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Supplemental Information

Divergent Levels of Marker Chromosomes in an hiPSC-Based Model of Psychosis

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SUPPLEMENTAL INFORMATION

SUPPLEMENTARY FIGURES

Figure S1. Related to Figure 1. Characterization of 9p24.1-carrier HFs.

(**A**) Bright field images of 9p24.1-carrier HFs from proband (DL3363) and mother (DL5459). There is no difference in HF morphology. Scale bar = 500 μm. (**B**) RT-qPCR for *GLDC* mRNA levels in HFs from proband (DL3363), his mother (DL5459), and his brother (DL6463). Data are represented as mean ± standard deviation. Data are represented as mean ± standard deviation from three technical replicates of each individual fibroblast line. Each individual has one fibroblast line. One-way ANOVA with Tukey's multiple comparison test, n.s.: not significant, *** $p < 0.001$.

Figure S2. Related to Figure 2. Characterization of 9p24.1-positive and 9p24.1-negative hiPSCs, including representative peaks of DNA fingerprinting from hiPSC lines and their original fibroblasts.

(**A**) Immunofluorescence of pluripotency markers, NANOG, OCT4, TRA1-81 and TRA-1-60 on 9p24.1-non carrier (NC) and 9p24.1-carrier (C) isogenic hiPSC lines generated from proband (DL3363) and mother (DL5459). Scale bar = 200 μm. (**B**) RT-PCR gels showing expression of the human pluripotency markers *NANOG, OCT4* and *LIN-28*. RT-PCR results from 9p24.1 non-carrier (NC1-3) and carrier (C1-3) hiPSCs from DL5459 and DL3363 are displayed. Both patient HF lines are displayed as a negative control. (**C-D**) PCR identification of 9p24.1 DUP/TRP in all of 9p24.1-positive and 9p24.1-negative isogenic hiPSCs generated from the proband (DL3363) and his mother (DL5459). (Primers used were ACTTCTGCCTCATTCTGGGTCTCT (forward TRP) and TTGATAGTCTTCTACAGCATCCATTGC (reverse duplication), with an expected size of 573bp.) (**E**) aCGH identification of 9p24.1 DUP/TRP in additional representative carrier and non-carrier isogenic hiPSCs generated from the mother (DL5459). (**F**) DL3363 fibroblasts and hiPSC line (DL3363 C2) derived from the fibroblasts share the same peaks in five different regions (**G**) DL5459 fibroblasts and hiPSC line (DL5459 C5) are matched but not with DL3363 (F) at the same region.

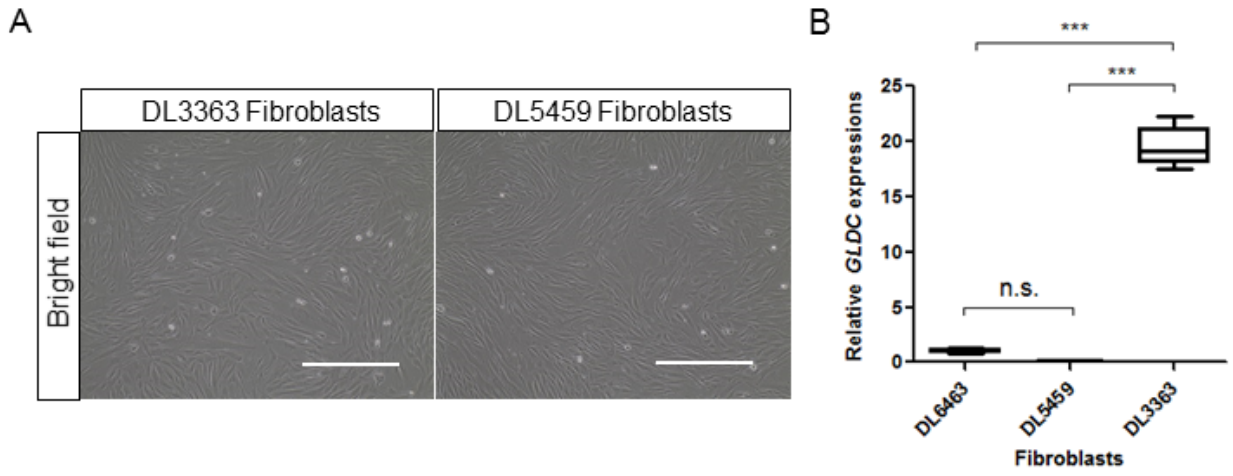
Figure S3. Related to Figure 3. Presence of marker elements detected by *GLDC* FISH from high passage hiPSCs derived from low and high marker element carrier lines.

