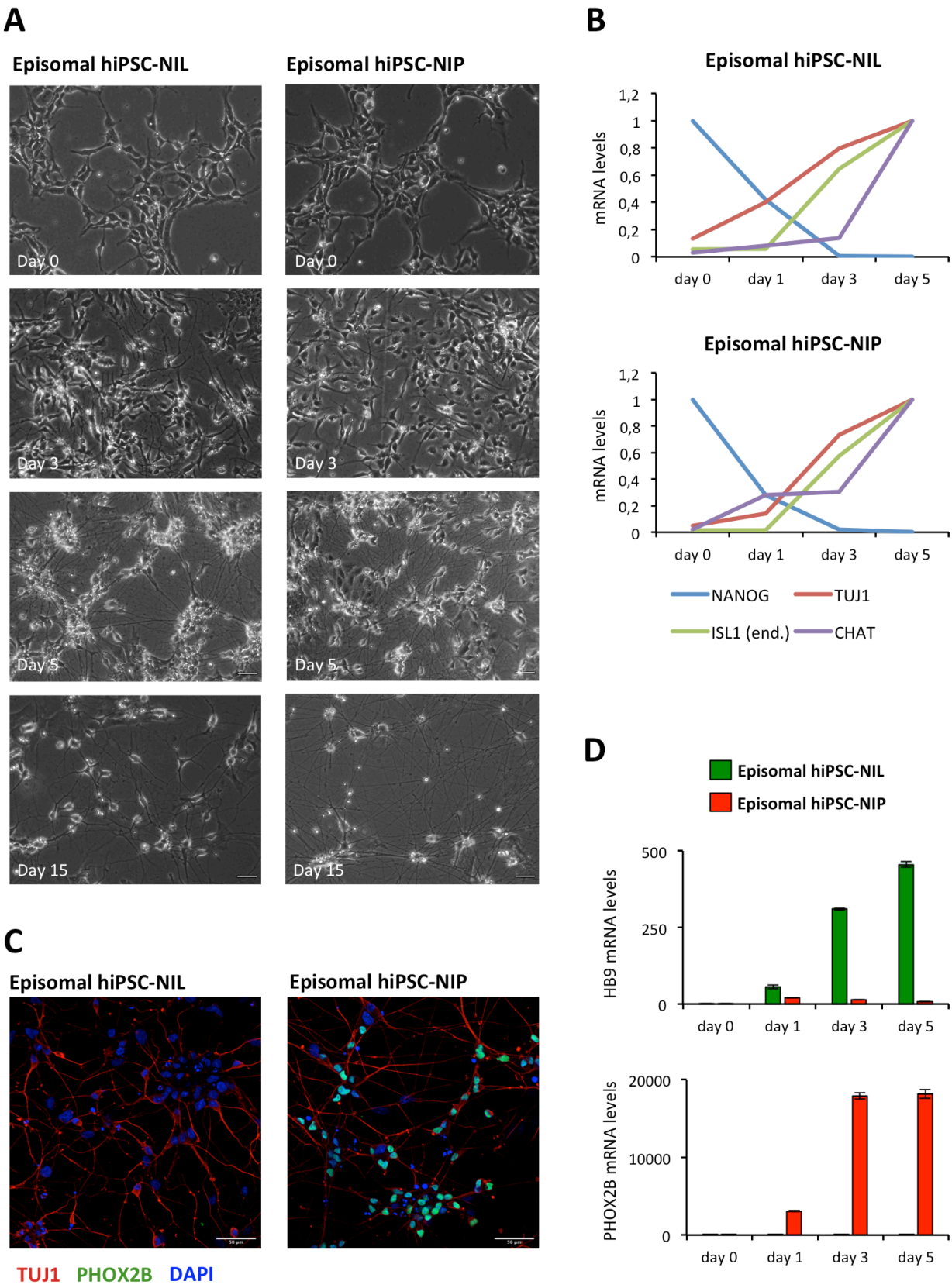
SUPPLEMENTARY FIGURES

Conversion of human induced Pluripotent Stem Cells (iPSCs) into functional spinal and cranial motor neurons using piggyBac vectors

