Supplementary Information

Structural insights into the novel HMW1C-like glycosyltransferases

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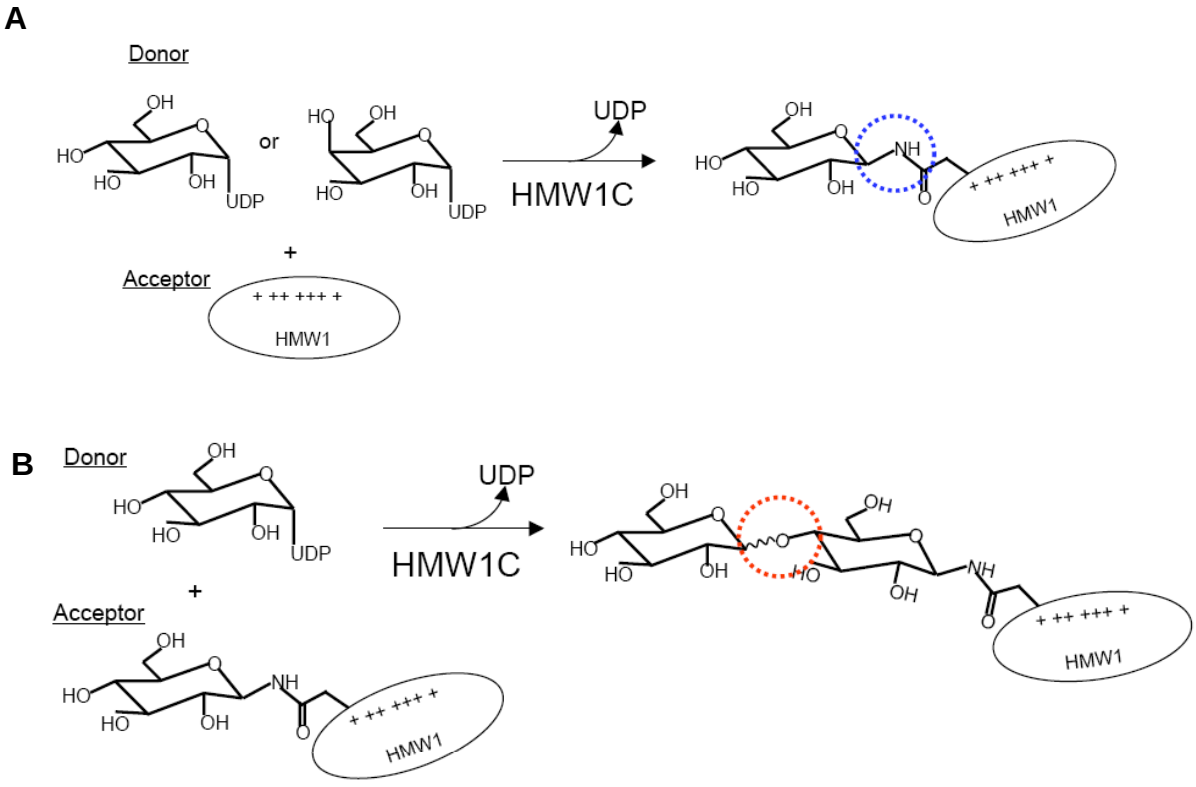

Figure S1. Schematics of HMW1 glycosylation by the versatile HMW1C. Glycosylation of the HMW1 adhesin is catalyzed by HMW1C, which creates two different types of bonds: (**A**) An N-glycosidic bond through the asparagine side chain (blue circle) of HMW1 and (**B**) An O-glycosidic bond (red circle) between an incoming glucose residue and a glucose residue already N-linked to HMW1. The stereochemistry of the linkage is not known.

