Supporting Information:

Efflux pump control alters synthetic gene circuit function

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SUPPORTING FIGURE LEGENDS

Figure S1. Dose-responses of mean fluorescence intensity for NR cells with the native copy of *PDR5* present at its endogenous locus (black) versus NR cells with endogenous *PDR5* knocked out (magenta). Dose-responses of mean fluorescence intensity (calculated using an average of 3 replicates) indicate that the presence/absence of PDR5 at its native locus does not affect the dose-response.

Figure S2. Fitting the NR model to the experimental data. NR ODE, NR analytic, and NR experimental mean dose-responses. The parameters for NR ODE and NR analytic were obtained partly from an earlier study and partly by using Matlab's curve fitting toolbox (see the Methods and the legend of Fig. 2): $a_x = a_z = 9.418$ nM h^{-1}, $l_z = 0.1$ nM h^{-1}, $b = 7.7877$ nM^{-1} h^{-1}, $C = 1.837$ [dox] h^{-1}, $d = 0.1313$ h^{-1}, $f = 1.551$ h^{-1}, $n = 2$, $\theta = 1.858$ nM, and $k = 0$ h^{-1}.

Figure S3. Dose-responses of NF fluorescence intensity mean after including the pump term, but without altering TetR synthesis rate. This plot shows that the pump term alone (red) is insufficient to capture all aspects of the NF dose-response change (parameters for NF are as in Figure S2 except $K = 100$ nM, h = 2, and $k = 200$ h^{-1}).

Figure S4. Dose-responses of fluorescence intensity mean without normalization. (A) NR series: NR (black), NRpump (red), NRpumpmutant (green and blue); **(B)** NF series (colors: as for the NR series).

Figure S5 Single-color and 2-color NF fluorescence mean dose-responses. Dose-responses of mean fluorescence intensity (calculated using an average of 3 replicates) indicate linearity up to saturation. The different slopes are most likely due to fusing mCherry to TetR, which can alter synthesis and degradation rates, as well as protein function.

Figure S6. Dose-responses of exponential growth rates. (A) Cells grow exponentially over 12 hours. Natural logarithms of cell densities (circles) are shown for NR (blue) and NRpump (cyan) strains. The dashed lines are linear fits to the experimental data with slopes of 0.26129, and 0.30217, respectively. **(B)** Exponential growth rates at increasing doxycycline concentrations are shown for NR (blue), NRpump (cyan), NF(red) and NFpump (magenta) strains.

SUPPORTING TABLE LEGEND

Table S1. Parameter sensitivity analysis for the NRpump model. The quality of the fits was measured using the L1-norm calculated from the difference between simulation results and experimental data for the 10 different doxycycline concentrations used in the dose response experiments:

