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Supplementary Information for

The nuclear ubiquitin ligase adaptor SPOP is a conserved regulator of C9orf72 dipeptide toxicity

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Other supplementary materials for this manuscript include the following:

Datasets S1 to S2

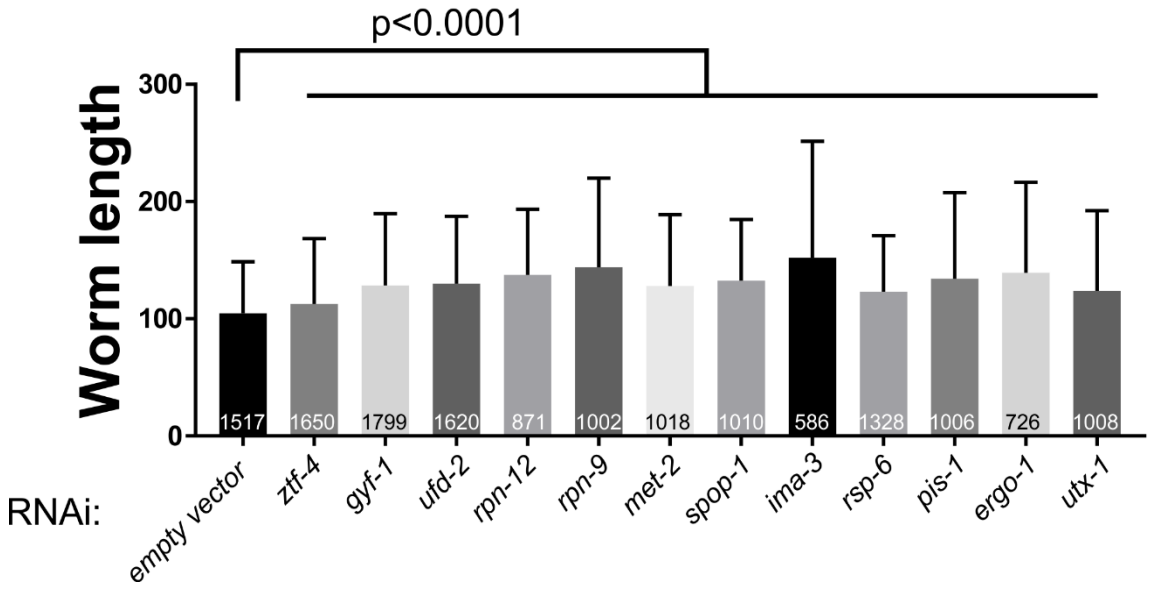


Fig. S1. Quantification of suppression of (PR)50-induced growth arrest by hits from the genome-wide RNAi screen. *drIs34* eggs were placed on either (empty vector) RNAi or the indicated gene-specific RNAi bacteria. After 7 days at 20º C, animals were washed off the plate and worm length was quantified as the event time-of-flight using a COPAS Biosort. Data shown are mean +/- S.D. The N for each sample is shown on each bar. P-values versus the empty vector control were calculated using a non-parametric one-way ANOVA with Dunn's multiple comparison post-hoc testing.

(GR)50

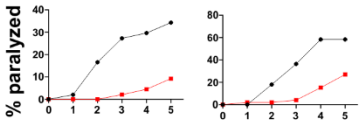
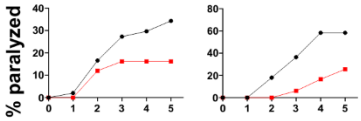
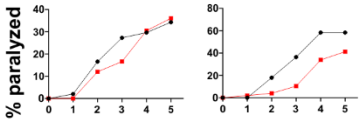
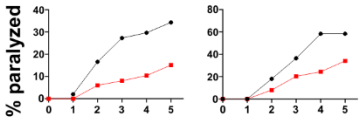
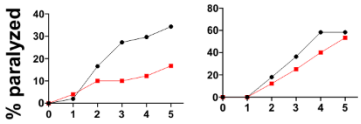
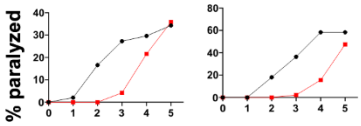
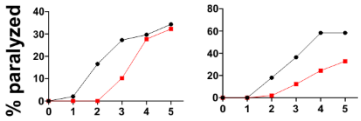
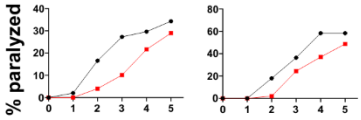
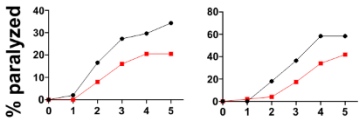
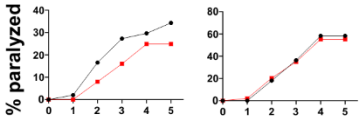
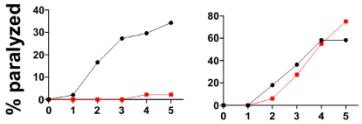
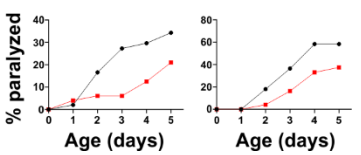
ev(RNAi) *gene(RNAi)*	Trial 1	Trial 2	# of trials with Log-rank significant p-value
ztf-4(RNAi)			2/2 p<0.05
gyf-1(RNAi)			1/2 p<0.05
ufd-2(RNAi)			1/2 p<0.05
rpn-12(RNAi)			1/2 p<0.05
rpn-9(RNAi)			1/2 p<0.05
met-2(RNAi)			1/2 p<0.05
spop-1(RNAi)			1/2 p<0.05
ima-3(RNAi)			0/2 p<0.05
rsp-6(RNAi)			1/2 p<0.05
pis-1(RNAi)			0/2 p<0.05
ergo-1(RNAi)			1/2 p<0.05
utx-1(RNAi)			1/2 p<0.05

