Supplemental information

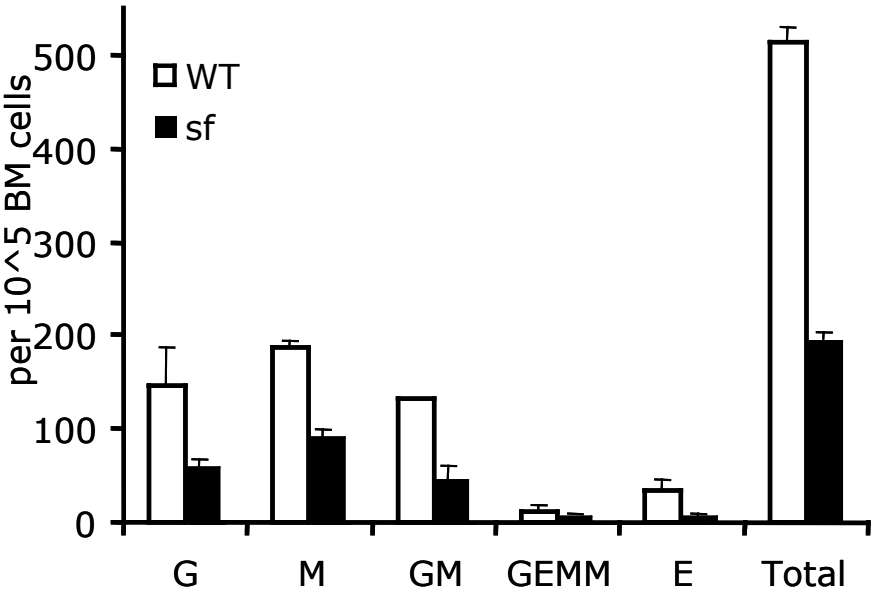

Fig. S1. CFU assay for progenitor cells in 3 weeks old WT and scurfy mice. E, erythroid; G, granulocyte; GEMM, granulocyte erythroid macrophage and megakaryocyte; GM, granulocyte and macrophage; M, macrophage. Data shown were means+/-S.D. of 3 independent experiments, each involving bone marrow from one mouse per group. Wt, wild-type, sf, scurfy.

