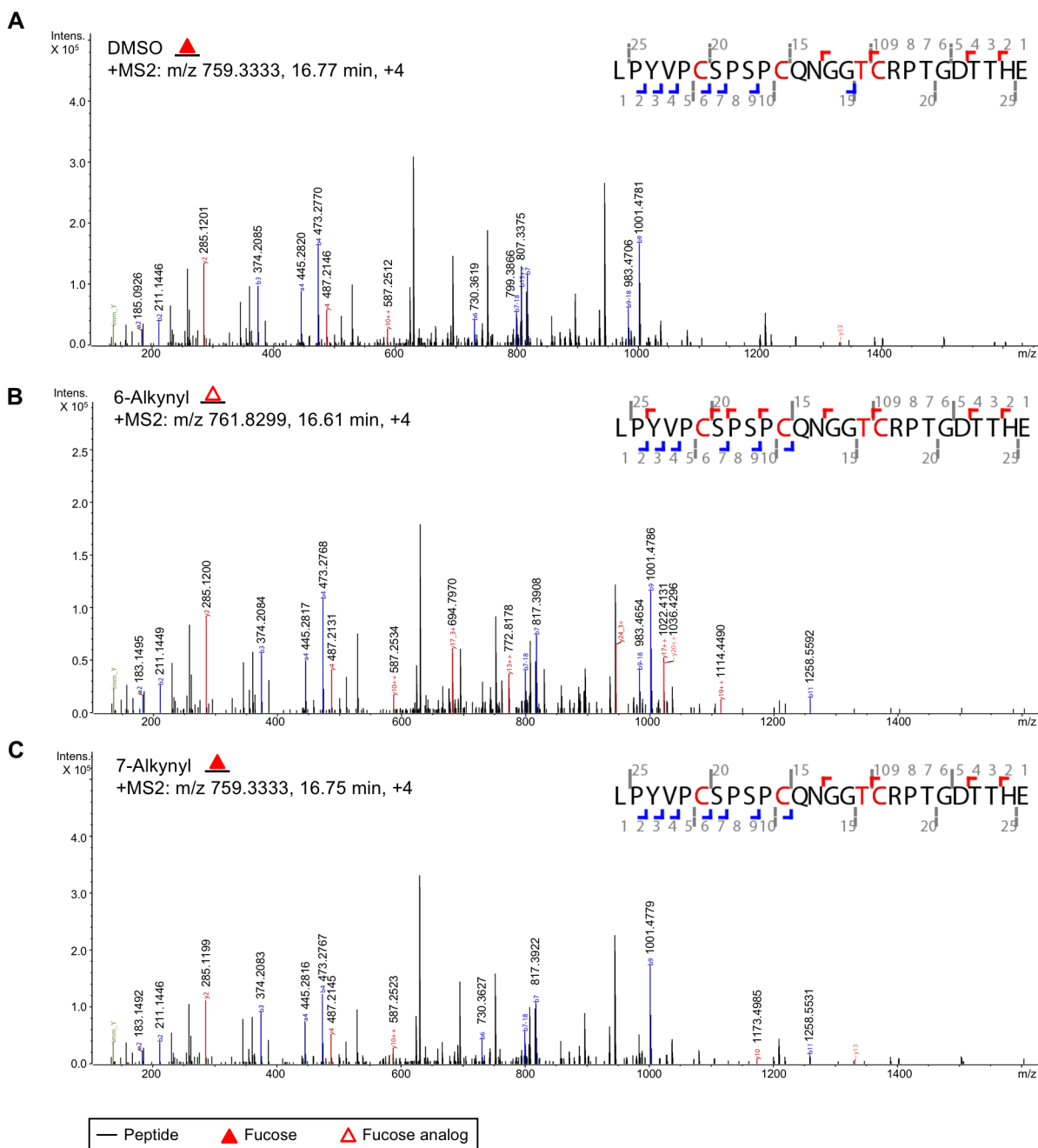
