Supporting Information

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SI Materials and Methods

Rotarod Test. For the rotarod test, mice were placed onto the ENV-577M Rotarod system (Med Associates) at a walking speed of 4 rpm. Then, the speed was increased to 40 rpm in 300 s. Latency for the animal to fall off the rotarod was recorded (1).

Grip Strength Test. Each individual's forelimbs were tested for grip strength by pulling on a wire attached to a Chatillon DFE-002 force gauge (Chatillon Force Measurement Systems). Five pulls were performed for each mouse. The mean of the recordings was determined.

Open-Field Test. Animals were placed in the center of a defined open-field region (43 × 43 cm) (Med Associates) and left without disruption for 30 min. The center zone was defined as a 10.2-cm^2 area equidistant from the peripheral walls. The tracking software (Activity Monitor version 4; Med Associates) recorded the exploratory behavior. The apparatus was cleaned with 70% ethanol before testing the next mouse.

Elevated Plus Maze Test. The test was conducted essentially as reported (2). A mouse with a high level of anxiety will spend more time in the closed arms rather than venture into the open arms. The apparatus consists of two closed arms (30 × 5 × 15 cm) with high walls and two open arms (30 × 5 × 2.5 cm) with low walls. The mice were placed individually in the center of the maze for a period of 5 min. A digital camera tracked the movements of the mouse with the video analyzed by ANY-maze software (Stoelting).

MotoRater Test. To record gait, we used the MotoRater (TSE Systems), a quantitative video system designed for kinematically evaluating rodent movement. Mice were filmed on a transparent runway with a ladder at the end, and a mirror system recorded gait from the bottom, left, and right sides of the runway. Mice were placed at the start of the runway behind a Plexiglas door. The door was removed to signal the beginning of the trial. Mice moved freely to the end of the runway where they climbed a ladder to reenter their cage. If necessary, mice were assisted in climbing the ladder. Each mouse was allowed three practice trials 24–48 h before testing. Trials were successful if the mouse walked three consecutive steps in the forward direction without pausing. If the mouse paused, it was lightly prodded until it began moving again. Mice unable to complete one successful trial after three attempts were excluded from the study. On test day, each mouse completed two successful trials. Mice unable to complete one successful trial after four attempts were excluded from the study. Between mice, the setup was cleaned with 70% ethanol. SIMI Motion software (SIMI Reality Motion Systems) was used to analyze the behavior. The 2D coordinates were tracked continually for at least three consecutive steps. The process was semiautomatic, and each frame was reviewed for tracking errors.

Forced Swim Test. The forced swim test, as originally described (3), assesses the tendency to give up attempting to escape from an unpleasant environment, whereby fewer attempts are interpreted as behavioral despair. The apparatus was a glass beaker (17.5 cm diameter, 24 cm high), filled with water (23–26 °C) to a height of 15 cm. The time mice spent floating (immobility time) during the last 4 out of 6 min as well as the latency to the first immobility episode were manually observed. A mouse was judged to be immobile when it ceased struggling and remained floating motionless in water, making only movements necessary to keep its head above water.

RNA Purification and Microarray. Gene-expression analysis was performed on cortex and hippocampus from 45 mice. RNA was purified with the NucleoSpin RNA isolation kit (no. 740955.250; Macherey-Nagel) following the manufacturer's protocol. Initial quantitation was conducted using a NanoDrop ND-1000 spectrophotometer. The quality of the RNA was inspected using the Agilent Bioanalyzer RNA 6000 Chip (Agilent Technologies). Samples with RNA integrity less than 7.5 were discarded. Finally, we used $n = 4$ for each group for analysis. The microarray was performed by the Gene Expression and Genomics core facility (NIA) and analyzed using DIANE 6.0 software as described before (2). A complete set of 880 canonic pathways and 2,392 chemical perturbation gene sets were obtained from the Molecular Signatures Database (MSigDB) (Broad Institute, MIT, Cambridge, MA). The complete set was tested for gene set enrichment using PAGE. For each pairwise comparison a pathway aggregation z-score was obtained from the gene-expression change z-ratio, and a t test P value to the whole array genes and FDR was computed using PAGE analysis software to test for the significance of the z-score obtained. Pathways per gene set were considered significant if they had more than three genes found in the array and a t test P value less than 0.05 with an FDR of not more than 0.3. The gene expression data has been deposited with GEO (GSE109055).

Splenocyte Cell Population by Flow Cytometry. The mice were killed, and the spleens were dissected to prepare splenocyte suspensions. For flow cytometry analysis, cells were washed by FACS buffer twice, blocked with TruStain FcX (no. 101320; BioLegend) for 10 min at RT, stained with the following fluorescence-labeled monoclonal antibodies: PerCP.Cy5.5 mouse anti-CD4 (no. 100434; BioLegend), APC anti- mouse CD8 (no. 100712; BioLegend), FITC anti-human/mouse CD11b (no. 101206; BioLegend), Brilliant Violet 510 anti-mouse CD11c (no. 117353; BioLegend), and PE anti-mouse CD19 (no. 115508; BioLegend) for 15 min at 4 °C, and then washed twice. Cells were analyzed by a FACSCanto II flow cytometer (BD Biosciences), and data analysis was performed with FlowJo software.

