Supplemental Material for the Submission

Cell cycle dependent transcription factors control the expression of yeast telomerase RNA.

Isabelle Dionne, Stéphanie Larose, Alain T. Dandjinou, Sherif Abou Elela and Raymund J. Wellinger

Supplemental Figure Legends

Supplemental Figure S1. Alignment of the *TLC1* promoter region obtained from different *Saccharomyces* "sensu stricto" species. The sequence from *S. cerevisiae* S288C (Scec), *S. cerevisiae* SK1 (Scek), *S. paradoxus* (Spar), *S. cariocanus* (Scar), *S. mikatae* (Smik), *S. kudriavzevii* (Skud), *S. bayanus* (Sbay) and *S. pastorianus* (Spas) were aligned according to the major 5'-end of Scec as indicated by +1. The numbering is indicated by vertical lines each 10 nucleotides according to Scec and dashes (-) denote gaps introduced to improve the alignment. Dashed underline nucleotides represent the coding sequence of *TLC1* (from +1 and downstream), *snR161* (-252 to -412) and *PDX3* (from -642 and upstream). The positions of the different TATA box-like sequence are indicated in italic bold (-75, -171 and -221). The positions of the MCB1/SCB1 (-108) and MCB2 (-120) are indicated in bold underlined. The consensus sequence (Cons) is shown at bottom with the uppercase, lower case and dots representing universally conserved, conserved (~ 75%) or variable nucleotides respectively. The conserved regions examined in this study are indicated by letters (A to G). The percent conservation is indicated below each region in brackets.

Supplemental Figure S2. (A) Schematic representation of the *TLC1* chromosomal locus and the flanking upstream genes. The start codon of *PDX3* as well as the 5'-ends of the mature *snR161* and *TLC1* RNAs are represented by arrows indicating the transcription direction. The identity of the conserved sequence motifs studied is indicated by letters. Some regions between the *PDX3* and *TLC1* genes are magnified at bottom. The position of the different 5'-ends of TLC1 detected by primer extension performed in B is indicated by arrowheads and the name of the deletion is indicated on the left. Stars (*) represent 5'-ends detected for specific deletions and the numbers indicated are relative to each deletion respectively and not to TLC1 WT 5'-end. **(B)** Primer extension analysis of TLC1 RNA derived from strains carrying different promoter deletions. The 5'-end was mapped using primer specific to TLC1 sequence. DNA sequencing of a cloned *TLC1* gene was performed with the same primer and included on the left as a marker. Black arrowhead (+1) represents the major TLC1 5'-end while white arrowheads represent minor 5'-ends.

