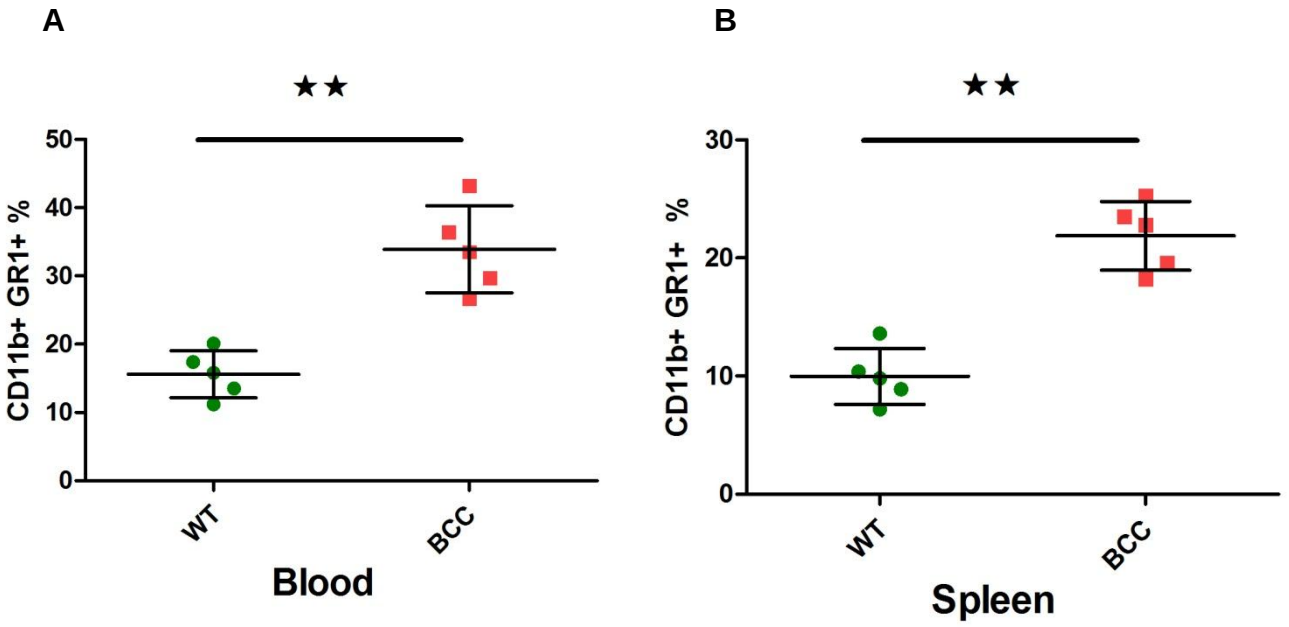
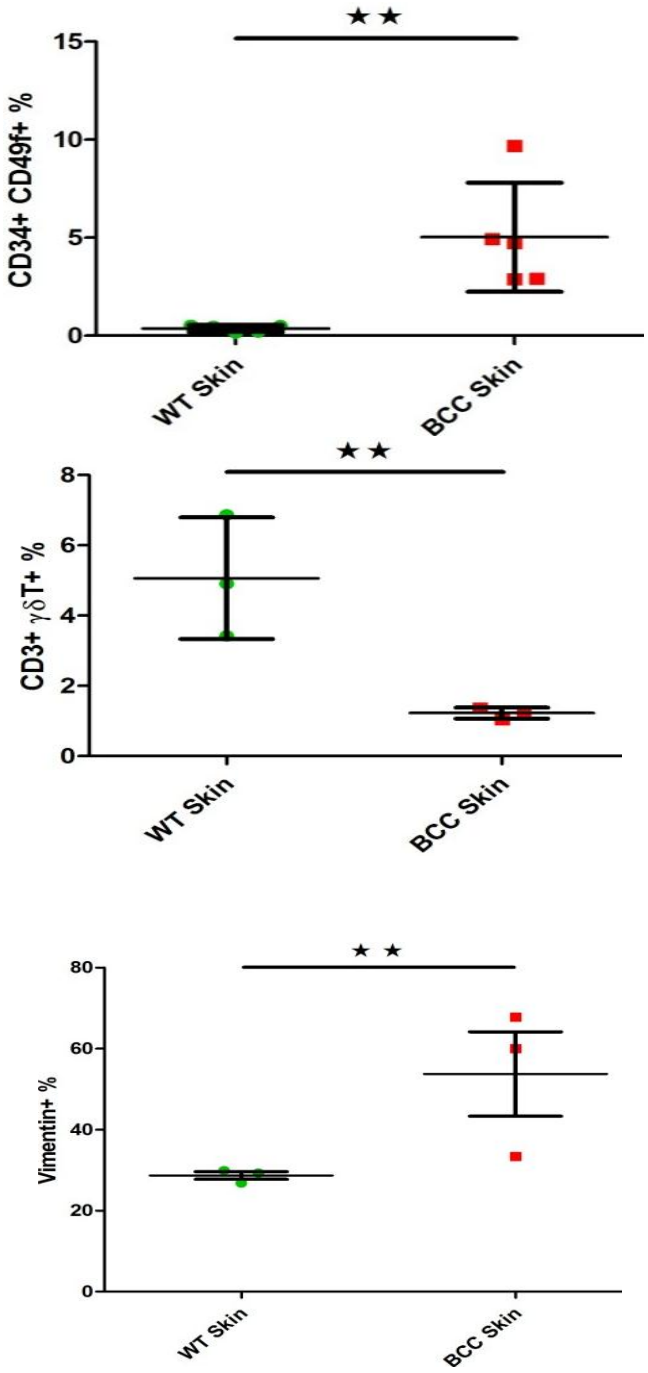
SUPPLEMENTARY DATA

