

Understanding repertoire sequencing data through a multiscale computational model of the germinal center

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Supplementary Information

Maintaining consistency among mutations

Supplementary Text 1. To ensure consistency of affinity values across the mutated sequences during the GC reaction, we store each combination of affinity and BcR sequence in a database. If a sequence is mutated, we determine its fate and, subsequently, update its affinity according to the following rules:

1. For a silent mutation in a region, we randomly select a nucleotide position, arbitrarily change said nucleotide and check that the corresponding amino acid is not changed. Otherwise, a different nucleotide position is selected.
2. For a lethal mutation in a FWR (replacement) we randomly select a nucleotide position and arbitrarily change the nucleotide at this position in a way that also changes the aminoacidic sequence. Subsequently, we check our database to ensure that this mutation was not already associated with a different type of mutation (neutral) from an earlier time point. If not, then the mutation is accepted we set the affinity of the B cell to zero and we update the database. Otherwise, we select a different nucleotide or we select a different position if none is viable until the process is successful. Finally, the database is updated.
3. For a neutral mutation in a FWR (replacement) a nucleotide position is randomly selected, said nucleotide is changed into a different nucleotide in a way that changes the corresponding aminoacid. The database is checked to confirm that the selected mutation was not associated with a lethal mutation at a previous time point. If not, then the mutation is accepted and stored as neutral, otherwise a new nucleotide is selected until the process is successful. Finally, the database is updated.

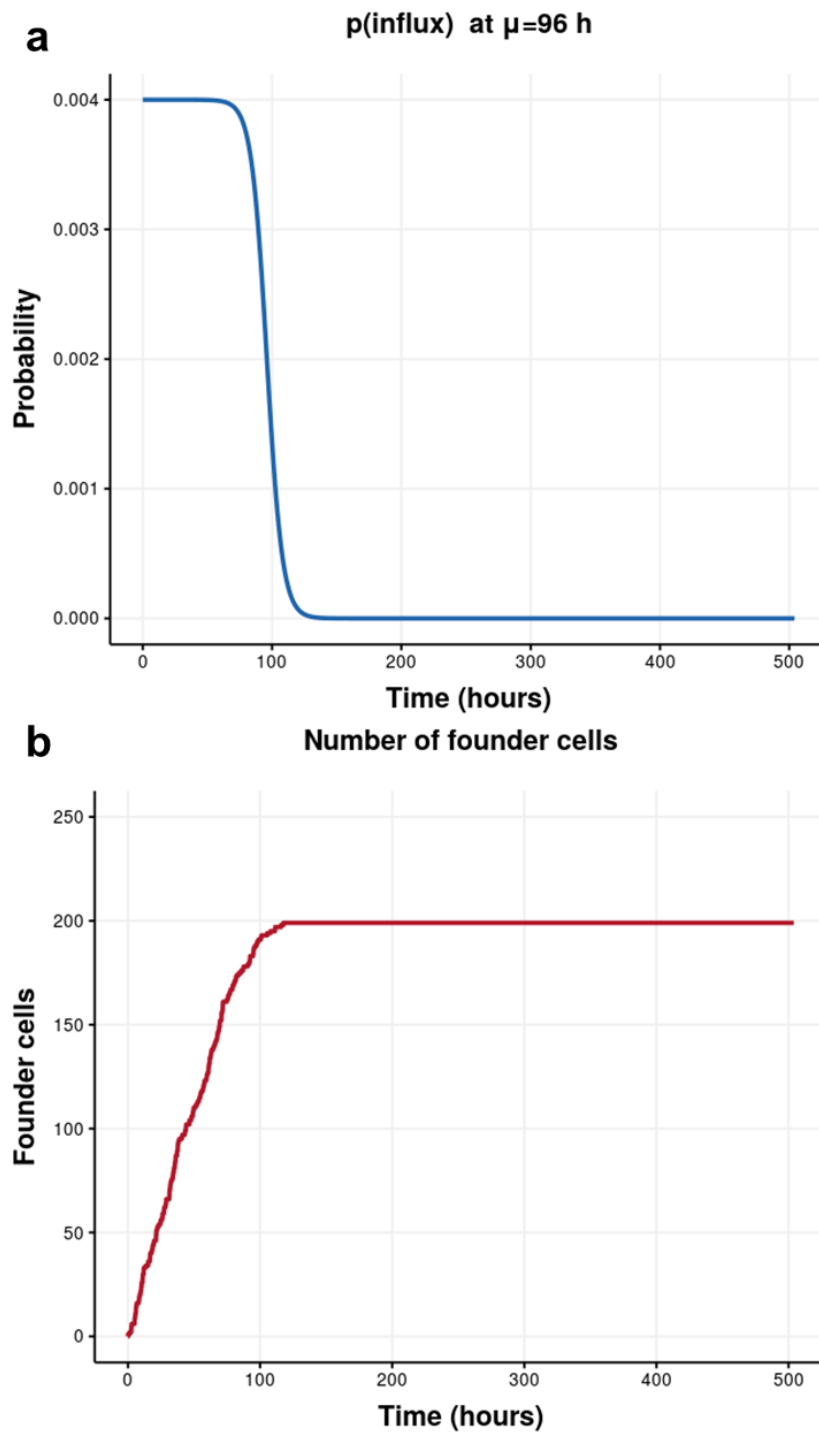
4. For an affinity changing mutation in a CDR (replacement) we randomly select a nucleotide position and change it to a different nucleotide such that this gives a change in the aminoacidic sequence. If a combination of identical CDR1, CDR2, and CDR3 amino acid sequences is present in the database then the affinity of the new daughter B cell is set to the affinity assigned to this combination in the database, otherwise a new affinity is determined from the shape space, based on the position of its mother cell or, in the case of back mutations, of its founder cell. Finally, the database is updated. It is important to take into account that subclones with different combinations of CDRs can have descendant subclones that share the same combination of CDRs between them (converge). This convergence can lead to a greater change in affinity than what could be expected from the normal distribution used in the shape space. This is because the affinity of a combination of CDRs is based on the affinity of the first mother cell whose descendant mutates into that combination, which can be very different from the affinity of another mother cell whose daughter B cell mutates into the same combination. This is something that may happen in a repertoire; e.g. Mathew and colleagues¹ reported an extreme case where a difference in two amino acids led to a nearly million-fold difference in the dissociation constant.

Software

Supplementary Text 2. The ABM is written in C++² version 17.

