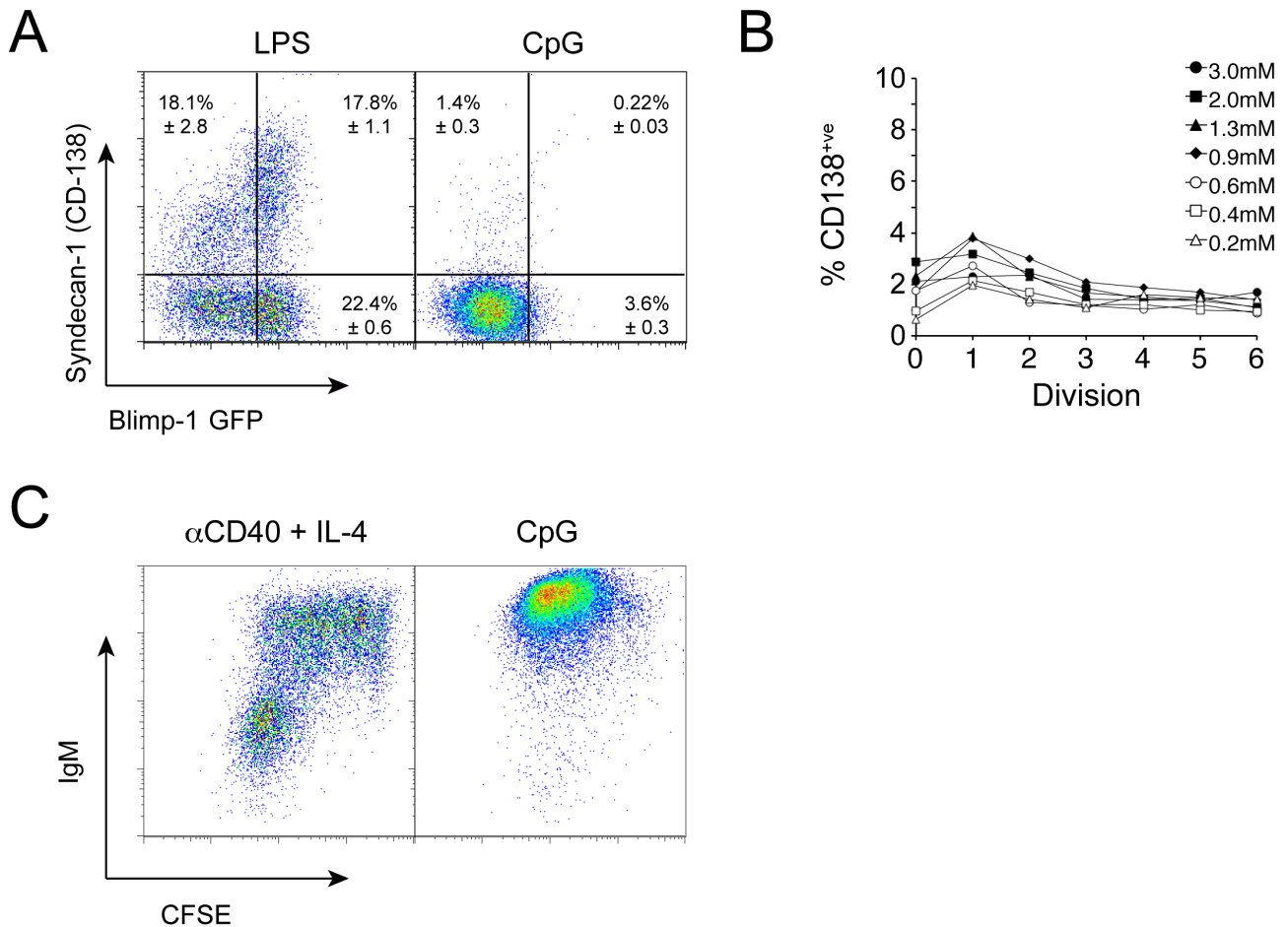


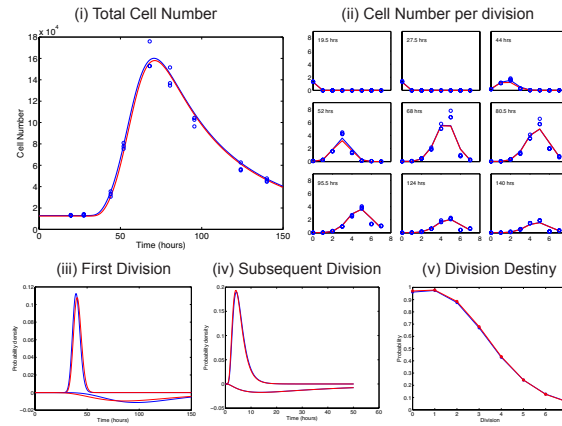
## Supplementary Material



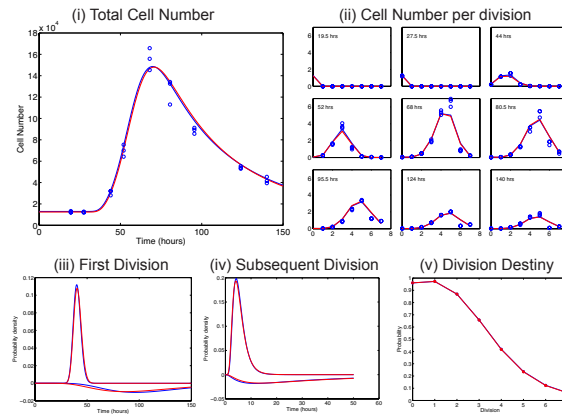
**Supplementary Figure S1: CpG stimulated B cells do not express the ASC markers Blimp-1 or CD138** – Small resting B cells were isolated from Blimp-1<sup>+GFP</sup> reporter mice and stimulated with either LPS (20 $\mu$ g/ml) or CpG (3mM). Four days post stimulation, B cells were analysed for expression of syndecan-1 (CD138) or Blimp-1 (GFP). Data represent mean  $\pm$ SEM of triplicate cultures.

