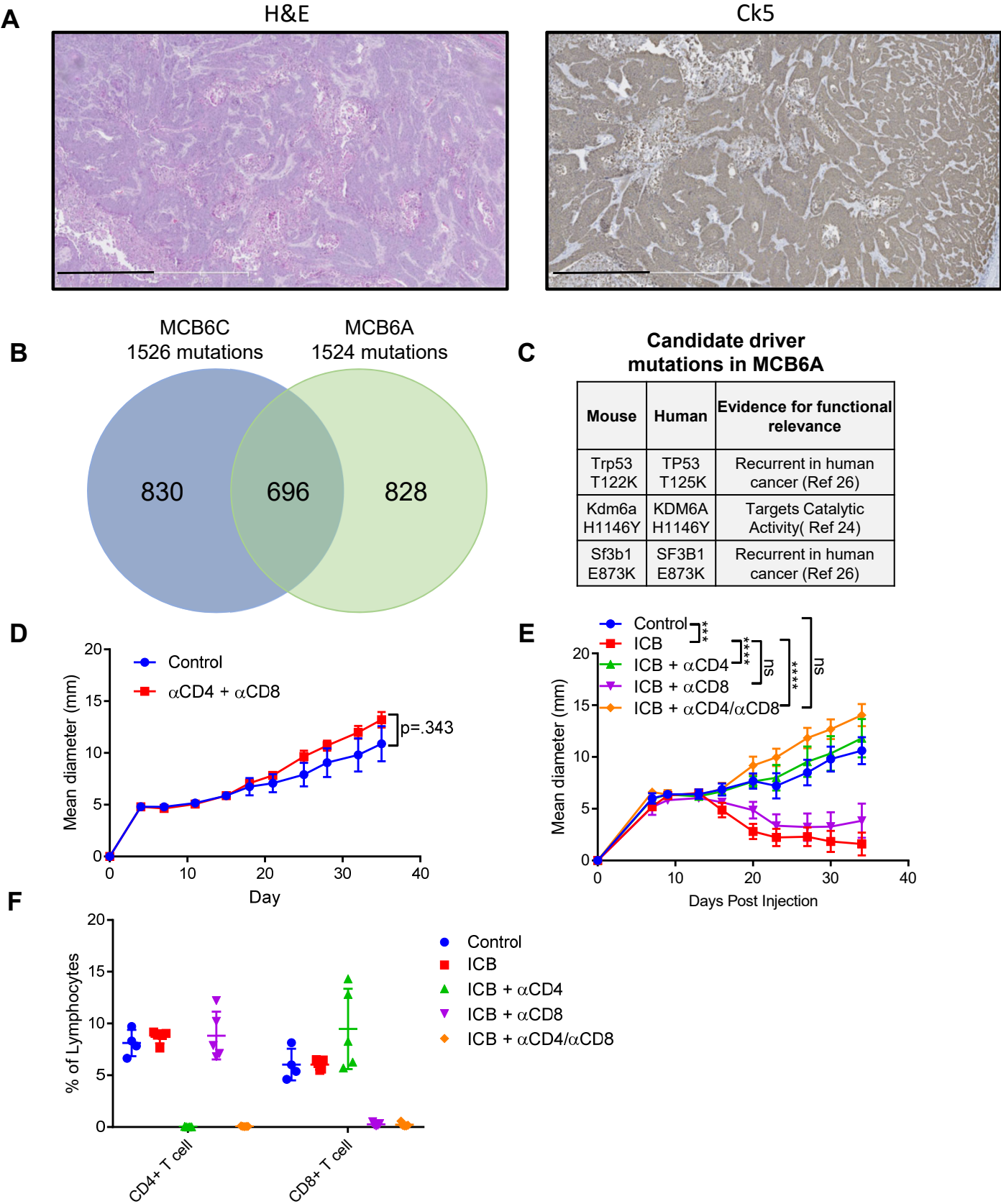
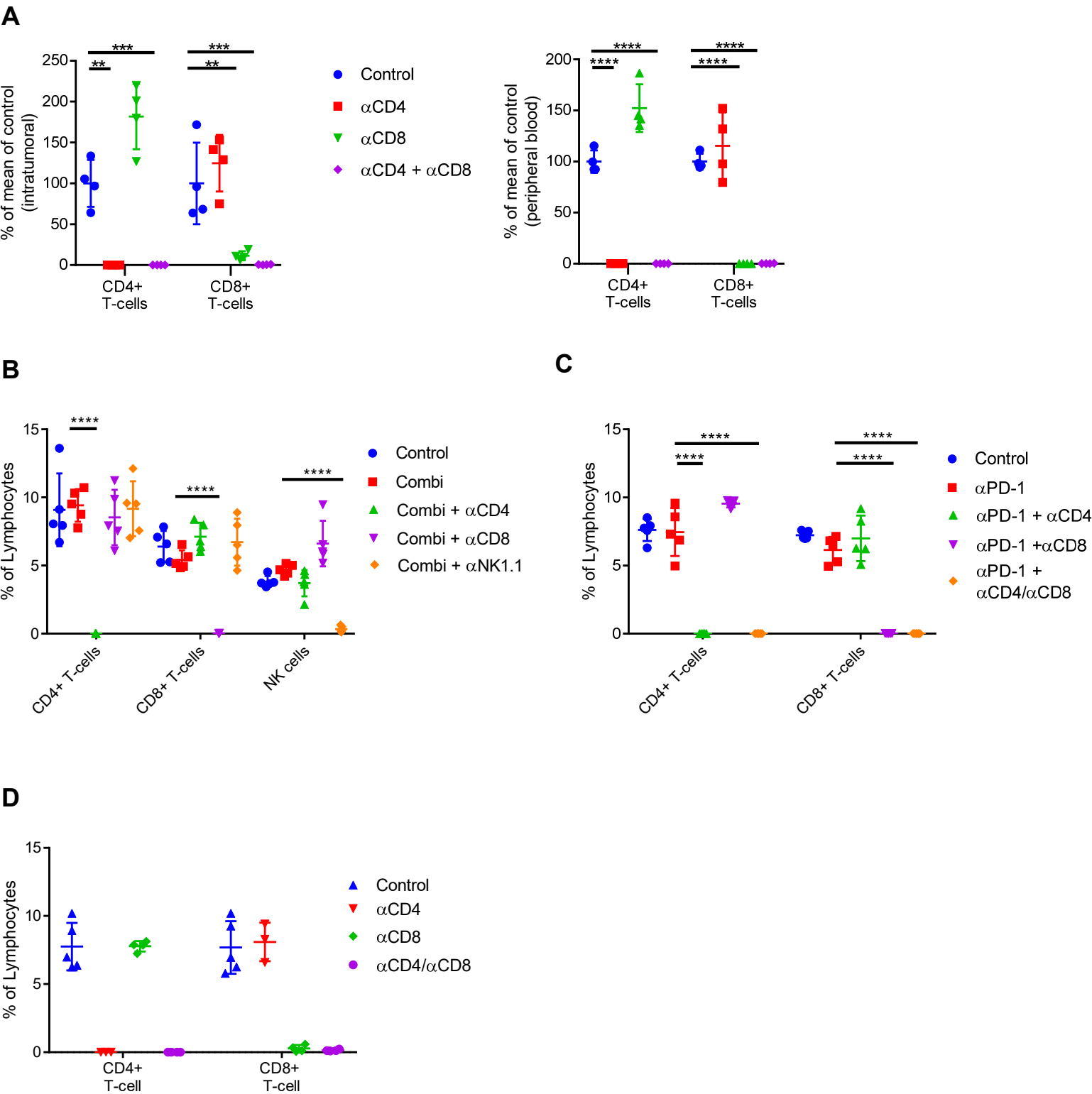


