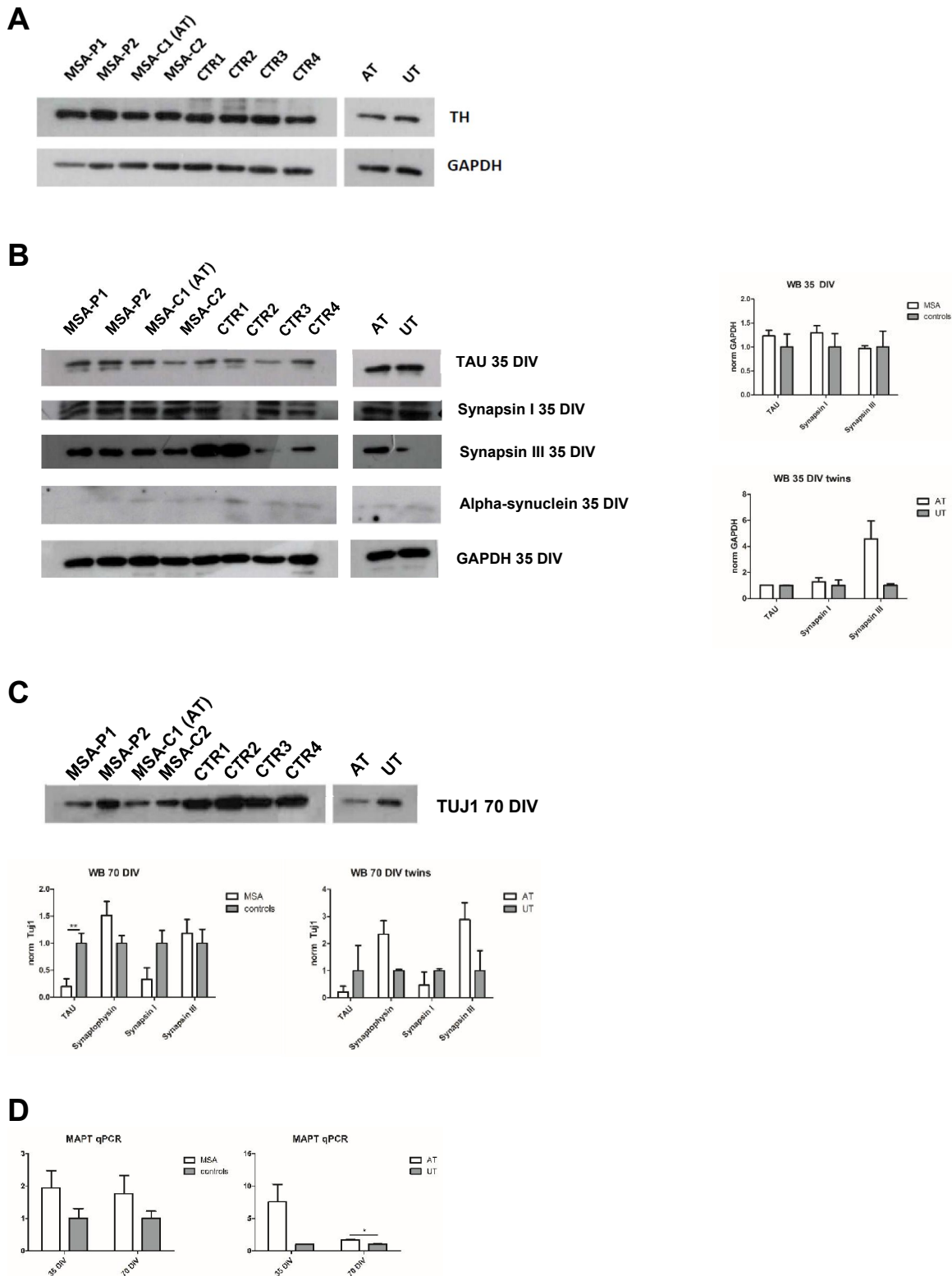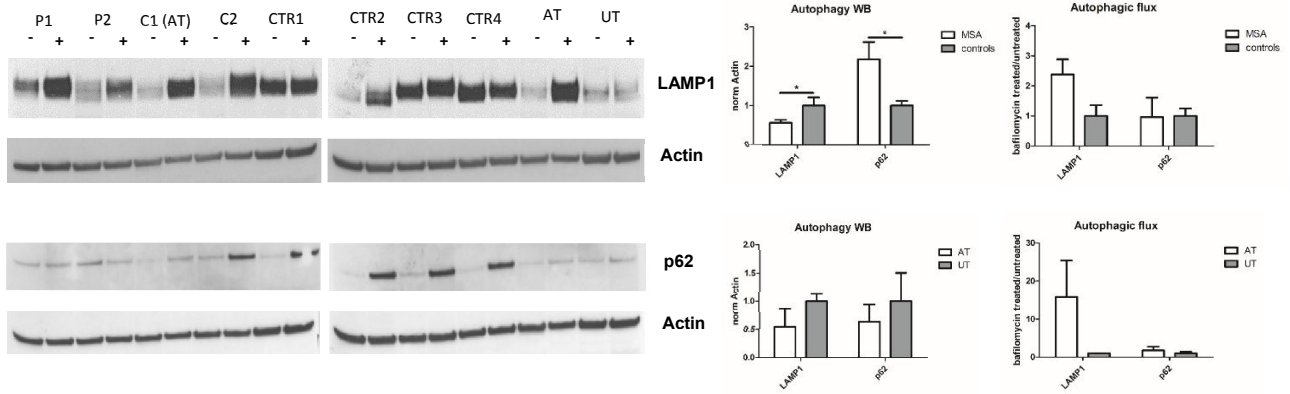# Supplemental Information

