

Supplementary Information to Accompany

Precision Modulation of Neurodegenerative Disease-Related Gene Expression in Human iPSC-Derived Neurons

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This PDF file includes:

Supplementary Figure S1

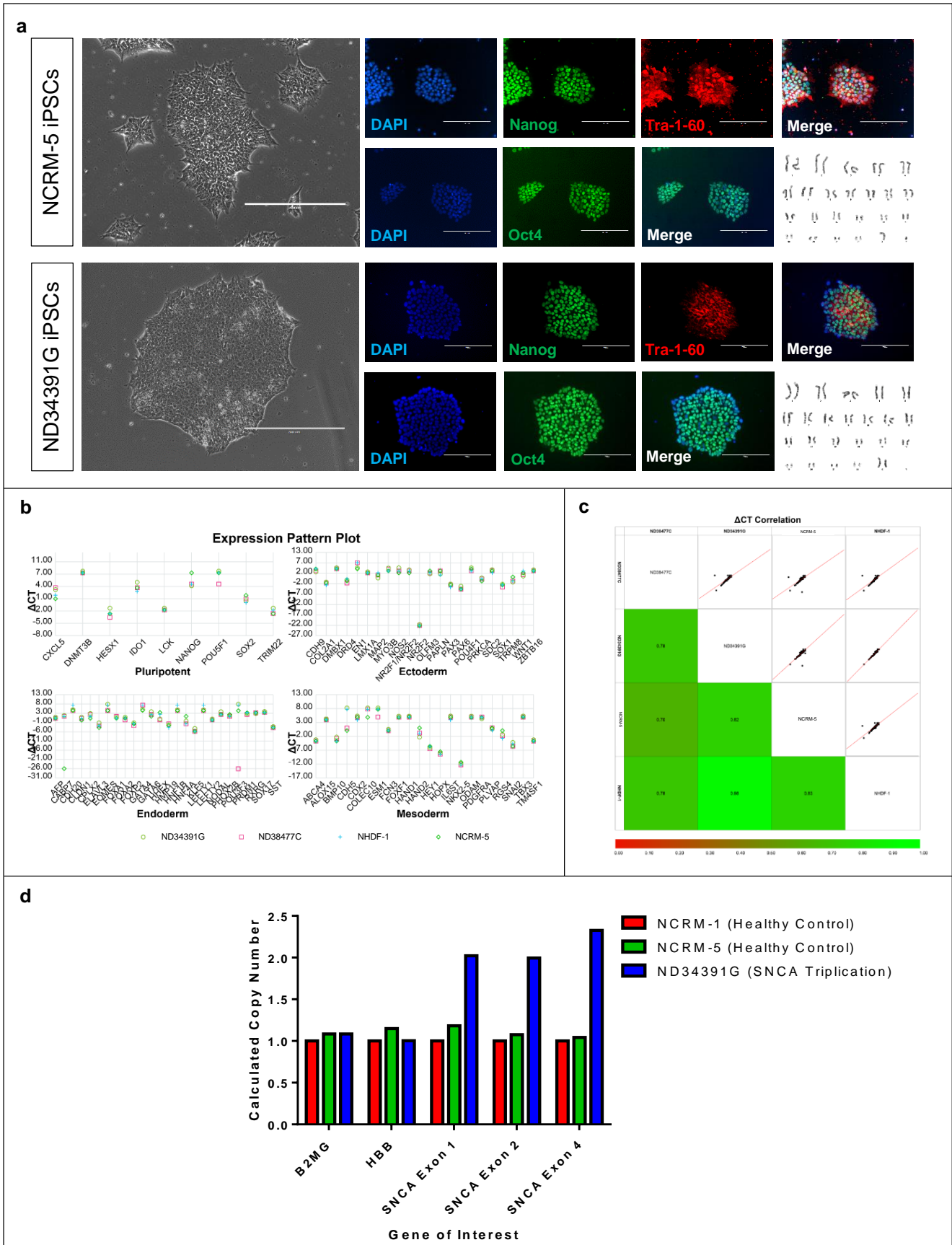
Supplementary Figure S2

Supplementary Table S1

Supplementary Table S2

Supplementary Table S3

Supplementary Methods



Supplementary Figure S1. iPSC Quality Control

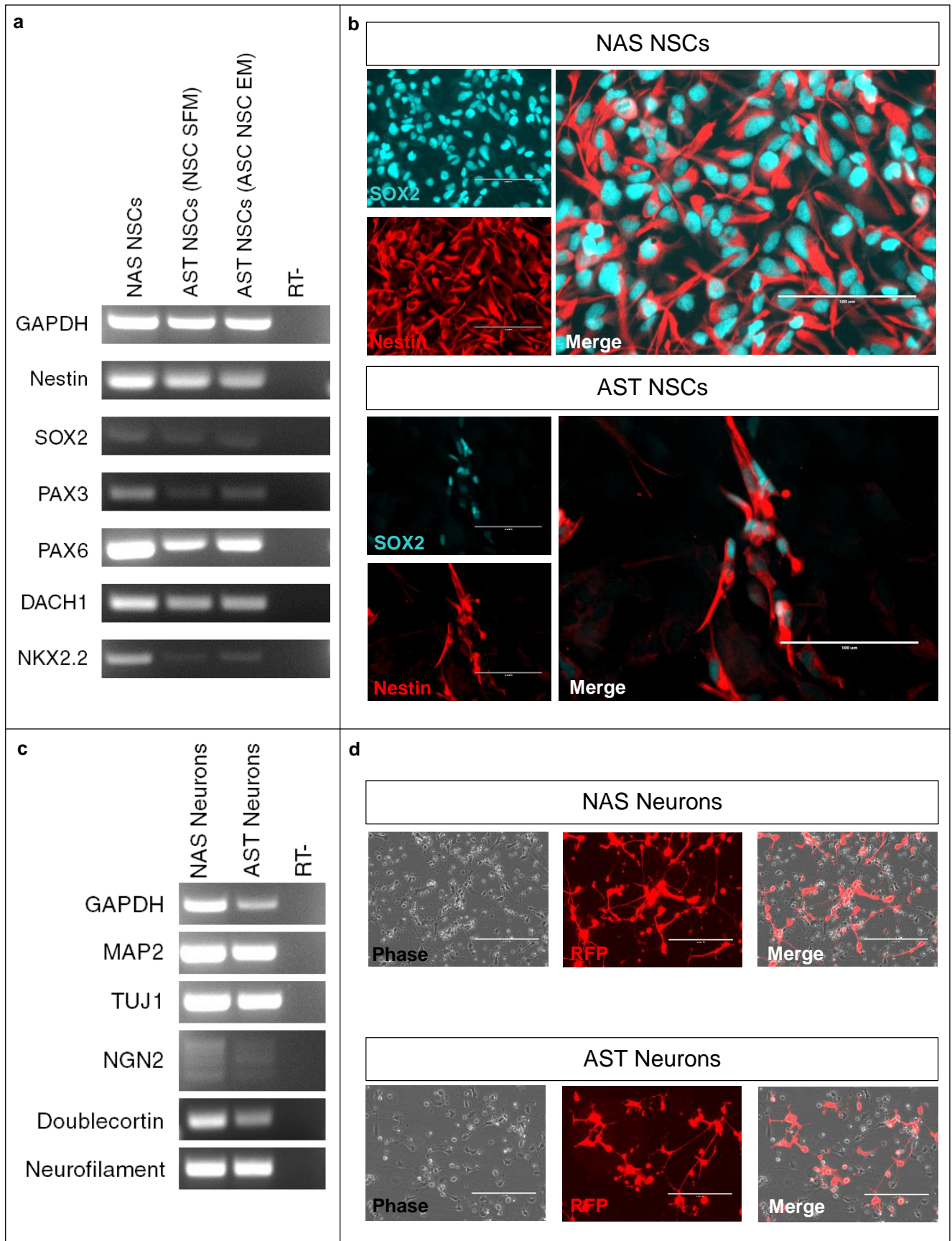
Supplementary Figure S1. iPSC Quality Control

(a) Morphology and pluripotency immunostaining of NCRM-5 (healthy control) and ND34391G (SNCA triplication) iPSCs. Phase images of live iPSCs are shown to the left (scale bars are 400 μm). Upper immunofluorescent micrographs of each cell line show (left to right) DAPI, Nanog, Tra-1-60 and merged images, respectively (scale bars are 200 μm). Lower immunofluorescent micrographs of each cell line show (left to right) DAPI, Oct4, and merged images, respectively (scale bars are 200 μm). To the right of each lower panel are the normal 46,XY and 46,XX g-banded karyotypes of NCRM-5 (healthy control) and ND34391G (SNCA triplication) iPSCs, respectively.

