

Identification of *Chlamydia pneumoniae* and NLRP3 inflammasome activation in Alzheimer's disease retina

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Supplementary Table 1. Detail neuropathological reports of human retinal and brain donors for histological study.

Donor	Sex	Race	Age at Death	Thal (A)	Braak (B)	CERAD (C)	CAA Score	Braak Stage	CDR Score	MMSE Score	APOE status	MOCA
NC1	F	W	93	3	2	3	0	3.5	1	27	e3/e2	n.a.
NC2	F	H	85	2	1	2	0	1.5	0	30	e3/e3	n.a.
NC3	M	H	81	3	1	2	0	1.5	0	23	e3/e4	23
NC4	F	W	99	1	2	1	0	3	0	29	e3/e3	n.a.
NC5	M	W	95	1	1	1	0	1	0	30	e3/e3	27
NC6	M	H	76	2	0	2	0	0	0	29	e3/e3	27
NC7	M	W	69	0	0	1	0	0	1	28	n.a.	n.a.
NC8	F	W	95	3	3	2	1	5	0	30	e3/e3	n.a.
NC9	F	W	95	1	0	0	0.5	1	0	30	n.a.	n.a.
NC10	F	W	98	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
NC11	F	n.a.	95	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
NC12	F	n.a.	91	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
NC13	M	W	77	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	30	n.a.	n.a.
NC14	F	W	58	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	30	n.a.	n.a.
NC15	M	W	84	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	30	n.a.	n.a.
NC16	M	W	70	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	30	n.a.	n.a.
NC17	F	W	88	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
NC18	M	W	74	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
NC19	M	W	87	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	30	n.a.	n.a.
NC20	M	B	80	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
NC21	F	W	86	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
MCI1	F	W	86	3	1	3	0	1.5	2	15	e3/e4	26
MCI2	F	W	93	3	2	2	2	4	3	11	e3/e3	n.a.
MCI3	M	W	93	2	0	2	0	0	3	19	e3/e2	n.a.
MCI4	M	W	97	2	3	3	1	5	1	28	e3/e3	n.a.
MCI5	F	B	94	2	1	2	0	2	0.5	29	e3/e3	23
MCI6	F	W	89	1	2	2	1	4	0.5	24	e3/e3	21
MCI7	M	W	83	2	3	2	1.5	3.5	0	26	n.a.	n.a.
MCI8	F	W	91	2	2	2	0	3	3	29	n.a.	n.a.
MCI9	F	W	98	3	3	2	2	5	2	15	n.a.	n.a.
MCI10	F	W	87	3	3	3	1.5	5.5	3	13	e3/e3	n.a.
MCI11	M	W	88	1	2	2	0	3	3	n.a.	n.a.	24
MCI12	M	H	80	3	3	2	n.a.	5	3	29	e3/e3	n.a.
MCI13	M	W	75	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
MCI14	M	W	90	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.

Donor	Sex	Race	Age at Death	Thal (A)	Braak (B)	CERAD (C)	CAA Score	Braak Stage	CDR Score	MMSE Score	APOE status	MOCA
MCI15*	M	H	85	0	1	3	1	1.5	0.5	n.a.	n.a.	n.a.
AD1	F	W	90	2	3	3	1	5	2	9	n.a.	n.a.
AD2	F	W	100	2	3	3	1	5.5	2	16	n.a.	n.a.
AD3	M	W	90	3	3	3	2	6	3	n.a.	n.a.	n.a.
AD4	M	W	88	2	3	2	1	5.5	1	4	e3/e4	18
AD5	F	W	87	2	3	3	1.5	5	3	16	e3/e4	n.a.
AD6	F	W	70	3	3	3	1.5	5	0.5	24	n.a.	n.a.
AD7	M	W	77	3	3	3	1	6	2	18	e3/e4	n.a.
AD8	F	W	66	3	3	3	0	6	3	2	e3/e3	n.a.
AD9	M	A	81	3	3	3	1	5	3	12	e4/e4	n.a.
AD10	F	H	99	3	3	3	1.5	4	3	23	e3/e3	n.a.
AD11	F	W	85	3	3	3	1.5	5.5	3	n.a.	e3/e3	n.a.
AD12	F	W	81	3	3	3	2	6	n.a.	4	n.a.	n.a.
AD13	M	W	83	3	2	3	2	4	1	18	n.a.	n.a.
AD14	M	H	65	3	3	3	1.5	5	3	n.a.	e4/e4	n.a.
AD15	F	H	92	2	3	2	2	5	3	9	n.a.	n.a.
AD16	F	A	88	2	3	3	1.5	5	3	4	n.a.	n.a.
AD17	M	W	66	3	3	3	1.5	5	3	19	n.a.	n.a.
AD18	F	W	86	3	3	2	3	5.5	3	18	e3/e4	n.a.
AD19	M	W	88	3	3	3	1.5	5.5	1	16	e2/e3	n.a.
AD20	F	A	93	2	2	2	1.5	3.5	3	17	n.a.	n.a.
AD21	F	W	94	3	3	3	0	5.5	3	n.a.	e3/e3	n.a.
AD22	F	H	81	3	3	3	1.5	5.5	3	12	e3/e3	n.a.
AD23	F	W	90	3	3	3	1	5.5	3	n.a.	e3/e4	n.a.
AD24	M	W	90	3	2	3	1	4	3	n.a.	e3/e4	1
AD25	F	W	93	2	3	3	1	5	3	n.a.	n.a.	n.a.
AD26	M	W	79	3	3	3	1.5	5	n.a.	n.a.	n.a.	n.a.
AD27	F	W	87	3	3	3	2	5.5	3	n.a.	e3/e4	9
AD28	M	W	88	2	2	2	0	3.5	n.a.	4	n.a.	n.a.
AD29	M	H	97	3	2	3	1	3	1	26	e3/e3	n.a.
AD30	M	W	90	3	3	2	2	5	2	18	n.a.	n.a.
AD31	M	W	85	3	2	3	1	4	n.a.	n.a.	n.a.	n.a.
AD32	M	W	99	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
AD33	M	n.a.	90	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
AD34	M	H	97	3	2	3	1	3	1	26	e3/e3	n.a.

AD, Alzheimer's disease dementia; MCI, mild cognitive impairment; NC, normal cognition; F, female; M, male; A, Asian; B, Black; H, Hispanic; W, White; A, A β plaque score modified from Thal; B, NFT stage modified from Braak; C, neuritic plaque score modified from CERAD; CAA, cerebral amyloid angiopathy; CDR, clinical dementia rating; MMSE, mini-mental state examination; MOCA: Montreal cognitive assessment; n.a., not available; APOE, apolipoprotein alleles. *Brain tissues only.

Supplementary Table 2. List of human donors whose postmortem retinas were used for mass spectrometry.

Donor	Sex	Race	Age at Death	Thal (A)	Braak (B)	CERAD (C)	CAA Score	Braak Stage	CDR Score	MMSE Score	APOE Status
AD1	F	W	93	2	3	3	1	5	3	n.a.	n.a.
AD2	M	W	88	2	3	2	1	5.5	1	4	e3/e4
AD3	F	B	94	3	3	3	0	5.5	3	n.a.	e3/e3
AD4	F	H	48	n.a.	n.a.	n.a.	2	5	n.a.	n.a.	n.a.
AD5	M	W	72	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
AD6	F	W	100	2	3	3	1	5.5	2	16	n.a.
NC1	M	H	81	3	1	2	0	1.5	0	23	e3/e4
NC2	F	W	75	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
NC3	F	W	72	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
NC4	M	W	69	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
NC5	F	W	79	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
NC6	M	W	85	0	1	3	1	1.5	0.5	n.a.	n.a.

Abbreviations: AD, Alzheimer's disease; APOE, apolipoprotein, B, Black; A (Thal), A β plaque score modified from Thal; B (Braak), NFT stage modified from Braak; C (CERAD), neuritic plaque score modified from CERAD; CAA, cerebral amyloid angiopathy; CDR, clinical dementia rating; CERAD, consortium to establish a registry for Alzheimer's disease; F, female; H, Hispanic; M, male; MMSE, mini-mental state examination; n.a., not available; NC, normal cognition; W, White. All human donor tissues obtained from UCI-ADRC.

Supplementary Table 3. List of human donors whose postmortem brains were used for mass spectrometry.

Diagnosis	Sex	Race	Age at Death	Plaque Stage	Braak Stage	Neuritic Plaque	MMSE Score	APOE Status
AD1	M	W	86	C	6	4	19	3/4
AD2	F	W	89	C	6	4	15	3/4
AD3	F	W	95	C	6	3	n.a.	2/3
AD4	F	W	93	C	6	4	13	3/3
AD5	F	W	88	C	5	4	10	3/3
AD6	M	W	92	C	5	4	12	3/3
AD7	M	H	86	C	5	4	n.a.	3/3
AD8	F	W	98	C	6	4	14	3/3
AD9	F	W	91	C	6	4	17	3/3
AD10	F	W	82	C	6	4	17	3/3
NC1	F	W	87	0	2	1	30	2/3
NC2	F	W	91	0	4	1	29	2/3
NC3	F	H/AI	95	0	2	1	n.a.	3/3
NC4	F	W	90	B	3	3	18	3/3
NC5	M	W	96	0	2	1	29	2/3
NC6	M	W	94	0	1	1	27	3/3
NC7	M	W	86	0	2	2	27	3/3
NC8	F	W	91	A	2	2	29	3/3

Abbreviations: AD, Alzheimer's disease; APOE, apolipoprotein; AI, American Indian; F, female; H, Hispanic; M, male; MMSE, Mini-Mental State Examination; n.a., not available; NC, normal cognition; Plaque Stage: 0, none; A, phase I-II; B, phase III; C, phase IV-V; Neuritic Plaque scores: 4, frequent NP; 3, moderate NP; 2, sparse NP; 1, no NPs. All human donor tissues obtained from UCI-ADRC.

Supplementary Table 4. Demographic data on human retinal donors for mass spectrometry analysis.

	NC	AD	F	<i>p</i>	
n = 12	6 (3F, 3M)	6 (4F, 2M)	-	-	
Age at Death (Years)	76.8 ± 5.9	82.5 ± 19.4	0.68	0.52	
Race	5W, 1H	4W, 1B, 1H	-	-	
PMI (Hours)	9.0 ± 5.1	7.8 ± 2.7	0.62	0.57	
MMSE Score (n = 3)	23.0 ± n.a	17.0 ± 1.4	-	-	
CDR Score (n = 6)	0.25 ± 0.35	2.25 ± 0.96	2.72	0.053	
Brain Neuropathology Severity Score (n = 8)	Braak stage (%)	I-II (100%)	31.03	<0.0001	
	ABC (amyloid, Braak, CERAD) (n = 6)	1.83 ± 0.71	2.67 ± 0.27	1.61	0.33
	Aβ plaque	1.76 ± 0.68	3.09 ± 1.26	1.90	0.14
	NFTs	1.15 ± 0.01	2.50 ± 0.96	3.45	0.0182
	NTs	0.22 ± 0.11	1.43 ± 0.43	5.91	0.0022
	Atrophy	0.55 ± 0.07	1.41 ± 0.97	2.16	0.08

Mean ABC scores were determined as follows: A, Aβ plaque score modified from Thal; B, NFT stage modified from Braak; C, neuritic plaque score modified from CERAD.

Group values are presented as mean ± standard deviation. F and *p*-values were determined using an unpaired Student t test. *p*-values presented in bold type demonstrate significance.

Abbreviations: Aβ, amyloid beta; AD, Alzheimer's disease; CDR, clinical dementia rating; CERAD, consortium to establish a registry for Alzheimer's disease; NC, normal cognition; MMSE, mini-mental state examination; NFTs, neurofibrillary tangles; NTs, neuropil threads; PMI, postmortem interval.

Supplementary Table 5. Demographic data on human brain donors for mass spectrometry analysis.

