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Supplementary Materials for

Aβ deposition is associated with increases in soluble and phosphorylated tau that precede a positive Tau PET in Alzheimer's disease

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This PDF file includes:

1. Supplementary Text

Methods: CSF P-tau assays

Both the P-tau181 and P-tau217 assays were performed on a streptavidin small spot plate using the Meso Scale Discovery (MSD) platform (Meso Scale Discovery, Rockville, MD, USA). Anti-P-tau217 antibody IBA413 was used as a capture antibody in the P-tau217 assay whereas anti-P-tau181 antibody AT270 was used as a capture antibody in the P-tau181 assay. Antibodies were conjugated with Biotin (Thermo Scientific, catalog number: 21329) or SULFO-TAG (MSD, catalog number: R91AO-1). The assays were calibrated using a recombinant tau (4R2N) protein that was phosphorylated in vitro using a reaction with glycogen synthase kinase-3 and characterized by mass spectrometry. The samples were thawed on wet ice, briefly vortexed, and diluted 1:8 in Diluent 35 (MSD, catalog number: R50AE) with the addition of a heterophilic blocking reagent to a concentration of 200µg/ml (Scantibodies Inc, catalog number: 3KC533). In order to perform the assays, MSD small-spot streptavidin coated plates (MSD, catalog number: L45SA) were blocked for 1 hour at room temperature with 200µl of 3% BSA in DPBS with 650rpm shaking on a plate shaker. The plates were then washed three times with 200µl of wash buffer (PBS + 0.05% Tween 20) and 25µl of biotinylated capture antibody (AT270 for P-tau181 or IBA413 for P-tau217) at 1µg/ml and 0.1 µg/ml respectively were added to the wells and incubated for 1 hour at room temperature with 650rpm shaking on a plate shaker. The plates were again washed three times with 200µl of wash buffer and 50µl of diluted calibrator or sample was added to each well and incubated for 2 hours at room temperature with 650rpm shaking on a plate shaker. The plates were then washed three times with 200µl of wash buffer and 25µl of SULFO-tagged LRL detection antibody was added at 3µg/ml for the P-tau181 and at 0.5µg/ml for the P-tau217 plates and incubated for 1 hour at room temperature with 650rpm shaking on a plate shaker. The plates were washed a final time with 200µl of wash buffer and 150µl of 2x MSD Read Buffer T with Surfactant (MSD, catalog number: R92TC) was added to each plate and read on the MSD SQ120 within 10 minutes of read buffer addition. Samples were analyzed in duplicates and the mean of duplicates were used in statistical analysis. Individual measurements all fell within 20% of the mean for QC and control samples.

Results: Associations between demographic factors and tau biomarkers

As expected (*Ossenkoppele et al, JAMA 2019*), higher age was associated with lower Tau PET in predefined regions-of-interest (ROIs), including the inferior temporal lobe (ITC) (rho=-0.26, P=0.0028), and a large neocortical meta-ROI (i.e. Braak V-VI) (rho=-0.37, P<0.0001) (the negative correlations were driven mainly by the symptomatic AD group). Older age was also associated with lower CSF levels of P-tau217 (rho=-0.18, p=0.036), but not significantly with CSF P-tau181 (p=0.12), or T-tau (P=0.30). Presence of the *APOE* ε4 allele correlated with higher levels of CSF P-tau217 (median [inter-quartile range, IQR] 165 [77-618] ng/L in non-carriers versus 458 [249-732] ng/L in carriers, P=0.003), CSF P-tau181 (156 [98-329] ng/l versus 273 [174-390] ng/l, P=0.008), and CSF T-tau (402 [293-527] ng/L versus 508 [362-593] ng/L, P=0.022), and greater Tau PET retention in ITC (1.24 [1.17-2.03] SUVR versus 1.44 [1.24-1.96] SUVR, P=0.024), but not Braak V-VI (P=0.22). There were no associations between tau biomarkers and sex (P=0.55-0.99) or education (P=0.27-0.98).

Results: CSF tau versus entorhinal Tau PET

We used Tau PET uptake in the ITC, since ITC is a reliable Tau PET region to study early neocortical tau deposition in AD (Johnson et al, Annals of Neurology 2016). Since very early accumulation of tau may also be seen in the entorhinal cortex, corresponding to Braak I-II stages of tau pathology (*Cho et el, Annals of Neurology 2016; Braak and Braak, Acta*

Neuropathologica 1991), we repeated key analyses using Tau PET uptake in the entorhinal cortex, even though this region might be more difficult to delineate using automatic segmentation methods like FreeSurfer due to difficulties in separating this structure from the adjacent meninges (*Xie et al, MICCAI 2016*). The results are shown in Supplementary Figure 8. In sum, the results were similar as for Tau PET in ITC. Entorhinal Tau PET uptake did not differ significantly between Aβ- CU and Aβ+ CU, but was increased in Aβ+ with mild cognitive deficits, and Aβ+ AD dementia. Using an a priori defined cut-point, most participants were either concordant negative or concordant positive for P-tau and entorhinal Tau PET. A large proportion was P-tau positive but Tau PET negative. There were more equal proportions of isolated T-tau positive and isolated PET positive participants. In CU who were entorhinal Tau PET-negative and also had an early CSF sample (13 Aβ- and 31 Aβ+), all Aβ- CU were negative on P-tau217, P-tau181 and T-tau in the early sample. Among the Aβ+ CU, seventeen (54.8%) were positive for P-tau217, eight (25.8%) for P-tau181, and three (9.7%) for T-tau. In a non-linear spline model, entorhinal Tau PET increased at Amyloid PET 0.723 (95% CI 0.696-0.773) SUVR, which was lower than for other Tau PET regions (and slightly before the threshold for Amyloid PET positivity), and closer to when CSF tau measures started to increase, but still later than the increase in P-tau217 (0.679 SUVR) and marginally later than the increase in P-tau181 (0.684 SUVR). The earliest increase in Tau PET in the entorhinal cortex may therefore happen shortly after changes in metabolism of soluble tau, as measured by CSF P-tau biomarkers. In a mediation analysis, P-tau217 mediated 46% (95% CI 25-75%) of the effect of Amyloid PET on entorhinal Tau PET. The mediation effect was stronger when using the P-tau217/T-tau ratio (69%, 95% CI 42-100%). This shows that a large proportion of the increased entorhinal Tau PET signal that is associated with Amyloid PET can be explained by increased phosphorylation status of soluble tau protein. These results confirm that P-tau markers (especially P-tau217) may reach abnormal levels before PHF deposition can be detected in AD, even when using a potentially very sensitive Tau PET region (entorhinal cortex) to quantify tau aggregation. The results were very similar with and without partial volume error correction (Supplementary Figure 8).

