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# Reporting Summary

Nature Research wishes to improve the reproducibility of the work that we publish. This form provides structure for consistency and transparency in reporting. For further information on Nature Research policies, see Authors & Referees and the Editorial Policy Checklist.

## Statistical parameters

When statistical analyses are reported, confirm that the following items are present in the relevant location (e.g. figure legend, table legend, main text, or Methods section).

| n/a | Confirmed | |
|---|---|---|
| ☐ | ☒ | The exact sample size (*n*) for each experimental group/condition, given as a discrete number and unit of measurement |
| ☐ | ☒ | An indication of whether measurements were taken from distinct samples or whether the same sample was measured repeatedly |
| ☐ | ☒ | The statistical test(s) used AND whether they are one- or two-sided<br>*Only common tests should be described solely by name; describe more complex techniques in the Methods section.* |
| ☒ | ☐ | A description of all covariates tested |
| ☒ | ☐ | A description of any assumptions or corrections, such as tests of normality and adjustment for multiple comparisons |
| ☒ | ☐ | A full description of the statistics including central tendency (e.g. means) or other basic estimates (e.g. regression coefficient) AND variation (e.g. standard deviation) or associated estimates of uncertainty (e.g. confidence intervals) |
| ☐ | ☒ | For null hypothesis testing, the test statistic (e.g. *F*, *t*, *r*) with confidence intervals, effect sizes, degrees of freedom and *P* value noted<br>*Give P values as exact values whenever suitable.* |
| ☒ | ☐ | For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings |
| ☒ | ☐ | For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes |
| ☐ | ☒ | Estimates of effect sizes (e.g. Cohen's *d*, Pearson's *r*), indicating how they were calculated |
| ☐ | ☒ | Clearly defined error bars<br>*State explicitly what error bars represent (e.g. SD, SE, CI)* |

*Our web collection on statistics for biologists may be useful.*

## Software and code

Policy information about availability of computer code

| | |
|---|---|
| Data collection | No custom software was used to collect data. Other programs used to collect and analyze data include: Licor (Image Studios version 2.1), Flow Jo (Version 8.7), GraphPad Prism (Version 7), IGV (Version 6), 4Peaks (version 1.8), Geneious (version 10), ImageJ with NeuronJ (1.4.2 by Erik Meijering), Nikon NIS Elements (Version 4.0). |
| Data analysis | RNA-seq data was processed with the bcbio pipeline and analyzed with the bcbioRNASeq R package. Detailed information on the source code and program versions (update version 06) used for analysis, including installation instructions, are available at our GitHub repo: https://github.com/mjsteinbaugh/eggan-es_derived_motor_neuron_knockdown-rnaseq-human<br>All methods used in the RNA-seq analysis are published in peer-reviewed journals.<br>bcbio and bcbioRNASeq source code are provided under an MIT license (https://github.com/hbc/bcbioRNASeq/blob/master/LICENSE).<br>Software URLs:<br>bcbio<br>http://bcbio-nextgen.readthedocs.io<br>https://github.com/bcbio/bcbio-nextgen<br>bcbioRNASeq<br>http://bioinformatics.sph.harvard.edu/bcbioRNASeq<br>https://github.com/hbc/bcbioRNASeq<br>http://dx.doi.org/10.12688/f1000research.12093.1<br><br>salmon |

https://combine-lab.github.io/salmon https://dx.doi.org/10.1038%2Fnmeth.4197
DESeq2
https://doi.org/doi:10.18129/B9.bioc.DESeq2 http://doi.org/10.1186/s13059-014-0550-8
Prism (version 7) was used to perform statistical analyses.

For manuscripts utilizing custom algorithms or software that are central to the research but not yet described in published literature, software must be made available to editors/reviewers upon request. We strongly encourage code deposition in a community repository (e.g. GitHub). See the Nature Research guidelines for submitting code & software for further information.

## Data

Policy information about availability of data

All manuscripts must include a data availability statement. This statement should provide the following information, where applicable:

- Accession codes, unique identifiers, or web links for publicly available datasets
- A list of figures that have associated raw data
- A description of any restrictions on data availability

The authors will make all data available to readers upon reasonable request. The RNA-seq data discussed in this publication have been deposited in NCBI's Gene Expression Omnibus61 and are accessible through GEO Series accession number GSE121569 (https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE121569). The patient spinal cord RNA-Seq is available through dbGAP (phs000747.v2.p1).

# Field-specific reporting

Please select the best fit for your research. If you are not sure, read the appropriate sections before making your selection.

