Supplementary Information for: Nonlinear signalling networks and cell-to-cell variability transform external signals into broadly distributed or bimodal responses

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1 Derivation of Equation 2 in the main text

A new random variable Y results from transformation of the existing random variable Z using a function $R(Z)$. The probability density function, *pdf*, of random variable Z is known and expressed as $f_Z(z)$. The goal is to obtain the *pdf* of Y, $f_Y(y)$ [1]. In our case, random variable Z represents cell-to-cell variability of the parameters that describe the response function $R(X)$, i.e. the input stimulus x, response threshold x_{50}, maximum response R_{max}, basal response β or response steepness H. For our purposes, the *pdf* $f_X(x)$ is a log-normal distribution.

The cumulative distribution function, CDF, of Y, can be expressed as:

$$F_Y(y) = P[Y \leq y] = P[R(Z) \leq y] = P[Z \in D_Y], \tag{1}$$

where the set $D_Y = z : R(Z) \leq y = z : z \leq r(y)$, where $r(y)$ is the inverse function of R. Equipped with these definitions we can express the CDF of Y in terms of the CDF of Z:

$$F_Y(y) = P[Z \leq r(y)] = F_Z(r(y)). \tag{2}$$

In order to obtain the *pdf* we simply differentiate the above expression to obtain:

$$f_Y(y) = \frac{d}{dy} F_Y(y) = f_Z(r(y)) \frac{d}{dy} r(y). \tag{3}$$

Assumptions behind the above equation, monotonicity of $R(z)$ and finite integral of f_z, are easily satisfied in a biological system such as a signalling network. The response function $R(z)$ is typically approximated by a sigmoidal Hill function:

$$y = R(x, \beta, \theta, R_{max}, H) = \beta + R_{max} \frac{x^H}{x_{50}^H + x^H}\,. \tag{4}$$

Parameter variability described by the function f_z is a probability density function which by definition sums up (integrates) to 1.

2 Conditions for the existence of bimodality in the presence of response threshold variability

In this section we derive conditions for existence of bimodal output distribution given log-normal distribution of the response threshold x_{50} with scale parameter μ_{x50} and shape parameter σ_{x50},

$$f_{X_{50}}(x_{50}) = \frac{1}{x_{50}\, \sigma_{x50} \sqrt{2\pi}} \exp\left[- \left(\log x_{50} - \mu_{x50}\right)^2 / \, 2\sigma_{x50}^2 \right], \quad x_{50} > 0, \tag{5}$$

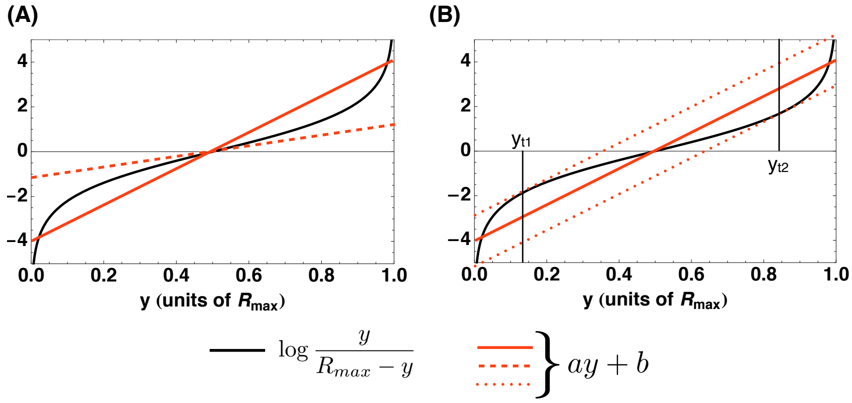

Figure S1: Graphical interpretation of conditions for the existence of bimodality. Equation 8 has solutions in terms of variable y when expressions on the left and the right hand side intersect. (A) First condition assures the slope of the linear function $ay+b$ on the left hand side of Eq. 8. Only lines with slopes larger than $4/R_{max}$ (solid red) are able to intersect the log function (solid black) three times. (B) Appropriate slope is yet not sufficient; y-intercept of the linear function, as quantified by b, has to be within certain bounds (Eq. 12).

and sigmoidal response modelled by Hill function (Eq. 4). Substituting the above into Eq. 3, we obtain:

$$f_{out}(\mathbf{y}) = \frac{1}{H\sigma_{x50}\sqrt{2\pi}} \frac{R_{max}}{(R_{max}-\mathbf{y})\mathbf{y}} \exp\left[-\frac{1}{2\sigma_{x50}^2}\left(\mu_{x50} - \log\left[x\left(\frac{R_{max}-\mathbf{y}}{\mathbf{y}}\right)^{\frac{1}{H}}\right]\right)^2\right]. \tag{6}$$

For ease of read, we set the type of the output y in bold.

