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Supplemental Information

Human Clinical-Grade Parthenogenetic ESC-Derived Dopaminergic Neurons Recover Locomotive Defects of Nonhuman Primate Models of Parkinson's Disease

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Supplemental Figures

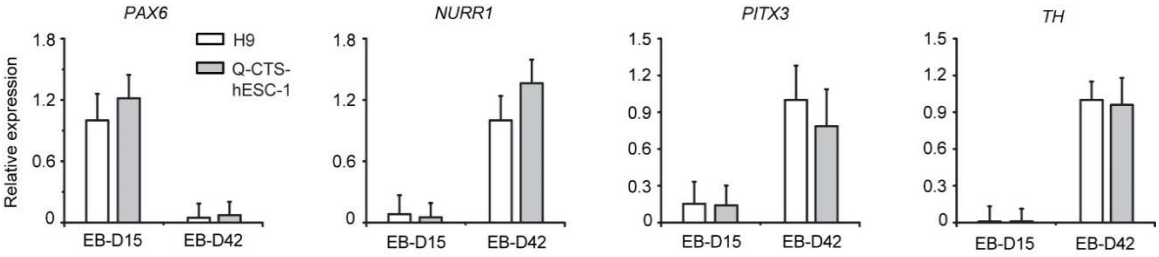
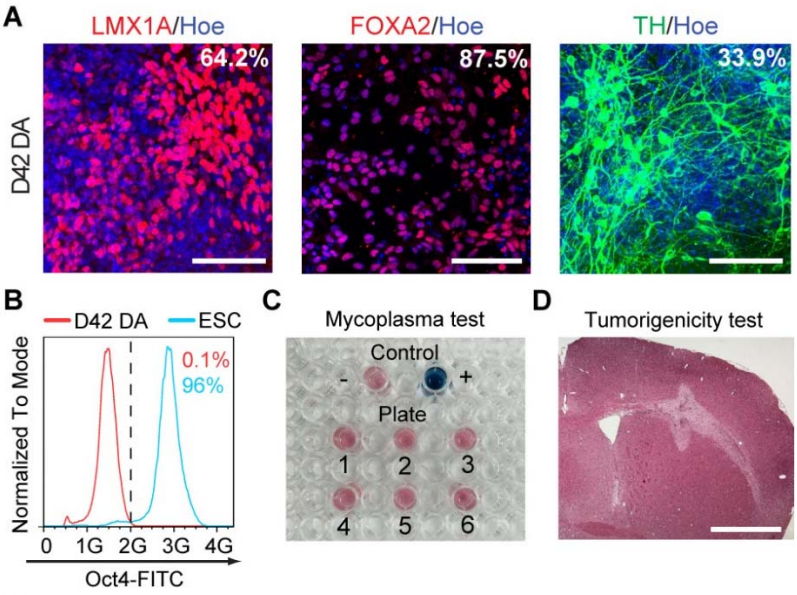


Figure S1. Gene expression analysis for key neural markers at day 15 and day 42 using EB protocol. Related to Figure 1. Error bars indicate SEM (compared to H9, Student's t-test), n = 3 independent repeated experiments.



E

Bacteria and Fungi	Mycoplasma	Endotoxin (EU/mL)	Survival rate	Tumorigenicity
Negtive	Negtive	≤0.5	92%	Negtive 0/8

Figure S2. Quality test of differentiated DA neurons. Related to Figure 1. (A) Immunofluorescence images for midbrain DA neuron markers at day 42 using FP protocol. Bars = 100 μm. (B) FACS analysis of the proportion of Oct4+ cells in DA neurons (shown as red line). ESCs stained with the same antibody were used as a staining control (shown as blue line). (C) The results of mycoplasma test of DA neurons in the all 6 plates. Blue color indicates a positive result and pink color indicates a negative result. (D) HE staining of brain tissue from SCID mice implanted with DA neurons for 5 weeks (n=8). Bars = 2 mm. (E) Summarized results of the biological safety assays of DA neurons.

