

## Supplementary Figure Legends

**Supplementary Figure S1. Characterization of Control and SNCA\_Tri NiPSCs.** Phase contrast (upper panels) and immunofluorescence (bottom panels) images. All cells were positive for the neural progenitor marker Nestin (red). Nuclei were counterstained with DRAQ5 (blue). Insets in phase contrast images are 5× digital zooms from the surrounding images.

**Supplementary Figure S2. Differentiation of the NiPSC lines.** (A) Schematic representation of the 30-day differentiation protocol to generate DAN: 10-day culture with FGF8 and SAG (DA1 Medium) for midbrain specification and greater than 20 days in the presence of BDNF, GDNF and cAMP (DA2 medium) for dopaminergic maturation. (B) Immunofluorescence of the differentiated NiPSCs (Control line) showing expression of Lmx1a, MAP2, Tuj1, Nurr1, TH and GIRK2, (DNA by DRAQ5 staining), confirming the generation of DAN.

**Supplementary Figure S3. Characterization of Control and SNCA\_Tri after DA1 differentiation.** Phase contrast (left) and immunofluorescence (center and right) images. Neural progenitor marker Nestin (center - green); early neuronal markers  $\beta$ III-Tubulin (center - red) and MAP2 (right - green); nuclei (DRAQ5 positive, blue, right). Insets in phase contrast images are 5× digital zooms.

**Supplementary Figure S4. Yield of dopaminergic neurons after DA2 differentiation.** TH immunofluorescence (data of Fig. 2C) for the extra cell lines used for dopaminergic quantification.

**Supplementary Figure S5. Electrophysiological characterization of differentiated NiPSCs.** Passive and active membrane properties obtained from cells that generated APs. The number of cells analyzed in each group is indicated in A. Cell membrane capacitance (A) and input

resistance ( $R_m$ ) **(B)** were estimated from integrals of current transients that were elicited by small hyperpolarizing voltage pulses (10 mV) from a holding potential of -70 mV after compensating the pipette's capacitance in cell-attached mode. **(C)** Peak amplitudes of APs elicited by 100 ms sustained current injection. **(D)** Maximum rates of rise estimated by measuring the peak of the first derivative of the membrane potential. The table below the figure presents the values obtained for each of the evaluated parameters.

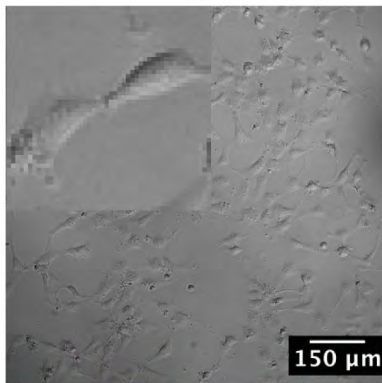
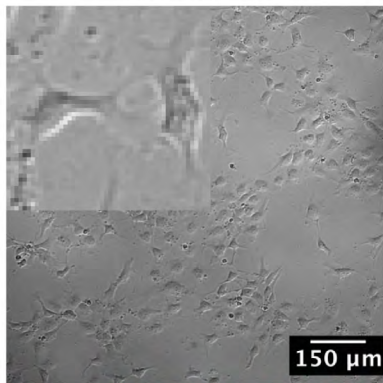
**Supplementary Figure S6. Long-term differentiation of NiPSCs from Control and SNCA\_Tri.** Phase contrast images of live NiSPC Control (left) and SNCA\_Tri (right) lines differentiated for 64 days.

**Supplementary Movie 1. DA2-differentiation of Control and SNCA\_Tri lines.** Control and patient cells plated in poly-L-lysine plus laminin-coated  $\mu$ -dishes at  $3.1 \cdot 10^2$  cells/mm<sup>2</sup>. Phase contrast images were taken at the indicated days of DA2 differentiation and in the same region of the dish. Images were compiled with Fiji and play at 1 frame/sec.

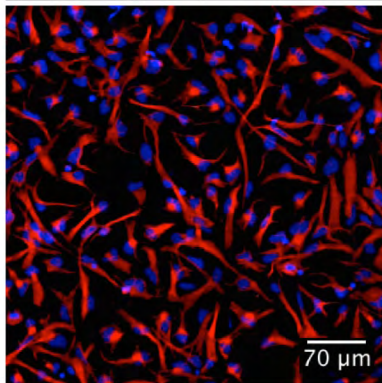
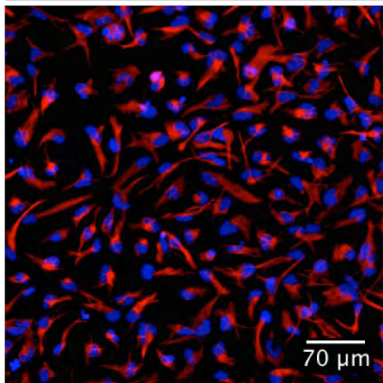
control 1

