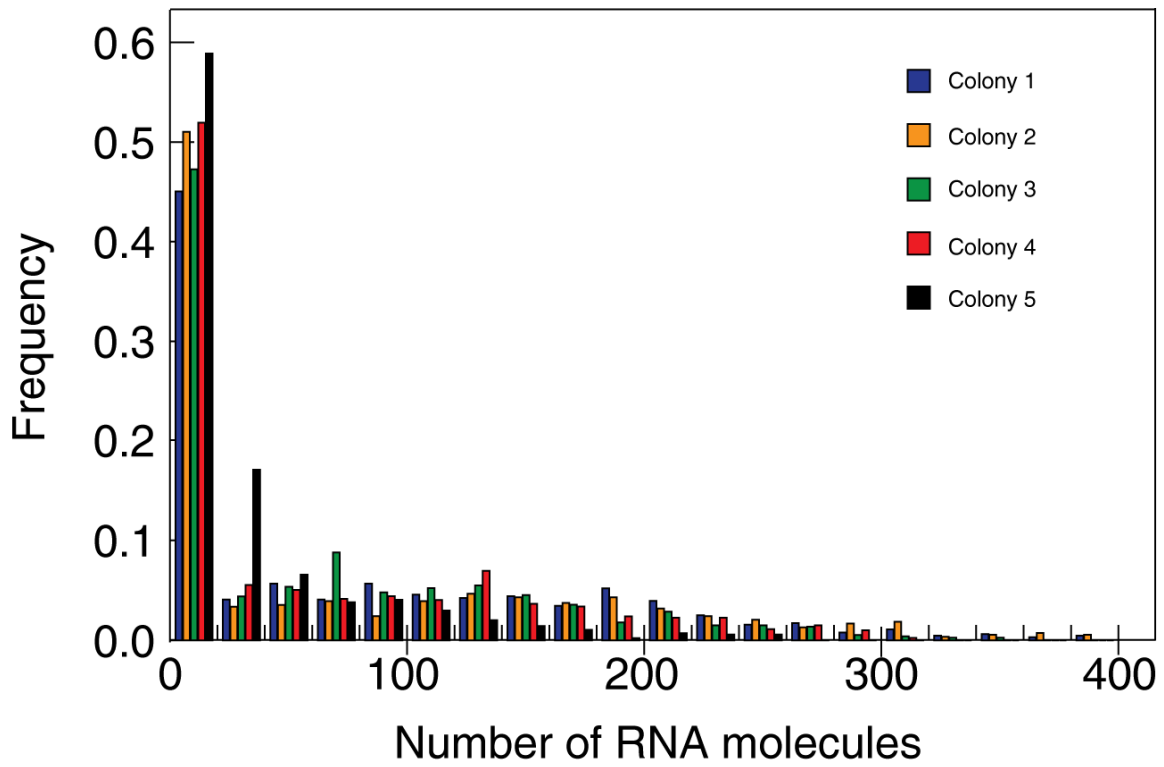
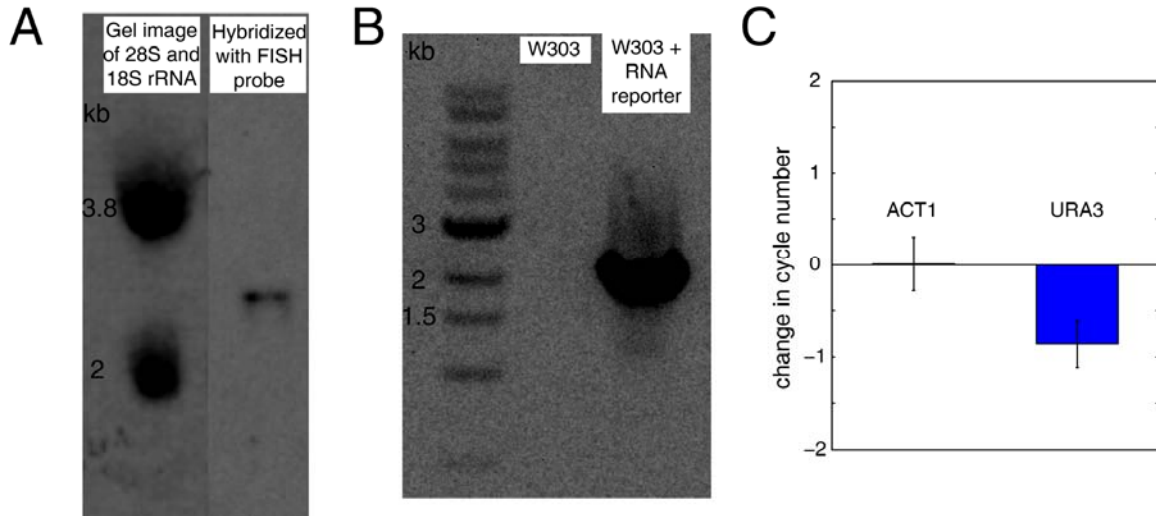


