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Supplementary Information for

Partial inhibition of the overactivated Ku80-dependent DNA repair pathway rescues neurodegeneration in *C9ORF72*-ALS/FTD

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This PDF file includes:

Supplementary text

Figs. S1 to S11

Tables S1 to S4

Other supplementary materials for this manuscript include the following:

Materials and methods

Motor Neuron Differentiation from iPSC Lines. Motor neurons were differentiated as described (Lopez-Gonzalez et al. 2016). Briefly, iPSCs were plated and expanded in mTSER1 medium (Stem Cell Technologies) in Matrigel-coated wells. Twenty-four hours after plating, the culture medium was replaced with neuroepithelial progenitor (NEP) medium, DMEM/F12, neurobasal medium at 1:1, 0.5X N2, 0.5X B27, 0.1 mM ascorbic acid (Sigma), 1X Glutamax (Invitrogen), 3 μM CHIR99021 (Tocris Bioscience), 2 μM DMH1 (Tocris Bioscience), and 2 μM SB431542 (Stemgent). After 6 days, NEPs were dissociated with accutase, split 1:6 into Matrigel-coated wells, and cultured for 6 days in motor neuron progenitor induction medium (NEP with 0.1 μM retinoic acid and 0.5 μM purmorphamine, both from Stemgent). Motor neuron progenitors were dissociated with accutase to generate suspension cultures. After 6 days, the cultures were dissociated into single cells, plated on laminin-coated plates/coverslips in motor neuron differentiation medium containing 0.5 μM retinoic acid, 0.1 μM purmorphamine, and 0.1 μM Compound E (Calbiochem) for 2 weeks and then in the same medium without Compound E for up to 4 months.

Western Blot Analysis. Fly heads were collected and homogenized with 2X Laemmli sample buffer (BioRad), and lysates from five fly heads were analyzed by SDS-PAGE. Motor neuron cultures were lysed with RIPA buffer (Thermo Scientific), and 20 μg of protein was separated by SDS-PAGE. Fly and motor neuron samples were immunoblotted with antibodies listed in *SI Appendix,* Table S6. After incubation, the membranes were washed with PBST and incubated with IRDye anti-rabbit or anti-mouse secondary antibodies (1:5000, LI-COR Biosciences).

Immunostaining. High-yield human motor neuron cultures from controls and *C9ORF72* carriers were fixed in 4% paraformaldehyde for 15 min and permeabilized with 0.3% Triton X-100 for 5 min. The cells were blocked with 5% bovine serum albumin for 30 min and incubated with the following primary antibodies overnight at 4ºC: goat anti-ChAT (1:200, Millipore, Cat. no. AB144P), rabbit anti-phosphorylated ATM (1:1000, Abcam, Cat. no. ab81292), rabbit anti Ku80 (1:500, Cell Signaling, Cat. no. 2753), and cleaved caspase 3 (1:200, Cell Signaling, Cat. no. 9661).

Comet Assay. A comet assay was done as described (Lopez-Gonzalez et al., 2016). Briefly, iPSCs derived neurons from *C9ORF72* line 26L6 and *Ku80* heterozygous lines 26L6-34E and 26L6-64A were dissociated to obtain single-cell suspensions. These cells were mixed with 1% low-gelling agarose solution and placed on glass slides and allowed to gel. These slides were gently submerged in lysis solution and left overnight at 4 °C. Slides were transferred to an electrophoresis solution, run at 0.6 V/cm for 25 min, removed, and submerged in rinse buffer for 30 min at room temperature. The slides were then incubated with 2.5 μg/ml SYBR Safe (Invitrogen) for 20 min and washed with distilled water. For each experimental condition, 100 cells were analyzed with Image J software and scored according to tail length and the percentage of DNA in the tail.

Genetic Analysis of iPSCs Lines. In order to analyze chromosome abnormalities, we performed genetic analysis using the kit from Stem Cell Technologies, which examines 8 most common karyotype abnormalities found in human embryonic stem cells and iPSCs. qPCR-based analysis was performed to examine the following chromosomes: 1q, 4p, 8q,

10p, 12p, 17q, 18q, 20q and Xp. In order to do the test, genomic DNA was extracted from control and *C9ORF72* iPSC lines as well as all the CRISPR-Cas9 lines used in this study. The qPCR was performed following manufacturer's instructions using 10 ng of genomic DNA per sample.

Short Tandem Repeat (STR) Analysis in iPSCs. Short tandem repeat analysis was performed using The PowerPlex® Fusion 6C System (Promega). Parental *C9ORF72* iPSC lines 26L6 and 27L11 as well as CRISPR-Cas9 modified lines 26L6-34E, 26L664A, 26Z90 and 27M91 were cultured and then genomic DNA was isolated to performed end point PCR following manufacturer's instructions. 1 ng of genomic DNA was amplified. Amplification products were then mixed with WEN Internal Lane Standard 500 and analyzed using an Applied Biosystems® 3500xL Genetic Analyzer and data analyzed with peak scanner software version 2 (Applied biosystems).

RNA Extraction and Quantitative Real-time PCR. Total RNA from fly brains or iPSC-derived neurons was extracted with the RNeasy Mini Kit (Qiagen) and reverse transcribed to cDNA with the TaqMan Reverse Transcription Kit (Applied Biosystems). Quantitative PCR was done with SYBR Green Master Mix (Applied Biosystems) Using primers listed in *SI Appendix,* Table S3. Ct values for each gene were normalized to GAPDH. Relative mRNA expression was calculated with the double delta Ct method.

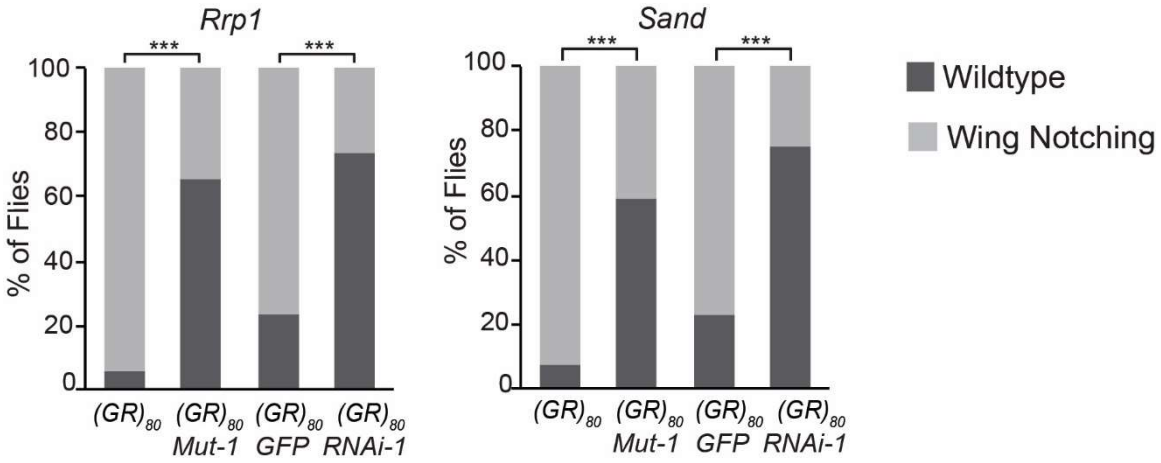


Fig. S1. Quantification of the effects of some suppressor genes on poly(GR)-induced loss of non-neuronal cells. $(GR)_{80}$-expressing flies with or without non-neuronal cell death were counted. For, w^{1118} or *UAS-GFP* flies crossed to *Vg-Gal4*/*CyO*; *UAS-*$(GR)_{80}$/*TM6,Tb* flies served as negative controls for genetic alleles or *UAS-RNAi* lines of each modifier gene, respectively. The *Drosophila* lines for modifier genes are described in Table S2. ***P<0.001 by chi-square analysis. 80-130 flies were analyzed for each genotype.

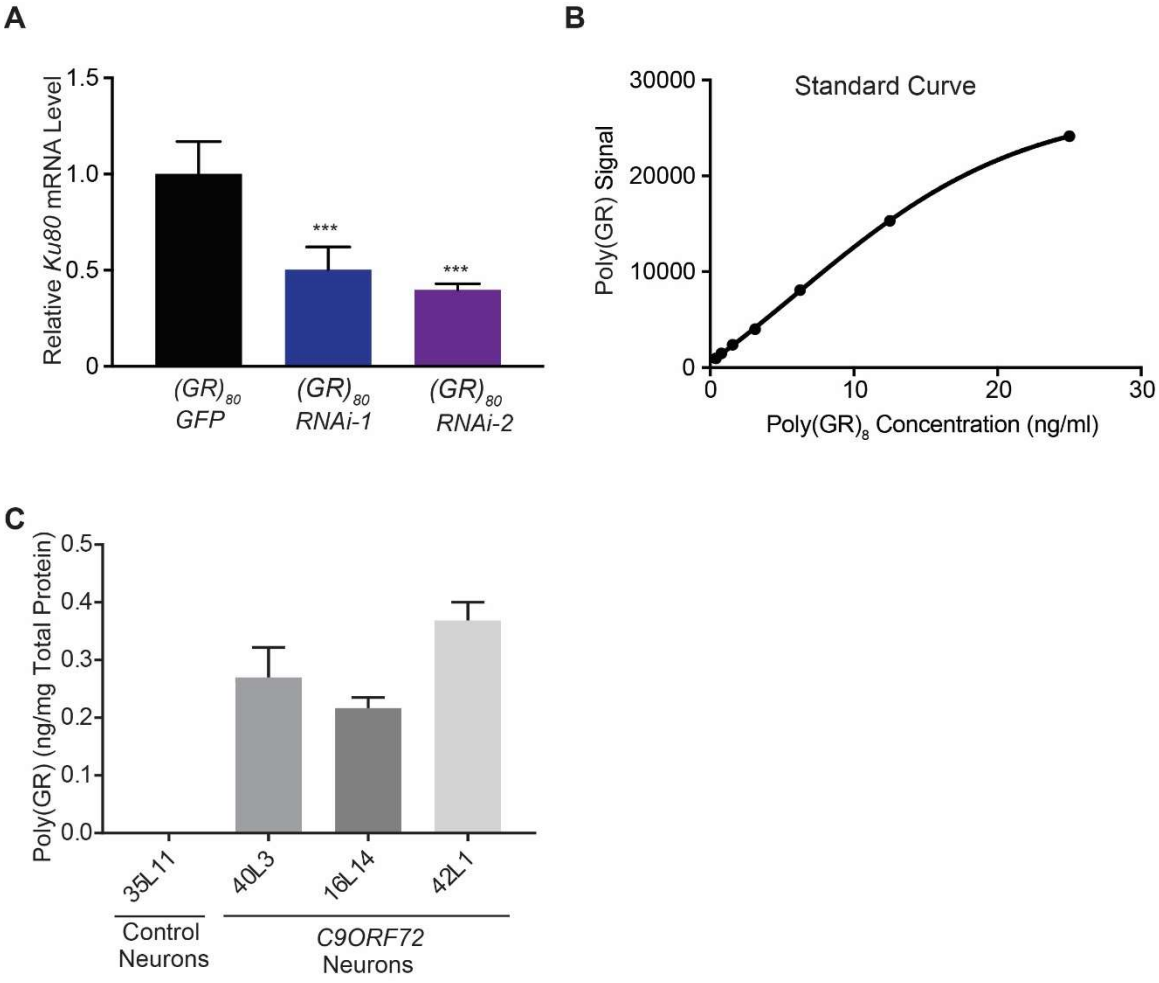


Fig. S2. Efficiency of *Ku80* RNAi in the fly eye and poly(GR) accumulation in *C9ORF72* iPSC-derived motor neurons. (A) The relative *Ku80* mRNA levels before and after RNAi knockdown in the fly eye. RNAi expression was driven by GMR-Gal4. Thus, the actual knockdown efficiency in photoreceptor neurons might be higher. Values are mean ± S.D. of 3 independent experiments. ***P<0.001 by one-way ANOVA. (B) Standard curve for poly(GR) ELISA measurement. (C) The expression level of Poly(GR) in 3 *C9ORF72* iPSC lines-derived motor neuron cultures. These cultures were of 2-month old and derived from two independent differentiations.