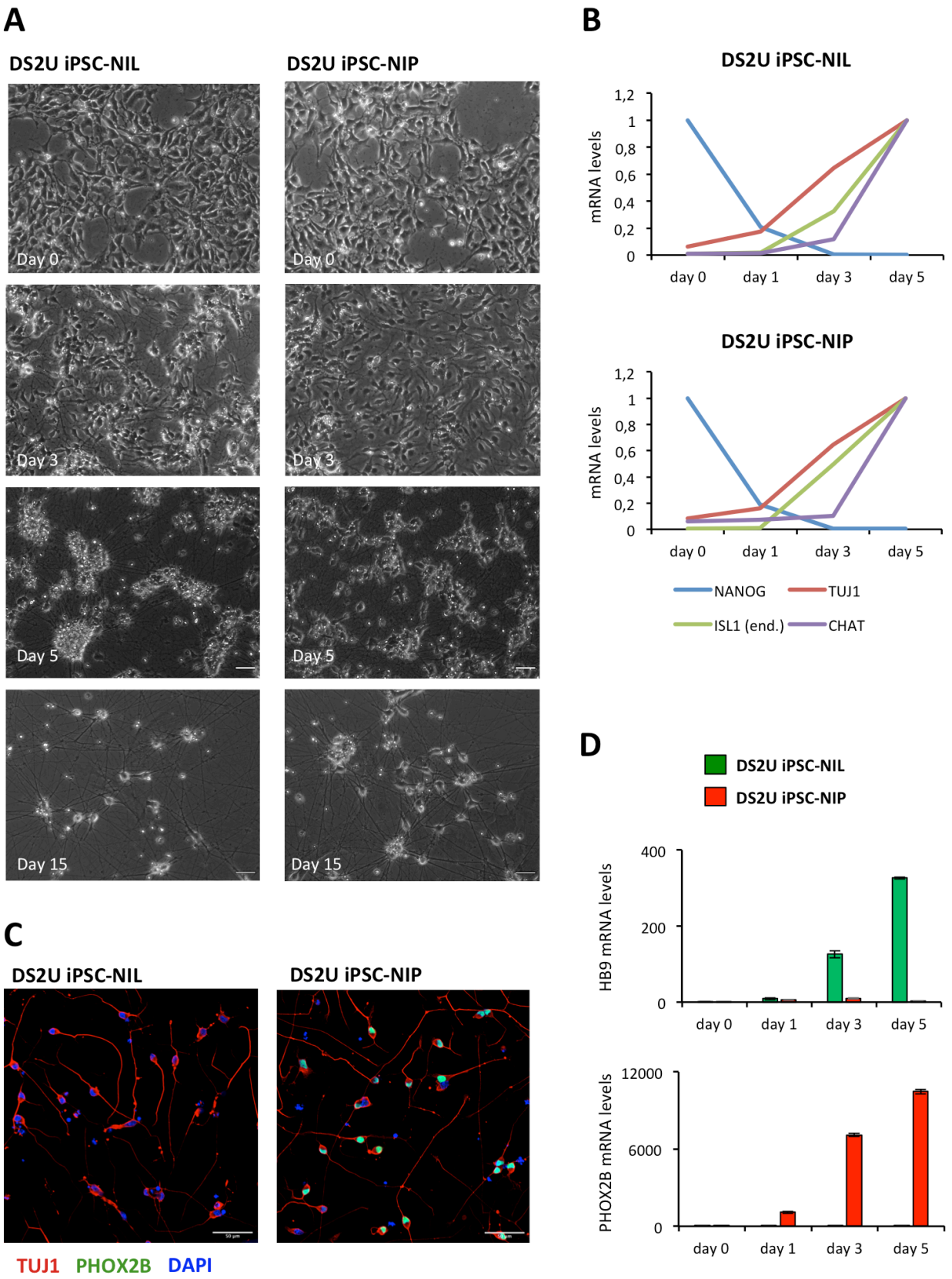
Maria G Garone[1], Valeria de Turris[2], Alessandro Soloperto[2], Carlo Brighi[2], Riccardo De Santis[3], Francesca Pagani[2,4], Silvia Di Angelantonio[2,4], Alessandro Rosa[1,2]

