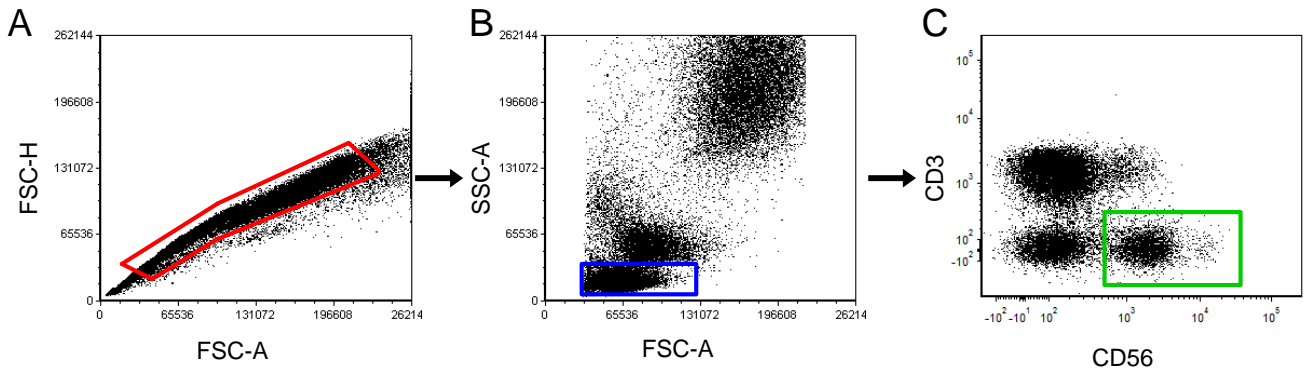


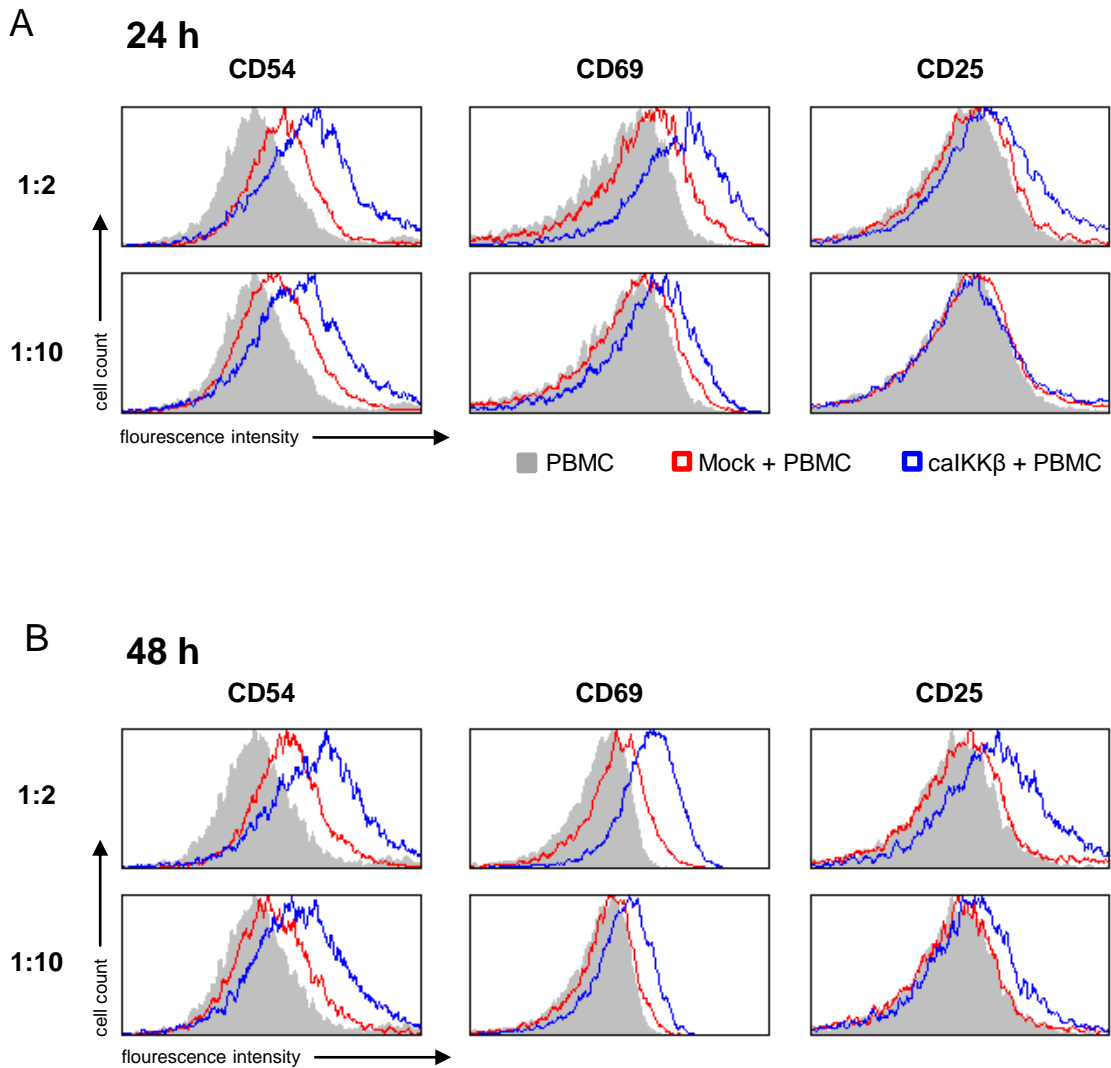
**Supplemental Figure 1** Electroporation efficiency of calKK $\beta$  DCs

