Mutant FUS causes DNA ligation defects to inhibit oxidative damage repair in Amyotrophic Lateral Sclerosis

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Genome damage and defective repair are etiologically linked to neurodegeneration. However, the specific mechanisms involved remain enigmatic. Here, we identify defects in DNA nick ligation and oxidative damage repair in a subset of amyotrophic lateral sclerosis (ALS) patients. These defects are caused by mutations in the RNA/DNA-binding protein FUS. In healthy neurons, FUS protects the genome by facilitating PARP1-dependent recruitment of XRCC1/DNA Ligase IIIα (LigIII) to oxidized genome sites and activating LigIII via direct interaction. We discover that loss of nuclear FUS caused DNA nick ligation defects in motor neurons due to reduced recruitment of XRCC1/LigIII to DNA strand breaks. Moreover, DNA ligation defects in ALS patient-derived iPSC lines carrying FUS mutations and in motor neurons generated therefrom are rescued by CRISPR/Cas9-mediated correction of mutation. Our findings uncovered a pathway of defective DNA ligation in FUS-linked ALS and suggest that LigIII-targeted therapies may prevent or slow down disease progression.
A myotrophic lateral sclerosis (ALS) is a neurodegenerative disease characterized by the selective and progressive death of upper and lower motor neurons. This leads to progressive muscle weakness and death of the patients usually occurs within two to five years after the onset of symptoms. In 10% of patients, there is a clear family history. The most prevalent genetic causes of familial ALS are mutations in the Superoxide Dismutase 1 (SOD1), TAR DNA Binding Protein-43 (TARDBP), Fused in Sarcoma (FUS) genes, and Chromosome 9 Open Reading Frame 72 (C9ORF72). Mutations in the gene encoding the RNA/DNA-binding protein Fused in Sarcoma (FUS) have been detected in ~5% of familial ALS patients.1, 2 These mutations are also found in a small subset (~1%) of sporadic ALS cases3–5. Most missense point mutations in FUS are clustered in the gene segment encoding the nuclear localization sequence (NLS) in the C-terminus and induce nuclear depletion and cytosolic aggregation of FUS6, 7. While arginine at position 521 (R mutated to G, H, or C) is most commonly mutated8, the P525L mutation is associated with aggressive juvenile-onset ALS9,10.

FUS is a multifunctional heterogeneous nuclear ribonucleoprotein (hnRNP) of the TET (TAF15, EWS, and TLS) family of RNA-binding proteins and it has been implicated in multiple aspects of RNA metabolism. It is unclear yet which of these functions of FUS is critical for neurodegeneration. In healthy neurons, FUS is predominantly localized in the nucleus, but it can shuttle between the nucleus and cytosol in response to various stimuli11,12. FUS also binds DNA and has been recently implicated in the maintenance of genome integrity, in particular the DNA damage response (DDR) signaling, induced by DNA double-strand breaks (DSBs). In response to DSB-inducing agents, FUS is phosphorylated by ataxia-telangiectasia mutated (ATM) and DNA-dependent protein kinase (DNA-PK), which are activated by DSBs13,14. FUS interacts with histone deacetylase 1 (HDAC1) in primary mouse cortical neurons, which may indirectly modulate the repair of DSBs by homologous recombination (HR) and non-homologous end joining (NHEJ). Furthermore, loss of FUS abrogated both HR and NHEJ efficiency in exogenomic vector-based assays15. In addition, impairment of poly(ADP-ribose) polymerase (PARP)-dependent DDR signaling due to mutations in the FUS NLS, which induces cytoplasmic FUS accumulation has been linked to ALS-related neurodegeneration16. However, it is yet unclear which function(s) of FUS is critical for preventing neurodegeneration.

Although FUS is associated with multiple genome repair pathways, its role in the DDR is not completely understood. Independent studies demonstrated that FUS is recruited to DNA damage tracks by microirradiation (MIr) at wavelengths of 405 nm or 351 nm (UVA), respectively, in a PARP1-dependent manner presumably via interaction with PAR groups17–19. However, the role of FUS in downstream repair reactions was not investigated. While MIr induces clusters of different types of DNA damage, including oxidized base lesions, single-strand breaks (SSBs), and DSBs, it is generally believed that UVA damage predominantly induces SSBs via elevated reactive oxygen species (ROS)20. Thus, recruitment of FUS at UVA laser tracks suggests its potential role in the repair of oxidative DNA damage, which has not been thoroughly investigated.

ROS, generated endogenously as a byproduct of cellular respiration, pose a critical challenge to the genome, especially in neurons due to their high metabolic/transcriptional activity and long lifespan21–23. Accumulation of ROS-induced oxidized DNA bases and SSBs in affected regions of the central nervous system is associated with degenerating neurons in ALS and other neurodegenerative diseases24,25. These lesions are repaired by evolutionarily conserved base excision (BER) and single-strand break repair (SSBR) pathways, in which the XRCC1/LigIII complex plays a critical role in sealing DNA nicks in the final repair step26–30.

In this study, we investigated the mechanism(s) responsible for the accumulation of SSBs in the neuronal genome after the loss of FUS. We have characterized the interaction of FUS with XRCC1/LigII and documented that FUS facilitates the PARP1 activity-dependent recruitment of XRCC1/LigIII to oxidative DNA damage sites. The connection between FUS function and DNA ligation defects was examined in multiple model systems, including CRISPR/Cas9-mediated FUS knockout (KO) cells, familial ALS patient-derived induced pluripotent stem cells (iPSCs) with FUS mutations, motor neurons differentiated from these patient-derived iPSCs, and spinal cord tissue with FUS pathology from ALS patients. Notably, both P525L and R521H mutations in FUS cause defects in DNA ligation, albeit via distinct mechanisms. The ligation defects in FUS KO cells and patient-derived iPSC lines were rescued by addition of wild-type (WT) FUS (but not mutant FUS) and by correcting the mutation by CRISPR/Cas9, separately. Together, our results provide important molecular insights into a previously unknown DNA ligation defect in FUS-associated ALS.

**Results**

**Loss of FUS induces SSB accumulation and ROS sensitization.** Although FUS is implicated in DDR, the effect of FUS deficiency on the repair of endogenous damage is unknown. To address this question, we first quantified the level of DNA SSBs vs DSBs by single cell electrophoresis (comet assay) in unstressed, FUS knockdown (KD) cells. Two independent FUS shRNAs were transfected into the neuroblastoma SH-SY5Y cell line and each shRNA induced ~70% depletion of FUS (Fig. 1a, b). The alkaline comet assay measures alkali-labile lesions, SSBs and DSBs, while the neutral comet assay exclusively measures DSBs. Thus, a comparison of tail moments in alkaline and neutral comet assays gives a relative measure of alkali-labile lesions and SSBs vs DSBs. FUS KD cells showed an ~sixfold increase in the alkaline tail moment, but a lower than 1.5-fold change in the neutral tail moment, compared to cells transfected with control shRNA (Fig. 1c). This result implies that most unrepaired DNA strand breaks that accumulated after FUS KD were alkali-labile lesions or SSBs. The small increase in DSB level could result secondarily from closely spaced alkali-labile lesions and/or SSBs.