Table S1. Amyloid plaque load and CSF T-tau in 5xFAD mice.

Plaque load (%)	**2 months N=10**	**4 months N=10**	**6 months N=10**	**12 months N=9**	**P-value (JT trend)**
Cortex	0.035 (0.00-0.14)	0.36 (0.22-0.42)[a]	0.50 (0.30-0.72)[a]	1.06 (0.77-1.59)[a]	< 0.001
Subiculum	0.62 (0.48-0.93)	2.38 (1.91-2.54)[a]	2.40 (2.20-3.49)[a]	4.85 (3.87-5.63)[a]	< 0.001
CSF T-tau (pg/ml)	**2 months N=10**	**4 months N=10**	**6 months N=10**	**12 months N=9**	***p*-value (Kruskal-Wallis H)**
5xFAD	318 (185-353)	593 (349-791)[a]	706 (593-951)[b]	726 (695-925)[c]	< 0.001
Non-tg	238 (170-466)	207 (106-255)	348 (181-463)	169 (117-266)	> 0.05 (0.123)

The top part of the table shows area (%) covered by ThioS positive amyloid plaques in 5xFAD mice at 2 (n = 10), 4 (n = 10), 6 (n = 10), and 12 (n = 9) month of age. Data are presented as median (inter-quartile range). The Jonckheere-Terpstra trend test was performed to study if amyloid plaque load was increased with age. If a statistically significant trend was found, *post hoc* analysis for group comparisons between the youngest group an all other groups were performed using the Mann-Whitney U test. P-values were corrected for multiple comparisons using the Bonferroni method ([a] $P < 0.00033$ vs. 2 months). The bottom part of the table shows CSF T-tau concentrations measured in 5xFAD mice and age-matched non-transgenic littermates at 2 (5xFAD: n = 10; non-tg: n = 9), 4 (5xFAD: n = 10; non-tg: n=10), 6 (5xFAD: n=10; non-tg: n=9), and 12 (5xFAD: n=9; non-tg: n=7) months of age. The Kruskal-Wallis H test was performed to study age-differences within groups. Statistically significant differences were found for 5xFAD mice, but not Non-tg mice. *Post hoc* analysis for group comparisons were performed using the Mann-Whitney U test in the 5xFAD group. *P*-values corrected for multiple comparisons using the Bonferroni method were: 2 vs. 4 months, $p < 0.0083$; 2 vs. 6 months, $p < 0.0017$; 2 vs. 12 months, $p < 0.00017$; 4 vs. 6 months, $p>0.05$, 4 vs 12 months, $p>0.05$, 6 vs 12 months, $p>0.05$.

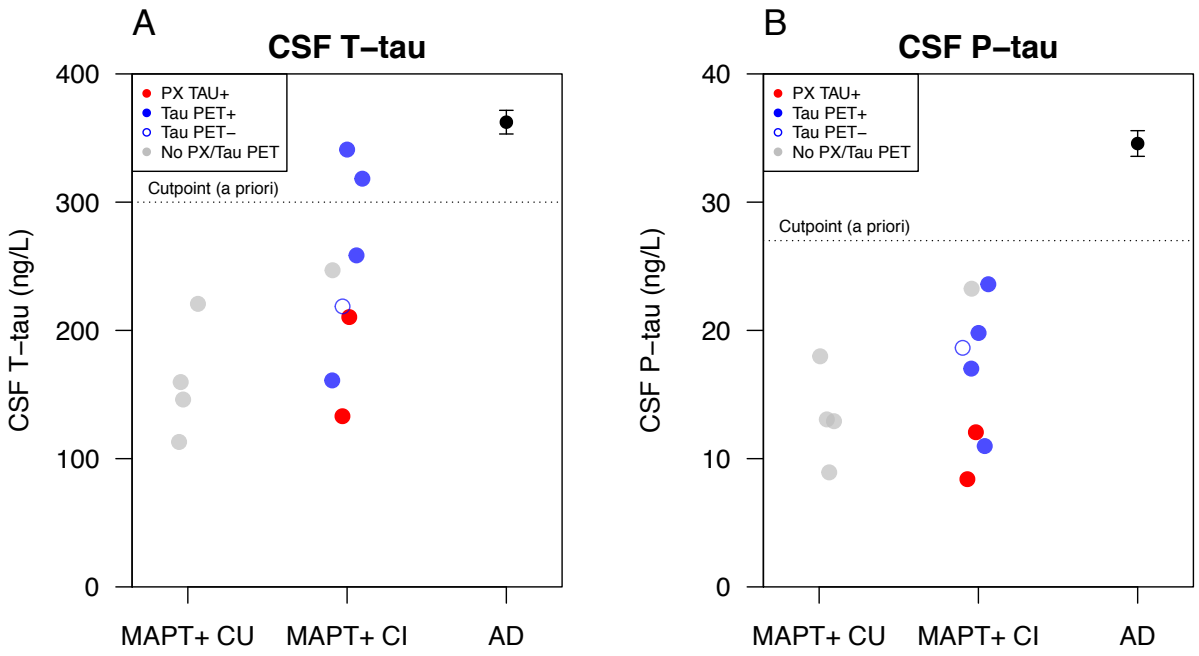

Fig. S1. CSF tau measures in *MAPT* mutation carriers.

