Supplemental Information

Human Stem Cell-Derived Dopamine Neurons Repair the Damaged Circuits and Restore Neural Function in a Mouse Model of Parkinson's Disease

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Figure S1

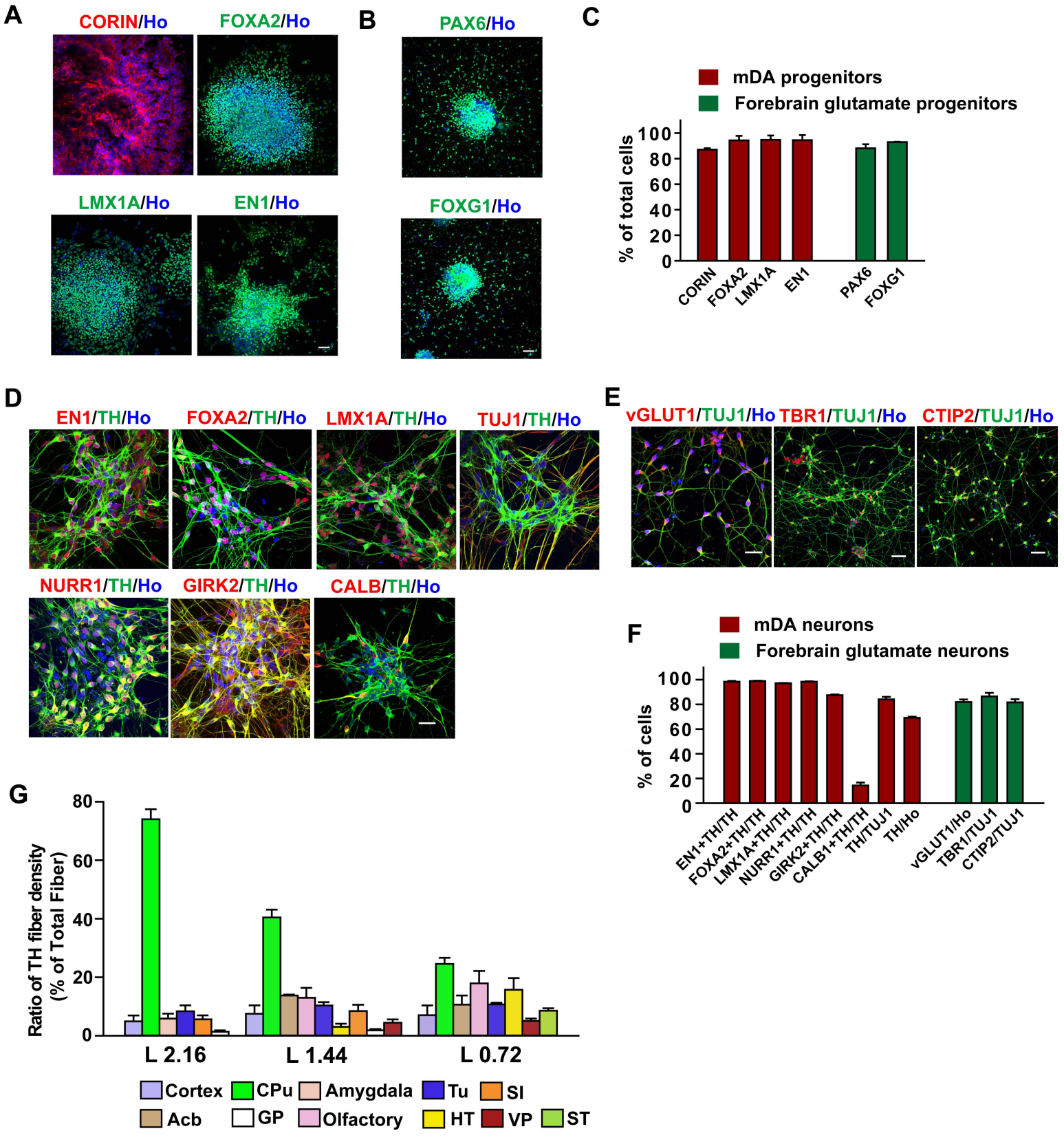
A
CORIN/Ho
FOXA2/Ho
LMX1A/Ho
EN1/Ho
B
PAX6/Ho
FOXG1/Ho
C
mDA progenitors
Forebrain glutamate progenitors
% of total cells
100
80
60
40
20
0
CORIN
FOXA2
LMX1A
EN1
PAX6
FOXG1
D
EN1/TH/Ho
FOXA2/TH/Ho
LMX1A/TH/Ho
TUJ1/TH/Ho
NURR1/TH/Ho
GIRK2/TH/Ho
CALB/TH/Ho
E
vGLUT1/TUJ1/Ho
TBR1/TUJ1/Ho
CTIP2/TUJ1/Ho
F
mDA neurons
Forebrain glutamate neurons
% of cells
100
80
60
40
20
0
EN1+TH/TH
FOXA2+TH/TH
LMX1A+TH/TH
NURR1+TH/TH
GIRK2+TH/TH
CALB1+TH/TH
TH/TUJ1
TH/Ho
vGLUT1/Ho
TBR1/TUJ1
CTIP2/TUJ1
G
Ratio of TH fiber density
(% of Total Fiber)
80
60
40
20
0
L 2.16
L 1.44
L 0.72
Cortex
CPu
Amygdala
Tu
SI
Acb
GP
Olfactory
HT
VP
ST

Figure S1. mDA and Glu neuron differentiation *in vitro* and TH fiber distribution *in vivo* (related to Figure 1).

(A-B) Immunostaining of day-32 cultures derived from hESCs shows markers for mDA progenitors (A) and forebrain glutamate progenitors (B). Ho, Hoechst. Scale bar = 25 μm. (C) Quantification of cellular differentiation presented in (A) and (B). (D-E) Immunostaining of day-42 cultures derived from hESCs shows markers for mDA neurons (D) and forebrain glutamate neurons (E). Scale bar = 25 μm. (F) Quantification of cellular differentiation presented in (D) and (E). (G) Quantification of the regional distribution of TH^+ fibers from wild type mice as in figure 1D. n = 5. TH^+ positive cell bodies in the cortex were included in our calculation. See supplemental Table 1 for abbreviations.

