

Fig. S1. Purification of Osh6-TAP from WT cells. After elution with TEV protease, eluates were loaded on SDS-PAGE and the gel was silver stained. Approximate positions of Ist2, Osh6-TAP and TEV are indicated. Experiment was performed once.

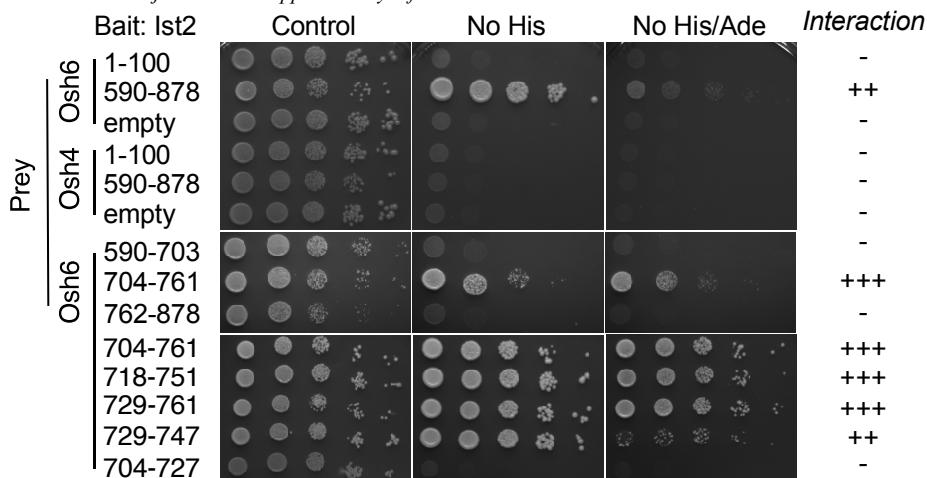
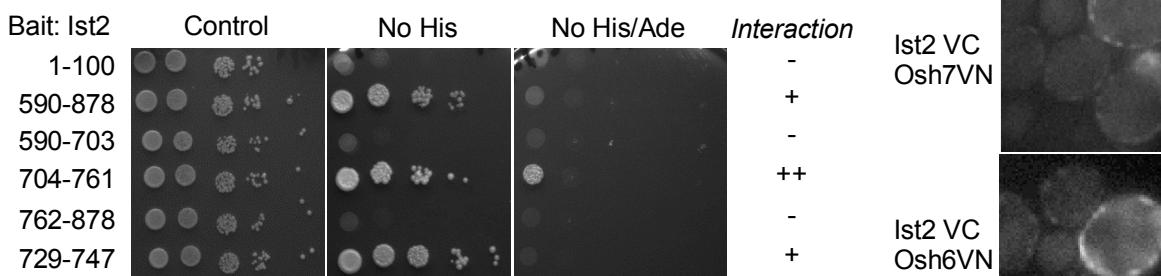
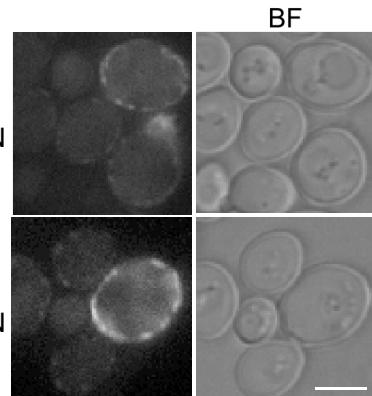
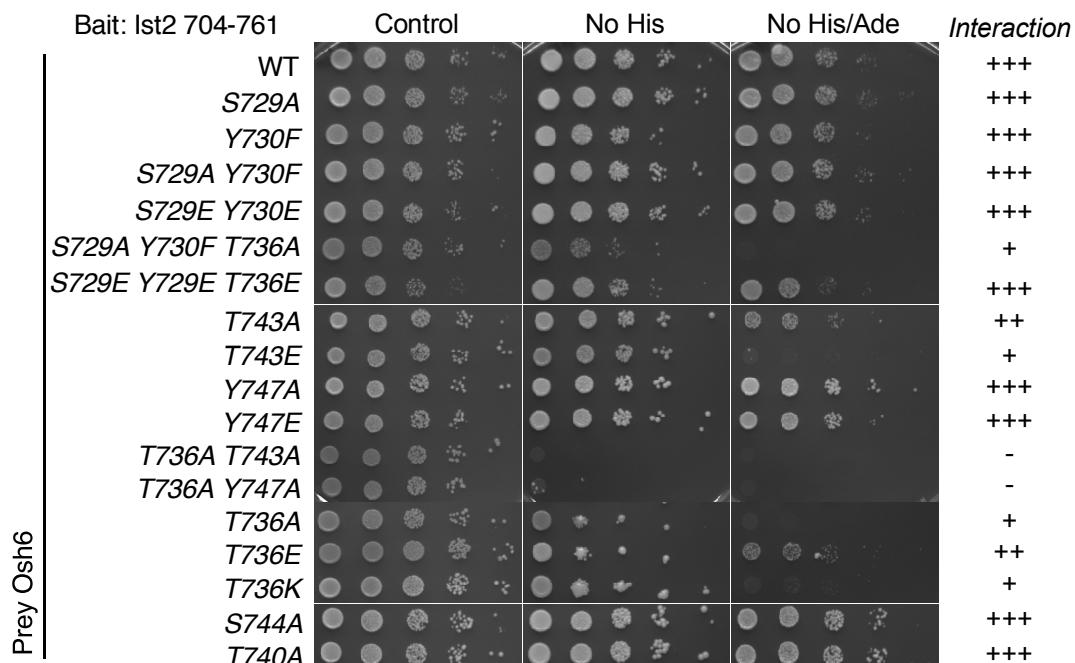
A**B****C****D**