(b) TaqMan hPSC Scorecard gene expression profiling of pluripotency, endodermal, mesodermal and ectodermal fate-specifying genes in NCRM-5 (healthy control) and ND34391G (SNCA triplication) iPSCs compared to other established iPSC lines, ND38477C (PARK2 mutant) and NHDF-1 (healthy control).

(c) Scatterplots and corresponding correlation coefficients of hPSC ScoreCard Ct values for NCRM-5 (healthy control) and ND34391G (SNCA triplication) iPSCs compared to ND38477C (PARK2 mutant) and NHDF-1 (healthy control) iPSCs.

(d) Validation of doubled SNCA copy number in ND34391G (SNCA triplication) iPSCs, compared to NCRM-5 (healthy control) and NCRM-1 (healthy control) iPSCs by Type-it CNV SYBR Green qPCR.



Supplementary Figure S2. Characterization of iPSC-Derived NSCs and Neurons

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(a) RT-PCR expression analysis of NSC markers in NCRM-5, normal alpha-synuclein (NAS) iPSC-derived NSCs and ND34391G, alpha-synuclein triplication (AST) iPSC-derived NSCs. Since AST NSCs were maintained in distinct NSC culture medium (ASC NSC EM), we evaluated whether transitioning them to the same medium as NAS NSCs (NSC SFM) would affect the expression of NSC markers. No such expression alterations were observed. RT- refers to PCR mastermix, including primers, in the absence of cDNA template.

(b) Immunostaining of NAS and AST iPSC-derived NSCs for the NSC markers, SOX2 and Nestin. Scale bars are 100 μ m.

(c) RT-PCR expression analysis of neuronal markers in NAS and AST iPSC-derived neurons. RT- refers to PCR mastermix, including primers, in the absence of cDNA template.

(d) NAS and AST iPSC-derived neurons Neon transfected with a CAG-driven tdTomato transfection control plasmid. Scale bars are 200 μ m.

Sequence Name	Forward	Reverse
SNCA Exon 1	CACCGCGCACCTCACTTCGCGTCG	AAACCGACGCGGAAGTGAGGTGCGC
SNCA Exon 1/2 SD	CACCGCCCGCTAACCTGTGTCGAA	AAACTTCGACGACAGGTTAGCGGGC
SNCA Exon 1/2 SA	CACCGTGAATTCTTTACACCACAC	AAACGTGTGGTGTAAAGGAATTCAC
SNCA Exon 2	CACCGAGCAGCCACAACCTCCCTCCT	AAACAGGAGGGAGTTGTGGCTGCTC
SNCA Exon 4	CACCGTGCTCCCTCCACTGTCTTCT	AAACAGAAGACAGTGGAGGGAGCAC
SNCA TSS1-1	CACCGTCGGAAGATTAGTTAAGCAC	AAACGTGCTTAACTAATCTTCCGAC
SNCA TSS1-2	CACCGAAGCCTTTGCTTTCTGTGCC	AAACGGCACAGAAAGCAAAGGCTTC
SNCA TSS1-3	CACCGTTCCTGGATCACACCAGAA	AAACTTCTGGTGTGATCCAGGAAC
SNCA TSS2-1 (Non-Template)	CACCGCTCCTCCTTCTCCTTCTCCT	AAACAGGAGAAGGAGAAGGAGGAGC
SNCA TSS2-1 (Template)	CACCGCGAGGAGAAGGAGAAGGAGG	AAACCTCCTTCTCCTTCTCCTCGC
SNCA TSS2-2	CACCGCCCCTCTCTTGGGCCCTTC	AAACGAAGGGGCCCAAGAGAGGGGC
SNCA TSS2-3	CACCGCACTTCCGCGTCGCGGCGCT	AAACAGCGCCGCGACGCGGAAGTGC
SNCA TSS3-1	CACCGAACCCCGCGCCAGCCACCCG	AAACCGGGTGGCTGGCGCGGGTTC
SNCA TSS3-2	CACCGCTCAGCTATCTACCCTGAGC	AAACGCTCAGGGTAGATAGCTGAGC
SNCA TSS3-3	CACCGAACAGCAGGCCCAAGTGTGA	AAACTCACACTTGGGCCTGCTGTTC
MAPT TSS-1	CACCGCGAAGAGGGCGCGTTCCTG	AAACAGGAACGCGCCCTCTTCGC
MAPT TSS-2	CACCGTGGGTGGCGGTGACTGCGA	AAACTCGCAGTCACCGCCACCCAC
MAPT TSS-3	CACCGAGCGGCGCTGCTGTTGGTGC	AAACGCACCAACAGCAGCGCCGCTC
HTT TSS-1	CACCGTTGCGTCCAGACGCTGCGC	AAACGCGCAGCGTCTGGGACGCAAC
HTT TSS-2	CACCGCGTCCATCTTGGACCCGTCC	AAACGGACGGTCCAAGATGGACGC
HTT TSS-3	CACCGTGAATGGGGCTCTGGGCCGC	AAACGCGGCCAGAGCCCCATTCAC
APP TSS1-1	CACCGTAACCCCAACGTCAAAGC	AAACGCTTTTGACGTTGGGGTTAC
APP TSS1-2	CACCGAGTGAAGCTTAAAGGAAAT	AAACATTTCTTTAAGCTTCACTC
APP TSS1-3	CACCGAGAGGTGGGGCAGGCGTTTC	AAACGAAACGCCTGCCCCACCTCTC
APP TSS2-1	CACCGCTTGAATCATCCGACCCCGC	AAACGCGGGGTCGGATGATTCAGC