A bimodal distribution exists only if the above function assumes two maxima and a minimum between them. In order to find these three extrema we analyse first derivative of Eq. 6:

$$\begin{aligned} \frac{d}{dy} f_{out}(\mathbf{y}) &= \frac{1}{H^2\sigma_{x50}^3\sqrt{2\pi}} \frac{R_{max}^2}{(R_{max}-\mathbf{y})^2\,\mathbf{y}^2} \exp\left[-(\ldots)^2\right] \\ &\times \left(\frac{2H\sigma_{x50}^2}{R_{max}}\mathbf{y} - \mu_{x50} - H\sigma_{x50}^2 + \log\left[x\left(\frac{R_{max}-\mathbf{y}}{\mathbf{y}}\right)^{\frac{1}{H}}\right]\right). \end{aligned} \tag{7}$$

The term with the exponent is always positive, therefore we only analyse the expression in brackets and search for its zeros. After expanding logarithm on the right hand side, we obtain:

$$\frac{2H^2\sigma_{x50}^2}{R_{max}}\mathbf{y} - H^2\sigma_{x50}^2 + H\left(\log x - \mu_{x50}\right) = \log\left[\frac{\mathbf{y}}{R_{max}-\mathbf{y}}\right], \tag{8}$$

which is a transcendental equation with a linear function of the form $ay+b$ on the left hand side. We solve this equation graphically as shown in Fig. S1, which results in two conditions, for the slope a (panel A) and for y-intercept b (panel B).

The slope $a = 2H^2\sigma_{x50}^2/R_{max}$ has to be larger than the smallest slope of the r.h.s. of Eq. 8, which is achieved at the inflection point at $y = R_{max}/2$:

$$\frac{d}{dy}\log\left[\frac{\mathbf{y}}{R_{max}-\mathbf{y}}\right]\Bigg|_{y=R_{max}/2} = \frac{4}{R_{max}}. \tag{9}$$

This way we obtain the first condition, necessary but not sufficient, for the existence of bimodal output distribution,

$$H^2\sigma_{x50}^2 > 2. \tag{10}$$

Note that the condition is independent of R_{max}.

Once we know the slope, we can search for the range of admissible y-intercepts that result in three intersections (Fig. S1). To achieve that, we first calculate argument y for which the slopes (first derivatives)

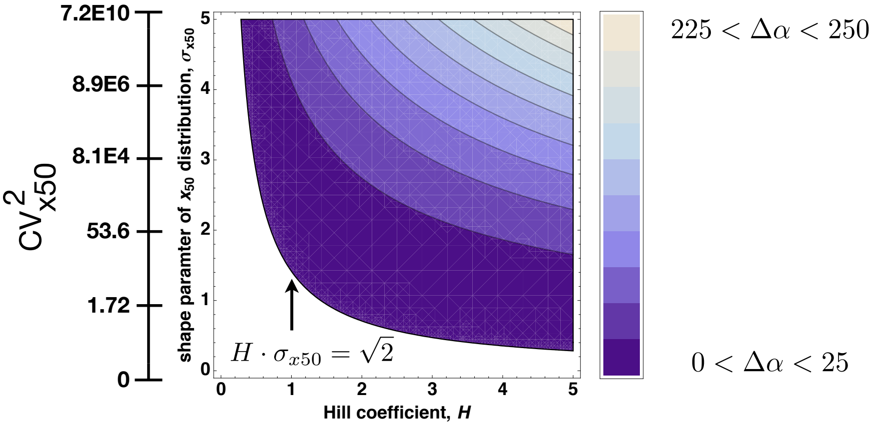

Figure S2: Bimodality region as function of Hill coefficient, H, and shape parameter of the x_{50} distribution, σ_{x50}. Colour coding indicates the width, $\Delta\alpha \equiv \alpha^+ - \alpha^-$, of admissible $\log(x/m_{x50})$ ratios that yield a bimodal distribution. The entire coloured region corresponds to the first condition, $H \cdot \sigma_{x50} > \sqrt{2}$.

of the left and the right hand side of Eq. 8 are equal; since the r.h.s. of Eq. 8 is symmetric around $R_{max}/2$, we obtain abscissae $y_{t1,t2}$ of two tangent points (cf. Fig. S1B),

$$\frac{2H^2\sigma_{x50}^2}{R_{max}} = \frac{R_{max}}{\mathbf{y}(R_{max} - \mathbf{y})} \quad \Longrightarrow \quad \mathbf{y_{t1,t2}} = \frac{R_{max}}{2}\left(1 \mp \sqrt{1 - \frac{2}{H^2\sigma_{x50}^2}}\right). \tag{11}$$

