SUPPLEMENTAL DATA

Title: Biphasic activation of WNT signaling facilitates the derivation of midbrain dopamine neurons from hESCs for translational use

Authors: Tae Wan Kim, Jinghua Piao, So Yeon Koo, Sonja Kriks, Sun Young Chung, Doron Betel, Nicholas D. Socci, Se Joon Choi, Susan Zabierowski, Brittany N. Dubose, Ellen J. Hill, Eugene V Mosharov, Stefan Irion, Mark Tomishima, Viviane Tabar, Lorenz Studer.

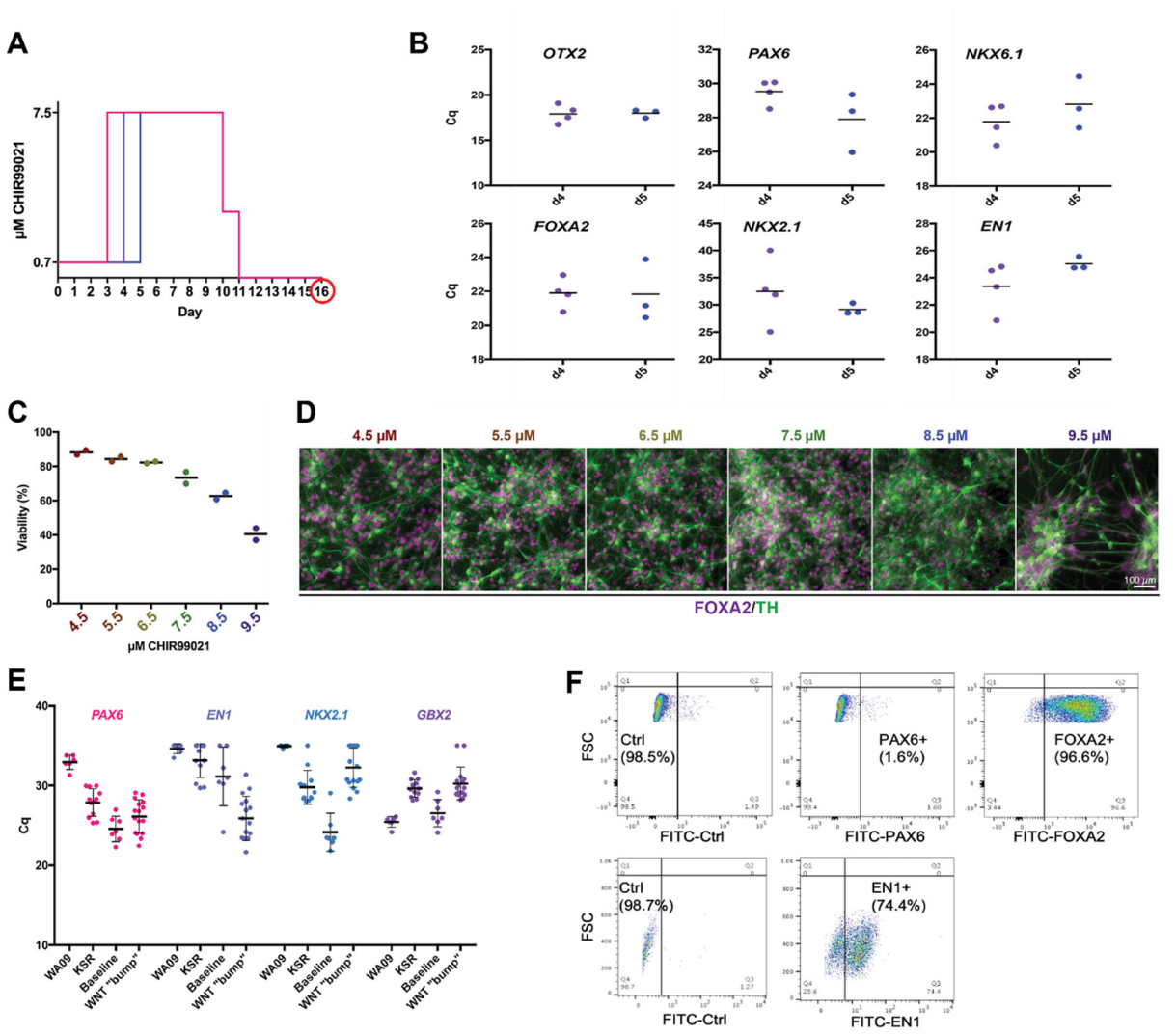

Figure S1. Optimizing timing and concentration of CHIR-boost. Related to Figure 1. (A) Testing the timing of "Chir-boosting" (addition of 7.5 μM CHIR99021). The "Boost" was initiated at day 3 (red), day 4 (purple) and day 5 (blue) following a time period of lower Chir (0.7 μM) through day 16. (B) Gene expression analysis for mDA neuron related *OTX2, FOXA2, EN1* and non mDA neuron genes (*PAX6, NKX6.1, NXK2.1*) following differential start of Chir boost as detailed in (A). Genes with high expression levels have low Cq values while genes with low expression have high values. (C) Dose response of Chir boost on total number of mDA neuron precursors. All experiments were "Chir boosted" at day 4. (D) Corresponding images analyzed expression of FOXA2 (purple) and TH (green) by immunocytochemistry (day 21). Scale bar = 100 μm. (E) Comparison of mRNA expression among WA09 (undifferentiated hESCs), KSR (the traditional Kriks et al. protocol (Kriks et al., 2011)), Baseline (Low (0.7μM)- Chir protocol) versus WNT bump (Chir- Boost protocol). Genes with high expression levels have low Cq values while genes with low expression have high values. (F) The flow plots for PAX6, FOXA2 and EN1 expression for negative control and Chir-boost conditions (compare to **Figure 1D**). Y-axis of Cq (B, E) showed ct values of mRNA expression. A low ct value means high level of gene expression and a high ct level shows low expression.