Mitochondrial Dysregulation and Impaired Autophagy in iPSC-Derived Dopaminergic Neurons of Multiple System Atrophy

Giacomo Monzio Compagnoni, Giulio Kleiner, Maura Samarani, Massimo Aureli, Gaia Faustini, Arianna Bellucci, Dario Ronchi, Andreina Bordoni, Manuela Garbellini, Sabrina Salani, Francesco Fortunato, Emanuele Frattini, Elena Abati, Christian Bergamini, Romana Fato, Silvia Tabano, Monica Miozzo, Giulia Serratto, Maria Passafaro, Michela Deleidi, Rosamaria Silipigni, Monica Nizzardo, Nereo Bresolin, Giacomo P. Comi, Stefania Corti, Catarina M. Quinzii, and Alessio Di Fonzo
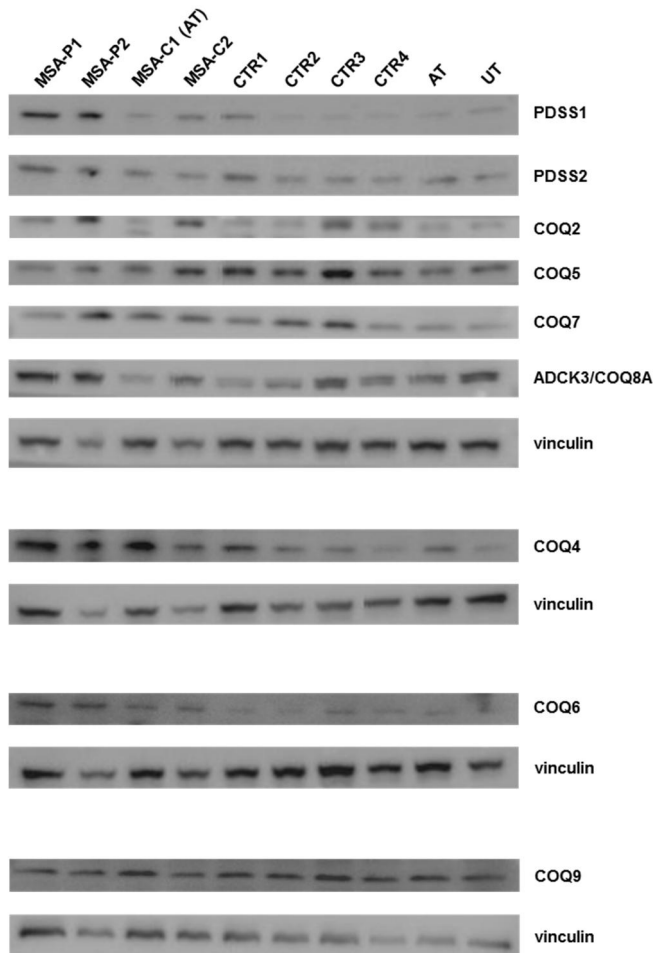
A