Fig. S2. Effect of (PR)50 RNAi screen hits on (GR)50 age-dependent paralysis phenotype. Paralysis assays were performed as previously described (63). N=50-80 animals per assay. EV(RNAi) control in black and gene specific RNAi in red. Each candidate gene was scored in two separate assays and the number of times the Log-rank Bonferroni-adjusted p-value was <0.05 is indicated.

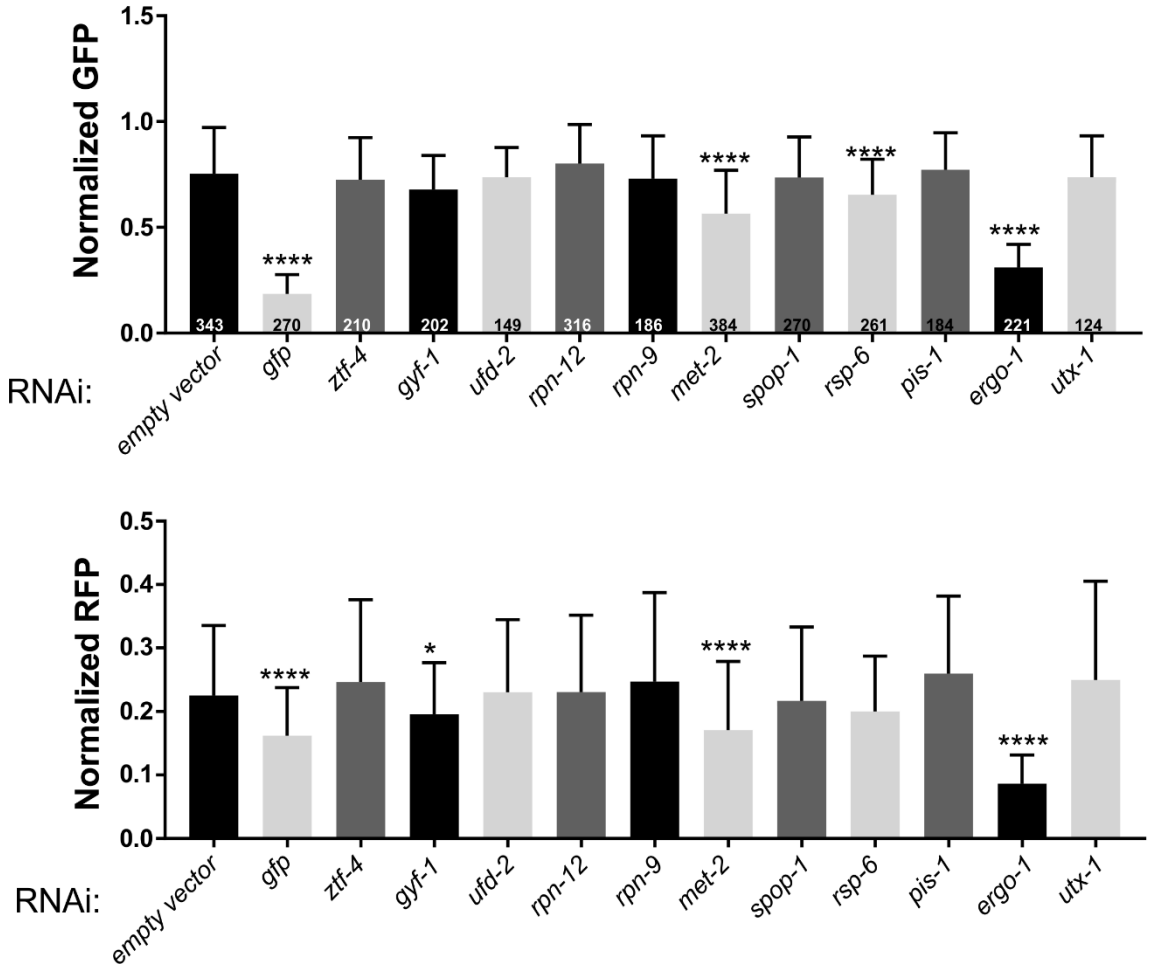


Fig. S3. Effect of RNAi screen hits on transgene expression. *drIs33* eggs were placed on either *empty vector(RNAi)* or the indicated gene-specific RNAi bacteria. After 7 days at 20°C, animals were washed off the plate and normalized GFP (GFP/TOF; A) and normalized RFP (RFP/TOF; B) from adult animals with TOF>400 were quantified with a COPAS Biosort. Data shown are mean +/- S.D. * - $p<0.05$, **** - $p<0.001$, non-parametric One-way ANOVA with Dunn's multiple comparisons test. The N for each sample is indicated within each bar.

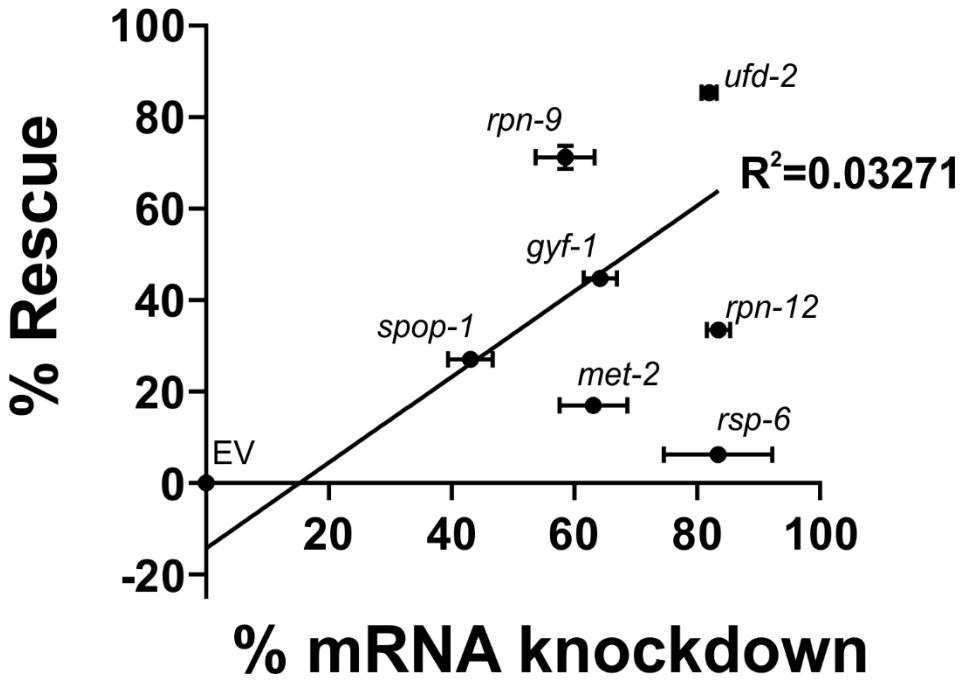


Fig. S4. Correlation between extent of mRNA knockdown and level of PR50 suppression for RNAi screen hits. '% Rescue' represents COPAS measured TOF normalized to the mean TOF of the empty vector control. '% mRNA knockdown' represents the level of mRNA reduction following RNAi of the indicated gene. Data shown are mean +/- SEM. For % mRNA knockdown, n=3. For % Rescue, n=2840-54763. R2 was calculated by fitting the data points to a simple linear regression function in GraphPad. Best fit values had a slope of 0.9368, with Y-intercept = -14.32 and X-intercept = 15.29.