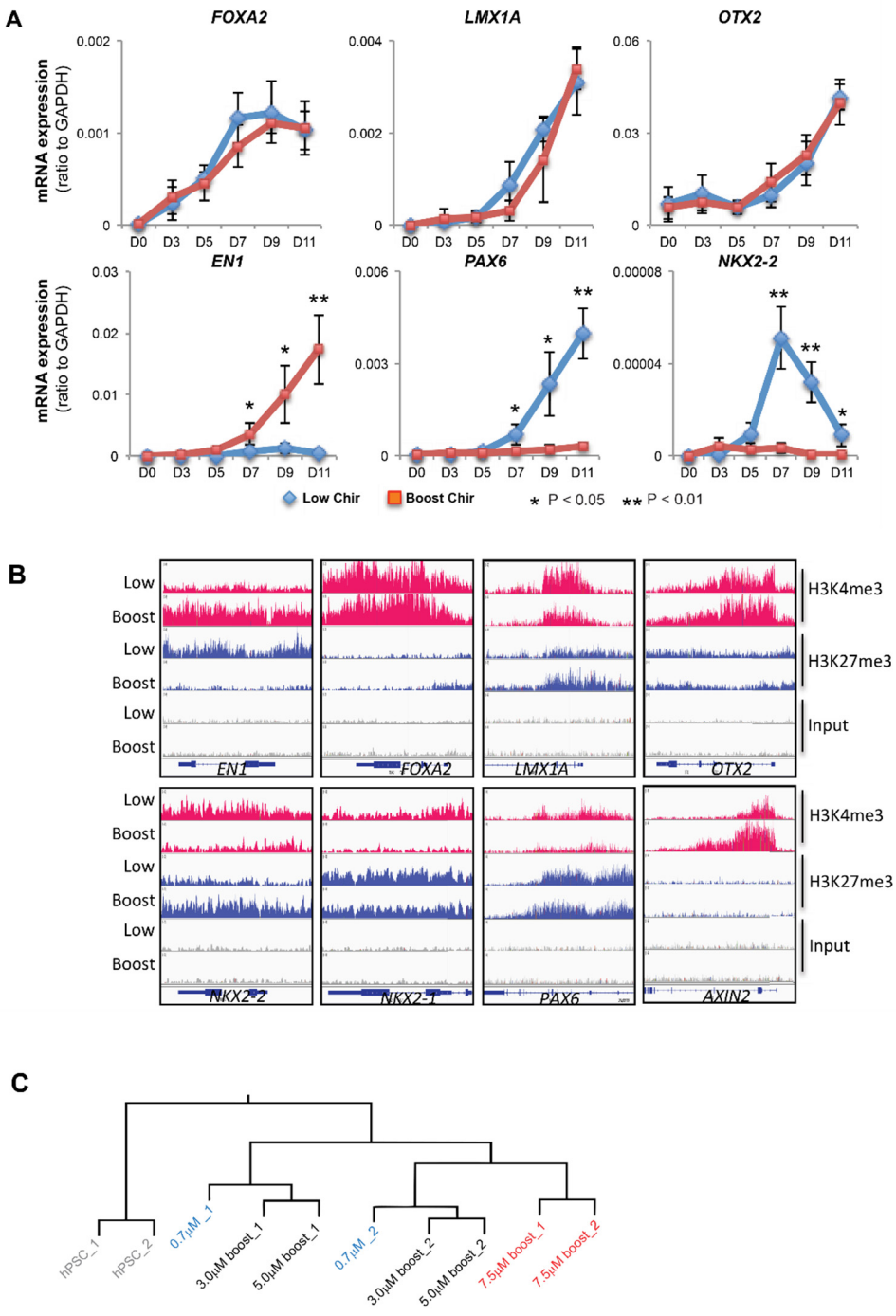

Figure S2. Gene expression and histone methylation analysis in CHIR-boost and low CHIR treated cells. Related to Figure 2 (A) Time course mRNA expression analysis during mDA differentiation in Low- (blue line) and Boost CHIR treated (red line) cells until day 11. (B) IGV views of ChIP-seq data for histone modification (H3K3me3 and H3K27me3) at day 11 of differentiation in Low- and Boost- Chir conditions. (C) Cluster dendrogram of all RNA-seq samples.