[1]*Department of Biology and Biotechnology Charles Darwin, Sapienza University of Rome, P.le A. Moro 5, 00185 Rome, Italy*
[2]*Center for Life Nano Science, Istituto Italiano di Tecnologia, Viale Regina Elena 291, 00161 Rome, Italy*
[3]*Laboratory of Stem Cell biology and Molecular Embryology, The Rockefeller University, 1230 York Avenue, New York, NY 10065, USA.*
[4]*Department of Physiology and Pharmacology, Sapienza University of Rome, P.le A. Moro 5, 00185 Rome, Italy*



Supplementary Figure S1: MN differentiation using Episomal hiPSCs. (A) Brightfield images of differentiating Episomal hiPSC-NIL (left) and Episomal hiPSC-NIP (right) at the indicated time points. Scale bar for all panels: 50 µm. (B) Analysis of the expression of the indicated markers in differentiating Episomal hiPSC-NIL (top) and Episomal hiPSC-NIP (bottom) cells by real time qRT-

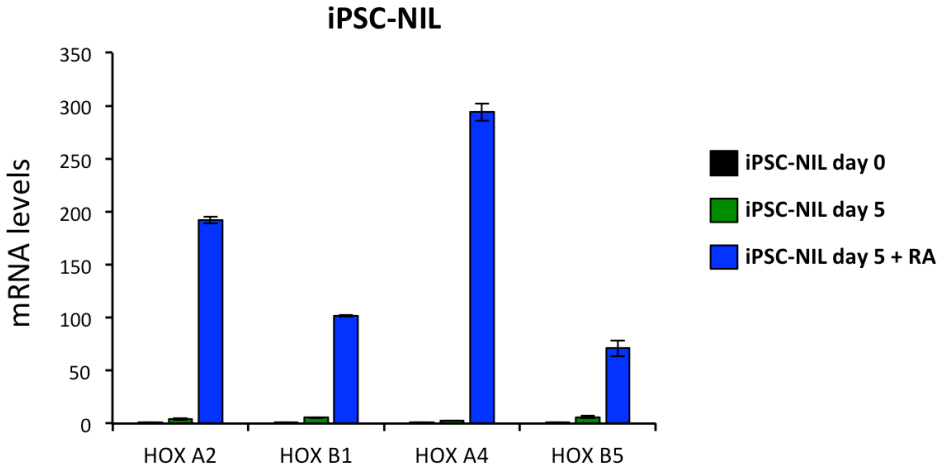
PCR. For each marker the time point with the highest expression has been used as calibrator sample. Primers used for ISL1 are specific for the endogenous gene. (C) Immunostaining for the pan-neuronal marker TUJ1 (red) and cranial MN marker PHOX2B (green) in differentiated (day 6) Episomal hiPSC-NIL (left) and Episomal hiPSC-NIP (right) cells. Nuclei are counterstained with DAPI. Scale bar for all panels: 50 μm. (D) Analysis of the expression of HB9 and PHOX2B in differentiating Episomal hiPSC-NIL (top) and Episomal hiPSC-NIP (bottom) cells by real time qRT-PCR. Day 0 has been used as the calibrator sample. PCR primers and methods are reported in De Santis et al., 2018[12].



Supplementary Figure S2: MN differentiation using DS2U iPSCs. (A) Brightfield images of differentiating DS2U-NIL (left) and DS2U-NIP (right) at the indicated time points. Scale bar for all panels: 50 µm. (B) Analysis of the expression of the indicated markers in differentiating DS2U-NIL (top) and DS2U-NIP (bottom) cells by real time qRT-PCR. For each marker the time point with the

highest expression has been used as calibrator sample. Primers used for ISL1 are specific for the endogenous gene. (C) Immunostaining for the pan-neuronal marker TUJ1 (red) and cranial MN marker PHOX2B (green) in differentiated (day 6) DS2U-NIL (left) and DS2U-NIP (right) cells. Nuclei are counterstained with DAPI. Scale bar for all panels: 50 µm. (D) Analysis of the expression of HB9 and PHOX2B in differentiating DS2U-NIL (top) and DS2U-NIP (bottom) cells by real time qRT-PCR. Day 0 has been used as the calibrator sample. PCR primers and methods are reported in De Santis et al., 2018[12].

