Habeck et al., http://www.jcb.org/cgi/content/full/jcb.201408088/DC1

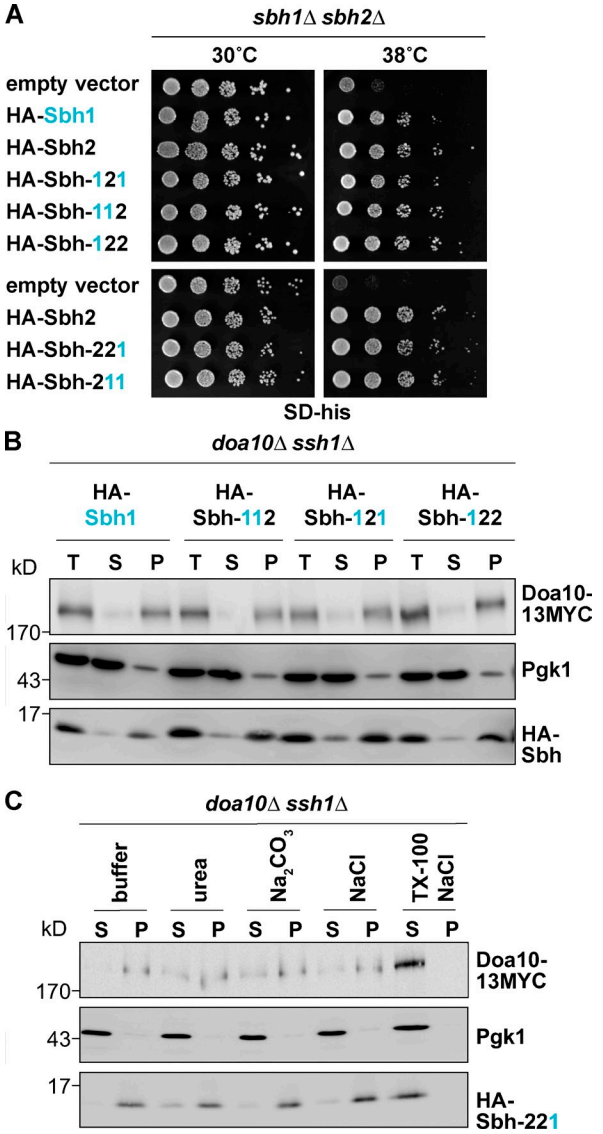

Figure S1. **Analysis of Sbh1/Sbh2 chimeric proteins.** (A) Suppression of growth defect of *sbh1Δ sbh2Δ* cells at high temperature by Sbh1/Sbh2 chimeric proteins. *sbh1Δ sbh2Δ* cells were transformed with an empty vector (p413MET25) or a p413MET25-based plasmid encoding HA-Sbh2 or the indicated Sbh1/Sbh2 chimeric protein. Serial dilutions (sixfold) of log-phase cells were spotted onto plates, and plates were incubated for 2–3 d at the indicated temperature. Blue numbers indicate the regions of the indicated chimeric Sbh protein that are from Sbh1. (B) Subcellular fractionation of *doa10Δ ssh1Δ* cells expressing the indicated Sbh1/Sbh2 chimeric protein. Sbh1/Sbh2 chimeric protein expressing *doa10Δ ssh1Δ* cells and Doa10-13MYC–expressing cells were mixed at a 7:1 ratio before lysis. Lysates were divided into a microsomal pellet (P) and a supernatant (S) fraction by centrifugation. Total (T) represents input fraction. (C) HA-Sbh-221 is an integral membrane protein in *doa10Δ ssh1Δ* cells. Subcellular fractionation of *doa10Δ ssh1Δ* cells expressing the HA-Sbh-221 protein as described in Fig. 1 E.

