Supporting Information

Kleinschmidt et al. 10.1073/pnas.1019175108

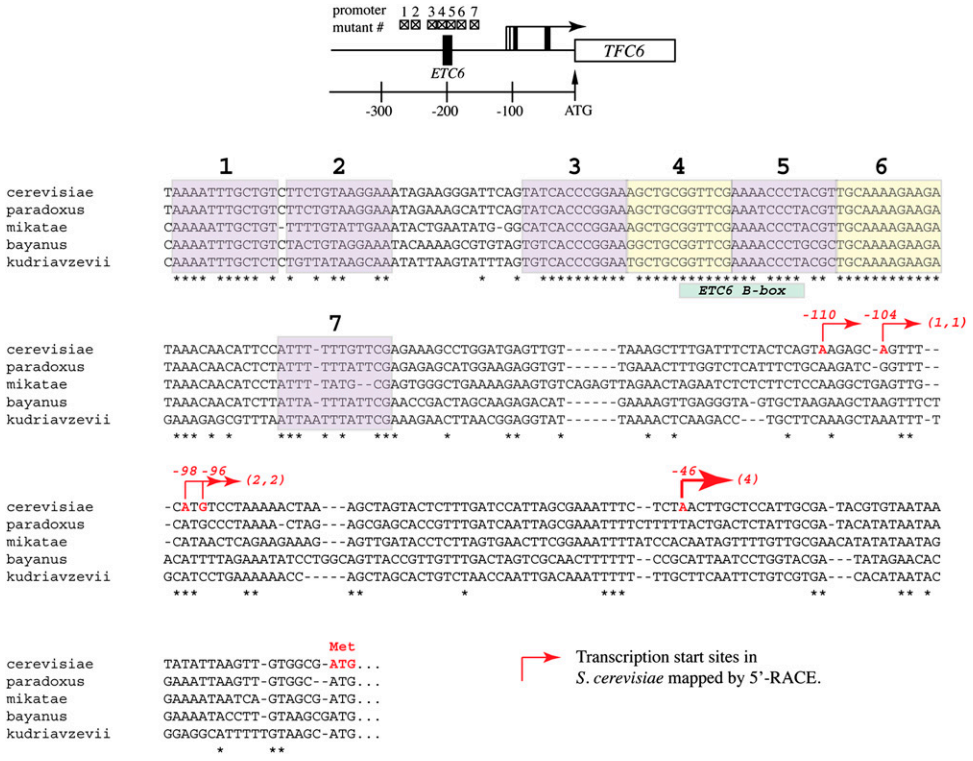

Fig. S1. Comparisons of the *TFC6* promoter region in budding yeast species. Sequences were aligned using Clustal software available through the *Saccharomyces* Genome Database website. Shaded boxes covering regions of highest homology were designated as sites 1–7 and were mutated as described in *Materials and Methods*; mutagenic oligonucleotides are listed in Table S3. Transcription start sites marked in red were determined by 5′-RACE analysis. The numbers above each arrow refer to the location of the start site relative to the start codon (Met), and the numbers in parentheses are the number of independent 5′-RACE clones obtained for that start site. The region of the *ETC6* B-box is marked by the green shading. Promoter mutants were constructed by site-directed mutagenesis of plasmid pDD1098, which contains the *TFC6-ESC2* intergenic region (from *Saccharomyces* Genome Database chromosome IV coordinates 1,198,718–1,199,357; http://www.yeastgenome.org/; accessed October 20, 2010) cloned into Bluescript SK+. Each mutant had a 12-bp region replaced with a DrdI restriction enzyme site (Quik-Change kit, Stratagene), which allowed scrambling of 10–12 bp within each region and initial restriction digest screening of mutants, which were verified by DNA sequencing.

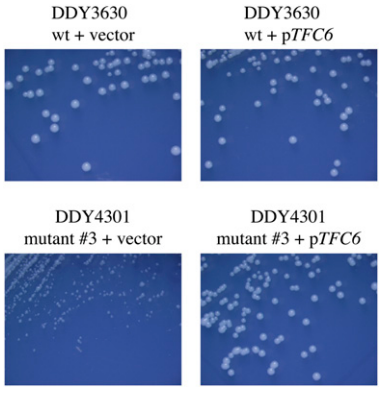

Fig. S2. *TFC6* promoter mutant 3 exhibits a slow growth phenotype due to insufficient *TFC6* expression. Wild-type (DDY3630) and promoter mutant 3 (DDY4301) strains were transformed with either *HIS3* vector (pRS413) or the same vector containing the *TFC6* gene (pDD1179) and streaked onto minimal media lacking histidine. The slow growth phenotype of strain DDY4301 is complemented by the *TFC6*-expressing plasmid. Identical complementation results were obtained using two independent promoter mutant 3 isolates and also strain DDY4114 (described in ref. 1), which has the *ETC6* site and the upstream region deleted.

