Supplementary Methods

RNA immunoprecipitation

Immunoprecipitation was carried out as described previously [36]. Briefly, N2a's were transfected with FLAG control or FLAG-tagged SRSF1 prior to fixing of ribonucleoprotein complexes and crosslinking with formaldehyde. Cell extracts were then subjected to anti-FLAG immunoprecipitation prior to the RT-qPCR (as described in section 4) analysis of SRSF1-crosslinked RNA molecules.

MSD-ELISA

DPR expression levels were detected via sandwich electrochemiluminescence ELISA Meso Scale Discovery platform (Meso Scale Technology) as described previously [79]. Briefly MSD multi-array 96-well plates were coated overnight at with 2µg/ml capture antibody specific for individual DPRs in TBST at 4^{o}C. After incubation antibody was removed and plates were washed with TBST and blocked with 3% BSA in TBST for 2 hours at RT. Standards and samples were added to plates at 50µl/well and were shaken at 700rpm for 2 hours. Detection antibodies were added at 25µl per well and were shaken at 700rpm for 2 hours at RT. After incubation plates were washed with TBST and 150µl read buffer per well was added to plates and read on QUICKPLEX SQ120 instrument.

Cycloheximide Assay

N2a cells were cultured as described. Cycloheximide (CHX) was added to N2a cells at a concentration of 0.5mg/ml for a period of 2h or 4h prior to harvest. Western blotting was subsequently performed as described.

Cell line	Sex (M/F)	Age at biopsy (years)	Disease duration (months)
Con-155	M	40	N/A
Con-209	F	69	N/A
Con-3050	M	65	N/A
C9-78	M	66	31.7
C9-183	M	50	27
C9-201	F	66	19.4

Table S1. Details of control and C9orf72 ALS skin biopsy donors.

Subject	Sex (M/F)	Age/Age-at-onset (years)	Age-at-death (years)	CSF/Plasma/ Both	Disease duration (months)
Con-SHF-030	M	64	N/A	Plasma	N/A
Con-SHF-034	M	51	N/A	Plasma	N/A
Con-SHF-052	F	58	N/A	Both	N/A
Con-SHF-175	M	56	N/A	Both	N/A
Con-SHF-180	M	52	N/A	CSF	N/A
Con-SHF-182	F	71	N/A	Both	N/A
Con-SHF-185	F	65	N/A	Both	N/A
Con-SHF-205	F	73	N/A	Both	N/A
Con-SHF-209	F	70	N/A	Both	N/A
Con-SHF-210	F	65	N/A	Plasma	N/A
Con-SHF-232	M	55	N/A	Plasma	N/A
C9-SHF-014	M	51	56	Plasma	60
C9-SHF-017	M	61	63	Both	30
C9-SHF-018	F	74	77	Plasma	45
C9-SHF-043	F	70	72	Both	31
C9-SHF-081	F	72	73	Plasma	12
C9-SHF-083	M	59	62	Both	36
C9-SHF-103	F	58	60	Both	32
C9-SHF-138	F	66	68	Both	27
C9-SHF-152	M	54	56	Both	29
C9-SHF-162	F	63	67	Both	56

Table S2. Details of control and C9orf72 ALS CSF and Plasma donors.

Subject	Sex (M/F)	Age-at-onset (years)	Age-at-death (years)	White/Grey matter/Both	Disease duration (months)
Con-129/94	M	N/A	63	Both	N/A
Con-025/95	M	N/A	67	Both	N/A
Con-178/95	F	N/A	63	Both	N/A
Con-293/91	F	N/A	65	Both	N/A
Con-072/92	F	N/A	76	Both	N/A
Con-071/92	M	N/A	75	Both	N/A
C9-098/02	F	61	64	Both	42
C9-081/09	M	56	59	Both	27
C9-041/04	M	62	64	Both	20
C9-063/08	F	67	69	Both	26
C9-046/10	F	63	67	Grey	43
C9-042/09	F	50	53	Grey	28
C9-040/11	F	72	76	Both	56

Table S3. Details of control and C9orf72 ALS white and grey matter donors.

Antibody	Species	Western blot dilution	Immunofluorescence dilution	Source	Cat. No.
ADA	Mouse	1:250-1,000	N/A	Santa Cruz	sc28346
ADA	Rabbit	1:1000	1:25-50	Proteintech	13328-1-AP
ADK	Rabbit	1:1000	N/A	Proteintech	66929-1-IG
Actin	Rabbit	1:3000	N/A	Proteintech	20536-1-AP
Biotin	Mouse	1:1000	N/A	Santa Cruz	sc101339
CD73	Rabbit	1:2000	N/A	Proteintech	12231-1-AP
HGPRT	Rabbit	1:2000	N/A	Proteintech	15059-1-AP
NQO-1	Rabbit	1:1000	N/A	Proteintech	11451-1-AP
P62	Mouse	1:1000	N/A	BD Biosciences	610833
P73	Mouse	1:2000	N/A	ThermoFisher	38C674.2
PNP	Mouse	1:1000	N/A	R&D Systems	MAB6486
V5-Tag	Mouse	N/A	1:1000	ThermoFisher	MA5-15253
XDH	Rabbit	1:2000	N/A	Proteintech	55156-1-AP

Table S4. Details of the primary antibodies used in this study.

Species	Western blot dilution	Immunofluorescence dilution	Excitation/Emission (nm)	Source	Cat. No.
Hoescht	N/A	1:2,000-10,000	350/461	ThermoFisher	H3570
Anti-Mouse	1:5,000	N/A	N/A	Abcam	ab97040
	1:2500	N/A	N/A	Cell Signalling	7076
Anti-Rabbit	1:5,000	N/A	N/A	Sigma-Aldrich	A0545
Fluorescent Anti-Mouse	N/A	1:1,000	499/520	ThermoFisher	A11001
Fluorescent Anti-Rabbit	N/A	1:1,000	579/603	ThermoFisher	A11011

Table S5. Details of the secondary antibodies used in this study.