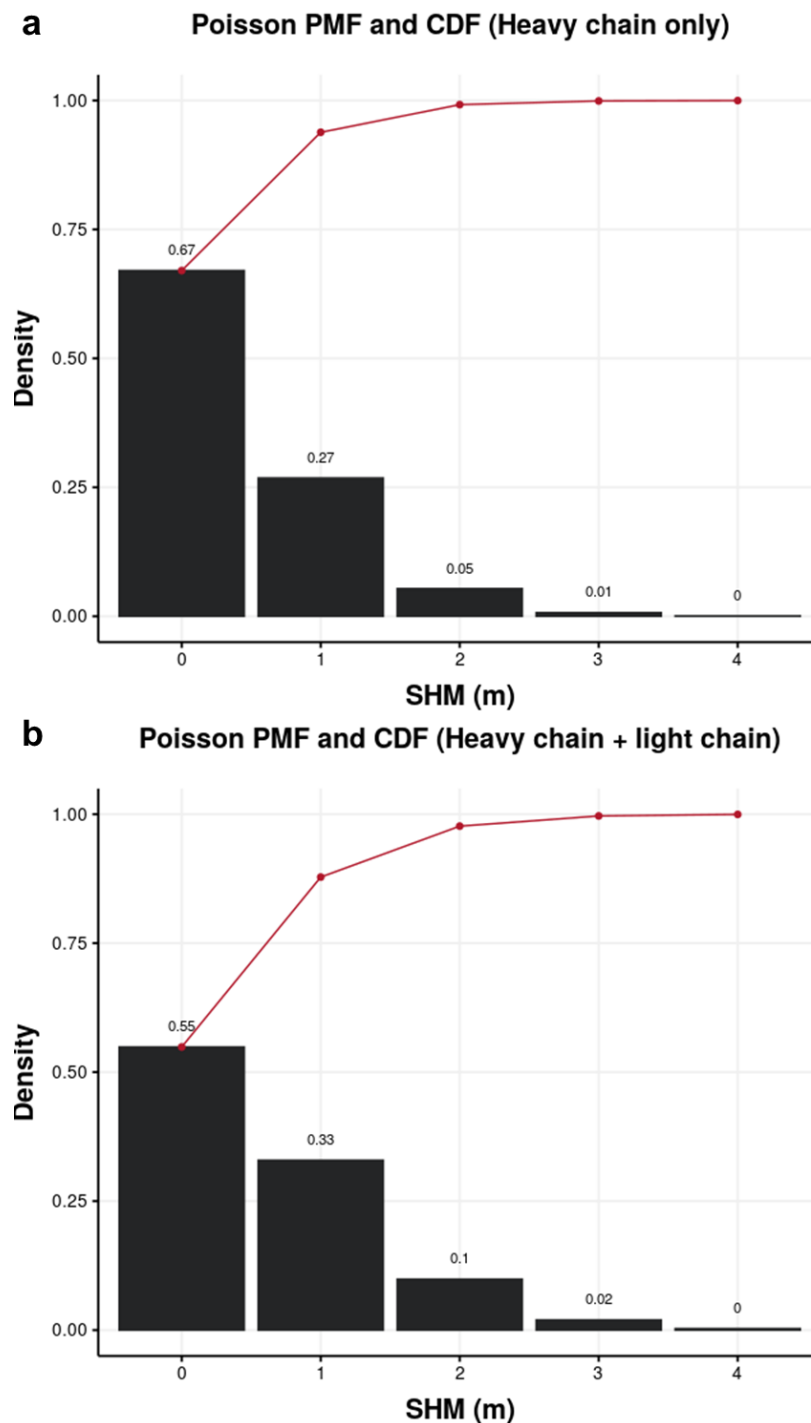
The analysis of the ABM output was done on R version 4.0.3³ using the packages Biostrings v.2.56⁴, dplyr v.1.0.0⁵, ggplot2 v.3.3.2⁶, ggbeeswarm_ v.0.6.0⁷, igraph v.1.2.5⁸, viridis v.0.6.1⁹, readr v.1.3.1¹⁰, seqinr v.3.6.1¹¹ and stringdist v.0.9.5.5¹². The analysis of the dataset from Attaf et al¹³ was done on R version 4.0.3 using the packages biomaRt v. 2.44.4¹⁴, dplyr v.1.0.0, EDASeq v. 2.28.0¹⁵, GenomicRanges v.1.40.0¹⁶, GEOquery v. 2.56.0¹⁷, ggplot2 v.3.3.2, gprofiler2 v.0.2.1¹⁸, org.Hs.eg.db v.3.11.4¹⁹, plyranges v. 1.8.0²⁰, Rsamtools v.2.4.0²¹, rtracklayer v.1.48.0²², Seurat v.4.1.0²³ and plyr v. 1.8.6²⁴.

Influx of founder cells



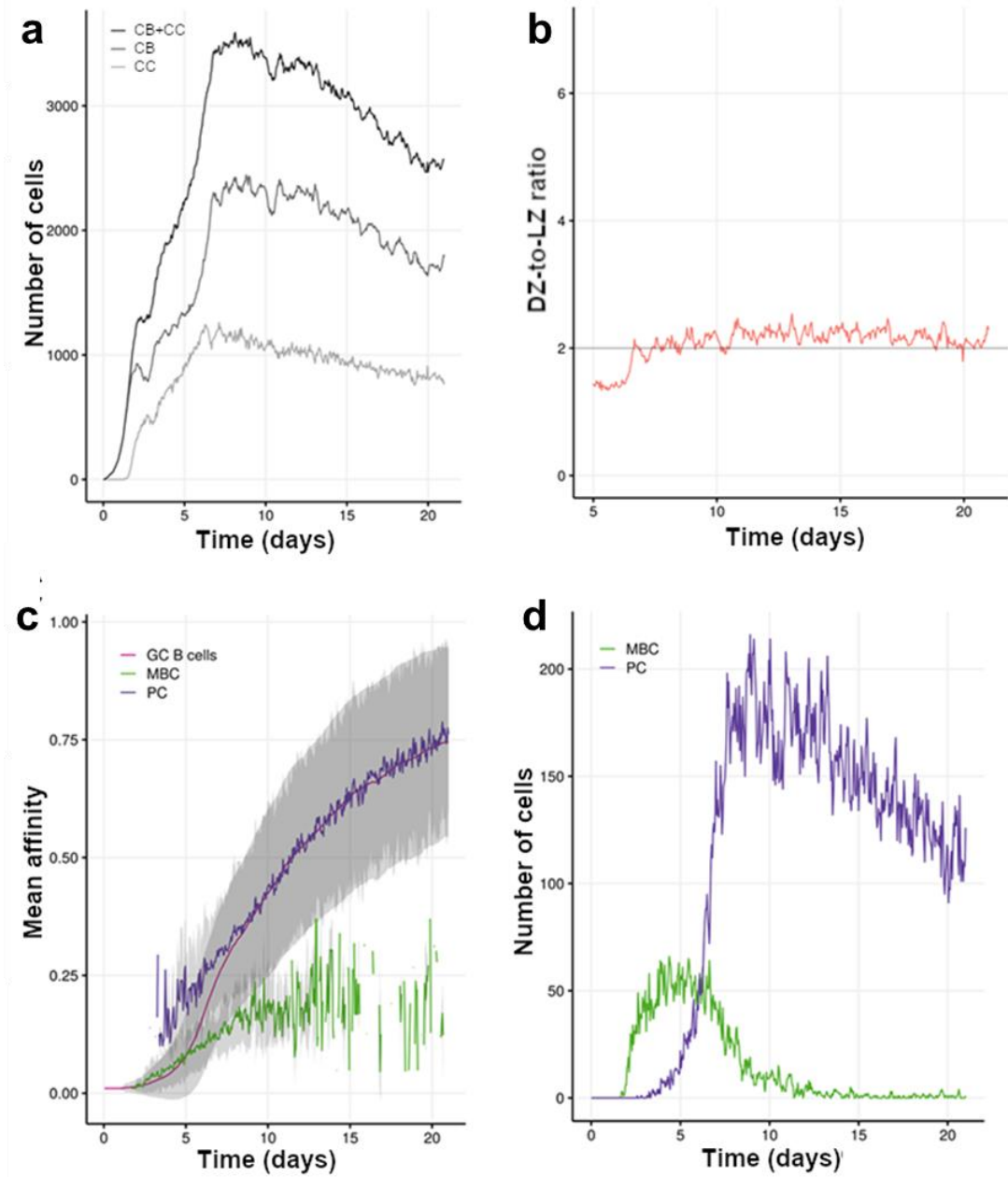
Supplementary Figure 1. (a) probability for a founder B cell to enter the GC reaction. (b) cumulative number of founder B cells that enter the GC in a single simulation, according to this probability. After approximately 96 hours the probability of influx quickly decreases until no new founder B cells enter the GC reaction.

