Stem Cell Reports, Volume *19*

Supplemental Information

ALS-related *p97* R155H mutation disrupts lysophagy in iPSC-derived motor neurons

Jacob A. Klickstein, Michelle A. Johnson, Pantelis Antonoudiou, Jamie Maguire, Joao A. Paulo, Steve P. Gygi, Chris Weihl, and Malavika Raman

ALS-related p97 R155H mutation disrupts lysophagy in iPSC-derived motor neurons

Supplemental Information

Supplementary Figure 1

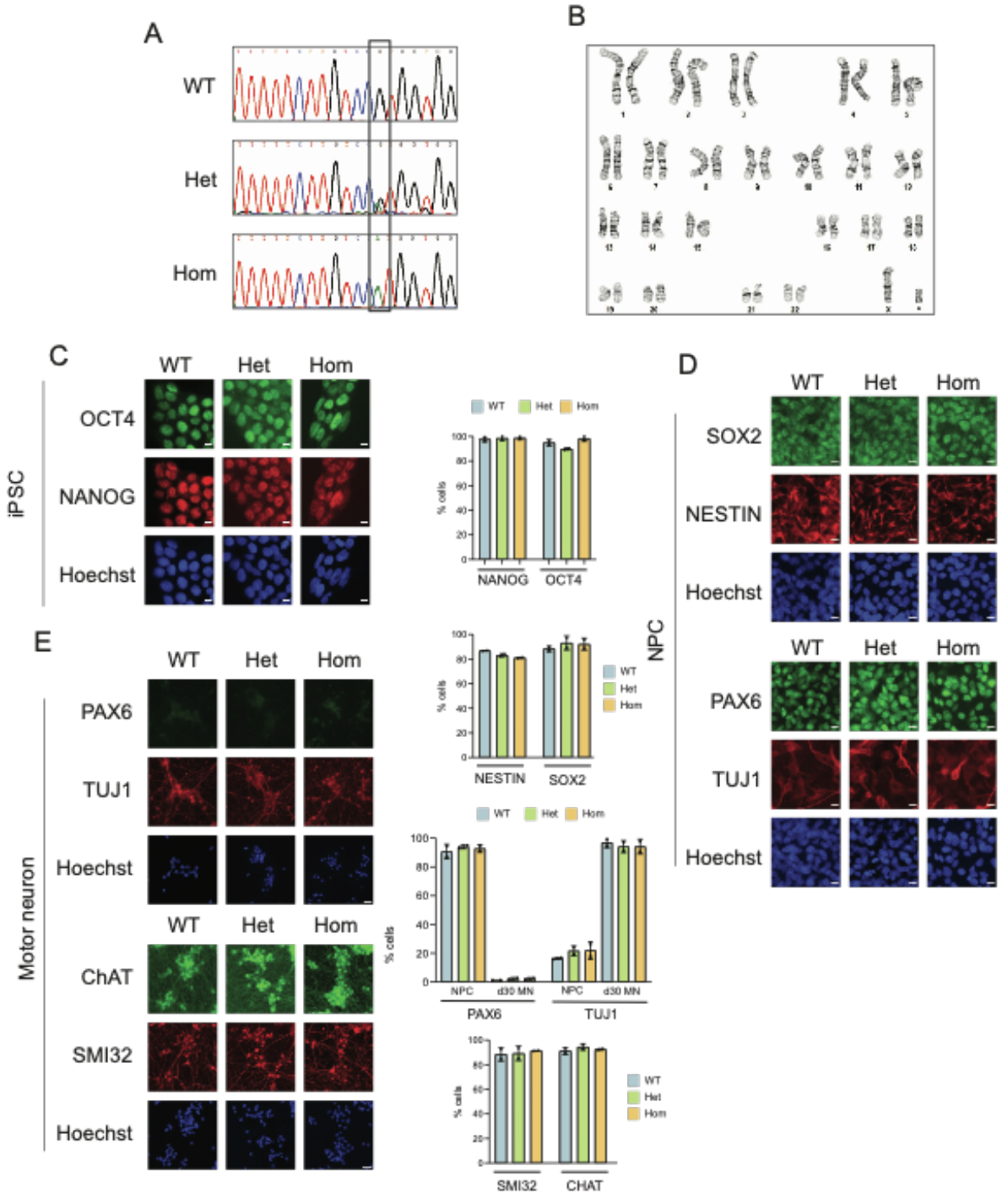

Figure 1S. p97 mutations do not affect motor neuron differentiation in the KOLF2.1 cell line

A. Sanger sequencing traces of KOLF2.1 iPSCs confirm WT, heterozygous, and homozygous R155H mutations in the endogenous p97 locus. B. Karyotype of the parental WT KOLF2.1 line shows no macroscopic genetic abnormalities. C. Representative images of iPSCs stained for pluripotency markers OCT4 and NANOG (left panels). Quantification of the percent of cells positive for each marker in each genotype (right). N = 2 independent experiments. Scale bar 10μm. D. Representative images of NPCs stained for NPC markers SOX2, NESTIN, and PAX6 as well as neuronal marker βIII-tubulin (TUJ1) (right panels). Quantification of the percent of cells positive for each marker (left middle graphs). N = 2 independent experiments. Scale bar 10μm. E. Representative images of motor neurons stained for the NPC marker PAX6, pan-neuronal marker βIII-tubulin (TUJ1), and motor neuron specific markers choline acetyl transferase (ChAT) and neurofilament heavy chain (SMI32) (left panels). Quantification of the percent of cells positive for each marker (left bottom two graphs). N = 2 independent experiments. Scale bar 10μm. All data expressed as means ± SEM.

