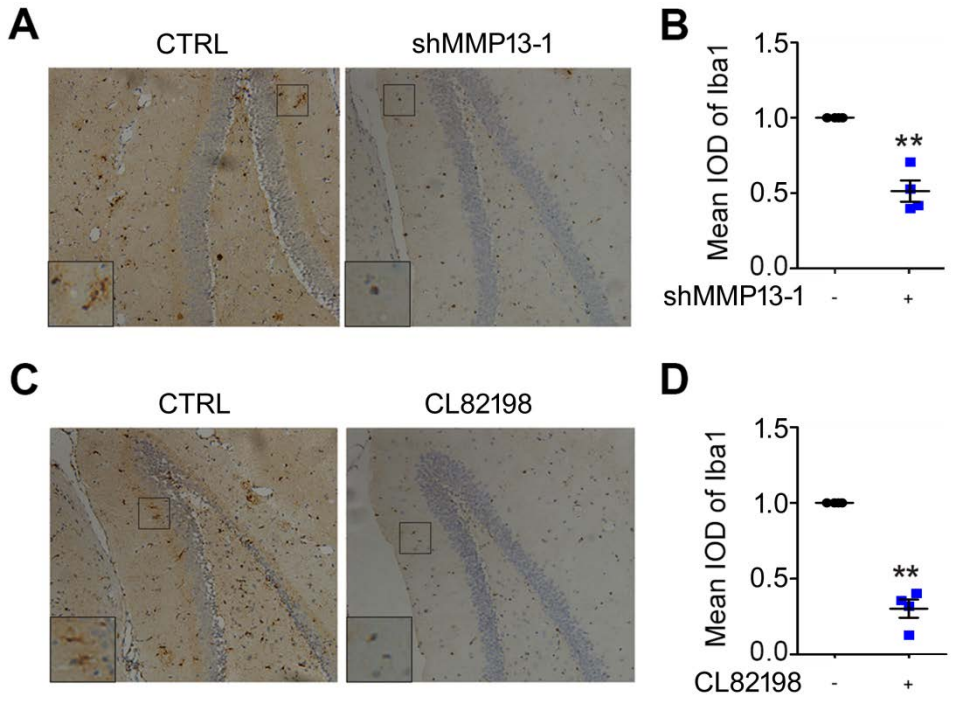
MMP13 inhibition rescues cognitive decline in Alzheimer transgenic mice via BACE1 regulation

Bing-Lin Zhu[1,#], Yan Long[1,#], Wei Luo[2], Zhen Yan[3], Yu-Jie Lai[1], Li-Ge Zhao[1], Wei-Hui Zhou[4], Yan-Jiang Wang[5], Lin-Lin Shen[5], Lu Liu[1], Xiao-Juan Deng[1], Xue-Feng Wang[1], Fei Sun[6], Guo-Jun Chen[1,*]



Supplementary Figure S1 MMP13 knockdown or CL82198 treatment reduces Iba1 expression in AD mice. (A, B) Representative immunohistochemistry images (A) and the mean integral optical density (IOD) of Iba1 (B) in the hippocampus of AD and AD-shMMP13-1 mice. **(C, D)** Representative immunohistochemistry images (C) and the mean IOD of Iba1 (D) in the hippocampus of AD and AD-CL mice. All values were normalized to AD mice (1.0) within each experiment. Data represent the mean ± SEM from 4 slices per genotype. Scale bar: 100 μm. **p<0.01 (ANOVA, n = 4).

Supplementary table 1 The clinical features of AD patients and control subjects from Sydney Brain Bank

Case #	Age	Gender	Postmortem delay (hour)	Cause of death	Disease duration (year)	Case characterisation
345	85	Female	23	Pneumonia	0	no significant neuropathology
603	101	Female	9	Cardiorespiratory failure	0	no significant neuropathology
759	93	Female	15	Gastrointestinal bleeding	0	no significant neuropathology
492	100	Female	3	Aspiration pneumonia	11	Alzheimer's disease
516	80	Female	32	Cardiorespiratory failure	10	Alzheimer's disease
678	91	Female	6	Cardiorespiratory failure	7	Alzheimer's disease

Supplementary table 2 The clinical features of AD patients and control subjects from NIH NeuroBiobank

HBS number	Sample number	sex	Age (years)	Postmortem interval (hours)	Clinical diagnosis	Neuropathological diagnosis
4431	1	female	68	23.7	myocardial infarction	essential normal brain
3861	2	female	81	14.5	COPD/pneumonia	essential unremarkable brain
3795	3	female	72	14	CA, overian/CA, peritoneal	essential normal brain
3740	4	female	88	14	leukemia	essential normal brain
3777	5	male	84	13.5	myoplastic syndrome/pancetopenia	essential normal brain
5072	6	male	83	19.5	COPD/seizure disorders/atrial fibrilation	no significant abnormalities
5265	7	male	80	18.75	normal	granulovascular degeneration and lipofuscin accumulation in hippocampal neurons consistent with age
4320	8	male	87	9.3	congestive hear failure	normal aging
4691	9	male	74	23.6	alcohol abuse	essential normal brain
3805	10	male	70	12	renal failure, acute/diabetes melitus	essential normal brain
3278	1	female	81	11.3	Alzheimer's Disease	definite(cerad)
3311	2	female	77	23	Alzheimer's Disease	braak 5

3342	3	female	88	12.4	Alzheimer's Disease	definite(cerad)
3478	4	male	88	15.5	Alzheimer's Disease	definite(cerad)/braak5
3512	5	male	76	13.8	Alzheimer's Disease	definite(cerad)/braak 6
3530	6	male	84	14.8	Alzheimer's Disease	definite(cerad)/braak5
3674	7	male	79	7.5	Alzheimer's Disease	definite(cerad)
3316	8	female	77	13	Alzheimer's Disease	braak 5
3329	9	female	74	14	Alzheimer's Disease	definite(cerad)/braak 6
3367	10	female	75	19.75	Alzheimer's Disease	definite(cerad)/braak5

Fig. 1C full blots

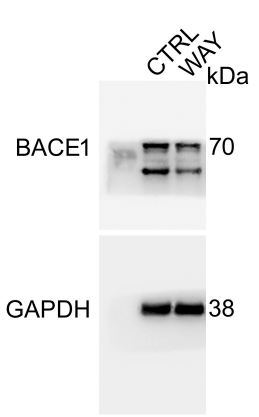


Fig. 1D full blots

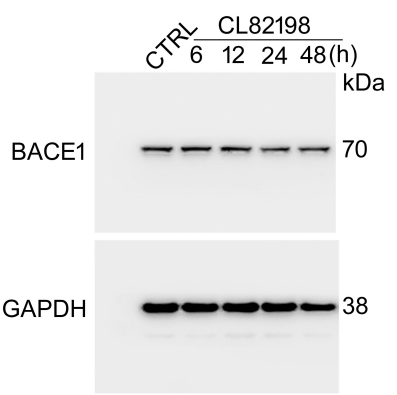
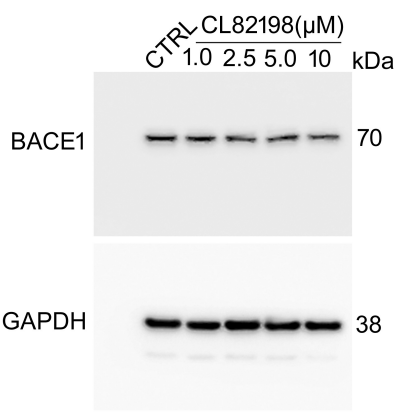