Fig. S2. Yeast two-hybrid analysis of Ist2 interaction with Osh6 and Osh7. **(A)** 10-fold serial dilutions of yeast cells expressing Ist2 fragments (first and last aa are indicated) fused to GAL4 DNA-binding domain (bait) and full-length Osh6 or Osh4 fused to GAL4 Activation domain (prey) on control, SD-His and SD-His-Ade reporter plates. Plates were incubated at 30°C for 3 days. **(B)** 10-fold serial dilutions of yeast cells expressing Ist2 fragments fused to GAL4 DNA-Binding domain (bait) and full-length Osh7 fused to GAL4 Activation domain (prey) on control, and SD-His reporter plate. Plates were incubated at 30°C for 3 days. **(C)** BiFC in diploid cells expressing endogenously tagged Osh7-VN and Ist2-VC or Osh6-VN and Ist2-VC, as indicated. Scale Bar = 5 μm. **(D)** 10-fold serial dilutions of yeast cells expressing Ist2 fragment aa704-761 fused to GAL4 DNA-Binding domain (bait) and different Osh6 mutants fused to GAL4 Activation domain (prey) on control and SD-His and SD-His-Ade reporter plates. Plates were incubated at 30°C for 3 days. Interaction score: (-) No growth detected in -His, (+) growth detected in -His, (++) weak growth detected in -His/-Ade, (+++) Strong growth detected in -His/-Ade. All plates are representative of 3 independent experiments.

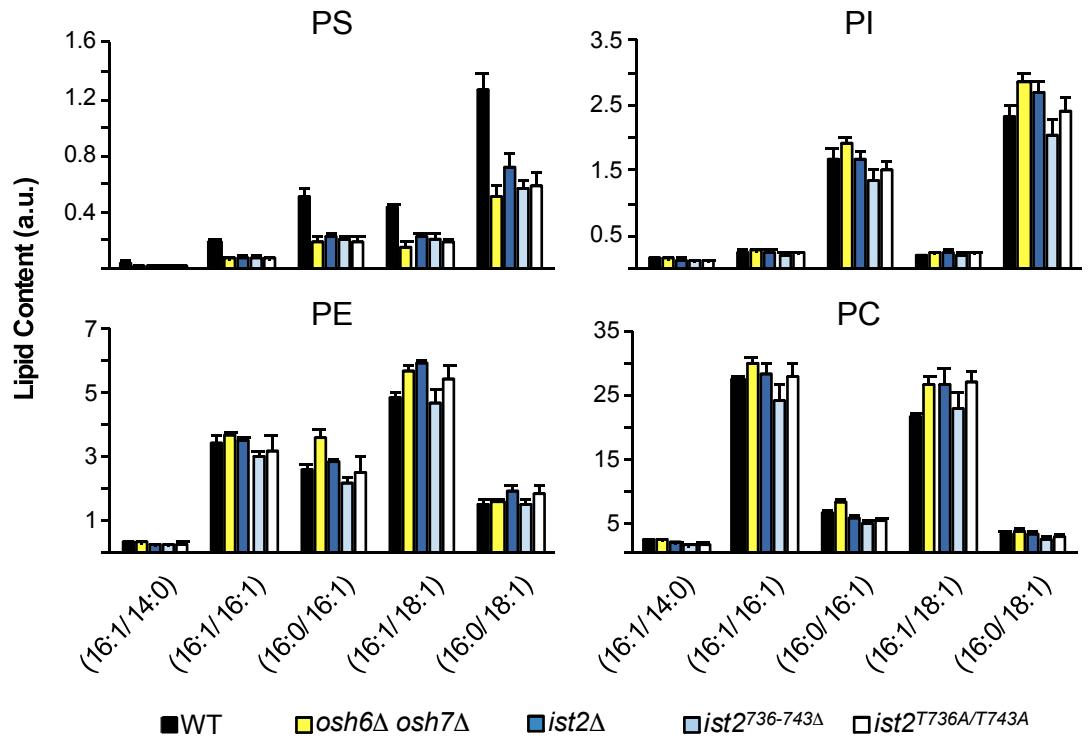


Fig. S3. Lipidomic analysis of total cellular content of PC, PE, PI and PS species in WT, *osh6Δ osh7Δ* and *ist2* mutant cells. Lipids were extracted from WT, *osh6Δ osh7Δ*, *ist2Δ*, *ist2^{736-743Δ}* and *ist2^{T736A/T743A}* (chromosomal deletion of 8 codons) and *ist2^{T736A/T743A}* (chromosomal substitution in 2 codons) cells, and analyzed by mass spectrometry. Data are mean±s.d. from 3 independent samples. Parentheses denote acyl chain length and saturation. The experiment was performed 3 times, yielding similar results.

