

Electrophysiological characterization of a diverse group of sugar transporters from *Trichoderma reesei*

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Supplementary information

Supplementary table S1. Accession numbers and references for the proteins in the phylogenetic tree shown in Fig. 1. Protein IDs for *T. reesei* transporters refer to QM6a genome version 2.0¹. RUT-C30 genome² versions of the protein were used for Tr_CRT1 (TrireRUT-C30_109243), Trire2_67752 (TrireRUT-C30_79984), Trire2_72383 (TrireRUT-C30_133083), Tr_XLT1 (TrireRUT-C30_33630), Trire2_123120 (TrireRUT-C30_26008) and Trire2_4774 (TrireRUT-C30_85251), as indicated in the table. Abbreviations: glc = D-glucose, fru = D-fructose, gal = D-galactose, mann = D-mannose. rha = L-rhamnose, sor = L-sorbose, xyl = D-xylose, ara = L-arabinose, cb = cellobiose, c3 = cellotriose, c4 = cellotetraose, lac = lactose, m2 = mannobiose, x2 = xylobiose, x3 = xylotriose, x3 = xylotetraose, mal = maltose, suc = sucrose, raf = raffinose, galUA = D-galacturonic acid, glcUA = D-glucuronic acid, cbA = cellobionic acid.

Organism	Transporter	Accession	Substrates	Reference
<i>Aspergillus flavus</i>	Afla_ST1	XP_001820343	cb, x2, x3	3
<i>Aspergillus nidulans</i>	Anid_LacpA	Q5B8D1		4
	Anid_LacpB	Q5B9G6	cb, lac	5–7
	Anid_CltA	Q5ATN3	cb	6
	Anid_Xtrd	Q5BGT0	glc, xyl, fru, gal, mann	8
	Anid_MstC/HxtB	Q5AYG1	glc, xyl, fru, gal, mann	6,9–11
<i>Aspergillus niger</i>	Anid_HxtC	C8VDD8	glc, fru, gal, mann	11
	Anid_MstA/HxtD	Q5ASJ3	glc	9,11
	Anid_HxtE	Q5BCD3	glc, fru, gal, mann	11
	Anid_MstE	AN5860		9
	Anid_ST15	XP_657617	x2, x3	3
	Anig_RhtA	XP_025455900	rha, fru	12
	Anig_XltA	XP_026623817	glc, xyl, gal, mann	13
	Anig_XltB	XP_025455052	xyl, mann, cb	13
	Anig_XltC	XP_025450664	glc, xyl, fru, gal, mann	13
	Anig_MstA	A2R055	glc, xyl, fru, mann	14,15
	Anig_MstG	XP_025453682	glc, gal, mann, suc	16
	Anig_MstH	XP_001396930	glc, fru, gal, mann, suc	16

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Organism	Transporter	Accession	Substrates	Reference
<i>Aspergillus oryzae</i>	Anig_GatA	A2R3H2	galUA	17,18
	Anig_CtA	A2R0P0	cb, c3, c4, c5	19
	Anig_1186134	XP_001396690	fru, sor	20
	Anig_296054	XP_001391049	fru, sor, mann	20,21
	Anig_mstC	XP_001399490.1	glc, mann, gal	21
	Anig_mstE	XP_001390118	mann, fru, gal	21
	Anig_An01g00850	XP_001388529.2	mann, fru, gal	21
	Anig_An11g09600	A2QXN5	mann	21
	Anig_An12g05820	A2QZQ8	suc	21
	Anig_An02g07610	XP_001399894	suc	21
	Anig_An03g01750	XP_001390077	mann	21
	Anig_An06g00620	XP_001390889	gal	21
	Ao_MalP	Q96WT5	mal	22
	Fgra_ST2	XP_011322622	cb, x2, x3	3
	Foxy_ST3	XP_018236089	x2, x3	3
<i>Fusarium oxysporum</i>	Nc_An25	Q7SEB3	xyl	23
<i>Neurospora crassa</i>	Nc_CDT-1	Q7SCU1	cb, c3, c4, lac, m2	7,24,25
	Nc_CDT-2	Q7SD12	cb, c3, lac, x2, x3, x4, m2	3,7,24–26
	Nc_GAT-1	Q7SD36	galUA, glcUA	27
	Nc_GLT-1	Q1K4S3	glc, xyl	28,29
	Nc_HGT-1	Q7S0I5	glc	29,30
	Nc_HGT-2	Q7S3Q4	glc	29
	Nc_CBT-1	Q7S555	cba	31,32
	Nc_XYT-1	Q7S5X9	xyl	28
	Nc_XAT-1	F5HC09	xyl, ara	28
	Nc_HXT-1	Q7SHD8	glc, fru, gal, sor, mann	33
	Nc_HXT-2	Q7S496	glc, fru, gal, sor	33
	Nc_NCU00809	Q7SEC5	cb	34
	Nc_LAT-1	Q7S4D2	glc, fru, gal, sor, mann, suc	28,35,36
	Nc_SUT-28	Q7S511		37
	Nc_RCO-3	Q92253		38
<i>Myceliophthora thermophila</i>	Mt_LAT-1	G2QFT5	ara	35
<i>Penicillium chrysogenum</i>	Pc_LacpA/ST	B6H4F0	cb	39,40
	Pc_AraT	B6HE12	ara	41
<i>Penicillium oxalicum</i>	Po_CdtC	EPS25673	cb	42
	Po_CdtD	EPS25817	cb	42
	Po_CdtG	EPS34431	cb, glc, lac	42,43
<i>Phanerochaete sordida</i>	Psor_hxt1	LC438459	glc, xyl, fru, mann	44
<i>Postia placenta</i>	Ppla_ST7	OSX60980	cb, x2, x3	3
<i>Thielavia terrestris</i>	Tter_ST/ST9	XP_003656976	cb	3,40
	Tter_ST13	XP_003650792	cb, x2, x3	3
	Tter_ST17	AEO63901	cb, x2, x3	3
<i>Trichoderma harzianum</i>	Thar_gtt1	Q8TFF4	glc	45
<i>T. reesei</i>	Tr_CRT1 ¹	ETS03552	cb, glc, lac	7,40,46
	Tr_HXT1	G0RQV6		47
	TRIRE2_46819	G0RFU7		

