

Boyle, ST *et al.* The chemokine receptor CCR6 facilitates the onset of mammary neoplasia in the MMTV-PyMT mouse model via recruitment of tumor-promoting macrophages.

**Additional File 1:
Supplementary Figures S1-S5**

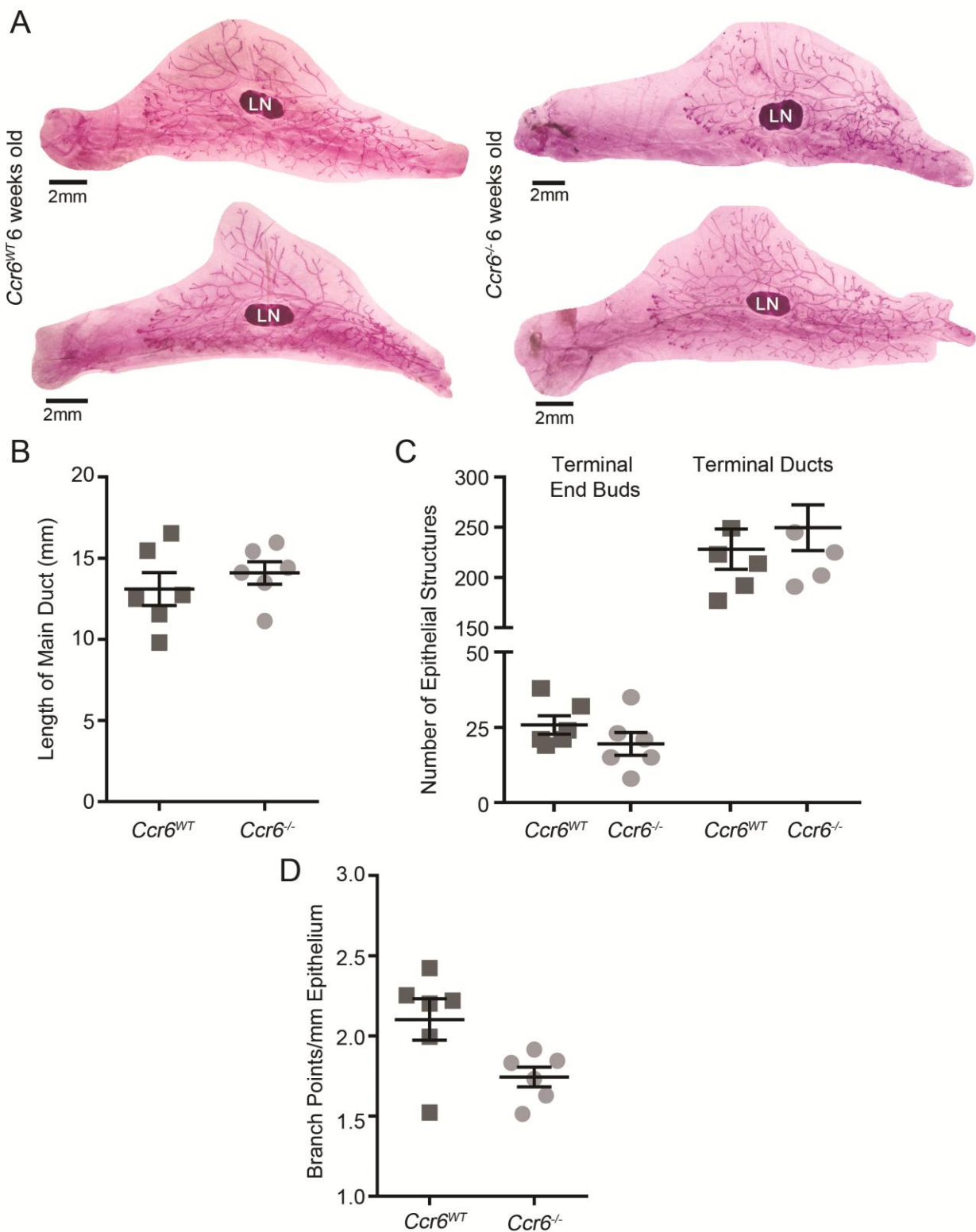


Figure S1: The role of CCR6 in normal mammary development

(a) Representative whole mount images of *Ccr6*^{WT} (n=6) and *Ccr6*^{-/-} (n=6) mice at 6 weeks of age showing development of mammary epithelium. LN=lymph node. (b-d) Quantitation of epithelial growth, by length of main duct (b), number of end structures (c) and branching (d).

