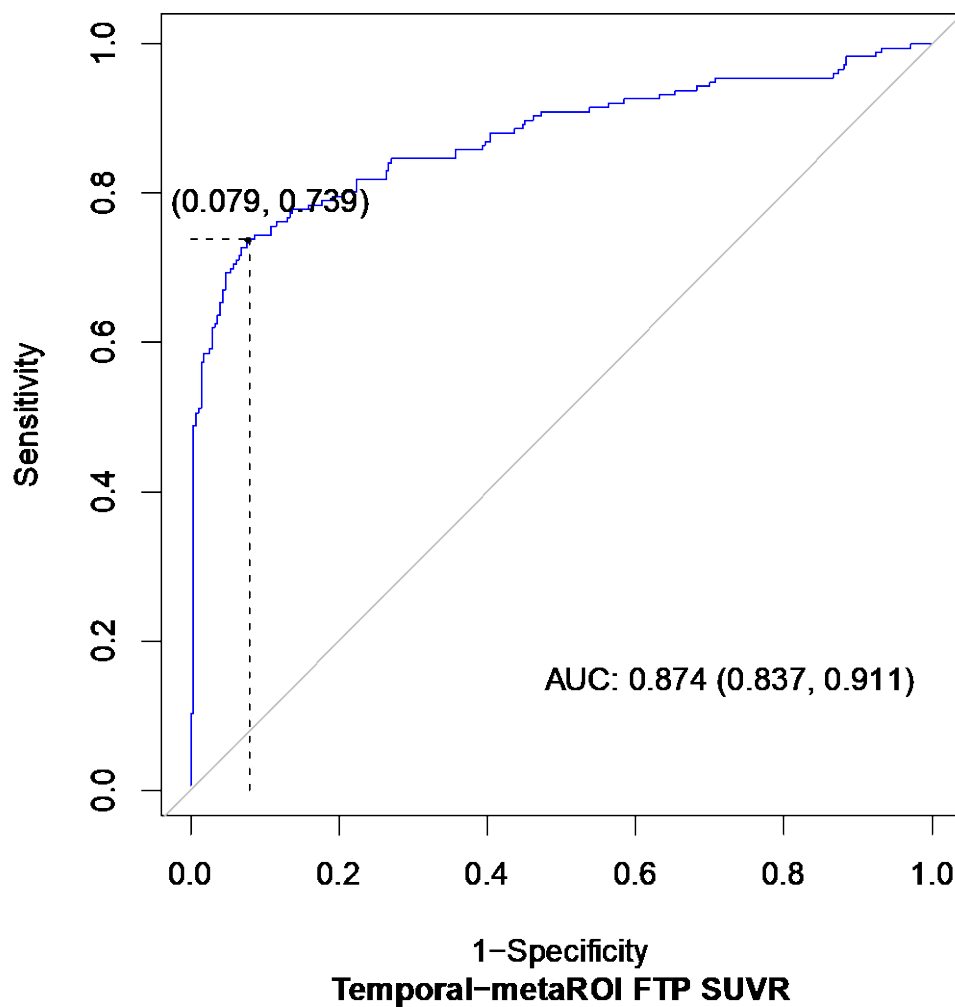


# Longitudinal Cognitive and Biomarker Measurements Support a Unidirectional Pathway in Alzheimer's Disease Pathophysiology

