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p75 neurotrophin receptor shapes the dynamics of adult hippocampal neurogenesis in Alzheimer's disease

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Abstract

Background Alzheimer's Disease (AD) is a neurodegenerative disorder primarily characterized by memory loss and cognitive decline. The AD-driven impairment of adult hippocampal neurogenesis - the process of generating new neurons in the dentate gyrus - is strongly implicated in this cognitive failure. Adult Neurogenesis is dependent on neurotrophin signaling, with the p75 pan-neurotrophin receptor (p75NTR) specific role to remain unclear under both physiological or pathological conditions. In the present study, we explore how p75NTR influences adult neurogenesis under both physiological and neurodegenerative conditions, focusing on AD.

Methods We used the amyloidogenic 5xFAD mouse model of AD, as well as p75NTR full and conditional knockout mice. Moreover, we have generated a 5xFAD/p75NTR knockout model to directly examine the connective role of p75NTR in adult neurogenesis and AD. We have tempo-spatially evaluated the impact of p75NTR, by performing 5-bromo-2'-deoxyuridine injections to detect neural stem cell proliferation and immunohistochemistry analysis for key neurogenic markers. Additionally, transcriptomic profiling identified p75NTR-dependent gene networks. To extend findings to humans and provide translational relevance, we investigated p75NTR effects in human induced Pluripotent Stem Cells-derived Neural Stem Cells (iPSCs-derived NSCs), depicting receptor's signaling in the presence of toxic Amyloid-β.

Results Deletion of p75NTR in mice led to reduced NSC proliferation, altered differentiation, and decreased survival of neurons in the dentate gyrus, while our results from conditional knockout lines suggest that p75NTR regulates these processes through mechanisms extending beyond NSCs. Under AD and specifically in 5xFAD mice, neurogenesis was transiently increased at early stages but subsequently declined. This compensatory response was absent in 5xFAD/p75NTR knock out mutants, which exhibited exacerbated deficits, indicating a p75NTR-dependent disease modification. Transcriptomic analyses revealed gene networks consistent with changes in proliferation, differentiation, and survival. Finally, in human iPSCs-derived NSCs, p75NTR expression was confirmed, and receptor inhibition significantly reduced amyloid-β–induced toxicity, pointing to conserved functions across species.

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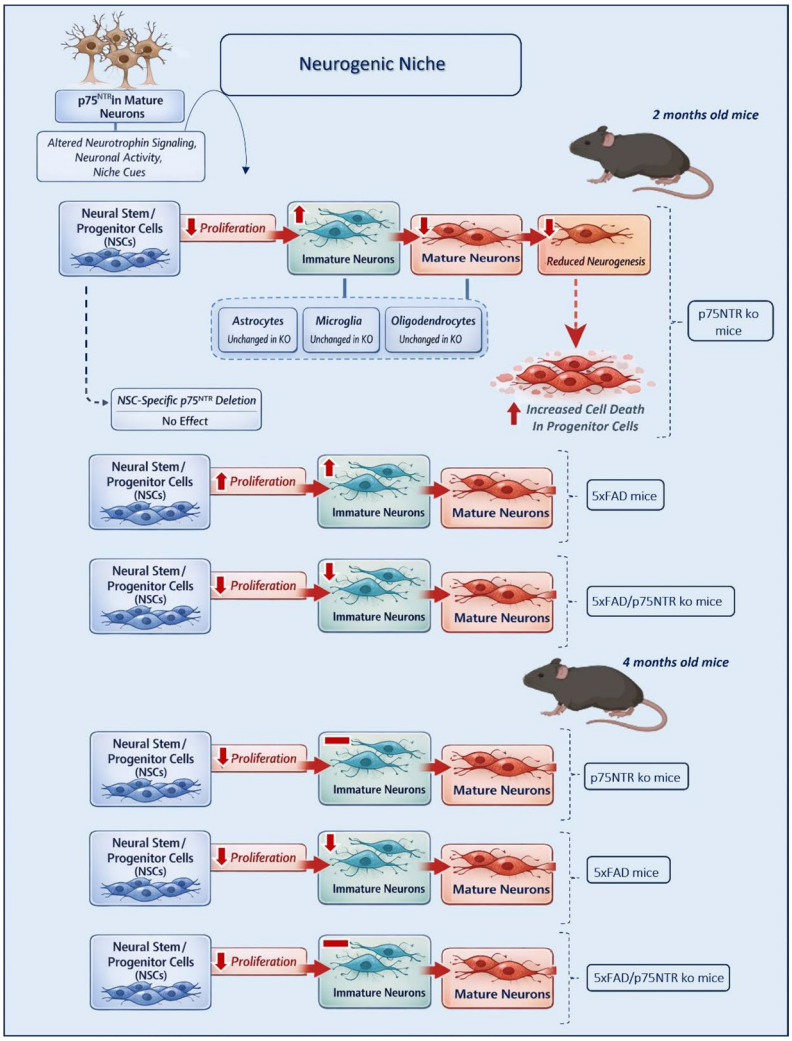
Conclusions Together, these findings support a significant role for p75NTR in regulating hippocampal neurogenesis under both physiological and AD-related conditions. By linking p75NTR function to both rodent and human neural stem cell responses, this study highlights that p75NTR is not only critical for maintaining neurogenesis but also represents a candidate target for future therapeutic exploration in AD.

Keywords Hippocampal adult neurogenesis, P75 neurotrophin receptor, Alzheimer's disease, Dentate gyrus, Neural stem cells, Human neural stem cells

Graphical abstract

p75NTR regulates adult hippocampal neurogenesis in vivo under physiological and Alzheimer's disease conditions.

Schematic summary of p75 neurotrophin receptor (p75NTR) function in the adult dentate gyrus of the hippocampus in vivo. Under physiological conditions, p75NTR supports neural stem cell (NSC) proliferation, proper neuronal differentiation and maturation through cell non-autonomous niche mechanisms, likely mediated by p75NTR expression in mature neurons. Deletion of p75NTR reduces NSC proliferation, leads to accumulation of immature neurons, impairs terminal neuronal maturation and increases overall cell death within the hippocampal neurogenic niche, whereas NSC-specific deletion has no effect, indicating extrinsic regulation rather than intrinsic stem cell or glial defects. In the 5xFAD mouse model of Alzheimer's disease (AD), early disease stages exhibit a compensatory increase in NSC proliferation and immature neuron production, which is lost in 5xFAD/p75NTR ko mice. By 4 months, proliferation and immature neuron production decline in 5xFAD mice, and p75NTR deletion further exacerbates these deficits, indicating its critical role during early AD, also highlighting p75NTR as a key regulator of hippocampal neurogenesis in vivo.



Background

Alzheimer's Disease (AD) is the most challenging neurodegenerative disease in terms of epidemiological penetrance and socioeconomical cost [1, 2]. It is characterized by progressive cognitive impairment along with amyloid-β (Aβ) plaques deposition, tau tangles and synaptic dysfunction [3, 4]. Despite decades of research strategies [5, 6], targeting these late-stage aggregates has largely failed in clinical trials and this has underscored the urgent need to identify novel therapeutic targets.

Among these emerging targets, the lifelong generation of new neurons in the dentate gyrus, meaning the adult hippocampal neurogenesis, has gained considerable attention. This process supports learning, memory, and emotional regulation by enabling Neural Stem Cells (NSCs) to proliferate, differentiate, and functionally integrate into neural circuits [7–10]. Additionally, impaired hippocampal neurogenesis has been linked to cognitive decline in AD [11–13], underscoring the importance of defining the mechanisms that regulate NSC proliferation and survival as a novel pharmacological approach for neurodegeneration.

Neurotrophins including NGF, BDNF, and NT3/4, are major regulators of neuronal survival and plasticity [14, 15]. They mainly act through Trk receptors, but all neurotrophins also bind with the p75 pan-neurotrophin receptor (p75NTR) [16, 17]. The last is broadly expressed during development [15, 18–20], however it becomes refined to specific brain regions during adulthood where it can promote either cell survival or cell death depending on cellular context and ligand availability [21–23]. As a multifactorial receptor, p75NTR, is gaining particular interest as a "fate decision protein" in stem cells, modulating their potency and differentiation [24].

Despite growing attention, p75NTR's role in adult hippocampal neurogenesis remains unclear, with contradictory results across genetic models [25–29]. Given the critical role of adult hippocampal neurogenesis in maintaining cognitive function, p75NTR has increasingly been recognized as a contributing factor to neurodegenerative disorders, alongside with its pleiotropic effects on neuronal functions [30]. Specifically in AD, p75NTR is upregulated in degenerating neurons and directly interacts with Aβ mediating either toxicity or protection [31–33]. This duality makes p75NTR both intriguing and challenging as a potential therapeutic target.

However, the precise role of p75NTR in regulating NSC fate in vivo, under both physiological and Alzheimer's disease conditions, remains poorly defined. Critically, it is unknown how its absence impacts the neurogenic process throughout AD progression, and whether its functions are conserved in human NSCs remains entirely unexplored.

To address these challenges, we used genetically modified mouse models at different ages to capture temporal aspects of AD pathology and neurogenesis. These included the amyloidogenic 5xFAD model [34], the full p75NTR knockout (p75NTRExIII) [firstly described by Lee et al. [35]], and a crossbred 5xFAD/p75NTR knockout line, as well as a Nestin-Cre–driven conditional knockout to study cell-autonomous effects in NSCs. In parallel, we used human induced pluripotent stem cell (hiPSC)-derived NSCs [36] to assess p75NTR's role in regulating proliferation and Aβ toxicity in a human context. Finally, we applied transcriptomics to identify gene networks regulated by p75NTR.

Through this combined in vivo models and human stem cell approach, we provide a detailed spatiotemporal characterization of p75NTR function in adult hippocampal neurogenesis under both physiological and AD conditions. Our findings highlight p75NTR as a regulator of hippocampal neurogenesis relevant to AD pathophysiology and as a candidate pathway for future therapeutic investigation.

Methods

Mice

Wild-type (WT), p75NTR ko, p75 fl/fl NestinCre, 5xFAD and 5xFAD/p75NTR ko male and female mice of different ages (2, 4 and 6-months old) were used. p75NTR ko mice (Ngfrtm1Jae ExonIII) and 5xFAD heterozygous mice (#002213 and #034848-JAX, respectively) were maintained on a C57BL/6 background. The p75fl/fl mice (ExonII floxed, Ngfrtm1a(EUCOMM)Wtsi) were crossed with Nestin-Cre transgenics. 5xFAD mice express human APP (Swedish K670N/M671L, Florida I716V, London V717I) and PSEN1 (M146L, L286V) mutations [34], and 5xFAD/p75NTR ko mice were generated by crossing 5xFAD and p75NTR heterozygotes. All animals were housed at IMBB-FORTH, Crete, under standard conditions and in compliance with Greek Government and FORTH ethics guidelines.

