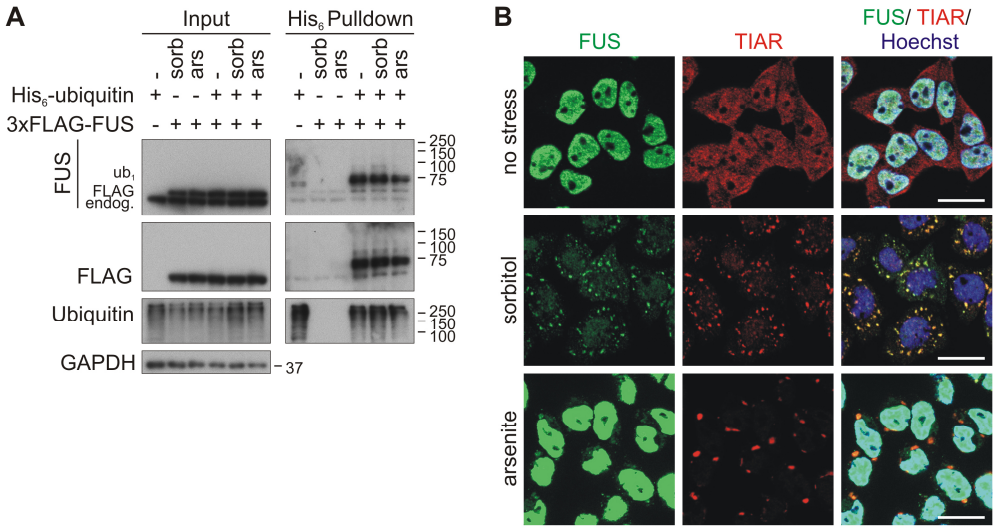
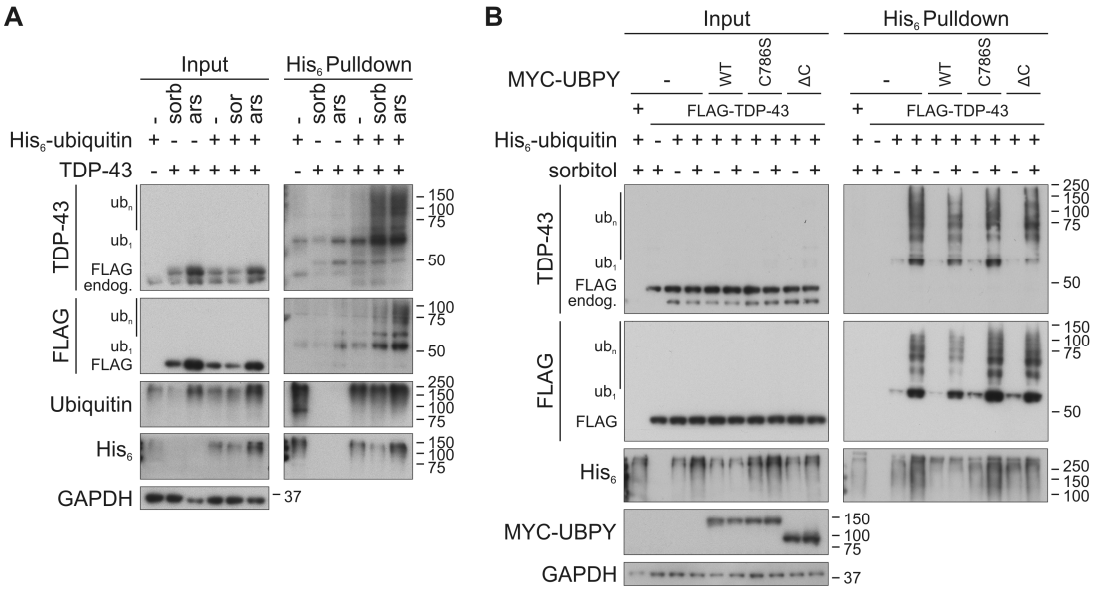
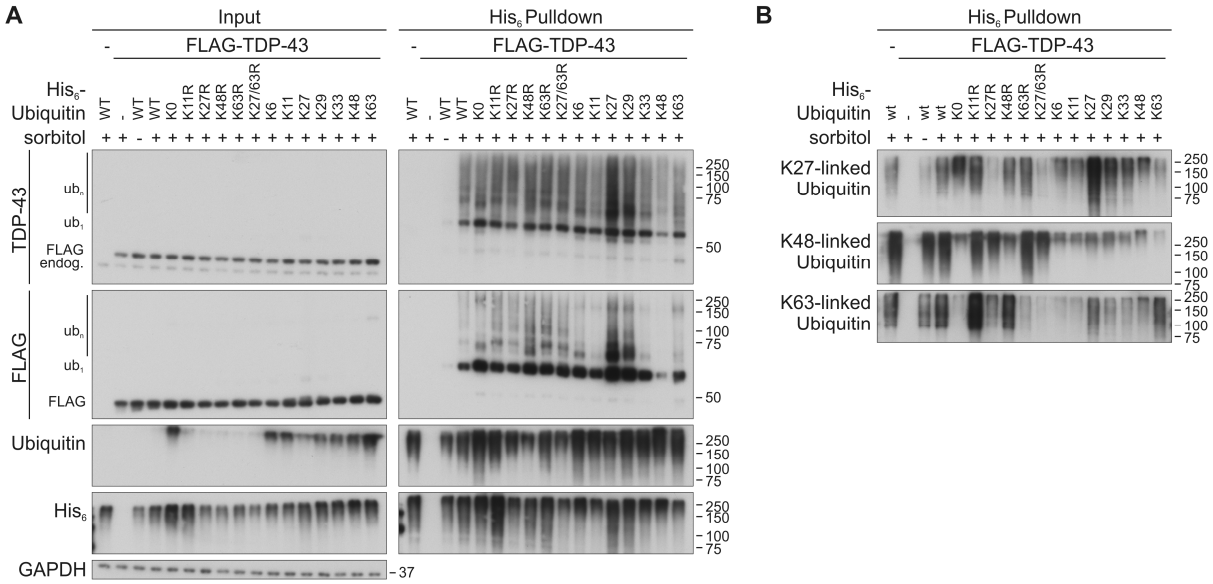


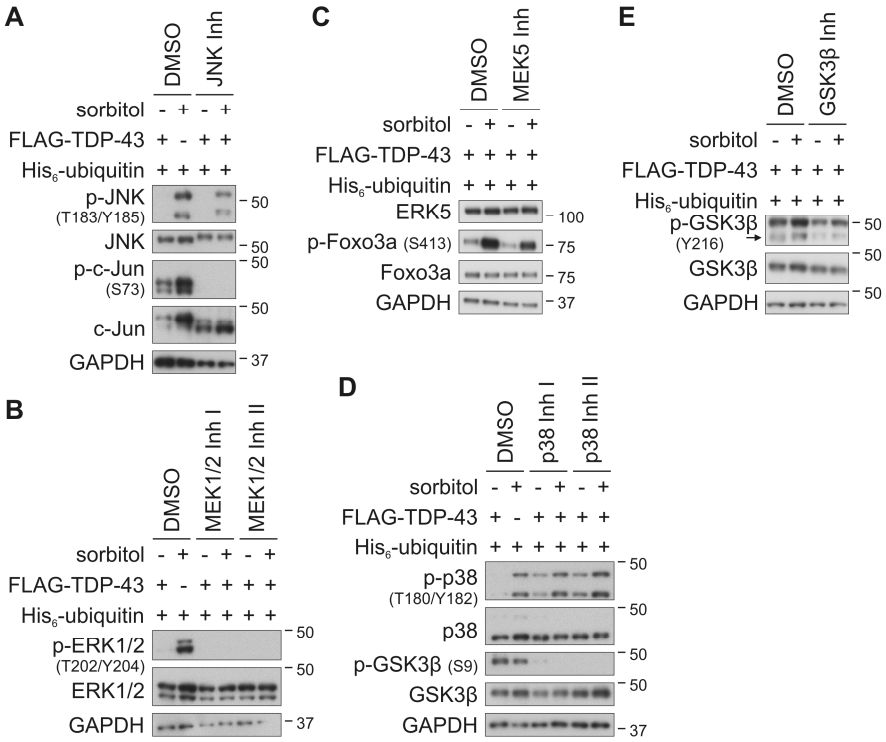
Supporting Figure S1. FUS ubiquitylation is not increased upon exposure to osmotic and oxidative stress. (*A*) HEK293E cells were transfected with 3xFLAG-FUS (+), His_6-ubiquitin (+), or 3xFLAG- and His_6-control vectors (-). The cells were treated either with control medium (-), sorbitol (sorb) or sodium arsenite (ars) for 1h, and lysed in 8M urea buffer. His_6-ubiquitin conjugated proteins were purified and total protein (Input) and eluates (His_6 Pulldown) were subjected to Western blotting analysis with antibodies detecting FUS, FLAG, ubiquitin and GAPDH. (*B*) Immunofluorescence analysis of endogenous FUS (green) and the SG marker TIAR (red) in HEK293E cells that were exposed to sorbitol, arsenite or control medium (no stress) for 1h. Cell nuclei were counterstained with Hoechst 33342 (blue). Scale bars are 20µm.



