Supplemental Methods

**Transgene constructions.** Expression of variant TDP-43 sequences was engineered by recovering the relevant sequences from mammalian expression constructs and cloning these into the Xho 1/Xba I sites of pCL35 using the primers: Xba1.eGFP F (5’ GCTCTAGACGCCACCATGGTGAGC) and TDP43.Xho1 R (5’ CCGCTCGAGCTACATTCCCAGCCAG). The eGFP::TDP-43 and eGFP::TDP-25 (i.e., TDP-43 residues 220-414) donor plasmids have been previously described (15), as has the eGFP::TDP-43 (D89E, D219E) caspase-uncleavable mutant (14).

Construction of the RRM1 and RRM2 deletion constructs was achieved using Stratagene’s QuikChange II XL kit to delete the relevant regions of a TDP-43 cDNA cloned within the eGFP vector. The RRM1 deletion construct consists of eGFP::TDP-43 lacking amino acids 106 to 175 (inclusive); primers: forward 5’ GAGCAGTCCAGAAAACATCCGATAAACTTCCAATCTAAAGCAAG, reverse 5’ CTTTGCTTAGAATTAGGAAGTTTATCGGATGTTTTCTGGACTGCTC. RRM2 deletion construct consists of eGFP::TDP-43 lacking amino acids 193 to 257 (inclusive); primers: forward 5’ GATGAGCCTTTTGAGAAGCAGAAAATCCAATGCCGAAC, reverse 5’ GTTCGGCCATTGGATTTCGACTGCTC. The respective sequences were then amplified and transferred to pCL35 using the Xbal.eGFP F and TDP-43.Xho1 R primers as above. The eGFP::TDP-43 C-terminal deletion (aa 1-257) was cloned into pCL35 from eGFP vector with Xba1.eGFP F primer and the reverse primer TDP-43 aa257.Xho1 R (5’ CCGCTCGAGTCATATATGAACGCTGATTCC).

The NLS1 (K82-84S) and caspase cleavage site (D89/219E) mutations were also introduced into eGFP::TDP-43 using Stratagene’s QuikChange II XL Site-Directed Mutagenesis Kit. K82-84S primers: Forward 5’ CCACTATCCAAAGATAACTCATCATCAATGGATGAGACAGATGC; Reverse 5’ GCATCTGTCTCATCCATTGATGAGTTATCTTTGAGATTG. D89E primers:
Forward 5' GAAAAATGGATGAGACAGAGGCTTCATCAGCAGTGAAAG; Reverse 5' CTTTCACTGCTGATGAAGCCTCTGTCTCATCCATTTTTTC. D219E primer: Forward 5' CGGGGATGTGATGGAGGTCTTCATCCCCAAG; Reverse 5' CTTGGGGATGAAGACCTCCATCACATCCCCG. The mutated eGFP::TDP-43 constructs were recloned into pCL35 as described above.

A construct expressing *C. elegans* TDP-1 was generated by recovering the genomic coding sequence of *tdp-1* by PCR and cloning this fragment into the Xba I and Sac I sites of pCL35 using the following primer sets:
Forward 5' CAATCTAGAATGGCCGACGAAACGCCGAAG
Reverse 5' CCTGAGCTCAATCACCATCCTGGTCCTCTCG

To generate a hTDP-43/TDP-1 fusion constructs, primers were made to the pCL35 vector outside the Xba I and Sac I cloning sites. Primers were also made to the fusion site contained roughly 20 bp of each respective protein; hence leaving a “tail” enabling subsequent PCR to join two disparate fragments (PCR stitching). The primers for hTDP-43::TDP-1 were:
Forward 5’ TTGGCCAAAGGACCCAAAGG
Reverse 5’ CACGTTCCTCACTGGCTGCCTTTG
Reverse fusion 5’
GCTGGGAAGGCCATAATCAGGGCCtaactgtctattgctattgtgcttagg

The tagged and untagged TDP-43 constructs used for the cell culture transfection studies have been previously described (12,13). All constructs were verified by sequencing.

**Immunoblotting:**
For *C. elegans* immunoblotting, 50 transgenic worms were picked into 15 µl lysis buffer (50mM Tris-HCl, pH 7.4, 1M NaCl, 1% Triton-X-100, 5mM EDTA) plus 1% SDS, PMSF, and protease and phosphatase inhibitor cocktails and snap frozen on dry ice. Samples were thawed, homogenized with a motorized pestle and sonicated. The samples were then mixed 1:1 with Laemmli’s buffer (with a final concentration of 5% β-Mercaptoethanol) and boiled for 5 minutes before loading to 10% Tris-Glycine gel (Novex). The procedure for washing, blocking and
visualization of bands follows that described in main text. Membranes probed with rabbit monoclonal anti-GFP (1:2000; Invitrogen), mouse monoclonal anti-TDP-43 (1:2000; Abnova), rabbit polyclonal anti-TDP-1 (1:1000; made "in-house") and mouse monoclonal anti-tubulin (1:5000; Sigma).

