Supplemental Material

Supplemental figures and tables

Figure S1

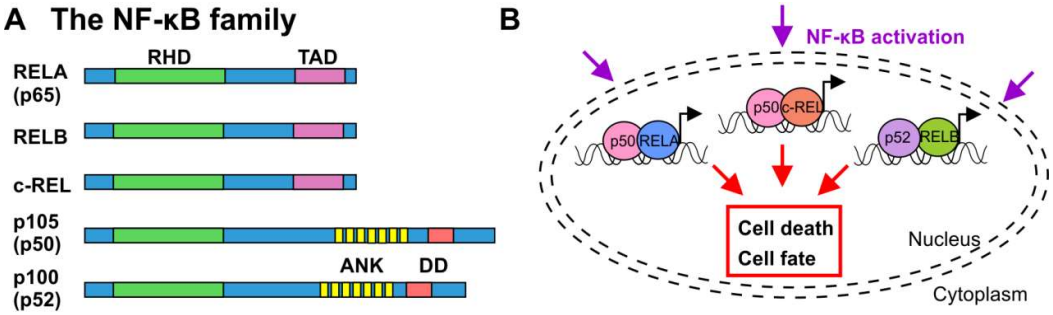

Figure S1. Schematic view on NF-κB family members and their potential effects on cell fate and survival. A) Schematic representation of the NF-κB family members. Relevant domains are indicated and alternative nomenclatures are provided in parenthesis. RHD: rel homology domain, TAD: transactivation domain, ANK: ankyrin repeats, DD: death domain. B) Schema showing NF-κB activation triggered during differentiation allowing nuclear translocation of a predominant NF-κB dimer or a particular combination of NF-κB dimers which would induce the gene expression program of a distinct cell fate, or it could result in cell death or in both.

