Multi-chromatic control of mammalian gene expression and signaling

Konrad Müller[1], Raphael Engesser[2,3], Simon Schulz[4], Thorsten Steinberg[4], Pascal Tomakidi[3,4], Cornelia C. Weber[5], Roman Ulm[6], Jens Timmer[2,3,7,8], Matias D. Zurbriggen[1] & Wilfried Weber[1,3,7]

[1]Faculty of Biology, University of Freiburg, Schänzlestrasse 1, 79104 Freiburg, Germany
[2]Physics Department, University of Freiburg, Hermann-Herder-Str. 3,79104 Freiburg, Germany
[3]BIOSS Centre for Biological Signalling Studies, University of Freiburg, Schänzlestrasse 18, 79104 Freiburg, Germany
[4]Department of Oral Biotechnology, University Hospital Freiburg, Hugstetterstrasse 55, 79106 Freiburg, Germany
[5]Present address: Novartis Pharma AG, 4002 Basel, Switzerland
[6]Department of Botany and Plant Biology, University of Geneva, Sciences III, CH-1211 Geneva 4, Switzerland
[7]Freiburg Centre for Biosystems Analysis (ZBSA), University of Freiburg, Habsburgerstrasse 49, 79104 Freiburg, Germany
[8]Freiburg Institute for Advanced Studies (FRIAS), University of Freiburg, Albertstrasse 19, 79104 Freiburg, Germany

Supplementary Information	Development and parameterization of the quantitative mathematical model
Supplementary Figure S1	Extended mode of function of the UVB-inducible gene expression system
Supplementary Figure S2	Dose-response characteristics of UVB-inducible gene expression
Supplementary Figure S3	Profile likelihood of the estimated parameters
Supplementary Figure S4	Effect of illumination on endothelial cell permeability
Supplementary Table S1	DNA sequence information
Supplementary Table S2	Fitted model parameters obtained by a maximum likelihood estimation

Supplementary Information

Development and parameterization of the quantitative mathematical model

1. Derivation of a mathematical ODE model

In this chapter a mathematical model based on a system of ordinary differential equations (ODE) is derived.

We assume that all etr8 operator is saturated with UVR8 which means every etr_8 is bound to a pair of UVR8 and forms an etr8-$(UVR8)_2$ complex. The UVR8 pair can be either in a closed state $UVR8_2^{closed}$ or in an open state $UVR8_2^{open}$. The transition between these two bound states is described by mass action kinetics

$$UVR8_2^{closed} \underset{k_{deact}}{\overset{k_{act}\, I_{UV}}{\rightleftharpoons}} UVR8_2^{open}$$

where the transition from $UVR8_2^{closed}$ to $UVR8_2^{open}$ is proportional to the intensity I_{UV} of the UVB light (Cell culture medium contains substances absorbing in the UVB region, we expect a decrease in light intensity according to Lambert-Beer's law. Consequently, absorption by the medium will result in a smaller k_{act}, but will not change the linear relation between the light intensity and the homodimer dissociation velocity) .This leads to the following equations for the concentrations of the two states of UVR8:

$$\frac{d[UVR8_2^{closed}](t)}{dt} = k_{deact}\left[UVR8_2^{open}\right] - k_{act}\ I_{UV}\ [UVR8_2^{closed}]$$

$$\frac{d[UVR8_2^{open}](t)}{dt} = -k_{deact}\left[UVR8_2^{open}\right] + k_{act}\ I_{UV}\ [UVR8_2^{closed}]$$

The transcription factor COP1(WD40)-VP16 binds to $UVR8_2^{open}$ and triggers the expression of the target mRNA. This activation can be described by using Monod kinetics

$$\frac{d[mRNA](t)}{dt} = k_{basal,mRNA} + k_{prod,mRNA}[UVR8_2^{open}]\frac{[VP16]^2}{K_m^2+[VP16]^2}.$$

$k_{basal,mRNA}$ is the basal promoter activity. The concentration of COP1(WD40)-VP16 is assumed to be constant. Therefore the fraction can be condensed into $k_{prod,mRNA}$

$$k_{prod,mRNA}\frac{[VP16]^2}{K_m^2+[VP16]^2} \longrightarrow k_{prod,mRNA}.$$

The mRNA expression can be turned off by the addition of erythromycin. This is modeled by introducing a factor $(1 - EM)$ to the mRNA production rate with $EM = 1$ when erythromycin is added and $EM = 0$ otherwise:

$$\frac{d[mRNA](t)}{dt} = k_{basal,mRNA} + k_{prod,mRNA}[UVR8_2^{open}](1 - EM)$$

The mRNA is subsequently transported to the cytoplasm for translation and secretion of the SEAP protein. These steps result in a time delay in the dynamics of the concentration of SEAP to the dynamics of the nuclear mRNA concentration. This time delay can be modeled by introducing a second volume compartment V_{cyt} for the mRNA in which the mRNA gets transported from the nucleus:

$$mRNA_{nuc} \xrightarrow{k_{mRNAnuc2cyt}} mRNA_{cyt} \xrightarrow{k_{deg,mRNAcyt}} \emptyset$$

The mRNA in the cytoplasm is linearly degraded with the rate $\mathrm{k_{deg,mRNAcyt}}$. This leads to the equations for the kinetic of the mRNA in the two compartments:

$$\frac{d[mRNA_{nuc}](t)}{dt} = k_{basal,mRNA} + k_{prod,mRNA}[UVR8_2^{open}](1 - EM) - k_{mRNAnuc2cyt}\,[mRNA_{nuc}]$$

$$\frac{d[mRNA_{cyt}](t)}{dt} = k_{mRNAnuc2cyt}\,[mRNA_{nuc}] - k_{\mathrm{deg},mRNAcyt}[mRNA_{cyt}]$$

SEAP is secreted into the medium. Therefore the production rate of SEAP is proportional to the number of cells N

$$\frac{d[SEAP](t)}{dt} = k_{tl,SEAP}\,\left[mRNA_{cyt}\right] N.$$

SEAP is very stable ($T_{1/2} > 500\ h$), thus the linear degradation rate can be neglected.