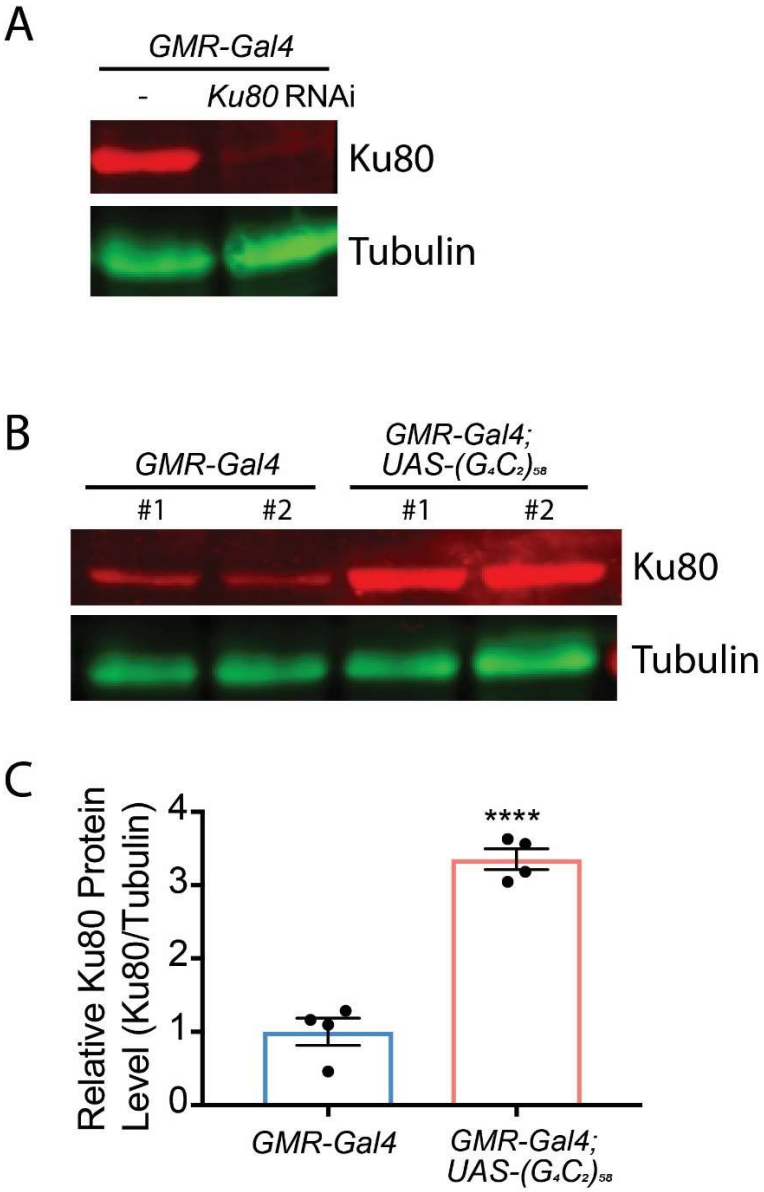


Fig. S3. The level of Ku80 protein is also increased in *$(G_4C_2)_{58}$* flies. (A) Western blot analysis of Ku80 protein levels in control (*GMR-Gal4/+*) and *Ku80* knockdown flies (*GMR-Gal4/+*; *UAS-Ku80 RNAi*) demonstrates the specificity of the Ku80 antibody. (B) A representative image of western blot analysis of Ku80 protein levels in control (*GMR-Gal4/GMR-Gal4*) and $(G_4C_2)_{58}$-expressing flies (*GMR-Gal4/GMR-Gal4*; *UAS-$(G_4C_2)_{58}$/+*). (C) Relative Ku80 protein levels for the indicated fly lines. *n*=4 independent experiments. All values are mean ± SEM. ****P<0.0001 by Student's t test.

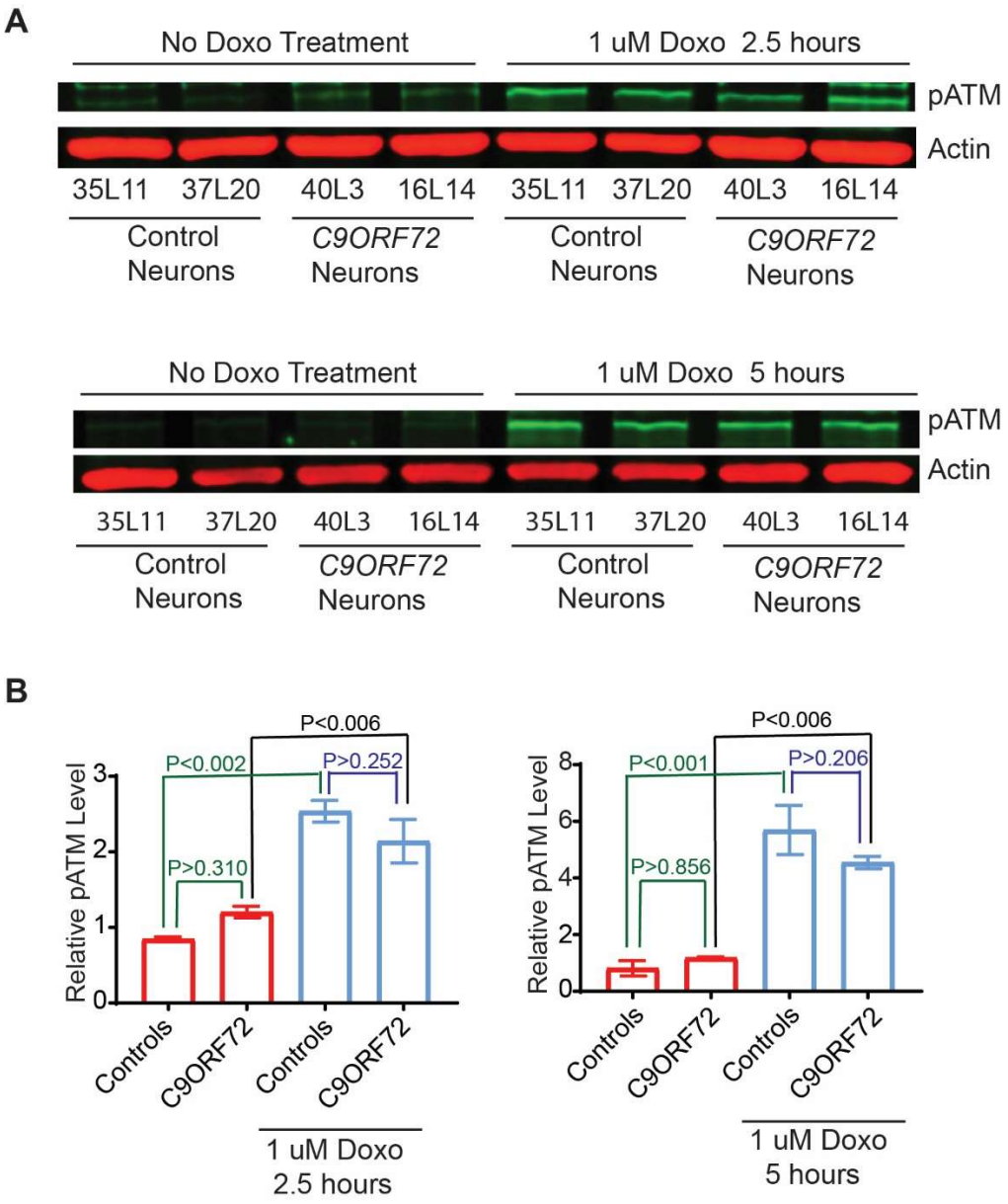


Fig. S4. DNA damage increased pATM levels in both control and *C9ORF72* iPSC-derived motor neurons. (A) Western blot analysis of 1-month-old neurons derived from two control and two *C9ORF72* iPSC lines. Neurons were treated with doxorubicin (1 μM) or left untreated. (B) Quantification of western blots shows a significant increase in pATM levels after 2.5 h and 5 h of doxorubicin treatment. Values are mean ± SEM of one differentiation of two control and two *C9ORF72* iPSC lines. *P* values were determined by one-way ANOVA and Tukey's test.

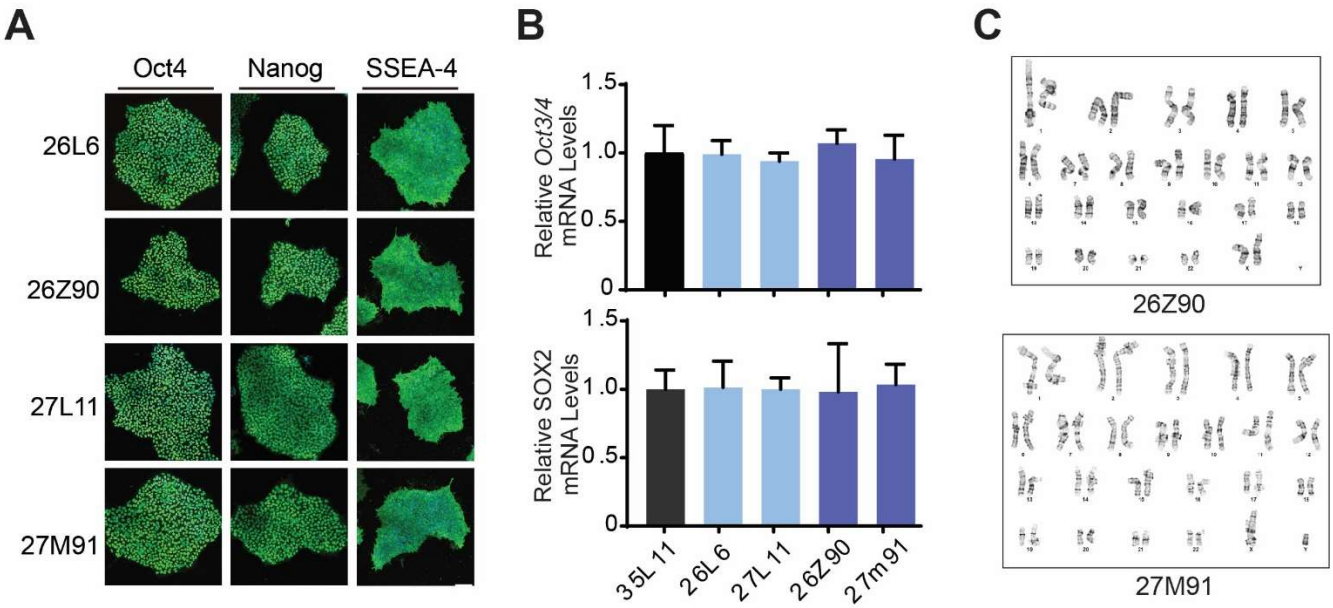


Fig. S5. Characterization of isogenic iPSC lines 26Z90 and 27M91 after CRISPR-Cas9 deletion of expanded G_4C_2 repeats from parental *C9ORF72* iPSC lines 26L6 and 27L11. (A) Expression of the pluripotency markers Oct-4, Nanog and SSEA-4 in iPSCs of different lines. Scale bar, 100 μm. (B) Quantitative RT-PCR analysis of expression levels of pluripotent stem cell markers SOX2 and Oct3/4. Values are mean ± SEM of 2 independent iPSC cultures shows no significant difference between different lines in the mRNA levels of *Oct3/4* (P=0.9990) and *SOX2* (P=0.9621). These P values were determined by one-way ANOVA. (C) Karyotype analysis of iPSC lines 26Z90 and 27M91 shows no chromosomal abnormalities.

