Supplementary Material

*At*FUT4 and *At*FUT6 are Arabinofuranose-specific Fucosyltransferases

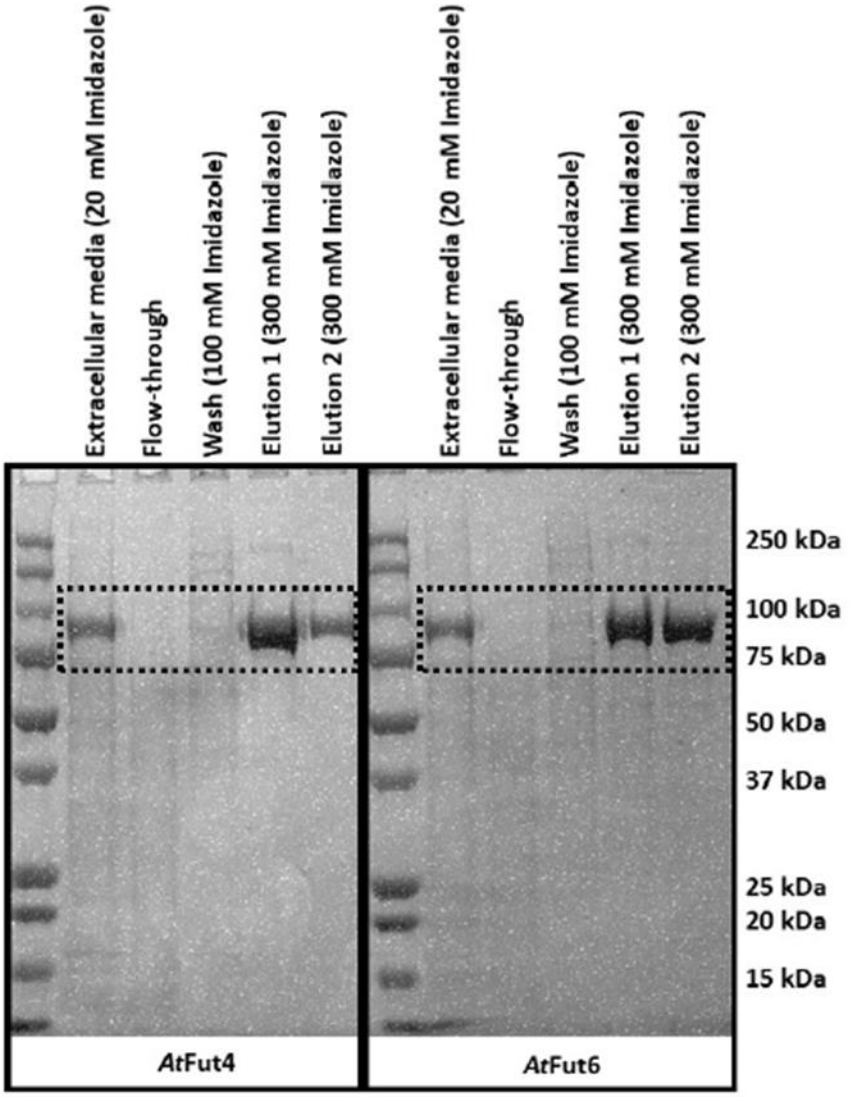
Maria J. Soto[1,2,3], Pradeep Kumar Prabahkar[2,4,5], Hsin-Tzu Wang[2,4,5], Jason Backe[2,4], Digantkumar Chapla[2,5], Max Bartetzko[6], Ian Black[2], Parastoo Azadi[2,5], Maria J. Pena[2,4], Fabian Pfrengle[6,7], Kelley W. Moremen[2,5], Breeanna R. Urbanowicz[2,4,5*], and Michael G. Hahn[2,3,4*]

Supplementary Table 1. Sequence of primers used to amplify truncated AtFUT4 and AtFUT6, and for the amplification of the native AtFUT4 and AtFUT6 promoter regions for subsequent GUS transformation. The underlined regions in the primers sequences for AtFUT4 and AtFUT6 specify the partial attB adapter sequences used in the first round of PCR amplification. Expression and secretion of the GFP-AtFUT4 and GFP-AtFUT6 fusion-proteins was determined by measuring the fluorescence of the recombinant proteins in the media used to transiently transfect the HEK293 cells. The concentration of secreted fusion protein in mg/L is shown in parentheses and was estimated based on GFP fluorescence (13.1 flourescence units = 1 mg/L).

