

Review Article

# The role of DNA damage response in amyotrophic lateral sclerosis

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Amyotrophic lateral sclerosis (ALS) is a rapidly disabling and fatal neurodegenerative disease. Due to insufficient disease-modifying treatments, there is an unmet and urgent need for elucidating disease mechanisms that occur early and represent common triggers in both familial and sporadic ALS. Emerging evidence suggests that impaired DNA damage response contributes to age-related somatic accumulation of genomic instability and can trigger or accelerate ALS pathological manifestations. In this review, we summarize and discuss recent studies indicating a direct link between DNA damage response and ALS. Further mechanistic understanding of the role genomic instability is playing in ALS disease pathophysiology will be critical for discovering new therapeutic avenues.

Amyotrophic lateral sclerosis (ALS) is a lethal degenerative motor neuron disease with a median survival of 2–4 years after diagnosis [1] and no available effective treatment [2]. Caused by loss of motor neurons in the motor cortex, brain stem and spinal cord, the worldwide annual incidence of ALS is approximately 1 per 50,000 live births and is expected to exponentially increase in the next 20 years [3]. Since it leads to severe disability with high fatality rate, there is an extensive socioeconomic burden alongside the unmet medical need [1,4,5]. Most likely, as for most neurodegenerative diseases, one of the reasons for the slow progression in the development of novel therapies in ALS is the fact that the underlying neurodegeneration may start decades before clinical diagnosis [6–10]. Thus, a better understanding of the disease mechanisms that appear early and represent common triggers in both familial (fALS) and sporadic (sALS) forms of ALS is required as to inform on early diagnostic/prognostic markers and therapies.

Although ALS is a mainly sporadic disease (90–95% of patients) [11], attention has been focused on the 5–10% of patients that have fALS where a gene-disease association can be made (Figure 1). Currently around 30 genes associated with fALS have been identified [12–16] and while a common molecular mechanism remains uncertain, recent evidence suggests that accumulation of genomic instability (GIN) – via impaired DNA damage recognition or defective DNA repair – is one of the hallmarks of ALS (Figure 2 and Table 1) [17].

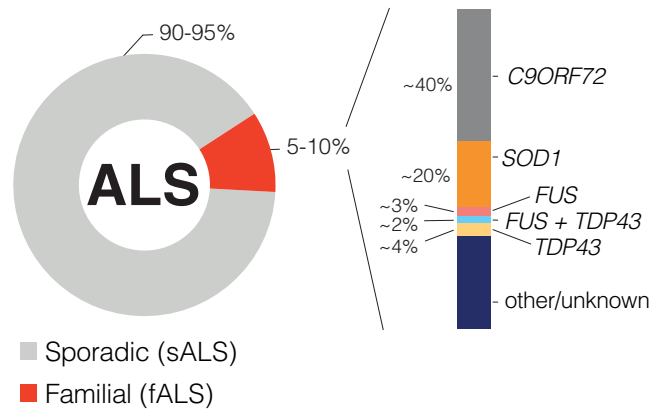
Without excluding the importance and relevance of other molecular mechanisms that have been extensively covered by others [18–21], in this review we will examine the evidence revealing a role for the DNA damage response (DDR) in ALS, by discussing some of the particular genes, proteins and cellular processes implicated at the intersection of the DDR and ALS.

## Sources of genomic instability and connection to ALS

DDR is starting to be recognized as a unifying mechanism in neurodegenerative disorders [22]. DNA damage can arise from both endogenous and exogenous sources and if not repaired will lead to the accumulation of GIN and ensuing pathologies [23]. To enable normal neuronal functions and survival, the DDR encompasses complex mechanisms that recognize DNA damage and signal for DNA damage repair [22,24]. Increasing evidence indicates that mature neurons are highly dependent on accurate DDR and that DNA damage accumulation accelerates in the normal human brain particularly after 40 years of age

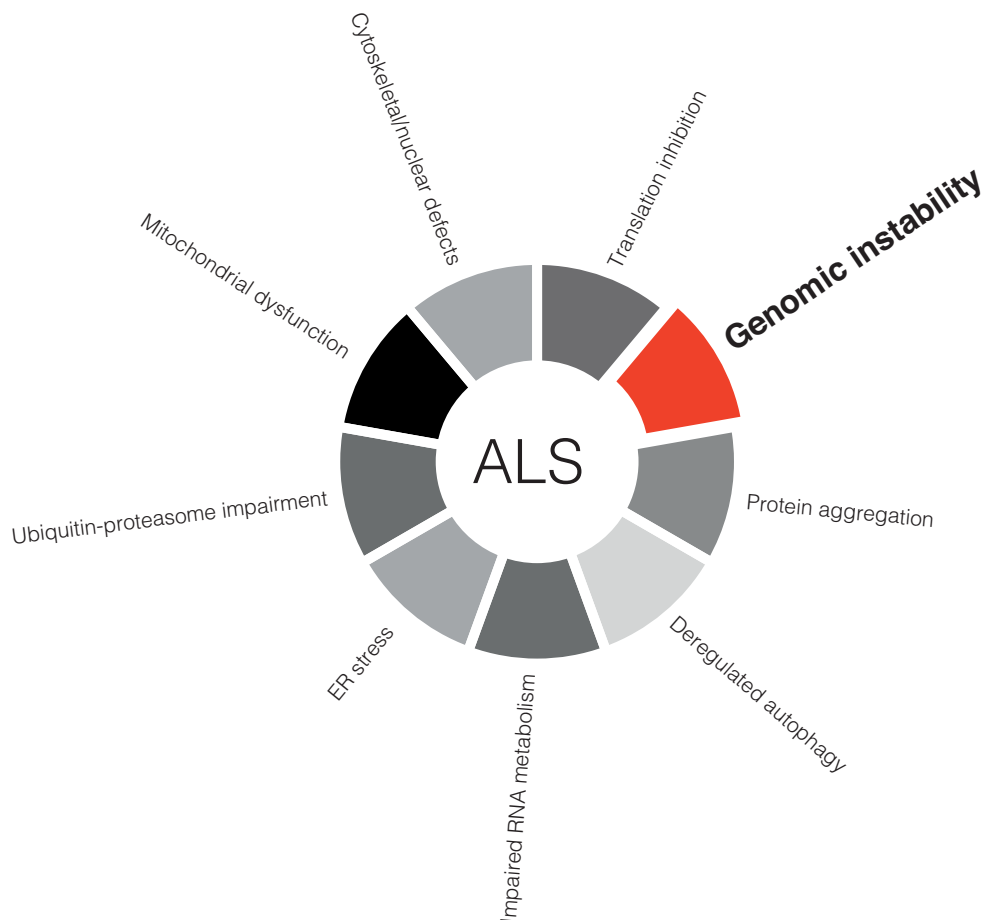
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**Figure 1. ALS patient stratification**

Although some genetic heterogeneity is observed across the world, literature suggests these are the approximate proportions of ALS patients with mutations in the represented genes. Table 1 highlights other genetic contributors and their links to DDR.



**Figure 2. Molecular hallmarks of ALS**

Current evidence suggests several underlying etiological factors in ALS. Genomic instability, caused by defective DNA damage signalling or DNA repair, toxic DNA repair, impaired clearance of endogenous genotoxic stressors (i.e. ROS), or due to imbalanced chromatin structure states, could be a unifying pathophysiological characteristic of the disease.

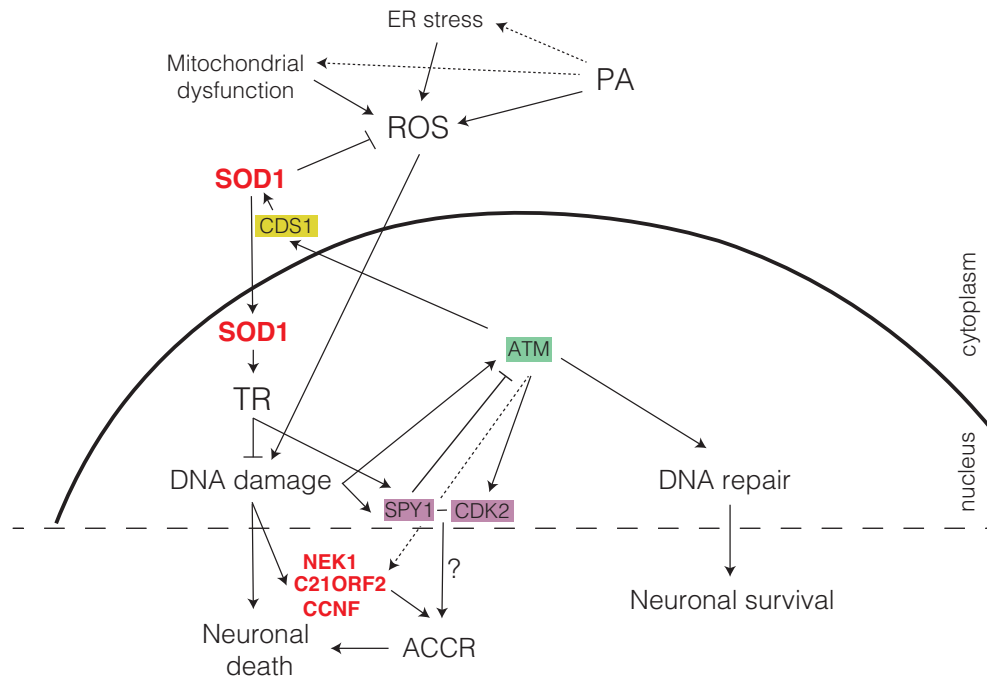
**Table 1 DDR associated mutations in ALS**

Gene	DDR link
<b>TDP-43</b>	<i>TAR DNA-binding protein 43</i> ; ALS-linked mutations [145]; impairs DDR in ALS [34]
<b>FUS/TLS</b>	<i>Fused in sarcoma</i> ; ALS-linked mutation [79,146]; impairs DDR in ALS [147]
<b>HNRNP</b>	<i>Heterogeneous nuclear ribonucleoprotein</i> ; modifies TDP43 [148,149]; associated with DDR [150]; hnRNP L recruits 53BP1 and BRCA1 in cancer [151]; hnRNP F,H, and K are related to ALS [152] and p53 recruitment [153]
<b>HNRNPA1</b>	ALS linked mutations [154]; telomere protection and telomerase activation [155]; regulated by TDP43 [156]
<b>HNRNPA2/B1</b>	ALS linked mutations [154]
<b>SARM1</b>	<i>Sterile alpha and TIR motif containing 1</i> ; ALS linked mutations [157]; <i>SARM1</i> deletion suppresses TDP43-linked ALS [158]
<b>PFN1</b>	<i>Profilin-1</i> ; mutated PFN1 aggregates and shifts TDP43 from nuclei to cytoplasm in ALS [159]
<b>UBQLN2</b>	<i>Ubiquilin-2</i> ; ALS-linked mutations [160]; interacts with TDP43 [161]
<b>CCNF</b>	<i>Cyclin F</i> ; ALS-linked mutations [162]; increases ubiquitinated TDP43 [162]
<b>ERBB4</b>	<i>Erb-B2 Receptor Tyrosine Kinase 4</i> ; interacts with TDP43 [163]; regulates p53-dependent DDR [164]; interacts with KAP1 for DDR [165]; activates p53 and p21 [166]
<b>SIGMAR1</b>	<i>Sigma nonopioid intracellular receptor 1</i> ; interacts with TDP43 [167]
<b>GLE1</b>	RNA export mediator; ALS-linked mutations [168]; interacts with TDP43 [169]; <i>GLE1</i> deletion increases phosphorylated H2AX, decreases BRCA1 and FANCD2 and increases ATR resulting in delayed DDR [170]
<b>SOD1</b>	<i>Superoxide dismutase</i> ; ALS-linked mutations [33]; protects DNA from oxidative stress damage in ALS [171]
<b>DAO</b>	<i>D-amino acid oxidase</i> ; ROS production [172]
<b>KIAA1563/ALS2</b>	<i>Alsin</i> ; ALS-linked mutations [154]; increases ROS in ALS [173]; regulates autophagy [174]
<b>C9ORF72</b>	Induces DNA damage in ALS [175]
<b>SETX</b>	<i>Senataxin</i> ; encodes a DNA/RNA helicase protein involved in DDR and RNA production in ALS4 (Juvenile ALS) [176,177]
<b>ATXN2</b>	<i>Ataxin-2</i> ; ALS-linked mutations [178]; R-loop suppressor [65]; affects R-loop in ALS [179]
<b>VCP</b>	<i>Valosin-containing protein</i> ; ALS-linked mutations [180]; facilitates 53BP1 recruitment for DSB repair [17,181]; causes p62 accumulation in ALS [182]
<b>NEK1</b>	<i>NIIMA-Related Kinase 1</i> ; mutation induces DNA damage in ALS and impairs ATM-mediated DDR [183]
<b>C21ORF2</b>	NEK1 interactor; involved in HR repair [48,157]
<b>MATR3</b>	<i>Matrin-3</i> ; activated by ATM and involved in the early stage of the DSB response [184]
<b>SQSTM1 /p62</b>	<i>Sequestosome-1</i> ; inhibits nuclear RNF168; an E3 ligase essential for histone H2A ubiquitination and DDR [185]
<b>TBK1</b>	<i>TANK-binding kinase 1</i> ; ALS-linked mutations [157]; an inducer of type-1 interferons; major role in autophagy and mitophagy [186]; cGAS/Sting/TBK1/IRF3 regulates p21 maintaining chromosomal stability [138]
<b>ELP3</b>	<i>Elongator complex protein 3</i> ; ALS-linked mutations [154]; binds to PCNA; linked to DNA replication and repair [187]
<b>TIA1</b>	<i>T-cell intracellular antigen 1</i> ; affects DDR; binds to p53 mRNA and controls p53 expression [188]; promotes phase separation and alters SG dynamics in ALS [15]

