SUPPLEMENTARY DATA

Staging of Neuropil Morphological Alterations in Alzheimer's Disease

Zhi-Yi Zhang, Xin-Meng Wang, Xin Ma, Yi Wang, Shuai-Deng Wang, Yi-Lin Peng, Kun Cheng, Yue-Ru Shen, Guo-Zhang Tang, Chen-Wei Wang, Jiang-Ning Zhou

SUPPLEMENTARY DATA

Supplementary Table 1. Demographics of the included cases, cases from China Brain Bank.

Case	Gender	Age at death	Brain weight (g)	Educational background	post-mortem delay (PMD, minutes)	APOE	Thal Plaque Phase	Braak NFT Stage	CERAD plaque score
1	Female	50	1247	Senior	1247	Ɛ3/Ɛ3	0	0	0
2	Male	57	1255	Senior	1255	Ɛ3/Ɛ3	0	0	0
3	Male	61	1248	Junior	1248	Ɛ3/Ɛ3	0	0	0
4	Male	53	1507	Junior	1507	Ɛ3/Ɛ3	0	0	0
5	Male	63	1209	Bachelor	1209	Ɛ3/Ɛ3	0	0	0
6	Male	74	1177	Primary	1177	Ɛ3/Ɛ3	0	0	0
7	Female	58	1135	Primary	1135	Ɛ3/Ɛ3	0	0	0
8	Male	62	1000	Bachelor	1000	Ɛ3/Ɛ3	2	0	0
9	Male	40	1557	Bachelor	1557	Ɛ3/Ɛ3	0	0	0
10	Female	53	1151	College	1151	Ɛ3/Ɛ3	0	0	0
11	Female	54	1042	Senior	1042	Ɛ3/Ɛ3	0	1	0
12	Male	77	1300	Senior	1300	Ɛ3/Ɛ3	0	1	0
13	Male	71	1240	Bachelor	1240	Ɛ3/Ɛ3	2	1	1
14	Female	85	1026	Bachelor	1026	Ɛ3/Ɛ4	0	1	0
15	Female	58	1419	College	1419	Ɛ3/Ɛ4	1	1	0
16	Female	47	1205	Junior	1205	Ɛ3/Ɛ3	0	1	0
17	Male	77	1188	College	1188	Ɛ3/Ɛ3	0	2	0
18	Male	80	1251	Primary	1251	Ɛ3/Ɛ3	1	2	0
19	Male	66	1440	College	1440	Ɛ3/Ɛ3	0	2	0
20	Male	58	1574	College	1574	Ɛ3/Ɛ4	0	2	0
21	Male	72	1177	College	1177	Ɛ3/Ɛ3	0	2	0
22	Male	73	1051	Junior	1051	Ɛ3/Ɛ3	0	2	0
23	Male	74	1179	Junior	1179	Ɛ3/Ɛ3	0	2	0
24	Female	77	1150	Senior	1150	Ɛ3/Ɛ3	1	2	1
25	Male	75	1613	Bachelor	1613	Ɛ3/Ɛ3	0	2	0
26	Male	65	1308	Junior	1308	Ɛ3/Ɛ3	0	2	0
27	Female	74	1211	Junior	1211	Ɛ3/Ɛ4	1	3	0
28	Female	84	1019	Primary	1019	Ɛ3/Ɛ3	3	3	1
29	Female	67	1162	Primary	1162	Ɛ3/Ɛ3	3	3	1
30	Male	89	1271	Bachelor	1271	Ɛ3/Ɛ3	2	3	1
31	Male	80	1333	Senior	1333	Ɛ3/Ɛ3	1	3	0
32	Female	88	967	Primary	967	Ɛ3/Ɛ3	3	3	0
33	Female	89	941	Primary	941	Ɛ3/Ɛ4	4	3	1
34	Male	83	1096	Senior	1096	Ɛ3/Ɛ4	4	3	1
35	Female	85	1107	Bachelor	1107	Ɛ3/Ɛ3	1	3	0
36	Male	87	1419	Bachelor	1419	Ɛ3/Ɛ3	1	3	0
37	Female	71	1411	Senior	1411	Ɛ3/Ɛ3	1	3	0
38	Male	87	1203	Bachelor	1203	Ɛ3/Ɛ3	0	3	0
39	Female	69	1227	Primary	1227	Ɛ3/Ɛ3	2	3	0
40	Male	75	1307	Senior	1307	Ɛ3/Ɛ3	0	3	0
41	Female	84	1070	Bachelor	1070	Ɛ3/Ɛ3	0	3	0
42	Male	84	1210	College	1210	Ɛ3/Ɛ4	1	4	1
43	Male	77	1040	Senior	1040	Ɛ3/Ɛ4	4	4	1
44	Female	80	1271	College	1271	Ɛ3/Ɛ3	3	4	2
45	Male	77	1231	Bachelor	1231	Ɛ3/Ɛ3	1	4	0
46	Male	90	1184	Bachelor	1184	Ɛ3/Ɛ3	4	4	1
47	Male	77	1295	Bachelor	1295	Ɛ3/Ɛ3	0	4	0
48	Female	88	1170	Primary	1170	Ɛ3/Ɛ3	5	4	1

49	Female	79	1110	Junior	1110	Ɛ4/Ɛ4	2	4	0
50	Female	89	941	College	941	Ɛ3/Ɛ3	4	5	3
51	Male	72	1396	Junior	1396	Ɛ3/Ɛ3	3	5	1
52	Female	84	1154	Primary	1154	Ɛ3/Ɛ4	5	5	1
53	Female	76	915	Bachelor	915	Ɛ3/Ɛ3	3	5	2
54	Female	82	980	Junior	980	Ɛ3/Ɛ4	3	5	2
55	Male	75	1193	Bachelor	1193	Ɛ3/Ɛ3	3	6	2
56	Male	88	1095	Bachelor	1095	Ɛ3/Ɛ3	3	6	2
57	Female	89	1115	Bachelor	1115	Ɛ3/Ɛ3	5	6	2
58	Female	95	995	Bachelor	995	Ɛ3/Ɛ4	5	6	2
59	Female	87	1195	College	1195	Ɛ3/Ɛ3	3	6	2
60	Male	86	1195	Senior	1195	Ɛ3/Ɛ4	4	6	2
61	Female	95	1290	Primary	1290	Ɛ3/Ɛ3	3	6	2

Supplementary Table 2. Demographics of the included cases, cases from Netherlands Brain Bank.

