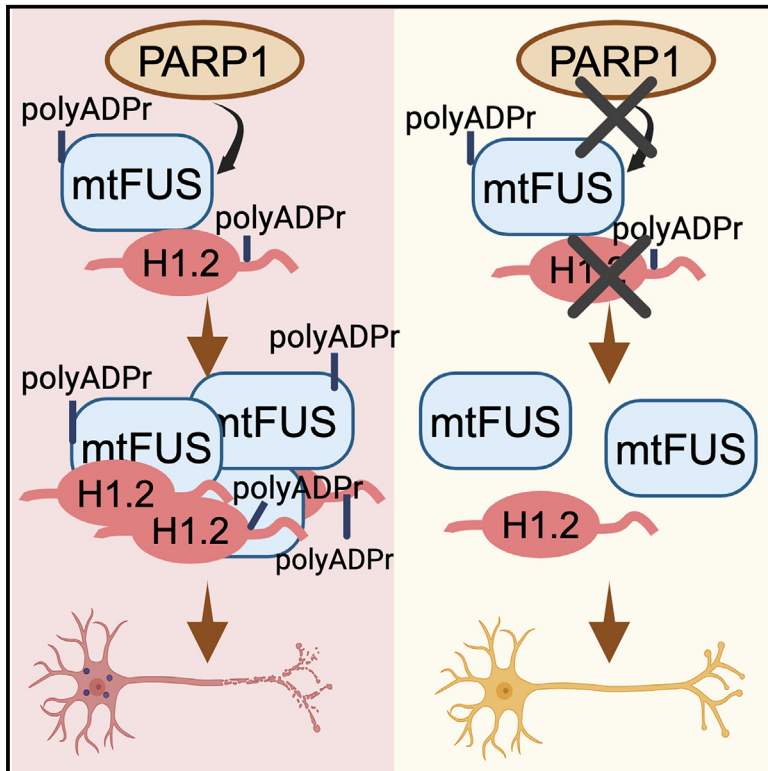


ALS-FUS mutations cause abnormal PARylation and histone H1.2 interaction, leading to pathological changes

Graphical abstract



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In brief

Alirzayeva et al. find that ALS-related mutant FUS gains interaction with PARP1 and histone H1.2. Reducing either PARP1 activity or H1.2 levels prevents pathological changes in human motor neurons and *C. elegans* models. These findings reveal a link between PARP1, H1.2, and FUS in ALS.

Highlights

- ALS-related mutant FUS gains enhanced interaction with PARP1 in human motor neurons
- ALS-FUS mutations cause abnormal PARylation and histone H1.2 interaction
- Reducing PARylation and H1.2 levels attenuates ALS-related changes in motor neurons
- H1.2 and PARP1 also lead to mutant FUS aggregation in *C. elegans*



Article

ALS-FUS mutations cause abnormal PARylation and histone H1.2 interaction, leading to pathological changes

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<https://doi.org/10.1016/j.celrep.2024.114626>

SUMMARY

The majority of severe early-onset and juvenile cases of amyotrophic lateral sclerosis (ALS) are caused by mutations in the *FUS* gene, resulting in rapid disease progression. Mutant *FUS* accumulates within stress granules (SGs), thereby affecting the dynamics of these ribonucleoprotein complexes. Here, we define the interactome of the severe mutant *FUS*^{P525L} variant in human induced pluripotent stem cell (iPSC)-derived motor neurons. We find increased interaction of *FUS*^{P525L} with the PARP1 enzyme, promoting poly-ADP-ribosylation (PARylation) and binding of *FUS* to histone H1.2. Inhibiting PARylation or reducing H1.2 levels alleviates mutant *FUS* aggregation, SG alterations, and apoptosis in human motor neurons. Conversely, elevated H1.2 levels exacerbate *FUS*-ALS phenotypes, driven by the internally disordered terminal domains of H1.2. In *C. elegans* models, knockdown of H1.2 and PARP1 orthologs also decreases *FUS*^{P525L} aggregation and neurodegeneration, whereas H1.2 overexpression worsens ALS-related changes. Our findings indicate a link between PARylation, H1.2, and *FUS* with potential therapeutic implications.

INTRODUCTION

Amyotrophic lateral sclerosis (ALS) is characterized by the selective degeneration of upper motor neurons in the motor cortex as well as the lower motor neurons in the brain stem and spinal cord.¹ The cause of ALS remains unknown in 90% of cases, but cumulative evidence suggests that sporadic ALS may result from complex interactions between genetic susceptibility and aging.² The remaining cases are hereditary and linked to mutations in one of over 30 distinct genes involved in different cellular processes.^{2,3} Many of these genes encode for RNA-binding proteins (RBPs) with low complexity domains, such as *FUS* (fused in sarcoma).^{2,3}

Several RBPs associated with ALS accumulate into stress granules (SGs), membraneless ribonucleoprotein organelles formed during cellular stress. Conditions such as oxidative stress elicit the assembly of cytoplasmic SGs, which are highly dynamic and rapidly disassemble upon removal of stress.^{4,5} SG assembly relies on dynamic interactions between RBPs harboring low-complexity domains, which drive their oligomerization.^{6,7} However, these domains are also prone to aggregation, leading to persistent and aberrant SGs.^{8,9} Alterations in

SGs have been implicated in various age-related neurodegenerative disorders characterized by protein aggregates, including Alzheimer's and Huntington's.^{10–12} The link is particularly compelling in ALS, where different ALS-associated proteins disrupt SG dynamics and disassembly.^{13–18}

The majority of severe early-onset and juvenile cases are caused by mutations in the *FUS* gene.¹⁹ Moreover, *FUS*-immunoreactive aggregates are also prevalent in sporadic ALS. *FUS* is widely expressed across tissues and has a role in various DNA and RNA processing steps, including DNA repair, transcription, RNA splicing, and nucleo-cytoplasmic RNA shuttling.^{20–25} Although predominantly localized within the nucleus, *FUS* shuttles between the nucleus and cytoplasm.²⁶ Almost all juvenile *FUS*-ALS cases exhibit mutations within the C-terminal nuclear localization signal of *FUS*.¹⁹ These mutations, including the most aggressive, P525L, lead to accumulation of *FUS* in the cytoplasm and SGs.^{27–29} The accumulation of *FUS* within SGs alters their composition and dynamics.^{30,31}

To gain insights into the pathogenesis caused by mutant *FUS*, we defined the protein interactome of *FUS*^{P525L} in human motor neurons derived from induced pluripotent stem cells (iPSCs). We found that mutant *FUS* exhibits increased interaction with



histone HIST1H1C/H1.2 in motor neurons compared to wild-type (WT) FUS. Knockdown of H1.2 mitigates neurodegeneration and SG alterations in both FUS-ALS human motor neurons and *C. elegans* models, whereas its overexpression hastens disease phenotypes. Importantly, mutant FUS also exhibits increased affinity to PARP1, the enzyme that catalyzes poly-ADP-ribosylation (PARylation). Conversely, inhibition of PARylation prevents the interaction between mutant FUS and H1.2, thereby attenuating ALS-related changes.

RESULTS

ALS-causing mutations promote FUS interaction with H1.2

We hypothesized that differences in the interactome of ALS-related mutant FUS variants can contribute to their pathological effects. ALS-related FUS mutations are dominant,³² and an anti-FUS antibody immunoprecipitated both WT and mutant FUS (Figures S1A–S1C). Thus, the expression of WT FUS in heterozygous cells from patients makes it challenging to quantify specific changes for mutant FUS. To overcome this limitation, we used established iPSCs generated from control iPSCs via transcription activator-like effector nucleases (TALEN)-directed mutagenesis.²⁸ These iPSCs carry the FUS^{P525L} mutation associated with juvenile ALS in homozygosis.²⁸

As reported previously, WT FUS is essentially located in the nucleus of control iPSCs and their differentiated motoneuronal counterparts²⁸ (Figure 1A). In contrast, mutant FUS^{P525L} is located in both the nucleus and cytoplasm²⁸ (Figure 1A). With paraformaldehyde fixation, we could not detect cytoplasmic granules of mutant FUS under physiological conditions (Figure 1A). However, methanol fixation allowed us to detect cytoplasmic focus-like structures of mutant FUS even in unstressed motor neurons, resembling disease-associated aggregation (Figure S1D).

Upon treatment with arsenite (oxidative stress),³³ mutant FUS^{P525L} accumulated into SGs, whereas WT FUS remained in the nucleus²⁸ (Figure 1A). We confirmed, using fluorescence recovery after photobleaching experiments, that the mobile fraction of SGs is higher in WT motor neurons than in FUS^{P525L} motor neurons (Figure S1E). To further examine alterations in SG dynamics, we monitored the rates of SG assembly and disassembly. We observed a higher number of SGs per cell upon arsenite treatment in FUS^{P525L/P525L} motor neurons compared with their isogenic control counterparts (Figure 1B). Upon removal of stress, FUS^{P525L/P525L} motor neurons exhibited a slower clearance of SGs compared with control motor neurons (Figure 1B). In contrast, we did not observe differences in the SG area between control and FUS^{P525L/P525L} motor neurons (Figure S1F). Although mutant FUS also resulted in slower disassembly of SGs in iPSCs (Figure 1C), they could clear SGs faster than their motoneuronal counterparts (Figure 1D). While we were unable to detect SGs in ALS iPSCs after 4 h of stress removal, approximately 50% of their motoneuronal counterparts contained SGs at this time point (Figure 1D). Despite a decrease in the percentage of motor neurons containing SGs over time, we continued to detect motor neurons with SGs even after 24 h of stress removal (Figure S1G).

These results support the theory that differentiation into motor neurons decreases their ability to prevent mutant FUS-related al-

terations. Indeed, expression of mutant FUS^{P525L} increased apoptosis in motor neurons compared to isogenic controls under normal conditions (Figure 1E). In contrast, mutant FUS did not induce apoptosis in undifferentiated iPSCs (Figure 1E). Together, these results demonstrate that the homozygous P525L mutation particularly affects motor neurons but not their parental iPSCs. Given that motor neurons carrying the homozygous P525L mutation exhibit alterations in SGs and neurodegeneration, these cells provide an invaluable tool to define ALS-related changes in the interactome of FUS protein.

Besides FUS itself, we detected other 99 significant interactors of FUS in iPSC-derived motor neurons in our proteomics experiments (Data S1). We then compared our datasets with previously reported interactors from several studies (Data S2).^{34–38} These studies performed analysis of ectopically expressed FUS fused to a tag protein (e.g., GFP) for immunoprecipitation (IP) in human cell lines (HEK293 and HeLa) or neuroblastoma cell lines.^{34–38} Despite differences in cell models, our study revealed a remarkable overlap of FUS interactors with previous datasets (Data S2). Among the 99 significant interactors of endogenous FUS identified in our proteomics analysis, 66 proteins have been reported previously as FUS interactors, including TAF15, HNRNP1, HNRNPUL1, HNRNPA2B1, PARP1, and the histone H1.2/HIST1H1C.^{34–38}

To define differences in the interactome between mutant and WT FUS, we compared FUS^{P525L/P525L} motor neurons with their isogenic controls (Data S1). We first confirmed that the anti-FUS antibody immunoprecipitates similar levels of endogenous FUS in both isogenic control and mutant FUS^{P525L/P525L} motor neurons (Figure 2A). However, we observed both loss and gain of interactions for mutant FUS^{P525L} compared to WT FUS (Figures 2B and 2C; Data S1). Specifically, mutant FUS^{P525L} exhibited decreased interaction with three proteins (HNRNPUL1, STAU2, and MYO5A) while gaining enhanced interaction with eight other proteins (H1.2/HIST1H1C, PARP1, EIF5B, TAF15, ZC3H4, CDK12, SNRNP200, and ABCF1) (Figures 2B and 2C; Data S1). Among them, histone H1.2/HIST1H1C displayed the highest binding intensity to FUS^{P525L} in motor neurons (Figure 2C). It is important to note that WT FUS also significantly interacted with proteins such as H1.2 and TAF15 when compared to the control FLAG antibody (Data S1), but the interaction was upregulated for mutant FUS (Figures 2B and 2C; Data S1). In contrast to motor neurons, H1.2/HIST1H1C was not among the proteins exhibiting differential binding intensity between WT and mutant FUS in undifferentiated iPSCs (Figure 2D). Along these lines, we observed that the interaction between FUS^{P525L} and HIST1H1C/H1.2 is significantly stronger in motor neurons compared to iPSCs (Figure 2E; Data S3).

