Supplemental Data

Heat Shock Induced Phosphorylation of TAR DNA Binding Protein 43 (TDP-43) by MAPK/ERK Kinase Regulates TDP-43 Function

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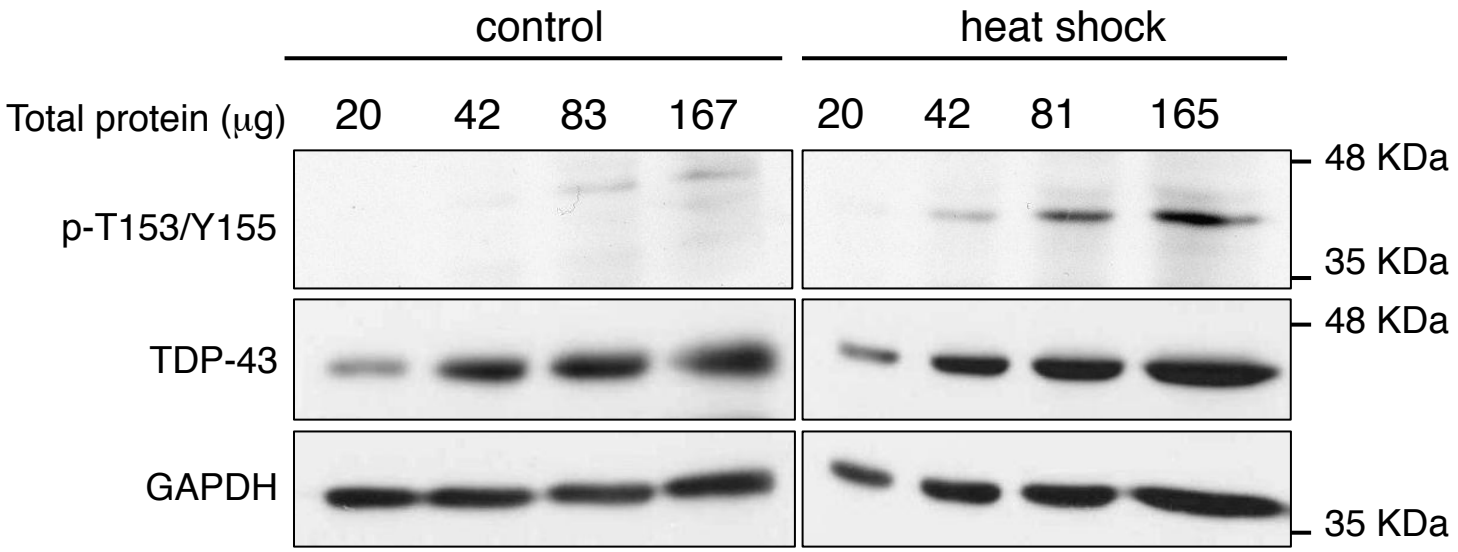
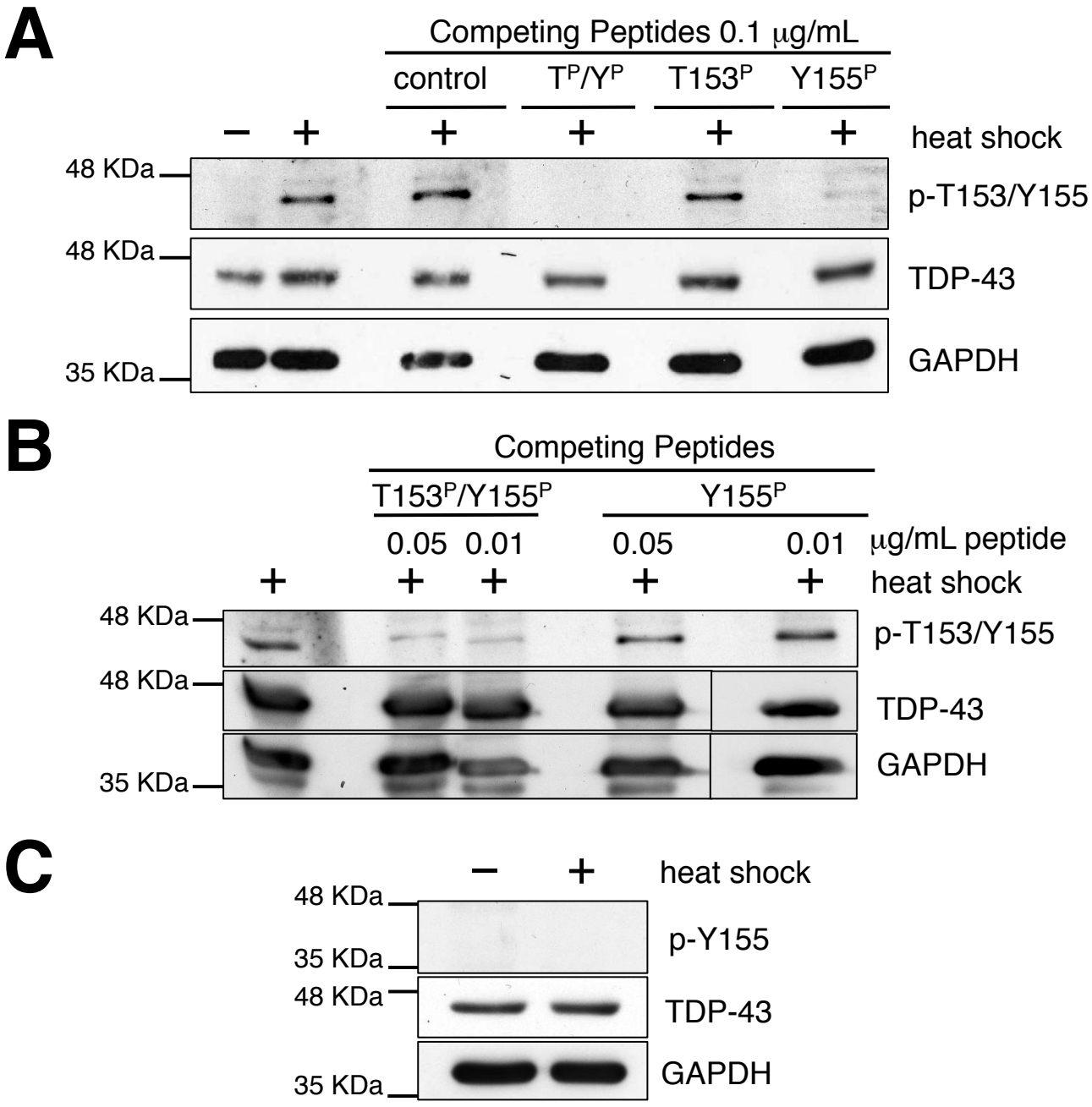
Supplemental Figures S1-S8

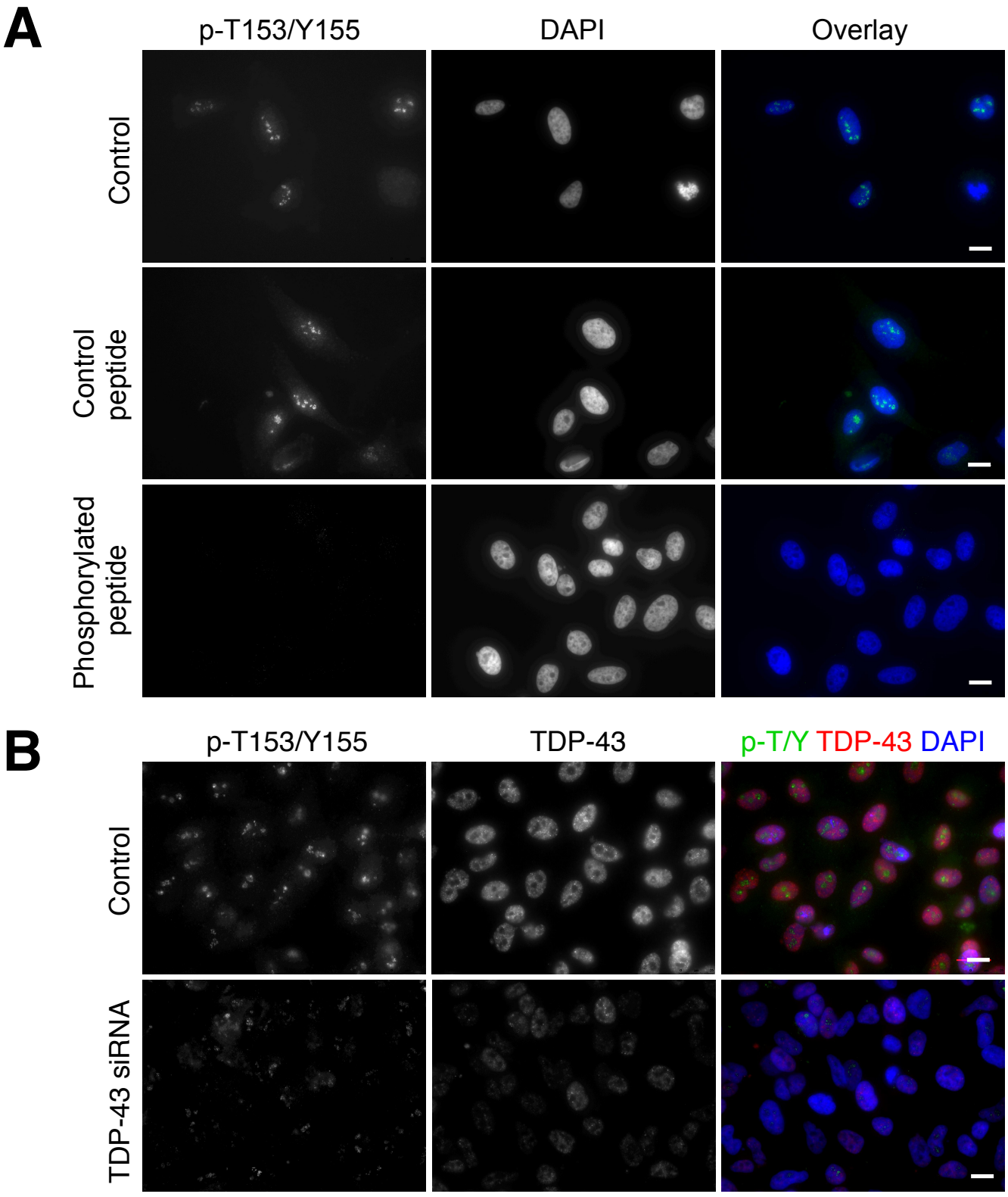
Supplemental Table



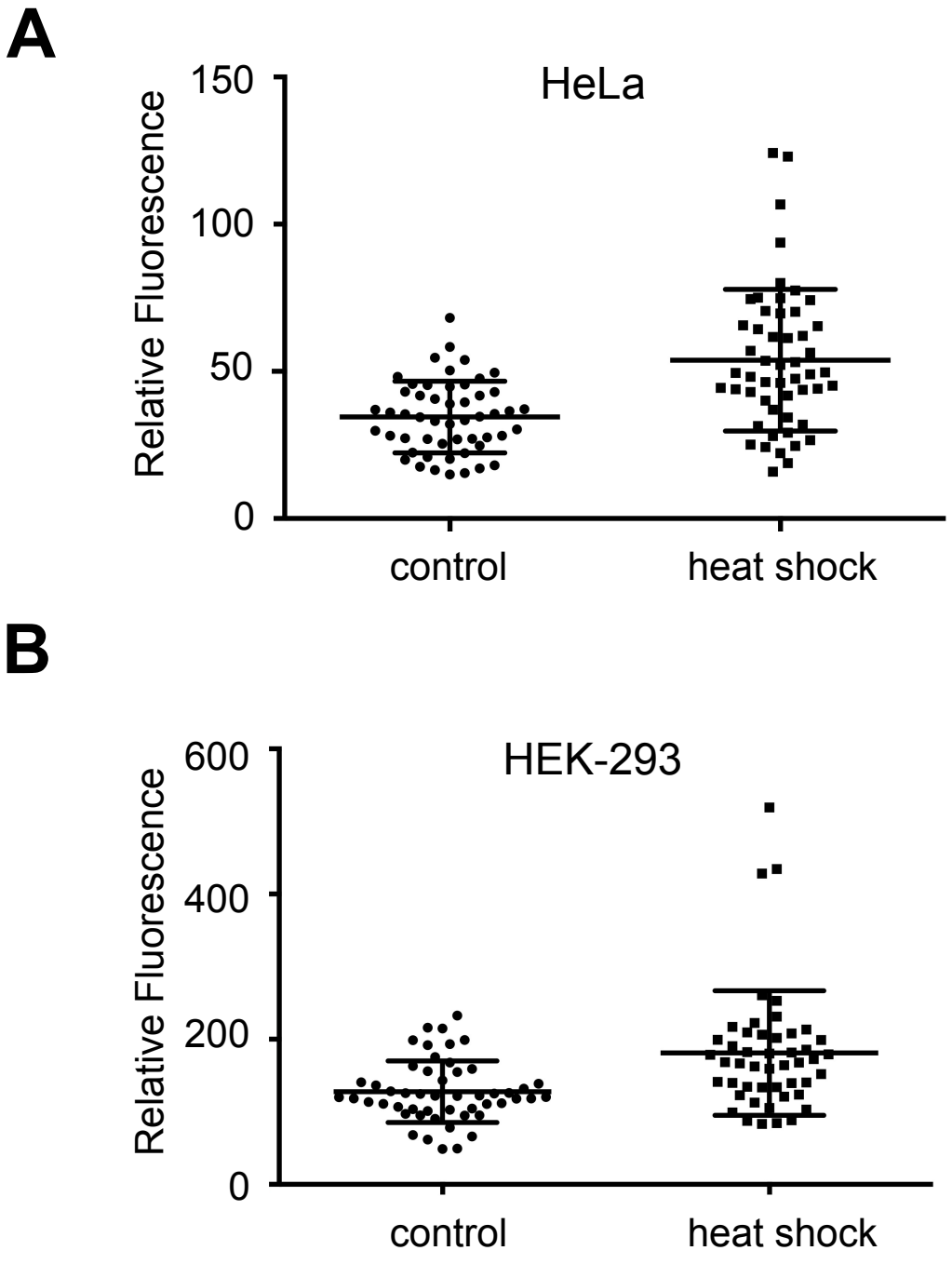
Supplemental Fig S1 p-T153/Y155-TDP-43-associated signal is not detected in control cells and