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Supplementary information

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Plasma MTBR-tau243 biomarker identifies tau tangle pathology in Alzheimer's disease

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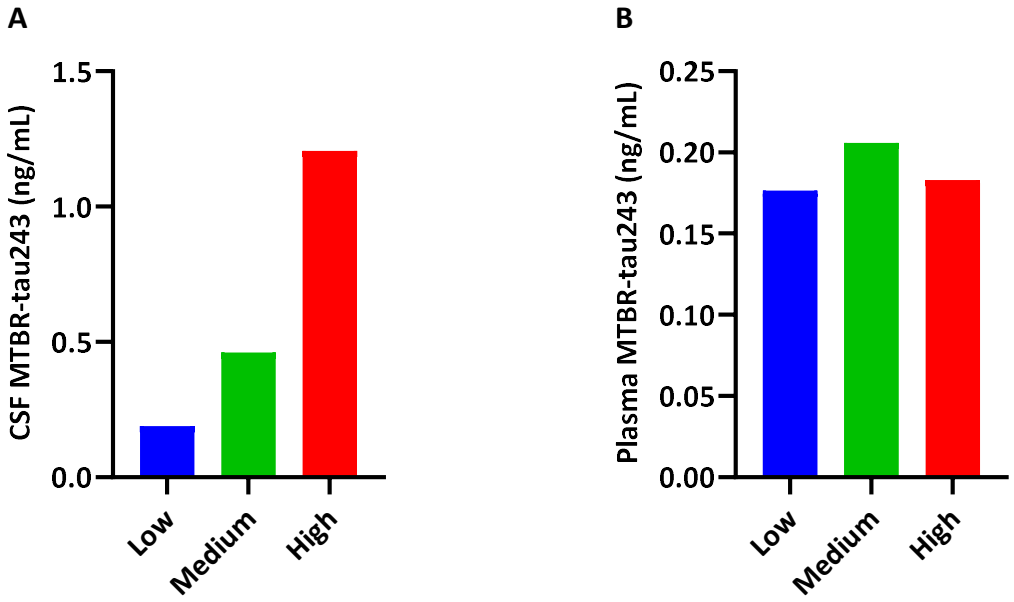
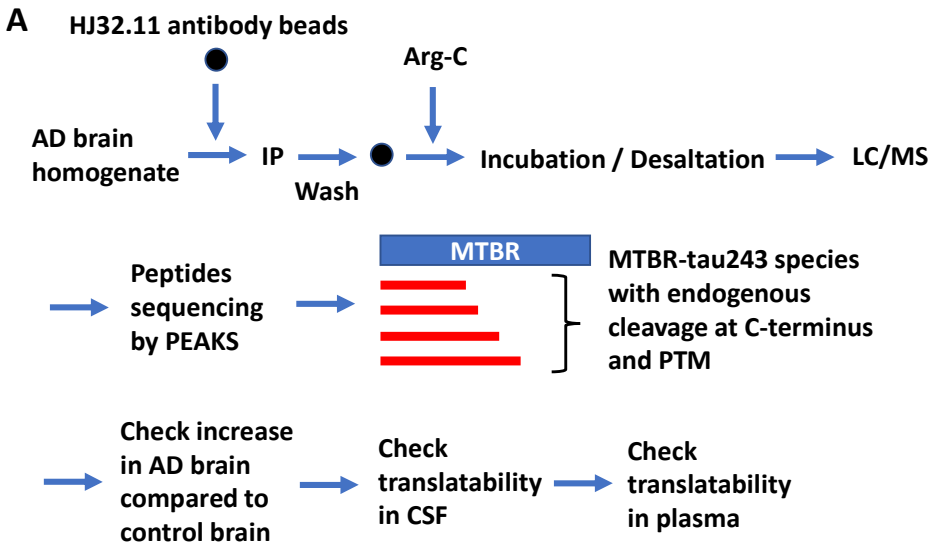


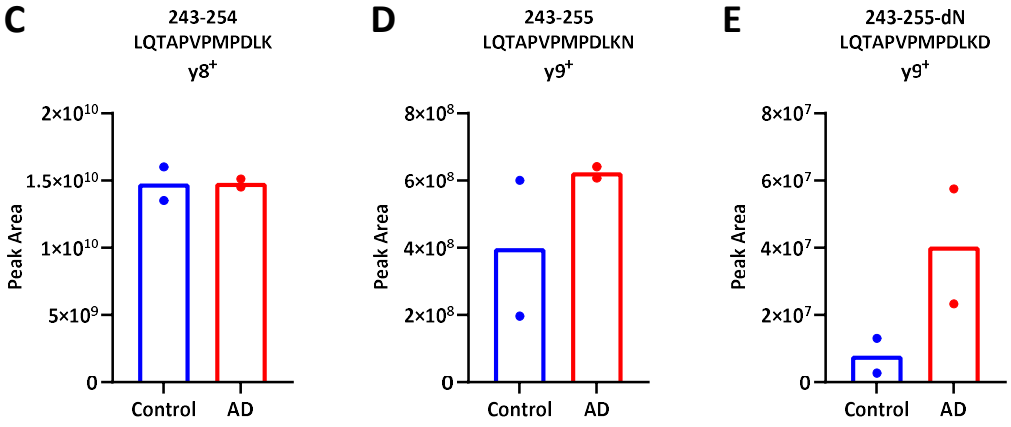
Supplementary Figure 1: MTBR-tau243 in matched plasma and CSF samples from three groups with different levels of tau-PET signal (low, medium, and high) measured by the tryptic digestion method.

(**A**) MTBR-tau243 levels in CSF samples from three groups with different levels of tau-PET signal (low, medium, and high). (**B**) MTBR-tau243 levels in plasma samples collected at the same time as the CSF samples from groups with different levels of tau-PET signal (low, medium, and high). The previously reported tryptic digestion method was used to quantify the tryptic peptide residue 243-254 (sequence: LQTAPVPMPDLK) for both CSF and plasma samples[20]. We found that this tryptic MTBR-tau243 increased as the tau-PET status changed from low to high only in CSF and not in plasma.