GLDC FISH images (600x magnification) in high mar (32.5%) carrier hiPSC line (DL5459 C5) at passage 22 and low mar (20.5%) carrier hiPSC line (DL 5459 C6) at passage 22.

Figure S4. Related to Figure 4. Characterization of NPCs differentiated from high and low mar 9p24.1 hiPSC lines.

9p24.1 NPCs generated from high mar (55%) and low mar (10%) hiPSC lines (DL5459 C5 and DL5459 C6) are not positive for astrocyte markers (GFAP and S100β (left)) or neuronal markers (MAP2AB (right)). Scale bar = 500μm.

SUPPLEMENTARY TABLES



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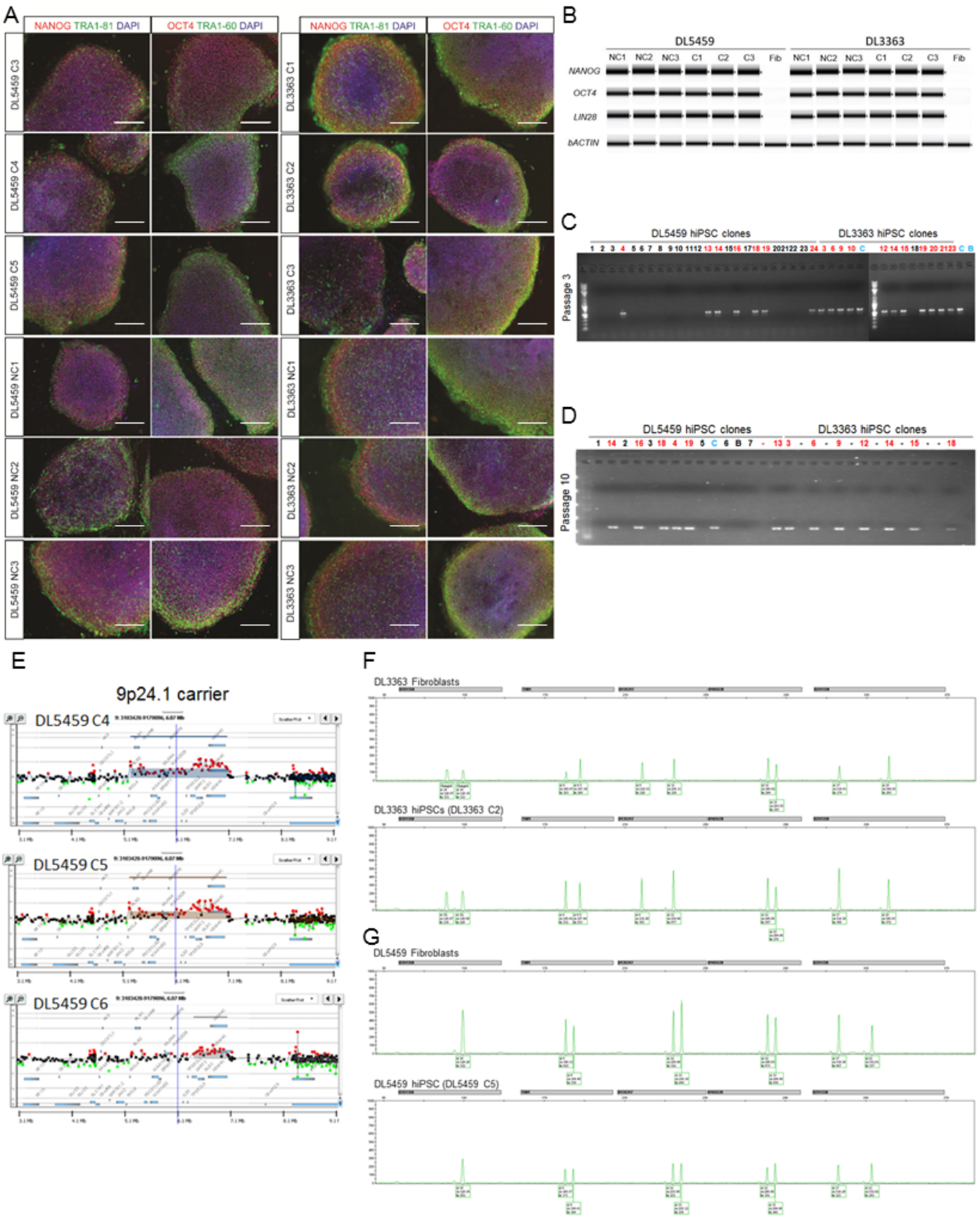