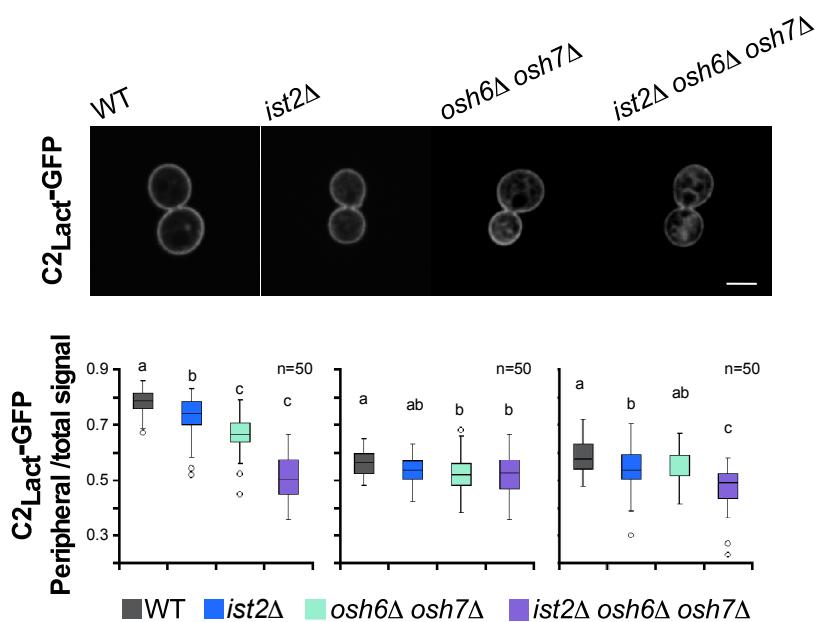


Fig. S4. Steady-state distribution of PS in WT, *ist2*^Δ and *osh6*^Δ *osh7*^Δ cells. Subcellular localization of C2_{Lact}-GFP in WT, *ist2*^Δ, *osh6*^Δ *osh7*^Δ and *osh6*^Δ *osh7*^Δ *ist2*^Δ cells was assessed by fluorescence microscopy (top panels). Quantification of relative C2_{Lact}-GFP peripheral signal, normalized to total cellular fluorescence, is shown in box plots for 50 individual cells (n=50) in 3 independent experiments. Each box encloses 50% of the data, median value is displayed as a line, top and bottom of the box mark the limits of \pm 25%. The lines extending from the top and bottom of each box mark the minimum and maximum values. Outlier are displayed as an individual point. Different letters ("a", "b" and "c") indicate significant differences between the means, "ab" denotes non-significant difference from either mean "a" or mean "b" (multiple comparison using one-way ANOVA, posthoc Tukey's test at p < 0.05).

