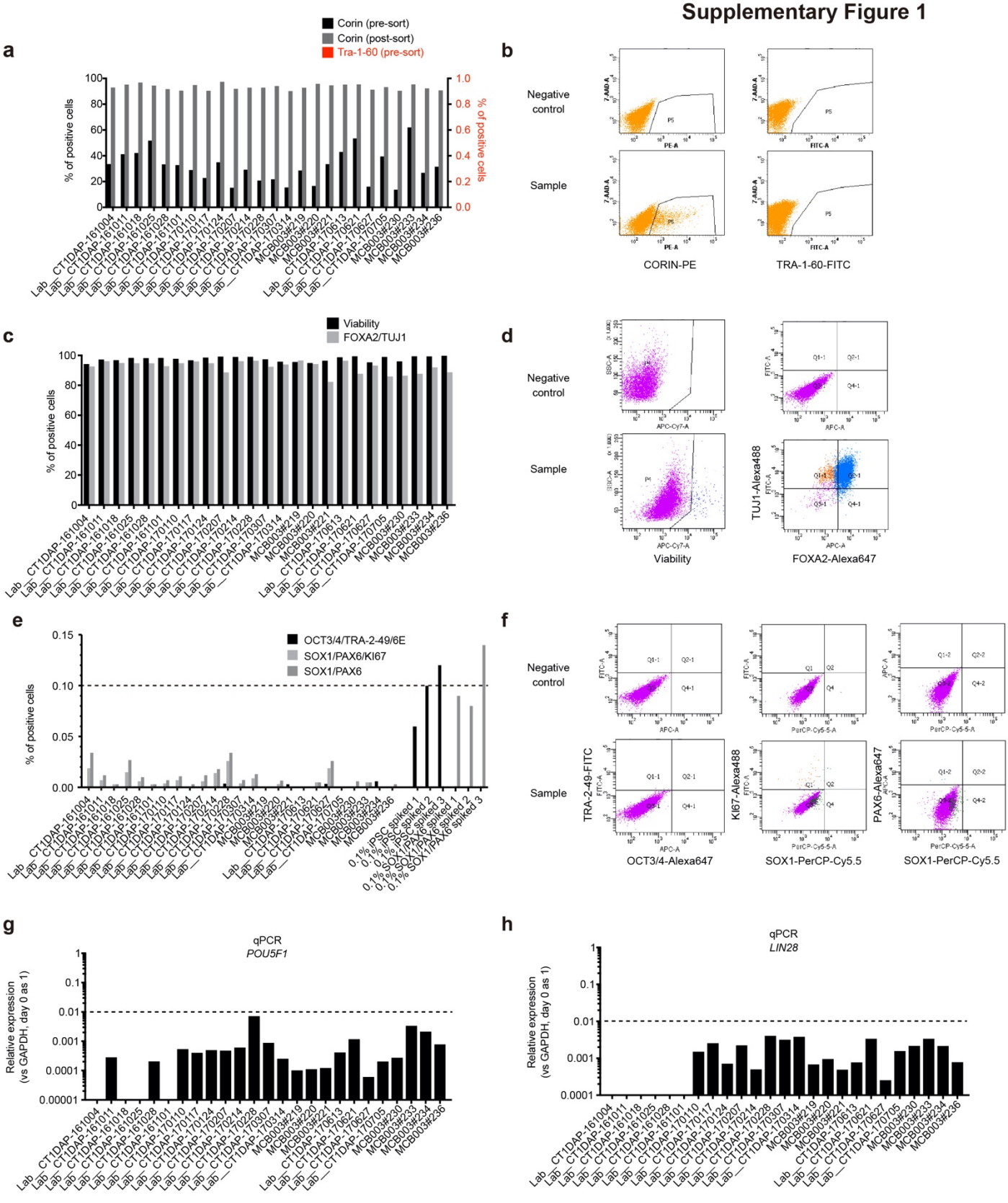
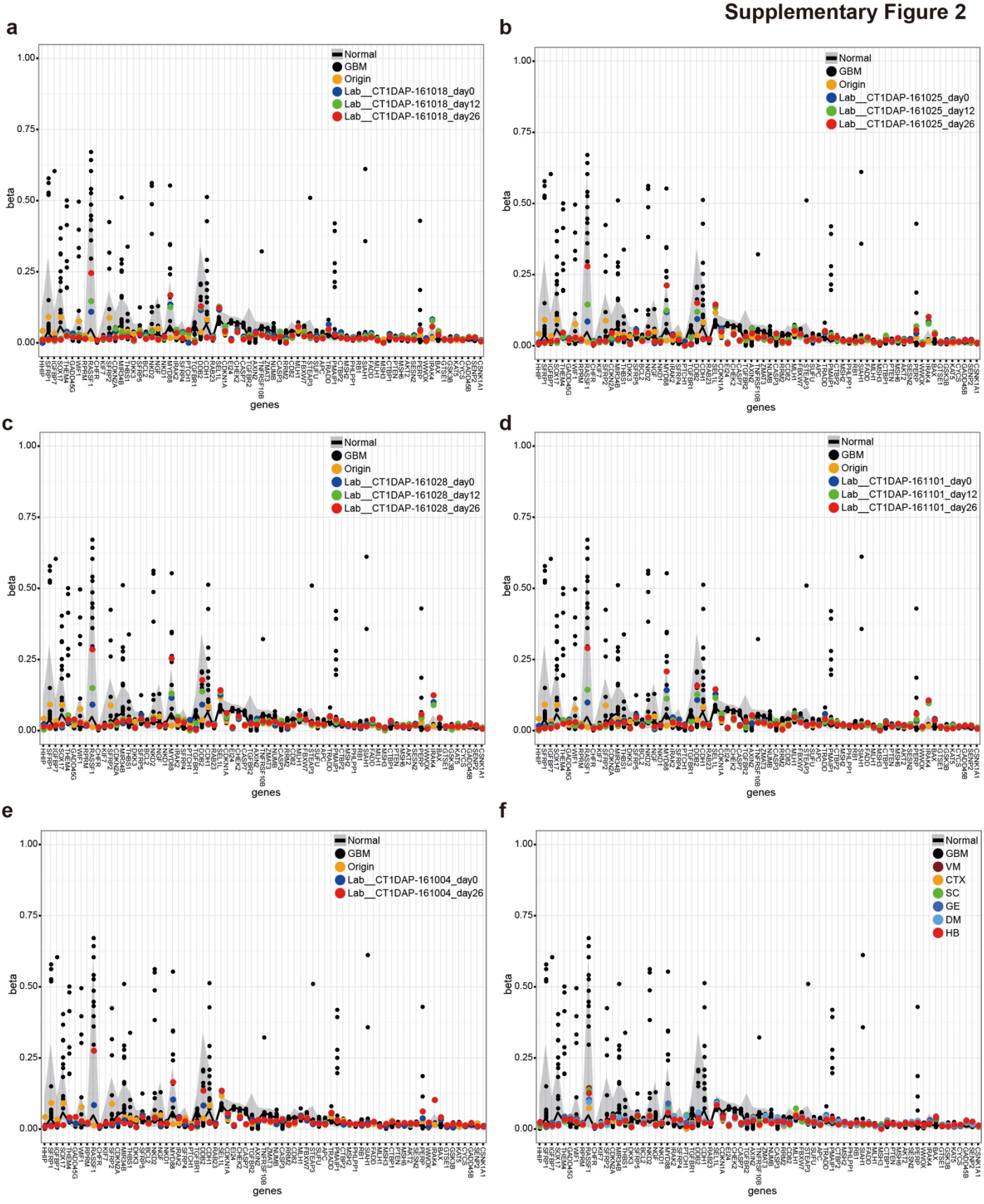
Supplementary Figure 1