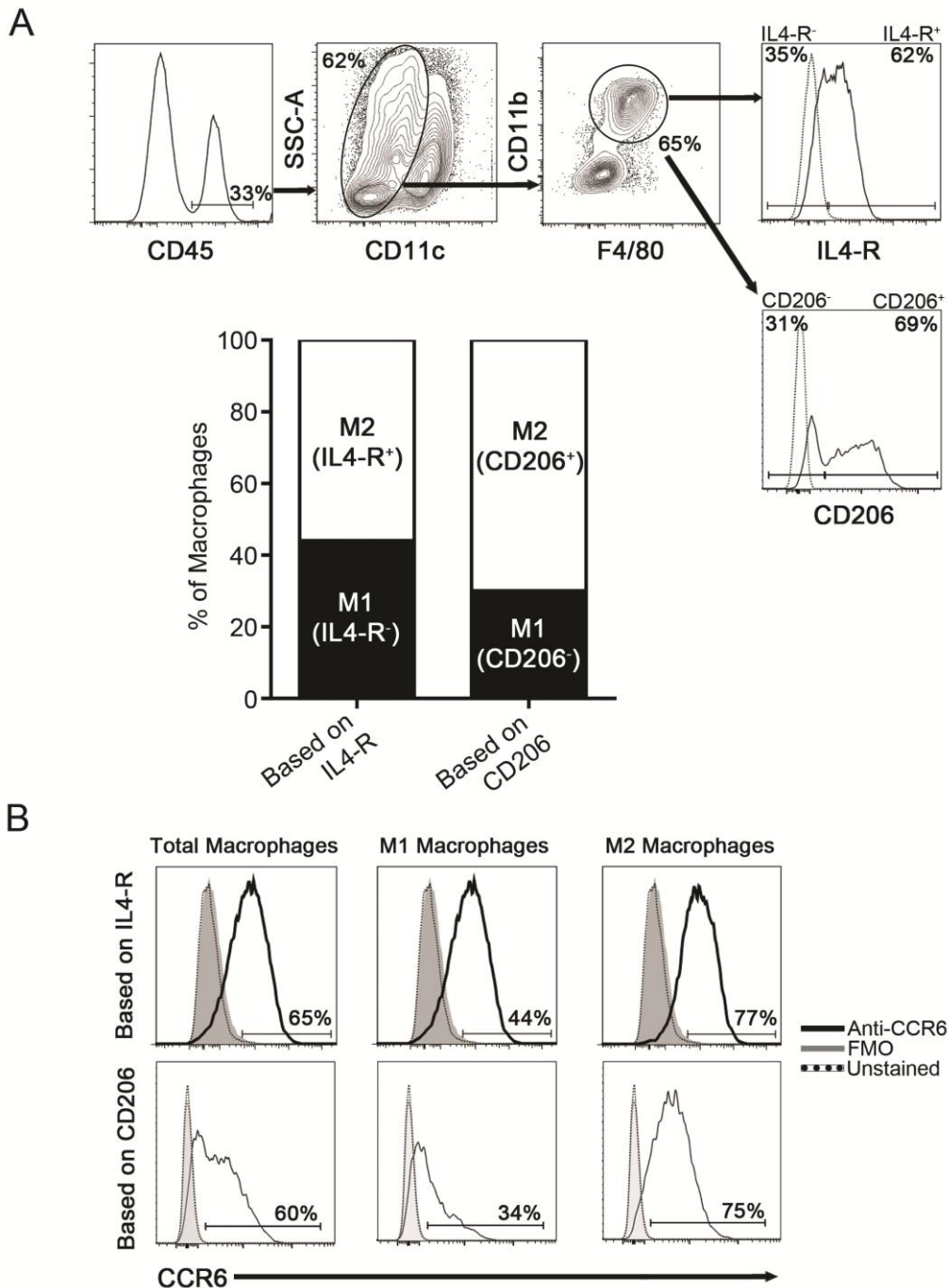


Figure S2: Analysis of infiltrating macrophages in MMTV-PyMT mammary tumors
(a) Analysis of M1 anti-tumor and M2 pro-tumor macrophages within the total macrophage population ($CD45^+CD11c^+CD11b^+F4/80^+$), based on IL4-R expression and CD206 expression as indicated, where M1 macrophages are classed as IL4-R or CD206-negative and M2 are IL4-R or CD206-positive. Dotted line=FMO control. M1 and M2 cell proportions (bottom) are presented as percentage of the total macrophage population. **(b)** Representative flow cytometry plots of CCR6 expression within the total, M1 and M2 macrophage populations as based on IL4-R and CD206 (gated as in (a)). **(a-b)** Representative results from 3 independent experiments.