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Organism	Transporter	Accession	Substrates	Reference
<i>Trichoderma virens</i>	Tr_STP1	G0RIJ1	glc, fru, gal, mann, cb, xyl, ara	46,48,49
	TRIIRE2_50618	G0RQZ2		
	Tr_STR1	G0RRR0	glc, xyl, fru, gal, mann, ara	13
	TRIIRE2_56684	G0RB79		
	Tr_STR3	G0RKF0	glc, xyl, fru, gal, mann	13
	TRIIRE2_62502	G0RKB2		
	Tr_XLTR1	G0RN44	glc, mann, fru, xyl	50
	TRIIRE2_65191	G0RNV9		
	TRIIRE2_67469	G0RSB2		
	TRIIRE2_67752 ¹	ETS01940	cb	51
	TRIIRE2_68812	G0RUI6		
	TRIIRE2_69957	G0RW02	xyl, mann, cb	52
	TRIIRE2_72383 ¹	ETR98700		
	TRIIRE2_76800	G0RFZ0		
	TRIIRE2_77517	G0RIF6		53
	TRIIRE2_79202	G0RN18		53
	TRIIRE2_82309	G0RWI3		
	Tr_XLT1 ¹	ETS04871	xyl	54
	TRIIRE2_106330	G0RHS6	galUA	18
	TRIIRE2_106556	G0RGR7		
	Tr_STR2	G0RH09	glc, xyl, fru, gal, mann	13
	TRIIRE2_121850	G0RJM5		
	TRIIRE2_4774 ¹	ETR99734		
	TRIIRE2_48444	G0RJZ0		
	TRIIRE2_55077	G0RA72		
	TRIIRE2_60945	G0RIF7		
	TRIIRE2_69026	G0RUM8	galUA	18
	TRIIRE2_69901	G0RW16		
	TRIIRE2_105260	G0RF04		
	TRIIRE2_59388	G0RFJ2		
	TRIIRE2_109677	G0RQ49		
	TRIIRE2_122013	G0RJY9		
	TRIIRE2_123809	G0RUN3		
	TRIIRE2_26160	G0RI84		
	TRIIRE2_27770	G0RRJ5		
	TRIIRE2_27939	G0RUM4		
	TRIIRE2_53903	G0R951		
	TRIIRE2_5656	G0RUS5		
	TRIIRE2_65153	G0RP99		
	TRIIRE2_65493	G0RPR8		
	TRIIRE2_67541	G0RSV0		
	TRIIRE2_69651	G0RVI9		
	TRIIRE2_76758	G0RFS1		
	TRIIRE2_77785	G0RJ14		
	TRIIRE2_80091	G0RQ60		
	Tv_ST16	XP_013958763	x2	3

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Organism	Transporter	Accession	Substrates	Reference
<i>Ustilago maydis</i>	Tv_SUT	XP_013949637	suc	55
	Um_Srt1	A0A0D1E1N5	suc	56,57
	Um_Hxt1	A0A0D1DTL9	glc, fru, gal, mann	58
<i>Amanita muscaria</i>	Am_MST1	O13411	glc, fru	59
<i>Botrytis cinerea</i>	Bc_FRT1	Q5XTQ5	glc, fru	60
<i>Colletotrichum graminicola</i>	Cg_Hxt1	D2TG02	glc, fru, mann	61
	Cg_Hxt2	D2TG03	glc, fru, gal, mann	61
	Cg_Hxt3	D2TG04	glc, fru, gal, mann	61
	Cg_Hxt5	D2TG06	glc, fru, gal, mann	61
	Cg_Mbt1	D2TG08	mal, raff	62
<i>Uromyces fabae</i>	Uf_HXT1	Q96VF4	glc, fru	63
<i>Puccinia striiformis</i>	Ps_HXT1	A0A0L0VC49	glc, fru, mann, xyl, ara, suc	64
	Gs_MST2	G3CES2	glc	65
<i>Geosiphon pyriformis</i>	Gp_MST1	A0ZXK5	glc, gal, mann	66

¹RUT-C30 version was used.

Supplementary table S2. K_m values (in mM) of monosaccharide transporters from filamentous fungi. H^+ indicates sugar/ H^+ symport activity. Values are in mM. Uncertainties were omitted for clarity. Abbreviations as in Supplementary table S1. nd = not determined.

Organism	Protein	H^+	glc	mann	gal	fru	xyl	ara	Reference
<i>A. muscaria</i>	MST1	x	0.46			4.2			59
<i>A. nidulans</i>	hxtB	x	15				0.54		6,10,11
<i>A. niger</i>	mstA	x	0.025	0.06		4.5	0.3		15
	mstG	x	0.5						16
	mstH	x	0.06						16
	xltA	nd	0.07				0.09		13
	xltB	nd					14		13
	xltC	nd	0.11				4.71		13
	1186134	x				0.066			20
	296054	x				0.1			20
<i>B. cinerea</i>	FRT1	x				1.1			60
<i>C. graminicola</i>	Hxt1	x	0.0206						61
	Hxt2	x	0.136						61
	Hxt3	x	0.013						61
	Hxt5	x	0.92						61
<i>G. pyriformis</i>	MST1	x	1.2						66
<i>Glomus</i> sp.	MST2	x	0.033						65
<i>M. thermophila</i>	LAT-1	x						29.39	35
<i>N. crassa</i>	An25	-					175.7		23
	HGT-1	nd	0.016 13						29
	HGT-2	nd	0.098 97						29
	GLT1	nd	18.42						29
	XAT-1	nd					18.17	61.93	28
	XYT-1	nd					7.58		28
	LAT-1	x						58.12	35
<i>P. chrysogenum</i>	AraT	x						0.13	41
<i>P. striiformis</i>	HXT1	x	0.059						64
<i>T. reesei</i>	STP1	-	0.007 38						This study, 49
	STR1	nd	0.01				5.7		13
	STR1	x	0.1461				0.0411	5.79	This study
	STR2	nd	0.05				6.18		13
	STR3	nd	0.06				2.19		13
	STR3	x	0.0579	0.1862	1.11		2.74		This study
	FRT1	x				13.95			This study
	MLT1	x	39.74						This study
	GLT1	-	3.04						This study
<i>U. fabae</i>	HXT1	x	0.36			1			63
<i>U. maydis</i>	Hxt1	x	0.018						58

Supplementary table S3. K_m (in mM) values of di-/trisaccharide and sugar acid transporters from filamentous fungi. H^+ indicates sugar/ H^+ symport activity. Values are in mM. Uncertainties were omitted for clarity. Abbreviations as in Supplementary table S1. nd = not determined.

