FILE S1

Supporting Materials & Methods

Plasmid construction details: pLND44-4 was created by cloning into pRS306 (SIKORSKI and HIETER 1989) a fusion PCR product amplified from the mix of a left and a right PCR reaction as templates. The left PCR reaction used primers XhoI_*MIP1*_F (GTCTCG*CTCGAG*CCCGTAATATGGTCGAAGGA) and *MIP1*_661Thr_R (AACCACG**t**ATTTTCCACAGCTCTTCTAGTGATT), and S288C genomic DNA as template. The right PCR reaction used primers *MIP1*_661Thr_F (AATCACTAGAAGAGCTGTGGAAAAT**a**CGTGGTT) and EagI_SCD5_R (CGAGAC*CGGCCG*GATGGCCTCTTTTCTGCTTG), and S288C genomic DNA as template. The fusion PCR product was digested with *Xho*I and *Eag*I (italicized and underlined in XhoI_*MIP1*_F and EagI_SCD5_R) and was ligated into similarly digested pRS306. The *MIP1*_661Thr_R and *MIP1*_661Thr_F primers are responsible for introducing one nucleotide difference (bold lower case) from the S288C genome. This change replaces the GCG codon for alanine at the 661st position of *MIP1* in S288C with a threonine codon (**a**CG in the *MIP1*_661Thr_F primer). Sequence verification of pLND44-4 revealed that the cloned insert contains one additional nucleotide change 987 base pairs downstream of the intended single nucleotide change at the 661st amino acid of *MIP1*. This mutation was most likely introduced by the PCR amplification. During the pop-in/pop-out strategy for *MIP1* allele replacement with pLND44-4, we made sure that the pop-out recombination excised this secondary mutation out of the genomic DNA.

pRS306-*MKT1*-D30G was also created by cloning into pRS306 a fusion PCR product. The left PCR reaction used primers mkt1-1-SpeI (GATC*ACTAGT*ACCACCAAAACAGCTCATCAA) and mkt1-D30G-1 (CATAATGGTTGACGTCTATAcC**t**AGGGTACAATTATTCAG), and S288C genomic DNA as template. The right PCR reaction used primers mkt1-D30G-2 (CTGAATAATTGTACCCTaG**g**TATAGACGTCAACCATTATG) and mkt1-2-XhoI (GATC*CTCGAG*ATCAAACAGCTGAGGAACTGGA), and S288C genomic DNA as template. The fusion PCR product was digested with *Spe*I and *Xho*I (italicized and underlined) and was ligated into similarly digested pRS306. The mkt1-D30G-1 and mkt1-D30G-2 primers are responsible for introducing two nucleotide differences (lower case letters) from the S288C genome. One nucleotide change (bold lower case) changes the G**A**T codon for aspartate at the 30th amino acid position of *MKT1* in S288C into a glycine codon (G**g**T in the mkt1-D30G-2 primer). The other nucleotide change (non-bold lower case) changes the wobble position of the CTG codon for leucine at the 29th amino acid position of *MKT1* into another leucine codon (CTa in the mkt1-D30G-2 primer). While preserving the amino acid sequence of the protein, this second nucleotide change introduces a new *Avr*II restriction site (boxed) in this *MKT1-30G* allele to simplify genotyping. The insert in pRS306-*MKT1*-D30G was sequence verified and contains only these two additional nucleotide changes compared to the S288C sequence of *MKT1*.

Allelic replacement details: The *SAL1* and *CAT5* allelic replacements were executed by two sequential transformations as described in detail elsewhere (GRAY *et al.* 2004). Briefly, the first transformation integrates the *URA3* marker into the targeted locus (*sal1-1* or *CAT5-91I*). The second transformation replaces *URA3* with a PCR product, which in our case was amplified from RM11-1a genomic DNA.

For the replacement of the *sal1-1* allele, the PCR product was generated with primers *SAL1*_F2 (TTTACCTATTCAACGAAGATGTCG) and *SAL1*_R2 (AGCTGATGGAAACTGCTGGA). The RM11-1a sequence between these two primers differs from the S288C sequence only in the stretch 5'-GGTGGGCTC-3' for codons 401 through 403 in RM11-1a versus 5'-GGGGGGGGTC-3' in S288C (Figure 6A). Thus, in addition to reverting the frameshift mutation in S288C, our allele replacement also introduces a different codon for the glycine amino acid in the 401st position of Sal1p (GGT in RM11 versus GGG in S288C).

For the replacement of the *CAT5-91I* allele, the PCR product was generated with primers *CAT5*_F (GCAGAGGCTTTTCCGTCTTA) and i*CAT5*_R (TTTATCAAACCGTTTTCCTTTCA). The RM11-1a sequence between these two primers differs from the S288C sequence at only three nucleotides. Only one is a non-synonymous SNP that changes the amino acid at the 91st position of the Cat1p (Figure 6C). The other two are silent SNPs that do not change the amino acid sequence of the protein. Interestingly, these two silent SNPs cluster very closely to the 91st amino acid position – one alters the 85th codon for lysine (AAA in S288C versus AAG in RM11-a, the other alters the 89th codon for lysine (AAG in S288C versus AAA in RM11-1a). Therefore, our *CAT5* allele replacement changes the only amino acid difference between the S288C and RM11-1a Cat5p, namely the one at the 91st position (Figure 6C).

Colony counting by ImageJ: In order to count *petite* and *grande* colonies with ImageJ, YEPDG plates were scanned as a transparency with an Epson Expression 1680 scanner and SF Launcher v.2.1.0 software. The scan resolution was 300 dots per inch without a filter to generate a 16→8 bit greyscale tiff file, which was manipulated further in ImageJ. Using the "MultiThresholder" plug-in filter in ImageJ, the tiff files were converted to black and white, binary images. The "Watershed" function was run consecutively twice to maximize the separation of merged colonies. The "Analyze Particles" function was then used to count the colonies. The use of BD Falcon, 150x15mm plates (catalog number 35 1058) allowed us to apply the "Analyze Particles" function to the entire area of a plate because these plates do not contain additional edges on the bottom. The settings for the "Analyze Particles" function were: size (in pixels) between 5 and infinity; circularity between 0.60 and 1.00; excluding colonies that lay on the edges of the analyzed area. The "Analyze Particles" function returns data on the size of each colony and the total number of colonies. To count the number of *petite* colonies, the distribution of colony size was generated and a cut-off pixel size that readily distinguished

between small and large colonies was determined. That cut-off was used to count the number of *petite* colonies. In optimizing this protocol, we determined that the correlation between manually versus ImageJ counted plates with a broad range of median *petite* frequencies was extremely high ($R^2 = 0.9980$, data not shown).

OD doubling time measurements: OD doubling times were measured at 660nm in 96-well plates using a Powerwave XS plate reader (BioTek, Winooski, VT) using a modification of the protocol in (TOUSSAINT and CONCONI 2006; VEATCH *et al.* 2009). To follow the growth rate of the same culture for a long period of time, a series of seven four-fold dilutions of each original culture were made in final volumes of 100µl. The plates were grown at 30° with high, continuous shaking for 48 hours with absorbance reads every 10 minutes. OD doubling times of individual wells were calculated for values of the path-length-unadjusted absorbance between 0.0625 and 0.25. The OD doubling time was calculated by linear regression of the $\log_2$ transformed ODs using all data points within this specified range. Each strain was analyzed in biological triplicate.

Literature Cited for Supporting Materials & Methods

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