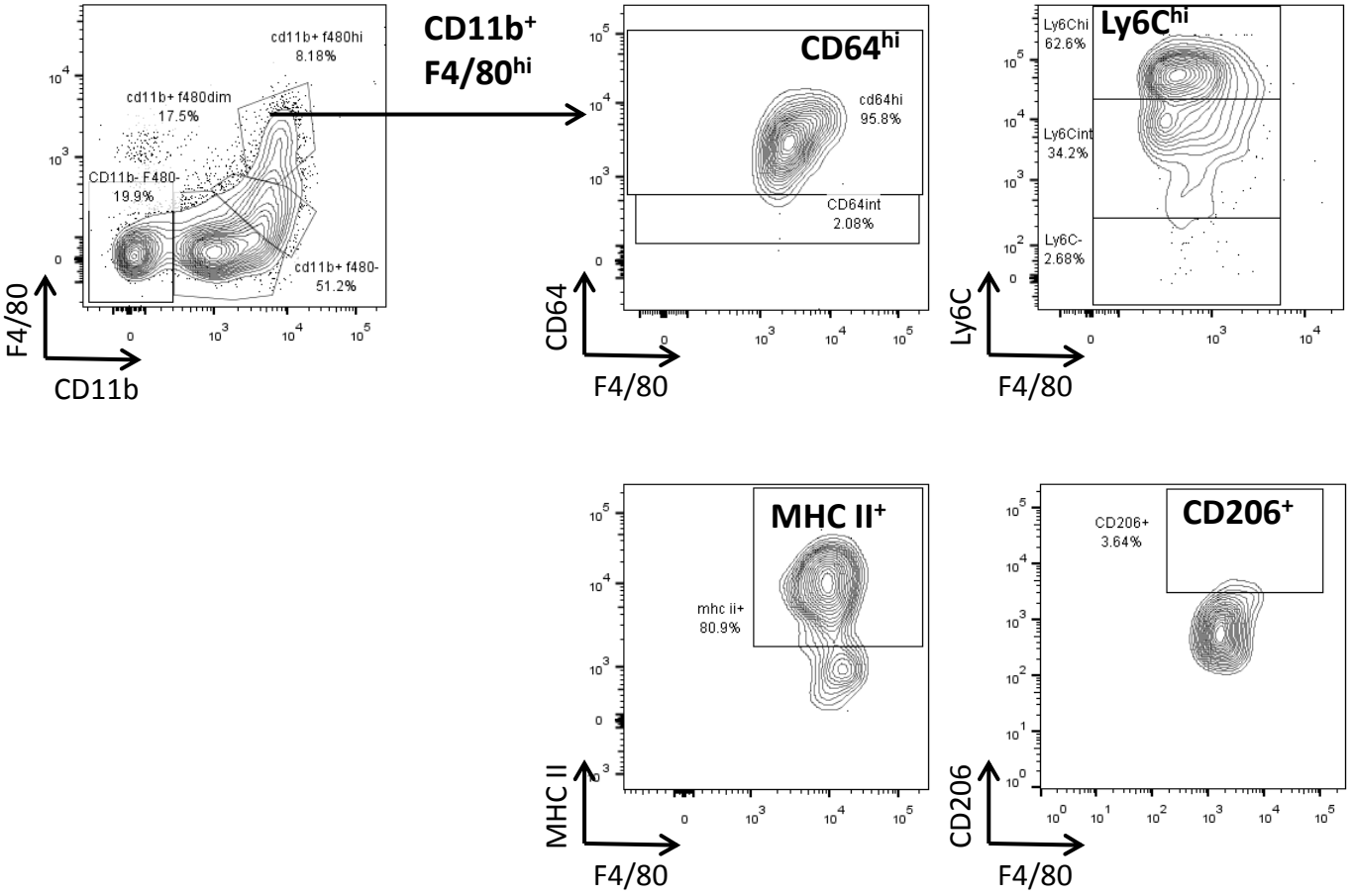
FACS gating strategy and representative plots

