Supporting Information

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SI Methods

Unless stated otherwise, all components of the tissue culture media and tissue culture reagents were from Invitrogen, and all growth factors were from Preprotech.

Human Tissue Samples. Human postmortem neuronal progenitor cell (NPC)-derived astrocytes have been described previously (1). Human skin fibroblast samples were obtained from Stephen J. Kolb (ALS/MND Clinic, Department of Neurology, The Ohio State University, Wexner Medical Center, Columbus, OH), as well as John Ravits (University of California, San Diego, School of Medicine) and P.J.S., and from established tissue banks as shown in Table S1 (Gibco and Coriell Institute). Informed consent forms were obtained from all subjects before sample collection.

Skin Fibroblast Isolation and Maintenance. Dissociation of skin biopsies and culture expansion of the fibroblasts was initiated within 30 min postsampling under sterile conditions. Briefly, 2-cm^2 cube skin samples were washed twice in PBS and placed in a 10-cm^2 dish with epidermal side down, whereas the s.c. layer was scraped off with a surgical scalpel; 0.5-cm^2 strips were cut and incubated with 25 mL of 0.05% trypsin/EDTA (Invitrogen) in a 50-mL conical tube at 37 °C for 45 min. After this, 20 mL of DMEM media containing 10% (vol/vol) FBS were added and cells were centrifuged at 350 × *g* for 4 min. The medium was aspirated and replaced with fresh DMEM containing 10% (vol/vol) FBS before plating in a six-well plate.

Conversion of Skin Fibroblasts to iNPCs. A total of 10^4 fibroblasts were seeded into one well of a six-well plate. One day after seeding, a mixture consisting of the retroviral vectors Oct3/4, Sox2, Klf4, and c-Myc was applied with a multiplicity of infection of 10 for each viral vector. The cells were incubated overnight in a final volume of 700-μL medium/viral vector. The following day, cells were washed 2× with PBS, and regular fibroblast medium (DMEM plus 10% FBS) was applied for 3 d. After this recovery time, the cells were washed 1× with PBS before 2 mL of NPC conversion medium consisting of DMEM/F12, 1% N2, 1% B27, 20 ng/mL FGF2, 20 ng/mL EGF, and heparin (5 μg/mL; Sigma-Aldrich) was added to the cells. This medium was changed every day thereafter. Once the cells change shape and form sphere-like structures, these can be collected with a pipette and expanded in an individual well of a six-well plate previously coated with human fibronectin (5 μg/mL; Millipore). Alternatively, the entire cell culture can be lifted with Accutase, centrifuged, resuspended in NPC conversion medium, and replated in two to three wells of a six-well plate. As soon as the NPC culture is established, we switch the medium to NPC medium consisting of DMEM/F12, 1% N2, 1% B27, and FGF2 (40 ng/mL) only.

Differentiation of iNPCs into Oligodendrocytes, Neurons, and Astrocytes. For differentiation into oligodendrocytes, NPCs were plated in NPC medium supplemented with FGF2 as described above, and differentiation to oligodendrocytes was started when cells were about 80% confluent. On day 0 of differentiation, NPC medium containing FGF2 was supplemented with 10 ng/mL PDGF-AA for 1 wk. After 1 wk, the medium was switched to oligodendrocyte progenitor cell differentiation medium (DMEM/F12 supplemented with 2% B27, 20 ng/mL PDGF-AA, and 20 ng/mL IGF1) for 2 wk, and IGF concentration was increased to 50 ng/mL during week 4 of differentiation.

To induce neuronal differentiation, iNPCs were plated in a 10cm dish and allowed to become confluent in NPC medium containing FGF2 as described above. Upon reaching confluence, the medium was switched to DMEM/F12 containing 1% N2, then Dorsomorphin (Tocris Bioscience, 1μM) and 2μM Retinoic acid was added for two days before Dorsomorphin was replaced by forskolin (Sigma, 5 μM). All drugs were added freshly every day for 10 d. Cells were then lifted and plated on fibronectin-coated coverslips for immunostaining.

For differentiation into motor neurons (MNs), we applied the protocol previously described (2).

For astrocyte differentiation, the iNPCs were seeded in NPC medium at low density in a fibronectin-coated 10-cm dish. The day after, the medium was switched to DMEM containing 10% FBS and 0.3% N2. Astrocytes were allowed to mature for at least 7 d.

