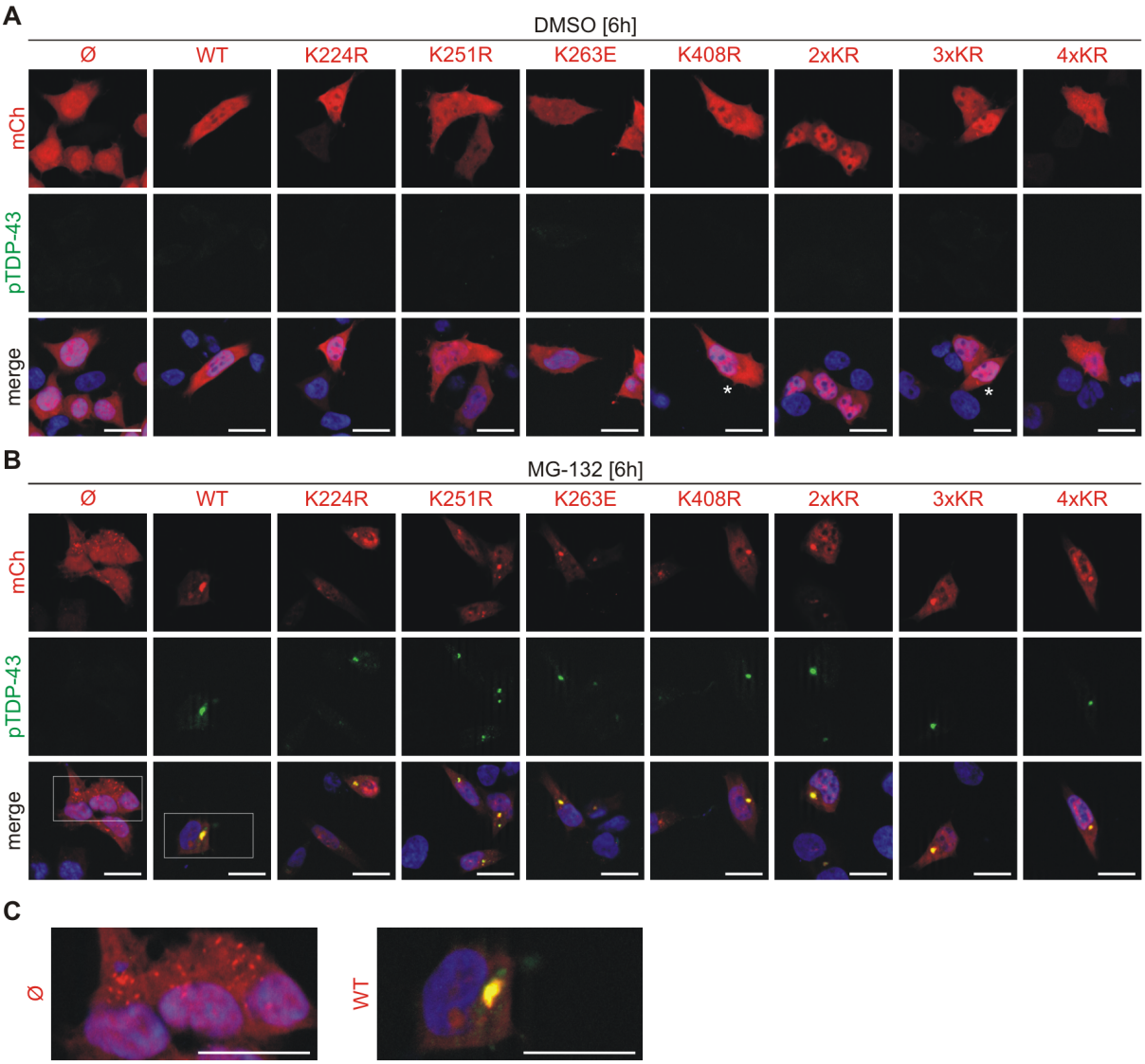
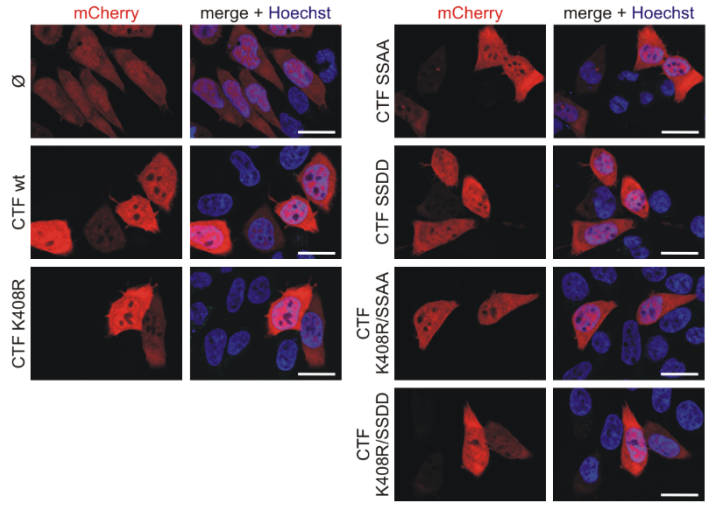
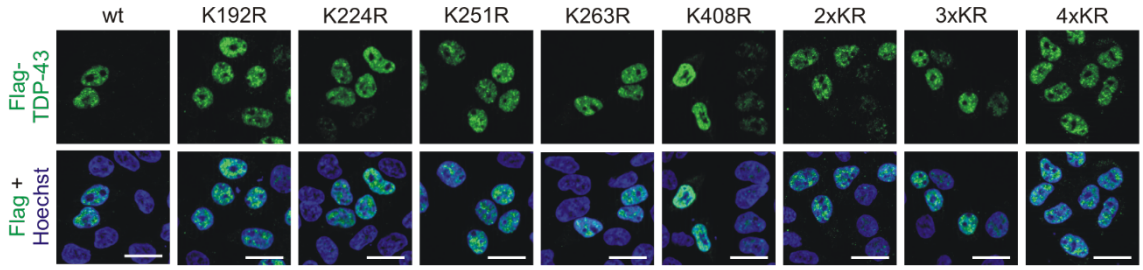