By evaluating r.h.s. of Eq. 8 at y_{t1} and y_{t2} we obtain the lower and upper bound for parameter $b = H(\log x - \mu_{x50}) - H^2\sigma_{x50}^2$,

$$\begin{aligned} a\mathbf{y} + b\Big|_{y=y_{t1}} &< \log\left[\frac{\mathbf{y}}{R_{max} - \mathbf{y}}\right]\Bigg|_{y=y_{t1}} \\ a\mathbf{y} + b\Big|_{y=y_{t2}} &> \log\left[\frac{\mathbf{y}}{R_{max} - \mathbf{y}}\right]\Bigg|_{y=y_{t2}}. \end{aligned} \tag{12}$$

Before spelling out the result we rewrite parameter b by noting that $\mu_{x50} = \log m_{x50}$, where m_{x50} is the median of the log-normal distribution of the response parameter x_{50}. Therefore b becomes:

$$b = H \log\frac{x}{m_{x50}} - H^2\sigma_X^2 \,. \tag{13}$$

The median of a probability distribution is a half-maximum point of the related cumulative distribution. In other words, the median is the “middle point” of the distribution. Therefore, the fraction x/m_{x50} is the ratio of the input signal x and the middle point of the x_{50} distribution.

From Eq. 12 we derive symmetric bounds for logarithm of this ratio:

$$\alpha^-(H, \sigma_{x50}) < \log\frac{x}{m_{x50}} < \alpha^+(H, \sigma_{x50}) \,, \tag{14}$$

where:

$$\alpha^{\pm}(H, \sigma_{x50}) \equiv \pm\sigma_{x50}\sqrt{H^2\sigma_{x50}^2 - 2} + \frac{1}{H}\log\left[H^2\sigma_{x50}^2 - 1 \mp H\sigma_{x50}\sqrt{H^2\sigma_{x50}^2 - 2}\right]. \tag{15}$$

Figure S2 illustrates graphically the range of H and σ_{x50} parameters (Eq. 10) and the range of $\log(x/m_{x50})$ ratios (Eq. 14) that need to be satisfied in order to obtain a bimodal output distribution.

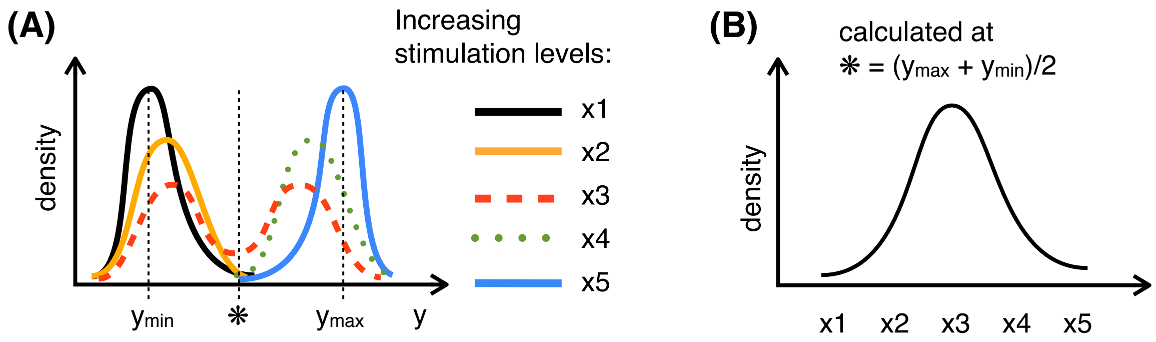

Figure S3: Schematic illustration of how to calculate x_{50} distribution from output distributions obtained for the range of inputs x. (A) First, create a function g_{cut} of input x, which assumes values of density (or frequency) for a particular output y. (B) We choose y at a half-distance between the peaks (the modes) of output distributions obtained for the saturating and basal levels of x. At $y = (R_{max} + \beta)/2$, the function g_{cut} depends only on parameters of x_{50} distribution (Eq. 17) which is assumed to be log-normal.