Figure S2

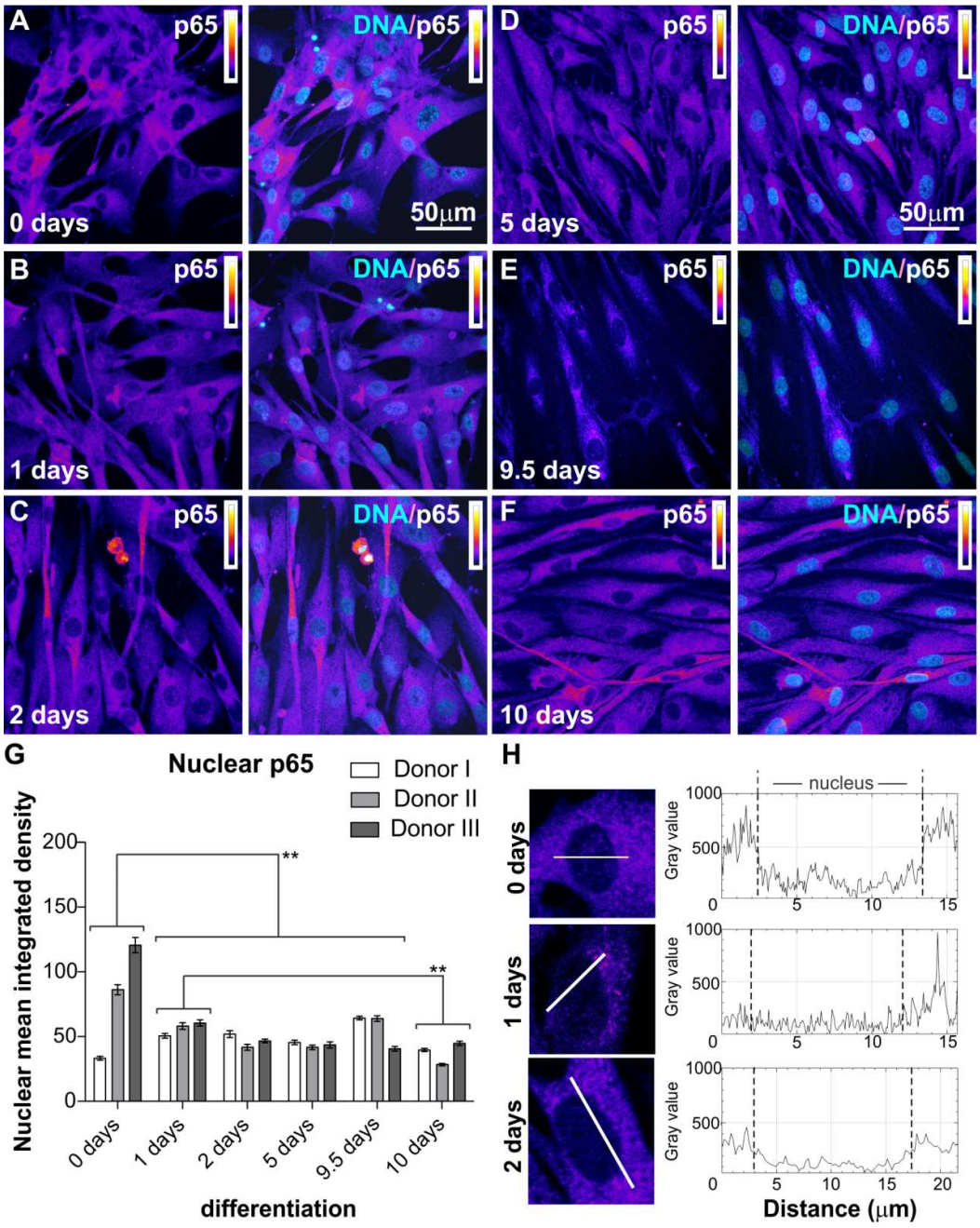

Figure S2. Immunocytochemical analysis of p65 (RELA). A-F) NCSC-derived NSCs labeled against RELA after 0, 1, 2, 5, 9.5 and 10 days of glutamatergic differentiation. Each panel shows RELA protein on the left-side and DNA co-localization with DAPI staining on the right-side. Intensity scale indicates white as highest intensity level and black as lowest intensity level. G) Quantification of immunocytochemical analyses showing nuclear mean integrated density of p65 (RELA) during early differentiation (mean ± SEM, n=3). Normality of the data was refuted using Shapiro-Wilk normality test. Non-parametric Kruskal-Wallis (***p≤0.001) and Bonferroni

corrected post-test (**p<0.01) revealed a significant nuclear translocation of NF-κB-p65 at day 0. H) Fluorescence intensity profiles measured at three different time points (0, 1, 2 days of differentiation), for different cells following transects as shown, to reveal the difference between the nuclear and cytoplasmic fluorescence. NCSC: neural crest-derived stem cells, NSCs: neural stem cells, SEM: Standard error of the mean.

Figure S3

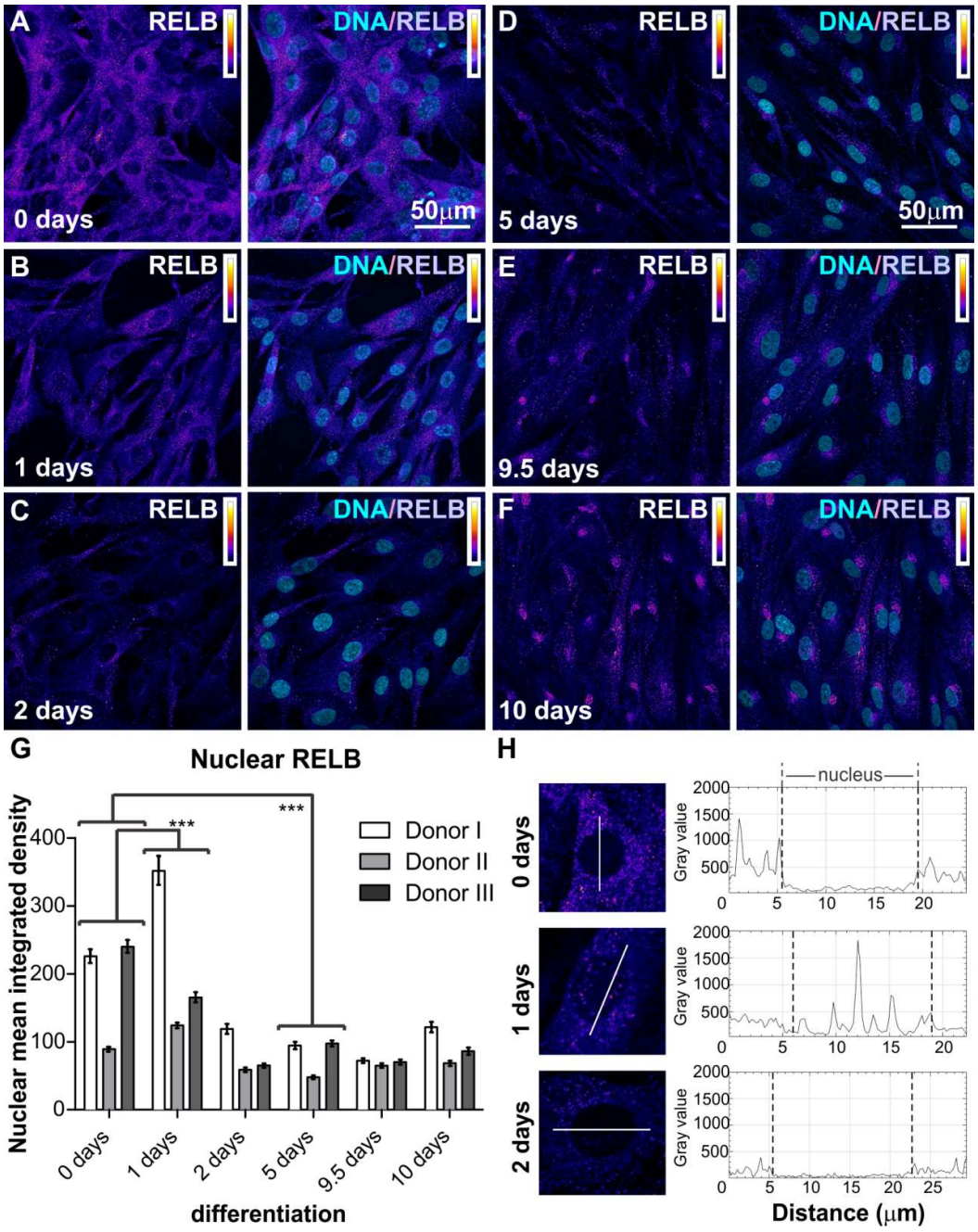

Figure S3. Immunocytochemical analysis of RELB. A-F) NCSC-derived NSCs labeled against RELB after 0, 1, 2, 5, 9.5 and 10 days of glutamatergic differentiation. Each panel shows RELB protein on the left-side and DNA co-localization with DAPI staining on the right-side. Intensity scale indicates white as highest and black as lowest intensity levels. G) Quantification of immunocytochemical analyses show nuclear mean integrated density of RELB during early differentiation (mean ± SEM, n=3). Normality of the data was refuted using Shapiro-Wilk normality test. Non-parametric Kruskal-Wallis (***p≤0.001) and Bonferroni corrected post-test

