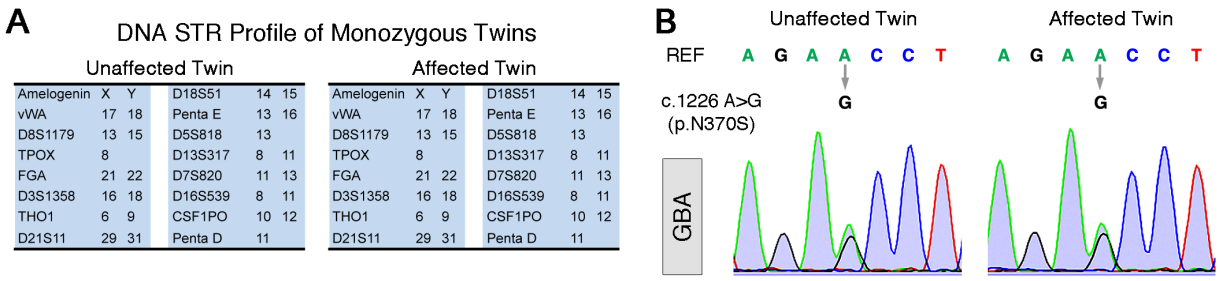
## SUPPLEMENTAL DATA

### Supplemental Figure Legends




| Unaffected Twin | | | | | | Affected Twin | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Amelogenin | X | Y | D18S51 | 14 | 15 | Amelogenin | X | Y | D18S51 | 14 | 15 |
| vWA | 17 | 18 | Penta E | 13 | 16 | vWA | 17 | 18 | Penta E | 13 | 16 |
| D8S1179 | 13 | 15 | D5S818 | 13 | | D8S1179 | 13 | 15 | D5S818 | 13 | |
| TPOX | 8 | | D13S317 | 8 | 11 | TPOX | 8 | | D13S317 | 8 | 11 |
| FGA | 21 | 22 | D7S820 | 11 | 13 | FGA | 21 | 22 | D7S820 | 11 | 13 |
| D3S1358 | 16 | 18 | D16S539 | 8 | 11 | D3S1358 | 16 | 18 | D16S539 | 8 | 11 |
| THO1 | 6 | 9 | CSF1PO | 10 | 12 | THO1 | 6 | 9 | CSF1PO | 10 | 12 |
| D21S11 | 29 | 31 | Penta D | 11 | | D21S11 | 29 | 31 | Penta D | 11 | |

**Figure S1. DNA Fingerprint and GBA N370S Validation in MZ Twins Discordant for PD, Related to Figure 1.**

(A) Fibroblasts were sent to Cell Line Genetics for DNA fingerprint. The DNA STR profiles of twins match each other, indicating they are monozygotic. (B) Sanger sequencing confirmed the heterozygous GBA mutation (c.1226 A>G) in twins.

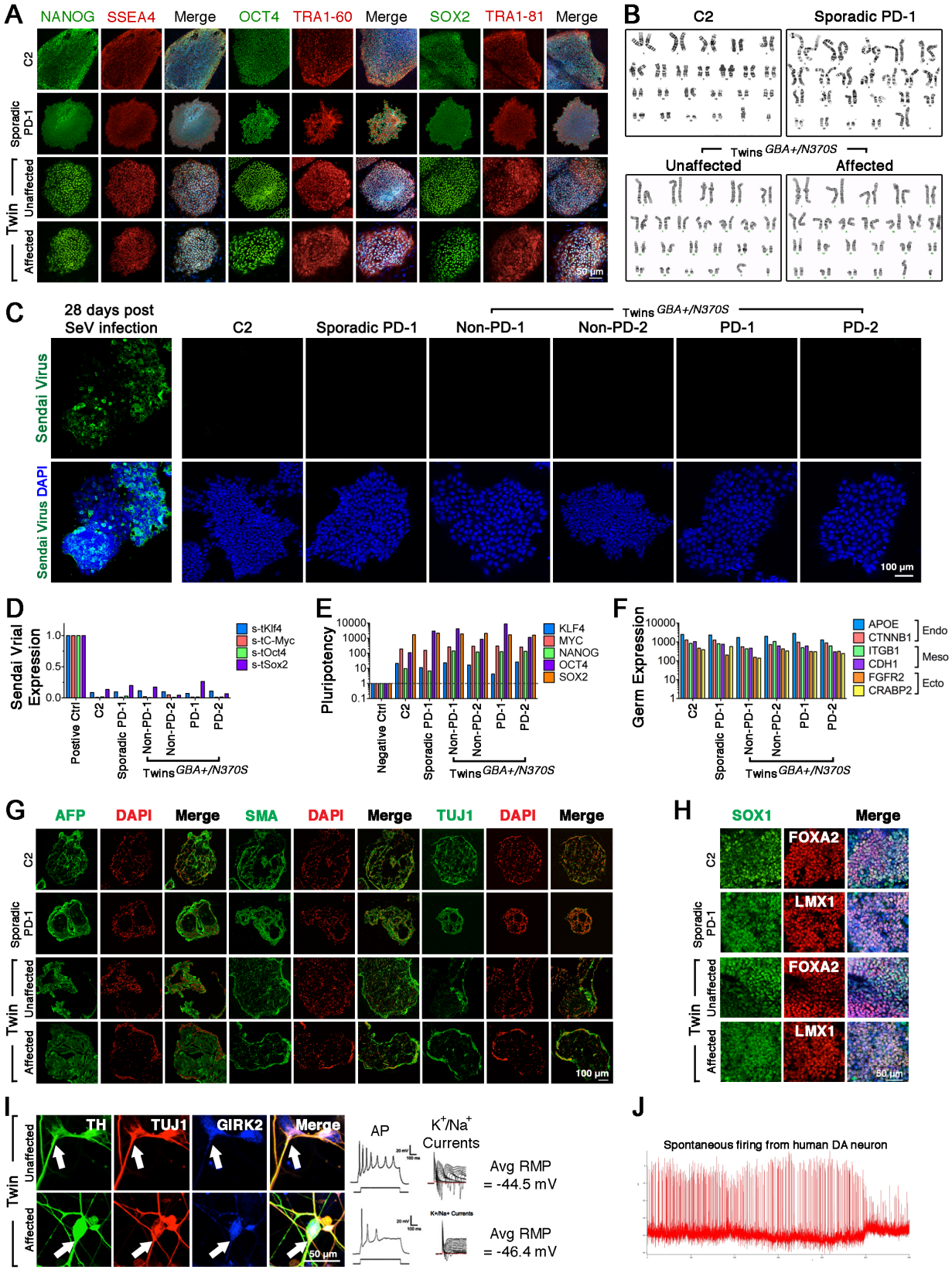

A
NANOG
SSEA4
Merge
OCT4
TRA1-60
Merge
SOX2
TRA1-81
Merge
C2
Sporadic PD-1
Twin
Unaffected
Affected
50 µm
B
C2
Sporadic PD-1
Twins GBA+/N370S
Unaffected
Affected
C
28 days post SeV infection
C2
Sporadic PD-1
Non-PD-1
Non-PD-2
PD-1
PD-2
Twins GBA+/N370S
Sendai Virus
Sendai Virus DAPI
100 µm
D
Sendai Vrial Expression
s-tKlf4
s-tC-Myc
s-tOct4
s-tSox2
Postive Ctrl
C2
Sporadic PD-1
Non-PD-1
Non-PD-2
PD-1
PD-2
Twins GBA+/N370S
E
Pluripotency
KLF4
MYC
NANOG
OCT4
SOX2
Negative Ctrl
F
Germ Expression
APOE
CTNNB1
ITGB1
CDH1
FGFR2
CRABP2
Endo
Meso
Ecto
G
AFP
DAPI
Merge
SMA
TUJ1
100 µm
H
SOX1
FOXA2
LMX1
50 µm
I
TH
TUJ1
GIRK2
Merge
AP
K+/Na+ Currents
Avg RMP = -44.5 mV
Avg RMP = -46.4 mV
50 µm
J
Spontaneous firing from human DA neuron