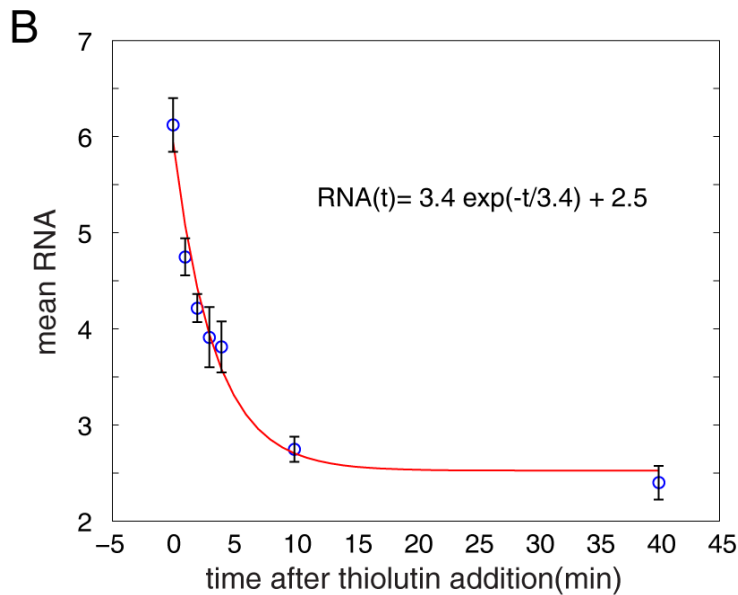
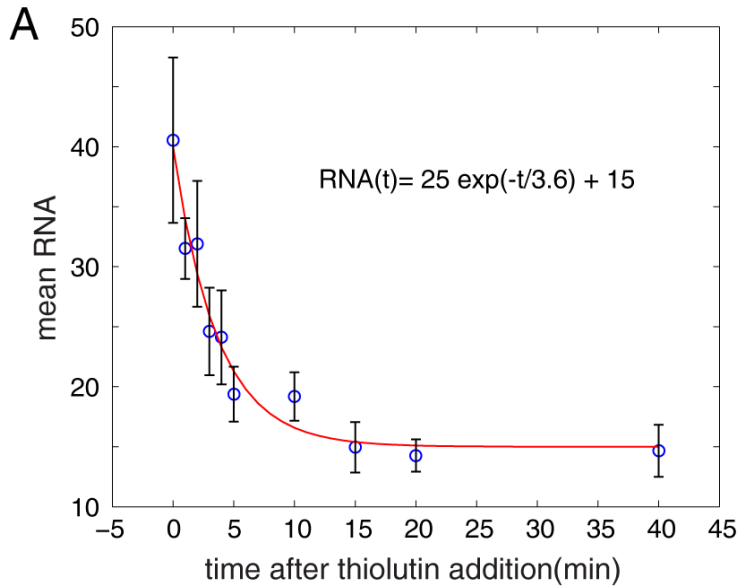
Supplementary Figure S1: Plot of total cell fluorescence versus RNA in the cell. Total cell fluorescence is directly proportional to the RNA count in the cells and can be used to estimate the number of RNAs in cells with high transcription. Background subtraction is done by subtracting the mean autofluorescence of cells with no RNA from the total fluorescence of the cells.



