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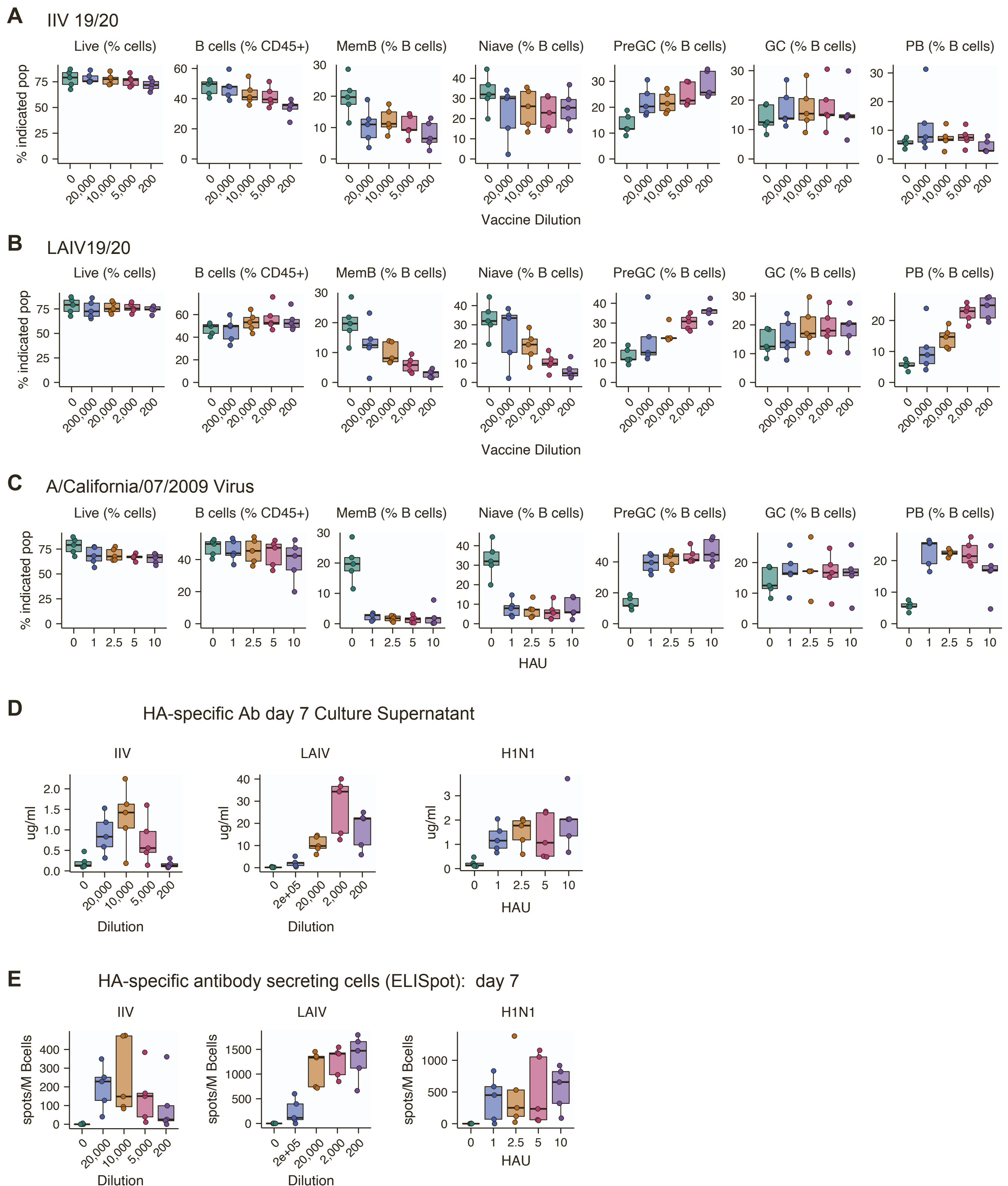
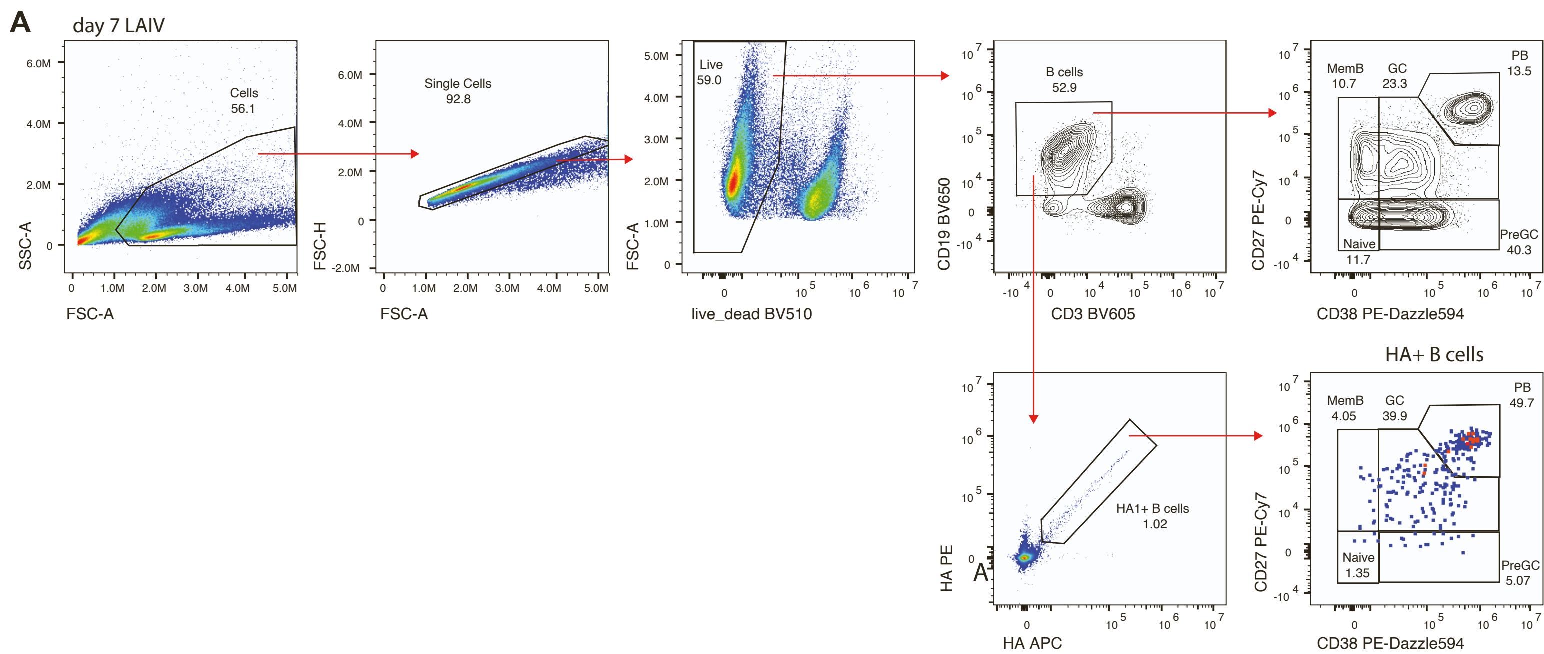
Supplemental information

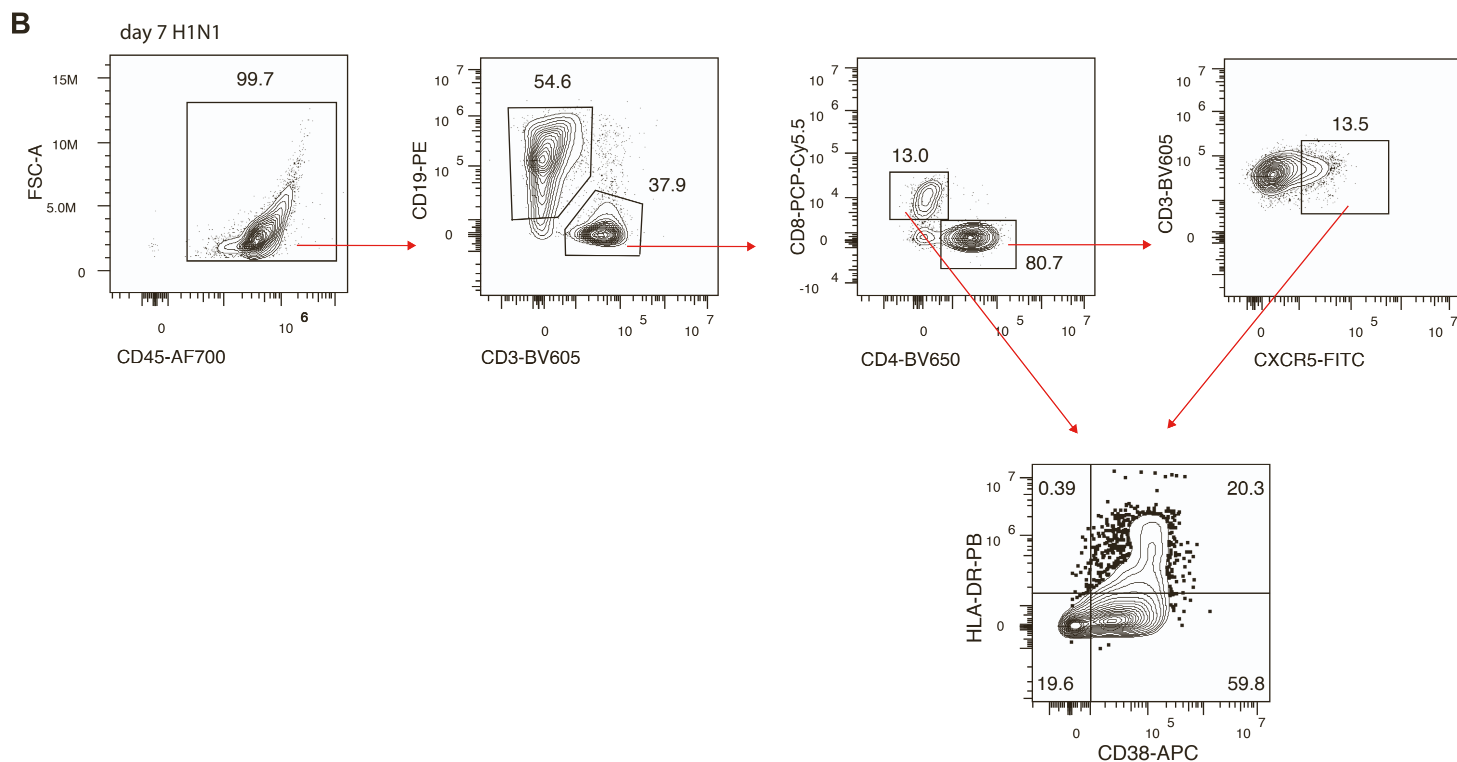
Influenza vaccine format mediates distinct cellular and antibody responses in human immune organoids

Jenna M. Kastenschmidt, Suhas Sureshchandra, Aarti Jain, Jenny