Figure S2

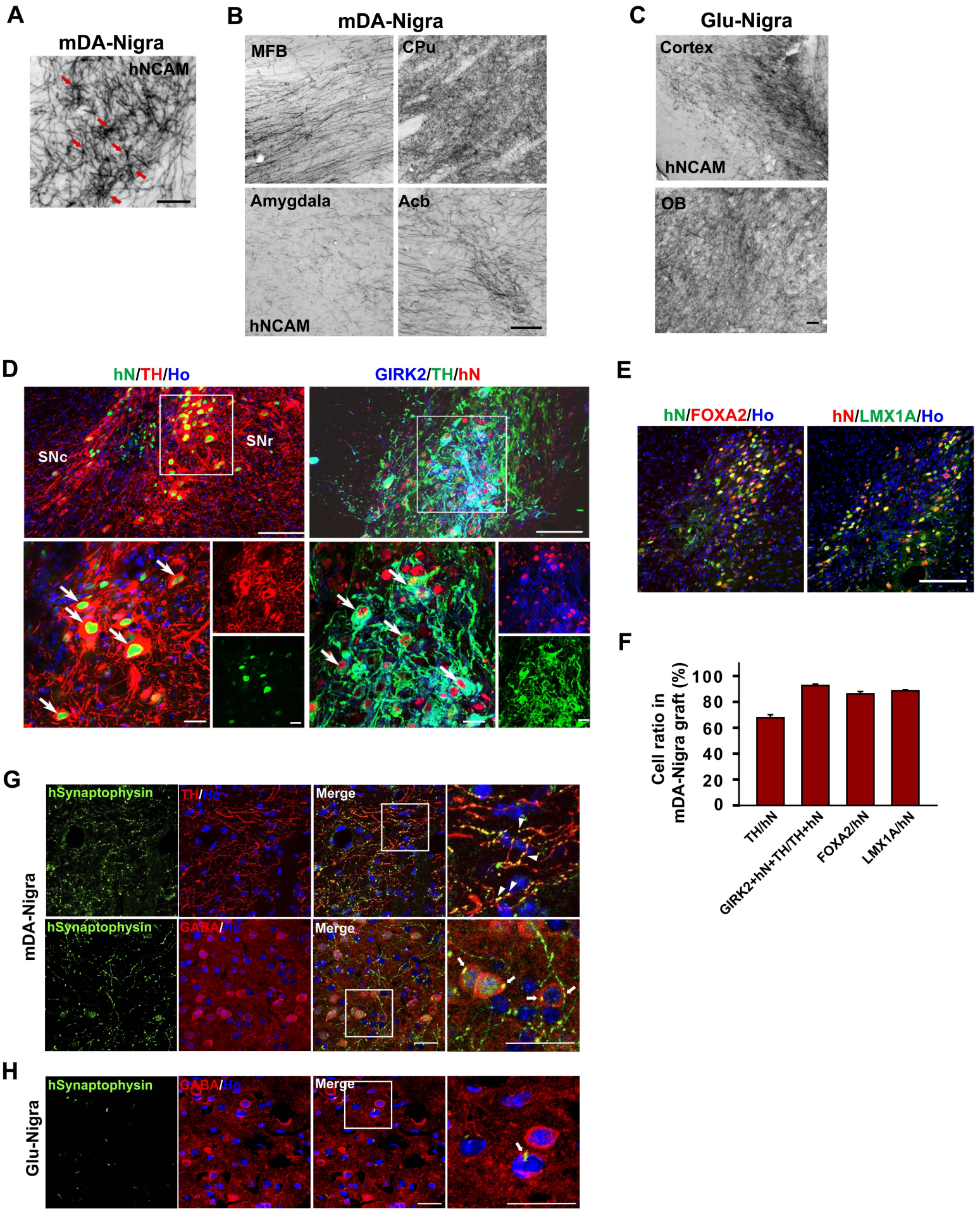
A
mDA-Nigra
hNCAM
B
mDA-Nigra
MFB
CPu
Amygdala
Acb
hNCAM
C
Glu-Nigra
Cortex
hNCAM
OB
D
hN/TH/Ho
GIRK2/TH/hN
SNc
SNr
E
hN/FOXA2/Ho
hN/LMX1A/Ho
F
Cell ratio in mDA-Nigra graft (%)
100
80
60
40
20
0
TH/hN
GIRK2+hN+TH/TH+hN
FOXA2/hN
LMX1A/hN
G
mDA-Nigra
hSynaptophysin
TH/Ho
Merge
hSynaptophysin
GABA/Ho
Merge
H
Glu-Nigra
hSynaptophysin
GABA/Ho
Merge

Figure S2. Survival, axonal projection, and synaptogenesis of nigrally transplanted mDA and Glu neurons (related to Figure 2).

(A) Immunohistochemistry images for hNCAM from PD mouse brain with nigrally grafted mDA neurons show distribution and arborization of $hNCAM^+$ fibers in CPu. The red arrows indicate bead-like structure of $hNCAM^+$ fibers. Scale bar = 50 μm. (B) Immunohistochemistry images from lesioned mouse brain with nigrally grafted mDA neurons show morphology of $hNCAM^+$ fibers in different brain regions. Scale bar = 125 μm. (C) Immunohistochemistry images for hNCAM from PD mouse brain with nigrally grafted Glu neurons show distribution and arborization of $hNCAM^+$ fibers in cortex and OB (olfactory bulb). Scale bar = 125 μm. See supplemental Table 1 for abbreviations. (D) Immunohistochemistry images show that the grafted TH positive cells in nigra co-express human nuclei (hN) and GIRK2. Boxed regions are magnified below. Scale bar = 100 μm for large images, Scale bar = 25 μm for magnified images. Arrows indicate double positive cells. (E) Immunohistochemistry images show that the grafted human nuclei (hN) positive cells in nigra co-express FOXA2 and LMX1A. Scale bar = 100 μm. (F) Quantification of cell identity presented in (D and E). (G) The mouse brain with nigrally grafted mDA neurons were stained for human-specific synaptophysin and TH (upper panel) or GABA (lower panel) in the host CPu. Boxed areas are magnified on the right. White arrowheads indicate co-localization of human-specific synaptophysin with TH along the TH fibers. White arrows indicate co-localization of human-specific synaptophysin with GABA around the GABA neuron cell body. (H) The mouse brain with nigrally grafted Glu neurons was stained for human-specific synaptophysin and GABA in the host CPu. Boxed areas are magnified on the right. White arrows indicate co-localization of human-specific synaptophysin with GABA around the GABA neuron cell body.

