Supplementary Table S1. Aptamer sequences.

Aptamer	**Parent aptamer**	**RNA sequence**
5-1.13	5-1[a]	5′GGGAGCUCAGAAUAAACGCUCAACUCCUGUAGUGAAGGCAGAGAAAGGUCGAUACGGACGGAAUGUGAUGGCCUUCGACAUCAGGCCCGGAUCCGGC
5-11.13	5-11[a]	5′GGGAGCUGAGAAUAAACGCUCAAAACUAGCAGGCAGAGAAGAGUGGGUGCGACCACAGGAUGUUAUGGCCUGUUCGACAUCAGGCCCGGAUCCGGC
5-14.13	5-14[a]	5′GGGAGCUGAGAAUAAACGCUCAACAGGAAACAGCAAGACAAACGAUGGGGAGCGUAAGACUGCGAGUGUCGGAUUCGACAUCAGGCCCGGAUCCGGC
5-18.13	5-18[a]	5′GGGAGCUGAGAAUAAACGCUCAAUAGGGAGAGAACUGUGUCAGAAUGUAGUGAACCAGACACGGAGUGGAGUAUUCGACAUCAGGCCCGGAUCCGGC
5-29.13	5-29[a]	5′GGGAGCUCAGAAUAAACGCUCAACUUGCUGCAGAGGGUCGAGAAUAUGUGUGACACUGCGUCGACGGGUUAAGUUCGACAUCAGGCCCGGAUCCGGC
5-1.2	5-1.13	5′GGAUCCAGGCAGAGAAAGGUCGAUACGGACGGAAUGUGAUGGCCUGGAUCCAAA
5-1.2m2	5-1.2	5′GGAUCCAGGCAGUGUAAGGUCGAUACGGACGGAAUGUGAUGGCCUGGAUCCAAA

[a] From Belmont and Niles (2010).

Supplementary Table S2. Oligonucleotides used for cloning and qPCR.

Oligonucleotide	**DNA sequence**
BJBOL111	5′GCGGTCGACTACAACATGGCTAGCATGACTGGTGGAC
BJBOL112	5′GCGCCTAGGTCAGTGGTGGTGGTGGTGGTGC
BJBOL233	5′ATTTATCGGAGTTGCAGTTGCGCC
BJBOL234	5′AACAAACACTACGGTAGGCTGCGA
BJBOL268	5′GCGCTCGAGAACATATGTCTAAAGGTGAAGAATTAT
BJBOL269	5′GCGTCTAGATTATTTGTACAATTCATCCATA
BJBOL295	5′GCGGTCGACTACAACATGTCTAGATTAGATAAAAGTA
BJBOL296	5′GCGCCTAGGTCAAGACCCACTTTCACATTTA
BJBOL410	5′GAGCAGATTGTACTGAGAGTGCACC
BJBOL411	5′TACGCATCTGTGCGGTATTTCACAC
BJBOL412	5′CAGAGACCAATCAGTAAAAATCAACGG
B**JBOL413**	5′GATCTTTTATGCTTGCTTTTCAAAAGGCC
ACT1F	5′GCCTTGGACTTCGAACAAGA
ACT1R	5′CCAAACCCAAAACAGAAGGA

Supplementary Table S3. Overlapping oligonucleotides used for PCR assembly of DNA templates for *in vitro* transcription.