is greatly upregulated in heat shock treated cells. Increasing amounts of cell lysate derived from control and heat shock treated HEK-293 cells analyzed by immunoblotting. Total protein was measured by Bradford analysis using a bovine serum albumin standard. Antibodies detecting phospho-independent TDP-43 and GAPDH were used as total TDP-43 and loading controls, respectively.



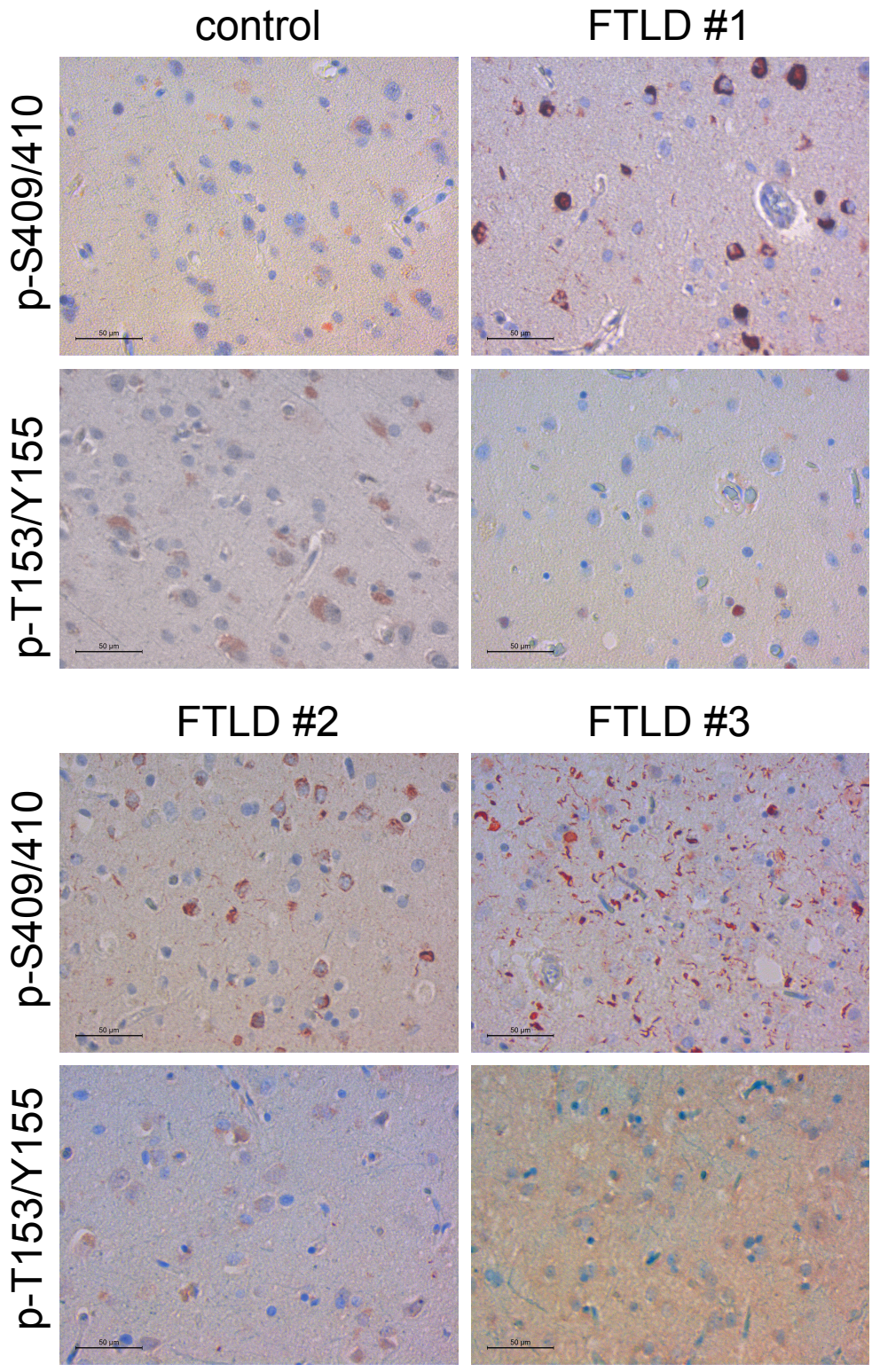
Supplemental Fig S2 p-T153/Y155-TDP-43 recognizes dual phosphorylation of TDP-43 