

Figure S1. Combined inhibition of the GSK3 β and p38MAPK pathways significantly improves *in vitro* hematopoietic stem cell expansion. Whole bone marrow cells were treated with 2.5 μ M SB216763 in combination with different concentrations of SB203580 (0.5, 1, 2, 2.5 μ M) for 9 days. (A) Cell morphology. Magnification, x40; scale bar, 1,000 μ m. Images were obtained using the confocal Leica DM RXA microscope. (B) LSK cells were analyzed by flow cytometry following treatment with 1 μ M SB203580 and 2.5 μ M SB216763. (C) Total cell numbers. (D) Lin⁻ cell frequency. (E) Relative LSK cell number. (F) Absolute LSK cell number. n=4. *P<0.05, **P<0.01. APC, allophycocyanin; PE, phycoerythrin; Lin, lineage; GSK3 β , glycogen synthase kinase 3 β ; Sca-1, stem cells-antigen-1.

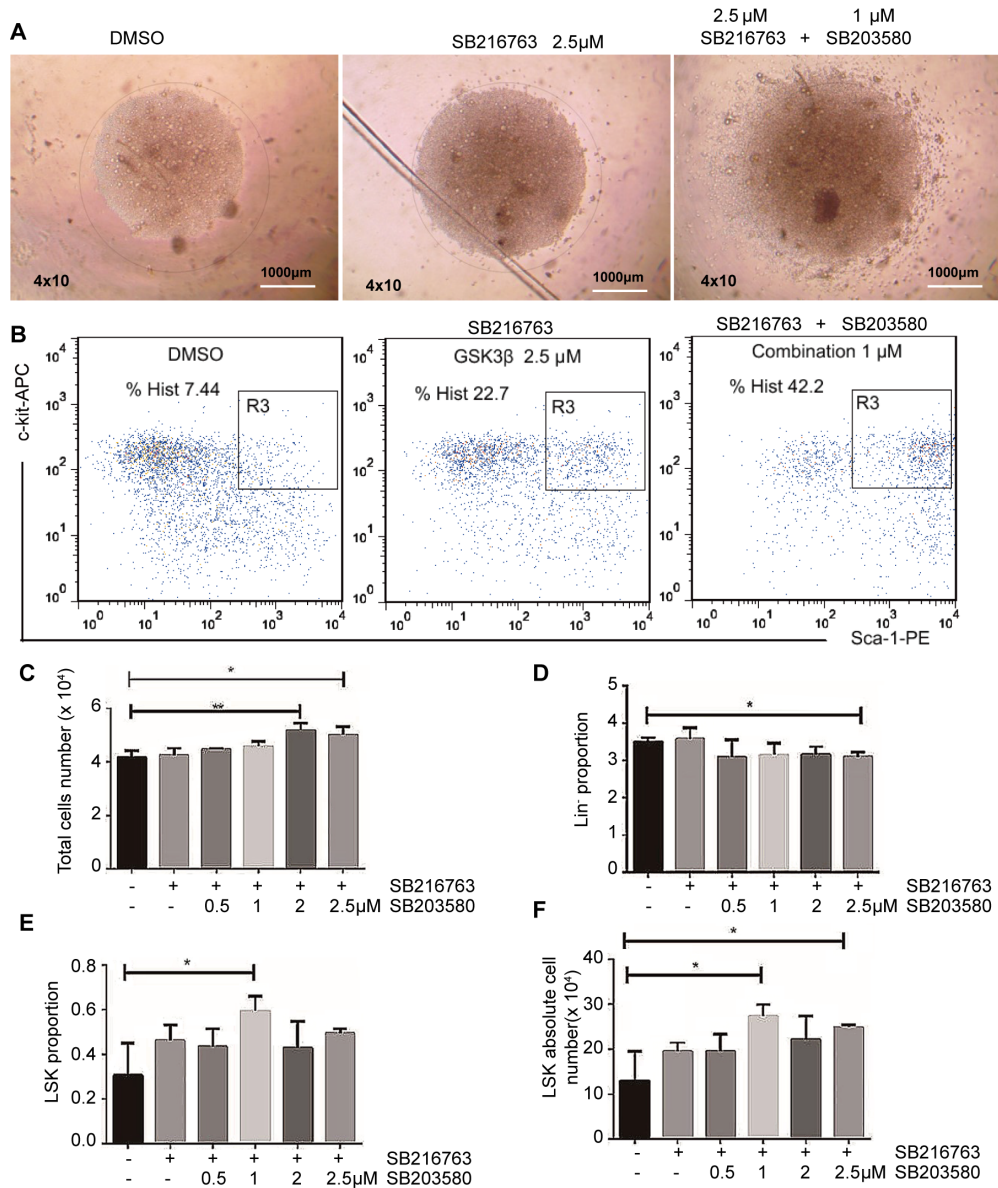


Figure S2. Selection of an optimal concentration of the glycogen synthase kinase 3 β pathway inhibitor CHIR99021 (50, 100, 500 or 1,000 nM). LSK cells were isolated after 9 days in culture and stained for surface expression using antibodies against Gr1, CD11b, Ter119, CD3e, B220, Sca-1, c-Kit and streptavidin, followed by flow cytometric analysis. (A) LSK flow cytometry dotplots. (B) Cell morphology. Magnification, x40; scale bar, 1,000 μ m. Images were obtained using the confocal Leica DM RXA microscope. (C) Total cell number. (D) Lin⁺ cell frequencies. (E) Lin⁺ cell numbers. (F) LSK frequencies. (G) LSK cell numbers. n=4. *P<0.05, **P<0.01, ***P<0.001. APC, allophycocyanin; PE, phycoerythrin; Lin, lineage; SS, side scatter; Sca-1, stem cells-antigen-1.

