Supplemental Information

Transient receptor potential melastatin 2 governs stress-induced depressive-like behaviors

Seung Yeon Ko, Sung Eun Wang, Han Kyu Lee, Sungsin Jo, Jinil Han, Seung Hoon Lee, Miyeon Choi, Hye-Ryeong Jo, Jee Young Seo, Sung Jun Jung and Hyeon Son

List of Supplemental Materials

SI Materials and Methods

Mice. TRPM2 heterozygous (*Trpm2*$^{+/-}$) mice were backcrossed into C57BL/6J inbred background over 10 generations. Heterozygous breeders were crossed to generate wild-type (*Trpm2*$^{+/+}$), heterozygous (*Trpm2*$^{+/-}$) and knockout (*Trpm2*$^{-/-}$) littermates, and PCR analysis determined each of the genotypes. All animals were maintained under a 12-h light/dark cycle with *ad libitum* access to food and water. All animal experiments were performed following protocols approved by the Institutional Animal Care and Use Committee of Hanyang University.

Behavioral Assessments. Mice were placed to the testing room 2 h before the start of each behavioral test and acclimated to the room conditions. All tests were conducted during the dark cycle of animal housing and in random order. After individual test session, the apparatus was cleaned with 70 % alcohol to remove any odor and trace of the previously tested mouse.

Locomotor Activity Test (LMA). Mice were placed in a corner of a white plastic box (50 × 50 × 20 cm) to initiate test session, and their movements were recorded for 5 min with a web camera (HD C310, Logitech, Switzerland) fixed over the apparatus. Total locomotor activity was measured using an ANY-maze video tracking system (Stoelting Co., IL, USA).

Sucrose Consumption Test (SCT). Mice were habituated for 48 h to 1 % (w/v) sucrose solution by providing the sucrose solution as the only drinking fluid. After 12−18 h water deprivation, the amount of sucrose solution consumed for 1 h was measured by comparing the bottle weight before and after the test.

Novelty Suppressed Feeding Test (NSFT). Three food pellets were placed in the center of a white plastic box (50 × 50 × 20 cm). After 24 h food deprivation, mice were placed in a corner of the box to initiate test session. Feeding latency was measured during 10 min period.

Forced Swim Test (FST). Mice were placed individually in a cylinder (height 30 cm, diameter 15 cm) with water (22 ± 1 °C, 12-cm depth) for 6 min, and the total period of immobility was measured.

Learned Helplessness Test (LHT). Mice were placed on a commercial shuttle box divided into two equal compartments by a central barrier (Gemini Avoidance System, San Diego Instruments, San Diego, CA, USA). Mice were given inescapable electric footshocks (180 scrambled footshocks, 0.3 mA intensity), and escape performance was tested 24 h later in the same chamber with 30 escape trials per mouse (25-sec maximum duration, 0.3 mA footshock amplitude). The shuttle door opens at the beginning of the shock and each trial is terminated when the mouse crosses into the nonshock compartment, or when a 25-sec duration is reached. Latencies to escape over first 10 escape trials and last 10 escape trials were analyzed.

Hippocampal Dissection. Mice were killed after the probe trial by cervical dislocation, and brains were removed from the skull. Brains were then chilled in ice-cold HBSS, and all further manipulations were performed on an ice-cooled plate. Whole hippocampus was dissected from the brain, and 500 μm-thick slices, transverse to the longitudinal axis, were cut with a Starrett tissue chopper. DG was

microdissected by hand under a dissecting microscope. Subregional boundaries were clearly visible under these conditions. Tissues were collected and then stored at −80 °C until use.

Malondialdehyde Measurement. As a measure of lipid peroxidation, malondialdehyde (MDA) levels were determined using Bioxytech MDA-586 Assay Kit (OxisResearch, Oregon) as described previously (1). Assays were performed according to the manufacturer's instructions. Briefly, hippocampal tissues were homogenized in the presence of 5 mM butylated hydroxytoluene. Homogenates were centrifuged at 3000 x g for 10 min at 4 °C and supernatant was collected. Free MDA in the supernatant was converted to a stable carbocyanine dye (maximum absorption at 586 nm) by chemical reaction with N-methyl-2-phenylindole. Absorbance of the supernatant was measured at 586 nm. MDA levels were normalized against the protein concentration.

Detection of PARP Enzymatic Activity. PARP enzymatic activity was detected using a cytochemical method as described previously (2). Cultured hippocampal neurons were fixed for 10 min in 95 % ethanol at −20 °C, permeabilized by 0.1 % (v/v) Triton X-100 in 100 mM Tris (pH 8.0) for 15 min and then incubated with a reaction mixture, containing 10 mM $MgCl_2$, 1 mM dithiothreitol, 30 μM biotinylated NAD^+ (BPS Bioscience) in 100 mM Tris (pH 8.0), for 30 min at 37 °C. A biotinylated NAD^+-free reaction mixture was used as a negative control. The cells were then incubated with FITC-conjugated streptavidin (1:100; Invitrogen) to detect incorporated biotin signals, a marker of PARP activity. Cells were imaged with

identical confocal microscope settings. Staining intensity of PARP activity was quantified using ImageJ software and expressed in arbitrary units.

Detection of Superoxide Levels. Superoxide levels in cultured hippocampal neurons were measured using dihydroethidium (DHE), a cell membrane-permeable superoxide-sensitive fluorescent dye (Molecular Probes) by following the procedure described previously (1), with slight modifications. Briefly, hippocampal neurons were incubated for 10 min at 37 °C in HBSS buffer (pH 7.4) containing 10 μM DHE in 24-well plates. The cells plated onto glass coverslips were washed twice with HBSS, which then was placed onto a microscope (Olympus IX71, Japan). Fluorescence intensity was detected as a marker of superoxide production. It was quantified using ImageJ software and expressed in arbitrary units.

Western Blot Analysis. Protein extract from hippocampal tissue or cultured hippocampal neurons was subjected to SDS-PAGE, transferred to PVDF membrane and incubated with antibodies. Extraction of nuclear protein was described previously (3). Primary antibodies were diluted in 1X TBS with 0.1 % (v/v) Tween-20 (for details, see Table S3). Specifically, anti-HDAC5(Ser279) was kindly given by Christopher W. Cowan (University of Texas Southwestern Medical Center, USA) (4), and anti-Prx2(Thr89) was kindly given by David S. Park (University of Ottawa, Canada) (5). Secondary antibodies were diluted in 1X TBS with 0.1 % (v/v) Tween-20 containing 5 % (w/v) non-fat dry milk, as follows: anti-rabbit IgG conjugated with HRP (1:2,000), anti-mouse IgG conjugated with HRP (1:2,000), anti-goat IgG conjugated with HRP (1:2,000). Blots were developed with enhanced

chemiluminescence western blotting detection system (ECL STAR; Dyne Bio, Korea). Optical density was measured using ImageJ software to quantify the blots.

Immunoprecipitation. Mouse hippocampus was homogenized in lysis buffer (Cell signaling) containing protease inhibitor and phosphatase inhibitor cocktail 2 and 3 (Sigma). Cdk5 was immunoprecipitated from hippocampal tissue lysates using anti-Cdk5 monoclonal antibody (Abcam, ab28441) or anti-Cdk5 polyclonal antibody (Santa Cruz Biotechnology, sc-173) in HNTG buffer (20 mM HEPES pH 7.5, 150 mM NaCl, 0.1 % Triton X-100 and 10 % glycerol) overnight at 4 °C. Anti-rabbit IgG (Millipore) or anti-mouse IgG (Millipore) was used as a negative control. Protein A-agarose beads (Roche) were added at 4 °C for 2 h followed by washing three times with HNTG buffer containing protease and phosphatase inhibitor cocktail. The bound proteins were denatured in 2X Laemmli sample buffer (126 mM Tris-HCl, 20 % glycerol, 4 % SDS, 0.02 % bromophenol blue), boiled 10 min, and analyzed by immunoblotting with anti-p35 antibody (Santa Cruz Biotechnology, sc-820). Prx2 was immunoprecipitated with anti-Prx2 monoclonal antibody (R&D Systems, MAB3489) in the same way as for Cdk5 immunoprecipitation.

Cdk5 Activity Assay. Cdk5 activity assay was performed using ADP-Glo^{TM} kinase assay kit according to manufacturer's protocol with slight modifications (Promega, Madison, WI). In brief, for endogenous kinase assays, endogenous Cdk5 was immunoprecipitated from hippocampal tissue extract using anti-Cdk5 monoclonal antibody (Abcam, ab28441) or anti-Cdk5 polyclonal antibody (Santa Cruz Biotechnology, sc-173) under non-denaturing conditions. For kinase reaction, immunoprecipitates were incubated with 20 μg Histone H1 and 50 μM ATP at room

temperature for 10 min. ADP was produced during Cdk5 kinase reaction and then converted to ATP, which was converted to light, by chemical reaction with kinase detection reagent. The luminescence was measured with a plate-reading luminometer and expressed in relative light units (RLU).

Hippocampal Primary Neuronal Cell Culture. Whole brains were collected from C57BL/6 mouse E14 embryos. Embryonic hippocampus was dissected in ice-cold Ca^{2+}/Mg^{2+}-free HBSS (Gibco), followed by removal of blood vessels and meninges. The hippocampal tissue was then incubated with 0.05 % trypsin-EDTA (Wel Gene, Korea) at 37 °C for 5−10 min, then dissolved in neurobasal (NB) medium (Gibco) containing 10 % (v/v) FBS (Wel Gene), 0.5 mM L-glutamine (Sigma), and 1 % 100X penicillin-streptomycin (Wel Gene). After centrifugation at 200 x g for 1 min, the pelleted cells were gently resuspended in the culture medium and plated at 40,000−50,000 cells per cm^2 on poly-L-lysine-coated (25 μg/mL in PBS; Sigma) and laminin-coated (10 μg/mL in PBS; Invitrogen) culture dishes. Hippocampal cultures were grown for 1 d in NB medium containing 10 % (v/v) FBS, 0.5 mM L-glutamine, and 1 % 100X penicillin-streptomycin. The medium was changed the following day to NB medium supplemented with 2 % (v/v) B27 (Gibco) serum-free supplement, 0.5 mM L-glutamine, and 1 % 100X penicillin-streptomycin antibiotic mixture. Cultures were maintained for 7−12 d at 37 °C in a 5 % CO_2/95 % air-humidified incubator. The neurons were used after 7−12 d.

Calcium Imaging. Cultured hippocampal neurons were loaded with the fluorescent Ca^{2+} indicator Fura-2 AM (5 μM; Molecular Probes, Eugene, OR, USA) for 40 min at 37 °C. The cells plated onto glass coverslips were mounted onto the recording

chamber, which then was placed onto an inverted microscope (Olympus IX70, Japan). The recording chamber was initially perfused with 2 mM Ca^{2+} bath, and test solutions including each drug, H_2O_2 (1 mM; JUNSEI, Japan) or KCl (50 mM; Sigma Aldrich, Germany), were applied by a gravity-driven multi-channel system (ALA-VM8, ALA Scientific Instruments, USA). Cells were illuminated with a lamp, and excitation wavelengths (340/380 nm) were selected by the Lambda 10-B (Shutter Instrument, Novato, CA). Intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$) was measured by microfluorometry with an ORCA-Flash2.8 Digital CMOS camera (HAMAMATSU, Japan) coupled to a microscope and software (MetaMorph® NX, Molecular Devices, USA) on a computer.

Hippocampal Slice Preparation and Patch-Clamp Recordings. Mice were decapitated, and the brain was rapidly removed from the skull and stored in ice-cold artificial cerebrospinal fluid (ACSF) containing (in mM): 124 NaCl, 3 KCl, 1.25 NaH_2PO_4, 1.3 $MgCl_2$, 1.3 $CaCl_2$, 26 $NaHCO_3$, and 10 glucose, oxygenated with 95 % O_2 and 5 % CO_2 (pH 7.4; 300mOsmol). Transverse 300-μm-thick hippocampal slices were cut using a vibrating microtome (Vibratome Series 1000; Technical Products International, St. Louis, MO). Before recordings, slices were recovered by incubating in ACSF (30 °C) which was continuously gassed with 95 % O_2 and 5 % CO_2. Individual slices were then transferred to an immersion-recording chamber on an upright, fixed-stage microscope equipped with infrared, differential interference contrast optics (Olympus BX50WI) and continuously perfused with oxygenated ACSF. Synaptic activity was recorded from visually identified DG granule cells using whole-cell patch-clamp recordings. Currents were recorded using an EPC-10 patch clamp amplifier (HEKA Elektronik, Lambrecht, Germany) and PatchMaster software

(HEKA Elektronik). Patch pipettes were pulled from borosilicate glass (1.5 mm outer diameter, 0.86 mm wall thickness; Sutter Instruments, Novato, CA) with a P-97 puller (Sutter Instruments), and the resistance of open tip was 4−7 MΩ. The intracellular pipette solution contained (in mM): 140 K^+ gluconate, 1 NaCl, 5 EGTA, 10 HEPES, 1 $MgCl_2$, 1 $CaCl_2$, 3 KOH, 2 ATP and 0.2 % biocytin (pH 7.15−7.3). The recording electrode was trained on DG granule cells using positive pressure. Once in whole cell configuration, the cell was held at −70 mV for equilibration of intracellular and recording pipette contents. Series resistance was monitored throughout the recordings, and recordings where the series resistance was remained < 25 MΩ and changed by ≤ 20 % during the recordings were only used for analysis.

Construction of shRNA-Expressing Vectors and Lentivirus Production. For gene silencing, shRNAs were cloned into the pLB lentiviral vector as previously described (6). The target sequences were the following: *Luciferase* shRNA (shLuc, control shRNA), 5'-CTTCGAAATGTCCGTTCGGTT-3'; *Cdk5* shRNA (shCdk5), 5'-GTACCCAGCTACAACATCCTT-3'; *Trpm2* shRNA (shTRPM2), 5'-GCTCATGG ATTCCCGAGAATA-3'. Lentivirus was produced by co-transfection of shLuc, shCdk5 or shTRPM2 with lentiviral packaging plasmids, pMD2.G and psPAX2, into 293T cells (ATCC, Manassas, VA, USA) by the calcium-phosphate method, as described by the RNAi Consortium (http://www.broadinstitute.org).

Viral-Mediated Gene Transfer. Mice were generally anesthetized with a mixture of Rompun (8.5 mg/kg) and Zoletil (17 mg/kg), and prepared for stereotaxic surgery. Lentiviral placements were confirmed by GFP fluorescence, which was coexpressed in each virus.

Genotyping and RT-PCR. To determine the mouse genotype, genomic DNA was isolated from mouse tail tissue using LaboPass^{TM} Tissue Genomic DNA Mini Prep Kit (Cosmo Genetech, Korea). PCR was carried out using the purified genomic DNA with the primer sets of PTRPM2-13F, PTRPM2-10R, and Pneo-5'a; sequences are PTRPM2-13F (5'-CTTGGGTTGCAGTCATATGCAGGC-3'), PTRPM2-10R (5'-GCCCTCACCATCCGCTTCACGATG-3'), and Pneo5'a (5'-GCCACACGCGTCAC CTTAATATGCG-3').

BrdU Administration and Quantitative Analysis. Mice were received BrdU injection (i.p., 50 mg/kg; Sigma) for 3 days of the last CUS period to examine survival of NPCs. Two weeks after BrdU injection, mice were perfused, and BrdU immunohistochemistry was performed. $BrdU^+$ cells colabeled with NeuN were counted in the GCL (three to four coronal 25 μm sections per animal) using a fluorescence confocal microscope (Leica Microsystems) at X400, plus video camera and LEICA IM50 software (Leica Microsystems, Wetzlar, Germany). $BrdU^+$ cells colabeled with GFAP were also quantified in DG. For analysis of NPCs proliferation, mice were administered an i.p. injection of BrdU (50 mg/kg; Sigma) on the last 3 days. One day after BrdU injection, mice were killed and transcardially perfused. The number of $BrdU^+$ cells was counted in the SGZ.

Immunohistochemistry and Immunocytochemistry. Mice were transcardially perfused with cold 4 % (w/v) paraformaldehyde (PFA) in PBS. Brains were postfixed in the same solution overnight at 4 °C and stored in 30 % (w/v) sucrose at 4 °C. Serial sections (25 μm) of the brains were cut coronally through the entire hippocampus. For

detection of BrdU, the sections were processed as described previously (7). For other types of immunofluorescent labeling, the sections were blocked with 3 % (w/v) bovine serum albumin (BSA) in PBS containing 0.3 % Triton X-100 for 1 h at room temperature and incubated with primary antibodies in the blocking solution overnight at 4 °C. Primary antibodies used for immunohistochemistry are described in Table S3. The sections were then incubated 2 h at room temperature with Alexa 488-conjugated (Invitrogen) and Cy3-labeled (Jackson ImmunoResearch) secondary antibodies diluted in PBS. The sections were mounted in Vectashield containing 4'-6-diamidino-2-phenylindole (DAPI; Vector Laboratories, Burlingame, CA) and visualized with a confocal microscope (Leica Microsystems, Wetzlar, Germany). Cultured hippocampal neurons were fixed with 4 % PFA in PBS for 20 min at room temperature. Fixed cells were washed with PBS and blocked with 10 % (v/v) normal goat serum containing 0.3 % Triton X-100. Cells were then stained with indicated primary and secondary antibodies followed by mounting in Vectashield containing DAPI.