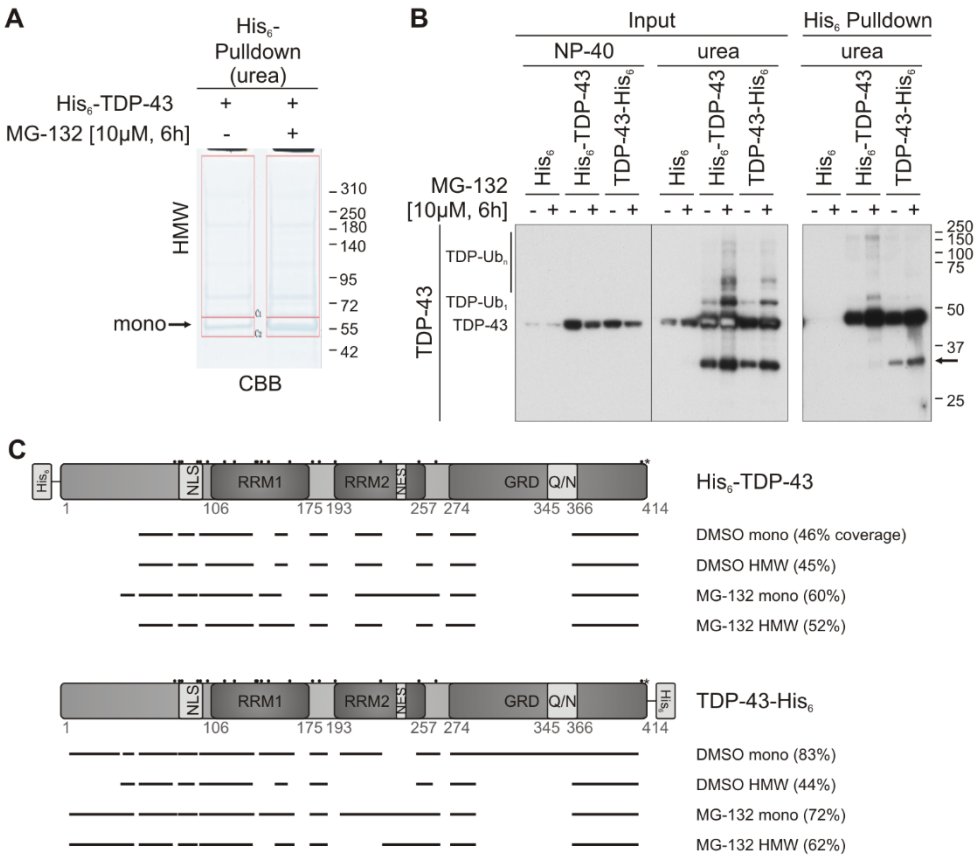

**Supporting Figure S1. Aggregate formation of mCherry-CTF lysine mutants upon proteasomal inhibition.** HEK293E cells were transfected with mCherry-CTF$^{WT}$, the indicated CTF lysine mutants or mCherry-control (Ø). Cells were treated either with DMSO (*A*) or 10µM MG-132 (*B*) for 6h to induce aggregate formation, followed by fixation with PFA and immunolabeling with rat-anti-phospho-TDP-43 (green). Merged images show nuclear counterstaining with Hoechst 33342 (blue). Scale bars correspond to 20µM. Asterisks (*) in (*A*) label examples for cells with aggresome-like inclusion. (*C*) Magnification of mCherry-Ø and –CTF$^{WT}$ expressing cells upon MG-132 treatment from (*B*) to illustrate the tiny aggregates formed of mCherry in comparison to aggresome-like aggregates formed of mCherry-CTF.



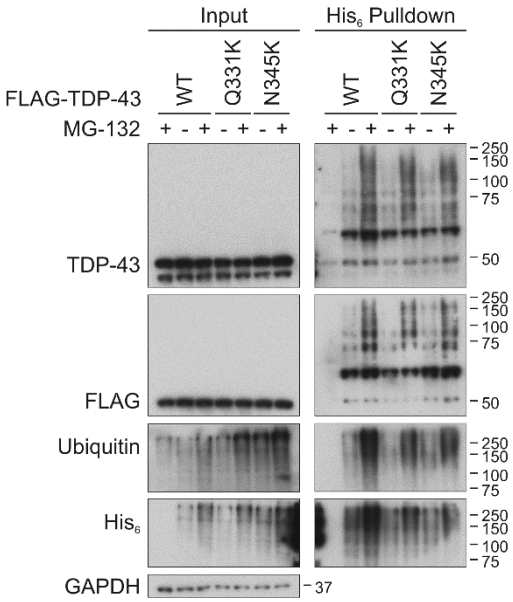

**Supporting Figure S2. Localization of mCherry-CTF phospho-mimic and –dead mutants.** HEK293E cells were transfected with mCherry-CTF$^{WT}$, -CTF$^{K408R}$, -CTF$^{SSAA}$, -CTF$^{SSDD}$ or the indicated double mutants. Cells were fixed after 72h, nuclei were counterstained with Hoechst33342 (blue) and epifluorescence of mCherry tag (red) was imaged. Scale bars correspond to 20µm.




**Supporting Figure S3. Localization of FLAG-TDP-43 C-terminal lysine mutants.** HEK293E cells were transfected with FLAG-TDP-43$^{WT}$ or the indicated C-terminal lysine mutants. The cells were fixed after 72h and overexpressed TDP-43 proteins were immunolabeled with mouse-anti-FLAG antibody (green), nuclei were counterstained with Hoechst 33342 (blue). Scale bars correspond to 20µM.