E. Hernandez-Davies, Rafael de Assis, Zachary W. Wagoner, Andrew M. Sorn, Mahina Tabassum Mitul, Aviv I. Benchorin, Elizabeth Levendosky, Gurpreet Ahuja, Qiu Zhong, Douglas Trask, Jacob Boeckmann, Rie Nakajima, Algimantas Jasinskas, Naresha Saligrama, D. Huw Davies, and Lisa E. Wagar

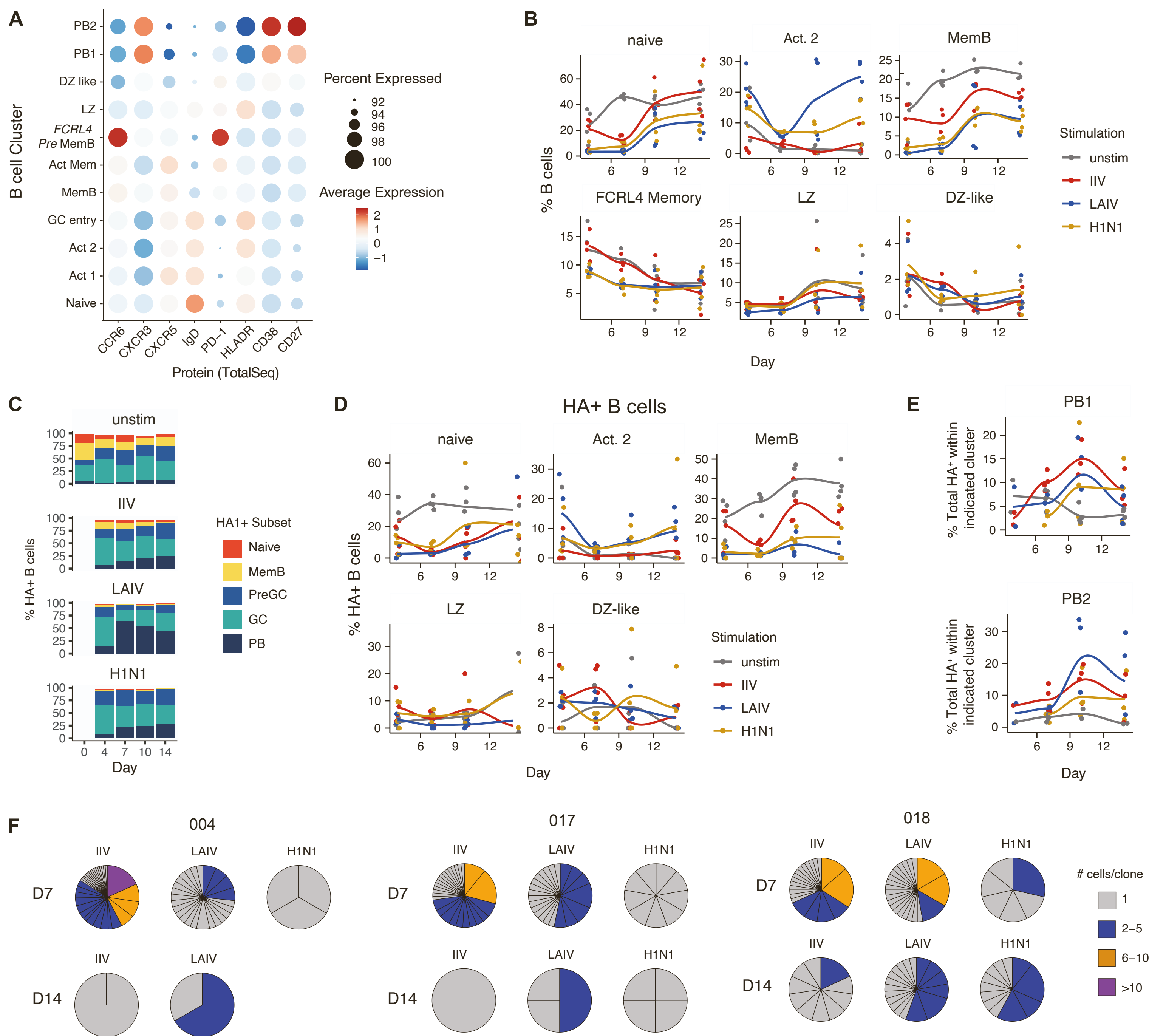


sFig 1. Influenza antigen dose titrations for immune organoid stimulation (Related to Figure 1).
(A-C) Flow cytometry analysis from immune organoids 7 days following stimulation with different doses of (A) 2019/20 influenza season inactivated influenza vaccine, (B) 2019/20 influenza season live attenuated influenza vaccine, or (C) A/California/07/2009 influenza virus. (D) HA-specific antibodies in culture supernatants, as measured by ELISA. (E) Quantification of HA+ antibody-secreting cells as measured by ELISpot. Values are expressed as the number of spots per 1 million B cells. n= 5 donors for all analyses. Box plots show the median, with hinges indicating the first and third quartiles and whiskers indicating the highest and lowest value within 1.5 times the interquartile range of the hinges.

