Supplementary Materials for

1,3-1,4-α-L-Fucosynthase that specifically introduces Lewis a/x antigens into type-1/2 chains

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Table S1. Hydrolysis of 3-fucosyllactose (3-FL) and *p*NP-Fuc by *Bb*AfcB variants

Enzyme	Specific activity[a] (mU/mg)	
	3-FL	*p*NP-Fuc
WT	5,100	0.18
Nucleophile mutants		
D703A	0.032	n.d.[b]
D703C	0.11	n.d.
D703G	0.30	n.d.
D703S	0.067	n.d.
Acid/base mutant		
E746A	0.74	n.d.
Gal-binding site mutants		
W742A	2.8	0.043
D807A	64	0.080
Disordered loop mutants		
D763A	1,500	0.17
D766A	1,600	0.15
D778A	1,900	0.16

[a]Only the specific activities were determined because the kinetic parameters could not be calculated due to the linear increase of the initial velocity up to 20 mM of the substrate.
[b]not determined.

For the hydrolysis of 3-FL, the WT enzyme (14 nM) and its mutants [D703 mutants (12 μM), W742A and E746A (17 μM), D763A and D778A (44 nM), D766A (14 nM), D807A (890 nM)] were incubated in reaction mixtures (40 μl) consisting of 100 mM MOPS buffer (pH 6.5) and 1 mM substrate at 30°C. The amounts of the liberated Fuc were determined using a fucose dehydrogenase-coupled method (23).

For the hydrolysis of *p*NP-Fuc, the enzymes (17 μM) were incubated in reaction mixtures (40 μl) consisting of 100 mM MOPS buffer (pH 6.5) and 1 mM substrate at 30°C. The amount of the liberated *p*NP was determined by measuring absorbance at 405 nm.

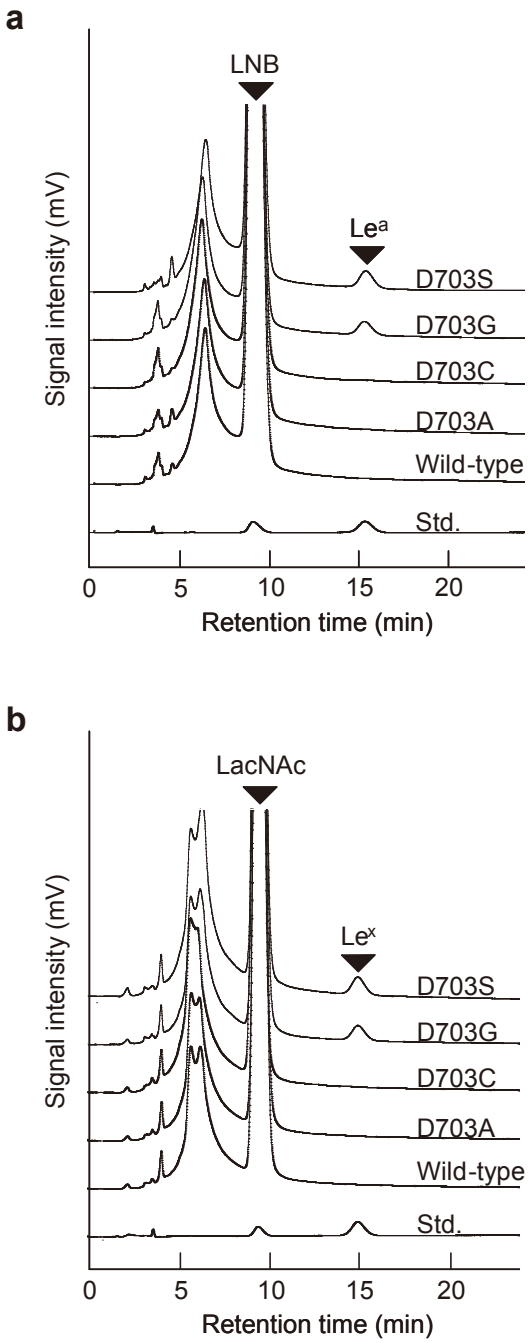

Figure S1. Syntheses of Le^a (**a**) and Le^x (**b**) trisaccharides by the *Bb*AfcB D703 mutants. The WT enzyme and D703 mutants (17 μM) were incubated in the reaction mixtures (50 μl) consisting of 100 mM MOPS (pH 7.0), 20 mM FucF (donor) and 100 mM LNB (**a**) or LacNAc (**b**) (acceptor) for 10 min at 30°C. The reaction products were analyzed by HPLC-UV detection. Standard sugars (Std.) are: lacto-*N*-biose I (LNB), *N*-acetyllactosamine (LacNAc), Lewis a (Le^a) and Lewis x (Le^x).