at T153 and Y155. (A) Immunoblots of cell lysate from heat shock treated HEK-293 to determine p-T153/Y155-TDP-43 specificity for dual phosphorylation at T153 and Y155. The first lane shows control treated cell lysate. Peptides corresponding to TDP-43 amino acid residues 148-161 (0.1 μg/ml) phosphorylated at single residues T153 or Y155 were used to compete with antibody detection of the heat shock-associated band. Non-phosphorylated and dual phosphorylated $T153^P/Y155^P$ (T^P/Y^P) peptides were used as negative and positive controls, respectively. (B) Lower concentrations of the $Y155^P$ peptide and the dual phosphorylated peptide $T153^P/Y155^P$ were compared for their ability to compete with antibody detection. (C) Immunoblot of cell lysates from control and heat shock treated HEK-293 cells using an antibody generated to recognize single residue phosphorylation at Y155 of TDP-43. Phospho-independent TDP-43 and GAPDH were used, respectively, as total TDP-43 and loading controls.



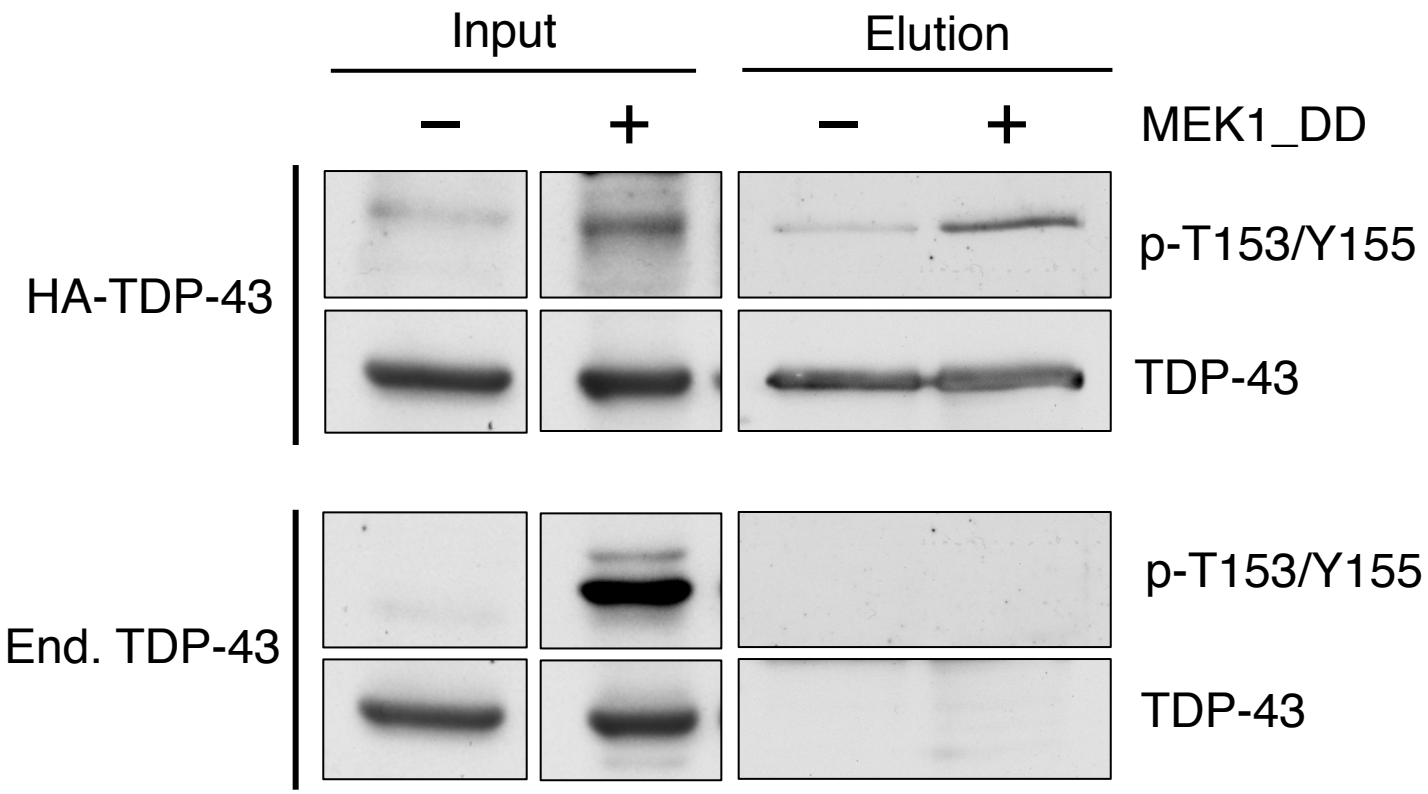
Supplemental Fig S3 Detection of p-T153/Y155 TDP-43 in nucleoli. (A) Fluorescence imaging of non-treated HeLa cells using p-T153/Y155-TDP-43 following the incubation of the antibody with the TDP-43 peptide a.a. 148-161 phosphorylated at T153/Y155 and with the corresponding non-phosphorylated peptide as control. (B) shRNA-mediated downregulation of TDP-43 in HeLa cells. TDP-43 expression levels were visualized by detection of total TDP-43. The nucleolar signal