Supplementary Information (SI)

SI Materials and Methods

Animals. SAMP8 males were compared to SAMR1 males, which are genetically related to SAMP8 but resistant to accelerated senescence (1). Both strains were kindly provided by Dr Mercè Pallàs from the Universitat de Barcelona. Crl:CD1 mice were purchased from Charles River (Les Oncins, France). All strains were housed under specific pathogen free (SPF) conditions on a 12 h light/dark cycles at Instituto de Salud Carlos III Facility where food and water were available ad libitum.

Tissue processing. BrdU was prepared in saline solution (0.9%) as previously described (2) and was injected intraperitoneally (at a dose of 50mg/kg of body weight) according to the different paradigms. Animals were anesthetized with isoflurane (Isoba vet, 100% w/w) and perfused with PB 0.1M and 4% PFA followed by 16 h postfixation in 4% PFA at 4°C. The brains were coronally sectioned in a vibratome (Leica Microsystems VT-1200-S). The resulting 40 µm free floating sections were collected sequentially generating antero-posterior reconstructions of the hippocampus conformed by 1 section every 320 µm of hippocampal structure.

NSPC isolation and treatments. NSPCs were isolated from the hippocampus of 2 months old Crl:CD1, SAMR1 or SAMP8 and maintained in culture on plates coated with poly-D-lysine (Sigma) and laminin (Invitrogen) as described in (3). For every experimental replicate, the DG from 6 animals were bulked and cultured for at least 8 passages. For BMP6 short stimulation treatment, 50.000 cells/MW24 were cultured for 6 hours on coverslips pre-treated with poly-D-lysisne and laminin in the presence of mitogens (FGF2 10ng/ml, EGF 10ng/ml) and the presence or absence of BMP6 (50 ng/ml). In some experiments, cells were exposed to Noggin (25ng/ml). Cells were then soaked 10 minutes in PFA 2% for fixation and immunocytochemistry. For cell growth kinetic analysis in the presence of mitogens and the presence or absence of BMP6, 250.000 cells/MW6 were cultured and quantified at 6 hours, 4, 7 and 10 days.

Proliferation, apoptosis and senescence assays. For all assays, 50.000 cells/MW24 were cultured on pre-treated coverslips for 4 days in the presence of mitogens (FGF2 10ng/ml, EGF 10ng/ml) and the presence or absence of BMP6 (50 ng/ml). For proliferation assays, cells were pulsed with 5µM BrdU or 5µM CldU prior to fixation in PFA 2%. For SA-b-galactosidase senescence assays, cells were fixed with glutaraldehyde 0.2% and incubated with X-gal (20mg/ml X-gal, 50mM K4Fe(CN)6, 50mM K3Fe(CN)6, 1M MgCl2, 0.2%NP40, PBS 1X) up to 48 hours. We used C17.2 cells treated with 200nM H_2O_2 during 4 days as positive control. For apoptosis assays, cells were fixed in PFA 2% and permeabilized for 2 minutes on ice using 0.1% Sodium Citrate/0.1% Triton X-100. Cell death was assessed by TUNEL staining, following the protocol supplied by the "In Situ Cell Death Detection Kit, TMR red kit" (12156792910, ROCHE). Cell quantifications were performed with Zeiss Axio Imager A1. Apoptosis was also measured by flow cytometry. We assessed cell death by treating SAMR1 and SAMP8 NSPCs with 0.01 µg/µl Propidium Iodide (PI, P4170 Sigma-Aldrich) and CF Blue-ANNEXIN V during 15 minutes in annexin binding buffer (Hepes 10mM, CaCl2 2.5mM, NaCl 140mM pH 7.4). PI/Annexin possitive cells were measured by flow cytometry using a BD

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FACSCANTO Flow CytometerTM. As positive control we used SAMR1 and SAMP8 NSPCs treated with 200mM H2O2 during 2 hours to induce cell death.

Immunostaining. Immunohistochemistry and immunocytochemistry were performed using standard procedures (4). Information about the antibodies is available upon request. After staining, all sections and cells were mounted and preserved with 50% Mowiol (Polysciences, 17951), 2.5% DABCO (Sigma, D2522). Tissue images are tiles of 2-4 µm z-stacks and cell images are single 1-2 µm z stacks both captured on a Leica Spectral SP5 confocal microscope with a 40x oil objective. Images were analysed with Image J.

Stereology and Statistical analysis. Stereology was performed by the analysis of 5 coronal sections, 40 µm each, separated 320 µm one to each other. Sampling started at first appearance of the infrapyramidal blade of the dentate gyrus. Antero-posterior Bregma coordinates of all 5 sections correspond approximately to -1.2 mm, -1.6 mm, -2 mm, -2,4 mm and -2,8 mm. Every quantification was normalized by the DG area of every section. Analysis of variance (ANOVA) was used for statistical analysis of differences between ages of the same strain. Post-hoc comparisons were performed using the Tukey test, and the Bonferroni correction was applied. Data were also analysed by 2-tailed Student t test (paired t test for statistical analysis of cell treatments and unpaired t test for analysis of both SAMR1 and SAMP8 strains of the same age). Data are presented as mean ± standard error of the mean (SEM) and n indicates the number of independent mice used per strain and age. A p value of <0.05 was considered as statistically significant.

Western blot. Dissected SAMR1 and SAMP8 DG were dissociated in TNE buffer supplemented with protease inhibitors (Protease Inhibitor Cocktail, Roche) on ice and total protein concentration was measured by bicinchoninic acid kit (Thermo Scientific). Lysates were run on 12% Tris-Gly gels at 100 mA for 2h. Gels were transferred to PVDF methanol-preactivated membranes (Millipore) at 4°C 80 V 2h. Membranes were blocked with 3 % BSA in TTBS buffer for 1 h and incubated in primary antibody overnight at 4°C. Information about the antibodies is available upon request. Proteins were detected by exposing the membrane to a Fuji-X-ray film and quantified by Image J.

qRT-PCR. The DG were dissected from mice, flash-frozen on dry ice and stored at -80°C. RNA was extracted using TriReagent (Sigma). qRT-PCR analysis was performed with a Roche Light Cycler 480 using SyBR Premix Ex Taq 2X (Takara). Primer sequences were designed and tested using Primer Express 3.0 software to obtain similar melting temperatures (∼58 °C) and are available upon request. Gene sequences were obtained from GenBank. All quantitative PCR primers were designed between exons with a resultant amplicon of less than 150 bp in length. Levels of RNA expression were determined according to the 2-ΔΔCt method.