Figure S3

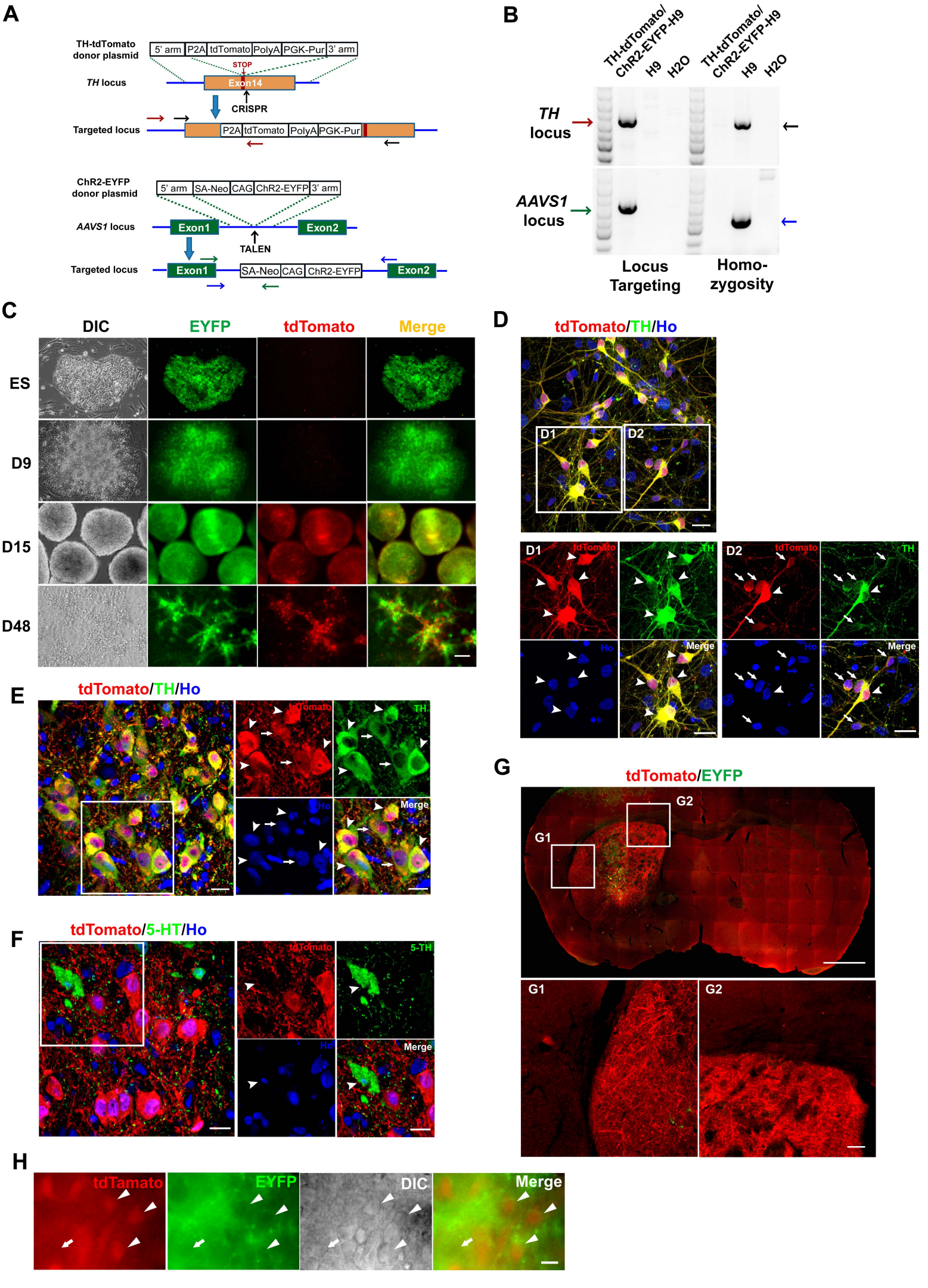
A
TH-tdTomato donor plasmid
5' arm
P2A
tdTomato
PolyA
PGK-Pur
3' arm
STOP
TH locus
Exon14
CRISPR
Targeted locus
ChR2-EYFP donor plasmid
SA-Neo
CAG
ChR2-EYFP
AAVS1 locus
Exon1
Exon2
TALEN
B
TH-tdTomato/ ChR2-EYFP-H9
H9
H2O
TH locus
AAVS1 locus
Locus Targeting
Homo-zygosity
C
DIC
EYFP
tdTomato
Merge
ES
D9
D15
D48
D
tdTomato/TH/Ho
D1
D2
E
tdTomato/TH/Ho
F
tdTomato/5-HT/Ho
5-TH
G
tdTomato/EYFP
G1
G2
H
tdTamato
EYFP
DIC
Merge

Figure S3. Establishment and characterization of TH-tdTomato/AAVS1-ChR2-EYFP hESC line (related to Figure 3).

