Supplementary information

An embeddable molecular code for Lewis X modification through interaction with fucosyltansferase 9

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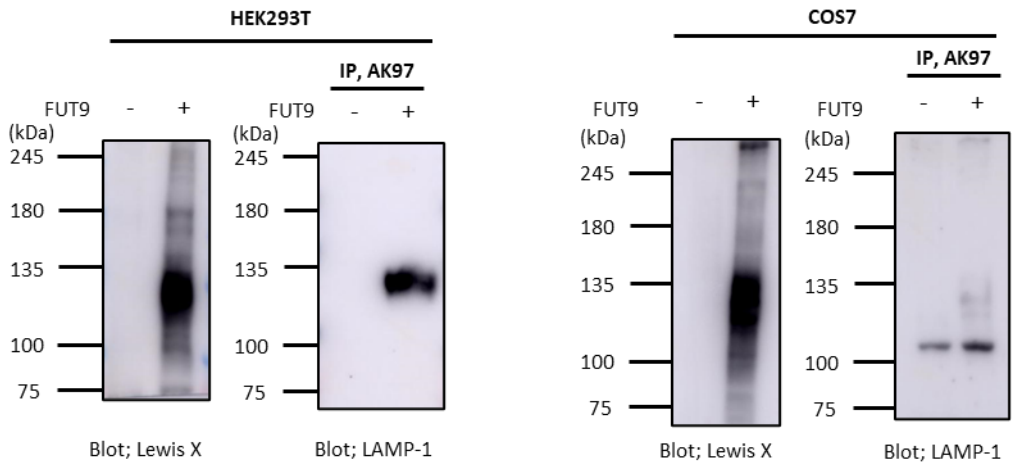
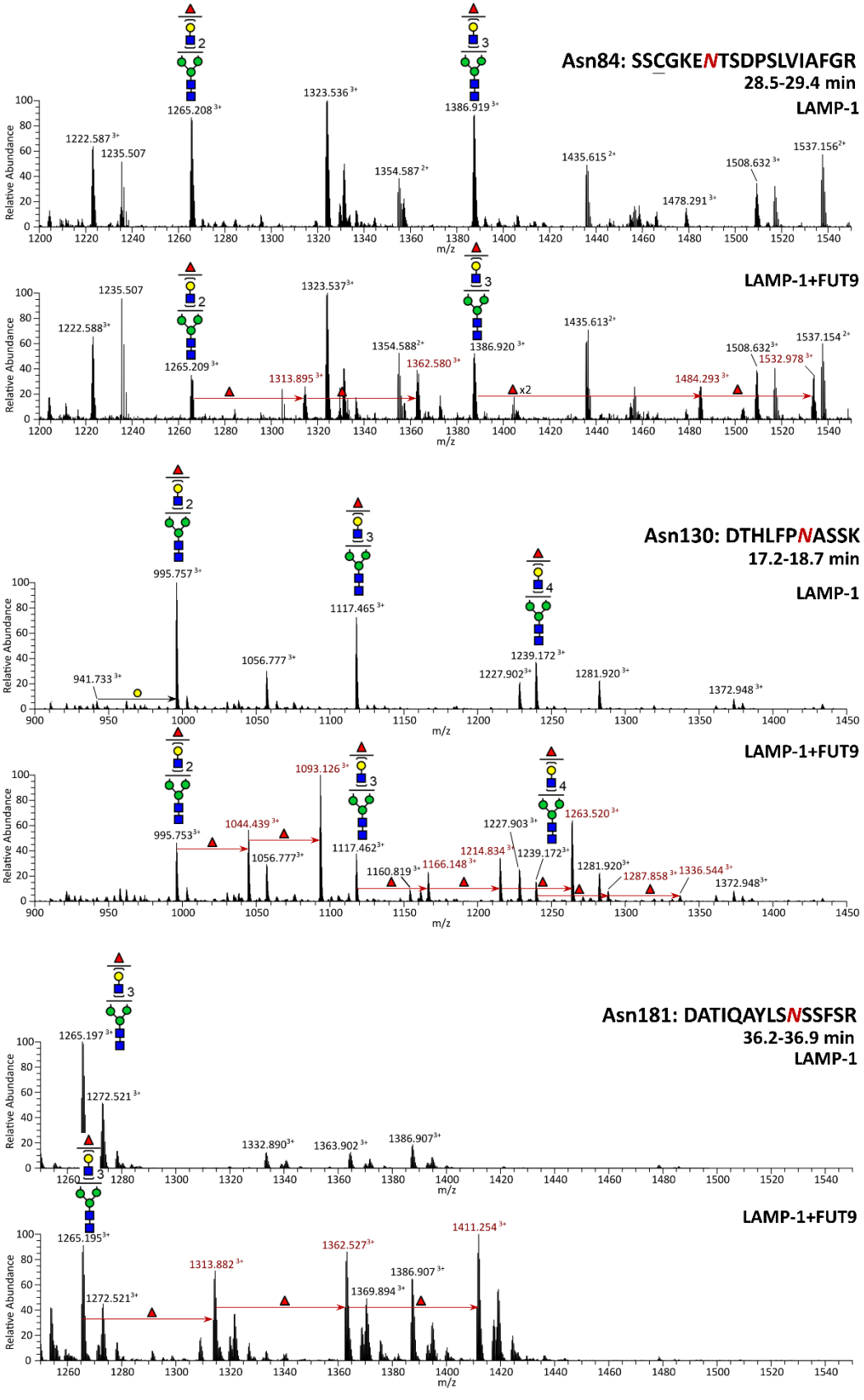
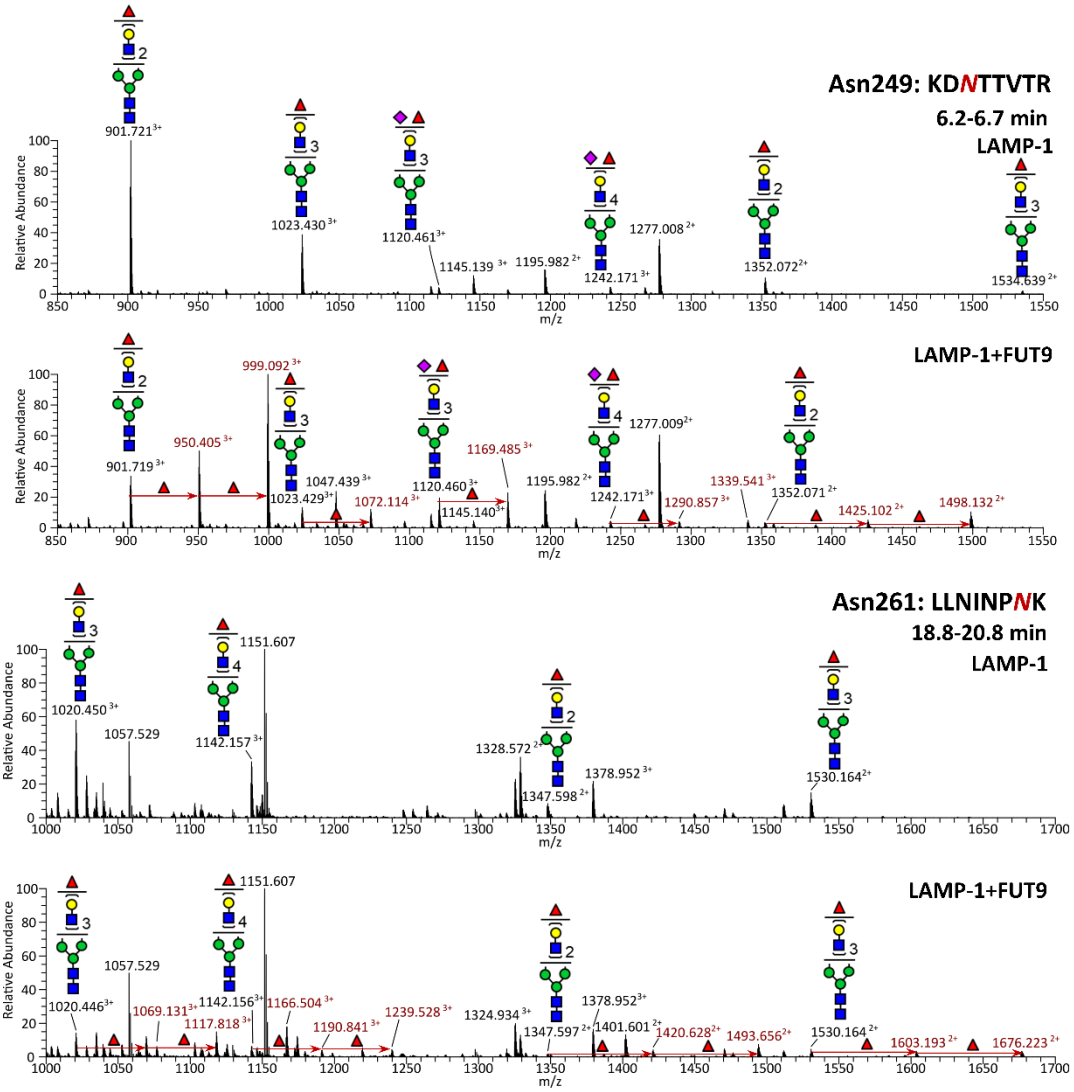