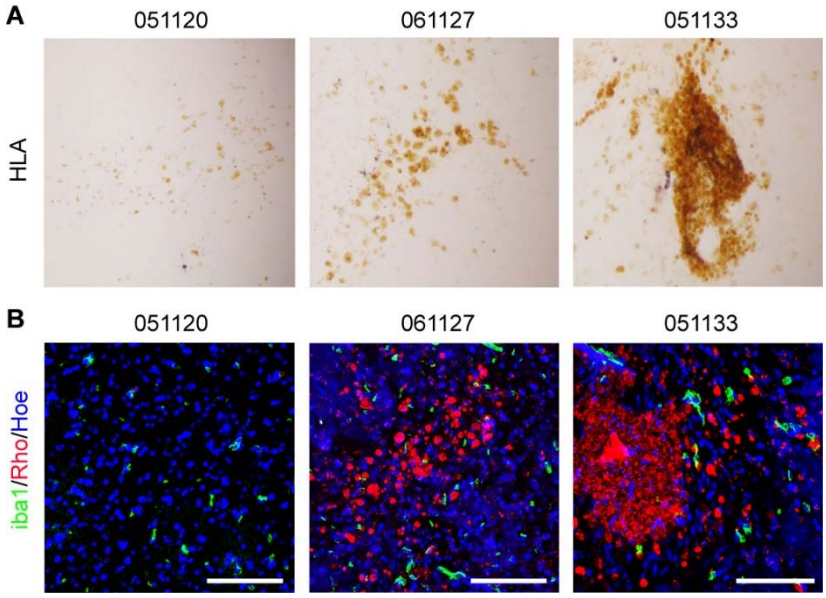


Figure S3. Histological analysis of immune response *in vivo* using DAB-developed HLA-DR staining (A) and Iba1 immunofluorescence staining (B). Related to Figure 3. Bars = 200 μm.

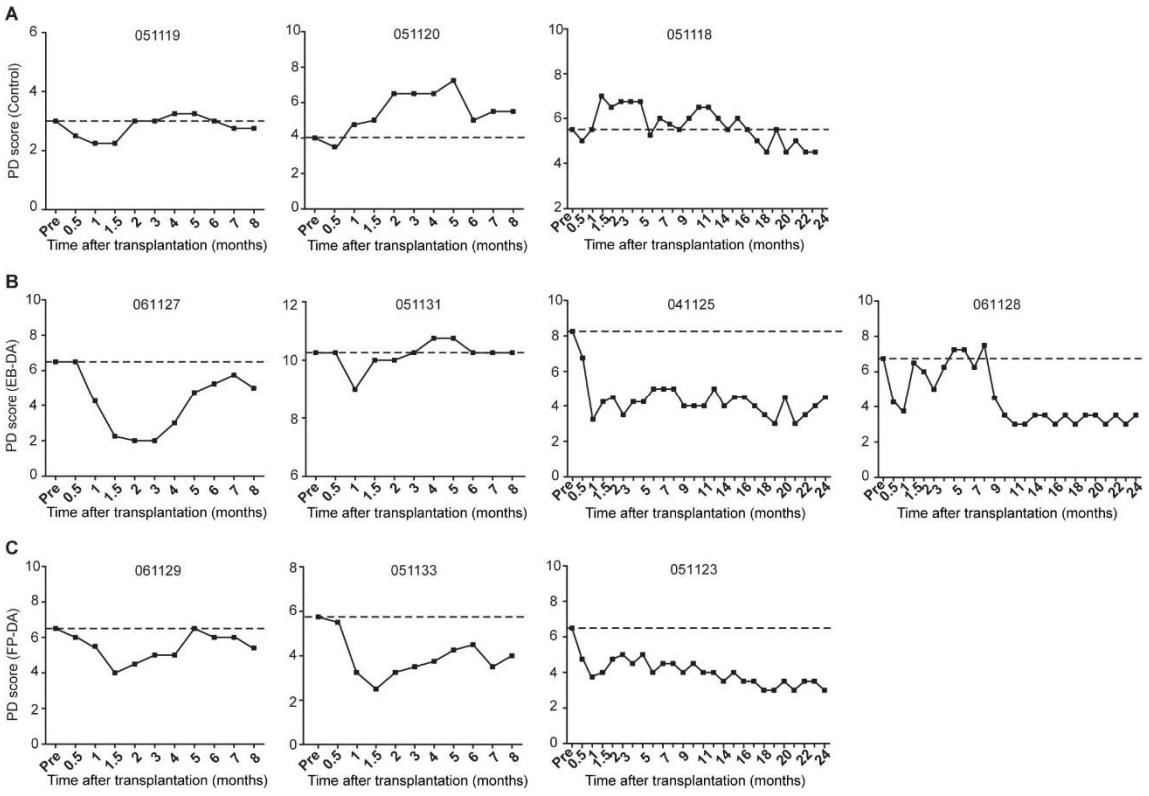


Figure S4. Behavior scores of monkeys in control group (A), EB-DA group (B) and FP-DA group (C). Related to Figure 4. Dotted lines are pre-transplantation baselines, showing the score at the 11th month after MPTP treatment.