Intracerebroventricular infusion. Infusion of 2-month old SAMR1 and SAMP8 animals was performed using Alzet osmotic mini-pumps (model 1007D and 1004, coupled to the brain infusion kit 3). The cannula was implanted at 0.0 anteroposterior, 0.7 mediolateral coordinates and was attached to the skull with Hystoacryl (B/Braun). Noggin (25 µg/mL, Sigma) or vehicle solution (NaCl 0,9%) was continuously infused at 0.5 µL/h for 7 days. Two different BrdU paradigms have been

used. In one experimental paradigm, animals were injected intraperitoneally with one injection of 50 mg BrdU/kg of body weight on the sixth day of infusion and were sacrificed 3 days after the injection. In a second paradigm, animals were injected in the same way but sacrificed 21 days after the injection. In the last paradigm, Noggin or vehicle solution were infused continuously to 6-month old animals for 28 days prior performance of behavioral tests. Ipsilateral hemispheres were sectioned on vibratome and analysed through immunohistochemistry.

Lentiviral injections. 5-month-old male SAMP8 and SAMR1 animals underwent stereotaxic surgery. 1.5 µL suspension of Lentiviral ORF particles expressing Noggin (Nog (Myc-DDK-tagged, from Origene technologies, Cat. No. MR225276L1V) or control particles expressing GFP (pLenti-C-mGFP, Origene technologies, Cat.No. PS100071V) were delivered into the DG (anterior-posterior: -2.0 mm, medial-lateral: ± 1.5 mm, dorsal-ventral: -2.0 mm). Six weeks post injection animals were subjected to behavioural assessments and sacrificed by transcardial perfusion-fixation. Afterwards, brains were extracted and processed for immunohistochemistry. For lentiviral validation**,** HEK293 cells were infected with 10^6 units of lentiviral particles and mRNA was isolated using TRIzol reagent (life technologies) according to the manufacturers' protocol. cDNA was synthetized using a PrimeScript RT reagent Kit (Perfect Real Time, Takara). Quantitative real time polymerase chain reactions was performed using EvaGreen (Solis Biodyne) and the following primer sequences (5'->3'): *Sdha* (for normalization) forward primer: AGAGGACAACTGGAGATGGCATT, reverse primer: AACTTGAGGCTCTGTCCACCAA; *Nog* forward primer: TGTGGTCACAGACCTTCTGC, reverse primer: GTGAGGTGCACAGACTTGGA; *Gfp* forward primer: AAGCAGCACGACTTCTTCAAGTC, reverse primer: AGACGTTGTGGCTGTTGTAGTTGT.

Behavioural assessments and Statistical analysis. To evaluate animal's general activity, the VersaMax Animal Activity Monitoring System was used. The apparatus consists in acrylic cages with infrared sensors. As the animal moves in the cage, it interrupts the infrared beams. The number of broken beams is correlated with the amount of movement in the cage. Animals were left in the cage for 5 minutes on two consecutive days. The elevated plus maze (Cibertec) was used to measure anxiety. It consists of a plus-shaped apparatus with two open and two enclosed arms, each with an open roof, elevated 40 cm from the floor. Each animal was tested in a 5 minutes single trial. Mouse was placed in the centre of the maze, in an open-arm direction. During each trial, the mouse moved freely along the apparatus. The movements of the animals were recorded and analysed with EthoVision XT software. Spatial learning and memory was assessed in a Morris Water Maze. The apparatus consists in a 1 meter diameter pool with a capacity of 120 litres, containing water at a temperature of 22±1°C, visual cues in the surface of the tank and an invisible methacrylate platform hidden in one of the virtual quadrant throughout the test. A 5 days protocol was applied for the acquisition phase, with an habituation trial (60 s withouth platform) the day before "day 1", two trials on day 1, three trials on day 2, and four trials per day on days 3 to 5 (60 s each trial, plus 20 s on the platform). On the last day, mice were subjected to a probe test consisting in a 60 s trial without the platform in the pool. The escape latency and the time spent in each quadrant were recorded with EthoVision XT software. To study differences between means over three or more time points in the water maze, a Repeated Measures ANOVA was used,

and to be able to study the interaction between the within-subject factor (time) and the between-subject factor (experimental group/treatment), a Mixed model ANOVA was used taking into account the following assumptions: the dependent variable was measured on a continuous scale, it was normally distributed and there was homogeneity of variances. If the normality and/or the homogeneity of variances assumptions were violated, a Friedman test was used followed by a post hoc Wilcoxon Signed-Ranked Test.

SI Supplementary figure legends

Figure S1. SOX2+ cells are depleted in the SGZ of SAMP8. (A and B) 2-m SAMP8 animals show a decrease in the number of SOX2+ cells compared to 2-m SAMR1 (*** $p < 0.001$) although both strains show an age-related reduction of this cell population (SAMR1: Δ $p < 0.05$; SAMP8 # $p < 0.05$). (C) 2-m SAMP8 animals exhibit a higher percentage of actively dividing (Ki67+) SOX2+ cells *vs* SAMR1 (* $p < 0.05$). (D) Total number of actively cycling SOX2+ cells in 2-m SAMP8 and SAMR1 strains (Abbreviations: GCL, granule cell layer; H, hilus; SGZ, subgranular zone. Scale bar, 200 µm).

Figure S2. BMP6 mRNA levels do not increase in the SEZ or CA1 of 2-m SAMP8. BMP6 mRNA levels are comparable between SAMR1 and SAMP8 strains at different ages in neurogenic (SEZ, subependymal zone) and non-neurogenic (hippocampal CA1) brain regions.

Figure S3. Mouse Crl:CD1 NSPC cultures and SAMR1/SAMP8 NSCP cultures are undifferentiated. (A) Crl:CD1 cultures express neural stem and progenitor cell markers (SOX2 and Nestin). (B) Differentiated cells of the neuronal (TuJ1), astroglial (GFAP) or oligodendroglial (MBP) lineages are absent from the cultures. (C) SAMR1 and SAMP8 cultures express neural stem and progenitor cell markers (SOX2 and Nestin) (Scale bar: 25 µm).

Figure S4. Mouse Crl:CD1 NSPC cultures do not udergo apoptosis or senescence upon BMP6 treatment. (A) Quantification of the percentage of apoptotic (TUNEL+) cells. We did not observe signs of increased apoptosis in the presence of 50 ng/ml BMP6 (average±SEM, n=4). (B) Representative images of the TUNEL+ cells (red). DAPI is shown in blue. (C) Representative images of SA-b-galalactosidase cells. We did not observe signs of increased senescence in Crl:CD1 NSPCs treated with BMP6 compared to control (lower panel). The c17.2 NSC cell line was treated with 200nM H_2O_2 for 4 d to induce senesce and was employed as a positive control for the SA-b-galalactosidase staining (Scale bar: 25 µm in B, 100 µm in C).