By performing co-immunoprecipitation (coIP) experiments using an antibody against H1.2, we confirmed that the interaction of mutant FUS^{P525L} with H1.2 increases in motor neurons compared with WT FUS (Figure 2F). Similarly, we observed elevated interaction between FUS and H1.2 in motor neurons from patient-derived iPSCs expressing other FUS mutations (R514S and R521C) in heterozygosis (Figures S2A and S2B). H1.2 is the only variant of the histone H1 family known to shuttle between the nucleus and cytoplasm.³⁹ Although H1.2 was more abundant in the nucleus, fractionation-based experiments

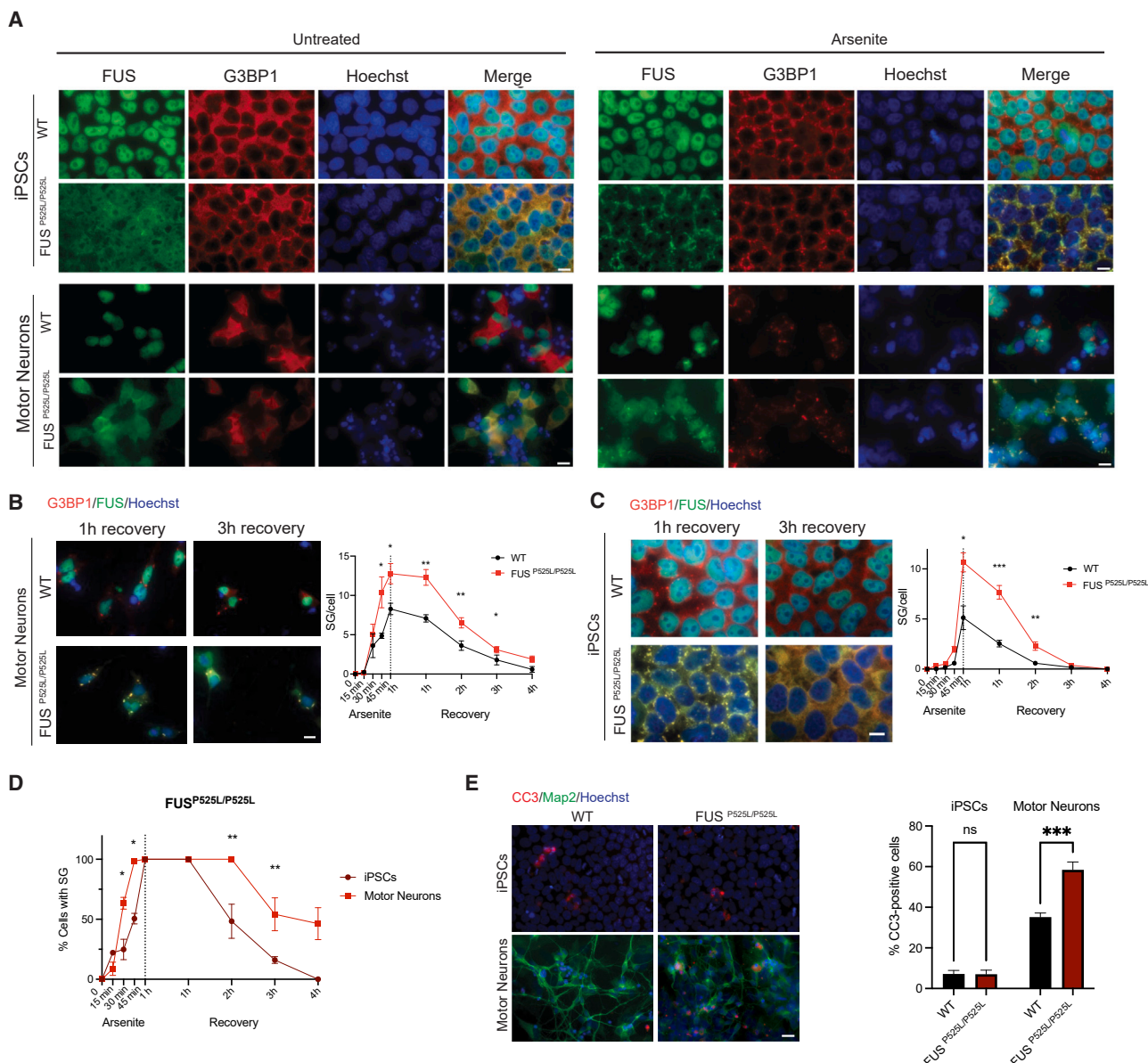


Figure 1. Motor neurons derived from FUS^{P525L/P525L} ALS-iPSCs exhibit disease-related phenotypes

(A) Immunocytochemistry of isogenic control wild-type (WT) and FUS^{P525L/P525L} ALS-iPSCs and their differentiated motoneuronal counterparts with an anti-FUS antibody. G3BP1 and Hoechst 33342 staining were used as markers of SGs and nuclei, respectively. When indicated, cells were treated with 500 μ M arsenite for 1 h. Representative of three independent experiments. Scale bars: 10 μ m.

(B) Immunostaining of G3BP1 and FUS for monitoring SG assembly and recovery in iPSC-derived motor neurons. Hoechst 33342 staining was used as a marker of nuclei. Scale bar: 10 μ m. Motor neurons were assessed during 500 μ M arsenite treatment and then after removal of arsenite stress at the indicated times. The graph represents the total number of G3BP1-positive SGs per cell (mean $\pm$ SEM, n = 100–120 total nuclei per condition from three independent experiments).

(C) SG assembly during 500 μ M arsenite treatment and recovery in undifferentiated iPSCs. Scale bar: 10 μ m. The graph represents the total number of G3BP1-positive SGs per cell (mean $\pm$ SEM, n = 700–800 total nuclei per condition from three independent experiments).

(D) The percentage of cells containing G3BP1-positive SGs upon 500 μ M arsenite treatment and removal of arsenite stress (mean $\pm$ SEM of at least 100 total nuclei per condition from three independent experiments).

(E) Immunostaining of WT and FUS^{P525L/P525L} iPSCs and their motor neuronal counterparts. Apoptotic cells were stained with anti-cleaved caspase-3 (CC3) antibody. MAP2 and Hoechst 33342 were used as markers of neurons and nuclei, respectively. Scale bar: 20 μ m. The graph represents the percentage of CC3-positive cells/total nuclei in iPSC-derived motor neurons (mean $\pm$ SEM of at least 200 cells per condition from three independent experiments).

Statistical comparisons were made by two-tailed Student's t test for unpaired samples. * p < 0.05, ** p < 0.01, *** p < 0.001; ns, not significant (p > 0.05). See also Figure S1.

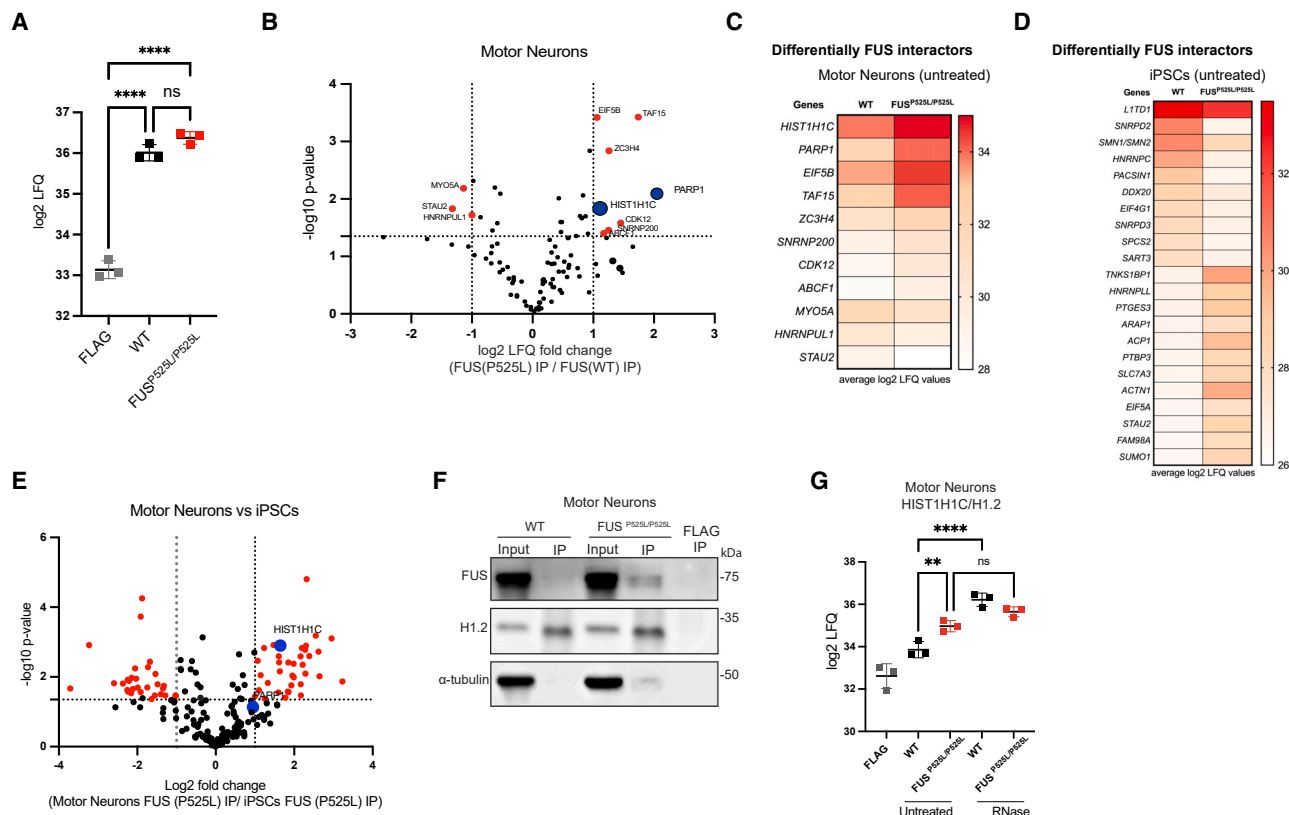


Figure 2. FUS interactome changes in motor neurons expressing mutant FUS

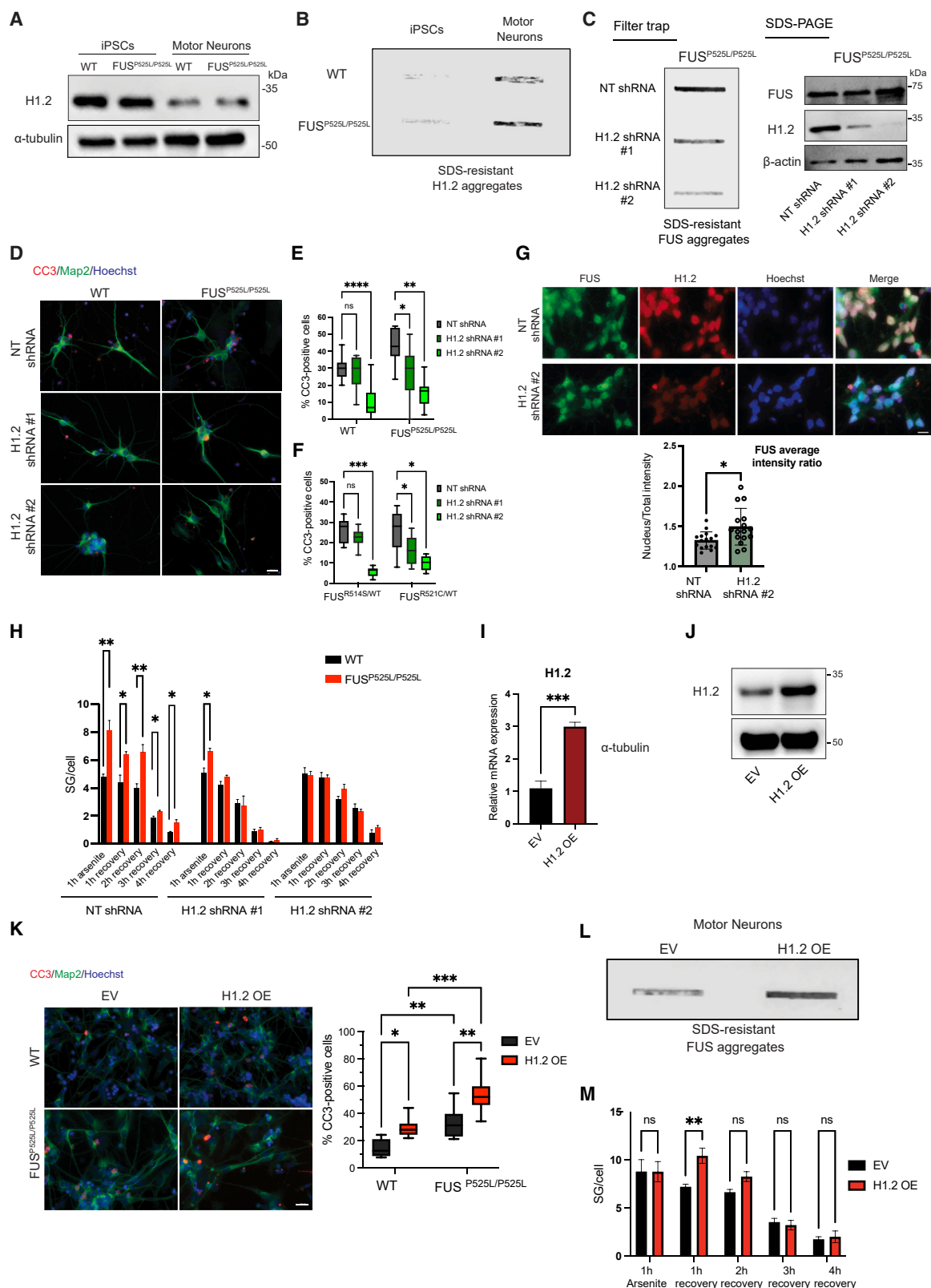
(A) Log₂ protein label-free quantification (LFQ) values of FUS levels after immunoprecipitation (IP) with anti-FUS antibody of isogenic WT (FUS^{WT/WT}) and mutant FUS^{P525L/P525L} motor neurons. IP with an anti-FLAG antibody is the negative control (mean ± SD, *n* = 3 biological replicates).
(B) Volcano plot of the FUS interactome comparing FUS^{P525L/P525L} and WT motor neurons. The $-\log_{10}(p)$ value of a two-sided *t* test is plotted against the log₂-transformed fold change of LFQ values from IP with the anti-FUS antibody.
(C) Heatmap of FUS interactors showing significantly different binding intensities between WT and mutant FUS in motor neurons (two-sided *t* test, false discovery rate [FDR] < 0.05 was considered significant).
(D) Heatmap of FUS interactors showing significantly different binding intensities between WT and mutant FUS in iPSCs (two-sided *t* test, FDR < 0.05 was considered significant).
(E) Volcano plot comparing the FUS interactome of FUS^{P525L/P525L} motor neurons with their parental iPSCs.
(B–E) *n* = 3 biological replicates; two-sided *t* test, false discovery rate < 0.05 was considered significant.
(F) Western blot with antibodies against FUS, H1.2, and α-tubulin following H1.2 IP in motor neurons. Representative of five independent experiments.
(G) Log₂ LFQ values of H1.2 levels after IP with the anti-FUS antibody in WT and mutant FUS^{P525L/P525L} motor neurons. IP with anti-FLAG antibody is the negative control (mean ± SD, *n* = 3 biological replicates).
Statistical comparisons were made by two-sided Student's *t* test for unpaired samples (A) or one-way ANOVA (G). ***p* < 0.01, *****p* < 0.001; ns, *p* > 0.05.
See also [Figure S2](#) and [Data S1–S3](#).

confirmed its presence in the cytoplasm ([Figure S2C](#)). Since mutant FUS localizes to both the nucleus and cytoplasm,²⁸ we investigated whether its enhanced association with H1.2 is specific to either of these cellular compartments. We observed that the interaction between mutant FUS and H1.2 occurs primarily within the nucleus ([Figure S2C](#)).

