

Supplementary file 1: FACS gating strategy

Two examples of the gating strategies are provided in the figures below. Briefly, all samples were gated to exclude debris, and doublets, then only the live fraction was considered. At this stage, the separation between cherry-niche labelled cells and unlabelled lung tissue was performed. Subsequent stainings were determined in the two matched samples and cell frequency was calculated either out of live cells or out of specific cell pools (such as CD45⁺ immune-cells). All gates were set based on the FMOs containing all antibodies minus the one to determine the background signal. Of note, lung tissue shows a high level of auto-fluorescence, which need to be considered when excluding dead cells. An example of the DAPI FMO showing this is represented in Figure 1b.

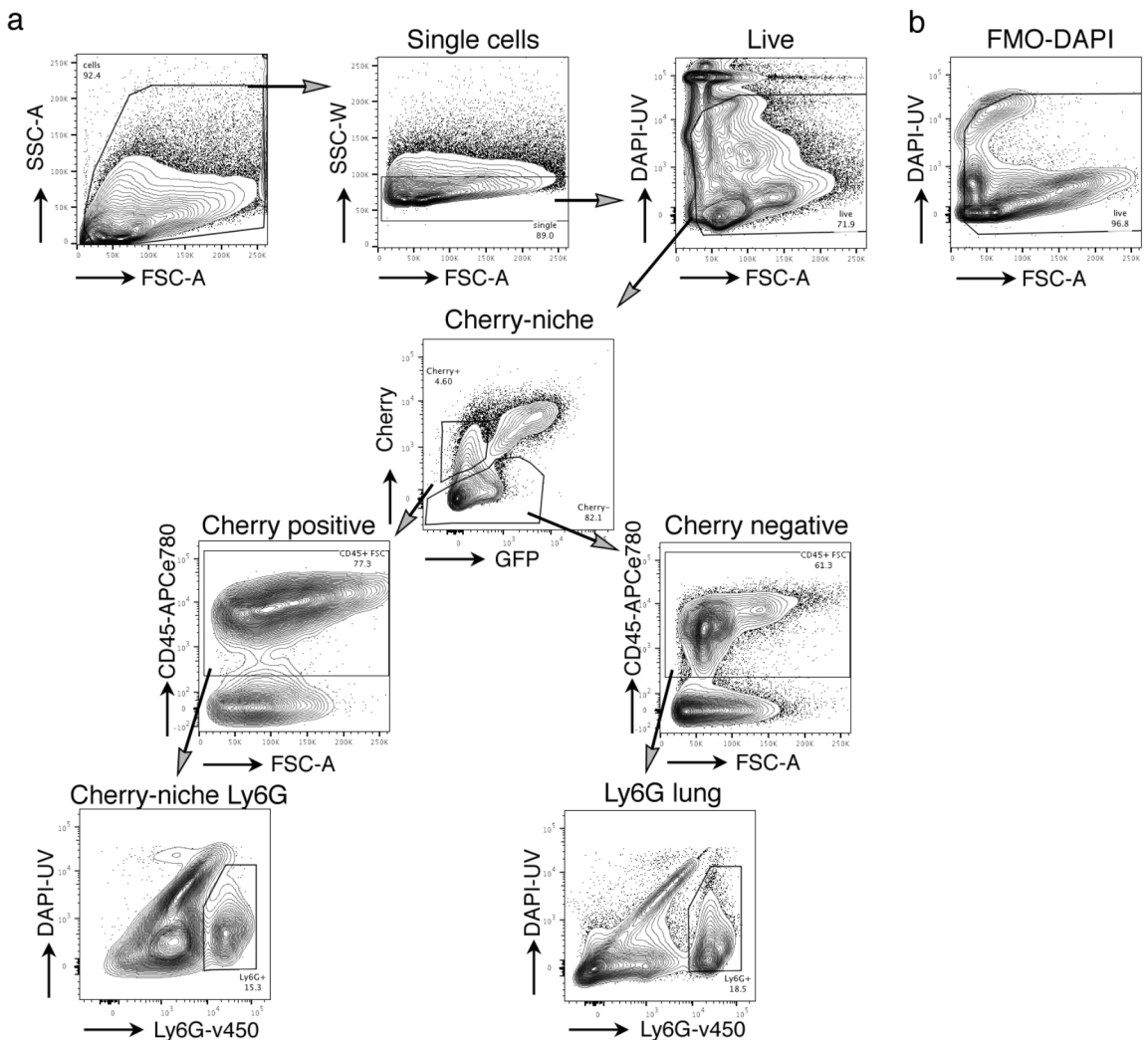


Figure 1. a. Example of gating strategy to determine CD45 positive immune cells and Ly6G positive neutrophils from either Cherry-niche labelled or unlabelled lung cells. **b.** example of an FMO (fluorescence minus one) containing all antibodies except DAPI, showing the level of lung auto-fluorescence in the UV channel.