(A) mDCs were either left untreated (grey filled histogram) or electroporated with EGFP RNA (green histogram). The GFP expression was measured 24 h after electroporation via flow cytometry. (The percentage of EGFP-positive cells is indicated within the histogram). (B) mDCs were electroporated either with calKK $\beta$  RNA or as a control were mock electroporated. The histograms show the upregulation of surface markers CD70, CD80 and CD40 on mock DCs (displayed in red) and calKK $\beta$  DCs (displayed in blue) 48 h after electroporation (the grey filled histograms display the isotype control).

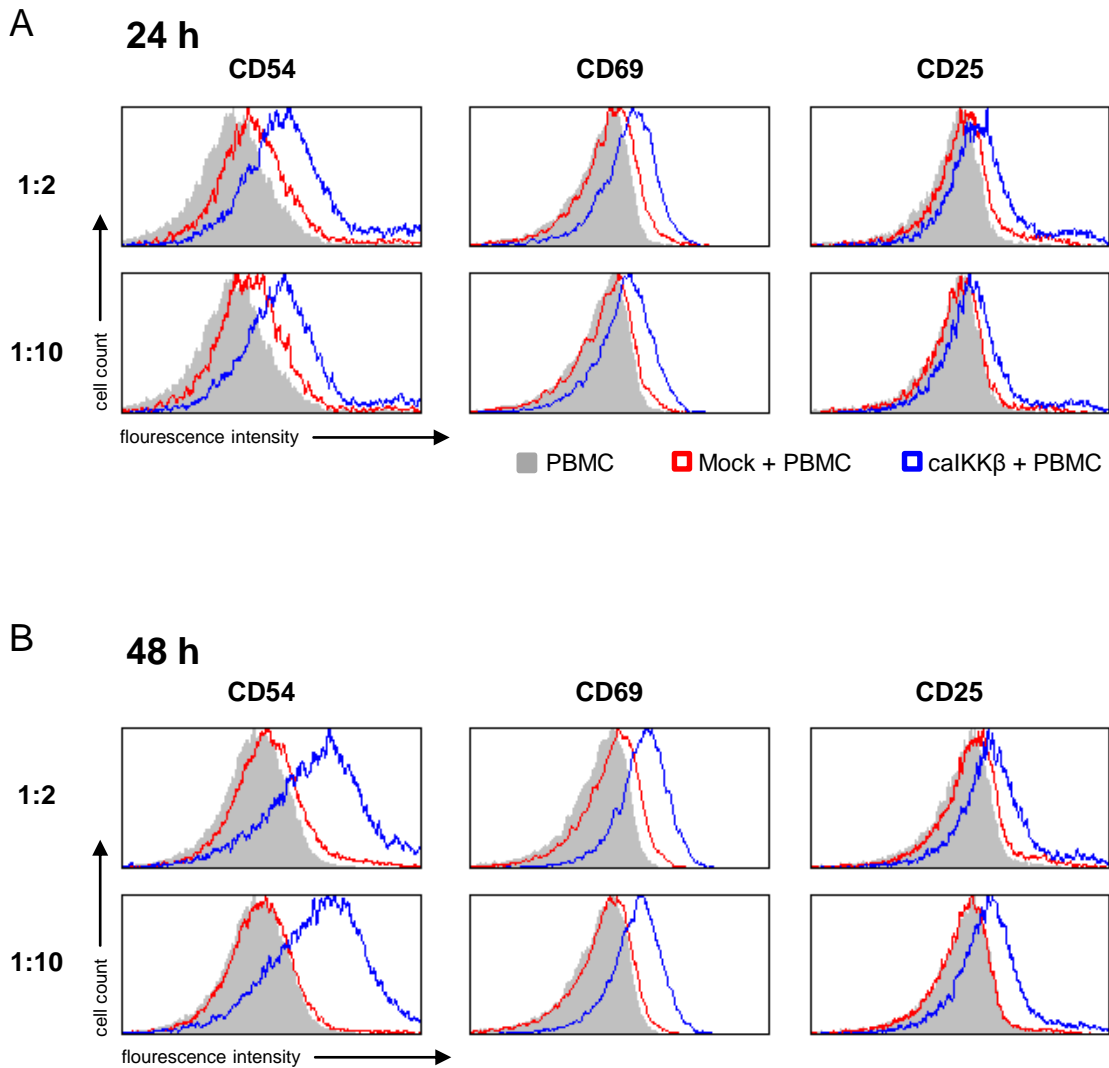


**Supplemental Figure 2.** Flow-cytometric gating strategy to identify NK cells

(A) DC/PBMC co-cultures were first gated on single and live cells (red gate). (B) Through forward and side scatter, lymphocytes were gated (blue gate). (C) Cells were stained with antibodies specific for CD56 and CD3 to finally gate on the CD56<sup>+</sup>/CD3<sup>-</sup> NK cells (green gate). Dot plots from a representative donor out of 4 independent donors are shown.