Dendritic Cells: CD45⁺CD11b⁺MHCII⁺CD11c⁺

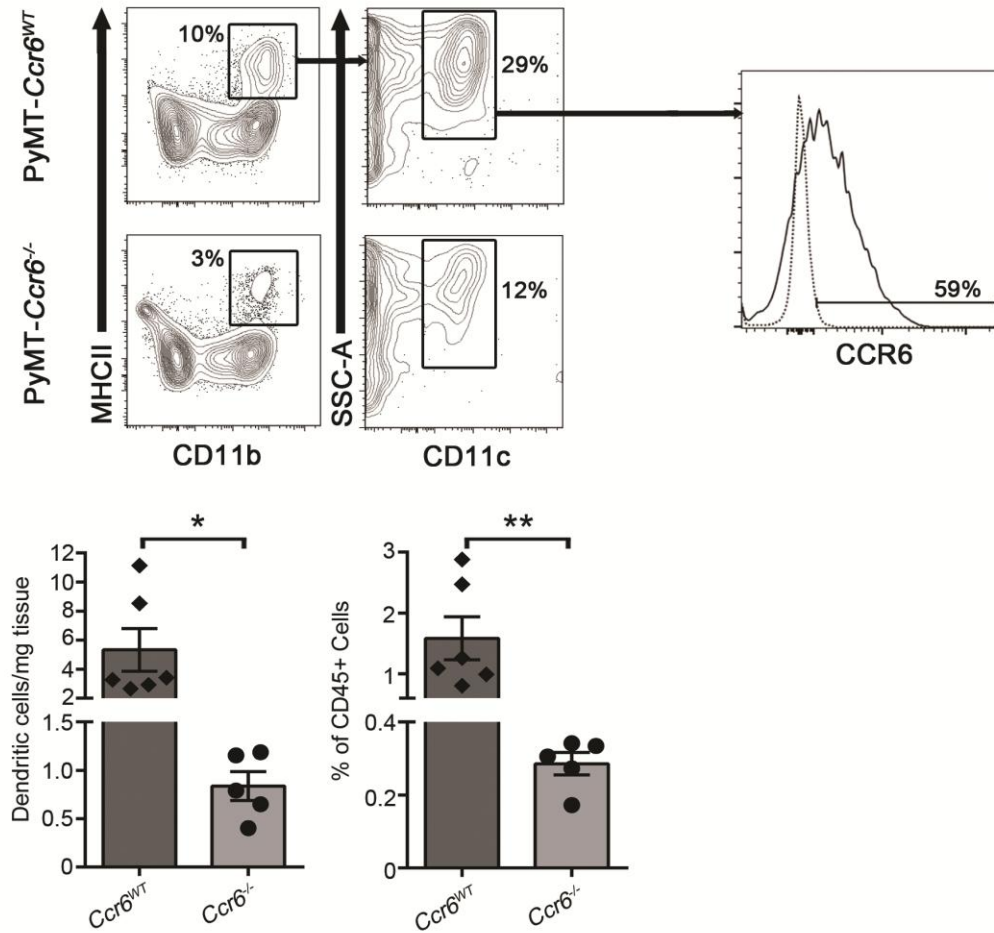


Figure S3: Infiltrating dendritic cells in MMTV-PyMT *Ccr6*^{WT} and *Ccr6*^{-/-} mammary tumors as determined by flow cytometry

All cells were gated for CD45⁺. MMTV-PyMT *Ccr6*^{WT} dendritic cells were assessed for CCR6 expression (right), and proportions and numbers of dendritic cells compared between MMTV-PyMT *Ccr6*^{WT} and *Ccr6*^{-/-} (bottom). Dotted line=FMO control. n= 5-7 mice per genotype.

