

# A Multiscale Spatial Modeling Framework for the Germinal Center Response

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## Abstract

29 The germinal center response or reaction (GCR) is a hallmark event of adaptive humoral  
30 immunity. Unfolding in the B cell follicles of the secondary lymph organs, a GC culminates in the  
31 production of high-affinity antibody-secreting plasma cells along with memory B cells. By  
32 interacting with follicular dendritic cells (FDC) and T follicular helper (Tfh) cells, GC B cells  
33 exhibit complex spatiotemporal dynamics. Driving the B cell dynamics are the intracellular signal  
34 transduction and gene regulatory network that responds to cell surface signaling molecules,  
35 cytokines, and chemokines. As our knowledge of the GC continues to expand in depth and in  
36 scope, mathematical modeling has become an important tool to help disentangle the intricacy of  
37 the GCR and inform novel mechanistic and clinical insights. While the GC has been modeled at  
38 different granularities, a multiscale spatial simulation framework – integrating molecular, cellular,  
39 and tissue-level responses – is still rare. Here, we report our recent progress toward this end  
40 with a hybrid stochastic GC framework developed on the Cellular Potts Model-based  
41 CompuCell3D platform. Tellurium is used to simulate the B cell intracellular molecular network  
42 comprising NF- $\kappa$ B, FOXO1, MYC, AP4, CXCR4, and BLIMP1 that responds to B cell receptor  
43 (BCR) and CD40-mediated signaling. The molecular outputs of the network drive the  
44 spatiotemporal behaviors of B cells, including cyclic migration between the dark zone (DZ) and  
45 light zone (LZ) via chemotaxis; clonal proliferative bursts, somatic hypermutation, and DNA  
46 damage-induced apoptosis in the DZ; and positive selection, apoptosis via a death timer, and  
47 emergence of plasma cells in the LZ. Our simulations are able to recapitulate key molecular,  
48 cellular, and morphological GC events including B cell population growth, affinity maturation,  
49 and clonal dominance. This novel modeling framework provides an open-source, customizable,  
50 and multiscale virtual GC simulation platform that enables qualitative and quantitative *in silico*  
51 investigations of a range of mechanistic and applied research questions in future.

52 **Key words:** B cells, germinal center, dark zone, light zone, affinity maturation, proliferative burst,  
53 chemotaxis

54

## Introduction

55 The adaptive humoral immune response is a vital component of host defense, where B cells  
56 terminally differentiate into plasma cells (PCs) that secrete antibodies specifically recognizing  
57 and neutralizing the invading foreign antigens. The B cell responses can be broadly classified  
58 into two types: T cell-dependent and independent, depending on whether helper T (Th) cells are  
59 involved (Nutt et al. 2015). In the T cell-independent response, naive B cells are activated  
60 directly via toll-like receptors (TLR) recognizing pathogen components such as  
61 lipopolysaccharide (LPS) or CpG DNA or via B cell receptors (BCR), without the assistance of  
62 Th cells (Fagarasan and Honjo 2000, Allman et al. 2019). The response is launched quickly and  
63 can occur within a few days of initial infection. Upon activation, B cells undergo clonal  
64 proliferation and differentiate into PCs, which secrete pentameric IgM molecules. While these  
65 antibodies provide initial protection, they are often polyclonal, not highly specific, and the IgM-  
66 secreting PCs are short-lived, thus unable to provide long-term immunity. In contrast, the T cell-  
67 dependent B cell response takes longer to develop, but through affinity maturation and class  
68 switch recombination (CSR) it can produce long-lived PCs that can provide life-long immunity  
69 with high-affinity IgG or other non-IgM antibody classes (Parker 1993). Additionally, memory B  
70 cells are generated during the primary response, which can quickly launch a secondary  
71 antibody response upon subsequent exposure to the same antigens (Inoue and Kurosaki 2024).

72

73 T cell-dependent B cell activation takes place primarily in the germinal centers (GC),  
74 which are specialized, often transient, microstructures formed in the B cell follicles of secondary  
75 lymphoid tissues such as lymph nodes and spleen in response to infection or immunization  
76 (Mesin et al. 2016, Stebegg et al. 2018, Young and Brink 2021, Victora and Nussenzweig 2022).  
77 GC B cells exhibit unique spatiotemporal dynamics (Allen et al. 2007a, Victora et al. 2010). A  
78 GC is polarized, containing two distinct, physically separated zones: the dark zone (DZ) and the  
79 light zone (LZ). In the DZ, B cells undergo clonal proliferative bursts, during which somatic

80 hypermutation (SHM) occurs. During SHM, the hypervariable regions of the genes encoding the  
81 immunoglobulin heavy chains and light chains are point-mutated by activation-induced cytidine  
82 deaminase (AID) at a high rate (Methot and Di Noia 2017). As a result, the BCR affinities of the  
83 participating B cell clones for the invading antigen are modified and diversified. During SHM  
84 those B cells incurring damaging mutations that prevent normal assembly of surface BCRs are  
85 killed via apoptosis in the DZ (Mayer et al. 2017, Stewart et al. 2018). After exiting the cell cycle  
86 following a proliferative burst, B cells migrate from the DZ to LZ under the chemoattractant force  
87 by CXCL13 secreted by follicular dendritic cells (FDCs) in the LZ (Allen et al. 2004, Cosgrove et  
88 al. 2020).

89

90 In the LZ, two main cell types participate in the positive selection of B cell clones  
91 harboring immunoglobulin (Ig) gene variants encoding relatively high-affinity antibodies: the  
92 residential FDCs and CD4<sup>+</sup> T follicular helper (Tfh) cells. These two cell types coordinate to  
93 provide key molecular signals for B cell activation, survival, DZ re-entry, proliferation, and  
94 differentiation (Vinuesa et al. 2010, Luo et al. 2018, Crotty 2019). FDCs are antigen-presenting  
95 cells (APCs), which previously encountered and engulfed pathogens and present the antigen  
96 epitopes on their cell surface. When B cells first encounter FDCs, their BCRs are activated by  
97 the surface antigens of FDCs. B cells then internalize the antigen-BCR complex and present the  
98 antigen epitopes on their own surface through major histocompatibility complex (MHC) II  
99 molecules to form peptide-MHCII complex (pMHCII). The density of pMHCII on the cell surface  
100 is proportional to the BCR affinity. Stronger BCR signaling also leads to higher PI3K-AKT-  
101 FOXO1 signal transduction (Hinman et al. 2007, Sander et al. 2015, Luo et al. 2018). When the  
102 B cells subsequently encounter Tfh cells, a complex mutual interaction occurs between the two  
103 cell types (Ise et al. 2018, Crotty 2019, Mintz and Cyster 2020). Tfh cells are activated via T cell  
104 receptors (TCR) liganded by pMHCII of the B cells, as well as by other cell surface signaling  
105 molecules such as inducible co-stimulator ligand (ICOSL) (Liu et al. 2015). Activated Tfh cells in

106 turn express surface CD40L which reciprocally activates B cells together with several secretory  
107 cytokines including interleukins (IL) 4, 10, and 21 (Reinhardt et al. 2009, Xin et al. 2018, Quast  
108 et al. 2022). CD40 signaling leads to NF- $\kappa$ B activation, increasing the chance of survival of B  
109 cells. In the presence of downregulated FOXO1, NF- $\kappa$ B elicits transient MYC activation that  
110 initiates the cell cycle (Luo et al. 2018). Only a small fraction of B cells is positively selected,  
111 which express CXCR4, the receptor for chemokine CXCL12, and migrate back to DZ where  
112 they undergo further proliferative bursts and SHM (Allen et al. 2004, Sander et al. 2015). Those  
113 B cells with weaker BCR affinity are more likely to undergo apoptosis in the LZ, as well as B  
114 cells that do not have a chance to encounter Tfh cells in the LZ. As a result of combined action  
115 of proliferation and SHM in the DZ and positive selection in the LZ, the overall BCR affinity of  
116 the GC B cell population for the antigen continues to improve. After many rounds of DZ-LZ  
117 cycles, a small fraction of B cells are affinity-matured and exit the GC as either long-lived  
118 antibody-secreting PCs or memory B cells.

119

120 The GC plays a critical role in the generation of long-term protective immunity, and this  
121 is relevant in both the context of natural infection and vaccination for infectious diseases such  
122 as COVID-19 (Laidlaw and Ellebedy 2022). If the GC is compromised or cannot be sufficiently  
123 induced due to genetic alterations, increased susceptibility to bacterial and viral infections will  
124 result. On the other hand, unintentional recognition of self-antigens and induction of GC can  
125 lead to autoimmune diseases such as systemic lupus erythematosus (Woods et al. 2015).  
126 Dysregulated B cell proliferation in GC can lead to lymphoma or other B cells-related leukemia  
127 (Mlynarczyk et al. 2019). GC also plays a role in antibody-mediated rejection of transplanted  
128 organs (Chong 2020). In addition, many environmental contaminants are immunotoxicants,  
129 some of which can suppress B cell activation and the humoral immune response, leading to  
130 increased susceptibility to infectious disease and cancer (Germolec et al. 2022). Therefore, a  
131 full mechanistic understanding of the complexity of GC is crucially important for sustaining

132 immune integrity and preventing or alleviating many pathological conditions.

133

134 Computational modeling has played a long-standing role in dissecting and  
135 understanding the complex dynamics of GC immune responses (Meyer-Hermann et al. 2009,  
136 Meyer-Hermann et al. 2012). The GC involves an elaborate interplay between many cell types,  
137 signaling molecules, transcription factors, and actuator genes (Verstegen et al. 2021). Key  
138 signal transduction and gene regulatory networks underpin the spatiotemporal dynamics of GC  
139 B cells and are crucial for the positive selection and ultimate formation of high-affinity PCs.  
140 Although there have been many efforts simulating the cellular dynamics and affinity maturation  
141 of GCs, cross-scale modeling that integrates molecular, cellular, and tissue-level actions in a  
142 spatial context has only begun to emerge recently and thus is still rare (Merino Tejero et al.  
143 2021a, Merino Tejero et al. 2021b, Merino Tejero et al. 2022). In this study, we presented a  
144 novel multiscale mathematical modeling framework of the GC developed in the CompuCell3D  
145 simulation environment that integrates the molecular network and spatiotemporal behaviors of  
146 GC B cells. The modeling framework provides an open-source, customizable, multiscale virtual  
147 GC platform that enables future *in silico* investigations of a range of questions both  
148 quantitatively and quantitatively, including B cell population turnover, BCR mutation rate, death  
149 timer, proliferative burst size, availability of Tfh cells, and effects of genetic and chemical  
150 perturbations.

151

152

## Methods

153 **1. Model structure**

154 **1.1 Cell types, cellular events, and interactions**

155 Four cell types are modeled in the framework: CXCL12-expressing reticular cells (CRCs)  
156 located in DZ, FDCs and Tfh cells in LZ, and B cells cycling between the DZ and LZ. For  
157 simplicity, CRC, FDC, and Tfh cells are treated as stationary. Key cellular events of B cells  
158 captured in the model include: (i) B cell volume growth, division, SHM, and apoptosis in the DZ;  
159 (ii) DZ-to-LZ B cell migration and simultaneous initiation of a cell death timer; (iii) interaction of B  
160 cells with FDCs in the LZ to determine BCR antigen affinity, interaction of B cells with Tfh cells  
161 in the LZ to make probabilistic decisions based on pMHC density on positive selection, survival,  
162 initiation of cell growth, and DZ re-entry of positively selected B cells, and death timer-triggered  
163 apoptosis of LZ B cells not positively selected.

164

165 **1.2 Molecular events in B cells**

166 The above cellular events are driven by an intracellular molecular network in B cells that  
167 responds to diffusive chemoattractants and signaling molecules from FDCs and Tfh cells. For  
168 simplicity, the following molecular species and regulatory events are included in the model (Fig.  
169 1). A DZ-to-LZ descending gradient of chemoattractant CXCL12 is established by CRCs in DZ  
170 (Bannard et al. 2013, Rodda et al. 2015), and an opposite gradient of chemoattractant CXCL13  
171 is established by FDCs in LZ (Wang et al. 2011, Cosgrove et al. 2020). In LZ, the contact of a B  
172 cell with an FDC will trigger BCR-mediated signal transduction, which leads to several signaling  
173 events in the modeled B cell: (1) re-expression on B cell surface of pMHCII, the density of which  
174 depends on BCR affinity for the antigen, (2) transient activation of AKT and downregulation of  
175 FOXO1, the extent of which depends on BCR affinity (Hinman et al. 2007, Sander et al. 2015,  
176 Luo et al. 2018), (3) once the BCR affinity reaches a threshold, a switch-like activation of NF- $\kappa$ B  
177 subtype RelA is triggered (Shinohara et al. 2014, Michida et al. 2020, Wibisana et al. 2022),

178 which in turn induces BLIMP1 (Heise et al. 2014, Roy et al. 2019), leading to terminal B cell  
179 differentiation into antibody-secreting PCs .

180

181 If a B cell expressing pMHCII encounters a Tfh cell, the B cell is stimulated via CD40  
182 signaling which activates another NF- $\kappa$ B subtype, cRel. cRel activation leads to at least two  
183 molecular signaling events. It activates BclxL which inhibits apoptosis, thus terminating the  
184 death timer (Zarnegar et al. 2004). With FOXO1 still downregulated, cRel also induces the  
185 expression of MYC (Luo et al. 2018). Upregulation of MYC triggers commitment to cell growth  
186 and initiates the cell cycle (Dominguez-Sola et al. 2012). MYC also activates AP4, which  
187 sustains B cell growth and division burst (Chou et al. 2016). With the B cell committed to growth  
188 and proliferation, as FOXO1 is re-expressed, CXCR4 is induced (Dominguez-Sola et al. 2015,  
189 Sander et al. 2015). As a result, the B cell migrates towards the DZ in response to the CXCL12  
190 gradient. In the DZ, shortly after each cell division, each of the two daughter cells incurs an  
191 independent point mutation of BCR which alters its affinity for the antigen with some  
192 probabilities (Faili et al. 2002, Sharbeen et al. 2012, Wang et al. 2017). The probability of a  
193 damaging mutation is encoded such that a fraction of B cell progeny dies by apoptosis in the DZ  
194 (Mayer et al. 2017, Stewart et al. 2018). The surviving B cells will continue to grow and divide as  
195 long as AP4 remains above a threshold (Chou et al. 2016). When AP4 drops below the  
196 threshold, the B cells exit the cell cycle followed shortly by downregulation of CXCR4 (Weber  
197 2018). With CXCR5 constitutively expressed (Allen et al. 2004, Victora et al. 2010), the B cells  
198 will be pulled by the CXCL13 gradient field into the LZ, repeating the DZ-LZ cycle.