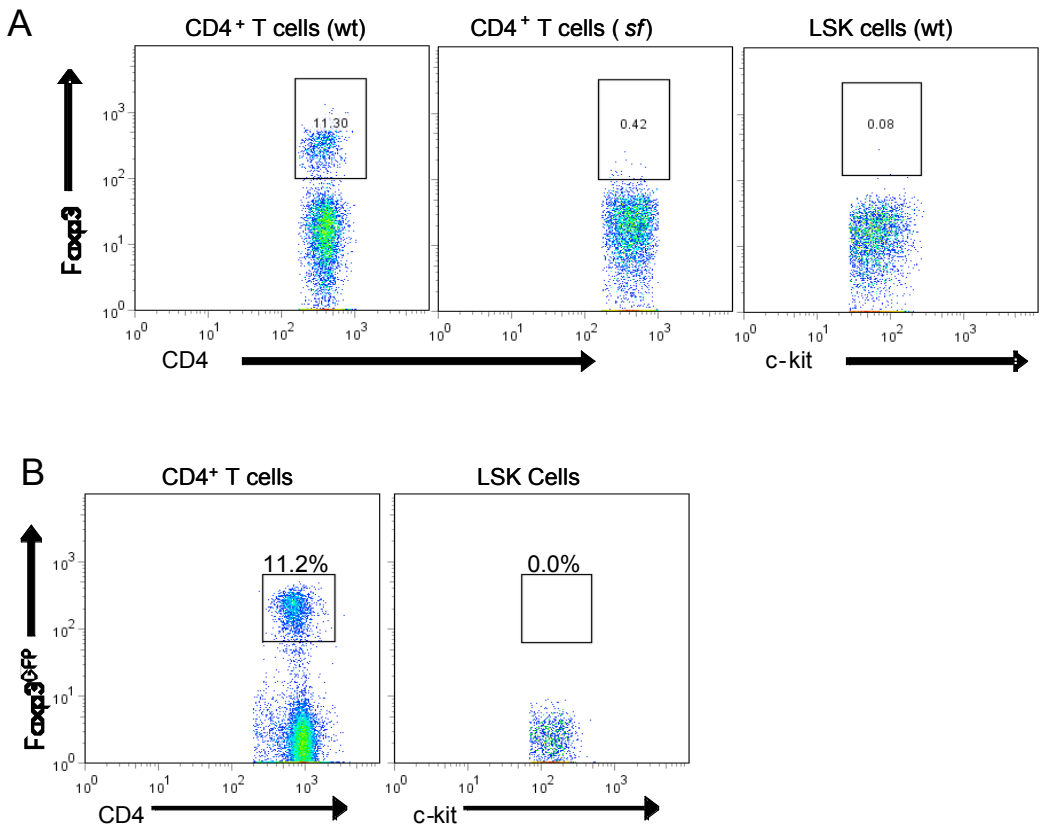

Fig S2. Foxp3 was not expressed in HSCs A. Foxp3 staining of $CD4^+$ splenocytes from wildtype and scurfy mice and $lin^-Sca\text{-}1^+c\text{-}kit^+$ cells (LSK) bone marrow cells from wildtype. B. GFP intensity of $CD4^+$ splenocytes and LSK cells from $Foxp3^{GFP}$ mice. Data shown are representative of at least 3 independent experiments.

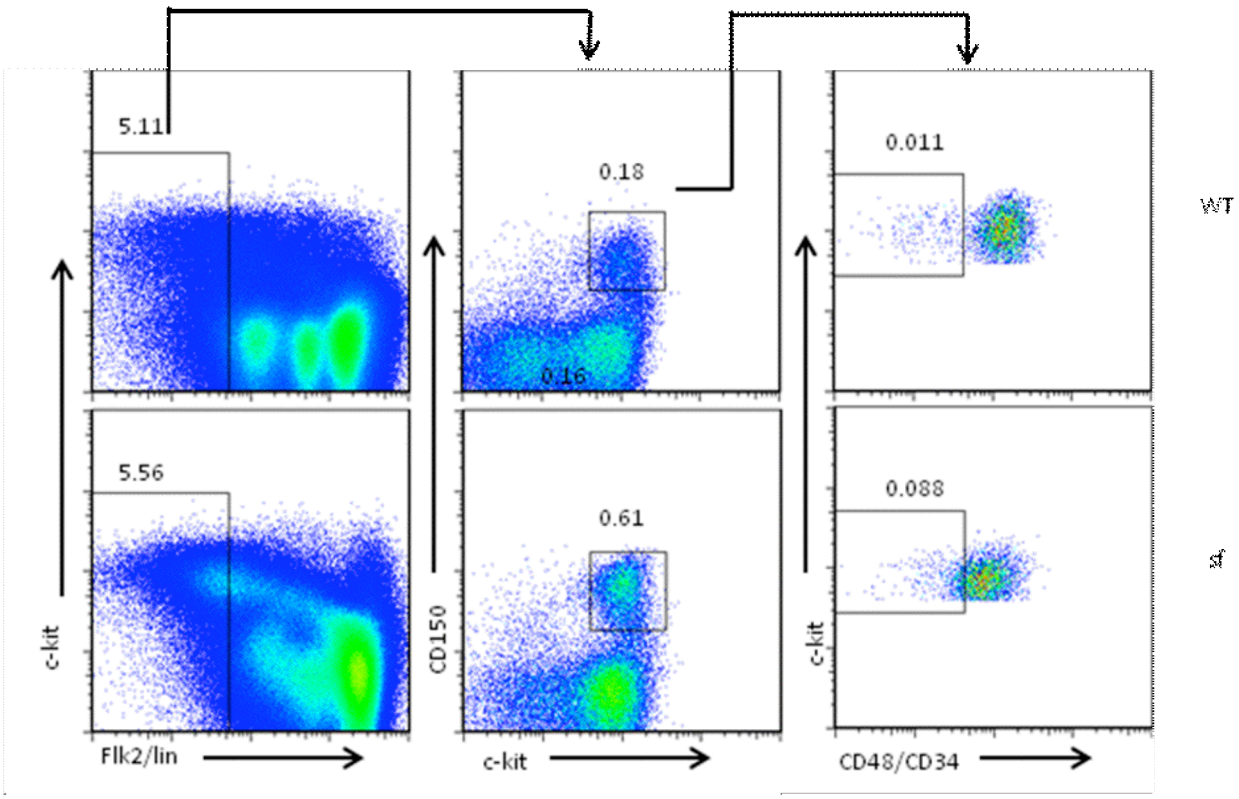

Fig. S3. Frequencies of HSC in bone marrow of the 3-weeks old WT and scurfy littermates as shown in Fig. 2a when bone marrow cells were gated without Sca-1 marker. Representative FACS profiles are presented using $Flk2^{-}$ $lin^{-}c\text{-}kit^{+}CD34^{-}$ $CD150^{+}CD48^{-}$ as HSC markers.