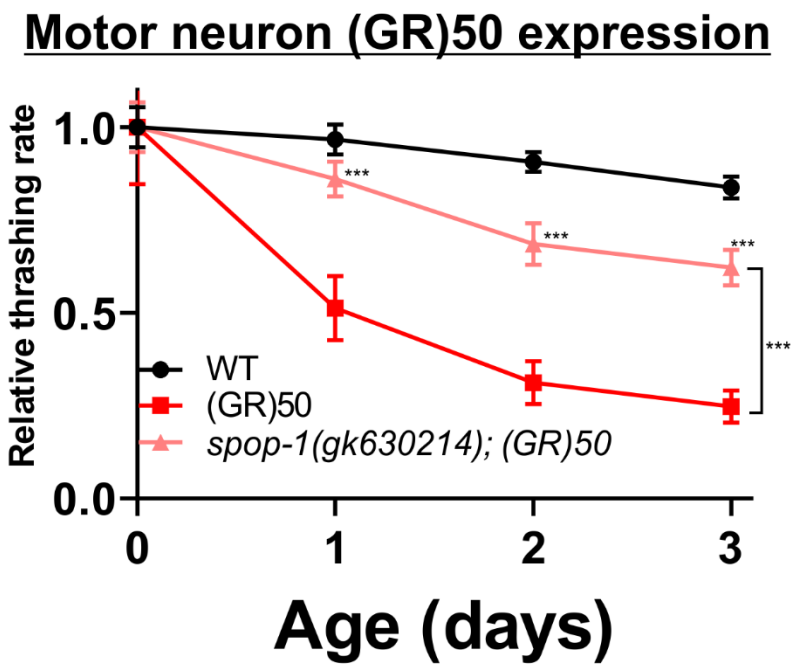


Fig. S5. *spop-1* functions in motor neurons to protect against motor neuron expressed (GR)50-GFP. WT or *drIs49* (*unc-47p*::3xFLAG-GR50-GFP) animals of the indicated genotype were grown at 25°C and L4 stage animals (Day 0) were picked to new plates (4 sets of plates with 10 animals each per genotype). At each time point, the number of thrashes were quantified as previously described (63). The experimenter was blinded to genotype. Data within genotypes were normalized to the thrashing rate at Day 0 and analyzed with a 2-way ANOVA with Tukey's multiple comparisons test to evaluate statistically significant differences among genotypes and specific days. Age variable – $F_{(3,153)}$ = 32.04, p<0.0001; Genotype variable – $F_{(3,153)}$ = 28.30, p<0.0001. Tukey's multiple comparison testing - **** - p<0.0001. Data shown are the mean ± S.D., N=10.

