Collected spleen, thymus, and ileocecal lymph node tissues (~2 g) were transported back to the lab in 10 mL tissue buffer (2 mM EDTA [Invitrogen AM9260G], 2mM L-glutamine [Gibco 25-030], 0.5% bovine serum albumin [BSA; Sigma-Aldrich A9418] in Hank's Balanced Salt Solution [HBSS; Gibco 14175]) in a gentleMACS C Tube (Miltenyi 130-093-237). In the lab, tissues were mechanically homogenized using a gentleMACS Octo Dissociator (Miltenyi 130-095-937) with the programed spleen – cells protocol. Cell suspensions were passed through a 100-micron nylon mesh cell strainer, and strainers were washed with 10 mL tissue buffer. Cells were pelleted by centrifugation at 300 xg for 5 min room temperature (RT). Cell pellets were resuspended in residual volume (<1 mL) and then incubated with 20 mL ACK lysis buffer (ThermoFisher A1049201) for 3 min to lyse red blood cells and centrifuged again at 300 xg for 5 min RT. Spleen cells were treated with ACK lysis buffer a second time. Cells were resuspended in 10 mL tissue buffer and passed through a 70-micron nylon mesh cell strainer. Cells were centrifuged 300 xg for 5 min RT and resuspended in tissue buffer.

Bone marrow cells were isolated by flushing ~30 mL tissue buffer through two rib bones using an 18 gauge needle with 10 mL syringe and collecting cells into a 50 mL conical as flushed out. Recovered cells were pelleted by centrifuging 300 xg for 5 min RT. Cells were then passed through cell strainers and treated with ACK lysis buffer as described above.

Cell quantity and viability was assessed using the Muse Count & Viability Assay Kit (Luminex MCH100102) with a Muse Cell Analyzer (Luminex 0500-3115). To further enrich for live cells, cell suspensions were processed through a Dead Cell Removal Kit (Miltenyi 130-090-101) according to manufacturer's protocol with a starting quantity of $5x10^7$ total cells as previously described [14]. Recovered cells were cryopreserved according to the 10X Genomics Sample Preparation Demonstrated Protocol in multiple aliquots of $1x10^7$ cells per vial.