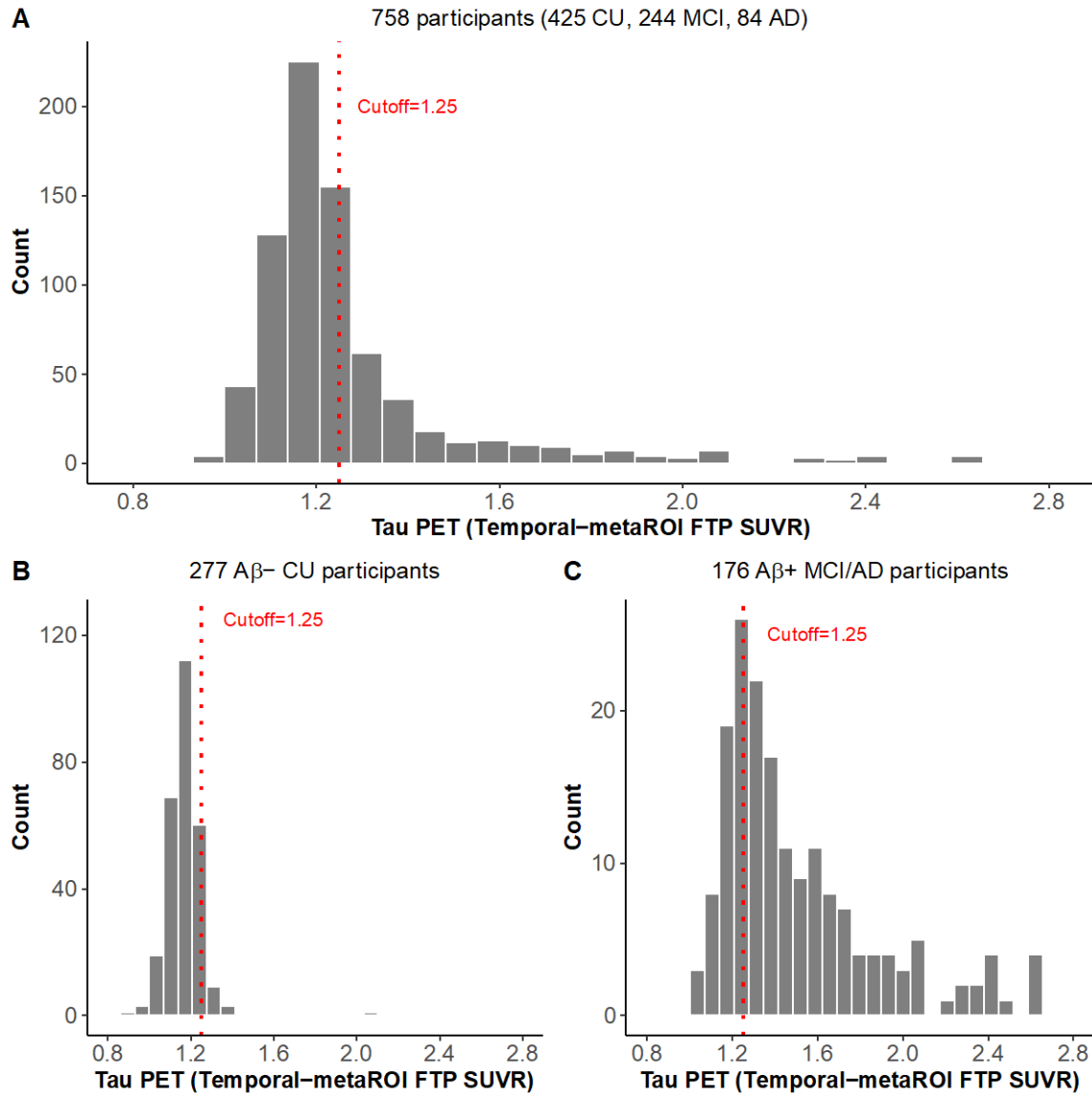
## Supplemental Information

### Cutoff of tau PET

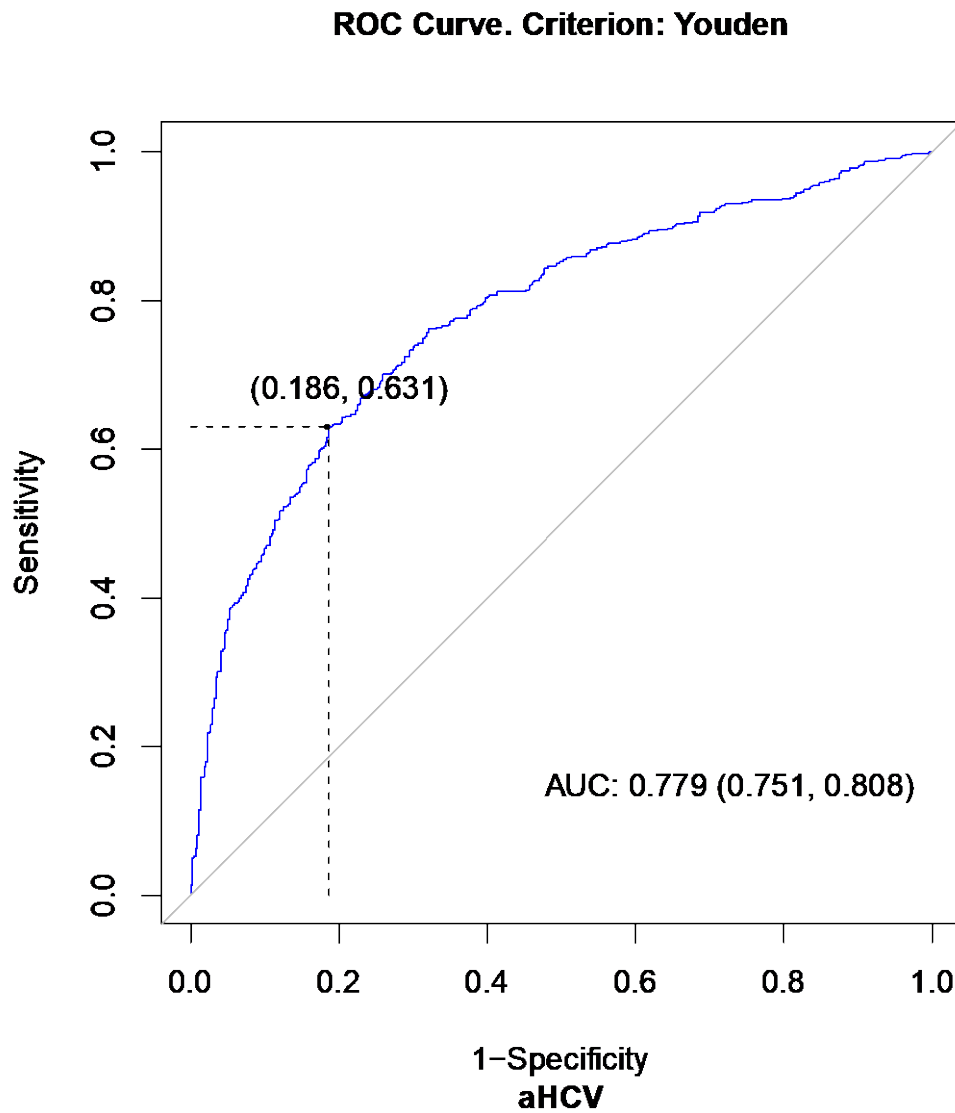
#### ROC Curve. Criterion: Youden



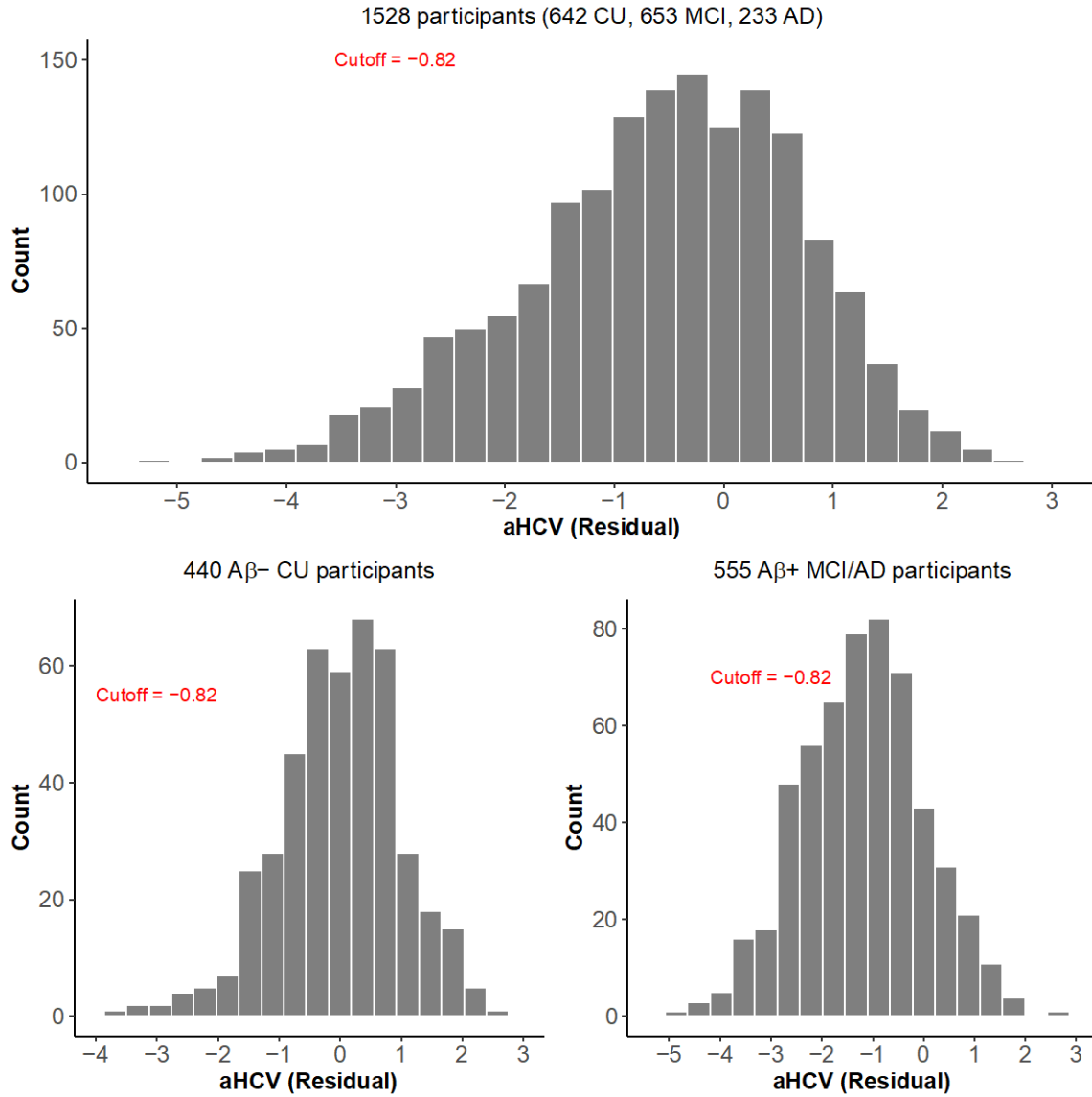
**Supplemental Fig. S1.** The ROC analysis using the Youden index classifying 277 A $\beta$ - ADNI CU participants and 176 A $\beta$ + ADNI MCI and AD patients as the endpoint to define the