SNCA\_Tri-C1

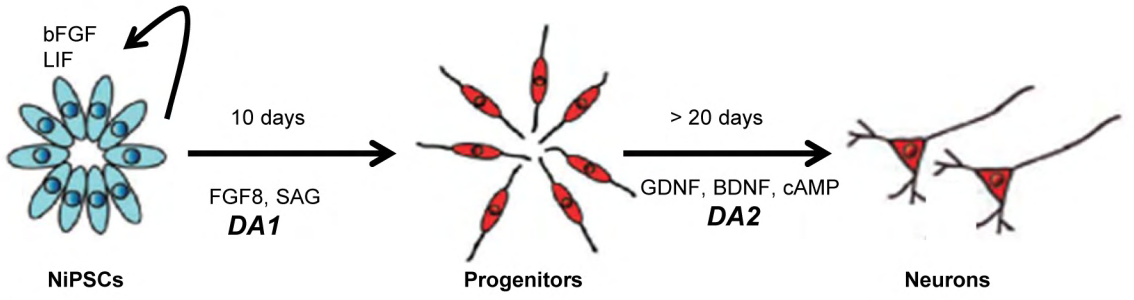
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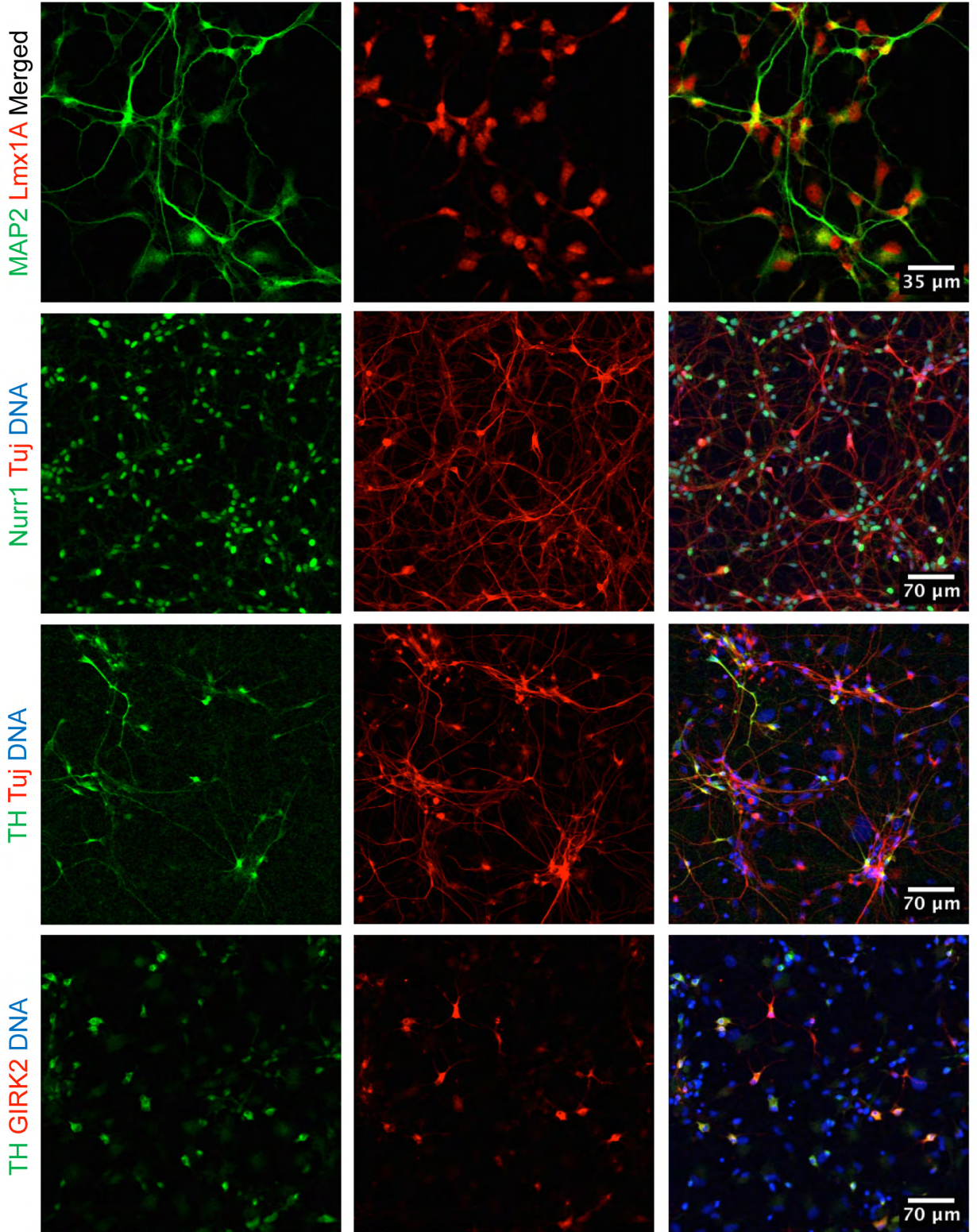
Nestin DNA

