

# A Commentary on TDP-43 and DNA Damage Response in Amyotrophic Lateral Sclerosis

Joy Mitra<sup>1,2</sup> and Muralidhar L Hegde<sup>1,2,3</sup> 

<sup>1</sup>Department of Radiation Oncology, Houston Methodist Research Institute, Houston Methodist, Houston, TX, USA. <sup>2</sup>Center for Neuroregeneration, Department of Neurosurgery, Houston Methodist Neurological Institute, Houston Methodist, Houston, TX, USA. <sup>3</sup>Department of Neuroscience, Weill Cornell Medical College, New York, NY, USA.

Journal of Experimental Neuroscience  
Volume 13: 1–5  
© The Author(s) 2019  
Article reuse guidelines:  
sagepub.com/journals-permissions  
DOI: 10.1177/1179069519880166



**ABSTRACT:** Amyotrophic lateral sclerosis (ALS) is a devastating, motor neuron degenerative disease without any cure. About 95% of the ALS patients feature abnormalities in the RNA/DNA-binding protein, TDP-43, involving its nucleo-cytoplasmic mislocalization in spinal motor neurons. How TDP-43 pathology triggers neuronal apoptosis remains unclear. In a recent study, we reported for the first time that TDP-43 participates in the DNA damage response (DDR) in neurons, and its nuclear clearance in spinal motor neurons caused DNA double-strand break (DSB) repair defects in ALS. We documented that TDP-43 was a key component of the non-homologous end joining (NHEJ) pathway of DSB repair, which is likely the major pathway for repair of DSBs in post-mitotic neurons. We have also uncovered molecular insights into the role of TDP-43 in DSB repair and showed that TDP-43 acts as a scaffold in recruiting the XRCC4/DNA Ligase 4 complex at DSB damage sites and thus regulates a critical rate-limiting function in DSB repair. Significant DSB accumulation in the genomes of TDP-43-depleted, human neural stem cell-derived motor neurons as well as in ALS patient spinal cords with TDP-43 pathology, strongly supported a TDP-43 involvement in genome maintenance and toxicity-induced genome repair defects in ALS. In this commentary, we highlight our findings that have uncovered a link between TDP-43 pathology and impaired DNA repair and suggest potential possibilities for DNA repair-targeted therapies for TDP-43-ALS.

**KEYWORDS:** Amyotrophic lateral sclerosis, TDP-43, DNA double-strand break repair, non-homologous end joining, DNA damage response, neurodegeneration

**RECEIVED:** September 12, 2019. **ACCEPTED:** September 13, 2019.

**TYPE:** Commentary

**FUNDING:** The author(s) disclosed receipt of the following financial support for the research, authorship, and/or publication of this article: Research in the Hegde Laboratory was supported by grants from the National Institute of Neurological Disorders and Stroke (NINDS) of the National Institute of Health (NIH) under the award number R01NS088645, and the Houston Methodist Research Institute funds. The content is solely the responsibility of the authors and does not necessarily represent the official views of the funding agencies.

**DECLARATION OF CONFLICTING INTERESTS:** The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

**CORRESPONDING AUTHOR:** Muralidhar L Hegde, Department of Radiation Oncology, Houston Methodist Research Institute, Houston Methodist, Houston, TX 77030, USA. Email: mlhegde@houstonmethodist.org

**COMMENT ON:** Mitra J, Guerrero EN, Hegde PM, et al. Motor neuron disease-associated loss of nuclear TDP-43 is linked to DNA double-strand break repair defects [published online ahead of print February 15, 2019]. *Proc Natl Acad Sci U S A*. pii: 201818415. doi:10.1073/pnas.1818415116. <https://www.ncbi.nlm.nih.gov/pubmed/30770445>.