Macrophages WT mBSA, gated on FMOs

Day 2

CD45^{+} live

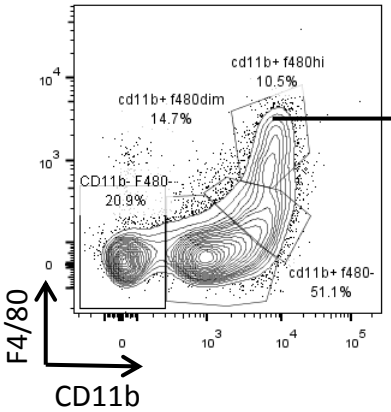


Gating strategy used to identify macrophage populations in the inflamed knee.
Images show representative plots of mBSA challenged joints collected at day two of AIA. A viability dye was used to exclude dead cells from the analysis. All samples were first gated on haematopoietic CD45^{+} live cells. Gates were set based on FMO (fluorescence minus one) controls excluding any background staining or autofluorescence. Macrophages were defined as CD11b^{+} F4/80^{hi} and then analysed for expression of CD64, Ly6C, MHC II and CD206.

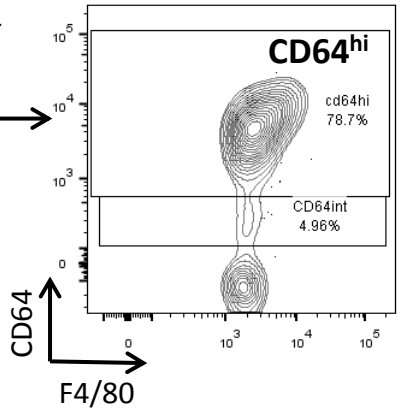
Macrophages IRF5 KO mBSA, gated on FMOs

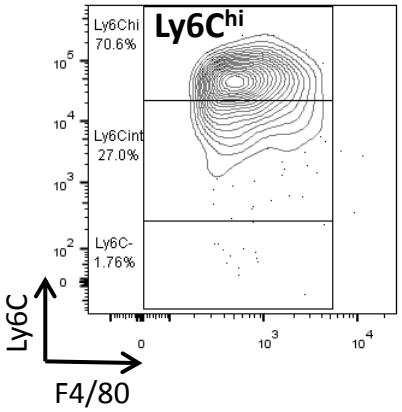
Day 2

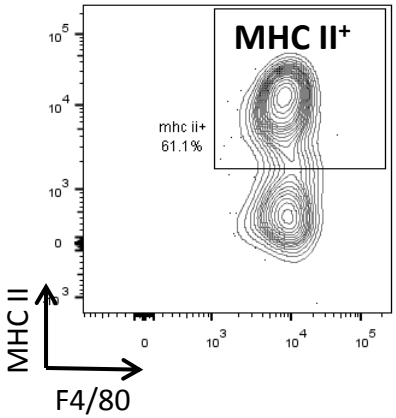
CD45^{+} live

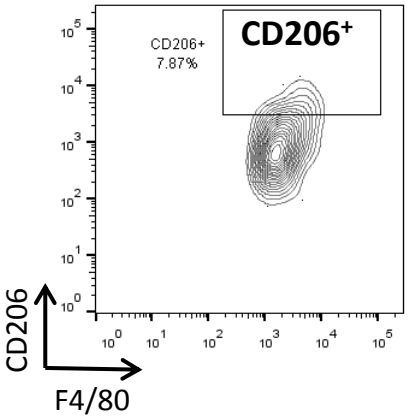