Number of somatic hypermutations during each B cell division



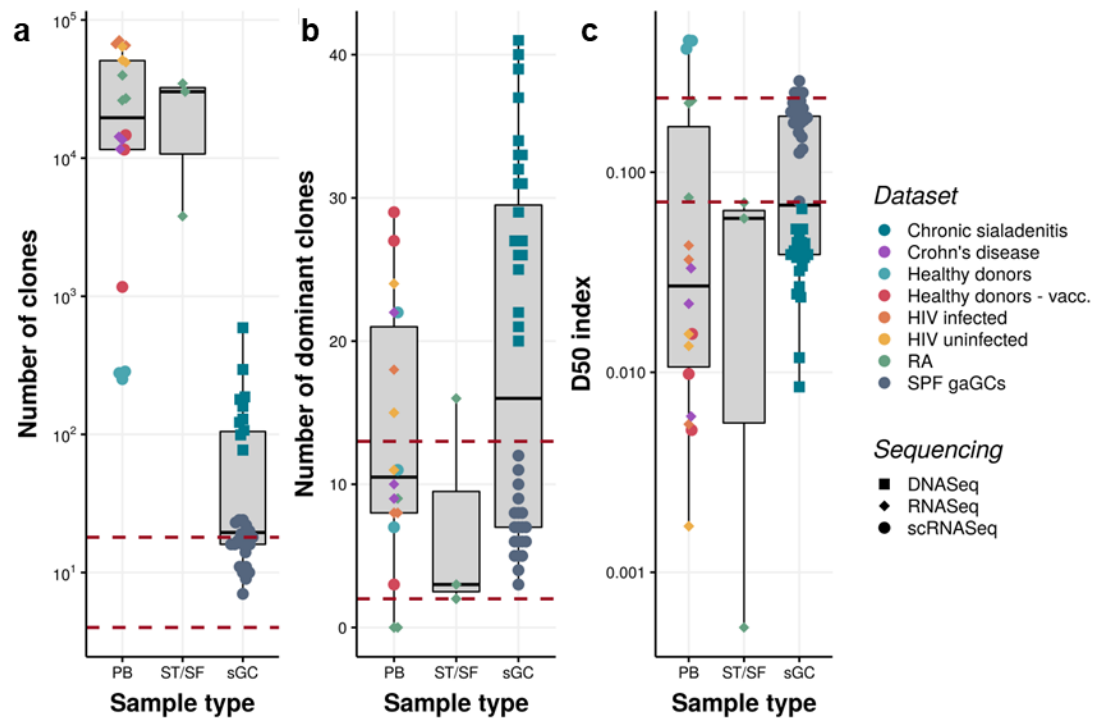
Supplementary Figure 2. Probability mass function (PMF, black bars) and cumulative distribution function (CDF, red line). **(a)** Number of BcR heavy chain somatic hypermutations assuming a heavy chain length of 400 nucleotides and a mutation rate of 1 mutation per 1000bp per B cell division²⁵. Number of SHM in heavy chain is $m \sim \text{Poisson}(\lambda=0.4)$ mutations per B cell division. In 33% of the B cell divisions, the BcR is mutated. **(b)** assuming a heavy chain and light chain with an approximate total length of 600 nucleotides, the BcR is mutated is 45% of the B cell divisions.

Overall GC dynamics



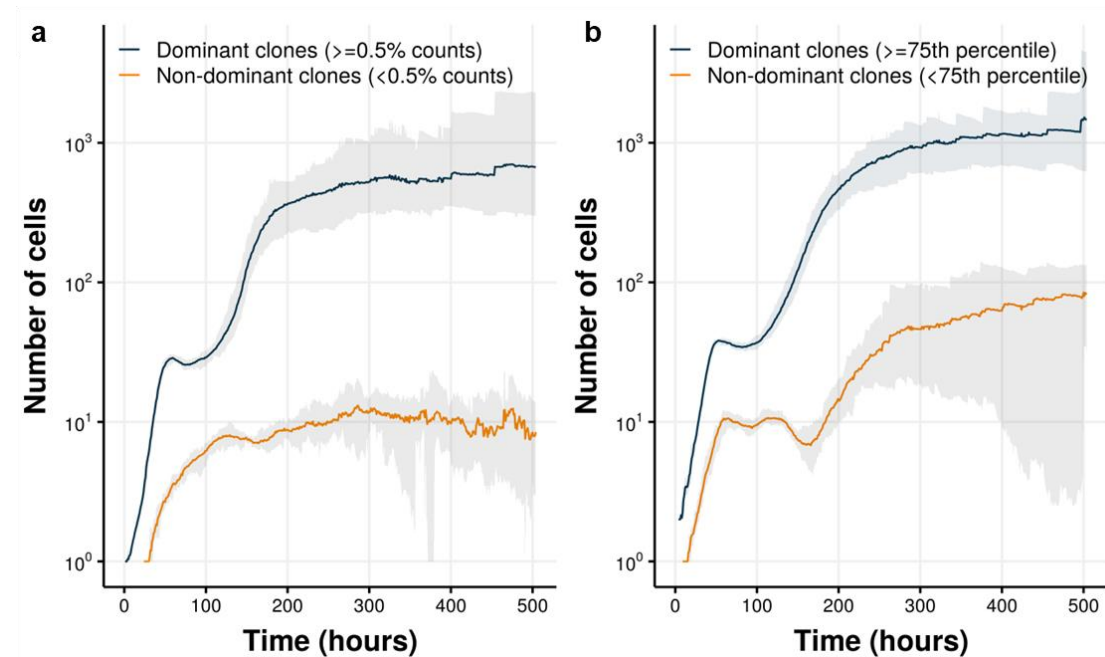
Supplementary Figure 3. Dynamics of the GC reaction observed from a single representative simulation of the eMS model of our previous computational model²⁶. **(a)** Number of centrocytes and centroblasts with a peak response after about 7.5 days²⁷⁻²⁹. **(b)** DZ-to-LZ ratio reflecting the transzone migration rates. This ratio has shown to be approximately 2³⁰. **(c)** Affinity maturation of the GC B cells, MBCs and PCs. Lines represent the average values for that cell type. Shadowed area represents the average value plus/minus its standard deviation. The interrupted lines show the time points at which no cells of that type are present in the GC. **(d)** Number of MBCs and PCs in the GC. Most MBCs are of low affinity and produced at an early stage during the GC reaction, in contrast to PCs that are mostly of higher affinities and produced mostly after the GC peak response in agreement with experimental data from Weisel and colleagues³¹.

