## Identification of residues important for substrate uptake in a glucose transporter from the filamentous fungus *Trichoderma reesei*

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## Supplementary data



**Figure S1.** Growth analysis of *S. cerevisiae* EBY.VW4000 cells expressing Stp1 or its variants with mannose, fructose or galactose as the sole carbon source. (a) Serial dilutions of yeast cells were spotted on a synthetic complete agar plate for 5 days with 1% mannose or 1% fructose as the sole carbon source. (b) Growth curves of yeast cells cultured on 1% galactose for the indicated time period. *S. cerevisiae* EBY.VW4000 cells displayed moderate background growth on the agar plate containing 1% galactose; growth in liquid medium was thus measured instead of that on plates.



**Figure S2** The effect of CCCP on the 2-NBDG uptake by EBY.VW4000 cells expressing Gxs1, a well-defined glucose/xylose-H<sup>+</sup> symporter from *Candida intermedia*. The *gxs1* expression cassette that contains TDH3 promoter, *gxs1* ORF and CYC1 terminator in order was integrated into the transformant genome. Yeast transformant expressing Gxs1 was cultured in SC medium with 1% maltose and 250  $\mu$ g/ml G418. The CCCP assay was performed under the same experimental conditions as that on Stp1. CCCP at the indicated final concentrations was added to Stp1-expressing yeast cells 5 min prior to the addition of 2-NBDG, and then the incubation was continued at 30 °C for 30 min and stopped by three washes with ice-cold buffer.