(***p<0.001) revealed a significant peak in nuclear translocation of NF-κB-RELB at day 0. H) Fluorescence intensity profiles measured at three different time points (0, 1 and 2 days of differentiation), for different cells following transects as shown, to elucidate the difference between the nuclear and cytoplasmic fluorescence. NCSC: neural crest-derived stem cells, NSCs: neural stem cells, SEM: Standard error of the mean.

Figure S4

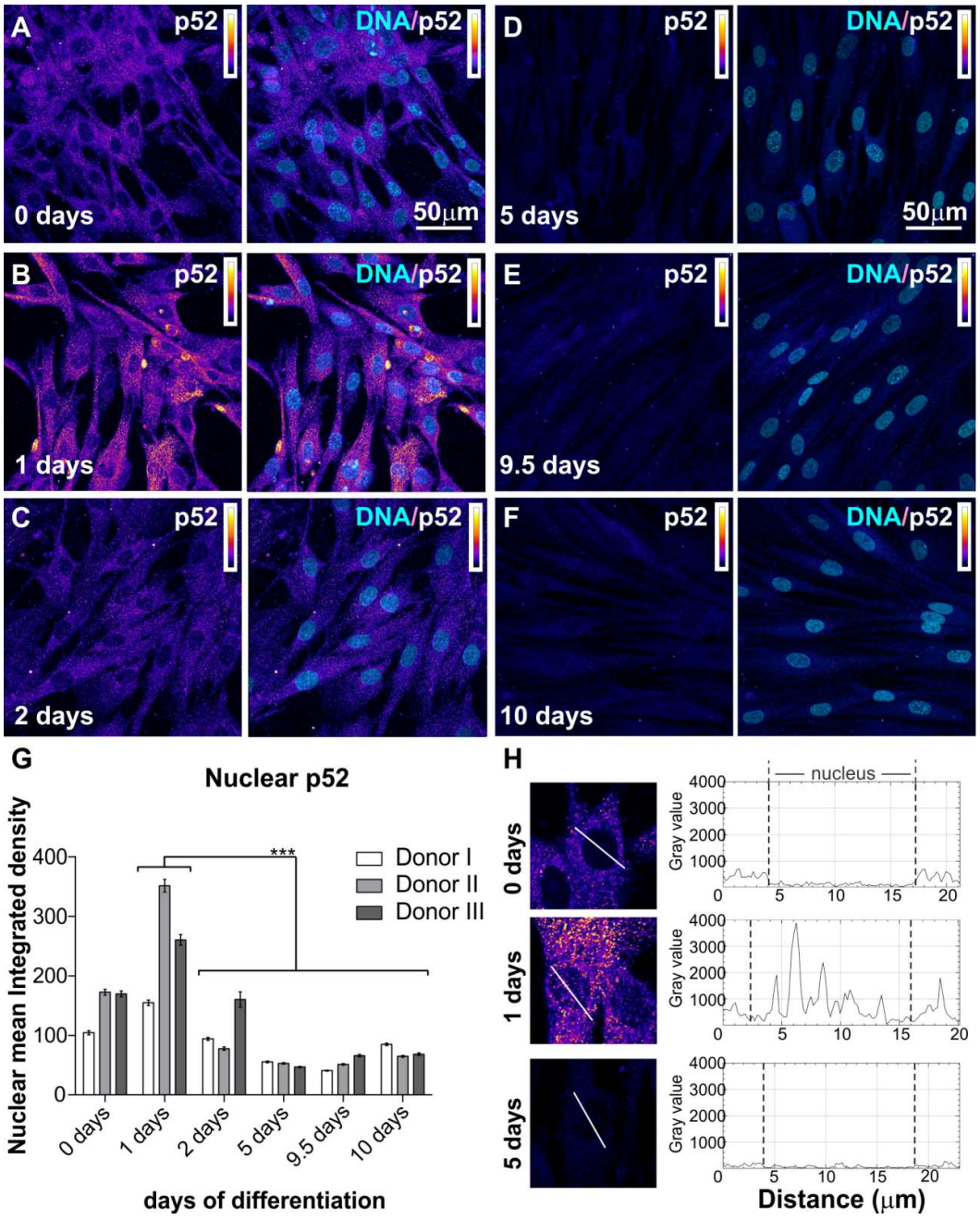

Figure S4. Immunocytochemical analysis of p52. A-F) NCSC-derived NSCs labeled against p52 after 0, 1, 2, 5, 9.5 and 10 days of glutamatergic differentiation respectively. Each panel shows p52 subunit on the left-side and the co-localization with DNA on the right-side. Intensity scale indicates white as highest intensity level and black as lowest intensity level. G) Quantification of immunocytochemical analyses showing nuclear mean integrated density of p52 during early differentiation (n=3, mean ± SEM). Normality of the data was refuted using Shapiro-Wilk normality test. Non-parametric Kruskal-Wallis (***p≤0.001) and Bonferroni corrected post-test

(***p<0.001) showed a significant peak of p52 at day 1 significantly different to all later time points (2-10 days). H) Fluorescence intensity profiles measured at different time points (0, 1 and 5 days of differentiation), for different cells following transects as shown, to clearly expose the difference between nuclear and cytoplasmic fluorescence. NCSC: neural crest-derived stem cells, NSCs: neural stem cells, SEM: Standard error of the mean.