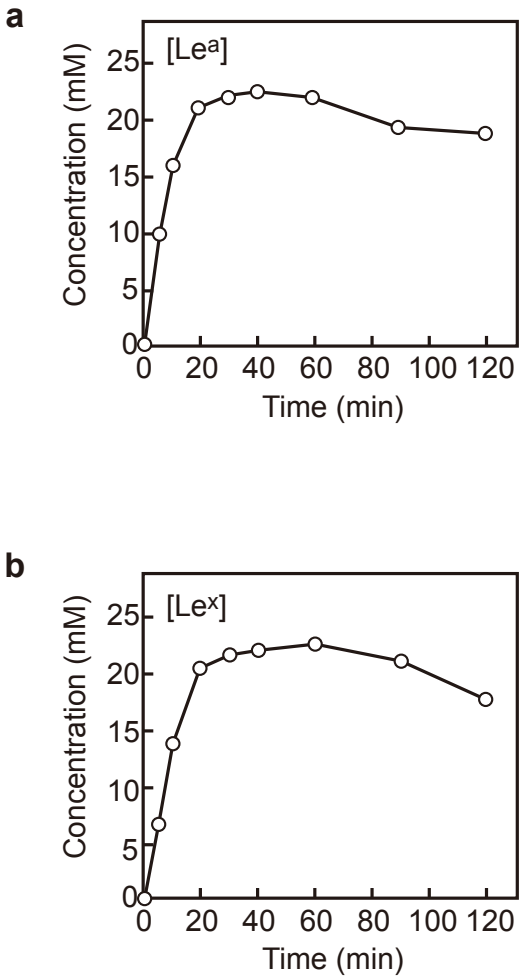

Figure S2. Time courses of the Le^a (**a**) and Le^x (**b**) syntheses catalyzed by the *Bb*AfcB D703S mutant. (**a**) The reaction was carried out in 100 mM MES buffer (pH 5.0) containing 40 mM FucF and 200 mM LNB for 120 min at 30^{o}C in the presence of the D703S mutant (17 μM). (**b**) A similar reaction was carried out for the synthesis of Le^x, except that 100 mM LacNAc was used. Aliquots were taken at the indicated times and analyzed by HPLC-CAD.

