

Organoid Polymer Functionality and Mode of *Klebsiella Pneumoniae* Membrane Antigen Presentation Regulates *Ex Vivo* Germinal Center Epigenetics in Young and Aged B Cells

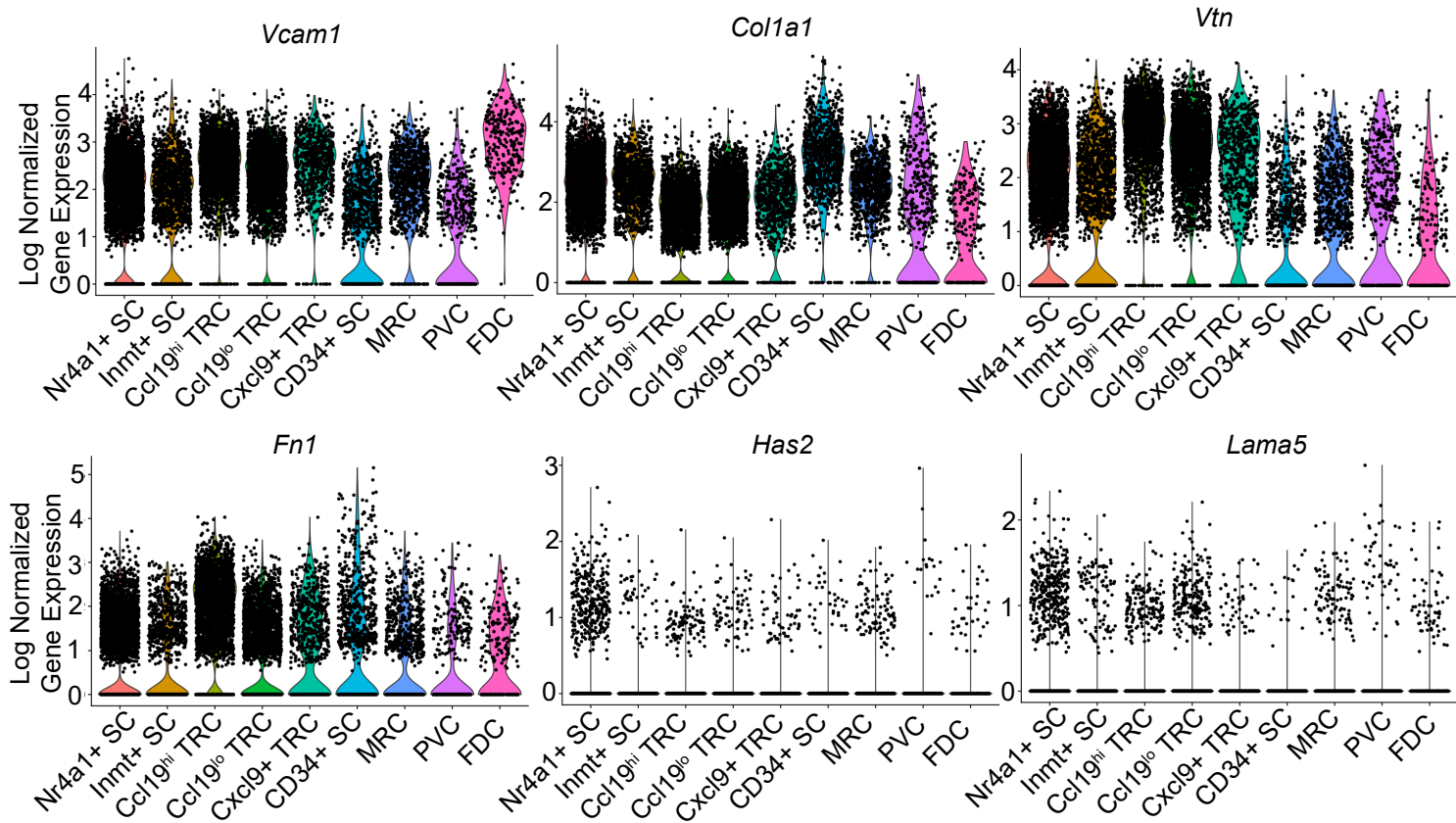
Pamela L. Graney^{1,2}, *Kristine Lai*^{1,2}, *Sarah Post*^{1,3}, *Ilana Brito*², *Jason Cyster*⁴, *Ankur Singh*^{1,2,5,6*}

