Supplemental information

Tables

Supplementary Table S1. List of primary antibodies used in this study

Antibody	Species	Isotype	Dilution	Cat.Nr.	Source
α-synuclein	mouse	IgG1	1:500	sc-12767	Santa Cruz Biotechnology, Dallas, TX, USA
α-synuclein	rabbit	IgG (H+L)	1:1000	2642S	Cell Signaling Technology, Danvers, MA, USA
β-actin	rabbit	IgG (H+L)	1:1000	5125S	Cell Signaling Technology, Danvers, MA, USA
CTIP2	rat	IgG2a	1:300	ab18465	Abcam, Cambridge, MA, USA
GABA	mouse	IgG1	1:500	A0310	Sigma-Aldrich, St. Louis, MO, USA
GABA	rabbit	IgG (H+L)	1:1000	A2052	Sigma-Aldrich, St. Louis, MO, USA
GAPDH	mouse	IgG1	1:1000	sc-32233	Santa Cruz Biotechnology, Dallas, TX, USA
GATA-4	rabbit	IgG (H+L)	1:400	MA5-32678	Thermo Fisher Scientific, Waltham, MA, USA
Nestin	mouse	IgG1	1:300	MAB1259	R&D Systems, Minnesota, MN, USA
OCT4	rabbit	IgG (H+L)	1:400	2750S	Cell Signaling Technology, Danvers, MA, USA
PAX6	mouse	IgG1	1:400	ab78545	Abcam, Cambridge, MA, USA
SOX2	rabbit		1:500	3579P	Cell Signaling Technology, Danvers, MA, USA
TRA-1-60	mouse	IgM	1:400	ab16288	Abcam, Cambridge, MA, USA
TUJ1	mouse	IgG2a	1:1000	ab78078	Abcam, Cambridge, MA, USA
TUJ1	rabbit	IgG (H+L)	1:1000	ab18207	Abcam, Cambridge, MA, USA
Vimentin	mouse	IgG1	1:400	ab8978	Abcam, Cambridge, MA, USA
DAPI					Thermo Fisher Scientific, Waltham, MA, USA

Abbreviations: COUP TF1-interacting protein 2 (CTIP2), γ-aminobutyric acid (GABA), GATA binding protein 4 (GATA-4), octamer-binding transcription factor 4 (OCT4), paired box protein Pax-6 (PAX6), SRY (sex determining region Y)-box 2 (SOX2), Podocalyxin (TRA-1-60), β-tubulin III (TUJ1), 4,6-diamidino-2-phenylindole (DAPI).

Supplementary Table S2. List of secondary antibodies used in this study

Target species	Host species	Target antigen	AlexaFluor	Dilution	Cat.Nr.	Source
mouse		IgG1	555		A21127	
mouse		IgG2a	488		A21131	
mouse		IgM	488		A21042	
rabbit	goat	IgG (H+L)	488	1:1000	A11034	Thermo Fisher Scientific, Waltham, MA, USA
rabbit		IgG (H+L)	555		A21428	
rat		IgG (H+L)	555		A21434	
mouse	horse	IgG (H+L)	na (Dot Blot)	1:2500	PI-2000-1	Vector Laboratories, Burlingame, CA, USA
rabbit	goat	IgG (H+L)		1:5000	PI-1000-1	

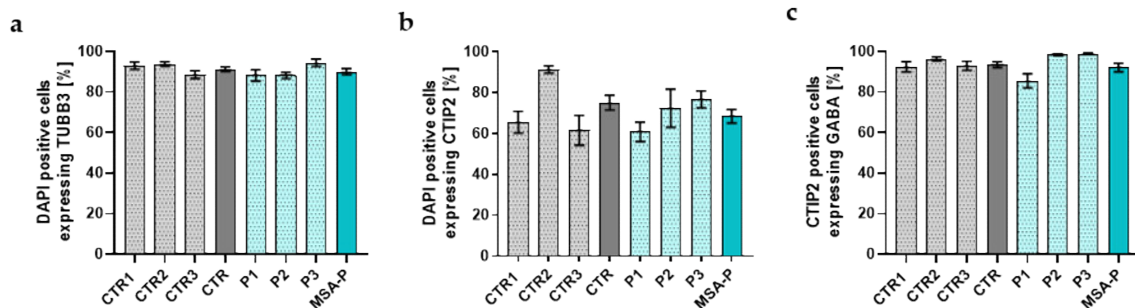**Supplementary Table S3. List of oligosequences used for quantitative RT-PCR analysis**