199

### 200 **1.3 Model assumptions and simplification**

201 The molecular and cellular events involved in the GC are complex. Here, for the purpose of  
202 modeling, several assumptions and simplifications are made.

203 (1) The mutual activation between B cells and Tfh cells is simplified to pMHCII density-

204 dependent CD40-cRel activation, as described above.

205 (2) Many factors involved in GC, including BCL6, BACH2, and IRF4, are not explicitly  
206 considered.

207 (3) Initial migration of B cells from the T/B cell border towards the LZ is not considered and  
208 neither is the Tfh cell migration into the LZ. CSR is thus not included as it is believed to  
209 occur primarily during pre-GC formation (Roco et al. 2019).

210 (4) For B cells returning to the DZ, a delay variable is introduced before the cell growth for the  
211 first cell cycle is initiated to reduce the chance of cell division in the LZ.

212 (5) The GC exit of PCs is modelled as deleting these cells from the simulation once they  
213 emerge.

214 (6) Formation of memory B cells is not considered.

215

## 216 **2. Construction of the computational model in CompuCell3D**

217 The GC model was constructed and simulated as a hybrid, agent-based stochastic model in  
218 CompuCell3D. CompuCell3D provides a flexible and customizable platform for simulating multi-  
219 cellular behaviors and interactions based on the Glazier-Graner-Hogeweg approach (Swat et al.  
220 2012). The Cellular Potts Model module in CompuCell3D was employed to simulate the physical  
221 properties and movements of individual B cells (Graner and Glazier 1992), while the molecular  
222 network operating in each individual B cell, as depicted in Fig. 1, was simulated by using the  
223 Gillespie's stochastic algorithm implemented in Tellurium conforming to the Antimony notation  
224 (Gillespie 1977, Choi et al. 2018). The CompuCell3D model consists of four files: an XML file, a  
225 Potts initialization file (PIF), and two Python script files. The XML file contains various "Plugins"  
226 and "Steppables" that define some default Potts model parameter values. The *Chemotaxis*  
227 plugin defines CXCL12 and CXCL13 as the chemoattractants, and the *DiffusionSolverFE*  
228 steppable designates that CXCL12 and CXCL13 are secreted by CRC and FDC respectively  
229 and specifies the parameter values for secretion, diffusion, and decay in the Medium. The PIF

230 file contains the initial coordinates of medium and cells where applicable. The steppable Python  
231 file contains the script that defines several steppable classes, including *GCR\_Steppable*,  
232 *MitosisSteppable*, *BCell\_GRNSteppable*, and *VisualizationSteppable*, and the Tellurium model.  
233 The main Python file contains the script that imports and registers all the steppables and runs  
234 the simulation.

235

## 236 **2.1 Initialization**

237 The model is initialized in the “start” section of *GCR\_Steppable*, including the generation of  
238 CRCs, FDCs, Tfh cells, and seeding B cells. Each B cell is assigned a Tellurium molecular  
239 network model named as *BCellNetwork*.

240

## 241 **2.2 Cell-cell contact and probabilistic decision-making**

242 To capture the physical contact between B cells, FDCs, and Tfh cells, the *NeighborTracker* and  
243 *PixelTracker* plugins are employed to identify the neighboring cells of each B cell. All  
244 neighboring cells of a B cell at a given moment are first identified by utilizing the  
245 *get\_cell\_neighbor\_data\_list()* function, then the specific cell type of each neighboring cell is  
246 identified by utilizing the *neighbor\_count\_by\_type()* function. Once a contact with an FDC is  
247 identified, the pMHCII level of the B cell is set proportional to its antigen-specific BCR affinity.  
248 Upon subsequent contact with a Tfh cell, the B cell can be positively selected based on a  
249 probability that is proportional to the pMHCII level. For the positively selected cell, the running  
250 death timer is terminated, cell cycle is committed, and DZ re-entry is initiated.

251

## 252 **2.3 SHM and probability of BCR affinity alteration**

253 Each of the two daughter cells of a dividing B cell has a probability of 0.3 to produce a  
254 damaging mutation that will result in cell death in the DZ. For the daughter cell that does not  
255 incur a damaging mutation, an SHM can either increase, decrease, or does not change the BCR

256 affinity, each with a probability of 1/3. The increment or decrement of the affinity alteration can  
257 be either 0.25 or 0.5 with equal probability. In general, the BCR affinity ranges between 0-10,  
258 but can be higher.

259

260 **3. Simulation data collection, storage, analysis, and model sharing**

261 Variables of each B cell are saved in a plain text file which is updated every 15 Monte Carlo  
262 steps (*mcs*). Saved variables include cell generation, mother ID, cell ID, BCR affinity, cell  
263 volume, X, Y, and Z-coordinates, and the molecular species levels in the Tellurium model, etc.  
264 The file is named in the format of “Generation\_mother ID\_cell ID.txt”. Analyzing the simulation  
265 results saved in the txt files was conducted in a separate Python script. The CompuCell3D  
266 model files, which contain the model parameter values, are available as Supplemental Material.

267

268

## Results

269 **1. Morphology of a simulated GC**

270 The morphological results of a representative simulation of the GC model are shown in Fig. 2.  
271 The simulated space dimension is 250x200x11 pixels, which can be considered as 250x200x11  
272 in  $\mu\text{m}$  in real space, to represent a slice of the GC to save computational time. The 2-D  
273 projection of the instantiated non-B cells on the X-Y plane is indicated in Fig. 2A, while along the  
274 Z dimension, these cells are distributed randomly in 3 of the 11 layers (results not shown).  
275 There are 45 CRCs distributed on the left half of the field, which becomes the future DZ, and 45  
276 FDCs and 36 Tfh cells distributed on the right half of the field, which becomes the future LZ. Tfh  
277 cells are located next to FDCs, reflecting the notion that they also express CXCR5 and are thus  
278 drawn to the LZ by chemoattractant CXCL13 (Breitfeld et al. 2000, Kim et al. 2001). Not all  
279 FDCs are surrounded by Tfh cells, mimicking the situation that the availability of Tfh cells is a  
280 limiting factor in the positive selection of LZ B cells (Meyer-Hermann et al. 2006, Allen et al.  
281 2007b, Meyer-Hermann 2007, Victora et al. 2010). CRCs and FDCs secrete CXCL12 and  
282 CXCL13 respectively, establishing two opposing chemoattractant fields and thus the polarity of  
283 the GC (Fig. 2B and 2C).

284

285 With the layout of residential cells and chemoattractant fields as above, a simulated GC  
286 that succeeds in producing B cells of high BCR/antibody affinities is shown as snapshots in Fig.  
287 2D and in Supplemental Video S1. For this simulation, the GC starts with 200 B cells (clones) of  
288 intermediate affinity of 5 (indicated by the color of the cells) between the DZ and LZ. Over a  
289 period of 40,000 Monte Carlo steps (mcs, where 100 mcs can be regarded approximately as 1  
290 hour in real time), both the number of total GC B cells and the fraction of high-affinity B cells  
291 increase, indicating successful GC population growth and affinity maturation. The 2-D  
292 trajectories of 3 select B cell lineage branches leading to different cell fates are shown in Fig. 2E.  
293 These trajectories cycle between the DZ and LZ for multiple rounds. The first trajectory ends

294 with cell death in the DZ due to damaging mutation during mitosis (left panel), the second  
295 trajectory also ends with cell death but in the LZ due to death timer (mid panel), and the last  
296 trajectory ends with differentiation into a PC in the LZ (right panel).

297

## 298 **2. Cellular events of GC B cells**

### 299 **2.1 B cell population dynamics**

300 In this section we performed an in-depth quantitative analysis of the GC B cell population  
301 dynamics with respect to time, location, and cell fates. For the GC simulation presented in Fig. 2,  
302 the total number of B cells ( $N_{Tot}$ ) increases rapidly from the initial 200 over a period of 40,000  
303 *mcs*, then approaches a steady-state size of about 2,500 cells through 72,000 *mcs* (equivalent  
304 to 30 days) without considering GC termination (Fig. 3A). The growth of the B cell population is  
305 not smooth – it proceeds in an uneven fashion due to random births and deaths occurring  
306 simultaneously. In the early stage of the GC, the numbers of B cells in the DZ ( $N_{DZ}$ ) and LZ ( $N_{LZ}$ )  
307 alternate in anti-phase, resulting from cyclic cell migration in unison between the two zones  
308 (Video S1). In the late stage,  $N_{DZ}$  is persistently greater than  $N_{LZ}$  with the  $N_{DZ}:N_{LZ}$  ratio stabilizing  
309 near 3:1 (Fig. 3A). The evolving B cell population in the GC is highly dynamic with constant  
310 turnover through several processes. Specifically, B cells (i) are born in the DZ out of proliferative  
311 bursts of clonal expansion, (ii) are cleared from the GC via apoptosis due to damaging BCR  
312 mutations in the DZ and, if not positively selected, in the LZ, and (iii) exit the GC as PCs. We  
313 next quantified the birth and death events.

314

#### 315 **2.1.1 B cell birth**

316 The number of B cells engaged in cell cycle increases over time and these cells are  
317 predominantly in the DZ (Fig. 3B). There are a small number of cell cycle-engaged B cells in the  
318 LZ, representing positively selected B cells that just initiate the cell cycle without much growing  
319 yet. Approaching the steady state, nearly 70% and 30% of DZ and LZ B cells, respectively, are

320 engaged in the cell cycle, while on average 65% of the overall B cell population is in the cell  
321 cycle (Fig. 3C). B cells are born predominantly in the DZ with only a negligible number of births  
322 in the LZ (Fig. 3D). The absolute birth rate increases over time approaching about 1100 births  
323 per 600 *mcs* (Fig. 3E). Cumulative births reach 150K in the entire 72,000 *mcs* period when two  
324 birth events are registered for each cell division (Fig. 3F). The mean cell generation increases  
325 almost linearly with time while the variability also progressively increases as more B cells are  
326 born (Fig. 3G). The DZ B cell volumes exhibit a biphasic distribution, reflecting that these cells  
327 are actively engaged in growth and division (Fig. 3G). In contrast, the LZ B cells exhibit a very  
328 narrow volume distribution consistent with the notion that they are mostly non-proliferating  
329 centrocytes.

330

### 331 **2.1.2 B cell death**

332 As the GC B cell population grows, the number of cell deaths increases and then approaches a  
333 steady state, where the total death rate is about 1000 deaths per 600 *mcs* (Fig. 3I). Although at  
334 the early time the DZ:LZ death rate ratio fluctuates dramatically as a result of randomness due  
335 to small numbers of cell deaths and DZ-LZ migration, the ratio stabilizes at about 2.5:1 at later  
336 time. The steady-state death turnover rate of the overall B cell population is slightly above 40%  
337 in 600 *mcs*, and the turnover rates in both zones are similar (Fig. 3J). Cumulatively, there are  
338 nearly 70,000 cell deaths, among which 70% occurred in the DZ and 30% in the LZ (Fig. 3K).

339

340 Further analysis showed that different types of cell deaths occur at different rates (Fig.  
341 3L). B cell death due to damaging BCR mutations occurs most often, comprising 65% of total  
342 deaths at steady state, followed by cell death due to no access to Tfh cells at 30%, while cell  
343 death for not being positively selected even after contacting Tfh cells (labelled as Neg selected)  
344 is only a small fraction of all death events (Fig. 3M). When a B cell cannot access Tfh cells,  
345 positive selection decisions cannot be made in time and the B cell will die when the default

346 death timer goes off. This type of death is only limited to B cells in the LZ initially, but as the GC  
347 population grows such that the space becomes more compact and thus crowded, such death  
348 also expands to the DZ when some of the B cells exiting the cell cycle do not have enough time  
349 to migrate through the densely populated DZ (Fig. 3N); however, these deaths in the DZ are  
350 only a small fraction (Fig. 3O).

351

## 352 **2.2 Affinity maturation and clonal dominance**

353 We next characterized the evolution of the BCR antigen affinities in the simulated GC. With all  
354 the 200 seeder B cells starting with an intermediate BCR affinity of 5 in this simulation, their  
355 clonal affinities initially drift to both higher and lower levels (Fig. 4A). However, the mean affinity  
356 increases progressively in a winding manner and then reaches a plateau at about 8.5. The  
357 variabilities of the BCR affinities, as defined by the 25-75% quantiles and 2.5-97.5% percentiles,  
358 also shift upward and then plateau along with the mean, despite that the affinities of some B  
359 cells reach as low as near 2 and as high as over 12 at times. PCs start to emerge shortly after  
360 20,000 *mcs*, with >10 affinity levels (Fig. 4A, 10 is defined as the threshold affinity to trigger  
361 terminal differentiation in the model). The production rate of PCs continues to increase albeit in  
362 a highly stochastic fashion and the total cumulative number of PCs produced at the end of GC  
363 reaches 2000 (Fig. 4B). Only a tiny fraction of the B cell population becomes PCs in each 600  
364 *mcs* time period, reaching as high as 2% at the end of simulation (Fig. 4C). The PC antibody  
365 affinities range from 10 to 12.5, occupying the right tail of the over affinity distribution (Fig. 4D).  
366 For those B cells that are positively selected, their mean affinity is 8.28, which is higher than the  
367 mean affinity 7.22 of those negatively selected cells. For those B cells that die due to no access  
368 to Tfh cells, their affinities cover a broader range on the high end, some of which reach 12.5.  
369 Among the initial 200 B cell clones, only 6 clones remain at the end (Fig. 4E), among which one  
370 single clone dominates, comprising over 60% of the total B cells at 72,000 *mcs*, followed by two  
371 other clones each comprising about 12%, while the remaining 3 clones are much smaller (Fig.