Case	**Gender**	**Age at death**	**Brain weight (g)**	**post-mortem delay (PMD, minutes)**	**APOE**	**Braak NFT Stage**	**diagnosis**
1	Female	69	1074	510	Ɛ3/Ɛ3	1	Non-demented control
2	Female	87	1275	480	Ɛ3/Ɛ3	1	Non-demented control
3	Female	82	1184	420	Ɛ3/Ɛ3	1	Non-demented control
4	Female	69	1074	510	Ɛ3/Ɛ3	1	Non-demented control
5	Female	89	1160	385	Ɛ3/Ɛ4	1	Non-demented control
6	Male	61	1360	340	Ɛ3/Ɛ4	1	Non-demented control
7	Female	92	1193	420	Ɛ3/Ɛ4	1	Non-demented control
8	Female	89	1160	385	Ɛ3/Ɛ4	1	Non-demented control
9	Male	92	1117	505	Ɛ3/Ɛ3	4	Alzheimer's disease
10	Male	90	1080	355	Ɛ3/Ɛ3	4	Alzheimer's disease
11	Female	90	950	251	Ɛ3/Ɛ3	4	Alzheimer's disease
12	Female	91	951	225	Ɛ3/Ɛ4	4	Alzheimer's disease
13	Female	86	995	200	Ɛ3/Ɛ4	4	Alzheimer's disease
14	Female	87	888	175	Ɛ3/Ɛ4	4	Alzheimer's disease
15	Female	74	904	365	Ɛ3/Ɛ3	5	Alzheimer's disease
16	Female	75	960	255	Ɛ3/Ɛ3	5	Alzheimer's disease
17	Male	59	1171	465	Ɛ3/Ɛ3	5	Alzheimer's disease
18	Female	76	971	265	Ɛ3/Ɛ4	5	Alzheimer's disease
19	Female	83	818	295	Ɛ3/Ɛ4	5	Alzheimer's disease
20	Female	85	1003	370	Ɛ3/Ɛ4	5	Alzheimer's disease
21	Female	83	818	295	Ɛ3/Ɛ4	5	Alzheimer's disease
22	Female	74	904	365	Ɛ3/Ɛ3	5	Alzheimer's disease
23	Male	95	1143	420	Ɛ3/Ɛ3	5	Alzheimer's disease
24	Female	75	960	255	Ɛ3/Ɛ3	5	Alzheimer's disease
25	Female	76	971	265	Ɛ3/Ɛ4	5	Alzheimer's disease
26	Female	85	1044	190	Ɛ3/Ɛ4	5	Alzheimer's disease
27	Female	70	920	270	Ɛ3/Ɛ3	6	Alzheimer's disease
28	Female	91	870	220	Ɛ3/Ɛ3	6	Alzheimer's disease
29	Female	70	876	495	Ɛ3/Ɛ3	6	Alzheimer's disease
30	Female	57	953	245	Ɛ4/Ɛ4	6	Alzheimer's disease
31	Female	86	1033	320	Ɛ4/Ɛ4	6	Alzheimer's disease

SUPPLEMENTARY DATA

Supplementary Table 3. False Discovery Rate (FDR) correction for multiple comparisons of fiber morphology across different Braak stages.

comparison_ label	Raw_ p	FDR_ p
Length of Horizontal fiber: Braak I vs Braak IV	＜0.0001	＜0.0001
Length of Horizontal fiber: Braak I vs Braak V	＜0.0001	＜0.0001
Length of Horizontal fiber: Braak I vs Braak VI	＜0.0001	＜0.0001
Length of Horizontal fiber: Braak IV vs Braak V	0.00011	0.00027
Length of Horizontal fiber: Braak IV vs Braak VI	0.65948	0.71444
Length of Horizontal fiber: Braak V vs Braak VI	0.00071	0.00153
Length of Vertical fiber: Braak I vs Braak IV	＜0.0001	＜0.0001
Length of Vertical fiber: Braak I vs Braak V	＜0.0001	＜0.0001
Length of Vertical fiber: Braak I vs Braak VI	＜0.0001	＜0.0001
Length of Vertical fiber: Braak IV vs Braak V	0.00171	0.00297
Length of Vertical fiber: Braak IV vs Braak VI	0.05911	0.07685
Length of Vertical fiber: Braak V vs Braak VI	0.00001	0.00002
Curvature of Horizontal fiber: Braak I vs Braak IV	0.11278	0.13964
Curvature of Horizontal fiber: Braak I vs Braak V	0.00005	0.00014
Curvature of Horizontal fiber: Braak I vs Braak VI	0.03510	0.05369
Curvature of Horizontal fiber: Braak IV vs Braak V	0.20170	0.23838
Curvature of Horizontal fiber: Braak IV vs Braak VI	0.32659	0.36919
Curvature of Horizontal fiber: Braak V vs Braak VI	0.95084	0.95084
Curvature of Vertical fiber: Braak I vs Braak IV	0.01169	0.01900
Curvature of Vertical fiber: Braak I vs Braak V	0.00158	0.00293
Curvature of Vertical fiber: Braak I vs Braak VI	＜0.0001	＜0.0001
Curvature of Vertical fiber: Braak IV vs Braak V	0.71105	0.73950
Curvature of Vertical fiber: Braak IV vs Braak VI	0.04084	0.05899
Curvature of Vertical fiber: Braak V vs Braak VI	0.05310	0.07266
Curvature of Horizontal fibere: Braak I vs Braak IV-VI	0.00092	0.00184
Curvature of Vertical fiber: Braak I vs Braak IV-VI	0.00058	0.00137

Supplementary Table 4. FDR correction for multiple comparisons of fiber morphology across different APOE genotypes.