Quantitative Real-Time PCR. Total RNA was isolated from mouse hippocampal tissue using Trizol reagent (Sigma). Reverse transcription of 1 μg of total RNA was performed with oligonucleotide deoxythymidine primer using Improm-II™ Reverse Transcription System (Promega). The resulting cDNA was used as a template for the amplification of target gene transcripts by real time PCR. Quantitative real-time PCR (qPCR) was performed on a CFX96 Touch™ Real-Time PCR Detection System (Bio-Rad Laboratories, CA, USA) using SensiFAST™ SYBR No-ROX mix (Bioline) according to the instructions of the manufacturer. The PCR primers are described in Table S4. All gene expression values were normalized to those of β-actin.

Microarray Analysis. The gene expression microarray dataset was obtained from the NCBI Gene Expression Omnibus (GEO, http://www.ncbi.nlm.nih.gov/geo/), accession number GSE53987. The dataset is described in detail in Lanz *et al*. (8). Briefly, frozen sections of human brain were dissected to obtain tissue samples of the hippocampus, Brodmann Area 46 and associative striatum. Total RNAs were isolated from the samples and microarray experiments were conducted with an Affymetrix platform (U133Plus-v2 Affymetrix whole genome microarray chips). The gene expression data were processed with Robust Multi-array Average (RMA). To identify differences in hippocampal gene expression between control subjects and subjects with MDD, a subset of the samples collected from the hippocampus were selected, and they were then filtered to exclude samples with low quality (brain pH $\leq$ 6.5 and RNA Integrity Number (RIN) $\leq$ 6.0) (9, 10). Demographic information for the subjects with MDD and matched controls is given in Table S5. The expression data were normalized by quantile normalization, and log2-transformed. Differentially expressed genes were identified using the LIMMA (Linear modeling of Microarray data) package (11). Because of the small sample size, the usual statistical criteria used in microarray analysis (i.e., false discovery rate, FDR < 0.05) were not appropriate for revealing differences between the groups. Hence, *p*-values were used to obtain potentially interesting genes (*p*-value < 0.05). All data analyses and visualization were conducted using R 3.4.3 (www.r-project.org).

Statistical Analysis. Unpaired two-tailed Student's *t* tests were used to compare 2-group data, as appropriate. Multiple comparisons were evaluated by one-way or two-way ANOVA and Bonferroni's *post hoc* test, when appropriate. Behavioral findings

were successfully replicated with mice from different litters and in several instances, across independent cohorts. Sample sizes for behavioral studies were determined based on similar work in the literature. All experiments were carried out at least three times, and data consistency was observed in repeated experiments. For all analyses $p < 0.05$ was considered statistically significant, and all data are presented as means ± SEM.

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Supplemental Tables

Table S1. Statistical parameters

Figure Number	Test Used	N		Descriptive States (Average, Variance)	P Value	Degrees Of Freedom Value
		Exact Value	Defined			
1B (*Trpm2*)	Student's t-test	**Home cage** $Trpm2^{+/+}$ (6); **CUS** $Trpm2^{+/+}$ (5)	Mice per group	Error bars are mean±SEM	p=0.0073	t(9)=−3.016
1C	Student's t-test	**Home cage** $Trpm2^{+/+}$ (6); **CUS** $Trpm2^{+/+}$ (4)	Mice per group	Error bars are mean±SEM	p=0.0326	t(8)=−2.1355
1D	Two-way ANOVA	**Home cage** $Trpm2^{+/+}$ (10), $Trpm2^{-/-}$ (7); **CUS** $Trpm2^{+/+}$ (10), $Trpm2^{-/-}$ (7)	Mice per group	Error bars are mean±SEM	Genotype x Stress interaction p=0.0156; Genotype p=0.0035; Stress p<0.0001 Bonferroni posttest: Home cage $Trpm2^{+/+}$ vs CUS $Trpm2^{+/+}$ p<0.001; CUS $Trpm2^{+/+}$ vs CUS $Trpm2^{-/-}$ p<0.001	Genotype x Stress interaction F(1,30)=6.578; Genotype F(1,30)=10.03; Stress F(1,30)=21.32
1E	Two-way ANOVA	**Home cage** $Trpm2^{+/+}$ (6), $Trpm2^{-/-}$ (6); **CUS** $Trpm2^{+/+}$ (6), $Trpm2^{-/-}$ (6)	Mice per group	Error bars are mean±SEM	Genotype x Stress interaction p=0.0257; Genotype p<0.0001; Stress p<0.0001 Bonferroni posttest: Home cage $Trpm2^{+/+}$ vs CUS $Trpm2^{+/+}$ p<0.001; Home cage $Trpm2^{+/+}$ vs Home cage $Trpm2^{-/-}$ p<0.05; CUS $Trpm2^{+/+}$ vs CUS $Trpm2^{-/-}$ p<0.001	Genotype x Stress interaction F(1,20)=5.805; Genotype F(1,20)=46.52; Stress F(1,20)=32.78
1F	Two-way ANOVA	**Home cage** $Trpm2^{+/+}$ (11), $Trpm2^{-/-}$ (10); **CUS** $Trpm2^{+/+}$ (11), $Trpm2^{-/-}$ (12)	Mice per group	Error bars are mean±SEM	Genotype x Stress interaction p=0.2442; Genotype p=0.0023; Stress p=0.9711 Bonferroni posttest: CUS $Trpm2^{+/+}$ vs CUS $Trpm2^{-/-}$ p<0.01	Genotype x Stress interaction F(1,40)=1.397; Genotype F(1,40)=10.62; Stress F(1,40)=0.001327
	Unpaired two-tailed t test	**Home cage** $Trpm2^{+/+}$ (11), $Trpm2^{-/-}$ (10); **CUS** $Trpm2^{+/+}$ (11), $Trpm2^{-/-}$ (12)	Mice per group	Error bars are mean±SEM	CUS $Trpm2^{+/+}$ vs CUS $Trpm2^{-/-}$ p=0.007	t(21)=−2.987
1G	Two-way ANOVA	**Home cage** $Trpm2^{+/+}$ (12), $Trpm2^{-/-}$ (15); **CUS** $Trpm2^{+/+}$ (11), $Trpm2^{-/-}$ (16)	Mice per group	Error bars are mean±SEM	Genotype x Stress interaction p=0.001; Genotype p<0.0001; Stress p=0.0015 Bonferroni posttest: Home cage $Trpm2^{+/+}$ vs CUS $Trpm2^{+/+}$	Genotype x Stress interaction F(1,50)=12.32; Genotype F(1,50)=55.24; Stress F(1,50)=11.26

					p<0.001; Home cage *Trpm2*$^{+/+}$ vs Home cage *Trpm2*$^{-/-}$ p<0.05; CUS *Trpm2*$^{+/+}$ vs CUS *Trpm2*$^{-/-}$ p<0.001	
1H	Two-way ANOVA	**Home cage** *Trpm2*$^{+/+}$ (13), *Trpm2*$^{-/-}$ (15); **CUS** *Trpm2*$^{+/+}$ (15), *Trpm2*$^{-/-}$ (15)	Mice per group	Error bars are mean±SEM	Genotype x Stress interaction p=0.0294; Genotype p<0.0001; Stress p=0.094 Bonferroni posttest: Home cage *Trpm2*$^{+/+}$ vs CUS *Trpm2*$^{+/+}$ p<0.05; Home cage *Trpm2*$^{+/+}$ vs Home cage *Trpm2*$^{-/-}$ p<0.001; CUS *Trpm2*$^{+/+}$ vs CUS *Trpm2*$^{-/-}$ p<0.001	Genotype x Stress interaction F(1,54)=5.006; Genotype F(1,54)=65.61; Stress F(1,54)=2.905
1I	Two-way ANOVA	**Home cage** *Trpm2*$^{+/+}$ (15), *Trpm2*$^{-/-}$ (14); **CUS** *Trpm2*$^{+/+}$ (13) *Trpm2*$^{-/-}$ (17)	Mice per group	Error bars are mean±SEM	Genotype x Stress interaction p=0.0092; Genotype p<0.0001; Stress p=0.0011 Bonferroni posttest: Home cage *Trpm2*$^{+/+}$ vs CUS *Trpm2*$^{+/+}$ p<0.001; CUS *Trpm2*$^{+/+}$ vs CUS *Trpm2*$^{-/-}$ p<0.001	Genotype x Stress interaction F(1,55)=7.286; Genotype F(1,55)=32.84; Stress F(1,55)=11.81
2A	Two-way ANOVA	***Trpm2***$^{+/+}$ Control (50), H_2O_2 500 µM (50), H_2O_2 1 mM (50), H_2O_2 10 mM (50); ***Trpm2***$^{-/-}$ Control (38), H_2O_2 500 µM (46), H_2O_2 1 mM (53), H_2O_2 10 mM (51)	Cells per group	Error bars are mean±SEM	Genotype x Durg interaction p<0.0001; Genotype p<0.0001; Durg p<0.0001 Bonferroni posttest: ***Trpm2***$^{+/+}$ Control vs 500 µM p<0.001; ***Trpm2***$^{+/+}$ Control vs 1 mM p<0.001; ***Trpm2***$^{+/+}$ Control vs 10 mM p<0.001; ***Trpm2***$^{-/-}$ Control vs 10 mM p<0.001	Genotype x Drug interaction F(3,380)=45.17; Genotype F(1,380)=1,057; Drug F(3,380)=107.6
2B	Two-way ANOVA	***Trpm2***$^{+/+}$ Control (50), H_2O_2 500 µM (50), H_2O_2 1 mM (50), DEXA 10 µM (50); ***Trpm2***$^{-/-}$ Control (50), H_2O_2 500 µM (50), H_2O_2 1 mM (50), DEXA 10 µM (50)	Cells per group	Error bars are mean±SEM	Genotype x Durg interaction p<0.0001; Genotype p<0.0001; Durg p<0.0001 Bonferroni posttest: ***Trpm2***$^{+/+}$ Control vs H_2O_2 500 µM p<0.001; ***Trpm2***$^{+/+}$ Control vs H_2O_2 1 mM p<0.001; ***Trpm2***$^{+/+}$ Control vs DEXA 10 µM p<0.001	Genotype x Drug interaction F(3,392)=96.43; Genotype F(1,392)=447.3; Drug F(3,392)=94.67
2C	Student's	*Trpm2*$^{+/+}$ (3);	Mice per	Error bars are	p=0.0219	t(4)=2.905

	t-test	*Trpm2*$^{-/-}$ (3)	group	mean±SEM		
3B	Two-way ANOVA	**Home cage** *Trpm2*$^{+/+}$ (5), *Trpm2*$^{-/-}$ (4); **CUS** *Trpm2*$^{+/+}$ (5) *Trpm2*$^{-/-}$ (5)	Mice per group	Error bars are mean±SEM	Genotype x Stress interaction p=0.0144; Genotype p=0.001; Stress p=0.0099 Bonferroni posttest: Home cage *Trpm2*$^{+/+}$ vs CUS *Trpm2*$^{+/+}$ p<0.01; CUS *Trpm2*$^{+/+}$ vs CUS *Trpm2*$^{-/-}$ p<0.001	Genotype x Stress interaction F(1,15)=7.659; Genotype F(1,15)=16.52; Stress F(1,15)=8.716
3C	Two-way ANOVA	**Home cage** *Trpm2*$^{+/+}$ (4), *Trpm2*$^{-/-}$ (4); **CUS** *Trpm2*$^{+/+}$ (4) *Trpm2*$^{-/-}$ (4)	Mice per group	Error bars are mean±SEM	Genotype x Stress interaction p=0.1168; Genotype p<0.0001; Stress p=0.0016 Bonferroni posttest: Home cage *Trpm2*$^{+/+}$ vs CUS *Trpm2*$^{+/+}$ p<0.01; Home cage *Trpm2*$^{+/+}$ vs Home cage *Trpm2*$^{-/-}$ p<0.05; CUS *Trpm2*$^{+/+}$ vs CUS *Trpm2*$^{-/-}$ p<0.001	Genotype x Stress interaction F(1,12)=2.857; Genotype F(1,12)=40.88; Stress F(1,12)=16.39
	Unpaired two-tailed t test	**Home cage** *Trpm2*$^{+/+}$ (4), *Trpm2*$^{-/-}$ (4); **CUS** *Trpm2*$^{+/+}$ (4) *Trpm2*$^{-/-}$ (4)	Mice per group	Error bars are mean±SEM	Home cage *Trpm2*$^{+/+}$ vs CUS *Trpm2*$^{+/+}$ p=0.0027; Home cage *Trpm2*$^{+/+}$ vs Home cage *Trpm2*$^{-/-}$ p=0.0245; CUS *Trpm2*$^{+/+}$ vs CUS *Trpm2*$^{-/-}$ p=0.0086	Home cage *Trpm2*$^{+/+}$ vs CUS *Trpm2*$^{+/+}$ t(6)=−4.905; Home cage *Trpm2*$^{+/+}$ vs Home cage *Trpm2*$^{-/-}$ t(6)=2.983; CUS *Trpm2*$^{+/+}$ vs CUS *Trpm2*$^{-/-}$ t(3)=6.168
4A (SYN1)	Two-way ANOVA	**DMSO** *Trpm2*$^{+/+}$ (3), *Trpm2*$^{-/-}$ (3); **Roscovitine** *Trpm2*$^{+/+}$ (3) *Trpm2*$^{-/-}$ (3)	Mice per group	Error bars are mean±SEM	Genotype x Durg interaction p=0.1304; Genotype p=0.0025; Durg p=0.0110 Bonferroni posttest: ***Trpm2***$^{-/-}$ DMSO vs Roscovitine p<0.05; **DMSO** *Trpm2*$^{+/+}$ vs *Trpm2*$^{-/-}$ p<0.01	Genotype x Drug interaction F(1,8)=2.840; Genotype F(1,8)=18.88; Drug F(1,8)=10.84
	Unpaired two-tailed t test	**DMSO** *Trpm2*$^{+/+}$ (3), *Trpm2*$^{-/-}$ (3); **Roscovitine** *Trpm2*$^{+/+}$ (3) *Trpm2*$^{-/-}$ (3)	Mice per group	Error bars are mean±SEM	***Trpm2***$^{-/-}$ DMSO vs Roscovitine p=0.0174; **DMSO** *Trpm2*$^{+/+}$ vs *Trpm2*$^{-/-}$ p=0.0003	***Trpm2***$^{-/-}$ DMSO vs Roscovitine t(4)=3.907 **DMSO** *Trpm2*$^{+/+}$ vs *Trpm2*$^{-/-}$ t(4)=−11.468
4A (HDAC5)	Two-way ANOVA	**DMSO** *Trpm2*$^{+/+}$ (3), *Trpm2*$^{-/-}$ (3); **Roscovitine** *Trpm2*$^{+/+}$ (3) *Trpm2*$^{-/-}$ (3)	Mice per group	Error bars are mean±SEM	Genotype x Durg interaction p=0.1016; Genotype p=0.1195; Durg p=0.0011 Bonferroni posttest: ***Trpm2***$^{-/-}$ DMSO vs Roscovitine p<0.01	Genotype x Drug interaction F(1,8)=3.420; Genotype F(1,8)=3.038; Drug F(1,8)=24.83