BrdU (5-bromo-2′-deoxyuridine) labelling

BrdU i.p. injections 100 mg/kg (10mgr/ml-Sigma, St. Louis, MO, USA, B5002) were performed for 5 days (1 injection per day). Analysis was performed either immediately after the BrdU injections (assessment of proliferation) or after 21 days (assessment of survival).

Tissue processing

Mice were anesthetized, trans-cardially perfused with saline and the brains have been dissected. The right hemispheres were further dissected and hippocampal specimens were stored in − 80 °C for RNA isolation. The left hemispheres were post-fixed by 4% paraformaldehyde (158127, Sigma) overnight at 4 °C. They were stored

in cryoprotective medium (15% sucrose/7,5% gelatine) at −80 °C, until they processed for coronal sections. Coronal sections of 40μM were cut in the dorsoventral axis of hippocampus (from bregma −4 mm to −1 mm).

Immunohistochemistry

Cryosections were permeabilized by immersing them in ice-cold acetone for 5 min at −20 °C and washed with 0.1% Triton X-100 in 1x PBS for 15 min followed by 0.3% Triton X-100 in 1x PBS for 30 min. Sections were then blocked for 1 h in 10% donkey serum (S30, Millipore, Burlington, MA, USA) containing 0.1% Triton X-100 and 0.1% BSA in 1x PBS and incubated overnight at 4 °C with the primary antibodies listed in Table 1. Slides were then washed and incubated with the appropriate fluorochrome-labeled secondary antibodies at room temperature (Table 1). Cell nuclei were visualized with Hoechst (1:10.000, H3570, Invitrogen, Carlsbad, CA, USA). Slides were covered with VECTASHIELD® Antifade mounting medium (VECTOR, Newark, CA, USA) and images were photographed via confocal microscopy (SP8 Leica, Wetzlar, Germany). For double labelling and the detection of BrdU-labeled nuclei, specimens have been previously incubated in 2 N HCl at 37 °C, followed by two rinses with PBS before blocking step.

Table 1 List of primary and secondary antibodies used in Immunohistochemistry-Immunofluorescence and Western blot assay

Antibody	Supplier	Catalogue Number	Dilution	Use
Anti-BrdU	Abcam	Ab1893	1:100	IHC, IF
Anti-Sox_2	Cell signaling	2748	1:100	IHC, IF
Anti-DCX	Abcam	207,175	1:100	IHC, IF
Anti-NeuN	Millipore	MAB377	1:100	IHC, IF
Anti-DCX	Santa Cruz	sc 271,390	1:50	IHC, IF
Anti-Tuj1	Biolegend	801,201	1:400	IHC, IF
Anti-GFAP	Proteintech	168,251	1:400	IHC, IF
Anti-Olig2	Millipore	AB9610	1:400	IHC, IF
Anti-Iba1	Synaptic Systems	234,017	1:400	IHC, IF
Anti-cleaved caspase3	Cell signaling	9661	1:300	IHC, IF
Anti-p75NTR	Biolegend	839,701	1:1000 1:100	WB, IHC, IF
Anti-p75NTR (MC192)	Abcam	ab6172	1:100	IP
Anti-RIP2	Enzo Life Sciences	ADI- AAP-460	1:1000	WB
Anti-TRAF6	Abcam	ab33915	1:2000	WB
Anti-actin	Santa Cruz Biotechnology	sc-47,778	1:2000,	WB
Anti-mouse Alexa Fluor 488	Invitrogen	A11029	1:500	IHC, IF
Anti-rabbit Alexa Fluor 555	Invitrogen	A10040	1:500	IHC, IF
Anti-sheep Alexa Fluor 647	Jackson ImmunoResearch	713-605-003	1:500	IHC, IF
HRP Anti-mouse IgG	Millipore	AP- 124P	1:5000	WB
HRP Anti-rabbit IgG	Invitrogen	65–6120	1:5000	WB

Cell counts and quantification

Cell counts and quantification are based on a modified unbiased stereology protocol. Seven out of every 10 adjacent sections were chosen (covering the whole DG area of the hippocampus) and processed for immunohistochemistry. The number of $BrdU^+$, $Sox2^+$, Dcx^+, $NeuN^+$, $caspase3^+$, $Tuj1^+$, $GFAP^+$, $Olig2^+$ and $Iba1^+$ cells was then counted under ×40 magnification under a fluorescent microscope (SP8 Leica) at the area of hippocampal DG of a total of 7 sections and the average number of cells was estimated. The mean was then multiplied with the total number of sections (75 per mouse) to estimate the total number of cells per DG.

Generation and culture of human neural stem cells (hNSCs)

iPSCs were reprogrammed from skin fibroblasts of two healthy human lines (SFC856-03–04, SFC841-03-01) that were kindly provided by Dr M. Z. Cader and used for the generation of neural progenitor cells, as previously described in [37]. Briefly, iPSCs colonies were cultured on mouse embryonic fibroblasts (MEFs), in a medium that consisted of DMEM-F12 (21331-020, Gibco, Carlsbad, CA, USA), 20% (v/v) Knockout Serum Replacement (10828028, Gibco), 1% non-essential amino acids (NEAA; 11–140-050, Gibco), 1% penicillin/streptomycin (PAA; 15140122, Gibco), 1% L- Glutamine (A2916801, Gibco), 1mM 2-Mercaptoethanol (31350010, Gibco) supplemented with 5 ng/ml FGF2 (100–18 C, Peprotech, Cranbury, NJ, USA). Next, iPSC colonies were detached from MEFs with 2 mg/mL collagenase IV (C1764, Sigma) and resuspended in the same medium without FGF2, supplemented with 1 μM Dorsomorphin (ab120843, Abcam, Cambridge, UK), 3 μM CHIR99021 (SML1046, Sigma), 10 μM SB-431542 (72232, Stem Cell Technologies, Vancouver, BC, Canada) and 0.5 μM Purmorphamine (72202, Stem Cell Technologies). Embryoid bodies (EBs) were formed and medium was changed to N2B27 medium (1:1 Neurobasal (21103-049, Gibco) and

DMEM-F12 medium, supplemented with N2 supplement (17502048, Gibco) and B27 supplement lacking vitamin A (12587010, Gibco), and 1% penicillin/streptomycin supplemented with the aforementioned small-molecules. On day 4, dorsomorphin and SB-431542 were removed, whereas 150μM L-Ascorbic acid (A4544, Sigma) was added to the medium. On day 6, the spheres were cut into smaller pieces and plated on Matrigel-coated plates (354263, Corning, NY, USA). When confluent, cells were split via treatment with accutase (A6964, Sigma). The identity of the cells was verified via immunocytochemistry for NESTIN (151 NB100-1604, BioTechne, Minneapolis, MN, USA). The generated NSCs can be expanded and cryopreserved enabling long-term, repetitive studies.

Immunoprecipitation and Immunoblotting

Cells were suspended in Pierce™ IP Lysis Buffer (87788, Thermo Fischer Scientific, Waltham, MA, USA) supplemented with protease inhibitors (539138, Calbiochem, Burlington, MA, USA) and phosphatase inhibitors (524629, Calbiochem). Lysates were pre-cleared for 1 h with protein G-plus Agarose beads (sc-2002, Santa Cruz Biotechnology, Dallas, TX, USA) and immunoprecipitated with p75NTR antibody overnight at 4 °C. Protein G-plus agarose beads were incubated with the lysates for 4 h at 4 °C via gentle shaking. Beads were collected via centrifugation, re-suspended in 2× SDS loading buffer and subjected to Western blot analysis against TRAF6 antibody. For immunoblot (IB) analysis, the beads were suspended in sodium dodecyl sulfate-loading buffer and separated through SDS-PAGE. Proteins were transferred to nitrocellulose membranes and blotted with the corresponding antibodies for RIP2, p75NTR, TRAF6 and Actin (Table 1). Immunoblots were developed using the ECL Western Blotting Kit (ThermoFisher Scientific), and Image analysis and quantification of band intensities were performed with ImageJ Software. For the immunoprecipitation assay, the analysis was derived from the immunoprecipitated fraction relative to the total fraction. For the identification of molecular weight of each protein we have used the Color Prestained Protein Standard, Broad Range (10–250 kDa) (P7719S, New England Biolabs, US).

Preparation of Aβ oligomers

Amyloid-β (1-42) peptide was purchased from AnaSpec (AS-20276, AnaSpec, Fremont, CA, USA) and prepared according to manufacturer's instructions. For Aβ treatment, Aβ oligomers were prepared according to previously described protocols [38, 39], and they were diluted in DMEM at the specified concentrations. Human NSCs were treated with 10μM for 48 h.

Cell tox assay

After 24 h of treatments, we used the CellTox™ Green Cytotoxicity Assay kit (G8742, Promega Corporation, Madison, WI, USA) to assess the survival of hNSCs in the presence or absence of p75NTR inhibitor MC-192 (2.5 ng/mL, ab6172, Abcam). AD pathology was mimicked by treating the human NSCs with Aβ-amyloid peptides (10μM of Aβ1–42 oligomers) and after 24 h we used cell tox assay for another 24 h. Dead cells were then counted with a fluorescent microscope at 485–500 nm Ex. CellTox assay reagents and Hoechst (1:10.000, H3570, Invitrogen) were added to each well for 30 min, and cells were imaged using a fluorescent microscope (Zeiss AXIO Vert A1, Zeiss, Oberkochen, Germany). Positive cells for cell tox reagent were normalized to reflect the total number of cells per image.

BrdU assay in hiPSCs-derived NSCs

iPSCs-derived NSCs were cultured on Matrigel for 24 h with or without treatment of p75NTR inhibitor (ab6172, abcam MC-192, 2,5ng/ml). After 24 h the cells were pulsed with 1μM BrdU for 4 h and fixed with 4% PFA for subsequent immunostaining for BrdU and Hoechst for nuclear labeling.

