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Supplemental information

Persistent mRNA localization defects and cell death in ALS neurons caused by transient cellular stress

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Figure S1

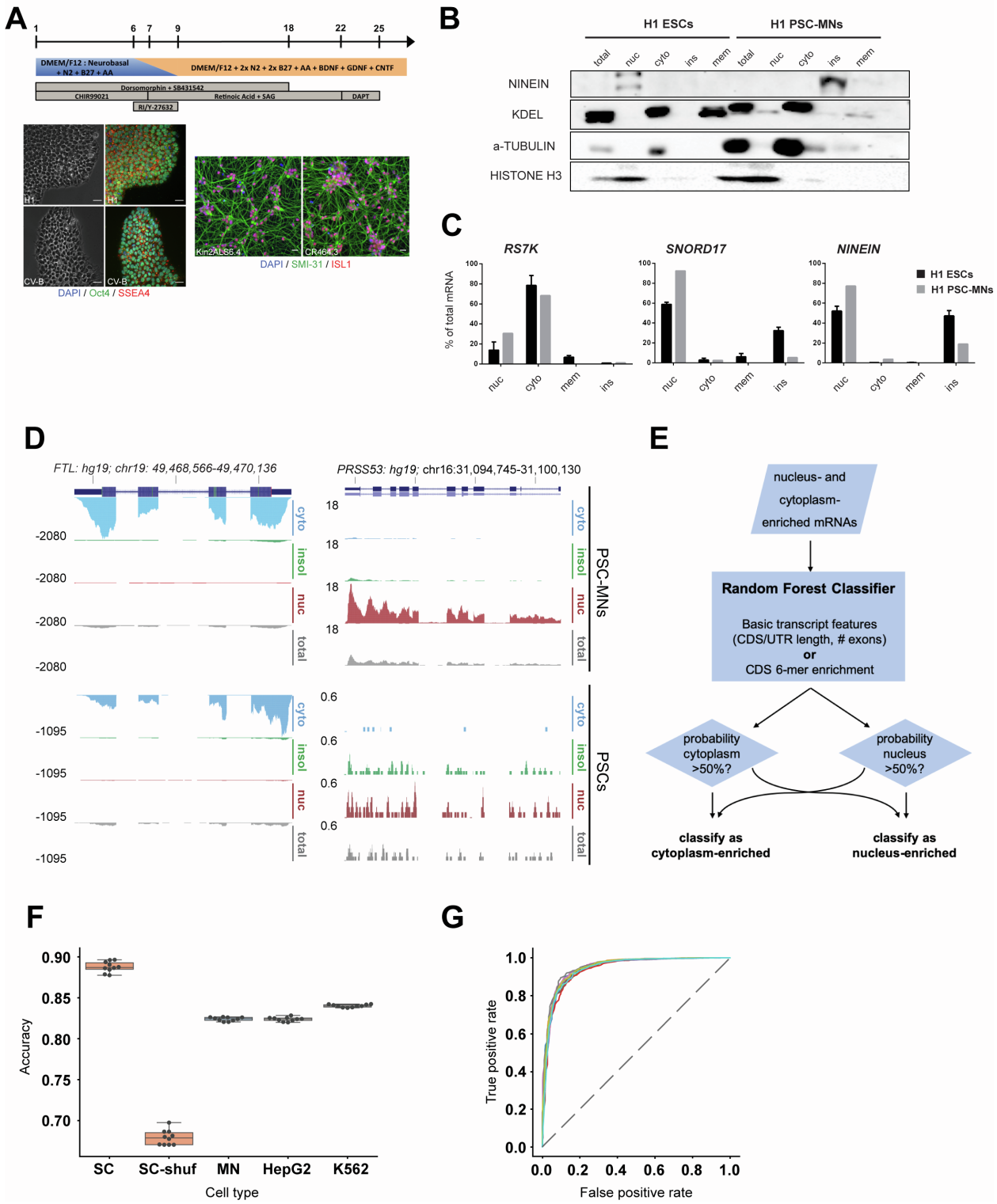

A
1
6
7
9
18
22
25
DMEM/F12 : Neurobasal + N2 + B27 + AA
DMEM/F12 + 2x N2 + 2x B27 + AA + BDNF + GDNF + CNTF
Dorsomorphin + SB431542
CHIR99021
Retinoic Acid + SAG
DAPT
RI/Y-27632
H1
H1
CV-B
CV-B
DAPI / Oct4 / SSEA4
Kin2ALS6.4
CR46.3
DAPI / SMI-31 / ISL1
B
H1 ESCs
H1 PSC-MNs
total
nuc
cyto
ins
mem
NINEIN
KDEL
a-TUBULIN
HISTONE H3
C
RS7K
SNORD17
NINEIN
% of total mRNA
H1 ESCs
H1 PSC-MNs
nuc
cyto
mem
ins
D
FTL: hg19; chr19: 49,468,566-49,470,136
PRSS53: hg19; chr16:31,094,745-31,100,130
cyto
insol
nuc
total
PSC-MNs
PSCs
E
nucleus- and cytoplasm-enriched mRNAs
Random Forest Classifier
Basic transcript features (CDS/UTR length, # exons)
or
CDS 6-mer enrichment
probability cytoplasm >50%?
probability nucleus >50%?
classify as cytoplasm-enriched
classify as nucleus-enriched
F
Accuracy
SC
SC-shuf
MN
HepG2
K562
Cell type
G
True positive rate
False positive rate

Figure S1. CeFRa-seq applied to PSC-derived motor neurons, related to Figure 1
(A) Schematic of motor neuron differentiation protocol and immunofluorescence staining of induced pluripotent stem cells (CV-B), embryonic stem cells (H1), and pluripotent stem cell-derived motor neurons (PSC-MNs) (Kin2ALS6, CR463.4. Pluripotent stem cells (PSCs) were stained with antibodies against Oct4 and SSEA4, PSC-MNs were stained with antibodies against phosphorylated neurofilament (SMI-31) and Islet1. Nuclei were stained using DAPI. Scale bars = 20 μm in all panels. **(B)** Western blot analysis of known fraction-enriched proteins in PSCs and PSC-MNs. **(C)** qRT-PCR analysis of known fraction-enriched transcripts in PSCs and PSC-MNs. **(D)** Genome browser tracks showing CeFra-seq read coverage for two sample transcripts, *FTL* (left) and *PRSS53* (right). **(E)** Schematic showing classification algorithm to predict transcripts with nuclear vs cytoplasmic enrichment. **(F)** Box plots showing accuracy of random forest classifier in correctly predicting nuclear vs. cytoplasmic localization across several cell lines. **(G)** Area under the curve for 10 iterations of the random forest classifier in predicting nuclear or cytoplasm-enriched transcripts in PSCs.