cutoff  $\geq 1.25$  for Temporal-metaROI FTP SUVR. AUC: 0.874 (95%CI-0.837,0.911).



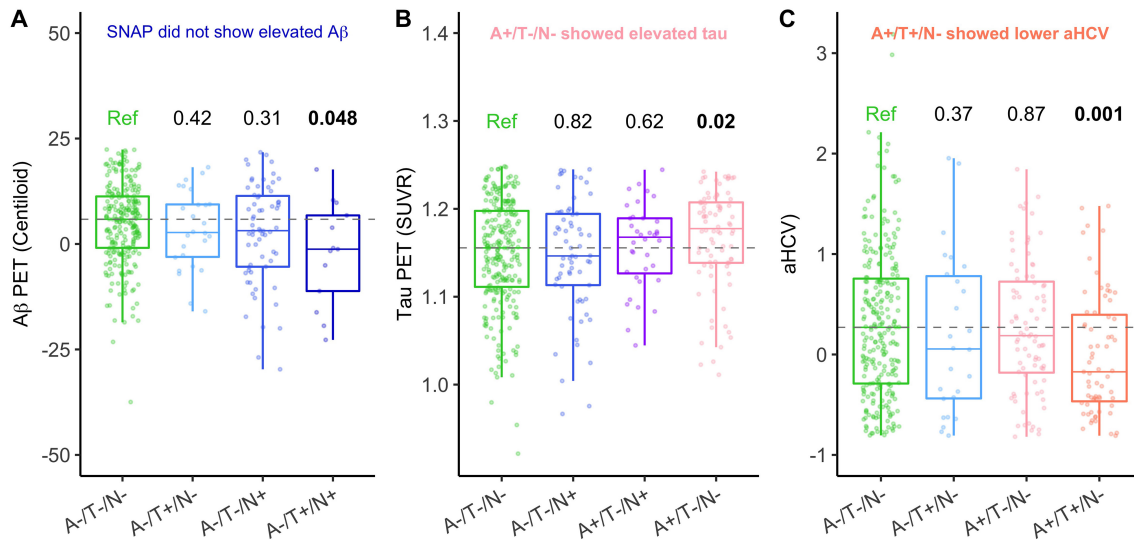
**Supplemental Fig. S2.** Histograms of Temporal-metaROI FTP SUVRs of (A) all 734 ADNI participants, (B) 271 A $\beta$ - ADNI CU participants and (C) 161 A $\beta$ + ADNI MCI and AD patients with tau PET scan. Red dotted line is the cutoff of Temporal-metaROI FTP SUVR 1.25.

