Figure S1

Procedure for the selection of target cells prior to analysis of four populations of peripheral blood nuclear cells (PBCs)

(A) For all retrieved cells, (a) PBCs were roughly gated to exclude aggregated cells. Cells were analyzed for “Area” (x-axis) and “Aspect ratio” (y-axis), as plotted in the bright field (Ch01). The “Area” feature indicates cell size, whereas the “Aspect ratio” indicates the ratio of the minor axis to the major axis (Area Ch01: 60–195 pixels, Aspect ratio Ch01 > 0.6). (b) Nuclear cells were gated by exclusion of erythrocytes and debris. Cells were analyzed for “Area” (x-axis) and Hoechst 33342 (y-axis). (c) Single-nuclear cells were analyzed using parameters of CD33 (x-axis) and side scatter (SSC, y-axis). Cells were roughly divided into three populations: SSC\textsuperscript{low} CD33−, SSC\textsuperscript{int} CD33+, and SSC\textsuperscript{int} CD33++ cells.

(B) For SSC\textsuperscript{low} CD33− cells (R1). (a) SSC\textsuperscript{low} CD33− cells were analyzed by CD3 (x-axis) and CD19 (y-axis). CD3+ CD19− cells (R2) are displayed in (b) and (c). CD19+ CD3− cells (R3) are displayed in (d) and (e).

Gated CD3+ cells (b) or CD19+ cells (d) are displayed in a plot with “Gradient RMS” (x-axis) and “Contrast” (y-axis) in the Ch07 based on Hoechst intensity. These plots are useful for determining focus quality in the nucleus. The next plots, focused CD3+ cells (c) and CD19+ cells (e) are displayed with “Area” Ch01 (x-axis) and Hoechst 33342 (y-axis) to exclude doublet cells and to select similar Hoechst intensities for all target cells.

(C) For SSC\textsuperscript{int} CD33++ cells (R4). (a) Extracted SSC\textsuperscript{int} CD33++ cells in A (c) were used to develop a plot using CD3− (x-axis) and CD19− (y-axis) to exclude other types of cells. The plots of (b) and (c) are displayed using the same axes as those described in B.

(D) For SSC\textsuperscript{int} CD33+ cells (R5). (a) Extracted SSC\textsuperscript{int} CD33+ cells in A (c) were used to develop a plot using CD3− (x-axis) and CD19− (y-axis) to exclude other types of cells. The plots of (b) and (c) are displayed using the same axes as those described in B.