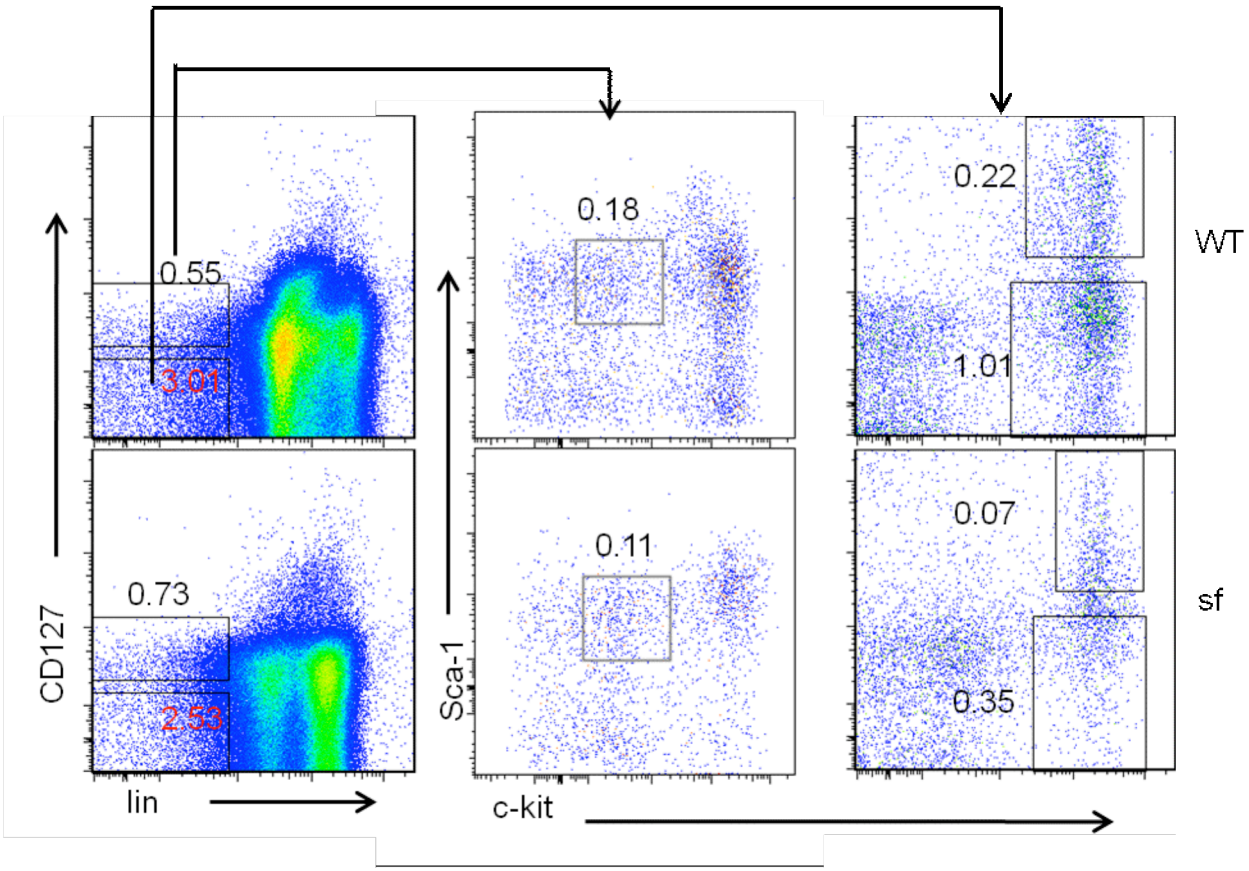

Fig. S4. Frequencies of progenitor cells in bone marrow of the 4-weeks old WT and scurfy littermates. The summary of frequencies and numbers of CLP and MP are presented in Figure 2C. Representative FACS profiles are presented. CLP is presented in middle panel using $lin^{-}CD127^{+}c\text{-}kit^{+/lo}Sca\text{-}1^{+/lo}$ as markers. MP is presented in right panel using $lin^{-}CD127^{-}c\text{-}kit^{+}Sca\text{-}1^{-}$ as markers.