Isolation of RNA & sequencing

Total RNA from biological triplicates (hippocampal specimens of p75NTR ko, 5xFAD, 5xFAD/p75NTR ko and WT mice of 2 months old, that have been dissected after the BrdU injections) was extracted using Trizol reagent (15596018, Thermo Scientific) as per the manufacturer's protocol. The quantity and quality of RNA samples were analyzed using Agilent RNA 6000 Nano kit with the bioanalyzer from Agilent. RNA samples with RNA integrity number (RIN) > 7, were used for library construction using the 3′ mRNA-Seq Library Prep Kit FWD for Illumina (QuantSeq-LEXOGEN, Vienna, Austria) as per the manufacturer's instructions. Amplification was controlled for obtaining optimal unbiased libraries across samples by assessing the number of cycles (14) required by qPCR. DNA High Sensitivity Kit for bioanalyzer was used to assess the quantity and quality of libraries, according to the manufacturer's instructions (Agilent, Santa Clara, CA, USA). Libraries were multiplexed and sequenced on an Illumina Nextseq 2000 at the genomics facility of IMBB FORTH according to the manufacturer's instructions.

Differential expression and GO enrichment analysis

The quality of the raw sequences in the output FASTQ files was assessed with the FastQC software [www.bioinformatics.babraham.ac.uk/projects/fastqc/]. Reads were aligned to the mouse (mm10) genome using the Hisat2 aligner (parameters used: hisat2 -p32 -x

$REFERENCE_GENOME -q fastq/$FILE_ID.fastq -S $FILE_ID.sam --score-min L,0,–0.5) [40]. The BAM files were sorted by genomic coordinates and indexed using samtools [41]. Htseq-count was utilized to summarize reads at the gene level (parameters used: htseq-count -f bam -s yes -i gene_id bam $FILE_ID.bam $REFERENCE_ANNOTATION > $COUNTS_DIR/NGS$FILE_ID) [42]. Differential expression analysis (DEA) was conducted using EdgeR [43]. Two designs were employed, both accounting for gender differences between the mice (gender included as a covariate). The first design also included disease and genotype as covariates (~ disease + genotype + gender), with healthy WT mice treated as the reference. The second design accounted for mouse line as a group covariate (~ group + gender, groups: WT, 5xFAD, p75NTR ko, 5xFAD/p75NTR ko), with WT mice as the reference. Differentially expressed genes (DEGs) were identified using a significance threshold of adjusted p-value (padj) < 0.05. Enrichment analysis was performed using the Metascape web tool (enrichment p-value set to 0.05) [44]. Gene Set Enrichment Analysis (GSEA) tool was also used, to analyze the functional changes in gene expression across genotype (p75NTR ko vs. WT) and between 5xFAD and 5xFAD/p75NTR ko samples, as specified by Broad GSEA package using default parameters [45]. GO-BP pathways were tested (https://www.gsea-msigdb.org/gsea/msigdb/mouse/genesets.jsp? collection=GO: BP) against a ranked lists of descending LFCs for both contrasts, and we selected some positive hits for display. The volcano and bubble plots were created in R with custom in-house scripts (available upon request).

Reverse transcriptase PCR & quantitative PCR

cDNA was synthesized by the total RNA, using the High-Capacity cDNA Reverse Transcription kit (4368814, Thermo Fisher) according to the supplier protocols. Primers for Ngfr, were designed using the NCBI Primer BLAST software (Forward: AGAGAAACTGCACAGC GACA, Reverse: CCATCACCCTTGAGGGCTTG), to detect an area inside the coding sequence of the gene. More specifically, the primer pair was designed as such to amplify specifically the area between exons V and VI (between TMD – Transmembrane Domain and DD – Death Domain). Primers for mouse GAPDH, were also designed to be used as a control sample (Forward: ATT GTCAGCAATGCATCCTG, Reverse: ATGGACTGTG GTCATGAGCC). To run the quantitative RT-PCR, we used 1 µL of cDNA (10 ng/µL) and the KAPA SYBR Fast kit (KK4601, Sigma) according to the supplier's instructions. The cycling program consisted of 20 s at 95 °C, followed by 40 cycles of 95 °C for 3 s and 60 °C for 30 s on a StepOne Real-Time PCR System (Thermo Fisher Scientific). After the completion of qPCR, a melt curve of the amplified products was performed. The housekeeping gene GAPDH was used to normalize the expression levels between the different conditions. Data were collected and analyzed using the StepOne Software v2.3 (Thermo Fischer Scientific).

Statistical analysis

All values are expressed as the mean ± SEM. Student's t-test was used for the comparison of two groups, and one-way or two-way ANOVA were used for multiple group comparisons. A $p < 0.05$ was considered to mark statistical significance. Statistical analysis was performed using GraphPad Prism 7 (GraphPad Software Inc., San Diego, CA, USA).

Results

p75NTR is a key regulator of NSC proliferation

To test the effect of p75NTR deficiency on the proliferation of NSCs in adult mice, we compared the number of BrdU and Sox2 positive cells at the DG of the hippocampus in WT and p75NTR ko mice. The deletion of p75NTR in the ko mice was identified by the absent expression of the receptor in the Basal Forebrain after immunohistochemical staining using primary antibody for p75NTR (Supplemental Fig. S1).

Coronal sections of hippocampal DG from WT and p75NTR ko mice were co-immunostained with BrdU and Sox2 primary antibodies and analysis showed that the number of proliferative NSCs was significantly decreased in the p75NTR ko mice [from 1011.25 ± 66.3 cells (SEM) to 530.5 ± 63.6 cells (SEM)] (Fig. 1A, B), corresponding to a 47.5% decrease. Thus, these results indicate that the expression of p75NTR is necessary for the proper proliferation of NSCs in the adult mice of 2 months old. By repeating this experimental approach at 4- and 6-months old mice, we observed that the deficiency of p75NTR in 4 months old mice, still has a significant effect on the proliferation rates reducing their numbers from 765 ± 38.2 cells (SEM) in WT to 489.5 ± 35 cells (SEM) in p75NTR ko mice (Fig. 1A, B). On the other hand, in 6 months old mice there were no significant differences since the proliferation of NSCs diminishes to such an extent (approximately around 75%) at this age that no significant effect can be detected.

Enhanced generation of immature neurons following p75NTR deficiency

Following the proliferation capacity of NSCs upon p75NTR deficiency, we explored the differentiation of NSCs of the hippocampal DG towards the production of immature neurons. At the age group of 2, 4 and 6 months old, we studied the number of Doublecortin (Dcx) positive cells, a protein marker depicting immature neurons. To our surprise, the analysis showed significantly increased number of immature neurons in the

