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Supplemental Information

NF-κB Activity Initiates Human ESC-Derived Neural Progenitor Cell Differentiation by Inducing a Metabolic Maturation Program

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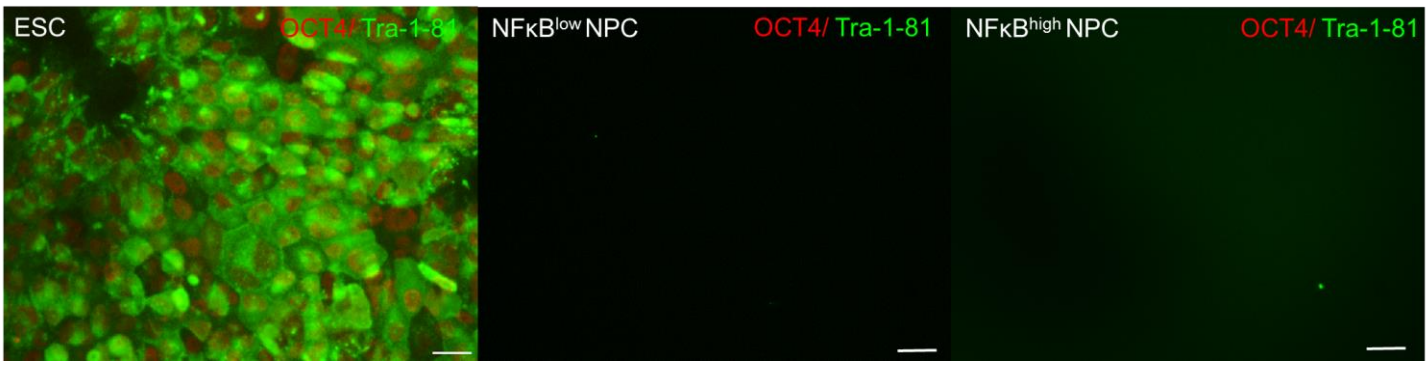
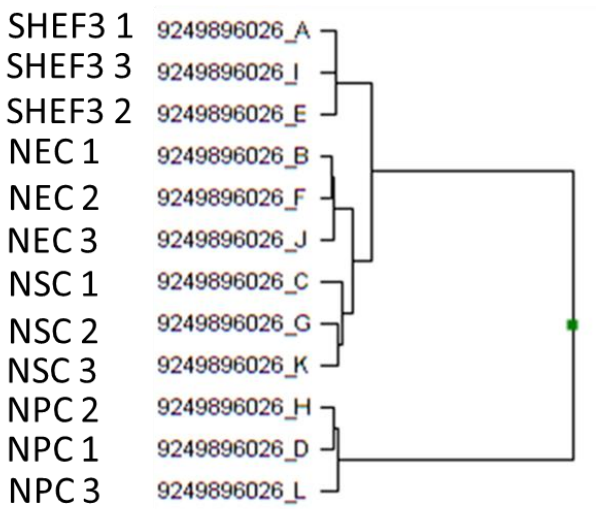
Figure S1.

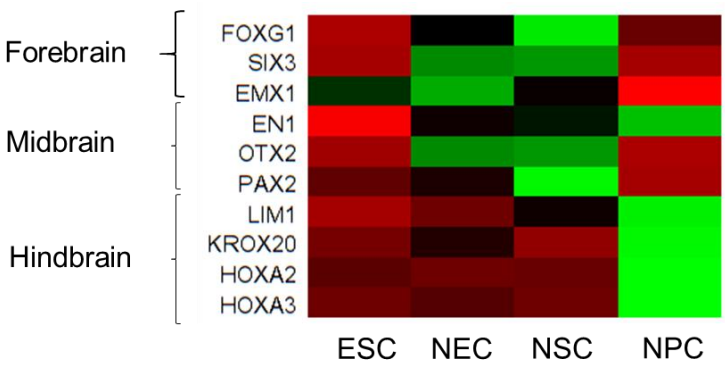
A.



B.



C.



D.