1. Nancy E. and Peter C. Meinig School of Biomedical Engineering, Cornell University, Ithaca, NY
2. Sibley School of Mechanical Engineering, Cornell University, Ithaca, NY
3. Biological and Biomedical Sciences, Cornell University, Ithaca, NY
4. Howard Hughes Medical Institute and Department of Microbiology and Immunology, University of California, San Francisco, San Francisco, CA
5. George W. Woodruff School of Mechanical Engineering, Georgia Institute of Technology, Atlanta, GA
6. Wallace H. Coulter Department of Biomedical Engineering, Georgia Institute of Technology and Emory University School of Medicine, Atlanta, GA

*Corresponding author: ankur.singh@gatech.edu

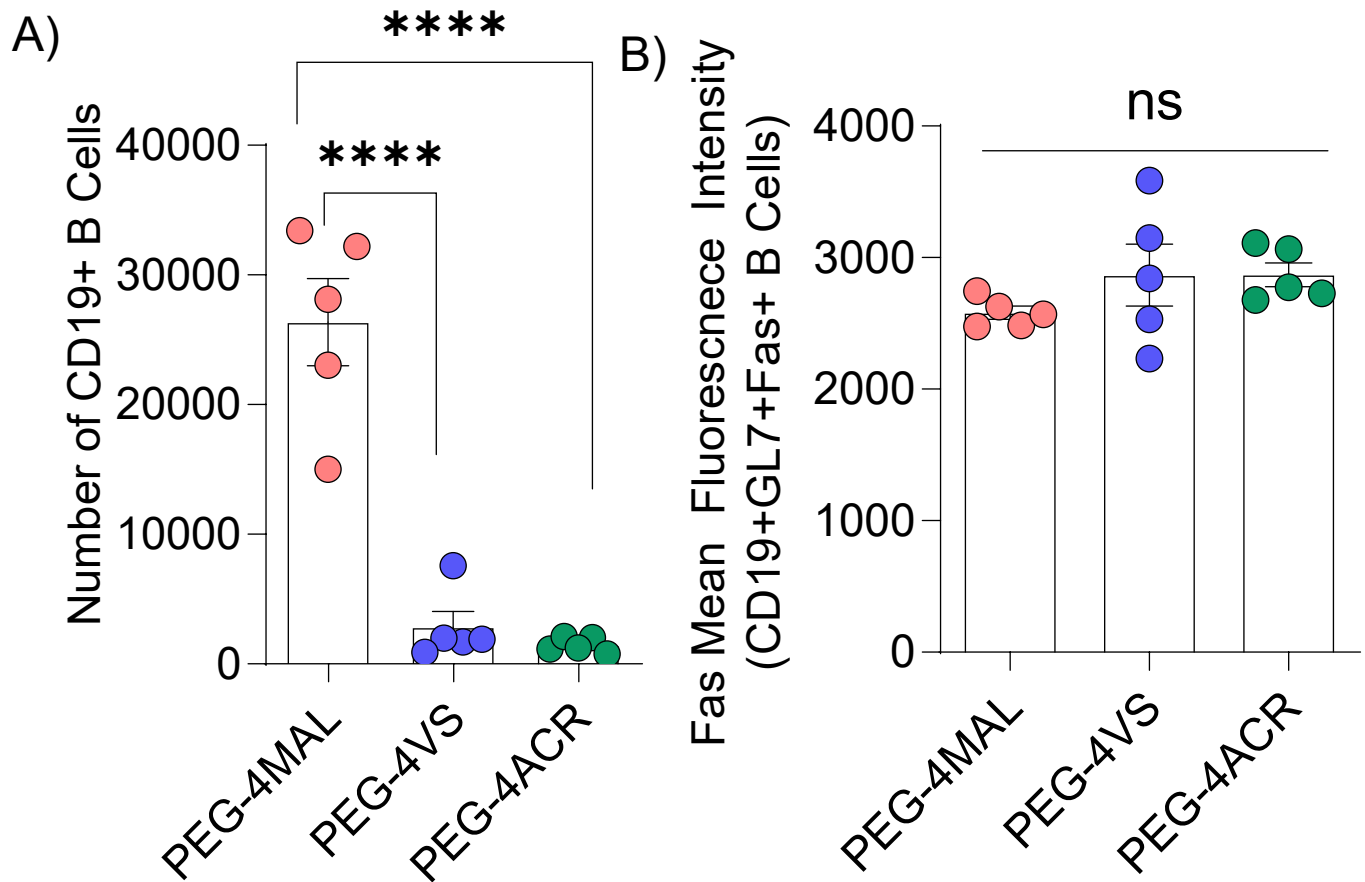
Keywords: hydrogel, B cell, bacteria, histone, EZH2, aging