☒ Life sciences ☐ Behavioural & social sciences ☐ Ecological, evolutionary & environmental sciences

For a reference copy of the document with all sections, see nature.com/authors/policies/ReportingSummary-flat.pdf

# Life sciences study design

All studies must disclose on these points even when the disclosure is negative.

| | |
|---|---|
| Sample size | No statistical methods were used to pre-determine sample sizes but our sample sizes are similar to those reported in previous publications including Egawa et al 2012 Sci Transl Med, Bilican et al 2012 PNAS, and Fujimori et al 2018 Nature Medicine |
| Data exclusions | No data were excluded from the analyses. |
| Replication | For the RNAi experiments, replication was achieved through multiple independent differentiations and through using two unique siRNAs for the control and siTDP-43 groups. For the iPS cell studies, we used multiple control cell lines and all available mutant TDP-43 lines for replication. For the STMN2 knockout studies, we generated knockout in two distinct hES cell lines for replication. Technical replication was used throughout for molecular studies. All attempts at replication were successful. |
| Randomization | No randomization was used. We compared specifically designated control samples and test samples through either treatment or genetic information. |
| Blinding | Blinding was used for the Sholl analysis for the researcher tracing the neurites. For other experiments, data collection and analysis were not performed blind to the conditions of the experiments. |

# Reporting for specific materials, systems and methods

### Materials & experimental systems

| n/a | Involved in the study |
|---|---|
| ☒ | ☐ Unique biological materials |
| ☐ | ☒ Antibodies |
| ☐ | ☒ Eukaryotic cell lines |
| ☒ | ☐ Palaeontology |
| ☒ | ☐ Animals and other organisms |
| ☒ | ☐ Human research participants |

### Methods

| n/a | Involved in the study |
|---|---|
| ☒ | ☐ ChIP-seq |
| ☐ | ☒ Flow cytometry |
| ☒ | ☐ MRI-based neuroimaging |

## Antibodies

Antibodies used

NCAM, BD Biosciences Alexa Fluor® 700 Mouse Anti-Human CD56, Cat # 557919, Clone B159, Lot 5020744
EpCAM, BD Biosciences PE Mouse Anti-Human EpCAM, Cat# 347198, Clone EBA-1, Lot 7174757
TDP-43, ProteinTech TDP-43 Antibody, Cat# 10782-2-AP, Polyclonal, Lot 00052103
TUJ1, R&D Systems Neuron-specific beta-III Tubulin NL493 Mab, Cat# NL1195G, Clone TuJ-1, Lot HGQ0116111
STMN2 (IF) Novus Biologicals Stathmin-2/STMN2 Antibody, Cat# NBP1-49461, Polyclonal, Lots C7 and D1
STMN2 (WB) R&D Systems Human/Mouse Stathmin-2/STMN2 Antibody, Cat# MAB6930, Clone 684433, Lot CFIL0114101
GAPDH, Millipore Anti-Glyceraldehyde-3-Phosphate Dehydrogenase Antibody, Cat# MAB374, Clone 6C5, Lot 2792998
MAP2, Abcam Anti-MAP2 antibody, Cat# ab5392, Polyclonal, Lot GR286806-6
GFP, Invitrogen Anti-GFP antibody, Cat# A10262, Polyclonal
Golgin97, Invitrogen Golgin-97 Monoclonal Antibody, Catalog # A-21270, Clone CDF4, GR230216-3
Hb9 DSHB 81.5C10, Cat# 81.5C10, Clone AB_2145209, Supernatant
Isl1, Abcam Anti-Islet 1 antibody, Cat# ab109517, Clone EP4182, Lot GR224258-5
TDP-43, gift from D Cleveland, Clone FL9
SOD1, Cell Signaling Technologies Anti-SOD1 antibody, Cat# 4266, Clone 71G8
Mouse IgG, Cell Signaling Technologies isotype control antibody, Cat# 5415, Clone G3A1
Secondary antibodies were purchased from Invitrogen conjugated to Alexa Fluor 488, 555, 594, and 647