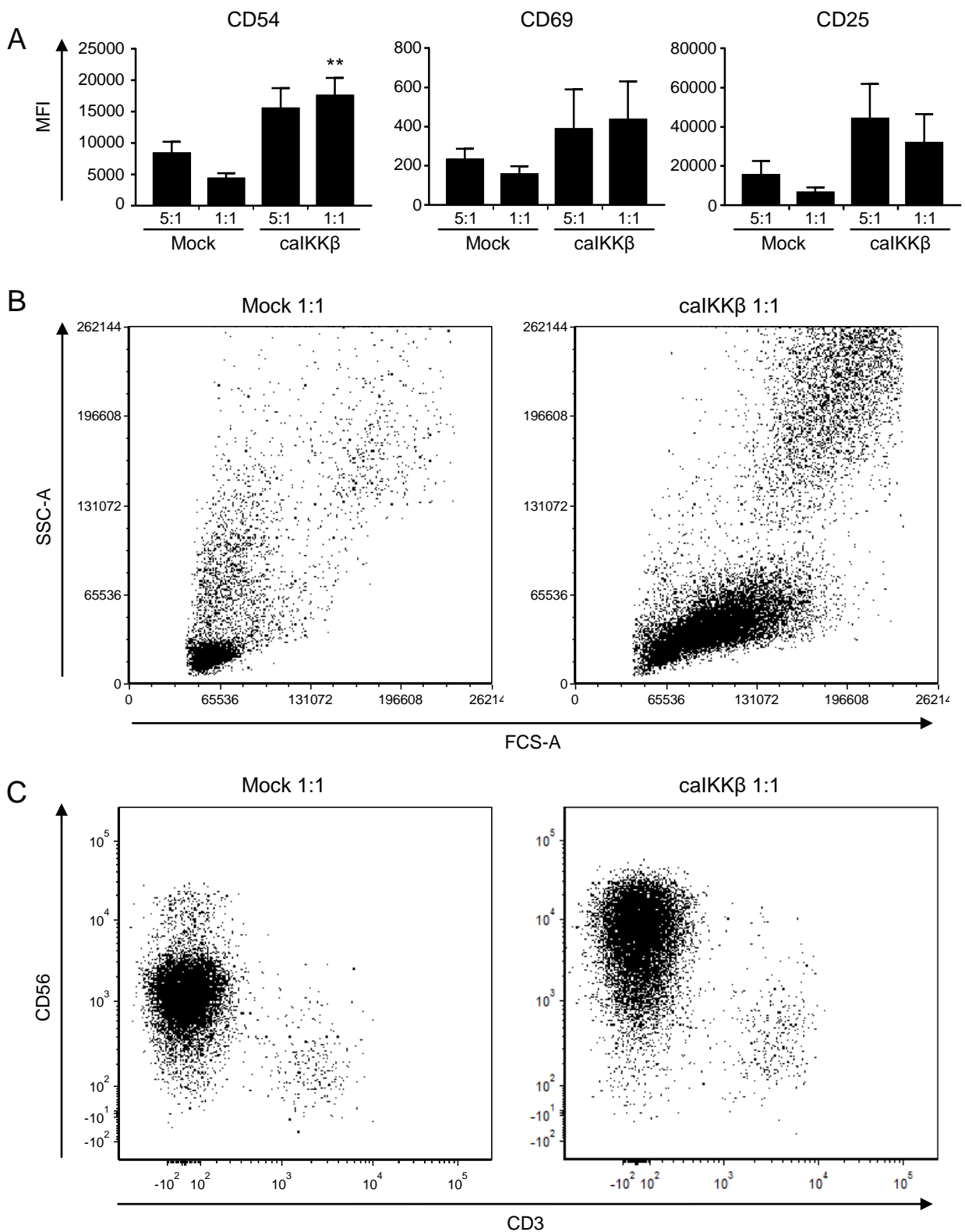


**Supplemental Figure 3.** Representative histograms corresponding to Fig 1A mDCs were electroporated either with calKK $\beta$  RNA or as a control were mock electroporated. Transfected DCs were co-cultured with PBMCs as described in Fig 1A. The histograms show the expression of the surface markers CD54, CD69, and CD25 after 24 h (**A**) and 48 h (**B**) on the NK cells (using the gating strategy shown in Supplemental Fig. S2). The grey filled histograms show NK cells from PBMCs cultured in absence of DC. NK cells from the mock-DC/PBMC co-culture are displayed as red histograms and NK cells from the calKK $\beta$  DC/PBMC co-culture as blue histograms. Histograms from a representative donor out of 4 independent donors are shown.