Comparison with blood, tissue and single GC Rep-Seq data



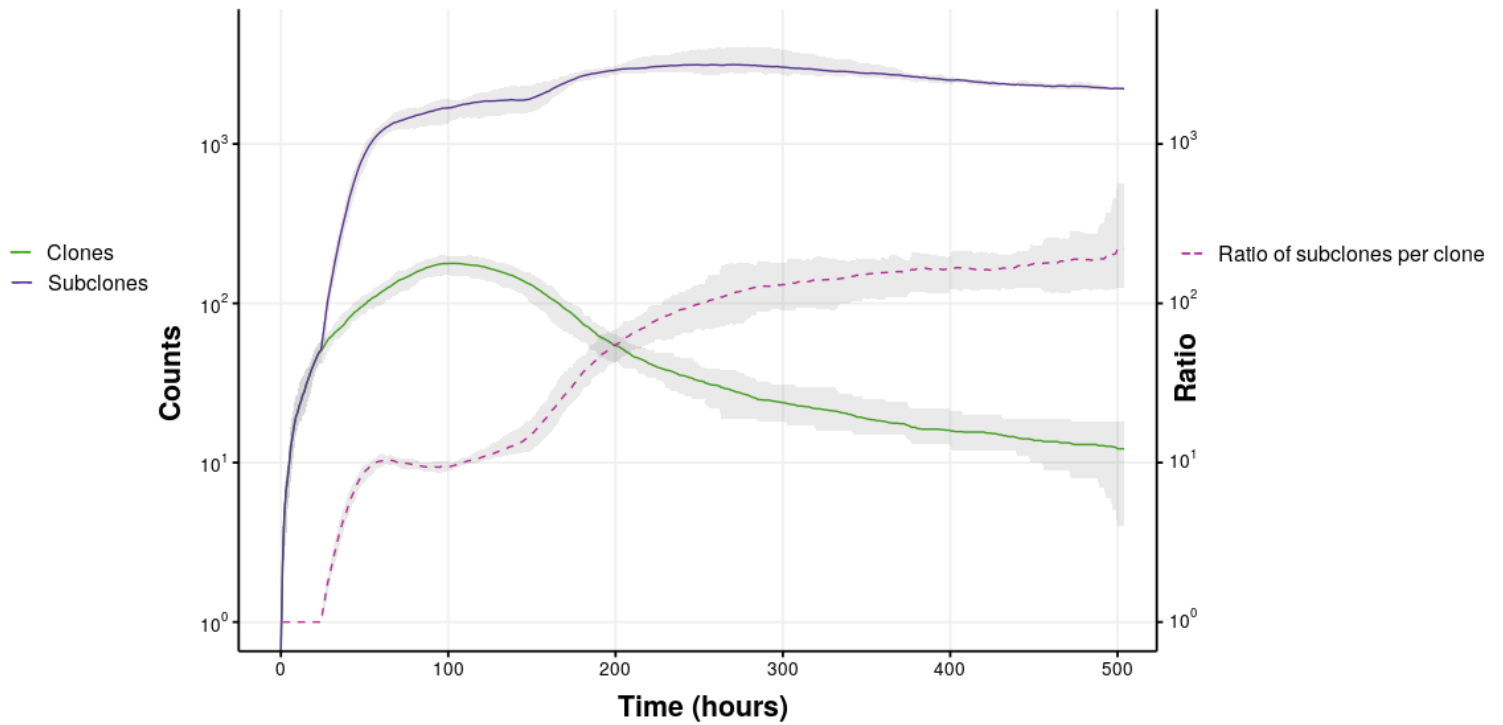
Supplementary Figure 4. Number of clones (a), number of dominant clones (b), and D50 values (c) determined from nine repertoire sequencing datasets. Dominant clones were defined the clones accounting for at least as 0.5% of the repertoire. The dashed red lines show the minimum and maximum values from nine simulations at day 21 of the simulation. SF: synovial fluid. ST: synovial tissue. sGC: single GC. Horizontal line: median. Box: 25th and 75th percentiles. Whiskers: 1.5 time the interquartile range.