of p-T153Y155-TDP-43 was compared in control and TDP-43 knock-down cells. The overlay images include DAPI staining to visualize the nuclear compartment. Bars, 10 μm.



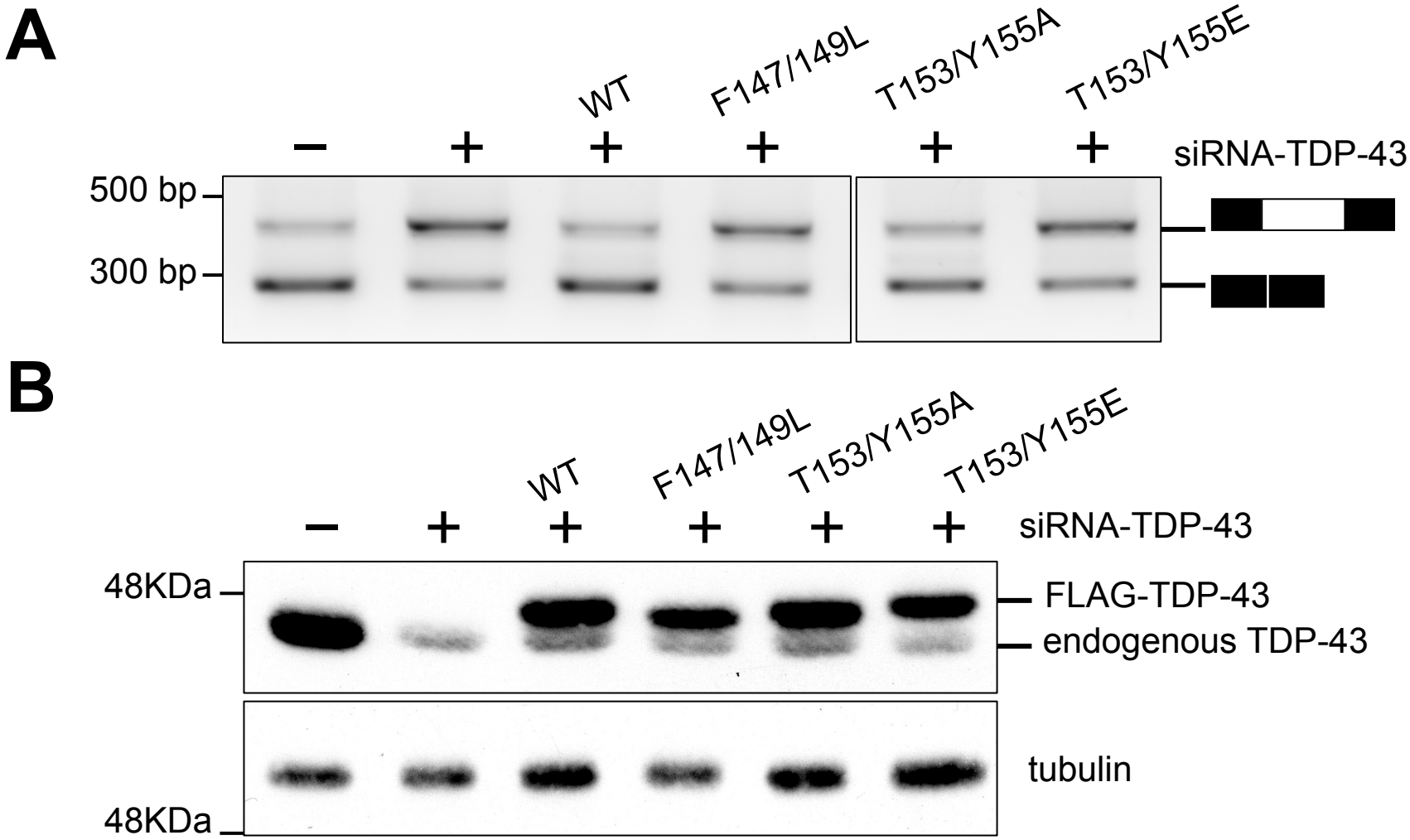
Supplemental Fig S4 Heat shock increases p-T153/Y155 levels as seen by immunofluorescence. Relative fluorescence values of p-T153/Y155 detection in HeLa (A), HEK-293 cells (B) exposed to heat shock or control conditions. Fluorescence in the nuclear and cytoplasmic compartments were