Supplemental Figure 1 (Sato et. al.)

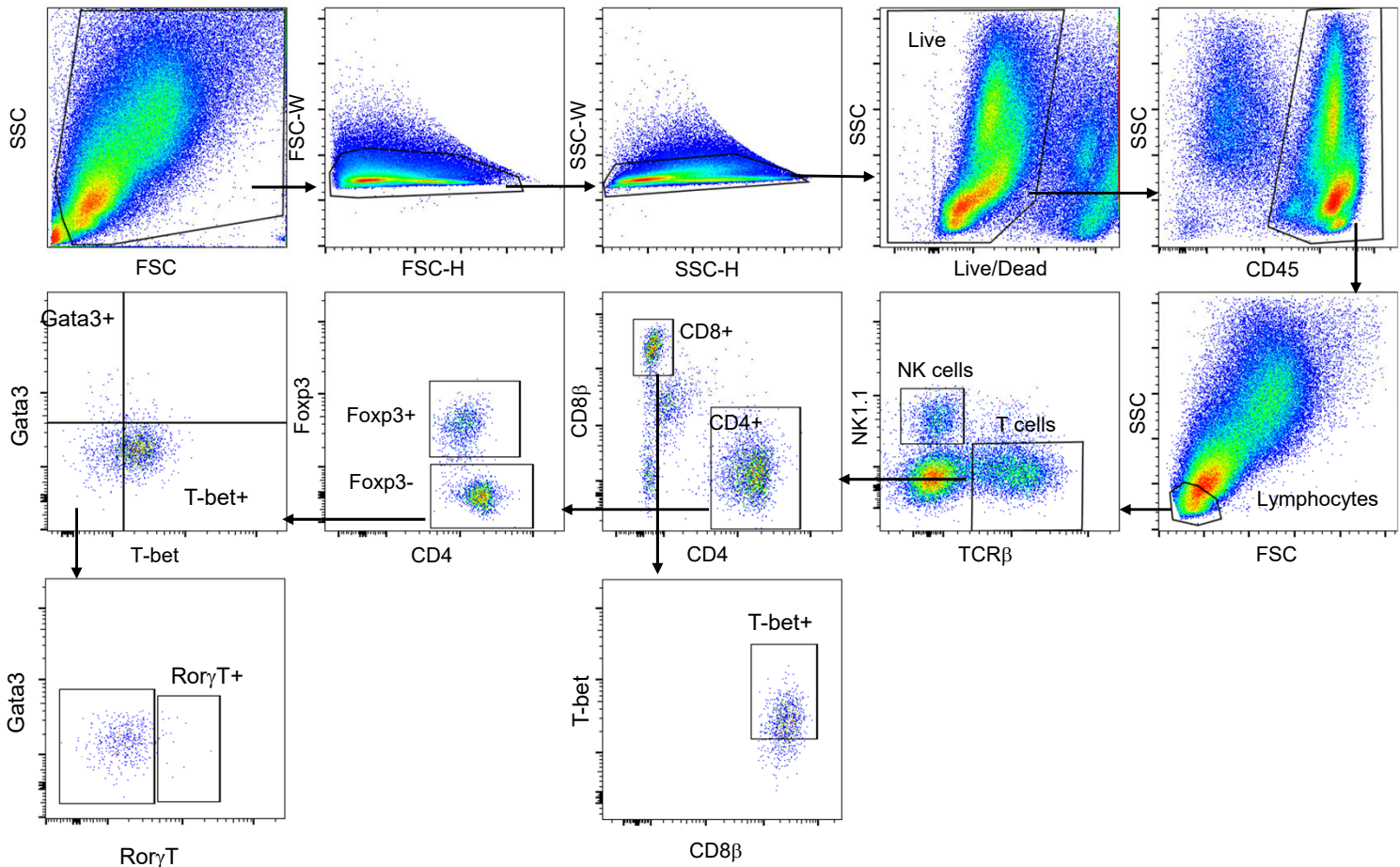


Supplemental Figure 1: Characterization of the MCB6A organoid line. A. Histologic and immuno-histochemical evaluation of s.c. MCB6A tumor collected 35 days after injection of organoid cells. Scale bar, 500 μ M. B. Comparison of mutations identified in MCB6A and MCB6C. C. Likely driver mutations in MCB6A. D. s.c growth of MCB6A organoids with and without combined CD4+ and CD8+ T-cell depletion. Data plotted as mean diameter \pm s.e.m, n = 4 mice per group. T-cell depletion was initiated on day -3 and repeated weekly throughout the experiment. Comparison is by 2 way ANOVA for repeated measures. E. MCB6A treated with combination ICB starting on day 9 and repeated every 3 days for a total of 6 treatments. Indicated T-cell depletions were initiated on day 7 and repeated weekly. Data plotted \pm s.e.m, n=8-12 mice per group, comparison by 2-way ANOVA for repeated measures. F. Confirmation of T-cell depletion in peripheral blood from a subset of mice from panel E on day 28. NS>0.05,*p<0.05, **p<0.01, ***p<0.001, ****p<0.0001.

