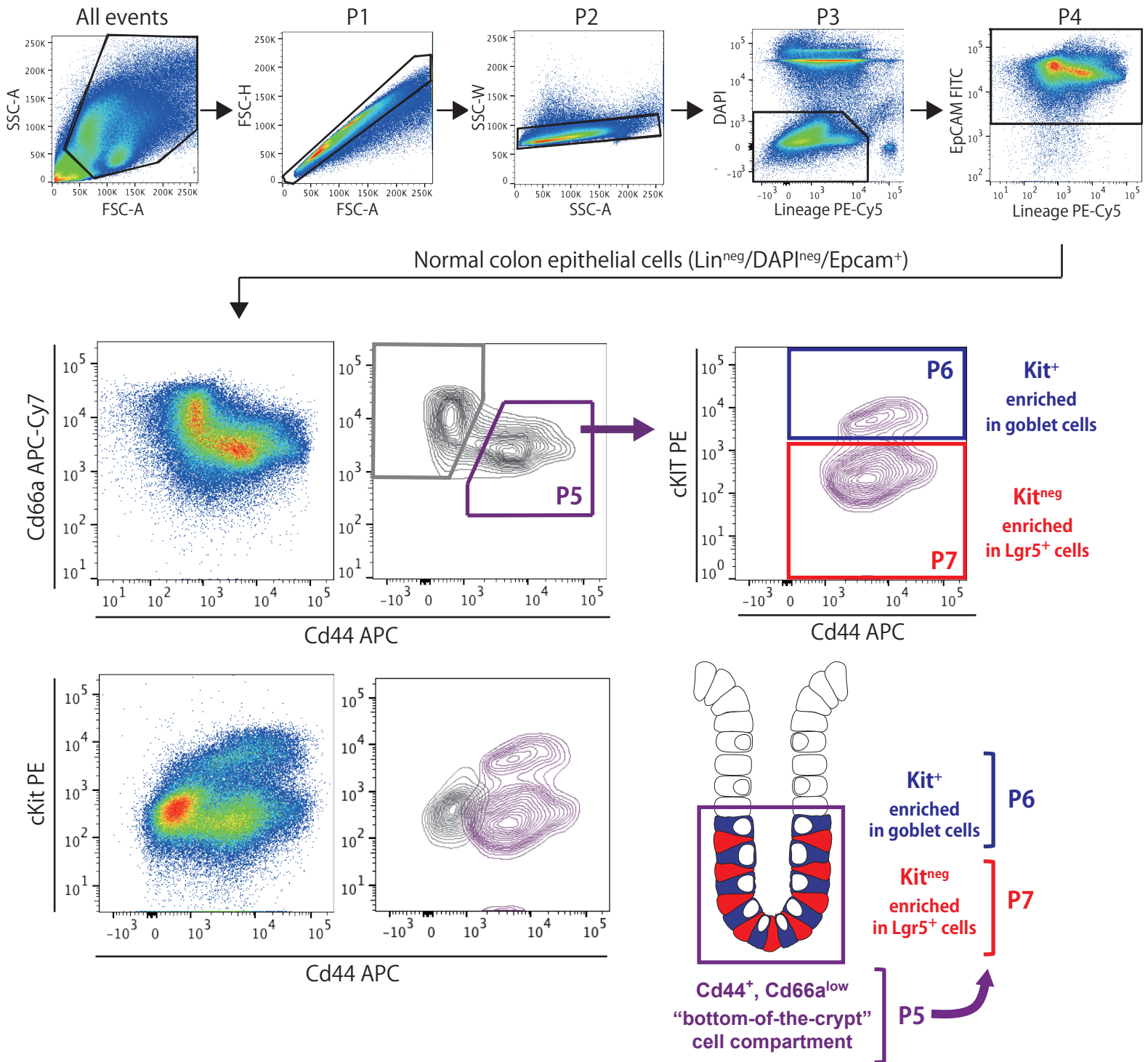


Flow Cytometry Gating Strategy



Supplementary Figure 1. Gating strategy for the differential purification by flow cytometry of distinct subtypes of epithelial cells from the “bottom-of-the-crypt” compartment of the mouse colon. Cell doublets were eliminated based on their differential distribution in forward-scatter (FSC) and side-scatter (SSC) profiles (P1-P2). Dead cells were eliminated by excluding DAPI⁺ cells (P3). Cells of hematopoietic and endothelial lineages (Lin) were eliminated by excluding cells that stained positive after labeling with a cocktail of antibodies (P3, Lineage) composed of anti-mouse Cd3 (clone 17A2; BD Biosciences), anti-mouse Cd45 (clone 30F-11; BD Biosciences), anti-mouse Cd16/Cd32 (clone 2.4G2; BD Biosciences) and anti-mouse Cd31 (clone 390; eBioscience). Epithelial cells were positively enriched by selecting Epcam⁺ cells (P4), based on their labeling with an anti-mouse Epcam-FITC (clone G8.8; Biolegend) antibody. “Bottom-of-the-crypt” ($\text{Cd44}^+, \text{Cd66a}^{\text{low}}$; P5) cells were then separated from “top-of-the-crypt” cells ($\text{Cd44}^{\text{neg}}, \text{Cd66a}^{\text{high}}$; P5) based on their differential labeling with anti-mouse Cd66a-APC/Cy7 (clone Mab-CC1; Biolegend) and anti-mouse Cd44-APC (clone IM7; Biolegend) antibodies. Finally, “bottom-of-the-crypt” cells were sorted into two groups based on their differential labeling with an anti-mouse Kit-PE antibody (clone 2B8; Biolegend): 1) Kit^+ cells (enriched in goblet cells); and 2) KIT^{neg} cells (enriched in Lgr5^+ colonic stem cells).