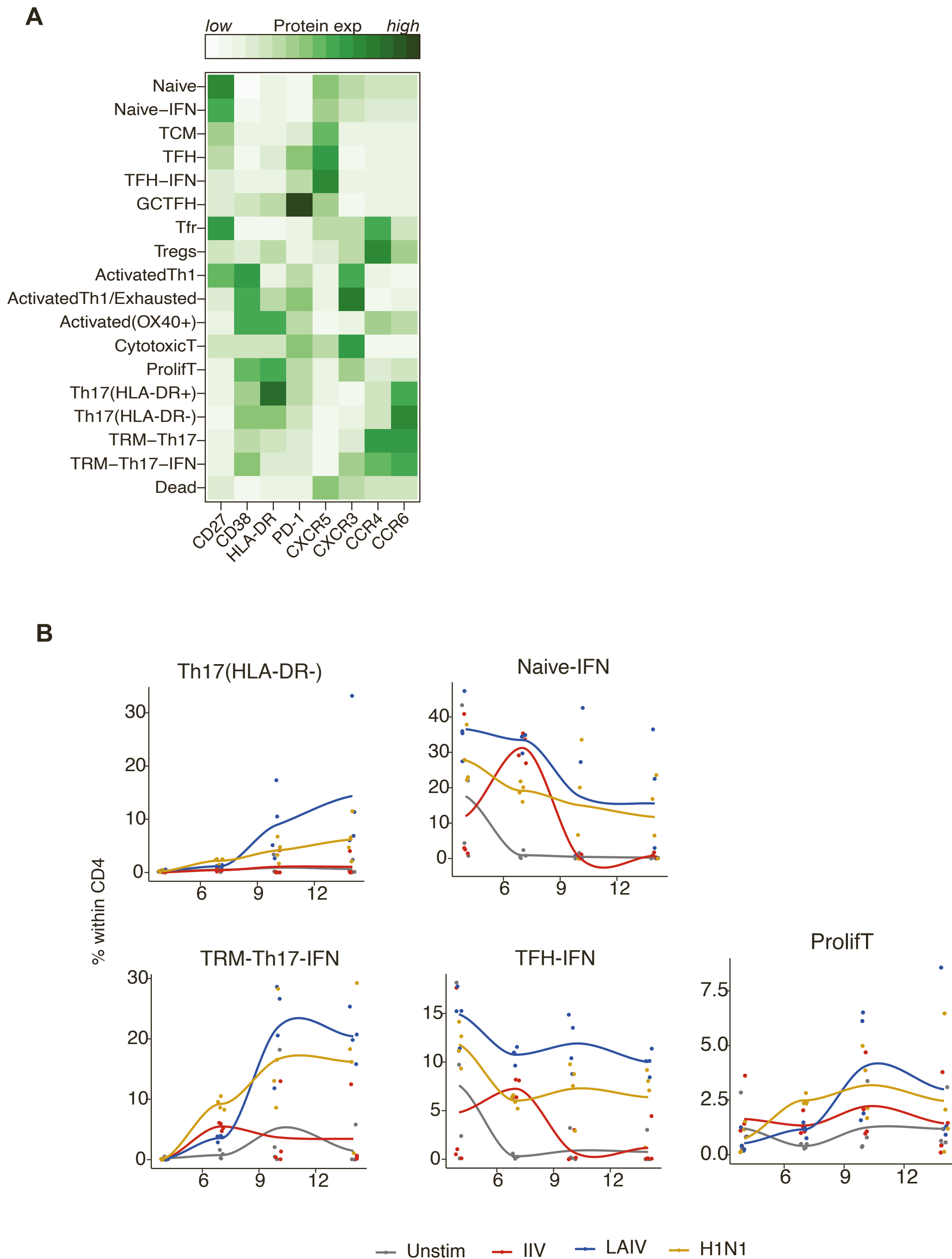




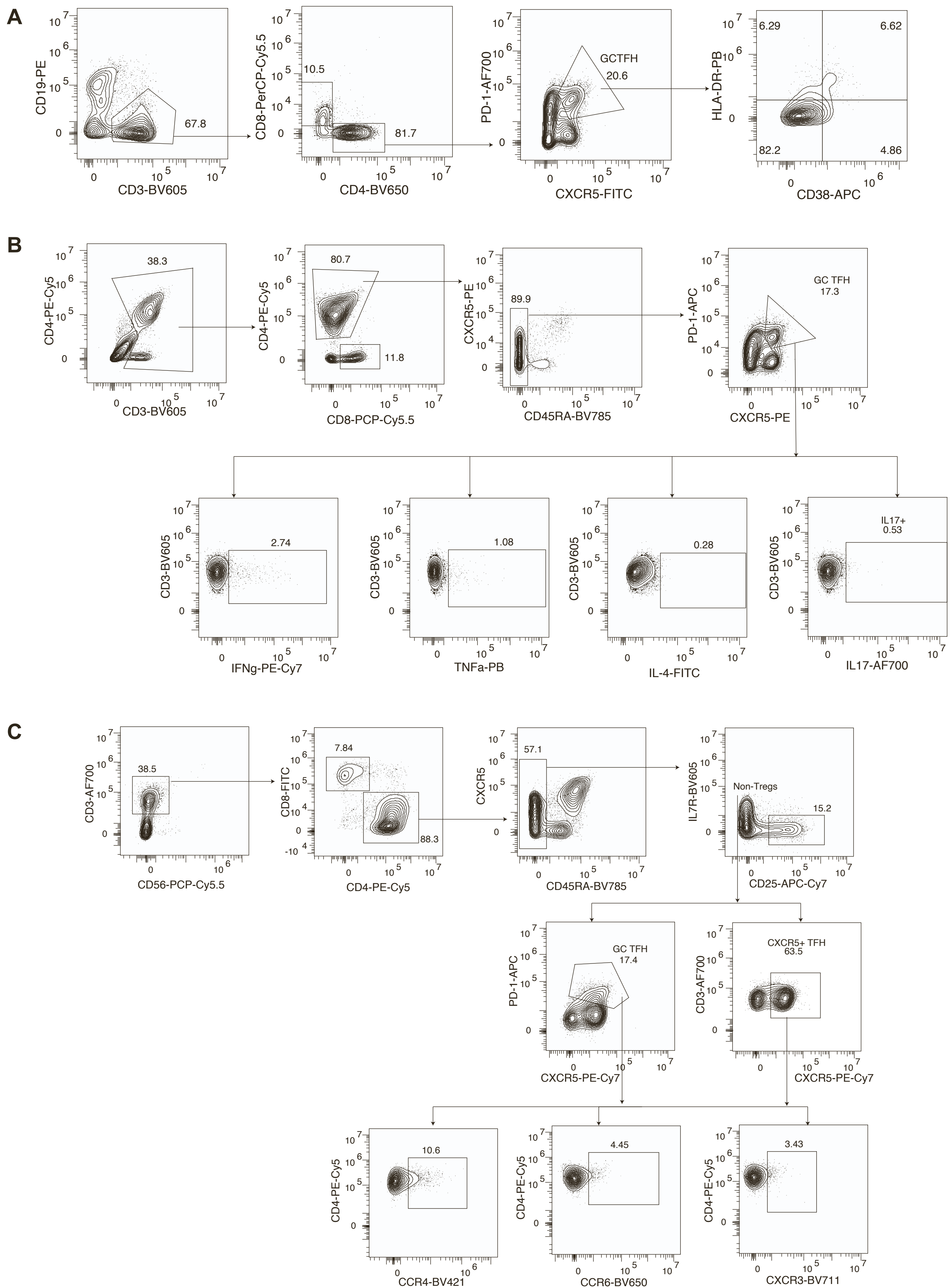
sFig 2. Flow cytometry gating strategies for tonsil and organoid derived B and T cells (Related to Figure 1). Representative data for organoids stimulated for 7 days with influenza antigens. (A) Gating strategy for B cell phenotyping. (B) Gating strategy for T cell phenotyping.