Primer	Fwd/Rev (F/R)	Sequence (5’-3’)
18s	F	ATGGCCGTTCTTAGTTGGTG
18s	R	CGCTGAGCCAGTCAGTGTAG
ADA	F	CTGCTGAACGTCATTGGCATGG
ADA	R	GGCGATCCTTTTGATAGCCTCC
ADA Drosophila	F	GGCATCAAAAGCCTATGTGATCT
ADA Drosophila	R	CGCGCACAACTTCAGGAAA
CD73	F	CTCCTCTCAATCATGCCGCT
CD73	R	TGGATTCCATTGTTGCGTTCA
GAPDH	F	CAACTTTGGTATCGTGGAAGGAC
GAPDH	R	ACAGTCTTCTGGGTGGCAGTG
HGPRT	F	CATTATGCTGAGGATTTGGAAAGG
HGPRT	R	CTTGAGCACACAGAGGGCTACA
HGPRT Drosophila	F	CCGTATCCAGCCTGATCCG
HGPRT Drosophila	R	ACTCCTGATCTCGGTGGTGA
Tub8	F	TGGGCCCGTCTGGACCACAA
Tub8	R	TCGCCGTCACCGGAGTCCAT
U1	F	CCATGATCACGAAGGTGGTT
U1	R	ATGCAGTCGAGTTTCCCACA
WPRE	F	CCCGTACGGCTTTCGTTTTC
WPRE	R	CAAACACAGAGAGCACACCACG

Table S6. Details of the primer pairs used in this study including 5’-3’ sequence.

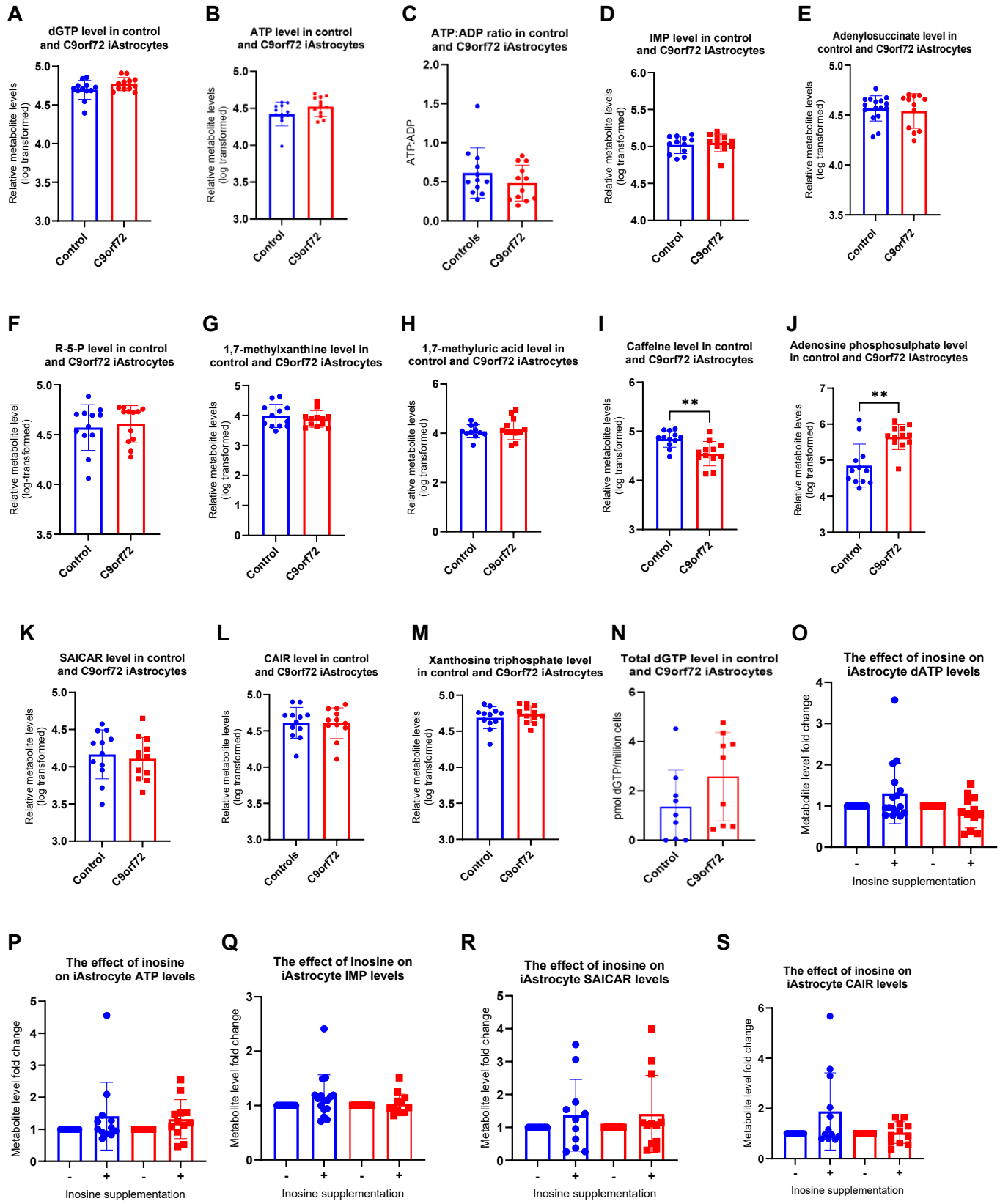
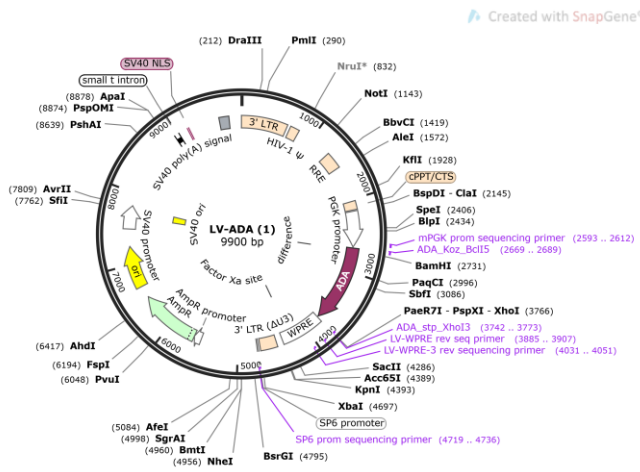

