Defining and Manipulating B cell Immunodominance Hierarchies to Elicit Broadly Neutralizing Antibody Responses Against Influenza Virus – supplementary information

Assaf Amitai[1,7,8], Maya Sangesland[2,8], Ralston M. Barnes[3], Daniel Rohrer[3], Nils Lonberg[3], Daniel Lingwood[2,9†], and Arup Chakraborty[1,2,4-7†]

[1]Department of Chemical Engineering, Massachusetts Institute of Technology, Cambridge, MA 02139

[2]Ragon Institute of MGH, MIT, and Harvard, Cambridge, MA 02139

[3]Bristol-Myers Squibb, 700 Bay Rd, Redwood City, California, 94063-2478

[4]Department of Physics, Massachusetts Institute of Technology, Cambridge, MA 02139

[5]Department of Biological Engineering, Massachusetts Institute of Technology, Cambridge, MA 02139

[6]Department of Chemistry, Massachusetts Institute of Technology, Cambridge, MA 02139

[7]Institute for Medical Engineering and Science, Massachusetts Institute of Technology, Cambridge, MA 02139

[8]These authors contributed equally

[9]Lead contact

[†] Address correspondence to: dlingwood@mgh.harvard.edu and arupc@mit.edu

Supplementary Tables

Index	Description	Size $\sigma_{i,i}$	Bead color in Figure S1
1	"Structural" atom of the immunogen. Not an epitope. The ferritin body is composed of such beads	0.8nm	-
2	Fc of the BCR	5nm	Blue
3	Hinge bead connecting the Fc bead to the two arms hinges	1nm	Orange
4	Hinge beads in the BCR arms	1.75nm	White
5	Fab	1.75nm	Yellow
6	Arm bead in the BCR	4.2nm	Magenta
7	Epitope beads	1.75nm	-

Table S1 **Dimensions of the elements constructing the coarse-grained models. Related to Figures 2, S1.** Description of the elements constructing the coarse-grained antibody model (Figure S1A-C) and the immunogens (Figure 1).

Bead index	1	2	3	4	5	6	7
1	-	1.275	1.275	1.275	1.275	1.275	1.275
2		-	3. 375	3. 375	3.375	3. 375	1.75
3			-	0.50	1.75	1.75	1.75
4				-	1.75	1.75	1.75
5					-	1.75	1.75
6						-	1.75
7							-

Table S2 **LJ interaction parameters. Related to Figures 2, S1.** Values of $\sigma_{i,j}$ in nm.

Parameters used in the simulation	**Value**
μ_0 : basal death rate	0.6 day^{-1}
$\mu_{\text{Memory B cells expand}}$: death rate of memory B cells during their expansion phase	0.01 day^{-1} (in the models where memory B cells expand (models 1-2). 0 day^{-1} (in the models where memory B cells do not expand (models 3).
β_0: basal birth rate	1.5 day^{-1}
β_{Expand}: birth rate of memory B cells during the expansion phase	0.42 day-1 (in the models where memory B cells expand (models 1-2). 0 day-1 (in the models where memory B cells do not expand (models 3).
N : germinal center capacity	5000
T_{Growth} : Growth phase duration	4 days
T_{comp}: Competitive phase duration	16 days
T_{Expand}	The same duration as the GC reaction T_{Growth}+ T_{comp}
F : Pulling force	1 pN
E_{Ag-mem}: interaction energy between the Ag and the immune complex on the FDC	$2k_BT$
E_{BCR-Ag}: initial interaction energy between the BCR and the Ag. When mutating, the bond energy this value change during the simulation	See sections "**Error! Reference source not found.**"
q (on-rate): activation rate for the encounter between the BCR and the Ag.	Determined by the MD simulations
x_b: the distance at which a bond breaks.	1nm
Number of BCR molecules per B cell	20
τ : the maximal search time of Ag by the BCR at the B cell protrusion in the immune synapse. After this time, the protrusion retracts with no Ag captured.	1 t.u
p : The probability that an Ag molecule will be in the searching radius of the BCR.	0.09
C : availability of T follicular help cells (TfhCs).	400
N_{NP}: the total number of immunogens (full virus/ HA-np / HA-trimr / SS-np) administrated in a shot	6×10^4
N_{IC}: the number of immune synapses.	3×10^3

P_{exit}: the probability of a B cell to exit the GC at each selection round	0.1
N_{seed} : the average number of B cells seeding a GC	200
A_{seed}: a B cell needs to capture at least that amount of Ag molecules to be considered for entry.	100 for all immunogens. The amount of antigen captured is in multiplications of 100.
μ : mean - parameter of the log-normal distribution	1
σ : sigma - parameter of the log normal distribution	0.5
a : offset- parameter of the log-normal distribution	-4
D: variability coefficient	0.05
f : On-target germline relative affinity - binding energy to Ag.	1 (for data in Figure 4A-D panels i, Figure 7A)
Precursor frequency: The number of clonal copies from each group: [On-target, off-target stem, head]	[7,7,7] Figure 4A-C panels i [7,7,0] Figure 4D-i [7,X,0] Figure 4D-ii-iii, Figure S3, Figure 5A. To create the phase diagram, we modify the number of copies (X) from each stem-specific off-target clone. [7,1,X] Figure 4A-C-ii-iii, Figure S5B. To create the phase diagram, we modify the number of copies (X) from each head-specific clone. [1,7,5] Figure 7A,B. [100,100,5] Figure S6 (first column). [100,500,5] Figure S6 (second column). [100,1000,5] Figure S6 (third column). [100,1500,5] Figure S6 (fourth column).

