Supporting Information

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SI Materials and Methods

Yeast Strains. The yeast strains used in this study are listed in Table S1. Gene disruptions/insertions used PCR fragments containing *KanMX*, *NAT*, or *URA3* amplified from pFA6a–*KanMX* (1), pAG25 (2), or pRS406 (3), respectively. The point mutation in the *KAT1* gene that corrected the −1 frameshift was introduced into the genome using a two-step procedure (4) generating SAY1746 (*KAT1+G*). Genomic manipulations were confirmed by locus-specific PCR and DNA sequencing. Sequences of the oligonucleotides used are available on request. SAY1597 (*MAT***a** *kat1*Δ, chromosome coordinates *KLLA0D488864–491819*) was generated in SAY572 (*MAT***a** *nej1*Δ). SAY1623 (*MAT*α *kat1*Δ) was obtained by crossing SAY1597 with SAY1476. SAY1628 (*MAT***a***::URA3 kat1*Δ) was obtained by crossing SAY1490 (*MAT***a***::URA3*) with SAY1623. SAY1639 (*MAT***a** *mre11*Δ *kat1*Δ) was obtained by crossing SAY559 (*mre11*Δ) with SAY1623. SAY1899 (*MAT***a***::URA3 cut1*Δ), SAY1871 (*MAT***a***::URA3 cut2*Δ), SAY1900 (*MAT***a***::URA3 cut2*Δ), and SAY1897 (*MAT***a***::URA3 cut1*Δ *cut2*Δ) were generated using a one-step procedure in SAY572 or SAY528 (*nej1*Δ*::hisG*).

Plasmids. A 1.1-kb PCR fragment containing the *URA3* gene from pRS306 was inserted immediately downstream of the *MAT***a***1* stop codon using a one-step gene-disruption–generating strain, 1488 (5). To generate the *mat***a***::URA3 cut1*Δ and *mat***a***::URA3 cut2*Δ and double mutations, a 3.7-kb *MAT***a***::URA3* PCR fragment starting in the *MAT***a***2* gene (328 bp from the **a***2* start codon) and ending downstream from the *MAT***a***1* gene (704 bp downstream from the **a***1* stop codon) was amplified using genomic DNA from SAY1488 as a template. The *MAT***a***::URA3* PCR fragment was cloned into the XbaI and SacI sites of pCXJ20, generating pFL15. Deletions in TIR-L and TIR-R of pFL15 (removing the 6-bp AccI sites) were introduced using site-directed mutagenesis. The resulting plasmids were linearized with SacI-XbaI and were used for gene replacement in vivo.

Plasmids with origins of replication and centromeres from *S. cerevisiae* do not replicate stably in *K. lactis*. To solve this problem, plasmid pEB155 was generated by cotransforming HindIII-XbaI linearized pCXJ18 with a PCR fragment from pGREG526 (6) containing 50 bp of homology with the cloning cassette of pCXJ18 into SAY172. The result was pCXJ18 containing a Drag&Drop cassette for generating galactose-regulated 13×Myc N-terminally tagged gene fusions that replicate stably in *K. lactis*. The p*GAL–KAT1+G* and p*GAL–KAT1* vectors were generated by Drag&Drop cloning (6), amplifying PCR fragments using the respective sequences, and cotransforming them with SalI-linearized pEB155 into strain SAY172. Site-directed mutagenesis of p*GAL–KAT1+G* generated the plasmids expressing the Kat1D310A, Kat1D377A, and Kat1E895A proteins. To obtain a vector expressing Kat1+G in *E. coli*, the ORF of Kat1+G was PCR amplified and cloned into the Sal1 sites of pGAT-2 (7). The resulting plasmid mediated expression of N-terminally GST–tagged Kat1+G. Mutations in the DDE motif were introduced using site-directed mutagenesis as described above. To obtain a vector detecting the −1 frameshift, two nucleotides in the *MalE* sequences in GM-1 plasmid were deleted (GM-1 is a +1 frameshift vector) (8). The resulting plasmid was digested with BamHI and EcoRI and combined with a 150-bp PCR fragment (BamHI–EcoRI) containing the putative *KAT1* slippery site. This cloning placed the *KAT1* slippery sequence between the *GST* and *MalE* genes with the *MalE* gene in the −1 frame compared with the *GST* gene. To construct the dual luciferase plasmids, the 0-frame plasmid (pJD375) and yeast HIV-1 plasmid (pJD376) were generously provided by J. D. Dinman, University of Maryland, College Park, MD (9). A 95-bp *KAT1* fragment including the slippery site was obtained by PCR. The fragment was digested with BamHI and cloned into BamHI-digested pJD375. To sequence the circular spacer, inverse PCR fragments were cloned into a T vector (Promega) and sequenced (Macrogen) using the T7 primer.