$$Q = \sum_{i=1}^{N} \left| GFP_{simulation} - GFP_{experiment} \right|_i .$$

The L1-norms of GFP mean and GFP CV for the parameter set before reducing the repressor synthesis rate (as in Figure 2A,B) appear in the left and right columns labeled "0", respectively. The L1-norms were then computed for each parameter in the model by independently varying them up or down twofold. An increase of two-times the parameter value from the main text appears in the column "+", and a decrease by half appears in the column "-". The best fits for GFP mean and GFP CV (lowest L1-norms) are shown in bold in each row. Most parameter changes that improved the fit of the GFP mean decreased the fit quality for the GFP CV and vice versa. The parameter changes that improved the fit (lower L1-norm compared to the "0" column) for both GFP mean and GFP CV are highlighted in yellow, and the overall best fit (a_x, -) is highlighted in green. Note that increasing parameter C (inducer influx rate multiplier) is equivalent to decreasing TetR synthesis rate (since intracellular inducer inactivates TetR).
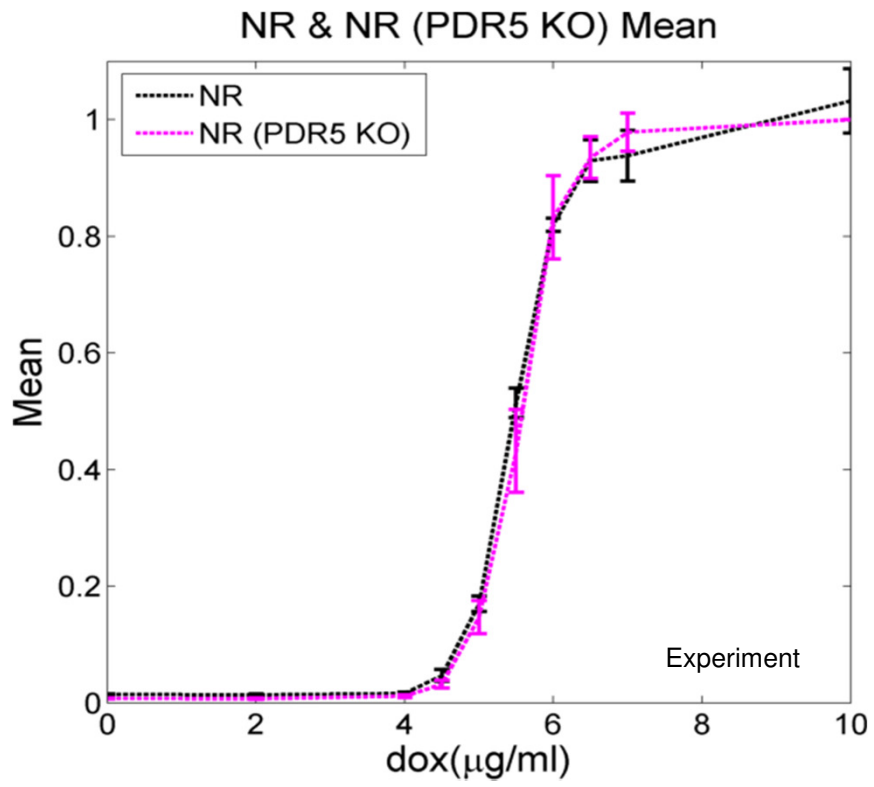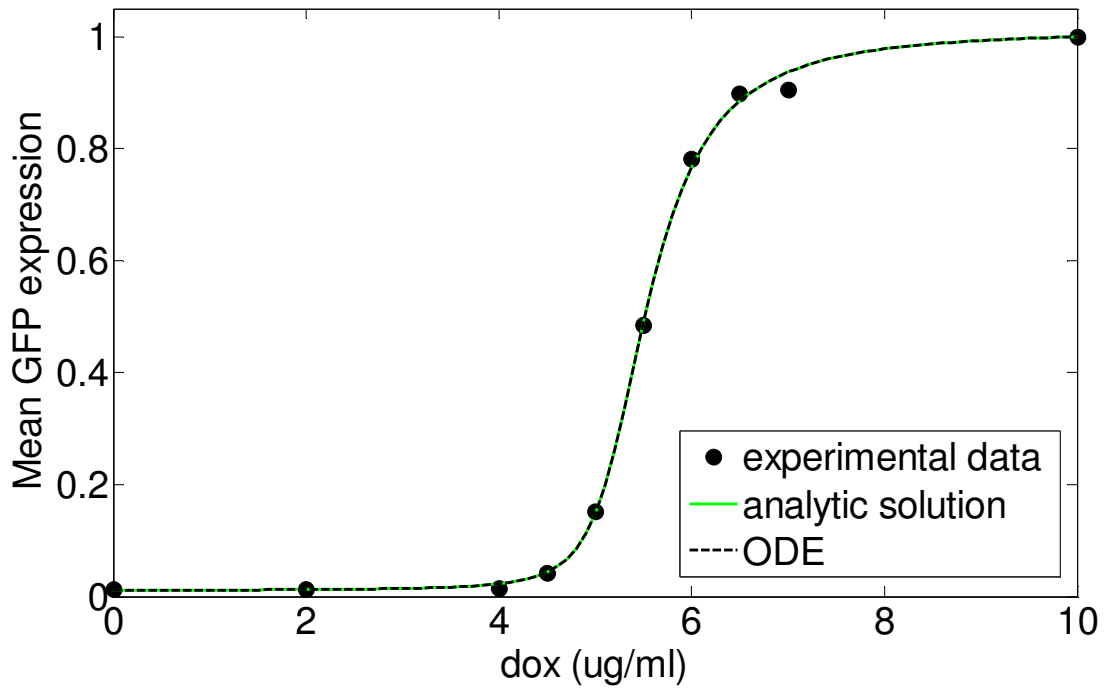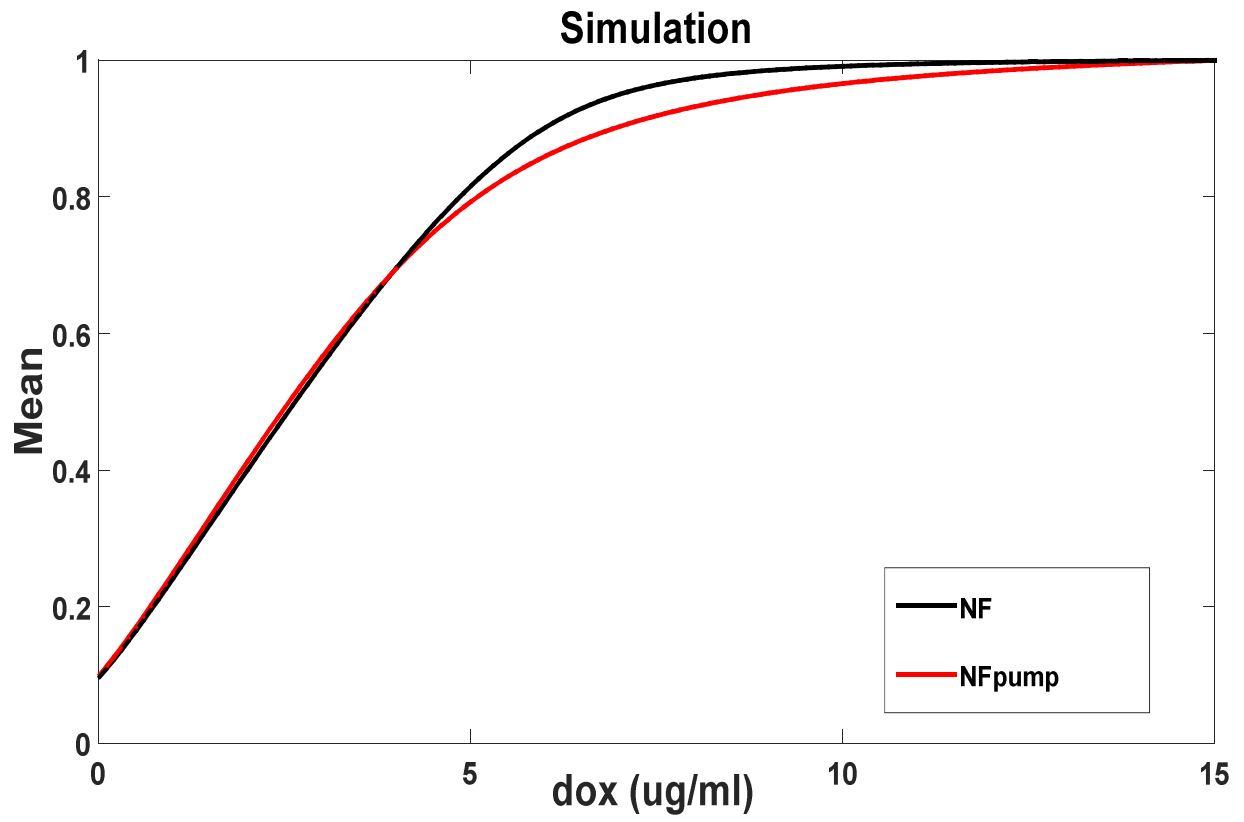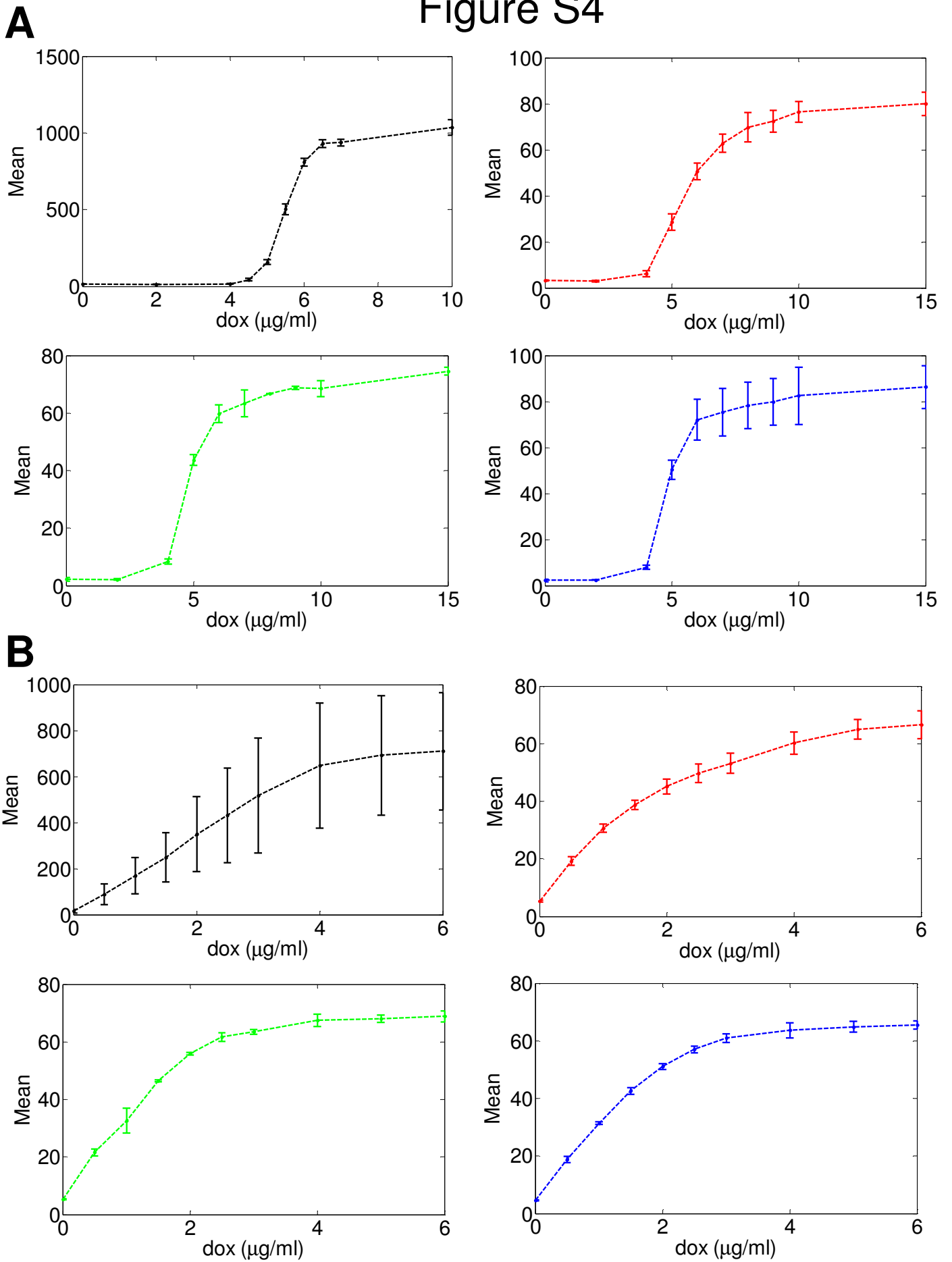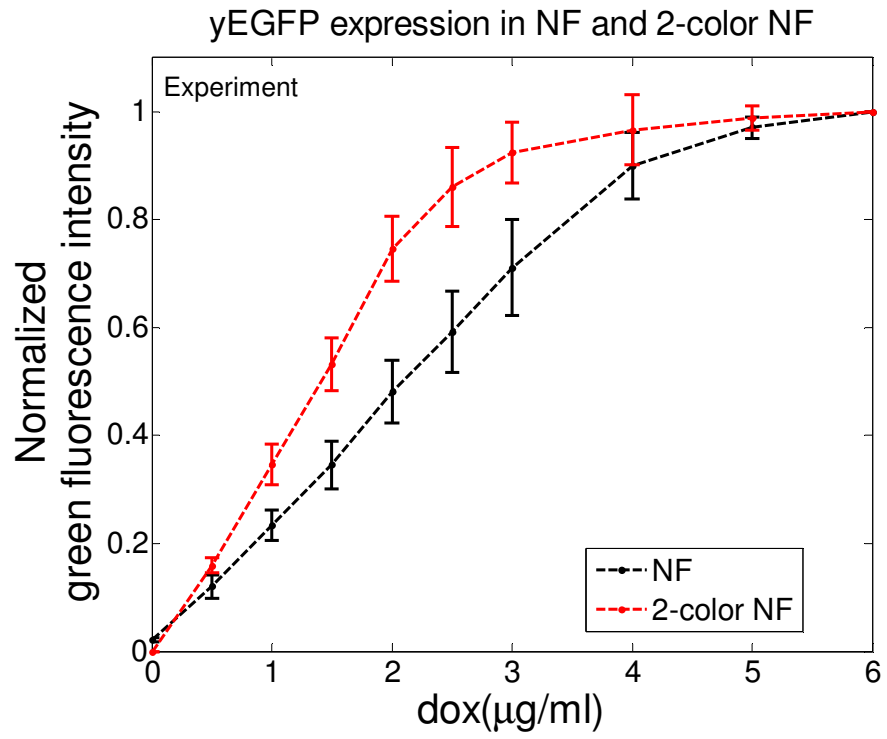
Figure S1

