Supplemental Methods

Native Gel Electrophoresis. Aβ1-42 oligomer preparations were mixed with equal volume of 2× native loading buffer (25 mM Tris-HCl, pH 7.4, 15% glycerol, 0.25% bromophenol blue) and vortexed. Samples (13.5 μg protein/lane) were electrophoresed under non-denaturing conditions at 150 V for 50 min (4 °C) using Tris-HEPES running buffer on 4-20% gradient Tris-HEPES precast gels (Solarbio, #PG42010-N). Unstained Protein Standard (RealTimes, #RTD6144) provided molecular weight calibration.

Silver staining. Following electrophoresis, gels were fixed on a shaker for 2 h at 55 rpm in 50% methanol, 12% acetic acid, 0.05% formaldehyde. After three washes with 35% ethanol (5 min each, 55 rpm), gels were sensitized in 0.02% sodium thiosulfate (2 min, 80 rpm) and washed with ultrapure water (3 × 5 min, 55 rpm). Staining was performed in 0.2% silver nitrate/0.076% formaldehyde for 20 min at 80 rpm, followed by two ultrapure water washes (1 min each, 55 rpm). Color development in 6% sodium carbonate/0.0004% sodium thiosulfate/0.05% formaldehyde (110 rpm) was terminated with 1.46% disodium EDTA (20 min, 110 rpm) when band intensity reached optimal contrast, followed by three washes with ultrapure water.

Thioflavin T Staining. Frozen sections were PBS-washed to remove OCT compound, then soaked in 50% ethanol (5 min). Sections were stained in freshly prepared 0.05% Thioflavin T (MedChemExpress, HY-D0218; PBS, pH 7.4) for 10 min at 22°C (dark), followed by three PBS rinses. After perimeter drying, sections were mounted with DAPI-free aqueous mounting medium. Images were acquired using an Olympus BX53 microscope (excitation/emission: 450/482 nm) with cellSens software. Plaque quantification was performed using ImageJ Fiji with threshold-based particle analysis.

Optogenetics. Two-month-old mice received unilateral stereotaxic injections of AAV-hSyn-ChR2(H134R)-mCherry (BrainVTA, 1×10^{13} vg/mL) into the dentate gyrus (DG) (coordinates: AP -2.1 mm, ML ±1.5 mm, DV -1.8 mm). A fiber optic cannula (Inper, 400 μm, NA 0.37) was implanted 0.1 mm dorsal to the injection site and secured with dental cement. After ≥2 weeks recovery, mice underwent 3-day habituation (30 min/day) to behavioral apparatus and fiber tethering. During experiment, awake mice received 473-nm laser stimulation (30 s ON/OFF cycles, constant, 1 mW) for 1 h via fiber-coupled laser (Inper). Mice were transcardially perfused at designated timepoints post-stimulation.