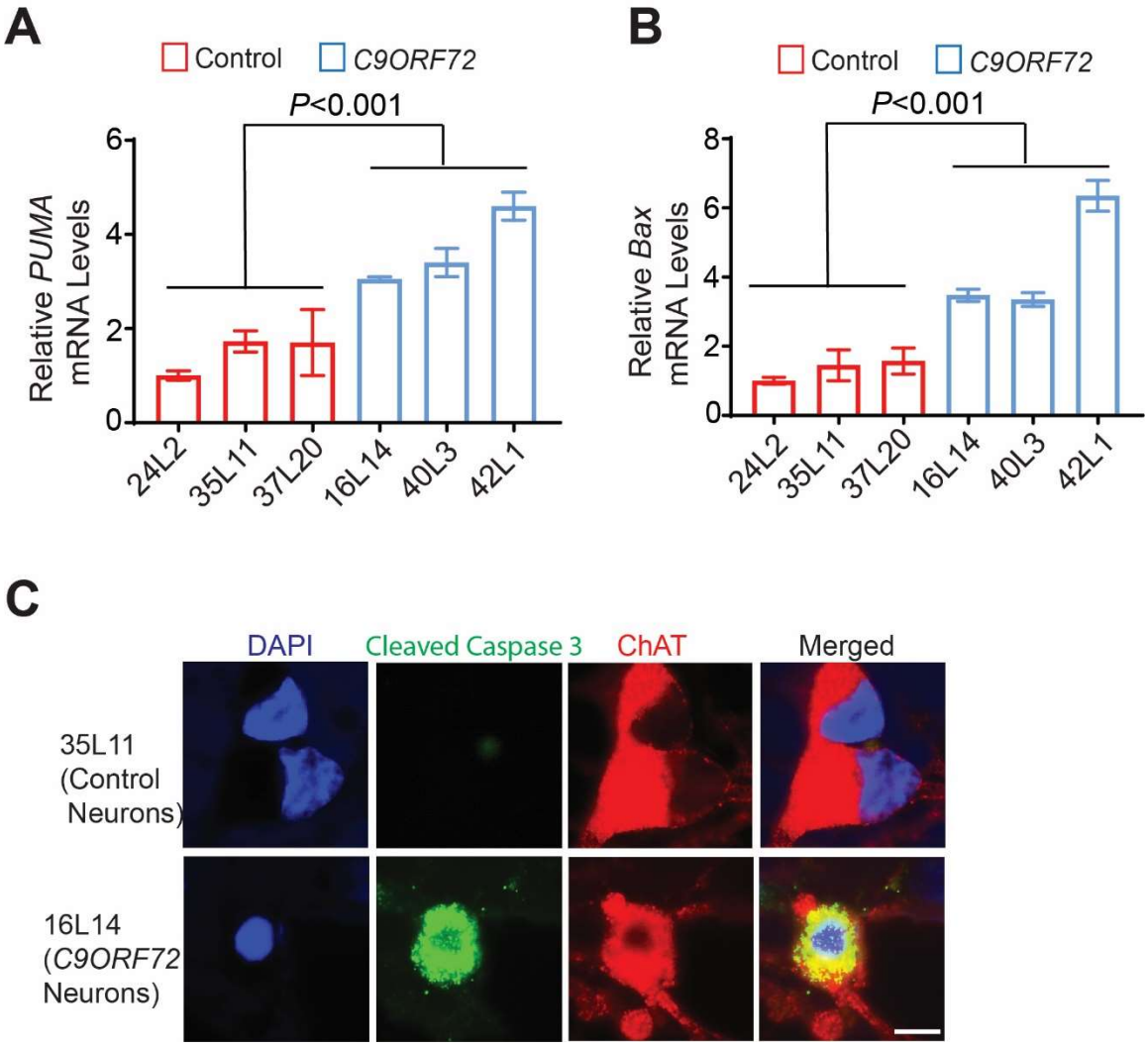


Fig. S6. Some apoptotic markers are upregulated in iPSC-derived motor neurons from *C9ORF72* carriers. (A) qPCR analysis of relative levels of PUMA mRNA. *(B)* qPCR analysis of relative mRNA levels of Bax. Values are mean ± SEM of neurons from two independent differentiations. Two-tailed Student's t test was used to compare 3 control subjects and 3 *C9ORF72* patients. Scale bar, 20 µm. (C) Cleaved caspase-3 and ChAT Immunostaining in iPSC-derived motor neurons. Scale bar, 10 µm.

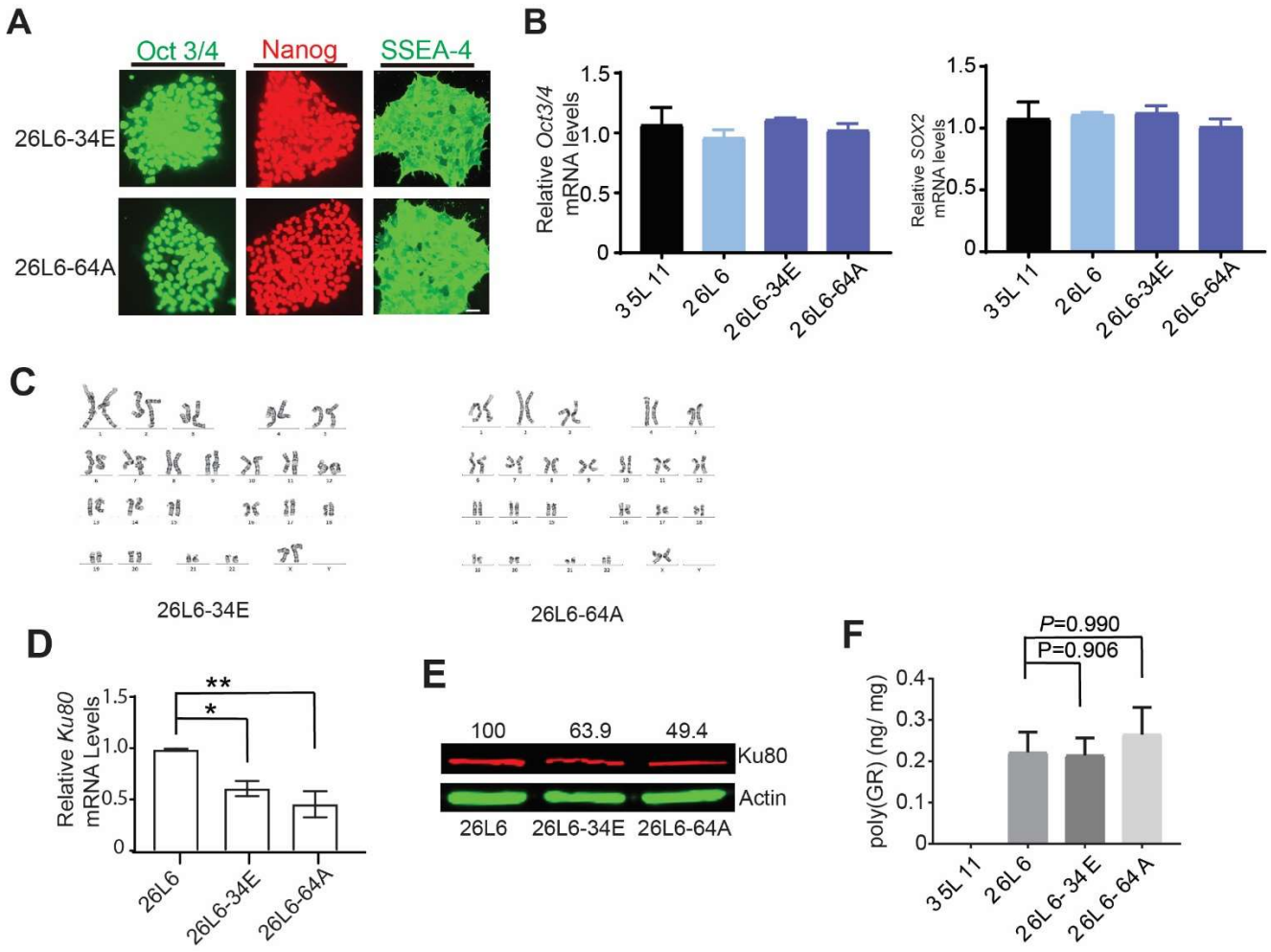


Fig. S7. Characterization of *Ku80* heterozygous knockout iPSC lines generated by CRISPR-Cas9 technology. (A) Immunostaining analysis of stem cell marker expression in heterozygous *Ku80* knockout lines. Scale bar 10 μm. (B) Genetic deletion of one copy of *Ku80* does not affect the expression levels of some stem cell marker mRNAs. Values are mean ± SEM of 2 independent iPSC cultures. There is no significant difference between different lines in the mRNA levels of *Oct3/4* (P=0.7358) and *SOX2* (P=0.6592). These P values were determined by one-way ANOVA. (C) Karyotype analysis of the two heterozygous *Ku80* knockout lines. (D) *Ku80* mRNA expression levels in *C9ORF72* iPSC lines. Values are mean ± SEM from two independent iPSC cultures. *P<0.05, **P<0.01 by one-way ANOVA and Tukey's test. (E) Ku80 western blot analysis of iPSCs from the *C9ORF72* carrier line 26L6 and heterozygous *Ku80* knockout lines. (F) ELISA analysis of poly(GR) levels in 2-month-old motor neurons derived from three independent differentiations. Values are mean ± SEM from three independent differentiations analyzed by one-way ANOVA and Tukey's test.

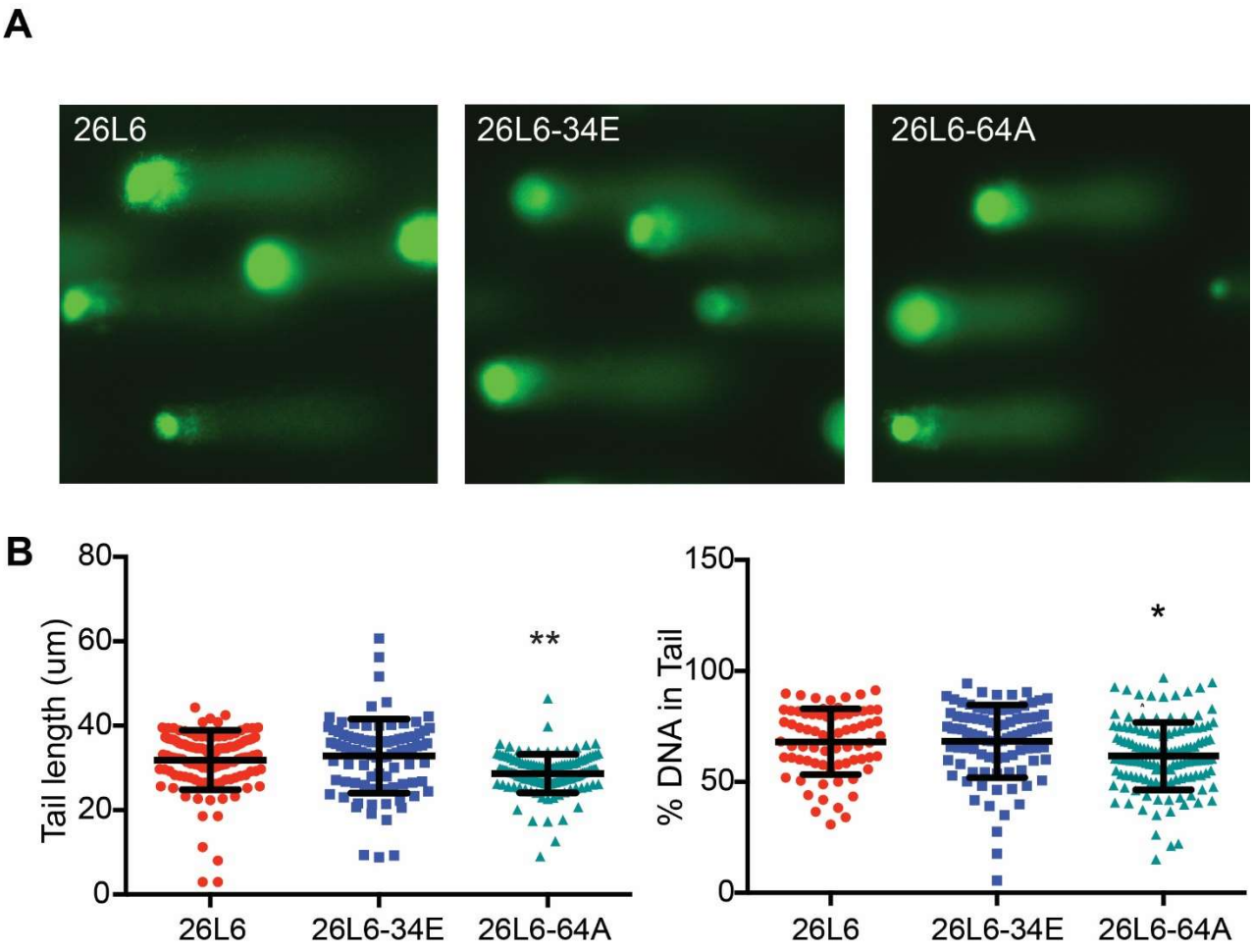


Fig. S8. Comet assay analysis. (A) Representative images of comet assay analysis of motor neurons differentiated from *C9ORF72* iPSC line 26L6 and its isogenic *Ku80* heterozygous knockout lines 26L6-34E and 26L6-64A. (B) Quantification of comet length tail and percentage of DNA in the tail in 2-month-old motor neurons from two independent differentiations. **P*<0.05. ***P*<0.01 by one-way ANOVA and Tukey's test.