Figure S5. BMP6 expression is not increased in SAMP8 NSPCs. (A) BMP6 mRNA levels were lower in SAMP8 *vs* SAMR1 NSPC cultures (average±SEM, n=5, right). Note that the expression of the Nestin gene was similar (average±SEM, n=5, left). (B) BMP6 mature protein and pre-protein levels as detected by SDS-PAGE and Western blot. *Left,* BMP6 pre-protein levels were lower in SAMP8 *vs* SAMR1 NSPC cultures (average±SEM BMP6 normalized to B-actin, n=3). The mature functional form of BMP6 was undetectable in both SAMP8 and SAMR1 NSPCs. Beta-actin is shown as a loading control. *Right,* Quantification of the BMP6 pre-protein levels normalized to Beta-actin.

Figure S6. SAMP8 NSPC cultures proliferate less and have an increased apoptotic death compared to SAMR1 cultures. (A) Total number of cells recovered at 4DIV after seeding 1.5 x 10^6 cells in growth medium (FGF2 20ng/ml, EGF 20 ng/ml). Note that SAMP8-NSPCs expand more slowly than SAMR1-NSPCs (average±SEM, n=9). (B) Percentage of BrdU+ cells in SAMP8-NSPC and SAMR1-NSPC cultures grown in the presence of mitogens (FGF2 10ng/ml, EGF 10ng/ml)(average±SEM, n≥4). (C) Percentage of Ki67+ cells in SAMP8-NSPC and SAMR1-NSPC cultures grown in the presence of mitogens (FGF2 10ng/ml, EGF 10ng/ml)(average±SEM, n≥4). (D) CldU/Ki67 in SAMP8-NSPC and SAMR1-NSPC cultures grown in the presence of mitogens (FGF2 10ng/ml, EGF 10ng/ml)(average±SEM, n=3). (E) Percentage of viable (AnnexinV-PI-), necrotic(AnnexinV-PI+), early (AnnexinV+PI-) and late (AnnexinV+PI+) apoptotic cells in SAMP8-NSPC and SAMR1-NSPC cultures grown in the presence of mitogens (FGF2 10ng/ml, EGF 10ng/ml) as measured by Flow cytometry (average±SEM, n=3).

Figure S7. SAMP8-NSPC and SAMR1-NSPC proliferation is not affected by Noggin. (A) Percentage of BrdU+ cells in SAMP8-NSPC and SAMR1-NSPC cultures grown in the presence of mitogens (FGF2 10ng/ml, EGF 10ng/ml) and the presence or absence of Noggin (25 ng/ml). Cells were fixed at 4DIV. Data are expressed as a fold relative to the SAMR1 untreated condition and correspond to the average±SEM, n=4. (B) Representative images of the GFAP (green) and BrdU (red) labelling in SAMP8 and SAMR1 NSPC cultures. The nuclear stain DAPI is shown in blue (Scale bar: 25 μm).

Figure S8. SAMP8-NSPCs and SAMR1-NSPCs respond to exogenously added BMP6 ligand. (A) Percentage of BrdU+ cells (left) and GFAP+ cells (right) in SAMP8-NSPC and SAMR1-NSPC cultures grown in the presence of mitogens (FGF2 10ng/ml, EGF 10ng/ml) and the presence or absence of BMP6 (50 ng/ml). Cells were fixed at 4DIV. Data correspond to the average±SEM, n=4. (B) Representative images of the GFAP (green) and BrdU (red) labelling in SAMP8 and SAMR1 NSPC cultures. The nuclear stain DAPI is shown in blue (Scale bar: 25 μm). (C) Percentage of Ki67+ cells in SAMP8-NSPC and SAMR1-NSPC cultures grown in the presence of mitogens (FGF2 10ng/ml, EGF 10ng/ml) and the presence or absence of BMP6 (50 ng/ml). Cells were fixed at 4DIV. Data correspond to the average±SEM, n=4.

Figure S9. Noggin infusion rescues the aberrant location of immature neurons in SAMP8. (A) Representative images of the amelioration in the abnormal location of DCX+ cells in 2-m SAMP8 animals treated with Noggin. (B) Number of DCX+ cells (left) and analysis of their location (right) in 2-m saline and Noggin infused SAMP8 (* $p< 0.05$). (Abbreviations: GCL, granule cell layer; SGZ, subgranular zone. Scale bar, 25 μm).

Figure S10. Morris Water Maze Probe trial. Data show the absolute time spent at the platform quadrant (PQ) of the pool and at the other three non-platform quadrants ($p< 0.05$; LV-Noggin-SAMP8 vs LV-GFP-SAMP8). SAMR1 and SAMP8 animals were injected with LV-Noggin and LV-GFP lentiviruses and Morris Water Maze was performed 6 weeks later.