Fig. 1E full blots



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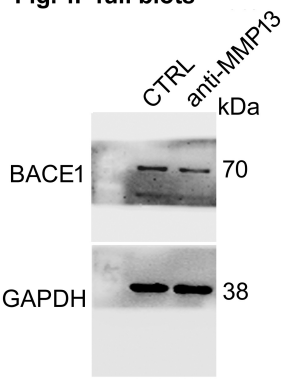


Fig. 1G full blots

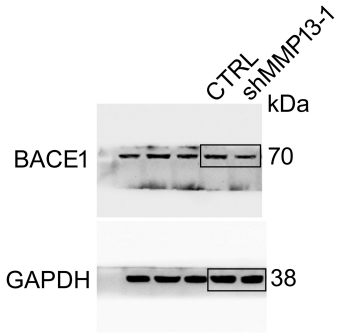


Fig. 1H full blots

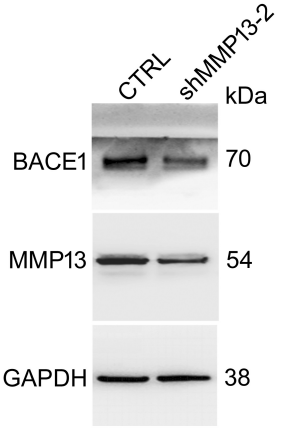


Fig. 1I full blots

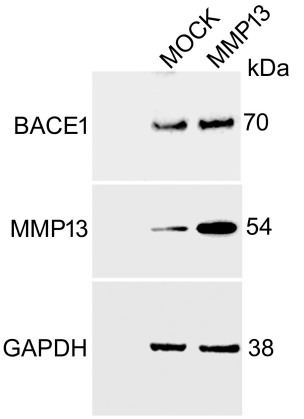


Fig. 1J full blots

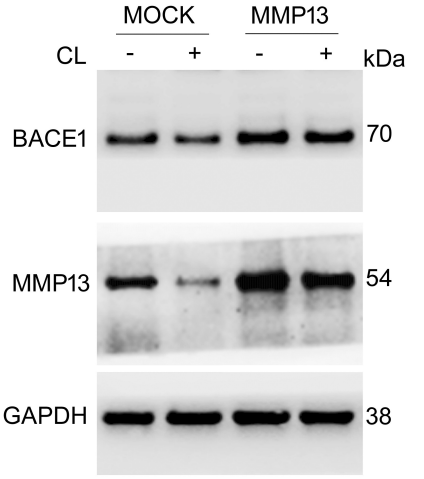


Fig. 1K full blots

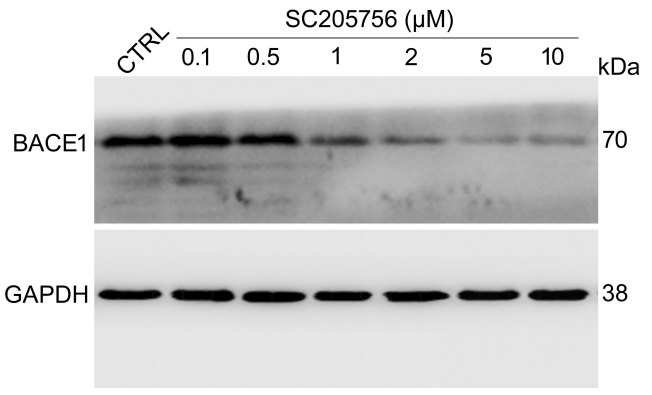


Fig. 1L full blots

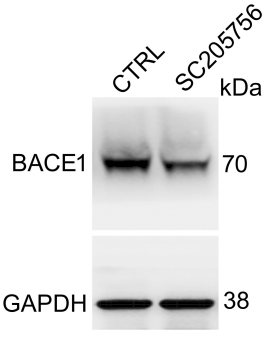
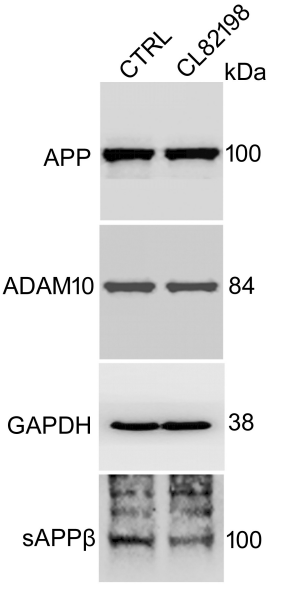