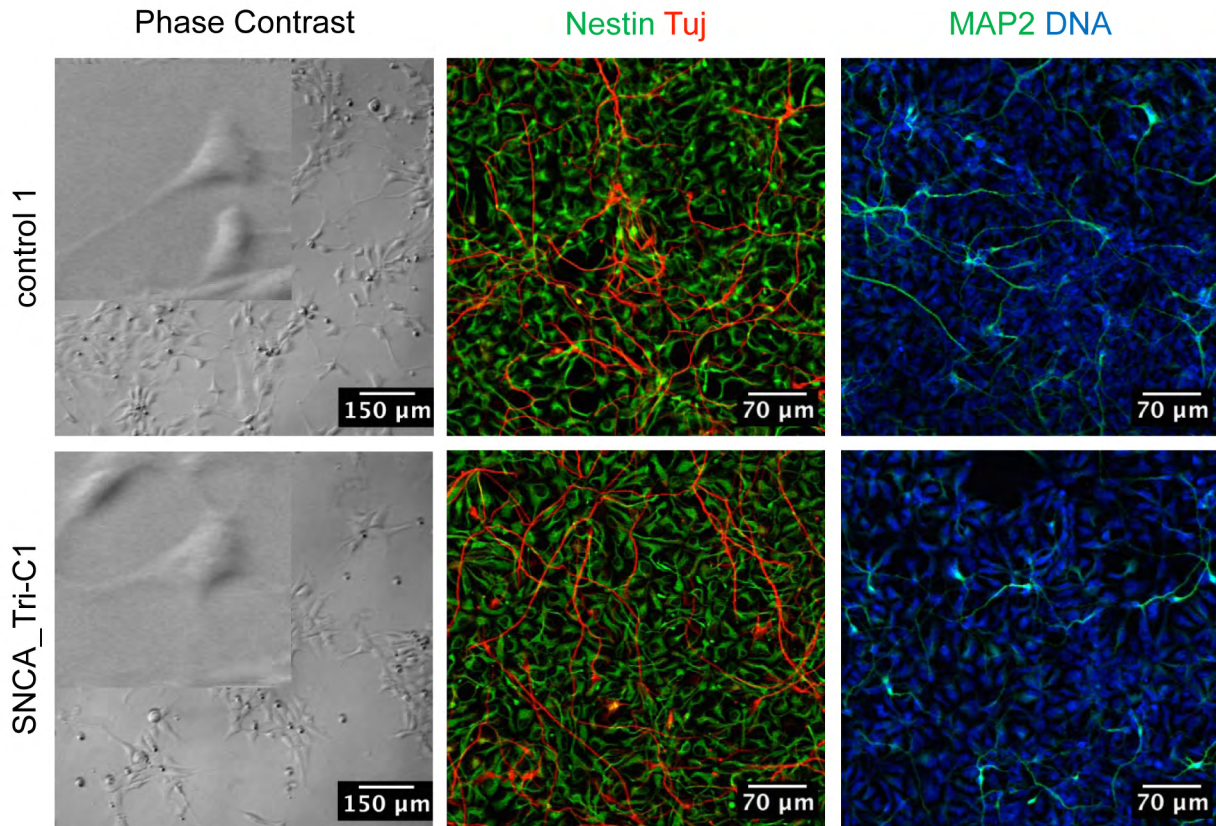


A

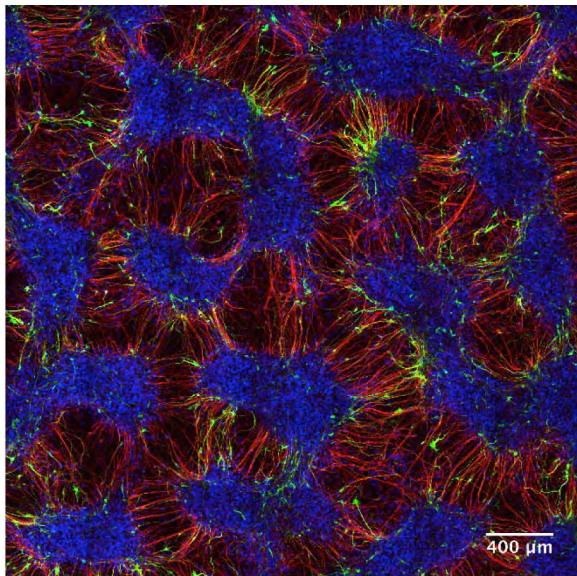


B

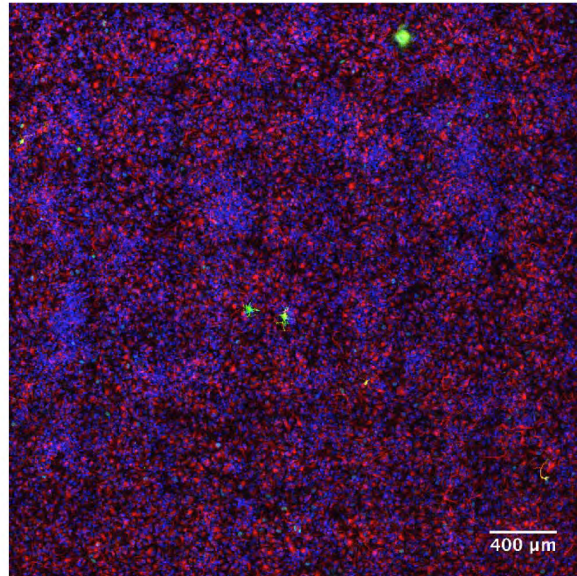




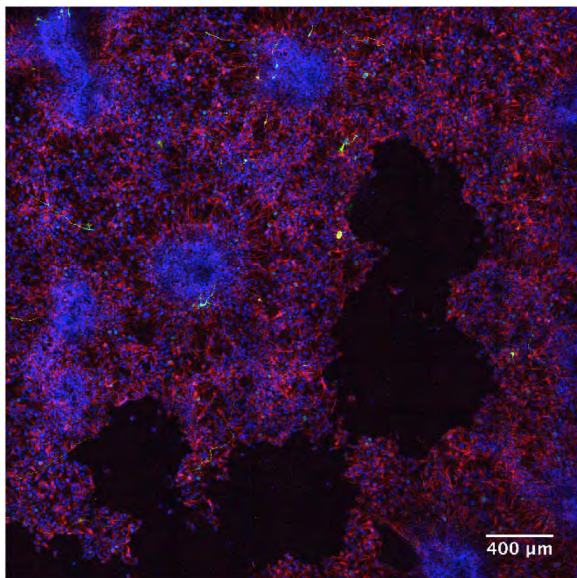
control 2



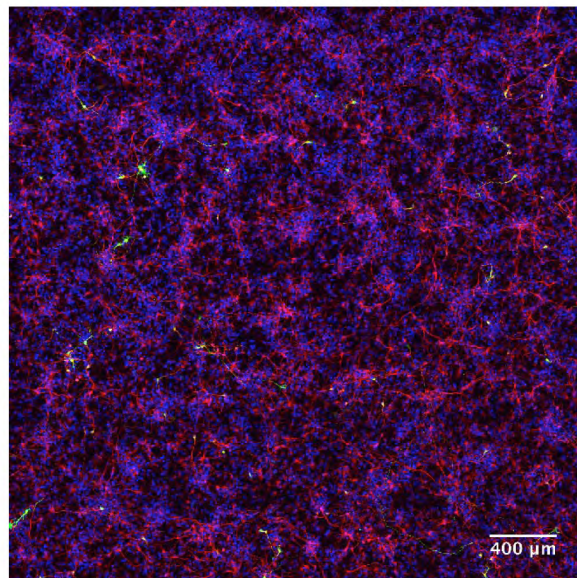
SNCA\_Tri-C1\_Scr



