**Isolation of Immune Cells from Intestinal Lamina Propria/Spleen and Flow Cytometry**

For isolation of intestinal lamina propria cells, the small and large intestines were dissected and placed immediately into ice cold PBS. After mesenteric fat and Peyer’s patches (small intestine) were removed, the intestines were longitudinally opened and luminal contents were washed out with cold PBS. Tissue pieces were washed for 10 min. in 1mM dithiothreitol (DTT)/PBS at room temperature on a rocker to remove mucus, followed by a wash for 25 min. in 10mM EDTA/30mM HEPES/PBS at 37C on a platform shaker (180 rpm) to remove epithelium. After a 2 min. wash in complete RPMI, tissue was digested in a 6-well plate for 1.5hrs. in complete RPMI with 150U/mL (small intestine) or 300U/mL (large intestine) collagenase VIII (Sigma) and 150ug/mL DNase (Sigma) in a cell culture incubator (5% CO2). Tissue digests were passed through a 100μm cell strainer and separated by centrifugation (1200g for 20 min.) using a 40/80% percoll gradient. Immune cells were collected at the 40/80% interface. For the spleen, the tissue was passed through a 100μm cell strainer and incubated in red cell lysis buffer (Sigma) for 8 min. at room temperature. Both spleen and intestine immune cells were washed with 0.5% BSA/PBS before staining and fixation (eBioscience Foxp3 / Transcription Factor Staining Buffer Set).

For flow cytometry staining, CD16/32 antibody (eBioscience) was used to block the non-specific binding to Fc receptors before surface staining. Immune cells isolated from intestinal lamina propria were stained with antibodies against the following makers: CD103 (PerCP-efluor710), CD11b (SuperBright645), CD11c (FITC), CD19 (FITC), CD3e (PE), CD4 (APC), CD45.2 (BV421), CD64 (APC-Cy7), CD8a (APC-e780), CSF1R (PE), Ly6C (APC), MHCII I-A/I-E (PE or PerCP-efluor710), TCRβ (PerCP-Cy5.5). For some panels, a lineage marker mix (Lin) contained TCRβ, B220, Ly6G and Siglec-F (PE-Cy7). Live and dead cells were discriminated by Live/Dead Fixable Aqua Dead Cell Stain Kit (Invitrogen).