[25]. With ageing, there is thus an even greater requirement for DDR, and failure to deal with GIN accumulation will eventually lead to increased neuronal loss. Paradoxically, the DDR is known to change and deteriorate with age [26]. GIN arises from the buildup of lesions, such as base modifications, abasic sites, single- or double-stranded breaks (SSBs; DSBs) [27,28]. DSBs are particularly deleterious and, if left unrepaired, are detrimental to cell survival. DSBs are repaired by homologous recombination (HR) and non-homologous end joining (NHEJ). Carried out exclusively in cells that are in S- or G2-phase of the cell cycle, HR is the preferential DSB repair pathway as it is a relatively error-free process. Because under physiological conditions neurons are outside of the replicative cell cycle in the quiescent G0-phase, even though error-prone, NHEJ is the primary repair pathway for DSBs. That being said, recent evidence suggests that in addition to classical NHEJ, neurons could employ transcription-coupled repair mechanisms utilizing mRNA as a template for homology directed repair [29].

Since mature neurons are post-mitotic non-replicating cells that are difficult to replace [30,31], unsanctioned neuronal loss will lead to neurodegeneration. Concomitantly, ageing also brings other imbalances that can accelerate such DDR-related processes [32].

In ALS, the endogenous sources contributing to deleterious accumulation of GIN are from both impaired removal of reactive metabolic genotoxins (i.e. reactive oxygen species; ROS) that can overwhelm DDR [33] and from the incapability to recognize or repair DNA damage [34,35]. Although in this review, we are focusing on endogenous sources of DNA damage, one must keep in mind the geographical heterogeneity of ALS that cannot be explained by genetic risk factors alone [12,36]. Thus, future research should consider environmental genotoxic influences that might also play a role in both sALS and fALS.



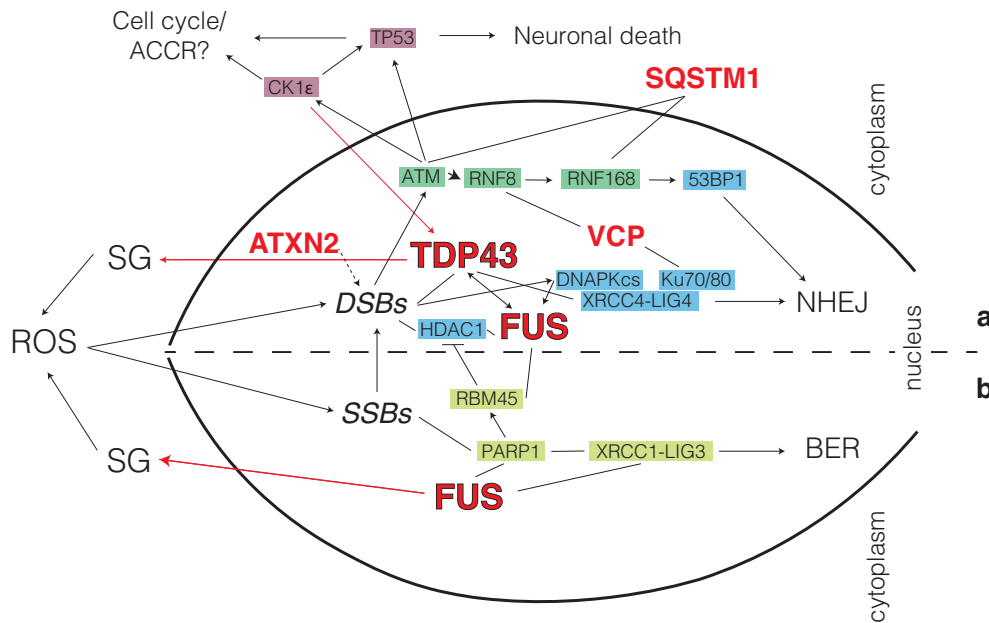
**Figure 3. SOD1 plays a dual role in DDR**

SOD1 nuclear translocation is ATM/CDS1-dependent. Once SOD1 enters the nucleus, it activates transcription (TR) of many genes that are involved in DDR or ROS defence. SOD1 regulates *SPY1* expression, which activates CDK2, a G1- to S-phases check point. Other cell cycle regulation (CCR) gene mutations (e.g. *NEK1*, *C21ORF2* and *CCNF*) are implicated in defective DDR, suggesting a role for atypical cell cycle re-entry (ACCR) in ALS. Mutated genes identified in ALS (red), homologous recombination (HR; green), atypical cell cycle checkpoint (AACR; purple) and ROS regulation (yellow). Dotted arrows are proposed, yet not completely proven, interactions.

## SOD1 and DNA damage in ALS

Superoxide dismutase 1 (SOD1) is a free radical scavenging enzyme that in the cytoplasm catalyzes the conversion of superoxide anions formed during mitochondrial respiration into hydrogen peroxide [37] and protects motor neurons – which are particularly prone to the toxic effects of mutant and dysfunctional SOD1 – against oxidative damage and neurodegeneration [33]. In both sALS and fALS, SOD1-induced neuronal toxicity occurs through gain-of-function mutations (Figure 1) [33,38] that lead to accumulation of injuries produced from the unscheduled free radical attack on pyrimidine and purine bases [39,40]. Secondly, in both sALS and fALS, SOD1 can be secreted as monomers into the extracellular space leading to cell death [41]. Unexpectedly, recent data show that independent from its catalytic function, SOD1 performs additional roles in the nucleus. In response to elevated ROS, in an ataxia-telangiectasia mutated (*ATM*; a core DDR gene [42]) dependent manner, CDP-diacylglycerol synthase 1 (CDS1) kinase phosphorylates SOD1 at S60 and S99 promoting rapid SOD1 translocation to the nucleus where it regulates the expression of a large set of genes involved in oxidative stress defence and DDR [43]. Furthermore, nuclear SOD1 increases *SpeedyA1* (*SPY1*) expression promoting cell survival and inhibiting damage-induced apoptosis. In ALS, pathologic SOD1–G93A cannot translocate to the nucleus and exercise its protective role via *SPY1* regulation [44]. *SPY1* is a nuclear protein that controls the transition between G1- and S-phases of the cell cycle via checkpoint-dependent kinase 2 (CDK2) activation [45]. In neurons, re-entry into cell cycle (CCR) is partly controlled by ATM, is atypical, and leads to neuronal death [30,46,47]. The observation that SOD1 can influence such decisions will require further investigation especially since other fALS genes, such as *NEK1*, *C21ORF2* and *CCNF* are also involved in cell cycle progression [48–51], suggesting CCR should be considered in ALS pathology.

Thus, SOD1 protection against DNA damage accumulation is bi-modal, with the first tier of defence being executed in the cytoplasm through ROS scavenging, and the second in the nucleus where it controls the expression of DDR-related genes (Figure 3).



**Figure 4. Role for TDP43 and FUS in maintaining genome stability in ALS**

Pathway choice is directed by the balance between TDP43 and FUS interaction at break sites. Simplified model for the role of TDP43 (a) and FUS (b) in DDR. Mutated genes identified in ALS (red), atypical cell cycle checkpoint (AACR; purple), non-homologous end-joining (NHEJ; blue), homologous recombination (HR; green) and base excision repair (BER; chartreuse). Dotted arrows are proposed, yet not completely proven, interactions.

## TDP43 mislocalization impairs DDR

Transactivation response DNA-binding protein 43 (TDP43) is a highly conserved nuclear protein that acts as transcription and splicing regulator as well as scaffold for nuclear bodies [52]. While in normal conditions TDP43 is primarily localized in the nucleus, in disease states it gets trapped in insoluble cytoplasmic inclusions (stress granules; SG; see Figure 4a) [53,54]. Although mutated TDP43 accounts for only approximately 4% of fALS cases (Figure 1), TDP43-SG accumulation is a pathology characteristic for ~95% of all ALS and ~50% of frontotemporal dementia (FTD) cases [21,55–57], as well as a secondary pathology in other neurodegenerative diseases, including Alzheimer's [58], Parkinson's [59] and Huntington's diseases [60,61].

Pathologic TDP43 mislocalization activates the mitochondrial unfolded protein response [62], elevates ROS levels and affects cytoplasmic-nuclear trafficking, eventually leading to increased neuronal stress and subsequent cell death [63,64]. Associated with such stress, GIN accumulation was described in sALS and fALS patients as well as in model organisms with orthologous TDP43 loss-of-function [34]. In addition, TDP43 cytoplasmic retention can be aggravated by other factors such as ataxin 2 (ATXN2), itself associated with DDR processes [65], thereby further increasing the risk of developing ALS [66]. Furthermore, TDP43 mislocalization and GIN accumulation maintain a vicious cycle via casein kinase 1ε (CK1ε) that has been shown to promote cytoplasmic accumulation of TDP43 [67]. Together with other CK1 isoforms, CK1ε is activated upon GIN build-up and controls several cellular processes linked to DNA damage signalling and repair, including apoptosis and cell cycle checkpoint control (Figure 4a) [68].

Although initially the connection between TDP43 dysfunction and the accumulation of GIN in ALS was thought to be a secondary feature, recent evidence shows that neuronal TDP43 plays an important direct role in DDR by controlling the nuclear recruitment of the XRCC4-DNA ligase 4 (LIG4) complex, critical for DSB repair via NHEJ [63]. In ALS/FTD, TDP43 nuclear exclusion incapacitates the transport of XRCC4/LIG4 leading to abortive NHEJ with consequent accumulation of toxic DSBs. The involvement of DSB repair in ALS/FTD is further substantiated by the observation that other proteins mutated in fALS such as valosin-containing protein (VCP)/p97 and sequestosome 1 (SQSTM1)/p62 are linked to NHEJ [69,70]. VCP has been shown to directly interact with the canonical NHEJ proteins Ku70/80 [69] as well as with ring finger proteins (RNF) 8/168 [71] to balance DNA repair pathway choice and promote cell survival. This process is done in close correlation with SQSTM1/p62 that via interactions with ATM, RAD50 and RNF168 also regulates the choice between HR and NHEJ in favour of the latter [70]. The TDP43/XRCC4

direct connection is somewhat unexpected as replicating cells have less of a requirement for TDP43 in NHEJ (Figure 4a). This should prompt a more detailed analysis of these pathways in neurons where the relationship between different DDR components might be rewired. Further studies will be required to look, for example, at the interplay between TDP43 and other NHEJ proteins, such as PAXX and XRCC4-like factor (XLF) [72] or the SHIELDIN complex [73]. Additionally, given its RNA-binding capabilities, TDP43 has been implicated in impeding DNA:RNA hybrids (R-loops) formation [17,74]. This places TDP43 squarely in the middle of both DSB repair and the transcriptional stress that neurons endure.

## FUS-mediated solid-to-liquid phase transition promotes DDR in ALS

Fused in sarcoma (FUS) is a nuclear ribonucleoprotein involved in a variety of cellular functions including transcription, protein translation and RNA splicing and transport [75,76]. Initially studied for its roles in cancer [77,78], it was later discovered that around 5% of fALS and 1% of sALS cases are associated with *FUS* mutations (Figure 1) [79].

Following oxidative damage, in a poly(ADP-ribose) polymerase (PARP1)-dependent manner, FUS facilitates the recruitment of XRCC1/LIG3 to SSBs and enhances LIG3 ligation activity thus promoting base excision repair (BER; Figure 4b) [80–83]. These interactions are, at least, partly based on the ability of FUS to rapidly traffic to the nucleus, as mutations in the nuclear localization sequence induce FUS aggregation, genomic instability, and consecutive neurodegeneration [84]. Additionally, in ATM and DNA-PKcs-dependent manner, FUS is involved in DSB repair by directly controlling the recruitment of histone deacetylases 1 (HDAC1) to chromatin [35]. The involvement of FUS in HDAC1 recruitment and activation is bimodal. Firstly, following DSB induction, FUS recruitment of HDAC1 promotes deacetylation and activation of NHEJ [85]. Secondly, in a PARP-dependent manner, FUS interacts with RNA-binding motif protein 45 (RBM45) and prevents excessive recruitment of HDAC1 [86].