Supplementary Figure 2

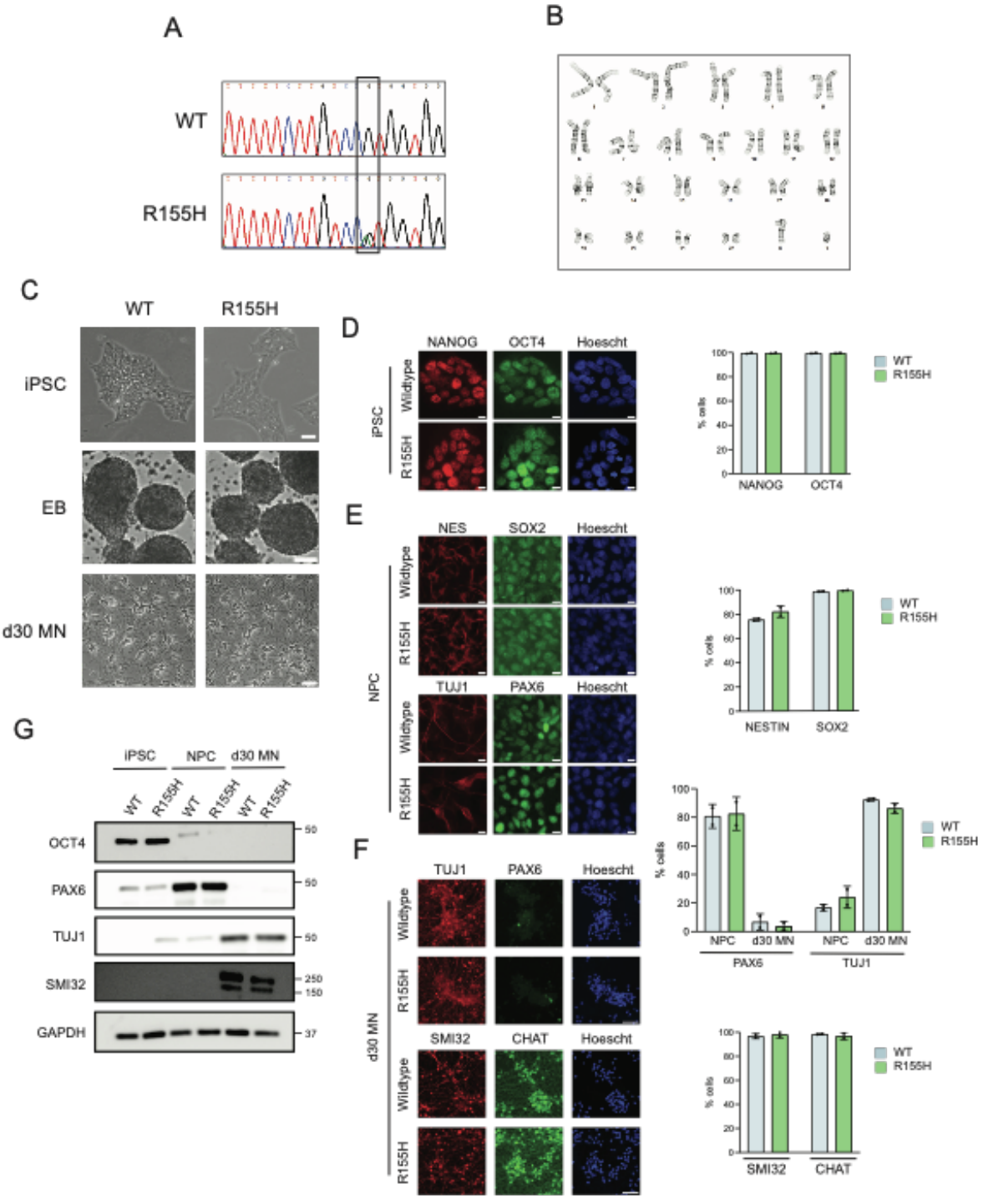

Figure 2S. Characterizing of 392.1 and p97 corrected IPSC lines and differentiation

A. Sanger sequencing traces of 392.1 iPSCs confirm WT and heterozygous R155H mutation in the endogenous p97 locus. B. Karyotype of 392.1 iPSCs. C. Phase contrast images of iPSC colonies (top panels), embryoid bodies (middle panels), and motor neurons (bottom panels). Scale bars 200μm (middle) and 50μm (top and bottom). D. Representative images of iPSCs stained for pluripotency markers OCT4 and NANOG (left panels). Quantification of the percent of cells positive for each marker in each genotype (right). N = 2 independent experiments. Data expressed as means ± SEM. Scale bar 10μm. E. Representative images of NPCs stained for NPC markers SOX2, NESTIN, and PAX6 as well as neuronal marker βIII-tubulin (TUJ1) (right panels). Quantification of the percent of cells positive for each marker (left middle graphs). N = 2 independent experiments. Scale bar 10μm. F. Immunoblot of iPSC, NPC, and neuronal markers in each cell type confirms identities. N = 3 independent experiments. G. Representative images of motor neurons stained for the NPC marker PAX6, pan-neuronal marker βIII-tubulin (TUJ1), and motor neuron specific markers choline acetyl transferase (ChAT) and neurofilament heavy chain (SMI32) (left panels). Quantification of the percent of cells positive for each marker (left bottom two graphs). N = 2 independent experiments. Scale bar 10μm. All data expressed as means ± SEM.