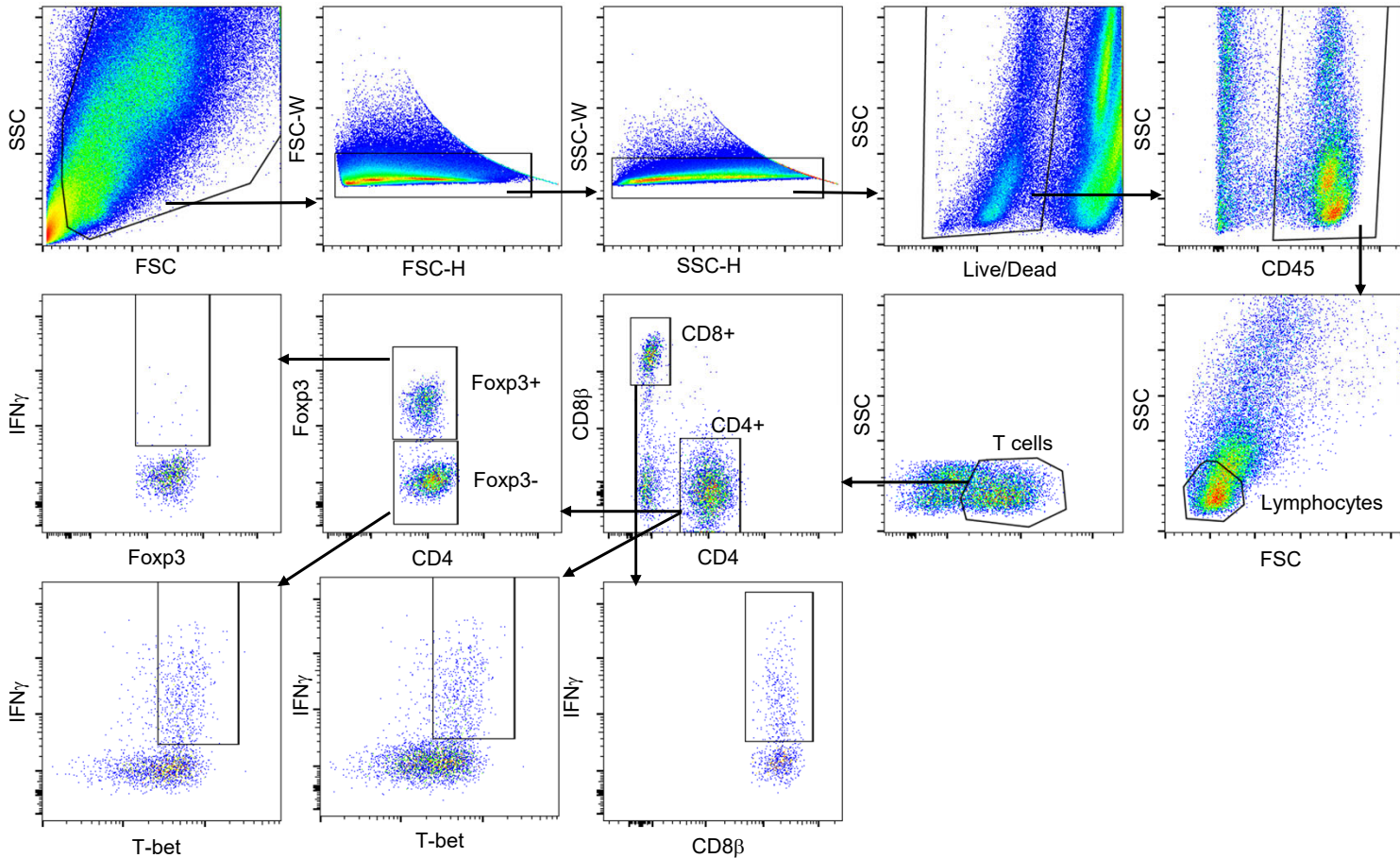


Supplemental Figure 2: Evaluation of lymphocyte depletion (related to Figure 2). A. Relative CD4+ and CD8+ T-cells in TIL and peripheral blood collected from tumor bearing mice on day 28 and treated with depletion antibodies administered as described in Figure 2A. B. Measurement of lymphocyte depletion on day 20 in peripheral blood of mice from