**Figure S2. Characterization and Neuronal Differentiations of Human iPS Cells, Related to Figure 1.**

To characterize iPS cells, we standardized a set of assays including endogenous pluripotent markers staining of Nanog, SEEA4, Oct4, Tra1-60, Sox2, Tra-1-81 (A), Karyotyping (B), the staining of Sendai virus (C), exogenous Sendai viral expression (D), endogenous pluripotent marker expression (E), three-germ layer differentiation using NanoString (F) and embryoid body formation (G) of control (C2), sporadic PD-1, non-PD twin, and PD-twin iPS cells, and immunostaining with anti-AFP (endoderm), anti-SMA (mesoderm), and anti-Tuj1 (ectoderm). Positive control, RNA from fibroblasts infected with virus for 24-48 hours; and negative control, RNA from never infected fibroblasts. (H - I) Twin-derived iPS cells were successfully induced into mDA progenitors and patterned into substantia nigra (A9) dopaminergic (mDA) neurons. At day 11, SOX1-positive cells also exhibited midbrain markers FOXA2 and LMX1. At day 35, many cells displayed neuritogenesis and branching. These differentiated cells were confirmed as A9 neurons by positive triple staining of TH, TUJ1, and GIRK2. (I) ~ 50-day differentiated neurons from both the unaffected twin and the affected twin fired multiple action potentials (APs) and presented voltage-gated Na+ and K+ currents with resting membrane potentials of ~ –45 mV. (J) Spontaneous firing from iPS cell-derived neurons. Scale bars, 50 μm (A, H, I); 100 μm (C, G).

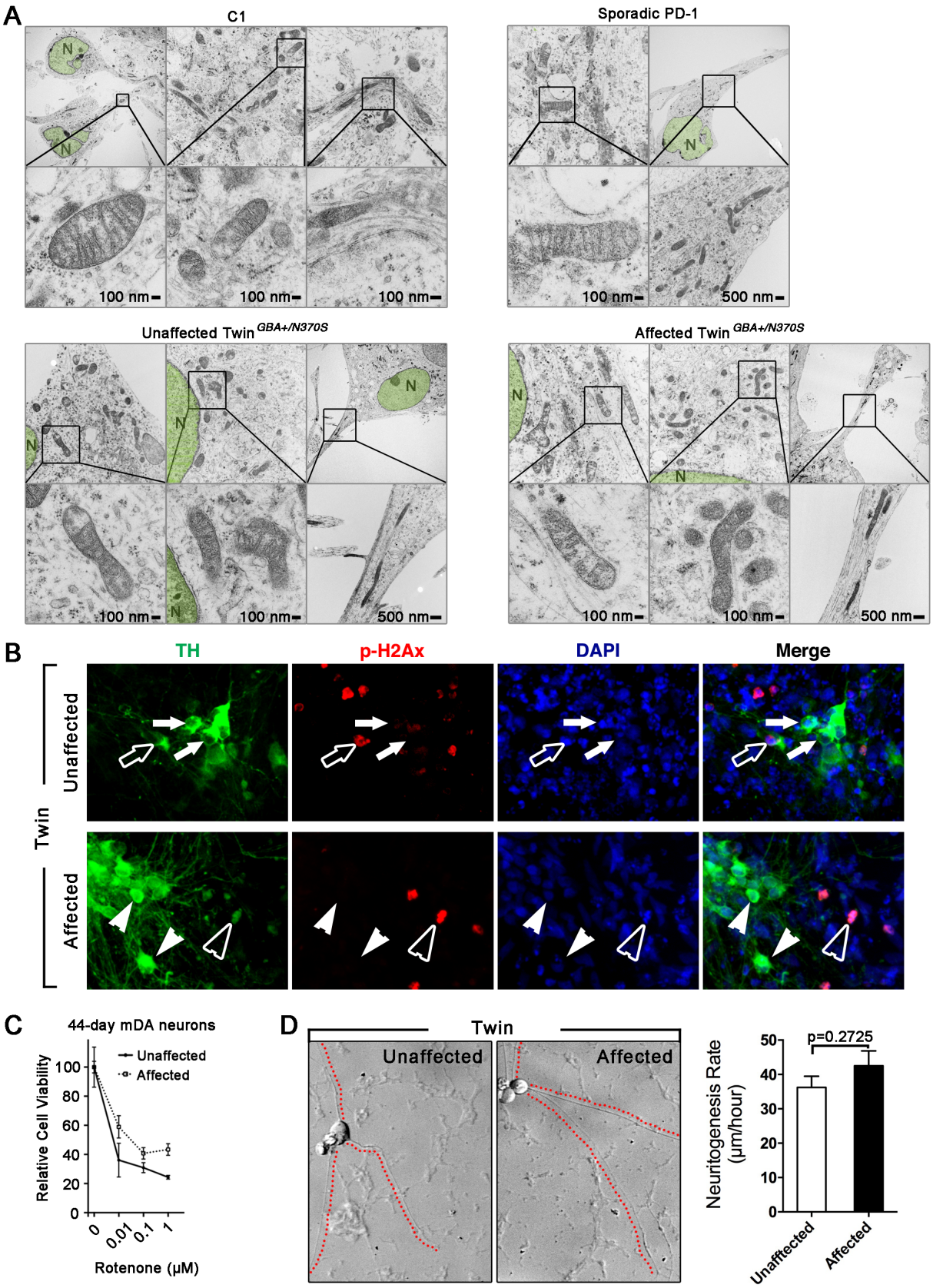

A
C1
Sporadic PD-1
Unaffected Twin GBA+/N370S
Affected Twin GBA+/N370S
N
100 nm
500 nm
B
TH
p-H2Ax
DAPI
Merge
Twin
Unaffected
Affected
C
44-day mDA neurons
Unaffected
Affected
Relative Cell Viability
100
80
60
40
20
0
0
0.01
0.1
1
Rotenone (μM)
D
Twin
Unaffected
Affected
Neuritogenesis Rate (μm/hour)
p=0.2725
50
40
30
20
10
0
Unaffected
Affected

**Figure S3. Mitochondrial Morphologies, Viability, and Transmission Electron Microscopic Analysis of iPS Cell-derived Neurons, Related to Figure 2.**

(A) Despite a genetic discrepancy in mitochondrial function, TEM analysis revealed normal mitochondrial morphologies in control, sporadic PD-1, non-PD twin and PD-twin iPS cell-derived neurons. Boxes highlight specific regions at higher magnification below each TEM image. N: Nuclear. Scale bars, 100 nm, 500 nm. (B) At ~33 days differentiation, the differentiated cells were stained with the antibodies against TH (green) and p-H2Ax (Red). Solid arrows or arrowheads indicate the viable mDA neurons negative for p-H2Ax, while hollow arrows and arrowheads indicate the dead mDA neurons positive for p-H2Ax. (C) Neuronal viability assay post rotenone treatment at three concentrations (0.01, 0.1, 1 μM). At ~44 days post differentiation, 50,000 $CD56^{+}/CD24^{+}/CD15^{-}/CD184^{-}$ cells were seeded into 96-well plate. After 1-day of recovery, cells were exposed to rotenone. Twenty-four hours later, the cell viability was determined by the PrestoBlue™ reagent assay (n = 6 wells per cell line). (D) The neurite outgrowth was captured in real time using VivaView® FL Incubator Fluorescence Microscope, and the neuritogenesis rate was quantitatively calculated (*t*-test, n = 6 cells captured by the microscope).