CD11b^{+} F4/80^{hi}





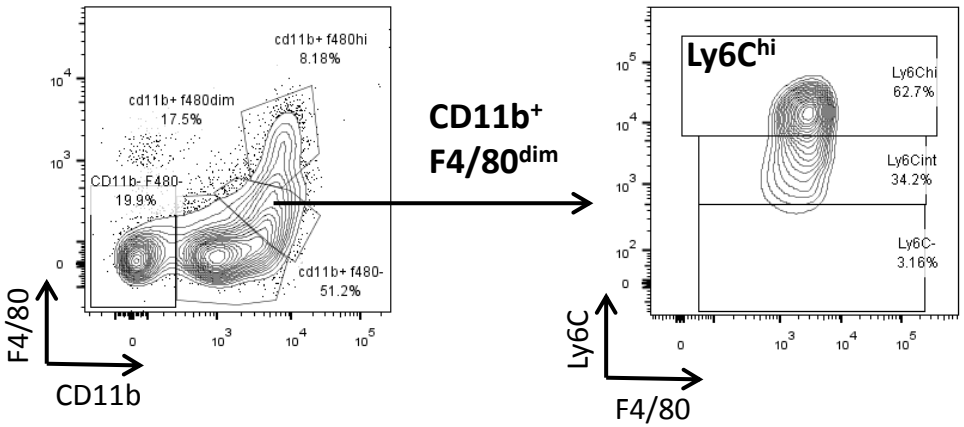




Monocytes WT mBSA, gated on FMOs

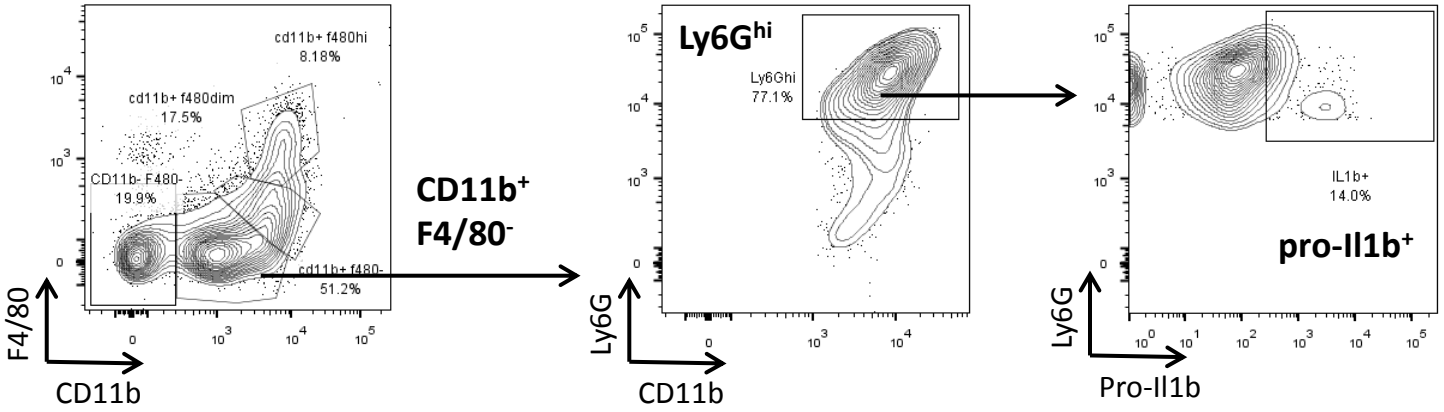
Day 2

CD45^{+} live



Neutrophils WT mBSA, gated on FMOs

CD45^{+} live



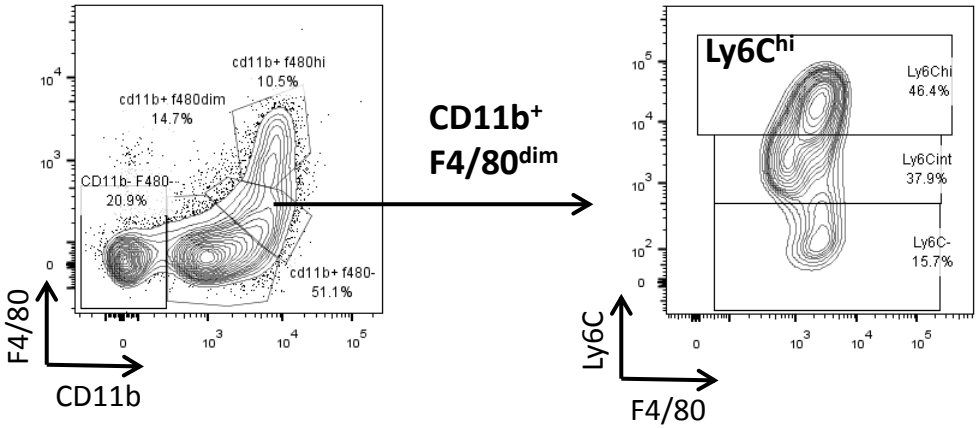
Gating strategy applied to detect monocytes and neutrophils in the challenged knee joint.

Plots are representative for mBSA knees collected at day two of AIA. Using a viability dye dead cells were excluded from the analysis. All samples were gated on haematopoietic CD45^{+} live cells before sub-gating. FMO controls were used to set all gates. Monocytes were defined as CD11b^{+} F4/80^{dim} and Ly6C expression was analysed. Neutrophils were generally defined as CD11b^{+} F4/80^{-} Ly6G^{hi} and pro-Il1beta allowed for identification of activated neutrophils.