(A) Schematic diagram of the genotyping strategy for TH-tdTomato/AAVS1-ChR2-EYFP hESC line. PCR primers for TH locus insertion or homozygosity are indicated by the red arrows and black arrows, respectively. PCR primers for *AAVS1* locus insertion or homozygosity are indicated by the green arrows and blue arrows, respectively. (B) PCR genotyping of the TH-tdTomato/AAVS1-ChR2-EYFP hESC line. The expected PCR product for correctly targeted *TH* locus or *AAVS1* locus are ~1200 bp (red arrow) or ~1000 bp (green arrow), respectively. Heterozygosity of the cell line is identified by the PCR product of ~1000 bp (black arrow) or ~650 bp (blue arrow) for *TH* locus or *AAVS1* locus, respectively. Those clones without ~1000 bp or ~650 bp PCR products are homozygous. The mother cell line H9 ESCs is included as control. TH-tdTomato/AAVS1-ChR2-EYFP hESC line is homozygous in both *TH* locus and *AAVS1* locus. (C) DIC and fluorescent images show expression of EYFP and tdTomato in the ESCs or during mDA neuron differentiation of TH-tdTomato/AAVS1-ChR2-EYFP hESCs. tdTomato is expressed in the mid (D15) and terminal (D48) stages, but not in the ES or early stage (D9) stages of mDA neuron differentiation. Scale bar = 100 μm. (D) Immunostaining of day 42 cultures derived from the above hESCs shows co-expression of tdTomato in TH^+ neurons. Boxed areas are magnified below. White arrowheads indicate neurons with high expression of tdTomato and TH. White arrows indicate neurons with low expression of tdTomato and TH. Scale bar = 20 μm. (E) Immunohistochemistry images show the expression of tdTomato in TH^+ neurons in the nigral mDA graft. Boxed areas are magnified right. White arrowheads and white arrows indicate neurons with high expression of tdTomato and TH or low expression of tdTomato and TH, respectively. Scale bar = 20 μm. (F) Immunohistochemistry images show 5-HT^+ serotonin neuron in the nigral mDA graft. Boxed areas are magnified right. White arrowheads indicate $tdTomato^-$ and 5-HT^+ neurons. Scale bar = 20 μm. (G) Coronal section away from the graft site immunostained for tdTomato and EYFP from the PD mouse brain striatally grafted with mDA neurons derived from TH-tdTomato/AAVS1-ChR2-EYFP hESCs, showing specific projection of mDA neuron in the CPu, but not neighboring brain regions. Scale bar = 1 mm. Boxed areas are magnified below. Scale bar = 100 μm. (H) DIC and fluorescent images of a slice from the PD mouse brain nigrally grafted with mDA neurons derived from TH-

tdTomato/AAVS1-ChR2-EYFP hESCs. White arrowheads indicate tdTomato^{+} mDA neurons, and white arrow indicates tdTomato^{-} non-mDA neurons in the EYFP+ graft. Scale bar = 50 μm.

Figure S4

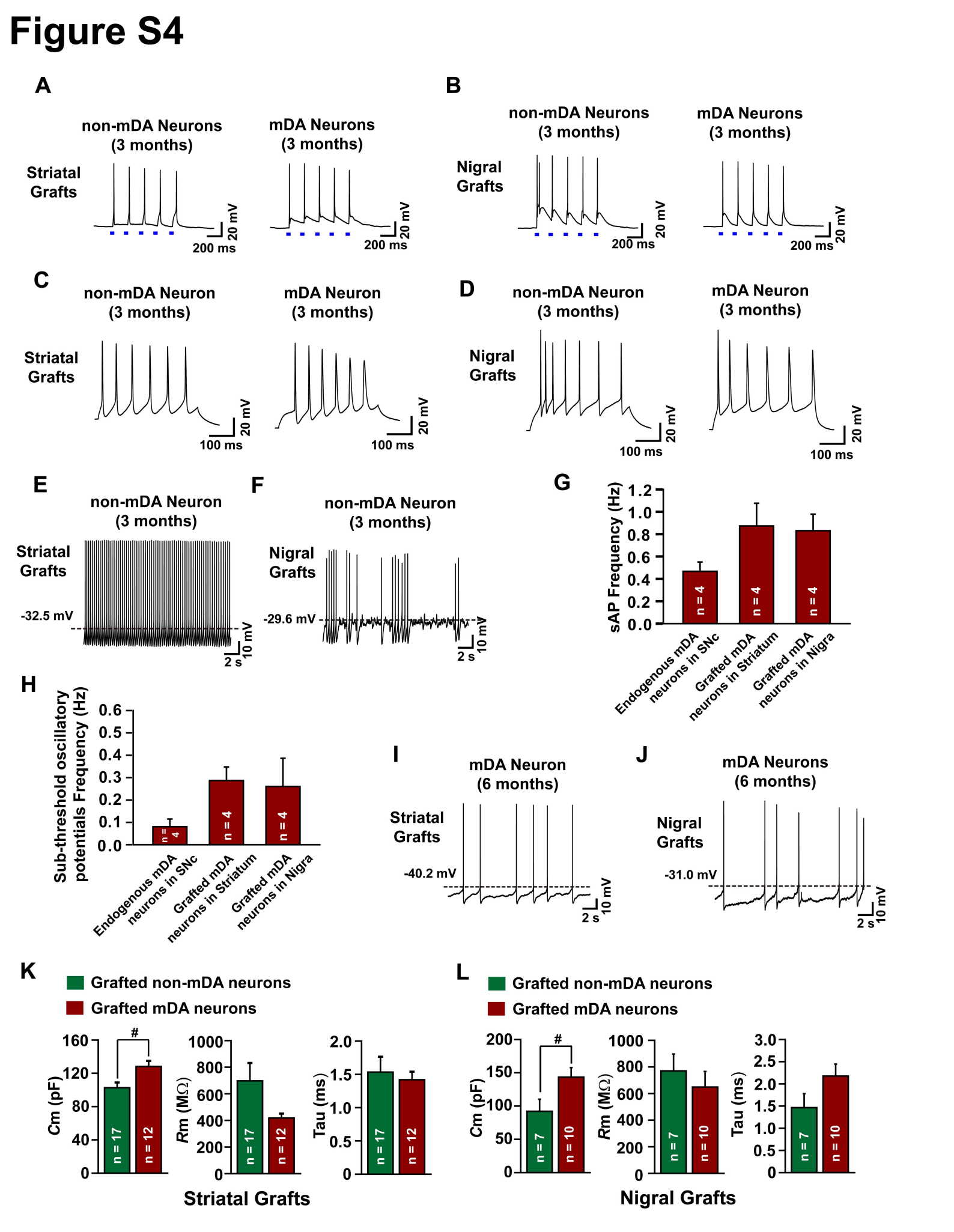
A
non-mDA Neurons
(3 months)
mDA Neurons
(3 months)
Striatal
Grafts
20 mV
200 ms
B
non-mDA Neurons
(3 months)
mDA Neurons
(3 months)
Nigral
Grafts
C
non-mDA Neuron
(3 months)
mDA Neuron
(3 months)
Striatal
Grafts
100 ms
D
Nigral
Grafts
E
non-mDA Neuron
(3 months)
Striatal
Grafts
-32.5 mV
10 mV
2 s
F
Nigral
Grafts
-29.6 mV
G
sAP Frequency (Hz)
n = 4
Endogenous mDA neurons in SNc
Grafted mDA neurons in Striatum
Grafted mDA neurons in Nigra
H
Sub-threshold oscillatory potentials Frequency (Hz)
I
mDA Neuron
(6 months)
Striatal
Grafts
-40.2 mV
J
mDA Neurons
(6 months)
Nigral
Grafts
-31.0 mV
K
Grafted non-mDA neurons
Grafted mDA neurons
Cm (pF)
Rm (MΩ)
Tau (ms)
n = 17
n = 12
Striatal Grafts
L
n = 7
n = 10
Nigral Grafts

