Cell Stem Cell, Volume *30*

Supplemental Information

SYF2 suppression mitigates neurodegeneration in models of diverse forms of ALS

Gabriel R. Linares, Yichen Li, Wen-Hsuan Chang, Jasper Rubin-Sigler, Stacee Mendonca, Sarah Hong, Yunsun Eoh, Wenxuan Guo, Yi-Hsuan Huang, Jonathan Chang, Sharon Tu, Nomongo Dorjsuren, Manuel Santana, Shu-Ting Hung, Johnny Yu, Joscany Perez, Michael Chickering, Tze-Yuan Cheng, Chi-Chou Huang, Shih-Jong James Lee, Hao-Jen Deng, Kieu-Tram Bach, Kamden Gray, Vishvak Subramanyam, Jeffrey Rosenfeld, Samuel V. Alworth, Hani Goodarzi, and Justin K. Ichida

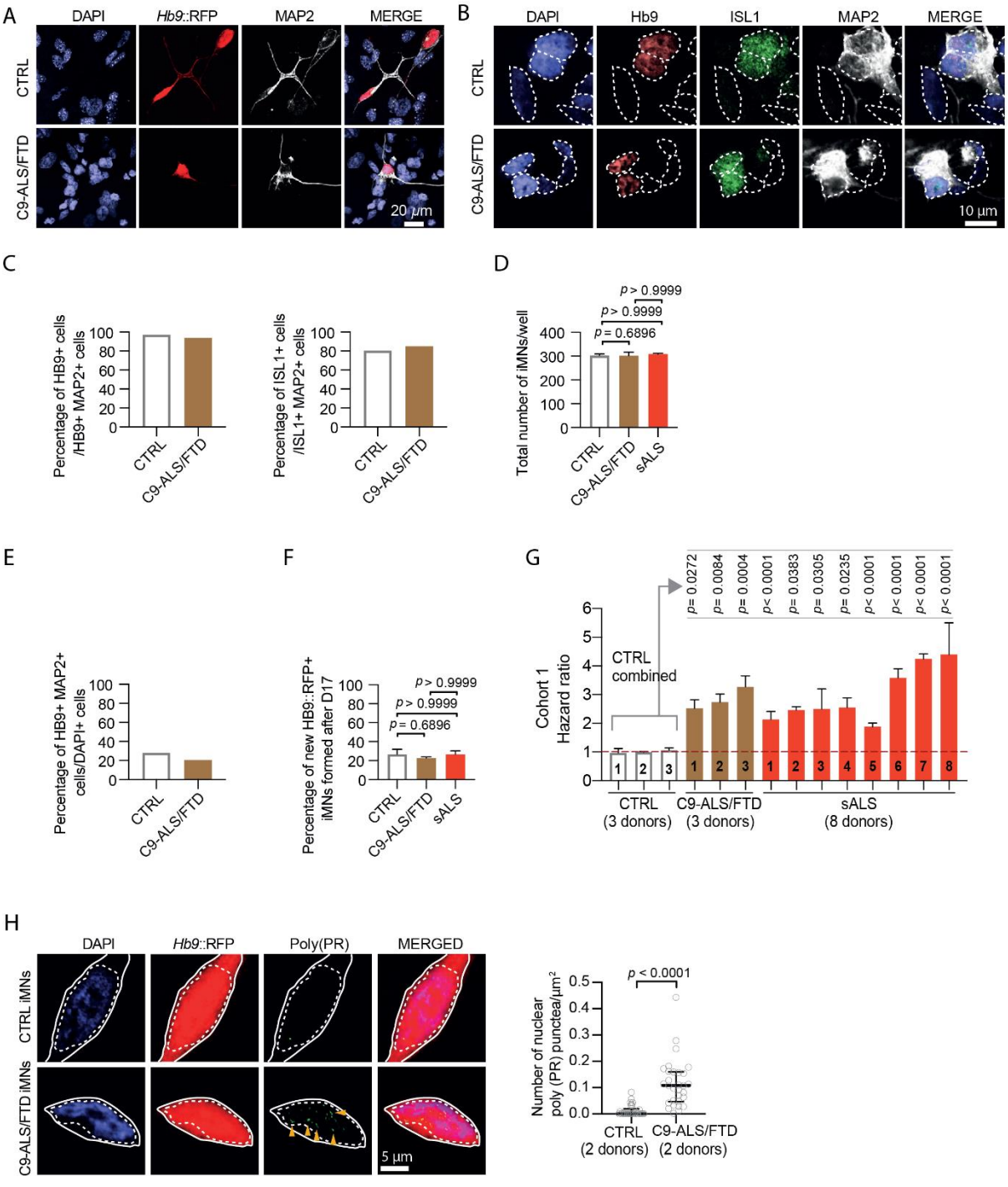


Figure S1, related to Figure 1. *C9ORF72* and sporadic ALS iMNs display neurodegenerative disease processes.

(A) Immunostaining showing control (CTRL) and *C9ORF72* ALS/FTD (C9-ALS/FTD) iMNs express the *Hb9*::RFP reporter (red) and Microtubule Associated Protein 2 (MAP2, white). Nuclei (blue) are labeled with DAPI. Scale bar = 20 μm.

(B) Immunostaining of endogenous Homeobox HB9 (HB9; red), Insulin gene enhancer protein (ISL1; green) and MAP2 (white) in CTRL and C9-ALS/FTD iMNs. Nuclei (blue) are labeled with DAPI. Dotted lines outline the nucleus. Scale bar = 10 μm.

(C) Quantification showing percentage of HB9+ neurons among MAP2+ neurons or ISL1+ neurons among MAP2+ neurons in CTRL and C9-ALS/FTD iMNs. Immunostaining was performed on day 17 of conversion. Neurons were quantified from 3 regions of a well from a CTRL line or a C9-ALS/FTD line.

(D) Quantification showing total numbers of iMNs/well in 96-well plates used for survival experiments from a CTRL line, a C9-ALS/FTD line and a sporadic ALS (sALS) line on day 17 of conversion. n=3 wells/line. Statistical significance (One-way ANOVA) was calculated comparing CTRL vs. C9-ALS/FTD and CTRL vs. sALS with Dunnett's multiple comparisons test. Unpaired t-test with *Bonferroni* correction for multiple comparison correction for C9-ALS/FTD vs. sALS.

(E) Quantification showing percentage of HB9 and MAP2 double positive neurons over DAPI+ cells in CTRL and C9-ALS/FTD iMNs. Immunostaining was performed on day 17 of conversion. Cells were quantified from 3 regions of a well from a CTRL line or a C9-ALS/FTD line.

(**F**) Quantification of *Hb9*::RFP+ iMNs formed after day 17 (D17) of conversion to the end of survival assay in CTRL, C9-ALS/FTD and sALS. The total number of iMNs present in a well on D17 was set to 100. n=3 wells/line from 1 CTRL, 1 C9-ALS/FTD and 1 sALS line. Statistical significance (One-way ANOVA) was calculated comparing CTRL vs. C9-ALS/FTD and CTRL

vs. sALS with Dunnett's multiple comparisons test. Unpaired t-test for C9-ALS/FTD vs. sALS with *Bonferroni* correction for multiple comparison correction.

(G) The hazard ratio (log-rank method) of iMNs from cohort 1: CTRL (N=3 donors), C9-ALS/FTD (N=3 donors), and sALS (N=8 donors) lines after withdrawal of neurotrophic factor supplementation. n=100 iMNs from 3 independent conversions/line. The hazard ratio = the hazard rate of each group relative to the hazard rate of the three CTRL lines in aggregate, which was set as 1 (red dotted line). Mean of biological replicates (independent iMN conversions) ± SEM The vertical *p*-values above each bar denote statistical significance (One-way ANOVA) comparing three CTRL lines in aggregate to each patient line individually after multiple comparison correction with Dunnett's multiple comparisons test.

(H) Immunostaining and quantification to determine endogenous poly(PR)+ punctae (green) in control (CTRL) or C9-ALS/FTD iMNs. Quantified values represent the average number of nuclear poly(PR)+ punctae per μm^2 in n=30 CTRL and n=30 C9-ALS/FTD iMNs from two CTRL and two C9-ALS/FTD lines (n=15 iMNs/line). iMNs were quantified from two independent iMN conversions per line. Each data point represents a single iMN. Mann-Whitney test. Median ± interquartile range. Solid and dotted lines outline the cell body and nucleus, respectively. Orange arrows mark several poly(PR)+ punctae. Scale bar = 5 μm.