Table S3 **Simulation parameters. Related to Figures 3-5, 7, S3, S5, S6.**

Supplementary Figures

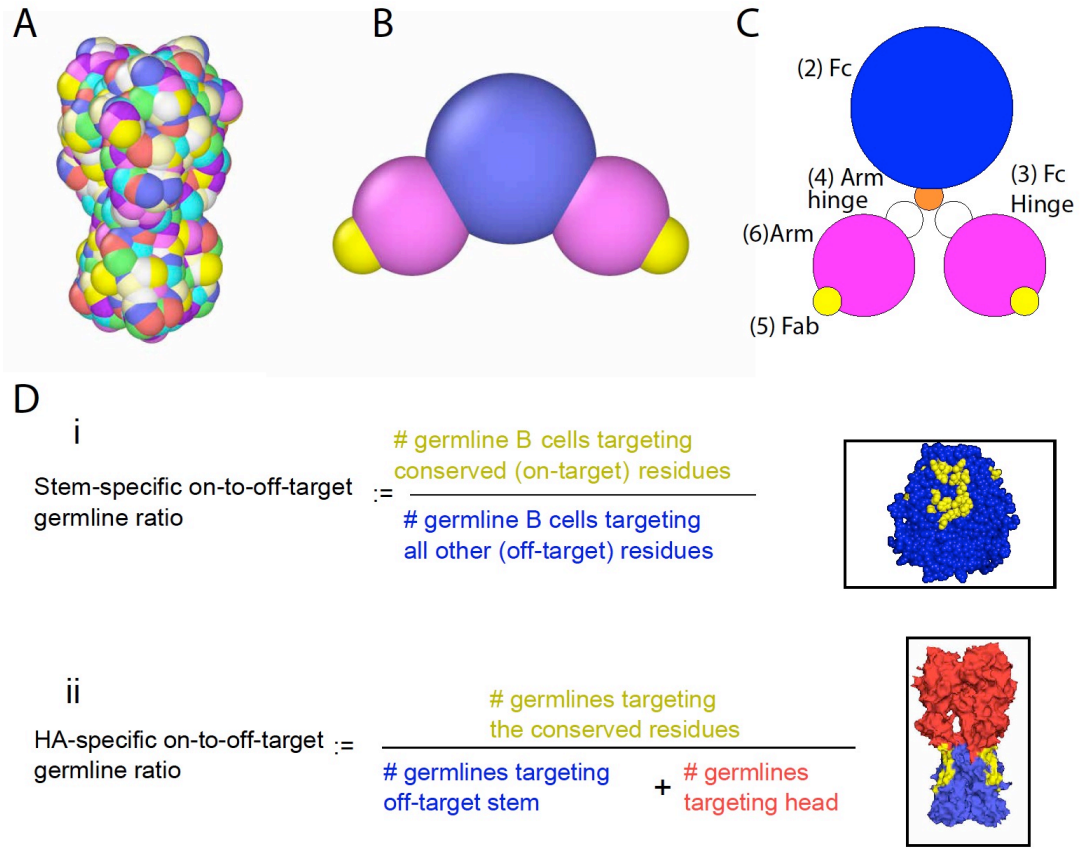
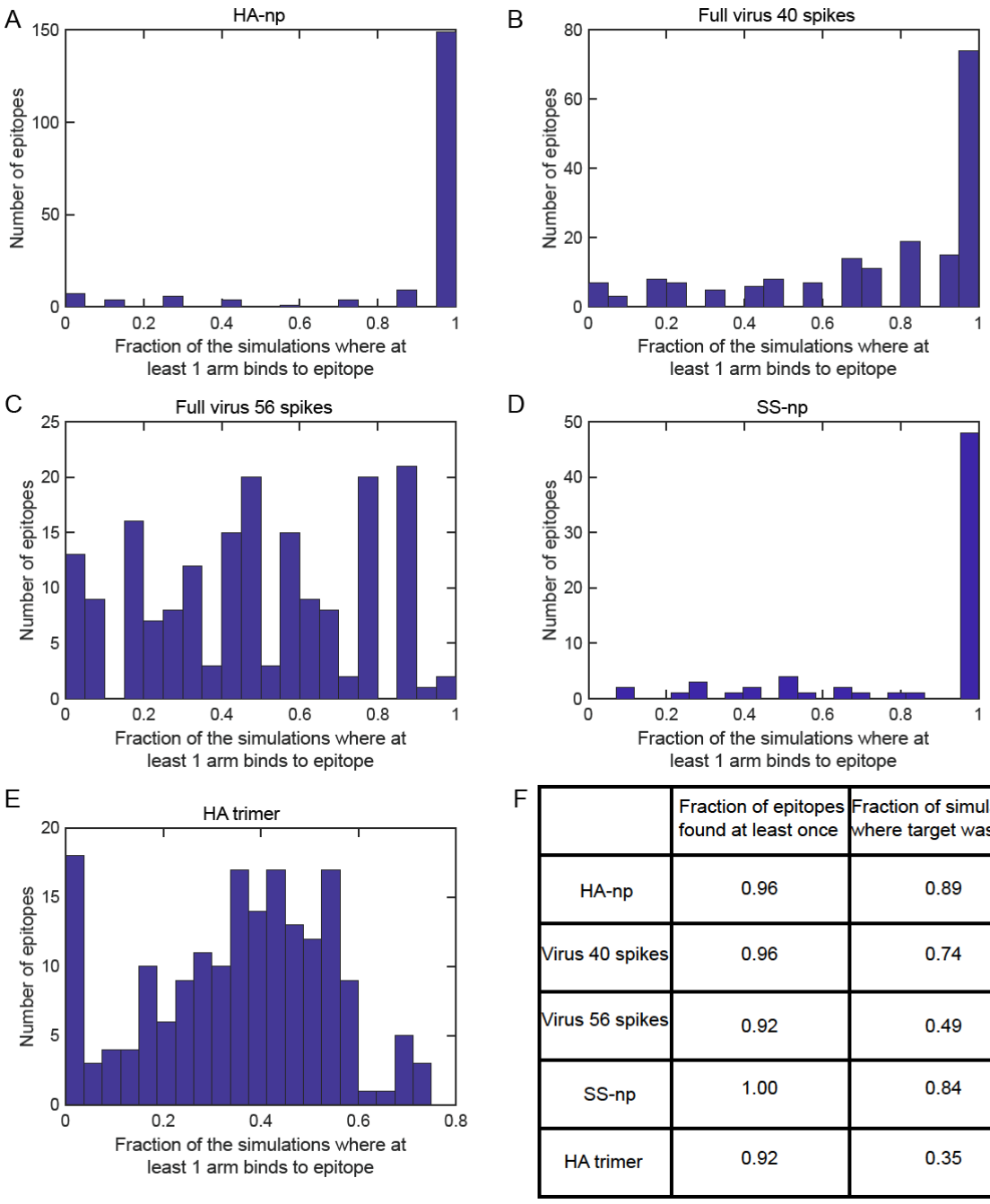


