Supplementary Information Appendix for:

Rewiring yeast sugar transporter preference through modifying a conserved protein motif

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Supplementary Methods Section

Growth rate measurements – All exponential growth rates were measured and calculated according to the method previously described using a Bioscreen C (Growth Curves USA, Piscataway, New Jersey) and a MATLAB script. The Bioscreen C measures online optical density for easy and accurate measurement of the growth curves of up to 200 strains at one time. Error was calculated based on biological triplicate in all cases. In all cases, the Bioscreen C was set to maintain a temperature of 30ºC, employ high continuous shaking, and to measure optical density every 10 minutes. A single carbon source per well was used in all experiments save one. Growth on xylose in the presence of increasing concentrations of glucose was measured for *C. intermedia* gxs1 Phe[38] Ile[39] Met[40].

It is important to note that the environment of the Bioscreen C does not support cultures reaching high optical density and observed values are below OD_{600} of 2. This does not reflect the optical densities reached in flasks, which typically approach OD_{600} of 10.

Transporter Cloning - Each of these transporters was functionally analyzed for conferred growth rate on xylose and glucose in *S. c. EX.12*. Genomic DNA and PCR were performed as previously described (36). Using this approach, open reading frames from *Scheffersomyces stipits*, *Debaryomyces hansenii*, *Yarrowia lipolytica*, *and Saccharomyces cerevisiae* were cloned using primers listed in **Table S4**. Mutant transporters and saturation library construction is described below and Primers are listed in **Tables S5** (saturation) and **S6** (point).

Saturation mutagenesis and point mutation – The Strategene Multi mutagenesis kit was used to generate saturation mutagenesis libraries at positions 38, 39, and 40 in *C.i.* GXS1. Each codon was replaced with the degenerate NNK sequence recommended for use when creating saturation mutagenesis libraries. It is important to note that the wild type codon was represented in the NNK library for both Val[38] and Leu[39] thus alternative

primers that did not contain the wild type sequence were designed. This subsequently necessitated the design of specific point mutation primers to access certain residues and the use of the Stratagene Quikchange kit. Some single point mutation primers were ordered to complete the saturation libraries. The Stratagene Quikchange mutagenesis kit was used to generate all rational single, double, and triple mutants. Primers are listed in **Tables S5** (saturation) and **S6** (point).

Supplementary Figure Legends

Figure S1 - *Maximum exponential growth rates for all cloned native and heterologous transporters.* Bar chart of growth rate (μ) calculated from growth curves of *S. cerevisiae EX.12* measured on a Bioscreen C. Carbon source profiling on five different sugars allows better functional classification than measuring only glucose and xylose. Error is standard deviation of biological triplicates. **A.** Transporters cloned in the initial study measured for the first time in *S. cerevisiae EX.12*. **B.** Novel transporters identified and characterized in this study. Abbreviations: *Empty – empty vector control strain. A.t. – Arabidopsis thaliana. C.i. – Candida intermedia. C.n. – Cryptococcos neoformans. D.h. – Debaryomyces hansenii. S.c. – Saccharomyces cerevisiae. S.s. – Scheffersomyces stipitis. Y.l. – Yarrowia lipolytica.*

Figure S2 - *High cell density cofermentation in* S. cerevisiae *EX.12.* Cells were inoculated at OD 20 in a mixture of 10 g/L glucose and 10 g/L xylose. Optical density, glucose, xylose, and ethanol concentration was measured over the length of the fermentation. Note that the triple mutant does not consume either xylose or glucose, nor is an appreciable amount of ethanol produced in this multiple knockout strain. **A.** Optical density over time. **B.** Glucose concentration in the media over time. **C.** Xylose concentration in the media over time. **D.** Ethanol concentration in the media over time.

Figure S3 – *High cell density cofermentation in* S. cerevisiae *YSX3*. Cells were inoculated at OD 20 in a mixture of 10 g/L glucose and 10 g/L xylose. Optical density, glucose, xylose, and ethanol concentration was measured over the length of the fermentation. Note that the triple mutant does not appreciably alter the fermentation dynamics in a strain that is expressing the full suite of transporters. **A.** Optical density over time.

B. Glucose concentration in the media over time. **C.** Xylose concentration in the media over time. **D.** Ethanol concentration in the media over time.

Figure S4 - *Growth curves of transporters of interest.* Optical density measurements from the Bioscreen C were plotted over time. Each line represents the growth curve for *S. cerevisiae EX.12* expressing a transporter on a particular carbon source. **A.** *D.h. 2D01474.* **B.** *S.s. RGT2.* **C.** *D.h. 2E01166.* **D.** *D.h. 2B05060.* **E.** *S.c. STL1.* **F.** *S.s. AUT1.*

Figure S5 - *Phylogenetic tree and growth rate.* Phylogram constructed in TreeView of a ClustalW multiple sequence alignment with the full amino acid sequences of all transporters. To the right of the phylogram is plotted the exponential growth rate of *S. cerevisiae EX.12* conferred by transporter expression. A blue line and a green line are placed across the chart to mark the upper limit of no growth for glucose and xylose, respectively. Note the most robust glucose growth phenotypes are clustered in the HXT family and related transporters. Some of the more desirable growth phenotypes for xylose growth are clustered in the transporters related to *C.i. GXS1* and *S.s. XUT3.*

Figure S6 - *Relatedness based on G-G/F-XXXG motif and growth rate data.* Phylogram constructed in TreeView of a ClustalW multiple sequence alignment of the G-G/F-XXG motif of each transporter. To the right of the phylogram is plotted the exponential growth rate of *S. cerevisiae EX.12* conferred by transporter expression. A blue line and a green line are placed across the chart to mark the upper limit of no growth for glucose and xylose, respectively. Arranging the transporters in this fashion remarkably clusters conferred phenotype better than basing the alignment on the whole amino acid sequence. This is further evidence of the influence the G-G/F-XXG motif has over monosaccharide uptake.

Figure S7 – *Carbon source profile comparison.* **A**. *C.i. GXS1* and mutants. **B**. *S.s. RGT2* and mutants. **C**. *S.c. HXT7* and mutants. Note that these values are maximum exponential growth rates, and therefore may produce different comparisons than the late-stage linear exponential portions of the growth curves.