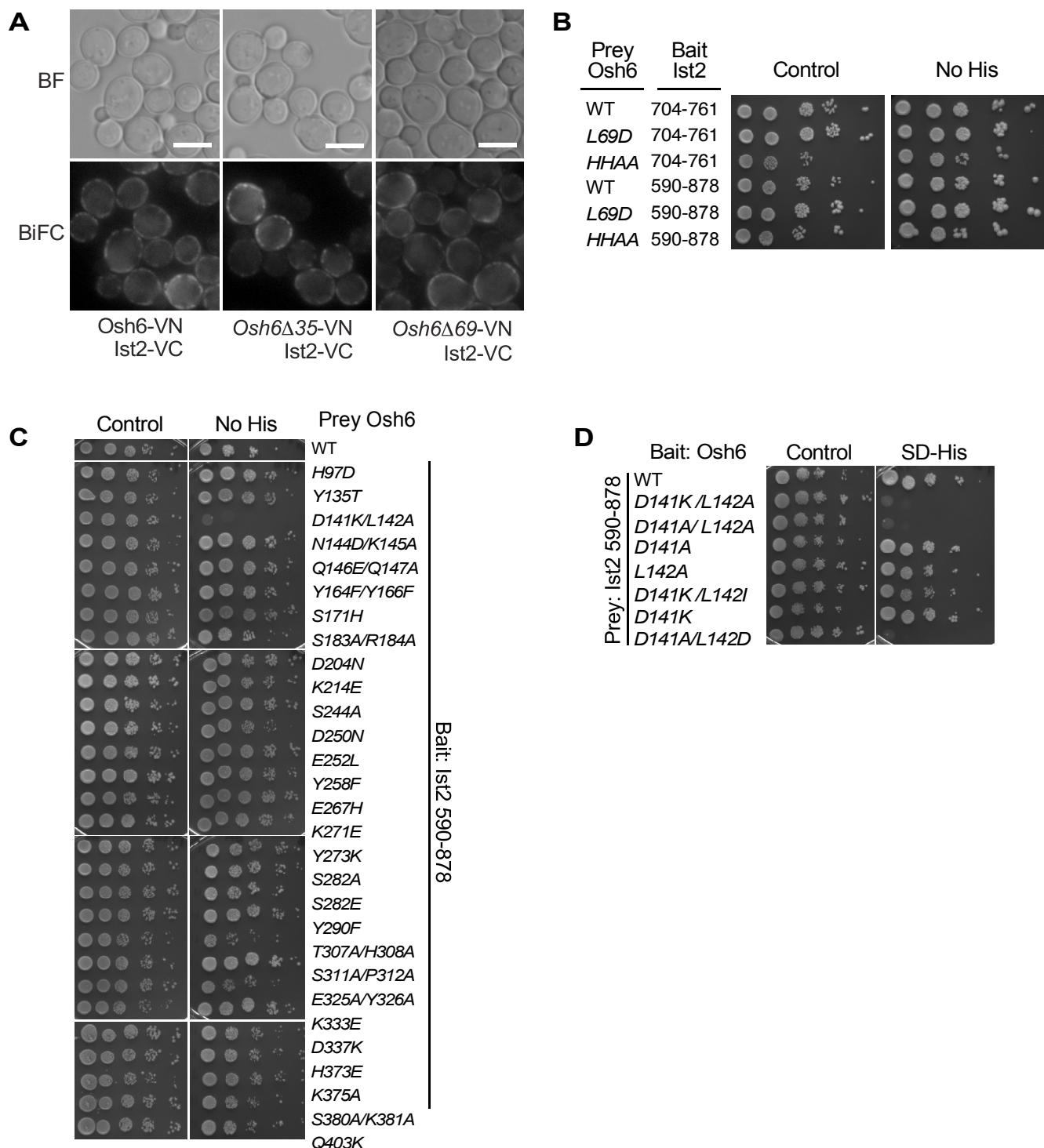


Fig. S5. Mapping Ist2 interaction site on Osh6. (A) Bimolecular fluorescence complementation (BiFC) between Osh6 WT, or two N-term deletion mutants Osh6 Δ 35 (lacking 35 N-term aa, which are disordered) or Osh6 Δ 69 (lacking the whole N-term lid that covers the lipid-binding pocket) fused to Venus N-term, and Ist2 fused to Venus C-term. Scale Bar= 5 μ m. (B-D) 10-fold serial dilutions of yeast cells (AH109) expressing Ist2 full-length cytosolic tail (aa 590-878) and Osh6 WT or selected mutants with substitutions in indicated aa, fused to GAL4 Activation domain (prey) or GAL4 DNA-Binding domain (bait), respectively. Osh6 mutants were selected based on structural and conservation analysis. Interaction of bait and prey proteins activates transcription of *HIS3* (controlled by GAL promoter) and allows growth in SD-His selective media. Results are representative of 3 independent experiments.