Supplementary Figure 1

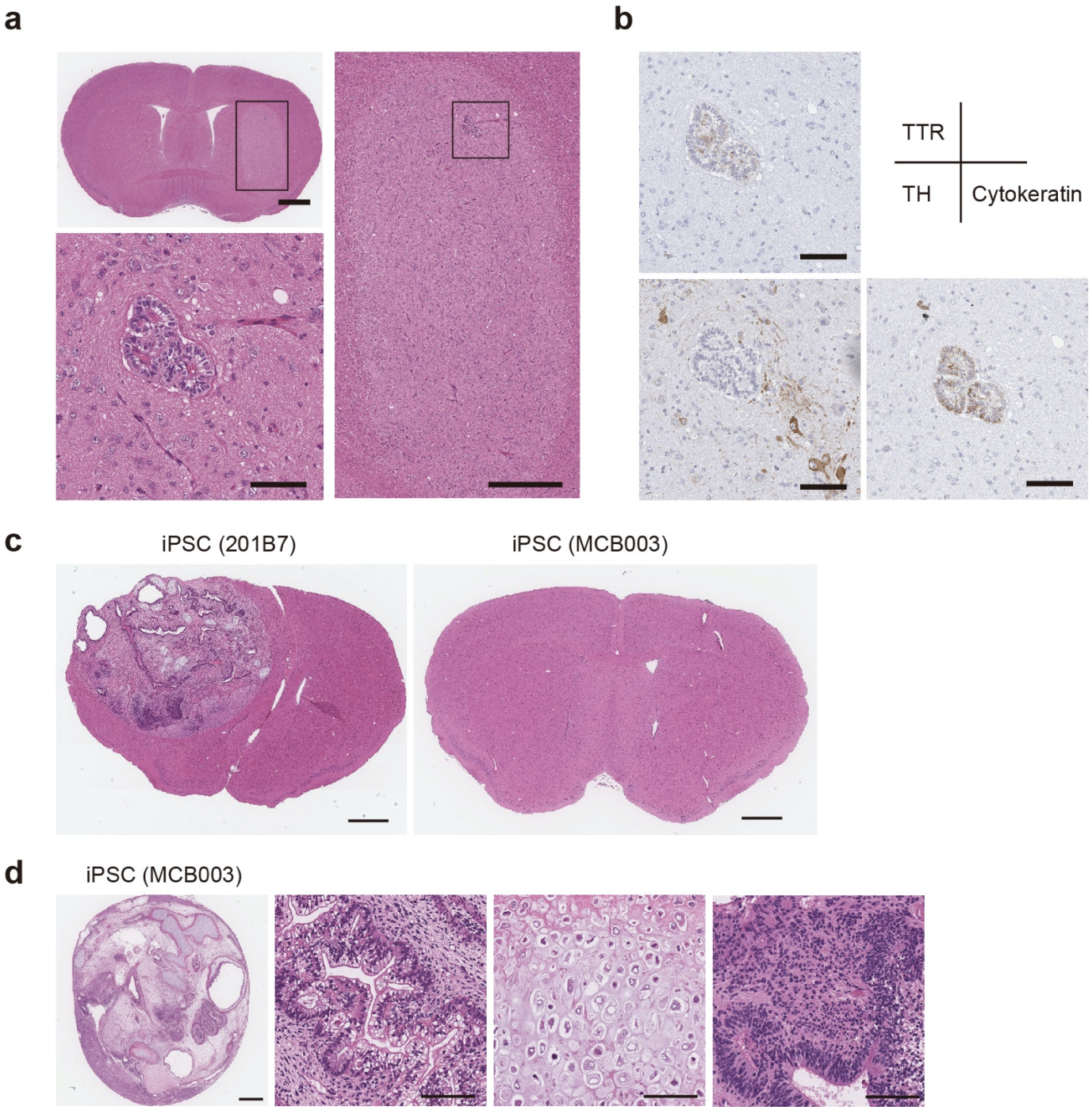
a) Flowcytometry results of CORIN positive (left Y axis, pre-and post-sorting) cells and TRA-1-60 positive (right Y axis) cells in 25 lots of DAPs on culture day 12. b) Representative dot plot images exemplifying the gating strategy of flowcytometry, corresponding to Supplementary Figure 1a. The positive gate was set so that less than 0.1% of cells were positive in negative control sample. c) Flowcytometry results of cell viability and FOXA2 and TUJ1 (efficacy markers) positive cells in 25 lots of DAPs on culture day 26. d) Representative dot plot images exemplifying the gating strategy of flowcytometry, corresponding to Supplementary Figure 1c. The positive gate was set so that less than 0.1% of cells were positive in negative control sample. e) Flowcytometry results of OCT3/4, TRA-2-49/6E, SOX1, PAX6, and KI67 (safety marker) positive cells 25 lots in DAPs on culture day 26. f) Representative dot plot images exemplifying the gating strategy of flowcytometry, corresponding to Supplementary Figure 1e. The positive gate was set so that less than 0.1% of cells were positive in negative control sample. g, h) Gene expressions of *POU5F1* (g) and *LIN28* (h) by RT-qPCR analysis in 25 lots of DAPs on culture day 26. The expression level of undifferentiated iPSCs was set to 1. i) In vitro detection of residual undifferentiated iPSCs in iPSC maintenance condition. Several spiking iPSCs were mixed with dissociated DAPs and cultivated for 14 days. Colonies of undifferentiated iPSCs were stained by alkaline phosphatase. Cells in 100% iPSC condition formed too many colonies, resulting in spontaneous differentiation. Bars=1 cm (n=3 independent experiments).



