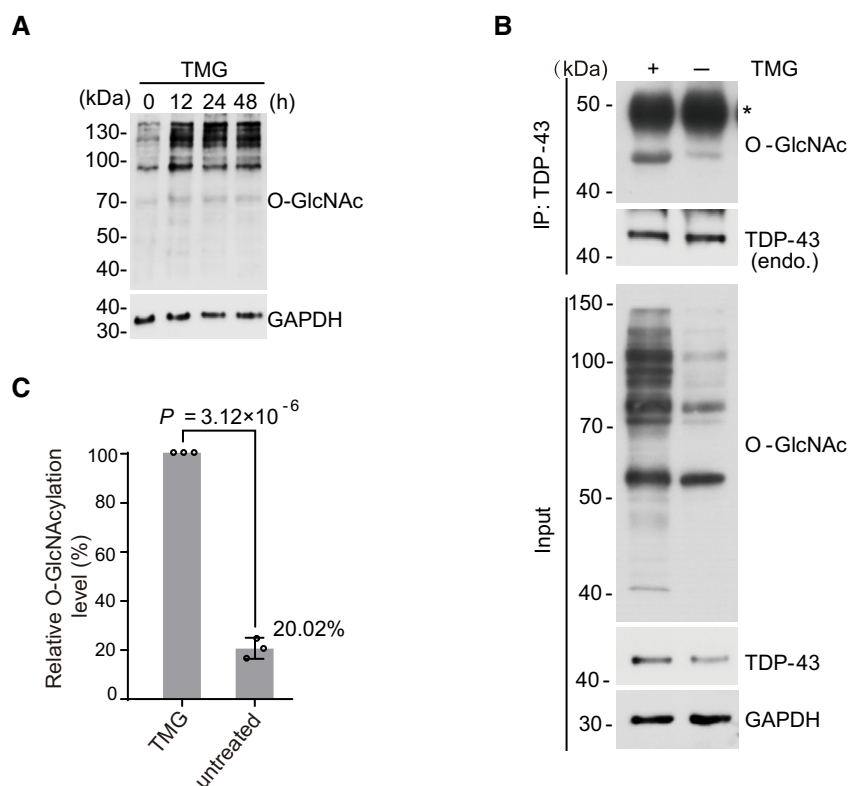


## Expanded View Figures



**Figure EV1. Measurement of the O-GlcNAcylation extent of TDP-43 in cellular context.**

- A SH-SY5Y cells were treated with 10  $\mu$ M TMG with the indicated times, and global O-GlcNAc levels were examined using the CTD110.6 antibody.
- B The O-GlcNAc levels of TDP-43 in SH-SY5Y cells treated with or without TMG for 48 h were examined. Endogenous (endo.) TDP-43 was indicated. The asterisk indicates heavy-chain bands.
- C Quantitative analysis was performed by comparing the relative O-GlcNAc levels of TDP-43 in TMG-treated vs. TMG-untreated cells in panel B from 3 biological replicates, data are presented as the mean  $\pm$  SD. The O-GlcNAc levels of TDP-43 in treated cells were set as 100%. Unpaired two-tailed t-test. P value is present. The number of 20.02% represents that the percentage of the O-GlcNAcylated modified TDP-43 in a physiological condition. Source data are available online for this figure.

Source data are available online for this figure.

**Figure EV2. Validation of O-GlcNAcylated modification on TDP-43.**

- A Mass spectra data show the original peptide sequences containing O-GlcNAcylation sites of TDP-43. Top: T199 site; bottom: S212, T233 sites.
- B Summary of three identified O-GlcNAcylated peptides of TDP-43 with significant ion scores by mass spectra analysis. "A score" represents the fidelity of MS.

Source data are available online for this figure.

