

Supplemental Material

The high capacity specific fructose facilitator ZrFfz1 is essential for the fructophilic behavior of *Zygosaccharomyces rouxii* CBS 732^T

Maria José Leandro^{a,b,#}, Sara Cabral^b, Catarina Prista^b, Maria C. Loureiro-Dias^b and Hana Sychrová^a

^aDepartment of Membrane Transport, Institute of Physiology AS CR, v.v.i., Prague, Czech Republic

^bCentre for Botany Applied to Agriculture (CBAA), Instituto Superior de Agronomia, University of Lisbon, Lisbon, Portugal

Address correspondence to Maria José Leandro, mjleandro@isa.ulisboa.pt

SUPPLEMENTAL MATERIAL LEGENDS

TABLE S1 Primers used in this study.

TABLE S2 Plasmids constructed in this study.

FIG S1 Values of V_{\max} of fructose and glucose transport in *Z. rouxii* strains CBS 732^T, UL4, F1 (*Zrffz1*Δ), F2 (*Zrffz2*Δ) and F1F2 (*Zrffz1*Δ *Zrffz2*Δ) grown in 2% glucose. Values result from GraphPad Prism Michaelis-Menten regression analysis of data from at least two independent experiments. Dotted lines represent values obtained for strain UL4.

FIG S2 Growth curves (in microplates) of *Z. rouxii* CBS 732^T (circles) and UL4 (triangles) strains in YNB medium supplemented with 2% fructose (filled symbols) or 2% glucose (empty symbols). Data are representative of at least two independent experiments.

FIG S3 Fructose and glucose consumption profiles of *Z. rouxii* strains CBS 732^T (A), UL4 (B), F1 (*Zrffz1*Δ; C), F2 (*Zrffz2*Δ; D), F1F2 (*Zrffz1*Δ *Zrffz2*Δ; E) and F1F2S1 (*Zrffz1*Δ *Zrffz2*Δ *Zrfsy1*Δ; F) grown in YNB + 10 g L⁻¹ fructose + 10 g L⁻¹ glucose. Data are representative of at least two independent experiments and error bars (SD) are shown.

FIG S4 Fructose and glucose consumption profiles of *Z. rouxii* strains F1F2 [pZEU] (A), F1F2 [pZGFP_*ZrFFZ2*] (B), F1F2 [pZEU_*ZrFFZ1*] (C), F1F2 [pZEU_*ZrFFZ2*] (D), F1F2 [pZCA_*ZrFFZ1*] (E) and F1F2 [pZCA_*ZrFFZ2*] (F) grown in YNB + 10 g L⁻¹ fructose + 10 g L⁻¹ glucose. Data are representative of at least two independent experiments and error bars (SD) are shown.

TABLE S1 Primers used in this study.

Primer	Sequence (5'-3')*
ZrFFZ1-Kan-F	5'- <u>CACAATATTTTCGATCATAACGGTTTCCACTGTCTTCAAAGGAAGAA</u> <u>TAAGGGTCATTA AAACTGATTTTTAAATTTCAAATTCGTACGCTGCAG</u> GTCGAC-3'
ZrFFZ1-Kan-R	5'- <u>TTTGTTAATTATAATAACAGAAAAAAGACAAATGAAAACAGAAATG</u> <u>TTATGAAATGAAATGAAATGAAATGACAACGTGCATAGGCCGCTAGT</u> GGATCTG-3'
ZrFFZ2-Kan-F2	5'- <u>TTGTTCCTTTCCGTGTTATTTTTCCGTATAGTTTTCCGTAGACTA</u> <u>TTGAACTCTTCACGGTACAACCCATAGAGAACAAAACACTATTTTCGT</u> ACGCTGCAGGTCGAC-3'
ZrFFZ2-Kan-R2	5'- <u>CATAAATATCTATCCATTACATTATCTCGAATTTGCTCCAAGTGGAC</u> <u>TGTCGTAAGTATCTTAATGAACATTTGTCATTGCCCTACTGTTGCATA</u> GGCCACTAGTGGATCTG-3'
ZrFSY1-Kan-F	5'- <u>CCGGTAAGTTCACCCATCCATATTTTTCAAGCTTCTCTAACAGGAT</u> <u>TTTTCAGATACGTAGAAGAAGTTGTGTCAAAGAATAACAGCGGTTTCG</u> TACGCTGCAGGTCGAC-3' (1)
ZrFSY1-Kan-R	5'- <u>TGAGAACCTGTAAAGAATCAACTGTTTTGACTCTCTTCGCTCCCC</u> <u>TATCCATTCTGTCTCTAATAGCTTAGTTTACCTCTTTTCAACCAGCATA</u> GGCCACTAGTGGATCTG-3' (1)
ZrFFZ1-361up-F	5'-TGGTCTCCTTTTCGTATGTGG-3'
ZrFFZ1-456d-R2	5'-CAGTATTTGTTAGTGTTAGCTTG-3'
ZrFFZ2-521up-F2	5'-TTTCCGTCGTGTGCGTATCTTTC-3'
ZrFFZ2-492d-R2	5'-AATTGGAGACTGAGCCTTTTCGC-3'
ZrFSY1-368-upF	5'-AGGATGATCACTGACTACTTGC-3' (1)
ZrFSY1-246d-R	5'-GCATCATGTAATACGACGACC-3' (1)
KANX-R1	5'-CTCTGGCGCATCGGGC-3'
KANX-F1	5'-CATTTGATGCTCGATGA-3'
ZrFFZ1-ZEU-F	5'-AACGACGGCCAGTGAATTCGAGCTCGGTACCCGGGGATCCAGAA GCATTGCCTTGAACCTG-3'