The number of cells is changing over time. We assume exponential cell growth with the growth rate k_{growth}. UVB-induced cytotoxicity is described by a first-order degradation rate proportional to the light intensity I_{UV}:

$$\frac{dN(t)}{dt} = k_{growth}\,N - k_{deg,cells}\,I_{UV}\,N$$

We assumed a doubling time of 14 hours corresponding to $\mathrm{k_{growth}} = 4.95\ \times 10^{-2}\mathrm{h}^{-1}$.

The used plasmids are transfected transiently into the cells and are not replicated during cell division. Therefore we have to introduce an equation for the gene dose (GD) of the transfected plasmids to describe the dilution caused by cell growth. This means the dilution rate $k_{dilution}$ equals the growth rate k_{growth}. The parameters $k_{basal,mRNA}$ and $k_{prod,mRNA}$ depend on the plasmid dose and have to be modified with the gene dose GD. By setting the initial gene dose to one, GD can be seen as dilution factor.

This leads to the following ODE model which was used for the further analysis in this study:

$$\frac{d[UVR8_2^{closed}](t)}{dt} = k_{deact}\left[UVR8_2^{open}\right] - k_{act}\ I_{UV}\ [UVR8_2^{closed}] \quad (1)$$

$$\frac{d[UVR8_2^{open}](t)}{dt} = -k_{deact}\left[UVR8_2^{open}\right] + k_{act}\ I_{UV}\ [UVR8_2^{closed}] \quad (2)$$

$$\frac{d[mRNA_{nuc}](t)}{dt} = GD\left(k_{basal,mRNA} + k_{prod,mRNA}[UVR8_2^{open}](1-EM)\right) - k_{mRNAnuc2cyt}\ [mRNA_{nuc}] \quad (3)$$

$$\frac{d[mRNA_{cyt}](t)}{dt} = k_{mRNAnuc2cyt}\ [mRNA_{nuc}] - k_{\mathrm{deg},mRNAcyt}[mRNA_{cyt}] \quad (4)$$

$$\frac{d[SEAP](t)}{dt} = k_{tl,SEAP}\left[mRNA_{cyt}\right] N \quad (5)$$

$$\frac{dN(t)}{dt} = k_{growth}\ N - k_{deg,cells}\ I_{UV}\ N \quad (6)$$

$$\frac{dGD(t)}{dt} = -k_{dilution}\ GD \quad (7)$$

2. Parameterization of the model by fitting to experimental data

2.1. Maximum Likelihood estimation

For the estimation of the unknown parameters we use the same approach as described in (26) which is briefly repeated at this point. Our system can be written in the following scheme

$$\frac{d\boldsymbol{x}(t)}{dt} = \boldsymbol{f}(\boldsymbol{x}(t), \boldsymbol{u}(t), \boldsymbol{p})$$

$$\boldsymbol{y}(t) = g(\boldsymbol{x}(t), \boldsymbol{s}) + \epsilon(t).$$

The first equation is the ODE system Eq. (1)-(7) written in vectorized form with the vector of internal states $\boldsymbol{x}(t)$, the set of dynamical parameters $\boldsymbol{p}$ and the model function $\boldsymbol{f}(\boldsymbol{x}(t), \boldsymbol{u}(t), \boldsymbol{p})$. The function $\boldsymbol{u}(t)$ is an external input, i.e. the intensity $I_{UV}(t)$ of the used UVB light in our system. The second equation connects the internal states $\boldsymbol{x}(t)$ with an observation $\boldsymbol{y}(t)$. The observation function g maps the internal state variables to the observable $\boldsymbol{y}(t)$. $\boldsymbol{s}$ are scaling parameters and $\epsilon(t)$ is the

measurement noise. To obtain a unique solution one needs also the initial concentrations $\boldsymbol{x}(0)$ of the internal states. The vector of initial concentrations can also depend on the dynamical parameters $\boldsymbol{p}$.

The measurement errors were modeled by an error model with a constant Gaussian error $\epsilon_0 \sim N(0, \sigma_0^2)$ with the variance σ_0^2.

With this error model we can calculate the likelihood function for a single experiment j:

$$L_j(\boldsymbol{y_j}|\boldsymbol{\theta_j}) = \frac{1}{\sqrt{2\pi}\,\sigma_0} \prod_{i=1}^{N_{data}} e^{-\frac{\left(y_{t_i} - g(x(t_i),s)\right)^2}{2\,\sigma_0^2}}$$

The vector $\boldsymbol{\theta_j} = (\boldsymbol{p}, \boldsymbol{x_j}(0), \boldsymbol{s_j}, \sigma_j)$ of all parameters depends on the conditions of the experiment j. $\boldsymbol{y_j} = (y_{t_1}, y_{t_2}, \dots, y_{t_{N_{data}}})$ is the vector of the measured data in the experiment j at the time points t_i.

The overall likelihood of multiple experiments is the product of the single likelihoods L_j over all experiments

$$L(\boldsymbol{y}|\boldsymbol{\theta}) = \prod_{j=1}^{N_{ex}} L_j(\boldsymbol{y}_j|\boldsymbol{\theta_j}).$$

$L(\boldsymbol{y}|\boldsymbol{\theta})$ is the probability of the data $\boldsymbol{y}$ given the parameters $\boldsymbol{\theta}$. The aim of a maximum likelihood estimation is to find the parameter set $\boldsymbol{\theta}$ that maximizes the likelihood function $L(\boldsymbol{y}|\boldsymbol{\theta})$

$$\boldsymbol{\theta_{opt}} = \underset{\boldsymbol{\theta}}{\operatorname{argmax}}(L(\boldsymbol{y}, \boldsymbol{\theta})).$$

Instead of maximizing the likelihood function $L(y|\theta)$ it is equivalent to minimize $\chi^2 = -2\log(L)$. For Gaussian distributed errors $\chi^2 = -2\ \log(L)$ is the sum of squared residuals with a second sum due to the error model:

$$\chi^2(\boldsymbol{\theta}, \mathbf{y}) = \sum_{\mathrm{i}} \mathrm{res}_{\mathrm{data,i}}^2 + \sum_{\mathrm{i}} \mathrm{res}_{\mathrm{error,i}}^2$$

Minimizing $\chi^2(\boldsymbol{\theta}, \mathbf{y})$

$$\boldsymbol{\theta_{opt}} = \arg\min_{\theta}\ \chi^2(\boldsymbol{\theta}, \mathbf{y})$$

is equivalent to a least squares problem.