**A**

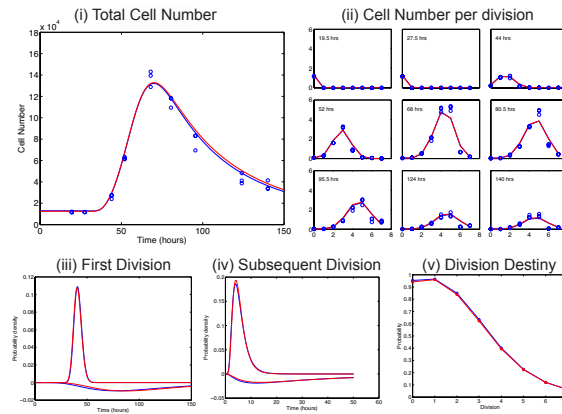
3mM CpG

**B**

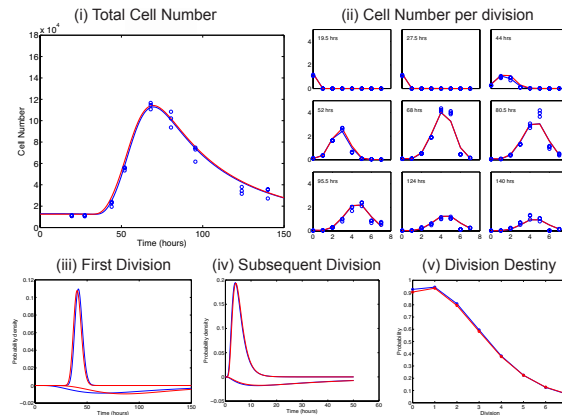
1.5mM CpG

**C**

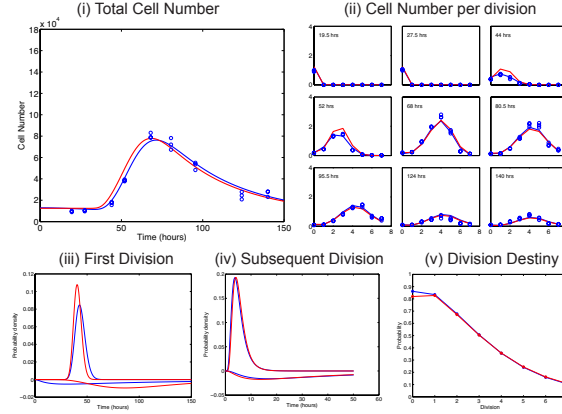
