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Supplemental Information

Reversal of Phenotypic Abnormalities by CRISPR/Cas9-Mediated Gene Correction in Huntington Disease Patient-Derived Induced Pluripotent Stem Cells

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SUPPLEMENTAL INFORMATION

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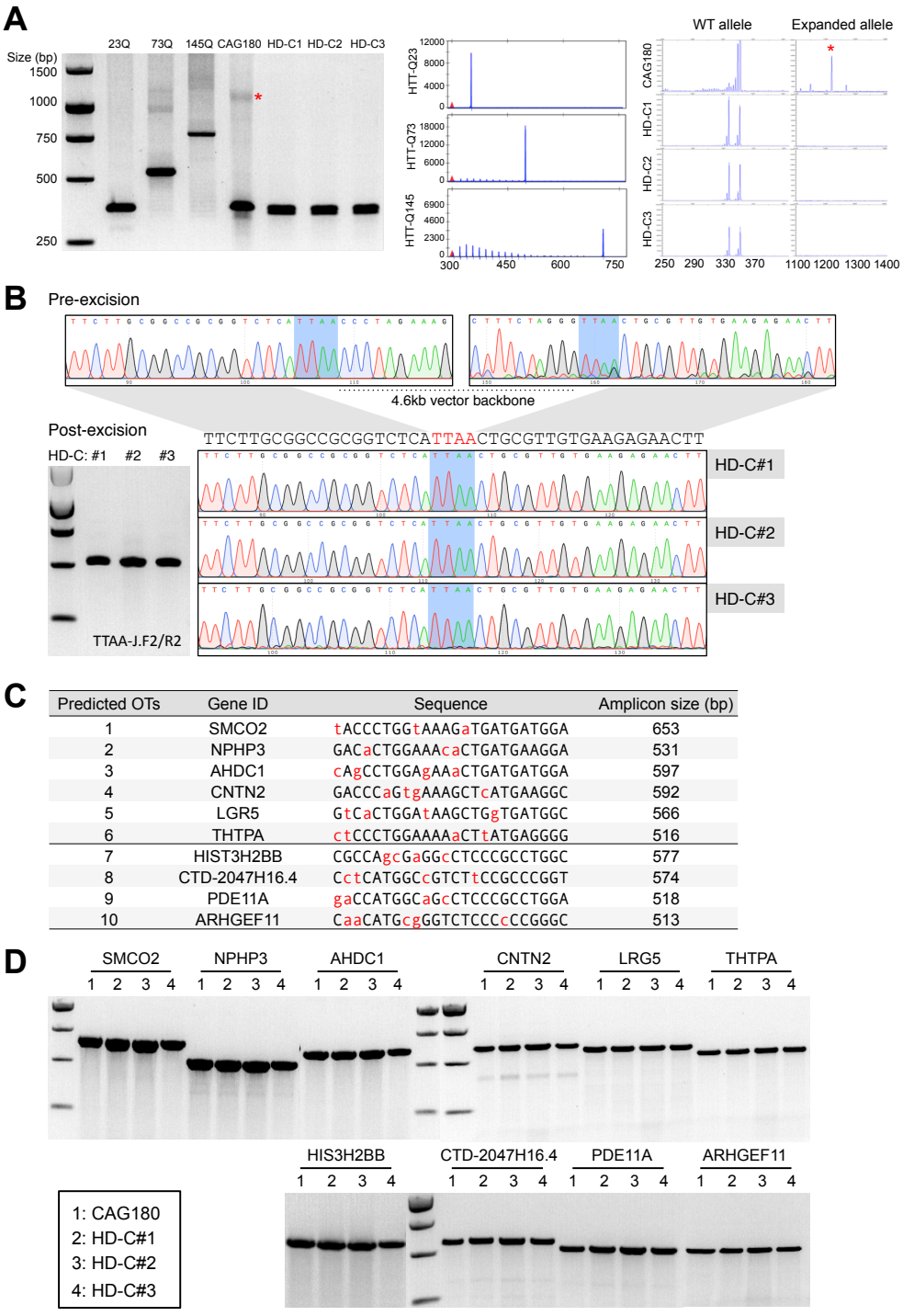
Figure S1. Fragment sizing, TTAA sequencing, Surveyor off-target analysis (**A**) PCR results and fragment analysis show different CAG sizes between WT allele and expanded allele; PCR results from full length HTT constructs with 23Q, 73Q, 145Q are shown for size comparison. (**B**) Sequence analysis of pre-excision and post-excision at TTAA site. (**C**) The predicted top 10 off-target (OT) sites; Red characters indicate mismatches compared to on-target sgRNA-a or sgRNA-b sequence. (**D**) The predicted off-target loci are amplified by PCR and analyzed by Surveyor assay. Related to Figure 1.



Predicted OTs	Gene ID	Sequence	Amplicon size (bp)
1	SMCO2	tACCCTGGtAAAGaTGATGATGGA	653
2	NPHP3	GACaCTGGAAAcaCTGATGAAGGA	531
3	AHDC1	cAgCCTGGAgAAaCTGATGATGGA	597
4	CNTN2	GACCCaGtgAAAGCTcATGAAGGC	592
5	LGR5	GtCaCTGGAtAAGCTGgTGATGGC	566
6	THTPA	ctCCCTGGAAAAaCTtATGAGGGG	516
7	HIST3H2BB	CGCCAgcGaGGcCTCCCGCCTGGC	577
8	CTD-2047H16.4	CctCATGGCcGTCTtCCGCCCGGT	574
9	PDE11A	gaCCATGGCaGcCTCCCGCCTGGA	518
10	ARHGEF11	CaaCATGcgGGTCTCCCcCCGGGC	513

Figure S2. PluriTest novelty and pluripotency scores for parental and corrected hiPSC lines. Related to Figure 2.