Supplementary Figure 3

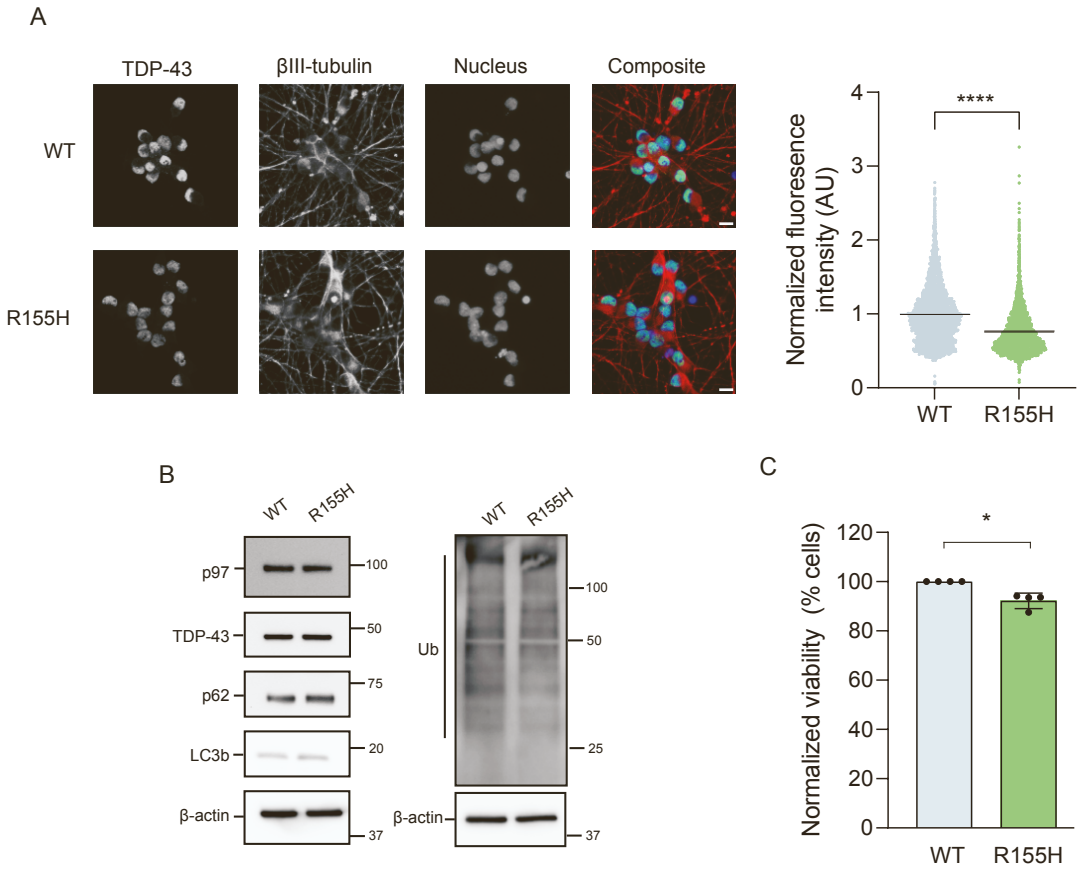

Figure 3S. Characterization of ALS phenotypes in 392.1 R155H motor neurons

A. Representative images of TDP-43 immunofluorescence in 392.1 motor neurons (left panels). Quantification of nuclear intensity of TDP-43 staining (right). N = >5000 cells per condition over 4 independent experiments. Data points represent individual cells with a line indicating the mean. Scale bar 10μm. B. Immunoblots of p97, TDP-43, autophagy markers (p62 and LC3b), and total ubiquitylated proteins (Ub) show no difference between genotypes. N = 3 independent experiments. C. Normalized viability of 392.1 motor neurons following 30 days of differentiation.

N = 4 independent experiments. All data expressed as means ± SEM unless otherwise indicated. *p<0.05, ****p<0.0001. unpaired t test (A, C).

Figure 4

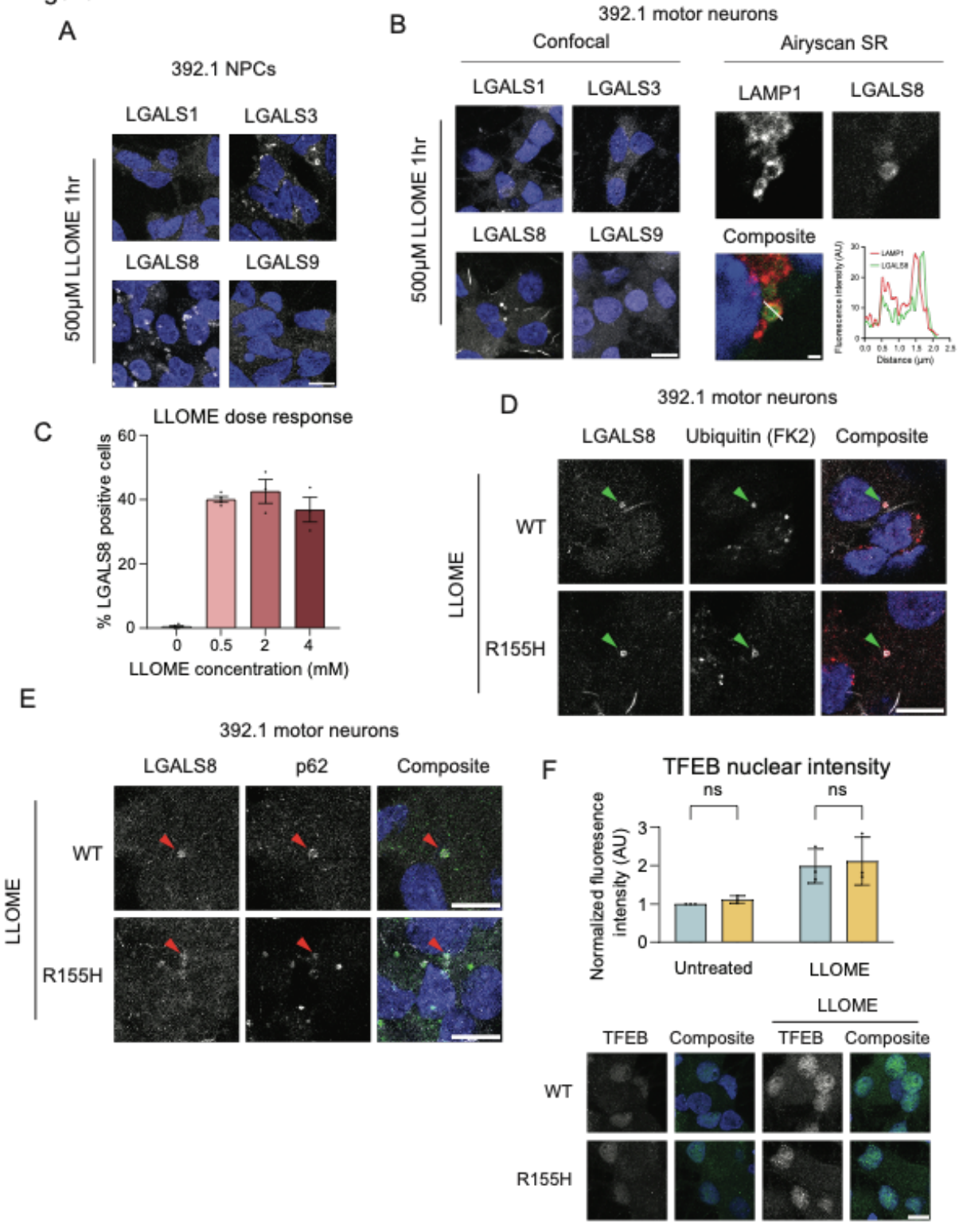
A
392.1 NPCs
LGALS1
LGALS3
LGALS8
LGALS9
500μM LLOME 1hr
B
392.1 motor neurons
Confocal
Airyscan SR
LGALS1
LGALS3
LAMP1
LGALS8
LGALS8
LGALS9
Composite
500μM LLOME 1hr
C
LLOME dose response
% LGALS8 positive cells
LLOME concentration (mM)
D
392.1 motor neurons
LGALS8
Ubiquitin (FK2)
Composite
WT
R155H
LLOME
E
392.1 motor neurons
LGALS8
p62
Composite
WT
R155H
LLOME
F
TFEB nuclear intensity
ns
ns
Normalized fluoresence intensity (AU)
Untreated
LLOME
LLOME
TFEB
Composite
TFEB
Composite
WT
R155H