Supplemental Figures

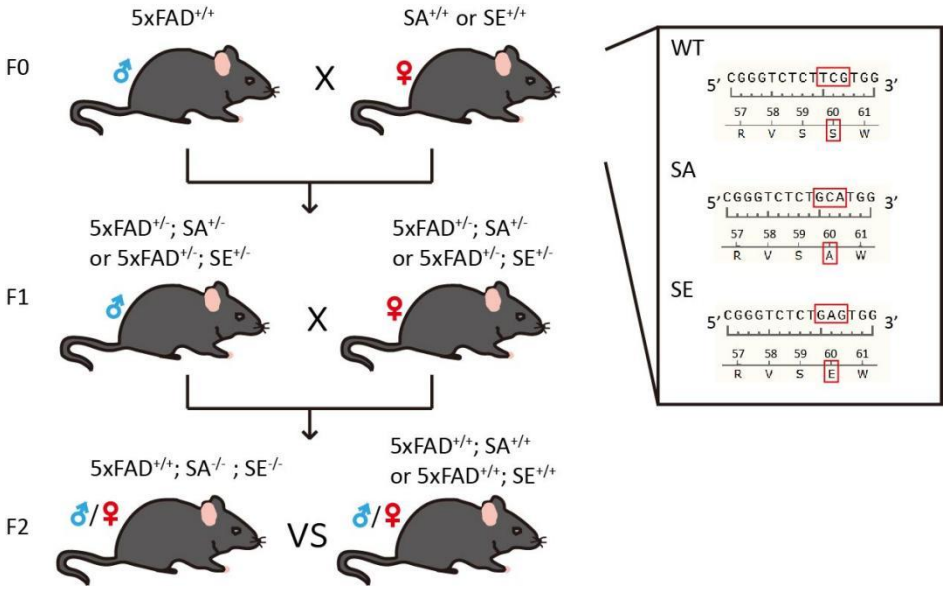

Fig. S1. Breeding strategy of 5xFAD/SA or 5xFAD/SE mice.

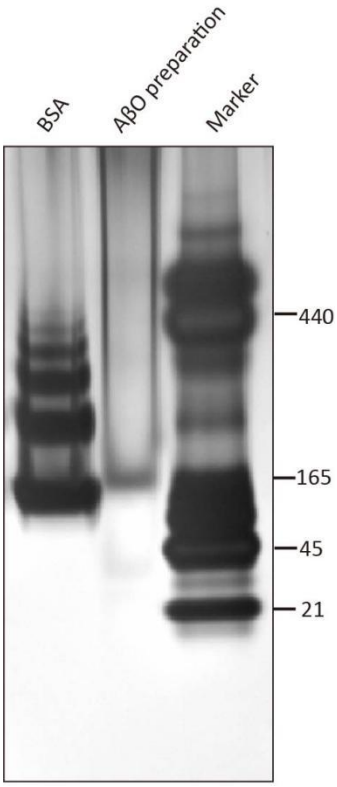

Fig. S2. Characterization of Aβ Oligomer Preparation.

Oligomeric composition (>96% of species >21 kDa) was confirmed by native PAGE using 4-20% gradient Tris-HEPES precast gels (Solarbio, #PG42010-N) with silver staining. Native protein markers (RealTimes, #RTD6144, kDa indicated) were included for molecular weight calibration. Bovine serum albumin (BSA) served as migration control. Densitometric analysis was used to quantify oligomer distribution profiles by ImageJ Fiji.