Supplementary Figure 2

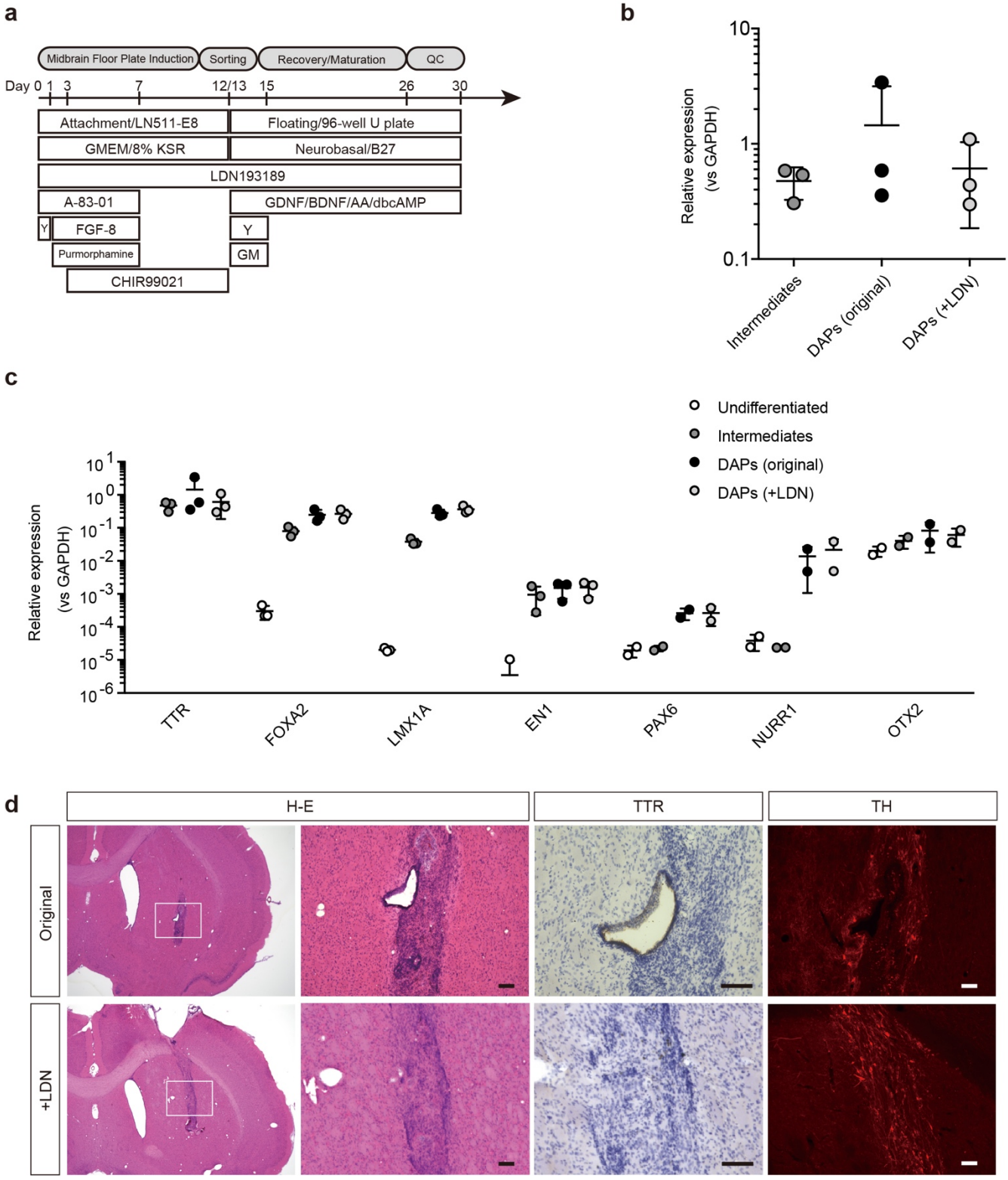
(a-f) Results of the methylation array analysis of 73 cancer-related genes in 5 samples used in the tumorigenicity study (a-e) and human fetal tissue (f) as a control. GBM, glioblastoma multiforme; VM, ventral mesencephalon; CTX, cortex; SC, spinal cord; GE, ganglionic eminence; DM, dorsal mesencephalon; HB, hindbrain.

Supplementary Figure 3



Supplementary Figure 3

a) Images of H-E staining of epithelium-like cell clusters found in the brain of NOG mice. Right is a magnification of upper left, and lower left is a magnification of right. Bars: left upper=1 mm, left lower=100 µm, and right=500 µm. b) Immunohistochemistry of the cell clusters. TTR, transthyretin; TH, tyrosine hydroxylase. Bars=100 µm. a-b) n=6 independent experiments. c) H-E staining of mouse brains injected with undifferentiated 201B7 (number of cell preparations=2 and number of animals=6) and MCB003 (number of cell preparations=2 and number of animals=7). Bars=1 mm. d) H-E staining of testis of NOG mice injected with undifferentiated MCB003 (number of cell preparations=1 and number of animals=6). Bar in leftmost panel=1 mm, others=100 µm.



Supplementary Figure 4

a) The modified culture protocol (+LDN). b) RT-qPCR results of the expression of TTR in intermediates (day 12) and the final product (DAPs) in the original and modified culture protocols. Values are the mean±SD (n=3 independent experiments). c) Gene expression patterns of dopaminergic neuron-related genes by RT-qPCR. Values are the mean±SD (n=3 independent experiments). d) H-E staining and immunohistochemistry of cells injected into the brain of a nude rat with the original (number of animals=7) or modified culture protocol (number of animals=6). Left images are magnified in the other images. Bars=100 μm. Number of cell

preparations=3.

Supplementary Table 1. Modifications between laboratory-grade and GMP-grade cell production

	Laboratory Use (ref. 8)	**Pre-clinical / Clinical trial**
Sorting Buffer	PBS (-) or HBSS (-)	PBS (-)
	2% FBS	2% FBS, gamma-ray irradiated
	50 μg/mL Penicillin/Streptomycin	80 μg/mL Gentamycin
Cell Dissociation Enzyme	1×Accumax	0.5×TrypLE Select (with 0.5 mM EDTA/PBS (-))
Dead Cell Exclusion	7-AAD	None
Anti-CORIN Antibody	Gift from KAN Research Institute	Phycoerythrin-conjugated antibody (Sumitomo Dainippon Pharma)
Negative control	Stained with 2nd antibody	Unstained sample or stained with PE-isotype control
Differentiation media	KSR	Gamma-ray irradiated KSR
	Neurobasal/B27 w/o vitamin A	Neurobasal/B27 w/o vitamin A (gamma-ray irradiated B27)
	2 mM L-glutamate	2 mM Glutamax
	50 μg/mL Penicillin/Streptomycin	80 μg/mL Gentamycin
Replating plate	Lipidure-coated 96-well plate (U-shaped)	PrimeSurface 96U plate (Sumitomo Bakelite)

Supplementary Table 2. Characterization of the human iPSC master cell bank (MCB003)