ZrFFZ1-ZEU-R	5'-TGATTACGCCAAGCTTGCATGCCTGCAGGTCGACTCTAGATCATG CACGACCAGTATGGCC-3'
ZrFFZ1-ZCA-F	5'-TGATTACGCCAAGCTTGCATGCCTGCAGGTCGACTCTAGAAGAAG CATTGCCTTGAACCTG-3'
ZrFFZ1-ZCA-R	5'-AACGACGGCCAGTGAATTCGAGCTCGGTACCCGGGGATCCTCAT GCACGACCAGTATGGCC-3'
ZrFFZ2-ZEU-F	5'-AACGACGGCCAGTGAATTCGAGCTCGGTACCCGGGGATCCAAAG GGGTTTGAAAGTAATCTC-3'
ZrFFZ2-ZEU-R	5'-TGATTACGCCAAGCTTGCATGCCTGCAGGTCGACTCTAGATTAAT GAACATTTGTCATTGC-3'
ZrFFZ2-ZGFP-R	5'-CAGTGAATAATTCTTCACCTTTAGACATTTTAATAAAGCTATGAACA TTTGTCATTGCCC-3'
ZrFFZ2-ZCA-F	5'-TGATTACGCCAAGCTTGCATGCCTGCAGGTCGACTCTAGAAAAGG GGTTTGAAAGTAATCTC-3'
ZrFFZ2-ZCA-R	5'-AACGACGGCCAGTGAATTCGAGCTCGGTACCCGGGGATCCTTAAT GAACATTTGTCATTGC-3'

*Sequences homologous to the *Z. rouxii* genes to be deleted are underlined. Sequences homologous to *Z. rouxii* genes to be cloned are in italics.

REFERENCE

1. **Leandro MJ, Sychrová H, Prista C, Loureiro-Dias MC.** 2013. ZrFsy1, a high-affinity fructose/H⁺ symporter from fructophilic yeast *Zygosaccharomyces rouxii*. PLoS One **8**:e68165.

TABLE S2 Plasmids constructed in this study.

Gene	Primers used to amplify genes	Plasmid	Constructed plasmid
<i>ZrFFZ1</i>	ZrFFZ1-ZEU-F and ZrFFZ1-ZEU-R	pZEU	pZEU_ZrFFZ1
	ZrFFZ1-ZCA-F and ZrFFZ1-ZCA-R	pZCA	pZCA_ZrFFZ1
<i>ZrFFZ2</i>	ZrFFZ2-ZEU-F and ZrFFZ2-ZEU-R	pZEU	pZEU_ZrFFZ2
	ZrFFZ2-ZEU-F and ZrFFZ2-ZGFP-R	pZGFP	pZGFP_ZrFFZ2
	ZrFFZ2-ZCA-F and ZrFFZ2-ZCA-R	pZCA	pZCA_ZrFFZ2