Figure S4. Electrophysiological examination of functional maturation of human mDA or non-mDA neurons grafted in nigra or striatum (related to Figure 3).

(A-D) Typical whole-cell path-clamp recording of blue light-induced action potentials (APs) (A and B) or current-induced APs (C and D) in striatally (A and C) or nigrally (B and D) grafted human mDA or non-mDA neurons at 3 months after transplantation. (E and F) Typical whole-cell path-clamp recording of spontaneous action potentials (sAPs) in striatally (E) or nigrally (F) grafted human non-mDA neurons at 3 months after transplantation. The dashed lines indicate threshold potentials. (G and H) The spontaneous action potential frequency (sAP) (G) and the sub-threshold oscillation potentials frequency (H) of endogenous SNc mDA neurons from mDA neuron reporter mice (DAT-Cre/Ai9), or striatally or nigrally grafted human mDA neurons at 3 months after transplantation were plotted. Data are represented as mean ± SEM. The sample number for statistics is indicated in the column. One-way ANOVA, $p > 0.05$. (I and J) Typical whole-cell path-clamp recording of spontaneous action potentials (sAPs) in striatally (I) or nigrally (J) grafted human mDA neurons at 6 months after transplantation. The dashed lines indicate threshold potentials. (K and L) The input resistance (Rm), membrane capacitance (Cm), and Tau of striatally (K) or nigrally (L) grafted human non-mDA and mDA neurons at 6 months after transplantation were plotted. Data are represented as mean ± SEM. The sample number for statistics is indicated in the column. Student-t test, $^{\#}p < 0.05$.

Figure S5

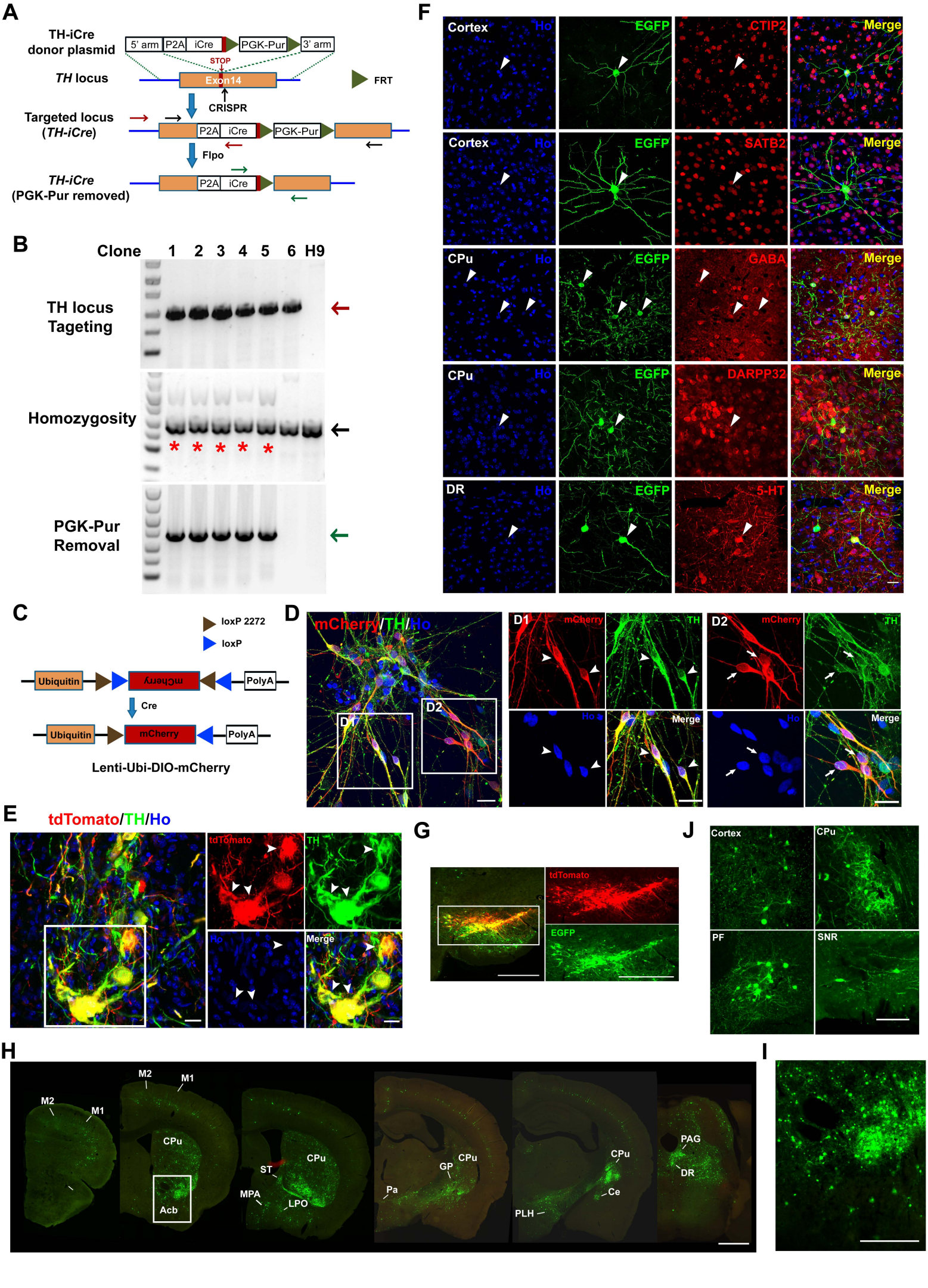
A
TH-iCre donor plasmid
5' arm
P2A
iCre
PGK-Pur
3' arm
STOP
TH locus
Exon14
FRT
CRISPR
Targeted locus (TH-iCre)
Flpo
TH-iCre (PGK-Pur removed)
B
Clone 1 2 3 4 5 6 H9
TH locus Tageting
Homozygosity
PGK-Pur Removal
C
loxP 2272
loxP
Ubiquitin
mCherry
PolyA
Cre
Lenti-Ubi-DIO-mCherry
D
mCherry/TH/Ho
D1
D2
E
tdTomato/TH/Ho
tdTomato
TH
Ho
Merge
F
Cortex
CPu
DR
Ho
EGFP
CTIP2
SATB2
GABA
DARPP32
5-HT
Merge
G
tdTomato
EGFP
J
Cortex
CPu
PF
SNR
H
M2
M1
CPu
Acb
ST
MPA
LPO
GP
Pa
PLH
Ce
PAG
DR
I