ELISA for Aβ. Mouse hippocampal extracts were prepared as previously reported (4). The accumulation of human $A\beta_{40}$ and $A\beta_{42}$ in these extracts was quantified using ELISA kits (no. KHB3442 and no. KHB3482, respectively; Thermo Fisher Scientific). $A\beta_{40}$ and $A\beta_{42}$ in APPsw-SH-SY5Y cell-culture medium were also measured with ELISA. pCAX APP swe/Ind (no. 30145; Addgene plasmid) was a gift from Dennis Selkoe and Tracy Young-Pearse (both at Brigham and Women's Hospital and Harvard Medical School, Boston). Cell viability was measured using CellTiter-Glo assay (no. G7572; Promega) according to the manufacturer's protocols.

Human Fibroblasts. Primary AD fibroblasts (AG07374) and age-matched control fibroblasts (AG09857) were purchased from Corriell Institute for Medical Research. They were grown in AmnioMAX II Complete Medium (no. 11269016; Gibco) and were protected from light. Cells were in passages 3–7 for experiments.

MitoSOX in Human Fibroblasts. The MitoSOX probe selectively reacts with the superoxide in the mitochondria and is used to measure mitochondrial-specific ROS. Two hundred thousand cells per milliliter in 12-well plates were treated with 1 mM NR

(provided by ChromaDex) for 24 h, or were not treated, and then were trypsinized, resuspended in medium, and stained with 5 μM MitoSOX (no. M36008; Invitrogen) for 30 min protected from light, to allow the dye to reach equilibrium. The cells were kept on a 37 °C heat block at all times. Then the fluorescence was measured with an Accuri C6 Flow Cytometer (Becton Dickinson). Analysis was performed with FlowJo software. Experiments were repeated three times.

ELISA for 8-oxo-dG. Primary AD fibroblasts (AG07374) and age-matched controls (AG09857) were cultured with or without 1 mM NR for 24 h. Cells were harvested, and DNA was extracted using the QIAamp DNA Mini Kit (no. 51304; QIAGEN). ELISA for 8-oxo-dG was performed using the HT 8-oxo-dG ELISA Kit II (no. 4380-192-K; TREVIGEN) following the kit instructions. Experiments were repeated three times.

1. Cheng A, et al. (2016) Mitochondrial SIRT3 mediates adaptive responses of neurons to exercise and metabolic and excitatory challenges. *Cell Metab* 23:128–142.
2. Sykora P, et al. (2015) DNA polymerase β deficiency leads to neurodegeneration and exacerbates Alzheimer disease phenotypes. *Nucleic Acids Res* 43:943–959.
3. Porsolt RD, Bertin A, Jalfre M (1977) Behavioral despair in mice: A primary screening test for antidepressants. *Arch Int Pharmacodyn Ther* 229:327–336.
4. Hou Y, et al. (2014) Smart soup, a traditional Chinese medicine formula, ameliorates amyloid pathology and related cognitive deficits. *PLoS One* 9:e111215.

