Additional file 2: Immunophenotypic analysis
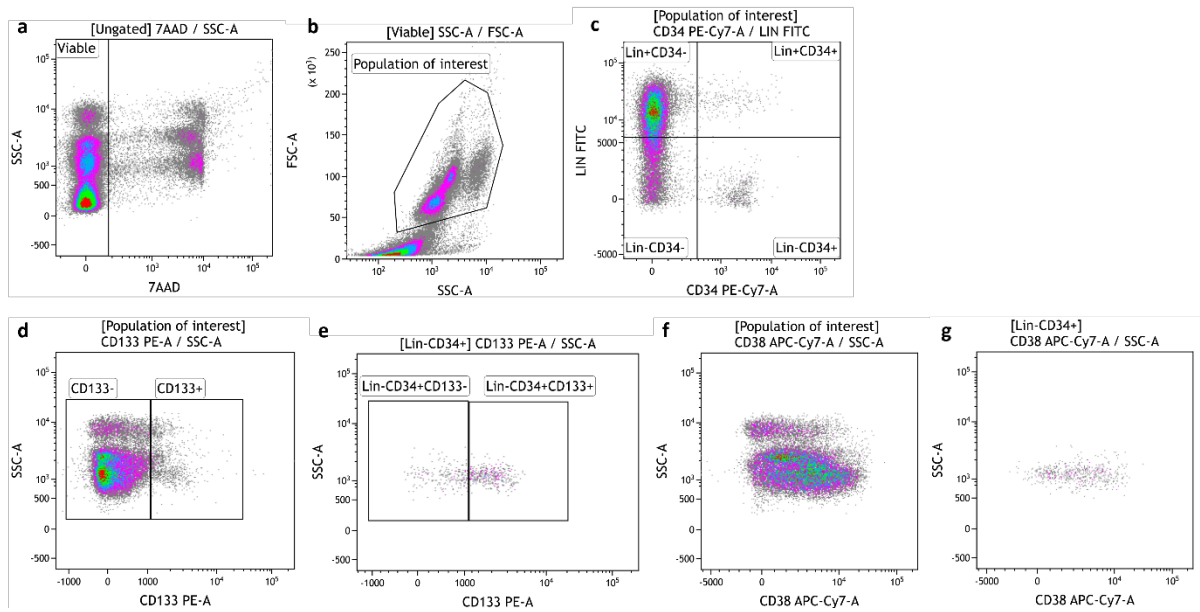

Fig. S1 Gating strategy for 4-colour HSPC-associated immunophenotypic analysis. A schematic illustration of the sequential gating strategy used for the HSPC-associated immunophenotypic analysis. (a) First, 7AAD-negative cells were identified using a 7AAD vs. side scatter (SSC-A) density plot. (b) Intact, viable cells were displayed on a forward scatter (FSC) vs. side scatter (SSC), using region "Population of interest." (c) Lin-CD34+ and Lin-CD34- HSPCs were visualized using a Lineage FITC vs. CD34 PE-Cy7 density plot (gated on "Population of interest"). (d) The CD133+/- boundary was created based on the negative population present in the sample. (e) Lin-CD34+ HSPCs expressing CD133 were identified using a CD133-PE-A vs SSC-A plot, gated on "Lin-CD34+", respectively. (f and g) CD38 is continuously expressed and therefore a negative/positive boundary was difficult to create.
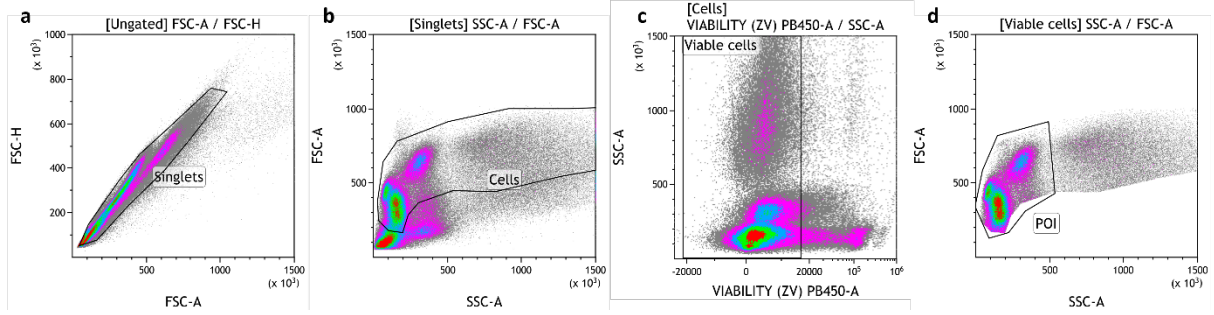
Fig. S2 Limited manual gating strategy for 8-colour HSPC-associated immunophenotypic analysis prior to import into Cytobank. A schematic illustration of the sequential gating strategy used for the HSPC-associated immunophenotypic analysis. (a) First, singlets were identified on an FSC-A vs FSC-H density plot. (b) "Cells" were then identified on an SSC-A vs FSC-A density plot gated on "Singlets." In plot (c), Viable cells were identified on a Viability (zombie violet (ZV)) vs SSC-A plot. Finally, the population of interest (POI) in (d) was then gated on "Viable cells" to identify the cells that were to be exported into Cytobank for analysis.