HeLa cell culture controls were seeded at 80 cells/µl in 6-well plates for 24hr then transfected with 1µg with pEGFP-C1 vector, pEGFP::TDP-43 or pEGFP::tdp-1 and grown for 48hr before harvesting for protein. 20µg protein loaded to 10% Tris-Glycine gel (Novex) and probed for GFP, TDP-43 and tdp-1 as above. Mouse monoclonal anti-GAPDH (1:10000; Biodesign).

**Fluorescent Microscopy:**

In addition, HeLa were seeded 80 cells/µl into 24-well plates onto sterile coverslips for 24hr. They were then transfected with 0.25µg of pEGFP-C1 vectors as above. After 48hr, cells were washed in PBS and fixed at 4°C in 4% PFA for 5 minutes before washing (6 times in PBS 0.2% Triton X100), permeabilized (10 minutes at RT in PBS 0.5% Triton X100) and washed overnight in PBS 0.2% Triton X100. Counterstaining was performed using Hoescht for 10 min at RT then washing (6 times in PBS 0.2% Triton X100). Images taken at 63 times magnification on Zeiss AxioImager Z1 with Apotome.
**Supplemental Figure 1.**

![Supplemental Figure 1](image)

**Supplemental Figure 1 legend.**

**Relative expression levels of smg-1(cc546ts) temperature inducible strains carrying neuronal eGFP::TDP-43 or eGFP::tdp-1 (A).** Lane 1: smg-1 (cc546ts); 2: Integrated strain CL1234(snb-1/GFP); 3: Extrachromosomal strain CL1625 (snb-1/eGFP::TDP-43); 4: Extrachromosomal strain CL1626 (snb-1/eGFP::TDP-43); 5: Extrachromosomal strain LEN144 (snb-1/eGFP::tdp-1_(cDNA)); 6: Extrachromosomal strain LEN145 (snb-1/eGFP::tdp-1_(cDNA)); 7: Extrachromosomal strain LEN146 (snb-1/eGFP::tdp-1_(cDNA)); 8: Extrachromosomal strain LEN148 (snb-1/eGFP::tdp-1_(cDNA)). **B.** HeLa transfected with pEGFP-C1 alone or carrying GFP::TDP-43 or GFP::tdp-1(cDNA). Both immunoblots are probed with (rabbit) anti-GFP (Invitrogen), (mouse) anti-TDP-43 (Abnova) and (rabbit) anti-tdp-1 (made in house). Loading control for *C. elegans* probed by (mouse) anti-tubulin (Sigma) and for HeLa with (mouse) anti-GAPDH (BioDesign).
Immunoblot of extracts from transgenic worms probed with anti-hTDP-43 monoclonal antibody M01. **Lane 1.** Control strain containing only transformation marker plasmids (CL1685). **Lane 2.** Transgenic strain expressing full-length hTDP-43 (CL1682). **Lane 3.** Transgenic strain expressing eGFP::hTDP-43 (CL1626). **Lane 4.** Transgenic strain expressing eGFP::hTDP-43 RRM1 deletion (CL1702). **Lane 5.** Transgenic strain expressing eGFP::hTDP-43 RRM2 deletion (CL1705). The absence of an hTDP-43 band is due to the deletion of the M01 epitope; probing a parallel blot with polyclonal anti-hTDP-43 body detects a band of the expected ~75 kd size. **Lane 6.** Transgenic strain expressing eGFP::hTDP-43 C-terminal deletion (CL1710). **Lane 7.** Transgenic strain expressing eGFP::hTDP-43 NLS1 mutant (CL1687). **Lane 8.** Transgenic strain expressing eGFP::hTDP-43 caspase site mutant (CL1688). Actin panel is from reprobing of this blot with anti-actin monoclonal antibody JLA20. (Lanes 1 &2 are also shown in Figure 2D).
Supplemental Figure 3 Legend.

Fluorescent images of HeLa cultures transfected with pEGFP-C1 constructs alone, or carrying TDP-43, tdp-1(cDNA), TDP-43aa1-270::tdp-1aa347-411 or TDP-43aa1-257 and counterstained with 1µg/ml Hoescht (Invitrogen). Addition of the C-terminus (aa347-411) of the C e tdp-1 restores the subnuclear localization of GFP tagged human TDP-43 that is disrupted with the deletion of its C-terminus (aa258-414). (Scale bar = 10µm).
Supplemental Figure 4 Legend. Fixed snb-1/hTDP-43 (dvls62) and wild type (strain N2) worms probed with polyclonal anti-TDP-43 antibody (red) and monoclonal anti-ubiquitin (mAb1510) (green), counterstained with DAPI (blue).

A. DIC image of nerve ring area of snb-1/hTDP-43 worm. B. Corresponding epifluorescence to panel A. No ubiquitinated inclusions are visible. (Small dots of green fluorescence are due to non-specific secondary antibody binding, see panel D.) C. Same image as panel C with red channel removed, illustrating lack of association of anti-ubiquitin staining with TDP-43 immunoreactivity. D. Wild
type worm probed with both antibodies; no anti-TDP-43 or anti-ubiquitin 
immunoreactivity detectable. Size bar = 10µM.