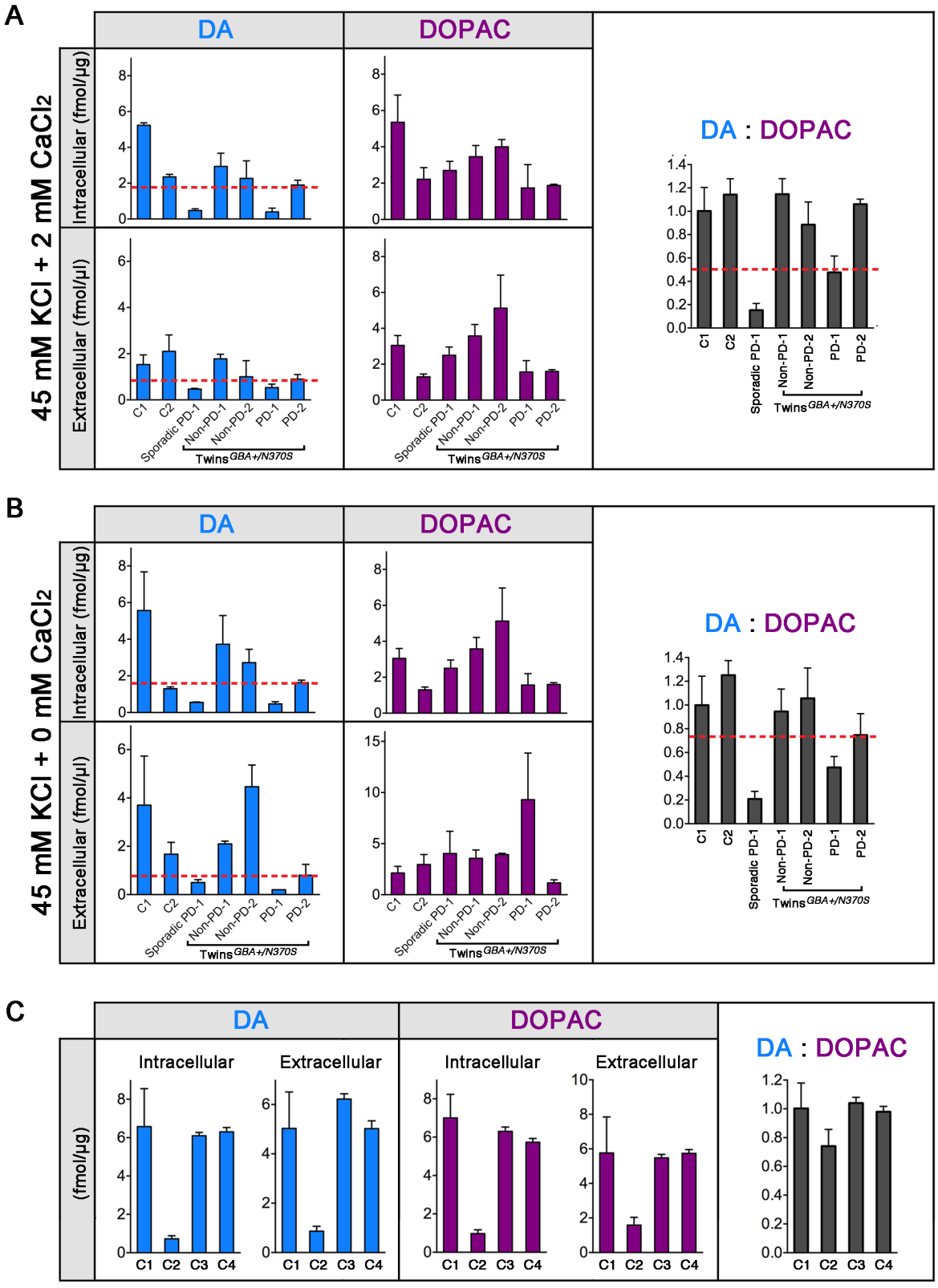

A
DA
DOPAC
DA : DOPAC
45 mM KCl + 2 mM CaCl2
Intracellular (fmol/µg)
Extracellular (fmol/µl)
C1
C2
Sporadic PD-1
Non-PD-1
Non-PD-2
PD-1
PD-2
Twins GBA+/N370S
B
DA
DOPAC
DA : DOPAC
45 mM KCl + 0 mM CaCl2
Intracellular (fmol/µg)
Extracellular (fmol/µl)
C1
C2
Sporadic PD-1
Non-PD-1
Non-PD-2
PD-1
PD-2
Twins GBA+/N370S
C
DA
DOPAC
DA : DOPAC
(fmol/µg)
Intracellular
Extracellular
Intracellular
Extracellular
C1
C2
C3
C4

**Figure S4. HPLC Analysis in iPS Cell-derived Neurons Treated with KCl Solution with and without $Ca^{2+}$, and in iPS Cell-derived Neurons of C3 and C4, Related to Figure 3.**

Compiled HPLC data show intra- and extracellular DA (left panels) and DOPAC (middle panels) concentrations between lines and DA:DOPAC (right panel) ratios in iPS cell-derived neurons treated with 45 mM KCl and 2 mM $CaCl_2$ (A), or 45 mM KCl only (B). The lower DA levels (below the dash red line) are indicative of a pathologic condition. (C) Compiled HPLC data show intra- and extracellular DA (left panels) and DOPAC (middle panels) concentrations between healthy control lines and DA:DOPAC (right panel) ratios. n = 3-6 biological replicates.

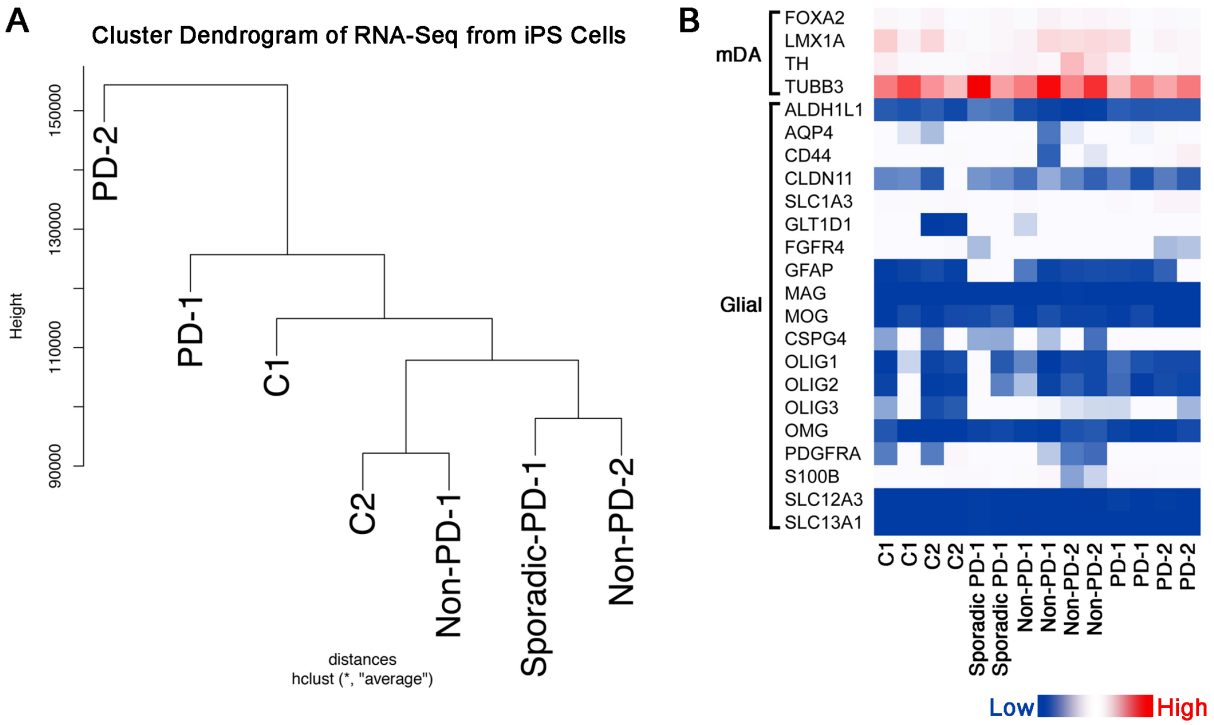



**Figure S5. RNA-seq Profile of 7 iPS Cell Lines and mDA Neuronal/Glial Genes Pattern in the Purified Neuronal Population, Related to Figure 4.**

(A) Hierarchical analysis of 7 iPS cell lines. (B) FPKM values of mDA neuronal and glial genes were extracted from the RNA sequencing analysisof purified neurons. Heat map generated in Microsoft's Excel.