Fig. 1M full blots



Full blots for Figure 1

Fig. 2A full blots

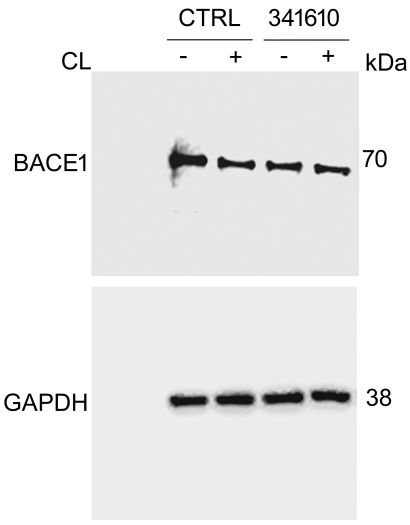


Fig. 2B full blots

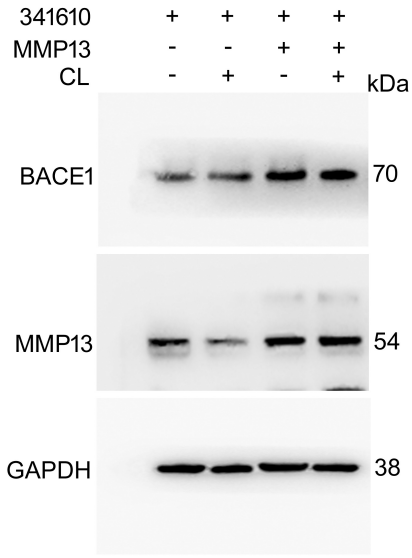


Fig. 2D ful blots

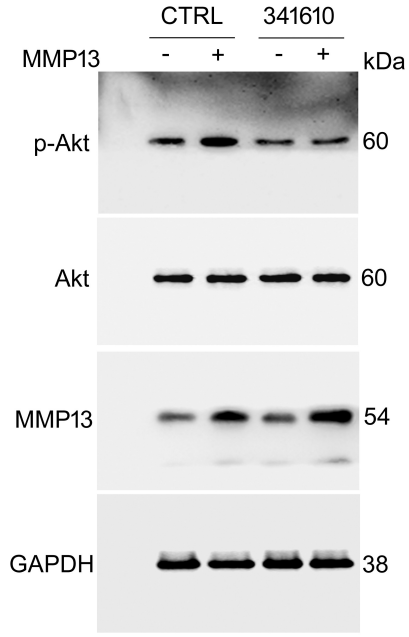


Fig. 2E full blots

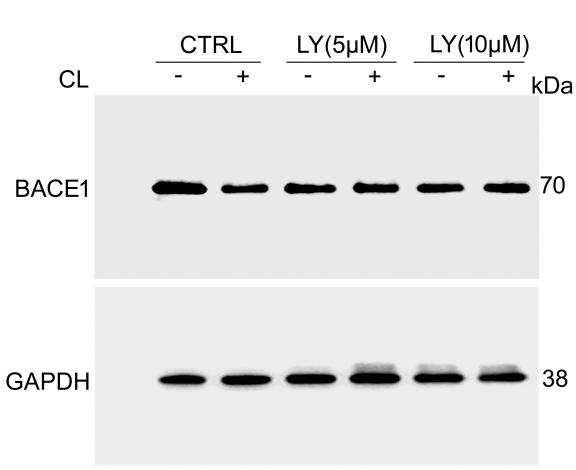


Fig. 2F full blots

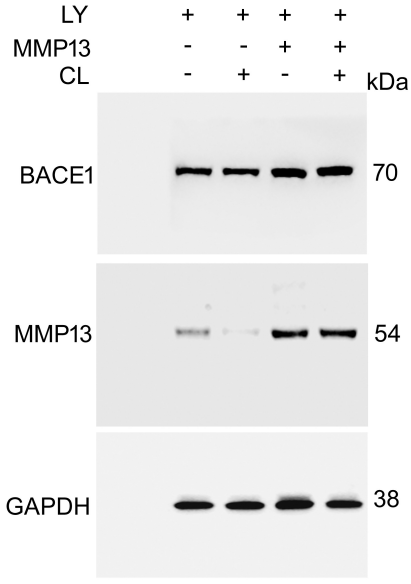


Fig. 2I full blots

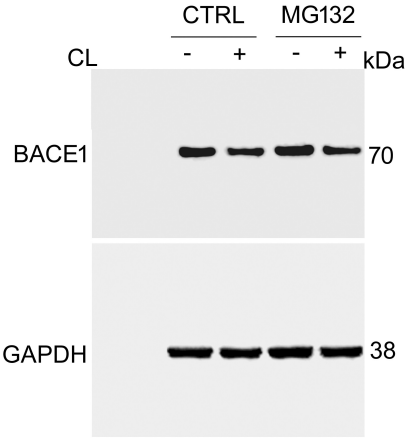


Fig. 2J full blots

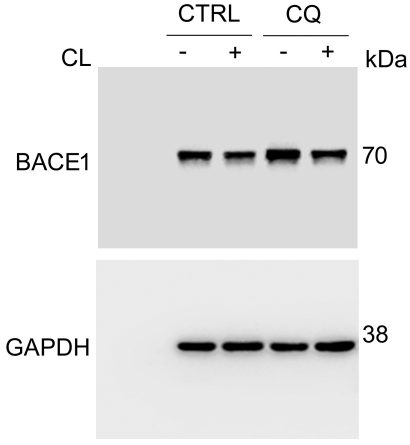
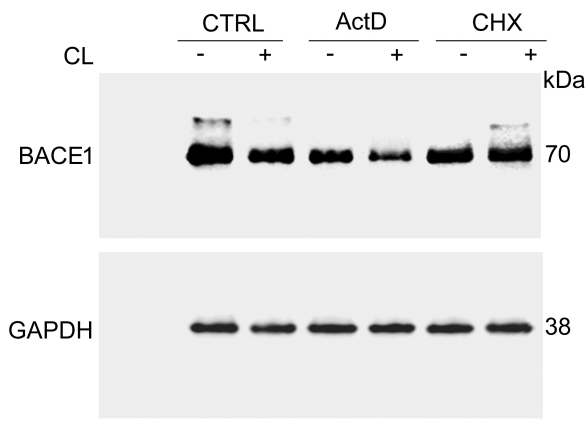