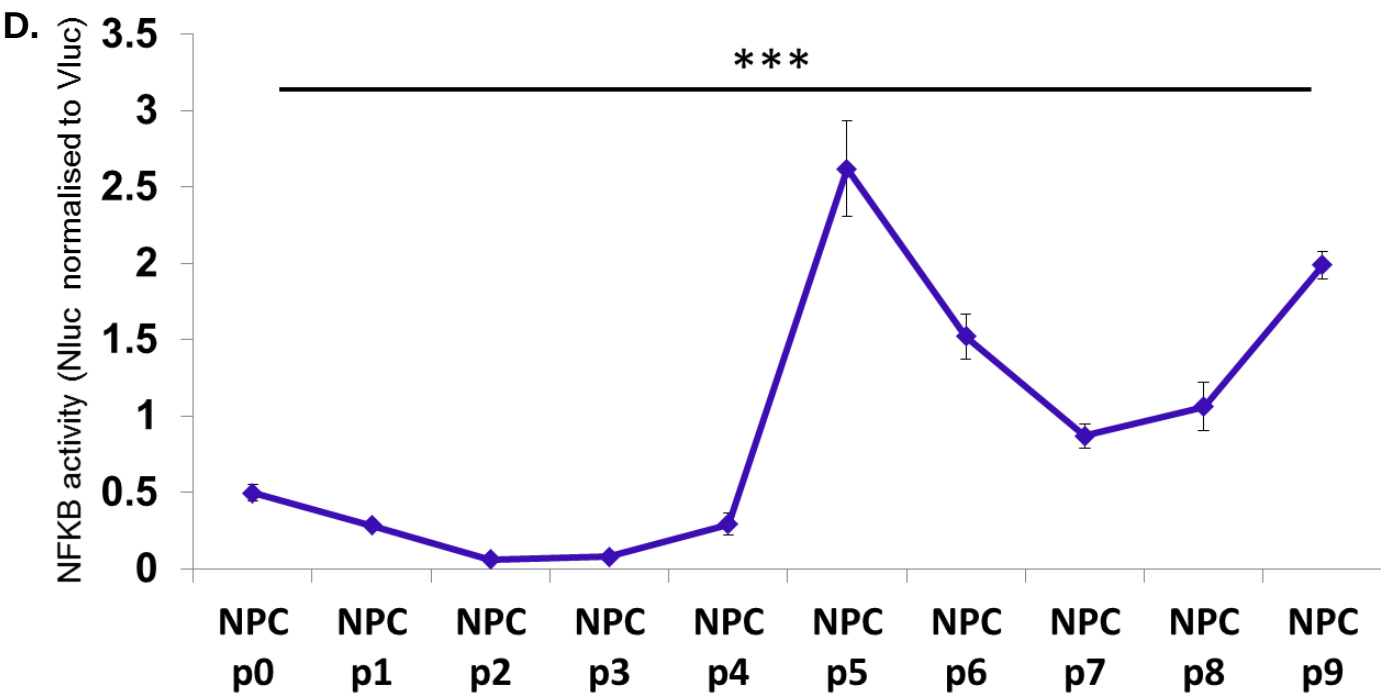
