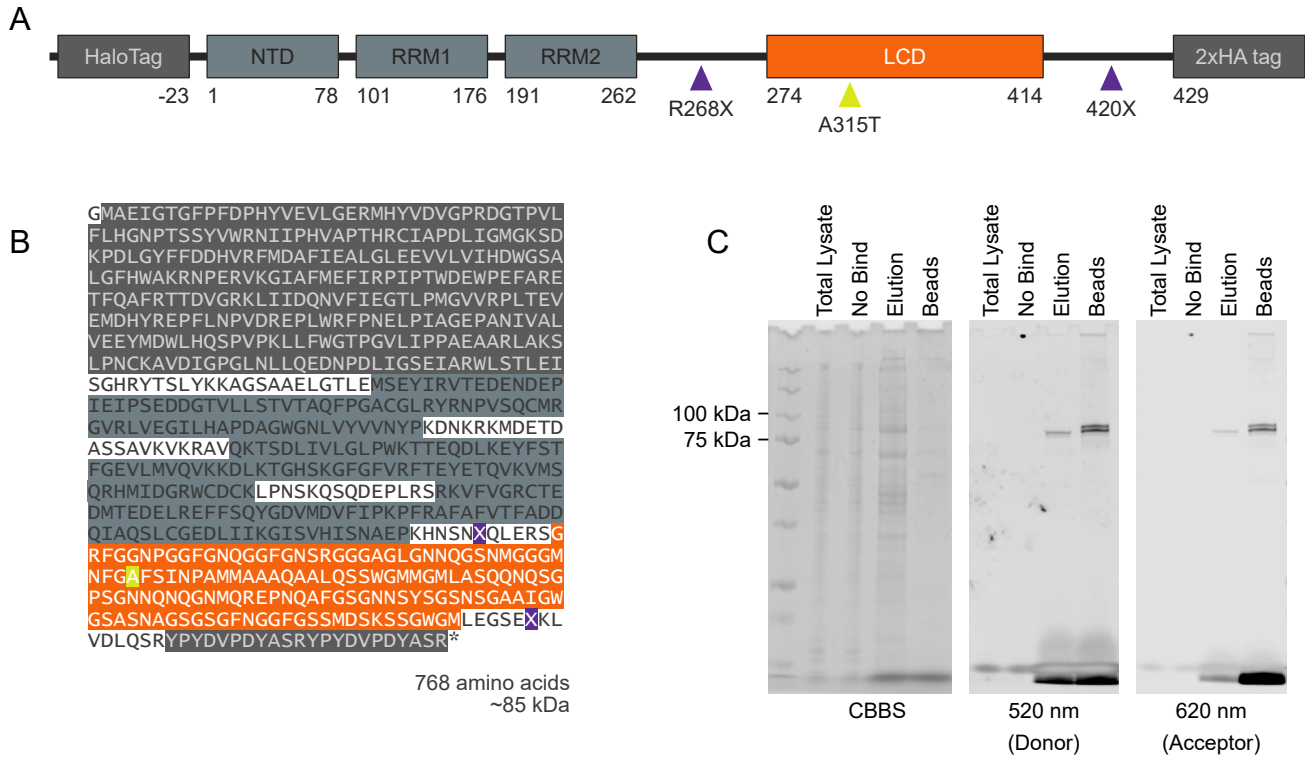




Supplemental Figure S1. Genetic code expansion in HEK293T cells for dye labeling of TDP-43

(A) Schematic of HALO-TDP43 with unnatural amino acids incorporated around LCD after elution via TEV protease cleavage. Boxes indicate TDP-43 regions and tags: the N-terminal domain (NTD), two RNA recognition motifs (RRM1 and RRM2), a low-complexity domain (LCD), a HaloTag, and a 2xHA tag. The last amino acid of HaloTag is the 23rd amino acid from the start of the TDP-43 sequence. Sites of unnatural amino acid (endo BCN-L-lysine) incorporation are indicated by violet triangles, where arginine 268 was replaced with unnatural amino acid (R268X), while the other site was incorporated at residue 420 (420X; the sixth residue after the end of the TDP-43 sequence, residue 414) in the linker before the 2xHA tag. The yellow triangle marks the site of the A315T mutation.

(B) The amino acid sequence of (A). The highlighted color corresponds to the regions in (A). X represents unnatural amino acid, and the A highlighted in yellow is alanine 315, which is mutated to threonine in the A315T mutant. The N-terminal glycine residue is left over from the TEV protease cleavage, and the asterisk marks the end of the sequence.

(C) Dye-labeling of HALO-TDP43. HEK293T cells expressing FLAG-tev-HALO-TDP43 incorporated with unnatural amino acids were lysed and incubated on anti-FLAG Dynabeads. The proteins were dye-labeled on beads and eluted via cleavage with TEV protease in naïve HEK293T cell lysate (note the multiple bands in the elution fraction of the CBB stain). Copper-free click chemistry-based dye labeling of endo BCN-L-lysine with tetrazine-Cy3 (Donor) and tetrazine-ATTO-647N (Acceptor) was confirmed by fluorescence imaging in gel via excitation at 520 nm and 620 nm, respectively, before staining with Coomassie Brilliant Blue (CBB). The two bands in the Elution fraction correspond to HALO-TDP43 with only one or two dyes incorporated (lower and upper bands, respectively). The Beads fraction indicates proteins that did not elute from the Dynabeads, and higher molecular weights correspond to dye-labeled but uncleaved FLAG-tev-HALO-TDP43.