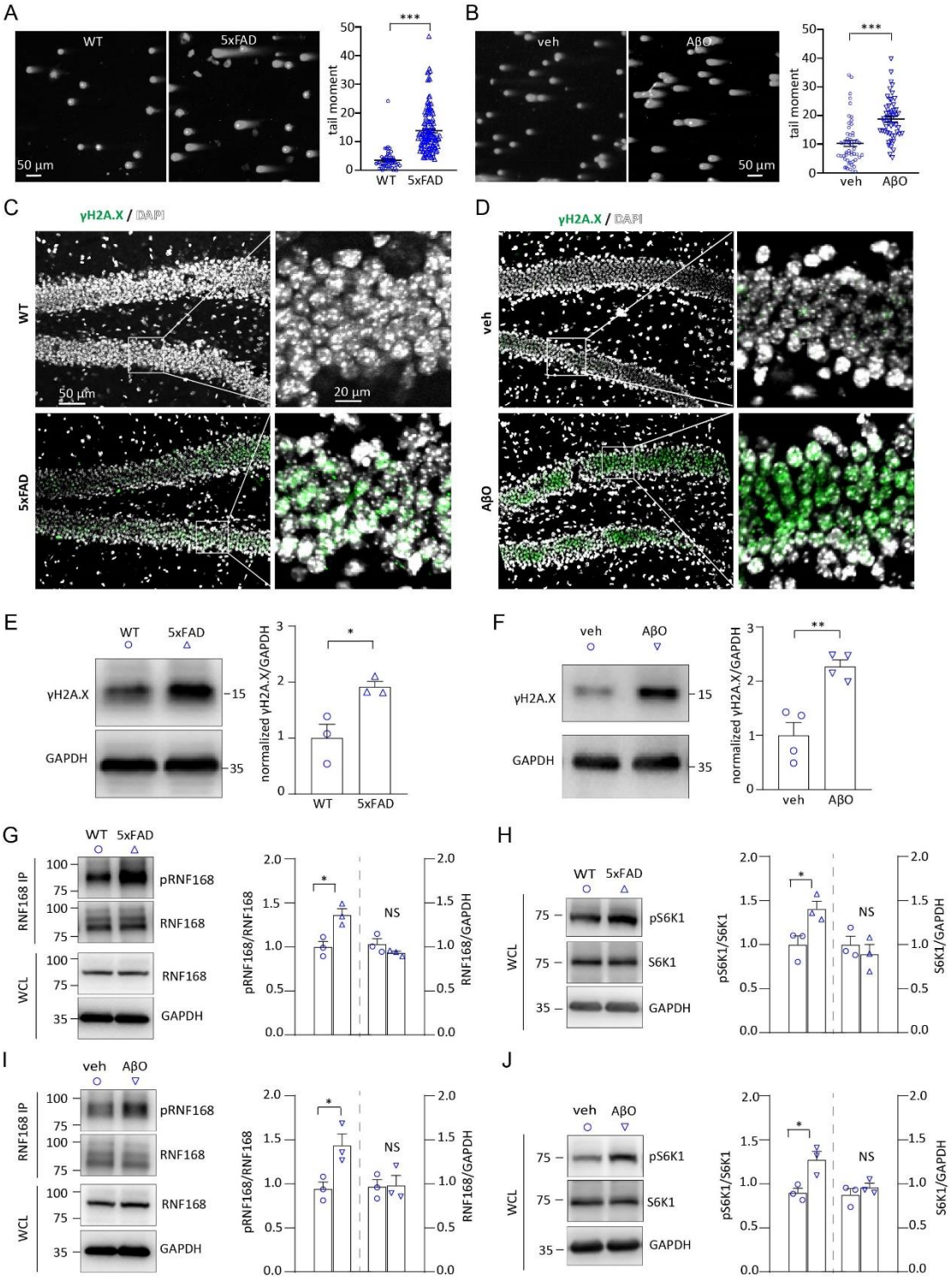

Fig. S3. Increased DSBs and impaired DNA damage response signaling in the hippocampi of Aβ-based mouse models of AD.

(A, B) Neutral comet assay showing DNA double-strand breaks (DSBs) in dissociated hippocampal dentate gyrus (DG) cells from two AD mouse models. (A) 5xFAD model: 4-5 month-old 5xFAD and age-matched wild-type (WT) mice. (B) AβO model: 2-3 month-old AβO-injected and vehicle-injected mice. Right panels show quantification of tail moment. Sample sizes: WT, n = 47; 5xFAD, n = 123; vehicle, n = 53; AβO, n =

49. Statistical analysis: Unpaired Student's t-test. Scale bars: 50 µm.

(C, D) Representative immunofluorescence images of γH2A.X staining in the hippocampal DG of two AD mouse models. (C) 5xFAD model: 4-5 month-old 5xFAD and WT mice. (D) AβO model: 2-3 month-old AβO-injected and vehicle-injected mice. Scale bars are provided in images.

(E, F) Immunoblot analysis of γH2A.X levels in the hippocampi. (E) 5xFAD model: 4-5 month-old 5xFAD and WT mice (n = 3). (F) AβO model: 2-3 month-old AβO-injected and vehicle-injected mice (n = 4). Left panels: Representative immunoblots. Right panels: Quantification. Protein ladders are labeled in kDa. Statistical analysis: Unpaired Student's t-test.

(G-J) Immunoblot analysis of hippocampal protein levels. (G, H) 5xFAD model: pRNF168, total RNF168, pS6K1, and total S6K1. (I, J) AβO model: pRNF168, total RNF168, pS6K1, and total S6K1. pRNF168 was detected using a specific anti-pRNF168 antibody after immunoprecipitation with an anti-RNF168 antibody. Left panels: Representative immunoblots. Right panels: Quantification. Sample sizes: n = 3. Statistical analysis: Unpaired Student's t-test. Data presentation: Mean ± SEM. *$p <$ 0.05, **$p < 0.01$, ***$p < 0.01$; NS, not significant.