Figure S2

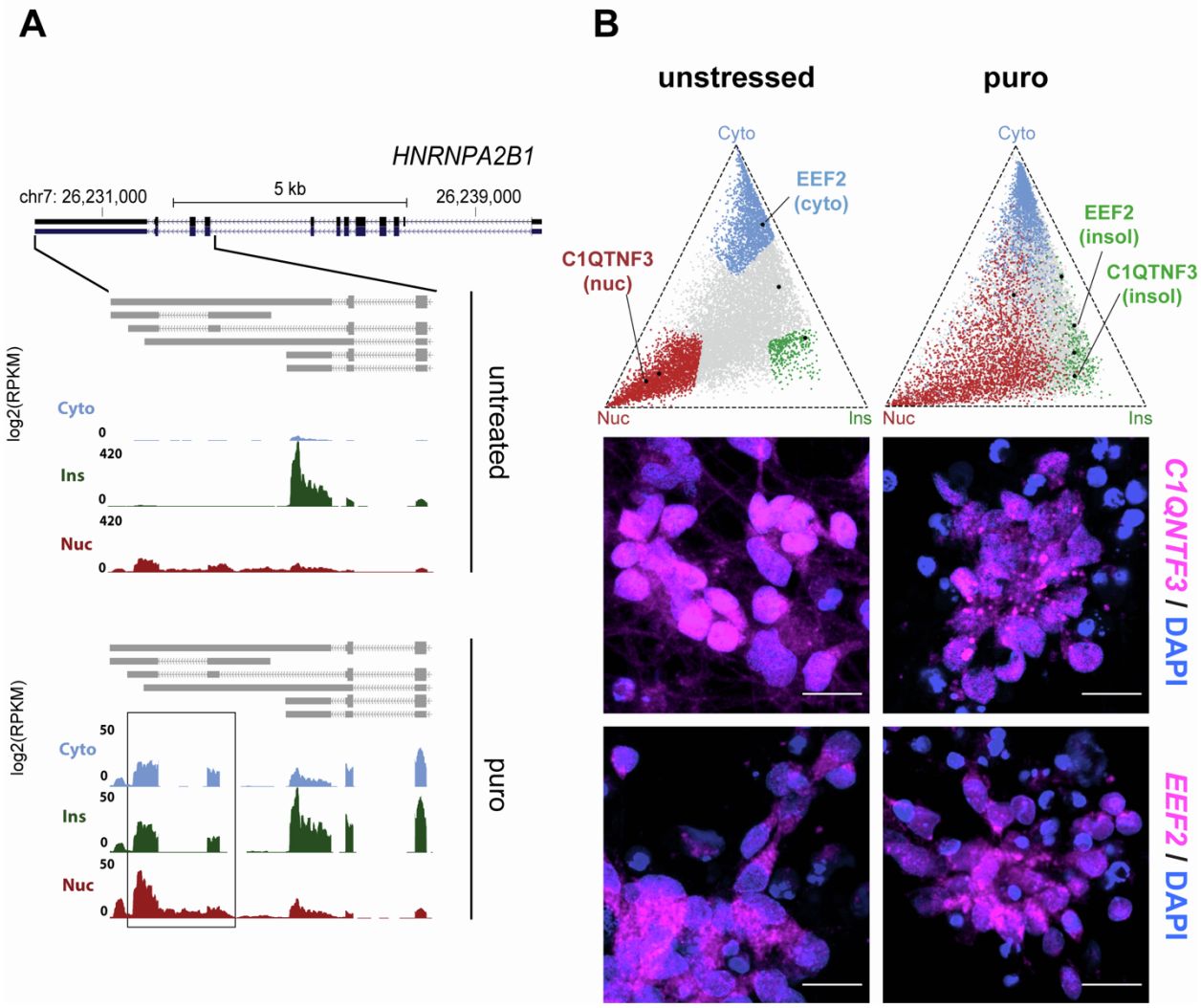


Figure S2. Stress-induced RNA localization changes in PSC-MNs, related to Figure 2

(A) Genome browser tracks showing CeFra-seq read coverage for *HNRNPA2B1* in unstressed and puromycin-stressed PSC-MNs. **(B)** RNA FISH staining of PSC-MNs that were either left untreated or treated with puromycin (10µg/ml) for 24h. Cells were stained with Stellaris probes against *C1QNTF3* or *EEF2*, respectively. Scale bars = 20 µm in all panels. Nuclei were stained using DAPI.