A

```
uniprot                                          TM
P52871    S. cere  (51) DEANGFRVDSLVVLFLSVGFIFSVIALHLLTKFTHII-----
P52870    S. cere  (44) DEATGLRVDPLVVLFLAVGFIFSVVALHVISKVAGKLF----
O43002    S. pomb  (62) DEASGFKVDPVVVMVLSVGFIASVFLLHIVARILKKFASE--
P38389    A. thal  (45) DDAPGLKISPNVVLIMSIGFIAFVAVLHVMGKLYFVK-----
Q9FKK1    A. thal  (67) DDAPGLKISPTVVLIMSLCFIGFVTALHVFGKLYLHKSGSQA
Q7JNZ0    D. mela  (64) DDSPGIKVGPVPVLVMSLLFIASVFMLHIWGKYNRS------
Q95XS2    C. eleg  (43) EDSTGLKIGPVPVLVMSLVFIASVFVLHIWGKFTRSRA----
Q8AVT7    X. laev  (60) EDSPGLKVGPVPVLVMSLLFIASVFMLHIWGKYTRS------
Q9CQS8    M. musc  (60) EDSPGLKVGPVPVLVMSLLFIAAVFMLHIWGKYTRS------
P60468    H. sapi  (60) EDSPGLKVGPVPVLVMSLLFIASVFMLHIWGKYTRS------
```

B

protein (uniprot ID)	predicted TM sequence	ΔG
Sbh1 (P52870)	PLVVLFLAVGFIFSVVALHVI	-1.07
Sbh2 (P52871)	SLVVLFLSVGFIFSVIALHLL	-1.28
Sbh2(S68A)	SLVVLFLAVGFIFSVIALHLL	-1.94
TCR-α (P01848)	VIGFRILLLKVAGFNLLMTL	+0.69

Figure S2. **A sequence alignment of C-terminal regions of Sec61 β subunits and hydrophobicity of Sbh1, Sbh2, and Sbh2(S68A) TM domains.** (A) Multiple sequence alignment of the C-terminal regions of Sec61 β subunits from different eukaryotic species. The alignment was generated with ClustalW2 (Larkin et al., 2007) and adjusted manually. The number of amino acid residues preceding the sequence shown is indicated in parentheses. The transmembrane (TM) domain of the *S. cerevisiae* Sec61 β subunits (P52870 and P52871) assigned according to the UniProt database is depicted as a black horizontal line above the alignment. P52870 and P52871 represent Sbh1 and Sbh2, respectively. The highly conserved proline residue at the cytosol–membrane interface and the highly conserved serine residue within the TM are highlighted in black. Species abbreviations are *S. cere, Saccharomyces cerevisiae*; *S. pomb, Schizosaccharomyces pombe*; *A. thal, Arabidopsis thaliana*; *D. mela, Drosophila melanogaster*; *C. eleg, Caenorhabditis elegans*; *X. laev, Xenopus laevis*; *M. musc, Mus musculus*; *H. sapi, Homo sapiens*. (B) Analysis of hydrophobicity of TM domains of Sbh1, Sbh2, and Sbh2(S68A). TM sequence assignments were taken from the UniProt database. ΔG values of TM sequences were calculated according to Hessa et al. (2007; http://dgpred.cbr.su.se/). Positive ΔG values predict an unfavorable free energy of membrane integration. The TM domain of the human T cell receptor α-chain (TCR-α), which was previously analyzed by Feige and Hendershot (2013), serves as an example of a TM sequence of low hydrophobicity.