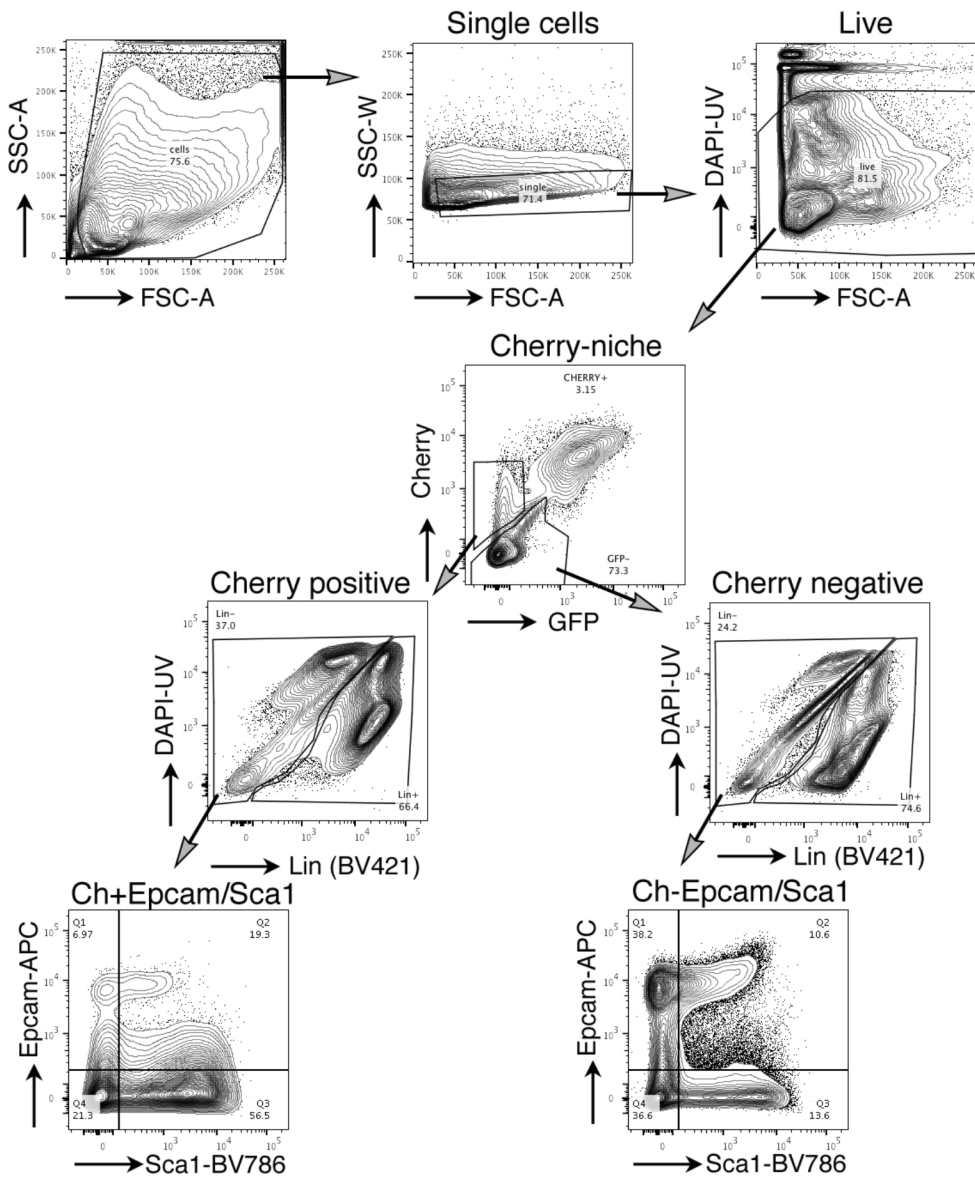


Figure 2. Example of gating strategy to determine Epcam and Sca1 positive cells from either Cherry-niche labelled or unlabelled lung cells.