372 4F). Additional simulations showed that the fractions of dominant clones may vary for each GC  
373 – in some cases a single clone absolutely dominates the GC, occupying nearly 90% of the B  
374 cells (Fig. S1A and S1B), while in other cases the GC can be co-inhabited by several clones  
375 with no single dominant clone (Fig. S1C and S1D).

376

### 377 **2.3 Inter-zonal migration and LZ residence time**

378 We next characterized the statistics of B cell migrations between the DZ and LZ. For the GC  
379 simulation presented in Fig. 2, there are a total of 33,440 B cells that enter from the DZ to the  
380 LZ, among which 54.6% die, 37.0% are positively selected and return to the DZ (thus making a  
381 full DZ-LZ-DZ round trip), 6.3% differentiate into PCs, and the rest remain in the LZ within the  
382 72,000 *mcs* timeframe of the simulation (Fig. 5A).

383

384 For the B cells that make a full DZ-LZ-DZ round trip, we calculated the durations they  
385 spend in different legs of the trip. The DZ-to-LZ migration time ( $T_{DL}$ ) is defined as the duration  
386 from the moment a B cell exits the cell cycle in the DZ and starts to migrate to the LZ to the  
387 moment the cell crosses the midline, i.e., the DZ/LZ border defined here.  $T_{DL}$  follows a right-  
388 skewed distribution, with the median, mean, and standard deviation (std) at 390, 438, and 205  
389 *mcs* respectively (Fig. 5B).  $T_{DL}$  is inversely correlated with the X-coordinate where the DZ-to-LZ  
390 migration is started (Fig. 5C), which is expected because migrations initiated further away from  
391 the DZ/LZ border will take a much longer time to complete than those initiated near the border.  
392 The variability of  $T_{DL}$  increases as DZ-to-LZ migrations start at later times, broadening to both  
393 shorter and longer durations (Fig. 5D). This increased variability can be attributed to the more  
394 spread-out X-coordinate of the migration start locations as the GC grows in size over time (Fig.  
395 5E).

396

397 Similarly, the LZ-to-DZ migration time ( $T_{LD}$ ) is defined as the duration from the moment a

398 B cell is positively selected in LZ and starts the DZ re-entry journey to the moment the cell  
399 crosses the midline.  $T_{LD}$  also follows a right-skewed distribution, with the median, mean, and std  
400 at 570, 581, and 153 mcs, respectively (Fig. 5F).  $T_{LD}$  is positively correlated with the X-  
401 coordinate where the LZ-to-LD migration is started (Fig. 5G), i.e., the migrations initiated further  
402 away from the DZ/LZ border take a longer time to complete than those initiated near the border.  
403 When the LZ-to-DZ migrations start at later times, the distribution of  $T_{LD}$  broadens to the long  
404 side, without dropping further on the short side (Fig. 5H). The broadening to longer durations  
405 can be attributed to the fact that as the GC grows there are more LZ-to-DZ migrations that are  
406 initiated at locations in the LZ further away from the midline (Fig. 5I). In comparison, the lower  
407 bound to the LZ-to-DZ migration time can be attributed to the locations of the Tfh cells in the LZ,  
408 where the ones nearest to the midline are at X-coordinate of about 160 (Fig. 2A), which is 35  
409 pixels ( $\mu$ M) away from the midline.

410

411 The LZ residence time ( $T_{LZ}$ ), which contains  $T_{LD}$ , is defined as the duration a B cell  
412 spends in the confine of the LZ before it reenters the DZ. Like the other two metrics,  $T_{DL}$  and  $T_{LZ}$ ,  
413  $T_{LZ}$  also follows a right-skewed distribution, with the median, mean, and std at 1065, 1105, and  
414 244 mcs respectively (Fig. 5J).  $T_{LZ}$  is positively correlated with the X-coordinate of the furthest  
415 reach of the B cells in LZ (Fig. 5K), and broadens to longer time as the GC progresses (Fig. 5L).  
416 There is no correlation between the LZ entry time and furthest reach in LZ (result not shown).

417

418 Lastly, we examined the DZ-LZ-DZ round trip time ( $T_{DLD}$ ) by summing  $T_{DL}$  and  $T_{LZ}$ . The  
419 median, mean, and std of the right-skewed distribution are 1395, 1432, and 260 mcs  
420 respectively (Fig. 5M). Examining the distributions of the components of  $T_{DLD}$  indicated that on  
421 average a B cell spends the least amount of time migrating from DZ to LZ, followed by a 1.3-fold  
422 longer time migrating from LZ back to DZ (Fig. 5N). A B cell spends a much longer time in LZ  
423 than in transition between the two zones, such that on average  $T_{LZ}$  is about 1.9 and 2.5-fold

424 longer than  $T_{DL}$  and  $T_{LD}$ , respectively. Overall, the round trip breaks down to 23% of the time  
425 spent on DZ-to-LZ migration and 77% on LZ residence among which 40% on LZ-to-DZ  
426 migration (Fig. 5O).

427

#### 428 **2.4 Proliferative burst and affinity**

429 B cells positively selected in the LZ initiate their proliferative burst by entering S phase while  
430 migrating back toward the DZ (Victora et al. 2010, Gitlin et al. 2014), and complete the first  
431 division of the proliferative burst primarily in the DZ. Since  $T_{LD} = 581 \pm 153$  mcs, a time delay of  
432 800 mcs for cell growth in the first cell cycle was introduced in the model to ensure that  
433 cytokinesis does not occur before B cells reach the DZ. Simulations confirmed that this is  
434 indeed the case for the first cell cycles of proliferative bursts of nearly all DZ-returning B cells,  
435 while all subsequent cycles in the bursts are started and completed within the DZ (Fig. 6A). In a  
436 proliferative burst, the length of the first cell cycle is  $1412 \pm 190$  and those of subsequent cycles  
437 are  $592 \pm 82$  mcs respectively (Fig. 6B). Putting these numbers into perspective, the subsequent  
438 cycles have an average length equivalent to nearly 6 hours. The variability of cell cycle length  
439 increases as the GC progresses, particularly to the long side (Fig. 6C), suggesting that in a  
440 more crowded space, more time is needed to allow “pixel copy” to occur in the CompuCell3D  
441 environment in order for cells to grow to the pre-cytokinesis volume. The X-coordinate locations  
442 at which cell cycles are initiated expand, especially for subsequent cycles in a proliferative burst  
443 (Fig. 6D). The average proliferative burst size, i.e., the number of cell divisions in each burst, is  
444  $2.19 \pm 0.88$  (Fig. 6E). While the majority of B cells that return to the DZ can mount a burst of 2  
445 divisions, some go on to divide up to 5 divisions. Examining the relationship between the affinity  
446 of each DZ-returning B cell and its burst size (Fig. 6F) revealed that while a high affinity does  
447 not guarantee a large burst size (due to the randomly produced damaging BCR mutations that  
448 would kill the daughter cells and thus terminate the burst prematurely), the affinity appears to be  
449 positively correlated with the highest achievable burst size. When the affinities are binned into

450 different intervals: <5, 5-6, 6-7, 7-8, 8-9, and >=10, the burst size at 95 percentile in each bin,  
451 which are likely the proliferations that contribute to the dominating B cells in the GC eventually,  
452 is positively associated with the affinity (Fig. 6G). The mean burst size in each affinity bin also  
453 exhibits a positive albeit less strong relationship with the affinity (Fig. 6H). Higher affinities  
454 correlate with longer LZ residence time (Fig. 6I and 6J), which is likely a result of association  
455 rather than causality, since improvement in affinity and GC population growth occur  
456 simultaneously over time, and a denser GC population at a later time tends to result in a longer  
457 navigating time through the LZ due to crowding (Fig. 5L).

458

### 459 **3. Molecular responses of B cells and their relationships with cellular phenotypes**

460 The above patterns of phenotypical behaviors of B cells are underpinned by the molecular  
461 network operating in each B cell responding to extracellular signaling cues in the GC, including  
462 chemoattractants CXCL12 and 13, BCR-mediated antigen signaling by engaging FDCs, and  
463 CD40 signaling by engaging Tfh cells (Fig. 1). Examining the steady-state GC population at  
464 700,000 *mcs* revealed that the expression or activity levels of the signaling molecules and  
465 genes in the 2352 B cells in the DZ and LZ are highly heterogenous (Fig. 7A). While there is no  
466 pMHCII expression in the DZ, a large fraction of B cells in the LZ expresses high pMHCII levels  
467 (Fig. 7B), and those expressing low pMHCII levels are B cells that either have just entered the  
468 LZ or are en route returning to the DZ with pMHCII being downregulated. A small fraction of B  
469 cells in the LZ exhibits high AKT levels as they interact with FDCs (Fig. 7C), which causes  
470 downregulation of FOXO1 (Fig. 7D). Signaling downstream of CD40 signaling are cRel and  
471 MYC, which are active in a small fraction of B cells in the LZ (Fig. 7E-7G). MYC activity induces  
472 AP4, which remains upregulated for an extended period of time, even after the B cells have  
473 returned to the DZ and MYC has been downregulated (Fig. 7H), to sustain proliferative bursts.  
474 The majority of B cells in the DZ express CXCR4 and the small fraction with CXCR4  
475 downregulated are B cells that have exited the cell cycle and are about to migrate to the LZ (Fig.

476 7I). There is no difference in CXCR5 expression in B cells between the two zones (Fig. 7A). A  
477 few cells in the LZ exhibit high RelA (Fig. 7A) and BLIMP1 (Fig. 7J) expression representing  
478 emerging PCs. The correlations between select pairs of the signaling molecules are shown in  
479 Fig. 8. There is a strong negative correlation between FOXO1 and AKT (Fig. 8A), positive  
480 correlations between cRel and CD40 (Fig. 8C), MYC and CD40 (Fig. 8D), MYC and cRel (Fig.  
481 8E), and AP4 and MYC (Fig. 8F) in B cells in the LZ, while CXCR4 is only expressed in high  
482 FOXO1-expressing B cells (Fig. 8B).

483

484 We next examined the relationship between the signaling molecules and phenotypical  
485 behaviors of B cells that are positively selected in the LZ and return to the DZ. Both the peak  
486 level (Fig. 9A) and duration of MYC expression (Fig. 9B), which is quantified by the area under  
487 the curve (AUC), are positively correlated with the BCR affinity of the cells. The correlation  
488 indicates that the strength of the affinity-dependent BCR signaling, which downregulates  
489 FOXO1 and upregulates pMHCII to enable Tfh-dependent CD40 signaling, is quantitatively  
490 transmitted to MYC. Although MYC is only transiently expressed in the positively selected B  
491 cells in the LZ, its encoding of affinity is relayed to AP4. By integrating the MYC signal, AP4,  
492 which has a longer half-life than MYC, can be activated for a much longer time as B cells  
493 migrate back to the DZ. Its peak level is positively correlated with the AUC of MYC in DZ-  
494 returning B cells (Fig. 9C). The 95<sup>th</sup> percentile burst size is positively correlated with the mean  
495 AP4 peak level in each affinity bin (Fig. 9D).

496

497 Lastly, the trajectory of a representative single B cell lineage branch that successfully  
498 makes it to a PC is presented (Fig. 10). As the seeding B cell and its progeny cycle between the  
499 DZ and LZ (Fig. 10A), multiple cell divisions (between 3-6 divisions) occur in each proliferative  
500 burst (Fig. 10B) with increasing cell generation (Fig. 10C). In this particular simulation result,  
501 nearly every cell division results in an increase in BCR affinity despite a few occasions when the

502 affinity decreases (Fig. 10D). Each time the B cell starts the trip to the LZ the countdown of the  
503 death timer is initiated, but in this case it never drops below the predefined death threshold of 50  
504 before the cell is rescued by positive selection where the death timer is reset (Fig. 10E). Every  
505 time the B cell moves into the LZ, pMHCII is re-expressed proportionally to the antigen-specific  
506 affinity after encounter with FDCs that triggers BCR signaling (Fig. 10F). BCR signaling  
507 transiently activates AKT (Fig. 10G) which downregulates FOXO1 transiently (Fig. 10H).  
508 Commitment to cell cycle after the B cell is positively selected allows upregulation of CXCR4 by  
509 FOXO1, which drives the B cell to return to the DZ, and CXCR4 is downregulated after the B  
510 cell exits the last cell cycle of a proliferative burst (Fig. 10I), which allows the cell to migrate to  
511 the LZ due to constitutively expressed CXCR5 (not shown). In the LZ, encounter with Tfh cells  
512 triggers transient activation of the CD40-cRel-MYC axis in the presence of downregulation of  
513 FOXO1 (Fig. 10J-10L). By integrating the MYC signal, AP4 is upregulated for an extended  
514 period of time, which lasts well into the DZ (Fig. 10M) to sustain the proliferative bursts. The  
515 proliferative bursts terminate when AP4 drops below a predefined threshold of 50. When the  
516 affinity increases past a predefined threshold of 10, the BCR signaling triggers RelA activation in  
517 a switch-like manner (Fig. 10N), which in turn activates BLIMP1 that drives the B cell to  
518 terminally differentiate into a PC (Fig. 10O). A representative result of a B cell lineage that ends  
519 in death in the DZ due to damaging BCR mutations is shown in Fig. S2A-S2C, where at the time  
520 the damaging mutation occurs the affinity drops to “-1” as an indication, and caspase 3 is  
521 upregulated to trigger apoptosis. A representative result of a B cell lineage that ends in death in  
522 the LZ because of not being positively selected is shown in Fig. S2D-S2F, where the death  
523 timer dips below the threshold of 50 thus triggering cell death.