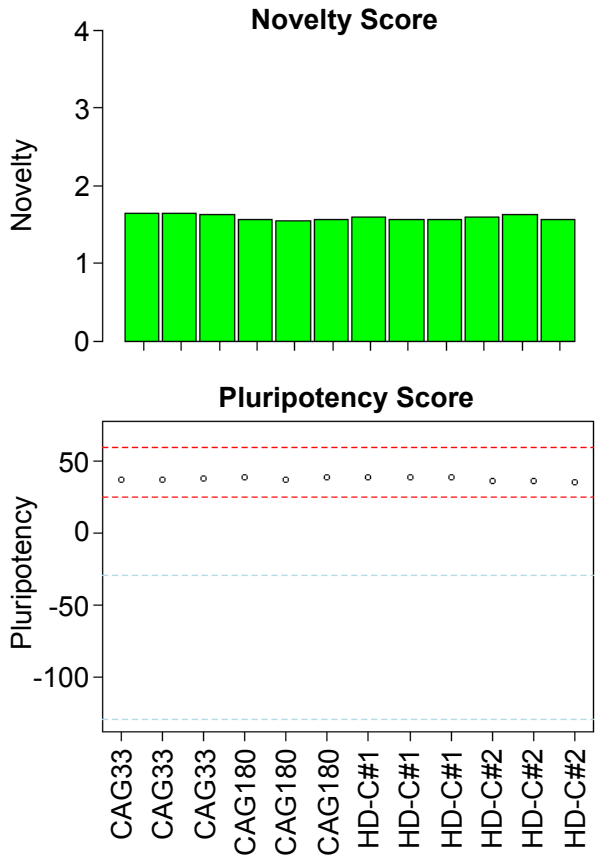


Figure S3. No aggregates in CAG180 hiPSC-derived neurons on Day 52. HEK293 cells overexpressed with HTT128Q N-fragment plasmid were used as positive control for EM48 staining (scale bar = 25μm). Related to Figure 3.

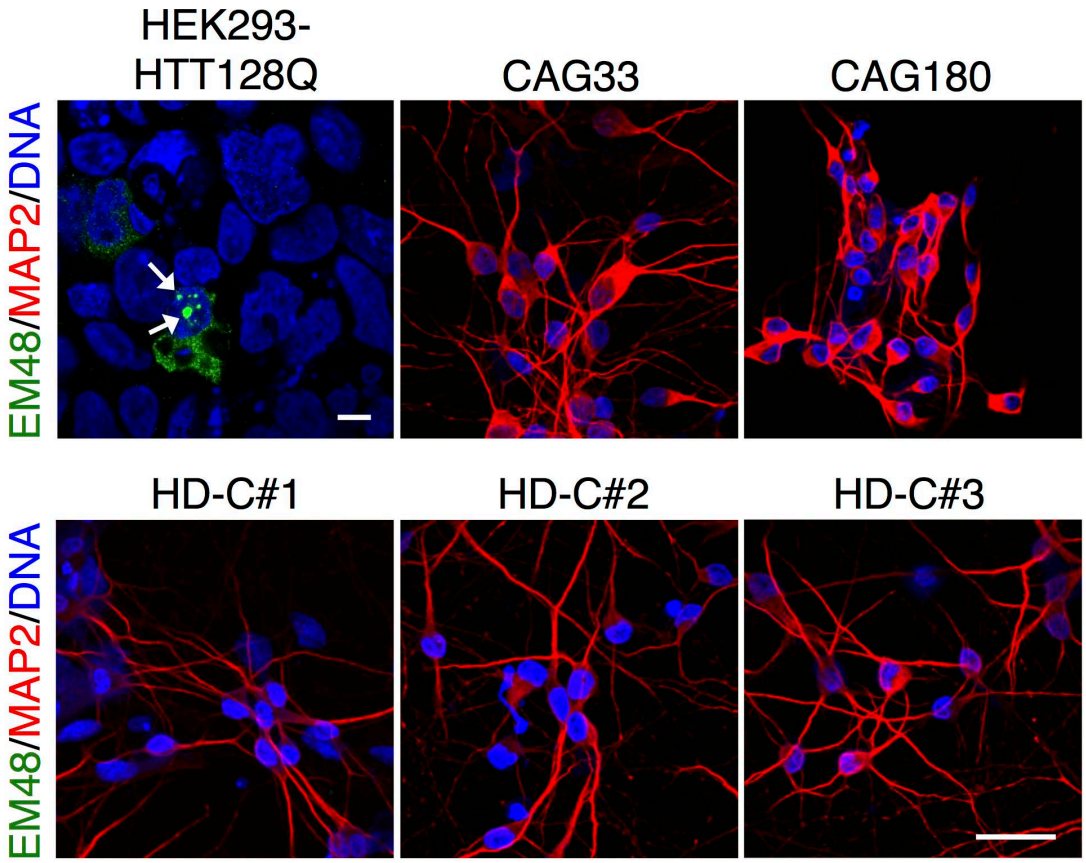


Figure S4. MEA recordings from hiPSC-derived neurons cultured in N2B27 versus BrainPhys media. (**A**) Mean and (**B**) max spike frequency, (**C**) the number of unresponsive channels, and (**D**) raster plots demonstrate the effect of culturing medium (N2B27 versus BrainPhys) on spiking activity. n = 3 independent biological replicates; values shown as mean±SEM. Related to Figure 4.