Human Donors		NC	AD	<i>t</i>	<i>p</i>
n = 18		8 (5F, 3M)	10 (7F, 3M)	-	-
Age at Death [Years] [§]		91.3 ± 3.6	90.0 ± 4.8	0.61	0.55
Race [No]		W (7) H/A (1)	W (9) H (1)	-	-
MMSE Score [§]		27.0 ± 4.1	14.6 ± 2.9	6.74	<0.0001
PMI [Hours] [§]		3.7 ± 0.8	5.3 ± 3.2	1.43	0.17
Brain Neuropathology Severity Score (n = 18)	Aβ-Plaque Stage (No; %)	None (6; 75%) Stage A (1; 12.5%) Stage B (1; 12.5%)	Stage C (10; 100%)	-	-
	Braak Stage (NO; %)	I-II (6; 75%) III-IV (2; 25%) V-VI (0)	V-VI (10; 100%)	-	-

Abbreviations: Aβ, Amyloid-β protein; Aβ-Plaque stages score: None, no Aβ plaque or amyloid plaque; A, mild, A1 Thal phases 1 or 2; B, moderate, A2 Thal phase 3; C, severe plaque pathology, A3 Thal phases 4 or 5; AD, Alzheimer's disease; Braak (NFT) stage scores; MMSE, mini-mental state examination; NC, normal cognition; NFT, neurofibrillary tangles; PMI, postmortem interval; Values are presented as mean ± SD. The *t* and *p* values were determined by Student's t-test.

Supplementary Table 6. List of antibodies for immunohistochemistry.

Antibodies	Source Species	Dilution	Application	Source	Catalog. #
<i>Primary antibody</i>					
Cp pAb	Rabbit	1:500	IF/IHC (DAB)	MyBioSource	MBS534621
Cp mAb	Mouse	1:50	IF	invitrogen	MA5-18183
Cp mAb	Mouse	1:200	IHC/DAB	invitrogen	MA5-18183
NLRP3 mAb	Rat	1:100	IF	R&D systems	MAB7578
Caspase-1 mAb	Rabbit	1:150	IF	R&D systems	MAB62156
ASC	Rabbit	1:300	IF		
N-GSDMD mAb	Rabbit	1:500	IF	Cell Signaling	36425
CCasp3 pAb	Rabbit	1:400	IF	Cell Signaling	9661
GFAP pAb	Goat	1:500	IF	Invitrogen	13-0300
Iba-1 mAb	Rabbit	1:400	IF	Wako	019-19741
Vimentin	Rabbit	1:350	IF	abcam	Ab92547
NeuN mAb	Rabbit	1:500	IF	abcam	Ab177487
<i>Secondary antibody</i>					
Cy3 (anti-mouse, anti-rat,)	Donkey	1:200	IF	Jackson ImmunoResearch Laboratories	715-165-150, 712-165-153
Cy5 (anti-rabbit)	Donkey	1:200	IF	Jackson ImmunoResearch Laboratories	711-175-152
Cy2 (anti-goat)	Donkey	1:200	IF	Jackson ImmunoResearch Laboratories	
HRP (anti-mouse, anti-rabbit)	Goat	-	IHC/DAB	DAKO invision	K4001, K4003

Abbreviations: ASC - apoptosis-associated speck like protein; Cp – *Chlamydia pneumoniae*; GFAP – glial fibrillary acidic protein; Iba1 – ionized calcium binding adaptor molecule 1; CCasp3 – Cleaved Caspase-3; DAB - 3,3-Diaminobenzidine; HRP - Horseradish peroxidase; IF – immunofluorescence; IHC – immunohistochemistry; mAb – monoclonal antibody; NGSDMD – Gasdermin D with cleaved N-terminus; NLRP3 - nucleotide-binding oligomerization domain-like receptor containing pyrin domain 3; pAb – polyclonal antibody.

Supplementary Table 7: Correlation of retinal Cp burden versus retinal and brain AD pathologies and cognition

Pearson's correlation (r_p) analysis: Retinal Cp versus retinal AD pathologies					
A β ₄₂	A β ₄₀	A β oligomers	pS396	PHF-1 tau	Oligo-tau (T22)
0.63****	0.65**	0.18	0.38*	0.54**	0.43**
n = 39	n = 21	n = 22	n = 44	n = 23	n = 43
Cit-tau	AT8-tau	MC-1 tau	NLRP3	Caspase-1	ASC
0.48**	0.02	0.04	0.70****	0.87****	0.60****
n = 37	n = 39	n = 43	n = 27	n = 27	n = 27
NGSDMD	CCasp3	IBA1	GFAP	Vimentin	S100 β
0.51**	0.73****	0.65****	0.70****	0.55**	0.36
n = 27	n = 25	n = 32	n = 28	n = 21	n = 6
Nissl loss	Atrophy				
-0.43*	0.75****				
n = 34	n = 23				
Spearman's correlation (r_s): Retinal Cp versus brain AD pathologies					
ABC	Braak Stage	A β plaques	NFTs	NTs	Gliosis
0.54****	0.54****	0.40**	0.54****	0.37**	0.40**
n = 60	n = 60	n = 60	n = 60	n = 60	n = 60
Atrophy	CAA				
0.48***	0.35**				
n = 60	n = 60				
Spearman's correlation (r_s): Retinal Cp versus cognition					
CDR	MMSE	MoCA			
0.43**	-0.53****	-0.56*			
n = 56	n = 50	n = 15			

Pearson's and Spearman's rank correlation analyses: p and r values determine the statistical significance and strength of each pairwise association between retinal Cp burden versus retinal and brain AD pathologies and cognition. p and r values presented in bold type with asterisk(s) depicts strong to very strong correlation.

Supplementary Table 8: Correlation of brain Cp burden versus brain AD pathologies and cognition

Spearman's correlation (r_s): Brain Cp versus Brain AD pathologies					
ABC	Braak Stage	A β plaques	NFTs	NTs	Gliososis
0.74**	0.72**	0.45	0.73**	0.75**	0.77***
n = 16	n = 16	n = 16	n = 16	n = 16	n = 16
Atrophy	CAA				
0.60*	0.57*				
n = 16	n = 16				
Spearman's correlation (r_s): Brain Cp versus cognition					
CDR	MMSE	MoCA			
0.50	-0.73**	-0.52			
n = 16	n = 14	n = 6			

Spearman's rank correlation analyses: p and r values determine the statistical significance and strength of each pairwise association between brain Cp burden versus brain AD pathologies and cognition. p and r values presented in bold type with asterisk(s) depicts strong to very strong correlation.

Supplementary Table 9. Upregulated and downregulated DEPs in AD versus NC brains from *Chlamydia* interactome.

Accession	Symbol	Description	FC	<i>p</i>	References
Upregulated in AD brain (32)					
Q13501	SQSTM1	Sequestosome-1	1.48	0.0001	1,2
Q08379	GOLGA2	Golgin subfamily A member 2	1.47	0.0190	3
O43291	SPINT2	Kunitz-type protease inhibitor 2	1.45	0.0439	1
P08670	VIM	Vimentin	1.45	0.0013	2
E9PND2	CSRP1	Cysteine and glycine-rich protein 1	1.37	0.0411	3
Q8N4Q1	CHCHD4	Mitochondrial intermembrane space import and assembly protein 40	1.36	0.0462	4
Q15796	SMAD2	Mothers against decapentaplegic homolog 2	1.36	0.0329	3
Q9Y676	MRPS18B	28S ribosomal protein S18b, mitochondrial	1.34	0.0069	4
O95817	BAG3	BAG family molecular chaperone regulator 3	1.34	0.0243	2
G3V1V0	MYL6	Myosin light polypeptide 6	1.34	0.0007	2
P06703	S100A6	Protein S100-A6	1.34	0.0167	1
A1L188	NDUFAF8	NADH dehydrogenase [ubiquinone] 1 alpha subcomplex assembly factor 8	1.28	0.0485	4
O75348	ATP6V1G1	V-type proton ATPase subunit G 1	1.28	0.0007	1
Q14019	COTL1	Coactosin-like protein	1.28	0.0026	1
P21333	FLNA	Filamin-A	1.27	0.0237	2
Q9UBI6	GNG12	Guanine nucleotide-binding protein G(I)/G(S)/G(O) subunit gamma-12	1.27	0.0195	1
P56385	ATP5ME	ATP synthase subunit e, mitochondrial	1.27	0.0103	4
P04792	HSPB1	Heat shock protein beta-1	1.27	0.0082	1,2
P04080	CSTB	Cystatin-B	1.26	0.0075	3
P17931	LGALS3	Galectin-3	1.26	0.0027	2
Q96HC4	PDLIM5	PDZ and LIM domain protein 5	1.25	0.0118	3
O75369	FLNB	Filamin-B	1.25	0.0429	3
Q6DD88	ATL3	Atlastin-3	1.24	0.0024	1,4
J3KN67	TPM3	Tropomyosin alpha-3 chain	1.24	0.0103	1-3
P61254	RPL26	60S ribosomal protein L26	1.22	0.0483	3
Q15691	MAPRE1	Microtubule-associated protein RP/EB family member 1	1.22	0.0003	4
Q12797	ASPH	Aspartyl/asparaginyl beta-hydroxylase	1.21	0.0205	1,4
Q9BQT9	CLSTN3	Calsyntenin-3	1.21	0.0171	4
Q9C0C2	TNKS1BP1	182 kDa tankyrase-1-binding protein	1.21	0.0054	2
Q09666	AHNAK	Neuroblast differentiation-associated protein AHNAK	1.22	0.0342	2,3
Q5TZA2	CROCC	Rootletin	1.22	0.0395	1
P05556	ITGB1	Integrin beta-1	1.20	0.0065	2
Downregulated in AD brain (52)					

Q9NZ01	TECR	Very-long-chain enoyl-CoA reductase	-2.05	0.0246	1
Q9NQC3	RTN4	Reticulon-4	-1.83	0.0039	1,3,4
Q9UGP8	SEC63	Translocation protein SEC63 homolog	-1.70	0.0089	1
Q6IA86	ELP2	Elongator complex protein 2	-1.62	0.0209	4
E9PK54	HSPA8	Heat shock cognate 71 kDa protein	-1.53	0.0411	1
Q9BVC4	MLST8	Target of rapamycin complex subunit LST8	-1.51	0.0263	4
O43760	SYNGR2	Synaptogyrin-2	-1.49	0.0208	1,3
P61204	ARF3	ADP-ribosylation factor 3	-1.47	0.0128	1
Q9P035	HACD3	Very-long-chain (3R)-3-hydroxyacyl-CoA dehydratase 3	-1.43	0.0372	1
P61225	RAP2B	Ras-related protein Rap-2b	-1.40	0.0007	1
Q8TCJ2	STT3B	Dolichyl-diphosphooligosaccharide--protein glycosyltransferase subunit	-1.39	0.0185	1,4
O15258	RER1	Protein RER1	-1.37	0.0263	1,4
O43747	AP1G1	AP-1 complex subunit gamma-1	-1.37	0.0196	1
O95716	RAB3D	Ras-related protein Rab-3D	-1.36	0.0000	1
P0CG08	GPR89B	Golgi pH regulator B	-1.36	0.0080	4
K7EJH8	ACTN4	Alpha-actinin-4	-1.33	0.0062	1,3
P63096	GNAI1	Guanine nucleotide-binding protein G(i) subunit alpha-1	-1.33	0.0034	3
Q92905	COPS5	COP9 signalosome complex subunit 5	-1.32	0.0015	4
O15144	ARPC2	Actin-related protein 2/3 complex subunit 2	-1.32	0.0133	1
P07099	EPHX1	Epoxide hydrolase 1	-1.32	0.0340	1
F2Z2X4	XPO4	Exportin-4	-1.31	0.0026	4
Q7Z7H5	TMED4	Transmembrane emp24 domain-containing protein 4	-1.31	0.0230	1
P15531	NME1	Nucleoside diphosphate kinase A	-1.28	0.0028	3
Q01581	HMGCS1	Hydroxymethylglutaryl-CoA synthase, cytoplasmic	-1.28	0.0119	4
O00410	IPO5	Importin-5	-1.28	0.0401	4
Q93050	ATP6V0A1	V-type proton ATPase 116 kDa subunit a isoform 1	-1.27	0.0015	1,4
Q9BXS5	AP1M1	AP-1 complex subunit mu-1	-1.27	0.0120	3,4
Q96F07	CYFIP2	Cytoplasmic FMR1-interacting protein 2	-1.27	0.0030	3
P42704	LRPPRC	Leucine-rich PPR motif-containing protein, mitochondrial	-1.27	0.0025	4
P60228	EIF3E	Eukaryotic translation initiation factor 3 subunit E	-1.26	0.0438	3
O43759	SYNGR1	Synaptogyrin-1	-1.26	0.0330	4
F8VXU5	VPS29	Vacuolar protein sorting-associated protein 29	-1.26	0.0234	1,3
P60981	DSTN	Destrin	-1.26	0.0406	3
P20645	M6PR	Cation-dependent mannose-6-phosphate receptor	-1.25	0.0005	1

