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Supplemental information

Human iPSC-derived neurons reveal early developmental alteration of neurite outgrowth in the late-occurring neurodegenerative Wolfram syndrome

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Supplemental Data

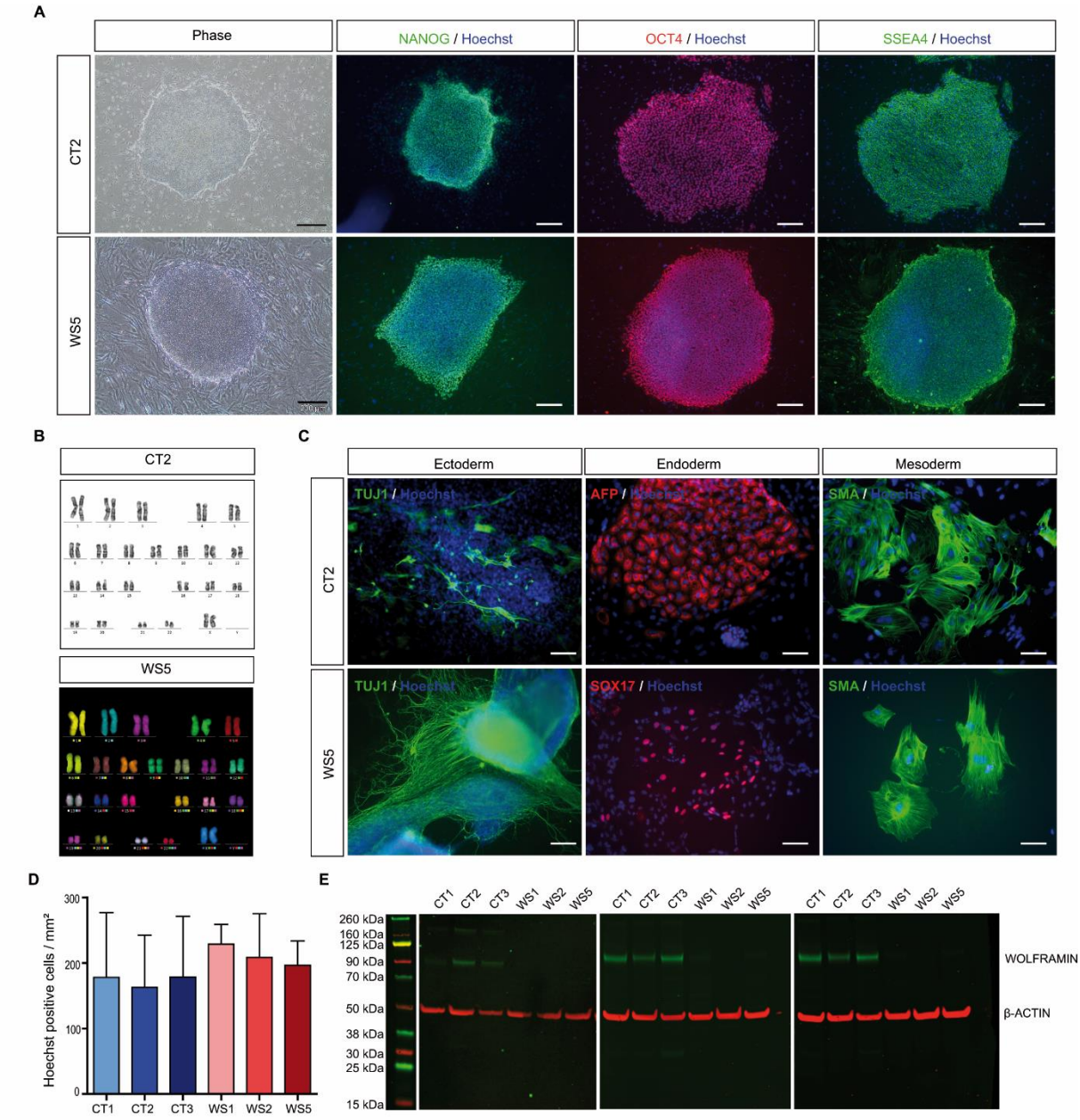


Figure S1: Characterization of CT2 and WS5 iPSC lines and quantification of the number of cells in CT and WS neuronal culture. A. Phase contrast images of CT2 and WS5 iPSCs and immunostaining of the pluripotency markers NANOG, OCT4 and SSEA4. Scale bars, 200 µm. **B.** G-banding and mFISH (46, XX). **C.** Three germ layer differentiation in *in vitro* embryoid bodies. Scale bars, 200 µm. **D.** Quantification of the number of cells (Hoechst) in CT and WS neuronal culture at DIV 14 from NSC stage. **E.** Western blot images of wolframin in CT and WS neurons at DIV 21 (related to Figure 1D for left panel; n = 3 biologically independent differentiations).