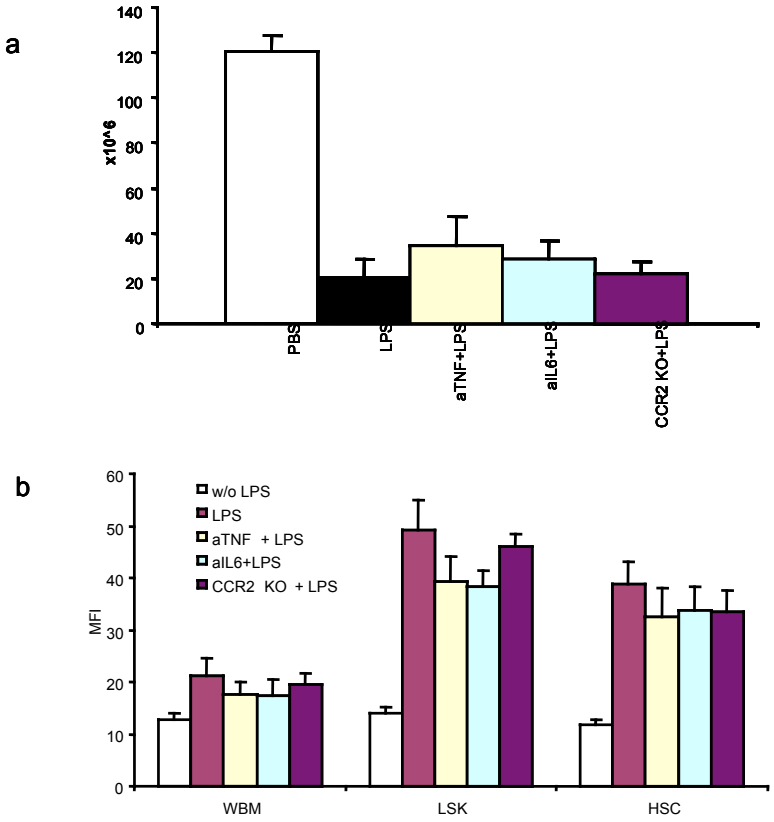

Fig. S5. Blockade of single cytokines was ineffective in preventing bone marrow hypocellularities (A) or mTOR activation in HSCs (B) induced by LPS. A. Similar as Fig. 3B, 6-8-week old WT or Ccr2$^{-/-}$ mice received PBS or LPS on days 0, 3 (0.3 mg per mice). At both time points, the WT mice were treated with 100 μg anti-IL6 or anti-TNF_ antibodies or control mouse IgG. Bone marrow cells were counted on day 7. n=3. B. WT or *Ccr2*$^{-/-}$ mice were treated with PBS (control) or 0.3 mg LPS. 2 hours after treatment, bone marrow cells were harvested and the levels of phosphorylated mTOR in whole bone marrow cells (BM), LSK and HSC were measured by flow cytometry. Shown the MFI ±SD. n=3.