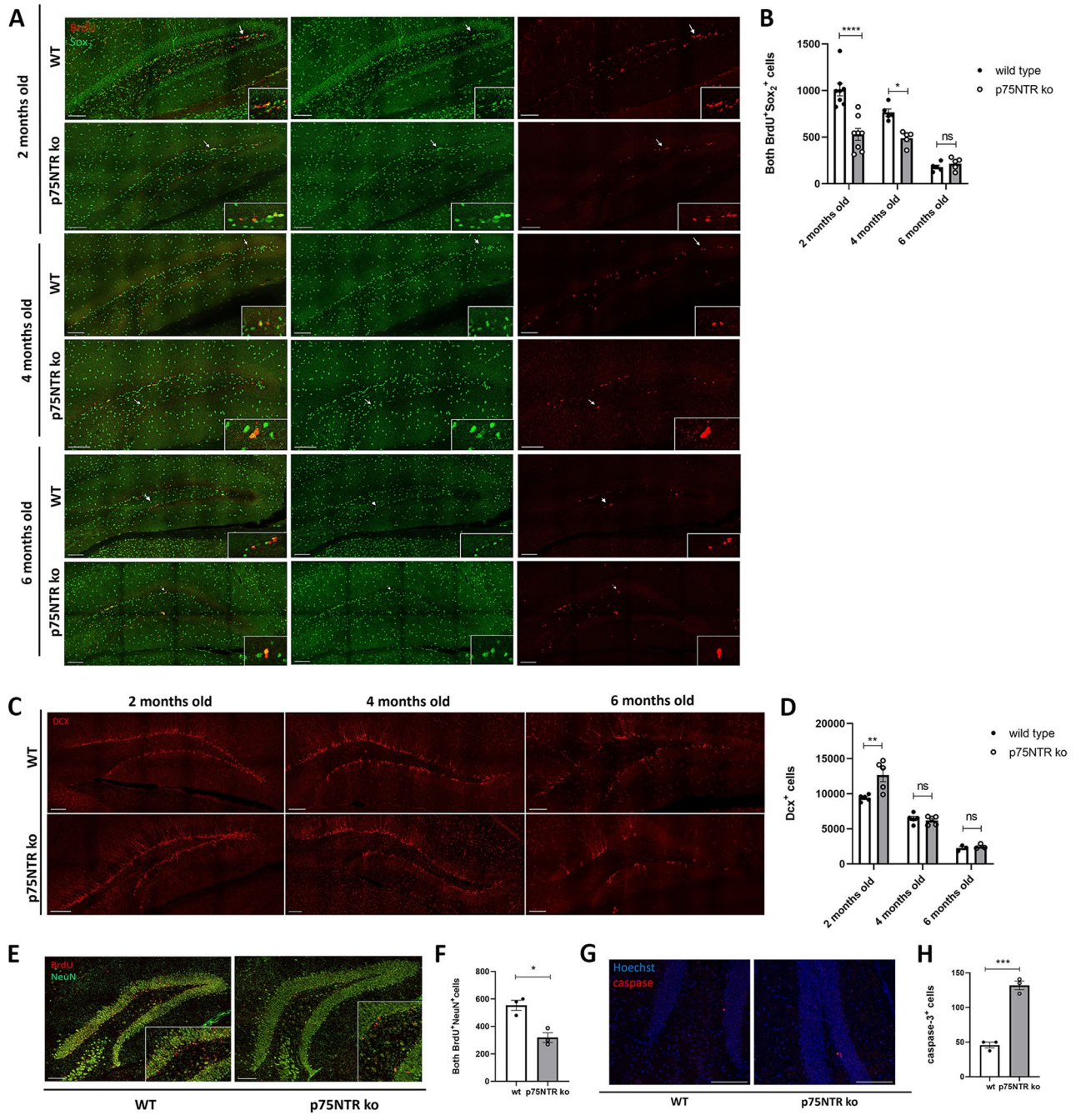


Fig. 1 The effects of p75NTR deficiency on the proliferation, differentiation and maturation of NSCs. **A** Coronal sections, of the hippocampal DG from 2 months old WT and p75NTR ko mice injected with BrdU for 5 days. Sections were co-immunostained for BrdU (red) and Sox2 (green). Scale bar, 100 µm. **B** Quantification of both $BrdU^+$ and $Sox2^+$ cells in injected mice (2mo -- $n=8$ for each genotype; 4mo & 6mo -- $n=5$ for each genotype). Data are presented as mean ± SEM. 2way ANOVA ****$p<0{,}0001$, *$p<0{,}0{,}5$ ns, no significant. **C** Coronal sections, of the hippocampal DG from 2 months old WT & p75NTR ko mice. Images depict Dcx (red) immunostained immature neurons. Scale bar, 100 µm. **D** Quantification of Dcx^+ cells in p75NTR ko & WT mice (2mo & 4mo -- $n=5$ for each genotype, 6mo -- $n=3$ for each genotype). Data are presented as mean ± SEM. 2way ANOVA**$p<0{,}005$, ns, no significant. **E** Coronal sections, of the hippocampal DG from 2 months old WT mouse. Images depict BrdU (red) & NeuN (green) immunostained mature neurons. Scale bar, 100 µm. **F** Quantification of both positive cells in 2 months old p75NTR ko & WT mice ($n=3$ p75NTR ko, $n=3$ WT). Data are presented as mean ± SEM. *$p<0.05$ (Student's t-test unpaired). **G** Coronal sections of the hippocampal DG from 2 months old WT mouse. Images depict caspase3^+ cells (red), Hoechst (blue). Scale bar, 100 µm. **H** Quantification of caspase3^+ cells in 2 months old WT & p75NTR ko mice ($n=3$ p75NTR ko, $n=3$ WT). Data are presented as mean ± SEM. ***$p<0.001$ (Student's t-test unpaired). BrdU, 5-bromo-2'-deoxyuridine; Sox2, SRY-Box Transcription Factor 2; Dcx, doublecortin; NeuN, Neuronal Nuclear marker

p75NTR ko mice compared to the WT [from 9410 ± 207 cells (SEM) to 1267.6 ± 949.7 cells (SEM) in p75NTR ko mice] (Fig. 1C, D). Furthermore, the area of Dcx^+ cells' processes was also significantly increased in p75NTR ko mice (Supplemental Fig. S2). Thus, the reduction of proliferation that was observed in 2 months old p75NTR ko mice resulted in the significant increase of immature neurons, indicating a robust maturation process forced by the lack of p75NTR expression. This receptor is known to regulate cell cycle progress [46, 47] and this action could explain the increased maturation rates in NSCs. Moreover, we studied the effect of p75NTR deletion in NSC differentiation in 4- and 6-months old mice (Fig. 1C, D), where we found no significant differences. As expected, the number of Dcx^+ cells in 6 months old mice was reduced compared to this of 2 months old mice (approximately around 75%) following the same pattern with the proliferation procedure.

p75NTR deficiency impairs survival and terminal neuronal maturation

To further evaluate the hypothesis of p75NTR-mediated cell cycle deterioration, we also investigated the production of fully mature neurons at 2 months old mice. For that reason, we pulsed mice with BrdU for the first 5 days of a 3-week period and after 21 days overall we sacrificed the mice and studied the number of $BrdU^+$ and $NeuN^+$ (Neuronal nuclear marker of mature neurons) cells that survived. Our analysis showed that the number of both positive cells in the DG was significantly decreased in p75NTR ko mice compared to the WT [from 553.3 ± 37.2 cells (SEM) to 319.17 ± 35.1 cells (SEM) in p75NTR ko mice] (Fig. 1E, F). Thus, p75NTR is necessary not only for proper differentiation of neuronal precursors to immature neurons, but it also determines the final number of fully mature neurons by driving immature neurons to "freezing" state. In order to specify if this decreased number of $NeuN^+$ cells is due to the inability of Dcx^+ neurons to further differentiate or cells are more vulnerable, we measured cell death in hippocampal DG. Analysis of apoptotic cell death using cleaved caspase-3 immunostaining revealed a significant increase in the total number of $caspase3^+$ cells in the adult hippocampus of p75NTR ko mice compared to the wt controls [from 45.83 ± 4.1 cells (SEM) to 131.87 ± 6.15 cells (SEM) in p75NTR ko mice] (Fig. 1G, H). In contrast, the number of $caspase3^+$ immature neurons (DCX^+) (WT 66.7 ± 33.3%, KO 40.2 ± 8.8%) and $caspase3^+$ mature neurons ($TUJ1^+$) (WT 33.3 ± 16.7%, KO 11.4 ± 5.8%) did not differ between genotypes (Supplemental Fig. S3). These data indicate that loss of p75NTR is associated with increased overall cell death in the hippocampal niche, while neuronal survival remains unaffected. Contradictory to receptor's well known pro-apoptotic effects, we here show that its expression is important for the proper differentiation and maturation of NSCs, by sustaining their survival rates in addition to cell cycle control.

Genes differential expression analysis confirmed the neurogenic capacity of p75NTR

To identify the gene networks underlying the role of p75NTR in adult neurogenesis, we performed RNA sequencing on hippocampal tissue obtained from WT mice, p75NTR ko, and a newly generated mouse line upon crossing of 5xFAD with the p75NTR ko mice, the 5xFAD/p75NTR ko (with the WT group serving as the reference). This bioinformatic analysis revealed 9 significantly upregulated and 33 significantly downregulated genes, as visualized in the volcano plot (Fig. 2A). Gene Ontology (GO) and pathway enrichment analyses revealed that these differentially expressed genes (DEGs) are significantly involved in biological processes such as the regulation of neurogenesis, axonogenesis, cell growth and cell migration (Fig. 2B), confirming the biological results from the in vivo studies. Genes and GOs are detailed in Supplemental Excel Table S1.

A heatmap representing the expression patterns of all 42 DEGs is shown in Fig. 2C, highlighting several genes with known roles in proliferation and migration. For instance, *Med1* is involved in the induction of cell proliferation and migration and regulates BDNF levels [48], while *Msl1* contributes to cell cycle progression [49]. *Fabp7* promotes proliferation and survival via the MEK/ERK signaling pathway [50], and deletion of *Fxr1* has been shown to impair neurogenesis by reducing cell proliferation in the dentate gyrus [51]. Additionally, *Cdh1* plays a critical role in regulating neurogenesis and cortical development [52], and, together with *Fzr*, promote neural stem cell differentiation in Drosophila [53]. Conversely, genes such as *Sv2a* were identified for their role in supporting NSC survival through the p53 signaling pathway [54].

It is of special notice that the *Ngfr* gene was found to be upregulated in both p75NTR ko and 5xFAD/p75NTR ko mice (Fig. 2A, C), indicating the important functions of this receptor since its expression levels are induced as a result of its loss of function. It could also be suggested that the receptor's intracellular part remaining after exon III deletion has potential signaling properties that contribute or opposing its extracellular functions. This upregulation was validated via qRT-PCR (Supplemental Fig. S4), with results presented as logarithmic fold change (logFC) relative to WT controls. To further explore the biological implications of these transcriptomic changes, we performed Gene Set Enrichment Analysis (GSEA), which revealed enrichment of GO Biological Process categories such as neural precursor cell proliferation, regulation of neuronal differentiation, and regulation of

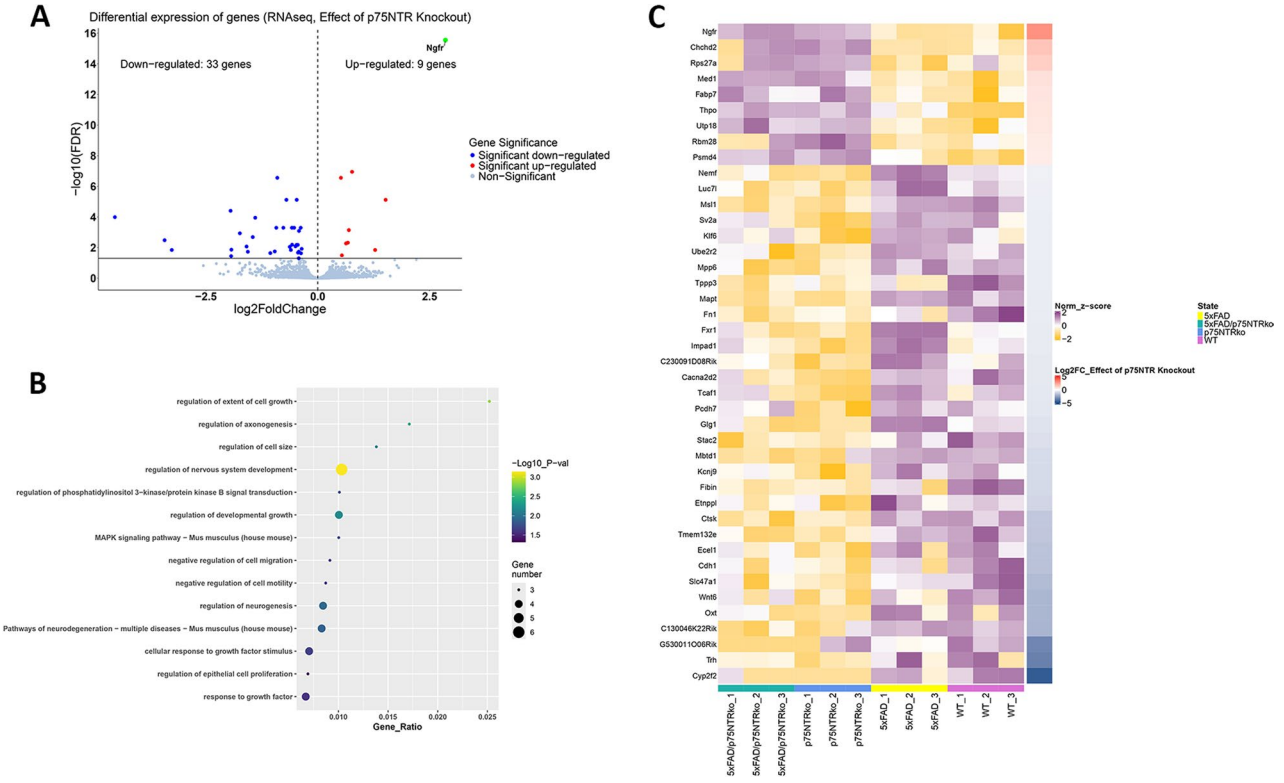


Fig. 2 Functional analysis of differential gene expression driven by the p75NTR ko genotype. **A** Volcano plot showing the number of Up (9) and Down (33) regulated genes (p adj < 0.05), pointing out the differential expression of Ngfr. Differential expression was determined using a Generalized Linear Model (EdgeR) identifying the main effect of the p75NTR ko genotype (p75NTR ko and 5xFAD/p75NTR ko mice, relative to WT and 5xFAD mice), adjusting for disease status and gender. **B** Bubble plot showing selected gene ontology (GO) and pathways significantly enriched by the complete list of significant DEGs (both upregulated and downregulated genes) identified in (**A**). Analysis was performed using Metascape (https://metascape.org). The x axis represents the gene ratio. Size of the bubble represents the numbers of DEGs found in each GO list. Coloration from yellow to black represents the – Log10_P value and depicts low (yellow) to high (black) enrichment scores (p adj < 0.05). **C** Heatmap showing the expression profile of all differentially expressed genes (p adj < 0.05) between samples with (5xFAD/p75NTR ko - green, p75NTR ko - blue) and without (5xFAD - yellow, WT - purple) the p75NTR receptor knocked out. Scaling was performed across genes using z-scores, to highlight relative expression differences. Fold changes are represented using the $\log_2$ fold change ($\log_2$FC) scale to reflect differential expression between conditions

apoptotic signaling in p75NTR ko mice compared to WT (Supplemental Fig. S5, Supplemental Excel Table S1).