APP TSS2-2 (NGG)	CACCG <u>AGGTGAGTCCTAGGACGCTG</u>	AAAC <u>CAGCGTCCTAGGACTCACCTC</u>
APP TSS2-2 (NAG)	CACCGCTGAGGCTCTAGAAAAGTC	AAACGACTTTTCTAGAGCCTCAGC
APP TSS2-3	CACCG <u>AGAGGGACGGTGCAGGATCA</u>	AAACTGATCCTGCACCGTCCCTCTC
APP TSS3-1	CACCG <u>CGGCGGCGGGGCTCAGAGCC</u>	AAACGGCTCTGAGCCCCGCGCCGC
APP TSS3-2	CACCG <u>ACCGCTGCCGAGGAAACTGA</u>	AAACTCAGTTTCTCGGCAGCGGTC
APP TSS3-3	CACCGCCACCGCCGCCGTCTCCCG	AAACCGGGAGACGGCGGGCGGTGGC
APP TSS4-1	CACCGCCA <u>ACTTCTAAGCTAACAA</u>	AAACTTGTTAGCTTAGAAGTTGGC
APP TSS4-2	CACCG <u>AACTTCTACCACGCACAGCA</u>	AAACTGCTGTGCGTGGTAGAAGTTC
APP TSS4-3	CACCG <u>CAGAGCTTCCATCCTCGGGA</u>	AAACTCCCGAGGATGGAAGCTCTGC
APP TSS5-1	CACCGCGTGTTCTCCAAAAAAGAG	AAACTCTTTTTTTGGAGAACACGC
APP TSS5-2	CACCG <u>CCCACCAGCCTATCCTTCTC</u>	AAACGAGAAGGATAGGCTGGTGGGC
APP TSS5-3	CACCG <u>TTAGAGATTGGACTTTCAGC</u>	AAACGCTGAAAGTCCAATCTCTAAC