Because SSBs are induced by ROS either directly or as BER intermediates of oxidized DNA bases32, we next tested the impact of decreased FUS expression on the cellular responses to oxidative stress. FUS KD SH-SY5Y cells were treated for 1 h with different concentrations of glucose oxidase (GO), which generates H₂O₂ in cellulo, mimicking endogenous ROS stress. MITT-based analysis of cellular metabolic activity, 24 h post-GO treatment showed significantly lower cell viability after FUS depletion (Fig. 1d). Similar results were obtained when the parental SH-SY5Y cells and the FUS KD derivatives were treated directly with H₂O₂, confirming the role of FUS in resistance to oxidative stress (Fig. 1e). In addition, clonogenic survival of oxidatively stressed HEK293 cells was significantly reduced in the absence of FUS, indicating that the protective effect of FUS against oxidative stress is not restricted to neuronal cells (Fig. 1f). We also examined the steady state level of cell viability and proliferation using the MTT and clonogenic data in unstressed control cells (Supplementary Figs. 1a, 1b). The MTT assays performed 24, 48, and 72 h after shRNA transfection revealed no significant change in cell survival, while the clonogenic assays represented as plating efficiency showed a moderate ~5% decrease in the average number of colonies formed. These data thus show that FUS KD...
did not significantly affect the survival of unstressed cells, but it only moderately affected cell proliferation. The presence of unrepairable DNA SSBs in neuronal genomes has been linked to neurodegeneration and is attributed to defective repair involving diverse mechanisms. To test whether the ROS sensitivity of FUS KD cells was due to impaired oxidative DNA damage repair, we evaluated the repair kinetics of alkali-labile lesions and SSBs induced by GO using alkaline comet assay. Repair was found to be significantly delayed in the FUS KD cells (Fig. 1g, h), indicating that oxidative DNA damage repair is impaired due to FUS deficiency.

**FUS forms a complex with PARP1, XRCC1, and LigIII.** To explore the involvement of FUS in repairing oxidative DNA damage, we first performed mass spectrometry analysis of oxidative stress-dependent interaction partners of FUS isolated by co-immunoprecipitation (IP) from GO-treated SH-SYSY cells with a FUS antibody, and detected the presence of PARP1 and XRCC1 (see Supplementary Data 1 for the list of proteins). Subsequently, analysis of Flag IPs from HEK293 cells expressing Flag-FUS revealed increased association of FUS with XRCC1, LigIII, and PARP-1, but not with other BER proteins (APE1, TDPI, PNKP, or FEN-1) in GO-treated cells (Fig. 2a). Notably, no association was observed with the DNA ligase complex, XRCC4/LigIV (Fig. 2a), involved in the repair of DSBs by NHEJ repair, or LigI (Supplementary Fig. 4c) in SH-SYSY cells. To investigate whether these associations also occur with endogenous FUS in neuronal cells, similar experiments were carried out with a FUS antibody and extracts from differentiated SH-SYSY cells.

![Fig. 1](image_url)

**Fig. 1** Loss of FUS induces SSB accumulation and ROS sensitization. **a, b** Immunoblot (IB) showing FUS knockdown (KD) by shRNAs. Total lysate were extracted from SH-SYSY cells 48 h after the transfection with two individual FUS shRNAs. β-actin was probed as loading control. Quantitation of relative band intensity of FUS shRNA vs Control shRNA shown in b. The error bars are standard deviation of experiments performed in triplicate (**p < 0.001, two-tailed unpaired Student’s t-test). **c** Alkaline and neutral comet assay of control vs FUS KD SH-SYSY cells. The quantitation of mean tail moment from 50 randomly selected nuclei is shown in the histogram. The error bars are standard deviation. (**p < 0.001, two-tailed unpaired Student’s t-test). **d, e** MTT-based viability analysis of FUS KD SH-SYSY cells, treated with increasing doses of GO or H2O2. The cells were incubated with GO for 1 h or H2O2 as indicated dose for 3 h, 48 h after the FUS shRNA transfection. MTT assay was performed 24 h after the treatment. The error bars are standard deviation of experiments performed in triplicate (**p < 0.001, two-tailed unpaired Student’s t-test). **f** Clonogenic survival analysis of FUS KD HEK293 cells after GO (100 ng/ml) treatment, as in Fig. 1c. The error bars are standard deviation of experiments performed in triplicate. **g, h** Repair kinetics of induced oxidative genome damage after FUS KD. Alkaline comet assay of control or FUS knockdown SH-SYSY cells at 0, 30, and 150 min post GO (100 ng/ml) treatment. Histogram represent quantitation of mean tail moment from 50 randomly selected nuclei. The error bars are standard deviation (**p < 0.05; ***p < 0.001, two-tailed unpaired Student’s t-test).
Fig. 2 FUS forms a complex with PARP1, XRCC1, and LigIII. a IB of Flag-FUS co-Immunoprecipitation (co-IP) from HEK293 cells for oxidative DNA damage repair proteins with or without GO treatment (100 ng/ml for 1 h). The IP was performed with anti-Flag antibody. b IB of endogenous FUS co-IP from SH-SY5Y cells for PARP1, XRCC1, and LigIII. The IP was performed with anti-FUS antibody. c Scheme of human iPSC line differentiation to motor neurons. Representative phase-contrast images of iPSC and differentiated motor neurons are shown. Scale bar = 50 μm. d Immunofluorescence (IF) staining of motor neuron markers. Representative images of motor neurons that stained Isl-1, MAP2, or βIII-tubulin indicated ~80% differentiation efficiency. Scale bar = 50 μm. e IB of endogenous FUS co-IP from differentiated motor neurons for PARP1, XRCC1, and LigIII. f Quantitation of IB band intensity in Fig. 2b, e to show level change of PARP-1, XRCC1, and LigIII in FUS IP after GO treatment. The error bars are standard deviation of experiments performed in triplicate. g PLA of FUS vs PARP1, XRCC1, or LigIII in SH-SY5Y and iPSC-derived motor neurons with or without GO treatment. Nuclei stained with DAPI. Scale bar = 5 μm. h Quantitation of PLA foci from 25 motor neuronal cells (**p < 0.01, two-tailed unpaired Student’s t-test). The error bars are standard deviation.
(Fig. 2b) and motor neurons (Fig. 2e), which were differentiated from human iPSCs (KYUO-DXR0109B line from ATCC) using previously reported protocol15 (Fig. 2c) that achieved up to ~80% efficiency of motor neuron differentiation detected with the neuronal marker MAP2 and specific motor neuron markers: Isl-1 and ChAT (Fig. 2d; Supplementary Fig. 2a). Under these conditions, we found that endogenous FUS associated with PARP-1 in addition LigIII and XRCC1, and that these associations were markedly enhanced in GO-treated cells (Fig. 2b–e, f). As hypothesized, FUS was also detected in reciprocal co-IPs performed with the XRCC1 antibody (Supplementary Fig. 2b). The oxidative stress-induced co-localization of FUS with PARP1, XRCC1, and LigIII was confirmed by proximity ligation assays (PLA) with differentiated SH-SY5Y cells (Fig. 2g, upper panels) and iPSC-deriv derived motor neurons (Fig. 2g, lower panels and Fig. 2h). To confirm the specificity of the antibodies used, we performed control PLA experiments of FUS vs XRCC1 and FUS vs LigIII in control and XRCC1 or LigIII siRNA transfected cells (Supplementary Figs. 2c, 2d and 2e). As expected, there was decreased co-localization after either XRCC1 or LigIII KD, confirming the specificity of the observed interactions. Furthermore, these results are consistent with previous studies showing that PARP1 facilitates optimal recruitment of FUS to DNA damage sites17–19 and suggest that FUS may be involved in a repair complex for oxidative DNA damage.