The numerical integration of the ODE was performed with CVODES (59). For optimization we used a trust region algorithm implemented in MATLAB (lsqnonlin) (60) with user supplied sensitivities which

were calculated together with the ODE system. To improve convergence and scan the parameters over orders of magnitude the optimization was done in logarithmic parameter space. To find the global optimum we performed multiple optimization runs were the initial parameter guess was chosen from a latin hypercube sampling of the parameter space.

To determine the parameter uncertainties in terms of confidence intervals we used the approach in (45) and calculated the profile likelihood for each parameter θ_j

$$\chi^2_{PL}(\theta_j) = \min_{\theta_{i\neq j}} \chi^2(\boldsymbol{\theta}, \mathbf{y}).$$

2.2 Implementation of the single experiments

The model was fitted to three independent experiments with different observations and experimental conditions. We used in all experiments the same initial conditions for all state variables. As initial conditions for UVR8 and mRNA we used the steady state without UVB illumination and cell growth. The total amount of the bound UVR8 pairs is set to one. This means $\left[UVR8_2^{open}\right]$ is the fraction of etr8 operator which is active and can recruit COP1(WD40)-VP16. At the time point zero of each experiment the medium was exchanged. Therefore the initial concentration of SEAP is assumed to be zero.

$[UVR8_2^{closed}](0) = 1$

$\left[UVR8_2^{open}\right](0) = 0$

$[mRNA_{nuc}](0) = \frac{k_{basal,mRNA}}{k_{mRNAnuc2cyt}}$

$\left[mRNA_{cyt}\right](0) = \frac{k_{mRNAnuc2cyt}[mRNA_{nuc}](0)}{k_{\mathrm{deg},mRNAcyt}} = \frac{k_{basal,mRNA}}{k_{\mathrm{deg},mRNAcyt}}$

$[SEAP](0) = 0$

$N(0) = 1$

$\mathrm{GD}(0) = 1$

Experiment 1: Light-inducible expression kinetics: Measurement of SEAP mRNA

In this experiment CHO-K1 cells were engineered for UVB-inducible SEAP expression. After 24 h, the medium was exchanged and the cells were illuminated for 6 h at 311 nm with the intensity of 2.7 µmol m^{-2} s^{-1} and were then either kept under 311 nm (i) , moved to darkness (ii) or were supplemented with erythromycin (iii).

The experimental conditions were modeled with

(i) $I_{UV} = 2.7\ \mu mol\ m^{-2}\ s^{-1}$ and $EM = 0$

(ii) $I_{UV} = 2.7\ \mu mol\ m^{-2}\ s^{-1}$ for $t < 6\ h$

$I_{UV} = \ \ 0\ \mu mol\ m^{-2}\ s^{-1}$ for $t > 6\ h$

$EM = 0$

(iii) $I_{UV} = 2.7\ \mu mol\ m^{-2}\ s^{-1}$ and $EM = 0$ for $t < 6\ h$

$I_{UV} = \ \ 0\ \mu mol\ m^{-2}\ s^{-1}$ and $EM = 1$ for $t > 6\ h$

As observation function we used

$$[mRNA_{t,measured}] = [mRNA_{nuc}](t).$$

The data were scaled to the measured basal expression level at the time point zero. As scaling factor we used 1 which means the simulated mRNA concentrations are considered in units of basal mRNA expression. The experimental data and the model fit are shown in Figure 2a.

Experiment 2: Light-inducible expression kinetics: Measurement of SEAP protein

In this experiment the same experimental conditions like in experiment 1 were used. The measured output was the SEAP activity. The observation function was defined by

$$[SEAP_{t,measured}] = [SEAP](t).$$

This means this experiment determines the scale of the simulated SEAP concentrations. The experimental data and the model fit are shown in Figure 2b.

Experiment 3: Dose-response characteristics of UVB-inducible gene expression

In this experiment CHO-K1 cells were transfected with pKM168, pKM115 and pKM081 for UVB-inducible SEAP expression. After 24 h the medium was exchanged and the cells were illuminated with UVB light with different UVB intensities I_{UV}. After 24 h the SEAP expression and the viability of the cells were measured.

The observation function for SEAP was defined by

$$[SEAP_{I_{UV},measured}] = s_{Exp3,SEAP}[SEAP](t = 24h,\ I_{UV}).$$

The observation function for the viability was defined by

$$viability_{I_{UV},measured} = 100 \cdot \frac{N(t=24h,\ I_{UV})}{N(t=24h,\ I_{UV}=0)},$$

which determines the fraction of the number of cells after illuminating with I_{UV} to the number of cells growing in the dark for 24 hours. The experimental data and the model fit are shown in Supplementary Figure S1.

2.3 Fitting results

Our system has in total 13 unknown parameters, therefrom 8 dynamical, 4 error and 1 scaling parameter. The parameters were estimated from 186 data points. We ran 1000 optimizations with different initial parameter guesses $\boldsymbol{\theta_0}$ which were chosen from of a latin hypercube sample. We sampled each parameter from 10^{-6} to 10^{+4} which are 10 orders of magnitude. More than 80 % of all optimization runs found the same optimal parameter set $\boldsymbol{\theta_{opt}}$ with a value of the objective function $\chi^2(\boldsymbol{\theta_{opt}}) = -2\ \log\big(L(\boldsymbol{\theta_{opt}})\big) = 1168.66$. This is strong evidence, that $\boldsymbol{\theta_{opt}}$ is the global optimum. At $\boldsymbol{\theta_{opt}}$ we calculated the profile likelihood for each parameter to determine the uncertainties of the estimated parameters. All parameters have finite confidence intervals and therefore are identifiable by the measured data. The estimated parameters and the obtained confidence intervals $[\sigma^-, \sigma^+]$ with a confidence level of 95 % are shown in Supplementary Table S2: The calculated profile likelihoods with indicated 95 % confidence levels can be found in Supplementary Figure S3.