Supplementary Table S1. sgRNA Oligonucleotides

Red: *Bbs* I compatible oligonucleotide overhangs

Underlined: inserted G nucleotides, canonical human U6 transcription start site

Black: sgRNA sequence

Primer Name	Forward	Reverse
ACTB qRT-PCR	CACAGAGCCTCGCCTTTG	GCGGCGATATCATCATCCAT
SNCA qRT-PCR	CCTTCTGCCTTTCCACCCT	TCCCTCCTTGGCCTTTGAAA
SNCA CHIP qPCR	AGATAGGGACGAGGAGCACG	CCCGCACGCACCTCACTT
MAPT qRT-PCR	CCTCGCCTCTGTGACTATC	CTCTTGGTCTTGGTGCATGG
HTT qRT-PCR	TCAGCTACCAAGAAAGACCGT	TTCATAGCGATGCCAGAA
APP qRT-PCR	GGTTTGGCACTGCTCCTG	CAGAACATGGCAATCTGGGG
B2MG qRT-PCR [†]	CTCACGTCATCCAGCAGAGA	AGTGGGGTGAATTCAGTGT
HBB qRT-PCR [†]	TTGGACCCAGAGGTTCTTTG	GAGCCAGGCCATCACTAAAG
SNCA Exon 1 qRT-PCR [†]	AAAGGCCAAGGAGGGAGTT	ATCCTAACCCATCACTCATGAAC
SNCA Exon 2 qRT-PCR	AAGGAGGGAGTTGTGGCTG	AGAACACCCCTCTTTTGTCTTTCC
SNCA Exon 4 qRT-PCR [†]	CCTGTGGATCCTGACAATGA	TGCAAGTTGTCCACGTAATGA
GAPDH RT-PCR [‡]	ACCACAGTCCATGCCATCAG	TCCACCACCTGTTGCTGTA
Nestin RT-PCR	GCGGGCTACTGAAAAGTTCC	GCTGAGGGACATCTTGAGGT
SOX2 RT-PCR	AGCATGGAGAAAACCCGGTA	TTTTGCGTGAGTGTGGATGG
PAX3 RT-PCR [†]	GAACACGTTGACAAAAGCA	GCACACAAGCAAATGGAATG
PAX6 RT-PCR [†]	AATAACCTGCCTATGCAACCC	AACTTGAAGTGAAGTGCACAC
DACH1 RT-PCR [†]	GTGGAAAACACCCCTCAGAA	CTTGTTCCACATTCACACCC
NKX2.2 RT-PCR [†]	TGCCCTCTCCTTCTGAACCTTGG	GCGAAATCTGCCACCAGTTG
MAP2 RT-PCR	CCTTCCTCCATTCTCCCTCC	TCCTGGGATAGCTAGGGGT
TUJ1 RT-PCR	TGGACATCTCTCAGGCCTG	ATGATGCGGTCCGGATACTC
NGN2 RT-PCR	CAGGCCAAAGTCACAGCAAC	GGCTCCTCCTCCTCTTCTTC
Doublecortin RT-PCR	ATTGCCTGTGGTCCGAAA	CATAGGACCAGGGCTCTTGG
Neurofilament RT-PCR	AGACCCTGGAAATCGAAGCA	TCACGTTGAGGAGGTCTTGG
TSS1 Isoform qRT-PCR	CAGCTCTGAAAGAGTGTGGTGT	TGCCACACCCTGTTTGGTTT
TSS2-1 Isoform qRT-PCR	CATTCGACGACAGTGTGGTG	TGCCACACCCTGTTTGGTTT
TSS2-2 Isoform qRT-PCR	GCAGAGGGACTCAGTGTGGT	TGCCACACCCTGTTTGGTTT
TSS3 Isoform qRT-PCR	TGAACCACACCCCGATGTGG	TGCCACACCCTGTTTGGTTT
Ex 1 TSS1 NTA qRT-PCR	TGTGATCCAGGAACAGCTGT	ACACACGCGAACTTCAAGAC
Ex 1 TSS2-1 NTA qRT-PCR	GAGCGGAGAACTGGGAGTG	GAAAAGGAGCGCACAGGAAG
Ex 1 TSS2-2 NTA qRT-PCR	GAGGAGTCGGAGTTGTGGAG	GATTTCCAAGACGCCCGTT
Ex 2 NTA qRT-PCR	CAAACAGGGTGTGGCAGAAG	TGAACAAGCACCAAAGTACA
Ex 3 NTA qRT-PCR	AAGGAGGGAGTGGTGCATG	ACTGGGCCACACTAATCACT

Supplementary Table S2. PCR Primers

[†]From Devine, M. J. *et al.* Parkinson's disease induced pluripotent stem cells with triplication of the alpha-synuclein locus. *Nature communications* 2, 440, doi:10.1038/ncomms1453 (2011).

