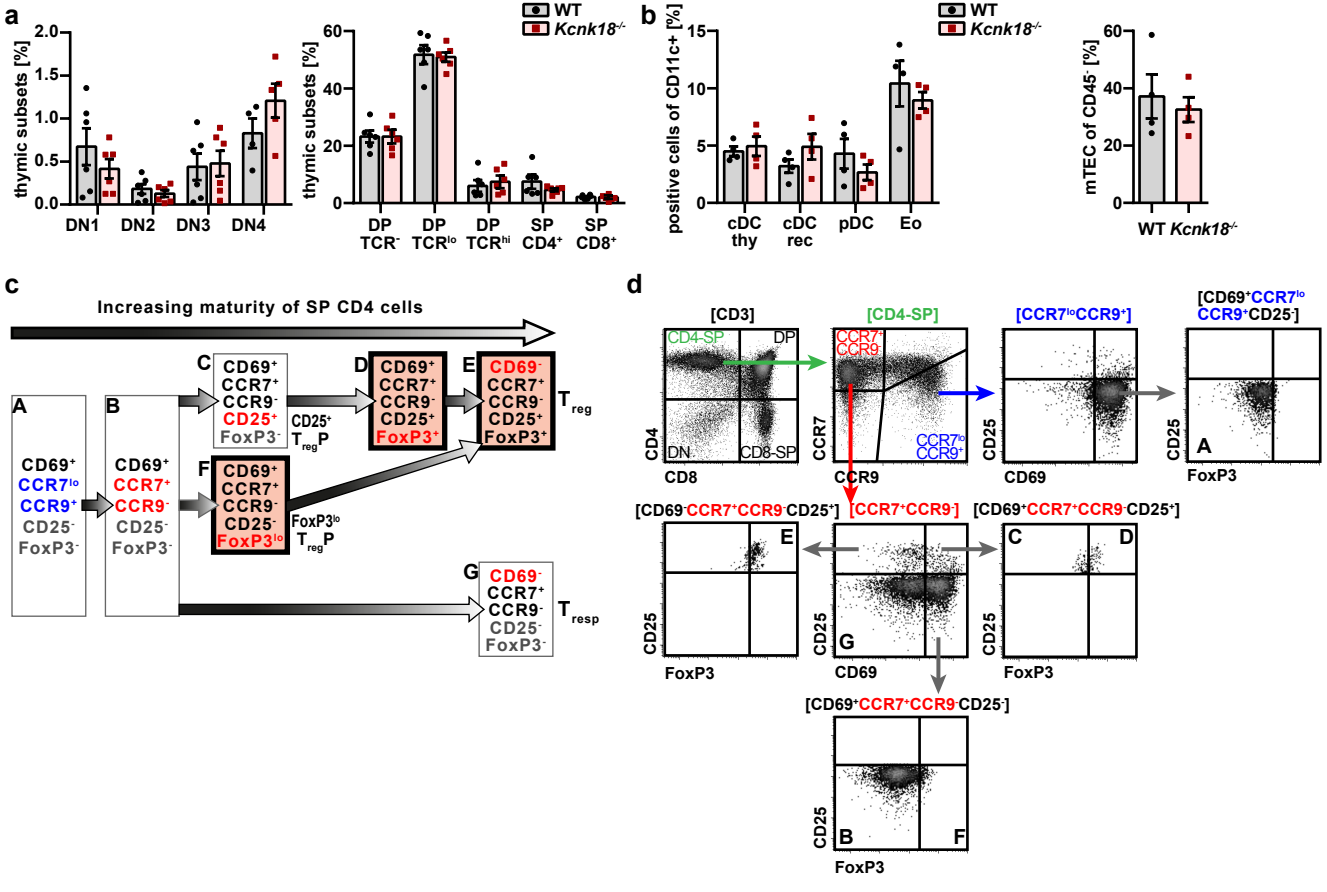

Supplementary figure 3 Major thymocyte and thymic APC subsets are not altered in *Kcnk18*$^{-/-}$ mice. a Flow cytometry analysis of thymocyte subsets (DN = double negative ($CD3^+CD4^-CD8^-$) , stage 1: $CD44^+CD25^-$, stage 2: $CD44^+CD25^+$, stage 3: $CD44^-CD25^+$, stage 4 : $CD44^+CD25^-$, DP = double positive ($CD3^+CD4^+CD8^+$), $CD4^+CD8^+TCR^-$, $CD4^+CD8^+TCR^{low}$, $CD4^+CD8^+TCR^{high}$, SP = single positive: SP4 – $CD4^+CD8^-TCR^{high}$, SP8 – $CD4^-CD8^+TCR^{high}$, in the thymus isolated from WT and *Kcnk18*$^{-/-}$ mice. (n = 6) **b** Frequencies of different antigen-presenting cell (APC) subsets (thymic resident cDCthy = $CD11c^{hi}CD11b^-CD8a^{hi}SIRP\alpha^-$, recirculating cDCrec = $CD11c^{hi}CD11b^+CD8a^{lo}SIRP\alpha^+$, plasmacytoid pDCs = $CD11c^{int}B220^+$, Eo = $CD11c^{int}$, $CD11b^+$, $SIRP\alpha^+$, mTEC = $CD45^-EpCAM^+BP\text{-}1^{lo}$) in the thymus analysed by flow cytometry. (n= 4) **c** Schematic overview of different tT_{reg} developmental stages in the thymus identified by indicated marker combinations. Highlighted are developmental stages with reduced cell numbers in *Kcnk18*$^{-/-}$ compared to WT mice. **d** Gating strategy for tT_{reg} developmental stages shown in c, quadrants A-G correspond to the respective subsets in c. Data are represented as mean ± SEM. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.