

## Supplementary Information

### **TDP-43 Overexpression Induces Cellular Dysfunction and ALS-Associated Transcriptional Changes**

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1  GGATCCGCCG CCACCATGAG CGAATACATC CGTGTTACCG AAGACGAAAA
51  TGACGAGCCA ATCGAAATCC CAAGCGAAGA CGATGGTACC GTGCTGCTGA
101 GCACCGTGAC CGCTCAGTTT CCAGGTGCCT GCGGCCTGCG TTACCGTAAC
151 CCGGTGAGCC AGTGCAATGC TGGTGTGCGC CTGGTGGAGG GCATCCTGCA
201 TGCTCCAGAT GCCGGCTGGG GTAACCTGGT GTACGTGGTG AACTATCCGA
251 AGGATAACAA GCGTAAAATG GACGAAACCG ATGCTAGCAG CGCCGTGAAG
301 GTGAAACGCG CGGTGCAGAA AACCAGCGAC CTGATTGTGC TGGGTCTGCC
351 GTGGAAGACC ACCGAGCAGG ATCTGAAAGA ATACTTCAGC ACCTTTGGTG
401 AGGTGCTGAT GGTGCAGGTG AAGAAAGACC TGAAGACCGG CCACAGCAAA
451 GGCTTCGGTT TTGTGCGTTT CACCGAGTAT GAAACCCAGG TGAAAGTGAT
501 GAGCCAGCGT CACATGATCG ACGGCCGCTG GTGCGATTGC AAGCTGCCGA
551 ACAGCAAACA GAGCCAGGAT GAACCGCTGC GTAGCCGCAA AGTGTTCTGT
601 GGTCGTTGCA CCGAGGACAT GACCGAGGAT GAACTGCGCG AATTCTTTAG
651 CCAGTACGGC GACGTGATGG ACGTGTTTCAT CCCGAAGCCG TTTCGCGCCT
701 TCGCCTTCGT GACCTTCGCC GACGATCAGA TTGCCCAGAG CCTGTGCGGC
751 GAGGACCTGA TCATTAAGGG CATCAGCGTG CACATTAGCA ACGCTGAGCC
801 GAAACACAAC AGCAACCGTC AGCTGGAACG TAGCGGCCGC TTCGGCGGTA
851 ACCCGGGCGG TTTCGGTAAT CAGGGCGGTT TTGGTAACAG CCGCGGCGGT
901 GCGCGCGGCC TGGGTAACAA CCAGGGTAGC AACATGGGTG GCGGTATGAA
951 CTTCGGCGCG TTTAGCATCA ACCCAGCTAT GATGGCGGCC GCTCAGGCCG
1001 CGCTGCAGAG CAGCTGGGGT ATGATGGGCA TGCTGGCCAG CCAGCAGAAC
1051 CAGAGCGGTC CGAGCGGCAA CAACCAGAAC CAGGGTAACA TGCAGCGTGA
1101 ACCGAACCAG GCGTTTGGCA GCGGTAACAA CAGCTATAGC GGTAGCAATA
1151 GCGGTGCTGC TATTGGTTGG GGTAGCGCGA GCAATGCTGG CAGCGGTAGC
1201 GGCTTCAACG GCGGTTTTTG CAGCAGCATG GATAGCAAGT CCTCCGGTTG
1251 GGGTATGTGA

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**Fig. S1** Sequence of synthetic TDP-43 gene (Genscript, Piscataway NJ). The open reading frame for TDP-43 starts with the highlighted ATG codon at base pair 16 and terminates with the highlighted TGA codon starting at base pair 1258. The full sequence of the pcDNA™5/FRT/TO plasmid is available from the manufacturer (Thermo Fisher, Waltham, MA).