p75NTR is affecting NSC proliferation in a cell non autonomous mechanism

In order to validate whether p75NTR expression specifically in NSCs is majorly controlling the adult hippocampal neurogenesis or if there are other cellular mechanisms derived from p75NTR expression in other cell types, we used an animal model, namely p75 fl/fl Nestin Cre. For that reason, we crossed mice with a specific deletion of p75NTR exonII with the Nestin-Cre mice, in order to delete the receptor only in Nestin positive cells (since Nestin is expressed only in NSCs). We counted again both $BrdU^+Sox2^+$ cells, at the DG of the hippocampus and the analysis showed that the number of proliferating NSCs remained unchanged in p75 fl/fl NestinCre mice compared to the WT (Fig. 3A, C). These results clearly show that p75NTR is affecting adult neurogenesis in a cell non autonomous mechanism.

p75NTR expressed in $Nestin^+$ cells does not affect the production of immature neurons

Furthermore, we analyzed if p75NTR specifically expressed in $Nestin^+$ cells has an impact in the production of immature neurons. Thus, we counted the number of Dcx^+ cells in the hippocampal DG of 2 months old p75 fl/fl NestinCre mice. Our results showed that there were no significant differences between the production of immature neurons in WT and p75 fl/fl NestinCre mice (Fig. 3B, D). In agreement with the aforementioned data about the proliferation of NSCs in this mouse model, we suggest that p75NTR expressed specifically in NSCs does not influence their proliferation, neither the production of immature neurons, indicating a cell non autonomous mechanism of action of the receptor in adult neurogenesis. Moreover, immunohistochemical analyses revealed no significant differences in astrocyte reactivity ($GFAP^+$), the number of oligodendrocyte lineage cells ($Olig2^+$), or microglia ($Iba1^+$) between genotypes, indicating that p75NTR deletion in $Nestin^+$ cells does not affect major

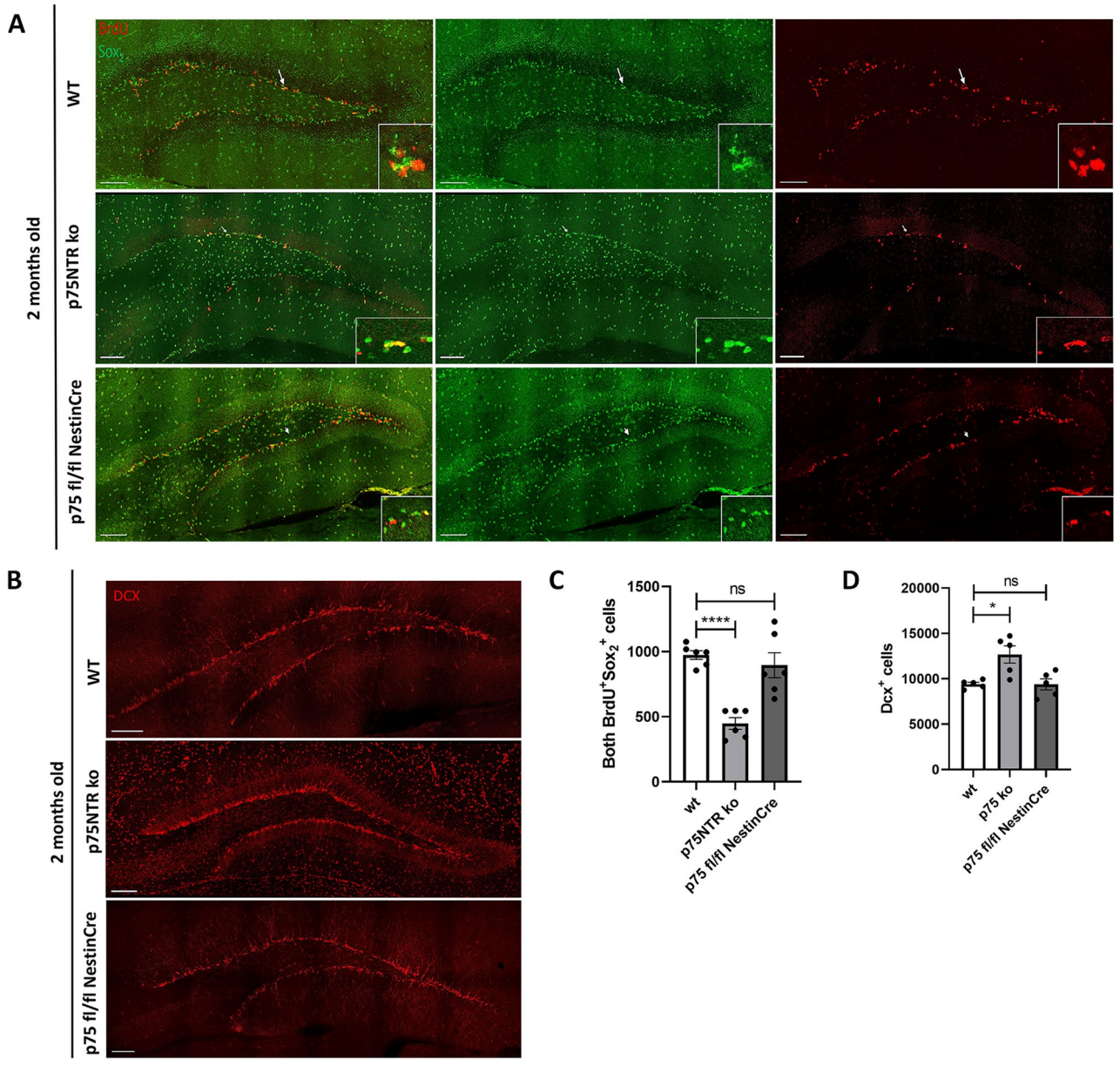


Fig. 3 p75NTR deficiency in p75 floxed/floxed NestinCre mice is not affecting the proliferation and differentiation of NSCs. **A** Coronal sections of the hippocampal DG from 2 months old WT and p75 fl/fl NestinCre mice injected with BrdU for 5 days. Sections were co-immunostained for BrdU (red) and Sox2 (green). Scale bar, 100 µm (**B**) Coronal sections of the hippocampal DG from 2 months old WT & p75 fl/fl NestinCre mice. Images depict Dcx (red) immunostained immature neurons. Scale bar, 100 µm. **C** Quantification of $BrdU^+$ and $Sox2^+$ cells in injected mice ($n=6$ for each genotype). Data are presented as mean ± SEM. p**** < 0,0001, ns, no significant (one way ANOVA). **D** Quantification of Dcx^+ cells in 2 months old WT & and p75 fl/fl NestinCre mice ($n=5$ for each genotype). Data are presented as mean ± SEM. *$p < 0,05$, ns, no significant (one way ANOVA). BrdU, 5-bromo-2′-deoxyuridine; Sox2, SRY-Box Transcription Factor 2; Dcx, doublecortin

non-neuronal cell populations in the adult hippocampus (Supplemental Fig. S6). Thus, the cell non-autonomous effects of p75NTR on adult hippocampal neurogenesis are unlikely to be mediated by alterations in astrocyte, oligodendrocytes or microglial populations, but rather by more subtle, functional changes within the neurogenic niche.

Increased proliferation rates of NSCs in 2 months old 5xFAD mice

In order to study the proliferation of NSCs under neurodegenerative conditions such as AD, we performed immunofluorescent staining of the coronal sections of hippocampal DG of 2 months old 5xFAD mice. Since there were no significant differences between 5xFAD homozygous vs. heterozygous mice (Supplemental Figure S7), we performed our studies with the heterozygous

mice (more commonly used in research studies). As it is shown in Fig. 4A and B, there is a statistically significant increase in the proliferation rates of NSCs in 2-months old 5xFAD mice when compared to the WT [from 997 ± 28.2 cells (SEM) to 1271.15 ± 80.8 cells (SEM) in 5xFAD mice]. Next, we studied the number of proliferative NSCs in 4 months old 5xFAD mice, and we observed that there is a significant decrease in the number of both $BrdU^+$ and $Sox2^+$ cells [from 765 ± 38.2 cells (SEM) to 475.5 ± 38.4 cells (SEM) in 5xFAD mice], as expected, since at this age most of the neurodegenerative effects of AD-like pathology have been described to be on their onset in this mouse strain (Fig. 4A, B). Additionally, 6 months old 5xFAD mice showed no significant differences, since in that age the number of proliferative NSCs is very low (Fig. 4A, B). The aforementioned results clearly show that AD background induces hippocampal neurogenesis at the initial stages, a phenomenon that is reversed at older age where AD pathology appears. We hypothesize that the increased number of NSCs observed in the 5xFAD background may represent a compensatory, homeostatic response to the neurodegenerative effects of AD-like pathology, such as the specific result of increased expression of Aβ, which could enhance signaling neurogenic mechanisms [55–58]. This response could potentially aim to counteract the neuronal loss typically seen at the onset

