Patient	iPSC	Diagnosis	Genotype	Age of onset	Sex	Age of Biopsy	Reprog. factors	Karyotype	Scorecard Assay	Silencing of Reprogramming factors	OCT4/ TRA-1-81 ICC	Reference
39	39b	FALS	*SOD1* $^{+/A4V}$	43y	F	44y	O,S,K	Normal	Normal	Yes	Normal	This report
RB9	RB9d	FALS	*SOD1* $^{+/A4V}$	45y	F	50y	O,S,K	Normal	Normal	Yes	Normal	This report
19	19f	FALS	*C9orf72* $(GGGGCC)_{>50}$	53y	F	54y	O,S,K	Normal	Normal	Yes	Normal	This report
RB8	RB8B	FALS	*C9orf72* $(GGGGCC)_{>50}$	49y	M	51y	O,S,K,cM	Normal	Normal	Yes	Normal	This report
15	15b	Healthy	N.A.	N.A.	F	49y	O,S,K	Normal	Normal	No	Normal	Boulting et al., 2011
17	17a	Healthy	N.A.	N.A.	F	72y	O,S,K	Normal	Normal	Yes	Normal	Boulting et al., 2011
20	20b	Healthy	N.A.	N.A.	M	56y	O,S,K	Normal	Normal	Yes	Normal	Boulting et al., 2011
11	11a	Healthy	N.A.	N.A.	M	37y	O,S,K	Normal	Normal	Yes	Normal	Boulting et al., 2011
18	18a, 18b	Healthy	N.A.	N.A.	F	48y	O,S,K	Normal	Normal	Yes	Normal	Boulting et al., 2011

Table S1, related to Figure 1, List of Patient-Specific iPSCs Used in This Study. All iPSCs were characterized by immunocytochemistry (ICC) for NANOG and TRA-1-81 and the pluripotency Scorecard assay (Bock et al., 2011), and had normal karyotypes. The expression of the reprogramming transgenes was evaluated by qRT-PCR. All data are available upon request. O: *OCT4*, S: *SOX2*, K: *KLF4*, cM: *cMYC*. N.A.: Non-applicable.

Table S2, related to Figure 3, List of genes found to be significantly altered in their expression levels in 39b-*SOD1*$^{+/A4V}$ relative to isogenic control motor neurons by RNA sequencing at an FDR 5%. (A) Upregulated in *SOD1*$^{+/A4V}$. **(B)** Downregulated in *SOD1*$^{+/A4V}$

Table S3, related to Figure 3, Gene enrichment analysis done by DAVID and GSET for upregulated and downregulated genes in 39b-*SOD1*$^{+/A4V}$ relative to isogenic control motor neurons. (A) DAVID**,** Upregulated in *SOD1*$^{+/A4V}$. **(B)** DAVID**,** Downregulated in *SOD1*$^{+/A4V}$. **(C)** GSET**,** Upregulated in *SOD1*$^{+/A4V}$. **(B)** GSET**,** Downregulated in *SOD1*$^{+/A4V}$

EXPERIMENTAL PROCEDURES

Cell culture

Cell cultures were maintained at 37^{C}, 5% CO^{2}. Human fibroblasts were cultured in KO-DMEM (Invitrogen), supplemented with 20% Earl's salts 199 (Gibco), 10% Hyclone (Gibco), 1x GlutaMax, (Invitrogen), and 100μM 2-mercaptoethanol and passaged by trypsinization (0.25% trypsin EDTA, Invitrogen). HuES and iPS cells were maintained on Matrigel (BD Biosciences) with mTeSR1 media (Stem Cell Technologies). Media was changed every 24 hours and lines were passaged by dispase (Gibco, 1mg/mL in hES media for 30min at 37^{C}). Human primary astrocytes were purchased from Sciencell Research Laboratories and grown according to supplier instructions.