.75mM CpG

**D**

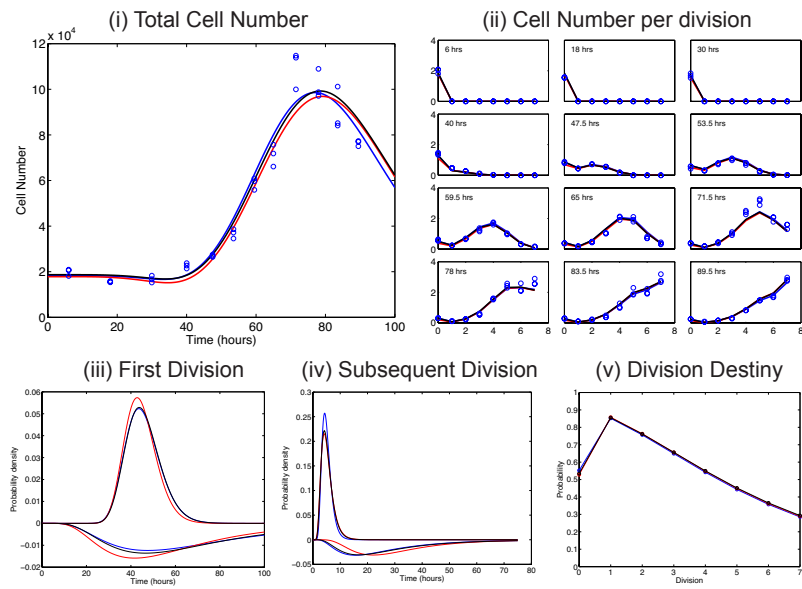
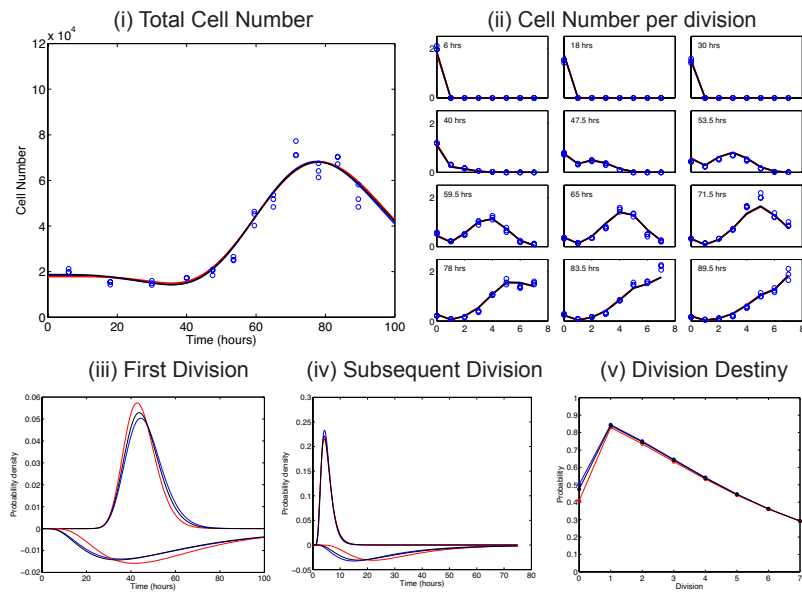
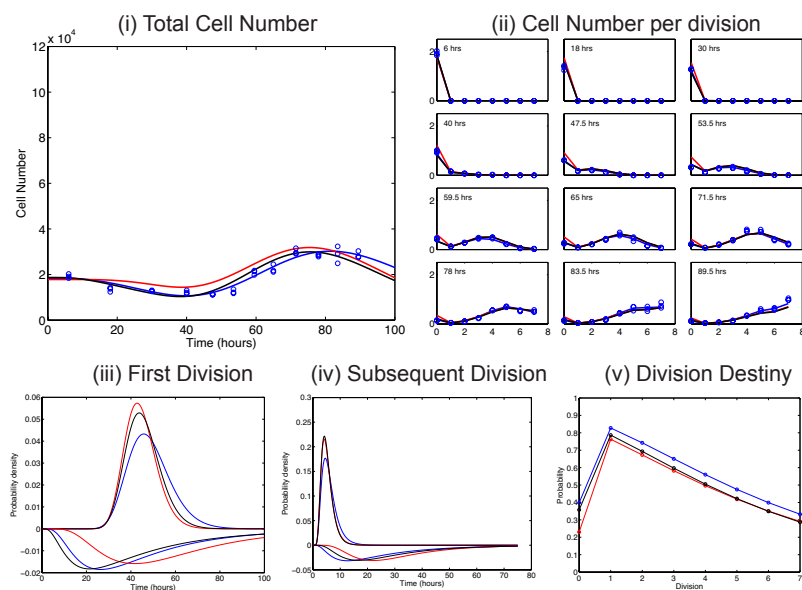
.38mM CpG