E9PFW3	AP2M1	AP-2 complex subunit mu	-1.25	0.0097	4
A0A0C4DGQ5	CAPNS1	Calpain small subunit 1	-1.25	0.0185	1,4
Q9BTE1	DCTN5	Dynactin subunit 5	-1.25	0.0007	4
P68104	EEF1A1	Elongation factor 1-alpha	-1.24	0.0061	1
P61106	RAB14	Ras-related protein Rab-14	-1.24	0.0051	1
M0QYN0	MYDGF	Myeloid-derived growth factor	-1.24	0.0056	1
P00338	LDHA	L-lactate dehydrogenase A chain	-1.23	0.0030	1
A0A2U3TZU2	GPI	Glucose-6-phosphate isomerase	-1.23	0.0199	1
Q9NZJ7	MTCH1	Mitochondrial carrier homolog 1	-1.23	0.0097	4
Q06136	KDSR	3-ketodihydrosphingosine reductase	-1.23	0.0235	1,4
P20340	RAB6A	Ras-related protein Rab-6A	-1.22	0.0222	1
P05388	RPLP0	60S acidic ribosomal protein P0	-1.21	0.0086	1
Q5VV89	MGST3	Microsomal glutathione S-transferase 3	-1.21	0.0104	3,4
O14617	AP3D1	AP-3 complex subunit delta-1	-1.21	0.0182	4
P61019	RAB2A	Ras-related protein Rab-2A	-1.21	0.0142	1
A0A0A0MRA8	EPB41L3	Band 4.1-like protein 3	-1.21	0.0006	4
O95782	AP2A1	AP-2 complex subunit alpha-1	-1.20	0.0010	4
Q08209	PPP3CA	Serine/threonine-protein phosphatase 2B catalytic subunit alpha	-1.20	0.0106	3,4

FC, fold change. $|FC| > 1.2$, $p < 0.05$.

Supplementary Table 10. Upregulated and downregulated DEPs in AD versus NC retinas from *Chlamydia* interactome.

Accession	Symbol	Description	FC	<i>p</i>	References
Upregulated in AD retina (40)					
A6NFX8	NUDT5	ADP-sugar pyrophosphatase	1.87	0.0302	3
Q08554	DSC1	Desmocollin-1	1.85	0.0450	1
P15531	NME1	Nucleoside diphosphate kinase A	1.75	0.0386	3
P04792	HSPB1	Heat shock protein beta-1	1.70	0.0054	1,2
Q14574	DSC3	Desmocollin-3	1.63	0.0042	4
P67936	TPM4	Tropomyosin alpha-4 chain	1.62	0.0060	1-3
J3KN67	TPM3	Tropomyosin alpha-3 chain	1.53	0.0174	1-3
P61204	ARF3	ADP-ribosylation factor 3	1.52	0.0344	1
P81605	DCD	Dermcidin	1.51	0.0276	1
A0A0B4J2C3	TPT1	Translationally-controlled tumor protein	1.50	0.0156	2
A0A087WYT3	PTGES3	Prostaglandin E synthase 3	1.50	0.0436	1
P20810	CAST	Calpastatin	1.49	0.0449	1,2
Q02413	DSG1	Desmoglein-1	1.45	0.0327	1
H3BUF6	ATXN2L	Ataxin-2-like protein	1.39	0.0008	2
Q01581	HMGCS1	Hydroxymethylglutaryl-CoA synthase, cytoplasmic	1.37	0.0013	4
Q9UBE0	SAE1	SUMO-activating enzyme subunit 1	1.36	0.0261	4
Q8TAA9	VANGL1	Vang-like protein 1	1.36	0.0230	4
E9PAV3	NACA	Nascent polypeptide-associated complex subunit alpha, muscle-specific	1.34	0.0175	1
O75348	ATP6V1G1	V-type proton ATPase subunit G 1	1.33	0.0451	1
O43852	CALU	Calumenin	1.33	0.0459	1
O60493	SNX3	Sorting nexin-3	1.32	0.0201	3
P13797	PLS3	Plastin-3	1.32	0.0322	3
O43399	TPD52L2	Tumor protein D54	1.32	0.0124	1
O95817	BAG3	BAG family molecular chaperone regulator 3	1.30	0.0225	2
P49354	FNTA	Protein farnesyltransferase/geranylgeranyltransferase type-1 subunit alpha	1.30	0.0426	4
P48507	GCLM	Glutamate--cysteine ligase regulatory subunit	1.29	0.0106	5
Q9NZ08	ERAP1	Endoplasmic reticulum aminopeptidase 1	1.28	0.0065	1
A0MZ66	SHTN1	Shootin-1	1.27	0.0264	3
P60981	DSTN	Destrin	1.27	0.0423	3
Q32MZ4	LRRFIP1	Leucine-rich repeat flightless-interacting protein 1	1.27	0.0087	1-4
P31946	YWHAB	14-3-3 protein beta/alpha	1.27	0.0163	1,4
O15355	PPM1G	Protein phosphatase 1G	1.27	0.0025	4
P54727	RAD23B	UV excision repair protein RAD23 homolog B	1.24	0.0431	4
Q8IYD1	GSPT2	Eukaryotic peptide chain release factor GTP-binding subunit ERF3B	1.24	0.0058	3
O75821	EIF3G	Eukaryotic translation initiation factor 3 subunit G	1.22	0.0077	3

P98172	EFNB1	Ephrin-B1	1.21	0.0325	4
Q13200	PSMD2	26S proteasome non-ATPase regulatory subunit 2	1.20	0.0168	1
O14974	PPP1R12A	Protein phosphatase 1 regulatory subunit 12A	1.20	0.0013	1
P27348	YWHAQ	14-3-3 protein theta	1.20	0.0333	1-3
Q99614	TTC1	Tetratricopeptide repeat protein 1	1.20	0.0464	2
Downregulated in AD retina (52)					
Q96KR6	FAM210B	Protein FAM210B, mitochondrial	-1.98	0.0101	4
P54819	AK2	Adenylate kinase 2, mitochondrial	-1.53	0.0040	4
Q5TZA2	CROCC	Rootletin	-1.49	0.0132	1
Q9NQC3	RTN4	Reticulon-4	-1.48	0.0076	1,3,4
Q8NBN7	RDH13	Retinol dehydrogenase 13	-1.46	0.0072	4
O95159	ZFPL1	Zinc finger protein-like 1	-1.44	0.0054	4
P61254	RPL26	60S ribosomal protein L26	-1.41	0.0324	3
Q9Y6A9	SPCS1	Signal peptidase complex subunit 1	-1.41	0.0052	1
Q96AG4	LRRC59	Leucine-rich repeat-containing protein 59	-1.41	0.0015	1,2,4
O94826	TOMM70	Mitochondrial import receptor subunit TOM70	-1.40	0.0057	4
P03928	MT-ATP8	ATP synthase protein 8	-1.38	0.0401	1
Q9Y5A9	YTHDF2	YTH domain-containing family protein 2	-1.36	0.0140	1
P24539	ATP5PB	ATP synthase F(0) complex subunit B1, mitochondrial	-1.35	0.0219	4
Q8N5G0	SMIM20	Small integral membrane protein 20	-1.35	0.0099	4
P56182	RRP1	Ribosomal RNA processing protein 1 homolog A	-1.34	0.0045	4
O95573	ACSL3	Long-chain-fatty-acid--CoA ligase 3	-1.32	0.0092	1
Q6ZNB6	NFXL1	NF-X1-type zinc finger protein NFXL1	-1.32	0.0080	1
B4DR61	SEC61A1	Protein transport protein Sec61 subunit alpha isoform 1	-1.32	0.0169	1
P35610	SOAT1	Sterol O-acyltransferase 1	-1.30	0.0051	4
P42167	TMPO	Lamina-associated polypeptide 2, isoforms beta/gamma	-1.30	0.0139	3
P28331	NDUFS1	NADH-ubiquinone oxidoreductase 75 kDa subunit, mitochondrial	-1.30	0.0249	4
Q9NP73	ALG13	Putative bifunctional UDP-N-acetylglucosamine transferase and deubiquitinase ALG13	-1.30	0.0136	4
P61026	RAB10	Ras-related protein Rab-10	-1.29	0.0048	1
O94874	UFL1	E3 UFM1-protein ligase 1	-1.29	0.0020	4
Q9BT22	ALG1	Chitobiosyldiphosphodolichol beta-mannosyltransferase	-1.28	0.0033	1,4
Q8TC12	RDH11	Retinol dehydrogenase 11	-1.27	0.0182	1
Q8TCJ2	STT3B	Dolichyl-diphosphooligosaccharide--protein glycosyltransferase subunit	-1.27	0.0100	1,4
Q9NVH1	DNAJC11	DnaJ homolog subfamily C member 11	-1.27	0.0179	4
H0YI09	TMT1A	Thiol Methyltransferase 1A	-1.27	0.0484	4
Q15154	PCM1	Pericentriolar material 1 protein	-1.25	0.0143	1
P60763	RAC3	Ras-related C3 botulinum toxin substrate 3	-1.24	0.0500	5
P63000	RAC1	Ras-related C3 botulinum toxin substrate 1	-1.24	0.0253	1,3,4

O94905	ERLIN2	Erlin-2	-1.24	0.0363	1
P49755	TMED10	Transmembrane emp24 domain-containing protein 10	-1.24	0.0396	1
O75396	SEC22B	Vesicle-trafficking protein SEC22b	-1.24	0.0062	1
Q99567	NUP88	Nuclear pore complex protein Nup88	-1.23	0.0022	3
P49792	RANBP2	E3 SUMO-protein ligase RanBP2	-1.23	0.0059	3
O00592	PODXL	Podocalyxin	-1.23	0.0072	1
Q9BWL3	C1orf43	Uncharacterized protein C1orf43	-1.23	0.0135	4
Q9NX20	MRPL16	39S ribosomal protein L16, mitochondrial	-1.22	0.0286	4
Q96CW1	AP2M1	AP-2 complex subunit mu	-1.22	0.0227	4
Q96EY7	PTCD3	Pentatricopeptide repeat domain-containing protein 3, mitochondrial	-1.22	0.0193	4
O15027	SEC16A	Protein transport protein Sec16A	-1.22	0.0004	3
Q9Y4P3	TBL2	Transducin beta-like protein 2	-1.21	0.0154	4
Q9Y5M8	SRPRB	Signal recognition particle receptor subunit beta	-1.20	0.0163	1
Q9NZ01	TECR	Very-long-chain enoyl-CoA reductase	-1.20	0.0093	1
Q7Z7H5	TMED4	Transmembrane emp24 domain-containing protein 4	-1.20	0.0453	1
Q9H0U4	RAB1B	Ras-related protein Rab-1B	-1.20	0.0476	4
Q9H0P0	NT5C3A	Cytosolic 5'-nucleotidase 3A	-1.20	0.0127	4
Q9Y2U8	LEMD3	Inner nuclear membrane protein Man1	-1.20	0.0015	4
P51153	RAB13	Ras-related protein Rab-13	-1.20	0.0364	1
O94973	AP2A2	AP-2 complex subunit alpha-2	-1.20	0.0002	4

FC, fold change. |FC| > 1.2, $p < 0.05$.

