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Supplemental Information

Rapid Germinal Center and Antibody Responses in Non-human Primates after a Single Nanoparticle Vaccine Immunization

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Supplemental Information

Figure S1. Evans blue drainage studies. Related to Figure 1.

Figure S2. LN FNA cell recovery and flow cytometry gating scheme for GC-T_{FH} and B_{GC} cells. Related to Figures 2 and 3.

Figure S3. eOD-GT8 60mer specific B cell lineages from draining LNs. Related to Figure 4.

Figure S4. RM BCR sequences with VRC01-class characteristics. Related to Figures 4 and 5.

Figure S5. Immunogen-specific blood $CXCR5^+$ CD4 T cell responses. Related to Figure 5.

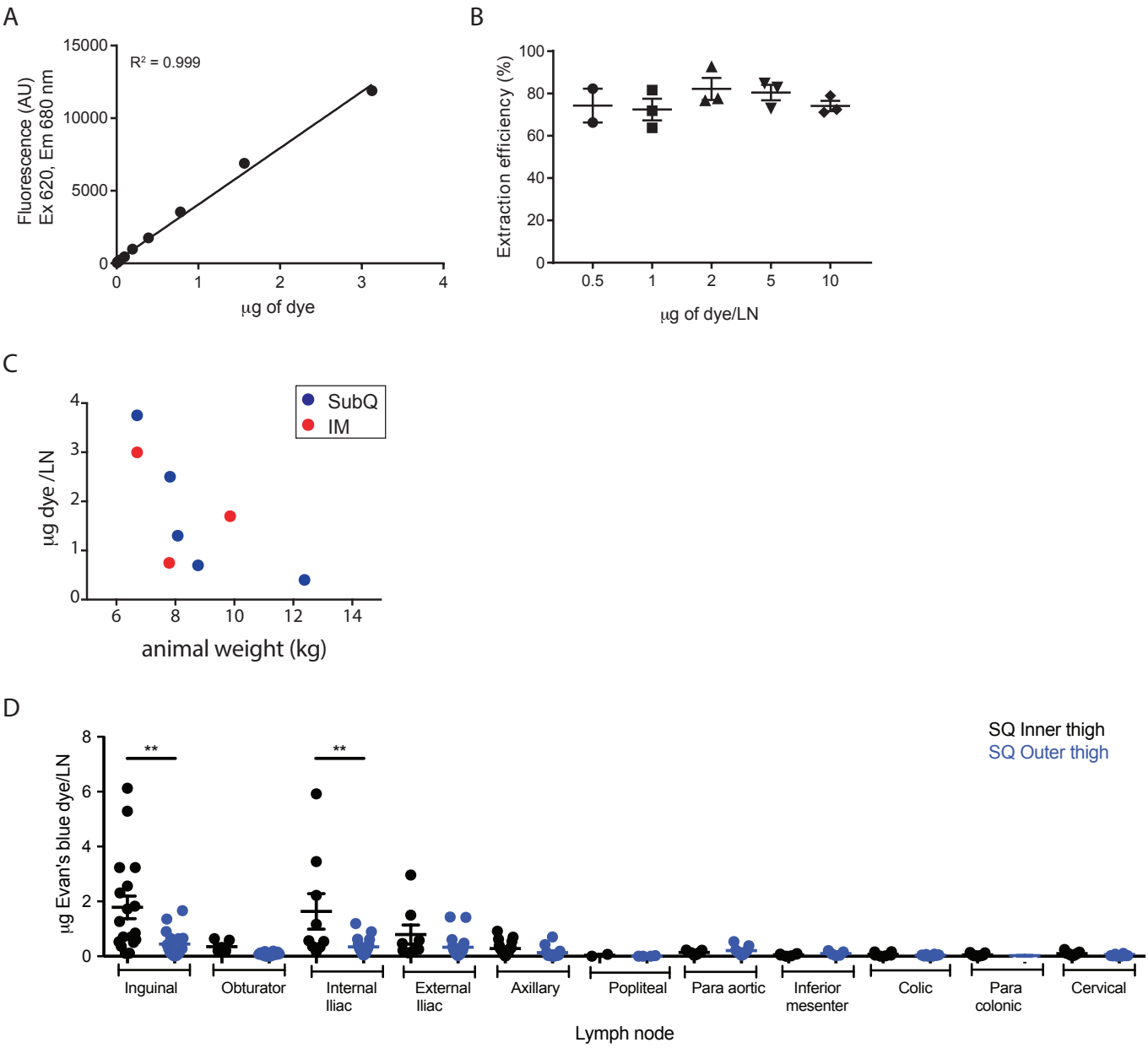


Figure S1. Evans blue drainage studies. Related to Figure 1.
A) Linear correlation of µg of dye extracted and fluorescence.
B) Consistence extraction efficiency over a range of dye amounts per LN.
C) Inverse correlation between animal weight and dye accumulation (Figure S1C).
D) Dye drainage after injection with ISCOMATRIX at either the inner or outer thigh.