Figure S1 **HA antigen-antibody models and B cell parameters**. **Related to Figure 2. (A)** Set of 184 distinct residues on the surface of HA. See also Methods Details. **(B-C)** Schematic representation of the B cell receptor. The large blue bead represents the Fc part of the BCR. The two magenta beads are the arms, and the yellow beads are the Fab section of the arms. The model also contains hinge beads between the Fc and the arms. For full description see "**Error! Reference source not found.**"). **(D)** Setting the germline precursor frequency**. (i)** For stem responses, the initial fraction of on-target precursors was defined as the ratio of on-target germline clones, to stem-specific off-target germline clones (=on/off ratio). See also Methods Details, "Setting clonal precursor frequency", Eq.(20). (ii) For HA responses, the initial fraction of the on-target precursor was defined as the ratio of on-target germline clones, to HA-specific off-target germline clones (=on/off ratio). The number of on-target and off-target-stem specific germlines is fixed to be 1.2 to represent the ratio measured in IGHV1-69 mice (Sangesland et al., 2019). To vary the on/off ratio, the number of head specific germlines is modified, see "Setting clonal precursor frequency" and Eq.(21).



F

	Fraction of epitopes found at least once	Fraction of simulations where target was found
HA-np	0.96	0.89
Virus 40 spikes	0.96	0.74
Virus 56 spikes	0.92	0.49
SS-np	1.00	0.84
HA trimer	0.92	0.35

Figure S2 **MD simulations efficiency. Related to Figure 2.** Histogram showing the fraction of simulations that finished with a successful binding event. For each immunogen we show the number of epitopes for which a certain fraction of the simulation ended in a successful binding event of a single arm: **(A)** HA-np; **(B)** full virus has 40 HA molecules at a spike spacing of 14.8 nm. [corresponding to the measured spike spacing on the influenza of 14 nm [(Harris et al., 2013)]; **(C)** full virus with 56 HA molecules on its surface; **(D)** SS-np; and **(E)** HA monomer. **(F)** The fraction of epitopes found at least once in all simulations, and the fraction of simulation were a targeted was found, computed over all epitopes per immunogen.

