Supporting Information

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SI Materials and Methods

Quantitative Model. We produce a kinetic model of the gene regulatory module controlling the germinal center (GC) pathway exit by using ordinary differential equations and based on the following additional knowledge: (*i*) BCL6 is a homodimer (1); (*ii*) IRF4 binds to DNA with the help of different transactivation partners (2); and (*iii*) BLIMP1 requires the cofactor LSD1 (3). As a consequence, we assume that each transcriptional interaction can be modeled by a Hill function with cooperative coefficient 2. The transcriptional strength of a transcription factor depends on its binding affinity to a promoter site and other factors such as the molecular environment or the ability to recruit binding cofactors. We assume that each transcription factor has the same binding affinity. The transcriptional activity is defined then in terms of the dissociation constant k and the maximum transcription rate σ.

Under these assumptions, the system's equations can be written in the following form:

$$\frac{dp}{dt} = \mu_p + \sigma_p \frac{k_b^2}{k_b^2 + b^2} + \sigma_p \frac{r^2}{k_r^2 + r^2} - \lambda_p p, \qquad [S1]$$

$$\frac{db}{dt} = \mu_b + \sigma_b \frac{k_p^2}{k_p^2 + p^2} \cdot \frac{k_b^2}{k_b^2 + b^2} \cdot \frac{k_r^2}{k_r^2 + r^2} - (\lambda_b + \mathrm{BCR})b, \qquad [S2]$$

and

$$\frac{dr}{dt} = \mu_r + \sigma_r \frac{r^2}{k_r^2 + r^2} + \mathrm{CD40} - \lambda_r r, \qquad [S3]$$

where p, b, and r stand for the protein levels of BLIMP1, BCL6, and IRF4, respectively, the μ's represent the basal production rate of each protein and the λ's stand for the degradation rates. The model implicitly assumes that the level of protein for each transcription factor is roughly proportional to the mRNA levels. BCR and CD40 denote the regulatory signals coming from both signaling pathways. Because BCL6 represses some of the genes associated with both signaling cascades, we postulate the following phenomenological form:

$$\mathrm{BCR} = bcr_0 \frac{k_b^2}{k_b^2 + b^2}, \qquad [S4]$$

and

$$\mathrm{CD40} = cd_0 \frac{k_b^2}{k_b^2 + b^2}. \qquad [S5]$$

Parameter Fitting. To gain a biologically realistic understanding of the qualitative behavior of the GC exit pathway, we first obtained a set of biochemical parameters for Eqs. **S1**–**S3** that is consistent with the expression levels of GC and plasma cells (PC). To constrain the model parameters, we used microarray gene expression datasets from normal, transformed, and experimentally manipulated human B cells related to the GC reaction (GEO accession no. GSE12195), and gene expression profiling of B lymphocytes and plasma cells (GEO accession no. GSE6691). The raw data from all of the datasets was processed by removing the noninformative probes and clustering the coherent ones by the Cleaner algorithm (4). The unified dataset was then gcrma summarized and quantile normalized (Gcrma and Limma packages from Bioconductor respectively). The final dataset includes 10 GC-related and 5 plasma cell-related gene expression profiles, allowing mRNA expression levels of BCL6, BLIMP1, and IRF4 to be used to compute the kinetic model parameters. Under the assumption that protein levels are roughly proportional to mRNA levels, when the cell is close to equilibrium, the ratio between the mean expression levels in GC B cells and plasma cells can be translated into three constraints that the parameters of Eqs. **S1**–**S3** must satisfy. We fit the rest of the parameters by using values for protein production and degradation extracted from the literature (Table S1).