B

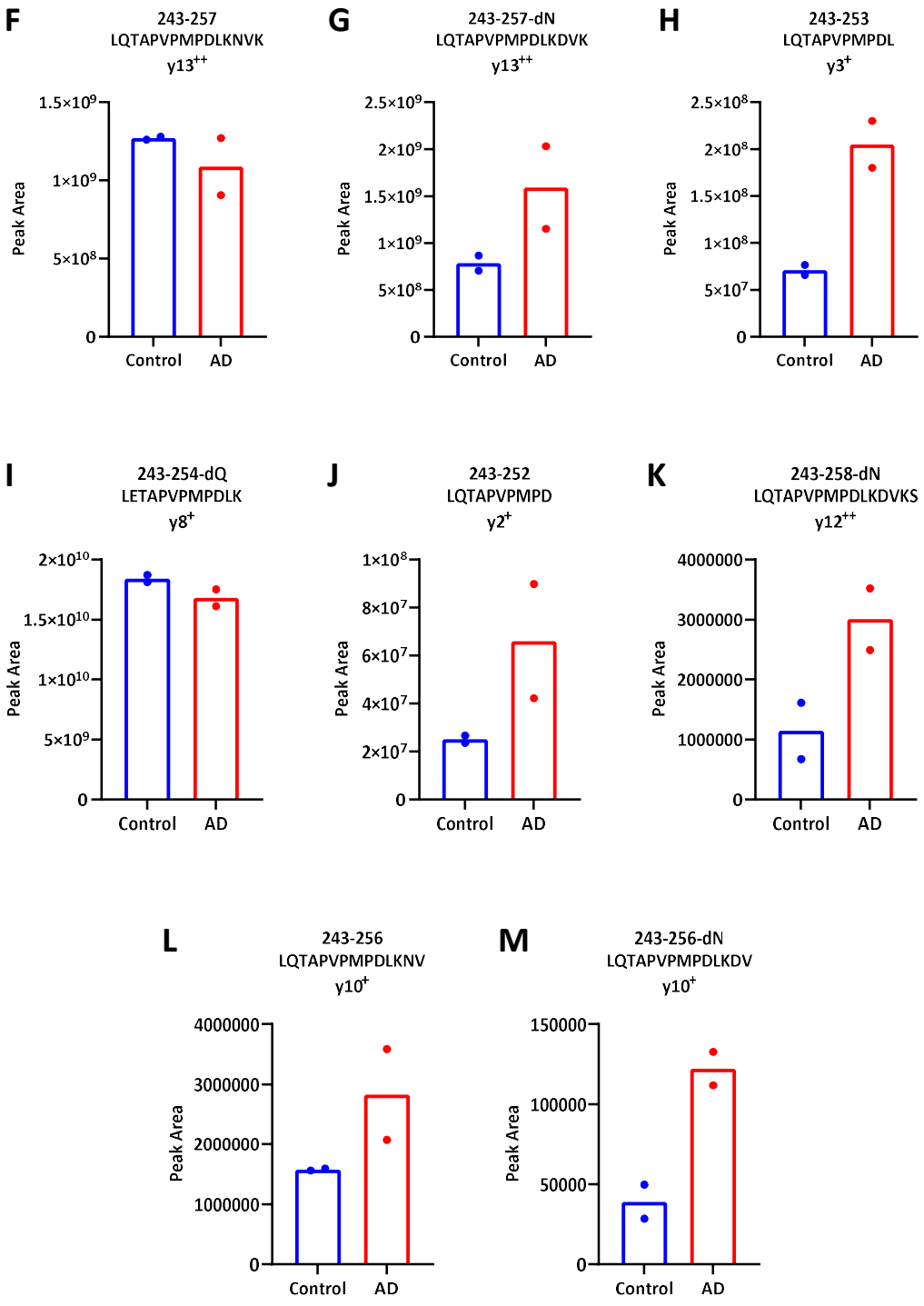
Peptide Sequence	Residues	Change in AD brain	Change in high tau-PET CSF
LQTAPVPMPDLK	243-254	NC	NC
LQTAPVPMPDLKN	243-255	+	NC
LQTAPVPMPDLKD	243-255-dN	+	+
LQTAPVPMPDLKNVK	243-257	NC	NC
LQTAPVPMPDLKDVK	243-257-dN	+	+
LQTAPVPMPDL	243-253	+	NC
LETAPVPMPDLK	243-254-dQ	NC	NC
LQTAPVPMPD	243-252	+	NC
LQTAPVPMPDLKDVKS	243-258-dN	+	+
LQTAPVPMPDLKNV	243-256	+	+
LQTAPVPMPDLKDV	243-256-dN	+	+



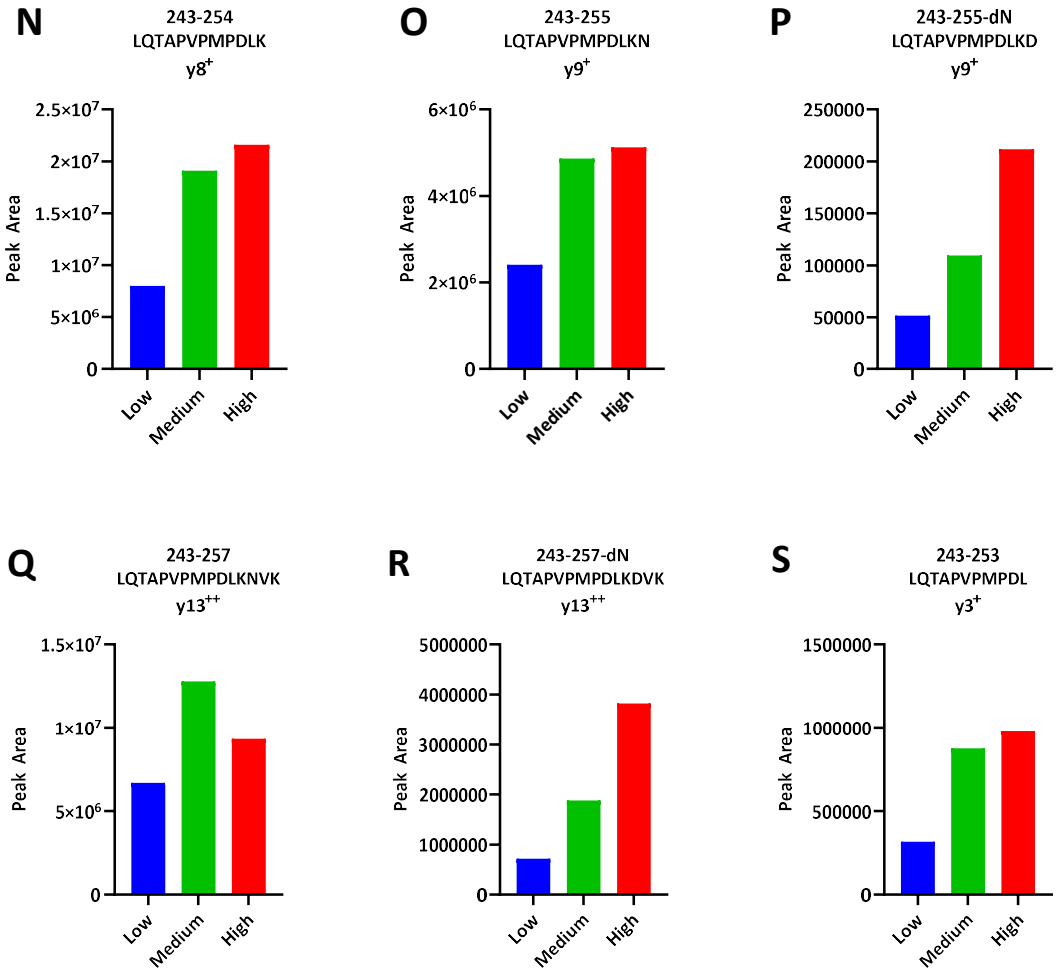
Supplementary Figure 2: Identification of endogenously-cleaved MTBR-tau243 species as a function of diagnosis and tau pathology burden in AD brain, CSF, and plasma samples.

