Cell Reports, Volume *28*

Supplemental Information

hESC-Derived Dopaminergic Transplants Integrate into Basal Ganglia Circuitry in a Preclinical Model of Parkinson's Disease

Andrew F. Adler, Tiago Cardoso, Sara Nolbrant, Bengt Mattsson, Deirdre B. Hoban, Ulla Jarl, Jenny Nelander Wahlestedt, Shane Grealish, Anders Björklund, and Malin Parmar

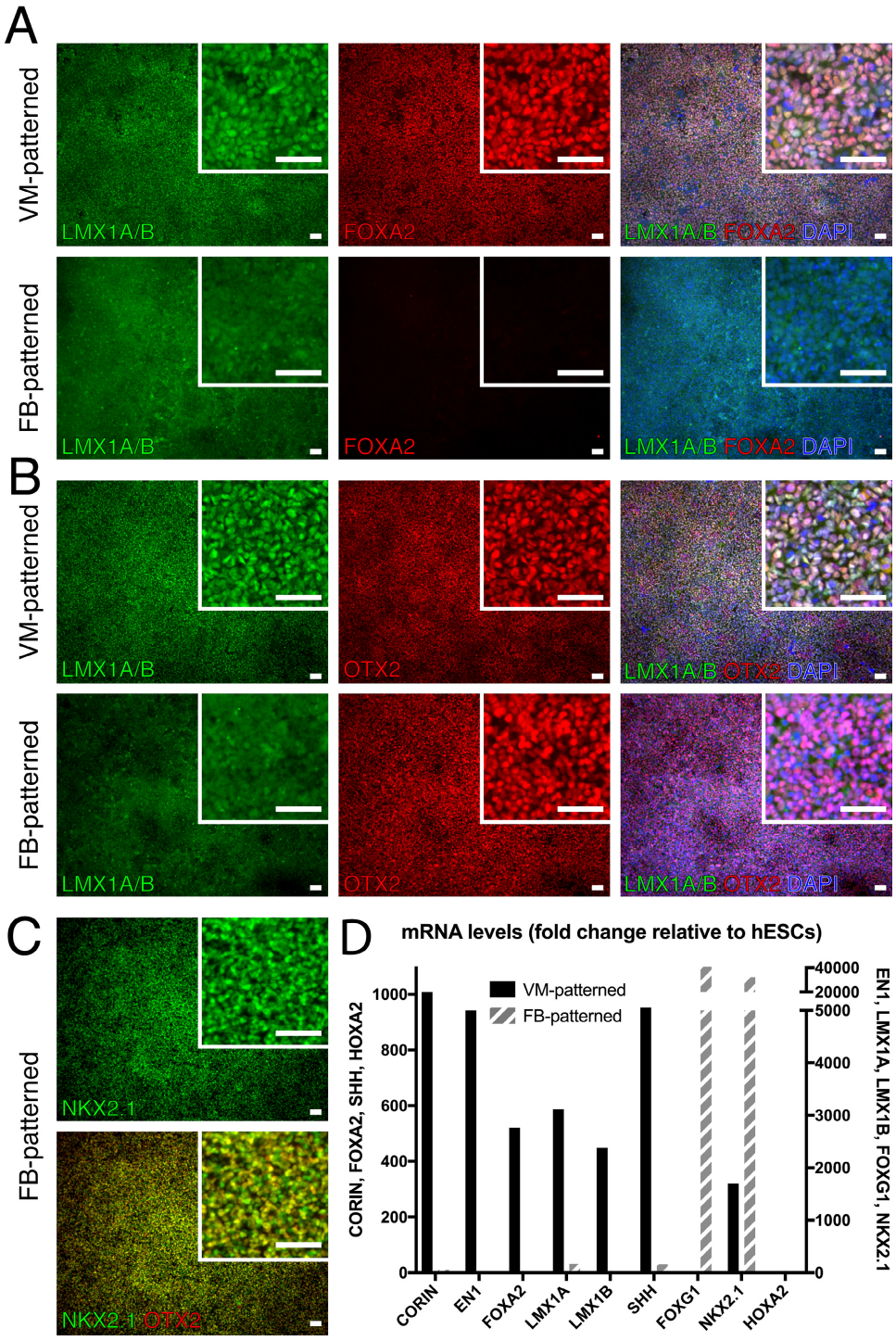

Figure S1. *In vitro* characterization of ventral midbrain- and ventral forebrain-patterned cell grafts (related to Fig. 1)