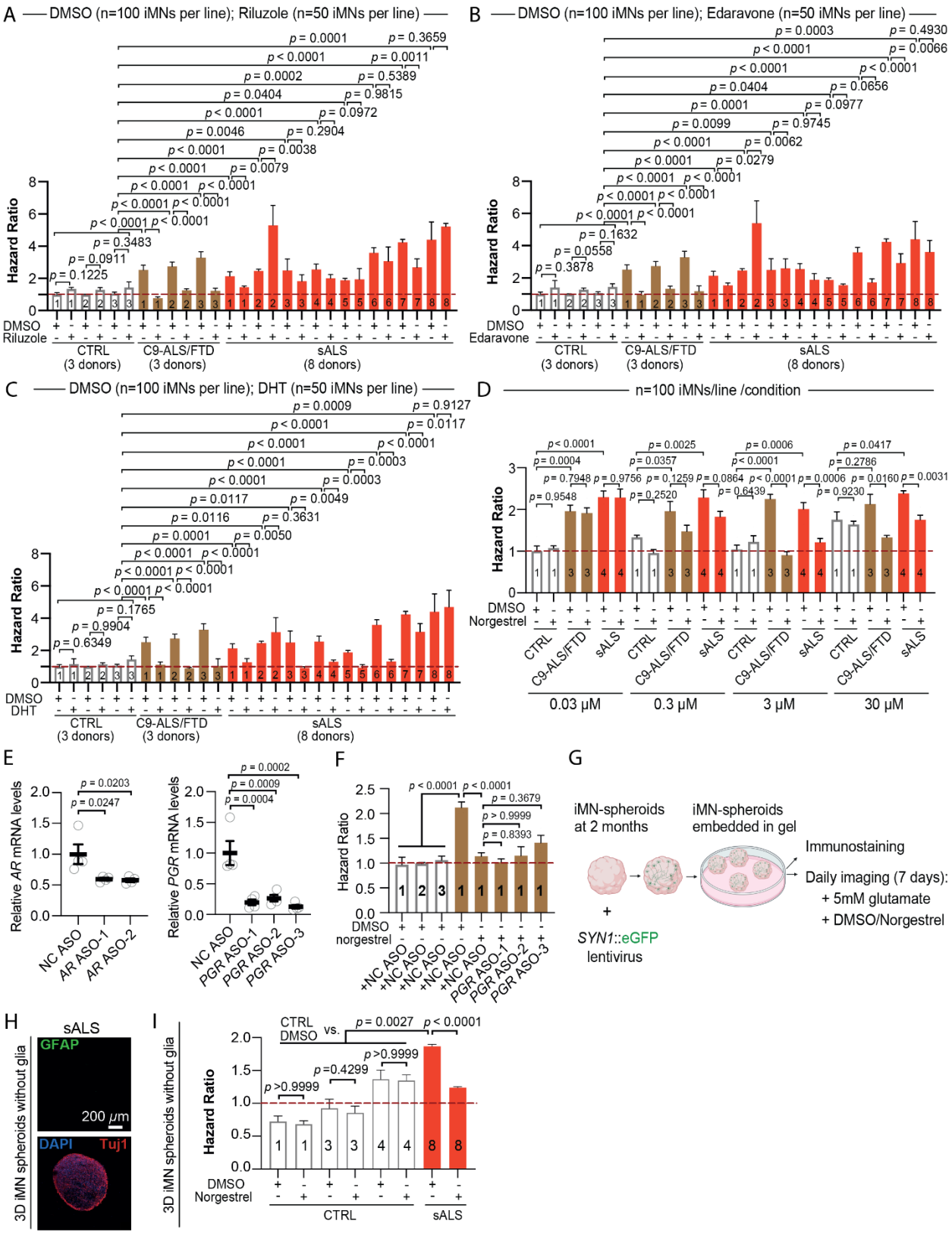

A
DMSO (n=100 iMNs per line); Riluzole (n=50 iMNs per line)
Hazard Ratio
DMSO
Riluzole
CTRL (3 donors)
C9-ALS/FTD (3 donors)
sALS (8 donors)
B
DMSO (n=100 iMNs per line); Edaravone (n=50 iMNs per line)
Hazard Ratio
DMSO
Edaravone
CTRL (3 donors)
C9-ALS/FTD (3 donors)
sALS (8 donors)
C
DMSO (n=100 iMNs per line); DHT (n=50 iMNs per line)
Hazard Ratio
DMSO
DHT
CTRL (3 donors)
C9-ALS/FTD (3 donors)
sALS (8 donors)
D
n=100 iMNs/line /condition
Hazard Ratio
DMSO
Norgestrel
CTRL
C9-ALS/FTD
sALS
0.03 µM
0.3 µM
3 µM
30 µM
E
Relative AR mRNA levels
NC ASO
AR ASO-1
AR ASO-2
Relative PGR mRNA levels
NC ASO
PGR ASO-1
PGR ASO-2
PGR ASO-3
F
Hazard Ratio
DMSO
norgestrel
+NC ASO
PGR ASO-1
PGR ASO-2
PGR ASO-3
G
iMN-spheroids at 2 months
iMN-spheroids embedded in gel
Immunostaining
Daily imaging (7 days):
+ 5mM glutamate
+ DMSO/Norgestrel
+
SYN1::eGFP lentivirus
H
sALS
3D iMN spheroids without glia
GFAP
200 µm
DAPI
Tuj1
I
3D iMN spheroids without glia
CTRL vs. DMSO
Hazard Ratio
DMSO
Norgestrel
CTRL
sALS

Figure S2, related to Figure 2. A phenotypic screen identifies androgens as broadly-effective rescuers of ALS iMN survival.

(A) The hazard ratio (log rank method) of iMNs from 3 control (CTRL), 3 *C9ORF72* ALS/FTD (C9-ALS/FTD), and 8 sporadic ALS (sALS) lines treated with DMSO or 3 μM riluzole after withdrawal of neurotrophic factor supplementation. n=100 iMNs from 3 independent conversions per line treated with DMSO and n=50 iMNs from 3 independent conversions per line treated with riluzole. The hazard ratio = the hazard rate of each group relative to the hazard rate of the three CTRL lines in aggregate treated with DMSO, which was set as 1 (red dotted line). Mean of biological replicates (independent iMN conversions) ± SEM. Statistical significance (One-way ANOVA) was calculated comparing the DMSO-treated condition for each ALS line to the riluzole-treated condition and the control lines + DMSO in aggregate. P-values were corrected for multiple comparisons using Dunnett's multiple comparisons test. Unpaired t-test for CTRL + DMSO vs. CTRL + riluzole with *Bonferroni* correction for multiple comparison correction.

(B) The hazard ratio (log rank method) of iMNs from 3 CTRL, 3 C9-ALS/FTD, and 8 sALS lines treated with DMSO or 3 μM edaravone after withdrawal of neurotrophic factor supplementation. n=100 iMNs from 3 independent conversions per line treated with DMSO and n=50 iMNS from 3 independent conversions per line treated with edaravone. The hazard ratio = the hazard rate of each group relative to the hazard rate of the three CTRL lines treated with DMSO in aggregate, which was set as 1 (red dotted line). Mean of biological replicates (independent iMN conversions) ± SEM. Statistical significance (One-way ANOVA) was calculated comparing the DMSO-treated condition for each ALS line to the edaravone-treated condition and the control lines + DMSO in aggregate. P-values were corrected for multiple comparisons using Dunnett's multiple

comparisons test. Unpaired t-test for CTRL + DMSO vs. CTRL + edaravone with *Bonferroni* correction for multiple comparison correction.

(C) The hazard ratio (log rank method) of iMNs from 3 CTRL, 3 C9-ALS/FTD, and 8 sALS lines treated with DMSO or 3 μM dihydrotestosterone (DHT) after withdrawal of neurotrophic factor supplementation. n=100 iMNs from 3 independent conversions per line treated with DMSO and n=50 iMNS from 3 independent conversions per line treated with DHT. The hazard ratio = the hazard rate of each group relative to the hazard rate of the three CTRL lines treated with DMSO in aggregate, which was set as 1 (red dotted line). Mean of biological replicates (independent iMN conversions) ± SEM. Statistical significance (One-way ANOVA) was calculated comparing the DMSO-treated condition for each ALS line to the DHT-treated condition and the control lines + DMSO in aggregate. P-values were corrected for multiple comparisons using Dunnett's multiple comparisons test. Unpaired t-test for CTRL+DMSO vs. CTRL+ DHT with *Bonferroni* correction for multiple comparison correction.

(D) The hazard ratio (log-rank method) of iMNs from 1 CTRL, 1 C9-ALS/FTD, and 1 sALS lines after withdrawal of neurotrophic factor supplementation and treatment with DMSO or norgestrel at 0.03 μM, 0.3 μM, 3 μM and 30 μM. n=100 iMNs from 3 independent conversions/line/condition. The hazard ratio = the hazard rate of each group relative to the hazard rate of CTRL line treated with DMSO at 0.03 μM, which was set as 1 (red dotted line). Mean of biological replicates (independent iMN conversions) ± SEM Statistical significance (One-way ANOVA) was calculated comparing the DMSO condition for each ALS line to the norgestrel condition and control line + DMSO for each dose. P-values were corrected for multiple comparisons using Dunnett's multiple comparisons test. Unpaired t-test for CTRL+ DMSO vs. CTRL+ norgestrel with *Bonferroni* correction for multiple comparison correction.

(E) mRNA levels of androgen receptor (*AR*) and progesterone receptor (*PGR*) (relative to *GAPDH*) in iMN cultures treated with 9μM negative control (NC) ASO, *AR* ASO-1, *AR* ASO-2, *PGR* ASO-1, *PGR* ASO-2, or *PGR* ASO-3 at Day 17 following ASO treatment on Day 14. Data are normalized to the NC ASO treated cultures. Each data point represents an independent culture from each treatment group. n=4 independent iMN conversions/line/condition. One-way ANOVA, mean ± SEM. P-values were corrected for multiple comparisons using Dunnett's multiple comparisons test.

(F) The hazard ratio (log-rank method) of iMNs from a C9-ALS/FTD line treated with 9 μM NC ASO, *PGR* ASO-1, *PGR* ASO-2, or *PGR* ASO-3 in the presence or absence of 3 μM norgestrel. n =85 iMNs from 3 independent conversions per treatment. The hazard ratio = the hazard rate of each group divided by the hazard rate of the CTRL lines treated with NC ASO and DMSO in aggregate, which was set as 1 (red dotted line). Statistical significance (One-way ANOVA) was calculated comparing the patient line treated with NC ASO and DMSO vs. 3 CTRL lines treated with NC ASO and DMSO in aggregate, and vs. the patient line treated with NC ASO and norgestrel. Another One-way ANOVA was performed to compare patient line + NC ASO vs. patient line + *PGR* ASO-1, *PGR* ASO-2, or *PGR* ASO-3, respectively within the norgestrel treated conditions. Mean (independent conversions) ± SEM. P-values were corrected for multiple comparisons using Dunnett's multiple comparisons test.

(G) Overview of the glia-free iMN spheroid survival assay used in (H) and (I).

(H) Immunostaining of Glial fibrillary acidic protein (GFAP) and beta-III Tubulin (Tuj1) in iMNs cultured as 3-dimensional (3D) spheroids used in (I). Scale bar = 200 μm.

(I) The hazard ratio (log-rank method) of iMNs cultured as 3-dimensional (3D) spheroids without mouse glia from 3 CTRL (CTRL-1, CTRL-3 & CTRL-5), and 1 sALS line (sALS-8) after

withdrawal of neurotrophic factor supplementation and treatment with DMSO or norgestrel at 3 μM. n=100 iMNs from 3 independent conversions/line/condition. The hazard ratio= the hazard rate of each group relative to the hazard rate of the 3 CTRL lines treated with DMSO in aggregate, which was set as 1 (red dotted line). Mean of biological replicates (independent iMN conversions) ± SEM Statistical significance (One-way ANOVA) was calculated comparing the DMSO condition for the sALS line to the norgestrel condition and control line + DMSO in aggregate. P-values were corrected for multiple comparisons using Dunnett's multiple comparisons test. Unpaired t-test for CTRL+ DMSO vs. CTRL+ norgestrel with *Bonferroni* correction for multiple comparison correction.