APOE 3/3 vs APOE 3/4	Raw_ p	FDR_ p
Length of Horizontal fiber - Braak I	0.02833	0.04533
Length of Horizontal fiber - Braak IV	0.07514	0.10930
Length of Horizontal fiber - Braak V	＜0.0001	＜0.0001
Length of Horizontal fiber - Braak VI	0.00014	0.00044
Length of Vertical fiber - Braak I	＜0.0001	＜0.0001
Length of Vertical fiber - Braak IV	＜0.0001	0.00023
Length of Vertical fiber - Braak V	＜0.0001	0.00040
Length of Vertical fiber - Braak VI	0.61763	0.65880
Curvature of Horizontal fiber - Braak I	0.00421	0.00841
Curvature of Horizontal fiber - Braak IV	0.00034	0.00090
Curvature of Horizontal fiber - Braak V	0.00563	0.01002
Curvature of Horizontal fiber - Braak VI	0.18945	0.23317
Curvature of Vertical fiber - Braak I	0.10876	0.14501
Curvature of Vertical fiber - Braak IV	0.00346	0.00790
Curvature of Vertical fiber - Braak V	0.54085	0.61812
Curvature of Vertical fiber - Braak VI	0.84761	0.84761

SUPPLEMENTARY DATA

Supplementary Table 5. FDR correction for multiple comparisons of neuropathological markers across different APOE genotypes.

APOE 3/3 vs APOE 3/4	Raw_p	FDR_p
Braak I_AT8	0.62676	0.62676
Braak IV_AT8	0.00022	0.00087
Braak V_AT8	0.23611	0.26982
Braak VI_AT8	0.02822	0.05643
Braak I_MBP	0.19312	0.23768
Braak IV_MBP	＜0.0001	0.00024
Braak V_MBP	0.25296	0.26982
Braak VI_MBP	0.09821	0.14285
Braak I_NFH	0.16161	0.21548
Braak IV_NFH	0.01622	0.03708
Braak V_NFH	＜0.0001	＜0.0001
Braak VI_NFH	0.06825	0.10920
Braak I_NFL	0.01340	0.03574
Braak IV_NFL	＜0.0001	＜0.0001
Braak V_NFL	0.00222	0.00710
Braak VI_NFL	0.04024	0.07154

Supplementary Table. 6 Omnibus likelihood ratio tests for Braak-by-APOE interaction across outcomes.

Outcome class	Outcome	LRT chi-square	df	P	q
Length	Horizontal fiber length	1.53	3	0.676	0.769
	Vertical fiber length	1.13	3	0.769	0.769
Curvature	Horizontal curvature	4.70	3	0.195	0.695
	Vertical curvature	1.14	3	0.767	0.769
Immunofluorescence	AT8 intensity	0.84	2	0.656	0.769
	MBP intensity	2.92	2	0.232	0.695
	NFH intensity	0.00	0	Not estimable	Not estimable
	NFL intensity	0.00	0	Not estimable	Not estimable

Omnibus Braak-by-APOE interaction tests were performed using likelihood ratio tests that compared the donor-aware full mixed-effects model with interaction to the corresponding reduced mixed-effects model without interaction. P values are unadjusted omnibus interaction P values derived from reduced-versus-full model comparison. q values were adjusted across all omnibus interaction tests in this table using the Benjamini-Hochberg procedure.

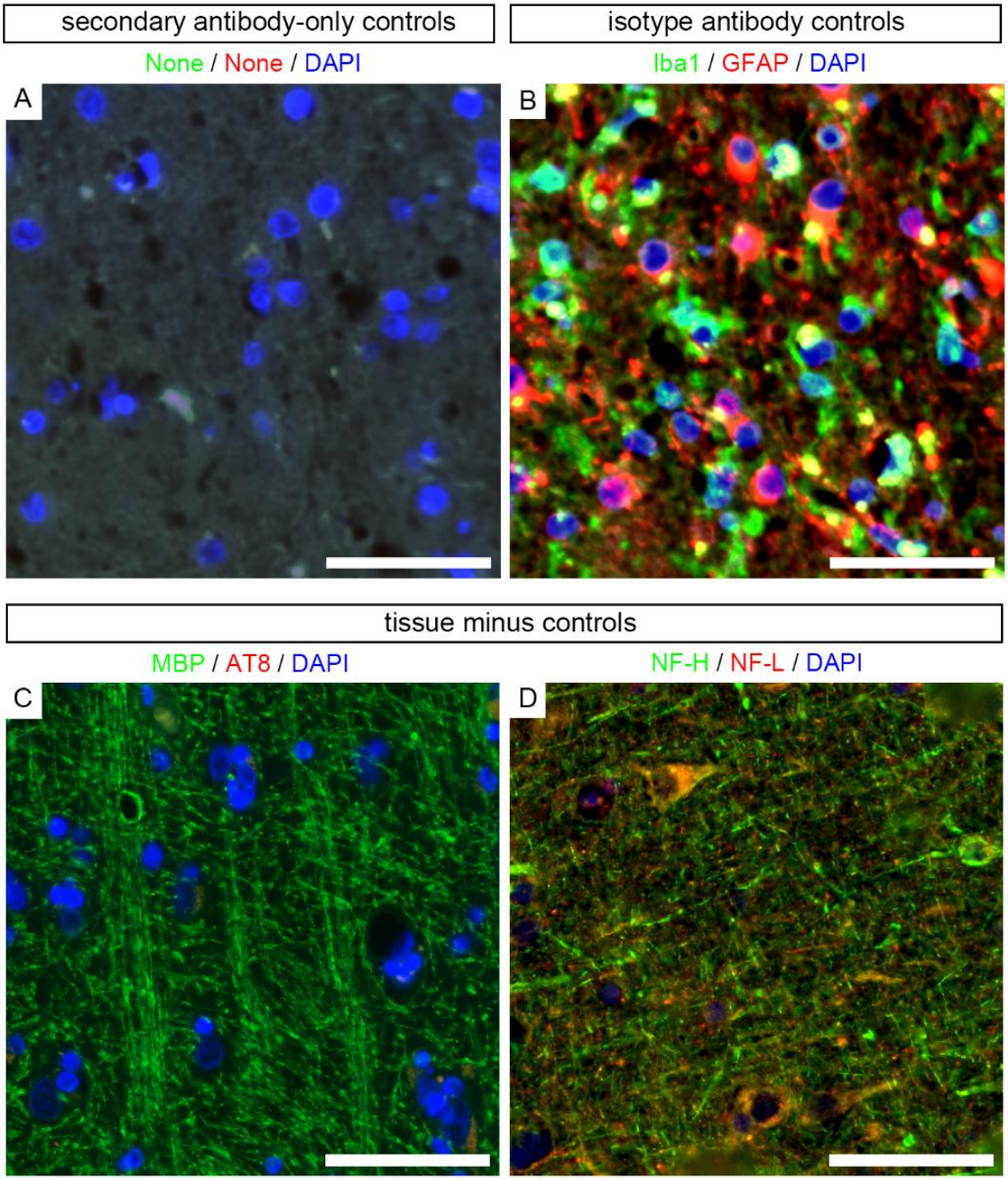
SUPPLEMENTARY DATA

Supplementary Table. 7 Full-model Braak-by-APOE interaction effect estimates across outcomes.

