

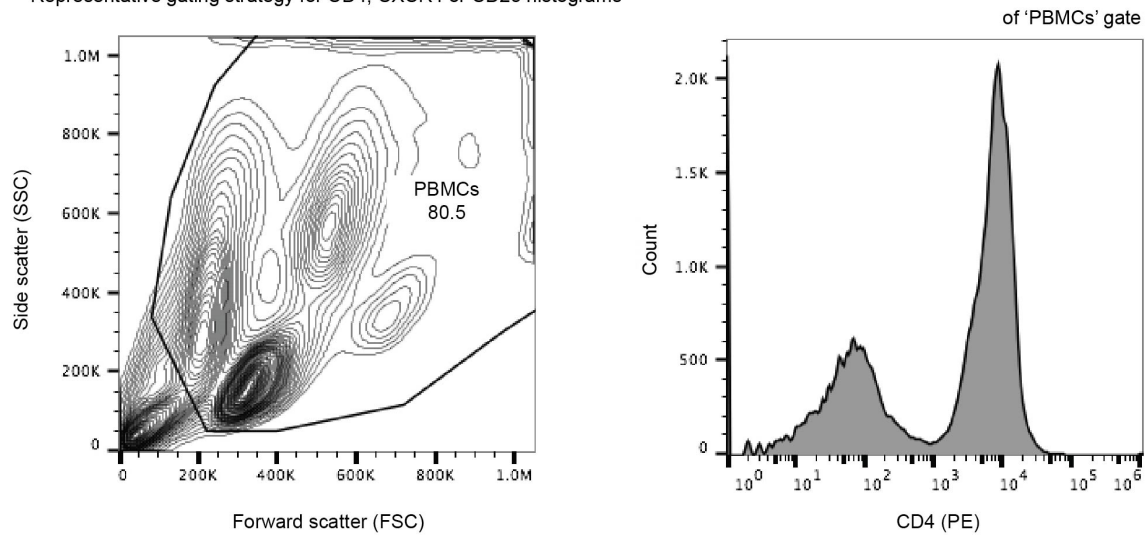
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CRISPR-Cas9 genome engineering of primary CD4⁺ T cells for the interrogation of HIV-host factor interactions

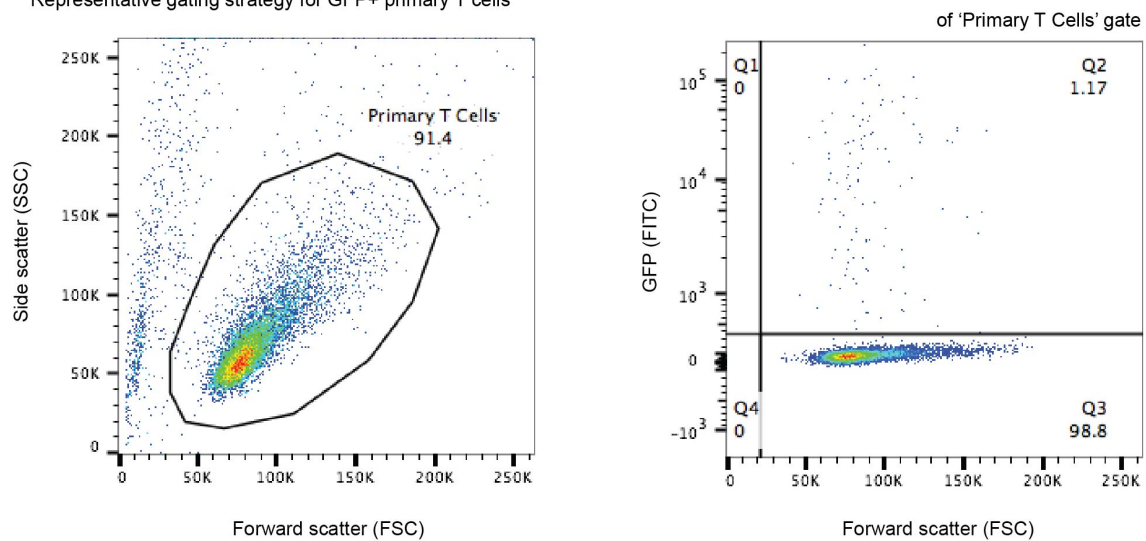
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A Representative gating strategy for CD4, CXCR4 or CD25 histograms



B Representative gating strategy for GFP+ primary T cells



Supplementary Figure 1

Gating strategy for flow cytometry analysis of immunostained and infected primary CD4⁺ T cells.

(a) Flow cytometry contour plot (left) and histogram (right) depicting cell size and CD4 levels on the cell surface of isolated PBMCs (Steps 32 and 47). A live cell gate ('PBMCs') is first applied to a forward versus side scatter contour plot before analysis of CD4 levels by histogram. Samples were run on an Attune NxT Flow cytometer and analyzed using FlowJo software v10.1 ($n > 100,000$ events). A similar strategy is used for the analysis of CD4, CD25, or CXCR4 levels in various cell populations before and after isolation, stimulation, and editing. **(b)** Flow cytometry dot plots depicting cell size (left) and GFP levels (right) of infected primary CD4⁺ T cells (Step 108). A live cell gate ('Primary T Cells') is first applied to a forward versus side scatter dot plot before analysis of GFP levels. An uninfected, GFP-negative control is first run to define a threshold for GFP gating. Samples were run on an Attune NxT Flow cytometer and analyzed using FlowJo software v10.1 ($n > 10,000$ events).