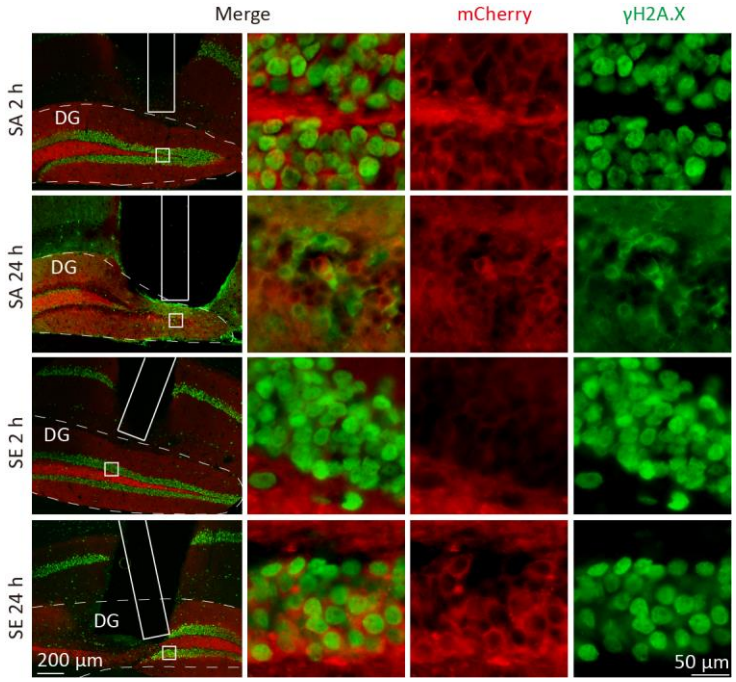

Fig. S4. Phospho-mimetic RNF168-SE mutant impairs DNA damage response (DDR) in activity-induced DSBs in the hippocampal DG region. SE (RNF168-SE phosphomimetic) or SA (RNF168-SA phospho-deficient) mice received unilateral stereotaxic intra-DG injections of ChR2-mCherry-expressing AAV. Neuronal activation and DSB induction were achieved by blue light stimulation (1 h, 30 s on/30 s off cycles, 1 mW) via optical fibers. Mice were perfused at indicated time points post-stimulation.

Representative immunofluorescence images of γH2A.X (marker of DSBs) and mCherry (ChR2 expression) in the hippocampal DG are shown. Left: optic fiber position (white outline). Right: magnified images show the DSB marker and ChR2 expression in DG neurons. Scale bars included.