	50		
Stp1	MADVHVADAPEGRKQSIGIHTTQDVEHIEAPITWKAYLMCAFASFGGIFEGYDSGYINGVNGSAIFYKAVEGIN	74	
XylE	.MNTQYNSSYIFSITLVATL <mark>G</mark> GLLFGYDTAVISGTVESLNTVFVAPQN	47	
GLUT1		53	
GLUT2		75	
GLUT3		51	
GLUT4		66	
ScHxt1	MNSTPDLISPQKSNSSNSYELESGRSKAMNTPEGKNESFHDNLSESQVQPAVAPPNTGKGVYVTVSICCVMVAF <mark>G</mark> GFI <mark>FGWDTGTI</mark> SGFVAQTDFLRRFGMKHH	104	
ScHxt2	MSEFATSRVESGSQQTSIHSTPIVQKLETDESPIQTKSEYTNAELPAKPIAAYWTVICLCLMIAF <mark>G</mark> GFV <mark>FGW</mark> DTGT <mark>I</mark> SGFVNQTDFKRRFGQMKS	95	
Consensu	Consensus g fg i		
115			
Stpl	ATKLSDSHTSLITSILSCGTFFGALIAGDVSDWIGRKWTVIIGCVIYMLGVVVQMITAPNHALGPIVAGRLIAGLGVGFESAIVILYMSEICPRKVRGALVAGY	178	
XylE	LSESAANSLLG <mark>FCVASALLGCIIGGALGC</mark> YCSNRF <mark>GR</mark> RDSLKIAAVLFFI <mark>S</mark> GVGSAWPELGFTSINPDNTVPIYLAGYVPEFVIY <mark>RIIGG</mark> IGVGLASMLS <b>PMYIAE</b> LAPAHIRGKLVSFN	167	
GLUT1	ESILPTTLTTLWSLSVAIFSVGGMIGSFSVGLFVNRFGRRNSMLMMNLLAFVSAVLMGFSKLGKSFEMLILGRFIIGVYCGLTTGFVPMYVGEVSPTALRGALGTLH	160	
GLUT2	PKPTPWAEEETVAAAQLITMLWSLSVSSFAVGCMTASFFCGWLGDTLGRIKAMLVANILSLVGALLMGFSKLGPSHILIIAGRSISGLYCGLISGLVPMYIGEIAPTALRGALGTFH	192	
GLUT3	APPSEVLLTSLWSLSVAIFSVGCMIGSFSVCLFVNRFGRRNSMLIVNLLAVIGGCFMGLCKVAKSVEMLILGRLVIGLFCGLCTGFVPMYIGEISPIALRGAFGTLN	158	
GLUT4	EGPSSIPPGTLTTLWALSVAIFSVGGMISSFLIGIISQWLGRKRAMLVNNVLAVLGGSLMGLANAAASYEMLILGRFLIGAYSGLTSGLVPMYVGEIAPTHLRGALGTLN	176	
ScHxt1	DGSHYLSKVRTGLIVSIFNIGCAIGGIVLAKLGDMYGRRIGLIVVVVIYTIGIIIQIASINKWYQYFIGRIISGLGVGGITVLSPMLISEVAPSEMRGTLVSCY	208	
ScHxt2	DGTYYLSDVRTGLIVGIFNIGCAFGGLTLGRLGDMYGRRIGLMCVVLVYIVGIVIQIASSDKWYQYFIGRIISGMGVGGIAVLSPTLISETAPKHIRGTCVSFY	199	
Consensu	is g gr r g g e p rg		
	179 186 214		
Stp1	QFCITIGILLASCVVYGTKDFTNTGAYRIPIALQFFWALILGGGLLFLPDSPRYFVKKGEIQKAINSLSRVRGOPEDSEYVONELAEIIANEEYERALIPSTTWFGSWANCFKGSLWTGKSNLRRTI	305	
XylE	QFATIFGQLLVYCVNYFIARSGDASWLNTDGWRYMFASECIPALLFLMLYTVPESPRWLMSRGKQEQAESILRKIMGNTLATQAVQEIKHSLDHGRKTGGRLLMFGVGVIV	279	
GLUT1	QLGIVVGILIAQVFGL.DSIMGNKDLWPLLLSIIFIPALLQCIVLPFCPESPRFLLINRNEENRAKSVLKKLRGTADVTHDLQEMKEESRQMMREKKVTILELFRSPAYRQPIL	273	
GLUT2	QLATVTGILISQIIGL.EFILGNYDIWHILLGLSGVRAILQSLLLFFOPESPRYLYIKLDEEVKAKQSLKRLRGYDDVTKDINEMRKEREEASSEQKVSIIQLFTNSSYRQPIL	305	
GLUT3	QLGIVVGILVAQIFGL.EFILGSEELWPLLLGFTILPAILQSAALPFCPESPRFLLINRKEEENAKQILQRLWGTCDVSQDIQEMKDESARMSQEKQVTVLELFRVSSYRQPII	271	
GLUT4	QLATVIGILIAQVLGL.ESLLGTASLWPLLLGLTVLPALLQLVLLPFCPESPRYLYIIQNLEGPARKSLKRLTGWADVSGVLAELKDEKRKLERERPLSLLQLLGSRTHRQPLI	289	
ScHxt1	QVMTILGIFLGYCTNFGTKNYSNSVCWRVPLGLCFAWALFMIGGMMFVPESPRYLVEAGRIDEARASLAKVNKCPPDHPYLQYELETIEASVEEMRAAGTASWGELFIGKPAMFQRTM	326	
ScHxt2	QIMITIGIFLGYCTNYGTKDYSNSVCWRVPLGLNFAFAIFMIAGMLMVFESFRFLVEKGRYEDAKRSLAKSNKVTIEDPSIVAEMDTIMANVETERLAGNASWGELFSNKGAILFRVI	317	
Consensusg i g., a p spr			
	315 320 324 433		
Stp1	LGTSLOW/CONTGVNFIFYYSTPFLOSTGA.IKNSFLISLIFSLVNVCSTPLSFWIVERFGRRTILIAGAFGMLICOFLVAIIGVTVGFNKTHPDPSNPDAALADNIPAVNAQIAFIAIFIFFASTWGFGA	436	
XylE	IGVMLSIFCOFVGINVVLYYAPEVFKILGASTDIALLQTIVGVINLTFTVLAIMTVDKFGRKPLQIIGALGMAIGMFSLGTAFYTQASGIVALLSMLFYVAAFAMSWGPVC	391	
GLUT1	IAVVLOLSCOLSGINAVFYYSTSIFEKAGVQQPVYATIGSGIVNTAFTVVSLFVVERAGRRTLHLIGLAGMAGCAILMTIALALLEQLPWMS	387	
GLUT2	VALMLHVACOFSGINGIFYYSTSIFOTAGISKPVYATIGVGAVNMVFTAVSVFIVEKAGRRSLFLIGMSGMFVCAIFMSVGLVLLNKFSWMS	419	
GLUT3	ISIVLOISCOLSCINAVFYYSTGIFKDAGVQEPIYATIGAGVVNTIFTVVSLFIVERAGRRTLHMIGLGGMAFCSTLMTVSLLLKDNYNGMSFVCIGAILVFVAFFEIGPGFIP	385	
GLUT4	IAVVLOLSCOLSGINAVFYYSTSIFETAGVGQPAYATIGAGVVNTVFTLVSVLLVERAGRRTLHLLGLAGMCGCAILMTVALLLLERVPAMS	403	
ScHxt1	MGIMIQSLCCLTGCNYFFYYGTIVFQAVGLSDSFETSIVFGVVNFFSTCCSLYTVDRFGRRNCLMWGAVGMVCCYVVYASVGVTRLWPNGQDQPSSKGAGNCMIVFACFYIFCFATTWAFIA	448	
ScHxt2	MGIMIQSLCOLTENNYFFYYGTIIFNAVGMKDSFQTSIVLGIVNFASTFVALYTVDKFGRRKCLLGGSASMAICFVIFSTVGVTSLYPNGKDQPSSKAAGNVMIVFTCLFIFFAISWAFIA	439	
Consensu	is qq g n yy us g n t v gr g m f p		
	437 460		
Stp1	WIVIGEIFPLPIRSRGVALSTASNWLWNTIISVITPYMVGTDKGNLKSSVFFIWGGLCTCAFVYSYFLIPEIKGLSLEQVDKMMEE.SSPRISSKWKPTITFAQMGTGEYIEKPTAEV	554	
XylE	WVLLS <mark>E</mark> IFPNAIRGKALAIAVAACWLANYFVSWTFPMMDKNSWLVAHFHNGFSYWIYGCMGVLAALFMWKFVP <mark>EIKG</mark> KTLEELEALWEPETKKTQQTATL	491	
GLUT1	MFIVAELFSQGPRPAAIAVAGFSNWTSNFIVGMCFQYVEQLCGPYVFIIFTVLLVLFFIFTYFKVPEIKGRTFDEIASGFRQGGASQSDKTPEELFHPLGADSQV	492	
GLUT2	WFMVAEFSQGPRPAALAIAAFSNWTCNFIVALCFQYIADFCGPYVFFLFAGVLLAFTLFTFFKVPETKGKSFEEIAAEFQKKSGSAHRPKAAVEMKFLGATETV	524	
GLUT3	WFIVAELFSQGFRPAAMAVAGCSNWTSNFLVGLLFPSAAHYLGAYVFIIFTGFLITFLAFTFFKVPETRGRTFEDITRAFEGQAHGADRSGKDGVMEMNSIEPAKETTTNV	496	
GLUT4	WFIVAELESQGPRPAAMAVAGFSNWTSNFIIGMGFQYVAEAMGPYVFLLFAVLLGFFIFTFLRVPETRGRTFDQISAAFHRTP.SLLEQEVKPSTELEYLGPDEND	509	
ScHxt1	VVVISECFPLRVKSKCMSIASAANNIWGFLISFFTPFITGAINFYYGYVFMGCMVFAYFYVFFFVPETKGLSLEEVNDMYAEGVLPWKSASWVPVSKRGADYNADDLMHDDOPFYKSLFSR	569	
ScHxt2	YVIVAESYPLRVKNRAMAIAVGANWIWGFLIGFFTPFITSAIGFSYGYVFMGCLVFSFFYVFFFVCETKGLTLEEVNEMYVEGVKPWKSGSWISKEKRVSEE	541	
Consensu	is e w et g		
What has a set and the set and			