Target	Primer sequence (5′ $\longrightarrow$ 3′)
iPSC characterization	
c-Myc	fwd: CCAGCAGCGACTCTGAGGA
	rev: GAGCCTGCCTCTTTTCCACAG
Ki67	fwd: AAGTACATGTGCCTGCTCGACCC
	rev: TGTGACATGTGCTTGTCAACTGCG
Nanog	fwd: CACCTATGCCTGTGATTTGTGGGC
	rev: AGAAGTGGGTTGTTTGCCTTTGGG
Oct4	fwd: GGAAGGAATTGGGAACACAAAGG
	rev: AACTTCACCTTCCCTCCAACCA
MSN characterization	
CTIP2	fwd: CTCCGAGCTCAGGAAAGTGTC
	rev: TCATCTTTACCTGCAATGTTCTCC
FOXP1	fwd: CCACGTGGAAGAATGCAGTGCG
	rev: GCATTGAGAGGTGTGCAGTAGGC
GAD67	fwd: AGATCAACAAATGCCTGGAACTGGC
	rev: GAGCCACCTTGTGTAGCTTTTCCC
MAP2	fwd: CAGGCAAAGGACAAAGTCTCTGACG
	rev: CGCCGAGGAGGGAGAATGGAGG
SST	fwd: GAGATCTGCTAACTCAAACCCGGC
	rev: TCGCTGAAGACTTGGAGGATTAGGG
***α*-Syn**	fwd: AAGAGGGTGTTCTCTATGTAGGC
	rev: GCTCCTCCAACATTTGTCACTT
GABA$_A$ receptor subunit	

α1	fwd: TGCAGCTTGGAGACAGGATT
	rev: TGAACCATCTTCCCCCTCTT
α2	fwd: AGAGGATGGACTTGGGATGG
	rev: AAGATTCGGGGCATAATTGG
α3	fwd: CACAAGTGTCGTTCTGGCTCA
	rev: TGGCACTGATACTCAAGGTGGT
α4	fwd: TCCGGTTTTCATGCAAAGGT
	rev: CTTCATTAAGGATAAGCCAGTGGAA
α5	fwd: GGTGTCCTTTTGGCTGAACC
	rev: GCCACTTTGGGCAGAGAGTT
α6	fwd: TTTCCCAGGTGTCTTTCTGGA
	rev: GGCACTGATGCTCAAAGTGG
β1	fwd: ATGCATCTGCAGCCAGAGTC
	rev: AGGGATCTTTGGCAGGGTCT
β2	fwd: CCCAAACCAAATGTCACTGC
	rev: TGGAACTGTCAACTTGCTTCAAA
β3	fwd: ATTGAAAGGCGCCATGTTTT
	rev: GGGTTGGTCCTAGGGAGAGG
γ1	fwd: GGAGATGGGGGATGATAGGC
	rev: ATCCCTTCCACCCAACACAC
γ2	fwd: TTGTCGAACAGGAGCTTGGA
	rev: GAAGGCAGTGGGGAAGAAGA
γ3	fwd: AACCAACCACCACGAAGAAGA
	rev: CCTCATGTCCAGGAGGGAAT
δ	fwd: GTCTTTGCTCTGCAGGATCG
	rev: CCAGGCCAAGGCTTTATTTC
GABA$_B$ receptor subunit	
GABBR1	fwd: AGATGACTGAGGCGGTGGA
	rev: TTCAGCCGCTTGGTTAGTTTC
GABBR2	fwd: GAGCAGATCCGCAACGAGTCAC
	rev: GACAGACGCCTCCAAACACCATC
ATP-regulated potassium (K_{ATP}) channels	
ABCC8	fwd: AACAACGGCTGCTTTGTGGACG
	rev: CAGGTTGTGCCCAGGGAAATGAAG
ABCC9	fwd: TTGAAGCAACCAGAAGTAGGAACAGG
	rev: GGCTGAAGAGAACAGGCATCTGTG
voltage-gated Ca^{2+}-channel subunit	
Ca$_V$ 1.2 (L-type)	fwd: CATTTGACGCCTTGATTGTTGTGGG
	rev: GTATGTTCAGCTGGGTTTACCTCGG
Ca$_V$ 1.3 (L-type)	fwd: CGGACCCCGTCCTCGAAGGA
	rev: CCTACGCGGATCGGGTTGGT
Ca$_V$ 2.1 (P-type)	fwd: CCAGAAACTTGCCCTACAGAAAGCC
	rev: CGGGTCCATTTCGTTATACAGGGC
Ca$_V$ 2.2 (N-type)	fwd: TGCTGTTCAGGAGCGCCACG
	rev: CGGTGGCATTGGCCTGCTCA