Figure S5. Establishment of TH-iCre hESC line and Rabies-mediated tracing of inputs to genetically labeled human and endogenous mDA neurons in mice (related to Figure 4).

(A) Schematic diagram of the genotyping strategy for TH-iCre hESC line. PCR primers for TH locus insertion or homozygosity are indicated by the red arrows and black arrows, respectively. PCR primer for PGK-Pur removal is indicated by the green arrows. (B) PCR genotyping of the TH-iCre hESC line. The expected PCR product for correctly targeted TH locus are ~1000 bp (red arrow). Homozygous clones are identified by the PCR product of ~1000bp (black arrow). Those clones without ~1000 bp PCR products are homozygous. The expected PCR product for PGK removal is ~750 bp (green arrow). The mother cell line (H9 ESCs) is included as control. Heterozygous clones in TH locus with PGK-Pur removal (red asterisks) are selected for experiments. (C) Schematic depiction of lentivirus encoding Cre-dependent expression of mCherry driven by the ubiquitin promoter (Lenti-Ubi-DIO-mCherry). (D) mDA neuron cultures derived from TH-iCre hESCs infected by Lenti-Ubi-DIO-mCherry show mCherry expression in the TH^+ mDA neurons. Boxed areas are magnified right. White arrowheads indicate co-expression of mCherry and TH in mDA neurons. White arrows indicate mCherry expressing neurons with low TH expression. Scale bar = 20 μm. (E) Immunohistochemistry images show co-expression of tdTomato and TH in striatally grafted mDA neurons derived from TH-iCre hESC line infected by AAV-DIO-TVA-2A-NLS-tdTomato 6 months after transplantation. Boxed areas are magnified right. White arrowheads indicate co-expressed neurons. Scale bar = 20 μm. (F) Immunohistochemistry images show $CTIP2^+$ or $SATB2^+$ neurons in cortex, $GABA^+$ or $DARPP32^+$ neurons in CPu, $5\text{-}HT^+$ neuron in DR connected to nigrally grafted human mDA neurons. White arrow heads indicate co-expressed neurons. Ho, Hoechst. Scale bar = 100 μm. (G) Rabies-mediated trans-synaptic tracing of endogenous mDA neurons. Confocal images show EGFP and tdTomato expressing neurons in the SNc of DAT-Cre mice. Scale bar = 0.5 mm. (H) Series of coronal sections show distribution of traced neurons ($EGFP^+/tdTomato^-$) for endogenous mDA neurons in DAT-Cre mice. Only the side ipsilateral to the graft site is shown. Scale bar = 1 mm. Boxed area is magnified in (I). (I) Magnified image shows patch-like distribution of labeled input neurons to endogenous mDA neurons. Scale bar = 0.5 mm. (J) High-magnified images of labeled input neurons to striatally grafted mDA neurons in different host brain regions. Scale

bar = 200 μm. See supplemental Table 1 for abbreviations.