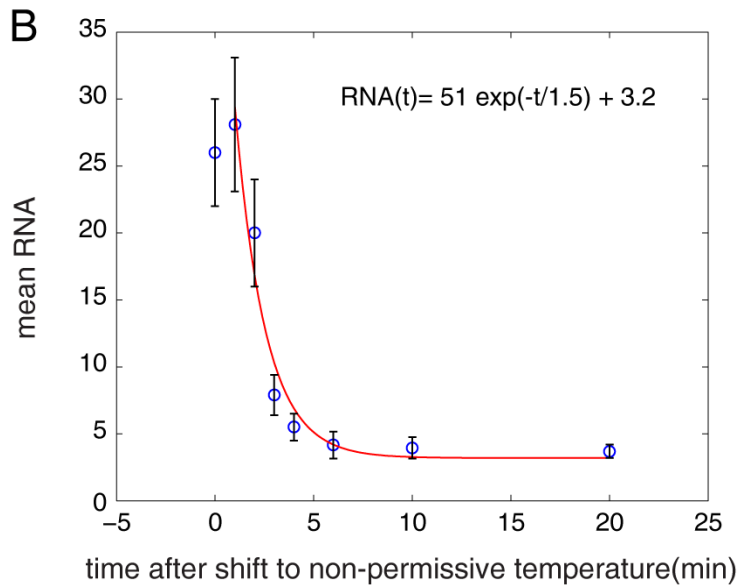
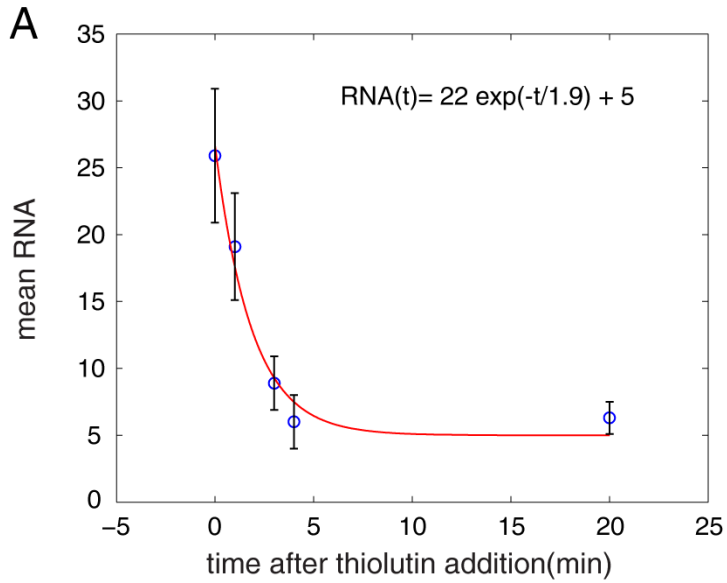
Supplementary Figure S2: RNA distributions obtained for five different integrants. Binsize is 20. The integrants are individual colonies obtained after plasmid integration of the construct into the rDNA array. They have the RNA reporter integrated at different positions on the rDNA array.