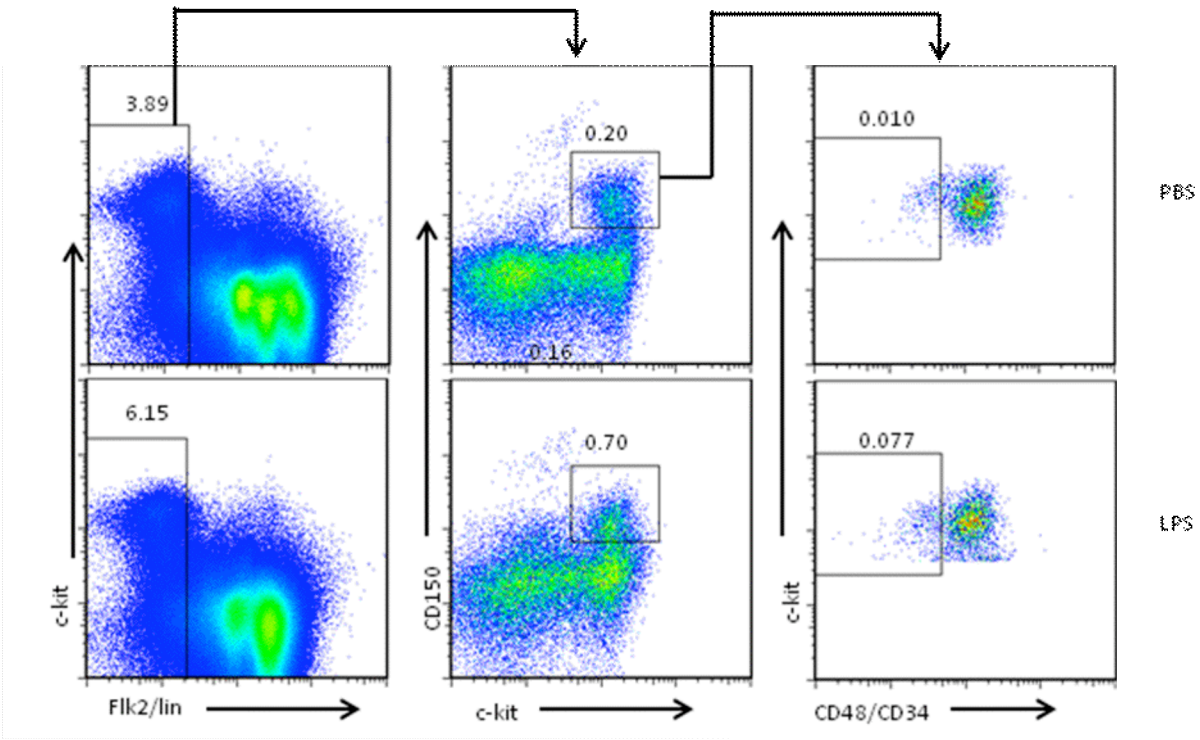

Fig. S6. Frequencies of stem and progenitor cells in bone marrow of the C57BL/6 mice treated with PBS or LPS. The bone marrow cells are gated without Sca-1 marker. Representative FACS profiles are presented using $Flk2^{-}$ $lin^{-}c\text{-}kit^{+}CD34^{-}$ $CD150^{+}CD48^{-}$ as HSC markers.