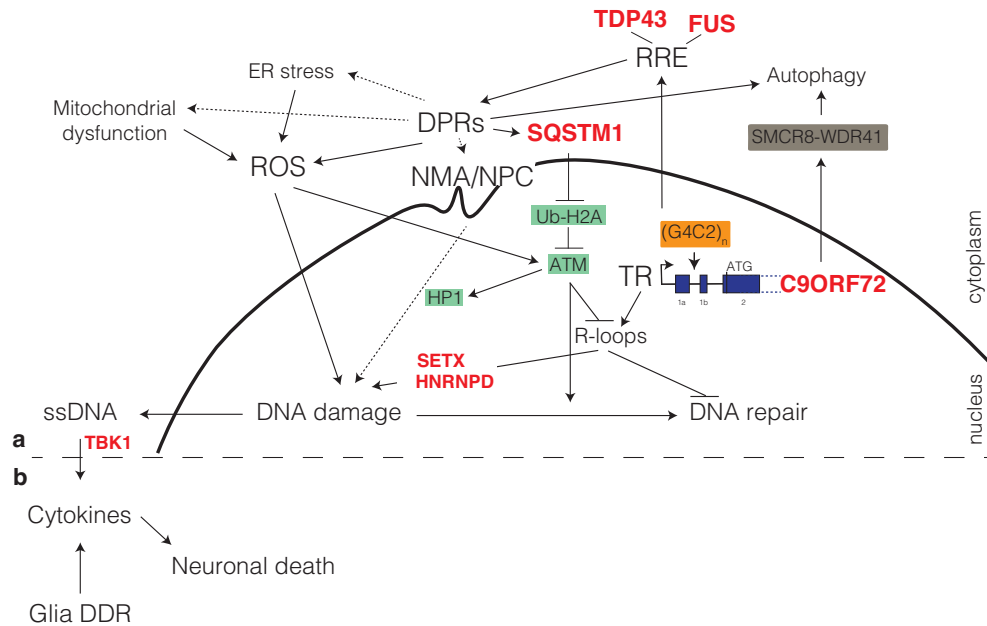
These data build a model in which FUS controls the choice between SSB repair and DSB repair pathways in healthy neurons. Further research will be required to specifically understand the connection between FUS and TDP43 in DDR as well as the requirement of HR versus microhomology mediated end-joining (alternative NHEJ; MMEJ). In some patients, ALS is evidenced to manifest on the basis of oligogenic rather than monogenic alterations, with summative effects from several DDR pathologies [87], as indicated in Figure 4.

## C9ORF72 repeat expansion and impaired DDR in ALS

Nucleotide repeat expansion (NRE) disorders encompass more than 20 human genetic diseases, most of which affect the nervous system, that arise from an expansion of a particularly unstable tandem of 3–12 DNA bases [88,89]. The deleterious effects of these NREs depend on the location of the repeat within the affected gene, its sequence, as well as the size of the repeat. *C9ORF72* ALS/FTD is caused by the expansion of a hexanucleotide GGGGCC (G4C2) track in the first intron of the *C9ORF72* gene [90].

G4C2–NREs are pathogenic through several non-exclusive mechanisms that can all influence the accumulation of DNA damage lesions and affect their repair (Figure 5A). Initially, transcription over G4C2 tracks is problematic and will lead to accumulation of R-loops [91,92] and accumulation of toxic DNA secondary structures, hairpins and G-quadruplexes, which require DDR to be resolved [93,94]. Intriguingly, mutations in the R-loop processing factors *senataxin* (*SETX*) and *HNRNPD* also lead to fALS [95,96]. Subsequently or in parallel, transcripts containing repeats can form RNA repeat expansion (RRE) foci that will bind and sequester various RNA-binding proteins such as TDP43, FUS, nucleophosmin (NPM1) or AP endonuclease (APE1), potentially altering their localization and DDR functions [91,97–105]. Finally, the G4C2–NREs are non-AUG (RAN) translated into dipeptide repeats (DPR)-containing proteins (poly-GR; -GP; -GA; -PR; -PA) that form inclusions throughout the brain of patients with ALS/FTD [106–109] and can lead to endoplasmic reticulum (ER) stress, mitochondrial dysfunction with ROS accumulation [110] and sequestration of DDR proteins [111,112]. Moreover, DPRs can accumulate at the nuclear membrane and the nuclear pore complex (NPC) to promote nuclear membrane abnormalities (NMA), impaired nuclear-cytoplasmic transport [113–115] and imbalanced chromatin states [116]. Furthermore, in a vicious feedback loop, the expanded G4C2 can interfere with the transcription and translation of the *C9ORF72* mRNA thus leading to decreased autophagy and further accumulation of DPRs [117].

Consequence of these pathologic mechanisms, *C9ORF72* ALS/FTD patients show increased GIN accumulation both in the brain [118] and spinal cord [97] where presence of DDR markers can be detected. One of the clearest evidences for a direct DDR deficiency in ALS comes from the observation that expressing RREs and/or DPRs results in elevated R-loop levels and DSBs build-up in rat neurons, human cells and *C9ORF72* ALS patient spinal cord tissues. This is as a result of the incapability of *C9ORF72*-ALS neurons to mount a suitable DDR signalling cascade



**Figure 5. DDR defects in ALS with C9orf72 mutations**

G4C2–NREs in the first intron of the *C9orf72* gene increases RRE, which impairs DDR through binding to RNA-binding proteins. Transcription over G4C2–NREs leads to R-loop formation and subsequent DNA damage accumulation. RAN translation produced DPRs that can increase ROS, induce nuclear membrane alterations (NMA) and may potentially sequester DDR proteins. NMA include structural and functional disturbances at the nuclear pore complexes (NPC) involving transport receptors. Abnormal nucleo-cytoplasmic transport of both RNA and proteins at NPC has been suggested to be, either related to molecule sequestrations by DPR and RRE or in parallel with other factors, a strong *C9orf72* disease modifier. G4C2–NREs also decreases *C9orf72* expression, which impairs autophagy and exacerbates DPRs accumulation. Mutated genes identified in ALS (red), homologous recombination (HR; green) and autophagy (brown). Dotted arrows are proposed, yet not completely proven, interactions.

which occurs due to defective ATM-mediated signalling that arises as a consequence of SQSTM1/p62 accumulation and impaired H2A ubiquitylation (Figure 5A). Most likely due to this ATM signalling problem, NHEJ seems to be up-regulated to toxic levels that can be rescued in fly models via Ku (NHEJ), APEX1 (BER) or ERCC1 (interstrand cross-link DNA repair) dysregulation [119]. Although more information is needed to understand where the NHEJ or other DNA repair dependent toxicity is coming from, such observation would be in line with similar mechanisms present in ATM deficient replicating cells [120].

Another important link between G4C2 expansion and DDR is the observation that DPR accumulation leads to imbalanced chromatin states with impact on DNA repair [121]. Poly-PR, for example, specifically binds DNA at heterochromatin, evicts HP1 $\alpha$  and causes abnormal histone H3 methylation leading to altered chromatin structure and NMA [116]. In response to endogenous DNA damage, to activate DDR, HP1 $\alpha$  is phosphorylated by ATM [122], while H3K9me3 is required to activate the acetyltransferase activity of TIP60 [123]. Moreover, DPR accumulation at the nuclear membrane can lead to nuclear membrane rupture with subsequent GIN [124,125] as well as bi-directional transport defects at the NPC resulting in impaired shuttling of RNA and proteins. Such transport disturbances might interfere with factors involved in DDR and DNA repair, further feeding a vicious circle of DNA damage with insufficient repair [57,84,126]. These mechanisms might also influence the onset of age-related ALS, as perturbed nucleo-cytoplasmic cargo delivery is itself a feature of the CNS ageing process [127]. Thus, because the NPC has been shown to play important roles in DNA repair and the organization of genome architecture [128] while in response to DNA damage chromatin undergoes dramatic genome-wide changes that are at the heart of DDR [129], further scrutiny will be required to apprehend the relationship between nuclear DPR accumulation at specific nuclear structures (i.e. NPC), imbalanced chromatin states and their link to DDR in ALS.

## Neuroinflammation and DDR in ALS

It must be highlighted that neurons do not live in isolation, and neurodegeneration is associated with microglial reactivity and activation of innate immune responses. Neuroinflammation is a common characteristic of ALS and comprises the stimulation of microglia, astrocytes and inflammatory T cells [130]. Upon activation, these cells secrete proinflammatory cytokines, such as tumour necrosis factor  $\alpha$ , interferon  $\gamma$ , and interleukin  $1\beta$  [131,132]. Typically, the innate immune response is activated by the presence of foreign cytoplasmic DNA via activation of cyclic GMP–AMP synthetase, (cGAS), and the cyclic dinucleotide receptor, stimulator of interferon genes (STING) [133]. Recently, more attention is being given to the link between accumulating GIN, the subsequent leakage of DNA in the cytoplasm and the activation of the cGAS–STING cascade [134]. In this model, ALS–GIN accumulating neurons can amass increasing amounts of single-stranded DNA (ssDNA) in the cytoplasm and promote neuroinflammation with production of cytokines and subsequent neuronal death (Figure 5B). Interestingly, haploinsufficiency in the STING activating kinase TANK-binding kinase (TBK1) [135] is associated with fALS and FTD [136,137] (Figure 5B). Within this pathway, TBK1 is important for several functions, including maintenance of chromosomal stability [138]. A functional cGAS/STING pathway is also known to be required for normal chromosomal segregation in cancer cells via a p21-dependent mechanism modulating G2/M transition [138]. The putative genome surveillance role in post-mitotic non-replicating cells is less clear.

Neuroinflammation with subsequent neurodegeneration can also result from a glia autonomous problem in dealing with DDR. Mutant human TDP43 expressed specifically in *Drosophila* glial cells causes DNA damage, elevated replication of retrotransposable elements (RTE) [139], and Gypsy endogenous retroviruses [140] and apoptosis in the nearby neurons. During their replication, the expression of RTE cDNA can lead to genome instability and accumulation of DSBs [141]. These studies highlight that TDP43 mutations in glial cells promote ALS progression, at least partly through impaired DDR signalling. Among glia, aberrant astrocyte function has also been implicated in ALS pathology which has been discussed extensively by others and merits further research [142–144]. Further studies will be required to better understand the relationship between DDR and neuroinflammation in ALS/FTD.

## Conclusion

ALS is one of the most common adult-onset neurodegenerative disorders. Currently, ALS is fatal and incurable with patients expected to survive ~2–4 years after diagnosis, revealing an urgent need for effective therapeutic strategies. Proof of DNA damage accumulation and DNA repair deficiency in both ALS initiation and progression is amassing, highlighting the fact that genomic instability is a hallmark of disease pathogenesis. Shedding light on the specific DDR mechanisms at play has important therapeutic potential.

## Summary

- Genomic instability is a hallmark of both sporadic and familial ALS with many ALS genes involved in recognition or repair of DNA damage.
- Outside of the nucleus SOD1 works to impede ROS accumulation and in the nucleus to influence DNA damage response via transcription regulation.
- TDP43 and FUS work mainly to balance the pathway choice between SSB repair and DSB repair.
- Expansion of a repeated G4C2 track in the *C9ORF72* gene leads to impaired ATM signalling.
- Genomic instability may be a starting point for neuroinflammation in ALS.

## Competing Interests

G.B. is a co-founder and consultant for Adrestia Therapeutics Ltd. The remaining authors declare no competing interests.



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## Author Contribution

G.B. conceptualized the review. G.B. and Y.S. wrote the review with help from A.J.C. and A.M.H. A.J.C. and A.M.H. implemented the response to reviewers with help from G.B. and Y.S.