SNCA\_Tri-C2

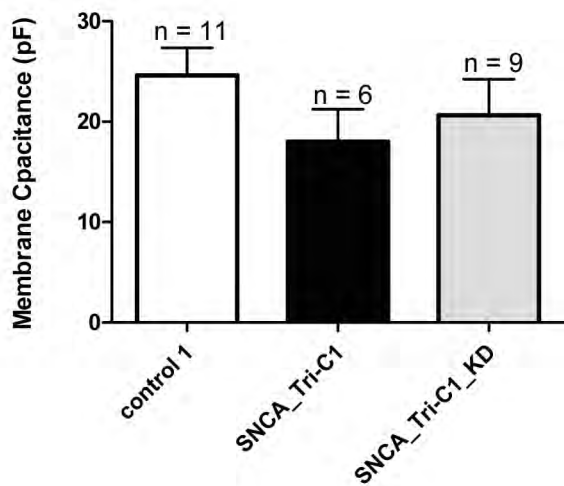


SNCA\_Tri-C2\_KD

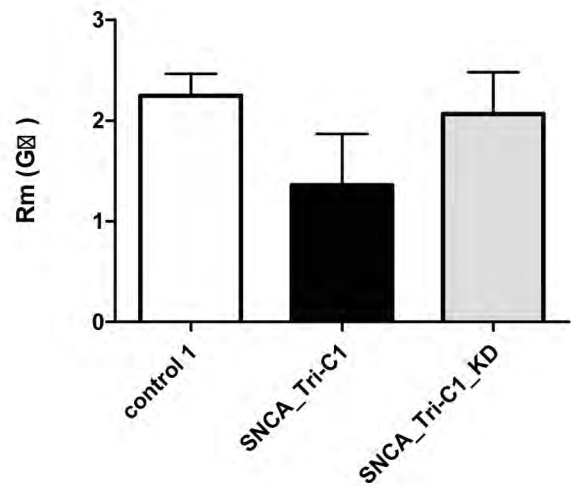


TH Tuji1 DNA

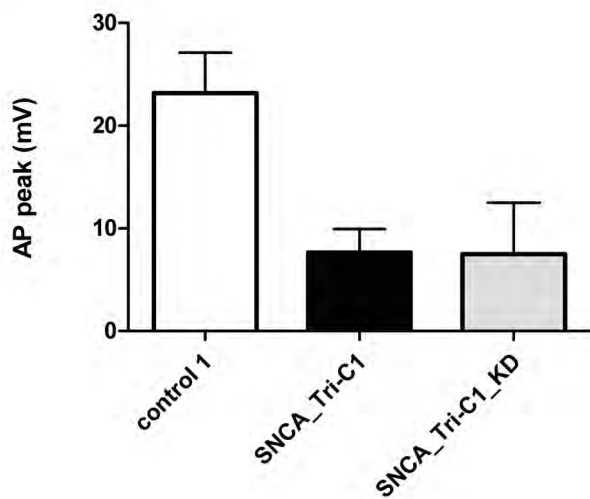
A



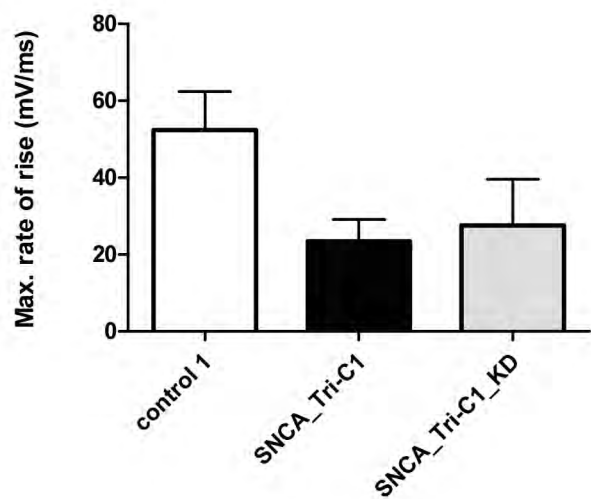
B



C

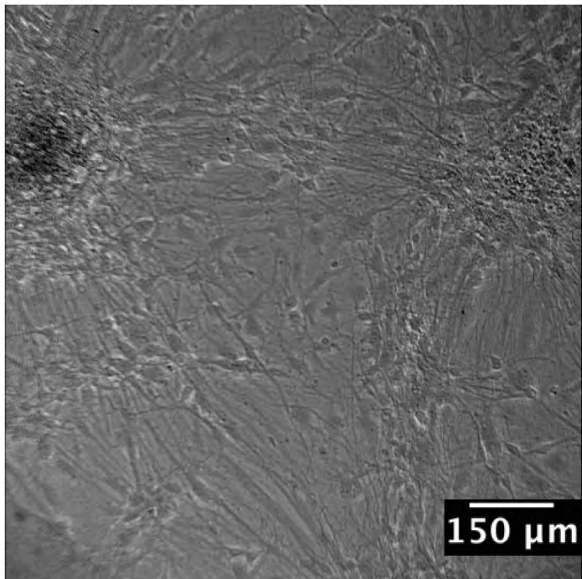