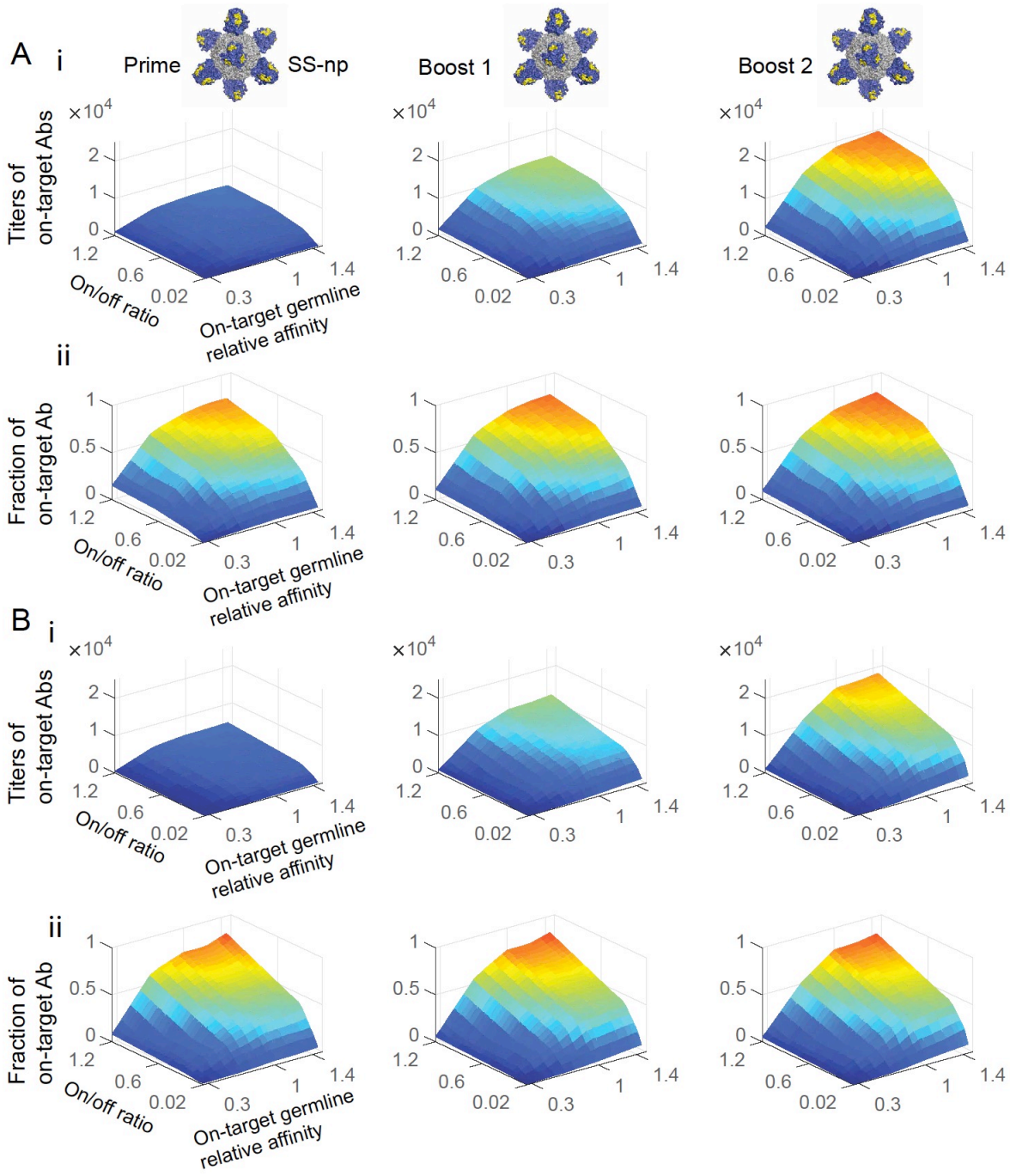


Figure S3 **Magnitude of the Group 1 bnAb epitope-specific antibody response and B memory established following sequential immunization by SS-np. Related to Figure 4.** (A) The number of captured Ag molecules needed for GC entry was set to 200 ($A_{seed} = 200$ see Table S3). This seeding threshold is a proxy for the threshold affinity for forming a productive GC. **(B) GC seeding antigen capture threshold set to 300 ($A_{seed} = 300$).** The initial fraction of on-target precursors was expressed as the ratio of on-target germline clones (specific for Group 1 bnAb target), to stem-specific off-target germline clones (targeting elsewhere on the stem) (=on/off ratio; see Methods Details "Setting clonal precursor frequency", Eq.(20)). The result of sequential immunization in silico was 3D-plotted as a function

of: the average on-target germline affinity (off-rate) relative to the off-target germline affinity (x-axis), the on/off ratio (y-axis), and on-target antibody response titer (z-axis). The color map (blue to red) corresponds to the total number of memory B cells targeting the conserved epitopes (Panels i), and the fraction of B cells targeting the conserved epitopes compared to all memory B cells elicited (Panels ii).