A
dGTP level in control and C9orf72 iAstrocytes
Relative metabolite levels (log transformed)
Control
C9orf72
B
ATP level in control and C9orf72 iAstrocytes
Relative metabolite levels (log transformed)
Control
C9orf72
C
ATP:ADP ratio in control and C9orf72 iAstrocytes
ATP:ADP
Controls
C9orf72
D
IMP level in control and C9orf72 iAstrocytes
Relative metabolite levels (log transformed)
Control
C9orf72
E
Adenylosuccinate level in control and C9orf72 iAstrocytes
Relative metabolite levels (log transformed)
Control
C9orf72
F
R-5-P level in control and C9orf72 iAstrocytes
Relative metabolite level (log-transformed)
Control
C9orf72
G
1,7-methylxanthine level in control and C9orf72 iAstrocytes
Relative metabolite levels (log transformed)
Control
C9orf72
H
1,7-methyluric acid level in control and C9orf72 iAstrocytes
Relative metabolite levels (log transformed)
Control
C9orf72
I
Caffeine level in control and C9orf72 iAstrocytes
**
Relative metabolite levels (log transformed)
Control
C9orf72
J
Adenosine phosphosulphate level in control and C9orf72 iAstrocytes
**
Relative metabolite levels (log transformed)
Control
C9orf72
K
SAICAR level in control and C9orf72 iAstrocytes
Relative metabolite levels (log transformed)
Control
C9orf72
L
CAIR level in control and C9orf72 iAstrocytes
Relative metabolite levels (log transformed)
Controls
C9orf72
M
Xanthosine triphosphate level in control and C9orf72 iAstrocytes
Relative metabolite levels (log transformed)
Control
C9orf72
N
Total dGTP level in control and C9orf72 iAstrocytes
pmol dGTP/million cells
Control
C9orf72
O
The effect of inosine on iAstrocyte dATP levels
Metabolite level fold change
Inosine supplementation
P
The effect of inosine on iAstrocyte ATP levels
Metabolite level fold change
Inosine supplementation
Q
The effect of inosine on iAstrocyte IMP levels
Metabolite level fold change
Inosine supplementation
R
The effect of inosine on iAstrocyte SAICAR levels
Metabolite level fold change
Inosine supplementation
S
The effect of inosine on iAstrocyte CAIR levels
Metabolite level fold change
Inosine supplementation

Figure S1. Metabolic analysis demonstrates aberrant purine metabolite output in C9orf72 iAstrocytes. Control and C9orf72 iAstrocyte (A) dGTP level, (B) ATP level, (C) ATP:ADP ratio, (D) IMP level, (E) adenylosuccinate level, (F) Ribose-5-phosphate (R-5-P) level, (G)1,7-methylxanthine level, (H) 1,7-methyluric acid level, (I) caffeine level, (J) adenosine phosphosulphate level, (K) succinylaminoimidazole carboxamide ribonucleotide (SAICAR) level, (L) 5-aminoimidazole-4-carboxylate ribonucleotide (CAIR) level and (M) xanthosine triphosphate level as measured via LC/MS. Control and C9orf72 iAstrocyte (N) dGTP level as determined via coupled click chemistry and DNA polymerase-based assay. (O) dATP level, (P) ATP level, (Q) IMP, (R) SAICAR and (S) CAIR as measured by LC/MS, +/- 7.5mM inosine. iAstrocyte metabolite levels in the presence of inosine were normalised to metabolite levels in the absence of inosine by fold change analysis. Data presented as mean and standard deviation of four biological replicates from three control and three C9orf72 iAstrocytes. Statistical analysis performed using unpaired t test (A, B, D, L and M), Mann-Whitney test (C, E, F, G, H, I, J and K), or Wilcoxon test (O, P, Q, R and S). $*P \leq 0.05$, $**P \leq 0.01$. Where P value is not indicated results were non-significant.

