SI Appendix:

Supplementary Methods

Supplementary Figures

Supplementary Tables

And full immunoblot data for:

The NRF2/ARE Pathway Negatively Regulates BACE1 Expression and Ameliorates Cognitive Deficits in Mouse Alzheimer's Models

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SI Methods

Human brain specimens. Inferior parietal lobule and cerebellum specimens from the brains of 5 AD patients and 5 control subjects that had been enrolled in the University of Kentucky Alzheimer's Disease Center Autopsy Program were used for this study. All AD patients met the clinical and the neuropathological diagnostic criteria for AD (1). Control subjects had no history or neuropathological signs of a brain disorder. Genders, ages, postmortem interval (PMI), Braak stages, and plaque densities of subjects are shown in *SI Appendix*, Table S1. At autopsy, tissue specimens were rapidly removed, frozen, and stored at -80°C.

Animals. We used C57BL/6J wild-type mice, *Nrf2*$^{-/-}$ mice, 5xFAD mice (human APP mutation: Swedish, Florida, and London; PS1 mutation: M146L and L286V) (2) and 3xTg-AD mice (APP Swedish, MAPT P301L, PSEN1 M146V) in this study. 5xFAD mice were purchased from Jackson laboratory, and the *Nrf2*$^{-/-}$ mice were obtained from RIKEN BioResource Center (Tsukuba, Japan). *Nrf2*$^{-/-}$mice were maintained on C57BL/6J background. 5xFAD-heterozygous mice were maintained on C57BL6/SJL. Thus, 5xFAD mice were backcrossed to the C57BL/6J background for at least 8 generations. The backcrossed 5xFAD mice were used to produce 5xFAD;*Nrf2*$^{-/-}$ mice. At the age of 9 months, WT (male, n=5; female, n=5), 5xFAD (male, n=5; female, n=4), *Nrf2*$^{-/-}$ (male, n=4; female, n=4), and 5xFAD;*Nrf2*$^{-/-}$ (male, n=4; female, n=3) mice were subjected to behavioral

tests, and at the end of testing the animals were sacrificed and their brains processed further biochemical and immunohistochemical analyses. In a separate experiment, non-backcrossed 5xFAD mice which have C57BL6/SJL background and 3xTgAD mice which had been backcrossed with C57BL/6 mice for 8 generations (3) were used for sulforaphane administration. Mice were maintained under a 12 h light-dark cycle with continuous access to food and water. 5xFAD (9-month-old, male, n=6-8 per group) and 3xTg-AD (7-month-old, male, n=6-8 per group) mice were injected intraperitoneally with 5 or 10 mg/kg sulforaphane (LKT laboratories) or vehicle (5% DMSO, 95% PBS) every other day for 2 months. 12-month-old C57BL/6J (male) and *Nrf2*$^{-/-}$mice (male) were injected intraperitoneally with 10 mg/kg sulforaphane or vehicle (0.67% DMSO, 99.33% PBS) every other day for one month (n=6-8 per group). This study was reviewed and approved by the Institutional Animal Care and Use Committee (IACUC) of Sungkyunkwan University.

Morris water maze test. A spatial memory test was performed as described in a previous report (4). The Morris water maze is a white circular pool (100 cm in diameter and 35 cm in height) with a featureless inner surface. The pool was filled with water and nontoxic water-soluble opaque white dye. The pool was divided into four quadrants of equal area. A platform (8 cm in diameter and 10 cm in height) was centered in one of the quadrants of the pool and submerged 1 cm below the water surface so that it was invisible at water level. The pool was located in a test room that contained various prominent visual cues. The location of each swimming mouse, from the start position to the platform, was monitored by a video tracking system (Ethovision system). The day before the beginning of the experiment, the mice were acclimated to swimming for 60 sec in the absence of the platform. The mice were then given two trial sessions each day for four consecutive days, with an inter-trial interval of 15 min; the escape latencies were recorded. This parameter was averaged for each session per mouse. Once the mouse located the platform, it was permitted to remain on it for 10 sec. If the mouse did not locate the platform within 120 sec, it was placed on the platform for 10 sec and then removed from the pool by the experimenter. On day 5, the probe test involved removing the platform from the pool. This test was performed with the cut-off time of 60 sec. The point of entry of the mouse into the pool and the location of the platform for escape remained unchanged between trial 1 and 2, but was changed each day thereafter.

Passive avoidance test. Testing began with training in which a mouse was placed in a light chamber; when the mouse crossed over to the dark chamber, it received a mild (0.25 mA/1 sec) a shock on the foot. This initial

latency to enter the dark (shock) compartment served as the baseline measure. During the probe trials, 24 hr after training, the mouse was again placed in the light compartment, and the latency to return to the dark compartment was measured as an index of passive fear avoidance.

Novel object recognition test. The novel object recognition test, based on the spontaneous tendency of rodents to explore a novel object more often than a familiar one, was performed as described previously (5). In brief, this task consists of habituation, familiarization, and test sessions performed on separate days. Mice were habituated to the empty open field (50 cm X 50 cm X 50 cm) for 10 min the day before the test. During the familiarization session, mice were allowed to freely explore two identical objects. On the next day, mice were placed into the same box containing one familiar object and one new object. The test was stopped when there has been a 20 sec exploration of both objects or when a 10 min period had elapsed. A discrimination index, calculated for each mouse, was expressed as the interaction time with the novel object divided by the interaction time with novel object and the familiar object x 100. The time spent exploring the object (nose pointing toward the object at a distance of less than 2 cm from the object) was recorded by hand by an observer blinded to the genotype and treatment history of the mice.

Immunohistochemistry. Mice were perfused transcardially with 4% paraformaldehyde (PFA) in PBS. Brains were removed and immersed in 4% PFA in PBS at 4℃ overnight and then cryoprotected by incubation in a 30% sucrose solution and stored at -80 ℃. Brains were sectioned at a thickness of 40 μm on a cryostat. The sections were blocked with 3% goat serum (Thermo) and incubated with antibodies 6E10 (Biolegend) or pS198 (#2567-1, Epitomics) at 4℃ overnight. The brain sections were then washed with PBS and incubated for 1h in the presence of anti-mouse IgG labeled rhodamine-red (Invitrogen) and anti-rabbit IgG labeled with Alexa Fluor-488 (Invitrogen). Nuclei of immune-labeled specimens were stained with 4', 6-diamidino-2-phenylindol (DAPI; Molecular probes, Karlsruhe). Aβ plaques in brains were visualized using Thioflavin-S staining. The brain sections were incubated in filtered 1% aqueous Thioflavin-S (Sigma) for 8 min at room temperature. Images were acquired using a confocal microscope LSM700 (Carl Zeiss). For light microscope images, fixed tissue embedded in paraffin was serially sectioned in 5 μm before immunohistochemical analysis. Slides were deparaffinized and rehydrated before heat-induced antigen retrieval. Antigens were detected using anti-Aβ (6E10, Biolegend) in conjunction with an HRP/DAB (ABC) detection kit (ab64259, abcam) according to the manufacturer's

instructions, and sections were counterstained with Harris hematoxylin. Images were acquired using Nikon ECLIPS TE-2000U and analyzed using ImageJ 1.48v software.

Cell lines. SH-SY5Y cells and HEK293T cells were purchased from ATCC, and *Nrf2*$^{-/-}$ MEFs and *Keap1*$^{-/-}$ MEFs were graciously gifted by Dr. Wakabayashi Nobunao and Dr. Masayuki Yamamoto (Tohoku Univ, Japan), respectively. HT22, mouse hippocampal neuronal cells, were kindly gifted by Dr. David Schubert (the Salk Institute, La Jolla, CA, USA).

Cell culture. Human SH-SY5Y neuroblastoma cells, HT22 cells, HEK293T cells and MEFs (wild-type, *Nrf2*$^{-/-}$, *Keap1*$^{-/-}$) were maintained in Dulbecco's Minimum Eagle's Medium (Thermo) supplemented with 10% (v/v) fetal bovine serum (Thermo) and 1% (v/v) penicillin/streptomycin (Invitrogen, Carlsbad, CA, USA). These cells were incubated at 37℃ in a humidified atmosphere containing 5% CO^2 (v/v). For transfection of SH-SY5Y and MEFs cells, cells were plated in 35 mm dishes, and plasmid DNA and siRNA (50-75 nM) were transfected using Lipofectamine 2000 (Invitrogen) according to manufacturer's protocol and then incubated with fresh medium for 24 h. Nrf2 plasmid DNA (pcDNA3-Myc-Nrf2: #21555) was purchased from Addgene. CMV13-3xFlag-Keap1 plasmid DNA were graciously provided by Dr. Masayuki Yamamoto (Tohoku Univ, Japan). Human NRF2-siRNA (sc-37030) and human KEAP1-siRNA (sc-43978) were purchased from Santa Cruz.

CRISPRi/dCas9 system. CRISPRi of the human *BACE1* promoter was performed as described (23). The ARE1 sgRNA (sequence: 5' – GATTGAGGGAGCAGGATGAAAGG-3') was cloned into *BstX*I and *Xho*I sites of CMV-puro-t2A-mCherry expression plasmid (Addgene #44248). The catalytically dead Cas9 (dCas) was obtained from Addgene (#44246). The efficacy of the inhibition of gene expression by CRISPRi/dCas9 was determined by real time RT-PCR and western blotting.