experiment shown if Figure 2C. C. Measurement of lymphocyte depletion on day 20 in peripheral blood of mice from experiment shown in Figure 2D. D. Measurement of lymphocyte depletion in peripheral blood collected on day 28 post-reinjection from experiment in Figure 2F.

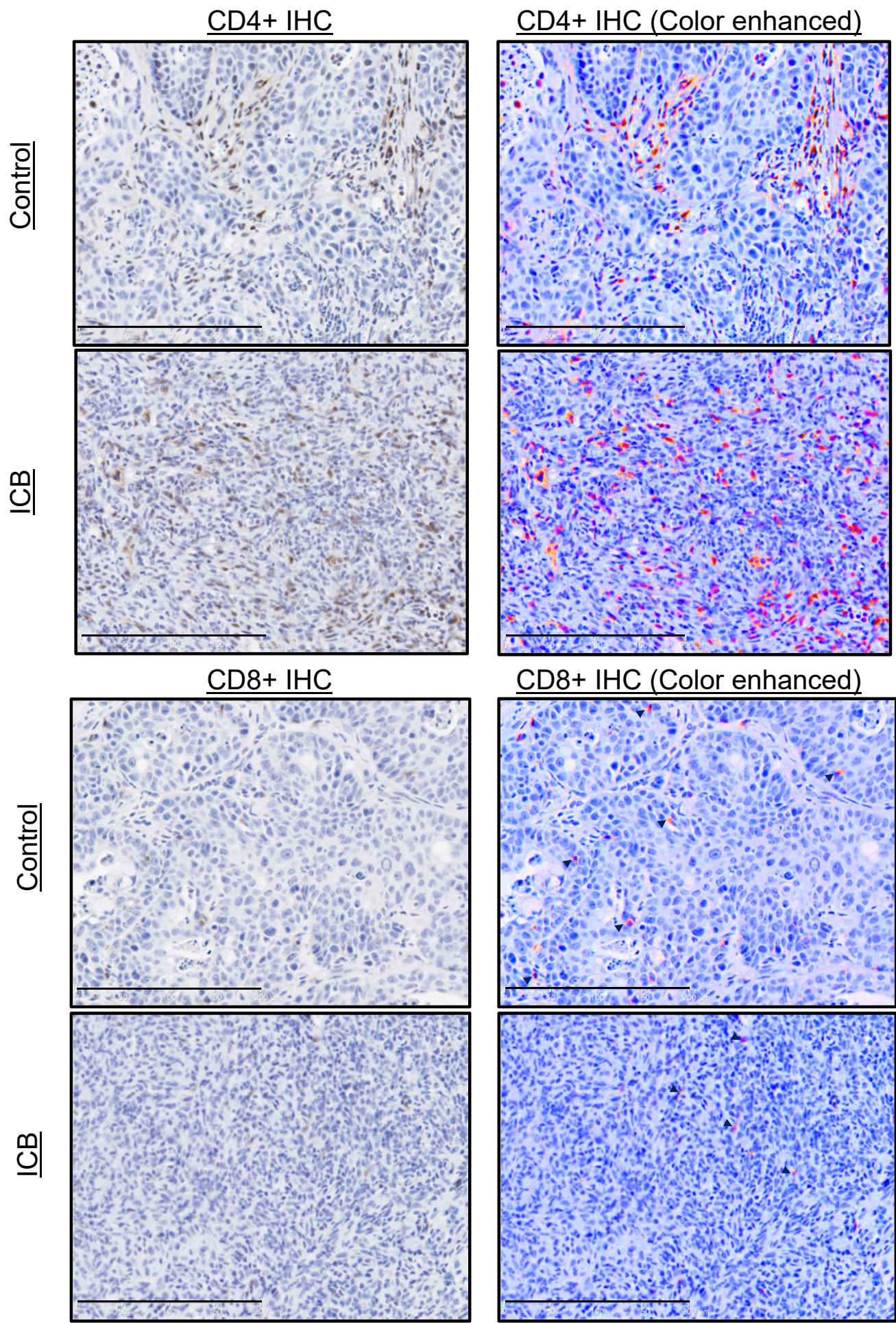
Gating strategy to detect NK cells, CD8+ T-cells and CD4+ T-cell subsets