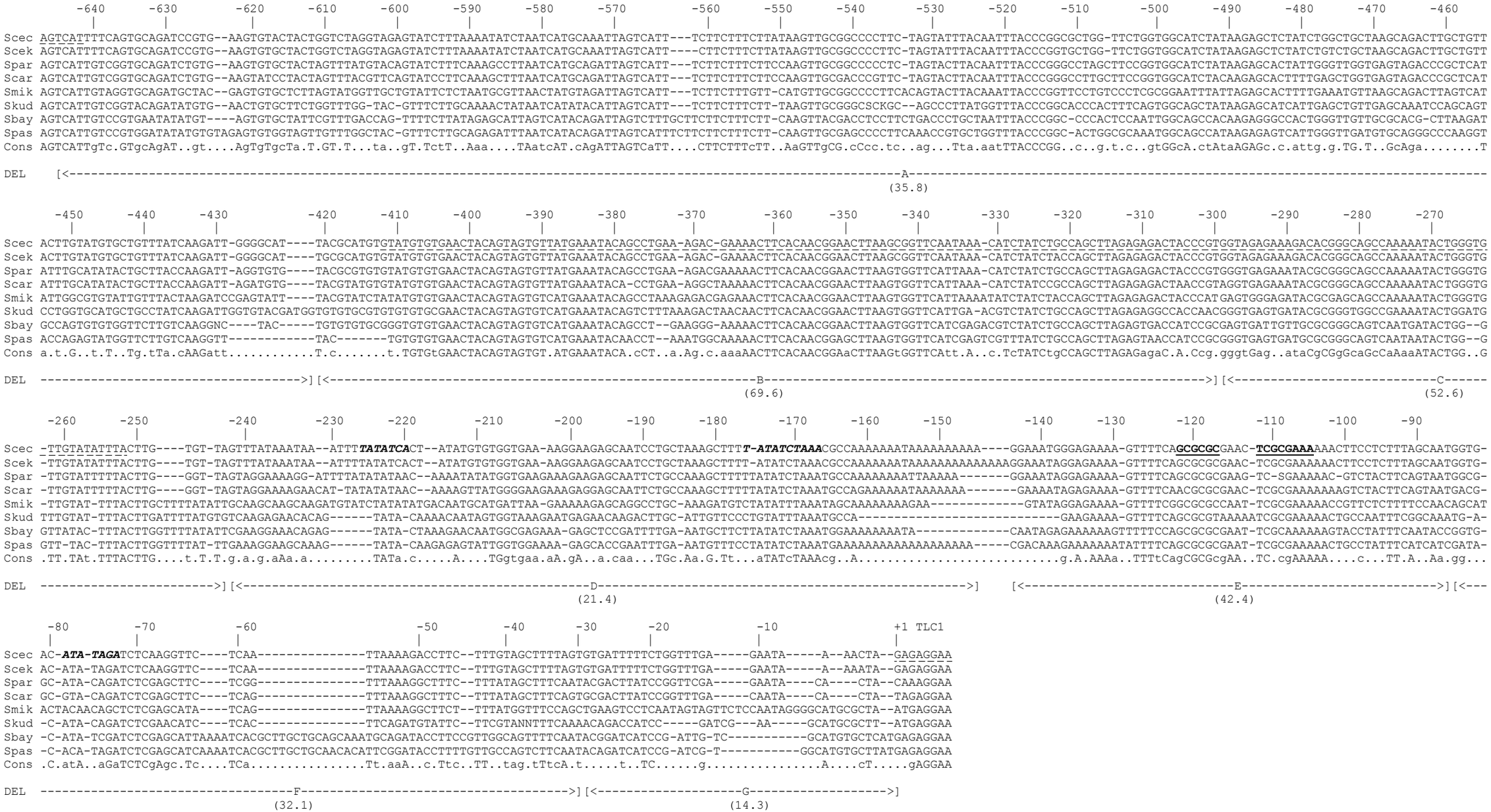
Supplemental Figure S3. RNA samples from strain BY4741 (WT), BY4741-swi4Δ and BY4741-swi6Δ were analyzed by qRT-PCR. The TLC1 RNA levels were measured and normalized against Act1. The WT value is arbitrarily set to 1.

Supplemental Figure S4. qRT-PCR analysis of Cln2 RNA levels at indicated time points for the same experiments as shown in Figure 5D. *CLN2* is a well-known cell cycle-regulated gene by SBF and serves here as a positive control for the synchronization and activation of the transcription. Average values of three independent biological replicates normalized against Act1 with standard deviation are shown as fold change over t = 0 (G1).