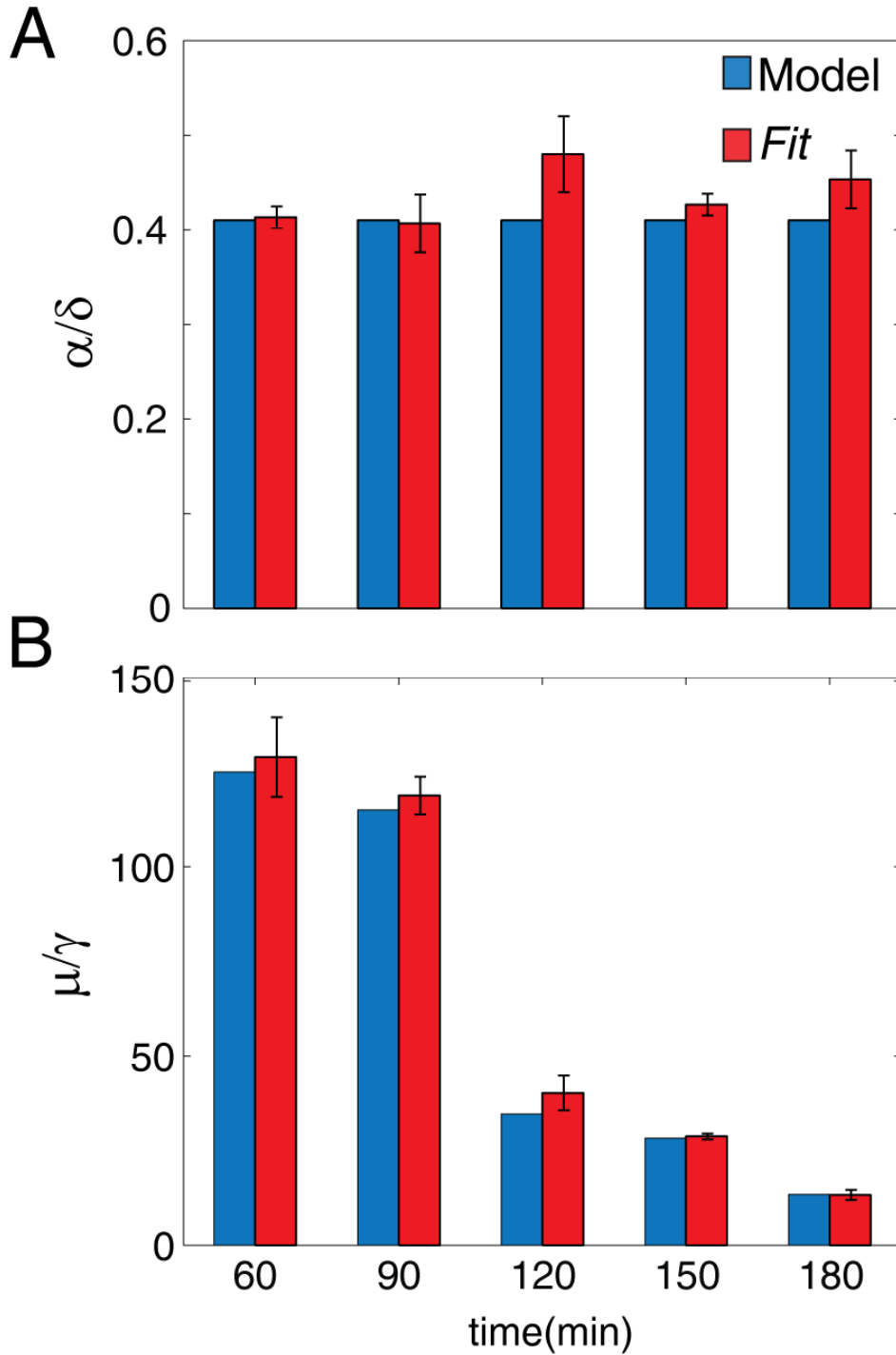
Supplementary Figure S3: (A) Northern blot analysis of total RNA isolated from W303 after integration of the plasmid. A single band is observed showing that the transcripts observed by FISH are full-lengths. (B) Analysis of PCR products obtained using a primer binding to the *AMP* sequence in the plasmid and another binding to the 35S coding region not included in the plasmid. (C) Quantitative PCR to determine the change in cycle number after plasmid integration. *ACT1* is used as a loading control.