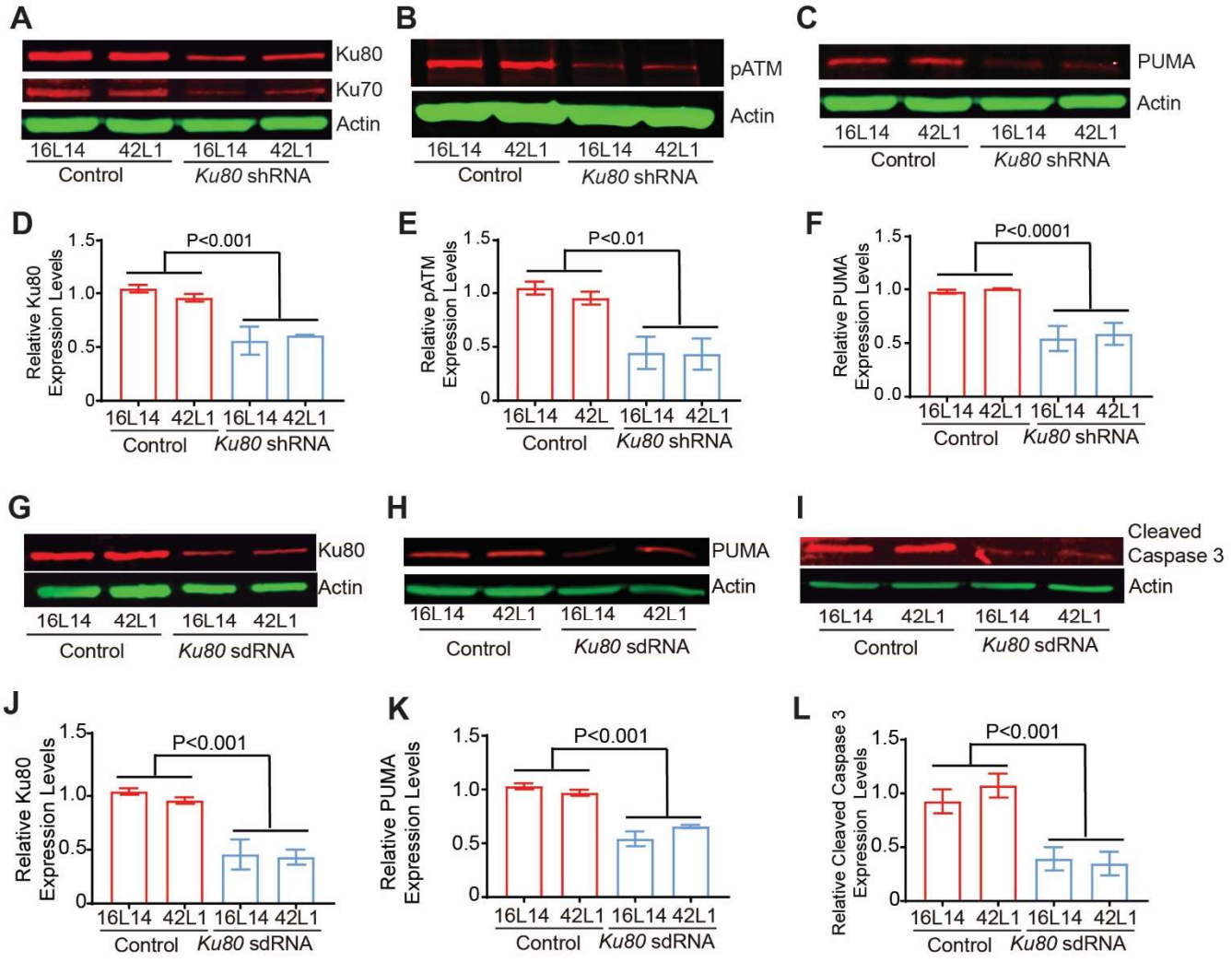


Fig S9. Knockdown of Ku80 mediated by small RNA rescues neurodegeneration in *C9ORF72*-ALS/FTD. (A–C) Western blot analysis of the relative levels of several proteins before and after sdRNA-mediated *Ku80* knockdown in neurons derived from two *C9ORF72* iPSC lines. (D–F) Quantification of western blot analysis from panels A–C. (G–I) Western blot analysis of the relative levels of several proteins before and after sdRNA-mediated *Ku80* knockdown in neurons derived from two *C9ORF72* iPSC lines. (J–L) Quantification of western blot analysis from panels G–I. For all quantifications, values are mean ± SEM of 2 independent differentiation experiments. Two-tailed Student's t test was used to compare two *C9ORF72* iPSC lines-derived neurons treated with control or *Ku80*-specific small RNAs.

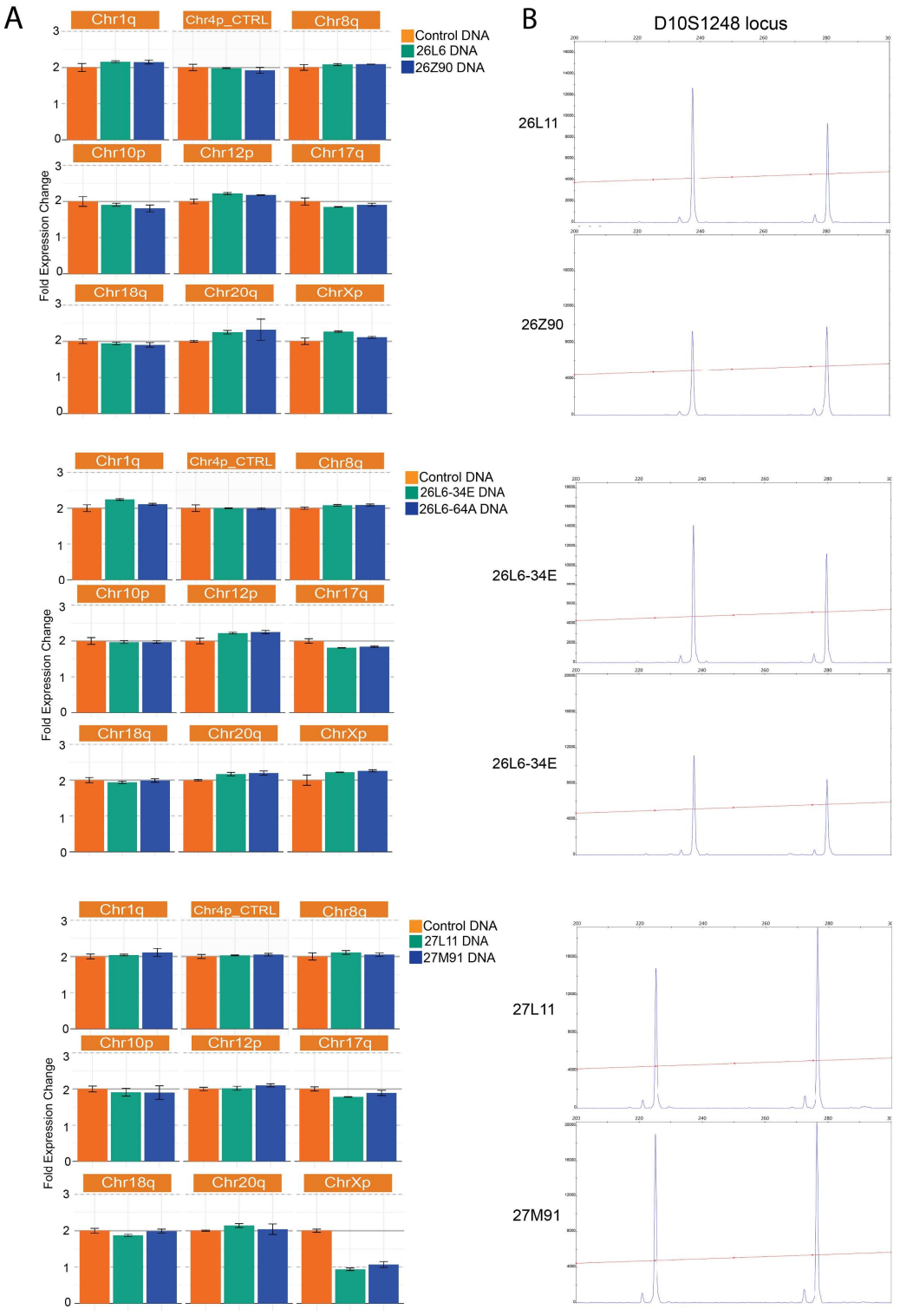


Fig S10. Genetic characterization and short tandem repeat analysis of CRISPR isogenic iPSCs lines**. (**A**)** qPCR based genetic analysis does not detect genetic abnormalities in iPSC lines 26L6, 26z90, 26L6-34E, 26L6-64A, 27L11 and 27m91. (B) Electropherogram showing the peaks of D10S1248 locus in lines listed above.

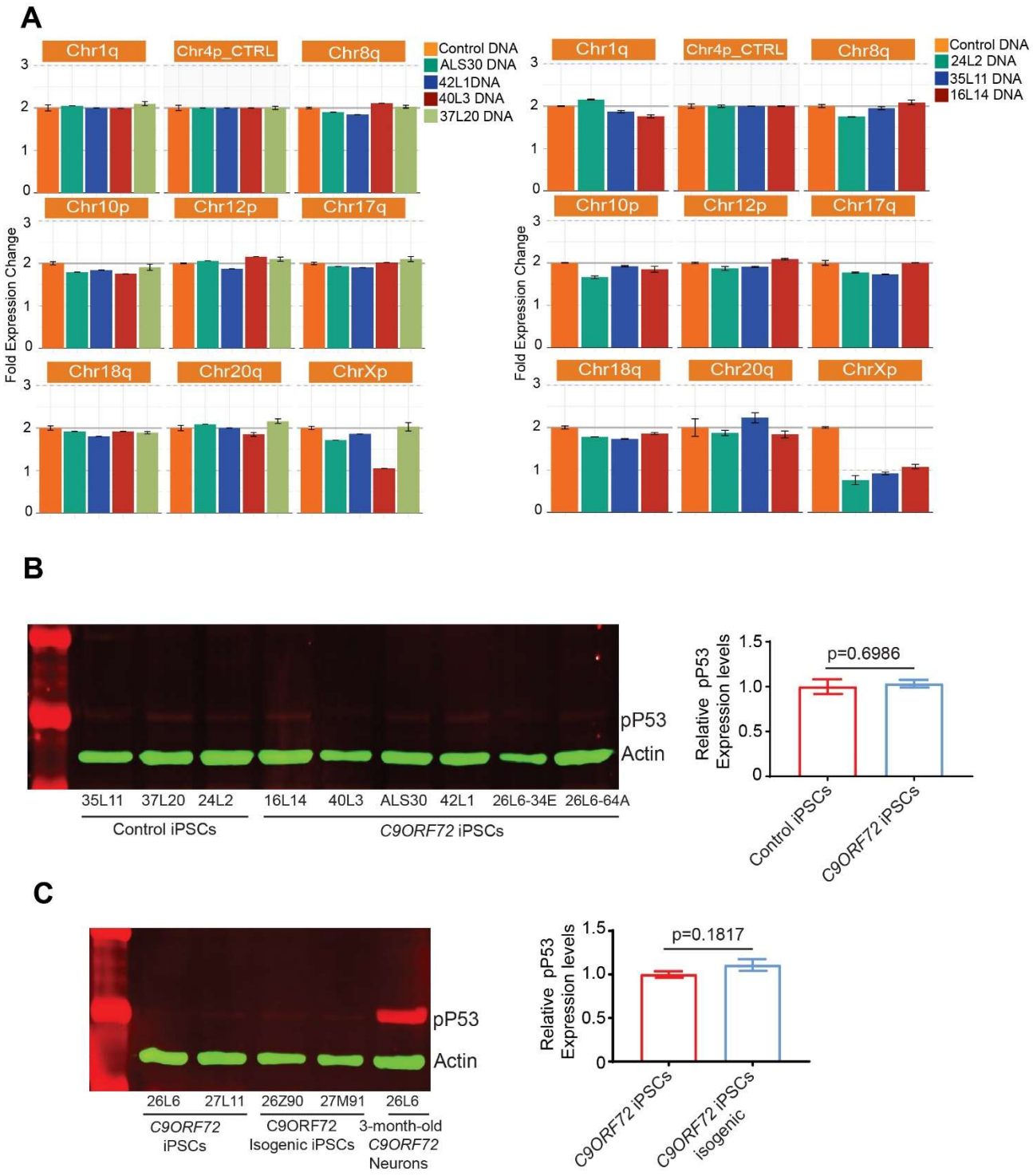


Fig S11. Further characterization of iPSC lines used in this study. (A) qPCR based genetic analysis did not detect genetic abnormalities in other iPSC lines used in this study. (B, C) phosphorylated P53 level is not elevated in any of iPSC lines used in this study. values are mean ± SEM. Two-tailed Student's t test was used for statistical analysis.