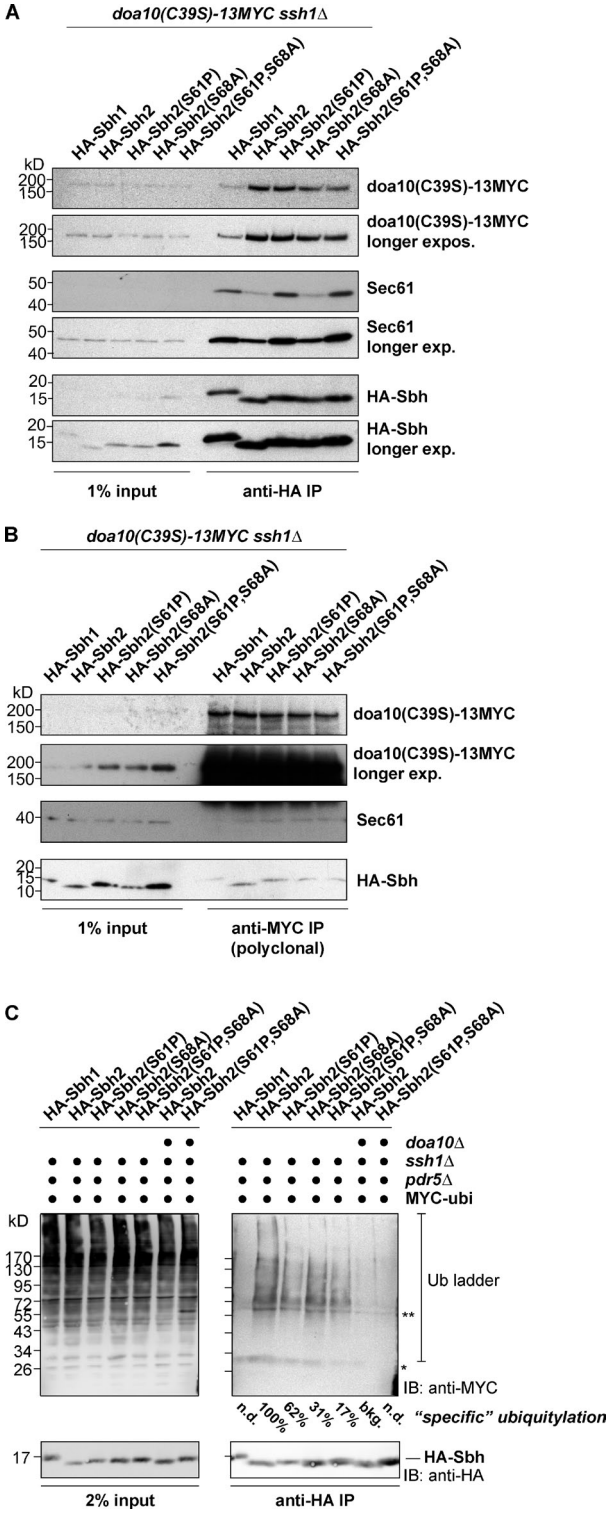

Figure S3. **Analysis of Doa10 interaction with Sec61 β subunits and ubiquitylation of WT and mutant Sec61 β subunits.** (A) Co-IP analysis to investigate the interaction between HA-tagged Sec61 β subunits (HA-Sbhs) and a MYC-tagged ligase-inactive Doa10 RING mutant (doa10(C39S)-13MYC). HA-Sbhs were ectopically expressed (low-copy plasmid; *MET25* promoter) in *doa10(C39S)-13MYC ssh1Δ* cells (DF5 strain background). HA-Sbhs were precipitated from digitonin-solubilized microsomes via anti-HA agarose beads, and precipitates were analyzed by immunoblotting with anti-MYC, anti-Sec61, or anti-HA antibodies. (B) Reciprocal co-IP analysis to investigate the interaction between Sec61 β subunits and doa10(C39S)-13MYC and Sec61. Co-IP was performed as in A except that the doa10(C39S)-13MYC protein was precipitated by incubation with an anti-MYC antibody and subsequent precipitation via protein A–Sepharose beads. exp., exposure. (C) In vivo ubiquitylation of WT and mutant HA-tagged Sec61 β subunits ectopically expressed (low-copy plasmid; *MET25* promoter) in indicated cells together with MYC-ubiquitin (MYC-ubi). Proteasome inhibitor MG132 (50 μM) was added 2 h before cell harvest. HA-tagged Sec61 β subunits were precipitated from cell lysates with anti-HA agarose beads. Precipitates were analyzed by immunoblotting with anti-HA and anti-MYC. Asterisks indicate cross-reactive bands (single and double asterisks indicate IgG light and heavy chains, respectively) recognized by the secondary antibody (anti–rabbit peroxidase). "Specific" ubiquitylation represents the background-corrected intensity of the ubiquitin ladder after anti-HA IP relative to the amount of the respective immunoprecipitated unmodified Sbh and was determined as follows: First, the amount of precipitated HA-Sbh was determined (for each HA-Sbh2, HA-Sbh2(S61P), HA-Sbh2(S68A), and HA-Sbh2(S61P,S68A)). Next, the ubiquitin ladder (Ub ladder) above the cross-reactive band for the IgG light chain (single asterisk) was quantitated whereby the ubiquitin ladder for HA-Sbh2 in *doa10Δ ssh1Δ* cells was defined as background (bkg.) and was subtracted from the other lanes. Specific ubiquitylation was then calculated by dividing the ratio between the background-corrected intensity of the ubiquitin ladder of an Sbh by the amount of its unmodified form. Specific ubiquitylation of HA-Sbh2 was set to 100%. IB, immunoblot.