Test Item	**Test Method**	**Criteria**	**Results**
Morphology	Microscopy observation	Human iPSC-like morphology	Complied
Cell count	Cell count	For information only	3.1×10^6 cells/tube
Viability	Cell count	For information only	93%
Growth Rate-Doubling	Cell count	For information only	14.6 hours
Undifferentiated markers	Flowcytometry	TRA-1-60 ≥ 90 %	97.6 %
		TRA-2-49 ≥ 90 %	100 %
		SSEA4 ≥ 90 %	100 %
Sterility	JP17[a] (direct method)	Complies	Complied
Mycoplasma	JP17 (nucleic acid amplification test)	Negative	Negative
Endotoxin	JP17 (LAL[b] turbidimetry test)	< 24 EU/mL	< 0.05 EU/mL
Karyotype	G-band	Normal	Normal
Viral testing	ICH Q5A	Negative	Complied

[a]The Japanese Pharmacopoeia Seventeenth Edition

[b]Limulous amoebocyte lysate

Supplementary Table 3. In-process testing for the manufacturing of DAPs

Process		**Test Items**	**Test Method**	**Criteria**
Prepared reagents (In-process test 1–6)		Sterility	Membrane filter	Negative
Expand iPSCs (In-process test 1–3)		Cell number	Cell counter	Required cell number
		Morphology	Microscopy	Colony formation
		Sterility	BacT/ALERT (Culture supernatant)	Negative
Differentiation	In-process test 5	Cell number	Cell counter	Required cell number
	In-process test 4–6	Morphology	Microscopy	Attached to the plate (test 3–5) Sphere formation (test 6)
		Sterility	BacT/ALERT	Negative
	In-process test 4, 6	Mycoplasma	qPCR	Negative
	In-process test 6	Endotoxin	LAL[a] turbidimetry test	≤ 1 EU/mL
	(In-process test 5, pre-sort)	Residual undifferentiated iPSCs	Flowcytometry: TRA-1-60	< 1%
	(In-process test 5, post-sort)	Purity of sorted cells	Flowcytometry: CORIN	> 90%

[a] Limulus Amebocyte Lysate

Supplementary Table 4. Shibata's gene list proposed by the PMDA

ABL1	CBFA2T3	ERCC4	GATA1	MEN1	NUP214	SH3GL1
ABL2	CBLB	ERCC5	GATA3	MET	NUP98	SMAD4
ACVR1B	CBLC	ERCC6	GNA11	MITF	PALB2	SMARCA4
AFF3	CCND1	ETV4	GNAQ	MLH1	PAX8	SMARCB1
AKAP9	CCND2	ETV6	GNAS	MLH3	PBRM1	SMO
AKT1	CCND3	EVI1	GOLGA5	MLL	PDE4DIP	SOCS1
AKT2	CDC73	EWSR1	GOPC	MLL2	PDGFB	SRGAP3
ALK	CDH1	EXT1	GPC3	MLL3	PDGFRA	SRSF2
APC	CDH11	EXT2	H3F3A	MLLT3	PDGFRB	SS18
ARHGEF12	CDK6	EZH2	HMGA1	MPL	PIK3CA	STAT3
ARID1A	CDKN2A	FAM123B	HMGA2	MSH2	PIK3R1	STK11
ARID2	CDKN2C	FANCA	HNF1A	MSH6	PIM1	SUFU
ASXL1	CDX2	FANCB	HRAS	MUTYH	PLAG1	SUZ12
ATF1	CEBPA	FANCC	IDH1	MYB	PML	SYK
ATM	CHEK1	FANCD2	IDH2	MYC	PMS2	TCF3
ATR	CHEK2	FANCE	IKZF1	MYCL1	POLE	TCL1A
ATRX	CIC	FANCF	IL2	MYCN	POLH	TET2
AXIN1	COL1A1	FANCG	IL7R	MYD88	PPARG	TFG
AXIN2	CREB1	FANCI	IRF4	MYST3	PPP2R1A	TLX1
BAP1	CREBBP	FANCJ	JAK2	NCOA2	PRKAR1A	TNFAIP3
BCL11A	CTNNB1	FANCL	JUN	NCOA4	PTCH1	TP53
BCL11B	CYLD	FANCM	KDM5C	NF1	PTEN	TPR
BCL2	DAXX	FANCP	KDM6A	NF2	PTPN11	TSC1
BCL3	DDB2	FBXW7	KDR	NFE2L2	RAD51C	TSC2
BCL6	DDIT3	FEV	KIT	NFKB2	RAF1	TSHR
BCOR	DDX5	FGFR1	KRAS	NIN	RB1	USP6
BCR	DDX6	FGFR1OP	LCK	NONO	REL	VHL
BHD	DEK	FGFR2	LMO2	NOTCH1	RET	WRN
BLM	DICER	FGFR3	MAF	NOTCH2	RNF213	WT1
BMPR1A	DNMT3A	FH	MAFB	NPM1	ROS1	XPA
BRAF	EGFR	FLCN	MAML2	NR4A3	RUNX1	XPC
BRCA1	ELK4	FLT3	MAP2K4	NRAS	SDHB	ZNF521
BRCA2	EP300	FOXL2	MDM2	NSD1	SDHD	
CARD11	ERBB2	FOXP1	MDM4	NTRK1	SETD2	
CARS	ERCC3	FUS	MED12	NTRK3	SF3B1	

Supplementary Table 5. The results of the amplicon sequencing

							Origin (PBMNC*)		day0		day12		day26	
Preparation	Chr**	Start	End	Ref	Alt	Gene name	coverage	alt_ratio***	coverage	alt_ratio	coverage	alt_ratio	coverage	alt_ratio
CT1DAP-161025	chr2	215657150	215657150	T	A	BARD1	69	13.00%	71	14.10%	68	20.60%	88	9.10%
CT1DAP-161028	chr3	195512734	195512734	T	G	MUC4	35	17.10%	29	24.10%	29	3.50%	28	14.30%
CT1DAP-161101	chr3	48719156	48719156	C	T	NCKIPSD	9911	0.10%	19609	0.20%	19451	0.10%	12491	0.10%

*Peripheral blood mononuclear cells

**Chromosome

***alteration ratio

Supplementary Table 6. Primer list of the amplicon sequencing

Gene_ID	sequence_l	pos_l	sequence_r	pos_r	size
BARD1	CCGGCTTGGACAACATAGAGAGACTC	215656838	GGAATTAAATTCTGCTGAATGGGTTGC	215657225	388
MUC4	GTGTCACCTGTGGATGCTGAGGAAA	195512650	GCATCTTCAGGTCACACCACCTCTC	195512906	257
NCKIPSD	CTTCTTCTTCTGCAGGGGGTTCAGG	48718986	TGGGCTGTGAAGGGTATGGTTTTCC	48719278	293

Supplementary Table 7. Animal numbers used in the tumorigenicity study

Differentiation number	**Animal number**			
	Control group		Sample group	
	Male	Female	Male	Female
CT1DAP-161004	5	5	2	2
CT1DAP-161011	5	5	12	12
CT1DAP-161018	5	5	7	7
CT1DAP-161025	5	5	8	8
CT1DAP-161028	5	5	6	6
CT1DAP-161101	0	0	5	5