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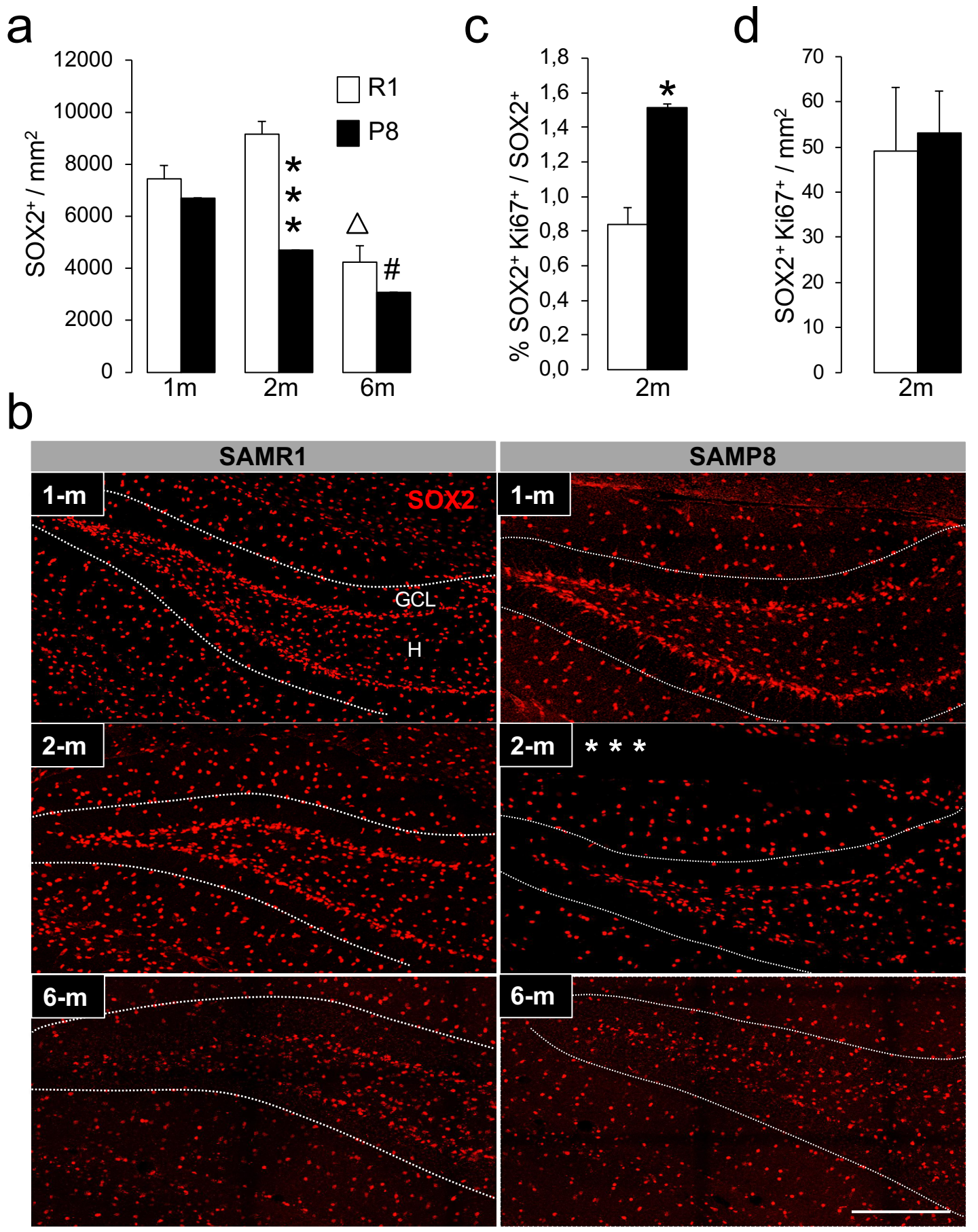
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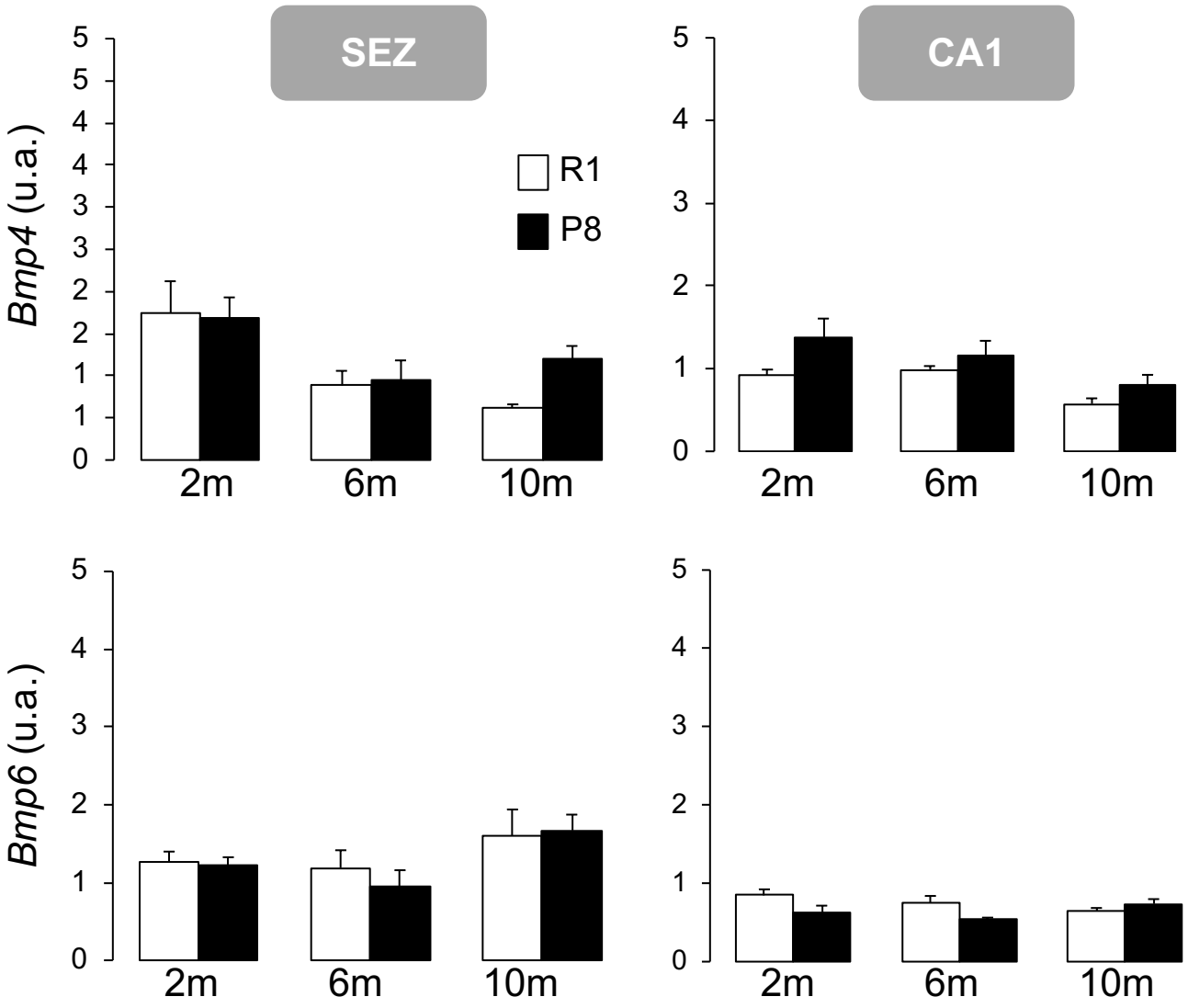
Supplementary Figure 2



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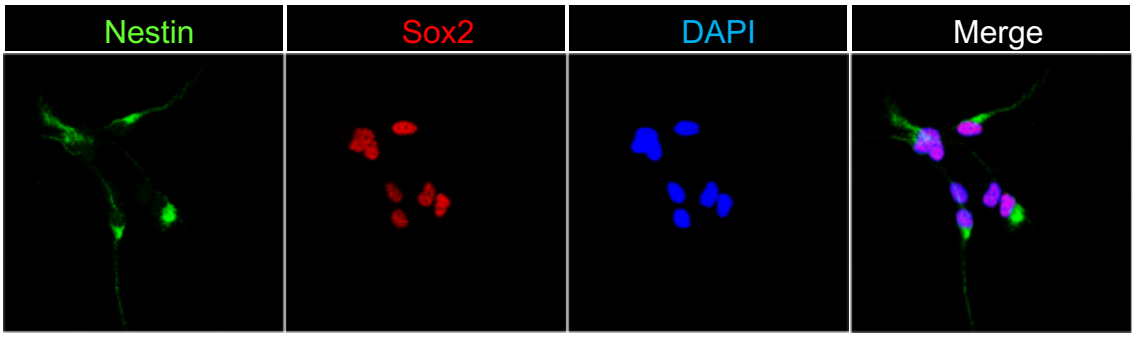