General Methods. Yeast media were prepared as described (10). Site-directed mutagenesis was performed according to the protocol outlined in the Stratagene QuikChange procedure and was confirmed by DNA sequencing. DNA blots for investigating mating-type switching were performed using a *MAT*-specific ^{32}P-labeled probe (AKO155/156). For mapping DSB formation in *mre11*Δ, a ^{32}P-labeled probe (EBO104/210) corresponding to the *MAT***a***1–MAT***a***2* intergenic region was used. DNA blots were visualized/quantified using a PhosphorImager (Fuji). Sequences of oligonucleotides used to generate probes for DNA blots and qPCR are given in Table S2. Lambda exonuclease digestion of genomic DNA was performed for 30 min at 37 °C using the buffer provided by the manufacturer (New England Biolabs).

ChIP. ChIP was performed as described (11) with the following modifications. Formaldehyde cross-linking was performed for 5 min at room temperature. Immunoprecipitation was performed with 10 μg/mL (final concentration) of anti–c-Myc antibody 9E11 (Santa Cruz). ChIP materials were analyzed by qPCR. Reactions were performed in triplicate using 300-nM primers (Table S2) and the iQ SYBR Green supermix and were analyzed on a CFX96 Real-Time system (Bio-Rad). The average of the triplicates from three independent experiments was normalized to 1% input. The fold-change over background or enrichment ratio was obtained by dividing the ratio from the primers amplifying the *MAT***a***1–MAT***a***2* intergenic region by the ratio obtained from the control locus (the *KAT1* ORF).

qPCR. For RT-qPCR, cultures were grown to exponential phase, and RNA was isolated by phenol extraction. RNA was reverse transcribed using a cDNA synthesis kit (Bio-Rad), and the cDNAs were quantified. The primers used for amplifying the *ACT1* control cDNA have been described previously (12). The primers used for amplifying the *KAT1* cDNA are shown in Table S2. The 2^-ΔΔCT formula was used for comparing the fold-change in *KAT1* expression.

Dual-Luciferase Assays. Dual-luciferase assays were performed as described (9). Luciferase activities were determined using 5 μg of lysate per sample using the Dual-Luciferase Assay System (Promega). Frameshift efficiencies were calculated using a method described previously (13).

Mating-Type Switching Assay. The assay for measuring switching rates was performed as described (5).

Expression of Frameshifted Fusion Protein. *E. coli* strain DE3 harboring *GST–KAT1-slippery site–MBP* and an empty expression vector was induced by the addition of 0.5 mM IPTG (5 h at 25 °C). The fusion protein was purified from 500 mL of culture using amylose resin (New England Biolabs). The purity and identity of eluted protein were confirmed by SDS/PAGE. The fusion protein resulting from the frameshift was purified further by SDS/PAGE; then the band was cut from the gel and sent for MS.

Phylogenetic Footprinting of *Kluyveromyces* Genomes. The draft genomes of *K. aestuarii*, *K. dobzhanskii*, and *K. wickerhamii* were collected in a public BLAST-searchable database (tools.scilifelab.se/adhoc) together with the completed *K. lactis* genome. The *K. marxianus* draft genome is available in GenBank (accession number AKFM00000000).

