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Supplementary information

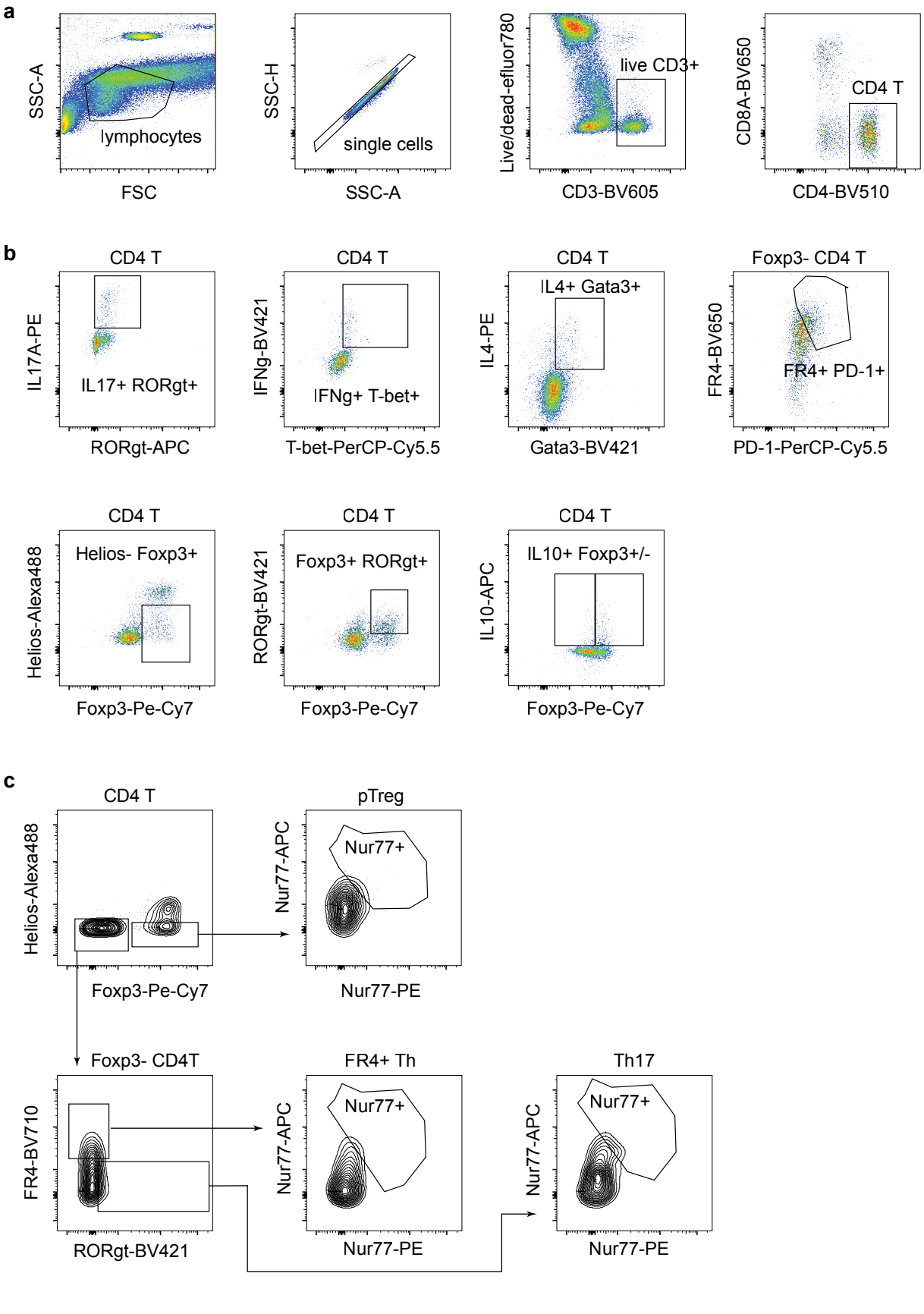
Mapping the T cell repertoire to a complex gut bacterial community