(**A**) Schematic of identification scheme for endogenously-cleaved MTBR-tau243 species in brain homogenate, cerebrospinal fluid (CSF), and plasma samples that varied in AD cases and controls. Tau species were immunoprecipitated from brain homogenate by HJ32.11 antibody-conjugated beads, then digested by Arg-C to obtain MTBR-tau species starting at residue 243 and ending at C-terminal endogenous cleavages. Endogenously-cleaved

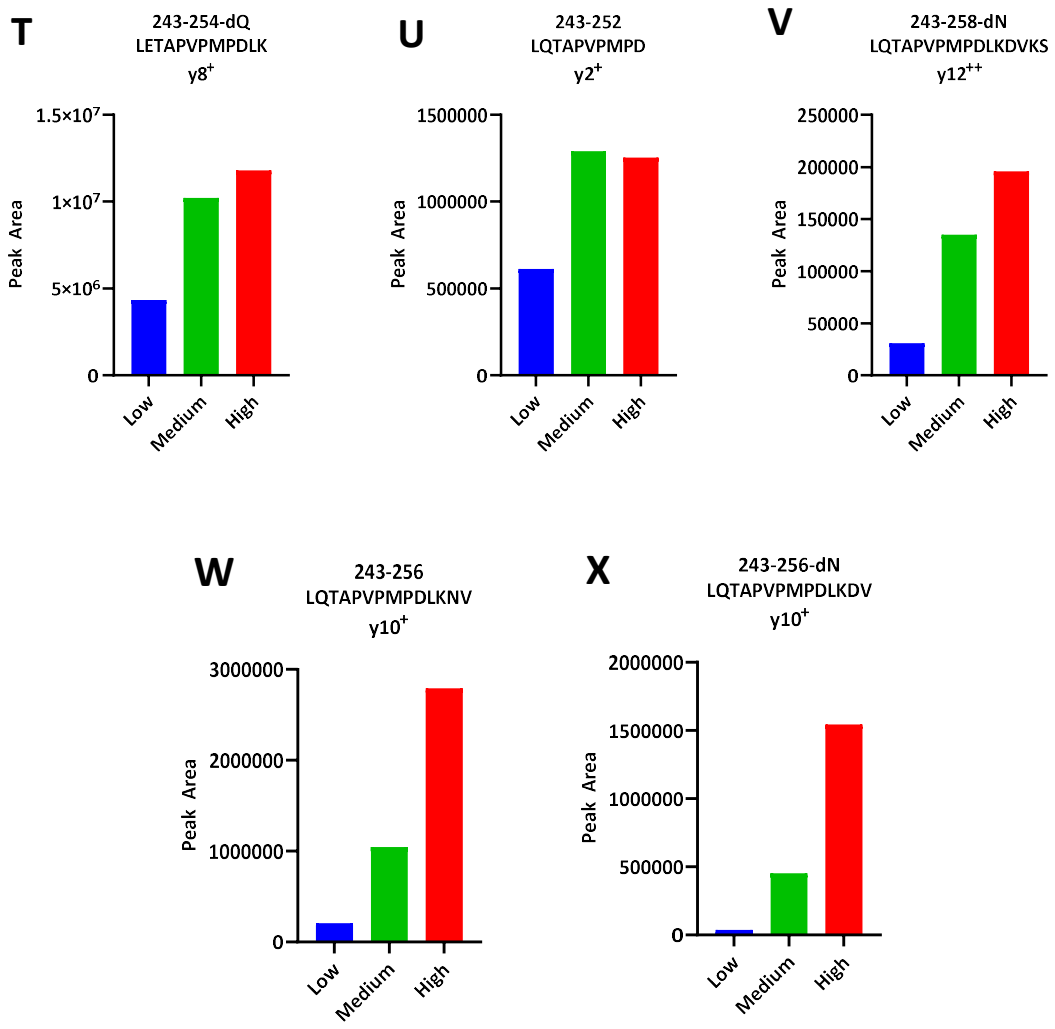
MTBR-tau243 peptides were identified by mass spectrometry (MS) analysis followed by sequencing with PEAKS X software (Bioinformatic Solutions). Levels of eleven endogenously-cleaved MTBR-tau243 peptides in brain homogenates from AD cases and controls were compared to determine whether there was a change or no change (NC). Next, endogenously-cleaved MTBR-tau243 species were quantified in CSF samples from three individuals with different levels of tau-PET signal (low, medium, and high) to investigate what species were associated with tau-PET signal. Finally, the selected endogenous-cleaved MTBR-tau243 species (sequence: LQTAPVPMPDLKDV) was quantified in matched plasma samples collected at the same time as the previously analyzed CSF samples. (**B**) Table summarizing the eleven endogenously-cleaved MTBR-tau243 species identified in AD brain homogenate. Residues “dN” and “dQ” indicate the deamidation of asparagine and glutamine, respectively. “+” and “NC” in table indicate either an increase or no change for each species, respectively, in the CSF sample from the individual with high tau-PET signal compared to the two individuals with lower tau-PET signal. (**C - M**) MS peak area of each endogenously-cleaved MTBR-tau243 species in control (n=2) and AD (n=2) brains. (**N - X**) MS peak area of each endogenously-cleaved MTBR-tau243 species in CSF samples from three individuals with different levels of tau-PET signal (low, medium, and high). The peptide residue 243-256-dN (**X**) was much higher in CSF from the individual with high tau-PET compared to the individual with medium tau-PET and was very low in the CSF from the individual with low tau-PET, suggesting that 243-256-dN was specific to the presence of AD tau aggregate pathology. Accordingly, this endogenously-cleaved MTBR-tau243 species (sequence: LQTAPVPMPDLKDV) was selected for characterization in plasma.