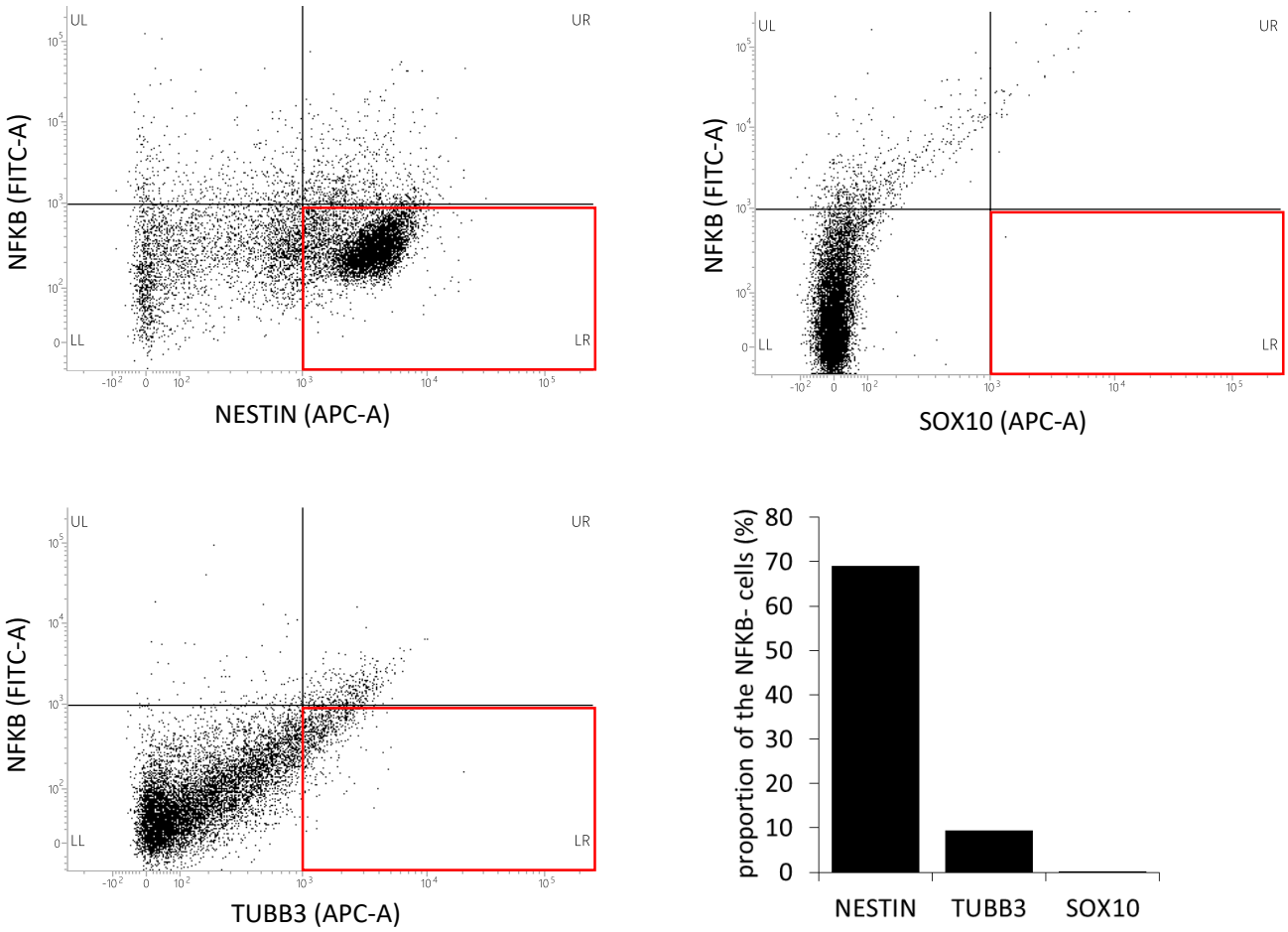