524

525

## Discussion

526 In the present study, we developed a multiscale spatial modeling framework for the GC in the  
527 CompuCell3D simulation platform. By integrating interactions across molecular, cellular, and  
528 tissue scales, the model captures key hallmark GC events, including cyclic migration of B cells  
529 between the DZ and LZ, proliferative burst, SHM, deaths due to damaging BCR mutation,  
530 positive selection, timed cell death, affinity maturation, clonal expansion and dominance, and  
531 loss of clonal diversity. These cellular behaviors are driven by simulating an underlying  
532 molecular network in individual B cells responding to BCR and CD40 activation via interacting  
533 with FDCs and Tfh cells, respectively. The molecular outputs of the network include CXCR4  
534 driving LZ-to-DZ chemotaxis, MYC and AP4 driving cell cycles, and caspase 3 driving apoptosis.  
535 While this multiscale model can be further tuned and elaborated to study diverse variables and  
536 conditions regulating GC outcomes, exploring these possibilities is beyond the scope of the  
537 present study. We focused on demonstrating the capability of the modeling framework by  
538 presenting a simulated GC that leads to affinity maturation, with qualitative and to some extent  
539 quantitative results that are commensurate with the primary literature.

540

### 541 1. GC B cell birth, death, and population dynamics

542 Emerging GCs are seeded with a few hundred B cell clones (Wittenbrink et al. 2010). Starting  
543 with 200 B cells, our GC simulation showed that the DZ:LZ ratio of the numbers of B cells  
544 oscillates at early time points (Fig. 3A). This occurs because there are not many B cells at this  
545 stage of the GC and these cells tend to migrate in sync between the two zones. As the GC B  
546 cell population approaches a steady state, the DZ:LZ ratio converges to a constant value, nearly  
547 2.6:1, in our simulation, which is comparable to the 2.15~2.2:1 ratio observed in both mouse  
548 and human GCs (Victora et al. 2012). The total number of B cells in the simulation can reach  
549 about 2500. Given that the modeled GC space contains only 11 pixels (equivalent to 11  $\mu\text{M}$ )  
550 along the Z-dimension to keep the simulation time tractable, we expect that when scaling up by

551 2-5 times to mimic the actual GC thickness of 20-50  $\mu$ m (Olivieri et al. 2013), the peak number  
552 of B cells will reach thousands to over 10,000, consistent with the estimated B cell counts in real  
553 GCs (Wittenbrink et al. 2010).

554

555 Our simulation showed that new B cells are born in the DZ, while cell deaths occur in  
556 both the DZ and LZ at an overall rate that eventually matches the birth rate as the GC  
557 approaches the steady state. Although the absolute number of deaths in the DZ is more than  
558 twice that in the LZ (Fig. 3I), the percentage death rates are comparable, at about 40-43% per  
559 600 mcs in both zones (Fig. 3J). These numbers are concordant with the nearly 50% death rate  
560 per 6 hours reported for the GCs in Peyer's patches in mice and GCs in mice immunized with 4-  
561 hydroxy-3-nitrophenylacetyl (NP)-conjugated ovalbumin (NP-OVA) or HIV-1 envelope antigen  
562 GT1.1 (Mayer et al. 2017). The steady-state death turnover rate of 50% per 6 hours is expected  
563 given that GC B cells proliferate with a cell-cycle length of 4-6 hours (Mintz and Cyster 2020,  
564 Victora and Nussenzweig 2022) and in our model the cell volume doubling time of proliferating  
565 B cells is parameterized at 500 mcs. The apoptotic deaths in the DZ are believed to occur in the  
566 late G1 phase, triggered by AID-induced BCR-damaging mutations during transcription,  
567 including stop codons, insertions and deletions in the Ig sequences, such that the synthesized  
568 BCR proteins fail to properly fold and be expressed on the cell surface (Mayer et al. 2017,  
569 Stewart et al. 2018).

570

571 In the LZ, the default fate of B cells is apoptosis if not positively selected regardless of  
572 their BCR affinity, and the apoptosis will occur when the death timer goes off, which is initiated  
573 after the B cells exit the cell cycle in the DZ (Heinzel et al. 2017, Mayer et al. 2017). Our  
574 simulations showed that only a small fraction of deaths in the LZ results from not being  
575 positively selected after contact with FDCs and Tfh cells, while the majority of the deaths are  
576 due to no access to Tfh at all (Fig. 3M). This result recapitulates the current notion that the

577 availability of Tfh cells is the limiting factor, not the competition for antigen, for positive selection  
578 (Meyer-Hermann et al. 2006, Allen et al. 2007b, Meyer-Hermann 2007, Victora et al. 2010).  
579 Interestingly, our simulations also suggested that at the advanced GC stage, a small fraction of  
580 cell deaths in the DZ may also result from the lack of access to Tfh if these cells do not have  
581 enough time to migrate through an increasingly densely populated DZ to reach the LZ (Fig. 3N  
582 and 3O). Whether deaths of such nature occur *in vivo* and to what extent remain to be tested  
583 experimentally.

584

585 The dynamics of the GC B cell population is determined primarily by the cell birth rate  
586 and death rate. The loss of B cells as a result of GC exit as memory and PCs is expected to be  
587 negligible because they only account for <3% of the GC B cell fates (Kräutler et al. 2017,  
588 Laidlaw et al. 2017, Holmes et al. 2020). Our simulation result is consistent with this estimate:  
589 PCs emerge at a fraction of only as high as 2% of the B cell population towards the end of the  
590 simulated GC (Fig. 4C). For a GC B cell population to grow or to avoid population collapse, the  
591 average proliferation rate has to be higher than the death rate. Absent any limiting factors, the  
592 cell population dynamics in the GC may operate as a positive feedback system, producing  
593 bistability of two alternative outcomes – expansion or regression – as predicted by previous  
594 mathematical models (Meyer-Hermann and Beyer 2004). In the expansion mode, as the overall  
595 affinities increase, the probability of cell death in the LZ owing to lack of positive selection  
596 decreases, thus more B cells will return to the DZ and proliferate with a larger burst size there,  
597 which results in a higher birth rate of progeny cells with potentially even higher affinities  
598 returning to the LZ, and the cycle repeats leading to GC B cell expansion. In the regression  
599 mode, the positive feedback works in the opposite direction, where lower affinities can lead to  
600 fewer B cells positively selected in the LZ and smaller burst size in the DZ, which eventually  
601 leads to GC regression. This all-or-none type of GC outcomes is consistent with the population  
602 bottleneck proposition (Zhang and Shakhnovich 2010) and suggests that there could be an

603 initial affinity threshold condition for those activated B cells that seed a GC, below which a  
604 tangible GC is unlikely to emerge and above which a GC will likely emerge and grow. This  
605 suggests that it may take B cells of some intermediate affinity to initiate a GC to produce optimal  
606 humoral immune outcomes, i.e., high production of high-affinity PCs. A GC starting with B cells  
607 of too low affinity will likely abort prematurely as argued above, while a GC starting with high-  
608 affinity B cells will likely grow but only to a small size before some B cells hit the affinity  
609 threshold that triggers terminal differentiation to PCs. This may also explain the observation that  
610 affinity selection for memory B cells is less stringent, and they are often formed and then exit the  
611 GC when their affinities are still low or intermediate levels, while the PCs are in general of high  
612 affinity (Smith et al. 1997, Phan et al. 2006, Shinnakasu et al. 2016, Kräutler et al. 2017, Viant et  
613 al. 2020). Upon secondary infection or booster immunization whether the recalled memory B  
614 cells directly differentiate to PCs or have to go through GCR again can be determined by many  
615 factors (Inoue et al. 2018, Valeri et al. 2022), and it is likely that their BCR affinity may play a  
616 role in this regard.

617

618 When a GC reaches a certain size, its growth could be restricted by multiple factors,  
619 before other GC-shutoff mechanisms such as antigen depletion and antibody feedback kick in.  
620 In the present study, we showed that the availability of Tfh cells is a limiting factor, where a  
621 higher fraction of B cells in the LZ die because of a lack of access to Tfh cells, thus increasing  
622 the overall death probability which balances out the increasing birth rate due to improved  
623 antigen affinity. That B cells become PCs once their affinities reach a threshold also helps the  
624 GC B cell population to reach an equilibrium by curbing further increase in the number of higher-  
625 affinity B cells and thus attenuating the positive feedback mechanism described above. Another  
626 limiting factor not considered in the present study is the limited nutritional and energetic  
627 resources that could restrict B cell proliferation once the GC has grown to a mature size  
628 (Wittenbrink et al. 2010).

629

630 To grow a GC the overall damaging BCR mutation-induced cell death probability cannot  
631 be higher than 50% for each cell division. Actually it has to be much lower than 50% because  
632 not all but only a small fraction of B cells arriving in the LZ are positively selected and return to  
633 DZ. In our model, we encoded a DZ death probability of 30% for each of the two daughter cells  
634 born from a cell division, which leads to a probability of 49% ( $0.7*0.7$ ) to double the number of B  
635 cells after each division, of 42% ( $2*0.3*0.7$ ) to keep the number of B cells constant, and of 9%  
636 ( $0.3*0.3$ ) to eliminate the proliferating B cell. The DZ re-entry probability was estimated to be  
637 between 10-30% through mathematical modeling and analysis of experimental data (Victora et  
638 al. 2010, Meyer-Hermann et al. 2012, Mesin et al. 2016). These values suggest that absent any  
639 DZ death, the average proliferative burst size has to be greater than 1.73-3.32 divisions to grow  
640 a GC. When taking into consideration DZ death, which is very significant, on par with LZ death  
641 (Mayer et al. 2017), the average burst size has to be much higher. The DZ re-entry probability  
642 depends on the BCR antigen affinity, thus it is likely that at the early stage of GC the re-entry  
643 probability is low and at the advanced stage it is high. In our model, the positive selection and  
644 thus DZ re-entry probability is set to be proportional to pMHCII, such that when the affinity is  
645 intermediate at 5, the probability is 50% and when the affinity approaches 10 or higher, the DZ  
646 re-entry probability is 100%. However, the overall DZ re-entry fraction is only 36% in our  
647 simulation (Fig. 5A), which can be attributed in part to the inaccessibility to Tfh cells. A re-entry  
648 fraction of 36% requires at least a burst size of 1.47 to grow the GC in the absence of DZ death.  
649 With a DZ death probability of 30% for each new born B cell, our model has an average burst  
650 size 2.2 (Fig. 6E), which is in general agreement with the estimated average of 2 divisions  
651 (Meyer-Hermann et al. 2012, Gitlin et al. 2014, Meyer-Hermann 2021) or 3 divisions per burst  
652 (Gitlin et al. 2015) in the literature.

653

654 **2. Affinity maturation, proliferative burst, clonal expansion and dominance**

655 While the average proliferative burst size is 2-3, each burst can vary between 1-6 divisions  
656 (Meyer-Hermann et al. 2012, Gitlin et al. 2014, Gitlin et al. 2015, Meyer-Hermann 2021). This  
657 range is quantitatively captured in the burst size distribution produced by our simulation (Fig. 6E).  
658 The right-tailed burst size distribution could result intrinsically and in part from the probabilistic  
659 damaging mutation-induced cell death after each cell division, which increases the chance of  
660 short bursts but limits the highest attainable number of divisions in a proliferative burst even for  
661 high-affinity B cells. GC B cells positively selected are guaranteed to divide once, while the  
662 number of additional divisions or the burst size is directly proportional to the amount of antigen  
663 captured by B cells from FDCs and presented to Tfh cells (Gitlin et al. 2014, Finkin et al. 2019).  
664 Our model reproduces this positive association (Fig. 6G-6H). Moreover, because of potential  
665 premature termination of a proliferative burst induced by damaging mutation, the affinity  
666 appears to be better correlated with the top attainable burst size than the average burst size.

667

668 The translation of BCR antigen affinity into burst size is mediated molecularly by two key  
669 transcription factors: MYC and one of its target genes AP4. Because of the transient nature of B  
670 cell interactions with FDCs and Tfh cells (Allen et al. 2007b) and the short half-life of MYC  
671 (Heinzel et al. 2017), MYC is only transiently expressed in a small fraction of LZ B cells (Calado  
672 et al. 2012, Dominguez-Sola et al. 2012). The MYC expression level is in direct proportion to the  
673 amount of antigen captured and dictates the proliferative burst size (Finkin et al. 2019). While  
674 MYC can initiate cell growth and cell cycle by driving LZ B cells into the S phase, its lasting  
675 effect on cell proliferation is mediated by AP4, which is induced by MYC in a delayed fashion in  
676 positively selected GC B cells and is sustained after the B cells re-enter the DZ (Chou et al.  
677 2016). Our model recapitulates the spatiotemporal dynamics of MYC by showing transient MYC  
678 expression in LZ B cells, its positive correlation with BCR antigen affinity, and sustained AP4  
679 expression.

680

681 Our model recapitulates a typical affinity maturation process along with GC growth (Fig.  
682 4A). The progressive increase in the mean affinity of the GC B cell population is not because all  
683 or the majority of the seeding B cell clones improve their affinities uniformly. Rather, in most  
684 cases, the mature GC B cell population is dominated by progenies of one or a few of the initial  
685 200 seeding clones (Fig. 4F, S1B, and S1D). This simulation result of terminal clonal dominance  
686 is consistent with the premise that GCs mature oligo-clonally (Kroese et al. 1987, Küppers et al.  
687 1993). More recently, using multiphoton microscopy and sequencing Tas and colleagues further  
688 revealed that a GC can start with tens to hundreds of distinct B cell clones but loses the clonal  
689 diversity over time, converging to one or a few parallelly expanding clones (Tas et al. 2016). The  
690 single dominant clone can constitute 10-100% of the final GC B cell population. They further  
691 showed that clonal dominance can be achieved through neutral competition, due to stochastic  
692 effect, even when all seeding B cells have equal affinity and cannot undergo SHM, a finding that  
693 can be explored with our model in the future.