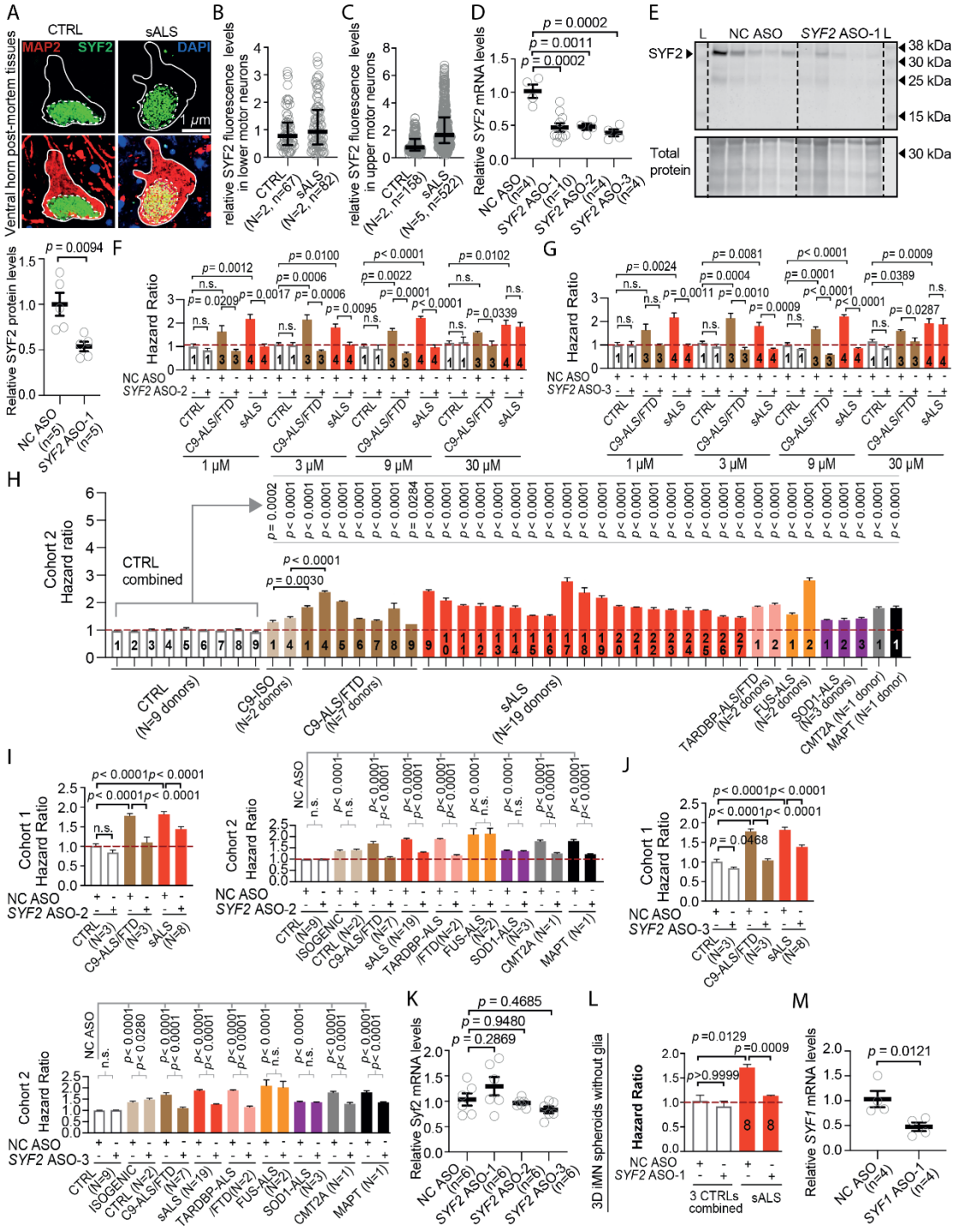


Figure S3, related to Figure 3. Suppression of spliceosome-associated genes mimics the broad-efficacy observed with androgens.

(A-C) Immunostaining (A) and quantification (B-C) to determine endogenous SYF2 levels in control (CTRL) or sporadic ALS (sALS) post-mortem tissues. Quantified values represent the total fluorescence levels of SYF2 per cell in MAP2+ neurons in the ventral horn of spinal cord from 2 CTRL and 2 sALS (B) or in CryM+ neurons in the motor cortex of 2 CTRL and 5 sALS (C). Each data point represents a single neuron. Median ± interquartile range. Solid and dotted lines outline the cell body and nucleus, respectively. Scale bar = 1 μm.

(D) mRNA levels of *SYF2* (relative to *GAPDH*) from iMN cultures treated with a negative control ASO (NC ASO), or *SYF2*-targeting ASOs (*SYF2* ASO-1, *SYF2* ASO-2, or *SYF2* ASO-3) at Day 17 following ASO treatment on Day 14 at 9 μM. Data are normalized to the NC ASO-treated cultures. Each data point represents an independent culture from each treatment group. n=10 independent iMN conversions from a CTRL line for the *SYF2* ASO-1 condition. n=4 independent conversions from a CTRL line for the rest of the conditions. One-way ANOVA. Mean ± SEM. P-values were corrected for multiple comparisons using Dunnett's multiple comparisons test.

(E) Immunoblot and quantifications of protein levels of SYF2 (relative total protein stain) from iMNs treated with 9 μM NC ASO or *SYF2* ASO-1 for 3 days. Each data point represents an individually-treated iMN culture. n=5 independent conversions per condition from a CTRL line. Unpaired t-test. Mean ± SEM.

(F-G) The hazard ratio (log-rank method) of iMNs from CTRL (N=1 donor), *C9ORF72* ALS/FTD (C9-ALS/FTD) (N=1 donor), and sALS (N=1 donor) lines after withdrawal of neurotrophic factor supplementation and treatment with NC ASO or *SYF2* ASO-2 (F)/*SYF2* ASO-3 (G) at 1 μM, 3 μM, 9 μM and 30 μM. n=100 iMNs from 3 independent iMN conversions/line/condition. The hazard ratio = the hazard rate of each group relative to the hazard rate of the CTRL line treated with NC ASO at 1 μM, which was set as 1 (red dotted line). Mean of biological replicates

(independent iMN conversions) ± SEM. Statistical significance (One-way ANOVA) was calculated comparing the NC ASO condition for each ALS line to the *SYF2* ASO-2 (F)/*SYF2* ASO-3 (G) condition and control line + NC ASO for each dose. P-values were corrected for multiple comparisons using Dunnett's multiple comparisons test. Unpaired t-test for CTRL+NC ASO vs. CTRL+*SYF2* ASO-2 (F)/*SYF2* ASO-3 (G) with *Bonferroni* correction for multiple comparison correction.

(H) The hazard ratio (log-rank method) of iMNs from cohort 2; CTRL (N=9 donors), *C9ORF72* isogenic control (C9-ISO) (N=2 donors), C9-ALS/FTD (N=7 donors), sALS (N=19 donors), TARDBP-ALS/FTD (N=2 donors), FUS-ALS (N=2 donors), SOD1 ALS (N=3 donors), CMT2A Charcot-Marie-Tooth disease (CMT2A, N=1 donor), and MAPT familial dementia (MAPT, N=1 donor) lines after withdrawal of neurotrophic factor supplementation. CTRL-1, 2, 3 and C9-ALS/FTD-1 were re-included from cohort 1 in cohort 2. n=100 iMNs from 4 independent conversions/line/condition. The hazard ratio = the hazard rate of each group relative to the hazard rate of the 9 CTRL lines in aggregate, which was set as 1 (red dotted line). Mean of biological replicates (independent iMN conversions) ± SEM The vertical *p*-values above each bar denote statistical significance (One-way ANOVA) comparing each patient line to the control lines in aggregate. P-values were corrected for multiple comparisons using Dunnett's multiple comparisons test. Unpaired t-test for C9-ISO-1 vs. C9-ALS/FTD-1 and C9-ISO-4 vs. C9-ALS/FTD-4 with *Bonferroni* correction for multiple comparison correction.

(I) The hazard ratio (log-rank method) of iMNs from cohort 1 (left panel); CTRL (N=3 donors), C9-ALS/FTD (N=3 donors), and sALS (N=8 donors) lines, and cohort 2 (right panel); CTRL (N=9 donors), C9-ISO (N=2 donors), C9-ALS/FTD (N=7 donors), sALS (N=19 donors), TARDBP-ALS/FTD (N=2 donors), FUS-ALS (N=2 donors), SOD1-ALS (N=3 donors), CMT2A (N=1

donor), and MAPT (N=1 donor) lines in aggregate treated with 9 µM NC ASO or *SYF2* ASO-2. n=100 iMNs from 4 independent conversions/condition/line. The hazard ratio = the hazard rate of each group relative to the hazard rate of the CTRL lines in aggregate treated with NC ASO, which was set as 1 (red dotted line). Mean of biological replicates (independent iMN conversions) ± SEM. Statistical significance (One-way ANOVA) was calculated comparing the NC ASO treated condition of each patient group to the *SYF2* ASO-2 treated condition and the CTRL lines + NC ASO in aggregate. P-values were corrected for multiple comparisons using Dunnett's multiple comparisons test. Unpaired *t*-test for CTRL+NC ASO vs. CTRL+ *SYF2* ASO-2 with *Bonferroni* correction for multiple comparison correction.

(J) The hazard ratio (log-rank method) of iMNs from cohort 1 (left panel); CTRL (N=3 donors), C9-ALS/FTD (N=3 donors), and sALS (N=8 donors) lines, and cohort 2 (right panel); CTRL (N=9 donors), C9-ISO (N=2 donors), C9-ALS/FTD (N=7 donors), sALS (N=19 donors), TARDBP-ALS/FTD (N=2 donors), FUS-ALS (N=2 donors), SOD1-ALS (N=3 donors), CMT2A (N=1 donor), and MAPT (N=1 donor) lines in aggregate treated with 9 µM NC ASO or *SYF2* ASO-3. n=100 iMNs from 4 independent conversions/condition/line. The hazard ratio = the hazard rate of each group relative to the hazard rate of the CTRL lines in aggregate treated with NC ASO, which was set as 1 (red dotted line). Mean of biological replicates (independent iMN conversions) ± SEM. Statistical significance (One-way ANOVA) was calculated comparing the NC ASO treated condition of each patient group to the *SYF2* ASO-3 treated condition and the CTRL lines + NC ASO in aggregate. P-values were corrected for multiple comparisons using Dunnett's multiple comparisons test. Unpaired *t*-test for CTRL+NC ASO vs. CTRL+ *SYF2* ASO-3 with *Bonferroni* correction for multiple comparison correction.

(K) mRNA levels of *Syf2* (relative to *Ppia*) from mouse glia treated with 9 µM NC ASO, *SYF2* ASO-1, *SYF2* ASO-2, or *SYF2* ASO-3 for 3 days. Data are normalized to the NC ASO-treated cultures. Each data point represents an independent culture from each treatment group. Mean ± SEM. n=6 independent mouse glia cultures/condition. One-way ANOVA. P-values were corrected for multiple comparisons using Dunnett's multiple comparisons test.

(L) The hazard ratio (log-rank method) of iMNs cultured as 3-dimensional (3D) spheroids without mouse glia from CTRL (N=3 donors), and sALS (N=1 donor) lines after withdrawal of neurotrophic factor supplementation and treatment with NC ASO or *SYF2* ASO-1 at 9 µM. n=100 iMNs from 3 independent conversions/line/condition. The hazard ratio= the hazard rate of each group relative to the hazard rate of the 3 CTRL lines treated with NC ASO in aggregate, which was set as 1 (red dotted line). Mean of biological replicates (independent iMN conversions) ± SEM Statistical significance (One-way ANOVA) was calculated comparing the NC ASO condition for the sALS line to the *SYF2* ASO condition and control line + NC ASO in aggregate. P-values were corrected for multiple comparisons using Dunnett's multiple comparisons test. Unpaired t-test for CTRL+ NC ASO vs. CTRL+ *SYF2* ASO-1.

(M) mRNA levels of *SYF1* (relative to *GAPDH*) from iMN cultures treated with 9 µM NC ASO or *SYF1*-targeting ASOs (*SYF1* ASO-1) at Day 17 following ASO treatment on Day 14. Data are normalized to the NC ASO treated cultures. Each data point represents an independent culture from each treatment group. n=4 independent iMN conversions from a CTRL line. Unpaired t-test. Mean ± SEM.