**E**

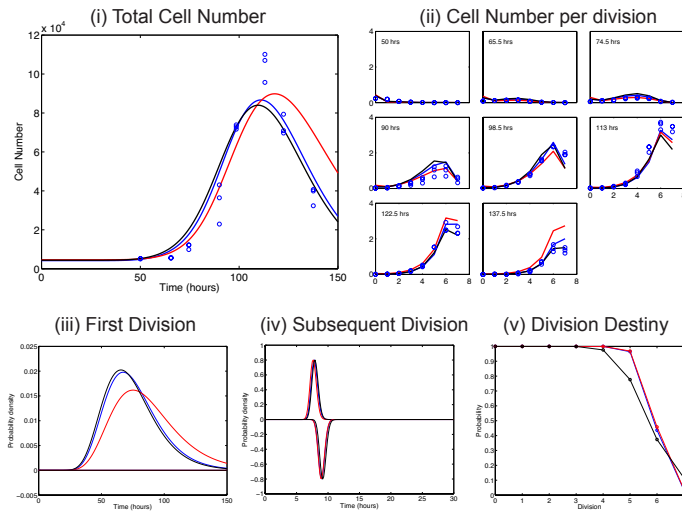
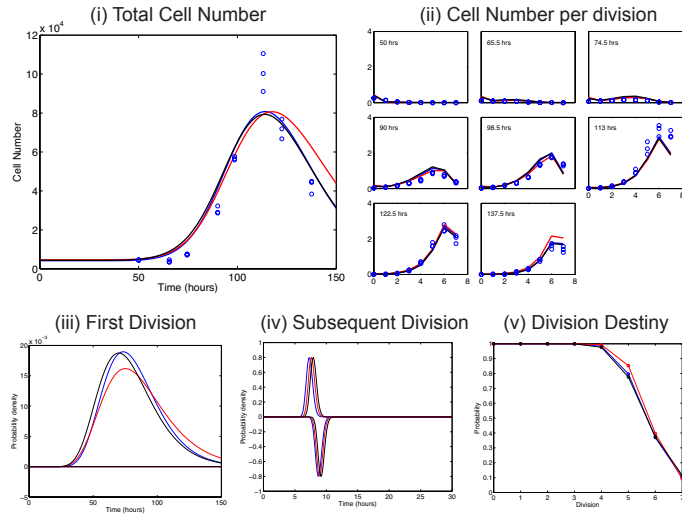
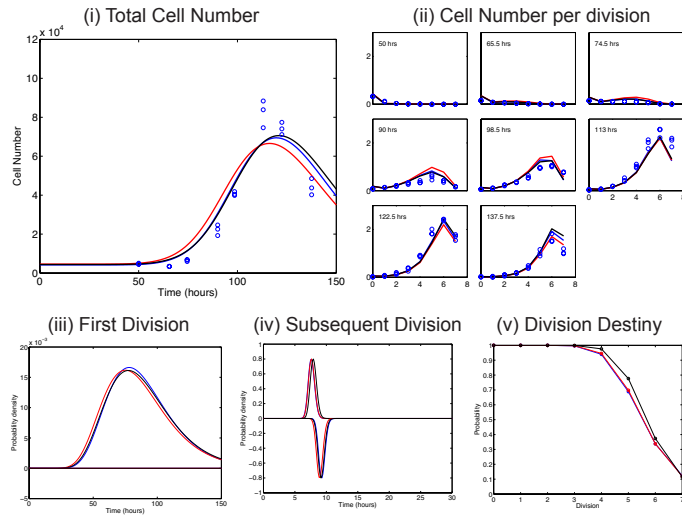
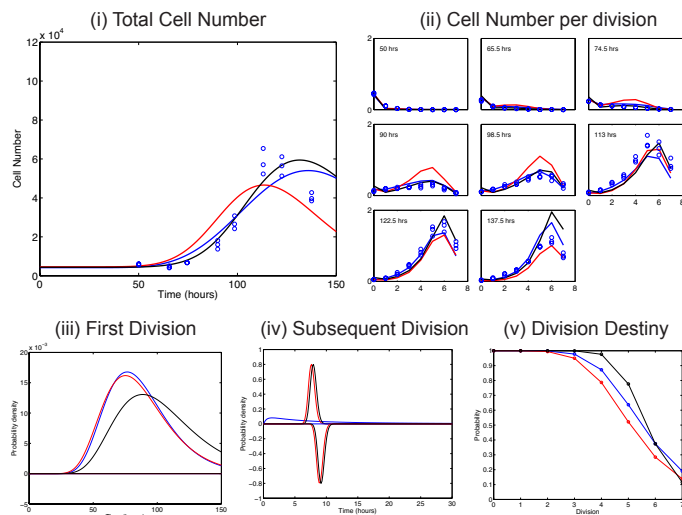
.19mM CpG