Average size of (non)dominant clones during the GC reaction



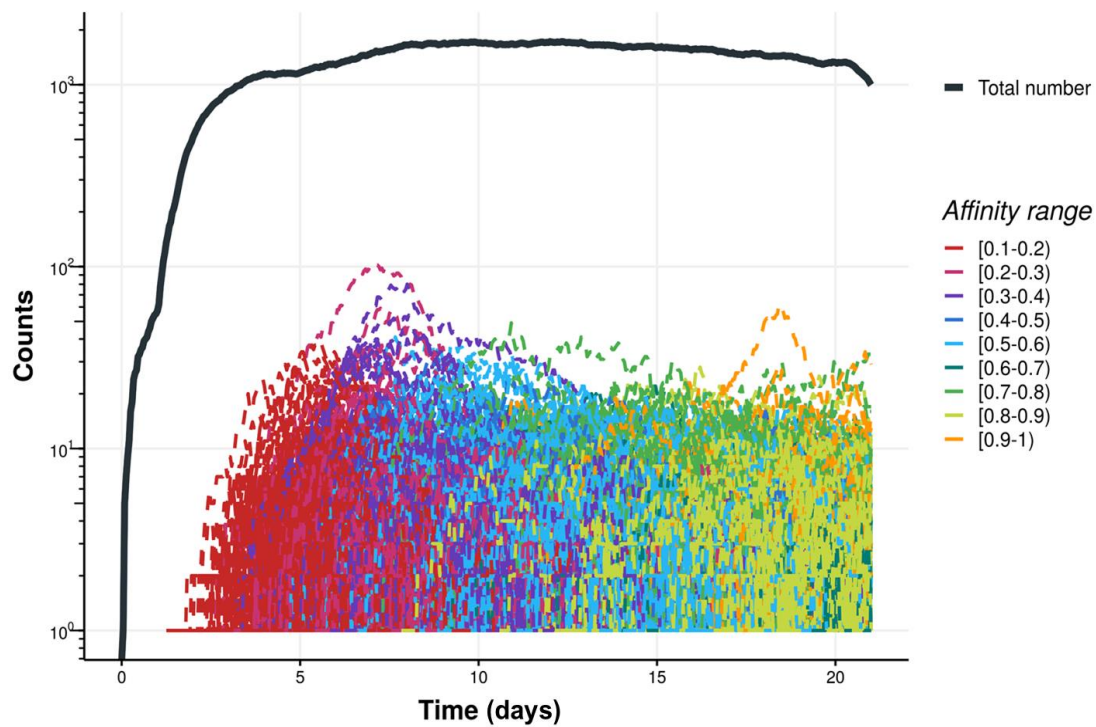
Supplementary Figure 5. Results from nine simulations showing the evolution of the mean clonal sizes of dominant clones (blue) and non-dominant clones (orange) during a 21-day GC reaction. Dominant clones were defined as those with abundances $\geq 0.5\%$ of the total counts (a) or higher or equal than the 75th percentile of clonal abundances (b). The shaded area represents the minimum and maximum of the mean values obtained from the simulations. When the number of clones within a group is low, changes on its clonal composition by group switching or by elimination lead to leaps in the trends. Our simulation suggests that the definition for dominant clones should be well-considered as it can have a relevant effect on their selection. The criteria in (a) depends on the total number of counts while the criteria in (b) instead depends on the distribution of the clonal abundances. In this case (b) is more restrictive than (a).

Number of (sub)clones during GC reaction



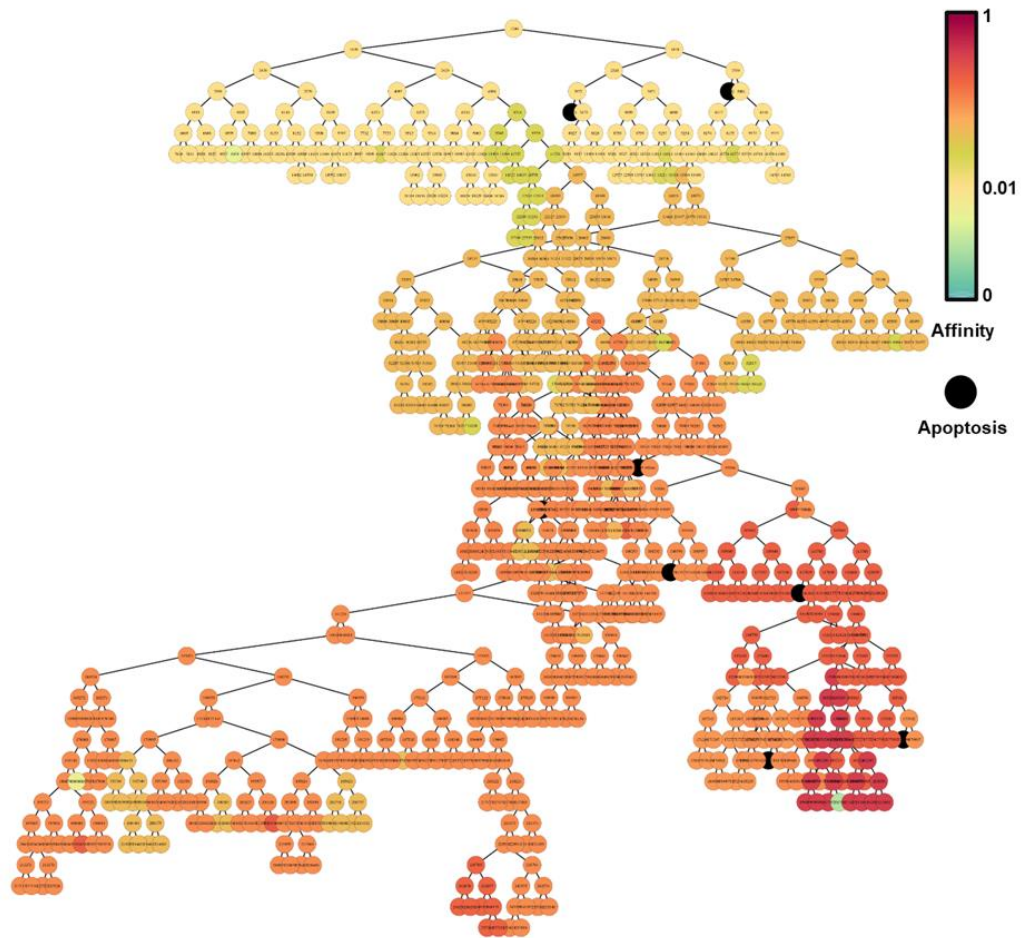
Supplementary Figure 6. Progression of the number of (sub)clones and the average ratio of subclones per clone in log10 scale during the GC reaction for nine repeated simulations. The number of clones initially increase as a result of the entry of founder B cells. Subsequently, the number of clones decrease due to clonal competition. Lines represent the average value while the shadowed area represents the interval of minimum and maximum values of the nine simulations at each time point.