Derivation of human fibroblasts and iPS cell generation

Human fibroblasts were generated from 3mm forearm dermal biopsies following informed consent as described previously (Dimos et al., 2008). Generation of iPS cells was done essentially as reported previously by retroviral transduction of *KLF4*, *SOX2*, *OCT4* and *(cMYC)* (Boulting et al., 2011). Retrovirus preparations were done at the Harvard Gene Therapy Initiative at Harvard Medical School (Boston, MA). For iPS cell derivation, 30,000 human fibroblasts were transduced at an MOI of 3-5 per gene, with viruses containing all three genes.

Scorecard assay

RNA samples were produced and analyzed as described previously (Bock et al., 2011) with minor modifications. Whole iPS and ES cell colonies were isolated by dispase treatment and plated in suspension in the presence of mTeSR1 media, cell aggregates (EBs) were allowed to form and 48hrs later EBs were switched to KOSR media without FGF (DMEM/F12, 10% KOSR, NEAA, Glutamax, and 100μM 2-mercaptoethanol). EBs were grown for a total of 16 days, at the end of which, cells were lysed and total RNA was extracted using Trizol (Invitrogen). Subsequently, RNA was analyzed on the NanoString nCounter using a custom codeset. The calculation of the iPS lines' lineage scores were performed according to our previously published protocol (Bock et al., 2011) using our published dataset for 20 human embryonic stem cell lines as a reference.

Motor neuron differentiation

MN differentiation was carried out as previously described (Boulting et al., 2011) with modifications (see also Figure S2). Briefly, pluripotent stem cell colonies were dissociated with accutase and single cells were plated in suspension in low-adherence dishes, at a 400K/ml density with 10μM ROCK inhibitor (Sigma, Y-27632) in mTeSR media for 24hrs. Embryoid bodies (EBs) were formed and media was gradually diluted (50% on day 3 and 100% on day 4) to KOSR (DMEM/F12, 10% KOSR) between days 1-4 and to a neural induction medium (NIM: DMEM/F12 with L-glutamine, NEAA, Heparin (2μg/ml), N2 supplement (Invitrogen) for days 5-24. From days 1-6 cells were cultured in the presence of SB431542 (10M, Sigma Aldrich) and Dorsmorphin (1μM, Stemgent), and from days 5-24 with BDNF (10μg/ml, R&D), ascorbic acid (AA, 0.4μg/ml, Sigma), Retinoic Acid (RA, 0.1μM, Sigma) and Smoothened Agonist 1.3 (SAG 1.3, 1μM, Calbiochem). At day 24 EBs were dissociated to single cells with Papain/DNase

(Worthington Bio) and plated onto poly-lysine laminin-coated chamber slides/plates/coverslips (BD Biosciences) for relevant experiments.

Motor neuron survival assay

After 24 days of differentiation, neuronal EBs were dissociated and 20K were plated on poly-D-lysine/Laminin coated 8-well chamber slides (BD biosciences) containing a confluent monolayer of primary cortical mouse glia. Primary glial preparations from P0-P2 mouse pups were generated as described previously (Boulting et al., 2011; Di Giorgio et al., 2008). Fresh glial preparations (<1 month, <2 passages) were used. Co-cultures were maintained in Neurobasal media (NB, Invitrogen), supplemented with B27 and N2 supplement (Invitrogen), 10μg/mL each of BDNF, GDNF, CNTF (R&D) and ascorbic acid (AA, 0.4μg/ml, Sigma) and fed every 2-3 days. Slides were fixed at various time points (3, 15, 20, 30 days), cultures were stained and cell numbers assessed. Whole-well images were quantified in a manner blinded to the genotype and condition of the experiment. Neuronal numbers on day 3 were set as 100% and numbers on subsequent time points were expressed as a percentage of day 3. To evaluate cell death, neuronal cultures were plated without glia on coverslips and live cells were assayed using the In Situ Cell Death Kit (Roche Diagnostics) according to manufacturer's instructions.