Given that FUS is an RBP, we investigated whether its interaction with H1.2 is direct or facilitated through RNA binding. To this end, we treated the samples with RNase A prior to IP of FUS protein ([Data S1](#)). Importantly, RNase treatment did not decrease the interaction between mutant FUS^{P525L} and H1.2, supporting the theory that these are likely protein-protein interactions ([Figure 2G](#); [Data S1](#)). In contrast, the lower interaction of WT FUS with H1.2 was upregulated upon removal of RNA ([Figure 2G](#)).

H1.2 promotes mutant FUS pathology in human motor neurons

The total levels of H1.2 were similar in motor neurons expressing mutant FUS compared to isogenic control motor neurons ([Figure 3A](#)). However, we observed increased aggregation of H1.2 in FUS^{P525L/P525L} motor neurons ([Figure 3B](#)). On the contrary, H1.2 did not form aggregates in undifferentiated FUS^{P525L/P525L} iPSCs, even when these cells express higher amounts of H1.2 compared to their motoneuronal counterparts ([Figures 3A](#) and [3B](#)). To determine whether H1.2 modulates mutant FUS-related changes, we used two independent short hairpin RNA (shRNA) constructs against this histone ([Figure 3C](#)). Knockdown of H1.2 was sufficient to decrease aggregation of mutant FUS^{P525L} without lowering the total levels of FUS ([Figure 3C](#)). Loss of



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H1.2 decreased apoptosis in both control and FUS^{P525L/P525L} motor neurons (Figures 3D and 3E). Similarly, H1.2 knockdown also led to decreased apoptosis in motor neurons expressing different FUS mutations in heterozygosis (Figure 3F). In addition to apoptosis, other cell death mechanisms can contribute to the neurodegeneration phenotype in ALS.⁴⁰ We found that H1.2 knockdown reduces global cell death rates in FUS^{P525L/P525L} motor neurons (Figure S3A). Then, we assessed whether lowering H1.2 levels influences the intracellular localization of FUS^{P525L}. Although mutant FUS was still partially localized to the cytoplasm of motor neurons, knockdown of H1.2 increased FUS localization in the nucleus (Figure 3G). Together, these results suggest that the elevated interaction of mutant FUS with H1.2 promotes FUS mislocalization in the cytosol, leading to pathological aggregation and neurodegeneration.

Importantly, arsenite treatment not only induced the accumulation of mutant FUS into SGs (Figure 1A) but also led to the colocalization of H1.2 foci with cytoplasmic FUS foci (Figure S3B). Both ALS motor neurons and their parental iPSCs exhibit higher numbers of SGs per cell upon arsenite treatment as well as slower SG disassembly upon removal of stress (Figures 3H, S3C, and S3D). However, knockdown of H1.2 reduced these alterations in SG dynamics in both ALS motor neurons and iPSCs (Figures 3H, S3C, and S3D). In correlation with the strongest knockdown levels induced by the second H1.2 shRNA construct (Figure 3C), this shRNA was the most efficient in reducing mutant FUS aggregation, neurodegeneration, and SG alterations (Figures 3C–3F and 3H), supporting a role of H1.2 in ALS-related changes.

To further define the effects of H1.2 levels, we generated cell lines that stably overexpress H1.2 (Figures 3I and 3J). We

observed that H1.2 overexpression induces cell death in both control and FUS^{P525L/P525L} motor neurons, but the effects were significantly more pronounced in ALS motor neurons (Figure 3K). Moreover, overexpression of H1.2 led to the accumulation of SDS-resistant aggregates of mutant FUS in motor neurons, suggesting that H1.2 might affect FUS solubility (Figure 3L). Increased levels of H1.2 did not alter the number of SGs assembled upon arsenite treatment in FUS^{P525L} motor neurons (Figure 3M). However, FUS^{P525L} motor neurons overexpressing H1.2 exhibited slower clearance of SGs 1 h after the removal of arsenite stress, although SG disassembly normalized at later recovery time points (Figure 3M). On the other hand, H1.2 overexpression even decreased SG assembly in FUS^{P525L} iPSCs, whereas it did not delay SG disassembly in these cells (Figure S3E).

FUS mutation enhances PARP1-FUS-H1.2 complex assembly

H1.2 is a subtype of the linker histone H1 family.⁴¹ H1 proteins undergo various post-translational modifications that regulate their protein-protein interactions and functional switches.^{39,42,43} H1 proteins are the main PARylation acceptor histones.⁴⁴ PAR residues are primarily added by PARP1,⁴⁵ an enzyme that interacts with WT FUS^{21,34,36–38,46–48} (Data S1). To assess the requirement of PARP1 for FUS-H1.2 interaction, we performed IP with an H1.2 antibody in PARP1 knockout human U2OS cells^{49,50} (Figure S4). Notably, knockout of PARP1 led to a pronounced decrease in the association between endogenous H1.2 and WT FUS protein (Figure 4A), suggesting that PARP1 contributes to this interaction.

Similar to H1.2, the interaction between PARP1 and FUS was upregulated in FUS^{P525L} motor neurons (Figures 2B, 2C, and 4B). The elevated association between mutant FUS and PARP1 was

Figure 3. H1.2 levels influence mutant FUS-related phenotypes

- (A) Western blot of H1.2 levels in control WT and FUS^{P525L/P525L} iPSCs and motor neurons. α -Tubulin is the loading control. Representative of three independent experiments.
- (B) Filter trap analysis of SDS-resistant H1.2 aggregates (detected by anti-H1.2 antibody) in FUS^{P525L/P525L} iPSCs and motor neurons. Representative of three independent experiments.
- (C) Filter trap of mutant FUS aggregates (detected by anti-FUS antibody) in FUS^{P525L/P525L} motor neurons upon H1.2 knockdown using two independent shRNA constructs. NT, non-targeting shRNA control. Right: western blot against FUS, H1.2, and β -actin loading control. Representative of three independent experiments.
- (D) Immunostaining of apoptotic cells with CC3 in motor neurons. MAP2 and Hoechst 33342 were used as markers of neurons and nuclei, respectively. Scale bar: 20 μ m. Representative of three independent experiments.
- (E and F) The percentage of CC3-positive cells/total nuclei in motor neurons (mean $\pm$ SEM of at least 100 cells per condition scored in three independent experiments).
- (G) Immunostaining of FUS and H1.2 in FUS^{P525L/P525L} motor neurons. Scale bar: 10 μ m. The graph represents the ratio of nuclear FUS signal intensity to total FUS signal intensity (mean $\pm$ SEM of at least 20 cells per condition scored in three independent experiments).
- (H) Total number of G3BP1-positive SGs per nuclei in isogenic control (WT) and FUS^{P525L/P525L} motor neurons. G3BP1 and Hoechst 33342 staining were used as markers of SGs and nuclei, respectively. Motor neurons were assessed after 1 h of 500 μ M arsenite treatment and then at the indicated times upon removal of arsenite stress (mean $\pm$ SEM of at least 90 cells per condition scored in three independent experiments).
- (I) Relative levels of H1.2 mRNA in FUS^{P525L/P525L} motor neurons with empty vector (EV) or H1.2 overexpression (OE) (mean $\pm$ SEM, $n = 6$ biological replicates).
- (J) Western blot against H1.2 and α -tubulin loading control. Representative of three independent experiments.
- (K) Immunostaining of motor neurons overexpressing H1.2 with anti-CC3, anti-MAP2, and Hoechst 33342. Scale bar: 20 μ m. Graph represents the percentage of CC3-positive cells/total nuclei in motor neuron cultures (mean $\pm$ SEM of at least 200 cells per condition scored in three independent experiments).
- (L) Filter trap analysis of SDS-resistant FUS aggregates (detected by FUS antibody) in H1.2-overexpressing motor neurons. Representative of four independent experiments.
- (M) Total number of G3BP1-positive SGs per nuclei in control and FUS^{P525L/P525L} motor neurons (mean $\pm$ SEM of at least 80 cells per condition scored in three independent experiments). G3BP1 and Hoechst 33342 staining were used as markers of SGs and nuclei, respectively. Motor neurons were assessed after 1 h of 500 μ M arsenite treatment and then upon removal of arsenite stress at the indicated times.
- Statistical comparisons were made by two-way ANOVA (E, F, K, and M) or two-tailed Student's *t* test for unpaired samples (G–I). * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$; ns, $p > 0.05$.
- See also Figure S3.

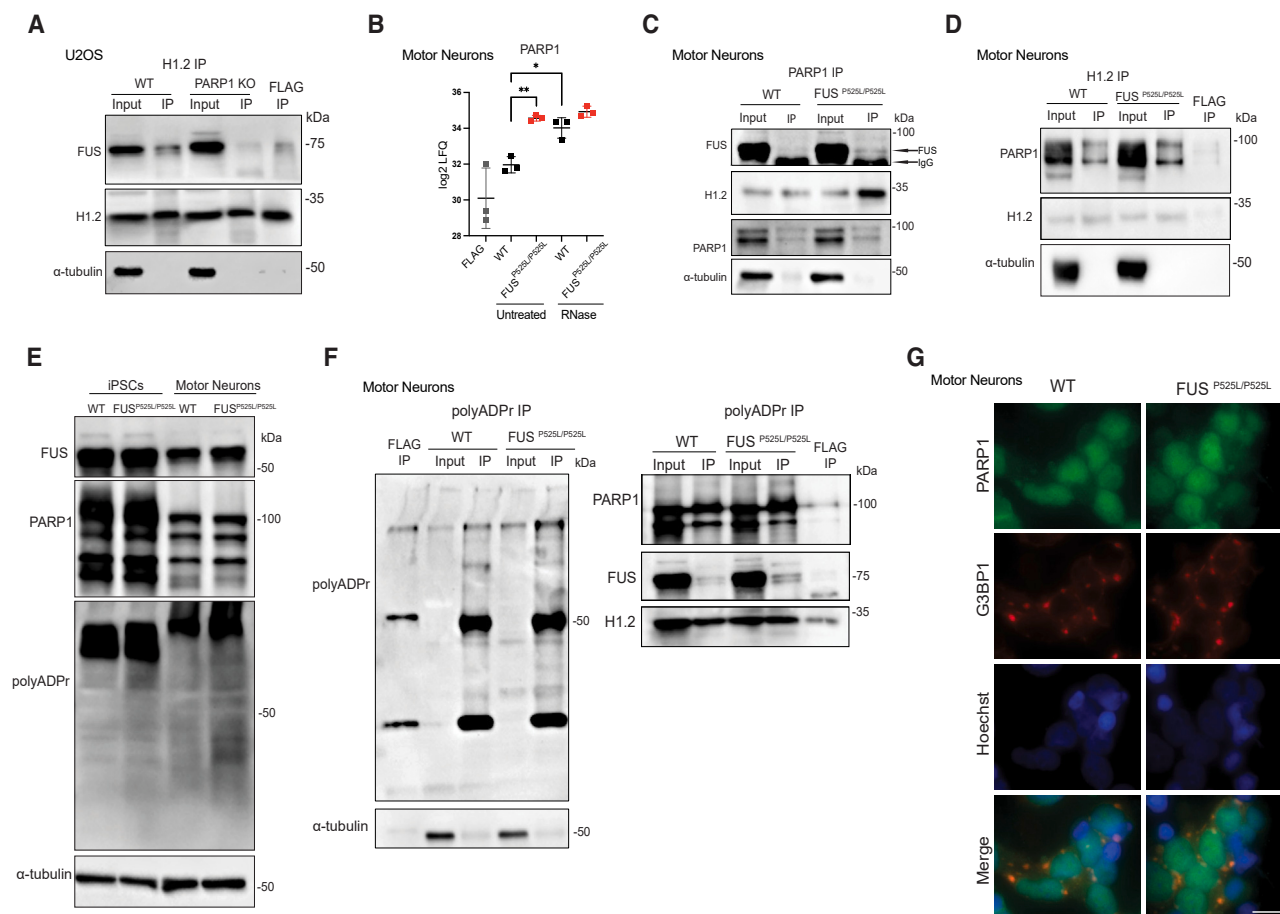


Figure 4. Mutant FUS increases the assembly of the PARP1-FUS-H1.2 complex

(A) Western blot with antibodies against FUS, H1.2, and α -tubulin after H1.2 IP in WT and PARP1 knockout (KO) U2OS cells. Representative of two independent experiments.

(B) Log2 protein LFQ values of PARP1 levels after IP with anti-FUS antibody of isogenic WT (FUS^{WT/WT}) and mutant FUS^{P525L/P525L} motor neurons (mean $\pm$ SD, $n = 3$ biological replicates). IP with anti-FLAG antibody is the negative control. Statistical comparisons were made by one-way ANOVA. * $p < 0.05$, ** $p < 0.01$.

(C) Western blot with antibodies against FUS, H1.2, and α -tubulin after PARP1 pull-down in motor neurons. Representative of three independent experiments.

(D) Western blot with antibodies against PARP1 and α -tubulin following H1.2 IP in motor neurons. Representative of three independent experiments.

(E) Western blot of total polyADPr (PARylated) proteins, PARP1, and FUS in iPSCs and their differentiated motoneuronal counterparts. Representative of three independent experiments.