3 Inferring x_{50} distribution from output distributions

Consider Eq. 6 as a function of the input level x instead of the output y (Fig. S3). After normalisation to 1, such a function becomes a *pdf*:

$$\begin{aligned} g_{cut}(\mathbf{x};\, y,\, m_{x50},\, \sigma_{x50}) \quad &= \quad \frac{1}{m_{x50}\sigma_{x50}\sqrt{2\pi}} \left(\frac{R_{max}-y}{y}\right)^{1/H} \\ &\times \quad \exp\left[-\frac{\sigma_{x50}^2}{2} - \frac{1}{2\sigma_{x50}^2}\left(\mu_{x50} - \log\left[\mathbf{x}\left(\frac{R_{max}-y}{y}\right)^{1/H}\right]\right)^2\right]. \end{aligned} \tag{16}$$

This equation has the output value y as a parameter and the remaining problem is the choice of y such that the *pdf* depends only on parameters determining the underlying log-normal distribution of x_{50}, thus $m_{x50} = \exp(\mu_{x50})$ and σ_{x50}. The dependency on H and R_{max} can be alleviated by setting $y = R_{max}/2$. Therefore, the expression in parentheses, $(R_{max} - y)/y$, becomes 1 which yields:

$$g_{cut}\left(\mathbf{x};\, m_{x50},\, \sigma_{x50}\right)\Big|_{y=R_{max}/2} = \frac{1}{m_{x_{50}}\sigma_{x_{50}}\sqrt{2\pi}} \exp\left[-\frac{\sigma_{x_{50}}^2}{2} - \frac{1}{2\sigma_{x_{50}}^2}\log^2\left[\frac{m_{x50}}{\mathbf{x}}\right]\right]. \tag{17}$$

The above result demonstrates how to obtain parameters of the underlying log-normal distribution of threshold parameter x_{50} in the Hill response model. First, values of experimental output distributions obtained for a range of input levels x need to be taken at y equal to half of the maximum response R_{max}. Such a set can be fitted to equation 17 to obtain m_{x50} and σ_{x50}. The function 17 is not the distribution of x_{50}, but describes how the midpoint of the output distribution depends on input x. From this dependency parameter values of the underlying x_{50} distribution can be inferred from experimental results. By taking values of distributions obtained from flow cytometry at half-maximal response, the threshold variability with least dependence on other parameters of the dose-response can be estimated.

4 Distribution of Hill parameters

We take a generic model of a two-level cascade (Fig. S4A) with the following Michaelis-Menten kinetics to demonstrate the log-normal character of parameter distributions in the Hill approximation of the dose response:

$$g_1 \to g_1p : \; [g_{IN}] \; v_1 \frac{[g_1]}{k_1 + [g_1]} \tag{18a}$$

$$g_1p \to g_1 : \; v_2 \frac{[g_1p]}{k_2 + [g_1p]} \tag{18b}$$

$$g_2 \to g_2p : \; [g_1p] \; v_3 \frac{[g_2]}{k_3 + [g_2]} \tag{18c}$$

$$g_2p \to g_2 : \; v_4 \frac{[g_2p]}{k_4 + [g_2p]} \tag{18d}$$

The distribution of Hill parameters results from a distribution of total protein levels: $[g_1]_{tot} = [g_1] + [g_1p]$ and $[g_2]_{tot} = [g_2] + [g_2p]$. We take the following parameters: $v_1 = 1.8$, $v_2 = 9.5$, $v_3 = 0.8$, $v_4 = 2.6$, $k_1 = 9$, $k_2 = 3.7$, $k_3 = 5.4$, $k_4 = 7.2$.