Figure 4S. Gene ontology of DEPs in R155H motor neurons reveal genetic background specific differences

A-C. Cluster dendrograms of identified proteins in each experiment indicated (top). Gene ontology terms for enriched and depleted DEPs for each experiment demonstrate divergent proteome changes between cell lines (bottom). D. Heatmap of mitochondrial proteins in KOLF2.1 homozygous motor neurons show wholesale depletion of electron transport chain (ETC) components. E. Validation of the depletion of ETC components TMEM70 and MT-ND2 and translocon components TOMM20 and TOMM70 in KOLF2.1 motor neurons. N = 3 independent experiments. F. Representative images of MitoTracker Red fluorescence in KOLF2.1 motor neurons. Scale bar 10μm. G. Quantification of fluorescence in F. The uncoupling agent carbonyl cyanide p-trifluoromethoxyphenylhydrazone (FCCP) was used to collapse the mitochondrial inner membrane potential as a positive control. N = 4 independent experiments. H. Quantification mitochondrial morphology using MitoTracker Red live cell imaging. All data expressed as means ± SEM. *p<0.05, **p<0.01, ***p<0.001; one-way ANOVA with Dunnett's multiple comparison test (G, H)

Supplementary figure 5

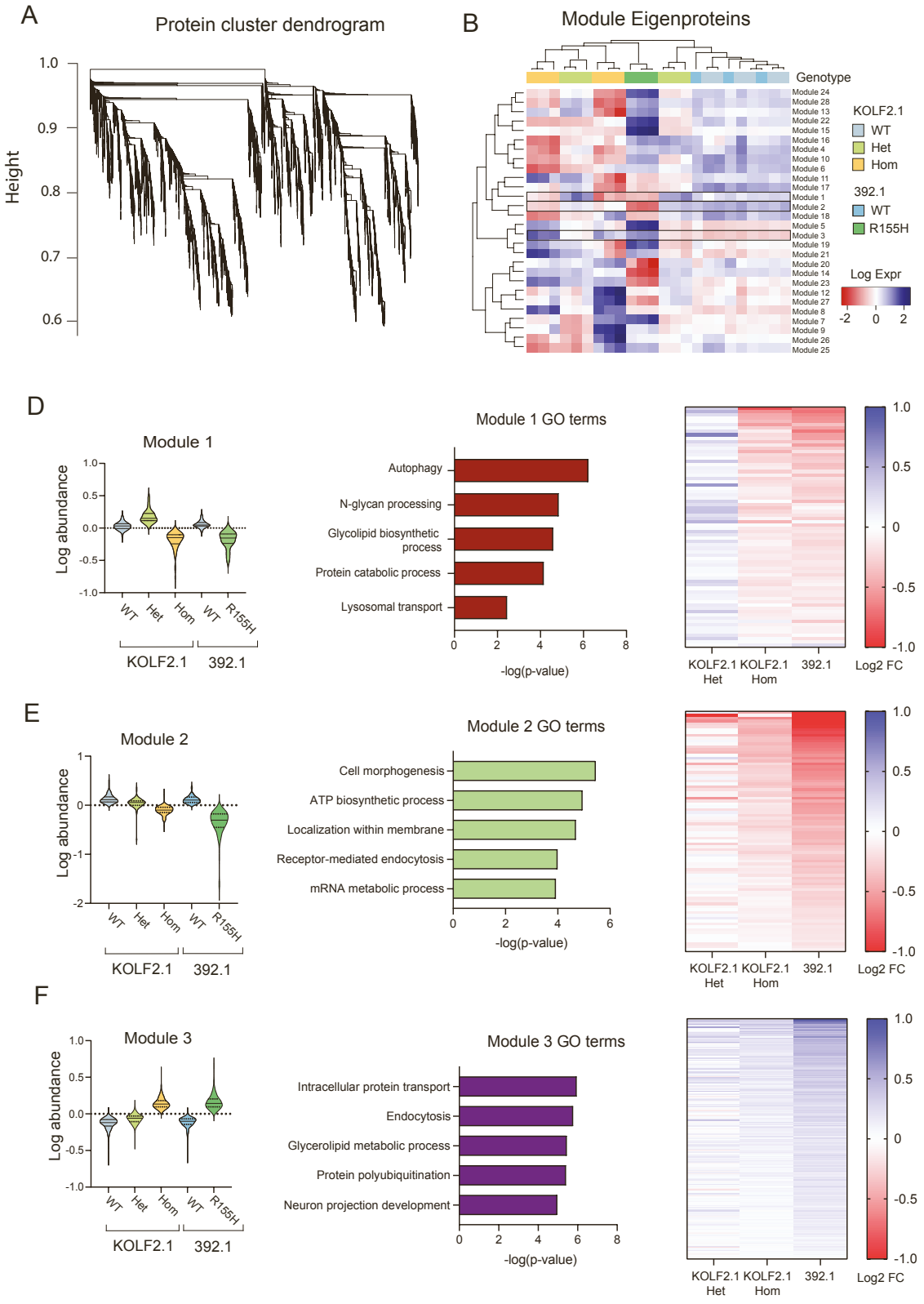

Figure 5S. Unbiased clustering analysis of KOLF2.1 and 392.1 motor neuron proteomes identify altered pathways related to autophagy and lysosome homeostasis

A. Weighted gene correlation network analysis (WGCNA) adapted for proteomics experiments was used to cluster all proteins into modules of distinct proteins which are altered in a similar manner between genotypes. B. Heatmap of module eigenproteins for each replicate in each genotype as depicted by color coding above the heatmap. Modules were ranked by concordance and average fold change to identify protein sets changing in a similar manner between cell lines. The top three modules are boxed within the heatmap. D-F. Violin plots of the relative log2 abundance of member proteins of the top three modules in motor neurons (left). Top 5 enriched gene ontology terms in each of the modules ranked by significance (middle). Heatmaps of the log2 fold change of member proteins in each module for each genotype expressed as mutant vs WT (right).