Fig. 2K full blots



Full blots for Figure 2

Fig. 3A full blots

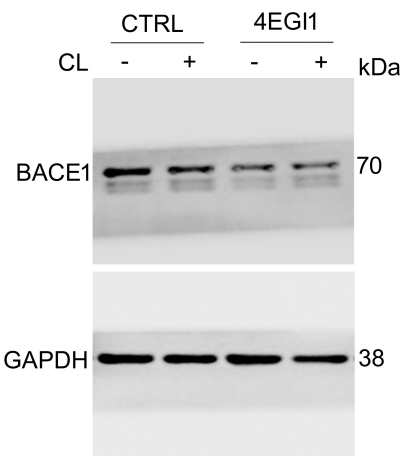


Fig. 3B full blots

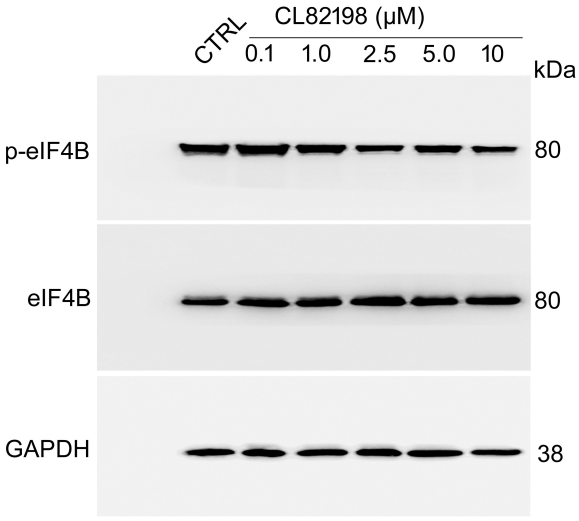


Fig. 3C full blots

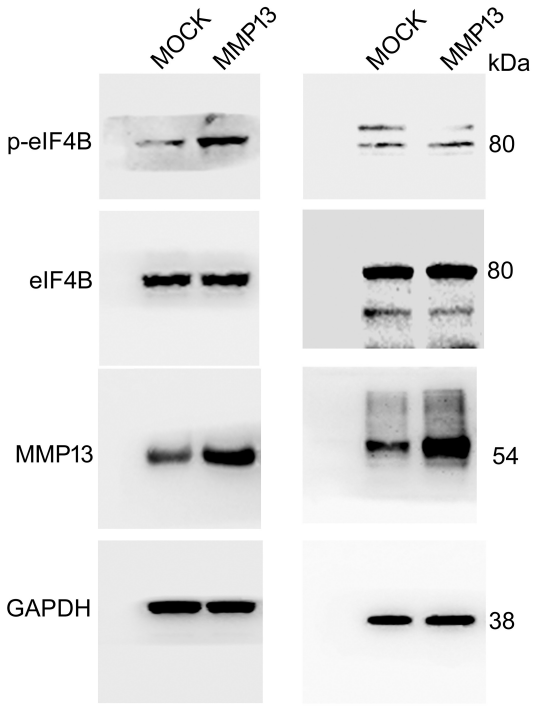


Fig. 3D full blots

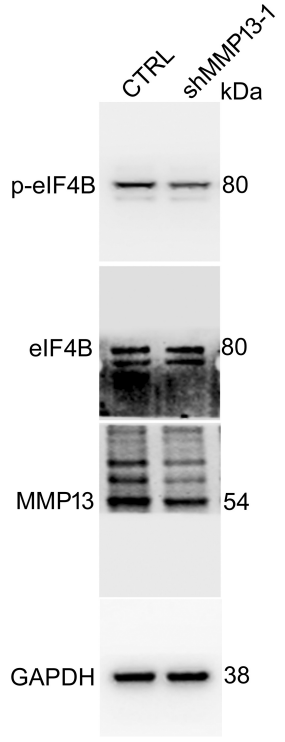


Fig. 3E full blots

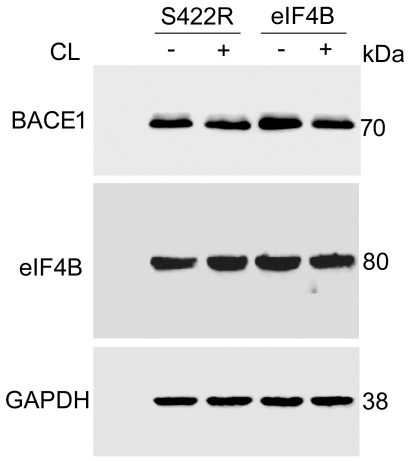


Fig. 3G full blots

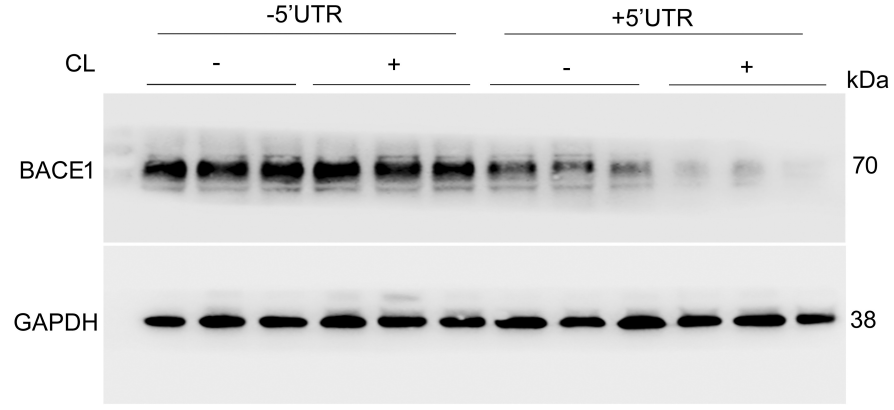


Fig. 3F full blots

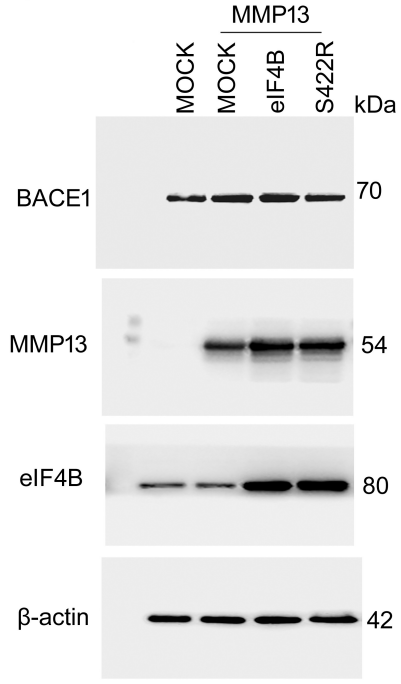
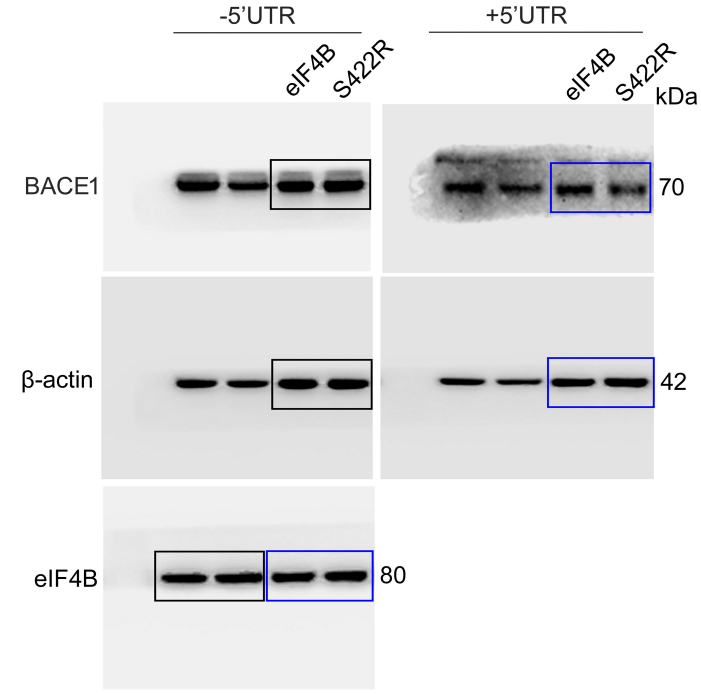