Electrophysiology recordings

MNs were plated at 20K cells/cm2 on lysine/laminin-coated coverslips, in the presence of primary mouse glial monolayers and allowed to mature for 2-4 weeks. MNs were identified by RFP fluorescence, after transduction with the HB9::RFP lentivirus (Marchetto et al., 2008). Whole-cell voltage-clamp or current-clamp recordings were made using a Multiclamp 700B (Molecular Devices) at room temperature (21-23^{C}). Data were digitized with a Digidata 1440A A/D interface and recorded using pCLAMP 10_software (Molecular Devices). Data were sampled at 20 kHz and low-pass filtered at 2 kHz. Patch pipettes were pulled from borosilicate glass capillaries on a Sutter Instruments P-97 puller and had resistances of 2-4 MΩ. The pipette capacitance was reduced by wrapping the shank with Parafilm and compensated for using the amplifier circuitry. Series resistance was typically 5-10 MΩ, always less than 15 MΩ, and compensated by at least 80%. Linear leakage currents were digitally subtracted using a P/4 protocol. Voltages were elicited from a holding potential of -80 mV to test potentials ranging from -80 mV to 30 mV in 10 mV increments. The intracellular solution was a potassium-based solution and contained K gluconate, 135; MgCl2, 2; KCl, 6; HEPES, 10; Mg ATP, 5; 0.5 (pH 7.4 with KOH). The extracellular was sodium-based and contained NaCl, 135; KCl, 5; CaCl2, 2; MgCl2, 1; glucose, 10; HEPES, 10, pH 7.4 with NaOH). Kainate and GABA were purchased from Sigma. For MEA recordings after 24 days of differentiation, equal numbers of MN cultures were plated on poly-D-lysine/laminin coated M768-GLx 12-well plates (Axion BioSystems) at typical densities of 40,000-80,000/ well, recorded after approximately 14 days using an Axion Maestro (Axion BioSystems) MEA device and analyzed using Axion Integrated Studio software as described in the companion manuscript, Wainger et al. Total spike counts were determined by summing total spikes recorded over one minute from the 64 electrodes in each well. Spike counts

were determined at baseline and at the listed time points after the application of 0.5 μM TTX (Sigma), 2mM DTT (Sigma), salubrinal 15 μM (Santa Cruz).

RNA preparation, RT-PCR and RNA sequencing

Total RNA was isolated from relevant cell types using Trizol LS (Invitrogen) according to manufacturer's instructions. MNs were differentiated as described above, MN cultures were plated on glial monolayers, infected with the Hb9::RFP virus on days 5-7 and FACS was used for purification of RFP MNs on day 15. A total of 300-1000ng was used to synthesize cDNA by reverse transcription according to the iSCRIPT kit (Bio-rad).Quantitative RT-PCR was then performed using SYBR green (Bio-Rad) and the iCycler system (Bio-rad). Quantitative levels for all genes were normalized to the average levels of 3 housekeeping genes: GAPDH/b-Actin/YWHAZ and expressed relative to the relevant control samples or the lowest expressing sample in the experiment (see figure legends). QPCR for retroviral and endogenous reprogramming genes was carried out as previously reported (Boulting et al., 2011). All primer sequences are available upon request. Human spinal cord RNA was purchased from Clonetech (#636530) and total human brain RNA was purchased from Applied Biosystems (#AM6050). Spinal cord RNA was pooled from 22 male/female Caucasians aged between 22-69, while total brain RNA was pooled from 23 male/female Caucasians aged between 23-86. For TTX (48hrs), kainate (48hrs) and DTT (2hrs) treatments, co-cultures were treated, RFP positive neurons were purified and RNA was subsequently isolated. For next-generation RNA sequencing, RNA integrity numbers (RIN) above 7.5, determined by bioAnalyzer, were used for library preparation. In brief, RNA sequencing libraries were generated from ~250ng total RNA using the illumina TruSeq RNA kit v2, according to the manufacturer's directions. Libraries were sequenced at the Harvard Bauer Core Sequencing facility on a HiSeq 2000. All FASTQ files were analyzed using FastQC software (v 0.10.1) to confirm that Phred scores were acceptable at all read positions (median Phred score>25 and lower quartile>20). The FASTQ files were aligned to the GRCh37/hg19 reference genome using Tophat (v 2.0.7). Duplicated reads were removed using Picard Tools MarkDuplicates (v 1.44). Differential expression testing was performed independently using two separate analysis packages: Cufflinks (v 2.1.1) and DESeq. The Cufflinks output was visualized with the cummeRbund R package using a false discovery rate of 0.05. For DESeq analysis, gene level annotation count files were first generated using the HTseq count Python script (v 0.5.4). DESeq analysis was performed using the methods recommended by the package authors. Gene Ontology term enrichment was determined for significantly differentially expressed genes at a false discovery rate cutoff of 0.05 using the Database for Annotation, Visualization and Integrated Discovery (DAVID) v6.7. Gene Set Enrichment Analysis (GSEA, Broad Institute) was performed by first creating a pre-ranked gene list of all genes included in differential expression testing ordered by log2 fold change. Analysis was performed using the GSEA preranked tool with the REACTOME and KEGG Pathway MSigDB collections.