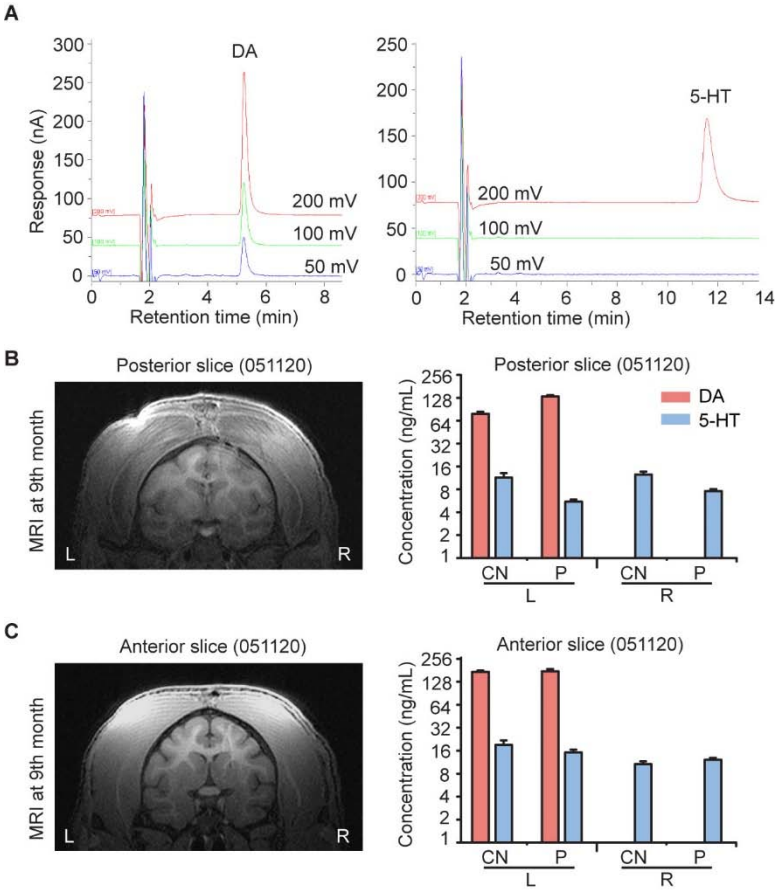


Figure S5. DA was detected in the brain from the control side but not the lesion side in the control monkey. Related to Figure 5. (A**)** HPLC analysis for DA and 5-HT (control). **(**B and C**)** left panels: MRI images of the monkey brain (051120, control) at 9th month. Right panels: Levels of DA in putamen (P) and caudate nucleus (CN). 5-hydroxytryptamine (5-HT) was measured as a control (n = 3 repeated experiments). Data were represented as mean ±SEM (n=3 repeated experiments)

Table S1. Biological Safety and Effectiveness Analysis of the Clinical-grade HPESCs-derived DA Cells[a]. Related to Figure 1.

Sterility and pathogen	**DA cells**
Cell morphology	Small adherent cells in monolayer, showing dendrite-like morphology
Isozyme analysis	Human origin
Short tandem repeats (STRs)	Expressing 16 STR loci, each STR locus has 1-2 alleles. STR data is consistent with its original ESCs.
Bacteria and fungi	Negative
Mycoplasma	Negative
[Exogenous virus test - *in vitro*][b]	
Cell observation#	Negative
Hemadsorption test	Negative
Hemagglutination test	Negative
[Exogenous virus test - *in vivo*]	
Cell inoculation in suckling mice	Survival rate 100%
Cell inoculation in adult mice	Survival rate 100%
Cell inoculation in guinea pigs	Survive, no tuberculosis
Cell inoculation in rabbits	Survive, no abnormality
Survival rate of 5- to 6-day-old chick embryos	Survival rate 80%
Hemagglutination test of 9- to 11-day-old chick embryo allantoic fluid	Negative
[Immune response test][c]	
Lymphocyte proliferation inhibition	Inhibition rate 36.8%
Th1 cells proliferation inhibition	Inhibition rate 45.3%