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## Abbreviations

ALS, amyotrophic lateral sclerosis; APE1, AP endonuclease 1; ATM, ataxia-telangiectasia mutated; ATXN2, Ataxin 2; BER, base excision repair; CCR, cell cycle re-entry; CDK2, checkpoint-dependent kinase 2; CDS1, CSP-diacylglycerol synthase 1; CK1 $\epsilon$ , casein kinase 1 $\epsilon$ ; DDR, DNA damage response; DRP, dipeptide-repeat proteins; DSB, DNA double-strand break; ER, endoplasmic reticulum; fALS, familial ALS; FTD, frontotemporal dementia; FUS, fused in sarcoma; G4C2, hexanucleotide GGGGCC; GIN, genomic instability; HDAC1, histone deacetylases 1; HNRNPD, heterogeneous nuclear ribonucleoprotein D; HR, homologous recombination; LIG4, ligase 4; MMEJ, microhomology mediated end-joining; NEK1, NIMA-related kinase 1; NHEJ, non-homologous end-joining; NMA, nuclear membrane abnormalities; NPM1, Nucleophosmin 1; NRE, nucleotide repeat expansion; PARP1, Poly (ADP-ribose) polymerase 1; RAN, Non-AUG; AUG is a start codon; RBM45, RNA-binding motif protein 45; RNF, Ring finger protein; ROS, reactive oxygen species; RRE, RNA repeat expansion; RTE, retrotransposable elements; sALS, Sporadic ALS; SETX, Senataxin; SG, stress granules; SOD1, superoxide dismutase 1; SPY1, SpeedyA1; SQSTM1, Sequestosome 1; SSB, single-stranded break; ssDNA, single-stranded DNA; STING, stimulator of interferon genes; TBK1, TANK-binding kinase 1; TDP43, transactivation response DNA-binding protein 43; VCP, Valosin-containing protein; XLF, XRCC4-like factor.

## References

- 1 del Aguila, M.A., Longstreth, W.T., McGuire, V., Koepsell, T.D. and van Belle, G. (2003) Prognosis in amyotrophic lateral sclerosis: A population-based study. *Neurology* **60**, 813–819
- 2 Hardiman, O., Al-Chalabi, A., Chio, A., Corr, E.M., Logroscino, G., Robberecht, W. et al. (2017) Amyotrophic lateral sclerosis. *Nat. Rev. Dis. Primers* **3**, 17071, <https://doi.org/10.1038/nrdp.2017.71>
- 3 Arthur, K.C., Calvo, A., Price, T.R., Geiger, J.T., Chiò, A. and Traynor, B.J. (2016) Projected increase in amyotrophic lateral sclerosis from 2015 to 2040. *Nat. Commun.* **7**, 12408, <https://doi.org/10.1038/ncomms12408>
- 4 López-Bastida, J., Perestelo-Pérez, L., Montón-Alvarez, F., Serrano-Aguilar, P. and Alfonso-Sanchez, J.L. (2009) Social economic costs and health-related quality of life in patients with amyotrophic lateral sclerosis in Spain. *Amyotroph Lateral Scler Off. Publ. World Fed Neurol. Res Group Mot. Neuron. Dis.* **10**, 237–243
- 5 Schepelmann, K., Winter, Y., Spottke, A.E., Claus, D., Grothe, C., Schröder, R. et al. (2009) Socioeconomic burden of amyotrophic lateral sclerosis, myasthenia gravis and facioscapulohumeral muscular dystrophy. *J. Neurol.* **257**, 15–23, <https://doi.org/10.1007/s00415-009-5256-6>
- 6 Kordower, J.H., Olanow, C.W., Dodiya, H.B., Chu, Y., Beach, T.G., Adler, C.H. et al. (2013) Disease duration and the integrity of the nigrostriatal system in Parkinson's disease. *Brain J. Neurol.* **136**, 2419–2431, <https://doi.org/10.1093/brain/awt192>
- 7 Schrag, A., Horsfall, L., Walters, K., Noyce, A. and Petersen, I. (2015) Prediagnostic presentations of Parkinson's disease in primary care: a case-control study. *Lancet Neurol.* **14**, 57–64, [https://doi.org/10.1016/S1474-4422\(14\)70287-X](https://doi.org/10.1016/S1474-4422(14)70287-X)
- 8 Jack, C.R., Knopman, D.S., Jagust, W.J., Petersen, R.C., Weiner, M.W., Aisen, P.S. et al. (2013) Tracking pathophysiological processes in Alzheimer's disease: an updated hypothetical model of dynamic biomarkers. *Lancet Neurol.* **12**, 207–216, [https://doi.org/10.1016/S1474-4422\(12\)70291-0](https://doi.org/10.1016/S1474-4422(12)70291-0)
- 9 Tabrizi, S.J., Reilmann, R., Roos, R.A., Durr, A., Leavitt, B., Owen, G. et al. (2012) Potential endpoints for clinical trials in premanifest and early Huntington's disease in the TRACK-HD study: analysis of 24 month observational data. *Lancet Neurol.* **11**, 42–53, [https://doi.org/10.1016/S1474-4422\(11\)70263-0](https://doi.org/10.1016/S1474-4422(11)70263-0)
- 10 Eisen, A., Kiernan, M., Mitsumoto, H. and Swash, M. (2014) Amyotrophic lateral sclerosis: a long preclinical period? *J. Neurol. Neurosurg. Psychiatry* **85**, 1232–1238, <https://doi.org/10.1136/jnnp-2013-307135>

- 11 Hardiman, O., Al-Chalabi, A., Chio, A., Corr, E.M., Logroscino, G., Robberecht, W. et al. (2017) Correction: Amyotrophic lateral sclerosis. *Nat. Rev. Dis. Primers* **3**, 17085, <https://doi.org/10.1038/nrdp.2017.85>
- 12 van Es, M.A., Hardiman, O., Chio, A., Al-Chalabi, A., Pasterkamp, R.J., Veldink, J.H. et al. (2017) Amyotrophic lateral sclerosis. *Lancet* **390**, 2084–2098, [https://doi.org/10.1016/S0140-6736\(17\)31287-4](https://doi.org/10.1016/S0140-6736(17)31287-4)
- 13 Renton, A.E., Chiò, A. and Traynor, B.J. (2013) State of play in amyotrophic lateral sclerosis genetics. *Nat. Neurosci.* **17**, 17–23, <https://doi.org/10.1038/nn.3584>
- 14 Smith, B.N., Topp, S.D., Fallini, C., Shibata, H., Chen, H.-J., Troakes, C. et al. (2017) Mutations in the vesicular trafficking protein annexin A11 are associated with amyotrophic lateral sclerosis. *Sci. Transl. Med.* **9**, eaad9157, <https://doi.org/10.1126/scitranslmed.aad9157>
- 15 Mackenzie, I.R., Nicholson, A.M., Sarkar, M., Messing, J., Purice, M.D., Pottier, C. et al. (2017) TIA1 Mutations in Amyotrophic Lateral Sclerosis and Frontotemporal Dementia Promote Phase Separation and Alter Stress Granule Dynamics. *Neuron* **95**, 808.e9–816.e9, <https://doi.org/10.1016/j.neuron.2017.07.025>
- 16 Cooper-Knock, J., Moll, T., Ramesh, T., Castelli, L., Beer, A., Robins, H. et al. (2019) Mutations in the Glycosyltransferase Domain of GLT8D1 Are Associated with Familial Amyotrophic Lateral Sclerosis. *Cell Rep.* **26**, 2298.e5–2306.e5, <https://doi.org/10.1016/j.celrep.2019.02.006>
- 17 Walker, C. and El-Khamisy, S.F. (2018) Perturbed autophagy and DNA repair converge to promote neurodegeneration in amyotrophic lateral sclerosis and dementia. *Brain* **141**, 1247–1262, <https://doi.org/10.1093/brain/awy076>
- 18 Balendra, R. and Isaacs, A.M. (2018) C9orf72-mediated ALS and FTD: multiple pathways to disease. *Nat. Rev. Neurol.* **14**, 544–558, <https://doi.org/10.1038/s41582-018-0047-2>
- 19 Hetz, C. and Saxena, S. (2017) ER stress and the unfolded protein response in neurodegeneration. *Nat. Rev. Neurol.* **13**, 477–491, <https://doi.org/10.1038/nrneurol.2017.99>
- 20 Paez-Colasante, X., Figueroa-Romero, C., Sakowski, S.A., Goutman, S.A. and Feldman, E.L. (2015) Amyotrophic lateral sclerosis: mechanisms and therapeutics in the epigenomic era. *Nat. Rev. Neurol.* **11**, 266–279, <https://doi.org/10.1038/nrneurol.2015.57>
- 21 Ling, S.-C., Polymenidou, M. and Cleveland, D.W. (2013) Converging Mechanisms in ALS and FTD: Disrupted RNA and Protein Homeostasis. *Neuron* **79**, 416–438, <https://doi.org/10.1016/j.neuron.2013.07.033>
- 22 Madabhushi, R., Pan, L. and Tsai, L.-H. (2014) DNA Damage and Its Links to Neurodegeneration. *Neuron* **83**, 266–282, <https://doi.org/10.1016/j.neuron.2014.06.034>
- 23 Jackson, S.P. and Bartek, J. (2009) The DNA-damage response in human biology and disease. *Nature* **461**, 1071–1078, <https://doi.org/10.1038/nature08467>
- 24 Maiuri, T., Stuart, C.E., Hung, C.L.K., Graham, K.J., Bazan, C.A.B. and Truant, R. (2019) DNA Damage Repair in Huntington's Disease and Other Neurodegenerative Diseases. *Neurotherapeutics* **16**, 948–956, <https://doi.org/10.1007/s13311-019-00768-7>
- 25 Lu, T., Pan, Y., Kao, S.-Y., Li, C., Kohane, I., Chan, J. et al. (2004) Gene regulation and DNA damage in the ageing human brain. *Nature* **429**, 883–891, <https://doi.org/10.1038/nature02661>
- 26 Watanabe, K., Ikuno, Y., Kakeya, Y., Ikeno, S., Taniura, H., Kurono, M. et al. (2019) Age-related dysfunction of the DNA damage response in intestinal stem cells. *Inflamm. Regen.* **39**, 8, <https://doi.org/10.1186/s41232-019-0096-y>
- 27 Blackford, A.N. and Jackson, S.P. (2017) ATM, ATR, and DNA-PK: The Trinity at the Heart of the DNA Damage Response. *Mol. Cell* **66**, 801–817, <https://doi.org/10.1016/j.molcel.2017.05.015>
- 28 Ciccia, A. and Elledge, S.J. (2010) The DNA damage response: making it safe to play with knives. *Mol. Cell* **40**, 179–204, <https://doi.org/10.1016/j.molcel.2010.09.019>
- 29 Welty, S., Teng, Y., Liang, Z., Zhao, W., Sanders, L.H., Greenamyre, J.T. et al. (2017) RAD52 is required for RNA-templated recombination repair in post-mitotic neurons. *J. Biol. Chem.* **293**, 1353–1362, <https://doi.org/10.1074/jbc.M117.808402>
- 30 Frade, J.M. and Ovejero-Benito, M.C. (2015) Neuronal cell cycle: the neuron itself and its circumstances. *Cell Cycle* **14**, 712–720, <https://doi.org/10.1080/15384101.2015.1004937>
- 31 de Anda, F.C., Madabhushi, R., Rei, D., Meng, J., Gräff, J., Durak, O. et al. (2016) Cortical neurons gradually attain a post-mitotic state. *Cell Res.* **26**, 1033–1047
- 32 Hou, Y., Dan, X., Babbar, M., Wei, Y., Hasselbalch, S.G., Croteau, D.L. et al. (2019) Ageing as a risk factor for neurodegenerative disease. *Nat. Rev. Neurol.* **15**, 565–581, <https://doi.org/10.1038/s41582-019-0244-7>
- 33 Rosen, D.R., Siddique, T., Patterson, D., Figlewicz, D.A., Sapp, P., Hentati, A. et al. (1993) Mutations in Cu/Zn superoxide dismutase gene are associated with familial amyotrophic lateral sclerosis. *Nature* **362**, 59–62, <https://doi.org/10.1038/362059a0>
- 34 Mitra, J., Guerrero, E.N., Hegde, P.M., Liachko, N.F., Wang, H., Vasquez, V. et al. (2019) Motor neuron disease-associated loss of nuclear TDP-43 is linked to DNA double-strand break repair defects. *Proc. Natl. Acad. Sci.* **116**, 4696–4705, <https://doi.org/10.1073/pnas.1818415116>
- 35 Wang, W.-Y., Pan, L., Su, S.C., Quinn, E.J., Sasaki, M., Jimenez, J.C. et al. (2013) Interaction of FUS and HDAC1 regulates DNA damage response and repair in neurons. *Nat. Neurosci.* **16**, 1383–1391, <https://doi.org/10.1038/nn.3514>
- 36 Kiernan, M.C., Vucic, S., Cheah, B.C., Turner, M.R., Eisen, A., Hardiman, O. et al. (2011) Amyotrophic lateral sclerosis. *Lancet* **377**, 942–955, [https://doi.org/10.1016/S0140-6736\(10\)61156-7](https://doi.org/10.1016/S0140-6736(10)61156-7)
- 37 Fridovich, I. (1997) Superoxide Anion Radical (O<sup>•</sup>-), Superoxide Dismutases, and Related Matters. *J. Biol. Chem.* **272**, 18515–18517, <https://doi.org/10.1074/jbc.272.30.18515>
- 38 Gagliardi, S., Cova, E., Davin, A., Guareschi, S., Abel, K., Alvisi, E. et al. (2010) SOD1 mRNA expression in sporadic amyotrophic lateral sclerosis. *Neurobiol. Dis.* **39**, 198–203, <https://doi.org/10.1016/j.nbd.2010.04.008>
- 39 Canugovi, C., Misiak, M., Ferrarelli, L.K., Croteau, D.L. and Bohr, V.A. (2013) The role of DNA repair in brain related disease pathology. *DNA Repair (Amst.)* **12**, 578–587, <https://doi.org/10.1016/j.dnarep.2013.04.010>