Organism	Protein	H^+	glc	CB	c3	lac	galUA	glcUA	suc	mal	raf	Reference
<i>A. niger</i>	GatA	nd					0.34					18
<i>C. graminicola</i>	Mbt1	-								8.7	0.31	62
<i>N. crassa</i>	CDT-1	x		0.004								24,67
	CDT-1	x		0.0044		0.0388						7
	CDT-1	x		0.0304	0.143	0.0454						This study
	CDT-2	-		0.0032								24,67
	GAT-1	x					0.0012					27
<i>T. reesei</i>	CRT1	x		0.0031		0.0629						7
	CRT1	x	9.26	0.0499		0.0831						This study
	GAT1	x					0.155	0.008				This study
	GAT2	x					0.015	0.031				This study
<i>T. virens</i>	SUT								1.5			55
<i>U. maydis</i>	Srt1	x							0.026			56
	Srt1 ¹								0.09			57
	Srt1 ²								0.12			57

¹Determined at pH 5.5

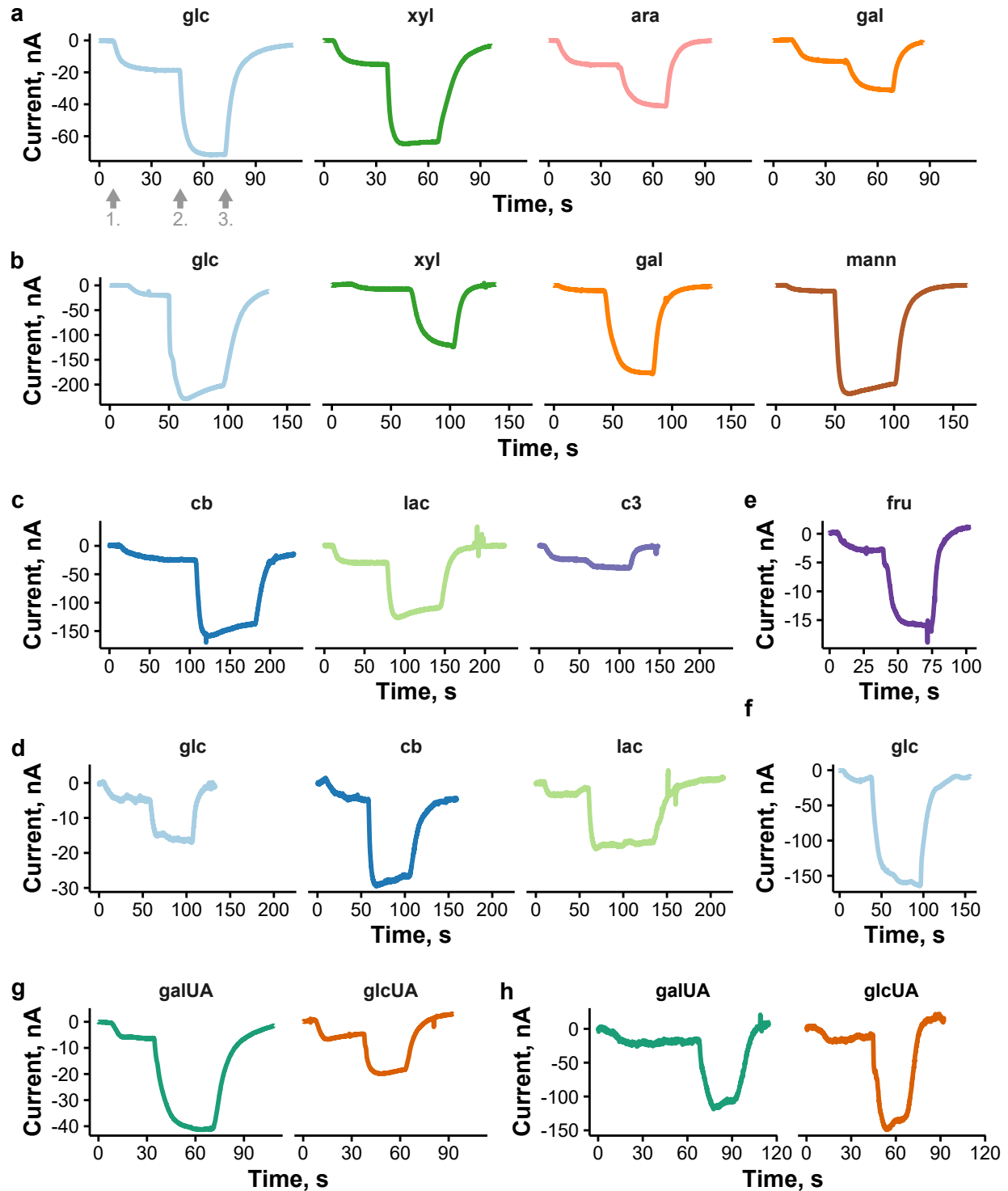
²Determined at pH 6.5

Supplementary table S4. Primers used in this study

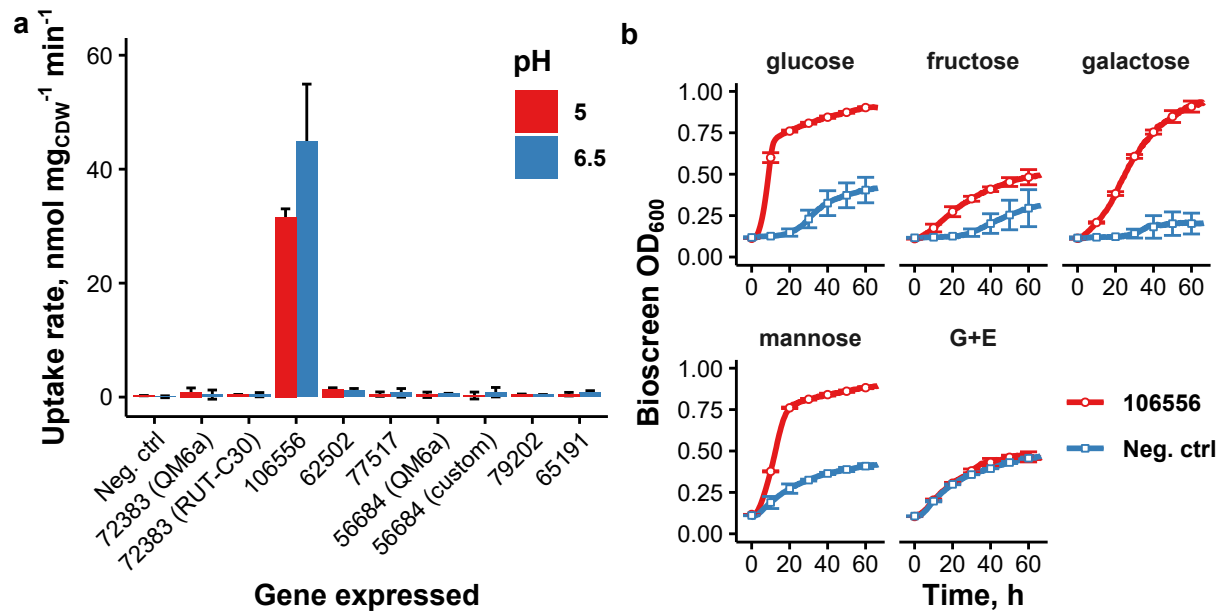
Name	Sequence
PP-203	CGCGGATCCATGGCCATCGCCATG
PP-204	CCCAAGCTTTTCAGTAACTGAAGTCAACG
PP-209	ATGCGGATCCATGGCTCCCTCTGCGGCGC
PP-210	ATGCGAATTCTTATACAGATGAACCGGAAACC
PP-211	ATGCTCTAGAATGTACTTGAAGCTCATCAGCG
PP-212	ATGCAAGCTTCTACTCTTGATGCAGCTCGGC
SaSS-19	GGTCAATGCAAGAAATACATATTTGGTCT
SaSS-20	CATTCTGACTATAAAATGAATAAACTAACTATTC
SaSS-57	ATTCGGGTGTTCTTGAGGCTGG
SaSS-75	AAATCTATAACTACAAAAACACATACAGGACAATGCGCTTCTTCAAGAACTATC
SaSS-76	TCAGTTAGCTAGCTGAGCTCGACTCTAGAGTTATACAGCCTTGTCGGCAGG
SaSS-95	TACAAAAGGATCCATGCGCTTCTTCAAGAACTATC
SaSS-96	CATGATCGAATTCTTATACAGCCTTGTCGGCAG
TPI1_P_long	GAAATTAATTAAAGCAATC
TPI1_T_long	GTATCGGTCAGTCATTAATAC

Supplementary table S5. Plasmids used in this study. The MoClo-based expression vector for yeast expression contained *PGK1* promoter, *ENO1* terminator and *CEN6/ARS4* origin of replication for low copy expression. QM6a version of the *T. reesei* genes was used unless otherwise specified. The manually annotated sequences are presented in Supplementary Fig. [S10](#).

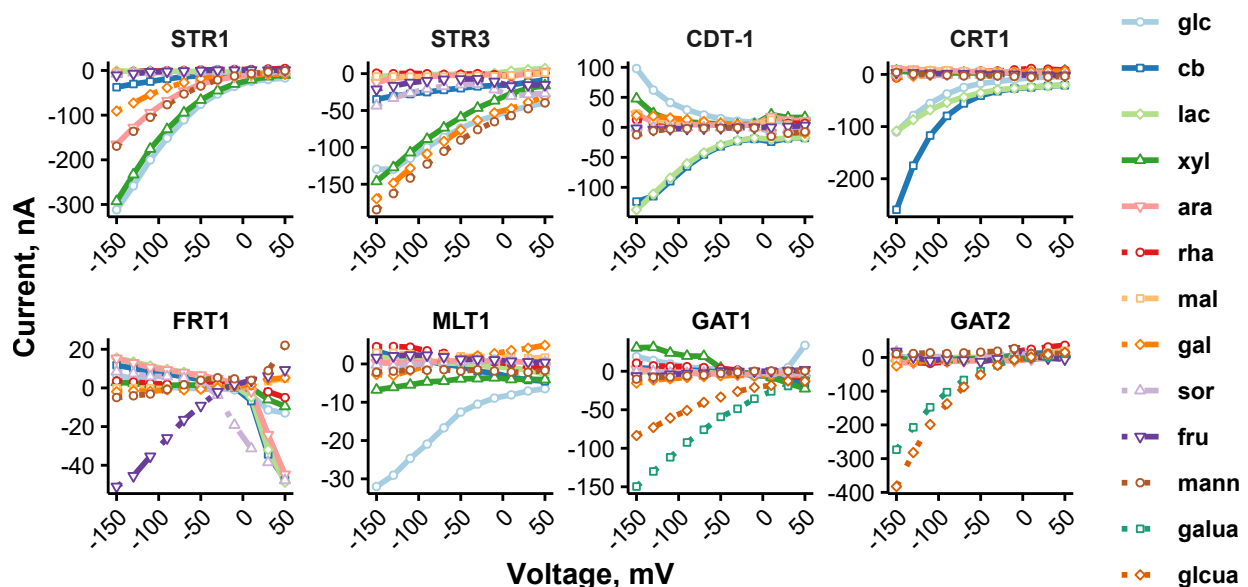
Name	Description	Reference
<i>S. cerevisiae</i> expression		
B2159	<i>P_{TPH1}</i> , <i>TPH1</i> , 2 μ m, amp ^R	68
pSS17	Trire2_72383 (QM6a version) in MoClo-based expression vector	This study