B

C

D

Figure S1. Related to Figures 2 and 4.
Neuronal markers in dopaminergic neurons. A) Western blot assessing high expression level of tyrosine hydroxylase in all neuronal lines at 70 DIV. B) WB for TAU, synapsin I, synapsin III, alpha-synuclein, GAPDH of neurons at 35 DIV and graphs showing related quantifications (values are normalized for GAPDH). C) WB for TUJ1 of neurons at 70 DIV and graphs showing quantifications of TAU, Synaptophysin, Synapsin I and Synapsin III normalized for TUJ1. D) qPCR performed on neurons at 35 and 70 DIV evaluating the expression of *MAPT*. AT=affected twin; UT=unaffected twin. Data are expressed as mean ± s.e.m. *=p<0.05 **=p<0.01
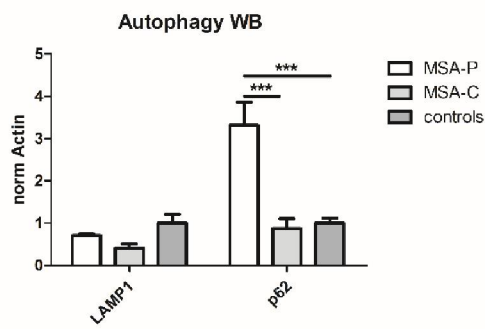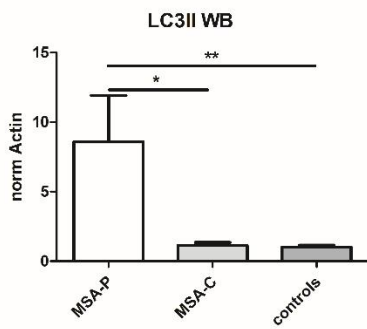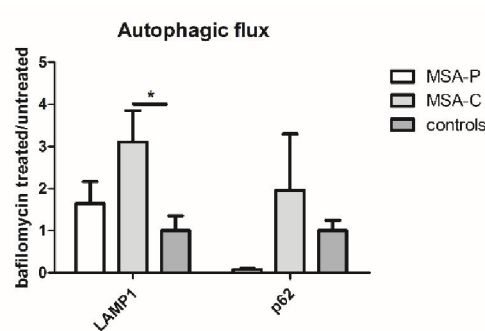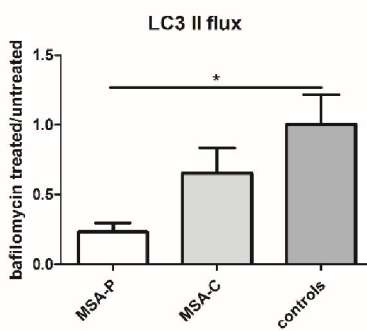
Figure S2. Related to Figures 5 and 6.
Autophagy and mitochondria in dopaminergic neurons. A) WB for LAMP1, p62 and Actin before (-) and after (+) treatment with bafilomycin (200 nM, 24 h) on iPSC-derived dopaminergic neurons at 35 DIV and graphs showing related quantifications at basal level and the autophagic flux. B) CoQ10 synthesis enzymes: WB for PDSS1, PDSS2, COQ2, COQ4, COQ5, COQ6, COQ7, ADCK3/COQ8A, COQ9 and Vinculin on iPSC-derived neurons at 35 DIV. AT=affected twin; UT=unaffected twin. Data are expressed as mean ± s.e.m. *=p<0.05