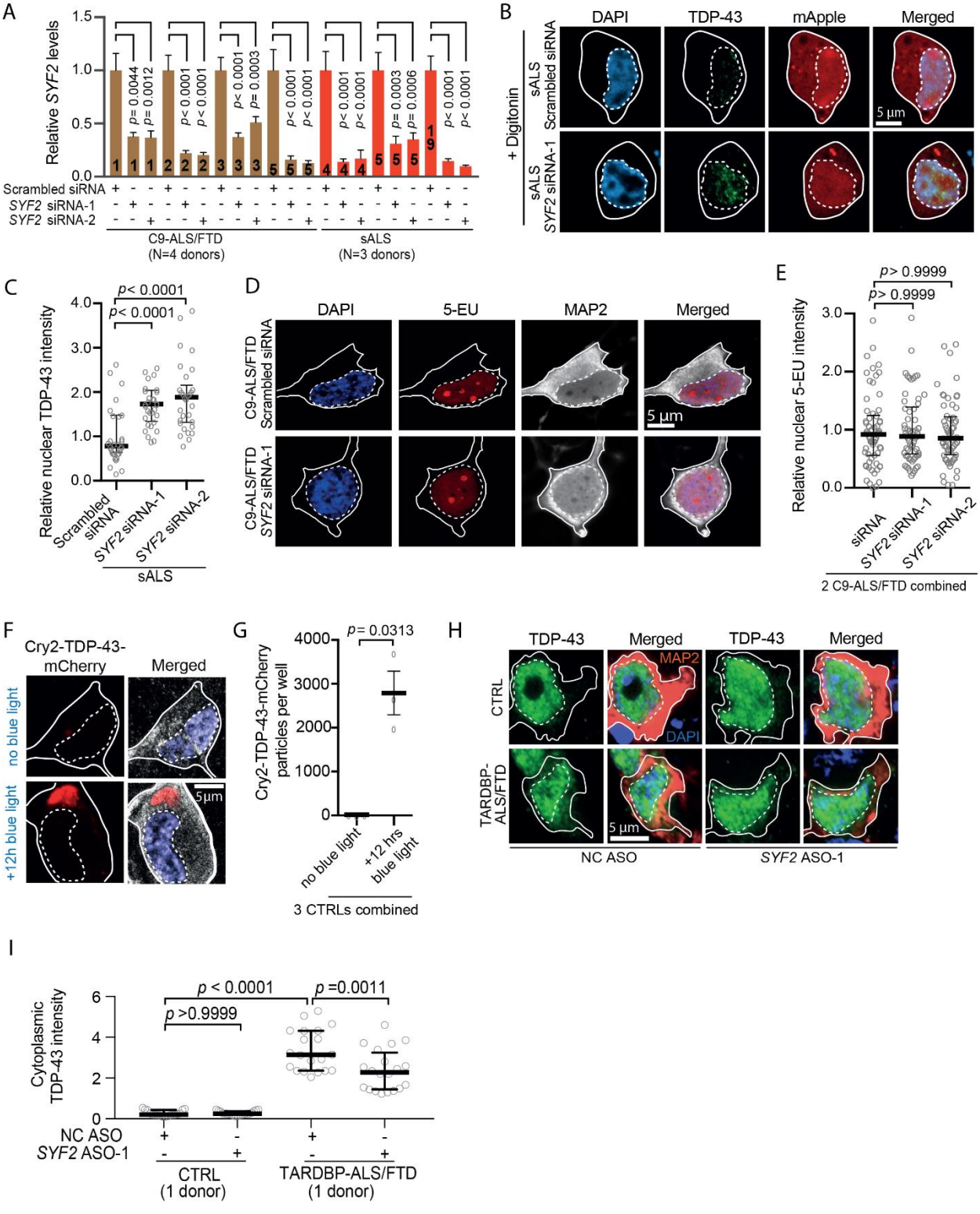

A
Relative SYF2 levels
1.5
1.0
0.5
0.0
p= 0.0044
p= 0.0012
p< 0.0001
p< 0.0001
p< 0.0001
p= 0.0003
p< 0.0001
p< 0.0001
p< 0.0001
p< 0.0001
p= 0.0003
p= 0.0006
p< 0.0001
p< 0.0001
1 1 1 2 2 2 3 3 3 5 5 5 4 4 4 5 5 5 19
Scrambled siRNA + - - + - - + - - + - - + - - + - - + - -
SYF2 siRNA-1 - + - - + - - + - - + - - + - - + - - + -
SYF2 siRNA-2 - - + - - + - - + - - + - - + - - + - - +
C9-ALS/FTD (N=4 donors)
sALS (N=3 donors)
B
DAPI
TDP-43
mApple
Merged
+ Digitonin
sALS Scrambled siRNA
sALS SYF2 siRNA-1
5 µm
C
Relative nuclear TDP-43 intensity
4.0
3.0
2.0
1.0
0.0
p< 0.0001
p< 0.0001
Scrambled siRNA
SYF2 siRNA-1
SYF2 siRNA-2
sALS
D
DAPI
5-EU
MAP2
Merged
C9-ALS/FTD Scrambled siRNA
C9-ALS/FTD SYF2 siRNA-1
5 µm
E
Relative nuclear 5-EU intensity
3.0
2.0
1.0
0.0
p> 0.9999
p> 0.9999
siRNA
SYF2 siRNA-1
SYF2 siRNA-2
2 C9-ALS/FTD combined
F
Cry2-TDP-43-mCherry
Merged
no blue light
+12h blue light
5µm
G
Cry2-TDP-43-mCherry particles per well
4000
3000
2000
1000
0
p= 0.0313
no blue light
+12 hrs blue light
3 CTRLs combined
H
TDP-43
Merged
TDP-43
Merged
CTRL
TARDBP-ALS/FTD
MAP2
DAPI
5 µm
NC ASO
SYF2 ASO-1
I
Cytoplasmic TDP-43 intensity
6
4
2
0
p < 0.0001
p =0.0011
p >0.9999
NC ASO + - + -
SYF2 ASO-1 - + - +
CTRL (1 donor)
TARDBP-ALS/FTD (1 donor)

Figure S4, related to Figure 4. *SYF2* suppression ameliorates TDP-43 pathology and dysfunction in ALS iMNs.

(A) mRNA levels of *SYF2* (relative to *MAP2* and *18S*) from *C9ORF72* ALS/FTD (C9-ALS/FTD, N=4 donors) and sporadic (sALS, N=3 donors) iMN cultures from treated with a scrambled siRNA or siRNAs targeting *SYF2* (*SYF2* siRNA-1, *SYF2* siRNA-2) at 3 nM. Data are normalized to the scrambled siRNA-treated condition for each line. Each data point represents an independent culture from each treatment group. n=10 independent iMN cultures/line/condition. One-way ANOVA. P-values were corrected for multiple comparisons using Dunnett's multiple comparisons test. Mean ± SEM.

(B, C) Representative images (B) and quantification (C) of immunostaining of total endogenous TDP-43 in sALS iMNs remaining one hour after 30 μg/ml digitonin treatment to selectively permeabilize the plasma membrane. iMNs were pretreated with a scrambled siRNA or two different *SYF2* siRNAs at 3nM. Quantified values represent the average of nuclear TDP-43 in n=30 sALS iMNs per condition from one sALS line (sALS-1). iMNs were quantified from two independent iMN conversions per group. For each group, the average nuclear intensity of TDP-43 at one hour was normalized to the average nuclear intensity of the same group at time zero in order to control for any possible differences in nuclear TDP-43 levels before digitonin treatment. The mApple reporter gene was constitutively expressed from the Dox-inducible *NGN2-ISL1-LHX3* cassette knocked into the *CLYBL* safe-harbor locus for motor neuron generation. Mann-Whitney test. P-values were corrected for multiple comparisons using Dunnett's multiple comparisons test. Median ± interquartile range. Scale bar = 5 μm.

(D, E) Representative images (D) and quantification (E) of nuclear 5-EU in C9-ALS/FTD (n= 2 donors) treated with a scrambled siRNA or one of the two different *SYF2* siRNAs at 3 nM.

Quantified values represent the average nuclear 5-EU intensity in C9-ALS/FTD lines. iMNs were quantified from two independent iMN conversions per group. The solid and dotted lines outline the cell body and nucleus, respectively. Kruskal-Wallis test. P-values were corrected for multiple comparisons using Dunnett's multiple comparisons test. Median ± interquartile range. Scale bar = 5 μm.

(F, G) Representative images (F) and quantification (G) of the size of cytoplasmic Cry2-TDP-43-mCherry punctae formed in CTRL iMNs (N= 3 donors) transduced with a lentivirus encoding this construct, and exposed to blue light for 12 hours. Three wells/line/condition were quantified. Unpaired t-test. Mean ± SEM. Solid and dotted lines outline the cell body and nucleus, respectively. Scale bar = 5 μm.

(H, I) Representative images (H) and quantification (I) of immunostaining of total endogenous TDP-43 in iMNs from CTRL (N=1 donor) and TARDBP-ALS/FTD (N=1 donor) lines treated with NC ASO or *SYF2* ASO-1 at 9 μM. Quantified values represent the average ratio of cytoplasmic TDP-43 intensity in 20 iMNs for CTRL and 20 iMNs for TARDBP-ALS/FTD per condition. iMNs were quantified from two independent iMN conversions/line/condition. Statistical significance (One-way ANOVA) was calculated comparing the NC ASO condition for TARDBP-ALS/FTD to the *SYF2* ASO-1 condition and to CTRL + NC ASO. P-values were corrected for multiple comparisons using Dunnett's multiple comparisons test. Unpaired t-test for CTRL+NC ASO vs. CTRL+*SYF2* ASO-1. Mean ± SEM. Solid and dotted lines outline the cell body and nucleus, respectively. Scale bar = 5 μm.