Figure S3

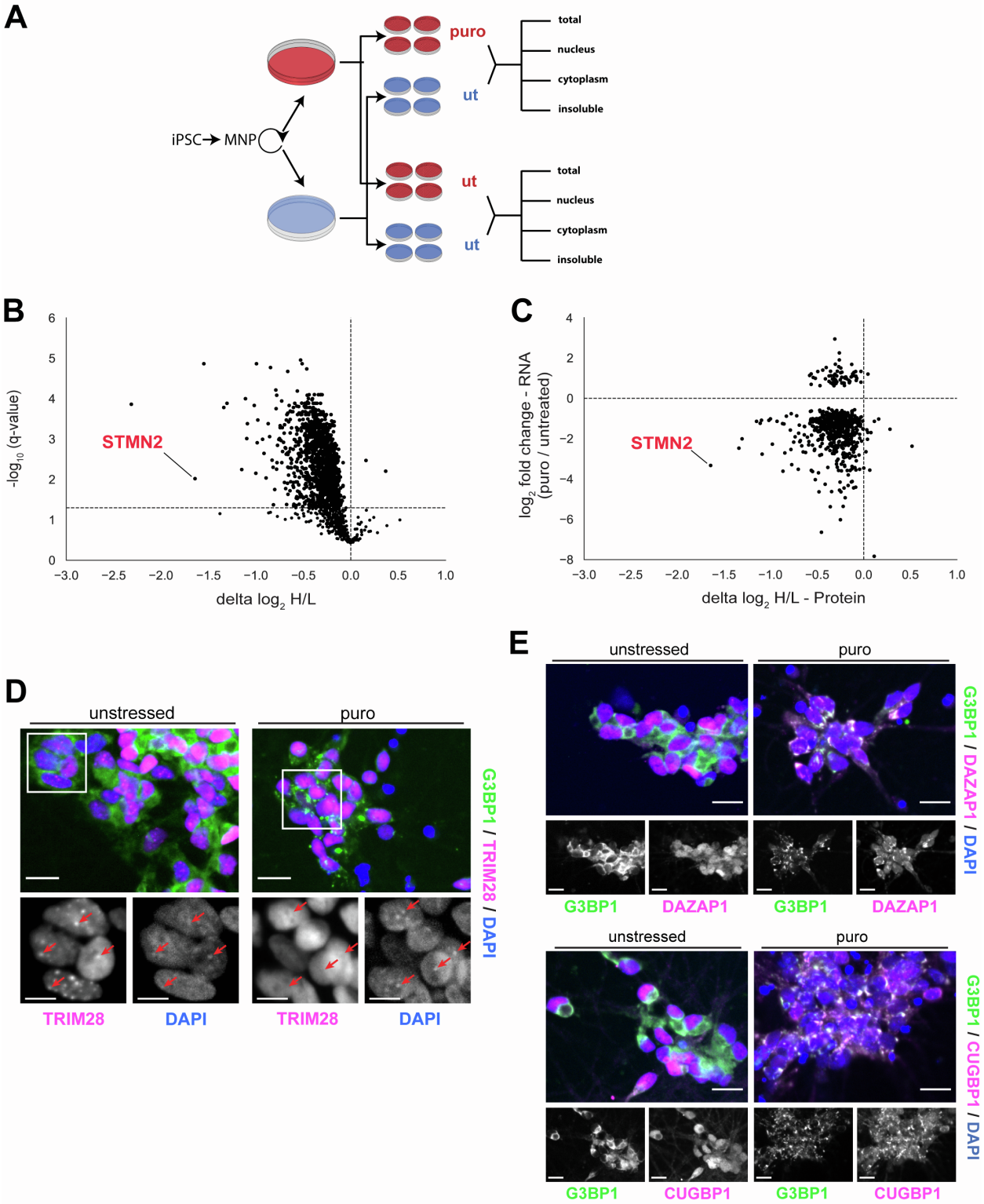