## SUPPLEMENTAL TABLES

**Table S1. Background of Subjects Studied in This Paper, Related to Figure 1.** Seven subjects—a man with a five-year history of PD (PD-1, 2), his MZ twin brother without PD, one sporadic PD patient (Sporadic PD-1), and four healthy subjects (C1, C2, C3, and C4)—were recruited for this study.

| ID | Gender | Age | Age of PD onset | Biopsy Site | Height | Weight (lbs) | Ethnicity | Significant medical Comorbidities | Family History of PD |
|---|---|---|---|---|---|---|---|---|---|
| 11C (C1) | M | 37 | | Left forearm | 6'1" | 170 | Caucasian (Not Hispanic or Latino) | None reported | None |
| 10001.130.01 (C2) | M | 54 | | Left Inner Arm | 6'4" | 220 | Caucasian (Not Hispanic or Latino) | None reported | Mother, maternal grandfather |
| 10005.433 (C3) | M | 36 | | Arm | 6'1" | 160 | African American | None reported | None |
| 10005.433 (C4) | M | 54 | | Arm | 5'10" | 190 | All Paternal and Maternal Parents: Western European and Ashkenazi Jew | None reported | None |
| 10005.117.01 (Sporadic PD-1) | F | 65 | 61 | Unknown | 5'3" | 110 | Paternal Grandfather: Western European; Paternal Grandmother: Not sure; Maternal Grandfather: Anglo-Saxon; Maternal Grandmother: Anglo-Saxon | None reported | Brother, paternal grandmother |
| 10001.197.01 (Unaffected Twin with GBA+/N370S) | M | 68 | | Right Upper Arm | 5'10" | 175 | Caucasian (Not Hispanic or Latino) | Gout; hyperlipidemia, | Monozygotic twin, brother; maternal aunt |
| 10001.198.01 (affected with GBA+/N370S) | M | 68 | 63 | Right Upper Arm | 5'10" | 162 | Caucasian (Not Hispanic or Latino) | Non-motor symptoms included REM sleep behavior disorder and hyposmia; Excellent response to levodopa monotherapy, and no other PD medications | Maternal aunt |

**Table S2. Summary of Whole Exome Sequencing in Fibroblasts, Related to Figure 1.**

Whole exomes (60× Germline) from a set of twins' skin fibroblasts were sequenced on an Illumina 2000/2500 V3 Instrument. Fibroblast of FB197 is derived from the unaffected twin, and FB198 is from the affected one.

| | | FB197 (Unaffected Twin) | FB198 (Affected Twin) |
|---|---|---|---|
| **Coding** | All_SNV | 17373 | 17325 |
| | All_known | 17149 | 17127 |
| | All_novel | 224 | 198 |
| | Known:ti/tv | 13040/4109 | 13025/4102 |
| | Known:ti/tv-ratio | 3.174 | 3.175 |
| | Novel:ti/tv | 162/62 | 146/52 |
| | Novel:ti/tv-ratio | 2.613 | 2.808 |
| | Silent | 9113 | 9076 |
| | Missense | 7919 | 7912 |
| | Nonsense | 55 | 52 |
| | Readthrough | 8 | 9 |
| | Homozygous | 7040 | 7036 |
| | Heterozygous | 10333 | 10289 |
| **Noncoding** | All_SNV | 21938 | 21928 |
| | All_known | 21585 | 21601 |
| | All_novel | 353 | 327 |
| | Known:ti/tv | 14952/6633 | 14979/6622 |
| | Known:ti/tv-ratio | 2.254 | 2.262 |
| | Novel:ti/tv | 234/119 | 219/108 |
| | Novel:ti/tv-ratio | 1.966 | 2.028 |
| | Silent | 0 | 0 |
| | Missense | 0 | 0 |
| | Nonsense | 0 | 0 |
| | Readthrough | 0 | 0 |
| | Homozygous | 8865 | 8888 |
| | Heterozygous | 13073 | 13040 |
| **Reads** | #Raw_Reads | 51030408 | 51746300 |
| | #Mapped_Reads | 50423337 | 51140553 |
| | #Bases_mapped_on_Target | 3560435732 | 3590646205 |
| | Avg_depth | 69.3 | 69.89 |
| | Median_depth | 56 | 57 |
| | D1 (%) | 99.9 | 99.8 |
| | D5 (%) | 99 | 98.8 |
| | D10 (%) | 96.8 | 96.5 |
| | D15 (%) | 93.2 | 93 |
| | D20 (%) | 88.7 | 88.5 |
| | %Mapped | 98 | 98 |
| | %Reads_mapped_on_target | 70 | 70 |

**Table S3. Overview of 82 PD SNVs Shared in Fibroblasts from Twins, Related to Figure 1.**

Cross-referencing with the previously published PD gene databases, we identified 82 PD-related SNVs including information on genetic locus, reference SNP ID numbers, chromosomal location, allele contrast, full gene name, missense, and odd ratio.