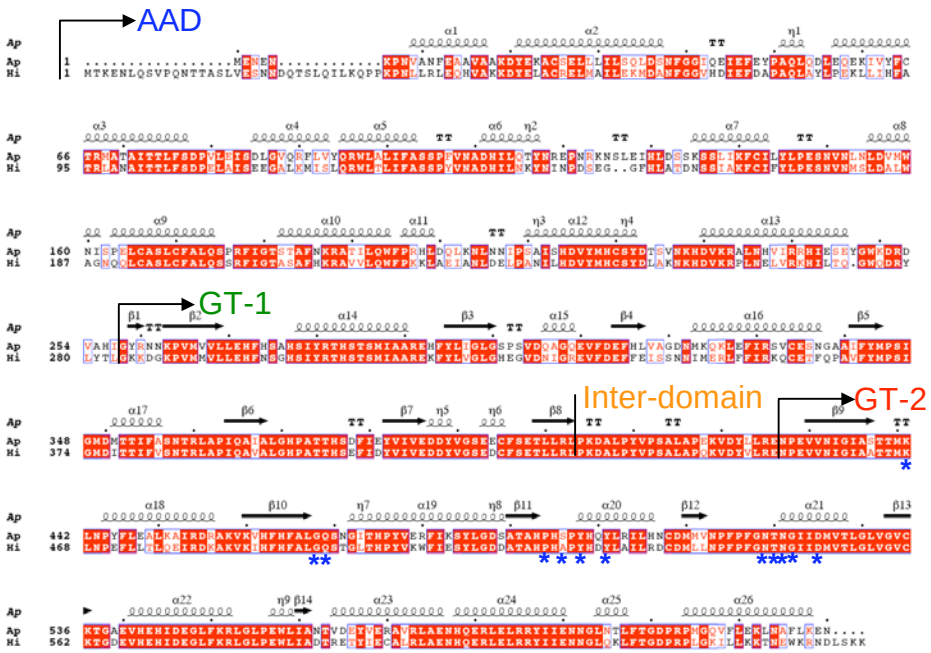

Figure S2. HMW1C sequence conservation and structure of ApHMW1C. (**A**) Sequence alignment of HMW1Cs from *A. pleuropneumoniae* (Ap) and *H. influenzae* (Hi). Secondary structural elements of ApHMW1C are indicated with α–helices and β-strands as springs and arrows, respectively. Identical and conserved residues are shown boxed, in red fill and red letters, respectively. Blue stars indicate residues involved in UDP binding.

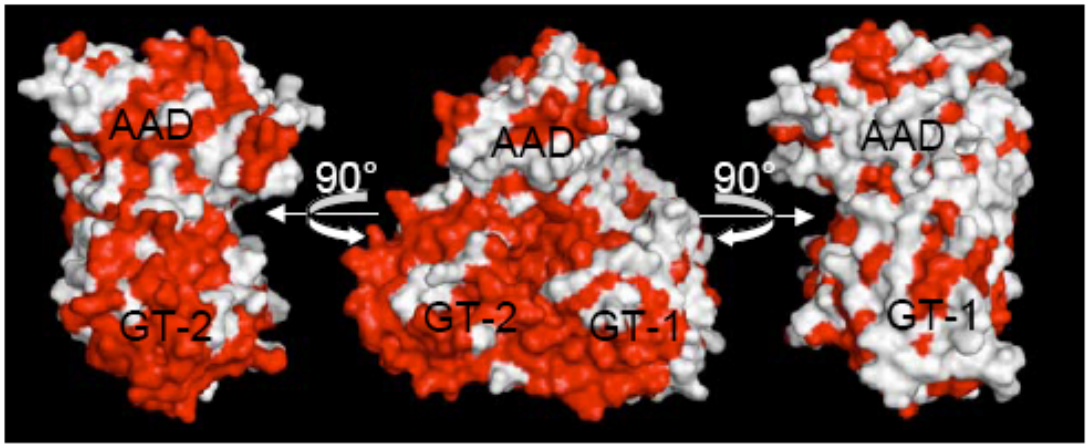

Figure S3. Location of identical residues (red) between ApHMW1C and HMW1C on the ApHMW1C surface. Although the two orthologs are highly homologous throughout their sequences, the molecular surfaces composed of AAD and GT-2 display more striking conservation than do the surfaces composed of ADD and GT1.