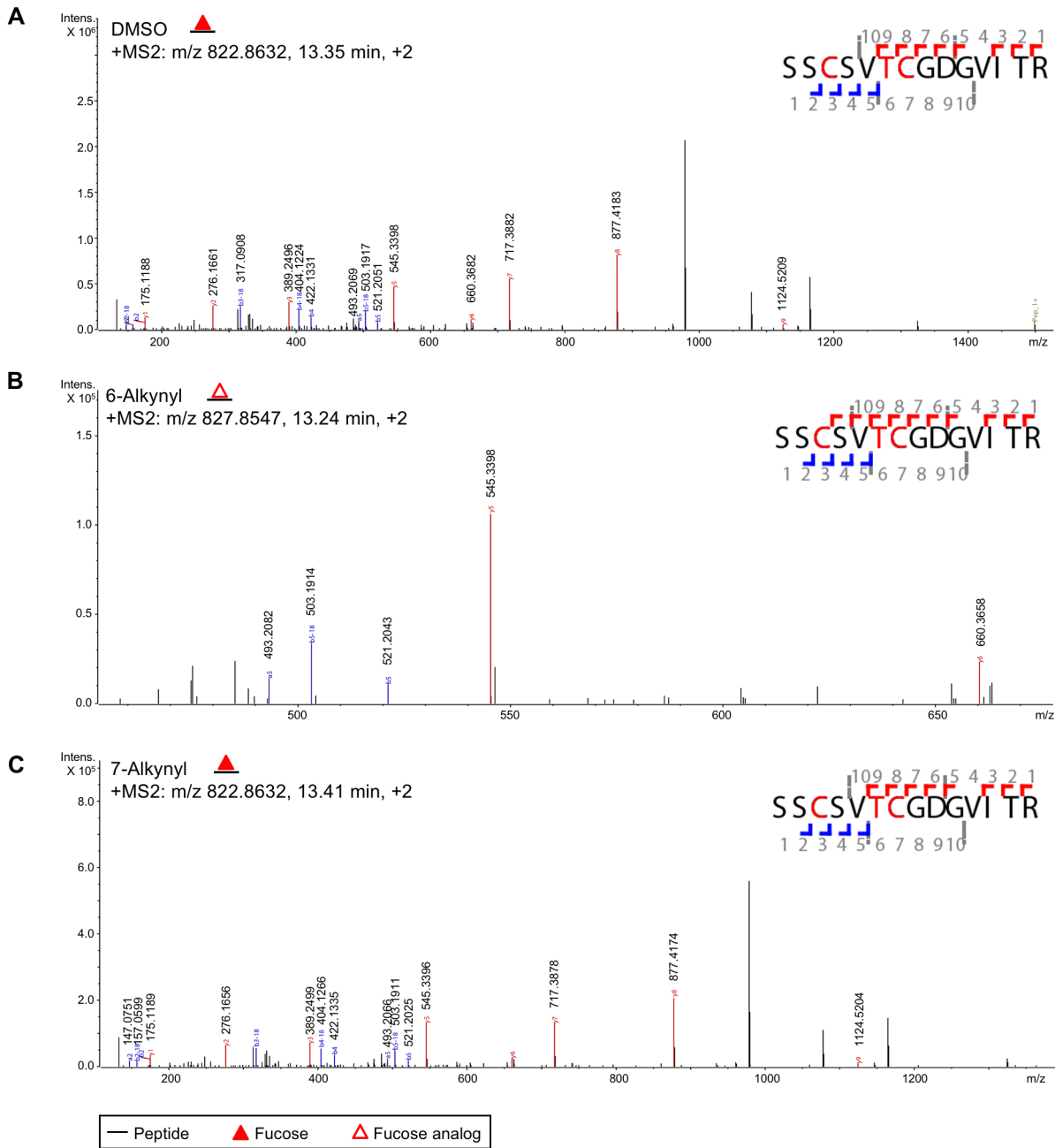