3. Predictions with the ODE model

3.1. Prediction of the promoter activity

For the prediction of the ON and OFF kinetics the activity of the target promoter (PA) was calculated. The activity of a single promoter is proportional to

$$PA_{prediction} = k_{basal,mRNA} + k_{prod,mRNA}[UVR8_2^{open}](1 - EM).$$

The promoter activity was simulated with the estimated parameter set $\boldsymbol{\theta_{opt}}$ with the experimental conditions used in experiment 1 and 2. The uncertainties of the estimated parameter where translated into a uncertainty of the prediction. As proposed in (46) this was done by simulating the predicted PA for all parameter sets along the profile likelihood profile of

each parameter. The predicted promoter activity is shown in Figure 2c, the shaded bands are indicating the 95 % prediction confidence interval.

3.2 Pulsed light

With the parameterized model it is possible to make predictions with more sophisticated light conditions, e.g. pulsed illumination. This is useful to find *in silico* optimal conditions leading to high gene induction while minimizing cytotoxic effects of the UVB light.

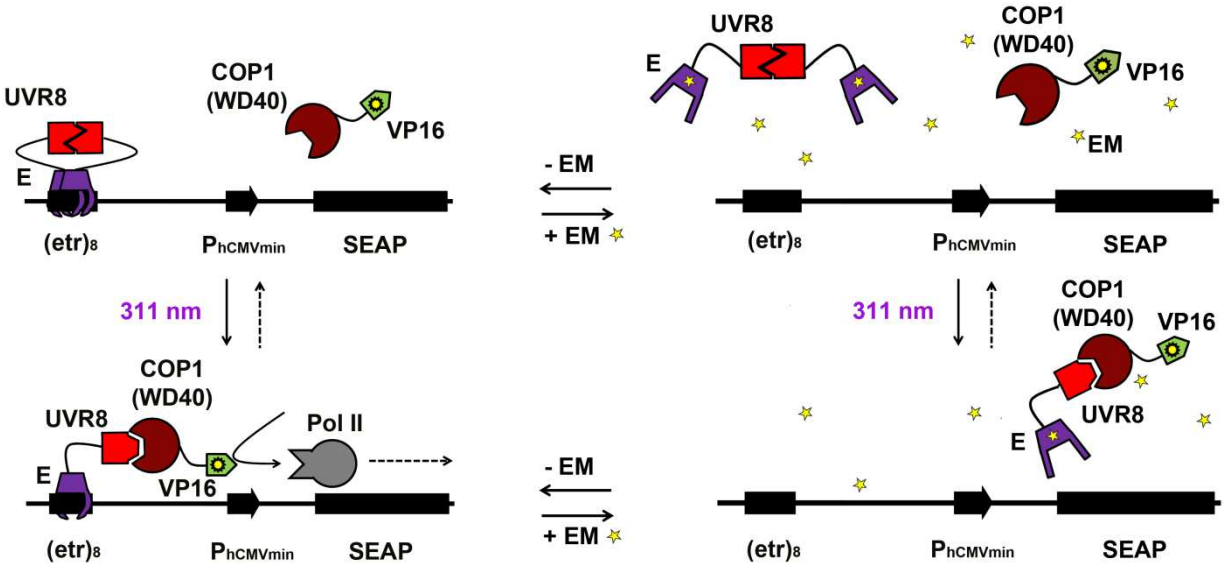
The pulsed light $I_{UV}(t)$ was simulated with a linear combination of Heaviside step functions which were approximated by the arctangent function. The system was simulated over 24 hours with pulses in 30 minutes cycles and pulse durations from 0 to 30 minutes. The predicted output was defined by

$[SEAP_{I_{UV}(t),prediction}] = s_{pulse}[SEAP]\big(t = 24h,\ \boldsymbol{\theta_{opt}},\ I_{UV}(t)\big)$ and

$$viability_{I_{UV}(t),prediction} = 100 \cdot \frac{N\big(t=24h,\ \boldsymbol{\theta_{opt}},\ I_{UV}(t)\big)}{N\big(t=24h,\ \boldsymbol{\theta_{opt}},\ I_{UV}=0\big)}.$$

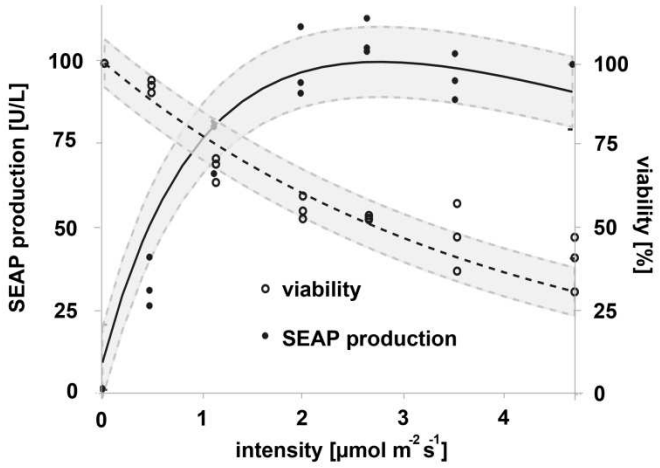
The scaling parameter s_{pulse} is necessary to scale the predicted SEAP production to the data which confirm the prediction. The resulting curves are shown in Figure 2d.