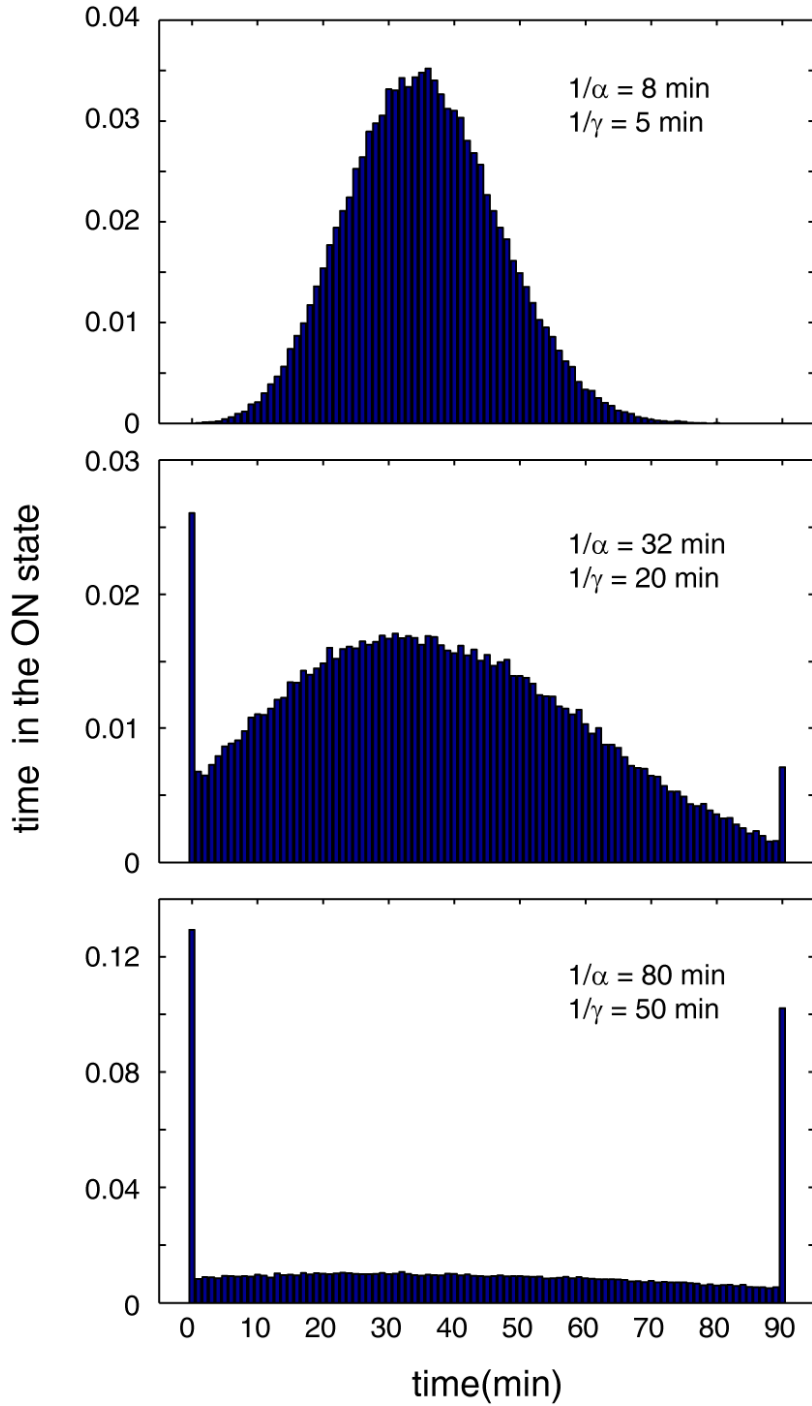
Supplementary Figure S4: Determination of reporter RNA degradation rate. The mean RNA is fitted to the equation $m = m_0 e^{-dt} + b$ where $m(t)$ is the mean RNA at different times after thiolutin addition. $m_0 + b$ is the initial amount of RNA before thiolutin addition. d is the rate constant for RNA decay and b is the amount of RNA transcription after thiolutin addition. The best fit is determined by the least square method with m_0 , d and b as free parameters. Plot shows the mean RNA level after addition of thiolutin (blue circles) for (A) O.D. = 0.6 and (B) OD = 1.5. The best fit is shown in red and error bars are shown in black. Error bars are the standard errors.