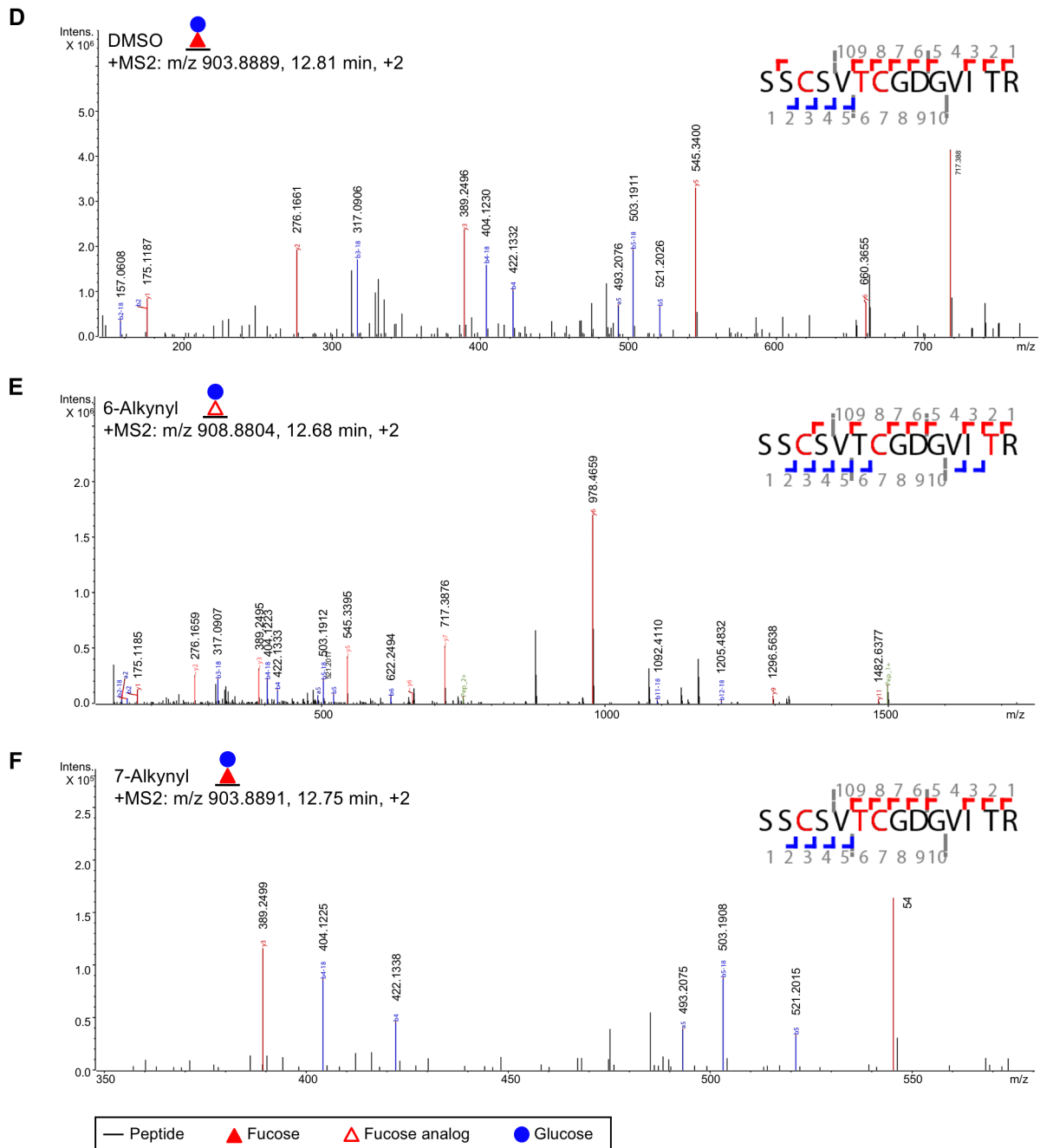
Supplementary Figure 1



Supplemental Figure 1. Mass spectra corresponding to EICs in Figure 2D. MS2 spectra of glycoforms of the peptide LPYVPCSPSPCQNGGTCRPTGDTT¹HE from mNOTCH1 EGF6 confirm the peptide assignment. **(A)** MS2 spectra of the peptide modified with Fuc in sample treated with DMSO. **(B)** MS2 spectra of the peptide modified with 6-Alk-Fuc in sample treated with peracetylated 6-Alk-Fuc. **(C)** MS2 spectra of the peptide modified with Fuc in sample treated with peracetylated 7-Alk-Fuc.