Outcome class	Outcome	Interaction term	Coefficient	95% CI	P	q
Length	Horizontal fiber length	Braak IV x APOE3/4	-4.91	-15.69 to 5.88	0.360	0.666
		Braak V x APOE3/4	1.11	-7.96 to 10.18	0.804	0.998
		Braak VI x APOE3/4	-0.18	-11.78 to 11.41	0.974	0.998
	Vertical fiber length	Braak IV x APOE3/4	-2.14	-21.02 to 16.74	0.819	0.998
		Braak V x APOE3/4	0.44	-15.51 to 16.40	0.955	0.998
		Braak VI x APOE3/4	-9.12	-29.43 to 11.19	0.367	0.666
Curvature	Horizontal curvature	Braak IV x APOE3/4	0.53	0.04 to 1.01	0.035	0.554
		Braak V x APOE3/4	0.25	-0.16 to 0.66	0.230	0.666
		Braak VI x APOE3/4	0.33	-0.19 to 0.84	0.204	0.666
	Vertical curvature	Braak IV x APOE3/4	0.12	-0.17 to 0.42	0.410	0.666
		Braak V x APOE3/4	0.05	-0.19 to 0.30	0.661	0.961
		Braak VI x APOE3/4	0.14	-0.17 to 0.46	0.360	0.666
Immunofluorescence	AT8 intensity	Braak IV x APOE3/4	0.04	-32.06 to 32.13	0.998	0.998
		Braak V x APOE3/4	10.90	-15.24 to 37.04	0.389	0.666
		Braak VI x APOE3/4	Not estimable	Not estimable	Not estimable	Not estimable
	MBP intensity	Braak IV x APOE3/4	12.00	-18.41 to 42.41	0.416	0.666
		Braak V x APOE3/4	20.66	-4.11 to 45.42	0.096	0.666
		Braak VI x APOE3/4	Not estimable	Not estimable	Not estimable	Not estimable
	NFH intensity	Braak IV x APOE3/4	Not estimable	Not estimable	Not estimable	Not estimable
		Braak V x APOE3/4	Not estimable	Not estimable	Not estimable	Not estimable
		Braak VI x APOE3/4	Not estimable	Not estimable	Not estimable	Not estimable
	NFL intensity	Braak IV x APOE3/4	Not estimable	Not estimable	Not estimable	Not estimable
		Braak V x APOE3/4	Not estimable	Not estimable	Not estimable	Not estimable
		Braak VI x APOE3/4	Not estimable	Not estimable	Not estimable	Not estimable

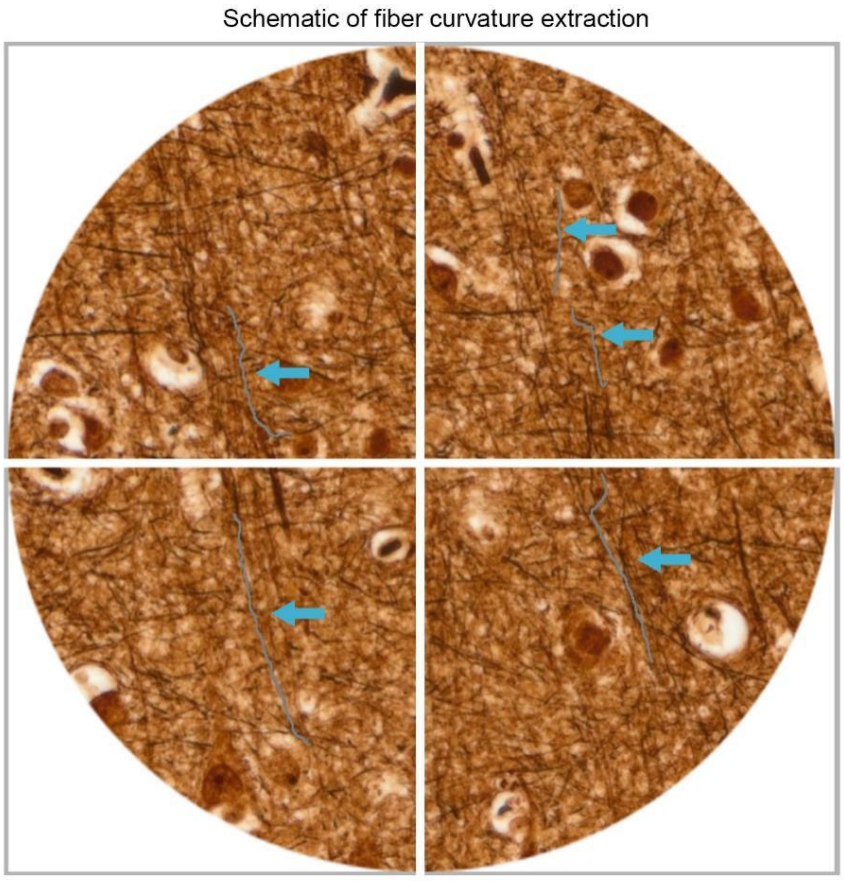
Interaction coefficients were estimated from the full donor-aware model value ~ braak_stage * apoe_genotype + (1 | sample_id). Coefficients represent additional mean differences for APOE3/4 at the indicated Braak stage beyond the additive Braak-stage and APOE-genotype main effects, relative to Braak I and APOE3/3. P values are coefficient-level interaction-term P values from the full model. q values were adjusted across all interaction coefficients in this table using the Benjamini-Hochberg procedure. Not estimable indicates that the corresponding coefficient and confidence interval could not be estimated from the available data.