Supplementary Table 8. Summary of pre-clinical *in vivo* studies of human iPSC-derived dopaminergic progenitors

Study Aim	**Host, Administration route**	**Duration**	**Number of animals**	**Number of cell preparation**	**Cells/ animal**	**Evaluation**	**Findings**
Tumorigenicity Biodistribution Toxicology	NOG mouse, unlesioned, Striatum	Life-long (52 weeks)	N=80; DAPs	6	2×10^5	Mortality/Morbidity, General health, Neurobehavioral tests, Hematology/Blood chemistry, Urinalysis, Necropsy, Histopathology	No effect on mortality/morbidity, animal observations or pathology. No graft overgrowth. Asymptomatic ectopic structures (choroid plexus epithelium-like cells) observed within graft. Little proliferative capacity (scattered Ki67+ cells, negative in ectopic structures).
			n=50; Control (Saline)				
Detection of residual iPSCs	NOG mouse, unlesioned, Subcutaneous with Matrigel	26 weeks	n=20; DAPs	7	6×10^5	Teratoma formation Tumor size Histology	No teratoma formation in every DAP group. Teratoma formation was observed in the positive control group (other iPS cell line and HeLa cells).
			n=10; 100% iPSCs				
			n=50; spiked with 10%~0.001% iPSCs				
			n=10; iPSC (201B7)				
			n=10; HeLa cells				
			n=10; Control (Matrigel)				
Efficacy	Nude rat, 6-OHDA lesioned, Striatum	20 weeks	n=8; DAPs	2	4×10^5	Behavioral test Histology	Methamphetamine-induced rotational behavior improved 16 weeks after transplantation. Human-derived dopaminergic neurons survived.
			n=6; Control (Saline)				
	Cynomolgus monkey, MPTP-lesioned, Putamen	8-24 weeks	n=3; DAPs	2	$1.5–2.0 \times 10^6$	Radiology (MRI) Histology	Grafts were detected in MRI images. Human-derived dopaminergic neurons survived. No ectopic structures of dopaminergic neurons survived in the graft.

Supplementary Table 9. 2 × 2 matrix for quality control of cellular products

	SAFETY		**EFFICACY**	
CELLULAR COMPONENTS	Unwanted cells	Undifferentiated iPSCs Early neural stem cells Transformed cells	Active cells	DA progenitor cells
NON-CELLULAR COMPONENTS	Process-derived impurities	Animal-derived materials Anti-CORIN antibody	Active ingredients	Dopamine
	Adventitious microbial agents	Bacteria Mycoplasma Endotoxin Virus		

Supplementary Table 10. PMDA requirements and solutions in this study

Issues		PMDA requirement	Solutions
Quality of cell product	Reagents	Meet Japanese standards of biological raw materials	Complied with requirement
	Residual iPSCs in final product	Assess the result of tumorigenicity study	Set criteria as OCT3/4/TRA-2-49/6E: <0.1% in flowcytometry and confirm no tumor formation
	Residual immature neural cells	Sox1/PAX6 positive cells should be evaluated	Set criteria as SOX1/PAX6: <0.1% in flowcytometry and confirm no tumor formation
	Residual vectors	To be confirmed in iPSCs	Confirm no residual plasmids in iPSCs and final product
	Genome/epigenome abnormality	No further request	WGS, SNP array / Methylation array
	Non-cell impurities	<120 μg/day (ICH M7)	Complied with requirement
Tumorigenicity/ Toxicity/ Biodistribution	Host species	NOG mice	NOG mouse / MPTP monkey
	Animal number per group	At least 10 animals for histological evaluation	Started 40 animals per group
	Observational period	Life-long (as long as possible)	Life-long (52 weeks after transplantation)
	Injection site	Same as clinical cases	Brain (striatum)
	Teratoma formation	Subcutaneous space of NOG mice, with Matrigel	Subcutaneous injection with Matrigel Testis injection
Efficacy study		No further request	6-OHDA nude rat / MPTP monkey

Supplementary Table 11. Assessment of viruses in the iPSC-derived master cell bank

Test Items	**Test Method**	**Results**
Infectivity	Co-culture with HEK293	Retrovirus was not detected
Electron Microscopic Observation	Electron microscopic observation	No extraneous agents observed in the 200 cell profiles examined
Reverse Transcriptase Activity	PCR (Polymerase chain reaction)	Pass
In vitro Assays	3 detector cell lines: MRC-5, Vero, and HeLa cells	Pass
In vivo Assays	Suckling mouse, adult mouse, guinea pigs and embryonated eggs	Pass
Cell Specific Virus Tests	PCR Target viruses: HSV 1/2 (Herpes Simplex Virus Type 1 & 2) B19 (Parvovirus B19) EBV (Epstein-Barr Virus) SV40 (Simian Virus 40) hCMV / HHV5 (Human Cytomegalovirus / Human Herpesvirus Type 5) HHV6 (Human Herpesvirus Type 6) HHV7 (Human Herpesvirus Type 7) HHV8 (Human Herpesvirus Type 8) HTLV (Human T Cell Leukemia Virus) HCV (Hepatitis C Virus) HAV (Hepatitis A Virus) HBV (Hepatitis B Virus) HIV-I (Human Immunodeficiency Virus Type 1) HIV-II (Human Immunodeficiency Virus Type 2) HBoV (Human Bocavirus) hMPV (Human metapneumovirus) lineage A and B WNV (West Nile Virus) VZV (VariCella Zoster Virus) HuPyV (Human Polyoma Virus) WUPyV (WU polyomavirus) KIPyV (KI polyomavirus) HPyV (Human polyomavirus) pathogen detection (MCPyV, HPyV6, HPyV7, TSPyV, HPyV9)	Negative