Supplementary Figure S1



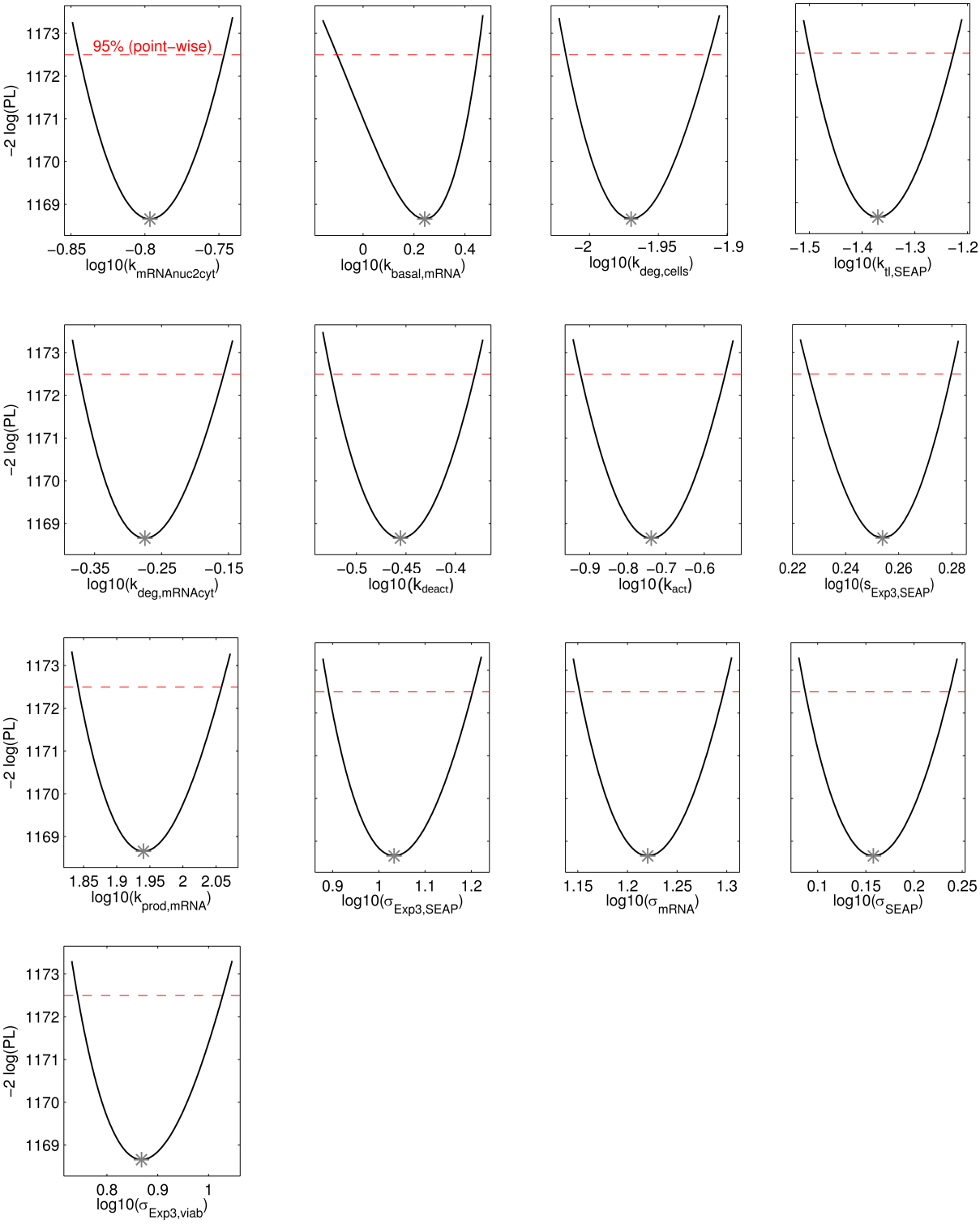
Extended mode of function of the UVB-inducible gene expression system. (Left) In the dark UVR8 is tethered to the operator sequence in the closed configuration but cannot interact with COP1(WD40). Upon illumination with 311 nm light, the UVR8 transits from the closed to the open state and recruits COP1(WD40)-VP16, to result in the activation of $P_{hCMVmin}$. Upon shifting to the dark, UVR8 spontaneously assumes the closed configuration, thereby resulting in a gradual shut-down of gene expression. **(Right)** Addition of erythromycin (EM) results in dissociation of E from its operator site resulting in shut-off of gene expression regardless of illumination.

Supplementary Figure S2



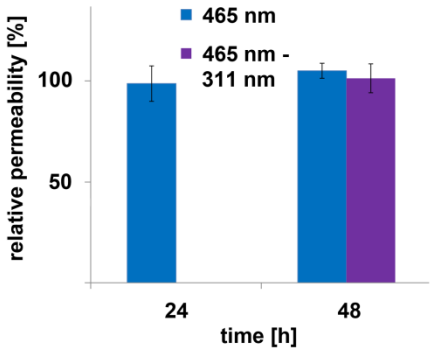
Dose-response characteristics of UVB-inducible gene expression. CHO-K1 cells were transfected with pKM168, pKM115 and pKM081 for UVB-inducible SEAP expression. After 24 h, the medium was exchanged and the cells were illuminated with UVB light at the indicated intensities for another 24 h prior to quantification of SEAP production and cell viability, using the WST-1 assay. SEAP production values are normalized to the highest observed intensity (100 %), whereas cell viability is normalized to the one of control cells incubated in the dark (100 %). The lines represent the fit of the model to the data points and the shaded error bands the error estimated by a simple error model with a constant Gaussian error.

Supplementary Figure S3



Profile likelihood of the estimated parameters. The solid lines indicate the profile likelihood, the optimal parameter set is marked with a grey star. The red dashed line marks the 95 % confidence level. The parameter axis is on a logarithmic scale.

Supplementary Figure S4



Effect of illumination on endothelial cell permeability. Endothelial cell monolayers were co-cultured with mock-transfected HEK293-T cells and illuminated as indicated. After 24 h and after 48 h the trans-layer permeation of fluorescently labeled dextran was quantified. The permeability is normalized to the control value obtained from monolayers of endothelial cells that were co-cultivated with mock-transfected HEK-293T cells and were incubated in the dark. Data are means ± SD (n=4).

Supplementary Table S1

DNA sequence information.