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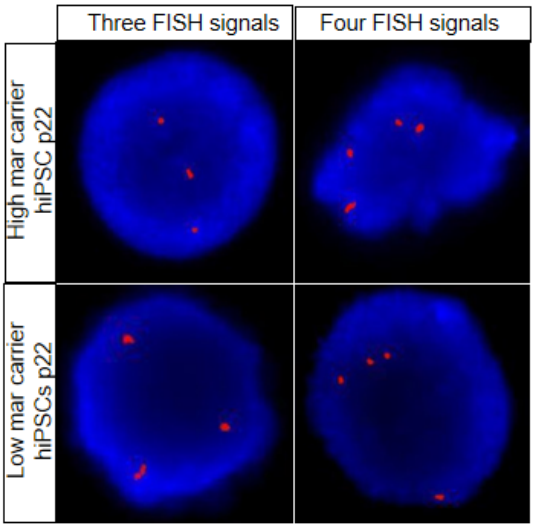

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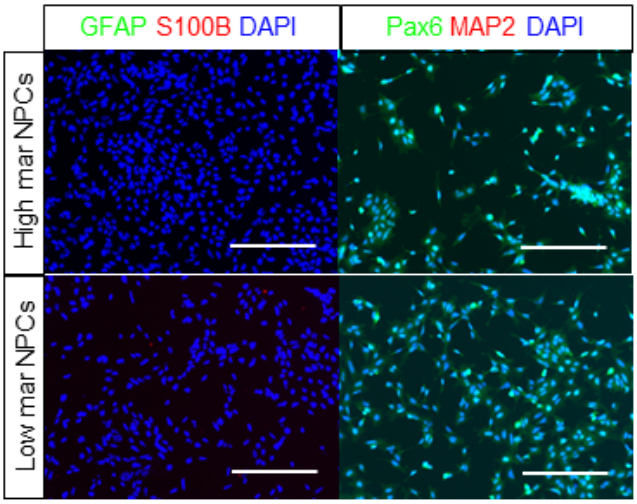
GLDC FISH images (600x magnification, images cropped and re-sized post-capture) in high mar (32.5%) carrier hiPSC line (DL5459 C5) at passage 22 and low mar (20.5%) carrier hiPSC line (DL 5459 C6) at passage 22.



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9p24.1 NPCs generated from high mar (55%) and low mar (10%) hiPSC lines (DL5459 C5 and DL5459 C6) are not positive for astrocyte markers (GFAP and S100β (left)) or neuronal markers (MAP2AB (right)). Scale bar = 500μm.

Table S1. Approximate correlation between mosaicism of the mar element in fibroblast lines by karyotype and early and late passage of lines with the level of mosaicism determined by *GLDC* FISH.

Cell line	Karyotype	Passage	# cells with one FISH signal	# cells with two FISH signal	# cells with three FISH signal	# cells with four FISH signal
DL3363 Fibroblasts	47,XY,+mar[8]/47,XY,t(3;12) (p21;q24.1),+mar /46,XY[7]	p5	0/200 (0.0%)	90/200 (45.0%)	75/200 (37.5%)	35/200 (17.5%)
		p20	4/200 (2.0%)	88/200 (44.0%)	47/200 (23.5%)	61/200 (30.5%)
DL5459 Fibroblasts	7,XX,+mar[4]/46,XX,t(1;18) (p22;q11.2)[2]/47,XX,t(1;18) (p22;q11.2),+mar[1] /46,XX[13]	p5	0/200 (0.0%)	91/200 (45.5%)	54/200 (24.0%)	55/200 (27.5%)
		p20	0/200 (0.0%)	109/200 (54.5%)	38/200 (19.0%)	53/200 (26.5%)

Table S2. Karyotypes of 9p24.1 DUP/TRP in 9p24.1-positive and 9p24.1-negative isogenic hiPSCs generated from the proband (DL3363) and mother (DL5459).