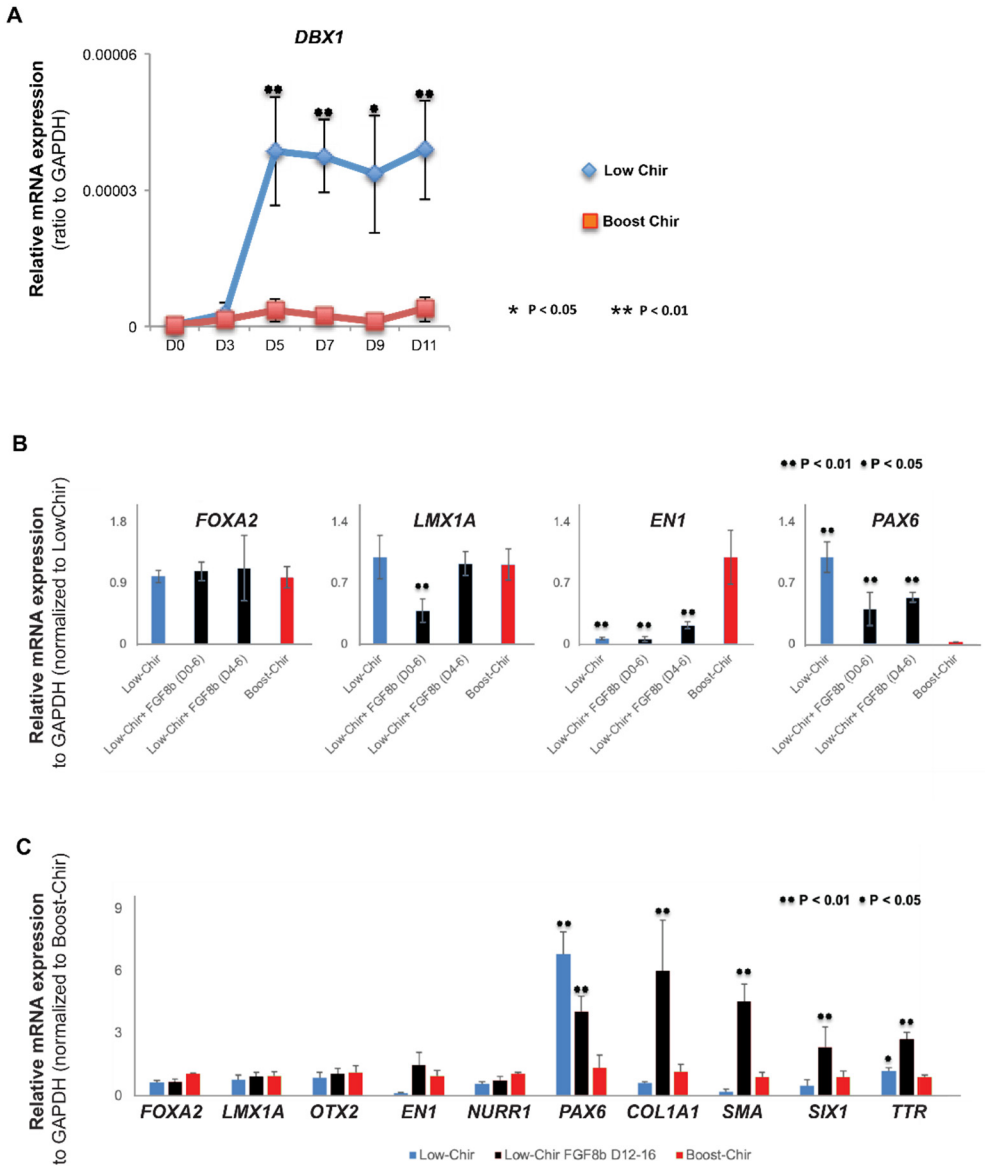

Figure S3. Gene expression analysis comparing Chir-boost condition versus Chir-Low condition with or without FGF8b treatment. Related to Figure 2. (A) mRNA expression of *DBX1* during mDA differentiation in CHIR-Low and CHIR-boost conditions, demonstrating Low-CHIR treated cells have induced DBX1 expression from day 5 of differentiation, while CHIR-BOOST treated cells show no such induction. **(**B) mRNA expression of mDA differentiated cells treated with Low-Chir +/- FGF8b treatment (D0-6; Day0-6, D4-6; Day4-6) versus Boost-Chir at day11 (Early exposure of FGF8b in Low Chir condition). (C) mRNA expression of mDA differentiated cells treated with Low- Chir +/- FGF8b treatment (D12-16; Day12-16), and Boost-Chir at day30 (Late exposure of FGF8 in Low Chir condition).

