Supplementary Information

Unconventional Source of Neurotoxic Protein Aggregation from Organelle Off-Target Bax△2 in Alzheimer's Disease

Biomolecules

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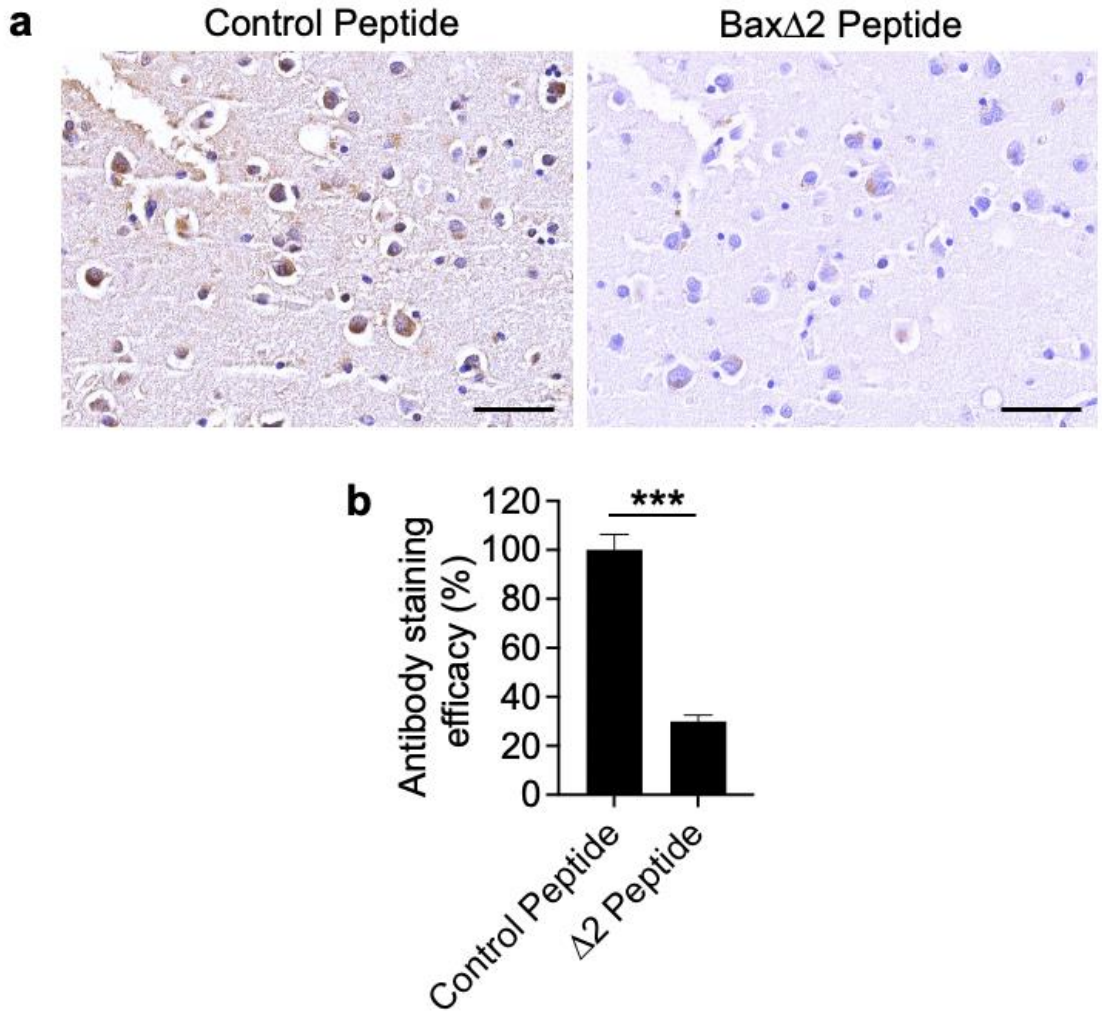
Fig. S1

Human brain RNA-seq databases

Data ID	Data type	Female (%)	AD (N)	NCI (N)
syn8612097	RNA-seq	64%	253	201
syn24175555	RNA-seq	75%	71	126