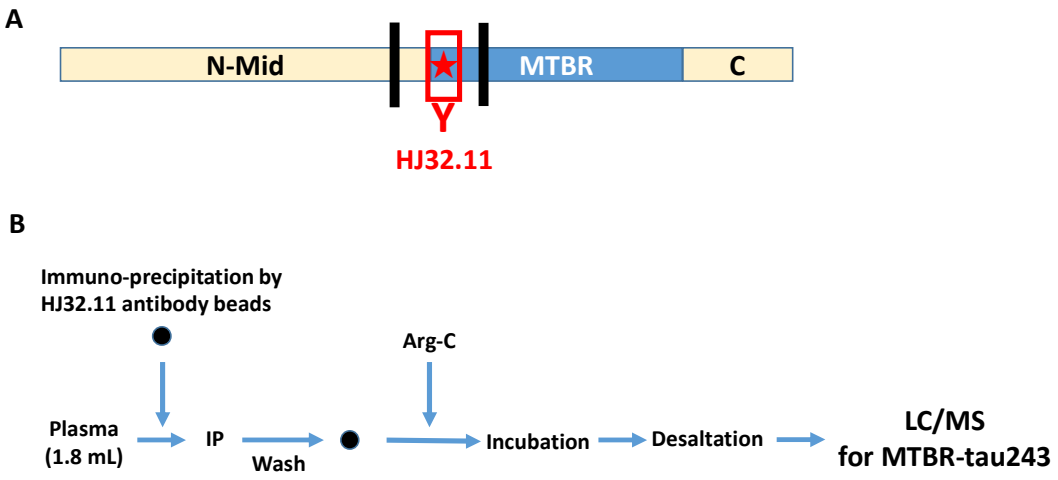
Supplementary Figure 2 (continued): Identification of endogenously-cleaved MTBR-tau243 species increasing in AD brain, CSF, and plasma samples.



Supplementary Figure 2 (continued): Identification of endogenously-cleaved MTBR-tau243 species increasing in AD brain, CSF, and plasma samples.



Supplementary Figure 2 (continued): Identification of endogenously-cleaved MTBR-tau243 species increasing in AD brain, CSF, and plasma samples.



Supplementary Figure 3: Schematic of analytical procedure of plasma eMTBR-tau243.

(**A**) Schematic of binding sites of the antibody used in this study and (**B**) the sample preparation scheme. Plasma eMTBR-tau243 was immunoprecipitated by the HJ32.11 antibody (generated by David Holtzman's lab). The HJ32.11 antibody was coupled to sepharose beads at 3 mg/gram. Plasma eMTBR-tau243 was immunoprecipitated from 1.4 mL plasma with 45 ug of the antibody. The sample was centrifuged, and 1.4 mL of the supernatant was mixed with 20 µL of solution containing 0.05 fmol/µL AQUA peptide (sequence: VAVVRTPPKSPSSAKSRLQTAPVPMPDLK$^{\#}$D*V, D*: deamidated asparagine, K$^{\#}$: 13C15N-labeled lysine) as internal standard. Tau species consisting primarily of N-terminal to mid-domain regions and late MTBR region (after 256 in R1) were not immunoprecipitated by HJ32.11 antibody because of the major truncations of tau. The immunoprecipitated eMTBR-tau species were digested by Arg-C (Promega) and desaltated by OASIS HLB micro-elution plate (Waters). The resulting eMTBR-tau243 peptide (sequence: LQTAPVPMPDLKD*V, D*: deamidated asparagine) and the corresponding isotopomer were analyzed by nanoAcquity ultra-performance LC system (Waters) coupled to Orbitrap Tribrid Eclipse mass spectrometer (Thermo Scientific) operating in parallel reaction monitoring mode. MS peaks were extracted using Quan Browser in Xculibur software (ver. 3.0.63) (Thermo Scientific) with 5 ppm mass tolerance. Microsoft Excel was used to calculate the plasma eMTBR-tau243 levels.

Supplementary Tables:

	Overall		CU-		CU+		MCI+		AD+	
	n	108	n	57	n	4	n	10	n	37
Age, years	108	76.1 (7.0)	57	78.0 (6.4)	4	77.8 (7.5)	10	73.9 (2.7)	37	73.6 (8.0)
Women, n	108	53 (49.1%)	57	34 (59.6%)	4	2 (50.0%)	10	6 (60.0%)	37	11 (29.7%)
APOE-e4 carriers, n	108	56 (51.9%)	57	15 (26.3%)	4	4 (100%)	10	7 (70.0%)	37	30 (81.1%)
Years of education	105	11.9 (3.39)	57	12.0 (3.30)	4	13.4 (4.82)	10	10.7 (2.50)	34	12.0 (3.64)
CSF Aβ42/40	108	0.779 (0.300)	57	1.03 (0.146)	4	0.460 (0.0949)	10	0.485 (0.0985)	37	0.499 (0.105)
CSF Aβ42/40 positivity, n	108	51 (47.2%)	57	0 (0%)	4	4 (100%)	10	10 (100%)	37	37 (100%)
Tau-PET (Braak I-VI global),SUVR	108	1.37 (0.472)	57	1.08 (0.0718)	4	1.29 (0.157)	10	1.41 (0.218)	37	1.82 (0.541)
Tau-PET positivity, n	108	41 (38.0%)	57	0 (0%)	4	1 (25.0%)	10	8 (80.0%)	37	32 (86.5%)
Plasma eMTBR-tau243 (fmol/L)	108	4.88 (8.45)	57	0.08 (0.11)	4	4.07 (5.47)	10	5.25 (3.69)	37	12.2 (10.7)
MMSE	108	24.7 (6.12)	57	28.9 (1.35)	4	26.3 (3.77)	10	24.5 (3.57)	37	18.0 (5.48)
Cortical thickness (by MRI)	94	2.57 (0.208)	51	2.70 (0.120)	4	2.60 (0.103)	9	2.51 (0.133)	30	2.36 (0.187)

Supplementary Data Table 1: The pilot BioFINDER-2 participants characteristics.