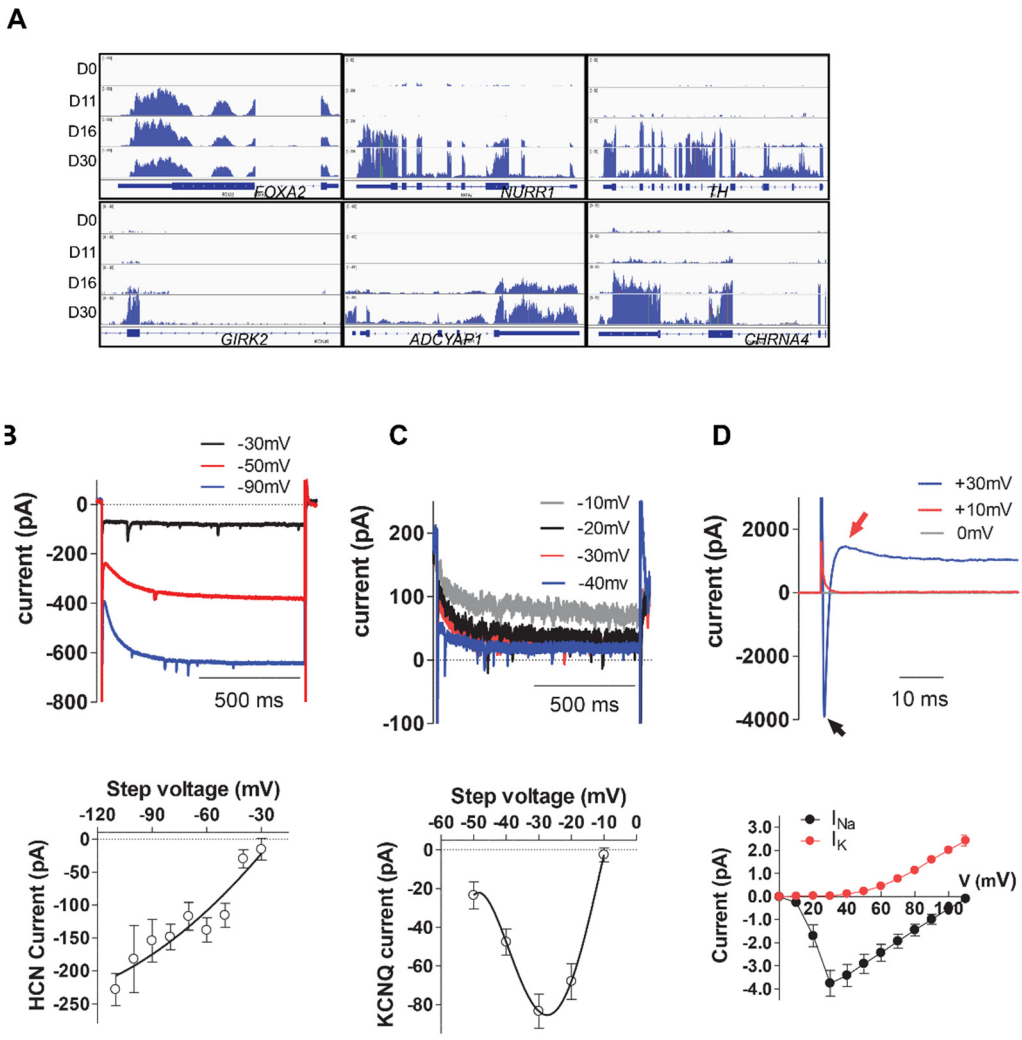

Figure S4. Molecular and functional analyses of mDA neurons under CHIR-boost. Related to Figure 3 (A) IGV views of selected mDA markers during differentiation. (B-D) Currents via specific channels recorded in voltage-clamp mode from hPSC-derived mDA neurons at day 60 and 75. Because no difference was observed between day 60 and DIV 75 neurons, data from them were pooled and are presented together (n=24). (B) *Upper:* Representative traces of HCN channel currents elicited by hyperpolarizing voltage step from - 40 mV to -20, -50 or -90 mV). *Lower:* Voltage dependence of HCN currents. (C) *Upper:* Representative traces of KCNQ channel currents elicited by hyperpolarizing voltage steps from -10 mV to -20, -30 or -40 mV. *Lower:* Voltage dependence of KCNQ currents. (D) *Upper:* Fast inward (Na^+, black arrow) and slow outward (K^+, red arrow) currents in response to voltage steps (Vh: -70 mV; 0, +10, and +30 mV). *Lower:* Voltage dependence of Na^+ (filled circles) and K^+ (red circles) currents.