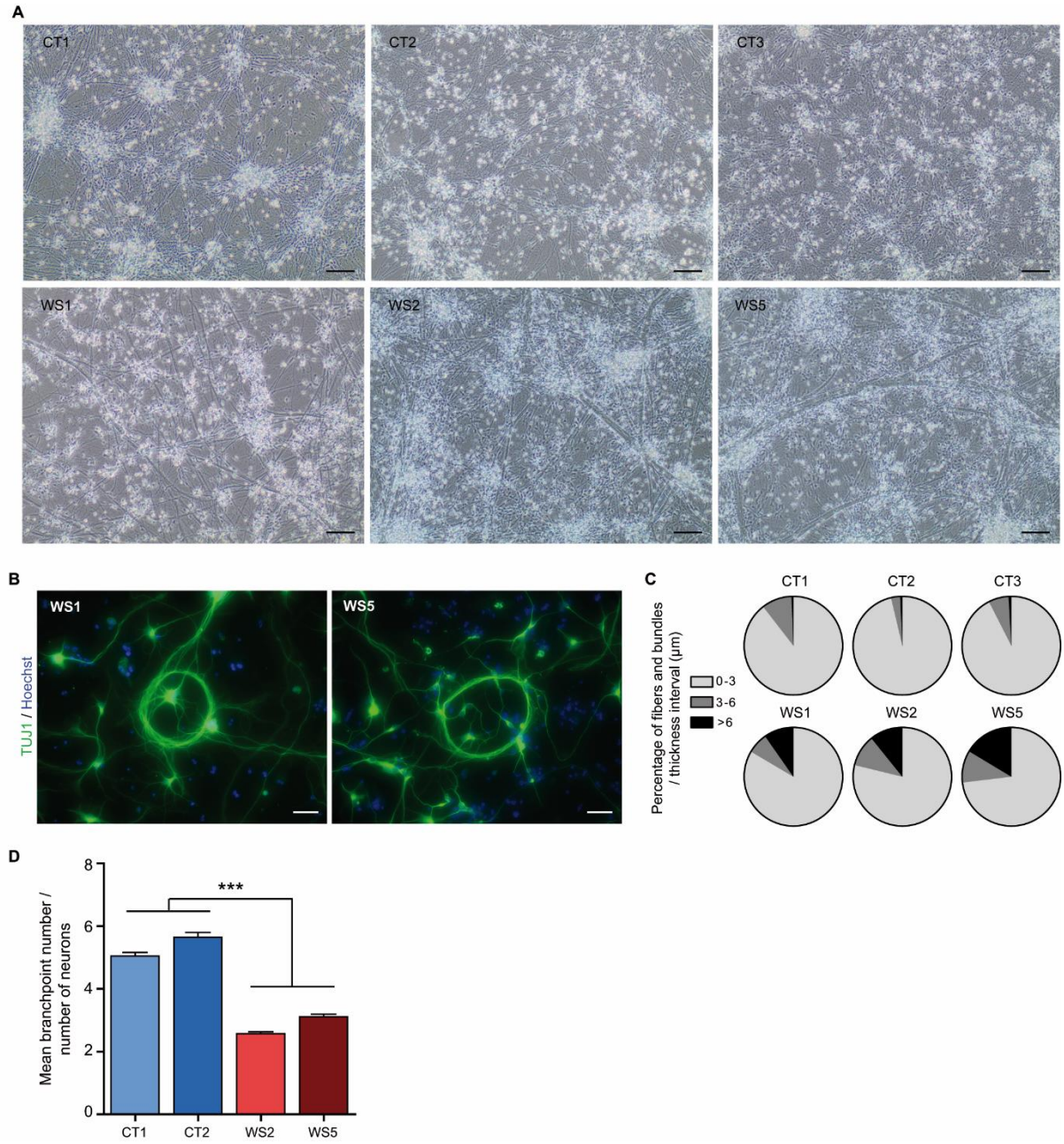


Figure S2: Abnormal neurite outgrowth in WS neurons. A. Phase contrast images of CT and WS neuronal network at DIV 14. Scale bars, 200 µm. **B.** TUJ1 immunostained loop structures. Scale bars, 50 µm. **C.** Proportion of isolated and bundled neurites for three thickness intervals (0 - 3; 3 - 6 and > 6 µm) in CT and WS neurons. **D.** Mean branchpoint number per number of neurons. Data are presented as mean ± SEM (n = 1 differentiation; 43 technical replicats; ***p < 0.001; Student's t-test).