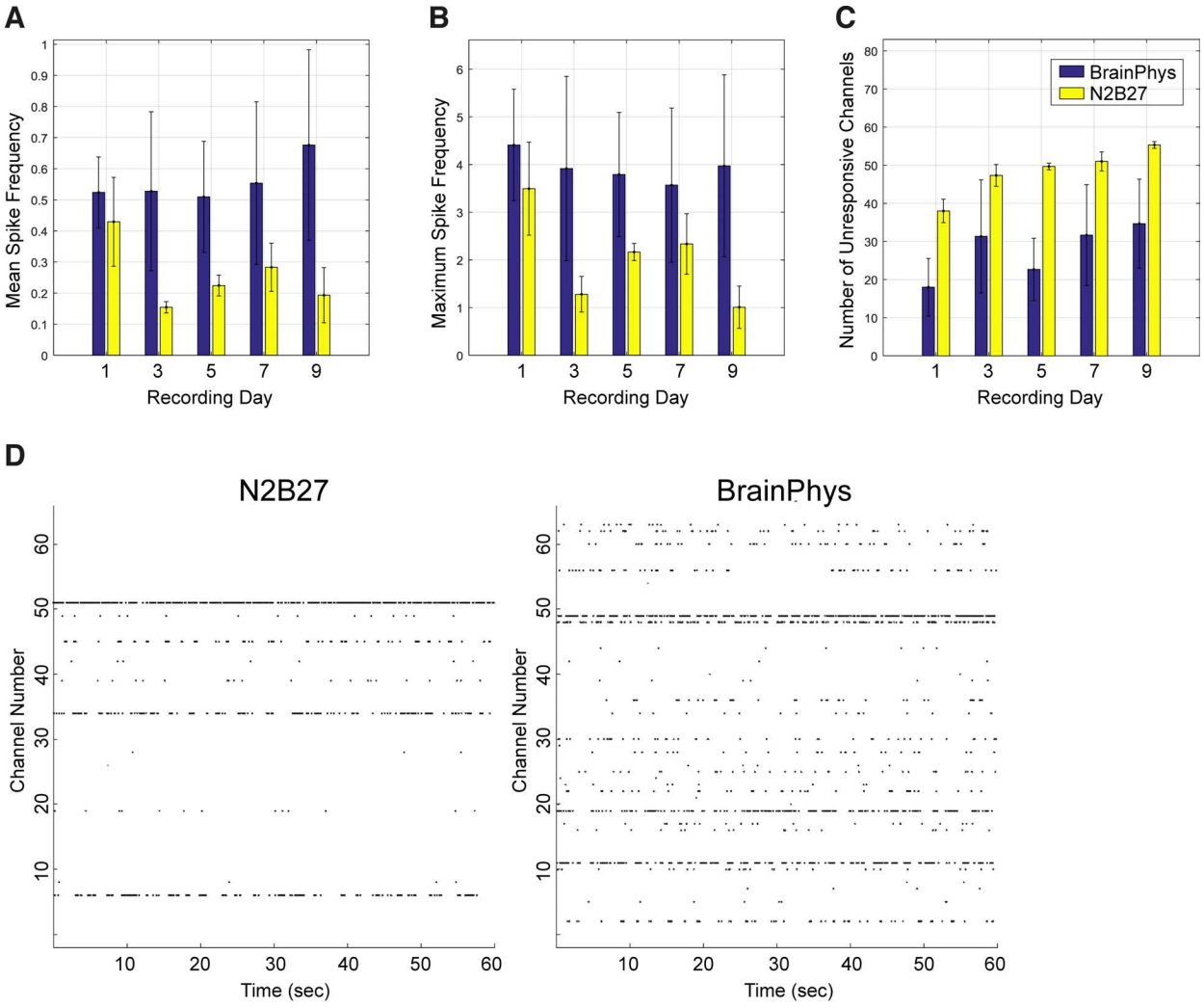


Figure S5. (**A**) Differentiation of HD hiPSCs and isogenic control hiPSCs into NPCs using the neuronal differentiation protocol described by Li and colleagues (Li et al., 2011) (scale bar = 25μm) (**B**) Significantly differentially enriched GO terms compared to CAG180 in NPCs. Related to Figure 6.