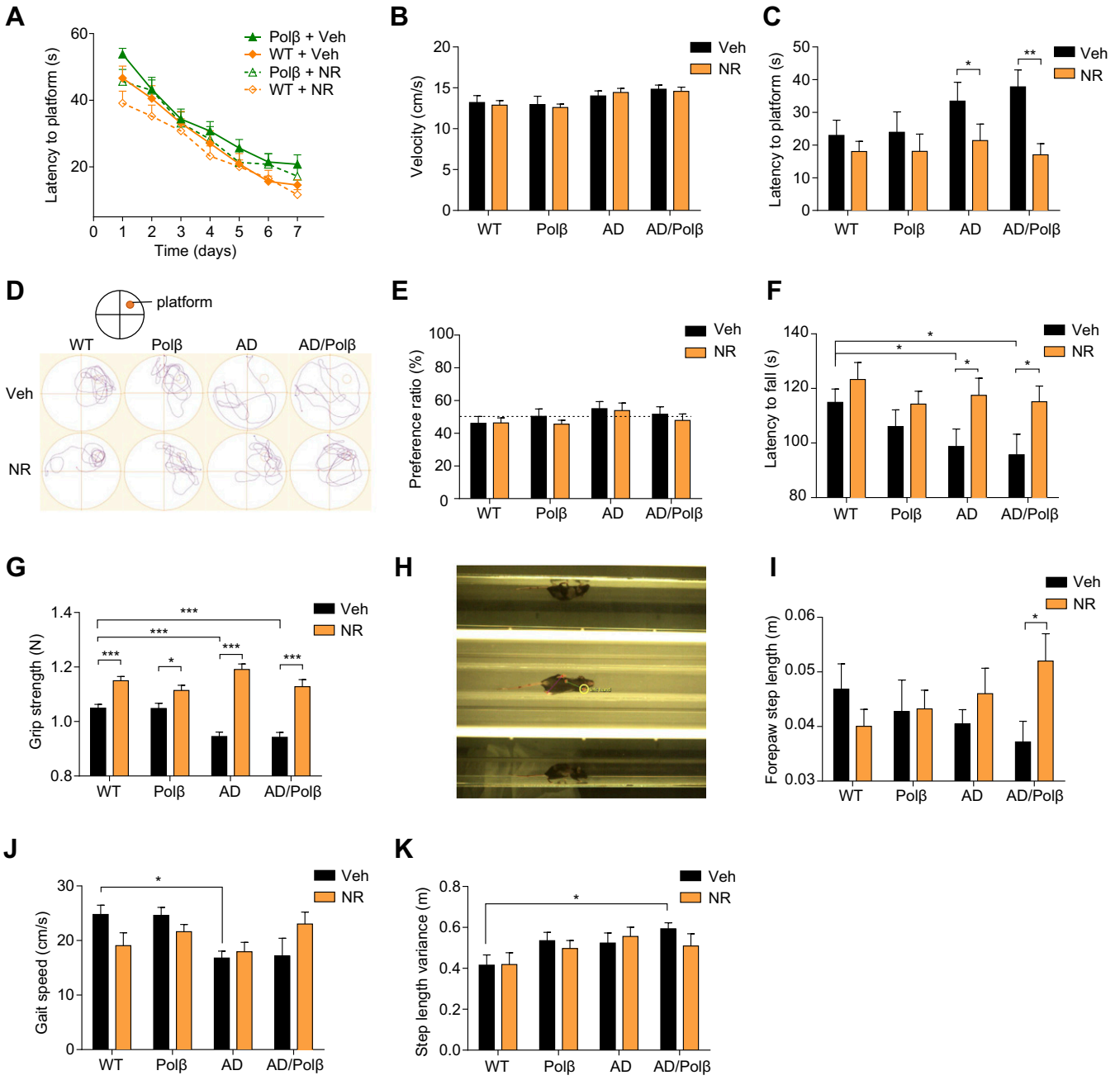

Fig. S1. NR improves memory and motor function in AD mice. (*A*) Latency to locating the platform during the training phase in the Morris water maze in WT and Polβ mice with or without NR treatment. (*B* and *C*) Velocity (*B*) and latency (*C*) to platform location in the probe trial in the Morris water maze in WT, Polβ, AD, and AD/Polβ mice with or without NR treatment. (*D*) Representative swimming paths in the probe trial of the Morris water maze of vehicle- or NR-treated WT, Polβ, AD, and AD/Polβ mice. The platform was located in the upright quadrant. (*E*) Effects of NR in the object-recognition test in WT, Polβ, AD, and AD/Polβ mice with two identical objects. (*F* and *G*) Effects of NR supplementation on the rotarod performance (*F*) and grip strength (*G*) tests. For *A*–*G*, $n = 17$ (WT + Veh), 16 (Polβ + Veh), 16 (AD + Veh), 16 (AD/Polβ + Veh), 13 (WT + NR), 12 (Polβ + NR), 14 (AD + NR), and 15 (AD/Polβ + NR) mice. (*H*) Representative MotoRater software analysis figures. Three directions can be shown together, and each paw of the mouse can be tracked throughout the analysis. (*I*–*K*) Gait performance: forepaw step length (*I*), gait speed (*J*), and step length variance (*K*) in the MotoRater test. $n = 8$ (WT + Veh), 8 (Polβ + Veh), 9 (AD + Veh), 8 (AD/Polβ + Veh), 9 (WT + NR), 8 (Polβ + NR), 10 (AD + NR), and 8 (AD/Polβ + NR) mice. Data are shown as mean ± SEM $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$.