Supplementary Figures

Figure S1

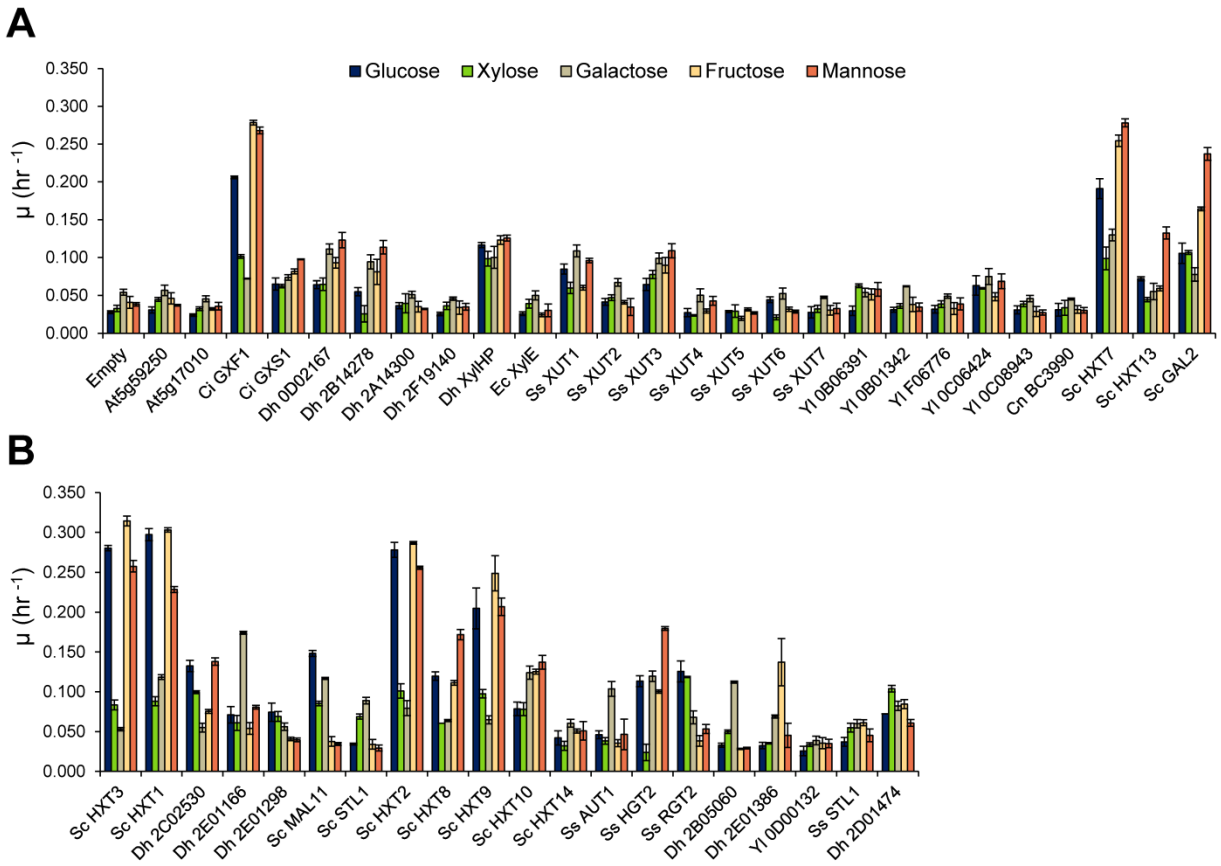
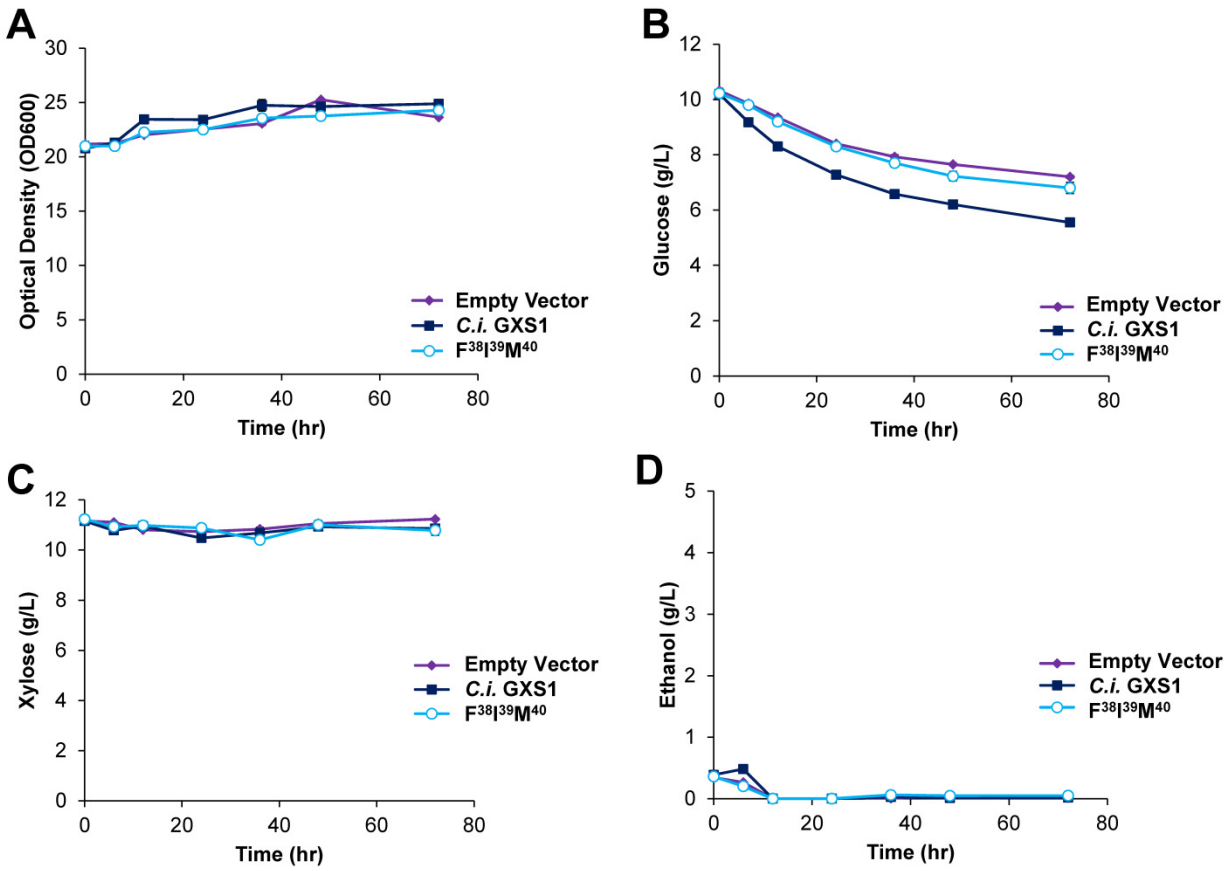