A

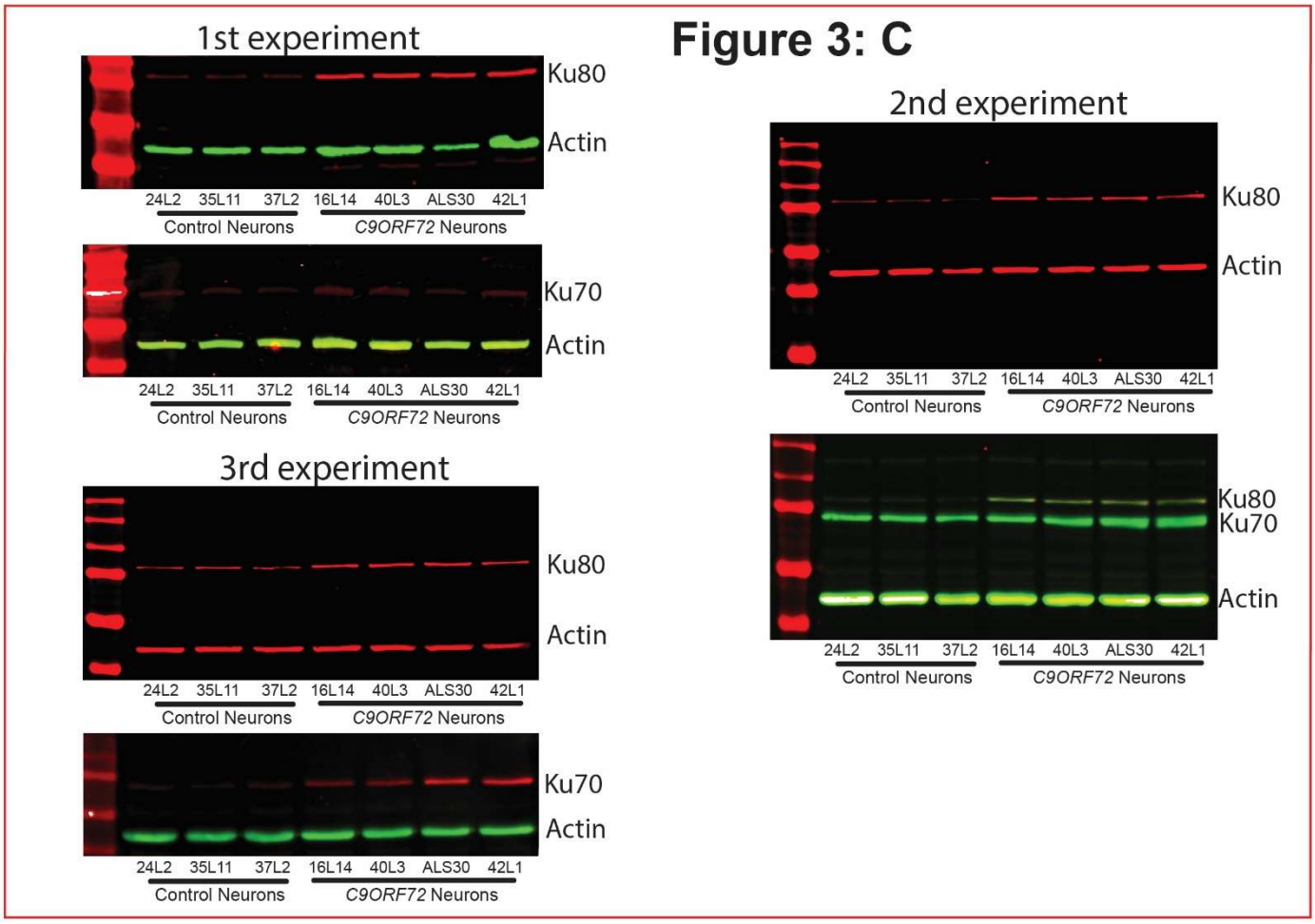


Figure 3: C
1st experiment
Ku80
Actin
24L2 35L11 37L2 16L14 40L3 ALS30 42L1
Control Neurons
C9ORF72 Neurons
Ku70
Actin
24L2 35L11 37L2 16L14 40L3 ALS30 42L1
Control Neurons
C9ORF72 Neurons
3rd experiment
Ku80
Actin
24L2 35L11 37L2 16L14 40L3 ALS30 42L1
Control Neurons
C9ORF72 Neurons
Ku70
Actin
24L2 35L11 37L2 16L14 40L3 ALS30 42L1
Control Neurons
C9ORF72 Neurons
2nd experiment
Ku80
Actin
24L2 35L11 37L2 16L14 40L3 ALS30 42L1
Control Neurons
C9ORF72 Neurons
Ku80
Ku70
Actin
24L2 35L11 37L2 16L14 40L3 ALS30 42L1
Control Neurons
C9ORF72 Neurons

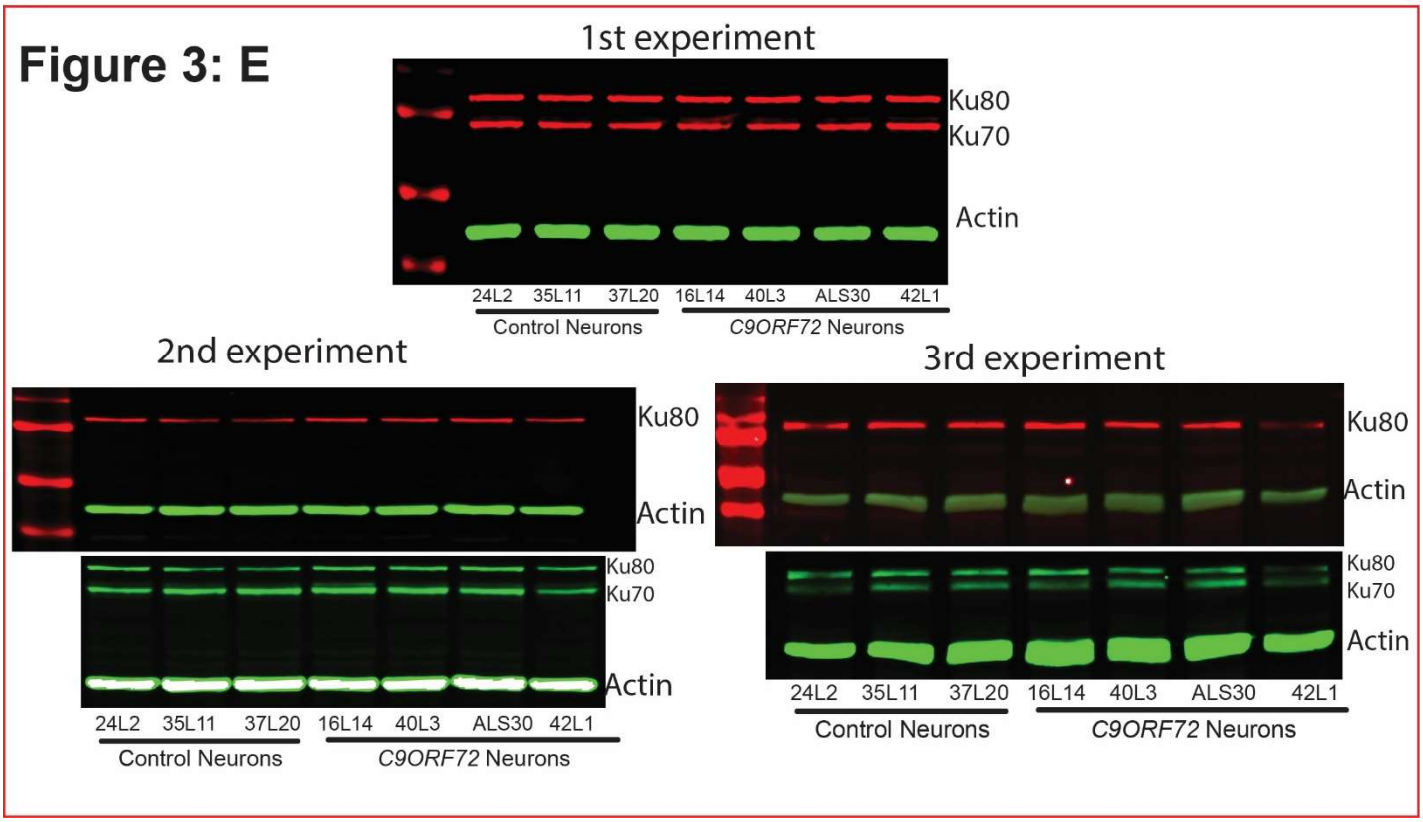


Figure 3: E
1st experiment
Ku80
Ku70
Actin
24L2 35L11 37L20 16L14 40L3 ALS30 42L1
Control Neurons
C9ORF72 Neurons
2nd experiment
Ku80
Actin
Ku80
Ku70
Actin
24L2 35L11 37L20 16L14 40L3 ALS30 42L1
Control Neurons
C9ORF72 Neurons
3rd experiment
Ku80
Actin
Ku80
Ku70
Actin
24L2 35L11 37L20 16L14 40L3 ALS30 42L1
Control Neurons
C9ORF72 Neurons