Designation	Nucleotide Sequence
pKM168 E-UVR8(12-381)	Upper case in 5’ -> 3’ direction, E and UVR8(12-381), underlined sequences in 5’ -> 3’ direction, restriction sites, *Not*I, *Eco*RI, *Kpn*I, *Bam*HI, *Hin*dIII 5’-...gcggccgcgaattcccaccATGCCCCGCCCCAAGCTCAAGTCCGATGACGAGGTACTCGAGGCCGCCACC GTAGTGCTGAAGCGTTGCGGTCCCATAGAGTTCACGCTCAGCGGAGTAGCAAAGGAGGTGGGGCTCTCCCGCGCAG CGTTAATCCAGCGCTTCACCAACCGCGATACGCTGCTGGTGAGGATGATGGAGCGCGGCGTCGAGCAGGTGCGGCA TTACCTGAATGCGATACCGATAGGCGCAGGGCCGCAAGGGCTCTGGGAATTTTTGCAGGTGCTCGTTCGGAGCATG AACACTCGCAACGACTTCTCGGTGAACTATCTCATCTCCTGGTACGAGCTCCAGGTGCCGGAGCTACGCACGCTTG CGATCCAGCGGAACCGCGCGGTGGTGGAGGGGATCCGCAAGCGACTGCCCCCAGGTGCTCCTGCGGCAGCTGAGTT GCTCCTGCACTCGGTCATCGCTGGCGCGACGATGCAGTGGGCCGTCGATCCGGATGGTGAGCTAGCTGATCATGTG CTGGCTCAGATCGCTGCCATCCTGTGTTTAATGTTTCCCGAACACGACGATTTCCAACTCCTCCAGGCACATGCGT CCGCGTACAGCggtaccggcgccggcgccGCTCCTCCTCGTAAGGTTCTTATCATCTCCGCTGGTGCTAGCCACTC CGTCGCTCTTCTCTCTGGTGACATTGTTTGTTCTTGGGGTCGAGGAGAGGATGGACAGTTAGGTCATGGCGATGCA GAGGATCGACCTTCTCCGACTCAGCTTAGCGCTTTAGATGGCCACCAAATTGTTTCCGTTACCTGTGGTGCTGATC ACACTGTTGCTTATTCACAATCAGGCATGGAAGTCTACAGTTGGGGATGGGGTGATTTTGGGAGATTAGGCCATGG TAACTCAAGCGACTTGTTTACTCCGCTACCAATCAAAGCATTGCACGGTATTCGGATCAAGCAGATTGCTTGTGGG GATAGTCATTGTTTGGCTGTCACTATGGAAGGAGAGGTCCAGAGTTGGGGCCGCAACCAGAATGGTCAACTTGGTC TGGGGGACACCGAAGATTCTCTAGTGCCTCAGAAGATTCAAGCCTTTGAGGGAATACGAATCAAAATGGTTGCTGC TGGTGCAGAACACACTGCTGCAGTTACAGAAGATGGTGACCTCTATGGATGGGGCTGGGGAAGATACGGAAATTTG GGATTAGGTGACCGGACTGACCGCTTAGTTCCTGAAAGAGTTACCTCTACTGGTGGTGAGAAAATGTCAATGGTTG CTTGTGGATGGCGGCACACAATATCAGTTTCCTACTCTGGAGCATTGTATACTTATGGATGGAGCAAATATGGACA GCTAGGACATGGAGACTTGGAGGATCACCTTATTCCTCACAAACTGGAAGCACTGAGCAACAGTTTTATCTCCCAG ATTTCGGGAGGTTGGAGACATACAATGGCATTGACTTCAGATGGAAAACTATATGGATGGGGTTGGAATAAGTTTG GACAAGTAGGAGTCGGCAATAATTTAGATCAGTGTTCTCCTGTGCAAGTGCGATTTCCCGATGATCAGAAAGTAGT TCAAGTCTCATGTGGATGGAGACATACCTTGGCTGTCACTGAAAGAAATAACGTGTTTGCTTGGGGTAGAGGTACA AATGGACAGCTCGGCATTGGAGAGTCGGTTGACAGGAACTTTCCCAAGATTATAGAGGCACTCAGCGTCGATGGAT AAggatccaagcttc...-3’
pKM115 COP1(WD40)-VP16	Upper case in 5’ -> 3’ direction, COP1(WD40) and VP16, underlined sequences in 5’ -> 3’ direction, restriction sites, *Not*I, *Bss*HII, *Bam*HI 5’-...gcggccgcgccaccATGTATAGCAACGGCCTTGCAGATTTTCAATCTGTGTTGACTACCTTCACTCGCTA CAGTCGTCTAAGAGTTATAGCAGAAATCCGGCATGGGGATATATTTCATTCAGCCAACATTGTATCAAGCATAGAG TTTGATCGTGATGATGAGCTGTTTGCCACTGCTGGTGTTTCTAGATGTATAAAGGTTTTTGACTTCTCTTCGGTTG TAAATGAACCAGCAGATATGCAGTGTCCGATTGTGGAGATGTCAACTCGGTCTAAACTTAGTTGCTTGAGTTGGAA TAAGCATGAAAAAAATCACATAGCAAGCAGTGATTATGAAGGAATAGTAACAGTGTGGGATGTAACTACTAGGCAG AGTCTTATGGAGTATGAAGAGCACGAAAAACGTGCCTGGAGTGTTGACTTTTCACGAACAGAACCATCAATGCTTG TATCTGGTAGTGACGACTGCAAGGTTAAAGTTTGGTGCACGAGGCAGGAAGCAAGTGTGATTAATATTGATATGAA AGCAAACATATGTTGTGTCAAGTACAATCCTGGCTCAAGCAACTACATTGCGGTCGGATCAGCTGATCATCACATC CATTATTACGATCTAAGAAACATAAGCCAACCACTTCATGTCTTCAGTGGACACAAGAAAGCAGTTTCCTATGTTA AATTTTTGTCCAACAACGAGCTCGCTTCTGCGTCCACAGATAGCACACTACGCTTATGGGATGTCAAAGACAACTT GCCAGTTCGAACATTCAGAGGACATACTAACGAGAAGAACTTTGTGGGTCTCACAGTGAACAGCGAGTATCTCGCC TGTGGAAGCGAGACAAACGAAGTATATGTATATCACAAGGAAATCACGAGACCCGTGACATCGCACAGATTTGGAT CGCCAGACATGGACGATGCAGAGGAAGAGGCAGGTTCCTACTTTATTAGTGCGGTTTGCTGGAAGAGTGATAGTCC CACGATGTTGACTGCGAATAGTCAAGGAACCATCAAAGTTCTGGTACTCGCTGCGggcgccggcgcgcgtACGAAA AACAATTACGGGTCTACCATCGAGGGCCTGCTCGATCTCCCGGACGACGACGCCCCCGAAGAGGCGGGGCTGGCGG CTCCGCGCCTGTCCTTTCTCCCCGCGGGACACACGCGCAGACTGTCGACGGCCCCCCCGACCGATGTCAGCCTGGG GGACGAGCTCCACTTAGACGGCGAGGACGTGGCGATGGCGCATGCCGACGCGCTAGACGATTTCGATCTGGACATG TTGGGGGACGGGGATTCCCCGGGTCCGGGATTTACCCCCCACGACTCCGCCCCCTACGGCGCTCTGGATATGGCCG ACTTCGAGTTTGAGCAGATGTTTACCGATGCCCTTGGAATTGACGAGTACGGTGGGTAGggggcgcgaggatcc...-3’
pKM081 etr_8-P_{CMVmin}-SEAP	Upper case in 5’ -> 3’ direction, etr_8, P_{CMVmin} and SEAP, underlined sequences in 5’ -> 3’ direction, restriction sites, *Nhe*I, *Eco*RI, *Sph*I, *Hpa*I 5’-...GATTGAATATAACCGACGTGACTGTTACATTTAGGGATTGAATATAACCGACGTGACTGTTACATTTAGG GATTGAATATAACCGACGTGACTGTTACATTTAGGGATTGAATATAACCGACGTGACTGTTACATTTAGGGATAAA