Product	**Oligonucleotide**	**DNA sequence**
5-1.13-**FLuc-A60**	SG178	5′TGGCCTTCGACATCAGGCCCGGATCCGGCAACACAAAACTCGAGAACATATGGAAGACGC
	SG179	5′GTAGTGAAGGCAGAGAAAGGTCGATACGGACGGAATGTGATGGCCTTCGACATCAGGCC
	SG180	5′ATCTAGGGAGCTCAGAATAAACGCTCAACTCCTGTAGTGAAGGCAGAGAAAGGTCGATAC
	SG181 [a]	5′*CTAATACGACTCACTATAGGG*AATTATCTAGGGAGCTCAGAATAAACGCTCAA
5-11.13-**FLuc-A60**	SG184	5′GGCCTGTTCGACATCAGGCCCGGATCCGGCAACACAAAACTCGAGAACATATGGAAGACG
	SG185	5′AGCAGGCAGAGAAGAGTGGGTGCGACCACAGGATGTTATGGCCTGTTCGACATCAGGCCC
	SG186	5′GGGAATTATCTAGGGAGCTGAGAATAAACGCTCAAAACTAGCAGGCAGAGAAGAGTGGG
	SG187 [a]	5′*CTAATACGACTCACTATAGGG*AATTATCTAGGGAGCTGAGAATAAACGCT
5-14.13-**FLuc-A60**	SG191	5′GGATTCGACATCAGGCCCGGATCCGGCAAAACACAAAACTCGAGAACATATGGAAGACGC
	SG192	5′AACAGCAAGACAAACGATGGGGAGCGTAAGACTGCGAGTGTCGGATTCGACATCAGGCCC
	SG193	5′AATTATCTAGGGAGCTGAGAATAAACGCTCAACAGGAAACAGCAAGACAAACGATGGGGA
	SG194 [a]	5′*CTAATACGACTCACTATAGGG*AATTATCTAGGGAGCTGAGAATAAACGCTCAA
5-18.13-**FLuc-A60**	SG251	5′CGGCAAAACACAAAACTCGAGAACATATGGAAGACGCCAAAAACATAAAGAAAGGCCCGG
	SG253	5′AACTGTGTCAGAATGTAGTGAACCAGACACGGAGTGGAGTATTCGACATCAGGCCCGGAT
	SG254	5′GGGAGCTGAGAATAAACGCTCAATAGGGAGAGAACTGTGTCAGAATGTAGTGAACCAGAC
	SG255 [a]	5′*CTAATACGACTCACTATAGGG*AATTATCTAGGGAGCTGAGAATAAACGCTCAATAGG
5-29.13-**FLuc-A60**	SG256	5′AGTTCGACATCAGGCCCGGATCCGGCAACACAAAACTCGAGAACATATGGAAGACGCCAA
	SG257	5′GAGGGTCGAGAATATGTGTGACACTGCGTCGACGGGTTAAGTTCGACATCAGGCCCGGAT
	SG259	5′AGGGAGCTCAGAATAAACGCTCAACTTGCTGCAGAGGGTCGAGAATATGTGTGACACTGC
	SG261 [a]	5′*CTAATACGACTCACTATAGGG*AATTATCTAGGGAGCTCAGAATAAACGCTCAACT
5-1.2-**FLuc-A60**	SG151 [a]	5′*CTAATACGACTCACTATAGGG*AATTATCTAGGATCCAGGCAGAGAAAGG
5-1.2m2-**FLuc-A60**	SG291 [a]	5′*CTAATACGACTCACTATAGGG*AATTATCTAGGATCCAGGCAGTGTAAGGTCGA
(Universal **reverse** primer)	SG137	5′TTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTACAATTTGGACTTTCCGCCCTTCTTGG

[a] Indicates outermost forward primer containing T7 promoter sequence (in italics).

Supplementary Table S4. 5′UTR sequences resulting when 5-1.2 **and its variants are transcriptionally fused to the TEF1 promoter.**

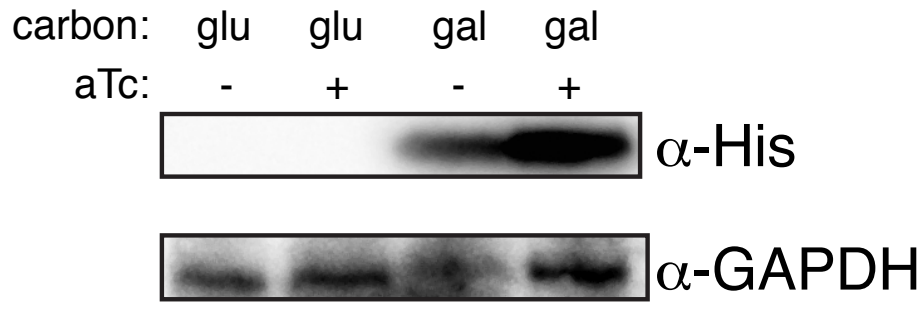
Aptamer	**RNA sequence**
5-1.2	AAUUAUCUAGGAUCCAGGCAGAGAAAGGUCGAUACGGACGGAAUGUGAU GGCCUGGAUCCAACACAAAACUCGAGAACAU*AUG*
5-1.2half	AAUUAUCUAAGAGAGAGACACCAAAGCGUGUGUGCGGACGGAAUGUGAU GGCCUGGAUCCAACACAAAACUCGAGAACAU*AUG*
5-1.4d	AAUUAUCUAGGAUCCAGGCAGAGAAAGGUCGAUACGGACGGAAUGUGGU GGCCUGGAUCCAAACACAAAACUCGAGAACAU*AUG*
5-1.4dhalf	AAUUAUCUAAGAGAGAGACACCAAAGCGUGUGUGCGGACGGAAUGUGGU GGCCUGGAUCCAAACACAAAACUCGAGAACAU*AUG*
5-1.30	AAUUAUCUAAGAUCCAGGCAGAGAAAGGUCGAUACGGACGGAAUGUGGU GGCCUGGAUCCAAACACAAAACUCGAGAACAU*AUG*
5-1.31	AAUUAUCUAAAAUCCAGGCAGAGAAAGGUCGAUACGGACGGAAUGUGGU GGCCUGGAUCCAAACACAAAACUCGAGAACAU*AUG*
5-1.32	AAUUAUCUAAAUUCCAGGCAGAGAAAGGUCGAUACGGACGGAAUGUGGU GGCCUGGAUCCAAACACAAAACUCGAGAACAU*AUG*
5-1.33	AAUUAUCUAAAUACCAGGCAGAGAAAGGUCGAUACGGACGGAAUGUGGU GGCCUGGAUCCAAACACAAAACUCGAGAACAU*AUG*