A



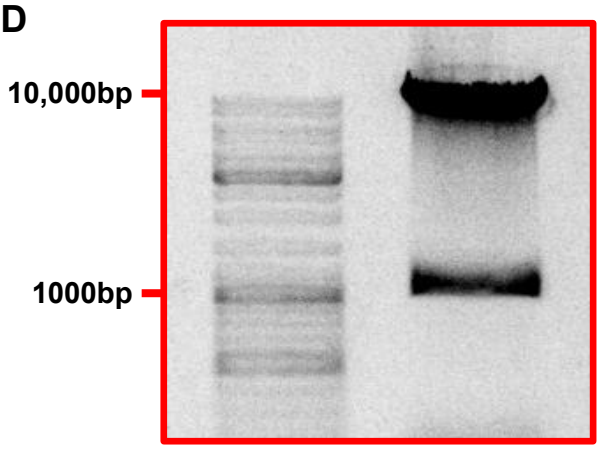
B



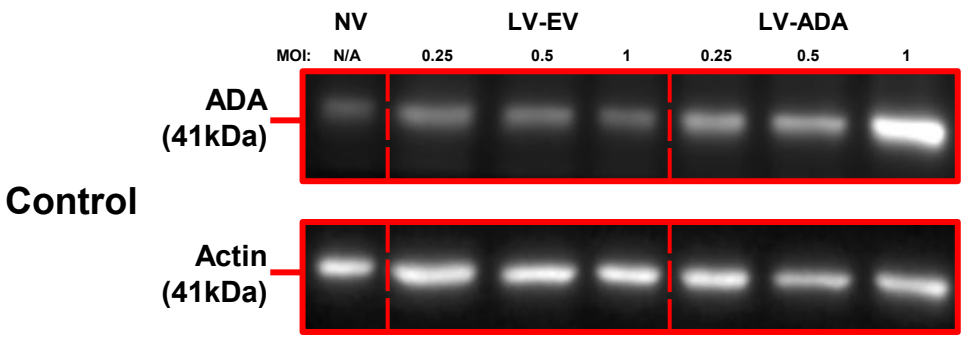
C

EnzymeC	Expected fragment size (bp)
BamHI/XhoI	8811 1089

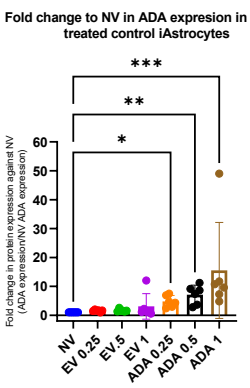
D



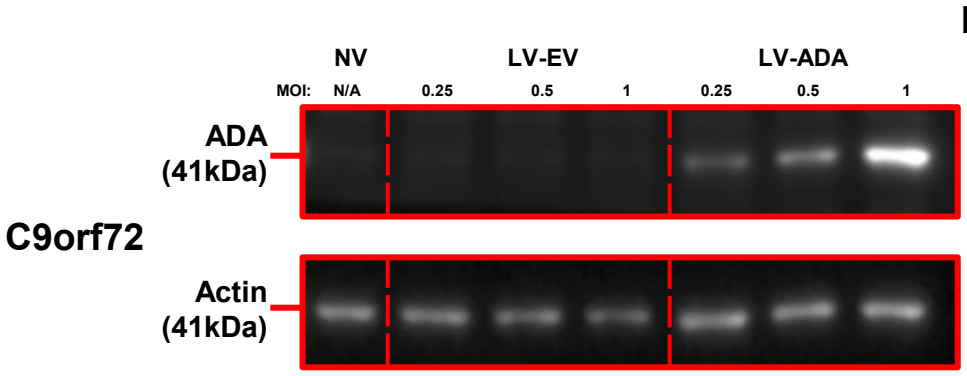
E



F



G



H

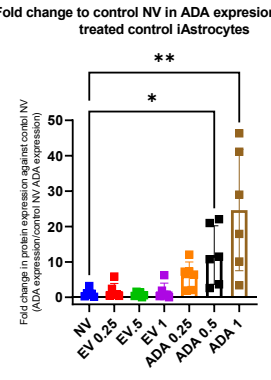
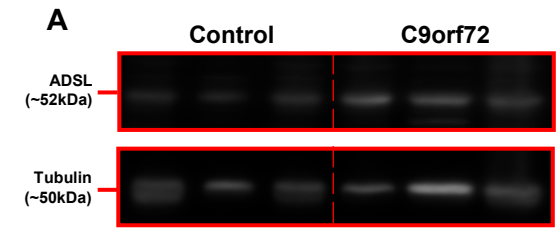
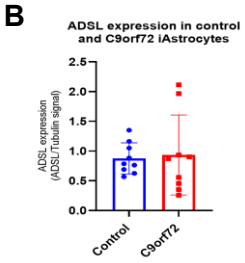
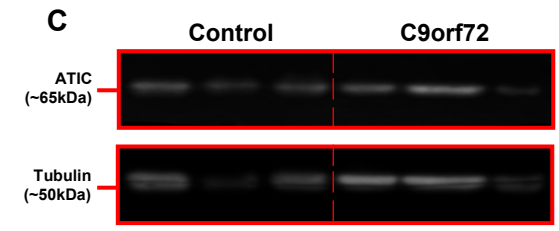
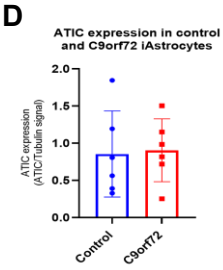


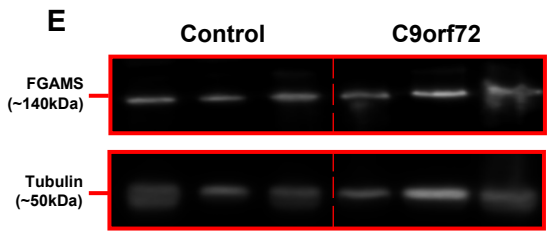
Figure S2. Plasmid maps of (A) SIN-PGK-cPPT-GDNF-WHV and (B) SIN-PGK-cPPT-GDNF-WHV containing adenosine deaminase (ADA) gene-of-interest plasmids that were used to generate lentivirus expressing empty vector (LV-EV) and lentivirus expressing ADA (LV-ADA). (C) Representative images and (D) densitometry analysis of ADA Western blot in control induced neural progenitor cell astrocytes (iAstrocytes) virally treated at MOIs 0.25, 0.5 and 1 with LV-EV and LV-ADA viral treatment. (E) Representative images and (F) densitometry analysis of ADA Western blot in C9orf72 iAstrocytes virally treated at MOIs 0.25, 0.5 and 1 with LV-EV and LV-ADA viral treatment. ADA expression in control virally treated lines measured as fold change to untreated iAstrocytes. ADA expression in C9orf72 virally treated lines measured as fold change to untreated matched control iAstrocytes. Data presented as mean and standard deviation of three biological replicates from three control and three C9orf72 iAstrocytes. Statistical analysis performed using Friedman test (D and F). $*P \leq 0.05$, $**P \leq 0.01$, $***P \leq 0.0001$. Where P value is not indicated results were non-significant.

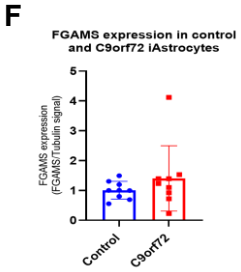

A
Control
C9orf72
ADSL
(~52kDa)
Tubulin
(~50kDa)

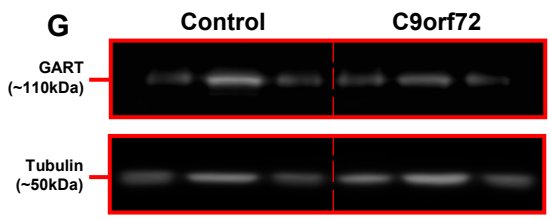

B
ADSL expression in control and C9orf72 iAstrocytes
ADSL expression (ADSL/Tubulin signal)
2.5
2.0
1.5
1.0
0.5
0.0
Control
C9orf72

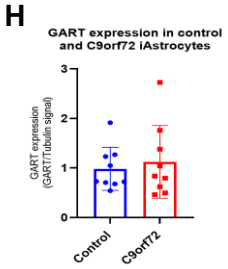

C
Control
C9orf72
ATIC
(~65kDa)
Tubulin
(~50kDa)