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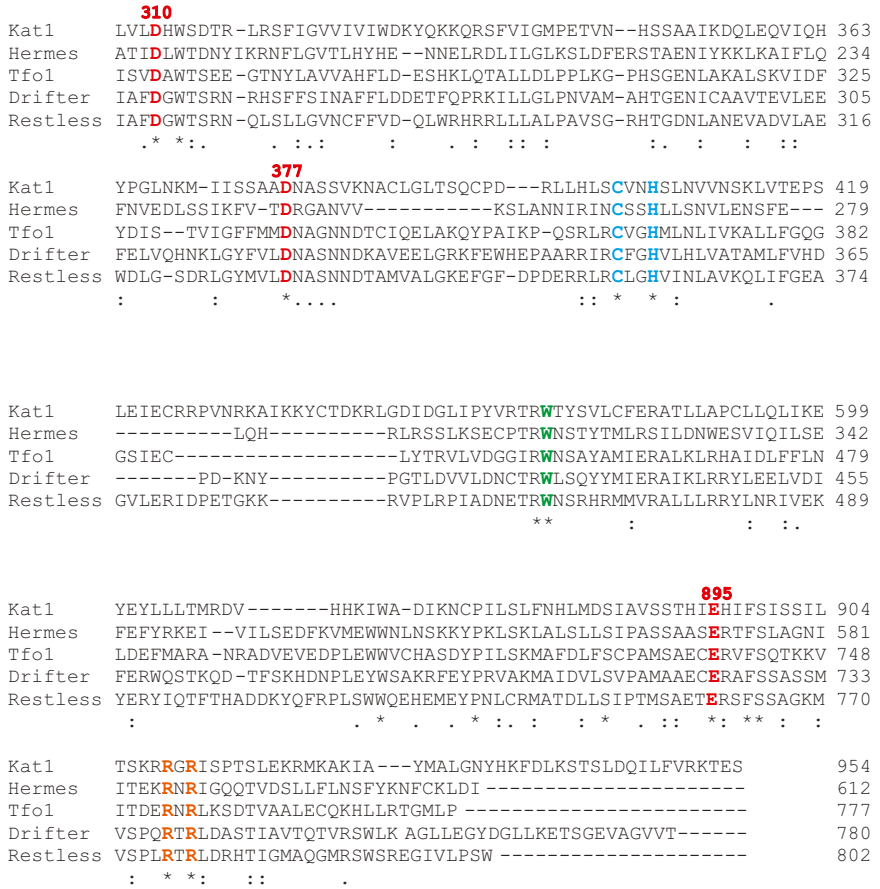

Fig. S1. Multiple sequence alignment (ClustalW) of *K. lactis* Kat1 and hAT transposases. The predicted Kat1 peptide resulting from the programmed frameshift was aligned with the hAT transposases TfoI (BAA32244, *Fusarium oxysporum*), Drifter (AAV28708, *F. oxysporum*), Restless (CAA93759, *Tolypocladium inflatum*), and Hermes (AAB60236, *Musca domestica*) (1–4). Resequencing of *KAT1* revealed four mutations leading to a different amino acid (phenylalanine 276 to leucine, histidine 411 to asparagine, alanine 661 to serine, and glycine 908 to arginine) compared with the published sequence (Genolevures), probably reflecting strain variation. A partial alignment is shown with regions displaying low sequence similarity omitted. Residues in red (changed by in vitro mutagenesis) represent the predicted DDE motif. Residues in blue represent a CXXH motif conserved among hAT transposases (5). The Trp residue labeled in green (Trp576 in Kat1) has been shown to be important for hairpin formation in Hermes (6). The two arginine residues labeled in orange in the C terminus of Kat1 are predicted to be critical for dimerization of hAT transposases (7).

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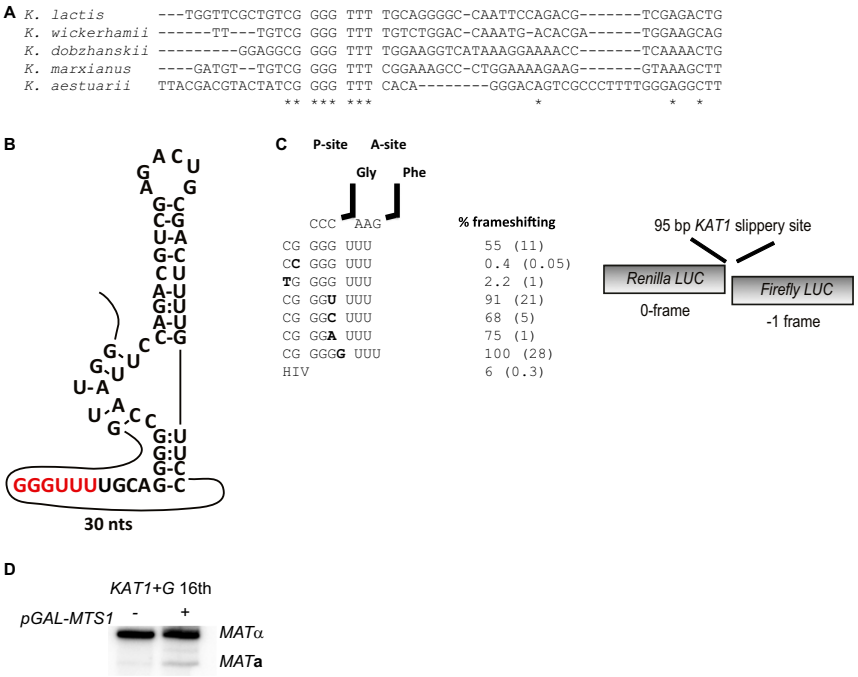