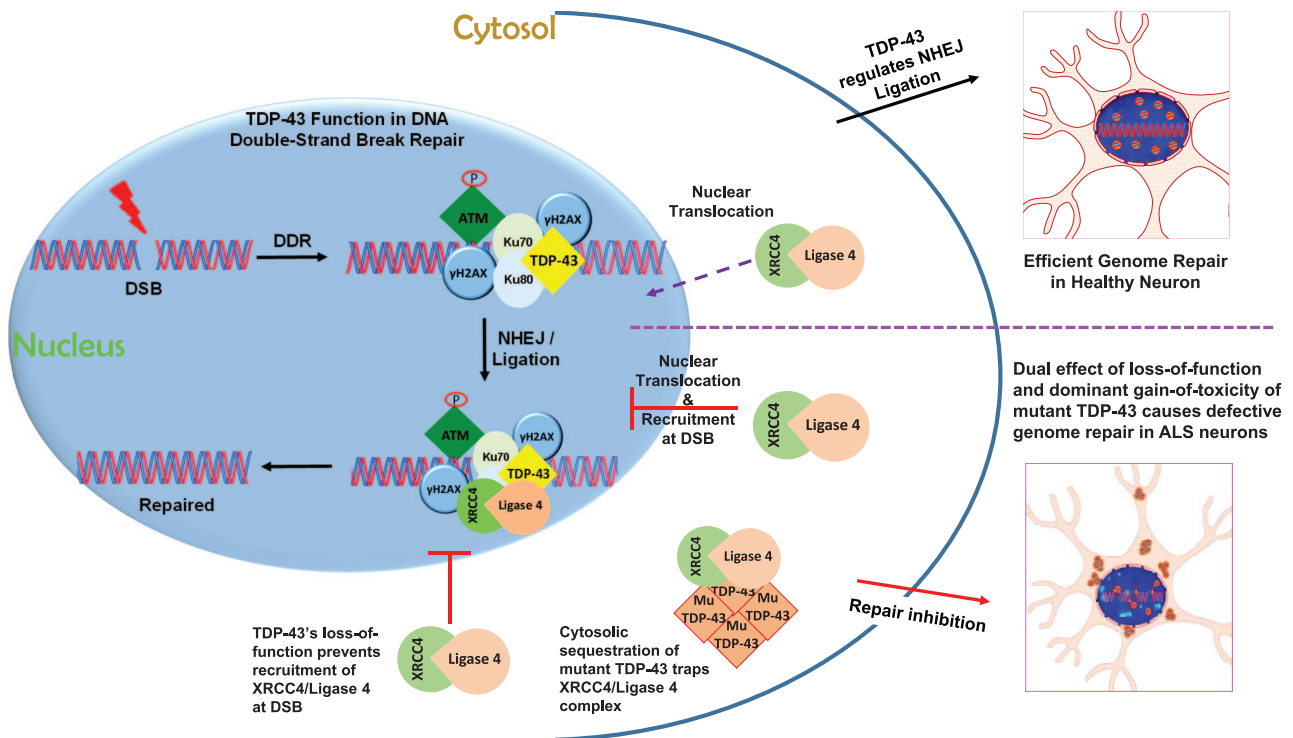
TDP-43 (TAR DNA-binding protein of 43 kDa) plays multifaceted roles in cellular functions and survival. Irrevocable nucleo-cytoplasmic translocation and aggregation are the hallmarks of TDP-43 pathology-associated neurodegenerative diseases, including amyotrophic lateral sclerosis (ALS) and frontotemporal lobar degeneration (FTD). Amyotrophic lateral sclerosis is a progressive neuro-muscular degenerative disease, with an average survival time of 3 to 5 years at diagnosis. However, a small fraction of ALS patients survive up to 20 years or longer, depending on the severity of disease progression and associated gene mutations. Amyotrophic lateral sclerosis is one of the most difficult diseases to diagnose, and no effective treatment is currently available. Complex involvement of multiple pathways and protein deregulations have hindered efforts to find a universal druggable target for the ALS group of diseases. Among various ALS subtypes, more than 90% of the cases have been linked to ALS-TDP-43, with a high degree of pathogenic overlap with other ALS-associated genes/factors in many subtypes.

TDP-43 is an RNA/DNA-binding protein of the hnRNP family. TDP-43 is predominantly a nuclear protein but shuttles between various cellular compartments/organelles using its bifurcated nuclear localization and nuclear export sequences, as well as several types of post-translational modifications. A decade of research since the discovery of the TDP-43's association

with ALS in 2006<sup>1</sup> identified important pathophysiological mechanisms, including (1) TDP-43 toxicity-mediated dysregulation of mRNA processing, small RNA biogenesis, and RNA-linked gene expression; (2) disease-linked pathogenic mutations, mostly localized in the C-terminal domain of TDP-43, induced its nucleo-cytoplasmic mislocalization/aggregation leading to activation of neuro-inflammatory and cell death mechanisms; and (3) TDP-43's association with stress granules and abnormal stress response mediated by TDP-43 toxicity. However, none of these protein- and/or RNA-linked disease mechanisms completely recapitulate human TDP-43-ALS pathology in animal models.