Supplementary Table 12: Materials and reagents used in this study

Product Name	Supplier	Product #	Specification
StemFit® AK03N Liquid A	Ajinomoto	–	400 mL
StemFit® AK03N Liquid B		–	100 mL
StemFit® AK03N Liquid C		–	2 mL
OTSUKA NORMAL SALINE	Otsuka Pharmaceutical	613	50 mL
OTSUKA DISTILLED WATER		1751	100 mL
ACTOSIN For injection (dibutyryl cyclic AMP)	Daiichi-Sankyo	–	300 mg
PE-anti CORIN antibody	Sumitomo Dainippon Pharma	–	1.0 mg/mL
Gentacin® 40	Takata Pharmaceutical	–	40 mg/1 mL
Ascorbic Acid	TOWA Pharmaceutical	–	500 mg
CultureSure® 10mmol/l Y-27632 solution, animal-derived free	FUJIFILM Wako Pure Chemical Corporation	039-24591	300 µL
CultureSure® 10mmol/l CHIR99021 DMSO Solution, Animal-derived-free		038-24681	300 µL
CultureSure® A-83-01		039-24111	2 mg
CultureSure® CHIR99021		034-23103	5 mg
CultureSure® DMSO		031-24051	10 mL
StemSure® 10mmol/l 2-mercaptoethanol solution (×100)		198-15781	100 mL
FGF8, human, recombinant, Animal-derived-free		067-06231	25 µg
Purmorphamine		166-23991	5 mg
BDNF, human, recombinant, Animal-derived-free		028-16451	10 µg
GDNF, human, recombinant, Animal-derived-free		070-06261	10 µg
0.5mol/L-EDTA (pH8.0)	nakalai tesque	06894-14	100 mL
D-PBS(-) without Ca and Mg	nakalai tesque	14249-24	500 mL
iMatrix-511MG	Nippi	892005	175 µg
BD sheath bag with DPBS and Filter	BD	660592	4.5 L
Accudrop Beads CTT	BD	625059	2.5 mL
Fetal Bovine Serum Gamma Irradiated by SER-TAIN™ Process Sourced in Australia	SAFC	12007C	500 mL
D-glucose solution, 45% in H2O, sterile-filtered, BioXtra, suitable for cell culture	SIGMA-ALDRICH®	G8769	100 mL
Sodium Pyruvate, 100 mM, sterile-filtered, BioReagent, suitable for cell culture	SIGMA-ALDRICH®	S8636	100 mL
Stemolecule™ LDN-193189	REPROCELL	04-0074	2 mg
B27 supplement (50×) without vitamin A	Thermo Fisher Scientific	12587	10 mL
Cell Therapy Systems TrypLE™ Select CTS™		A12859	100 mL
Cell Therapy Systems GlutaMAX™- I CTS™ (100×)200mM		A12860	100 mL
GMEM Glasgow Minimum Essential Medium (1×), liquid + L-Glutamine-TPB		11710	500 mL
KNOCKOUT™ SR Serum Replacment for ESCs/iPSCs (KSR)		10828	500 mL
MEM Non-Essential Amino Acids Solution (100×)		11140	100 mL
Neurobasal® medium (1×), liquid -L-Glutamine		21103	100 mL
Petri Dish for Cell/Tissue Culture 90φ	Sumitomo Bakelite	MS-13900S	90 (φ) × 20 (H) mm
			Culture area: 57 cm^2
MultiWell Plate for Cell/Tissue Culture 6F with lid		MS-80060S	culture area: 9.2 cm^2
			well volume: 16 mL
PrimeSurface®Plate 96U		MC-9096UI	U-shaped bottom
			well volume: 0.3 mL
15 mL Centrifuge Tube		MS-56150S	19 (φ) × 118 (L) mm
50 mL Centrifuge Tube		MS-56500S	30 (φ) × 115 (L) mm
2 mL Pipette		MS-66020S	Volume: 2 mL
			Scale: 0.05 mL
5 mL Pipette		MS-66052S	Volume: 5 mL
			Scale: 0.1 mL
10 mL Pipette		MS-66102S	Volume: 10 mL
			Scale: 0.1 mL
25 mL Pipette		MS-66252S	Volume: 25 mL
			Scale: 0.2 mL
50 mL Pipette		MS-66500S	Volume: 50 mL
			Scale: 2 mL
Cell Scraper S		MS-93101S	10 mm width, 160 mm length
0.5 mL Slim Tube		MS-4701WS	10.5 (φ) × 46 (L) mm
Reagent Reservoir		MS-62803	-
Lock Stand		MS-78000	5/case
BOTTLE, 250ML,.45MM,PS,W/CAP,S	Corning	430281	2/Bag, 24/Case
BOTTLE, 500ML,.45MM,PS,W/CAP,S		430282	2/Bag, 24/Case
ep Dualfilter T.I.P.S® 2 – 20 µL	Eppendorf	0030 077.539	10 × 96 Eppendorf Tips
ep Dualfilter T.I.P.S® 2 – 200 µL		0030 077.555	10 × 96 Eppendorf Tips
ep Dualfilter T.I.P.S® 50 – 1000 µL		0030 077.571	10 × 96 Eppendorf Tips
ep Dualfilter T.I.P.S® 0.1 – 5 mL		0030 077.580	5 × 24 Eppendorf Tips
1.5 mL tube	WATSON	T119-7155CS	100/case
Nunc CryoTube 1.0 mL, Starfoot, External Thread Writing area	ThermoFisher Scientific	375353	50/BAG, 500/CASE
Tube 5mL 12x75mm RBtm PP 500cs	Corning	352063	BAG: 25, CASE: 500
Tube 5mL 12x75mm RBtm w/Strain		352235	BAG: 25, CASE: 500
Kit BD Cytopeia Fluidic 100 um Bag Ster1	BD Biosciences	660535	1kit/box
Assy Waste Line Extension 76 in SVC	BD	661183	–
O-RING VITON FOR PRESSURE VESSELS		645266	1 EA
Conn 4 Shutoff Male 1/16in		649677	0 EA
Conn 4 Shutoff Female 1/16in		649676	0 EA
Cell container (inner tube)	JMS	Custom	PET/PE film
Cell container (outer tube)		Custom	
Sterile Acrodisc® Syringe Filters with Supor® Membrane	Pall Life Sciences	4612	50 /PK

Supplementary Table 13. Antibody list

Antigen	Host species	Supplier	Cat No.	Applications	Dilution
LMX1A	Rabbit	Millipore	AB10533	IF	1:2,000
FOXA2	Goat	R&D	AF2400	IF	1:500
NURR1	Rat	Gifted from KAN laboratory	–	IF,IHC	1:1,000
TH	Rabbit	Millipore	AB152	IF,IHC	1:400
				DAB	1:5,000
OCT3/4	Mouse	Santa Cruz Biotechnology	sc-5279	IF	1:200
NANOG	Goat	R&D	AF1997	IF	1:500
SOX1	Goat	R&D	AF3369	IF,IHC	1:100
KI67	Rabbit	Novocastra	NCLKi67p	IF,IHC	1:1,000
KU80	Rabbit	Cell Signaling	2180	IHC	1:200
PAX6	Mouse	BD Pharmingen	561462	IF	1:500
TTR	Rabbit	Dako	A0002	IF, DAB	1:1,000
		abcam	ab92469	IF, DAB	1:250
HNA	Mouse	Millipore	MAB1281	IF	1:400
Pan-Cytokeratin (AE1/AE3)	Mouse	Dako	IS053	DAB	1 Drop
GFAP	Rabbit	Dako	Z0334	IHC	1:200
Secondary antibodies					
Anti-Mouse IgG (H+L) Highly Cross-Adsorbed Secondary Antibody, Alexa Fluor 488	Donkey	ThermoFisher	A21202	IF	1:400
Anti-Rat IgG (H+L) Highly Cross-Adsorbed Secondary Antibody, Alexa Fluor 488	Donkey	ThermoFisher	A21208	IF	1:400
Anti-Goat IgG (H+L) Cross-Adsorbed Secondary Antibody, Alexa Fluor 488	Donkey	ThermoFisher	A11055	IF	1:400
Anti-Rabbit IgG (H+L) Highly Cross-Adsorbed Secondary Antibody, Alexa Fluor 488	Donkey	ThermoFisher	A21206	IF	1:400
Anti-Rabbit IgG (H+L) Highly Cross-Adsorbed Secondary Antibody, Alexa Fluor 594	Donkey	ThermoFisher	A21207	IF	1:400
Anti-Goat IgG (H+L) Cross-Adsorbed Secondary Antibody, Alexa Fluor 594	Donkey	ThermoFisher	A11058	IF	1:400
Anti-Mouse IgG (H+L) Highly Cross-Adsorbed Secondary Antibody, Alexa Fluor 647	Donkey	ThermoFisher	A31571	IF	1:400
Conjugated antibodies					
FITC-conjugated TRA-1-60	Mouse	BD	560380	FCM	1:10
PE-conjugated CORIN	Mouse	Sumitomo Dainippon Pharma	–	FCM	1:30,000
Alexa488-conjugated TUJ1	Mouse	BD	560381	FCM	1:5
Alexa488-conjugated KI67	Mouse	BD	561165	FCM	1:20
Alexa647-conjugated OCT4	Mouse	BD	560329	FCM	1:5
Alexa647-conjugated PAX6	Mouse	BD	562249	FCM	1:20
PerCP-Cy5.5-conjugated SOX1	Mouse	BD	561549	FCM	1:20
FITC-conjugated TRA-2-49	Mouse	Millipore	FCMAB133F	FCM	1:20