Figure S5

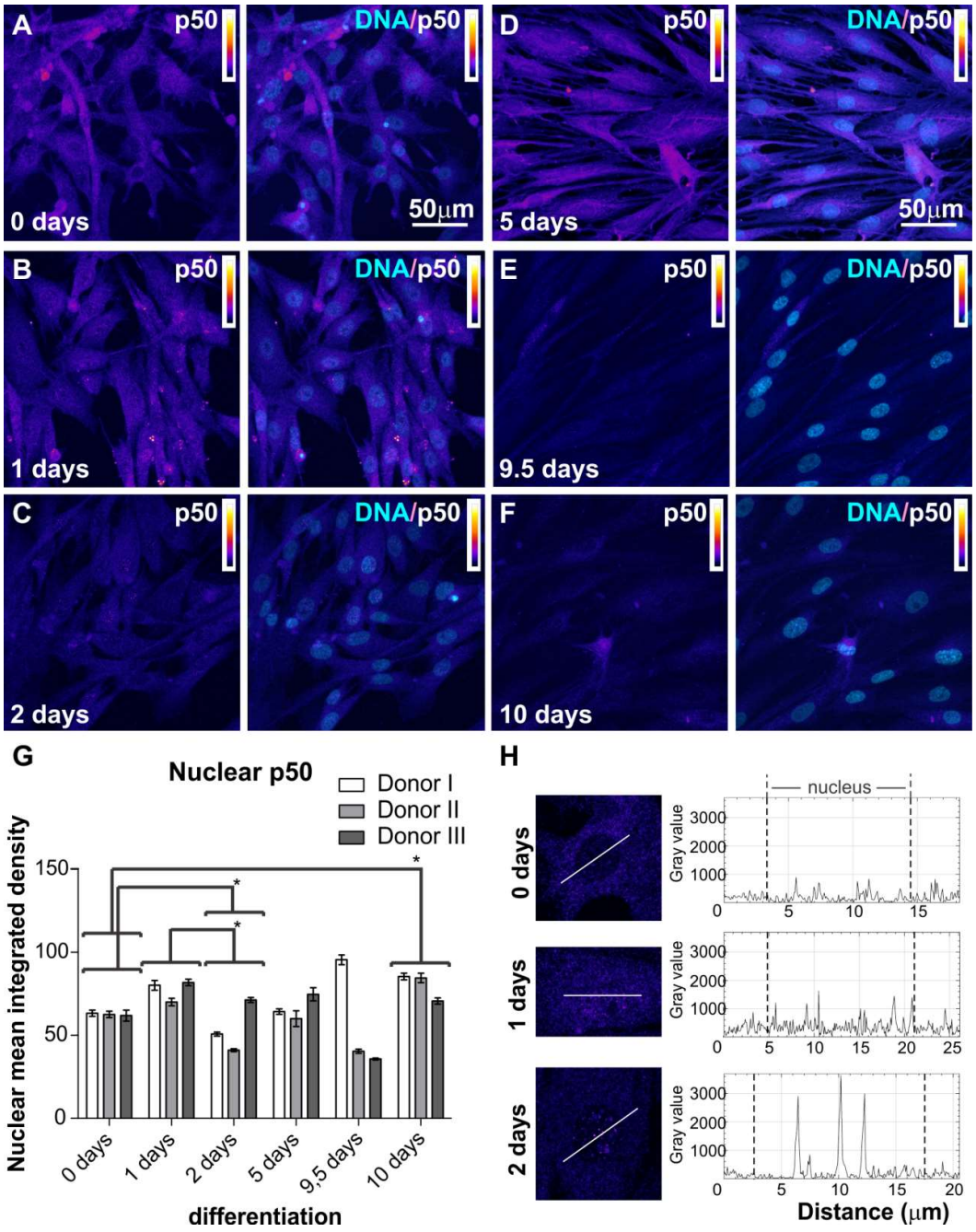

Figure S5. Immunocytochemical analysis of p50. A-F) NCSC-derived NSCs labeled against p50 after 0, 1, 2, 5, 9.5 and 10 days of glutamatergic differentiation. Each panel shows p50 protein on the left-side and the co-localization with DNA staining on the right-side. Intensity scale indicates white as highest and black as lowest intensity levels. G) Quantification of immunocytochemical analyses showing nuclear mean integrated density of p50 during early differentiation (n=3, mean ± SEM). Normality of the data was refuted using Shapiro-Wilk normality test. Non-parametric Kruskal-Wallis (***p≤0.001) and Bonferroni corrected post-test (*p<0.05) showed some significant

differences between day 0 and 2 and 10 days, and between day 1 and day 2. H) Fluorescence intensity profiles measured at different time points (0, 1 and 2 days of differentiation), for different cells following transects as shown, to clearly expose the difference between nuclear and cytoplasmic fluorescence. NCSC: neural crest-derived stem cells, NSCs: neural stem cells, SEM: Standard error of the mean.