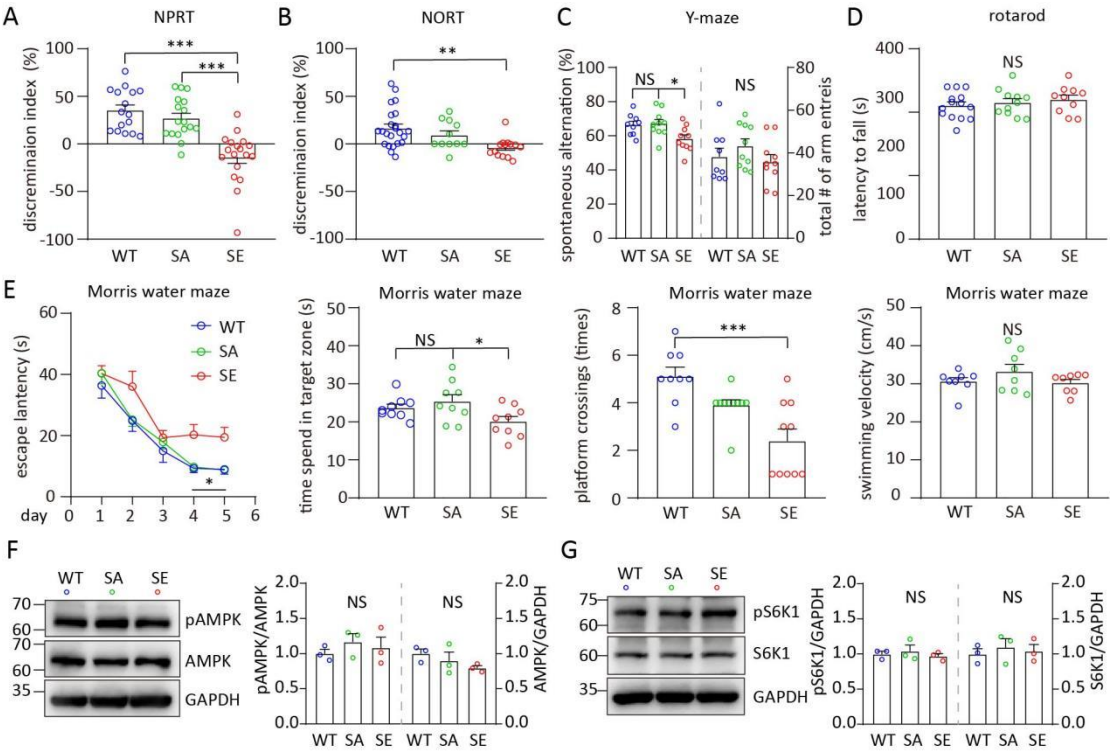

Fig. S5. Phospho-mimetic RNF168-SE mutant impairs learning and memory.

A battery of behavioral and motor function tests was performed in 8-10-week-old wild-type (WT), RNF168-SE (phosphomimetic), and RNF168-SA (phospho-deficient) mice.

Tests performed: (A) Novel position recognition, (B) Novel object recognition (C) Y-maze spontaneous alternation, (D) Rotarod motor coordination, and (E) Morris water maze: Left panel, Escape latency during training (repeated two-way ANOVA with Tukey's post-hoc test). Right panels, Probe trial metrics (time in target zone, platform crossings, swimming velocity; one-way ANOVA with Tukey's post-hoc test). (F) Representative immunoblots and quantification of pAMPK (Thr172) levels normalized to total AMPK in wild-type (WT), RNF168-S60A (SA), and RNF168-S60E (SE) mice. (G) Phospho-S6K1 (Thr389) immunoblots normalized to total S6K1. Bar charts show pAMPK/AMPK and pS6K1/S6K1 ratios. GAPDH served as loading control for all blots. Statistical analysis: Data are presented as mean ± SEM. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$; NS, not significant.