Fig. S2. Identification of a conserved slippery site and a pseudoknot and analysis of the frameshifting activity. (*A*) Multiple sequence alignment (ClustalW) using the DNA sequences from the overlaps between the two long *KAT1* ORFs from the species indicated on the left. Bases that are identical in all five species are marked by asterisks. For the slippery site, spaces indicate zero-frame codons. The sequences have been trimmed in their 3′ ends. (*B*) The KnotInFrame software (1) predicted the RNA structure shown with a free energy of −9.4 kcal/mol. Red bases represent the slippery site. (*C*) Frame-shifting activity analyzed using a dual-luciferase assay (2). A 95-bp sequence including the *KAT1* slippery site was cloned between the renilla and firefly *luciferase* genes. The mutations introduced are shown in bold letters. The results are the averages of three to five independent experiments; SDs from the means are shown in parentheses. (*D*) Plasmid alone or p*GAL-MTS1* was introduced into the *KAT1+G* strain from the 16th passage (Fig. 1*E*) and subjected to DNA blot analysis.

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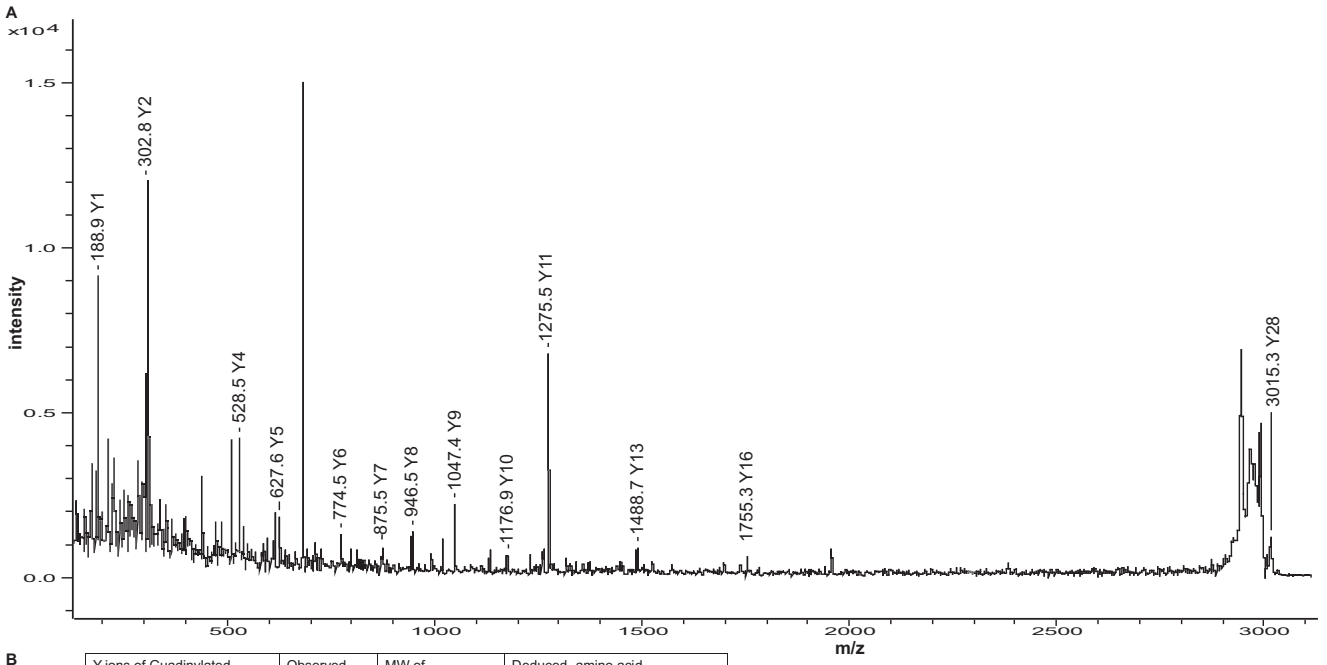
2. Harger JW, Dinman JD (2003) An in vivo dual-luciferase assay system for studying translational recoding in the yeast *Saccharomyces cerevisiae*. *RNA* 9(8):1019–1024.