	CGATTGAATATAACCGACGTGACTGTTACATTTAGGGATTGAATATAACCGACGTGACTGTTACATTTAGGGATTG AATATAACCGACGTGACTGTTACATTTAGGGATTGAATATAACCGACGTGACTGTTACATTTAGGgatgctagcCC TATATAAGCAGAGCTCGTTTAGTGAACCGTCAGATCGCCTGGAGACGCCATCCACGCTGTTTTGACCTCCATAGAA GACACCGGGACCGATCCAGCCTccgcggccccggtaccgaattcgagctcgcccggggatcctctagagtcagctt cctgcATGCTGCTGCTGCTGCTGCTGCTGGGCCTGAGGCTACAGCTCTCCCTGGGCATCATCCCAGTTGAGGAGGA GAACCCGGACTTCTGGAACCGCGAGGCAGCCGAGGCCCTGGGTGCCGCCAAGAAGCTGCAGCCTGCACAGACAGCC GCCAAGAACCTCATCATCTTCCTGGGCGATGGGATGGGGGTGTCTACGGTGACAGCTGCCAGGATCCTAAAAGGGC AGAAGAAGGACAAACTGGGGCCTGAGATACCCCTGGCCATGGACCGCTTCCCATATGTGGCTCTGTCCAAGACATA CAATGTAGACAAACATGTGCCAGACAGTGGAGCCACAGCCACGGCCTACCTGTGCGGGGTCAAGGGCAACTTCCAG ACCATTGGCTTGAGTGCAGCCGCCCGCTTTAACCAGTGCAACACGACACGCGGCAACGAGGTCATCTCCGTGATGA ATCGGGCCAAGAAAGCAGGGAAGTCAGTGGGAGTGGTAACCACCACACGAGTGCAGCACGCCTCGCCAGCCGGCAC CTACGCCCACACGGTGAACCGCAACTGGTACTCGGACGCCGACGTGCCTGCCTCGGCCCGCCAGGAGGGGTGCCAG GACATCGCTACGCAGCTCATCTCCAACATGGACATTGACGTGATCCTAGGTGGAGGCCGAAAGTACATGTTTCGCA TGGGAACCCCAGACCCTGAGTACCCAGATGACTACAGCCAAGGTGGGACCAGGCTGGACGGGAAGAATCTGGTGCA GGAATGGCTGGCGAAGCGCCAGGGTGCCCGGTATGTGTGGAACCGCACTGAGCTCATGCAGGCTTCCCTGGACCCG TCTGTGACCCATCTCATGGGTCTCTTTGAGCCTGGAGACATGAAATACGAGATCCACCGAGACTCCACACTGGACC CCTCCCTGATGGAGATGACAGAGGCTGCCCTGCGCCTGCTGAGCAGGAACCCCCGCGGCTTCTTCCTCTTCGTGGA GGGTGGTCGCATCGACCATGGTCATCATGAAAGCAGGGCTTACCGGGCACTGACTGAGACGATCATGTTCGACGAC GCCATTGAGAGGGCGGGCCAGCTCACCAGCGAGGAGGACACGCTGAGCCTCGTCACTGCCGACCACTCCCACGTCT TCTCCTTCGGAGGCTACCCCCTGCGAGGGAGCTCCATCTTCGGGCTGGCCCCTGGCAAGGCCCGGGACAGGAAGGC CTACACGGTCCTCCTATACGGAAACGGTCCAGGCTATGTGCTCAAGGACGGCGCCCGGCCGGATGTTACCGAGAGC GAGAGCGGGAGCCCCGAGTATCGGCAGCAGTCAGCAGTGCCCCTGGACGAAGAGACCCACGCAGGCGAGGACGTGG CGGTGTTCGCGCGCGGCCCGCAGGCGCACCTGGTTCACGGCGTGCAGGAGCAGACCTTCATAGCGCACGTCATGGC CTTCGCCGCCTGCCTGGAGCCCTACACCGCCTGCGACCTGGCGCCCCCCGCCGGCACCACCGACGCCGCGCACCCG GGTTAAc...-3′
pKM085 Gal4(1-65)-VVD-p65	Upper case in 5′ -> 3′ direction, Gal4(1-65), VVD and p65 transactivation domain, underlined sequences in 5′ -> 3′ direction, restriction sites *Kpn*I, *Not*I 5′-...ggtaccGCCACCATGAAGCTGCTGTCCTCCATCGAGCAGGCCTGTGACATCTGCCGGCTGAAGAAACTGA AGTGCTCCAAAGAAAAGCCCAAGTGCGCCAAGTGCCTGAAGAACAACTGGGAGTGCCGGTACAGCCCCAAGACCAA GCGGTCCCCTCTGACCAGAGCCCACCTGACCGAGGTGGAATCTCGGCTGGAAAGACTGGAAcggtctatcgccacc cggtccCACACCCTGTATGCCCCTGGCGGCTACGACATCATGGGCTACCTGATCCAGATCATGAAGCGGCCCAACC CCCAGGTGGAACTGGGCCCTGTGGATACCTCTGTGGCCCTGATCCTGTGCGACCTGAAGCAGAAAGACACCCCCAT CGTGTACGCCTCCGAGGCCTTCCTGTACATGACCGGCTACTCCAACGCCGAGGTGCTGGGCCGGAACTGCAGATTC CTGCAGTCCCCTGACGGCATGGTCAAGCCTAAGTCCACCCGGAAATACGTGGACTCTAACACCATCAACACCATGC GGAAGGCCATCGACCGGAACGCTGAGGTGCAGGTGGAAGTCGTGAACTTCAAGAAGAACGGCCAGCGCTTCGTGAA TTTCCTGACCATGATCCCCGTGCGGGACGAGACAGGCGAGTACAGATACTCCATGGGCTTCCAGTGCGAGACAGAG ctgcagtacccctacgacgtgcccgactacgccgagttcCAGTACCTGCCTGACACCGACGACCGGCACCGGATCG AGGAAAAGCGGAAGCGGACCTACGAGACATTCAAGTCCATCATGAAGAAGTCCCCCTTCTCCGGCCCCACCGACCC TAGACCTCCACCTAGAAGAATCGCCGTGCCCTCCAGATCCTCCGCCTCCGTGCCTAAACCTGCCCCCCAGCCTTAC CCCTTCACCTCCAGCCTGTCTACCATCAACTACGATGAGTTCCCTACCATGGTGTTCCCCAGCGGCCAGATCTCTC AGGCCTCTGCTCTGGCTCCAGCCCCTCCTCAGGTGCTGCCTCAGGCTCCTGCTCCTGCACCAGCTCCAGCCATGGT GTCTGCACTGGCTCAGGCACCAGCACCCGTGCCTGTGCTGGCTCCTGGACCTCCACAGGCTGTGGCTCCTCCAGCT CCTAAGCCTACACAGGCTGGCGAGGGCACCCTGTCTGAAGCTCTGCTGCAGCTGCAGTTCGACGACGAGGACCTGG GTGCCCTGCTGGGCAACTCTACCGATCCTGCCGTGTTCACCGACCTGGCCTCCGTGGACAACTCTGAGTTCCAGCA GCTGCTGAACCAGGGCATCCCTGTGGCCCCTCATACCACCGAGCCCATGCTGATGGAATACCCCGAGGCCATCACC CGGCTCGTGACAGGTGCTCAGAGGCCTCCTGATCCCGCTCCAGCACCACTGGGAGCACCTGGCCTGCCTAATGGAC TGCTGTCCGGCGACGAGGATTTCTCCTCTATCGCCGACATGGACTTCTCCGCCCTGCTGTCCCAGATCTCCTCCGA CTACAAGGACGACGACGACAAATGAgcggccgc...-3′