Autophagy Western Blot

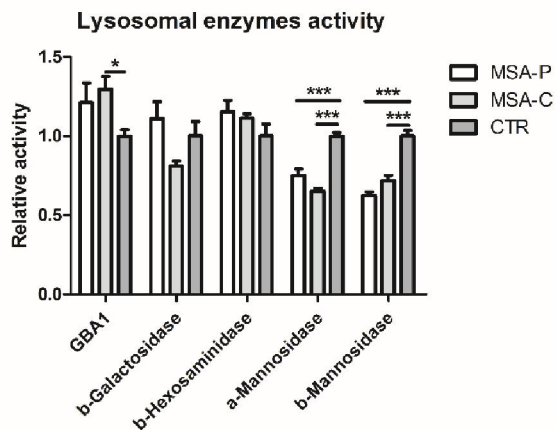**Autophagic flux Western Blot**

Lysosomal enzymes activity
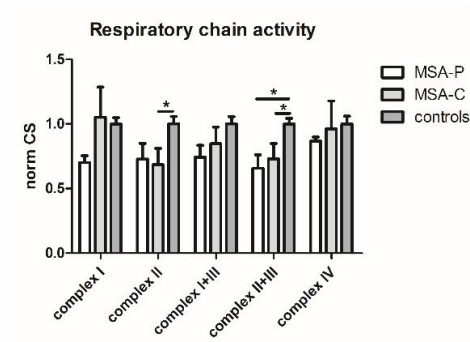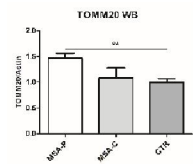

Figure S3. Related to Figure 5.
Graphs showing the results of autophagy-related experiments, separating MSA-P and MSA-C. Data are expressed as mean ± s.e.m. *=p<0.05; **=p<0.01; ***=p<0.001