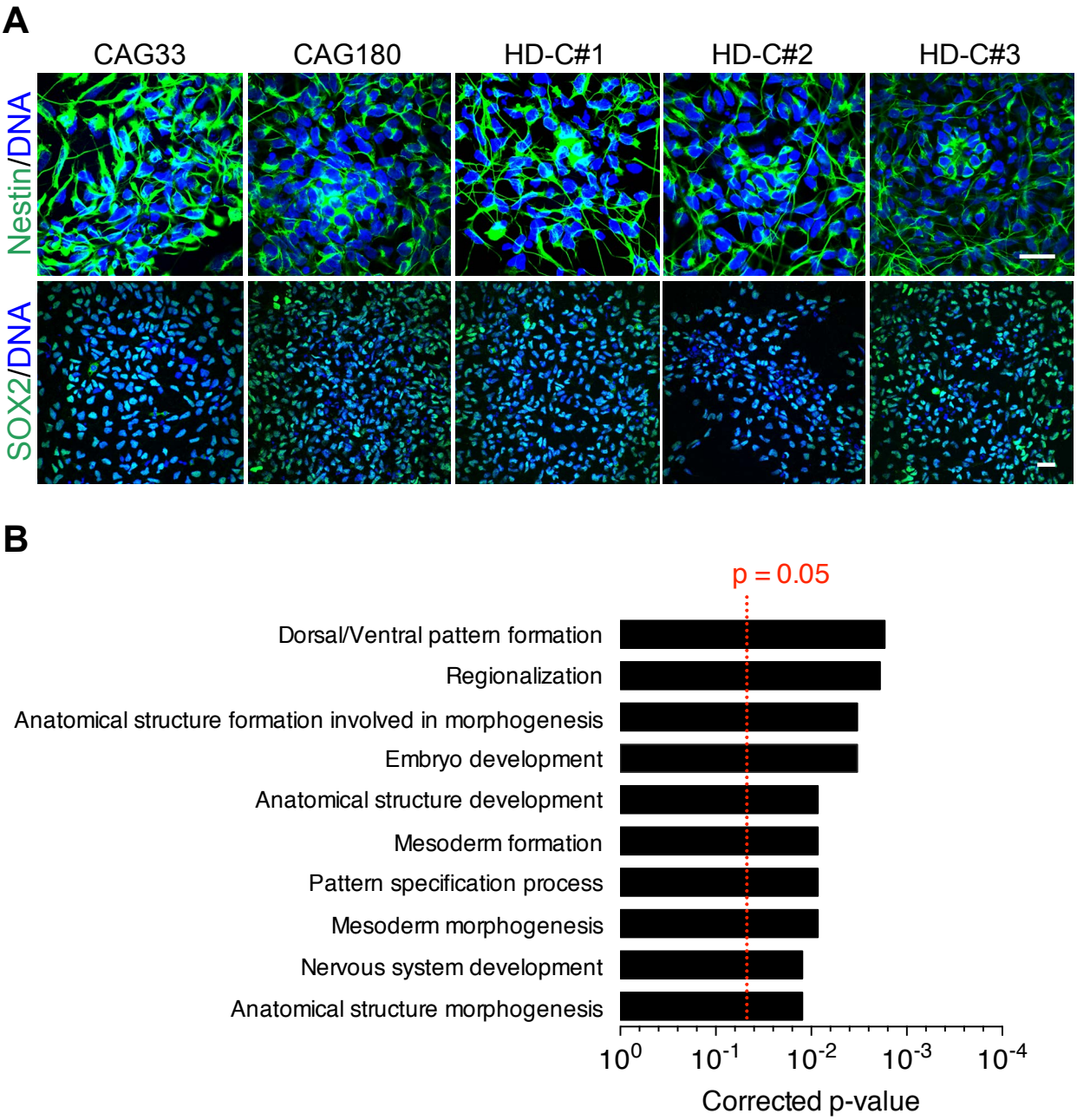


Table S1. Sequences of oligos for CRISPR-Cas9n cloning. Related to Figure 1.

Name	Sequence (5'→3')
sgRNA-a-Forward	caccgGACCCTGGAAAAGCTGATGA
sgRNA-a-Reverse	aaacTCATCAGCTTTTCCAGGGTCc
sgRNA-b-Forward	caccgCGCCATGGCGGTCTCCCGCC
sgRNA-b-Reverse	aaacGGCGGGAGACCGCCATGGCGc

Table S2. Primer sequences for Surveyor off-target analysis. Related to Figure S1.

Name	Forward (5'→3')	Reverse (5'→3')
SMCO2	CTGCCTCAGCTTTCCTCTGT	AGAGCCACAAGGGCTTAACA
NPHP3	GCAGCCCCATAAAACTACCA	AAGACCTGAACCAGCAATTCA
AHDC1	CTCCAACTACACACCGCAGA	ATGAAGTCACAGGGGTCTCG
CNTN2	GGTACCGAGATGAAGCTGGA	CCAGGGATGGGTGCTTCTAA
LGR5	AGCAAACCTACGTCTGGACA	TCTCCCTCCTCCCAAAATGA
THTPA	GACATCAGCAGCAGTGGAAG	TGTAGGTTCCGCTGTGAGTT
HIST3H2BB	ACCCTTTCTTGATCGTGTGG	CCAGCGATGACGTAGAACAA
CTD-2047H16.4	CTCCTCTAGCTCCCAATGCA	GGGACACATCTGCAGAACTG
PDE11A	AACTGGGAATACTGGTGGGG	GTAGGTCCTGTTCACGTGGA
ARHGEF11	AAACATGTGGAAGCGGTCAC	AGAGGGGAGGAAGAAAGTGC

Table S3. Sequences of primers for qRT-PCR analysis. Related to Figures 2, 3, 6, and 7.