Note: AD, Alzheimer's disease; NCI: None Cognitive impair

Fig. S2



Caption: a Immunostaining of human brain tissues with anti-BaxΔ2 antibody in the presence of Baxα (control) or BaxΔ2 antigen peptide. Scale bars, 50 μm. **b** Quantitation of (a) using Cell-Profiler software and analyzed by non−parametric statistical analysis, *** $p < 0.001$

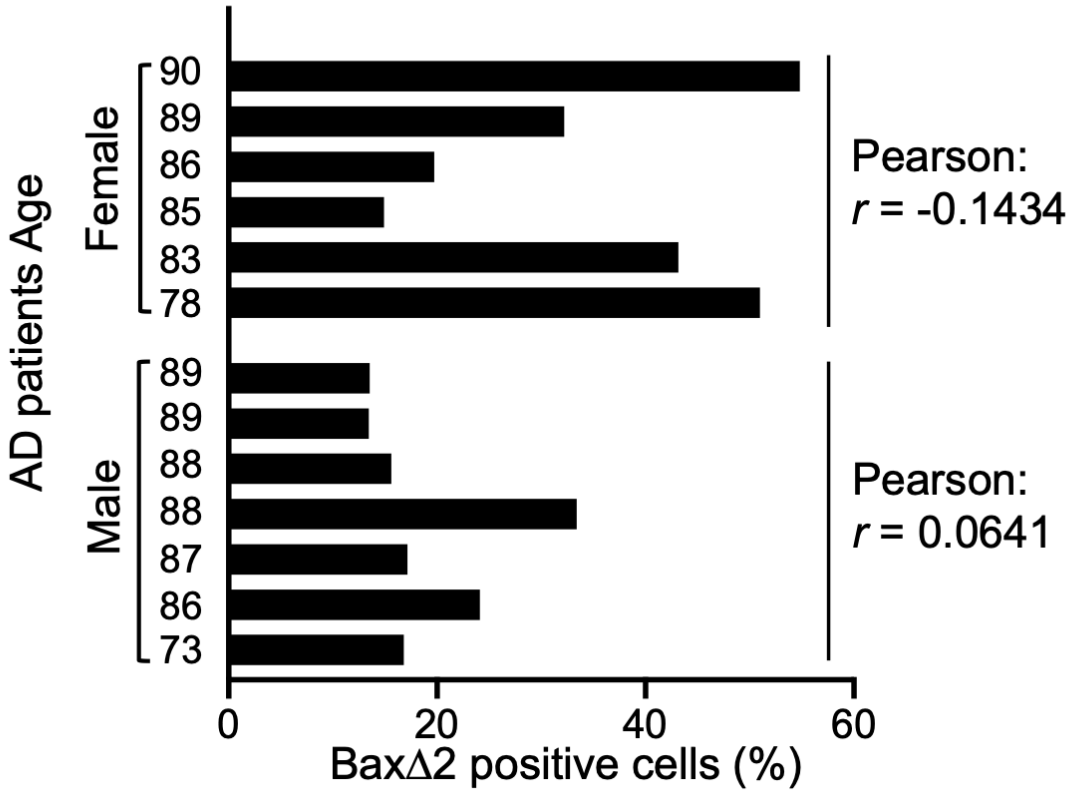
Fig. S3

Human Brain Tissue information

Patient	Source	Gender	APOE genotype	Age death	Braaksc	Cogdx
1	RADC	M	33	77	2	1
2	RADC	F	33	87	4	1
3	RADC	F	33	79	3	1
4	RADC	F	33	82	1	1
5	RADC	M	33	72	0	1
6	Biochain	M	N/A	54	N/A	1
7	Biomax	M	N/A	38	N/A	1
8	Biomax	F	N/A	18	N/A	1
9	Biomax	M	N/A	15	N/A	1
10	RADC	M	33	88	4	4
11	RADC	F	33	90	3	4
12	RADC	F	34	86	5	4
13	RADC	F	33	89	4	4
14	RADC	F	NA	78	5	4
15	RADC	M	34	87	5	4
16	RADC	M	34	89	6	4
17	RADC	F	44	83	5	4
18	RADC	M	33	86	5	4
19	RADC	M	23	89	4	4
20	Biochain	M	N/A	73	N/A	4
21	Biochain	F	N/A	85	N/A	4
22	Biochain	M	N/A	88	N/A	4