Mitochondrial transport assays and EM analysis

After 24 days of differentiation, neuronal EBs were dissociated and 20K were plated on poly-D-lysine/laminin-coated 35 mm glass bottom culture dishes (MatTek Corporation) or coverslips (BD-Biosciences). Cultures were infected with *Hb9::*RFP lentivirus 5 days after dissociation and MNs were selected based on expression of RFP. On days 23-26, MNs were stained with MitoTracker® Green FM (50nM, Invitrogen) and transferred to a custom observation chamber mounted on the stage of the microscope. Live microscopy of mitochondrial transport was performed with a Nikon Eclipse Ti equipped with an automated stage and In Vivo Scientific incubator. Mitochondrial movements were recorded for 5 minutes with 4-second time-lapse intervals using NIS-Elements (Nikon) using a 63x lens. Kymographs were generated from each video using NIS-Elements Analyzing Software (Nikon). Mitochondria were considered motile if they traveled faster than 0.017 μm/second. The average distance between mitochondria was calculated excluding motile mitochondria, and the total unoccupied space was divided by total process length analyzed to yield the proportion of processes unoccupied by mitochondria. Average distance was measured using the top portion of each kymograph using NIS-Elements Analyzing Software (Nikon). For Electron Microscopy analysis, MN cultures were fixed with 2.5% glutaraldehyde-2% paraformaldehyde in 0.1M sodium cacodylate buffer (pH 7.4) and maintained at 4°C O/N. Cultures were then postfixed in 1% OsO4-1.5% KFeCN6 for 30 min, washed in water 3x and incubated in 1% aqueous uranyl acetate for 30mn followed by 2x washes in water and subsequent dehydration in grades of alcohol (5min each; 50%, 70%, 95%, 2x 100%). Cells were then embedded in plastic and ~60nm thick sections were cut, picked up onto copper grids, stained with lead citrate and analyzed in a JEOL 1200EX Transmission Electron Microscope. At least 3 independent differentiation experiments were analyzed in each case and pictures were taken by a technician blinded for sample IDs.

XBP1 splicing assay

300ng of RNA was used to generate cDNA. PCR reactions were set up using 2μl of cDNA and premixed *Ampligold Taq* Polymerase (Applied. Biosystems), at 66^{C} annealing. PCR products were analyzed on a 2% low-melting agarose gel. The ratio of spliced/unspliced bands was quantified using Image J software. Primers available upon request.

Gene targeting

Zinc finger nucleases (ZFNs) were constructed using either the OPEN method as described previously (Maeder et al., 2008) or a modified version of OPEN that uses antibiotic resistance for the selection as previously described (Sander et al., 2011). Briefly, pools of ZF pre-selected zinc finger (ZF) domains were ligated together to create a combinatorial library of three-3 finger proteins. A bacterial two-hybrid-based selection system was used to interrogate the ZF library for proteins that could bind the appropriate target sequences of interest. ZF proteins that bound the target sequence were cloned into a mammalian expression vector and fused to heterodimeric FokI nuclease protein domains to construct ZFNs. Active ZFNs capable of inducing a double strand break at the desired locus were identified by screening pairs of nucleases for the capability to induce characteristic indel mutations at the SOD1 target site in sequencing the locus of ZFN-treated HEK 293s. 2.5 million iPS cells of the 39b cell line were accutased and nucleofected,

