Cell Reports

Supplemental Information

Yeast Replicator: A High-Throughput Multiplexed Microfluidics Platform for Automated Measurements of Single-Cell Aging

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SUPPLEMENTAL DATA

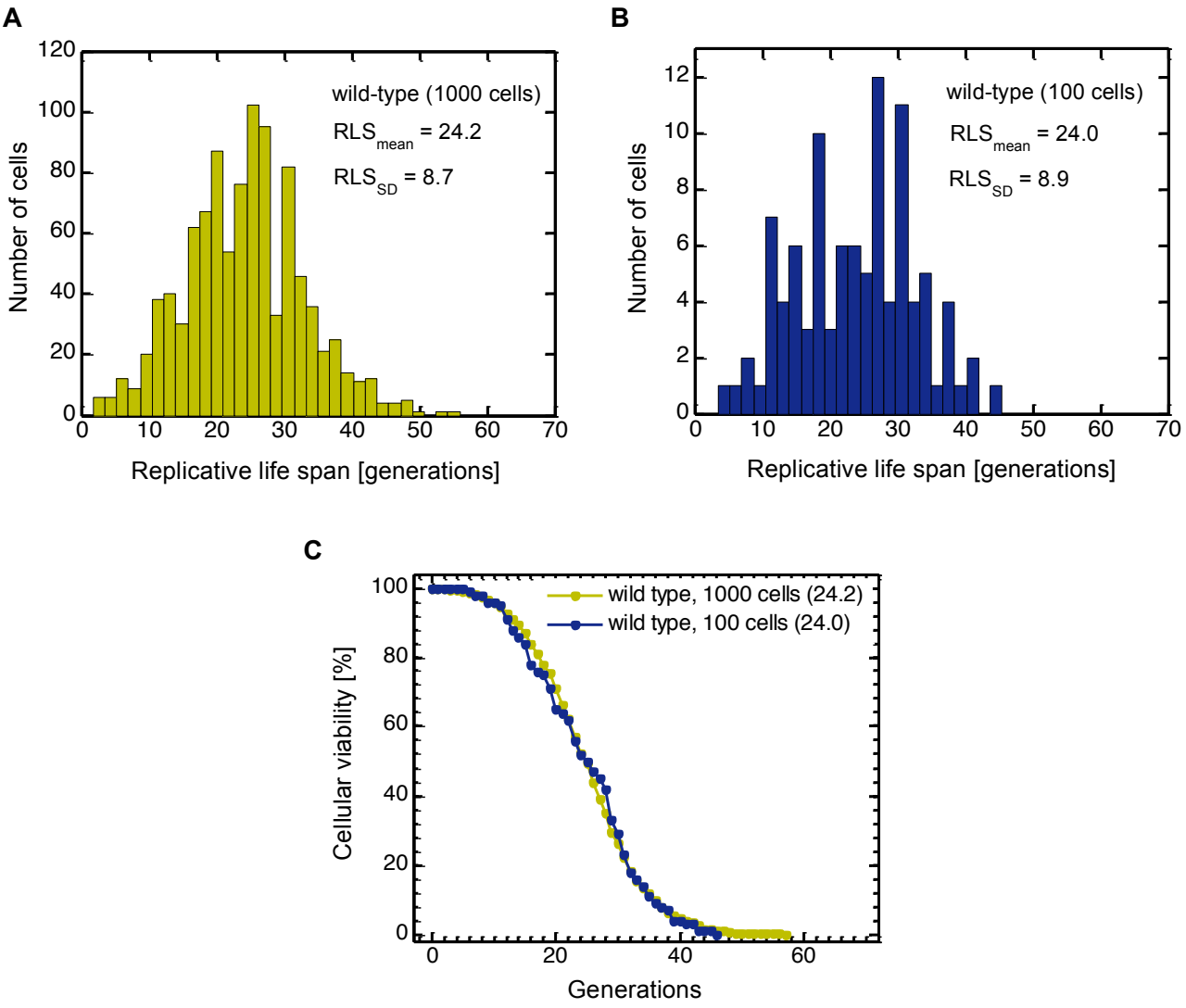

Figure S1. Replicative life spans and viability curves for 1000 and 100 cells, Related to Figure 3. A-B. Histogram distributions of single-cell replicative life spans for 1000 (**A**) and 100 (**B**) mother cells analyzed. 100 cells were randomly picked from 1000 cells by using MATLAB's *rand* function. **C.** Cellular viability as a function of number of generations for the two cases.

