

Supplementary Data

Validation of the α 1ACT#2 Antibody

For the validation of the α 1ACT#2 antibody, the exon 47 of the *CACNA1A* gene, including the epitope sequence MERRVPGPARSESPRAC as well as the polyQ repeat expansion from cDNA synthesized from SCA6 neurons (SCA6-2), was subcloned into the pENTRY TOPO cloning vector system (Thermo Fisher). The following primers were used for the polymerase chain reaction: 5'-CACCCACGTGTCCTATTCCCCTG-3' and 5'-TTAGCACCAATCATCGTCACTCT-3'. Polyplus transfection reagent (jetPei) was used to transfect human embryonic kidney (HEK) cells according to the manufacturer's instructions. Two days after transfection, transfected HEK cells and nontransfected controls were either lysed by using RIPA lysis and extraction buffer (Thermo Fisher) or fixed with ice-cold 4% PFA solution.

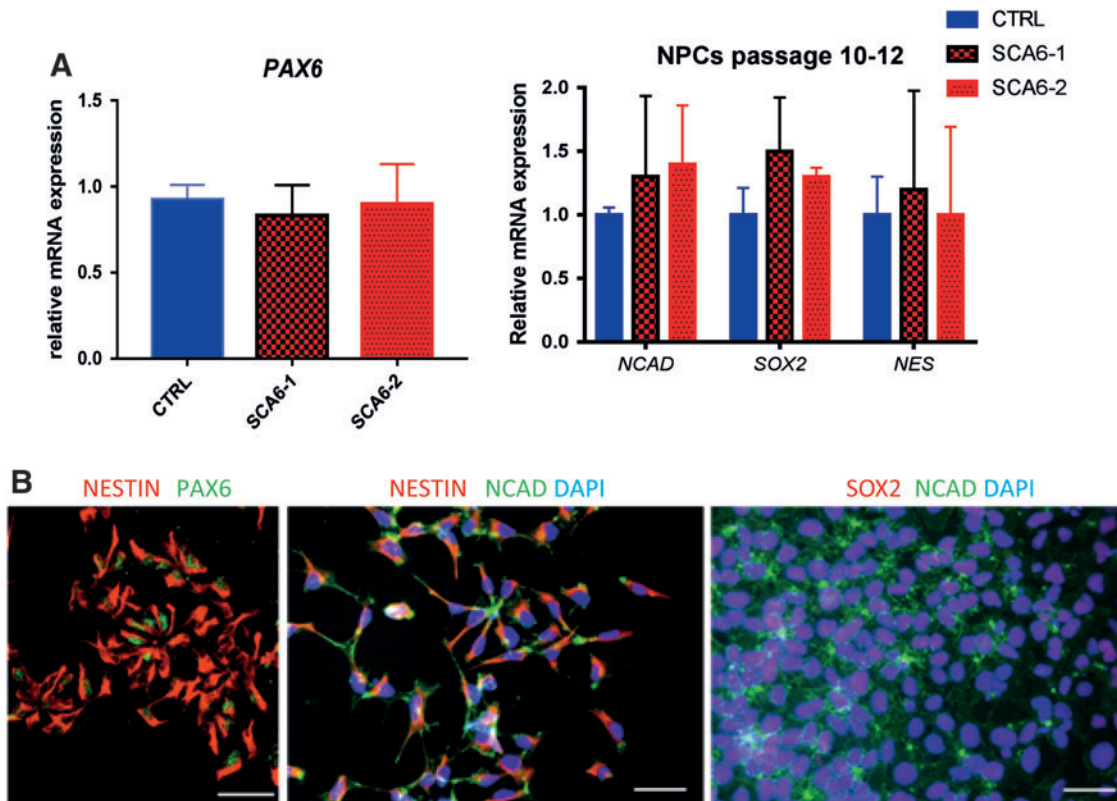
For Western blot analysis, 18 μ g of total lysate was separated in a 10% polyacrylamide gel electrophoresis (PAGE) and blotted on PVDF membrane. After 1 h of blocking using 5% milk solution with 0.05% Tween20, the membrane was incubated either with the affinity-purified fraction of

α 1ACT#2 antibody (1:5,000) or with preimmune rabbit serum overnight at 4°C. After washes and secondary antibody incubation, the membrane was revealed by chemiluminescence reaction by using Super Signal West Dura (Thermo Fisher), and digital imaging was performed.