Figure S1. Immunocytochemical and transcriptomic characterisation of differentiating hESC. **(A)**(Left-right): Feeder-free hESC NFκB^{low} and NFκB ^{high} NPC expression of the pluripotency genes; transcription factor OCT4 and cell surface marker, Tra 1-81, scale bar = 20µm, related to Fig. 1A-D.**(B)** Hierarchical clustering of biological replicates from the microarray of ESC, NEC, NRPC and NPC, relates to Fig. 1E. **(C)** Analysis of regional markers in differentiating cultures, relating to Fig. 1E. (**D**) Continuous monitoring of NFKB activity at each NPC passage (n=3 ± s.e.m. ***p≤0.001, statistical test : one way ANOVA), related to Fig 3C.

Figure S2.

A.



B.

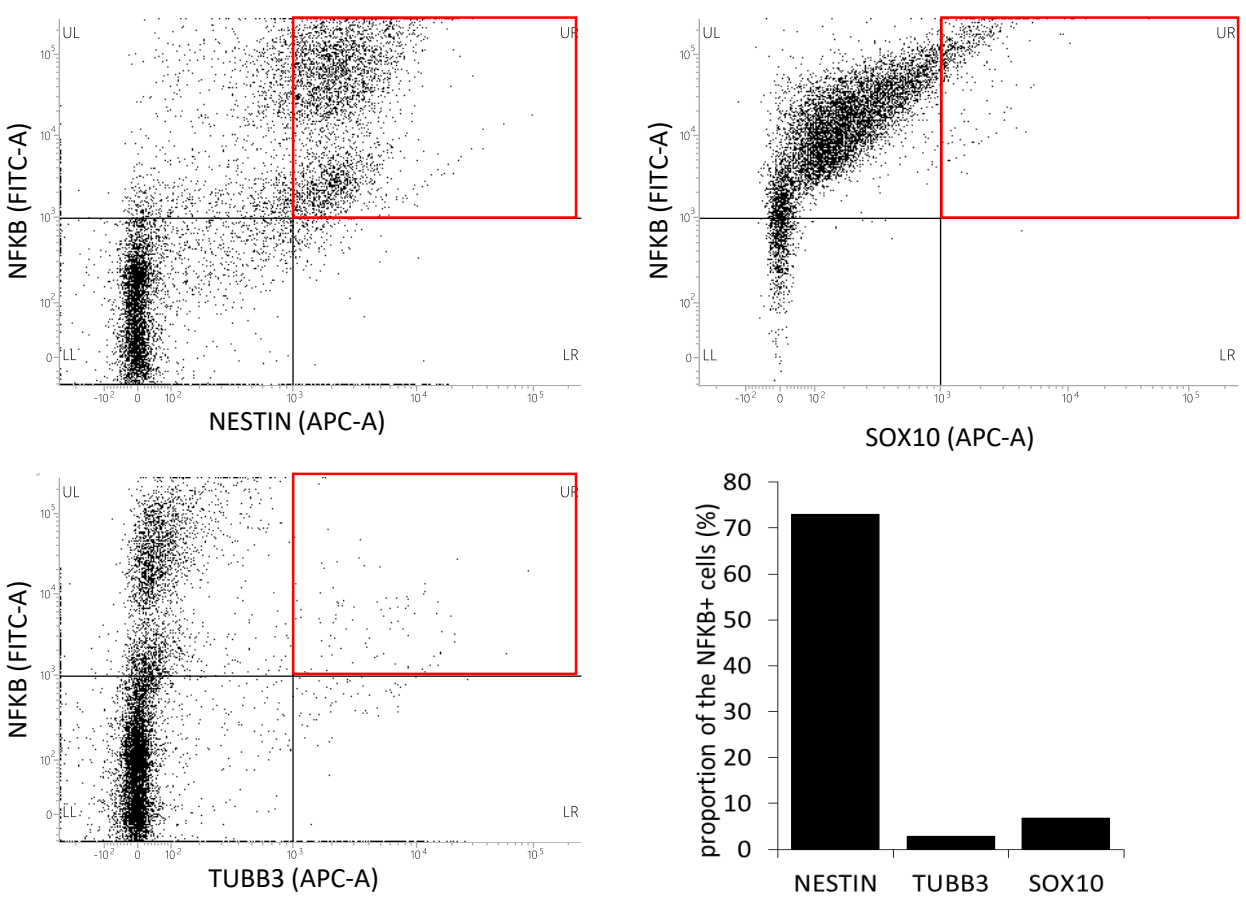


Figure S2. FACS estimation of different neural lineages in NPC.
(A) Populations **of** NFκB^{low} and NFκB^{high} NPC **(B)** analysed for expression neural stem cell marker Nestin, terminal differentiation marker TUBB3 and SOX10, a marker of neural crest cells. Related to Fig 3.

Figure S3.