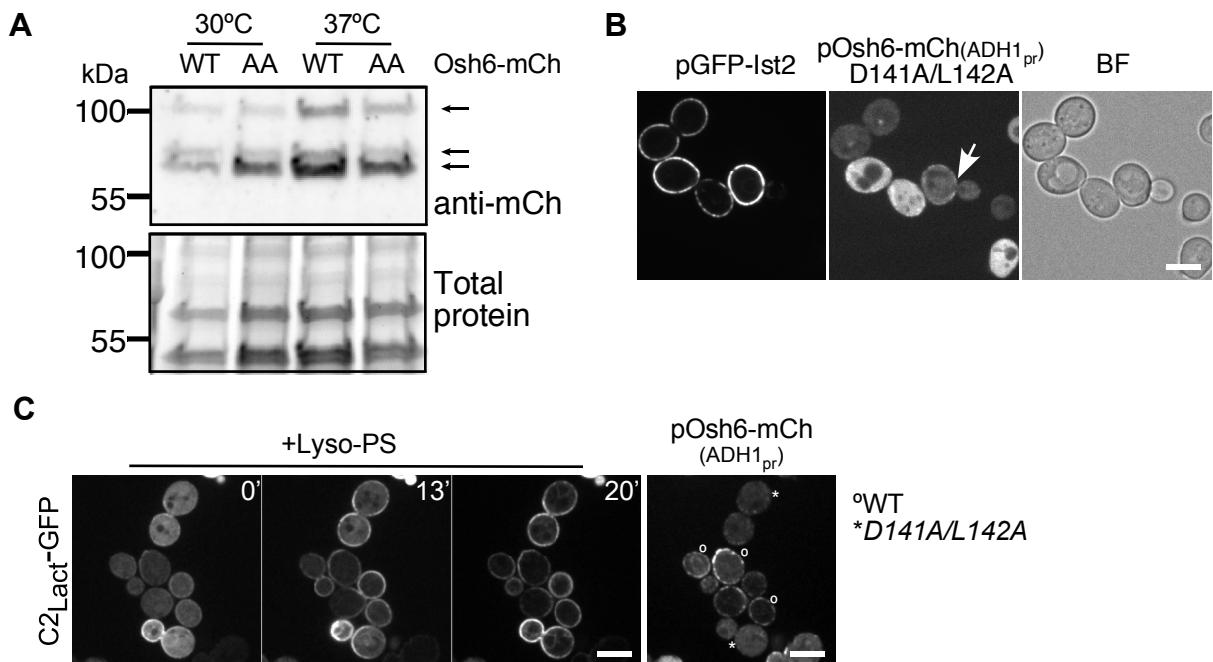


Fig. S6. Mutations of D141 and L142 do not affect the stability of Osh6 and still permit some interaction with Ist2. (A) Western blot analysis of whole cell TCA precipitates showing mCherry-tagged Osh6 and Osh6^{D141A/L142A} protein levels at 30 °C and 37 °C. Experiment was repeated 2 times. (B) GFP-Ist2 and Osh6^{D141A/L142A}-mCherry, expressed from low-copy plasmids in *ist2Δ osh6Δ osh7Δ* cells were visualized using fluorescence microscopy. (C) Imaging of C2_{Lact}-GFP over time in a mix of two *cho1Δ osh6Δ osh7Δ* strains (lacking endogenous PS), expressing Osh6-mCherry WT ([°]) or D141A/L142D mutant (*) from the medium-level ADH1-promotor. For identification, cells expressing WT Osh6 were labelled with CMAC. Time (in min) after addition of lyso-PS is indicated. Osh6-mCherry signal is shown in the right panel at t=0 min. Representative images from two independent experiments are shown. Scale Bar = 5 μm.

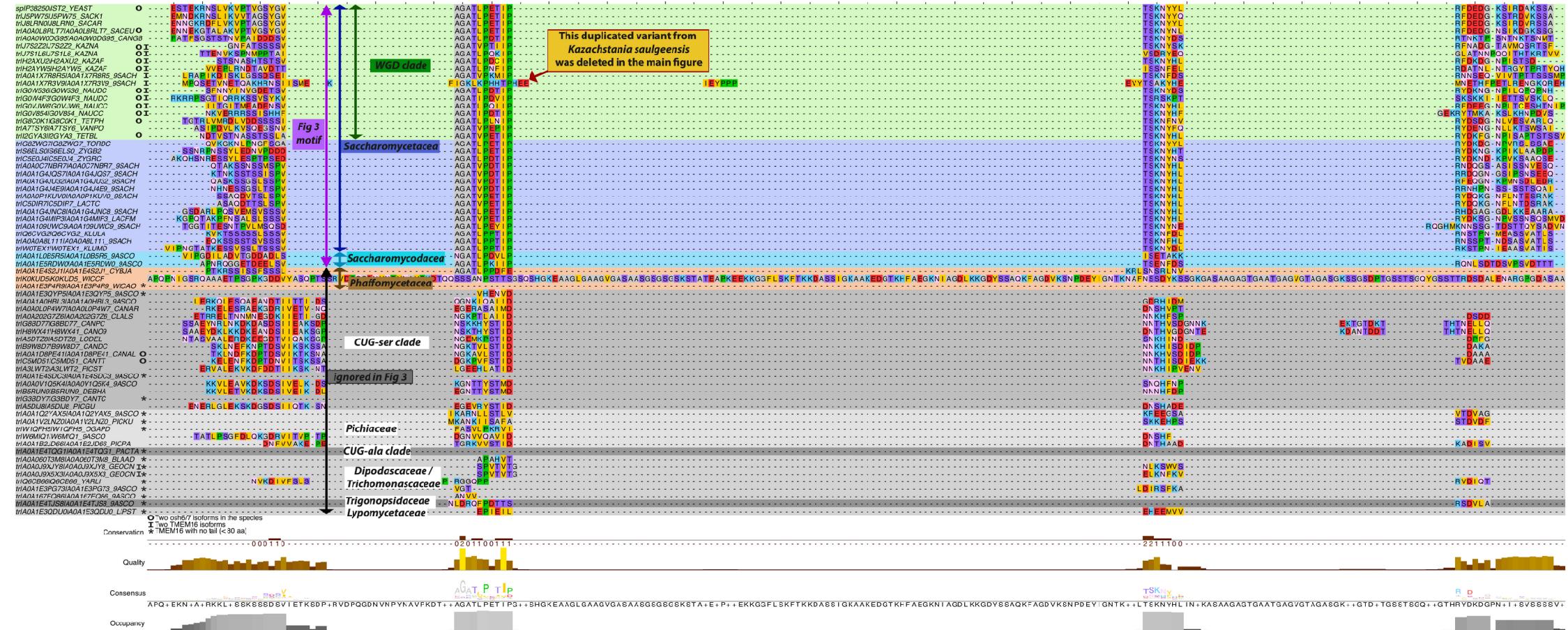


Figure S7

Fig. S7. Related to Fig. 3 and Fig. 7C: Alignment of the tail region of 68 TMEM16 homologous sequences from 62 *Saccharomycotina* species. Color coding is explained in the figure and is the same as in Fig. 7C. Conservation score and heatmap on a brown to yellow scale are shown at the bottom. Asterisks indicate homologues with a short cytosolic tail (< 80 aa). WGD denotes whole genome duplication (green); ‘O’: two *OSH6* genes, ‘I’: two *IST2* genes.