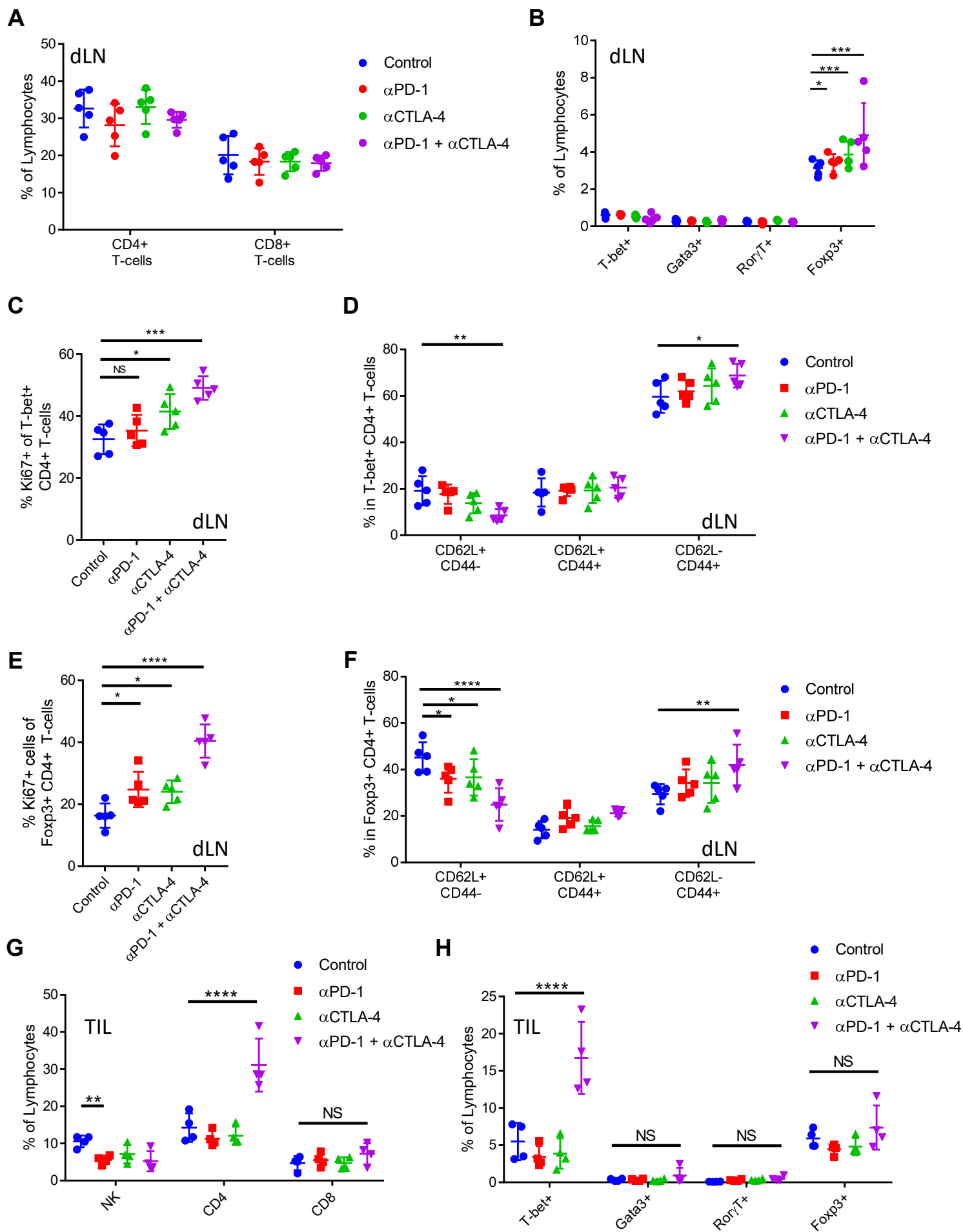
Gating strategy to detect IFN γ expressing T-cells