Data are presented as mean (SD). Values in square brackets indicate the % in total number within the group.

Abbreviations: Aβ, amyloid β; AD+, Alzheimer's disease dementia Aβ-positive; CSF, cerebrospinal fluid; CU-, cognitively unimpaired Aβ-negative; CU+, cognitively unimpaired Aβ-positive; MCI+, mild cognitive impairment Aβ-positive; MMSE, Mini-Mental State Examination; eMTBR-tau243, endogenously-cleaved microtubule binding region containing residue 243; MRI, magnetic resonance imaging; PET, positron emission tomography; SUVR, standardized uptake value ratio.

	Overall		CU-		CU+		Very mild AD+		AD+	
	n	55	n	15	n	14	n	18	n	8
Age, years	55	73.2 (6.1)	15	71.1(4.1)	14	73.9 (6.3)	18	75.0 (6.3)	8	71.9 (8.2)
Women, n	55	24 [43.6 %]	15	7 [46.7 %]	14	8 [57.1 %]	18	8 [44.4 %]	8	1 [12.5 %]
APOE-e4 carriers, n	55	28 [50.9 %]	15	2 [13.3 %]	14	7 [50.0 %]	18	15 [83.3 %]	8	4 [50.0 %]
CSF Aβ42/40	55	0.1166 (0.0395)	15	0.1712 (0.0207)	14	0.0998 (0.0195)	18	0.0912 (0.0212)	8	0.1007 (0.0218)
CSF Aβ42/40 positivity, n	55	40 [72.7 %]	15	15 [0 %]	14	14 [100 %]	18	18 [100 %]	8	8 [100 %]
Plasma eMTBR-tau243 (fmol/L)	55	17.8 (33.0)	15	1.0 (1.0)	14	10.6 (28.5)	18	18.5 (31.2)	8	60.2 (42.3)

Supplementary Data Table 2: The pilot Knight ADRC participants characteristics.

Data are presented as mean (SD). Values in square brackets indicate the % in total number within the group.

Abbreviations: Aβ, amyloid β; AD+, Alzheimer's disease dementia Aβ-positive; CSF, cerebrospinal fluid; CU-, cognitively unimpaired Aβ-negative; CU+, cognitively unimpaired Aβ-positive; eMTBR-tau243, endogenously-cleaved microtubule binding region containing residue 243.

Diagnostic groups	eMTBR-tau243	%p-tau217	%p-tau205
CU+ - CU-	0.235	<0.001	<0.001
MCI+ - CU-	<0.001	<0.001	<0.001
AD+ - CU-	<0.001	<0.001	<0.001
PD/DLB - CU-	0.967	0.314	0.495
PSP/CBS - CU-	0.999	0.86	0.942
VaD - CU-	0.993	0.587	0.53
FTD - CU-	0.991	0.997	0.987
MCI- - CU-	0.956	0.908	0.057
other - CU-	0.981	0.298	0.335
MCI+ - CU+	<0.001	<0.001	<0.001
AD+ - CU+	<0.001	<0.001	<0.001
PD/DLB - CU+	1	0.773	0.998
PSP/CBS - CU+	1	0.989	1
VaD - CU+	1	1	1
FTD - CU+	1	1	0.483
MCI- - CU+	0.986	<0.001	0.336
Other - CU+	1	0.981	1
AD+ - MCI+	<0.001	<0.001	<0.001
PD/DLB - MCI+	0.588	<0.001	<0.001
PSP/CBS - MCI+	0.954	0.051	0.124
VaD - MCI+	1	0.671	0.941
FTD - MCI+	1	0.661	0.02
MCI- - MCI+	<0.001	<0.001	<0.001
Other - MCI+	0.813	0.003	0.029
PD/DLB - ADD	<0.001	<0.001	<0.001
PSP/CBS - ADD	<0.001	<0.001	<0.001
VaD - ADD	0.005	0.001	0.054
FTD - ADD	0.281	0.008	0
MCI- - ADD	<0.001	<0.001	<0.001
Other - ADD	<0.001	<0.001	<0.001
PSP/CBS - PD/DLB	1	1	1
VaD - PD/DLB	1	1	0.996
FTD - PD/DLB	1	1	0.748

MCI- - PD/DLB	1	0.829	1
Other - PD/DLB	1	1	1
VaD - PSP/CBS	1	1	0.999
FTD - PSP/CBS	1	1	0.835
MCI- - PSP/CBS	1	0.988	1
Other - PSP/CBS	1	1	1
FTD - VaD	1	1	0.52
MCI- - VaD	1	0.844	0.973
Other - VaD	1	1	1
MCI- - FTD	0.999	1	0.783
Other - FTD	1	1	0.628
Other - MCI-	1	0.754	0.997

Supplementary Table 3: Summary of p-values in comparison of plasma biomarker levels by diagnostic groups the validation BioFINDER-2 cohort

Statistical differences were assessed using ANCOVA for each plasma biomarker independently, adjusted for age and sex followed by Tukey-corrected post hoc pairwise comparisons (two-sided analysis). Abbreviations: AD+, Alzheimer's disease dementia with Aβ-pathology; CBS, corticobasal syndrome; CU-, cognitively unimpaired without Aβ-pathology; CU+, cognitively unimpaired with Aβ-pathology; DLB, dementia with Lewy bodies; FTD, frontotemporal dementia; MCI-, mild cognitive impairment without Aβ-pathology; MCI+, mild cognitive impairment with Aβ-pathology; PD, Parkinson's disease; PSP, progressive supranuclear palsy; VaD, vascular dementia.