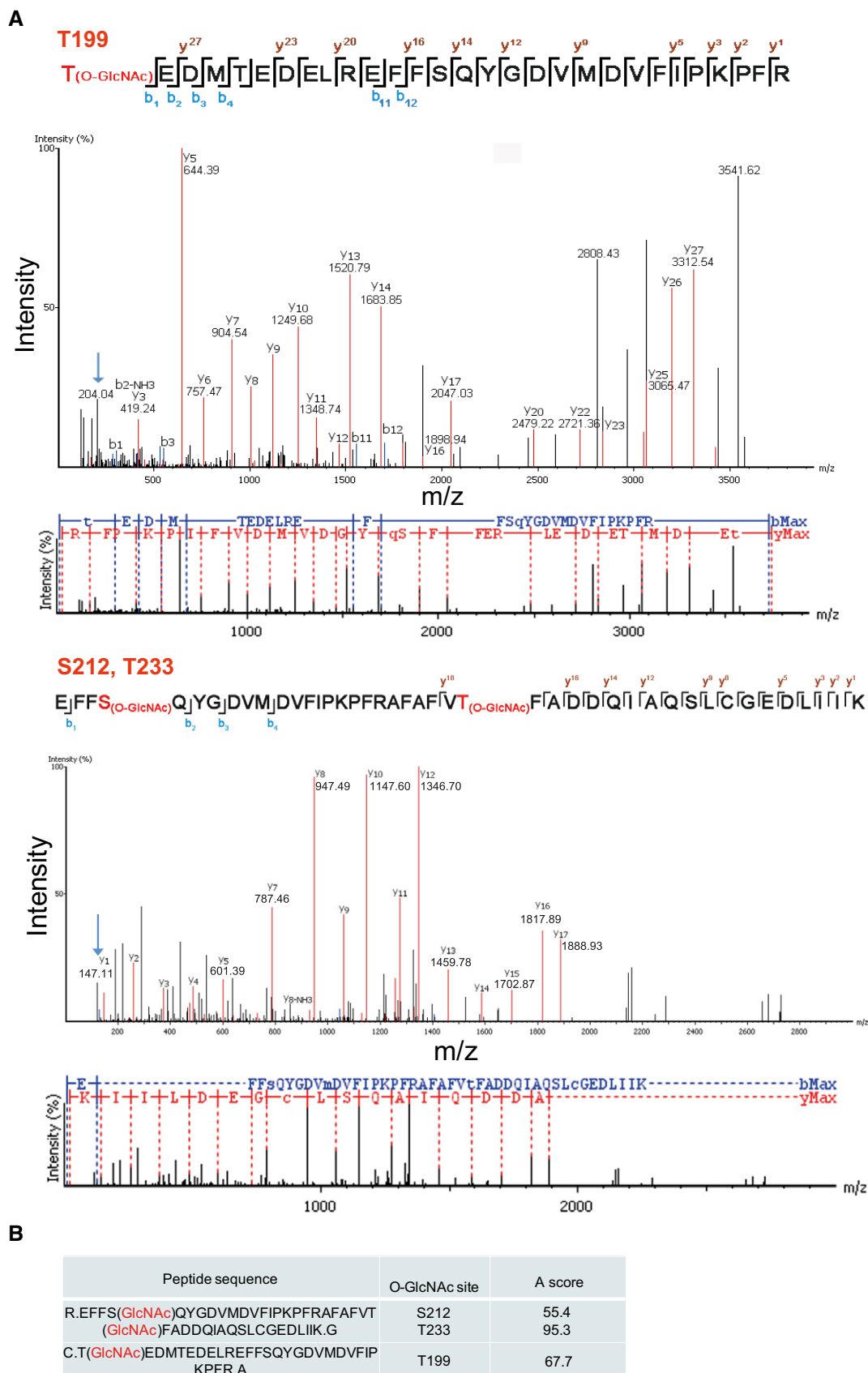
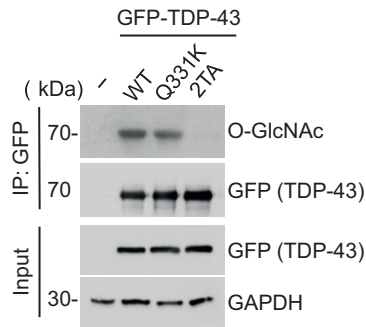
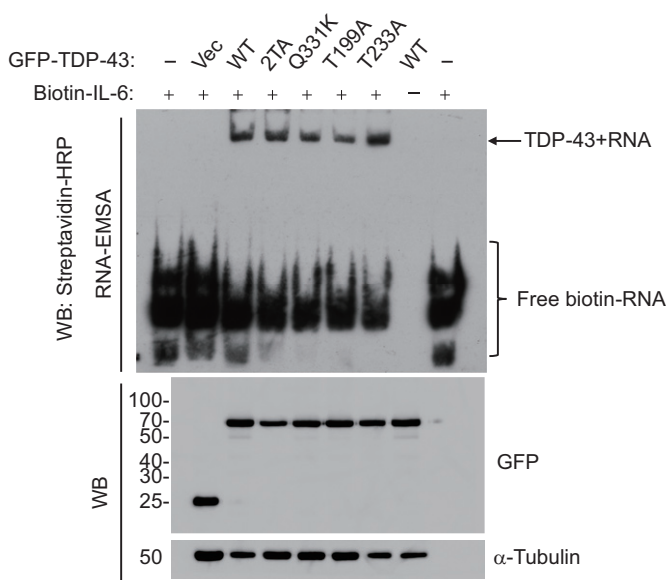


Figure EV2.