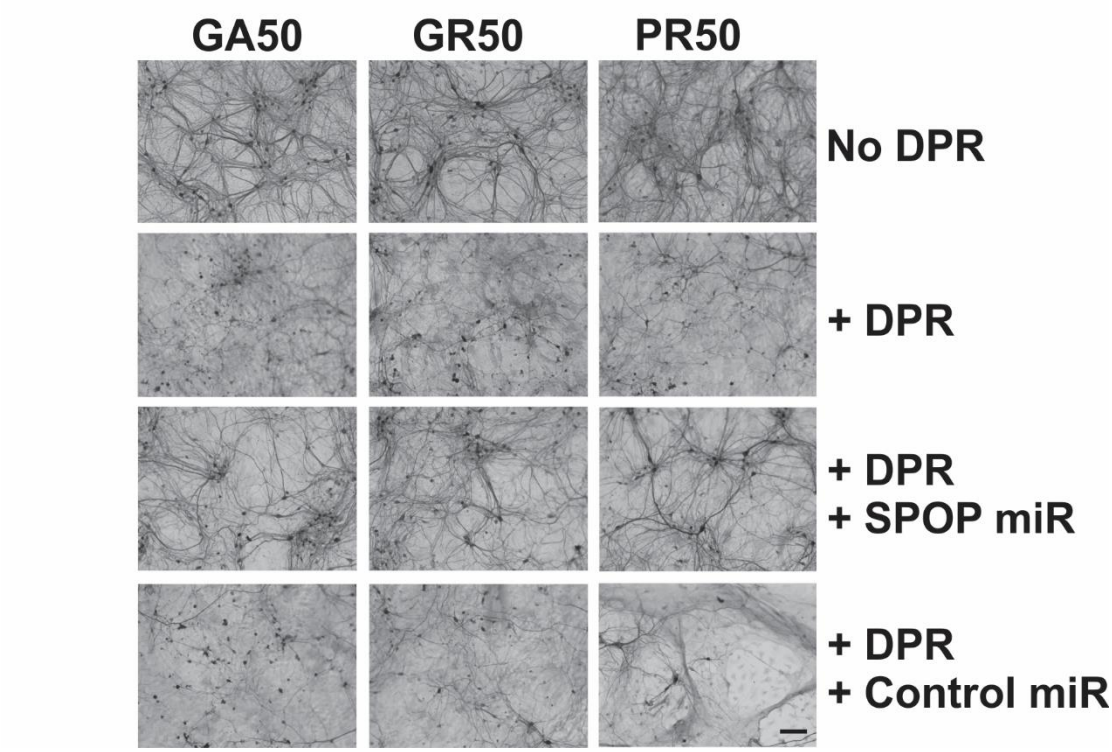


Fig. S6. SPOP knockdown inhibits DPR-induced toxicity in mammalian neurons. Representative images of mixed spinal cord cultures immunocytochemically stained with SMI-32 (a monoclonal antibody that recognizes a non-phosphorylated epitope of 168 kD and 200 kD neurofilament), an avidin conjugated secondary antibody, and Vectastain ABC kit (Vector Laboratories). Cells with soma larger than 20 microns reliably identify motor neurons. Images of cultures from four experimental conditions are presented: 1) no DPR, 2) expression of DPRs (e.g., GA50, GR50 or PR50), 3) DPRs plus SPOP miRNA, and 4) DPR plus control miRNA. In comparison with the no DPR condition, expression of individual DPRs leads to a clear reduction of SMI-32 labeled cells. Knockdown of SPOP with the specific miRNA (but not the non-targeting control miRNA), promotes partial rescue from DPR toxicity.