Immunofluorescence. Cells were fixed with 4% paraformaldehyde for 15 min and washed 3× with Tris-buffered saline (TBS) before the blocking solution consisting of TBS with 10% donkey serum, 0.1% Triton X-100, and 0.1% Tween-20 was applied for 1 h. All primary antibodies were diluted in blocking solution and their dilution and provider are listed in Table S2. Incubation of the primary antibody was performed overnight at 4 °C. The next day, cells were washed 3× in TBS before the secondary antibody and DAPI diluted in blocking solution was applied for 1.5 h at room temperature.

RNA Isolation and RT-PCR/Quantitative RT-PCR. RNA was harvested using the RNAeasy Mini kit (Qiagen), and total RNA was reverse-transcribed with RT2 First Strand Kit (SABiosciences) according to the manufacturer's instructions. PCR was performed with PCR master mix from Promega. Real-time quantitative PCR reactions were performed using RT2 Real-Time SYBR Green/Rox PCR Master Mix (SABiosciences), and PCR arrays were run on an ABI Prism 7000. Primer sequences used can be found in Table S3.

ES Motor Neuron Differentiation. Mouse embryonic stem cells expressing GFP under the MN-specific promoter HB9 (HBG3 cells; kind gift from Tom Jessell, Columbia University, New York) were cultured on primary mouse embryonic fibroblasts (Millipore). For differentiation into MNs, cells were lifted with trypsin and resuspended in DFK10 culture medium consisting of knockout DMEM/F12, 10% knockout serum replacement, 1% N2, 0.5% L-glutamine, 0.5% glucose (30% in water), and 0.0016% 2-mercaptoethanol. The cells were plated on nonadherent Petri dishes to allow formation of embryoid bodies. After 1 d of recovery, 2 μM retinoic acid (Sigma) and 2 μM purmorphamine (Calbiochem) were added freshly every day with new medium. After 5 d of differentiation, the embryoid bodies were dissociated and sorted for GFP on a BD FACSVantage/DiVa sorter.

Coculture of Motor Neurons and Astrocytes. Human astrocytes were plated in 96-well plates coated with human fibronectin (2.5 μg/mL; Millipore) at a density of 10,000 per well. Two days later, FACS-sorted GFP-positive MNs were resuspended in MN media consisting of DMEM/F12, 5% horse serum, 2% N2, 2% B27 plus GDNF (Invitrogen; 10 ng/mL), BDNF (Invitrogen; 10 ng/mL), and CNTF (Invitrogen; 10 ng/mL) and added to the astrocytes at a density of 10,000 per well. Each plate was scanned every day with the fully automated IN CELL 6000 confocal plate reader to capture GFP-positive cells. The IN CELL developer and analyzer software were used to create whole-well

pictures and count MNs. For the monoculture of MNs, 96-well plates were coated with poly-ornithine/laminin as previously described (1), and 10,000 FACS-sorted GFP-positive MNs were seeded in each well and monitored in the same manner as the cocultures.

Statistical Analyses. Statistical analysis was performed by one-way ANOVA unpaired *t* test for mean differences between the average of all converted control lines versus each individual ALS line (GraphPad Prizm Software). All experiments were performed minimum in triplicate, quadruplicate, or more.

1. Haidet-Phillips AM, et al. (2011) Astrocytes from familial and sporadic ALS patients are toxic to motor neurons. *Nat Biotechnol* 29(9):824–828.

2. Hester ME, et al. (2011) Rapid and efficient generation of functional motor neurons from human pluripotent stem cells using gene delivered transcription factor codes. *Mol Ther* 19(10):1905–1912.

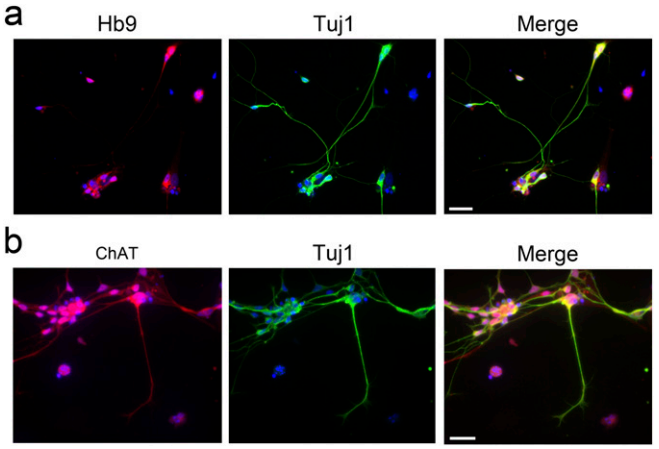

Fig. S1. Differentiation of iNPCs into MNs. Immunofluorescence for motor neuron-specific HB9 (*A*) and ChAT (*B*) red and pan-neuronal Tuj1 in green indicates successful differentiation into MNs. Blue, DAPI. (Scale bar: 100 μm.)