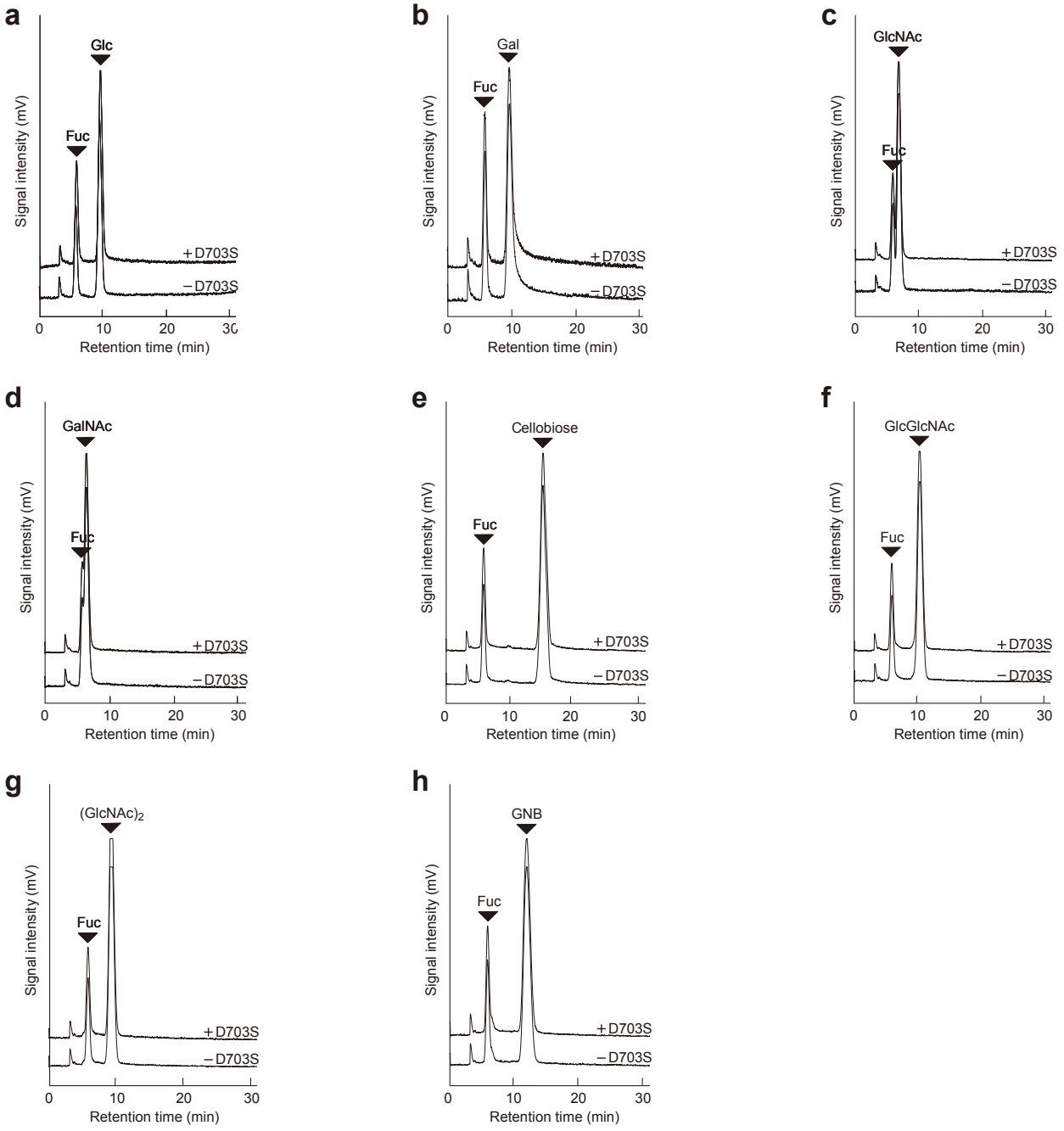

Figure S3. The acceptor specificity of the *Bb*AfcB D703S glycosynthase. The reaction was carried out in 100 mM MES buffer (pH 5.0) containing 40 mM FucF and 100 mM of each acceptor for 40 min at 30^{o}C in the presence and absence of the enzyme (17 μM). The reaction products were analyzed by HPLC-CAD. The acceptors used were: Glc (**a**), Gal (**b**), GlcNAc (**c**), GalNAc (**d**), cellobiose (**e**), 2-acetamide-2-deoxy-4-*O*-(β-glucosyl)-glucose (GlcGlcNAc) (**f**), *N,N'*-diacetylchitobiose [$(GlcNAc)_2$] (**g**) and galacto-*N*-biose (GNB) (**h**). The peaks of Fuc and acceptor are indicated.

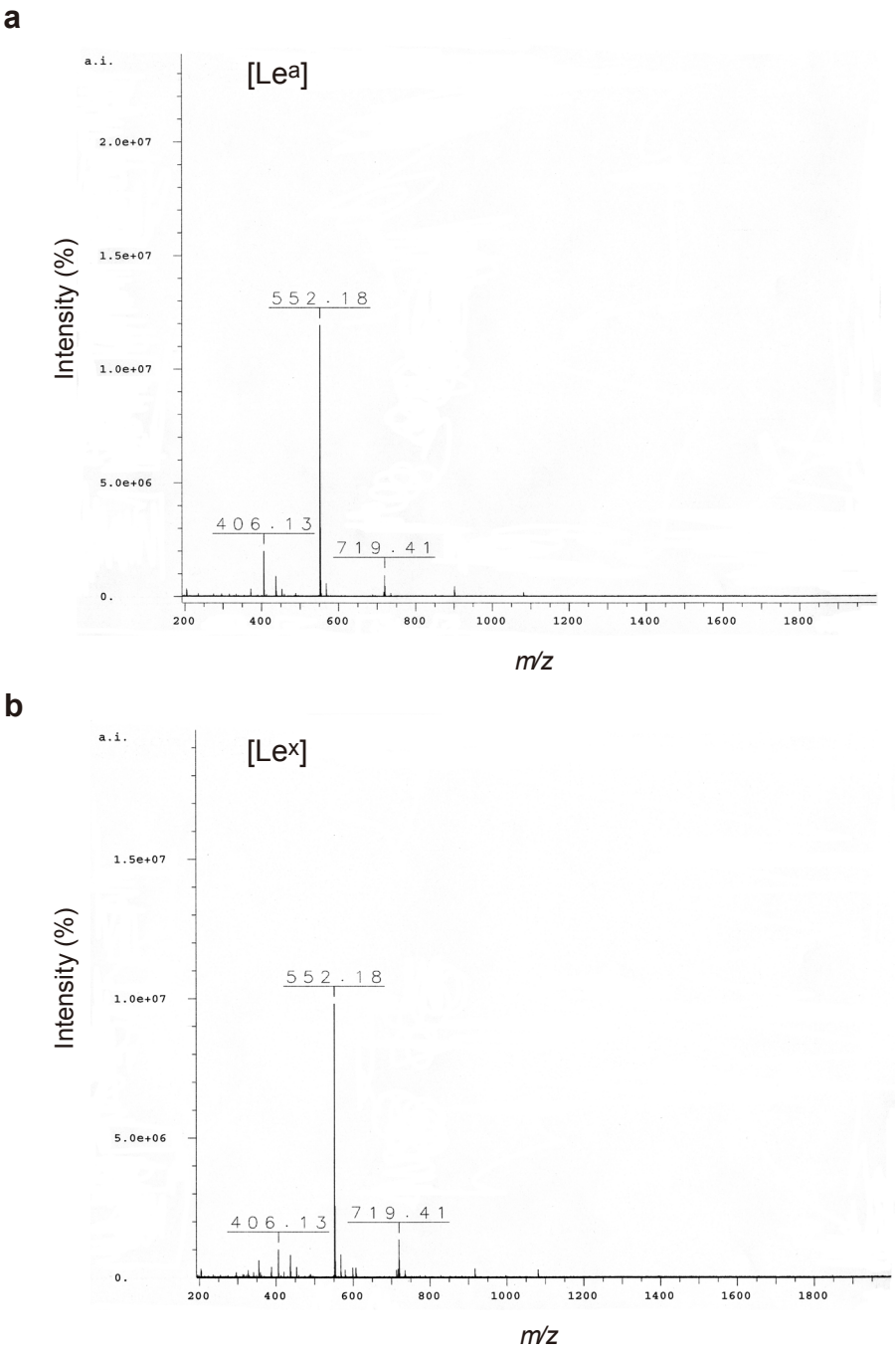

Figure S4. ESI-MS analysis of the purified products (Le^a and Le^x). The syntheses of Le^a (**a**) and Le^x (**b**) trisaccharides were performed as described in the legend of **Figure 1**. The products were purified and subjected to ESI-MS analysis (calculated for the sodium adduct $[M+Na]^+$ 552.19, observed 552.18).

