Supplementary Figures

Contents:

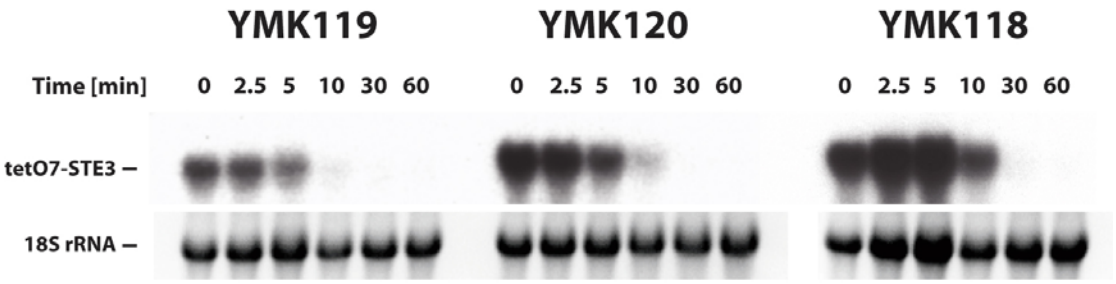

Figure S1. Northern analysis to compare transcript levels and kinetics of repression for *STE3* in three tetOFF strains.
The tetO7-*CYC1* promoter was inserted upstream of *STE3* (retaining its 5'UTR) in the three prototype tetOFF strains YMK118 (derived from CEN-PK), YMK119 (BY4741) and YMK120 (W303). Cultures were grown to mid-log phase in YPD and dox was added to 4µg/ µl (T0). Aliquots of cells were collected at intervals and RNA was analysed by northern blotting with probes for *STE3* (GTTGTAGCGAGCGATAC) and 18S rRNA (CATGGCTTAATCTTTGAGAC).

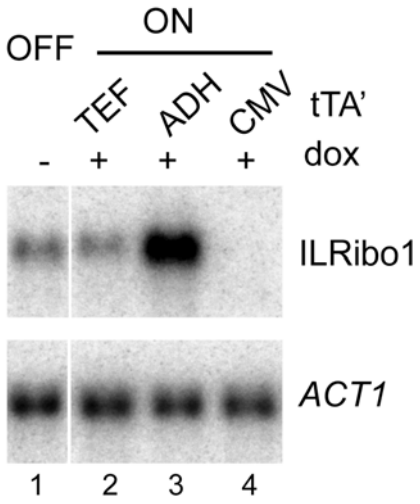

Figure S2. Effect of tTA′ promoter strength on reporter expression.

The level of the intronless ILRibo1 reporter in tetON strains expressing the tTA' activator from different promoters. TetON strains with integrated ILRibo1 reporter gene and expressing tTA' on a centromeric plasmid under the control of the *ADE1* (lane 2), *TEF2* (lane 3) or CMV (lane 4) promoter were grown in SD minimal medium (SD-trp) in the presence of doxycline (4µg/µl) to induce transcription (steady-state level). The tetOFF ILRibo1 strain grown in SD medium in the absence of doxycylin (lane 1) was used to compare the level of reporter expression in the two systems. Northern hybridization was performed using the 3HA probe to detect the ILRibo1 reporter and the *ACT1* probe against the endogenous *ACT1* mRNA as a control. (See Supplemental material 1 for probe sequences)

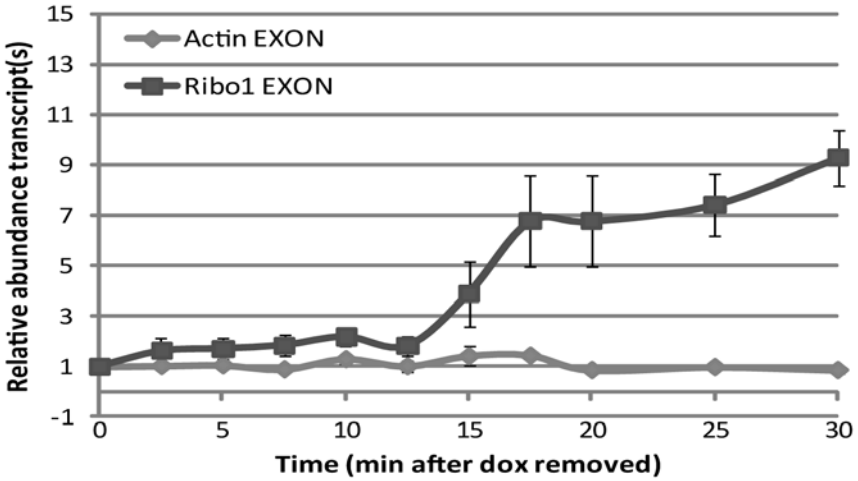

Figure S3. Reproducibility.