Emerging studies in recent years indicate that DNA strand breaks, including the most lethal form of double-strand breaks (DSBs), transiently occur as a physiological constraint or prerequisite during neural development and functioning,<sup>2</sup> particularly for regulation of neuronal gene expression, chromatin remodeling, and neuron maturation.<sup>3</sup> The evolutionary significance of such DNA break formations during normal physiological processes and whether these DNA breaks are formed as a by-product or as a prerequisite is still controversial; however, their efficient and timely repair is critically important in maintaining genomic integrity and fidelity. Unraveling of the intricate connection between genome instability and neurodegeneration in recent studies underscores the need to





**Figure 1.** Novel role of TDP-43 in non-homologous end joining (NHEJ)-mediated DNA double-strand break (DSB) repair in neuronal genome. In response to DSB formation, TDP-43 is rapidly recruited at the DSB sites together with early DNA damage response (DDR) factors, such as phospho-ATM and  $\gamma$ H2AX, and classical NHEJ repair proteins such as Ku heterodimer. TDP-43 plays a critical role in regulating the rate-limiting final step of break ligation by recruiting the XRCC4/Ligase 4 complex at DSB sites in healthy neurons. However, in ALS, TDP-43's nucleo-cytoplasmic mislocalization/aggregation traps the XRCC4/Ligase 4 in the cytosol and thus inhibiting their translocation to nucleus. Non-homologous end joining repair inhibition leads to persistent accumulation of unrepaired DSB damage and genomic instability in ALS neurons. ALS indicates amyotrophic lateral sclerosis.

examine aspects of genome repair and maintenance under neurological conditions, particularly in those associated with defects in nucleic acid binding proteins such as TDP-43.

Our recent study uncovered a critical role for TDP-43 in the non-homologous end joining (NHEJ)-mediated repair of DSBs in regulating the recruitment of classical NHEJ repair factors at break sites in response to genomic insults.<sup>4</sup> We discovered that nuclear clearance pathology of TDP-43 in spinal motor neurons causes DNA DSB repair defects in ALS. Non-homologous end joining is considered a predominant DSB repair pathway in post-mitotic neurons that lack homologous recombination (HR)-mediated repair machinery. We showed that the absence of functional TDP-43 impaired DSB ligation via the XRCC4-DNA Ligase 4 (Lig4) complex in the neuronal genome. These novel findings (schematically represented in Figure 1) highlight previously unexplored functions of TDP-43 in DNA metabolism and a key pathological mechanism for TDP-43-induced cytotoxicity in ALS neurons. In this commentary, we discuss the implications of these new findings and explore the potential of the DNA repair pathway as a therapeutic target to ameliorate genome instability in affected neurons. We also discuss the unanswered questions in this area and future perspectives.

### Etiological Linkage of TDP-43 Proteinopathy With Genome Damage Accumulation and Altered DNA Damage Response Signaling in ALS-TDP-43

A typical TDP-43 proteinopathy in ALS as well as other TDP-43-associated neurodegenerative diseases is characterized by tau-negative and ubiquitin-positive inclusions in the neuronal cytosol. The inclusion bodies contain aggregated and/or fragmented forms of TDP-43. This is accompanied by the corresponding decrease in functional TDP-43 from the neuronal nuclei. Several pathogenic mutations, mostly in the C-terminal prion-like domain of TDP-43, are linked to both sporadic and familial ALS. We recently reported a strong correlation between persistent accumulation of unrepaired DSBs and DNA damage response (DDR) activation as measured by the increased accumulation of DDR/DSBR markers such as p-53BP1 (S1778) and  $\gamma$ H2AX, and TDP-43 proteinopathy in ALS-TDP-43 patients' spinal cord tissues.<sup>4</sup> A similar linkage of TDP-43's loss of function and increased DNA strand break accumulation and altered DDR was consistently observed in multiple *in vitro* and *in vivo* ALS models, including TDP-43-depleted human-induced pluripotent stem cell (iPSC)-derived motor neurons, CRISPR/Cas9-mediated TDP-43 knockout (KO) neurons, and a *Caenorhabditis elegans* strain expressing a

functionally dead TDP-1 (ortholog of human TDP-43) mutant. This was the first report linking TDP-43 pathology with genome instability and suggested a crucial role of TDP-43 in DNA strand break repair in neurons.