1. Simms TA, et al. (2008) TFIIIC binding sites function as both heterochromatin barriers and chromatin insulators in Saccharomyces cerevisiae. *Eukaryot Cell* 7:2078–2086.

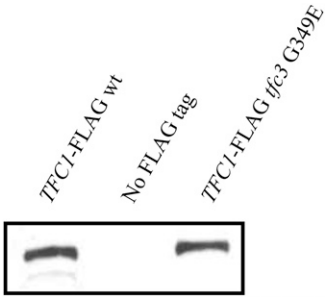

Fig. S3. Tfc1p levels are not significantly affected by the *tfc3*-G349E temperature-sensitive mutation. Whole-cell extracts from *TFC1*-3×FLAG strains were prepared, and equivalent protein amounts were immunoprecipitated with anti-FLAG antibody. The immunoprecipitates were subjected to Western blot analysis with anti-FLAG primary antibody. No significant difference was seen in the wild-type versus *tfc3*-G349E mutant.

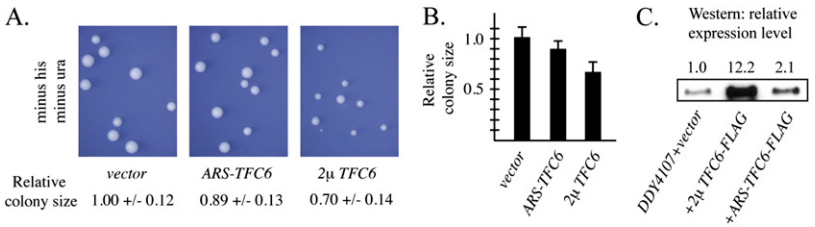

Fig. S4. Tfc6p inhibition of its own promoter is dose-dependent. (*A*) Strain DDY4403 containing the *TFC6* promoter driving *URA3* was transformed with empty *HIS3* vector, low-copy *ARS-CEN-TFC6* plasmid, or high-copy 2μ-*TFC6* plasmid. Platings and colony size determinations were as in Fig. 3 and as described in *Materials and Methods*. (*B*) Bar graph represents the measurement of at least 30 colonies from three separate images. (*C*) Western blot analysis to estimate the relative level of overexpression of *TFC6*. The same plasmids used in *A* and *B* were modified to contain the identical triple FLAG epitope that is integrated as a single copy in the haploid strain DDY4107 (pDD1244 = 2μ *TFC6*-3×FLAG and pDD1245 = ARS-CEN *TFC6*-3×FLAG). DDY4107 was then transformed either with empty *HIS3* vector (pRS413) to measure normal Tfc6p levels or with each of the *TFC6*-FLAG plasmids. Total protein extracts from each strain were prepared and equal protein amounts were immunoprecipitated with anti-FLAG M2 monoclonal antibody (Sigma), and the concentrated immunoprecipitates were analyzed by Western blotting using the same antibody. Relative amounts were assessed by scanning the blot on a Pharmacia Typhoon 8600 PhosphorImager on the chemiluminescent setting and by using ImageQuant software to measure the relative signals.

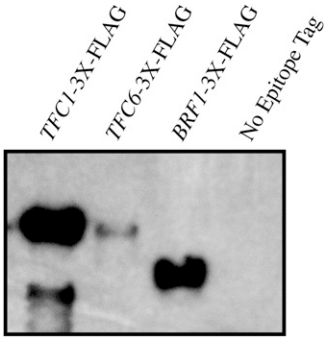

Fig. S5. Western blot analysis of yeast strains containing identical carboxyl-terminal triple-FLAG epitope tags on *TFC1* (DDY4381), *TFC6* (DDY4107), or *BRF1* (DDY844). Total protein extracts from each strain were prepared, and equal protein amounts were immunoprecipitated with anti-FLAG M2 monoclonal antibody (Sigma), and the concentrated immunoprecipitates were analyzed by Western blotting using the same antibody. Tfc6 protein levels are limiting compared with Tfc1p and Brf1p levels.