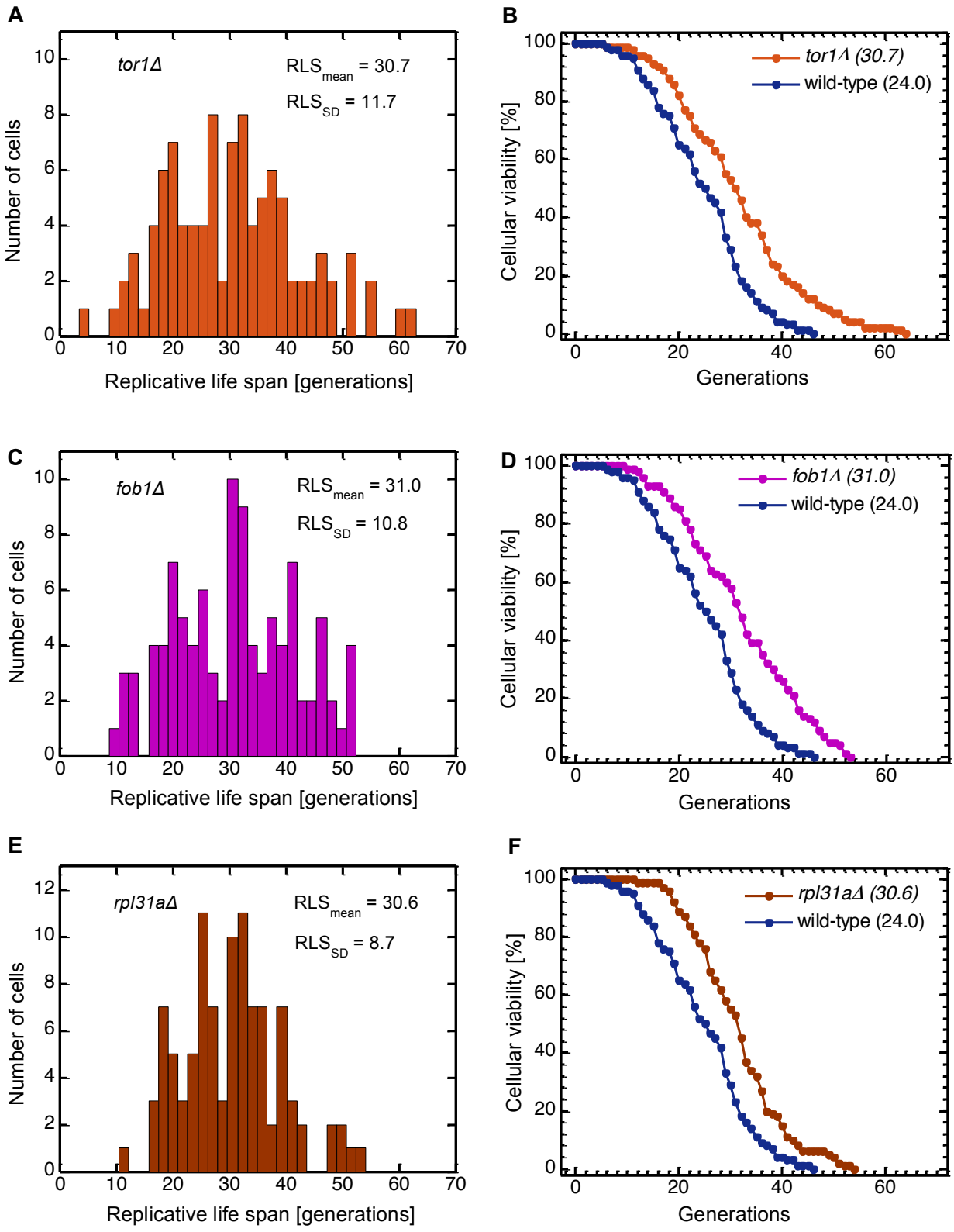

Figure S2. Canonical gene deletion strains compared to wild type, Related to Figure 3. Distributions of replicative life spans (left) and viability curves (right). N=100 cells.