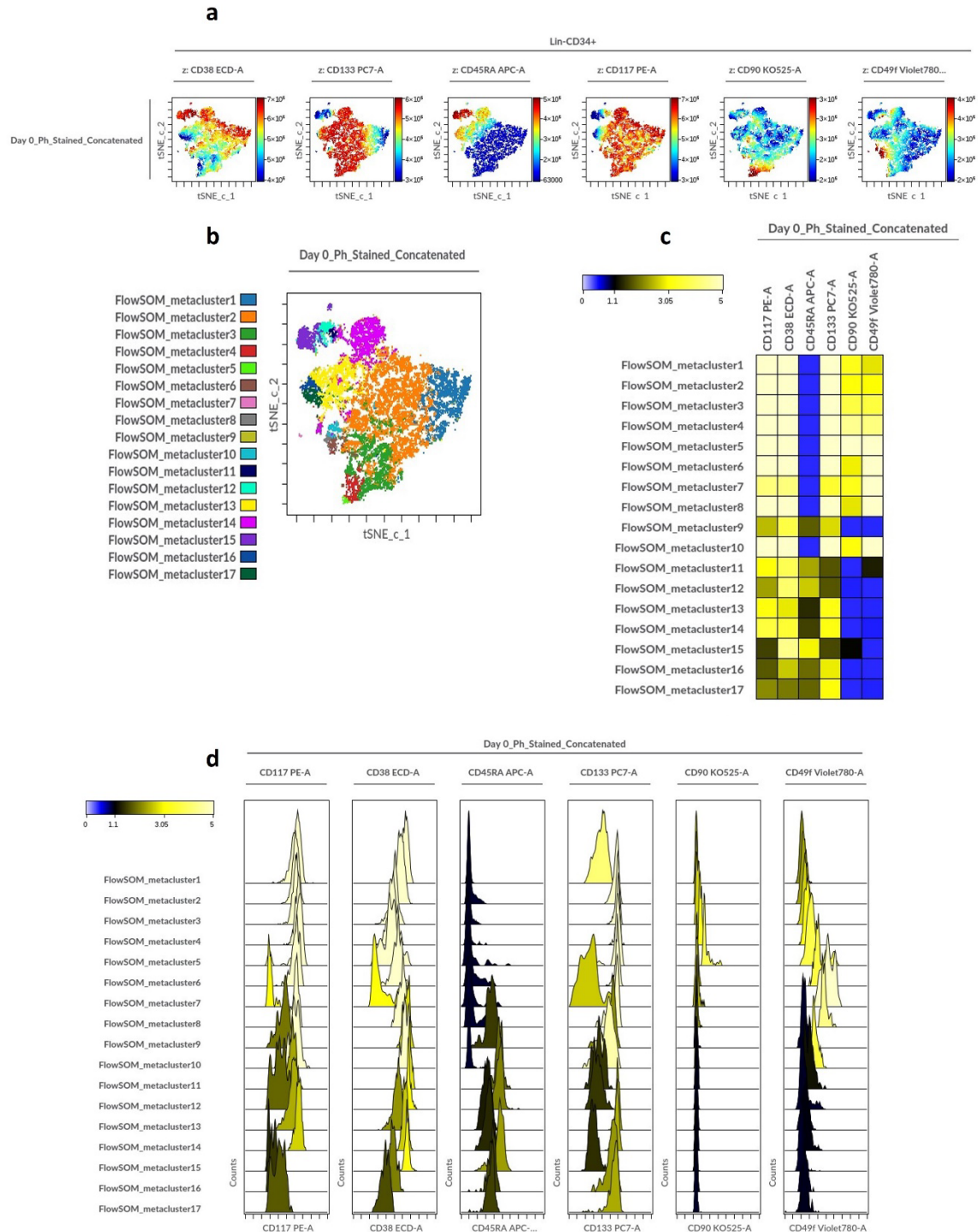
Fig. S3 A visual representation of the D0 immunophenotype (concatenated). (a) tSNE plots, (b) FlowSOM metaclusters, (c) heatmaps, and (d) histograms visually showing the immunophenotypic profile of Lin-CD34+ cells on D0. Files from five individual donors were concatenated before import into Cytobank analysis software (n=5).