Cell line	Genotype	Karyotype
DL3363 hiPSCs	9p24.1 carrier	C1 (TCW6), p12: 47,XY+mar[2]/46,XY[18] C2 (TCW14), p16: 47,XY+mar1[6]/46,XY[13] C3 (TCW15), p16: 47,XY+mar[4]/46,XY[16]
	Isogenic 9p24.1 non-carrier	NC1 (kjbB), p12: 46,XY NC2 (kjbC), p12: 46,XY NC3 (kjbD), p12: 46,XY
DL5459 hiPSCs	9p24.1 carrier	C1 (TCW4), p13: 47,XX+mar[4]/ 46,XX[16] C2 (TCW13), p12: 47,XX+mar[10]/ 46,XX,t(1;3)(q21;q21)[2]/ 46,XX[8] C3 (TCW18), p12: 47,XX+mar[12]/46,XX[8] C4 (kjb3), p12: 47,XX+mar[8]/46,XX[12] C5 (kjb6), p12: 47,XX+mar[11]/46,XX[9] C6 (kjb7), p12: 47,XX+mar[2]/46,XX[18]
	Isogenic 9p24.1 non-carrier	NC1 (TCW1), p12: 46,XX NC2 (TCW3), p12: 46,XX NC3 (TCW6), p12: 46,XX

Table S3. Approximate correlation between mosaicism of the mar element in genotype-positive hiPSC lines with the level of mosaicism determined by Chr 9 and/or *GLDC* FISH.

FISH probe	Cell line	Karyotype	# cells with one FISH signal	# cells with two FISH signal	# cells with three FISH signal	# cells with four FISH signal
Chr9	DL3363 C1	47,XY+mar[2] /46,XY[18]	0/200 (0.0%)	187/200 (93.5%)	13/200 (6.5%)	N/A
	DL5459 C5	47,XX+mar[11] /46,XX[9]	3/200 (1.5%)	99/200 (49.5%)	98/200 (49.0%)	N/A
GLDC	DL3363 C1	47,XY+mar[2] /46,XY[18]	4/200 (2.0%)	154/200 (77.0%)	29/200 (14.5%)	13/200 (6.5%)
	DL3363 C2	47,XY+mar[6] /46,XY[13]	0/200 (0.0%)	108/200 (54.0%)	77/200 (38.5%)	15/200 (7.5%)
	DL5459 C5	47,XX+mar[11] /46,XX[9]	0/200 (0.0%)	11/200 (56.0%)	71/200 (35.5%)	17/200 (8.5%)
	DL5459 C6	47,XX+mar[2] /46,XX[18]	0/200 (0.0%)	135/200 (67.5%)	56/200 (28.5%)	9/200 (4.5%)

Table S4. Changes in the level of mosaicism determined by *GLDC* FISH in early and late passage of hiPSC lines.

Cell line	Passage	# cells with one FISH signal	# cells with two FISH signals	# cells with three FISH signals	# cells with four FISH signals
DL5459 C5	p12	0/200 (0.0%)	112/200 (56.0%)	71/200 (35.5%)	17/200 (8.5%)
	p22	0/200 (0.0%)	115/200 (57.5%)	58/200 (29.0%)	27/200 (13.5%)
DL5459 C6	p12	0/200 (0.0%)	135/200 (67.5%)	56/200 (28.0%)	9/200 (4.5%)
	p22	2/200 (1.0%)	157/200 (78.5%)	28/200 (14.0%)	13/200 (6.5%)

SUPPLEMENTAL METHODS

Description of patients

Based on Structured Clinical Interviews for DSM-IV (SCID) (Spitzer et al., 1994) and a review of hospital records, the 9p24.1-carrier male proband (DL3363) met criteria for a DSM-IV (Association, 1994) diagnosis of schizoaffective disorder and his 9p24.1-carrier mother (DL5459) met DSM-IV criteria for bipolar disorder with psychotic features. This family cohort also includes two non-carrier relatives, a full brother (DL6463) and maternal half-sister of the proband (DL5754), who did not meet criteria for psychosis or a schizophrenia-related personality disorder based on a SCID interview and the Structured Interview for Schizotypal Symptoms (Kendler, 1989), respectively.