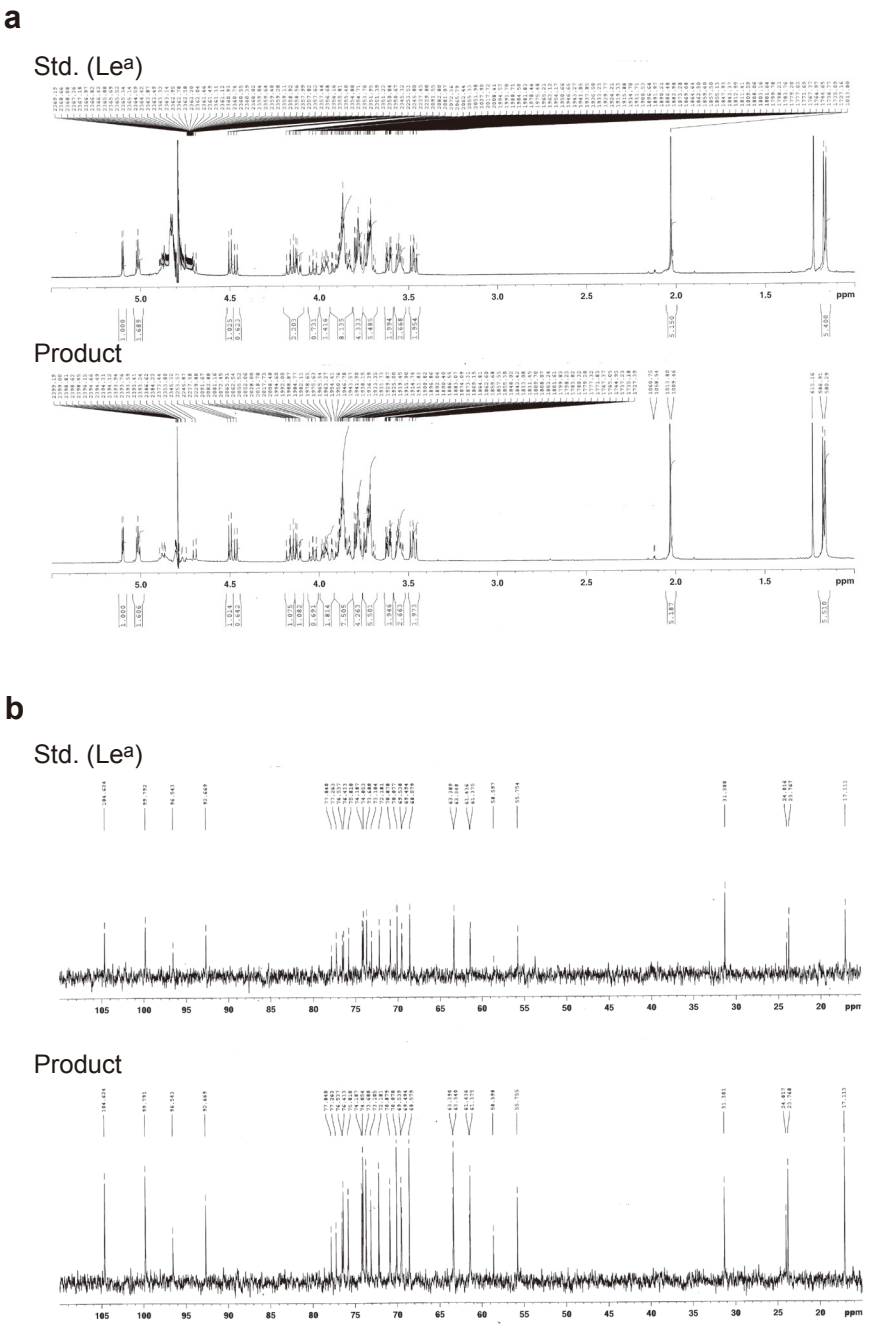

Figure S5. NMR analysis of the purified product (Le^a). The ^{1}H (**a**) and ^{13}C (**b**) NMR spectra of the purified product (lower panels) were compared with those of the authentic Le^a (upper panels). The spectra were measured in D_2O using 2-methyl-2-propanol as the internal standard.