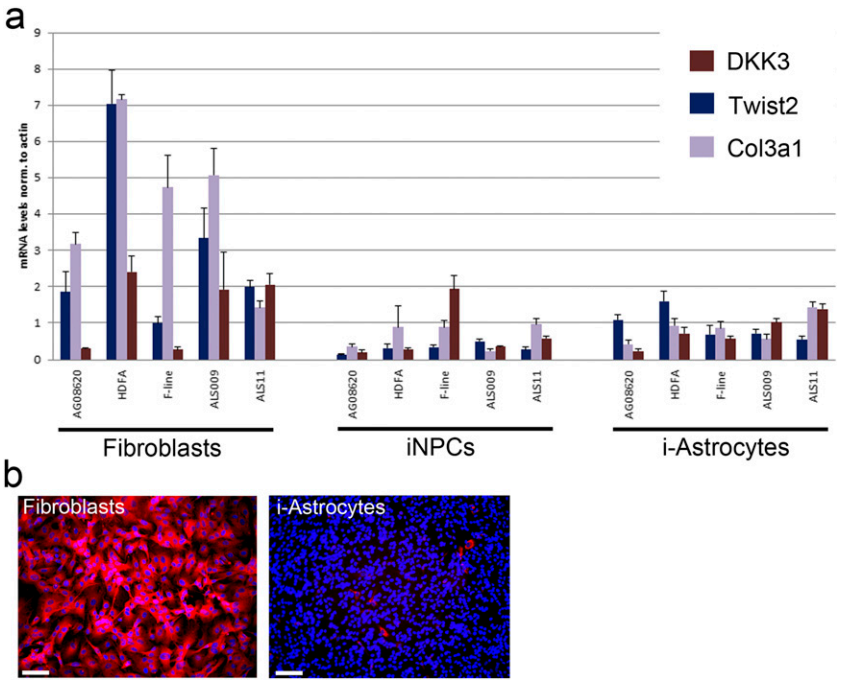

Fig. S2. Down-regulation of fibroblast genes in induced NPCs (iNPCs) and neuronal progenitor cells and subsequent differentiation into astrocytes (i-astrocytes). (*A*) Quantitative (q) RT-PCR revealed reduced expression of the fibroblast genes *Twist2*, *DKK3*, and *Col3a1* in iNPCs and i-astrocytes. (*B*) Immunostaining for the fibroblast marker Col1 demonstrates strong down-regulation of its expression in iNPCs compared with fibroblasts.

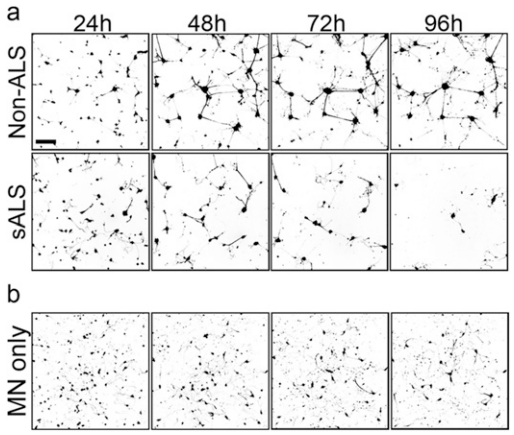

Fig. S3. Representative MN growth and death on control or ALS i-astrocytes. (*A*) At 24 h postseeding, MNs have settled on the astrocyte monolayer and start to extend neurites. At 48 h, MNs are well established and look morphologically similar in the presence of control or ALS i-astrocytes. By 72 h, the first indications of reduced cell numbers and neurite retractions are observed. After 96 h in coculture, the differences in number of MNs on ALS i-astrocytes and non-ALS i-astrocytes become highly significant. (*B*) Representative pictures of monocultures of MNs during the same time course.