Supplementary Fig. 1. FUT9-dependent Lewis X formation on LAMP-1 in HEK293T and COS7 cells.

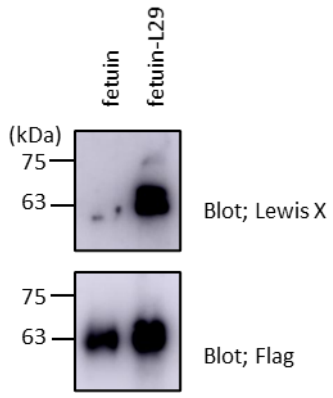
Lysates from HEK293T or COS7 cells transfected with or without FUT9 were subjected to immunoblot analysis using the anti-Lewis X antibody, AK97. The cell lysates were also immunoprecipitated with AK97 (IP) and subjected to immunoblot analysis with anti-LAMP-1 antibody.


Asn84: SSCGKENTSDPSLVIAFGR
28.5-29.4 min
LAMP-1
LAMP-1+FUT9
Asn130: DTHLFPNASSK
17.2-18.7 min
LAMP-1
LAMP-1+FUT9
Asn181: DATIQAYLSNSSFSR
36.2-36.9 min
LAMP-1
LAMP-1+FUT9
Relative Abundance
m/z
x2



Supplementary Fig. 2. Comparisons of the LC-MS profiles of N-glycosylated peptides from LAMP-1 produced in wild-type and FUT9-expressing CHO-K1 cells.

The MS profiles of tryptic peptides containing Asn84, Asn130, Asn181, Asn249, and Asn261 showed the increment of fucose in FUT9-expressing cells comparing to wild-type. The MS spectra were averaged according to the time ranges labeled in the figures, respectively. The glycan structures were identified by Byonic (Protein Metrics Inc.) and manually assigned using Xcalibur Software.



Supplementary Fig. 3. FUT9-dependent Lewis X modification of fetuin dependent upon the L29 sequence.

The recombinant fetuin glycoproteins with or without a C-terminal L29 sequence tag was subjected to immunoblot analysis using anti-Lewis X AK97 and anti-Flag antibodies.