BCR Subnetwork. Because we want to understand the dynamics of BCR signaling when decoupled from CD40 signaling, we assume that the levels of IRF4 are much smaller than k_r and, therefore, the protein levels do not change substantially during the early response phase. The dynamical equations reduce to:

$$\frac{dp}{dt} = \mu_p + \sigma_p \frac{k_b^2}{k_b^2 + b^2} - \lambda_p p, \qquad [S6]$$

and

$$\frac{db}{dt} = \mu_b + \sigma_b \frac{k_p^2}{k_p^2 + p^2} \cdot \frac{k_b^2}{k_b^2 + b^2} - \left(\lambda_b + bcr_0 \frac{k_b^2}{k_b^2 + b^2}\right) b. \qquad [S7]$$

Steady-state exploration of the BCL6 and BLIMP1 expression levels, at different levels of protein synthesis, degradation, and BCR stimulation, shows that the system becomes bistable when the ratio between the rate of protein synthesis, σ_b, and the rate of protein degradation, λ_b, is higher than a critical value $\sigma_b / \lambda_b > 20$. In the bistable regime, the BCR signaling module (Eqs. **S6** and **S7**) has three stationary points: two stable and one unstable.

CD40 Subnetwork. In the absence of other signals acting upon the system, the dynamic response of IRF4 can be modeled by Eq. **S3**, which we rewrite here for convenience:

$$\frac{dr}{dt} = \mu_r + \mathrm{CD40} + \sigma_r \frac{r^2}{k_r^2 + r^2} - \lambda_r r, \qquad [S8]$$

where μ_r is IRF4 basal transcription rate and CD40 represents the strength of the signal created after CD40 stimulation and resulting in an enhanced IRF4 transcription rate. Note that CD40 depends on BCL6 (Eq. **S5**), but in the absence of signals acting directly on BCL6, we can assume that μ_r + CD40 is approximately constant during the early response.

Quantitative steady-state analysis for different levels of IRF4 protein synthesis and degradation shows another bistable regime. The conditions for bistability can be written as follows:

$$\beta = \frac{\mu_r + \mathrm{CD40} + \sigma_r}{\lambda_r k_r} > \sqrt{3}, \qquad [S9]$$

$$\beta^3 - (\beta^2 - 3)^{\frac{3}{2}} + 9\beta < \frac{27}{2} \frac{\sigma_r}{\lambda_r k_r}, \qquad [S10]$$

and

$$\beta^3 + (\beta^2 - 3)^{\frac{3}{2}} + 9\beta > \frac{27}{2}\frac{\sigma_r}{\lambda_r k_r}, \qquad \text{[S11]}$$

where μ_r, σ_r, and CD40 represent the basal, self-induced, and CD40-mediated IRF4 transcription rate, λ_r is the IRF4 degradation rate, and k_r is the IRF4 dissociation constant.

The bistability constraints can be written as a function of a dimensionless parameter β that includes all of the relevant IRF4 kinetic parameters. β has a straightforward interpretation as a measurement of the ratio of all IRF4 synthesis contributions (basal, induced, and CD40-stimulated transcription) to degradation contributions (degradation rate and dissociation constant to the IRF4 self-promoting binding site).

Parameter Values Used in the Simulations. ***Time units.*** The unit of time has been set to be equal to a typical protein half-life:

- BCL6 is very stable in B cells. Its half-life has been reported to be between 4 and 6 h in unstimulated cells and 1 h after BCR cross-linking (5).
- BLIMP1 has a half-live of 4 h in long-lived plasma cells in the bone marrow of mice (6).

We take the average of these two half-lives as our unit of time, $t_0 = 4$ h. In this unit, the degradation rate of BCL6 and BLIMP1 is 1. We assume a similar value for IRF4.

Dissociation constant. The dissociation constant of a transcription factor to DNA strongly depends on the DNA binding site, the ability to recruit the help of cofactors and the biological context. IRF4 binds weakly to DNA without the help of cofactors. The dissociation constant for the interaction of IRF4, its cofactor PU.1, and DNA has been determined to be $\approx 10^{-7}$ M (7).

The dissociation constant of Blimp-1 binding to a binding to its site on the c-*myc* gene was found to be $\approx 2 \cdot 10^{-9}$ M (8).