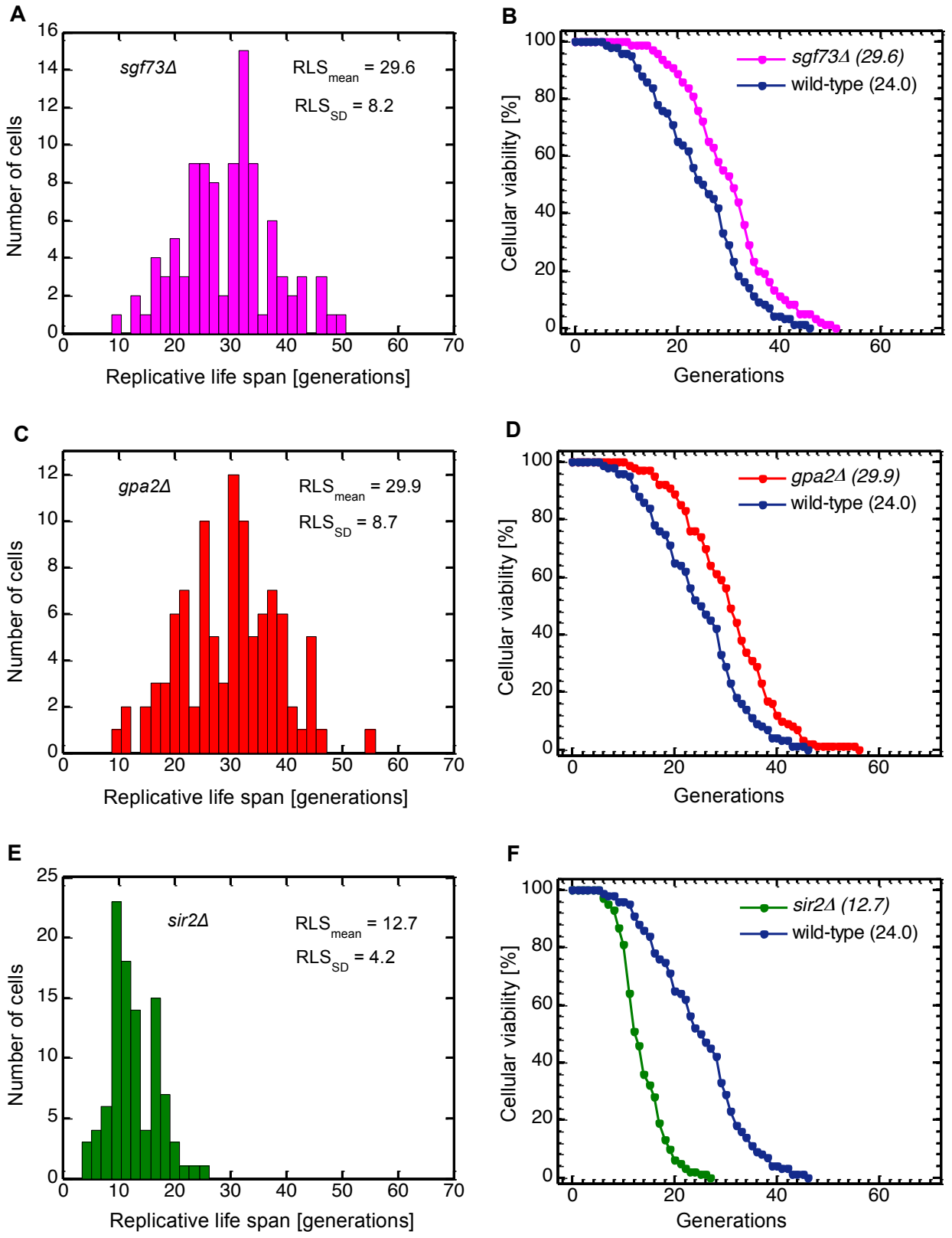

Figure S3. Canonical gene deletion strains compared to wild type, Related to Figure 3. Distributions of replicative life spans (left) and viability curves (right). N=100 cells.