Fig. 3H full blots



Full blots for Figure 3

Fig. 4A (a) full blots

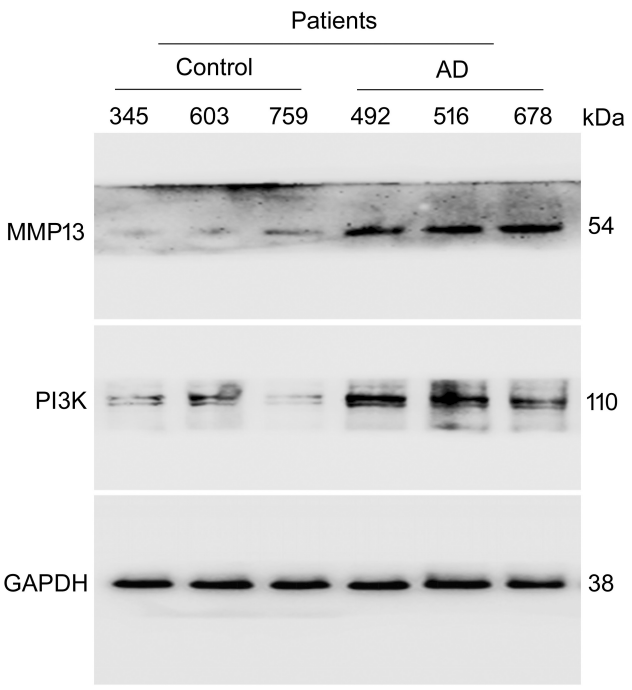


Fig. 4A(c) full blots

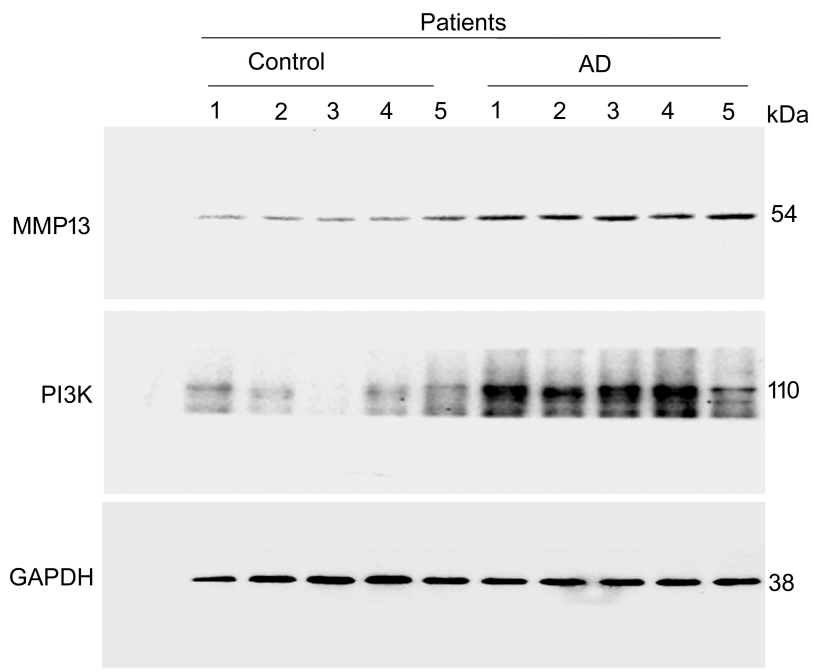