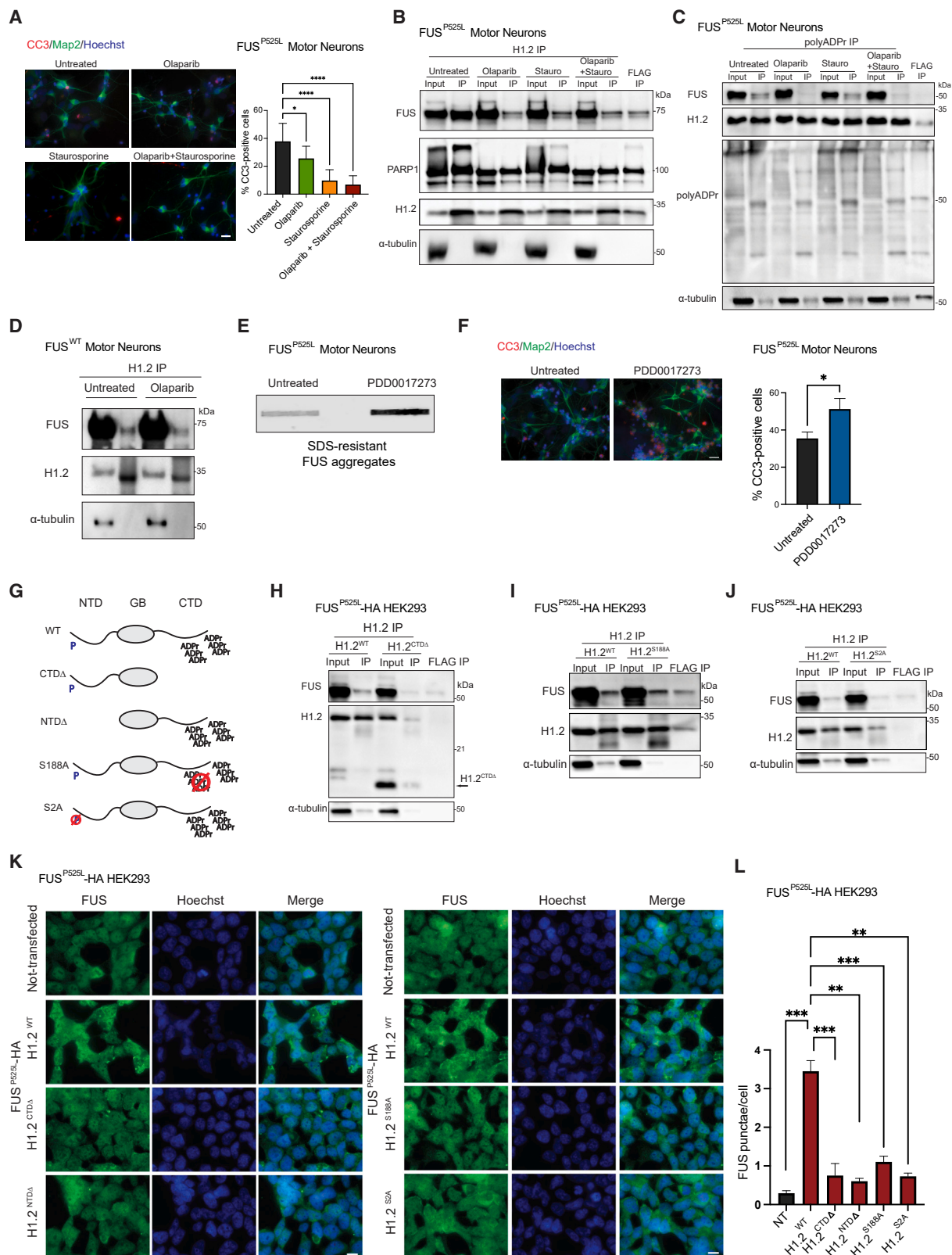
(F) Western blot with antibodies against polyADPr, PARP1, FUS, H1.2, and α -tubulin after IP of PARylated proteins. Representative of three independent experiments.

(G) Immunostaining of PARP1 in FUS^{WT/WT} and mutant FUS^{P525L/P525L} motor neurons treated with 500 μ M arsenite for 1 h. G3BP1 and Hoechst 33342 were used to stain SGs and nuclei, respectively. Representative of four independent experiments. Scale bar: 10 μ m.

See also [Figure S4](#) and [Data S1](#).

not mediated by RNA ([Figure 4B](#)). To further confirm the elevated interaction between PARP1, H1.2, and mutant FUS, we performed coIP experiments followed by western blotting. IP with the anti-PARP1 antibody demonstrated an increased interaction between PARP1 and mutant FUS^{P525L} compared with WT FUS ([Figure 4C](#)), supporting our proteomics data with the FUS antibody. Using either PARP1 or H1.2 antibodies for coIP, we found increased binding of PARP1 enzyme to H1.2 in FUS^{P525L/P525L} motor neurons compared to isogenic controls ([Figures 4C and 4D](#)). Together, these results indicate that PARP1, H1.2, and FUS may form a complex whose assembly is upregulated by mutant FUS.

The PARP1 enzyme is responsible for the majority of PARylation events within a cell.⁵¹ We found that the total levels of PARylation are upregulated in the whole-cell lysates of FUS^{P525L/P525L} motor neurons compared to isogenic controls ([Figure 4E](#)). IP of PARylated proteins revealed increased PARylation of FUS and PARP1 itself in motor neurons expressing mutant FUS ([Figure 4F](#)). Although H1.2 was heavily PARylated, we did not detect an increase upon FUS mutation ([Figure 4F](#)). The PARylation activity of PARP1 is also associated with SG assembly and dynamics.^{52,53} In addition, PARP1 protein itself is a constituent of SGs in different cell types.^{6,54,55} Along these lines, we observed PARP1 within



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arsenite-induced SGs of both control and FUS^{P525L/P525L} motor neurons (Figure 4G).

ALS-associated phenotypes arising from mutant FUS are PARylation dependent

While our results indicate that PARP1 is necessary for FUS-H1.2 interaction (Figure 4A), it remained unclear whether the elevated PARylation induced by mutant FUS contributes to the enhanced formation of FUS-H1.2-PARP1 complexes in FUS^{P525L} motor neurons. Thus, we asked whether post-transcriptional modifications such as PARylation influence FUS-H1.2-PARP1 assembly and ALS-related changes.

In addition to PARylation, our proteomics data revealed phosphorylation of H1.2 on its Ser2 in FUS^{P525L} motor neurons (Figure S5A; Data S4). Given that protein kinase C (PKC) activity on H1 histones is increased in the spinal cord of ALS patients,⁵⁶ we targeted both PARP1 and PKC activity. To inhibit PKC activity, we used a low concentration of staurosporine, as higher amounts can induce cell death.⁵⁷ For PARylation inhibition, we used olaparib, which outcompetes nicotinamide adenine dinucleotide (NAD⁺) for binding to the catalytically active domain of the PARP1 enzyme.⁵⁸ Importantly, treatment with either olaparib or staurosporine, alone or in combination, decreased apoptosis in FUS^{P525L} motor neurons (Figure 5A). Likewise, these treatments also reduced global cell death rates (Figure S5B).

To assess whether the beneficial effects of olaparib and staurosporine ensue from changes in the interaction between mutant FUS^{P525L} and H1.2, we performed IP experiments. Both olaparib and staurosporine treatments, whether administered separately or in combination, reduced FUS^{P525L}-H1.2 binding in motor neurons (Figure 5B). Since staurosporine can have off-target effects,⁵⁹ we tested a different PKC inhibitor (Gö 6983)⁶⁰ and observed that it also decreases FUS^{P525L}-H1.2 association (Figure S5C).

In addition to FUS^{P525L}-H1.2 interaction, inhibition of either PARylation or PKC-dependent phosphorylation weakened H1.2-PARP1 binding (Figure 5B). However, only olaparib treatment, but not staurosporine, globally decreased PARylation levels (Figure 5C). Moreover, PARylation of mutant FUS was also specifically diminished by olaparib treatment (Figure 5C), indicating that PKC inhibition modulates disease-related changes independent of PARP1 activity. Since olaparib did not decrease the PARylation levels of H1.2 (Figure 5C), our results suggest that the upregulated assembly of the FUS-H1.2-PARP1 complex in FUS^{P525L} motor neurons may be associated with elevated PARylation of mutant FUS. Indeed, olaparib treatment did not reduce the basal interaction between WT FUS and H1.2 in control motor neurons (Figure 5D). However, we cannot rule out that other changes induced by elevated PARylation promote the elevated interaction of H1.2 with mutant FUS.

To further investigate the detrimental effects of increased PARylation in FUS^{P525L} motor neurons, we administered PDD0017273 to inhibit poly(ADP-ribose) glycohydrolase (PARG) enzyme, which cleaves PARylation/PAR chains. Notably, PDD0017273 treatment increased FUS aggregation and apoptosis in FUS^{P525L/P525L} motor neurons (Figures 5E and 5F). We observed only a slight increase in the association between mutant FUS and H1.2 upon PARG inhibitor treatment in FUS^{P525L} motor neurons (Figure S5D), likely because their interaction is already upregulated at the basal level in these cells. To further assess whether the PARG inhibitor increases the binding between mutant FUS and H1.2, we used human U2OS cells overexpressing FUS^{P525L}. Indeed, we found that PDD0017273 treatment promotes FUS-H1.2 association in these cells (Figure S5E).

While chemical inhibitors are effective tools for modulating PARylation and phosphorylation on a global scale, they cannot

Figure 5. Post-translational modifications contribute to the effects of H1.2 in FUS-ALS pathology

(A) Immunostaining of CC3 in FUS^{P525L/P525L} motor neurons treated with 10 μ M olaparib for 24 h or 20 nM staurosporine for 3 h. Map2 and Hoechst 33342 were used as markers of neurons and nuclei, respectively. Scale bar: 20 μ m. The graph represents the percentage of CC3-positive cells (mean $\pm$ SEM of at least 200 cells per condition from three independent experiments). Statistical comparisons were made by one-way ANOVA with multiple-comparisons analysis. * p < 0.05, *** p < 0.0001.

(B) Western blot with antibodies against FUS, H1.2, PARP1, and α -tubulin after IP of H1.2 in FUS^{P525L/P525L} motor neurons treated with 10 μ M olaparib for 24 h or 20 nM staurosporine for 3 h. Representative of four independent experiments.

(C) Western blot with antibodies against FUS, H1.2, polyADPr, and α -tubulin after IP of polyADPr (PARylated) proteins in motor neurons treated with 10 μ M olaparib for 24 h or 20 nM staurosporine for 3 h. Representative of three independent experiments.

(D) Western blot with antibodies against FUS, H1.2 and α -tubulin after IP of H1.2 WT FUS motor neurons treated with 10 μ M olaparib for 24 h. Representative of two independent experiments.

(E) Filter trap analysis of mutant FUS aggregates (detected by anti-FUS antibody) in FUS^{P525L/P525L} motor neurons treated with 1 μ M PDD0017273 for 24 h. Representative of two independent experiments.

(F) Immunostaining of CC3 in FUS^{P525L/P525L} motor neurons treated with 1 μ M PDD0017273 for 24 h. MAP2 and Hoechst 33342 were used as markers of neurons and nuclei, respectively. Scale bar: 20 μ m. The graph represents the percentage of CC3-positive cells (mean $\pm$ SEM of at least 200 cells per condition from two independent experiments). Statistical comparisons were made by two-tailed Student's t test for unpaired samples. * p < 0.05.

(G) Schematic of H1.2 domains and post-translational modifications targeted in this study: N-terminal domain (NTD), globular domain (GB), and C-terminal domain (CTD).

(H–J) Western blot with antibodies against FUS, H1.2, and α -tubulin after H1.2 IP in HEK293 cells transfected with FUS P525L-hemagglutinin (HA) and the indicated H1.2 mutant variants.

The images are representative of three independent experiments.

(K) Immunostaining of FUS in FUS P525L-HA HEK293 cells overexpressing H1.2^{WT}, H1.2^{CTDA}, H1.2^{NTDA}, H1.2^{S188A}, or H1.2^{S2A}. Hoechst 33342 was used as a marker for nuclei. Scale bar: 10 μ m. Representative of two independent experiments.

(L) Number of FUS granules per cell (mean $\pm$ SEM of at least 100 cells per condition from two independent experiments).

Statistical comparisons were made by one-way ANOVA (A and L) or by two-tailed Student's t test for unpaired samples (F). ** p < 0.01, *** p < 0.001.

See also Figure S5 and Data S4.

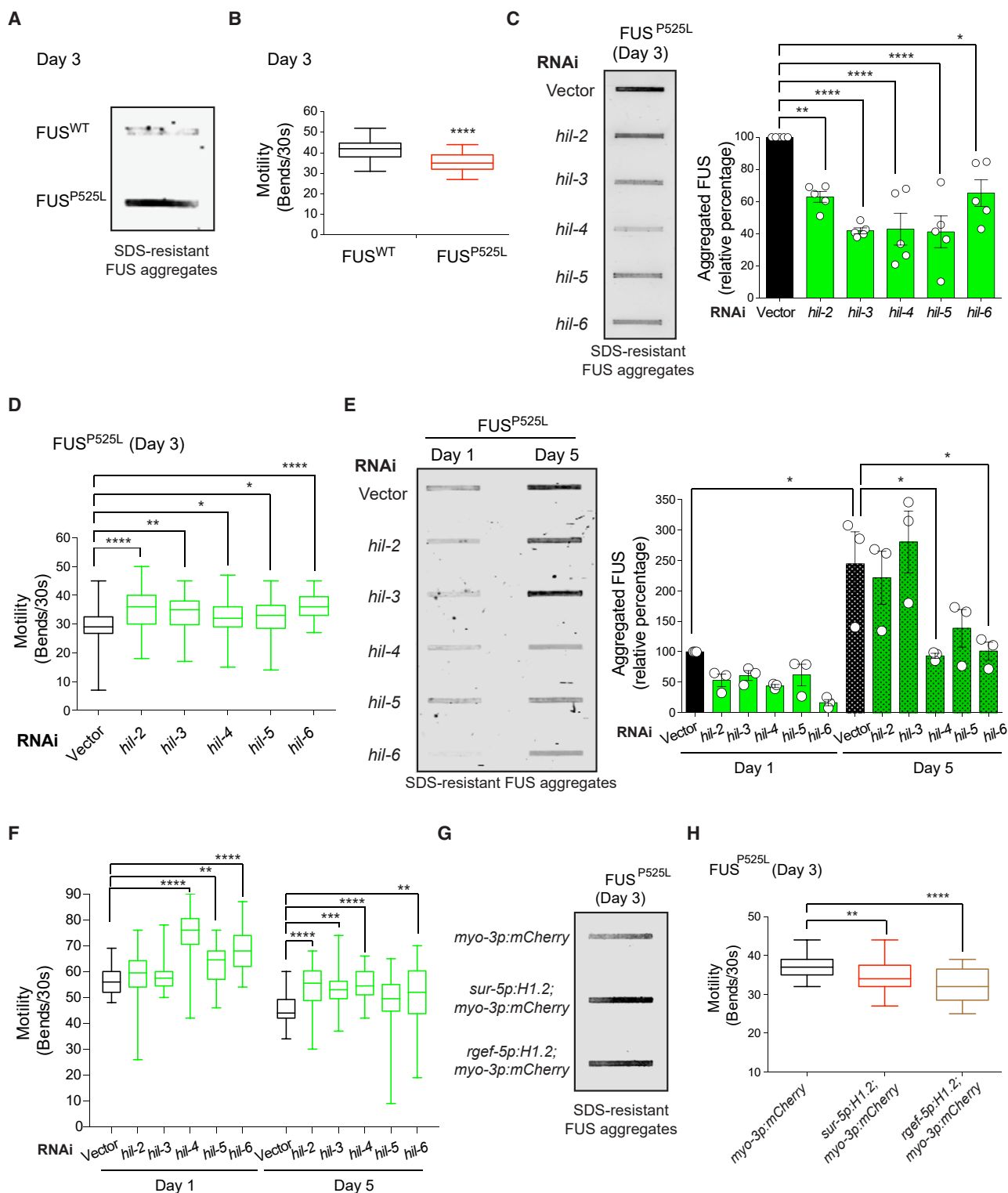


Figure 6. Knockdown of H1.2 worm orthologs ameliorates pathological changes in FUS^{P525L}-ALS *C. elegans*

(A) Filter trap assay of SDS-resistant FUS aggregates (detected by anti-FUS antibody) in day-3 adult *C. elegans* expressing human WT FUS or mutant FUS^{P525L} in neurons. Representative of four independent experiments.