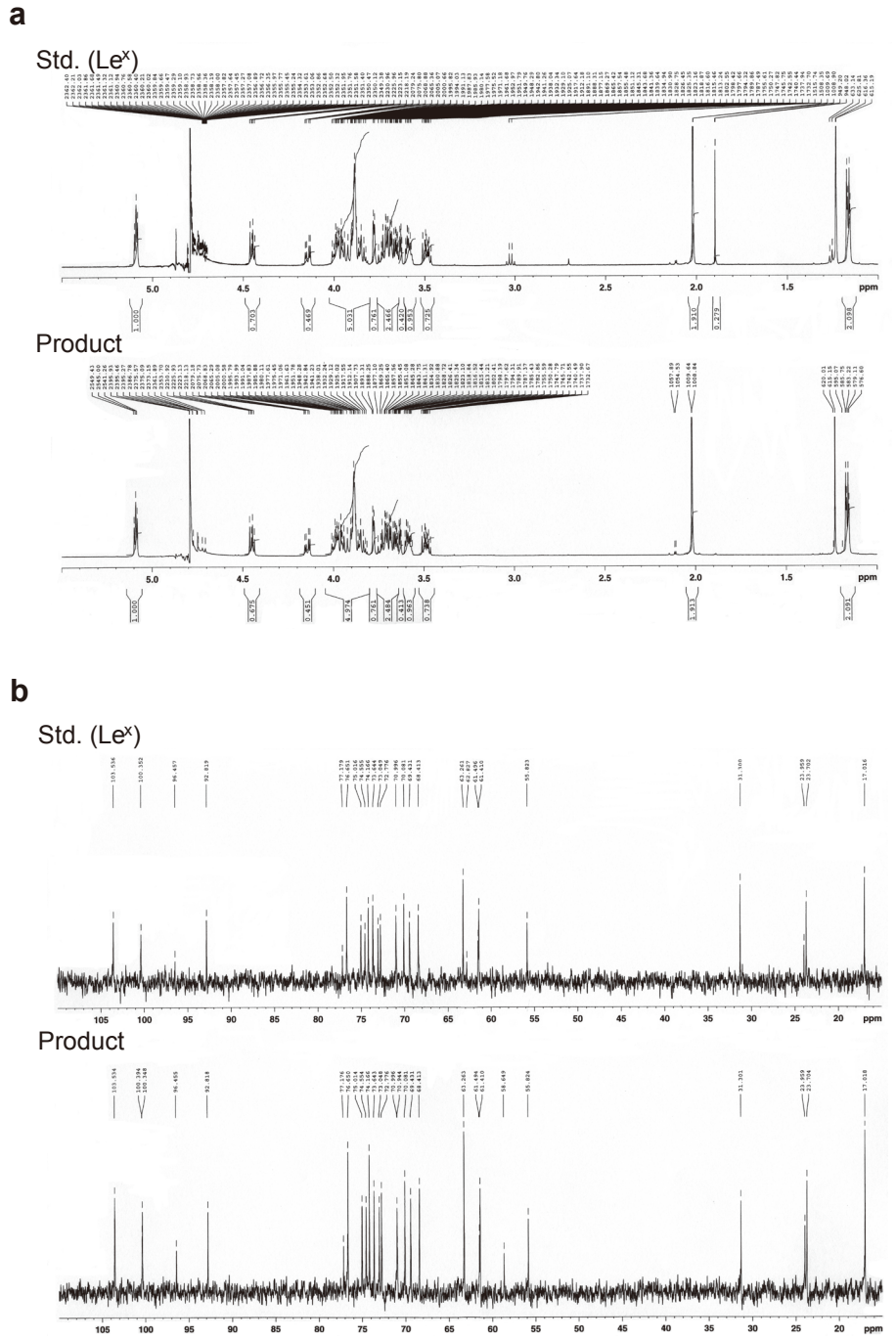

Figure S6. NMR analysis of the purified product (Le^x). The ^{1}H (**a**) and ^{13}C (**b**) NMR spectra of the purified product (lower panels) were compared with those of the authentic Le^x (upper panels). The spectra were measured in D_2O using 2-methyl-2-propanol as the internal standard.

