

Supplementary Figure 1. Collection method for fetal intestinal sample bank. Uninterrupted small intestine sections were divided into equal thirds and internal contents (meconium) cryopreserved for either genomic DNA extraction (in RNAlater) or bacterial isolation (in 50% v/v glycerol). Remaining intestinal tissue from all three sections was pooled and washed with EDTA to recover epithelium (preserved in RNAlater for subsequent RNAseq analysis) and enzymatically digested to isolate lamina propria cells (for immediate analysis by flow cytometry). Internal kidney punch biopsies and surgical environmental swabs served as procedural or environmental controls. Extraction buffer, pre-moistened swabs, and pre-moistened swabs held in the surgical room air for 30 seconds served as technical negative controls.



Supplementary Figure 2. Gating strategy for T cell profile assessment. Gating strategy for identification of PLZF⁺ CD161⁺ CD4⁺ $\alpha\beta$ T cells. Cells were gated on 1- lymphocytes, 2- singlets, 3- live cells expressing TCR β , 4- CD4 expressing cells that were excluded of the dominant invariant chain expressed on mucosa-associated invariant T cells, V α 7.2. 5- PLZF⁺ CD161⁺ or PLZF⁺ CD161⁻ cells. All gating was set on mesenteric lymph node (MLN) internal controls and when available, splenic internal controls (SPL).

Supplementary Figure 3



Supplementary Figure 3. Gating strategy for antigen presenting cell phenotypes. Gating strategy for identification of fetal splenic antigen presenting cells. Cells were gated on 1-lymphocytes, 2-singlets, 3-live, 4-lineage (CD3, CD56, CD20, CD19)⁻ and CD45⁺, 5-HLA-DR⁺ cells.