Luciferase assay. HEK 293T cells (for human promoter) and HT22 cells (for mouse promoter) were transfected with the wild-type or mutant reporter plasmids (250 ng) and control Renilla luciferase plasmid (pRLTK-ΔARE, 6.25 ng) in 12 or 24 well plates by liposome-mediated transfection (Lipofectamin 2000, Life Technologies). The pRLTK-ΔARE vector expresses Renilla luciferase and contains deleted ARE in the thymidine

kinase (TK) promoter region of pRLTK. After 24 hr of transfection, cells were treated for 24 h with vehicle (DMSO) or sulforaphane. Cells were lysed in passive lysis buffer (Promega) and luciferase activity was measured using a Dual-Luciferase-Reporter System (Promega) with a luminometer (Berthold Technologies). Firefly luciferase activity was normalized by measuring the pRLTK-ΔARE activity from a co-transfected reporter vector. A human *BACE1* promoter vector (pB1PA) was kindly provided by Dr. Weihong Song (6). For mouse *Bace1* promoter cloning, we inserted the wild-type mouse *Bace1* promoter region (from -1380 to +220 bp; PCR-amplified from mouse BAC library, Invitrogen) into the *Kpn*I and *Sac*I sites of the pGL3-Basic vector (Promega), and generated a mutant ARE3 containing *Bace1* promoter by site-directed mutagenesis. Also, we inserted the wild-type mouse *Bace1-AS* promoter region (from -602 to -22 bp) into the *Sac*I and *Nhe*I sites of the pGL3-Basic vector, and obtained a *Bace1-AS* promoter construct containing mutated ARE1 by site-directed mutagenesis. The mutations were confirmed by DNA sequencing. The oligonucleotide sequences used to generate each ARE mutant construct are listed in *SI Appendix*, Table S2.

Quantitative real-time PCR. Total RNA were extracted from brain tissue samples or cultured cells after homogenization in total RNA extraction reagent (RNAiso plus, Takara) according to the manufacturer's protocol; DNase Ⅰ (Promega) was used to eliminate DNA contamination. We used 500 ng of each sample for the first strand cDNA synthesis (PrimeScript 1st strand cDNA Synthesis Kit, Takara). Then, we performed real-time RT-PCR using CFX connect (Biorad). PCR amplification was performed using SYBR Premix Ex TaqTMII (Takara). The PCR conditions for all genes were as follows: 95℃ for 30 sec; 40 cycles of 95℃ for 5 sec; and 60℃ for 30 sec. We calculated differences between the Ct values for experimental and reference genes (18s rRNA) as ΔΔCt. The qRT-PCR primer sequences are listed in *SI Appendix*, Table S3.

Western blot. Protein extracts were prepared from cells and tissues using a T-per extraction buffer mixture (Thermo) containing 1x protease cocktail and phosphatase inhibitors (Thermo). For Aβ detection, brain tissues were homogenized in urea-sodium dodecyl sulfate (SDS) buffer (8 M Urea, 4% SDS, T-per; 25 mM bicine, 150 mM sodium chloride; pH 7.6). Protein concentrations were measured using a BCA protein assay kit (Thermo). Proteins (20 μg) were separated by SDS polyacrylamide gel electrophoresis and transferred to polyvinylidene difluoride membranes. Membranes were blocked in 5% non-fat milk for 1 hr at room temperature, and incubated overnight at 4℃ with antibodies raised against BACE1 (D10E5, Cell Signaling Technology), NRF2 (H-300, Santa

Cruz), KEAP1 (H-190, Santa Cruz or D6B12, Cell signaling Technology), HO-1 (ab13248, Abcam or ADI-SPA-895, Enzo Life Sciences), NQO1(H-90, Santa Cruz), APP full-length (APP fl) or Aβ (6E10, Biolegend), APP-CTF (Biolegend), Presenilin 1 (D39D1, Cell Signalling Technology), Presenilin 2 (ab51249, Abcam), Nicastrin (D65G7, Cell Signalling Technology), Aph-1 (#PA1-2010, Invitrogen), PEN2 (ab18189, Abcam), β-actin (A5316, Sigma) , pS198 (#2567-1, Epitomics), or pT217 (#2502-1, Epitomics). PHF-1 and TG5 antibodies were kindly gifted by Peter Davies. Membranes were then washed and incubated with peroxide-conjugated anti-mouse or anti-rabbit secondary antibodies (Millipore) for 1 hr at room temperature. Protein bands were visualized using ECL solution (Pierce). Densitometric quantification of Western blot results was performed using ImageJ 1.48v (NIH).

Chromatin Immunoprecipitation (ChIP). Sulforaphane-treated SH-SY5Y cells were cross-linked in 10 ml of 1% formaldehyde for 10 min at room temperature, followed by quenching with 1/20 volume of 2.5 M glycine solution for 5 min. Cells were washed twice with cold PBS containing protease inhibitors. Nuclear extracts were prepared in cell lysis buffer (50 mM HEPES, 1% SDS, 10 mM EDTA, pH 7.5), and sonicated to fragment chromatin using a Bioruptor (Diagenode, 3×5 min, 30 sec on/30 sec off). Proteins of interest were immunoprecipitated in ChIP dilution buffer (0.01% SDS, 1% Triton X-100, 16.7 mM Tris-HCl pH 8.0, 1.2 mM EDTA, 167 mM NaCl and 1x protease inhibitors) using NRF2 antibody (H-300, Santa Cruz). Cross-linking was reversed by incubating overnight at 65℃, and DNA was isolated using a DNA purification kit (GeneAll, Korea). DNA purity was analyzed by qPCR. Primer sequences used for ChIP-qPCR analysis are listed in *SI Appendix*, Table S4. For NRF2 ChIP-qPCR in brain tissues, brains were collected from C57BL6J treated with vehicle or sulforaphane (10 mg/kg, every other day for one month). Cerebral hemispheres were minced using mortar and pestle with liquid nitrogen, and then cross-linked in 10 ml 1% formaldehyde (Sigma) for 15 min at room temperature, followed by quenching with stop buffer (Qiagen) for 5 min. Minced tissues were washed twice with cold PBS with protease inhibitors (150 x g, 5 min, 4℃). Nuclear extracts were prepared by homogenizer in ChIP lysis buffer (Qiagen). Chromatin fragmentation was performed by sonication, using the Bioruptor (Diagenode, 3 x 5 min, 30 sec on/30 sec off). NRF2 was immunoprecipitated using 10 μl of NRF2 antibody (ab62352, Abcam), cross-linking was reversed overnight at 65℃, and DNA was isolated using DNA purification kit (Qiagen).

BACE1 and γ-secretase activity assays. The activity of BACE1 in cells and mouse brain tissues were determined using a commercially available kit (Abcam, ab65357). Briefly, Cells and cerebral cortex were

homogenized with Extraction buffer, and then incubate with Reaction buffer and β-secretase substrate at 37 ℃ for 1 hour. For measuring BACE1 activity, samples were read in a fluorescent plate reader (Biotek) with Ex/ Em = 335/495 nm. Luciferase based γ-secretase reporter assay were carried out as previously described (7).

ELISA. Levels of Aβ1-40 and Aβ1-42 were measured using sandwich ELISA kits (IBL) according to the manufacturer's instructions. Cerebral cortex was isolated and homogenized with T-per extraction buffer (Thermo) and subjected to Aβ measurement with the use of a human Aβ42 or Aβ40 ELISA kit.

Reactive oxygen species detection. To measure ROS, wild-type, *Nrf2*$^{-/-}$, or *Keap1*$^{-/-}$ MEF cells were incubated with 5 μM of H_2DCFDA (Sigma) during 30 min at 37℃ of 4 hr stimulation with 1 mM NAC (N-acetyl-L-cysteine, Sigma). SH-SY5Y cells were transfected with 50 nM of NRF2 or KEAP1 siRNA using Lipofectamine 2000 (Invitrogen) according to manufacturer's instructions. After 24 hr, transfected cells were incubated with H_2DCFDA and NAC. The total cellular intensity of DCFDA was detected with flow cytometry analysis using FACS (Guava Easycyte Flow cytometer, Merck Millipore).

Statistical analysis. All statistical analyses were performed with Prism8 (GraphPad Software, San Diego, CA), using two-tailed Student's t test, one-way ANOVA with Tukey's or two-way ANOVA. Data are expressed as mean ± SEM. Groups were considered significantly different when $p < 0.05$ (*$p < 0.05$, **$p < 0.01$, and ***$p < 0.001$).

SI Figures

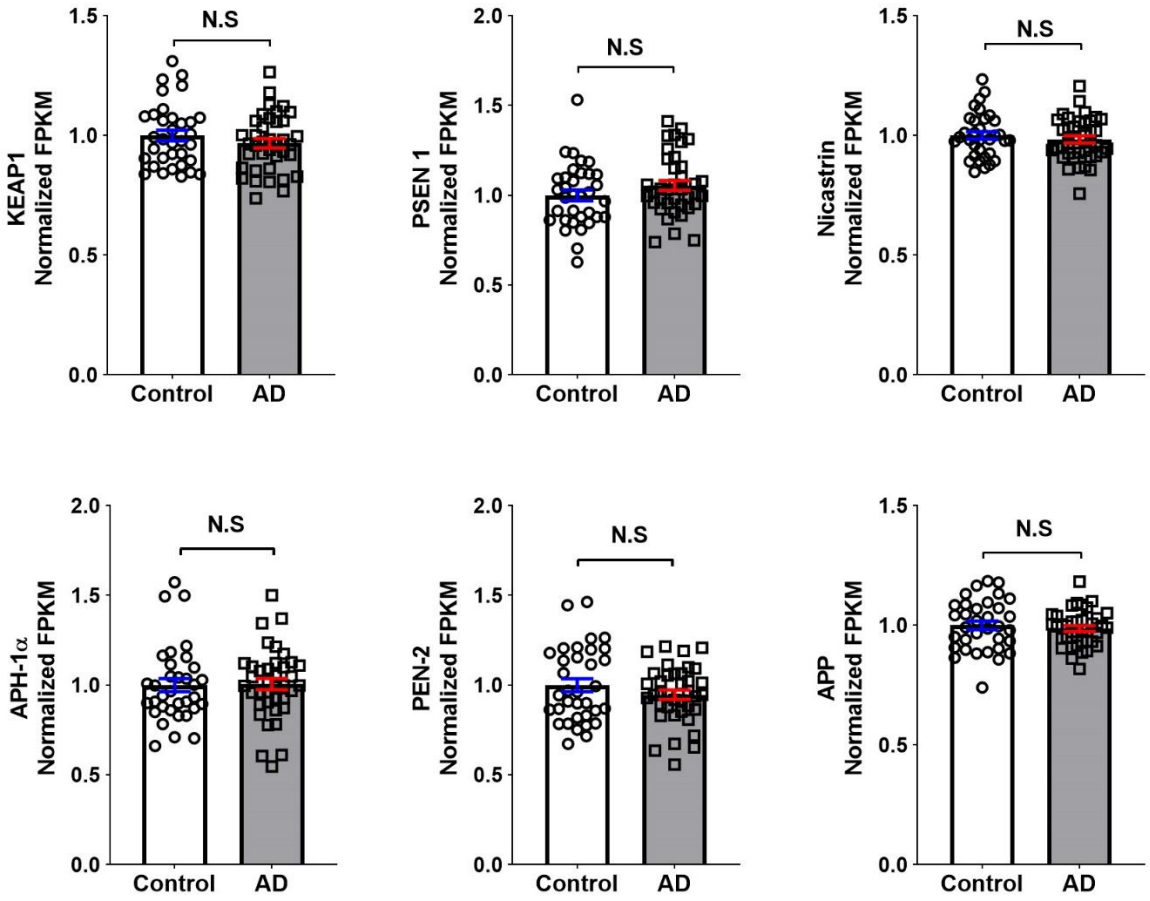


Fig. S1. Statistical analysis of RNA Seq data.