Figure S2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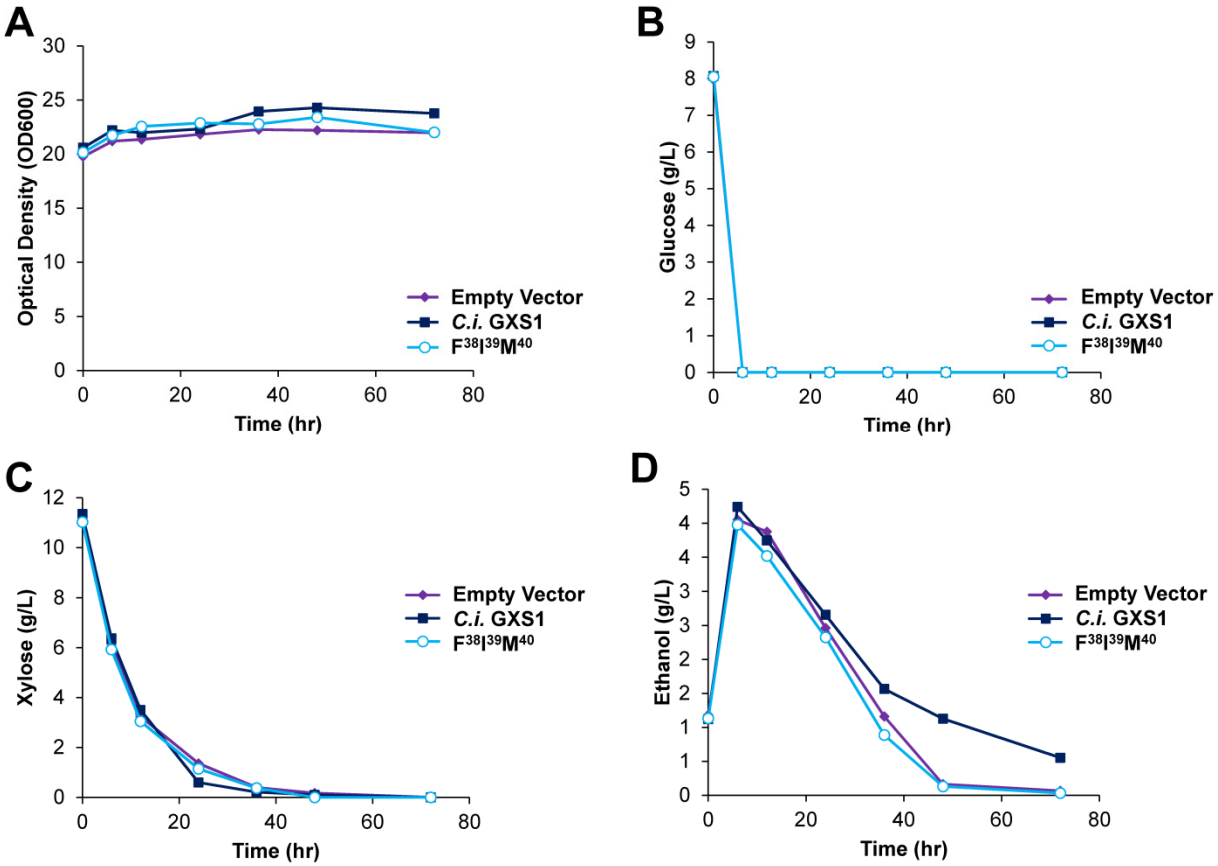
Optical Density (OD600)
Time (hr)
Empty Vector
C.i. GXS1
F38I39M40
B
Glucose (g/L)
Time (hr)
Empty Vector
C.i. GXS1
F38I39M40
C
Xylose (g/L)
Time (hr)
Empty Vector
C.i. GXS1
F38I39M40
D
Ethanol (g/L)
Time (hr)
Empty Vector
C.i. GXS1
F38I39M40

Figure S3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Optical Density (OD600)
Time (hr)
Empty Vector
C.i. GXS1
F38I39M40
B
Glucose (g/L)
Time (hr)
Empty Vector
C.i. GXS1
F38I39M40
C
Xylose (g/L)
Time (hr)
Empty Vector
C.i. GXS1
F38I39M40
D
Ethanol (g/L)
Time (hr)
Empty Vector
C.i. GXS1
F38I39M40