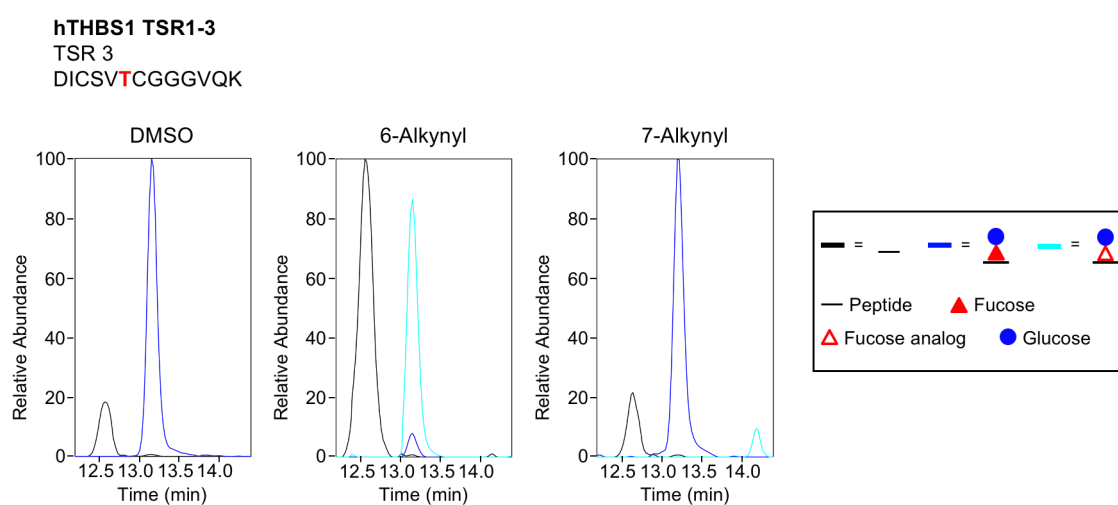
Supplementary Figure 2





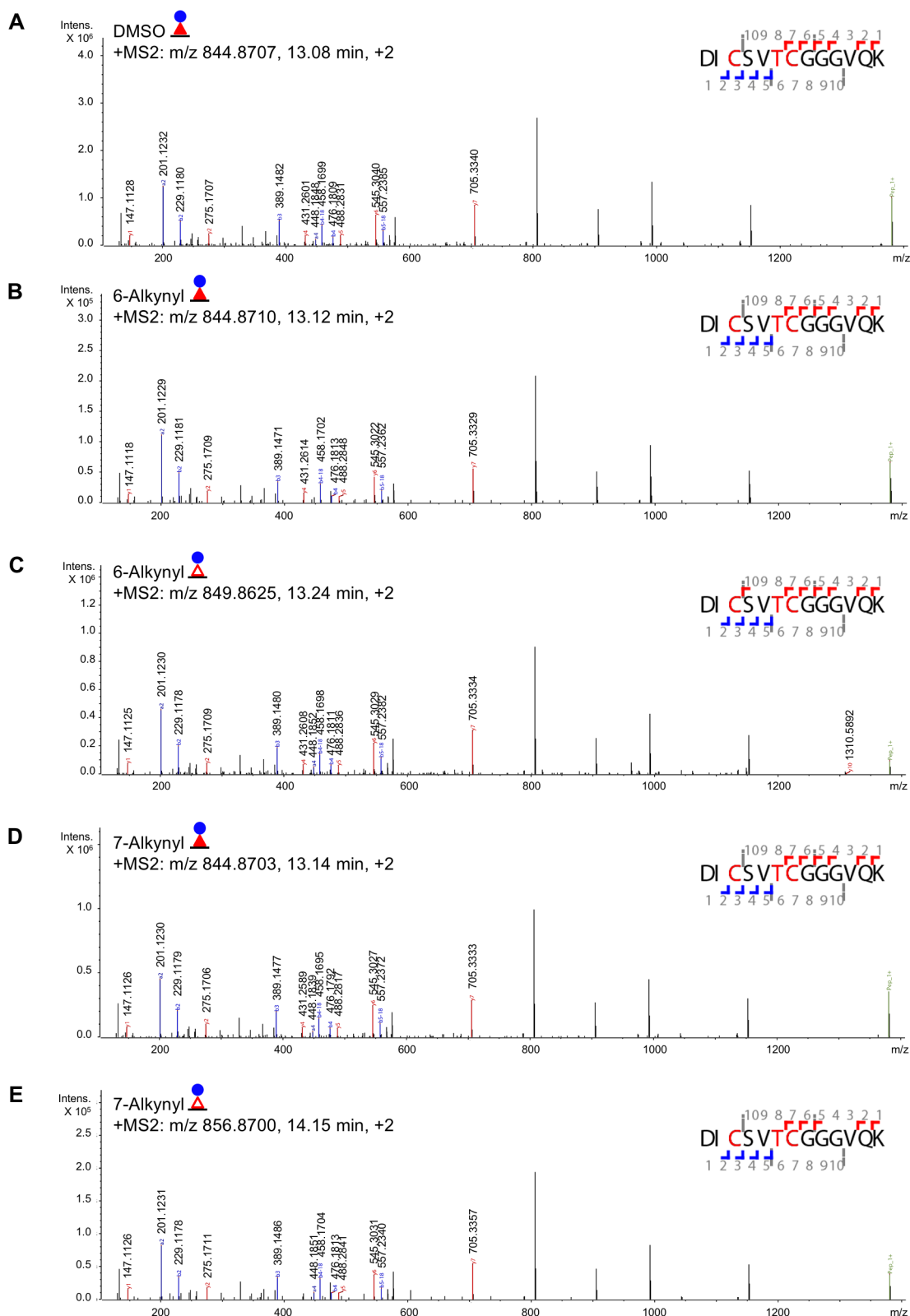
Supplemental Figure 2. Mass spectra corresponding to EICs in Figure 2F. MS2 spectra of glycoforms of the peptide SSCSVTCGDGVI TR from hTHBS1 TSR2 confirm the peptide assignment. (A) MS2 spectra of the peptide modified with Fuc monosaccharide in sample treated with DMSO. (B) MS2 of the peptide modified with 6-Alk-Fuc monosaccharide in sample treated with peracetylated 6-Alk-Fuc. (C) MS2 spectra of the peptide modified with Fuc monosaccharide in sample treated with peracetylated 7-Alk-fuc. (D) MS2 spectra of the peptide modified with Glc-Fuc disaccharide in sample treated with DMSO. (E) MS2 of the peptide modified with Glc-6-Alk-Fuc disaccharide in sample treated with peracetylated 6-Alk-Fuc. (F) MS2 spectra of the peptide modified with Glc-Fuc disaccharide in sample treated with peracetylated 7-Alk-Fuc.