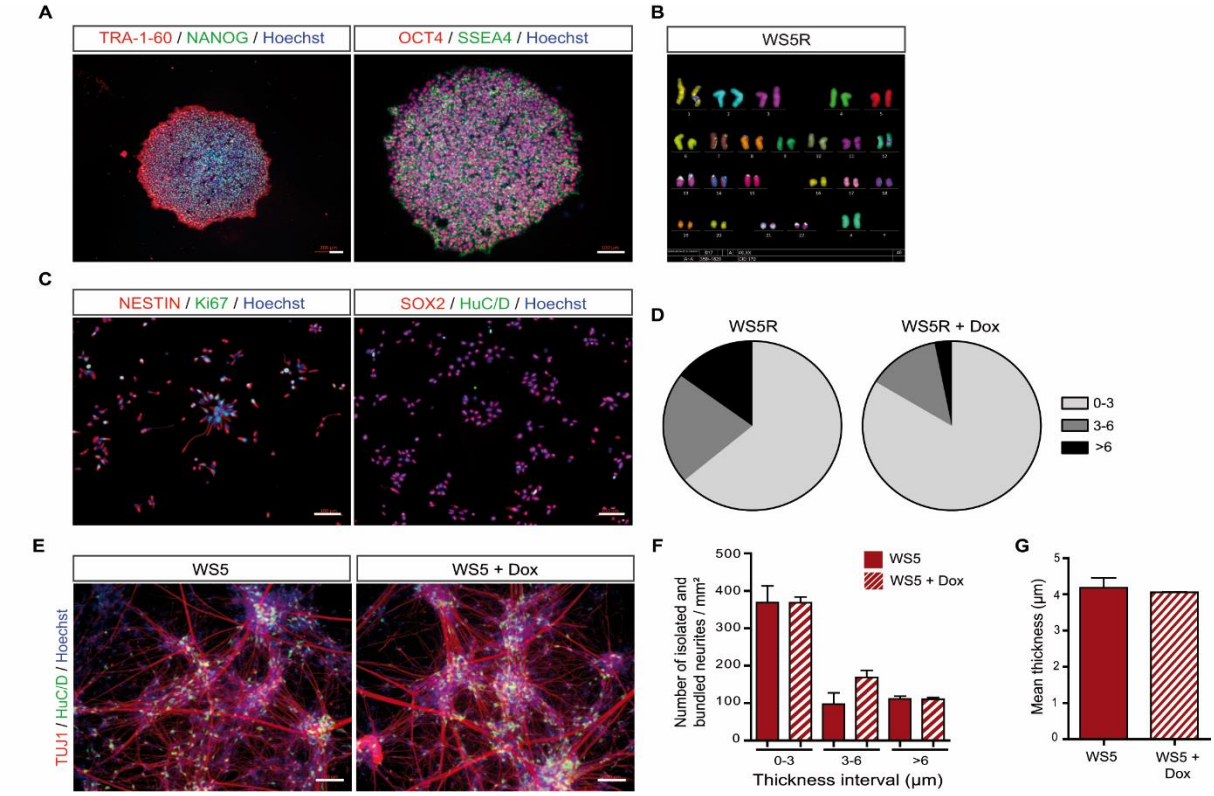
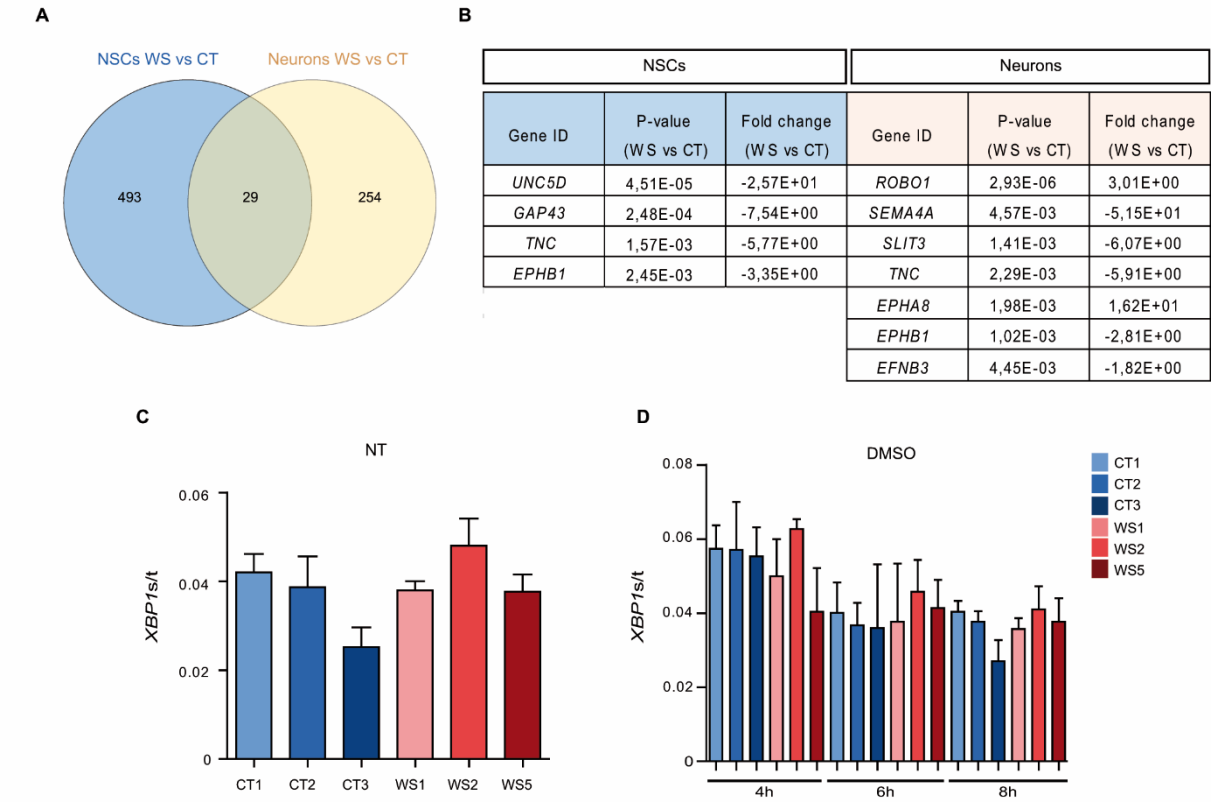