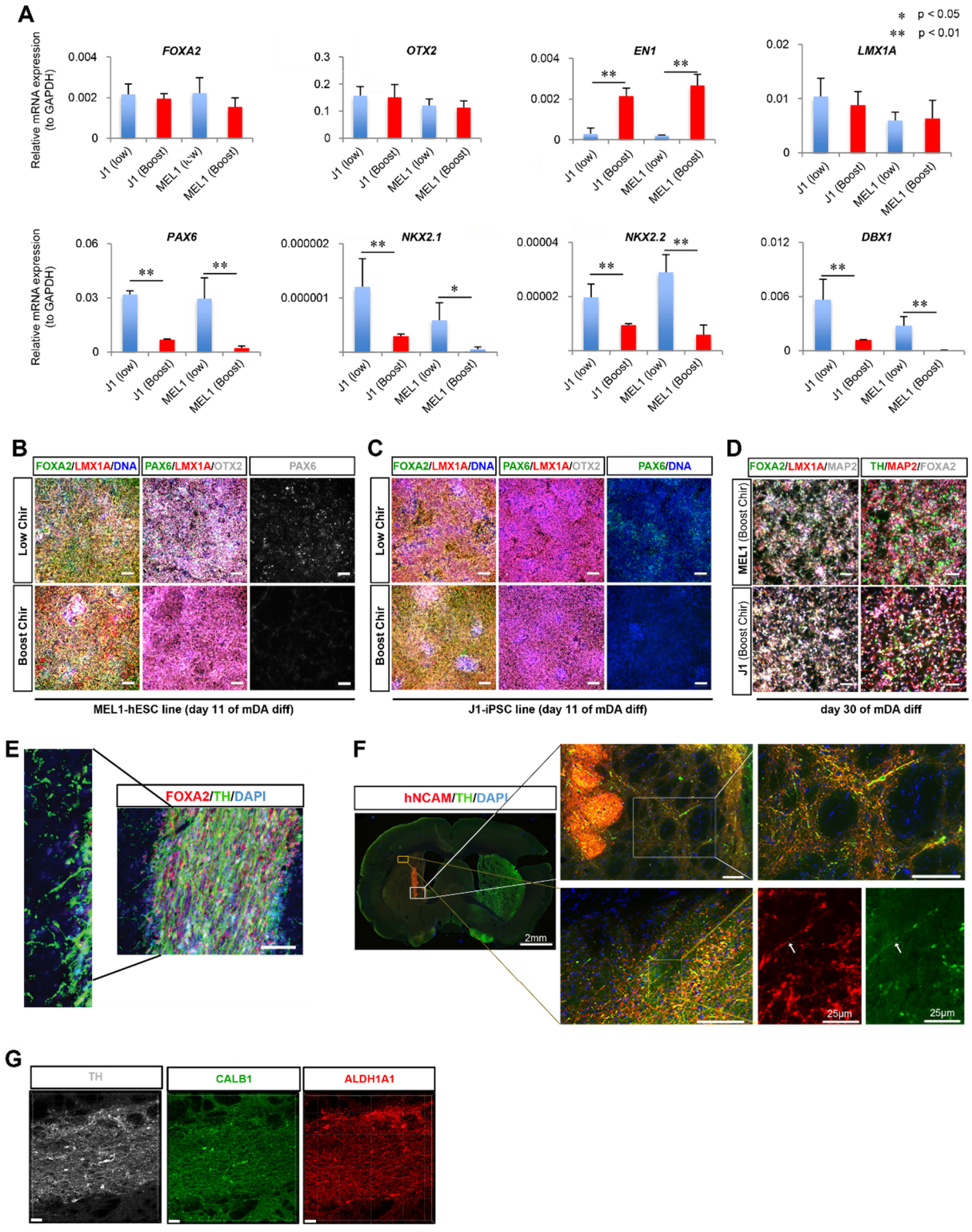

Figure S5. Validation of CHIR-boost protocol across independent hPSCs (MEL1 hESC and J1 iPSC lines), and graft survival and characterization studies *in vivo*. Related to Figure 2 and Figure 5 (A) mRNA expression analysis at day 11 of mDA neuron differentiation using Low-

and Boost- Chir conditions in J1 and MEL1 hPSCs. (B, C) Immunocytochemistry at day 11 of mDA neuron differentiation using Low- and Boost- Chir conditions in J1 and MEL1 hPSCs (D) Immunocytochemistry of mDA neuron differentiation at day 30 in Chir-Boost condition. (E) Short term *in vivo* survival of cryopreserved mDA neuron precursor grafts indicating robust graft survival expressing mDA markers TH and FOXA2 in unlesioned rat striatum. (F) Human TH+ fibers, which were identified with the co-labeling with hNCAM (human specific cytoplasmic marker) and TH, were projected from the graft. (G) Representative image to illustrate distribution of ALDH1A1+ and CALB1+ mDA subtypes within the graft. Scale bars = 100 μm in (B-F) and 50 μm in (G) unless indicated in pictures.

Table S1 – Sequences of qRT-PCR primers used in this study (related to the Star Methods)