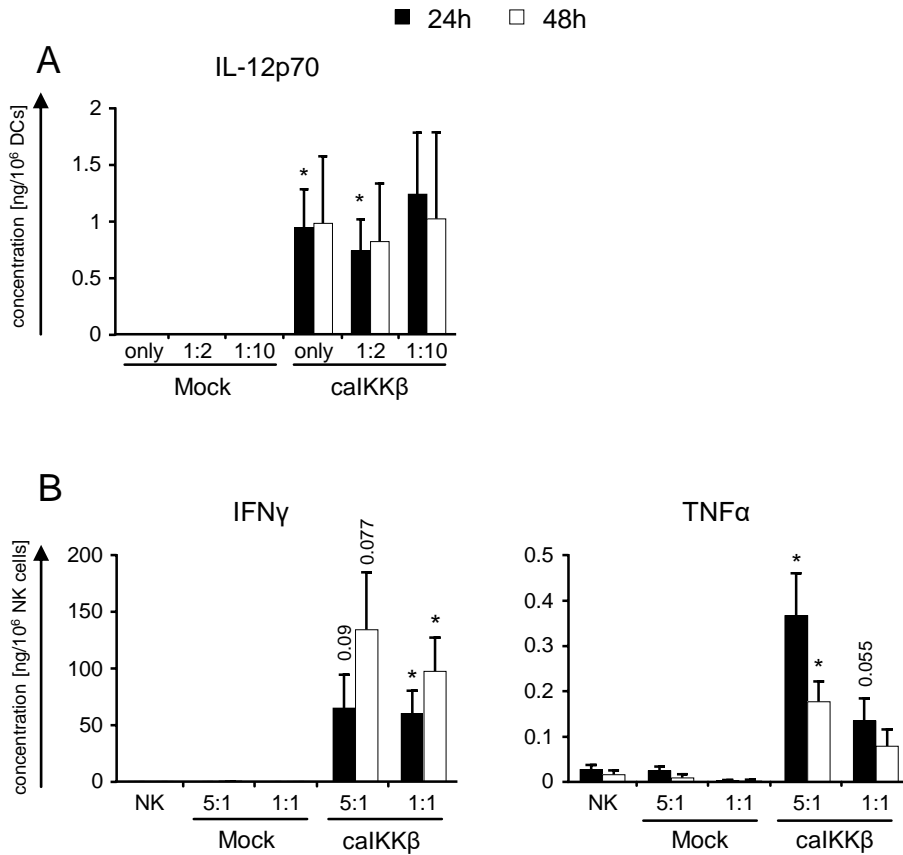
**Supplemental Figure 4.** *Representative histograms corresponding to Fig 1B*

mDCs were electroporated either with calKK $\beta$  RNA or as a control were mock electroporated. Transfected DCs were co-cultured with NK cells as described in Fig 1B. The histograms show the upregulation of surface markers CD54, CD69, and CD25 after 24 h (**A**) and 48 h (**B**) on NK cells (using the gating strategy shown in Supplemental