Fig. 4A(d) full blots

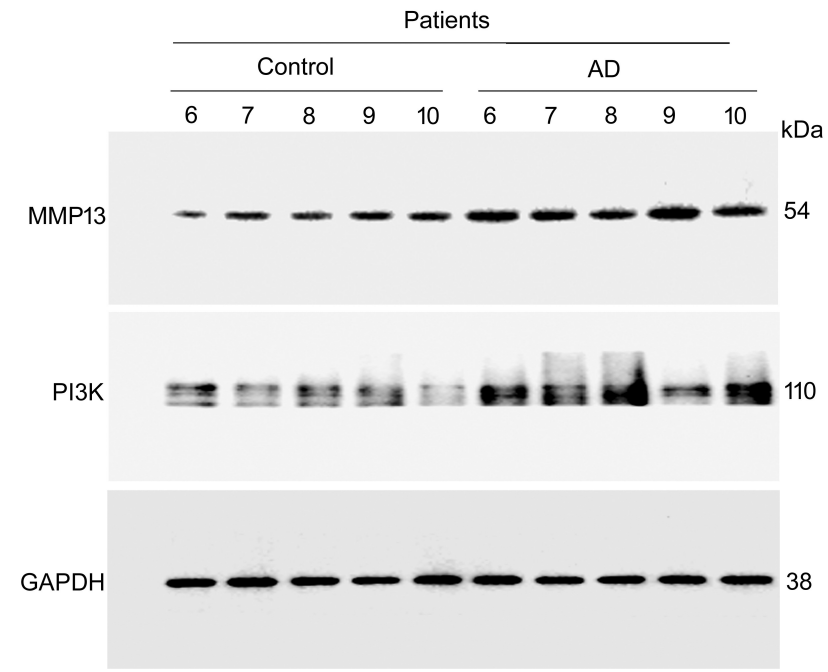


Fig. 4C full blots

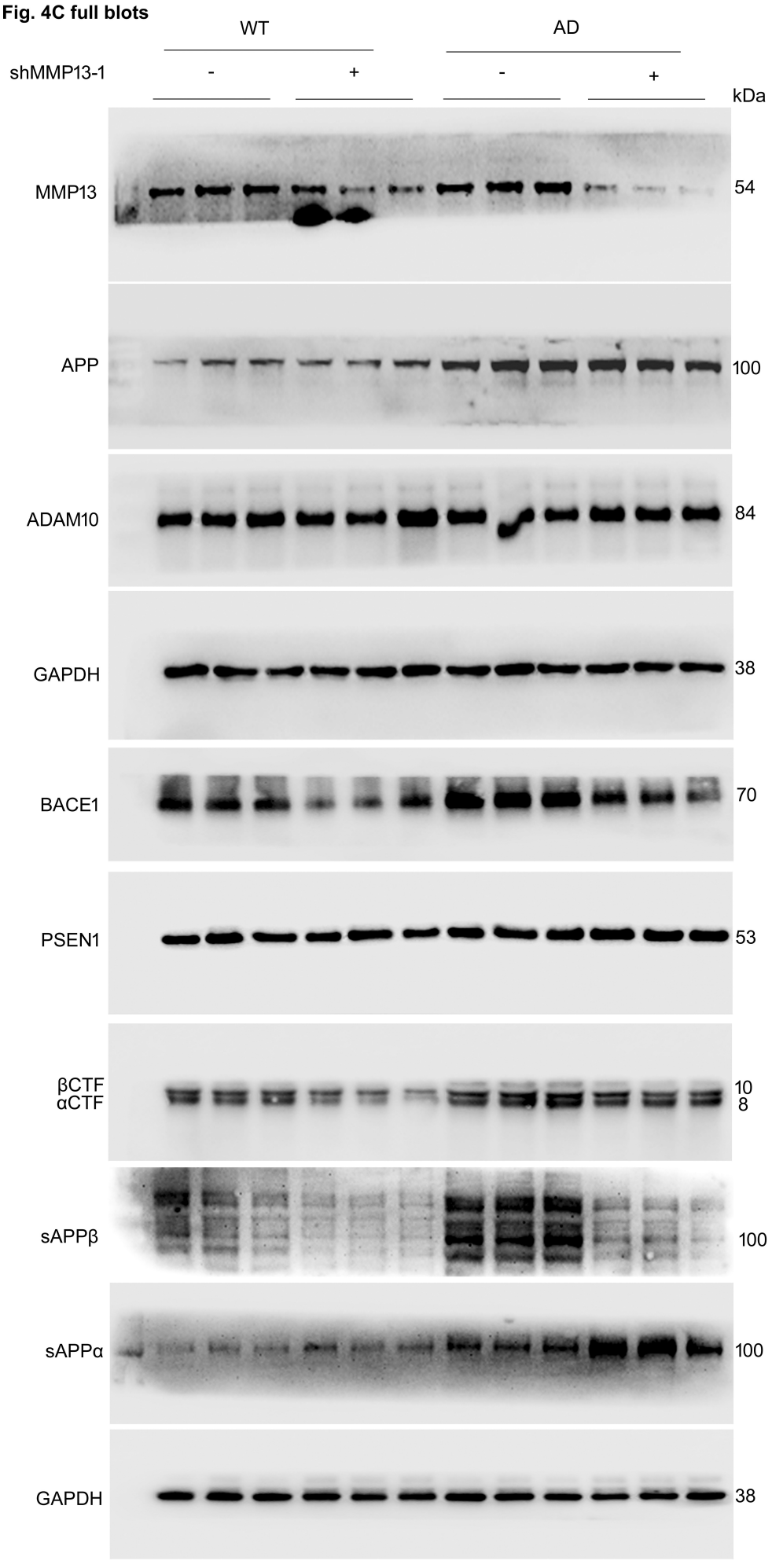
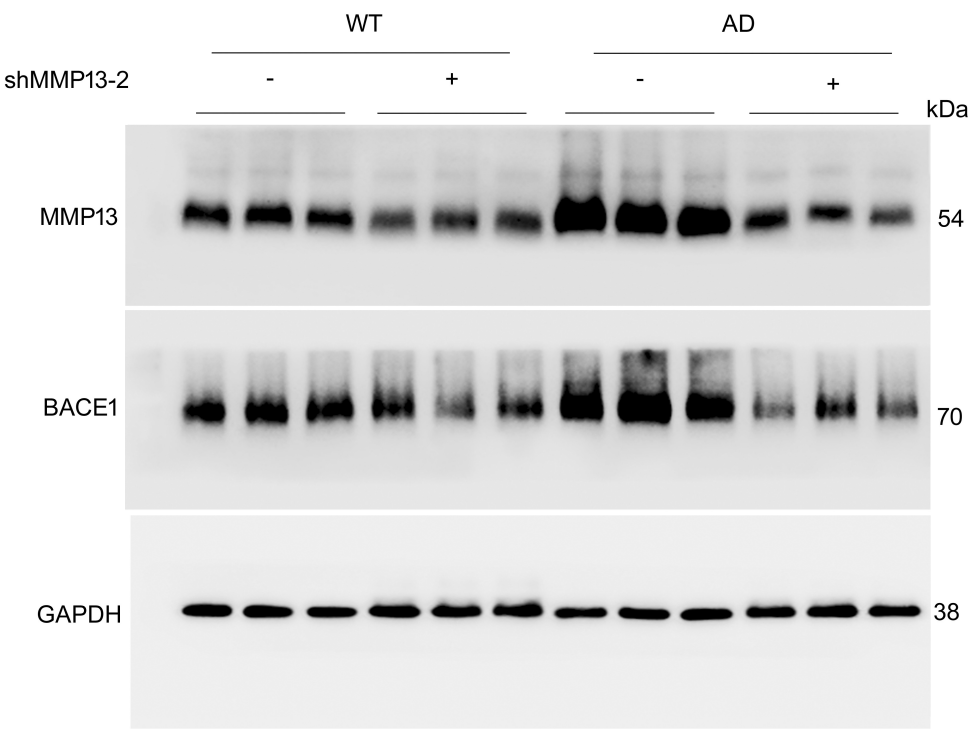


