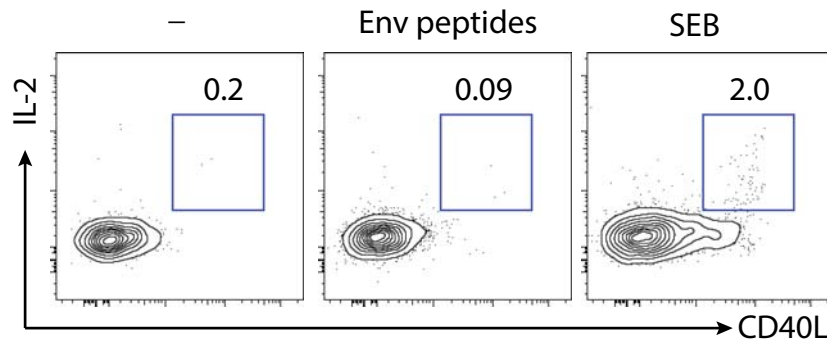


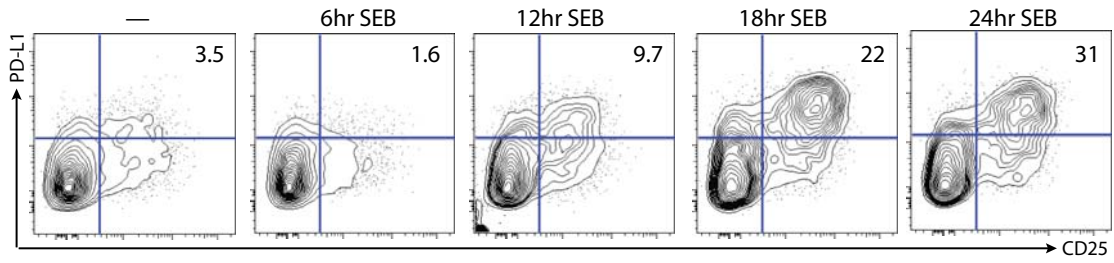
Supplemental Materials



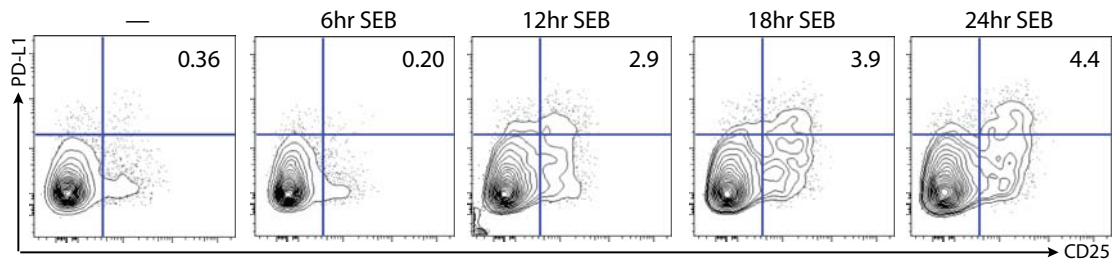
Supplemental Figure 1: Failure of ICS assay to detect antigen specific GC Tfh cells.

CXCR5^{hi} PD1^{hi} GC Tfh cells were gated. IL2 expression by GC Tfh cells in an ICS assay of spleen cells. Cells incubated with no exogenous antigen added are marked by "-". Alternatively, cells were incubated with a pool of overlapping, consensus clade C Env peptides (Env peptide) or SEB. Data are representative of 8 immunized RM.

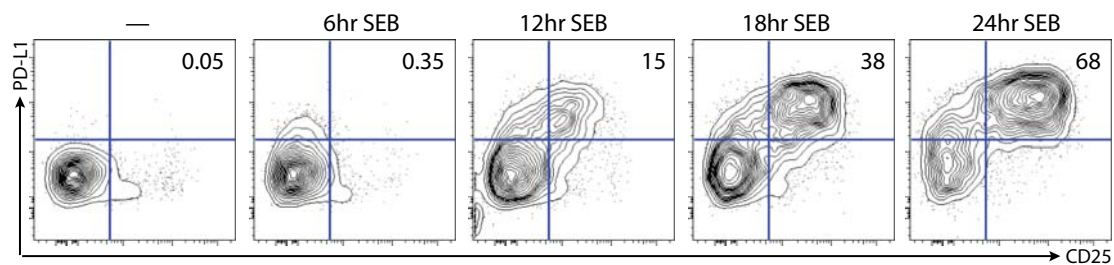
A Human Tonsil, CXCR5⁺ Tfh.