(B) Thrashing movements over a 30-s period in FUS^{WT} and FUS^{P525L} worms at day 3 of adulthood (FUS^{WT} *n* = 44 worms from three independent experiments, FUS^{P525L} *n* = 45).

(legend continued on next page)

discriminate whether these post-translational modifications have a specific effect on H1.2 to regulate ALS-related changes. Previous studies indicate that Ser188 is a major PARylation site of H1.2.^{61,62} Although it does not completely abolish PARylation of H1.2, *in vivo* and *in vitro* experiments have demonstrated that mutation of this site (S188A) decreases H1.2 PARylation.⁶² Thus, we generated HEK293 cells expressing the S188A H1.2 mutant variant (Figure 5G). To assess the effects of PKC-mediated phosphorylation, we generated a Ser2 phospho-null H1.2 mutant (S2A). In addition, we aimed to understand the role of the N-terminal domain (NTD) and C-terminal domain (CTD) of H1.2 in the context of FUS-ALS (Figure 5G). There are two main reasons that raise the importance of these domains: (1) both the NTD and CTD of H1.2 are intrinsically disordered, which might promote FUS aggregation, and (2) the phospho target Ser2 is located in the NTD, while the PAR target Ser188 as well as other potential PARylation sites are part of the CTD.⁶² The distinct H1.2 constructs were co-expressed with FUS^{P525L} in human HEK293 cells (Figure 5G). Since most H1.2 antibodies recognize the NTD region of H1.2, we were not able to detect an H1.2^{NTDΔ} signal by western blotting, although we could detect H1.2^{CTDΔ} (Figure S5F). Importantly, CTD-deficient H1.2 had decreased interaction with FUS^{P525L}, indicating the importance of this internally disordered domain in FUS pathology (Figure 5H). On the other hand, PAR-less (S188A) and S2 phospho-null H1.2 variants did not exhibit changes in FUS interaction compared with WT H1.2 (Figures 5I and 5J). Moreover, the PAR-less S188A H1.2 did not affect PARylation of mutant FUS (Figure S5G).

In line with our results in neurons, overexpression of WT H1.2 triggered cytoplasmic FUS^{P525L} aggregation in HEK293 cells (Figures 5K and 5L). In contrast, overexpression of PAR-less (S188A) and S2 phospho-null H1.2 variants resulted in lower numbers of FUS^{P525L} aggregates compared with WT H1.2 (Figures 5K and 5L). Likewise, overexpression of H1.2 lacking the CTD or NTD did not trigger the accumulation of FUS^{P525L} into foci, further supporting a role of disordered terminal domains of H1.2 in FUS aggregation (Figures 5K and 5L). Collectively, our data establish a link between PARylation and mutant FUS-H1.2-PARP1 interactions in ALS-related changes in human cell models.

Knockdown of H1.2 or PARP1 orthologs prevents disease-related changes in FUS-ALS *C. elegans*

To examine the influence of H1.2 on ALS-related changes *in vivo*, we used *C. elegans* expressing human FUS^{P525L} throughout the nervous system.⁶³ These animals exhibit pathological phenotypes of ALS, including mutant FUS aggregation and subsequent neurodegeneration, as reflected by loss of motility (Figures 6A and 6B).^{63–65} Thus, we asked whether knockdown of H1.2 orthologs prevents these phenotypes in FUS^{P525L}-expressing worms. To avoid potential developmental effects, we initiated the RNAi treatment when the animals reached adulthood. Knockdown of H1.2 orthologs did not affect the lifespan of worms expressing WT FUS and even slightly extended the lifespan of FUS^{P525L}-expressing worms (Figure S6; Data S5). Notably, single knockdown of any of the *C. elegans* orthologs of H1.2 (i.e., *hil-2*–*hil-6*) reduced mutant FUS^{P525L} aggregation in day-3 adult worms (Figure 6C). Moreover, knockdown of H1.2 orthologs after development ameliorated deficits in coordinated movement of FUS^{P525L} worms at day 3 of adulthood (Figure 6D).

The levels of aggregated FUS^{P525L} increased with age (Figure 6E), supporting the theory that aging also promotes mutant FUS aggregation in *C. elegans*.⁶³ Thus, we analyzed the effects of the H1.2 worm orthologs in older worms. In contrast to day-3 adult worms, knockdown of H1.2 orthologs such as *hil-2* or *hil-3* lost its efficacy in reducing FUS aggregation when the animals reached day 5 of adulthood (Figure 6E). However, RNAi against *hil-4* or *hil-6* retained the ameliorative effects on mutant FUS aggregation in 5-day-old worms (Figure 6E). These data suggest that knockdown of specific H1.2 orthologs, such as *hil-4* and *hil-6*, is more effective to prevent FUS aggregation during aging compared to other orthologs. Importantly, knockdown of either *hil-4* or *hil-6* during adulthood also attenuated motility deficits in day-5 adult FUS^{P525L} worms (Figure 6F). However, we observed that FUS^{P525L} worms treated with *hil-2* and *hil-3* RNAi also exhibit increased motility at day 5 (Figure 6F), likely due to the beneficial effects of these RNAi in preventing FUS aggregation at earlier ages (Figure 6C).

To further confirm the role of H1.2 in ALS-related changes, we overexpressed human H1.2 in FUS^{P525L} *C. elegans* models. Ubiquitous overexpression of human H1.2 through the

(C) Filter trap of SDS-resistant FUS aggregates in day-3 adult FUS^{P525L} worms on knockdown of H1.2 *C. elegans* orthologs (*hil-2*–*hil-6*). Representative of five independent experiments. The graph represents the relative percentage values of aggregated FUS^{P525L} levels to control vector RNAi (mean ± SEM, *n* = 5 independent experiments).

(D) Motility assay in day-3 adult FUS^{P525L} worms on knockdown of H1.2 worm orthologs (vector RNAi *n* = 62 worms from three independent experiments, *hil-2* RNAi *n* = 54, *hil-3* RNAi *n* = 62, *hil-4* RNAi *n* = 50, *hil-5* RNAi *n* = 61, *hil-6* RNAi *n* = 37).

(E) Filter trap of SDS-resistant FUS aggregates in day-1 and day-5 adult FUS^{P525L} worms on knockdown of H1.2 *C. elegans* orthologs. Representative of three independent experiments. The graph represents the relative percentage values of aggregated FUS^{P525L} levels to day-1 adult worms + vector RNAi (mean ± SEM, *n* = 3 independent experiments).

(F) Motility assay in day-1 and day-5 adult FUS^{P525L} worms on knockdown of H1.2 worm orthologs (*n* = 50 worms per condition from three independent experiments).

(G) Filter trap of FUS aggregates in day-3 adult FUS^{P525L} worms upon whole-body (*sur-5p*) or neuron-specific (*rgef-1*) expression of human H1.2. Representative of three independent experiments.

(H) Motility assay in day-3 adult FUS^{P525L} worms upon whole-body or neuron-specific expression of human H1.2 (*n* = 33 worms per condition from three independent experiments).

In (B), (D), (F), and (H), the boxplots represent the 25th–75th percentiles, the line depicts the median, and the whiskers show the minimum–maximum values. In all experiments, RNAi was initiated after development. Statistical comparisons were made by two-tailed Student's *t* test for unpaired samples (B and H) or one-way ANOVA with Tukey's multiple-comparisons test (C–F and I). **p* < 0.05, ***p* < 0.01, ****p* < 0.001, *****p* < 0.0001.

See also Figure S6.

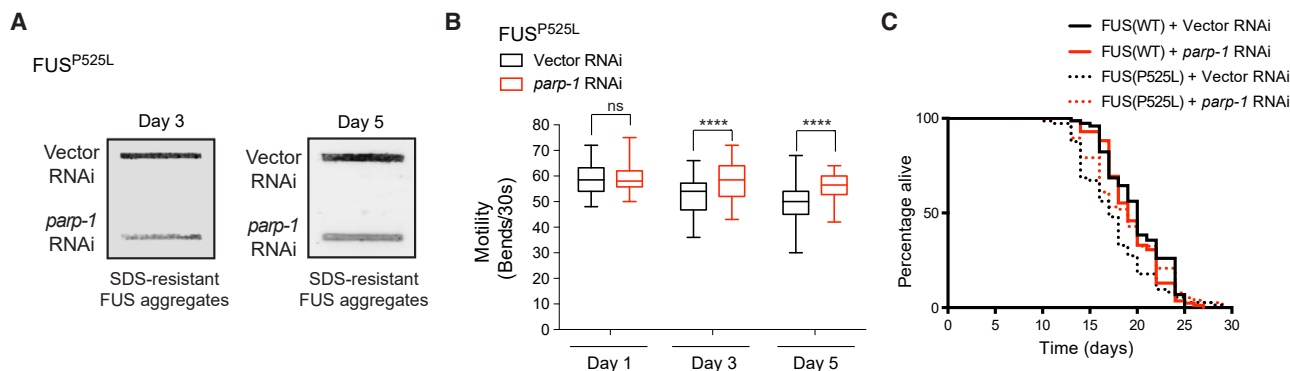


Figure 7. Knockdown of *parp-1* prevents disease-related changes in FUS^{P525L}-ALS *C. elegans*

(A) Filter trap analysis of SDS-resistant FUS^{P525L} aggregates (detected by anti-FUS antibody) in FUS^{P525L}-expressing *C. elegans* at day 3 (left) and day 5 (right) of adulthood on knockdown of *parp-1*. Representative of three independent experiments.

(B) Thrashing movements over a 30-s period in FUS^{P525L} worms on *parp-1* RNAi at day 1, 3, and 5 of adulthood ($n = 50$ worms per condition from three independent experiments). The boxplots represent the 25th–75th percentiles, the line depicts the median, and the whiskers show the minimum–maximum values. Statistical comparisons were made by one-way ANOVA, Tukey’s multiple comparisons test. **** $p < 0.0001$; ns, $p > 0.05$.

(C) FUS^{P525L}-expressing worms are short lived compared to FUS^{WT}-expressing worms ($p < 0.001$). Knockdown of *parp-1* extends the lifespan of FUS^{P525L}-expressing worms ($p < 0.05$) but does not extend the lifespan of control worms expressing WT FUS ($p = 0.2323$). FUS^{WT} + vector RNAi (mean $\pm$ SEM): 19.97 days $\pm$ 0.38, FUS^{WT} + *parp-1* RNAi: 19.40 $\pm$ 0.32, FUS^{P525L} + vector RNAi: 17.50 $\pm$ 0.45, FUS^{P525L} + *parp-1* RNAi: 18.86 $\pm$ 0.48. p -values: two-sided log rank test, $n = 96$ worms/condition. In all experiments, RNAi was initiated after development. Data S5 contains statistical analysis and replicate data of independent lifespan experiments.

pan-tissue *sur-5* promoter hastened mutant FUS aggregation and motility deficits (Figures 6G and 6H). Overexpression of H1.2 through the neuron-specific *rgef-1* promoter was sufficient to aggravate FUS^{P525L} aggregation in neurons and subsequent loss of motility (Figures 6G and 6H), indicating that intracellular levels of H1.2 can directly regulate mutant FUS aggregation in neurons.

Finally, we assessed whether *parp-1*, the worm ortholog of *PARP1*, also influences mutant FUS aggregation *in vivo*. Consistent with our findings in human motor neurons, knockdown of *parp-1* during adulthood reduced FUS^{P525L} aggregation in *C. elegans* (Figure 7A). Along these lines, loss of *parp-1* mitigated motility deficits in FUS-ALS worms at days 3 and 5 of adulthood (Figure 7B). Moreover, knockdown of *parp-1* after development extended the short lifespan of FUS^{P525L}-expressing worms but did not increase the lifespan of worms expressing WT FUS (Figure 7C; Data S5).

DISCUSSION

Although H1.2 has been implicated in neurodegenerative disorders such as Alzheimer’s disease and Parkinson’s disease,^{66–70} its association with ALS remained unexplored. We found that H1.2 levels influence mutant FUS aggregation, SG dynamics, and neurodegeneration in ALS motor neurons, suggesting a potential role for this specific H1 subtype in FUS-ALS pathology.