- 40 Slupphaug, G., Kavli, B. and Krokan, H.E. (2003) The interacting pathways for prevention and repair of oxidative DNA damage. *Mutat. Res. Fundam. Mol. Mech. Mutagen.* **531**, 231–251, <https://doi.org/10.1016/j.mrfmmm.2003.06.002>
- 41 Kabashi, E., Valdmanis, P.N., Dion, P. and Rouleau, G.A. (2007) Oxidized/misfolded superoxide dismutase-1: the cause of all amyotrophic lateral sclerosis? *Ann. Neurol.* **62**, 553–559, <https://doi.org/10.1002/ana.21319>
- 42 Shiloh, Y. and Ziv, Y. (2013) The ATM protein kinase: regulating the cellular response to genotoxic stress, and more. *Nat. Rev. Mol. Cell Biol.* **14**, 197–210, <https://doi.org/10.1038/nrm3546>
- 43 Tsang, C.K., Liu, Y., Thomas, J., Zhang, Y. and Zheng, X.F.S. (2014) Superoxide dismutase 1 acts as a nuclear transcription factor to regulate oxidative stress resistance. *Nat. Commun.* **5**, 3446, <https://doi.org/10.1038/ncomms4446>
- 44 Wang, X.-D., Zhu, M.-W., Shan, D., Wang, S.-Y., Yin, X., Yang, Y.-Q. et al. (2019) Spy1, a unique cell cycle regulator, alters viability in ALS motor neurons and cell lines in response to mutant SOD1-induced DNA damage. *DNA Repair (Amst.)* **74**, 51–62, <https://doi.org/10.1016/j.dnarep.2018.12.005>
- 45 Porter, L.A., Kong-Beltran, M. and Donoghue, D.J. (2003) Spy1 Interacts with p27 Kip1 to Allow G 1 /S Progression. *Mol. Biol. Cell* **14**, 3664–3674, <https://doi.org/10.1091/mbc.e02-12-0820>
- 46 Ye, W. and Blain, S.W. (2010) S phase entry causes homocysteine-induced death while ataxia telangiectasia and Rad3 related protein functions anti-apoptotically to protect neurons. *Brain J. Neurol.* **133**, 2295–2312, <https://doi.org/10.1093/brain/awq139>
- 47 Kruman, I.I., Wersto, R.P., Cardozo-Pelaez, F., Smilenov, L., Chan, S.L., Chrest, F.J. et al. (2004) Cell Cycle Activation Linked to Neuronal Cell Death Initiated by DNA Damage. *Neuron* **41**, 549–561, [https://doi.org/10.1016/S0896-6273\(04\)00017-0](https://doi.org/10.1016/S0896-6273(04)00017-0)
- 48 Fang, X., Lin, H., Wang, X., Zuo, Q., Qin, J. and Zhang, P. (2015) The NEK1 interactor, C21ORF2, is required for efficient DNA damage repair. *Acta Bioch. Bioph. Sin.* **47**, 834–841, <https://doi.org/10.1093/abbs/gmv076>
- 49 Pelegri, A.L., Moura, D.J., Brenner, B.L., Ledur, P.F., Maques, G.P., Henriques, J.A.P. et al. (2010) Nek1 silencing slows down DNA repair and blocks DNA damage-induced cell cycle arrest. *Mutagenesis* **25**, 447–454, <https://doi.org/10.1093/mutage/geq026>
- 50 Wang, Z., Liu, P., Inuzuka, H. and Wei, W. (2014) Roles of F-box proteins in cancer. *Nat. Rev. Cancer* **14**, 233–247, <https://doi.org/10.1038/nrc3700>
- 51 Bai, C., Richman, R. and Elledge, S.J. (1994) Human cyclin F. *EMBO J.* **13**, 6087–6098, <https://doi.org/10.1002/j.1460-2075.1994.tb06955.x>
- 52 Chen-Plotkin, A.S., Lee, V.M.-Y. and Trojanowski, J.Q. (2010) TAR DNA-binding protein 43 in neurodegenerative disease. *Nat. Rev. Neurol.* **6**, 211–220, <https://doi.org/10.1038/nrneuro.2010.18>
- 53 Winton, M.J., Igaz, L.M., Wong, M.M., Kwong, L.K., Trojanowski, J.Q. and Lee, V.M.-Y. (2008) Disturbance of Nuclear and Cytoplasmic TAR DNA-binding Protein (TDP-43) Induces Disease-like Redistribution, Sequestration, and Aggregate Formation. *J. Biol. Chem.* **283**, 13302–13309, <https://doi.org/10.1074/jbc.M800342200>
- 54 Igaz, L.M., Kwong, L.K., Xu, Y., Truax, A.C., Uryu, K., Neumann, M. et al. (2008) Enrichment of C-Terminal Fragments in TAR DNA-Binding Protein-43 Cytoplasmic Inclusions in Brain but not in Spinal Cord of Frontotemporal Lobar Degeneration and Amyotrophic Lateral Sclerosis. *Am. J. Pathol.* **173**, 182–194, <https://doi.org/10.2353/ajpath.2008.080003>
- 55 Lagier-Tourenne, C. and Cleveland, D.W. (2009) Rethinking ALS: the FUS about TDP-43. *Cell* **136**, 1001–1004, <https://doi.org/10.1016/j.cell.2009.03.006>
- 56 Smethurst, P., Sidle, K.C.L. and Hardy, J. (2015) Review: Prion-like mechanisms of transactive response DNA binding protein of 43 kDa (TDP-43) in amyotrophic lateral sclerosis (ALS). *Neuropath. Appl. Neurol.* **41**, 578–597, <https://doi.org/10.1111/nan.12206>
- 57 Hergesheimer, R.C., Chami, A.A., de Assis, D.R., Vourc'h, P., Andres, C.R., Corcia, P. et al. (2019) The debated toxic role of aggregated TDP-43 in amyotrophic lateral sclerosis: a resolution in sight? *Brain* **142**, 1176–1194, <https://doi.org/10.1093/brain/awz078>
- 58 Masters, C.L., Simms, G., Weinman, N.A., Multhaup, G., McDonald, B.L. and Beyreuther, K. (1985) Amyloid plaque core protein in Alzheimer disease and Down syndrome. *Proc. Natl. Acad. Sci.* **82**, 4245–4249, <https://doi.org/10.1073/pnas.82.12.4245>
- 59 Spillantini, M.G., Schmidt, M.L., Lee, V.M.-Y., Trojanowski, J.Q., Jakes, R. and Goedert, M. (1997)  $\alpha$ -Synuclein in Lewy bodies. *Nature* **388**, 839–840, <https://doi.org/10.1038/42166>
- 60 Davies, S.W., Turmaine, M., Cozens, B.A., DiFiglia, M., Sharp, A.H., Ross, C.A. et al. (1997) Formation of Neuronal Intranuclear Inclusions Underlies the Neurological Dysfunction in Mice Transgenic for the HD Mutation. *Cell* **90**, 537–548, [https://doi.org/10.1016/S0092-8674\(00\)80513-9](https://doi.org/10.1016/S0092-8674(00)80513-9)
- 61 DiFiglia, M., Sapp, E., Chase, K.O., Davies, S.W., Bates, G.P., Vonsattel, J.P. et al. (1997) Aggregation of Huntingtin in Neuronal Intranuclear Inclusions and Dystrophic Neurites in Brain. *Science* **277**, 1990–1993, <https://doi.org/10.1126/science.277.5334.1990>
- 62 Wang, P., Deng, J., Dong, J., Liu, J., Bigio, E.H., Mesulam, M. et al. (2019) TDP-43 induces mitochondrial damage and activates the mitochondrial unfolded protein response. *PLoS Genet.* **15**, e1007947, <https://doi.org/10.1371/journal.pgen.1007947>
- 63 Guerrero, E.N., Mitra, J., Wang, H., Rangaswamy, S., Hegde, P.M., Basu, P. et al. (2019) Amyotrophic lateral sclerosis-associated TDP-43 mutation Q331K prevents nuclear translocation of XRCC4-DNA ligase 4 complex and is linked to genome damage-mediated neuronal apoptosis. *Hum. Mol. Genet.* **28**, 2459–2476, <https://doi.org/10.1093/hmg/ddz062>
- 64 Wang, W., Wang, L., Lu, J., Siedlak, S.L., Fujioka, H., Liang, J. et al. (2016) The inhibition of TDP-43 mitochondrial localization blocks its neuronal toxicity. *Nat. Med.* **22**, 869–878, <https://doi.org/10.1038/nm.4130>
- 65 Abraham, K.J., Chan, J.N.Y., Salvi, J.S., Ho, B., Hall, A., Vidya, E. et al. (2016) Intersection of calorie restriction and magnesium in the suppression of genome-destabilizing RNA-DNA hybrids. *Nucleic Acids Res.* **44**, 8870–8884, <https://doi.org/10.1093/nar/gkw752>
- 66 Elden, A.C., Kim, H.-J., Hart, M.P., Chen-Plotkin, A.S., Johnson, B.S., Fang, X. et al. (2010) Ataxin-2 intermediate-length polyglutamine expansions are associated with increased risk for ALS. *Nature* **466**, 1069–1075, <https://doi.org/10.1038/nature09320>
- 67 Gu, J., Hu, W., Tan, X., Qu, S., Chu, D., Gong, C.-X. et al. (2019) Elevation of casein kinase 1 $\epsilon$  associated with TDP-43 and tau pathologies in Alzheimer's disease. *Brain Pathol. Zurich Switz* **30**, 283–297, <https://doi.org/10.1111/bpa.12775>
- 68 Knippschild, U., Krüger, M., Richter, J., Xu, P., García-Reyes, B., Peifer, C. et al. (2014) The CK1 Family: Contribution to Cellular Stress Response and Its Role in Carcinogenesis. *Front. Oncol.* **4**, 96, <https://doi.org/10.3389/fonc.2014.00096>