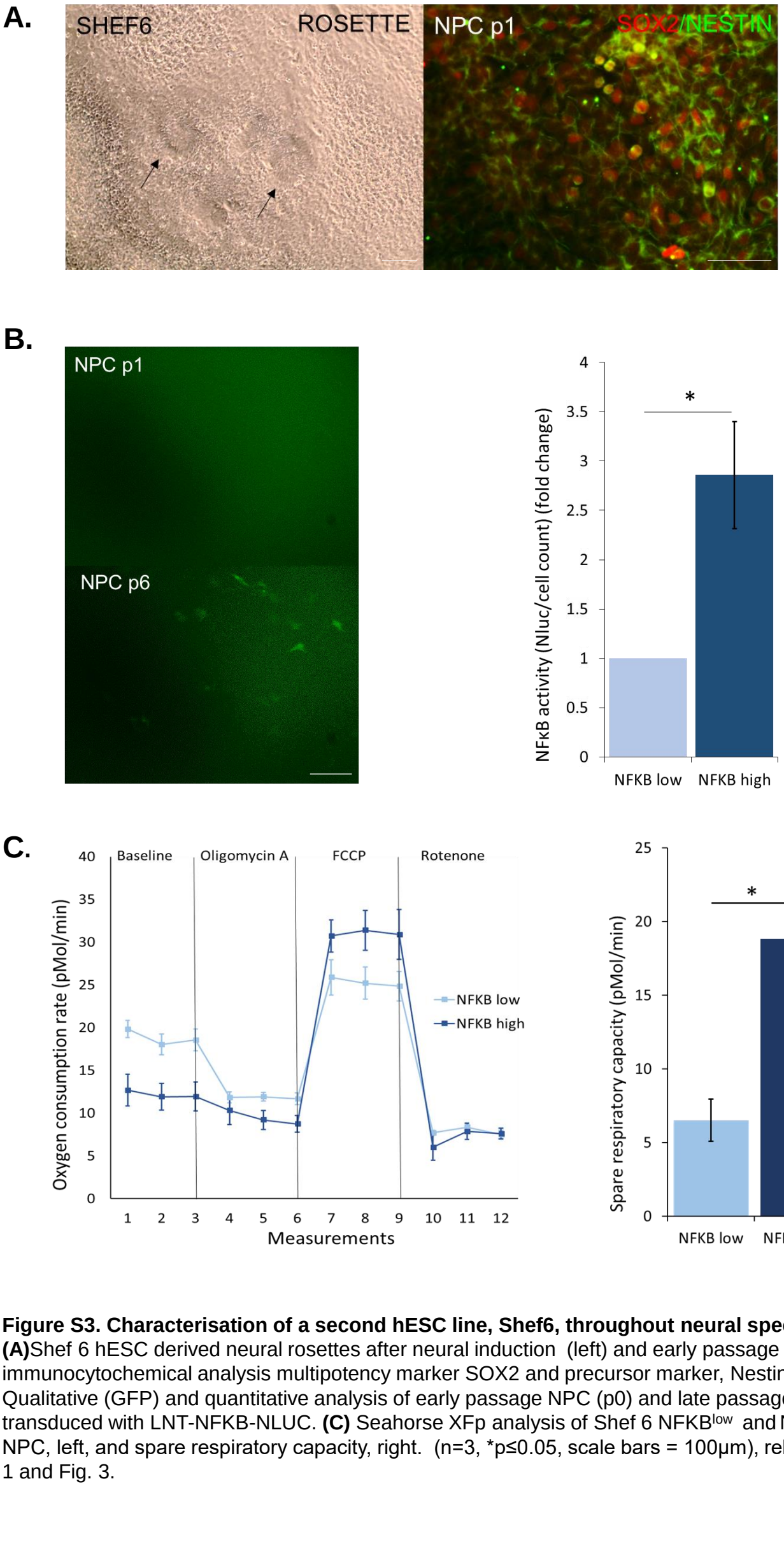


Figure S3. Characterisation of a second hESC line, Shef6, throughout neural specification. **(A)**Shef 6 hESC derived neural rosettes after neural induction (left) and early passage NPCs (p1) immunocytochemical analysis multipotency marker SOX2 and precursor marker, Nestin (right). **(B)** Qualitative (GFP) and quantitative analysis of early passage NPC (p0) and late passage NPC transduced with LNT-NFKB-NLUC. **(C)** Seahorse XFp analysis of Shef 6 $NFKB^{low}$ and $NFKB^{high}$ NPC, left, and spare respiratory capacity, right. (n=3, *p≤0.05, scale bars = 100μm), related to Fig. 1 and Fig. 3.

Figure S4.