Supporting Figure S2. Analysis of the involvement of candidate enzymes in osmotic stress induced TDP-43 ubiquitylatoin. (*A*) HeLa cells that lack endogenous expression of the E3 ligase Parkin were transfected with FLAG-TDP-43 (+), His_6-ubiquitin (+) or empty control vectors (-), as indicated. The cells were exposed to osmotic (sorb) oxidative (ars) stress or control medium (-) for 1h, followed by lysis with urea buffer. Ubiquitylated proteins were isolated by His_6 Pulldown with Ni-NTA agarose. Eluates (His_6 Pulldown) and were analyzed by Western blot together with total protein lysates (Input) with antibodies detecting TDP-43, FLAG- and His_6-tagged proteins, ubiquitin and GAPDH. (*B*) HEK293E cells expressing FLAG-TDP-43 and His_6-ubiquitin were co-transfected with MYC-UBPY WT, catalytically inactive C786S or a mutant lacking the whole C-terminus (ΔC) or empty control vectors (-). The cells were treated with sorbitol (+) or control medium (-) for 1h and the urea cell lysates were subjected to His_6-ubiquitin pulldown. Western blotting analysis of total protein (Input) and eluates (His_6 Pulldown) was performed with antibodies against TDP-43, FLAG-, His_6- and MYC-tagged proteins, and GAPDH as loading control.



Supporting Figure S3. Osmotic stress increases K27-linked poly-ubiquitylated TDP-43. (*A*) HEK293E cells expressing FLAG-TDP-43 were co-transfected with His6-ubiquitin lysine mutants, in which either one of seven lysines was mutated to arginine (KxR mutants) or all but the named lysine residue was exchanged (Kx mutants). Ubiquitin^{K0} had all lysines mutated to arginine. The cells were stressed with sorbitol for 1h, followed by lysis with 8M urea buffer and isolation of His_6-ubiquitylated proteins with Ni-NTA purification. Total proteins (Input) and eluates (His_6 Pulldown) were analyzed by Western blot with antibodies detecting TDP-43, FLAG, ubiquitin, His_6 and GAPDH. (*B*) His_6-ubiquitin eluates from (A) were analyzed with K27-, K48- and K63-linkage specific ubiquitin antibodies.



Supporting Figure S4. Confirmations of kinase pathway inhibition. (A-E) Total protein lysates from the experiment shown in Fig. 7 were analyzed for efficient kinase pathway inhibition by the inhibitors for JNK (A), MEK1/2 (B), MEK5 (C), p38 (D) and GSK3β (E). Western blotting analysis with the indicated phosphorylation-specific and total protein antibodies against the targeted and downstream kinases showed sorbitol-induced activation and successful inhibition of the different pathways. Phosphorylation of c-Jun was completely abolished upon JNK inhibition, as well as ERK1/2 phosphorylation under MEK1/2 inhibited conditions (A+B). GSK3β activity is negatively controlled by $p38^{MAPK}$ signaling so $p38^{MAPK}$ inhibition removed completely the inactivating phosphorylation at S9 (D). The inhibition of MEK5 was verified by detection of reduced Foxo3a phosphorylation (C), and activity of GSK3β was also reduced as could be seen by decrease of Y216 phosphorylation (E).