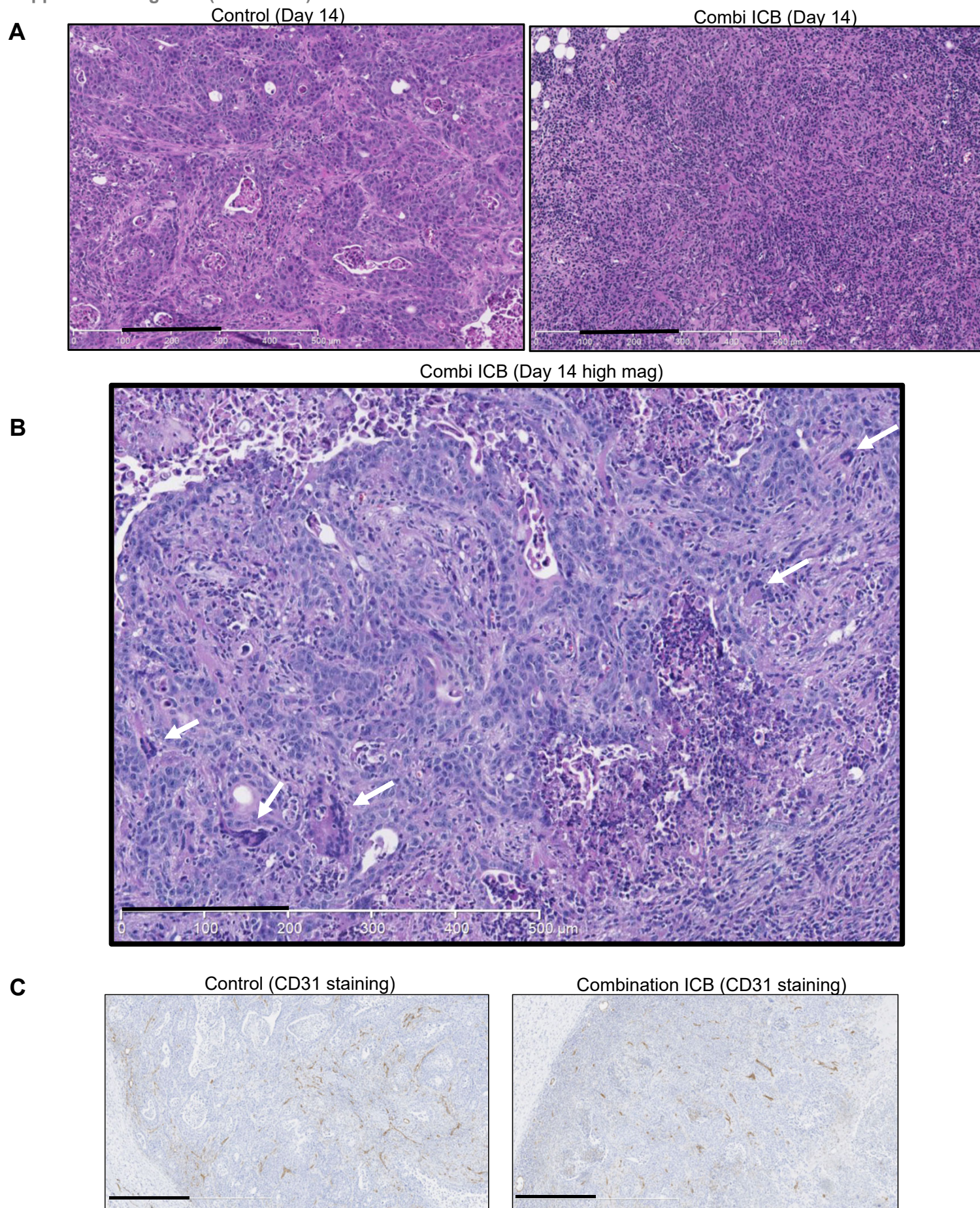
Supplemental Figure 3: Gating strategies for lymphocyte evaluation.



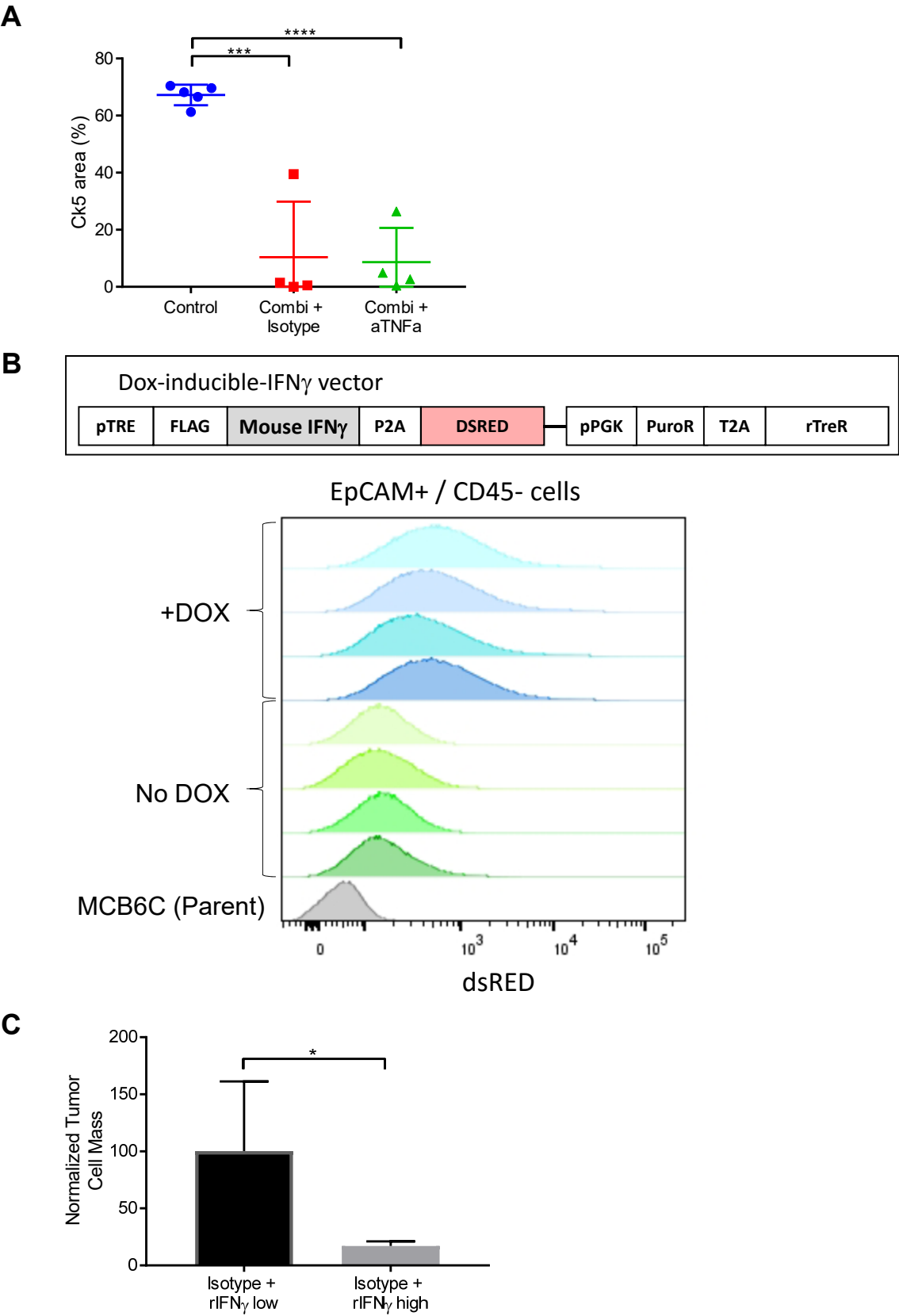
Supplemental Figure 4: Immunohistochemical analysis of CD4+ or CD8+ T-cells in MCB6C tumors on day 14, five days after ICB initiation. Arrowhead indicates CD8+ T-cell in the tumor. Images on right enhanced with contrast and hue adjustment in Adobe Photoshop such that stained T-cells appear pink. Scale bar = 200 μ M