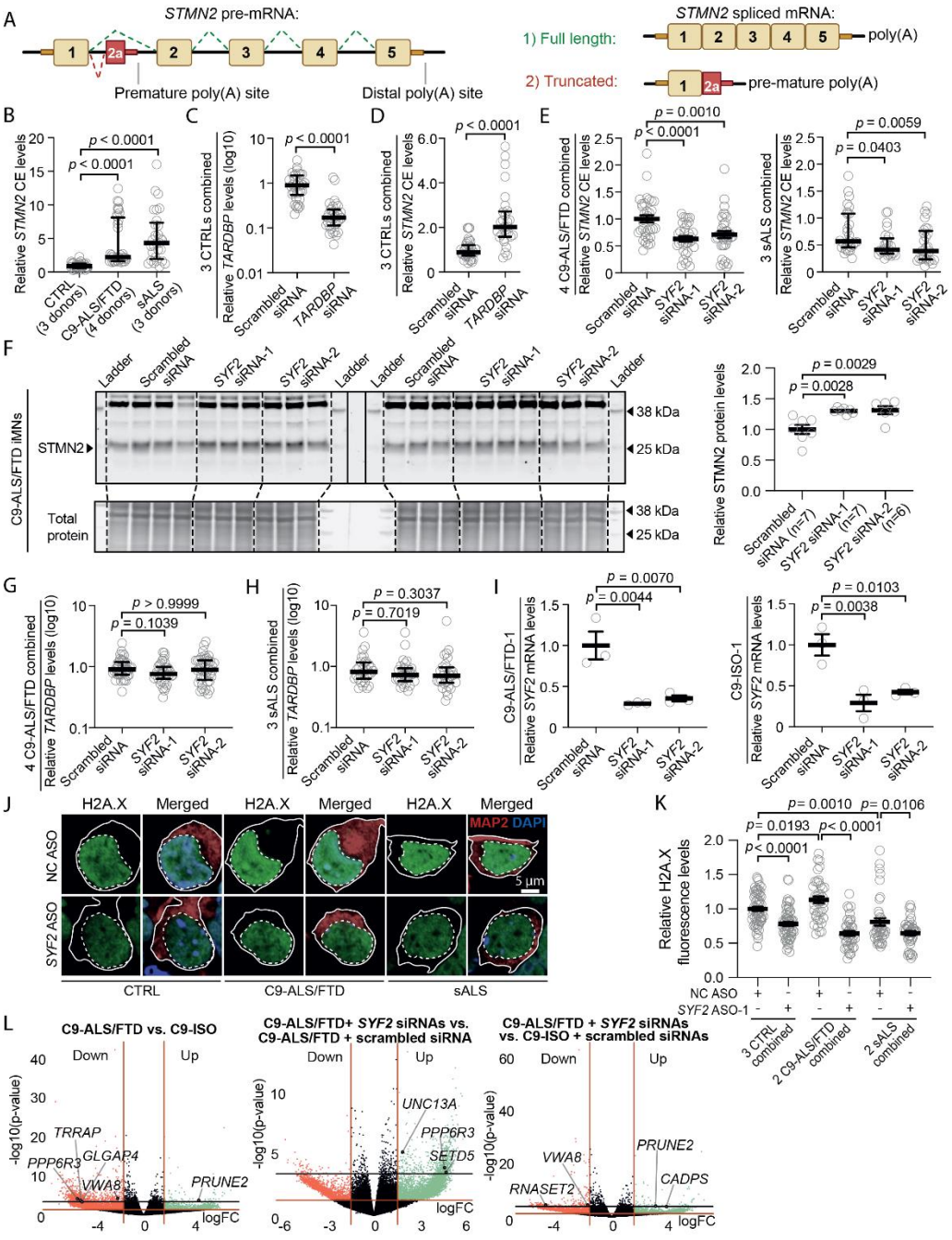


Figure S5, related to Figure 5. *SYF2* suppression increases STMN2 function in ALS iMNs.

(A) The splicing of *STMN2* pre-mRNA into full length mRNA or truncated mRNA. Green dashed lines denote splicing events that result in full length mRNA and red dashed lines denote splicing events that result in truncated mRNA.

(B) mRNA levels of *STMN2* cryptic exon (CE) (relative to non-cryptic exon-containing *STMN2*) from 3 CTRL (CTRL-1, CTRL-3 & CTRL-7), 4 C9-ALS/FTD (C9-ALS/FTD-1 to C9-ALS/FTD-3 & C9-ALS/FTD-5), and 3 sALS (sALS-4, sALS-5 & sALS-19) iMN cultures in aggregate. Data are normalized to the CTRL cultures. Each data point represents an independent iMN culture. n=10 independent conversion per line per condition. Mann-Whitney test. Median ± interquartile range.

(C) mRNA levels of *TARDBP* (relative to *18S*) from 3 CTRL lines (CTRL-1, CTRL-3 & CTRL-7) treated with a scrambled siRNA or siRNA targeting *TARDBP* at 3 nM in aggregate. Data are normalized to the scrambled siRNA-treated cultures. Each data point represents an independent iMN culture from each treatment group. n=12 independent conversion per line per condition. Mann-Whitney-test. Median ± interquartile range.

(D) mRNA levels of *STMN2* cryptic exon (*STMN2* CE, relative to non-cryptic exon-containing *STMN2*) from 3 CTRL lines (CTRL-1, CTRL-3 & CTRL-7) treated with a scrambled siRNA or siRNA targeting *TARDBP* at 3 nM in aggregate. Data are normalized to the scrambled siRNA-treated cultures. Each data point represents an independent iMN culture from each treatment group. n=12 independent conversion per line per condition. Mann-Whitney-test. Median ± interquartile range.

(E) mRNA levels of *STMN2 CE* (relative to non-cryptic exon-containing *STMN2*) from 4 C9-ALS/FTD patient lines (C9-ALS/FTD-1 to C9-ALS/FTD-3 & C9-ALS/FTD-5) and 3 sALS lines (sALS-4, sALS-5 & sALS-19) treated with a scrambled siRNA or one of the two *SYF2* siRNAs at 3 nM in aggregate. Data are normalized to the scrambled siRNA-treated cultures. Each data point

represents an independent iMN culture from each treatment group. n=10 independent conversion per line per condition. One-way ANOVA., mean ± SEM for C9-ALS/FTD and Kruskal-Wallis test, median ± interquartile range for sALS.

(F) Immunoblot and quantifications of protein levels of STMN2 (relative to total protein stain) from C9-ALS/FTD iMNs treated with 3 nM scrambled siRNA or one of the two *SYF2* siRNAs. Each data point represents an individually-treated iMN culture. n=7 independent conversions for scrambled siRNA and *SYF2* siRNA-1 condition, n=6 for *SYF2* siRNA-2 condition. One-way ANOVA. P-values were corrected for multiple comparisons using Dunnett's multiple comparisons test. Mean ± SEM

(G) mRNA levels of *TARDBP* (relative to *18s*) from 4 C9-ALS/FTD patient lines treated with a scrambled siRNA or one of the two *SYF2* siRNAs at 3 nM in aggregate. Data are normalized to the scrambled siRNA-treated cultures. Each data point represents an independent iMN culture from each treatment group. n=10 independent conversion per line per condition. Kruskal-Wallis test. Median ± interquartile range.

(H) mRNA levels of *TARDBP* (relative to *18s*) from 3 sALS patient lines treated with a scrambled siRNA or siRNAs targeting *SYF2* at 3 nM in aggregate. Data are normalized to the scrambled siRNA-treated cultures. Each data point represents an independent iMN culture from each treatment group. n=10 independent conversion per line per condition. Kruskal-Wallis test. Median ± interquartile range.

(I) mRNA levels of *SYF2* (relative to *MAP2* and *18s*) in C9-ALS/FTD-1 and C9-ISO-1 glia-free iMNs treated with a scrambled siRNA or one of the two *SYF2* siRNAs. Data are normalized to the scrambled siRNA condition for each line. n=3 independent conversions/line/condition. One-way ANOVA. Mean ± SEM

(J-K) Representative images (J) and quantification (K) of immunostaining of nuclear H2A.X fluorescence levels in iMNs from 3 CTRL (CTRL-1, CTRL-2 & CTRL-3), 2 C9-ALS/FTD (C9 ALS-FTD-1 & C9-ALS/FTD-2), and 2 sALS (sALS-4 & sALS-5) lines treated with NC ASO or a *SYF2*-targeting ASO (*SYF2* ASO-1) at 9 μM. Quantified values represent the average fluorescence levels of H2A.X in 60 iMNs for CTRL (N=3 donors), 40 iMNs for C9-ALS/FTD (N=2 donors) and 40 iMNs for sALS (N=2 donors) per condition in aggregate (20 iMNs/line). The NC ASO condition in CTRL iMNs was set as 1. iMNs were quantified from two independent iMN conversions per group. Statistical significance (One-way ANOVA) was calculated by comparing ALS (C9-ALS/FTD or sALS) + NC ASO to the *SYF2* ASO-1 condition and to CTRL + NC ASO. P-values were corrected for multiple comparisons using Dunnett's multiple comparisons test. Mean ± SEM. Unpaired t-test for CTRL+ NC ASO vs. CTRL+ *SYF2* ASO-1. Solid and dotted lines outline the cell body and nucleus, respectively. Scale bar = 5 μm.

(L) Volcano plots from alternative splicing analysis using ASpli. Aligned reads from the bulk RNAseq were assessed based on the bin-coverage signals. Bins with a log fold change of log2(3) and a corresponding p-≤0.05 were considered statistically significant. Higher coverage indicates lower cryptic events. From left to right: comparison between C9-ALS/FTD and C9-ISO iMNs (red means down-regulated in C9-ALS/FTD, green means up-regulated in C9-ALS/FTD), comparison between C9-ALS/FTD iMNs treated with *SYF2* siRNAs and C9-ALS/FTD iMNs treated with scrambled siRNA (red means down-regulated in *SYF2* siRNA treated condition, green means up-regulated in *SYF2* siRNA treated condition), comparison between C9-ALS/FTD iMNs treated with *SYF2* siRNAs and C9-ISO iMNs treated with scrambled siRNA (red means down-regulated in *SYF2* siRNA treated C9-ALS/FTD iMNs, green means up-regulated in *SYF2* siRNA treated C9-

ALS/FTD iMNs). Genes whose splicing patterns that were previously described as associated with ALS were marked with dots with pink fill/black outline[44–46]. Black line marks p=0.05.