- 69 van den Boom, J., Wolf, M., Weimann, L., Schulze, N., Li, F., Kaschani, F. et al. (2016) VCP/p97 Extracts Sterically Trapped Ku70/80 Rings from DNA in Double-Strand Break Repair. *Mol. Cell* **64**, 189–198, <https://doi.org/10.1016/j.molcel.2016.08.037>
- 70 Hewitt, G., Carroll, B., Sarallah, R., Correia-Melo, C., Ogrodnik, M., Nelson, G. et al. (2016) SQSTM1/p62 mediates crosstalk between autophagy and the UPS in DNA repair. *Autophagy* **12**, 1917–1930, <https://doi.org/10.1080/15548627.2016.1210368>
- 71 Singh, A.N., Oehler, J., Torrecilla, I., Kilgas, S., Li, S., Vaz, B. et al. (2019) The p97-Ataxin 3 complex regulates homeostasis of the DNA damage response E3 ubiquitin ligase RNF 8. *EMBO J.* **38**, e102361, <https://doi.org/10.15252/embj.2019102361>
- 72 Balmus, G., Barros, A.C., Wijnhoven, P.W.G., Lescale, C., Hasse, H.L., Borowiak, K. et al. (2016) Synthetic lethality between PAXX and XLF in mammalian development. *Gene. Dev.* **30**, 2152–2157, <https://doi.org/10.1101/gad.290510.116>
- 73 Dev, H., Chiang, T.-W.W., Lescale, C., de Krijger, I., Martin, A.G., Pilger, D. et al. (2018) Shieldin complex promotes DNA end-joining and counters homologous recombination in BRCA1-null cells. *Nat. Cell Biol.* **20**, 954–965, <https://doi.org/10.1038/s41556-018-0140-1>
- 74 Hill, S.J., Mordes, D.A., Cameron, L.A., Neuberger, D.S., Landini, S., Eggen, K. et al. (2016) Two familial ALS proteins function in prevention/repair of transcription-associated DNA damage. *Proc. Natl. Acad. Sci.* **113**, E7701–E7709, <https://doi.org/10.1073/pnas.1611673113>
- 75 Ratti, A. and Buratti, E. (2016) Physiological functions and pathobiology of TDP-43 and FUS/TLS proteins. *J. Neurochem.* **138**, 95–111, <https://doi.org/10.1111/jnc.13625>
- 76 Guerrero, E.N., Wang, H., Mitra, J., Hegde, P.M., Stowell, S.E., Liachko, N.F. et al. (2016) TDP-43/FUS in motor neuron disease: Complexity and challenges. *Prog. Neurobiol.* **145–146**, 78–97, <https://doi.org/10.1016/j.pneurobio.2016.09.004>
- 77 Crozat, A., Åman, P., Mandahl, N. and Ron, D. (1993) Fusion of CHOP to a novel RNA-binding protein in human myxoid liposarcoma. *Nature* **363**, 640–644, <https://doi.org/10.1038/363640a0>
- 78 Rabbitts, T.H., Forster, A., Larson, R. and Nathan, P. (1993) Fusion of the dominant negative transcription regulator CHOP with a novel gene FUS by translocation t(12;16) in malignant liposarcoma. *Nat. Genet.* **4**, 175–180, <https://doi.org/10.1038/ng0693-175>
- 79 Vance, C., Rogelj, B., Hortobagyi, T., Vos, K.J.D., Nishimura, A.L., Sreedharan, J. et al. (2009) Mutations in FUS, an RNA Processing Protein, Cause Familial Amyotrophic Lateral Sclerosis Type 6. *Science* **323**, 1208–1211, <https://doi.org/10.1126/science.1165942>
- 80 Patel, A., Lee, H.O., Jawerth, L., Maharana, S., Jahnel, M., Hein, M.Y. et al. (2015) A Liquid-to-Solid Phase Transition of the ALS Protein FUS Accelerated by Disease Mutation. *Cell* **162**, 1066–1077, <https://doi.org/10.1016/j.cell.2015.07.047>
- 81 Mastrocola, A.S., Kim, S.H., Trinh, A.T., Rodenkirch, L.A. and Tibbetts, R.S. (2013) The RNA-binding protein fused in sarcoma (FUS) functions downstream of poly(ADP-ribose) polymerase (PARP) in response to DNA damage. *J. Biol. Chem.* **288**, 24731–24741, <https://doi.org/10.1074/jbc.M113.497974>
- 82 Wang, H., Rangaswamy, S., Kodavati, M., Mitra, J., Guo, W., Guerrero, E.N. et al. (2019) RT2 PCR array screening reveals distinct perturbations in DNA damage response signaling in FUS-associated motor neuron disease. *Mol. Brain* **12**, 103, <https://doi.org/10.1186/s13041-019-0526-4>
- 83 Wang, H., Guo, W., Mitra, J., Hegde, P.M., Vandoorne, T., Eckelmann, B.J. et al. (2018) Mutant FUS causes DNA ligation defects to inhibit oxidative damage repair in Amyotrophic Lateral Sclerosis. *Nat. Commun.* **9**, 3683, <https://doi.org/10.1038/s41467-018-06111-6>
- 84 Naumann, M., Pal, A., Goswami, A., Lojewski, X., Japtok, J., Vehlow, A. et al. (2018) Impaired DNA damage response signaling by FUS-NLS mutations leads to neurodegeneration and FUS aggregate formation. *Nat. Commun.* **9**, 335, <https://doi.org/10.1038/s41467-017-02299-1>
- 85 Miller, K.M., Tjeertes, J.V., Coates, J., Legube, G., Polo, S.E., Britton, S. et al. (2010) Human HDAC1 and HDAC2 function in the DNA-damage response to promote DNA nonhomologous end-joining. *Nat. Struct. Mol. Biol.* **17**, 1144–1151, <https://doi.org/10.1038/nsmb.1899>
- 86 Gong, J., Huang, M., Wang, F., Ma, X., Liu, H., Tu, Y. et al. (2017) RBM45 competes with HDAC1 for binding to FUS in response to DNA damage. *Nucleic Acids Res.* **45**, 12862–12876, <https://doi.org/10.1093/nar/gkx1102>
- 87 Kuuluvainen, L., Kaijola, K., Mönkäre, S., Laaksovirta, H., Jokela, M., Udd, B. et al. (2019) Oligogenic basis of sporadic ALS: The example of SOD1 p.Ala90Val mutation. *Neurol. Genet.* **5**, e335, <https://doi.org/10.1212/NXG.0000000000000335>
- 88 Paulson, H. (2018) Handbook of Clinical Neurology. *Handb. Clin. Neurol.* **147**, 105–123, <https://doi.org/10.1016/B978-0-444-63233-3.00009-9>
- 89 Zhao, X.-N. and Usdin, K. (2015) The Repeat Expansion Diseases: The dark side of DNA repair. *DNA Repair (Amst.)* **32**, 96–105, <https://doi.org/10.1016/j.dnarep.2015.04.019>
- 90 Mossevelde, S.V., van der Zee, J., Cruets, M. and Broeckhoven, C.V. (2017) Relationship between C9orf72 repeat size and clinical phenotype. *Curr. Opin. Genet. Dev.* **44**, 117–124, <https://doi.org/10.1016/j.gde.2017.02.008>
- 91 Haeusler, A.R., Donnelly, C.J., Periz, G., Simko, E.A.J., Shaw, P.G., Kim, M.-S. et al. (2014) C9orf72 nucleotide repeat structures initiate molecular cascades of disease. *Nature* **507**, 195–200, <https://doi.org/10.1038/nature13124>
- 92 Reddy, K., Schmidt, M.H.M., Geist, J.M., Thakkar, N.P., Panigrahi, G.B., Wang, Y.-H. et al. (2014) Processing of double-R-loops in (CAG)·(CTG) and C9orf72 (GGGGCC)·(GGCCCC) repeats causes instability. *Nucleic Acids Res.* **42**, 10473–10487, <https://doi.org/10.1093/nar/gku658>
- 93 Fratta, P., Mizielińska, S., Nicoll, A.J., Zloh, M., Fisher, E.M.C., Parkinson, G. et al. (2012) C9orf72 hexanucleotide repeat associated with amyotrophic lateral sclerosis and frontotemporal dementia forms RNA G-quadruplexes. *Sci. Rep.-U.K.* **2**, 1016, <https://doi.org/10.1038/srep01016>
- 94 Reddy, K., Zamiri, B., Stanley, S.Y.R., Macgregor, R.B. and Pearson, C.E. (2013) The disease-associated r(GGGGCC)<sub>n</sub> repeat from the C9orf72 gene forms tract length-dependent uni- and multimolecular RNA G-quadruplex structures. *J. Biol. Chem.* **288**, 9860–9866, <https://doi.org/10.1074/jbc.C113.452532>
- 95 Bennett, C.L., Dastidar, S.G., Ling, S.-C., Malik, B., Ashe, T., Wadhwa, M. et al. (2018) Senataxin mutations elicit motor neuron degeneration phenotypes and yield TDP-43 mislocalization in ALS4 mice and human patients. *Acta Neuropathol.* **136**, 425–443, <https://doi.org/10.1007/s00401-018-1852-9>
- 96 Taylor, J.P., Brown, R.H. and Cleveland, D.W. (2016) Decoding ALS: from genes to mechanism. *Nature* **539**, 197–206, <https://doi.org/10.1038/nature20413>
- 97 Farg, M.A., Konopka, A., Soo, K.Y., Ito, D. and Atkin, J.D. (2017) The DNA damage response (DDR) is induced by the C9orf72 repeat expansion in Amyotrophic Lateral Sclerosis. *Hum. Mol. Genet.* **26**, 2882–2896, <https://doi.org/10.1093/hmg/ddx170>

- 98 Almeida, S., Gascon, E., Tran, H., Chou, H.J., Gendron, T.F., Degroot, S. et al. (2013) Modeling key pathological features of frontotemporal dementia with C9ORF72 repeat expansion in iPSC-derived human neurons. *Acta Neuropathol.* **126**, 385–399, <https://doi.org/10.1007/s00401-013-1149-y>
- 99 Donnelly, C.J., Zhang, P.-W., Pham, J.T., Haeusler, A.R., Heusler, A.R., Mistry, N.A. et al. (2013) RNA toxicity from the ALS/FTD C9ORF72 expansion is mitigated by antisense intervention. *Neuron* **80**, 415–428, <https://doi.org/10.1016/j.neuron.2013.10.015>
- 100 Lagier-Tourenne, C., Baughn, M., Rigo, F., Sun, S., Liu, P., Li, H.-R. et al. (2013) Targeted degradation of sense and antisense C9orf72 RNA foci as therapy for ALS and frontotemporal degeneration. *Proc. Natl. Acad. Sci. U.S.A.* **110**, E4530–E4539, <https://doi.org/10.1073/pnas.1318835110>
- 101 Lee, Y.-B., Chen, H.-J., Peres, J.N., Gomez-Deza, J., Attig, J., Stalekar, M. et al. (2013) Hexanucleotide repeats in ALS/FTD form length-dependent RNA foci, sequester RNA binding proteins, and are neurotoxic. *Cell Rep.* **5**, 1178–1186, <https://doi.org/10.1016/j.celrep.2013.10.049>
- 102 Mizielinska, S., Lashley, T., Norona, F.E., Clayton, E.L., Ridler, C.E., Fratta, P. et al. (2013) C9orf72 frontotemporal lobar degeneration is characterised by frequent neuronal sense and antisense RNA foci. *Acta Neuropathol.* **126**, 845–857, <https://doi.org/10.1007/s00401-013-1200-z>
- 103 Cooper-Knock, J., Walsh, M.J., Higginbottom, A., Highley, J.R., Dickman, M.J., Edbauer, D. et al. (2014) Sequestration of multiple RNA recognition motif-containing proteins by C9orf72 repeat expansions. *Brain* **137**, 2040–2051, <https://doi.org/10.1093/brain/awu120>
- 104 Conlon, E.G., Lu, L., Sharma, A., Yamazaki, T., Tang, T., Shneider, N.A. et al. (2016) The C9ORF72 GGGGCC expansion forms RNA G-quadruplex inclusions and sequesters hnRNP H to disrupt splicing in ALS brains. *Elife* **5**, e17820, <https://doi.org/10.7554/eLife.17820>
- 105 Conlon, E.G., Fagegaltier, D., Agius, P., Davis-Porada, J., Gregory, J., Hubbard, I. et al. (2018) Unexpected similarities between C9ORF72 and sporadic forms of ALS/FTD suggest a common disease mechanism. *Elife* **7**, e37754, <https://doi.org/10.7554/eLife.37754>
- 106 Ash, P.E.A., Bieniek, K.F., Gendron, T.F., Caulfield, T., Lin, W.-L., DeJesus-Hernandez, M. et al. (2013) Unconventional translation of C9ORF72 GGGGCC expansion generates insoluble polypeptides specific to c9FTD/ALS. *Neuron* **77**, 639–646, <https://doi.org/10.1016/j.neuron.2013.02.004>
- 107 Gendron, T.F., Bieniek, K.F., Zhang, Y.-J., Jansen-West, K., Ash, P.E.A., Caulfield, T. et al. (2013) Antisense transcripts of the expanded C9ORF72 hexanucleotide repeat form nuclear RNA foci and undergo repeat-associated non-ATG translation in c9FTD/ALS. *Acta Neuropathol.* **126**, 829–844, <https://doi.org/10.1007/s00401-013-1192-8>
- 108 Mori, K., Weng, S.-M., Arzberger, T., May, S., Rentzsch, K., Kremmer, E. et al. (2013) The C9orf72 GGGGCC Repeat Is Translated into Aggregating Dipeptide-Repeat Proteins in FTD/ALS. *Science* **339**, 1335–1338, <https://doi.org/10.1126/science.1232927>
- 109 Zu, T., Liu, Y., Banez-Coronel, M., Reid, T., Pletnikova, O., Lewis, J. et al. (2013) RAN proteins and RNA foci from antisense transcripts in C9ORF72 ALS and frontotemporal dementia. *Proc. Natl. Acad. Sci.* **110**, E4968–E4977, <https://doi.org/10.1073/pnas.1315438110>
- 110 Lopez-Gonzalez, R., Lu, Y., Gendron, T.F., Karydas, A., Tran, H., Yang, D. et al. (2016) Poly(GR) in C9ORF72-Related ALS/FTD Compromises Mitochondrial Function and Increases Oxidative Stress and DNA Damage in iPSC-Derived Motor Neurons. *Neuron* **92**, 383–391, <https://doi.org/10.1016/j.neuron.2016.09.015>
- 111 Solomon, D.A., Stepto, A., Au, W.H., Adachi, Y., Diaper, D.C., Hall, R. et al. (2018) A feedback loop between dipeptide-repeat protein, TDP-43 and karyopherin- $\alpha$  mediates C9orf72-related neurodegeneration. *Brain* **141**, 2908–2924, <https://doi.org/10.1093/brain/awy241>
- 112 Nihei, Y., Mori, K., Werner, G., Arzberger, T., Degeneration GC for FL, Alliance BBB et al. (2019) Poly-glycine-alanine exacerbates C9orf72 repeat expansion-mediated DNA damage via sequestration of phosphorylated ATM and loss of nuclear hnRNPA3. *Acta Neuropathol.* **139**, 99–118, <https://doi.org/10.1007/s00401-019-02082-0>
- 113 Freibaum, B.D., Lu, Y., Lopez-Gonzalez, R., Kim, N.C., Almeida, S., Lee, K.-H. et al. (2015) GGGGCC repeat expansion in C9orf72 compromises nucleocytoplasmic transport. *Nature* **525**, 129–133, <https://doi.org/10.1038/nature14974>
- 114 Jovičić, A., Mertens, J., Boeynaems, S., Bogaert, E., Chai, N., Yamada, S.B. et al. (2015) Modifiers of C9orf72 dipeptide repeat toxicity connect nucleocytoplasmic transport defects to FTD/ALS. *Nat. Neurosci.* **18**, 1226–1229, <https://doi.org/10.1038/nn.4085>
- 115 Zhang, C.-Z., Spektor, A., Cornils, H., Francis, J.M., Jackson, E.K., Liu, S. et al. (2015) Chromothripsis from DNA damage in micronuclei. *Nature* **522**, 179–184, <https://doi.org/10.1038/nature14493>
- 116 Zhang, Y.-J., Guo, L., Gonzales, P.K., Gendron, T.F., Wu, Y., Jansen-West, K. et al. (2019) Heterochromatin anomalies and double-stranded RNA accumulation underlie C9orf72 poly(PR) toxicity. *Science* **363**, eaav2606, <https://doi.org/10.1126/science.aav2606>
- 117 Boivin, M., Pfister, V., Gaucherot, A., Ruffenach, F., Negroni, L., Sellier, C. et al. (2020) Reduced autophagy upon C9ORF72 loss synergizes with dipeptide repeat protein toxicity in G4C2 repeat expansion disorders. *EMBO J.* **39**, e100574, <https://doi.org/10.15252/embj.2018100574>
- 118 Walker, C., Herranz-Martin, S., Karyka, E., Liao, C., Lewis, K., Elsayed, W. et al. (2017) C9orf72 expansion disrupts ATM-mediated chromosomal break repair. *Nat. Neurosci.* **20**, 1225–1235, <https://doi.org/10.1038/nn.4604>
- 119 Lopez-Gonzalez, R., Yang, D., Pribadi, M., Kim, T.S., Krishnan, G., Choi, S.Y. et al. (2019) Partial inhibition of the overactivated Ku80-dependent DNA repair pathway rescues neurodegeneration in C9ORF72 -ALS/FTD. *Proc. Natl. Acad. Sci.* **116**, 9628–9633, <https://doi.org/10.1073/pnas.1901313116>
- 120 Balmus, G., Pilger, D., Coates, J., Demir, M., Sczaniecka-Clift, M., Barros, A.C. et al. (2019) ATM orchestrates the DNA-damage response to counter toxic non-homologous end-joining at broken replication forks. *Nat. Commun.* **10**, 87, <https://doi.org/10.1038/s41467-018-07729-2>
- 121 Kramer, N.J., Haney, M.S., Morgens, D.W., Jovičić, A., Couthouis, J., Li, A. et al. (2018) CRISPR-Cas9 screens in human cells and primary neurons identify modifiers of C9ORF72 dipeptide-repeat-protein toxicity. *Nat. Genet.* **50**, 603–612, <https://doi.org/10.1038/s41588-018-0070-7>
- 122 Dinant, C. and Luijsterburg, M.S. (2009) The Emerging Role of HP1 in the DNA Damage Response. *Mol. Cell. Biol.* **29**, 6335–6340, <https://doi.org/10.1128/MCB.01048-09>
- 123 Sun, Y., Jiang, X., Xu, Y., Ayrapetov, M.K., Moreau, L.A., Whetstone, J.R. et al. (2009) Histone H3 methylation links DNA damage detection to activation of the tumour suppressor Tip60. *Nat. Cell Biol.* **11**, 1376–1382, <https://doi.org/10.1038/ncb1982>
- 124 Earle, A.J., Kirby, T.J., Fedorchak, G.R., Isermann, P., Patel, J., Iruvanti, S. et al. (2019) Mutant lamins cause nuclear envelope rupture and DNA damage in skeletal muscle cells. *Nat. Mater.* **19**, 464–473, <https://doi.org/10.1038/s41563-019-0563-5>
- 125 Frost, B., Bardai, F.H. and Feany, M.B. (2016) Lamin Dysfunction Mediates Neurodegeneration in Tauopathies. *Curr. Biol.* **26**, 129–136, <https://doi.org/10.1016/j.cub.2015.11.039>

