Supplemental Figures

FIG. S1. The theoretical fragment pattern of the FAT10-conjugated peptide. This pattern indicates that not only the target peptide but also the FAT10 fragment would generate b/y ions.

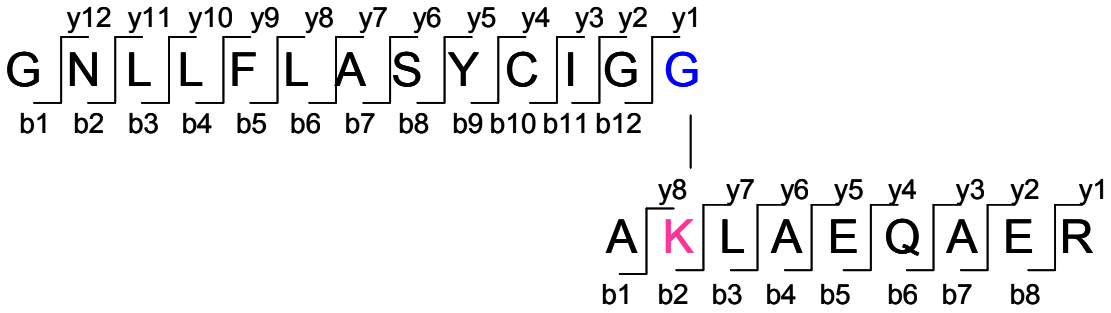

FIG. S2. The expression of the proteins G-FAT10 and G-FAT10-ΔGG in stable HeLa cell lines. The cell lysates were probed by western blot with the anti-FAT10 antibody. WT, wild type FAT10; ΔGG, FAT10ΔGG.