Respiratory chain activity

TOMM20 WB

mtDNA content

Respiratory chain WB
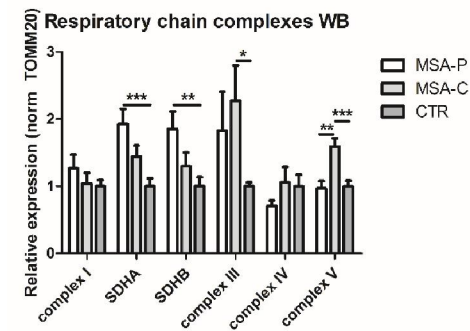
CoQ10 dosage
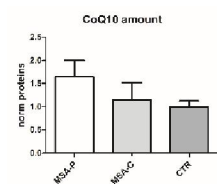
CoQ10 biosynthesis enzymes WB
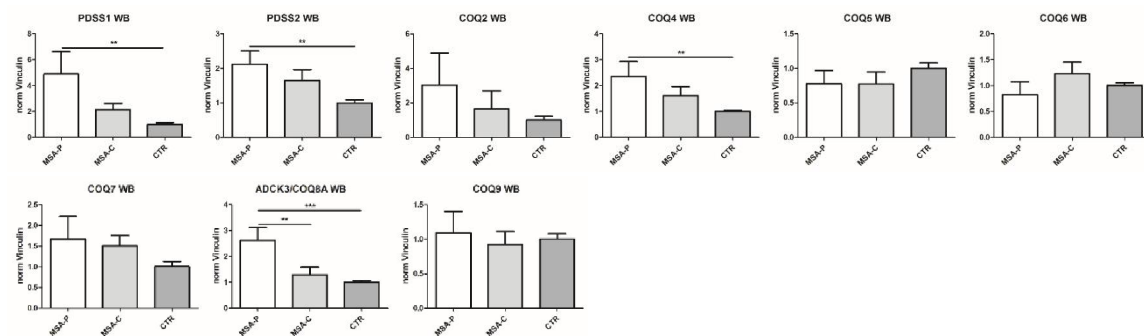
Figure S4. Related to Figure 6.
Graphs showing the results of mitochondria-related experiments, separating MSA-P and MSA-C. Data are expressed as mean ± s.e.m. *=p<0.05; **=p<0.01; ***=p<0.001
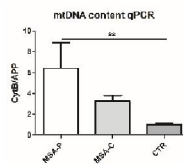
Autophagy WB
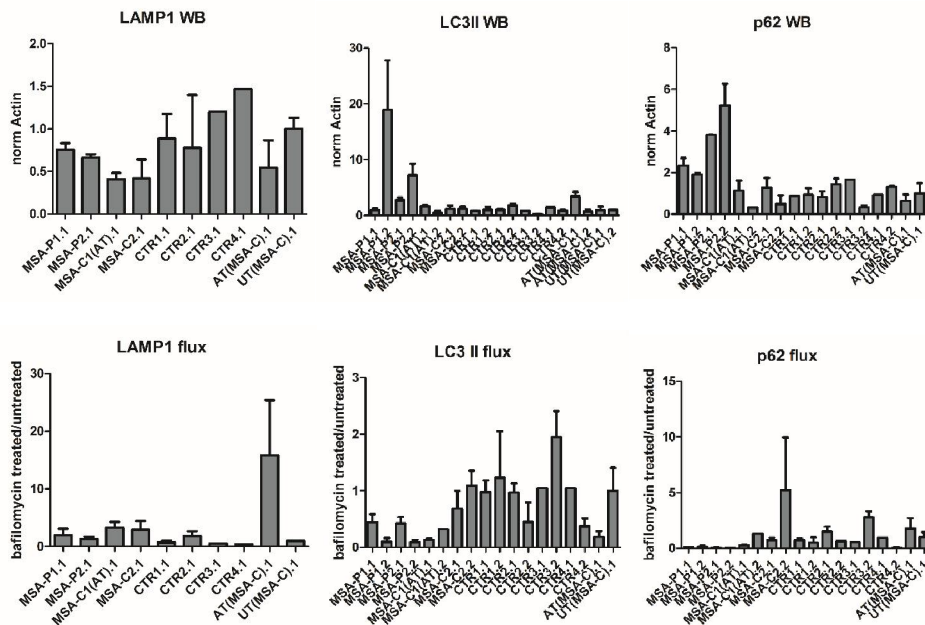
Lysosomal enzymes activity
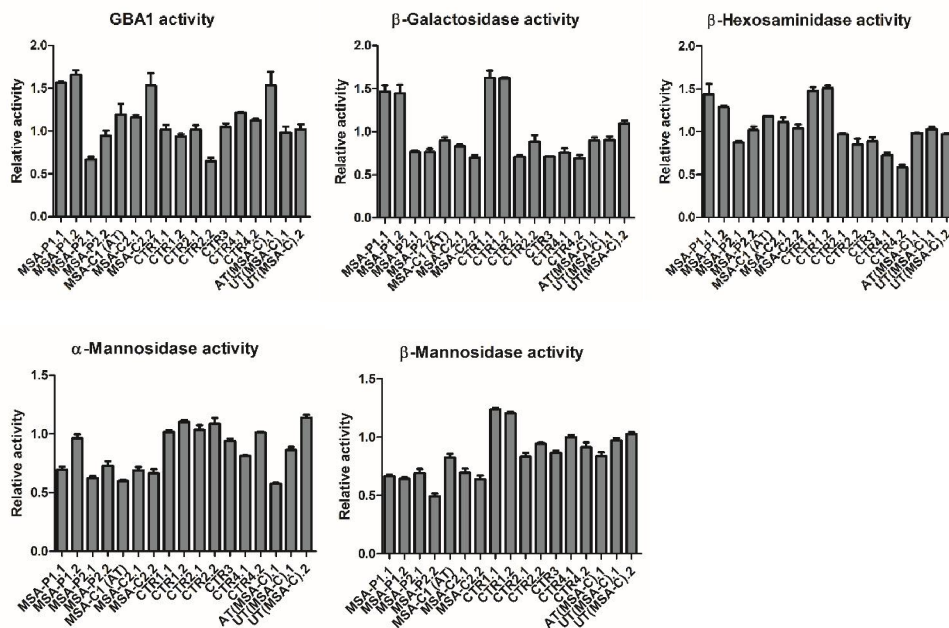
Figure S5. Related to Figure 5.
Autophagy-related experiments, showing the results for each clone.