KEAP1, γ-secretase complex (*PSEN1*, *Nicastrin*, *APH-1α*, and *PEN-2*), and *APP* normalized FPKM in control (n=34) and AD (n=38).

All values are the mean ± SEM; by two-tailed Student's t test (N.S, nonsignificant).

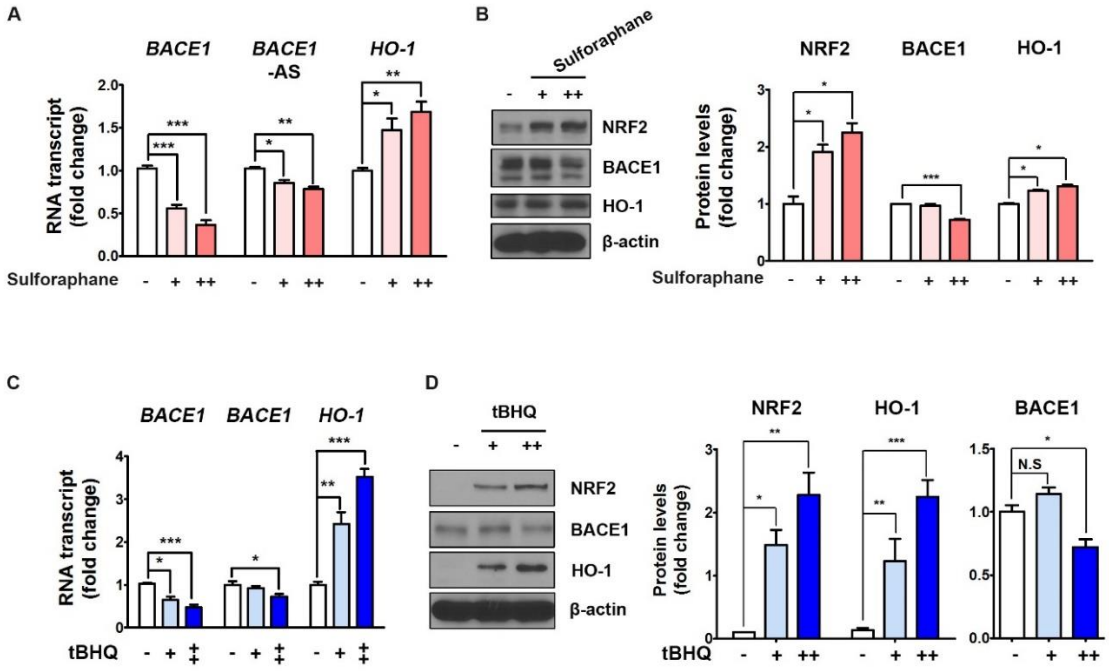


Fig. S2. Pharmacological activation of NRF2 reduces the expression of *BACE1*.

(A) Treatment with sulforaphane reduced *BACE1*, *BACE1-AS*, and *HO-1* transcript levels in SH-SY5Y cells in a dose-dependent manner; cells were treated with DMSO (-: vehicle) or sulforaphane (+, 500 nM; ++, 1 μM) for 24 hr.

(B) Protein levels of BACE1, HO-1 and NRF2 with the same conditions as described for panel A (left, representative blot; right, summary graph).

(C) The expression of NRF2 and its target gene HO-1 were upregulated by tBHQ treatment, whereas *BACE1* and *BACE1-AS* expression were decreased in SH-SY5Y cells. Cells were treated with DMSO (control) or tBHQ (+, 2.5 μM; ++, 5 μM) for 24 hr.

(D) Protein levels of BACE1, HO-1 and NRF2 with the same conditions described for panel C (left, representative blot; right, summary graph). Values are the means ± SEM**;** *$p < 0.05$, **$p < 0.01$, and ***$p < 0.001$; one-way ANOVA with Tukey's**.** (N.S, not significant).

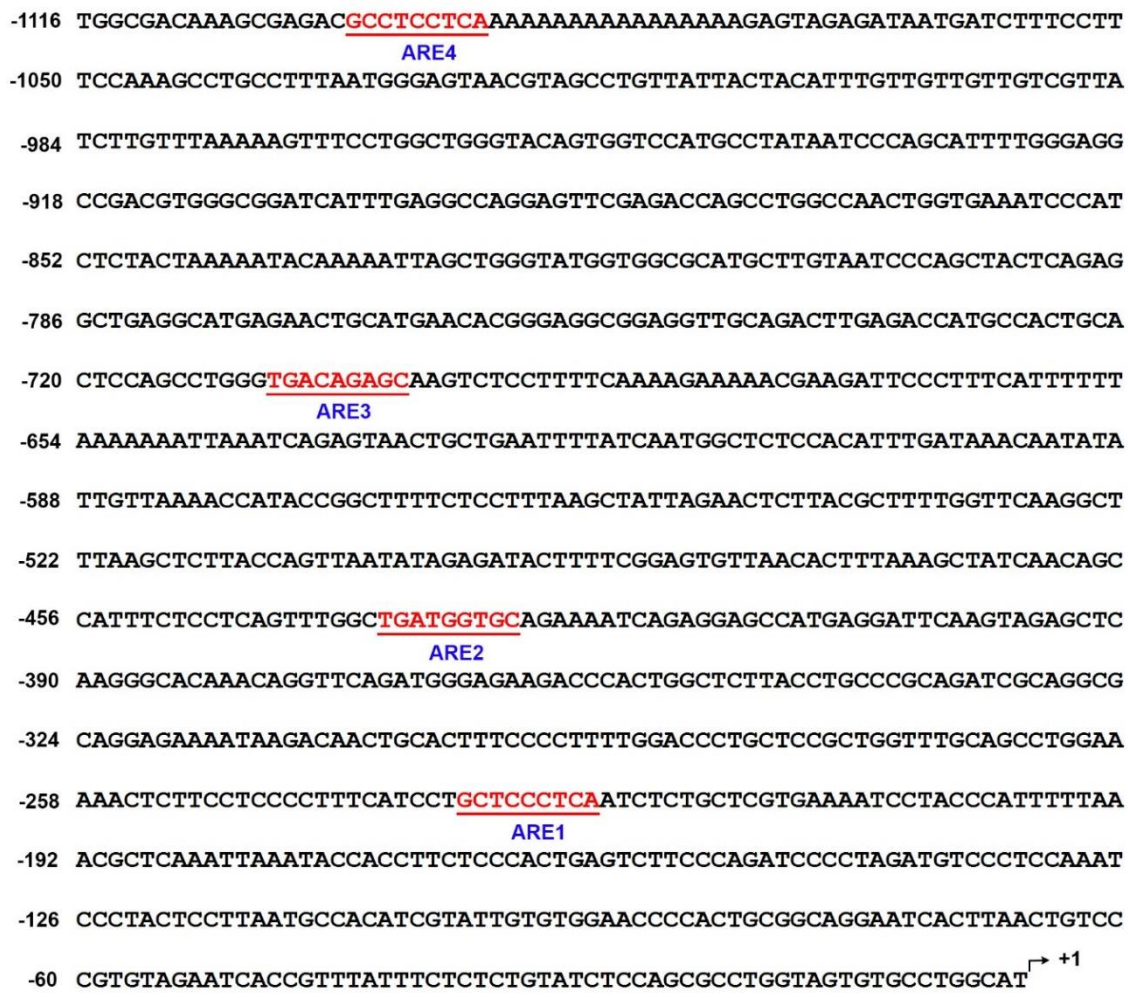


Fig. S3. Sequence of the human *BACE1* gene promoter.

Four AREs, TGAnnnnGC, are present in the human BACE1 promoter. The sequence (GenBank accession number: AY162468) extends from the transcription start site +1 to -1116bp. This promoter is located at Chr11: 117,311,460-117,322,629.