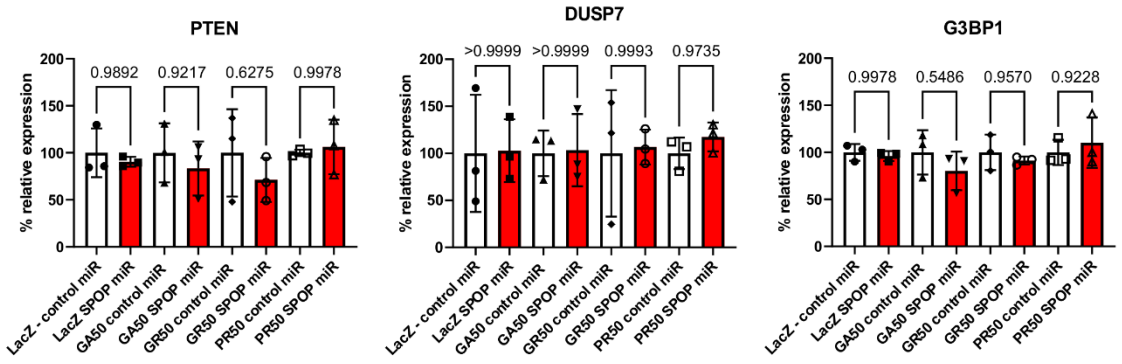


Fig. S7. Quantification of band intensities from Fig. 4C. Band intensities were normalized to the mean of the control miR for each DPR. Data shown are mean ± S.D., n=3. P-values were calculated by a one-way ANOVA with Holm-Šidák multiple comparison test. PTEN – $F_{(7,16)}$ = 0.5304, p=0.7991; DUSP7 – $F_{(7,16)}$ = 0.06932, p=0.9992; G3BP1 – $F_{(7,16)}$ = 0.7596, p=0.6281.

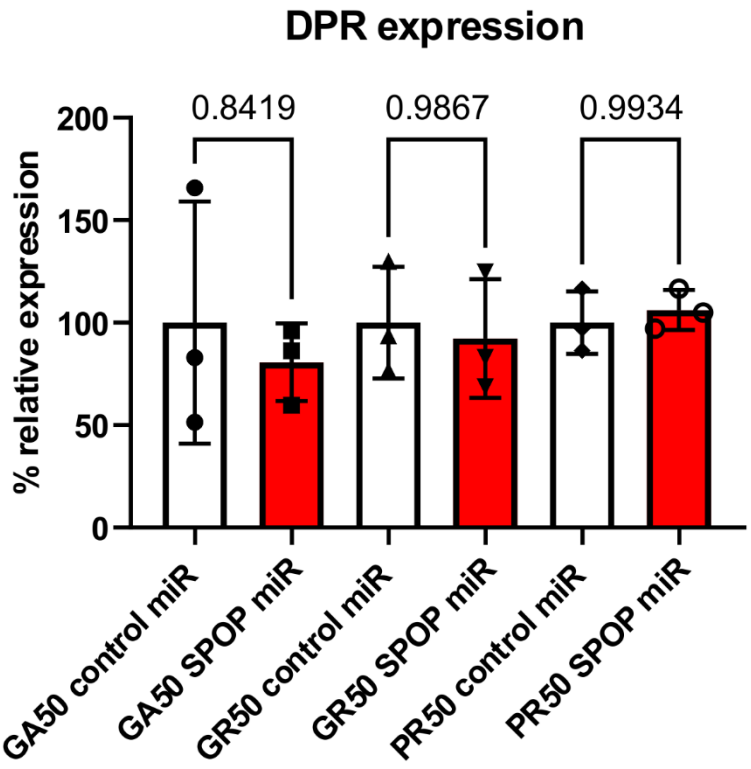


Fig. S8. Quantification of band intensities from Fig. 4D. Band intensities were normalized to the mean of the control miR for each DPR. Data shown are mean ± S.D., n=3. P-values were calculated by a one-way ANOVA with Holm-Šidák multiple comparison test. $F_{(5,12)}$ = 0.2496, p=0.9320.