For immunofluorescence analysis of the transfected HEK cells, we proceeded as described in the main Material and Methods section. Although the specificity of the epitope sequence for the *CACNA1A* gene was confirmed by database analysis and the detected specificity of the α 1ACT#2 antibody in western blot (WB) analysis, we observed an unexplained immunoreactivity in human induced pluripotent stem cell (iPSC)-derived astrocytes (data not shown).

Reference

- Eigentler A, S Boesch, R Schneider, G Dechant and R Nat. (2013). Induced pluripotent stem cells from friedreich ataxia patients fail to upregulate frataxin during in vitro differentiation to peripheral sensory neurons. *Stem Cells Dev* 22:3271–3282.

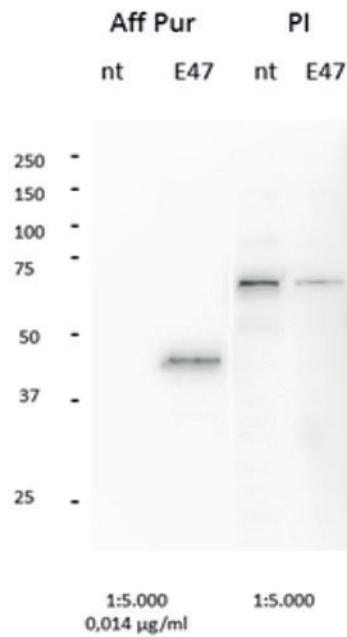


SUPPLEMENTARY FIG. S1. Characterization of SCA6 neural progenitor cells. **(A)** The early neural marker *PAX6* is expressed in NPCs at passage 1–2, and no differences were observed in *PAX6* expression between genotypes by qRT-PCR. Again, control and patient lines showed no significant differences for the expression of *SOX2*, *NESTIN*, and *NCAD* in NPCs at passage 10–12 (used for generating the neurons analyzed in this study). Experiments were repeated with cell populations generated by two different neural induction methods (see the Materials and Methods section) and showed no significant differences between lines or differentiation protocols. Expression levels are normalized to control NPCs. Each bar represents mean \pm s.e.m. from three independent experiments for each induction method. One-way ANOVA test was performed. **(B)** Representative immunostainings showing co-expression of the neural markers *PAX6* and Nestin in SCA6 NPCs at passage 2 (*left*) and of N-Cadherin, Nestin, and *SOX2* in SCA6 NPCs at passage 10–12 (*right*). Scale bars 100 μ m. NPCs, neural progenitor cells.