Fig. 4D full blots



Full blots for Figure 4A, 4C, 4D

Fig. 4F full blots

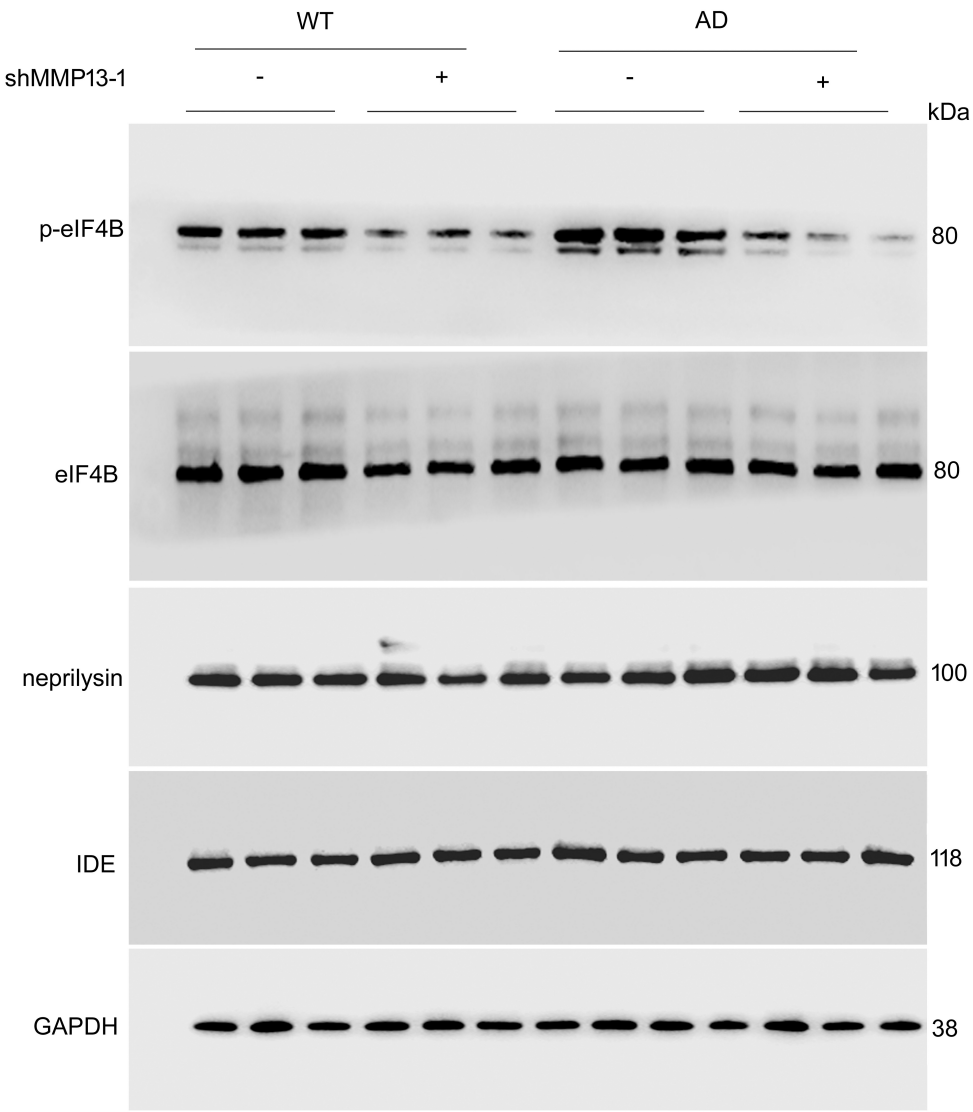


Fig. 5B full blots

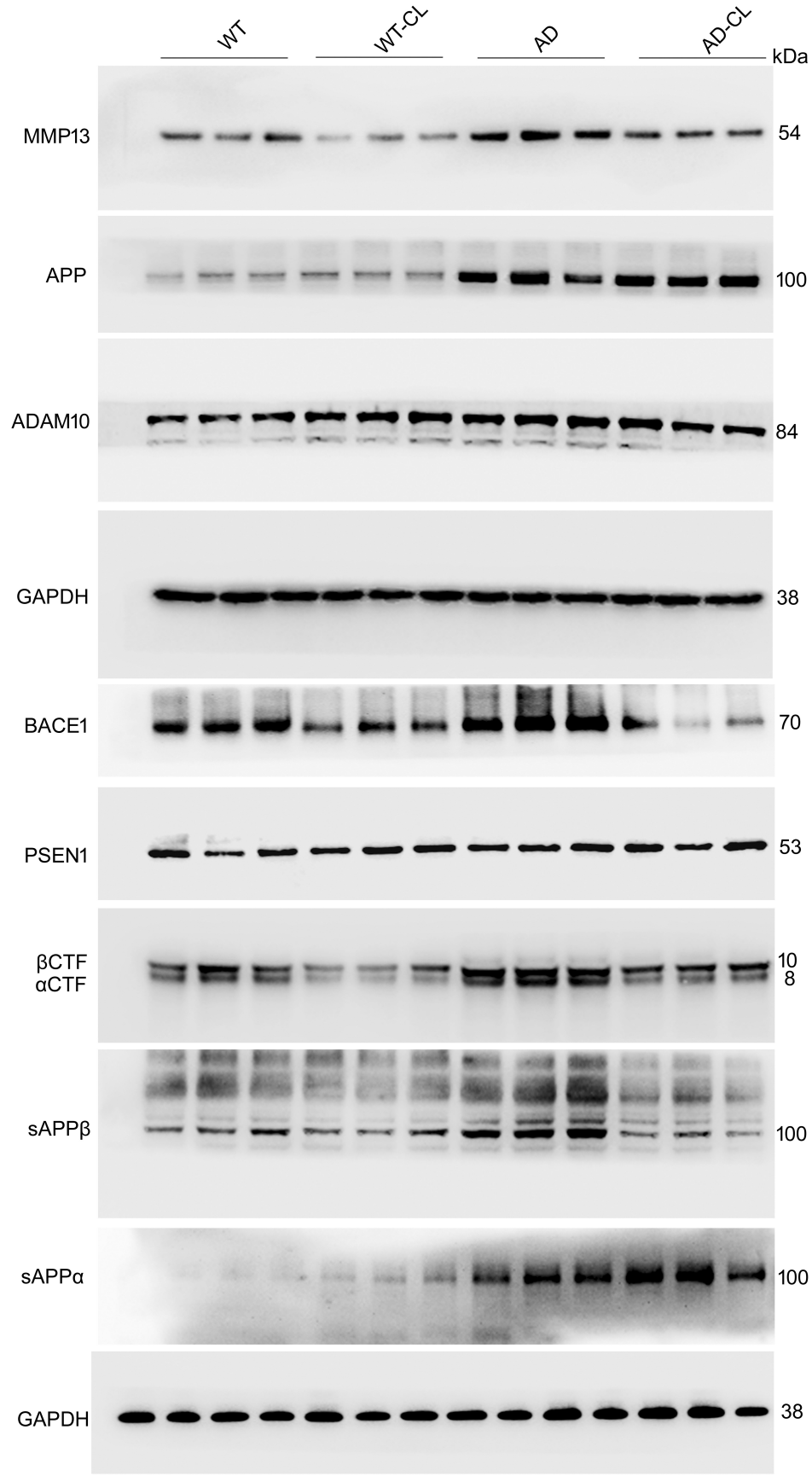
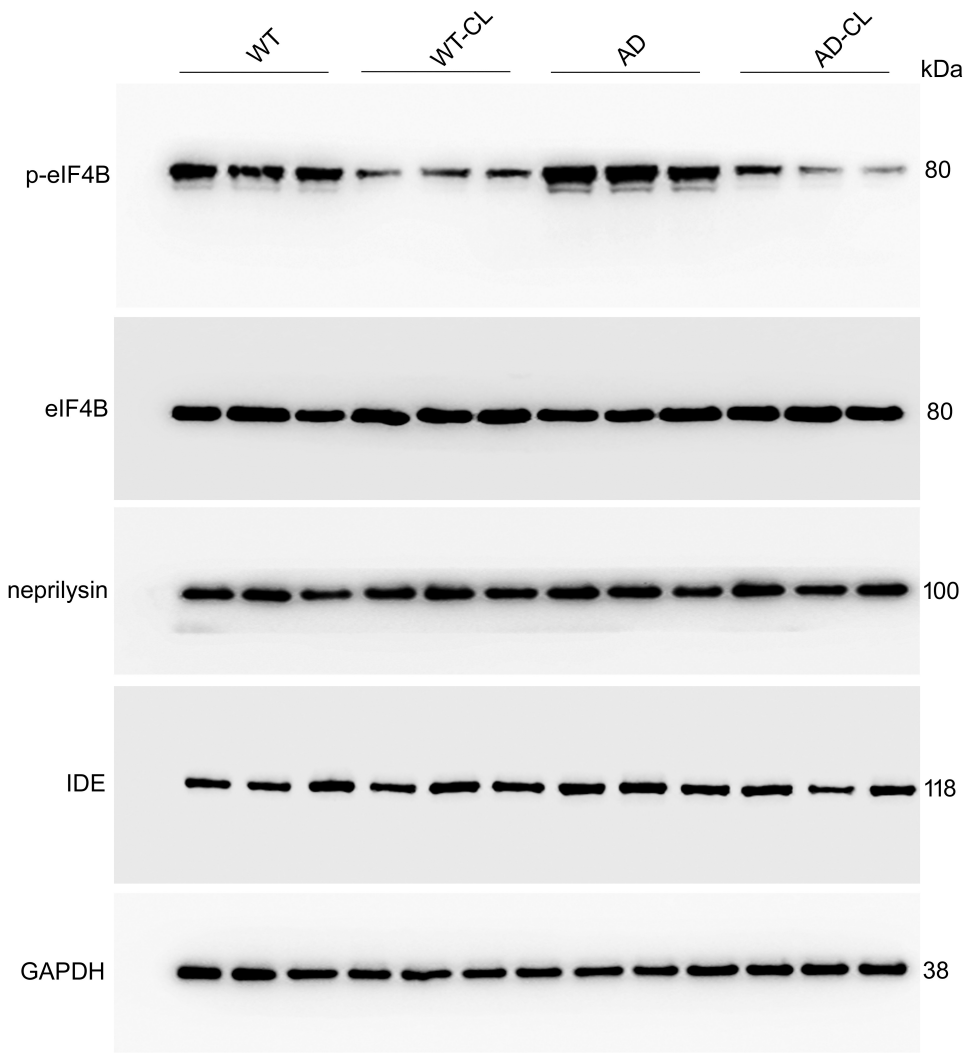