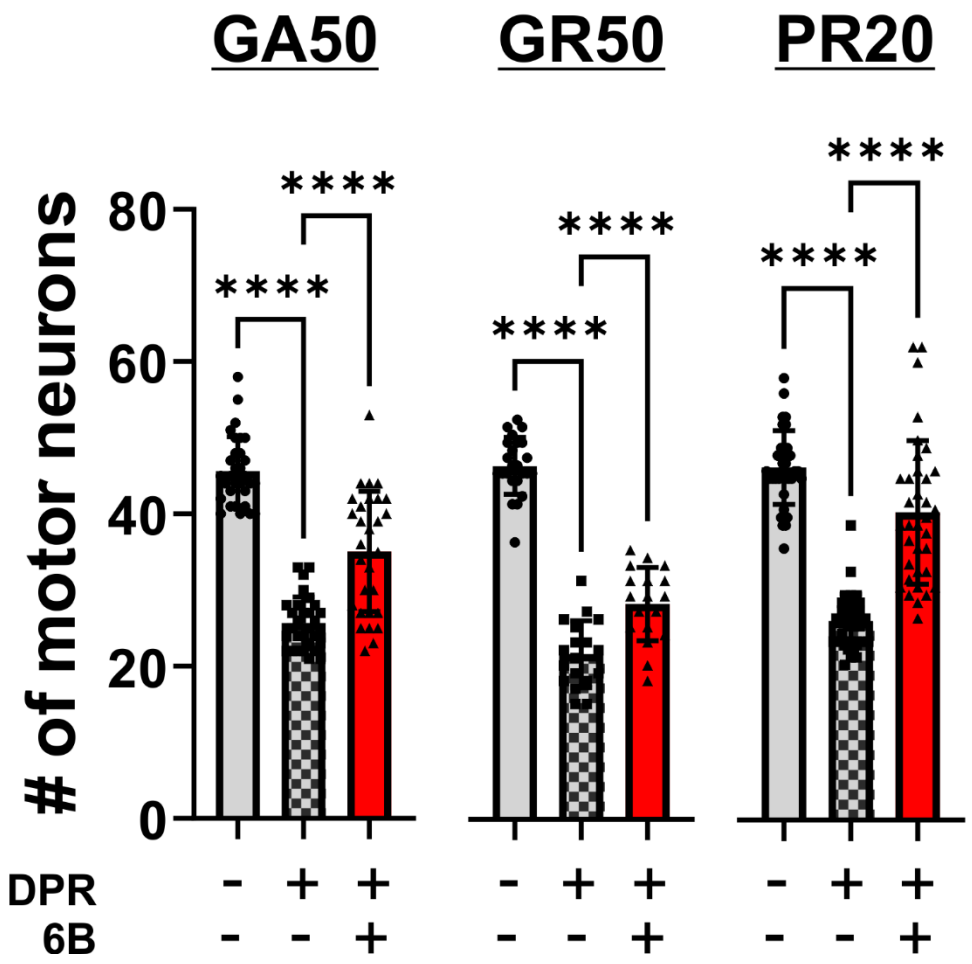


Fig. S9. The SPOP inhibitor, compound 6B protects against DPR toxicity.

Days in vitro 14 spinal cord neuron cultures were infected with HSV engineered to express the GA(50), GR(50), LacZ, or exposed to bath applied synthetic PR20 and were treated with either 6B (10 μM) or vehicle once. Five days later, cultures were processed for immunocytochemistry and motor neuron counts were obtained. By one-way ANOVA, DPRs lead to a statistically significant reduction in motor neuron number (compared with LacZ expressing cultures) and application of 6B leads to a statistically significant protection against DPR toxicity (e.g., GA(50) group, $F_{(2,88)}$ = 97.09, $p < 0.0001$; GR(50) group, $F_{(2,57)}$ = 190.2, $p < 0.0001$; PR20 group, $F_{(2,102)}$ = 90.49, $p < 0.0001$). Pairwise comparisons were made with Tukey's multiple comparisons test. **** - p<0.0001.