We measured CSF T-tau (panel A) and P-tau181 (panel B) in 12 individuals with *MAPT* (the gene encoding for tau protein) mutations, recruited in Lund (N=2) and at the Memory and Aging Clinic at University of California San Francisco (N=10). Different *MAPT* variants were represented, including P243L, R317W, R406W and others. Ten individuals had cognitive impairment (CI) and four were cognitively unimpaired (CU). CSF T-tau and P-tau181 were measured with fully automated CSF Elecsys® assays, as described previously (*Hansson et al, Alzheimers Dement. 2018*). Five of the individuals had been tested with Tau PET (using 18F-flortaucipir, see *Tsai et al Alzheimers Res Ther 2019* for details on PET methods), and four of these had a positive scan, defined as positive uptake in the inferior temporal cortex. Two individuals had gone to autopsy, and in both of these the pathological report indicated tau pathology as the primary pathological finding. All twelve *MAPT* mutation carriers were Aβ-negative, defined as high CSF Aβ42/Aβ40 ratio (>0.055). For comparison, the figure also includes reference data for a group of AD patients (N=229, with mild cognitive impairment or dementia, all Aβ-positive; data are mean and standard error). All *MAPT* mutation carriers had CSF T-tau and P-tau levels below the mean levels in AD patients. All except two patients had levels (for CSF T-tau) below cut-points for T-tau (300 ng/L) and P-tau181 (27 ng/L), as recently defined (*Hansson et al, Scientific Reports, in press*). Note that the absolute concentrations quantified differ between the Elecsys® assays and other assays used in the main part of the paper.

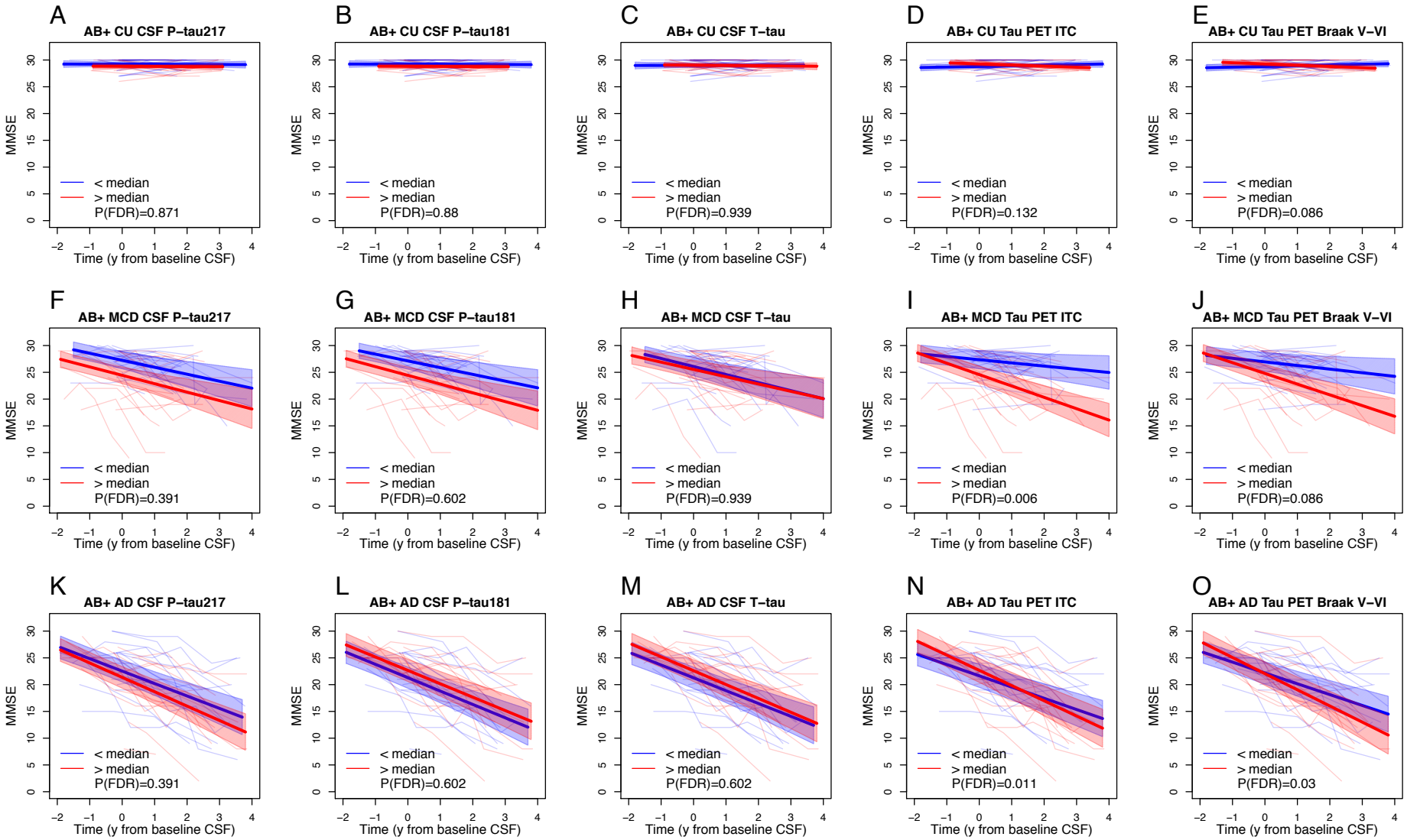

Fig. S2. Tau biomarkers and longitudinal cognition.

Tau biomarkers were used as predictors of longitudinal MMSE in linear-mixed effects models, with adjustment for age, sex, education, and time from baseline MMSE to tau biomarker testing (date of lumbar puncture or Tau PET scan). Models were tested separately in Aβ+ cognitively unimpaired (CU, panels A-E), Aβ+ mild cognitive deficits patients (MCD, panels F-J), and Aβ+ AD dementia patients (panels K-O). FDR-corrected P-values are shown for effects of baseline tau measure on longitudinal cognition. Tau biomarkers were tested as continuous predictors, but the results are visualized by trajectories for high and low (divided by median) levels.