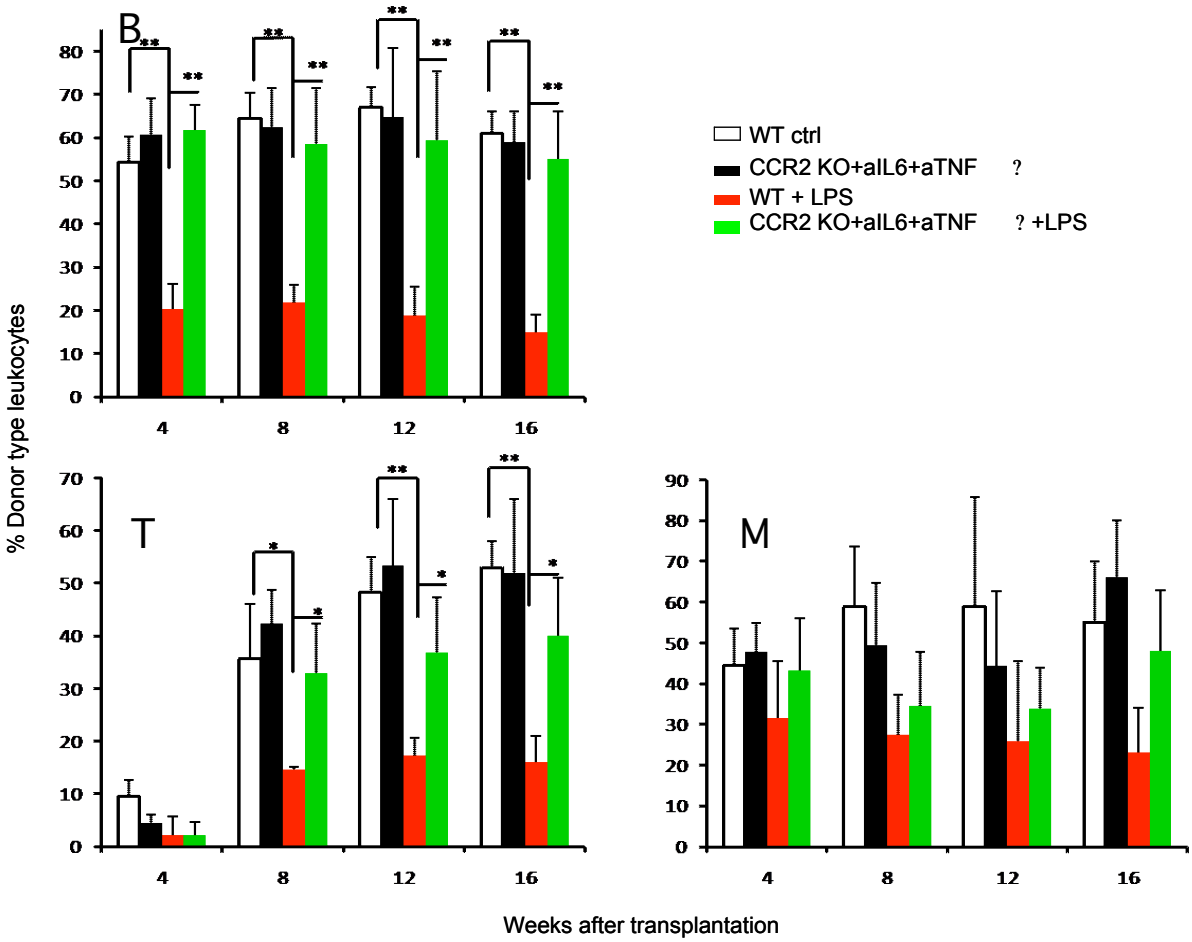

Fig. S7. LPS treatment impaired the HSC's long-term reconstitution of T and B cells through inflammatory cytokines. WT or IL6 and TNF_-treated $Ccr2^{-/-}$ mice were treated with PBS or LPS twice. 4 days after the second treatment, mice were sacrificed and bone marrow cells were isolated. $5x10^5$ bone marrow cells, mixed with equal number of recipient-type (CD45.1) bone marrow cells, were transplanted into lethally irradiated CD45.1 C57BL/6 recipients. Reconstitution ratios in the recipient peripheral blood by the donor cells were monitored at 4, 8 12 and 16 weeks after transplantation. B, $B220^+$ B lymphocytes; T, CD3 T lymphocytes; M, $Mac\text{-}1^+$ myeloid cells. Shown mean ± S.D. n=10. *, $p<0.05$; **, $p<0.01$.

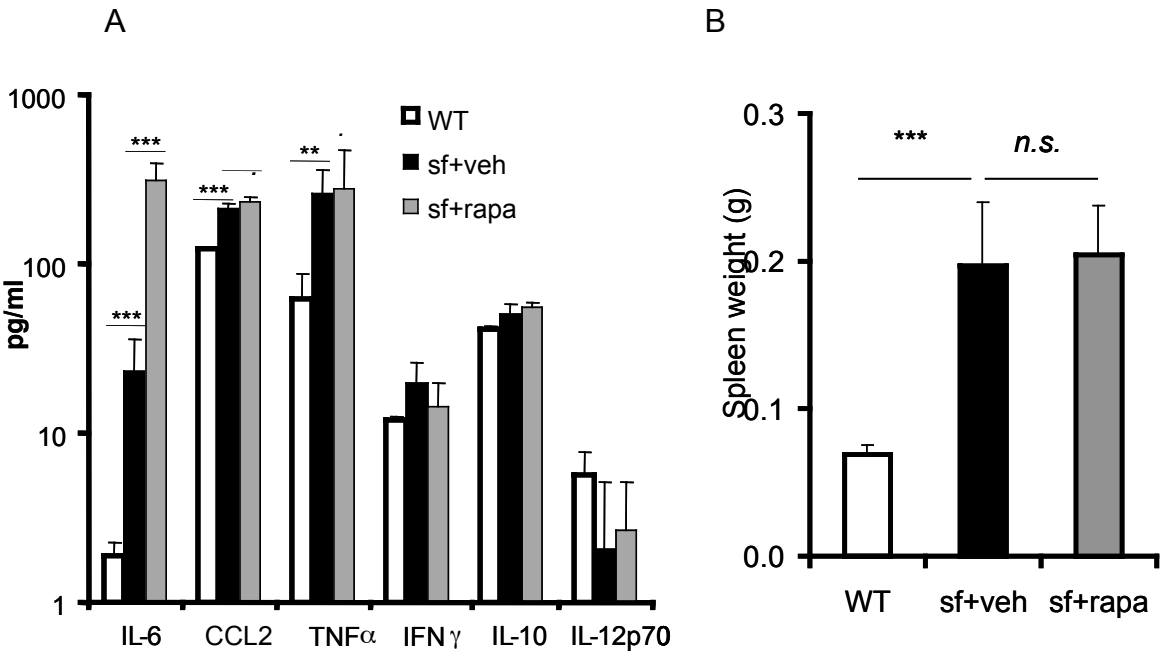

Fig. S8. Rapamycin treatment does not reduce the production of inflammatory cytokines (A) or splenomegaly (B) in scurfy mice. A. Mouse sera were collected after two weeks of treatment and the given cytokines were measured by multiple cytokine bead arrays. Data shown were means±S.D. (n=3). B. Spleen weight. Data shown are means±S.D. (n=3) of the weight of spleens from the Scurfy mice treated with either vehicle or rapamycin. Data for WT littermates were included as controls.