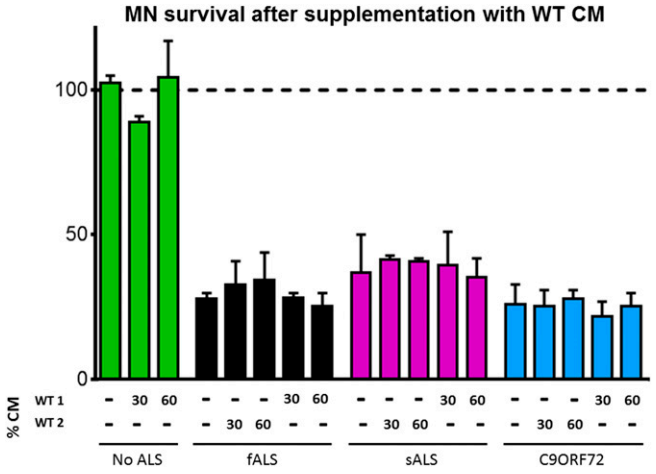

Fig. S4. Medium supplementation does not abolish ALS i-astrocyte toxicity. Quantification of the percentage of MNs alive after 96 h of coculture with control or ALS i-astrocytes supplemented either with 0%, 30%, or 60% conditioned medium from two different control cocultures (WT1, AG08620; WT2, 155). No significant effect on toxicity was observed upon supplementation.

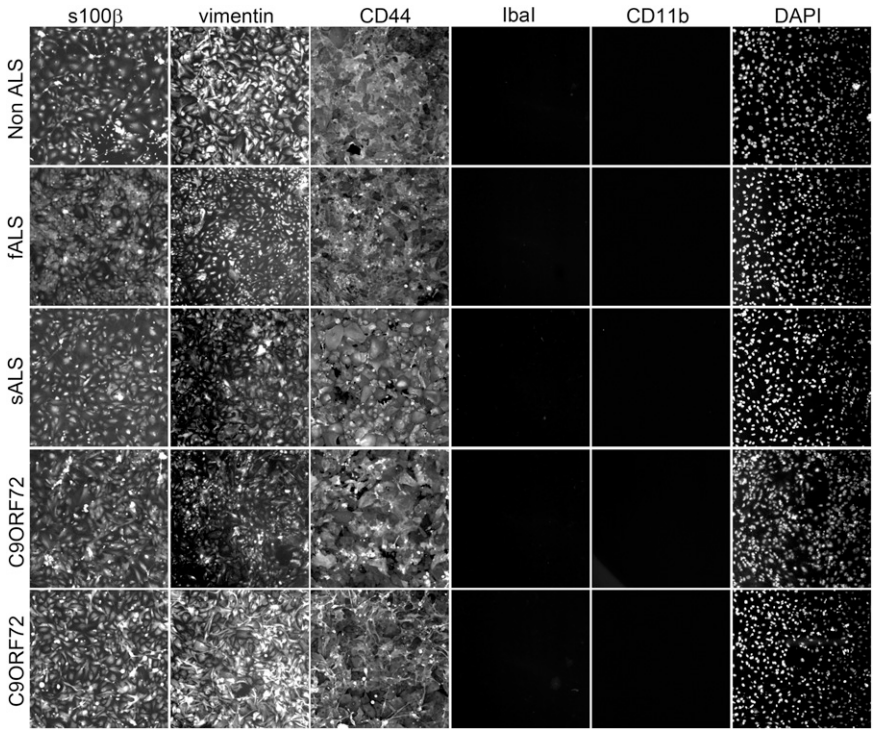

Fig. S5. Similar expression levels of astrocyte markers in various i-astrocyte lines. Representative pictures of immunostainings performed in the 96-well plate after 96 h of coculture. All i-astrocyte lines express high levels of the astrocytic markers s100β, vimentin, and CD44, whereas the microglial markers Iba1 and CD11b were completely absent. DAPI was used to visualize nuclei.