Supplementary Figure 3



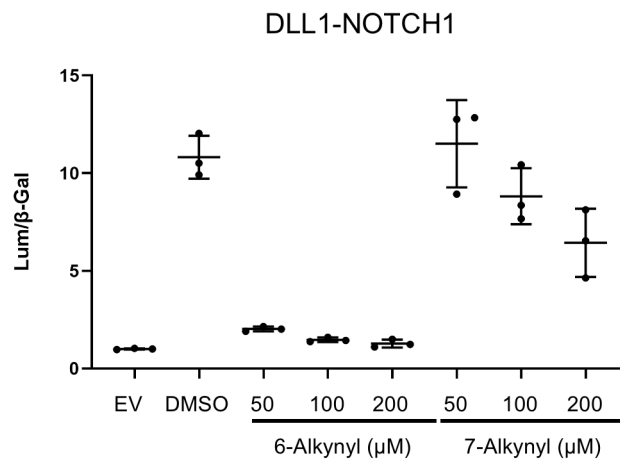
Supplemental Figure 3. 6-Alk-Fuc but not 7-Alk-Fuc is incorporated into TSR3 of hTHBS1 TSR1-3. HEK293T cells were transfected with hTHBS1 TSR1-3 plasmid and incubated with 50 μ M peracetylated 6-Alk-Fuc or 7-Alk-Fuc for 3 days. hTHBS1 TSR1-3 was purified from medium and analyzed by mass spectrometry as described in Methods. An EIC of the different glycoforms of a peptide from hTHBS1 TSR3 was prepared. Black line, unmodified; blue line, Glc-Fuc modified; aqua line, Glc-6-Alk-Fuc or Glc-7-Alk-Fuc.