The dissociation constant of the BCL6 BTB domain and its corepressor $SMRT^{1414-1441}$ has been measured, a 2.5-fold stronger affinity than the equivalent for the affinity to another one of its corepressors *N*-$CoR^{1351-1383}$ (1).

We take the intermediate value 10^{-8} M to be the dissociation constant of BCL6, IRF4, and BLIMP1 to their DNA binding sequences. Defining the unit of concentration as $C_0 = 10^{-8}$ M, the dissociation constants take the value of 1.

The rest of the parameters are fitted by using microarray gene expression datasets as described in the text.

Parameter Sensitivity Analysis for Bistability. We investigate the range of parameters that allow for the existence of bistable solutions in the expression of at least one gene. Because of the complexity of studying simultaneous variations of 12 parameters, we have studied independently the variation of each parameter around the fitted solution presented in the Table S1.

Table S2 provides the ranges of parameters that admit bistability. The fourth column presents the sensitivity coefficient for bistability, defined as parameter/Δparameter, where Δparameter is the range where bistability in at least one gene is observed. As it can be seen in the table, the parameters controlling the dynamics of BCL6 and BLIMP1 can take a wide range of values without substantial modification of the bistable switch. However, the parameters regulating IRF4 expression are constrained to small ranges, according to the analytical condition for bistability Eqs. **S9**–**S11**.

Fig. S2 shows a bifurcation study for BCL6. Each subplot shows the BCL6 homeostatic levels as we change the values of each parameter. In each subplot, only one parameter is changed, whereas the rest remained fixed. The kinetic parameters associated to BCL6 and BLIMP1 show a monotonic behavior, i.e., each parameter value is characterized by a single BCL6 homeostatic level. On the contrary, λ_r, μ_r, and σ_r show a regime where two stable solutions can be found for the same parameter value. In this regime, the regulatory network behaves as a bistable switch that allows cells to leave the germinal center and differentiate into plasma cells.

Fig. S5: Comparison with Extended Models. To understand the role of additional interactions reported in the literature, we define three extended models that include an additional double-negative feedback loop between BLIMP1 and PAX5 (extended model 1), a positive induction of BCL6 mediated by PAX5 and repression of PAX5 by BLIMP1 (extended model 2), and a double-positive loop between BLIMP1 and IRF4 (extended model 3). p, b, r, and x stand, respectively, for BLIMP1, BCL6, IRF4, and PAX5.

Extended model 1. Additional double-negative feedback loop between BLIMP1 and PAX5.

$$\frac{dp}{dt} = \mu_p + \sigma_p \frac{k_b^2}{k_b^2 + b^2}\frac{k_x^2}{k_x^2 + x^2} + \sigma_p \frac{r^2}{k_r^2 + r^2} - \lambda_p p,$$

$$\frac{db}{dt} = \mu_b + \sigma_b \frac{k_p^2}{k_p^2 + p^2} \cdot \frac{k_b^2}{k_b^2 + b^2} \cdot \frac{k_r^2}{k_r^2 + r^2} - (\lambda_b + \mathrm{BCR})b,$$

$$\frac{dr}{dt} = \mu_r + \sigma_r \frac{r^2}{k_r^2 + r^2} + \mathrm{CD40} - \lambda_r r,$$

and

$$\frac{dx}{dt} = \mu_x + \sigma_x \frac{k_p^2}{k_p^2 + p^2} - \lambda_x x.$$

Extended model 2. BLIMP1 represses PAX5, and PAX5 activates BCL6.