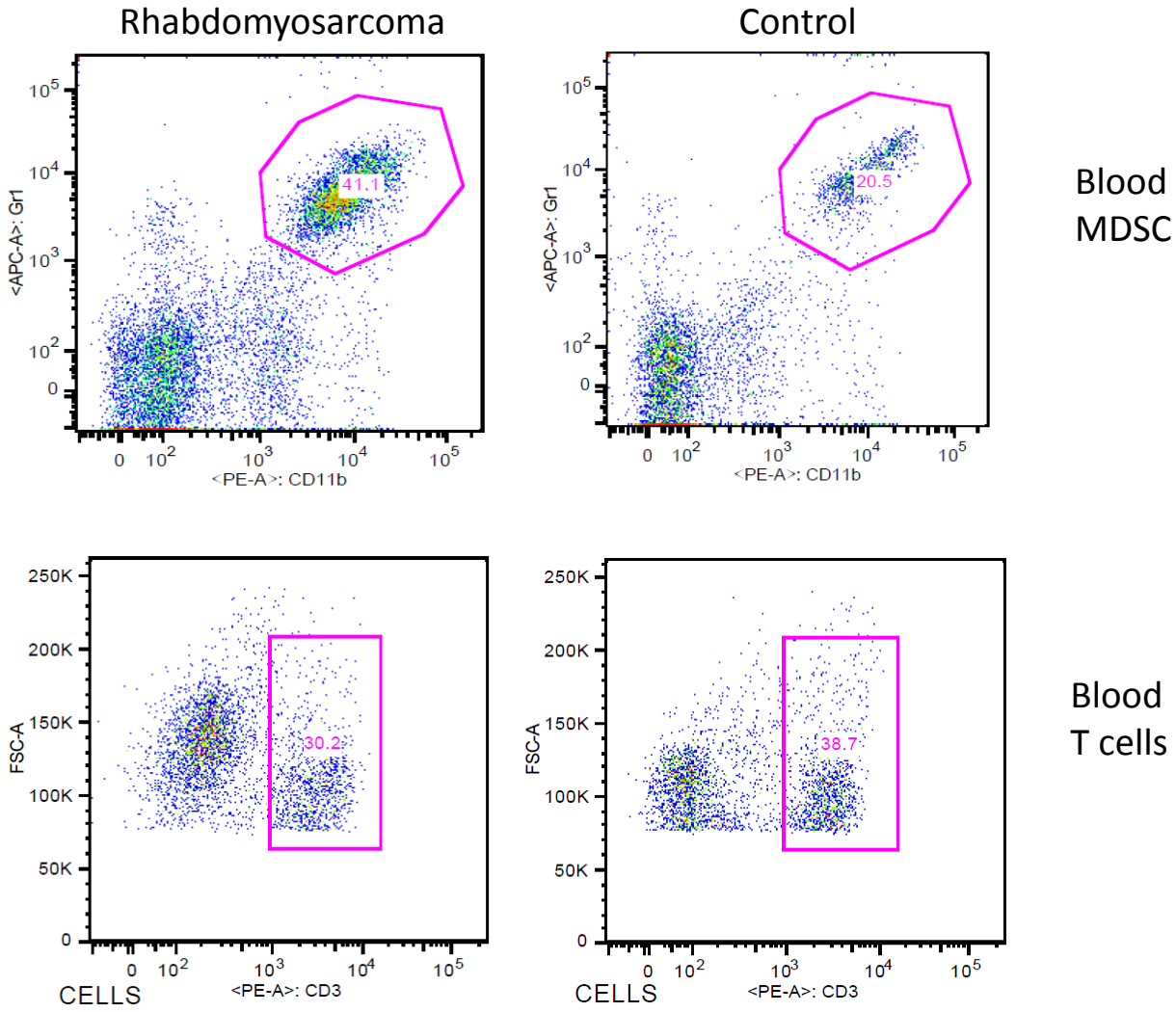
Supplementary Figure 1- Flow cytometry analyses of $CD11b^{+}Gr1^{+}$ cells in peripheral blood and spleen of mice. Peripheral blood and spleen were collected from wt or skin tumor bearing mice, and non-blood cells were analyzed for expression of CD11b/Gr1 using flow cytometry. **A** shows $CD11b^{+}Gr1^{+}$ cells from peripheral blood and **B** shows $CD11b^{+}Gr1^{+}$ cell population from spleen.