Human subjects work was approved by the McLean Hospital Institutional Review Board. Written informed consent was obtained from all subjects. One hour before the procedure a topical analgesic was locally applied to the skin where the biopsy was obtained in order to minimize any discomfort from the procedure. The surface was cleansed and the area to be biopsied was injected with 1% xylocaine with epinephrine intradermally. A 4mm punch biopsy was taken; antibiotic ointment and a bandage were applied. The family quad was biopsied at McLean Hospital. 4mm dermal punch biopsies were collected and shipped overnight at rt in HF media (DMEM (Life Technologies) + 20% FBS (Gemini) + Antibiotic-Antimycotic (Life Technologies)) to Icahn School of Medicine at Mount Sinai.

HF culture

Biopsies were digested at 37°C for 12 hrs in enzyme digestion media (DMEM (Life Technologies) + 20% FBS (Gemini) + 1% collagenase (Life Technologies) + 1 unit/ml

dispase (Sigma)), washed twice and plated in a minimal media volume of HF media on 0.1% gelatin coated plates. Media was changed every 72 hrs. HFs grew from biopsy to confluency in 1-2 weeks and were then split 1:3 approximately every 7 days. HFs were cultured on plates treated with 0.1% gelatin (in milli-Q water) for a minimum of 30 min and grown in HF media.

hiPSC derivation

Replicating but recently confluent HFs were transfected once with Cytotune Sendai virus expressing *OCT4*, *SOX2*, *KLF4* and *cMYC* (Life Technologies) and after 3-5 days of recovery, with TrypleE (Life Technologies) and re-plated onto a 10-cm dish containing 1 million mouse embryonic fibroblasts (mEFs). Cells were switched to HUES media (DMEM/F12 (Invitrogen), 20% Knockout-Serum Replacement (Invitrogen), 1x Glutamax (Invitrogen), 1x NEAA (Invitrogen), 1x 2-mercaptoethanol (Sigma) and 20 ng/ml FGF2 (Invitrogen)) and fed every 2-3 days. Single hiPSC colonies were manually picked and clonally plated onto 24-well mEF plates. hiPSCs were either maintained on mEFs in HUES media or on Matrigel (BD) in mTeSR media (Stemcell Technologies). At early passages, hiPSCs were split via manual passaging. At higher passages, hiPSCs could be enzymatically passaged with 100mM EDTA in PBS (Gibco). hiPSCs were fed every 72 hrs using HUES media containing StemBeads FGF2 (StemCultures). Cells were frozen in cold freezing media (50% HUES media, 40% FBS, 10% DMSO) or Synthafreeze (Life Technologies).

NPC differentiation

NPCs were differentiated using a 7-day neural induction protocol (Life Technologies). Briefly, $2.5x10^5$ – $3x10^5$ collagenase-passaged (1mg/ml in DMEM) (Sigma) hiPSCs from

mTeSR were plated per well of a Matrigel-coated 6-well plate and cultured in PSC Neural Induction Medium (Life Technologies). On day 7, NPCs were harvested using Accutase, gently pipetted, passed through a 100μm strainer, washed and re-plated in Neural Expansion Media (Life Technologies).

RT-qPCR experiments

Gene expression analysis was performed on HFs and hiPSCs. Cells were washed with PBS and lysed with RLT buffer (Qiagen). The total RNA was extracted with RNeasy mini kit (Qiagen, # 74106) with on-column DNase I digestion (Qiagen, #79254). For standard qPCR, RNA was reverse transcribed into complementary DNA (cDNA) with iScript™ cDNA Synthesis kit (Bio-Rad). cDNA was used as template for the quantitative PCR using a Quantstudio Real-Time PCR system (Applied Biosystems) with *GLDC* TaqMan gene expression assay, Hs01580591_m1 (ThermoFisher). Expression was analyzed using the ΔΔCt method. qPCR results were normalized to *GAPDH* expression, and the values of uninduced fibroblasts were set to 1. Three replicates were used to determine the standard deviation.

aCGH

To further determine the size, genomic extent and gene content for the rearrangement, we designed a tiling-path oligonucleotide microarray spanning the long arm of chr 9. A custom, 4X180K, Agilent Technologies (Santa Clara, CA) microarray was designed using the Agilent e-array website (http://earray.chem.agilent.com/earray/). Probe labeling and hybridization were performed according to the manufacturer's protocol with modifications.