FUS activates LigIII for DNA ligation via direct interaction. XRCC1 binds to and stabilizes LigIII, generating the ligation complex that is critical for efficient SSB repair36, particularly in post-mitotic cells, such as neurons, that lack repair subpathways completed by the replicative DNA ligase, Lig37,38. Following our observation that FUS associates with both XRCC1 and LigIII in cellulo, we wondered whether FUS interacts directly with one or both of these proteins. In pull-down assays, PARP1, XRCC1, and LigIII as well as the XRCC1/LigIII complex were specifically retained on glutathione beads liganded by GST-FUS, indicating that FUS interacts with both subunits of the XRCC1/LigIII complex (Fig. 3a). To further evaluate the specificity of FUS–XRCC1 interaction, we performed a broad domain mapping analysis and identified that Glycine-rich region (aa268–aa355) of FUS is the major region involved in XRCC1 binding (Supplementary Fig. 3). The C-terminal aa465–aa526 also exhibited weak binding, while the N-terminal aa1–aa267 did not show detectable binding activity.

To address the functional impact of these interactions, we incubated XRCC1/LigIII with a nick-containing Cy3-labeled duplex oligonucleotide (Fig. 3b, top) prior to separation of the ligated product and unligated substrate oligonucleotides by denaturing gel electrophoresis39. FUS significantly stimulated ligation efficiency (Fig. 3b, c) with ~fivefold increase at a 1:2 molar ratio of XRCC1/LigIII to FUS. To further evaluate the enzymatic mechanism of this activation, we analyzed the Michaelis-Menten kinetic parameters and found a threefold decrease in $K_m$ of LigIII but no significant change in $k_{cat}$ in the presence of FUS, indicating enhanced substrate affinity or loading (Fig. 3d–g). Overall, the catalytic efficiency of LigIII was enhanced fourfold by FUS (Fig. 3g).

DNA ligation defects in ALS patients with FUS pathology. We next investigated whether there was a correlation between FUS pathology and DNA ligation defects in the spinal cord tissue of ALS patients (see Methods for details). Of the 4 control and 10 ALS samples screened by immunoblotting for monomeric vs oligomeric FUS levels, 2 ALS samples (P-6 and P-7) showed >60% reduction in FUS monomer levels (Supplementary Figs. 5a, b). The immunoblots also showed an increase in higher mobility bands corresponding to FUS oligomers. Immunohistochemistry (IHC) of control and ALS spinal cord tissue sections using FUS antibody showed clear evidence for significant cytosolic accumulation of FUS in ALS spinal cord (Supplementary Fig. 5c), similar to the FUS pathology previously demonstrated in ALS-FUS patients1,2. Analysis of genomic DNA integrity by LA-PCR detected a ~twofold higher occurrence of strand breaks in P-6 and P-7 ALS spinal cord tissue relative to C-2 and C-3 (Supplementary Fig. 5d). In accord with these observations, DNA ligation capacity in P-6 and P-7 spinal cord tissue extracts was reduced, compared to C-2 and C-3 extracts (Supplementary Figs. 5e, f), even though the levels of XRCC1 and LigIII were comparable in the control and ALS samples (Supplementary...
Figs. 5g and 5h). The clinical characteristics of all samples are provided in Supplementary Table 1. These data reveal a strong correlation between FUS pathology and DNA ligation defects that is consistent with our cell culture data.

Defective DNA repair in ALS patient-derived iPSC line. Several FUS mutations, mostly familial, have been linked to ALS1-2. To examine the impact of FUS mutations on oxidative DNA damage repair, we cultured human fibroblast lines and iPSC lines derived
from a healthy control individual and two patients with familial ALS carrying either a R521H or a P525L mutant versions of FUS (Fig 5a)35. We first compared nuclear and cytoplasmic FUS levels in the fibroblasts by immunoblotting. In the FUS–P525L cell line, there was a ~threefold increase in cytoplasmic FUS level and a reduction in nuclear FUS levels relative to the control. In contrast, the R521H cell line had only a small increase in cytoplasmic FUS (Fig. 5b, c). However, both mutant fibroblasts had a comparable increase in the steady state levels of genomic DNA strand breaks, as determined by LA-PCR amplification of a 10.4 kb segment of the hprt gene (Fig. 5d, e).

To evaluate the impact of FUS mutations on DNA damage repair in motor neurons, the mutant iPSC lines were induced to differentiate into motor neurons (Fig. 2e)35. Motor neuron differentiation efficiency was confirmed by immunofluorescent staining with MAP2, β-tubulin III, and motor neuron-specific markers Isl-1, ChAT (Supplementary Figs. 6a, b). Similar to the observations in the iPSC lines from which they are differentiated, the motor neurons derived from the FUS–P525L mutant line showed significant cytoplasmic FUS accumulation compared to WT FUS motor neurons, while the FUS–R521H motor neurons showed only moderate cytoplasmic accumulation (Fig. 5f). To evaluate SSB repair kinetics in patient-derived motor neurons, cells were treated with GO for 1 h and evaluated 30, 60 or 180 min later. LA-PCR analysis of isolated genomic DNA showed significantly delayed repair of DNA strand breaks in both R521H and P525L mutant motor neurons compared to the control WT cells (Fig. 5g, h). Furthermore, while the untreated mutant FUS motor neurons had only slightly higher numbers of TUNEL-positive cells compared with the WT motor neurons, this difference was increased following incubation with H2O2, indicating a defect in DNA damage-induced repair (Fig. 5i, j).