A



B

Y ions of Guadinylated peptide of 2973,6 Daltons	Observed MW (Daltons)	MW of expected amino acid (s)	Deduced amino acid
Y1-Y2	113,9	114,1	Asn (N)
Y2-Y4	225,7	226,1	Glu+Pro (E+P)
Y4-Y5	99,1	99,1	Val (V)
Y5-Y6	146,9	147,1	Phe (F)
Y6-Y7	101	101	Thr (T)
Y7-Y8	71	71,1	Ala (A)
Y8-Y9	100,9	101	Thr (T)
Y9-Y10	129,5	129,1	Glu (E)
Y10-Y11	98,6	99,1	Val (V)
Y11-13	213,2	212,2	Asp+Pro (D+P)
Y13-Y16	266,6	267, 3	Ile+Pro+Gly or Leu+Pro+Gly (I+P+G or L+P+G)

GIHYGSLSGFLQ**GPIPDVETATFVPEN**K 2.97kDa

Fig. S3. MS/MS-analysis of the peptide covering the frameshift. (*A*) MS/MS analysis of the 2.97-kDa peptide obtained from the GST–Kat1 slippery site–MBP fusion protein. Before analysis, the peptide was derivatized with guanidine, resulting in the conversion of lysine residues to homo arginine, adding 42 Da in mass. The mass of the resulting Y ions is indicated. The *x* axis shows the molecular weight in daltons. (*B*) The table shows the observed mass of amino acids obtained by subtracting the mass of neighbor Y ions from each other. Also shown are the expected molecular weights of the predicted amino acids. The predicted peptide from the tandem-slippage hypothesis is shown next to the table; the confirmed protein sequence is shown in bold.