**Cutoff of adjusted hippocampal volume (aHCV)**

**Supplemental Fig. S3.** The ROC analysis using the Youden index classifying ADNI 440 A $\beta$ - CU participants and 555 A $\beta$ + MCI and AD patients as the endpoint to define the cutoff  $\leq -0.82$  cm<sup>3</sup> for aHCV. AUC: 0.78 (95%CI-0.751,0.808).



**Supplemental Fig. S4.** Histograms of aHCVs of (A) all 1528 ADNI participants, (B) 440 Aβ- ADNI CU participants and (C) 555 Aβ+ ADNI MCI and AD patients with aHCV data. Red dotted line is the cutoff of aHCV -0.82.



**Supplemental Fig. S5.** Baseline A $\beta$ , tau and neurodegeneration of A-, T- and N- groups. Comparison of baseline (A) A $\beta$  PET of A- groups, (B) tau PET of T- groups, and (C) aHCV of N- groups with the reference group (A-/T-/N-). Values on the top of the bar indicates the p values of the comparisons with the reference.

**Supplemental Table S1.** Comparisons of baseline A $\beta$  PET, tau PET, aHCV and PACC, and slopes of aHCV and PACC of different A/T/N profiles with the reference group (Ref: A-/T-/N-).

	Baseline A $\beta$ PET (Centiloid)			Baseline tau PET (SUVR)		
	Median (IQR)	Comparison with Ref		Median (IQR)	Comparison with Ref	
		Estimate (95%CI)	<i>p</i> value		Estimate (95%CI)	<i>p</i> value
A-/T-/N- (Ref)	5.9 (12.2)	-----	-----	1.15 (0.09)	-----	-----
A-/T+/N-	2.7 (12.4)	-1.7 (-5.5, 2.3)	0.42	1.29 (0.06)	0.14 (0.12, 0.16)	<b>&lt;0.001</b>
A-/T-/N+	3.2 (16.8)	-1.8 (-4.6, -1.2)	0.31	1.15 (0.08)	0.00 (-0.02, 0.02)	0.82
A-/T+/N+	-1.23 (17.9)	-7.1(-13.5, -0.3)	<b>0.048</b>	1.32 (0.06)	0.17 (0.13, 0.20)	<b>&lt;0.001</b>
A+/T-/N+	53.0 (51.0)	50.4 (41.0, 61.8)	<b>&lt;0.001</b>	1.17 (0.06)	0.01 (-0.01, 0.03)	0.62
A+/T-/N-	43.1 (36.7)	40.8 (36.1, 46.1)	<b>&lt;0.001</b>	1.18 (0.07)	0.02 (0.004, 0.03)	<b>0.02</b>
A+/T+/N-	71.5 (50.4)	68.8 (61.0, 77.1)	<b>&lt;0.001</b>	1.33 (0.14)	0.20 (0.18, 0.22)	<b>&lt;0.001</b>
A+/T+/N+	78.7 (60.1)	75.5 (66.6, 85.5)	<b>&lt;0.001</b>	1.46 (0.30)	0.31 (0.26, 0.37)	<b>&lt;0.001</b>

**Supplemental Table S2.** Comparisons of baseline aHCV and PACC of different A/T/N profiles with the reference group (Ref: A-/T-/N-).

	Baseline aHCV (cm <sup>3</sup> )			Baseline PACC		
	Median (IQR)	Comparison with Ref		Median (IQR)	Comparison with Ref	
		Estimate (95%CI)	<i>p</i> value		Estimate (95%CI)	<i>p</i> value
A-/T-/N- (Ref)	0.27(1.04)	-----	-----	0.59 (4.04)	-----	-----
A-/T+/N-	0.05 (1.22)	-0.14 (-0.46, 0.15)	0.37	-0.43 (5.33)	-0.30 (-1.66, 1.18)	0.75
A-/T-/N+	-1.23 (0.67)	-1.66 (-1.88, -1.47)	<b>&lt;0.001</b>	-1.40 (5.93)	-1.51 (-2.50, -0.42)	<b>0.007</b>
A-/T+/N+	-2.45(1.51)	-2.63 (-3.14, -2.07)	<b>&lt;0.001</b>	-6.07 (6.58)	-5.86 (-8.70, -3.29)	<b>&lt;0.001</b>
A+/T-/N+	-1.42(0.60)	-1.82 (-2.07, -1.57)	<b>&lt;0.001</b>	-1.43 (5.84)	-1.93 (-3.27, -0.68)	<b>0.007</b>
A+/T-/N-	0.19 (0.91)	-0.01 (-0.19, 0.16)	0.87	-0.19 (3.95)	-0.32 (-1.08, 0.44)	0.58
A+/T+/N-	-0.17 (0.86)	-0.30 (-0.49, -0.12)	<b>0.001</b>	-1.26 (5.80)	-2.22 (-3.15, -1.24)	<b>&lt;0.001</b>
A+/T+/N+	-1.48 (0.82)	-1.82 (-2.03, -1.62)	<b>&lt;0.001</b>	-8.13 (7.63)	-8.18 (-9.69, -6.65)	<b>&lt;0.001</b>