Furthermore, the alkaline comet assay showed deficient oxidative DNA damage repair in motor neurons with FUS mutations (Supplementary Fig. 6c).

**Correction of FUS mutations rescue DNA ligation defects**

Consistent with the accumulation of DNA damage and delayed DNA repair, the mutant FUS motor neurons exhibited a >50% reduction in DNA nick ligation efficiency, compared with control WT neurons (Fig. 6c, lanes 2, 4, and 6). Again, both R521H and P525L showed comparably impaired ligation efficiency. To directly attribute this DNA ligation defect to the FUS mutations, we corrected the mutation in the iPSC line using the CRISPR/Cas9 knock-in technology. The reversal of R521H to H521R and we corrected the mutation in the iPSC line using the CRISPR/Cas9 knock-in technology. The reversal of R521H to H521R and P525L to L525P was confirmed by sequencing, and we verified the pluripotent self-renewal capacity by embryonic body formation analysis (Fig. 6a; Supplementary Figs. 7a, 7b and 7c)35. The origin of the isogenic controls was confirmed by SNP analysis (Supplementary Table 2)35. These isogenic control lines were differentiated to motor neurons and FUS distribution and DNA ligation capacity were examined. As expected, correcting FUS mutations rescued the nuclear FUS cleavage phenotype in iPSCs (Supplementary Fig. 7d) and motor neurons (Fig. 6b). Furthermore, as shown in Fig. 6c, d, the correction of the FUS mutation completely rescued the ligation defect. To further test whether the mutation correction reverted the delayed repair of DNA strand breaks, we performed LA-PCR-based DNA integrity measurement at early (30 min) and late (180 min) time points after GO treatment (Fig. 6e). The data at 30 min showed a comparable level of DNA damage induced by GO. At 180 min, DNA integrity was mostly restored in mutation-corrected cells (~90% DNA integrity), whereas the mutant cells still showed significantly reduced (~60%) DNA integrity, confirming that the observed ligation defect and delayed repair are indeed caused by the FUS mutations. Altogether, these results confirm that the R521H and P525L mutant versions of FUS fail to enhance nick ligation, leading to the accumulation of unrepaired DNA strand breaks in motor neurons.

**Dominant negative activity of the R521H FUS mutation**

While the ligation defect in mutant FUS–P525L cells can be attributed to the increased nuclear clearance of the mutant FUS protein, nuclear level of the FUS–R521H mutant was only slightly reduced. This suggests that the reduced DNA ligation in cells expressing FUS–R521H occurs by a different mechanism. To address this question, we examined the DNA damage recruitment and repair complex formation of FUS–R521H and FUS–R521C mutants. GFP-tagged WT and mutant FUS proteins were ectopically expressed in HEK293 cells. While GFP–FUS–WT and GFP–FUS–R521H were predominantly localized in the nucleus (Fig. 7c), the FUS–R521C mutant exhibited a few clear cytoplasmic aggregates (Fig. 7c). Recruitment of the GFP fusion proteins to MIR-induced DNA damage tracks was monitored in live cells. A 365 nm low-intensity laser was used to generate predominantly oxidative damage in the laser track.20,45 Both FUS mutants showed significantly reduced recruitment at the damage track (Fig. 7a, b, Supplementary Movie 1, 2 and 3).

We next examined the association of WT and FUS–R521H proteins with SSB repair proteins using the PLA. Flag-tagged WT and mutant FUS proteins were expressed in SH-SY5Y cells at comparable levels (Supplementary Fig. 8a). PLA of Flag vs XRCCI, LigIII, or PARP-1 in GO-treated SH-SY5Y cells showed a significantly reduced association (~80%) of mutant FUS with XRCCI, LigIII, and PARP-1 compared with WT FUS (Supplementary Figs. 8b, c). The reduced association of FUS–R521H with SSB proteins was also observed in iPSCs (Fig. 7d, e). Thus, the FUS–R521H mutant is defective in recruitment to damage sites and repair complex formation despite being present in the nucleus.

Two independent approaches were employed to determine whether the effect of the FUS–R521H allele is solely due to haploinsufficiency of functional FUS or a dominant negative activity of the FUS–R521H protein. First, we optimized ~50% transient KD of FUS in WT human motor neurons using antisense oligonucleotides (ASOs) and measured the LigIII...
**Fig. 4** DNA ligation defects in CRISPR/Cas9-mediated FUS KO cells. 

**a** Scheme of CRISPR/Cas9 targeting of FUS. Images indicate stable expression of GFP-Cas9 in HEK293 cells. Scale bar = 50 µm.

**b** IB of endogenous FUS, PARP1, XRCC1 and LigIII in FUS WT and FUS KO HEK293 cells. β-actin was probed as loading control.

**c, d** In vitro DNA nick ligation activity assay. XRCC1 IP complex from nuclear extract of GO-treated FUS WT and FUS KO HEK293 cells, was incubated with or without purified FUS. d Quantitation of ligation activity (**p < 0.01, two-tailed unpaired Student’s t-test). 

**e, f** IF of ectopically expressed Flag-FUS WT and Flag-FUS P525L mutant in HEK293 cells. Endogenous FUS depleted using UTR-specific shRNA. IF was performed by anti-Flag antibody 48 h after the co-transfection of FUS shRNA with either Flag-FUS WT or Flag-FUS P525L plasmid. DAPI staining indicates nucleus. Scale bar = 5 µm.

**g** IB of cell extract used in **f** to confirm the comparable level of PARP1, XRCC1, and LigIII in WT or mutant FUS expressing cells. β-actin was probed as loading control. All error bars are standard deviation of experiments performed in triplicate.
activity in XRCC1 IP isolated from control vs 50% FUS KD cells. 50% KD was confirmed by both mRNA quantification (Fig. 7f) and immunoblotting of protein level (Fig. 7g, h). Incubation with 15 nM ASOs for one week caused ~50% KD of FUS in motor neurons. The 50% FUS KD cells showed a moderate (~20%) reduction in LigIII activity (Fig. 7i, j; Lane 1 vs 2). We then compared the LigIII activity in the patient-derived R521H mutant FUS cell line with the FUS KD line. Theoretically, the heterozygous mutant line derived from an ALS patient is expected to have 50% WT and 50% mutant FUS. However, the ligase activity in mutant motor neurons was significantly lower compared to both WT and 50% KD lines. Quantitation of the ligase activity from three independent experiments showed that the ligase activity in the mutant cells was ~50% lower compared to WT cells and it was reduced an additional ~30% compared to FUS KD cells (Fig. 7j). These data are in line with a dominant negative effect of mutant FUS on the LigIII activity, rather than with only haploinsufficiency.