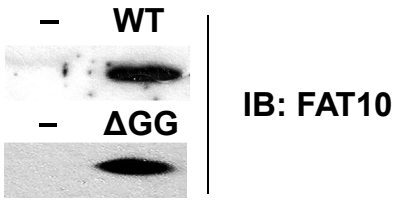

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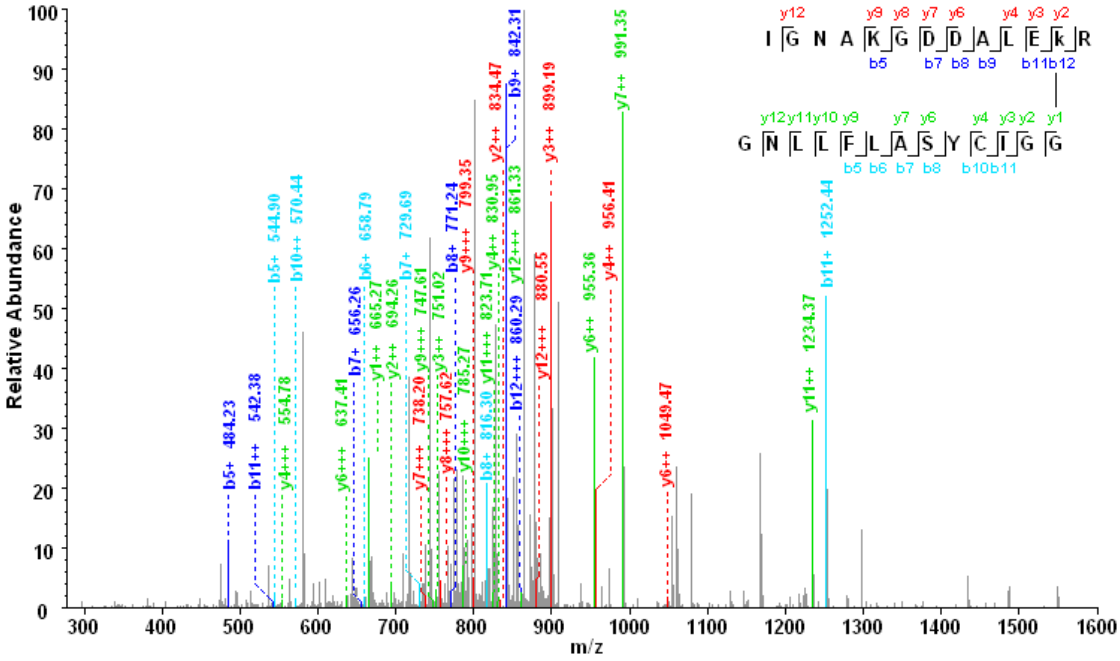
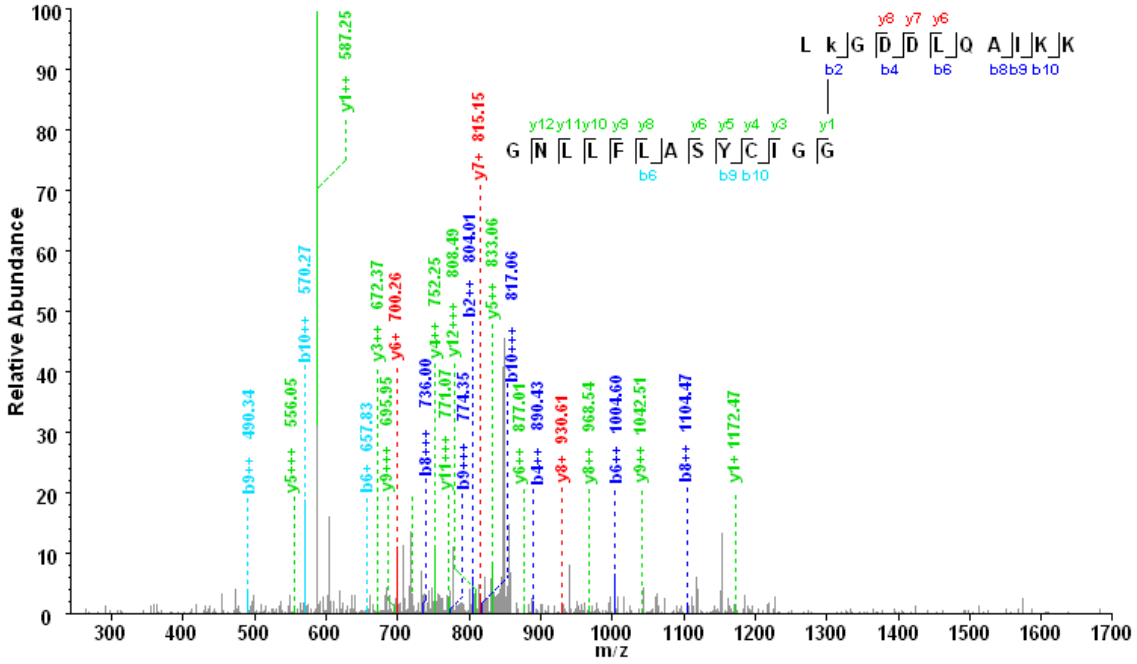
FIG. S3. MS/MS spectra of FAT10-conjugated peptides and sites.