SUPPLEMENTARY DATA



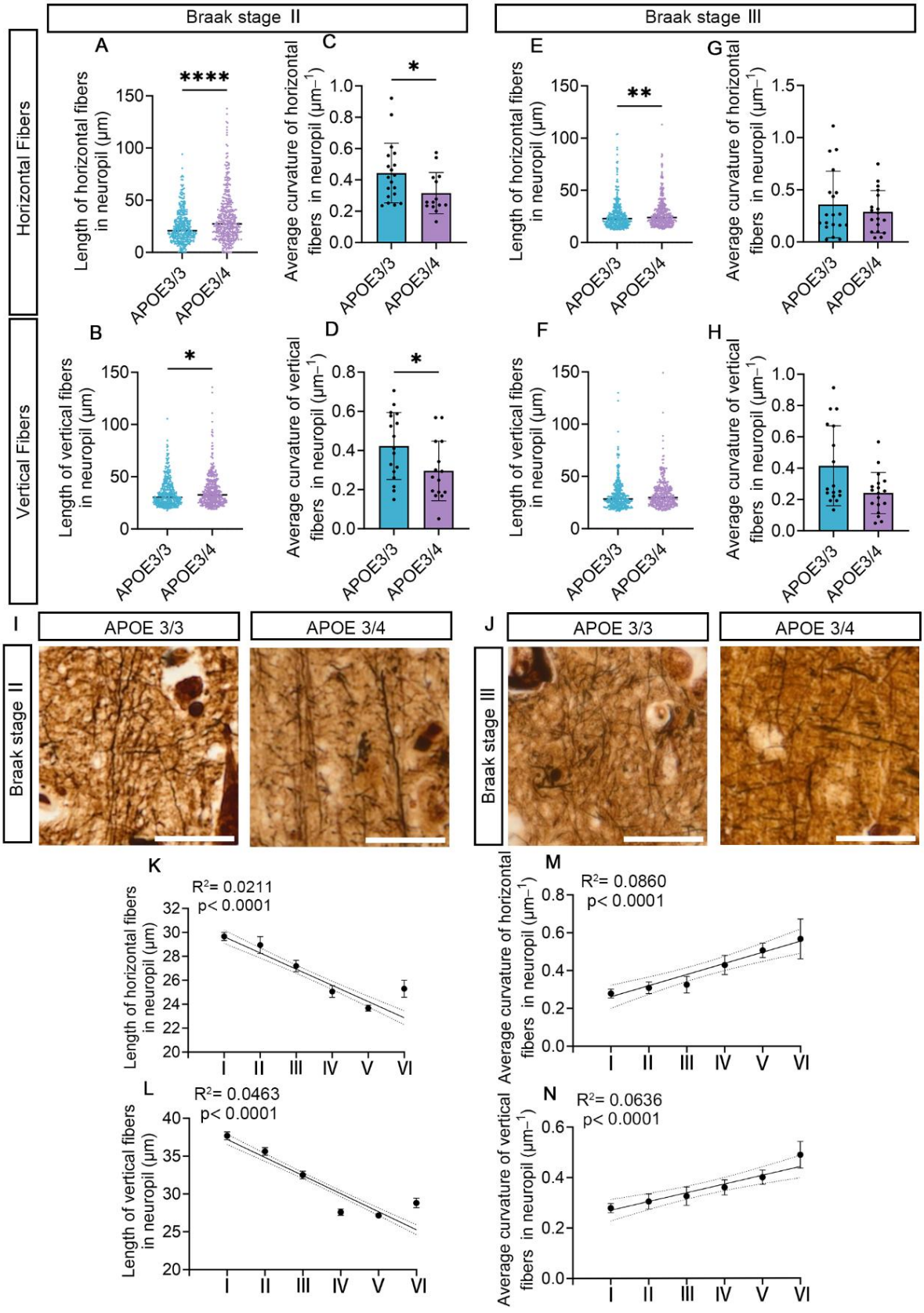
Supplementary Figure 1. Validation of antibody specificity and assessment of background staining.
A. Representative image of secondary antibody-only controls. **B.** Isotype antibody controls, matched for Iba1 (rabbit, 019-19741, Wako, Japan, 1:1000) and GFAP (mouse, G3893, Sigma-Aldrich, USA, 1:1000) channels. **C.** Healthy human brain sections utilized as tissue controls, showing vibrant staining for MBP, AT8. **D.** Healthy human brain sections utilized as tissue controls, showing vibrant staining for NF-H, NF-L. Nuclei were counterstained with DAPI. Scale bars, 50μm.

SUPPLEMENTARY DATA



Supplementary Figure 2. Schematic of fiber curvature extraction.
The field of view is divided into four quadrants. Blue arrows and gray tracings highlight representative target fibers selected for curvature quantification.