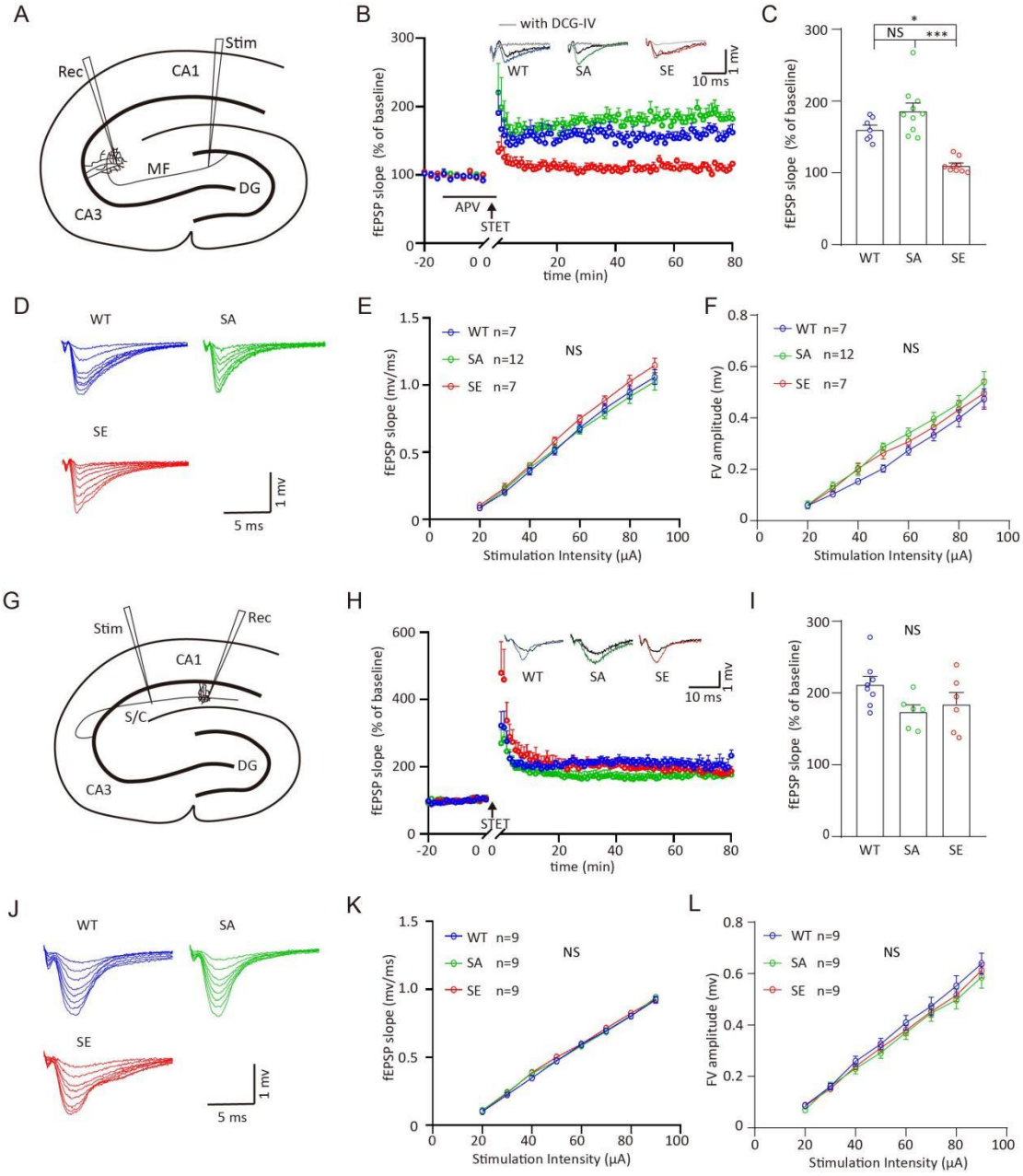

Fig. S6. The phospho-mimetic RNF168-SE mutant impairs the long-term potentiation (LTP) of mossy fiber-CA3 pyramidal neuron synapses.

A, G) Schematic drawings of the hippocampal slices showing the positions of stimulation and recording electrodes for fEPSP recordings. **B)** Mossy fiber fEPSP recordings in the CA3 stratum lucidum were performed while stimulating mossy fibers. LTP was induced using four trains of high-frequency stimulation (HFS). fEPSPs were recorded in the presence of D-APV (25 μM) for 30 min before and during HFS delivery to exclude LTP contributions from associational-commissural synapses. At the end of the recording, the mGluR2 agonist DCG-IV (1 μM) was perfused to confirm that fEPSPs were evoked by mossy fiber synapses. Representative EPSP traces are shown in insets: black (before HFS), colored (after HFS), and gray (with DCG-IV). Scale bars are indicated in the chart. **C)** Statistical analysis of the fEPSP slope during the last 10

minutes of B, using the Kruskal-Wallis test with Dunn's post-hoc multiple comparisons. **D, E, F)** Input-output (I/O) curves for basal transmission at mossy fiber-CA3 synapses were analyzed using repeated one-way ANOVA. **H)** Schaffer collateral fEPSP recordings in the CA1 stratum lucidum were performed while stimulating Schaffer collaterals. LTP was induced using four trains of high-frequency stimulation. Representative fEPSP traces are shown in insets: black (before HFS), colored (after HFS). Scale bars are indicated in the chart. **I)** Statistical analysis of the fEPSP slope during the last 10 min of H, using one-way ANOVA. **J, K, L)** Input-output (I/O) curves for basal transmission at Schaffer collateral-CA1 synapses were analyzed using repeated one-way ANOVA. $*p < 0.05$, $**p < 0.01$, $***p < 0.001$; NS, not significant.