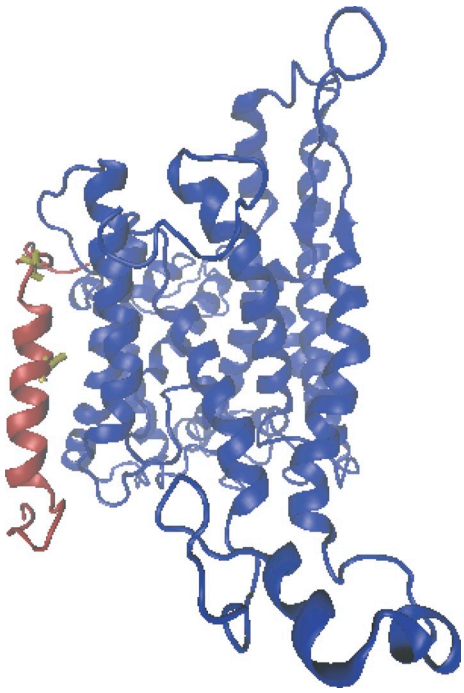

Figure S4. **Modeled Sec61 complex with Sbh2.** The protein 3D structure of *S. cerevisiae* Sec61 (in blue; UniProt ID P32915) in complex with an *S. cerevisiae* Sbh2 fragment comprising Sbh2 residues 52–88 (in red; Sbh2 residues S61 (top) and S68A (bottom) are depicted in yellow; UniProt ID P52871) was modeled with Modeller 9.11. (Eswar et al., 2006) and visualized with VMD (Visual Molecular Dynamics; Humphrey et al., 1996). The cryo-EM structure of the mammalian Sec61 complex (Protein Data Bank code 2WWB) was used as a template (Becker et al., 2009). Change in binding affinity between Sec61 and Sbh2 caused by single-site mutations within Sbh2 sequence was evaluated using the BeAtMuSiC program (Dehouck et al., 2013). In accordance with the experimental data, the point mutation of Sbh2 Ser61 to proline was predicted to decrease the binding free energy (ΔΔGBind = −0.5 kcal/mol), whereas all the other point mutations at residue 61 were predicted to increase the binding free energy. Thus, S61P mutant appears specifically to increase its binding affinity to Sec61. Unlike S61P mutation, no mutations of the serine residue at position 68 in the Sbh2 sequence resulted in a decrease in binding free energy. Therefore, mutations of the residue at position 68 in Sbh2 were not predicted to increase its binding affinity to Sec61.