Figure S6

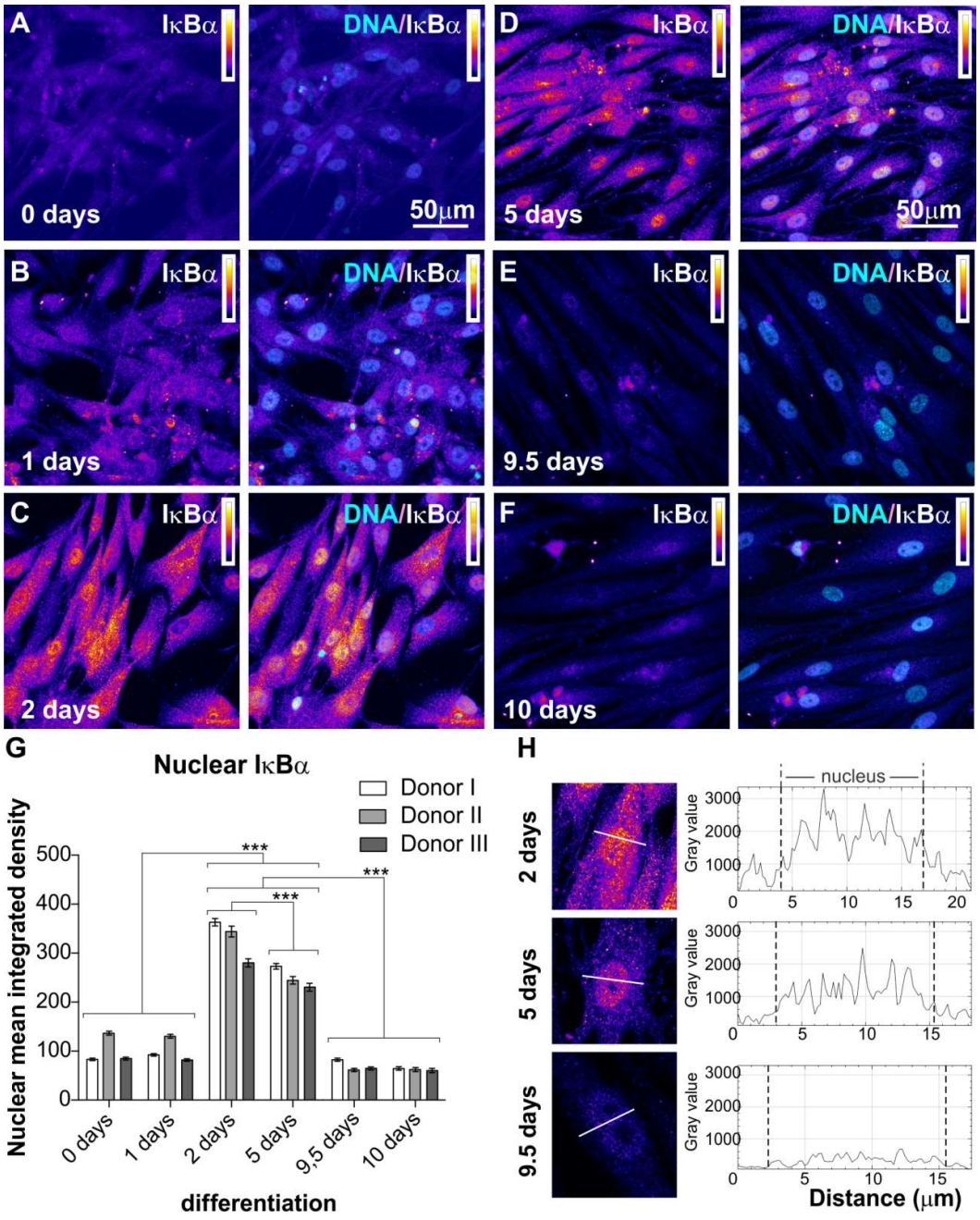

Figure S6. Immunocytochemical analysis of IκB*α*. A-F) NCSC-derived NSCs labeled against IκB*α* after 0, 1, 2, 5, 9.5 and 10 days of neuronal glutamatergic differentiation respectively. Each panel shows on the left c-REL and co-localization with DNA on the right. Intensity scale indicates white as highest intensity level and black as lowest. G) Quantification of immunocytochemical analyses showing nuclear mean integrated density for IκB*α* during early differentiation (n=3, mean ± SEM). Normality of the data was refuted using Shapiro-Wilk normality test. Non-parametric Kruskal-Wallis (***p≤0.001) and Bonferroni corrected post-test (***p<0.001) revealed a

significantly increased nuclear translocation of IκBα at days 2 and 5. H) Fluorescence intensity profiles measured at three different time points (2, 5 and 9.5 days of differentiation), for different cells following transects as shown, in order to clearly reveal the difference between the nuclear and cytoplasmic fluorescence. NCSC: neural crest-derived stem cells, NSCs: neural stem cells, SEM: Standard error of the mean.