$$\frac{dp}{dt} = \mu_p + \sigma_p \frac{k_b^2}{k_b^2 + b^2} + \sigma_p \frac{r^2}{k_r^2 + r^2} - \lambda_p p,$$

$$\frac{db}{dt} = \mu_b + \sigma_{b1} \frac{x^2}{k_x^2 + x^2} + \sigma_{b2} \frac{k_p^2}{k_p^2 + p^2} \cdot \frac{k_b^2}{k_b^2 + b^2} \cdot \frac{k_r^2}{k_r^2 + r^2} - (\lambda_b + \mathrm{BCR})b,$$

$$\frac{dr}{dt} = \mu_r + \sigma_r \frac{r^2}{k_r^2 + r^2} + \mathrm{CD40} - \lambda_r r,$$

and

$$\frac{dx}{dt} = \mu_x + \sigma_x \frac{k_p^2}{k_p^2 + p^2} - \lambda_x x.$$

Extended model 3. Double-positive loop between IRF4 and BLIMP1.

$$\frac{dp}{dt} = \mu_p + \sigma_p \frac{k_b^2}{k_b^2 + b^2} + \sigma_p \frac{r^2}{k_r^2 + r^2} - \lambda_p p,$$

$$\frac{db}{dt} = \mu_b + \sigma_b \frac{k_p^2}{k_p^2 + p^2} \cdot \frac{k_b^2}{k_b^2 + b^2} \cdot \frac{k_r^2}{k_r^2 + r^2} - (\lambda_b + \mathrm{BCR})b,$$

and

$$\frac{dr}{dt} = \mu_r + \sigma_r \frac{p^2}{k_p^2 + p^2} + \mathrm{CD40} - \lambda_r r.$$

1. Ahmad KF, et al. (2003) Mechanism of SMRT corepressor recruitment by the BCL6 BTB domain. *Mol Cell* 12:1551–1564.
2. Brass AL, Zhu AQ, Singh H (1999) Assembly requirements of PU.1-Pip (IRF-4) activator complexes: inhibiting function in vivo using fused dimers. *EMBO J* 18: 977–991.
3. Su S-T, et al. (2009) Involvement of histone demethylase LSD1 in Blimp-1-mediated gene repression during plasma cell differentiation. Mol Cell Biol 29:1421–1431.
4. Alvarez MJ, Sumazin P, Rajbhandari P, Califano A (2009) Correlating measurements across samples improves accuracy of large-scale expression profile experiments. *Genome Biol* 10:R143.
5. Niu H, Ye BH, Dalla-Favera R (1998) Antigen receptor signaling induces MAP kinase-mediated phosphorylation and degradation of the BCL-6 transcription factor. *Genes Dev* 12:1953–1961.
6. Shapiro-Shelef M, et al. (2003) Blimp-1 is required for the formation of immunoglobulin secreting plasma cells and pre-plasma memory B cells. *Immunity* 19:607–620.
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8. Kuo TC, Calame KL, Lymphocyte-Induced Maturation Protein B (2004) B lymphocyte-induced maturation protein (Blimp)-1, IFN regulatory factor (IRF)-1, and IRF-2 can bind to the same regulatory sites. *J Immunol* 173:5556–5563.