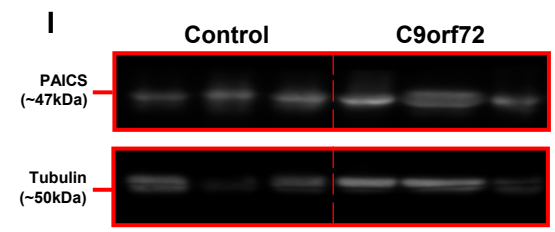

D
ATIC expression in control and C9orf72 iAstrocytes
ATIC expression (ATIC/Tubulin signal)
2.0
1.5
1.0
0.5
0.0
Control
C9orf72

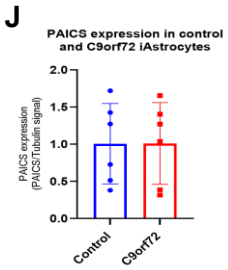

E
Control
C9orf72
FGAMS
(~140kDa)
Tubulin
(~50kDa)

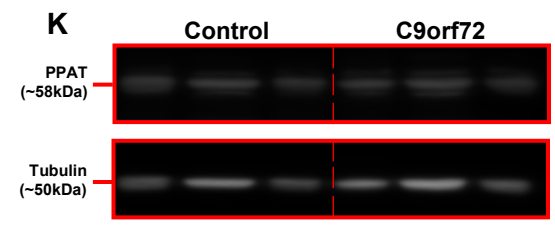

F
FGAMS expression in control and C9orf72 iAstrocytes
FGAMS expression (FGAMS/Tubulin signal)
5
4
3
2
1
0
Control
C9orf72


G
Control
C9orf72
GART
(~110kDa)
Tubulin
(~50kDa)


H
GART expression in control and C9orf72 iAstrocytes
GART expression (GART/Tubulin signal)
3
2
1
0
Control
C9orf72


I
Control
C9orf72
PAICS
(~47kDa)
Tubulin
(~50kDa)


J
PAICS expression in control and C9orf72 iAstrocytes
PAICS expression (PAICS/Tubulin signal)
2.0
1.5
1.0
0.5
0.0
Control
C9orf72


K
Control
C9orf72
PPAT
(~58kDa)
Tubulin
(~50kDa)

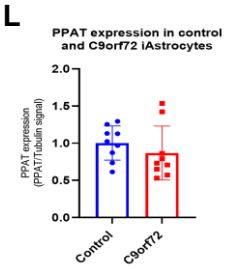

L
PPAT expression in control and C9orf72 iAstrocytes
PPAT expression (PPAT/Tubulin signal)
2.0
1.5
1.0
0.5
0.0
Control
C9orf72

Figure S3. DNPB enzyme expression. (A) Representative images and (B) densitometry analysis of adenylosuccinate lyase (ADSL) Western blot in control and C9orf72 iAstrocytes. (C) Representative images and (D) densitometry analysis of 5-Aminoimidazole-4-carboxamide ribonucleotide formyl-transferase (ATIC) Western blot in control and C9orf72 iAstrocytes. (E) Representative images and (F) densitometry analysis of formylglycinamide ribonucleotide amidotransferase (FGAMS) Western blot in control and C9orf72 iAstrocytes. (G) Representative images and (H) densitometry analysis of phosphoribosylglycinamide formyltransferase/phosphoribosylglycinamide synthetase/phosphoribosylaminoimidazole synthetase (GART) Western blot in control and C9orf72 iAstrocytes. (I) Representative images and (J) densitometry analysis of phosphoribosylaminoimidazole carboxylase/phosphoribosylaminoimidazole succinocarboxamide synthetase (PAICS) Western blot in control and C9orf72 iAstrocytes. (K) Representative images and (L) densitometry analysis of amidophosphoribosyltransferase (PPAT) Western blot in control and C9orf72 iAstrocytes. Data presented as mean and standard deviation of three biological replicates from two or three control and C9orf72 iAstrocytes. Statistical analysis performed using Mann-Whitney test (B, F, H and L) or unpaired t test (D and J). Where *P* value is not indicated results were non-significant. Uncropped Western images can be found in Supplementary Material.