PET	Plasma	β[95%CI]	p-value	p_{diff}	R^2
Tau-PET positive only					
Tau-PET – Braak I-VI (n=172)	eMTBR-tau243	0.68 [0.57,0.80]	<0.001	Ref.	0.45
	%p-tau217	0.57 [0.44,0.70]	<0.001	0.002	0.33
	%p-tau205	0.50 [0.36,0.64]	<0.001	<0.001	0.22
Tau-PET – Braak I-II (n=280)	eMTBR-tau243	0.57 [0.47,0.67]	<0.001	Ref.	0.32
	%p-tau217	0.51 [0.41,0.61]	<0.001	0.12	0.29
	%p-tau205	0.38 [0.26,0.49]	<0.001	0.008	0.15
Tau-PET – Braak III-IV (n=190)	eMTBR-tau243	0.67 [0.56,0.78]	<0.001	Ref.	0.46
	%p-tau217	0.57 [0.45,0.69]	<0.001	0.004	0.35
	%p-tau205	0.45 [0.31,0.58]	<0.001	<0.001	0.20
Tau-PET – Braak V-VI (n=122)	eMTBR-tau243	0.64 [0.48,0.79]	<0.001	Ref.	0.36
	%p-tau217	0.47 [0.30,0.64]	<0.001	<0.001	0.20
	%p-tau205	0.34 [0.16,0.52]	<0.001	<0.001	0.10
Aβ-positive only					
Tau-PET – Braak I-VI (n=436)	eMTBR-tau243	0.74 [0.68,0.81]	<0.001	Ref.	0.54
	%p-tau217	0.68 [0.61,0.75]	<0.001	<0.001	0.45
	%p-tau205	0.60 [0.52,0.68]	<0.001	<0.001	0.34
Tau-PET – Braak I-II (n=436)	eMTBR-tau243	0.72 [0.66,0.79]	<0.001	Ref.	0.53
	%p-tau217	0.69 [0.63,0.76]	<0.001	0.147	0.49
	%p-tau205	0.56 [0.48,0.64]	<0.001	<0.001	0.31
Tau-PET – Braak III-IV (n=436)	eMTBR-tau243	0.75 [0.68,0.81]	<0.001	Ref.	0.55
	%p-tau217	0.69 [0.62,0.76]	<0.001	<0.001	0.48
	%p-tau205	0.60 [0.52,0.67]	<0.001	<0.001	0.34
Tau-PET – Braak V-VI (n=436)	eMTBR-tau243	0.70 [0.63,0.77]	<0.001	Ref.	0.47
	%p-tau217	0.64 [0.56,0.71]	<0.001	<0.001	0.39
	%p-tau205	0.57 [0.49,0.65]	<0.001	<0.001	0.30
Aβ-PET (n=328)	eMTBR-tau243	0.54 [0.45,0.64]	<0.001	Ref.	0.29
	%p-tau217	0.71 [0.63,0.79]	<0.001	<0.001	0.49
	%p-tau205	0.43 [0.33,0.53]	<0.001	0.014	0.19
All participants					
Tau-PET – Braak I-VI (n=649)	eMTBR-tau243	0.72 [0.67,0.77]	<0.001	Ref.	0.56
	%p-tau217	0.64 [0.58,0.70]	<0.001	<0.001	0.49
	%p-tau205	0.54 [0.47,0.60]	<0.001	<0.001	0.35
Tau-PET – Braak I-II (n=649)	eMTBR-tau243	0.74 [0.69,0.79]	<0.001	Ref.	0.57
	%p-tau217	0.69 [0.64,0.75]	<0.001	0.016	0.55
	%p-tau205	0.52 [0.45,0.58]	<0.001	<0.001	0.32
Tau-PET – Braak III-IV (n=649)	eMTBR-tau243	0.74 [0.69,0.79]	<0.001	Ref.	0.59
	%p-tau217	0.67 [0.61,0.72]	<0.001	<0.001	0.52
	%p-tau205	0.54 [0.48,0.61]	<0.001	<0.001	0.35
Tau-PET – Braak V-VI (n=649)	eMTBR-tau243	0.68 [0.62,0.73]	<0.001	Ref.	0.50
	%p-tau217	0.59 [0.53,0.65]	<0.001	<0.001	0.43
	%p-tau205	0.51 [0.44,0.57]	<0.001	<0.001	0.31
Aβ-PET (n=483)	eMTBR-tau243	0.63 [0.56,0.71]	<0.001	Ref.	0.39
	%p-tau217	0.81 [0.76,0.87]	<0.001	<0.001	0.67
	%p-tau205	0.48 [0.40,0.56]	<0.001	<0.001	0.28

Supplementary Table 4: Simple associations between plasma biomarkers and PET continuous measures in the validation BioFINDER-2 cohort

Linear regression models were used for each outcome (plasma biomarker) and predictor (PET) with age and sex as covariates. Plasma biomarkers were log_{10}-transformed. Significant differences of β from eMTBR-tau243 were calculated for each PET measure using bootstrapping (n=1,000 without replacement; p_{diff}<0.05). Aβ-positivity was assessed by CSF Aβ42/40 or Aβ-PET (>1.033 SUVR). Tau positivity was assessed for each region independently using previously validated thresholds (Braak I-VI: >1.22, Braak I-II: >1.38, Braak III-IV: >1.36 and Braak V-VI: >1.22). CSF: cerebrospinal fluid; SUVR: standardized uptake value ratio.