	Unpaired two-tailed t test	**DMSO** *Trpm2*$^{+/+}$ (3), *Trpm2*$^{-/-}$ (3); **Roscovitine** *Trpm2*$^{+/+}$ (3) *Trpm2*$^{-/-}$ (3)	Mice per group	Error bars are mean±SEM	***Trpm2***$^{+/+}$ DMSO vs Roscovitine p=0.0438; ***Trpm2***$^{-/-}$ DMSO vs Roscovitine p=0.0154; **DMSO** *Trpm2*$^{+/+}$ vs *Trpm2*$^{-/-}$ p=0.0312	***Trpm2***$^{+/+}$ DMSO vs Roscovitine t(4)=2.906; ***Trpm2***$^{-/-}$ DMSO vs Roscovitine t(4)=4.0559; **DMSO** *Trpm2*$^{+/+}$ vs *Trpm2*$^{-/-}$ t(4)=−3.256
4B (SYN1)	Two-way ANOVA	**Home cage** *Trpm2*$^{+/+}$ (3), *Trpm2*$^{-/-}$ (3); **CUS** *Trpm2*$^{+/+}$ (3) *Trpm2*$^{-/-}$ (3)	Mice per group	Error bars are mean±SEM	Genotype x Stress interaction p=0.0583; Genotype p<0.0001; Stress p=0.0017 Bonferroni posttest: Home cage *Trpm2*$^{+/+}$ vs CUS *Trpm2*$^{+/+}$ p<0.01; Home cage *Trpm2*$^{+/+}$ vs Home cage *Trpm2*$^{-/-}$ p<0.01; CUS *Trpm2*$^{+/+}$ vs CUS *Trpm2*$^{-/-}$ p<0.001	Genotype x Stress interaction F(1,8)=4.875; Genotype F(1,8)=68.23; Stress F(1,8)=21.38
	Unpaired two-tailed t test	**Home cage** *Trpm2*$^{+/+}$ (3), *Trpm2*$^{-/-}$ (3); **CUS** *Trpm2*$^{+/+}$ (3) *Trpm2*$^{-/-}$ (3)	Mice per group	Error bars are mean±SEM	Home cage *Trpm2*$^{+/+}$ vs CUS *Trpm2*$^{+/+}$ p=0.0483; Home cage *Trpm2*$^{+/+}$ vs Home cage *Trpm2*$^{-/-}$ p=0.0371; CUS *Trpm2*$^{+/+}$ vs CUS *Trpm2*$^{-/-}$ p=0.0028	Home cage *Trpm2*$^{+/+}$ vs CUS *Trpm2*$^{+/+}$ t(2)=4.385; Home cage *Trpm2*$^{+/+}$ vs Home cage *Trpm2*$^{-/-}$ t(2)=−5.047; CUS *Trpm2*$^{+/+}$ vs CUS *Trpm2*$^{-/-}$ t(4)=−6.540
4B (HDAC5)	Two-way ANOVA	**Home cage** *Trpm2*$^{+/+}$ (3), *Trpm2*$^{-/-}$ (3); **CUS** *Trpm2*$^{+/+}$ (3) *Trpm2*$^{-/-}$ (3)	Mice per group	Error bars are mean±SEM	Genotype x Stress interaction p=0.0773; Genotype p=0.0008; Stress p=0.1420 Bonferroni posttest: CUS *Trpm2*$^{+/+}$ vs CUS *Trpm2*$^{-/-}$ p<0.01	Genotype x Stress interaction F(1,8)=4.105; Genotype F(1,8)=27.44; Stress F(1,8)=2.653
	Unpaired two-tailed t test	**Home cage** *Trpm2*$^{+/+}$ (3), *Trpm2*$^{-/-}$ (3); **CUS** *Trpm2*$^{+/+}$ (3) *Trpm2*$^{-/-}$ (3)	Mice per group	Error bars are mean±SEM	Home cage *Trpm2*$^{+/+}$ vs CUS *Trpm2*$^{+/+}$ p=0.0215; CUS *Trpm2*$^{+/+}$ vs CUS *Trpm2*$^{-/-}$ p=0.0024	Home cage *Trpm2*$^{+/+}$ vs CUS *Trpm2*$^{+/+}$ t(4)=3.667; CUS *Trpm2*$^{+/+}$ vs CUS *Trpm2*$^{-/-}$ t(6)=−6.848
4D	Unpaired two-tailed t test	**Home cage** *Trpm2*$^{+/+}$ (4), *Trpm2*$^{-/-}$ (4); **CUS** *Trpm2*$^{+/+}$ (3) *Trpm2*$^{-/-}$ (3) (All values from repeatedly measurement were included in statistical test; n = 66)	Mice per group	Error bars are mean±SEM	Home cage *Trpm2*$^{+/+}$ vs Home cage *Trpm2*$^{-/-}$ p<0.0001; Home cage *Trpm2*$^{+/+}$ vs CUS *Trpm2*$^{+/+}$ p<0.0001; CUS *Trpm2*$^{+/+}$ vs CUS *Trpm2*$^{-/-}$ p<0.0001	Home cage *Trpm2*$^{+/+}$ vs Home cage *Trpm2*$^{-/-}$ t(103)=−4.140; Home cage *Trpm2*$^{+/+}$ vs CUS *Trpm2*$^{+/+}$ t(103)=5.302; CUS *Trpm2*$^{+/+}$ vs CUS *Trpm2*$^{-/-}$ t(108)=−5.925
4E	Unpaired two-tailed t	**Home cage** *Trpm2*$^{+/+}$ (4),	Mice per group	Error bars are mean±SEM	Home cage *Trpm2*$^{+/+}$ vs Home cage	Home cage *Trpm2*$^{+/+}$ vs Home cage

	test	*Trpm2*$^{-/-}$ (4); **CUS** *Trpm2*$^{+/+}$ (3) *Trpm2*$^{-/-}$ (3) (6 to 8 cells per group)			*Trpm2*$^{-/-}$ p<0.0001; Home cage *Trpm2*$^{+/+}$ vs CUS *Trpm2*$^{+/+}$ p=0.0233; CUS *Trpm2*$^{+/+}$ vs CUS *Trpm2*$^{-/-}$ p<0.0001	*Trpm2*$^{-/-}$ t(12)=−7.887; Home cage *Trpm2*$^{+/+}$ vs CUS *Trpm2*$^{+/+}$ t(6)=3.023; CUS *Trpm2*$^{+/+}$ vs CUS *Trpm2*$^{-/-}$ t(10)=−18.922
5D (Cdk5)	Two-way ANOVA	***Trpm2***$^{+/+}$ shLuc (3), shCdk5 (3); ***Trpm2***$^{-/-}$ shLuc (3), shCdk5 (3)	Mice per group	Error bars are mean±SEM	Genotype x Knockdown interaction p=0.3287; Genotype p=0.1442; Knockdown p=0.0001 Bonferroni posttest: shLuc *Trpm2*$^{+/+}$ vs shCdk5 *Trpm2*$^{+/+}$ p<0.01; shLuc *Trpm2*$^{-/-}$ vs shCdk5 *Trpm2*$^{-/-}$ p<0.01	Genotype x Knockdown interaction F(1,8)=1.082; Genotype F(1,8)=2.62; Knockdown F(1,8)=45.32
	Unpaired two-tailed t test	***Trpm2***$^{+/+}$ shLuc (3), shCdk5 (3); ***Trpm2***$^{-/-}$ shLuc (3), shCdk5 (3)	Mice per group	Error bars are mean±SEM	shLuc *Trpm2*$^{+/+}$ vs shLuc *Trpm2*$^{-/-}$ p=0.0256; shLuc *Trpm2*$^{+/+}$ vs shCdk5 *Trpm2*$^{+/+}$ p=0.0284; shLuc *Trpm2*$^{-/-}$ vs shCdk5 *Trpm2*$^{-/-}$ p=0.0018	shLuc *Trpm2*$^{+/+}$ vs shLuc *Trpm2*$^{-/-}$ t(4)=−3.468; shLuc *Trpm2*$^{+/+}$ vs shCdk5 *Trpm2*$^{+/+}$ t(4)=3.356; shLuc *Trpm2*$^{-/-}$ vs shCdk5 *Trpm2*$^{-/-}$ t(4)=7.331
5D (p35)	Two-way ANOVA	***Trpm2***$^{+/+}$ shLuc (3), shCdk5 (3); ***Trpm2***$^{-/-}$ shLuc (3), shCdk5 (3)	Mice per group	Error bars are mean±SEM	Genotype x Knockdown interaction p=0.0923; Genotype p=0.1153; Knockdown p=0.0004 Bonferroni posttest: shLuc *Trpm2*$^{-/-}$ vs shCdk5 *Trpm2*$^{-/-}$ p<0.01	Genotype x Knockdown interaction F(1,8)=3.654; Genotype F(1,8)=3.121; Knockdown F(1,8)=33.48
	Unpaired two-tailed t test	***Trpm2***$^{+/+}$ shLuc (3), shCdk5 (3); ***Trpm2***$^{-/-}$ shLuc (3), shCdk5 (3)	Mice per group	Error bars are mean±SEM	shLuc *Trpm2*$^{+/+}$ vs shCdk5 *Trpm2*$^{+/+}$ p=0.0137; shLuc *Trpm2*$^{-/-}$ vs shCdk5 *Trpm2*$^{-/-}$ p=0.0123	shLuc *Trpm2*$^{+/+}$ vs shCdk5 *Trpm2*$^{+/+}$ t(4)=4.203; shLuc *Trpm2*$^{-/-}$ vs shCdk5 *Trpm2*$^{-/-}$ t(4)=4.337
5D (SYN1)	Two-way ANOVA	***Trpm2***$^{+/+}$ shLuc (3), shCdk5 (3); ***Trpm2***$^{-/-}$ shLuc (3), shCdk5 (3)	Mice per group	Error bars are mean±SEM	Genotype x Knockdown interaction p=0.0601; Genotype p=0.1803; Knockdown p=0.0014 Bonferroni posttest: shLuc *Trpm2*$^{-/-}$ vs shCdk5 *Trpm2*$^{-/-}$ p<0.01	Genotype x Knockdown interaction F(1,8)=4.789; Genotype F(1,8)=2.155; Knockdown F(1,8)=22.74
	Unpaired two-tailed t test	***Trpm2***$^{+/+}$ shLuc (3), shCdk5 (3); ***Trpm2***$^{-/-}$ shLuc (3), shCdk5 (3)	Mice per group	Error bars are mean±SEM	shLuc *Trpm2*$^{+/+}$ vs shLuc *Trpm2*$^{-/-}$ p=0.0064; shLuc *Trpm2*$^{-/-}$ vs shCdk5 *Trpm2*$^{-/-}$ p<0.0001	shLuc *Trpm2*$^{+/+}$ vs shLuc *Trpm2*$^{-/-}$ t(4)=−5.234; shLuc *Trpm2*$^{-/-}$ vs shCdk5 *Trpm2*$^{-/-}$ t(4)=30.183
5D	Two-way	***Trpm2***$^{+/+}$	Mice per	Error bars are	Genotype x	Genotype x

(HDAC5)	ANOVA	shLuc (3), shCdk5 (3); ***Trpm2***$^{-/-}$ shLuc (3), shCdk5 (3)	group	mean±SEM	Knockdown interaction p=0.003; Genotype p=0.0097; Knockdown p=0.0002 Bonferroni posttest: shLuc *Trpm2*$^{+/+}$ vs shLuc *Trpm2*$^{-/-}$ p<0.01; shLuc *Trpm2*$^{-/-}$ vs shCdk5 *Trpm2*$^{-/-}$ p<0.001	Knockdown interaction F(1,8)=17.62; Genotype F(1,8)=11.42; Knockdown F(1,8)=42.45
	Unpaired two-tailed t test	***Trpm2***$^{+/+}$ shLuc (3), shCdk5 (3); ***Trpm2***$^{-/-}$ shLuc (3), shCdk5 (3)	Mice per group	Error bars are mean±SEM	shLuc *Trpm2*$^{+/+}$ vs shLuc *Trpm2*$^{-/-}$ p=0.005; shLuc *Trpm2*$^{-/-}$ vs shCdk5 *Trpm2*$^{-/-}$ p=0.0029	shLuc *Trpm2*$^{+/+}$ vs shLuc *Trpm2*$^{-/-}$ t(4)=−5.588; shLuc *Trpm2*$^{-/-}$ vs shCdk5 *Trpm2*$^{-/-}$ t(4)=6.472
5F	Two-way ANOVA	***Trpm2***$^{+/+}$ shLuc (13), shCdk5 (16); ***Trpm2***$^{-/-}$ shLuc (14), shCdk5 (13)	Mice per group	Error bars are mean±SEM	Genotype x Knockdown interaction p=0.0015; Genotype p=0.0383; Knockdown p=0.0698 Bonferroni posttest: shLuc *Trpm2*$^{+/+}$ vs shLuc *Trpm2*$^{-/-}$ p<0.001; shLuc *Trpm2*$^{-/-}$ vs shCdk5 *Trpm2*$^{-/-}$ p<0.01	Genotype x Knockdown interaction F(1,52)=11.19; Genotype F(1,52)=4.52; Knockdown F(1,52)=3.428
5G	Two-way ANOVA	***Trpm2***$^{+/+}$ shLuc (12), shCdk5 (17); ***Trpm2***$^{-/-}$ shLuc (14), shCdk5 (13)	Mice per group	Error bars are mean±SEM	Genotype x Knockdown interaction p=0.0026; Genotype p=0.7032; Knockdown p=0.1292 Bonferroni posttest: shLuc *Trpm2*$^{+/+}$ vs shLuc *Trpm2*$^{-/-}$ p<0.05; shLuc *Trpm2*$^{-/-}$ vs shCdk5 *Trpm2*$^{-/-}$ p<0.01	Genotype x Knockdown interaction F(1,52)=10.03; Genotype F(1,52)=0.1467; Knockdown F(1,52)=2.377
5H	Two-way ANOVA	***Trpm2***$^{+/+}$ shLuc (8), shCdk5 (8); ***Trpm2***$^{-/-}$ shLuc (8), shCdk5 (8)	Mice per group	Error bars are mean±SEM	Genotype x Knockdown interaction p=0.4445; Genotype p=0.9258; Knockdown p=0.0007 Bonferroni posttest: shLuc *Trpm2*$^{-/-}$ vs shCdk5 *Trpm2*$^{-/-}$ p<0.01	Genotype x Knockdown interaction F(1,28)=0.6017; Genotype F(1,28)=0.0088; Knockdown F(1,28)=14.41
	Unpaired two-tailed t test	***Trpm2***$^{+/+}$ shLuc (8), shCdk5 (8); ***Trpm2***$^{-/-}$ shLuc (8), shCdk5 (8)	Mice per group	Error bars are mean±SEM	shLuc *Trpm2*$^{+/+}$ vs shCdk5 *Trpm2*$^{+/+}$ p=0.014; shLuc *Trpm2*$^{-/-}$ vs shCdk5 *Trpm2*$^{-/-}$ p=0.0319	shLuc *Trpm2*$^{+/+}$ vs shCdk5 *Trpm2*$^{+/+}$ t(11)=−2.916; shLuc *Trpm2*$^{-/-}$ vs shCdk5 *Trpm2*$^{-/-}$ t(7)=−2.672
S2B	Two-way ANOVA	**Hippocampus** *Trpm2*$^{+/+}$ (2), *Trpm2*$^{-/-}$ (2); **DG** *Trpm2*$^{+/+}$ (3)	Mice per group	Error bars are mean±SEM	Genotype x Sub-region interaction p=0.0011; Genotype p<0.0001; Sub-region p=0.0016	Genotype x Sub-region interaction F(1,7)=28.01; Genotype F(1,7)=89.63; Sub-