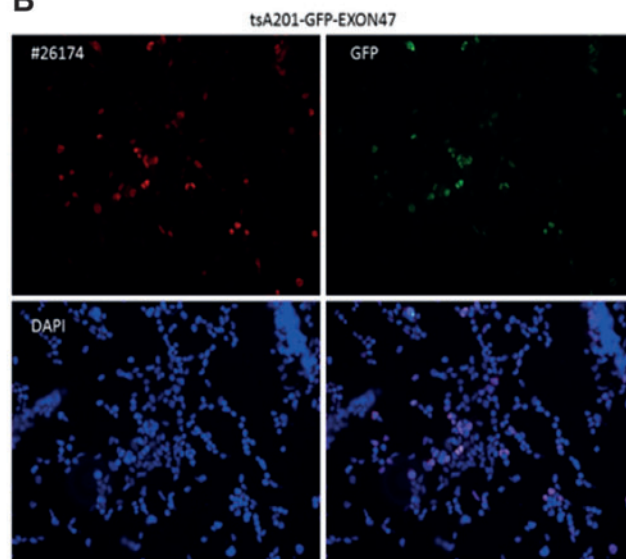
A Gel 10%

samples 18 μ g total lysate

#26174

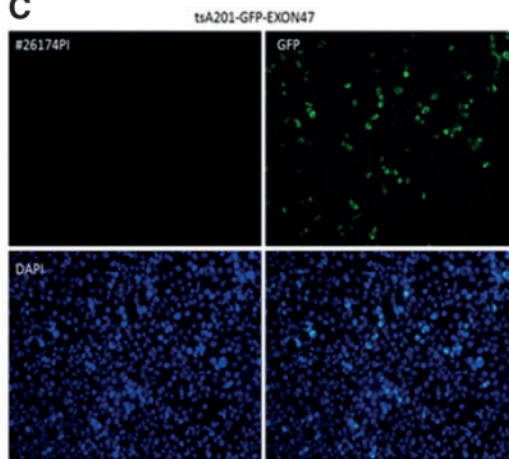


B

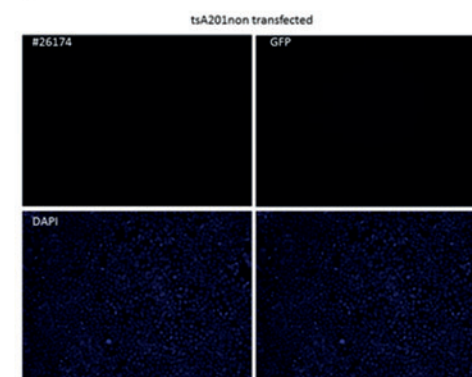


5min exposure

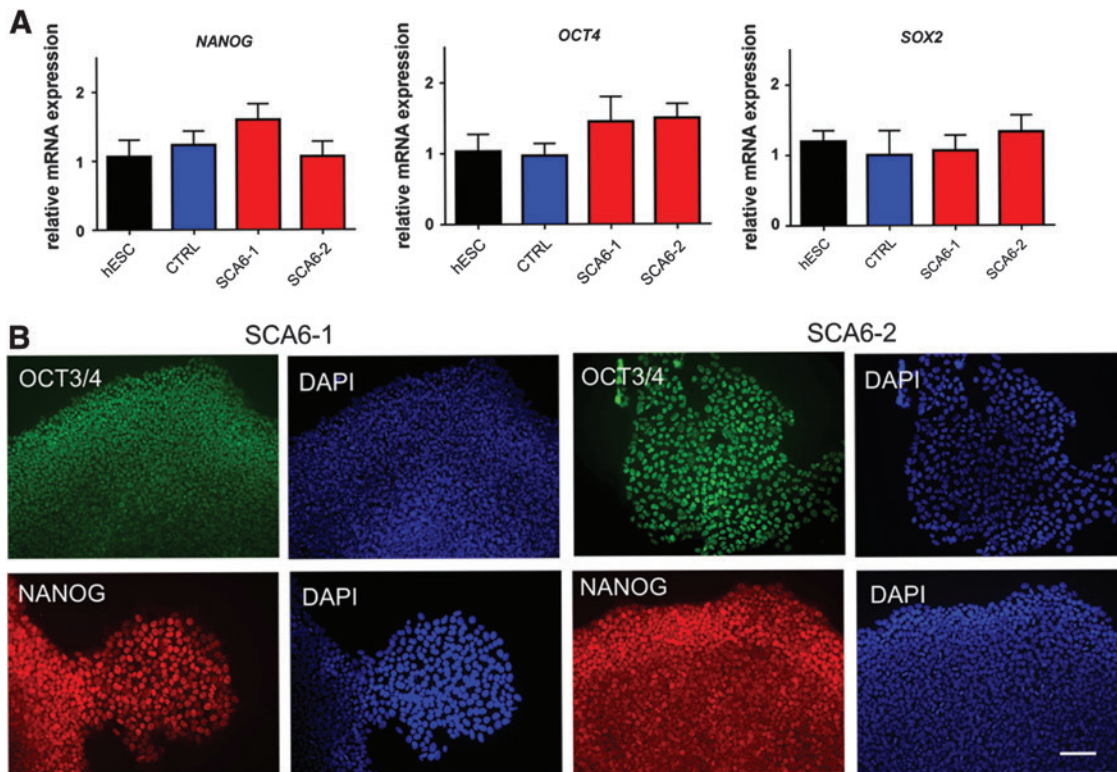
C



D



SUPPLEMENTARY FIG. S2. Characterization of the α 1ACT#2 antibody. (A) Western blot analysis demonstrating the specificity of the α 1ACT#2 antibody. Cell lysates extracted from HEK cells either nt or transiently transfected with the recombinant exon 47 (E47) of the *CACNA1A* gene containing the α 1ACT#2 epitope were analyzed. The *left panel* shows the bands obtained with the affinity purified (Aff. Pur.) α 1ACT#2 antibody, whereas in the *right panel* the PI rabbit serum was used as a negative control. A single specific band is detected by the α 1ACT#2 antibody at the expected molecular weight in lysates containing the recombinant epitope (*left panel*), whereas only nonspecific bands are revealed by the PI serum. (B) Representative immunostainings of HEK cells expressing the recombinant exon 47 of the *CACNA1A* gene fused to GFP demonstrate overlapping of immunostaining using the α 1ACT#2 antibody and the GFP signal. (C) No specific staining is observed with the PI serum in HEK cells expressing the recombinant exon 47 of the *CACNA1A* gene fused to GFP. D. α 1ACT#2 immunoreactivity is absent in nt HEK cells. HEK, human embryonic kidney; nt, nontransfected; PI, preimmune.



