Supporting information description:

Figure S1 Transmembrane segment prediction for sugar transporters.

Figure S2 Predicting the Xltr1p structure using 4GBY as template (silver) with SwissModel and using 6H7D_A as template with MODELLER predicted by HHpred as template (green)

Figure S3 ConSurf analysis of the evolutionary conservation of the amino acids in Xltr1p.

Figure S4 The interaction analysis between Xltr1p and xylose molecule by ligplot.

Figure S5 Predicted transmembrane structures of mutants: a. I299A b. F300A c. N326W d. N326Y. Original amino acid residues were displayed in green color, and mutated amino acid residues were displayed in red color.

Figure S6 Kinetics of glucose/ $_{D}$-xylose consumption($q_{glucose}$, q_{xylose}) for Xltr1p (square), $N326F^{Xltr1}$ (triangle) and BSW4PP(circle). For kinetics for glucose, the cells were cultivated in SD medium with 20 g/L $_{D}$-xylose at 30 °C for 24h, 60h, and 72h with an initial OD=0.1. For kinetics for $_{D}$-xylose, the cells were cultivated in SD medium with 20 g/L $_{D}$-xylose at 30 °C for 30, 60, and 120 minutes with an initial OD=20.

Figure S7 The result on docking study on Xltr1p or its variant N326F with glucose. The origin amino acid residue was displayed in green color, and mutated amino acid residue was displayed in red color.

Table S1 Sequence similarities among transporters used in this study.

Table S2 Primers used in this study.

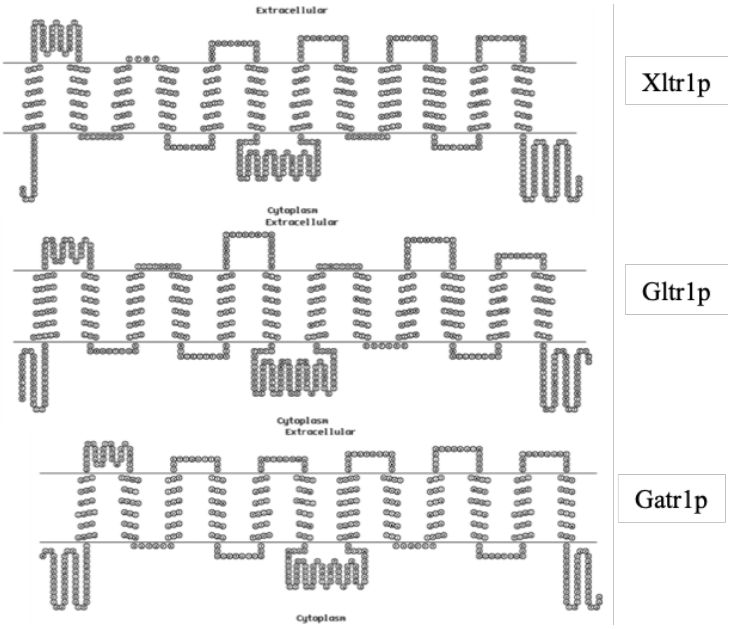
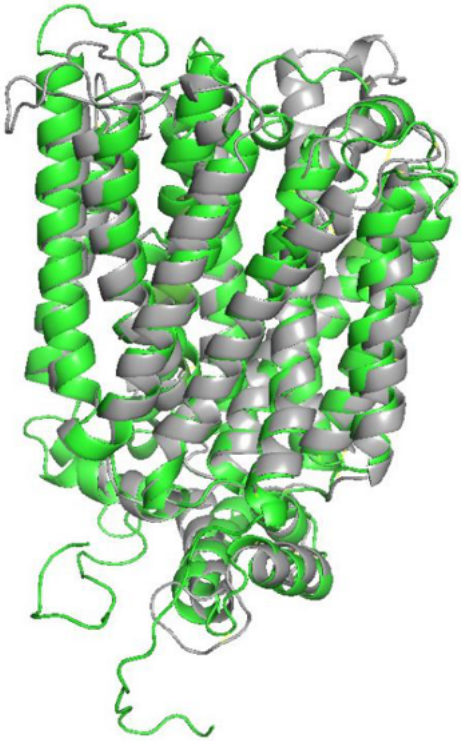


Figure S1 Transmembrane segment prediction for sugar transporters.