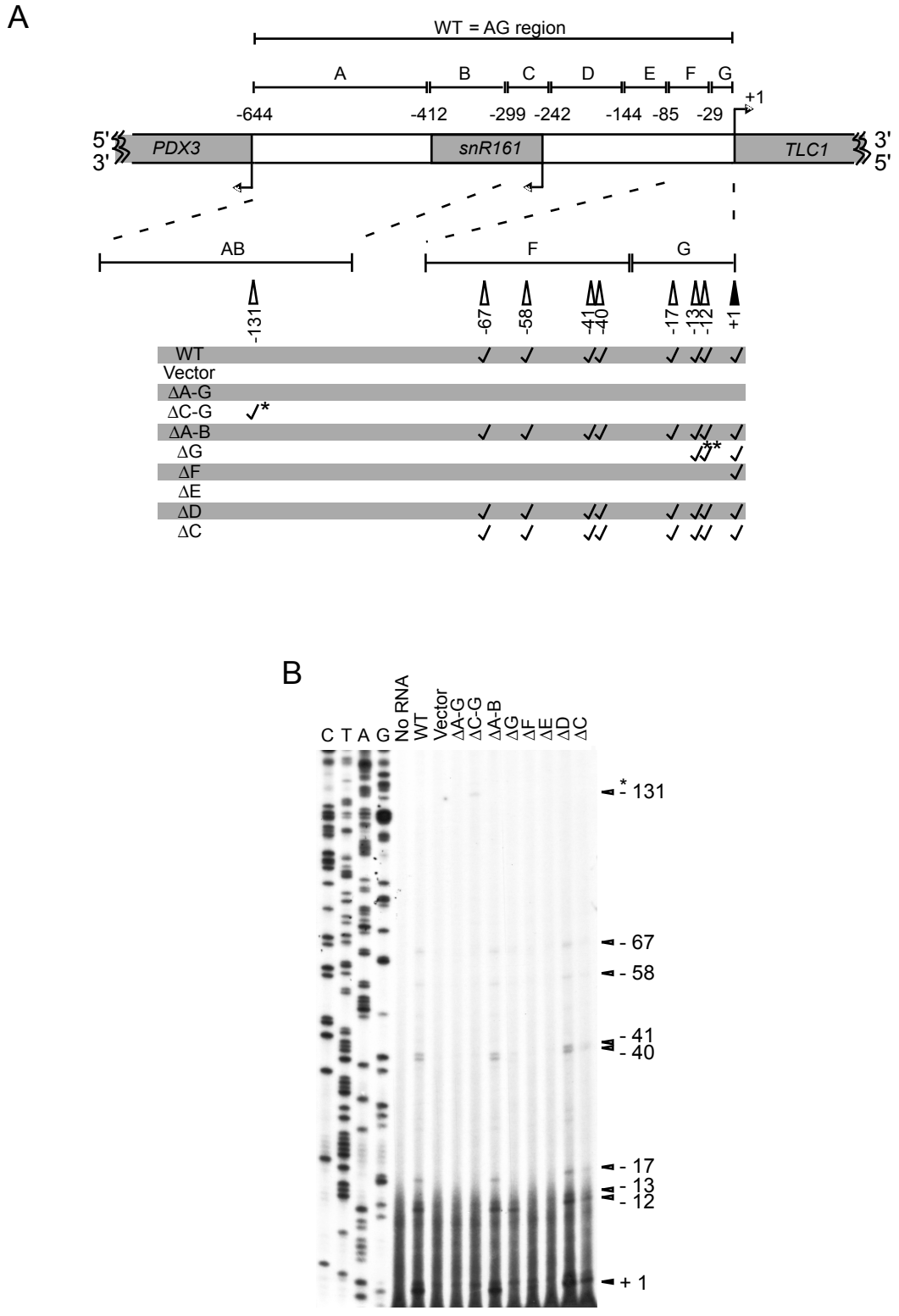
Table S1. Primers used in this study

Name	Sequence (5'→ 3')
SPE-931TLC1FWD[a]	GGGTACACTAGTAGCCTTTCTAGAGGTTCC (SpeI)
SPE-298TLC1FWD[a]	GGGTACACTAGTGGTAGAGAAAGACACGG (SpeI)
SPE+1TLC1FWD[a]	GGGTACACTAGTAGAGAGGAAGATAGGTACC (SpeI)
-6TLC1FWD[a]	GAGAGGAAGATAGGTACC
-35TLC1FWD[a]	GTGATTTTTCTGGTTTGAG
-91TLC1FWD[a]	GGTGACATATAGATCTCAAGG
-150TLC1FWD[a]	GGAAATGGGAGAAAAGTTTTC
-248TLC1FWD[a]	GTTTATAAATAAATTTTATATCAC
-305-224TLC1REV[a]	**GTGATATAAAATTTATTTATAAAC**ACGGGTAGTCTCTCTAAG
-298+20TLC1REV[a]	**GGGTACCTATCTTCCTCTCT**ACGGGTAGTCTCTCTAAG
-241-122TLC1REV[a]	**AACTTTTCTCCCATTTCC**TAACACAAGTAAATATACAACACCC
-150-70TLC1REV[a]	**CCTTGAGATCTATATGTCACC**TTTTTTTTTTATTTTTTTGGCG
-87-7TLC1REV[a]	**TTCTCAAACCAGAAAAATCAC**ATTGCTAAAGAGGAAGTTTTTTCGCG
-28+20TLC1REV[a]	**GGGTACCTATCTTCCTCTCT**ACTAAAAGCTACAAAGAAGGTC
+1502TLC1ECOREV[a]	AACAGAATTCGGGAAGGTAAATACCACC (EcoRI)
ADH1 TERM FWD[a]	CCGGAATTCGAGCTCAAGCTTTGGACTTCTTCGCC
ADH1 TERM REV[a]	ATCCGCGGACTAGTGGCCGGTAGAGGTGTGG
TLC1-A-FOR[b]	CATTTTCAGTGCAGATCCGTGA
TLC1-B-REV[b]	**CAT**ACGGGTAGTCTCTCTAAGCTGG
TLC1-C-FOR[b]	GGTAGAGAAAGACACGGGCAGC
TLC1-G-REV[b]	**CAT**TAGTTTTATTCTCAAACCAGAA
TLC1-D-FOR[b]	GTTTATAAATAAATTTTATATC