694

### 695 **3. Inter-zonal migration**

696 Beltman et al. analyzed time-lapsing imaging data of GCs and revealed that B cells move at a  
697 net speed of 0.2-0.3  $\mu\text{m}/\text{min}$  toward the LZ to produce a DZ-to-LZ migration time of a few hours  
698 (Beltman et al. 2011). In our simulation the DZ-to-LZ migration time is about  $438 \pm 205 \text{ mcs}$  (Fig.  
699 5B), consistent with that estimated by Beltman. The LZ-to-DZ migration time in our simulation is  
700  $581 \pm 205 \text{ mcs}$  (Fig. 5F) which is about 33% longer than the DZ-to-LZ migration time. The longer  
701 time is consistent with the experimental observations (Victora et al. 2010), and could be  
702 attributed to the fact that B cells returning to the DZ have to move against the much heavier  
703 incoming traffic of B cells migrating from DZ to LZ. The overall LZ residence time is  $1105 \pm 244$   
704  $\text{mcs}$  (Fig. 5J). These travel times can be tuned by varying the positions and distributions of  
705 CRCs and FDCs/Tfh cells in the DZ and LZ respectively, the CXCL12 and 13 gradients, and the

706 chemotaxis strength parameters in the model.

707

#### 708 **4. Existing computational GC models and improvements by our modeling framework**

709 Many mathematical GC models have been developed in the past two decades using a variety of  
710 computational approaches, including deterministic, stochastic, agent-based, and hybrid ones  
711 implemented in programming languages such as C++, C, MATLAB, and R. Focusing on various  
712 aspects of the GCR and simulating at various biological scales, these models have aided our  
713 understanding of this long known phenomenon, explored possible modes of mechanisms,  
714 predicted GC-associated disease outcomes, and explored optimal design of vaccination  
715 schemes. A prominent one among these efforts is the agent-based modeling framework  
716 *hyphasma* pioneered by Meyer-Hermann and colleagues that is implemented in C++ (Meyer-  
717 Hermann et al. 2009, Meyer-Hermann et al. 2012, Robert et al. 2017). The base model uses  
718 stochastic agent-based approach to simulate the movement of each cell as diffusion on a 3D  
719 equidistant lattice. Simulating the GC at the cell and tissue levels, the base model and  
720 subsequent iterations have been used to explore a range of mechanistic questions, generating  
721 novel and useful insights some of which have been validated experimentally.

722

723 GC Issues explored with earlier versions of these models include: signaling vs.  
724 chemotaxis modes of action (Beyer et al. 2002, Meyer-Hermann 2002, Meyer-Hermann and  
725 Beyer 2002), requirement of DZ re-entry for GC development and affinity maturation (Meyer-  
726 Hermann and Maini 2005a), whether affinity maturation is driven by competition for antigen or  
727 Tfh cells, GC termination resulting from antigen depletion (Meyer-Hermann et al. 2006, Meyer-  
728 Hermann 2007), persistent random walk of B cells observed with two-photon imaging and the  
729 requirement of active chemotaxis for maintenance of GC zonation (Meyer-Hermann and Maini  
730 2005b, Figge et al. 2008, Binder and Meyer-Hermann 2016), and affinity-dependent proliferative  
731 burst size enabling large, high-affinity GC B cell populations (Meyer-Hermann 2014), Issues

732 explored with later iterations of these models include: model variants informed by FOXO1, MYC,  
733 and mTOR signaling dynamics on clonal dominance and independent control of B cell selection  
734 and division fate decisions (Meyer-Hermann 2021), the effects of periodic cycling of antigen  
735 immune complex in FDCs on GC development (Arulraj et al. 2021b), differences in the lifetime  
736 of individual GCs resulting from variations in antigen availability and founder cell composition  
737 (Arulraj et al. 2021a), contribution of GC-GC interactions to variability in the timing of individual  
738 GC maturation (Arulraj et al. 2022a), different mechanisms of GC shutdown (Arulraj et al.  
739 2022b), the effects of kinetic rates of BCR-antigen binding on antigen uptake by B cells and GC  
740 dynamics and outputs (Lashgari et al. 2022), and the evolution of clonal diversity and  
741 dominance and the modulating effects of antigen amount, Tfh cell availability, and seeding B  
742 cell affinity (Meyer-Hermann et al. 2018, Garg et al. 2023),

743

744 Besides the seminal work by Meyer-Hermann and colleagues, many others also  
745 modeled the GC with various simulation approaches including deterministic, stochastic, and  
746 agent-based ones. With deterministic models, Zhang and Shakhnovich explored the parameter  
747 space of mutation rate, selection strength, and initial antigen affinity for maximizing affinity  
748 maturation (Zhang and Shakhnovich 2010), Chan et al. showed that the feedback from receptor  
749 downregulation induced by the CXCL12 and 13 fields may explain the spontaneous interzonal  
750 and intrazonal oscillations of B cells (Chan et al. 2013), and Reshetova et al. revealed there is a  
751 limited correlation between the size and antigen affinity of GC B cell subclones and B cells with  
752 highest affinity can reside in low-abundance subclones (Reshetova et al. 2017). Beltman et al.  
753 constructed a stochastic model that recapitulated the persistent random walk of B cell  
754 movement in the GC and the small preference for DZ-to-LZ migration (Beltman et al. 2011).  
755 Molari et al. developed stochastic and deterministic models to show that the average GC B cell  
756 affinity is determined non-monotonically by the antigen dosage, and clonal dominance and  
757 limited diversity can be achieved over time (Molari et al. 2020). Using stochastic models and

758 analytical solutions Molari et al. studied the probability for a B cell lineage to surpass the  
759 population bottleneck as a function of the antigen concentration and initial B cell population size  
760 (Molari et al. 2021). More recently Yan et al. developed a spatiotemporal stochastic model to  
761 understand the determinants of GC size and found there is a critical GC volume to achieve best  
762 performance (Yan et al. 2022). With an agent-based modeling framework of Basic Tonsil Unit,  
763 Hawkins et al. showed that the persistent random walk of B cells could be an emergent outcome  
764 of mobile but morphologically rigid B cells in a GC of dense cellularity, where cells are  
765 constantly competing for space (Hawkins et al. 2011). Using a spatiotemporally resolved  
766 stochastic model similar to the agent-based model by Meyer-Herman et al, Wang et al. showed  
767 the importance of efficient Tfh cell delivery for affinity maturation, suggesting that antagonism  
768 between BCR signaling and Tfh cells may accelerate affinity maturation (Wang et al. 2016).  
769 Using an agent-based model, Amitai explored the GC population dynamics and diversity of  
770 clonal dominance with either birth- or death-limited selection (Amitai et al. 2017).

771

772 In addition to probing basic GC biology, computational GC models have also been used  
773 to help optimize the design of vaccination schemes. Using a stochastic agent-based model,  
774 Wang et al. predicted that for GCs to produce cross-reactive antibodies against different antigen  
775 variants, sequential rather than simultaneous vaccination with several antigen variants is  
776 preferred, and the *in silico* prediction was validated in mice vaccinated with variant gp120  
777 constructs of the HIV envelope protein (Wang et al. 2015, Wang 2017). Meyer-Hermann  
778 showed that a feedback imposed by preexisting antibodies or memory B cells can mask the  
779 immunodominant epitopes to diversify GCs toward less frequent epitopes to help generate  
780 broadly neutralizing antibodies (Meyer-Hermann 2019). Garg et al. developed a stochastic GC  
781 model to explain and predict that passive immunization can promote and optimize GCR by  
782 tuning the administered external antibodies to control antigen availability such that only high-  
783 affinity B cells prevail (Garg et al. 2019). With an agent-based GC model, Yang et al. interpreted

784 why 3 doses of mRNA vaccine against the original SARS-CoV-2 strain are required to develop  
785 anti-Omicron neutralizing antibodies, which involves enhanced antigen availability and  
786 immunodominant epitope masking after the 2<sup>nd</sup> dose, and expansion of memory B cells  
787 targeting subdominant epitopes by the 3<sup>rd</sup> dose (Yang et al. 2023). Adapting the same agent-  
788 based model, Bhagchandani et al. explained why a particular two-shot extended-prime regimen  
789 of immunization against HIV is effective in producing high-titer antibodies; the model predicted  
790 that it was because the antigen delivered in the second dose can be captured more efficiently  
791 as immune complexes, which was verified by experiments (Bhagchandani et al. 2023).

792

793 While these models above investigated the complex processes of affinity maturation and  
794 B cell population dynamics, no molecular networks were included in them to drive the B cell  
795 behaviors and GC evolution. Quantitative multiscale mathematical models of GC dynamics have  
796 been proposed as predictive frameworks to translate basic immunological knowledge to  
797 practical challenges (Verstegen et al. 2021, Vaidehi Narayanan and Hoffmann 2022). In more  
798 recent years, modeling efforts in this direction have emerged. Merino Tejero and colleagues  
799 have developed multiscale GC models integrating molecular and cellular responses (Merino  
800 Tejero et al. 2021a, Merino Tejero et al. 2021b). Implemented in C++ language, they combined  
801 the agent-based model developed by (Meyer-Hermann et al. 2012) and ODE-based gene  
802 regulatory network model comprising BCL6, IRF4, and BLIMP1 developed in (Martínez et al.  
803 2012). They used the model to study the role of affinity-based CD40 signaling and asymmetric B  
804 cell division in temporal switch from memory B cell to PC differentiation and DZ-to-LZ ratio.  
805 Lately they adapted the model to examine the oncogenic effects of genetic alteration of the  
806 above key transcription factors on GC-originated diffuse large B cell lymphoma (Merino Tejero  
807 et al. 2022). More recently, the model was used to explore the relationship between clonal  
808 abundance and affinity as well as affinity variability within B cells from the same clone, with an  
809 attempt to make sense of repertoire sequencing data (García-Valiente et al. 2023).

810

811        In comparison, the multiscale spatial GC modeling framework we developed here in the  
812        CompuCell3D platform further integrates across the molecular, cellular, and tissue scales and  
813        offers several improvements. The framework allows the molecular network to drive multiple  
814        cellular behaviors, including B cell growth, division, chemotaxis, survival/death, and PC  
815        differentiation, which in turn collectively drive GC tissue pattern formation; reciprocally the cell-  
816        to-cell interactions between B cells and FDCs and Tfh cells drive the responses of the molecular  
817        signaling network in B cells. Novel cross-scale strengths include cell cycle and FOXO1-  
818        dependent CXCR4 expression driving DZ-reentry chemotaxis, MYC and AP4-dependent cell  
819        growth and division burst, and RelA and BLIMP1-dependent PC differentiation. As more  
820        molecular species are added to the network, additional cross-scale integrations will become  
821        available. Because the simulated B cells comprise multiple pixels, the model allows  
822        recapitulation of B cell morphology during chemotaxis and volume growth during cell cycle as  
823        well as better mimicking of cell-cell interaction. For future iterations of the model, the  
824        CompuCell3D platform can easily include paracrine signaling by ILs and other cytokines  
825        secreted by B cells, Tfh cells, and FDCs. Last but not least, with the modular plugins and  
826        systems biology markup language (SBML) support, the CompuCell3D platform allows a more  
827        structured construction of the GC model that will facilitate future model sharing and integration.

828

## 829        **5. Limitations and future iterations**

830        As an initial effort to establish the multiscale GC modeling framework on the CompuCell3D  
831        platform, we simulated the GC to the mature stage where the B cell population approaches a  
832        steady state. While persistent GCs can exist for months or years under chronic infections such  
833        as certain viral infections (Bachmann et al. 1996, Kasturi et al. 2011, Adachi et al. 2015), and in  
834        the Peyer's patch for mucosal immunity (Reboldi and Cyster 2016), in most other infection or  
835        immunization scenarios, GCs eventually regress in 3-4 weeks with mechanisms not well

836 understood. Some GC terminations may be because the antigens stored in FDCs are depleted,  
837 changes in the signaling nature of Tfh cells and FDCs, or antibodies produced by the departed  
838 PCs circulate back into the GC and block antigen presentation (Zhang et al. 2013, Arulraj et al.  
839 2021c, Arulraj et al. 2022b). In addition, in the current model, memory B cells are not included  
840 as a cell fate option. Since like PCs, memory B cells only constitute a very small fraction of the  
841 GC B cells fates (Arulraj et al. 2021c), its exclusion is not expected to affect the overall model  
842 behavior. Nonetheless, future iterations of the GC model will include the self-termination and  
843 memory B cell formation as driven by the IRF4-BCL6-BLIMP1 network (Martínez et al. 2012),  
844 and if needed, CSR, which is believed to occur primarily during pre-GC formation (Roco et al.  
845 2019).

846

847 A small caveat of the current model, as currently implemented on the CompuCell3D  
848 platform, is that when the cell density is too high such that the DZ is packed, there is a small  
849 chance that some dividing B cells vanish in the DZ when their volumes are too small (as  
850 represented by the small left tail of the DZ B cell volume distribution in Fig. 3H). This issue can  
851 be prevented in future iterations by imposing a limiting resource for cell growth and division to  
852 control DZ cell density. B cells are highly mobile in both the DZ and LZ with a mobility pattern  
853 observing persistent random walk (Allen et al. 2007b, Schwickert et al. 2007, Beltman et al.  
854 2011). While we did not analyze the B cell mobility in this regard, the CompuCell3D platform,  
855 which is based on the Cellular Potts Model that follows the Boltzmann law (Graner and Glazier  
856 1992), is capable of simulating persistent random walk (Aponte-Serrano 2021). In our case, the  
857 *temperature* parameter and local CXCL12 and 13 distribution patterns in the LZ and DZ can be  
858 optimized, along with reducing the directional chemotactic forces, to help accentuate the  
859 random-walk effect. As indicated above, it was previously showed that the persistent random  
860 walk of B cells could be an emergent behavior of B cells in a crowded GC environment  
861 (Hawkins et al. 2011), thus it will be interesting to inspect our GC model in this regard.