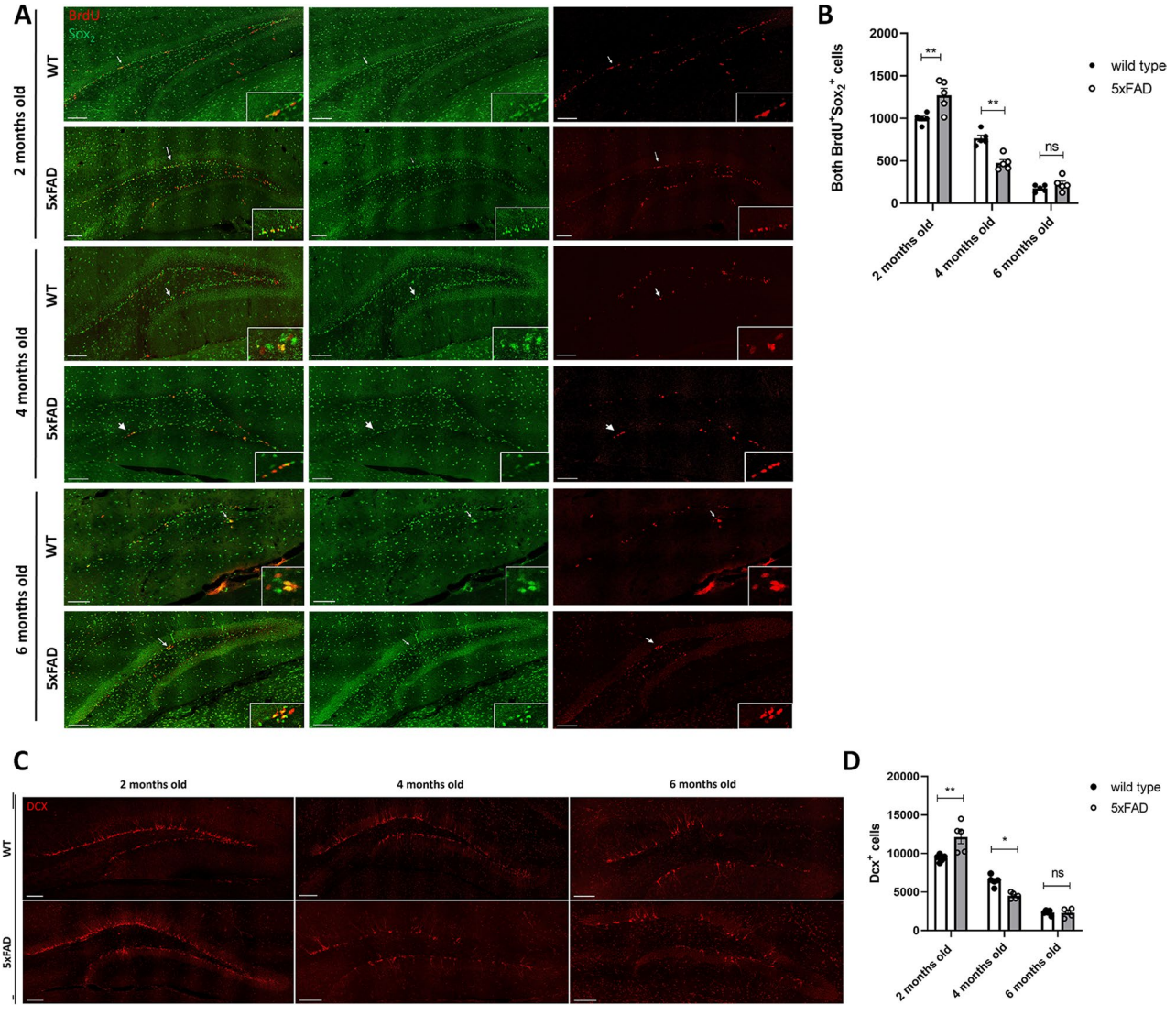


Fig. 4 Proliferation and differentiation of NSCs, under Alzheimer's Disease. **A** Coronal sections, of the hippocampal DG from 2 months old WT and 5xFAD mice injected with BrdU for 5 days. Sections were co-immunostained for BrdU (red) and Sox2 (green). Scale bar, 100 μm. **B** Quantification of $BrdU^+$ and $Sox2^+$ cells in injected mice (2mo, 4mo & 6mo -- $n = 5$ for each genotype). Data are presented as mean ± SEM. 2way ANOVA $^{**}p < 0{,}005$, ns, no significant. **C** Coronal sections, of the hippocampal DG from 2 months old WT & 5xFAD mice. Images depict Dcx (red) immunostained immature neurons. Scale bar, 100 μm. **D** Quantification of Dcx^+ cells in WT & 5xFAD mice (2mo, 4mo & 6mo -- $n = 5$ for each genotype). Data are presented as mean ± SEM. 2way ANOVA $^{**}p < 0{,}005$, $^{*}p < 0{,}05$, ns, no significant. BrdU, 5-bromo-2′-deoxyuridine; Sox2, SRY-Box Transcription Factor 2; Dcx, doublecortin

of the disease, which generally occurs around 4 months of age [38].

2 months old 5xFAD mice present increased number of immature neurons

In order to assess the production of immature neurons in terms of AD, we measured the Dcx^+ cells in 2 months old 5xFAD mice. By comparing the Dcx^+ cells in the DG of the hippocampus of WT and 5xFAD mice, we concluded that there is a significant increase between the two groups [from 9410 ± 210 cells (SEM) to 12128.25 ± 847.8 cells (SEM) in 5xFAD mice] (Fig. 4C, D). These results confirm that AD phenotype affects the proliferation of NSCs and it has also a significant impact in the production of new immature neurons, further proposing a general regulatory mechanism of neurogenesis upon Aβ changes. Taking into consideration, the number of immature neurons in 4 months old 5xFAD mice, we observed a significant decrease in the number of Dcx^+ cells [from 6469.6 ± 310.2 cells (SEM) to 4511.2 ± 180.6 cells (SEM) in 5xFAD mice], as it was expected due to the progress of the neurodegenerative effects of AD (Fig. 4C, D). As it was previously shown in WT mice, the 6 months old 5xFAD mice had no differences, emphasizing on the really low number of immature neurons at this age (Fig. 4C, D).

Deficiency of p75NTR in 2 months old 5xFAD mice regulates the proliferation of NSCs

Based on the above results, we tested the outcome of p75NTR deletion in the 5xFAD mice. We generated double transgenic mice, the 5xFAD/p75NTR ko and by following the same experimental protocols and measuring the number of both $BrdU^+Sox2^+$ cells, we revealed that in 2 months old 5xFAD/p75NTR ko mice there was a significant decrease in the proliferation rates of NSCs when compared to the WT mice, almost at the same levels as in p75NTR ko mice [from 997 ± 28.2 cells (SEM) to 702 ± 40.5 cells (SEM) in 5xFAD/p75NTR ko mice] (Fig. 5A, B). This novel result confirms the crucial role of p75NTR for the proliferation of NSCs, even in the AD

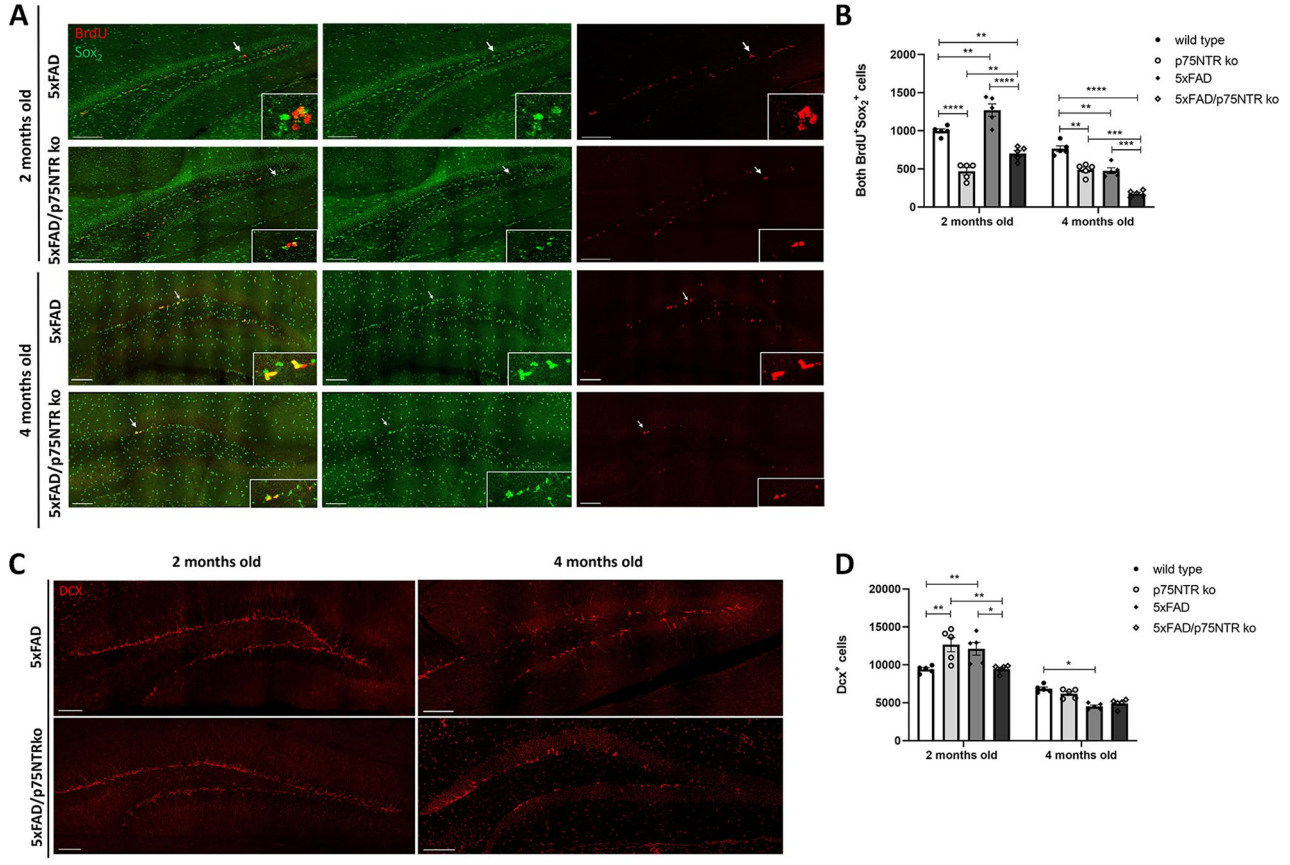


Fig. 5 The effects of p75NTR deficiency on the proliferation and differentiation of NSCs, under Alzheimer's Disease. **A** Coronal sections, of the hippocampal DG from 2 months old 5xFAD/p75NTR ko mouse injected with BrdU for 5 days. Sections were co-immunostained for BrdU (red) and Sox2 (green). Scale bar, 100 μm (**B**) Quantification of $BrdU^+$ and $Sox2^+$ cells in injected mice. (2mo & 4mo - $n = 5$ for each genotype). Data are presented as mean ± SEM. 2way ANOVA****$p < 0{,}0001$, ***$p < 0{,}001$, p**$< 0{,}005$. **C** Coronal sections, of the hippocampal DG from 2 months old WT, p75NTR ko, 5xFAD and 5xFAD/p75NTR ko mice. Image depicts Dcx (red) immunostained immature neurons. Scale bar, 100 μm. **D** Quantification of Dcx^+ cells in WT, p75NTR ko, 5xFAD, and 5xFAD/p75NTR ko mice (2mo & 4mo - $n = 5$ for each genotype). Data are presented as mean ± SEM. 2way ANOVA**$p < 0{,}005$, *$p < 0{,}05$. BrdU, 5-bromo-2′-deoxyuridine; Sox2, SRY-Box Transcription Factor 2; Dcx, doublecortin

background. Furthermore, it is of special notice that this p75NTR-mediated inhibition of NSC proliferation counteracts the 5xFAD genotype, where an elevated NSC proliferation was observed.