Ca$_v$ 2.3 (R-type)	fwd: GTGGCCCTGGGGTTCATCTTCCATA
	rev: CAGGATGCCACTGAGGACCACGA
Ca$_v$ 3.1 (T-type)	fwd: TCAGCCTCCCCCTGAGCGTG
	rev: TTCTGCAGGACCGCATGCCG
Ca$_v$ 3.2 (T-type)	fwd: GTCACTCTGCTGCTGGATACGC
	rev: TCAGGTTGTTGTTCCTGACAAAGGC
Ca$_v$ 3.3 (T-type)	fwd: ATCGACTACACCCTGTGCTTCCG
	rev: GACGTAGTCGAAGAGTTTGTGGGC
reference genes	
B2M	fwd: TGCCTGCCGTGTGAACCATGT
	rev: TGCGGAATCTTCAAACCTCCATGA
GAPDH	fwd: AGCCACATCGCTCAGACACCAT
	rev: CAGGCGCCCAATACGACCAAAT
β-actin	fwd: CATGTACGTTGCTATCCAGGC
	rev: CTCCTTAATGTCACGCACGAT

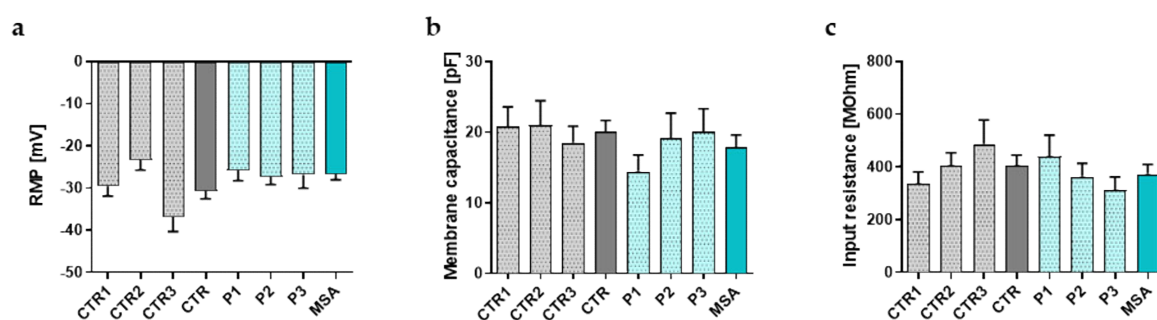# Figures

Supplementary Figure S1

Supplementary Figure S1: Quantification of immunocytochemistry stainings of the mature MSNs at day 70 (± 3 days) of differentiation, confirming the expression of (a) TUBB3 and (b) CTIP2 in 88-95% and 61-95% of all cells analyzed, respectively, as well as (c) GABA in 86-99% of the CTIP2-positive cells. Each cell line used in this study was analyzed separately and is shown separately.
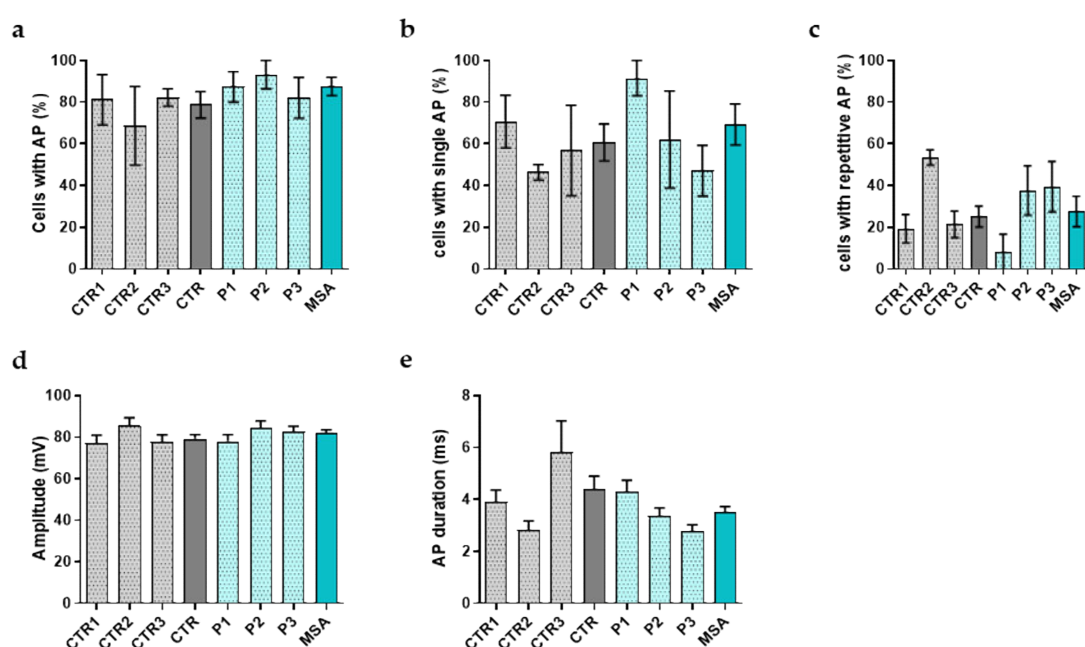
Supplementary Figure S2