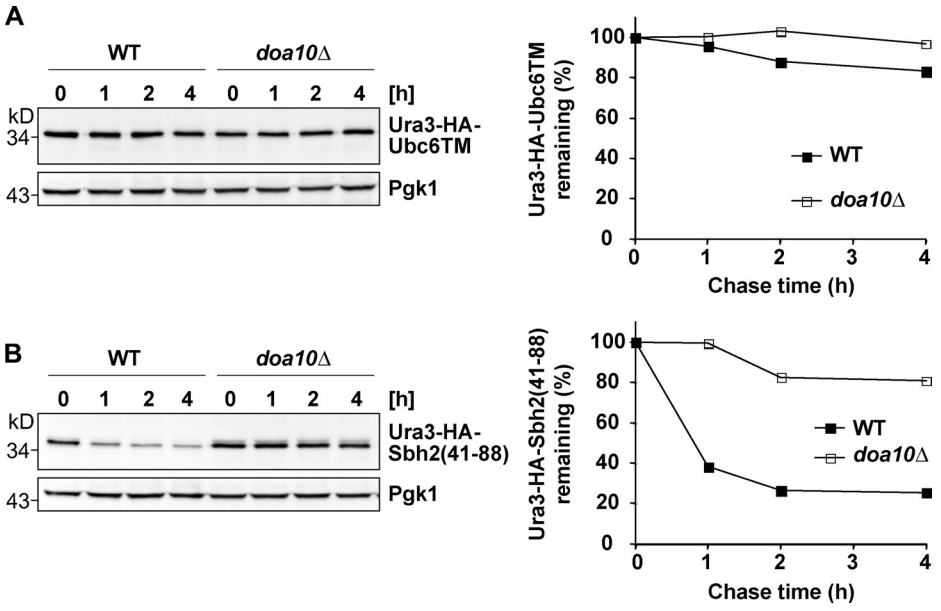

Figure S5. **Comparison of stability of Ura3-HA-Ubc6TM and Ura3-3HA-Sbh2(aa 41–88) fusion proteins.** (A) Degradation of ectopically expressed Ura3-HA-Ubc6TM fusion protein (low-copy plasmid; *MET25* promoter) in WT and *doa10Δ* cells (DF5 strain background). The Ubc6TM segment consists of the C-terminal membrane anchor sequence of Ubc6 plus 18 residues preceding the TM (Kreft and Hochstrasser, 2011). chx chase analysis was performed as in Fig. 1 B. Pgk1 served as a loading control. (right) Quantification of the gel. Ura3-HA-Ubc6TM levels at $t = 0$ h were set to 100%. (B) Degradation of ectopically expressed Ura3-HA-Sbh2(aa 41–88) (low-copy plasmid; *MET25* promoter) in WT and *doa10Δ* cells as in A. (right) Quantification of the gel. Ura3-HA-Sbh2(aa 41–88) levels at $t = 0$ h were set to 100%. The data shown in A and B are in each case from a single representative experiment out of two repeats.

Table S1. **Plasmids used in this study**

Name	Description	Source
***S. cerevisiae* expression plasmids**		
p413GPD	Amp^R; *CEN HIS3* with *ScGPD* promoter and *CYCI* terminator	Mumberg et al., 1995
p413MET25	Amp^R; *CEN HIS3* with *ScMET25* promoter and *CYCI* terminator	Mumberg et al., 1994
p416GPD	Amp^R; *CEN URA3* with *ScGPD* promoter and *CYCI* terminator	Mumberg et al., 1995
p416MET25	Amp^R; *CEN URA3* with *ScMET25* promoter and *CYCI* terminator	Mumberg et al., 1994
p413MET25-HA-Sbh1 (STK05-4-4)	p413MET25 with HA-Sbh1; *CEN HIS3*	This study
p413MET25-HA-Sbh2 (STK05-1-2)	p413MET25 with HA-Sbh2; *CEN HIS3*	This study
p413MET25-HA-Sbh2(S61P) (STK05-2-3)	p413MET25 with HA-Sbh2(S61P); *CEN HIS3*	This study
p413MET25-HA-Sbh2(S68A) (STK05-5-8)	p413MET25 with HA-Sbh2(S68A); *CEN HIS3*	This study
p413MET25-HA-Sbh2(S61P,S68A) (STK05-5-9)	p413MET25 with HA-Sbh2(S61P,S68A); *CEN HIS3*	This study
p413MET25-HA–Sbh-122 (STK05-9-1)	p413MET25 with HA–Sbh-122; *CEN HIS3*; Sbh-122 contains Sbh1 aa 1–53 and Sbh2 aa 61–88	This study