Monocytes IRF5 KO mBSA, gated on FMOs

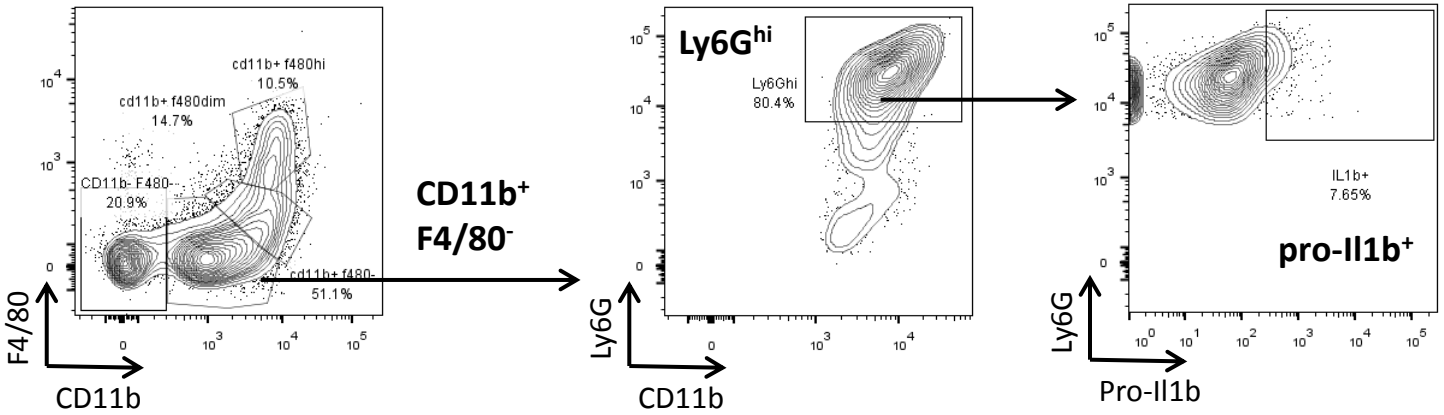
Day 2

CD45^{+} live



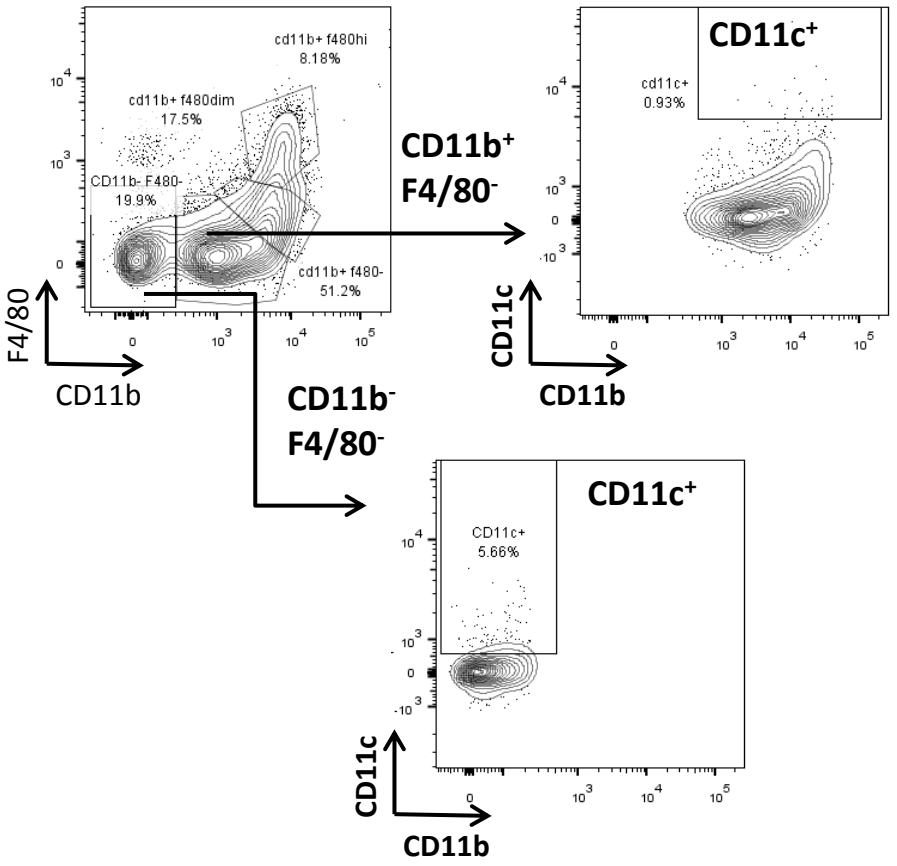
Neutrophils IRF5 KO mBSA, gated on FMOs

CD45^{+} live



DCs WT mBSA, gated on FMOs

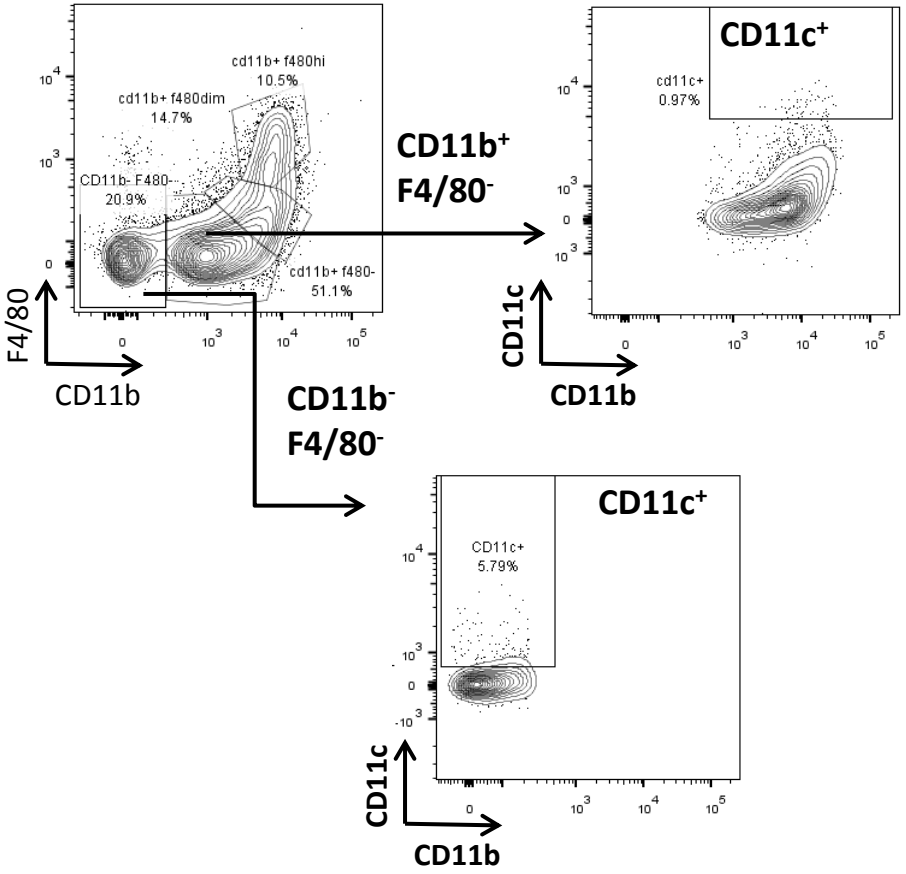
CD45$^+$ live



DCs IRF5 KO mBSA, gated on FMOs

Day 2

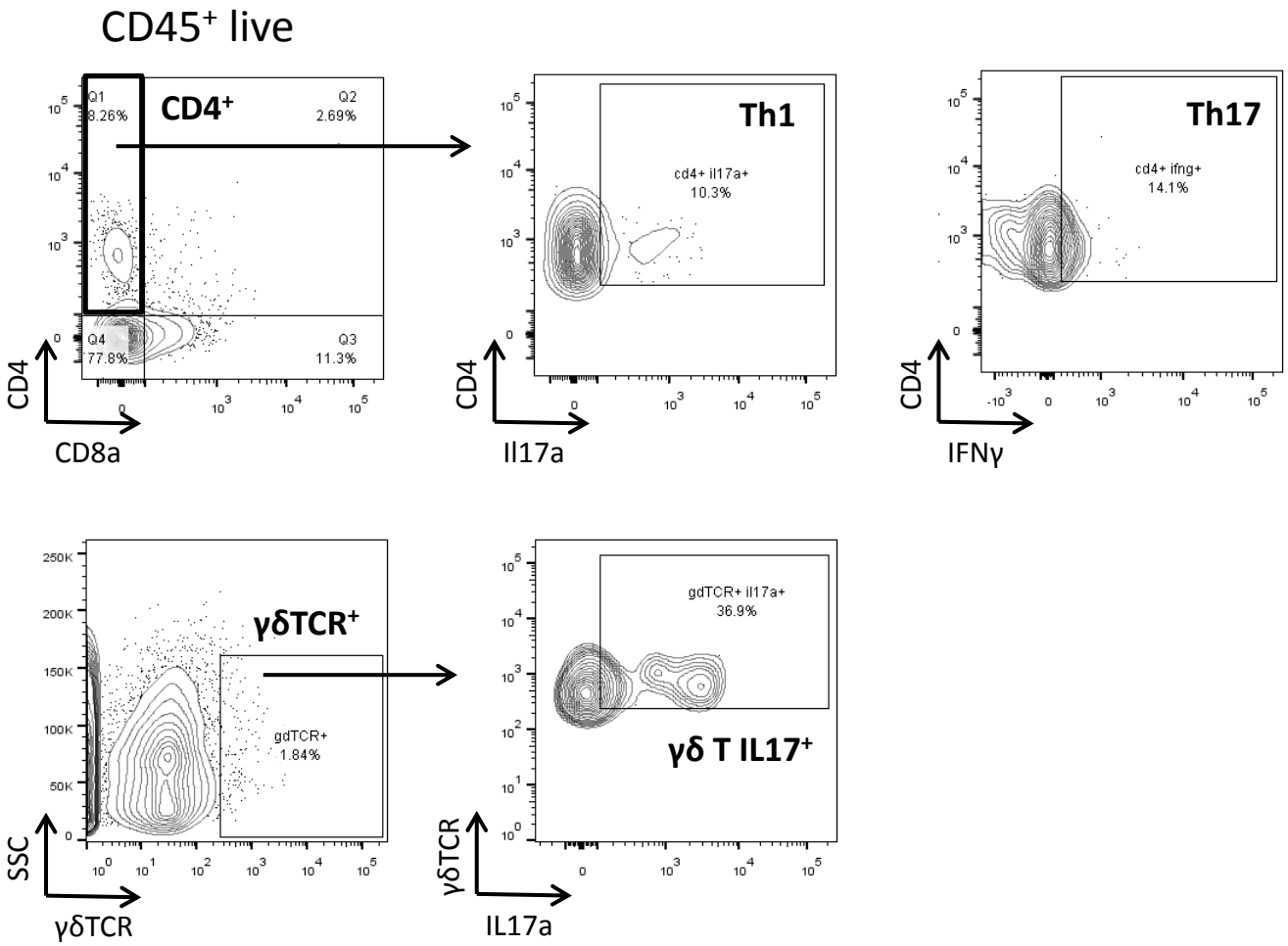
CD45$^+$ live



Representative FACS plots for the dendritic cell gating strategy.
Images shown are from mBSA knees collected after two days of AIA. Samples were gated on haematopoietic CD45$^+$ live cells prior to further gating. Gates were set based on background staining in FMO controls. Two DC populations were identified based on their CD11c expression in either the CD11b$^+$ F4/80$^-$ or CD11b$^-$ F4/80$^-$ gates.

T-cells WT mBSA, gated on FMOs, day 7

Day 7



Gating strategy used to identify T-cell subsets in the inflamed knee.
Images shown are representative plots of mBSA challenged joints collected at day seven of AIA. A viability dye was used to exclude dead cells from the analysis and all samples were first gated on haematopoietic CD45$^+$ live cells. Gates were set based on FMO controls. **A.** T-cells were gated as CD4$^+$ CD8a$^-$ and then further gated on IL17a and IFNγ expression to identify T-cell subsets. **B.** IL17a producing γδ T-cells were first gated on γδTCR expression and then IL17a staining.

T-cells IRF5 KO mBSA, gated on FMOs, day 7

Day 7