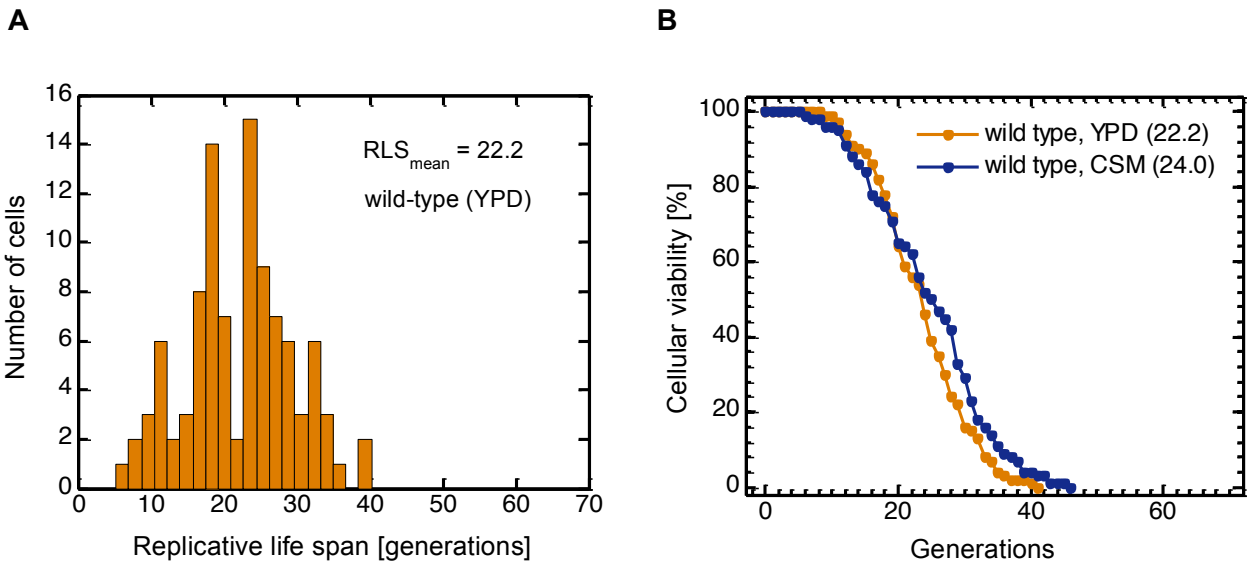

Figure S4, Lifespan measurements in rich media, Related to Figure 3. A. Histogram distribution of single-cell replicative life spans for one hundred wild type mother cells tracked in rich YPD media. Mean and SD of the distribution are 22.2 and 7.3. **B.** Cellular viability curve of the YPD-grown cells compared to the curve obtained from mother cells grown in CSM minimal media. Both media types contained 2% glucose.