Table S1. Yeast strains

Strain number	Genotype	Source
DDY3	*MAT***a** *ADE2 his3-11 leu2-3,112 lys2Δ trp1-1 ura3-1*	Donze laboratory
DDY232	*MATα ADE2 his-3–11 leu2-3,112 LYS2 trp1-1 ura3-1 rpc31-236 hmr*Δ	Donze laboratory
DDY237	*MATα ADE2 his-3–11 leu2-3,112 LYS2 trp1-1 ura3-1 tfc3-G349E hmr*Δ	Donze laboratory
DDY246	*MATα ade2-1 his-3–11 leu2-3,112 LYS2 trp1-1 ura3-1 rpc160-Δ1::HIS3 p-rpc160-112*	Donze laboratory
DDY247	*MAT***a** *ade2-1 his-3–11 leu2-3,112 LYS2 trp1-1 ura3-1 rpc160-Δ1::HIS3 p-rpc160-112*	Donze laboratory
DDY261	*MATα ADE2 his-3–11 leu2-3,112 LYS2 trp1-1 ura3-1 tfc3-G349E hmr*ΔI	Donze laboratory
DDY269	*MATα ADE2 his-3–11 leu2-3,112 lys2Δ trp1-1 ura3-1 rpc31-236 hmr*ΔI	Donze laboratory
DDY416	*MAT***a** *ADE2 his-3–11 leu2-3,112 lys2Δ trp1-1 ura3-1 brf1Δ::HIS3 p-brf1 II.9 hmr*Δ	Donze laboratory
DDY420	*MATα ADE2 his-3–11 leu2-3,112 lys2Δ trp1-1 ura3-1 brf1Δ::HIS3 p-brf1 II.6 hmr*Δ	Donze laboratory
DDY844	*MATα ADE2 his4-519 leu2-3,112 LYS2 trp1-1 ura3-52 BRF1-3×FLAG-KanMX*	Donze laboratory
DDY3453	*MAT***a** *ADE2 his3-11 leu2-3,112 lys2Δ trp1-1 ura3-1 etc6Δ::URA3*	Donze laboratory
DDY4107	*MAT***a** *ADE2 his3-11 leu2-3,112 lys2Δ trp1-1 ura3-1 TFC6:3×FLAG:KanMX*	Donze laboratory
DDY4274	*MAT***a** *ADE2 his3-11 leu2-3,112 lys2Δ trp1-1 ura3-1 tfc6* promoter mutant 1	This study
DDY4297	*MAT***a** *ADE2 his3-11 leu2-3,112 lys2Δ trp1-1 ura3-1 tfc6* promoter mutant 2	This study
DDY4300	*MAT***a** *ADE2 his3-11 leu2-3,112 lys2Δ trp1-1 ura3-1 tfc6* promoter mutant 3	This study
DDY4304	*MAT***a** *ADE2 his3-11 leu2-3,112 lys2Δ trp1-1 ura3-1 tfc6* promoter mutant 4	This study
DDY4306	*MAT***a** *ADE2 his3-11 leu2-3,112 lys2Δ trp1-1 ura3-1 tfc6* promoter mutant 5	This study
DDY4309	*MAT***a** *ADE2 his3-11 leu2-3,112 lys2Δ trp1-1 ura3-1 tfc6* promoter mutant 6	This study
DDY4312	*MAT***a** *ADE2 his3-11 leu2-3,112 lys2Δ trp1-1 ura3-1 tfc6* promoter mutant 7	This study
DDY4077	*MAT***a** *ADE2 his3-11 leu2-3, 112 LYS2 trp1-1 ura3-1 etc6 boxB TFC1-3×FLAG::KanMX*	This study
DDY4376	*MAT***a** *ADE2 his3-11 leu2-3, 112 lys2Δ trp1-1 ura3-1 tfc3-G349E TFC1-3×FLAG::KanMX*	This study
DDY4381	*MATα ADE2 his3-11 leu2-3, 112 lys2Δ trp1-1 ura3-1 TFC1-3×FLAG::KanMX*	This study
DDY4403	*MATα/MAT***a** *ADE2/ADE2 his3Δ1/his3Δ1 leu2Δ0/leu2Δ0 LYS2/lys2Δ0 met15Δ0/MET15 TRP1/TRP1 ura3Δ0/ura3Δ0 TFC6/tfc6Δ::URA3*	This study
DDY4520	*MATα/MAT***a** *ade2-1/ADE2 his3-11/his3-11 leu2-3,112/leu2-3, 112 LYS2/lys2Δ trp1-1/trp1-1 ura3-1/ura3-1 TFC6/tfc6Δ::URA3*	This study
DDY4521	*MATα/MAT***a** *ade2-1/ADE2 his3-11/his3-11 leu2-3,112/leu2-3, 112 LYS2/lys2Δ trp1-1/trp1-1 ura3-1/ura3-1 TFC6/etc6 b-box-tfc6Δ::URA3*	This study