Respiratory chain activity
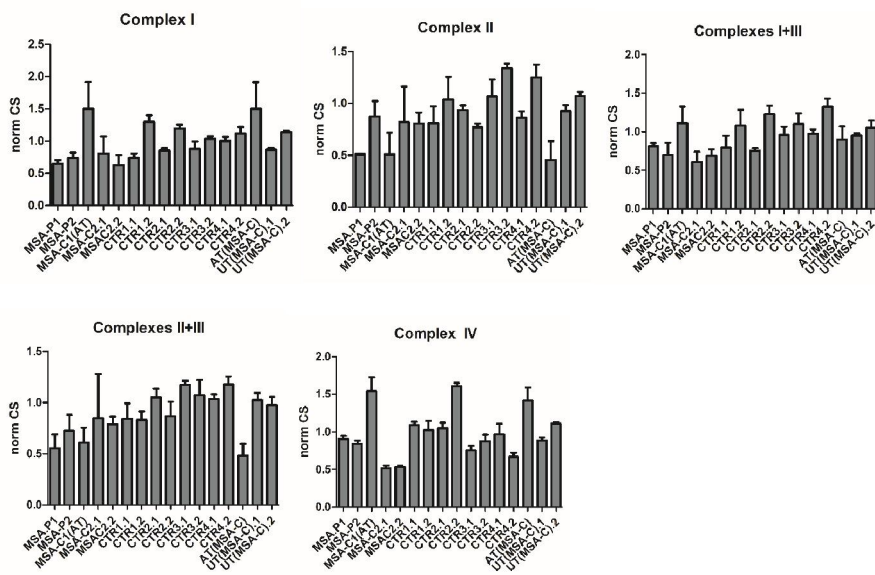
Respiratory chain complexes WB
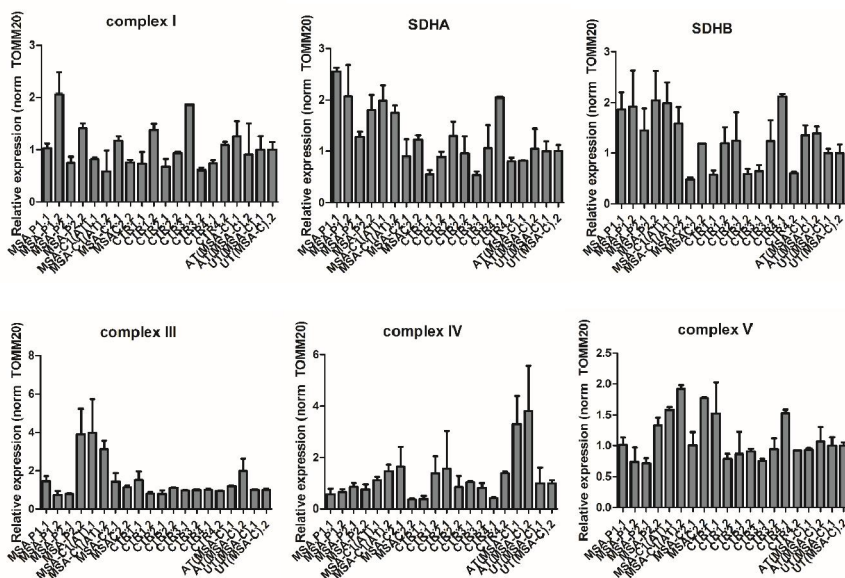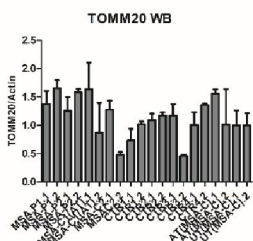
TOMM20 WB

Figure S6. Related to Figure 6.
Mitochondria-related experiments (respiratory chain activity, respiratory chain complexes WB, TOMM20 WB), showing the results for each clone.

mtDNA content
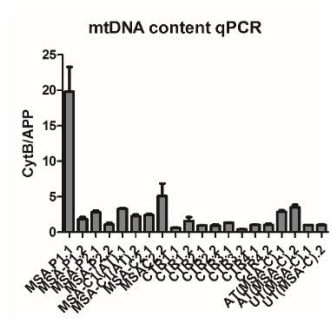
CoQ10 amount
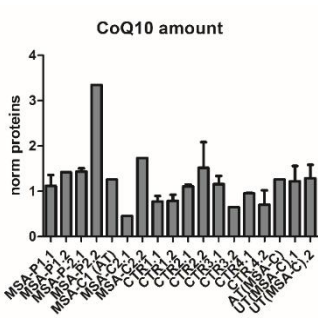
CoQ10 biosynthesis enzymes WB
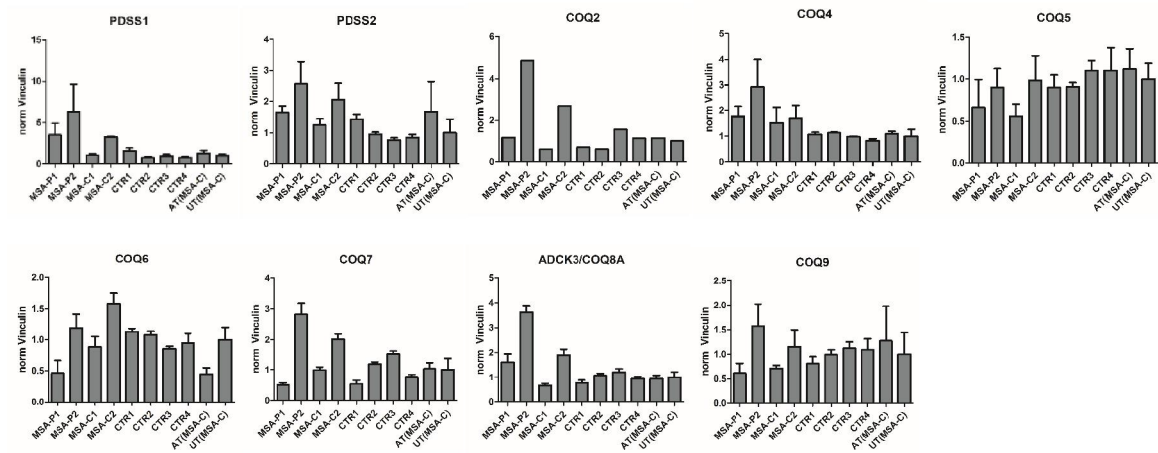
Figure S7. Related to Figure 6.
Mitochondria-related experiments (mtDNA content, CoQ10 amount, CoQ10 biosynthesis enzymes WB), showing the results for each clone.