A



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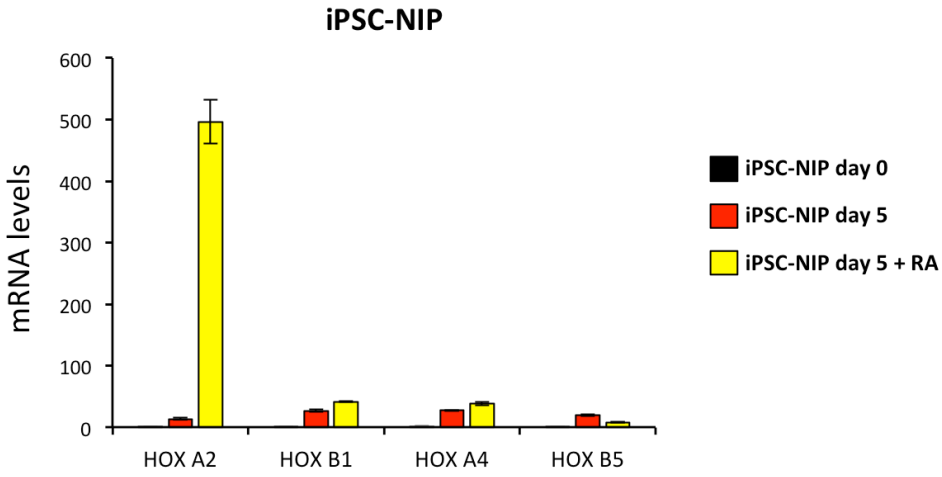
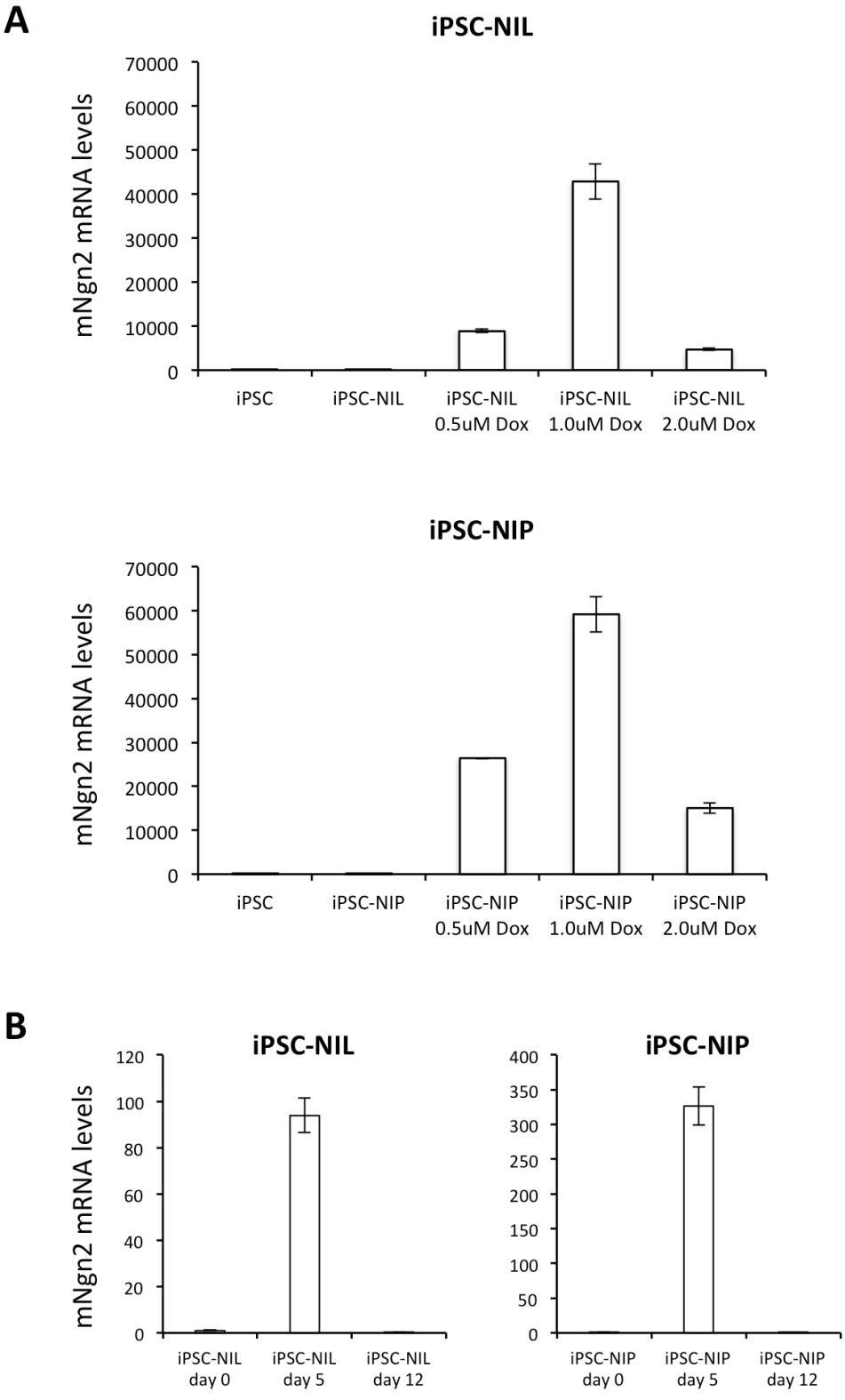