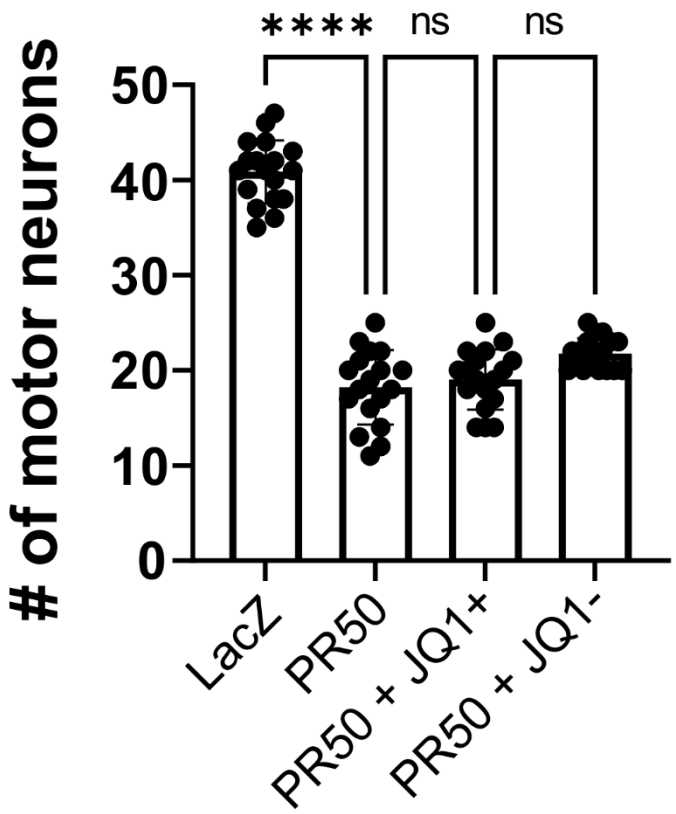


Figure S10. The BRD domain inhibitor JQ1+ and its inactive isomer JQ1- do not alter PR50 toxicity in mammalian neurons. Days in vitro 14 spinal cord neuron cultures were infected with HSV engineered to express either PR50 or LacZ and were treated with either JQ1+ or JQ1- (100 nM) once. Five days later, cultures were processed for immunocytochemistry and motor neuron counts were obtained. Neither JQ1+ nor JQ1- significantly altered the toxicity of PR50. $F_{(3,68)}$ = 212.4, $p < 0.0001$. Tukey's multiple comparison testing - **** - $p<0.0001$, n.s. – $p=0.8533$ (PR50 vs PR50 + JQ1+); $p=0.0518$ (PR50 JQ1+ vs. PR50 JQ1-).

Table S1. Strains used in this study

Strain name	Genotype	Reference
OG736	*drIs28 [myo-3p::3XFLAG-GR50-gfp; myo-3p:dsRed2]*	(21)
OG755	*drIs34 [myo-3p::3XFLAG-PR50-gfp; myo-3p:dsRed2]*	(21)
OG919	*met-2(n4256); drIs34*	This study
OG922	*rsp-6(ok798); drIs34*	This study
VC40422	*spop-1(gk630214)*	(64)
OG1053	*spop-1(gk630214) drIs34*	This study
OG1054	*spop-1(gk630214); drIs28*	This study
OG1055	*spop-1(dr26(CRISPR inserted gfp))*	This study
OG1057	*spop-1(dr28); drIs34*	This study
OG1153	*drIs49 [unc-47p::3XFLAG-GR50-gfp; unc-47p::gfp; myo-3p::mCherry]*	This study
OG1158	*spop-1(dr95(W195G)); drIs34*	This study
OG1159	*spop-1(dr26 dr96(W195G))*	This study
OG1168	*spop-1(dr100(F197V); drIs34*	This study
OG1169	*spop-1(dr26 dr101(F197V))*	This study
OG1171	*spop-1(gk630214); drIs49*	This study