Subclone dynamics



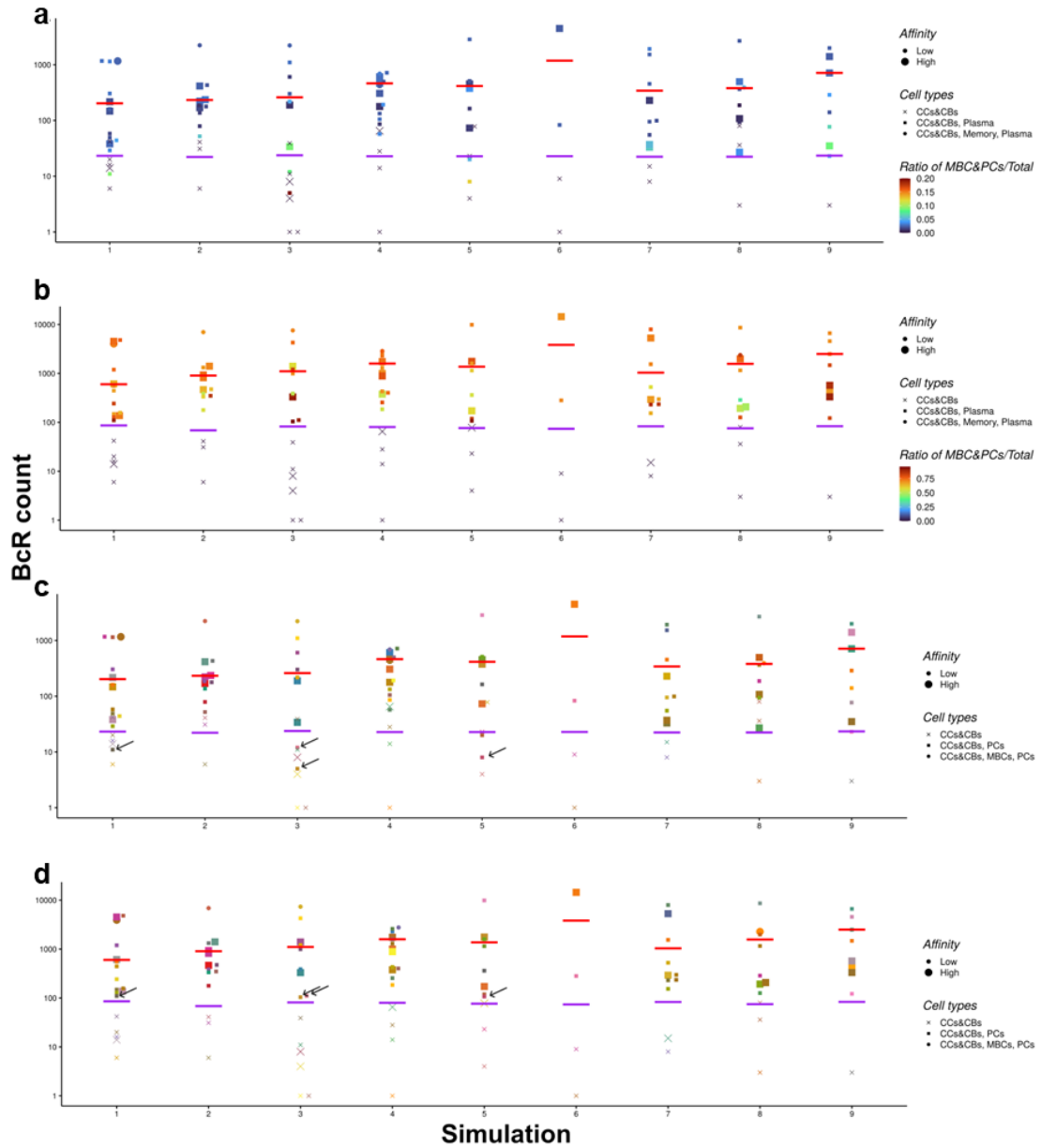
Supplementary Figure 7. Abundance and affinity of subclones, defined as B cells that share the same Fab NT sequence, during a 21-day GC reaction (representative simulation). Only subclones with a frequency larger than 3 counts at any timepoint are included in this plot. Each colored line represents a subclone with a unique BcR. When a subclone is created by SHM it will start as a single B cell that will, subsequently, proliferate. The abundance of each subclone at any timepoint does not exceed 100 copies. This is due to the fact that a mutation of one subclone B cell will, by definition, create a new subclone. Consequently, a SHM reduces the subclone count with one. This balance between proliferation and SHM prevents large subclonal frequencies. It also accounts for the relatively constant of about 2000 subclones with a frequency larger than 3 counts at any timepoint during the GC reaction (black line).

Single B cell lineage tree



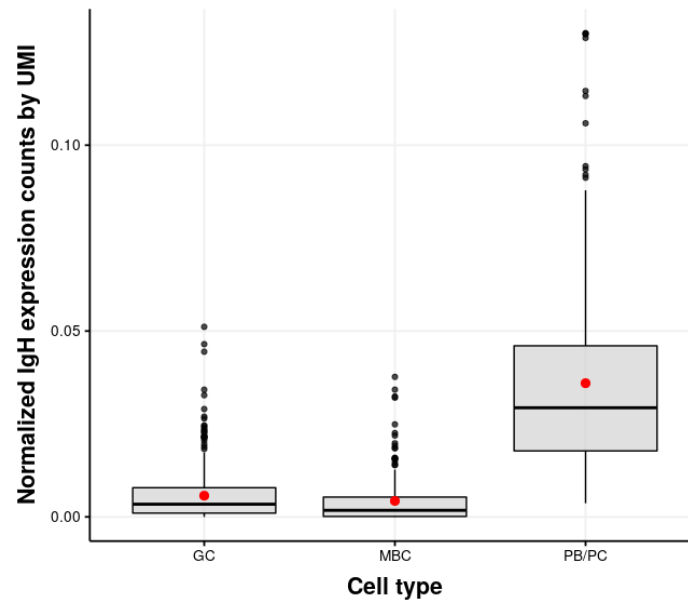
Supplementary Figure 8. Example of the division of a single unmutated founder B cell during a 21-day GC reaction. During each division two daughter B cells are generated. SHM may change the affinity of each B cell. The color of a cell denotes its affinity value; from 0 (blue: very low affinity), to 0.01 (yellow: low affinity, initial affinity of the founder cell) to 1 (red: high affinity) or its functionality (black: lethal mutation, non-functional BcR). The division trees for all founder B cells are shown in the main text (Figure 5). Every cell is labeled according to their ID number.