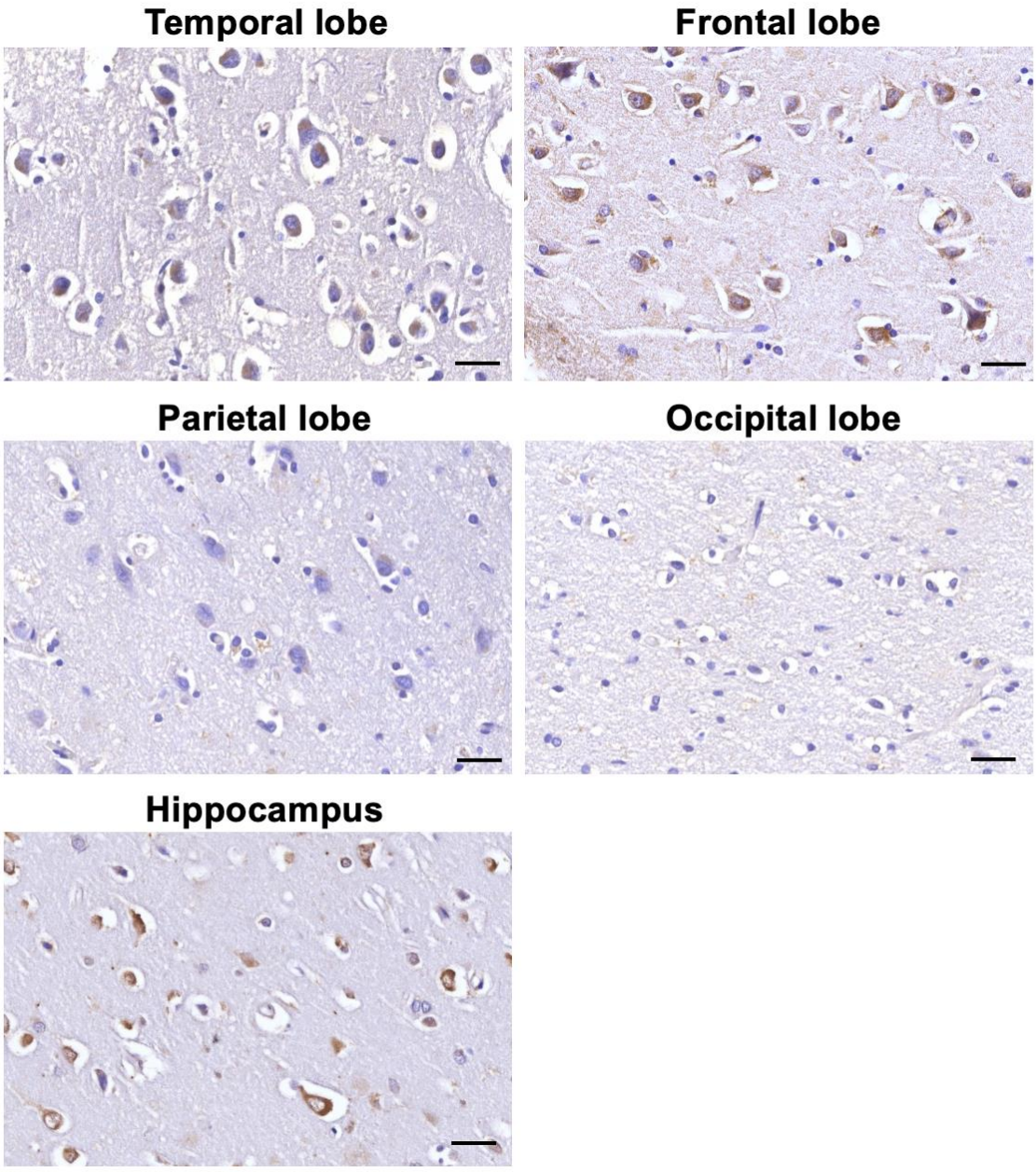
Note: RADC, Rush Alzheimer's Disease Center.
Braaksc, Braak stages: stage 1 and 2 indicate NFTs confined mainly to the entorhinal region of the brain; stage 3 and 4 indicate involvement of limbic regions such as the hippocampus; stage 5 and 6 indicate moderate to severe neocortical involvement.
Codgx, Clinical Consensus Diagnosis, 1 = non-AD, 4 = AD.