Fig. S2). The grey filled histograms show NK cells that were cultured alone. NK cells from the mock-DC/NK cell co-culture are displayed as red histograms and NK cells from the calKK $\beta$  DC/NK cell co-culture as blue histograms. Histograms from a representative donor out of 4 independent donors are shown.



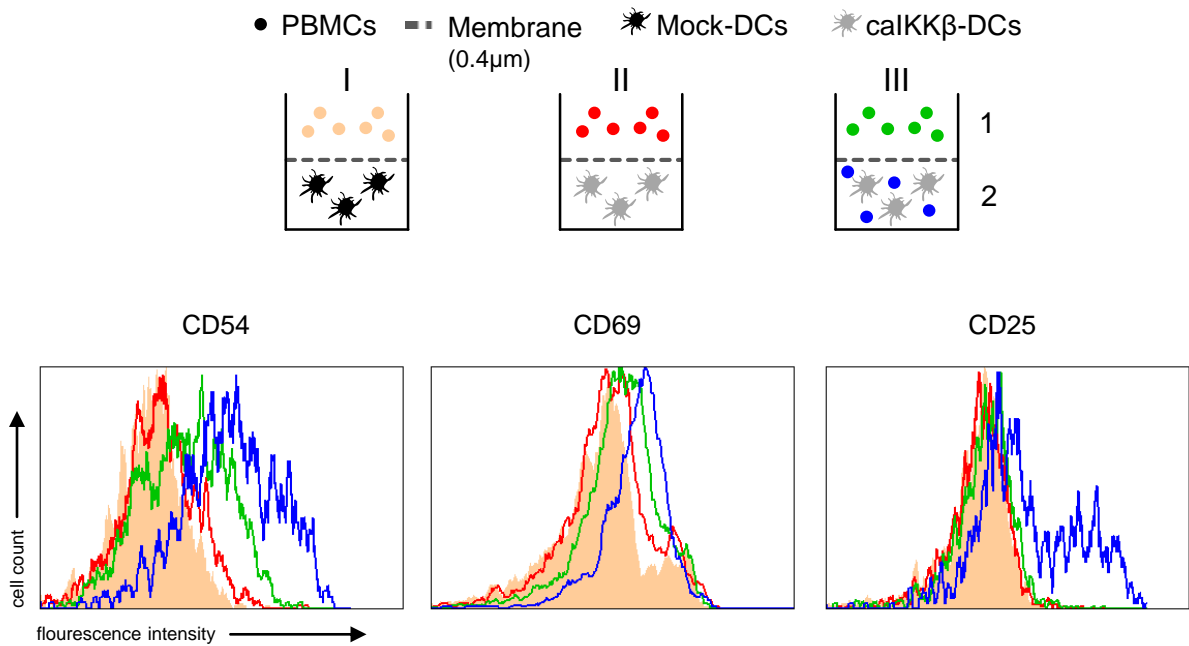
**Supplemental Figure 5. NK-cells maintain activation by stimulation with calKKβ-transfected DCs up to one week**