Supplementary figure 6

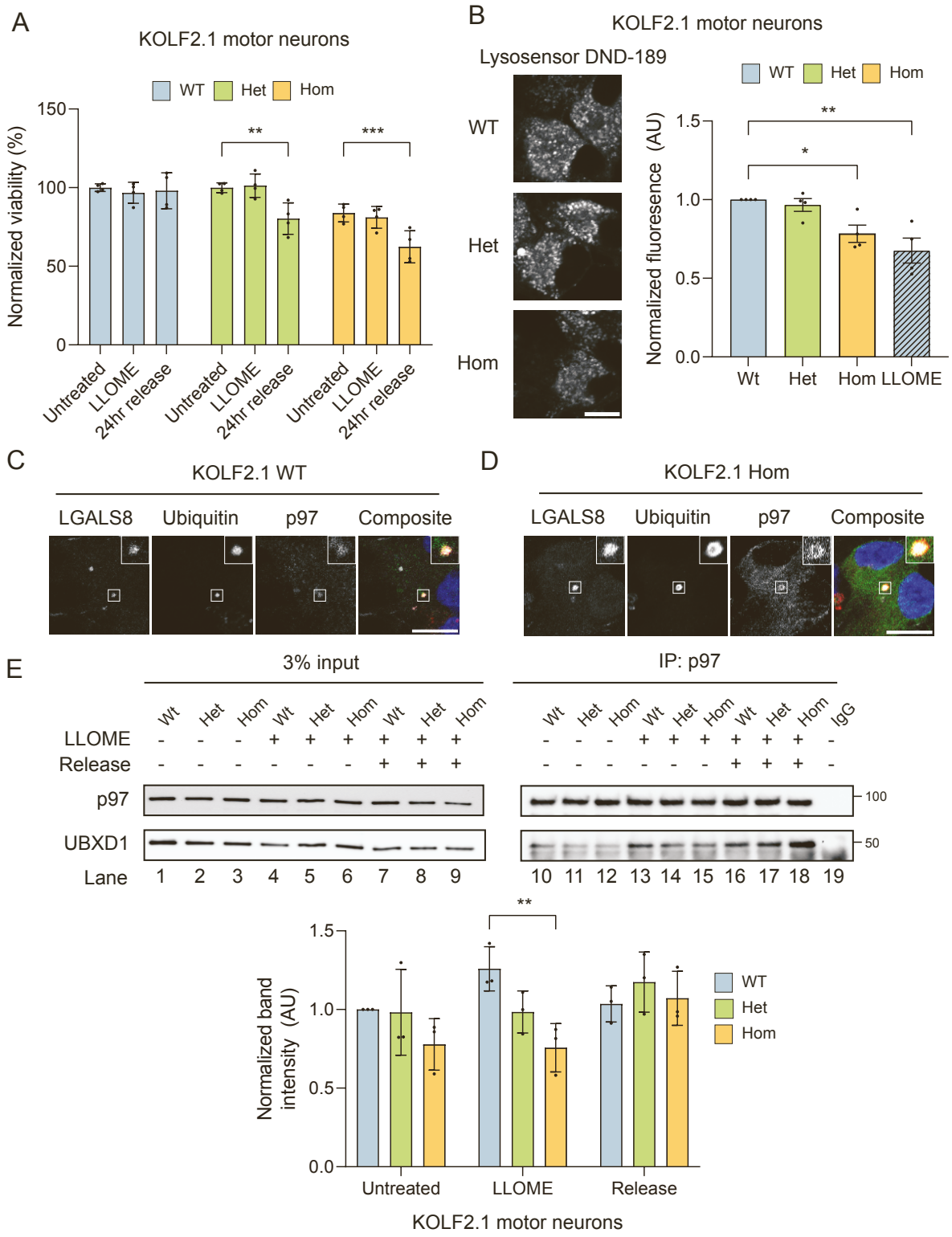

Figure 6S. Mutant p97 reduces neuronal resilience to LLOME treatment, increases lysosomal pH, and disrupts association to UBXD1 following lysosomal damage

A. Normalized viability in untreated, LLOME treated or after release in KOLF2.1 motor neurons. N = 4 independent experiments. B. Representative images of Lysosensor DND-189 live cell imaging in KOLF2.1 motor neurons (left). Quantification of DND-189 fluorescence in untreated and LLOME treated cells. N = 4 independent experiments. Scale bar 5 μm. C-D. Representative images of wildtype (C) and R155H p97 (D) KOLF2.1 motor neurons co-stained with LGALS8, p97, and ubiquitin showing colocalization. N = 3 independent experiments. Scale bars 10μm. E. Immunoblot of endogenous p97 immunoprecipitation in KOLF2.1 motor neurons before, during, and after LLOME treatment (top). Release condition represents 5 hours of recovery. Quantification of UBXD1 band intensities normalized to immunoprecipitated p97 (left). N = 4 independent experiments. All data expressed as means ± SEM. **p<0.01, ***p<0.001; two-way ANOVA with Dunnett's (A, E) or Sidak's (B) multiple comparison test