D

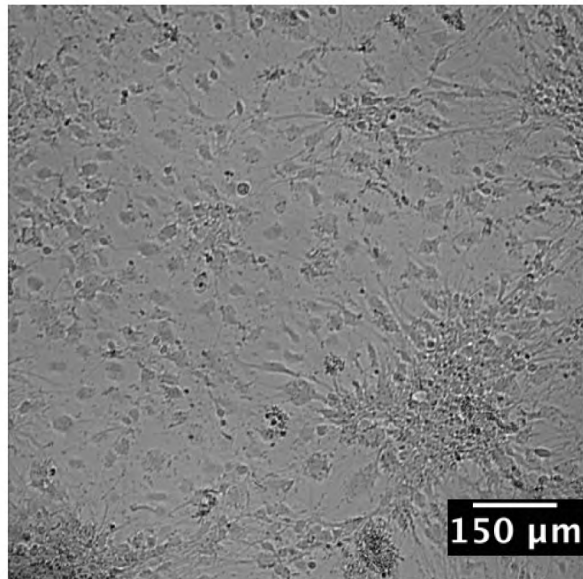


	control 1	SNCA_Tri-C1	SNCA_Tri-C1_KD
Membrane Capacitance (pF)	25 ± 3	17 ± 4	21 ± 4
Rm (GΩ)	2.3 ± 0.2	1.2 ± 0.6	2.3 ± 0.7
AP peak (mV)	23 ± 4	8 ± 2	8 ± 5
Max. Rate of Rise (mV/ms)	52 ± 10	20 ± 6	27 ± 14