Th17 cells proliferation inhibition	Inhibition rate 43.9%
Treg cells proliferation promotion	Promotion rate 3.99%
TNF-α secretion inhibition test	Inhibition rate 35.4%
[Biological effectiveness test]	
Tuj1 expression assay by immunofluorescence	Positive, while its original ESCs is negative
EN1 expression assay by immunofluorescence	Positive, while its original ESCs is negative
Tuj1+ proportion assay by FACS	99.8%
Foxa2 expression assay by PCR	Positive, while its original ESCs is negative
Lmx1A expression assay by PCR	Positive, while its original ESCs is negative
GIRK2 expression assay by PCR	Positive, while its original ESCs is negative
DAT expression assay by PCR	Positive, while its original ESCs is negative
Electrophysiology assay by patch-clamp	Exhibiting Na +, K + currents and action potential, while its original ESCs is negative
[Pluripotent cells residuals]	
TRA-1-60+ proportion assay by FACS	0.1%
SSEA-4+ proportion assay by FACS	0.1%
OCT4 expression assay by immunofluorescence	Negative, while its original ESCs is positive
SSEA-4 expression assay by immunofluorescence	Negative, while its original ESCs is positive
TRA-1-60 expression assay by immunofluorescence	Negative, while its original ESCs is positive
TRA-1-81 expression assay by immunofluorescence	Negative, while its original ESCs is positive
OCT4 expression assay by PCR	Negative, while its original ESCs is positive
Nanog expression assay by PCR	Negative, while its original ESCs is positive
Rex1 expression assay by PCR	Negative, while its original ESCs is positive
Teratoma formation in SCID mice	Six weeks after cell inoculation in SCID mice, no teratoma formation was observed,

	while its original ESCs is positive
[Cell activity test]	
Cell survival rate	98.3%
Cells in sub-G1 phase	-
Cells in G0/G1 phase	74.9%
Cells in S phase	16.9%
Cells in G2/M phase	8.2%
[Tumorigenicity test]	
Nude mice inoculated	Negative
Soft agar clone formation assay	Negative
Telomerase activity assay	361 TPG units per 10000 cells
Endotoxin assay	≤0.5 EU/mL
Bovine serum albumin residuals	10.7 ng/mL

[a]This table is translated from NIFDC report (NO. SH201701242).

[b]Human 2BS cells, monkey Vero cells and human MRC-5 cells were used in all the experiments.

[c]The ratio of DA cells to PBMC is 1: 5.

Table S2. Group Information of All the Monkeys. Related to Figure 2.

Group	NO. of monkeys	Time for euthanasia
Control	051119	9 months post graft
	051120	9 months post graft
	051118	N.E.[a]
EB-DA	061127	9 months post graft
	051131	9 months post graft
	041125	N.E.
	061128	N.E.
FP-DA	061129	9 months post graft
	051133	9 months post graft
	051123	N.E.

[a]"N.E." means not be euthanized.

Table S3. Blood Biochemistry Analysis of 6 Monkeys after Cell Transplantation for 9 Months. Related to Figure 2.

No.	ALT (U/L)	AST (U/L)	BUN (mM)	CRE (μM)	LDH (U/L)	NSE (ng/mL)	AFP (ng/mL)	CEA (ng/mL)	Timing
051119	41.2	25	4.96	113.9	173	0.45	0.81	0.67	Pre-MPTP
	55.8	24.4	5.79	125.5	198	0.67	0.69	0.45	Pre-grafting
	52.5	25.9	7.79	108.6	173	0.5	0.76	0.26	Post-grafting
051120	70.8	42.1	6.93	101.1	242	0.41	0.35	0.35	Pre-MPTP
	43.5	28.2	6.39	102.4	203	0.45	0.39	0.52	Pre-grafting
	41.8	33.9	6.68	112.2	214	0.78	0.42	0.76	Post-grafting
061127	44.2	34.1	9.25	136.6	247	0.24	4	0.16	Pre-MPTP
	108.2	40.1	5.67	128.6	233	0.3	5.64	0.18	Pre-grafting
	47.5	37.7	8.8	120.6	246	0.15	3.85	0.27	Post-grafting
061129	40.8	17.6	6.79	104.6	192	0.43	0.78	0.33	Pre-MPTP
	48.2	22	4.84	125	198	0.46	0.83	0.46	Pre-grafting
	44.8	39.8	4.73	98.4	261	0.37	0.4	0.57	Post-grafting
051131	86.8	45.7	13.27	172	243	0.76	5.15	0.63	Pre-MPTP
	43.2	25.6	6.19	143.2	147	0.15	2.58	0.36	Pre-grafting
	55.5	47.7	7.05	139.2	160	0.26	2.55	0.16	Post-grafting
051133	51.5	27.9	8.08	124.1	214	0.21	1.55	0.21	Pre-MPTP
	32.2	41.8	3.41	115.7	309	0.38	1.48	0.32	Pre-grafting
	62.5	50.4	5.33	102	257	0.88	1.1	0.46	Post-grafting
Range	5-40	5-40	2.88-7.2	44-133	40-120	<15	<10	<5	

ALT: alanine aminotransferase; AST: aspartate aminotransferase; BUN: blood urea nitrogen; CRE: creatinine; LDH: lactate dehydrogenase; NSE: neuron specific enolase; AFP: α-fetoprotein; CEA: carcinoembryonic antigen.