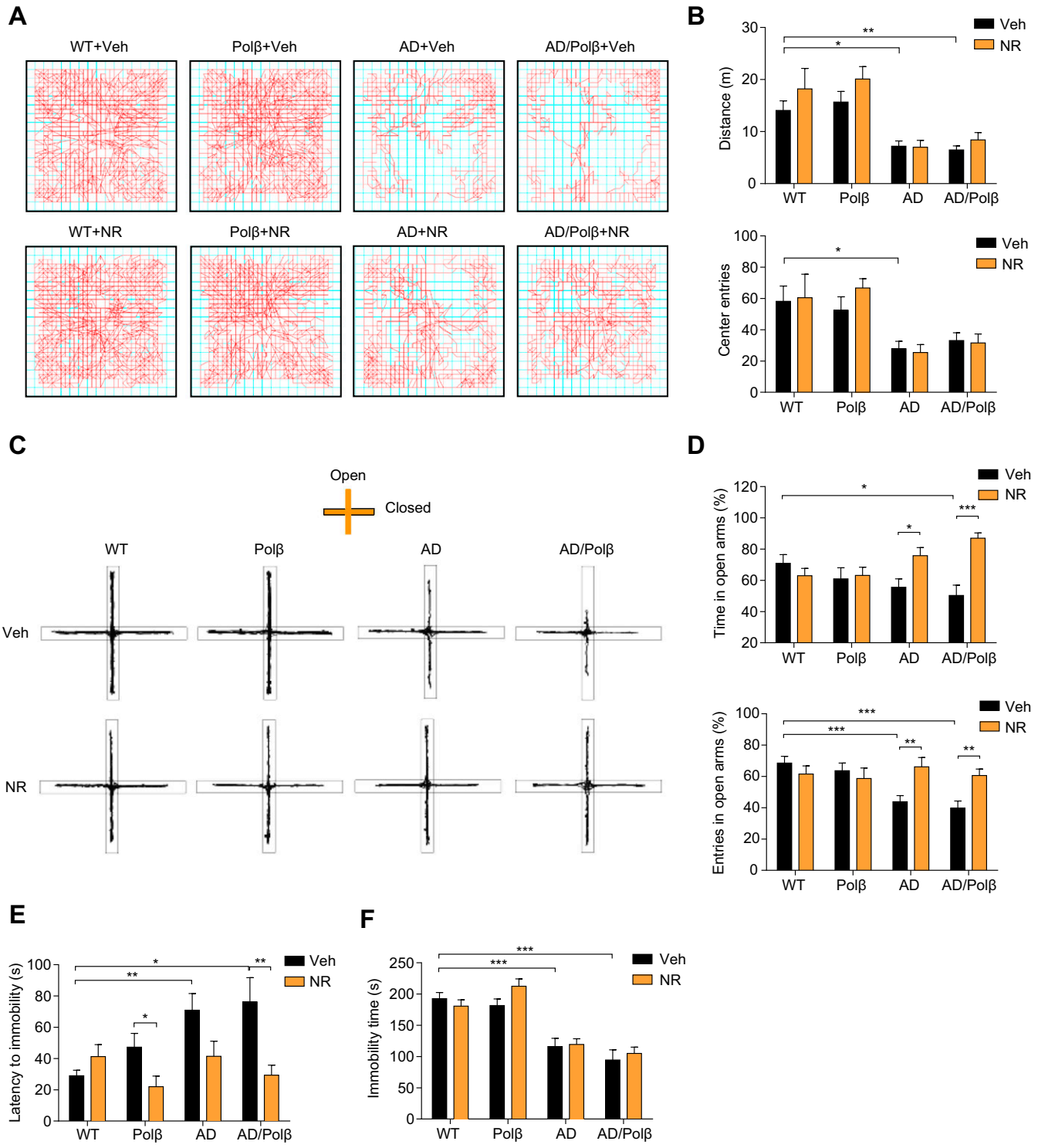

Fig. S2. NR decreases anxiety but not depression in AD and AD/Polβ mice. (*A*) Representative figures of the open-field test in WT, Polβ, AD, and AD/Polβ with or without NR treatment. (*B*) Distance traveled and center entries in the open-field test in WT, Polβ, AD, and AD/Polβ mice with or without NR treatment. (*C* and *D*) Representative elevated plus maze figures (*C*) and time (*D*, *Upper*) and entries (*Lower*) in the open arms of WT, Polβ, AD, and AD/Polβ mice with vehicle or NR treatment. (*E* and *F*) Effects of NR treatment in depression behavior measured by the forced swim test in WT, Polβ, AD, and AD/Polβ mice with vehicle or NR treatment. In all panels, $n = 17$ (WT + Veh), 16 (Polβ + Veh), 16 (AD + Veh), 16 (AD/Polβ + Veh), 13 (WT + NR), 12 (Polβ + NR), 14 (AD + NR), and 15 (AD/Polβ + NR) mice. Data are shown as mean ± SEM. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$.

