

Yeast Trk1 potassium transporter gradually changes its affinity in response to both external and internal signals

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Supplementary material

Table S1. List of yeast strains

Name	Genotype	Reference
BY4741	<i>MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0</i>	EUROSCARF
BYT2	<i>trk2Δ::loxP</i> , derived from BY4741	[25]
BYT12	<i>trk1Δ::loxP trk2Δ::loxP</i> , derived from BY4741	[48]

[25] Petrezselyova, S.; Ramos, J.; Sychrova, H. Trk2 transporter is a relevant player in K⁺ supply and plasma-membrane potential control in *Saccharomyces cerevisiae*. *Folia Microbiol.* **2011**, *56*, 23-28.

[48] Petrezselyova, S.; Zahradka, J.; Sychrova, H. *Saccharomyces cerevisiae* BY4741 and W303-1A laboratory strains differ in salt tolerance. *Fungal Biol.* **2010**, *114*, 144-150.

Table S2. List of plasmids

Plasmid	Relevant features	Reference
<i>Empty multicopy plasmids</i>		
pRS316	<i>URA3</i> , CEN6ARS4	[49]
YEpl352	<i>URA3</i> , 2 μ origin	[50]
<i>Empty centromeric plasmid</i>		
YCp352	YEpl352-CEN6ARS4 <i>URA3</i> ,	This study
<i>Multicopy plasmids with TRK1*</i>		
pScTRK1	<i>ScTRK1</i> in YEpl352	Papouskova (unpublished)
pGRU1ScTRK1	GFP-tagged <i>ScTRK1</i> <i>URA3</i> , 2 μ origin	[24]
<i>Centromeric plasmids with TRK1*</i>		
pCScTRK1	pScTRK1-CEN6ARS4	This study
pCScTRK1-GFP	pGRU1ScTRK1-CEN6ARS4	This study
<i>Centromeric plasmids pCScTRK1 and pCSTRK1-GFP bearing indicated mutations in TRK1*</i>		
pCScTRK1_L949P		This study
pCScTRK1_L949A		This study
pCScTRK1_L949E		This study
pCScTRK1_L949R		This study
pCScTRK1_L949S		This study
pCScTRK1_L81P		This study
pCScTRK1_F820P		This study
pCScTRK1_L1115P		This study
pCScTRK1-GFP_L949P		This study
pCScTRK1-GFP_L949A		This study
pCScTRK1-GFP_L949E		This study
pCScTRK1-GFP_L949R		This study
pCScTRK1-GFP_L81P		This study
pCScTRK1-GFP_F820P		This study
pCScTRK1-GFP_L1115P		This study

**TRK1* expressed under the control of weak and constitutive *S. cerevisiae* *NHA1* promoter

- [49] Sikorski, R.; Hieter, P. A system of shuttle vectors and yeast host strains designed for efficient manipulation of DNA in *Saccharomyces cerevisiae*. *Genetics* **1989**, *122*, 19-27.
- [50] Hill, J.; Myers, A.; Koerner, T.; Tzagoloff, A. Yeast/*E. coli* shuttle vectors with multiple unique restriction sites. *Yeast* **1986**, *2*, 163-167.
- [24] Zimmermannova, O.; Felcmanova, K.; Rosas-Santiago, P.; Papouskova, K.; Pantoja, O.; Sychrova, H., Erv14 cargo receptor participates in regulation of plasma-membrane potential, intracellular pH and potassium homeostasis via its interaction with K⁺-specific transporters Trk1 and Tok1. *Biochim. Biophys. Acta* **2019**, *1866*, 1376-1388.