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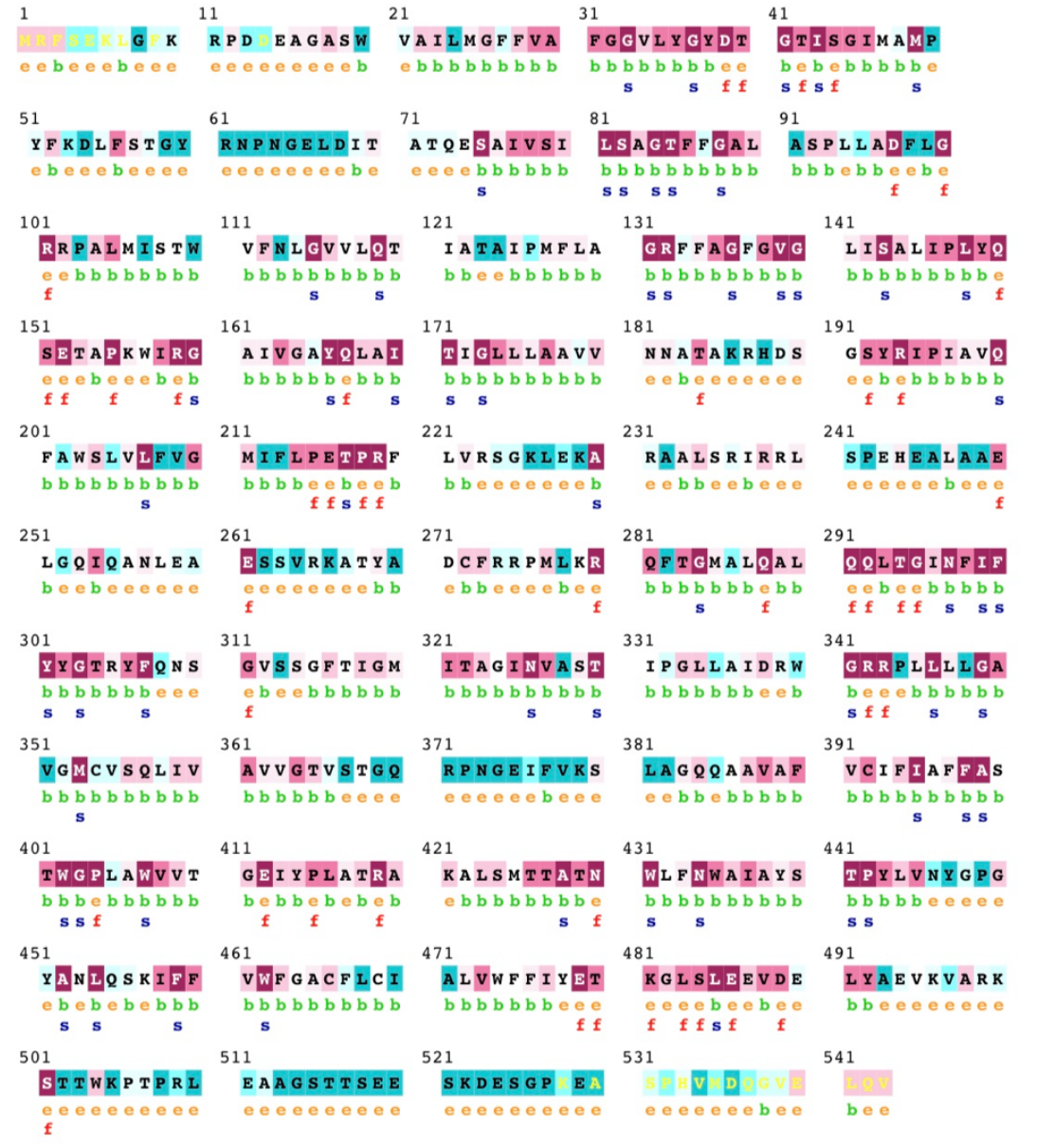