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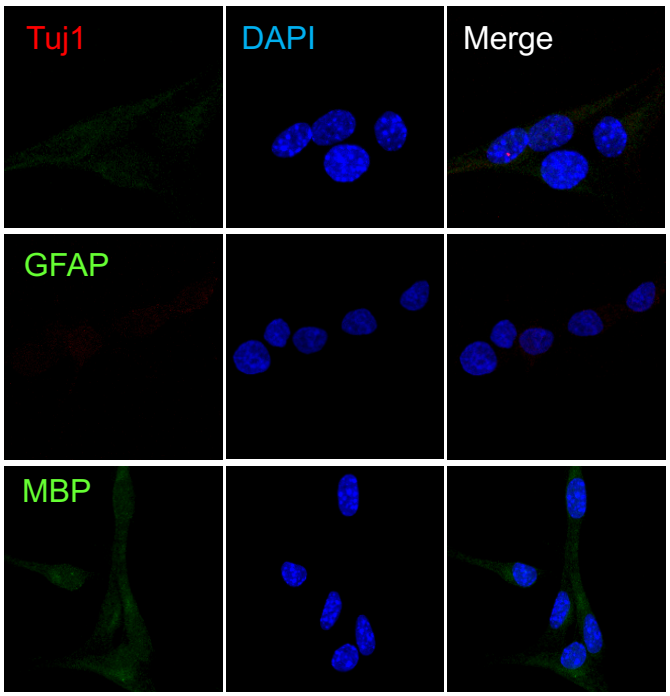

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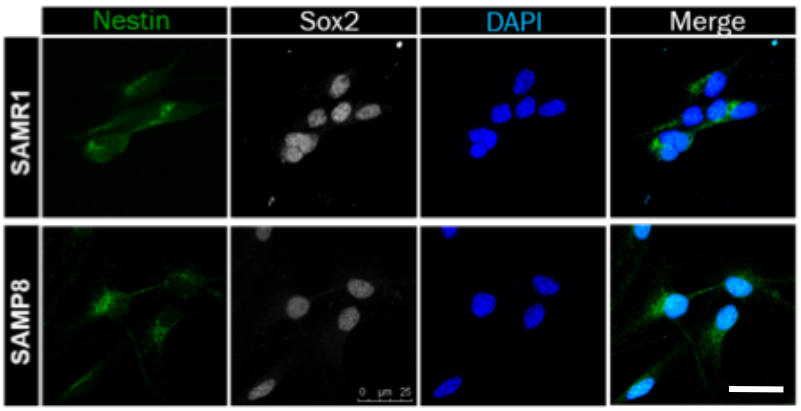

Supplementary Figure 4

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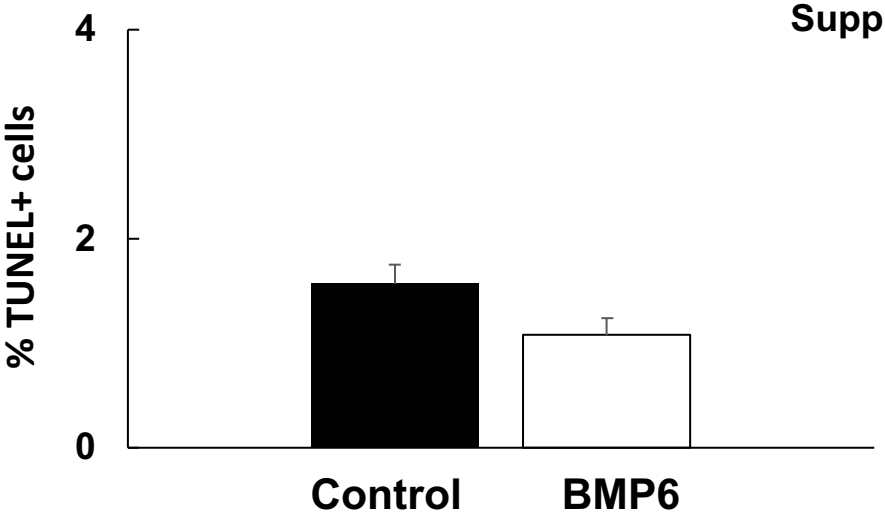

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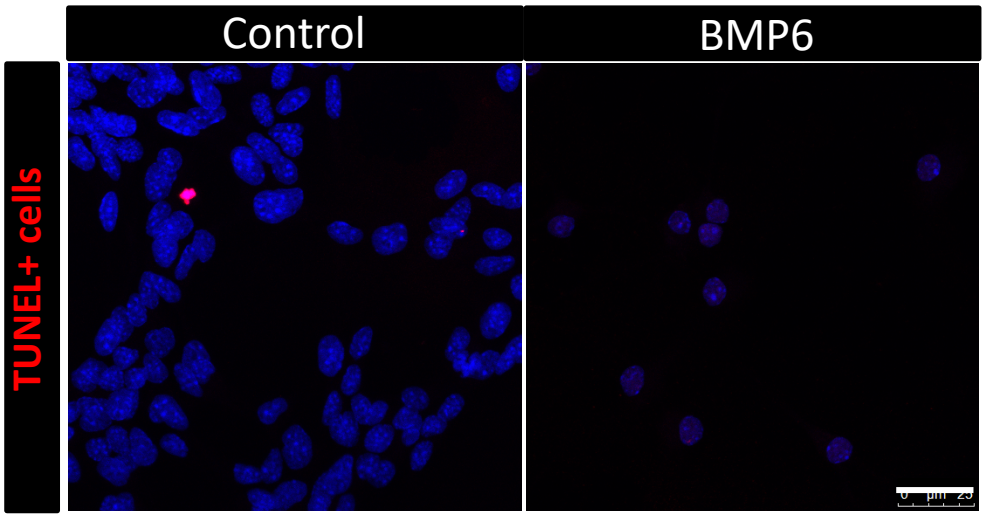