[‡]From Applied StemCell

NTA = nascent transcript analysis

Tissue	p1@ SNCA	p4@ SNCA	p2@ SNCA	p3@ SNCA	p5@ SNCA	p6@ SNCA	p7@ SNCA	p9@ SNCA	p13@ SNCA	p14@ SNCA	p11@ SNCA
pons_pool1.CNhs1064 0.10033-101E6	79.9324 06	9.58373 235	13.6619 163	12.5744 006	9.44779 288	1.42736 439	0.54375 786	1.56330 386	0.74766 706	0.06796 973	0.13593 947
temporal_lobe_donor1	43.5892 424	7.96319 508	15.3041 918	13.1761 705	8.34961 532	3.08819 975	1.46591 24	0.41741 619	0.31546 597	0	0.04532 766
occipital_lobe_donor1. CNhs11784.10073- 102A1	65.7565 812	14.3073 364	15.4143 245	11.1455 19	8.26080 485	6.10271 852	2.20761 605	0.29811 117	0.48849 162	0	0.04610 483
middle_temporal_gyrus	115.584 053	36.7767 44	13.8258 436	6.22162 963	3.59471 934	3.59471 934	1.10606 749	0.96780 905	0	0	0
cerebellum_adult	70.3582 958	7.61169 421	3.85809 095	3.54718 65	2.46485 108	0.20909 513	0.41819 027	0.17223 538	0.03685 975	0.09851 587	0
frontal_lobe_pool1.CNh s10647.10040-101F4	85.1530 206	29.5028 309	21.7789 138	8.35702 507	5.38141 766	5.63466 084	0.37986 478	0.31655 398	0.25324 318	0.12662 159	0.06331 08
Astrocyte_cerebral_cort ex	2.73940 055	0	0.59466 298	0.48337 17	0.93322 061	0.09541 2	0.09124 962	0	0	0	0
putamen_donor10196. CNhs12324.10176- 103C5	85.9422 277	28.5950 374	9.26981 981	3.77077 416	2.35673 385	0.78557 795	1.88538 708	0.94269 354	0	0	0
substantia_nigra_donor 10252.CNhs12318.101 58-103A5	51.0288 916	0.86003 75	6.73696 041	11.8971 854	9.03039 374	1.00337 708	1.29005 625	0.57335 833	0.14333 958	0	0
brain_donor1.CNhs117 96.10084-102B3	71.0941 527	15.0105 853	16.2688 624	13.0421 726	7.00566 172	2.64127 723	0.49476 319	0.34014 969	0.03092 27	0.06184 54	0.09276 81
neuroblastoma_cell_lin e:CHP- 134.CNhs11276.10508 -107D4	1.87961 824	0.75490 974	0.08526 291	0.53552 421	0.64473 385	0	0	0.04263 146	0	0	0
medulla_oblongata_ad ult	37.9362 635	3.28738 217	5.69165 17	6.87921 876	5.85174 193	0.56672 861	0.44997 085	0.27651 946	0.33160 675	0	0.02069 88
diencephalon	44.5102 447	6.79366 893	2.57690 89	7.02793 337	6.32514 003	0.70279 334	0.23426 445	0.46852 889	0	0	0
Neutrophils	14.3473 056	0.24966 901	1.63105 405	0.13813 85	0.13813 85	1.13534 337	0	0	0	0	0
nucleus_accumbens_p ool1.CNhs10644.10037 -101F1	62.4950 36	20.4248 936	12.3320 112	7.06521 476	3.72529 506	3.46837 816	0.44960 458	0.89920 915	0.19268 768	0.06422 923	0

corpus_callosum_pool1 .CNhs10649.10042- 101F6	21.5009 614	0.87621 473	2.62864 419	10.5145 767	8.15553 709	0.60661 02	0.06740 113	0.06740 113	0.33700 566	0	0
pineal_gland_adult	12.5558 421	9.97704 201	0.84070 931	1.47612 02	0.33628 372	0.26785 092	0	0	0	0	0
olfactory_region	160.829 743	37.7853 011	24.5442 981	18.7311 749	9.36558 745	5.49017 195	5.32869 631	0.80737 823	0.48442 694	0	0.16147 565
astrocytoma_cell_line:T M- 31.CNhs10742.10425- 106D2	0	0	0	0	0	0	0	0	0	0	0
amygdala_adult	92.9991 9	18.1233 287	19.8775 938	8.68901 604	3.88260 155	5.02214 948	1.97410 27	0.35167 354	0	0.07033 471	0.07033 471
pituitary_gland_adult	11.6473 797	5.57712 261	1.44179 376	4.28717 043	1.21413 157	0.34149 329	0.30348 28	0.11383 11	0	0	0
occipital_cortex_adult	41.0920 071	14.1696 576	12.7526 919	4.25089 729	5.66786 305	0	0	0	0	0	0
Astrocyte_cerebellum	1.81347 645	0	0.17248 514	0.38601 063	0.31345 472	0.04104 035	0	0	0.10448 491	0	0
hippocampus_adult	103.330 534	19.9073 4	14.7426 059	9.06178 543	6.62111 949	2.87339 636	1.07863 188	0.99953 48	0	0	0
paracentral_gyrus_pool 1.CNhs10642.10035- 101E8	77.6006 847	18.7079 109	15.9388 7	6.55114 571	4.99778 126	3.91717 991	0.33768 792	0.67537 585	0.13507 517	0	0
Neurons	8.70761 463	0	1.69042 134	4.27426 276	3.35939 854	0.05583 876	0.21264 625	0	0	0	0
medial_frontal_gyrus_a dult	84.1764 771	25.6959 772	13.2910 227	7.08854 544	1.77213 636	3.54427 272	2.65820 454	0.88606 818	0	0.88606 818	0
medial_temporal_gyrus _adult	101.171 077	37.3702 393	20.1515 527	6.54809 102	4.61396 092	4.17421 033	2.71464 215	1.23450 365	0	0.06259 547	0
dura_mater_donor1.CN hs10648.10041-101F5	19.7773 994	1.01098 527	3.22251 556	1.32691 817	0.50549 264	0.06318 658	0.31593 29	0.12637 316	0	0.06318 658	0.06318 658
Meningeal_Cells	1.82413 897	0.23158 924	0	0.18269 705	0.14532 002	0	0	0	0	0	0
cerebral_meninges	111.228 396	39.0506 007	27.6425 6	7.67848 89	6.14279 112	8.77541 589	3.07139 556	0.65815 619	0	0.21938 54	0.21938 54
Neural_stem_cells	3.88873 291	0	0	3.38054 366	2.07914 512	0	0	0	0	0	0