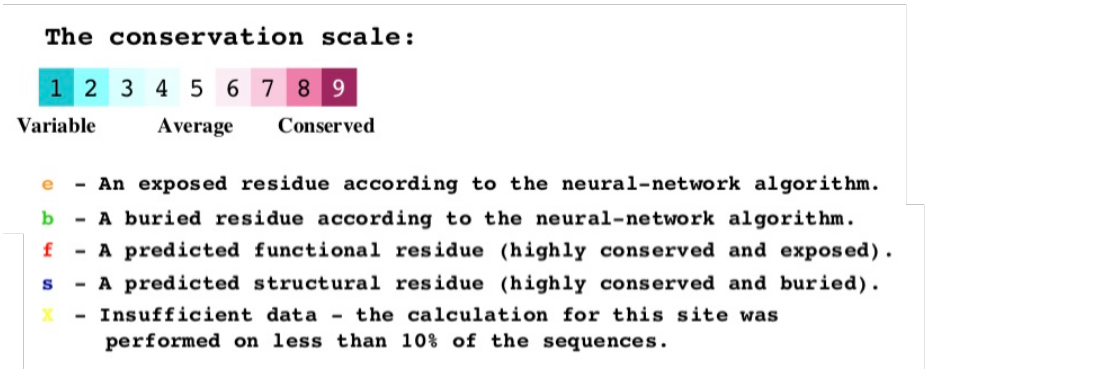


Figure S3 ConSurf analysis of the evolutionary conservation of the amino acids in Xltr1p.