	DAPI/TUJ1 35 DIV	TUJ1/TH 35 DIV	DAPI/MAP2 50 DIV
MSA-P1	91 %	83 %	87 %
MSA-P2	87 %	55 %	88 %
MSA-C1	94 %	70 %	88 %
MSA-C2	88 %	86 %	90 %
CTR1	92 %	81 %	84 %
CTR2	96 %	78 %	89 %
CTR3	89 %	94 %	87 %
CTR4	92 %	92 %	84 %
UT	86 %	70 %	84 %

Table S1. Related to Figure 2.

Percentage of DAPI/TUJ1 co-staining and TUJ1/TH co-staining at 35 DIV and of DAPI/MAP2 co-staining at 50 DIV in iPSC-derived neurons.

	% aqueous phase of total sphingolipids
P2 D35	22,18
P2 D70	18,67
C2 D35	22,33
C2 D70	16,17
ctrl D35	18,57
ctrl D70	20,57

Table S2. Related to Figure 3.

Radioactivity associated with the aqueous phases expressed as % of the total radioactivity associated with sphingolipid composition of iPSC-derived neurons.

	GM3	GM2	GM1	GD3	GD1a	GD1b	GT1b	GQ1b
P2 D35	0,147	0,120	0,179	0,600	0,458	0,208	0,232	0,030
P2 D70	0,045	0,106	0,208	0,313	0,498	0,165	0,219	0,025
C2 D35	0,081	0,056	0,165	0,661	0,395	0,221	0,236	0,045
C2 D70	0,061	0,099	0,161	0,471	0,391	0,156	0,178	0,024
ctrl D35	0,191	0,188	0,374	0,783	0,606	0,257	0,293	0,034
ctrl D70	0,311	0,135	0,150	0,393	0,444	0,173	0,155	0,030

Table S3. Related to Figure 3.

Radioactivity expressed as nCi/mg of cellular proteins associated with mono- and poly-gangliosides.

Antibody	Manufacturer	Code	Dilution
Pluripotent Stem Cell 4-Marker Immunocytochemistry Kit	Thermo Fisher	A24881	1:100
TUJ1	Abcam	ab18207	1:250
TH	R&D Systems	MAB7566	20 µg/ml
TH	Thermo Scientific	PA5-17800	1:100
GIRK2	Abcam	ab65096	1:100
MAP2	Sigma-Aldrich	M9942	1:100
Alexa 488 anti-mouse	Life Technologies	A11001	1:500
Alexa 488 anti-goat	Jackson ImmunoResearch	705-545-003	1:500
Alexa 488 anti-rabbit	Life Technologies	A11034	1:500
Alexa 568 anti-mouse	Life Technologies	A11004	1:500
Alexa 568 anti-rabbit	Life Technologies	A11011	1:500

Table S4. Related to Experimental Procedures.