quantified by ImageJ. Mean and standard deviation values are shown. Statistical significance values for HeLa and HEK-293 were $P<0.001$.



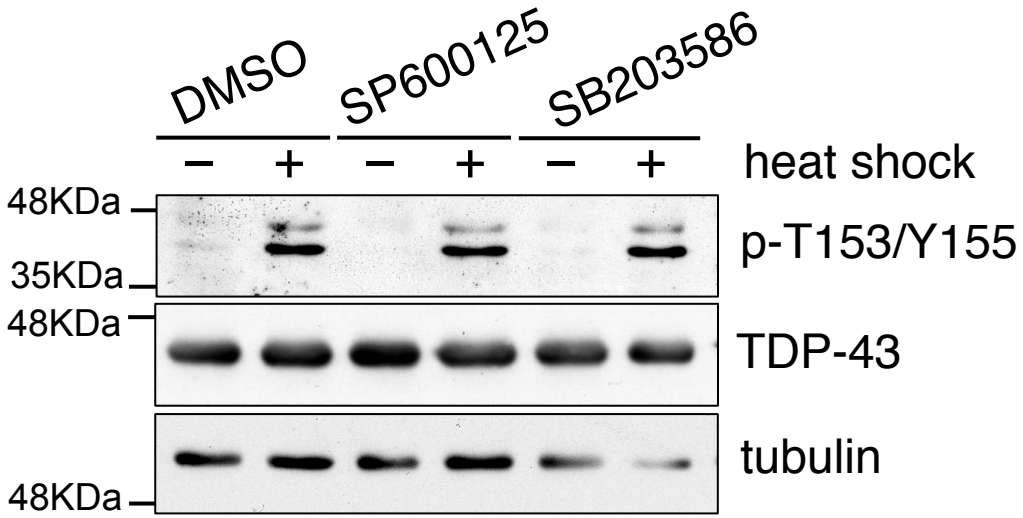
Supplemental Fig S5 Immunohistochemical analysis of p-T153/Y155 in frontotemporal lobar degeneration. Brain histological sections immunostained with p-T153/Y155 and p-S409/410 TDP-43 as control for TDP-43 pathology. Frontal cortex and dentate gyrus paraffin sections from control and frontotemporal lobar degeneration cases.