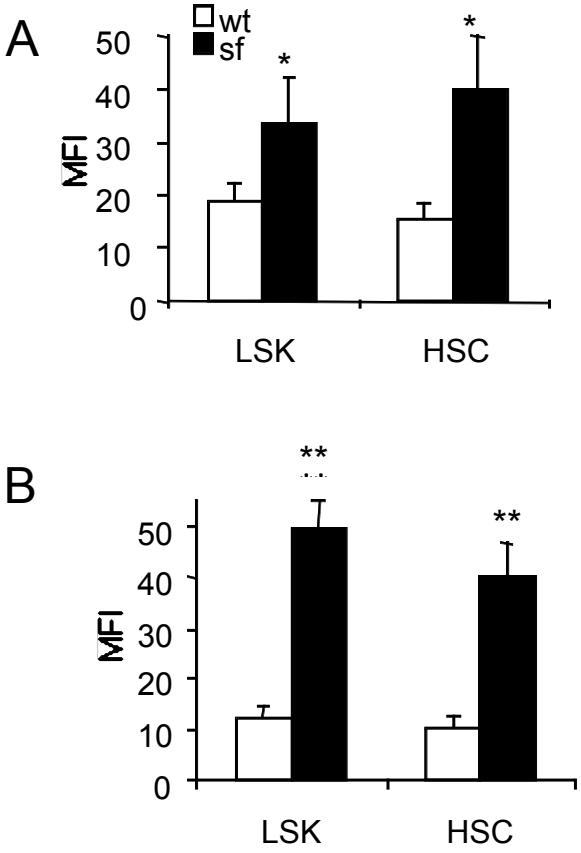

Fig. S9. Hyperactivity of mTOR in HSCs of the Scurfy mice. Bone marrow cells from 3 weeks old Scurfy mice and their littermate controls were stained with antibodies specific for phosphorylated mTOR (a) and phosphorylated S6 (b). The mean fluorescences±S.D (n=4, from two independent experiments, each involving 2 mice per group.) are presented. *, $p<0.05$; **, $p<0.01$.

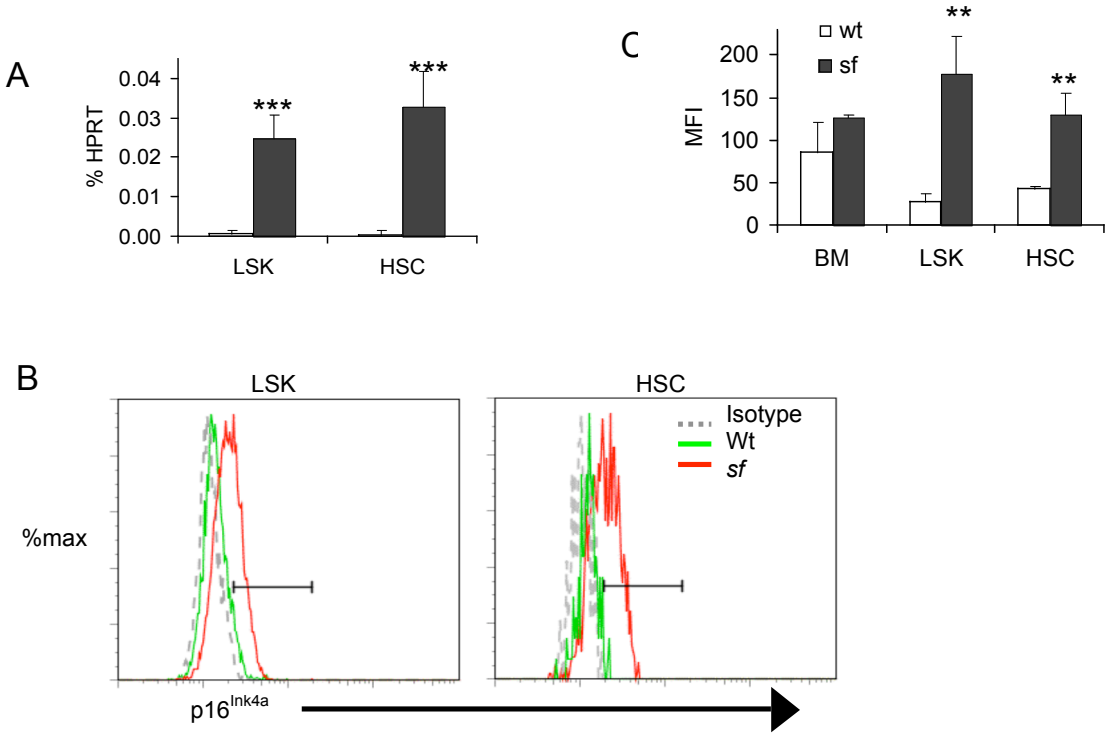

Fig. S10. Premature expression of senescence the marker $p16^{Ink4a}$ in HSC from the Scurfy mice. A. The levels of *p16Ink4a* mRNA in LSK and HSCs from 21 days old Scurfy mice and their littermate control, as determined by RT-PCR. Data shown are means and S.D. from three independently sorted HSC per group. B-C. The intracellular staining of $p16^{Ink4a}$ on LSK and HSCs from 3 week old Scurfy mice and their littermates. b. The representative histogram, depicting expression of $p16^{Ink4a}$. c. Summary of mean fluorescence intensity of $p16^{Ink4a}$. Data shown are mean ± S.D. n=4 (from two independent experiments, each involving 2 mice per group.). **, $p<0.01$; ***, $p<0.001$.