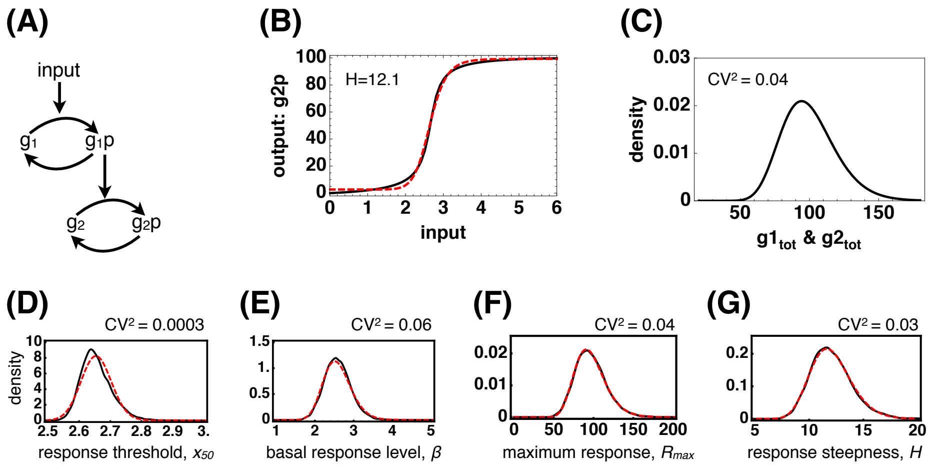

Figure S4: Distribution of parameters in Hill response model. (A) A generic two-level cascade. (B) Solid black line – steady-state dose-response of g_2p to input stimulation from analytical solution for $[g_1]_{tot} = [g_2]_{tot} = 100$; dashed red – fit of the Hill response function (Eq. 4), $\beta = 2.7$, $R_{max} = 96.6$, $x_{50} = 2.6$, $H = 12.1$. (C) Assumed log-normal distribution of g_1 and g_2 total levels with the mean 100 and the standard deviation 20. (D-G) Solid black line – distributions of response threshold x_{50}, basal response β, maximum response R_{max}, and response steepness H, respectively. Steady-state dose-responses were calculated for a range of inputs from the analytical solution with total $g_{1,2}$ levels sampled from the log-normal distribution shown in panel C. Then, in order to obtain parameter distributions, Hill functions were fitted to every dose-response. Dashed red line – fit of a log-normal distribution.

5 Fitting parameters of the Hill response model to experiments

The theoretical prediction of distributions shown in Figure 5C (main text) is based on fitting Hill response parameters to flow cytometry results shown in panel B of that figure. Steepness coefficient H is obtained by fitting Hill curve to normalised mean fluorescence intensity (nMFI) of ODD-GFP (Fig. S5A and Table S2). Other parameters are assumed to vary according to distributions fitted to flow cytometry data.

The variability in basal level β is assumed to follow a distribution of fluorescence intensity for unstimulated case, i.e. DMOG = $0mM$ (Fig. S5B and Table S3). The maximum response R_{max} varies as the distribution for the maximum stimulation in our experiments, i.e. DMOG = $4mM$ (Fig. S5C). We correct this distribution for the variability in basal level by subtracting the mean and variance of the latter (Table S4).

The threshold x_{50}, the only parameter that we assume to give rise to widening of the output distribution at intermediate stimuli level, is obtained according to procedure described in Section 3: values of ODD-GFP distributions for all DMOG stimuli are taken at approximately half maximum fluorescence intensity (Fig. S5D and Table S5).

We used the following probability density functions for the fitting procedure:

Log-normal distribution with shape parameter σ and scale parameter μ:

$$f_{LN}(x;\mu,\sigma) = \frac{1}{x\sqrt{2\pi}\sigma} e^{-\frac{(\ln x-\mu)^2}{2\sigma^2}} \tag{19}$$

Gamma distribution with shape parameter k and scale parameter θ:

$$f_G(x;k,\theta) = \frac{1}{\Gamma(k)\theta^k} x^{k-1} e^{-x/\theta} \tag{20}$$

Weibull distribution with shape parameter k and scale parameter λ:

$$f_W(x;k,\lambda) = \frac{k}{\lambda}\left(\frac{x}{\lambda}\right)^{k-1} e^{-(x/\lambda)^k} \tag{21}$$