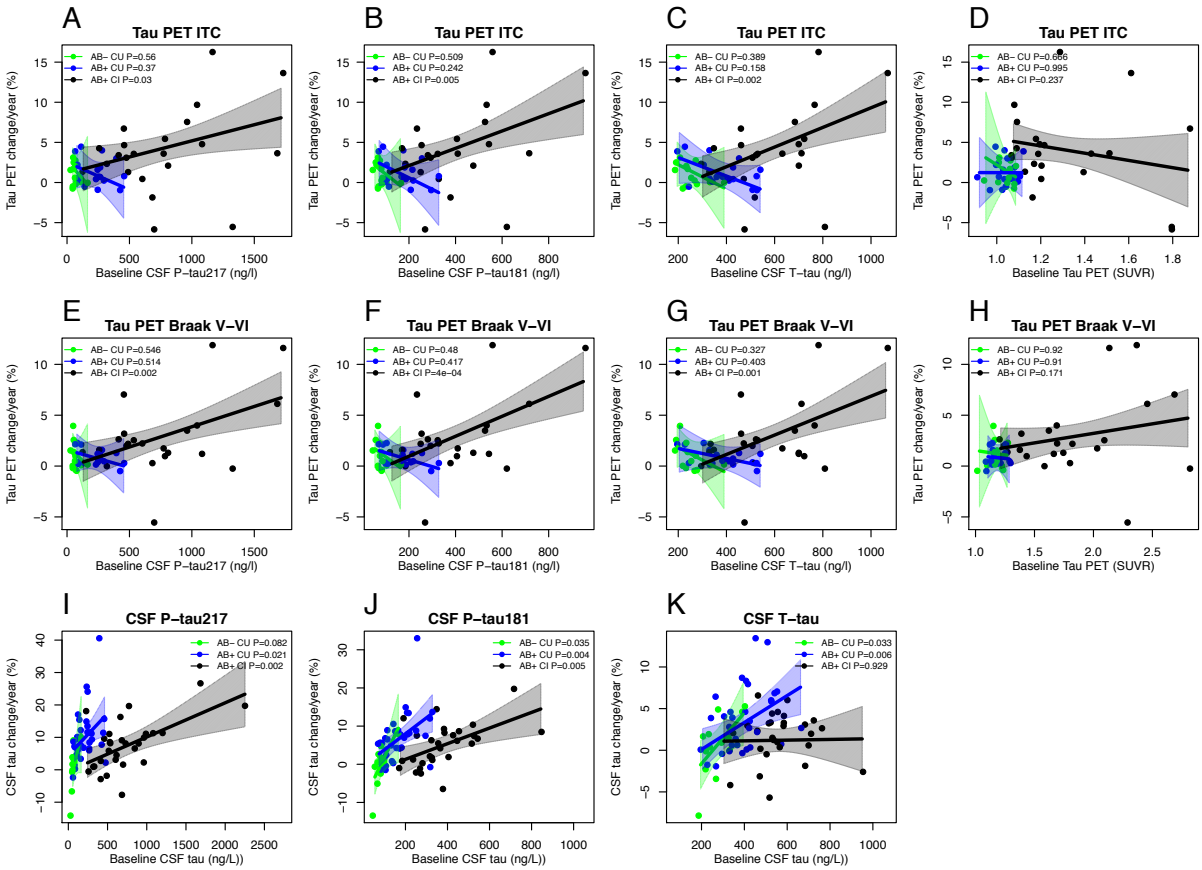

Fig. S3. Associations between baseline tau biomarkers and longitudinal tau biomarkers.

Panels A-H: Longitudinal change in Tau PET by baseline CSF P-tau217 (panels A, E), P-tau181 (panels B, F), T-tau (panels C, G), and baseline (same region) Tau PET (panels D, H). Panels I-K: Longitudinal change in CSF tau (% change per year) by baseline CSF tau (same tau measure). Associations were tested in linear regression models adjusted for age, sex and Aβ by impairment status, contrasting Aβ- cognitively unimpaired (CU), Aβ+ CU and Aβ+ cognitively impaired (CI, defined as Clinical Dementia Rating score 0.5-3). Note that all CI individuals were combined for this analysis (rather than separating mild cognitive deficits and dementia) due to the small number of individuals with longitudinal Tau PET data.

