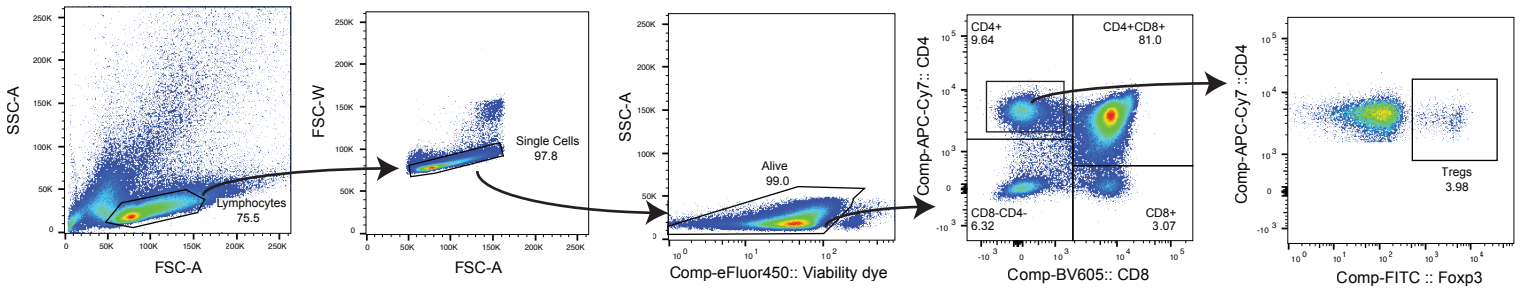
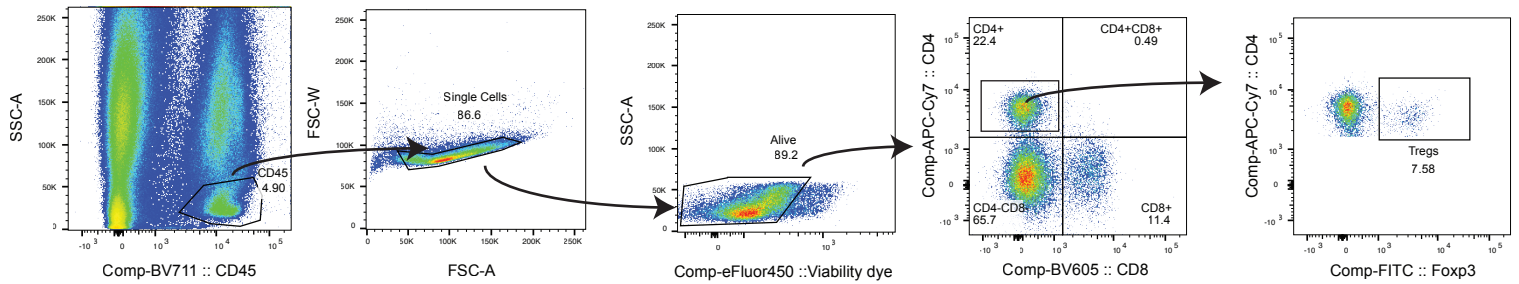


a

Gating strategy for Tregs in thymus (and other immune organs)

**b**

Gating strategy for Tregs in VAT (and other non-immune organs)

**Supplementary Data 1. Sequential gating strategies used for FACS analysis and sorting of Tregs from different tissues.**

a, shows gating strategy for thymus (also used for lymph nodes and spleen); **b**, shows gating strategy for fat tissue (also used for placenta, lung and kidney). In immune tissues (**a**), lymphocytes were initially gated by FSC-A vs SSC-A for cell size and granularity. In non-immune tissues (**b**), the first gate was based on CD45⁺ expression to only include hematopoietic cells; samples were then gated by FSC-A vs FSC-W to only include single cells. Subsequently, and to exclude dead cells, live cells were identified and gated by using the viability dye (eBioscience). Finally, CD4⁺ T cells were gated from a CD4⁺ versus CD8⁺ cell blot, from which the Tregs were identified as Foxp3⁺ CD4⁺ T cells.