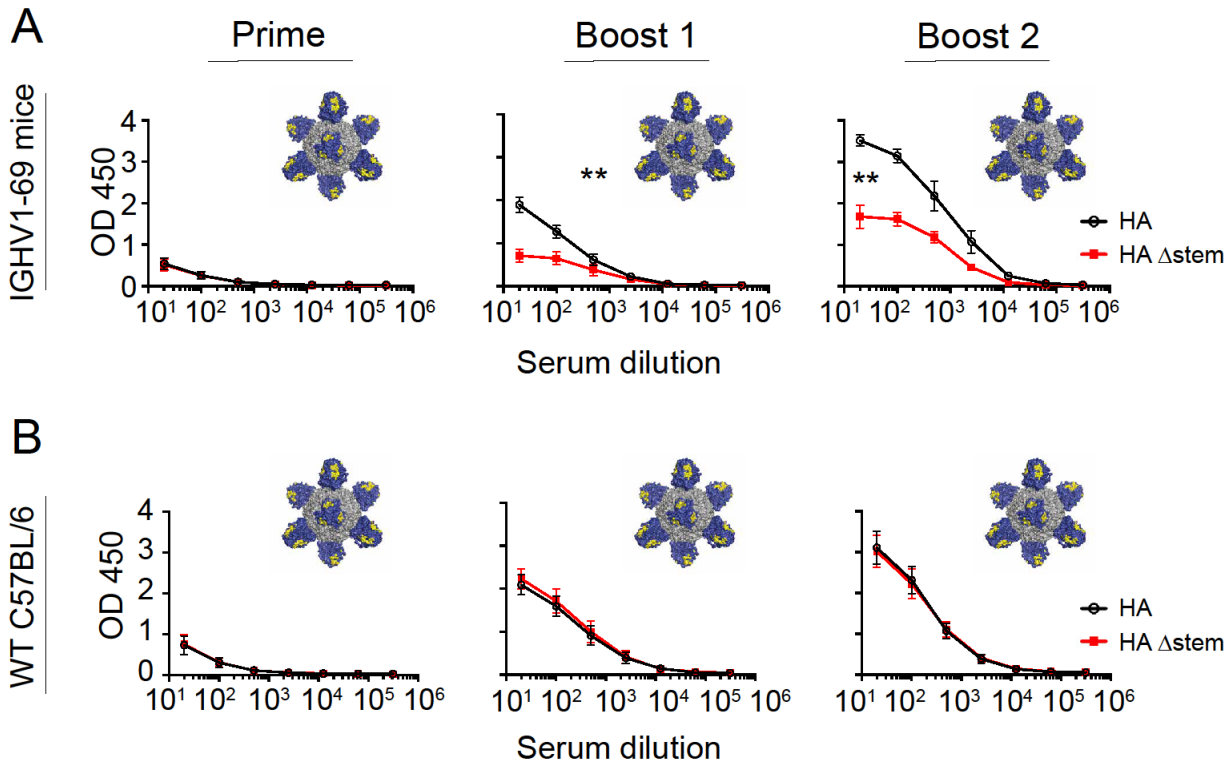


Figure S4 **Elicitation of serum antibodies targeting the Group 1 bnAb epitope. Related to Figure 5.** Usage of the human V_H gene IGHV1-69 naturally endows antibody repertoire with germline B cells specific for the Group 1 bnAb epitope (Sangesland et al., 2019). We sequentially immunized IGHV1-69 mice **(A)** and wildtype C57BL/6 **(B)** with SS-np at weeks 0, 3, and 6 and sampled for blood at weeks 2 (post-prime), 5 (post-boost 1), and 8 (post-boost 2) as previously described (Sangesland et al., 2019). Epitope-targeting in the serum antibody response was assessed by binding of serum IgG to HA (black line) vs HAΔstem (I45R, T49R) (red line). Mean and SEM values for reactivity to HA vs HAΔstem are shown for each time point (n=5 animals per regimen) and AUC values were compared using the method of Hanley and McNeil (Hanley and McNeil, 1983) which was applied to test the two-sided null hypothesis that the there is no difference between the curve areas (**P<0.002).