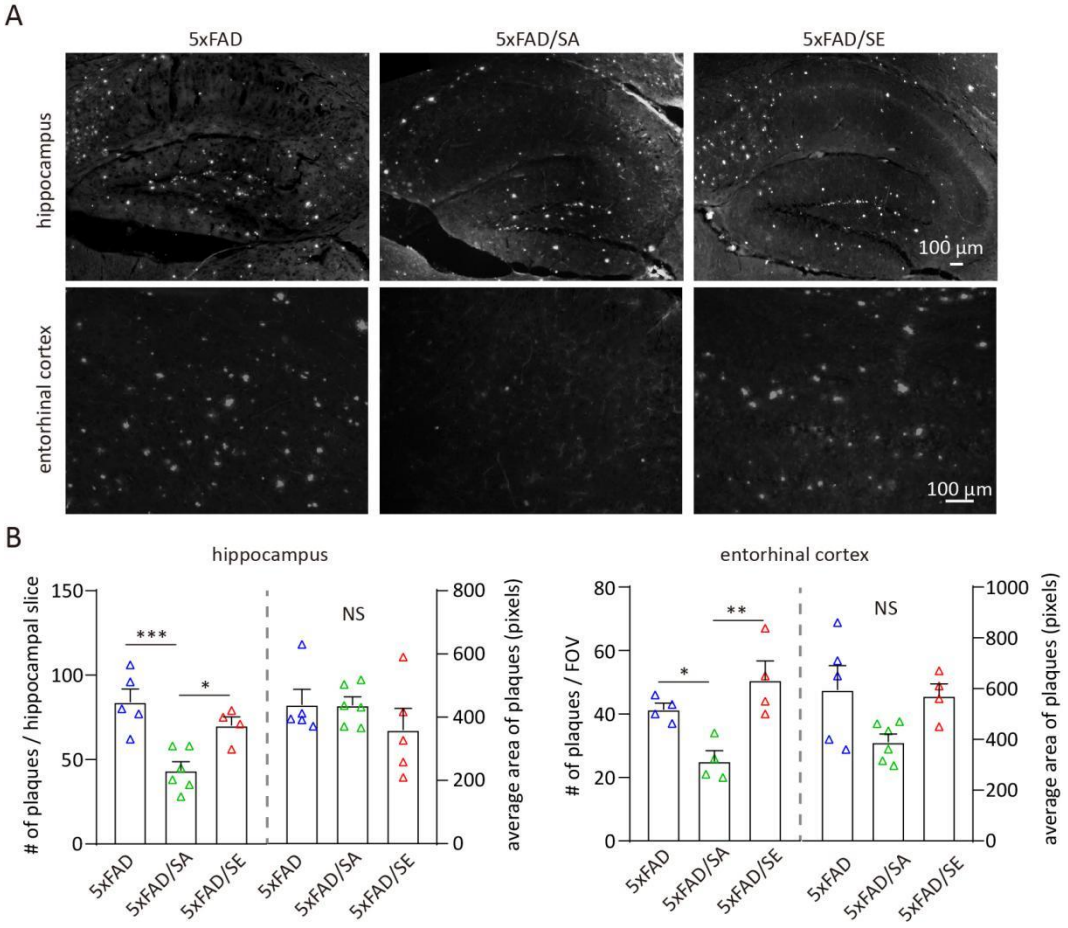

Fig. S7. RNF168 S60A mutant alleviates Aβ plaque pathology in 5xFAD mice. (A) Representative fluorescent images of Thioflavin T-labeled amyloid plague in sagittal sections of the hippocampus and the entorhinal cortex of 4-5-month-old 5xFAD, 5xFAD/SA and 5xFAD/SE mice under a 10× objective. (B) Statistical bar-chart showing levels of number and average area of Aβ plaques (5xFAD: n = 4-5; 5xFAD/SA: n = 4-6; 5xFAD/SE: n = 4).