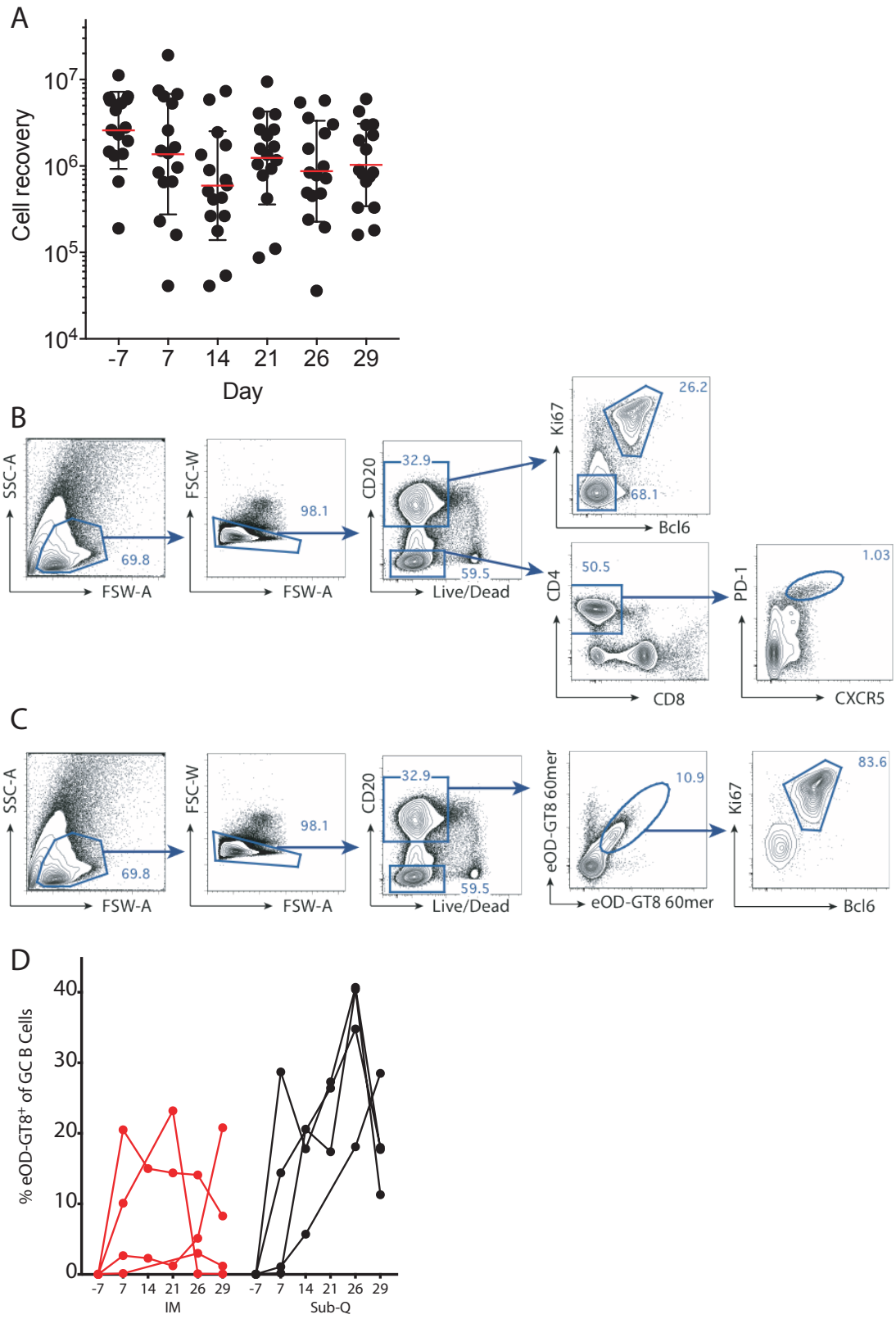


Figure S2. LN FNA cell recovery and Flow cytometry gating scheme for GC-T_{FH} and B_{GC} cells. Related to Figures 2 and 3.
A) Total cell recovery from left and right axillary LN FNAs at time points before (day -7) and after (days 7, 14, 21, 26, 29) immunization.
B) Identification of B_{GC} cells (top) as $CD20^+KI67^+BCL6^+$ cells and GC-T_{FH} cells (bottom) as $CD4^+CXCR5^+PD1^{high}$ cells from LN fine needle aspirate samples.
C) Identification of eOD-GT8 60mer Ag-specific B cells as $CD20^+$eOD-GT8 $60mer^+$eOD-GT8 $60mer^+$ cells from LN fine needle aspirate samples. Within the eOD-GT8 60mer Ag-specific B cell population $Ki67^+Bcl6^+$ B_{GC} cells were gated.
D) Plot of frequencies of eOD-GT8$^+$ of GC B cells. Flow cytometry is shown in Fig 3F.