Enzyme/Gene	Amino Acid Truncation	Primer Sequence 5' - 3'	GFP Fluorescence
*At*FUT4_F	Δ_54	AACTTGTACTTTCAAGGCAACGACGAATCCGAAACA	1423 (108.62 mg/L)
*At*FUT4_R		ACAAGAAAGCTGGGTCCTATAACTCATCAAAAAGCT	
*At*FUT6_F	Δ_43	AACTTGTACTTTCAAGGCAACGACTTCAACAACCAAC	1028 (77.8 mg/L)
*At*FUT6_R		ACAAGAAAGCTGGGTCCTATAACTCATCAAATAGCTTA	
AtFUT4::GUS_F		AAGCTTTTGTGCTCGCTTGAATCAGAAG	N/A
AtFUT4::GUS_R		GGATCCGTTGACTTTTAGTTTGTGAAGATGATT	
AtFUT6::GUS_F		GGATCCCTTCAAACCAAAAGCTCTG	N/A
AtFUT6::GUS_R		AAGCTTATTTTACAAATCGAAACAG	

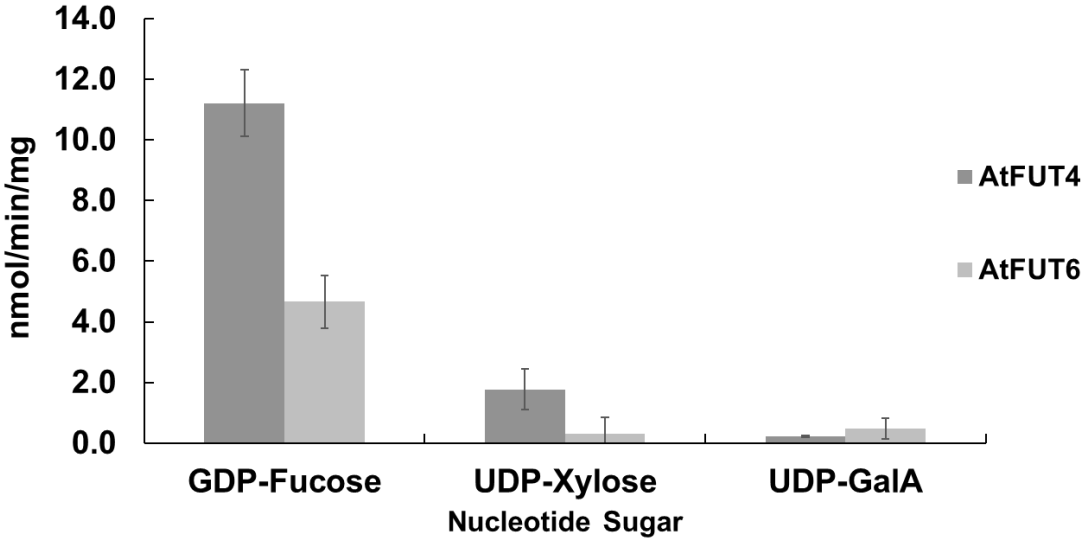
Supplementary Table 2. ^{1}H NMR assignments of arabinotriose incubated with GDP-Fuc and GFP-*At*FUT4. (A.) Reaction without the enzyme (control) and after (B) incubation with the enzyme. [1]Chemical shifts are reported in ppm relative to internal dimethyl sulfoxide, δ_H 2.721.

Residue	**H1**	**H2**	**H3**	**H4**	**H5$_{a,b}$**	**H6**
Chemical Shift (ppm)[1]						
A.		**α-L-Ara*f*-(1,5)-α-L-Ara*f*-(1,5)-α-L-Ara*f***				
T-α-l-Ara*f*	5.090	4.13	3.95	4.09	3.83-3.72	
5-α-l-Ara*f*	5.084	4.13	4.22	3.89	3.80 7.7	
5-α-l-Ara*f*	5.258	4.03	4.03	4.23	3.86-3.76	
5-β-l-Ara*f*	5.294	4.09	4.00	4.23	3.86-3.76	
B.		**α-L-Fuc*p*-(1,2)-α-L-Ara*f*-(1,5)-α-L-Ara*f*-(1,5)-α-L-Ara*f***				
T-α-l-Fuc*p*	5.076	3.79	3.89	3.8	4.08	1.24
2-α-l-Ara*f*	5.196	4.14	4.10	4.10	3.835-3.724	
5-α-l-Ara*f*	5.084	4.13	4.22	3.89	3.80 7.7	
5-α-l-Ara*f*	5.258	4.03	4.03	4.23	3.86-3.76	
5-β-l-Ara*f*	5.294	4.09	4.00	4.23	3.86-3.76	



Supplementary Figure 1. Purification of GFP-AtFUT4 and GFP-AtFUT6 using immobilized metal affinity chromatography (IMAC).

SDS-PAGE of purification of GFP-AtFUT4 and GFP-AtFUT6. Respective protein bands are highlighted in the dashed boxes. All buffers contain 50 mM HEPES and 400 mM NaCl, pH 7.2, and the concentration of imidazole used during the purification is indicated.



Supplementary Figure 2. Hydrolysis of GDP-Fucose, UDP-Xylose, and UDP-GalA by GFP-*At*FUT4 and GFP-*At*FUT6.

Enzyme hydrolysis activity for *At*FUT4, and *At*FUT6 was measured based on the production of GDP or UDP using the GDP-Glo and UDP-Glo assay kits in the absence of acceptor substrates. Enzyme assays consisted of 150 ng of enzyme and 100 μM of nucleotide sugars and were incubated for 20 minutes.