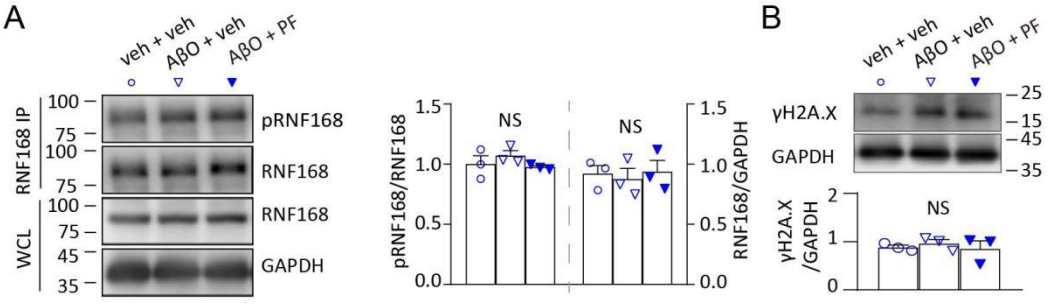

Fig. S8. Hippocampal DG injection of AβO does not induce DSBs in other hippocampal areas outside the DG. (A) Representative immunoblot images and

statistical bar-chart showing levels of phosphorylated RNF168 (pRNF168) and total RNF168. (B) Levels of γH2A.X, a marker of DSBs, and statistical analysis in areas outside the DG in vehicle + vehicle, AβO + vehicle, and AβO + PF mice. Data was analyzed using one-way ANOVA. NS indicates not significant.

Supplementary table 1. Antibodies used in this study.

Antibodies	Catalogue Number	Company	Dillution	Validation
anti-pRNF168	AP1219	ABclonal Technology	1/1000	Datasheet from manufacturer
anti-RNF168	A3556	ABclonal Technology	1/1000	Datasheet from manufacturer
anti-pS6K1	9205	Cell Signaling Technology	1/1000	Datasheet from manufacturer
anti-S6K1	SC-8418	Santa Cruz	1/1000	Datasheet from manufacturer
anti-pAMPK	2535	Cell Signaling Technology	1/3000	Datasheet from manufacturer
anti-AMPK	5831	Cell Signaling Technology	1/3000	Datasheet from manufacturer
anti-γH2A. X	9718S	Cell Signaling Technology	1/1000 (WB) 1/200 (IF)	Datasheet from manufacturer
anti-H2A	ab18255	Abcam	1/1000	Datasheet from manufacturer
anti-53BP1	ab172580	abcam	1/200	Datasheet from manufacturer
anti-Ub (FK2)	04-263	Millipore	1/1000	Datasheet from manufacturer
anti-GAPDH	ET1601-4	HuaAn	1/10000	Datasheet from manufacturer

HRP-conjugated Affinipure rabbit anti-Rabbit IgG (H+L)	SA00001-2	Proteintech	1/10000	Datasheet from manufacturer
Goat Anti-Mouse IgG H&L/HRP	bs-40296G-HRP	Bioss	1/10000	Datasheet from manufacturer
Anti-DDDDK(FLAG)-tag	M185-3L	MBL	1/10000	Datasheet from manufacturer
Anti-DYKDDDDK tag, Mouse IgG2b antibody(HRP)	016-303-005	AlpVHHs	1/10000	Datasheet from manufacturer

Supplementary table 2. Drugs used in this study.

Designation	Source of reference	Identifiers	Additional information
Beta-Amyloid 1-42	Genicbio	Cat. #: HA-42-T-1	0.1 mM final concentration
D-AP5	MedChemExpress	Cat. #: HY-100714A	50 μM final concentration
DCG-IV	MedChemExpress	Cat. #: HY-101335	1 μM final concentration
Metformin	GLPBIO	Cat. NO.: GC60245	100 mg/kg final concentration in mouse
PF-4708671	MedChemExpress	Cat. #: HY-15773	10 mg/mL final concentration
thioflavin-T	MedChemExpress	Cat. #: 2390-54-7	0.5% final concentration