In the format provided by the authors and unedited

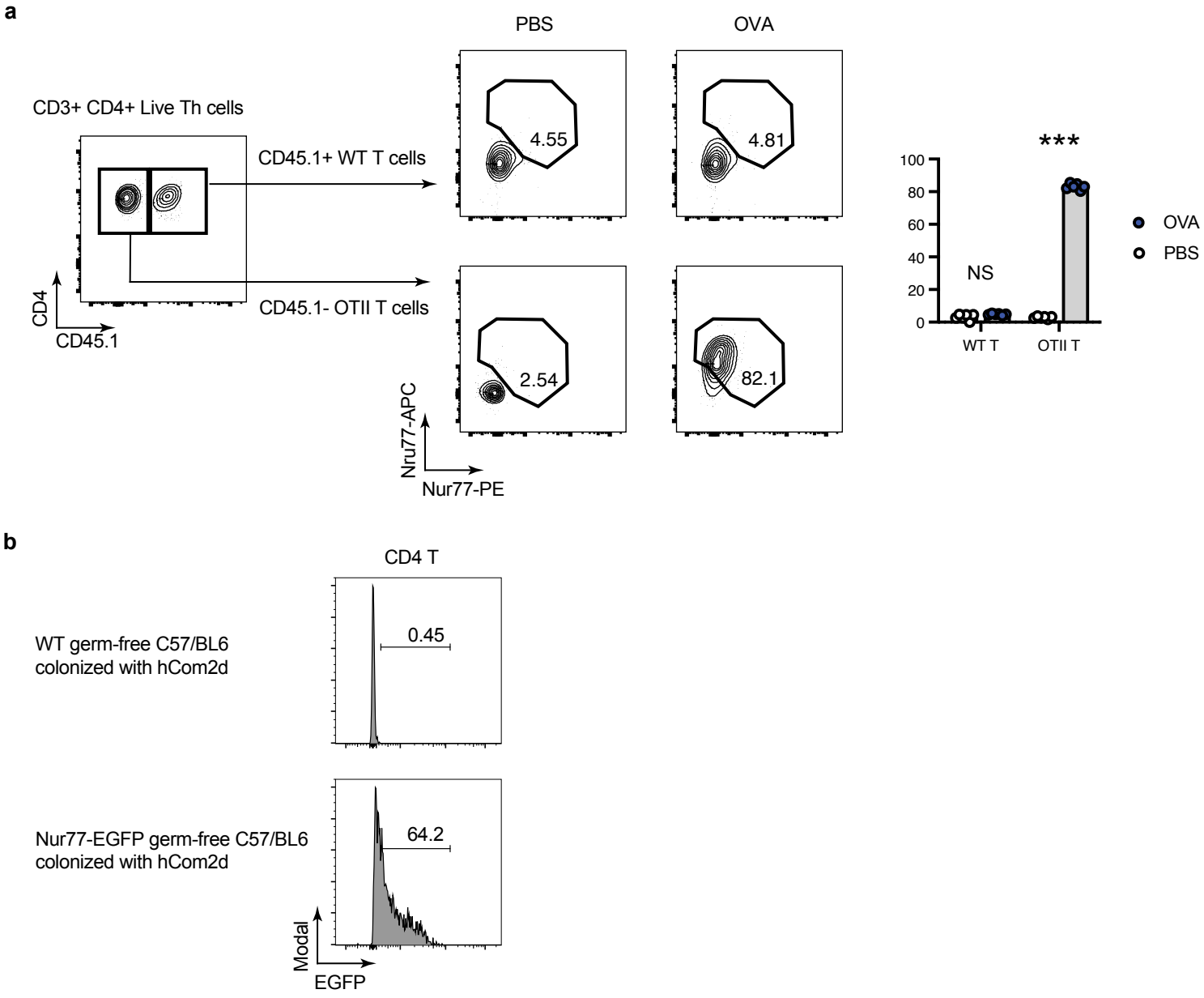
Supplementary Figure 1: Gating strategy for T cell in flow cytometry analysis. a-c, Representative gating strategy for (**a**) CD4+ T cells, (**b**) subsets of CD4+ T cells, or (**c**) the mixed lymphocyte assay.

Supplementary Figure 2: Establishment of the mixed lymphocyte assay. a, Endogenous Nur77 expression sensitively detects antigen-stimulated T cells in the mixed lymphocyte assay. We mixed an equal number of CD45.1- OTII T cells and CD45.1+ wild-type T cells, and then cocultured the T cell pool with OVA or PBS using dendritic cells for antigen presentation. TCR stimulation was monitored by using two antibodies specific to Nur77. *n* = 6 mice per group from one experiment. **b**, Nur77-EGFP mice are not suitable for the mixed lymphocyte assay. We generated germ-free Nur77-EGFP mice by Caesarean rederivation. We colonized germ-free wild-type or Nur77-EGFP mice with hCom2d and analyzed freshly isolated CD4+ T cells. >64% of the CD4+ T cells were already EGFP-positive before restimulation by a bacterial strain. We concluded that with this level of background, it would be too difficult to measure restimulated T cells in an ex vivo coculture experiment. **c**, The mixed lymphocytes assay detects MHCII-dependent colonist-specific T cell responses. We isolated T cells from SFB+ Taconic or SFB- Jackson mice. T cells were cocultured with PBS, SFB or *Staphylococcus epidermidis* (a non-community strain) using MHCII+ DCs, MHCII- DCs or a no DC control. Nur77 levels were monitored to measure TCR stimulation in Th17 cells. *n* = 5, 3, 3, 3 mice per group from one experiment. p-values were calculated using a two-sided t-test by comparison to PBS treatment as a negative control. ***p = 0.005. NS > 0.05. Data shown are mean ± standard deviation.