**Supplemental Table S3.** Comparisons of aHCV slope and PACC slope of different A/T/N profiles with the reference group (Ref: A-/T-/N-).

	aHCV slope			PACC slope		
	Estimate (SE)	Comparison with Ref		Estimate (SE)	Comparison with Ref	
		Estimate (95%CI)	<i>p</i> value		Estimate (95%CI)	<i>p</i> value
A-/T-/N- (Ref)	-0.10(0.02)	-----	-----	0.12 (0.16)	-----	-----
A-/T+/N-	-0.20 (0.06)	-0.11 (-0.24, 0.02)	0.11	-0.30 (0.49)	-0.42 (-1.43, 0.59)	0.42
A-/T-/N+	-0.00 (0.05)	0.10 (-0.01, 0.20)	0.07	-0.32 (0.37)	-0.44 (-1.22, 0.35)	0.27
A-/T+/N+	-0.01 (0.09)	0.08 (-0.09, 0.26)	0.34	-0.64(0.63)	-0.76 (-2.04, 0.52)	0.24
A+/T-/N+	-0.12(0.07)	-0.02 (-0.16, 0.11)	0.72	-0.35 (0.44)	-0.47 (-1.39, 0.45)	0.32
A+/T-/N-	-0.12 (0.04)	-0.03 (-0.11, 0.05)	0.50	0.10 (0.28)	-0.02 (-0.66, 0.62)	0.96
A+/T+/N-	-0.22 (0.04)	-0.13 (-0.21, -0.04)	<b>0.003</b>	-0.72 (0.32)	-0.85 (-1.54, -0.15)	<b>0.02</b>
A+/T+/N+	-0.26 (0.04)	-0.16 (-0.25, -0.07)	<b>&lt;0.001</b>	-1.26 (0.33)	-1.39 (-2.10, -0.67)	<b>&lt;0.001</b>

### Results using alternative T and N thresholds: Comparisons of A $\beta$ , tau, neurodegeneration and cognition of different A/T/N groups

The characteristics of different A/T/N groups defined by alternative cutoffs for FTP SUVR and aHCV can be found in Supplemental table S4. Compared to the A/T/N profiles defined in Table 1 in the main text, slightly more A-/T+/N- (6% Vs. 4%), A-/T+/N+ (4% Vs. 2%) and A+/T-/N+ (7% Vs. 6%) individuals but less A+/T-/N- (11% Vs. 14%) individuals were defined according to the alternative lenient cutoffs for FTP SUVR and aHCV.



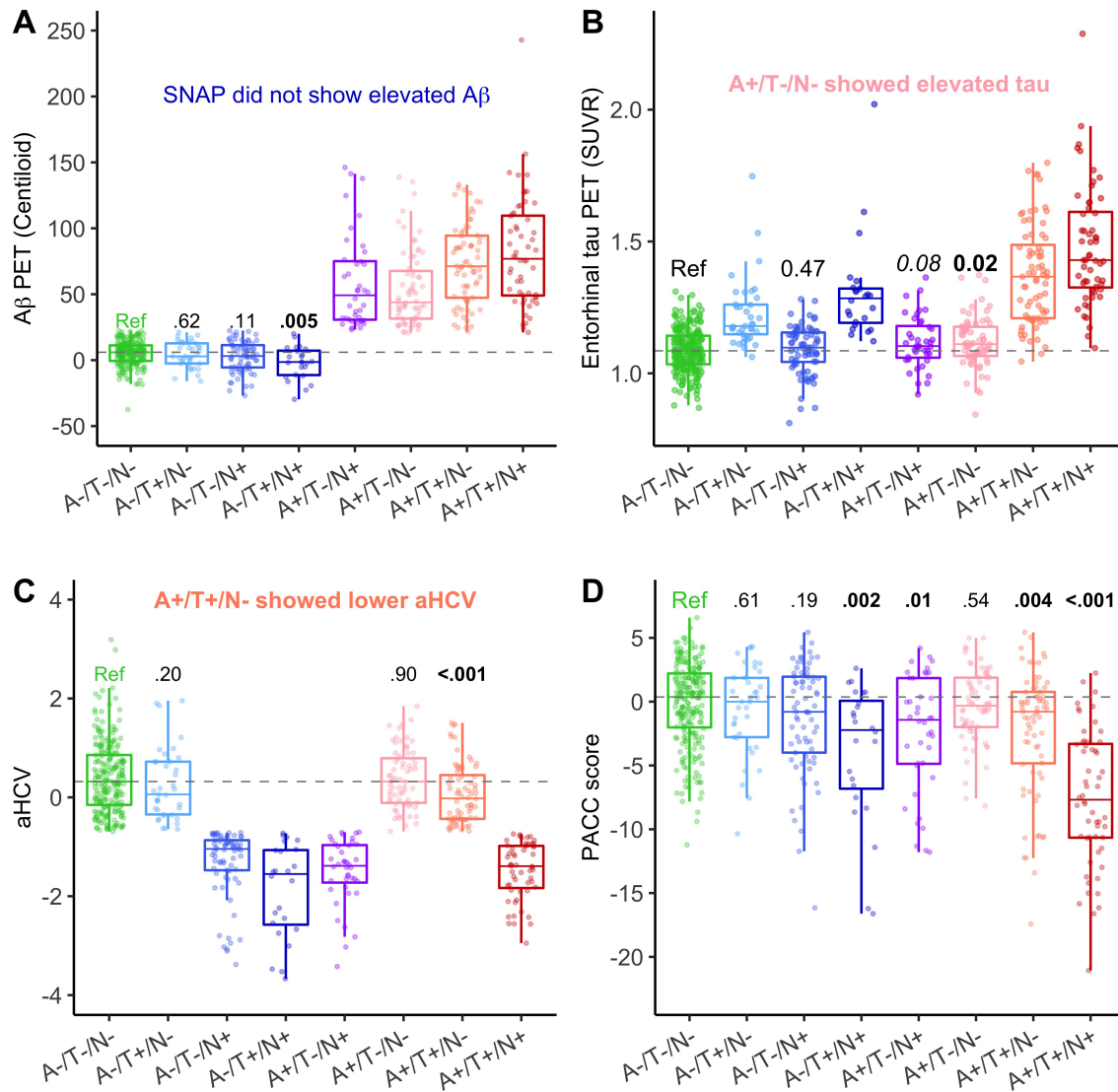
**Supplemental Table S4.** Demographic characteristics of participants in different A/T/N profiles defined by alternative cutoffs