Supplementary Table S5. Summary of the regulatory behavior observed when using 5-1.2 **and** 5-1.31 **within the endogenous yeast 5′UTRs indicated. FLuc and vYFP were used as reporters.**

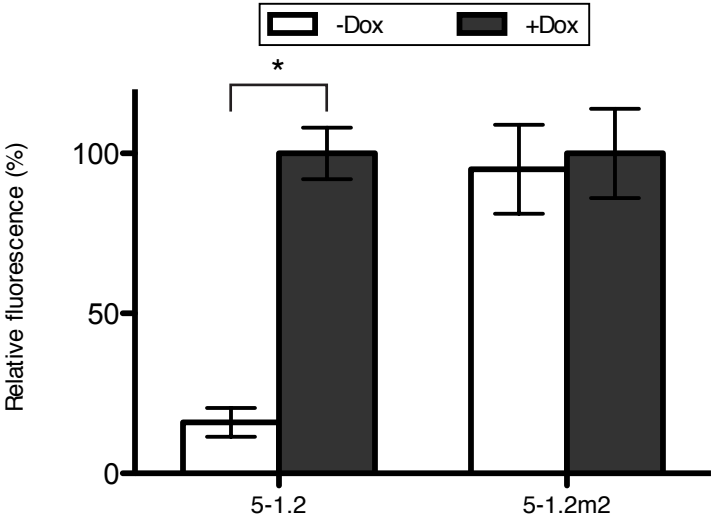
	%Repression					
Promoter/5′ UTR	**5-1.2 FLuc**	**5-1.31 vYFP**	**5-1.31 FLuc**	**+TDH3 5′UTR 5-1.2 FLuc**	**Length before aptamer (nt)**	**%(G+C)**
TEF1	87	85	85	95	9	11
ADH1	77	77	89	84	44	34
PGK1	81	84	93	85	46	24
P**YK1**	94	84	91	80	33	27
TDH3	97	84	95	N.D.	45	29
TEF2	96	88	92	89	28	29
TPI1	61	71	95	94	36	25

Supplementary Table S6. Oligonucleotide primers and probes used in qPCR of polysome fractions.

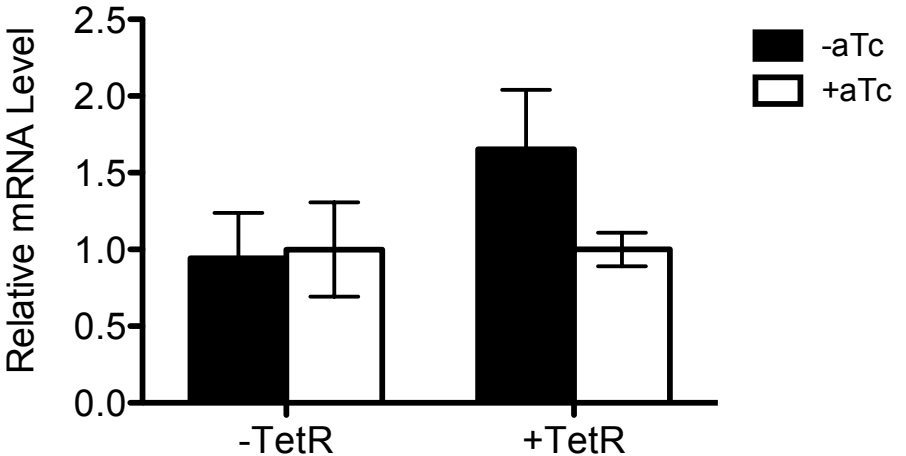
	Sequence
Firefly luciferase	
Primer #1	5′TCCTCTGACACATAATTCGCC
Primer #2	5′GCTATTCTGATTACACCCGAGG
Probe	5′HEX/TCCAGATCC/ZEN/ACAACCTTCGCTTCAAA/IABkFQ
vYFP	
Primer #1	5′CACCTTCAAACTTGACTTCAGC
Primer #2	5′TGTGTTTTGCTAGATACCCAGATC
Probe	5′6-FAM/TTTCTTGAA/ZEN/CATAACCTTCTGGCATGGC/IABkFQ
ACT1	
Primer #1	5′GGCAGATTCCAAACCCAAAAC
Primer #2	5′TCGAACAAGAAATGCAAACCG
Probe	5′Cy5/ACGAAAGATTCAGAGCCCCAGAAGC/IAbRQSp