Supplementary Table 11: Pearson's correlation analyses of markers for retinal inflammasome, cell degeneration, and gliosis with retinal AD pathology markers

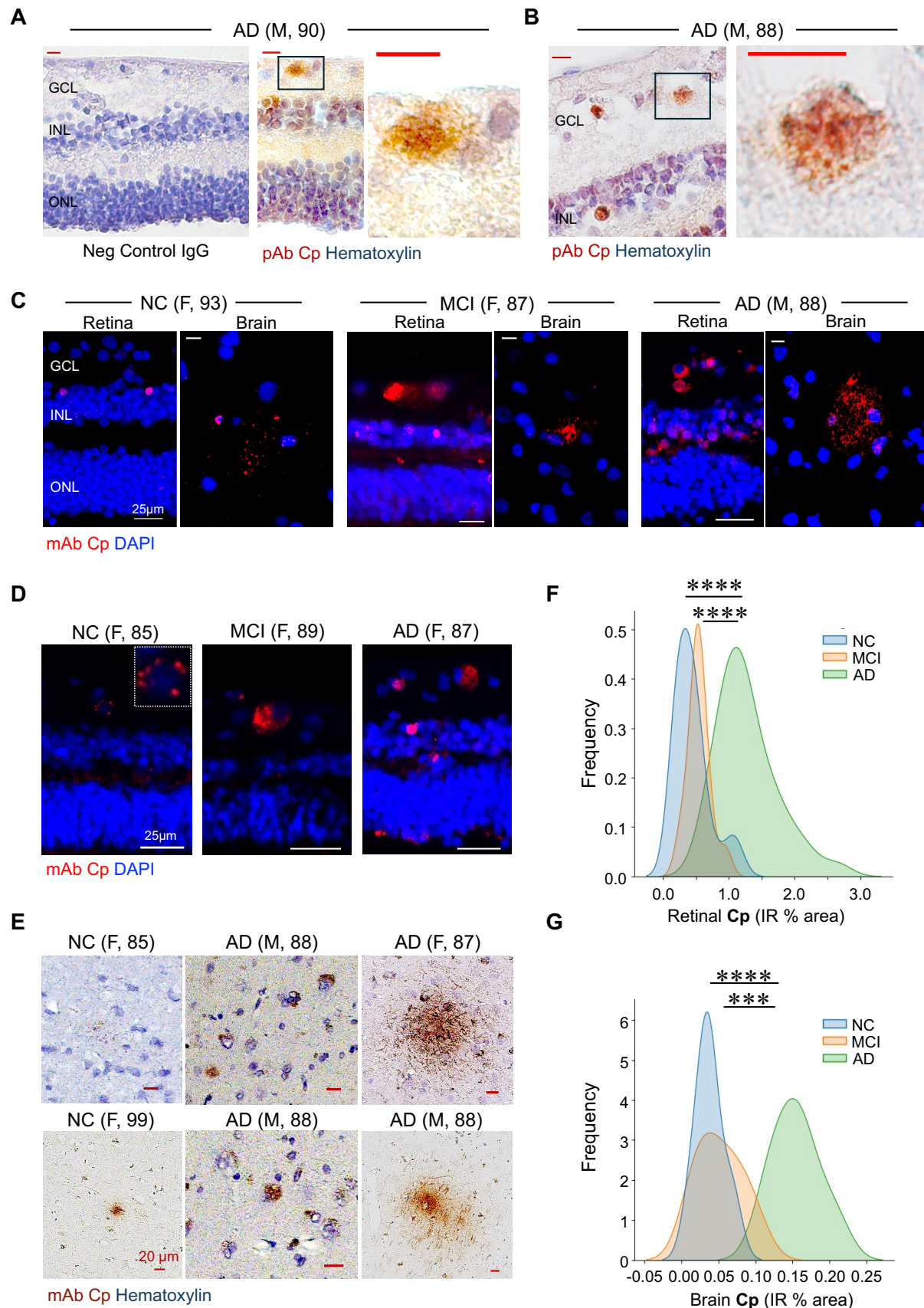
		Retinal inflammasome			Retinal degeneration		Retinal gliosis		
		NLRP3	ASC	Caspase-1	NGSDMD	CCasp3	Iba1	GFAP	Vimentin
Retinal Amyloidosis	A β ₄₂	0.81**** n = 19	0.40 n = 19	0.70*** n = 19	0.64** n = 19	0.77*** n = 17	0.85**** n = 20	0.65** n = 20	0.56* n = 15
	A β oligomer	0.41 n = 9	-0.40 n = 9	0.39 n = 9	-0.22 n = 8	0.32 n = 7	0.46 n = 9	-0.15 n = 10	0.05 n = 8
Retinal Tauopathy	PHF-1	0.30 n = 13	0.31 n = 13	0.19 n = 13	0.28 n = 13	0.30 n = 12	0.18 n = 15	0.60* n = 13	0.21 n = 7
	pS396	0.40* n = 25	0.44* n = 25	0.32 n = 25	0.38 n = 24	0.53** n = 25	0.31 n = 27	0.43* n = 24	0.19 n = 12
	MC-1	0.23 n = 24	-0.21 n = 24	-0.12 n = 24	-0.12 n = 20	0.06 n = 21	0.26 n = 26	0.14 n = 24	-0.06 n = 13
	Oligo-tau	0.70*** n = 24	0.44* n = 24	0.60** n = 24	0.77**** n = 23	0.80**** n = 23	0.69**** n = 27	0.60** n = 24	0.60* n = 14
Retinal Gliosis	Iba1	0.77**** n = 25	0.42* n = 25	0.65*** n = 25	0.39 n = 24	0.69*** n = 23	-	0.65*** n = 24	0.81** n = 9
	GFAP	0.91**** n = 23	0.71*** n = 23	0.77**** n = 23	0.68*** n = 26	0.85**** n = 21	0.65*** n = 24	-	0.42 n = 11
	Vimentin	0.84** n = 10	0.16 n = 10	0.78** n = 10	0.54 n = 11	0.58 n = 8	0.81** n = 9	0.42 n = 11	-
Retinal Inflammasome	NLRP3	-	0.59** n = 27	0.83**** n = 27	0.74**** n = 23	0.80**** n = 23	0.77**** n = 25	0.91**** n = 23	0.84** n = 10
	ASC	0.59** n = 27	-	0.70**** n = 27	0.58** n = 23	0.76**** n = 23	0.42* n = 25	0.71*** n = 23	0.16 n = 10
	Caspase-1	0.83**** n = 27	0.70**** n = 27	-	0.57** n = 23	0.80**** n = 23	0.65*** n = 25	0.77**** n = 23	0.78** n = 10
Retinal Degeneration	NGSDMD	0.74**** n = 23	0.58** n = 23	0.57** n = 23	-	0.75**** n = 21	0.39 n = 24	0.68*** n = 26	0.54 n = 11
	CCasp3	0.80**** n = 23	0.76**** n = 23	0.80**** n = 23	0.75**** n = 21	-	0.69*** n = 23	0.85**** n = 21	0.58 n = 8
	Atrophy	0.86**** n = 14	0.62* n = 14	0.82*** n = 14	0.60* n = 14	0.86*** n = 11	0.72** n = 15	0.72** n = 15	0.87* n = 6

Pearson's correlation analyses: *p* and *r* values determine the statistical significance and strength of each pairwise association between markers of retinal inflammasome, degeneration, and gliosis versus retinal AD-associated pathologies, inflammasomes, gliosis, and degeneration markers. *p* and *r* values presented in bold type with asterisk(s) depicts strong to very strong correlation.

Supplementary Table 12: Spearman's correlation analyses of markers for retinal inflammasome, cell degeneration, and gliosis with brain AD pathology and cognition

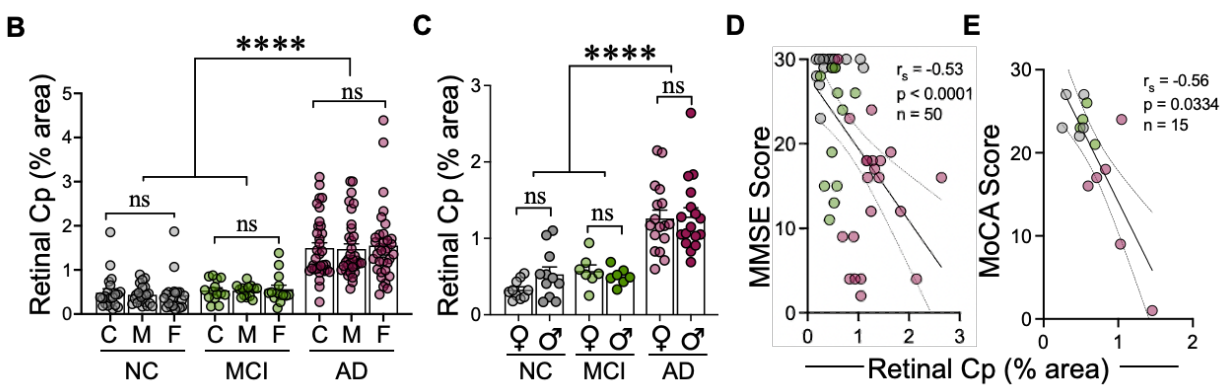
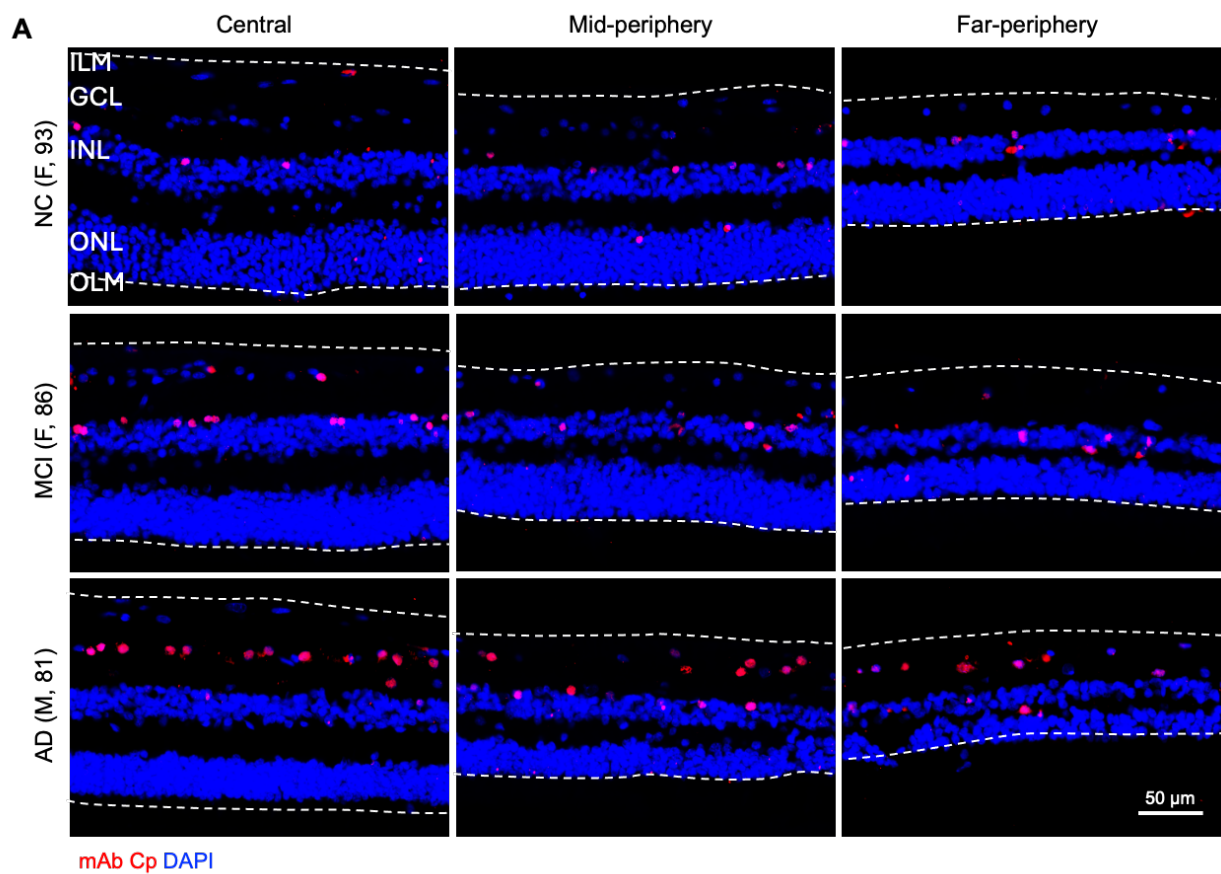
	Retinal inflammasome			Retinal degeneration		Retinal gliosis		
	NLRP3	ASC	Caspase-1	NGSDMD	CCasp3	Iba1	GFAP	Vimentin
Brain Aβ plaque	0.34 n = 27	0.25 n = 27	0.34 n = 27	0.21 n = 25	0.42* n = 25	0.28 n = 29	0.39* n = 26	0.38 n = 18
ABC	0.67*** n = 27	0.35 n = 27	0.56** n = 27	0.43* n = 25	0.72**** n = 25	0.49** n = 29	0.71**** n = 26	0.62** n = 18
NFTs	0.64*** n = 27	0.53** n = 27	0.65*** n = 27	0.49* n = 25	0.78**** n = 25	0.56** n = 29	0.59** n = 26	0.43 n = 18
Braak	0.68*** n = 27	0.60*** n = 27	0.63** n = 27	0.55** n = 25	0.72**** n = 25	0.49** n = 30	0.78**** n = 26	0.46 n = 18
Brain atrophy	0.47* n = 27	0.27 n = 27	0.41* n = 27	0.23 n = 25	0.45* n = 25	0.36 n = 29	0.45* n = 26	0.16 n = 18
CAA	0.54** n = 27	0.31 n = 27	0.47* n = 27	0.29 n = 25	0.59** n = 25	0.49** n = 29	0.39 n = 26	0.35 n = 17
CDR	0.66*** n = 27	0.26 n = 27	0.55** n = 27	0.37 n = 25	0.62** n = 25	0.66*** n = 28	0.51** n = 26	0.68** n = 16
MMSE	-0.66*** n = 25	-0.49* n = 25	-0.58* n = 25	-0.50* n = 25	-0.69*** n = 22	-0.58** n = 26	-0.48* n = 26	-0.59** n = 18
MoCA	-0.76 n = 7	-0.33 n = 7	-0.60 n = 7	-0.41 n = 6	-0.45 n = 8	-0.31 n = 7	-0.49 n = 6	-0.80 n = 4

Spearman's rank correlation analyses: p and r values determine the statistical significance and strength of each pairwise association between markers of retinal inflammasome, degeneration, and gliosis versus brain AD-pathology markers. p and r values presented in bold type with asterisk(s) depicts strong to very strong correlation.