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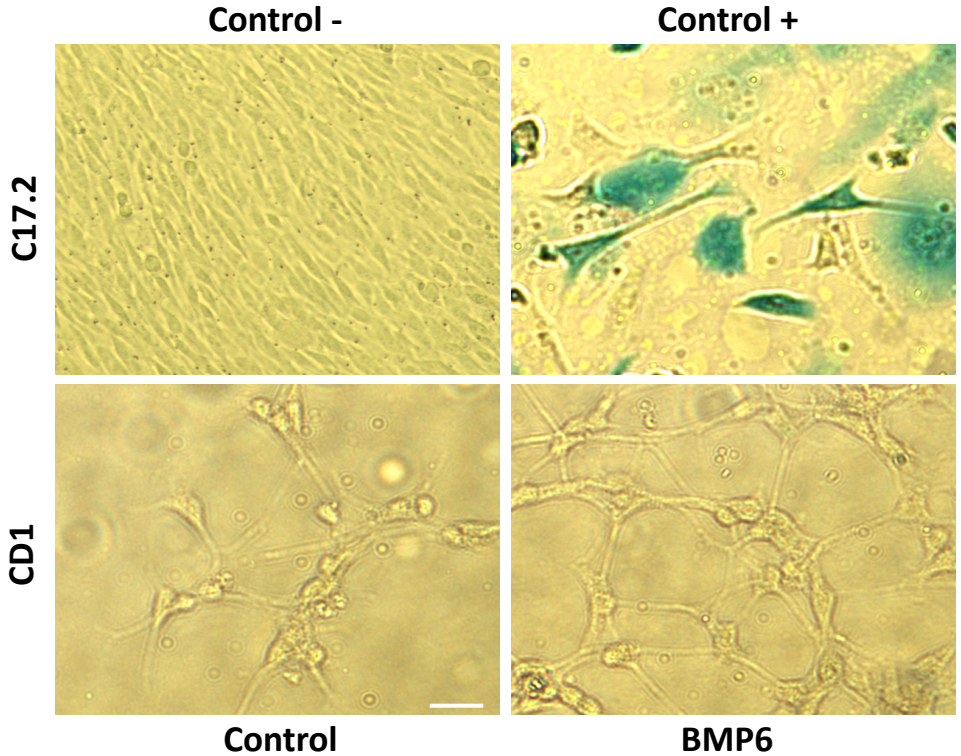

Supplementary Figure 5

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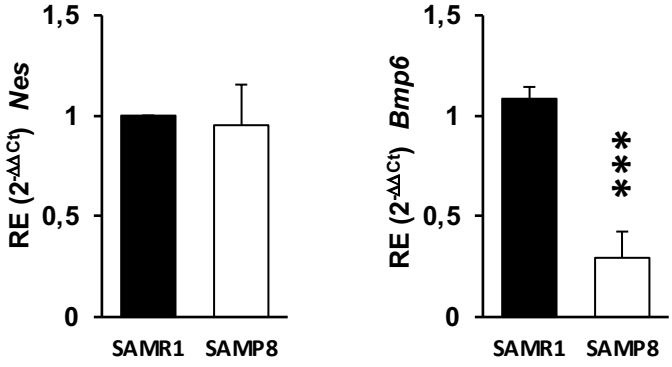

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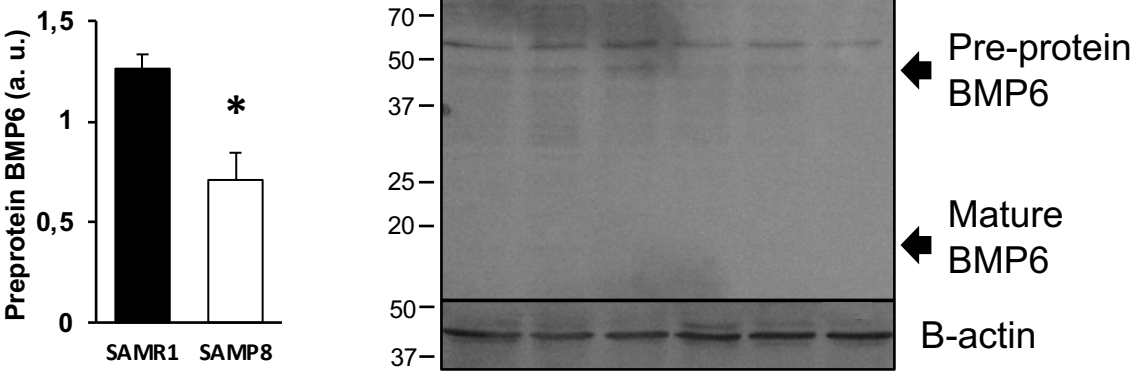
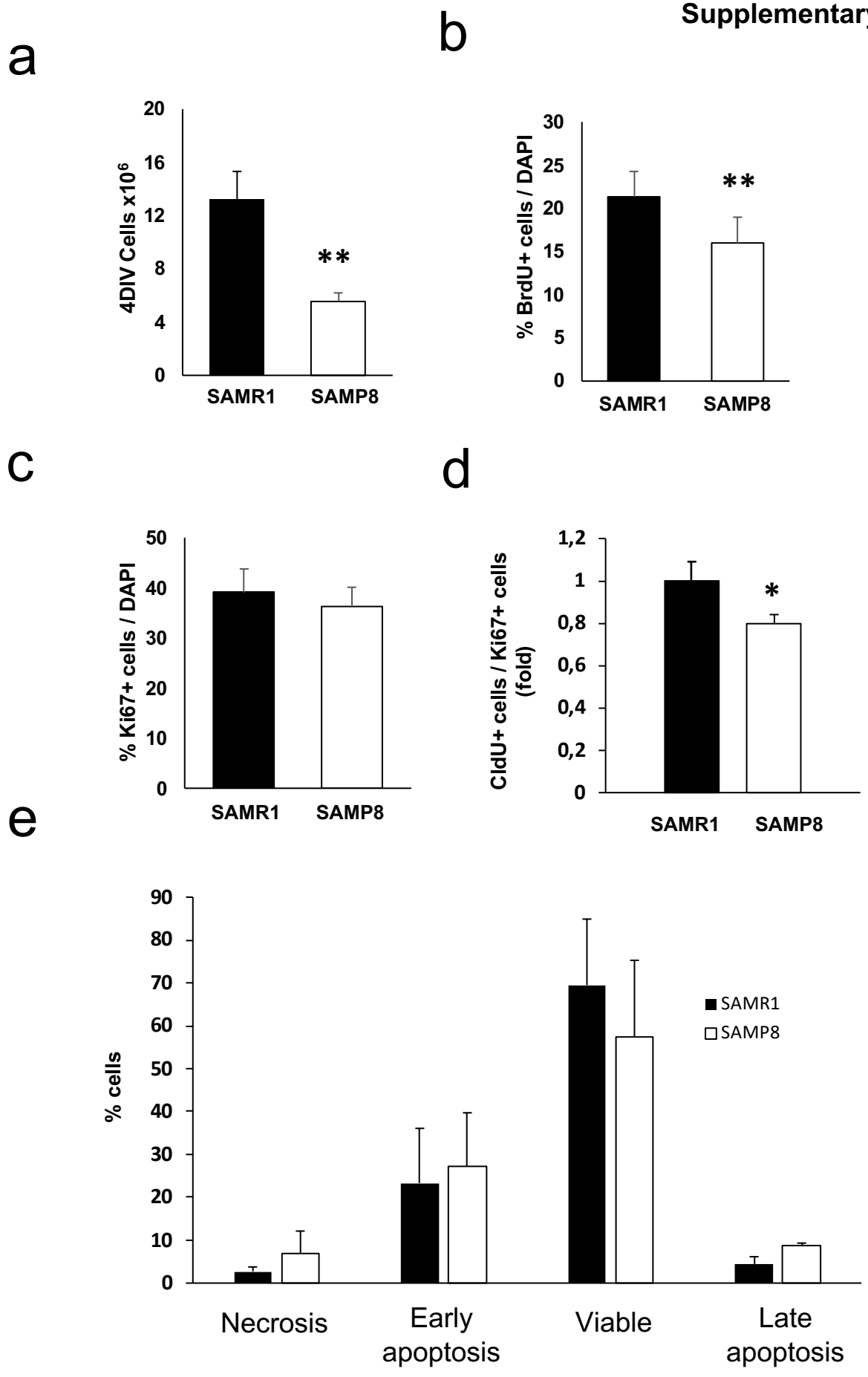