### Crucial Role of TDP-43 in Classical NHEJ Pathway of DSB Repair

#### *Selective interaction with DDR and NHEJ factors*

TDP-43 was first shown to interact with NHEJ factor Ku70 by mass spectrometry analyses by Taylor and colleagues,<sup>5</sup> which led us to investigate the possible role of TDP-43 in DNA damage repair. TDP-43 exhibited several-fold increase in its *in cell* association with DDR factors  $\gamma$ H2AX, p-ATM, and p-53BP1 in response to DSB induction in multiple cell types including human iPSC-derived motor neurons. In addition, TDP-43 also interacts with the DSB repair protein-linked NHEJ pathway, including Ku, DNA-PKcs, DNA polymerase  $\lambda$ , XRCC4, and Lig4. All these interactions were enhanced on DSB damage induction, suggesting their specificity. Selective interaction of TDP-43 with the XRCC4-Lig4 complex, but not with the XRCC1/DNA Ligase 3 complex, suggested its specific role in NHEJ-mediated DSB repair. Furthermore, we observed TDP-43's preferential DNA damage-dependent association with DNA polymerase  $\lambda$  (but not with DNA polymerase  $\mu$ ), which is required for short-gap filling for XRCC4-Lig4-mediated DSB ligation.<sup>4</sup>

#### *Recruitment at DSB damage sites*

Our studies using laser microirradiation (to induce DSBs) coupled with live cell imaging, damaged DNA immunoprecipitation, and proximity ligation assays (PLA) revealed that TDP-43 is rapidly recruited at DSB sites in neuronal genome and is retained until the completion of repair. In fact, TDP-43's association/dissociation at DSB sites closely followed a key NHEJ factor Ku70. Importantly, laser ablation was performed using a 405-nm laser with 100% output, which generated primarily DSBs. Notably, laser ablation at lower output power, which induces mostly non-DSB damages, did not induce any detectable TDP-43 recruitment in the damage track. TDP-43's affinity to blunt-ended DNA oligos *in vitro* further confirmed direct binding of TDP-43 to DSBs. Altogether, the early recruitment of TDP-43 at DSB sites and its sustained presence until the repair completion suggest a vital role of TDP-43 in DSB repair/DDR signaling.

#### *Loss of TDP-43 inhibits NHEJ*

Neutral Comet analysis and DSB marker  $\gamma$ H2AX/p-53BP1 foci kinetics to monitor the disappearance of etoposide-induced DSB damage in cells with/without TDP-43 first revealed significantly reduced DSB repair capacity in neurons lacking TDP-43. Performing reproducible and consistent

downregulation of TDP-43 in neurons is a challenge, both due to transfection consistency of si/shRNAs and lethality of neurons that lack more than 60% of endogenous TDP-43. To overcome this, we generated a doxycycline-inducible TDP-43 KO system using the CRISPR/Cas9 technique in a neuronal cell line to monitor the effect of TDP-43 depletion in a timely manner. Notably, dose-dependent depletion of TDP-43 by the conditional *TARDBP* gene KO proportionally yielded DNA DSBs in the nuclear genome, even under unstressed conditions. Consistently, the neuronal viability was proportionately affected, which was further enhanced synergistically when treated with DSB-inducing drugs or irradiation.

To directly test the effect of loss of TDP-43 on NHEJ, we employed a reporter-based *in situ* NHEJ assay by transfecting the inducible TDP-43 KO cells with I-SceI NHEJ reporter plasmids. Significantly reduced green fluorescent protein (GFP) readout in TDP-43 KO cells compared with control cells confirmed that loss of TDP-43 caused DSB repair inhibition via the NHEJ pathway in the neuronal genome.

#### *Mechanistic role of TDP-43 in NHEJ: Regulation of recruitment and function of XRCC4-DNA Lig4 complex*

Given that DSB ligation is the final critical step in the NHEJ pathway to restore genomic integrity, and the selective interaction of TDP-43 with the XRCC4-Lig4 complex, we investigated whether TDP-43 plays a role in the recruitment and/or function of the NHEJ ligation complex at the break sites. Complementary experimental approaches, namely chromatin immunoprecipitation (ChIP), PLA, and co-immunoprecipitation (co-IP), firmly established the role of TDP-43 in regulating the recruitment of XRCC4 and Lig4 at DSB sites. Consistently, loss of TDP-43 inhibited the recruitment of XRCC4-Lig4 at  $\gamma$ H2AX. However, TDP-43 depletion did not affect assembly of XRCC4, XRCC4-like factor (XLF), and Lig4 in response to genome damage. These *in cellulo* results were recapitulated by *in vitro* ligation assays using XRCC4 co-IP complexes in the presence or absence of TDP-43. TDP-43 downregulation had significant effects on Lig4 activity, which was rescued by supplementing the reaction mixture with recombinant TDP-43. Furthermore, TDP-43 directly interacted with XRCC4-Lig4 in the absence of DNA *in vitro*. Interestingly, our follow-up study with ALS-associated mutant TDP-43 (Q331K) variant revealed that mutant TDP-43 mislocalized in the cytosol and trapped XRCC4, preventing its DNA damage-dependent nuclear translocation, eventually affecting Lig4 recruitment and activity.<sup>6</sup>