SUPPLEMENTARY FIG. S3. Characterization of SCA6 iPSC lines. **(A)** Expression levels of *NANOG*, *OCT3/4*, and *SOX2* determined by qRT-PCR in SCA6 iPSCs are not different from expression levels in the control lines AE iPSC line 8 and hESC line 207-KI [1]. Expression levels are normalized to the mean expression in hESCs. Each bar represents mean \pm s.e.m. from three independent experiments. One-way ANOVA test performed. **(B)** Representative immunostainings of SCA6 iPSC colonies showing almost uniform expression of the pluripotency markers *NANOG* and *OCT3/4* in the DAPI-positive nuclear compartment. Scale bars 100 μ m. hESC, human embryonic stem cell; iPSCs, induced pluripotent stem cells; qRT-PCR, quantitative real-time polymerase chain reaction; s.e.m., standard error of the mean.

SUPPLEMENTARY TABLE S1. LIST OF TAQMAN GENE EXPRESSION ASSAYS USED IN THIS STUDY

<i>Gene name</i>	<i>Taqman Assay code</i>	<i>Gene name</i>	<i>Taqman Assay code</i>
<i>AFP</i>	Hs00173490_m1	<i>NANOG</i>	Hs04399610_g1
<i>BRACHIURY</i>	Hs00610080_m1	<i>OCT3/4</i>	Hs03005111_g1
<i>BTG1</i>	Hs00982890_m1	<i>SOX2</i>	Hs01053049_s1
<i>CACNA1A</i>	Hs01579431_m1	<i>SYN-1</i>	Hs00199577_m1
<i>CTIP2</i>	Hs00256257_m1	<i>SYP</i>	Hs00300531_m1
<i>EOMES</i>	Hs00172872_m1	<i>TAF1</i>	Hs00936234_m1
<i>FOXG1</i>	Hs01850784_s1	<i>EN1</i>	Hs00154977_m1
<i>GATA4</i>	Hs00171403_m1	<i>EN2</i>	Hs00171321_m1
<i>GATA6</i>	Hs00232018_m1	<i>OTX2</i>	Hs00222238_m1
<i>GBX2</i>	Hs00230965_m1	<i>PCP2</i>	Hs00418270_m1
<i>GRN</i>	Hs00963707_g1	<i>TH</i>	Hs00165941_m1
<i>LHX5</i>	Hs00223612_m1	<i>VACHT</i>	Hs00268179_s1
<i>MAP-TAU</i>	Hs00902194_m1	<i>TBR1</i>	Hs00232429_m1
<i>MATH1</i>	Hs00944192_s1	<i>SATB2</i>	Hs00392652_m1