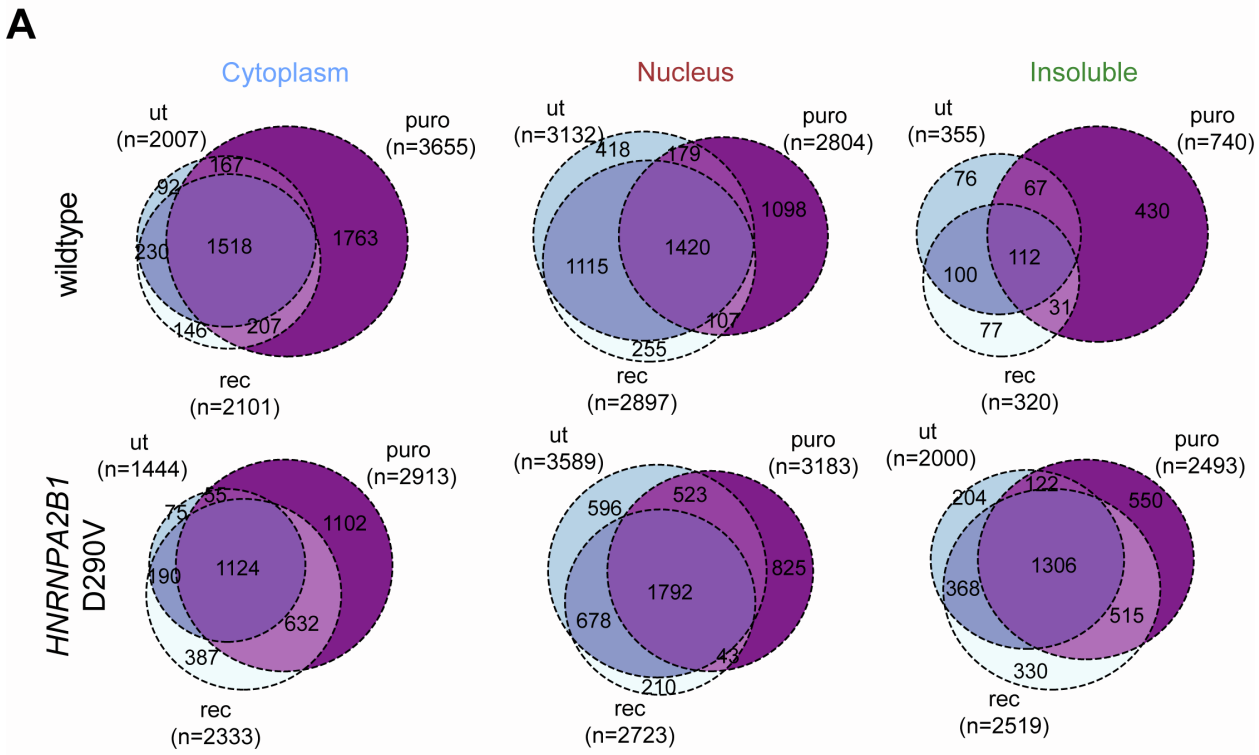