Figure S2

Figure S3

Figure S4

Figure S5

yEGFP expression in NF and 2-color NF

Figure S6
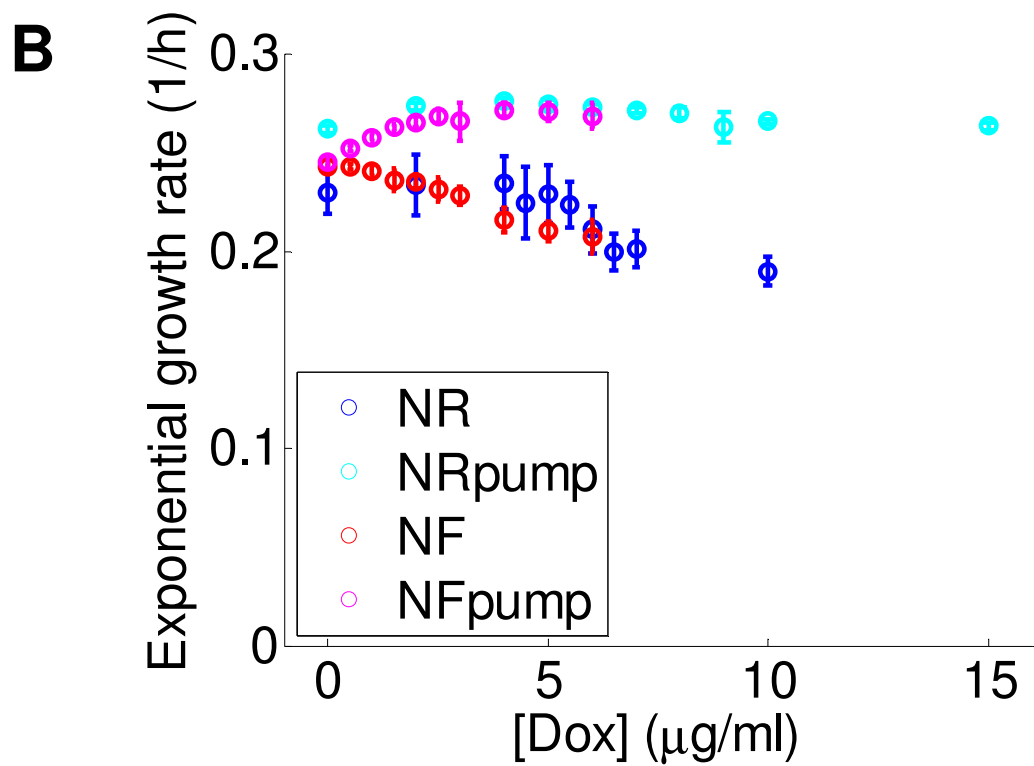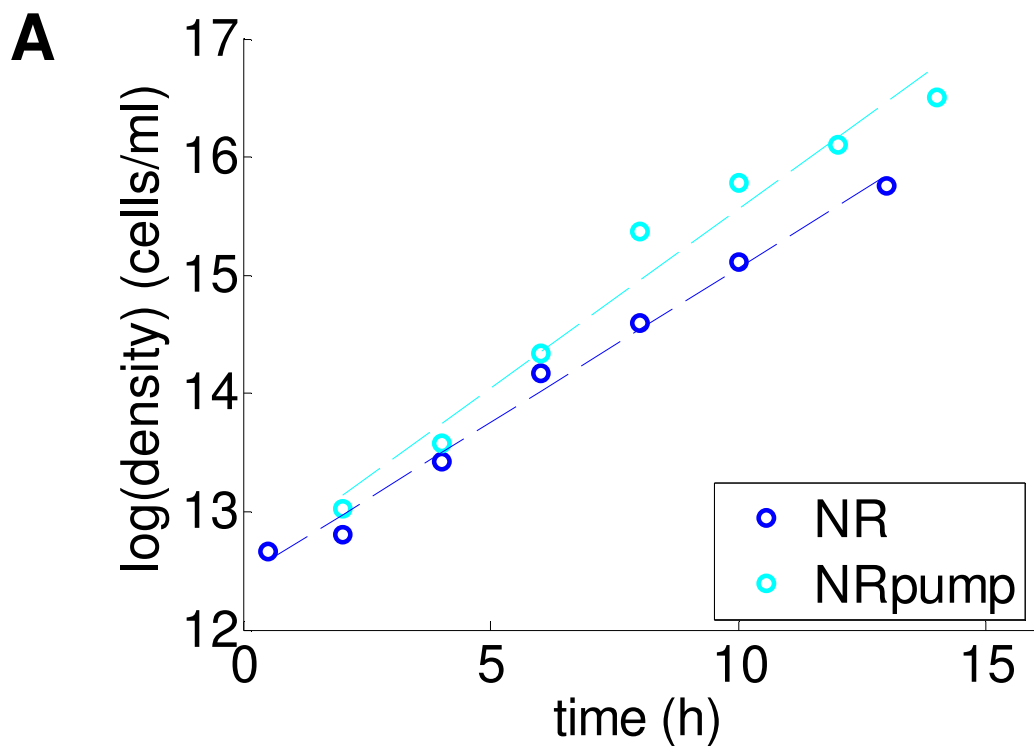
Table S1

Parameter	L1-Norm Mean GFP			L1-Norm CV GFP		
	-	**0**	**+**	**-**	**0**	**+**
a_x	**1.0715**	1.5933	4.5405	**0.2153**	0.3458	0.4646
a_z	**1.5756**	1.5933	1.6195	**0.3314**	0.3458	0.4759
l_z	**1.5111**	1.5933	1.7143	0.6053	**0.3458**	0.3525
$d_{x=d_z}$	1.7538	1.5933	**1.4083**	**0.3169**	0.3458	0.6501
d_y	**1.4894**	1.5933	1.7860	0.3475	**0.3458**	0.3469
b	1.9763	1.5933	**1.3179**	0.3842	**0.3458**	0.3694
n	**1.4777**	1.5933	1.6173	0.3494	**0.3458**	0.3459
C	4.4718	1.5933	**1.0398**	0.5375	0.3458	**0.2418**
k	**1.4667**	1.5933	1.7125	0.3597	0.3458	**0.3368**
h	3.4265	1.5933	**0.7894**	0.4109	**0.3458**	0.4054
K	2.0369	1.5933	**1.1830**	0.3835	**0.3458**	0.3815
$theta_z$	1.6111	1.5933	**1.4244**	0.3487	**0.3458**	0.3660