(A) At day 14 of differentiation, hESC-derived VM-patterned progenitors co-expressed ventral midbrain markers LMX1A/B and FOXA2, while FB-patterned progenitors did not, and **(B)** both cell sources expressed the anterior marker OTX2. **(C)** At day 21 of differentiation, FB-patterned progenitors expressed the ventral forebrain marker NKX2.1. **(D)** qPCR analysis at day 14 of differentiation (two days before grafting) revealed VM- and FB-patterned cells adopted ventral midbrain and ventral forebrain fates, respectively, without expression of the hindbrain marker *HOXA2*.

Scale bars represent 100 µm. DAPI = 4′,6-diamidino-2-phenylindole; EN1 = homeobox protein engrailed-1; FB = forebrain; FOXA2 = forkhead box A2; HOXA2 = homeobox A2; LMX1A/B = LIM homeobox transcription factor 1 alpha/beta; NKX2.1 = NK2 homeobox 1; OTX2 = orthodenticle homeobox 2; SHH = sonic hedgehog; VM = ventral midbrain

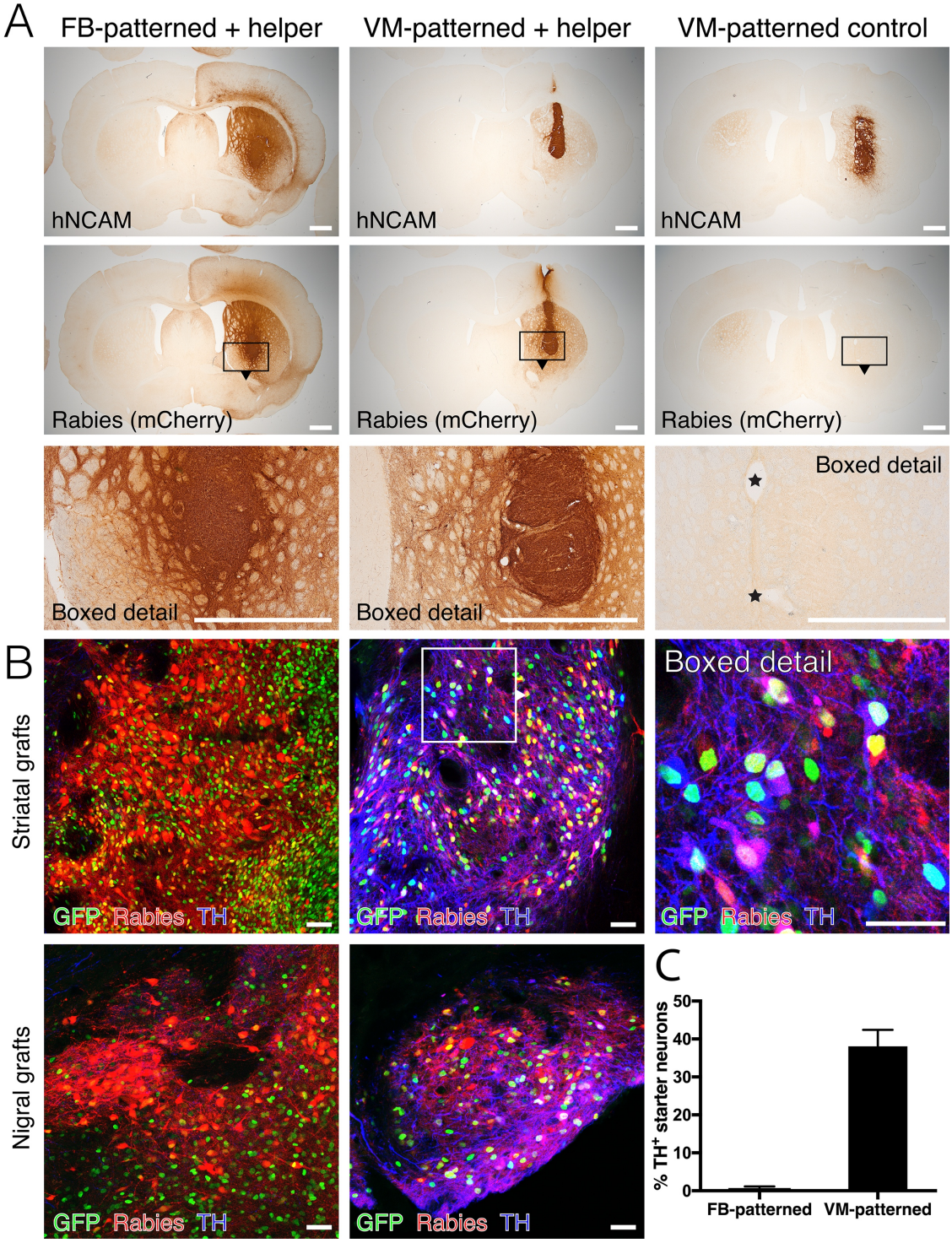

Figure S2. EnvA-pseudotyped ΔG-rabies tracing starter cell characterization (related to Fig. 4)
(A) hNCAM and ΔG-rabies (mCherry) staining reveals both FB- and VM-patterned progenitor cells generate neuron-rich grafts, but only animals grafted with cells pre-transduced with the rabies helper construct