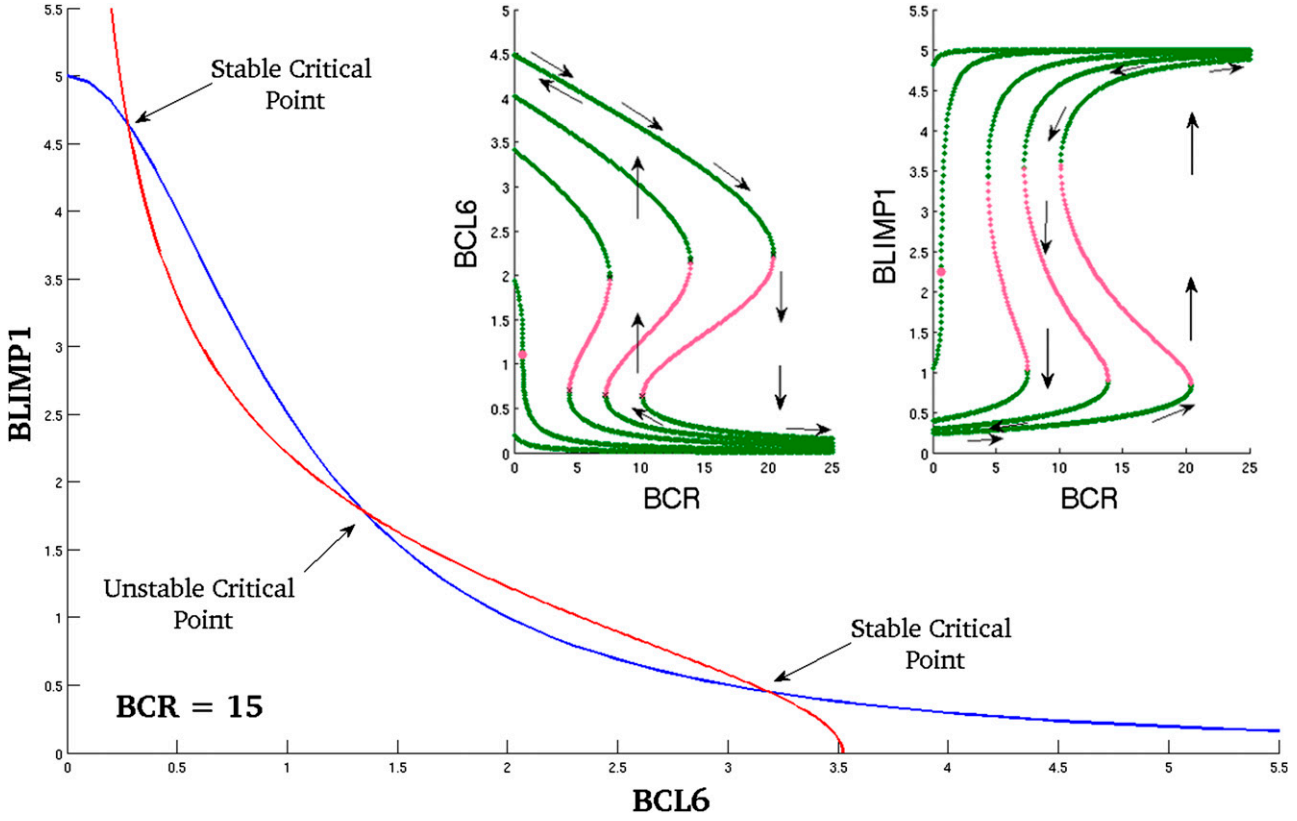

Fig. S1. Nullclines and trajectories of the BCR signaling subsystem. The red and blue line show the nullclines of the BCR signaling module. The intersections of the nullclines are the critical points: two stable and one unstable. Inset: Hysteresis curves on the BCR signaling reaction for different values of BCL6 transcription and degradation. The green and pink points indicate respectively stable and unstable equilibrium points. Black arrows indicate the evolution of a cell after an increase and decrease of BCR signaling.