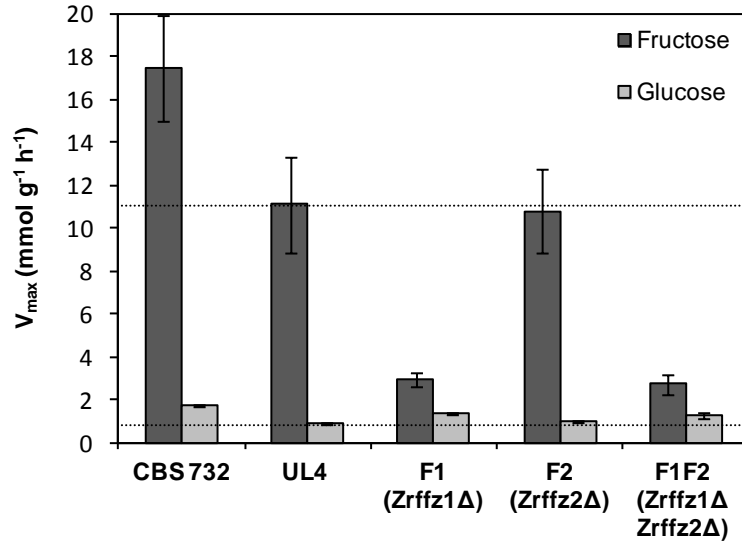


FIG S1 Values of V_{max} of fructose and glucose transport in *Z. rouxii* strains CBS 732^T, UL4, F1 ($Zrffz1\Delta$), F2 ($Zrffz2\Delta$) and F1F2 ($Zrffz1\Delta Zrffz2\Delta$) grown in 2% glucose. Values result from GraphPad Prism Michaelis-Menten regression analysis of data from at least two independent experiments. Dotted lines represent values obtained for strain UL4.

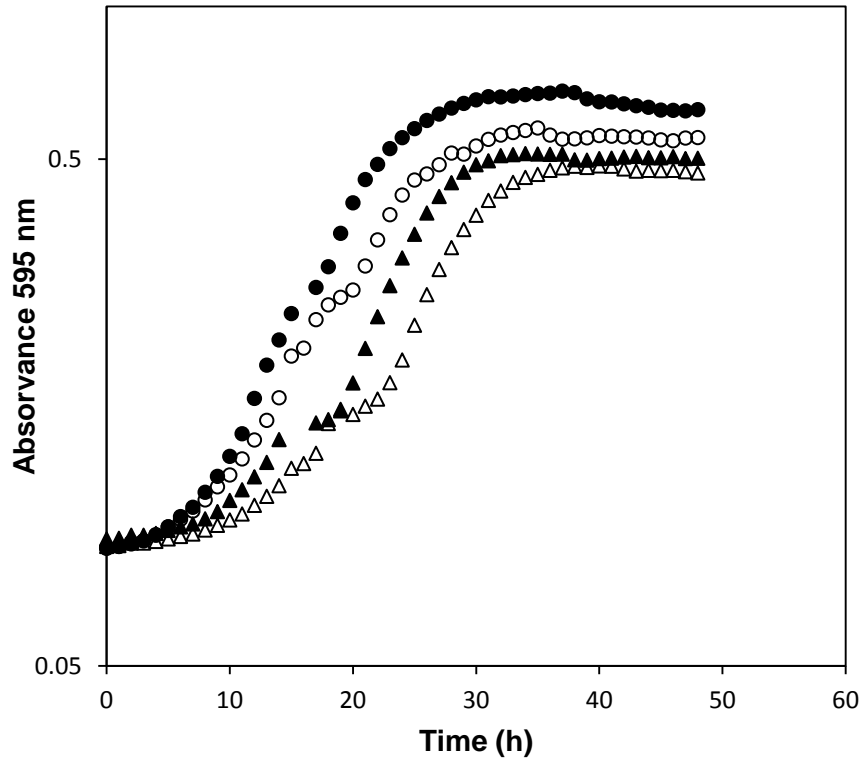


FIG S2 Growth curves (in microplates) of *Z. rouxii* CBS 732^T (circles) and UL4 (triangles) strains in YNB medium supplemented with 2% fructose (filled symbols) or 2% glucose (empty symbols). Data are representative of at least two independent experiments.