Fig. S4



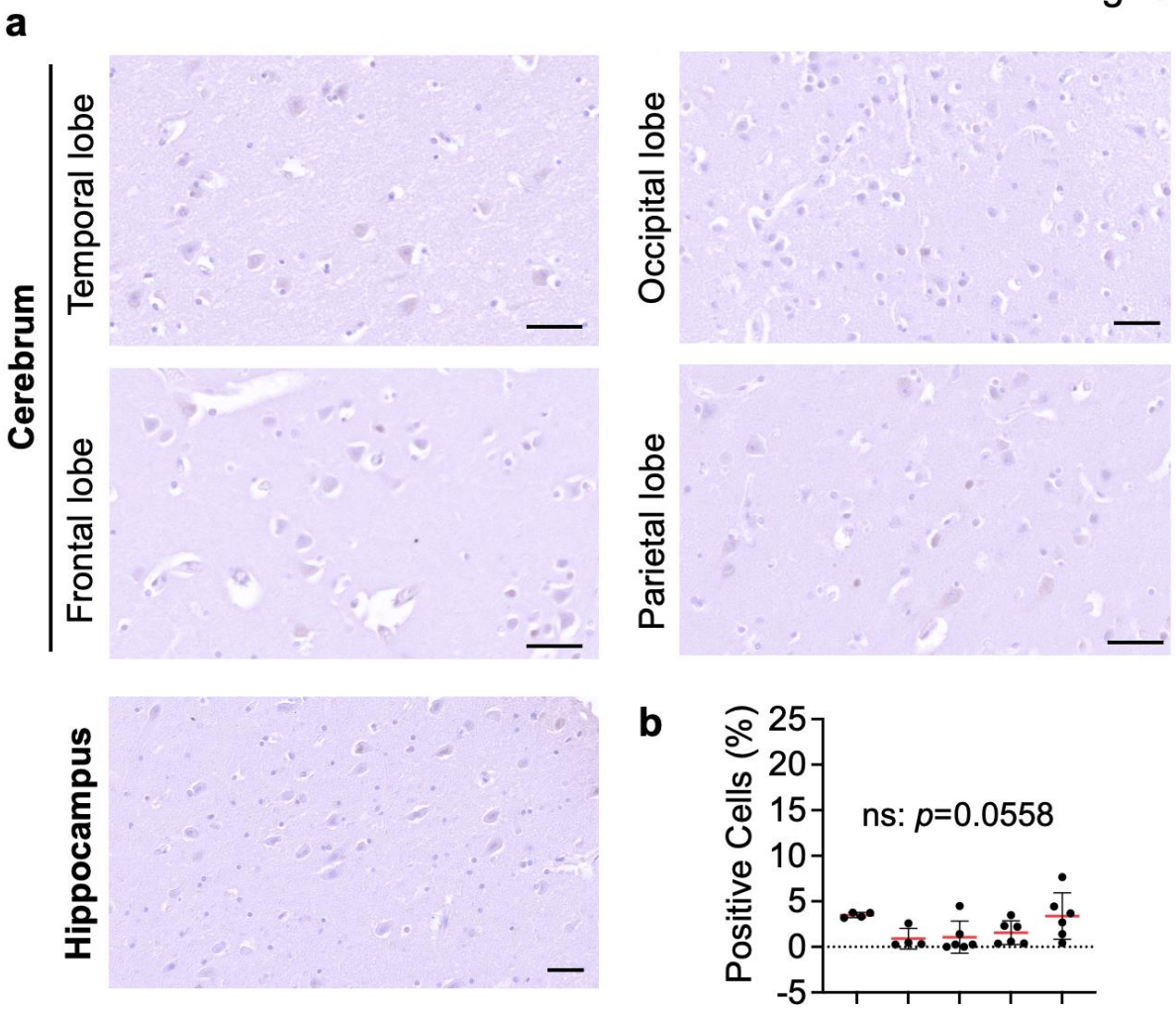
Caption: Correlation histogram graph of BaxΔ2 positivity and AD patients' age. Pearson numbers indicate the correlation between the X (BaxΔ2 positive cells) and Y (AD female or male patients' age) axes.