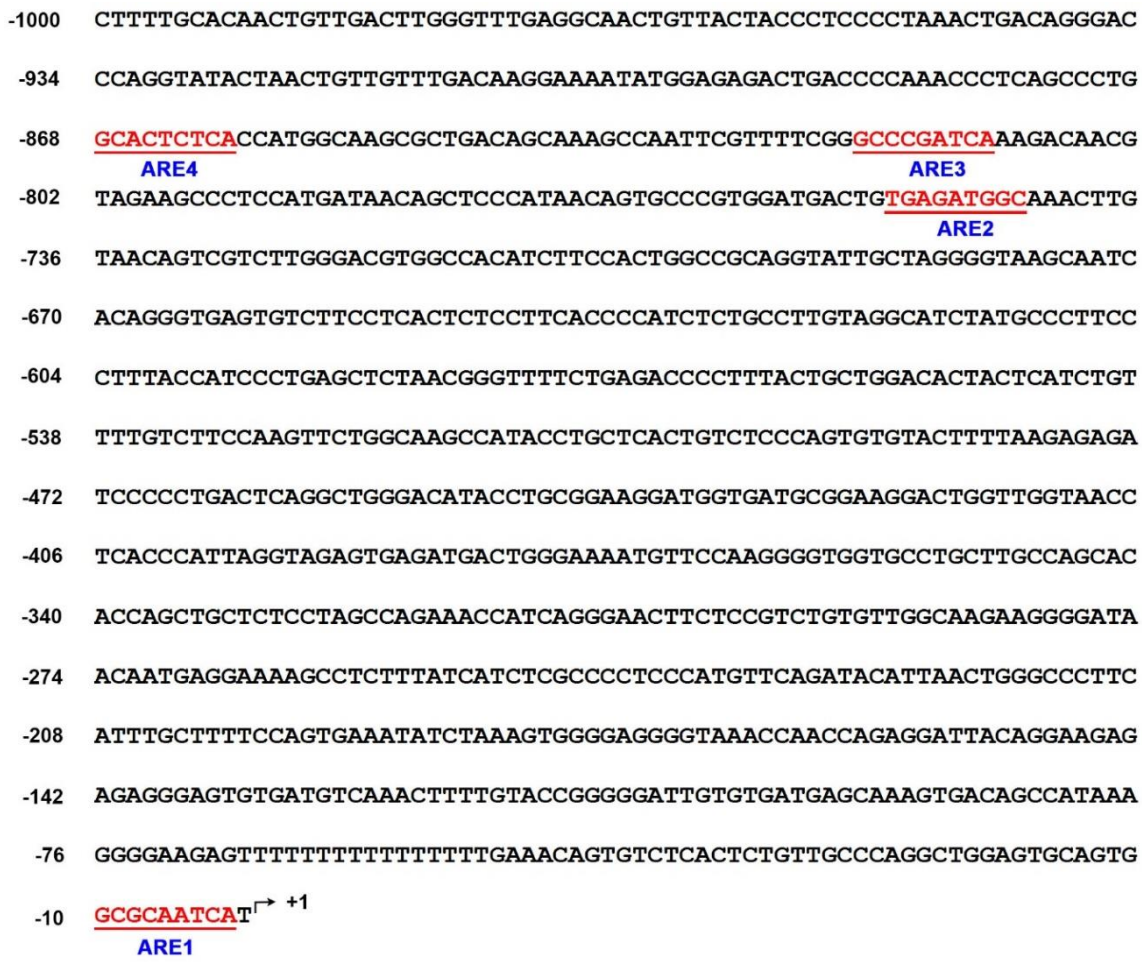


Fig. S4. Sequence of the human *BACE1-AS* gene promoter.

Four AREs are present in the human *BACE1-AS* gene promoter. The sequence shown here is located in chr11:117,290,346-117,291,345.

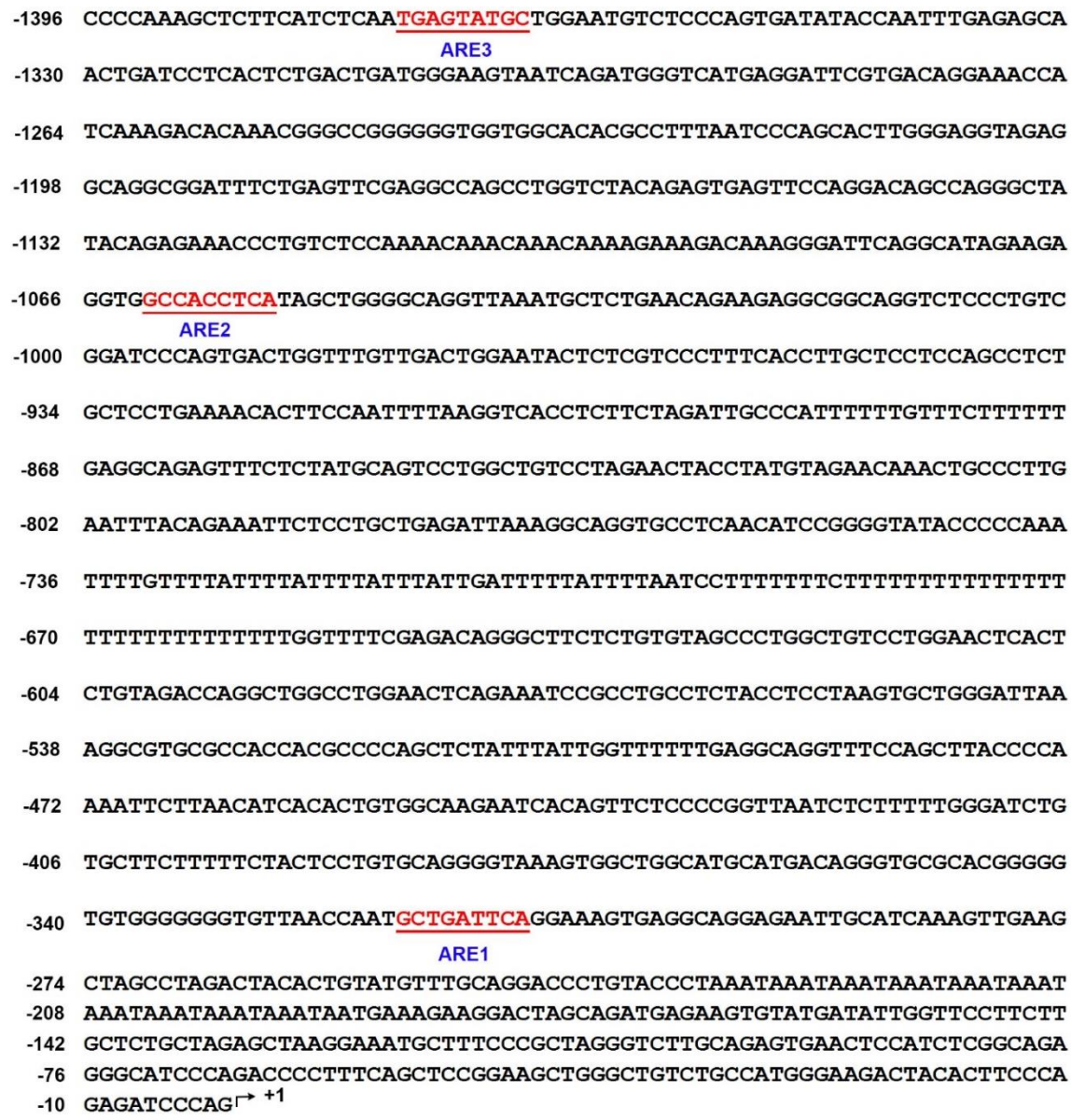


Fig. S5. Sequence of the mouse *Bace1* gene promoter.

Three AREs are present in the mouse *Bace1* gene promoter. The sequence shown here is located in chr9:45,837,150-45,838,544.

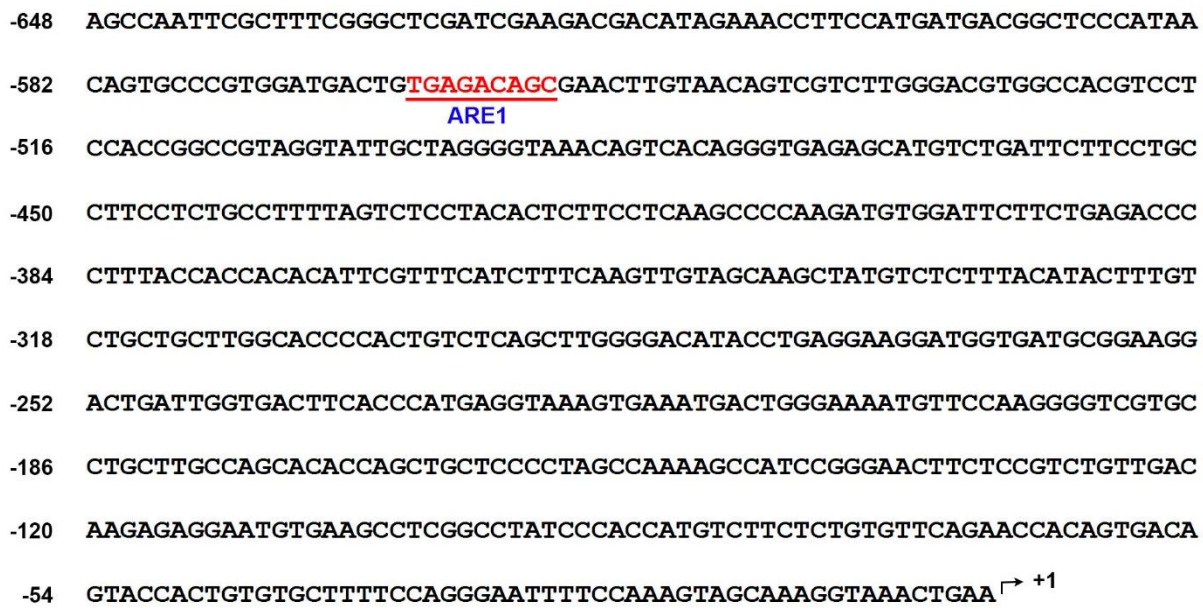


Fig. S6. Sequence of the mouse *Bace1-AS* gene promoter.

One ARE is present in the mouse *Bace1-AS* gene promoter. The sequence shown here is located in chr9:45,858,782-45,859,429.