mDCs were electroporated either with calKKβ RNA or as a control were mock electroporated. Transfected DCs were co-cultured with fresh autologous NK cells at a ratio of 5:1 (A) and 1:1 (A-C) and incubated for one week. (A) Surface marker expressions of CD54, CD69, and CD25 on NK cells were determined through flow cytometry. The average mean fluorescence intensity (MFI) of 4 different donors with SEM is shown; for original data see Supplemental table S11. P-values were calculated using the paired student's t-test, \*\*  $P \leq 0.01$ . (B) Co-cultures were first gated on single and live cells and NK cells are presented through forward and side scatter. (C) Cells were stained with antibodies against CD3 and CD56. Dot plots from a representative donor out of 4 independent donors are shown (B+C).



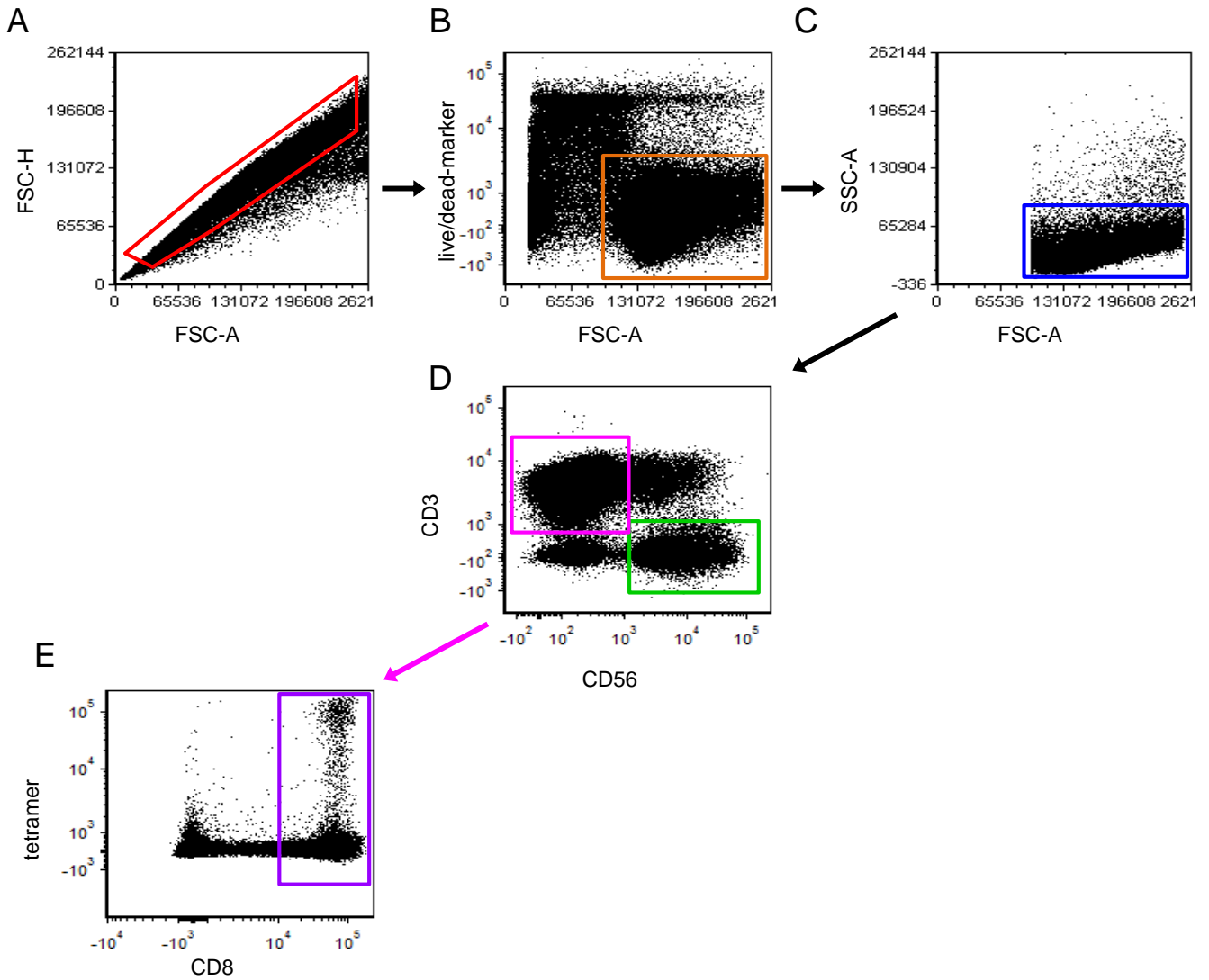
**Supplemental Figure 6.** Secretion of pro-inflammatory cytokines corresponding to Fig 2 normalized to 10<sup>6</sup> DCs (A) or to 10<sup>6</sup> NK cells (B)