pSS27	Trire2_72383 (RUT-C30 version) in MoClo-based expression vector	This study
pSS35	STR3/Trire2_62380 (RUT-C30 version) in MoClo-based expression vector	This study
pSS36	Trire2_106556 in MoClo-based expression vector	This study
pSS14	Trire2_47710 in MoClo-based expression vector	This study
pSS40	Trire2_62502 in MoClo-based expression vector	This study
pSS16	Trire2_50894 in MoClo-based expression vector	This study
pSS29	Trire2_77517 in MoClo-based expression vector	This study
pSS51	Trire2_56684 (QM6a version) in MoClo-based expression vector	This study
pSS30	Trire2_56684 (manually annotated) in MoClo-based expression vector	This study
pSS18	Trire2_79202 in MoClo-based expression vector	This study
pSS32	Trire2_67469 (manually annotated) in MoClo-based expression vector	This study
pSS31	Trire2_65191 in MoClo-based expression vector	This study
pSS87	Trire2_69026 in B2159	This study
pSS49	CEL1a/Trire2_120749 in MoClo-based expression vector	This study
<i>X. laevis</i> expression		
pol1	<i>P_{T7}</i> , <i>X. laevis</i> β -globin 5' and 3' UTR, poly(A) tract, Amp ^R	69
pMJB08	pol1 with additional tags and modified multiple cloning site	70
pSS79	pol1 modified to be compatible with the MoClo system	Havukainen <i>et al.</i> , submitted
B7287	Trire2_72383 (QM6a version) in pMJB08	This study
pSS81	STR3/Trire2_62380 (RUT-C30 version) in pSS79	This study
pSS106	Trire2_50618 in pSS79	This study
pSS80	Trire2_106556 in pSS79	This study
pSS108	Trire2_62502 in pSS79	This study
pSS107	STR1/Trire2_50894 in pSS79	This study
B7290	Trire2_77517 in pMJB08	This study
pSS82	Trire2_67752 (RUT-C30 version) in pSS79	This study
pSS110	Trire2_56684 (manually annotated) in pSS79	This study
pSS83	CRT1/Trire2_3405 (RUT-C30 version) in pSS79	This study
B7291	Trire2_79202 (manually annotated) in pMJB08	This study
pSS113	Trire2_69957 in pSS79	This study
pSS111	Trire2_67469 (manually annotated) in pSS79	This study
pSS109	Trire2_65191 in pSS79	This study
pSS114	Trire2_69026 in pol1	This study
pSS84	Trire2_106330 in pSS79	This study
pSS104	CDT-1/NCU00801 in pSS79	This study
pSS105	CDT-2/NCU08114 in pSS79	This study