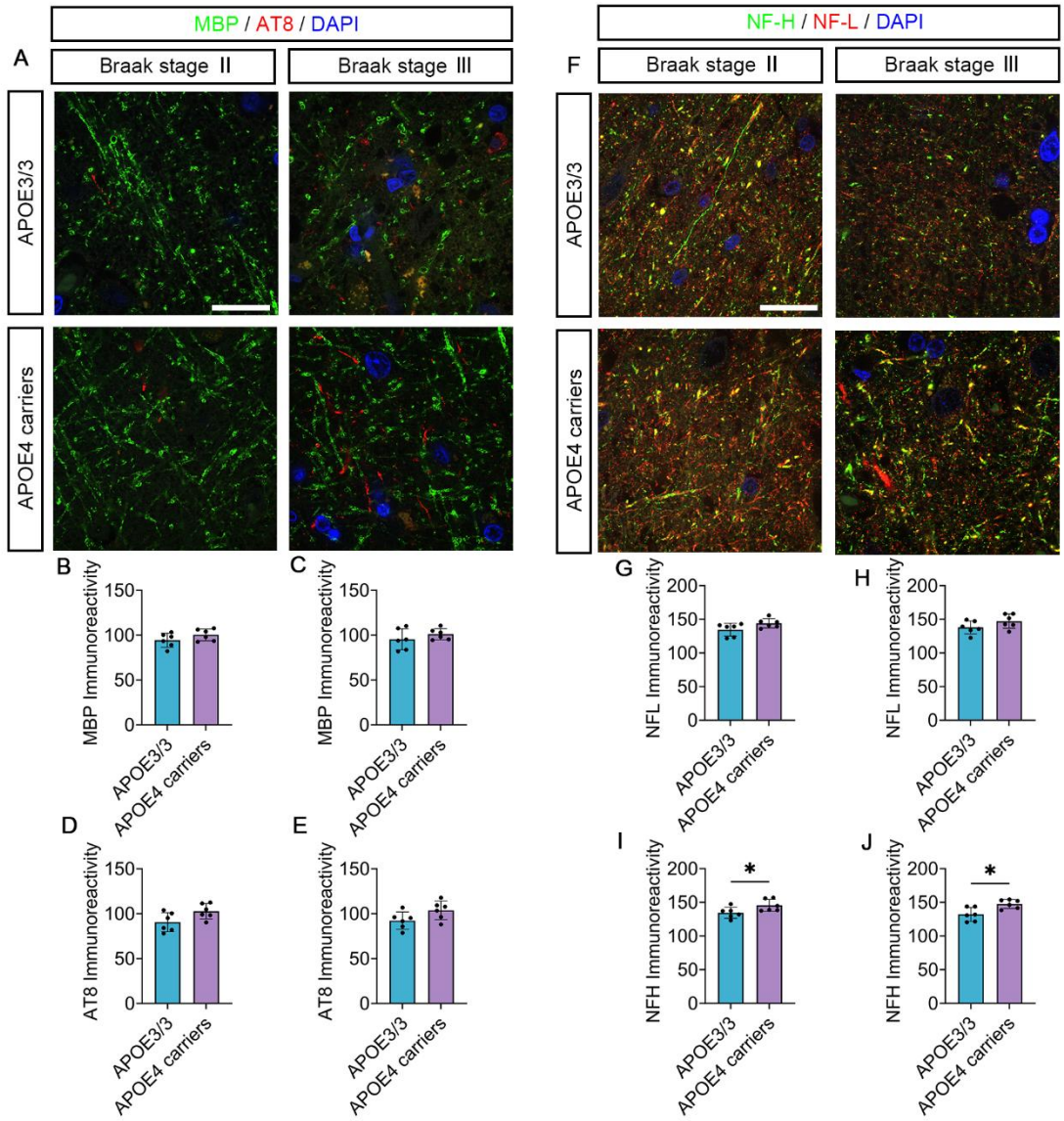
SUPPLEMENTARY DATA



SUPPLEMENTARY DATA

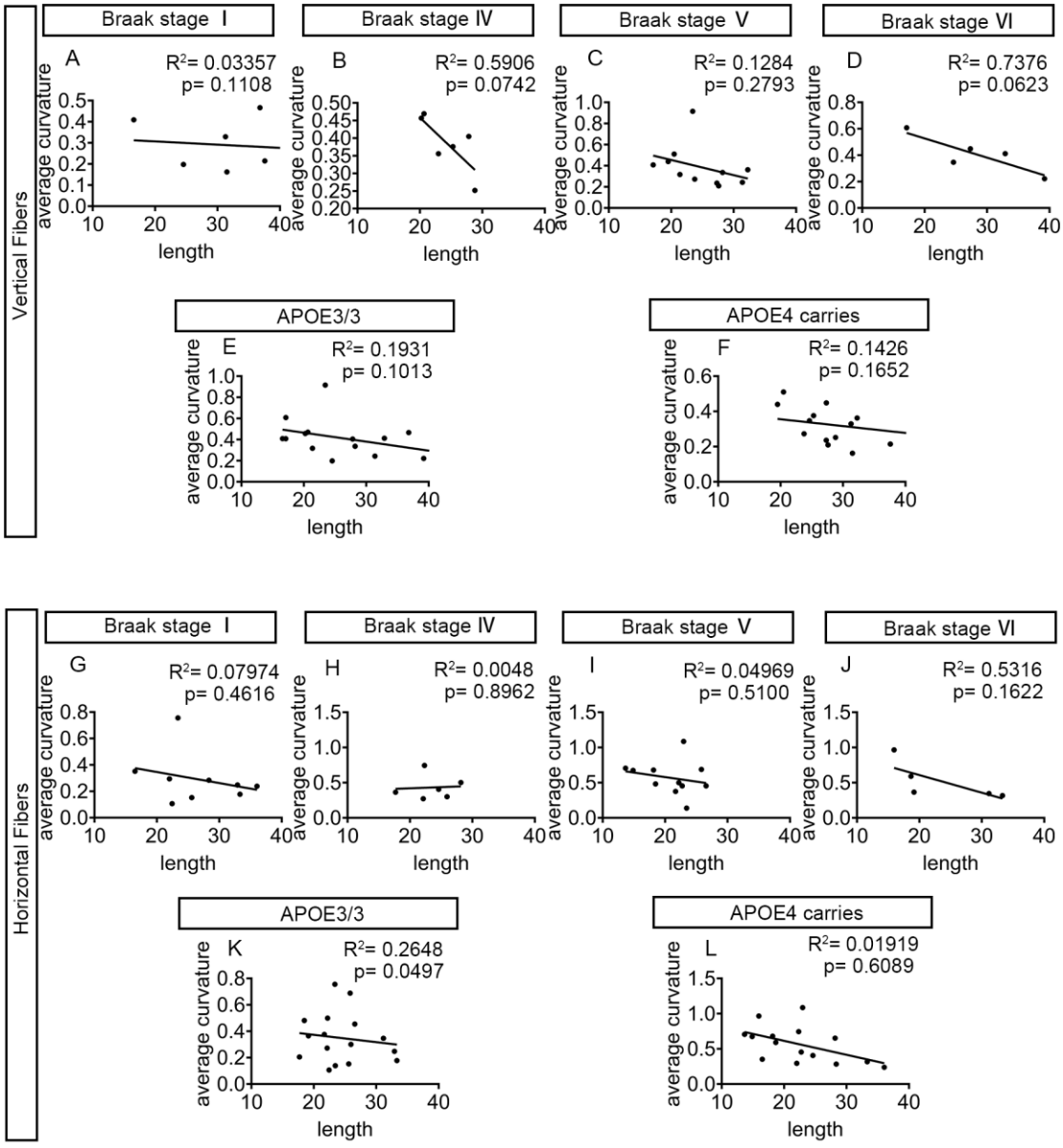
Supplementary Figure 3. Progressive morphological degeneration of neuronal fibers across varying Braak stages and the alterations in intermediate stages.
A– D. Quantitative comparisons of morphological parameters between APOE3/3 and APOE3/4 at Braak stage II. Graphs detail the length of horizontal (**A**) and vertical fibers (**B**), as well as the average curvature of horizontal (**C**) and vertical fibers (**D**). **E– H.** Corresponding quantitative comparisons of the fiber length (**E, F**) and average curvature (**G, H**) between APOE3/3 and APOE3/4 at Braak stage III. The brain samples from 8 donors were statistically analyzed, 4 at Braak stage II (2 each for APOE3 and APOE4), 4 at Braak stage III (2 each for APOE3 and APOE4). Each data point represents a single ROI. For fiber length analysis, 30–40 slices were evaluated per donor with 2 ROIs per slice; the lengths of 100–200 individual fibers within each ROI were averaged to obtain the final ROI value. For average curvature analysis, 4–5 slices were evaluated per donor with 2 ROIs per slice; the curvatures of 3–6 individual fibers within each ROI were averaged to obtain the final ROI value. Statistical significance was determined using unpaired *t-tests*. $*p < 0.05$, $**p < 0.01$, $***p < 0.001$, $****p < 0.0001$. **I– J** Examples curvature of neuropil fibers across Braak stage and APOE genotype (MTG). Scale bar, 20 μm. **K– N.** Linear regression analyses correlating Braak stages (I to VI) with the morphological parameters of neuropil fibers, including the length of horizontal (**K**) and vertical fibers (**L**), and the average curvature of horizontal (**M**) and vertical fibers (**N**). The solid lines represent the linear best fit, and the dotted lines delineate the 95% confidence intervals.