A. IPI00001734.3 Phosphoserine aminotransferase



B. IPI00477313.3 Isoform C2 of Heterogeneous nuclear ribonucleoproteins



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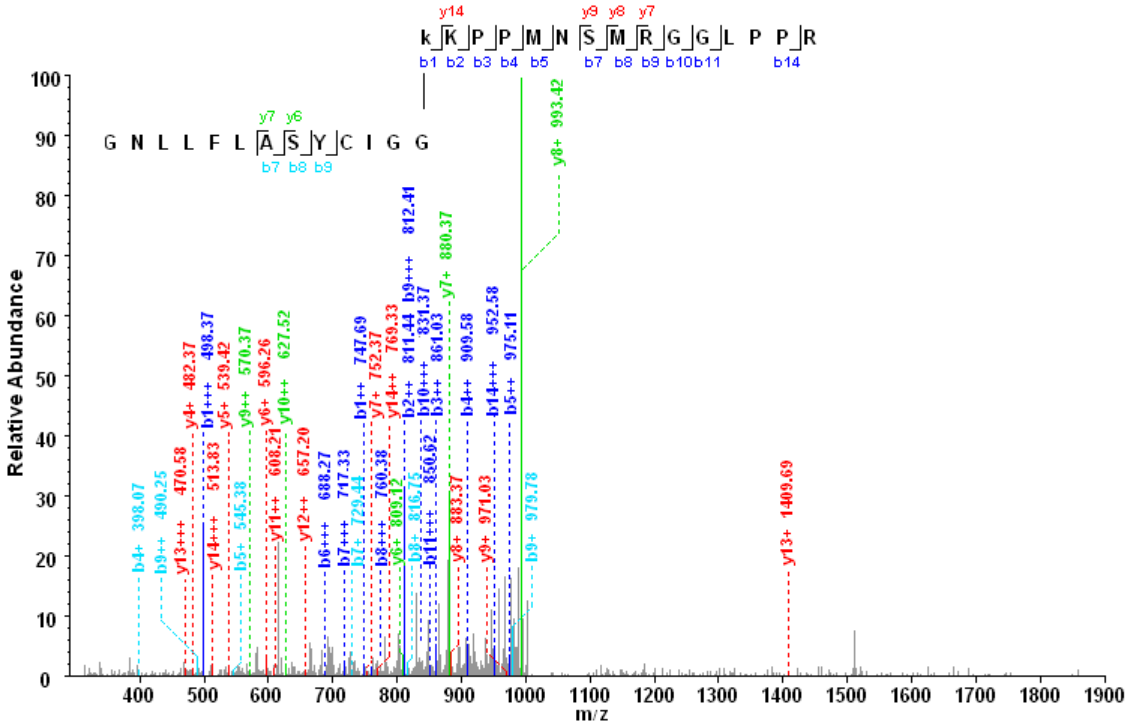
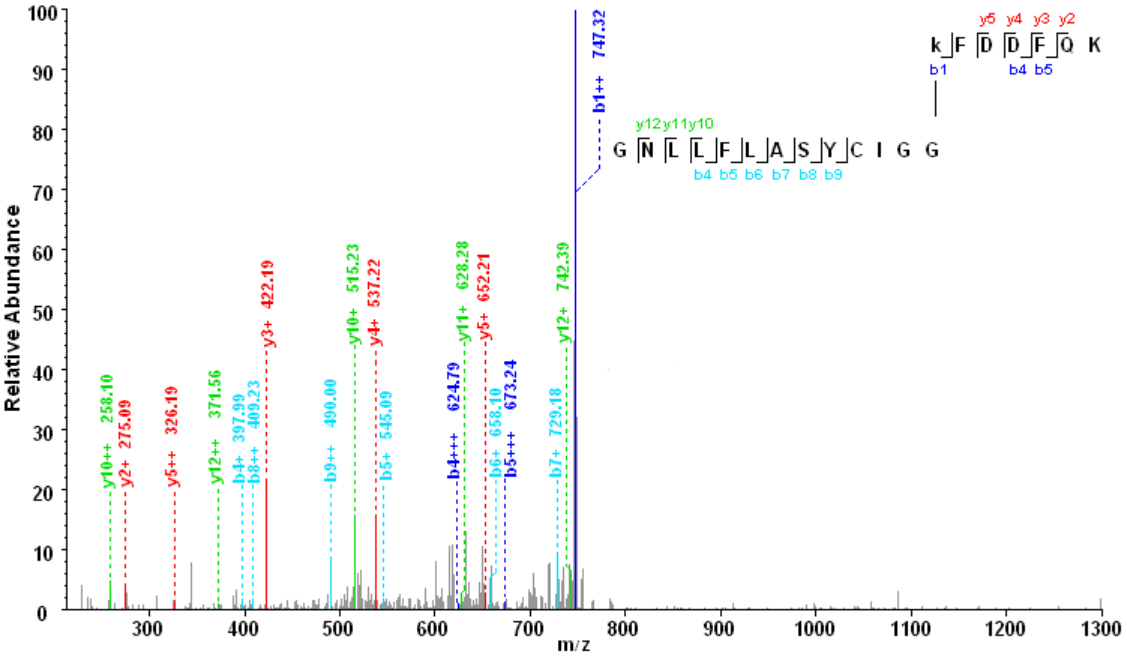
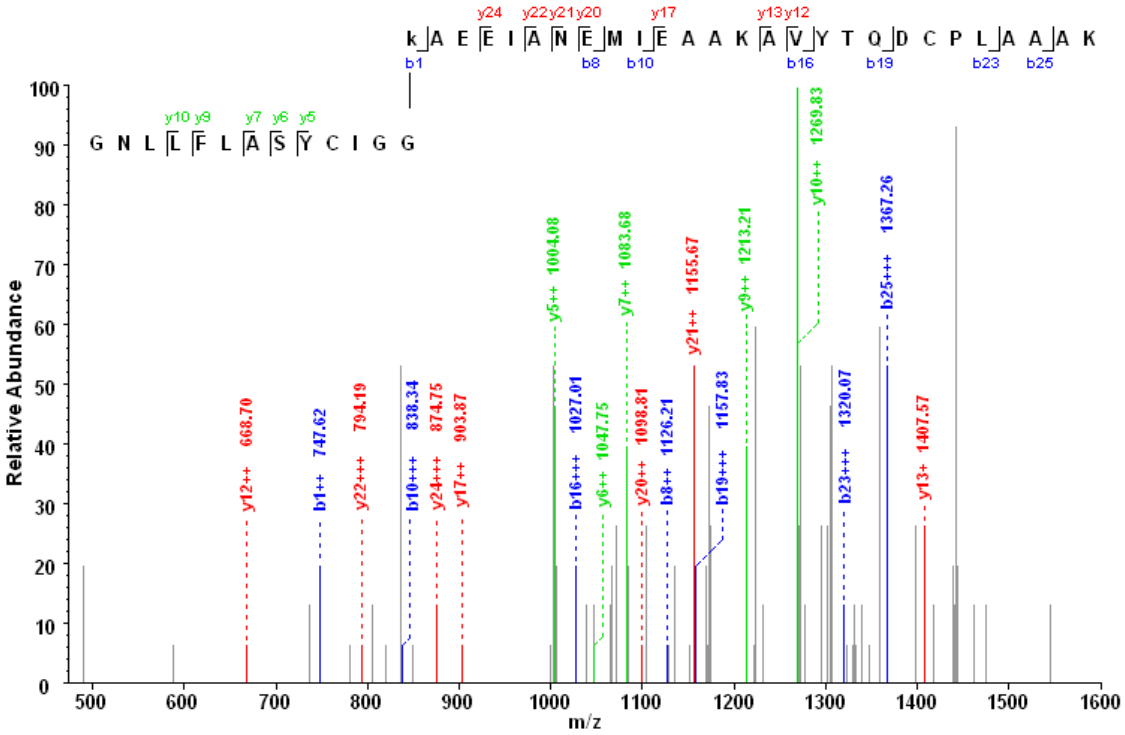
C. IPI00009841.6 RNA-binding protein EWS isoform 1