Figure S4

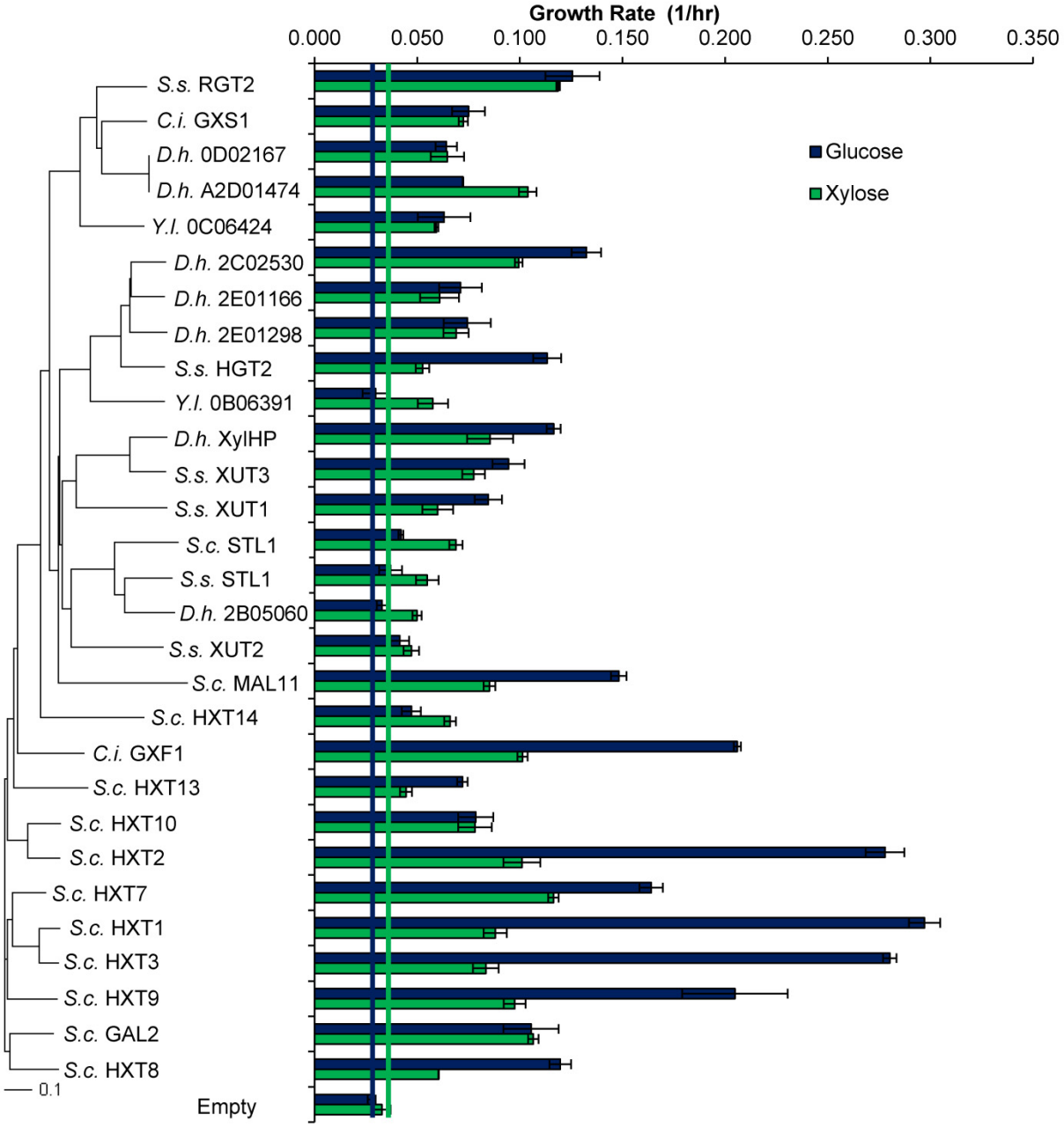
Growth Rate (1/hr)
0.000
0.050
0.100
0.150
0.200
0.250
0.300
0.350
Glucose
Xylose
S.s. RGT2
C.i. GXS1
D.h. 0D02167
D.h. A2D01474
Y.l. 0C06424
D.h. 2C02530
D.h. 2E01166
D.h. 2E01298
S.s. HGT2
Y.l. 0B06391
D.h. XylHP
S.s. XUT3
S.s. XUT1
S.c. STL1
S.s. STL1
D.h. 2B05060
S.s. XUT2
S.c. MAL11
S.c. HXT14
C.i. GXF1
S.c. HXT13
S.c. HXT10
S.c. HXT2
S.c. HXT7
S.c. HXT1
S.c. HXT3
S.c. HXT9
S.c. GAL2
S.c. HXT8
0.1
Empty

Figure S5

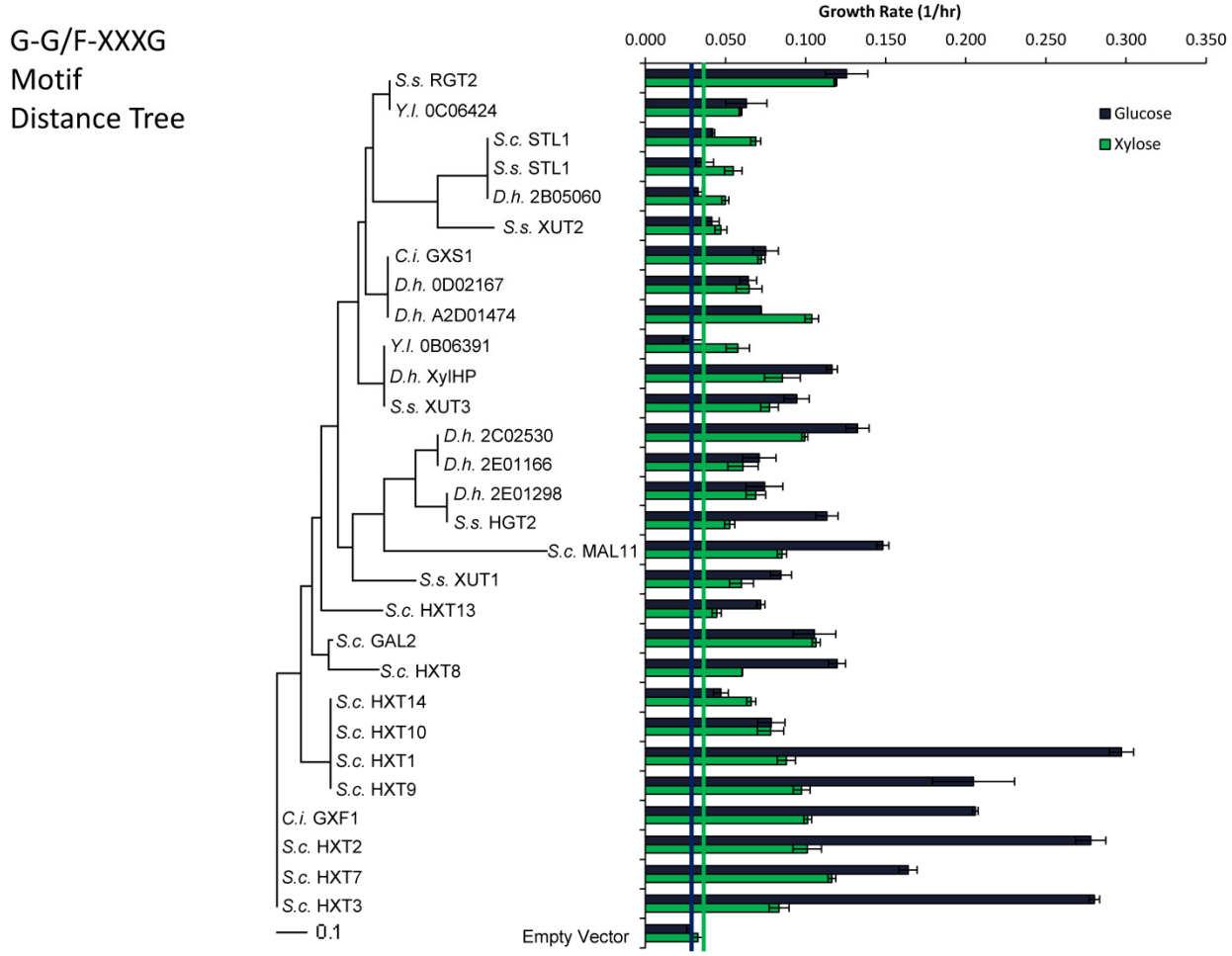
G-G/F-XXXG
Motif
Distance Tree
Growth Rate (1/hr)
0.000
0.050
0.100
0.150
0.200
0.250
0.300
0.350
Glucose
Xylose
S.s. RGT2
Y.l. 0C06424
S.c. STL1
S.s. STL1
D.h. 2B05060
S.s. XUT2
C.i. GXS1
D.h. 0D02167
D.h. A2D01474
Y.l. 0B06391
D.h. XylHP
S.s. XUT3
D.h. 2C02530
D.h. 2E01166
D.h. 2E01298
S.s. HGT2
S.c. MAL11
S.s. XUT1
S.c. HXT13
S.c. GAL2
S.c. HXT8
S.c. HXT14
S.c. HXT10
S.c. HXT1
S.c. HXT9
C.i. GXF1
S.c. HXT2
S.c. HXT7
S.c. HXT3
0.1
Empty Vector