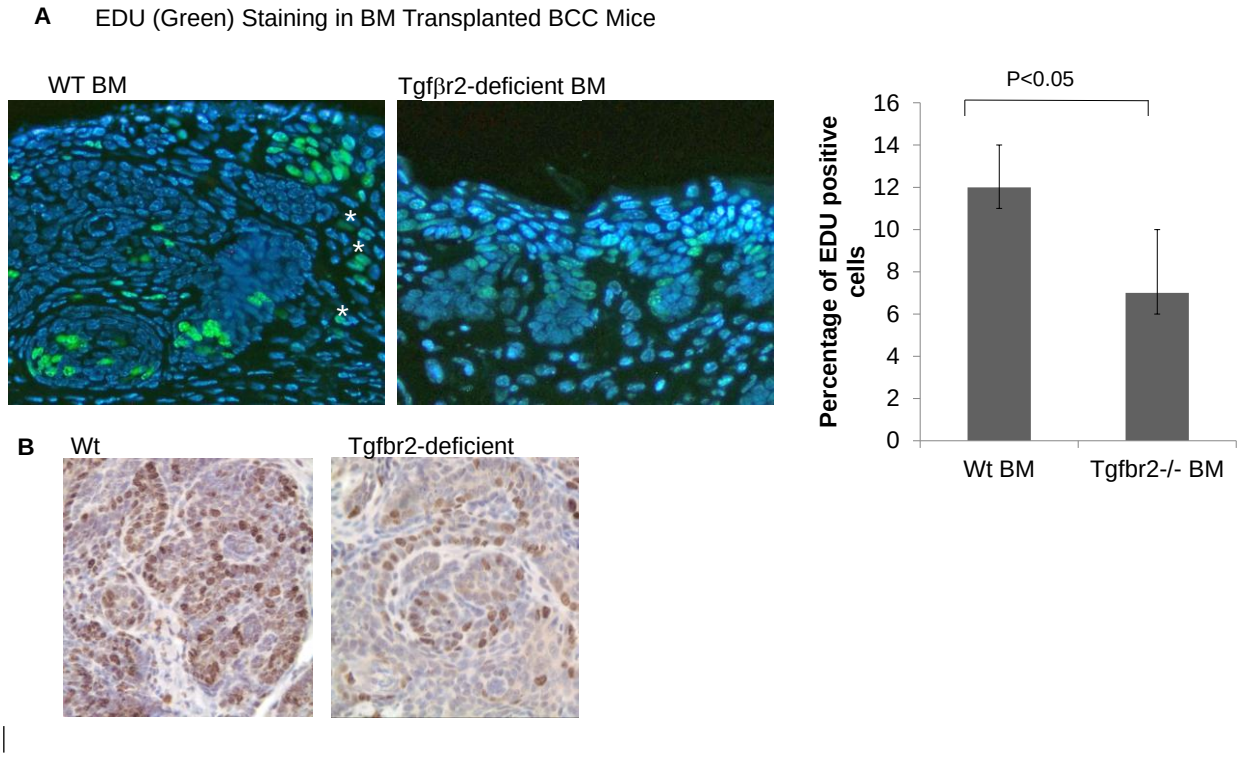
Supplementary Figure 2- Flow cytometry analyses of hair follicle stem cell ($CD34^{+}CD49f^{+}$), γδ-T cell ($CD3^{+}\gamma\delta T^{+}$) and fibroblast ($vimentin^{+}$) populations in BCC-bearing skin tissues. P values below 0.05 (indicated by **) were regarded as statistically significant.