Supplemental Figure 5: ICB monotherapy effects on T-cell subsets in TIL and dLN. A. CD4+ and CD8+ T-cells in dLN plotted as a percent of lymphocytes on day 14. ICB was initiated on day 9 and repeated on day 12. Data are shown as mean \pm s.d, n=5 mice. B. Same as A, but showing indicated CD4+ T-cell subsets. C. Same as A, but showing percent Ki67 positive cells in CD4+ T-bet+ population. D. Same as A, but showing proportion of T-bet+ CD4+ T cells with each of the indicated phenotypes. E. Same as A, but showing percent Ki67 positive cells in CD4+ Foxp3+ population. F. Same as A, showing proportion of Foxp3+ CD4+ T-cells with each of the indicated phenotypes. G. T-cell subsets in TIL on day 14, mean \pm s.d, n=4 mice. I. CD4+ T-cell subsets in TIL on day 14, mean \pm s.d. All statistical analysis by Student's t-test. NS>0.05, *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001.



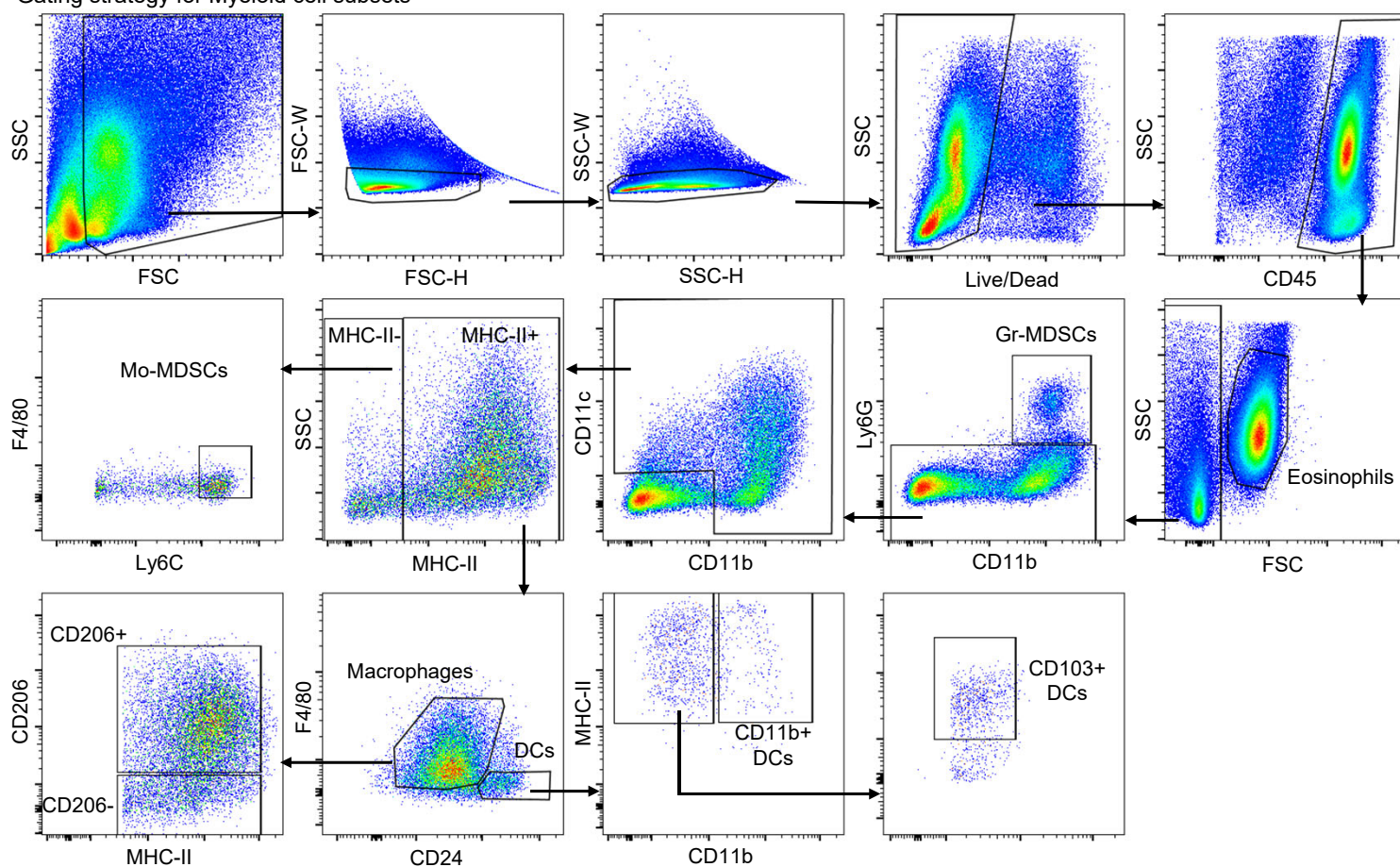
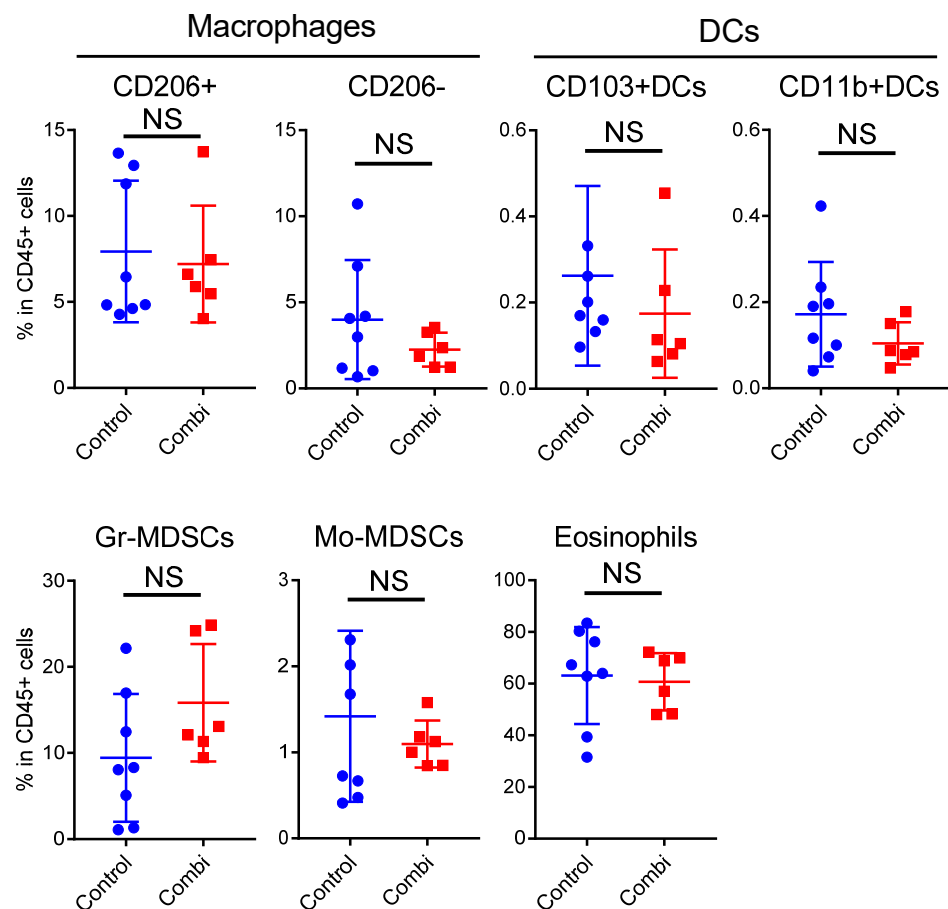
Supplemental Figure 6: Histologic evaluation of ICB treated tumors. A. H&E staining of MCB6C tumor sections obtained on day 14, five days after initiation of combination ICB. Images obtained with NanoZoomer-XR (10X). Scale bar 200 μ m. B. Similar to A, but 20X and white arrows indicate giant cells, scale bar 200 μ m. C. CD31 IHC staining of MCB6C tumor sections obtained on day 14, five days after initiation of combination ICB. Images obtained with NanoZoomer-XR (5X), scale bar 500 μ m.



Supplemental Figure 7: IFN γ mediates ICB activity and is sufficient to inhibit tumor growth (related to Figure 5). A. Ck5 staining of MCB6C tumor sections obtained 5 days after initiation of combination ICB with and without TNF α neutralization. TNF α neutralization antibody was administered on days 8 and 11 post MCB6C injection. Quantification and comparison of Ck5 staining as described in figure 5B. B. dsRED expression as a surrogate for rIFN γ expression in epithelial compartment of tumors described in Figure 5D. For flow analysis, day 34 tumors from the IFN γ neutralizing groups were used so that adequate epithelial cells were available for evaluation. dsRED in tumors cells from 4 mice in each group is compared to background signal from non-transduced MCB6C tumor cells. C. Tumor cell mass calculated by multiplying total mass by %Ck5 positivity (see Figure 5E, F). Comparison by Student's t-test.

A

Gating strategy for Myeloid cell subsets

**B**

Supplemental Figure 8: Evaluation of intratumoral myeloid cells. A. Gating strategies for myeloid cell evaluation. B. Quantification of myeloid lineages in tumor as proportion of total leukocytes