Supplemental Materials

Supplemental Methods

DDAB Transfection and Induction of S2 cells

Drosophila S2 cells were cultured in Express Five SFM medium containing L-glutamine (16mM) and 1% penicillin/streptomycin (all from Invitrogen). Cells were maintained at 28°C in ventilated polycarbonate Erlenmeyer flasks (Corning) under constant rotation (100 rpm, Bench top Orbital Shaker Incubator, Ratek Instruments). Dimethyldioctadecylammonium bromide (DDAB; Sigma-Aldrich), a lipid based agent, was used for S2 cell transfection. Transfection was done during passage 5-10. DNA was incubated with DDAB for 20 min and added to S2 cells (10^6/mL) at a final DNA concentration of 400 ng/mL. After three days of cell growth, protein production was induced by adding 500 µM $CuSO_4$ to the cell culture volume. Cells were left to grow for four days before the supernatant was harvested. Afterwards supernatant was centrifuged at 16,000g for 15 minutes at 4°C.

Purification of the bispecific Tand-scFv$_{Sca\text{-}1+GPIIb/IIIa}$ and Tand-scFv$_{Sca\text{-}1+Mutant}$

Fast Protein Lipid Chromatography (FPLC) purification was performed in order to separate and purify proteins from the S2 cell supernatant using ion-chromatography. Immobilised Metal Ion Affinity Chromatography was used to covalently bind histidine to Sepharose chelated copper. Firstly, supernatant was filtered using 0.45 µm membrane polyvinylidene difluoride (PVDF) filter paper with a vacuum system. The cell supernatant was applied to a chelating Sepharose fast flow column (GE Healthcare) with a flow rate of 5 mL/min. After the protein had bound to the copper in the column, unspecific proteins were washed out. This was done using washing buffer containing 50 mM NaH_2PO_4 (Amresco) and 300 mM NaCl (Sigma-Aldrich) with a pH of 8. Following this, an elution process with Imidazole was performed to remove the desired protein from the column. This process was achieved by loading 50 mM NaH_2PO_4, 250 mM Imidazole (Merck) and 300 mM NaCl with a pH of 8. After the antibody was

collected, the column was washed with 0.5 M Ethylenediaminetetraacetic acid (EDTA; Sigma-Aldrich) to remove the copper from the column.

Prior to further purification, the supernatant was dialyzed against PBS and filtered with 0.22 µm membrane PVDF filter paper using a vacuum system. To increase antibody purity, a second purification was performed using a BioLogic DuoFlow FPLC machine (Biorad). The filtered supernatant was applied to a 5 mL Ni-NTA Agarose Column (Life Technologies). The supernatant was then washed at a flow rate of 5 mL/min using washing buffer. Unspecific proteins were washed off the column using 50 mM NaH_2PO_4, 20 mM Imidazole and 300 mM NaCl. Elution was performed using 50 mM NaH_2PO_4, 250 mM Imidazole and 300 mM NaCl with a pH of 8. The desired fractions of antibody were collected, dialyzed against PBS at 4 °C overnight and stored at -80°C. Protein concentrations were quantified using the Pierce™ BCA Protein Assay Kit (Thermo Scientific) per the manufacturer's instructions.