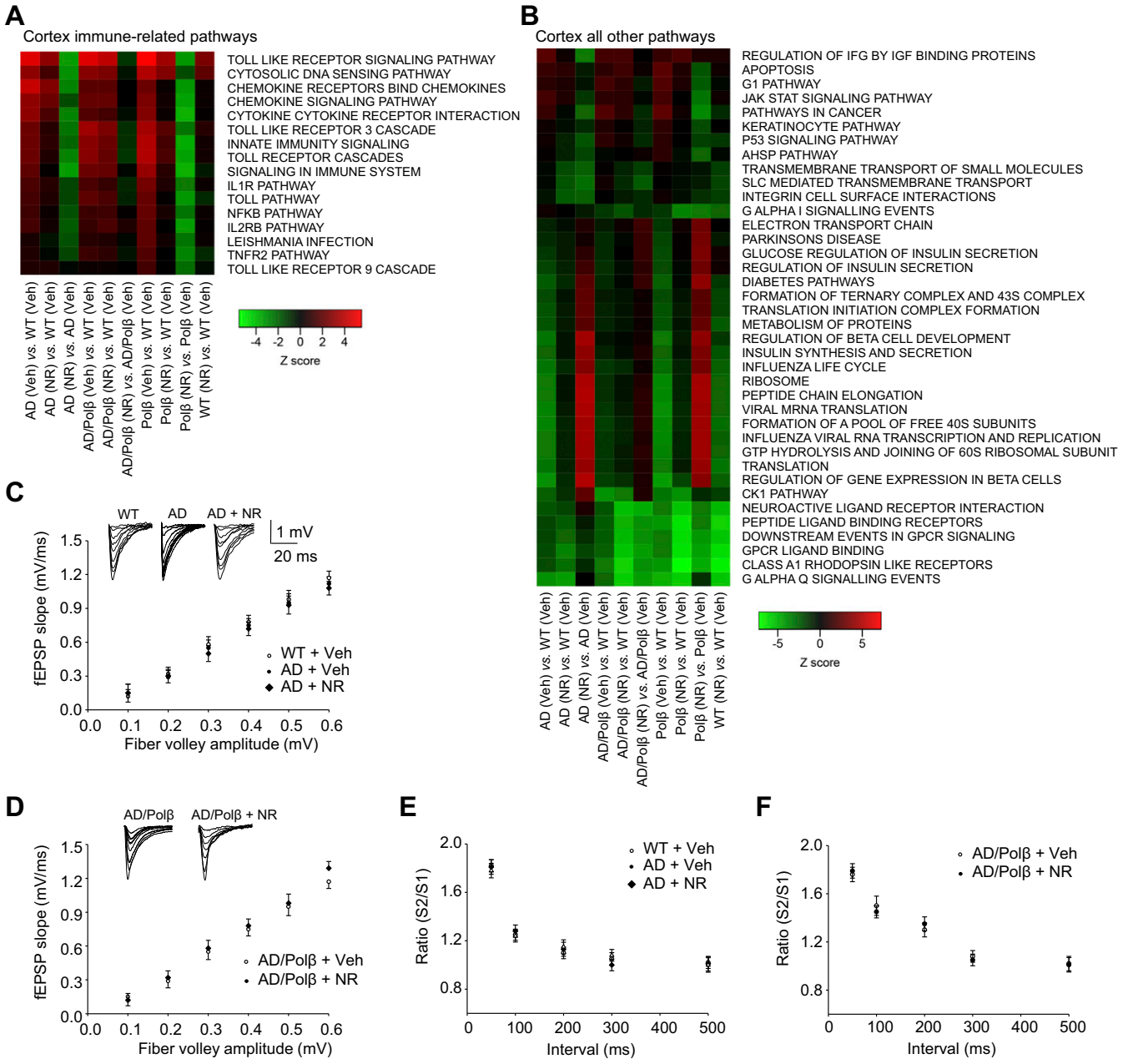

Fig. S3. Microarray analysis in mouse cortex and electrophysiology analysis. (*A* and *B*) Pathways in the cortex of NR- and vehicle-treated mice of each genotype were compared. Pathways that showed ≥1.5-fold change in at least one genotype are shown. We have fixed the order of the samples but allowed the clustering of the pathways based on row means. In cortex, immune-related pathways were the most abundant. We divided the pathways into immune-related pathways (*A*) and all other pathways (*B*). (*C*–*F*) Electrophysiology analysis. (*C* and *D*) Input–output curves in which the postsynaptic responses (fEPSP) are plotted as a function of increasing intensity of presynaptic stimulation (fiber volley amplitude). (*E* and *F*) Results of paired-pulse facilitation analysis, a measure of excitatory neurotransmitter (glutamate) release from presynaptic terminals. For *C*–*F*, values are the mean and SEM of determinations made on eight hippocampal slices from at least five different mice.