		Trpm2$^{-/-}$ (4)			Bonferroni posttest: Hippocampus *Trpm2*$^{+/+}$ vs Hippocampus *Trpm2*$^{-/-}$ p<0.001; DG *Trpm2*$^{+/+}$ vs DG *Trpm2*$^{-/-}$ p<0.05; Hippocampus *Trpm2*$^{+/+}$ vs DG *Trpm2*$^{+/+}$ p<0.001	region F(1,7)=24.95
S2F (block2)	Unpaired two-tailed t test	**Home cage** *Trpm2*$^{+/+}$ (15), *Trpm2*$^{-/-}$ (15)	Mice per group	Error bars are mean±SEM	Home cage *Trpm2*$^{+/+}$ vs Home cage *Trpm2*$^{-/-}$ p=0.0355	Home cage *Trpm2*$^{+/+}$ vs Home cage *Trpm2*$^{-/-}$ t(28)= −2.2097
S2F (block3)	Unpaired two-tailed t test	**Home cage** *Trpm2*$^{+/+}$ (15), *Trpm2*$^{-/-}$ (15)	Mice per group	Error bars are mean±SEM	Home cage *Trpm2*$^{+/+}$ vs Home cage *Trpm2*$^{-/-}$ p=0.0089	Home cage *Trpm2*$^{+/+}$ vs Home cage *Trpm2*$^{-/-}$ t(28)= −2.8135
S2G (block2)	Unpaired two-tailed t test	**Home cage** *Trpm2*$^{+/+}$ (15), *Trpm2*$^{-/-}$ (15)	Mice per group	Error bars are mean±SEM	Home cage *Trpm2*$^{+/+}$ vs Home cage *Trpm2*$^{-/-}$ p=0.0352	Home cage *Trpm2*$^{+/+}$ vs Home cage *Trpm2*$^{-/-}$ t(28)= −2.2139
S2G (block3)	Unpaired two-tailed t test	**Home cage** *Trpm2*$^{+/+}$ (15), *Trpm2*$^{-/-}$ (15)	Mice per group	Error bars are mean±SEM	Home cage *Trpm2*$^{+/+}$ vs Home cage *Trpm2*$^{-/-}$ p=0.00923	Home cage *Trpm2*$^{+/+}$ vs Home cage *Trpm2*$^{-/-}$ t(28)= −2.7966
S2H (block4)	Unpaired two-tailed t test	**Home cage** *Trpm2*$^{+/+}$ (15), *Trpm2*$^{-/-}$ (15)	Mice per group	Error bars are mean±SEM	Home cage *Trpm2*$^{+/+}$ vs Home cage *Trpm2*$^{-/-}$ p=0.00875	Home cage *Trpm2*$^{+/+}$ vs Home cage *Trpm2*$^{-/-}$ t(19)= −2.9218
S4B	Student's t-test	*Trpm2*$^{+/+}$ (3); *Trpm2*$^{-/-}$ (3)	Mice per group	Error bars are mean±SEM	p=0.0090	t(4)=−3.873
S4D	Two-way ANOVA	**Home cage** *Trpm2*$^{+/+}$ (5), *Trpm2*$^{-/-}$ (5); **CUS** *Trpm2*$^{+/+}$ (4) *Trpm2*$^{-/-}$ (5)	Mice per group	Error bars are mean±SEM	Genotype x Stress interaction p=0.0371; Genotype p<0.0001; Stress p=0.0041 Bonferroni posttest: Home cage *Trpm2*$^{+/+}$ vs CUS *Trpm2*$^{+/+}$ p<0.01; Home cage *Trpm2*$^{+/+}$ vs Home cage *Trpm2*$^{-/-}$ p<0.05; CUS *Trpm2*$^{+/+}$ vs CUS *Trpm2*$^{-/-}$ p<0.001	Genotype x Stress interaction F(1,15)=5.231; Genotype F(1,15)=40.27; Stress F(1,15)=11.46
S4H	Student's t-test	*Trpm2*$^{+/+}$ (5); *Trpm2*$^{-/-}$ (5)	Mice per group	Error bars are mean±SEM	p=0.02897	t(8)=−2.212
S4I	Student's t-test	*Trpm2*$^{+/+}$ (5); *Trpm2*$^{-/-}$ (5)	Mice per group	Error bars are mean±SEM	p=0.02680	t(8)=−2.262
S5A (Cdk5)	Student's t-test	*Trpm2*$^{+/+}$ (2); *Trpm2*$^{-/-}$ (2)	Mice per group	Error bars are mean±SEM	p=0.0172	t(2)=−5.250
S5A (p25/p35)	Student's t-test	*Trpm2*$^{+/+}$ (2); *Trpm2*$^{-/-}$ (2)	Mice per group	Error bars are mean±SEM	p=0.0353	t(2)=3.560
S5B	Student's t-test	*Trpm2*$^{+/+}$ (3); *Trpm2*$^{-/-}$ (3)	Mice per group	Error bars are mean±SEM	p=0.0069	t(4)=−4.191
S5C	Student's t-test	*Trpm2*$^{+/+}$ (5); *Trpm2*$^{-/-}$ (5)	Mice per group	Error bars are mean±SEM	p=0.0016	t(8)=−4.144
S6A (Cdk5)	Student's t-test	*Trpm2*$^{+/+}$ (3); *Trpm2*$^{-/-}$ (3)	Mice per group	Error bars are mean±SEM	p=0.0033	t(4)=−5.198
S6A (p35)	Student's t-test	*Trpm2*$^{+/+}$ (3); *Trpm2*$^{-/-}$ (3)	Mice per group	Error bars are mean±SEM	p=0.0002	t(4)=−10.784
S6B (GSK3β)	Student's t-test	*Trpm2*$^{+/+}$ (7); *Trpm2*$^{-/-}$ (7)	Mice per group	Error bars are mean±SEM	p=0.0324	t(12)=−2.032

S7A (p25/p35)	One-way ANOVA	***Trpm2***$^{+/+}$ Control (4); H_2O_2 700 μM (3); H_2O_2 1 mM (4); H_2O_2 10 mM (3)	Mice per group	Error bars are mean±SEM	p=0.0003 Bonferroni posttest: *Trpm2*$^{+/+}$ Control vs 1 mM p<0.05; *Trpm2*$^{+/+}$ Control vs 10 mM p<0.001	F(3,10)=16.62
S7B (SYN1)	One-way ANOVA	***Trpm2***$^{+/+}$ Control (3); H_2O_2 700 μM (3); H_2O_2 1 mM (3); H_2O_2 10 mM (3)	Mice per group	Error bars are mean±SEM	p<0.0001 Bonferroni posttest: ***Trpm2***$^{+/+}$ Control vs 1 mM p<0.05; ***Trpm2***$^{+/+}$ Control vs 10 mM p<0.001	F(3,8)=33.72
S7B (HDAC5)	One-way ANOVA	***Trpm2***$^{+/+}$ Control (3); H_2O_2 700 μM (3); H_2O_2 1 mM (3); H_2O_2 10 mM (3)	Mice per group	Error bars are mean±SEM	p=0.017 Bonferroni posttest: ***Trpm2***$^{+/+}$ Control vs 10 mM p<0.05	F(3,8)=6.274
S8A	Student's t-test	*Trpm2*$^{+/+}$ (3); *Trpm2*$^{-/-}$ (3)	Mice per group	Error bars are mean±SEM	p=0.0004	t(4)=8.933
S8B	Two-way ANOVA	**DMSO** *Trpm2*$^{+/+}$ (3), *Trpm2*$^{-/-}$ (3); **Roscovitine** *Trpm2*$^{+/+}$ (3) *Trpm2*$^{-/-}$ (3)	Mice per group	Error bars are mean±SEM	Genotype x Durg interaction p=0.6927; Genotype p=0.5582; Durg p=0.0022 Bonferroni posttest: ***Trpm2***$^{+/+}$ DMSO vs Roscovitine p<0.05; ***Trpm2***$^{-/-}$ DMSO vs Roscovitine p<0.05	Genotype x Drug interaction F(1,8)=0.168; Genotype F(1,8)=0.3731; Drug F(1,8)=19.53
	Unpaired two-tailed t test	**DMSO** *Trpm2*$^{+/+}$ (3), *Trpm2*$^{-/-}$ (3); **Roscovitine** *Trpm2*$^{+/+}$ (3) *Trpm2*$^{-/-}$ (3)	Mice per group	Error bars are mean±SEM	***Trpm2***$^{+/+}$ DMSO vs Roscovitine p=0.0354; ***Trpm2***$^{-/-}$ DMSO vs Roscovitine p=0.0346	***Trpm2***$^{+/+}$ DMSO vs Roscovitine t(4)=−3.123; ***Trpm2***$^{-/-}$ DMSO vs Roscovitine t(4)=−3.149
S9B (SYN1)	One-way ANOVA	No infection (2); shLuc (2); shTRPM2 1 (2), 5 (2), 25 (2)	Mice per group	Error bars are mean±SEM	p=0.0096 Bonferroni posttest: No infection vs shTRPM2 25 p<0.05	F(4,5)=11.61
S9B (HDAC5)	One-way ANOVA	No infection (3); shLuc (2); shTRPM2 1 (3), 5 (2), 25 (2)	Mice per group	Error bars are mean±SEM	p=0.0064 Bonferroni posttest: No infection vs shTRPM2 5 p<0.05; No infection vs shTRPM2 25 p<0.05	F(4,7)=9.198
S10A (NR1)	Student's t-test	*Trpm2*$^{+/+}$ (7); *Trpm2*$^{-/-}$ (7)	Mice per group	Error bars are mean±SEM	p=0.0064	t(12)= −2.918
S10A (NR2B)	Student's t-test	*Trpm2*$^{+/+}$ (6); *Trpm2*$^{-/-}$ (6)	Mice per group	Error bars are mean±SEM	p=0.0042	t(10)= −3.275

S10A (GluR2)	Student's t-test	*Trpm2*$^{+/+}$ (6); *Trpm2*$^{-/-}$ (6)	Mice per group	Error bars are mean±SEM	p=0.0001	t(10)= −5.444
S10A (Shank2)	Student's t-test	*Trpm2*$^{+/+}$ (6); *Trpm2*$^{-/-}$ (6)	Mice per group	Error bars are mean±SEM	p=0.0017	t(10)= −3.824
S11A	Student's t-test	shLuc (2); shCdk5 (2)	Mice per group	Error bars are mean±SEM	p=0.0285	t(2)=−4.004
S13B	Unpaired two-tailed t test	**CUS *Trpm2*$^{+/+}$** shLuc (7), shCdk5 (7); **CUS *Trpm2*$^{-/-}$** shLuc (6), shCdk5 (8)	Mice per group	Error bars are mean±SEM	CUS shLuc *Trpm2*$^{-/-}$ vs CUS shCdk5 *Trpm2*$^{-/-}$ p=0.0635	CUS shLuc *Trpm2*$^{-/-}$ vs CUS shCdk5 *Trpm2*$^{-/-}$ t(12)=2.045
S13C	Unpaired two-tailed t test	**CUS *Trpm2*$^{+/+}$** shLuc (7), shCdk5 (7); **CUS *Trpm2*$^{-/-}$** shLuc (6), shCdk5 (8)	Mice per group	Error bars are mean±SEM	CUS shLuc *Trpm2*$^{-/-}$ vs CUS shCdk5 *Trpm2*$^{-/-}$ p=0.0298	CUS shLuc *Trpm2*$^{-/-}$ vs CUS shCdk5 *Trpm2*$^{-/-}$ t(12)=−2.464
S13D	Unpaired two-tailed t test	**CUS *Trpm2*$^{+/+}$** shLuc (7), shCdk5 (7); **CUS *Trpm2*$^{-/-}$** shLuc (6), shCdk5 (8)	Mice per group	Error bars are mean±SEM	CUS shLuc *Trpm2*$^{+/+}$ vs CUS shLuc *Trpm2*$^{-/-}$ p=0.0017; CUS shLuc *Trpm2*$^{-/-}$ vs CUS shCdk5 *Trpm2*$^{-/-}$ p=0.0004	CUS shLuc *Trpm2*$^{+/+}$ vs CUS shLuc *Trpm2*$^{-/-}$ t(11)=4.134; CUS shLuc *Trpm2*$^{-/-}$ vs CUS shCdk5 *Trpm2*$^{-/-}$ t(12)=−4.789
S13E	Unpaired two-tailed t test	**CUS *Trpm2*$^{+/+}$** shLuc (7), shCdk5 (7); **CUS *Trpm2*$^{-/-}$** shLuc (6), shCdk5 (8)	Mice per group	Error bars are mean±SEM	CUS shLuc *Trpm2*$^{+/+}$ vs CUS shLuc *Trpm2*$^{-/-}$ p=0.014; CUS shLuc *Trpm2*$^{-/-}$ vs CUS shCdk5 *Trpm2*$^{-/-}$ p=0.0085	CUS shLuc *Trpm2*$^{+/+}$ vs CUS shLuc *Trpm2*$^{-/-}$ t(7)=3.254; CUS shLuc *Trpm2*$^{-/-}$ vs CUS shCdk5 *Trpm2*$^{-/-}$ t(8)=−3.466

Table S2. Experimental schedule for the CUS procedure in mice

Stressors / Duration	Day
Restraint / 1 h	1, 6, 10, 15, 18
Light on / Overnight	2, 7, 9, 16, 24
Cage tilt / Overnight	1, 11, 15, 20, 26
Cage rotation (overcrowding) / 1 h	2, 9, 14, 22, 28
Wet bedding (overcrowding) / Overnight	5, 12, 14, 18, 22
Light off / 3 h	4, 7, 11, 14, 16, 17, 21, 22, 24
Strobe / Overnight	6, 10, 17, 21, 23, 27
Cold (4 °C) / 1 h	3, 5, 13, 16, 20, 27
Food deprivation / Overnight	3, 8, 13, 19, 25, 28
Overcrowding / Overnight	4, 9, 22
Different partner / 3 h	8, 12, 19, 23, 26

Table S3. Primary antibodies used in western blotting and immunohistochemistry

Antigen	Manufacturer	Host, Clonality	Catalog number	Dilution
TRPM2	Novus Biologicals	Rabbit polyclonal	NB110-82364	1:1,000
PAR	Santa Cruz Biotechnology	Mouse monoclonal	sc-71848	1:1,000
Cdk5	Abcam	Mouse monoclonal	ab28441	1:1,000
Cdk5	Santa Cruz Biotechnology	Rabbit polyclonal	sc-173	1:1,000
p35/p25	Santa Cruz Biotechnology	Rabbit polyclonal	sc-820	1:1,000
Spectrin αII	Santa Cruz Biotechnology	Mouse monoclonal	sc-46696	1:1,000
p-Synapsin1 (Ser553)	Santa Cruz Biotechnology	Goat polyclonal	sc-12913	1:1,000
Synapsin1	Cell Signaling Technology	Rabbit monoclonal	#5297	1:1,000
p-HDAC5 (Ser279)	A kind gift from Christopher W. Cowan (University of Texas Southwestern Medical Center, USA)			1:1,000
HDAC5	Cell Signaling Technology	Rabbit polyclonal	#2082	1:500
Nrf2	Santa Cruz Biotechnology	Rabbit polyclonal	sc-13032	1:1,000
LaminB1	Abcam	Rabbit polyclonal	ab16048	1:1,000
p-CaMKII (Thr286)	Cell Signaling Technology	Rabbit monoclonal	#12716	1:1,000
CaMKII	Cell Signaling Technology	Rabbit polyclonal	#3362	1:1,000
p-mTOR (Ser2448)	Cell Signaling Technology	Rabbit polyclonal	#2971	1:1,000
mTOR	Cell Signaling Technology	Rabbit monoclonal	#2983	1:1,000
p-PKD (Ser744/748)	Cell Signaling Technology	Rabbit polyclonal	#2054	1:500
PKD	ABM	Rabbit polyclonal	Y021126	1:1,000

p-ERK1/2 (Thr202/Tyr204)	Cell Signaling Technology	Mouse monoclonal	#9106	1:1,000
ERK1/2	Cell Signaling Technology	Rabbit polyclonal	#9102	1:1,000
p-Akt (Ser473)	Cell Signaling Technology	Rabbit polyclonal	#9271	1:1,000
Akt	Cell Signaling Technology	Rabbit polyclonal	#9272	1:1,000
p-GSK3β (Ser9)	Cell Signaling Technology	Rabbit polyclonal	#9336	1:1,000
GSK3β	Cell Signaling Technology	Rabbit monoclonal	#9315	1:1,000
p-Prx2 (Thr89)	A kind gift from David S. Park (University of Ottawa, Canada)			1:500
Prx2	R&D Systems	Mouse monoclonal	MAB3489	1:1,000
PSD95	Cell Signaling Technology	Rabbit polyclonal	#2507	1:1,000
NR1	Upstate	Mouse monoclonal	05-432	1:1,000
NR2B	Chemicon	Rabbit polyclonal	AB1557P	1:1,000
GluR1	Abcam	Rabbit polyclonal	Ab31232	1:1,000
GluR2	Millipore	Rabbit polyclonal	AB1768-I	1:1,000
Shank2	Santa Cruz Biotechnology	Rabbit polyclonal	sc-30192	1:500
βactin	Santa Cruz Biotechnology	Mouse monoclonal	sc-47778	1:2,000
GFP	Roche	Mouse monoclonal	11814460001	1:200
TRPM2	Novus Biologicals	Rabbit polyclonal	NB500-242	1:200
MAP2	Sigma Aldrich	Mouse monoclonal	M1406	1:500
BrdU	Abcam	Rat monoclonal	ab6326	1:300
NeuN	Chemicon	Mouse monoclonal	MAB377	1:200

GFAP	Dako	Rabbit polyclonal	Z0334	1:200
Calbindin	Santa Cruz Biotechnology	Mouse monoclonal	sc-365360	1:100

Table S4. Primer sequences for qPCR

Gene	5’ ------ Forward ------ 3’	5’ ------ Reverse ------ 3’
β-actin	AAGGCCAACCGTGAAAAGAT	GTGGTACGACCAGAGGCATAC
Trpm2	TTGGGGCCATTCTGCTCTTC	AGACGTCCTTCATCATCCGC
Trpm7	GGTTCCTCCTGTGGTGCCTT	CCCCATGTCGTCTCTGTCGT
Trpc3	GCCAAGCGACGGAGGAATTA	CAGCACACTGGGGTTCAGTT
Trpc5	GGAGATAAAGGAAATGTGGGATGGT	AATAGTTGCCAGGTAGAGGGAGT
Trpa1	TGCTGCAGAAAAAAATCAAGTTGA	CCTTGGCTGAGAAGAAACTTTACACT
Iba1	CAGACTGCCAGCCTAAGACA	AGGAATTGCTTGTTGATCCC
HMGB1	GGCTGACAAGGCTCGTTATG	GGGCGGTACTCAGAACAGAA
MMP9	CTTCTGGCGTGTGAGTTTCCA	ACTGCACGGTTGAAGCAAAGA
TNFα	TGGCCCAGACCCTCACACTCAG	ACCCATCGGCTGGCACCACT
COX-2	CCAGCACTTCACCCATCAGTT	ACCCAGGTCCTCGCTTATGA
IL-6	ATGGATGCTACCAAACTGGAT	TGAAGGACTCTGGCTTTGTCT

Table S5. Demographic information between the subjects with MDD and the controls

	Control	MDD	*p*-value
n	12	8	
Gender			1.000[a]
Male	6	4	
Female	6	4	
Age (years)			1.000[a]
≤ 50	7	5	
> 50	5	3	
PMI (hours) (mean ± SEM)	18.3 ± 5.3	21.7 ± 6.1	0.219[b]
pH (mean ± SEM)	6.7 ± 0.2	6.7 ± 0.3	0.799[c]
RIN (mean ± SEM)	7.5 ± 0.7	7.7 ± 0.7	0.588[b]

[a]Fisher's exact test; [b]Student's t-test; [c]Mann-Whitney test
MDD = Major depressive disorder; PMI = Postmortem interval; RIN = RNA Integrity Number

Supplemental Figure Legends

Figure S1. TRPM2 levels are increased in the hippocampus of patients with MDD. (A) Using the criteria described in Materials and Methods, we identified a total of 1,198 differentially-expressed genes (DEGs) in patients with MDD. Of these genes, 700 were up-regulated and 498 were down-regulated. The top 100 significantly up- or down-regulated genes are displayed in the heat-map. Blue, low expression; red, high expression. Red arrow indicated TRPM2. **(B)** Microarray analysis of post-mortem MDD hippocampal samples confirms the alterations in oxidative stress-sensitive TRP channels in the MDD hippocampus. **(C−G)** Box plots displaying the data shown in S1B. TRPM2 **(C)** is up-regulated in post-mortem hippocampal tissue from subjects with MDD. The expression of TRPM7 **(D)**, TRPA1 **(E)**, TRPC3 **(F)** and TRPC5 **(G)** is not changed. Using appropriate sample selection criteria, we performed gene expression analyses on 20 hippocampus samples from 8 patients with MDD and 12 controls. As shown in Table S5, there were no statistically significant differences in demographic characteristics between subjects and controls.