Figure S7

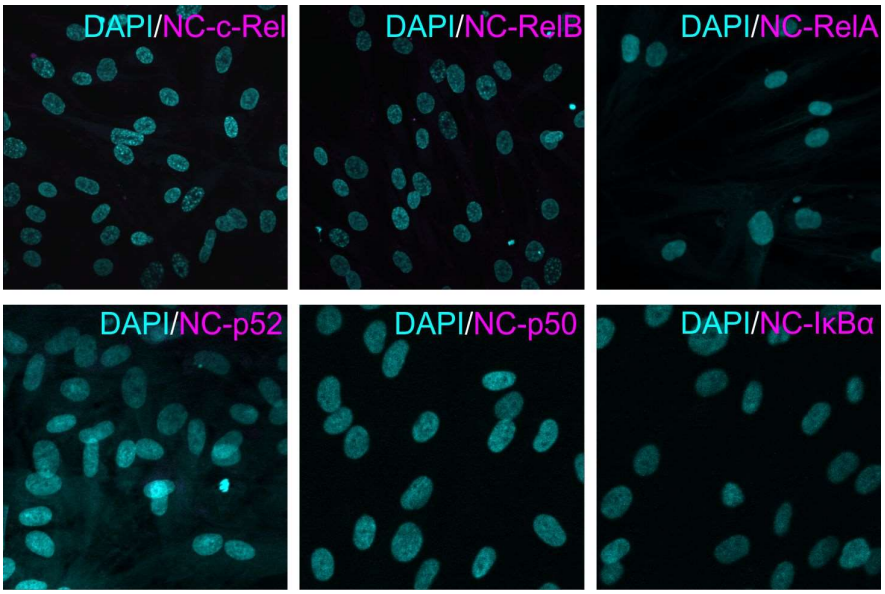

Figure S7. Negative controls for the immunocytochemical analysis of NF-κB subunits. NCSC-derived NSCs at day 0 of neuronal glutamatergic differentiation were treated for immunocytochemical analysis, without the addition of the first antibody. In cyan the nucleus is stained by DAPI and the magenta staining should depict the reaction of the second antibody, due to the absence of the first antibodies no detectable staining was observed. NC: negative control, NCSC: neural crest-derived stem cells, NSCs: neural stem cells.