control 1



SNCA\_Tri-C1





**Supplementary Table 1 – Designations and etiology of the lines used in this study**

Patient ID		Clone ID	Alternative ID	Reprogramming method	Age at biopsy	Gender	Disease
PI-1754	SNCA_Tri	clone 1	C7	retroviral integrating four factors <sup>1</sup>	42 yrs	male	clinical description <sup>2</sup>
PI-1754	SNCA_Tri	clone 2	1754-MIT	lentiviral integrating single vector containing all four factors <sup>3</sup>	42 yrs	male	clinical description <sup>2</sup>
PI-1761	sibling control	control 1	C1	retroviral integrating four factors <sup>1</sup>	46 yrs	female	healthy
PI-1815	healthy control	control 2	C7	retroviral integrating four factors <sup>1</sup>	62 yrs	male	healthy

<sup>1</sup> Takahashi K, Tanabe K, Ohnuki M, et al. Induction of pluripotent stem cells from adult human fibroblasts by defined factors. *Cell* 2007, **131**:861-872.

<sup>2</sup> Byers B, Cord B, Nguyen HN, et al. SNCA triplication Parkinson's patient's iPSC-derived DA neurons accumulate  $\alpha$ -synuclein and are susceptible to oxidative stress. *PloS one* 2011, **6**:e26159.

<sup>3</sup> Somers A, Jean JC, Sommer CA, et al. Generation of transgene-free lung disease-specific human induced pluripotent stem cells using a single excisable lentiviral stem cell cassette. *Stem cells* 2010, **28**:1728-1740.

**Supplementary Table 2 – Full differential expression profile of Parkinson's disease-related genes in SNCA\_Tri vs both the control and SNCA\_Tri\_KD lines**