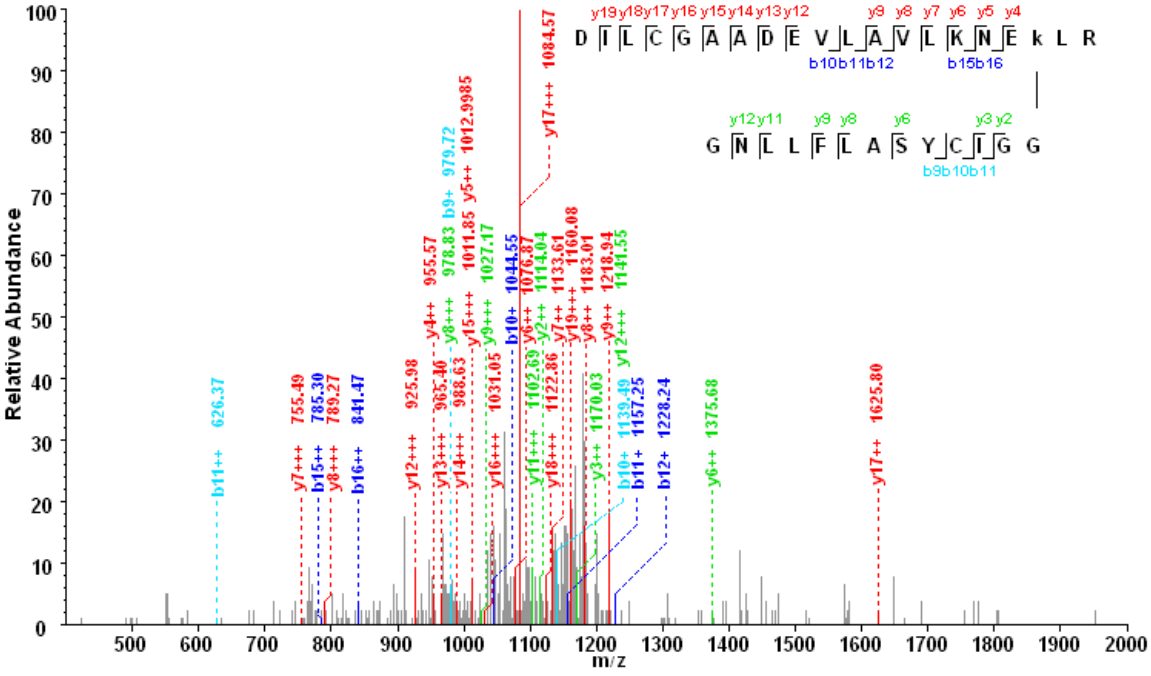
D. IPI00844215.1 Isoform 1 of Spectrin alpha chain