Outcome	N	β_{MTBR} [95%CI]	p-value $_{eMTBR\text{-}tau243}$	$\beta_{\%p\text{-}tau217}$ [95%CI]	p-value $_{\%p\text{-}tau217}$	$\beta_{\%p\text{-}tau205}$ [95%CI]	p-value $_{\%p\text{-}tau205}$	R^2
Tau-PET positive only								
Tau-PET – Braak I-VI	172	0.63 [0.43,0.83]	**<0.001**	-0.09 [-0.31,0.14]	0.448	0.14 [-0.01,0.29]	0.075	0.49
Tau-PET – Braak I-II	172	0.33 [0.08,0.57]	**0.010**	0.35 [0.07,0.62]	**0.013**	-0.21 [-0.40,-0.03]	**0.023**	0.25
Tau-PET – Braak III-IV	172	0.57 [0.35,0.78]	**<0.001**	0.04 [-0.19,0.28]	0.717	0.09 [-0.07,0.25]	0.253	0.43
Tau-PET – Braak V-VI	172	0.61 [0.40,0.83]	**<0.001**	-0.15 [-0.39,0.08]	0.195	0.15 [-0.01,0.3]	0.070	0.45
Aβ-positive only								
Tau-PET – Braak I-VI	436	0.50 [0.39,0.60]	**<0.001**	0.18 [0.07,0.29]	**0.002**	0.12 [0.03,0.21]	**0.008**	0.58
Tau-PET – Braak I-II	436	0.46 [0.35,0.56]	**<0.001**	0.3 [0.19,0.41]	**<0.001**	0.05 [-0.04,0.14]	0.295	0.56
Tau-PET – Braak III-IV	436	0.5 [0.40,0.60]	**<0.001**	0.21 [0.10.,0.32]	**<0.001**	0.11 [0.02,0.20]	**0.016**	0.59
Tau-PET – Braak V-VI	436	0.47 [0.36,0.58]	**<0.001**	0.14 [0.03,0.26]	**0.017**	0.12 [0.03,0.22]	**0.010**	0.52
Aβ-PET	328	0.08 [-0.03,0.2]	0.162	0.67 [0.55,0.79]	**<0.001**	-0.05 [-0.16,0.05]	0.328	0.50
All participants								
Tau-PET – Braak I-VI	649	0.55 [0.46,0.64]	**<0.001**	0.16 [0.07,0.26]	**0.001**	0.12 [0.05,0.19]	**0.001**	0.57
Tau-PET – Braak I-II	649	0.48 [0.39,0.56]	**<0.001**	0.33 [0.24,0.42]	**<0.001**	0.02 [-0.05,0.09]	0.506	0.60
Tau-PET – Braak III-IV	649	0.55 [0.46,0.63]	**<0.001**	0.20 [0.11,0.3]	**<0.001**	0.10 [0.03,0.17]	**0.005**	0.60
Tau-PET – Braak V-VI	649	0.53 [0.43,0.63]	**<0.001**	0.12 [0.02,0.22]	**0.022**	0.12 [0.05,0.20]	**0.002**	0.50
Aβ-PET	483	0.07 [-0.01,0.14]	0.091	0.76 [0.67,0.84]	**<0.001**	-0.02 [-0.08,0.05]	0.643	0.68

Supplementary Table 5: Combined plasma models for predicting continuous PET measures in the validation BioFINDER-2 cohort

Multivariate linear regressions were used for each outcome including all three plasma biomarkers as predictors with age and sex as covariates. Plasma biomarkers were $\log_{10}$-transformed. Predictors that were significant for each model ($p<0.05$) are shown in bold.

Outcome	Model	AUC	p_{DeLong}
Aβ-positive only			
Tau-PET – Braak I-VI	eMTBR-tau243	0.92 [0.88, 0.96]	Ref.
	%p-tau217	0.86 [0.82, 0.91]	0.002
	%p-tau205	0.83 [0.78, 0.89]	<0.001
Tau-PET – Braak I-II	eMTBR-tau243	0.87 [0.83, 0.91]	Ref.
	%p-tau217	0.83 [0.79, 0.88]	0.080
	%p-tau205	0.75 [0.70, 0.80]	<0.001
Tau-PET – Braak III-IV	eMTBR-tau243	0.93 [0.90, 0.96]	Ref.
	%p-tau217	0.89 [0.86, 0.93]	0.046
	%p-tau205	0.85 [0.80, 0.89]	<0.001
Tau-PET – Braak V-VI	eMTBR-tau243	0.95 [0.92, 0.97]	Ref.
	%p-tau217	0.88 [0.83, 0.93]	0.001
	%p-tau205	0.88 [0.82, 0.93]	0.004
Aβ-PET	eMTBR-tau243	0.77 [0.70, 0.83]	Ref.
	%p-tau217	0.93 [0.89, 0.98]	<0.001
	%p-tau205	0.78 [0.71, 0.85]	0.694
All participants			
Tau-PET – Braak I-VI	eMTBR-tau243	0.92 [0.88, 0.96]	Ref.
	%p-tau217	0.88 [0.84, 0.93]	0.008
	%p-tau205	0.85 [0.81, 0.90]	0.003
Tau-PET – Braak I-II	eMTBR-tau243	0.88 [0.85, 0.91]	Ref.
	%p-tau217	0.87 [0.84, 0.90]	0.512
	%p-tau205	0.77 [0.73, 0.82]	<0.001
Tau-PET – Braak III-IV	eMTBR-tau243	0.94 [0.92, 0.97]	Ref.
	%p-tau217	0.93 [0.90, 0.95]	0.296
	%p-tau205	0.88 [0.84, 0.92]	0.001
Tau-PET – Braak V-VI	eMTBR-tau243	0.94 [0.91, 0.98]	Ref.
	%p-tau217	0.89 [0.84, 0.95]	<0.001
	%p-tau205	0.88 [0.83, 0.93]	0.004

Supplementary Table 6: Accuracy of plasma biomarkers to assess tau- and Aβ-PET positivity in the validation BioFINDER-2 cohort

AUCs for each plasma biomarker predicting Aβ- and tau-PET positivity in different regions using ROC curves. Significant differences of AUCs from eMTBR-tau243 were calculated for each PET measure the De Long test. Aβ-positivity was assessed by CSF Aβ42/40 or Aβ-PET (>1.033 SUVR). Tau positivity was assessed for each region independently using previously validated thresholds (Braak I-VI: >1.22, Braak I-II: >1.38, Braak III-IV: >1.36 and Braak V-VI: >1.22). Abbreviations: AUC: area under the curve; CSF: cerebrospinal fluid; ROC: receiver operating characteristic curves; SUVR: standardized uptake value ratio.