Supplementary Figure 1. Cp inclusions in AD retina and paired-brain tissues.

A-B. Representative peroxidase-based images (DAB brown, hematoxylin nuclei blue) of retinal cross-sections from AD patients. **A.** Left image exhibit no immunoreaction when used negative (Neg) control IgG, whereas staining with Cp polyclonal antibody (pAb) depicts the presence of Cp inclusions in GCL and INL (middle image). Cp inclusions in higher magnification is shown (right image). **B.** AD retinal cross sections depict the presence of Cp inclusions in the GCL and INL. Higher magnification, right image. Scale bars: 10 μ m. **C.** Representative fluorescence images of retinal and paired-brain (Area 9 located in the dorsolateral prefrontal cortex) cross-sections from MCI and AD patients versus NC individuals, depicting the presence of Cp inclusions (red), with specific Cp monoclonal antibody (mAb). DAPI (blue) stained nuclei. Scale bars: 25 μ m (retina) and 10 μ m (brain cross-sections). **D.** Representative fluorescence images of retinal cross-sections from MCI and AD patients versus NC individuals (the same subjects which were stained using peroxidase-based (DAB) method, see **Fig. 1E**), depicting the presence of Cp inclusions (red), stained with specific Cp mAb. DAPI (blue) stained nuclei. Scale bars: 25 μ m. **E.** Representative peroxidase-based (DAB) images of brain cross-sections from AD patients versus NC individuals depicting the presence of Cp inclusions (brown) stained with Cp mAb and hematoxylin. Scale bars: 20 μ m. **F-G.** Gaussian distribution curves display the frequency of **(F)** Retinal and **(G)** Brain Cp % IR area in individuals with premortem clinical diagnoses of NC, MCI, and AD. *** $p < 0.001$ and **** $p < 0.0001$, by one-way ANOVA and Tukey's post hoc multiple comparison test. Ganglion cell layer (GCL); Inner nuclear layer (INL); Outer nuclear layer (ONL).



Supplementary Figure 2. Distribution of Cp inclusions across retinal subregions and sex.

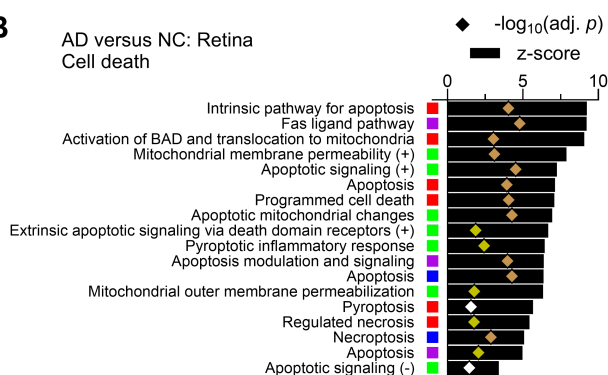
A. Representative fluorescence images of retinal cross-sections from MCI and AD patients versus NC individuals depicting the distribution of Cp inclusions (red) across three retinal subregions (central, mid-periphery, and far-periphery). DAPI (blue) stained nuclei. White dotted lines display the analyzed area, between the inner limiting membrane (ILM) and outer limiting membrane (OLM). Scale bar: 50 μ m. **B-C.** Scatter plots display quantitative-IHC analysis of retinal Cp % IR area in **(B)** three retinal subregions (C - Central; M - Mid periphery and F - Far periphery) among NC (N=21), MCI (N=14), and AD (N=34) subjects, and **(C)** male and female subjects of same cohort (NC=10M, 11F, MCI=7M, 7F, AD=17M, 17F). **D, E.** Pearson correlation (r_p) analyses between retinal Cp burden and **(D)** MMSE, and **(E)** MoCA cognitive score. Statistics: Data from individual subjects (circles) as well as group means $\pm$ SEMs are shown. ♀ = female; ♂ = Male. **** $p < 0.0001$, by one-way ANOVA and Tukey's post hoc multiple comparison test. ns = non-significant. Ganglion cell layer (GCL); Inner nuclear layer (INL); Outer nuclear layer (ONL).

A

	RTN4	TOMM70	NDUFS1	RAB10	RER1	STT3B	RDH11	LRPPRC	AP2M1	TECR	TMED4	AP2A2	AP1M1	AP2A1	HSPB1	TPM3	ATP6V1G1	TPD52L2	BAG3	LRRFIP1	TNKS1BP1	FC p
Retina	-1.48 ★ ★	-1.40 ★ ★	-1.30 ★	-1.29 ★ ★	-1.28 0.0901	-1.27 ★ ★	-1.27 ★	-1.22 0.0601	-1.22 ★	-1.20 ★ ★	-1.20 ★	-1.20 ★ ★ ★	-1.12 ★	-1.11 ★	1.70 ★ ★	1.53 ★	1.33 ★	1.32 ★	1.30 ★	1.27 ★ ★	1.17 ★ ★	
Cerebral cortex	-1.83 ★ ★	-1.12 ★ ★ ★	-1.15 ★	-1.16 ★	-1.37 ★	-1.39 ★	-1.18 ★	-1.27 ★ ★	-1.25 ★ ★	-2.05 ★	-1.31 ★	-1.15 ★	-1.27 ★	-1.20 ★ ★	1.27 ★ ★	1.24 ★	1.28 ★ ★ ★	1.10 ★	1.34 ★	1.26 0.0516	1.21 ★ ★	

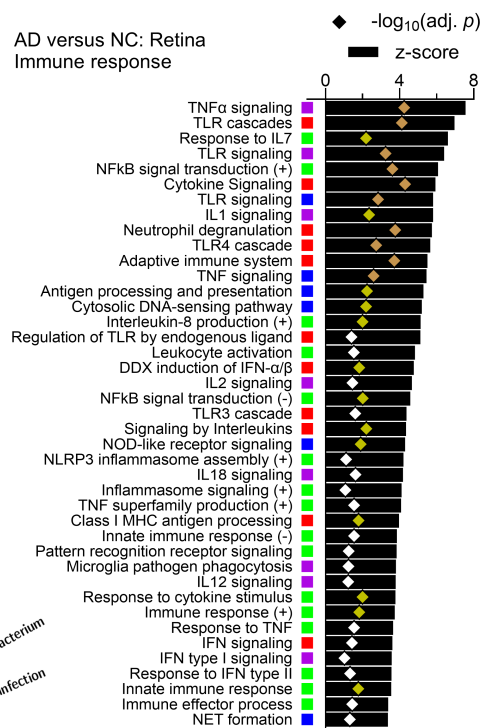
B

AD versus NC: Retina
Cell death

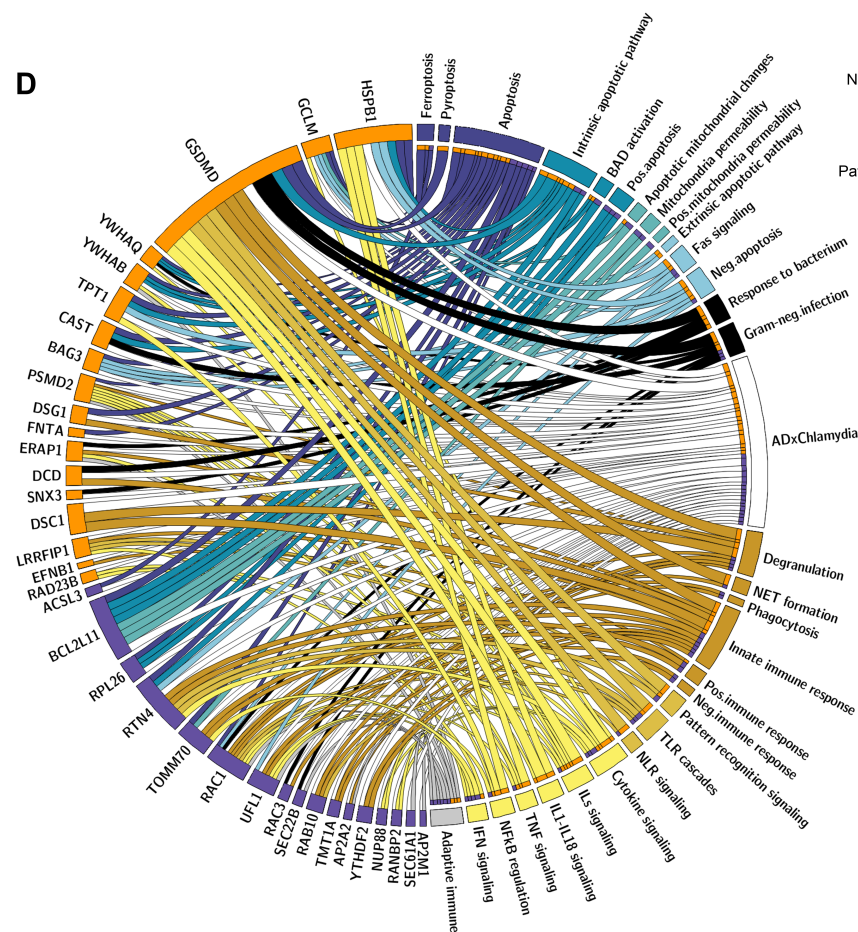


C

AD versus NC: Retina
Immune response



D



Legend:

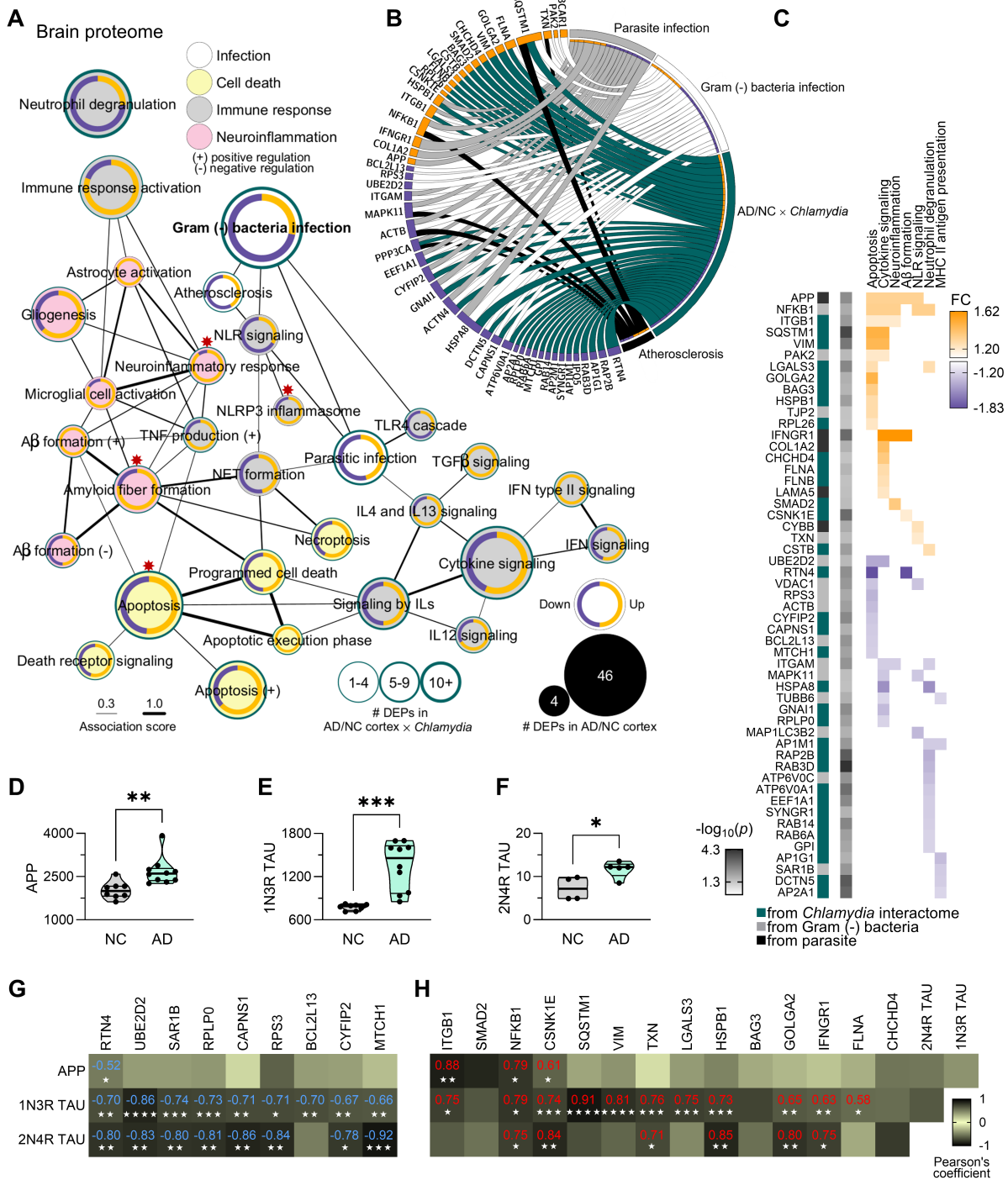
- GO BP
- Reactome
- KEGG
- WikiPath
- (+) positive regulation
- (-) negative regulation
- $\diamond$ $p < 0.01$
- $\blacklozenge$ $p < 0.001$
- $\blacklozenge$ $p < 0.0001$