Table S2. Oligonucleotides used in this study

Primer	Sequence	Description
TL118	GAGTCAGTGAGCGAGGAAGC	*C. elegans* feeding RNAi clone sequencing primer
OG1454	TACCGTACTACACGCATCGCGAGC	*rsp-6(ok798)* genotyping
OG1455	CGAGAGCGGGAGCGACTCTTCGAG	*rsp-6(ok798)* genotyping
OG1456	CGCGTTTATTGAAATCATTTCGCG	*rsp-6(ok798)* genotyping
OG1457	ACGATCACGTTCTTGAGGAGATCG	*rsp-6(ok798)* genotyping
OG1458	GCAGGAAAGATGTATTCGACAAATATGC	*rsp-6(ok798)* genotyping
OG1449	GTGAATCACTTGAGGGACTTTCAC	*met-2(n4256)* genotyping
OG1450	GTCCAACCATAATAGCAATCTGAGC	*met-2(n4256)* genotyping
OG1451	GATGAAGACGTGCTAGTCGCTGC	*met-2(n4256)* genotyping
OG1452	CCTGGTTCACCAGGCATTGTCGGAG	*met-2(n4256)* genotyping
OG1453	CAGGTCTTCGAGTTGCCTGTCGCC	*met-2(n4256)* genotyping
OG1534	AGATCTCCAGTCGCCTTTTC	*spop-1(dr28)* guide RNA 1
OG1535	CGGCATGGGTAACGATGAAG	*spop-1(dr28)* guide RNA 2
OG1537	AATTAACAAATAAATATATTTGAATTTAAGATAAATAAGTGTAAATTTAATTTACAAATACTGTAAACGA	*spop-1(dr28)* repair oligo
OG1528	AGCTCGTGGCAAGTGTGCATAC	*spop-1(dr28)* genotyping
OG1529	GTTTCCCGATCCACTCGAGACCTG	*spop-1(dr28)* genotyping
OG1459	GTGCCGAAAACAGTCTGGCAATGG	*spop-1(gk630214)* genotyping
OG1460	GAGATCCACTTCTTCCGGTTGCTG	*spop-1(gk630214)* genotyping
OG1461	CAGTCTGGCAATGGACAAAC	*spop-1(gk630214)* genotyping
OG1462	CCGGTTGCTGAAAATTGGTGTC	*spop-1(gk630214)* genotyping
OG1536	GGCTGTACAAGCACTACGGG	*spop-1(dr26)*-gfp gRNA
OG1530	TTTAGAGCACTAGCAACTCAGCAAACTCCTCCCGTAATGAGTAAAGGAGAAGAACTTTTC	GFP PCR with *spop-1* overlaps, CRISPR repair template
OG1531	GTGTTTCGGCCGTTTCTTGGGTGGCTGTACAAGCACTTTGTATAGTTCATCCATGCCATG	GFP PCR with *spop-1* overlaps, CRISPR repair template
OG1532	GAAGATGGAAGCGTTCAACTAGCA	*spop-1::gfp* genotyping
OG1533	GAGATTCAAGACATTTCGATGAC	*spop-1::gfp* genotyping
OG1819	GATTTGTACAGGGAAAGGAT	W195G / F197V gRNA
OG1820	CACAGCGGGCTTATCGATTTGTACAGGGAAAGGATGGAGGATTCAAAAAATTCATTCGACGAGATTTTTTGTT	W195G repair oligo
OG1822	GCCGCGAAGAAATGGGCGAAG	W195G genotyping
OG1823	CCTGTCACGTTTACTGTTTCGGC	W195G genotyping
OG1824	CTGAAATCTTCCACGTTTTCCGCCG	W195G genotyping
OG1825	CGGCTACCACAGACACCTGAAAATTG	W195G genotyping
OG1821	AGGAGACGAAAGCTATGGAATCACAGCGGGCTTATCGCTTTGTCCAAGGTAAAGACTGGGGAGTTAAAAAATTCATTCGACGAGATTTTTTGTTGGATGA	F197V repair oligo
OG1826	ATGCGAAACGAGAGGAGACGAAAGCTATG	F197V genotyping
OG1827	AATCTATCTCCAGGAAGAAGCC	F197V genotyping
OG1828	TCACAGCGGGCTTATCGATTTG	F197V genotyping
OG1829	AACAGTCTGGCAATGGACAAAC	F197V genotyping
OG1479	CCTGGAGGCTTGCTGAAGGCTGTATGCTGTATAGCACCAGCTCTCAGCAAGTTTTGGCCACTGACTGACTTGCTGAGCTGGTGCTATACAGGACACAAGGCCTGTTACTAGCACTCACATGGAACAAATGGCCT	SPOP miR 1 sense
OG1480	CCGGAGGCCATTTGTTCCATGTGAGTGCTAGTAACAGGCCTTGTGTCCTGTATAGCACCAGCTCAGCAAGTCAGTCAGTGGCCAAAACTTGCTGAGAGCTGGTGCTATACAGCATACAGCCTTCAGCAAGCCTCCAGGCCGG	SPOP miR antisense
OG1481	CCTGGAGGCTTGCTGAAGGCTGTATGCTGAGGAGAACTTCACTACCTTGAGTTTTGGCCACTGACTGACTCAAGGTAGAAGTTCTCCTCAGGACACAAGGCCTGTTACTAGCACTCACATGGAACAAATGGCCT	SPOP miR 2 sense
OG1482	CCGGAGGCCATTTGTTCCATGTGAGTGCTAGTAACAGGCCTTGTGTCCTGAGGAGAACTTCTACCTTGAGTCAGTCAGTGGCCAAAACTCAAGGTAGTGAAGTTCTCCTCAGCATACAGCCTTCAGCAAGCCTCCAGGCCGG	SPOP miR 2 antisense
OG1485	GCTCTTAAGGCTAGAGTACTT	p1006 miR vector sequencing
OG1486	GGAATTGATCCTTATCGATT	p1006 miR vector sequencing

Dataset S1 (separate file). Summary statistics for all featured and replicate paralysis assays.

Dataset S2 (separate file). Raw data files for Fig. 1-5 and Fig. S1-S10