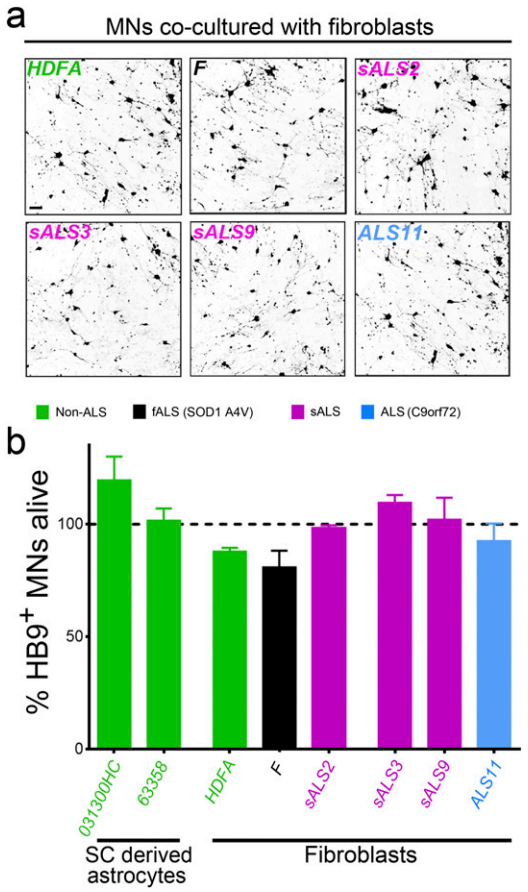

Fig. S6. ALS fibroblasts are nontoxic to MNs. (*A*) Representative images of MNs after 96 h of coculture with fibroblasts from ALS patients or controls. (*B*) Quantification of the percentage of MNs alive after 96 h of coculture with non-ALS astrocytes or with fibroblasts from ALS patients or non-ALS controls. Similar levels of survival were found between all groups. Data are representative of $n = 5$ experiments performed in triplicate. (Scale bar: 100 μm.)

Table S1. Summary of the demographic information on the astrocytes and fibroblast lines used in this study

Cell line	Diagnosis	Mutation	Age, y	Sex	Astrocytes source	Source
031300HC	Non-ALS	—	N/A	N/A	Brain	Fred Gage (Salk Institute, La Jolla, CA)
63358	Non-ALS	—	87	Male	Spinal cord	National Disease Research Interchange
4944MA	fALS	*SOD1*^{A4V}	57	Male	Spinal cord	National Disease Research Interchange
090322	sALS	Unknown	70	Male	Spinal cord	National Disease Research Interchange
HDFA	Non-ALS	—	N/A	N/A	Skin	Invitrogen
AG08620	Non-ALS	—	64	Female	Skin	Coriell Institute
155	Non-ALS	—	42	Male	Skin	P.J.S.
170	Non-ALS	—	65	Male	Skin	P.J.S.
fALS	fALS	*SOD1*^{A4V}	40	Male	Skin	R.A.S.
sALS2	sALS	Unknown	60	Male	Skin	S.J.K.
sALS3	sALS	Unknown	81	Female	Skin	S.J.K.
sALS9	sALS	Unknown	61	Female	Skin	S.J.K.
ALS11	sALS	C9ORF72 expansion	52	Male	Skin	S.J.K.
ALS78	fALS	C9ORF72 expansion	68	Male	Skin	P.J.S.
ALS183	fALS	C9ORF72 expansion	51	Male	Skin	P.J.S.

N/A, not available.

Table S2. Antibodies used in this study

Antibody	Source	Species	Dilution used
Pax6	Abcam	Mouse	1:200
Nestin	Millipore	Mouse	1:500
S-100β	Swant	Rabbit	1:500
GFAP	Millipore	Rabbit	1:200
Vimentin	Santa Cruz	Goat	1:200
CD44	BD Pharmigen	Mouse	1:1,000
Tuj1	Covance	Rabbit	1:500
MBP	Millipore	Rabbit	1:200
Collagen 1	Cedarlane	Rabbit	1:200
Hb9	Abcam	Rabbit	1:200
ChAT	Millipore	Rabbit	1:200
CD11b	BD Pharmigen	Mouse	1:50
Iba1	Wako	Rabbit	1:500

Table S3. Primers sequences used for semiquantitative and quantitative RT-PCR

Gene	Forward primer (5′–3′)	Reverse primer (5′–3′)	Use
Col3a1	CCCTGGATCTCCAGGATAC	GTCCGGGTCTACCTGATTC	RT-PCR
DKK3	GTGCACCGAGAAATTCAC	ACAGTCCTCGTCGATGATG	RT-PCR
Twist2	GAAGTCGAGCGAAGATGG	AAGGCCTCGTTGAGCGACTG	RT-PCR
β-Actin	CTGGCACCACACCTTCTACA	AGGTCTCAAACATGATCTGGGT	RT-PCR
Aqp4	GCACCAGGAAGATCAGCATCG	GAGACCATGACCAGCGGTAAG	qRT-PCR
IGFBP3	GCCGTAGAGAAATGGAAGACAC	AGAGGCTGCCCATACTTATC	qRT-PCR
NCAN	CTACAGGCTGCCTTTGAG	TACGGTCGCCATAGCAAC	qRT-PCR
NKX2-2	CTTCTACGACAGCAGCGACAAC	GACTTGGAGCTTGAGTCCTGAG	qRT-PCR
β-Actin	CTGGCACCACACCTTCTACA	AGGTCTCAAACATGATCTGGGT	qRT-PCR