In a complementary approach, we used cell lines that ectopically express WT and mutant FUS at a comparable level (~2-fold greater than endogenous) following induction with doxycycline (Supplementary Figs. 8d, e) and measured
LigIII activity in XRCC1 IPs. The inducible human H9 embryonic stem cells (hH9-ESCs) contained WT and R521H mutant FUS in a safe harbor locus. Expression of WT FUS increased LigIII activity by ~20% whereas expression of FUS R521H decreased ligation activity by about 25% compared to control cells (Supplementary Figs. 8f, b). This clearly shows that mutant FUS can reduce the LigIII activity, despite the fact that endogenous FUS was present. The ability of FUS R521H to reduce ligation activity in the presence of comparable levels of endogenous WT FUS protein indicates that the mutant FUS...
reduces LigIII-dependent ligation, at least in part, by acting in a dominant negative manner.

**FUS facilitates PARP-1-dependent recruitment of XRCC1/LigIII.** FUS was previously shown to be rapidly recruited to UVA irradiation-induced DNA damage tracks in a PARP-dependent fashion\(^{17-19}\). PARP1 also facilitates recruitment of XRCC1/LigIII to damage sites\(^{28-30}\). To further dissect the role of FUS in this early oxidative DNA damage response, we examined the effect of FUS depletion on XRCC1 recruitment to MIR-induced DNA damage tracks. GFP-XRCC1, which was ectopically expressed in
Fig. 7 Dominant negative activity of the R521H FUS mutation. a, b Recruitment of GFP-FUS at laser ablation track by live cell imaging. Representative images of ectopic GFP-FUS WT or R521H/C mutant following laser ablation. GFP-FUS WT or R521H/C was transfected into HEK293 cells and subjected to laser ablation 48 h after the transfection. The recruitment of GFP fluorescence at laser tracks was monitored by live cell imaging (see movie clip in Supplementary Movie 1, 2 and 3 for live imaging). Scale bar = 1 μm. c Quantification of GFP-FUS track intensity from 15 cells is shown in histogram. The error bars are standard deviation. d Representative IF images of GFP-FUS WT or R521H/C mutant in HEK293 cells without laser treatment. Scale bar = 5 μm. e PLA of GFP vs XRCC1, LigIII and PARP1 in iPSC carrying GFP-FUS WT or FUS R521H after GO treatment. Representative images shown in d and average number of PLA foci from 25 cells were quantified in e. The error bars are standard deviation (*p < 0.05, two-tailed unpaired Student’s t-test). Scale bar = 5 μm. f Quantification of FUS mRNA analyzed by RT-PCR in antisense oligonucleotide-incubated motor neurons at indicated concentrations. The error bars are standard deviation of experiments performed in triplicate. g, h IB showing FUS KD in f Histogram shows quantitation of IB band intensity (h). The error bars are standard deviation of experiments performed in triplicate. i, j in vitro DNA nick ligation activity assay. XRCC1 IP complex from GO-treated motor neurons with FUS WT, FUS KD, and FUS R521H, and quantified. The error bars are standard deviation of experiments performed in triplicate (*p < 0.05, two-tailed unpaired Student’s t-test)

HEK293 cells, was rapidly recruited to the laser track within 30 s and was retained until 300 s as expected (Fig. 8a; Supplementary Movie 4). Depletion of FUS by shRNA significantly delayed XRCC1 recruitment and reduced the total amount of XRCC1 that was recruited (Fig. 8a, b; Supplementary Movie 5).

In addition, there was a marked reduction in the oxidative stress-dependent association of PARP-1 and XRCC1 in FUS KD cells measured by PLA (Fig. 8c, d) and reduced levels of LigII and XRCC1 in PARP1 IP from fibroblasts expressing either FUS-R521H or FUS-P525L (Fig. 8e, f). To test whether the FUS–XRCC1 interaction is modulated by PARylation, we examined the proteins co-immunoprecipitated by a FUS antibody from extracts cells treated with or without the PARP1 inhibitor, AG-14361 (Supplementary Fig. 9a). The levels of both XRCC1 and LigIII were markedly reduced in the FUS IPs from AG-14361-treated cells. Similarly, the PLA signals for FUS vs PARP-1 were reduced in AG-14361-treated cells (Supplementary Fig. 9b). These data suggest that although FUS interacts directly with XRCC1 in a binary fashion in vitro, the interaction is promoted by PARP-1 activity in cells. To investigate this further, we performed an in vitro ADP-ribosylation assay using purified PARP1 protein together with NAD+ and octameric oligonucleotide as described previously14. PARylated PARP1 was detected by immunoblotting with PAR antibody (Supplementary Fig. 9c). Surprisingly, when we added purified FUS protein to the reaction, the auto-PARylation level of PARP-1 was increased by ~10-fold. The PAR antibody detected a second lower mobility band that corresponded in size to FUS, suggesting that FUS may be PARylated by PARP-1 in vitro. To test the effect of PARP-1 activity on the FUS–XRCC1 interaction, we performed GST affinity pull-down in the presence of PARP-1 and NAD+ and found that PARylation enhanced the in vitro interaction of FUS and XRCC1 (Supplementary Fig. 9d). Together, these data show that PARP-1 and its PARylation activity enhance the interaction of FUS with XRCC1, which is critical for the recruitment of XRCC1/LigIII at oxidatively damaged genomic DNA (schematically shown in the Fig. 8g).

Discussion

In this study, we identified FUS as a critical component of the oxidative genome damage repair complex. ROS generate SSBs both directly and also indirectly during the repair of oxidized bases by BER26. PARP1 acts as the SSB sensor that recruits other SSB proteins, including XRCC1/LigIII, in a PARylation-dependent manner26,28,29. The basic BER/SSBR pathway involves four key reactions: (1) excision of the base lesion by a DNA glycosylase, (2) end-processing at the SSBs to generate 3′-OH and 5′-phosphate ends, compatible with gap-filling synthesis and ligation, (3) gap-filling by a DNA polymerase, and (4) final nick sealing by a DNA ligase33. Nick sealing is the critical rate-limiting step in both BER and SSBR. In contrast to cycling cells that utilize both LigI and XRCC1/LigIII to complete BER and SSBR, XRCC1/LigIII is the predominant activity in post-mitotic cells such as motor neurons35. Our comprehensive in cellulo and in vitro studies reveal stable and direct interaction between FUS and XRCC1/LigIII that enhances DNA nick ligation to protect the genome from oxidative damage and show that loss of FUS function results in DNA nick ligation defects, significantly reduced SSB repair efficiency, and cellular vulnerability to oxidative insults.