In contrast to motor neurons, the interaction of FUS with H1.2 does not increase in FUS^{P525L}-expressing iPSCs relative to control iPSCs. Additionally, the interaction between FUS^{P525L} and H1.2 is significantly lower in iPSCs compared to their motoneuronal counterparts. Along these lines, we found that mutant FUS^{P525L} does not induce apoptosis in undifferentiated iPSCs. We speculate that the elevated binding of H1.2 with FUS^{P525L}

in motor neurons contributes to the more pronounced effects of mutant FUS expression in motor neurons compared to iPSCs. An intriguing question raised by these findings is how iPSCs prevent elevated binding of mutant FUS with H1.2. Previous research demonstrated that pluripotent stem cells such as iPSCs have increased mechanisms to maintain protein homeostasis (proteostasis), thereby preventing the misfolding and aggregation of disease-related proteins.^{71–73} However, iPSCs lose their elevated proteostasis capacity during differentiation.^{71–73} Thus, this enhanced proteostasis capacity of iPSCs may prevent conformational changes in aggregation-prone mutant FUS that promote its interaction with histone H1.2.

In addition to H1.2, the interaction of FUS with PARP1 was also upregulated for mutant FUS in motor neurons. Multiple studies have previously reported an interaction of FUS with PARP1.^{21,34,36–38,46–48} In human HEK293 cells, either WT FUS or mutant FUS^{P525L} interacts with both PARP1 and H1.2 when FUS accumulates into condensates.³⁷ In contrast, free FUS protein does not interact with PARP1 or H1.2³⁷. These findings demonstrate that the accumulation of FUS into condensates can change its interactome, which may be particularly relevant for understanding how H1.2 and PARP1 regulate ALS-related changes.

Our results collectively suggest that the increased interaction of mutant FUS with H1.2 and PARP1 contributes to ALS-related changes through various interconnected mechanisms. The role of PARylation in the assembly of FUS condensates and its translocation to the cytoplasm has been studied extensively. Elevated PAR levels promote FUS-FUS interactions, leading to the assembly of FUS condensates.⁷⁴ PARP1 and FUS can form co-condensates at DNA damage sites to facilitate DNA repair.⁴⁸ However, PARP1 activation eventually triggers the translocation of WT FUS from the nucleus to the cytoplasm under oxidative

stress.^{22,48} Thus, we hypothesize that the upregulated interaction of PARP1 with mutant FUS may contribute to the increased cytoplasmic location of mutant FUS and subsequent formation of pathological FUS aggregates.

Previous studies have shown that PARP1 interacts with H1.2.^{62,75,76} We found that the elevated interaction of PARP1 with mutant FUS promotes its interaction with H1.2, suggesting that PARP1 activity may also influence mutant FUS-related phenotypes by involving H1.2. Interestingly, H1.2 is the only variant of the histone H1 family reported to shuttle from nucleus to cytoplasm, a process that can act as a signaling pathway to initiate apoptosis.³⁹ This shuttling of H1.2 is regulated by post-translational modifications such as ubiquitination and PARP1-mediated PARylation.³⁹ Since inhibition of PARP1-mediated PARylation decreases mutant FUS-H1.2-PARP1 interactions and prevents apoptotic phenotypes, it is possible that the pro-apoptotic activity of H1.2 depends on the interaction with PARylated FUS. Additionally, the effects of H1.2 on SG alterations and FUS aggregation may also contribute to the cell death phenotype. Altogether, our data indicate that H1.2 and PARP1 could be potential therapeutic targets to decrease FUS-related aggregation and neurodegeneration.

Limitations of the study

In this study, we defined changes in the interactome of FUS by comparing isogenic controls with motor neurons expressing mutant FUS^{P525L} in homozygosis;²⁸ however, FUS mutations in patients are typically heterozygous.³² Importantly, we also observed elevated interaction between FUS and H1.2 in motor neurons expressing mutant FUS in heterozygosis. Likewise, knockdown of H1.2 reduced SG alterations and apoptosis in heterozygous motor neurons. However, as WT FUS could retain mutant FUS in the nucleus and ameliorate some of its pathological effects,²⁸ we cannot exclude the possibility that some part of the interactome alterations and subsequent cellular effects observed in homozygous mutant FUS motor neurons are influenced by the absence of WT FUS.

While our data suggest that elevated PARylation of mutant FUS promotes its interaction with H1.2, further experiments are needed to confirm a direct effect of FUS PARylation on this interaction. For instance, biochemical experiments inducing PARylation of recombinant FUS and assessing its interaction with H1.2 *in vitro* could provide valuable insights. Moreover, identifying the PARylation sites of mutant FUS and determining whether mutations at these sites influence its interaction with H1.2 would further elucidate whether the interaction between FUS and H1.2 is directly mediated by PAR.

STAR★METHODS

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SUPPLEMENTAL INFORMATION

Supplemental information can be found online at <https://doi.org/10.1016/j.celrep.2024.114626>.

ACKNOWLEDGMENTS

This work was supported by the Else Kröner-Fresenius-Stiftung (2021-EKSE.95), the Köln Fortune Program/Faculty of Medicine/University of Cologne (Project #177/2022), the Center for Molecular Medicine Cologne (C16), the Deutsche Forschungsgemeinschaft (Germany's Excellence Strategy-CE-CAD, EXC 2030-390661388), and the German Academic Exchange Service (DAAD, 91862831 fellowship to T.T.P.D.). We thank the CECAD Proteomics Facility for contributions and guidance regarding proteomics experiments. We thank the CECAD Imaging Facility and Christian Jüngst for support with confocal microscopy and any related experiments (FRAP). We are grateful to Alessandro Rosa and Irene Bozzoni for providing the iPSC lines. We thank Ivan Matic for providing advice regarding PARylation. We are also grateful to I. Ahel for providing the PARP1 knockout U2OS cell line.

AUTHOR CONTRIBUTIONS

H.A. performed most of the experiments, data analyses, and interpretation. R.L., S.K., F.H., Y.N., W.H.Z., T.T.P.D., M.W., and H.J.L. conducted a subset of the experiments and analyses presented. D.V. supervised the project. H.A. and D.V. wrote the manuscript. All authors discussed the results and reviewed the manuscript.

DECLARATION OF INTERESTS

The authors declare no competing interests.

Received: November 29, 2023

Revised: May 13, 2024

Accepted: July 30, 2024

Published: August 19, 2024

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STAR★METHODS

KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Antibodies		
FUS	Abcam	Cat# ab154141; RRID:AB_2885092
Histone H1.2	Abcam	Cat# ab17677; RRID:AB_2117984
PARP1	Proteintech	Cat# 66520-1-Ig; RRID:AB_2881883
PAR/pADPr	Bio-Techne	Cat# 4335-MC-100; RRID:AB_2572318
α -tubulin	Sigma	Cat# T6199; RRID:AB_477583
β -actin	Abcam	Cat# ab8226; RRID:AB_306371
G3BP1	BioZol	Cat# MBL-RN048PW; RRID:AB_10794608
Map2	Sigma	Cat# M1406; RRID:AB_477171
Cleaved-caspase-3	Cell Signaling	Cat# 9661S; RRID:AB_2341188
HRP AP Donkey Anti-Mouse IgG (H + L)	Jackson Immuno Research	Cat# 715-035-150; RRID:AB_2340770
HRP AP Donkey Anti-Rabbit IgG (H + L)	Jackson Immuno Research	Cat #715-035-152; RRID:AB_10015282
IRDye 680RD Donkey Anti Mouse IgG	LI-COR Biosciences	Cat# 926-68072; RRID:AB_10953628
IRDye 680RD Donkey Anti-Rabbit IgG	LI-COR Biosciences	Cat# 926-68073; RRID:AB_10954442
Alexa Fluor 568 F(ab') ₂ Fragment of Goat Anti-Rabbit IgG (H + L)	LifeTechnologies	Cat# A-21069; RRID:AB_2535730
Alexa Fluor 488 Goat anti Rabbit IgG (H + L)	LifeTechnologies	Cat# A-11029; RRID:AB_2534088
Bacterial and virus strains		
Non-targeted control pLKO.1-puro Empty Vector	Sigma Aldrich	SHC016V-1EA
HIST1H1C shRNA#1 5'CAAGGTTGCGAAGCCCAAGAA 3'	Sigma Aldrich	TRCN0000442081
HIST1H1C shRNA#2 5'GCACTCTGGTGCAAACGAAAG 3'	Sigma Aldrich	TRCN0000443007
OP50	CGC	N/A
<i>E. coli</i> HT115	CGC	N/A
Chemicals, peptides, and recombinant proteins		
Staurosporine	Abcam	Cat# ab120056
PDD0017273	Sigma Aldrich	Cat# SML1781
Sodium Arsenite	Sigma Aldrich	Cat# 106277
Olaparib	Cayman Company	Cat# 10621
IMMOBILON Western Chemilum HRP Substrate	Merck	Cat# WBKLS0100
RNase A	ThermoScientific	Cat# EN0531
Protein A microbeads	Miltenyi Biotec	Cat# 130-071-001
Complete Mini protease inhibitor cocktail	Roche	Cat# 11836153001
Geltrex	ThermoFisher	Cat# A1413302
mTeSR1	StemCell Technologies	Cat# 85850
Dispase	StemCell Technologies	Cat# 07913
Accutase	LifeTechnologies	Cat# A1110501
Dulbecco's modified Eagle's medium (DMEM)	LifeTechnologies	Cat# 41965039
DMEM/F12	LifeTechnologies	Cat# 11320074
Non-Essential Amino Acids (NEAA)	LifeTechnologies	Cat# 11140035
Glutamax	LifeTechnologies	Cat# 35050038
Penicillin/Streptomycin solution	LifeTechnologies	Cat# 15070063
ROCK-inhibitor	Abcam	Cat# ab120129
PBS	LifeTechnologies	Cat# 14190169

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REAGENT or RESOURCE	SOURCE	IDENTIFIER
Puromycin	LifeTechnologies	Cat# A1113803
Neurobasal medium	LifeTechnologies	Cat# 21103049
N2	LifeTechnologies	Cat# 175020-01
B27	LifeTechnologies	Cat# 12587-010
Retinoic acid	Sigma Aldrich	Cat# R2625
Smoothed agonist (SAG)	Sigma Aldrich	Cat# 566661
LDN-193189	Miltenyi Biotech	Cat# 130-103-925
SB-431542	Miltenyi Biotech	Cat# 130-105-336
SU-5402	Sigma Aldrich	Cat# SML0443
DAPT	Sigma Aldrich	Cat# D5942
Poly-L-Ornithine	Sigma Aldrich	Cat# P3655
Laminin	Invitrogen	Cat# 23017-015
Brain-derived neurotrophic factor (BDNF)	Peprtech	Cat# 450-02
Glial cell-derived neurotrophic factor (GDNF)	Peprtech	Cat# 450-10
Hygromycin	Invitrogen	Cat# 10687-10
Fugene HD	Promega	Cat# E2311
Hoechst 33342	LifeTechnologies	Cat# H3570
ProLong Gold Antifade mounting solution	LifeTechnologies	Cat# P36941
SYBR Green Supermix	BioRad	Cat# 1725275
Propidium iodide	Sigma	Cat# 81845
Gö 6983	Sigma	Cat# G1918
Critical commercial assays		
BCA protein assay	ThermoFisher Scientific	Cat# 1074-1395
qScript Flex cDNA synthesis kit	Quantabio	Cat# 95049
LentiStarter 3.0 kit	SBI	Cat# LV060A-1
Deposited data		
Proteomics raw data of FUS interactome in motor neurons upon ALS-related mutation	This study	ProteomeXchange: PXD047271
Experimental models: Cell lines		
Control hiPSCs #1, isogenic line of FUS ^{P525L/P525L}	Lenzi et al. ²⁸	N/A
FUS ^{WT/R514S} hiPSCs	Lenzi et al. ²⁸	N/A
FUS ^{WT/R521C} hiPSCs	Lenzi et al. ²⁸	N/A
FUS ^{P525L/P525L} , generated from Control hiPSCs #1 via TALEN	Lenzi et al. ²⁸	N/A
U2OS cells	Gibbs-Seymour et al. ⁵⁰	N/A
U2OS PARP1 KO cells	Gibbs-Seymour et al. ⁵⁰	N/A
HEK293T/17	ATCC	Cat# CRL-11268
SH-SY5Y	ATCC	Cat# CRL-2266
Experimental models: Organisms/strains		
ZM5838 (<i>hpls223[rgef-1p::FUSWT::GFP]</i>)	Murakami et al. ⁶³	N/A
ZM5844 (<i>hpls233[rgef-1p::FUSP525L::GFP]</i>)	Murakami et al. ⁶³	N/A
DVG265 (<i>hpls233[rgef-1p::FUSP525L::GFP]</i> ; <i>sur-5p::H1.2</i>)	This study	N/A
DVG267 (<i>hpls233[rgef-1p::FUSP525L::GFP]</i> ; <i>rgef-1p::H1.2</i>)	This study	N/A
Oligonucleotides		
Primers for plasmid construction. See Table S1.	Integrated DNA Technologies	N/A
Primers for qPCR experiments. See Table S2.	Integrated DNA Technologies	N/A

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REAGENT or RESOURCE	SOURCE	IDENTIFIER
Recombinant DNA		
pL4440:parp1	This study	N/A
pcDNA3.1 HA-FUS P525L	Dormann et al. ⁷⁷	N/A
L4440	Addgene (Fire Lab)	Cat# 1654
pCDH-MCS-T2A-Puro-MSCV	SBI	Cat# CD522A-1
pCDH-MCS-T2A-Puro-MSCV: H1.2 HIST1H1C	This study	N/A
pCDH-MCS-T2A-Puro-MSCV: H1.2 S2A	This study	N/A
pCDH-MCS-T2A-Puro-MSCV: H1.2 S188A	This study	N/A
pCDH-MCS-T2A-Puro-MSCV: H1.2 NTD del	This study	N/A
pCDH-MCS-T2A-Puro-MSCV: H1.2 CTD del	This study	N/A
GFP-Fusion HR Targeting Vector	System Biosciences	Cat# HR120PA-1
pDV027 rgef-1p:H1.2	This study	N/A
sur-5p:H1.2	This study	N/A
pL4440:parp1	This study	N/A
pcDNA3.1 HA-FUS P525L	Dormann et al. ⁷⁷	N/A
L4440	Addgene (Fire Lab)	Cat# 1654
Software and algorithms		
FIJI (ImageJ)	Schindelin et al. ⁷⁸	https://fiji.sc
Zen 3.2 (blue edition)	Carl Zeiss AG	http://www.zeiss.com/microscopy/int/products/microscope-software/zen-lite.com
Graphpad PRISM version 9.4.1	GraphPad Software, Inc.	http://www.graphpad.com
Adobe Illustrator CC (2018)	Adobe Inc.	https://www.adobe.com/products/illustrator.html
SnapGene	GSL Biotech LLC	https://www.snapgene.com/
MaxQuant v.1.5.3.8	Cox et al. ⁷⁹	https://www.maxquant.org
Ortholist2	Kim et al. ⁸⁰	http://ortholist.shaye-lab.org
Ensembl	Martin et al. ⁸¹	ensembl.org

RESOURCE AVAILABILITY

Lead contact

Further information and requests for resources and reagents should be directed to the lead contact, David Vilchez (dvilchez@uni-koeln.de).