Gene Symbol	Gene Name	Gene Description	Fold Change SNCA_Tri vs Control	p value	Fold Change SNCA_Tri vs SNCA_Tri_KD	p value	action
ALDH1A1	ALDC	Aldehyde dehydrogenase 1 family, member A1	1.14	0.295	1.05	0.637	Oxidative Stress
APC	BTPS2	Adenomatous polyposis coli	-1.19	0.762	1.60	0.248	Signal Transduction
APP	ABETA	Amyloid beta (A4) precursor protein	1.12	0.614	1.06	0.686	Signal Transduction
ATP2B2	PMCA2	ATPase, Ca <sup>++</sup> transporting, plasma membrane 2	-9.29	0.241	2.02	0.269	Ion Transport
ATXN2	ATX2	Ataxin 2	1.31	0.210	1.01	0.849	Parkin Substrate
ATXN3	ATX3	Ataxin 3	1.26	0.294	1.30	0.250	Parkin Substrate
BASP1	CAP-23	Brain abundant, membrane attached signal protein 1	-1.30	0.354	1.96	0.064	Transcription
BDNF	MGC34632	Brain-derived neurotrophic factor	-1.39	0.424	2.13	0.090	Anti-Apoptosis
CADPS	CADPS1	Ca <sup>++</sup> -dependent secretion activator	-1.58	0.007	8.22	5.3x10 <sup>-5</sup>	Ion Transport
CASP1	ICE	Caspase 1, apoptosis-related cysteine peptidase	1.14	0.295	1.05	0.637	Pro-Apoptosis
CASP3	CPP32	Caspase 3, apoptosis-related cysteine peptidase	1.01	0.874	1.68	0.003	Pro-Apoptosis
CASP7	CMH-1	Caspase 7, apoptosis-related cysteine peptidase	-1.06	0.435	-1.12	0.151	Mitochondria
CASP8	ALPS2B	Caspase 8, apoptosis-related cysteine peptidase	3.78	0.018	-1.16	0.552	Pro-Apoptosis
CASP9	APAF-3	Caspase 9, apoptosis-related cysteine peptidase	1.28	0.145	-1.16	0.162	Pro-Apoptosis
CDC27	ANAPC3	Cell division cycle 27 homolog (S. cerevisiae)	1.15	0.361	1.11	0.453	Ubiquitination
CDC42	CDC42Hs	Cell division cycle 42 (GTP binding protein, 25kDa)	1.20	0.442	1.19	0.295	Cytoskeleton
CDH8	Nbla04261	Cadherin 8, type 2	-1.56	0.467	4.47	0.107	Cell Adhesion
CHGB	SCG1	Chromogranin B (secretogranin 1)	-1.76	0.027	2.95	0.018	Secretion
CUL2	MGC131970	Cullin 2	1.07	0.622	1.20	0.274	Ubiquitination
CXXC1	CFP1	CXXC finger protein 1	1.25	0.293	1.03	0.759	Ion Transport
D4S234E	D4S234	DNA segment on chromosome 4 (unique)	-1.12	0.813	2.52	0.141	Signal Transduction
DDC	AADC	Dopa decarboxylase (aromatic L-amino acid decarboxylase)	-2.07	0.078	7.01	0.005	Signal Transduction
DLK1	DLK	Delta-like 1 homolog (Drosophila)	-1.65	0.017	-1.36	0.006	Signal Transduction
DRD2	D2DR	Dopamine receptor D2	-14.08	0.116	1.00	0.847	Signal Transduction
EGLN1	C1orf12	Egln9 homolog 1 (C. elegans)	1.10	0.504	1.34	0.100	Ion Transport
FBXO9	FBX9	F-box protein 9	-1.01	0.965	-1.05	0.955	Ubiquitination
FGF13	FGF-13	Fibroblast growth factor 13	-2.07	0.002	-1.30	0.109	Signal Transduction
FN1	CIG	Fibronectin 1	2.01	0.181	-1.66	0.065	Cell Adhesion
GABBR2	GABABR2	Gamma-aminobutyric acid (GABA) B receptor, 2	-3.60	0.022	-2.96	0.018	Signal Transduction
GBE1	GBE	Glucan (1,4-alpha-), branching enzyme 1	1.22	0.257	1.46	0.069	Ion Transport
GPR37	EDNRBL	G protein-coupled receptor 37	1.12	0.637	-1.06	0.916	Parkin Substrate
GRIA3	GLUR-C	Glutamate receptor, ionotropic, AMPA 3	-1.57	0.168	1.08	0.477	Ion Transport
HSPA4	HS24	Heat shock 70kDa protein 4	1.32	0.003	1.13	0.032	Heat Shock Response
HTR2A	5-HT2A	5-hydroxytryptamine (serotonin) receptor 2A	1.07	0.567	1.05	0.637	Signal Transduction
KCNJ6	GIRK-2	Potassium inwardly-rectifying channel	-3.17	3.1x10 <sup>-5</sup>	-2.59	3.1x10 <sup>-4</sup>	Ion Transport
LRRK2	PARK8	Leucine-rich repeat kinase 2	-6.09	0.087	1.16	0.562	Ubiquitination
MAPK9	JNK2	Mitogen-activated protein kinase 9	-1.10	0.805	1.16	0.482	Pro-Apoptosis
MAPT	TAU	Microtubule-associated protein tau	-2.54	0.171	4.61	0.112	Cytoskeleton
NCOA1	F-SRC-1	Nuclear receptor coactivator 1	1.14	0.512	1.20	0.404	Transcription
NEFL	NF-L	Neurofilament, light polypeptide	-1.84	0.008	1.13	0.003	Cytoskeleton
NFASC	NF	Neurofascin	-3.84	0.019	5.55	0.136	Cell Adhesion
NR4A2	NURR1	Nuclear receptor subfamily 4, group A, member 2	-7.79	0.030	-3.97	0.040	Signal Transduction
NRXN3	MGC176711	Neurexin 3	-1.38	0.692	6.35	0.114	Cell Adhesion
NSF	SKD2	N-ethylmaleimide-sensitive factor	-1.01	0.997	1.38	0.073	Ion Transport