Supplemental Experimental Procedures

Quantitative PCR Analysis

RNA was extacted from cells using TRIZOL (Invitrogen), cDNA was synthesized with oligo (dT) primers (Promega) and the SuperScript III Reverse Transcriptase kit (Invitrogen) according to manufacturer's protocols. PCR was performed with ExTaq (Takara), and qPCR was performed with SYBR Green (TOYOBO), with primer sets as described previously.

Immunofluorescence

Adherent cells on glass coverslips were rinsed with PBS (phosphate-buffered saline) for 3 times, then fixed with 4% paraformaldehyde for 30 min. Cells were treated with 0.3% Triton X-100 and 10% normal donkey serum for 60 min before they were incubated at 4ºC overnight in primary antibodies: Sox1 (R&D, AF3369, 1:2000), PAX6 (Covance, PRB-278P, 1:400), OTX2 (Proteintech, 13497-1-ap, 1:1000), TH (Millipore, AB152, 1:400; immunostar, 22941, 1:2000), TUJ1 (Covance, MMS-435P, 1:1000), NCAM (Santa-Cruz, SC-106, 1:100), FOXA2 (R&D, AF2400, 1:50), LMX1A (Millipore, ab10533, 1:2000), Girk2 (Alomone labs, APC-006-25, 1:300), HLA-DR (BD, 347360, 1:200), Iba1 (Wako, 019-19741, 1:400). Secondary fluorescent antibodies purchased from Jackson ImmunoResearch (Cy3-AffiniPure Donkey Anti-Mouse IgG (H+L), 715-165-151; Alexa Fluor 488 AffiniPure Donkey Anti-Rabbit IgG (H+L), 711-545-152; Cy3-AffiniPure Donkey Anti-Goat IgG (H+L), 705-165-147; Alexa Fluor 488 AffiniPure Donkey Anti-Goat IgG (H+L), 705-545-147) were used at 1:500 for 1 h at room temperature. Coverslips were mounted and visualized with Zeiss 710 fluorescent scope. Negative controls without primary antibody were performed in all experiments to monitor nonspecific staining.

Electrophysiological Recordings

Whole-cell patch-clamp recordings in either voltage- or current-clamp mode were conducted to measure the voltage-activated sodium/potassium currents or action potentials, which were recorded using an Axopatch 200B or MultiClamp 700A amplifier (Molecular Devices). The electric signals were filtered at 2-10 kHz, digitized at 20-100 kHz (Digidata 1322A; Molecular Devices) and further analyzed using pClamp version 9.2 software (Molecular Devices). The intracellular fluid contained (in mM) 130 K^+-gluconate, 20 KCl, 10 HEPES, 0.2 EGTA, 4 Mg_2ATP, 0.3 Na_2GTP, and 10 Na^+-phosphocreatine (at pH 7.3, 310 mOsm), and the pipette ranged from 2.0-4.0 MΩ. The extracellular fluid consisted of (in mM) 124 NaCl, 3.3 KCl, 2.4 $MgSO_4$, 1.2 KH_2PO_4, 26 $NaHCO_3$, 2.5 $CaCl_2$ and 10 glucose (pH 7.4, 310 mOsm). The transmitter receptor blocker TTX (1 μM) were used in the bath solution for the detection of action potentials and spontaneous action potentials.

Blood Collection and Examination

Veinal blood samples were taken from each monkey's lower limb. Blood serum samples were collected after blood clotting and centrifugation (4000 rpm, 5 min) and preserved in -80ºC refrigerator for a variety of biochemical examination. Blood examination of all the monkeys were performed pre-MPTP lesion, pre-grafting and 9 m post-grafting. The examination items contained alanine aminotransferase (ALT), aspartate aminotransferase (AST), blood urea nitrogen (BUN), creatinine (CRE), lactate dehydrogenase (LDH), neuron specific enolase (NSE), α-fetoprotein (AFP), carcinoembryonic antigen (CEA).

Cell Counts

To estimate the number of survival cells within the graft, the hNCAM+ cells co-labeled with Rhodamine or Hoechst were counted with hNCAM immunostained sections using Imaris Image Analysis Software (ver. 9.0, Bitplane). TH+ cells within the graft core were counted with TH-DAB immunohistochemistry images. The quantification was performed every 8-10th section where the graft was identifiable.

Mycoplasma and Endotoxin Test

Mycoplasma test and Endotoxin test was performed using PlasmoTest™ Reagent Kit (Invivogen) and ToxinSensor™ Gel Clot Endotoxin Assay Kit (Genscript), respectively, according to manufacturer's protocols.