Strain	Genotype
AL002	*MATα*, *MATα, his3Δ, leu2Δ, LYS2, met15Δ, ura3Δ*
TZY10a	*MATα, his3Δ, leu2Δ, LYS2, met15Δ, ura3Δ, ho::HIS5-*P_{GAL1}*-YFP*
TZY54a	*MATα, his3Δ, leu2Δ, LYS2, met15Δ, ura3Δ, ho::HIS5-*P_{SOD1}*-mCherry*
BY4741	*MATa*, *MATα, his3Δ, leu2Δ, LYS2, met15Δ, ura3Δ*
BY4742	*MATα*, *MATα, his3Δ, leu2Δ, lys2, MET15, ura3Δ*
fob1Δ	*MATa*, *MATα, his3Δ, leu2Δ, lys2, MET15, ura3Δ, fob1Δ*
sgf73Δ	*MATa*, *MATα, his3Δ, leu2Δ, lys2, MET15, ura3Δ, sgf73Δ*
tor1Δ	*MATa*, *MATα, his3Δ, leu2Δ, lys2, MET15, ura3Δ, tor1Δ*
sir2Δ	*MATa*, *MATα, his3Δ, leu2Δ, lys2, MET15, ura3Δ, sir2Δ*
gpa2Δ	*MATa*, *MATα, his3Δ, leu2Δ, lys2, MET15, ura3Δ, gpa2Δ*
rpl31aΔ	*MATa*, *MATα, his3Δ, leu2Δ, lys2, MET15, ura3Δ, rpl31aΔ*

Table S1. Yeast strains used in this study, Related to Experimental Procedures. All *S. cerevisiae* strains used have the BY genetic background.

SUPPLEMENTAL EXPERIMENTAL PROCEDURES

Experimental characterization of the phenotypic switching rates with flow cytometry:

The phenotypic switching rates between the OFF and ON states of the bimodal GAL network is extracted from experimental FACS data as previously described (Acar et al., 2008; Peng et al., 2015). For this, the experimentally-obtained number of ON and OFF cells, N_{OFF} and N_{ON}, are fed into the following coupled differential equations:

$$\begin{cases} \frac{dN_{ON}}{dt} = \gamma N_{ON} - r_{OFF} N_{ON} + r_{ON} N_{OFF} \\ \frac{dN_{OFF}}{dt} = \gamma N_{OFF} + r_{OFF} N_{ON} - r_{ON} N_{OFF} \end{cases}$$

where γ is the growth rate of the cells, and r_{OFF} and r_{ON} are the ON-to-OFF and OFF-to-ON switching rates, respectively. These differential equations were solved analytically to obtain the fraction of ON cells, f_{ON} :

$$f_{ON}(t) = \frac{r_{ON}}{r_{ON} + r_{OFF}} + \left(f_{ON}(t=0) - \frac{r_{ON}}{r_{ON} + r_{OFF}} \right) e^{-(r_{ON} + r_{OFF})t}$$

Using two different initial states $f_{ON}(t=0)$ and the corresponding final states after 22 hours $f_{ON}(t=22h)$, we numerically solved the above equation for r_{OFF} and r_{ON}. The two different initial states were experimentally obtained by growing yeast cells in duplicate in [0.5% glucose (OFF history)] or [0.5% glucose + 2% galactose (ON history)] respectively for 22 hours, and the fraction of ON cells were measured using FACS. The cells were then grown in induction media, consisting of [0.5% glucose + 2% galactose], for another 22 hours, and the post-induction fraction of ON cells were measured using FACS. A cutoff for ON cells was selected based on FACS measurements performed on uninduced cells (i.e., without galactose). The experimental f_{ON} results obtained from these measurements were used to solve the above equation for r_{OFF} and r_{ON}. The following table shows the results obtained from our measurements.

OFF history	experiment 1	$f_{ON}(t=0) = 0\%$	$f_{ON}(t=22h) = 10.15\%$
OFF history	experiment 2	$f_{ON}(t=0) = 0\%$	$f_{ON}(t=22h) = 8.8\%$
OFF history	average 1&2	$f_{ON}(t=0) = 0\%$	$f_{ON}(t=22h) = 9.47\%$
ON history	experiment 1	$f_{ON}(t=0) = 19.73\%$	$f_{ON}(t=22h) = 30.37\%$
ON history	experiment 2	$f_{ON}(t=0) = 19.69\%$	$f_{ON}(t=22h) = 31.98\%$
ON history	average 1&2	$f_{ON}(t=0) = 19.71\%$	$f_{ON}(t=22h) = 31.17\%$
Switching rate	ON => OFF	$r_{OFF} = 1.2 * 10^{-12}\ h^{-1}$	
Switching rate	OFF => ON	$r_{ON} = 0.0055\ h^{-1}$	