Table S1: List of proteins identified by mass spectrometry analysis of Osh6-TAP complex purified from wild-type yeast

[Click here to Download Table S1](#)

Table S2. Yeast strains used in this study.

Name	Alias	Background	Genotype	Origin/ Reference
BY4742	WT	BY4742	<i>MATα his3Δ1 leu2Δ0 lys2Δ0 ura3Δ0</i>	Euroscarf
BY4741	WT		<i>Mata his3Δ1 leu2Δ0 met15Δ0 ura3Δ0</i>	Euroscarf
VAY2817	OSH6-TAP	BY4742	<i>MATα his3Δ1 leu2Δ0 lys2Δ0 ura3Δ0 OSH6-TAP::KanMX</i>	This study
Ist2-GFP	IST2-GFP	BY4741	<i>MATA his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 IST2-GFP::HIS3</i>	Huh et al., 2003
Osh6-GFP	OSH6-GFP	BY4741	<i>MATA his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 OSH6-GFP::HIS3</i>	Huh et al., 2003
VAY2802	OSH6-GFP ist2Δ	BY4741	<i>MATα his3Δ1 leu2Δ0 lys2Δ0 ura3Δ0 ist2Δ::hphNT1 OSH6-GFP::KanMX</i>	This study
VAY3652	OSH6-GFP scs2Δ scs22Δ	BY	<i>MAT? his3Δ1 leu2Δ0 scs2Δ::KanMX Δscs22::KanMX Osh6-GFP::HIS3</i>	This study
VAY2811	IST2-VC	BY4742	<i>MATα his3Δ1 leu2Δ0 lys2Δ0 ura3Δ0 IST2-VC::KanMX</i>	This study
VAY2695	ist2Δ590-VC	BY4742	<i>MATα his3Δ1 leu2Δ0 lys2Δ0 ura3Δ0 IST2Δ590-VC::KanMX</i>	This study
VAY2829	OSH6-VN	BY4741	<i>MATA his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 OSH6-VN::HIS3</i>	This study
VAY2969	OSH7-VN	BY4741	<i>MATA his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 OSH7-VN::HIS3</i>	This study
AH109	AH109	AH109	<i>MATA trp1-901 leu2-3,112 ura3-52 his3-200 gal4Δ gal80Δ LYS2::GAL1_{UAS}-GAL1_{TATA}-HIS3, GAL2_{UAS}-GAL2_{TATA}-ADE2 URA3::MEL1_{UAS}-MEL1_{TATA}-lacZ</i>	James et al., 1996
VAY2784	ist2Δ	BY4742	<i>MATα his3Δ1 leu2Δ0 lys2Δ0 ura3Δ0 ist2Δ::hphNT1</i>	This study
MdPY08	cho1Δ	BY4742	<i>MATα his3Δ1 leu2Δ0 lys2Δ0 ura3Δ0 cho1Δ::kanMX</i>	This study
SGAY1635	osh6Δ osh7Δ	BY4742	<i>MATα can1Δ::STE2pr-LEU2 lyp1Δ ura3Δ0 leu2Δ0 his3Δ1 met15Δ0 osh6Δ::HYGMX osh7Δ::natNT2</i>	Maeda et al., 2013
SGAY7039	cho1Δ osh6Δ osh7Δ	BY4742	<i>MATα can1Δ::STE2pr-LEU2 lyp1Δ ura3Δ0 leu2Δ0 his3Δ1 met15Δ0 cho1Δ::KanMX osh6Δ::hphNT1 osh7Δ::natNT2</i>	Maeda et al., 2013
ACY406	ist2Δ osh6Δ osh7Δ	SGAY1635	<i>MATA can1Δ::STE2pr-LEU2 lyp1Δ ura3Δ0 leu2Δ0 his3Δ1 met15Δ0 ist2Δ::HISMX6 osh6Δ::hphNT1 osh7Δ::natNT2</i>	This study
MdPY01	ist2Δ osh6Δ osh7Δ	ACY406	<i>MATA can1Δ::STE2pr-LEU2 lyp1Δ ura3Δ0 leu2Δ0 his3Δ1 met15Δ0 ist2Δ::kanMX osh6Δ::hphNT1 osh7Δ::natNT2</i>	This study
MdPY04	ist2Δ590-743Δ	BY4742	<i>MATα his3Δ1 leu2Δ0 lys2Δ0 ura3Δ0 ist2Δ590-743Δ</i>	This study
MdPY06	ist2Δ590-743Δ	BY4742	<i>MATα his3Δ1 leu2Δ0 lys2Δ0 ura3Δ0 ist2Δ590-743Δ</i>	This study
MdPY07	cho1Δ ist2Δ590-743Δ	MdPY08	<i>MATα his3Δ1 leu2Δ0 lys2Δ0 ura3Δ0 cho1Δ::kanMX ist2Δ590-743Δ</i>	This study

Table S3. Plasmids used in this study.