TLC1-E-FOR[b]	GGAAATGGGAGAAAAGTTTTCA
TLC1-F-FOR[b]	GGTGACATATAGATCTCAAGGT
TLC1-EFA[b, d]	GGAAATGGGAGAAAAGTTTTCAGCGCGCGAACTCGCGAAAAAACTTCC TCTTTAGCAATATG
TLC1-ERA[b, d]	**CAT**ATTGCTAAAGAGGAAGTTTTTTCGCGAGTTCGCGCGCTGAAAACTT TTCTCCCATTTCC
ADH1-FORW[b, d]	ACACAGAATTCAGAAGCTTTGGACTTCTTC
ADH1-REV[b, d]	ATGACGAATTCGGGCATGCCGGTAGAG
TLC1-33-49[c]	CAGCCATTGACATTTTC
LACZ2[c]	GCAAGGCGATTAAGTTGGG
CYC1-FOR[d]	CCTACCTGAATCTAAAATTCCC
CYC1-REV[d]	CATTATTAATTTAGTGTGTGTATTTGTG
CAR1-FA[d]	TTAGCGGTAGCCGCCGAGGGGTCTAAAGAGTA
CAR1-RA[d]	TACTCTTTAGACCCCTCGGCGGCTACCGCTAA
ACT1 coding For[d]	TCCGGTGATGGTGTTACTCA
ACT1 coding Rev[d]	ATTCTCAAAATGGCGTGAGG
TLC1-DFA[d]	GTTTATAAATAAATTTTATATCACTATATGTGTGGTGAAAAGGAAGAGCA ATCCTGCTAAAGCTTTTATATCTAAACGCCAAAAAAATAAAAAAAAAA
TLC1-DRA[d]	TTTTTTTTTTATTTTTTTGGCGTTTAGATATAAAAGCTTTAGCAGGATTGC TCTTCCTTTTCACCACACATATAGTGATATAAAATTTATTTATAAAC
MCB1/2b-Rev[e]	GGTCTTTTAATTGAGAACCTTG**AGATCT**ATATGTCACCATTGCTAAAGAG GAAGTTTTTTCCAGAGTTCCCGAGCTGAAAACTTTTCTCCCATTTCC
MCB1mutF[e]	GTTTTCAGCGCGCGAACTGGCTAAAAAACTTCCTCTTTAG
MCB1mutR[e]	CTAAAGAGGAAGTTTTTTAGCCAGTTCGCGCGCTGAAAAC
MCB2mutF-2[e]	GGGAGAAAAGTTTTCAGCTCGGGAACTGGCTAAAAAACTTC
MCB2mutR-2[e]	GAAGTTTTTTAGCCAGTTCCCGAGCTGAAAACTTTTCTCCC