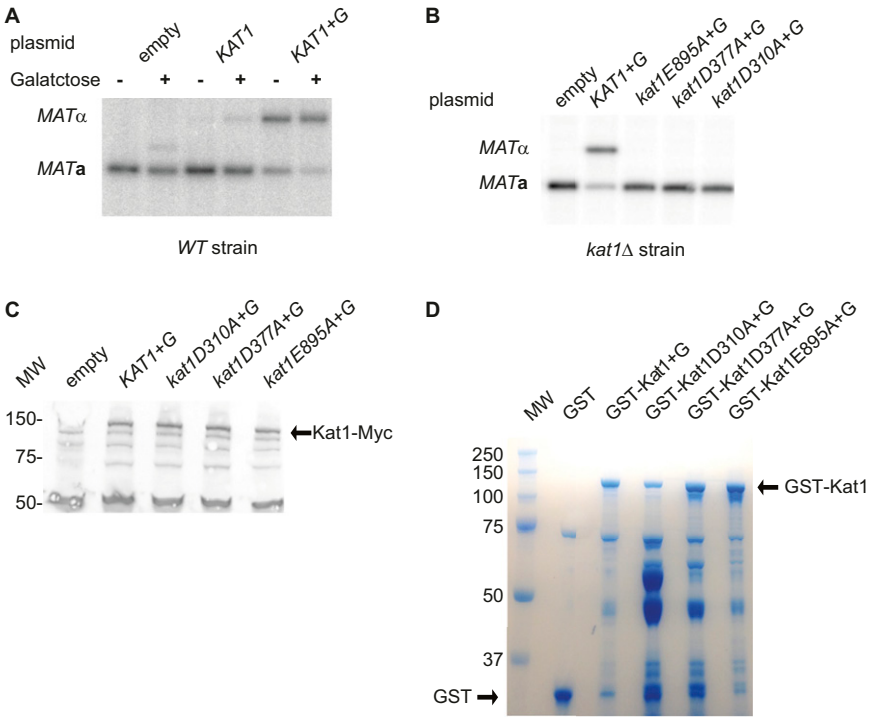


Fig. S4. Hyperactivity of Kat1+G and identification of the DDE motif in Kat1. (*A*) DNA blot analysis from a WT (SAY572) strain containing plasmid alone (empty) or plasmids overexpressing p*GAL–KAT1* or p*GAL–KAT1+G*, as indicated. Glucose (–) or galactose (+) was used as the carbon source. (*B*) DNA blot analysis from a *kat1*Δ strain (SAY1597) transformed with empty vector or plasmids expressing, Kat1+G, kat1D310A+G, kat1D377A+G, and kat1E895A+G, as indicated. (*C*) Protein blot analysis using protein extracts from SAY572 containing the indicated plasmids. An anti-Myc antibody (9E11) was used to detect expression of Myc-tagged Kat1 and DDE mutants. Molecular weight in kilodaltons is shown at the left; the Kat1–Myc bands are indicated at the right. (*D*) Coomassie-stained polyacrylamide gel showing expression of the indicated fusion proteins. GST alone and full-length GST–Kat1+G are indicated together with a molecular-weight marker.

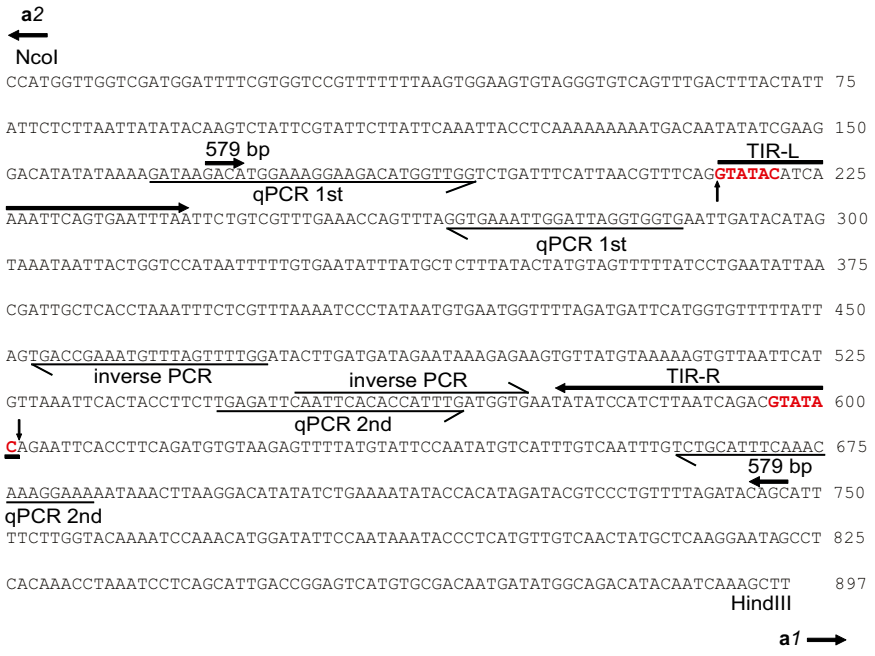


Fig. S5. DNA sequence of the *a1–a2* intergenic region. The DNA sequence of the region between the NcoI–HindIII sites in the a*1*–a*2* intergenic region is shown. The boundaries of the 579-bp fragment used as a substrate for Kat1 in vitro are shown as short arrows. The TIRs are depicted by long arrows. The AccI sites, deleted in the *cut1*Δ and *cut2*Δ mutants, are colored in red. Short vertical arrows indicate the suggested sites cleaved by Kat1. Primers used for ChIP followed by qPCR and for inverse PCR are indicated by harpoons. See Table S2 for exact sequences.