Figure S3: Characterization of WS5 rescue (WS5R) iPSCs, NSCs and neurons. A. Characterization of WS5R iPSCs by immunostaining of the pluripotency markers TRA-1-60, NANOG, OCT4, SSEA4. Scale bars, 100 µm**. B.** mFISH chromosome analysis of WS5R iPSC line showing a normal karyotype (46, XX). **C.** Immunostaining of the neural (NESTIN and SOX2), the neuronal (HuC/D) and the proliferation (Ki67) markers in WS5R-derived NSCs. Scale bars, 100 µm. **D.** Proportion of isolated and bundled neurites per each thickness interval (0 - 3; 3 - 6 and > 6 µm) of WS5R neurons at DIV 21. **E.** TUJ1 and HuC/D immunostaining of the parental WS5 neurons at DIV 21 under doxycycline (Dox) treatments. Scale bars, 100 µm. **F.** Quantification of the number of isolated and bundled neurites per mm^2 for each thickness interval (0 - 3; 3 - 6 and > 6 µm) at DIV 21 in the parental WS5 neurons treated with doxycycline. Data are presented as mean ± SEM (n = 3 biologically independent differentiations; No statistical difference was observed using one-way ANOVA with Dunnett's post hoc test). **G.** Mean thickness of the parental WS5 neurons at DIV 21 under doxycycline treatments. Data are presented as mean ± SEM (n = 3 biologically independent differentiations; No statistical difference was observed using Student's t-test).