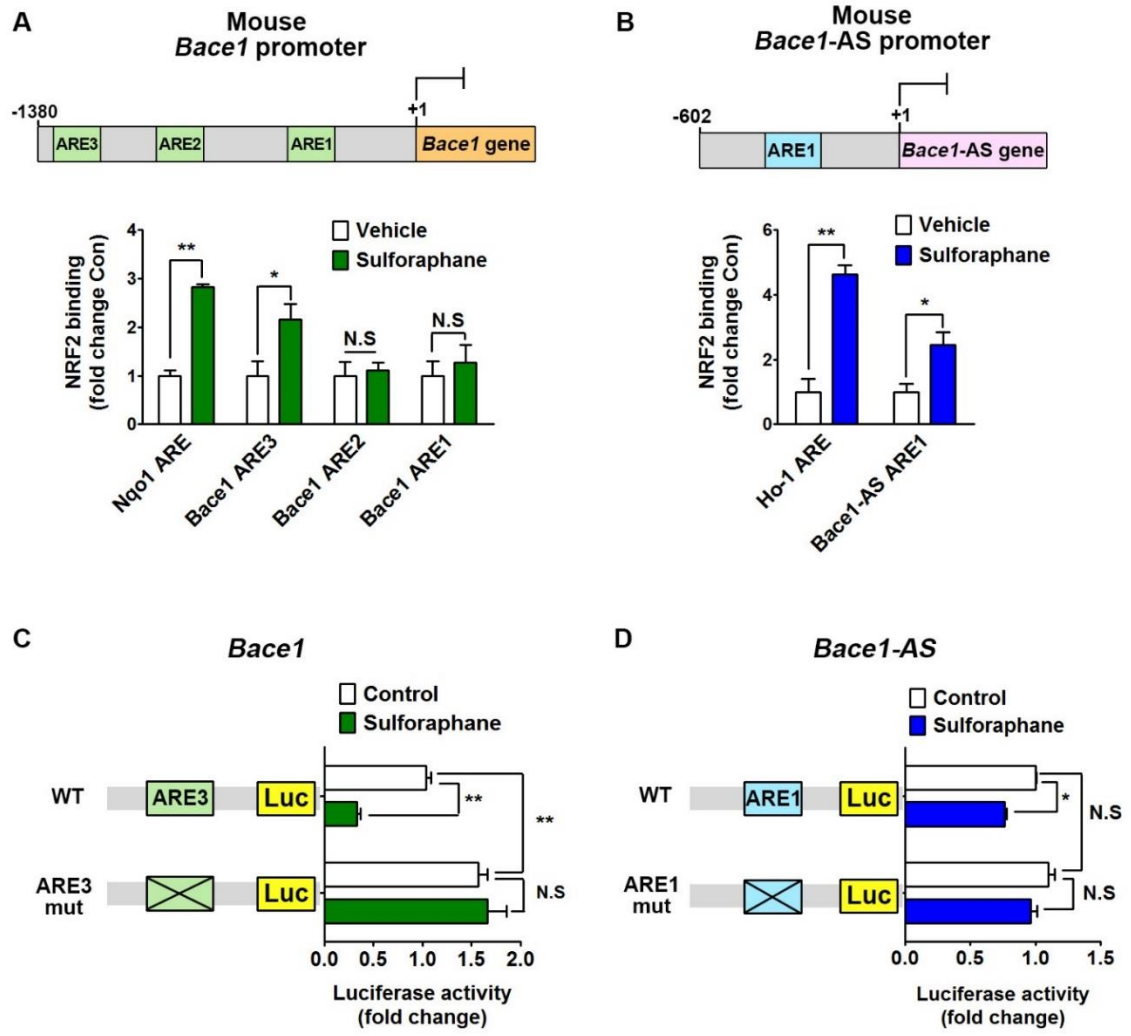


Fig. S7. NRF2 binds to AREs of mouse *Bace1* and *Bace1-AS* genes.

(A and B) NRF2 binding affinities in mouse *Bace1* gene promoter regions in brains of 12-month-old C57BL/6J that had been treated with sulforaphane (10 mg/kg) or vehicle every other day for one month. Fragmented chromatin was immunoprecipitated with NRF2 antibody and quantified with real-time RT-PCR. NRF2 binding affinity was increased in the ARE of mouse *Nqo1* promoter and the ARE3 of mouse *Bace1* promoter by 2- to 3-fold, whereas NRF2 did not bind to ARE1 and ARE2 in the mouse *Bace1* promoter (A). NRF2 ChIP assay in mouse *Bace1-AS* promoter was performed with same samples used in panel A. NRF2 binding affinity was increased in the ARE of mouse *Ho-1* promoter and mouse *Bace1-AS* promoter in response to sulforaphane treatment (B).

(C and D) HT22 cells were transfected with firefly luciferase plasmids carrying the WT or mutant ARE3 of mouse *Bace1* promoter and the WT or mutant ARE1 of *Bace1-AS* promoter. Cells were then treated with sulforaphane (500 nM) or vehicle for 24 hr. Sulforaphane treatment significantly decreased both WT *Bace1* promoter activity,

but had no effect on the mutant ARE3 of mouse *Bace1* promoter (C) and the mutant ARE1 mouse *Bace1-AS* promoter (D).

Values are the mean ± SEM. *$p < 0.05$ and **$p < 0.01$; one-way ANOVA with Tukey's**.** (N.S, not significant).

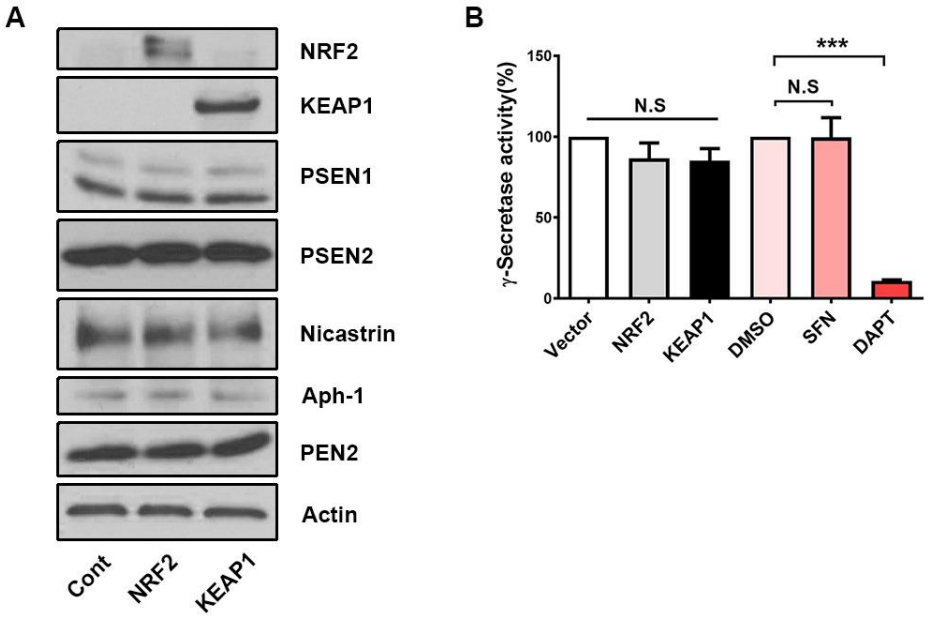
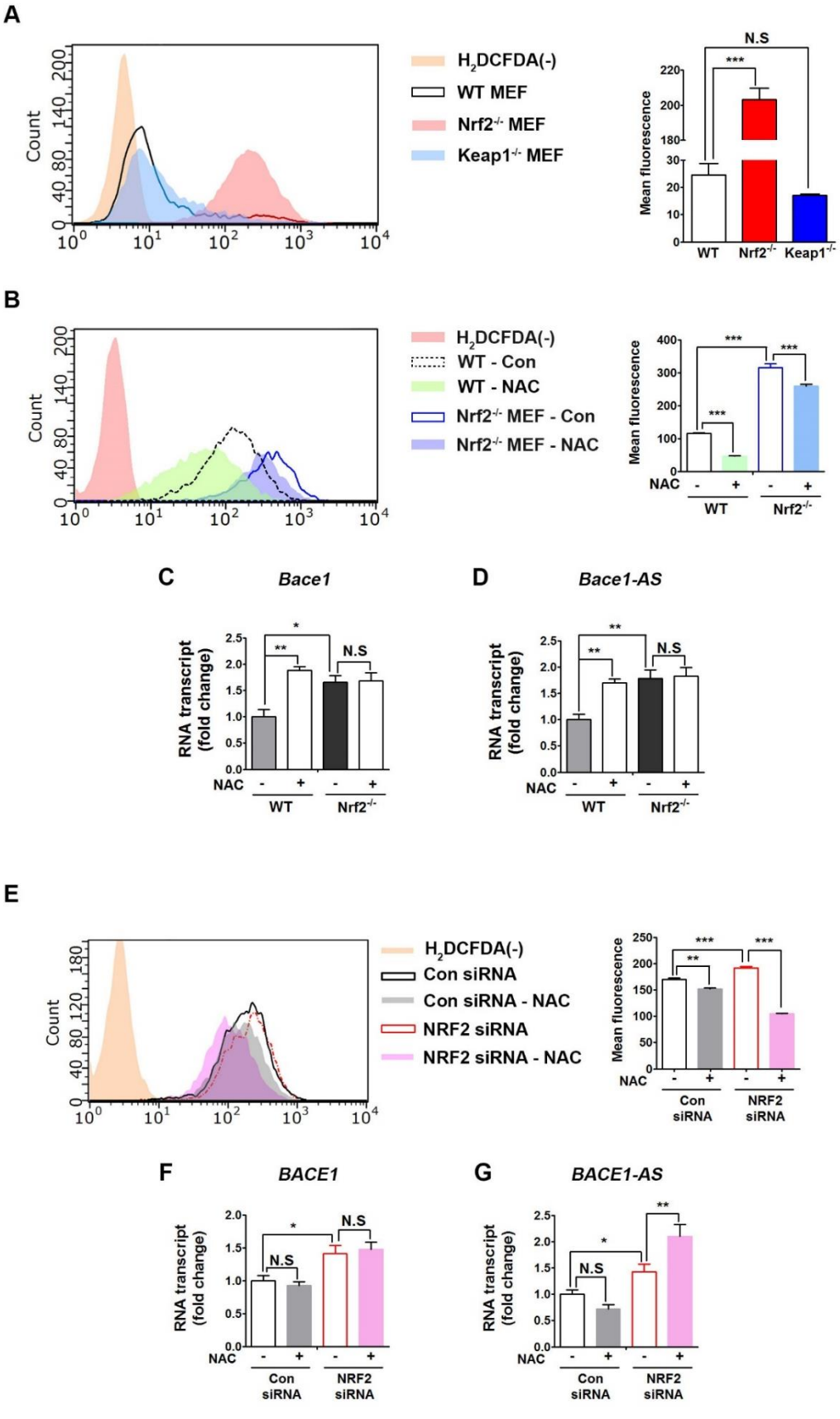


Fig. S8. NRF2 do not affect the γ-secretase.