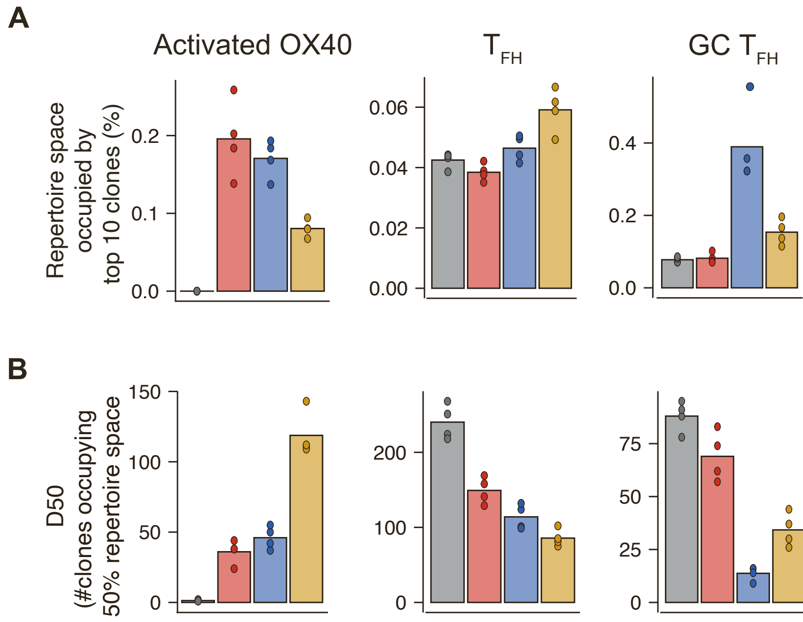
sFig 3. Single-cell analysis of B cells from influenza antigen (Related to Figures 3 and 4).
(A) Total-seq antibodies were used to quantify select proteins used for defining classical B cell subsets. Bubble size indicates the proportion of cells expressing the protein and color indicates the average expression. B cell cluster labels were manually assigned based on gene and protein expression patterns. Data represent all B cells aggregated from n=4 donors. (B) Frequency of HA+ B cells occupying additional scRNAseq clusters (corresponding to Figure 3c) on days 4, 7, 10, and 14 post-stimulation with different influenza antigen modalities. (C) Protein-level phenotypes of HA+ B cells over the course of organoid culture as defined by flow cytometry. Naive (CD38-CD27-), MemB (CD38-CD27+), PreGC (CD38+CD27-), GC (CD38+CD27+) and plasmablast (CD38+++CD27+) phenotypes are shown. Data represent the average of n=12 donors for each time point and stimulation. (D) Frequency of HA+ B cells occupying additional scRNAseq clusters (corresponding to Figure 3g) on days 4, 7, 10, and 14 post-stimulation with different influenza antigen modalities. (E) HA+ B cell occupancy within plasmablast subsets. (F) BCR clonal lineage analysis of HA+ B cells from influenza-stimulated organoids. Each set of pie charts represents an individual donor. Wedge color represents the number of B cells within a given clonal lineage and wedge size indicates the proportion of the repertoire. n=4 donors for all data shown.



sFig 4. scRNAseq analysis of CD4 T cells from tonsil organoids (Related to Figure 5).
(A) Heatmap of average protein expression across individual scRNAseq clusters of CD4 T cells in tonsil organoids. Protein levels were quantified based on Total-seq antibody detection. Values here represent the average for all donors, stimulation, and time points sampled. Color represents the magnitude of protein expression. (B) Frequencies of additional T cell clusters within cells sampled by scRNAseq over time for each stimulation (n=4 donors).



sFig 5. Flow cytometry gating strategies for identifying T cell subsets (Related to Figure 5).
(A) Gating strategy for identifying activated GC T_{FH} in tonsil organoids. (B) Gating strategy for identifying cytokine profiles (IFNγ, TNFα, IL-4, and IL-17) of GC T_{FH} in tonsil organoids (C) Gating strategy for identifying Th1-, Th2-, and Th17-like profiles using surface chemokine receptor expression on GC T_{FH}.



sFig 6. Repertoire analysis of T cell subsets (Related to Figure 5).
Bar graphs comparing (A) repertoire space occupied by the top 10 T cell clones and (B) D50 metric – the number of clones occupying 50% of the repertoire within activated (OX40+) T cells, TFH, and GC TFH on day 7 post-stimulation.