All strains are isogenic to *S. cerevisiae* W-303 except for DDY4403, which is in the S288C background (BY4743 parent). Most experiments were confirmed with at least three independent isolates, and only those isolates depicted in each of the figures are listed. Strains 4403, 4520, and 4521 contain the *URA3* ORF expressed from the *TFC6* promoter as described in the main text and *Materials and Methods*.

Table S2. Plasmids

		Source
pRS406	*URA3* vector	1
pRS413	ARS-CEN *HIS3* vector	1
pRS423	2μ *HIS3* vector	2
pDD1098	*TFC6-ESC2* intergenic region (~640 bp) in Bluescript SK+	3
pDD1179	*TFC6* in pRS413, ARS-CEN *HIS3*	This study
pDD1184	*TFC6* promoter mutant 1	This study
pDD1185	*TFC6* promoter mutant 2	This study
pDD1186	*TFC6* promoter mutant 3	This study
pDD1187	*TFC6* promoter mutant 4	This study
pDD1188	*TFC6* promoter mutant 5	This study
pDD1189	*TFC6* promoter mutant 6	This study
pDD1190	*TFC6* promoter mutant 7	This study
pDD1197	*TFC6* in pRS423, 2μ *HIS3*	This study
pDD1221	*TFC4* in pRS423, 2μ *HIS3*	This study
pDD1226	*TFC1* in pRS423, 2μ *HIS3*	This study
pDD1228	*TFC3* in pRS423, 2μ *HIS3*	This study
pDD1234	*ADH1* promoter-*TFC6* in pRS413, ARS-CEN *HIS3*	This study
pDD1244	*TFC6*-3×FLAG in pRS423, 2μ *HIS3*	This study
pDD1245	*TFC6*-3×FLAG in pRS413, ARS-CEN *HIS3*	This study

1. Sikorski RS, Hieter P (1989) A system of shuttle vectors and yeast host strains designed for efficient manipulation of DNA in Saccharomyces cerevisiae. *Genetics* 122:19–27.
2. Christianson TW, Sikorski RS, Dante M, Shero JH, Hieter P (1992) Multifunctional yeast high-copy-number shuttle vectors. *Gene* 110:119–122.
3. Simms TA, et al. (2008) TFIIIC binding sites function as both heterochromatin barriers and chromatin insulators in Saccharomyces cerevisiae. *Eukaryot Cell* 7:2078–2086.

Table S3. Oligonucleotides

TFC6 promoter mutagenesis*

DDO-1135	CATTTCTTTTCTTATATGCTACAAT*GACCGGGTAGTC*CTTCTG TAAGGAAATAGAAGG	TFC6 promoter mutant 1 top
DDO-1136	CCTTCTATTTCCTTACAGAAG*GACTACCCGGTC*ATTGTA GCATATAAGAAAAGAAATG	TFC6 promoter mutant 1 bottom
DDO-1137	GCTACAATAAAATTTGCTGTC*GACGTGCCTGTC*ATAGAA GGGATTCAGTATCACC	TFC6 promoter mutant 2 top
DDO-1138	GGTGATACTGAATCCCTTCTAT*GACAGGCACGTC*GA CAGCAAATTTTATTGTAGC	TFC6 promoter mutant 2 bottom
DDO-1139	GTAAGGAAATAGAAGGGATTCAG*GACACAAATGTC*AGCT GCGGTTCGAAAACCCTAC	TFC6 promoter mutant 3 top
DDO-1140	GTAGGGTTTTCGAACCGCAGCT*GACATTTGTGTC*CTGAA TCCCTTCTATTTCCTTAC	TFC6 promoter mutant 3 bottom
DDO-1141	GGATTCAGTATCACCCGGAA*GACGTATTGGTC*AAAACC CTACGTTGCAAAAGAAG	TFC6 promoter mutant 4 top
DDO-1142	CTTCTTTTGCAACGTAGGGTTTT*GACCAATACGT* *C*TTCCGGGTGATACTGAATCC	TFC6 promoter mutant 4 bottom
DDO-1143	CACCCGGAAAGCTGCGGTTCG*GACCAAAGCGTC*TGC AAAAGAAGATAAACAACATTC	TFC6 promoter mutant 5 top
DDO-1144	GAATGTTGTTTATCTTCTTTTGCA*GACGCTTTGGTC*CGA ACCGCAGCTTTCCGGGTG	TFC6 promoter mutant 5 bottom
DDO-1145	CGGTTCGAAAACCCTACGT*GACCCCCTCGTC*TAAAC AACATTCCATTTTTTGTTCG	TFC6 promoter mutant 6 top
DDO-1146	CGAACAAAAAATGGAATGTTGTTTA*GACGAGGGGGT* *C*ACGTAGGGTTTTCGAACCG	TFC6 promoter mutant 6 bottom
DDO-1147	GCAAAAGAAGATAAACAACATTCC*GACTTTTGTGTC*A GAAAGCCTGGATGAGTTG	TFC6 promoter mutant 7 top
DDO-1148	CAACTCATCCAGGCTTTCT*GACACAAAAGTC*GGAATG TTGTTTATCTTCTTTTGC	TFC6 promoter mutant 7 bottom