Our data in fibroblasts obtained from familial ALS patients with the R521H and P525L FUS mutations, and iPSCs/motor neurons derived from these fibroblasts indicate that the two familial mutants, R521H and P525L, cause ligation defects by distinct mechanisms: the DNA damage-dependent association of nuclear FUS-R521H with PARP1 and XRCC1/LigIII is reduced, whereas FUS-P525L nuclear levels are low due to its aberrant cytoplasmic localization. These cells showed a significant defect in DNA ligation, which was rescued by addition of recombinant FUS or by correcting the genomic FUS mutations. Furthermore, the R521H mutant inhibited LigIII-dependent joining activity in a dominant negative fashion. The dominant nature of the toxic gain-of-function of FUS mutants observed in our study is consistent with recent FUS KO and mutant transgenic mice studies. While a heterozygous FUS KO mice was viable and did not develop strong ALS-like phenotype46, the expression of a mutant FUS transgene induced selective motor neuron degeneration in mice47.

Our study provides specific molecular insights into a previously undescribed DNA repair defect linked with FUS-associated neurodegeneration. It was reported that PARP is involved in forming liquid compartments of FUS at DNA damage sites, and aberrant phase transition of the liquid to solid-like FUS aggregates could be involved in the disease onset46. Other studies showed recruitment of FUS to DNA damage sites in a PARP1-dependent manner via its affinity for PAR17,18. Our data show that once FUS is recruited at damage sites it facilitates the recruitment of XRCC1/LigIII. Interestingly, we observed a direct interaction between FUS and PARP-1 in vitro. Although FUS was previously shown to bind to PAR groups17,18, its direct binding with PARP-1 has never been shown. Our study thus documents a direct binding of FUS to PARP-1, XRCC1 and LigIII, which is enhanced by the PARylation activity of PARP-1. It is likely that PAR on auto-PARylated PARP-1 provides the initial signal for the recruitment of FUS–XRCC1–LigIII to the PARP-1-bound-damage sites, with direct binding stabilizing these interactions. Our in vitro PARylation data also indicate that FUS may be PARylated by PARP-1, consistent with previous observation of mass spec screening49, whose functional role in genome maintenance needs to be investigated.
To further evaluate the biological implications of these findings in ALS patients, we analyzed spinal cord tissue of sporadic ALS patients. The increased DNA damage together with reduced DNA ligation activity in these tissues broadly correlated with FUS pathology shown in immunoblotting and IHC studies, supporting our in vitro findings. Together these data suggest that low LigIII activity and SSB repair defects may be a common pathological mechanism of FUS-dependent ALS (schematically represented in Fig. 9).
The hypothesis of defective DNA repair in ALS was postulated as early as 1982 by Bradley et al., who proposed that abnormal DNA in ALS may arise from deficiency of an isozyme of a DNA repair enzyme. Subsequent studies observed abnormal activity of DNA repair components, including APE1, DNA glycosylase OGG1, mitochondrial DNA polymerase γ, and PARP1, in ALS patients or mouse models, strongly supporting the model for impaired oxidative DNA damage repair in ALS. In line with our findings, a recent study reported that FUS pathology relies on PARP, which can cause axonal degeneration in ALS patient-derived motor neurons.

Notably, while previous studies suggested a role of FUS in the repair of DSBs, the relative presence of DSB vs non-DSB damage caused by loss of FUS or FUS mutations was not known. One study showed co-localization of FUS with γ-H2AX at laser (405 nm) ablation sites to suggest its possible presence at DSB sites. Another report showed the presence of FUS at UVA (351 nm) damage sites to suggest its linkage to oxidative damage. It is important to note that laser ablation does not specifically induce a single type of damage; rather, it forms both DSB and non-DSB damage in proximity to laser track. Thus, accurately attributing the co-localization data to a specific type of damage is not possible. Our alkaline and comet assay data demonstrate that loss of FUS predominantly caused SSBs, rather than DSBs. A small fraction of DSBs may be generated secondarily from closely spaced bi-stranded SSBs and from repair intermediates of other oxidative lesions. This result was consistent with our previous observation that neurodegenerative brain tissue accumulated significantly more SSBs than DSBs. Since the brain is generally protected by the blood–brain barrier, endogenous ROS-induced genome damage is likely the most critical threat to neuronal cells. The major lesions induced by ROS include oxidized bases/sugar fragments, AP sites, and SSBs. Most of these lesions are repaired by the BER and SSBR pathways, which are largely dependent on LigIII in non-dividing and terminally differentiated cells. The loss of nuclear LigIII function may have more profound effect in post-mitotic cells unlike in cycling cells, due to lack of back up ligases in neurons. LigI, which is primarily involved in replication-associated LP-BER, and whose level is very low in post-mitotic cells did not, in contrast to LigIII, specifically associate with FUS. Consistently, our data showed specific association of FUS with LigIII but not with LigI or LigIV. Thus, defects in LigIII function caused by FUS abnormalities are likely to contribute to genomic instability and neuronal cell death in ALS. It is also reasonable to speculate that while loss of a small fraction of spinal cord neurons in the CNS results in motor phenotype, other tissues may be more tolerant to loss/dysfunction of a small fraction of cells.

Finally, although our understanding of the pathological and biochemical changes in ALS has increased, there is still no cure.

**Fig. 9** Mutant FUS induces DNA ligation defects to inhibit oxidized damage repair. A model showing the involvement of FUS for optimal DNA nick ligation in healthy neurons to facilitate efficient oxidative genome damage repair, and how loss of functional FUS in ALS leads to DNA nick ligation defects. FUS is required for PARP-1-dependent recruitment of XRCC1/LigIII complex at oxidative DNA damage sites. Two familial FUS mutations impair DNA nick ligation by distinct mechanisms. Substantial cytoplasmic localization of P525L FUS causes DNA ligation defect due to loss of functional FUS from nucleus. The R521H/C FUS fails to form the repair complex with XRCC1/LigIII and PARP-1 and is not recruited at damaged site. Unrepaired DNA SSBs caused by impaired DNA nick ligation, together with secondary DSBs that may be generated from closely placed single-strand breaks, may substantially contribute to neurodegeneration in familial FUS-ALS patients.
Currently available treatments only temporarily slow disease progression and do not prevent neuronal death. This shortcoming underscores the need for a mechanism-driven approach to effectively prevent onset and delay progression. The identification of a defect in DNA nick ligation that likely contributes to the pathological changes in FUS-linked ALS opens avenues for generative disease that enhance DNA LigIII activity and/or DNA repair.