Supplementary Figure 4



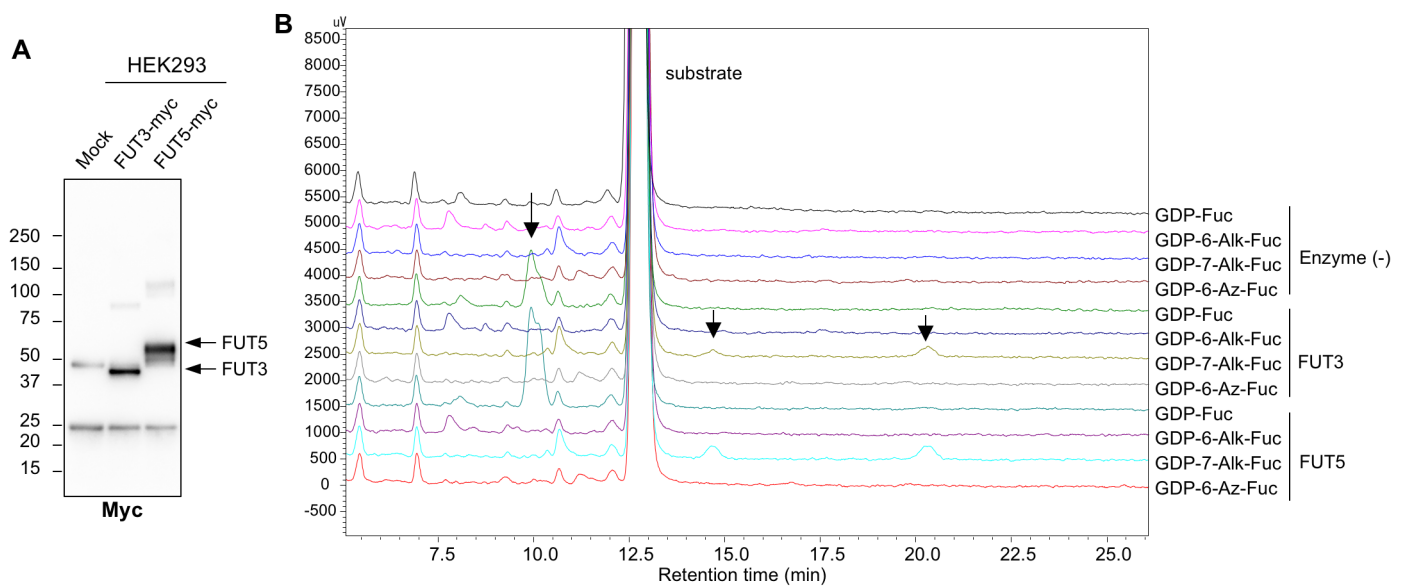
Supplemental Figure 4. Mass spectra corresponding to EICs in Supplemental Figure S3. MS2 spectra of glycoforms of the peptide DICSVTCTGGGVQK from hTHBS1 TSR3 confirm the peptide assignment. **(A)** MS2 spectra of the peptide modified with Glc-Fuc disaccharide in sample treated with DMSO. **(B)** MS2 of the peptide modified with Glc-Fuc disaccharide in sample treated with peracetylated 6-Alk-Fuc. **(C)** MS2 spectra of the peptide modified with Glc-6-Alk-Fuc disaccharide in sample treated with peracetylated 6-Alk-Fuc. **(D)** MS2 spectra of the peptide modified with Glc-Fuc disaccharide in sample treated with peracetylated 7-Alk-Fuc. **(E)** MS2 of a peptide modified with glucose-7-Alk-Fuc disaccharide in sample treated with peracetylated 7-Alk-Fuc.