Chromatin immunoprecipitation

DDO-305	CGTGCCGGTGAAACATATATGTCT	Chromosome XVI tF(GAA)P2 ChIP top
DDO-306	CAAGTTCAAGAACCAACTTTCCGC	Chromosome XVI tF(GAA)P2 ChIP bottom
DDO-307	GCACTAGTTGATTCTTGTTCCAACAG	Chromosome VII tK(CUU)G1 ChIP top
DDO-308	CCGTTTTTCCCCAGAGCACTTTTA	Chromosome VII tK(CUU)G1 ChIP bottom
DDO-705	ATTATTACACGTATCGCAATGG	ETC6 ChIP top
DDO-1093	CTTCTGGAATCACCGGTCATC	ETC6 ChIP bottom
DDO-1023	CCATATACATATCCATATCTAATC	GAL LOCUS ChIP top
DDO-1024	ATAACCATAAAAGCTAGTATTGTAG	GAL LOCUS ChIP bottom
DDO-1215	GCCATTCTCTTATCTTCCAAG	ETC4 ChIP top
DDO-698	AAGTAAGGTTTGCATATGCGG	ETC4 ChIP bottom
DDO-1343	GTCTTAGTTTGATTGAGCGACAAG	ETC5/RNA170 ChIP top
DDO-1344	AAACAAGGGTTGTGGAGTATGC	ETC5/RNA170 ChIP bottom
DDO-1345	TAGCAGTTTATGTACGCATTTTAAAAGC	ZOD1 ChIP top
DDO-1346	TGCTGTCTTATTCCCTAGTGTC	ZOD1 ChIP bottom
DDO-1402	TACGACATCAAAGTCGCCGAG	Chromosome XII tR(CCG)L ChIP top
DDO-1403	ATTGACAGCCCTTACGCGAAG	Chromosome XII tR(CCG)L ChIP bottom

Knockout and insertion†

DDO-792	CAACTCATCCAGGCTTTCTCGAACAAAAAATGGAATGTTGTTTATC TTCTTTT**GCAGATTGTACTGAGAGTG**	ETC6 delete URA3 KO TOP
DDO-793	ATTTGCTGTCTTCTGTAAGGAAATAGAAGGGATTCAGTATCACCCG GAAAG**CTCTCCTTACGCATCTGTGCGG**	ETC6 delete URA3 KO BOTTOM
DDO-1201	CTCTAACTTGCTCCATTGCGATACGTGTAATAATATATTAAGTT GTGGCGATGTCGAAAGCTACATATAAG	TFC6 del URA3 ORF top
DDO-1202	GTCAACAATAGTTCAATGTCACAAATTGTATTTATTACGTAA AGTCCATCTTAGTTTTGCTGGCCGCATC	TFC6 del URA3 ORF bottom

ChIP, chromatin immunoprecipitation.

*Bases in italic correspond to the DrdI site described in *Materials and Methods*.

†Bases in boldface correspond to the pRS416 plasmid sequence adjacent to *URA3*. Underlined bases correspond to the start and end of the *URA3* ORF.