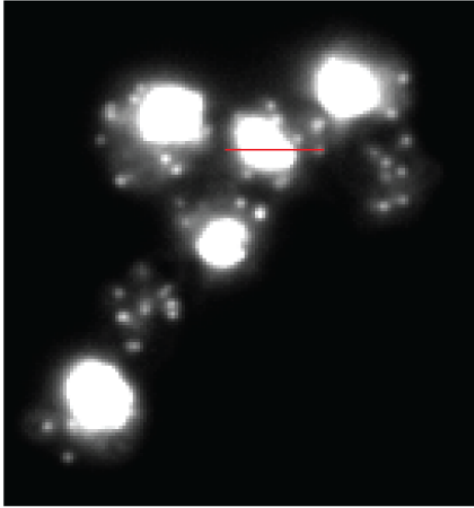
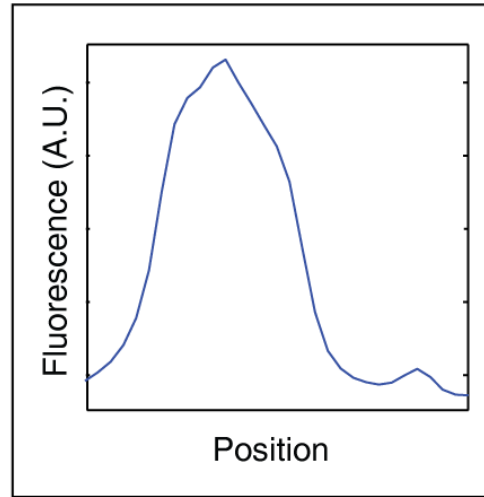
Supplementary Figure S5: Comparison of reporter RNA degradation rate obtained using thiolutin inhibition and temperature inactivation. The mean RNA is fitted to the equation $m = m_0 e^{-dt} + b$ where $m(t)$ is the mean RNA at different times after thiolutin addition or shift to non-permissive temperature. Plot shows the mean RNA level after addition of thiolutin (blue circles) for **(A)** thiolutin addition at 25°C and **(B)** shift to non-permissive temperature (38°C) (the first time point has been omitted in the fit to reduce transient effects). The best fit is shown in red and error bars are shown in black. Error bars are the standard errors.



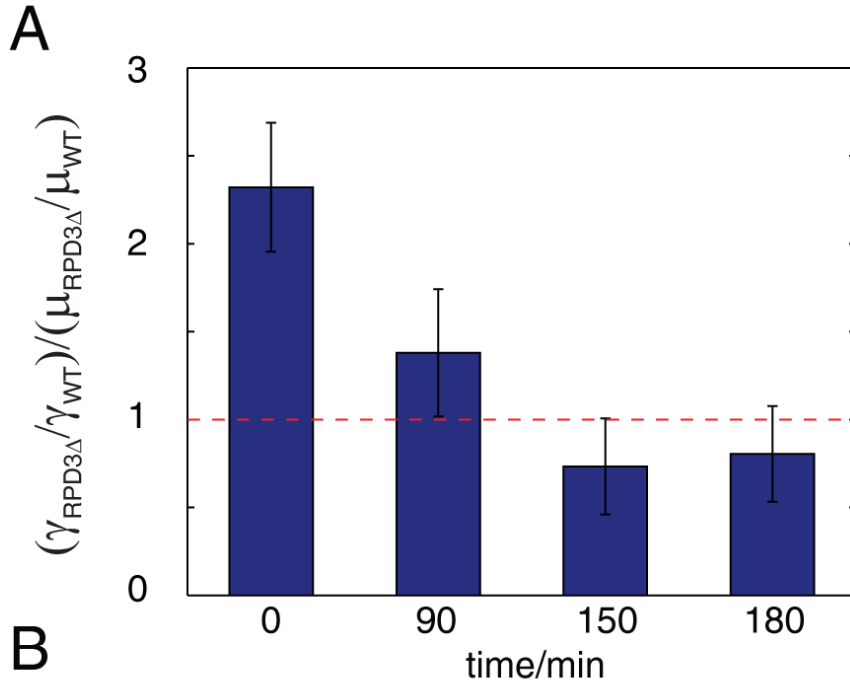
Supplementary Figure S6: (A) Activation rates used in the model (blue) and activation rates obtained by fitting to the steady state equation for the two state model (red) versus time. (B) Burst sizes used in the model (blue) and burst sizes obtained by fitting to the steady state equation for the burst model (red) versus time.