- 126 Zhong, Y., Wang, J., Henderson, M.J., Yang, P., Hagen, B.M., Siddique, T. et al. (2017) Nuclear export of misfolded SOD1 mediated by a normally buried NES-like sequence reduces proteotoxicity in the nucleus. *ELife* e23759, <https://doi.org/10.7554/eLife.23759>
- 127 D'Angelo, M.A., Raices, M., Panowski, S.H. and Hetzer, M.W. (2009) Age-Dependent Deterioration of Nuclear Pore Complexes Causes a Loss of Nuclear Integrity in Postmitotic Cells. *Cell* **136**, 284–295, <https://doi.org/10.1016/j.cell.2008.11.037>
- 128 Bukata, L., Parker, S.L. and D'Angelo, M.A. (2013) Nuclear pore complexes in the maintenance of genome integrity. *Curr. Opin. Cell Biol.* **25**, 378–386, <https://doi.org/10.1016/j.ccb.2013.03.002>
- 129 Hauer, M.H. and Gasser, S.M. (2017) Chromatin and nucleosome dynamics in DNA damage and repair. *Gene. Dev.* **31**, 2204–2221, <https://doi.org/10.1101/gad.307702.117>
- 130 Phillips, T. and Robberecht, W. (2011) Neuroinflammation in amyotrophic lateral sclerosis: role of glial activation in motor neuron disease. *Lancet Neurol.* **10**, 253–263, [https://doi.org/10.1016/S1474-4422\(11\)70015-1](https://doi.org/10.1016/S1474-4422(11)70015-1)
- 131 Hanisch, U.-K. and Kettenmann, H. (2007) Microglia: active sensor and versatile effector cells in the normal and pathologic brain. *Nat. Neurosci.* **10**, 1387–1394, <https://doi.org/10.1038/nn1997>
- 132 Mantovani, A., Sica, A., Sozzani, S., Allavena, P., Vecchi, A. and Locati, M. (2004) The chemokine system in diverse forms of macrophage activation and polarization. *Trends Immunol.* **25**, 677–686, <https://doi.org/10.1016/j.it.2004.09.015>
- 133 Sun, L., Wu, J., Du, F., Chen, X. and Chen, Z.J. (2012) Cyclic GMP-AMP Synthase Is a Cytosolic DNA Sensor That Activates the Type I Interferon Pathway. *Science* **339**, 786–791, <https://doi.org/10.1126/science.1232458>
- 134 Li, T. and Chen, Z.J. (2018) The cGAS-cGAMP-STING pathway connects DNA damage to inflammation, senescence, and cancer. *J. Exp. Med.* **215**, 1287–1299, <https://doi.org/10.1084/jem.20180139>
- 135 Tanaka, Y. and Chen, Z.J. (2012) STING specifies IRF3 phosphorylation by TBK1 in the cytosolic DNA signaling pathway. *Sci. Signal.* **5**, ra20, <https://doi.org/10.1126/scisignal.2002521>
- 136 Pottier, C., Bieniek, K.F., Finch, N., van de Vorst, M., Baker, M., Perkersen, R. et al. (2015) Whole-genome sequencing reveals important role for TBK1 and OPTN mutations in frontotemporal lobar degeneration without motor neuron disease. *Acta Neuropathol.* **130**, 77–92, <https://doi.org/10.1007/s00401-015-1436-x>
- 137 Freischmidt, A., Wieland, T., Richter, B., Ruf, W., Schaeffer, V., Müller, K. et al. (2015) Haploinsufficiency of TBK1 causes familial ALS and fronto-temporal dementia. *Nat. Neurosci.* **18**, 631–636, <https://doi.org/10.1038/nn.4000>
- 138 Basit, A., Cho, M.-G., Kim, E.-Y., Kwon, D., Kang, S.-J. and Lee, J.-H. (2020) The cGAS/STING/TBK1/IRF3 innate immunity pathway maintains chromosomal stability through regulation of p21 levels. *Exp. Mol. Med.* **52**, 643–657, <https://doi.org/10.1038/s12276-020-0416-y>
- 139 Krug, L., Chatterjee, N., Borges-Monroy, R., Hearn, S., Liao, W.-W., Morrill, K. et al. (2017) Retrotransposon activation contributes to neurodegeneration in a Drosophila TDP-43 model of ALS. *PLoS Genet.* **13**, e1006635, <https://doi.org/10.1371/journal.pgen.1006635>
- 140 Chang, Y.-H. and Dubnau, J. (2019) The Gypsy Endogenous Retrovirus Drives Non-Cell-Autonomous Propagation in a Drosophila TDP-43 Model of Neurodegeneration. *Curr. Biol. Cb* **29**, 3135.e4–3152.e4, <https://doi.org/10.1016/j.cub.2019.07.071>
- 141 Wallace, N.A., Belancio, V.P. and Deininger, P.L. (2008) L1 mobile element expression causes multiple types of toxicity. *Gene* **419**, 75–81, <https://doi.org/10.1016/j.gene.2008.04.013>
- 142 Yamanaka, K. and Komine, O. (2018) The multi-dimensional roles of astrocytes in ALS. *Neurosci. Res.* **126**, 31–38, <https://doi.org/10.1016/j.neures.2017.09.011>
- 143 Rostalski, H., Leskelä, S., Huber, N., Katisko, K., Cajanus, A., Solje, E. et al. (2019) Astrocytes and Microglia as Potential Contributors to the Pathogenesis of C9orf72 Repeat Expansion-Associated FTL and ALS. *Front. Neurosci. -Switz* **13**, 486, <https://doi.org/10.3389/fnins.2019.00486>
- 144 Pehar, M., Harlan, B.A., Killoy, K.M. and Vargas, M.R. (2018) Role and Therapeutic Potential of Astrocytes in Amyotrophic Lateral Sclerosis. *Curr. Pharm. Design* **23**, <https://doi.org/10.2174/1381612823666170622095802>
- 145 Sreedharan, J., Blair, I.P., Tripathi, V.B., Hu, X., Vance, C., Rogelj, B. et al. (2008) TDP-43 Mutations in Familial and Sporadic Amyotrophic Lateral Sclerosis. *Science* **319**, 1668–1672, <https://doi.org/10.1126/science.1154584>
- 146 Kwiatkowski, T.J., Bosco, D.A., LeClerc, A.L., Tamrazian, E., Vanderburg, C.R., Russ, C. et al. (2009) Mutations in the *FUS/ALS* Gene on Chromosome 16 Cause Familial Amyotrophic Lateral Sclerosis. *Science* **323**, 1205–1208, <https://doi.org/10.1126/science.1166066>
- 147 Wang, H. and Hegde, M.L. (2019) New Mechanisms of DNA Repair Defects in Fused in Sarcoma-Associated Neurodegeneration: Stage Set for DNA Repair-Based Therapeutics? *J. Exp. Neurosci.* **13**, 1–5, <https://doi.org/10.1177/1179069519856358>
- 148 Appocher, C., Mohagheghi, F., Cappelli, S., Stuani, C., Romano, M., Feiguin, F. et al. (2017) Major hnRNP proteins act as general TDP-43 functional modifiers both in Drosophila and human neuronal cells. *Nucleic Acids Res.* **45**, gkx477, <https://doi.org/10.1093/nar/gkx477>
- 149 Gittings, L.M., Foti, S.C., Benson, B.C., Gami-Patel, P., Isaacs, A.M. and Lashley, T. (2019) Heterogeneous nuclear ribonucleoproteins R and Q accumulate in pathological inclusions in FTL-FUS. *Acta Neuropathol. Commun.* **7**, 18, <https://doi.org/10.1186/s40478-019-0673-y>
- 150 Haley, B., Paunesku, T., Protić, M. and Woloschak, G.E. (2009) Response of heterogeneous ribonuclear proteins (hnRNP) to ionising radiation and their involvement in DNA damage repair. *Int. J. Radiat. Biol.* **85**, 643–655, <https://doi.org/10.1080/09553000903009548>
- 151 Hu, W., Lei, L., Xie, X., Huang, L., Cui, Q., Dang, T. et al. (2019) Heterogeneous nuclear ribonucleoprotein L facilitates recruitment of 53BP1 and BRCA1 at the DNA break sites induced by oxaliplatin in colorectal cancer. *Cell Death. Dis.* **10**, 550, <https://doi.org/10.1038/s41419-019-1784-x>
- 152 Geuens, T., Bouhy, D. and Timmerman, V. (2016) The hnRNP family: insights into their role in health and disease. *Hum. Genet.* **135**, 851–867, <https://doi.org/10.1007/s00439-016-1683-5>
- 153 Decorsière, A., Cayrel, A., Vagner, S. and Millevoi, S. (2011) Essential role for the interaction between hnRNP H/F and a G quadruplex in maintaining p53 pre-mRNA 3'-end processing and function during DNA damage. *Gene Dev.* **25**, 220–225, <https://doi.org/10.1101/gad.607011>
- 154 Chia, R., Chiò, A. and Traynor, B.J. (2018) Novel genes associated with amyotrophic lateral sclerosis: diagnostic and clinical implications. *Lancet Neurol.* **17**, 94–102, [https://doi.org/10.1016/S1474-4422\(17\)30401-5](https://doi.org/10.1016/S1474-4422(17)30401-5)