Considering the results derived by the 4 months old mice, we observed for once more, a decreased number of proliferative NSCs in the 5xFAD/p75NTR ko mice, showing again the necessity of p75NTR expression [from 765 ± 38.2 cells (SEM) to 176 ± 17.7 cells (SEM) in 5xFAD/p75NTR ko mice] (Fig. 5A, B). Moreover, if we compare the number of proliferative NSCs in the 5xFAD and 5xFAD/p75NTR ko mice, of both ages 2- and 4- months old, we can conclude that the expression of p75NTR is necessary for the proliferation of NSCs under AD both during the onset as well as the progression of the disease (Fig. 5A, B).

p75NTR deficiency in 2 months old 5xFAD mice does not affect the production of immature neurons

Having the above in mind, we analyzed the number of Dcx^+ cells upon p75NTR deletion in 2 months old 5xFAD mice. The number of Dcx^+ cells in the DG of the hippocampus of 5xFAD/p75NTR ko mice was reduced compared to p75NTR ko and the 5xFAD mice, and was almost the same as this of the WT mice of the same age (Fig. 5C, D). So, p75NTR deletion is sufficient to abolish the increase in NSC proliferation rates of 5xFAD and also to affect the production of immature neurons and decrease the number of Dcx^+ cells, compared to p75NTR ko and 5xFAD mice [from 12128.25 ± 847.8 cells (SEM) in 5xFAD mice to 9448.25 ± 243.2 cells (SEM) in 5xFAD/p75NTR ko mice] (Fig. 5C, D).

Comparing the number of Dcx^+ cells, in 4 months old mice, we observed that although there is a decreased number of immature neurons in the 5xFAD/p75NTR ko mice, this number is not statistically different from that one of the 5xFAD model (Fig. 5C, D). Taking into consideration the significantly reduced number of Dcx^+ cells at the age of 4 months old, we assume that any potential effects of receptor's deletion are minimized.

Gene set enrichment analysis revealed genes implicated in p75NTR-dependent effects under AD

To quantitatively evaluate the gene networks associated with the role of p75NTR in AD, we performed RNA sequencing on hippocampal tissue obtained from 5xFAD and 5xFAD/p75NTR ko mice. This analysis identified five significantly upregulated and one downregulated gene, as illustrated in the volcano plot (Supplemental Fig. S8).

Among the upregulated genes, *Wdfy1*, which interacts with TLR4, is known to promote neurogenesis and facilitate the recruitment of TLR3 and TLR4 signaling adaptors that mediate neural stem cell differentiation and the production of type I interferons and inflammatory cytokines [59]. Thbs4, another upregulated gene, is implicated in the migration of newly formed neurons from the rostral migratory stream (RMS) to the olfactory bulb [60]. In contrast, the downregulated gene *Hes5* plays a critical role in maintaining NSCs, regulating neuronal differentiation, and controlling the timing of the transition between neurogenesis and gliogenesis during mammalian neocortical development [61]. To further investigate the functional relevance of p75NTR in the context of AD, we conducted GSEA which revealed that pathways related to neurogenesis, cellular differentiation, and Wnt signaling were enriched in 5xFAD/p75NTR ko samples, whereas pathways associated with cell death, inflammation, DNA damage, stress responses, and regulation of lipid biosynthesis were predominantly enriched in 5xFAD samples (Supplemental Fig. S8). Genes are detailed in Supplemental Excel Table S2.

p75NTR expression in hiPSCs-derived NSCs

Although the use of humanized mouse models as tools for studying human diseases is still meaningful, the translational outcome of any research work demands testing in human tissue or cells. To fulfil this approach, we have explored the p75NTR-dependent effects on neurogenesis using human iPSCs-derived NSCs. This methodology allows also the validation of mouse results, and provides a novel platform for drug screening of new compounds with neuroprotective and/or neurogenic effects against AD. We have generated NSCs from two different iPSC lines, derived from healthy individuals (named as 841, 856). To study the role of p75NTR in human NSCs, we firstly detected its expression in NSC lysates with Western Blot analysis. Apart from p75NTR, NSCs were found to express the p75NTR intracellular interactors RIP2 and TRAF6. Furthermore, the actual interaction of p75NTR with the aforementioned TRAF6 protein also confirmed with co-IP (Fig. 6A) indicating an active state of p75NTR signaling in these cells.

Inhibition of p75NTR rescues Aβ induced toxicity in human iPSCs – derived NSCs

Following the detection and signaling properties of p75NTR, we were interested in investigating the necessity of p75NTR on NSC survival under healthy and AD relevant conditions. To that end, we blocked the activity of p75NTR by using a p75NTR specific neutralizing antibody (2,5ngr/ml, MC-192) which has been extensively used as a selective pharmacological inhibitor of p75NTR activation. AD pathology was mimicked by treating the cells with Aβ peptides (specifically the Aβ1–42 oligomers) that are known to have a toxic effect on cells. The results of cell death measurement using the Celltox assay, provide clear evidence that p75NTR negatively influences human NSC survival after treatment with Aβ peptides

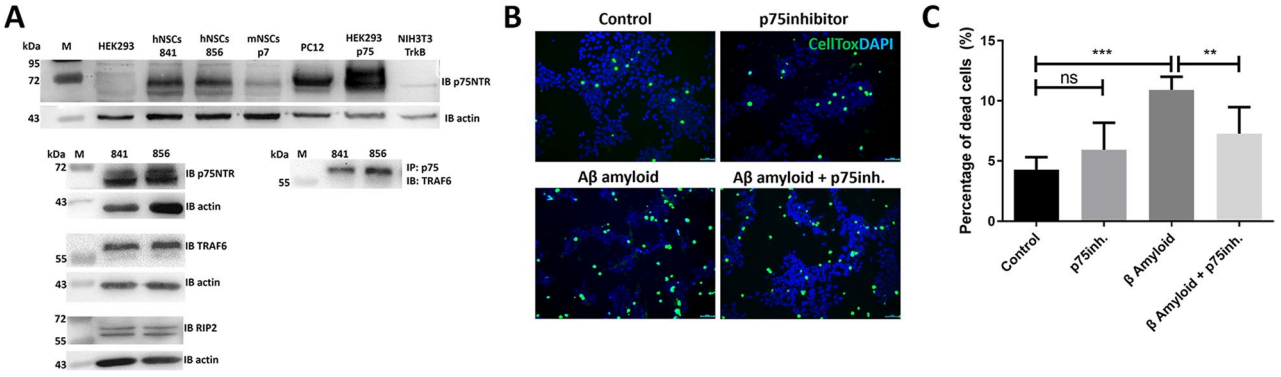


Fig. 6 p75NTR expression and function on human iPSCs - derived NSCs. **A** Representative blots ($n=3$) determined via Western Blot showing the expression of p75NTR, RIP2 and TRAF6 proteins in lysates of two iPSCs-derived NSCs lines (841, 856). Co-IP showing the interaction of p75NTR with TRAF6 protein. HEK293T cells, mNSCs p7 (mouse NSCs from postnatal day 7), PC12 (pheochromocytoma of the rat adrenal medulla), HEK293T cells transiently transfected with p75NTR plasmid and NIH3T3 cells expressing TrkB receptor were used like control samples. kDa, molecular weight; M, Marker for molecular weights, p75NTR (70–75 KDa), actin (42 KDa), TRAF6 (60KDa), RIP2 (61KDa). **B** Dead cells (green) upon inhibition of p75NTR and/or treatment with aβ oligomers (50 μm scale bar). **C** Graph showing the percentage of dead cells (Celltox labeled cells/total number of HOECHST$^+$ cells) $n=3$ from different NSCs lines (841, 856) (one way ANOVA $^{**}p<0{,}05$, $^{***}p<0{,}005$, ns, no significant)

indicating a regulatory role of p75NTR in human NSC pathology of AD (Fig. 6B, C).

Inhibition of p75NTR has no effect in proliferation of human NSCs

In continuation of the p75NTR-mediated cell survival effects, we also investigated the effect of p75NTR inhibition on NSC proliferation under healthy conditions. BrdU assay in NSCs, revealed no alteration in the proliferation rate of the cells when p75NTR is inhibited, suggesting that p75NTR is not actively involved at least in human NSC proliferation (Supplemental Fig. S9). This finding is in agreement with our results in mice studies, since it proves the cell non autonomous effects of this receptor to NSC proliferation.

Discussion

The consistent failure of amyloid and tau targeting therapies in AD [1, 2, 5] underscores the critical need to reveal and target pathogenic events, such as the impaired adult hippocampal neurogenesis [62, 63]. The p75 neurotrophin receptor is of particular interest due to its complex role and its regulation under AD [64, 65], but its function in regulating adult neurogenesis under neurodegenerative diseases is minimally explored.

In the present study, we investigated the relationship between p75NTR and adult hippocampal neurogenesis, not only under physiological conditions but also in AD-related pathology, revealing a critical role for p75NTR in maintaining neurogenic niche integrity.