Breakpoint junction sequencing

Sample-specific primers for PCR were designed at the apparent boundaries of the DUP/TRP segments as inferred from the aCGH result marked by a transition from normal copy number to copy number gain. Long-range PCR reactions were performed using TaKaRa LA Taq (Clontech, Mountain View, CA). PCR products were sequenced by Sanger sequencing methodology.

Karyotype analysis

Karyotyping was performed by Wicell Cytogenetics (Madison, WI). This laboratory has established and verified the test's accuracy and precision.

FISH

The Vysis CEP 9 probe for 9p11q11 (#06J36-019, Abbott Molecular) was hybridized hiPSCs; signal patterns in two hundred interphase nuclei were reported. The Empire Genomics red probe (GLDC-20-OR) that maps to *GLDC* at 9p24.1 was hybridized to HFs, hiPSCs and NPCs; signal patterns in two hundred interphase nuclei were reported. This test was developed and its performance characteristics determined by the Cytogenetic Laboratory of WiCell Research Institute (Madison, WI). This laboratory has established and verified the test's accuracy and precision.

Immunohistochemistry

Cells were fixed in 4% paraformaldehyde in PBS at 4°C for 15 min. hiPSCs and NPCs for cytosol protein detections were permeabilized at room temperature (rt) for 15 min in 0.5% Triton in PBS, while hiPSCs for surface marker staining were not permeabilized. Cells were blocked in 10% donkey serum with 0.1% Triton at rt for 30 min. The following primary antibodies and dilutions were used: 1:500; rabbit anti-Foxp2 (Abcam,

ab16046), 1:100; mouse anti-Nestin (Abcam, ab22035), 1:60; rabbit anti-Pax6 (Abcam, ab5790), 1:2000; chicken anti-GFAP (Aves Lab, GFAP), 1:1000; mouse anti-S100B (Sigma, S2532), and 1:400; mouse anti-MAP2AB (Sigma, M1406). Secondary antibodies were used at 1:300; Alexa donkey 488 and 568 anti-rabbit, mouse, or chicken (Life Technologies). To visualize nuclei, cells were stained with 0.5 μg/ml DAPI (4',6-diamidino-2-phenylindole). Images were acquired using an Olympus IX51 Fluorescence Microscope.

DNA fingerprinting

The short tandem repeat (STR) profile assays were performed. We used AmpFLSTR® Identifiler® Plus PCR Amplification Kit (ThermoFisher) and GeneScan™ 500 LIZ™ dye Size Standard (ThermoFisher) and followed the manufacturer's recommendation to obtain the results. These samples were blinded by labeling as numbers and identified matched lines.

DNA fingerprinting for cells used in this study:

Locus	**DL3363**	**DL5459**

Amelogenin	X	Y	X	X
D5S818	12	13	11	13
FGA	22	25	21	25
D8S1179	13	14	13	14
D21S11	29	31	29	30
D7S820	11	12	9	12
CSF1PO	11	13	10	11
D3S1358	14	16		16
TH01	6	9.3	8	9
D13S317	8	12	12	13
D16S539	12	13	12	13
D2S1338	17	23	17	21
D19S433	14	14.2	13	14.2
vWA	16	17	16	17
TPOX	9		8	9
D18S51	13	20	13	16
Confirmed Samples	DL3363 Fibroblasts (p7)		DL5459 Fibroblasts (p7)	
	DL3363 C1 hiPSCs (p16)		DL5459 C5 hiPSCs (p17)	
	DL3363 C2 hiPSCs (p15)		DL5459 C6 hiPSCs (p17)	
	DL3363 NC3 hiPSCs (p15)		DL5459 NC1 hiPSCs (p14)	

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