Figure S6

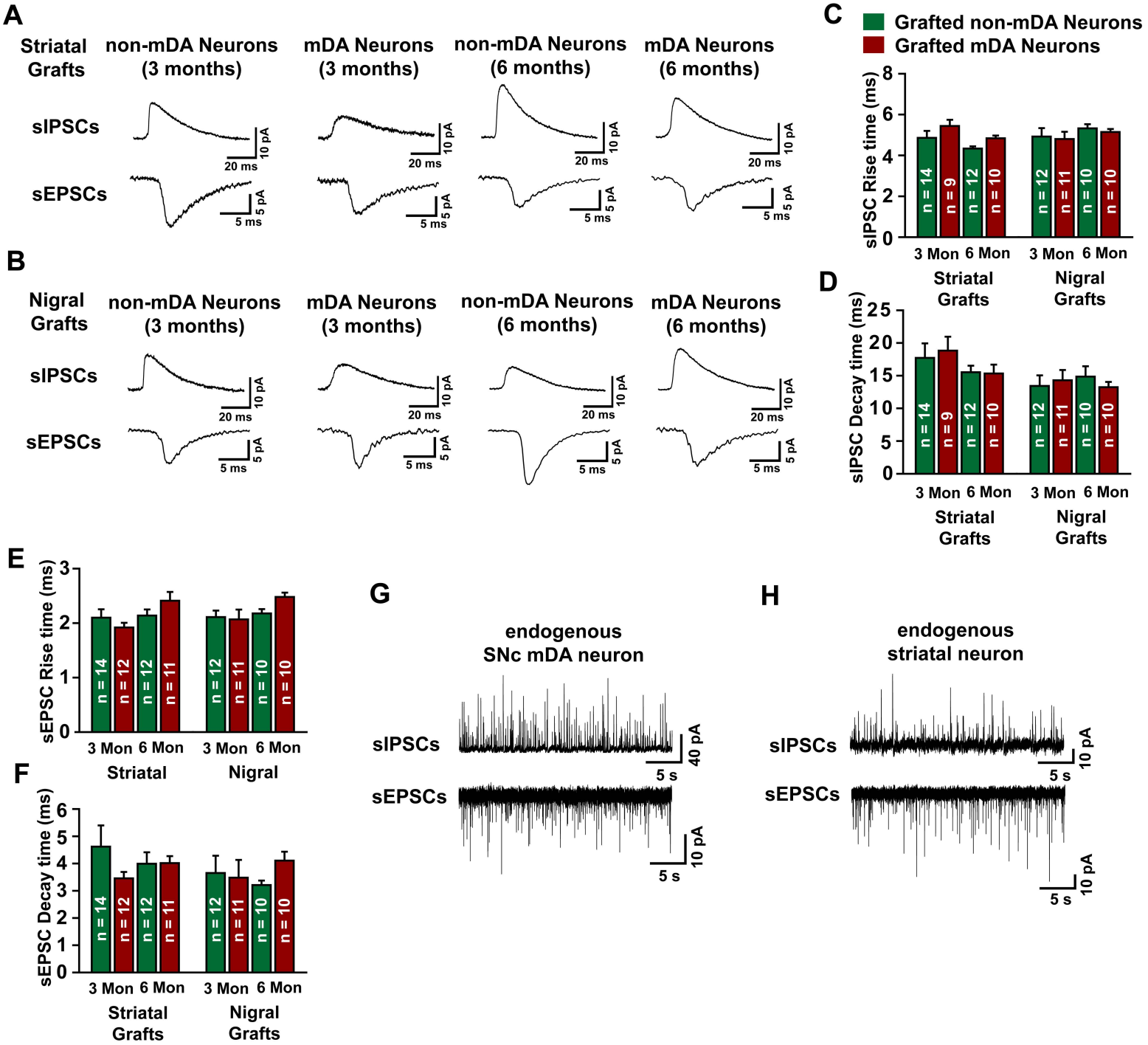
A
Striatal Grafts
non-mDA Neurons (3 months)
mDA Neurons (3 months)
non-mDA Neurons (6 months)
mDA Neurons (6 months)
sIPSCs
sEPSCs
20 ms
10 pA
5 ms
5 pA
B
Nigral Grafts
non-mDA Neurons (3 months)
mDA Neurons (3 months)
non-mDA Neurons (6 months)
mDA Neurons (6 months)
sIPSCs
sEPSCs
C
Grafted non-mDA Neurons
Grafted mDA Neurons
sIPSC Rise time (ms)
n = 14
n = 9
n = 12
n = 10
n = 12
n = 11
n = 10
n = 10
3 Mon
6 Mon
Striatal Grafts
Nigral Grafts
D
sIPSC Decay time (ms)
E
sEPSC Rise time (ms)
F
sEPSC Decay time (ms)
G
endogenous SNc mDA neuron
sIPSCs
sEPSCs
40 pA
10 pA
5 s
H
endogenous striatal neuron
sIPSCs
sEPSCs
10 pA
5 s

Figure S6. Functional inputs to endogenous neurons and the kinetics of sIPSCs and sEPSCs in grafted neurons (related to Figure 5).

(A and B) Representative traces from spontaneous excitatory postsynaptic currents (sEPSCs) and spontaneous inhibitory postsynaptic currents (sIPSCs) in striatal (A) or nigral (B) grafted non-mDA or mDA neurons in the brain slices at 3 or 6 months after transplantation. (C-F) Quantitative analysis of isolated sEPSCs and sIPSCs in A and B. Data are represented as mean ± SEM. The sample number for statistics is indicated in the column. One-way ANOVA followed by Holm-Sidak post hoc test. $p > 0.05$. (G and H) Typical whole-cell path-clamp recording of sEPSCs and sIPSCs in endogenous lateral SNc mDA (G) or striatal neurons (H) of the brain slices from wild type SCID mice.