**Table S3. Overview of 82 PD SNVs Shared in Fibroblasts from Twins, Related to Figure 1.**

| Genetic locus | Polymorphism | Location (hg18) | Allele contrast | Gene | Missense | OR (95% CI) |
|---|---|---|---|---|---|---|
| GBA | rs76763715 | chr1:155204239 | A vs. G | glucosidase, beta, acid | N360T | 3.40 (1.50-7.40) |
| *MTHFR* | rs1801131 | chr1:11777063 | C vs. A | methylenetetrahydrofolate reductase (NAD(P)H) | E429G | 0.96 (0.87-1.06) |
| *ARNT* | rs2228099 | chr1:149075513 | G vs. C | aryl hydrocarbon receptor nuclear translocator | - | 1.05 (0.95-1.16) |
| *ATP13A2* | rs3738815 | chr1:17187289 | A vs. G | ATPase type 13A2 | - | 0.98 (0.89-1.07) |
| *SLC41A1* | rs823156* | chr1:204031263 | G vs. A | solute carrier family 41, member 1 | - | 0.86 (0.81-0.91) |
| *PINK1* | rs45530340 | chr1:20832817 | T vs. C | PTEN induced putative kinase 1 | - | 1.03 (0.86-1.24) |
| *EPHX1* | rs1051740 | chr1:224086256 | C vs. T | epoxide hydrolase 1, microsomal (xenobiotic) | Y113H | 1.01 (0.90-1.12) |
| *MTR* | rs1805087 | chr1:235115123 | G vs. A | 5-methyltetrahydrofolate-homocysteine methyltransferase | D919G | 1.04 (0.95-1.13) |
| *EIF2B3* | rs263978 | chr1:45135579 | T vs. C | eukaryotic translation initiation factor 2B, subunit 3 gamma, | - | 0.99 (0.89-1.09) |
| *ELAVL4* | rs2494876 | chr1:50439102 | T vs. C | ELAV (embryonic lethal, abnormal vision, Drosophila)-like 4 (Hu antigen D) | P270S | 1.03 (0.87-1.21) |
| *GPX7* | rs1970951 | chr1:52845042 | T vs. C | glutathione peroxidase 7 | - | 1.07 (0.88-1.31) |
| *LRP8* | rs3820198 | chr1:53565239 | G vs. T | low density lipoprotein receptor-related protein 8 | D46E | 1.06 (0.99-1.13) |
| *GLIS1* | rs797906* | chr1:53963283 | A vs. C | GLIS family zinc finger 1 | - | 1.05 (1.02-1.09) |
| *TTC22* | rs1147984 | chr1:55039433 | C vs. T | tetratricopeptide repeat domain 22 | - | 1.04 (0.91-1.18) |
| *USP24* | rs487230 | chr1:55313762 | T vs. C | ubiquitin specific peptidase 24 | V2468A | 0.91 (0.82-1.02) |
| *NDUFB8* | rs1800662 | chr10:102279068 | A vs. C | NADH dehydrogenase (ubiquinone) 1 beta subcomplex | - | 1.03 (0.91-1.16) |
| *GSTO1* | rs4925 | chr10:106012779 | A vs. C | glutathione S-transferase omega 1 | A112D | 0.92 (0.82-1.03) |
| *GSTO2* | rs156697 | chr10:106029175 | C vs. T | glutathione S-transferase omega 2 | N114D | 0.96 (0.89-1.03) |
| *TH* | rs6356 | chr11:2147527 | A vs. G | tyrosine hydroxylase | V112M | 1.01 (0.94-1.09) |
| *GRIN2B* | rs1806201 | chr12:13608775 | T vs. C | glutamate receptor, ionotropic, N-methyl D-aspartate 2B | - | 0.97 (0.88-1.07) |
| *LRRK2* | rs10878245 | chr12:38918058 | A vs. G | leucine-rich repeat kinase 2 | - | 0.99 (0.93-1.06) |
| *LRRK2* | rs7955902 | chr12:38931524 | A vs. C | leucine-rich repeat kinase 2 | - | 1.01 (0.94-1.08) |
| *LRRK2* | rs7966550 | chr12:38974962 | C vs. T | leucine-rich repeat kinase 2 | - | 0.94 (0.86-1.02) |
| *LRRK2* | rs1427263 | chr12:39000101 | G vs. T | leucine-rich repeat kinase 2 | - | 0.95 (0.87-1.03) |
| *LRRK2* | rs11176013 | chr12:39000140 | A vs. G | leucine-rich repeat kinase 2 | - | 1.05 (0.91-1.21) |
| *LRRK2* | rs11564148 | chr12:39000168 | A vs. T | leucine-rich repeat kinase 2 | S1647T | 1.21 (1.10-1.33) |
| *LRRK2* | rs10878371 | chr12:39002527 | T vs. C | leucine-rich repeat kinase 2 | - | 0.95 (0.87-1.04) |
| *LRRK2* | rs10878405 | chr12:39028521 | A vs. G | leucine-rich repeat kinase 2 | - | 1.07 (1.00-1.15) |
| *LRRK2* | rs3761863 | chr12:39044919 | T vs. C | leucine-rich repeat kinase 2 | M2397T | 0.94 (0.87-1.01) |
| *MTIF3* | rs7669 | chr13:26907851 | T vs. C | mitochondrial translational initiation factor 3 | D266E | 0.95 (0.86-1.06) |
| *GCH1* | rs841* | chr14:54380242 | T vs. C | GTP cyclohydrolase 1 | - | 0.94 (0.88-1.00) |
| *TP53BP1* | rs2602141* | chr15:41511938 | C vs. A | tumor protein p53 binding protein 1 | K1141Q | 1.05 (0.98-1.11) |
| *B2M* | rs7151* | chr15:42797304 | C vs. G | beta-2-microglobulin | - | 0.91 (0.80-1.02) |
| *CYP19A1* | rs10046 | chr15:49290278 | T vs. C | cytochrome P450, family 19, subfamily A, polypeptide 1 | UTR-3 | 1.01 (0.92-1.11) |
| *CYP1A1* | rs1048943 | chr15:72800038 | G vs. A | cytochrome P450, family 1, subfamily A, polypeptide 1 | I462L | 0.83 (0.64-1.09) |
| *CYP1A2* | rs2470890 | chr15:72834479 | C vs. T | cytochrome P450, family 1, subfamily A, polypeptide 2 | - | 0.95 (0.88-1.03) |
| *IREB2* | rs954144 | chr15:76517646 | T vs. C | iron-responsive element binding protein 2 | UTR-5 | 0.97 (0.85-1.12) |
| *LRRK1* | rs11853661 | chr15:99415837 | T vs. C | leucine-rich repeat kinase 1 | - | 0.96 (0.87-1.05) |
| RAI1 | rs11649804 | Chr17:17637480 | C vs. A | retinoic acid induced 1 | P165T | 0.866 (0.82 - 0.92) |
| SREBF1 | rs11868035 | Chr17:17655826 | G vs. A | sterol regulatory element binding transcription factor 1 | UTR-3 | 0.851 (0.80 - 0.90) |
| C17orf39 | rs2955355 | Chr17:17889200 | A vs. G | chromosome 17 open reading frame 39 | - | 0.887 (0.84 - 0.94) |
| *NOS2* | rs1060826 | chr17:23113994 | A vs. G | nitric oxide synthase 2, inducible | - | 1.03 (0.95-1.12) |
| *PLEKHM1* | rs11012 | chr17:40869224 | A vs. G | leckstrin homology domain containing, family M | UTR-3 | 0.79 (0.75-0.85) |
| *CRHR1* | rs1396862 | chr17:41258778 | T vs. C | corticotropin releasing hormone receptor 1 | - | 0.78 (0.72-0.85) |
| *CRHR1* | rs16940665 | chr17:41263677 | C vs. T | corticotropin releasing hormone receptor 1 | - | 0.78 (0.72-0.85) |
| *CRHR1* | rs16940676 | Chr17:41266817 | G vs. A | corticotropin releasing hormone receptor 1 | - | *0.773 (0.72 - 0.83)* |
| *CRHR1* | rs1876830 | Chr17:41267133 | C vs. T | corticotropin releasing hormone receptor 1 | - | 0.773 (0.72 - 0.83) |
| *MAPT* | rs1052553 | chr17:41429726 | G vs. A | microtubule-associated protein tau | - | 0.79 (0.75-0.84) |
| *NSF* | rs183211 | rs16940676 | A vs. G | N-ethylmaleimide-sensitive factor | - | 0.81 (0.77-0.86) |
| *NSF* | rs199533 | chr17:42184098 | T vs. C | N-ethylmaleimide-sensitive factor | - | 0.77 (0.74-0.81) |
| *NDUFV2* | rs906807 | chr18:9107867 | T vs. C | NADH dehydrogenase (ubiquinone) flavoprotein 2, | V29A | 0.95 (0.85-1.07) |
| *ICAM1* | rs5498 | chr19:10256683 | G vs. A | intercellular adhesion molecule 1 | K469E | 0.97 (0.89-1.07) |
| *NR4A2* | rs12803* | chr2:156890171 | T vs. G | nuclear receptor subfamily 4, group A, member 2 | UTR-3 | 1.03 (0.98-1.08) |
| *GIGYF2* | rs3816334 | chr2:233417050 | A vs. G | GRB10 interacting GYF protein 2 | - | 1.01 (0.92-1.11) |
| *COMT* | rs4818 | chr22:18331207 | G vs. C | catechol-O-methyltransferase | - | 1.08 (0.98-1.18) |
| *COMT* | rs4680 | chr22:18331271 | A vs. G | catechol-O-methyltransferase | V158M | 0.96 (0.89-1.04) |
| *ADORA2A* | rs5751876* | chr22:23167301 | T vs. C | adenosine A2a receptor | - | 0.99 (0.95-1.03) |
| *DRD3* | rs6280* | chr3:115373505 | G vs. A | dopamine receptor D3 | G9S | 1.03 (0.99-1.07) |
| LARP1B | rs2306054 | Chr4:129231631 | A vs. G | La ribonucleoprotein domain family, member 1B | - | 0.892 (0.84 - 0.94) |
| LARP1B | rs12645577 | Chr4:129320093 | A vs. G | La ribonucleoprotein domain family, member 1B | R660H | 0.893 (0.84 - 0.95) |
| *BST1* | rs3213710* | chr4:15326419 | G vs. A | bone marrow stromal cell antigen 1 | - | 0.89 (0.85-0.92) |
| *UCHL1* | rs5030732* | chr4:40954390 | A vs. C | ubiquitin carboxyl-terminal esterase L1 | S18Y | 0.94 (0.91-0.98) |
| FAM47E | rs1542096 | Chr4:77423524 | T vs. C | family with sequence similarity 47, member E | - | 0.891 (0.84 - 0.94) |
| *UNC5C* | rs2241743 | chr4:96310547 | C vs. T | UNC5C unc-5 homolog C (C. elegans) | - | 1.02 (0.94-1.11) |
| *SOD2* | rs4880* | chr6:160033862 | C vs. T | superoxide dismutase 2, mitochondrial | V16A | 1.03 (0.99-1.08) |
| *PARK2* | rs1801582* | chr6:161727845 | G vs. C | parkinson protein 2, E3 ubiquitin protein ligase (parkin) | V380L | 0.96 (0.89-1.03) |
| *PARK2* | rs4709583 | chr6:162542294 | T vs. C | parkinson protein 2, E3 ubiquitin protein ligase (parkin) | - | 0.97 (0.79-1.19) |
| *VEGFA* | rs2010963 | chr6:43846328 | C vs. G | vascular endothelial growth factor A | UTR-5 | 1.04 (0.93-1.15) |
| *PLA2G7* | rs1051931* | chr6:46780902 | T vs. C | phospholipase A2, group VII (platelet-activating factor acetylhydrolase | V379A | 0.99 (0.95-1.03) |
| KLHL7 | rs15775 | Chr7:23131226 | C vs. T | kelch-like 7 (Drosophila) | - | 0.888 (0.84 - 0.94) |
| *ABCB1* | rs1128503 | chr7:87017537 | T vs. C | ATP-binding cassette, sub-family B (MDR/TAP), member 1 | - | 0.97 (0.88-1.08) |
| *PON1* | rs662* | chr7:94775382 | G vs. A | paraoxonase 1 | Q192R | 0.96 (0.93-0.99) |
| *FGF20* | rs1721100 | chr8:16894869 | C vs. G | fibroblast growth factor 20 | UTR-3 | 0.96 (0.89-1.04) |
| *NAT2* | rs1041983 | chr8:18302075 | T vs. C | N-acetyltransferase 2 (arylamine N-acetyltransferase) | - | 1.04 (0.94-1.15) |
| *NAT2* | rs1801280 | chr8:18302134 | C vs. T | N-acetyltransferase 2 (arylamine N-acetyltransferase) | I114T | 1.14 (0.88-1.49) |
| *NAT2* | rs1799929 | chr8:18302274 | T vs. C | N-acetyltransferase 2 (arylamine N-acetyltransferase) | - | 0.97 (0.87-1.09) |
| *NAT2* | rs1799930 | chr8:18302383 | A vs. G | N-acetyltransferase 2 (arylamine N-acetyltransferase) | R197Q | 1.05 (0.95-1.17) |
| *NAT2* | rs1208 | chr8:18302596 | G vs. A | N-acetyltransferase 2 (arylamine N-acetyltransferase) | R268K | 1.03 (0.87-1.22) |
| *LMX1B* | rs2277158 | chr9:128493050 | G vs. A | LIM homeobox transcription factor 1, beta | - | 0.93 (0.81-1.08) |
| *LMX1B* | rs13295990 | chr9:128495408 | C vs. G | LIM homeobox transcription factor 1, beta | - | 0.94 (0.82-1.08) |
| *MAOA* | rs6323 | chrX:43475980 | G vs. T | monoamine oxidase A | - | 1.09 (0.95-1.26) |
| *MAOB* | rs1799836 | chrX:43512943 | G vs. A | monoamine oxidase B | - | 1.09 (1.00-1.19) |