parietal_lobe_adult	69.2888 2	20.9982 92	12.8330 07	9.44064 942	5.66328 581	1.88777 613	1.11230 554	0.40104 407	0.26768 516	0	0.04756 186
caudate_nucleus_adult	38.0868 796	13.2861 208	3.54296 554	7.08593 109	5.31444 831	0.88574 139	0.88574 139	0.88574 139	0	0	0
thalamus_adult	35.8642 624	3.62100 759	3.98643 402	7.45314 486	6.98940 185	0.51716 498	0.77660 021	0.25858 249	0.05171 65	0	0
postcentral_gyrus_pool 1.CNhs10638.10032- 101E5	66.6278 196	18.7452 405	12.7599 181	5.91954 962	3.15709 313	2.43359 262	0.52618 219	0.59195 496	0.06577 277	0	0.06577 277

Supplementary Table S3. CAGE Data

From website: <http://fantom.gsc.riken.jp/zenbu/gLyphs/#config=b-IMGb5IG53ntH8qgNeChB;loc=hg19::chr19:36307745..36428760+>

Blue: SNCA expression from TSSs identified by CAGE tags in relevant tissues

Pink: Predominant SNCA TSS, as identified by CAGE and validated by our qRT-PCR data

SUPPLEMENTARY METHODS

iPSC Culture

ND34391G (SNCA triplication) iPSCs are distributed through the NINDS Repository Fibroblasts and iPSCs Collection and were obtained as a feeder-based live culture from the Coriell Institute. For feeder-based culture, ND34391G iPSCs were maintained on CF-1 mouse embryonic fibroblasts (Global Stem) in DMEM/F12 (10565-018, Life Technologies) supplemented with 20% KnockOut Serum Replacement (Life Technologies), 1X GlutaMAX (Life Technologies), 1X MEM non-essential amino acids (Life Technologies), 0.1 mM beta mercaptoethanol (Sigma-Aldrich) and 10 ng/mL bFGF (R&D Systems). During feeder-based culture, ND34391G iPSCs were passaged with the StemPro EZPassage tool (Life Technologies) and supplemented with 10 μ M ROCK inhibitor (Tocris Bioscience) for the first 24 hours after passaging. For feeder-free adaptation, ND34391G iPSCs were passaged with collagenase IV (Stem Cell Technologies) onto BD Matrigel Matrix Growth Factor Reduced (BD Biosciences)-coated plates in E8 medium (Life Technologies), supplemented with 10 μ M ROCK inhibitor for the first 24 hours after passaging.

NCRM-5 (healthy control) and NCRM-1 (healthy control) iPSCs are distributed through RUDCR Infinite Biologics at Rutgers University and were obtained as feeder-free cryostocks from the NIH Center for Regenerative Medicine (NIH CRM). ND38477C (PARK2 mutant) iPSCs are distributed through the NINDS Repository Fibroblasts and iPSCs Collection and were obtained as a feeder-free cryostock from the Coriell Institute. NHDF-1 (healthy control) iPSCs were likewise obtained as a feeder-free cryostock from the James Martin Stem Cell Facility. Cells were thawed onto Matrigel-coated plates in E8 medium, supplemented with 10 μ M ROCK inhibitor for the first 24 hours after thawing.

For maintenance of feeder-free cultures, 100% E8 medium was replaced daily. When cells reached 70% confluence, they were passaged with 0.5 mM EDTA (Life Technologies) in calcium- and magnesium-free DPBS (Life Technologies). Cells were seeded at a subcultivation ratio between 1:6 and 1:24 in E8 medium, supplemented with 10 μ M ROCK inhibitor for the first 24 hours after passaging.