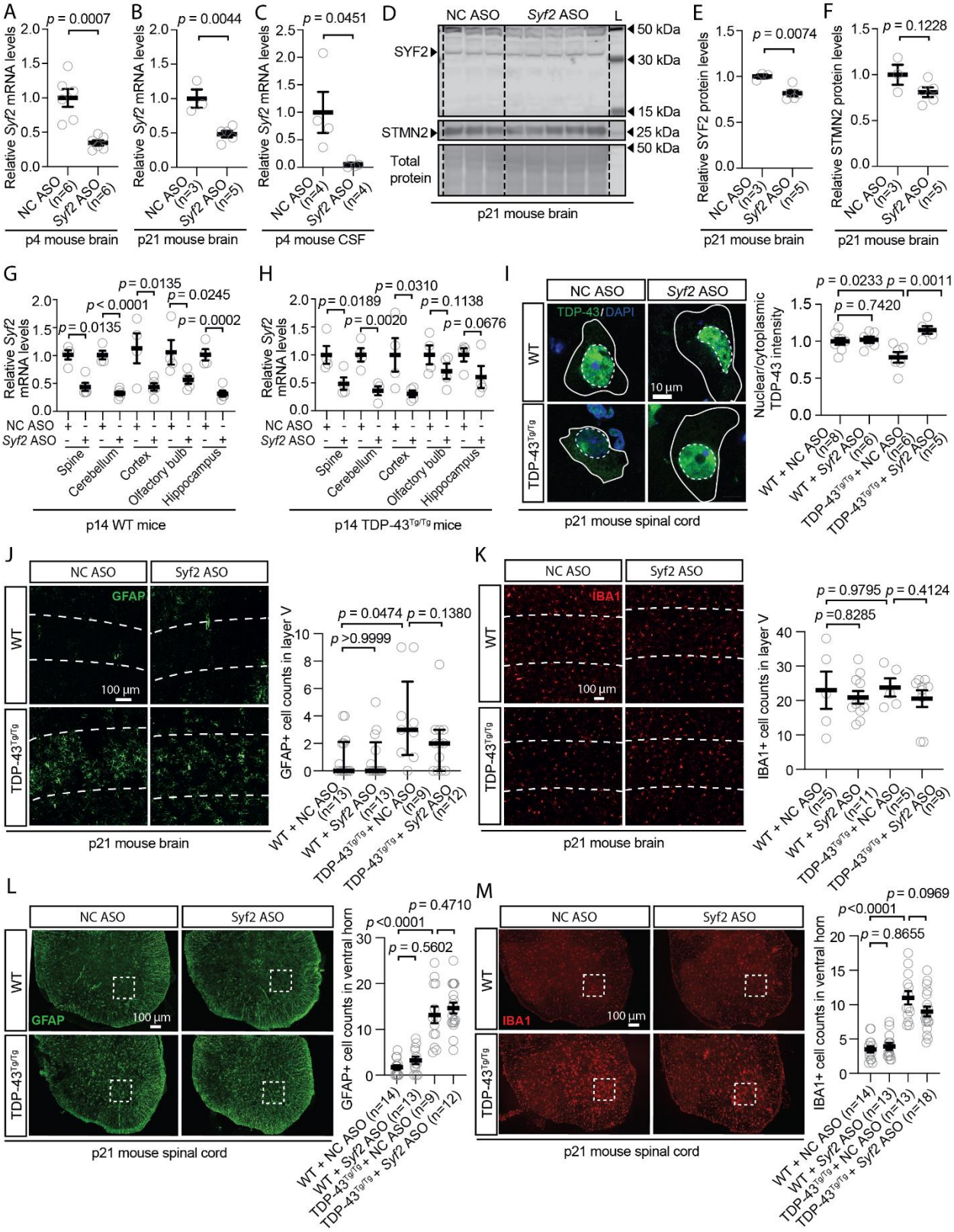

A
p = 0.0007
Relative Syf2 mRNA levels
NC ASO (n=6)
Syf2 ASO (n=6)
p4 mouse brain
B
p = 0.0044
Relative Syf2 mRNA levels
NC ASO (n=3)
Syf2 ASO (n=5)
p21 mouse brain
C
p = 0.0451
Relative Syf2 mRNA levels
NC ASO (n=4)
Syf2 ASO (n=4)
p4 mouse CSF
D
NC ASO
Syf2 ASO
L
SYF2
STMN2
Total protein
50 kDa
30 kDa
15 kDa
25 kDa
50 kDa
p21 mouse brain
E
p = 0.0074
Relative SYF2 protein levels
NC ASO (n=3)
Syf2 ASO (n=5)
p21 mouse brain
F
p = 0.1228
Relative STMN2 protein levels
NC ASO (n=3)
Syf2 ASO (n=5)
p21 mouse brain
G
p = 0.0135
p < 0.0001
p = 0.0135
p = 0.0245
p = 0.0002
Relative Syf2 mRNA levels
NC ASO
Syf2 ASO
Spine
Cerebellum
Cortex
Olfactory bulb
Hippocampus
p14 WT mice
H
p = 0.0310
p = 0.0189
p = 0.0020
p = 0.1138
p = 0.0676
Relative Syf2 mRNA levels
NC ASO
Syf2 ASO
Spine
Cerebellum
Cortex
Olfactory bulb
Hippocampus
p14 TDP-43Tg/Tg mice
I
NC ASO
Syf2 ASO
TDP-43/DAPI
WT
TDP-43Tg/Tg
10 µm
p21 mouse spinal cord
p = 0.0233
p = 0.0011
p = 0.7420
Nuclear/cytoplasmic TDP-43 intensity
WT + NC ASO (n=8)
WT + Syf2 ASO (n=6)
TDP-43Tg/Tg + NC ASO (n=6)
TDP-43Tg/Tg + Syf2 ASO (n=5)
J
NC ASO
Syf2 ASO
GFAP
WT
TDP-43Tg/Tg
100 µm
p21 mouse brain
p = 0.0474
p = 0.1380
p >0.9999
GFAP+ cell counts in layer V
WT + NC ASO (n=13)
WT + Syf2 ASO (n=13)
TDP-43Tg/Tg + NC ASO (n=9)
TDP-43Tg/Tg + Syf2 ASO (n=12)
K
NC ASO
Syf2 ASO
IBA1
WT
TDP-43Tg/Tg
100 µm
p21 mouse brain
p = 0.9795
p = 0.4124
p =0.8285
IBA1+ cell counts in layer V
WT + NC ASO (n=5)
WT + Syf2 ASO (n=11)
TDP-43Tg/Tg + NC ASO (n=5)
TDP-43Tg/Tg + Syf2 ASO (n=9)
L
NC ASO
Syf2 ASO
WT
GFAP
100 µm
TDP-43Tg/Tg
p21 mouse spinal cord
p = 0.4710
p <0.0001
p = 0.5602
GFAP+ cell counts in ventral horn
WT + NC ASO (n=14)
WT + Syf2 ASO (n=13)
TDP-43Tg/Tg + NC ASO (n=9)
TDP-43Tg/Tg + Syf2 ASO (n=12)
M
NC ASO
Syf2 ASO
WT
IBA1
100 µm
TDP-43Tg/Tg
p21 mouse spinal cord
p = 0.0969
p<0.0001
p = 0.8655
IBA1+ cell counts in ventral horn
WT + NC ASO (n=14)
WT + Syf2 ASO (n=13)
TDP-43Tg/Tg + NC ASO (n=13)
TDP-43Tg/Tg + Syf2 ASO (n=18)

Figure S6, related to Figure 6. *Syf2* suppression ameliorates TDP-43 pathology, neurodegeneration, and motor dysfunction in TDP-43 mice.

(A) mRNA levels of *Syf2* (relative to *Ppia*) in whole brain lysate of wild-type mice treated with 50 µg negative control (NC) ASO (n=6 mice) or ASO targeting *Syf2* (*Syf2* ASO) (n=6 mice) at postnatal day 4 (p4) after ASO injection at p1. Each data point represents one mouse. Unpaired t-test. Mean ± SEM.

(B) mRNA levels of *Syf2* (relative to *Ppia*) in whole brain lysate of wild-type mice treated with 50 µg NC ASO (n=3 mice) or *Syf2* ASO (n=5 mice) at postnatal day 21 (p21) after ASO injection at p1. Each data point represents one mouse. Unpaired t-test. Mean ± SEM.

(C) mRNA levels of *Syf2* (relative to *Ppia*) from the cerebrospinal fluid (CSF) of wild-type mice treated with 50 µg NC ASO (n=4 mice) or *Syf2* ASO (n=4 mice) at postnatal day 4 (p4) after ASO injection at p1. Each data point represents one mouse. Unpaired t-test. Mean ± SEM.

(D-F) Immunoblot of SYF2 protein levels (D, E) and STMN2 protein levels (D, F) in whole brain lysate of wild-type mice treated with 50 µg NC ASO (n=3 mice) or *Syf2* ASO (n=5 mice) at p21 after ASO injection at p1. The values are calculated as the relative intensity of SYF2 or STMN2 normalized to total protein stain. Statistical significance was calculated comparing NC ASO-treated vs. *Syf2* ASO-treated mice. Each data point represents one mouse. Unpaired t-test. Mean ± SEM.

(G) mRNA levels of *Syf2* (relative to *Ppia*) in lysates prepared from spine, cerebellum, cortex, olfactory bulb and hippocampus of wild-type (WT) mice treated with 50 µg negative control (NC) ASO (n=4 mice) or *Syf2* ASO (n=5 mice) at postnatal day 14 (p14) after ASO injection at p1. Each data point represents one mouse. Unpaired t-test. Mean ± SEM.

(H) mRNA levels of *Syf2* (relative to *Ppia*) in lysates prepared from spine, cerebellum, cortex, olfactory bulb and hippocampus of TDP-43$^{Tg/Tg}$ mice treated with 50 μg negative control (NC) ASO (n=4 mice) or *Syf2* ASO (n=4 mice) at postnatal day 14 (p14) after ASO injection at p1. Each data point represents one mouse. Unpaired t-test. Mean ± SEM.

(I) Representative images and quantification of the nuclear:cytoplasmic ratio of total TDP-43 in motor neurons in the ventral horn of the lumbar spinal cord of p21 wild-type (WT) mice treated with a negative control (NC) ASO (n=8 mice), WT mice treated with a *Syf2* ASO (n=6 mice), TDP-43$^{Tg/Tg}$ mice treated with a NC ASO (n=6 mice), TDP-43$^{Tg/Tg}$ mice treated with a *Syf2* ASO (n=5 mice) at p1. Each gray circle represents the average nuclear:cytoplasmic ratio of total TDP-43 across three sections in one mouse. Statistical analysis (One-way ANOVA) was calculated by comparing the NC ASO treated TDP-43$^{Tg/Tg}$ mice to the *Syf2* ASO conditions, and to WT mice + NC ASO. P-values were corrected for multiple comparisons using Dunnett's multiple comparisons test. Unpaired t-test for WT mice + NC ASO vs. WT mice + *Syf2* ASO. Mean ± SEM Scale bar = 10 μm. White solid lines and dashed lines mark the cell body and the nucleus, respectively.

(J) Representative images and quantification of the numbers of GFAP+ cells in layer V of the motor/somatosensory cortex of p21 WT mice injected with a NC ASO (n=13 mice), WT mice injected with a *Syf2* ASO (n=13 mice), TDP-43$^{Tg/Tg}$ mice injected with a NC ASO (n=9 mice), TDP-43$^{Tg/Tg}$ mice injected with a *Syf2* ASO (n=12 mice) at p1. Layer V (white dotted lines) was identified with CryM staining. Exposure time was reduced to allow visualization of individual GFAP+ cells in TDP-43$^{Tg/Tg}$ mice. Each gray circle represents the average number of GFAP+ cell counts in a 200 x 200 μm square/ section for 4 sections in one mouse. Statistical significance (Kruskal-Wallis test) was calculated comparing the NC ASO treated TDP-43$^{Tg/Tg}$ mice to the *Syf2* ASO condition, and to WT mice + NC ASO. P-values were corrected for multiple comparisons

using Dunnett's multiple comparisons test. Mann-Whitney test for WT mice + NC ASO vs. WT mice + *Syf2* ASO. Median ± interquartile range. Scale bar = 100 μm.

(K) Representative images and quantification of the numbers of IBA1+ cells in layer V of the motor/somatosensory cortex of p21 WT mice injected with a NC ASO (n=5 mice), WT mice injected with a *Syf2* ASO (n=11 mice), TDP-43$^{Tg/Tg}$ mice injected with a NC ASO (n=5 mice), TDP-43$^{Tg/Tg}$ mice injected with a *Syf2* ASO (n=9 mice) at p1. Layer V was identified with CryM staining. Each gray circle represents the average number of Iba1+ cell counts in a 500 x 500 μm square/ section for 1 section in one mouse. Statistical significance (One-way ANOVA) was calculated comparing the NC ASO treated TDP-43$^{Tg/Tg}$ mice to the *Syf2* ASO condition, and to WT mice + NC ASO. P-values were corrected for multiple comparisons using Dunnett's multiple comparisons test. Unpaired t-test for WT mice + NC ASO vs. WT mice + *Syf2* ASO. Mean ± SEM. Scale bar = 100 μm.