Figure S8

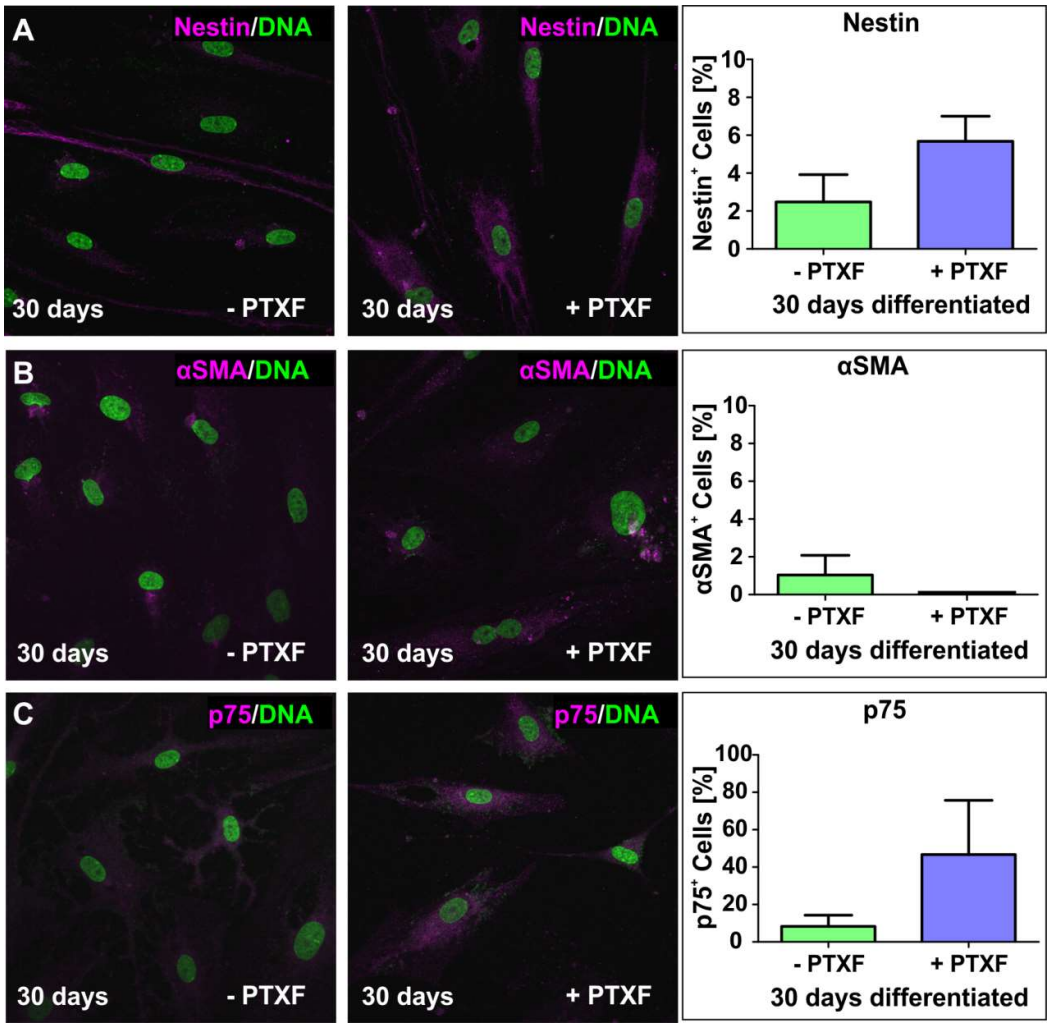

Figure S8. Immunocytochemical analysis of different cell markers after 30 days of glutamatergic neuronal differentiation in the absence or presence of pentoxifylline. A-B) Neuronally differentiated NCSC-derived NSCs labeled against Nestin, a stemness marker. C) Quantified Nestin⁺ cells shown in percentage suggests a higher tendency of Nestin for the PTXF-treated differentiated- NCSC-derived NSCs (5,68% ± 1,33%), compared to the untreated differentiated NCSC-derived NSCs (2,48% ± 1,44%), however it is not significantly relevant according to non-parametric Kruskal-Wallis test, p=0,2683. D-E) Neuronal differentiated NCSC-derived NSCs labeled against αSMA. F) Quantification showing percentage of αSMA⁺ cells which is cero for the differentiated NCSC-derived NSCs treated with PTXF and it is 1,04% (±1,04) in control neurons. No significant difference was observed according to non-parametric Kruskal-Wallis test, p=0,3173. G-H) Differentiated NCSC-derived NSCs labeled against p75, a human oligodendrocyte marker. I) Quantification of the percentage of p75⁺ cells indicates a lower amount of p75⁺ cells (8,25% ± 6,03%), present in the control neurons and a higher tendency of positive

cells in the differentiated NCSC-derived NSCs treated with PTXF (46,67% ± 29,06%), however no significant difference according to non-parametric Kruskal-Wallis test, p=0,3758. PTXF: pentoxifylline, NCSC: neural crest-derived stem cells, NSCs: neural stem cells, αSMA: alpha smooth muscle actin.

