<0.0001
	bvFTD vs PSP, CBS, *MAPT*-FTD	28 vs 46	0.707	0.5810 to 0.8336	0.0029
	PSP, CBS, *MAPT* -FTD vs Control	46 vs 98	0.568	0.4582 to 0.6784	0.1869

Supplementary Fig. 10. Sporadic bvFTD containing FTLD-tau, FTLD-TDP, FTLD-FUS may be separated from Control, AD and other tauopathies with CSF pT181/T181. (A) Sporadic bvFTD (bvFTD) containing FTLD-tau, FTLD-TDP, FTLD-FUS has higher CSF Aβ 42/40 ratio and lower CSF pT181/T181 than Control, AD, and tauopathies containing CBS, PSP, and *MAPT*-FTD (P301L and R406W mutation carriers). *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001. (B) Diagnostic values of CSF pT181/T181 in separating bvFTD from other cohort. Sporadic bvFTD can be separated from Control (AUC = 0.752) and PSP, CBS, and *MAPT*-FTD (AUC = 0.707).