Materials availability

All unique/stable reagents generated in this study are available from the [lead contact](#) upon request without restrictions.

Data and code availability

- The mass spectrometry proteomics data have been deposited to the ProteomeXchange Consortium (<https://proteomecentral.proteomexchange.org>) via the PRIDE partner repository (ProteomeXchange: PXD047271). All data reported in this paper will be shared by the [lead contact](#) upon request.
- This paper does not report original code.
- Any additional information required to reanalyze the data reported in this paper is available from the [lead contact](#) upon request.

EXPERIMENTAL MODEL AND STUDY PARTICIPANT DETAILS

Human cell lines

HEK293T/17 (CRL-11268) and SH-SY5Y (Cat# CRL-2266) cells were obtained from the America Type Culture Collection (ATCC) (CRL-11268). Control and *PARP1* knockout U2OS lines were a gift from I. Ahel.⁵⁰ All the human cell lines were cultured in Dulbecco's modified Eagle's medium (DMEM) (LifeTechnologies, 41965039), supplemented with 1x Non-Essential Amino Acids (NEAA) (LifeTechnologies, 11140035), 1x Glutamax (LifeTechnologies, 35050038), 10% heat-inactivated fetal bovine serum, and 1x Penicillin/Streptomycin solution (LifeTechnologies, 15070063). Cells were split when they reached 80% confluency using Trypsin/EDTA 0.25%

(LifeTechnologies, 25200072). All cell lines were tested for mycoplasma contamination at least once every 3 weeks, and no mycoplasma contamination was detected.

iPSCs

Control iPSC (FUS^{WT/WT}), isogenic homozygous FUS^{P525L/P525L} iPSC, and heterozygous FUS^{WT/R521C} and FUS^{WT/R514S} iPSC lines were kindly provided by I. Bozzoni and A. Rosa (Sapienza University of Rome). These iPSC lines were established and characterized for pluripotency in ref. ²⁸. Briefly, control iPSCs were derived from a donor and checked for the absence of mutation in *FUS*.²⁸ FUS^{P525L/P525L} iPSCs were generated from control iPSCs using TALEN (transcription activator-like effector nucleases)-directed mutagenesis.²⁸ FUS^{WT/R521C} and FUS^{WT/R514S} iPSC lines were generated from donors with ALS.²⁸ All iPSC lines were cultured on Geltrex (ThermoFisher, A1413302) using mTeSR1 (Stem Cell Technologies, 85850) at 37°C, 5% CO₂ conditions. The medium was changed daily. When iPSC confluence reached approximately 80%, the cells were split using 2 mg mL⁻¹ dispase (Stem Cell Technologies, 07913) and colonies were scraped using a glass pipette. For lentiviral infection or differentiation experiments, we used single-cell splitting with Accutase (LifeTechnologies A1110501) for 3 min at 37°C, followed by washing with DMEM/F12 containing ROCK-inhibitor (Abcam, ab120129, 1:5000) and centrifugation at 300xg for 2 min. The supernatant was removed, and cell pellets were diluted in fresh growth media before being plated on freshly Geltrex-coated tissue culture plates. iPSC lines were tested for mycoplasma contamination at least once every 3 weeks, and no mycoplasma contamination was detected.

C. elegans strains and maintenance

C. elegans strains were grown and maintained on standard nematode growth media (NGM) seeded with *E. coli* (OP50) bacteria. The ZM5838 (*hpls223[rgef-1p::FUSWT::GFP]*) and ZM5844 (*hpls223[rgef-1p::FUSP525L::GFP]*) strains were kindly provided by P. St George-Hyslop.⁶³ The DVG265 (*hpls223[rgef-1p::FUSP525L::GFP]; sur-5p::H1.2*), and DVG267 (*hpls223[rgef-1p::FUSP525L::GFP]; rgef-1p::H1.2*) strains were generated in this study. All experiments were carried out using hermaphrodite adult worms at the age indicated in the corresponding figures.

METHOD DETAILS

Lentivirus production

To generate lentiviral particles, *HIST1H1C/H1.2* was PCR-amplified from cDNA to include 5' NheI and 3' NotI restriction sites (primers: 5'-GTGCTAGCGATGTCCGAGACTGCT-3', 5'-GTGCGGCCGCCTATTTCTTCTTG-3') and cloned into pCDH-MCS-T2A-Puro-MSCV cDNA cloning and expression vector (SBI, CD522A-1). The construct was sequence-verified. The LentiStarter 3.0 kit (SBI, LV060A-1) was used to produce lentivirus particles containing HIST1H1C/H1.2. First, HEK293T cells were cultured in 10cm cell-culture-treated plates in fresh growth medium until confluency reached 50–70%. The transfection mixture containing 0.8 mL DMEM, 20 μL pPACKH1-plasmid mix, 24 μL PureFection reagent, and 2 μg plasmid with HIST1H1C/H1.2 was prepared, vortexed and incubated at room temperature for 15 min. The mixture was then added to the cells, and the plate was incubated overnight at 37°C. On the following day, the transfection medium was replaced with fresh growth medium. The medium was collected 48 h post-transfection and centrifuged at 3,000xg for 15 min to pellet cell debris. The viral supernatant was mixed with PEG-it in a 1:5 (v/v) ratio and stored at 4°C overnight. To precipitate the virus, the supernatant/PEG-it mixture was centrifuged at 1,500xg for 30 min at 4°C. The viral pellet was resuspended in cold PBS (1:100 of original supernatant volume) (LifeTechnologies, 14190169), and aliquots were stored at –80°C until further use. Lentivirus (LV)-non-targeting shRNA, HIST1H1C shRNA #1 (TRCN0000442081: 5'-CAAGGTTGCGAAGCCCAAGAA-3' and HIST1H1C shRNA #2 (TRCN0000443007: 5-GCACTCTGGTGCAAACGAAAG-3') in pLKO.1-puro vector were obtained from Mission shRNA (Sigma-Aldrich).

Transduction of iPSCs

iPSCs were dissociated into single cells using Accutase and plated in Geltrex-coated 12-well plates. For transduction of viral particles to overexpress *HIST1H1C/H1.2*, we used LentiStarter 3.0 kit (SBI, LV060A-1). The transduction mixture containing 2.5 μL TransDux, 100 μL TransDux MAX Enhancer, 400 μL of fresh growth media and 7 μL of virus was added to cells. The medium was replaced with fresh growth medium the next day. After 2 days, iPSCs were treated with 2 μg mL⁻¹ puromycin (LifeTechnologies, A1113803) to select for transduced cells. For knockdown experiments, shRNA lentiviral particles (Sigma-Aldrich) were mixed with growth medium and added to the cells. After 2 days, transduced iPSCs were selected using 2 μg mL⁻¹ puromycin.

G3BP1-GFP reporter cells

G3BP1-GFP reporter cell lines were generated via Crispr/Cas9 mediated knock-in of control and FUS^{P525L/P525L} iPSCs. The G3BP1-GFP donor plasmid was generated by cloning 1.5 kb homology arms of G3BP1 into the GFP-Fusion HR Targeting Vector (System Biosciences, HR120PA-1) using Gibson assembly. Primers G3BP1-5'HA-GFP_FWD and G3BP1-5'HA-GFP_REV were used to amplify the 5' homology arm, spanning intron 11 and exon 12 of G3BP1. 3'HA-G3BP1-GFP_FWD and 3'HA-G3BP1-GFP_REV primers were used to amplify the 3' homology arm of G3BP1, spanning the 3' untranslated region of G3BP1. GuideRNA (gRNA) was generated by combining crRNA Sequence 1 (Integrated DNA Technologies (IDT):/A1TR1/rUrCrArUrGrArGrArUrUrCrArCrUrGrCrGrGrUrUrUrArGrArGrCrUrArUrGrCrU/A1TR2/), targeting the sequence of interest, with tracrRNA (IDT Alt-R,

#1072532). Crispr/Cas9 mediated knock-in of GFP to the c-terminus of G3BP1 was performed via ribonucleoprotein transfection (RNP) of control and FUS^{P525L/P525L} iPSCs with gRNA, *S.p.* Cas9 Nuclease (IDT Alt-R) and the G3BP1-GFP donor plasmid via FuGENE transfection reagent (Promega). Monoclonal colonies were expanded and successful knock-in of G3BP1-GFP was validated via genotyping and sequencing.

Differentiation of iPSCs into motor neurons

Motor neurons were differentiated from iPSC lines using a monolayer differentiation protocol previously described by Hill et al.⁸² iPSCs were initially plated on Geltrex and cultured in mTeSR1 medium until they reached confluence. Differentiation started with the change of mTeSR1 to neuron differentiation media which consisted of DMEM/F12:neurobasal 1:1 (Life Technologies, 11320074, 21103049) supplemented with 1x NEAA, 1x Glutamax, 1x B27 (Life Technologies, 12587-010), and 1x N2 (Life Technologies, 175020-01). From day 0 to day 6, the neuron differentiation media was further supplemented with 1 μ M retinoic acid (Sigma Aldrich, R2625), 1 μ M smoothened agonist (Sigma Aldrich, 566661), 0.1 μ M LDN-193189 (Miltenyi Biotech, 130-103-925), and 10 μ M SB-431542 (Miltenyi Biotech, 130-105-336). Continuing from day 7 to day 14, the neuron differentiation medium was modified to include 1 μ M retinoic acid, 1 μ M smoothened agonist, 4 μ M SU-5402 (Sigma Aldrich, SML0443), and 5 μ M DAPT (Sigma Aldrich, D5942).

Subsequently, the differentiated motor neurons were detached using Accutase and plated onto poly-L-ornithine-coated (Sigma Aldrich, P3655) and laminin-coated (Invitrogen, 23017-015) plates in neurobasal media supplemented with NEAA, Glutamax, N2, B27, 10 ng mL⁻¹ brain-derived neurotrophic factor (BDNF) (Peprotech, 450-02) and 10 ng mL⁻¹ glial cell-derived neurotrophic factor (GDNF) (Peprotech, 450-10).

Generation and transfection of mutant H1.2 constructs

The H1.2-Puro-MSCV plasmid was used as a template to generate domain-specific deletions and amino acid mutations. Putative N-terminal domain (NTD) and C-terminal domain (CTD) sequences were identified using the UniProt database. For amplification of S2A point mutation as well as CTD and NTD deletion inserts, primers were designed to flank the regions of interest, with NheI and NotI sites at the ends (S2A: 5'-GTGCTAGCATGGCCGAGACTGCTCCTG-3', CTD: 5'-GTGCGGCCGCGAGTTTAAAGGAGCCAG-3', NTD: 5'-GTGCTAGCGATGGGTCCCCCGGTG-3'). To introduce the S188A mutation, primers with 5' phosphate modifications were employed for PCR-amplification of the H1.2-Puro-MSCV plasmid. Subsequently, the resulting amplicons were ligated using T4 ligase overnight at 16°C (5'-GAAAGCTGCCAAAGCTGCTGCTAAG-3', 5'-CTATTCTTCTTGG-3').

HEK293 cells were grown in 12 well-plate and transfected with plasmids once they reached 70% confluency. To this end, we added a mixture of 1 μ g plasmid DNA and 3 μ L Fugene HD (Promega, E2311) following the manufacturer's instructions. After 24 h of incubation, cells were treated with 100 μ g mL⁻¹ hygromycin (Invitrogen, #10687-10) and 2 μ g mL⁻¹ puromycin for two days. Antibiotic-resistant cells were expanded and harvested for further experiments. The pcDNA3.1 HA-FUS P525L plasmid was a gift from D. Dormann.⁷⁷

Immunocytochemistry

Cells were fixed using 4% paraformaldehyde diluted in PBS for 20 min at room temperature. Alternatively, cells were fixed with 100% ice-cold methanol for 20 min at -20°C when indicated in the corresponding figure legend. Following fixation, cells were washed with 1x PBS and permeabilized for 10 min using 0.2% Triton X-100 diluted in 1x PBS, followed by a 10-min blocking step with 3% BSA diluted in 1x PBS. Coverslips were then incubated with primary antibody diluted in the blocking solution for 2 h at room temperature (mouse anti-FUS (Abcam, ab154141, 1:100), rabbit anti-G3BP1 (BioZol, MBL-RN048PW, 1:500), mouse anti-MAP2 (Sigma, M1406, 1:300), rabbit anti-H1.2 (Abcam, ab17677, 1:200), mouse anti-PARP1 (Proteintech, 66520-1-Ig, 1:100) and rabbit anti-cleaved-caspase-3 (Cell Signaling, #9661 S, 1:300)). Subsequently, cells were subjected to three washes with 1x PBS and incubated with fluorescent secondary antibodies (Alexa Fluor 488 Goat anti-Mouse IgG (H + L) (LifeTechnologies, A-11029, 1:300), Alexa Fluor 568 F(ab')₂ Fragment of Goat Anti-Rabbit IgG (H + L) (LifeTechnologies, A-21069, 1:300)) and Hoechst 33342 (LifeTechnologies, #H3570) diluted in 3% BSA in 1x PBS for 30 min at room temperature. After staining, cells underwent three washes with 1x PBS, one with distilled H₂O, and one with 100% ethanol before being left to dry in the dark. Finally, coverslips were mounted onto microscope slides using ProLong Gold Antifade mounting solution (LifeTechnologies, P36941).