Currently, genetic manipulation in *C. elegans* has emerged as a useful disease model to study ALS-associated neurodegeneration. In this study with a *C. elegans* model harboring truncated TDP-1, we documented higher mortality rates at larval stages, compared with wild-type nematodes, when challenged with



irradiation. Furthermore, etoposide-treated mutant worms exhibited reduced genome repair rates in vivo and also in vitro plasmid-based DSB repair assays compared with that of wild-type worms. These results revealed that although TDP-1 is not an essential protein for *C. elegans* to survive, it plays important role in DSB repair in response to genome damage insults.

In summary, our findings, for the first time, demonstrate that TDP-43 participates in the DDR/DNA damage repair and its nuclear clearance in spinal motor neurons causes DSB repair defects in ALS. The key highlights of this study are as follows: (1) in addition to its RNA processing and miRNA biogenesis functions, TDP-43 acts as a critical component of the NHEJ pathway for DSB repair; (2) TDP-43 rapidly recruits and persists at DSB sites until repair completion and functions to recruit DSB ligation factor XRCC4-Lig4 complex at the break sites; and (3) loss of functional TDP-43 or its nucleo-cytoplasmic mislocalization causes persistent accumulation of unrepaired DSBs and DDR activation contributing to neuronal apoptosis. These new paradigms that uncover a link between TDP-43 pathology and impaired DNA repair suggest potential avenues for DNA repair-targeted therapies for TDP-43-ALS and related motor neuron diseases.

### Unanswered Questions and Future Directions

Given that TDP-43 is a multi-functional protein, our novel findings not only provided insights into a previously undescribed DNA repair defect linked with TDP-43-associated neurodegeneration but also raised an important opportunity for exploring DNA repair-targeted therapeutic avenues. This requires a comprehensive understanding of molecular mechanisms involved, as well as the implications of these defects in neuronal and glial functions in the central nervous system. Future studies should address the following areas. (1) The role of TDP-43 in maintaining genome integrity in non-neuronal brain cells, for example, glia and astrocytes. Furthermore, comparing the DNA repair role of TDP-43 in post-mitotic vs cycling cells may reveal important mechanistic insights about selective vulnerability of neurons in ALS. (2) Does genome instability and altered DDR signaling contribute to neuro-inflammation in ALS? Neuro-inflammation has been identified as a major factor for neuronal cell death in ALS and several other neurodegenerative diseases.<sup>7</sup> Reports have shown that TDP-43 aggregation induces cellular oxidative stress and inflammatory responses; however, neuro-inflammation has been found to cause TDP-43 mislocalization.<sup>8</sup> (3) Does TDP-43 act as a scaffolding factor for assembling non-coding RNAs at genome damage sites? Long non-coding RNAs and microRNAs have been shown to regulate DDR signaling.<sup>9,10</sup> Recent next-generation sequencing studies identified several DNA damage-induced small RNAs, which may play critical roles in endogenous DSB repair.<sup>11</sup> Because TDP-43 acts as a partner in the DROSHA/DICER complex for miRNA biogenesis,<sup>12</sup> it

will be interesting to elucidate the TDP-43 broader or indirect roles in DNA repair via its RNA binding functions. Furthermore, the role of RNA/DNA-binding proteins in DNA repair has been recently highlighted in an increasing number of studies. We previously documented the involvement of hnRNP-U, a member of the hnRNP family, which acted as a switch allowing DSB repair to proceed in preference to oxidative/single-strand break (SSB) repair processes.<sup>13,14</sup> More recently, we discovered that another RNA/DNA-binding protein, fused in sarcoma/translocated in liposarcoma (FUS/TLS), linked to the ALS-FUS subtype, participated in DNA SSB repair by facilitating PARP-1-mediated recruitment and in functioning of the XRCC1/Ligase 3 complex.<sup>15</sup> It is intriguing that these RNA binding proteins influence the final DNA break/sealing step of repair, which raises the possibility of their structural and functional roles in maintaining optimal DNA repair enzyme processivity/stability at break sites. Further investigation along these lines may add new dimensions to our knowledge of genome repair and their defects in neurodegenerative diseases and allow us to develop clinically effective strategies to ameliorate genome instability in ALS-TDP-43.