p413MET25-HA-Sbh-121 (STK05-8-5)	p413MET25 with HA-Sbh-121; *CEN HIS3*; Sbh-121 is Sbh1 with Sbh1 residues aa 54–75 replaced by Sbh2 residues aa 61–82	This study
p413MET25-HA-Sbh-112 (STK05-9-9)	p413MET25 with HA-Sbh-112; *CEN HIS3S; S*bh-112 is Sbh1 with Sbh1 residues aa 77–82 replaced by Sbh2 residues aa 84–88	This study
p413MET25-HA-Sbh-221 (STK08-1-5)	p413MET25 with HA-Sbh-221; *CEN HIS3*; Sbh-221 is Sbh2 with Sbh2 residues aa 84–88 replaced by Sbh1 residues aa 77–82	This study
p413MET25-HA-Sbh-211 (STK05-5-5)	p413MET25 with HA-Sbh-211; *CEN HIS3*; Sbh-221 is Sbh2 with Sbh2 residues aa 61–88 replaced by Sbh1 residues aa 54–82	This study
p413MET25-Ura3-HA-Sbh2(aa 41–88) (STK05-1-3)	p413MET25 with Ura3-HA-Sbh2(aa 41–88); *CEN HIS3*	This study
p413MET25-Ura3-HA-Sbh2(aa 57–88) (STK05-1-8)	p413MET25 with Ura3-HA-Sbh2(aa 57–88); *CEN HIS3*	This study
p413MET25-Ura3-HA-Sbh1(aa 50–82) (STK05-1-7)	p413MET25 with Ura3-HA-Sbh1(aa 50–82); *CEN HIS3*	This study
p416MET25-HA-Sbh1 (STK06-5-3)	p416MET25 with HA-Sbh2; *CEN URA3*	This study
p416MET25-HA-Sbh2 (STK05-7-3)	p416MET25 with HA-Sbh2; *CEN URA3*	This study
p416MET25-HA-Sbh2(S61P) (STK06-5-4)	p416MET25 with HA-Sbh2(S61P); *CEN URA3*	This study
p416MET25-HA-Sbh2(S68A) (STK06-5-5)	p416MET25 with HA-Sbh2(S68A); *CEN URA3*	This study
p416MET25-HA-Sbh2(S61P,S68A) (STK06-5-6)	p416MET25 with HA-Sbh2(S61P,68A); *CEN URA3*	This study
p413GPD-HA-Sbh2 (STK05-4-5)	p413GPD with HA-Sbh2; *CEN HIS3*	This study
p413GPD-FLAG-Sbh2 (STK06-1-2)	p413GPD with FLAG-Sbh2; *CEN HIS3*	This study
pTEF2-HA-mCherry-Sbh2 (STK05-5-3)	pTEF2 with HA-mCherry-Sbh2; *CEN URA3*	This study
YEp105	Amp^R; *2μ TRP1* with MYC-ubiquitin ORF under control of *CUP1* promoter; *CYCI* terminator	Ellison and Hochstrasser, 1991
pCUP1-MYC-Ub (*URA3)* (STK07-8-7)	YEp105 with marker swapped (*TRP1* → *URA3*) using marker swap plasmid pTU10; *2μ URA3*	This study
Others		
pAG32	Amp^R; template for PCR amplification of hphMX4 marker cassette for gene deletion	Goldstein and McCusker, 1999
pTU10	Chloramphenicol^R; marker swap plasmid (TRP1 → URA3)	Cross, 1997