NSCs			Neurons		
Gene ID	P-value (WS vs CT)	Fold change (WS vs CT)	Gene ID	P-value (WS vs CT)	Fold change (WS vs CT)
UNC5D	4,51E-05	-2,57E+01	*ROBO1*	2,93E-06	3,01E+00
GAP43	2,48E-04	-7,54E+00	*SEMA4A*	4,57E-03	-5,15E+01
TNC	1,57E-03	-5,77E+00	*SLIT3*	1,41E-03	-6,07E+00
EPHB1	2,45E-03	-3,35E+00	*TNC*	2,29E-03	-5,91E+00
			EPHA8	1,98E-03	1,62E+01
			EPHB1	1,02E-03	-2,81E+00
			EFNB3	4,45E-03	-1,82E+00

Figure S4: Gene expression analysis between CT and WS NSCs and neurons. A. Venn diagram summarizing the number of DEGs specific or common to NSCs and neurons. **B.** Table of axon guidance genes deregulated in WS NSCs and neurons. **C. and D.** Controls of thapsigargin (TG) treatments. Quantification of *XBP1s* expression normalized to total *XBP1* (*XBP1t*) in non-treated (**C**) and in DMSO treated (**D**) CT and WS NSCs after 4, 6 and 8 hours. Data are presented as mean ± SEM (n = 3 biologically independent experiments; *p<0.05; No statistical difference was observed using one-way ANOVA with Dunnett's post hoc test).