**Supplementary Figure S2: Cyton fitting to CpG titration data** - In each case the blue line show fits obtained from the cyton model with no constraints while the red lines show the fits obtained with all the parameters for death and division times constrained to be equal for all CpG concentrations. (A) - 3mM, (B) - 1.5mM, (C) - 0.75mM, (D) - 0.38mM and (E) - 0.19mM (i) Total cell number as a function of time, here the blue points represent triplicate experimental observations. (ii) Cell numbers per division at different time points, again the blue points are triplicate experimental observations. (iii) Probability density functions for the division times of undivided cells and the negative probability density function of time to die for undivided cells. (iv) Subsequent division probabilities - Probability density functions for the division times of divided cells and the negative probability density function of time to die for divided cells. (v) Division destiny - Probability that the cells are division capable as a function of division.

**A**15 $\mu$ g/ml LPS**B**5.6 $\mu$ g/ml LPS**C**1.9 $\mu$ g/ml LPS

**Supplementary Figure S3: Cyton fitting to LPS titration data** - In each case the blue line show fits obtained from the cyton model with no constraints while the black lines show the fits obtained with all the parameters for death and division times constrained to be equal for all concentrations, and the red lines show fits obtained where all the parameters associated with times to death and division, apart from time to initial division, are fixed for all concentrations. (A) - 15 $\mu$ g/ml, (B) 5.6 $\mu$ g/ml and (C) 1.9 $\mu$ g/ml. (i) Total cell number as a function of time, here the blue points represent triplicate experimental observations. (ii) Cell numbers per division at different time points, again the blue points are triplicate experimental observations. (iii) Probability density functions for the division times of undivided cells and the negative probability density function of time to die for undivided cells. (iv) Subsequent division probabilities - Probability density functions for the division times of divided cells and the negative probability density function of time to die for divided cells. (v) Division destiny - Probability that the cells are division capable as a function of division.

**A**20 $\mu$ g/ml  $\alpha$ CD40**B**10 $\mu$ g/ml  $\alpha$ CD40**C**5 $\mu$ g/ml  $\alpha$ CD40**D**2.5 $\mu$ g/ml  $\alpha$ CD40

**Supplementary Figure S4: Cyton fitting to  $\alpha$ CD40 titration data** - In each case the blue line show fits obtained from the cyton model with no constraints while the black lines show the fits obtained with all the parameters for death and division times constrained to be equal for all concentrations, and the red lines show fits obtained where all the parameters associated with times to death and division, apart from time to initial division, are fixed for all concentrations. **(A)** - 20 $\mu$ g/ml, **(B)** 10 $\mu$ g/ml, **(C)** 5 $\mu$ g/ml and **(D)** 2.5 $\mu$ g/ml. **(i)** Total cell number as a function of time, here the blue points represent triplicate experimental observations. **(ii)** Cell numbers per division at different time points, again the blue points are triplicate experimental observations. **(iii)** Probability density functions for the division times of undivided cells and the negative probability density function of time to die for undivided cells. **(iv)** Subsequent division probabilities - Probability density functions for the division times of divided cells and the negative probability density function of time to die for divided cells. **(v)** Division destiny - Probability that the cells are division capable as a function of division.