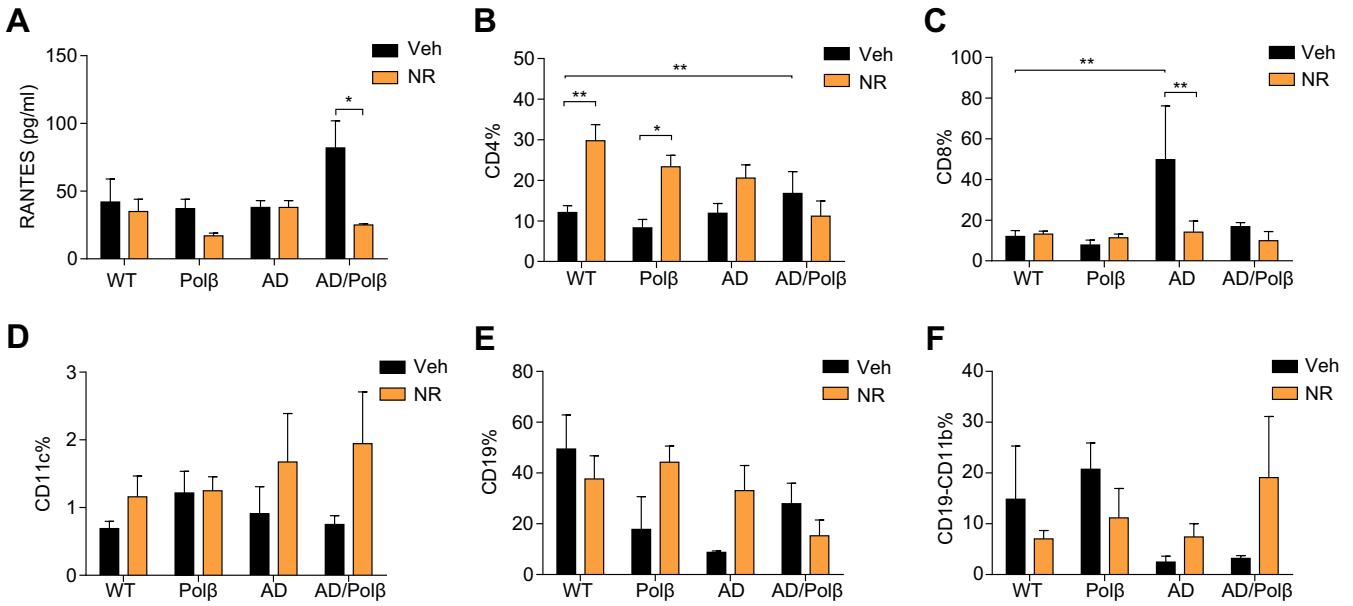

Fig. S4. NR decreases RANTES level and restores the spleen cell population to the WT level. (*A*) The RANTES level in mouse plasma detected by multiplex cytokine array. $n = 11$ (WT + Veh), 10 (Polβ + Veh), 11 (AD + Veh), 12 (AD/Polβ + Veh), 9 (WT + NR), 10 (Polβ + NR), 8 (AD + NR), and 9 (AD/Polβ + NR) mice. (*B–F*) Splenocyte cell population distribution by flow cytometry in WT, Polβ, AD, and AD/Polβ mice with vehicle or NR treatment. Data are shown as mean ± SEM. $*P < 0.05$, $**P < 0.01$.