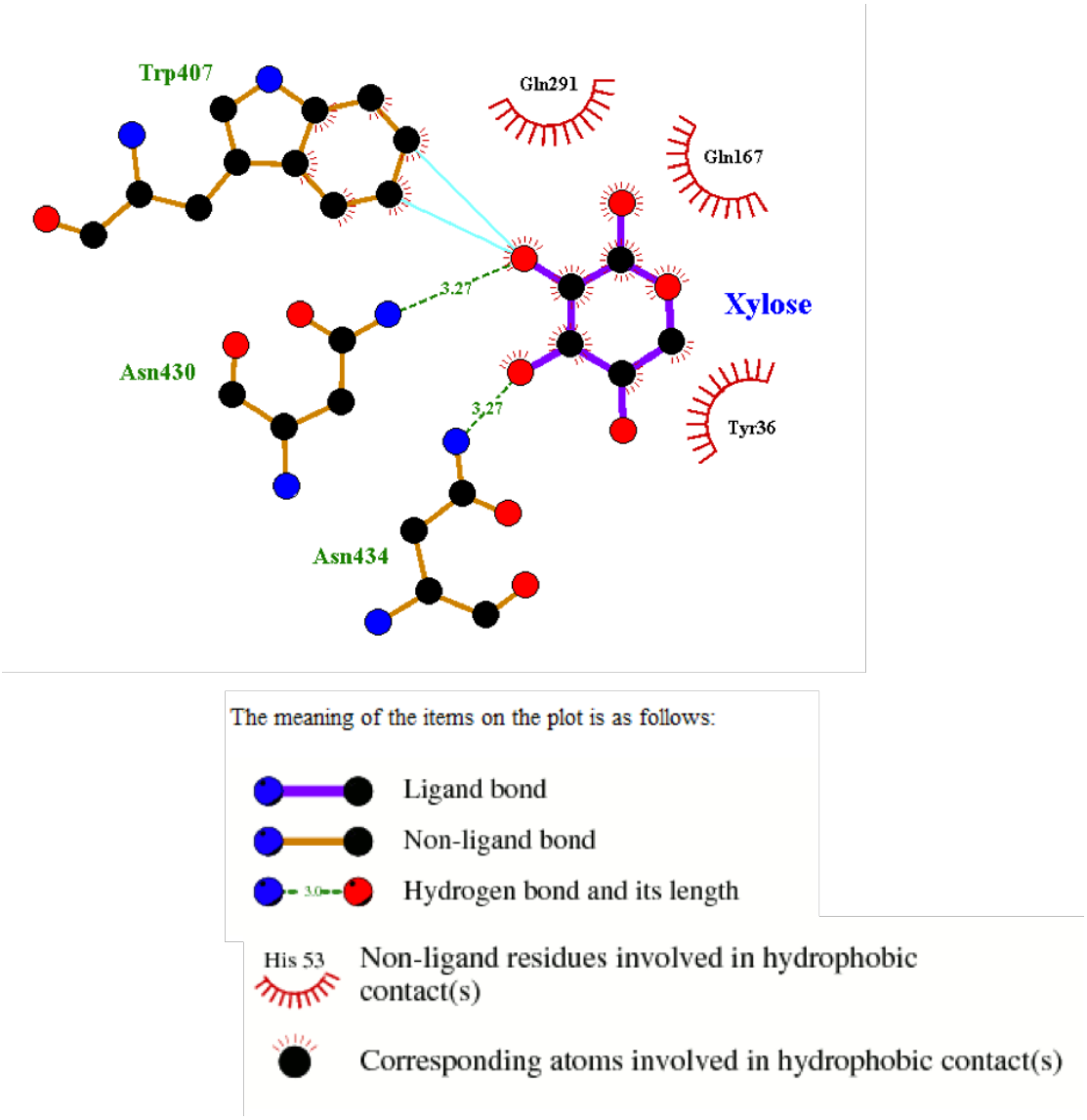
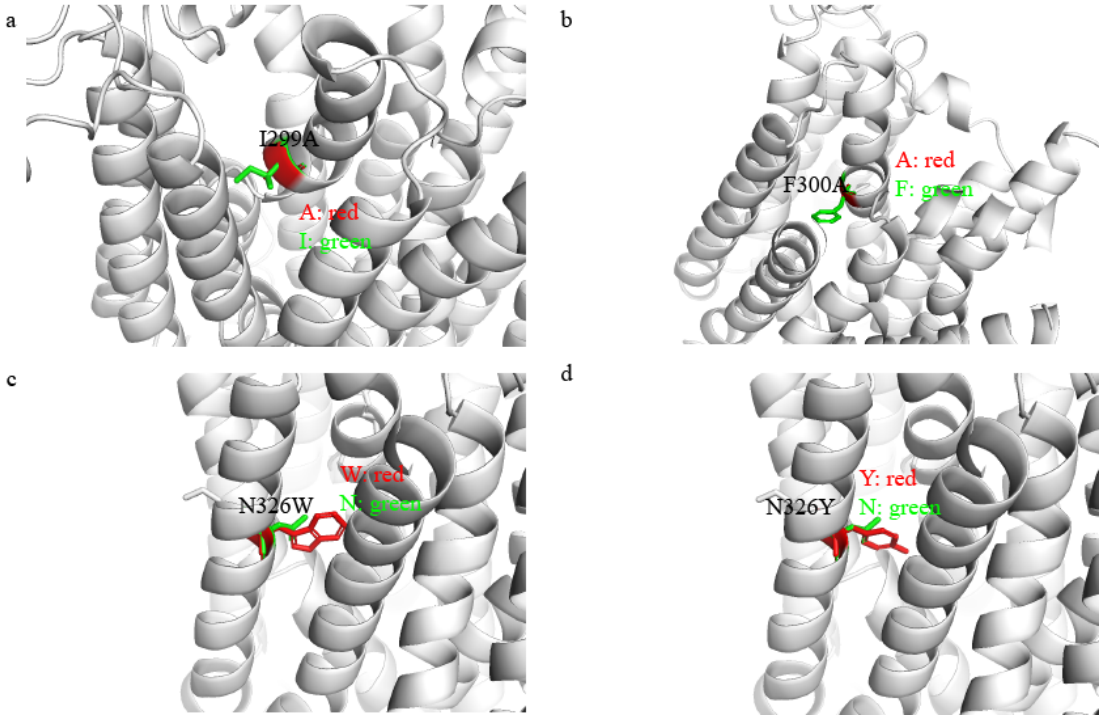