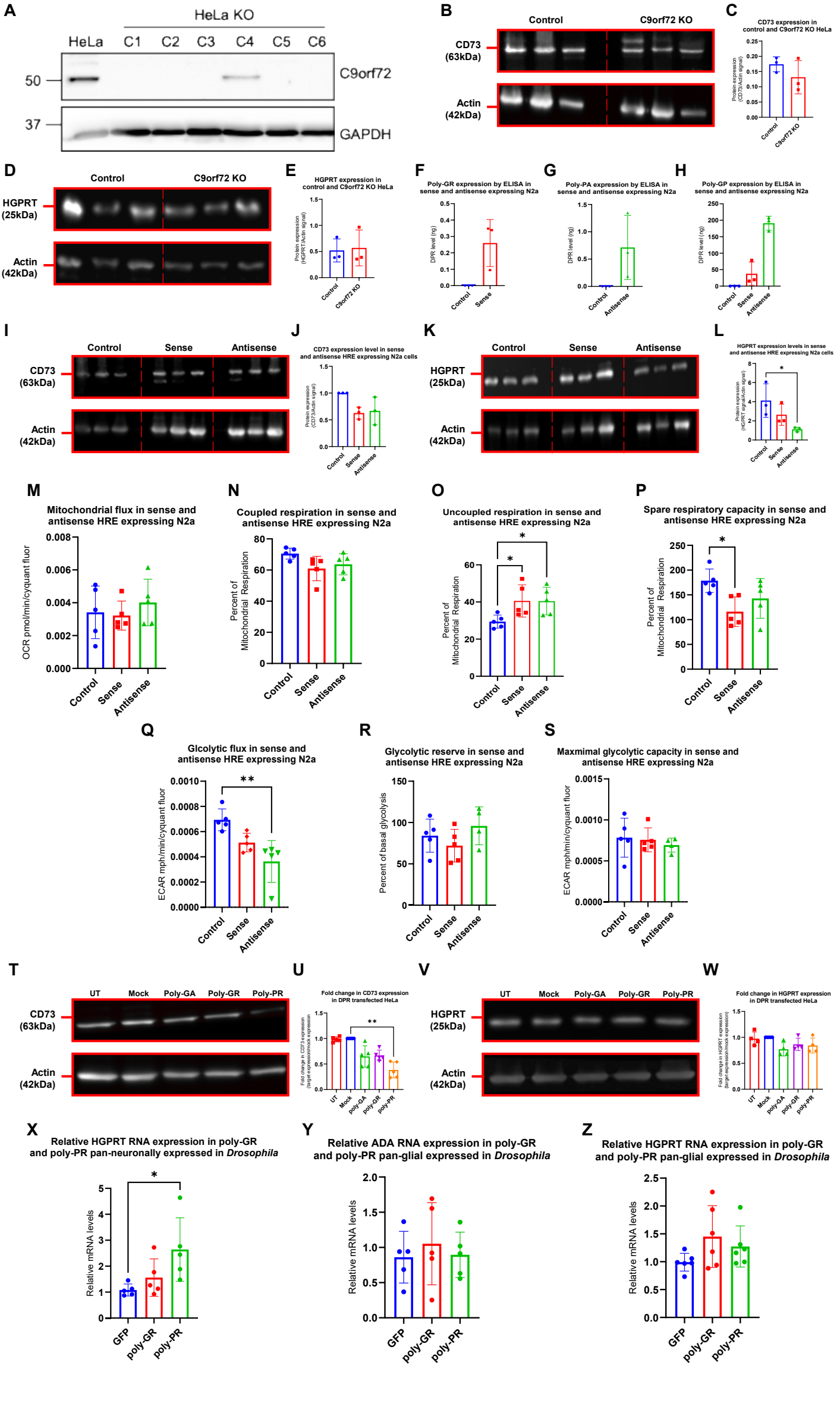

A
HeLa KO
HeLa
C1
C2
C3
C4
C5
C6
50
37
C9orf72
GAPDH
B
Control
C9orf72 KO
CD73 (63kDa)
Actin (42kDa)
C
CD73 expression in control and C9orf72 KO HeLa
Protein expression (CD73/Actin signal)
Control
C9orf72 KO
D
Control
C9orf72 KO
HGPRT (25kDa)
Actin (42kDa)
E
HGPRT expression in control and C9orf72 KO HeLa
Protein expression (HGPRT/Actin signal)
Control
C9orf72 KO
F
Poly-GR expression by ELISA in sense and antisense expressing N2a
DPR level (ng)
Control
Sense
G
Poly-PA expression by ELISA in sense and antisense expressing N2a
DPR level (ng)
Control
Antisense
H
Poly-GP expression by ELISA in sense and antisense expressing N2a
DPR level (ng)
Control
Sense
Antisense
I
Control
Sense
Antisense
CD73 (63kDa)
Actin (42kDa)
J
CD73 expression level in sense and antisense HRE expressing N2a cells
Protein expression (CD73/Actin signal)
Control
Sense
Antisense
K
Control
Sense
Antisense
HGPRT (25kDa)
Actin (42kDa)
L
HGPRT expression levels in sense and antisense HRE expressing N2a cells
Protein expression (HGPRT signal/Actin signal)
Control
Sense
Antisense
M
Mitochondrial flux in sense and antisense HRE expressing N2a
OCR pmol/min/cyquant fluor
Control
Sense
Antisense
N
Coupled respiration in sense and antisense HRE expressing N2a
Percent of Mitochondrial Respiration
Control
Sense
Antisense
O
Uncoupled respiration in sense and antisense HRE expressing N2a
Percent of Mitochondrial Respiration
Control
Sense
Antisense
P
Spare respiratory capacity in sense and antisense HRE expressing N2a
Percent of Mitochondrial Respiration
Control
Sense
Antisense
Q
Glcolytic flux in sense and antisense HRE expressing N2a
ECAR mph/min/cyquant fluor
Control
Sense
Antisense
R
Glycolytic reserve in sense and antisense HRE expressing N2a
Percent of basal glycolysis
Control
Sense
Antisense
S
Maxmimal glycolytic capacity in sense and antisense HRE expressing N2a
ECAR mph/min/cyquant fluor
Control
Sense
Antisense
T
UT
Mock
Poly-GA
Poly-GR
Poly-PR
CD73 (63kDa)
Actin (42kDa)
U
Fold change in CD73 expression in DPR transfected HeLa
Fold change in CD73 expression (target expression/mock expression)
UT
Mock
poly-GA
poly-GR
poly-PR
V
UT
Mock
Poly-GA
Poly-GR
Poly-PR
HGPRT (25kDa)
Actin (42kDa)
W
Fold change in HGPRT expression in DPR transfected HeLa
Fold change in HGPRT expression (target expression/mock expression)
UT
Mock
poly-GA
poly-GR
poly-PR
X
Relative HGPRT RNA expression in poly-GR and poly-PR pan-neuronally expressed in Drosophila
Relative mRNA levels
GFP
poly-GR
poly-PR
Y
Relative ADA RNA expression in poly-GR and poly-PR pan-glial expressed in Drosophila
Relative mRNA levels
GFP
poly-GR
poly-PR
Z
Relative HGPRT RNA expression in poly-GR and poly-PR pan-glial expressed in Drosophila
Relative mRNA levels
GFP
poly-GR
poly-PR