Abbreviations:

- IFN interferon
- IL interleukin
- NET neutrophil extracellular trap
- NFkB nuclear factor kappa B subunit
- NLR NOD-like receptor
- TLR Toll-like receptor
- TNF tumor necrosis factor

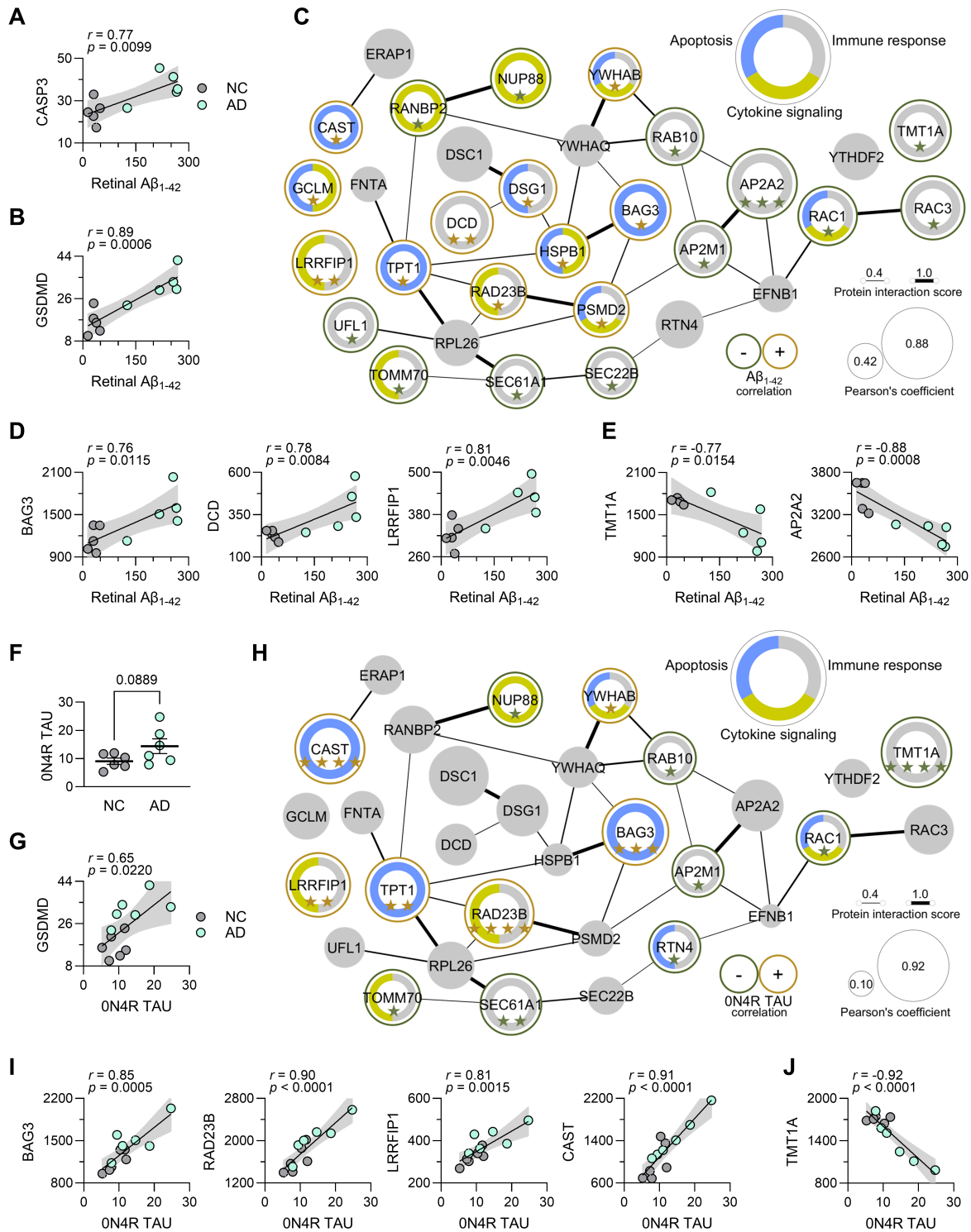
Supplementary Figure 3. Cell death and immune response pathways, and association with *Chlamydia* infection in the human retina and cerebral cortex.

A. Heatmaps of shared *Chlamydia*-interactome differentially expressed proteins (DEPs) in AD retina and cerebral cortex [Fold Change (FC) and p]. The expression of 5 downregulated (RTN4, STT3B, AP2M1, TECR, TMED4) and 5 upregulated (HSPB1, TPM3, LRRFIP1 [t-test], BAG3, ATP6V1G1) proteins were within cutoffs for DEPs ($|FC| > 1.2$ and $p < 0.05$) in both the retina and the cortex. Additional proteins, whose expression was within cutoffs for DEPs ($|FC| > 1.2$ and $p < 0.05$) for either the retina or the cortex, were found. **B-C.** Gene ontology (GO) analysis of DEPs related to **(B)** cell death and **(C)** immune response in human AD ($n = 6$) versus NC retina ($n = 6$). The analysis was carried out in Metascape and included the GO Biological Processes (BP), Reactome, Kyoto Encyclopedia of Genes and Genomes (KEGG) and WikiPathways databases. Bar and symbol graphs represent z-scores and Benjamini-Hochberg adjusted p -values from Metascape analysis, respectively. Range of p -values are presented as color-coded symbols. **D.** Chord diagram displays the association of *Chlamydia*-interacting proteins with pathways related to cell death (blue gradient outer segments and ribbons), immune response (yellow gradient and grey outer segments and ribbons), as well as Gram (-) bacterial infection (white outer segment and ribbons). For proteins, orange and purple outer segments indicates upregulation and downregulation, respectively.



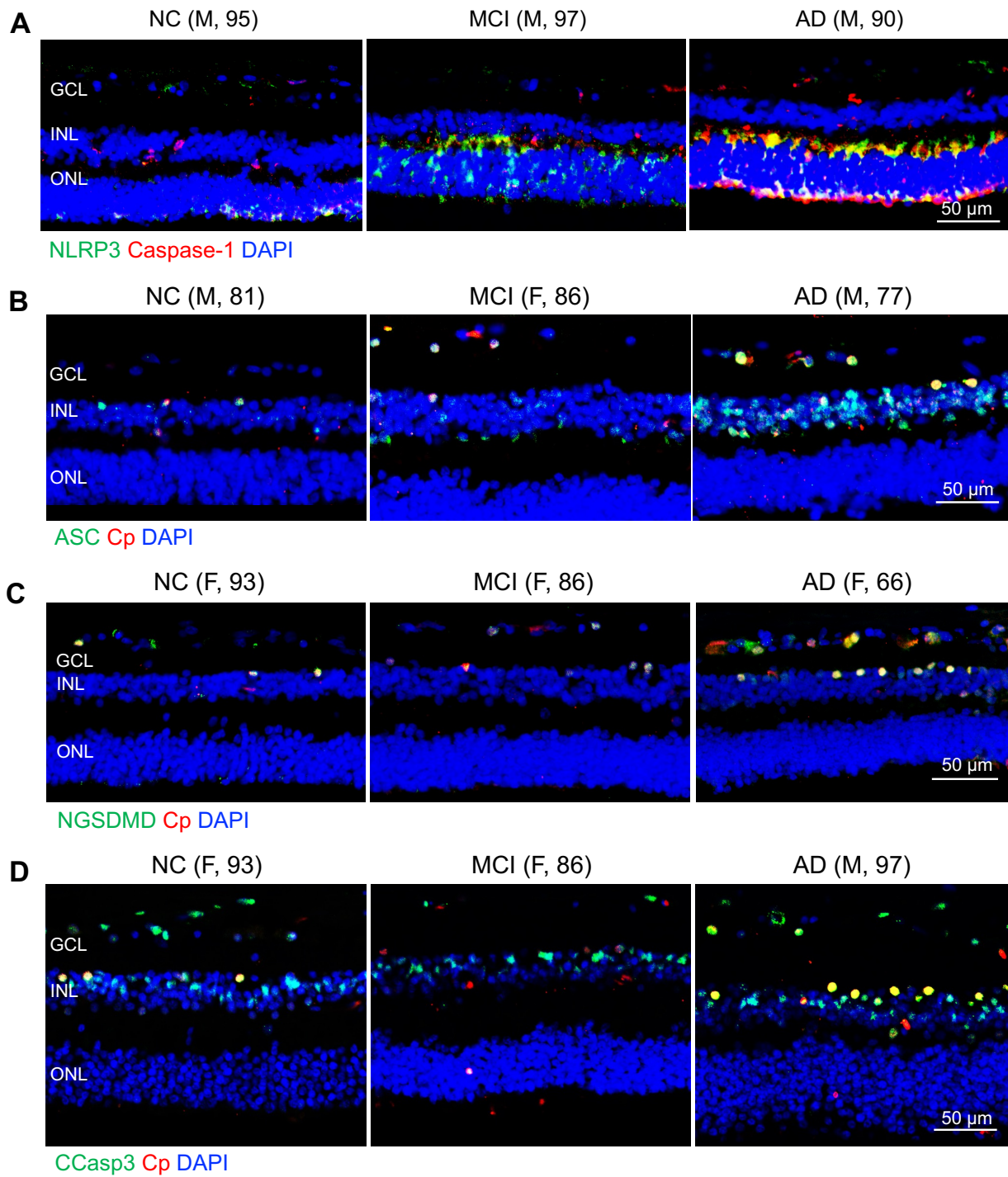
Supplementary Figure 4. Cell death and immune response pathways, and association with *Chlamydia* infection in the cerebral cortex.