## SUPPLEMENTAL EXPERIMENTAL PROCEDURES

### Subjects, Genotyping, Whole Exome Sequencing

Seven subjects—a man with a five-year history of PD (PD-1, 2), his monozygotic twin brother without PD, one sporadic PD patient (Sporadic PD-1), and four healthy control subjects (C1, C2, C3 and C4)—signed the consent form for participation in this study. Their clinical information and any family history of PD are listed in **Table S1**. Fibroblasts from their skin biopsies were grown on 0.1% gelatin-coated plates in FM10 media. DNA was harvested from fibroblasts by QIAamp mini DNA kit (Qiagen) and screened for known PD genes mutations using Columbia Udall genotyping array (Marder et al., 2010). Whole exomes (60× Germline) were sequenced on an Illumina 2000/2500 V3 Instrument at Columbia Genome Center. The paired-end reads (read size: 101bp) were mapped to the human reference genome NCBI build 37 using bwa (version 0.5.9), allowing up to five mismatched, inserted or deleted bases (indels). The alignment was refined using GATK (version 1.6) by performing local multiple sequence alignment around inferred putative indels and known ones from 1000 genomes project, and base quality score recalibration. Then, germline SNVs and indels were called for samples. In total, we expected about 17,000 to 20,000 coding SNVs, and an average transition to transversion ratio of 3.3 for known coding variants and 2.9 for novel coding variants, which would indicate low false discovery rate.

### Transgene-free iPS Cell Generation and Maintenance

In addition to an already well-characterized control iPS cell line (C1, Harvard Stem Cell Institute), six iPS cell lines were generated from the skin fibroblasts of the aforementioned four

subjects at the New York Stem Cell Foundation (NYSCF) Research Institute. Each MZ twin had two clones from different batches of reprogramming. Using CytoTune® iPS Sendai reprogramming protocol (Life Technologies), we reprogrammed fibroblasts into transgene-free iPS cells. Fibroblasts were plated at $5.0\times 10^4$ cells/12-well well. Viruses coding Oct4, Sox2, Klf4, and c-Myc were thawed on ice, and suspended in FM10 media according to specific virus lot titers (MOI=3). 500 μl virus suspension was added to each well. Twenty-four hours post-infection, 500 μl HuESM was added to each well. 48 hours post-infection and every day thereafter, media was exchanged with HuESM, until clones were picked or FAC-sorted, typically around 10 days. Each colony was picked as a clone, and each clone was expanded for karyotyping (Cell Line Genetics, Madison, WI) at around passage 8. Clones with normal chromosome profile were chosen for this experiment.

All cells were cultured in 37°C, 5% CO2 incubators. Newly infected iPS cells were cultured on irradiated mouse embryonic fibroblasts (MEF) feeder cells (GlobalStem) plated at 2 ~ $2.5\times 10^4$ cells/$cm^2$ and fed with HuESM. Then, cells were adapted (passaged) to Geltrex-coated (Life Technologies) dishes (23 μg protein/$cm^2$) and fed with Freedom Media (Life Technologies)—referred to in the paper as feeder-free conditions. Media was exchanged daily and spontaneous differentiation was removed. During passaging, cells were dissociated with Accutase (Life Technologies) and re-plated at 0.5 - $1\times 10^5$ cells/6 well well. Cells were treated with 10 μM Rho kinase–specific inhibitor (Y-27632, Stemgent, MA) for 24 hours after passaging.

**Characterization of iPS Cells**

All the reprogrammed iPS cell colonies underwent immunostaining for pluripotency markers: Nanog, OCT4, SOX2, SSEA4, Tra-1-60, and Tra-1-81. Viral and endogenous pluripotent genes expression were analyzed using two custom probe sets designed by NanoString Technologies and used with the nCounter Gene Expression Assay (NanoString). Assays were carried out at up to 12 samples at a time, per the NanoString protocol. Samples of 10,000 cells were lysed and RNA hybridized to custom specific molecularly-barcoded reporter and capture probes before processing on the nCounter Prep-station (NanoString). Processed samples were then loaded into an nCounter Digital Analyzer (NanoString) and scanned at high resolution (about 800 frames/sample). nCounter Analyzer data were analyzed on NanoString's nSolver software, normalizing counts to three housekeeping genes, ACTB, POLR2A, and ALAS1. Probe sets include a Pluripotency Codeset of 25 genes indicative of pluripotency – both endogenous and residual viral – and a Lineage Codeset including multiple targets indicative of separate germ layers is previously described (Kahler et al., 2013).

Embryoid bodies (EBs) were formed by adding cell suspension of $1.0\times 10^6$ cells in EB Media to an ultra-low attachment 6-well plate. Cells were allowed to aggregate at the bottom of the well by gravity and media is exchanged every 48 hours. EBs were harvested after 14 - 21 days for cryosection (Columbia Pathology Core Lab) and stained against three germ layers markers (AFP, SMA and TUJ1). RNA extracted from EBs was also analyzed by differentiation scorecard (NanoString).