Dominant clones in nine repeated simulations



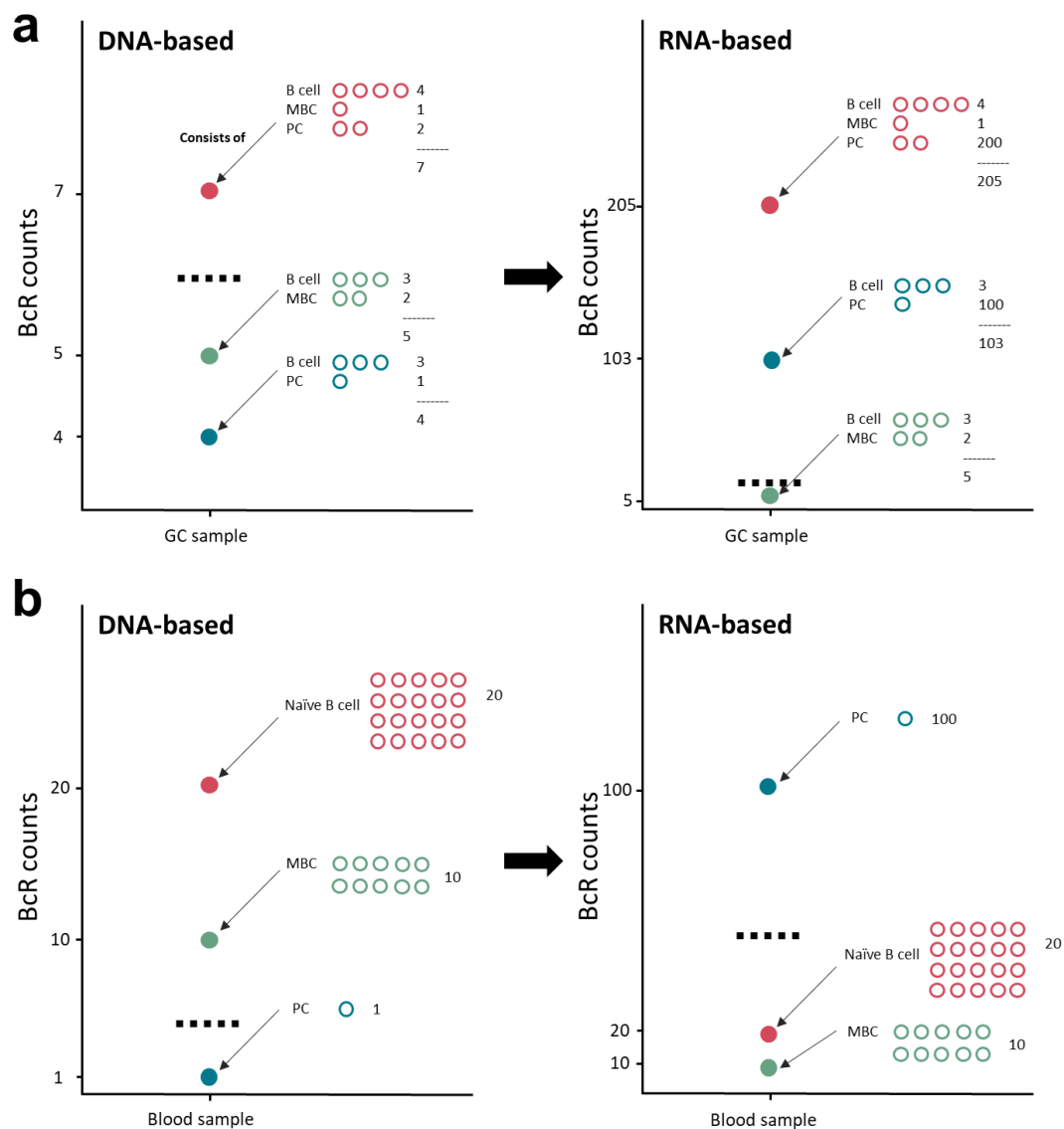
Supplementary Figure 9. (a, c) DNA-seq and (b, d) RNA-seq repertoires at day 21 of the GC reaction generated by nine repeated simulations. Each dot represents a clone, some of which are a mixture of B cells, MBCs and/or PCs. Colors in (a) and (b) indicate the percentage of PCs within each clone, while colors in (c) and (d) represent each clone. The size of the symbol represents the median affinity of that clone (small: affinity < 75th percentile). The horizontal lines denote the 75th percentile (red) and 0.5% (purple) thresholds that define dominant clones. In (c) and (d) the arrows point to some of the clones that can be selected as dominant or not depending on the sequencing method.

Determination of BcR RNA levels



Supplementary Figure 10. Normalized IgH expression counts per cell type (GC, MBC, PB/PC). The red spots represent the mean value for each cell type. To determinate the relation between BcR levels and cell type, we processed the human tonsils datasets from Attaf and coworkers¹³, following their methods, using the default Seurat workflow²³ to determinate the cell type based on expression markers. For every cell, we calculated the sum of all the IgH genes UMI counts normalized by the total cell UMI count. Then we compared the IgH expression between the different groups.

BcR counts in DNA-based vs RNA-based scenarios



Supplementary Figure 11. Conceptual illustration of DNA-based vs RNA-based clonal size inference for three clones and a threshold for selecting the dominant clones in (a) a single GC sample and (b) peripheral blood. In the DNA-based scenario, every cell type contributes equally to the calculation of the clonal size. In the RNA-based scenario, PCs contribute up to 100 times greater than the other cell types, changing the inferred clonal sizes, distorting the results and the selection of dominant clones over the threshold. The correlation between the clonal size and its PC composition in GCs helps to reduce this change. However, in peripheral blood this correlation does not need to happen and therefore dominant clones can be wrongly selected in the RNA-based scenario.

GC ABM and gene regulatory network

Parameters for the model

Supplementary Table 1. Main parameters of the GC model. Source and additional information for these parameters related to the GC ABM can be found in previous literature³²⁻³⁶. Parameters of the ODE model of the GRN³⁷ are normalized by a unit of time (t_0) and concentration (C_0). p=BLIMP1, b=BCL6, r=IRF4.

Category	Parameter name	Value	Unit	Concept
<i>Space discretization</i>				
	dimension	3	dimensions	Lattice Dimensions
	dt	0.002	hour	Time resolution
	tmin	0	hour	Beginning of the simulation
	tmax	504	hour	Maximum duration of the simulation
	dx	5	μm	Lattice Constant of space grid
	radius	160	μm	Radius of GC
	zoneRatioGC	0.5		Ratio that determines the position of DZ in Germinal Center
<i>Initialization</i>				
	InitialNumberSC	300	cell	Initial Number Stromal cells
	InitialNumberTC	250	cell	Initial Number T-cells
	InitialNumberCB	0	cell	Initial Number CBs (founder cells)
	InitialNumberFDC	200	cell	Initial Number FDCs
	macrophage	6	cells/hour	Rate of macrophage transport of dead cells
	DendriteLength	40		Length FDC dendrites / dx (number of positions)
	AgAmountperFDC	3000	Antigens	Presented Antigen per FDC
	Avogadro_constant	6.02E+23	mol ⁻¹	Number of Avogadro
<i>Affinity</i>				
	Gamma	2.8		Width of gaussian affinity weight function
	eta	2		Exponent of the hamming distance
	expMin	5.5	1/mol	Conversion of shape space affinity to (1/mol)
	expMax	9.5	1/mol	Conversion of shape space affinity to (1/mol)
	Nmax	9	residue	Maximum number of residues in one dimension
<i>Dynamic update of chemokine receptors</i>				
	CXCL13crit	8.00E-11	mol	Critical CXCL13 concentration for desensitization
	CXCL13recrit	6.00E-11	mol	Critical CXCL13 concentration for resensitization
	CXCL12crit	6E-09	mol	Critical CXCL12 concentration for desensitization
	CXCL12recrit	4E-09	mol	Critical CXCL12 concentration for resensitization
<i>Chemotaxis</i>				
	chemmax	10		Maximum weight of chemotaxis to random polarity
	chemosteep	1.00E+10	mol/L	Steepness of weight reduction with chemokine gradient
	chemohalf	2.00E-11	L/mol	Chemokine gradient of half weight
	chemo_dx	5	μm	Lattice Chemokine Constant
<i>PersistentLengthTime(Cell Type)</i>				
	Bcell_tp	1.5	hour	B-Cell Persistent Time average