Name	F/R[a]	Sequence (5'→3')	Name	F/R[a]	Sequence (5'→3')
OCT4	F	AGTTTGTGCCAGGGTTTTTG	CHCHD2	F	GCTTCAGTGGAGGAAGTAATG
	R	ACTTCACCTTCCCTCCAACC		R	TGATGTCACCCTGGTTCT
LIN28	F	GCGGGCATCTGTAAGTGGTT	KIAA1239	F	GGACACATCAACCCTCTTATTA
	R	GGTGAACTCCACTGCCTCAC		R	CTCATGTAGCCAGCCATAAG
PAX6	F	AATAACCTGCCTATGCAACCC	JRID1D	F	CCTCTTCTCCGACTTCTATCT
	R	AACTTGAACTGGAACTGACACAC		R	GAAGTGAGACTGGGCTTTG
SOX1	F	ATGCACCGCTACGACATGG	GPM6A	F	GCGAATCTACTGAGCTGAAC
	R	CTCATGTAGCCCTGCGAGTTG		R	GTTGGCAGACAGAACCATAA
FOXG1	F	TACTACCGCGAGAACAAGCA	CLDN10	F	ACTAAGCATGTGGGAGTTATTT
	R	TCACGAAGCACTTGTTGAGG		R	GGGATGTCCTTAACCCATTTAT
MAP2	F	AAAGCTGATGAGGGCAAGAA	EIF1AY	F	CGCCATGCCCAAGAATAA
	R	GGCCCCTGAATAAATTCCAT		R	TCCTCTTTAAACACCAACTCTC
SYP	F	TCTTGAGCAAGGCAAGAAGTGGGA	OPN1SW	F	TCACCATTCCTTCATTCTTCTC
	R	CTGCCCAAACCCAGCCATTGTAAA		R	CTTCCCACACACCATCTTC
NKX2.1	F	CGCATCCAATCTCAAGGAAT	ZIC4	F	CCATCCTTCCCTTCATTCAC
	R	TGTGCCCAGAGTGAAGTTTG		R	GGACCAGCACATCCTTATTT
GAD65	F	GGGAATTGGCAGACCAACCACAAA	CSAG3A	F	CACCAACACCAAAGAGGTT
	R	TCAGCCAGTCTGCTGCTAATCCAA		R	GTCAGAGTGGCTGGATAGT
NESTIN	F	TGGCAAAGGAGCCTACTCCAAGAA	LGR6	F	GGTTCCATAACAACAACATCAA
	R	ATCGGGATTCAGCTGACTTAGCCT		R	CAGAGATAGTGTGTGGAGTTTAG
ACTIN	F	GGCATGGGTCAGAAGGATTC	SPATA8	F	GCAATCAGCTGGCTCTATATC
	R	CACACGCAGCTCATTGTAGAAG		R	CCATTCCAGGACTAGCATAAC
SPON2	F	CTTTCCCAACCTTGCTTCT			
	R	CTGGACGATGAAGGACAATC			

[a] Primer orientation: F, forward; R, reverse

Table S4. Summary of capture statistics for whole exome sequencing. Related to Figure 1.

Category	Sample ID	Mean Read Length	Total Reads	After Removing Identical Reads	Unique (%)	Mapped reads	Mapping (%)
Parental iPSC clone	CAG180	188	89,275,348	69,634,771	78%	69,425,867	99.7%
Isogenic corrected clones	HD-C#1	184	76,298,896	60,276,128	79%	59,974,747	99.5%
	HD-C#2	192	84,908,852	65,379,816	77%	65,118,297	99.6%
	HD-C#3	192	94,346,489	75,477,191	80%	75,175,282	99.6%

Table S5: Sequence Variants in the gene-corrected hiPSC clones by whole exome sequence analysis. Related to Figure 1.

	Sample	HD-C#1	HD-C#2	HD-C#3
Number of SNVs	Total	6	2	14
	Intergenic	0	0	0
	Intronic	3	1	6
	Exonic	2	1	7
	UTRs	1	0	0
	ncRNA	0	0	1
	Up/downstream	0	0	0

Table S6. SNVs detected by whole exome sequencing. Related to Figure 1.

HD-C#1	HD-C#2	HD-C#3	Chr	Position	Gene	Location	Ref (CAG180)	Alt
		SNV	1	32131000	COL16A1	intronic	C	T
		SNV	1	179533782	NPHS2	intronic	G	T
		SNV	2	103061805	IL18RAP	intronic	G	T
SNV			2	170382223	KLHL41	utr_3	T	A
		SNV	4	81123529	PRDM8	exonic	A	T
SNV			7	134719493	AGBL3	exonic	A	G
	SNV		8	96276030	C8orf37	intronic	C	T
SNV			10	124337239	DMBT1	intronic	G	A
SNV			12	52886627	KRT6A	exonic	C	T
		SNV	13	79928669	RBM26	exonic	C	T
		SNV	15	42057270	MGA	intronic	A	T
		SNV	15	81199146	CEMIP	exonic	C	A
		SNV	16	25172530	LCMT1	intronic	G	A
		SNV	16	58577599	CNOT1	exonic	G	T
		SNV	17	4936878	SLC52A1	exonic	G	T
		SNV	17	72322909	KIF19	intronic	C	A
		SNV	19	17301918	MYO9B	exonic	C	A
		SNV	19	51391447	KLKP1	intronic_nc	G	C
		SNV	20	238447	DEFB132	exonic	G	T
SNV			21	45743643	PFKL	intronic	C	A
SNV			22	30981293	PES1	intronic	G	A
	SNV		22	41573572	EP300	exonic	C	T

Supplemental Experimental Procedures

Selection cassette excision

To remove the *piggyBac* cassette, 1×10^6 corrected hiPSCs were transfected with 2 μg piggyBac Excision-Only Transposase vector (System Biosciences, PB220PA-1) using NEON Transfection System. 72 hr after transfection, 0.2 μM of 1-(2-deoxy-2-fluoro-1-D-arabinofuranosyl)-5-iodouracil (FIAU, Sigma) was used to eliminate piggyBac-containing clones for 5 days. Resistant colonies were picked and expanded, and further screened by junction PCR using F1/R1 (F1-5'GCACCTCGCTGGAACTTAAT-3'; R1-5'GTGTCTGCAGGCTCAAAGAG-3') and F2/R2 (F2-5'ACTTACCGCATTGACAAGCACG-3' and R2-5'CCACAGTTCCACACCAAAGAGC-3') primers.