	<b>Normal</b>	<b>SNAP</b>			<b>AD continuum</b>			
A/T/N groups	A-/T-/N-	A-/T+/N-	A-/T-/N+	A-/T+/N+	A+/T-/N+	A+/T-/N-	A+/T+/N-	A+/T+/N+
No. (%) of A/T/N	229(38%)	37(6%)	73(12%)	24(4%)	40(7%)	68(11%)	72(12%)	56(9%)
No. (%) of MCI	52(23%)	12(32%)	27(37%)	12(50%)	14(35%)	14(21%)	29(40%)	44(79%)
Age (year)	69(8)	72(6)	77(10)	79(9)	82(9)	72(10)	74(9)	78(7)
Education, y	16(2)	18(4)	18(5)	18(3)	16(5)	17(3)	16(2)	16(4)
No. (%) of females	136(59%)	23(62%)	29(40%)	9(38%)	15(38%)	33(49%)	49(68%)	26(46%)
No. (%) of APOE-ε4	49(21%)	4(11%)	9(12%)	6(25%)	15(38%)	34(50%)	39(54%)	27(48%)
<b>121 participants with ≥ 2 Aβ PET scans</b>								
A/T/N groups	A-/T-/N-	A-/T+/N-	A-/T-/N+	A-/T+/N+	A+/T-/N+	A+/T-/N-	A+/T+/N-	A+/T+/N+
No. (%) of A/T/N	51(42%)	7(6%)	10(8%)	4(3%)	5(4%)	14(12%)	15(12%)	15(12%)
FU Visits Median (IQR, range)	2 (0, 2-3)	2 (1, 2-3)	2 (0, 2-3)	2 (0, 2-2)	2 (0, 2-2)	2 (0, 2-3)	2 (0, 2-2)	2 (0, 2-3)
Duration of FU, year Median (IQR, range)	2.0 (0.2, 0.8-4.0)	3.1(1.6, 1.1-4.1)	2.0(0.4, 1.1-3.7)	2.0(0.1, 1.8-2.1)	2.1(0.1, 1.9-3.7)	1.9(0.1, 1.6-3.0)	2.0(0.1, 0.8-3.0)	2.0(0.4, 1.0-3.3)
<b>185 participants with ≥ 2 tau PET scans</b>								
A/T/N groups	A-/T-/N-	A-/T+/N-	A-/T-/N+	A-/T+/N+	A+/T-/N+	A+/T-/N-	A+/T+/N-	A+/T+/N+
No. (%) of A/T/N	50(29%)	8(3%)	11(6%)	5(2%)	16(9%)	32(22%)	38(18%)	25(12%)
FU Visits Median (IQR, range)	2 (0, 2-5)	2 (0, 2-4)	2 (0, 2-4)	2 (1, 2-3)	2 (0, 2-3)	2 (0, 2-4)	2 (1, 2-4)	2 (1, 2-4)
Duration of FU, year Median (IQR, range)	1.3 (1.0, 0.6-3.8)	1.1(0.3, 0.9-3.1)	1.0(0.5, 0.7-2.9)	1.9(0.9, 0.81-2.3)	1.0(0.3, 0.6-2.1)	1.0(0.5, 0.7-3.3)	1.1(1.0, 0.8-4.0)	1.5(1.0, 1.0-3.1)
<b>218 participants with ≥ 2 MRI scan</b>								
A/T/N groups	A-/T-/N-	A-/T+/N-	A-/T-/N+	A-/T+/N+	A+/T-/N+	A+/T-/N-	A+/T+/N-	A+/T+/N+
No. (%) of A/T/N	68(31%)	12(6%)	18(8%)	8(4%)	16(7%)	31(14%)	38(17%)	27(12%)
FU Visits Median (IQR, range)	2 (1, 2-5)	2 (0.25, 2-4)	2 (0, 2-4)	2 (0.25, 2-3)	2 (0, 2-3)	2 (0, 2-4)	2 (0, 2-3)	2 (1, 2-4)
Duration of FU, year Median (IQR, range)	1.9 (1.0, 0.9-43.8)	1.8(1.2, 1.1-4.1)	1.2(0.9, 1.0-3.6)	1.2(0.9, 1.0-2.0)	1.1(0.2, 0.9-2.1)	1.1(0.6, 0.9-3.3)	1.1(0.4, 1.0-4.0)	1.6(0.9, 1.0-3.2)
<b>299 participants with ≥ 2 PACC scores</b>								
A/T/N groups	A-/T-/N-	A-/T+/N-	A-/T-/N+	A-/T+/N+	A+/T-/N+	A+/T-/N-	A+/T+/N-	A+/T+/N+
No. (%) of A/T/N	99(33%)	17(6%)	33(11%)	12(4%)	25(8%)	37(12%)	43(14%)	33(11%)
FU Visits Median (IQR, range)	2 (1, 2-5)	2 (1, 2-4)	2 (0, 2-4)	2 (1, 2-3)	2 (1, 2-3)	2 (0, 2-4)	2 (0.5, 2-4)	2 (1, 2-4)
Duration of FU, year Median (IQR, range)	2.0 (1.0, 0.9-3.8)	2.0(1.0, 1.0-4.1)	1.1(1.0, 0.9-3.6)	1.3(1.0, 1.0-2.1)	1.1(1.0, 0.9-3.7)	1.1(1.0, 0.8-3.3)	1.1(1.0, 0.9-4.0)	1.5(1.0, 1.0-3.5)