Figure S6

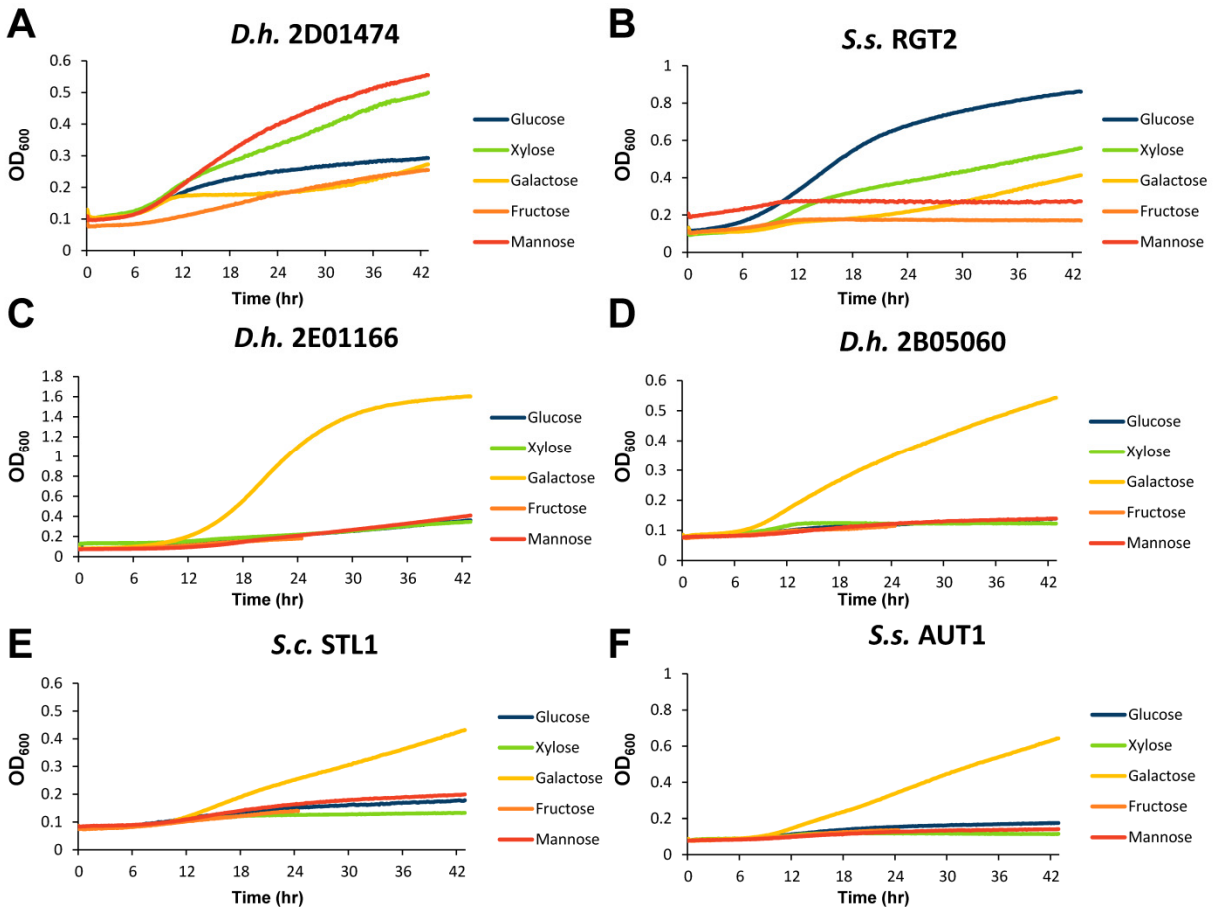
A
D.h. 2D01474
B
S.s. RGT2
C
D.h. 2E01166
D
D.h. 2B05060
E
S.c. STL1
F
S.s. AUT1
OD600
Time (hr)
Glucose
Xylose
Galactose
Fructose
Mannose

Figure S7

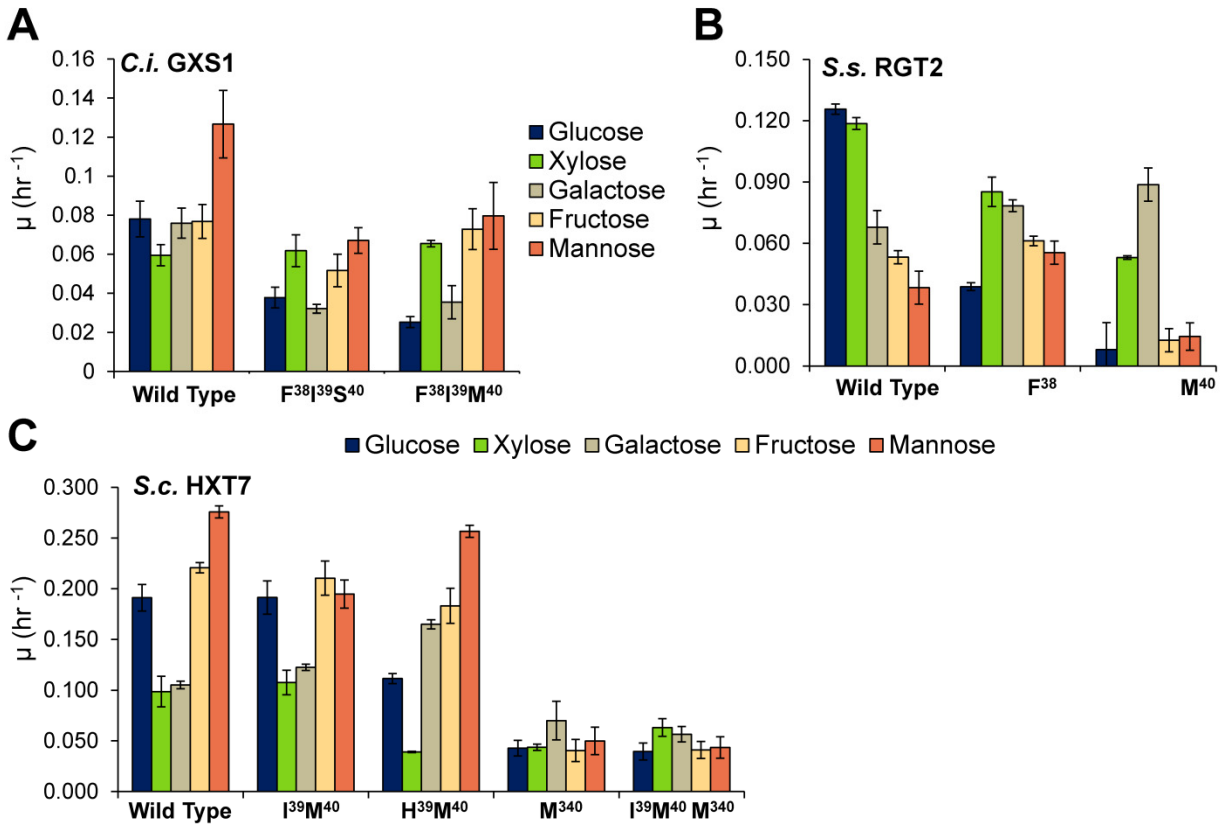
A
C.i. GXS1
μ (hr -1)
0.16
0.14
0.12
0.1
0.08
0.06
0.04
0.02
0
Wild Type
F38I39S40
F38I39M40
Glucose
Xylose
Galactose
Fructose
Mannose
B
S.s. RGT2
μ (hr -1)
0.150
0.120
0.090
0.060
0.030
0.000
Wild Type
F38
M40
C
Glucose
Xylose
Galactose
Fructose
Mannose
S.c. HXT7
μ (hr -1)
0.300
0.250
0.200
0.150
0.100
0.050
0.000
Wild Type
I39M40
H39M40
M340
I39M40 M340