Table S3. List of used oligonucleotides

Oligonucleotide	Sequence (5'-3')
2μ-CEN6ARS4 exchange	
2μ-CEN6ARS4_for	cggcatcagagcagattgtactgagagtgaccataacgcGGGTCCTTTTCATCACGTGC
2μ-CEN6ARS4_rev	tgctccttccttgcttcttctgttcggagattaccgCTTAGGACGGATCGCTTGCC
URA3_for	GTATATTCTCCAGTAGATAGG
CEN6ARS4_rev	GTAAGTTACAGGCAAGCGATCCG
Site-directed mutagenesis	
ScTRK1_L949P_for	GCTATAGAGTCCTTGTCGGC <u>CCG</u> TTTCAATCTGTTAGCACAAG
ScTRK1_L949P_rev	CTTGTGCTAACAGATTGAAAC <u>CGG</u> GCCGACAAGGACTCTATAGC
ScTRK1_L949A_for	GCTATAGAGTCCTTGTCGGC <u>GCG</u> TTTCAATCTGTTAGCACAAG
ScTRK1_L949A_rev	CTTGTGCTAACAGATTGAAAC <u>GCG</u> GCCGACAAGGACTCTATAGC
ScTRK1_L949E_for	GCTATAGAGTCCTTGTCGGC <u>GAG</u> TTTCAATCTGTTAGCACAAG
ScTRK1_L949E_rev	CTTGTGCTAACAGATTGAAAC <u>CTC</u> GCCGACAAGGACTCTATAGC
ScTRK1_L949R_for	GCTATAGAGTCCTTGTCGGC <u>CGG</u> TTTCAATCTGTTAGCACAAG
ScTRK1_L949R_rev	CTTGTGCTAACAGATTGAAAC <u>CCG</u> GCCGACAAGGACTCTATAGC
ScTRK1_L949S_for	GCTATAGAGTCCTTGTCGGC <u>TCG</u> TTTCAATCTGTTAGCACAAG
ScTRK1_L949S_rev	CTTGTGCTAACAGATTGAAAC <u>CGA</u> GCCGACAAGGACTCTATAGC
ScTRK1_L81P_for	GATACATTGATACAC <u>CCG</u> TTTTTAGCAGCGGG
ScTRK1_L81P_rev	CCCCTGCTAAAAAC <u>CGG</u> TGTATCAATGTATC
ScTRK1_F820P_for	CTACATGGTGGGGAC <u>CT</u> TGGACAGCAATGAG
ScTRK1_F820P_rev	CTCATTGCTGTCCA <u>AGG</u> TCCCCACCATGTAG
ScTRK1_L1115P_for	CAAACTTTAATATATTTGCAATT <u>CCT</u> TTTGAAATTGTTAGCGCTTACGG
ScTRK1_L1115P_rev	CCGTAAGCGCTAACAATTTCAAA <u>AGGA</u> ATTGCAAATATATTAAAGTTTG

*underlined: codon for substituting amino acid

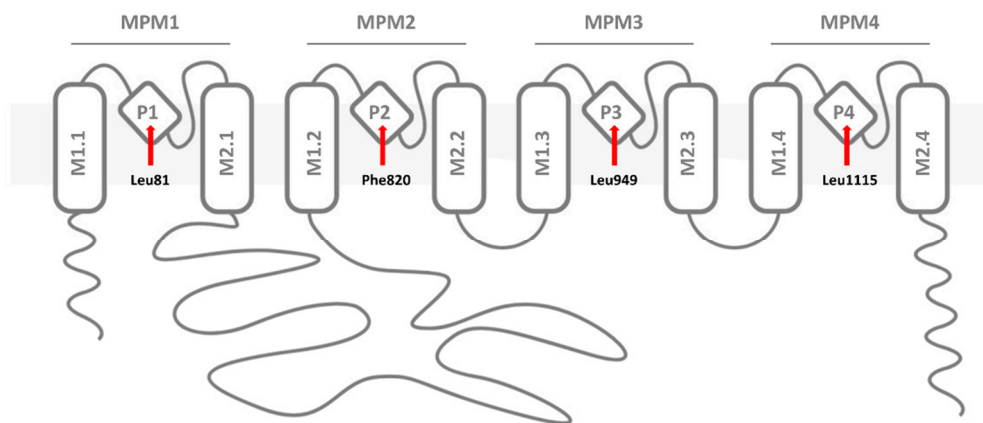


Figure S1: Schematic representation of the predicted transmembrane topology of *ScTRK1*. Highlighted are positions of residues studied in this work.

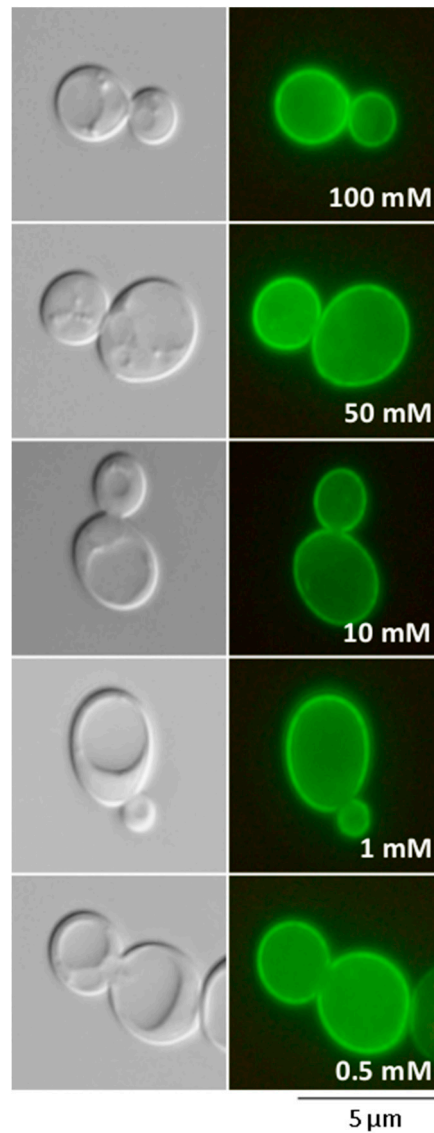


Figure S1. Localization of GFP-tagged Trk1. Cells were grown in YNB-F supplemented with indicated KCl concentrations to $OD_{600} \sim 1$, and visualized using Olympus Bx53 microscope and captured by Olympus DP73 camera.