Outcomes	Plasma	β[95%CI]	p-value	p_{diff}	R^2
Tau-PET positive only					
Cortical thickness (n=159)	eMTBR-tau243	0.45 [0.31, 0.58]	<0.001	Ref.	0.26
	%p-tau217	0.33 [0.18, 0.47]	<0.001	0.018	0.17
	%p-tau205	0.20 [0.05, 0.35]	<0.001	<0.001	0.1
Early cognitive dysfunction (mPACC; n=143)	eMTBR-tau243	0.45 [0.29, 0.61]	<0.001	Ref.	0.18
	%p-tau217	0.35 [0.19, 0.52]	<0.001	0.016	0.11
	%p-tau205	0.32 [0.15. 0.49]	<0.001	0.006	0.08
Global cognitive dysfunction (MMSE; n=160)	eMTBR-tau243	0.46 [0.31, 0.60]	<0.001	Ref.	0.22
	%p-tau217	0.38 [0.23, 0.53]	<0.001	0.052	0.16
	%p-tau205	0.32 [0.16, 0.48]	<0.001	0.004	0.12
Aβ-positive only					
Cortical thickness (n=413)	eMTBR-tau243	0.46 [0.38, 0.54]	<0.001	Ref.	0.29
	%p-tau217	0.40 [0.31, 0.48]	<0.001	0.028	0.24
	%p-tau205	0.37 [0.28, 0.45]	<0.001	0.006	0.21
Early cognitive dysfunction (mPACC; n=445)	eMTBR-tau243	0.60 [0.53, 0.68]	<0.001	Ref.	0.38
	%p-tau217	0.51 [0.43, 0.60]	<0.001	0.034	0.28
	%p-tau205	0.48 [0.40, 0.57]	<0.001	0.008	0.26
Global cognitive dysfunction (MMSE; n=481)	eMTBR-tau243	0.59 [0.51, 0.66]	<0.001	Ref.	0.36
	%p-tau217	0.51 [0.43, 0.59]	<0.001	0.012	0.28
	%p-tau205	0.48 [0.40, 0.56]	<0.001	0.002	0.26
All participants					
Cortical thickness (n=522)	eMTBR-tau243	0.47 [0.40, 0.54]	<0.001	Ref.	0.36
	%p-tau217	0.41 [0.33, 0.48]	<0.001	0.100	0.30
	%p-tau205	0.35 [0.28, 0.43]	<0.001	0.004	0.27
Early cognitive dysfunction (mPACC; n=556)	eMTBR-tau243	0.63 [0.56, 0.69]	<0.001	Ref.	0.46
	%p-tau217	0.58 [0.51, 0.65]	<0.001	0.052	0.39
	%p-tau205	0.52 [0.45, 0.59]	<0.001	0.008	0.34
Global cognitive dysfunction (MMSE; n=593)	eMTBR-tau243	0.60 [0.54, 0.67]	<0.001	Ref.	0.40
	%p-tau217	0.54 [0.46, 0.61]	<0.001	0.014	0.32
	%p-tau205	0.50 [0.43, 0.57]	<0.001	0.004	0.29

Supplementary Table 7: Individual associations between plasma biomarkers and AD-downstream effects in the validation BioFINDER-2 cohort

Linear regression models were used for each outcome (cortical thickness and early and global cognitive dysfunction) and predictor (plasma biomarkers) with age and sex (and years of education for cognitive outcomes) as covariates. Plasma biomarkers were log_{10}-transformed. Participants having non-AD diagnosis were excluded from all these analyses. Significant differences of β from eMTBR-tau243 were calculated for each outcome measure using bootstrapping (n=1,000 without replacement; p_{diff}<0.05). Aβ-positivity was assessed by CSF Aβ42/40 or Aβ-PET (>1.033 SUVR). Tau positivity was assessed for the global region using previously validated thresholds (Braak I-VI: >1.22). Abbreviations: CSF: cerebrospinal fluid; MMSE: Mini-Mental State Examination; mPACC: modified preclinical Alzheimer's cognitive composite; SUVR: standardized uptake value ratio.

Outcome	N	β_{MTBR} [95%CI]	p-value $_{eMTBR\text{-}tau243}$	$\beta_{\%p\text{-}tau217}$ [95%CI]	p-value $_{\%p\text{-}tau217}$	$\beta_{\%p\text{-}tau205}$ [95%CI]	p-value $_{\%p\text{-}tau205}$	R^2
Aβ-positive only								
Cortical thickness	427	0.37 [0.23, 0.51]	**<0.001**	0.03 [-0.11, 0.18]	0.669	0.10 [0.02, 0.22]	0.099	0.29
Early cognitive dysfunction (mPACC)	461	0.47 [0.34, 0.59]	**<0.001**	0.03 [-0.10, 0.16]	0.632	0.14 [0.03, 0.25]	**0.010**	0.37
Global cognitive dysfunction (MMSE)	499	0.44 [0.32, 0.57]	**<0.001**	0.03 [-0.11, 0.16]	0.687	0.16 [0.05, 0.26]	**0.004**	0.36
All participants								
Cortical thickness	636	0.40 [0.28, 0.51]	**<0.001**	-0.02 [-0.15, 0.10]	0.703	0.09 [0.01, 0.18]	0.065	0.34
Early cognitive dysfunction (mPACC)	678	0.43 [0.33, 0.54]	**<0.001**	0.00 [-0.12,0.12]	0.991	0.16 [0.08, 0.25]	**<0.001**	0.37
Global cognitive dysfunction (MMSE)	720	0.48 [0.37, 0.59]	**<0.001**	-0.03 [-0.15, 0.09]	0.622	0.15 [0.06, 0.23]	**0.001**	0.34
Tau-positive only								
Cortical thickness	166	0.55 [0.30, 0.80]	**<0.001**	-0.06 [-0.33, 0.22]	0.683	0.11 [-0.07,0.3]	0.233	0.25
Early cognitive dysfunction (mPACC)	152	0.46 [0.18, 0.75]	**0.002**	-0.11 [-0.43, 0.20]	0.474	-0.09 [-0.12, 0.30]	0.413	0.16
Global cognitive dysfunction (MMSE)	169	0.42 [0.15, 0.69]	**0.002**	-0.02 [-0.32, 0.27]	0.892	-0.05 [0.15, 0.25]	0.616	0.20

Supplementary Table 8: Combined plasma models for predicting atrophy and cognition in the validation BioFINDER-2 cohort

Multivariate linear regressions were used for each outcome including all three plasma biomarkers as predictors with age and sex (and years of education for cognitive outcomes) as covariates. Plasma biomarkers were log_{10}-transformed. Predictors that were significant for each model ($p<0.05$) are shown in bold. Participants having non-AD diagnosis were excluded from all these analyses.