Figure S2. Molecular and behavioral characterization of *Trpm2*$^{-/-}$ mice. (A) PCR analysis for genotyping showed disruption of the *Trpm2* gene in *Trpm2*$^{-/-}$ mice. Lanes 1 − 2, PCR products from *Trpm2*$^{+/+}$; lanes 3 − 4, from *Trpm2*$^{+/-}$; lanes 5 − 6, from *Trpm2*$^{-/-}$ mice. **(B)** *Trpm2* mRNA levels were quantitated by real-time PCR. Expression of *Trpm2* mRNA was not detected in the whole hippocampus and DG of *Trpm2*$^{-/-}$ mice (n = 2 to 4 per group; genotype x sub-region interaction $F_{1,7} = 28.01$, $p = 0.0011$, two-way ANOVA followed by Bonferroni posttest). **(C)** Average speed, **(D)** total distance travelled and **(E)** time in the center zone were scored for locomotor activities over each 5 min period (n = 15 to 17 per group). CUS did not have any effects on locomotor activities of *Trpm2*$^{+/+}$ and *Trpm2*$^{-/-}$ mice. **(F)** Average speed, **(G)** total distance travelled and **(H)** time in the center zone were re-analyzed over 1 min blocks. Time segmentation showed that average speed and total distance travelled were slightly higher in *Trpm2*$^{-/-}$ mice than *Trpm2*$^{+/+}$ mice during the initial block of testing (n = 15 to 17 per group, unpaired two-tailed t test). Data are means ± SEM. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$. Other statistical parameters with significant differences are listed in Table S1.

Figure S3. Expression of TRPM2 in mouse hippocampal neurons. (A) Representative confocal images of DG neurons labeled with DAPI (blue), MAP2 (green) and TRPM2 (red). Co-localization of TRPM2 and MAP2 represents TRPM2 expression in *Trpm2*$^{+/+}$ DG neurons. Scale bar, 50 μm. **(B)** Representative confocal images of neural progenitor cells (NPCs) in the hippocampal DG labeled with TRPM2 (green) and BrdU (red). A double-labeled NPC was marked with *a white arrow* in the SGZ of DG. Scale bar, 20 μm. **(C)** High power confocal image of a cell (white arrow in B). A merged image indicates expression of TRPM2 in proliferating BrdU^{+} cells. The bottom and right panels showed images merged across the z- and x-axis, respectively. **(D)** Representative confocal images of cultured hippocampal neurons labeled with DAPI (blue), MAP2 (green) and TRPM2 (red). Co-localization of TRPM2 and MAP2 represents TRPM2 expression in *Trpm2*$^{+/+}$ mouse embryo hippocampal primary cultures. Scale bar, 20 μm.

Figure S4. TRPM2 deficiency enhances neurogenesis, but has no effect on astrocyte differentiation and inflammation in the adult mouse hippocampus. (A) Experimental paradigm for proliferation of NPCs. Mice were sacrificed 1 day after the last BrdU administration. **(B)** Proliferating $BrdU^+$ cells (red) in the SGZ of DG (left). TRPM2 deficiency increased the number of $BrdU^+$ cells in the SGZ (right) (n = 3 per group, student's t test). Scale bar, 200 μm. **(C)** Timeline of experimental procedures for CUS and BrdU administration to evaluate survival of NPCs. **(D)** Mature neurons were double-labeled (white arrow) with $NeuN^+$ (green) $BrdU^+$ (red) in the granule cell layer (GCL) of DG (left). CUS significantly reduced the survival of NPCs in $Trpm2^{+/+}$ mice, but not in $Trpm2^{-/-}$ mice (right) (n = 4 to 5 per group; genotype x stress interaction $F_{1,15} = 5.231$, $p = 0.0371$, two-way ANOVA followed by Bonferroni posttest). Scale bar, 50 μm. **(E)** Experimental paradigm for survival of NPCs. Mice were sacrificed 12 days after the last BrdU administration. **(F)** NPCs differentiated to astrocytes were double-labeled with $GFAP^+$ (green) $BrdU^+$ (red) in the DG (left). There was no significant difference in the ratio of NPCs to astrocyte differentiation between $Trpm2^{+/+}$ and $Trpm2^{-/-}$ mice (right) (n = 3 per group). Scale bar, 100 μm. **(G)** Hippocampal mRNA levels of genes related to glia and inflammation were quantitated by real-time PCR. There were no significant differences between $Trpm2^{+/+}$ and $Trpm2^{-/-}$ mice (n = 2 to 3 per group). **(H)** Mature granule cells were double-labeled with $BrdU^+$ (red) and $calbindin_{D28k}{}^+$ (green) in the GCL of the DG (left). Increased colocalization of BrdU with $calbindin_{D28k}$ was seen in $Trpm2^{-/-}$ mice (right) (n = 5 per group). Scale bar, 20 μm. **(I)** The GCL area was outlined on mice hippocampal section (left). Quantification of the GCL volume (right). The volume of GCL was higher in $Trpm2^{-/-}$ mice than $Trpm2^{+/+}$ mice (n = 5 per group). Scale bar, 200 μm. Data are means ± SEM. *$p < 0.05$, **$p < 0.01$, ***$p <$ 0.001. Other statistical parameters with significant differences are listed in Table S1.

Figure S5. TRPM2 deficiency enhances Cdk5 activity. (A) Representative immunoblots of the hippocampal DG lysates (left). TRPM2 deficiency increased Cdk5 protein levels and reduced cleavage of p35 to p25 quantified as the p25/p35 ratio (right) (n = 2 per group, Student's t test). **(B)** Endogenous Cdk5 was immunoprecipitated from the hippocampi of *Trpm2*$^{+/+}$ and *Trpm2*$^{-/-}$ mice, and immunoblots were probed with the indicated antibodies (left). Cdk5 and p35 interaction was enhanced in *Trpm2*$^{-/-}$ mice (right) (n = 3 per group, Student's t test). **(C)** Cdk5 activity was detected by *in vivo* kinase assay (RLU, relative light units) (n = 4, 3 for *Trpm2*$^{+/+}$ and *Trpm2*$^{-/-}$ mice, respectively, Student's t test). Data are means ± SEM. *$p < 0.05$, **$p < 0.01$. Other statistical parameters with significant differences are listed in Table S1.

Figure S6. Expression of Cdk5 and other protein kinases in the mouse hippocampal neurons. (A) Representative immunoblots of the lysates from hippocampal primary cultures (left). Cdk5 and p35 protein levels were increased in *Trpm2*$^{-/-}$ neurons (right) (n = 3 per group, student's t test). **(B)** Immunoblots of phosphorylated and total forms of CaMKII, mTOR, PKD, ERK1/2, Akt and GSK3β in the adult mouse hippocampal DG (left). There were no significant differences between *Trpm2*$^{+/+}$ and *Trpm2*$^{-/-}$ mice except GSK3β (right) (n = 7 to 8 per group, Student's t test). Data are means ± SEM. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$. Other statistical parameters with significant differences are listed in Table S1.

Figure S7. TRPM2 deficiency blocks H_2O_2-induced p35 cleavage and the decrease in Cdk5-specific phosphorylation. (A) Representative immunoblots of the lysates from hippocampal primary cultures treated with H_2O_2 for 30 min (left). p35 cleavage was dose-dependently triggered by H_2O_2 only in *Trpm2*$^{+/+}$ neurons, but not in *Trpm2*$^{-/-}$ neurons (right) (n = 3 to 4 per group). **(B)** Representative immunoblots of the lysates from mice hippocampal primary neurons treated with H_2O_2 for 30 min (left). A dose-dependent decrease in Cdk5-specific phosphorylation of SYN1 and HDAC5 was observed in *Trpm2*$^{+/+}$ neurons, but not in *Trpm2*$^{-/-}$ neurons (right) (n = 3 per group). Data are means ± SEM. *p < 0.05, ***p < 0.001. Other statistical parameters are listed in Table S1.

Figure S8. TRPM2 mediates the stress-induced ROS response via Cdk5. (A) Immunoblots of total and phosphorylated Prx2 in the hippocampus (left). TRPM2 deficiency reduced Cdk5-specific phosphorylation of Prx2 (right) (n = 3 per group, Student's t test). **(B)** Immunoblots of PAR in mice hippocampal primary cultures treated with roscovitine (50 μM; Calbiochem) for 12 h (left). Roscovitine induced PAR formation in cultured neurons from both *Trpm2*$^{+/+}$ and *Trpm2*$^{-/-}$ mice (right) (n = 3 per group, unpaired two-tailed t test). Data are means ± SEM. *$p < 0.05$, ***$p < 0.001$. Other statistical parameters are listed in Table S1.

Figure S9. Knockdown of TRPM2 increases Cdk5-specific phosphorylation. (A) Lentiviral vector expressing small hairpin RNAs (shRNAs) targeted against mouse TRPM2 (lenti-shTRPM2). **(B)** Representative immunoblots of the lysates from mice hippocampal primary neurons infected with lenti-shTRPM2 (left). Cdk5-specific phosphorylation of SYN1 and HDAC5 was increased in the presence of lenti-shTRPM2 in a dose-dependent manner (right) (n = 2 per group, one-way ANOVA followed by Bonferroni posttest). Data are means ± SEM. *$p < 0.05$. Other statistical parameters with significant differences are listed in Table S1.

Figure S10. TRPM2 deficiency increases the expression of synaptic molecules in the hippocampus. (A) Representative immunoblot images of hippocampal synaptic molecules. **(B)** Quantification of the immunoblots in A. SYN1, NR1, NR2B, GluR2 and Shank2 protein levels were elevated in the hippocampi of *Trpm2*$^{-/-}$ mice (n = 6 to 8 per group, Student's t test). Data are means ± SEM. **$p < 0.01$, ***$p < 0.001$. Other statistical parameters with significant differences are listed in Table S1.

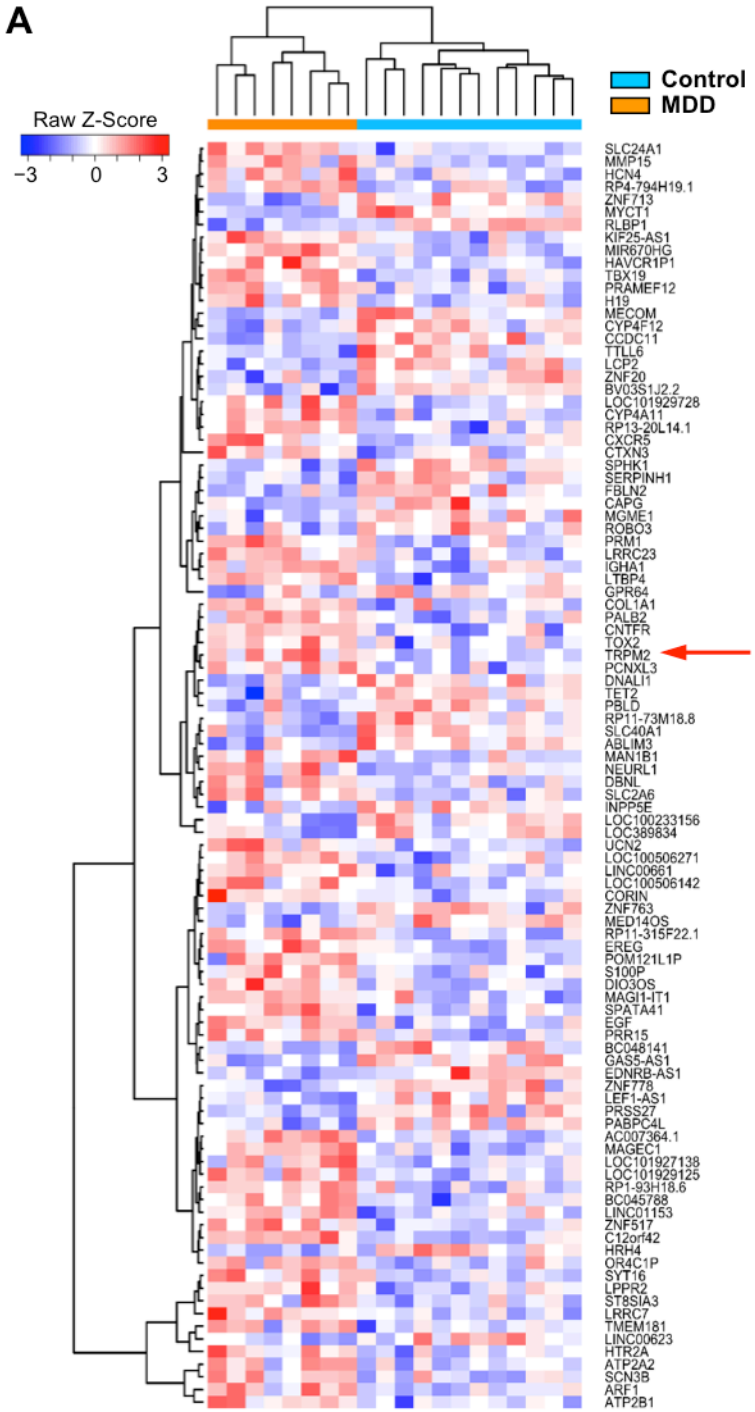
Figure S11. Knockdown of Cdk5 increases PAR expression. (A) Representative immunoblots of the lysates from mice hippocampal primary cultures infected with lenti-shCdk5 (left). Quantitative analysis showed Cdk5 deficiency increased PAR formation (right) ($n = 2$ per group, student's t test). Data are means ± SEM. *$p < 0.05$. Other statistical parameters with significant differences are listed in Table S1.

Figure S12. Locomotor activities in lenti-shLuc- and lenti-shCdk5-infused mice. **(A)** Average speed, **(B)** total distance travelled and **(C)** time in the center zone were scored in each 5 min period (n = 12 to 17 per group). Disruption of Cdk5 expression in the DG did not have any effects on locomotor activities of *Trpm2*$^{+/+}$ and *Trpm2*$^{-/-}$ mice. Data are means ± SEM.

Figure S13. Knockdown of Cdk5 blocks antidepressant-like behaviors in *Trpm2*$^{-/-}$ mice under CUS. (A) Timeline of the experimental procedures. **(B)** SCT. Knockdown of Cdk5 had no effect on sucrose consumption under CUS in either genotype (n = 6 to 8 per group). **(C)** NSFT. Knockdown of Cdk5 increased latency to feed in *Trpm2*$^{-/-}$ mice under CUS (n = 6 to 8 per group, unpaired two-tailed t test). **(D)** FST. Knockdown of Cdk5 increased immobility time in *Trpm2*$^{-/-}$ mice under CUS (n = 6 to 8 per group, unpaired two-tailed t test). **(E)** LHT. Knockdown of Cdk5 increased escaped latency in *Trpm2*$^{-/-}$ mice under CUS (n = 6 to 8 per group, unpaired two-tailed t test). **(F)** Average speed, **(G)** total distance travelled and **(H)** time in the center zone were scored in each 5 min period (n = 6 to 8 per group). Disruption of Cdk5 expression in the DG did not have any effect on locomotor activity of *Trpm2*$^{+/+}$ and *Trpm2*$^{-/-}$ mice under CUS. Data are means ± SEM. *p < 0.05, **p < 0.01, ***p < 0.001. Other statistical parameters are listed in Table S1.

Figure S14. Schematic diagram

Chronic stress-induced increases in ROS lead to activation of DNA repair enzyme, PARP, resulting in the production of TRPM2 activator, ADPR. TRPM2-dependent calcium influx causes calpain activation, aberrant hyperactivation of Cdk5, and impaired ROS scavenging system; this in turn leads to accumulation of ROS and provides an autoregulatory feed-forward loop.