862

863        In the current model the two daughter cells split the parent cell's volume by following a  
864        lognormal distribution. While this approach implemented some degree of cell division  
865        asymmetry, in future iterations asymmetrical cell divisions can be better implemented based on  
866        experimental studies which showed asymmetrical segregation of pMHCII among the two  
867        daughter cells, which plays a role in B cell fate decision-making (Thaunat et al. 2012), and was  
868        implemented in recent models (Merino Tejero et al. 2021a, Merino Tejero et al. 2021b). In the  
869        current model the length of each cell cycle in a proliferative burst is targeted as a constant. It  
870        has been shown that the S phase, which constitutes the major portion of the cell cycles of  
871        proliferating B cells, can be shortened by regulating replication fork progression, while the  
872        relative order of replication origin activation is preserved (Gitlin et al. 2015). The degree of S-  
873        phase shortening depends on the interaction strength of B cells with Tfh cells which in turn  
874        depends on BCR antigen affinity. Therefore, positively selected high-affinity GC B cells, upon  
875        returning to the DZ, will proliferate not only with a larger burst size but also with accelerated cell  
876        cycles. This could be a mechanism to compensate for the tendency of longer DZ residence time  
877        of high-affinity B cells due to more cell divisions such that they can return to the LZ sooner.  
878        Future iterations of the model may consider to incorporate affinity-dependent cell cycle  
879        shortening.

880

881        The molecular network model running in each B cell uses the Gillespie's stochastic  
882        simulation algorithm, with some of the molecular switching actions implemented as hybrid, rule-  
883        based events. For simplicity and parsimony, several genes known to participate in GCR and B  
884        cell terminal differentiation are not included in the current implementation of the GC model,  
885        including BCL6, IRF4, BACH2, and PAX5. BCL6 is upregulated in antigen-engaged B cells in  
886        the early stage of GC formation, before these cells migrate back into the intrafollicular space  
887        and cluster in the GC (Kitano et al. 2011). There does not appear to be cyclic BCL6 expression

888 between the DZ and LZ. Since our current model starts with B cells seeding the GC, not  
889 including the initial interactions at the T/B border, the absence of BCL6 in the molecular network  
890 should not affect the simulation results. BCL6, IRF4, BACH2, BLIMP1, and PAX5 form coupled  
891 positive or double-negative feedback loops underpinning multistability-based binary decision-  
892 making in B cells (Bhattacharya et al. 2010, Méndez and Mendoza 2016). In future iterations,  
893 the hybrid stochastic and rule-based approach of molecular network simulation can be updated  
894 by implementing relevant intracellular feedback circuits that enable bi- and multistability. To this  
895 end, single-cell RNA sequencing data of GC cells can be integrated to the molecular network  
896 model to parameterize the molecular abundance of the gene transcripts (Holmes et al. 2020).

897

898 There are a number of other considerations that can be potentially added in future  
899 iterations as well. Paracrine signals mediated by ILs and other cytokines secreted by B cells,  
900 Tfh cells and FDCs may be considered to better recapitulate the tissue-level molecular signaling  
901 milieu in the GC. Mobility of Tfh cells and their intracellular signal transduction and gen  
902 regulatory network can be included. The shapes and locations of residential CRCs and FDCs  
903 and as a result the spatial patterns of CXCL12 and 13 gradients can be fine-tuned according to  
904 immunohistochemistry data, which may ultimately affect the morphology and polarity of GCs.

905

## 906 **6. Conclusions**

907 In conclusion, we have developed a multiscale spatial computational modeling framework for  
908 GC simulation in CompuCell3D. The current model is capable of recapitulating GC features that  
909 are both qualitatively and to some extent quantitatively consistent with the literature. Given the  
910 complexity of GCR, simulations in such a modeling framework can help investigate a range of  
911 research questions on this hallmark event of high-affinity antibody production in response to  
912 viral infection and vaccination. Upon further extension and refinement, this open-source  
913 modeling framework may also help research in the area of autoimmunity and lymphoma when

914 the GC goes awry due to genetic or environmental disruptions. Lastly, the GC modeling  
915 framework may also be utilized towards building the digital twins of the human immune system  
916 for precision medicine (Lubenbacher et al. 2022).

917

918

### Acknowledgements

919 This research was supported in part by NIEHS Superfund Research grant P42ES04911 and  
920 Emory Synergy grant. We would like to thank Dr. James P. Sluka for his technical assistance  
921 with CompuCell3D.

922

923

### Conflict of Interest

924 The authors declare that the research was conducted in the absence of any commercial or  
925 financial relationships that could be construed as a potential conflict of interest.

926

927

### Author Contributions

928 QZ conceived the model structure with inputs from CDS and NEK. DPM constructed and  
929 simulated the model in CompuCell3D. DPM and QZ conducted the parameter justification and  
930 estimation, and wrote the Python code for formal analysis of simulation results. DPM and QZ  
931 wrote the initial draft and revised the manuscript. CDS and NEK critically reviewed and revised  
932 the manuscript. All authors contributed to the article and approved the submitted version.

933

934

## Figure Legends

935 **Figure 1.** Schematic illustration of a simplified intracellular molecular network of GC B cells and  
936 different B cell outcomes driven by key molecules as indicated. Pointed arrowhead:  
937 stimulation/activation, blunted arrowhead: inhibition, and dotted arrow head: regulation in either  
938 direction.

939

940 **Figure 2. Morphology of a representative simulated GC.** **(A)** 2-D distributions of CRCs,  
941 FDCs, and Tfh cells in the DZ (left) and LZ (right) with X-Y coordinates indicated. **(B-C)**  
942 Concentration gradients of chemoattractants CXCL12 and CXCL13 respectively. White contour  
943 lines: isolines of equal concentrations. **(D)** Snapshots of simulated GC at various *mcs* indicated.  
944 Colors of B cells denote BCR/antibody affinity as indicated by the colormap. **(E)** 2-D trajectories  
945 of B cells in three select lineages leading respectively to damaging mutation-induced apoptosis  
946 in DZ (left panel), death timer-triggered apoptosis in the LZ for not positively selected (mid  
947 panel), and emergence of a PC (right panel). Dot color denotes *mcs* time as indicated by the  
948 colormap.

949

950 **Figure 3. Quantitative analyses of B cell population dynamics in a simulated GC.** **(A)**  
951 Numbers of B cells in the DZ, LZ, and total, and DZ:LZ birth ratio as indicated at a given time.  
952 **(B)** Numbers of B cells engaged in cell cycle in DZ, LZ, and total as indicated. **(C)** Fractions of B  
953 cells in the DZ, LZ, and total that are engaged in cell cycle. **(D)** The X-coordinate and time at  
954 which cytokinesis events occur. **(E)** Numbers of B cells born every 600 *mcs* in the DZ, LZ, and  
955 total as indicated. **(F)** Numbers of cumulative cell births in the DZ, LZ, and total as indicated. **(G)**  
956 Mean, interquartile, 2.5-97.5<sup>th</sup> percentile, minimum and maximum generations of GC B cells as  
957 indicated. **(H)** Distributions of last volumes of B cells in the DZ and LZ as indicated before they  
958 divide, die, or differentiate into PCs. **(I)** Numbers of B cell deaths in every 600 *mcs* in the DZ, LZ,  
959 and total, and DZ:LZ death ratio as indicated. **(J)** Fractions of B cells in the DZ, LZ, and total

960 that die in every 600 *mcs* as indicated. **(K)** Numbers of cumulative cell deaths in the DZ, LZ, and  
961 total as indicated. **(L)** Numbers of B cell deaths in every 600 *mcs* in total, due to damaging BCR  
962 (lethal) mutation, not being positively (Neg) selected after contacting Tfh cells, or no access to  
963 Tfh cells as indicated. **(M)** Percentage composition of B cell deaths in every 600 *mcs* due to  
964 lethal mutation, negative selection, or no access to Tfh cells as indicated. **(N)** The X-coordinate  
965 and time at which cell deaths occur. **(O)** Distributions of X-coordinate at which cell deaths occur  
966 due to lethal mutation, negative selection, or no access to Tfh cells as indicated.

967

968 **Figure 4. Evolution of B cell affinity maturation and clonal dominance.** **(A)** Mean,  
969 interquartile, 2.5-97.5<sup>th</sup> percentile, minimum and maximum BCR affinities of GC B cells as  
970 indicated. Gray dots denote the time and antibody affinities of PCs when they emerge. **(B)**  
971 Numbers of PCs produced every 600 *mcs* and cumulative numbers of PCs produced as  
972 indicated. **(C)** Fractions of B cells that differentiate into PCs in every 600 *mcs*. **(D)** Distributions  
973 of BCR/antibody affinities in B cells or PCs as indicated. **(E)** Evolution of B cell clone size as  
974 represented by the number of progeny B cells descending from each of the initial 200 clones. **(F)**  
975 Muller plot of evolution of the clonal fractions of the GC B cells.

976

977 **Figure 5. Quantitative analyses of inter-zonal migration.** **(A)** Fractions of fates of B cells that  
978 have entered the LZ. **(B)** Distribution of DZ-to-LZ migration time ( $T_{DL}$ ) with mean  $\pm$  std indicated.  
979 **(C)** Relationship between  $T_{DL}$  and X-coordinate of DZ-to-LZ migration start location. **(D)**  
980 Relationship between  $T_{DL}$  and DZ-to-LZ migration start time. **(E)** Relationship between DZ-to-LZ  
981 migration start time and location. **(F)** Distribution of LZ-to-DZ migration time ( $T_{LD}$ ) with mean  $\pm$   
982 std indicated. **(G)** Relationship between  $T_{LD}$  and X-coordinate of LZ-to-DZ migration start  
983 location. **(H)** Relationship between  $T_{LD}$  and LZ-to-DZ migration start time. **(I)** Relationship  
984 between LZ-to-DZ migration start time and location. **(J)** Distribution of LZ residence time ( $T_{LZ}$ )  
985 with mean  $\pm$  std indicated. **(K)** Relationship between  $T_{LZ}$  and X-coordinate of LZ furthest reach

986 of the B cells. **(L)** Relationship between  $T_{LZ}$  and LZ entry time. **(M)** Distribution of DZ-LZ-DZ  
987 round trip time ( $T_{DLD}$ ) with mean  $\pm$  std indicated. **(N)** Overlay of distributions of  $T_{DL}$ ,  $T_{LD}$ ,  $T_{LZ}$ , and  
988  $T_{DLD}$ . **(N)** Distributions of  $T_{DL}$ ,  $T_{LD}$ , and  $T_{LZ}$  as fractions of  $T_{DLD}$ .

989

990 **Figure 6. Quantitative analyses of proliferative bursts and BCR affinities of GC B cells. (A)**  
991 Relationship between the X-coordinates of cell cycle start location and cytokinesis location for  
992 the first and subsequent cycles as indicated in a proliferative burst. **(B)** Distributions of cell cycle  
993 lengths of the first and subsequent cycles in a proliferative burst. **(C)** Relationship between cell  
994 cycle length and start time of the first and subsequent cycles in a proliferative burst. **(D)**  
995 Relationship between cell cycle start location and start time of the first and subsequent cycles in  
996 a proliferative burst. **(E)** Distribution of proliferative burst size. **(F)** Relationship between the  
997 proliferative burst size and affinity of DZ-returning B cells. **(G)** Association between the 95<sup>th</sup>  
998 percentile proliferative burst size and mean affinity of DZ-returning B cells in each affinity bin as  
999 indicated. **(H)** Association between the mean proliferative burst size and mean affinity of DZ-  
1000 returning B cells in each affinity bin as indicated. **(I)** Relationship between the LZ residence time  
1001 and affinity of DZ-returning B cells. **(J)** Association between the mean LZ residence time and  
1002 mean affinity of DZ-returning B cells in each affinity bin as indicated.

1003

1004 **Figure 7. Molecular response profiles of GC B cells. (A)** Violin plots of expression/activity  
1005 levels of signaling molecules in DZ and LZ B cells as indicated at 70,000 *mcs*. **(B)** Simulated  
1006 “immunohistochemistry” staining of signaling molecules as indicated in GC B cells at 70,000  
1007 *mcs*.

1008

1009 **Figure 8. Correlations between key signaling molecules in DZ and LZ B cells as indicated**  
1010 **at 70,000 *mcs*.** Red and green dots denote DZ and LZ B cells respectively as shown in (A).

1011

1012 **Figure 9. Relationships between signaling molecules and phenotypical behaviors of DZ-  
1013 returning B cells. (A)** Violin plots of MYC peak levels in different affinity bins. **(B)** Violin plots of  
1014 MYC AUC level in different affinity bins. **(C)** Correlation between AP4 peak level and MYC AUC  
1015 level. **(D)** Correlation between the 95<sup>th</sup> percentile proliferative burst size and mean AP4 peak  
1016 level in each affinity bin.

1017

1018 **Figure 10. Trajectory of a single B cell lineage branch that successfully makes it to a PC  
1019 with molecular and cellular variables as indicated.**

1020

1021 **Figure S1. (A-B)** Evolution of B cell clone sizes and clonal fractions respectively for a GC  
1022 simulation resulting in single-clone dominance. **(C-D)** Evolution of B cell clone sizes and clonal  
1023 fractions respectively for a GC simulation resulting in multi-clone dominance.

1024

1025 **Figure S2. (A-C)** Trajectory of a single B cell lineage branch that ends up in death in the DZ  
1026 due to damaging BCR mutation. **(D-F)** Trajectory of a single B cell lineage branch that ends up  
1027 in death in the LZ due to not being positively selected and the death timer counts down below a  
1028 threshold level. Molecular and cellular variables are indicated.