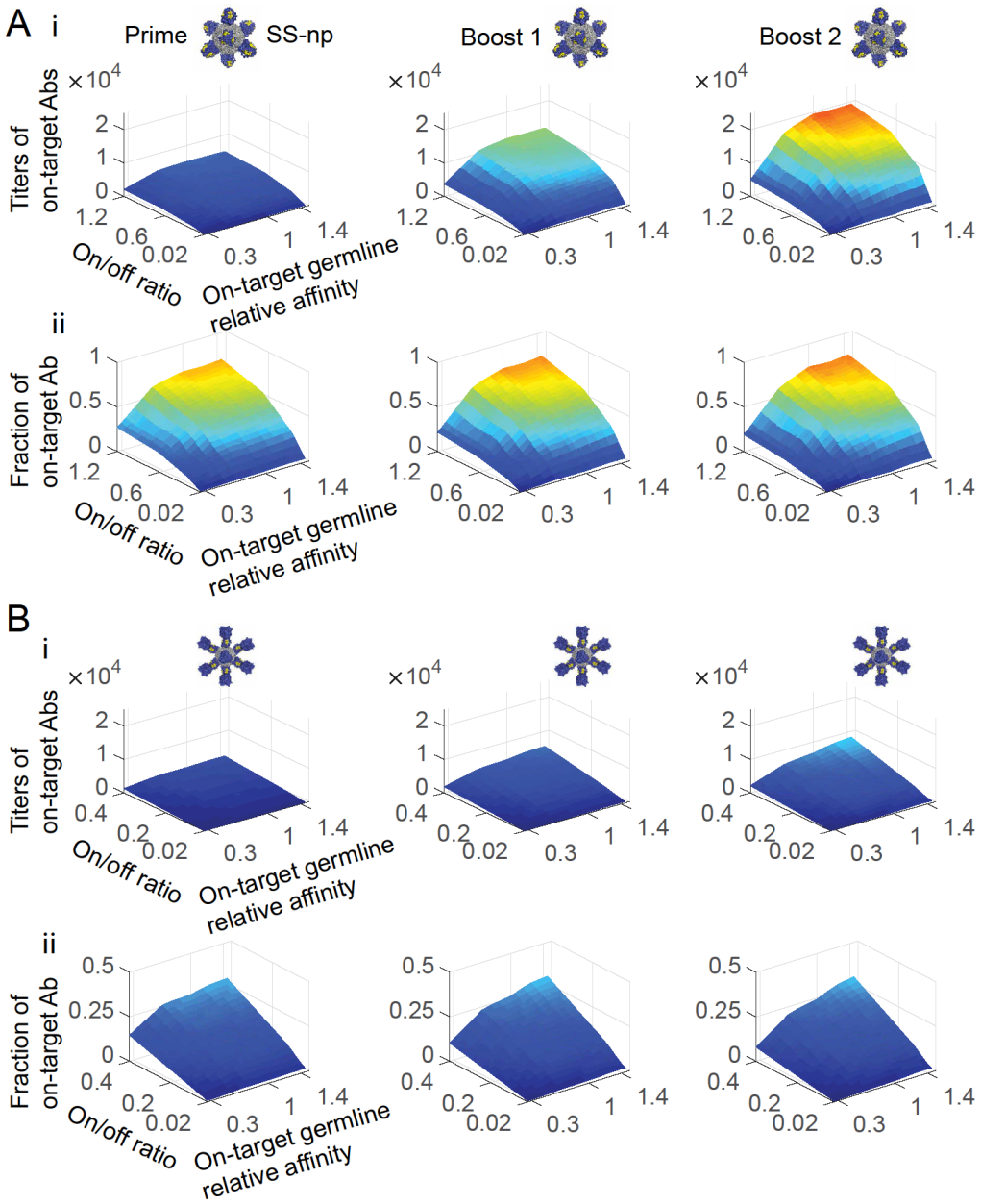


Figure S5 **The magnitude of the on-target response elicited by different HA geometries as a function of precursor frequency and affinity. Simulation of model 3 (no memory B cell proliferation). Related to Figure 4. (A-B)** The initial fraction of on-target precursors was expressed as the ratio of on-target germline clones (specific for Group 1 bnAb target), to stem-specific off-target germline clones (=on/off ratio) (see Methods Details "Setting clonal precursor frequency", Eq.(20)), or to HA-specific off-target germline clones (=on/off ratio) (see also Methods Details "Setting clonal precursor frequency", Eq.(21)). The result of sequential immunization in silico plotted as a function of: the average on-target germline affinity (off-rate) relative to the off-target germline affinity (x-axis), the on/off ratio(y-axis), and on-target antibody response titer (z-axis). (**Panels i**) The color map (blue to red) corresponds to the number of memory B cells targeting the conserved epitopes elicited by the SS-np (A) or HA-np (B). (**Panels ii**) The fraction of B cells targeting the conserved epitopes compared to all memory B cells created during the simulation, elicited by the SS-np (A) or HA-np (B).