Supplementary Figure 3- Typical flow cytometry profiles for MDSC and T cells in blood from rhabdomyosarcoma (RMS)-bearing mice (left) or control mice (right). Single cells were isolated from RMS or the control muscles, and MDSC population ($Gr1^+$ $CD11b^+$) and T cells ($CD3^+$) were analyzed by flow cytometry. We observed an increase in MDSC in blood (shown here), spleen and tumors as well as a decrease in T cell number (blood T cell shown here). Similar to BCCs, we observed an increase in expression of *Tgfb2* and TGFβ target gene pai1 (data not shown). These data indicate that the regulation mechanism of MDSC in RMS is similar to that in BCCs.

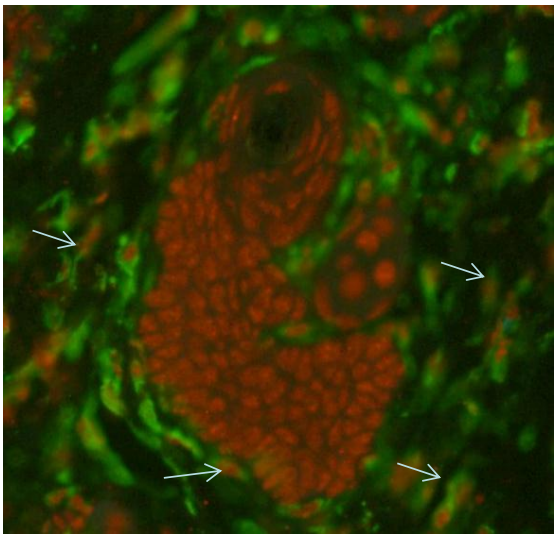


Supplementary Figure 4- EDU labeling and ki-67 staining of skin tissues from mice receiving wt or Tgfβr2 deficient bone marrow cells. To examine cell proliferation in K14-CreER/R-26SmoM2^{YFP} mice receiving wild type (wt) or Tgfβr2 deficient bone marrow cells (BM), we injected EDU 14 hours before mouse sacrifice and performed EDU labeling analysis (**A**). EDU positive cells were in green and DAPI stained nuclei. Please note EDU positive cells in mice receiving wt bone marrow cells (indicated by *) but not in mice receiving TGFβR2-deficient bone marrow cells. Also, EDU labeling was stronger in tissues from mice receiving wt bone marrow cells. The difference between the two groups of mice were significant (p value<0.05). Similarly, tumor-bearing mice receiving Tgfbr2 deficient bone marrow cells had low ki-67 positive cells (**B**).