1029

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1031

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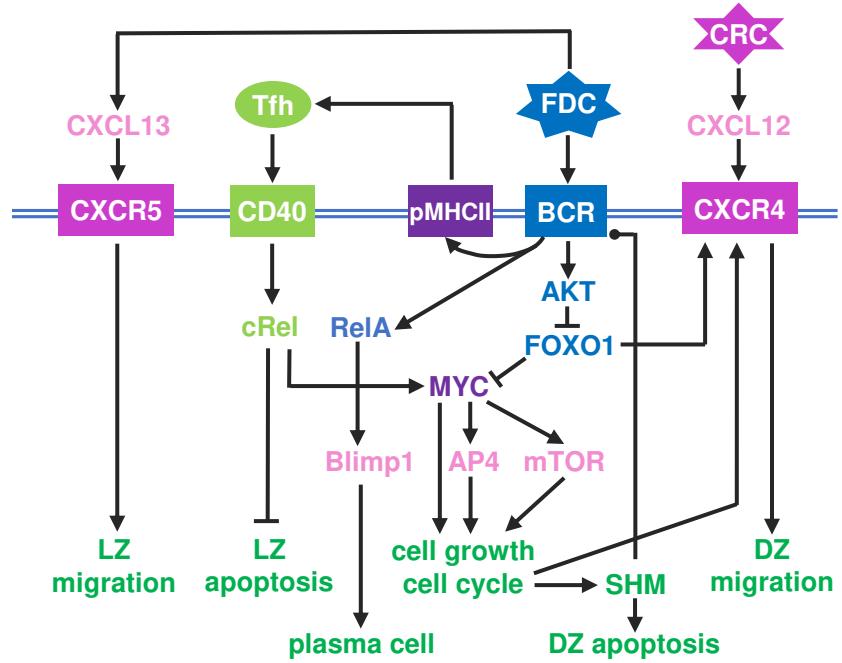
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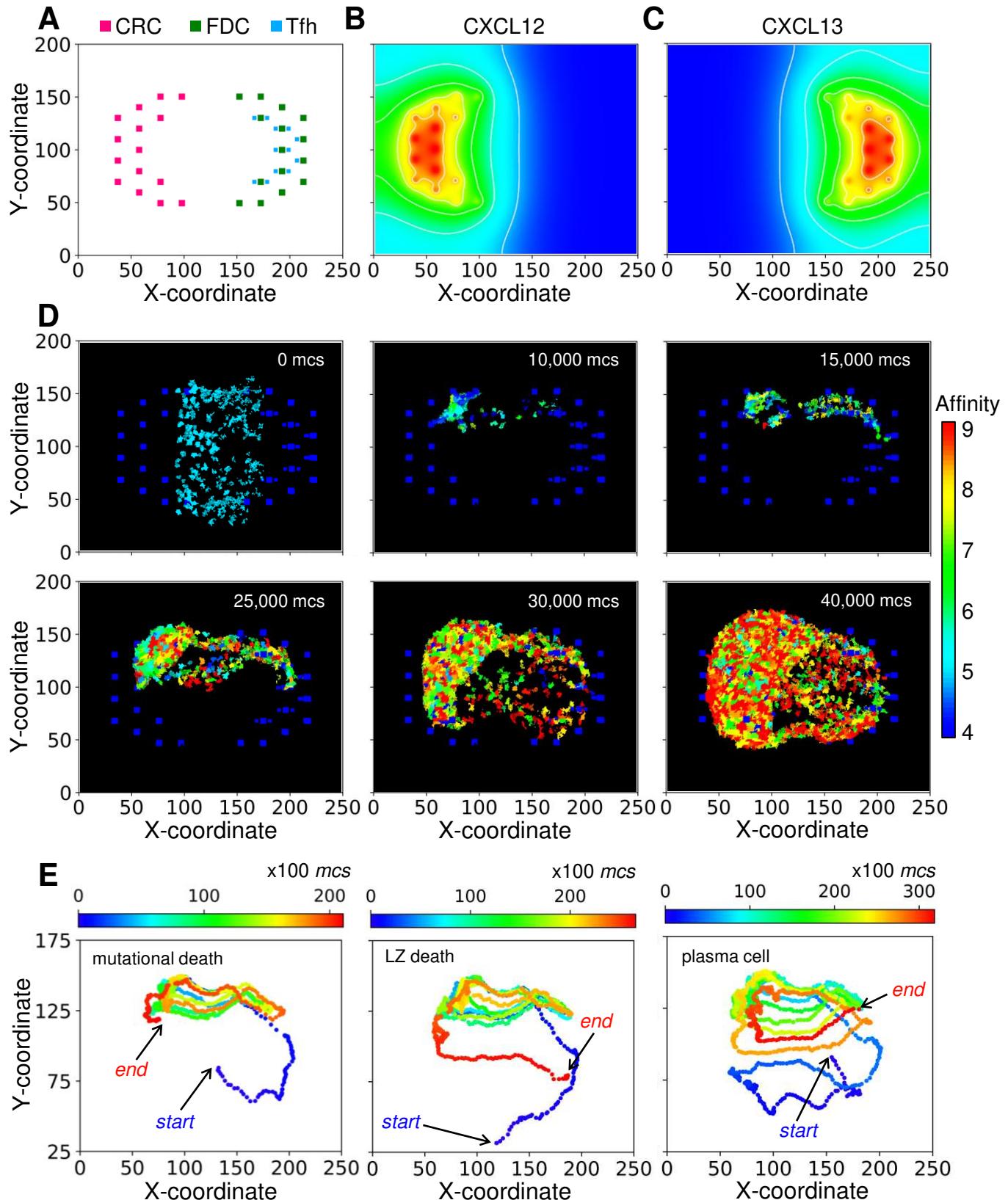
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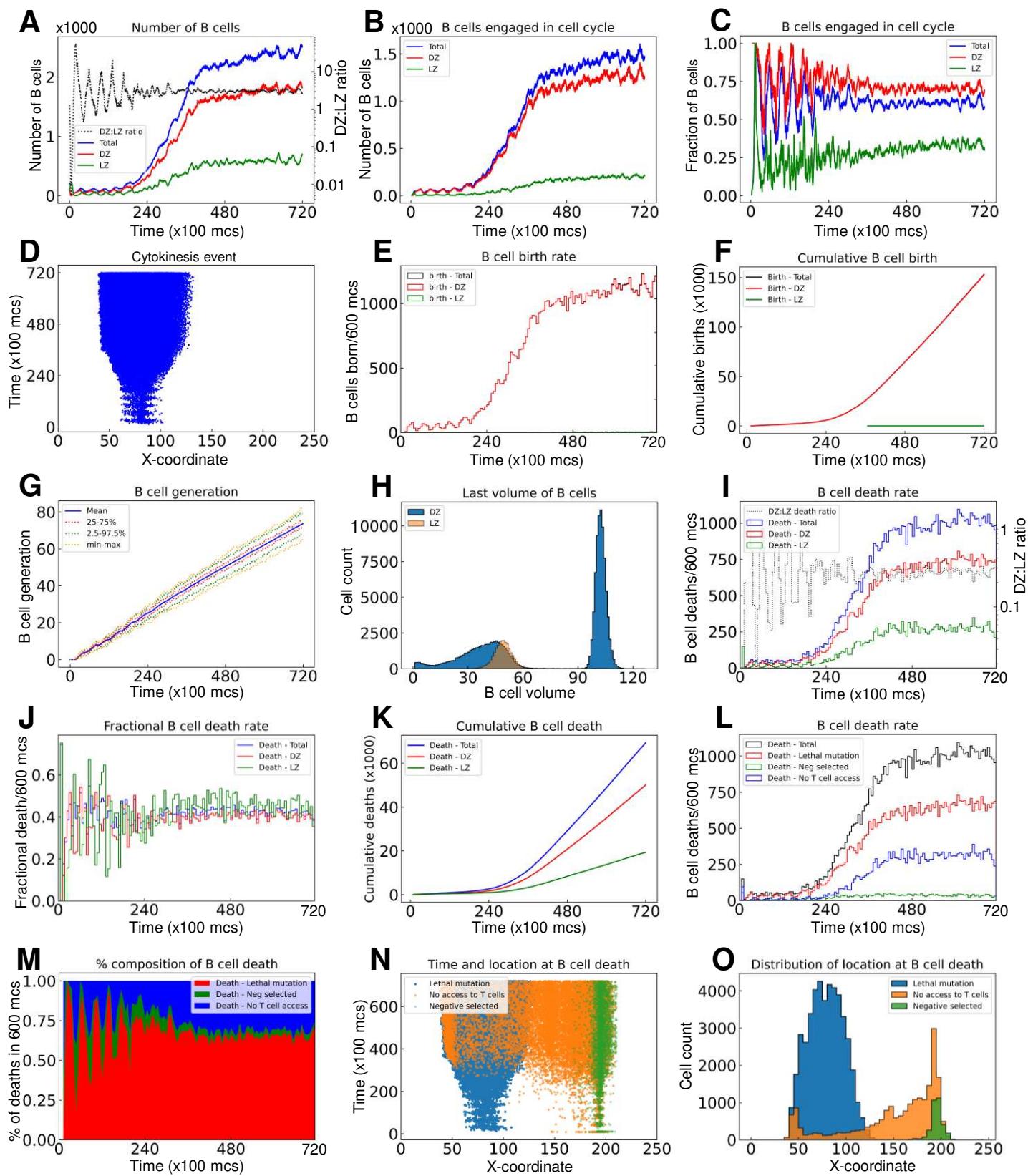
**Figure 1**