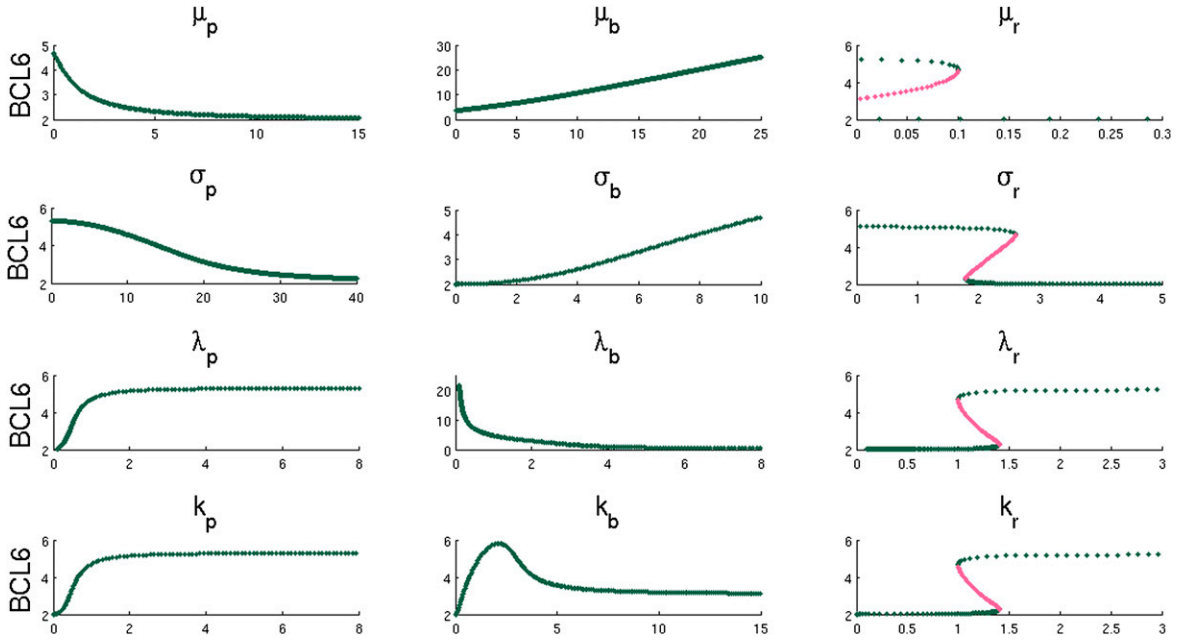

Fig. S2. BCL6 bifurcation study showing BCL6 homeostatic levels for all systems parameters. Only the kinetic parameters associated to IRF4 dynamics (λ_r, μ_r, σ_r, and k_r) show a regime where bistable solutions can be found, supporting the idea of the critical role of IRF4 in the transition from germinal centers to plasma cell. The green and pink points indicate respectively stable and unstable equilibrium points.