using Human Stem Cell Kit II and program A-023, with 1 μg of ZFN plasmid and 5μg of targeting plasmid. After nucleofection the cells were plated on matrigel with mTesr and ROCK inhibitor. After 48hrs puromycin selection was applied for 1 week after which surviving colonies were passaged and gDNA was extractedPCR was used to confirm proper targeting of the cassette. To remove the puromycin cassette 2.5 million cells were nucleofected with 1μg of a mammalian expression plasmid containing hygromycin and 5μg of a mammalian expression plasmid containing the FLPo recombinase and plated on matrigel with mTeSR and ROCK inhibitor. Twenty four hours after nucleofection, hygromycin was added for 48 hours. Colonies were allowed to expand for 1 week then picked and genomic DNA was extracted. Sequencing of the genomic DNA was used to confirm removal of the puromycin cassette. SOD1 expression was verified by qPCR after RNA extraction and cDNA synthesis. PshAI digestion along with sequencing of the qPCR product demonstrated loss of expression of the mutant allele. Copy number qPCR using primer SOD1cnF and SOD1cnR was performed as described previously (D'Haene et al., 2010) to rule out random integration events. Primer sequences are available upon request.

Genome sequencing and analysis.

DNA samples were derived from the parental 39b cell line and the gene corrected clone using phenol chloroform extraction. The sequencing libraries were made with 50ng genomic DNA using the Illumina Nexterra DNA kit. Deep (30×) WGS was performed using the Illumina HiSeq 2500 Platform (500 bp library, 101 bp reads). All subsequent alignments and analysis were performed with hg19 as a reference. To investigate whether there were changes in copy number due to the cell line transformation, we used Genome STRiP (Handsaker et al., NG, 2011) to extract and process the read depth signal from the aligned sequencing data. Using a genome alignability mask of size 101, we segmented the genome into non-overlapping windows each containing 100Kb of uniquely alignable base positions (based on a read length of 101bp). In each window, we computed normalized read depth by counting the read fragments aligned within that window with a minimum mapping quality of 10 and normalized the counts based on genome-wide sequencing depth and correcting for sequencing coverage bias due to local G+C content. This was done separately for the parental and derived cell lines. To look for regions of copy number change, we evaluated the ratio of normalized read depth in the derived cell line compared to the parental cell line in each window. To find rare coding SNPs in ALS genes, we annotated coding variants called by Haplotype caller with SNPeff (Abecasis et al., 2012). SNPs classified as missense, silent, or nonsense were retained. We then integrated allele frequencies for the European population from the thousand genomes project (Cingolani et al., 2012). Variants were selected that overlapped target genes for ALS. To find variants that differed between cell lines, we compared the genotypes of both lines in a stringent manner similar to the methodologies used to discover de novo mutations. For a variant to be confidently different between cell lines, we required a read depth of at least 2 and a likelihood score (PL) of at least 30 across both lines. Homozygous variants were required to have no more than 5% of the reads observed from the alternate allele, while heterozygous variants were required to have at

least 30% of reads observed from the less frequent allele and at most 70% of the reads from the more frequent allele. To examine the off target effects of the designed nuclease, variants within the top 12,000 potential off target nuclease cut sites were selected from this filtered set of confident variants.

Nanostring karyotyping

Karyotyping was undertaken using the Nanostring nCounter Human Karyotype Panel (Nanostring Technologies, USA) and performed as per the manufacturers instructions. In brief, the protocol is as follows: 600 ng of genomic DNA was Alu1 digested at 37 °C for 2 hours, before being denatured at 95 °C for 5 minutes. To prevent renaturing samples were kept on ice. A total of 300 ng of Alu1-digested DNA per sample was mixed with hybridization buffer, capture and reporter codes. Following a 16 hour incubation at 65 °C, samples were transferred to a Nanostring Prep station where hybridized DNA was bound to an imaging cartridge before imaging. Using reference samples, a copy number was calculated for each chromosome following normalization of the data using nSolver (Nanostring Technologies, USA) and Microsoft Excel.

Treatments with small molecules and siRNAs.