Figure S4 The interaction analysis between Xltr1p and xylose molecule by ligplot.



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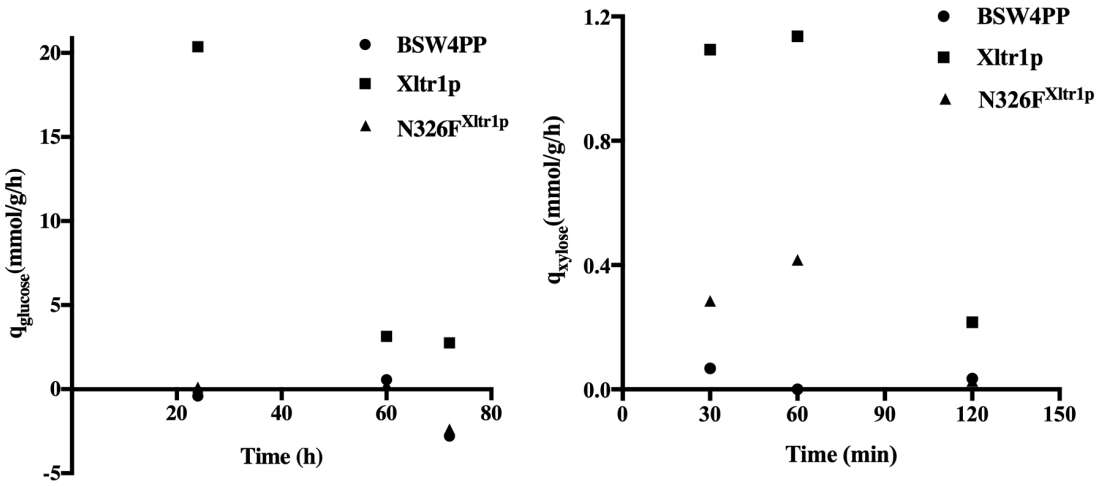

Figure S6 Kinetics of glucose/ D-xylose consumption($q_{glucose,}$ q_{xylose}) for Xltr1p(square), $N326F^{Xltr1}$ (triangle) and BSW4PP(circle). For kinetics for glucose, the cells were cultivated in SD medium with 20 g/L D-xylose at 30 °C for 24h, 60h, and 72h with an initial OD=0.1. For kinetics for D-xylose, the cells were cultivated in SD medium with 20 g/L D-xylose at 30 °C for 30, 60, and 120 minutes with an initial OD=20.

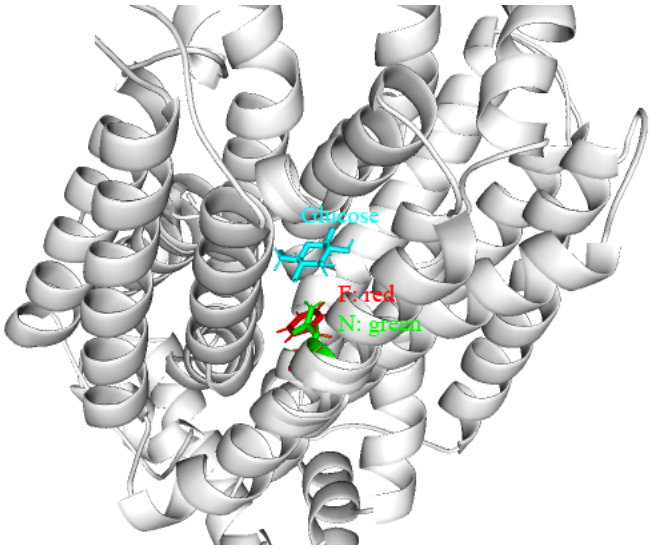

Figure S7 The result on docking study on Xltr1p or its variant N326F with glucose. The origin amino acid residue was displayed in green color, and mutated amino acid residue was displayed in red color.

