Supplementary file 4







Supplementary file 4. Flow cytometry gating strategy.

(A) Sequential gating strategy for CD44- and CD25-expressing double-negative (DN) cells from *Cic*^{FLAG/FLAG} mice.

(B) Sequential gating strategy used for CD24^{hi}TCR β^{lo} immature single-positive (ISP) cells and T cells expressing CD69 and TCR β .

- (C) Sequential gating strategy for DP, CD4 SP, CD8 SP, and DN cells expressing CD44 and CD25.
- (D) Sequential gating strategy for T cells from TCR transgenic mice.
- (E) Sequential gating strategy for Nur77 expression analysis in double-positive (DP) cells.
- (F) Sequential gating strategy for CD5 expression analysis in DP, CD4 SP, and CD8 SP (TCR β^{hi}) cells.
- (G) Sequential gating strategy for CD69⁺ and CD69⁻ DP cells.
- (H) Sequential gating strategy for analysis of T cells from mixed bone marrow (BM) chimeric mice.
- (I) Sequential gating strategy for GFP⁺ DP cells.
- (J) Sequential gating strategy for $\gamma\delta T$ cells expressing CD24, CD44, T-bet, and ROR γt .
- (K) Sequential gating strategy for splenic T cells expressing CD44 and CD62L.
- (L) Sequential gating strategy for CD73⁺CD24¹⁰CD4⁺ T cells.

(M) Sequential gating strategy for CD4⁺ non-Treg and Treg Vβ2 chain. Other Vβ chains were gated with the same gating strategy.