**Midbrain Neuronal Differentiation and Characterization**

Dopaminergic neuronal differentiation procedure consists of 11-day neural induction and dopaminergic neuronal pattering, as described previously (Kriks et al., 2011). Slightly different

from their protocol, iPS cells at a higher density of 1.5 $\times 10^6$ were seeded when initializing the neuronal differentiation. After 11 days, cultures were passaged into plates coated sequentially with 1:10 Poly-L-ornithine solution in water (P4957, Sigma Aldrich) and 0.333 mg/ml Matrigel matrix (3542777, BD Biosciences). Differentiated cells were stained with SOX1 (mDA progenitors marker), FOXA2, LMX1 (midbrain markers), TUJ1 (neuronal marker), TH (dopaminergic neuronal marker), and GIRK2 (substantia nigra neuronal marker). The electrical characteristics of ~ 45-day differentiated cells on 13-mm diameter coverslips (sterile, thermanox plastic, Thermo Scientific, NY) were studied by the whole-cell patch clamp recordings (Nestor et al., 2013).

**Electrophysiology and Multi-electrode Array Recordings**

Whole-cell patch clamp was performed on neurons plated on 13 mm plastic coverslips (Thermanox, Thermo) from days 50-56. Recordings were performed at 22°C under continuous perfusion (1 ml $min^{-1}$) with a bath solution containing: NaCl (119 mM), KCl (5 mM), HEPES (20 mM), glucose (30 mM), $MgCl_2$ (2 mM), $CaCl_2$ (2 mM) and glycine (0.001 mM). The osmolality of the solution was adjusted to 310 mOsm with sucrose, and the pH was adjusted to 7.3 with KOH. Glass micropipettes with resistances between 8.5-9.5 MΩ were backfilled with an intracellular solution containing (in mM): 130 mM K-gluconate, 10 mM KCl, 2 mM Mg-ATP, 0.2 mM Li-GTP, 0.6 mM $CaCl_2$, 5 mM $MgCl_2$, 0.6 mM Ethylene glycol-bis(2-aminoethylether)-N,N,N′,N′-tetraacetic acid (EGTA), and 5 mM HEPES titrated to a pH of 7.1 and an osmolality of 310. Cells were viewed with a BX51W1 microscope using DIC optics (Olympus) and recordings were made using an Axon Multiclamp 700B amplifier (signals were filtered at 1 kHz and sampled at 10 kHz) and a Digidata 1440a acquisition system, with pClamp 10 software

(Molecular Devices). Data analysis was performed using Clampfit 10 software (Molecular Devices, Sunnyvale, CA) and Matlab 8.0 (MathWorks, Natick, MA). To verify the presence of action potentials current clamp steps were given: 0 pA for 100 ms, steps from -60 pA to +120 pA, 20 pA each, for 1 second. To test for the presence of $Na^+$ and $K^+$ currents, voltage-clamp steps were given from a starting potential of -70 mV: -90 mV to +20 mV in 10 mV increments and 1 second in duration.

Both twins' neurons were plated in 4-wells each of a 12-well MEA plate from Axion Biosystems. Each well contained an 8 x 8 grid of 30 nm circular nanoporous platinum electrodes embedded in the cell culture substrate, with a pole-to-pole electrode spacing of 200 μM. In preparation for cell seeding, the wells were treated with 0.1% polyethylenimine (PEI) in sodium borate buffer, pH 8.4. Wells were then coated in laminin (6 μg/mL) and neurons were plated at 650,000 neurons/well dotted on the electrode grid. Cells were then fed every other day using standard culture media. Extracellular recordings of spontaneous action potentials were performed in culture medium at 37°C using a Maestro MEA system and AxIS software (Axion Biosystems). Data were sampled at rate of 12.5 kHz with a hardware frequency bandwidth of 200-5000 Hz, and filtered again in software using a 200-2500 Hz single-order Butterworth band-pass filer to remove high frequency noise before spike detection. The threshold for spike detection was set to 5.25 times the rolling standard deviation of the filtered field potential on each electrode. Ten-minute recordings were used to calculate average spike rate for the well, and the number of active electrodes in a well ("Active Electrodes"), which was defined as the number with spike rates ≥ 0.5/s. Spike time stamps were exported to Neuroexplorer (NEX Technologies) for creation of spike raster plots.

**Immunohistochemistry and Western Blot**

Immunohistochemistry: Cells were fixed with 4% PFA for 10 minutes and blocked with blocking buffer (PBS with 0.25% TritonX-100, 2% bovine serum albumen, and 1% sodium azide) for 30 minutes. Cells were then stained with primary antibody diluted according to manufacturer recommendation in blocking buffer overnight at 4°C. Cells were washed thrice with 0.1% PBST and stained with secondary antibody (1:400 in blocking buffer) for two hours at 25°C. Cells were washed thrice again with 0.1% PBST and either mounted with Aqua-Mount (Thermo) or kept in PBS, protected from light. Images were taken on an Olympus IX71 inverted microscope or a Zeiss LSM 5 Pascal laser scanning microscope.

Western Blot: Cells were harvested in Pierce RIPA (Thermo Fisher Scientific) with 1% protease and 1% phosphatase inhibitors (Thermo Fisher Scientific) at 5 - 10× $10^6$ cells/ml. Samples were sonicated for 1 minute then centrifuged at full speed for 10 minutes. Pellets were discarded. Protein concentrations were calculated using Pierce BSA Protein assay (Thermo Fisher Scientific) and a BioTech plate reader per manufacturer instructions. Protein concentrations were standardized to 20 ng protein/16.6 μl RIPA. 6× loading buffer (Morganville Scientific) was added to each sample and boiled at 100°C for 5 minutes. 20 μl was loaded into each well of a 4 - 12% Bis-Tris polyacrylamide gel (Life Technologies). Samples with SeeBlue® Pre-stained Protein Standard (Life Technologies) were run for 35 minutes at 200 V. Proteins are transferred to nitrocellulose membrane using the iBlot Nitrocellulose transfer kit (Invitrogen).

Protein-bound membranes were then blocked for 30 minutes with 5% non-fat dry milk (BioRad) in 0.1% tris-buffered saline with tween (TBST). Membranes were then incubated with primary antibody diluted per recommendation in blocking buffer overnight at 4°C on a shaker.

Membranes were washed thrice with TBST and treated with HRP-conjugated secondary antibodies diluted 1:3000 in blocking buffer for two hours. Membranes were washed thrice with TBST and once with tris-buffered saline (TBS) and membranes are rinsed with 2 ml chemiluminescent solution (GE Healthcare Amersham, or Thermo SuperSignal West Femto for weaker signals). Membranes were immediately read in a Kodak Image Station 4000mm PRO. Protein levels were quantified with Carestream Molecular Imaging Software, using β-Actin or α-Tubulin for loading control. Below are the primary antibodies used in this study.

| Antibody | Species | IF | WB | Source | Catalog |
|---|---|---|---|---|---|
| AFP | Rabbit | 1:1000 | | DAKO | A000829 |
| FOXA2 | Rabbit | 1:1000 | | Abcam | ab40874 |
| FOXA2 | Goat IgG | 1:100 | | Santa Cruz | sc-6554 |
| GBA | Mouse IgG2a | 1:200 | 1:800 | Abnova | H00002629-M01 |
| GIRK2 | Rabbit | 1:100 | | Alomone Labs | APC-006 |
| LMX-1 | Rabbit | 1:1000 | | Millipore | AB10533 |
| MAP2 | Chicken | 1:5000 | | Abcam | Ab5392 |
| MAO-B | Mouse IgG1κ | | 1:200 | MABN304 | Millipore |
| NANOG | Rabbit | 1:100 | | Cell Signaling Tech | #4903 |
| NESTIN | Mouse IgG1 | 1:500 | | Millipore | mab5326 |
| OCT-4 | Rabbit | 1:250 | | Stemgent | 09-0023 |
| p-H2Ax | Mouse | 1:2000 | | Millipore | 05-636 |
| PITX3 | Rabbit | 1:400 | | Abcam | ab30734 |
| SeV | Rabbit | 1:500 | | MBL International | PD029 |
| SOX-1 | Goat IgG | 1:100 | | R&D | AF3369 |
| SOX-2 | Rabbit | 1:250 | | Stemgent | 09-0024 |
| SSEA4 | Mouse IgG3 | 1:250 | | Abcam | MC813 |
| TH | Rabbit | 1:500 | | Pelfreez | P40101 |
| TH | Sheep | 1:500 | | Pelfreez | p60101 |
| TRA-1-60 | Mouse IgM | 1:250 | | Millipore | MAB4360 |
| TRA-1-81 | Mouse IgM | 1:250 | | Millipore | MAB4381 |
| TUJ1 | Mouse IgG2a | 1:1000 | | Covance | MMS-435P |
| V5 | Mouse IgG2aκ | 1:200 | | Life Tech | R960-25 |
| α-SMA | Mouse IgG2a | 1:1000 | | DAKO | M085101 |
| α-Synuclein | Mouse IgG1 | 1:300 | | Abcam | Ab1903 |
| α-Synuclein | Rabbit | | 1:1000 | Cell Signaling Tech | #2642S |
| α-Tubulin | Mouse IgG1 | | 1:2000 | Abcam | ab7291 |
| β-Actin | Rabbit | | 1:1000 | Abcam | ab8227 |