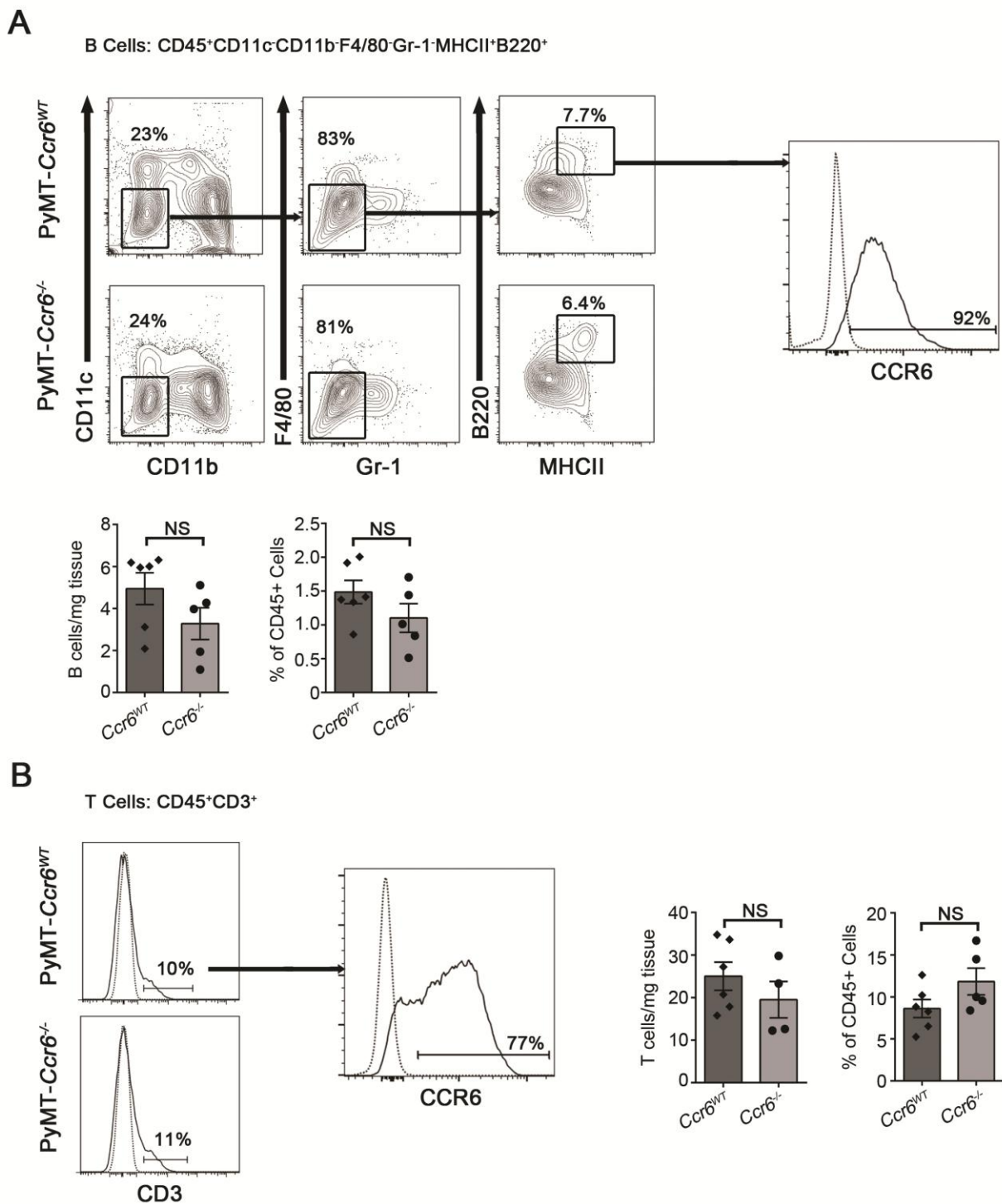


Figure S4: Infiltrating B and T immune cells in MMTV-PyMT *Ccr6*^{WT} and *Ccr6*^{-/-} mammary tumors as determined by flow cytometry

All cells were gated for CD45⁺. (a) MMTV-PyMT *Ccr6*^{WT} B cells were assessed for CCR6 expression (right), and proportions and numbers of B cells compared between MMTV-PyMT *Ccr6*^{WT} and *Ccr6*^{-/-} (bottom). (b) MMTV-PyMT *Ccr6*^{WT} T cells were assessed for CCR6 expression (middle), and proportions and numbers of T cells compared between MMTV-PyMT *Ccr6*^{WT} and *Ccr6*^{-/-} (right). Dotted lines=FMO controls. n= 5-7 mice per genotype.

