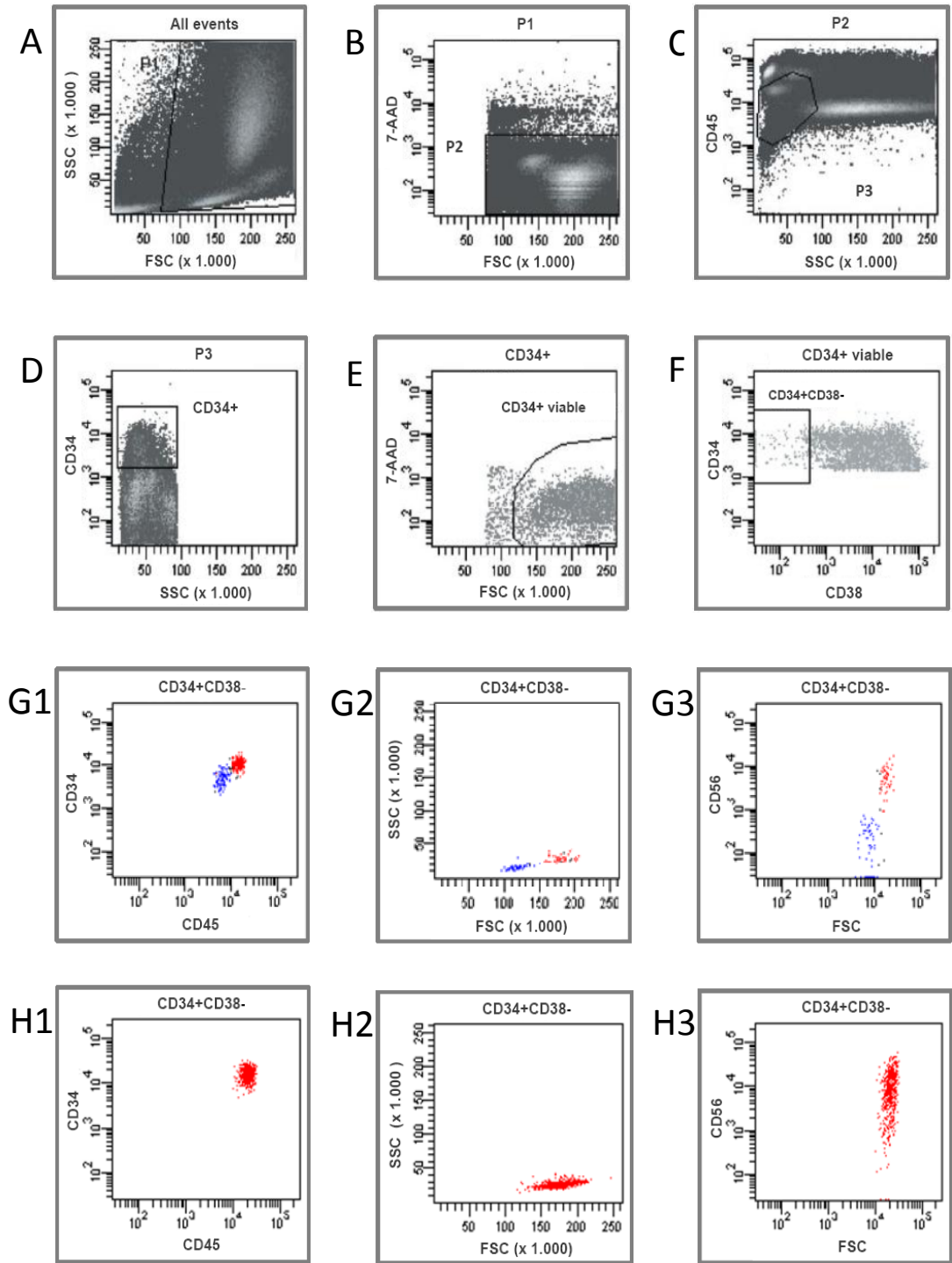


SUPPLEMENTAL MATERIAL

Supplemental Figure 1.



Supplemental Figure 1:

Gating strategy to identify normal and leukemic CD34⁺CD38⁻ cells by multiparameter

flowcytometry (MPFC). MNCs from bone marrow or peripheral blood were separated by Ficoll centrifugation (data not shown). Cells were labeled with fluorescent antibodies as described previously.¹⁴ A-F: gating of CD34⁺CD38⁻ cells. A gate was set subsequently around all mononuclear cells (A), 7-AAD- (viable) cells (B), CD45^{dim} blast population (and lymphocytes) (C), CD34⁺ population (D), followed by a second viability check (E). The CD34⁺CD38⁻ compartment was determined as 1% of all CD38 expressing cells (F).

G1-3 and H1-3: gating of the CD34⁺CD38⁻ subpopulations. The red and blue populations refer to the same populations. Two different cell patterns (G and H) were observed when the CD34⁺CD38⁻ cells were back gated in a CD34CD45 plot: either two distinct cell populations were visible, consisting of a population with relatively high CD34CD45 expression (CD34^{high}CD45^{high}) and a population with relatively low CD34CD45 expression (CD34^{low}CD45^{low}) (G1) or one population was visible, analogous with the CD34^{high}CD45^{high} population in G1 (H1). Next, when back gated in a FSC/SSC plot the CD34^{high}CD45^{high} and CD34^{low}CD45^{low} were FSC^{high}SSC^{high} and FSC^{low}SSC^{low} respectively (G2 and H2).

By FISH analysis the red cells were predominantly Ph positive and therefore considered candidate leukemic stem cells while the blue populations consisted of mostly Ph negative cells and considered normal stem cells (data not shown).

The Philadelphia positive populations expressed one or more aberrant markers (CD7, CD11b or CD56). Expression of CD56 by the different populations is shown in figures G3 and H3.