Supplementary figure 7

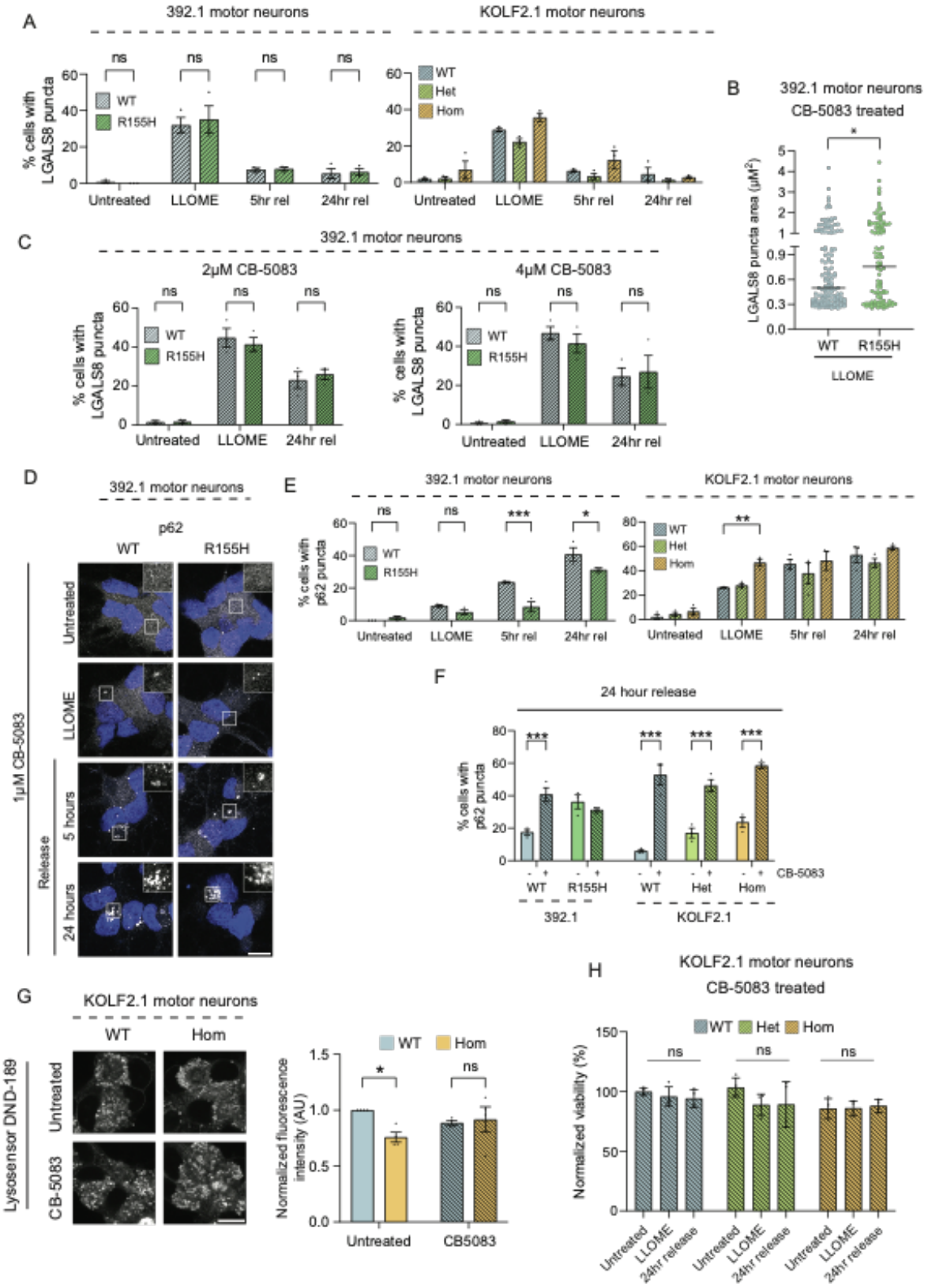

Figure 7S. p97 inhibition rescues lysosome defects in motor neurons

A. Quantification of LGALS8 puncta in 392.1 (left) and KOLF2.1 (right) motor neurons co-treated with LLOME and CB-5083. N = 3 independent experiments. B. Quantification of LGALS8 puncta area. Data expressed as individual LGALS8 puncta; lines represent means. C. Quantification of cells with LGALS8 puncta in 392.1 motor neurons treated with 2μM (left) and 4μM CB-5083 (right). N = 3 independent experiments. D. Representative images p62 puncta in 392.1 motor neurons before, during, and after LLOME treatment. Scale bar 10μm. E. Quantification of p62 puncta in 392.1 (left) and KOLF2.1 (right) motor neurons co-treated with LLOME and CB-5083. N = 3 independent experiments. F. Comparison of p62 puncta clearance at 24 hours release in motor neurons treated with LLOME (solid bars) or co-treated with LLOME and CB-5083 (shaded bars). N = 3 independent experiments. G. Representative images of Lysosensor DND-189 live-cell imaging in KOLF2.1 motor neurons. Quantification of DND-189 fluorescence in untreated (solid bars) and CB-5083 treated (shaded bars) motor neurons. N = 4 independent experiments. Scale bar 10μm. H. Normalized viability of KOLF2.1 motor neurons treated with CB-5083 in untreated, LLOME treated, or after release. Release condition represents 24 hours of recovery. All data expressed as means ± SEM unless otherwise indicated. ns – nonsignificant, *p<0.05, **p<0.01, ***p<0.001; two-way ANOVA with Sidak's (A-left, C, E-left, F, G) or Dunnett's (A-right, E-right, H) multiple comparison test or unpaired t-test (B)

Supplementary Methods

Antibodies and chemicals

Antibodies for LGALS1 (11858-1-AP), LGALS3 (14979-1-AP), PAX6 (12323-1-AP), TDP-43 (10782-2-AP), ATF4 (10835-1-AP), TMEM70 (20388-1-AP), TFEB (13372-1-AP), and MT-ND2 (19704-1-AP) were procured from ProteinTech Group. Antibodies for SOX2 (3579T), NANOG (4903T), LC3B (3868T), and Bip (3177T) were procured from Cell Signaling Technologies. Additional antibodies used were TUJ1 (T8660 Sigma), ChAT (ab181023 Abcam), SMI32 (801702 BioLegend), LGALS8 (AF1305 RND Systems), LGALS9 (AF2045 RND Systems), p97 (A300-589A Bethyl (IP) and 612183 BD Biosciences (IF)), ubiquitin (FK2) (04-263 EMD Millipore), LAMP1 (sc-20011 Santa Cruz), GAPDH (sc-47724 Santa Cruz), NESTIN (NBP1-92717SS Novus Biologicals), OCT4 (653701 BioLegend), TOMM20 (sc-17764 Santa Cruz), TOMM70 (sc-390545 Santa Cruz), and p62 (GP62-C Progen Biotechnik). Alex Fluor-conjugated secondary antibodies were from Molecular Probes. Primary antibodies were used at 1:100 for immunofluorescence studies and 1:1000 for immunoblotting. Secondary antibodies were used at 1:5000 for immunofluorescence and immunoblot studies. SB431532 (S1067), Y-27632 (S1049), and SAG1 (S7779) were obtained from Selleck Chem. LDN193189 (SML0559), valproic acid (P4543), BrdU (B9285), PLO (P3655), laminin (L2020), and fibronectin (F2006) were obtained from Sigma-Aldrich. CHIR99021 (4423) was obtained from Tocris. Retinoic acid (sc-200898) was obtained from Santa Cruz. Compound E (565790) was obtained from EMD Millipore. GDNF (450-10), BDNF (450-02), and NT-3 (450-03) were obtained from PeproTech. LLOME (16008) and CB-5083 (19311) were obtained from Cayman Chemicals.