E. IPI00027442.4 Alanyl-tRNA synthetase



F. IPI00420014.2 Isoform 1 of U5 small nuclear ribonucleoprotein 200 kDa helicase



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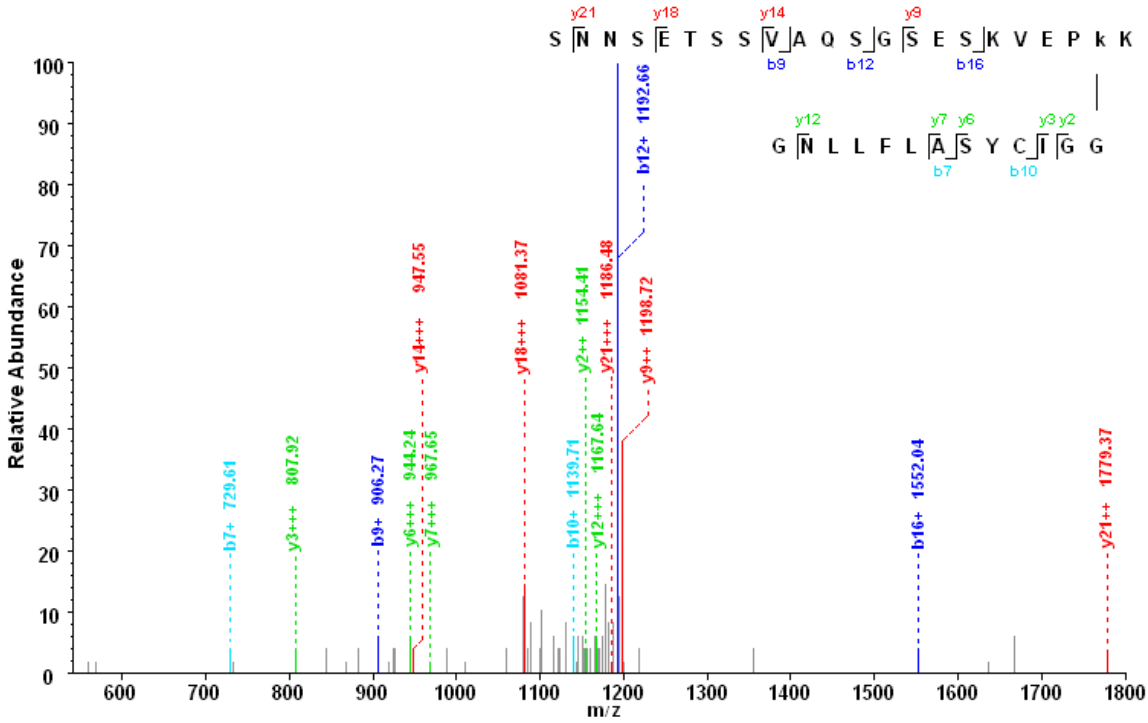
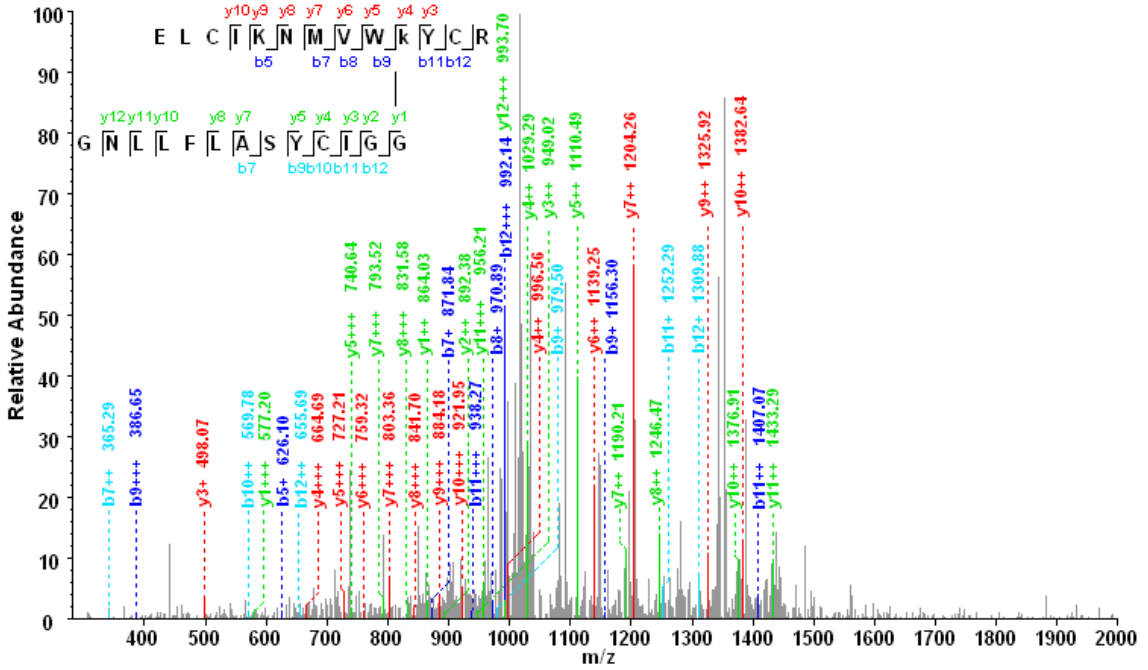
G. IPI00221325.3 E3 SUMO-protein ligase RanBP2



