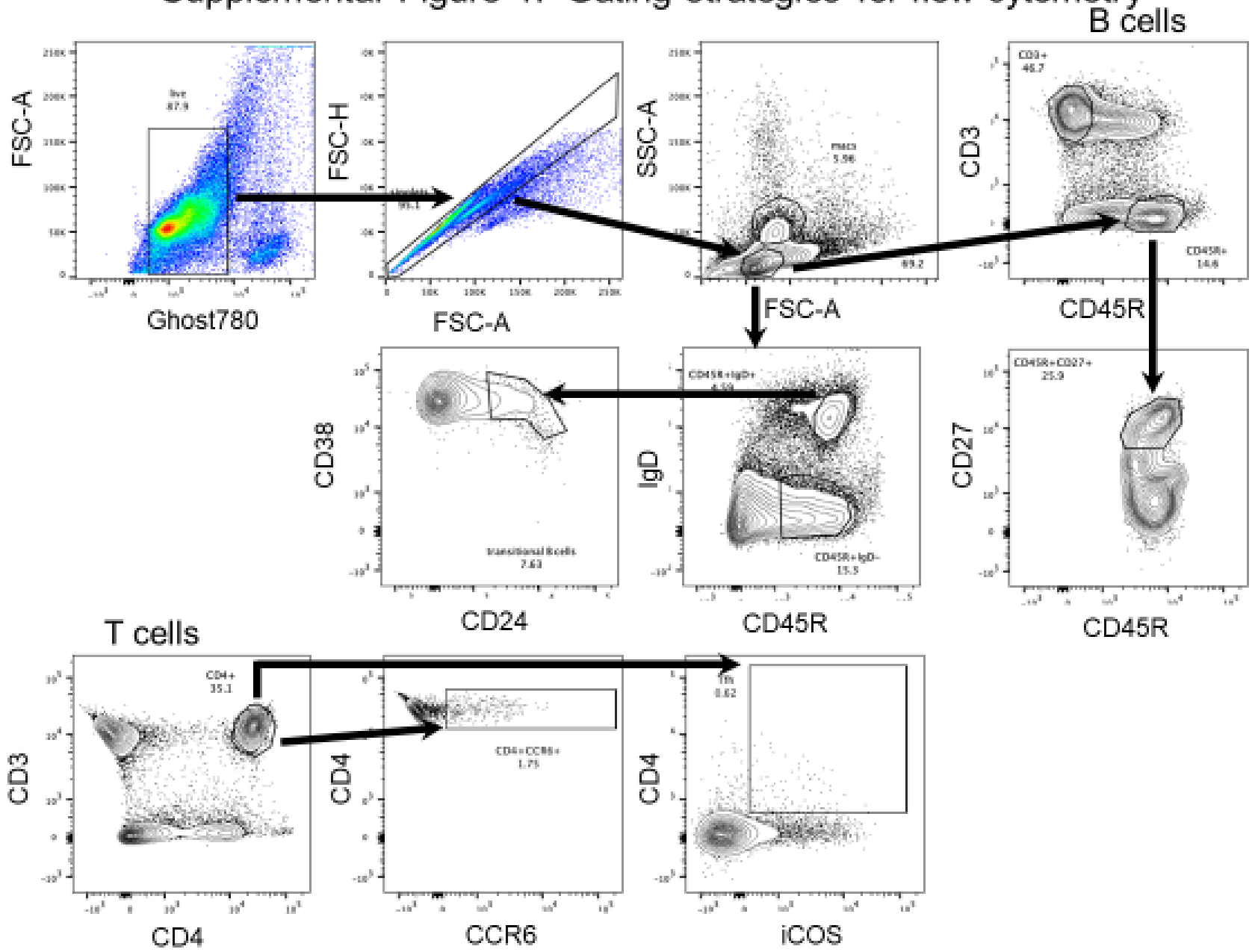
Supplemental Figure 1. Gating strategies for flow cytometry



Supplemental Figure 1. Gating strategies for flow cytometry. Several different staining panels were utilized to optimize the limited clone and fluorphore availability for anti-rat antibodies. In all cases, cells were first gated for negative Ghost780 staining to remove dead cells, then gated on the diagonal formed by forward scatter area (FSC-A) and height (FSC-H) to include only singlets. Next cells were gated by forward (FSC-A) and side scatter (SSC-A) to identify the lymphocyte gate. From here, strategies diverged, depending on the stain used for particular cell subsets. For total B cells, gated lymphocytes were visualized as CD3 versus CD45R and gates drawn for B cells and T cells, then B cells were gated for CD45R and CD27, with the double positives cells identified as activated B cells (CD45R+CD27+). Additionally, lymphocytes were gated for IgD versus CD45R to obtain CD45R+IgD+ cells, that were further gated for CD38 and CD24 to identify transitional B cells. For T cells, after the lymphocyte gate, cells were gated for CD3 and CD4, with the double positive cells further gated for CCR6 (CD4+CCR6+ T cells) or iCOS (CD4+iCOS+ T cells).