

Figure 3. Correlation of the SP phenotype with expression of HSC surface antigens in mouse bone marrow cells using violet excitation. Cells were stained with Hoechst 33342 alone (**A,B,C**), or in combination with c-Kit-PE-Cy5 and Sca-1-PE (**D,E,F**). Samples were analyzed on a FACSVantage SE/FACSDiVa equipped with an Innova 302C krypton-ion laser providing 100 mW of 407 nm excitation. Optical filter configuration was the same as described in **Fig. 1** (see also **Notes 1, 12** and **13**). 500,000 events were collected. For the Hoechst 33342/c-Kit/Sca-1 sample, the Hoechst 33342 staining profile is shown in (**D**), and the cell surface antigen staining profile is displayed as c-Kit (y-axis) vs. Sca-1 (x-axis), with the region KS defining co-expression of both antigens (**E**). When cells within the SP region were displayed on a c-Kit vs. Sca-1 plot, the majority showed co-expression of both c-Kit and Sca-1 (**F**). The sample stained with Hoechst 33342 alone served as a control (**A, B** and **C**).

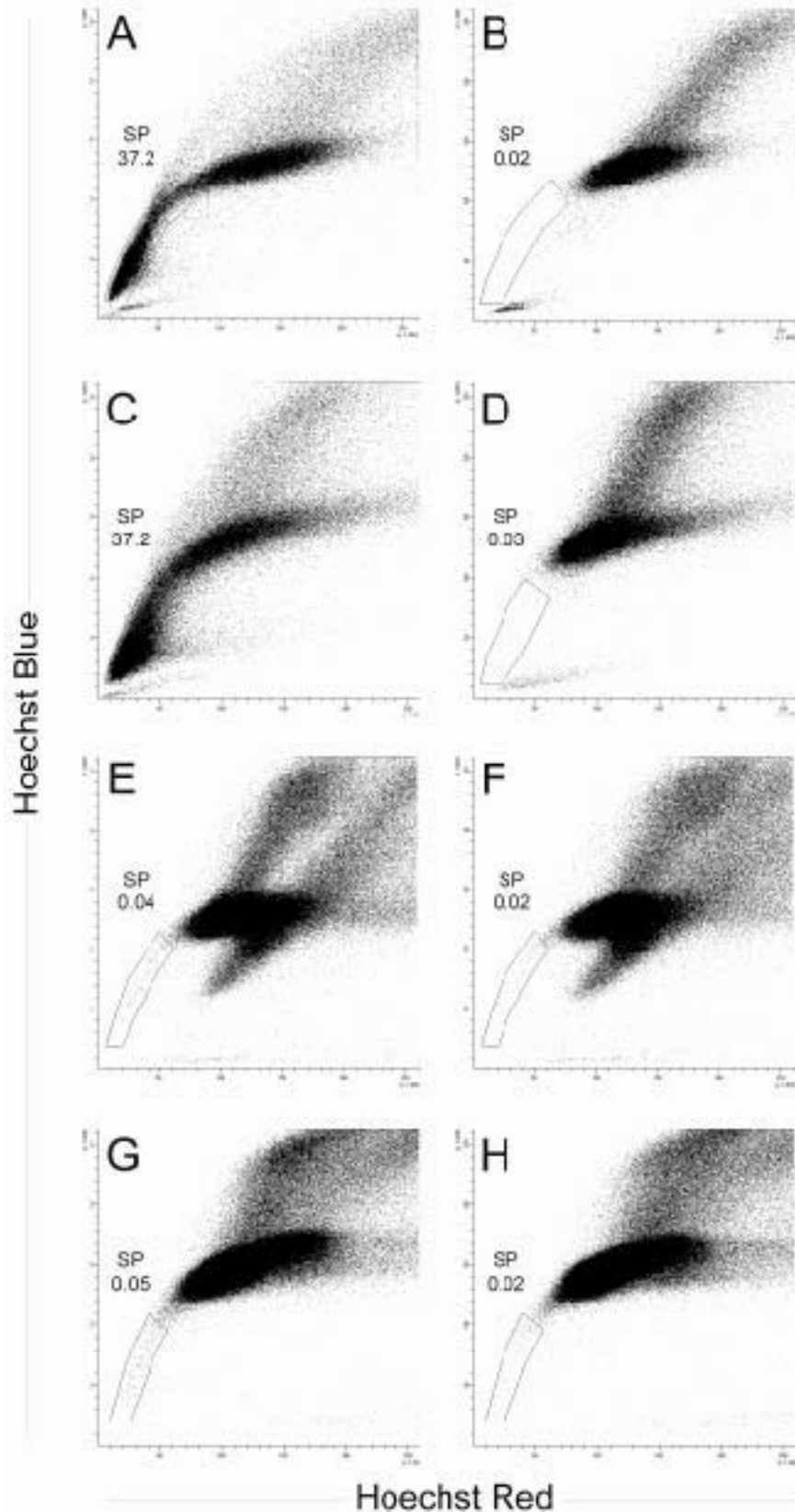


Figure 6. Direct correlation of ABCG2 activity and the SP phenotype. A549 human lung carcinoma cells, expressing high levels of ABCG2, were stained with 5 $\mu\text{g}/\text{mL}$ of Hoechst 33342 at 37°C for 90 min, in the absence (A,C) or presence (B,D) of 1 μM FTC. Samples were analyzed on a BD LSR with 8 mW of UV excitation (A,B), and on a FACSVantage SE/FACSDiVa with 50 mW of violet excitation (C,D). 100,000 events were collected. As previously reported by Scharenberg *et al.* (24), FTC reduced the number of cells within the SP region by inhibiting ABCG2 efflux activity. C57BL/6 mouse bone marrow cells were stained with Hoechst 33342 in the absence (E,G) or presence (F,H) of 1 μM FTC. Samples were analyzed on a BD LSR with 8 mW of UV excitation (E,F), and on a FACSVantage SE/FACSDiVa with 100 mW of violet excitation (G,H). 400,000 live (PI-negative) cells were analyzed. FTC reduced the number of the cells within the SP region.