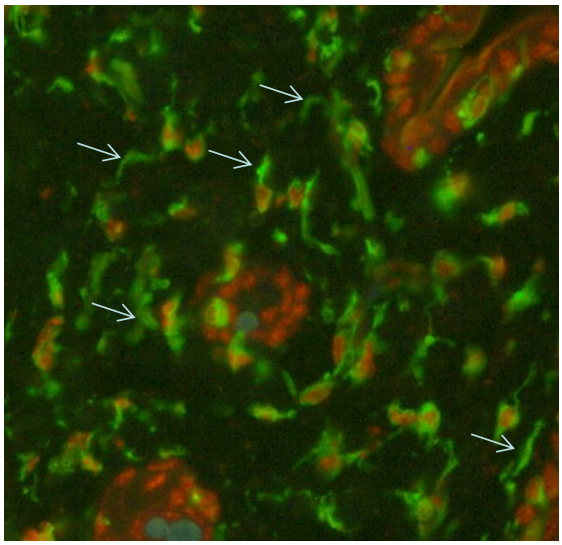


Supplementary Figure 5- Reduced phospho-SMAD2 in skin tissues from mice receiving Tgfβr2 deficient bone marrow cells. Phospho-SMAD2 was detected by immunofluorescent staining with specific antibodies in red and vimentin positive cells in green. In mice receiving wt bone marrow cells (such as mouse #467), SMAD-2 phosphorylation was detected in most vimentin positive cells (indicated by arrows). In contrast in mice receiving Tgfβr2 deficient bone marrow cells (such as mouse # 477), many vimentin positive cells did not have phospho-SMAD2 staining (indicated by arrows).

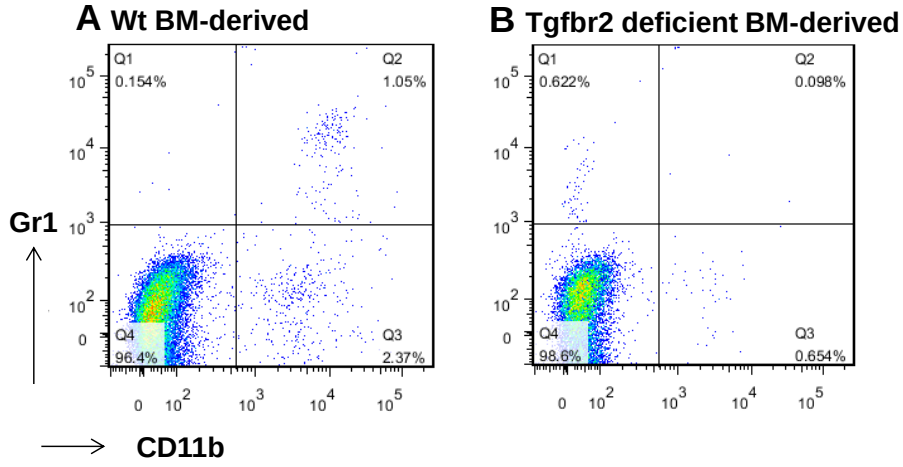
Mouse# 467 (wt BM)

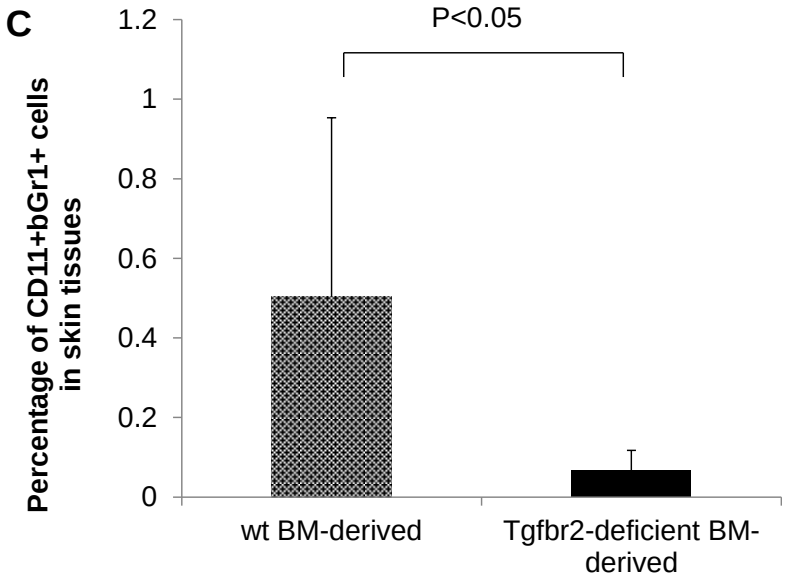


Mouse #477 (Tgfβr2 ko BM)