H. IPI00743335.3 Isoform 1 of Myosin-Ic



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I. IPI00221091.9 40S ribosomal protein S15a

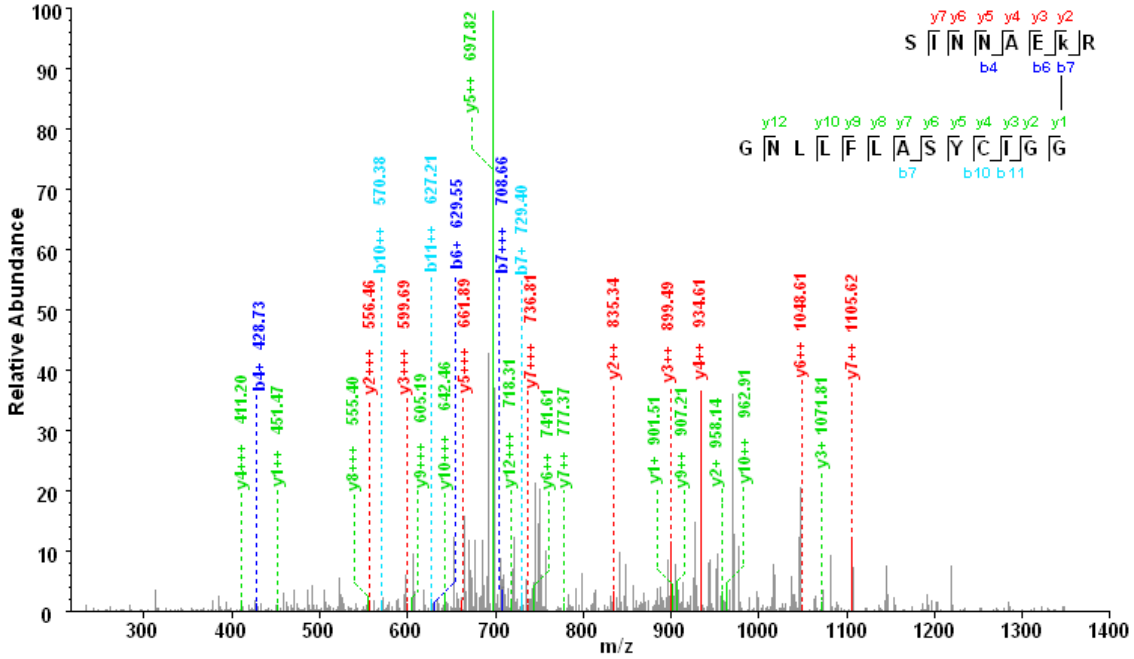

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FIG. S4. Examination of a six amino acid window adjacent to the FATylated sites. A, sequence logo presenting the potential preservation at the FATylation site.

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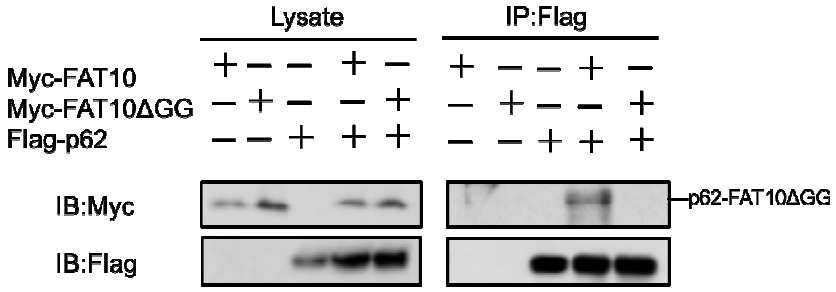
FIG. S5 FAT10 modifies p62 in a co-immunoprecipitation assay. The HEK293 cells were transfected with the indicated plasmids and treated with TNF-α, IFN-γ and MG132. After 24 h, the cells were collected. The Flag antibody was used to immunoprecipitate p62. The immunoprecipitates were then detected with the anti-Myc-HRP antibody.



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