**Supporting Figure S4. Identification of TDP-43 ubiquitinylation sites with LC-MS/MS analysis.** HEK293E cells were transfected with $His_6$-TDP-43 or TDP-43-$His_6$ as indicated. In (+) conditions, ubiquitinylation was stabilized by proteasomal inhibition with MG-132 for 6h; (-) conditions are controls. The cells were harvested and separated into a soluble NP40 and insoluble urea fraction. 6xHis-tagged TDP-43 species were purified with Ni-NTA agarose. (*A*) Affinity purified $His_6$-TDP-43 samples were separated with SDS-PAGE, followed by coomassie brilliant blue (CBB) staining of the gel. The indicated mono-TDP-43 (mono) and higher molecular weight smear (HMW) were further subjected to mass spectrometry. (*B*) Western blot analysis of total protein (Input) and NP40- and urea eluates (Pulldown) with antibodies detecting TDP-43. $TDP\text{-}Ub_1$ - mono-ubiquitinylated TDP-43; $TDP\text{-}Ub_n$ - multi-ubiquitinylated TDP-43; arrow labels affinity-enriched 35kDa CTF (*C*) Schematic overview of $His_6$-TDP-43 (upper panel) and TDP-43-$His_6$ (lower panel), including the lysine residues as well as localization of the peptide fragments that were identified by mass spectrometry (visualizing MS coverage of TDP-43).




**Supporting Figure S5. Ubiquitinylation of clinical TDP-43 mutants introducing lysine residues.** HEK293E cells expressing the indicated FLAG-TDP-43 constructs and $His_6$-Ubiquitin were subjected to proteasomal inhibition with MG-132, followed by lysis with 8M urea buffer. $His_6$-Ubiquitin conjugated proteins were purified with Ni-NTA agarose and analyzed by Western blot using antibodies against TDP-43, FLAG, ubiquitin, $His_6$ and GAPDH.