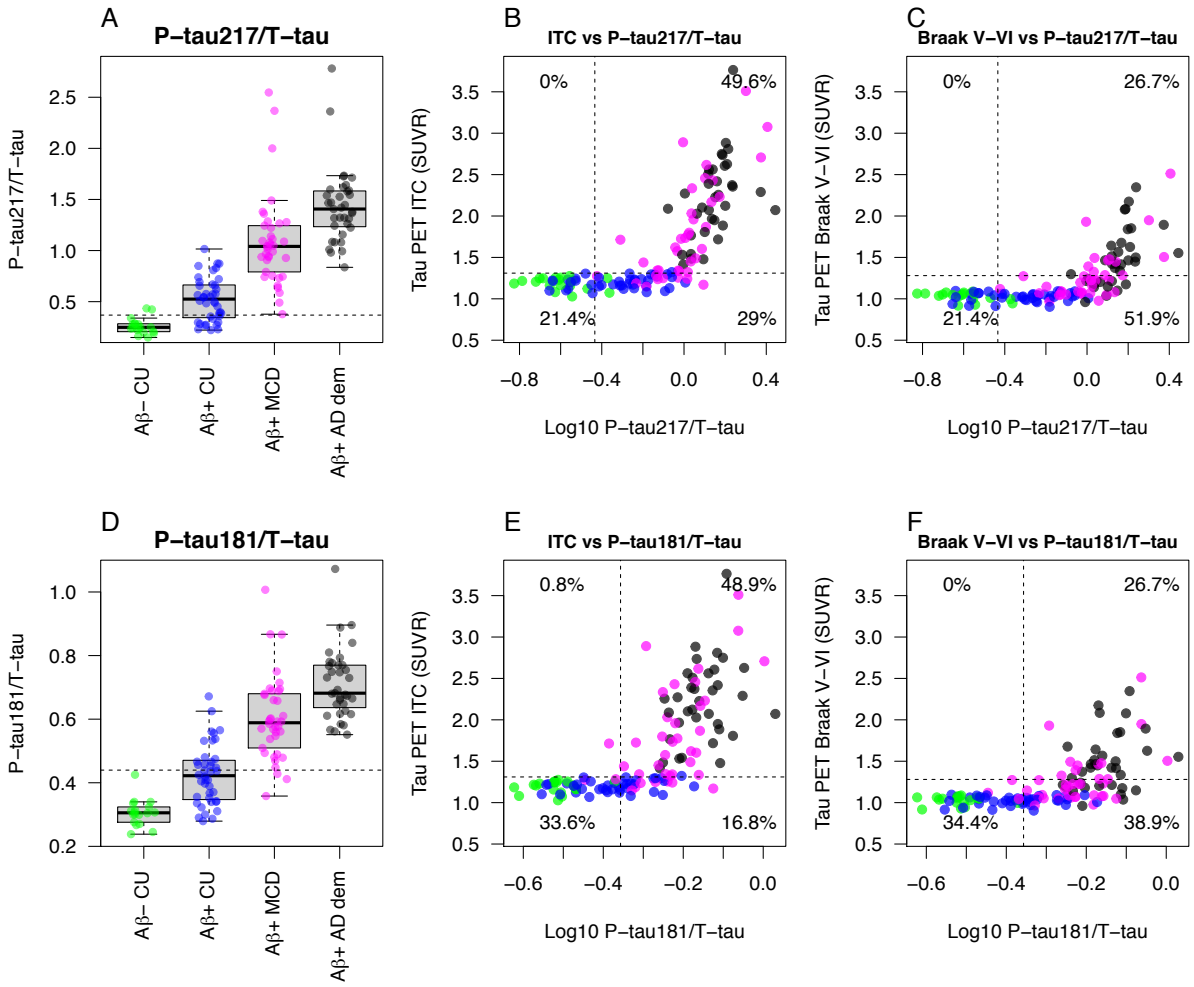

Fig. S4. CSF P-tau217/T-tau and P-tau181/T-tau ratios by Aβ and cognitive impairment.

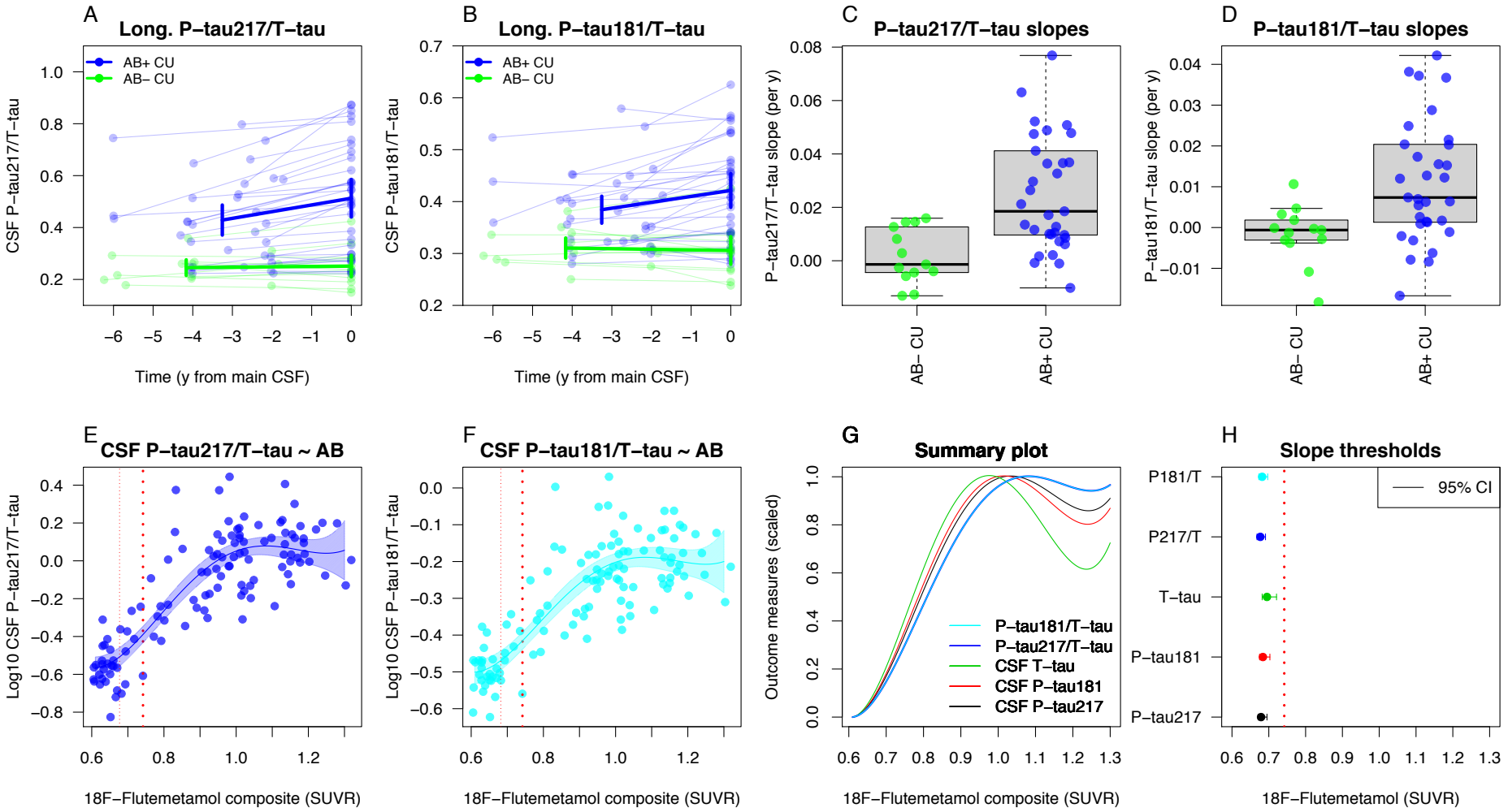

Fig. S5. CSF P-tau/T-tau ratios over time and by continuous Amyloid PET load.

Panels A-D show longitudinal CSF P-tau217/T-tau and P-tau181/T-tau ratios in CU with an additional early sample, preceding the main CSF sample by 2-6 years. Slope differences were tested in linear regression models, adjusted for age, sex, and time span between the two lumbar punctures. CSF P-tau217/T-tau and CSF P-tau181/T-tau ratios increased significantly more in Aβ+ CU than in Aβ- CU individuals (P-tau217/T-tau,

difference: β=0.0236 per year, P=0.0036; P-tau181/T-tau, difference: β=0.013 per year, P=0.014). Panels E-F: CSF P-tau/T-tau ratios in relation to global cortical ^{18}F-flutemetamol load. The solid lines are fits from spline models of tau biomarkers on ^{18}F-flutemetamol. The thick dotted lines show an a priori ^{18}F-flutemetamol threshold (0.743 SUVR). The thin dotted lines indicate the ^{18}F-flutemetamol level where tau biomarkers are significantly increased from baseline. Panel G: A summary plot of the models in E-F, with all biomarkers on a common scale ranging from 0 (baseline levels) to 1 (the mean levels in the top 10 percentiles). For reference, the summary plot also includes corresponding models with CSF P-tau217, P-tau181 and T-tau (from main Figure 3). The summary plot shows that all CSF tau biomarker levels increase early (prior to the a priori threshold for ^{18}F-flutemetamol). The CSF P-tau/T-tau ratios have parallel curves (overlapping in the summary plot). In contrast to the individual CFS tau biomarkers, the ratios show less tendency to decrease at high ^{18}F-flutemetamol levels. Panel H: Thresholds for the slopes with 95% CI. All biomarkers were log10-transformed to facilitate the fit of the spline models. CU, cognitively unimpaired.