Table S1

Exponential growth rate values for each cloned transporter.

			Glucose		Xylose		Galactose		Fructose		Mannose	
	GeneID	GenBank	μ (1/hr)	st. dev	μ (1/hr)	st. dev	μ (1/hr)	st. dev	μ (1/hr)	st. dev	μ (1/hr)	st. dev
Empty	-	-	0.028	0.002	0.033	0.004	0.054	0.004	0.041	0.008	0.038	0.002
At5g59250	836043		0.031	0.004	0.045	0.003	0.057	0.007	0.046	0.008	0.037	0.001
At5g17010	831564		0.024	0.002	0.032	0.002	0.046	0.004	0.032	0.002	0.036	0.005
Ci GXF1		AJ937350.1	0.206	0.002	0.101	0.003	0.072	0.000	0.278	0.003	0.268	0.004
Ci GXS1		AJ875406.1	0.065	0.008	0.062	0.002	0.074	0.004	0.082	0.003	0.098	0.000
Dh 0D02167	2901237		0.064	0.005	0.065	0.008	0.111	0.007	0.093	0.007	0.123	0.010
Dh 2B14278	2913528		0.055	0.005	0.026	0.011	0.094	0.009	0.081	0.017	0.114	0.009
Dh 2A14300	8998057		0.036	0.004	0.040	0.013	0.051	0.004	0.035	0.007	0.032	0.001
Dh 2F19140	8999011		0.026	0.002	0.036	0.005	0.046	0.002	0.034	0.009	0.035	0.004
Dh XylHP	50419288		0.116	0.003	0.098	0.010	0.100	0.014	0.123	0.006	0.126	0.004
Ec XylE	948529		0.026	0.002	0.039	0.006	0.050	0.006	0.024	0.002	0.030	0.009
Ss XUT1	4839826		0.085	0.007	0.060	0.007	0.109	0.008	0.060	0.003	0.096	0.003
Ss XUT2	4852047		0.042	0.005	0.047	0.004	0.067	0.005	0.041	0.002	0.035	0.011
Ss XUT3	4851844		0.064	0.008	0.078	0.005	0.099	0.007	0.090	0.010	0.109	0.009
Ss XUT4	4840896		0.027	0.005	0.024	0.001	0.050	0.009	0.029	0.003	0.043	0.006
Ss XUT5	4840252		0.029	0.001	0.029	0.008	0.020	0.003	0.031	0.002	0.027	0.002
Ss XUT6	4841106		0.044	0.004	0.021	0.003	0.053	0.007	0.032	0.003	0.029	0.002
Ss XUT7	4851701		0.028	0.008	0.032	0.005	0.048	0.001	0.030	0.006	0.033	0.007
Yl 0B06391	2907283		0.030	0.006	0.063	0.002	0.054	0.006	0.051	0.007	0.058	0.009
Yl 0B01342	2906708		0.031	0.003	0.036	0.003	0.062	0.001	0.038	0.010	0.035	0.005
Yl F06776	2908504		0.032	0.005	0.039	0.004	0.049	0.003	0.033	0.008	0.039	0.008
Yl 0C06424	2909312		0.063	0.013	0.059	0.001	0.075	0.010	0.048	0.005	0.069	0.010
Yl 0C08943	2909701		0.031	0.005	0.039	0.003	0.046	0.004	0.029	0.007	0.027	0.003
Cn BC3990	4935064		0.031	0.008	0.034	0.010	0.045	0.001	0.032	0.005	0.030	0.004
Sc HXT7	851943		0.191	0.013	0.099	0.015	0.130	0.008	0.254	0.008	0.278	0.005
Sc HXT13	856640		0.072	0.003	0.045	0.003	0.055	0.011	0.059	0.003	0.132	0.008
Sc GAL2	856640		0.105	0.013	0.107	0.003	0.078	0.009	0.164	0.003	0.237	0.008
Sc HXT3	851946		0.280	0.003	0.083	0.006	0.053	0.002	0.314	0.006	0.258	0.007
Sc HXT1	856494		0.297	0.008	0.088	0.006	0.118	0.003	0.303	0.003	0.229	0.004
Dh 2C02530	8998297		0.132	0.007	0.099	0.002	0.055	0.006	0.075	0.002	0.138	0.005
Dh 2E01166	2902950		0.071	0.010	0.061	0.010	0.174	0.002	0.054	0.007	0.081	0.002
Dh 2E01298	2902912		0.074	0.011	0.069	0.006	0.056	0.005	0.041	0.002	0.040	0.002
Sc MAL11	853207		0.148	0.004	0.085	0.003	0.117	0.001	0.038	0.006	0.034	0.002
Sc STL1	852149		0.034	0.001	0.069	0.003	0.089	0.004	0.034	0.006	0.029	0.004
Sc HXT2	855023		0.278	0.009	0.101	0.009	0.079	0.009	0.287	0.002	0.256	0.002
Sc HXT8	853216		0.120	0.005	0.060	0.000	0.064	0.001	0.111	0.003	0.172	0.007
Sc HXT9	853236		0.205	0.026	0.098	0.005	0.065	0.005	0.249	0.022	0.207	0.011
Sc HXT10	850536		0.079	0.009	0.078	0.008	0.124	0.008	0.125	0.003	0.137	0.009
Sc HXT14	855398		0.042	0.009	0.032	0.006	0.061	0.005	0.051	0.003	0.051	0.012
Ss AUT1	4836720		0.046	0.005	0.038	0.004	0.104	0.009	0.036	0.004	0.046	0.019
Ss HGT2	4836632		0.113	0.007	0.024	0.010	0.120	0.007	0.100	0.002	0.179	0.002
Ss RGT2	4840859		0.126	0.013	0.119	0.001	0.068	0.008	0.038	0.007	0.053	0.006
Dh 2B05060	2913215		0.033	0.003	0.050	0.002	0.112	0.001	0.028	0.001	0.029	0.001
Dh 2E01386	2902914		0.032	0.004	0.035	0.001	0.069	0.002	0.137	0.030	0.045	0.015
Yl 0D00132	2910370		0.026	0.006	0.034	0.002	0.039	0.005	0.035	0.007	0.035	0.005
Ss STL1	4838168		0.037	0.006	0.055	0.006	0.060	0.005	0.061	0.004	0.045	0.008
Dh 2D01474	2901237		0.072	0.000	0.104	0.004	0.082	0.006	0.085	0.006	0.061	0.004