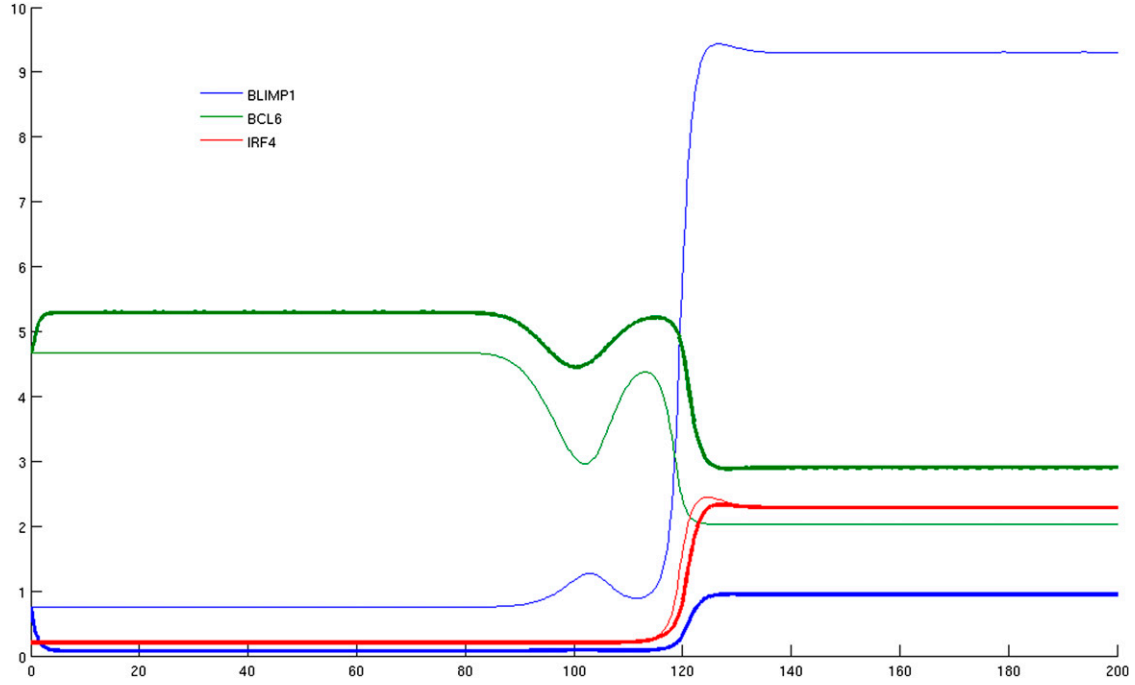

Fig. S3. Hypothetic scenario for memory B-cell differentiation where IRF4 regulatory program has been partially abrogated after BCR and CD40 stimulation at the GC. Specifically, elimination of IRF4-mediated BLIMP1 activation in Eq. **S1** leads to a homeostatic state (thick lines) after stimulation at the GC, comparable to a memory cell: low levels of BLC6 and IRF4 and absence of BLIMP1 expression. For comparison, the steady-state levels in a typical plasma cell are also shown (thin lines).

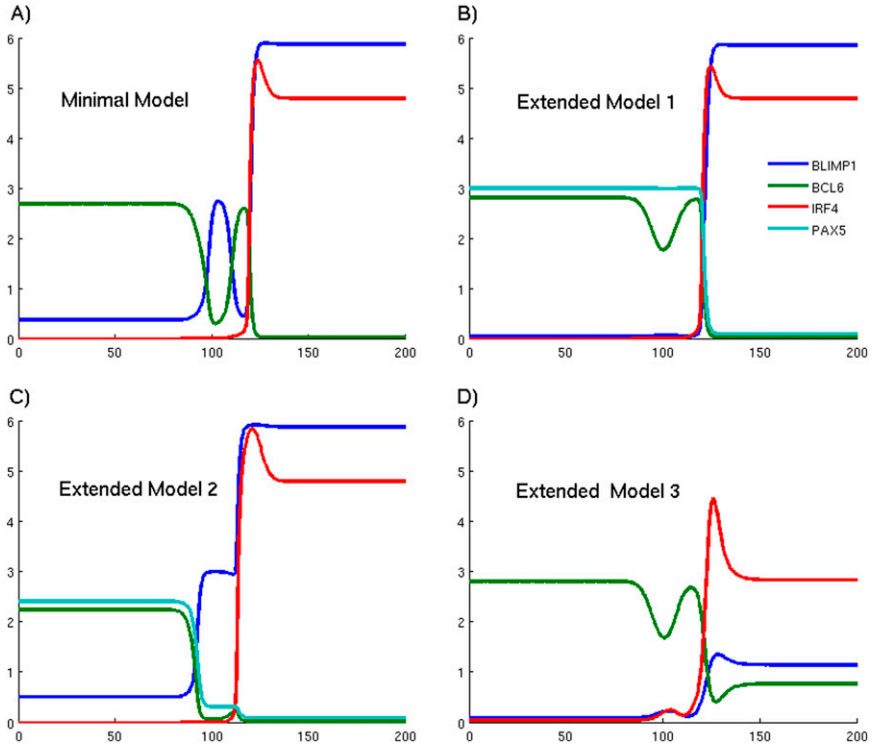

Fig. S4. Comparison of the minimal model (*A*) with extended models 1–3 (*B–D*). Simulations show qualitative agreement between all models.