Figure S9

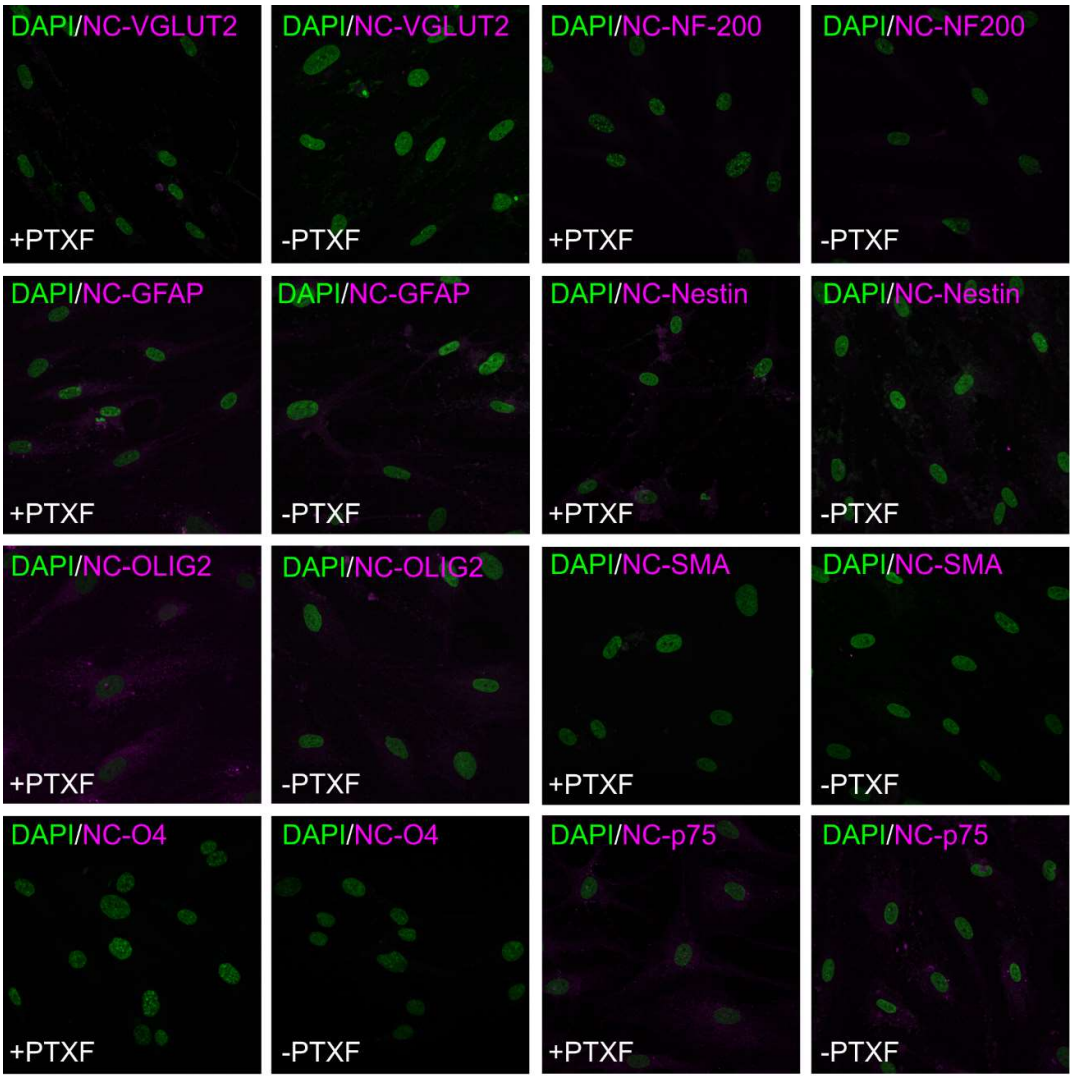

Figure S9. Negative controls for the immunocytochemical analysis of cell differentiation markers. NCSC-derived NSCs after 30 days of neuronal glutamatergic differentiation in the absence or presence of pentoxifylline were treated for immunocytochemical analysis, without the addition of the first antibody. In green the nucleus is stained by DAPI and the magenta staining should depict the reaction of the second antibody, due to the absence of the first antibodies no detectable staining was observed. NC: negative control, NCSC: neural crest-derived stem cells, NSCs: neural stem cells, PTXF: pentoxifylline.

Figure S10

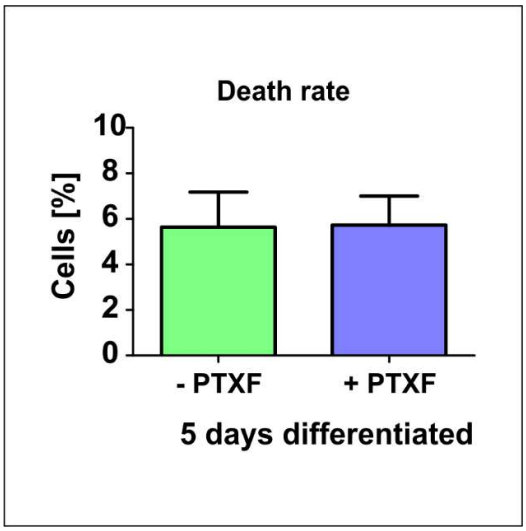

Figure S10. Death rate quantified for NCSC-derived NSCs differentiated for 5 days in the absence or presence of PTXF (n=3, mean ± SEM). No significant differences were determined using non-parametric Mann Whitney test, p=0,8728.

Table S1. Primers sequences for quantitative polymerase chain reaction.

Target	Primer sequence 5'-3'
Fw- *NG2* (transmembrane proteoglycan nerve-glia antigen*)	CATCCCACTAGAGGCGCAAA
Rev- *NG2*	CCCAGGAGAGTGGGGAAGTA
Fw-*MBP* (Myelin basic protein)	GCGTCACAGAAGAGACCCTC
Rev-*MBP*	CTCTGTGCCTTGGGAGGAAG
Fw-*PDGFRA* (Platelet derived growth factor receptor alpha)	GAAGAAAACAACAGCGGCCTT
Rev-*PDGFRA*	TGTACAACCCTGTGTGGGC
Fw-*RPLP0* (Ribosomal Protein Lateral Stalk Subunit P0)	TGGGCAAGAACACCATGATG
Rev-*RPLP0*	AGTTTCTCCAGAGCTGGGTTGT
Fw-*GAPDH* (Glyceraldehyde-3-phosphate dehydrogenase)	CATGAGAAGTATGACAACAGCCT
Rev-*GAPDH*	AGTCCTTCCACGATACCAAAGT

*also known as chondroitin sulfate proteoglycan type 4 (*CSPG4*).

Supplemental materials and methods section

Extended detailed methods

Neuronal differentiation

For neuronal differentiation, NCSCs from three to six donors were expanded and dissociated as described above. Cells were re-suspended in Dulbecco's modified Eagle's medium (DMEM) high glucose (Sigma-Aldrich) containing 2 mM L-glutamine (Sigma-Aldrich), penicillin/streptomycin (1x, Sigma-Aldrich), 10% Fetal Calf Serum (Sigma-Aldrich) and plated at a density of $5x10^4$ cells per 24 well plate followed by cultivation at 37°C, 5% CO_2 and atmospheric O_2 in a humidified incubator for 2 days. Moreover, 1μM dexamethasone (Sigma-Aldrich), 2 μM insulin (Sigma-Aldrich), 500 μM 3-isobutyl-1-methylxanthine (Sigma-Aldrich), 200 μM indomethacin (Sigma-Aldrich) and 200 μM ethanol were added to the medium to induce neuronal differentiation (neuronal induction medium, NIM) according to (Muller *et al.*, 2015). After 9 days of differentiation cells were induced with 0.5μM retinoic acid (Sigma Aldrich) and 1x N-2 supplement (Gibco, Darmstadt, Germany). Subsequently, the medium was changed by removing half of the volume, followed by addition of fresh pre-warmed NIM containing 1x N-2 supplement (Muller *et al.*, 2015).

Immunocytochemistry

Differentiated NCSC-derived NSCs were fixed in phosphate-buffered 4% paraformaldehyde (pH 7.4) for 15 minutes at room temperature (RT) followed by 3 wash steps in phosphate-buffered saline (1xPBS). Cells were permeabilized with 0.02% Triton X-100 and blocked using 5% of appropriate serum or 3% bovine serum albumin for 30 minutes at RT, followed by incubation with primary antibodies for 1 hour at RT.