Analysis of piggyBac TTAA site post-excision

The genomic region flanking the TTAA site (piggyBac transposase excision site) from pre and post-excision clones were PCR amplified and sequenced with primers TTAA.J-F2 5' CCTGTCCTGAATTCACCGAGGG3' and TTAA.J-R2 5'CCTGCAGACCAACTTAGGCTTAGA3' with KOD Xtreme (Novagen, #71975). Amplicons were visualized on 1% agarose gel on the Geldoc XR system (Bio-Rad) and analyzed by Sanger sequencing.

Surveyor assay for off-target analysis

To predict potential off-target effects, guides sequences sgRNA-a and sgRNA-b (**Table S2**) were analyzed using the CasOT script (Xiao et al., 2014) and the top ranked hits were selected for screening. Selected genomic region were amplified with Platinum Taq polymerase (Invitrogen, # 10966-083) and amplicons were then mixed in a 1:1 ratio of Parental (CAG180):Corrected (HD-C#1, #2 or #3) to a final volume of 20μl for surveyor assay following the manufacturer's instructions (IDT, #706020).

Fragment sizing analysis

The RNA of parental CAG180 and the corrected HD-C#1, #2, and #3 hiPSC lines were extracted using the RNeasy Plus kit (QIAGEN, #74136) and cDNA converted using the Takara-RT kit (#RR037A) following the manufacturer's instructions. HTT constructs with 23Q, 73Q, and 145Q (Coriell, #CH00022, CH00023,and CH00024) were used for tract length comparison. To amplify the CAG tract, 100ng of cDNA or plasmid DNA was used as template DNA with KOD Xtreme (Novagen, #71975) supplemented with 8% DMSO (final v/v). Cycling conditions were as follows: initial denaturation at 96°C for 5 mins, followed by 7 cycles of 96°C for 45s, 70°C for 30s, 72°C for 2 mins; and 33 cycles of 96°C for 45s, 58°C for 30s and 72°C for 2mins and a final elongation at 72°C for 10mins. Primers used for amplification span exons 1-6 of HTT cDNA and are 6-FAM conjugated L33FAM-5'-CGAGTCCCTCAAGTCCTTCC-3' and unconjugated R390-5'-TTCCATAGCGATGCCCAGAA-3'. Amplicons were visualized on 1% agarose gel on the Geldoc XR system (Bio-Rad) and sent to Axil Scientific Pte Ltd for fragment sizing with the GeneScan™ 1200 LIZ® dye Size Standard. Files were analyzed using Genemapper (Applied Biosystems).

Whole Exome Sequencing

One microgram of high-molecular weight DNA per sample was used for exome capture with Agilent Technologies SureSelectXT^{TM} All Human Exon V6 Kit. The exon capture kit targets 60 Mb that allows capture of 99% of RefSeq, CCDS, GENCODE, HGMD, OMIM exons. DNA was sheared using Covaris M220 Focused-ultrasonicator (Covaris Inc., Woburn, MA, USA) to target an average fragment size of 200 bp. Shearing was followed by end repair, ligation of adapters, nick repair, purification, size selection and final amplification prior to exome capture as per SureSelect protocol. The amplified DNA was cleaned with Ampure XP reagent (Agencourt, Boston, USA) and the DNA was eluted in 30 ml low TE buffer. The libraries were quantified using a Qubit 2.0 Fluorometer (Life Technologies, Carlsbad, CA, USA). The exome library was used for emulsion PCR on Ion Chef System (Life Technologies, Carlsbad, CA, USA) following the manufacturer's protocol. Each library was sequenced on an Ion Proton instrument (Life Technologies, Carlsbad, CA, USA) using one ION PI chip. For the HD-C#1, HD-C#2, HD-C#3 and CAG180 samples, 14.0 Gb, 16.3 Gb, 18.1 Gb and 16.7 Gb were sequenced with an average read length of 184 bp, 192 bp , 192 bp and 188bp, respectively. An average coverage of 164× (HD-C#1), 187× (HD-C#2), 208× (HD-C#3) and 195× (CAG180) was achieved per base over the exome with 97% of the bases covered at least 20×. Sequence reads were aligned to the human reference genome [Human GRCh37 (hg19) build] using Torrent Mapping Alignment Program (TMAP) from the Torrent Suite (v5.0.2). PCR duplicates in the BAM file were identified by the Filter Duplicates plugin (v5.0) and removed. The variants were called using the Torrent Variant Caller (TVC) plugin (v5.0.2). The variants were imported into Ion Reporter (v5.2), where each variant was annotated using the "annotate single sample variants" workflow.

Immunoblotting

Cells were lysed with RIPA buffer (Sigma Aldrich) containing complete protease inhibitor Cocktail Tablets (Roche) and protein concentration was measured using the Bradford Assay (Bio-Rad). Samples were denatured at 70°C for 10 min in 4× NuPAGE sample buffer and 10× NuPAGE reducing agent (Life Technologies). A total of 30 µg of protein per sample was separated on NuPAGE 3%-8% Tris-Acetate (for HTT/Calnexin) or NuPAGE 4%-12% Bis-Tris (for CHCHD2/Calnexin) gradient gels at 100 V for 3 h followed by transfer to nitrocellulose membrane at 120 V for 1.5 h at room temperature. The following primary antibodies were used for detection: anti-total HTT (Millipore, MAB2166), anti-mutant HTT [1C2] (Millipore, MAB1574), anti-mutant HTT [MW1] (Developmental Studies Hybridoma Bank), anti-Calnexin (Sigma, C4731), and anti-CHCHD2 (Proteintech). Alexa Fluor 680 goat anti-mouse and Alexa Fluor 790 goat anti-rabbit (Life Technologies) were used as secondary antibodies. Membranes were imaged using the Li-Cor Odyssey infrared imaging system and quantified by ImageJ software.