All yeast expression plasmids are low-copy (*CEN*) plasmids, except for plasmids YEp105 and pCUP1-MYC-Ub (URA3), which are high-copy (*2μ*) plasmids. We received plasmid pTEF2-mCherry-Sbh2 from J. Weissman (University of California, San Francisco, San Francisco, CA), which was used to generate plasmid pTEF2-HA-mCherry-Sbh2.

Table S2. **Yeast strains used in this study, with relevant markers, plasmids, and origin of strains**

Name	Relevant genotype	Source
***S. cerevisiae* strains DF5 strain background**		
MHY500	MATa *his3-200 leu2-3,112 ura3-52 lys2-801 trp1-1 gal2*	Chen et al., 1993
MHY551	MATa *his3-200 leu2-3,112 ura3-52 lys2-801 trp1-1 gal2 ubc7::LEU2*	Chen et al., 1993
MHY553	MATa *his3-200 leu2-3,112 ura3-52 lys2-801 trp1-1 gal2 ubc6-Δ1::HIS3 ubc7::LEU2*	Chen et al., 1993
MHY1310	MATa *his3-200 leu2-3,112::LEU2-Deg1-lacZ ura3-52 lys2-801 trp1-1*	Hochstrasser laboratory[a]; unpublished data
SKY218	MATa *his3-200 leu2-3,112 ura3-52 lys2-801 trp1-1 gal2 ubc6-Δ1::HIS3*	Sommer and Jentsch, 1993
MHY3339	MATa *his3-200 leu2-3,112::LEU2-Deg1-lacZ ura3-52 lys2-801 trp1-1 doa10-C39S*	Kreft and Hochstrasser, 2011
SKY165	MATa *his3-200 leu2-3,112::LEU2-Deg1-lacZ ura3-52 lys2-801 trp1-1 doa10Δ::kanMX4*	This study
SKY166	MATa *his3-200 leu2-3,112::LEU2-Deg1-lacZ ura3-52 lys2-801 trp1-1 doa10Δ::hphMX4*	Kreft and Hochstrasser, 2011
SKY361	MATa *his3-200 leu2-3,112 ura3-52 lys2-801 trp1-1 gal2 doa10::doa10(C39S)-13MYC ssh1Δ::kanMX4*	This study
SKY472	MATa *his3-200 leu2-3,112 ura3-52 lys2-801 trp1-1 gal2 ssh1Δ::hphMX4*	This study
SKY473	MATa *his3-200 leu2-3,112 ura3-52 lys2-801 trp1-1 gal2 ubc7::LEU2 ssh1Δ::hphMX4*	This study
SKY474	MATa *his3-200 leu2-3,112 ura3-52 lys2-801 trp1-1 gal2 ubc6-Δ1::HIS3 ubc7::LEU2 ssh1Δ::hphMX4*	This study
SKY475	MATa *his3-200 leu2-3,112 ura3-52 lys2-801 trp1-1 gal2 ubc6-Δ1::HIS3 ssh1Δ::hphMX4*	This study
***S. cerevisiae* strains YPH499 strain background**		
MHY2923	MATα *his3-Δ200 leu2-Δ1 ura3-52 lys2-801 trp1-Δ63 doa10-1-1319myc13-His3MX6*	Hochstrasser laboratory[a]; unpublished data
***S. cerevisiae* strains S288C strain background**		
BY4741 (=MHY2972)	MATa *his3Δ1 leu2Δ0 met15Δ0 ura3Δ0*	Tong et al., 2001
MHY3032	MATa *his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 hrd1Δ::kanMX*	Tong et al., 2001
MHY3033	MATa *his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 doa10Δ::kanMX*	Tong et al., 2001
SKY247	MATa *his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 ssh1Δ::kanMX*	Tong et al., 2001
SKY249	MATa *his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 sbh2Δ::kanMX*	Tong et al., 2001
SKY252	MATa *his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 doa10Δ::kanMX hrd1Δ::kanMX*	This study
SKY261	MATa *his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 doa10Δ::hphMX4 ssh1Δ::kanMX*	This study
SKY266	MATa *his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 sbh1Δ::kanMX ssh1Δ::kanMX*	This study
SKY269	MATa *his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 sbh2Δ::kanMX ssh1Δ::kanMX*	This study
SKY286	MATa *his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 sbh1Δ::kanMX sbh2Δ::hphMX4*	This study
SKY298	MATa *his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 get2Δ::kanMX*	Tong et al., 2001
SKY386	MATa *his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 hrd1Δ::kanMX ssh1Δ::hphMX4*	This study
SKY388	MATa *his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 pdr5Δ::kanMX ssh1Δ::hphMX4*	This study
SKY483	MATa *his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 doa10Δ::KlLEU2 pdr5Δ::kanMX ssh1Δ::hphMX4*	This study
***S. cerevisiae* strains W303 strain background**		
W303-1a (=SKY335)	MATa *ade2-1 ura3-1 his3-11,15 trp1-1 leu2-3,112 can1-100*	Rothstein laboratory[b]
SKY336	MATa *ade2-1 ura3-1 his3-11,15 trp1-1 leu2-3,112 can1-100 cdc48-3*	Ye et al., 2001
YWO1407	MATα *ade2-1 ura3-1 his3-11,15 trp1-1 leu2-3,112 can1-100 prc1-1 SEC61myc9::TrpMX*	Schäfer and Wolf, 2009
SKY316	MATα *ade2-1 ura3-1 his3-11,15 trp1-1 leu2-3,112 can1-100 prc1-1 SEC61myc9::TrpMX ssh1Δ::hphMX4*	This study

D.H. Wolf (University of Stuttgart, Stuttgart, Germany) provided yeast strain YWO1407, which was used to generate yeast strain SKY316.
[a]Yale University, New Haven, CT.
[b]Columbia University, New York, NY.

References

Becker, T., S. Bhushan, A. Jarasch, J.P. Armache, S. Funes, F. Jossinet, J. Gumbart, T. Mielke, O. Berninghausen, K. Schulten, et al. 2009. Structure of monomeric yeast and mammalian Sec61 complexes interacting with the translating ribosome. *Science*. 326:1369–1373. http://dx.doi.org/10.1126/science.1178535

Chen, P., P. Johnson, T. Sommer, S. Jentsch, and M. Hochstrasser. 1993. Multiple ubiquitin-conjugating enzymes participate in the in vivo degradation of the yeast MAT alpha 2 repressor. *Cell*. 74:357–369. http://dx.doi.org/10.1016/0092-8674(93)90426-Q

Cross, F.R. 1997. 'Marker swap' plasmids: convenient tools for budding yeast molecular genetics. *Yeast*. 13:647–653. http://dx.doi.org/10.1002/(SICI)1097-0061(19970615)13:7<647::AID-YEA115>3.0.CO;2-#

Dehouck, Y., J.M. Kwasigroch, M. Rooman, and D. Gilis. 2013. BeAtMuSiC: Prediction of changes in protein-protein binding affinity on mutations. *Nucleic Acids Res.* 41(W1):W333–W339. http://dx.doi.org/10.1093/nar/gkt450

Ellison, M.J., and M. Hochstrasser. 1991. Epitope-tagged ubiquitin. A new probe for analyzing ubiquitin function. *J. Biol. Chem.* 266:21150–21157.