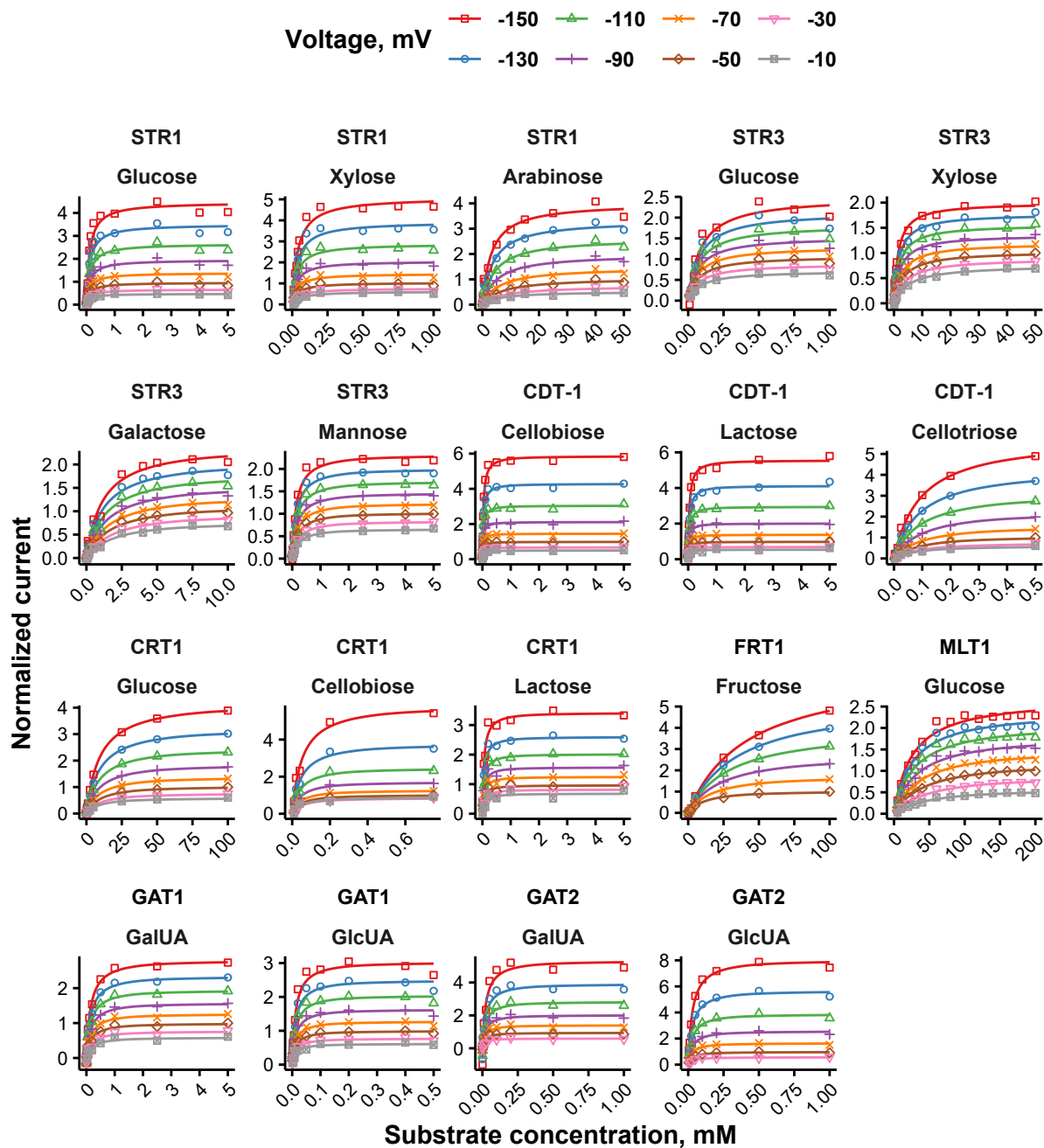
Supplementary figure S1. Current trace recordings of the identified symporters. (a–h) Traces of STR1 (a), STR3 (b), CDT-1 (c), CRT1 (d), Trire2_50618 (FRT1) (e), Trire2_67469 (MLT1) (f), Trire2_106330 (GAT1) (g) and Trire2_69026 (GAT2) (h) for selected sugars. Arrows in panel a indicate the timeline of the experiment: 1. perfusion with ND-96 (pH 5.5), 2. perfusion with the same buffer with sugar, 3. perfusion with ND-96 (pH 7.4) to return to baseline. Same steps can be identified in each subpanel. The oocytes were clamped at -50 mV and the sugars were in 5 mM concentration except for celotriose (c3) in panel c and D-glucose in panel f, which were in 10 μ M and 200 mM concentrations, respectively. Abbreviations as in Supplementary table S1. The figure was created with ggplot2 package for R^{71,72}.