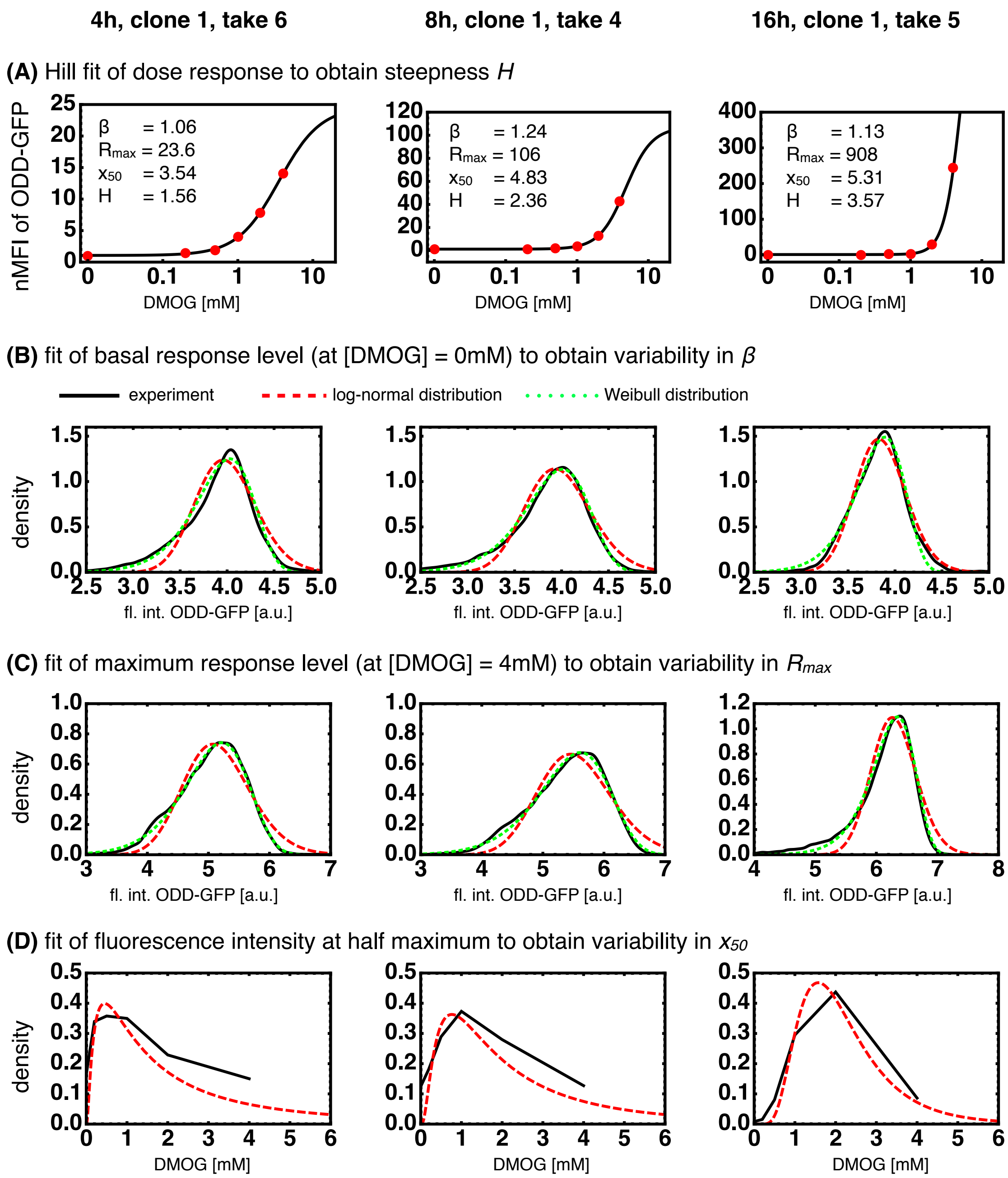

Figure S5: Fitting parameters of Hill response model to single flow cytometry measurements shown in Figure 5B (main text). (A) Red points – normalised median fluorescence intensity (nMFI, Eq. 5, main text) of ODD-GFP; solid line – fitted Hill response function (Eq. 4). Fitting parameters are β, R_{max}, x_{50} and H that correspond to basal level, maximum response, response threshold and response steepness, respectively. Fitting parameters are listed in Table S2. (B) Fits to ODD-GFP fluorescence intensity distributions for unstimulated case, which is a proxy of variability in β. (C) Fits to ODD-GFP fluorescence intensity distributions for maximum stimulation case, which is a proxy of variability in R_{max}. (B-D) Solid black line – experimental data. (B & C) Dashed red – log-normal distribution, dotted green – Weibull distribution. Weibull distribution was a better fit based on a smaller value of Akaike information criterion (AIC) as listed in Tables S3 & S4. (D) Fits to values of ODD-GFP fluorescence intensity distributions at approximately half maximum of the distance between medians of distributions recorded for DMOG $= 0mM$ and DMOG $= 4mM$, i.e. at $10^{4.4}$, $10^{4.5}$, 10^{5} for 4, 8 and 16h, respectively. Dashed red – $g_{cut}(x)$ distribution from Eq. 17 from which we obtain μ_{x50} and σ_{x50} of the underlying log-normal threshold distribution as listed in Table S5.

Table S1: Parameters of the response function, Eq. 4, fitted to averages over 3-4 biological repeats and shown in Fig. 5A, main text.

Time	Parameter	Estimate	Std.Err.	95% CI
4h	β	1.06	0.0785	(0.724, 1.40)
	R_{max}	23.5	5.69	(-0.958, 48.0)
	x_{50}	5.43	1.63	(-0.157, 12.4)
	H	1.42	0.124	(0.881, 1.95)
8h	β	1.18	0.153	(0.524, 1.84)
	R_{max}	101	40.5	(-73.7, 275)
	x_{50}	5.07	1.42	(-1.03, 11.2)
	H	2.45	0.263	(1.32, 3.58)
16h	β	1.16	0.131	(0.592, 1.72)
	R_{max}	394	56.1	(152, 635)
	x_{50}	4.24	0.326	(2.83, 5.64)
	H	3.47	0.154	(2.81, 4.14)

Table S2: Parameters of the response function, Eq. 4, fitted to a single experiment (Fig.S5A). We use fitted values of the steepness parameter H to predict ODD-GFP distributions in Fig. 5C, main text.