A
iPSC → MNP
puro
ut
ut
ut
total
nucleus
cytoplasm
insoluble
total
nucleus
cytoplasm
insoluble
B
STMN2
-log10 (q-value)
delta log2 H/L
C
STMN2
log2 fold change - RNA (puro / untreated)
delta log2 H/L - Protein
D
unstressed
puro
G3BP1 / TRIM28 / DAPI
TRIM28
DAPI
TRIM28
DAPI
E
unstressed
puro
G3BP1 / DAZAP1 / DAPI
G3BP1
DAZAP1
G3BP1
DAZAP1
unstressed
puro
G3BP1 / CUGBP1 / DAPI
G3BP1
CUGBP1
G3BP1
CUGBP1

Figure S3. Stress-induced protein localization changes in PSC-MNs, related to Figure 2

(A) Schematic showing experimental design for SILAC quantitative mass spectrometry analysis of fraction-enriched proteins. **(B)** Volcano plot showing changes in total protein levels for all detected proteins in response to puromycin stress. **(C)** Scatterplot showing log_2 fold changes at the RNA and protein level for all detected transcripts/proteins in response to puromycin stress. **(D)** Immunofluorescence staining of PSC-MNs that were either left untreated or treated with puromycin (10μg/ml) for 24h. Cells were stained with antibodies against G3BP1 and DAZAP1 or CUGBP1, respectively. Nuclei were stained using DAPI. Scale bars = 20 μm in top panels, 10μm in bottom panels. **(E)** Immunofluorescence staining of PSC-MNs that were either left untreated or treated with puromycin (10μg/ml) for 24h. Cells were stained with antibodies against G3BP1 and TRIM28. Heterochromatin foci are indicated by solid yellow arrowheads. Nuclei were stained using DAPI. Scale bars = 20 μm in top panels, 10μm in magnified insert panels.

Figure S4


A
Cytoplasm
Nucleus
Insoluble
wildtype
HNRNPA2B1 D290V
ut (n=2007)
puro (n=3655)
rec (n=2101)
167
92
1518
1763
230
146
207
ut (n=3132)
puro (n=2804)
rec (n=2897)
418
179
1098
1420
1115
107
255
ut (n=355)
puro (n=740)
rec (n=320)
76
67
430
112
100
31
77
ut (n=1444)
puro (n=2913)
rec (n=2333)
75
55
1102
1124
190
632
387
ut (n=3589)
puro (n=3183)
rec (n=2723)
596
523
825
1792
678
43
210
ut (n=2000)
puro (n=2493)
rec (n=2519)
204
122
550
1306
368
515
330

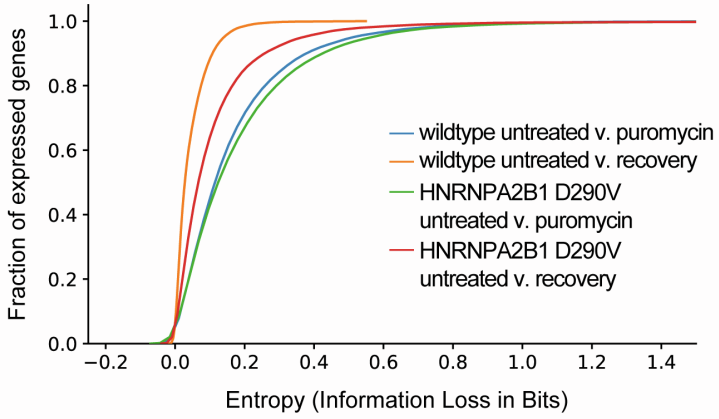

B
Fraction of expressed genes
1.0
0.8
0.6
0.4
0.2
0.0
−0.2
0.0
0.2
0.4
0.6
0.8
1.0
1.2
1.4
Entropy (Information Loss in Bits)
wildtype untreated v. puromycin
wildtype untreated v. recovery
HNRNPA2B1 D290V untreated v. puromycin
HNRNPA2B1 D290V untreated v. recovery

Figure S4, Persistent stress-induced localization changes in ALS neurons, related to Figure 4
(A) Venn diagrams showing overlap between sets of fraction-enriched transcripts in unstressed (left), puromycin-stressed (center) and recovery (right) conditions in wildtype (top) PSC-MNs and PSC-MNs harboring a mutation in *HNRNPA2B1* (bottom). **(B)** Cumulative distribution plots showing relative entropies for all detected transcripts in the indicated comparisons between two conditions.