A. GO network (Metascape) of pathways related to infection, cell death, immune response and neuroinflammation. The size of the nodes represents the number of DEPs in AD versus NC cortex, with the inner ring showing the proportion of these DEPs that are downregulated (purple) or upregulated (orange) in AD. The green border and its thickness represent the number of DEPs that interact with *Chlamydia* inclusion in each pathway. The thickness of edges represents the shared DEPs (association score) between pathways. Red asterisks indicate pathways (NLRP3 inflammasome, apoptosis, neuroinflammatory response, and amyloid fiber formation) that were further explored and validated. **B.** Chord diagram displays the association between cell death, immune response-related DEPs in AD cortex and *Chlamydia* (interactome), Gram (-) bacteria and parasitic infections, and atherosclerosis. For DEPs, orange and purple outer segments indicate upregulation and downregulation, respectively. **C.** Heatmaps of DEPs [FC and $-\log_{10}(p)$] in AD cortex for selected pathways. Only proteins connected to Gram (-) bacteria and parasitic infections (Metascape analysis) and *Chlamydia* infection (*Chlamydia* interactome) are shown for each pathway. **D-F.** Mass spectrometry (MS) quantitation of **(D)** Amyloid beta precursor protein (APP), **(E)** one N-terminal domain and three microtubule-binding repeat domains (1N3R) and **(F)** two N-terminal domains and four microtubule-binding repeat domains (2N4R) isoforms of TAU in cerebral cortex of human AD (n = 10) and NC retina (n = 8). **G-H.** Heatmaps of **(G)** negative and **(H)** positive Pearson's correlation coefficients analysis (r) between *Chlamydia* interactors and APP, 1N3R TAU, and 2N4R TAU, quantified by MS. Correlation statistics: $*p < 0.05$, $**p < 0.01$, $***p < 0.001$, and $****p < 0.0001$.



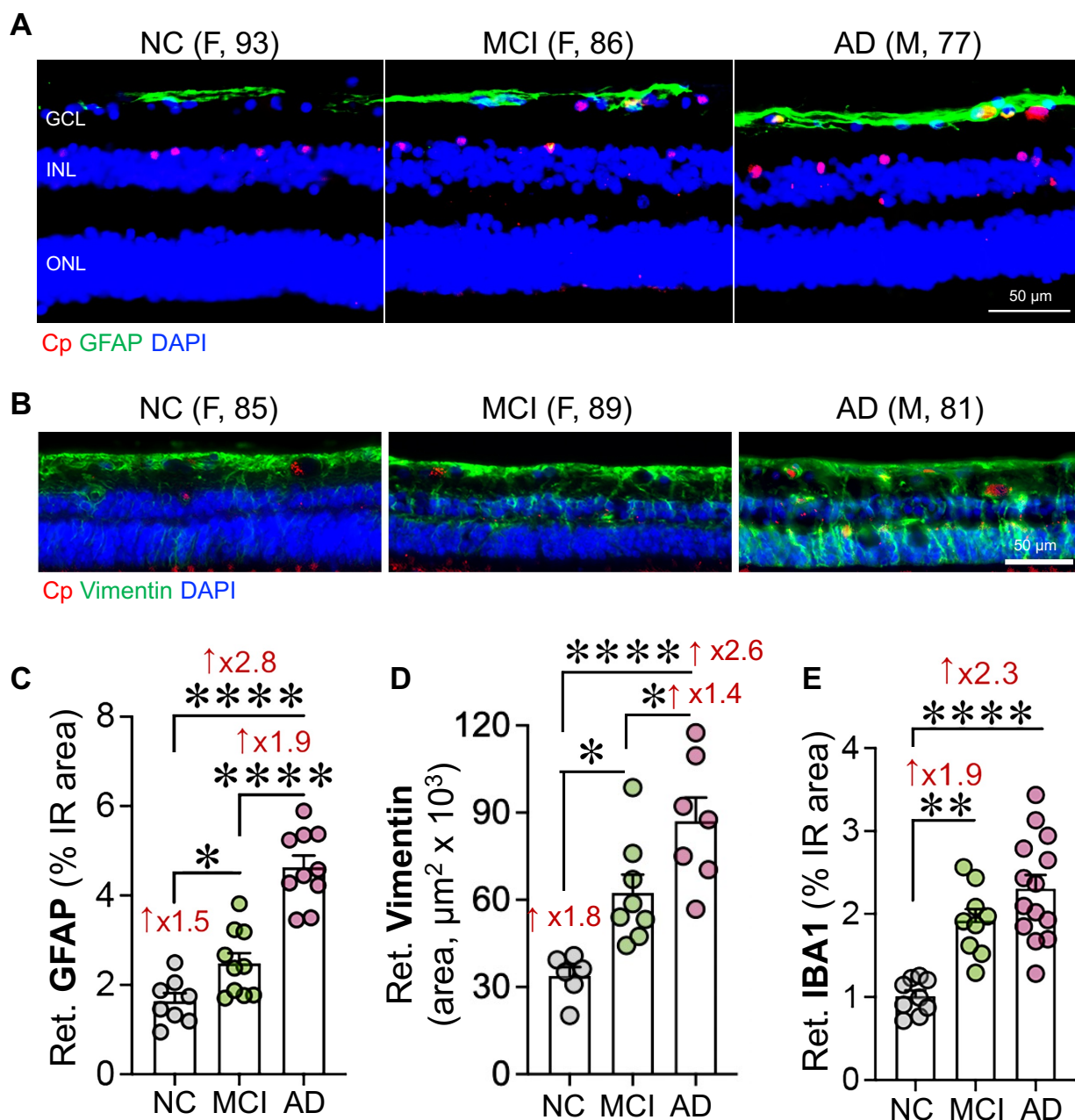
Supplementary Figure 5. Correlation of *Chlamydia* inclusion interactors with amyloid deposits and neurofibrillary tangle burden in the AD retina.

A-B. Pearson's correlation coefficient analysis of A β_{1-42} levels with (A) Caspase-3 (CASP3) and (B) Gasdermin D (GSDMD). Retinal A β_{1-42} was quantified by using sandwich enzyme-linked immunosorbent assay (ELISA) from human AD (n = 5) and NC retina (n = 5). (C) Protein interaction network (String v12.0) of *Chlamydia* interactors and their correlation with A β_{1-42} levels. Node size represents the Pearson's correlation coefficient (r). Dark green and orange node borders indicate negative and positive correlation with A β_{1-42} levels, respectively. Inner ring categorizes the protein's role into apoptosis (blue), immune response (grey) and/or cytokine signaling (olive). The thickness of edges between nodes represents the protein interaction score from String v12.0. Correlation statistics: * $p < 0.05$, ** $p < 0.01$, and *** $p < 0.001$. **D-E.** Pearson's correlation coefficients analysis between several *Chlamydia* interactors and retinal A β_{1-42} levels. Interactors with strongest (D) positive and (E) negative correlations are shown. **F.** Mass spectrometry quantitation of 0N4R isoform of TAU [absence of N-terminal insert (0N) and presence of four microtubule-binding repeat domains (4R)] in human AD (n = 6) and NC retina (n = 6). **G.** Pearson's correlation coefficient analysis of 0N4R TAU with GSDMD. **H.** The same protein interaction network of *Chlamydia* interactors showing their correlation to 0N4R TAU. Node size represents the Pearson's correlation coefficient (r). Dark green and orange node borders indicate negative and positive correlation with 0N4R TAU, respectively. Inner ring categorizes the protein's role in apoptosis (blue), immune response (grey), and/or cytokine signaling (olive). The thickness of edges between nodes represents the protein interaction score from String v12.0. Correlation statistics: * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, and **** $p < 0.0001$. **I-J.** Pearson's correlation coefficients analysis between several *Chlamydia* interactors and 0N4R TAU quantitated in the retina by MS. Interactors with strongest (I) positive and (J) negative correlations are shown.



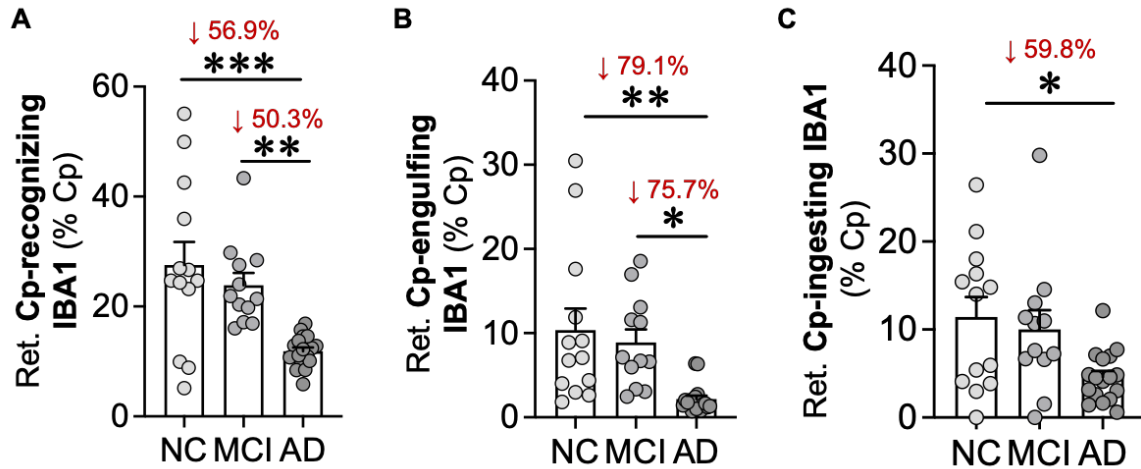
Supplementary Figure 6. Retinal Cp Colocalized with retinal NLRP3 inflammasome components, early apoptosis, and cellular pyroptosis markers.

A-D. Representative images of retinal cross-sections from MCI and AD patients versus NC controls stained with (A) NLRP3 (green) and caspase-1, (red), (B) ASC (green) and Cp (red), (C) NGSDMD (green) and Cp (red), (D) CCasp3 (green) and Cp (red) with DAPI (blue) nuclear staining. Colocalization are shown in yellow. Scale bars: 50 μ m.



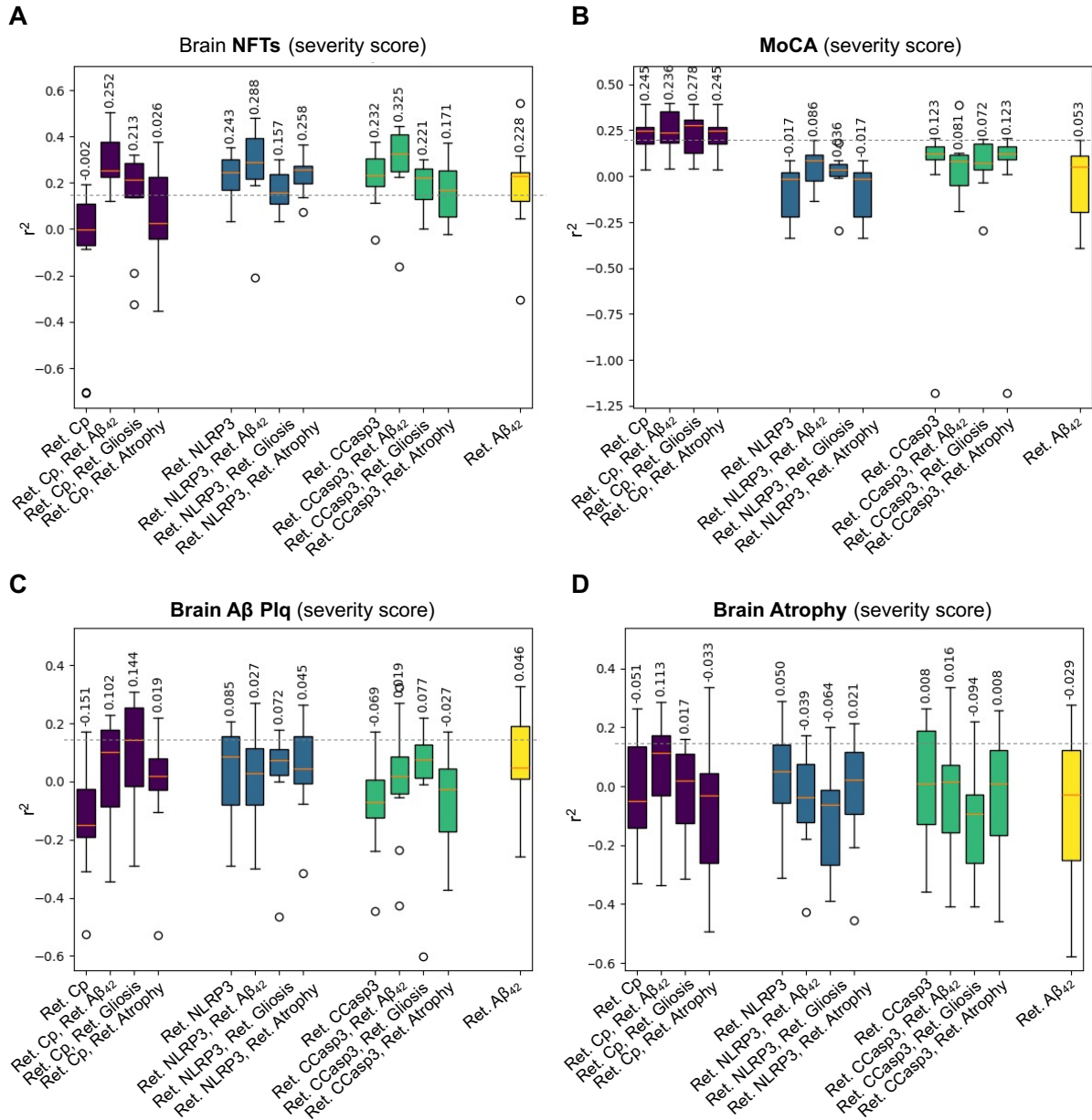
Supplementary Figure 7. Association of retinal Cp with retinal gliosis.