B

Symbol	up/down	logFC	P-Value
TRPM2	up	0.21	0.004
TRPM7	down	−0.04	0.755
TRPA1	up	0.03	0.713
TRPC3	down	−0.02	0.826
TRPC5	up	0.16	0.077

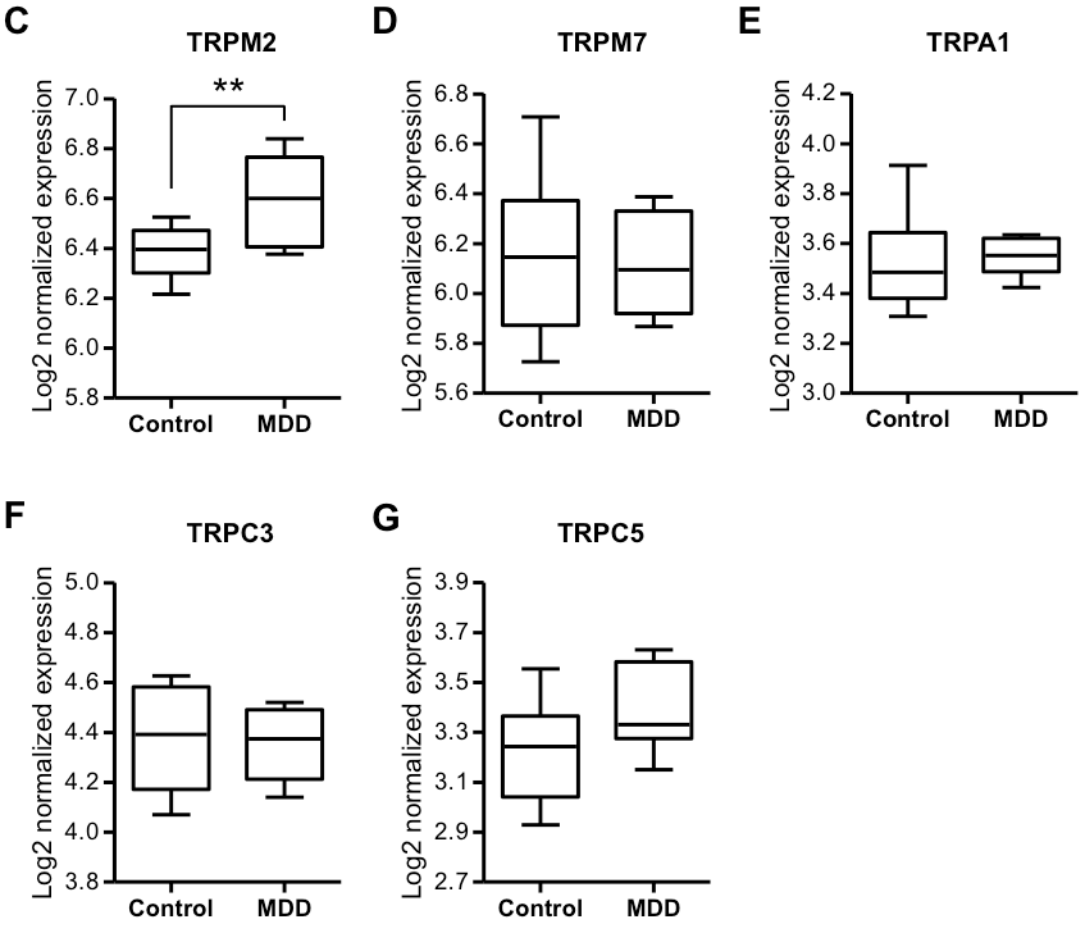
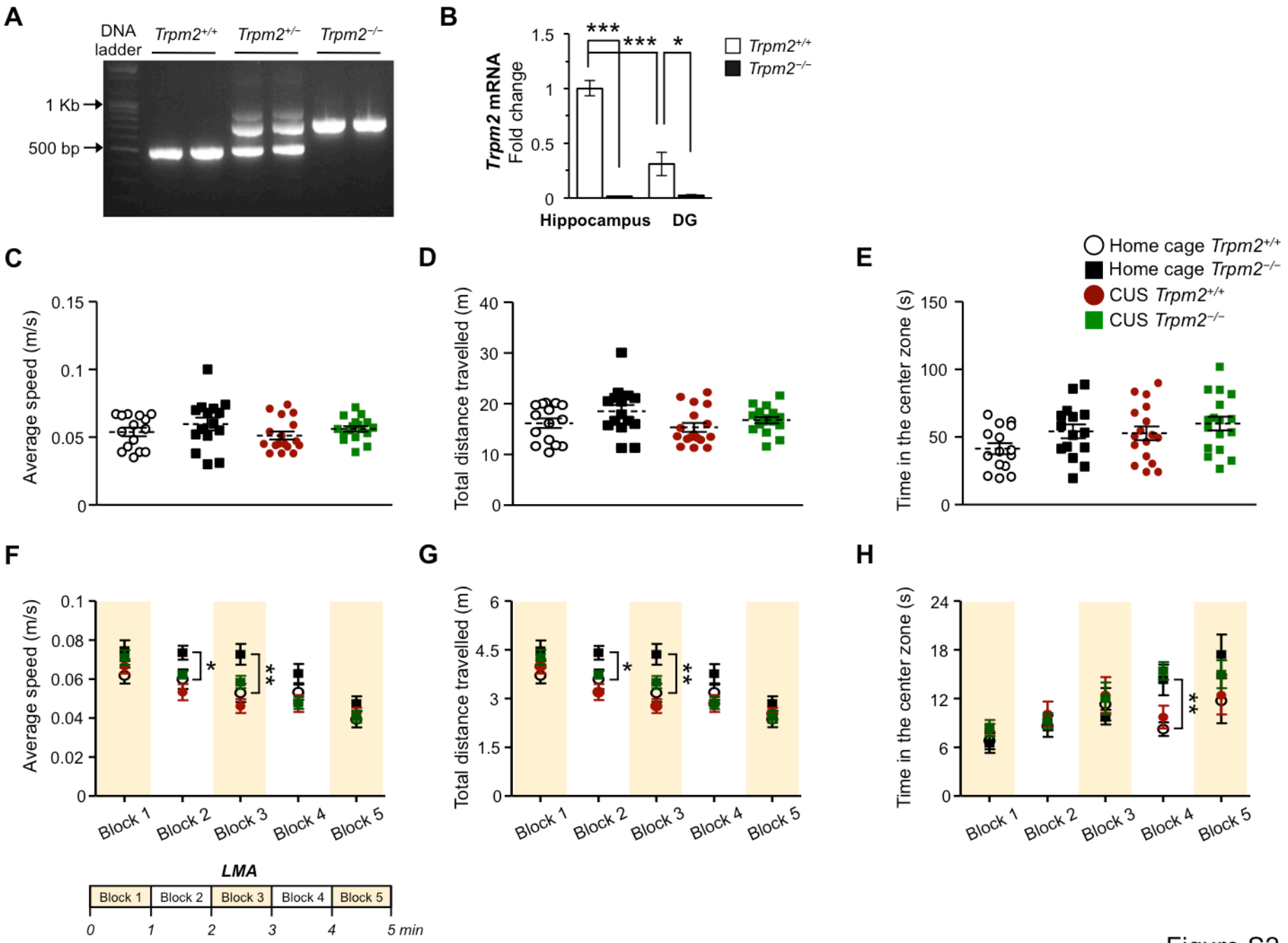


Figure S1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DNA ladder
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Trpm2+/−
Trpm2−/−
1 Kb
500 bp
B
Trpm2 mRNA Fold change
1.5
1
0.5
0
***
***
*
Trpm2+/+
Trpm2−/−
Hippocampus
DG
C
Average speed (m/s)
0.15
0.1
0.05
0
D
Total distance travelled (m)
40
30
20
10
0
E
Home cage Trpm2+/+
Home cage Trpm2−/−
CUS Trpm2+/+
CUS Trpm2−/−
Time in the center zone (s)
150
100
50
0
F
Average speed (m/s)
0.1
0.08
0.06
0.04
0.02
0
*
**
KO
Block 1
Block 2
Block 3
Block 4
Block 5
LMA
Block 1
Block 2
Block 3
Block 4
Block 5
0
1
2
3
4
5 min
G
Total distance travelled (m)
6
4.5
3
1.5
0
*
**
KO
Block 1
Block 2
Block 3
Block 4
Block 5
H
Time in the center zone (s)
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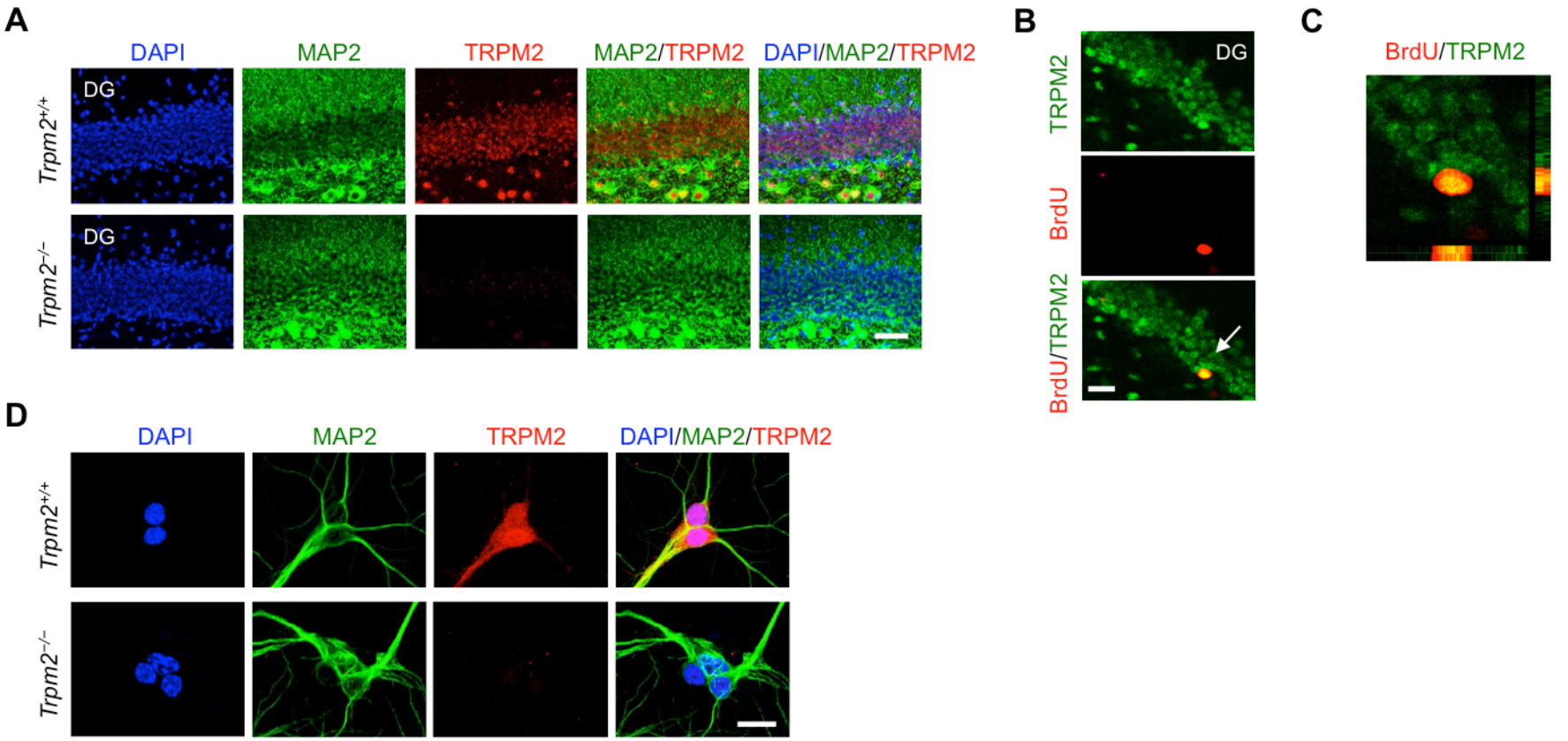
18
12
6
0
**
KO
Block 1
Block 2
Block 3
Block 4
Block 5

Figure S2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MAP2
TRPM2
MAP2/TRPM2
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DG
DG
B
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BrdU
BrdU/TRPM2
DG
C
BrdU/TRPM2
D
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MAP2
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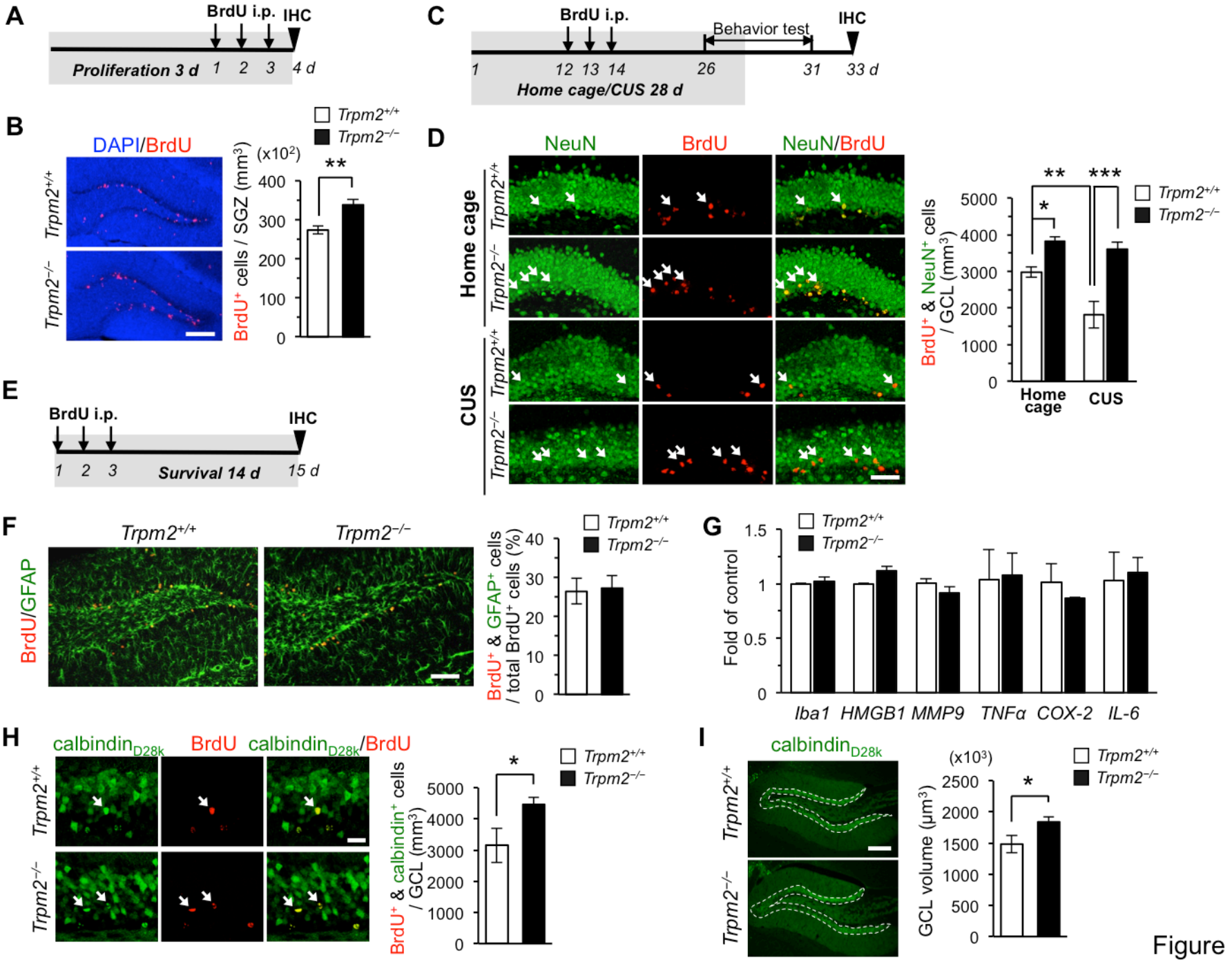
DAPI/MAP2/TRPM2
Trpm2+/+
Trpm2−/−