Abbreviation: Aβ=amyloid-β; PACC=preclinical Alzheimer's cognitive composite; A=Aβ;

T=tau; N= neurodegeneration; SNAP=suspected non-Alzheimer's pathology; FU=follow-up.

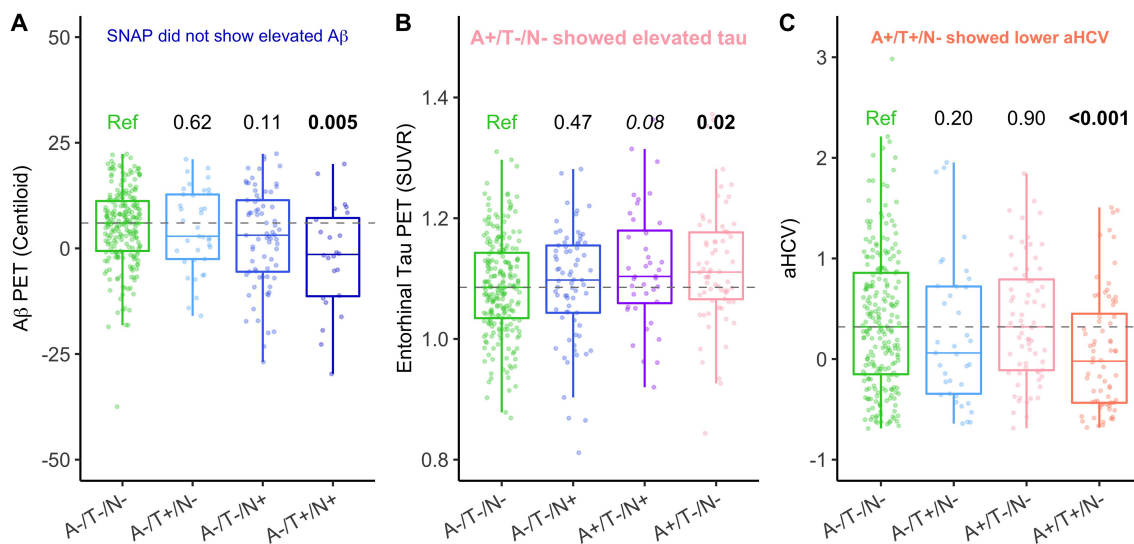
Cross-sectional continuous biomarker levels are shown in Supplemental fig. S6, and these were elevated according to the classification schemes. The results were substantially the same as used the initial cutoffs of FTP SUVR and aHCV to define T+/- and N+/- . Among A- participants, the A-/T+ individuals did not show higher A $\beta$  PET and A-/T+/N+ individuals even had significantly lower A $\beta$  PET, perhaps due to atrophy (Supplemental Fig. S6A and Fig. S7A). Among N- participants, A+/T+/N- individuals had lower aHCV (Estimate = -0.31 [95%CI, -0.49 - -0.14],  $p < 0.001$ , Supplemental Fig. S6C and Fig. S7C); whereas A-/T-/N+ individuals did not show either higher A $\beta$  or tau (Supplemental Fig. S6A-B and Fig. S7A-B).



**Supplemental Fig. S6.** Baseline  $A\beta$ , tau, neurodegeneration and cognition of different A/T/N groups defined by alternative cutoffs. Comparison of baseline (A)  $A\beta$  PET, (B) tau PET, (C) aHCV, (D) PACC of SNAP and AD continuum groups with the reference (Ref) group (A-/T-/N). Notes: The box plot whiskers extend to the lowest and highest data points within 1.5 times the IQR from the lower and upper quartiles. The dots represent individual points of each A/T/N group. Gray dashed lines represent the median values of the reference. Values on the top of the bar indicates the p values of the comparisons with the reference.