Fig. S5



Caption: Immunostaining of Bax∆2 proteins in different brain regions from a 73−year−old male AD patient. Scale bars, 50 μm.

Fig. S6

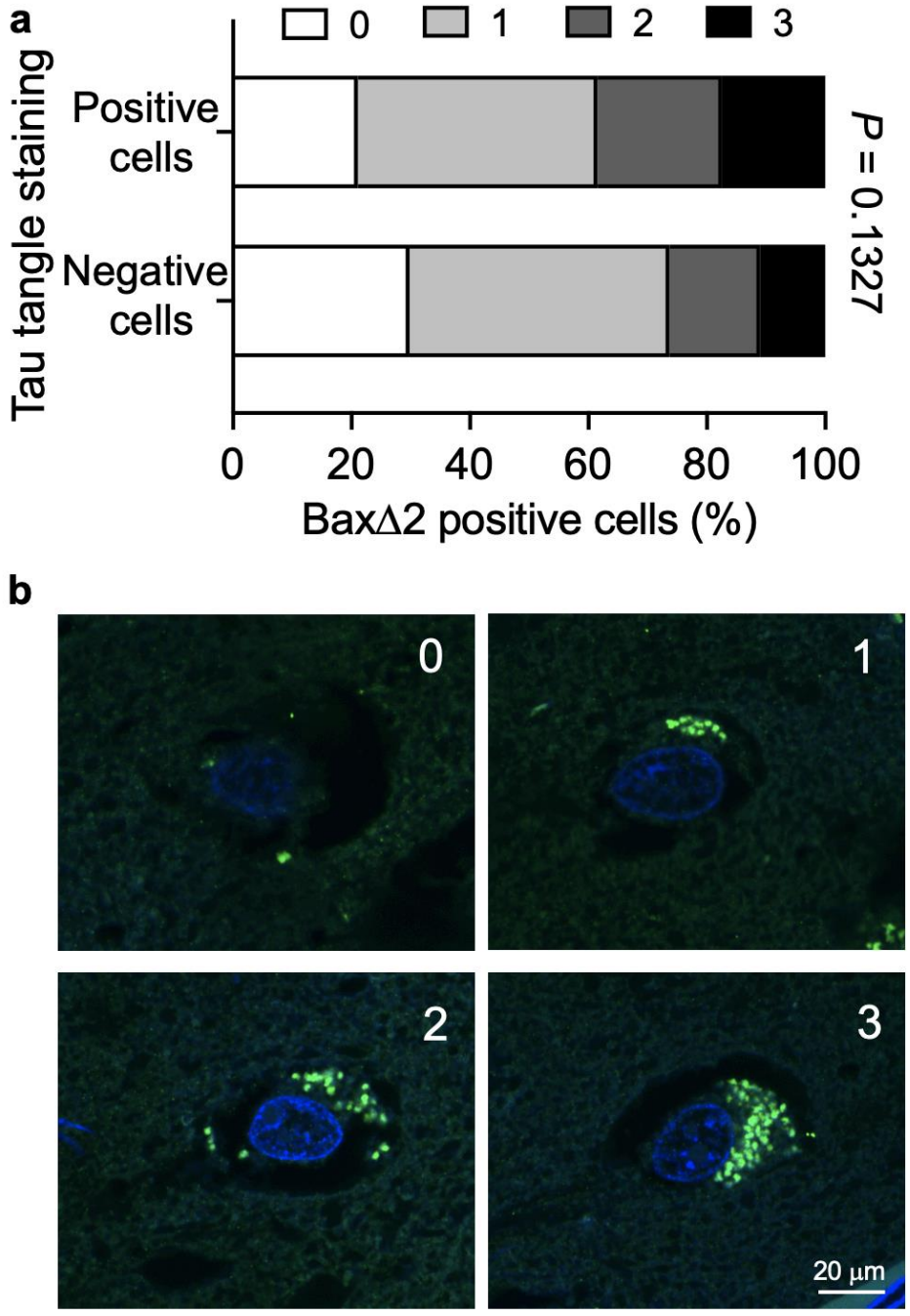


c

Anatomic site	Age	Sex
Temporal lobe	45	F
Temporal lobe	27	M
Frontal lobe	18	F
Frontal lobe	38	M
Parietal lobe	45	M
Parietal lobe	23	F
Parietal lobe	54	M
Occipital lobe	23	M
Occipital lobe	45	F
Occipital lobe	27	F
Hippocampus	47	M
Hippocampus	28	F
Hippocampus	41	M