Immunofluorescence staining

Cells grown on coverslips were fixed with 4% paraformaldehyde (PFA) for 15 min at room temperature. The cells were permeabilized using 0.3% Triton X-100 for 20 min at room temperature, then blocked with 3% normal donkey serum containing 0.1% Triton X-100 in DPBS for 1 h at room temperature, before being stained with primary antibodies at 4°C overnight. The following primary antibodies and dilutions were used: anti-OCT4 (Santa Cruz, 1:500), anti-Foxg1 (Abcam, 1:500), anti-Nestin (Millipore, 1:2000), anti-ASM-1 (Millipore, 1:1000), anti-AFP

(Millipore, 1:150), anti-TUJ1 (Covance, 1:1000), anti-SOX2 (Santa Cruz, 1:500), anti-ZO-1 (Zymed, 1:200), anti-MAP2 (Millipore, 1:2000), anti-SYP (Abcam, 1:1000), anti-GABA (Sigma, 1:1000), and anti-HTT (mEM48, Millipore, 1:100). After three washes in PBS containing 0.1% Triton X-100, the cells were incubated with the appropriate secondary antibodies for 1 h at room temperature, washed three times with PBS containing 0.1% Triton X-100, and incubated with 1 µg/mL 4', 6-diamidino-2-phenylindole (DAPI, Sigma Aldrich) for 10 min. Images were acquired using an Olympus FV1000 inverted confocal microscope.

RNA isolation, cDNA synthesis and Quantitative PCR

Cells were lysed using RLT Plus Buffer and RNA was purified using the RNeasy Plus Mini (QIAGEN) according to the manufacturer's instructions. For all samples, cDNA was generated using PrimeScript® RT reagent Kit (TAKARA). Quantitative real-time PCR was performed on the StepOnePlus™ or QuantStudio 6 Flex Real-Time PCR System (Applied Biosystems) using primers listed in **Table S3**. Relative gene expression levels were analyzed using the Comparative CT Method (ΔΔCT Method).

Three germ layers differentiation

To form embryonic bodies (EBs), hiPSCs were dissociated into clumps using Dispase, and cultured on low-attachment tissue culture plates in KSR medium (DMEM/F12 with 20% knockout serum replacer, 1% Glutamax, 1% NEAA, and 0.1 mM 2-Mercaptoethanol) and medium was changed every two days for a total of seven days. EBs were then harvested onto Matrigel-coated coverslips and left to spontaneously differentiate for nine days in KSR medium before fixation and staining.

Differentiation of hiPSCs

NPC differentiation: hiPSCs were induced into NPCs according to a previously published protocol (Li et al., 2011). Briefly, hiPSCs at approximately 20% confluence were treated with N2B27 media (DMEM-F12/Neural Basal medium 1:1 with 1% N2, 2% B27, 1% pen/strep/glutamine, 10 ng/mL hLIF, and 5 µg/mL BSA) containing 3 µM CHIR99021 (Tocris), 2 µM SB431542 (Sigma), and 0.1 µM compound E (Millipore) for the first seven days. The culture was then split 1:3 for the next six passages using Accutase without compound E on Matrigel-coated plates. Cells between passage 3 and passage 6 were used for experiments.

Forebrain neuronal differentiation: hiPSCs were differentiated into forebrain neurons using an established protocol (Delli Carri et al., 2013) incorporating some modifications from other published protocols (Maroof et al., 2013; Xu et al., 2013). hiPSCs maintained in feeder-free cultures were disaggregated using 1 mg/mL Dispase, then washed in mTeSR-1 medium, and cell clumps were cultured on uncoated petri dishes in N2B27 medium (DMEM-F12/Neural Basal medium 1:1 with 1% N2, 2% B27, 1% non-essential amino acids, and 2 mM L-glutamine) supplemented with 10 µM Y-27632 (Sigma Aldrich) for 8 h. Then cell aggregates were collected and plated on dishes pre-coated with 10 µg/mL poly-L-ornithine (Sigma Aldrich) and 10 µg/mL laminin in N2B27 medium supplemented with 100 nM LDN193189 (Stemgent),10 µM SB-431542 (Sigma Aldrich), and 2 µM XAV939 (Stemgent). From Day 5, 200 ng/mL SHH (R&D) was added to the differentiated cells. After 15 days culture, cells were passaged with a cell

scraper at a split ratio of 1:1. These cells, hereon referred to as neural progenitor cells (NPCs), were cultured in N2B27 medium supplemented with 20 ng/mL BDNF (R&D), 2 µM XAV939, and 200 ng/mL SHH for five days. For neuronal differentiation, the NPCs were first cultured in N2B27 medium supplemented with 20 ng/mL BDNF (R&D) and 20 ng/mL GDNF for seven days, then cultured in N2B27 medium supplemented with BDNF (20 ng/mL), GDNF (20 ng/mL), 10 µM DAPT (Sigma-Aldrich), and 0.2 mM ascorbic acid (STEMCELL Technologies) for another seven days. In the final step of neuronal differentiation and maturation, cells were dissociated with Accutase and filtered with a 40-µm cell strainer (BD Bioscience) to obtain single cells. The differentiated cells were seeded onto plates pre-coated with 10 µg/mL poly-L-ornithine and 4 µg/mL laminin at a density of 50,000 – 100,000 cells/cm^2 and cultured for a further 2 – 3 weeks in N2B27 medium supplemented with BDNF (20 ng/mL), GDNF (20 ng/mL), cAMP (N6,2′-O-Dibutyryladenosine 3′,5′-cyclic monophosphate, Sigma Aldrich, 0.5 mM), and ascorbic acid (0.2 mM). Half the medium was replaced with fresh medium every 3 – 4 days for the terminally differentiated neurons.