**Supplementary Table S1 - Parameter values of lognormal curves fitted to CpG <sup>3</sup>H data**

[CpG] mM	$\mu$	95% C.I.	$\sigma$	95% C.I.	Area (x10 <sup>3</sup> )	95% C.I.
<b>3.00</b>	39.82	+0.54, -0.52	7.79	+0.52, -0.45	296	+16,-15
<b>2.00</b>	39.94	+0.53, -0.50	8.03	+0.57, -0.44	284	+14,-13
<b>1.00</b>	40.30	+1.13, -0.83	8.66	+1.86, -0.86	261	+22,-20
<b>0.75</b>	40.45	+0.64, -0.55	8.47	+0.56, -0.49	278	+14,-14
<b>0.56</b>	40.23	+0.64, -0.55	8.37	+0.65, -0.52	253	+13,-14
<b>0.42</b>	40.82	+0.56, -0.50	8.65	+0.54, -0.47	228	+10,-10
<b>0.32</b>	42.67	+0.04, -1.20	9.25	+1.12, -0.84	199	+15,-14
<b>0.24</b>	44.29	+2.30, -1.70	12.41	+2.58, -1.51	132	+14,-12
<b>0.18</b>	41.23	+1.35, -1.00	7.94	+1.05, -0.76	69	+8,-8

**Supplementary Table S2 - Parameter values of lognormal curves fitted to LPS <sup>3</sup>H data**

[LPS] (μg/ml)	$\mu$	95% C.I.	$\sigma$	95% C.I.	Area (x10 <sup>3</sup> )	95% C.I.
<b>50</b>	58.49	+1.907,-1.634	21.533	+2.072, -1.446	390	+26,-22
<b>15</b>	62.412	+2.080, -2.032	23.015	+3.081, -2.119	358	+30,-24
<b>5.56</b>	59.620	+2.610, -2.469	22.428	+2.888, -2.205	291	+27,-26
<b>1.86</b>	61.888	+2.688, -2.343	22.611	+3.558, -2.608	242	+24,-21

**Supplementary Table S3 – Parameter values of lognormal curves fitted to CD40 stimulation <sup>3</sup>H data**

[αCD40] (μg/ml)	$\mu$	95% C.I.	$\sigma$	95% C.I.	Area(x10 <sup>3</sup> )	95% C.I.
<b>10.0</b>	60.5	+1.3,-1.2	19.0	+2.2,-1.6	136	+ 9.2, -6.5
<b>3.3</b>	73.6	+3.5, -2.7	24.9	+5.7, -4.0	112	+1.0,-6.8
<b>1.1</b>	81.6	+7.8,-4.6	37.2	+8.9,-5.6	61	+7.1, -5.0



**Supplementary Table S4 – Optimal parameter values for cyton fit to CpG data with all parameters associated with times to division and death constrained to be equal for all stimulus concentrations.**

	$\mu_{\text{div}}^0$ (hour)	$\sigma_{\text{div}}^0$ (hours)	$\mu_{\text{die}}^0$ (hours)	$\sigma_{\text{die}}^0$ (hours)	$\mu_{\text{div}}^{1+}$ (hours)	$\sigma_{\text{div}}^{1+}$ (hours)	$\mu_{\text{die}}^{1+}$ (hours)	$\sigma_{\text{die}}^{1+}$ (hours)	$\gamma_0$	$\mu_{\text{dest}}$	$\sigma_{\text{dest}}$	$N$
3.0 mM	40.93	3.74	115.47	53.48	5.56	2.68	62.79	83.17	0.97	4.25	1.14	12447.6
1.5 mM									0.96	4.16	1.15	
0.75 mM									0.94	4.00	1.18	
0.38 mM									0.90	3.77	1.24	
0.19 mM									0.82	3.03	1.48	