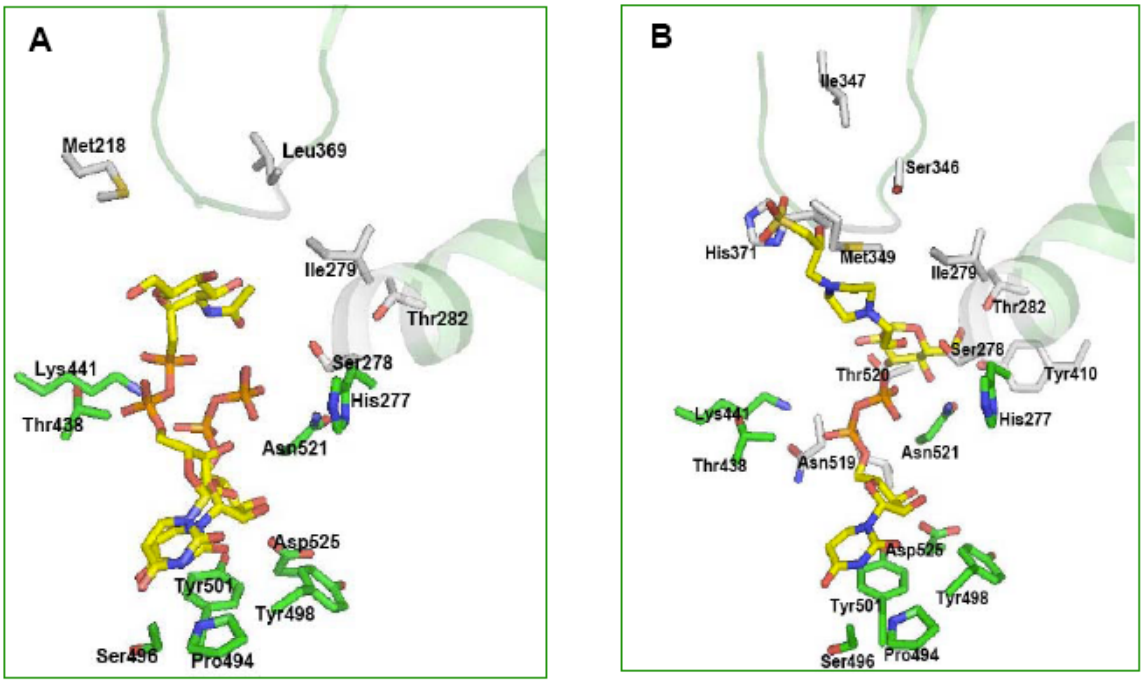

Figure S4. Active sites of ApHMW1C and putative sugar-binding sites. (**A**) Superimposition of UDP-GlcNAc analog of XcOGT onto the ApHMW1C::UDP complex active site. (**B**) Superimposition of ADP-glucose-HEPPSO of EcGS onto the ApHMW1C::UDP complex active site. For simplicity, the ADP moiety is omitted.

Supplemental Experimental Procedures

Glycosylation assays of ApHMW1C and data analysis

Reaction mixtures containing 50 mM HEPES pH 8.0, 50 mM KCl, 5 mM $MgCl_2$, 0.5 mM UDP-Glucose, 3.2 μM His-HMW1ct (Choi et al., 2010), 0.2 mM β-NADH, 2 mM phosphoenolpyruvate (PEP), 2.5 U lactate dehydrogenase (LDH), and 1.75 U pyruvate kinase (PK) were pre-incubated at room temperature for 10 min. Reactions were started by adding ApHMW1C and ApHMW1C variants to a final concentration of 0.9 μM, and the initial glycosylation rates were measured by monitoring the decrease of absorbance at 340 nm for 1 min. One unit was defined as the amount of enzyme that produced 1 nmol of UDP per minute in standard assay conditions.

The apparent K_M and V_{max} values were determined by fitting the initial rates to the Michaelis-Menten equation ($v = V[A]/(K_M + [A])$) using the Enzyme Kinetic Module version 1.3 integrated into SigmaPlot ver 10.0.1 (Systat).

Reference

Choi, K. J., Grass, S., Paek, S., St Geme, J. W., 3rd, and Yeo, H. J. (2010). *PLoS ONE* ***5***, e15888