Media Composition

Base media	Media type	Component	Concentration
PIE base	Induction media	SB-431542	10uM
		LDN-193189	1uM
		CHIR-99021	3uM
	Induction-patterning media	SB-431542	10uM
		LDN-193189	1 μM
		CHIR-99021	3 μM
		Retinoic acid	2 μM
		SAG1	1 μM
	Expansion media	CHIR-99021	3 μM
		Valproic acid	5 μM
		Retinoic acid	0.5 μM
		SAG1	1 μM
	Patterning media	Retinoic acid	2 μM
		SAG1	1 μM
3M base	Maturation media	Retinoic acid	1 μM
		SAG1	0.5 μM
		BDNF	20 ng/mL
		GDNF	20 ng/mL
		NT-3	10 ng/mL
		Compound E	0.2 μM
		Laminin	1 μg /mL

Table 1

Base media	Component	Concentration	Source	Identifier

Patterning, induction, expansion base (PIE)	DMEM/F12	50%	Cytiva	SH30023.FS
	Neurobasal plus	50%	Thermo	A3582901
	N-2 supplement	0.5x	Thermo	17502048
	B27 plus supplement	0.5x	Thermo	A3582801
	GlutaMax	1x	Thermo	35050061
	NEAA	1x	Thermo	11140050
	Penicillin/streptomycin	1x	Caisson	PSL01
	Insulin	5ug/mL	Sigma	91077C
	ß-mercaptoethanol	100 µM	Gibco	21985023
Motor neuron maturation media (3M) base	Neurobasal plus	100%	Thermo	A3582901
	N-2 supplement	0.5x	Thermo	17502048
	B27 plus supplement	0.5x	Thermo	A3582801
	GlutaMax	1x	Thermo	35050061
	NEAA	1x	Thermo	11140050
	Penicillin/streptomycin	1x	Caisson	PSL01
	Insulin	5 µg /mL	Sigma	91077C
	ß-mercaptoethanol	100 µM	Gibco	21985023

Table 2

Western blot

Cells were removed from plates using ReLeSR (IPSCs), 0.05 % trypsin-EDTA (NPCs), or pipetting (motor neurons), centrifuged at 600 x g for 5 minutes, and supernatant removed. Cell pellets were stored at -80 ºC until used. Pellets were lysed in RIPA buffer (150 mM NaCl, 50 mM Tris Cl, 0.5 % sodium deoxycholate, 0.1 % SDS, 1% NP-40) supplemented with 1x

HALT protease inhibitor (PI-78425 Thermo) and incubated on ice for 10 minutes. Lysates were sonicated at 15% power for 5 seconds, 3 times with 2 second pauses. Lysates were then centrifuged at 21,000 x g at 4 ºC for 15 minutes to pellet cell debris. Clarified lysates were transferred to clean microcentrifuge tubes and either frozen at -80ºC or used immediately for further assays. Total protein was measured using the BCA assay (23225 Thermo). Protein levels between samples were equalized, mixed with 5x Laemmli buffer (250 mM tris, 25 % ß-mercaptoethanol, 50 % glycerol, 20% SDS, 0.05% bromophenol blue), and boiled for 5 minutes at 95C. An equal amount of sample was loaded and separated on a 12% bis-tris gel and transferred to PVDF membranes (1620177 Bio-Rad) using a Bio-Rad cassette at 70 volts for 2 hours. Membranes were blocked in 5 % milk in tris buffered saline containing 0.1% tween 20 (TBS-T) for 1 hour at room temperature. Primary antibodies were diluted in 2 % BSA (A-420-500 GoldBio) in TBS-T to the indicated concentration and incubated with membranes overnight at 4C with rocking. Membranes were washed 3 times with TBS-T for 10 minutes each while rocking at room temperature. Secondary antibodies were diluted in 2% BSA in TBS-T and incubated with membranes at 1:5000 for 1 hour at room temperature with rocking. Membranes were washed 3 times as described previously, developed with Clarity ECL substrate (1705061 Bio-Rad), and imaged with a ChemiDoc MP Imaging system (BioRad). Blot densities were analyzed using ImageJ.

LLOME treatment

LLOME was reconstituted to 500mM in sterile DMSO and aliquoted for storage at -20 ºC. LLOME was diluted in fresh media immediately before being added to cells. Cells were incubated at the indicated concentrations for the indicated times after which they were washed

once with PBS and fixed or fresh media was added for release time points. CB-5083 was reconstituted to 10 mM in sterile DMSO and aliquoted for storage. Fresh aliquots were thawed before each experiment and added directly to complete media to a final concentration of 1μM.

Slides were imaged with either Nikon TI2 Eclipse epifluorescent microscope 40X or 100X objectives using NIS acquisition software (Figures 1, 2, S1, and S2) or Zeiss LSM880 confocal microscope 63X oil objective using ZenBlue software (all others). Image analysis was performed using a modified version of the AggreCount ImageJ macro[75] or custom ImageJ macros.

Immunofluorescence

Starting on day 9 of differentiation, cells were plated on acid-washed (1 M HCl 4 hours with rocking) # 1.5 glass coverslips that were triple coated (PLO/laminin/fibronectin). Cells were maintained as previously described. Cells were fixed in ice cold 4 % PFA (15710-S Electron Microscopy Sciences) diluted in PBS for 20 minutes. Coverslips were washed once in PBS and incubated in blocking buffer (2 % BSA, 0.2 % Triton-X100 (Figures 1, 2, S1, S2, and S3) or 2 % BSA, 0.4 % saponin (Figures 4, 5, 6, 7, S6, and S7) in PBS) for 1 hour at room temperature. Primary antibodies were diluted to the indicated concentrations in blocking buffer and coverslips were incubated overnight at 4 ºC in a humidified chamber. Coverslips were then washed once in blocking buffer and incubated in secondary antibodies diluted to the indicated concentrations in blocking buffer for 1 hour at room temperature. The secondary antibody solution was replaced with Hoechst diluted in PBS and incubated for 5 minutes at room temperature. Coverslips were washed once with PBS and mounted to slides with ProLong Gold antifade mounting media (P36930 Invitrogen).

Immunoprecipitation

Pellets were lysed in mammalian cell lysis buffer (50 mM Tris Cl, 150 mM NaCl, 0.2 % NP-40, HALT), incubated on ice for 10 minutes, and centrifuged at 21,000 x g for 15 minutes at 4 ºC. Clarified lysate was transferred to clean microcentrifuge tubes and total protein was measured using the BCA assay. A small portion of the lysate was set aside as the input, and equal amounts of protein were added to washed protein G beads (20398 Thermo) with the indicated antibody (0.5 µg antibody/ 500 µg of lysate). Samples were incubated at 4 ºC overnight while rocking. They were centrifuged at 400 x g for 1 minute at 4C, supernatant was removed, and beads were washed with lysis buffer 3 times. 2X Laemmli buffer was added to samples and boiled for 5 minutes at 95 ºC. Samples were centrifuged at 400 x g for 1 minute to pellet the beads and remaining supernatant was analyzed by western blot as previously described.