Supplemental Figures

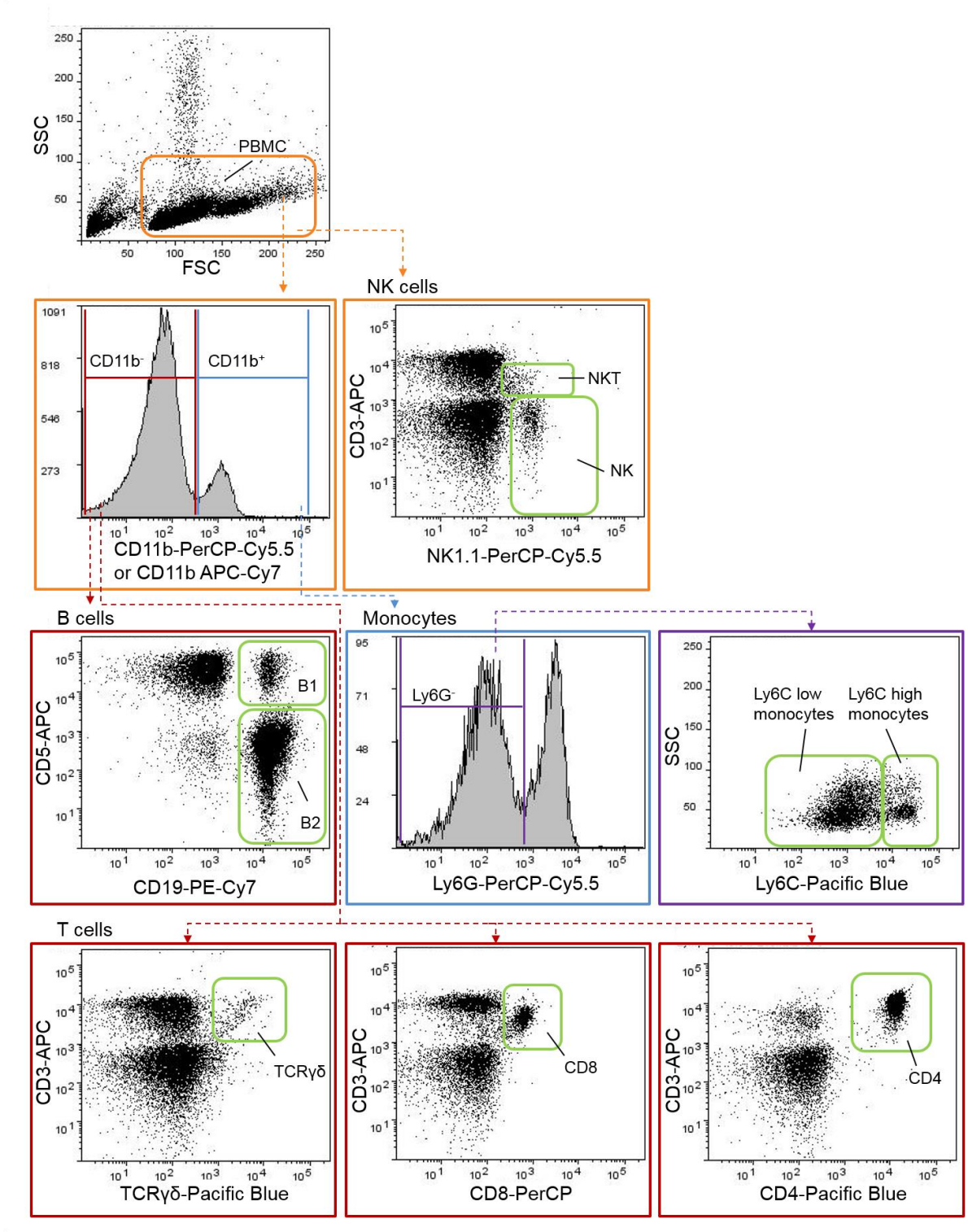

Figure S1: Gating strategy to characterize PBMCs.

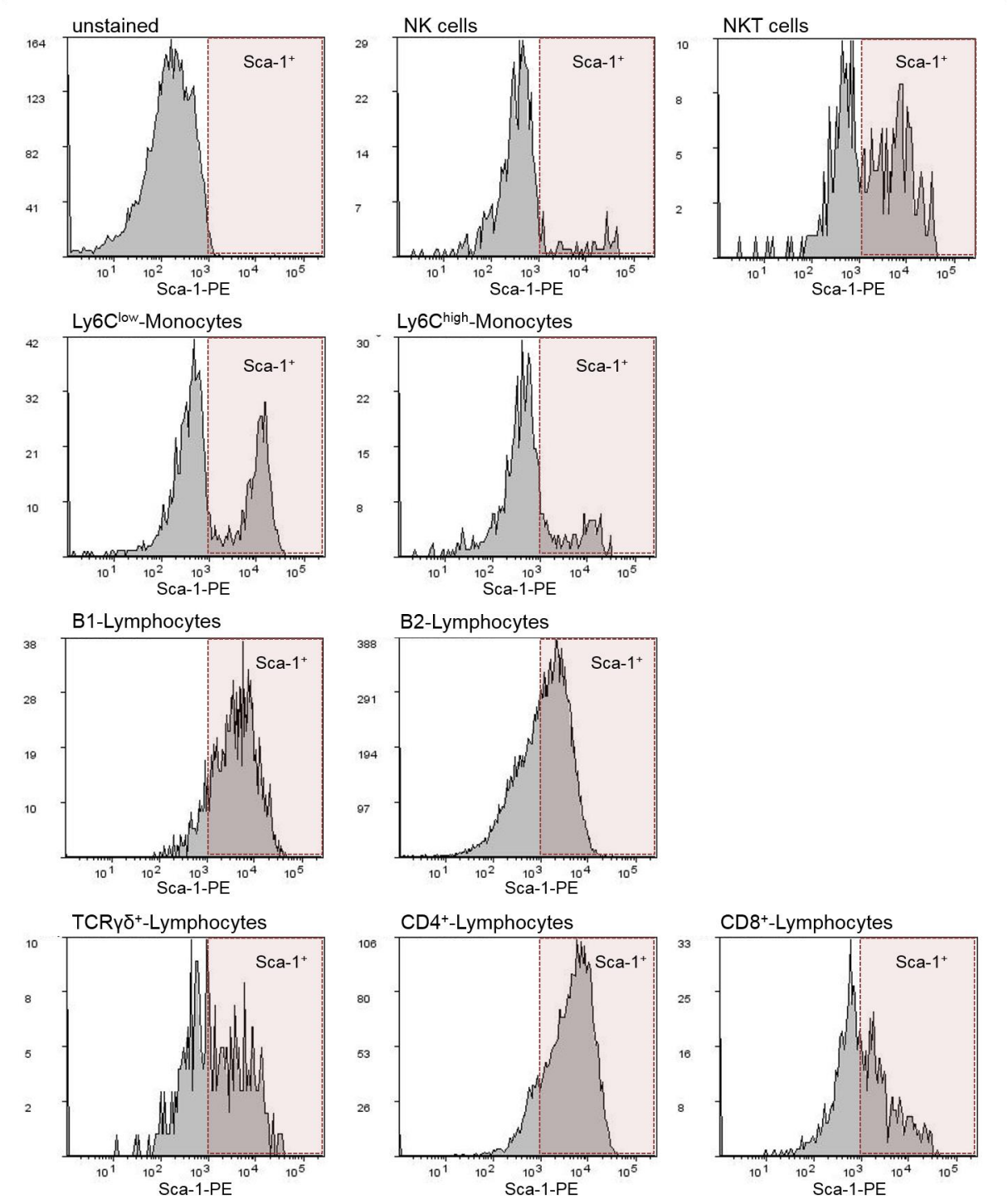

Figure S2: Gating strategy to characterize Sca-1^{+}-PBMCs. In a last step Sca-1 expression on the PBMC subpopulations was determined using the herein presented gating strategy.