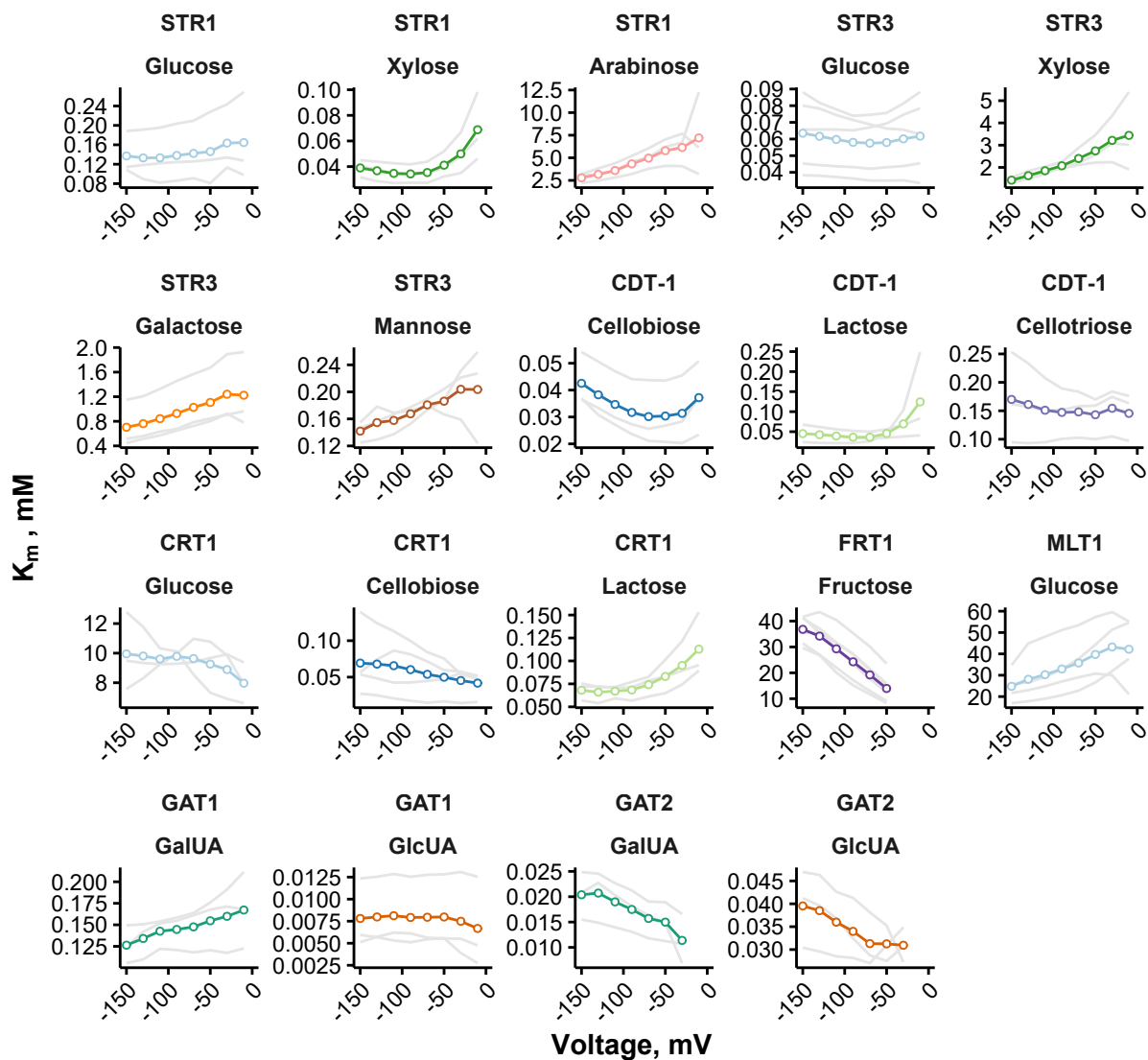
Supplementary figure S2. Identification of transport activity of Trire2_106556 (GLT1). **(a)** Representative experiment showing D-glucose uptake rates of uncharacterized *T. reesei* transporters which did not function as symporters in oocytes. D-glucose concentration was 5 mM and the assay was done in two different pH values. Error bars present standard deviation from two technical replicates. **(b)** Representative experiment showing growth curves of strain expressing Trire2_106556 and negative control strain at various monosaccharide carbon sources. The carbon sources were at 0.5% concentration, except for glycerol and ethanol (G+E), which were at 2% concentration. Error bars present standard deviation between 3 technical replicates, and they and points are shown only for every 10 h for clarity. The figure was created with `ggplot2` package for R^{71,72}.



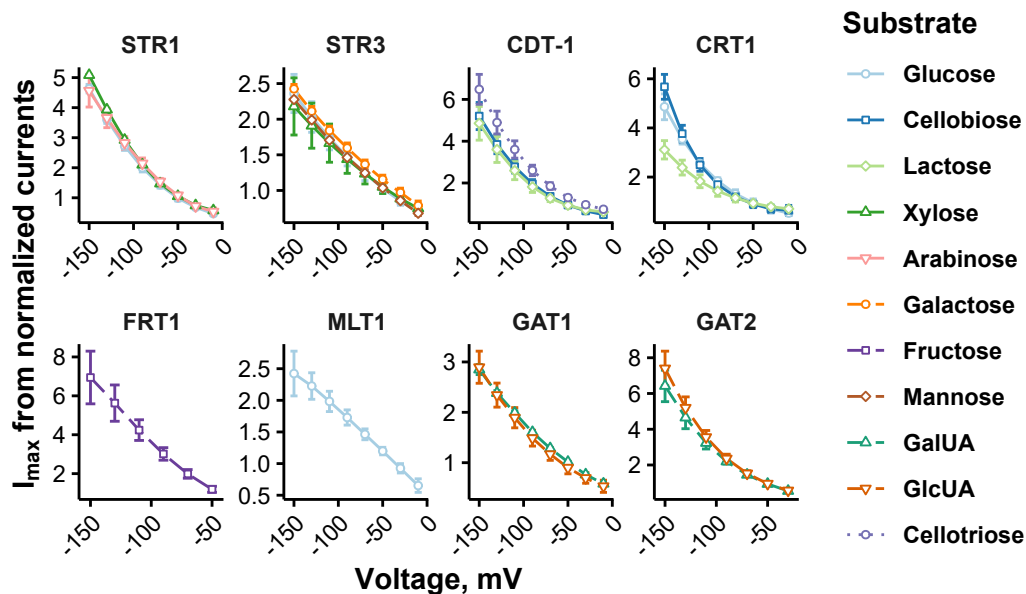
Supplementary figure S3. Representative I-V curves for each of the identified symporters. Sugars were at 5 mM concentration. Abbreviations as in Supplementary table S1. The figure was created with `ggplot2` package for R^{71,72}.