Table S2

Kinetics values calculated from radiolabeled xylose uptake.

Gene in p414-TEF	K_M (mM)	V_{MAX} (nmol min^{-1} $gDCW^{-1}$)
C.i. GXS1	0.0256 ± 0.0659	7.23 ± 0.6
C.i. GXS1 $F^{38}I^{39}M^{40}$	0.721 ± 0.116	15.01 ± 2.38

Table S3

Form of the G-G/F-XXXG motif for all cloned transporters.

	GG?	X1	X2	X3
At5g59250	Yes	L	L	F
At5g17010	Yes	L	L	Y
Ci GXF1	Yes	F	V	F
Ci GXS1	Yes	V	L	F
Dh 0D02167	Yes	V	L	F
Dh 2B14278	Yes	F	L	Y
Dh 2A14300	SG	M	M	F
Dh 2F19140	No	-	-	-
Dh XylHP	Yes	L	L	F
Ec XylE	Yes	L	I	F
Ss XUT1	Yes	L	V	Y
Ss XUT2	AF	I	L	F
Ss XUT3	Yes	L	L	F
Ss XUT4	No	-	-	-
Ss XUT5	HP	T	I	M
Ss XUT6	GF	L	L	F
Ss XUT7	No	-	-	-
Yl 0B06391	Yes	L	L	F
Yl 0B01342	Yes	M	L	F
Yl F06776	GV	F	L	F
Yl 0C06424	Yes	I	L	F
Yl 0C08943	Yes	L	L	Y
Cn BC3990	GV	W	L	F
Sc HXT7	Yes	F	V	F
Sc HXT13	Yes	F	L	P
Sc GAL2	Yes	F	M	F
Sc HXT3	Yes	F	V	F
Sc HXT1	Yes	F	I	F
Dh 2C02530	SG	L	M	F
Dh 2E01166	SG	L	M	F
Dh 2E01298	SG	M	M	F
Sc MAL11	TL	V	M	E
Sc STL1	GF	S	L	F
Sc HXT2	Yes	F	V	F
Sc HXT8	Yes	F	M	S
Sc HXT9	Yes	F	I	F
Sc HXT10	Yes	F	I	F
Sc HXT14	Yes	F	I	F
Ss AUT1	No	-	-	-

Ss HGT2	SG	M	M	F
Ss RGT2	Yes	I	L	F
Dh 2B05060	GF	S	L	F
Dh 2E01386	GF	S	L	F
Yl 0D00132	N	-	-	-
Ss STL1	GF	S	L	F
Dh 2D01474	Y	V	L	F

Table S4

Primers used for cloning putative transporters.

Name	*Target ORF*	*Orientation*	*Tm*	*R. Enz.*	*Sequence*
EY194	ScHXT3	F	55	XmaI	TATTCCCCCGGGatgaattcaactccagatttaatatctcc
EY208	ScHXT3	R	64	ClaI	CGGTATCCATCGATttatttcttgccgaacattttctt
EY265	HXT1	F	64	XmaI	TATTCCCCCGGGatgaattcaactcccgatctaatatc
EY266	HXT1	R	64	ClaI	CGGTATCCATCGATttatttcctgctaaacaaactcttg
EY554	SsHGT2	F	60	SpeI	GGACTAGTatgagctacgaagataaactcg
EY555	SsHGT2	R	60	SalI	TATTCCGTCGACttaaggcttttcctcagaactt
EY558	Dh2C02530	F	60	SpeI	GGACTAGTatgggttacgaagataaattagtg
EY559	Dh2C02530	R	60	SalI	TATTCCGTCGACttaagtcatgtgagaagtatcgc
EY560	Dh2E01166	F	61	SpeI	GGACTAGTatgggatatgaagaaaagttgg
EY561	Dh2E01166	R	59	SalI	TATTCCGTCGACtcaagcaatgtgatctgc
EY562	Dh2E01298	F	61	SpeI	GGACTAGTatgggatacgaagataaattactagg
EY563	Dh2E01298	R	60	SalI	TATTCCGTCGACctaagcaatatggacagcactag
EY564	ScMAL11	F	60	SpeI	GGACTAGTatgaaaaatatcatttcattggtaag
EY565	ScMAL11	R	60	SalI	TATTCCGTCGACttaacatttatcagctgcatttaat
EY566	ScSTL1	F	60	SpeI	GGACTAGTatgaaggatttaaaattatcgaattt
EY567	ScSTL1	R	60	SalI	TATTCCGTCGACtcaaccctcaaaatttgct
EY568	SsRGT2	F	60	SpeI	GGACTAGTatgggtttagaagacagtgct
EY569	SsRGT2	R	61	SalI	TATTCCGTCGACctatacagaagcttcttcaacttcag
EY572	SsAUT1	F	61	XmaI	TATTCCCCCGGGatgagtgctgacgaaaaagtc
EY573	SsAUT1	R	61	XhoI	TATTCCCTCGAGctactcgacataagagacttctgg
EY574	HXT8	F	61	XmaI	TATTCCCCCGGGatgactgatcgtaaaaccaactt
EY575	HXT8	R	61	XhoI	TATTCCCTCGAGctaaaacattcttttgtagaagggtt
EY576	HXT2	F	62	XmaI	TATTCCCCCGGGatgtctgaattcgctactagcc
EY577	HXT2	R	63	XhoI	TATTCCCTCGAGttattcctcggaaactctttttc
EY578	HXT9	F	62	XmaI	TATTCCCCCGGGatgtccggtgttaataatacatcc
EY579	HXT9	R	62	XhoI	TATTCCCTCGAGttagctggaaaagaacctcttg
EY580	HXT10	F	60	XmaI	TATTCCCCCGGGatggttagttcaagtgtttcca
EY581	HXT10	R	60	XhoI	TATTCCCTCGAGttatttactatcaacaataactaatggtgtac
EY582	HXT14	F	61	XmaI	TATTCCCCCGGGatgactgctcagattccgtat
EY583	HXT14	R	61	XhoI	TATTCCCTCGAGctactccggttcaaatattttattg
EY644	SsSTL1	F	61	SpeI	GGACTAGTatggcatatcttgattggttaac
EY645	SsSTL1	R	62	XmaI	TATTCCCCCGGGctaggctgctttaggtttttctg
EY646	DhE01386	F	61	SpeI	GGACTAGTatgtataaaatatggtcaaaaactaacact
EY647	DhE01386	R	61	XmaI	TATTCCCCCGGGttaaacttccgcaggcttaa
EY648	DhB05060	F	63	SpeI	GGACTAGTatggctttaaaaatcttttctagaacc
EY649	DhB05060	R	63	XmaI	TATTCCCCCGGGttaagcattaggagttaagataccttctg
EY650	Yl0D00132	F	63	SpeI	GGACTAGTatggtttttggacgagaaaaag
EY651	Yl0D00132	R	63	XmaI	TATTCCCCCGGGttaaacgaactcggcagtgt
EY700	DhA2D01474	F	55	XmaI	TATTCCCCCGGGatgggtttagaagataatgc
EY701	DhA2D01474	R	56	XhoI	TATTCCCTCGAGttagactgaagtggtttcaat