Supplementary Table 14. DNA Primer List

Gene name	Primer Fw	Gene name	Primer Rv
AADC_Hu_Fw	AGCCCCTACTTCTTCGCCTA	AADC_Hu_Rv	GAGCCAGTCCATCATCACAG
ALCAM_Hu_Fw	CCGTGTCATGCACAATATCTGC	ALCAM_Hu_Rv	CTTTAGTCCTTCAACCTCCTGC
ALDH1A1_Hu_Fw	ATGCTTCCGAGAGGGGGCGA	ALDH1A1_Hu_Rv	CCCAACCTGCACAGTAGCGCA
BLBP_Hu_Fw	GTCTGTTGTTAGCCTGGATGGAGAC	BLBP_Hu_Rv	AGTGGCGAACAGCAACCACATCA
BRN3a_Hu_Fw	AGCAAGCAGCCTCACTTTGC	BRN3a_Hu_Rv	CTTGAAAGGATGGCTCTTGC
CALB1_Hu_Fw	GGCTCACGTATTACCCACAGA	CALB1_Hu_Rv	GAAGCCACTGTGGTCAGTATCA
CORIN_Hu_Fw	CACAGCCAGGGTCTGGTGGAATGCAG	CORIN_Hu_Rv	GAGAGCTACCACCACATGAATCAAGG
DAT_Hu_Fw	TCATCGCCACATCCTCCA	DAT_Hu_Rv	ACCAGCTCACGGTCCTTCTC
EN1_Hu_Fw	AGCGCAGGGCACCAAATAC	EN1_Hu_Rv	GGACGATCCGAATAACGTGTG
EN2_Hu_Fw	GGTCTACTGTACGCGCTACTCG	EN2_Hu_Rv	CTTTGTTCGGGTTCTTCTTCTTTG
FN1_Hu_Fw	AAAAAGACAGACGAGCTTCCCCAACT	FN1_Hu_Rv	GGGTGACGAAAGGGGTCTTTTGA
FOXA2_Hu_Fw	TTCAGGCCCGGCTAACTCT	FOXA2_Hu_Rv	AGTCTCGACCCCCACTTGCT
GAPDH_Hu_Fw	GGTCGGAGTCAACGGATTTG	GAPDH_Hu_Rv	TCAGCCTTGACGGTGCCATG
GBX2_Hu_Fw	GGTAACTTCGACAAGGCGGAGG	GBX2_Hu_Rv	GGTCGTCTTCCACCTTTGACTCG
GFAP_Hu_Fw	GTTCTCTCGGAGTATCTGG	GFAP_Hu_Rv	GATATCCCACCTCATAAAAACC
GLI1_Hu_Fw	GGTCCCATCAGGGAGGAAAG	GLI1_Hu_Rv	TCGTCCAAGCTGGAGAGGTC
GSC_Hu_Fw	GAGGAGAAAGTGGAGGTCTGGTT	GSC_Hu_Rv	CTCTGATGAGGACCGCTTCTG
HES5_Hu_Fw	GCACATTTGCCTTTTGTGAA	HES5_Hu_Rv	CACACTCAGGAGCCTTTTGG
HOXA2_Hu_Fw	GGATGAAGGAGAAGAAGGCGG	HOXA2_Hu_Rv	CTGCCATCGGCGATTTCCAGG
HOXB4_Hu_Fw	GCGCAAAGTTCACGTGAGCAC	HOXB4_Hu_Rv	GGAACCAGATCTTGATCTGGCG
KCNJ6_Hu_Fw	GCTCGAAGCTCCTACATCACC	KCNJ6_Hu_Rv	CTCTTTGGCACTAAGGGATGG
LMX1A_Hu_Fw	GATCCCTTCCGACAGGGTCTC	LMX1A_Hu_Rv	GGTTTCCCACTCTGGACTGC
LMX1B_Hu_Fw	TGTGCAAGGGTGACTACGAGAA	LMX1B_Hu_Rv	TTCATGTCCCCATCTTCATCCT
LRP4_Hu_Fw	CAGGAGGTGGTAGTGGATAC	LRP4_Hu_Rv	GACGATCAAGGTTCTCCCAG
LRTM1_Hu_Fw	ATTGCCACTTGCTCGGTCTT	LRTM1_Hu_Rv	TCCTTTCCCTTCCAGGTGTCT
MAP2_Hu_Fw	GGATCAACGGAGAGCTGAC	MAP2_Hu_Rv	TCAGGACTGCTACAGCCTCA
MKI67_Hu_Fw	AAGCCCTCCAGCTCCTAGTC	MKI67_Hu_Rv	GCAGGTTGCCACTCTTTCTC
NANOG_Hu_Fw	GGCTCTGTTTTGCTATATCCCCTAA	NANOG_Hu_Rv	CATTACGATGCAGCAAATACGAGA
NESTIN_Hu_Fw	AACTCCCGGCTGCAAACAC	NESTIN_Hu_Rv	GGACTGGGAGCAAAGATCCA
Neurogenin2_Hu_Fw	CAGGCCAAAGTCACAGCAAC	Neurogenin2_Hu_Rv	CCGAGCAGCACTAACACGTC
NKX2.1_Hu_Fw	AACCAAGCGCATCCAATCTCAAGG	NKX2.1_Hu_Rv	TGTGCCCAGAGTGAAGTTTGGTCT
NKX6.1_Hu_Fw	ATCTTCTGGCCCGGAGTG	NKX6.1_Hu_Rv	TCTTCCCGTCTTTGTCCAAC
NR4A2_Hu_Fw	CGAAACCGAAGAGCCCACAGGA	NR4A2_Hu_Rv	GGTCATAGCCGGGTTGGAGTCG
OLIG2_Hu_Fw	TGCCAGGTTCTCCCCTGAGGC	OLIG2_Hu_Rv	AGTCGTCGCAGCTTTCGCAGG
OTX2_Hu_Fw	CATGCGAGAGGAGGTGGCAC	OTX2_Hu_Rv	CCCGAGCTGGAGATGTCTTC
PAX2_Hu_Fw	GACCAAAGTTCAGCAGCCTTTC	PAX2_Hu_Rv	CAGGATCCCATTGATGGAGTAG
PAX6_Hu_Fw	ACCCATTATCCAGATGTGTTTGCCCGAG	PAX6_Hu_Rv	ATGGTGAAGCTGGGCATAGGCGGCAG
PITX3_Hu_Fw	GGGCCAGGAGCACAGCGACTCA	PITX3_Hu_Rv	GCTGCCGCCGCTGCTTCTTTTT
POU5F1_Hu_Fw	AGACCATCTGCCGCTTTGAG	POU5F1_Hu_Rv	GCAAGGGCCGCAGCTT
SHH_Hu_Fw	GATGTCTGCTGCTAGTCCTCGTC	SHH_Hu_Rv	TTTGGGGTGCCTCCTCTTC
SIX3_Hu_Fw	CCGGAAGAGTTGTCCATGTTC	SIX3_Hu_Rv	CGACTCGTGTTTGTTGATGGC
SNCA_Hu_Fw	GTGTGACAGCAGTAGCCCAGAAG CAGTG	SNCA_Hu_Rv	CCTTCTTCATTCTTGCCCAACTGGTCC
SOX1_Hu_Fw	GCGGAGCTCGTCGCATT	SOX1_Hu_Rv	GCGGTAACAACTACAAAAAACTTGTAA
SOX17_Hu_Fw	CGCTTTCATGGTGTGGGCTAAGGACG	SOX17_Hu_Rv	TAGTTGGGGTGGTCCTGCATGTGCTG
T_Hu_Fw	ATGGAGGAACCCGGAGACA	T_Hu_Rv	TGAGGATTTGCAGGTGGACA
TBR1_Hu_Fw	GCCTTTCTCCTTCTATCATGCTC	TBR1_Hu_Rv	GTCAGTGGTCGAGATAATGGGA
TH_Hu_Fw	GCAGTTCTCGCAGGACATTG	TH_Hu_Rv	CGGCACCATAGGCCTTCA
TPH2_Hu_Fw	TCAGCTACTTGGCAGCTCAAC	TPH2_Hu_Rv	CTTGCCACTTTCGGTAGCAG
TUBB3_Hu_Fw	TGATGAGCATGGCATCGAC	TUBB3_Hu_Rv	GGCCTGAAGAGATGTCCAAA
VMAT2_Hu_Fw	TGGCTTTGTGCTCTTCTGG	VMAT2_Hu_Rv	CCAAAGTTCGGAGCTATGAGTC
WNT1_Hu_Fw	GAACTGTCCCACTGCTCCAG	WNT1_Hu_Rv	GCGGAGGTGATAGCGAAGA
WNT5A_Hu_Fw	TTCGCCCAGGTTGTAATTGA	WNT5A_Hu_Rv	GCAGAGAGGCTGTGCTCCTA
ZBTB16_Hu_Fw	CCACCCCTACGAGTGTGAGT	ZBTB16_Hu_Rv	CTCAAAGGGCTTCTCACCTG