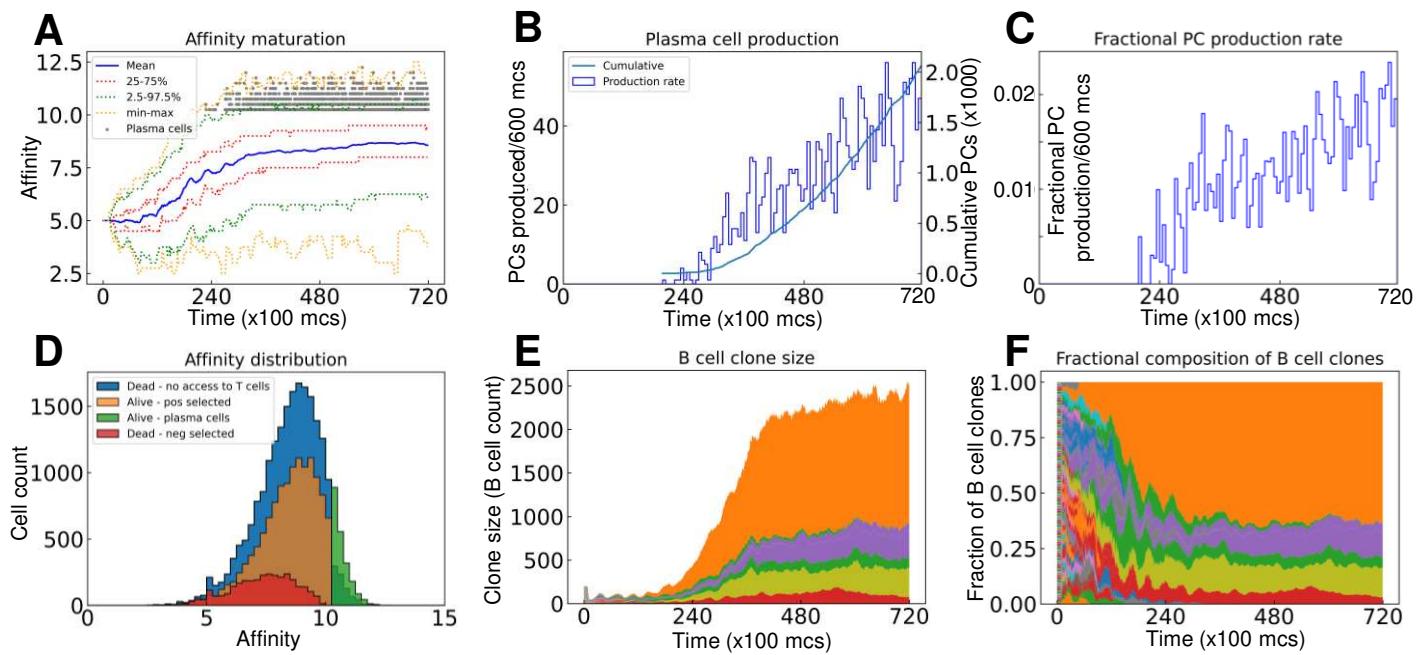
**Figure 2**



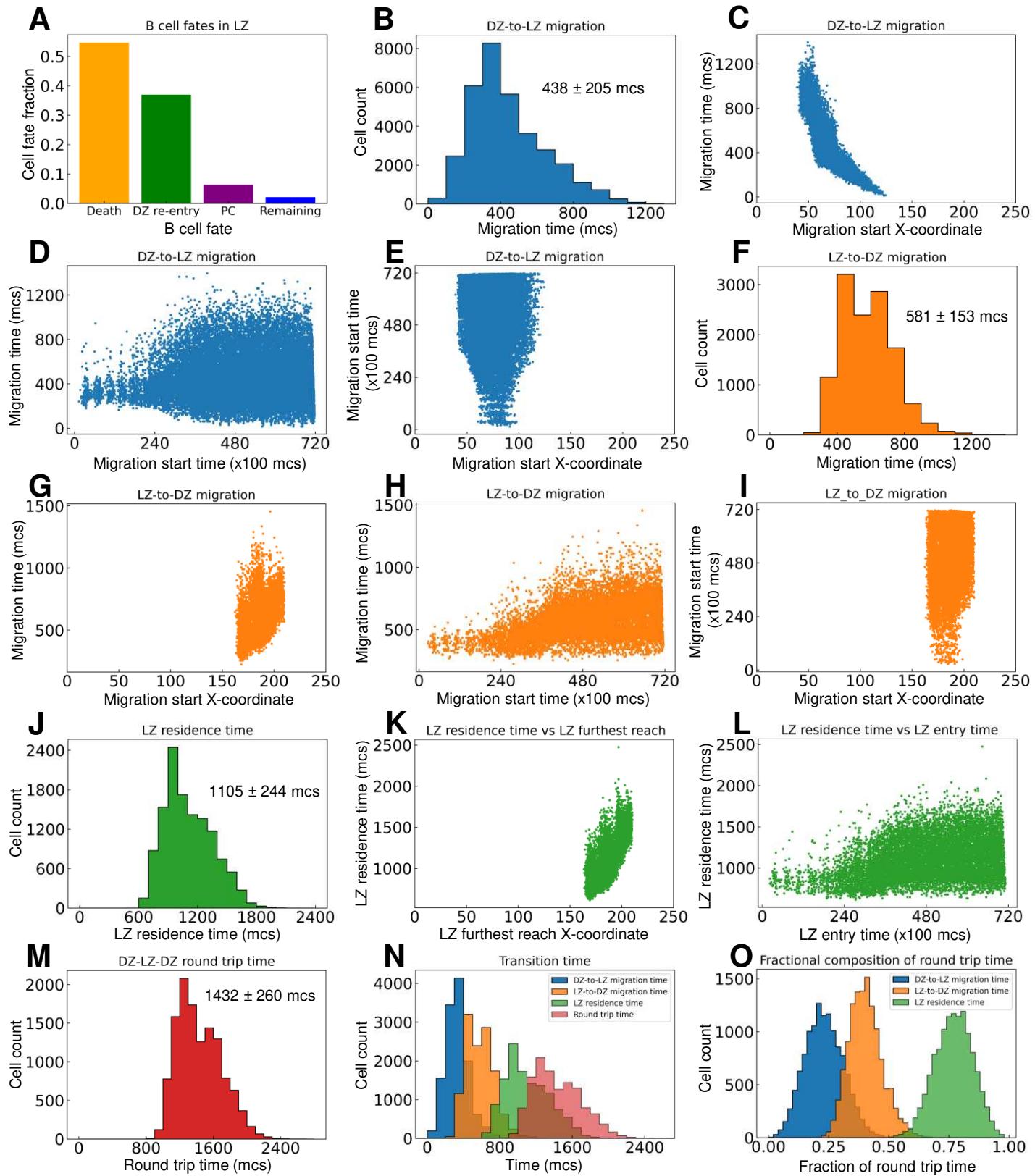
## Figure 3



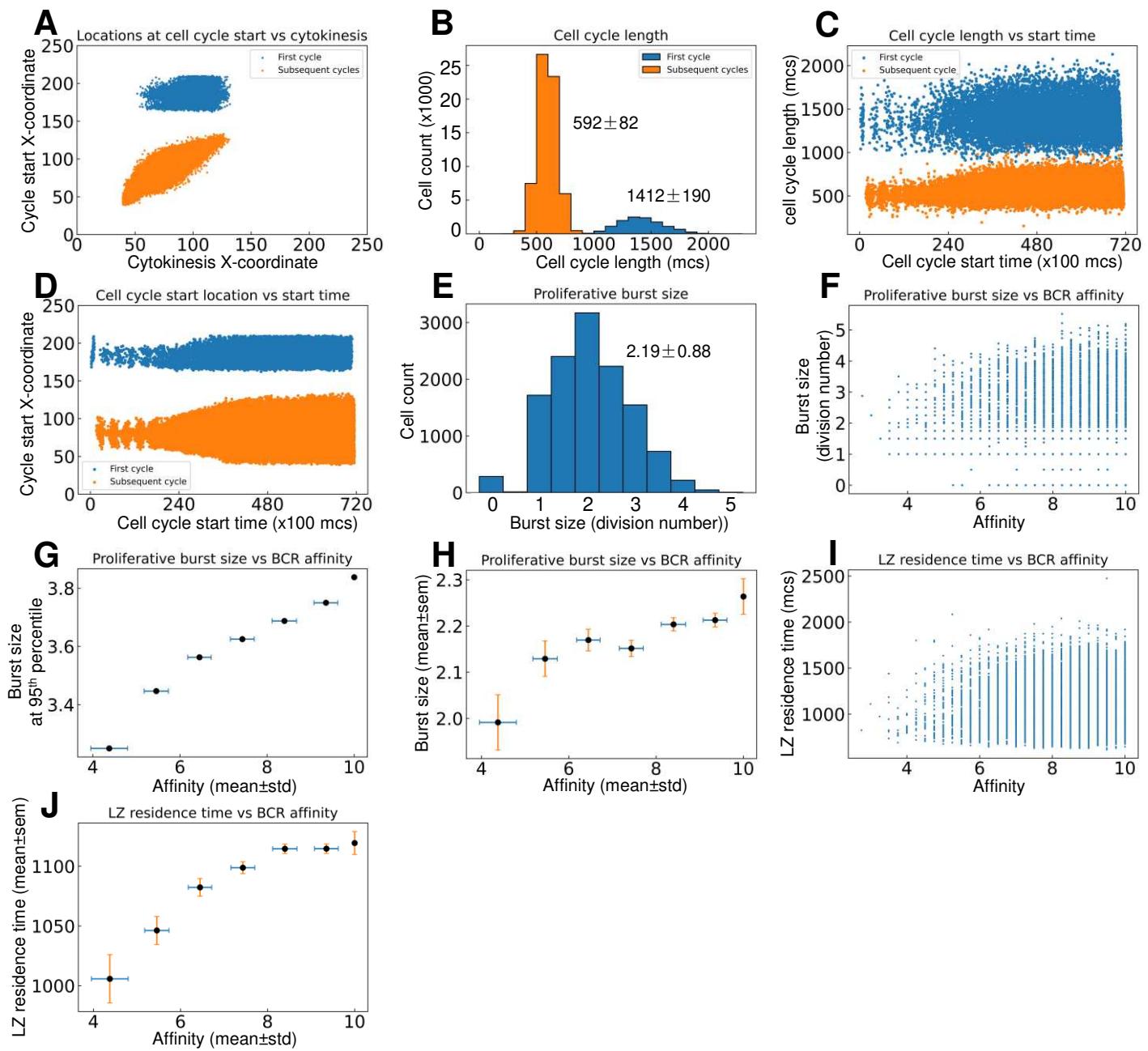
**Figure 4**



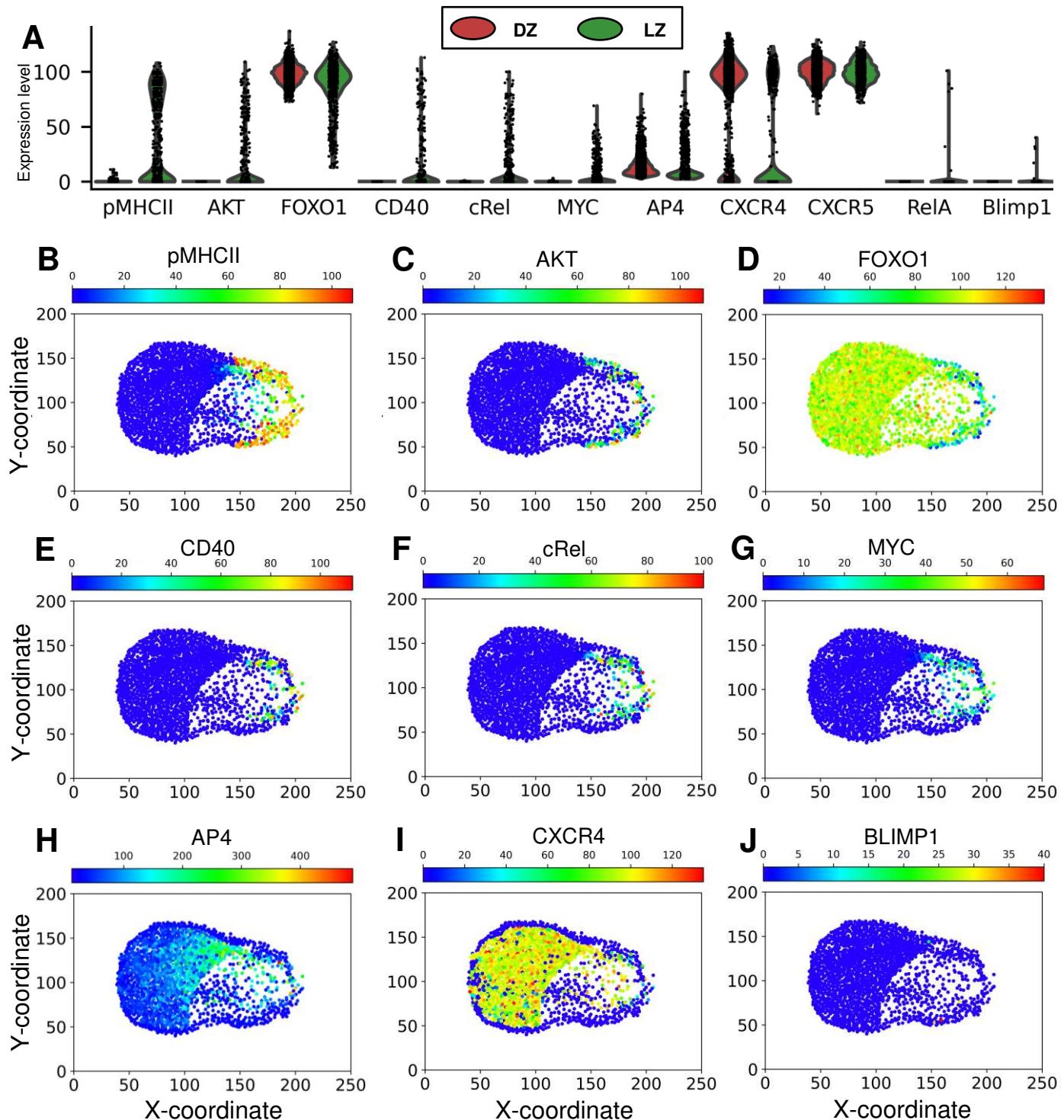
## Figure 5



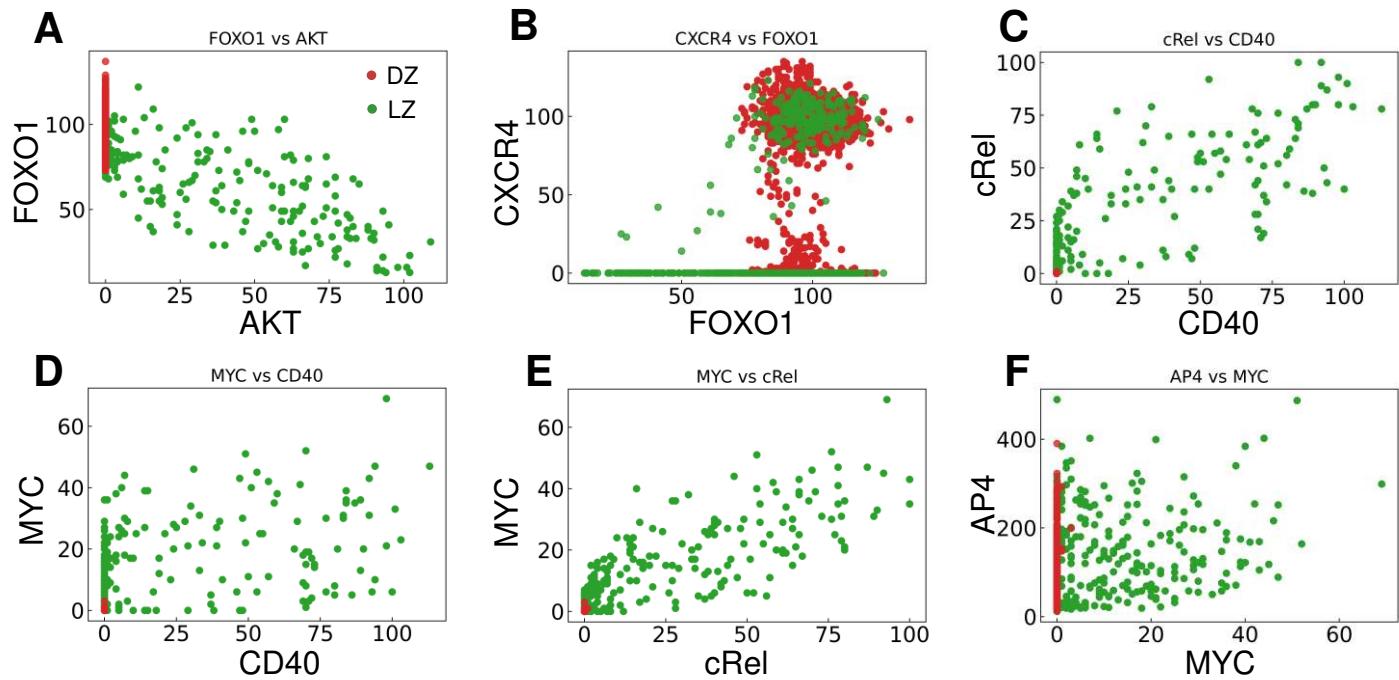
## Figure 6



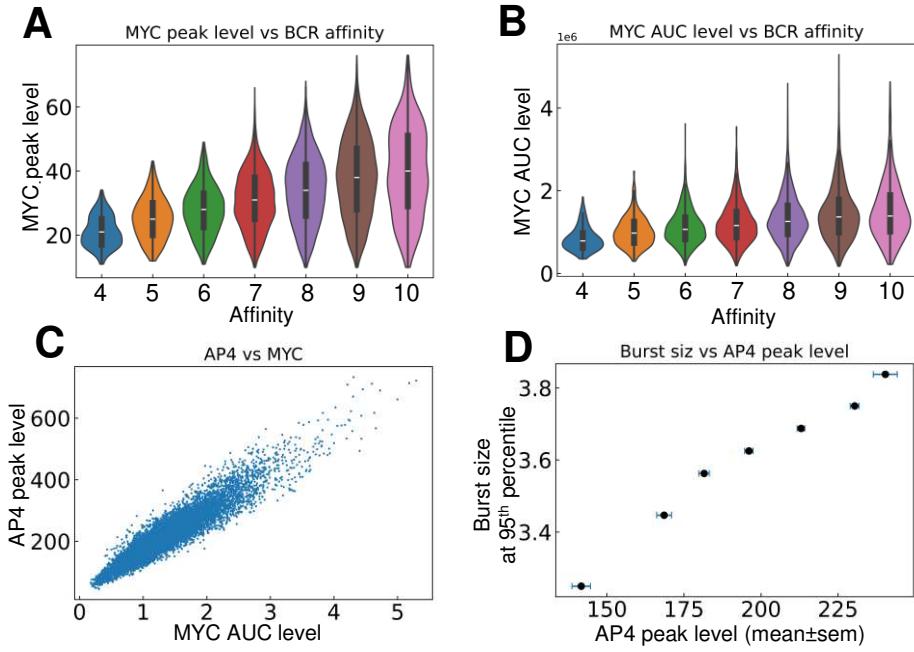
**Figure 7**



**Figure 8**



**Figure 9**



## Figure 10