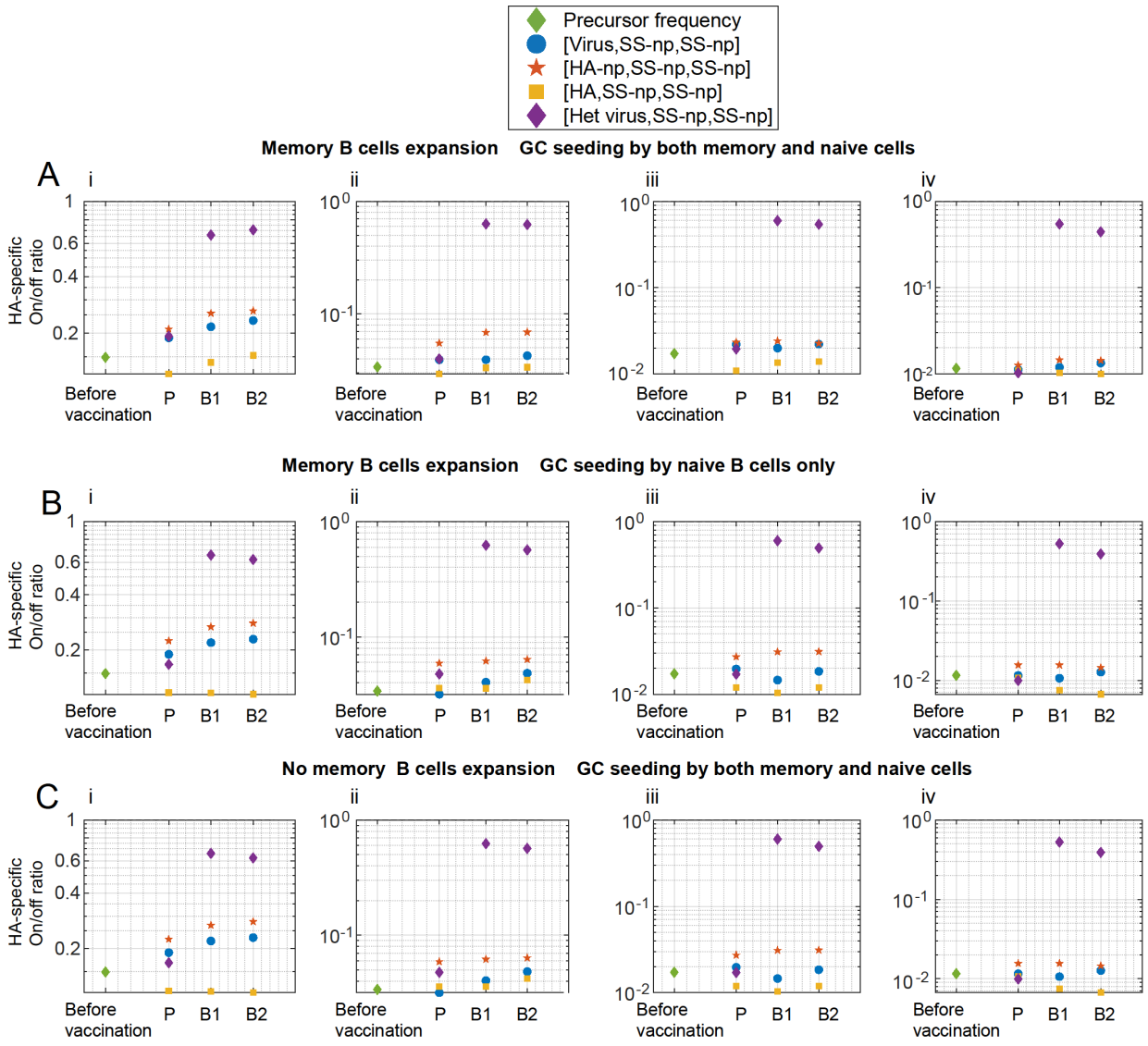


Figure S6 **Pre-exposure to different HA geometries shapes the memory recall response elicited by SS-np. Related to Figure 7.** In silico immune exposure regimens where the prime [P] is one of the following: NC99 H1N1 virus (strain matched to SS-np) (blue full circles); HA-np (red pentagram); HA trimer (yellow squares); or heterologous virus (CA09) (purple diamonds). SS-np was then sequentially immunized (boost [B1] 1 and then boost 2 [B2]). Depicted is the on-to-off HA-specific ratio of mature B cells (a proxy for Ab titers) following each immunization regimen. This ratio is also shown prior to the immune challenge (green diamond). **(A)** A model where memory B cells expand outside of the GC in an Ag dependent manner. The GC can be seeded by both memory and naïve B cell. The reaction was studied as a function of different germline precursor frequencies. The on-to-off stem-specific ratio is: **(i)** 0.175 **(ii)** 0.035 **(iii)** 0.018 **(iv)** 0.011. **(B)** A model where memory B cells expand outside of the GC in an Ag dependent manner. The GC can only be seeded by naïve B cells. The marker color for the different immunogens and germline frequencies at each column are the same as in (A). **(C)** A model where memory B cells do not expand outside of the GC in an Ag dependent manner. The GC can be seeded by both memory and naïve B cell.