A-B. Representative images of retinal cross-sections from MCI and AD patients versus NC controls stained with (A) Cp (red) and GFAP (green) and (B) Cp (red) and vimentin (green) with DAPI (blue) nuclear staining. **C-E.** Quantitative IHC analysis of retinal macrogliosis (C) astroglia (GFAP) and (D) Müller Glia (vimentin), (E) microglia (IBA1) % IR area in individuals with premortem clinical diagnoses of NC (n = 6-9), MCI (n = 8-10), and AD (n = 7-14). Scale bar: 50 μm . Statistics: Data from individual subjects (circles) as well as group means $\pm$ SEMs are shown. Fold changes are shown in red. * $p < 0.05$, ** $p < 0.01$, and **** $p < 0.0001$, by one-way ANOVA and Tukey's post hoc multiple comparison test.



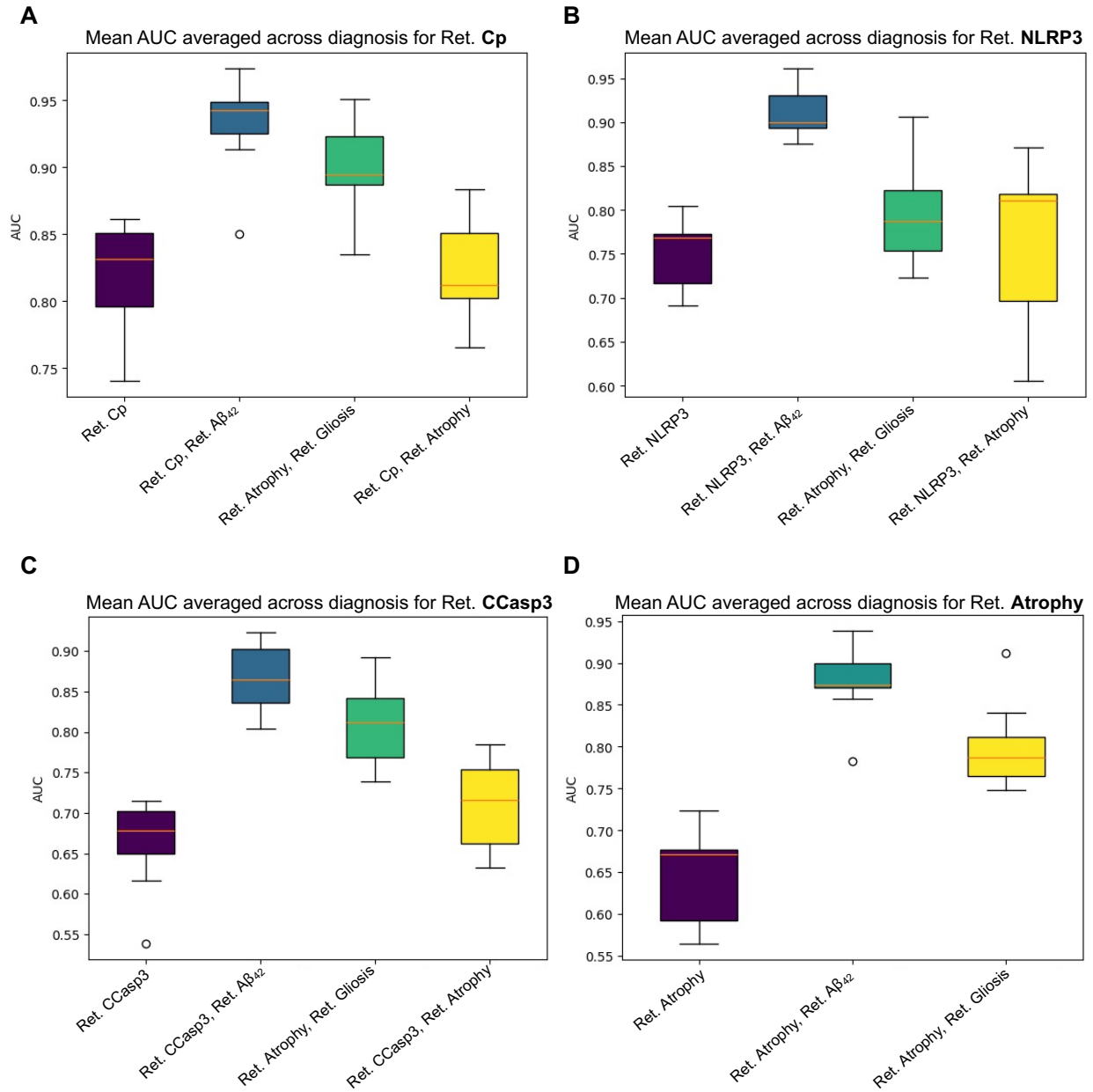
Supplementary Figure 8. Analysis of retinal microglia in association with retinal Cp.

A-C. Scatter plot of quantitative IHC analysis of retinal microgliosis (IBA1) per retinal Cp % IR area in three microglial stages for Cp phagocytosis (**A**) Cp-recognizing microglia, (**B**) Cp-engulfing microglia, and (**C**) Cp-ingesting microglia in individuals with premortem clinical diagnoses of NC ($n = 13$), MCI ($n = 12$), and AD ($n = 17$). Statistics: Data from individual subjects (circles) as well as group means $\pm$ SEMs are shown. Percentage (%) changes are shown in red. * $p < 0.05$, ** $p < 0.01$, and *** $p < 0.001$, by one-way ANOVA and Tukey's post hoc multiple comparison test.



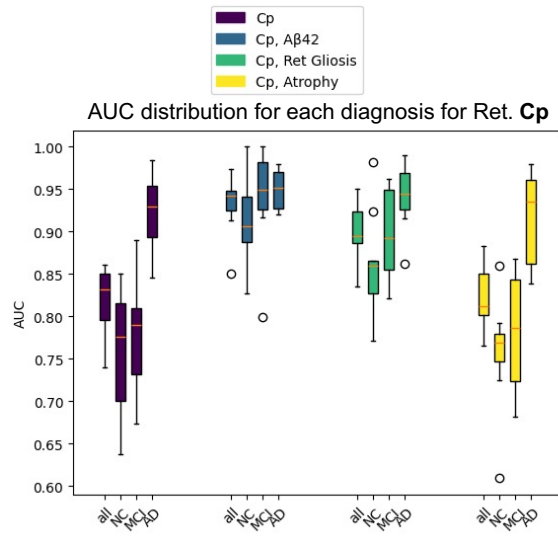
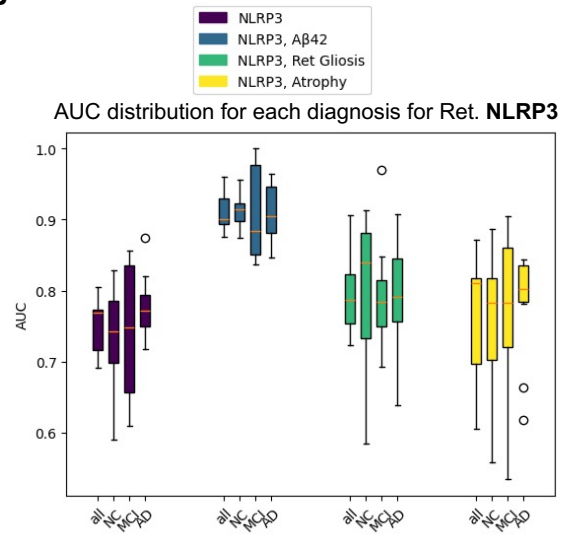
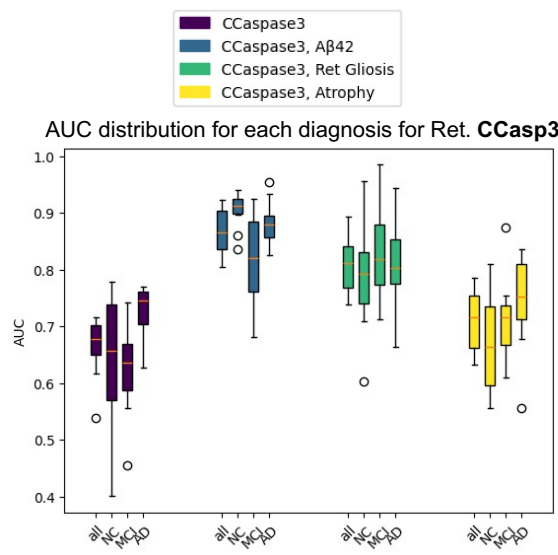
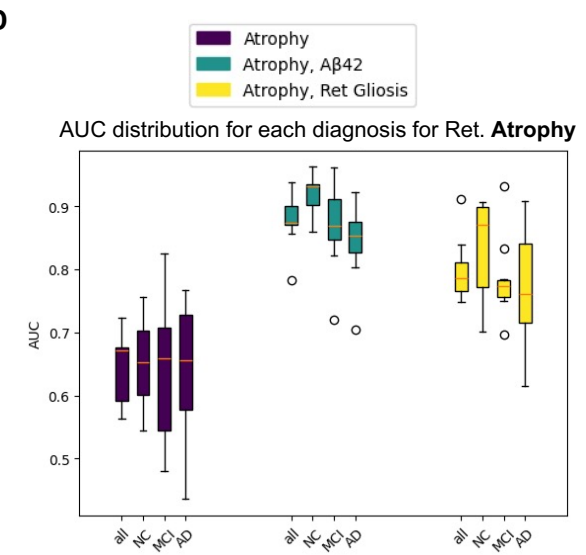
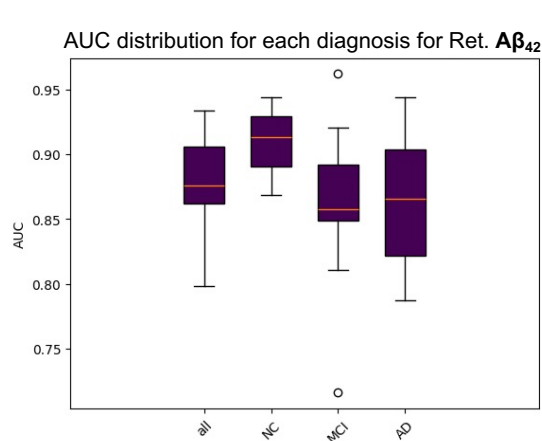
Supplementary Figure 9. Prediction of brain AD pathologies by retinal Cp, NLRP3, cleaved caspase-3, and Aβ₄₂.

A-D. Machine learning algorithm with a Random Forest regressor using 80 estimators was trained on the data to predict several brain pathologies and cognitive status, including (A) brain NFT severity score, (B) Montreal cognitive assessment (MoCA) score, (C) total brain Aβ plaques severity score, and (D) brain atrophy severity score. The distributions show the spread of models trained on different folds of the 5x2 cross-validation. The mean r^2 for each model is shown at the top of each box plot. Only models performing with variance coefficient $r^2 > 0.15$ were retained.



Supplementary Figure 10. The AUC box plots across all diagnostic groups.

A-D. Box plots representing the AUC measure for (A) retinal Cp, (B) retinal NLRP3, (C) retinal CCaspase3, and (D) retinal atrophy for all diagnostic groups combined. For each model, AUC was measured either individually or combined with retinal A β_{42} , or retinal gliosis (IBA1, GFAP, and Vimentin), or retinal atrophy.

A**B****C****D****E**

Supplementary Figure 11. The AUC box plots across each diagnostic groups.

Box plots representing the AUC measure for (A) retinal Cp, (B) retinal NLRP3, (C) retinal CCasp3, (D) retinal atrophy, and (E) retinal A β_{42} for each diagnostic groups NC, MCI and AD. For each model, AUC was measured either individually or combined with retinal A β_{42} , or retinal gliosis (IBA1, GFAP, and Vimentin), or retinal atrophy.

References:

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