Primary and secondary antibodies used for ICC

Antibody	Manufacturer	Code	Dilution
TUJ1	Sigma	T8660	1:1000
Alpha-synuclein	BD transduction laboratories	610787	1:1000
LAMP1	Abcam	ab25630	1:875
P62	Millipore	MABN130	1:1200
LC3	Cell Signaling	2775	1:1000
TOMM20	Sigma	HPA011562	1:750
OXPHOS (containing antibodies detecting NDUFB8, SDHB, UQCRC2, MTCO2 and ATP5A)	Abcam	ab110411	1:1000
SDHA	Invitrogen	459200	1:1000
TAU	Cell Signaling	4019	1:1000
Synaptophysin	Synaptic System	101011	1:5000
Synapsin I	Synaptic System	106103	1:2000
Synapsin III	Synaptic System	106303	1:2000
ADCK3/CABC1	Thermo Scientific	PA5-13906	1:1000
PDSS1	Sigma	AV46195	1:1000
PDSS2	Abcam	ab88817	1:1000
COQ2	Abnova	H00027235-M03	1:1000
COQ4	Abcam	ab167182	1:1000
COQ5	Thermo Scientific	PA5-26327	1:1000
COQ6	Abcam	ab128652	1:1000
COQ7	Thermo Scientific	PA5-25774	1:1000
COQ9	Thermo Scientific	PA5-24816	1:1000
Actin	Sigma	A2066	1:1200
Vinculin	Abcam	ab18058	1:5000
GAPDH	Sigma-Aldrich	G8795	1:5000
Secondary mouse HRP	Dako	P0260	1:3200
Secondary rabbit HRP	Dako	P0217	1:2700
Secondary goat HRP	Dako	P0160	1:4000
Secondary mouse HRP	Sigma	A0545	1:2000
Secondary rabbit HRP	Sigma	A9044	1:2000

Table S5. Related to Experimental Procedures.

Primary and secondary antibodies used for WB

Gene	Code / probe sequence / primer sequence
OCT4 assay	Hs01895061
NANOG assay	Hs02387400
TUBB3 assay	Hs00801390
MAP2 assay	Hs00258900
MAPT assay	Hs00902192
MAPT assay	Hs00902312
18S assay	Hs99999901
GAPDH assay	Hs99999905
TH forward primer	CGGGCTTCTCGGACCAGGTGTA
TH reverse primer	CTCCTCGGCGGTGTACTCCACA
18S forward primer	GCTTAATTTGACTCAACACGGGA
18S reverse primer	AGCTATCAATCTGTCAATCCTGTC
ACTB forward primer	ACGGCTCCGGCATGTGCAAG
ACTB reverse primer	TGACGATGCCGTGCTCGATG
CYTB forward primer	GCCTGCCTGATCCTCCAAAT
CYTB reverse primer	AAGGTAGCGGATGATTCAGCC
APP forward primer	TGTGTGCTCTCCCAGGTCTA
APP reverse primer	CAGTTCTGGATGGTCACTGG
CYTB probe	CACCAGACGCCTCAACCGCCTT (VIC-MGB)
APP probe	CCCTGAACTGCAGATCACCAATGTGGTAG (FAM-MGB)

Table S6. Related to Experimental Procedures.

TaqMan assays, probes and primers sequences used for RT-PCR

	Manufacturer	Code
DMEM high glucose	Euroclone	ECM0728L
Neurobasal medium	gibco	21103-049
Knockout Serum Replacement	gibco	10828-028
N-2 Supplement (100X)	gibco	17502-048
B27 Supplement (50X)	gibco	17504-001
Glutamax	gibco	35050-061
NEAA	gibco	11140-035
Beta-mercaptoethanol	gibco	31350-010
Penicillin/Streptomicin	Euroclone	ECB3001D
Amphotericin	Euroclone	ECM0009D
Accutase	Euroclone	ECB3056D
cAMP	Sigma	D0260
BDNF	Peprotech	450-02
GDNF	Peprotech	450-10
TGF-beta	Sigma	SRP171
FGF8b	RD systems	423-F8
SAG	Calbiochem	566660
SB431542	Sigma	54317
LDN	StemMACS	LDN-193189
CHIR 99021	Sigma	SML1046
Purmorphamine	Calbiochem	540220
Ascorbic acid	Sigma	A92902

Table S7. Related to Experimental Procedures.

Reagents used for neuronal differentiation