Validation

All antibodies have their respective source company and clone number and are validated for the applications used within this manuscript. This information is available on the manufacturers publicly available datasheets, which we have provided links for in here:
http://www.bdbiosciences.com/us/applications/research/stem-cell-research/hematopoietic-stem-cell-markers/human/negative-markers/alexa-fluor-700-mouse-anti-human-cd56-b159/p/557919
http://www.bdbiosciences.com/us/applications/research/stem-cell-research/cancer-research/human/pe-mouse-anti-human-epcam-eba-1/p/347198
https://ptglab.com/products/TARDBP-Antibody-10782-2-AP.htm?gclid=EAIaIQobChMI3KPwl8WY3gIVT8DICh1vdwe5EAAYASAAEgJcBvD_BwE#datasheet
https://resources.rndsystems.com/pdfs/datasheets/nl1195g.pdf
https://www.novusbio.com/products/stathmin-2-stmn2-antibody_nbp1-49461
https://resources.rndsystems.com/pdfs/datasheets/mab6930.pdf
http://www.emdmillipore.com/US/en/product/Anti-Glyceraldehyde-3-Phosphate-Dehydrogenase-Antibody-clone-6C5,MM_NF-MAB374
https://www.abcam.com/map2-antibody-ab5392.html
https://www.thermofisher.com/antibody/product/GFP-Tag-Antibody-Polyclonal/A10262
https://www.thermofisher.com/antibody/product/Golgin-97-Antibody-clone-CDF4-Monoclonal/A-21270
http://dshb.biology.uiowa.edu/81-5C10_2
https://www.abcam.com/islet-1-antibody-ep4182-ab109517.html
http://www.pnas.org/content/pnas/suppl/2010/07/12/1008227107.DCSupplemental/pnas.201008227SI.pdf
https://www.cellsignal.com/products/primary-antibodies/sod1-71g8-mouse-mab/4266
https://www.cellsignal.com/products/primary-antibodies/mouse-g3a1-mab-igg1-isotype-control/5415?site-search-type=Products&N=102236+4294956287&Ntt=mouse+igg&fromPage=plp
https://www.thermofisher.com/us/en/home/life-science/antibodies/secondary-antibodies/fluorescent-secondary-antibodies/alexa-fluor-secondary-antibodies.html

## Eukaryotic cell lines

Policy information about cell lines

Cell line source(s)

HUES3 Hb9::GFP is a human embryonic stem cell line derived at Harvard University study.
WA01 is a human embryonic stem cell line derived at the University of Wisconsin Madison
The control iPS cell lines (11a, 15b, 17a, 18a, 20b) and two TDP-43 lines (36a Q343R and 47d) were generated in our lab with fibroblasts under IRB approved protocols of collaborative study with Dr. Chris Henderson at the ALS Clinic at Columbia University.
The TDP-43 lines (RB20a) was generated in our lab with fibroblasts from a collaborative study with Dr. Robert Brown through ongoing IRB approved protocol in collaboration with Dr. Merit Cudkowicz and Dr. James Berry at the MGH Neurological Research Institute.
The TDP-43 line with the M337V mutation was a generous gift from Dr. Christopher Shaw

Authentication

Specific point mutations were confirmed by PCR amplification followed by Sanger sequencing.

Mycoplasma contamination

Weekly, we both specifically and randomly check cultures within the lab for mycoplasma contamination using the MycoAlert kit (Lonza). All cell lines used in this study were tested and none tested positive.

Commonly misidentified lines
(See ICLAC register)

No commonly misidentified cell lines were used.

# Flow Cytometry

## Plots

Confirm that:

☒ The axis labels state the marker and fluorochrome used (e.g. CD4-FITC).

☒ The axis scales are clearly visible. Include numbers along axes only for bottom left plot of group (a 'group' is an analysis of identical markers).

☒ All plots are contour plots with outliers or pseudocolor plots.

☒ A numerical value for number of cells or percentage (with statistics) is provided.

## Methodology

| | |
|---|---|
| Sample preparation | Differentiated cultures were dissociated to single cells using accutaseTM treatment for 1 hour inside a 5% CO2 / 37ºC incubator. Repeated (10-20 times) but gentle pipetting with a 1000µL Pipetman® was used to achieve a single cell preparation. Cells were spun down, washed 1x with PBS and resuspended in sorting buffer (1x cation-free PBS 15mM HEPES at pH 7 (Gibco®), 1% BSA (Gibco®), 1x penicillin-streptomycin (Gibco®), 1 mM EDTA, and DAPI (1µg/mL). Cells were passed through a 45µm filter immediately before FACS analysis and purification. |
| Instrument | The BD FACS Aria II cell sorter (SORP) was used. |
| Software | FlowJo was used to analyze the data. |
| Cell population abundance | The purity of samples was determined post sort using microscopy. |
| Gating strategy | Forward and side scatter was used to resolve cells from debris with doublet discrimination by forward scatter area vs forward scatter width. DAPI signal was then used to determine cell viability, and differentiated cells not exposed to MN patterning molecules (RA and SAG) were used as negative controls to gate for green fluorescence. For lines not containing the Hb9::GFP reporter, single cell sunspensions were incubated with antibodies against NCAM (BD Bioscience, BDB557919, 1:200) and EpCAM (BD Bioscience, BDB347198, 1:50) for 25 minutes in sorting buffer, then washed once with PBS 1x and resuspended in sorting buffer. The population that was negative for EpCAM but positive for NCAM was sorted. We can provide additional figures exemplifying all the gating strategies if the ones provided are insufficient. |

☒ Tick this box to confirm that a figure exemplifying the gating strategy is provided in the Supplementary Information.