Table S1 Sequence similarities among transporters used in this study

Sequence Similarity	XylE	Gal2	Mgt01596	Gatr1p	Gltr1p
Xltr1p	28.57%	35%	37.55%	35.17%	29.18%

Table S2 Primers used in this study

Name of Primer	Nucleotide Sequence
D39A-F	GCCACTGGCACCATTTCCGG
D39A-R	ATATCCGTAGAGGACACCGC
R132A-F	GCCTTCTTTGCCGGCTTC
R132A-R	ACCAGCCAGGAACATGGGG
Q288A-F	GCGGCGCTGCAGCAGCTGA
Q288A-R	CAGGGCCATGCCGGTGAAC
Q291A-F	GCGCAGCTGACGGGCATCAAC
Q291A-R	CAGCGCCTGCAGGGCCATG
Q292A-F	CAGGCGCTGACGGGCATCAAC
Q292A-R	CAGCGCCTGCAGGGCCATG
F298A-F	GCCATCTTCTACTACGGGACGCGG
F298A-R	GTTGATGCCCGTCAGCTGC
I299A-F	TTCGCCTTCTACTACGGGACGCGG
F299A-R	GTTGATGCCCGTCAGCTGC
F300A-F	GCCTACTACGGGACGCGGTA
F300W-F	TGGTACTACGGGACGCGGTA
F300Y-F	TACTACTACGGGACGCGGTA
F300X-R	GATGAAGTTGATGCCCGTCAG
Y301A-F	GCCGGGACGCGGTATTTCCAG
Y301-R	GTAGAAGATGAAGTTGATGCCCGTC

Y302A-F	TACGCGACGCGGTATTTCCAG
Y302-R	GTAGAAGATGAAGTTGATGCCCGTC
N326A-F	GCCGTCGCCTCCACCATCC
N326A-R	GATGCCCGCCGTGATCATG
N326F-F	TTCGTCGCCTCCACCATCC
N326W-F	TGGGTCGCCTCCACCATCC
N326Y-F	TACGTCGCCTCCACCATCC
N326X-R	GATGCCCGCCGTGATCATG
W407A-F	GCGGTCGTCACCGGCGAAATC
W407A-R	CGCGAGGGGGCCCCAGG
N434A-F	GCCTGGGCCATTGCCTACTC
N434A-R	GAAGAGCCAGTTTGTGGCCG
Gal2-F	ATGGCAGTTGAGGAGAACAATATGC
Gal2-R	TTATTCTAGCATGGCCTTGTACCAC
Xltr1-F	ATGCGTTTCTCCGAGAAGCTC
Xltr1-R	TTACACTTGAAGTTCAACTCCTTGGTC
Gatr1-F	ATGGGATTCTTGAACAAAAAAGCCG
Gatr1-R	TTATTTCTCATCCTCGTGAGCGTGC
Gltr1-F	ATGGCAGACGCTGATCTCAAAC
Gltr1-R	TCATGCAGCCCCCGTAGAC
yeGFP-F	ATGTCTAAAGGTGAAGAATTATTCACTG
yeGFP-R	TTATTTGTACAATTCATCCATACCATGG

Note:

1. “F” represents forward primer, and “R” represents reverse primer.

2. F300X-R/N326X-R represents the reverse primer for following mutated amino acid residues:

F300A, F300W, F300Y; N326A, N326F, N326W, and N326Y.