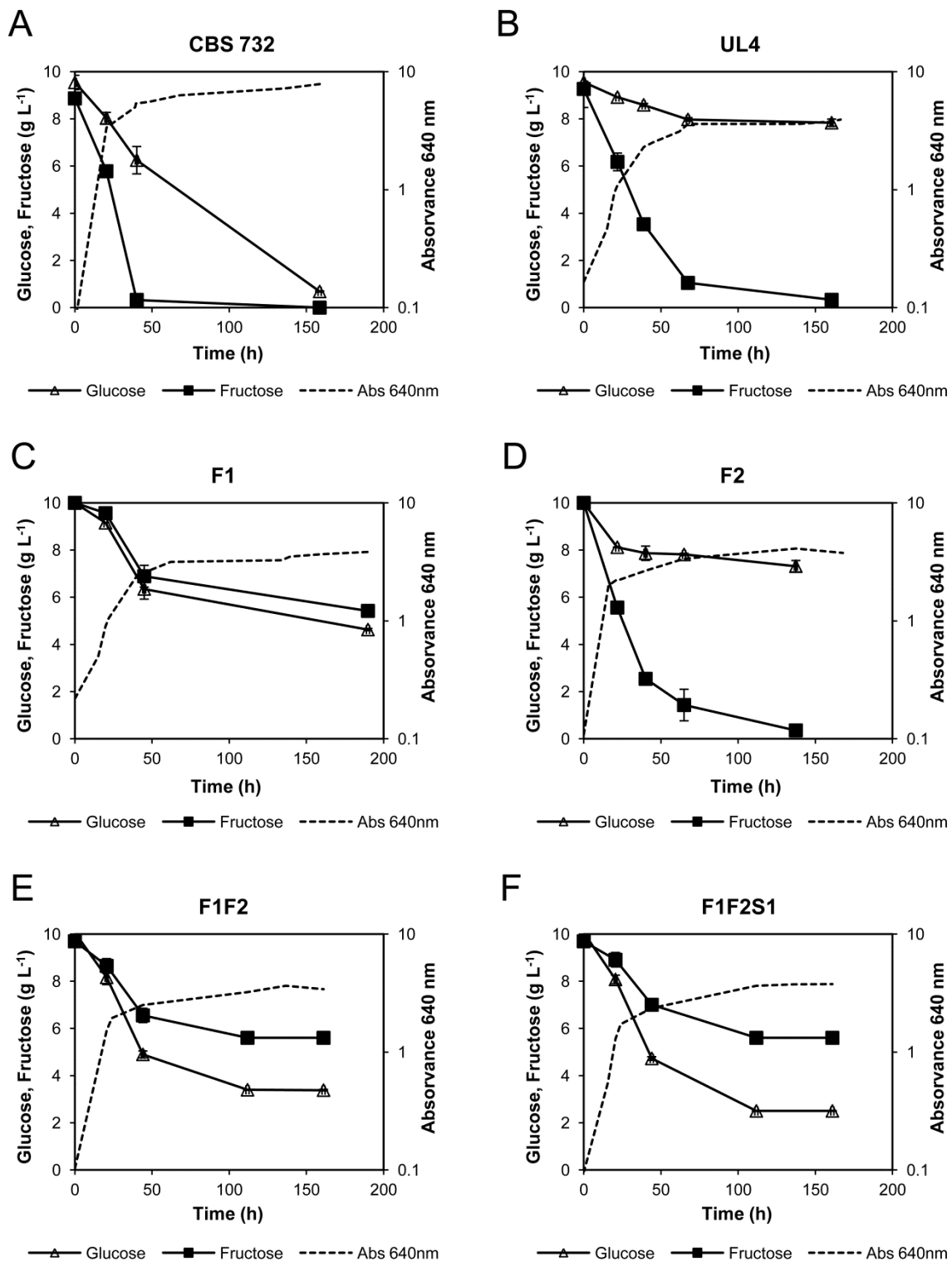


FIG S3 Fructose and glucose consumption profiles of *Z. rouxii* strains CBS 732^T (A), UL4 (B), F1 (*Zrffz1Δ*; C), F2 (*Zrffz2Δ*; D), F1F2 (*Zrffz1Δ Zrffz2Δ*; E) and F1F2S1 (*Zrffz1Δ Zrffz2Δ Zrfsy1Δ*; F) grown in YNB + 10 g L⁻¹ fructose + 10 g L⁻¹ glucose. Data are representative of at least two independent experiments and error bars (SD) are shown.

