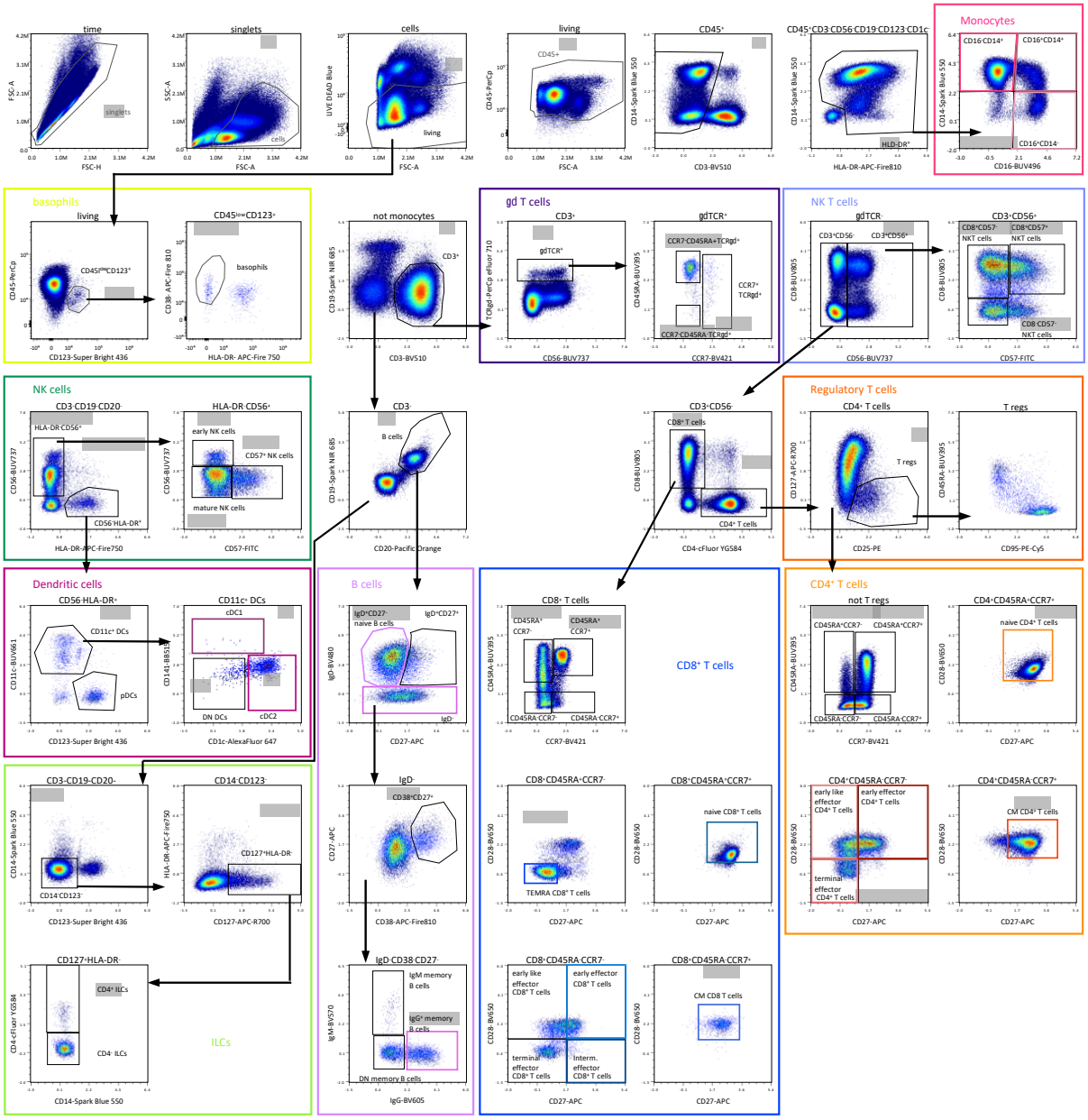

Supplemental Figure 2: Manual gating strategy used to identify the main cellular subsets. The arrows describe the relationship of the cells across the plots. FlowAI was used to clean the flow cytometry data from anomalies in flow rate, signal acquisition, and dynamic range (Monaco, G. *et al.* flowAI: automatic and interactive anomaly discerning tools for flow cytometry data. *Bioinformatics* 32, 2473–2480 (2016)). Thereafter doublets, debris, and dead cells were excluded. Basophils (yellow) were marked as $CD45^{low}CD123^{+}HLA\text{-}DR^{-}CD38^{+}$. Leukocytes were defined as $CD45^{+}$. Monocytes were then classified by the exclusion of lymphocyte markers as $CD45^{+}CD3^{-}CD56^{-}CD19^{-}CD123^{-}CD1c^{-}$ followed by CD14 and CD16 expression as classical ($CD14^{+}CD16^{-}$), intermediate ($CD14^{+}CD16^{+}$), and non-classical ($CD14^{-}CD16^{+}$). Subsequently, monocytes were excluded from further analysis of $CD45^{+}$ cells (not-gate). The further gating strategy is often based on the use of not-gates. Next, via the expression of CD3 γδ T cells (purple) and NKT cells (lilac) were defined as $CD3^{+}TCR\ \gamma\delta^{+}$ subsets and $CD3^{+}CD56^{+}$ subsets, respectively. $CD3^{+}CD56^{-}$ cells were further separated into $CD4^{+}$, $CD8^{+}$, $CD4^{+}CD8^{+}$ T cells. Regulatory T cells (T_{regs}) were identified from the $CD4^{+}$ population as $CD127^{low/-}CD25^{hi}$ cells, and CD95 and CD45RA expressions were used to classify these cells (red) further. CCR7, CD45RA, CD27, and CD28 expressions were used for in-depth characterization of memory/effector CD4 and CD8 T cell subsets (orange and blue, respectively). $CD3^{-}CD19^{+}CD20^{+}$ cells were defined as B cells (violet) and further gated as $IgD^{+}CD27^{-}$ naïve B cells, $IgD^{+}CD27^{+}$ B cells, or $IgD^{-}CD27^{+}CD38^{+}$ plasmablasts or IgD^{-} memory B cells based on IgG or IgM expression. $CD3^{-}CD19^{-}CD20^{-}$ cells were further defined as $HLA\text{-}DR^{-}CD56^{+}$ NK cells with early NK ($CD56^{hi}CD57^{-}$), mature NK ($CD56^{+}CD57^{-}$), and terminal NK ($CD56^{+}CD57^{+}$) cell subsets (green) or $CD123^{-}CD14^{-}HLA\text{-}DR^{-}CD127^{+}$ ILCs (light green). The latter can be further characterized by CD4 expression. Dendritic cells (DCs, pink) were identified first by gating on $CD3^{-}CD19^{-}CD20^{-}CD56^{-}HLA\text{-}DR^{+}$, and from there, $CD123^{+}$ (pDCs) and $CD11c^{+}$ DCs were identified. $CD11c^{+}$ DCs were further divided into CD141 expressing cDC1s and CD1c expressing cDC2s. All data presented is derived from frozen PBMCs of one healthy donor.