Supplementary Figure S1, Related to Fig. 1

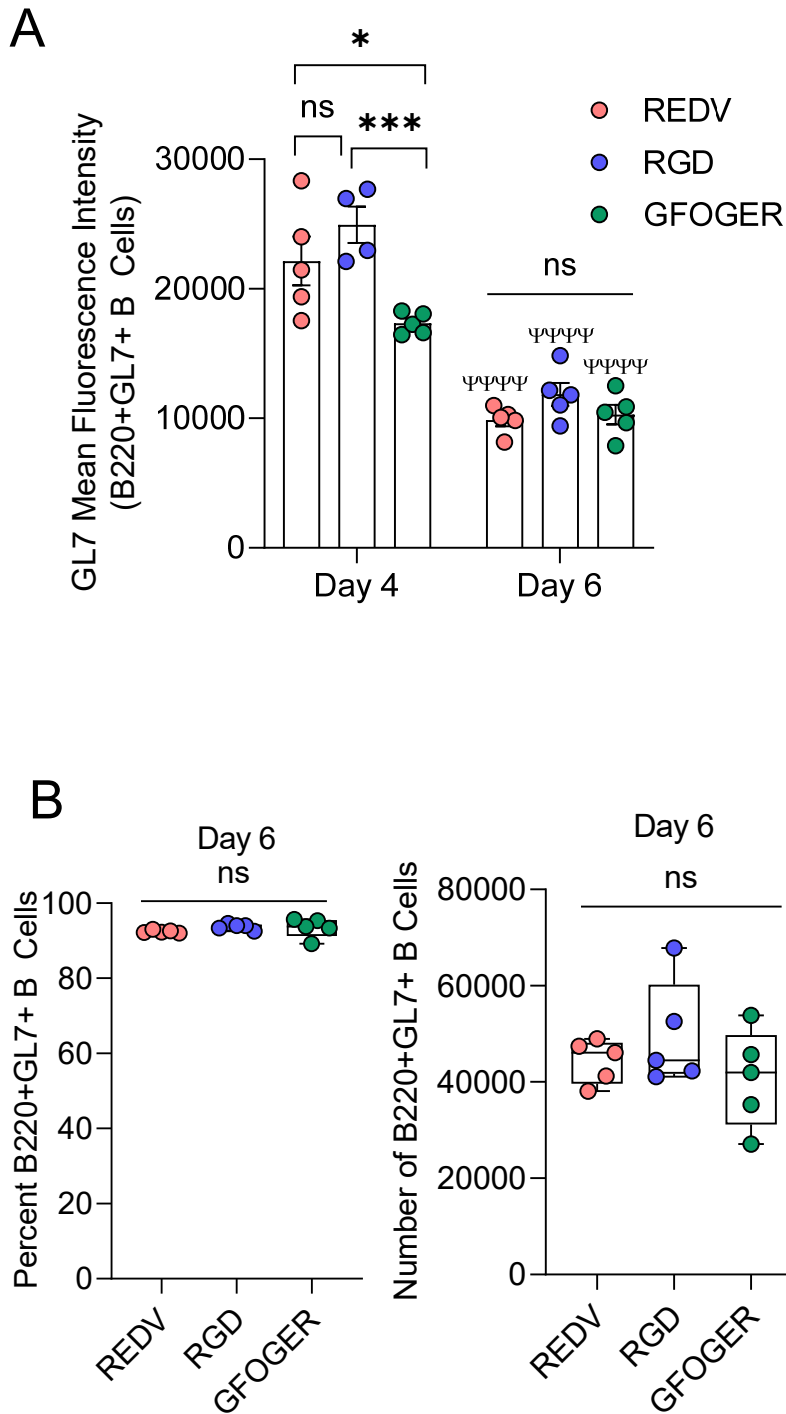


Supplementary Figure S1: Single-cell RNA sequencing of nine uniquely identified clusters of peripheral lymph node non-endothelial stromal cells reveals the expression of mRNA encoding for proteins that bind to integrins. Violin plots represent the log-normalized gene expression (unique molecular identifier counts) for each cell grouped by the cluster.