Supplementary Table 15. Test items evaluated in the general toxicity and tumorigenicity studies.

Test Items	**Tendency**
Clinical signs	Twice a day on the day of transplantation (before administration, about 4 hours after administration); once a day on other days
Body weight	Once a week
Food consumption	Once a week
General physical condition and behavior: modified Irwin's method	Before transplantation, 3, and 12 months after transplantation
Ophthalmological examination	Before transplantation, 3, and 12 months after transplantation
Urinalysis: color, pH, protein, glucose, ketone bodies, bilirubin, occult blood, urobilinogen	Before transplantation, 3, and 12 months after transplantation
Hematology: red blood cell count, white blood cell count, hematocrit value, hemoglobin concentration, platelet count, mean corpuscular volume, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration, reticulocyte count, white blood cell types (eosinophil count, basophil count, neutrophil count, monocyte count, and lymphocyte count). When necessary, Wright-stained blood smears were also evaluated.	Upon necropsy or emergency euthanasia (when possible)
Blood chemistry: aspartate transaminase, alanine transaminase, alkaline phosphatase, creatine kinase, total bilirubin, total protein, albumin, globulin, total cholesterol, triglyceride, glucose, urea nitrogen, creatinine, inorganic phosphate, calcium, sodium, potassium, chloride, protein fraction (albumin ratio, α1-globulin ratio, α2-globulin ratio, β-globulin ratio, γ-globulin ratio, and A/G ratio)	Upon necropsy or emergency euthanasia (when possible)
Necropsy	On the next day after the observation period, death or emergency euthanasia
Organ weight:	Upon necropsy Target organs/tissues: lungs (including the bronchial tubes), submandibular/sublingual glands, liver (including the gallbladder), heart, kidneys, testes, epididymides, prostate gland, seminal vesicles, ovaries, uterus, brain, spleen, thymus, pituitary gland, thyroid/parathyroid glands, adrenal glands.
Histopathology tests: H-E and immunohistochemistry staining (human cell-specific antibody, KU80; proliferation-specific antibody, KI67; dopaminergic neuron-specific antibody, TH; epithelial cell-specific antibody, pan-cytokeratin; choroid plexus-specific antibody, TTR)	Target organs/tissues: trachea, lungs (including the bronchial tubes), tongue, submandibular/sublingual glands, esophagus, stomach (forestomach, Glandular stomach), small intestine (duodenum, jejunum, ileum), large intestine (cecum, colon, rectum), pancreas, liver, gallbladder, aorta, heart, kidneys, bladders, testes, epididymides, prostate gland, seminal vesicles, ovaries, uterus, vagina, brain (site of administration, cerebrum, cerebellum, pons, medulla oblongata), spinal cord, sciatic nerves, sternum/sternal bone marrow, femur/femoral bone marrow, submandibular lymph nodes, mesenteric lymph nodes, spleen, thymus, pituitary gland, thyroid/parathyroid glands, adrenal glands, eye balls/optic nerves, lacrimal glands, Harderian glands, skeletal muscles (quadriceps muscles), mammary glands, skin (ventral), skin (dorsal), sites of gross abnormalities.