**Methods**

***Cell lines, cell culture, and tissue origin***

Human neuroblastoma SH-SYSY (ATCC) and HEK293 (ATCC) were expanded in Dulbecco’s modified Eagle’s medium (DMEM)/F12 or DMEM (HyClone), respectively, with 10% fetal bovine serum (HyClone) and 100 U/ml each of penicillin and streptomycin (HyClone). Human iPSCs (ATCC and VIB-KU Leuven) were maintained on GelTrex LDEV-Free, hESC-Qualified, Reduced Growth Factor Baseline Human Fibroblast Medium ( GibcoTM) in Essential 8 medium ( GibcoTM). [35]

Human fibroblasts (VIB-KU Leuven) were grown in DMEM/F12 medium containing 10% fetal bovine serum, 1% MEM non-essential amino acids (Gibco), and 1.6% Sodium Bicarbonate (Corning). All cell lines were cultured in an incubator at 37 °C in 5% CO2.

Human iPSCs and matched control cord tissue cords were obtained as de-identified specimens from the Department of Veteran’s Affairs (VA) Biorepository, USA. Studies on human tissues were conducted in accordance with the ethics board standards at the Department of Veteran’s Affairs (VA) and the institutional review boards at the Houston Methodist Research Institute (Houston, Texas).

*cryo* XT platform (Ventana Medical Systems, Inc.). The primary rabbit anti-FUS (Cat # A300–302A) antibody was purchased from Bethyl Laboratories, Inc. Mouse anti-Poly (ADP-Ribose) Polymer antibody was purchased from Sigma-Aldrich. Mouse anti-FLAG antibody was purchased from Bethyl Laboratories, Inc. Rabbit anti-PARP1 antibody was purchased from Proteintech. Co-immunoprecipitation was performed according to the manufacturer’s instructions [37, 58]. Briefly, plasmids were transfected into SH-SY5Y or HEK293 cells with DNA transfection kit (Trevigen), according to the manufacturer’s instructions. Cell proliferation was measured using a microplate reader (BioRad Model 680) at an absorbance of 570 nm. For the clonogenic survival assay, GO treatment was performed at various concentrations, then defined numbers of control or FUS shRNA transfected cells were plated in 6-well plates. After 5 days, cells were stained for 30 min with crystal violet (0.1%) and survival fractions were calculated [56].

**Antibodies, plasmids, shRNAs, and siRNAs.**

Rabbit anti-FUS (Cat # A300–302A) antibody was purchased from Bethyl Laboratories, Inc. Rabbit anti-PARP1 antibody (Cat # sc-25780) was purchased from Santa Cruz. Rabbit anti-XRC1 antibody (Cat # ab134056) was purchased from Abcam. Mouse anti-FLAG antibody (A8592) was purchased from Sigma-Aldrich. Mouse anti-XRCC1 antibody (Cat # TA500880) was purchased from Origin. Mouse anti-LigIII antibody (Cat # ab5877) was purchased from Abcam. Mouse anti-Poly (ADP-Ribose) Polymer antibody (Cat # ab 14459) was purchased from Abcam. Fluorescent secondary antibodies, Alexa Fluor 488 anti-mouse (Cat # A28175), and Texas Red anti-rabbit antibody (Cat # T-2767) were purchased from Life Technologies. The concerned antibodies were diluted at 1:1000 for western blotting, 1:500 for immunofluorescence and 1:100 for PLA. GST-FUS plasmid (pGE6X-P) and GST-FUS domain polypeptide-expression plasmid were purchased from Addgene. aa266–526 from Addgene. The aa2–267 FUS polypeptide was cloned into pGE6X-P1 vector as per standard protocol. FUS coding sequence from GST-FUS plasmid was re-cloned into pCDNA3.1 vector as a C-terminal Flag-FUS construct, and Flag-FUS-R212H, Flag-FUS-P252L, GFP-FUS-R251C, and GFP-FUS-P252L mutants were then generated using a QuickChange II XL Site-Directed Mutagenesis kit (Agilent Technologies), following the manufacturer’s instructions. FUS-GFP and GFP-XRC1 were gifts from Dr. Lawrence J. Hayward (University of Massachusetts Medical School, Worcester, MA.) and Dr. Li Lan (University of Pittsburgh, Pittsburgh, PA), respectively. FUS shRNA plasmids were purchased from Sigma. XRCC1 siRNA was purchased from Dharmacon and LigIII siRNA was purchased from Sigma [35].

Human spinal cord tissue sample preparation. Human postmortem spinal cord tissue of cervical region from ALS patients and age-matched controls were obtained as de-identified specimens from the Department of Veteran’s Affairs (VA) Biorepository, USA. The patient clinical features are listed in Supplementary Table 1. All animals were housed in specific pathogen-free (SPF) environment. HEK293 cells were cultured in DMEM containing 10% FBS, 1% MEM non-essential amino acids (Gibco), and 1% fetal bovine serum. HEK293 cells were transfected with pCDNA3.1 vector as a C-terminal Flag-FUS construct, and Flag-FUS-R521H, Flag-FUS-P526, and aa465–526 were purchased from Addgene. The aa1–526 FUS polypeptide was cloned into pGE6X-P1 vector as per standard protocol. FUS coding sequence from GST-FUS plasmid was re-cloned into pCDNA3.1 vector as a C-terminal Flag-FUS construct, and Flag-FUS-R212H, Flag-FUS-P252L, GFP-FUS-R251C, and GFP-FUS-P252L mutants were then generated using a QuickChange II XL Site-Directed Mutagenesis kit (Agilent Technologies), following the manufacturer’s instructions. For motor neuron differentiation, cells were then kept for over 7 days in a medium containing BDNF, GDNF, 10 ng/ml Glial-Like derived neurotrophic factor (GDNF, from Peprotech). Cell proliferation was measured using a microplate reader (BioRad Model 680) at an absorbance of 570 nm. For the clonogenic survival assay, GO treatment was performed at various concentrations, then defined numbers of control or FUS shRNA transfected cells were plated in 6-well plates. After 5 days, cells were stained for 30 min with crystal violet (0.1%) and survival fractions were calculated [56].

**Single cell gel electrophoresis (Comet) Assay.**

Alkaline or neutral comet assays were performed using Comet Assay kits (Trevigen), according to the manufacturer’s instructions. The nuclear DNA was stained with SYBR green dye for 10 min and visualized with a fluorescent microscope (Zeiss Axio observer). Tail moment was measured by CASP software.