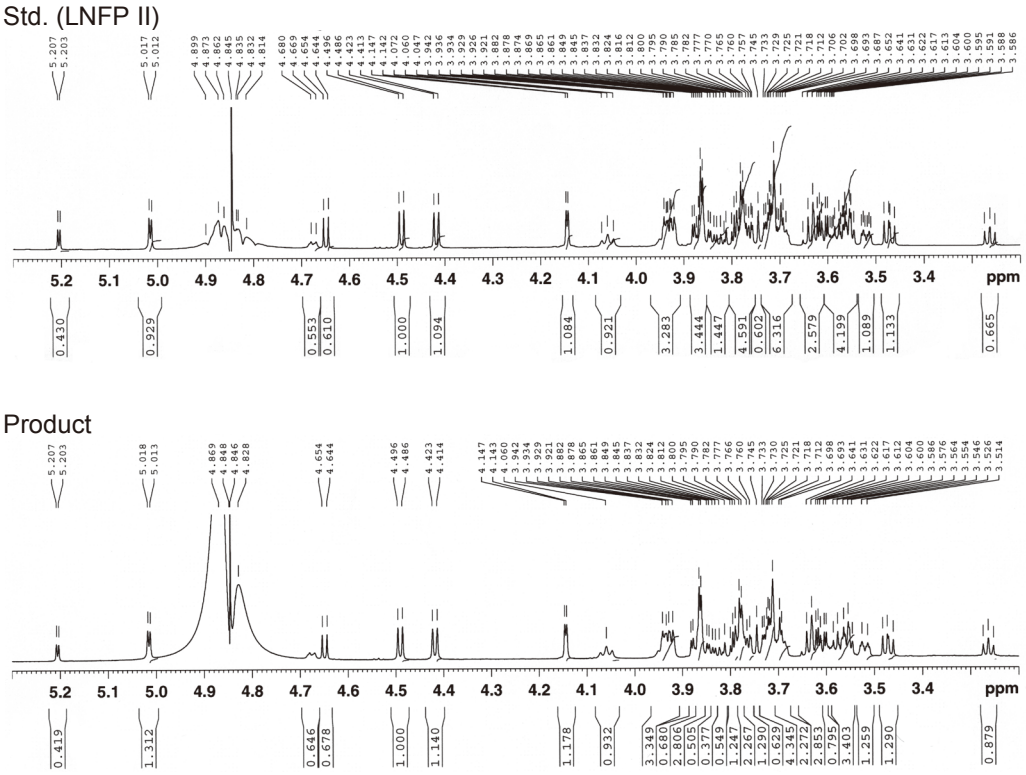

Figure S7. ^{1}H NMR analysis of the purified product (LNFP II). The reaction was carried out in 100 mM MES buffer (pH 5.0) containing 40 mM FucF and 100 mM LNT at 30^{o}C in the presence of 17 μM D703S. The spectra were obatined in D_2O using 2-methyl-2-propanol as the internal standard. Upper panel, authentic LNFP II; lower panel, purified product.

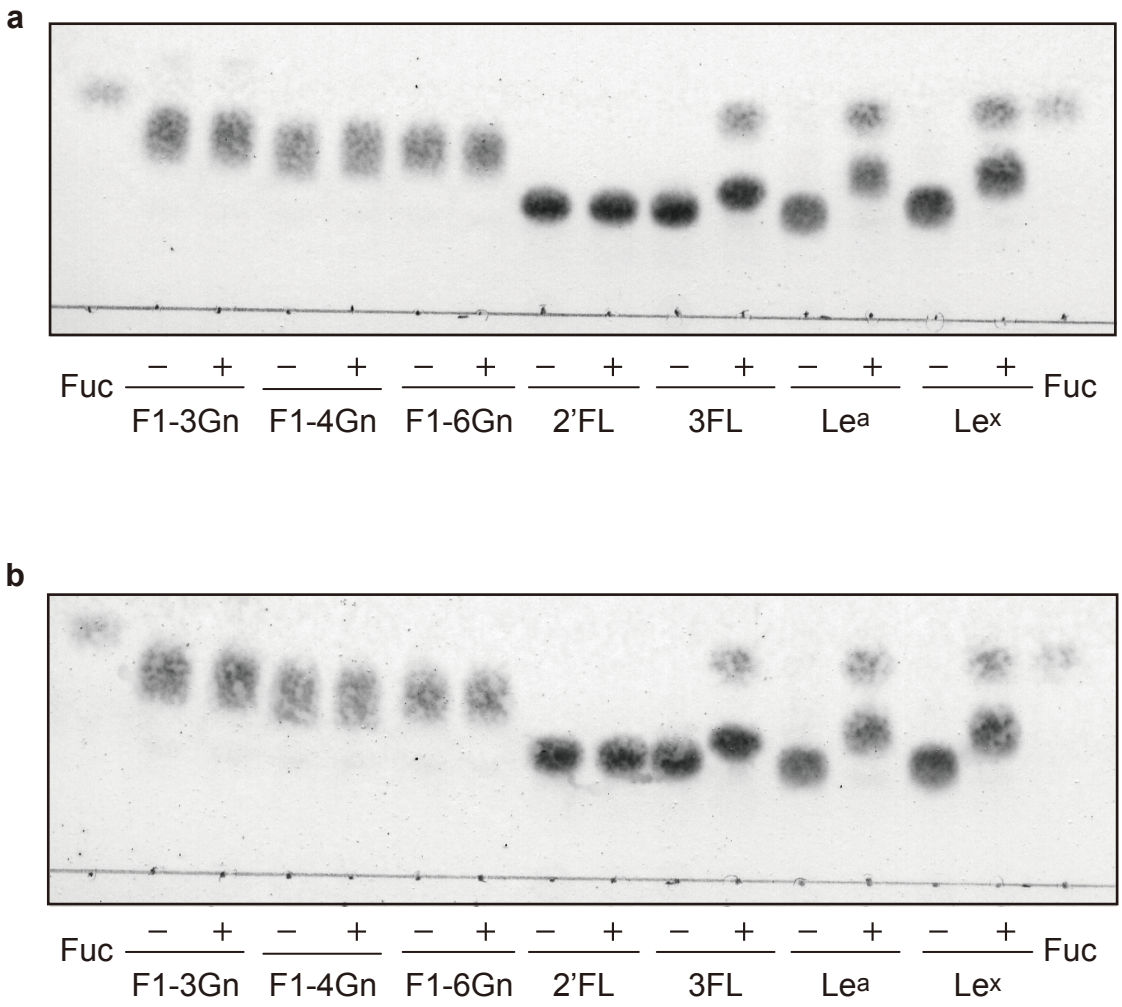

Figure S8. Substrate specificities of *Bb*AfcB (**a**) and *Bi*AfcB (**b**). The reactions were carried out in 100 mM MOPS buffer (pH 6.5) containing 6 mM substrates at 30°C for 60 min in the presence and absence of the enzyme (*Bb*AfcB; 1.1 μM, *Bi*AfcB; 0.47 μM). The reaction products were analyzed by TLC (Silica Gel 60, Merck) using a solvent system of 1-butanol/acetic acid/water (2:1:1, by volume). The sugars were visualized as described previously (Ashida, H., Miyake, A., Kiyohara, M., Wada, J., Yoshida, E., Kumagai, H., Katayama, T., and Yamamoto, K. (2009). Glycobiology **19**, 1010-1017). The substrates used were: Fucα1-3GlcNAc (F1-3Gn), Fucα1-4GlcNAc (F1-4Gn), Fucα1-6GlcNAc (F1-6Gn), 2'-fucosyllactose (2'-FL), 3-fucosyllactose (3-FL), Lewis a (Le^a) and Lewis x (Le^x).