Table S5

Primers used for saturation mutagenesis of C. intermedia GXS1.

Name	*Target A.A.*	*Orientation*	*Tm*	*Kit Used*	*Sequence*
EY630	GXS1Sat40	F	79	Sat Multi	tttgctgcttctggtggtgtccttNNKggatacgatactggtacta
EY675	GXS1 39LSat	F	79	Sat Multi	tttgctgcttctggtggtgtcnnkttcggatacgatactggtact
EY676	GXS1 38VSat	R	79	Sat Multi	gtctttttgctgcttctggtggtnnkcttttcggatacgatactggtac
EY711	GXS1 38Sat	F	79	Sat Multi	gtctttttgctgcttctggtggtyrkcttttcggatacgatactggtac
EY712	GXS1 39LSat	F	79	Sat Multi	tttgctgcttctggtggtgtcvvgttcggatacgatactggtact
EY723	GXS139DDK	F	78	Sat Multi	tttgctgcttctggtggtgtcDDKttcggatacgatactggtact
EY727	GXS1 38Y	F	78	Quik	gctgcttctggtggttatcttttcggatacgatac
EY728	GXS1 38Y	R	78	Quik	gtatcgtatccgaaaagataaccaccagaagcagc
EY729	GXS1 38C	F	78	Quik	gctgcttctggtggttgtcttttcggatacgatac
EY730	GXS1 38C	R	78	Quik	gtatcgtatccgaaaagacaaccaccagaagcagc
EY731	GXS1 38H	F	78	Quik	gctgcttctggtggtcatcttttcggatacgatac
EY732	GXS1 38H	R	78	Quik	gctgcttctggtggtcatcttttcggatacgatac
EY733	GXS1 39H	F	78	Quik	tgcttctggtggtgtccatttcggatacgatactg
EY734	GXS1 39H	R	78	Quik	cagtatcgtatccgaaatggacaccaccagaagca
EY735	GXS1 39M	F	78	Quik	gctgcttctggtggtgtcatgttcggatacgatactggt
EY736	GXS1 39M	R	78	Quik	accagtatcgtatccgaacatgacaccaccagaagcagc

Table S6

Primers used for point mutations.

Name	*Target A.A.*	*Orientation*	*Tm*	*Sequence*
EY702	SsRGT2-F40M	F	78	cagccttcggtggtatccttatgggttatgacactggt
EY703	SsRGT2-F40M	R	78	accagtgtcataacccataaggataccaccgaaggctg
EY737	GXS1 FLS	F	78	tttttgctgcttctggtggtttcctttctggatacg
EY738	GXS1 FLS	R	78	cgtatccagaaaggaaaccaccagaagcagcaaaaa
EY739	GXS1 FIS	F	78	ttttgctgcttctggtggtttcatttctggatacgatactgg
EY740	GXS1 FIS	R	78	ccagtatcgtatccagaaatgaaaccaccagaagcagcaaaa
EY741	GXS1 FIM	F	78	ttttgctgcttctggtggtttcattatgggatacgatactgg
EY742	GXS1 FIM	R	78	ccagtatcgtatcccataatgaaaccaccagaagcagcaaaa
EY747	ScHXT7M340	F	78	ctatgattcaatctctacaacaattgacaggtatgaactatttcttctactatggtactactatttt
EY748	ScHXT7M340	R	78	aaaatagtagtaccatagtagaagaaatagttcatacctgtcaattgttgtagagattgaatcatag
EY749	ScHXT7 FHM	F	78	catgatcgcctttggtggtttccatatgggttgggatactggtaccattt
EY750	ScHXT7 FHM	R	78	aaatggtaccagtatcccaacccatatggaaaccaccaaaggcgatcatg
EY753	ScHXT10 63M	F	78	ctgatgattgcctttggtggattcattatgggttgggatacagg
EY754	ScHXT10 63M	R	78	cctgtatcccaacccataatgaatccaccaaaggcaatcatcag
EY760	SsRGT2-38F	F	78	gttcgcagccttcggtggtttccttttcggtta
EY761	SsRGT2-38F	R	78	taaccgaaaaggaaaccaccgaaggctgcgaac
EY766	ScHXT7-FIM	F	78	catgatcgcctttggtggtttcattatgggttgggatactggtaccattt
EY767	ScHXT7-FIM	R	78	aaatggtaccagtatcccaacccataatgaaaccaccaaaggcgatcatg