Supplemental Figure 5



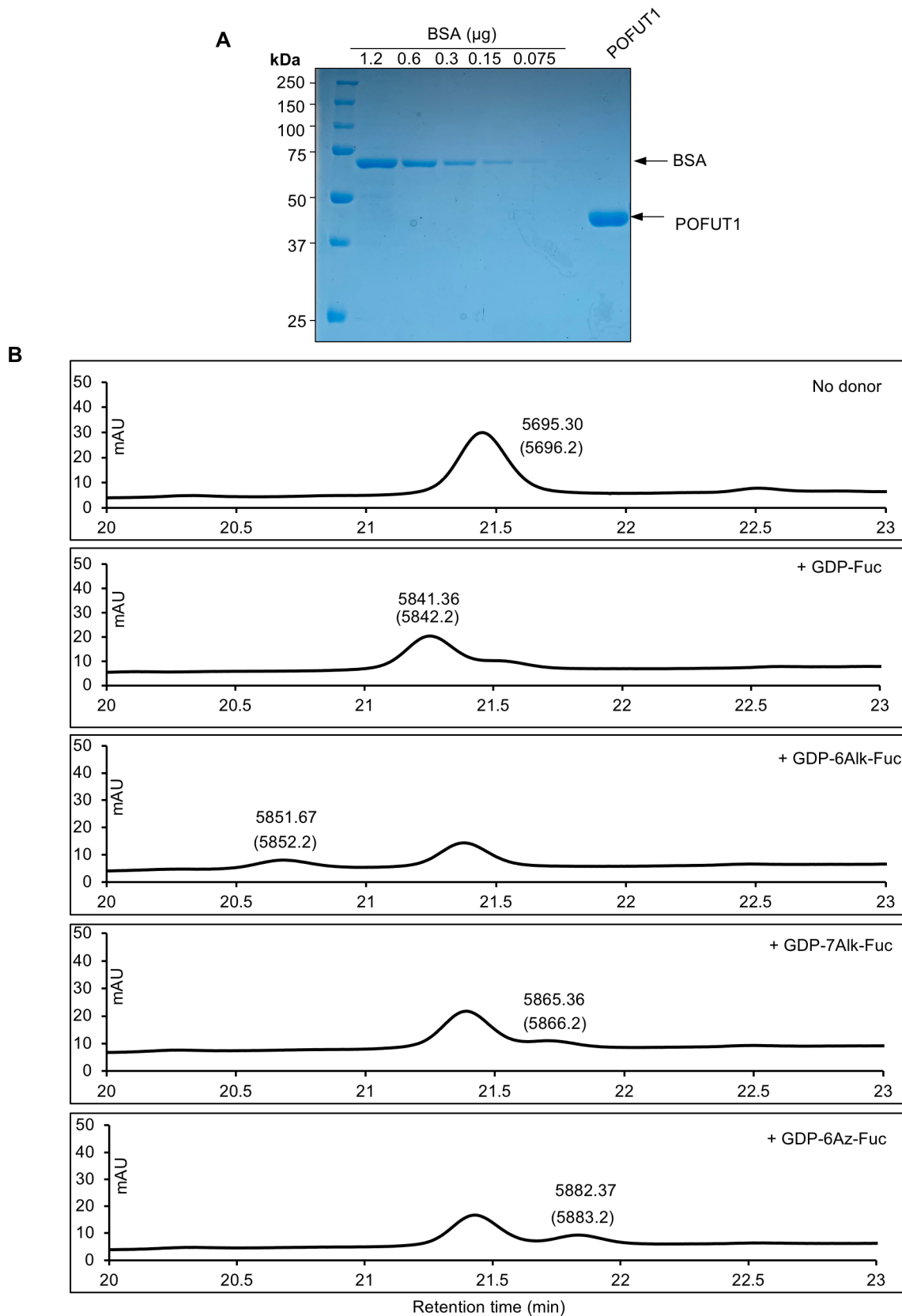
Supplemental Figure 5. 6-Alk-Fuc but not 7-Alk-Fuc inhibits DLL1-mediated Notch signaling. NIH3T3 cells expressing mNOTCH1 were co-cultured with L-cells expressing DLL1 to initiate Notch signaling. Cells were incubated with 50 μM, 100 μM or 200 μM 6-Alk-Fuc or 7-Alk-Fuc for 24 h before testing. Cells transfected with empty vector (EV) were used as a negative control for Notch signaling. Cells expressing mNOTCH1 and treated with DMSO were used as a positive control. All plots represent mean ± SD. Lum, luminescence; β-Gal, β-galactosidase.