(A) Protein levels of 5 different γ-secretase components (PSEN1, PSEN2, Nicastrin, Aph-1, or PEN2) were determined in SH-SY5Y cells transfected with control vector, *NRF2* or *KEAP1* cDNA clone expression plasmid. (B) Measurement of γ-secretase activity using luciferase-based assay. γ-secretase activity is not changed in HEK293T cells overexpressing NRF2 or KEAP1 compared to control vector. Treatment with sulforaphane (SFN, 5μM) for 24h did not affect the γ-secretase activity, whereas DAPT treatment (5μM) for 24h significantly reduced the γ-secretase activity. All values are the mean ± SEM; $^{***}p<0.001$, by one-way ANOVA with Tukey's. n=3 separate experiments.


A
Count
H2DCFDA(-)
WT MEF
Nrf2-/- MEF
Keap1-/- MEF
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WT
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H2DCFDA(-)
WT - Con
WT - NAC
Nrf2-/- MEF - Con
Nrf2-/- MEF - NAC
NAC
C
Bace1
RNA transcript (fold change)
D
Bace1-AS
E
Con siRNA
Con siRNA - NAC
NRF2 siRNA
NRF2 siRNA - NAC
F
BACE1
G
BACE1-AS

Fig. S9. NRF2-mediated inhibition of *BACE1* and *BACE1-AS* expression is independent of ROS.

(A) Evaluation of the ROS levels in WT, *Nrf2*$^{-/-}$, and *Keap1*$^{-/-}$ MEF cells. The ROS level were quantified using the ROS-sensitive fluorescent probe H_2DCFDA.

(B) Evaluation of the ROS levels in NAC-treated WT and *Nrf2*$^{-/-}$ MEF cells. WT and Nrf2$^{-/-}$ MEF cells were treated with 1 mM NAC for 4 hr. NAC treatment significantly decreased the ROS level in both cells.

(C and D) Relative RNA transcript levels of *Bace1* (C) and *Bace1-AS* (D) were examined by real time RT-PCR after the same treatments described for panel B. The NAC treatment significantly increased the transcript levels of *Bace1* and *Bace1-AS* in WT MEF cells, but these effects were lost in *Nrf2*$^{-/-}$ MEF cells.

(E) Evaluation of the ROS level in NAC-treated SH-SY5Y cells after transfection with control siRNA or NRF2 siRNA. NAC treatment significantly reduced ROS levels.

(F and G) Transcript levels of *BACE1* (F) and *BACE1-AS* (G) were examined after the same treatments described for panel (E).

Values are the means ± SEM**.** *$p < 0.05$, **$p < 0.01$, and ***$p < 0.001$; one-way ANOVA with Tukey's**.** (N.S, not significant).

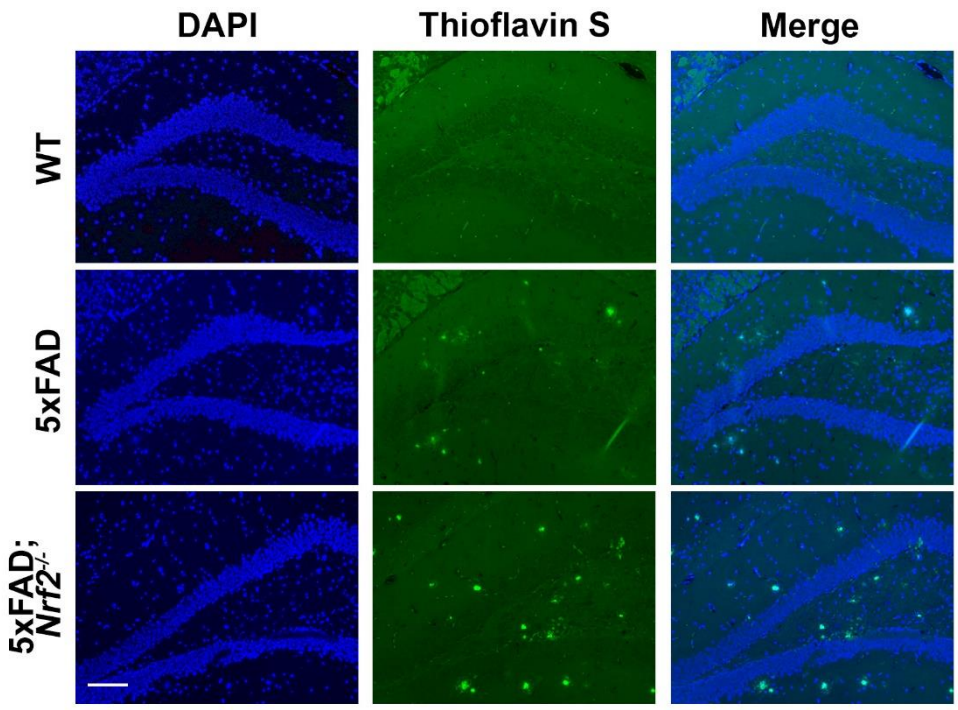


Fig. S10. *Nrf2* deficiency increases Aβ plaque loads in 5xFAD mice.

Staining of Aβ plaques with thioflavin S in the hippocampus of WT, 5xFAD, and 5xFAD;*Nrf2*$^{-/-}$ mice. (Scale bar, 100 μm).

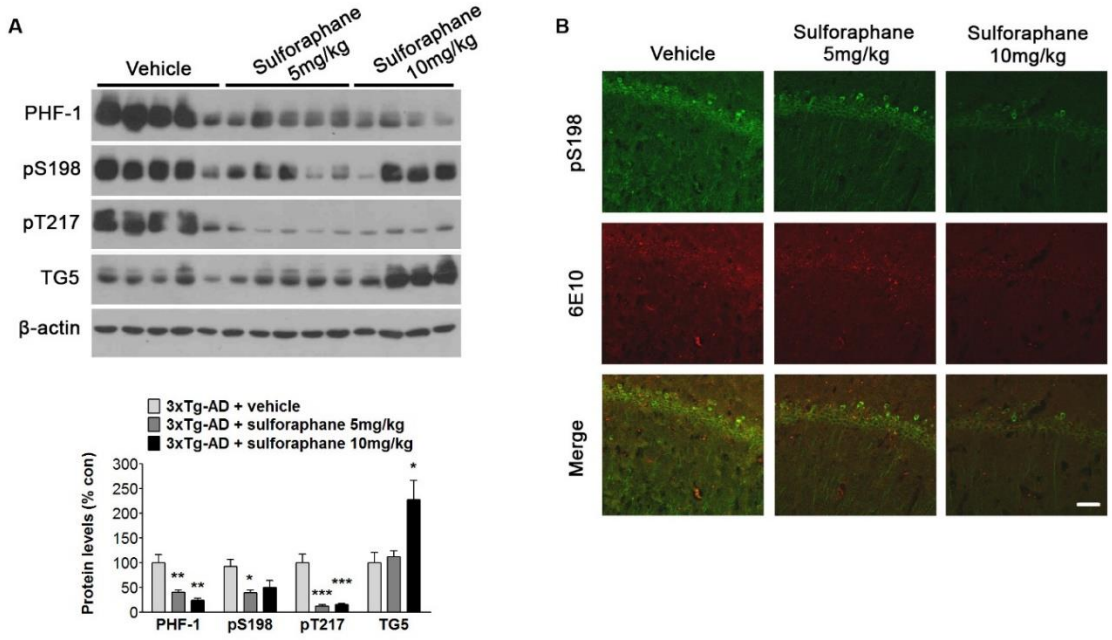


Fig. S11. Administration of sulforaphane reduces Tau pathology in 3xTg-AD mice.

Seven-month-old 3xTg-AD mice were treated with vehicle or sulforaphane (5 or 10 mg/kg) every other day for 2 months.

(A) Proteins in cerebral cortical tissue homogenates were immunoblotted using antibodies against pTau (phospho Ser396/Ser404, PHF-1; phospho Ser198, pS198; phospho Thr217, pT217) or total Tau (TG5). Phosphorylated Tau levels were reduced in sulforaphane-injected 3xTg-AD mice. (upper, representative; lower, summary graph).

(B) Images showing immunoreactivity with pTau (pS198) and Aβ (6E10) antibodies in region CA1 of the hippocampus of 3xTg-AD mice that had been treated for 2 months with vehicle, 5 mg/kg sulforaphane or 10 mg/kg sulforaphane. (Scale bar, 100 μm).

Values are the mean ± SEM**.** *$p < 0.05$, **$p < 0.01$, and ***$p < 0.001$; one-way ANOVA with Tukey's.

SI Tables

Table S1. Clinical data on AD patients and non-demented control subjects.

Patients	Age (years)	Gender	PMI(h)	Neuritic Plaques	Cause of death	Braak stage
Control						
1	86	Female	2.25	7.6	Unknown	2
2	91	Female	4.00	10.4	Unknown	1
3	81	Male	2.00	13.4	Pulmonary embolism	2
4	87	Male	2.40	0.2	Prostate cancer	2
5	82	Male	2.10	1.2	Cognestive heart failure, pneumonia	1
6	74	Male	4.00	0.0	Cognestive heart failure	1
7	79	Male	1.75	16.2	Bladder cancer	2
Mean±S.D.	82.86± 5.64		2.643± 0.9489	7± 6.663		
Alzheimer's disease						
1	83	Male	4.00	24.6	Aspiration pneumonia	6
2	86	Female	4.25	23.4	Bowel obstruction	6
3	78	Male	3.75	34.2	Unknown	6
4	81	Male	3.00	17.4	Unknown	6
5	86	Female	3.25	19.4	Respiratory infection	6
6	74	Male	3.00	27.2	Fall	6
7	84	Male	4.50	34.8	Unknown	6
Mean±S.D.	81.71± 4.424		3.679± 0.6075	25.86± 6.736		

The numbers of neuritic plaques per 2.35 mm^2 microscopic field were counted in Aβ antibody-stained sections of inferior parietal lobule (the value for each subject is the mean of counts of the 5 most involved fields in each section). PMI, postmortem interval.