B

Figure 4: A

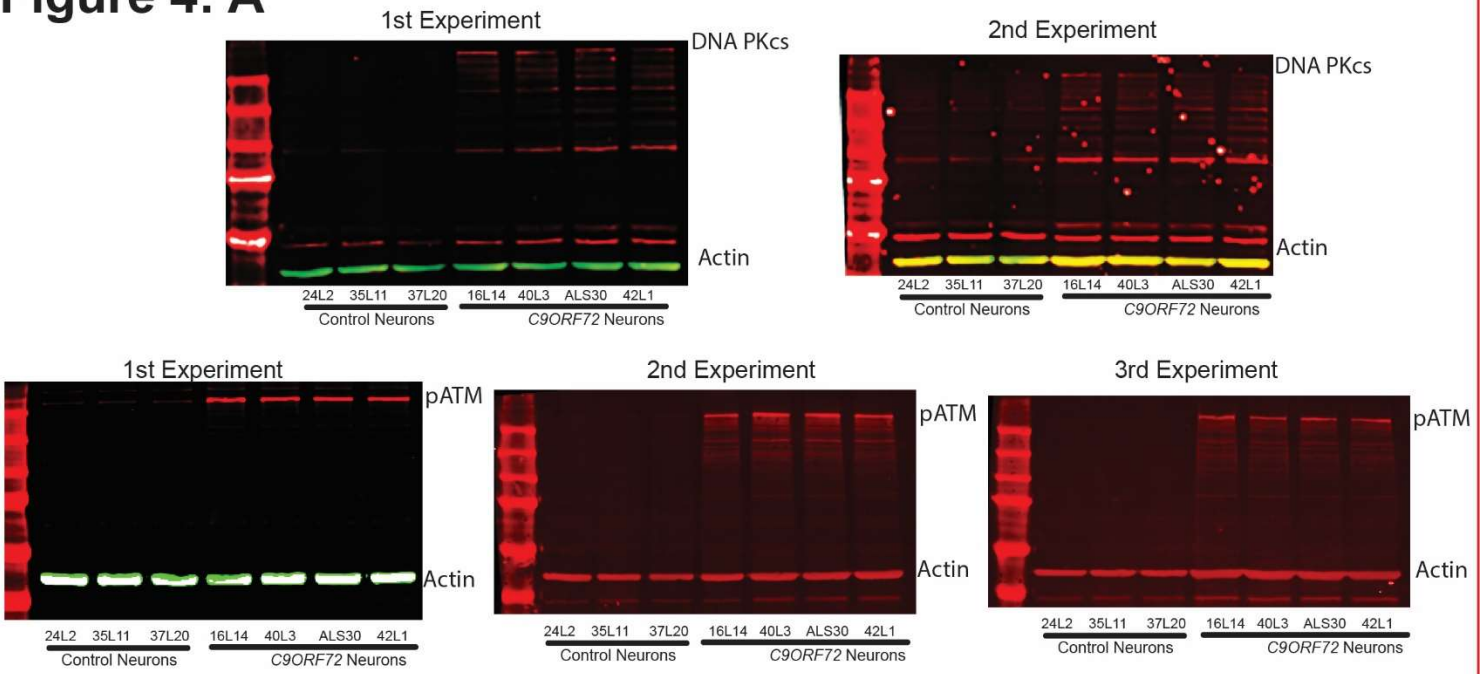
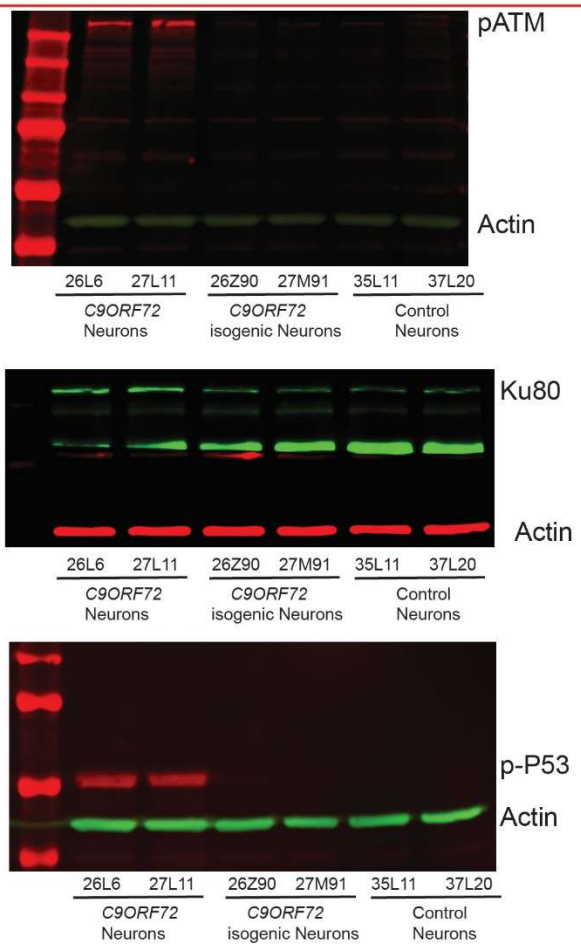


Figure 4: E



C

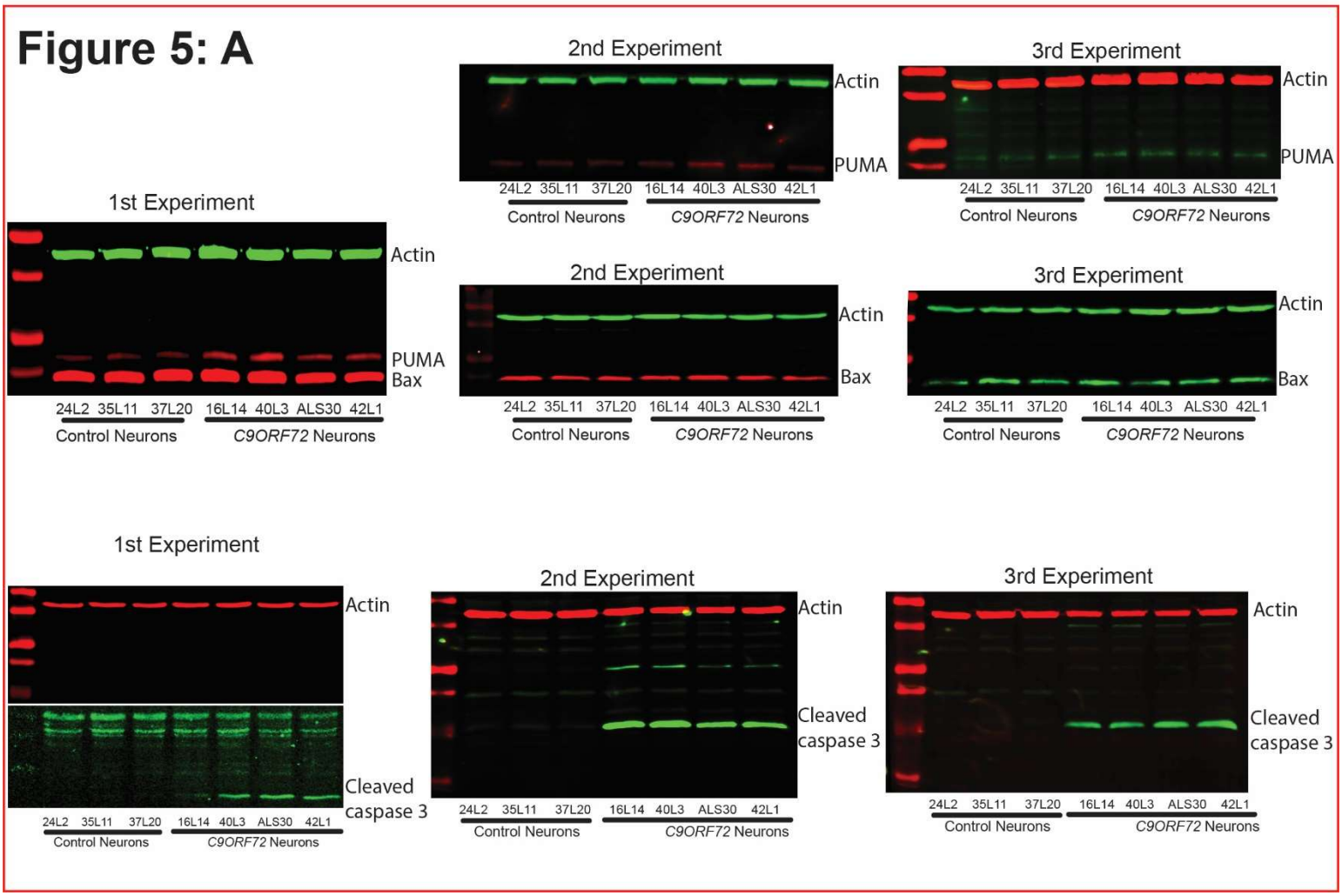
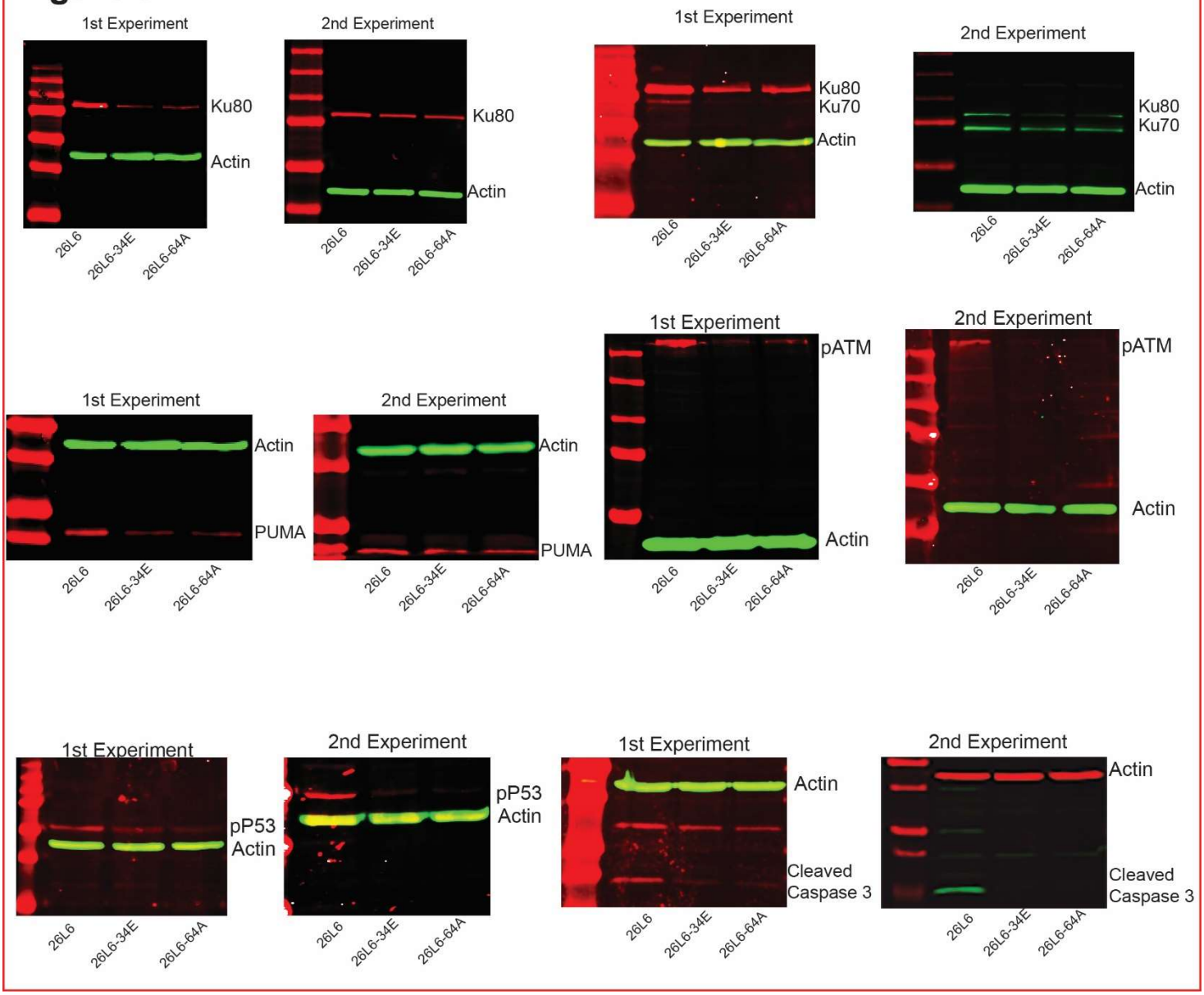