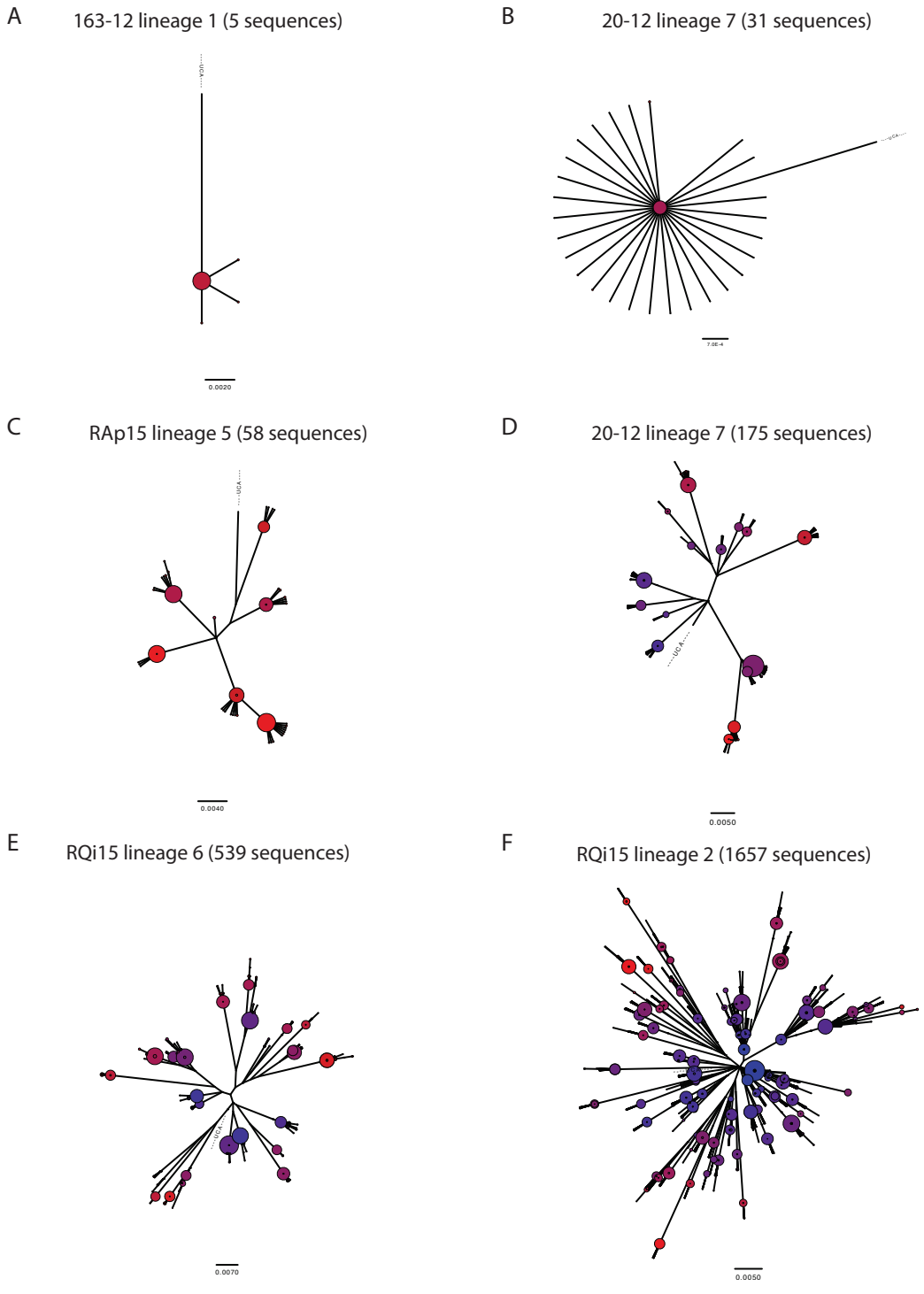
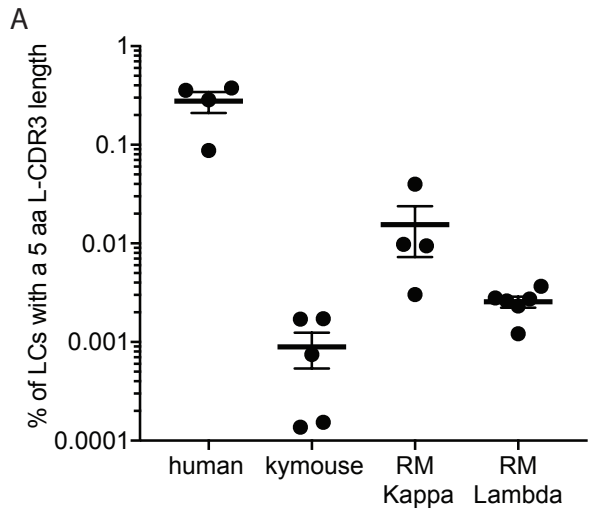
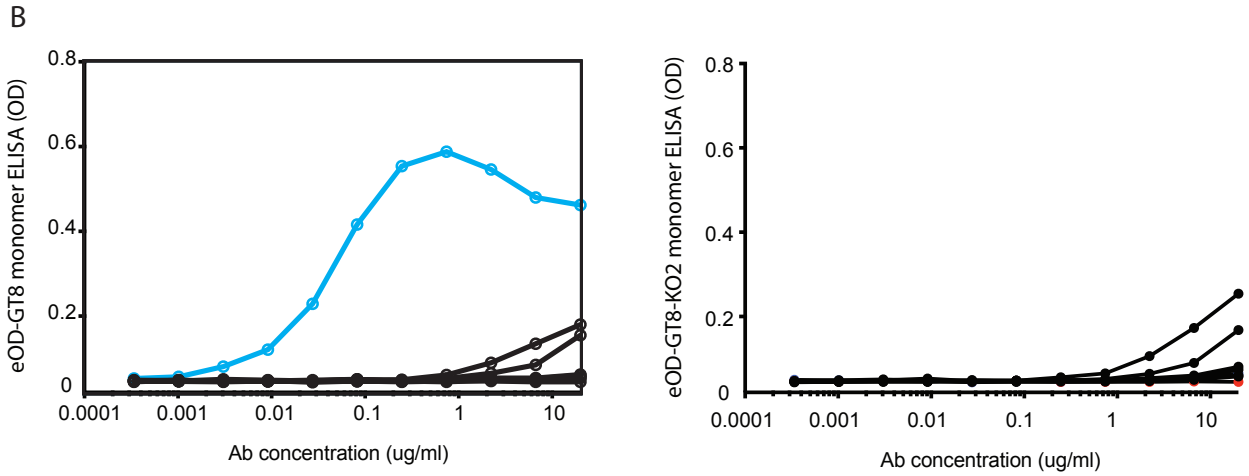


Figure S3. eOD-GT8 60mer specific B cell lineages from draining LNs. Related to Figure 4.
A-F) Examples of the diversity found within a single eOD-GT8 60mer-specific B cell lineage. The unmutated common ancestor (UCA) of the lineage is indicated by a dashed line. Circle size indicates the number of reads for each variant, and circle color indicates the mutation count.