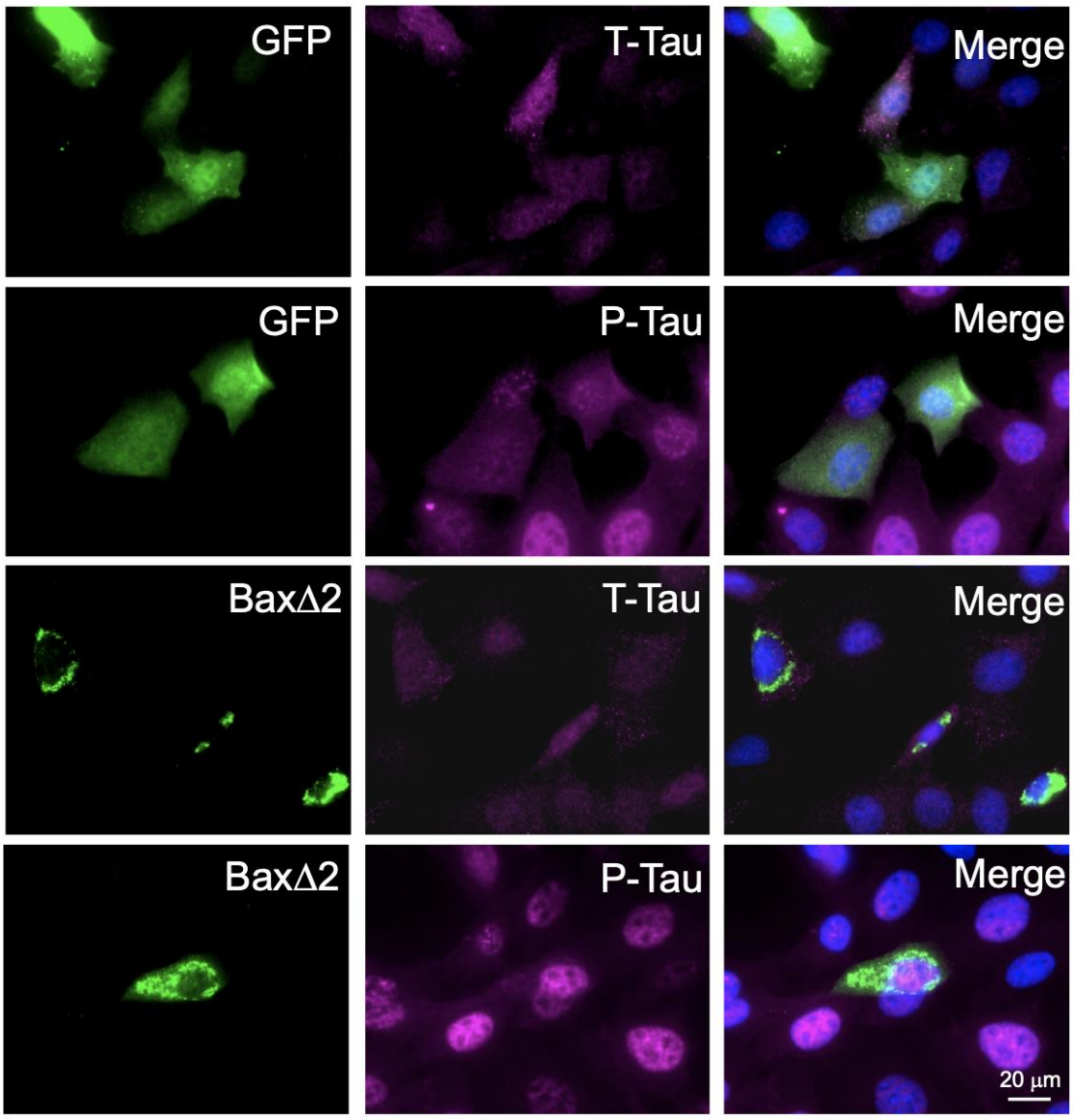
Caption: a Immunostaining of Bax∆2 proteins in different regions of human normal brains. scale bars, 50 μm. **b** Quantitation of (a). **c** Tissue source information for (a) experiments.