**Supplementary Table S5 – Optimal parameter values for the unconstrained cyton fit to the CpG data**

	$\mu_{\text{div}}^0$ (hour)	$\sigma_{\text{div}}^0$ (hours)	$\mu_{\text{die}}^0$ (hours)	$\sigma_{\text{die}}^0$ (hours)	$\mu_{\text{div}}^{1+}$ (hours)	$\sigma_{\text{div}}^{1+}$ (hours)	$\mu_{\text{die}}^{1+}$ (hours)	$\sigma_{\text{die}}^{1+}$ (hours)	$\gamma_0$	$\mu_{\text{dest}}$	$\sigma_{\text{dest}}$	$N$
3.0 mM	39.84	3.58	115.99	40.95	5.74	2.70	63.69	84.85	0.96	4.20	1.15	12920.10
1.5 mM	40.51	3.60	118.78	45.89	5.57	2.55	64.10	92.96	0.96	4.16	1.15	
0.75 mM	41.22	3.70	110.91	57.36	5.61	2.86	59.41	81.78	0.95	4.05	1.17	
0.38 mM	42.08	3.67	110.36	72.04	5.39	2.73	62.35	86.67	0.93	3.84	1.22	
0.19 mM	43.44	4.78	275.25	500.00	5.33	2.83	64.25	77.20	0.86	3.08	1.46	

**Supplementary Table S6 - AIC values for cyton testing of CpG data**

Model	Number of free parameters	AIC
Unconstrained	56	2363
Constrained times	24	2310

**Supplementary Table S7 – Optimal parameter values for a cyton fit to LPS data in which all parameters associated with division and death time are constrained to be constant for all stimulus concentrations.**

	$\mu^0_{\text{div}}$ (hours)	$\sigma^0_{\text{div}}$ (hours)	$\mu^0_{\text{die}}$ (hours)	$\sigma^0_{\text{die}}$ (hours)	$\mu^{1+}_{\text{div}}$ (hours)	$\sigma^{1+}_{\text{div}}$ (hours)	$\mu^{1+}_{\text{die}}$ (hours)	$\sigma^{1+}_{\text{die}}$ (hours)	$\gamma_0$	$\mu_{\text{dest}}$	$\sigma_{\text{dest}}$	$N$
15	44.48	7.19	63.10	35.61	5.34	2.28	32.66	17.82	0.53	3.41	1.97	17823.0
5									0.41	3.08	2.05	
1.5									0.23	2.25	2.20	

**Supplementary Table S8 – Optimal parameter values for a cyton fit to LPS data in which all parameters associated with time to initial division and time to death and division in subsequent divisions are constrained to be constant for all stimulus concentrations.**

	$\mu^0_{\text{div}}$ (hours)	$\sigma^0_{\text{div}}$ (hours)	$\mu^0_{\text{die}}$ (hours)	$\sigma^0_{\text{die}}$ (hours)	$\mu^{1+}_{\text{div}}$ (hours)	$\sigma^{1+}_{\text{div}}$ (hours)	$\mu^{1+}_{\text{die}}$ (hours)	$\sigma^{1+}_{\text{die}}$ (hours)	$\gamma_0$	$\mu_{\text{dest}}$	$\sigma_{\text{dest}}$	$N$
15	45.66	7.82	72.56	42.53	5.30	2.19	30.63	21.50	0.53	3.38	1.99	18685.1
5			65.38	48.29					0.47	3.20	2.02	
1.5			51.17	45.36					0.36	2.57	2.13	

**Supplementary Table S9 – Optimal parameter values for a cyton fit to LPS data in which all parameters are unconstrained.**

	$\mu^0_{\text{div}}$ (hours)	$\sigma^0_{\text{div}}$ (hours)	$\mu^0_{\text{die}}$ (hours)	$\sigma^0_{\text{die}}$ (hours)	$\mu^{1+}_{\text{div}}$ (hours)	$\sigma^{1+}_{\text{div}}$ (hours)	$\mu^{1+}_{\text{die}}$ (hours)	$\sigma^{1+}_{\text{die}}$ (hours)	$\gamma_0$	$\mu_{\text{dest}}$	$\sigma_{\text{dest}}$	$N$
15	45.55	7.91	78.12	48.96	5.12	1.79	29.70	21.86	0.55	3.34	1.97	18297.5
5	46.51	8.23	67.41	49.34	5.24	2.04	28.69	21.18	0.50	3.26	2.01	
1.5	48.54	9.68	50.41	37.08	6.14	2.91	29.59	25.79	0.40	3.03	2.19	

**Supplementary Table S10 - AIC values for cyton testing of LPS data**

<b>Model</b>	<b>Number of free parameters</b>	<b>AIC</b>
<b>Unconstrained</b>	<b>34</b>	<b>1412</b>
<b>Constrained times</b>	<b>18</b>	<b>1515</b>
<b>Constrained times other than death of undivided cells</b>	<b>22</b>	<b>1429</b>