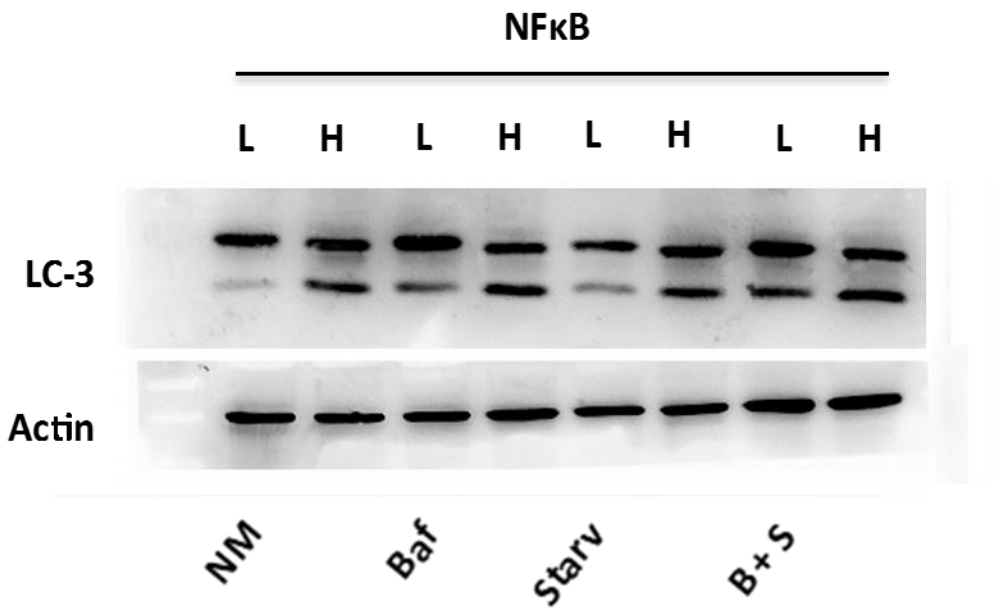


Figure S4. Autophagy gene, LC3-II is activated in NFκB^{high}-NPC.
Comparison of LC3I and LC3II in NFκB^{high}- and NFκB^{low}-NPC populations in normal media conditions, after treatment with bafilomycin A, under starvation conditions or with both starvation and bafilomycin A treatment, related to Fig. 4Ai .

Figure S5.