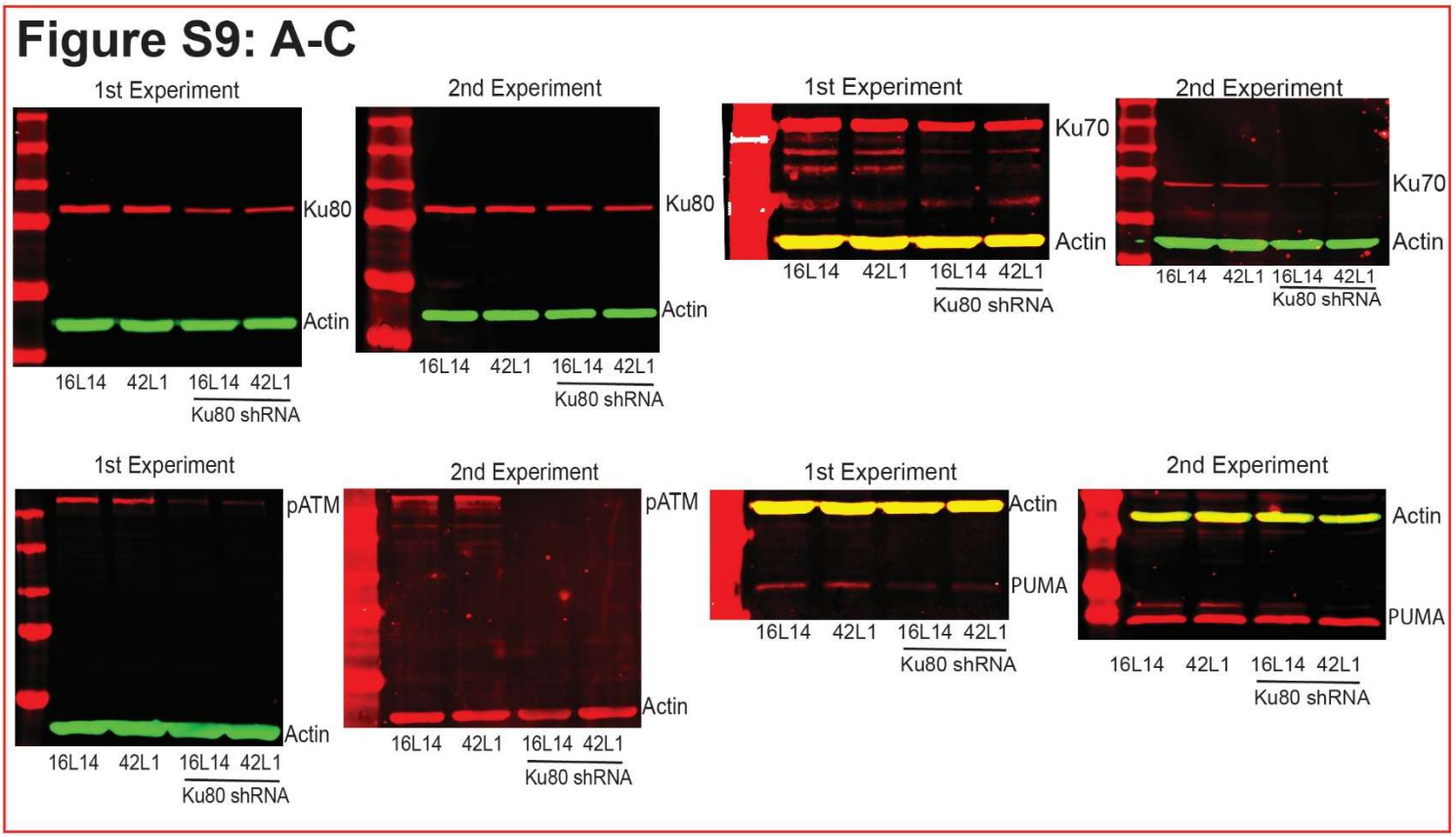
Figure 5: A
1st Experiment
Actin
PUMA
Bax
24L2 35L11 37L20 16L14 40L3 ALS30 42L1
Control Neurons
C9ORF72 Neurons
2nd Experiment
3rd Experiment
1st Experiment
Actin
Cleaved caspase 3
2nd Experiment
3rd Experiment

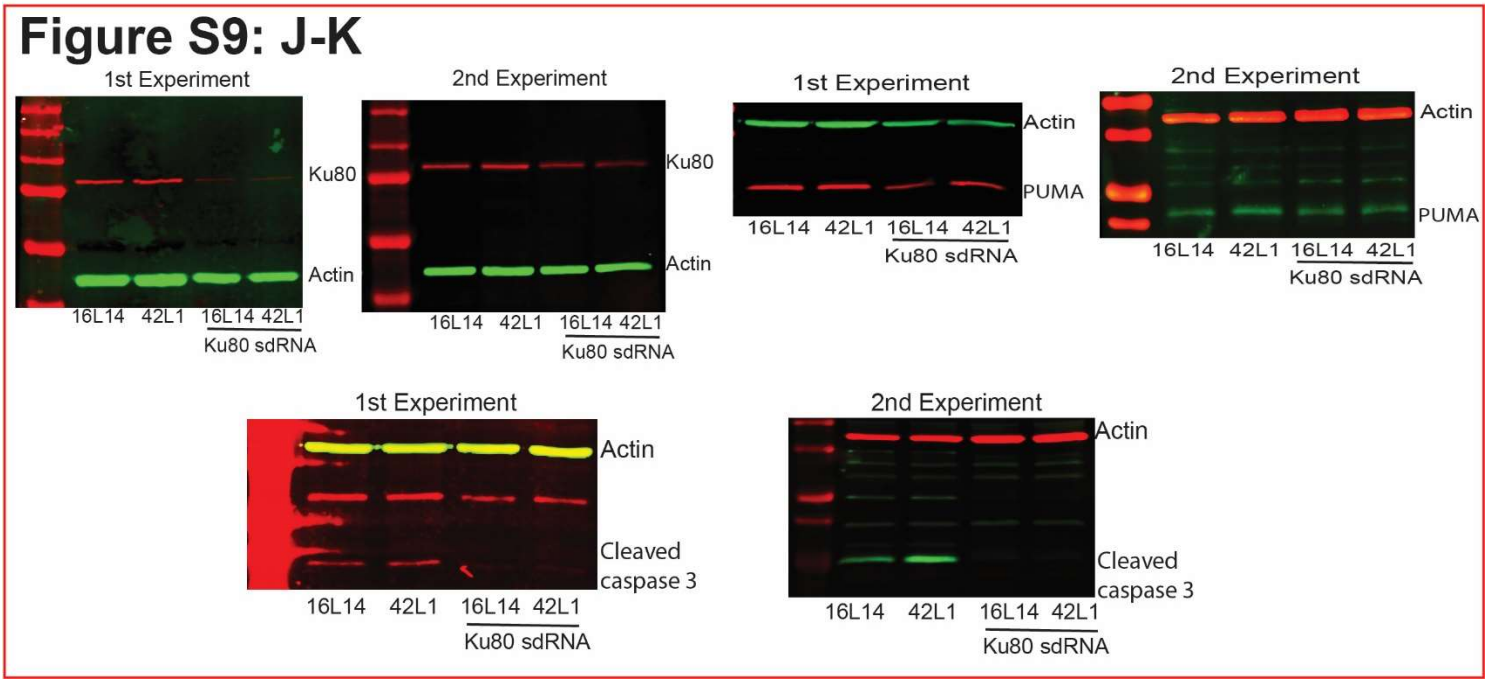
D

Figure 6: F-H


1st Experiment
2nd Experiment
1st Experiment
2nd Experiment
Ku80
Actin
Ku80
Actin
Ku80
Ku70
Actin
Ku80
Ku70
Actin
26L6
26L6-34E
26L6-64A
1st Experiment
2nd Experiment
1st Experiment
2nd Experiment
Actin
PUMA
Actin
PUMA
pATM
Actin
pATM
Actin
1st Experiment
2nd Experiment
1st Experiment
2nd Experiment
pP53
Actin
pP53
Actin
Actin
Cleaved Caspase 3
Actin
Cleaved Caspase 3

E





FigS12. Immunoblot data from: (A) figure 3, (B) figure 4, (C) figure 5, (D) figure 6 and (E) figure S9.

Table S1. Deficiency(*Df*) modifiers from the primary screen

BL Stock #	Deficiency Name	Locus Deleted	Phenotype
90	*Df(2L)C144*	22F4--23C3	Suppressor
744	*Df(2L)M24F-B*	24E1--25A2	Suppressor
4959	*Df(2L)C'*	h35--40A1	Suppressor
4961	*Df(2R)Kr10*	60E10--60F5	Suppressor
5330	*Df(2L)ed1*	24A2--24D4	Suppressor
6338	*Df(2L)BSC6*	26D3--26F7	Suppressor
6507	*Df(2L)drm-P2*	23F3--24A2	Suppressor
7548	*Df(2R)Exel6066*	53F8--54B6	Suppressor
8045	*Df(2R)ED1612*	42A13--42E6	Suppressor
8904	*Df(2L)ED4651*	23B8--23F3	Suppressor
8906	*Df(2L)ED678*	29F5--30B12	Suppressor
8908	*Df(2L)ED94*	21E2--21E3	Suppressor
8918	*Df(2R)ED3683*	55C2--56C4	Suppressor
9266	*Df(2L)ED1473*	39B4--40A5	Suppressor
9610	*Df(2L)BSC180*	23B7--23C3	Suppressor
9615	*Df(2L)BSC188*	26F1--27A2	Suppressor
24626	*Df(2L)ED50001*	21A1--21B1	Suppressor
24652	*Df(2L)ED441*	27A1--27E1	Suppressor
25430	*Df(2R)BSC597*	58A2--58F1	Suppressor
26542	*Df(2L)BSC690*	35D4--35D4	Suppressor
282	*Df(2R)X58-12*	58D1--59A1	Lethal
7494	*Df(2L)Exel6008*	22F4--23A3	Lethal
7783	*Df(2L)Exel7011*	22E1--22F3	Lethal
741	*Df(2R)M41A10*	h38R--h46	Enhancer
1072	*Df(2R)X1*	46C2;47A1	Enhancer
1469	*Df(2L)J39*	31C--32E5	Enhancer
6478	*Df(2L)BSC17*	30C3--30F1	Enhancer
8469	*Df(2L)BSC50*	30F5--31B1	Enhancer
23691	*Df(2R)BSC308*	52B5--52D15	Enhancer
24335	*Df(2R)BSC267*	44A4--44F1 44A4--44C4	Enhancer
24933	*Df(2R)BSC429*	51C2--51D1	Enhancer
25428	*Df(2R)BSC595*	47A3--47F1	Enhancer
25705	*Df(2R)BSC630*	41D3--41F11	Enhancer
25741	*Df(2R)BSC651*	51C5--51E2	Enhancer
26782	*Df(2L)lt109*	40F7--H36	Enhancer
27582	*Df(2R)BSC821*	57D10--57E6	Enhancer
29988	*Df(2R)BSC865*	59A4--59B7	Enhancer
30590	*Df(2R)BSC885*	57D2--57D10	Enhancer

Table S2. *Drosophila* lines used in this study

***Drosophila* lines**	**Souces**
$w^{[1118]}$	Yang et al., 2015
UAS-GFP	Yang et al., 2015
UAS-$(GR)_{80}$	Yang et al., 2015
UAS-$(GR)_{80}$-Control	Yang et al., 2015
Vg-Gal4/Cyo; UAS-(GR)80/TM6B	Yang et al., 2015
GMR-Gal4	BDSC(#9146)
Tub-$Gal80^{ts}$	BDSC(#7019)
GMR-Gal4, $Gal80^{ts}$, UAS-$(GR)_{80}$	Generated for this work
GMR-Gal4, $Gal80^{ts}$;UAS-$(GR)_{80}$-Control	Generated for this work
Dificiency Kit for 2nd Chromosome	BDSC(Identified locus were listed in figureS1)