Supplementary Figure 6



Supplementary Figure 7

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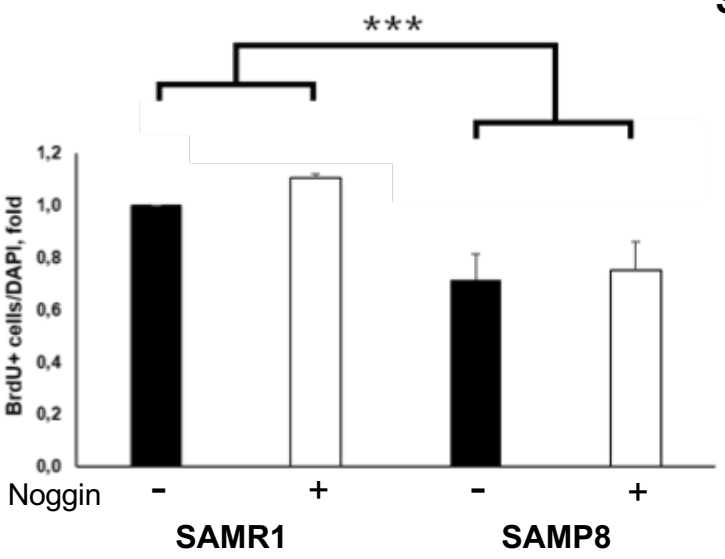

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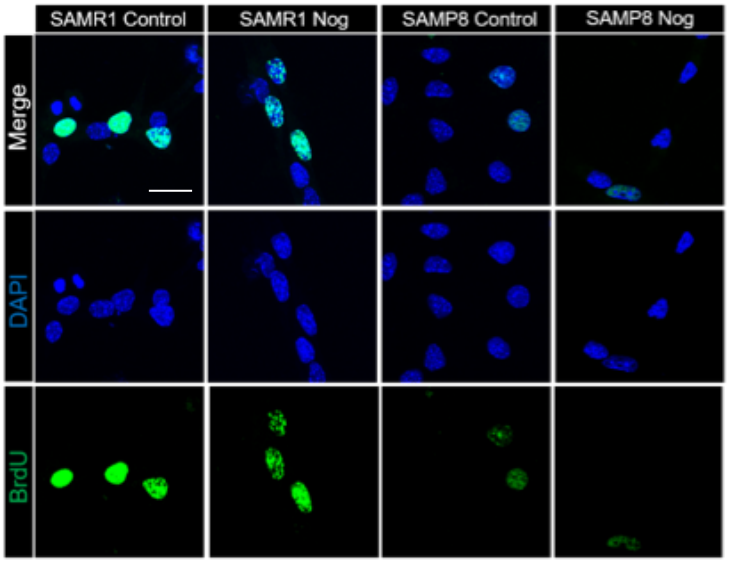

Supplementary Figure 8

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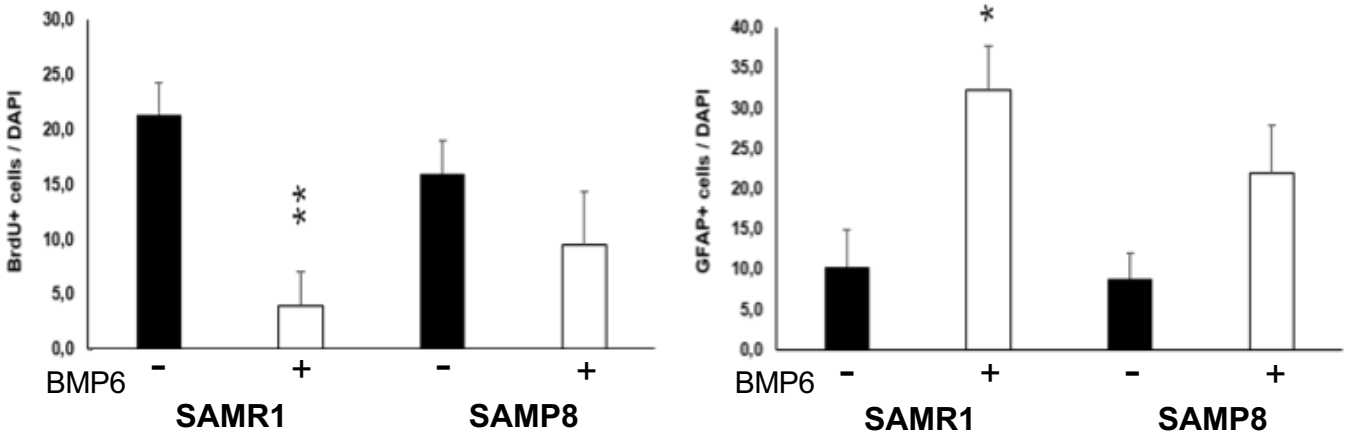

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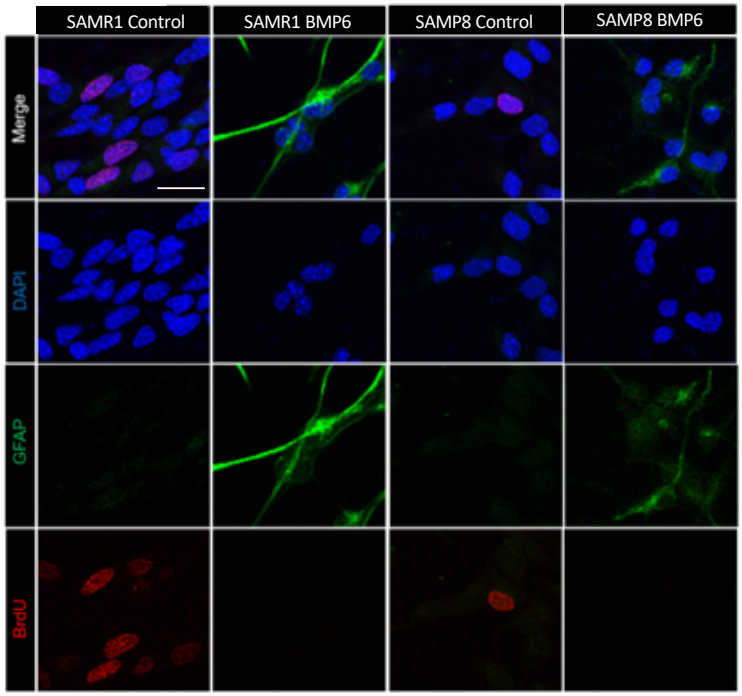