Supplementary Figure 6



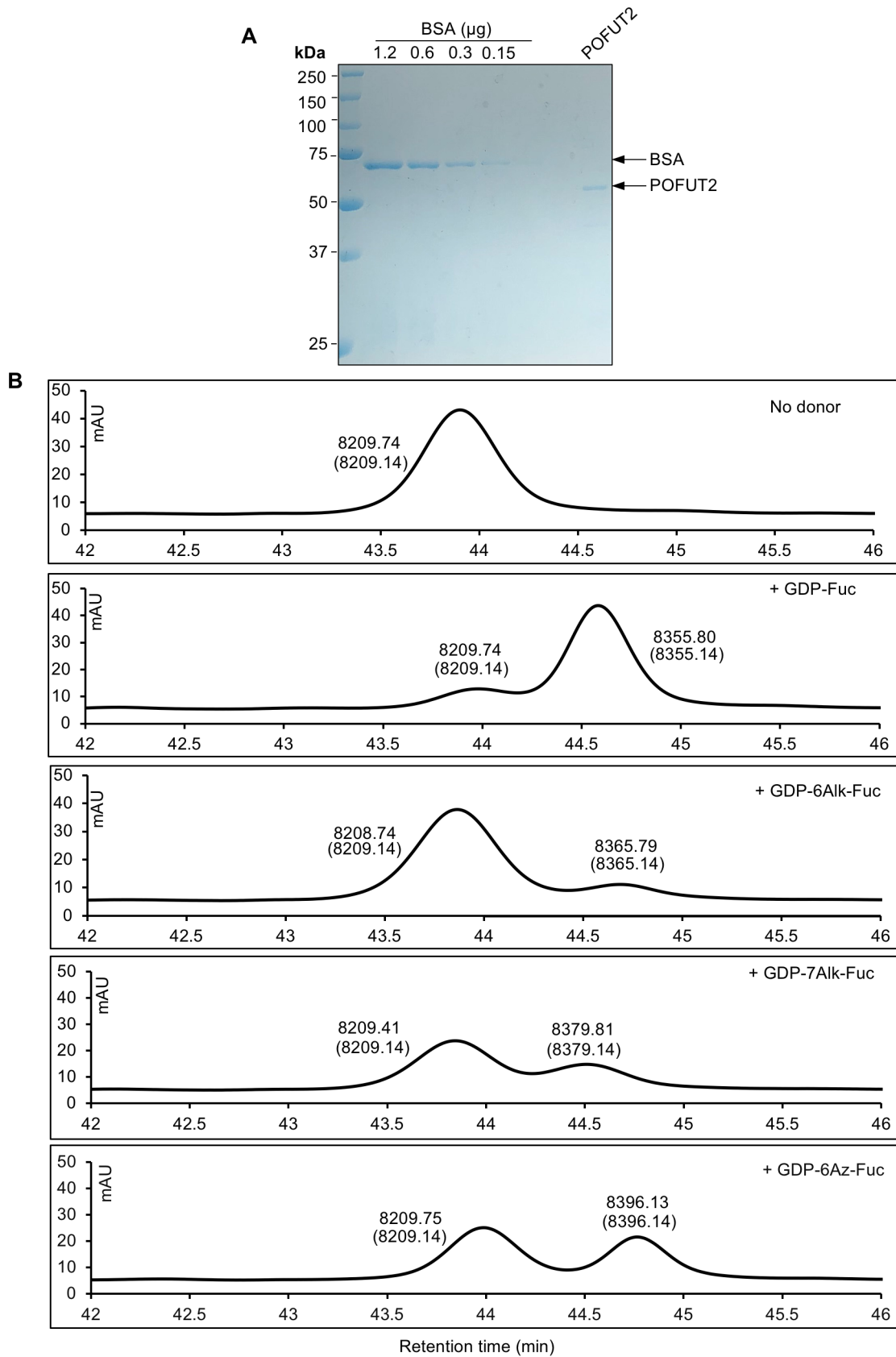
Supplementary Figure 6. In vitro activity assay of FUT3 and FUT5. (A) Full length myc-tagged human FUT3 and FUT5 were overexpressed in HEK293 cells, and immunopurified with anti-myc Ab and western blotted with the same Ab. **(B)** The purified enzymes were incubated with acceptor substrate (GGnGGnbi-PA) and donor substrate (GDP-Fuc or GDP-Fuc analog), and the reaction mixtures were analyzed by reversed-phase HPLC. Arrows indicate the products of enzymatic reactions.

Supplementary Figure 7



Supplementary Figure 7. In vitro activity assay of POFUT1. (A) His-tagged human POFUT1 lacking C-terminal RDEF sequence (POFUT1) proteins were expressed in HEK293T cells, purified from the medium by Ni-NTA agarose, and quantified by Coomassie brilliant blue staining. (B) The purified enzyme was incubated with acceptor substrate (human factor IX EGF1) and donor substrate (GDP-Fuc or GDP-Fuc analog), and the reaction mixtures were analyzed by reversed-phase HPLC. The elution profiles are shown, and determined masses (Da) are indicated. The predicted masses (Da) of proteins are indicated in parentheses.

Supplementary Figure 8



Supplementary Figure 8. In vitro activity assay POFUT2. (A) The flag-tagged mouse POFUT2 (POFUT2) was expressed in HEK293T cells and purified by DDDDK-tagged protein purification gel from cell lysates. POFUT2 enzyme was quantified by Coomassie brilliant blue staining. (B) The purified enzyme was incubated with acceptor substrate (TSP1-TSR3) and donor substrate (GDP-Fuc or GDP-Fuc analog), and the reaction mixtures were analyzed by reversed-phase HPLC. The elution profiles are shown, and determined masses (Da) are indicated. The predicted masses (Da) of proteins are indicated in parentheses.