Gene	**Forward (5' to 3')**	**Reverse (5' to 3')**
GAPDH	ATGTTCGTCATGGGTGTGAA	AGGGGTGCTAAGCAGTTGGT
EN1	CTG GGT GTA CTG CAC ACG TT	GCT TGT CCT CCT TCT CGT TC
FOXA2	CCGACTGGAGCAGCTACTATG	TACGTGTTCATGCCGTTCAT
LMX1A	CAGCAGCAAGATCAGCAGAA	AGGGGTTCATGATTCCTTCC
OTX2	AGA GGA GGT GGC ACT GAA AA	GCT GTT GTT GCT GTT GTT GG
PAX6	GAAGGGCCAAATGGAGAAGA	GGTGCTGAAACTACTGCTGATA
NXK2-1	ATG TGG TCC GGA GGC AGT	TGC TTT GGA CTC ATC GAC AT
NXK2-2	CAG CGA CAA CCC GTA CAC	GAC TTG GAG CTT GAG TCC TGA
NURR1	CGCTTCTCAGAGCTACAGTTAC	TGGTGAGGTCCATGCTAAAC
PITX3	CCGTGTCCTGCCCTTATG	CGTGCTGTTTGGCTTTGAG
GIRK2	GATGGGAAACTGTGCCTGAT	CTCCGAGGTCTGTTTGGATTT
ADCYAP1	CGGAAACAAATGGCTGTCAAG	TAAGCTATTCGGCGTCCTTTG
CHRNA4	GAACCAGATGATGACCACGAA	GGATGGAGGTGACATTCTCATAG
SNCA	CACAGGAAGGAATTCTGGAAGATA	GCTTCAGGTTCGTAGTCTTGAT
COL1A1	CTAAAGGCGAACCTGGTGAT	TCCAGGAGCACCAACATTAC
TTR	GGTGAATCCAAGTGTCCTCTG	GTGTCATCAGCAGCCTTTCT

Gene	**From QuantiTect (Qiagen)**
HOXA2	QT00092960
HOXB1	QT00017080
AXIN2	QT00037639
DBX1	QT02394021
BARHL1	QT00214599
BARHL2	QT00063042
PITX2	QT00015785
PAX2	QT00028385
PAX5	QT00021399
PAX8	QT00070693
FGF8	QT00010990
SIX1	QT00010584
SMA	QT00088102