For ER stress induction, human fibroblasts and human astrocytes were treated with Dithiothreitol (DTT) (2mM) (Bio-Rad Laboratories) for 2hrs washed with PBS and either fixed, stained and evaluated or trypsinized for RNA/protein collection. For analysis of SOD1 protein, MN cultures were treated with vehicle or 1μM MG132 for 48hrs. For assessing the role of XBP1 (s14913) and ATF4 (s1702) 40nM of siRNA (Ambion) was transfected into MN cultures using siRNA-Select in Optimem on days 10/20 and knockdown levels and survival were assessed on day 30.

Immunocytochemistry

Cell cultures were fixed in 4% PFA for 15minutes at 4°C, permeabilized with 0.2% Triton-X in PBS for 45 minutes and blocked with 10% donkey serum in PBS-T (Triton 0.1%). Cells were then incubated in primary antibody overnight and secondary antibodies for 1 hour in 2% donkey serum in PBS-T after several washes in between. DNA was visualized by a Hoechst stain. The following antibodies were used: Primary antibodies used in this study are TRA1-81 (1:500, Chemicon, MAB4381), Nanog (1:500, R&D, AF 1997), Islet1 (1:200, DSHB, 40.2D6), HB9 (1:100, DSHB, MNR2 81.5C10-c), ChAT (1:100, Chemicon, AB144P), TUJ1 (1:1000, Sigma, T2200), MAP2 (1:10000, Abcam ab5392), BrdU (3H579, Santa Cruz Biotechnology, sc-70441), Ki67 (1:400, Abcam, ab833), GFP (1:500, Life Technologies, A10262), SOD1 (1:2000, Agrisera #AS09 540), Hoxa5 and FOXP1 (courtesy of Susan Morton, Jessell lab). Secondary antibodies used (488, 555, 594, and 647) were AlexaFluor (1:1000, Life Technologies) and DyLight (1:500, Jackson ImmunoResearch Laboratories).

Chick embryo transplants

HUES3 Hb9::GFP+ MNs were differentiated and one EB was placed into the neural tube of a stage 15 chick embryo using a tungsten needle. Embryos were harvested after 5 days, fixed and sectioned in paraffin. Sections were immunostained with antibodies specific for GFP (rabbit,

Abcam) and an HRP-conjugated anti-rabbit antibody (Vector labs) and counterstained with hematoxylin.

Western Blots

For analysis of Phospho-eIF2α protein, cells were lysed in RIPA buffer (150mM Sodium Chloride; 1% Triton X-100; 0.5% sodium deoxycholate; 0.1% SDS; 50 mM Tris pH 8.0) containing protease and phosphatase inhibitors (Roche) for 20 min on ice, and centrifuged at high speed. Samples containing 20μg of protein were separated by SDS-PAGE (NuPAGE®, Life Technologies) and transferred to nitrocellulose membranes. Membranes were probed with anti-Phospho-eIF2α antibody (#3597, Cell Signaling Technology), and anti-α–Tubulin (abcam, ab4074) and anti-eIF2α (Cell Signaling Technology, #9722) antibodies were used as loading and normalizing controls, respectively. For analysis of SOD1 protein, detergent-soluble fractions were prepared using RIPA buffer and detergent-insoluble fractions were obtained using UREA buffer (8M UREA; 4% CHAPS; 40 mM Tris; 0.2% Bio-Lyte® 3/10 ampholyte). 5μg of detergent-soluble and equivalent volumes of detergent-insoluble protein samples were separated by SDS-PAGE (Bio Rad Laboratories), transferred to PDVF membranes and probed with anti-SOD1 antibody (Agrisera #AS09 540) and anti-α–Tubulin (Sigma Aldrich # T6199). For mitochondrial biogenesis analysis, 6ug of detergent soluble protein samples were analyzed using the MitoBiogenesis™ Western Blot Cocktail (ab123545).

Statistical analysis

Statistical significance was assessed by a standard Students T test (1 tail & 2 tail); $P<0.05$ was considered significant. Two-tailed, unpaired tests were used except to confirm specific hypotheses, in which case one-tailed, unpaired tests were used. Error bars represent ±s.e.m, unless otherwise stated.

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