Electrophysiology

WT and homozygous KOLF2.1 motor neurons were perfused in normal artificial cerebral spinal fluid (nACSF) containing (in mM) 126 NaCl, 26 NaHCO3, 1.25 NaH2PO4, 2.5 KCl, 2 CaCl2, 2 MgCl2, and 10 dextrose (300–310 mosM) and bubbled with 95% O2-5% CO2. Neurons were recorded under physiological temperature maintained at 33°C (in-line heater; Warner Instruments) and perfused at a high flow rate (~4 ml/min) throughout the experiment. Input-output curves were generated as previously described[76,77]. Whole cell patch clamp recordings on visually identified motor neurons were performed in the current-clamp configuration using an intracellular recording solution containing (in mM) 130 K-

gluconate, 10 KCl, 4 NaCl, 10 HEPES, 0.1 EGTA, 2 Mg-ATP, and 0.3 Na-GTP (pH = 7.25, 280–290 mosM). The resting membrane potential and input resistance was calculated using Ohm's law in response to a −100-pA current injection. The presence of spontaneous action potentials was evaluated during a 2-min baseline recording period. The number of action potentials generated in response to a series of 500-ms current injections from 10 to 150 pA in 10-pA steps was measured to generate input-output curves. Series resistance and whole cell capacitance were continually monitored and compensated throughout the course of the experiment. Recordings were eliminated from data analysis if series resistance increased by >20%. For all electrophysiology experiments, data acquisition was carried out using an Axopatch 200B (Axon Instruments) and PowerLab hardware and software (ADInstruments). Data analysis was performed using analysis scripts developed in-house using Python.

qPCR

Total RNA from KOLF2.1 iPSCs, NPCs and d30 motor neurons was harvested and purified using the Quick RNA miniprep kit (R1054 Zymo) following the manufacturer guidelines. Complementary DNA (cDNA) was created for each sample from equals amounts of total RNA using the iScript cDNA synthesis kit (1708890 BioRad). Real-time PCR was performed from cDNA on a StepOnePlus Real-Time PCR System (Applied Biosystems) using the PowerUp SYBR Green Master Mix (A25741 Thermo). Results were quantified using the ΔΔCT method. Signals from samples were normalized against the housekeeping genes, GAPDH or 18s. Primers sequences are as follows:

18s Forward 5’-GGCCCTGTAATTGGAATGAGTC-3’
18s Reverse 5’-CCAAGATCCAACTACGAGCTT
Oct4 Forward 5’-GAAACCCACACTGCAGATCA-3’
Oct4 Reverse 5’-CGGTTACAGAACCACACTCG-3’

Pax6 Forward 5'-CCCACACTCTTTATCTCTCACTC-3'
Pax6 Reverse 5'-AGTTGCTGGTGAGAGTTTTCT-3'
ChAT Forward 5'-CCTGCAGTGCATGCGACAC-3'
ChAT Reverse 5'-AAACTGCTGCACAATGGCCT-3'

TMT proteomic sample preparation

Samples were collected by aspirating media and pipetting cells off with ice cold PBS. Cells were spun at 600 x g for 5 minutes at 4 ºC, the supernatant was removed, and cells were stored at -80 ºC. Cell pellets were lysed with 8 M urea supplemented with 1x HALT in 200 mM EPPS (E0276 Sigma) by pipetting up and down 10 times. Samples were passed through a 26g syringe 10 times to shear membranes and centrifuged at 21,000 x g for 10 minutes to pellet debris. The supernatant was transferred to clean microcentrifuge tubes and total protein was estimated using the BCA assay. 100 µg of protein was aliquoted from each sample and the volumes were made equal with lysis buffer. Samples were reduced with 5 mM TCEP for 30 minutes, alkylated with 14 mM iodoacetamide for 30 minutes, and the reaction was quenched with 5 mM DTT for 15 minutes all at room temperature in the dark. Protein was precipitated by adding 400 µL methanol, 100 µL chloroform, and 300 µL water and centrifuging 21,000 x g for 2 minutes at room temperature. The organic and aqueous layers were aspirated, and the remaining protein was allowed to air dry for 5-10 minutes. The protein precipitate was resuspended in 100 µL of 200 mM EPPS. 1 µg/µg of Lys-C (129-02541 Wako) was added to each sample and incubated overnight at room temperature with shaking. Samples were further digested with 1 µg/µg trypsin (90305 Thermo) per sample for 6 hours at 37 ºC with shaking. 30 µL of anhydrous acetonitrile was added to each sample. Samples were labeled with 10-plex TMT labeling reagents (90110 Thermo) at 1:10 for 1 hour at room temperature. The labeling reaction was quenched with 10uL 5% hydroxylamine in 200mM EPPS for 15 minutes. TMT-

labeled peptides from each sample were combined in equal amounts. The pooled sample was dried under vacuum.

The sample was resuspended in 5% formic acid for 15 minutes. The peptide mix was desalted using C18 solid-phase extraction (SPE) (WAT036945 Waters). This sample was then fractionated using off-line basic pH reverse-phase fractionation via high performance liquid chromatography (HPLC). The peptide mix was fractionated in 96 fractions which were combined into a total of 24 fractions. These fractions were further desalted using STAGE tips made from C18 resin and p200 pipette tips and died under vacuum. They were reconstituted in 5% acetonitrile / 1% formic acid for LC-MS/MS processing. An Orbitrap Lumos mass spectrometer coupled with a Proxeon NanoLC-1000 UHPLC was used for data collection. Proteins were identified using all entries from the Human UniProt Database (2018). Peptide-spectrum matches were adjusted to a 1% false discovery rate. Raw protein abundances were determined by summing reporter ion counts across all matching peptides. The abundances were adjusted for protein loading and scaled to generate a relative abundance measurement.

Proteomic data analysis

Log_2 fold changes and adjusted p values were calculated using a modified linear modeling for microarray data as an empirical Bayes procedure using a custom R script[78]. Weighted gene co-expression analysis (WGCNA) was performed as previously described using a modified version of the WGCNA R script[41]. In brief, relative abundances were normalized using sample loading normalization and internal reference scaling to eliminate variation between proteomics experiments. Normalized abundances were used to build a topological overlap distance matrix which was clustered using the TOM-based dissimilarity

method. Modules were identified via a dynamic tree cutting algorithm. These modules were ranked based via concordance between cell line (KOLF2.1 Hom and 392.1 R155H) and average $\log_2$ FC. The three highest ranked modules were used for gene ontology analysis using Metascape to identify enriched pathways[79].