express mCherry upon injection of EnvA-pseudotyped mCherry ΔG-rabies virus. Stars indicate rabies injection sites for a 6 week VM-patterned control graft. **(B)** Analysis of intrastriatal and intranigral graft-derived "starter" cells, expressing both mCherry (ΔG-rabies) and nuclear GFP (rabies helper construct), reveals that **(C)** 38 ± 4.3 % of VM-patterned starter neurons were TH^+ (mean + SEM, VM-patterned grafts, n = 8 animals; FB-patterned grafts, n = 11 animals).

Scale bars represent 1 mm (A), 50 µm (B). FB = forebrain; GFP = green fluorescent protein; hNCAM = human neural cell adhesion molecule; TH = tyrosine hydroxylase; VM = ventral midbrain

	Forward	Reverse
ACTB	CCTTGCACATGCCGGAG	GCACAGAGCCTCGCCTT
CORIN	CATATCTCCATCGCCTCAGTTG	GGCAGGAGTCCATGACTGT
EN1	CGTGGCTTACTCCCCATTTA	TCTCGCTGTCTCTCCCTCTC
FOXA2	CCGTTCTCCATCAACAACCT	GGGGTAGTGCATCACCTGTT
FOXG1	TGGCCCATGTCGCCCTTCCT	GCCGACGTGGTGCCGTTGTA
GAPDH	TTGAGGTCAATGAAGGGGTC	GAAGGTGAAGGTCGGAGTCA
HOXA2	CGTCGCTCGCTGAGTGCCTG	TGTCGAGTGTGAAAGCGTCGAGG
LMX1A	CGCATCGTTTCTTCTCCTCT	CAGACAGACTTGGGGCTCAC
LMX1B	CTTAACCAGCCTCAGCGACT	TCAGGAGGCGAAGTAGGAAC
NKX2.1	AGGGCGGGGCACAGATTGGA	GCTGGCAGAGTGTGCCCAGA
SHH	CCAATTACAACCCCGACATC	AGTTTCACTCCTGGCCACTG

Table S1. Primer sequences (related to STAR Methods)