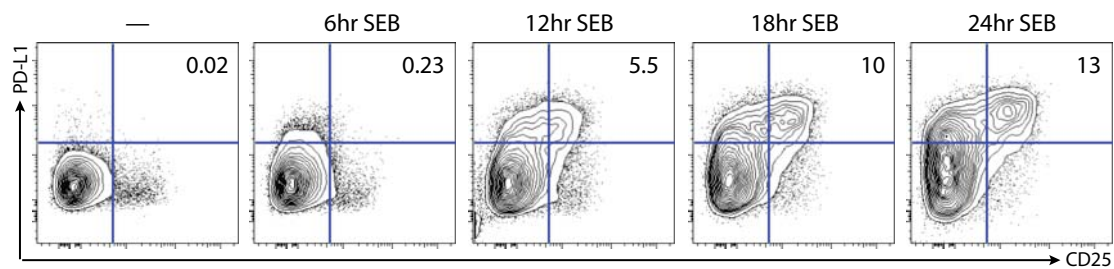
B Human Tonsil, CXCR5⁻ effector CD4 T cells.



C Human PBMC, CXCR5⁺ Tfh.



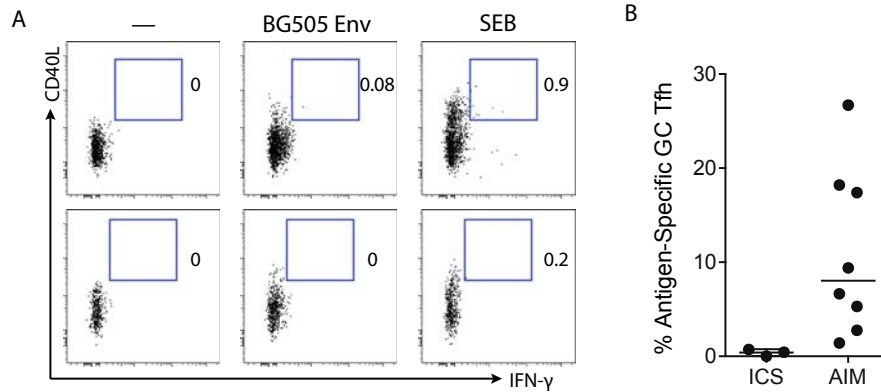
D Human PBMC, CXCR5⁻ effector CD4 T cells.



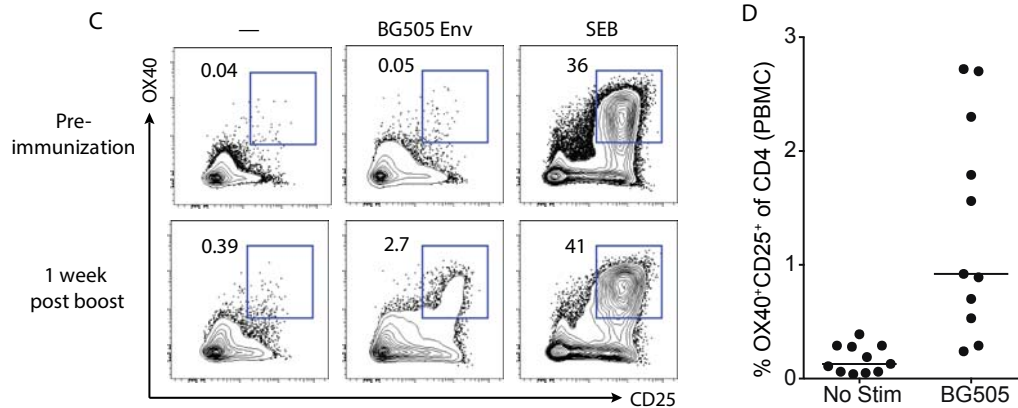
Supplemental Figure 2: Kinetics of PD-L1 and CD25 expression following SEB stimulation in human tonsil and PBMC.

Kinetics of PD-L1 and CD25 surface expression on human tonsil CXCR5⁺ Tfh (A) and CXCR5⁻ effector CD4 T cells (B), and human PBMC CXCR5⁺ Tfh (C) and CXCR5⁻ effector CD4 T cells (D), following stimulation with SEB over the course of 24 hours.

ICS assay of GC Tfh cells from LN



AIM assay of CD4 T cells from PBMC



Supplemental Figure 3: Failure of ICS assay to detect antigen-specific GC Tfh cells and AIM detection of immunogen-specific CD4 T cells in blood.

Failure of ICS assay to detect antigen-specific GC Tfh cells (A and B). A. Expression of CD40L and IFN γ in an ICS assay by PD1^{hi}CXCR5^{hi} GC Tfh cells. B. Raw quantitation of antigen-specific GC Tfh cells by ICS (TNF⁺CD40L⁺ n=3) and AIM (CD25⁺OX40⁺ n=8) assays. These are the same data shown in Figure 4B, but without the background subtracted. AIM detection of immunogen-specific CD4 T cells in blood (C and D). C. BG505 Env-specific responses detected with the AIM assay among total CD4 T cells from PBMC of immunized animals. PBMC from a pre-immunization time point and 1 week post the 4th immunization time point are shown. D. Raw frequency of BG505 Env-specific responses in the AIM assay by total CD4 T cells from macaque PBMC one week post 4th immunization.