SUPPLEMENTARY DATA



Supplementary Figure 4. Quantification of AT8 and MBP levels, and NF-L and NF-H levels in APOE3/3 and APOE4 carriers stratified by Braak stage.
A. Representative double-label immunofluorescence images showing MBP and AT8 expression. Scale bar, 20 μm. **B– C.** Quantitative analysis of MBP immunoreactivity across APOE genotypes at Braak stage II (**b**) and III (**c**). **D– E.** Quantitative analysis of AT8 immunoreactivity across APOE genotypes at Braak stage II (**D**) and III (**E**). **F.** Representative double-label immunofluorescence images showing NFH and NFL expression. Scale bar, 20 μm. **G– H.** Quantitative analysis of NF-L immunoreactivity across APOE genotypes at Braak stage II (**G**) and III (**H**). **I– J.** Quantitative analysis of NF-H immunoreactivity across APOE genotypes at Braak stage II (**I**) and III (**J**). The brain samples from 8 donors were statistically analyzed, including 4 samples at Braak stage II (2 each for APOE3 and APOE4), 4 at Braak stage III (2 each for APOE3 and APOE4). Each data point represents a single ROI. One ROI was selected from each slice, with three slices analyzed per donor. Statistical significance was determined using unpaired *t-tests*. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, ****$p < 0.0001$.

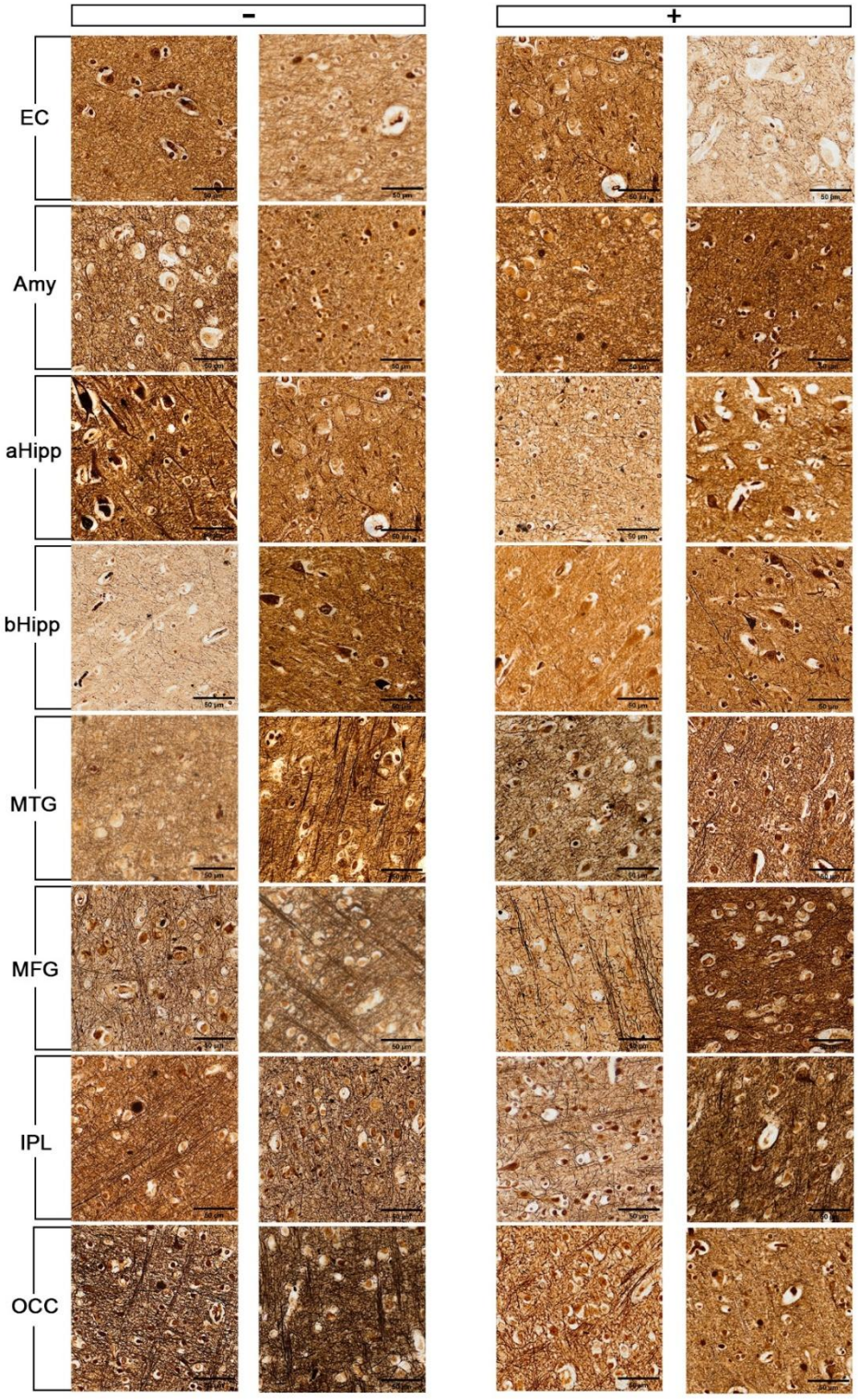
SUPPLEMENTARY DATA



Supplementary Figure 5. Correlation analysis between neuropil fiber length and curvature across different Braak stages and APOE genotypes.