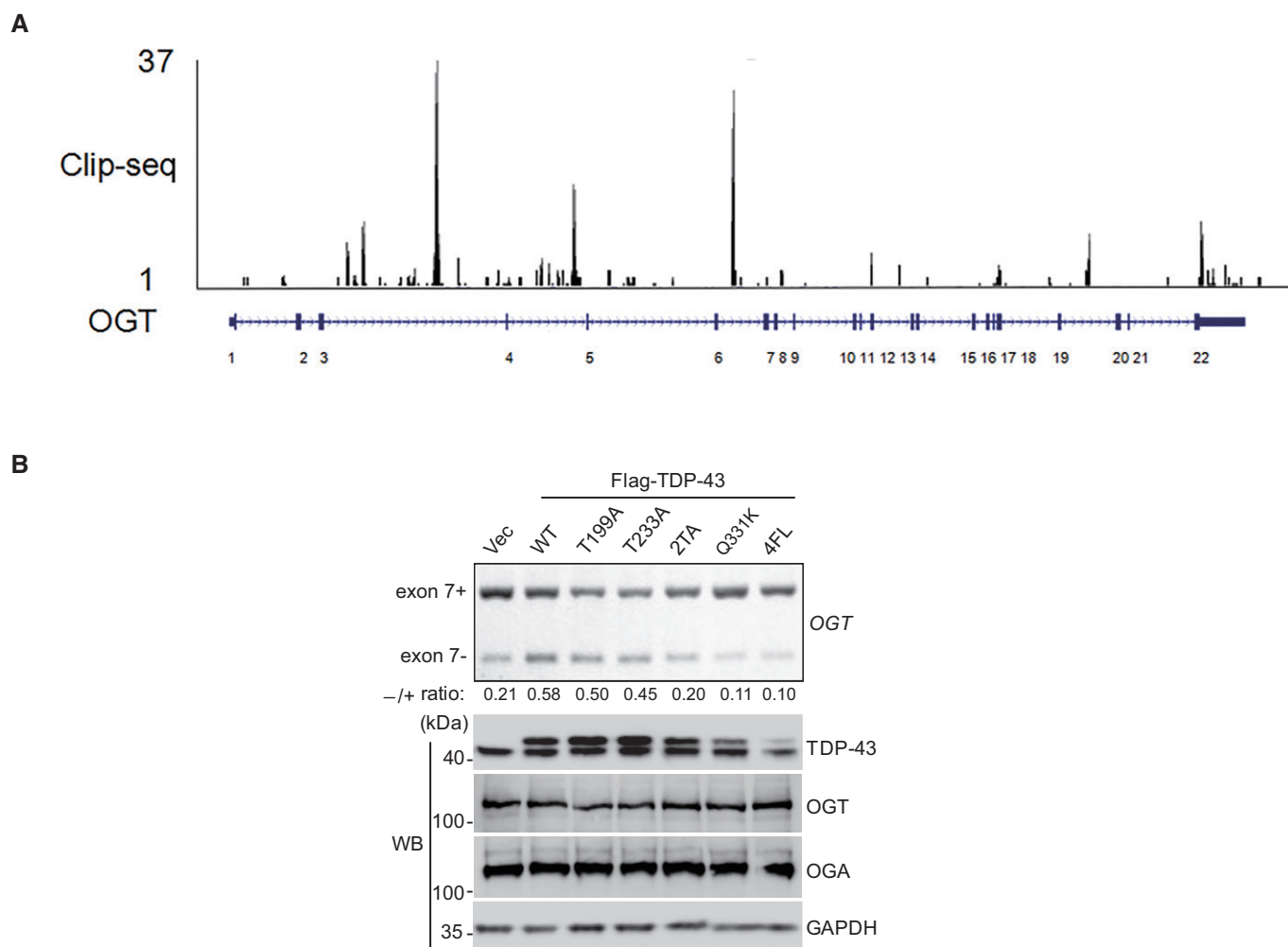
**Figure EV3. ALS-linked Q331K mutant of TDP-43 does not show obvious change of O-GlcNAcylation.**

Cells expressing the indicated forms of GFP-TDP-43 were subjected to immunoprecipitation, and the O-GlcNAcylated levels of TDP-43 were examined. Source data are available online for this figure.



**Figure EV4. O-GlcNAcylation deficient mutations do not affect the RNA-binding ability of TDP-43.**

RNA-EMSA was performed using total cell lysate from the indicated GFP-TDP-43 transfected HEK 293T cells and biotin-labeled IL-6 RNA probe (top panel). The indicated GFP-TDP-43 proteins were examined by immunoblotting (bottom panel). Source data are available online for this figure.



**Figure EV5. TDP-43 affects OGT mRNA splicing.**

A CLIP-Seq data show the RNA-binding sites on OGT of TDP-43. The numbers shown in y-axis represent the intensity of signals, and the numbers shown in x-axis represent exons of OGT.

B RNA splicing status of OGT at exon 7 was examined by PCR analysis using specific primers flanking exon 7. The number represented the ratios of exon 7 exclusion (–) vs. inclusion (+) (top panel). TDP-43, OGT, and OGA protein levels were examined by immunoblotting (bottom panel).

Source data are available online for this figure.