Bcell_tp_stddev	0 hour	B-Cell Persistent Time stddev
Tcell_tp	1.7 hour	T Cell persistence time
Tcell_tp_stddev	0 hour	T Cell persistence time Standard deviation
Plasmacell_tp	0.75 hour	Plasma and Memory B Cell persistence time
Plasmacell_tp_stddev	-1 hour	Plasma and Memory B Cell persistence time Standard deviation

Speed(Cell Type)

Bcell_speed	7.5 $\mu\text{M} / \text{min}$	B-Cell Speed
Tcell_speed	10 $\mu\text{M} / \text{min}$	T-Cell Speed
Plasmacell_speed	3 $\mu\text{M} / \text{min}$	Plasma and Memory B cell speed

Duration of cell cycle phases (according to Gaussian distribution with parameters)

c_G1	2.5 hour	Phase G1 of cell cycle
c_G1_stddev	1 hour	Stddev of phase G1 of cell cycle
c_S	1.5 hour	Phase S of cell cycle
c_S_stddev	1 hour	Stddev of phase S of cell cycle
c_G2	2.5 hour	Phase G2 of cell cycle
c_G2_stddev	1 hour	Stddev of phase G2 of cell cycle
c_M	0.5 hour	Phase M of cell cycle
c_M_stddev	1 hour	Stddev of phase M of cell cycle

Differentiation rates

StartDifferentiation	72 hour	Time when differentiation can start
difDelay	6 hour	Delay cell differentiation after TC selection
DeleteAgInFreshCC	TRUE Boolean	Retained Ag is deleted in fresh CC
Ccdif_delay_stddev	0 hour	Standard deviation for delay to differentiation.
pApoCC	0	% Casp3+ LZ cells per hr. used as (apoptosis rate)
pApoCB	0	% Casp3+ DZ cells per hr. used as (apoptosis rate)
tolight	0.1	Rate for differentiation of centroblasts to centrocytes

Dynamic number of divisions

pMHCdepHill	2	p-MHC dependent division number Hill (Hill coef. n_P)
pMHCdepMin	1	p-MHC dependent division number Hill (Hill coef. P_Min)
pMHCdepMax	6	p-MHC dependent division number Hill (Hill coef. P_Max)
pMHCdepK	9	p-MHC dependent division number Hill (Hill coef. K_P)
NoMutFounderCells	FALSE Boolean	Founder cells do not mutate
nDiv	12 divisions	Number of divisions of founder cells present at time 0
nDiv_stddev	0 divisions	Stddev of Number of divisions of founder cells present at time 0
nDivinflow	6 divisions	Number of divisions of founder cells that enter during the process
rateCBinflow	2 cells/hour	Rate of inflow of CBs (founder cells)
smoothnessStopCBinflow	6	Smoothness of the stop of inflow CB (-1 = no)
timeStopCBinflow	96 hour	Time when CB inflow stops (effective time depends on the smoothness)

Mutation and division

pmutB4StartMut	0	Probability of mutation before the start of the mutation period
StartMutation	24 hour	Start of the mutation period
lambda	0.4	Lambda value of the Poisson formula for the probability of mutations after 24 hours
alpha_FWR1	0.17	Probability of a mutation happening in FWR1

alpha_FWR2	0.17	Probability of a mutation happening in FWR2
alpha_FWR3	0.29	Probability of a mutation happening in FWR3
alpha_FWR4	0.05	Probability of a mutation happening in FWR4
alpha_CDR1	0.1	Probability of a mutation happening in CDR1
alpha_CDR2	0.03	Probability of a mutation happening in CDR2
alpha_CDR3	0.19	Probability of a mutation happening in CDR3
beta_FWR1	0.73	Probability of a R mutation in FWR1
beta_FWR2	0.67	Probability of a R mutation in FWR2
beta_FWR3	0.73	Probability of a R mutation in FWR3
beta_FWR4	0.6	Probability of a R mutation in FWR4
gamma_CDR1	0.79	Probability of a R mutation in CDR1
gamma_CDR2	0.76	Probability of a R mutation in CDR2
gamma_CDR3	0.75	Probability of a R mutation in CDR3
delta	0.5	Probability of a lethal R mutation in the FWRs, always leading to apoptosis
polarityIndex	1	Assymetric Distribution of Ag
widthPI	0.04	Coefficient of variation around Polarity Index
pDivideAgAssymmetric	0.72	Probability to divide Ag assymmetrically to daughter B-cell

Selection steps

testDelay	0.02	hour	Time gap between TFHC-CC binding tests
collectionFDCperiod	0.7	hour	Duration of CC collection of Antigen by serial encounters with FDC
agSaturation	20	threshold	Ag saturation per FDC fragment in units of threshold. 1:constant
pSel	0.04		Probability to be selected by FDC
tcTime	0.6	hour	Duration of CC-Tc contact
tcRescueTime	0.5	hour	Minimum duration of TC-CC-polarization for CC-rescue
Ag_threshold	1.00E-08	mol	Threshold Ag-concentration for binding CC

Antibody/BcR

kon	1.00E+06	1/(mol*h)	k_on for building immune complex
koff	0.001	1/s	k_off for dissociation of immune complex (in /s):
BCR_length	4	dimension	Dimensions in the sspace space

ODE system

μ_p	1.00E-6	C ₀ /t ₀	Basal transcription rate
μ_b	2	C ₀ /t ₀	Basal transcription rate
μ_r	0.1	C ₀ /t ₀	Basal transcription rate
σ_p	9	C ₀ /t ₀	Maximum induced transcription rate
σ_b	100	C ₀ /t ₀	Maximum induced transcription rate
σ_r	2.6	C ₀ /t ₀	Maximum induced transcription rate
κ_p	1	C ₀	Dissociation constant
κ_b	1	C ₀	Dissociation constant
κ_r	1	C ₀	Dissociation constant
λ_p	1	1'/t ₀	Degradation rate
λ_b	1	1'/t ₀	Degradation rate
λ_r	1	1'/t ₀	Degradation rate
bcr0	1	1/t ₀	Maximum BCR signal

cd0	50 C_0/t_0	Maximum CD40 signal
C₀	1.00E-8 M	Concentration unit
t₀	4 h	Time unit

Supplementary Table 2. Results at day 21 from nine repeated simulations.

Simulation	Clones	Dominant clones				D50	
		DNA-based		RNA-based			
		75th percentile	0.5%	75th percentile	0.5%	DNA-based	RNA-based
1	18	5	13	5	13	0.11	0.11
2	14	4	13	4	11	0.07	0.07
3	15	4	8	4	9	0.13	0.13
4	17	4	15	4	13	0.24	0.24
5	11	3	8	3	9	0.09	0.09
6	4	1	2	1	2	0.25	0.25
7	11	3	9	3	9	0.18	0.18
8	11	3	10	3	9	0.09	0.09
9	9	2	7	2	8	0.22	0.22
Average	12	3	9	3	9	0.15	0.15

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