Figure S4. C9-HRE gain-of-function model further characterisation. (A) C9orf72 expression in HeLa *C9orf72* knockout clones C1, C2, C3, C5 and C6. HeLa clone C5 was used in this study. (B) Representative images and (C) densitometry analysis of *C9orf72*-KO HeLa ecto-5'-nucleotidase (CD73) Western blot. (D) Representative images and (E) densitometry analysis of *C9orf72*-KO HeLa hypoxanthine-guanine phosphoribosyltransferase (HGPRT) Western blot. (F) Glycine-arginine (poly-GR), (G) proline-alanine (poly-PA) and (H) glycine-proline (poly-GP) level measured by Meso Scale Discovery (MSD) ELISA in 38-repeat sense and 39-repeat antisense hexanucleotide repeat expansion (HRE) expressing N2a, presented relative to control background expression. (I) Representative images and (J) densitometry analysis of 38-repeat sense and 39-repeat antisense HRE expressing N2a CD73 Western blot analysed as fold change to matched control. (K) Representative images and (L) densitometry analysis of 38-repeat sense and 39-repeat antisense HRE expressing N2a HGPRT Western blot. (M) Mitochondrial respiration, (N) coupled respiration, (O) uncoupled respiration, (P) spare respiratory capacity, (Q) glycolytic flux, (R) glycolytic reserve and (S) glycolytic maximal capacity in 38-repeat sense and 39-repeat antisense HRE expressing N2a. (T) Representative images and (U) densitometry analysis of 36-repeat glycine alanine (poly-GA), poly-GR and proline-arginine (poly-PR) expressing HeLa CD73 Western blot as fold change to JetPRIME mock transfection. (V) Representative images and (W) densitometry analysis of 36-repeat poly-GA, poly-GR and poly-PR expressing HeLa HGPRT Western blot as fold change to JetPRIME mock transfection. (X) HGPRT relative mRNA expression in 1000-repeat poly-GR and poly-PR pan-neuronally expressing *Drosophila*. (Y) Adenosine deaminase (ADA) relative mRNA expression in 1000-repeat poly-GR and poly-PR pan-glial expressing *Drosophila*. (Z) HGPRT relative mRNA expression in 1000-repeat poly-GR and poly-PR pan-glial expressing *Drosophila*. Data presented as mean and standard deviation of three to six biological replicates. Statistical analysis by unpaired t-test (C and E), Kruskal-Wallis test (J and Q), ordinary one-way ANOVA (L, M, N, O, P, R, S, X, Y and Z) or Friedman test (U and W). $*P \leq 0.05$, $**P \leq 0.01$. Where P value is not indicated results were non-significant. Uncropped Western images can be found in Supplementary Material.

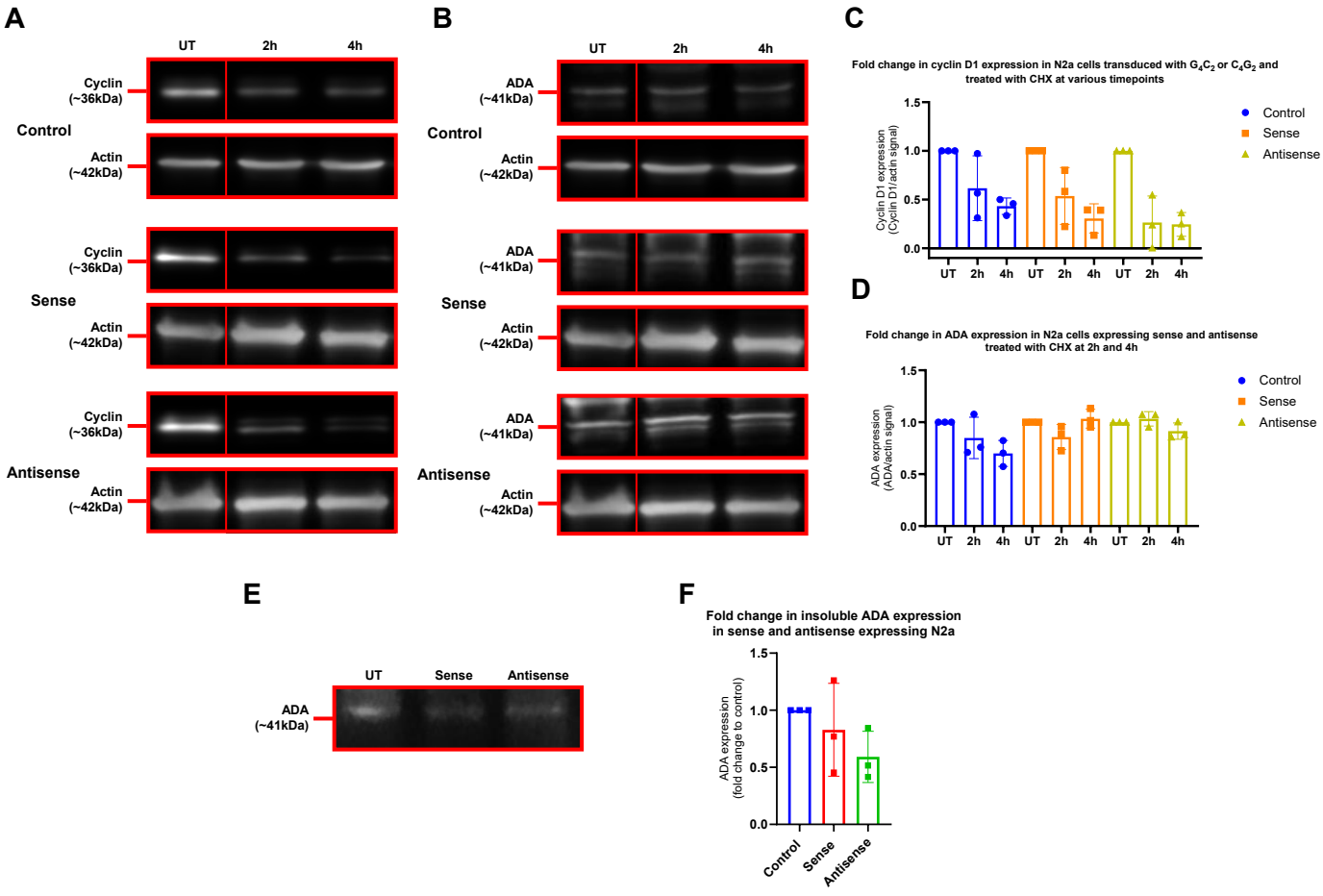
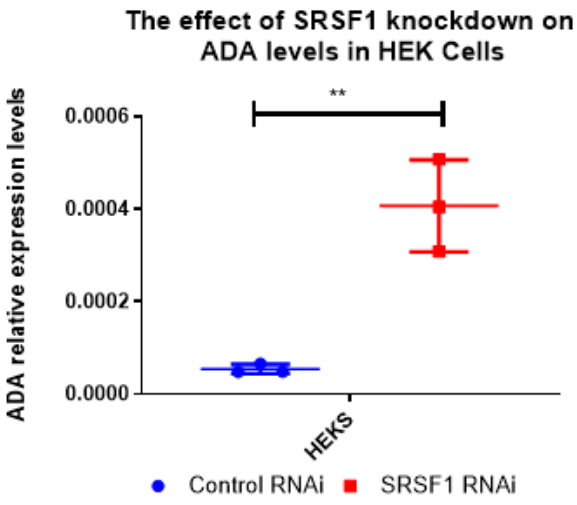
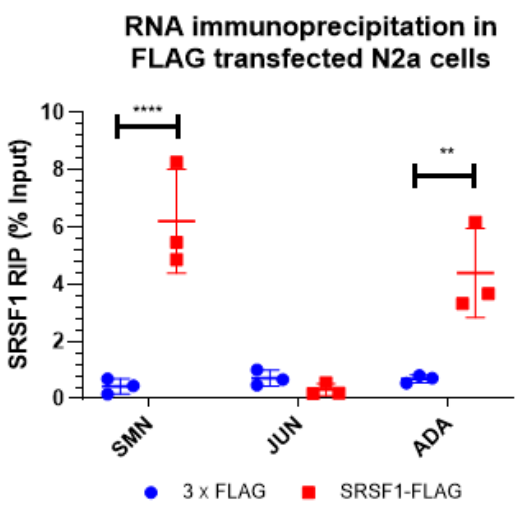