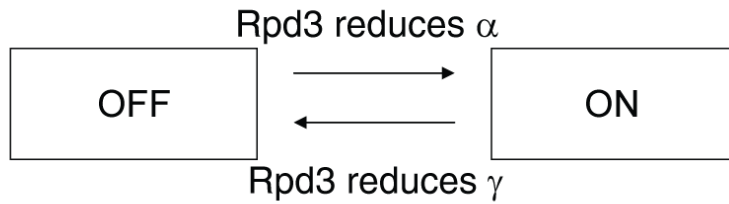
Supplementary Figure S7: Computed dwell times of cells in the ON state per cell cycle of 90 minutes for different switching rates.

A**B**

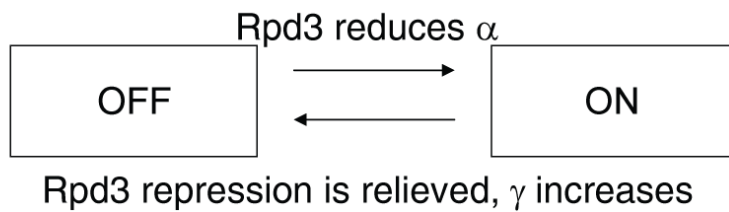
Supplementary Figure S8: (A) Fluorescence image of cells that had been fixed and hybridized with fluorescent probes. (B) This plot shows the fluorescence intensity as a function of position along the red line in (A). The exposure time has been chosen such that bright transcription sites are not saturating and individual RNAs can be resolved. As seen from the graph, the fluorescence of the transcription site is much higher than that of the single RNA.



Low cell density



High cell density



Supplementary Figure S9: (A) The burst size of WT normalized by the burst size of the *rpd3Δ* strain. (B) Possible model of Rpd3 regulation.

Time (min)	0	60	90	120	150	180
Mean RNA	65 ± 15	43 ± 9	41 ± 9	13 ± 3	13 ± 2	6 ± 1
α/δ	0.42 ± 0.07	0.41 ± 0.07	0.38 ± 0.06	0.35 ± 0.05	0.47 ± 0.06	0.43 ± 0.07
μ/β	190 ± 20	130 ± 20	120 ± 20	35 ± 9	28 ± 5	13 ± 2

Table 1: Summary of the mean RNA, α/δ and μ/β for WT strain at different times during diauxic shift.

Time (min) /O.D.	0	90	150	180
Mean RNA	68 ± 16	56 ± 13	28 ± 6	13 ± 2
α/δ	0.84 ± 0.06	0.8 ± 0.1	0.79 ± 0.07	0.9 ± 0.08
μ/β	82 ± 10	83 ± 14	39 ± 6	17 ± 2

Table 2: Summary of the mean RNA, α/δ and μ/β for *rpd3Δ* strain at different times during diauxic shift.