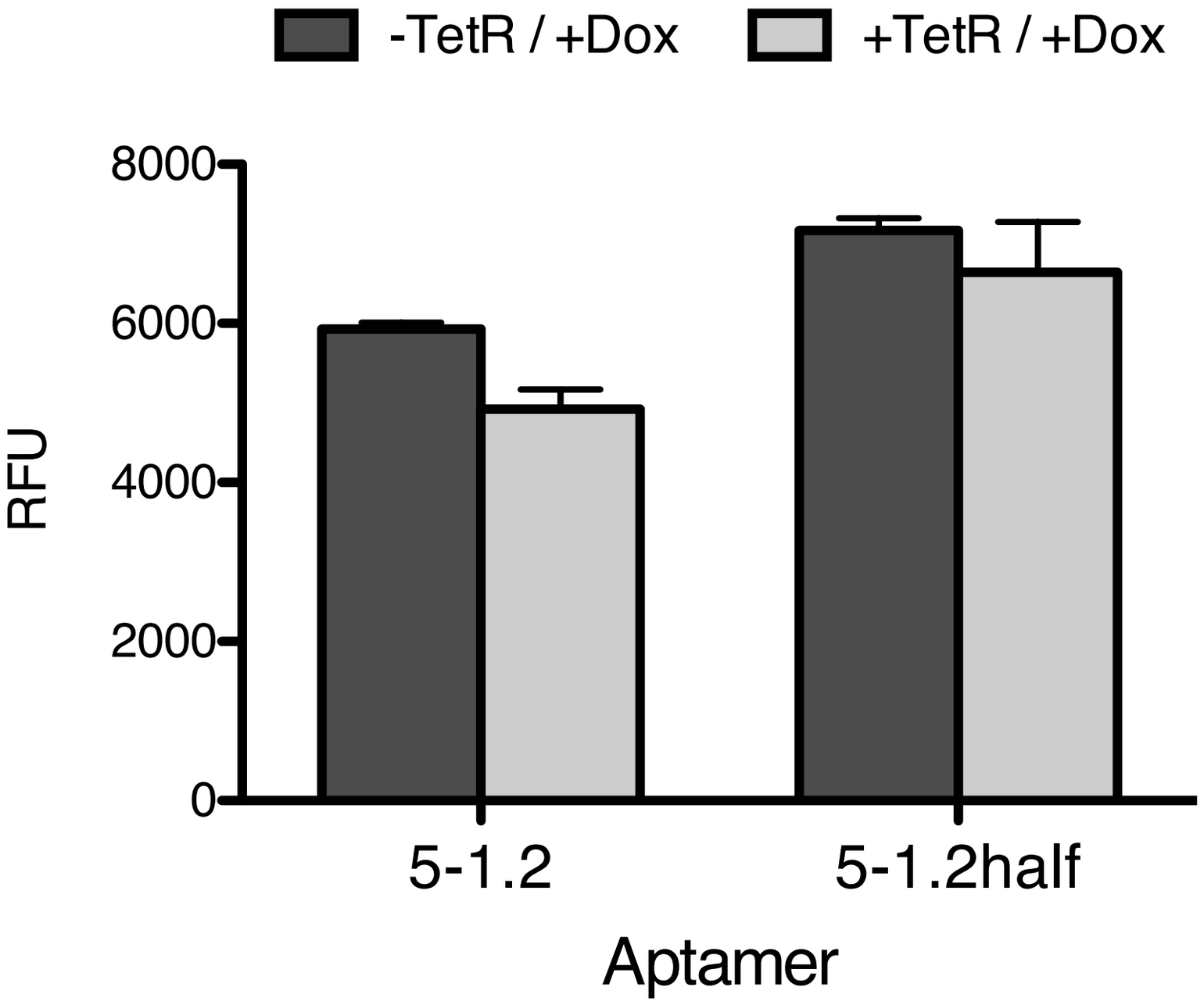
Supplementary Figure S1: Western blot measurement of TetR protein. The addition of aTc does not decrease TetR expression. Yeast cells were grown in the presence or absence of aTc plus either glucose or galactose (to repress or induce TetR expression, respectively). Anti-His_6 tag antibody was used to detect His_6-tagged TetR. Sample loading was verified by GAPDH detection with an anti-GAPDH antibody.