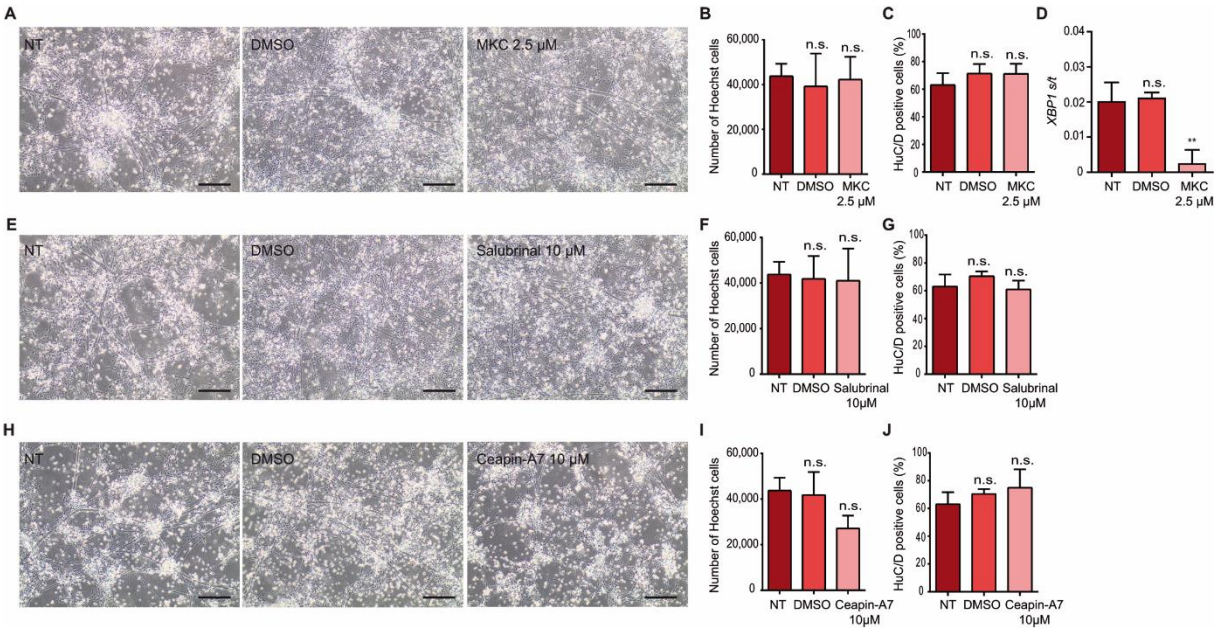


Figure S5: Effect of UPR pathways inhibition on the abnormal neurite outgrowth in WS5 neurons. **A.** Phase contrast images of WS5 neurons at DIV 18 treated or not with 2.5 µM MKC-8866 (MKC) or control DMSO. Scale bars, 200 µm. **B.** and **C.** Quantification of the number of cells (Hoechst) (**B**) and of the percentage of neurons (HuC/D) (**C**) after MKC treatment. **D.** Analysis of spliced *XBP1* (*XBP1s*) expression normalized to total *XBP1* (*XBP1t*) in WS5 neurons treated with 2.5 µM MKC or control DMSO. **E.** Phase contrast images of WS5 neurons at DIV 18 treated or not with 10 µM salubrinal or control DMSO. Scale bars, 200 µm. **F.** and **G.** Quantification of the number of cells (Hoechst) (**F**) and of the percentage of neurons (HuC/D) (**G**) after salubrinal treatment. **H.** Phase contrast images of WS5 neurons at DIV 18 treated or not with 10 µM ceapin-A7 or control DMSO. Scale bars, 200 µm. **I.** and **J.** Quantification of the number of cells (Hoechst) (**I**) and of the percentage of neurons (HuC/D) (**J**) after ceapin-A7 treatment. For all analyses, data are presented as mean ± SEM (n = 3 biologically independent differentiations; **p < 0.01; one-way ANOVA with Dunnett's post hoc test).

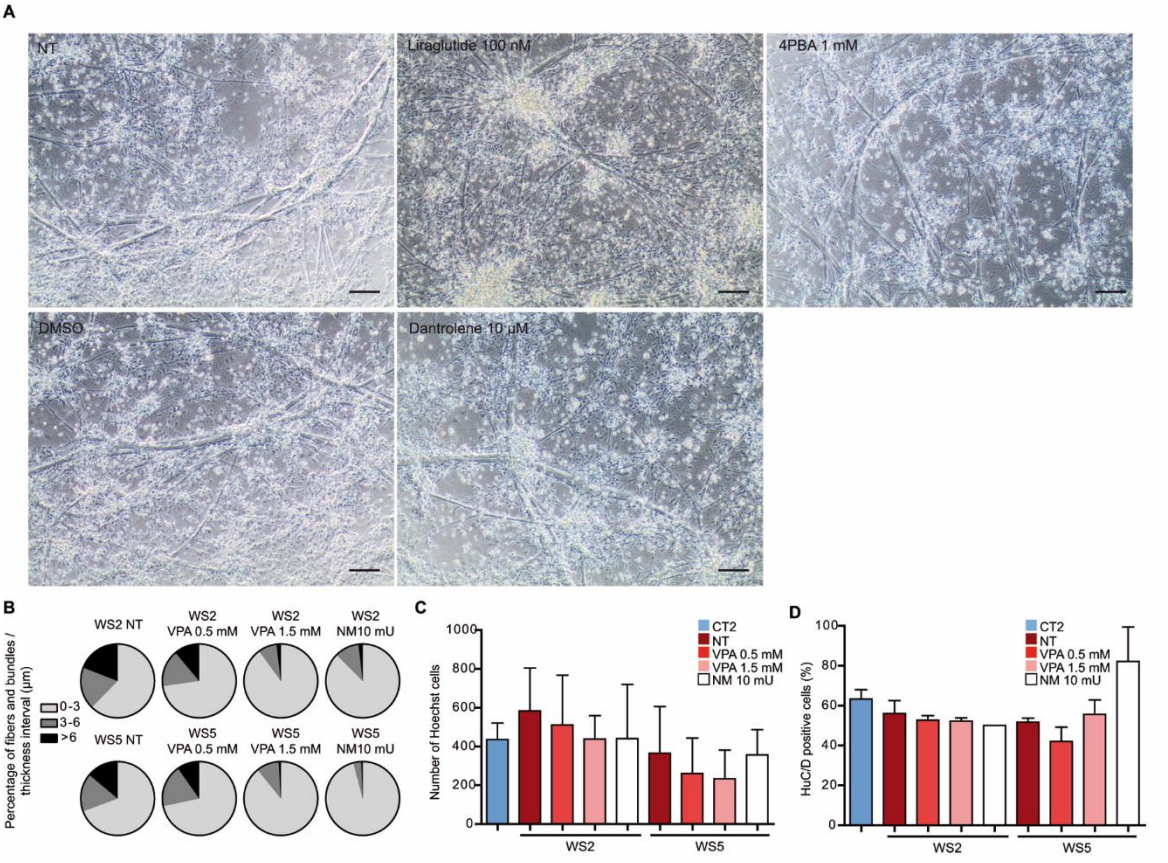


Figure S6: Effect of drugs treatments on the neurite outgrowth defect in WS5 neurons. A. Phase contrast images of WS5 neurons at DIV 18 treated or not (NT) with 10 µM dantrolene, 100 nM liraglutide or 1 mM 4-phenylbutyric acid (4PBA). Scale bars, 200 µm. **B.** Repartition profile of the percentage of isolated and bundled neurites per thickness intervals after VPA treatments. **C.** and **D.** Quantification of the number of cells (Hoechst) (**C**) and of the percentage of neurons (HuC/D) (**D**) after VPA treatments. Data are presented as mean ± SEM (n = 3 biologically independent differentiations; *p<0.05; No statistical difference was observed using one-way ANOVA with Dunnett's post hoc test, data are compared to CT2 cells).