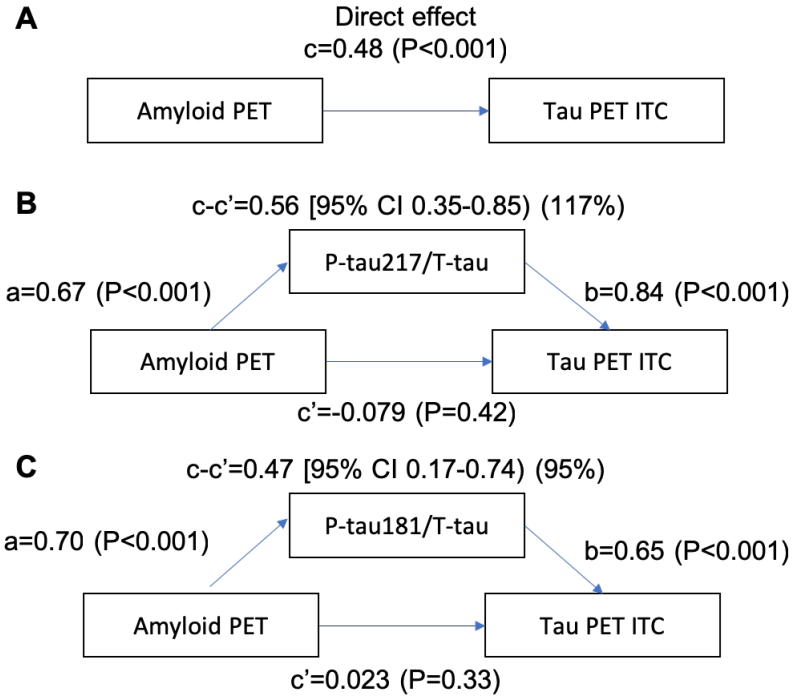

Fig. S6.CSF tau ratios as statistical mediators of the relationship between Amyloid PET and Tau PET.

Mediation analysis of the relationship between Amyloid PET, CSF tau ratios and Tau PET in inferior temporal cortex (ITC). Amyloid PET was the global cortical ^{18}F-flutemetamol uptake (the direct effect [c] on Tau PET is shown in panel A). Analyses are shown with CSF P-tau217/T-tau (panel B) and CSF P-tau181/T-tau (panel C) as mediators. Both CSF tau ratios were strong mediators of the relationship between Amyloid PET and Tau PET. The mediated effect is designated [c-c']. The remaining effect of Amyloid PET on Tau PET after adjusting for the mediator is designated [c']. The direct effect of Amyloid PET on the mediator is [a], and the direct effect of the mediator on Tau PET is [b]. These analyses included individuals who were CU or who had mild cognitive deficits, to focus the analyses on the effects of Aβ on tau in early stages of AD. To facilitate model comparisons, all models use continuous standardized (z-scores) data for biomarkers. CU, cognitively unimpaired.