Figure S7

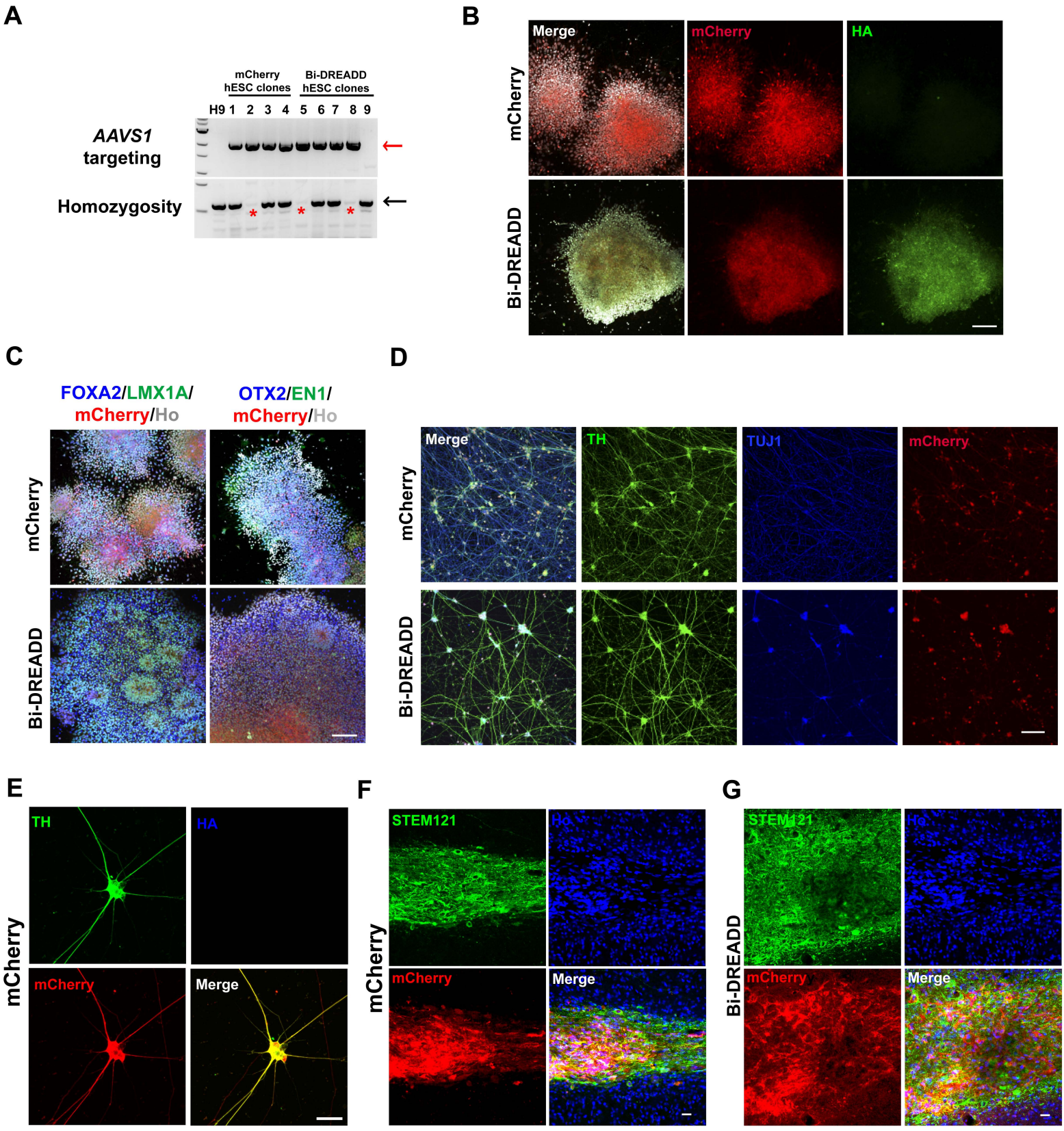
A
mCherry hESC clones
Bi-DREADD hESC clones
H9 1 2 3 4 5 6 7 8 9
AAVS1 targeting
Homozygosity
B
Merge
mCherry
HA
mCherry
Bi-DREADD
C
FOXA2/LMX1A/ mCherry/Ho
OTX2/EN1/ mCherry/Ho
mCherry
Bi-DREADD
D
Merge
TH
TUJ1
mCherry
mCherry
Bi-DREADD
E
TH
HA
mCherry
Merge
mCherry
F
STEM121
Ho
mCherry
Merge
mCherry
G
STEM121
Ho
mCherry
Merge
Bi-DREADD

Figure S7. Establishment and characterization of mCherry- and Bi-DREADD-expressing hESC lines (related to Figure 7).

(A) PCR genotyping of mCherry- or Bi-DREADD-expressing hESC clones. The expected PCR products for correctly targeted *AAVS1* locus are ~2000bp (red arrows). Homozygous clonesare identified by those without PCR products of ~650 bp (black arrow) in homozygosity test, and clones with ~650 bp PCR products are heterozygous. Homozygous clones (red asterisk) are selected for experiments. (B) Immunostaining shows expression of mCherry, hM_3Dq-mcherry or HA-tagged KORD in mCherry or Bi-DREADD hESCs. Scale bar = 50 μm. (C) Immunostaining for mDA neuron progenitor markers at day 16 of differentiation from mCherry or Bi-DREADD hESCs. Ho, Hoechst. Scale bar = 50 μm. (D) Immunostaining of day 42 cultures derived from mCherry- or Bi-DREADD-expressing hESCs shows markers for mDA neurons. Scale bar = 50 μm. (E) Immunostaining shows co-expression of TH, mCherry, but not HA-tagged KORD in mDA neurons at day 42 of differentiation from mCherry hESCs. Scale bar = 50 μm. (F and G) Immunohistochemistry images show that the nigrally grafted mDA neurons derived from mCherry hESCs (F) or Bi-DREADD hESCs (G) co-expressed transgene mCherry or hM_3Dq-mCherry and human STEM121. Scale bar = 20 μm.

Table S1. Abbreviation list of anatomical areas in text and figure legends (related to Figures 1, 2, 4, S1, S2 and S5).

Abbreviation	Anatomical area
Acb	Accumbens nucleus
AcbC	Accumbens nucleus core
AcbSh	Accumbens nucleus shell
AI	Agranular insular cortex
Amy	Amygdala
AOV	Anterior olfactory nucleus, ventral part
APT	Anterior pretectal nucleus
ASt	Amygdala striatal transition area
Ce	Central nucleus of the amygdala
CPu	Caudate putamen
DR	Dorsal raphe nucleus
DTT	Dorsal tenia tecta
EA	Extended amygdala
FrA	Frontal association cortex
G	Geniculate nucleus
GP	Globus pallidus
Hipp	Hippocampus
HT	Hypothalamus
IPAC	Interstitial nucleus of the posterior limb of the anterior
LPO	Lateral preoptic nucleus
M	Motor cortex
M1	Primary motor cortex
M2	Secondary motor cortex
MD	Mediodorsal thalamic nucleus
MFB	Medial forebrain bundle
MnR	Median raphe nucleus
MPA	Medial preoptic area
mRt	Mesencephalic reticular formation
OB	Olfactory bulb

Pa	Paraventricular hypothalamic nucleus
PAG	Periaqueductal gray
PB	Parabrachial nucleus
PF	Parafascicular thalamic nucleus
PLH	Peduncular part of lateral hypothalamus
Pn	Pontine reticular nucleus
PO	Preoptic area
PrCnF	Precuneiform area
SI	Substantia innominata
SN	Substantia nigra
SNC	Substantia nigra, pars compacta
SNR	Substantia nigra, pars reticularis
ST	Bed nucleus of the stria terminalis
STh	Subthalamic nucleus
Tu	Olfactory tubercle
VM	Ventromedial thalamic nucleus
VMH	Ventromedial hypothalamic nucleus
VP	Ventral pallidum
VS	Ventral striatum
ZI	Zona incerta