**Transfection, immunoblotting, co-immunoprecipitation, and immunofluorescence.**

Plasmids were transfected into SH-SY5Y or HEK293 cells with Lipofectamine 2000 (Invitrogen), per the manufacturer’s instructions. Immunoblotting, co-immunoprecipitation, and immunofluorescence were performed as regularly [56, 57]. For immunoblotting, cell lysates extracted with lysis buffer (Fisher) containing the protease inhibitor cocktail (Roche) were loaded into 4–12% Bis-Tris precast gels (Bio-Rad) for electrophoresis. Following transferring onto the nitrocellulose membrane and incubating with primary and secondary antibodies, protein signal was detected by adding chemiluminescence reagents (Thermo) and visualized by X-ray film in dark room. Co-immunoprecipitation was performed using 2 μg of antibodies for 1 mg of total cell lysate and protein G sepharose (Sigma) to wash down immunocomplexes followed by washing with NP-40 buffer. For immunofluorescence, cells grown on chamber slides were fixed with 4% paraformaldehyde for 15 min, followed by permeabilization in 0.5% Triton X-100 for 10 min. After incubating with primary antibodies overnight and fluorescent labeled secondary antibodies for 2 h, immunofluorescence images were captured with Zeiss Axios Observer fluorescent microscope.

**In situ proximity ligation assay.**

A Duolink PLA kit (Sigma) was used for the in situ PLA assay, following the manufacturer’s instructions [28, 38]. Briefly, cells grown in chamber slides were fixed with 3.5% formaldehyde for 15 min at 37 °C, permeabilized with 0.5% TritonX-100 for 10 min, and then incubated with primary antibodies overnight. Subsequently, cells were incubated at 37 °C with PLA probes for 1 h, with ligase for 30 min, and with polymerase for 100 min. Slides were stained with DAPI and mounted using Mounting Medium containing DAPI and PLA signals was visualized with a fluorescent microscope (Zeiss Axios observer). The negative control was tested by incubating with IgG.
Long amplicon PCR and PCR products quantitation. Genomic DNA was isolated using the Qiaamp DNA Blood Kit (Qiagen) and purified using MicroSpin S-400HR (GE Healthcare). PCR products were quantified using the Qubit dsDNA BR Assay Kit (Thermo Fisher Scientific) and visualized on a 2% agarose gel. The quantification was performed using the Qubit 3.0 Fluorometer (Thermo Fisher Scientific).

To probe the amount of PCR products, the DNA was quantified using the Quant-iT PicoGreen dsDNA Quantitation Kit (Life Technologies). The fluorescence was detected using a Typhoon FLA 9000 Variable Imaging System (GE Healthcare).

Protein purification and GST pull-down. GST-FUS and GST-fused FUS domain polypeptides were expressed in the bacterial cell strain BL21 strain (Merck Millipore) and purified using glutathione-Sepharose 4B beads (GE Healthcare). The purified proteins were loaded into 96-well plates and used for GST pull-downs.

In vitro ligation activity assay and kinetic activity analysis. The ligation activity assay was performed using forward primer 5′-TTGGAATTA CGCCTGTAAACGACG-3′ and reverse primer 5′-CTGATGTTTCCGATGTT-3′. The PCR cycles were finished in 250 bp of the 5′PRP gene, which was amplified using forward primer 5′-TGTCCGATTAGTGAAGG-3′ and reverse primer 5′-CTGATATTGGTTCCGATGTT-3′. PCR products were separated in agarose gel and visualized by Gel Logic 2200 imaging system (Kodak).

In vitro ligation activity assay and kinetic activity analysis. The ligation activity assay was performed using forward primer 5′-GCGAGCAAGGACGGATGACGCTG-3′ and reverse primer 5′-ACCGATGATGTACGATAGTTGAT-3′. The PCR cycles were finished in 250 bp of the 5′PRP gene, which was amplified using forward primer 5′-TGTCCGATTAGTGAAGG-3′ and reverse primer 5′-CTGATATTGGTTCCGATGTT-3′. PCR products were separated in agarose gel and visualized by Gel Logic 2200 imaging system (Kodak).

Antisense oligonucleotide-mediated FUS knockdown. Scrambled ASO and ASO for FUS were purchased from Exiqon (Vedbaek, Denmark), and the delivery into motor neurons was performed by adding sterile water dissolved-ASOs into culture medium of motor neuronal cells from the 20th day of differentiation from iPSC and consist maintaining ASOs for one week.

Generation of CRISPR/Cas9-based FUS knockout cell line. Single-guide RNA (sgRNA) against the FUS gene was designed by screening the target sequence with the online tool http://www.broadinstitute.org/rnai/public/analysis-tools/sgrna-design. One high-score sgRNA target sequence was detected in exon 4 which was annealed with the indicated antibodies.

In situ apoptosis detection. TUNEL staining was used to analyze apoptosis in motor neurons. TUNEL Assay Kit-In situ BrdU Red DNA Fragmentation (Abcam) NeuronTACS detection kit (TREVGEN) was used for the staining. Apoptotic motor neurons seeded on chamber slides were detected following H2O2 treatment, according to the manufacturer’s instructions. Images were taken by fluorescent microscope (Zeiss Axios observer).

ADP-ribosylation assay in vitro. The ADP-ribosylation assay was performed as published protocol with modification. Briefly, 1 μg human PARP1 purified protein was incubated with 1 μM NAD⁺ (Sigma) and 5 μM activator oligonucleotide (5′-GAATTTCC-3′) in reaction buffer for 15 min at 37 °C, in a total reaction volume of 25 μl. Samples were then loaded in NuPAGE 4–12% Bis-Tris Protein Gels (Invitrogen) for 5 min at 95°C. The ADP-ribosylation was detected by western blotting by probing anti-Poly (ADP-Ribose) Polymer antibody (abcam).

Statistical analysis. A minimum of three independent experiments based on three different differentiation batches was always performed. Statistical analysis was performed using Microsoft excel or graphpad prism software. Results were analyzed for significant differences using ANOVA procedures and Student’s t-tests, with p < 0.05 considered statistically significant. Data availability. The data that support the findings of this study are available from the corresponding author upon reasonable request.

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**Author contributions**

H.W. designed and performed the majority of experiments and co-wrote the manuscript. M.L.H. designed and supervised the study, analyzed and interpreted the data, and co-wrote and prepared the final manuscript. J.M. generated the CRISPR/Cas FUS KO cell line. P.M.H. contributed to purification of recombinant proteins. B.E.E. assisted in XRCC1 and LigIII knockdown assays. S.M. provided expert inputs on oxidative genome repair and commented on the manuscript. W.G., T.V., and L.V.D.B. contributed to the ALS patient-derived fibroblasts, iPSCs and the motor neuron differentiation protocol and commented on the manuscript. A.E.T. provided XRCC1/LigIII protein complex purified from insect cells, provided expert input on DNA ligation assays, and commented on the manuscript. All authors discussed the results and provided comments.

**Additional information**

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**Competing interests:** The authors declare no competing interests.

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