Figure S3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IHC
Proliferation 3 d
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2
3
4 d
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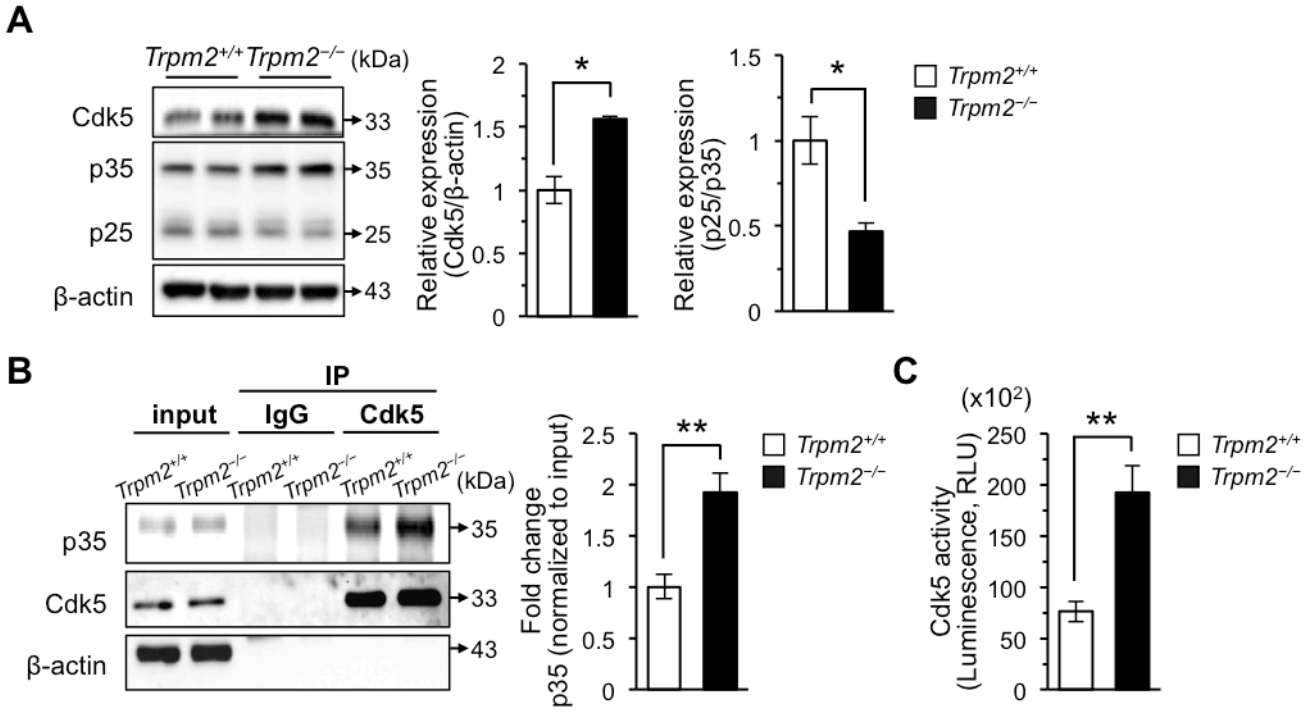
BrdU i.p.
Behavior test
IHC
1
12
13
14
26
31
33 d
Home cage/CUS 28 d
B
DAPI/BrdU
Trpm2+/+
Trpm2−/−
BrdU+ cells / SGZ (mm3)
(x102)
**
D
NeuN
BrdU
NeuN/BrdU
Home cage
CUS
BrdU+ & NeuN+ cells / GCL (mm3)
*
**
***
Home cage
CUS
E
BrdU i.p.
IHC
1
2
3
Survival 14 d
15 d
F
Trpm2+/+
Trpm2−/−
BrdU/GFAP
BrdU+ & GFAP+ cells / total BrdU+ cells (%)
G
Fold of control
Iba1
HMGB1
MMP9
TNFα
COX-2
IL-6
H
calbindinD28k
BrdU
calbindinD28k/BrdU
BrdU+ & calbindin+ cells / GCL (mm3)
I
calbindinD28k
GCL volume (μm3)
(x103)

Figure S4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Trpm2+/+ Trpm2−/− (kDa)
Cdk5
p35
p25
β-actin
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35
25
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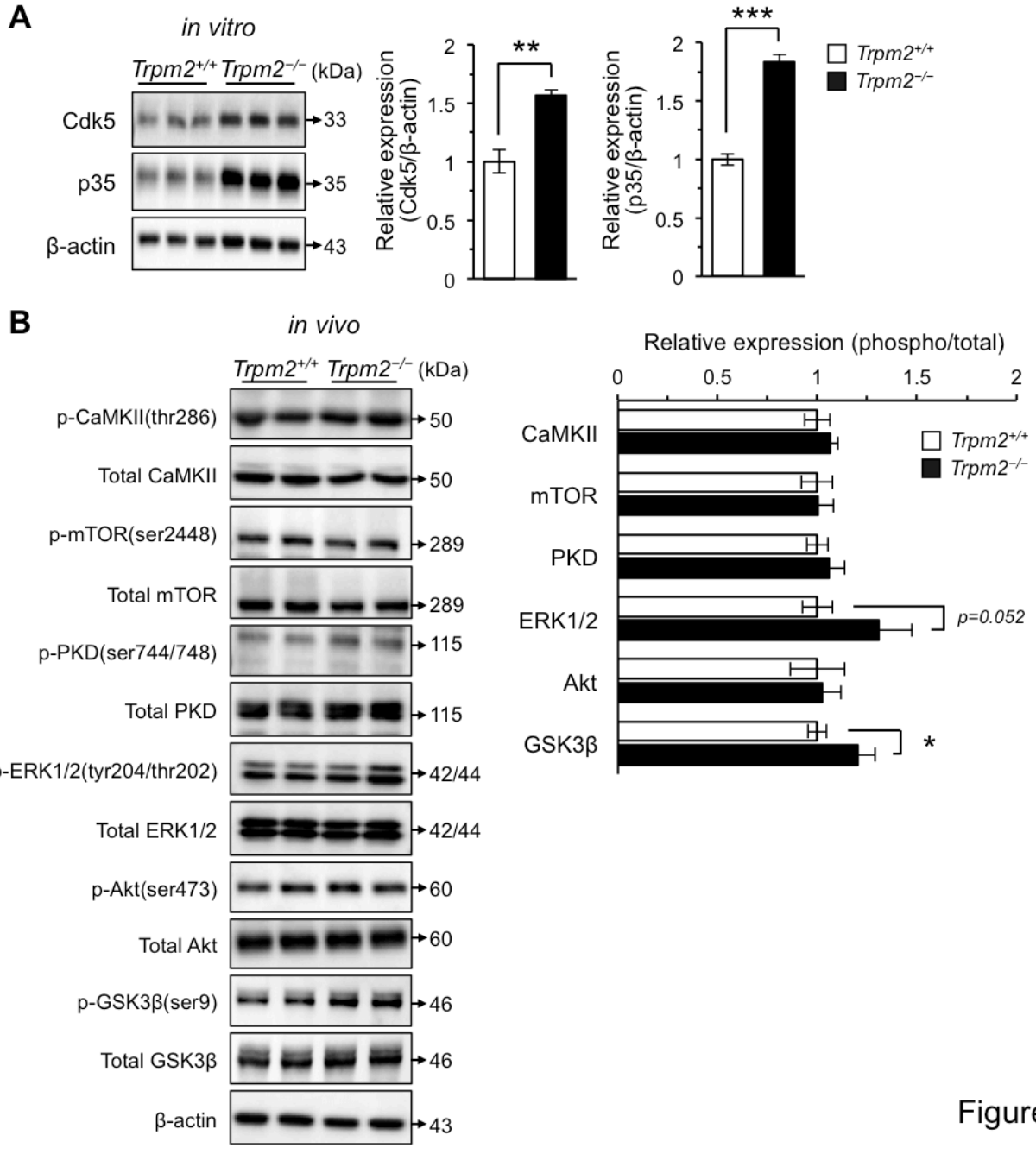
Relative expression (Cdk5/β-actin)
Relative expression (p25/p35)
Trpm2+/+
Trpm2−/−
B
IP
input
IgG
Cdk5
(kDa)
p35
Cdk5
β-actin
Fold change p35 (normalized to input)
C
(x10²)
Cdk5 activity (Luminescence, RLU)

Figure S5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in vitro
Trpm2+/+ Trpm2−/− (kDa)
Cdk5
p35
β-actin
33
35
43
Relative expression (Cdk5/β-actin)
Relative expression (p35/β-actin)
**
***
Trpm2+/+
Trpm2−/−
B
in vivo
Trpm2+/+ Trpm2−/− (kDa)
p-CaMKII(thr286)
Total CaMKII
p-mTOR(ser2448)
Total mTOR
p-PKD(ser744/748)
Total PKD
p-ERK1/2(tyr204/thr202)
Total ERK1/2
p-Akt(ser473)
Total Akt
p-GSK3β(ser9)
Total GSK3β
β-actin
50
50
289
289
115
115
42/44
42/44
60
60
46
46
43
Relative expression (phospho/total)
CaMKII
mTOR
PKD
ERK1/2
Akt
GSK3β
p=0.052
*

Figure S6

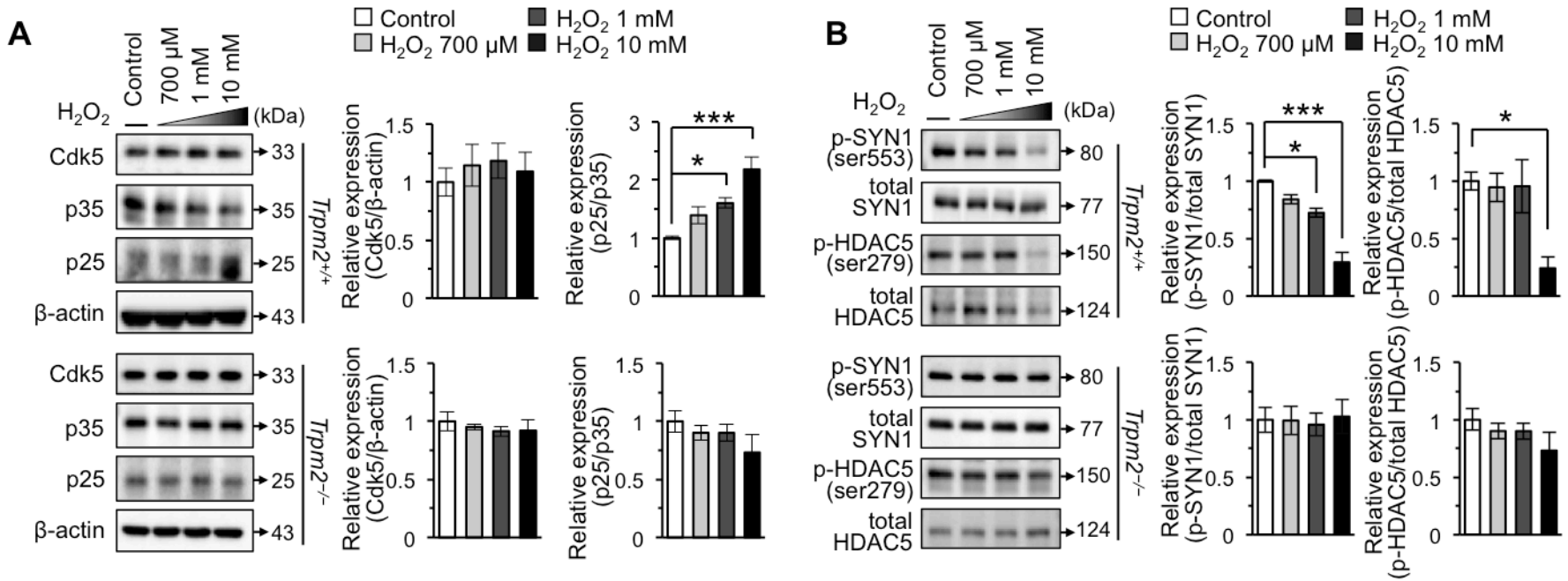
A
Control
700 μM
1 mM
10 mM
H2O2
(kDa)
Cdk5
p35
p25
β-actin
33
35
25
43
Trpm2+/+
Trpm2−/−
Control
H2O2 700 μM
H2O2 1 mM
H2O2 10 mM
Relative expression (Cdk5/β-actin)
Relative expression (p25/p35)
***
*
B
p-SYN1 (ser553)
total SYN1
p-HDAC5 (ser279)
total HDAC5
80
77
150
124
Relative expression (p-SYN1/total SYN1)
Relative expression (p-HDAC5/total HDAC5)

Figure S7

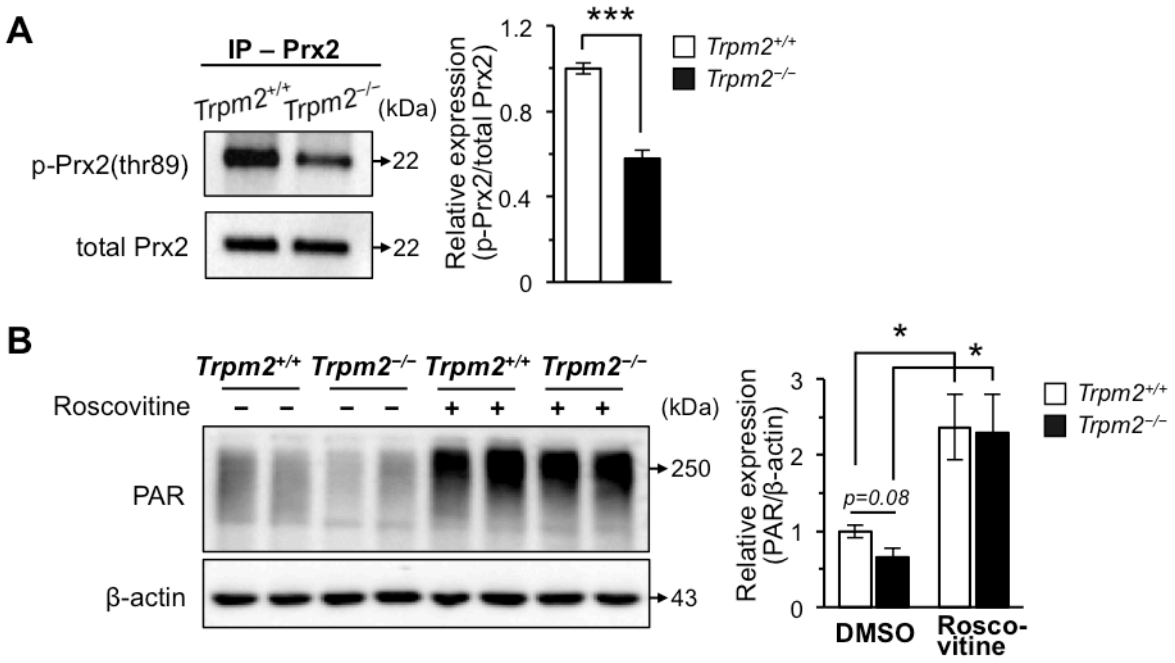
A
IP – Prx2
Trpm2+/+ Trpm2−/− (kDa)
p-Prx2(thr89)
22
total Prx2
22
Relative expression (p-Prx2/total Prx2)
1.2
0.8
0.4
0
***
Trpm2+/+
Trpm2−/−
B
Trpm2+/+ Trpm2−/− Trpm2+/+ Trpm2−/−
Roscovitine
− − − − + + + +
(kDa)
PAR
250
β-actin
43
Relative expression (PAR/β-actin)
3
2
1
0
p=0.08
*
*
DMSO
Rosco-vitine
Trpm2+/+
Trpm2−/−

Figure S8

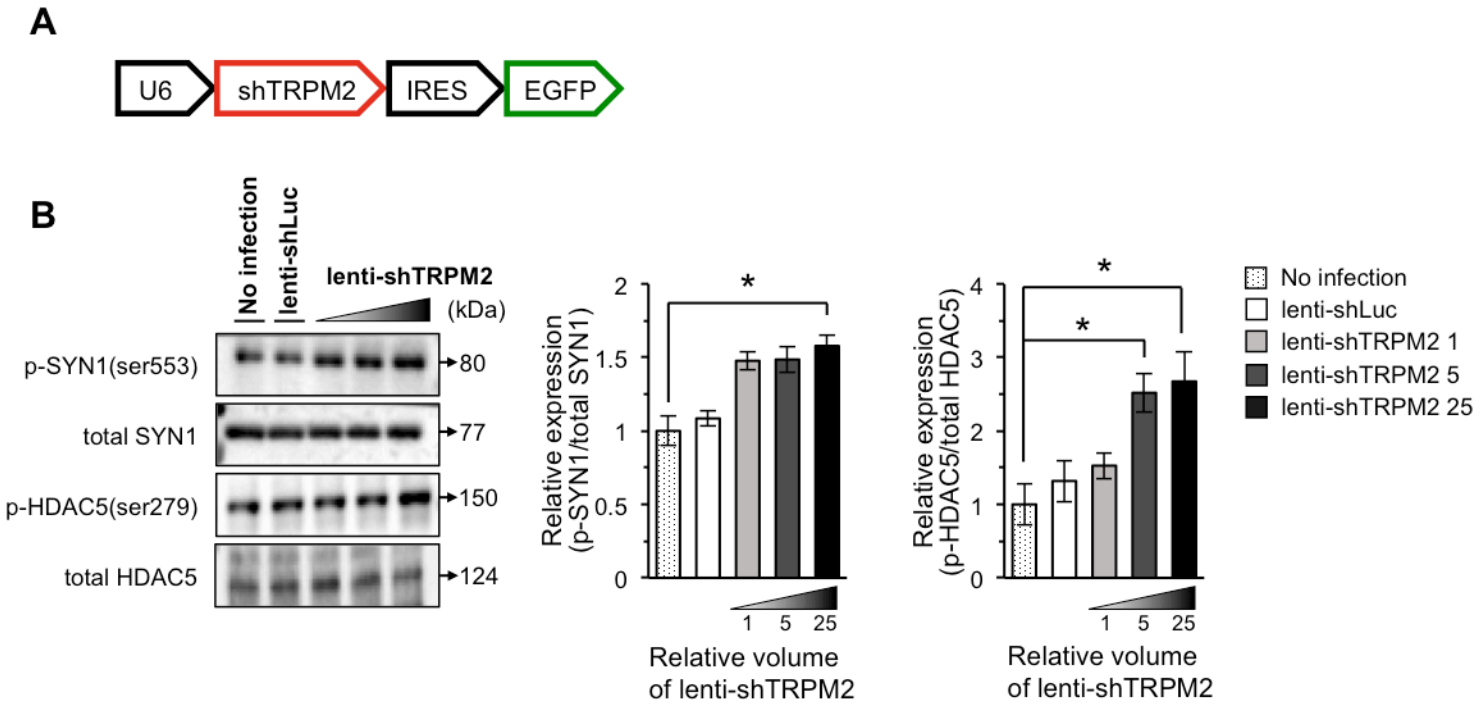
A
U6
shTRPM2
IRES
EGFP
B
No infection
lenti-shLuc
lenti-shTRPM2
(kDa)
p-SYN1(ser553)
80
total SYN1
77
p-HDAC5(ser279)
150
total HDAC5
124
Relative expression (p-SYN1/total SYN1)
Relative volume of lenti-shTRPM2
Relative expression (p-HDAC5/total HDAC5)
Relative volume of lenti-shTRPM2
No infection
lenti-shLuc
lenti-shTRPM2 1
lenti-shTRPM2 5
lenti-shTRPM2 25