In p75NTR ko mice, we observed decreased numbers of proliferative neural stem cells, accumulation of immature neurons and a reduction in newly generated mature neurons, while the number of dead immature neurons and mature neurons remained unchanged. Notably, total caspase3-positive cells were increased, suggesting that p75NTR may support the survival of early progenitors. In agreement to our observations, Catts et al. have also observed increased cell death, which likely affects neuronally committed precursor cells and/or immature neurons, while Meier et al. showed that p75NTR is required for the survival of neuronal progenitors (specifically Tbr2$^+$ progenitor cells) [25, 66]. Since we did not observe significant differences in the number of dying immature neurons, our findings strongly suggest that the elevated cell death in p75NTR ko mice originates primarily from the early neural stem cells and progenitors within the neurogenic niche (cells expressing either SOX2 or Tbr2 markers).

Importantly, conditional deletion of p75NTR in Nestin$^+$ NSCs had no effect on NSC proliferation or immature neuron production, indicating that p75NTR regulates adult hippocampal neurogenesis through cell non-autonomous mechanisms. Furthermore, analyses of astrocytes, oligodendrocytes and microglia revealed no changes in glial abundance or reactivity at two months of age, excluding overt glial remodeling as a contributor to the observed phenotype.

Although p75NTR is expressed in multiple hippocampal cell types [67–72] our data argue against a major contribution from astrocytes, oligodendrocytes, or microglia at this stage. We observed no changes in the number or reactivity of these glial populations, nor evidence of inflammatory or gliotic transcriptional signatures. Together with the absence of a phenotype following NSC-specific p75NTR deletion, these findings suggest that the neurogenic impairment arises from altered niche signaling rather than intrinsic stem cell or glial defects. Given the established role of mature hippocampal neurons in regulating adult neurogenesis

Table 2 Summary of the in vivo results

Age Markers		**p75NTR ko vs. WT**	**5xFAD vs. WT**	**5xFAD/p75NTR ko vs. 5xFAD**
2 months old	BrdU/Sox_2	↓	↑	↓
	Dcx	↑	↑	↓
4 months old	BrdU/Sox_2	↓	↓	↓
	Dcx	Not changed	↓	Not changed
Age Markers		**WT vs. p75NTR ko**	**WT vs. 5xFAD**	**6mo WT vs. 2mo WT**
6 months old	BrdU/Sox_2	Not changed	Not changed	↓
	Dcx	Not changed	Not changed	↓

through activity-dependent and neurotrophin-mediated mechanisms, as well as the known multifunctional role of p75NTR in these processes, mature neurons represent a likely mediator of the observed effects [64, 73, 74]. The pleiotropic p75 signaling could provide a regulatory mechanism to mediate differential effects on several cell types of hippocampal region, especially under pathological stimuli. Thus, more detailed studies are necessary to decipher this receptor signaling properties in each cell type in association with the network effects.

Loss of p75NTR in these non-stem cell populations may disrupt trophic signaling and neuronal activity within the niche, leading to reduced NSC proliferation and an accumulation of immature neurons. Moreover, this observed accumulation may reflect a compensatory but ultimately ineffective attempt to maintain neuronal output, resulting in impaired maturation of newly generated neurons also consistent with the established role of p75NTR in the regulation of cell-cycle exit [46, 47, 75, 76].

Transcriptomic analyses further support a niche-dependent role for p75NTR. Key proliferative and survival mediators, including Fabp7 and Fxr1, were downregulated, providing a potential molecular explanation for the depletion of the $SOX2^+$ progenitor pool and the observed increase in cell death [50]. Genes involved in cytoskeletal organization and adhesion (Mapt, Tppp3 and Cdh1) [77–79] as well as niche signaling (Fn1, Wnt6, and Thpo) [80–82] were also dysregulated, consistent with impaired maturation of immature neurons. Notably, markers of glial reactivity, microglial activation and synaptic density remained stable, indicating that overall hippocampal homeostasis is preserved. While these profiling data are descriptive, they serve as a crucial hypothesis generating resource, identifying specific candidate genes and pathways that may underlie the observed phenotypes.

A particularly intriguing finding was the upregulation of the *Ngfr* transcript, which is actually the deleted gene, in our knockout models. Interestingly, this mouse line still expresses a truncated p75NTR isoform lacking the neurotrophin-binding domain [83, 84]. Thus, this upregulation could be explained by a feedback loop of the cellular system to regain p75NTR functions, indicating its importance in homeostasis. This truncated isoform may mimic proteolytically cleaved intracellular domains of p75NTR, which can possess independent signaling capacity [84, 85].

Under AD context, in the 5xFAD model, we observed a transient increase in neurogenesis at 2 months, which we interpret as a compensatory response to initial Aβ pathology. This aligns with evidence that monomeric or low-dose Aβ can promote neurogenesis and neuronal survival [55–58]. By 4 months, as Aβ oligomerization and plaque deposition progress [34, 86] this compensatory response fails, leading to the characteristic decrease in neurogenesis, consistent with the established toxic effects of aggregated Aβ oligomers.

Crucially, p75NTR is essential for the early compensatory response to Aβ, as its deletion in 2-month-old 5xFAD/p75NTR ko mice abolished the increase in NSC proliferation. This reveals a protective role where p75NTR acts as an Aβ sensor to maintain neurogenic output [31, 33], contrasting its well-described role in mediating Aβ toxicity at later disease stages [32]. However, in 5xFAD/p75NTR ko mice, Aβ cannot act through deleted p75NTR, resulting in loss of neuroprotection. Therefore, p75NTR deletion does not simply create an additive impairment but rather ablates a specific protective pathway, leading to a premature failure of neurogenic compensation. This dual role aligns with the context-dependent nature of p75NTR signaling and positions it as a key molecule linking early pathological cues to regenerative performance. At the age of 4 months, the combined insults of AD pathology and p75NTR deficiency caused a severe neurogenic collapse, exceeding the deficit in 5xFAD mice alone. However, p75NTR's influence on neuronal differentiation was lost at this later stage, indicating that its primary impact on differentiation is restricted to earlier, pre-symptomatic phases of the disease.

All the results from our in vivo experiments are summarized in Table 2.

The translational relevance of our findings is strongly supported by our studies in human iPSC-derived NSCs from healthy donors. We confirmed for the first time to our knowledge, that p75NTR signaling is active in human NSCs and that its inhibition exacerbated Aβ-induced

toxicity, indicating a regulatory role of p75NTR in NSC pathology of AD. A limitation of this approach is that NSCs from healthy individuals may not fully recapitulate the molecular landscape of the AD brain. To further validate the pathophysiological significance of our findings, future work should utilize iPSCs derived from AD patients or isogenic lines with edited AD-risk genes (e.g., APP, PSEN1) to model more accurately the disease-specific environment.

Given the context-dependent roles of p75NTR revealed by our study, future therapeutic strategies will need to move towards selective modulation of its signaling pathways. The receptor's duality suggests that promoting its pro-survival outputs while attenuating its pro-apoptotic signals could be strongly beneficial.

This aligns with growing interest in developing synthetic neurotrophin mimetics that achieve receptor selectivity, as recently reviewed by Zota et al. (2025) [87]. The feasibility of this approach is demonstrated by compounds like BNN27, which targets p75NTR and TrkA and shows efficacy in AD models [38], and more directly by the p75NTR-modulating ligand LM11A-31, which has progressed to clinical trials for Alzheimer's disease [88]. Our findings therefore provide a strong rationale for developing p75NTR-modulating therapies for Alzheimer's disease, underscoring the potential of targeting neurogenic decline.

Conclusions

In summary, our research establishes p75NTR as a critical regulator of adult hippocampal neurogenesis and a key mediator of the brain's compensatory response to early AD pathology. We demonstrate that p75NTR functions through a cell non-autonomous mechanism to regulate the NSC proliferation, differentiation and survival. The observation that p75NTR loss exacerbates neurogenic deficits in AD models provides a new framework for understanding neurogenic decline. While p75NTR has dual roles in cell survival and apoptosis, its pharmacological manipulation may offer a novel strategy to restore neurogenesis and mitigate disease progression. Future studies using AD patient-derived stem cells and in vivo modulation of the receptor will be critical to determine the therapeutic feasibility of p75NTR.

Abbreviations

AD	Alzheimer's disease
NGF	Nerve Growth Factor
BDNF	Brain Derived Neurotrophic Factor
NT3/4	Neurotrophin 3/4
p75NTR	p75 Neurotrophin Receptor
BrdU	5-bromo-2'-deoxyuridine
Sox2	SRY-Box Transcription Factor 2
Dcx	doublecortin
NeuN	Neuronal Nuclear marker
Tuj1	class III beta-tubulin marker
GFAP	Glial Fibrillary Acidic Protein marker
Olig2	Oligodendrocyte Transcription Factor 2 marker
Iba1	Ionized calcium-binding adaptor molecule 1 marker
Aβ	Amyloid beta
APP	Amyloid precursor protein
PSEN1	presenilin-1
WT	Wild type
KO	Knock out
hiPSCs	human induced Pruripotent stem cells
NSCs	Neural stem cells
DG	Dentate Gyrus
TMD	Transmembrane Domain
DD	Death Domain
GO	gene ontology
DEA	Differential expression analysis
GSEA	Gene Set Enrichment Analysis
DEGs	differentially expressed genes
qRT PCR	quantitative Real Time Polymerase Chain Reaction
Ngfr	Nerve growth factor Receptor

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s13195-026-01989-7.

Supplementary Material 1.

Supplementary Material 2.

Supplementary Material 3.

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Authors' contributions

M.A.P.: conception and design of the study, acquisition and interpretation of the data, drafting the text, MAP, KC, MP: in vivo experiments, in vitro experiments, analysis of results, I.C.: conception and design of the study, drafting the manuscript, supervision, funding, E.T., K.M., M.L.: RNA-seq analysis.

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Data availability

The RNA-seq datasets generated and analyzed during the current study are available in the GEO repository GSE296390. All materials are available upon request. Supplementary information is available for this paper.

Declarations

Ethics approval and consent to participate

All procedures were performed under the approval of Veterinary Directorate of Prefecture of Heraklion (Crete) and carried out in compliance with Greek

Government guidelines and the guidelines of FORTH ethics committee and were performed in accordance with approved protocols from the Federation of European Laboratory Animal Science Associations (FELASA) and Use of Laboratory animals [License number: EL91-BIOexp-02), Approval Code: 360667, Approval Date: 29/11/2021 (active for 3 years)]. All research activities strictly adhered to the EU adopted Directive 2010/63/EU on the protection of animals used for scientific purposes.

Competing interests
The authors declare no competing interests.

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