Supplemental Fig S6 Tagged TDP-43 is phosphorylated by MEK in human cells. Immunoblot analysis to detect the levels of p-T153/Y155 in hemagglutinin-tagged (HA) TDP-43 following immunoprecipitation from tetracycline-induced HEK-293 cells stably expressing HA-TDP-43. Cells were transfected with control GFP or GFP-MEK_DD, a construct expressing constitutively active MEK1. As a control, the total levels of tagged TDP-43 are shown in the cell lysate (Input) and eluted samples. Lower panels show phosphorylation (p-T153/Y155) and total levels of endogenous TDP-43.



Supplemental Fig S7 siRNA-rescue experiments to analyze the effect of T153/Y155 mutations on CFTR exon 9 splicing. (A) siRNA-resistant wild-type (WT) and mutant TDP-43 constructs expressed in siRNA treated HeLa cells. A non-targeting siRNA was used as control. Relative exon inclusion of the CFTR exon 9 mini-gene was visualized on ethidium bromide-stained 2% agarose gel following RT-PCR. The relative levels of exon inclusion were quantified by ImageJ and used as a measure of TDP-43 activity. (B) Representative immunoblot showing expression levels of siRNA-resistant FLAG-tagged TDP-43: wild-type (WT), the RNA binding-deficient mutant F147/149L, and T153/Y155 substitutions to Ala and Glu. Constructs were expressed in siRNA-treated HeLa cells with the CFTR exon 9 mini-gene reporter. The slightly lower molecular weight band shows endogenous TDP-43. Tubulin was used as loading control.



Supplemental Fig S8 p-T153/Y155 induction is not affected in the presence of inhibitors of JNK and p38 Immunoblots of SH-SY5Y cells treated with control (DMSO), JNK inhibitor SP600125 (10 μM) and p38 MAP kinase inhibitors SB203586 (20 μM). Inhibitors were added for 1 hour prior to heat shock or incubated at 37 °C, as control. Phospho-independent TDP-43 and tubulin were used, respectively, as total TDP-43 and loading controls.

Supplemental Table: Oligonucleotide sequences used in cloning and site-directed mutagenesis

Oligonucleotide Name	Sequence
pQTDPBam_FW	5’-CGCGGATCCTCTGAATATATTCGGGTAACCG-3’
SacTDPend_RV	5’-GATCGAGCTCCTACATTCCCCAGCCAG-3’
Bam_102_FW	5’-CGCGGATCCAAAACATCCGATTTAATAG-3’
Sac_269_RV	5’-GATCGAGCTCCTACTGTCTATTGCTATTGTG-3’
T153AY155A_FW	5’-CTTTGTTCGTTTTGCGGAAGCTGAAACACAAGTG-3’
T153AY155A_RV	5’-CACTTGTGTTTCAGCTTCCGCAAAACGAACAAAG-3’
T153EY155E_FW	5’-CTTTGTTCGTTTTGAGGAAGAAGAAACACAAGTG-3’
T153EY155E_RV	5’-CACTTGTGTTTCTTCTTCCTCAAAACGAACAAAG-3’
W113A_FW	5’-ATAGTGTTGGGTCTCCCAGCGAAAACAACCGAACAGGAC-3’
W113A_RV	5’-GTCCTGTTCGGTTGTTTTCGCTGGGAGACCCAACACTAT-3’
W113F_FW	5’-ATAGTGTTGGGTCTCCCATTCAAAACAACCGAACAGGAC-3’
W113F_RV	5’-GTCCTGTTCGGTTGTTTTGAATGGGAGACCCAACACTAT-3’
W172A_FW	5’-CATATGATAGATGGACGAGCGTGTGACTGCAAACTTCC-3’
W172A_RV	5’-GGAAGTTTGCAGTCACACGCTCGTCCATCTATCATATG-3’
W172F_FW	5’-CATATGATAGATGGACGATTCTGTGACTGCAAACTTCC-3’
W172F_RV	5’-GGAAGTTTGCAGTCACAGAATCGTCCATCTATCATATG-3’
Mek1DD_FW	5’-GCGGGCAGCTCATCGACGACATGGCCAACGACTTCGTGGGCACAAGGTCC-3’
Mek1DD_RV	5’-GGACCTTGTGCCCACGAAGTCGTTGGCCATGTCGTCGATGAGCTGCCCGC-3’
shT2_FW	5’-CCGGAAGCAAAGCCAAGATGAGCCTCTCGAGAGGCTCATCTTGGCTTTGCTTTTTTTG-3’
shT2_RV	5’-AATTCAAAAAAAGCAAAGCCAAGATGAGCCTCTCGAGAGGCTCATCTTGGCTTTGCTT-3’