Table S2. Site directed mutagenesis primer sequence.

		Primer 5'- 3'
Human		
BACE1 ARE1	WT	CCCCTTTCATCCTGCTCCCTCAATCTCTGCTCGTG
	Mutant	CCCCTTTCATCCTGCTCCC**AA**AATCTCTGCTCGTG
BACE1 ARE2	WT	TCCTCAGTTTGGCTGATGGTGCAGAAAATCAGAGGA
	Mutant	TCCTCAGTTTGGCT**C**ATGGTG**G**AGAAAATCAGAGGA
*BACE1*ARE3	WT	CCAGCCTGGGTGACAGAGCAAGTCTCCTTT
	Mutant	CCAGCCTGGGT**TT**CAG**TT**CAAGTCTCCTTT
BACE1 ARE4	WT	GCGAGACGCCTCCTCAAAAAAAAAAAAAAAAAGAG
	Mutant	GCGAGACGCCTCC**AA**AAAAAAAAAAAAAAAAGAG
Mouse		
Bace1 ARE1	WT	GTTAACCAATGCTGATTCAGGAAAGTGAGGCAGC
	Mutant	GTTAACCAAT**TA**TGAT**GTC**GGAAAGTGAGGCAGC
Bace1 ARE2	WT	GAAGAGGTGGCCACCTCATAGCTGGGGCAG
	Mutant	GAAGAGGTG**TA**CACC**AAA**TAGCTGGGGCAG
Bace1 ARE3	WT	CTTCATCTAATGAGTATGCTGGAATGTCTC
	Mutant	CTTCATCTAA**GTC**GTAT**TA**TGGAATGTCTC
Bace1-AS ARE1	WT	CGTGGATGACTGTGAGACAGCGAACTTGTAAC
	Mutant	CGTGGATGACTG**AA**AGACAGCGAACTTGTAAC

Square boxes indicate ARE sites in each primer, and bold sequences indicate mutated sequence in each ARE sites.

Table S3. qRT-PCR primer information.

Primer name	Sequence (Forward)	Sequence (Reverse)	References
Human			
BACE1	GGCGGGAGTGGTATTATGA	CTTGGGCAAACGAAGGTT	
BACE1-AS	TACCATCTCTTTTACCCCCATCCT	AGCTGCAGTCAAATCCATCAA	(8)
HO-1	GGGCCAGCAACAAAGTGCAAGATT	TCGCCACCAGAAAGCTGAGTGTAA	(9)
NRF2	AAACCAGTGGATCTGCCAAC	GACCGGGAATATCAGGAACA	(10)
KEAP1	ATTGGCTGTGTGGAGTTGC	CAGGTTGAAGAACTCCTCTTGC	
18S	GCAATTATTCCCCATGAACG	GGGACTTAATCAACGCAAGC	
Mouse			
Bace1	CTTTGTGGAGATGGTGGAC	GTTACTACTGCCCGTGTC	
Bace1-AS	GTAGGCAGGGAAGCTAGTACTGA	AGAGGCTTGCAGTCCAGTTC	(11)
Ho-1	CTGTGAACTCTGTCCAATG	AACTGTGTCAGGTATCTCC	(12)
Nrf2	CCATTTACGGAGACCCACCGCCTG	CTCGTGTGAGATGAGCCTCTAAGCGG	(13)
Nqo1	CATTCTGAAAGGCTGGTTTGA	TTTCTTCCATCCTTCCAGGAT	(13)
Keap1	CCCATGGAAAGGCTTATTGAGTTC	GAAGTGCATGTAGATATACTCCC	(13)
18S	GCCTCCTCCTCCTCTCTC	GCTACTGGCAGGATCAACC	

Table S4. ChIP assay primer information.

Primer name	Sequence (Forward)	Sequence (Reverse)
Human		
BACE1 ARE1	ACAGGTTCAGATGGGAGAAGACC	AGGAGTAGGGATTTTGGAGGGAC
BACE1 ARE2	ACCATACCGGCTTTTCTGCT	TGTGCCCTTGAGGCTCTACT
BACE1 ARE3	TTGCAGACTTCAGACCATGC	GCCTTGAACCAAAAGCGTTA
BACE1 ARE4	AGAGCTTGCAGTGAGCTGAGA	TACCCAGCCAGGAAACTTTTTA
BACE1-AS ARE1	GCCATAAAGGGGAAGAGTTTTT	ACTTTAACTCAGGACGGTGGAGG
BACE1-AS ARE2	AACGTAGAAGCCCTCCATGAT	TTGCCATCTCACAGTCACC
BACE1-AS ARE3	AAGCGCTGACAGCAAAGC	GTCATCCACGGGCACTGT
BACE1-AS ARE4	TGTTTGACAAGGAAAATATGGAGA	GCTTGCCATGGTGAGAGTG
HO-1 ARE	CCCTGCTGAGTAATCCTTTCCCGA	ATGTCCCGACTCCAGACTCCA
Mouse		
Bace1 ARE1	GGGGTGTTAACCAATGCTG	CAGGGTCCTGCAAACATAC
Bace1 ARE2	CCCTGTCTCCAAAACAAACAAAC	CATTTAACCTGCCCCAGCTATG
Bace1 ARE3	TAAGCTATCAGGGCCCCAAAG	GGCCCGTTTGTGTCTTTGATG
Bace1-AS ARE1	CATGATGACGGCTGGCATAAC	GGAAGGCAGGAAGAATCAGAC
Nqo1 ARE	GCAGTTTCTAAGAGCAGAACG	GTAGATTAGTCCTCACTCAGCCG

Dataset S1. Representative full immunoblots

Original immunoblot images

Figure 1G

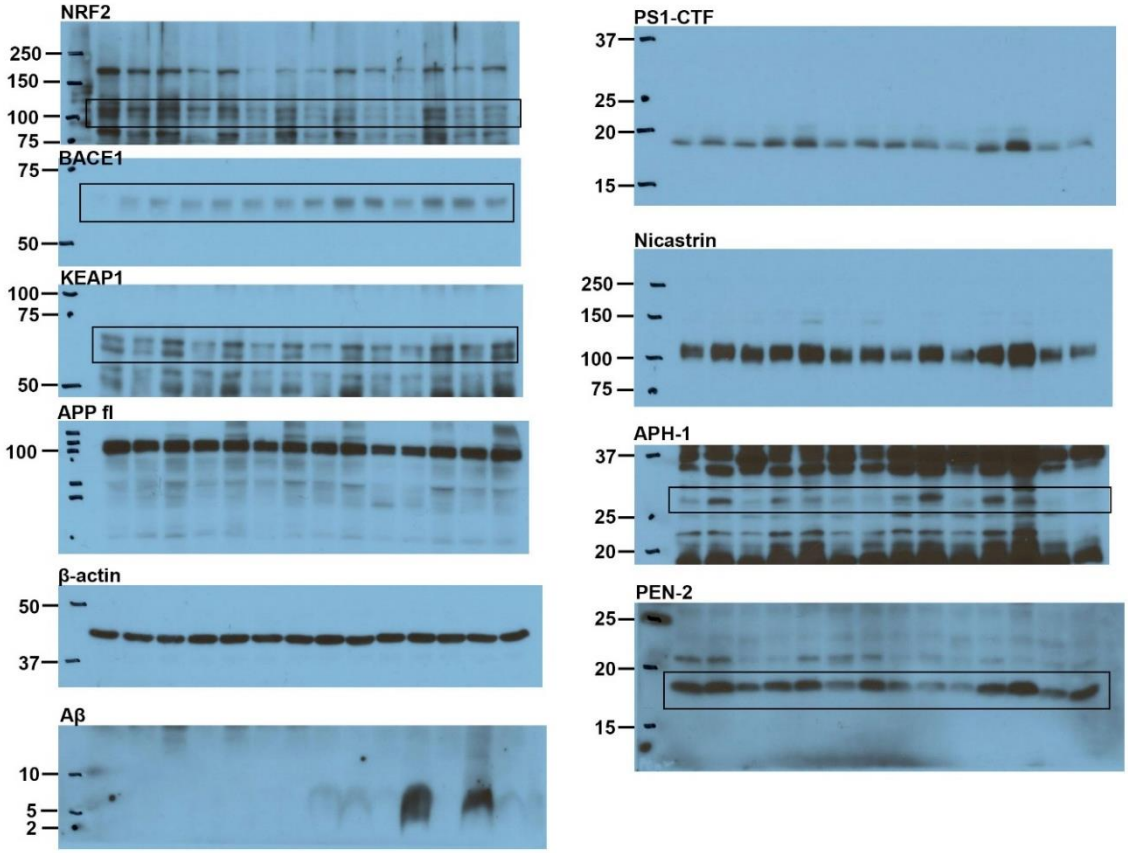


Figure 2B

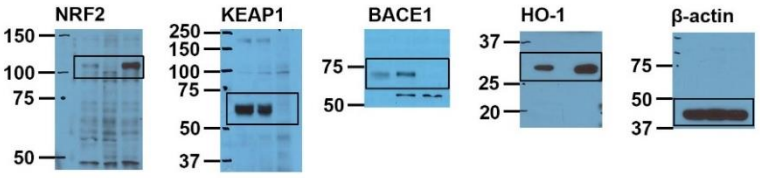


Figure 2D

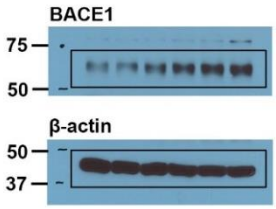
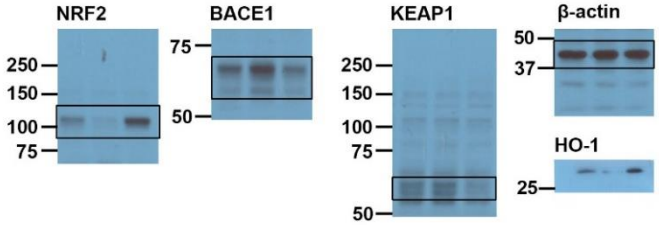