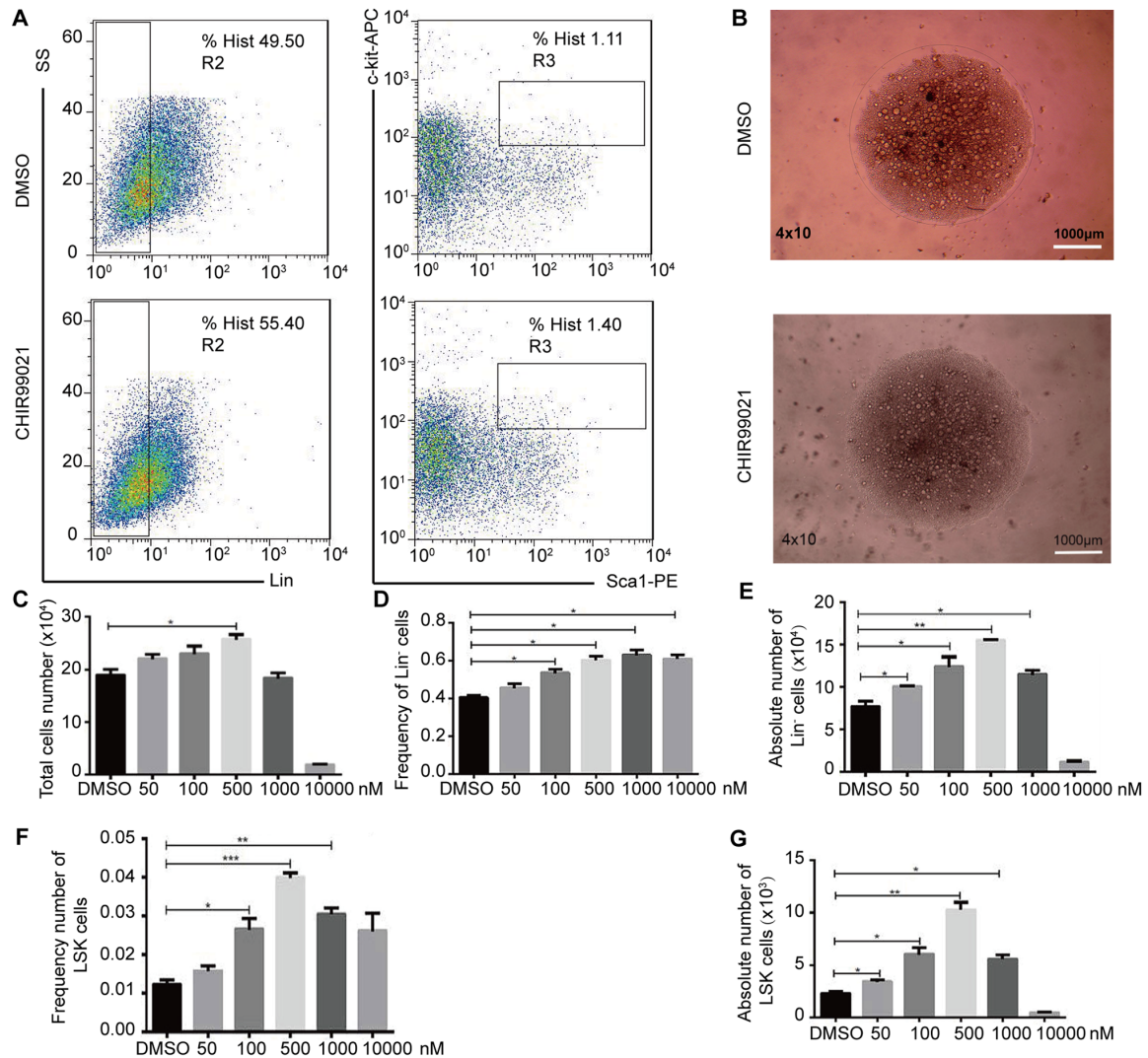


Figure S3. Selection of an optimal concentration of the glycogen synthase kinase 3 β pathway inhibitor SB216763. Hematopoietic stem cells were stained following SB216763 (1, 1.5, 2, 2.5 or 3 μ M) for 9 days in culture and analyzed via flow cytometry. (A) Cell morphology. Magnification, x40; scale bar, 1,000 μ m. Images were obtained using the confocal Leica DM RXA microscope. (B) Flow cytometric plots for LSK cells following SB216763. (C) Total cell numbers following SB216763 (1, 1.5, 2, 2.5 or 3 μ M). (D) Proportion of Lin⁻ cells following SB216763. (E) Proportion and (F) absolute LSK cell numbers following SB216763. n=4. *P<0.05, **P<0.01. APC, allophycocyanin; PE, phycoerythrin; Lin, lineage; Sca-1, stem cells-antigen-1.