SUPPLEMENTARY TABLE S2. LIST OF PRIMARY ANTIBODIES USED IN THIS STUDY

<i>Protein</i>	<i>Company</i>	<i>Catalog No.</i>	<i>Application</i>	<i>Dilution</i>
Map2	Sigma	M4403	ICC	1:1000
Tuj 1	Promega	6712a	ICC	1:1000
vGlut1	Synaptic Systems	135304	ICC	1:500
vGlut2	Synaptic Systems	135403	ICC	1:500
Gad67	Millipore	MAB540B	ICC	1:500
Ca _v 2.1	Synaptic Systems	152 203	ICC	1:2000
Nanog	AbCam	109250	ICC	1:250
N-Cadherin	BD Biosciences	610920	ICC	1:2000
Nestin	Santa Cruz	21247	ICC	1:200
Sox2	Santa Cruz	20088	ICC	1:100
Pax6	Santa Cruz	32766	ICC	1:100
MAP-TAU	Dako	A0024	ICC	1:4000

SUPPLEMENTARY TABLE S3. EXPRESSION LEVELS OF DIFFERENTIATION MARKERS IN SCA6 iPSCs, IN THEIR DERIVATIVES OBTAINED AFTER SPONTANEOUS DIFFERENTIATION, AND IN NEURAL PROGENITOR CELLS

<i>Differentiation markers</i>	<i>Undifferentiated SCA6 iPSCs</i>		<i>Spontaneous in vitro differentiation</i>		<i>Neural progenitor cells</i>
	ΔCt	<i>SD</i>	ΔCt	<i>SD</i>	ΔCt
<i>AFP</i>	ND		5.8	1.6	ND
<i>GATA4</i>	13.2	2.3	5.1	0.9	ND
<i>GATA6</i>	ND		6.6	0.7	ND
<i>EOMES</i>	12.2	2.1	5.3	0.4	ND
<i>BRACHIUARY</i>	ND		9.1	0.6	ND

qRT-PCR analysis reveals that early differentiation mesendodermal markers are not expressed or very low expressed in SCA6 iPSCs, as well as in neural progenitor cells. Expression of the same markers is markedly increased during spontaneous in vitro differentiation (see the Materials and methods section). Gene expression levels are expressed as ΔCt values, relative to *GAPDH*. High ΔCt values indicate low gene expression levels. Ct values from three independent experiments. iPSCs, induced pluripotent stem cells; ND, not detected; qRT-PCR, quantitative real-time polymerase chain reaction; SD, standard deviation.

SUPPLEMENTARY TABLE S4. EXPRESSION OF NEURONAL SPECIFICATION AND PATTERNING MARKERS IN SCA6 AND CONTROL CULTURES AT WEEK 5 OF NEURONAL DIFFERENTIATION

Markers	SCA6-1		SCA6-2		CTRL	
	Mean Δ Ct	SD	Mean Δ Ct	SD	Mean Δ Ct	SD
Purkinje cell/progenitor markers						
<i>PCP2</i>	12.9	1.4	ND		ND	
<i>LHX5</i>	ND		ND		ND	
<i>MATH1</i>	ND		ND		ND	
Patterning markers						
<i>OTX2</i>	6.6	1.6	4.3	1.6	5.2	1.6
<i>FOXP1</i>	3.3	1.5	2.4	2.9	4.5	1.2
<i>EN1</i>	5.8	2.7	5.2	3.1	6.3	1.7
<i>EN2</i>	5.3	1.6	6.8	2.4	7.8	2.1
<i>GBX2</i>	4.7	0.4	3.9	1.2	5.4	1.4
Neuronal specification markers						
<i>TH</i>	7.3	1.6	9.4	2.4	8.1	1.5
<i>VACHT</i>	8.9	0.9	9.7	1.5	9.8	1.9
<i>TBR1</i>	2.6	0.9	2.1	0.7	2.8	1.7
<i>SATB2</i>	4.7	1.4	4.3	2.2	5.6	1.6
<i>CTIP2</i>	5.3	2.6	6.5	0.9	7.6	1.2

Expression levels were determined by qRT-PCR and are shown as mean Δ Ct values, relative to *GAPDH*. Low Δ Ct values indicate high gene expression levels. Ct values were obtained from ≥ 3 independent experiments.