Eswar, N., B. Webb, M.A. Marti-Renom, M.S. Madhusudhan, D. Eramian, M.Y. Shen, U. Pieper, and A. Sali. 2006. Comparative protein structure modeling using Modeller. *Curr. Protoc. Bioinformatics*. Chapter 5(Suppl. 15.):6.

Feige, M.J., and L.M. Hendershot. 2013. Quality control of integral membrane proteins by assembly-dependent membrane integration. *Mol. Cell*. 51:297–309. http://dx.doi.org/10.1016/j.molcel.2013.07.013

Goldstein, A.L., and J.H. McCusker. 1999. Three new dominant drug resistance cassettes for gene disruption in *Saccharomyces cerevisiae. Yeast*. 15:1541–1553. http://dx.doi.org/10.1002/(SICI)1097-0061(199910)15:14<1541::AID-YEA476>3.0.CO;2-K

Hessa, T., N.M. Meindl-Beinker, A. Bernsel, H. Kim, Y. Sato, M. Lerch-Bader, I. Nilsson, S.H. White, and G. von Heijne. 2007. Molecular code for transmembrane-helix recognition by the Sec61 translocon. *Nature*. 450:1026–1030. http://dx.doi.org/10.1038/nature06387

Humphrey, W., A. Dalke, and K. Schulten. 1996. VMD: visual molecular dynamics. *J. Mol. Graph.* 14:33–38: 27–28. http://dx.doi.org/10.1016/0263-7855(96)00018-5

Kreft, S.G., and M. Hochstrasser. 2011. An unusual transmembrane helix in the endoplasmic reticulum ubiquitin ligase Doa10 modulates degradation of its cognate E2 enzyme. *J. Biol. Chem.* 286:20163–20174. http://dx.doi.org/10.1074/jbc.M110.196360

Larkin, M.A., G. Blackshields, N.P. Brown, R. Chenna, P.A. McGettigan, H. McWilliam, F. Valentin, I.M. Wallace, A. Wilm, R. Lopez, et al. 2007. Clustal W and Clustal X version 2.0. *Bioinformatics*. 23:2947–2948. http://dx.doi.org/10.1093/bioinformatics/btm404

Mumberg, D., R. Müller, and M. Funk. 1994. Regulatable promoters of *Saccharomyces cerevisiae*: comparison of transcriptional activity and their use for heterologous expression. *Nucleic Acids Res.* 22:5767–5768. http://dx.doi.org/10.1093/nar/22.25.5767

Mumberg, D., R. Müller, and M. Funk. 1995. Yeast vectors for the controlled expression of heterologous proteins in different genetic backgrounds. *Gene*. 156:119–122. http://dx.doi.org/10.1016/0378-1119(95)00037-7

Schäfer, A., and D.H. Wolf. 2009. Sec61p is part of the endoplasmic reticulum-associated degradation machinery. *EMBO J.* 28:2874–2884. http://dx.doi.org/10.1038/emboj.2009.231

Sommer, T., and S. Jentsch. 1993. A protein translocation defect linked to ubiquitin conjugation at the endoplasmic reticulum. *Nature*. 365:176–179. http://dx.doi.org/10.1038/365176a0

Tong, A.H., M. Evangelista, A.B. Parsons, H. Xu, G.D. Bader, N. Pagé, M. Robinson, S. Raghibizadeh, C.W. Hogue, H. Bussey, et al. 2001. Systematic genetic analysis with ordered arrays of yeast deletion mutants. *Science*. 294:2364–2368. http://dx.doi.org/10.1126/science.1065810

Ye, Y., H.H. Meyer, and T.A. Rapoport. 2001. The AAA ATPase Cdc48/p97 and its partners transport proteins from the ER into the cytosol. *Nature*. 414:652–656. http://dx.doi.org/10.1038/414652a