Since different numbers of DC and NK cells were present in the co-cultures, we normalized the cytokine secretion to cell numbers. (A) The average cytokine concentration of IL-12p70 from Fig 2A was normalized to 10<sup>6</sup> DCs by dividing by the actual DC concentration in 10<sup>6</sup> per ml (see Materials & Methods section for details). (B) The average cytokine concentration of IFN $\gamma$  and TNF $\alpha$  was normalized to 10<sup>6</sup> NK cells (see Materials & Methods section for details).



**Supplemental Figure 7. Representing histograms corresponding to Fig. 3**

mDCs were electroporated either with calKKβ RNA or as a control were mock electroporated. Transfected DCs were used in a transwell assay as described in Fig. 3A. The histograms show the expression of the surface markers CD54, CD69 and CD25 on NK cells (using the gating strategy shown in Supplemental Fig. S2) for each condition (I-III) as described in Fig 3A. NK cells from condition I are displayed as beige filled histograms, NK cells from condition II as red, NK cells from condition III.1 as green and NK cells from condition III.2 as blue histograms. Histograms from a representative donor out of 4 (I) or 5 (II and III) independent donors are shown.



**Supplemental Figure 8.** Flow-cytometric gating strategy used for Fig. 4 to identify NK cells and CD8<sup>+</sup> T cells

(A) DC/PBMC co-cultures were first gated on single cells (red gate). (B) Co-cultures were then gated on live cells (orange gate). (C) Through forward and side scatter, lymphocytes were gated (blue gate). (D) Cells were gated according to CD56 and CD3 expression for NK cells (CD56<sup>+</sup>/CD3<sup>-</sup>; green gate) and T cells (CD3<sup>+</sup>/CD56<sup>-</sup>; pink gate). (E) T cells were next gated on CD8<sup>+</sup> T cells to determine the percentage of tetramer-binding cells in relation to all CD8<sup>+</sup> cells. Dot plots from a representative donor out of 4 independent donors are shown.