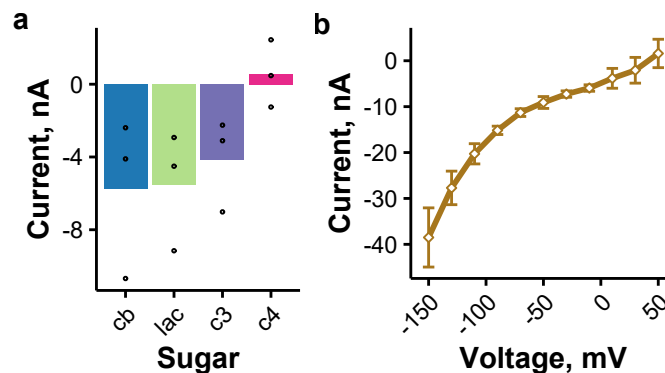
Supplementary figure S4. Substrate-induced currents at different voltages for sugar and sugar acid substrates of the identified symporters. Currents were normalized to the highest value at -50 mV. Values obtained at voltages below 0 mV are shown, except for Trire2_50618 (FRT1) and Trire2_69026 (GAT2), for which values obtained below -30 mV and -10 mV are shown, respectively. Representative experiments are shown. The figure was created with `ggplot2` package for R^{71,72}.



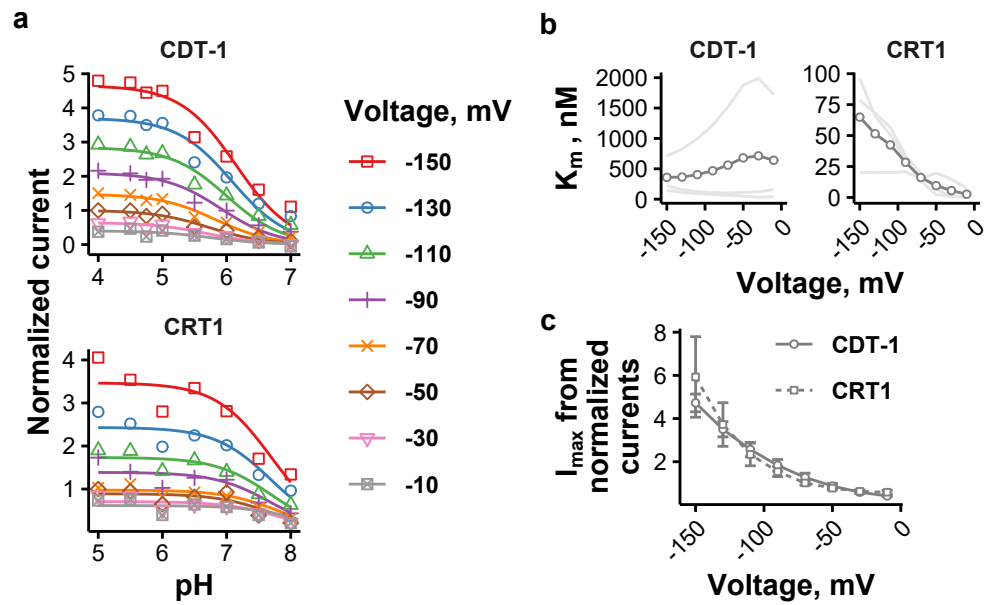
Supplementary figure S5. Voltage-dependence of K_m values. Colored lines and points present average K_m value across experiments. Light gray lines present results from individual experiments ($n \geq 3$). Values obtained at voltages below 0 mV are shown, expect for Trire2_50618 (FRT1) and Trire2_69026 (GAT2), for which values obtained below -30 mV and -10 mV are shown, respectively. The figure was created with ggplot2 package for R ^{71,72}.



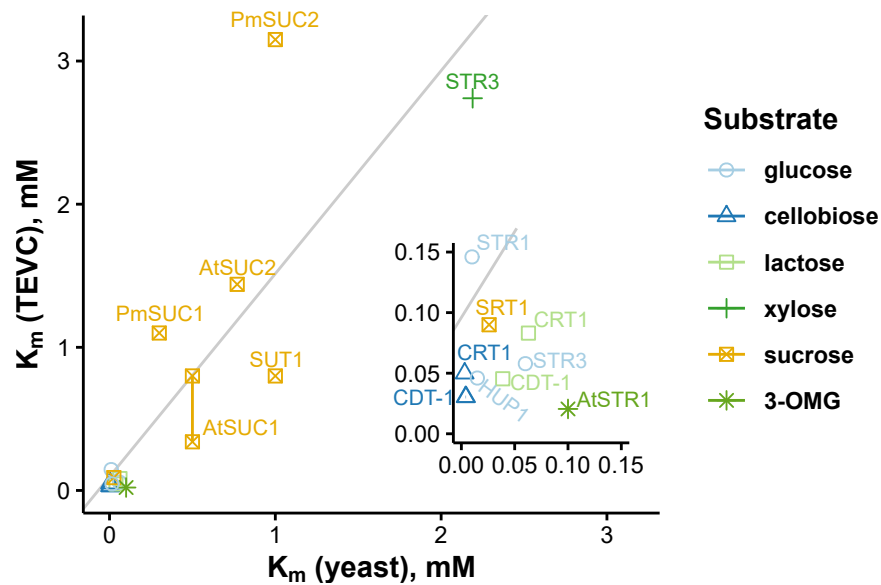
Supplementary figure S6. Voltage-dependence of $I_{\max}$ values for each transporter. $I_{\max}$ values were calculated from currents normalized to highest value at -50 mV (see Supplementary Fig. S4). Values obtained at voltages below 0 mV are shown, except for Trire2_50618 (FRT1) and Trire2_69026 (GAT2), for which values obtained below -30 mV and -10 mV are shown, respectively. Points and error bars present the mean and standard deviation between individual experiments ($n \geq 3$). The figure was created with ggplot2 package for R^{71,72}.



Supplementary figure S7. Further selectivity testing with CDT-1 and CRT1. (a.) Selectivity plots of CDT-1 with cellobiose, lactose, cellotriose or cellotetraose in 10 μ M concentration at -50 mV. Points present results from individual experiments and bars their average ($n = 3$). Abbreviations as in Supplementary table S1. (b.) Current traces for CRT1 with 1 mM α -sophorose. Points and error bars present mean and standard deviation between oocytes ($n = 3$). The figure was created with ggplot2 package for R^{71,72}.



Supplementary figure S8. Voltage-dependence of pH-dependence kinetics. **(a.)** Currents induced by 5 mM cellobiose at different voltages as a function of pH. Representative experiments are shown. **(b.)** Voltage-dependence of K_m value for protons. Points and dark lines present average K_m values across experiments, while light gray lines present results from individual experiments ($n = 3$). Voltages below 0 mV are shown. **(c.)** Voltage-dependence of I_{max} for protons. Points present average I_{max} values calculated from currents normalized to highest value at -50 mV (see panel **a**), while error bars present their standard deviation ($n = 3$). Voltages below 0 mV are shown. The figure was created with ggplot2 package for R^{71,72}.