Supplementary Table S2

Fitted model parameters obtained by a maximum likelihood estimation

Parameter	$\boldsymbol{\theta_{opt,i}}$	$\boldsymbol{\sigma^-}$	$\boldsymbol{\sigma^+}$	Unit
k_{deact}	0.3503	0.2980	0.4166	h^{-1}
k_{act}	0.1826	0.1193	0.2851	$h^{-1} \cdot (\mu mol\ m^{-2}\ s^{-1})^{-1}$
$k_{basal,mRNA}$	1.7462	0.7918	2.8190	$[mRNA_{nuc}](0) \cdot h^{-1}$
$k_{prod,mRNA}$	87.182	69.449	114.335	$[mRNA_{nuc}](0) \cdot h^{-1} \cdot (\mu mol\ m^{-2}\ s^{-1})^{-1}$
$k_{mRNAnuc2cyt}$	0.1597	0.1431	0.1793	h^{-1}
$k_{\mathrm{deg},mRNAcyt}$	0.5312	0.4231	0.6965	h^{-1}
$k_{tl,SEAP}$	0.0426	0.0317	0.0594	$\mathrm{U/L} \cdot \left[mRNA_{cyt}\right](0)^{-1} \cdot h^{-1}$
$k_{deg,cells}$	0.01072	0.0096	0.0122	$h^{-1} \cdot (\mu mol\ m^{-2}\ s^{-1})^{-1}$
$s_{Exp3,SEAP}$	1.7943	1.6835	1.9045	1
σ_{SEAP}	1.4382	1.2221	1.7244	U/L
σ_{mRNA}	16.6106	14.179	19.807	$[mRNA_{nuc}](0)$
$\sigma_{Exp3,SEAP}$	10.7874	7.7870	15.945	U/L
$\sigma_{Exp3,viab}$	7.3798	5.5220	10.658	%

σ^- and σ^+ indicate the 95% point-wise confidence interval obtained by exploiting the profile likelihood.

Supplementary References

26. Muller, K., Engesser, R., Metzger, S., Schulz, S., Kampf, M.M., Busacker, M., Steinberg, T., Tomakidi, P., Ehrbar, M., Nagy, F. *et al.* (2013) A red/far-red light-responsive bi-stable toggle switch to control gene expression in mammalian cells. *Nucleic Acids Res*.

45. Raue, A., Kreutz, C., Maiwald, T., Bachmann, J., Schilling, M., Klingmuller, U. and Timmer, J. (2009) Structural and practical identifiability analysis of partially observed dynamical models by exploiting the profile likelihood. *Bioinformatics*, **25**, 1923-1929.

46. Raue, A., Becker, V., Klingmuller, U. and Timmer, J. (2010) Identifiability and observability analysis for experimental design in nonlinear dynamical models. *Chaos*, **20**, 045105.

59. Hindmarsh, A.C., Brown, P.N., Grant, K.E., Lee, S.L., Serban, R., Shumaker, D.E. and Woodward, C.S. (2005) SUNDIALS: Suite of nonlinear and differential/algebraic equation solvers. *Acm T Math Software*, **31**, 363-396.

60. Coleman, T.F. and Li, Y.Y. (1996) An interior trust region approach for nonlinear minimization subject to bounds. *Siam J Optimiz*, **6**, 418-445.