Fig. 5D full blots



Full blots for Figure 4F and Figure 5

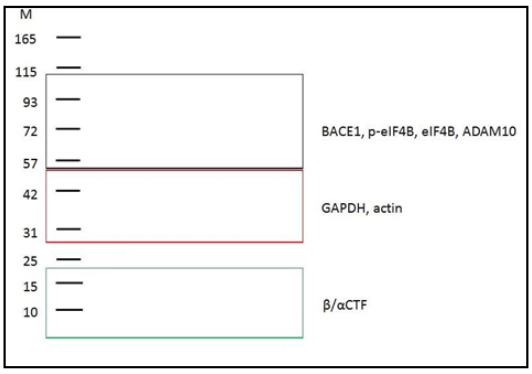
The WB images with the ladders have been placed in revised PDF file. In addition, there are a couple of technical issues we would like to address regarding the data, please refer to the following for detailed description.

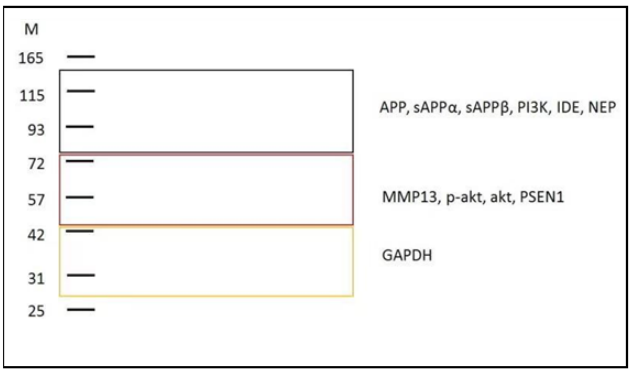
Except BACE1 in Fig. 1B, which is the major focus of this study and is shown in full-membrane, the other protein bands have been pre-cut from the full PVDF membrane before probing.

We think the major work of our study deals with large amount of Western blotting (WB) experiments, which is unlike most of the papers published in Brain. One way to improve our working efficiency is to pre-cut the full PVDF membrane into different parts.

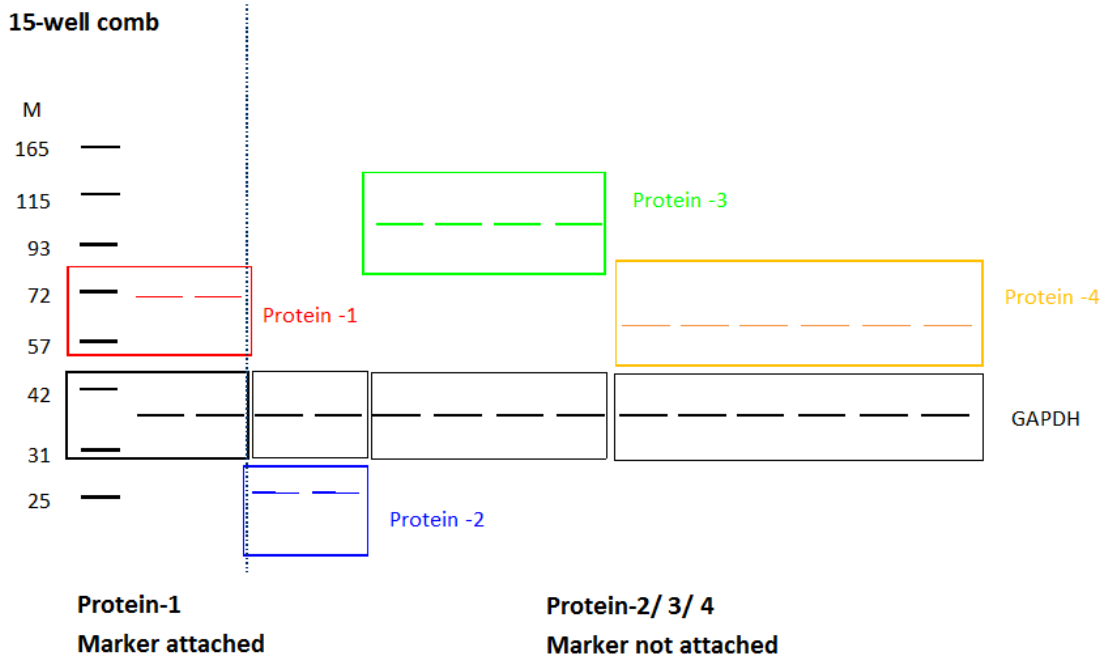
Considering the internal reference for a target protein, it might be time and effort consuming to probe a protein on a full membrane. For example, after we have probed ADAM10 on the full membrane, the subsequent probing of the internal reference GAPDH/β-actin would be possible only after we have stripped the same membrane. This process would result in possible compromise of the band quality and sometimes failure. Although the internal reference can be probed by another round of gel-running using the same samples, this does not guarantee the re-productivity due to the possible technique issues ranging from sampling to gel running and membrane transferring.

Alternatively, cutting the PVDF membrane would allow us to probe at least two proteins at the same time and to solve this problem quickly. Please refer to the following diagrams that show how we have cut the membrane. Since the WB methods of all proteins in our study have been previously reported, and the corresponding molecular weight (MW) can be easily identified, there would be no difficulties in cutting the membrane according to the visible markers.





In the revised file, the ladder is indeed in position (blank lane on the left) but not visible in most cases. As we have known, it is impossible to have an image that contains the clear ladder and the protein band, unless the previously separate ladder image and protein image are merged. This involves an additional signal capturing and merging processes. To save time, we just focused on protein bands and left the ladder in the blank lane. Also, a few blots do not have the ladders attached. This has resulted from another way of cutting. Sometimes several proteins of different interest share one set of marker. In the figure shown below, protein-1 is probed for other use, while protein-2/-3/-4 is used for the current study. Since the MW of protein-2/-3/-4 is known from literature, we were sure that protein-2/-3/-4 is the target protein.



In short, we realized that (1) the WB images are not in full-membrane, and (2) most ladders are not visible but shown in blank lanes and some are not attached with protein bands. Meanwhile, we assure that these are indeed raw data. We believe that these routine WB techniques for this single paper in the past 7 years, have been widely used by peer researchers in the area.