(L) Representative images and quantification of the numbers of GFAP+ cells in the ventral horns of lumbar spinal cords of p21 WT mice injected with a NC ASO (n=14 mice), WT mice injected with a *Syf2* ASO (n=13 mice), TDP-43$^{Tg/Tg}$ mice injected with a NC ASO (n=9 mice), TDP-43$^{Tg/Tg}$ mice injected with a *Syf2* ASO (n=12 mice) at p1. Each gray circle represents the average number of GFAP+ cell counts in one 200 x 200 μm square (white dotted lines) drawn on the left and right ventral horns. 1 section was analyzed for one mouse. Statistical significance (One-way ANOVA) was calculated comparing the NC ASO treated TDP-43$^{Tg/Tg}$ mice to the *Syf2* ASO condition, and to WT mice + NC ASO. P-values were corrected for multiple comparisons using Dunnett's multiple comparisons test. Unpaired t-test for WT mice + NC ASO vs. WT mice + *Syf2* ASO. Mean ± SEM. Scale bar = 100 μm.

(M) Representative images and quantification of the numbers of IBA1+ cells in the ventral horns of lumbar spinal cords of p21 WT mice injected with a NC ASO (n=14 mice), WT mice injected with a *Syf2* ASO (n=13 mice), TDP-43$^{Tg/Tg}$ mice injected with a NC ASO (n=13 mice), TDP-43$^{Tg/Tg}$ mice injected with a *Syf2* ASO (n=18 mice) at p1. Each gray circle represents the average number of Iba1+ cell counts in one 200 x 200 μm square (white dotted lines) drawn on the left and right ventral horns. 1 section was analyzed per mouse. Statistical significance (One-way ANOVA) was calculated comparing the NC ASO treated TDP-43$^{Tg/Tg}$ mice to the *Syf2* ASO condition, and to WT mice + NC ASO. P-values were corrected for multiple comparisons using Dunnett's multiple comparisons test. Unpaired t-test for WT mice + NC ASO vs. WT mice + *Syf2* ASO. Mean ± SEM. Scale bar = 100 μm.

ALS2
ANG
C9ORF72
CHCHD10
CHMP2B
DCTN1
ERBB4
FIG4
FUS
HNRNPA1
MATR3
NEFH
OPTN
PFN1
PRPH
SETX
SIGMAR1
SMN1
SOD1
SPG11
SQSTM1
TARDBP
TBK1
TRPM7
TUBA4A
UBQLN2
VAPB
VCP

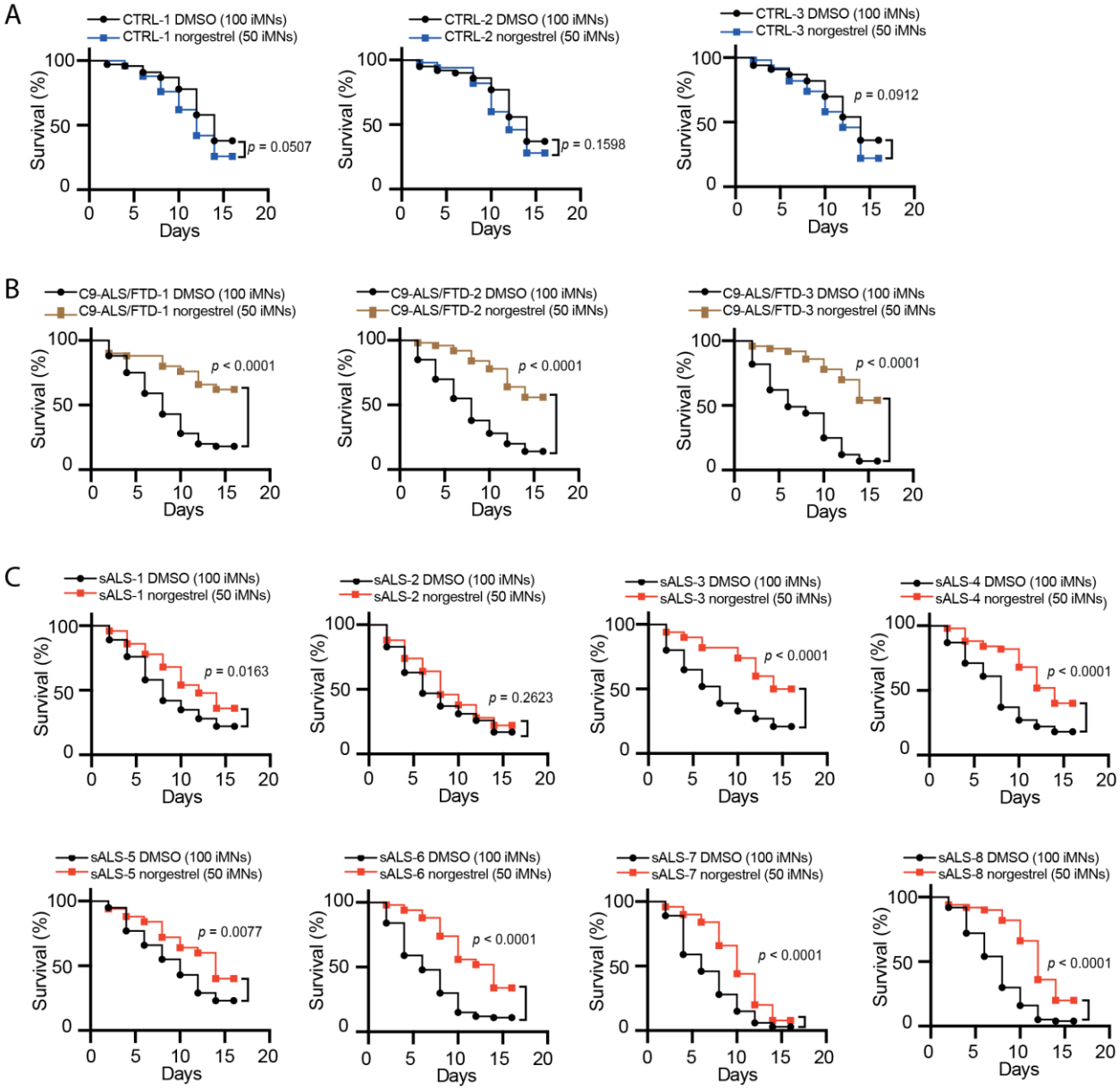
Table S2. Related to Figure 1. All ALS-related genes screened for rare variants in the sporadic ALS lines used in the study.

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Oligonucleotides		
qPCR primer for *SYF2* (ASO experiment), Forward	This paper	GGCTATAGCTGCATCCGAGG
qPCR primer for *SYF2* (ASO experiment), Reverse	This paper	ACGAGCTTCATTCCGCATCA
qPCR primer for human *SYF2* (siRNA experiment), Forward	This paper	GGCTATAGCTGCATCCGAGG
qPCR primer for human *SYF2* (siRNA experiment), Reverse	This paper	ACGAGCTTCATTCCGCATCA
qPCR primer for mouse *Syf2*, Forward	This paper	TGTAATGGCGGCTGTGACTG
qPCR primer for mouse *Syf2*, Reverse	This paper	CGAGCTTCATTCCGCTTCAGA
qPCR primer for human *18s*, Forward	This paper	GTAACCCGTTGAACCCCATT
qPCR primer for human *18s*, Reverse	This paper	CCATCCAATCGGTAGTAGCG
qPCR primer for human *STMN2*, Forward	Melamed et al.[61]	AGCTGTCCATGCTGTCACTG
qPCR primer for human *STMN2*, Reverse	Melamed et al.[61]	GGTGGCTTCAAGATCAGCTC
qPCR primer for human *STMN2 cryptic exon*, Forward	Melamed et al.[61]	GGACTCGGCAGAAGACCTTC
qPCR primer for human *STMN2 cryptic exon*, Reverse	Melamed et al.[61]	GCAGGCTGTCTGTCTCTCTC
qPCR primer for mouse *Stmn2*, Forward	Guerra San Juan et al.[62]	AGAAGCTGATCCTGAAGATGG
qPCR primer for mouse *Stmn2*, Reverse	Guerra San Juan et al.[62]	TTCGCAGGAACAAGGAACT
qPCR primer for human *TARDBP*, Forward	Melamed et al.[61]	TCATCCCCAAGCCATTCAGG
qPCR primer for human *TARDBP*, Reverse	Melamed et al.[61]	TGCTTAGGTTCGGCATTGGA
qPCR primer for human *MAP2*, Forward	This paper	GGAACCAACTCTCTCTGGATTT
qPCR primer for human *MAP2*, Reverse	This paper	GCATTCTCTCTTCAGCCTTCT
qPCR primer for *PGR*, Forward	This paper	CAGGTCTACCCGCCCTATCT
qPCR primer for *PGR*, Reverse	This paper	TAGTTGTGCTGCCCTTCCAT
qPCR primer for *AR*, Forward	This paper	TTACGGGGACATGCGTTTGG
qPCR primer for *AR*, Reverse	This paper	AGTTTCTTCAGCTTCCGGGC
qPCR primer for *GAPDH*, Forward	This paper	CGAGATCCCTCCAAAATCAA
qPCR primer for *GAPDH*, Reverse	This paper	GTCTTCTGGGTGGCAGTGAT
qPCR primer for mouse *PPIA*, Forward	This paper	TCCTGGACCCAAAACGCTCC
qPCR primer for mouse *PPIA*, Reverse	This paper	CCATGGCAAATGCTGGACCA

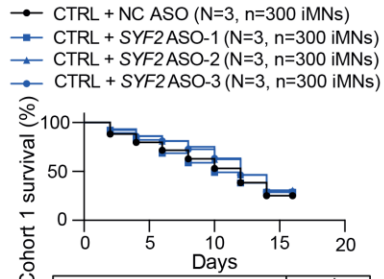
DNA Common primer, TDP-43 genotyping	Wils et al.[51]	TGAAATCCGGGT GGTATTGG
DNA WT primer, TDP-43 genotyping	Wils et al.[51]	GGTGAGTTTAACC TTCAAGGGCT
DNA Transgene primer, TDP-43 genotyping	Wils et al.[51]	AGCTTGCTAGCGG ATCCAGAC
Negative control ASO (9 μM)	This paper	mG*mC*mG*mA*m C*T*A*T*A*C*G*C* G*C*A*mA*mU*mA* mU*mG
SYF2 ASO-1 (9 μM)	This paper	mC*mU*mU*mC*mU *C*C*T*C*T*T*G*C* C*G*mC*mA*mC*m A*mU
SYF2 ASO-2 (9 μM)	This paper	mG*mC*mU*mG*m A*G*T*G*A*G*A*A* G*G*C*mA*mU*mG* mG*mA
SYF2 ASO-3 (9 μM)	This paper	mG*mA*mG*mU*m G*A*G*A*A*G*G*C* A*T*G*mG*mA*mC* mU*mA
SYF1 ASO-1 (9 μM)	This paper	mC*mC*mU* mC*mU*G* T*G*T* A*G*G* T*G*T* mU*mG*mA* mU*mG
Scramble siRNA (3 nM)	ThermoFisher Scientific	Cat# 4390846
TARDBP siRNA (3 nM)	ThermoFisher Scientific	Cat# s530937
SYF2 siRNA-1 (3 nM)	ThermoFisher Scientific	Cat# s24813
SYF2 siRNA-2 (3 nM)	ThermoFisher Scientific	Cat# s24811
AR ASO-1 (9μM)	This paper	mG*mC*mC*mA*mU *C*T*G*G*T*C*G*T* C*C*mA*mC*mG*m U*mG
AR ASO-2 (9μM)	This paper	mU*mG*mC*mU*m G*C*T*G*C*C*T*T* C*G*G*mA*mU*mA* mC*mU
PGR ASO-1 (9μM)	This paper	mG*mC*mC*mC*m U*T*C*C*A*T*T*G*C *C*C*mU*mC*mU*m U*mA
PGR ASO-2 (9μM)	This paper	mG*mC*mA*mG*m U*C*C*G*C*T*G*T* C*C*T*mU*mU*mU* mC*mU
PGR ASO-3 (9μM)	This paper	mG*mG*mU*mG*m U*C*C*G*A*G*G*T* C*T*G*mG*mC*mU* mC*mC

