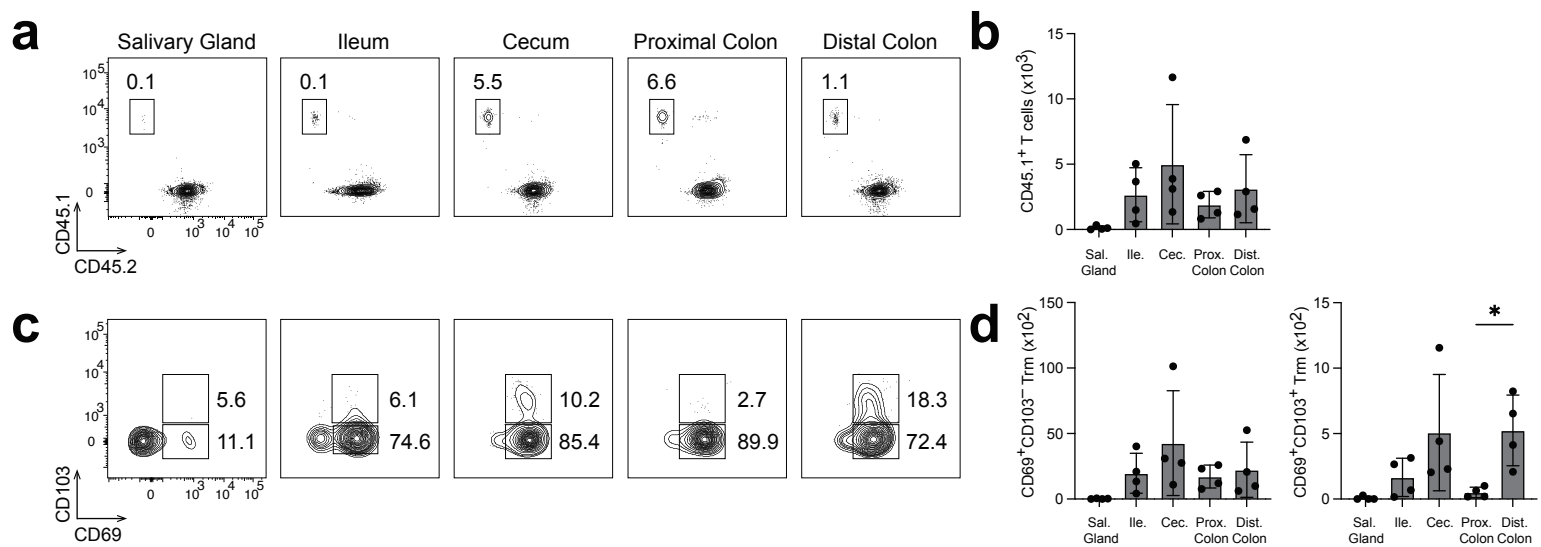
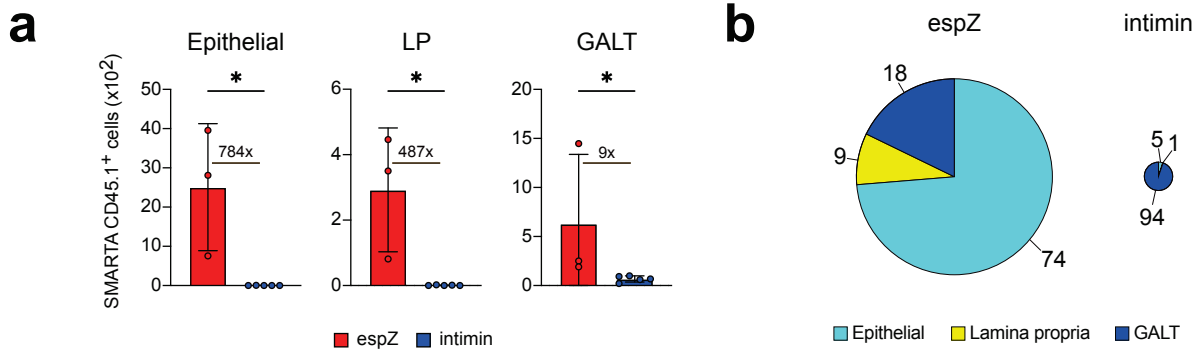


Barrier immune memory is programmed by intestinal epithelial cell presentation of cytosol-delivered bacterial antigens

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Supplementary Figure 1. Regional distribution and tissue-residency phenotype of SMARTA CD45.1⁺ T cells at day 42 post-infection. a, Representative flow cytometry plots showing recovery of CD45.1⁺ SMARTA T cells from salivary glands (pooled sublingual and submandibular glands), ileum, cecum, proximal colon, and distal colon at day 42 post-*C. rodentium* espZ FL infection. Cells were gated on live CD4⁺TCRβ⁺ lymphocytes and subdivided into CD45.1⁺ (transferred SMARTA) and CD45.2⁺ (host) populations. b, Quantification of total CD45.1⁺ T cell numbers recovered from each tissue. c, Representative flow cytometry plots showing CD69 and CD103 expression on CD45.1⁺ SMARTA T cells from the tissues in a. CD69⁺CD103⁺ double-positive Trm are most abundant in the cecum and distal colon, consistent with sites of *C. rodentium* colonization, whereas CD69⁺ T cells in the proximal colon predominantly lack CD103 expression. d, Quantification of CD69⁺CD103⁻ (left) and CD69⁺CD103⁺ (right) Trm numbers across tissues. Data are mean ± s.d. with individual mice shown; n = 4 mice per tissue for both b and d. Data are from one experiment representative of 2 independent experiments. One-way ANOVA with Tukey's post hoc test; **P* < 0.05.



Supplementary Figure 2. Microanatomical distribution of SMARTA T cells in espZ-gp66 and intimin-gp66 *Cr*-infected mice at day 42 post-infection. LSFM was performed on mid-distal colons from mice infected with espZ-gp66 or intimin-gp66 *C. rodentium* at day 42 post-infection. a, Quantification of CD45.1⁺ SMARTA T cells ($\times 10^2$) identified in epithelial, lamina propria (LP), and gut-associated lymphoid tissue (GALT) compartments. Data are mean $\pm$ s.d. with individual mice shown; n = 3 espZ-gp66 and 5 intimin-gp66 mice. Data are from one experiment representative of 2 independent experiments. Unpaired two-sided Student's t-test; * $P < 0.05$. b, Pie charts depicting the percentage of CD45.1⁺ SMARTA T cells distributed across epithelial, LP, and GALT compartments. Pie chart areas are scaled to total CD45.1⁺ SMARTA T cell counts per mouse.