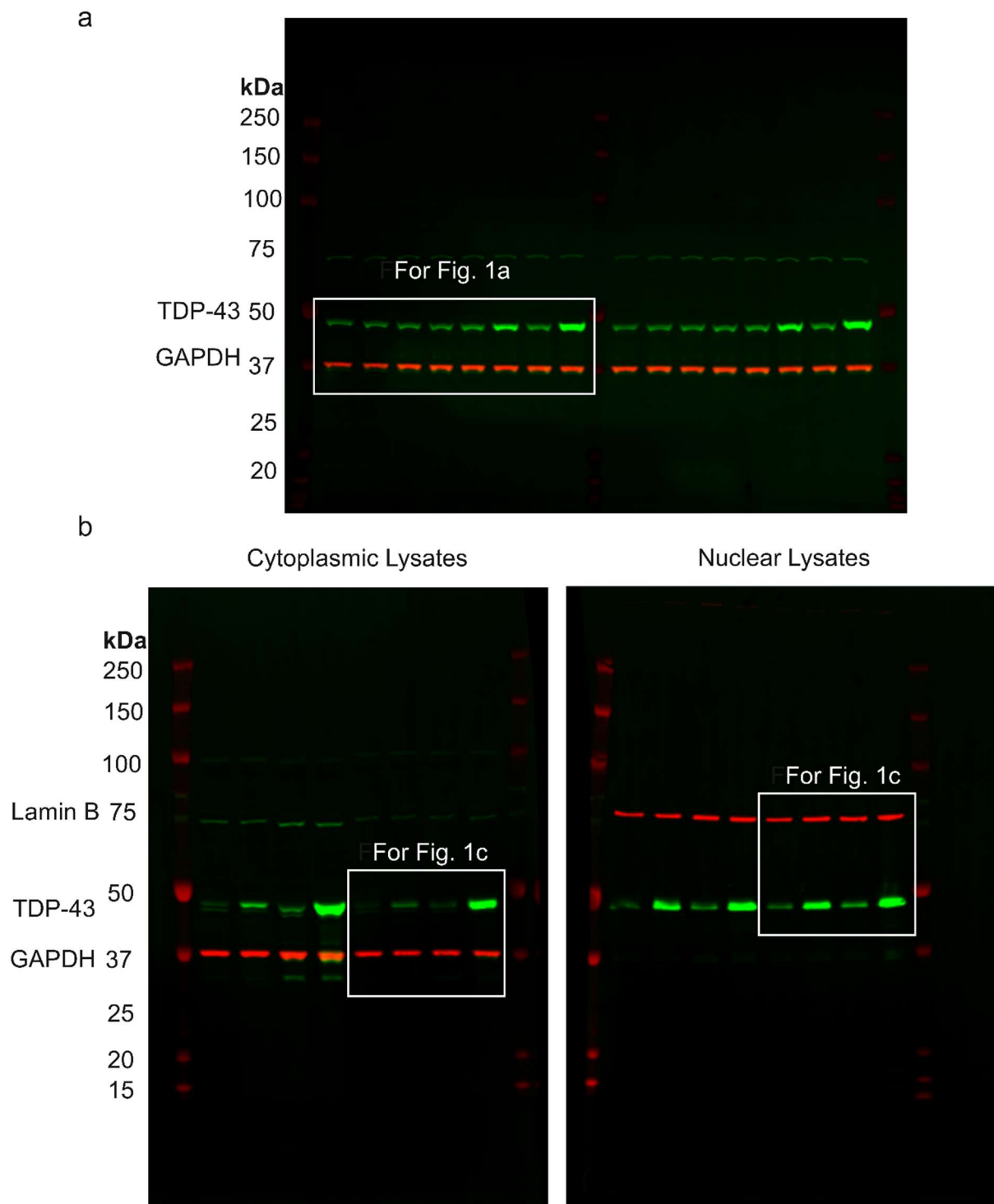


**Fig. S2** Uncropped western blot membranes of **a.** RIPA lysates from Fig. 1a. **b.** Cytoplasmic and nuclear fractions from Fig. 1c. Excess lanes are from duplicate treatments and were not used for the final figure.