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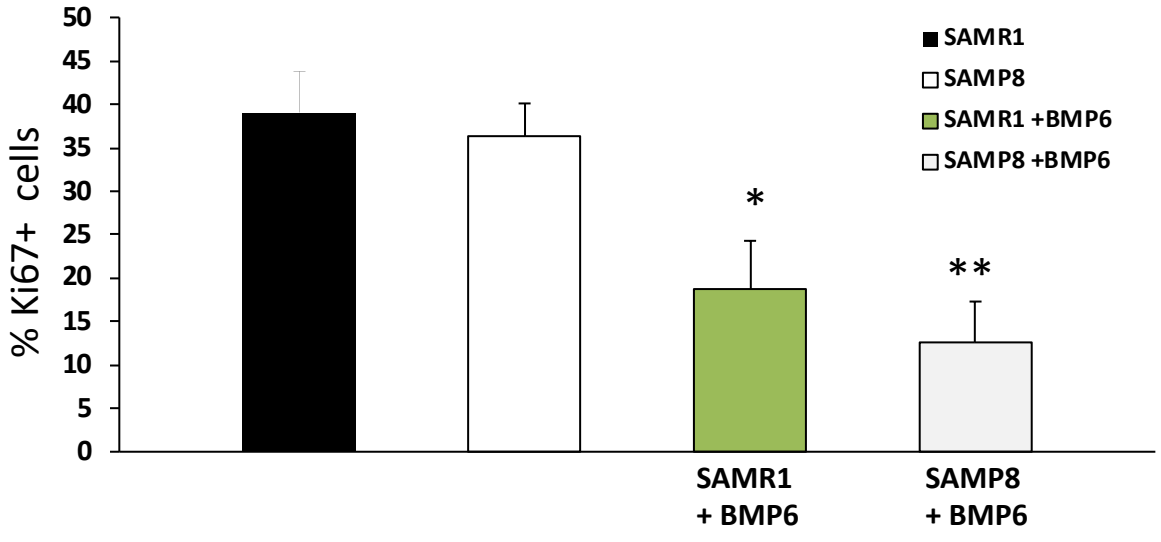

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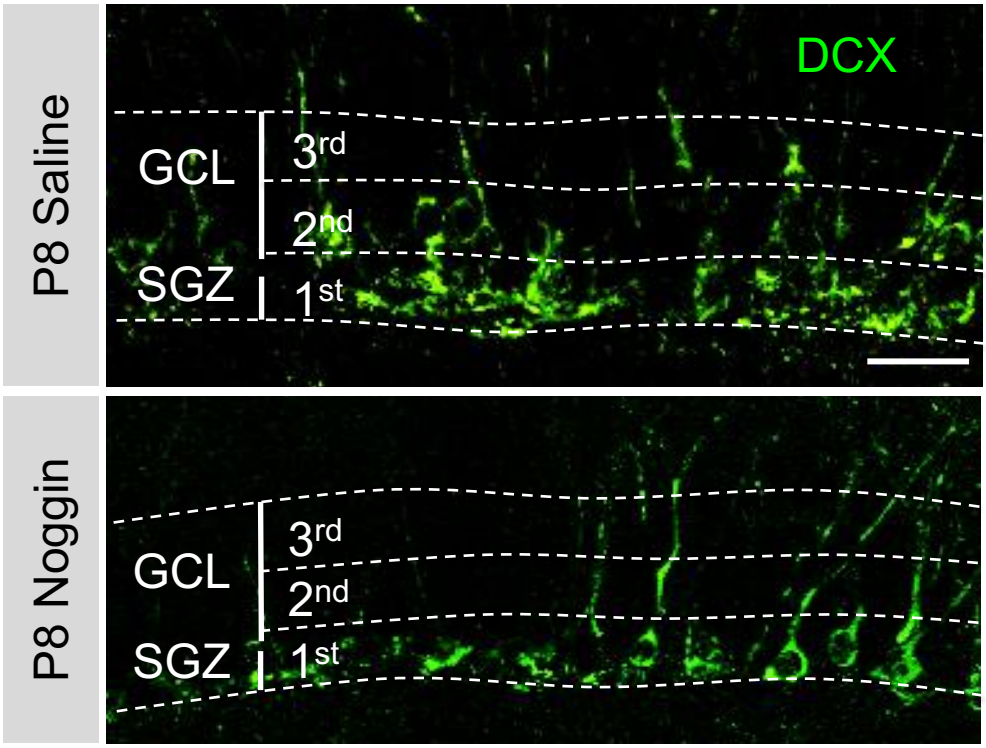
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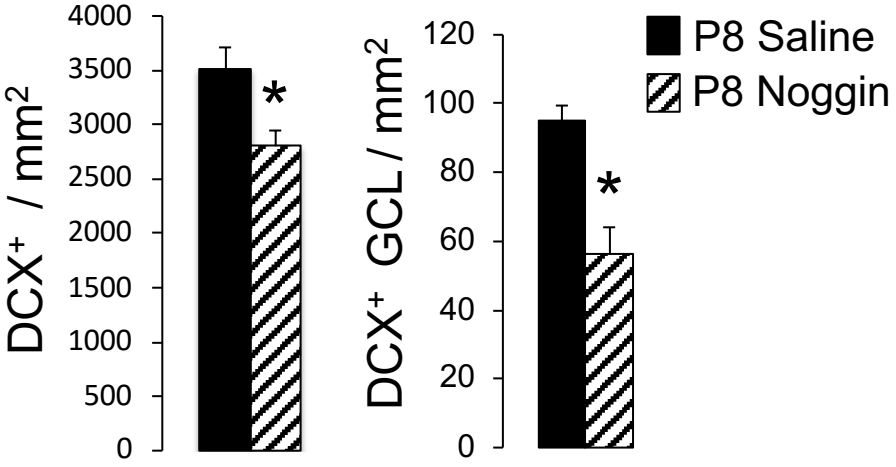
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Supplementary Figure 10