Plasmid Name	Alias	Description	Origin/Reference
pAK75	GFP-Ist2	<i>IST2_pGFP-IST2</i> (full-length), CEN, HIS3 (pUG34-Based).	Kral et al., 2014
pAK76	GFP-Ist2RL	<i>IST2_pGFP-IST2</i> with randomized linker [596-917], CEN, HIS3 (pUG34-Based).	Kral et al., 2014
pAK81	GFP-IstL240	<i>IST2_pGFP-IST2</i> with 100 aa deletion in cytosolic linker, CEN, HIS3 (pUG34-Based).	Kral et al., 2014
pAK77	GFP-IstL140	<i>IST2_pGFP-IST2</i> with 200 aa deletion in cytosolic linker, CEN, His3 (pUG34-Based).	Kral et al., 2014
pAK84	GFP-IstL058	<i>IST2_pGFP-IST2</i> with 135 aa deletion in cytosolic, CEN, His3 (pUG34-Based).	Kral et al., 2014
pJMD_01	GFP-Ist2RL ^[729-747wt]	57 bp of <i>IST2</i> WT [729-747] inserted in the randomized linker of pAK76.	This study
pJMD_02	GFP-Ist2[TT->AA]	T736A T743A Quikchange mutagenesis of pAK75.	This study
pJMD_07	BFP-Ist2	<i>IST2_pBFP-IST2</i> full-length, CEN, HIS3 (pUG34-Based).	This study
pJMD_08	BFP-Ist2[705-762]	<i>IST2_pBFP-IST2</i> [codon705 to 762], CEN, HIS3 (pUG34-Based).	This study
pJMD_12	BFP-Ist2RL ^[718-751wt]	102 nt of <i>IST2</i> WT [729-747] inserted in the randomized linker of pJMD_21	This study
pJMD_21	BFP-Ist2RL	<i>IST2_pBFP-IST2</i> with randomized linker [596-917], CEN, HIS3 (pUG34-Based).	This study
pRS315-Osh6-mCh	pADH1 Osh6-mCherry (L)	<i>ADH1_pOSH6-mCherry</i> , CEN, LEU2 (pRS315-based).	Maeda et al., 2013
pAC100	pADH1 OSH6-mCherry (U)	<i>ADH1_pO SH6-mCherry</i> , CEN, URA3 (pRS315-based); marker swap	This study
pVA111	pCYC1 Osh6-mCherry	<i>CYC1_pO SH6-mCherry</i> , CEN, URA3 (pRS316-based); OSH6 subcloned	This study
pJMD_22	pADH1 _p Osh6 L141A D142A	Quikchange mutagenesis of pAC100	This study
pJMD_23	pCYC1 _p Osh6 L141A D142A	Quikchange mutagenesis of pVA111	This study
pJMD_24	pADH1 _p Osh6 L141K D142A	Quikchange mutagenesis of pAC100	This study
pJMD_25	pCYC1 Osh6 L141K D142A	Quikchange mutagenesis of pVA111	This study
pC2 _{Lact} -GFP	C2 _{Lact} -GFP	GPD _p C2 _{Lact} -GFP, CEN, URA3 (pRS416-based)	Yeung et al., 2008
pAC107	C2 _{Lact} -GFP (LEU)	GPD _p C2 _{Lact} -GFP, CEN, LEU2 (pRS416-based)	Lipp et al., 2019
pJMD_26	crRNA Ist2 736-743	crRNA targeting <i>IST2</i> codons 736-743, URA, 2 μ ori, based on pUD628 (Addgene Plasmid #103018)	This study
pUDC175	fnCpf1	<i>TEF1_pFnCPF1</i> , CEN, NatMX (p414TEF1 Backbone) Addgene Plasmid #103019	Swiat et al., 2017
pVA117	pRS415-ADH _p Osh6Δ35-VN	ADH _p Osh6Δ35 (Lipp et al., 2019) subcloned into pRS415-VN using SacI/BamHI digestion	This study
pVA119	pRS415-ADH _p Osh6Δ69-VN	ADH _p Osh6Δ69 (Lipp et al., 2019) subcloned into pRS415-VN using SacI/BamHI digestion	This study

Table S4. Plasmids constructed for the yeast two-hybrid assay.