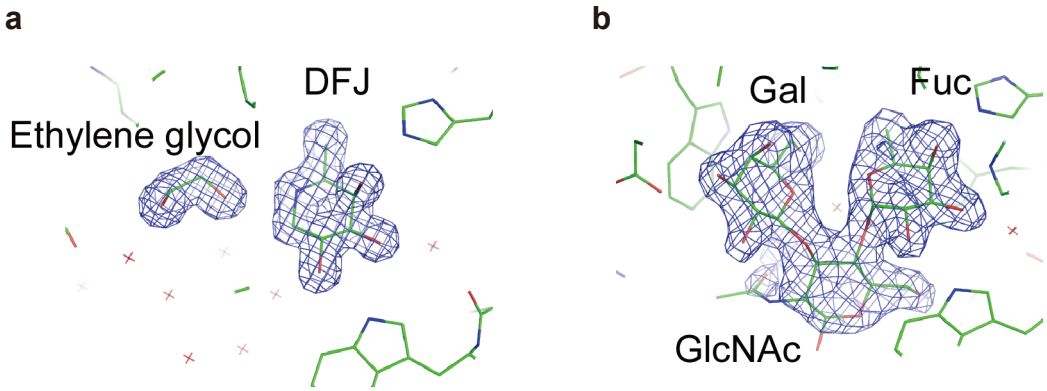

Figure S9. $|F_o| - |F_c|$ omit electron density maps of WT-DFJ-EG (**a**) and D172A/E217A-LNFP II (**b**) complex. The maps were contoured at 3 σ.

a

b

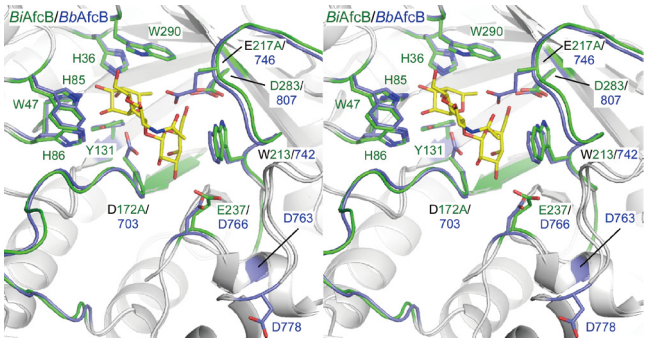

Figure S10. (a) Sequence alignment of *Bi*AfcB and *Bb*AfcB. The secondary structure of *Bi*AfcB is indicated above the sequences. Two mobile loops (173-182 and 215-220) and one disordered loop (236-254) of *Bi*AfcB are boxed. The residues important for catalysis, Fuc binding and Gal binding are indicated by magenta, black and cyan circles, respectively. **(b)** A structural model built for *Bb*AfcB (aa 491-1039) (cyan) and the comparison of the model with *Bi*AfcB (D172A/E217A-LNFP II complex) (protein in green, ligand in yellow) at the catalytic pockets (stereoview). The *Bb*AfcB model was constructed using SWISS-MODEL server [Arnold, K., Bordoli, L., Kopp, J., and Schwede, T. (2006). *Bioinfomatics* **22**,195-201]. The numbers of the residues are indicated.