Pluripotency Immunostaining

Immunostaining was performed as described in the methods section of the main article. The following antibodies were used for pluripotency immunostaining: Nanog (PeproTech, 500-P236), Tra-1-60 (Millipore, MAB4360), Oct4 (Cell Signaling, 2750), goat anti-rabbit

IgG Alexa Fluor 488 (Life Technologies, A-11034) and goat anti-mouse IgM 555 (Life Technologies, A-21426).

TaqMan hPSC Scorecard Analysis

For TaqMan hPSC Scorecard (Life Technologies) analysis, 20 μ L of cDNA, made with 1 μ g of RNA from each iPSC line (prepared as described in the methods section of the main article), was diluted in 610 μ L of nuclease-free water. 630 μ L of 2X TaqMan Fast Advanced Master Mix was added and 10 μ L of the reaction mixture was loaded per well into the plate, using a fresh tip for each well. Cycling was performed on a StepOnePlus Real-Time PCR System (Life Technologies) under fast cycling conditions with the following program: 50°C for 2 min, 95°C for 20 sec and 40 cycles of 95°C for 1 sec and 60°C for 20 sec. Data were analyzed using hPSC Scorecard Analysis Software (Life Technologies).

CNV Quantification

Quantification of SNCA copy number was performed using Type-IT CNV SYBR Green (Qiagen) qPCR assays, per the manufacturer's instructions. Primers for CNV quantification were designed both within and outside the triplication region. Primer sequences are provided in Supplementary Table S2.

Generation, Maintenance and Characterization of iPSC-derived NSCs

NAS (normal alpha-synuclein) NSCs were derived from NCRM-5 iPSCs by the NIH CRM, using PSC Neural Induction Medium (Life Technologies) per the manufacturer's instructions. NAS NSCs were maintained in StemPro NSC SFM (Life Technologies), containing Knockout DMEM, 1X StemPro Neural Supplement, 1X GlutaMAX, 20 ng/mL EGF and 20 ng/mL bFGF. AST (alpha-synuclein triplication) NSCs were derived from ND34391G iPSCs via the Applied StemCell NSC Generation Service, using the Chambers protocol (Chambers et al. 2009). AST NSCs were maintained in Applied StemCell NSC Expansion Medium, containing 1:1 Neurobasal (Life Technologies) and DMEM/F-12 (11320-033, Life Technologies), 1X B27 supplement (Life Technologies), 1X N2 supplement (Life Technologies), 1X GlutaMAX, and 20 ng/mL bFGF (Peprotech). All NSCs were grown on Geltrex LDEV-Free Reduced Growth Factor Basement Membrane Matrix (Life Technologies)-coated plates, prepared by applying a dilution of 60 μ L of Geltrex in 12 mL of DMEM/F12 (11320-074, Life Technologies) at tissue culture dish-dependent coating volumes (i.e. 1 mL per well of 6-well tissue culture dish), and equilibrating at 37°C, 5% CO₂ for one hour prior to cell seeding. Notably, variability

introduced by the derivation of NSCs has been minimized to the extent possible by using protocols based on chemically defined medium for neural induction in adherent culture format. In addition, expression of NSC markers was evaluated in AST NSCs transitioned to StemPro NSC SFM medium, and no alteration in NSC marker expression was detectable (Supplementary Fig. S2). All NSCs were passaged with StemPro Accutase Cell Dissociation Reagent (Life Technologies) and quenched with 5 volumes of medium per 1 volume of Accutase applied. Cells were seeded at a subcultivation ratio between 1:3 and 1:6. NSCs could be maintained for more than 50 passages, while retaining NSC marker expression and neuronal differentiation capacity.

iPSC-derived NSCs were characterized by their expression of NSC markers, including Nestin, SOX2, PAX3, PAX6, DACH1 and NKX2.2 (Supplementary Fig. S2). Primers for RT-PCR of NSC markers are provided in Supplementary Table S2. In addition, iPSC-derived NSCs were co-immunostained with Nestin (BD Biosciences, 611658) and SOX2 (Cell Signaling, 3579P) primary antibodies with goat anti-mouse IgG Alexa Fluor 555 (Life Technologies, A-21424) and goat anti-rabbit IgG Alexa Fluor 488 (Life Technologies, A-11034) secondary antibodies, respectively (Supplementary Fig. S2).