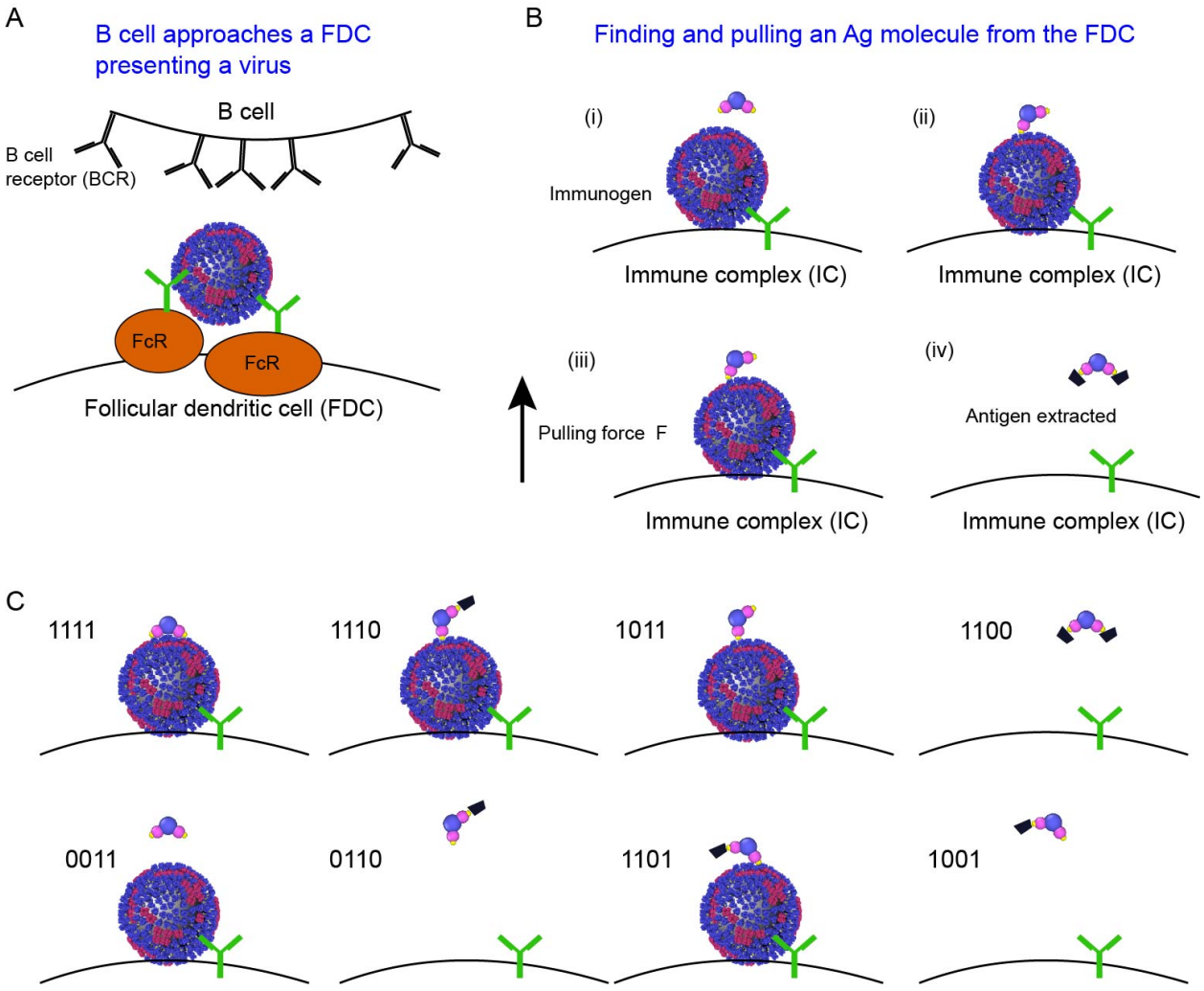


Figure S7. **Extraction of influenza antigens. Related to STAR Methods. (A)** A B cell approaches an FDC to capture antigen (Ag) (the full virus is presented as a generic example). The FDC presents an immune complex holding an immunogen. The attachment of the virus to the FDC is mediated by low-affinity antibodies (green) bound to receptors, called Fc Receptors (orange, FcR), on the FDC surface. **(B)** The extraction of antigen from an FDC by a B cell: (b-i) A protrusion containing the BCR extends to touch the surface of the FDC displaying Ag molecules. (b-ii) Depending on the on-rate of the first arm, and Ag availability, the BCR may encounter Ag molecules to which it can attach. (b-iii) Once a BCR-Ag bond has formed, the B cell pulls on it by applying force mediated by actin. (b-iv) Extraction of Ag from FDC can occur. **(C)** Illustrated are all the possible states of the BCR and the Ag molecules. The notation is explained in the Methods section (see subsection "Estimating the Ag capture probabilities").