**Fluorescence-activated Cell Sorting**

We performed the FACS using a combination of surface markers (CD133, a stem/progenitor marker; CD56, a nerve cell adhesion molecule; CD15 and CD184, NSC markers; and CD24, a cell differentiation antigen). Differentiated cell cultures were dissociated with Accutase, neutralized with an equal volume of media and transferred into 15 ml conical tube. After spinning at 800 RPM for 5 minutes, the cell pellet was re-suspended in FACS buffer (DPBS + 0.5% BSA Fraction V, 2mM EDTA, 20mM Glucose, and 100U/mL PenStrep), and filtered through a BD Falcon polypropylene 12 × 75 mm tube with cell strainer cap. Cell suspensions were then incubated in antibody cocktail (CD133-PE (Cat. 130-080-801) from Miltenyi Biotec; CD184-APC (Cat. 555976), CD24-PE (Cat. 555428), CD56-V450 (Cat. 560360), CD15-AF488 (Cat. 560172), from BD, diluted 1:100 in FACS buffer) for 15 minutes at 25°C protected from light. Excess antibodies were washed off and cells were re-suspended in 1-3 ml FACS buffer prior to flow cytometry. To determine the efficiency of the protocol, we used CytoSpin™ Cytocentrifuge (Thermo Fisher Scientific) to deposit $1.0\times 10^5$ cells onto a gelatin-coated glass slide for staining.

**Transmission Electron Microscopic Analysis**

$2.0\times 10^5$ FAC-sorted cells were plated in Lab-Tek permanox chamber slide w/cover (Thermo Scientific Nunc, NY). After 2-3 days recovery, cells were fixed with 0.5% glutaraldehyde in 0.1 M Sorenson's buffer (pH 7.2) for 1 hour. Samples were examined using a JEOL JEM-1200 EXII transmission electron microscope, and imaged at 5,000×, 25,000× and 100,000× magnifications using an ORCA-HR digital camera (Hamamatsu) by a Diagnostic

Service provider at the Department of Pathology and Cell Biology, Columbia University (Shang et al., 2014).

**High-performance Liquid Chromatography**

Neuronal differentiation cultures were FAC-sorted between 32 - 45 days of differentiation to obtain a pure population of mature dopaminergic neurons. These cells were then kept in culture on Poly-L-ornithine solution and Matrigel matrix in 24-well plates (~50 - 200K neurons per well) for a week before being collected in 1.2 M perchloric acid (PCA) and frozen for high-performance liquid chromatography. For each line, three conditions were tested (at least 3 biological triplicates): the addition of Tyrode's solution (118.6 mM NaCl, 3 mM KCl, 2.7 mM HEPES Na Salt, 3.3 mM HEPES salt, 1.2 mM $MgCl_2 \cdot 6H_2O$, 2 mM $CaCl_2$, 10 mM D-Glucose, pH7.2 - 7.4), KCl solution (80 mM NaCl, 40 mM KCl, 2.7 mM HEPES Na Salt, 3.3 mM HEPES salt, 1.2 mM $MgCl_2 \cdot 6H_2O$, 2 mM $CaCl_2$, 10 mM D-Glucose, pH7.2 - 7.4), and KCl without calcium solution (80 mM NaCl, 40 mM KCl, 2.7 mM HEPES Na Salt, 3.3 mM HEPES salt, 1.2 mM $MgCl_2 \cdot 6H_2O$, 10 mM D-Glucose, pH7.2 - 7.4) for five minutes prior to collection. Both media and lysate samples were collected separately for each condition per line. Using the ESA Coulochem II Multi-Electrode Detector—an HPLC system—dopamine and DOPAC levels were determined on a customized program named Igor using a sensitivity range of 2-10nA (Mosharov et al., 2009). The results were then statistically analyzed using Microsoft Excel through normalization of cell counts and conversion of peak reads to numerical expressions that allowed for comparison among cell lines.

**RNA-Seq and Real-time PCR Assay**

RNA was isolated from 7 iPS cell lines and FAC-sorted cells of 14 samples (biological duplicates for each cell line, 7 cell lines in total) using RNeasy Micro Kit (QIAGEN). Quality control of the RNA was carried out with the Agilent Bio-analyzer, Qubit 2.0 at the MPSR of Columbia University. 100 ng of RNA with RIN ≥ 9 were used for generating mRNA-focused libraries using TruSeq RNA Sample Preparation Kit v2 and sequencing on an Illumina 2000/2500 V3 Instrument offered by the Columbia Genome Center. The read depth was 30 million reads with single end, 100bp reads. The reads were mapped to a reference genome (Human: NCBI/build37.2) using Tophat (version 2.0.4) with 4 mismatches (--read-mismatches = 4) and 10 maximum multiple hits (--max-multihits = 10). The relative abundance (aka expression level) of genes and splice isoforms were evaluated using cufflinks (version 2.0.2) with default settings. We test for differentially expressed genes under various conditions using DEseq. The differential expression signature and differential pathways enrichment were identified with a statistical significance ($P < 0.01$). To specifically discover the profile of genes involved in DA synthesis, storage, release, re-uptake, metabolism, and PD pathogenesis in both twins, the average FPKM (Fragments Per Kilobase Of Exon Per Million Fragments Mapped) value from four biological replicates of each twin and their ratio of FPKM (198 affected twin vs. 197 unaffected twin) were calculated. Positive/negative fold change mean an up-regulation and a down-regulation, respectively. 2-fold change was used as an arbitrary cut-off point in this analysis.

Total RNAs from isolated neurons of both twins were reverse transcribed into cDNA using the Promega GoScript Reverse Transcription system. Once the cDNA was obtained, the samples were then run in the Applied Biosystems StepOnePlus Real-Time PCR machine using SYBR Select Master Mix combined with a forward primer

(GGAGACCCTAAACCATGAGATG) and a reverse primer (TGACTGAACCCAAAGGCAC) in the exon 8 region of *MAOB* gene. Using StepOne v2.2.2 Software, we recorded Ct values to quantitatively calculate the *MAOB* gene expression. Pre-designed primer set for *TBP* gene (FW primer: TGCACAGGAGCCAAGAGTGAA; RV primer: CACATCACAGCTCCCCACCA) was used as an internal control.

**GBA-lentiviral Infection**

Neuronal differentiation cultures from both twins (two lines for each twin) were FAC-sorted between 32-45 days of differentiation to obtain a pure population of mature dopaminergic neurons. 250 μl of cell suspension (~ $6.0\times 10^5$ cells) were treated with 250 μl of lenti-virus 7.2 *wild-type* GBA (1: 1 ratio), and the mixture was kept on Poly-L-ornithine solution and Matrigel matrix in 4-well plate. After 3-7 days, these cells were collected for immunohistochemistry, Western blot analysis, or HPLC assay. Methods were kept identical between experiments for reproducibility and effective comparison.

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