Mouse Negative control ASO (*in vivo-50 µg*)	This paper	/52MOErC/*/i2MOErC/ /i2MOErT/ /i2MOErA/ /i2MOErT/ A*G*G*A*C*T*A*T*C*C*/i2MOErA//i2MOErG/ /i2MOErG/* /i2MOErA/*/32MOErA/
Mouse *SYF2* ASO (*in vivo- 50µg*)	This paper	/52MOErT/*/i2MOErC//i2MOErC//i2MOErA/ /i2MOErC/A*A*C*T*T*C*C*T*G*G*/i2MOErT/ /i2MOErG//i2MOErA/*/i2MOErT/*/32MOErT/

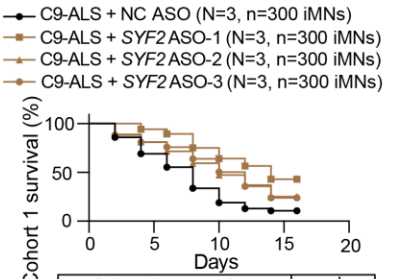
Table S6. Related to Key Resources Table. Oligonucleotides used in the study.


A
CTRL-1 DMSO (100 iMNs)
CTRL-1 norgestrel (50 iMNs)
p = 0.0507
CTRL-2 DMSO (100 iMNs)
CTRL-2 norgestrel (50 iMNs)
p = 0.1598
CTRL-3 DMSO (100 iMNs)
CTRL-3 norgestrel (50 iMNs
p = 0.0912
B
C9-ALS/FTD-1 DMSO (100 iMNs)
C9-ALS/FTD-1 norgestrel (50 iMNs)
p < 0.0001
C9-ALS/FTD-2 DMSO (100 iMNs)
C9-ALS/FTD-2 norgestrel (50 iMNs)
p < 0.0001
C9-ALS/FTD-3 DMSO (100 iMNs
C9-ALS/FTD-3 norgestrel (50 iMNs
p < 0.0001
C
sALS-1 DMSO (100 iMNs)
sALS-1 norgestrel (50 iMNs)
p = 0.0163
sALS-2 DMSO (100 iMNs)
sALS-2 norgestrel (50 iMNs)
p = 0.2623
sALS-3 DMSO (100 iMNs)
sALS-3 norgestrel (50 iMNs)
p < 0.0001
sALS-4 DMSO (100 iMNs)
sALS-4 norgestrel (50 iMNs)
p < 0.0001
sALS-5 DMSO (100 iMNs)
sALS-5 norgestrel (50 iMNs)
p = 0.0077
sALS-6 DMSO (100 iMNs)
sALS-6 norgestrel (50 iMNs)
p < 0.0001
sALS-7 DMSO (100 iMNs)
sALS-7 norgestrel (50 iMNs)
p < 0.0001
sALS-8 DMSO (100 iMNs)
sALS-8 norgestrel (50 iMNs)
p < 0.0001
Survival (%)
Days

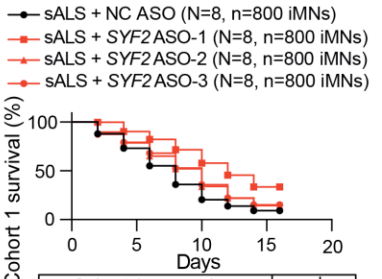
D



Cohort 1 comparison	*p*-value
+NC ASO vs +*SYF2* ASO1	>0.9999
+NC ASO vs +*SYF2* ASO2	0.0627
+NC ASO vs +*SYF2* ASO3	0.1335

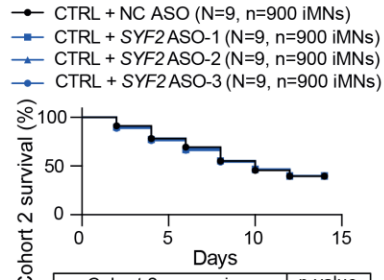


Cohort 1 comparison	*p*-value
+NC ASO vs +*SYF2* ASO1	<0.0001
+NC ASO vs +*SYF2* ASO2	<0.0001
+NC ASO vs +*SYF2* ASO3	<0.0001

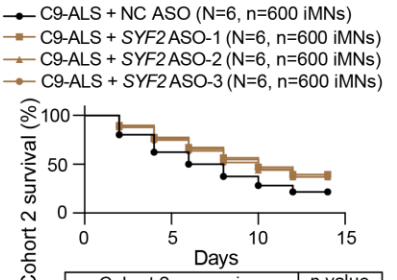


Cohort 1 comparison	*p*-value
+NC ASO vs +*SYF2* ASO1	<0.0001
+NC ASO vs +*SYF2* ASO2	<0.0001
+NC ASO vs +*SYF2* ASO3	<0.0001

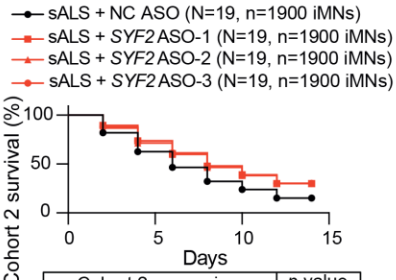
E



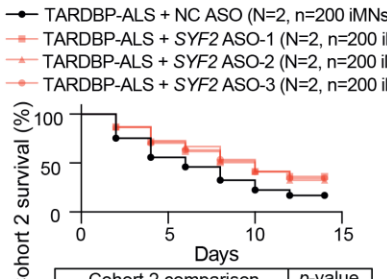
Cohort 2 comparison	*p*-value
+NC ASO vs +*SYF2* ASO1	>0.9999
+NC ASO vs +*SYF2* ASO2	>0.9999
+NC ASO vs +*SYF2* ASO3	0.1179



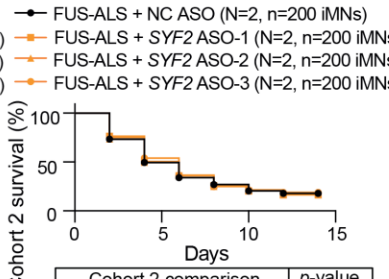
Cohort 2 comparison	*p*-value
+NC ASO vs +*SYF2* ASO1	<0.0001
+NC ASO vs +*SYF2* ASO2	<0.0001
+NC ASO vs +*SYF2* ASO3	<0.0001



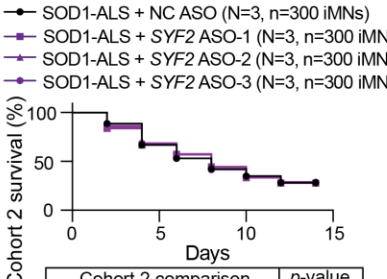
Cohort 2 comparison	*p*-value
+NC ASO vs +*SYF2* ASO1	<0.0001
+NC ASO vs +*SYF2* ASO2	<0.0001
+NC ASO vs +*SYF2* ASO3	<0.0001



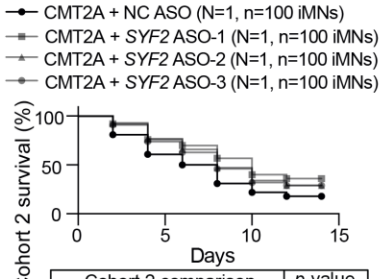
Cohort 2 comparison	*p*-value
+NC ASO vs +*SYF2* ASO1	<0.0001
+NC ASO vs +*SYF2* ASO2	<0.0001
+NC ASO vs +*SYF2* ASO3	<0.0001



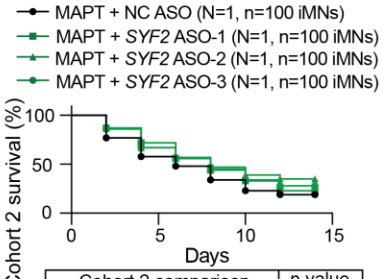
Cohort 2 comparison	*p*-value
+NC ASO vs +*SYF2* ASO1	0.9939
+NC ASO vs +*SYF2* ASO2	0.8556
+NC ASO vs +*SYF2* ASO3	0.6555



Cohort 2 comparison	*p*-value
+NC ASO vs +*SYF2* ASO1	0.9067
+NC ASO vs +*SYF2* ASO2	0.8706
+NC ASO vs +*SYF2* ASO3	0.8765



Cohort 2 comparison	*p*-value
+NC ASO vs +*SYF2* ASO1	<0.0001
+NC ASO vs +*SYF2* ASO2	<0.0001
+NC ASO vs +*SYF2* ASO3	<0.0001



Cohort 2 comparison	*p*-value
+NC ASO vs +*SYF2* ASO1	0.1444
+NC ASO vs +*SYF2* ASO2	0.0139
+NC ASO vs +*SYF2* ASO3	0.0625

Data S1. Related to Figures 2, 3 & Figures S2, S3. Kaplan-Meier survival curves for iMNs treated with DMSO, norgestrel, negative control ASO, or *SYF2* ASOs.

Kaplan–Meier survival curves of iMNs from 3 control (CTRL) (A), 3 C9-ALS/FTD (B), and 8 sALS (C) treated with DMSO or 3 μM norgestrel after withdrawal of neurotrophic factor supplementation (each line shown in a separate graph, CTRL 1-3, C9-ALS/FTD 1-3, and sALS 1-8). n=100 iMNs per line for DMSO treatment and n=50 iMNs per line for norgestrel treatment. iMNs quantified from three biologically independent iMN conversions per line per condition. Log-rank test. Statistical significance calculated using the entire survival course.

In addition, Kaplan–Meier survival curves of iMNs from cohort 1 donors (D); 3 control (CTRL), 3 C9-ALS/FTD, and 8 sALS lines, and cohort 2 donors (E); 9 CTRL, 6 C9-ALS/FTD, 19 sALS, 2 TARDBP, 2 FUS, 3 SOD1, 1 CMT2A and 1 MAPT in aggregate treated with 9 μM NC ASO, SYF2 ASO-1, SYF2 ASO-2, or SYF2 ASO-3 (each genotype shown in a separate graph). n=100 iMNs per treatment/line. iMNs quantified from 4 independent iMN conversions per line per condition. Log-rank test. Statistical significance calculated using the entire survival course.