Fig. S7



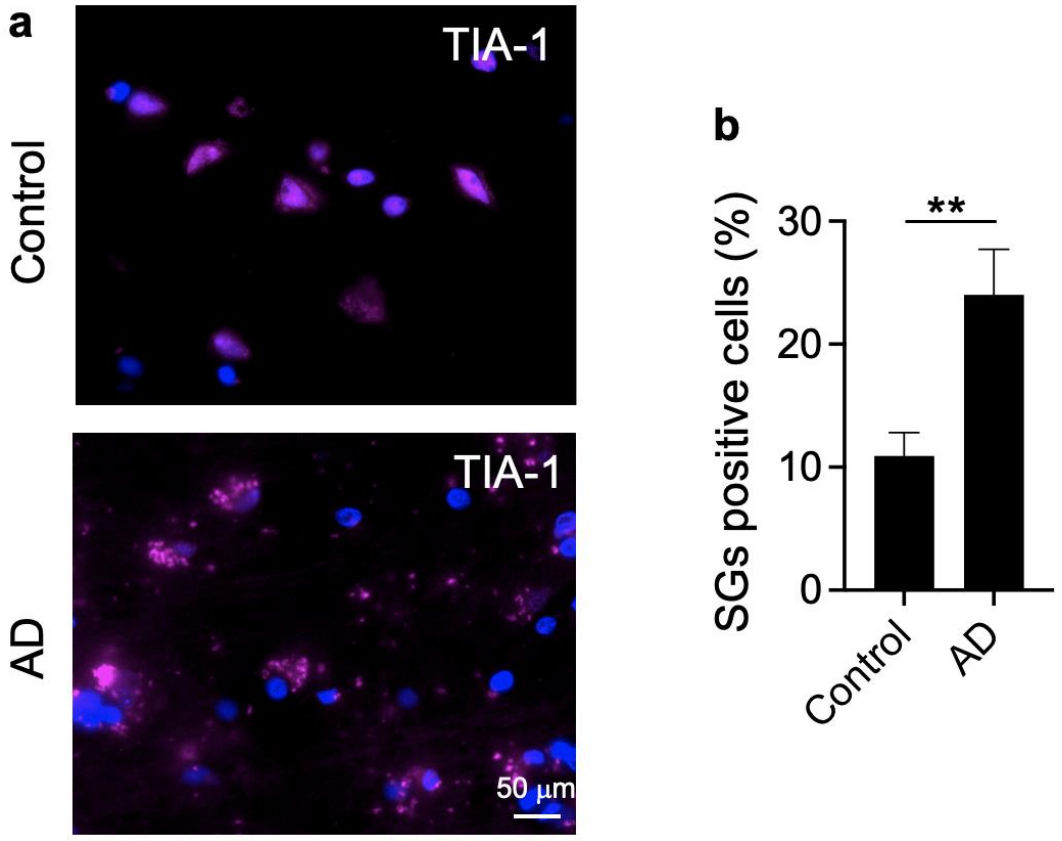
Caption: a Quantitation of correlations between BaxΔ2 positive cells and Tau positivity from stained tissue samples in Figure 4. Tau−positive cells (n=60) and Tau negative cells (n=60) were analyzed in the quantitation. BaxΔ2 positivity was classified into 4 different levels: 0, 1, 2, 3. Typical images for each score are shown in (**b**). Parametric statistical analysis was performed, $p = 0.1327$.

Fig. S8



Caption: HT22 cells were transfected with GFP, BaxΔ2 followed by immunofluorescent staining for BaxΔ2 (green) and T−Tau or P−Tau (T205) (purple), Nucleus was stained as blue. Scale bars, 20 μm.

Fig. S9



Caption: a Immunofluorescent staining of non−AD and AD human brain tissues with SGs marker TIA−1 (purple). Scale bars, 50 μm. **b** Quantitation of (a) analyzed by parametric statistical analysis, ** $p < 0.01$