C

Gene name	Primer forward	Primer reverse
HOX A2	TGGATGAAGGAGAAGAAGGCGG	TCGGCGATTTCCAGGGATTCTT
HOX B1	AGAGAAACCCACCCAAGACAGG	AAGAGAAGAACCCAGCCCAGAC
HOX A4	AAACTGCCCAACACCAAGATGC	GGCTCTGAGTTTGTGCTTTCCC
HOX B5	TCACCGAAATAGACGAGGCCAG	AATATTTGCGGAGTCTGCCCCT

Supplementary Figure S3: HOX gene expression. (A) Analysis of the expression of four different HOX genes (HOX A2, HOX B1, HOX A4, HOX B5) after 5 days of differentiation of iPSC-NIL and iPSC-NIL + RA cells by real time qRT-PCR. IPSC-NIL at day 0 has been used as the calibrator sample. (B) Analysis of the expression of four different HOX genes (HOX A2, HOX B1, HOX A4, HOX B5) after 5

days of differentiation of iPSC-NIP and iPSC-NIP + RA cells by real time qRT-PCR. IPSC-NIP at day 0 has been used as the calibrator sample. (C) PCR primer pairs.



Supplementary Figure S4: Doxycycline induction analysis. (A) Analysis by real time qRT-PCR of the expression of exogenous Ngn2 in iPSC-NIL (top) and iPSC-NIP (bottom) cells untreated or cultured for 24 h in presence of doxycycline at different concentration (0.5 µM, 1.0 µM, 2.0 µM). Ngn2 was analyzed with primers specific for the exogenous mouse gene. The parental iPSC line, devoid of NIL and NIP constructs, has been included in the analysis as a control. Expression of the transgenes

in iPSC-NIL and iPSC-NIP was neglectable in absence of doxycycline. iPSC-NIL and iPSC-NIP at day 0 have been used as calibrator samples. (B) Analysis by real time qRT-PCR of the expression of exogenous Ngn2 in differentiating iPSC-NIL (left) and iPSC-NIP (right) cells at the indicated time points of the protocol. Ngn2 was analyzed with primers specific for the exogenous mouse gene. iPSC-NIL and iPSC-NIP at day 0 have been used as calibrator samples. PCR primers and methods are reported in De Santis et al., 2018[12].