Figure S9

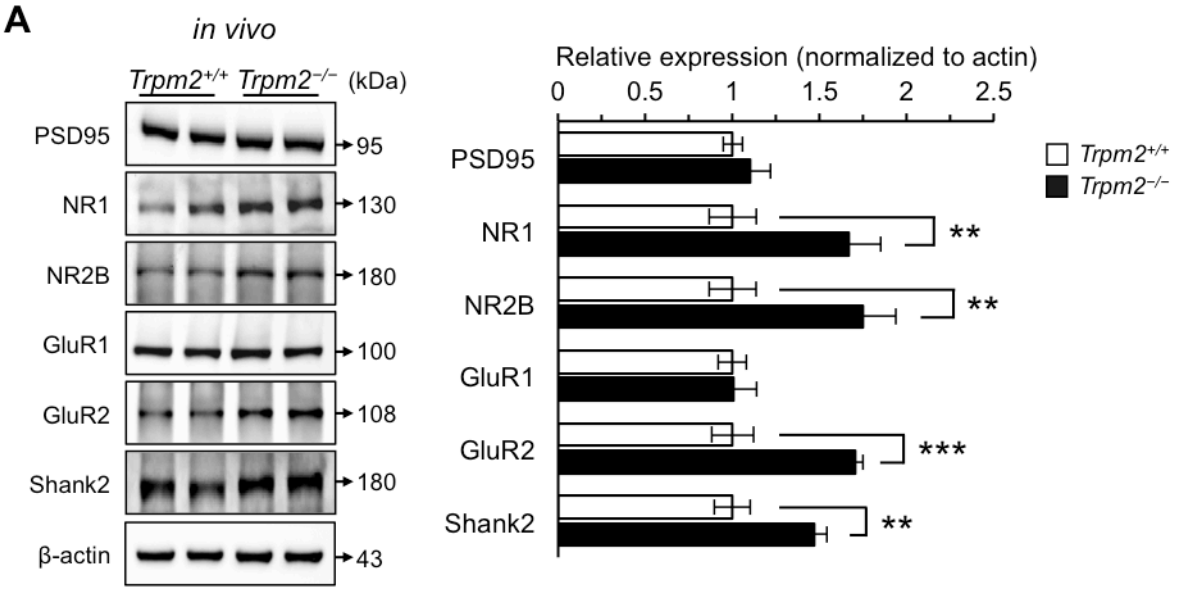
A
in vivo
Trpm2+/+ Trpm2−/− (kDa)
PSD95
NR1
NR2B
GluR1
GluR2
Shank2
β-actin
95
130
180
100
108
180
43
Relative expression (normalized to actin)
0
0.5
1
1.5
2
2.5
Trpm2+/+
Trpm2−/−
**
**
***
**

Figure S10

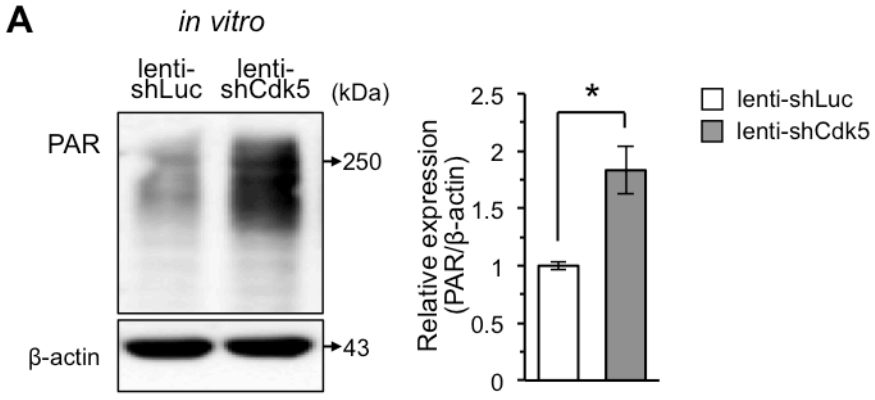
A
in vitro
lenti-shLuc
lenti-shCdk5
(kDa)
PAR
250
β-actin
43
Relative expression (PAR/β-actin)
2.5
2
1.5
1
0.5
0
*
lenti-shLuc
lenti-shCdk5

Figure S11

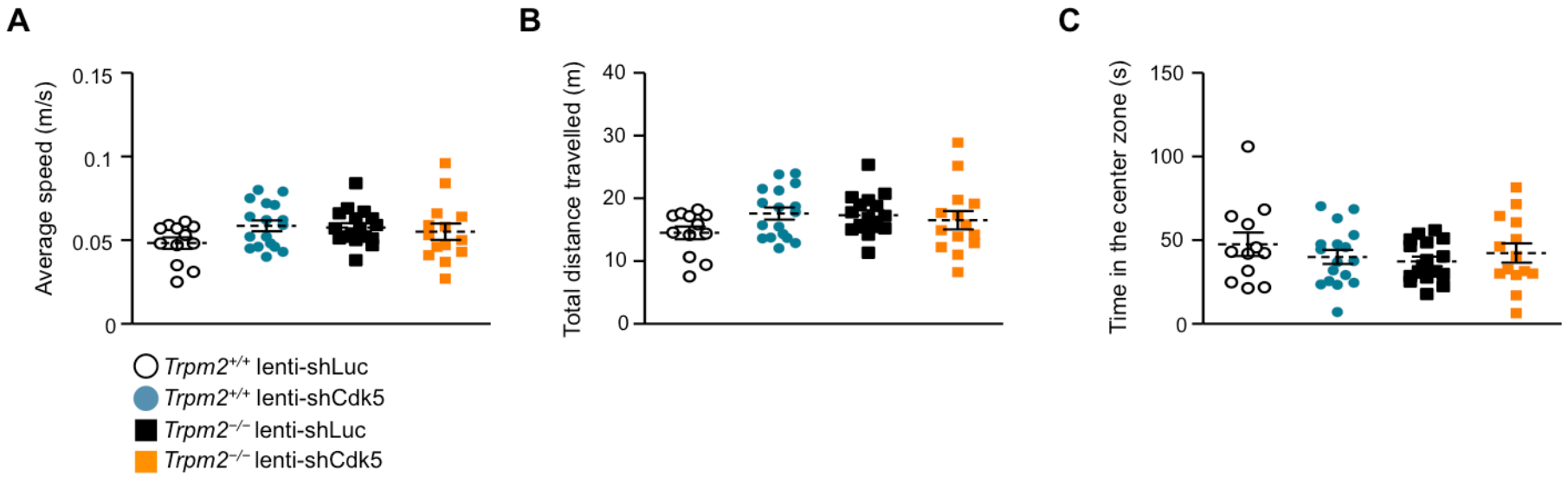
A
B
C
Average speed (m/s)
0.15
0.1
0.05
0
Total distance travelled (m)
40
30
20
10
0
Time in the center zone (s)
150
100
50
0
Trpm2+/+ lenti-shLuc
Trpm2+/+ lenti-shCdk5
Trpm2−/− lenti-shLuc
Trpm2−/− lenti-shCdk5

Figure S12

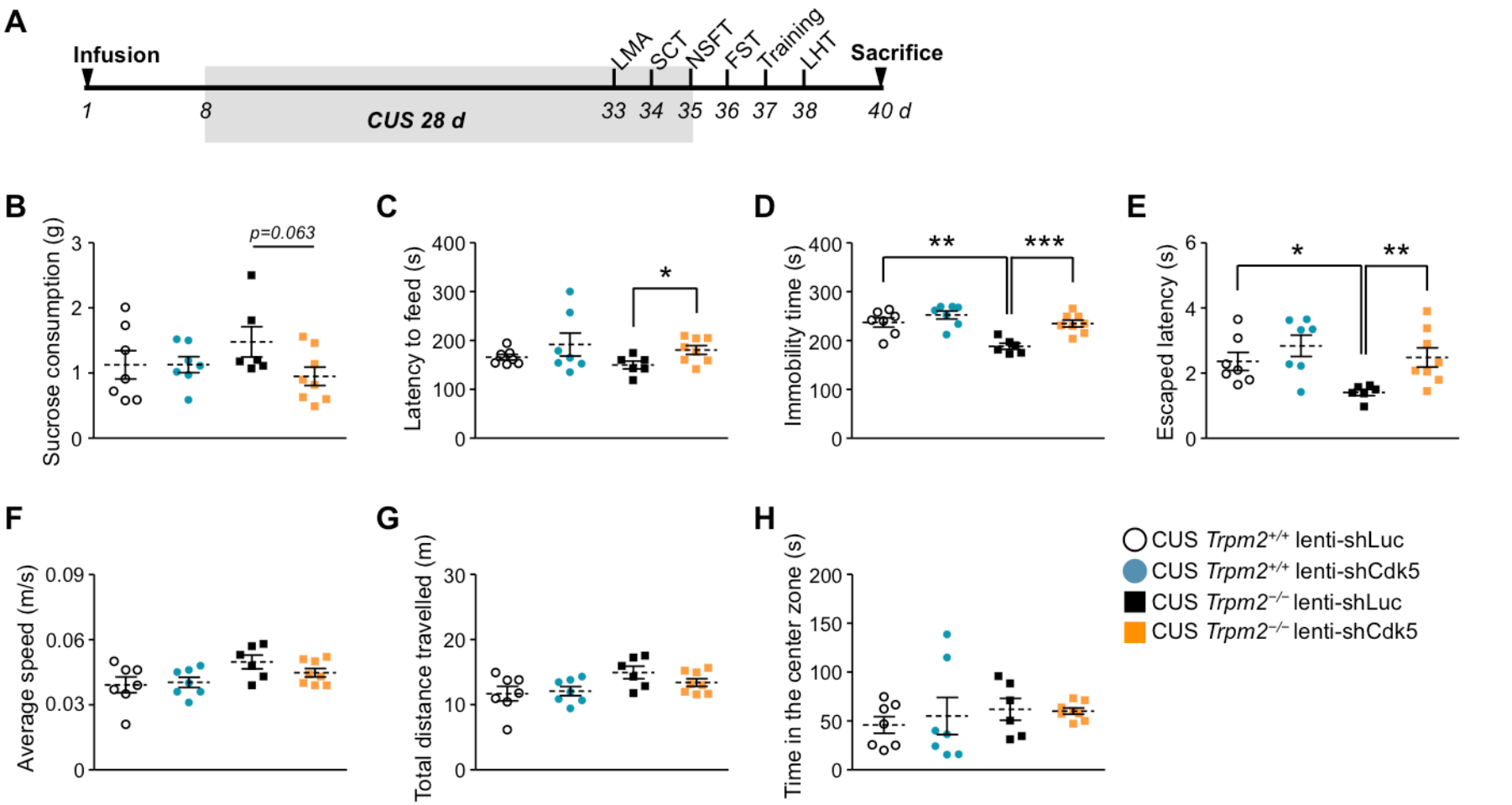
A
Infusion
LMA
SCT
NSFT
FST
Training
LHT
Sacrifice
1
8
CUS 28 d
33
34
35
36
37
38
40 d
B
Sucrose consumption (g)
p=0.063
C
Latency to feed (s)
D
Immobility time (s)
E
Escaped latency (s)
F
Average speed (m/s)
G
Total distance travelled (m)
H
Time in the center zone (s)
CUS Trpm2+/+ lenti-shLuc
CUS Trpm2+/+ lenti-shCdk5
CUS Trpm2−/− lenti-shLuc
CUS Trpm2−/− lenti-shCdk5

Figure S13

Schematic diagram

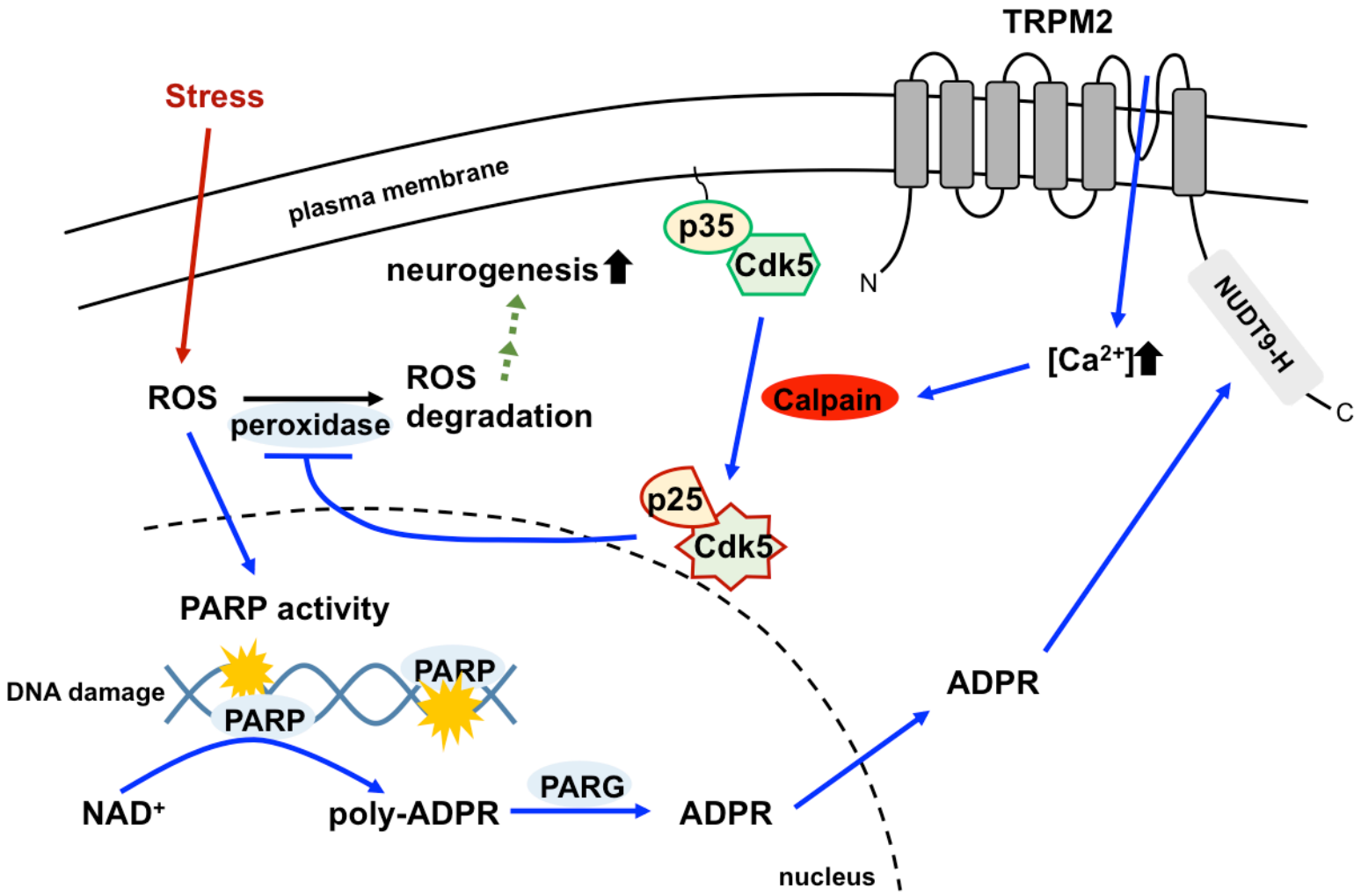

Figure S14