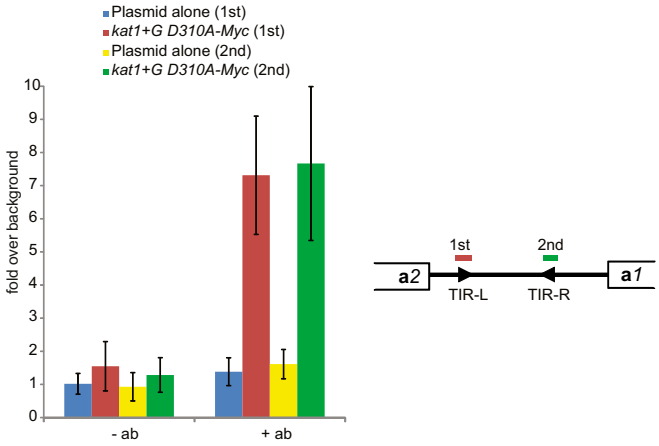


Fig. S6. Kat1 binds the *MAT*a locus in vivo. ChIP was performed from strain SAY572 containing empty plasmid or p*GAL–Myc–kat1D310A+G* using an anti-Myc antiserum (9E11). A catalytically inactive form of Kat1+G was used because expression of the active protein resulted in switching to the *MAT*α genotype. After ChIP, DNA associated with the *MAT***a***1–MAT***a***2* intergenic region was analyzed by qPCR using two specific pairs of primers (Table S2) indicated by colored bars in the schematic diagram of the locus. The two primer pairs are only about 500 bp apart and therefore may amplify the same precipitated DNA fragment. Mock immunoprecipitations, in which the 9E11 antiserum was omitted (no antibody) are included. The *y* axis is enriched relative to the control locus. Error bars represent the SEM from three independent experiments.

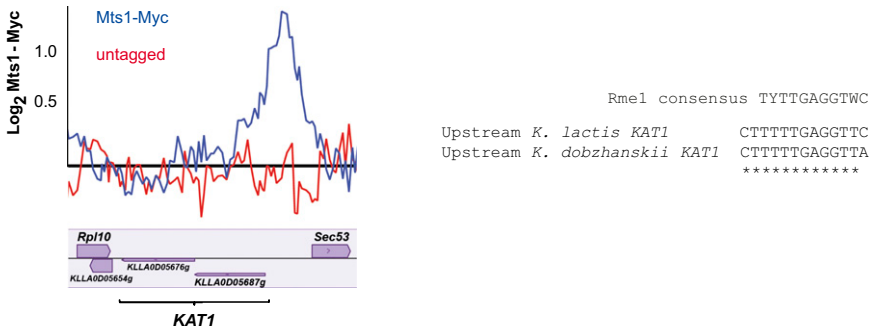


Fig. S7. A putative Kat1 consensus site and binding of Mts1 to the *KAT1* regulatory region. (*Left*) An *MTS1–Myc* strain was subjected to ChIP-on-chip analysis (1) and analyzed using MochiView software (Johnson Lab). The *y* axis shows the $\log_2$ (fold enrichment) ratio of the *MTS1–Myc* strain to the input (blue) and an untagged control strain (red). The genomic location is shown on the *x* axis. (*Right*) A putative Mts1-binding site in the *KAT1* regulatory region in *K. lactis* and *K. dobzhanskii* with the consensus *S. cerevisiae* Rme1-binding site on top.

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Table S1. Yeast strains used in this study