C

	Repeat 1		Repeat 2	
	Wk -1 ID_{50}			
	HxB2	HxB2 N276A	HxB2	HxB2 N276A
283_12	<10	<10	<10	<10
RIp15	<10	<10	<10	<10
128_12	<10	<10	<10	<10
RKp15	<10	<10	<10	<10
RAp15	<10	<10	<10	<10
RQi15	<10	<10	<10	<10
20_12	<10	<10	<10	<10
163_12	<10	<10	<10	<10

	Repeat 1		Repeat 2	
	Wk 4 ID_{50}			
	HxB2	HxB2 N276A	HxB2	HxB2 N276A
	<10	<10	<10	<10
	<10	<10	<10	<10
	<10	<10	<10	<10
	<10	<10	<10	<10
	<10	<10	<10	<10
	<10	<10	<10	<10
	<10	<10	<10	<10
	<10	<10	<10	<10

Controls IC_{50} (µg/mL)	VRC01	0.01	0.009	0.01	0.005
	Den3	>50	>50	>50	>50

Figure S4. RM BCR sequences with VRC01-class characteristics. Related to Figures 4 and 5.
A) Frequency of LCs with a L-CDR3 length of 5 aa in unimmunized RMs compared to healthy humans and unimmunized human-Ig transgenic (Kymouse) mice (from (Sok et al., 2016)). Points represent individual animals. Sequencing depth was 0.6-1.8x10^6 sequences per sample.
B) ELISA data for recombinant RM Abs with VRC01-class characteristics (synthesized as human IgG). Black points are RM Abs (n=6). Blue (human VRC01-class naive B cell) and red (Vaccinia specific Ab) were used as controls.
C) Neutralization data for HxB2 HIV Env sequence psuedoviruses, with (HxB2) and without the N276 glycan at the CD4 binding site (NxB2 N276A) by serum before and after eOD-GT8 immunization. The eOD-GT8 sequence was originally developed from the HxB2 HIV Env sequence. Two assays are shown. The human bnAb VRC01 and dengue virus were used as controls.

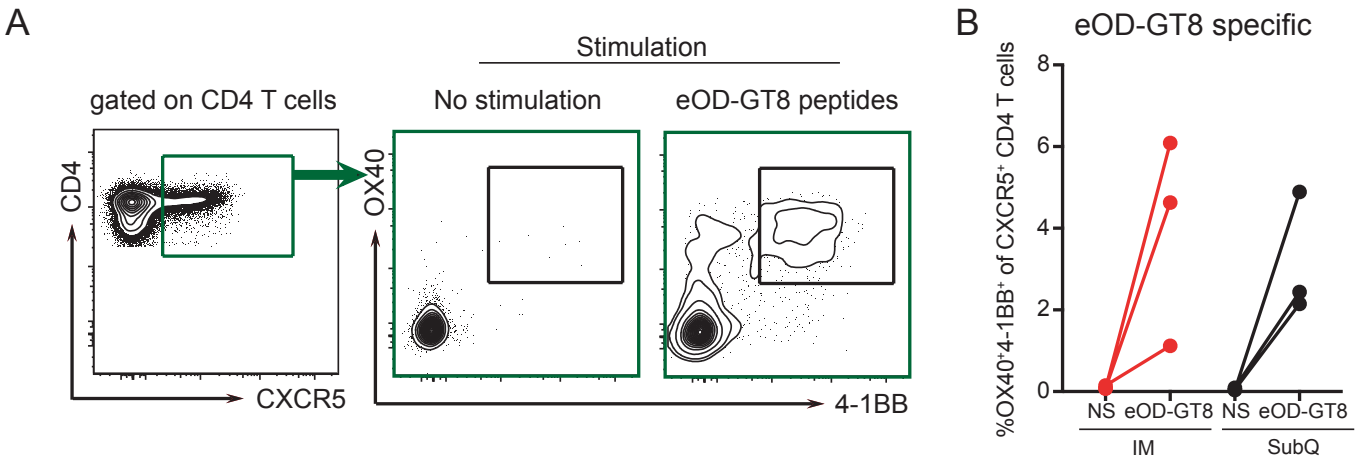


Figure S5. Immunogen-specific blood CXCR5$^+$ CD4 T cell responses. Related to Figure 5.
A) eOD-GT8-specific CXCR5$^+$ CD4$^+$ blood Tfh cell responses in PBMC using the AIM$_{OB}$ assay. One week after immunization. 'NS', not stimulated. 'eOD-GT8', eOD-GT8 peptide pool.
B) Frequency of eOD-GT8 AIMOB+ cells after SubQ or IM immunization.