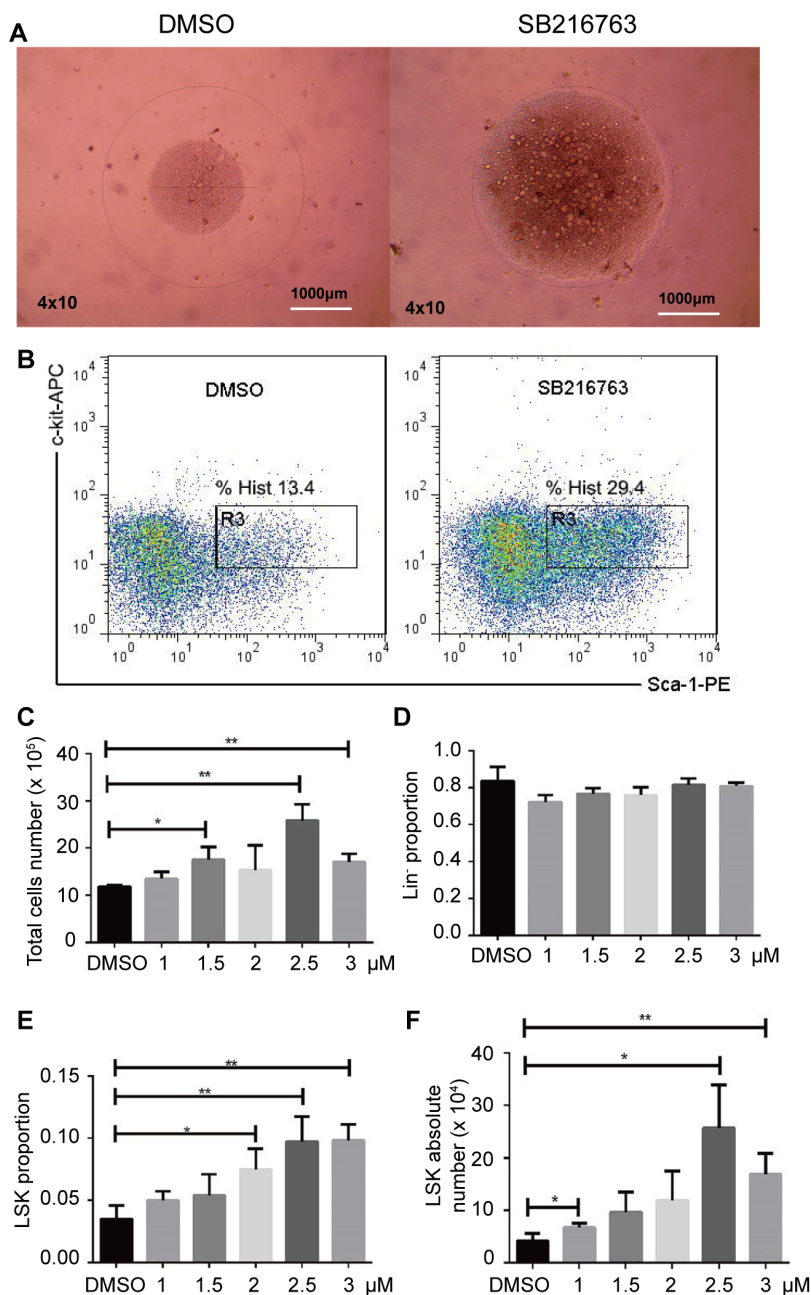


Figure S4. Effect of DMSO on cells. To exclude the influence of DMSO on cells, morphological changes of cells, total cell numbers, Lin⁺ cell frequencies, Lin⁺ cell numbers, LSK cell numbers and LSK cell frequencies were observed under a microscope. NS, not significant. (A) Cell morphology. Magnification, x40; scale bar, 1,000- μ m. Images were obtained using a confocal Leica DM RXA microscope. (B) Lin⁺ cell frequencies. (C) Total cell numbers. (D) Absolute of LSK cell numbers. (E) LSK cell frequencies. (F) Absolute of Lin⁺ cell numbers.

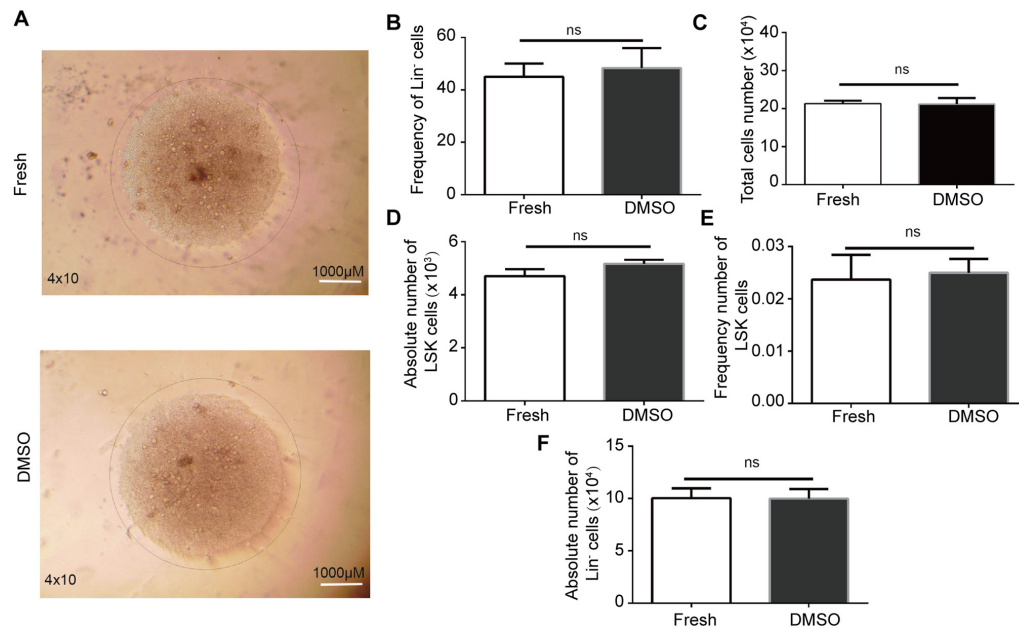


Figure S5. Assessment of the effects of signaling inhibitor combinations of hematopoietic stem cell expansion *in vitro*. The results of the group A and DMSO are presented in Fig. S4. LSK cells were sorted and treated with the indicated combinations of inhibitors presented in Table I for 9 days, after which the cell morphology, as well as the ratios and numbers of LSK/Lin⁻ cells were assessed. (A) Cell morphology. Magnification, x40; scale bar, 1,000- μ m. Images were obtained using a confocal Leica DM RXA microscope. (B) Total cell numbers. (C) Lin⁻ cell frequencies. (D) Lin⁻ cell numbers. (E) LSK cell numbers. (F) LSK cell frequencies. n=4. *P<0.05 vs. B. For abbreviations of group names see Table I. n=4. Lin, lineage.