Time	Parameter	Estimate	Std.Err.	95% CI
4h	β	1.06	0.168	(0.339, 1.79)
	R_{max}	23.6	4.74	(3.21, 44.0)
	x_{50}	3.54	0.899	(-0.329, 7.41)
	H	1.56	0.192	(0.737, 2.39)
8h	β	1.24	0.245	(0.181, 2.29)
	R_{max}	106	44.5	(-85.4, 297)
	x_{50}	4.83	1.52	(-1.70, 11.4)
	H	2.36	0.308	(1.03, 3.68)
16h	β	1.13	0.125	(0.595, 1.67)
	R_{max}	908	304	(-399, 2214)
	x_{50}	5.31	0.746	(2.10, 8.51)
	H	3.57	0.163	(2.87, 4.27)

Table S3: Fitting parameters of the basal response level, [DMOG] = 0 mM (Fig. S5B). The distributions are given by Eqs. 19-21. AIC denotes Akaike information criterion based on which a better fitting distribution is chosen.

Time	Distribution	AIC	Parameter	Estimate	Std.Err.	95% CI	CV^2
4h	log-normal	-1230	σ, shape μ, log-scale	0.0812 1.38	0.000821 0.00101	(0.0796, 0.0828) (1.380, 1.384)	0.0066
	gamma	-1270	k, shape θ, scale	152 0.0263	2.93 0.000509	(146, 157) (0.0253, 0.0273)	0.0066
	Weibull	-1920	k, shape λ, scale	13.7 4.05	0.0736 0.00192	(13.6, 13.9) (4.045, 4.052)	0.0079
8h	log-normal	-1450	σ, shape μ, log-scale	0.0887 1.38	0.000744 0.000910	(0.0873, 0.0902) (1.375, 1.378)	0.0079
	gamma	-1510	k, shape θ, scale	127 0.0312	2.0 0.000494	(123, 131) (0.0303, 0.0322)	0.0079
	Weibull	-2370	k, shape λ, scale	12.4 4.03	0.0444 0.00142	(12.3, 12.5) (4.034, 4.039)	0.0096
16h	log-normal	-1690	σ, shape μ, log-scale	0.0708 1.35	0.000384 0.000470	(0.0701, 0.0716) (1.346, 1.348)	0.0050
	gamma	-1770	k, shape θ, scale	200 0.0192	2.0 0.000193	(196, 204) (0.0189, 0.0196)	0.0050
	Weibull	-1920	k, shape λ, scale	15.8 3.91	0.0715 0.00136	(15.6, 15.9) (3.905, 3.911)	0.0061

Table S4: Fitting parameters of the maximum response level, [DMOG] = 4 mM (Fig. S5C).

Time	Distribution	AIC	Parameter	Estimate	Std.Err.	95% CI	CV^2
4h	log-normal	-1590	σ, shape μ, log-scale	0.107 1.64	0.000907 0.00111	(0.105, 0.109) (1.633, 1.638)	0.011
	gamma	-1690	k, shape θ, scale	88.1 0.0584	1.36 0.000905	(85.4, 90.8) (0.0566, 0.0602)	0.011
	Weibull	-2970	k, shape λ, scale	10.6 5.27	0.0242 0.00139	(10.52, 10.62) (5.26, 5.27)	0.013
	Weibull corrected (*)	–	k, shape λ, scale	2.65 1.26	–	–	0.16
8h	log-normal	-1520	σ, shape μ, log-scale	0.109 1.71	0.00106 0.00129	(0.107, 0.111) (1.706, 1.711)	0.012
	gamma	NA	k, shape θ, scale	– –	– –	– –	– –
	Weibull	-2840	k, shape λ, scale	10.4 5.67	0.0287 0.00185	(10.3, 10.4) (5.665, 5.672)	0.014
	Weibull corrected (*)	–	k, shape λ, scale	3.37 1.70	–	–	0.11
16h	log-normal	-1130	σ, shape μ, log-scale	0.0584 1.84	0.000577 0.000706	(0.0572, 0.0595) (1.837, 1.839)	0.0034
	gamma	-1160	k, shape θ, scale	294 0.0214	5.6 0.000410	(283, 305) (0.0206, 0.0222)	0.0034
	Weibull	-2140	k, shape λ, scale	18.9 6.36	0.0727 0.00157	(18.8, 19.1) (6.361, 6.367)	0.0043
	Weibull corrected (*)	–	k, shape λ, scale	10.5 2.53	–	–	0.01

(*) R_{max} is corrected for the variability in basal level by subtracting the mean and variance of β. We use the corrected values of shape and scale parameters to sample the responses shown in Fig. 5C in the main text.