Supplementary figure S9. Relationship between K_m values obtained from yeast and *X. laevis* oocytes with TEVC. Points present values for independent transporters and the gray line represents linear fit for the data ($R^2 = 0.7269$). Two TEVC K_m values were available for AtSUC1, and thus the points were joined with colored line. Inset shows transporters with K_m below 0.15 mM. Error bars were omitted for clarity. Data was available for the following transporters: *T. reesei* CRT1⁷, STR1¹³ and STR3¹³; *N. crassa* CDT-1^{7,24}; *U. maydis* SRT1^{56,57}; *Solanum tuberosum* SUT1^{73,74}; *Arabidopsis thaliana* STP1^{75,76} SUC1⁷⁷⁻⁷⁹ and SUC2^{77,80}; *Chlorella kessleri* HUP1^{81,82}; *Plantago major* SUC1^{79,83} and SUC2^{79,84}. *T. reesei* STR1 K_m for D-xylose was excluded from the analysis (see Results and Discussion). K_m values obtained at -50 mV were used for transporters analyzed in this study (CRT1, STR1, STR3 and CDT-1) and for HUP1, whereas the K_m values for UmSRT1 and AtSUC2 were obtained at -130 and -137 mV, respectively, and those of *A. thaliana* and *P. major* SUC1 and SUC2 were the average of values obtained at the smallest and highest test voltages^{57,78-80,82}. Abbreviations: At = *A. thaliana*, Pm = *P. major*, 3-OMG = 3-O-methyl-D-glucose. The figure was created with ggplot2 package for R^{71,72}.

- a** MASSGIEKEMASADGVDIQSSKDVTTGGVQDVGAGDFSHDQRLVIESARAAAAKEQSMTLLQGIKLYPKAIAWSI
LISTCIVMEGYDVS LVNNFYAFPQFNEKYGELYPDGTYQVPARWQSGLSNGATVGEIIGLFINGFVSEFGRKTVM
GCLILVAAFTAIFFTAPNVETLLVAEILCGIPWGIFQTITVTYASEVCPVALRGYLT TYVNFCWGLGQEIGIGVIYA
MLKRNDWAYRIPYGLQWMWPLPLFIAIYFAPESPWVLVRQGKAEEAKKSLLRLTSLNRETD FADETVAMMVHTTA
LEEKMTAGATYWDCFKGVDLRRTEIVCMTWAIQNLSGNAFSNYSTYFLQQAGLSTKDSYSFALGQYAINMVG VFGAW
GLMTMGVGRRSLYLYGLCGLCSMLFILGLFLGLVPESHRRREGSLATGSIMVVWALFYQLTVGTVCYSLVGELSSRRLQ
IKTVVLARNLYNIVGIVTNVLT PYMLNPKAWDWSNYTGFFWGGICFCCIVYTYFRLPEPRGRTF AELDVLF EKKIPA
RKFSSTKVDVFHESIDEKVMHQYD GIMKTHVEKISFV
- c** MDSSKVT DHDIVQDVVHDESPVKVTIPNCMTASRMQAALLRENPHIFRNSFLKLYGCIFVGYLCSATNGFDSNTFGG
LSAIPQFTRYFGITTQNGQLVAALYVIGNIAGSFVAGPCSDTYGRRVGMVAGSAICVIGAVLQTASTTLSMLMGGRF
ILGMGAVLVQTAGPSYV VEMSYPKYRAQLTGAYQACFFLGTIVSTWLEFGLNHAKTTLSYPWRLPLAIQGLPSVMIL
CAVWFIPESPRWC VQHGRVDEAKAILIKYHGDGDPDALVANLELQEMIEVIQMDGSDKRWWDYRCLFDTRAARYRTF
LVLCIAWFGELDLPPTSYYFPLMVKTVGITDVNTQLLLNAIQTPVMAVSALCGLSVVHKFGRRKLLMFESSAGMSVSI
AIITACTALQAGRPVGGTGIAFLYIFLIVFAFAWTPMQSLYPVEVLSFNSRAKGMAFLAFMNNAVKMNTYVPPIA
IANSWKYYFLYVFWDAFGVIVIIYFFVETRDWSLEEIEDLFQAKNPVKASLEKKRISVAYDGTIAHVPDGRDDV
- b** MYLKLISAGFSFFVAGVNDGAIGALIPYFIRDYNVTTAIVSSVYGANFLGWLFAAITNTHLRQYLDLGAMLALGAAF
QIAAAHALRSWEPPFGLFVVTFWLVSVGQAFQDTHANSWVASAVPKGAHRWLAFIHAMYMAGCLVGPVFSTAVASAGE
VSRWYLFYTFPLGLGV MNLVLT CVAFRDTLGLQKRSSASE**SQGRTLGETAAAEP**SNEAVSRNKEATQLIKKTASTPS
VWLLSLFFFFYLGSVLTAGGWVVEYLVNVRHG NLSQMGYVPAGFN GGGLLGRLLLAEP THRFGERRMVFLYVVASIG
LQLIFWLVPNIVAASIAVSLIGFFTGPLFPTGISLGSKLFPSDIHSTALPLVFVFAQLGGSLFPIITGVVSADAGVK
VLQPM LIALLT VTAISWLLVPSPKTSNAELHQE

Supplementary figure S10. Manually annotated *T. reesei* transporter sequences used in this study. Residues which are not present in the QM6a annotation are in bold. **(a)** Amino acid sequence of Trire2_67469 (MLT1) was extended based on the sequence of putative maltose permease from *T. parareesei* (GenBank OTA04610.1). The part that is missing from the QM6a gene corresponds to nucleotides 61,612–61,586 in scaffold 21 (minus-strand), and is adjacent to the start codon of the QM6a gene. **(b)** Amino acid sequence of Trire2_56684 was extended based on the sequence of MFS permease from *T. guizhouense* (OPB43417). The part that is missing from the QM6a gene corresponds to nucleotides 62,906–63,007 (plus-strand), and is adjacent to the start codon of the QM6a gene. **(c)** Amino acid sequence of Trire2_79202 that was obtained from cDNA. The insertion corresponds to the absence of the second intron from the QM6a sequence. The figure was created with Inkscape software (version 1.01, <https://inkscape.org/>).

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