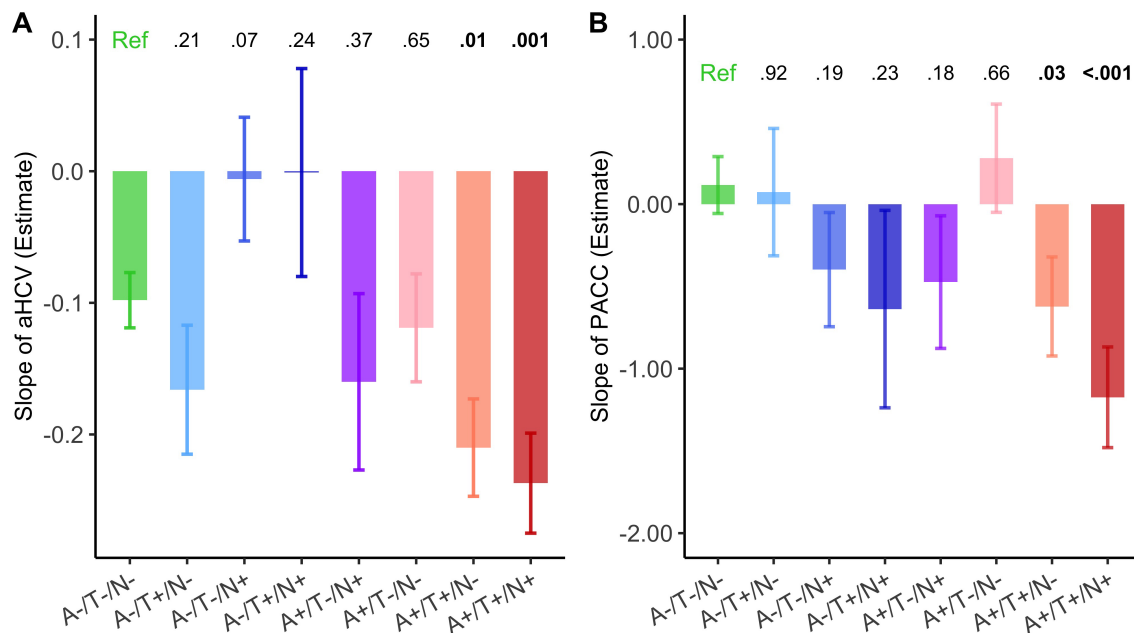
The lenient cutoff of FTP SUVR defined 16 and 4 initial (20/84, 24%) A+/T-/N- individuals as A+/T+/N- and A+/T-/N+ respectively, and the new A+/T-/N- individuals did not show significantly higher Temporal-metaROI FTP SUVR than the reference group, but still had higher entorhinal FTP SUVR (Estimate = 0.03 [95%CI, 0.01- 0.05],  $p=0.02$ , Supplemental Fig. S6B and Fig. S7B) than the reference group, which was overall consistent with findings with the original thresholds.

In addition, PACC scores of A-/T-/N+ individuals were not significantly lower than the reference group any more due to the lenient cutoff of aHCV (Supplemental Fig. S6D).



**Supplemental Fig. S7.** Baseline A $\beta$ , tau and neurodegeneration of A-, T- and N- groups defined by alternative cutoffs. Comparison of baseline (A) A $\beta$  PET of A- groups, (B) tau PET of T- groups, and (C) aHCV of N- groups with the reference group (A-/T-/N-). Gray dashed lines represent the median values of the reference. Values on the top of the bar indicates the p values of the comparisons with the reference.

The comparisons of rates of aHCV and PACC were substantially the same as used the initial cutoffs of FTP SUVR and aHCV to define T+/- and N+/. Results of LME models showed that both the A+/T+/N+ and A+/T+/N- groups showed greater decline than the reference group in aHCV (A+/T+/N- Vs. A-/T-/N-: Estimate = -0.112[95%CI, -0.196 - -0.069], SE=0.046, p=0.01; A+/T+/N+ Vs. A-/T-/N-: Estimate = -0.139[95%CI, -0.225 - -0.054], SE=0.044, p<0.001, Supplemental Fig. S8A) and PACC (N=299, A+/T+/N- Vs. A-/T-/N-: Estimate = -0.74[95%CI, -1.42 - -0.06], SE=0.35, p=0.03; A+/T+/N+ Vs. A-/T-/N-: Estimate = -1.29[95%CI, -1.98 - -0.60], SE=0.35, p<0.001; Supplemental Fig. S8B).



**Supplemental Fig. S8.** Longitudinal changes of neurodegeneration and cognition over time of different A/T/N groups defined by alternative cutoffs. Comparisons of slopes of (A) aHCV decreases in 218 participants with longitudinal MRI data, (F) PACC cognitive score decline in 299 participants with longitudinal cognitive data. Error bars reflect standard error of estimated slope in linear mixed effects models analyses. Values on the top of the bar indicates the p values of the comparisons with the reference. Ref=the reference group; aHCV=adjusted hippocampal volume; PACC=Preclinical Alzheimer Cognitive Composite.

**A $\beta$ , tau and neurodegeneration in relation to each other among 76 participants with concurrent longitudinal A $\beta$  PET, tau PET and MRI scans**

Among 76 participants with longitudinal A $\beta$  PET, tau PET and MRI scans all available at follow-up, neither baseline high FTP SUVR nor low aHCV was associated with subsequent A $\beta$  PET increases. However, high baseline A $\beta$  PET (but not low aHCV) was associated (Estimate = 0.079[95%CI, 0.031-0.126], p=0.003) with subsequent tau PET increase. In addition, subsequent longitudinal aHCV decreases were significantly associated with high baseline tau PET (Estimate = -0.067[95%CI, -0.133 - -0.001], p=0.04), but only marginally with high A $\beta$  PET (Estimate = -0.053[95%CI, -0.113 - 0.007], p=0.07) and no interaction between A $\beta$  PET and tau PET (Estimate = 0.049[95%CI, -0.010 - 0.108], p=0.10) was found.