Fluorescence recovery after photo bleaching (FRAP)

FRAP experiments for the endogenously tagged G3BP-copGFP isogenic and FUS P525L mutant motor neurons were performed on a Zeiss LSM 980 Airyscan2 using a 63 $\times$ objective. The motor neurons were treated with 500 μ M arsenite 45 min prior to the start of imaging and the motor neurons remained in the presence of the arsenite throughout the FRAP experiments at 37°C and 5% CO₂. SGs of interest were first monitored for 15 s to establish the baseline signal, after which a 2 $\times$ 2 μ m circular region over the target granule was bleached using 10 $\times$ 0.5 ms pulses of the laser at 100% power at 488 nm. The recovery of the granule was then monitored with an image taken every 5 s for a total measurement time of 6 min. The average pixel intensity for the bleached region over time was extracted using the Zeiss analysis software, with the data of the different replicates combined into a single dataset for a given condition. The resulting recovery curves were used to calculate the half recovery time $t_{1/2}$ and to determine whether there was a difference between the percentile mobile (M) fraction of the SGs.

Propidium iodide staining

Cells on coverslips were stained with 2 $\mu\text{g mL}^{-1}$ propidium iodide (Sigma, #81845) and Hoechst 33342 (Life Technologies, #H3570) for 1.5 h. After that, coverslips were mounted using FluorSave Reagent (Calbiochem, #345789).

Monitoring SG disassembly

To monitor the dynamics of stress granule (SG) disassembly, cells were seeded on coverslips coated with Geltrex. For SG formation, cells were initially treated with 500 μM sodium arsenite (Sigma Aldrich, 106277). In recovery experiments, cells were washed twice with 1x PBS and then fed with fresh growth media. At the indicated time points, cells were fixed with 4% paraformaldehyde, followed by immunostaining as described above. The quantification of SG numbers per cell and the percentage of cells containing SGs was manually performed using FIJI (ImageJ).

Western blot

Whole-cell lysates were separated by SDS-PAGE and transferred to PVDF membranes (Merck, IPVH00010). Membranes were blocked with 3% BSA and incubated with primary antibody overnight at 4°C. The following antibodies were used for western blot experiments: mouse anti-FUS (Abcam, ab154141, 1:1000), rabbit anti-H1.2 (Abcam, ab17677, 1:2000), mouse anti-PARP1 (Proteintech, 66520-1-Ig, 1:1000), mouse anti- α -tubulin (Sigma, T6199, 1:5,000) and mouse β -actin (Abcam, ab8226, 1:5,000) antibodies. To detect poly ADP-ribosylation, we used a monoclonal mouse anti-PAR/pADPr antibody specific for polyADPr polymers ranging from 2 to 50 units (Bio-Techne, 4335-MC-100, clone #10HA, 1:1000). After incubation with secondary antibodies (HRP AP Donkey Anti-Mouse IgG (H + L) (Jackson Immuno Research, #715-035-150, 1:10000), HRP AP Donkey Anti-Rabbit IgG (H + L) (Jackson Immuno Research, #715-035-152, 1:10000), the membranes were developed using IMMOBILON Western Chemilum HRP Substrate (Merck, WBKLS0100).

Immunoprecipitation

Cells were lysed in RIPA lysis buffer (50 mM Tris-HCl pH 7.5, 150 mM NaCl, 1% sodium deoxycholate, 1 mM EDTA, 1% Triton X-100) supplemented with Complete Mini protease inhibitor cocktail (Roche, 11836153001). Lysates were sonicated for 10 s at 30% intensity using a Sonicator (Bandelin) and then passed through a syringe with a 27 G needle. Protein concentrations were determined using a standard BCA protein assay (ThermoFisher Scientific, 1074–1395). For subsequent Western blot analyses, 100 μg of whole-cell lysate was set aside as input. For immunoprecipitation experiments, 400 μg of lysate was incubated overnight at 4°C with 1 μg of monoclonal antibody or 2 μg of polyclonal antibody. The antibodies used for immunoprecipitation experiments were: monoclonal mouse anti-FUS (Abcam, ab154141), polyclonal rabbit anti-H1.2 (Abcam, ab17677), polyclonal rabbit anti-PARP1 (Proteintech, 66520-1-Ig), and polyclonal rabbit anti-PAR/pADPr (BioTechne, 4335-MC-100). For RNase experiments, samples were pre-treated with 1 μL of RNase A (ThermoScientific, EN0531) on ice before antibody incubation. Protein A microbeads (Milenyi Biotech, 130-071-001) were added to the samples (50 μL for monoclonal and 100 μL for polyclonal antibody) and incubated for 1 h on an overhead shaker at 4°C. μMACS columns (Milenyi Biotech, 130-042-701) were set up in the magnet at 4°C. The columns were pre-cleared with 200 μL of ice-cold RIPA lysis buffer. The lysate/antibody/bead mixtures were loaded onto the columns and washed three times with 200 μL of ice-cold Wash Buffer I (50 mM Tris-HCl, pH 7.5, 150 mM NaCl, 5% glycerol, 0.05% Triton X-100) and five times with 200 μL of ice-cold Wash Buffer II (50 mM Tris-HCl, pH 7.5, 150 mM NaCl). For western blot analysis, samples were eluted with 75 μL of boiled 2x Laemmli buffer.

For label-free quantitative proteomics analysis, following the washing step, beads were treated with 25 μL of Digestion buffer (2 M Urea, 7.5 mM AmBic, 1 mM DTT, 5 ng mL^{-1} trypsin) for 30 min at room temperature. After treatment, peptides were eluted twice with 50 μL of Elution buffer (2 M Urea, 7.5 mM AmBic, 5 mM CAA) and incubated overnight at room temperature in the dark for full digestion. The next day, 100% formic acid was added to the samples to reach a final concentration of 4%, followed by centrifugation at full speed for 5 min. The supernatant was separated and samples were stage-tipped for label-free quantitative proteomics at the CECAD Proteomics Core Facility. The resulting supernatant was separated, and the samples were subjected to stage-tipping for label-free quantitative proteomics at the CECAD Proteomics Core Facility. The raw data of proteomics mass spectrometry were analyzed in Maxquant v.1.5.3.8 (ref. ⁷⁹).

Nucleus/cytosol fractionation

SH-SY5Y cells were harvested in cold DPBS. Following centrifugation, the cell pellets were suspended in a cold cytoplasmic extraction buffer (10 mM HEPES (pH 7.9), 10 mM KCl, 0.1 mM EDTA, 0.1 mM EGTA, 1 mM DTT, 1 mM PMSF, and a protease inhibitor cocktail). The cells were allowed to swell on ice for 15 min, then 10% Nonidet P-40 was added to the lysis buffer to achieve a final concentration of 0.5% (v/v). The tubes were vortexed for 10 s and then centrifuged at 4 °C at 14000 rpm for 5 min, resulting in the cytosolic extract. The nuclear pellets were washed with cold DPBS supplemented with 1 mM DTT, 1 mM PMSF, and a protease inhibitor cocktail. Following centrifugation at 4 °C at 4000 rpm for 5 min, the pellets were resuspended in a cold nuclear extraction buffer (20 mM HEPES (pH 7.9), 400 mM NaCl, 1 mM EDTA, 1 mM EGTA, 1 mM DTT, 1 mM PMSF, and a protease inhibitor cocktail). The pellets were placed on ice and subjected to intermittent vortexing for a duration of 1 h. The nuclear extract was centrifuged at 4°C for 10 min at 15,000 rpm, resulting in the nuclear fraction.

Quantitative RT-PCR

The total RNA of motor neurons was isolated using RNAbee (Tel-Test Inc.). cDNA was generated using qScript Flex cDNA synthesis kit (Quantabio, 95049). SybrGreen real-time qPCR experiments were performed with a 1:20 dilution of cDNA on a CFC384 Real-Time System (Bio-Rad). The following primers were used for *HIST1H1C/H1.2* (5'-TCAAGAGCCTGGTGAGCAAG-3', 5'-TCTTAGGTTTGGTTCCGCCC-3'). Data were analyzed with the comparative $2\Delta\Delta C_t$ method using the geometric mean of ACTB (5'-CTGGCACCCAGCACAAATG-3', 5'-CCGATCCACACGGAGTACTTG-3') and GAPDH (5'-GCACCGTCAAGGCTGAGAAC-3', 5'-GGATCTCGCTCCTGGAAGATG-3') as housekeeping genes.

Generation of H1.2-overexpressing *C. elegans* strains

For the generation of DVG265 (*hpls233[rgef-1p::FUSP525L::GFP]*; *sur-5p::H1.2*) and DVG267 (*hpls233[rgef-1p::FUSP525L::GFP]*; *rgef-1p::H1.2*), a DNA plasmid mixture containing 70 ng μL^{-1} of H1.2 plasmids and 20 ng μL^{-1} of *myo-3p::mCherry* plasmids were injected into the gonads of adult ZM5844 hermaphrodite worms by standard methods.⁸³ The corresponding control strain was generated by microinjecting ZM5844 worms with 20 ng μL^{-1} of *myo-3p::mCherry* plasmid alone.

RNAi *C. elegans* experiments

For RNAi experiments, worms were fed *E. coli* (HT115) containing an empty vector L4440 or expressing double-stranded RNAi. *hil-2*, *hil-3*, *hil-4*, *hil-5*, *hil-6* RNAi constructs were obtained from the Vidal RNAi library. RNAi constructs for *parp-1* were cloned into L4440 vector using SacI and XbaI restriction enzymes (primers: 5'-GAGCTCATGGCTTCGAAGAATGTTTCG-3' and 5'-TCTAGATTAAAGATGACGAGCATGGTG-3'). All RNAi constructs were verified by Sanger sequencing.

Lifespan assay

For lifespan experiments, synchronized larvae by egg-laying protocol were fed and raised on OP50 *E. coli* at 20 °C.⁸⁴ Once the worms reached adulthood, they were transferred to plates with HT115 *E. coli* carrying empty vector or RNAi clones. Adult worms ($n = 96$ per condition) were scored every day or every other day.⁸⁴ From the initial adult worm population, worms that were lost or burrowed into the medium, as well as those showing 'protruding vulva' or undergoing bagging were censored. We used GraphPad PRISM software (version 6.0h) to calculate median lifespan and generate lifespan graphs. Mean lifespan and statistical analysis was performed using OASIS software (version 1) with p values calculated using the log rank (Mantel-Cox) method.⁸⁵ The p values refer to experimental and control animals in a single experiment. Each graph in the main text represents a single experiment, and Data S5 provides detailed statistical analysis and replicate data.

Motility assay

At the ages indicated in the corresponding figures, worms were transferred to 20 μL of M9 buffer on a microscope slide. After 20 s of adaptation, the number of body bends (i.e., change in direction of the bend at the mid-body) was counted for 30 s.^{64,65}

Filter trap assay

At day 3 of adulthood, worms were collected with M9 buffer. Worm pellets were lysed with RIPA lysis buffer, which was supplemented with Complete Mini protease inhibitor cocktail (Roche, 11836153001), and then homogenized through glass bead disruption using the Precellys 24 tissue homogenizer. Human cells were lysed in RIPA lysis buffer (50 mM Tris-HCl pH 7.5, 150 mM NaCl, 1% sodium deoxycholate, 1 mM EDTA, 1% Triton X-100) supplemented with a Complete Mini protease inhibitor cocktail. Lysates were sonicated for 10 s at 30% intensity using Sonicator (Bandelin) and subsequently passed through a syringe with a 27 G needle. Protein concentrations were quantified using a standard BCA protein assay (ThermoFisher Scientific, 1074–1395).

Both worm and human cell samples were centrifuged at 8000 $\times g$ for 5 min. The resulting supernatants were collected in separate tubes and then supplemented with 0.5% SDS. Lysates were loaded onto a cellulose acetate membrane (VWR, 516–5020) and assembled in a dot-blot apparatus (Bio-Rad, 1706542). The membrane was washed with 0.2% SDS and the retained proteins were assessed by immunoblotting with primary antibodies (anti-FUS (Abcam, ab154141, 1:1000), rabbit anti-H1.2 (Abcam, ab17677, 1:2000) followed by secondary antibodies (IRDye 680RD Donkey Anti-Mouse IgG (Li-cor, 926–68072, 1:10,000), IRDye 680RD Donkey Anti-Rabbit IgG (Li-cor, 926–68073, 1:10,000)). Total cell lysates were also analyzed by SDS-PAGE to determine protein expression levels.

QUANTIFICATION AND STATISTICAL ANALYSIS

Cell images were analyzed using FIJI (ImageJ).⁷⁸ Stress granules, protein foci and Hoechst positive-nuclei were quantified manually. Stress granule to cell ratio was quantified in Microsoft Excel. The total and nuclear signal intensity was measured by FIJI, the ratio was quantified in Excel. We used GraphPad Prism version 9.4.1 for statistical analysis of all the data, except lifespan experiments that were analyzed with GraphPad PRISM version 6.0h and OASIS software (version 1). The statistical details of experiments are indicated in the corresponding figure legends. The raw data of proteomics mass spectrometry was analyzed using Maxquant v. 1.5.3.8 (ref. ⁷⁹) by the CECAD Proteomics Facility. We identified worm orthologues of human linker histone H1 family employing comparative genomic analysis tool OrthoList2 (ref. ⁸⁰) and Ensembl genome browser.

For experiment using human cell lines, cells with similar confluence were split and equal amounts of cells were transferred to new plates for experiments. The plates were randomly assigned to the different treatment conditions. The samples were collected and lysed in random order. Data collection and analysis were not randomized. For *C. elegans* experiments, worms were synchronized by picking young hermaphrodite adults and let them lay eggs for 6 h. These young hermaphrodites were randomly picked from our maintenance plates. After egg laying for 6 h, larvae were raised until adulthood and adult worms were then randomly assigned to the different treatment conditions. No statistical methods were used to predetermine sample size. No animals or data points were excluded from the analyses.