

Supplemental Figure Legends

Supplemental Figure 1. Flow charts of two scenarios to analyze cytokine signaling in murine HSCs based on the current phospho-flow procedure.

Supplemental Figure 2. CD150⁻ CD41⁻ cells and CD150⁺ CD41⁻ cells are highly purified from Sca1⁺ enriched cells. Percentages of CD150⁻ CD41⁻ cells and CD150⁺ CD41⁻ cells in Sca1⁺-enriched cells are presented as mean \pm s.d. (n=9). The sorted cells were reanalyzed using the same setting of the same sorter. A purity above 95% was routinely obtained from all the independent sorting experiments. Results of a representative experiment are shown.

Supplemental Figure 3. SCF signaling study in LK, LK Sca1⁻, and LK Sca1⁺ cells.

Sca1⁻ and Sca1⁺ cells were separated using AutoMACS. The purified cells were starved and stimulated with 1 or 10 ng/ml of SCF (dose study), or 10 ng/ml of SCF for 10 or 30 minutes (time-course study) as described in Figure 5 in parallel with LK cells. Levels of phosphorylated ERK1/2 (pERK1/2) were measured and quantified as described in Figure 5. The Lin cocktail includes CD3, CD4, CD8, Ter119, B220 and Gr1.

Supplemental Figure 4. A schematic picture illustrating the relative strengths of SCF, TPO, GM-CSF signaling in HSCs, MPPs, and LK cells.