**Table S1** Western blot reagents and antibodies

|                             | Company      | Catalog number |
|-----------------------------|--------------|----------------|
| <b>Primary Antibodies</b>   |              |                |
| Rb TDP-43                   | Protein Tech | 12892-I-AP     |
| Ms TDP-43                   | Protein Tech | 67345-1-Ig     |
| Ms GAPDH                    | Protein Tech | 60004-1-Ig     |
| Ms Lamin-B1                 | Protein Tech | 66095-1-Ig     |
| <b>Secondary Antibodies</b> |              |                |
| Anti- Rabbit 800CW          | LI-COR       | 926-32211      |
| Anti-Mouse 680RD            | LI-COR       | 925-68070      |

**Table S2** Sample details of ALS and non-neurological control patients used in the analysis  
(Separate excel file)**Table S3** List of primers used for qPCR assays

| Target Gene | Type | Sequence (5'-3')       |
|-------------|------|------------------------|
| Beta-Actin  | F    | CACCATTGGCAATGAGCGGTTC |
|             | R    | AGGTCTTTGCGGATGTCCACGT |
| CDC23       | F    | AACTGCTGCTTATTGCGGGC   |
|             | R    | CTTGCCAGGGTATAGGCAT    |
| DKC1        | F    | TCACCACCAAAGGAGAAGCA   |
|             | R    | GTCGCAGGTAGAGATGACCG   |
| ELP1        | F    | GTCCAGGGAATCCTCAGTGC   |
|             | R    | CCATCCTCTGGGAGAAAGCC   |
| NCAPD3      | F    | AATATGGCCTTCATGCCGCT   |
|             | R    | TGGGGCCAGCTCTTCTTTAG   |
| NUP85       | F    | CGCTATGGAGGAGCTCGATG   |
|             | R    | ATACCAGCATCTCCCCTGGA   |
| NUP88       | F    | TCTGGGACCCACGATGATCT   |
|             | R    | GGAATCTGGGGTTTCAGCCA   |
| PTS *       | F    | CGCGAGCCACCGATTGTA     |
|             | R    | CTCTCCATGTACTGTCACCAC  |
| RBM12       | F    | CGCAGCTTTCCCCGTCTAAA   |
|             | R    | GACCTTGCAAACGGATGACC   |
| SAFB **     | F    | ACTCTGGGGATGGACAGATAG  |
|             | R    | CTCCAGGGATGGCTCTTCAAT  |
| SREBF2      | F    | TGTCCTGAGCGTCTTTGTGA   |
|             | R    | GGCAGGTTTGTAGGTTGCCG   |
| TBC1D1 ***  | F    | GAATGCCAGGAACCTCCACA   |
|             | R    | ACGGTAGGAGATGTTTCGTTC  |
| VAMP5       | F    | TGCGTAACAACCTTCGGCAAG  |
|             | R    | GGGCCAGGTTCTGTGTAGTC   |

\*PTS Primer set recognizes loss of exon 4, \*\* SAFB Primer set recognizes loss of exon 4, \*\*\* TBC1D1 Primer set recognizes loss of exon 13. Exon numbering is from the spliceSeq program.

**Table S4** Differential gene expression summary of 24-and 48-hour timepoints (**Separate excel file**)

**Table S5** Differential gene expression analysis from 24 hours post induction with tetracycline. Sample 2-x are HEK293-EV and Sample 4-x are HEK293-TDP cells (**Separate excel file**)

**Table S6** Gene Ontology analysis summary 24-and 48-hour timepoints (**Separate excel file**)

**Table S7** Gene Ontology analysis from 24 hours post induction with tetracycline, significant pathways and genes changed in HEK293-TDP cells compared to HEK293-EV cells (**Separate excel file**)

**Table S8** SpliceSeq splicing analysis summary of 24-and 48-hour timepoints (**Separate excel file**)

**Table S9** SpliceSeq splicing analysis from 24 hours post induction with tetracycline, significant splice event in HEK293-TDP cells compared to HEK293-EV cells, with >25% dPSI (**Separate excel file**)

**Table S10** Differential gene expression analysis of 24 hour induced HEK293-TDP cells, compared to induced HEK293-EV cells and ALS patient samples, compared to non-neurological patient samples (**Separate excel file**)