**Figure S3** Sequence alignment of Stp1 with its homologues including the xylose/H<sup>+</sup> symporter XylE from *E. coli*, human GLUT1-GLUT4, and Hxt1 and Hxt2 from *S. cerevisiae*. The multiple sequence alignments was performed with ClustalW, and the result was constructed with DNAMAN. The residues in Stp1 described in this study are indicated with their corresponding residue numbers.



expressing the respective variants fused with C-terminal GFP. Yeast cells expressing GFP alone were used as the control, in which fluorescence of GFP was distributed in the cytoplasmic space. (b) Analysis of fluorescence intensity of yeast cells expressing WT Stp1 and the indicated variants fused with C-terminal GFP. The yeast cells cultured in synthetic complete medium containing 1% maltose as the sole carbon source were collected, resuspended in a 25-mM phosphate buffer at pH 5.2 and subjected to fluorescence measurement.



**Figure S5** Individual mutation of the aromatic residues including Tyr 324, Trp 461 and Trp 437 in the vicinity of the substrate-binding site of Stp1 nearly abolished its glucose transport capability. (a) Top view of the modelled structure of Stp1 with the crystal structure of *E. coli* XylE as template. The N and C domains are coloured grey and tan, respectively, and the intracellular helices connecting these two domains are coloured yellow. Phe 50, Tyr 324, Trp 461 and Trp 437 are highlighted in magenta. (b) Glucose-mediated growth of *S. cerevisiae* EBY.VW4000 expressing Stp1 and its variants as indicated. Serial dilutions of the yeast cells were spotted on a synthetic complete agar plate for 8 days with 1% glucose as the sole carbon source.



**Figure S6** The effect of dose-dependent inhibition by glucose on 2-NBDG uptake by Stp1. Yeast cells expressing Stp1 were incubated with 100  $\mu$ M 2-NBDG plus excess glucose as indicated for 4 h and then subjected to fluorometric analysis after washing with ice-cold phosphate buffer.



**Figure S7** Quantitative analysis of the 2-NBDG-specific fluorescent signals in the cytoplasmic and membrane fractions of yeast cells expressing the variants as indicated after a 5-min incubation with 2-NBDG.