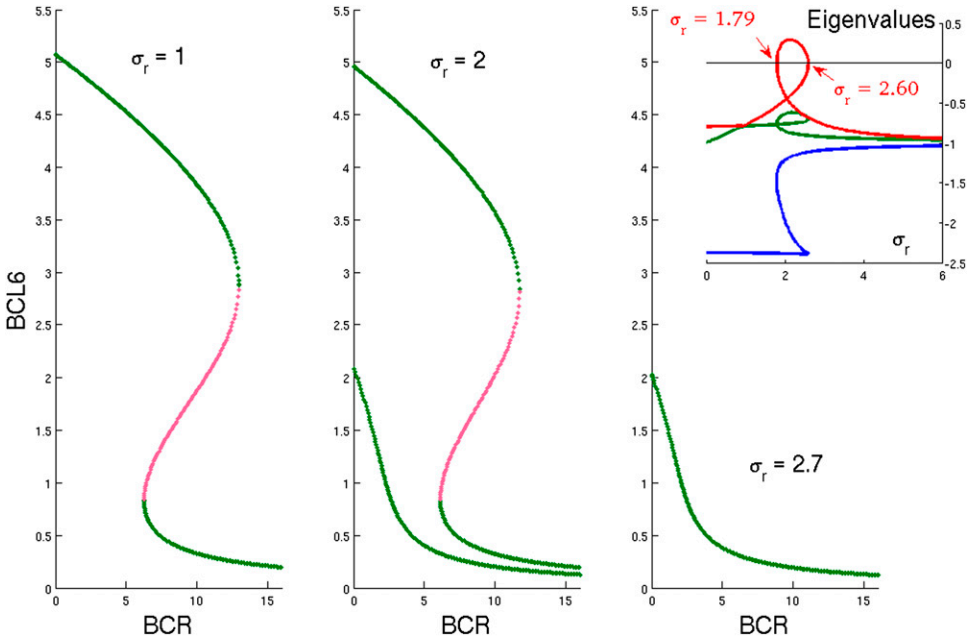

Fig. S5. Bifurcation analysis of the homeostatic levels of a GC cell after BCR stimulation for different values of IRF4 self-induced transcription rate, σ_r. As described in the main text, the appearance of bistability and hysteresis is linked to changes in a parameter β, which roughly describes the ratio of IRF4 production versus degradation (Eq. **S9**). In physiologically normal GC B cells, β is only increased through CD40-mediated stimulation; however, in aberrant cells, β may be modified by additional pathological mechanisms, such as an increase of the self-induced IRF4 production rate. This situation is shown in this figure where it is observed that outside a certain range of transcription rates only one branch is available. The disappearance of the second branch, representing a different cellular phenotype, implies that the cell has lost the ability to dynamically access it and, thus, the normal transit from GC to PC is abrogated. The range of parameters for which this happens is linked to the sign of the eigenvalues (*Inset*): When one eigenvalue becomes positive, both branches can be present simultaneously.

Table S1. Parameter values used in Eqs. S1–S3

Parameter	Value	Units	Description
μ_p	10^{-6}	C_0/t_0	Basal transcription rate
μ_b	2		
μ_r	0.1		
σ_p	9		Maximum induced transcription rate
σ_b	100		
σ_r	2.6		
k_p	1	C_0	Dissociation constant: ligand concentration that produces
k_b	1		half of the maximum induced transcription rate
k_r	1		
λ_p	1	$1/t_0$	Degradation rate
λ_b	1		
λ_r	1		
bcr_0	0–10	C_0/t_0	Range of BCR-induced degradation of BCL6
cd_o	0–1		Range of CD40-induced transcription of IRF4
$C_0 = 10^{-8}$ M			
$t_0 = 4$ h			

Table S2. Bistability related parameter sensitivity analysis

Parameter	Min value	Max value	Sensitivity coefficient,* %	Protein regulated
μ_p	0	>100	<10	Parameters controlling BLIMP1 dynamics
σ_p	0	>100	<9	
λ_p	>0	>100	<1	
k_p	>0	>100	<1	
μ_b	0	>100	<2	Parameters controlling BCL6 dynamics
σ_b	0	$>10^2$	<1	
λ_b	>0	>100	<1	
k_b	>0	>100	<1	
μ_r	0	0.101	100	Parameters controlling IRF4 dynamics
σ_r	1.79	2.62	32	
λ_r	0.99	1.40	41	
k_r	1	1.40	40	

*The parameter sensitivity coefficient for bistability is defined as the ratio between the parameter used in the model (Table S1) and the range of parameters within which the systems is bistable, i.e., parameter/Δparameter.