- 155 Sui, J., Lin, Y.-F., Xu, K., Lee, K.-J., Wang, D. and Chen, B.P.C. (2015) DNA-PKcs phosphorylates hnRNP-A1 to facilitate the RPA-to-POT1 switch and telomere capping after replication. *Nucleic Acids Res.* **43**, 5971–5983, <https://doi.org/10.1093/nar/gkv539>
- 156 Deshaies, J.-E., Shkreta, L., Moszczynski, A.J., Sidibé, H., Semmler, S., Fouillen, A. et al. (2018) TDP-43 regulates the alternative splicing of hnRNP A1 to yield an aggregation-prone variant in amyotrophic lateral sclerosis. *Brain* **141**, 1320–1333, <https://doi.org/10.1093/brain/awy062>
- 157 Registry, P., Group, S., Registry, S., Consortium, F.S., Consortium, S., Group, N.S. et al. (2016) Genome-wide association analyses identify new risk variants and the genetic architecture of amyotrophic lateral sclerosis. *Nat. Genet.* **48**, 1043–1048
- 158 White, M.A., Lin, Z., Kim, E., Henstridge, C.M., Altamira, E.P., Hunt, C.K. et al. (2019) Sarm1 deletion suppresses TDP-43-linked motor neuron degeneration and cortical spine loss. *Acta Neuropathol. Commun.* **7**, 166, <https://doi.org/10.1186/s40478-019-0800-9>
- 159 Matsukawa, K., Hashimoto, T., Matsumoto, T., Ihara, R., Chihara, T., Miura, M. et al. (2016) Familial Amyotrophic Lateral Sclerosis-linked Mutations in Profilin 1 Exacerbate TDP-43-induced Degeneration in the Retina of *Drosophila melanogaster* through an Increase in the Cytoplasmic Localization of TDP-43. *J. Biol. Chem.* **291**, 23464–23476, <https://doi.org/10.1074/jbc.M116.729152>
- 160 Deng, H.-X., Chen, W., Hong, S.-T., Boycott, K.M., Gorrie, G.H., Siddique, N. et al. (2011) Mutations in UBQLN2 cause dominant X-linked juvenile and adult-onset ALS and ALS/dementia. *Nature* **477**, 211–215, <https://doi.org/10.1038/nature10353>
- 161 Renaud, L., Picher-Martel, V., Codron, P. and Julien, J.-P. (2019) Key role of UBQLN2 in pathogenesis of amyotrophic lateral sclerosis and frontotemporal dementia. *Acta Neuropathol. Commun.* **7**, 103, <https://doi.org/10.1186/s40478-019-0758-7>
- 162 Williams, K.L., Topp, S., Yang, S., Smith, B., Fifita, J.A., Warraich, S.T. et al. (2016) CCNF mutations in amyotrophic lateral sclerosis and frontotemporal dementia. *Nat. Commun.* **7**, 11253, <https://doi.org/10.1038/ncomms11253>
- 163 Takahashi, Y., Uchino, A., Shioya, A., Sano, T., Matsumoto, C., Numata-Uematsu, Y. et al. (2019) Altered immunoreactivity of ErbB4, a causative gene product for ALS19, in the spinal cord of patients with sporadic ALS. *Neuropathology* **39**, 268–278, <https://doi.org/10.1111/neup.12558>
- 164 Icli, B., Bharti, A., Pentassuglia, L., Peng, X. and Sawyer, D.B. (2012) ErbB4 localization to cardiac myocyte nuclei, and its role in myocyte DNA damage response. *Biochem. Biophys. Res. Commun.* **418**, 116–121, <https://doi.org/10.1016/j.bbrc.2011.12.144>
- 165 Gilmore-Hebert, M., Ramabhadran, R. and Stern, D.F. (2010) Interactions of ErbB4 and Kap1 Connect the Growth Factor and DNA Damage Response Pathways. *Mol. Cancer Res.* **8**, 1388–1398, <https://doi.org/10.1158/1541-7786.MCR-10-0042>
- 166 Arasada, R.R. and Carpenter, G. (2005) Secretase-dependent Tyrosine Phosphorylation of Mdm2 by the ErbB-4 Intracellular Domain Fragment. *J. Biol. Chem.* **280**, 30783–30787, <https://doi.org/10.1074/jbc.M506057200>
- 167 Fukunaga, K., Shinoda, Y. and Tagashira, H. (2015) The role of SIGMAR1 gene mutation and mitochondrial dysfunction in amyotrophic lateral sclerosis. *J. Pharmacol. Sci.* **127**, 36–41, <https://doi.org/10.1016/j.jpsh.2014.12.012>
- 168 Kaneb, H.M., Folkmann, A.W., Belzil, V.V., Jao, L.-E., Leblond, C.S., Girard, S.L. et al. (2015) Deleterious mutations in the essential mRNA metabolism factor, hGle1, in amyotrophic lateral sclerosis. *Hum. Mol. Genet.* **24**, 1363–1373, <https://doi.org/10.1093/hmg/ddu545>
- 169 Aditi, X.X.X., Glass, L., Dawson, T.R. and Wenthe, S.R. (2016) An amyotrophic lateral sclerosis-linked mutation in GLE1 alters the cellular pool of human Gle1 functional isoforms. *Adv. Biological Regul.* **62**, 25–36, <https://doi.org/10.1016/j.bior.2015.11.001>
- 170 Okamura, M., Yamanaka, Y., Shigemoto, M., Kitadani, Y., Kobayashi, Y., Kambe, T. et al. (2018) Depletion of mRNA export regulator DBP5/DDX19, GLE1 or IPPK that is a key enzyme for the production of IP6, resulting in differentially altered cytoplasmic mRNA expression and specific cell defect. *PLoS ONE* **13**, e0197165, <https://doi.org/10.1371/journal.pone.0197165>
- 171 Bordoni, M., Pansarasa, O., Dell'Orco, M., Crippa, V., Gagliardi, S., Sproviero, D. et al. (2019) Nuclear Phospho-SOD1 Protects DNA from Oxidative Stress Damage in Amyotrophic Lateral Sclerosis. *J. Clin. Med.* **8**, 729, <https://doi.org/10.3390/jcm8050729>
- 172 Kondori, N.R., Paul, P., Robbins, J.P., Liu, K., Hildyard, J.C.W., Wells, D.J. et al. (2018) Focus on the Role of D-serine and D-amino Acid Oxidase in Amyotrophic Lateral Sclerosis/Motor Neuron Disease (ALS). *Front. Mol. Biosci.* **5**, 8, <https://doi.org/10.3389/fmolb.2018.00008>
- 173 Cai, H., Lin, X., Xie, C., Laird, F.M., Lai, C., Wen, H. et al. (2005) Loss of ALS2 Function Is Insufficient to Trigger Motor Neuron Degeneration in Knock-Out Mice But Predisposes Neurons to Oxidative Stress. *J. Neurosci.* **25**, 7567–7574, <https://doi.org/10.1523/JNEUROSCI.1645-05.2005>
- 174 Gautam, M., Jara, J.H., Sekerkova, G., Yasvoina, M.V., Martina, M. and Özdinler, P.H. (2016) Absence of alsin function leads to corticospinal motor neuron vulnerability via novel disease mechanisms. *Hum. Mol. Genet.* **25**, 1074–1087, <https://doi.org/10.1093/hmg/ddv631>
- 175 Konopka, A. and Atkin, J. (2018) The Emerging Role of DNA Damage in the Pathogenesis of the C9orf72 Repeat Expansion in Amyotrophic Lateral Sclerosis. *Int. J. Mol. Sci.* **19**, 3137, <https://doi.org/10.3390/ijms19103137>
- 176 Tripolszki, K., Török, D., Goudenège, D., Farkas, K., Sulák, A., Török, N. et al. (2017) High-throughput sequencing revealed a novel SETX mutation in a Hungarian patient with amyotrophic lateral sclerosis. *Brain Behav.* **7**, e00669, <https://doi.org/10.1002/brb3.669>
- 177 Chen, Y.-Z., Bennett, C.L., Huynh, H.M., Blair, I.P., Puls, I., Irobi, J. et al. (2004) DNA/RNA Helicase Gene Mutations in a Form of Juvenile Amyotrophic Lateral Sclerosis (ALS4). *Am. J. Hum. Genet.* **74**, 1128–1135, <https://doi.org/10.1086/421054>
- 178 Sproviero, W., Shatunov, A., Stahl, D., Shoai, M., van Rheenen, W., Jones, A.R. et al. (2017) ATXN2 trinucleotide repeat length correlates with risk of ALS. *Neurobiol. Aging* **51**, 178.e1–178.e9, <https://doi.org/10.1016/j.neurobiolaging.2016.11.010>
- 179 Zhao, D.Y., Gish, G., Braunschweig, U., Li, Y., Ni, Z., Schmitges, F.W. et al. (2016) SMN and symmetric arginine dimethylation of RNA polymerase II C-terminal domain control termination. *Nature* **529**, 48–53, <https://doi.org/10.1038/nature16469>
- 180 Koppers, M., van Blitterswijk, M.M., Vlam, L., Rowicka, P.A., van Vught, P.W.J., Groen, E.J.N. et al. (2012) VCP mutations in familial and sporadic amyotrophic lateral sclerosis. *Neurobiol. Aging* **33**, 837.e7–837.e13, <https://doi.org/10.1016/j.neurobiolaging.2011.10.006>
- 181 Acs, K., Luijsterburg, M.S., Ackermann, L., Salomons, F.A., Hoppe, T. and Dantuma, N.P. (2011) The AAA-ATPase VCP/p97 promotes 53BP1 recruitment by removing L3MBTL1 from DNA double-strand breaks. *Nat. Struct. Mol. Biol.* **18**, 1345–1350, <https://doi.org/10.1038/nsmb.2188>
- 182 Ayaki, T., Ito, H., Fukushima, H., Inoue, T., Kondo, T., Ikemoto, A. et al. (2014) Immunoreactivity of valosin-containing protein in sporadic amyotrophic lateral sclerosis and in a case of its novel mutant. *Acta Neuropathol. Commun.* **2**, 172, <https://doi.org/10.1186/s40478-014-0172-0>
- 183 Higelin, J., Catanese, A., Semelink-Sedlacek, L.L., Oetzuerk, S., Lutz, A.-K., Bausinger, J. et al. (2018) NEK1 loss-of-function mutation induces DNA damage accumulation in ALS patient-derived motoneurons. *Stem Cell Res.* **30**, 150–162, <https://doi.org/10.1016/j.scr.2018.06.005>

- 184 Salton, M., Lerenthal, Y., Wang, S.-Y., Chen, D.J. and Shiloh, Y. (2010) Involvement of Matrin 3 and SFPQ/NONO in the DNA damage response. *Cell Cycle* **9**, 1568–1576, <https://doi.org/10.4161/cc.9.8.11298>
- 185 Wang, Y., Zhu, W.-G. and Zhao, Y. (2016) Autophagy substrate SQSTM1/p62 regulates chromatin ubiquitination during the DNA damage response. *Autophagy* **13**, 212–213, <https://doi.org/10.1080/15548627.2016.1245262>
- 186 Oakes, J.A., Davies, M.C. and Collins, M.O. (2017) TBK1: a new player in ALS linking autophagy and neuroinflammation. *Mol. Brain* **10**, 5, <https://doi.org/10.1186/s13041-017-0287-x>
- 187 Li, Q., Fazly, A.M., Zhou, H., Huang, S., Zhang, Z. and Stillman, B. (2009) The Elongator Complex Interacts with PCNA and Modulates Transcriptional Silencing and Sensitivity to DNA Damage Agents. *PLoS Genet.* **5**, e1000684, <https://doi.org/10.1371/journal.pgen.1000684>
- 188 Díaz-Muñoz, M.D., Kiselev, V.Y.U., Novère, N.L., Curk, T., Ule, J. and Turner, M. (2017) Tia1 dependent regulation of mRNA subcellular location and translation controls p53 expression in B cells. *Nat. Commun.* **8**, 530, <https://doi.org/10.1038/s41467-017-00454-2>