Supplementary Figure S2: Spontaneous calcium (Ca^{2+}) signaling of the MSNs derived from the patients with MSA-P and the healthy controls. Intracellular Ca^{2+} transients are presented as ratios of the fluorescence signals obtained at 340 and 380 nm (F_{340}/F_{380}). (**a**) Basal intracellular Ca^{2+} levels of all cell lines. (**b**) Percentage of cells exhibiting spontaneous Ca^{2+} transients, (p = 0.0251, nonparametric Mann–Whitney test) (**c**) frequency (p = 0.0047, nonparametric Mann–Whitney test) and (**d**) amplitudes of these transients. Each cell line was analyzed separately and is shown separately. Data are presented as means ± SEM.
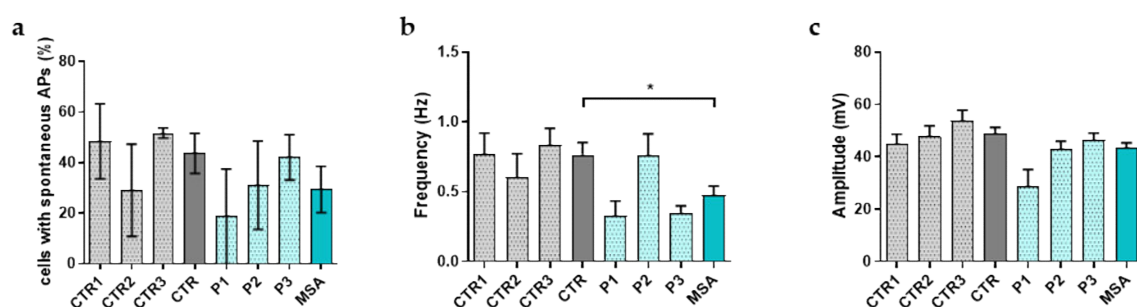
Supplementary Figure S3

Supplementary Figure S3: Resting membrane potential (**a**), membrane capacitance (**b**), and input resistance (**c**) of each cell line measured by voltage-gated patch-clamp recordings. Data are presented as means ± SEM.
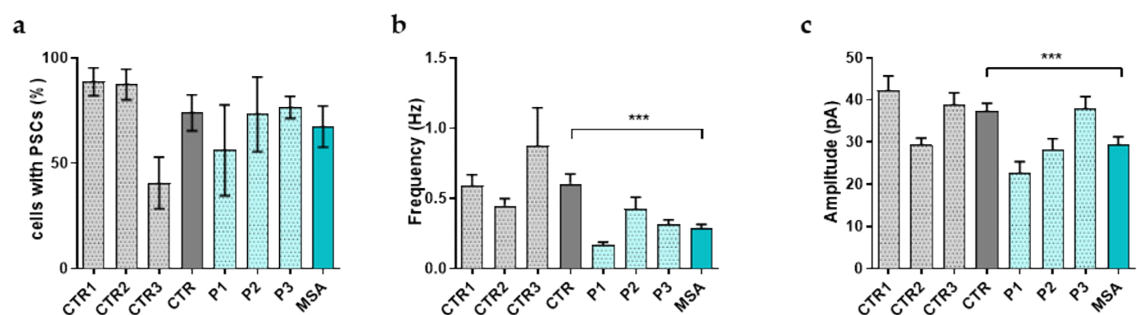
Supplementary Figure S4

Supplementary Figure S4: (**a**) Percentage of cells able to spike action potentials (APs) evoked by depolarizing current pulses, (**b**) percentage of cells that produced a single evoked AP, and (**c**) percentage of cells with repetitive firing. (**d**) Amplitude and (**e**) duration of evoked APs measured by voltage-gated patch-clamp recordings for each cell line. Data are presented as means ± SEM.

Supplementary Figure S5

Supplementary Figure S5: Properties of spontaneous APs in the MSNs from the MSA-P patients and the healthy controls. (**a**) Percentage of neurons with spontaneous APs, (**b**) frequency ($p = 0.0291$, nonparametric Mann–Whitney test), and (c) amplitudes of spontaneous APs in all cell lines used in this study. Data are presented as means ± SEM.

Supplementary Figure S6

Supplementary Figure S6: Characteristics of miniature postsynaptic currents (mPSCs) in the MSNs from the MSA-P patients and the healthy controls. (**a**) Percentage of neurons showing mPSCs, (**b**) frequency ($p = 0.0007$, non-parametric Mann–Whitney test), and (c) amplitudes of mPSCs ($p < 0.0001$, nonparametric Mann–Whitney test) in all cell lines used in this study. Data are presented as means ± SEM.