**Supplementary Table S11 – Optimal parameter values for a cyton fit to the CD40 stimulation data in which all parameters associated with division and death times are constrained to be equal for all stimulus concentrations**

	$\mu^0_{\text{div}}$ (hours)	$\sigma^0_{\text{div}}$ (hours)	$\mu^0_{\text{die}}$ (hours)	$\sigma^0_{\text{die}}$ (hours)	$\mu^{1+}_{\text{div}}$ (hours)	$\sigma^{1+}_{\text{div}}$ (hours)	$\mu^{1+}_{\text{die}}$ (hours)	$\sigma^{1+}_{\text{die}}$ (hours)	$\gamma_0$	$\mu_{\text{dest}}$	$\sigma_{\text{dest}}$	$N$
20 $\mu\text{g}$ $\text{ml}^{-1}$	87.01	27.87	327.73	55.11	7.67	0.50	8.96	0.50	1.00	6.93	0.50	4618.7
10 $\mu\text{g}$ $\text{ml}^{-1}$									1.00	6.71	0.69	
5 $\mu\text{g}$ $\text{ml}^{-1}$									1.00	6.35	0.85	
2.5 $\mu\text{g}$ $\text{ml}^{-1}$									1.00	5.69	1.07	

**Supplementary Table S12 –Optimal parameter values for a cyton fit to the CD40 stimulation data in which all parameters other than those associated with time to first division are constrained to be equal for all divisions**

	$\mu^0_{\text{div}}$ (hours)	$\sigma^0_{\text{div}}$ (hours)	$\mu^0_{\text{die}}$ (hours)	$\sigma^0_{\text{die}}$ (hours)	$\mu^{1+}_{\text{div}}$ (hours)	$\sigma^{1+}_{\text{div}}$ (hours)	$\mu^{1+}_{\text{die}}$ (hours)	$\sigma^{1+}_{\text{die}}$ (hours)	$\gamma_0$	$\mu_{\text{dest}}$	$\sigma_{\text{dest}}$	$N$
20 $\mu\text{g}$ $\text{ml}^{-1}$	74.20	21.92	327.72	55.12	7.97	0.50	9.21	0.50	1.00	6.55	0.79	4394.7
10 $\mu\text{g}$ $\text{ml}^{-1}$	79.39	23.71										
5 $\mu\text{g}$ $\text{ml}^{-1}$	89.06	27.84										
2.5 $\mu\text{g}$ $\text{ml}^{-1}$	104.00	35.04										

**Supplementary Table S13 – Optimal parameter values for a cyton fit to the CD40 stimulation data in which all parameters other than starting cell number**

	$\mu^0_{\text{div}}$ (hours)	$\sigma^0_{\text{div}}$ (hours)	$\mu^0_{\text{die}}$ (hours)	$\sigma^0_{\text{die}}$ (hours)	$\mu^{1+}_{\text{div}}$ (hours)	$\sigma^{1+}_{\text{div}}$ (hours)	$\mu^{1+}_{\text{die}}$ (hours)	$\sigma^{1+}_{\text{die}}$ (hours)	$\gamma_0$	$\mu_{\text{dest}}$	$\sigma_{\text{dest}}$	$N$
20 $\mu\text{g}$ $\text{ml}^{-1}$	76.17	22.37	327.91	54.99	7.85	0.50	9.00	0.50	1.00	6.90	0.50	4180.9
10 $\mu\text{g}$ $\text{ml}^{-1}$	82.31	23.16	327.60	57.29	7.33	0.50	8.77	0.50	1.00	6.59	0.75	
5 $\mu\text{g}$ $\text{ml}^{-1}$	88.91	26.75	327.86	54.77	7.63	0.50	9.30	0.50	1.00	6.32	0.87	
2.5 $\mu\text{g}$ $\text{ml}^{-1}$	87.36	26.56	329.30	51.23	23.91	56.88	32.24	0.50	1.00	6.11	1.07	

**Supplementary Table S14 - AIC values for cyton testing of CD40 stimulation data**

<b>Model</b>	<b>Number of free parameters</b>	<b>AIC</b>
<b>Unconstrained</b>	<b>45</b>	<b>1660</b>
<b>Constrained times</b>	<b>21</b>	<b>1700</b>
<b>All but time to first division constrained</b>	<b>18</b>	<b>1639</b>