A– F. Scatter plots illustrating the relationship between mean fiber length (x-axis) and mean curvature (y-axis) for vertical fibers stratified by Braak stages (I, IV, V, VI; **A– D**) and APOE genotypes (APOE3/3 and APOE4 carriers; **E– F**). **G– L.** Scatter plots showing the relationship between mean fiber length and mean curvature for horizontal fibers stratified by Braak stages (**G– J**) and APOE genotypes (**K– L**). Each data point represents an individual subject. The coordinates for each point were derived by calculating the arithmetic mean of the length and curvature of neuropil fibers identified within the ROI for that sample. Linear regression lines, R^2 values, and p-values are indicated within each plot.

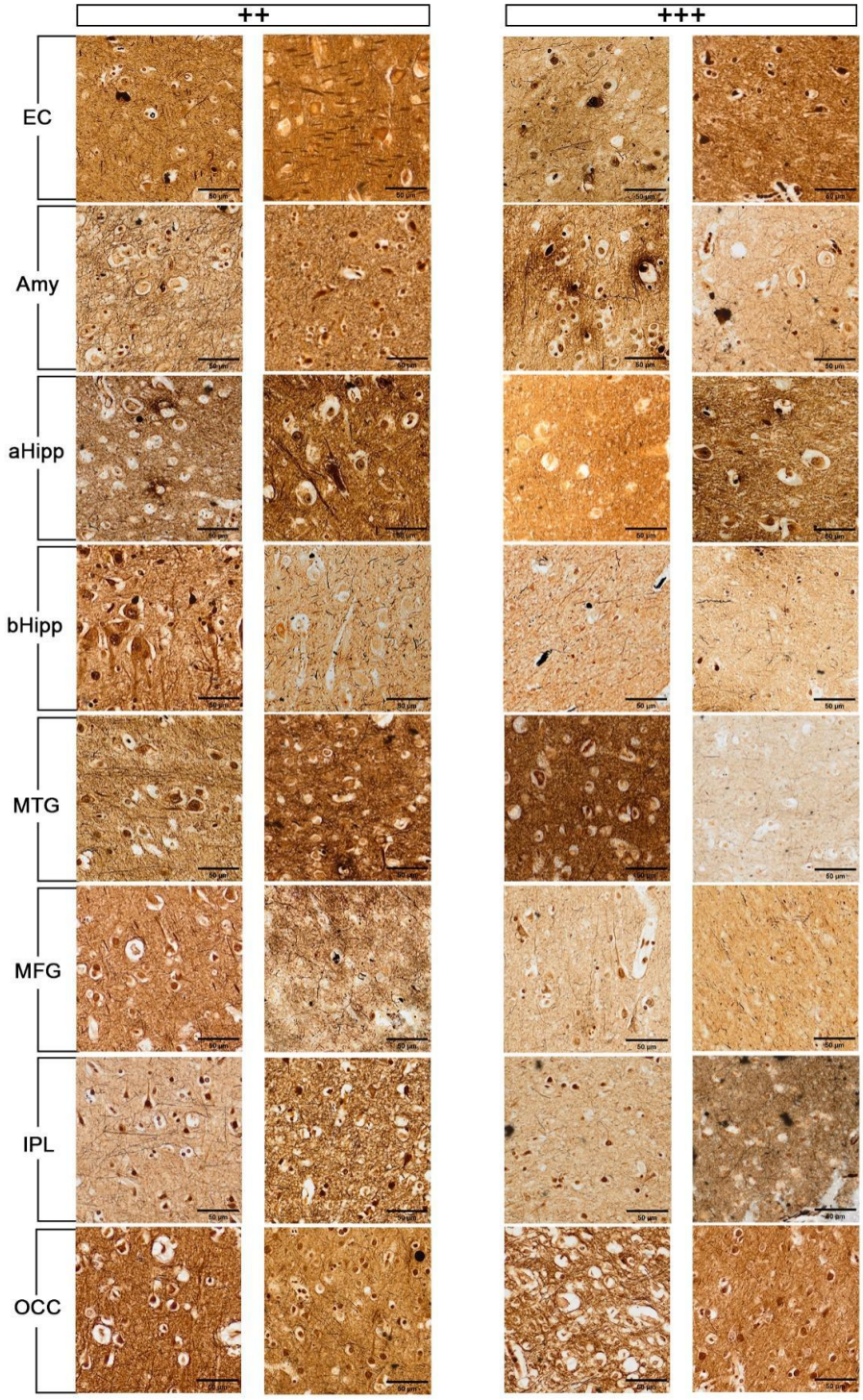
SUPPLEMENTARY DATA



Supplementary Figure 6. Inter-case consistency of the four-stage neuropil disruption staging system across multiple brain regions.
Representative microphotographs illustrating the morphological grading system of neuropil disruption classified into four stages: None (-), Low (+). Two corresponding images from two independent patient samples are presented side-by-side for each specific grade within each distinct brain region. Scale bar, 50 μm.

SUPPLEMENTARY DATA

SUPPLEMENTARY DATA



Supplementary Figure 7. Inter-case consistency of the four-stage neuropil disruption staging system across multiple brain regions.
Representative microphotographs illustrating the morphological grading system of neuropil disruption classified into four stages: Moderate (++), High (+++). Two corresponding images from two independent patient samples are presented side-by-side for each specific grade within each distinct brain region. Scale bar, 50 μm.