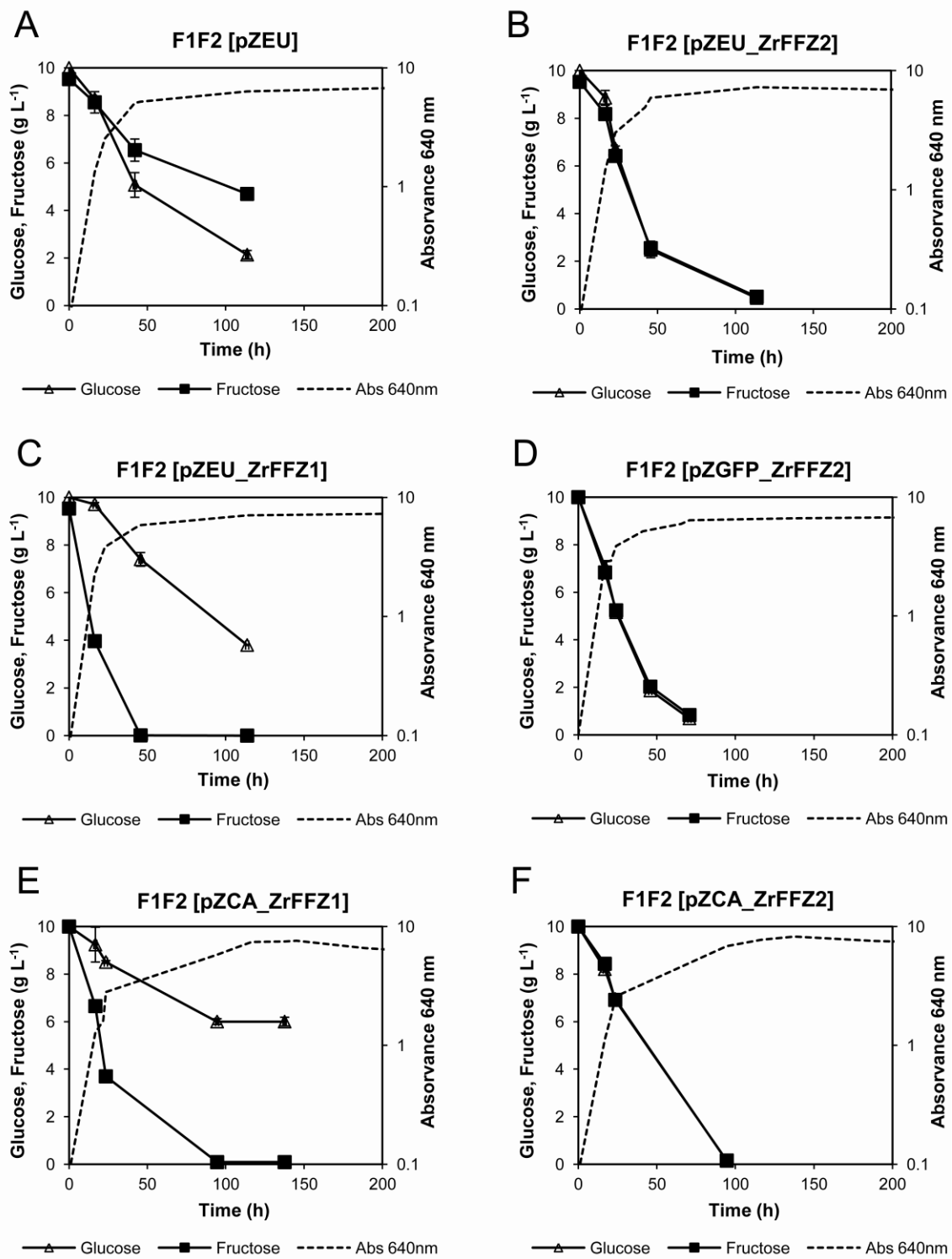


FIG S4 Fructose and glucose consumption profiles of *Z. rouxii* strains F1F2 [pZEU] (A), F1F2 [pZGFP_ZrFFZ2] (B), F1F2 [pZEU_ZrFFZ1] (C), F1F2 [pZEU_ZrFFZ2] (D), F1F2 [pZCA_ZrFFZ1] (E) and F1F2 [pZCA_ZrFFZ2] (F) grown in YNB + 10 g L⁻¹ fructose + 10 g L⁻¹ glucose. Data are representative of at least two independent experiments and error bars (SD) are shown.