RT-QPCR analysis of endogenous ACT1 transcripts that are unaffected by dox provides a measure of the reproducibility of this technique during the time course of an experiment. This shows derepression following removal of dox from the medium at T0. The data represent three technical replicates with error bars indicating standard deviation.

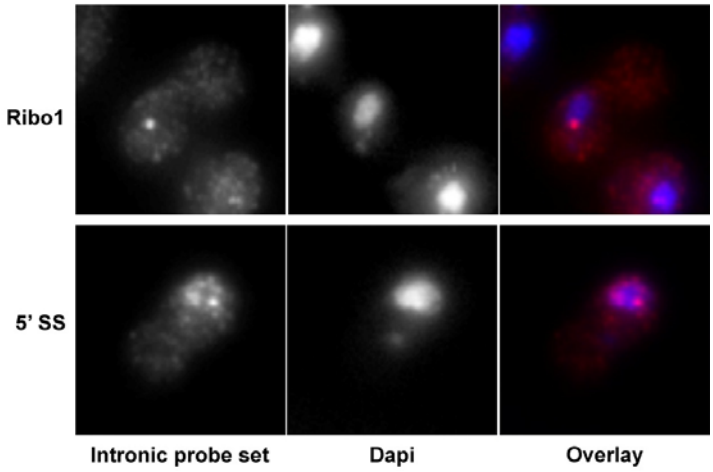

Figure S4. Detection of single molecules of the Ribo1 and mutant 5'SSRibo1 transcripts in Tet OFF strains.
Strains expressing the wild-type Ribo1 and mutant 5'SSRibo1 reporters were hybridized in situ with the intronic pool of probes. Ribo1 pre-mRNA is seen as a single nuclear focus, while the 5'SS mutant pre-mRNA displays several foci accumulating at the nuclear periphery.

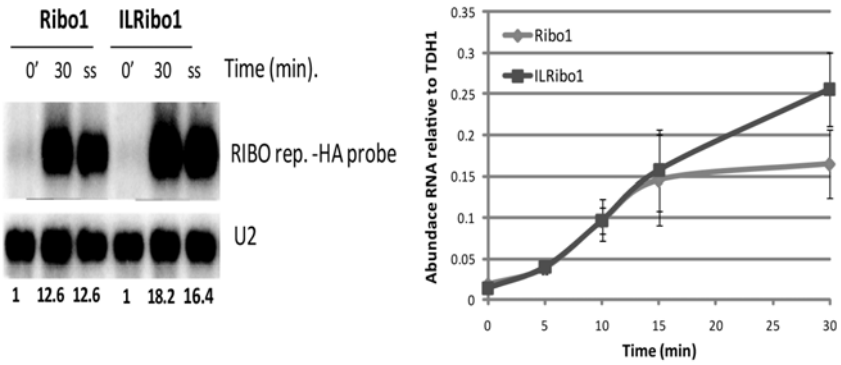

Figure S5. Induction of the RiboSys reporters in the tetON strains analysed by northern blot or RT-qPCR comparison with levels of *TDH1* transcript.
A. Northern blot analysis of Ribo1 and intronless Ribo1 (ILRibo1) mRNA levels measured immediately prior to dox addition (0), 30 min after dox addition (30) or after reaching the steady state induced level (SS). Numbers indicate relative levels of signal compared to 0 min. B. Comparison of RiboSys reporter transcripts with endogenous *TDH1* transcripts, analysed by RT-qPCR. Numbers on the Y-axis indicate level of Ribo1 transcripts relative to *TDH1* transcripts. The RT reaction was performed with random hexamers. QPCR was performed using Exon2F and Exon2R primers for the reporter and TDH1F and TDH1R primers for the *TDH1* mRNA. The ratio was calculated according to the following equation:

$$\frac{Exon2\,primerefficiency^{-(CpExon2)}}{TDH1 primerefficiency^{-(CpTDH1)}}$$

3HA probe	CATAGTCCGGGACGTCATAG
U2 probe	TGAAGAAACCATGAGCGAAGAAA
TDHF primer	GGTATGGCTTTCAGAGTCCCA
TDHR primer	AGACAACGGCATCTTCGGTG