NTRK2	TRKB	Neurotrophic tyrosine kinase, receptor, type 2	1.08	0.669	1.24	0.388	Signal Transduction
OPA1	MGM1	Optic atrophy 1 (autosomal dominant)	1.05	0.703	1.49	0.084	Anti-Apoptosis
PAN2	FLJ39360	Poly(A) specific ribonuclease subunit homolog (S. cerevisiae)	1.07	0.678	-1.12	0.687	Ubiquitination
PARK2	PRKN	Parkinson protein 2, E3 ubiquitin protein ligase (parkin)	-1.20	0.879	1.00	0.716	Ubiquitination
PARK7	DJ-1	Parkinson protein 7	1.23	0.008	-1.03	0.493	Parkin Complex
PINK1	PARK6	PTEN induced putative kinase 1	1.08	0.738	1.19	0.477	Mitochondria
PPID	CYP-40	Peptidylprolyl isomerase D	1.28	0.009	-1.06	0.322	Anti-Apoptosis
PRDX2	NKEFB	Peroxiredoxin 2	1.29	0.010	1.08	0.166	Inflammation
PSEN2	AD4	Presenilin 2	-1.09	0.623	1.05	0.658	Pro-Apoptosis
PTEN	PTEN1	Phosphatase and tensin homolog	-1.03	0.970	1.33	0.311	Pro-Apoptosis
RGS4	RGP4	Regulator of G-protein signaling 4	-6.69	0.045	1.05	0.637	Signal Transduction
RTN1	NSP	Reticulon 1	-8.31	0.165	2.40	0.141	-
S100B	S100	S100 calcium binding protein B	-2.06	0.362	2.38	0.276	Ion Transport
38596	PNUTL1	Septin 5	-2.15	0.077	2.74	0.119	Cytoskeleton
SKP1	EMC19	S-phase kinase-associated protein 1	1.12	0.608	1.37	0.227	Ubiquitination
SLC18A2	VMAT2	Solute carrier family 18 (vesicular monoamine), member 2	-1.22	0.356	1.12	0.489	Transporters
SLC25A4	AAC1	Solute carrier family 25 (mitochondrial carrier), member 4	-1.07	0.759	1.17	0.357	Mitochondria
SLC6A3	DAT	Neurotransmitter transporter, dopamine, member 3	1.14	0.295	1.05	0.637	Signal Transduction
SLIT1	SLIL1	Slit homolog 1 (Drosophila)	-1.12	0.867	4.83	0.155	Ion Transport
SNCA	PARK1	Synuclein, alpha (non A4 component of amyloid precursor)	1.85	0.029	4.68	0.005	-
SPEN	SHARP	Spen homolog, transcriptional regulator (Drosophila)	1.02	0.820	1.01	0.869	Signal Transduction
SRSF7	SFRS7	Serine/arginine-rich splicing factor 7	1.06	0.692	-1.18	0.226	Ion Transport
STUB1	UBOX1	STIP1 homology and U-box containing protein 1	1.14	0.443	1.07	0.629	Parkin Complex
SV2B	KIAA0735	Synaptic vesicle glycoprotein 2B	-1.51	0.433	1.05	0.637	Synaptic Vesicles
SYNGR3	MGC20003	Synaptogyrin 3	-1.51	0.478	1.99	0.207	Synaptic Vesicles
SYT1	SVP65	Synaptotagmin I	-1.24	0.890	1.16	0.566	Synaptic Vesicles
SYT11	SYT12	Synaptotagmin XI	-2.33	0.008	1.35	0.249	Synaptic Vesicles
TCF7L2	TCF4	Transcription factor 7-like 2 (T-cell specific, HMG-box)	1.23	0.008	-1.25	0.008	Anti-Apoptosis
TH	TYH	Tyrosine hydroxylase	-10.16	8.8x10 <sup>-5</sup>	-5.54	4.2x10 <sup>-5</sup>	Signal Transduction
TPBG	M6P1	Trophoblast glycoprotein	-2.13	0.008	-2.21	0.008	Cell Adhesion
UBA1	UBA1A	Ubiquitin-like modifier activating enzyme	-1.19	0.189	1.17	0.231	Ubiquitination
UBB	UBA52	Ubiquitin B	1.09	0.289	1.11	0.057	Ubiquitination
UBE2I	UBC9	Ubiquitin-conjugating enzyme E2I	1.38	0.058	1.02	0.803	Ubiquitination
UBE2K	UBC1	Ubiquitin-conjugating enzyme E2K	1.04	0.735	1.07	0.612	Ubiquitination
UBE2L3	UbcM4	Ubiquitin-conjugating enzyme E2L 3	1.32	0.095	-1.01	0.953	Ubiquitination
UCHL1	PARK5	Ubiquitin carboxyl-terminal esterase L1	-1.90	0.033	1.06	0.626	Ubiquitination
USP34	FLJ43910	Ubiquitin specific peptidase 34	1.03	0.766	-1.08	0.954	Ubiquitination
VAMP1	VAMP-1	Vesicle-associated membrane protein 1 (synaptobrevin 1)	-1.04	0.999	-1.42	0.171	Mitochondria
VDAC3	HD-VDAC3	Voltage-dependent anion channel 3	-1.10	0.727	1.02	0.798	Ion Transport
YWHAZ	YWHAD	Tyrosine 3-monooxygenase activation protein	1.07	0.005	1.18	0.003	Inflammation

**Supplementary Table 3 - Primary antibodies used for immunocytochemistry**

<b>Antibody</b>	<b>Vendor</b>	<b>Cat. No.</b>	<b>Dilution</b>
Nestin	Santa Cruz	SC-21247	1:800
MAP2	Sigma	M4403	1:1000
$\beta$ III-tubulin	Sigma	T8660	1:3000
Lmx1a	Santa Cruz	sc-134990	1:100
Nurr1	Santa Cruz	sc-990	1:200
Tyrosine hydroxylase	Millipore	AB152	1:1000
Tyrosine hydroxylase	Sigma	T2928	1:1000
GIRK2	Abcam	ab30738	1:100
GABA	Sigma	A2052	1:1000
$\alpha$ -Synuclein	BD	610787	1:400
LC3	Nanotools	0231-100/LC3-5F10	1:200
LAMP2	Abcam	Ab25631	1:100