Strain	Genotype	Source or reference
SAY172	*MAT***a** *RAD5+* W303 (*S. cerevisiae*)	P. Kaufman*
SAY1476	*MATα ade1 leu2 trp1 metA1 or META1 uraA1*	S.U.Å. laboratory
SAY528	*MAT***a** *leu2 lysA1 metA1 nej1Δ::hisG trp1 uraA1*	S.U.Å. laboratory
SAY559	*MAT***a** *leu2 lysA1 metA1 mre11Δ::KanMX trp1 uraA1*	(1)
SAY572	*MAT***a** *leu2 lysA1 metA1 nej1Δ::LEU2 trp1 uraA1*	(1)
SAY752	*MAT***a** *leu2 lysA1 metA1 or META1 mts1Δ::LEU2 trp1 uraA1*	(2)
SAY1488	*MAT***a***::URA3 leu2 lysA1 metA1 nej1Δ::LEU2 trp1 uraA1*	(3)
SAY1489	*MAT***a** *HMR***a***::URA3 lysA1 leu2 metA1 nej1Δ::LEU2 trp1 uraA1*	(3)
SAY1490	*MAT***a***::URA3 leu2 lysA1 metA1 trp1 uraA1*	(3)
SAY1597	*MAT***a** *kat1Δ::NAT* (*KLLA0D488864-491819*) *lysA1 leu2 metA1 nej1Δ::LEU2 trp1 uraA1*	This study
SAY1623	*MATα ade1 kat1Δ::NAT leu2 metA1 or META1 trp1 uraA1*	This study
SAY1628	*MAT***a***::URA3 kat1Δ::NAT leu2 lysA1 metA1 or META1 trp1 uraA1*	This study
SAY1639	*MAT***a** *kat1Δ::NAT leu2 lysA1 metA1 or META1 mre11Δ::KanMX trp1 uraA1*	This study
SAY1746	*MAT***a** *KAT1+G leu2 lysA1 metA1 nej1Δ::LEU2 trp1 uraA1*	This study
SAY1871	*MAT***a***::URA3-cut2Δ lysA1 trp1 leu2 metA1 uraA1 nej1::LEU2*	This study
SAY1897	*MAT***a***::URA3-cut1, 2Δ lysA1 trp1 leu2 metA1 uraA1 nej1::hisG*	This study
SAY1899	*MAT***a***::URA3-cut1Δ lysA1 trp1 leu2 metA1 uraA1 nej1::hisG*	This study
SAY1900	*MAT***a***::URA3-cut2Δ lysA1 trp1 leu2 metA1 uraA1*	This study

*University of Massachusetts Medical School, Worcester, MA.

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3. Barsoum E, Rajaei N, Aström SU (2011) RAS/cyclic AMP and transcription factor Msn2 regulate mating and mating-type switching in the yeast *Kluyveromyces lactis*. *Eukaryot Cell* 10(11): 1545–1552.

Table S2. Primers used for generation of probes, ChIP, RT-qPCR, and deletion of *KAT1*

Primer name	Sequence	Locus	Application
Naghmeh21	TGCATGTCTGGGTTTGACAT	*KAT1* ORF 5′ part	RT-qPCR + control primer for ChIP
Naghmeh22	TAGAACTGGGCTCGGTGACT	*KAT1* ORF 5′ part	RT-qPCR + control primer for ChIP
Naghmeh25	AAACGGGATGGACACAACAT	*KAT1* ORF 3′ part	RT-qPCR
Naghmeh26	TTTGGTCATCGATGGTTTCA	*KAT1* ORF 3′ part	RT-qPCR
Naghmeh121	GATAAGACATGGAAAGGAAGACATGG	*MAT***a***1-a2* intergenic region	ChIP for TIR-L
Naghmeh122	CACCACCTAATCCAATTTCACC	*MAT***a***1-a2* intergenic region	ChIP for TIR-L
Naghmeh142	TGAGATTCAATTCACACCATTTG	*MAT***a***1-a2* intergenic region	ChIP for TIR-R
Naghmeh143	TTTCCTTTGTTTGAAATGCAGA	*MAT***a***1-a2* intergenic region	ChIP for TIR-R
Naghmeh139	CCAAAACTAAACATTTCGGTCA	*MAT***a***1-a2* intergenic region	Inverse PCR
EBO211	CAATTCACACCATTTGATGGTG	*MAT***a***1-a2* intergenic region	Inverse PCR
AKO155	GTCTGGATGTGTTAAATTGTTGG	Flanking *MAT***a** and *MATα*	DNA blot/*MAT*
AKO156	CTGAAATGTAGAACATCCAACTTTG	Flanking *MAT***a** and *MATα*	DNA blot/*MAT*
EBO104	GATCGGATCCGACTCCGGTCAATGCTGAGG	*MAT***a***1-a2* intergenic region	DNA blot /**a***1-a2*
EBO210	GGTTGGTCGATGGATTTTCGTGG	*MAT***a***1-a2* intergenic region	DNA blot /**a***1-a2*
Jiang254	TTACAATCATACGTAAATAATACATATAGATCCGCTT-GGCAAAAACTAGGCGGATCCCCGGGTTAATTAA	Upstream of *KAT1* + *NATMX*	Deletion of *KAT1*
Jiang255	TTTTACAGGGTTTTTTCAATACTAAGTCTGTGACGCT-GCCAGAATAAATGTCATCGATGAATTCGAGCTC	Downstream of *KAT1* + *NATMX*	Deletion of *KAT1*