Target genes	**Short name in figures**	**Souces**	**Identifier**	**Stock List Description**
nAChRalpha6		BDSC	BL9686	*nAChRalpha6[DAS2]*
nAChRalpha6		BDSC	BL25835	*y[1] v[1]; P{y[+t7.7] v[+t1.8]=TRiP.JF01853}attP2*
Bka		BDSC	BL31629	*y[1] v[1]; P{y[+t7.7] v[+t1.8]=TRiP.JF01415}attP2*
CG12769		BDSC	BL26769	*y[1] v[1]; P{y[+t7.7] v[+t1.8]=TRiP.JF02333}attP2*
CG12769		BDSC	BL13261	*y[1] w[67c23]; P{y[+mDint2] w[BR.E.BR]=SUPor-P}CG12769[KG03851]*
Dbr		BDSC	BL59280	*y[1] w[*]; Mi{y[+mDint2]=MIC}dbr[MI13343]*
Dbr		BDSC	BL43222	*y[1] sc[*] v[1]; P{y[+t7.7] v[+t1.8]=TRiP.GL01567}attP40*
Ercc1	*RNAi-1*	BDSC	BL36906	*y[1] sc[*] v[1]; P{y[+t7.7] v[+t1.8]=TRiP.GL01110}attP2*
Ercc1	*RNAi-2*	VDRC	V12622	*w[1118]; P{GD4103}v12622*
FKBP59		BDSC	BL28349	*y[1] v[1]; P{y[+t7.7] v[+t1.8]=TRiP.JF02985}attP2*
FKBP59		BDSC	BL35612	*y[1] sc[*] v[1]; P{y[+t7.7] v[+t1.8]=TRiP.GL00453}attP2*
Ku80	*RNAi-1*	BDSC	BL27710	*y[1] v[1]; P{y[+t7.7] v[+t1.8]=TRiP.JF02790}attP2*
Ku80	*RNAi-2*	VDRC	V37110	*w[1118]; P{GD1712}v37110*
L(2)gl	*RNAi-1*	BDSC	BL31089	*y[1] v[1]; P{y[+t7.7] v[+t1.8]=TRiP.JF01553}attP2*
L(2)gl	*RNAi-2*	BDSC	BL31517	*y[1] v[1]; P{y[+t7.7] v[+t1.8]=TRiP.JF01073}attP2*
Lig		BDSC	BL18242	*w[1118]; PBac{w[+mC]=RB}lig[e04268]/CyO*
Lig		BDSC	BL14943	*y[1]; P{y[+mDint2] w[BR.E.BR]=SUPor-P}lig[KG08209]/CyO; ry[506]*
Lig		BDSC	BL61857	*y[1] v[1]; P{y[+t7.7] v[+t1.8]=TRiP.HMJ23346}attP40*
Lilli		BDSC	BL5726	*lilli[A17-2] cn[1] bw[1]/CyO*
Lilli		VDRC	V106142	*P{KK102916}VIE-260B*
Odd		BDSC	BL28295	*y[1] v[1]; P{y[+t7.7] v[+t1.8]=TRiP.JF02925}attP2*
Odd		BDSC	BL34328	*y[1] sc[*] v[1]; P{y[+t7.7] v[+t1.8]=TRiP.HMS01315}attP2/TM3, Sb[1]*
Rbp9		BDSC	BL25775	*w[*]; Rbp9[Delta1]/CyO*
Rbp9		BDSC	BL28669	*y[1] v[1]; P{y[+t7.7] v[+t1.8]=TRiP.JF03084}attP2*
Rrp1	*Mut-1*	BDSC	BL10213	*w[1118]; PBac{w[+mC]=PB}Rrp1[c00695]/CyO*
Rrp1	*RNAi-1*	BDSC	BL35420	*y[1] sc[*] v[1]; P{y[+t7.7] v[+t1.8]=TRiP.GL00343}attP2*
Sand	*Mut-1*	BDSC	BL17895	*w[1118]; PBac{w[+mC]=RB}sand[e00867]*
Sand	*RNAi-1*	BDSC	BL25853	*y[1] v[1]; P{y[+t7.7] v[+t1.8]=TRiP.JF01874}attP2*
Sec5		BDSC	BL27526	*y[1] v[1]; P{y[+t7.7] v[+t1.8]=TRiP.JF02676}attP2*
Sec5		BDSC	BL50556	*y[1] v[1]; P{y[+t7.7] v[+t1.8]=TRiP.GLC01676}attP2*
Snx1		BDSC	BL29177	*w[1118]; Mi{ET1}Snx1[MB11025]*
Snx1		BDSC	BL38301	*y[1] sc[*] v[1]; P{y[+t7.7] v[+t1.8]=TRiP.HMS01763}attP40*
Srp54		BDSC	BL30533	*y[1] sc[*] v[1]; P{y[+t7.7] v[+t1.8]=TRiP.HM05224}attP2*
Srp54		BDSC	BL55254	*y[1] sc[*] v[1]; P{y[+t7.7] v[+t1.8]=TRiP.HMC03941}attP40*
Wek		BDSC	BL30471	*b[1] wek[RAR14] pr[1] cn[1] bw[1]/CyO*
Wek		BDSC	BL35680	*y[1] sc[*] v[1]; P{y[+t7.7] v[+t1.8]=TRiP.GLV21045}attP2*
βggt-II	*RNAi-1*	BDSC	BL50516	*y[1] v[1]; P{y[+t7.7] v[+t1.8]=TRiP.GLC01634}attP40*
βggt-II	*RNAi-2*	BDSC	BL34902	*y[1] sc[*] v[1]; P{y[+t7.7] v[+t1.8]=TRiP.HMS01247}attP2*
Tefu(dATM)	*Mut-1*	BDSC	BL29888	*w[1118]; Mi{ET1}tefu[MB09945]/TM6C, Sb[1]*
Tefu(dATM)	*Mut-2*	BDSC	BL8626	*w[*]; P{ry[+t7.2]=neoFRT}82B tefu[atm-6] e[1]/TM6B, Tb[1]*
Tefu(dATM)	*RNAi-1*	BDSC	BL31635	*y[1] v[1]; P{y[+t7.7] v[+t1.8]=TRiP.JF01422}attP2*

Bloomington *Drosophila* Stock Center (BDSC)
Vienna *Drosophila* Resource Center (VDRC)

Table S3. List of primers and oligonucleotides used in this study.

Primer **qPCR**	**Sequence**
Ku80 forward	CCCCAATTCAGCAGCATATT
Ku80-reverse	CCTTCAGCCA GACTGGAGAC
PUMA-forward	GCCCAGACTGTGAATCCTGT
PUMA-reverse	TCCTCC CTCTTCCGAGATTT
BAX-forward	TTTGCTTCAGGGTTTCATCC
BAX-reverse	CAGTT GAAGTTGCCGTCAGA
GAPDH-forward	CTGAACGGCTGTGATGGGAT
GAPDH-reverse	GGCATGGACTGTGGTCATGAG
Drosophila Ku80-forward	CCTTGTACCTCCTGGTGCAT
Drosophila Ku80-reverse	GGGCAGTAGCACAACCATTT
Primer to verify CRISPR/Cas9 deletion	
Ku80 forward	GAGGTCTGGTTGTCCTGCTC
Ku80-reverse	TGCCTCCCAACCTCTCAGTA
gRNA for C9ORF72 CRISPR/Cas9	
Upstream target oligonucleotides	5'-CACCGAACTCAGGAGTCGCGCGCT-3'
	5'-AAACAGCGCGCGACTCCTGAGTTC-3'
Downstream target oligonucleotides	5’-CACCGCGGGGCGGGGCTGCGGTTG-3’
	5’-AAACCAACCGCAGCCCCGCCCCGC3’.
gRNA for ku80 CRISPR/Cas9	
Upstream target oligonucleotides	5'-TACTGATCCCCACCAGAAAG-3'
Downstream target oligonucleotides	5'-AATCCAACCAGGTTCTCAAC-3’

Table S4. Summary of Quality control assays for *C9ORF72* iPSCs lines edited by the CRISPR/Cas9 technology.

iPSC line name	Gene edited	Modification	Karyotyping test	Pluripotency markers	STR	qPCR based Genetic analysis of chromosomes:
26L6-34E	*Ku80*	Deletion	G banding at passage 12*	-qPCR analysis -Immunostaining	Fusion 6C^{TM}, locus multiplex system	1q, 4p, 8q, 10p, 12p, 17q, 18q, 20q and Xp
26L6-64A	*Ku80*	Deletion	G banding at passage 12*	-qPCR analysis -Immunostaining	Fusion 6C^{TM}, locus multiplex system	1q, 4p, 8q, 10p, 12p, 17q, 18q, 20q and Xp
26Z90	G_4C_2 repeats	Deletion	G banding at Passage 39	-qPCR analysis -Immunostaining	Fusion 6C^{TM}, locus multiplex system	1q, 4p, 8q, 10p, 12p, 17q, 18q, 20q and Xp
27M91	G_4C_2 repeats	Deletion	G banding at Passage 48	-qPCR analysis -Immunostaining	Fusion 6C^{TM}, locus multiplex system	1q, 4p, 8q, 10p, 12p, 17q, 18q, 20q and Xp

Abbreviations:

qPCR: quantitative polymerase chain reaction, STR: Short tandem repeat analysis

Note:

*: the passage number is after CRISPR/Cas9 deletion was made.

Table S5. Summary of iPSCs lines used in this study

iPSC line name	Source	Clinical Diagnosis	Karyotyping test	Pluripotency Markers tests	*In vitro* 3 germ layers differentiation	qPCR based Genetic analysis of chromosomes:
Control						
24L2	Skin fibroblasts	Healthy control	G banding at passage #14	-qPCR -Immunostaining	-Endoderm -Mesoderm -Ectoderm	1q, 4p, 8q, 10p, 12p, 17q, 18q, 20q and Xp
35L11	Skin fibroblasts	Healthy control	G banding at passage #13	-qPCR -Immunostaining	-Endoderm -Mesoderm -Ectoderm	1q, 4p, 8q, 10p, 12p, 17q, 18q, 20q and Xp
37L20	Skin fibroblasts	Healthy control	The passage # for the test is unknown	-qPCR -Immunostaining	-Endoderm -Mesoderm -Ectoderm	1q, 4p, 8q, 10p, 12p, 17q, 18q, 20q and Xp
C9ORF72						
16L14	Skin fibroblasts	FTD	G banding at passage #12	-qPCR -Immunostaining	-Endoderm -Mesoderm -Ectoderm	1q, 4p, 8q, 10p, 12p, 17q, 18q, 20q and Xp
40L3	Skin fibroblasts	FTD/ALS	G banding at passage #8	-qPCR -Immunostaining	-Endoderm -Mesoderm -Ectoderm	1q, 4p, 8q, 10p, 12p, 17q, 18q, 20q and Xp
ALS30	Skin fibroblasts	ALS	The passage # for the test is unknown	-Immunostaining	-Endoderm -Mesoderm -Ectoderm	1q, 4p, 8q, 10p, 12p, 17q, 18q, 20q and Xp
42L1	Skin fibroblasts	Clinical normal	G banding at passage #14	-qPCR -Immunostaining	-Endoderm -Mesoderm -Ectoderm	1q, 4p, 8q, 10p, 12p, 17q, 18q, 20q and Xp
26L6	Skin fibroblasts	Clinical normal	G banding at passage #14	-qPCR -Immunostaining	-Endoderm -Mesoderm -Ectoderm	1q, 4p, 8q, 10p, 12p, 17q, 18q, 20q and Xp
27L11	Skin fibroblasts	FTLD-TDP Type B	G banding at passage #17	-qPCR -Immunostaining	-Endoderm -Mesoderm -Ectoderm	1q, 4p, 8q, 10p, 12p, 17q, 18q, 20q and Xp

Abbreviations:

FTD: Frontotemporal dementia

ALS: Amyotrophic lateral sclerosis

FTLD: Frontotemporal lobar degeneration

FTLD-TDP: FTLD with motor neuron disease and TDP-43 immunoreactive inclusions

qPCR: quantitative polymerase chain reaction

Table S6. List of antibodies used in this study.

Antibody	Dilution	Vendor
rabbit anti-Ku80	1:1000	Cell Signaling, Cat. no. 2753
mouse anti-Ku70	1:1000	Cell Signaling, Cat. no. 4104
rabbit anti-phosphorylated ATM	1:1000	Abcam, Cat. no. ab81292
rabbit anti-phosphorylated P53 (Ser15)	1:1000	Cell Signaling, Cat. no. 9284
rabbit DNA PKcs	1:1000	Abcam, Cat. no. ab70250
rabbit anti-cleaved caspase 3	1:1000	Cell Signaling, Cat. no. 9661
rabbit anti-BAX	1:1000	Abcam, Cat. No. 32503
rabbit anti-PUMA	1:1000	Proteintech, Cat. no. 55120-1AP
mouse anti-β-actin	1:3000	Sigma-Aldrich, Cat. no. A2228
mouse anti-α-Tubulin	1:2000	Sigma, Cat. no. T6199

References

1. Lopez-Gonzalez R*, et al.* (2016) Poly(GR) in C9ORF72-related ALS/FTD compromises mitochondrial function and increases oxidative stress and DNA damage in iPSC-derived motor neurons. *Neuron* 92:383–391.