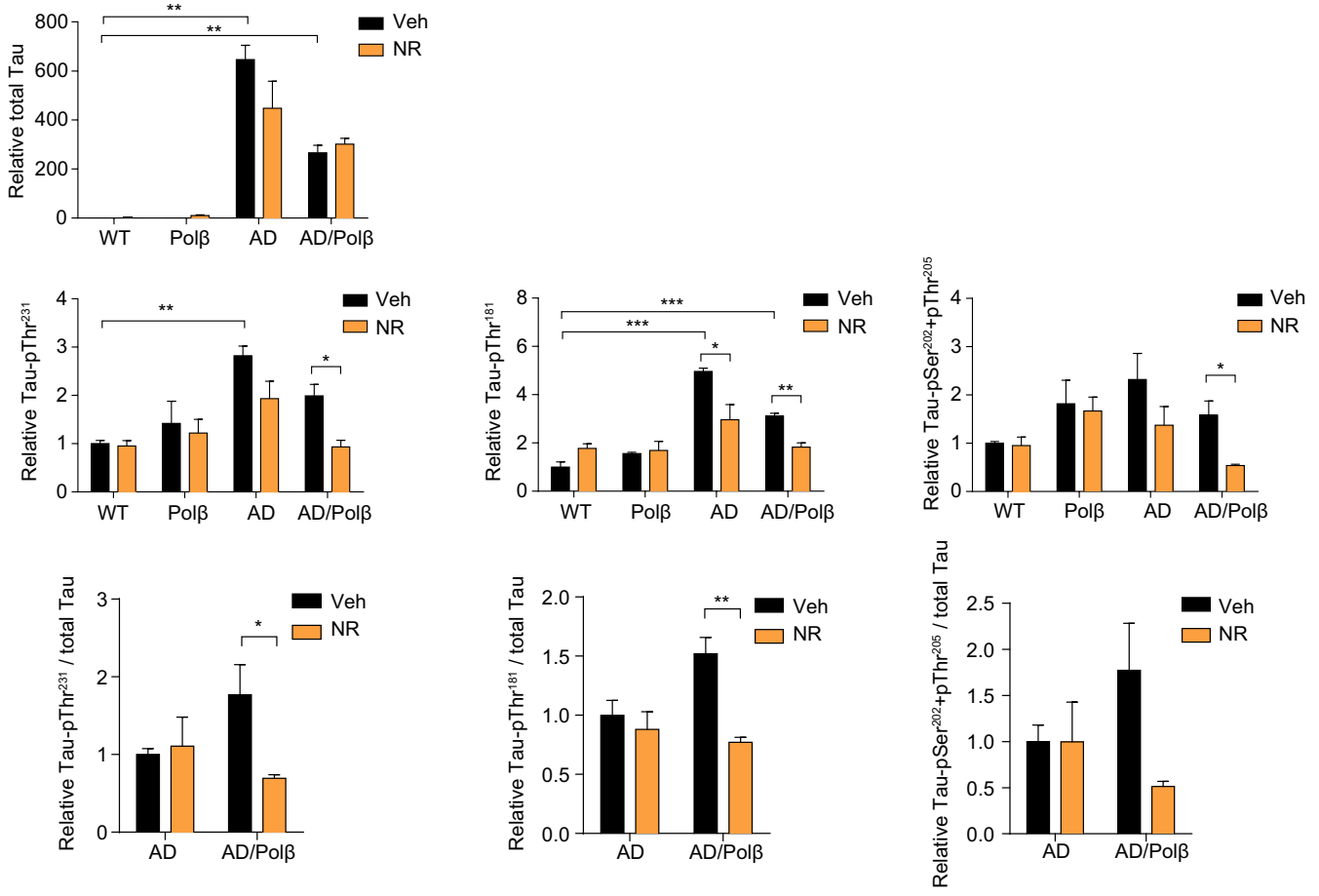

Fig. S5. Tau phosphorylation is decreased after NR treatment in mouse models. Quantification of immunoblots from Fig. 4*D* of the tau proteins from the hippocampus of WT, Polβ, AD, and AD/Polβ mice after treatment with vehicle or NR. Data are shown as mean ± SEM. $*P < 0.05$, $**P < 0.01$, $***P < 0.001$.