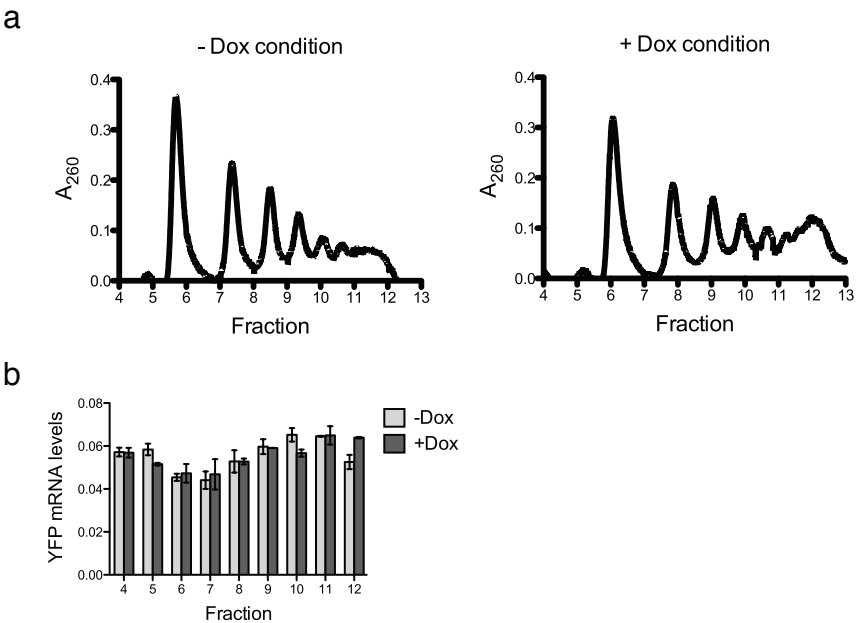
Supplementary Figure S2: Constitutively expressed TetR represses translation. TetR expressed constitutively in yeast from the *TDH3* promoter is able to repress translation of vYFP controlled by **5-1.2**, but not **5-1.2m2**. The addition of Dox relieves vYFP repression. Data represent the mean ± s.d. of six experiments. A two-tailed, unpaired t-test was used to calculate the significance ($\alpha = 0.005$) of the difference between induced and uninduced conditions.
*, $P = 7.1 \times 10^{-10}$.



Supplementary Figure S3: Quantitative PCR measurement of FLuc mRNA. The addition of aTc does not increase the steady-state level of FLuc mRNA, indicating that regulation by TetR/aTc occurs at the level of translation. For each condition (with or without TetR), values indicate the amount of FLuc mRNA in the presence of 1 μM aTc relative to the amount of mRNA in the absence of aTc. The data represent the mean ± s.d. of six experiments.



Supplementary Figure S4. Removal of **5-1.2** aptamer structure has little effect on basal expression. Flow cytometry measurements show expression levels of aptamer-regulated vYFP. The aptamer located within the *5'UTR* of vYFP and the expression status of TetR are indicated. In all cases, cells were grown in the presence of Dox.



Supplementary Figure S5. Polysome profiles of aptamer-containing mRNA are consistent across separate experiments. Growth of yeast and subsequent polysome separation and analysis were performed independently of the experiment shown in Figure 7. **(a)** Polysomes were fractionated from yeast expressing both TetR and a 5-1.2-containing vYFP reporter mRNA, which were grown in the absence and presence of Dox. Polysome profiles for both the - Dox and + Dox growth conditions are shown. **(b)** Quantitative PCR measurements of relative amount of reporter mRNA within each polysome fraction under - Dox and + Dox conditions. Error bars indicate range of values for technical duplicates.