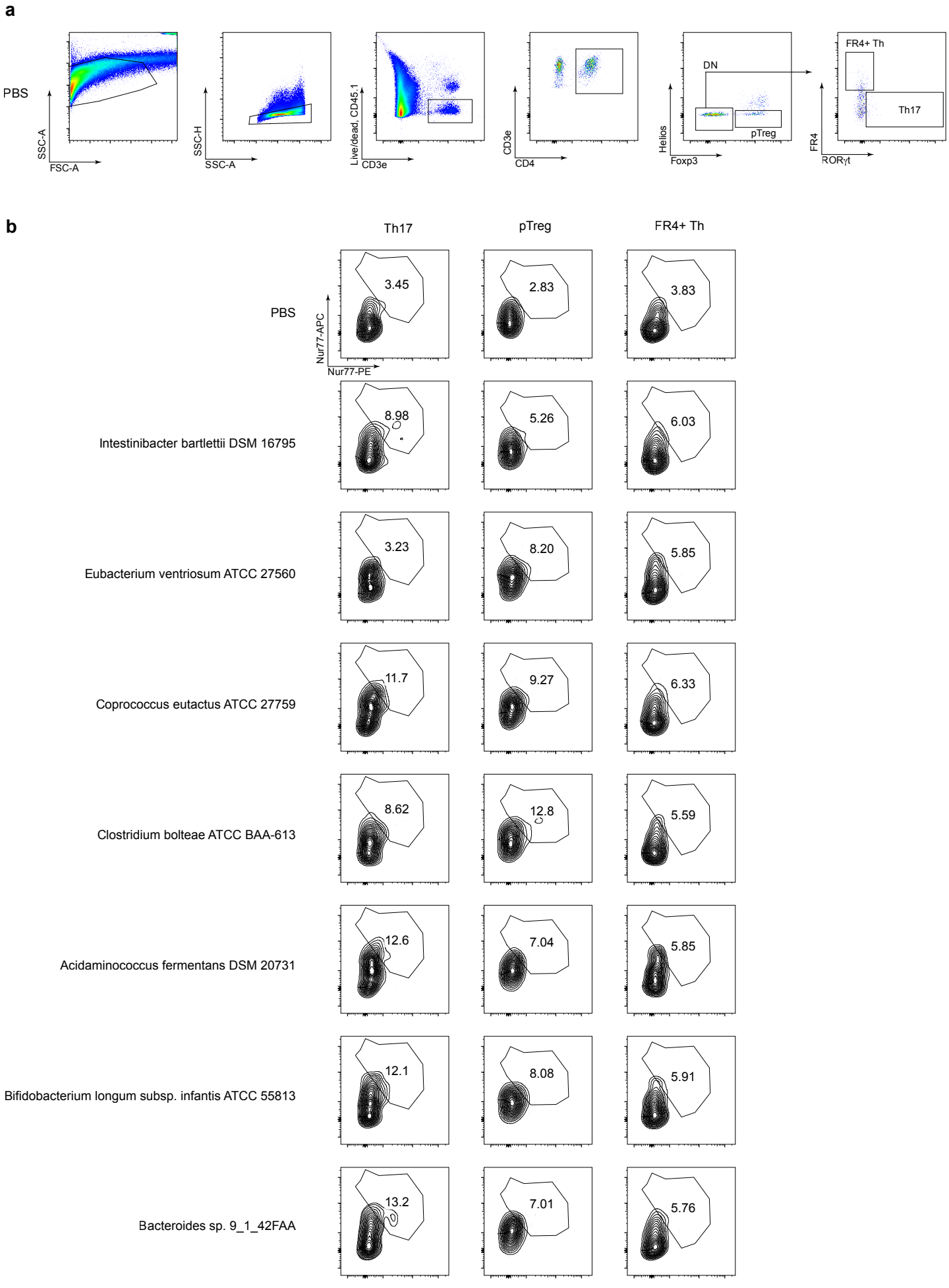
Supplementary Figure 3: Representative flow cytometry images in Fig. 2. a, Immune cells from hCom1d-colonized CD45.2$^+$ mice were pooled and co-cultured with each strain in hCom1d, along with CD45.1$^+$ dendritic cells purified with magnetic beads. After 4 hours, T cells were fixed, stained for Nur77 expression, and analyzed by flow cytometry. A representative gating strategy for Th cell subsets in the PBS sample is shown. **b,** Representative gating strategy for Nur77 staining is shown for multiple samples.



Supplementary Figure 1



Supplementary Figure 2



Supplementary Figure 3