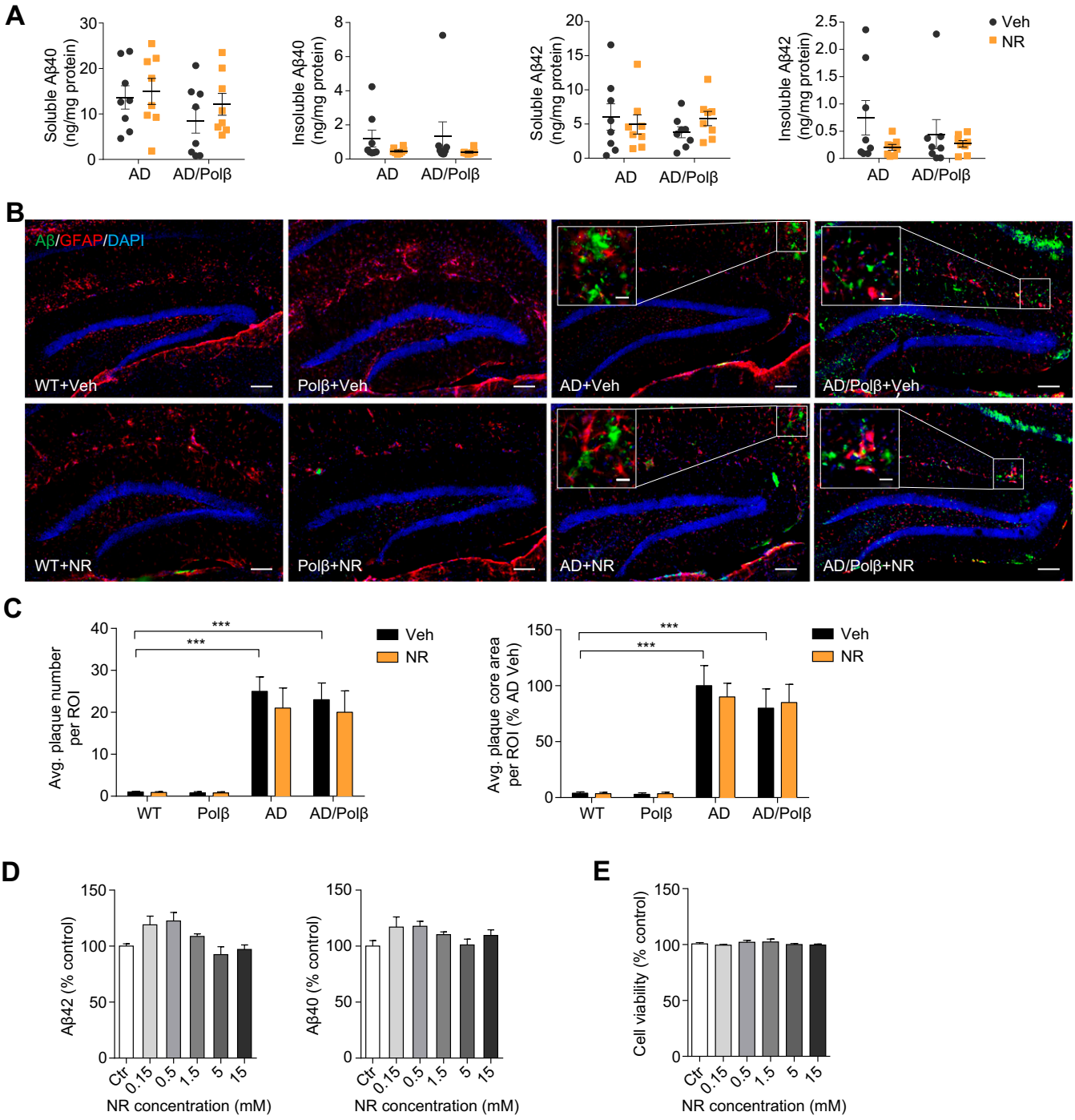

Fig. S6. Aβ is not changed after NR treatment in AD and AD/Polβ mice or in APPsw-SH-SY5Y cells. (*A*) ELISA for soluble and insoluble $A\beta_{40}$ and $A\beta_{42}$ in the hippocampus of AD and AD/Polβ mice treated with vehicle or NR. $n = 8$ mice per group. (*B*) Representative images of 6E10 (green), GFAP (red), and DAPI (blue) staining of hippocampal regions from WT, Polβ, AD, and AD/Polβ mice treated with vehicle or NR. (Scale bars, 100 μm; *Inset*, 20 μm.) (*C*) Quantification of average plaque number (*Left*) and plaque core area (*Right*) from sections as in *B*. $n = 5$ mice per group. (*D*) ELISA for $A\beta_{40}$ (*Left*) and $A\beta_{42}$ (*Right*) levels in APPsw-transfected SH-SY5Y cells with or without 0.15–15 mM NR treatment for 24 h. (*E*) Cell viability by CellTiter-Glo assay in APPsw-transfected SH-SY5Y cells with or without 1 mM NR treatment for 24 h. For *D* and *E*, experiments were repeated three times. Data are shown as mean ± SEM. $^*P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$.

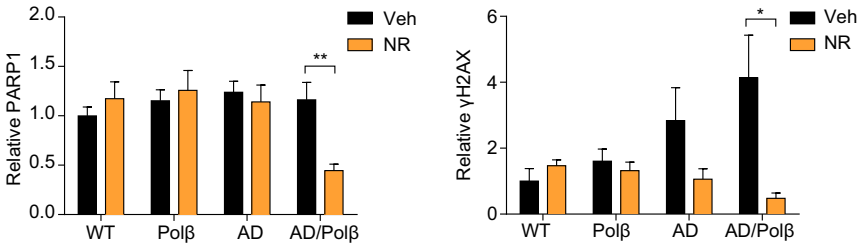

Fig. S7. Effects of NR augmentation on designated antigen levels of AD and AD/Polβ mice. Quantification of immunoblots from Fig. 5*A* of the indicated proteins from the hippocampus of WT, Polβ, AD, and AD/Polβ mice after 6 mo of treatment with vehicle or NR. Data are shown as mean ± SEM. $*P < 0.05$, $**P < 0.01$.

Table S1. Comparison of NR effects in 3xTgAD and 3xTgAD/Polβ

NR's effect	AD mice	AD/Polβ mice
Cognition		
Learning	↑	↑↑
Memory		
Spatial memory in water maze	↑	↑↑
Recognition memory	↑	↑
Y-maze spatial memory	↑	↑
Fear memory	↑	↑↑
LTP	↑	↑↑↑
Anxiety	↓	↓↓
Motor function		
Rotarod	↑	↑
Grip strength	↑	↑
Gait	–	↑
Tau		
Phosphorylated tau	↓	↓
Inflammation		
Neuroinflammation by staining	↓	↓↓
IL-6	↓	↓
TNFα	–	↓
RANTES	–	↓
MCP-1	–	↓
IL-1β	–	↓
MIP-1α	–	↓
IL-10	–	↑
DNA repair		
DNA damage	↓	↓↓
Apoptosis	–	↓
Sirtuins		
SIRT3	–	↑
SIRT6	–	↑