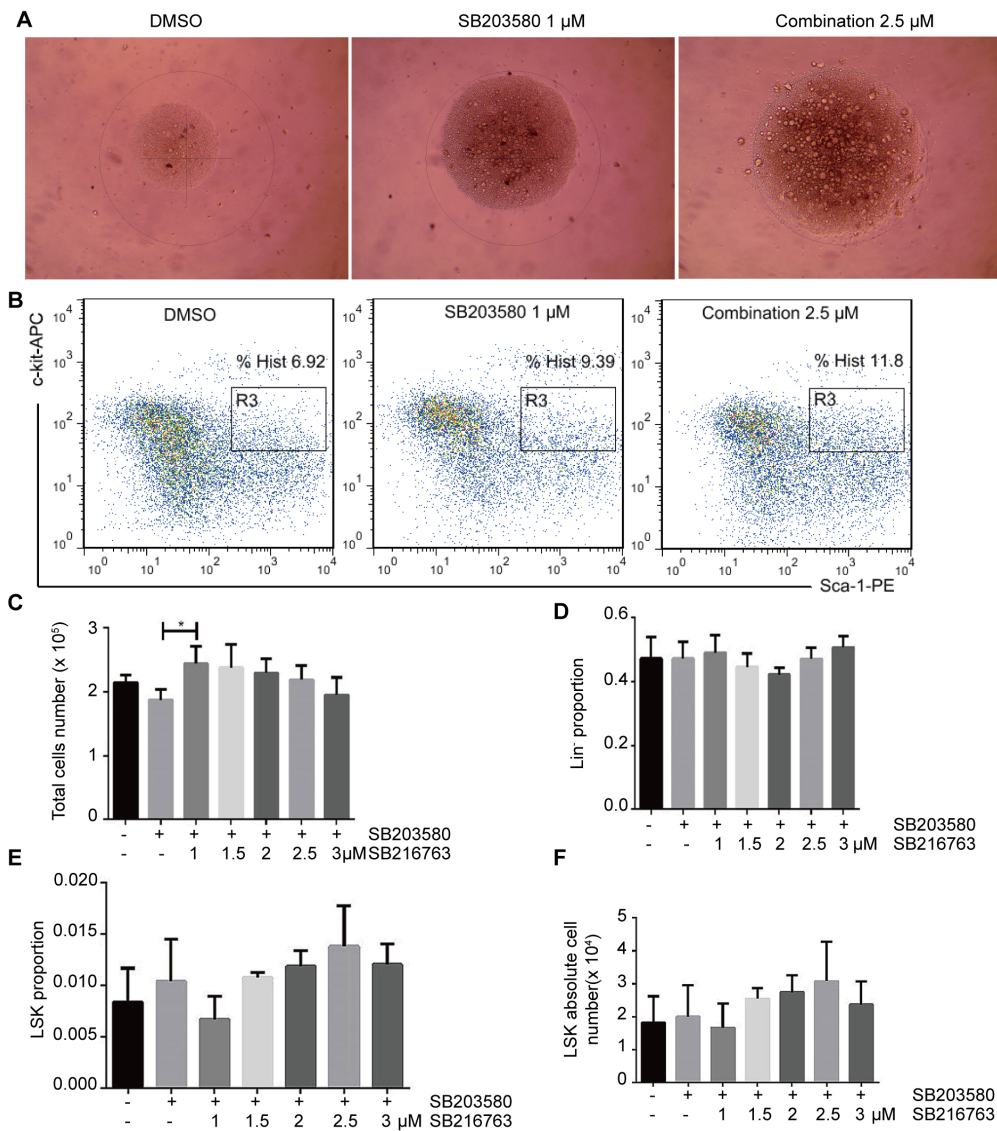


Figure S6. Assessment of the effects of DMSO on HSC expansion compared with freshly isolated HSCs *in vitro*. In order to eliminate the effect of solvents on cells, LSK cells were sorted and treated with or without equal volume DMSO for 9 days. (A) Cell morphology. Magnification, x40; scale bar, 1,000- μ m. Images were obtained using a confocal Leica DM RXA microscope. (B) Total cell number. (C) Frequency of Lin⁺ cells. (D) Absolute of Lin⁺ cells. (E) Absolute of LSK cell. (F) Frequency of LSK cell. n=4. Fresh is group A in Table I. *P<0.05. HSC, hematopoietic stem cells; NS, non-significant; Lin, lineage.