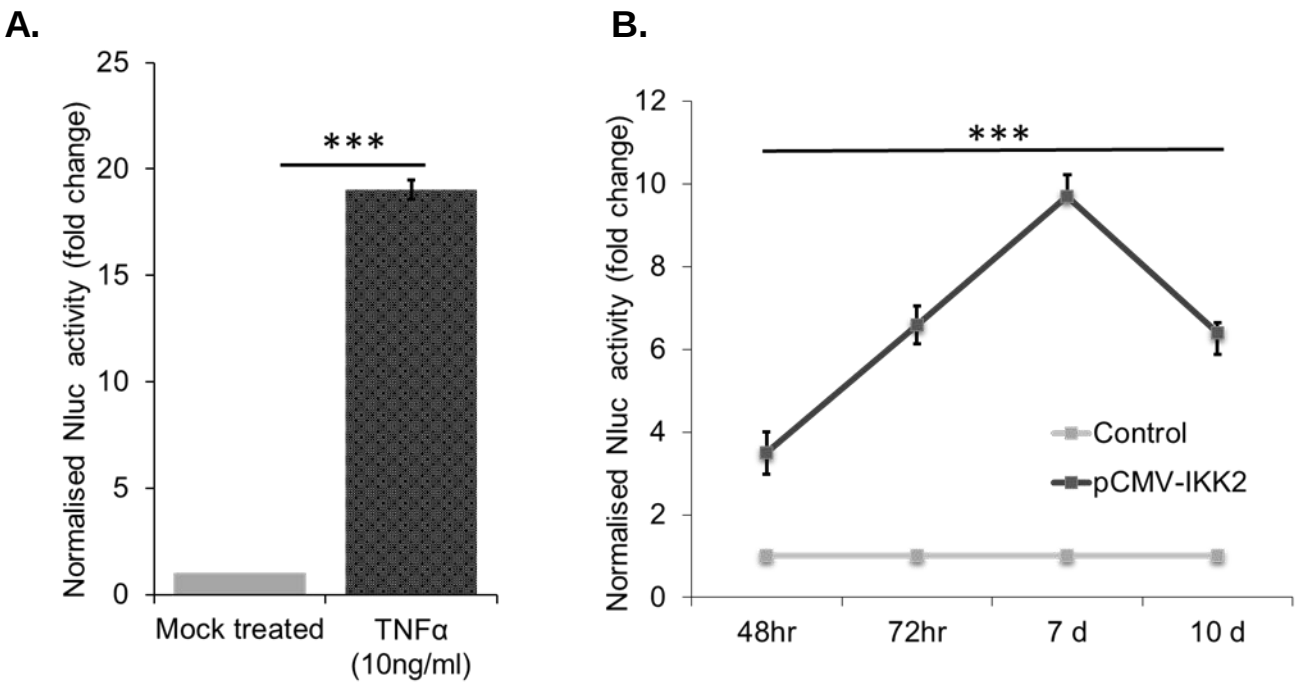


Figure S5. Validation of NFκB agonists in NFκB^{low}-NPC. Confirmation of (**A**) TNFα and (**B**) IKK2 stimulation of the NFκB-NLuc/ reporter in NPC (All luminometry; n=3, ***p≤0.001), related to Fig. 6.

Figure S6.

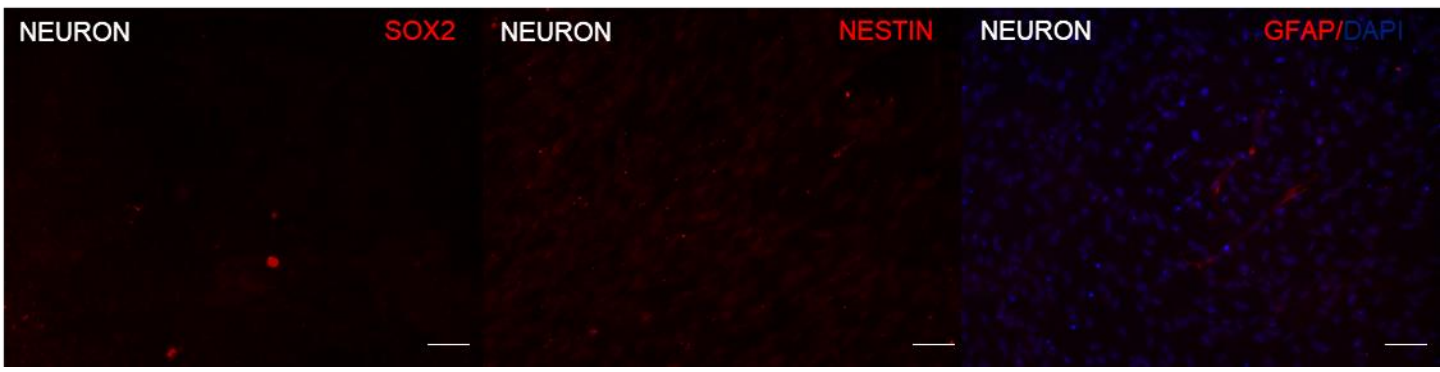


Figure S6. Immunocytochemical analysis of terminally matured NFκB^{high}-NPC.
Left-right: SOX2, Nestin and GFAP protein expression after terminal differentiation, related to Fig. 1A-D. Scale bar :100μm

Gene	Primer sequence Forward (5'-3')	Primer sequence Reverse (5'-3')
LIN28A	AAGCGCAGATCAAAAGGAGA	CTGATGCTCTGGCAGAAGTG
NESTIN	GGCAGCGTTGGAACAGAG	CATCTTGAGGTCGCCAGCT
MAP2	CTCAGCACCGCTAACAGAGG	CATCTTGAGGTCGCCAGCT
SOX2	GACCAGCTCGCAGACCTACAT	TGGAGTGGGAGGAAGAGGTA
PABPC4	GCTCAGGGAAGGCCTCCAT	GAGCGCTCAGCAGCAGCAACAG
NQO1	GGGCAAGTCCATCCCAACTG	GCAAGTCAGGGAAGCCTGGA
NFκB1	TGCCAACAGATGGCCCATAC	TGTTCTTTTCACTAGAGGCACCA
GLUT1	AACTCTTCAGCCAGGGTCCAC	CACAGTGAAGATGATGAAGAC
HO-1	CGGGACCTGACTGACTACC	TGAAGGTAGTTTCGTGGATGC
ATP synthase	CTTGACCTTCTTTGCGGCTC	CGCACGGACAGCATCTTTG
UCP2	CCCCGAAGCCTCTACAATGG	CTGAGCTTGGAATCGGACCTT
GAPDH	GAAGGTGAAGGTCGGAGTC	GAAGATGGTGATGGGATTTC

Table S1. qPCR primer list