Multielectrode array (MEA) recordings

After 46 days of differentiation, neurons were dissociated and re-plated on 0.1% polyethylenimine (PEI, Sigma-Aldrich) coated 12-well MEA plates (Axion Biosystems) at a density of 140,000 cells/well. The following day, complete medium was changed with N2B27 medium or Brainphys medium (BrainPhys™ Neuronal Medium with 1% N2 and 2% NeuroCult™ SM1, STEMCELL Technologies) supplemented with 20 ng/mL BDNF, 20 ng/mL GDNF, 0.5 mM cAMP, and 0.2 mM ascorbic acid. For cell maintenance, 50% medium was exchanged with fresh medium every 3 – 4 days. Spontaneous neuronal activity was observed and recorded at 37°C for 5 min every other day using the Maestro MEA system (Axion Biosystems). For all recordings, a neural spikes analog mode was applied along with 12.5 kHz sampling frequency and median referencing. The recorded traces were high-pass filtered (200Hz – 3 kHz) offline, and spike detection was performed using a threshold of 6 standard deviation (SD) above noise levels (Axion Integrated Studio software, AxIS). Timestamps files were subsequently imported and analyzed using custom-written Matlab scripts (R2015b). Mean and max spike frequency, the number of unresponsive channels, and raster plots were chosen to characterize the extracellular activity recorded with the MEA.

Whole-cell patch clamp recordings

Whole-cell patch clamp recordings were performed as described previously (Ma et al., 2015). In brief, recordings were made with electrodes (6-8 MΩ) pulled from borosilicate glass (World Precision Instruments, Inc.) and filled with the internal solution (in mM): 120 K-gluconate, 9 KCl, 10 KOH, 4 NaCl, 10 HEPES, 1 EGTA, 2 Mg_2ATP, 0.4 Na_3GTP (pH 7.3, and 290 mOsm). For 2-photon fluorescence imaging, Alexa Fluor 594 (5 µg/ml; Invitrogen) was included in the internal solution. Recordings were made at room temperature (25°C) from cells bathed in an external solution containing (in mM): 127 NaCl, 2.6 KCl, 23.8 $NaHCO_3$, 0.77 NaH_2PO_4, 2 $MgCl_2$, 2.5 $CaCl_2$, and 10 glucose. Recordings were made with a Multiclamp 700B amplifier, filtered at 2 kHz, digitized at 10 kHz with a Digidata 1440A, and acquired/analyzed with PCLAMP software (all from Molecular Devices). Series resistance ranged from 10-20 MΩ and was monitored throughout the recordings. The resting membrane potential (RMP) of hiPSC derived cells was

determined immediately after breakthrough in the whole-cell patch clamp mode, taking into account a liquid junction potential of 11.8 mV (Barry and Lynch, 1991). Action potentials were evoked by depolarizing current steps (10 pA; 1 s). To facilitate comparison across cells during these current clamp measurements, steady currents were applied as needed to maintain the RMP at -80 mV. Membrane capacitance and input resistance were determined, under voltage clamp, from responses to a 5 mV hyperpolarization.

Neural rosette formation assay

hiPSCs were dissociated into single cells using Accutase, and 4.5×10^6 cells were seeded into AggreWell™800 Plates to form neural aggregates. Cells were cultured in STEMdiff™ Neural Induction Medium and three-quarters of the medium was changed every four days. On Day 5, neural aggregates were harvested and transferred into Poly-L-Ornithine/Laminin coated plates. On Day 10, cells were fixed using 4% PFA and stained with antibodies against ZO-1 and Nestin.

Growth factor withdrawal assay

hiPSC-derived neurons were dissociated and re-plated on coverslips on Day 34 of differentiation. After 6 days of culture in N2B27 medium with 20 ng/mL BDNF, 20ng/mL GDNF, 0.2 mM ascorbic acid and 0.5mM cAMP, cells were switched to N2B27 medium supplemented with 50 ng/mL BDNF or N2B27 medium only for 48 hrs. Cells were then fixed with 4% PFA and used for TUNEL staining (In Situ Cell Death Detection Kit, Fluorescein, Roche) following the manufacturer's instructions.

Microarray analysis

Total RNA was purified from hiPSCs and hiPSC-derived NPCs following the double extraction protocol: RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction (Trizol Invitrogen) followed by a Qiagen RNeasy clean-up procedure. Total RNA integrity was assessed by Agilent Bioanalyzer and the RNA Integrity Number (RIN) was calculated; all RNA samples had perfect RIN 10. Biotinylated cRNA was prepared from 300 ng of total RNA using the Epicentre TargetAmp Nano-g Biotin-aRNA Labelling kit for Illumina system. Labelled cRNAs (750 ng) were hybridized onto the Illumina® HumanHT-12-v4 Expression BeadChip at 58°C for 16 h; the arrays were then washed and stained using the Illumina Wash Protocol and then scanned using a BeadArray Scanner 500GX at the BSF Microarray Facility. The images were analyzed using GenomeStudio Gene Expression v 1.9.0 according to the instructions provided by Illumina.

References

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