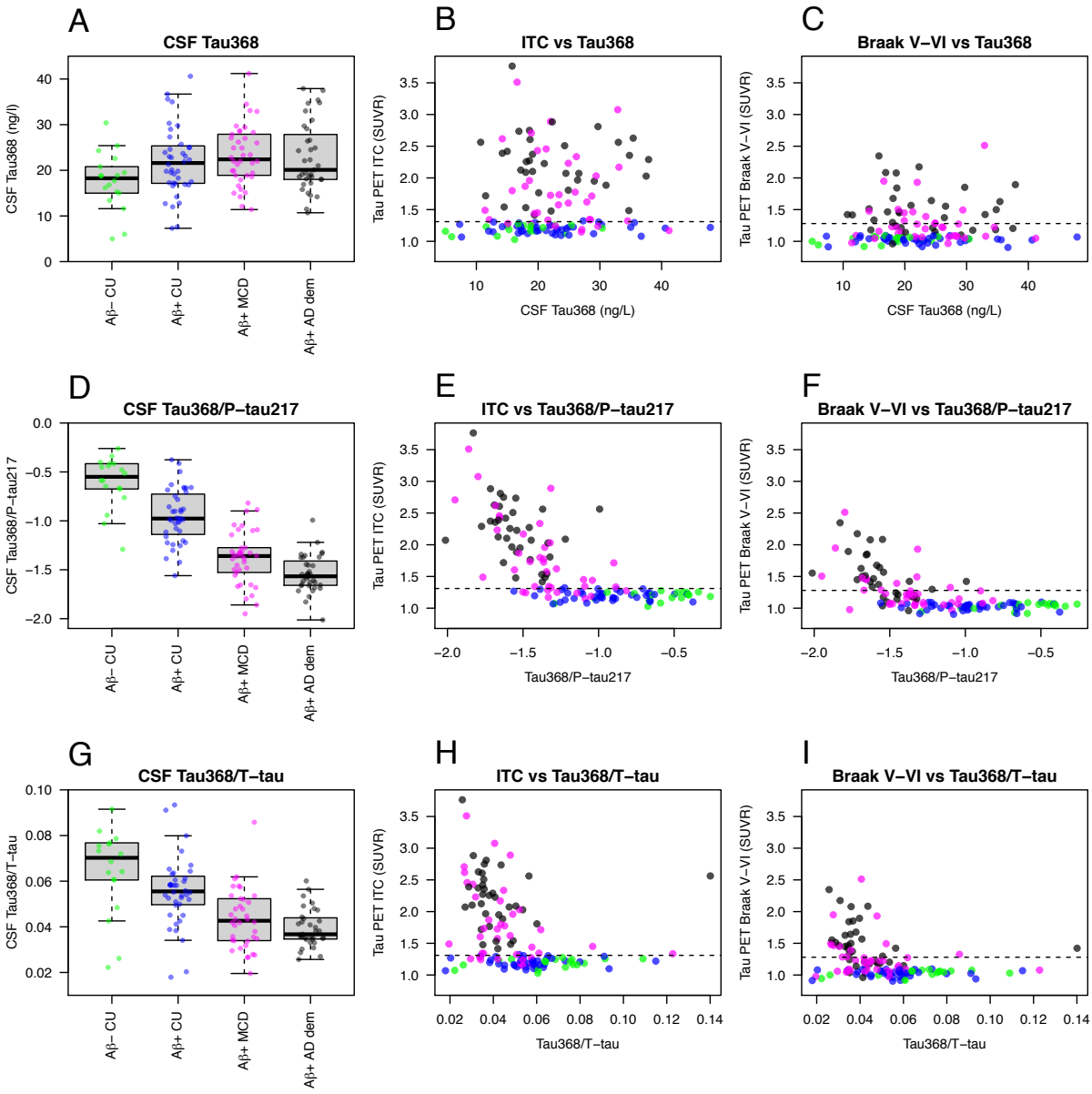

Fig. S7. CSF levels of truncated tau (Tau368).

The figure shows data on a C-terminally truncated fragment of tau (Tau368), used alone (panels A-C) or in ratios with P-tau217 (panels D-F) or T-tau (panels G-I). The CSF Tau368 biomarker was tested in relation to diagnostic groups (panels A, D, and G), using linear regression models adjusted for age and sex. Aβ+ CU did not differ significantly from Aβ- CU in CSF Tau368 (P=0.053). Aβ+ individuals with MCD or AD dementia had higher CSF Tau368 than Aβ- CU (P=0.0039, P=0.012), but did not differ from Aβ+ CU (P=0.47, P=0.75), and there was no difference between Aβ+ MCD or AD dementia (P=0.79). The CSF Tau368/P-tau217 ratio differed significantly between all groups (P≤0.0023). The CSF Tau368/T-tau ratio did not differ between Aβ- CU and Aβ+ CU (P=0.12) but was reduced in Aβ+ MCD or AD dementia compared to both Aβ- and Aβ+ CU CU (P≤0.0082). The CSF Tau368/T-tau ratio did not differ between Aβ+ MCD and AD dementia (P=0.44). The CSF Tau368 biomarker was also plotted in relation to Tau PET uptake, sampled in inferior temporal cortex (ITC, panels B, E, and H), and in Braak V-VI regions (panels C, F and I). CU, cognitively unimpaired; MCD, mild cognitive deficits; dem, dementia.

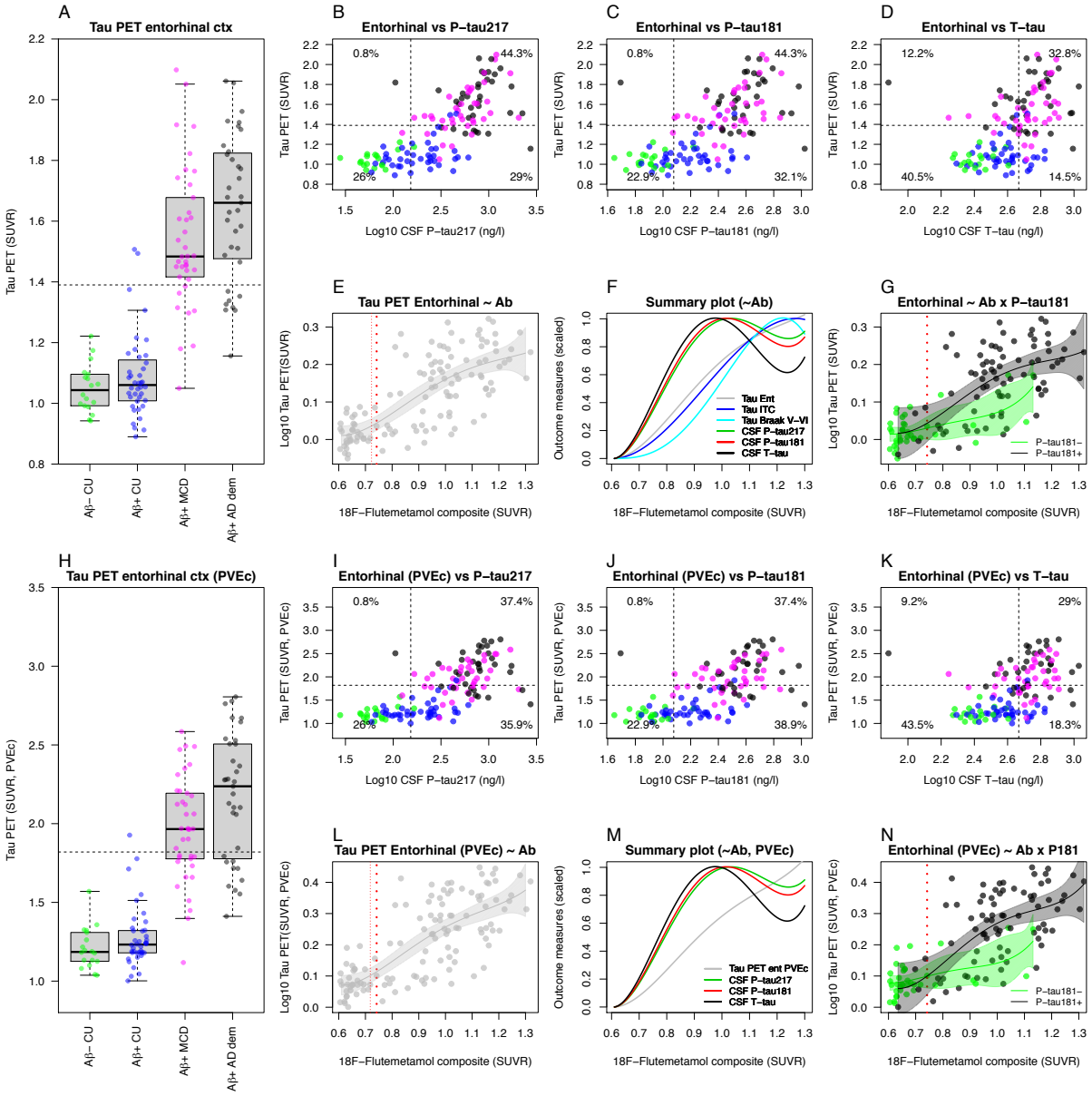

Fig. S8. Entorhinal Tau PET analyses.