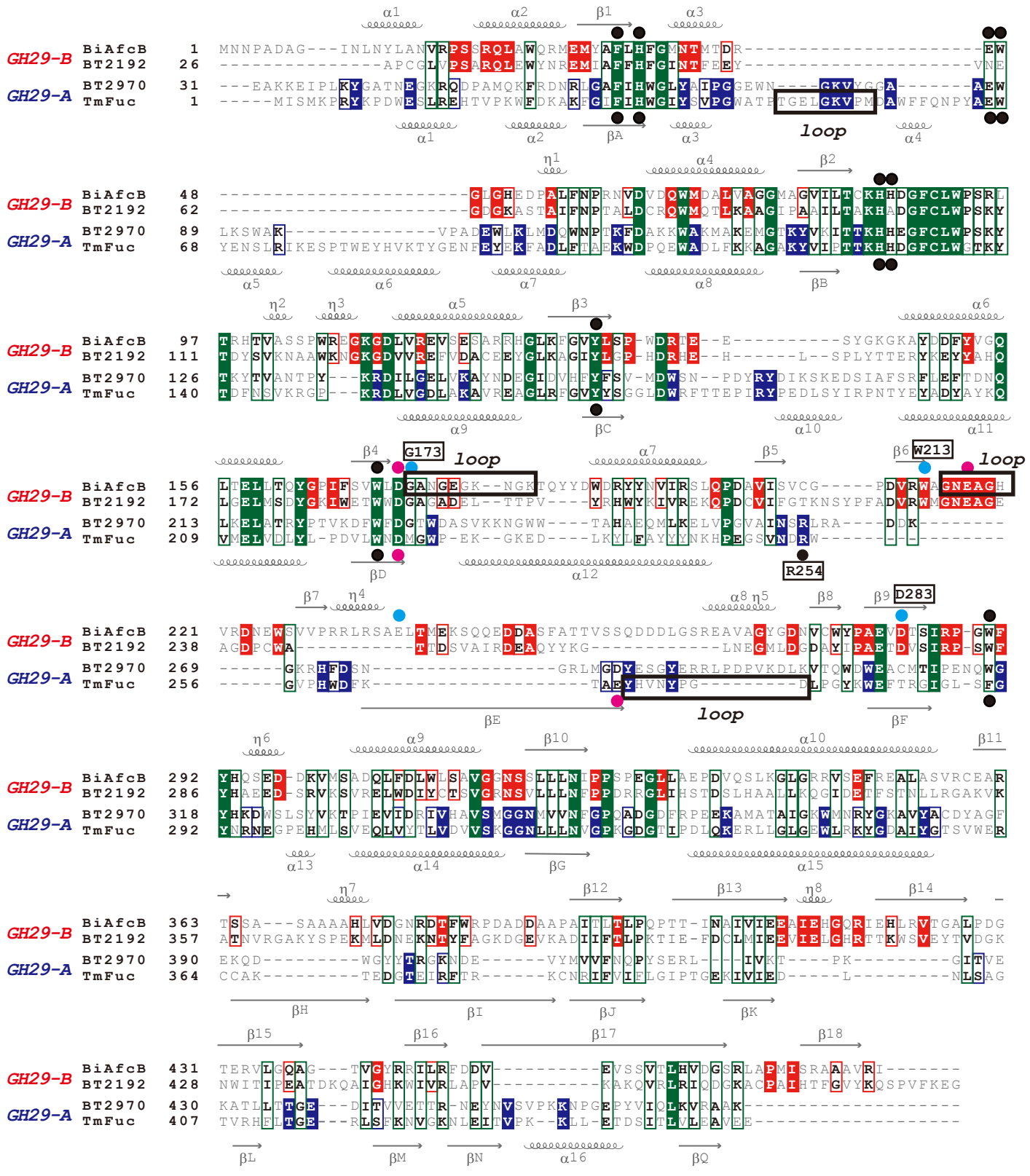

Figure S11. Sequence alignment of enzymatically characterized, structure-solved GH29-A and GH29-B members. The secondary structures of *Bi*AfcB and *Tm*Fuc are indicated above and below the sequences. The residues conserved in GH29-A, GH29-B and the both are shown in blue, red and green, respectively. Two mobile loops (173-182 and 215-220 in *Bi*AfcB, 47-55 and 267-274 in *Tm*Fuc) are boxed. The residues important for catalysis (for *Bi*AfcB and *Tm*Fuc), Fuc-binding (for *Bi*AfcB and *Tm*Fuc) and Gal-binding (for *Bi*AfcB) are indicated by magenta, black and cyan circles, respectively. G173, W213 and D283 of *Bi*AfcB and R254 of *Tm*Fuc are indicated. See also Figure 5 and Supplementary Figure S12.

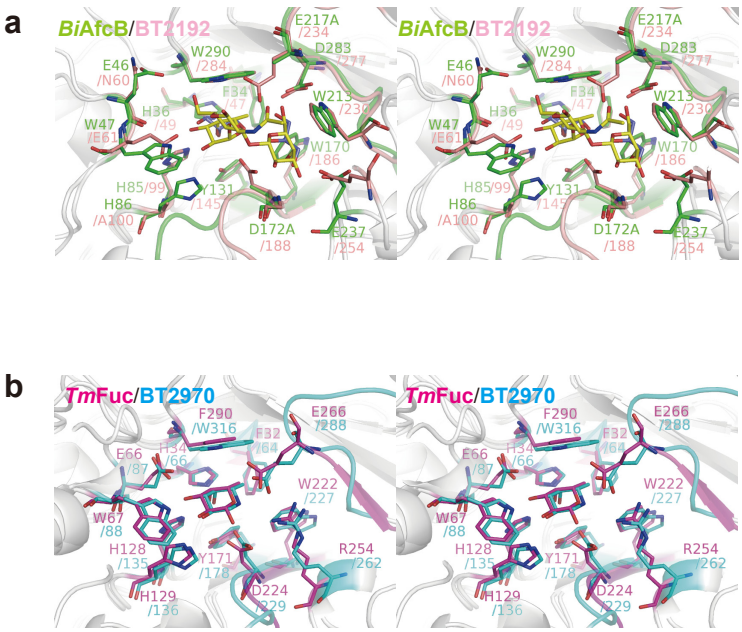

Figure S12. Conservation of active-site structures in the respective subfamilies. Enzymatically characterized members were used for comparison. **(a)** GH29-B: *Bi*AfcB (D172A/E217A-LPFP II complex: protein in green, ligand in yellow) vs. BT2192 (glycerol complex: protein in pink, ligand in violet, PDB code 3EYP), **(b)** GH29-A: *Tm*Fuc (Fuc complex: magenta, PDB code 1ODU) vs. BT2970 (DFJ complex: cyan, PDB code 2XIB). The numbers of the residues are indicated. See also Figure 5 and Supplementary Figure S11.