Table S5: Fitting parameters of the response threshold distribution (Fig. S5D). We fit g_{cut} distribution given by Eq. 17 to obtain parameters μ_{x50} and σ_{x50}, which are scale and shape parameters of the log-normal distribution that determines x_{50} variability.

	Results of the fitting:				Calculated stats of the x_{50} distribution:			
Time	Parameter	Estimate	Std.Err.	95% CI	Mean	Median	StDev	CV^2_{x50}
4h	σ_{x50} μ_{x50}	1.15 -0.793	0.204 0.343	(0.58, 1.71) (-1.75, 0.16)	0.87	0.45	1.44	2.72
8h	σ_{x50} μ_{x50}	0.932 -0.269	0.138 0.197	(0.55, 1.32) (-0.82, 0.28)	1.18	0.76	1.39	1.38
16h	σ_{x50} μ_{x50}	0.482 0.453	0.033 0.034	(0.39, 0.57) (0.36, 0.55)	1.77	1.57	0.90	0.26

Table S6: Evaluation of conditions 1 and 2 (Eqs. 10 & 14) using parameters calculated from experimental data: H – response steepness, σ_{x50} – shape parameter of x_{50} distribution, $m_{x50} = e^{\mu_{x50}}$ – median of x_{50} distribution, where μ_{x50} is the scale parameter of x_{50} distribution. The minimum and maximum value of DMOG limit the range of treatment for which bimodality can arise.

	Estimated from the experiment:				Condition 1:	Condition 2:	
	H	shape, σ_{x50}	$m_{x50} = e^{\mu_{x50}}$	CV^2_{x50}	$H^2\,\sigma^2_{x50} > 2$	$[\mathrm{DMOG}]_{min}$	$[\mathrm{DMOG}]_{max}$
4h	1.56	1.15	0.45	2.72	3.22	0.32	0.64
8h	2.36	0.93	0.76	1.38	4.82	0.38	1.55
16h	3.57	0.48	1.57	0.26	2.97	1.40	1.76

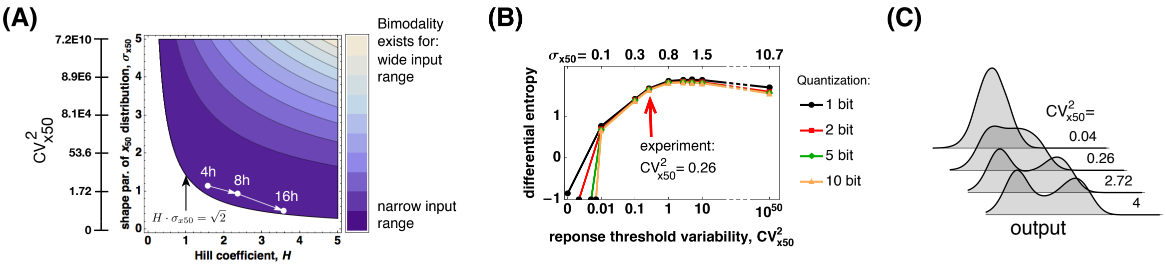

Figure S6: Numerical quantification of bimodal regime from experimental data. (A) Bimodality region as function of Hill coefficient, H, and shape parameter of the x_{50} distribution, σ_{x50}. Colour coding indicates the width of admissible input range that yields a bimodal distribution (cf. Fig. S2). Points indicate H and σ_{x50} estimated from experiments. (B) Predicted differential entropy of output distributions at 16h after treatment with [DMOG] $= 1.6nM$ (median of the threshold distribution) for a range of threshold variability. Steepness $H = 3.57$ (Table S2); β is Weibull-distributed with $k = 15.8$ and $\lambda = 3.91$ (Table S3); R_{max} is Weibull-distributed with $k = 10.5$ and $\lambda = 2.53$ (Table S4); the median of the threshold distribution $m_{x50} = 1.6$. Since the distributions are sampled numerically, Shannon entropy is calculated for decreasing histogram bin sizes (or increasing quantisations) to give the best approximation of the entropy of the continuous density. Threshold variability $CV^2_{x50} = 0.26$ ($\sigma_{x50} = 0.48$) is estimated from the experiment at 16h post-DMOG (Table S5). (C) Predicted ODD-GFP distributions for some values of CV^2_{x50} from panel B.

References

[1] S. Miller and D. Childers, *Probability and Random Processes*. With Applications to Signal Processing and Communications, Academic Press, 2012.