Plasmid Name	Insert	Mutation	Construction
pJMD100	<i>OSH4</i>	-	<i>OSH4</i> coding region inserted in the BamHI and Xhol restrictions sites of pGBK7 (Chien et al., 1991).
pJMD101	<i>OSH6</i>	-	<i>OSH6</i> coding region inserted in the BamHI and Xhol restrictions sites of pGBK7 (Chien et al., 1991).
pJMD102	<i>OSH7</i>	-	<i>OSH7</i> coding region inserted in the BamHI and Xhol restrictions sites of pGBK7 (Chien et al., 1991).
pJMD103	<i>OSH6</i>	<i>H97D</i>	Plasmid pJMD103 to pJMD138 were generated by Quikchange site-directed mutagenesis of pJMD101.
pJMD104	<i>OSH6</i>	<i>Y135T</i>	
pJMD105	<i>OSH6</i>	<i>D141K_L142A</i>	
pJMD106	<i>OSH6</i>	<i>N144D_K145A</i>	
pJMD107	<i>OSH6</i>	<i>Q146E_Q147A</i>	
pJMD108	<i>OSH6</i>	<i>Y164F_Y166F</i>	
pJMD109	<i>OSH6</i>	<i>S171H</i>	
pJMD110	<i>OSH6</i>	<i>S183A_R184A</i>	
pJMD111	<i>OSH6</i>	<i>D204N</i>	
pJMD112	<i>OSH6</i>	<i>K214E</i>	
pJMD113	<i>OSH6</i>	<i>S244A</i>	
pJMD114	<i>OSH6</i>	<i>D250N</i>	
pJMD115	<i>OSH6</i>	<i>E252L</i>	
pJMD116	<i>OSH6</i>	<i>Y258F</i>	
pJMD117	<i>OSH6</i>	<i>E267H</i>	
pJMD118	<i>OSH6</i>	<i>K271E</i>	
pJMD119	<i>OSH6</i>	<i>Y273K</i>	
pJMD120	<i>OSH6</i>	<i>S282A</i>	
pJMD121	<i>OSH6</i>	<i>S282E</i>	
pJMD122	<i>OSH6</i>	<i>Y290F</i>	
pJMD123	<i>OSH6</i>	<i>T307A_H308A</i>	
pJMD124	<i>OSH6</i>	<i>S311A_P312A</i>	
pJMD125	<i>OSH6</i>	<i>E325A_Y326A</i>	
pJMD126	<i>OSH6</i>	<i>K333E</i>	
pJMD127	<i>OSH6</i>	<i>D337K</i>	
pJMD128	<i>OSH6</i>	<i>H373E</i>	
pJMD129	<i>OSH6</i>	<i>K375A</i>	
pJMD130	<i>OSH6</i>	<i>S380A_K381A</i>	
pJMD131	<i>OSH6</i>	<i>Q403K</i>	
pJMD132	<i>OSH6</i>	<i>D141K_L142A</i>	
pJMD133	<i>OSH6</i>	<i>D141A_L142A</i>	
pJMD134	<i>OSH6</i>	<i>D141A</i>	
pJMD135	<i>OSH6</i>	<i>L142A</i>	
pJMD136	<i>OSH6</i>	<i>D141K_L142I</i>	
pJMD137	<i>OSH6</i>	<i>D141K</i>	
pJMD138	<i>OSH6</i>	<i>D141A_L142D</i>	

Table S4 continued. Plasmids constructed for the yeast two-hybrid assay.

Name	Insert	Mutation	Construction
pJMD139	Ist2_1-100	-	Ist2 coding region from amino acid position 1 to 100 inserted in the BamHI and Xhol restriction sites of pGADT7 (Chien et al., 1991).
pJMD140	Ist2_590-878	-	Ist2 coding region from amino acid position 590 to 878 inserted in the BamHI and Xhol restriction sites of pGADT7 (Chien et al., 1991).
pJMD141	Ist2_590-703	-	Ist2 coding region from amino acid position 590 to 703 inserted in the BamHI and Xhol restriction sites of pGADT7 (Chien et al., 1991).
pJMD142	Ist2_762-878	-	Ist2 coding region from amino acid position 762 to 878 inserted in the BamHI and Xhol restriction sites of pGADT7 (Chien et al., 1991).
pJMD143	Ist2_704-761	-	Ist2 coding region from amino acid position 704 to 761 inserted in the BamHI and Xhol restriction sites of pGADT7 (Chien et al., 1991).
pJMD144	Ist2_718-751	-	Ist2 coding region from amino acid position 718 to 751 inserted in the BamHI and Xhol restriction sites of pGADT7 (Chien et al., 1991).
pJMD145	Ist2_729-761	-	Ist2 coding region from amino acid position 729 to 761 inserted in the BamHI and Xhol restriction sites of pGADT7 (Chien et al., 1991).
pJMD146	Ist2_729-747	-	Ist2 coding region from amino acid position 729 to 747 inserted in the BamHI and Xhol restriction sites of pGADT7 (Chien et al., 1991).
pJMD147	Ist2_704-727	-	Ist2 coding region from amino acid position 704 to 747 inserted in the BamHI and Xhol restriction sites of pGADT7 (Chien et al., 1991).
pJMD148	Ist2_704-761	S729A	Plasmid pJMD148 to pJMD164 were generated by Quikchange site-directed mutagenesis of pJMD143.
pJMD149	Ist2_704-761	Y730F	
pJMD150	Ist2_704-761	S729A Y730F	
pJMD151	Ist2_704-761	S729E Y730E	
pJMD152	Ist2_704-761	S729A Y730F T736A	
pJMD153	Ist2_704-761	S729E Y730E T736E	
pJMD154	Ist2_704-761	T743A	
pJMD155	Ist2_704-761	T743E	
pJMD156	Ist2_704-761	Y747A	
pJMD157	Ist2_704-761	Y747E	
pJMD158	Ist2_704-761	T736A T743A	
pJMD159	Ist2_704-761	T736A Y747A	
pJMD160	Ist2_704-761	T736A	
pJMD161	Ist2_704-761	T736E	
pJMD162	Ist2_704-761	T736K	
pJMD163	Ist2_704-761	S744A	
pJMD164	Ist2_704-761	T740A	