Figure 2F



Original immunoblot images (Continued)

FIgure 2H

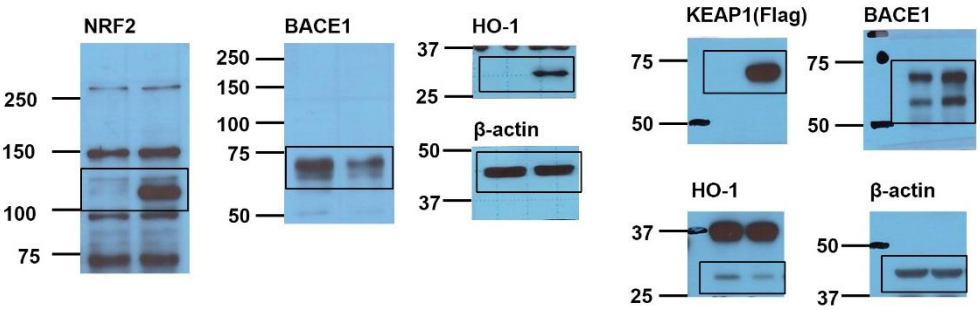


Figure 3G

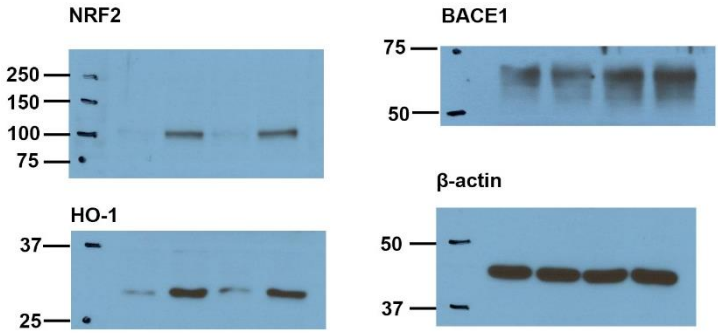


Figure 4D

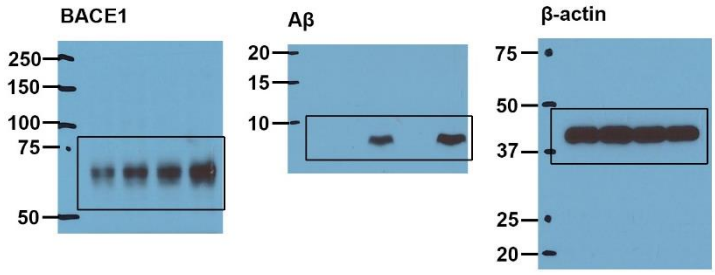
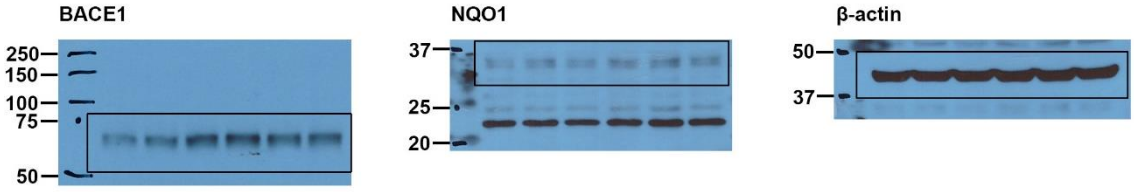
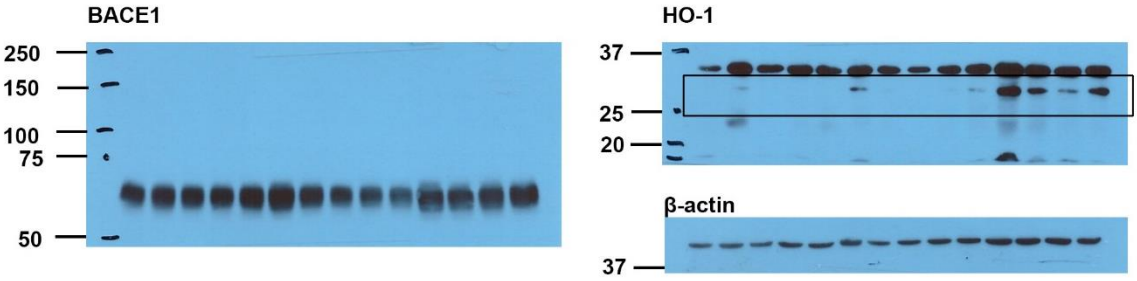


Figure 5D

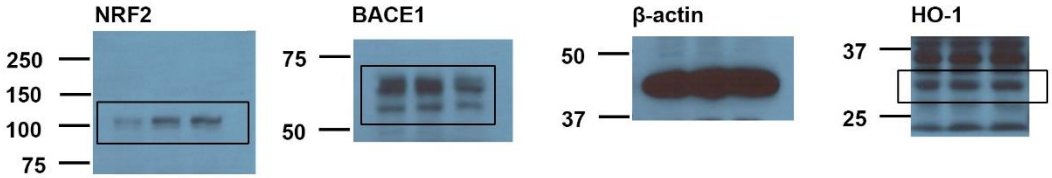


Original immunoblot images (Continued)

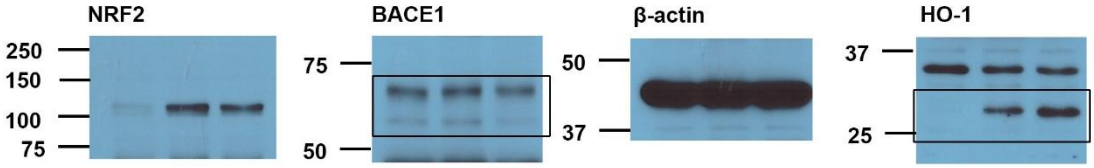
Figure 6D



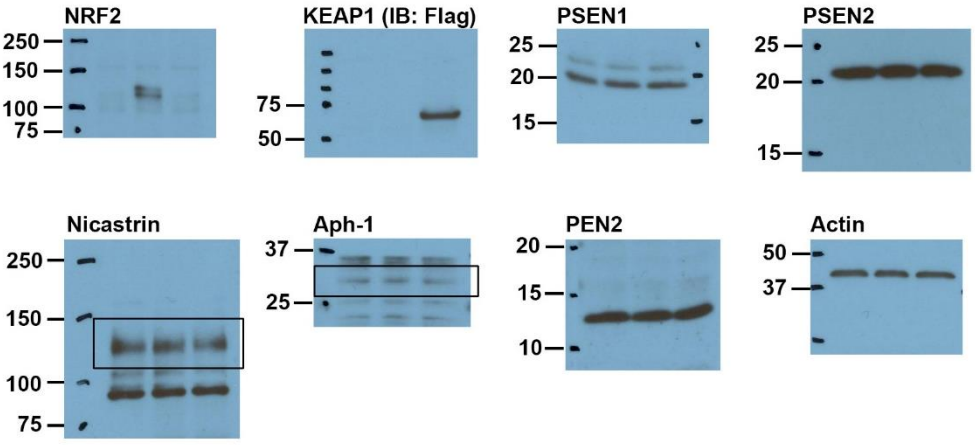
Supplementary Figure 2B



Supplementary Figure 2D

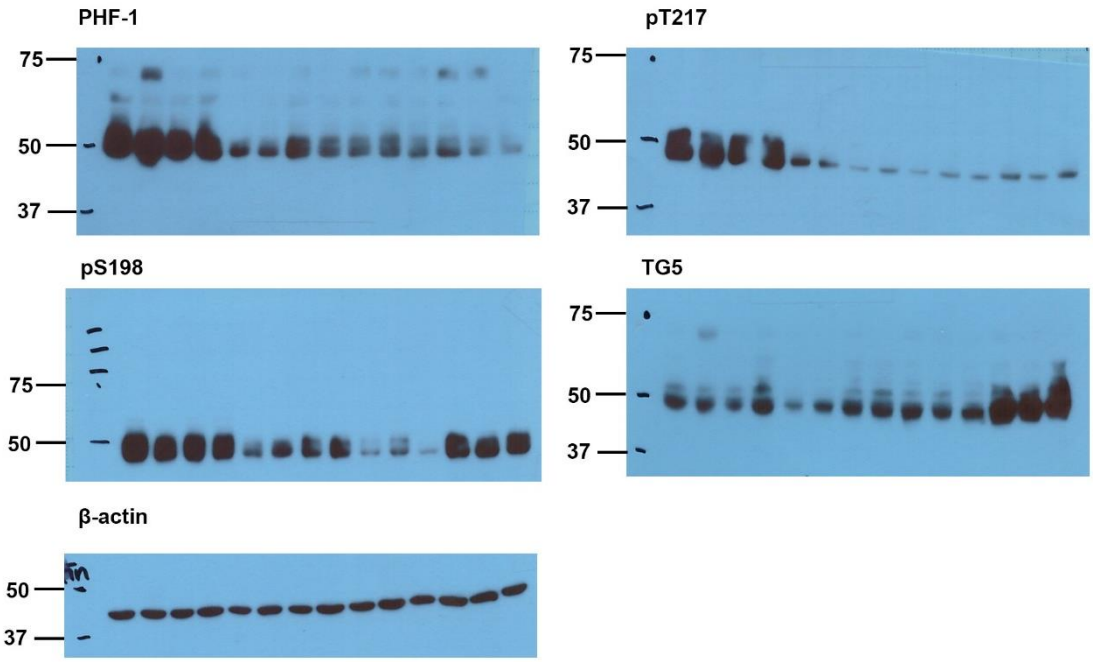


Supplementary Figure 8A



Original immunoblot images (Continued)

Supplementary Figure 11A



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