Panel A: Entorhinal tau PET uptake did not differ significantly between Aβ- CU to Aβ+ CU (β=0.249, P=0.40), but increased in Aβ+ MCD (β=1.604, P<0.001 versus Aβ- CU; β=1.575, P<0.001 versus Aβ+ CU), and Aβ+ AD dementia (β=1.678, P<0.001 versus Aβ- CU; β=1.657, P<0.001 versus Aβ+ CU, β=0.418, P=0.069 versus Aβ+ MCD). The dashed line indicate an priori cut-point for positivity (SUVR 1.39), defined in independent populations of CU (at mean plus two standard deviations) (*Ossenkoppele et al, JAMA 2019*). Panels B-D: CSF tau biomarkers and entorhinal Tau PET in Aβ- CU (green), Aβ+ CU (blue) subjects, Aβ+ mild cognitive deficits (magenta) and Aβ+ AD dementia patients (black). The dashed lines indicate a priori cut-points. The percentages indicate proportion of subjects in each quadrant. CSF tau biomarker data were log10-transformed for visualization purposes. The majority of cases had concordant CSF and Tau PET status. Within the discordant cases, the majority had abnormal CSF P-tau and normal Tau PET (but discordance for CSF T-tau and Tau PET was evenly distributed). Panel E: Entorhinal Tau PET in relation to global cortical ^{18}F-flutemetamol load. The solid line is a fit from a spline model for Tau PET on ^{18}F-flutemetamol. The thick dotted line shows an a priori ^{18}F-flutemetamol threshold (0.743

SUVR). The thin dotted line indicates the ^{18}F-flutemetamol level where the Tau PET uptake is significantly increased from baseline (0.723 [95% CI 0.696-0.773] SUVR). Panel F: A summary plot of different CSF and PET models, including entorhinal Tau PET, with all biomarkers on a common scale ranging from 0 (baseline levels) to 1 (the mean levels in the top 10 percentiles). The summary plot shows that all CSF tau biomarker levels increase early and have steeper slopes of increase than the Tau PET measures, including entorhinal tau. All biomarkers were log10-transformed to facilitate the fits of the spline models. Panel G: Spline models stratified by CSF P-tau181 status, showing that most increase in entorhinal Tau PET by ^{18}F-flutemetamol occurs in CSF P-tau181 positive individuals (although there are few CSF P-tau181 negative cases with positive ^{18}F-flutemetamol, making the estimates in the CSF P-tau181 group uncertain). Panels H-N show similar analyses but using partial volume error corrected (PVEc) data for entorhinal Tau PET uptake. Panel H: Entorhinal tau PET uptake did not differ significantly between Aβ- CU to Aβ+ CU (β=0.338, P=0.25), but increased in Aβ+ MCD (β=1.701, P<0.001 versus Aβ- CU; β=1.648, P<0.001 versus Aβ+ CU), and Aβ+ AD dementia (β=1.695, P<0.001 versus Aβ- CU; β=1.697, P<0.001 versus Aβ+ CU, β=0.508, P=0.027 versus Aβ+ MCD). The dashed line indicates an priori cut-point for positivity (SUVR 1.82), defined in independent populations of CU (at mean plus two standard deviations) (*Ossenkoppele et al, JAMA 2019*). CU, cognitively unimpaired; MCD, mild cognitive deficits; dem, dementia.

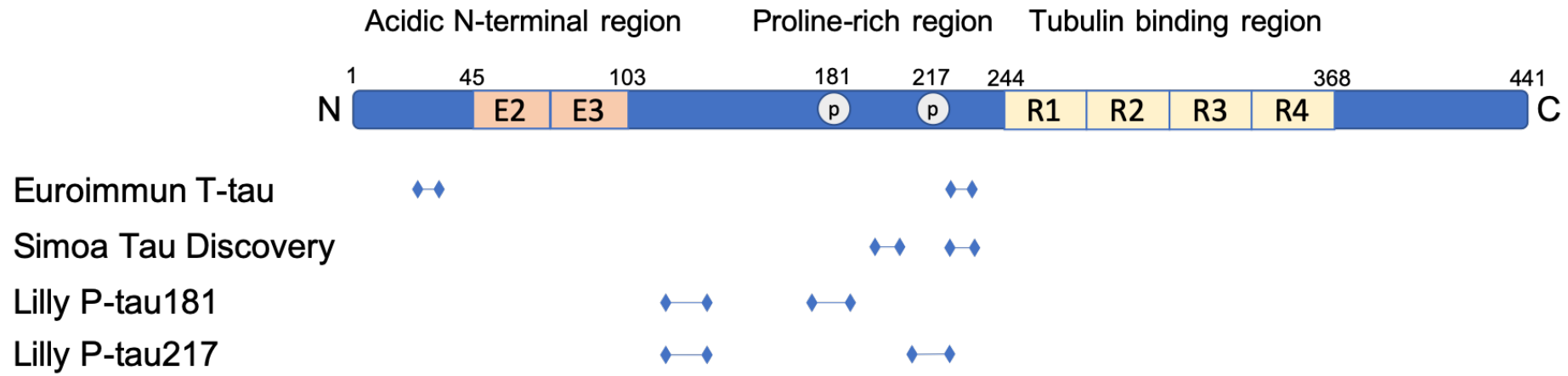

Fig. S9. Epitopes for anti-tau antibodies.

The cartoon shows epitopes for the main antibodies used in the study for T-tau and P-tau. The Simoa Tau Discovery assay was used for mouse CSF T-tau. The antibodies used in the Simoa® assay recognize epitopes 207–214 and 174–184 of the murine sequence (which correspond to epitopes 218–225 and 185–195 of the human sequence).