A
UT
2h
4h
Cyclin
(~36kDa)
Control
Actin
(~42kDa)
Cyclin
(~36kDa)
Sense
Actin
(~42kDa)
Cyclin
(~36kDa)
Antisense
Actin
(~42kDa)
B
UT
2h
4h
ADA
(~41kDa)
Control
Actin
(~42kDa)
ADA
(~41kDa)
Sense
Actin
(~42kDa)
ADA
(~41kDa)
Antisense
Actin
(~42kDa)
C
Fold change in cyclin D1 expression in N2a cells transduced with G4C2 or C4G2 and treated with CHX at various timepoints
Cyclin D1 expression
(Cyclin D1/actin signal)
1.5
1.0
0.5
0.0
UT
2h
4h
UT
2h
4h
UT
2h
4h
Control
Sense
Antisense
D
Fold change in ADA expression in N2a cells expressing sense and antisense treated with CHX at 2h and 4h
ADA expression
(ADA/actin signal)
1.5
1.0
0.5
0.0
UT
2h
4h
UT
2h
4h
UT
2h
4h
Control
Sense
Antisense
E
UT
Sense
Antisense
ADA
(~41kDa)
F
Fold change in insoluble ADA expression in sense and antisense expressing N2a
ADA expression
(fold change to control)
1.5
1.0
0.5
0.0
Control
Sense
Antisense

Figure S5. Gain-function-mechanisms do not affect ADA post-translational regulation. Representative images of (A) cyclin D1 and (B) adenosine deaminase (ADA) in control, 38-repeat sense and 39-repeat antisense HRE expressing Neuro2a (N2a) treated with cycloheximide (CHX) for 2h and 4h. Densitometry analysis of (C) cyclin D1 and (D) ADA in control, 38-repeat sense and 39-repeat antisense HRE expressing N2a treated with CHX for 2h and 4h presented as fold change to control. (E) Representative images and (F) densitometry analysis of ADA in insoluble fraction of 38-repeat sense and 39-repeat antisense HRE expressing N2a ADA Western blot presented as fold change to control. Data presented as mean and standard deviation of three biological replicates.

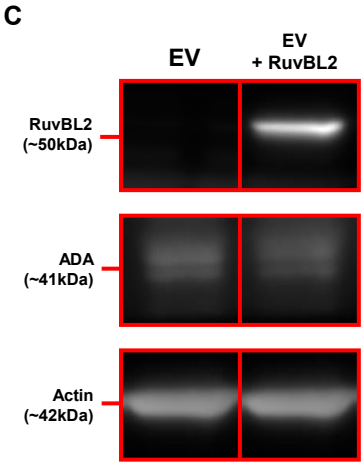
A


The effect of SRSF1 knockdown on ADA levels in HEK Cells
ADA relative expression levels
0.0006
0.0004
0.0002
0.0000
**
HEKS
Control RNAi
SRSF1 RNAi

B


RNA immunoprecipitation in FLAG transfected N2a cells
SRSF1 RIP (% Input)
10
8
6
4
2
0
****
**
SMN
JUN
ADA
3 x FLAG
SRSF1-FLAG

C


EV
EV + RuvBL2
RuvBL2 (~50kDa)
ADA (~41kDa)
Actin (~42kDa)

D

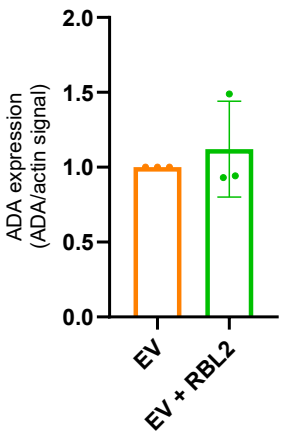

ADA expression in empty vector and RuvBL2 overexpression Hela
ADA expression (ADA/actin signal)
2.0
1.5
1.0
0.5
0.0
EV
EV + RBL2

Figure S6. SRSF1 binds and elevates ADA expression, RuvBL2 does not affect ADA expression alone. (A) Relative RNA expression of adenosine deaminase (ADA) in control and serine/arginine-rich splicing factor 1 (SRSF1) shRNA transfected HEK293T (B) RNA immunoprecipitation of survival motor neuron protein (SMN), jun proto-oncogene, AP-1 transcription factor subunit (JUN) and ADA from FLAG and SRSF1 transfected Neuro2a (N2a). (C) ADA Western blot representative images and (D) densitometry analysis of HeLa transfected with empty vector (EV) and EV + RuvB-like 2 (RuvBL2) presented as fold change to control. Data presented as mean and standard deviation of three biological replicates. Analysis performed by unpaired t-test (A), two-way ANOVA (B) or Wilcoxon test (D). *$P \leq 0.05$, **$P \leq 0.01$, ****$P \leq 0.0001$. Where P value is not indicated results were non-significant. Uncropped Western images can be found in Supplementary Material.