Supplementary Figure S2, Related to Fig 2

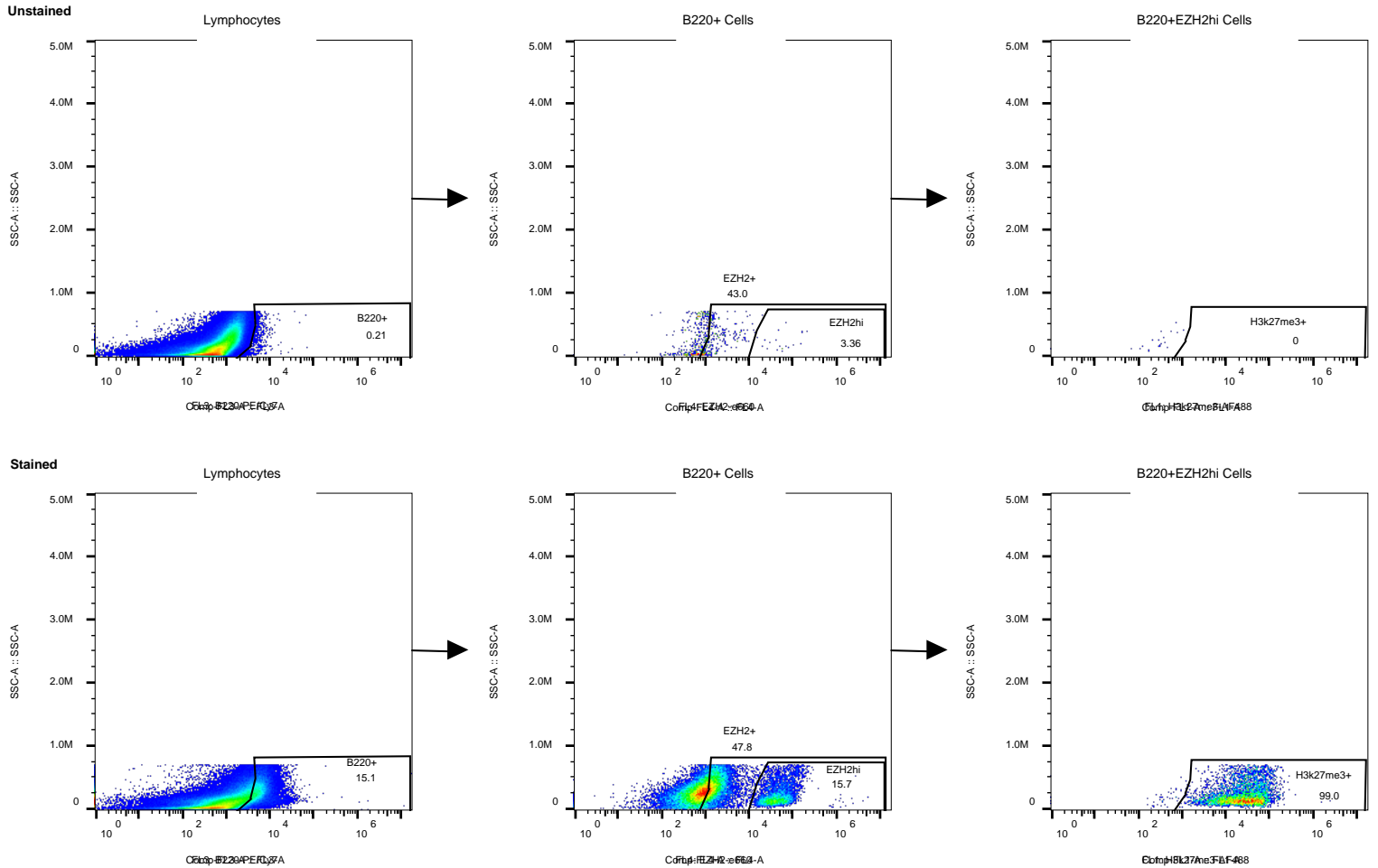


Supplementary Figure S2: Effect of four-arm PEG end-modification chemistry on (A) percent CD19+ B cells, and (B) Fas expression in CD19+GL7+ B cells. All data represent mean \pm S.E.M. and were analyzed via one-way ANOVA with Tukey's posthoc multiple comparisons test. **** $p < 0.0001$, ns=not significant; N=5.



Supplementary Figure S3: Kinetics of GL7 expression in germinal center B cells in response to integrin ligand presentation. A) Change in GL7 intensity over time in response to integrin-binding peptides. B) Effect of the integrin-binding peptide on germinal center B cells (B220+GL7+) on day 6 of organoid culture. Data represent mean \pm S.E.M. Significance determined via one-way (A) or two-way (B) ANOVA with Tukey's posthoc multiple comparisons test; N=5. * $p < 0.05$, *** $p < 0.001$, ns=not significant; $\Psi\Psi\Psi\Psi p < 0.0001$ relative to day 4.

Supplementary Figure S4, Related to Figure 4



Supplementary Figure S4: Gating of EZH2+ and EZH2hi H3k27me3+ B cells isolated from ex vivo germinal center organoids. Representative gating for unstained (top) and stained (bottom) samples showing B220+ B cells, B220+ EZH2hi B cells, and B220+ EZH2hi H3k27me3+ B cells. The unstained sample is a pooled sample of cells obtained by collecting 10 μ L of cell suspension from each experimental sample. A small number of cells in the unstained samples that appear within the B220+EZH2hi gate could be due to minor autofluorescence of some of the cells within the sample.