Table S1: Information on iPS cell lines.

iPSC lines	Source of iPSC	Mutations in *WFS1* gene	Sex	Clinical features
WS1	New York Stem Cell Foundation	c.1230-1233delCTCT [p.Val412fs*440]; c.2171C>T [p.Pro724Leu]	Male	Diabetes mellitus; optic atrophy; cognitive disabilities
WS2	New York Stem Cell Foundation	c.1230-1233delCTCT [p.Val412fs*440]	Female	Diabetes mellitus; optic atrophy; cognitive disabilities; diabetes insipidus
WS5	I-Stem	c.1060-1062delTTC [p.Phe354del]; c.1676C>A [p.Ala559Asp]	Female	Diabetes mellitus; optic atrophy
CT1	I-Stem	None	Male	N/A
CT2	I-Stem	None	Female	N/A
CT3	I-Stem	None	Female	N/A

Table S2: List of antibodies, hosts, providers and dilutions.

Application	Antibody	Host	Provider	Dilution
Immunostaining	OCT4	Rabbit	Cell Signaling Technology	1/200
	TRA-1-60	Mouse	Cell Signaling Technology	1/1000
	NANOG	Rabbit	Cell Signaling Technology	1/800
	SSEA4	Mouse	Cell Signaling Technology	1/500
	TUJ1 (neuron-specific class III beta-tubulin)	Chicken	Millipore	1/1000
	SOX17	Goat	R&D systems	1/200
	SMA	Mouse	Agilent Dako	1/100
	AFP	Mouse	Cell Signaling Technology	1/100
	NESTIN	Rabbit	Millipore	1/500
	Ki67	Mouse	Agilent Dako	1/500
	HuC/D (Elav family members HuC, HuD)	Mouse	ThermoFisher Scientific	1/250
	CUX2 (Cut-Like Homeobox 2)	Rabbit	Abcam	1/500
	Alexa Fluor™ 555 Donkey anti-rabbit	Donkey	ThermoFisher Scientific	1/1000
	Alexa Fluor™ 555 Goat anti-chicken	Goat	ThermoFisher Scientific	1/1000
	Alexa Fluor™ 555 Goat anti-mouse	Goat	ThermoFisher Scientific	1/1000
	Alexa Fluor™ 488 Chiken anti-rabbit	Chicken	ThermoFisher Scientific	1/1000
	Alexa Fluor™ 488 Goat anti-mouse	Goat	ThermoFisher Scientific	1/1000
Western-Blot	WOLFRAMIN	Rabbit	Cell Signaling Technology	1/500
	β-ACTIN	Mouse	Li-Cor Biosciences	1/5000
	IRDye 800CW Donkey anti-rabbit	Donkey	Li-Cor Biosciences	1/10000
	IRDye 800CW Donkey anti-mouse	Donkey	Li-Cor Biosciences	1/10000

Table S3: List of primers used for PCR and qPCR.

Gene	Primer	5'-3' Sequence
18S	Forward	GAGGATGAGGTGGAACGTGT
	Reverse	TCTTCAGTCGCTCCAGGTCT
XBP1	Forward	GAAGCCAAGGGGAATGAAGT
	Reverse	GGGAAGGGCATTTGAAGAAC
UNC5D	Forward	ATACCCCAATGCCAAAGGC
	Reverse	TTCAAAATGACAGCAGATGGGC
SLIT3	Forward	GCGATTTGGAGATCCTTACCCT
	Reverse	AGTCGCAGTACAGGTGGTTG
TNC	Forward	TCTGATGGGGGTGGATGGAT
	Reverse	CTTCTCTGCGGTCCCCAAAT
EPHB1	Forward	TCTTCGAGCCCAACCAGAAC
	Reverse	TCTGTGTAGATGCGATGGGC
SEMA4A	Forward	CTTGGTGGATGGGATGCTCTA
	Reverse	GGAGGAAGTTGTCGGTCTTG