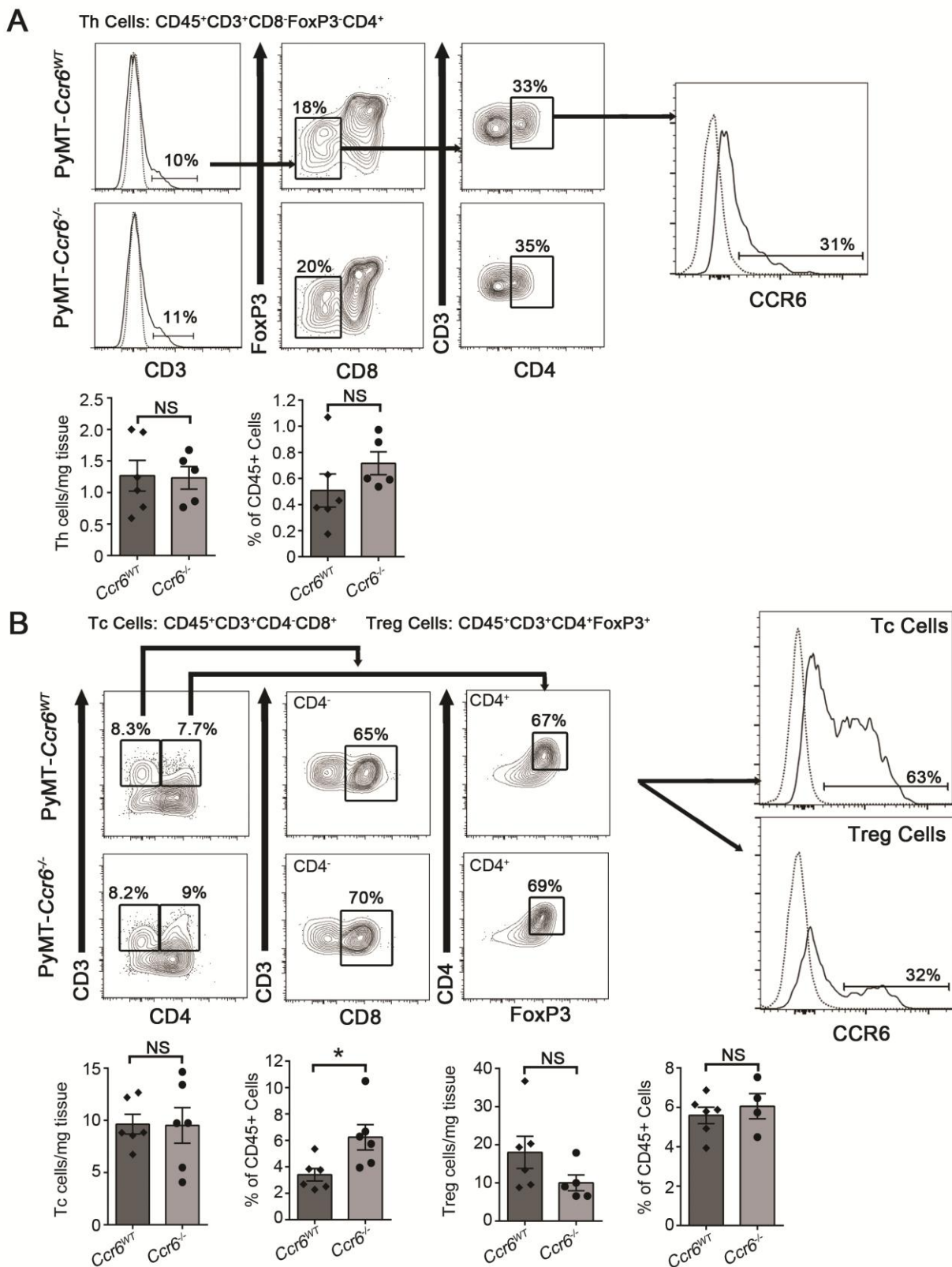


Figure S5: Infiltrating T cell subsets in MMTV-PyMT *Ccr6*^{WT} and *Ccr6*^{-/-} mammary tumors as determined by flow cytometry

All cells were gated for $CD45^+$. **(a)** MMTV-PyMT *Ccr6*^{WT} helper T cells (Th) were assessed for CCR6 expression (right), and proportions and numbers of Th cells compared between MMTV-PyMT *Ccr6*^{WT} and *Ccr6*^{-/-} (bottom). **(b)** MMTV-PyMT *Ccr6*^{WT} cytotoxic T cells (Tc) and regulatory T cells (Treg) were assessed for CCR6 expression (right), and proportions and numbers of Tc/Treg cells compared between MMTV-PyMT *Ccr6*^{WT} and *Ccr6*^{-/-} (bottom). Dotted lines=FMO controls. n= 5-7 mice per genotype.