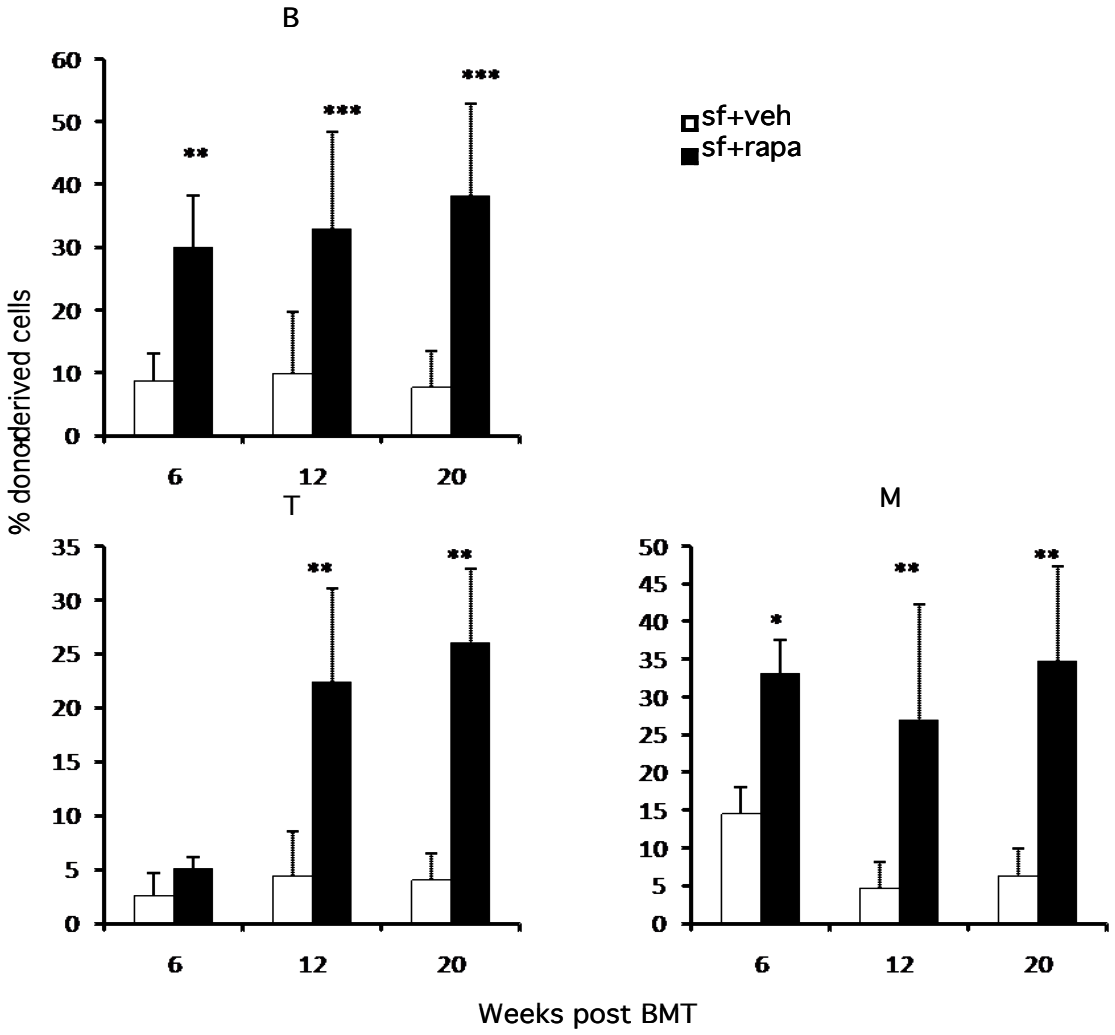

Fig. S11. Short-term treatment of rapamycin in the Scurfy mice increased their HSC function. Data shown are the % of CD45.2^{+} donor (Scurfy) bone marrow-derived cells in the peripheral blood at various time points after bone marrow transplantation. B cells are defined as B220^{+}; T cells are defined as CD3^{+}, while myeloid cells are defined as CD11b^{+} cells. Data shown are means+/-S.D., n=15 from 3 independent donors per group. *, $p<0.05$; **, $p<0.01$; ***, $p<0.001$.