### Acknowledgements

The authors thank Drs K.S. Rao (INDICASAT, Panama), R. Garruto (Binghamton University, New York), N. Liachko and B. Kraemer (University of Washington, Seattle), P. Wu (University of Texas Medical Branch, Galveston), P. Taylor (St Jude Children's Research Hospital, Memphis), and S. Mitra (Houston Methodist Research Institute, Houston) for their collaboration in the original study.

### Author Contributions

J.M. and M.L.H. conceived and wrote the commentary.

### ORCID iD

Muralidhar L Hegde  <https://orcid.org/0000-0001-7333-8123>

### REFERENCES

- Guerrero EN, Wang H, Mitra J, et al. TDP-43/FUS in motor neuron disease: complexity and challenges. *Prog Neurobiol.* 2016;145-146:78-97.
- Su Y, Ming GL, Song H. DNA damage and repair regulate neuronal gene expression. *Cell Res.* 2015;25:993-994.
- Madabhushi R, Gao F, Pfenning AR, et al. Activity-induced DNA breaks govern the expression of neuronal early-response genes. *Cell.* 2015;161:1592-1605.
- Mitra J, Guerrero EN, Hegde PM, et al. Motor neuron disease-associated loss of nuclear TDP-43 is linked to DNA double-strand break repair defects [published online ahead of print February 15, 2019]. *Proc Natl Acad Sci U S A.* doi:10.1073/pnas.1818415116.
- Freibaum BD, Chitta RK, High AA, Taylor JP. Global analysis of TDP-43 interacting proteins reveals strong association with RNA splicing and translation machinery. *J Proteome Res.* 2010;9:1104-1120.
- Guerrero EN, Mitra J, Wang H, et al. 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 [published online ahead of print April 1, 2019]. *Hum Mol Genet.* doi:10.1093/hmg/ddz062.

7. McCauley ME, Baloh RH. Inflammation in ALS/FTD pathogenesis. *Acta Neuropathol.* 2019;137:715-730.
8. Correia AS, Patel P, Dutta K, Julien JP. Inflammation induces TDP-43 mislocalization and aggregation. *PLoS ONE.* 2015;10:e0140248.
9. Thapar R. Regulation of DNA double-strand break repair by non-coding RNAs. *Molecules.* 2018;23:E2789. doi:10.3390/molecules23112789.
10. Wei W, Ba Z, Gao M, et al. A role for small RNAs in DNA double-strand break repair. *Cell.* 2012;149:101-112. doi:10.1016/j.cell.2012.03.002.
11. Bonath F, Domingo-Prim J, Tarbier M, Friedlander MR, Visa N. Next-generation sequencing reveals two populations of damage-induced small RNAs at endogenous DNA double-strand breaks. *Nucleic Acids Res.* 2018;46:11869-11882. doi:10.1093/nar/gky1107.
12. Kawahara Y, Mieda-Sato A. TDP-43 promotes microRNA biogenesis as a component of the Drosha and Dicer complexes. *Proc Natl Acad Sci U S A.* 2012;109:3347-3352. doi:10.1073/pnas.1112427109.
13. Hegde ML, Dutta A, Yang C, et al. Scaffold attachment factor A (SAF-A) and Ku temporally regulate repair of radiation-induced clustered genome lesions. *Oncotarget.* 2016;7:54430-54444. doi:10.18632/oncotarget.9914.
14. Hegde ML, Banerjee S, Hegde PM, et al. Enhancement of NEIL1 protein-initiated oxidized DNA base excision repair by heterogeneous nuclear ribonucleoprotein U (hnRNP-U) via direct interaction. *J Biol Chem.* 2012;287:34202-34211. doi:10.1074/jbc.M112.384032.
15. Wang H, Guo W, Mitra J, et al. Mutant FUS causes DNA ligation defects to inhibit oxidative damage repair in amyotrophic lateral sclerosis. *Nat Commun.* 2018;9:3683. doi:10.1038/s41467-018-06111-6.