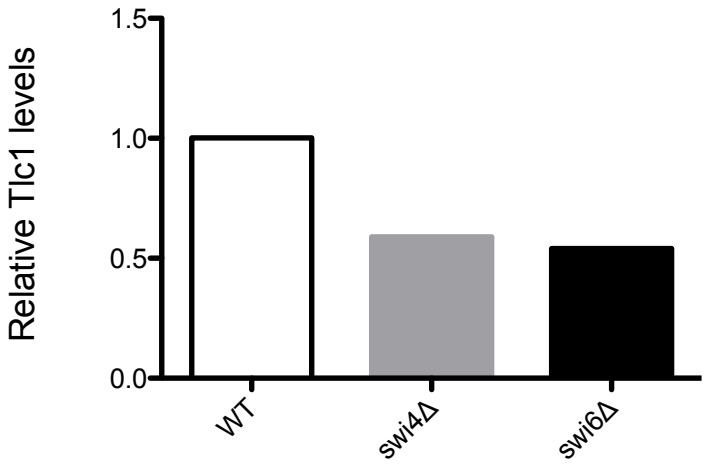
[a]Oligonucleotides used for PCR deletion strategy. Underlined sequences are restriction sites added for subsequent cloning (corresponding restriction enzyme is in parenthesis), bold nucleotides include sequences located on both sides of the deleted region.
[b]Oligonucleotides used for PCR amplification of *TLC1* promoter region, bold nucleotides in the reverse primers are added sequences corresponding to the "start codon ATG".
[c]Oligonucleotides used for primer extension and Northern blot analysis.
[d]Oligonucleotides used for PCR amplification of *CYC1* promoter regulated by different TLC1 promoter regions.
[e]Oligonucleotides used for site-directed mutagenesis; underlined nucleotides correspond to the mutations introduced in the MCB sequence.



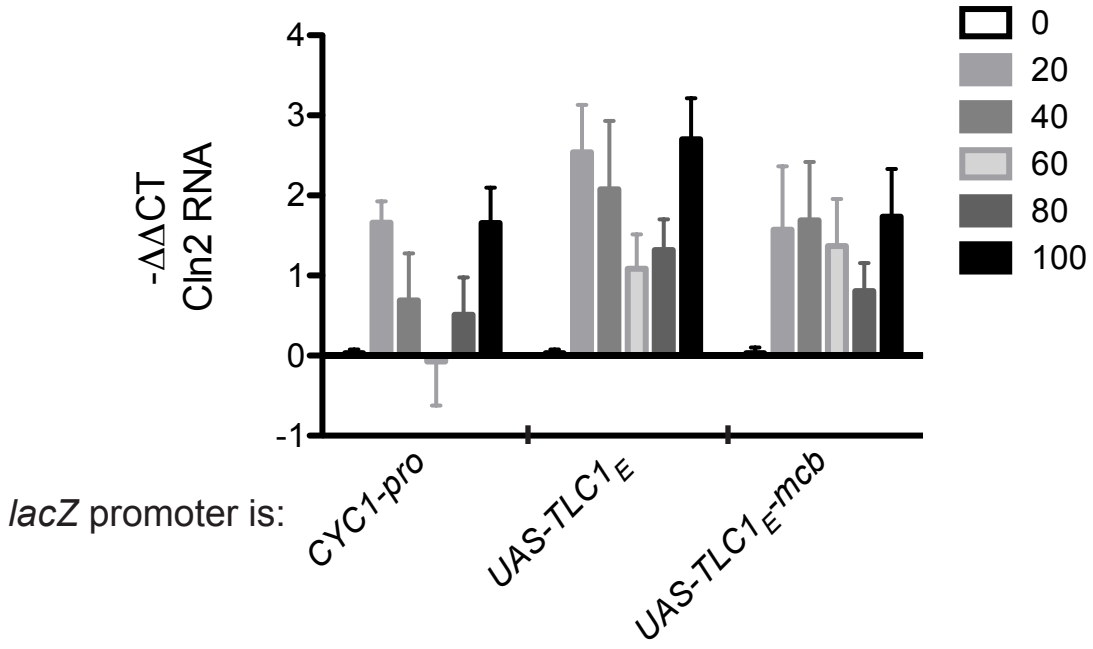
Dionne et al., Supplemental Figure S1



Dionne et al., Supplemental Figure S2



Dionne et al., Supplemental Figure S3



Dionne et al., Supplemental Figure S4