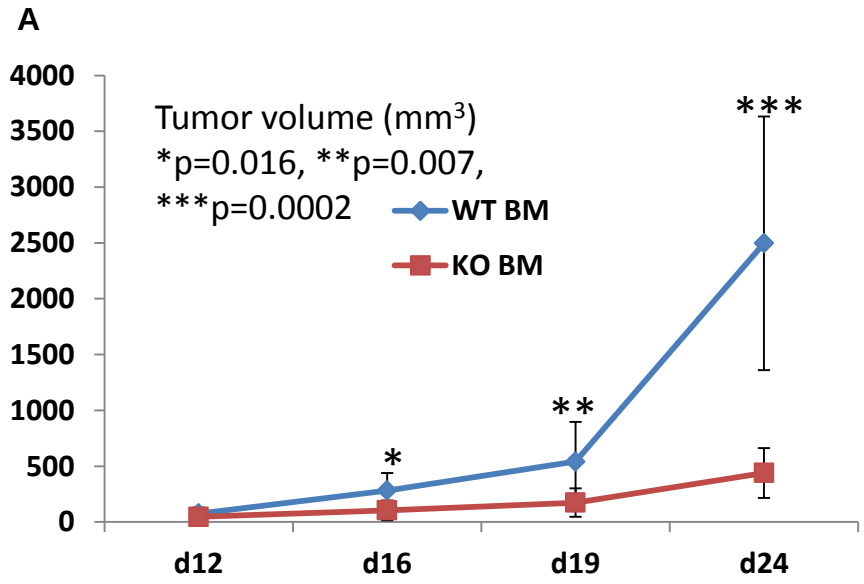


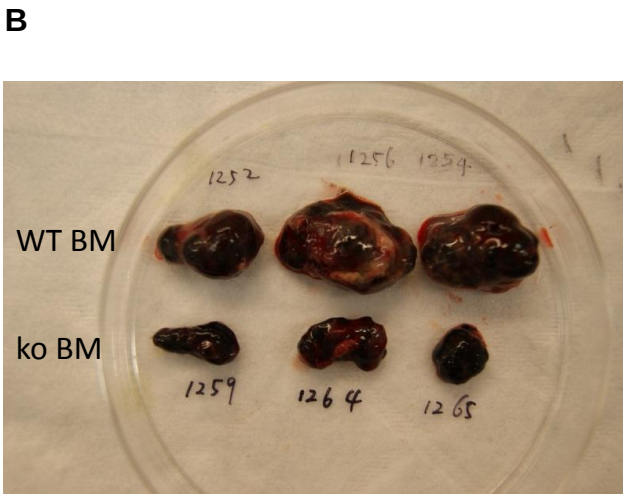
Supplementary Figure 6- Percentage of $CD11b^{+}Gr1^{+}$ cells in skin tissues from tumor-bearing mice receiving wt or Tgfbr2 deficient bone marrow cells. MDSC population in mouse skin tissues was detected by expression of CD11b and Gr1. Single cells from skin tissues were stained with CD11b and Gr1 antibodies and analyzed by flow cytometry. $P< 0.05$ was regarded as statistically significant (Mann-Whitney U test). **A** and **B** show typical flow cytometry profiles from the two groups of mice, and **C** shows the summary.

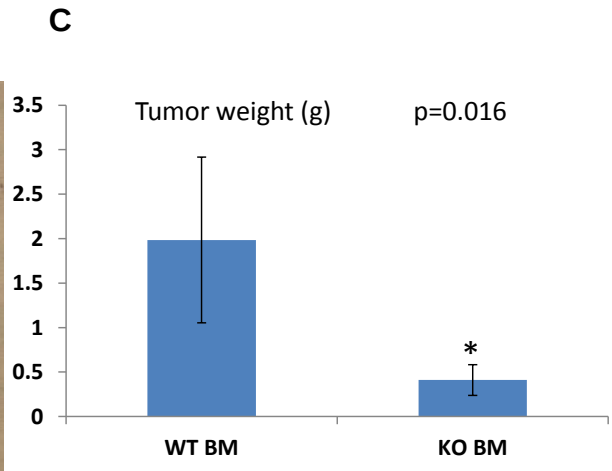




Supplementary Figure 7- Effects of Tgfbr2 deletion in bone marrow-derived cells for melanoma tumor growth. B16F10 cells were subcutaneously injected into C57B/6L mice whose bone marrows were either wild type or Tgfbr2 deficient. The engraftment of the bone marrow cells was over 90% four weeks after bone marrow transplantation. Tumor size was measured twice a week. At the end of the study, tumors were weighted. **A** shows the tumor growth curve from mice with wild type bone marrow or mice with Tgfbr2 deficient bone marrow; **B** shows the pictures of the tumors and **C** shows the tumor weights at the end of the study; and.

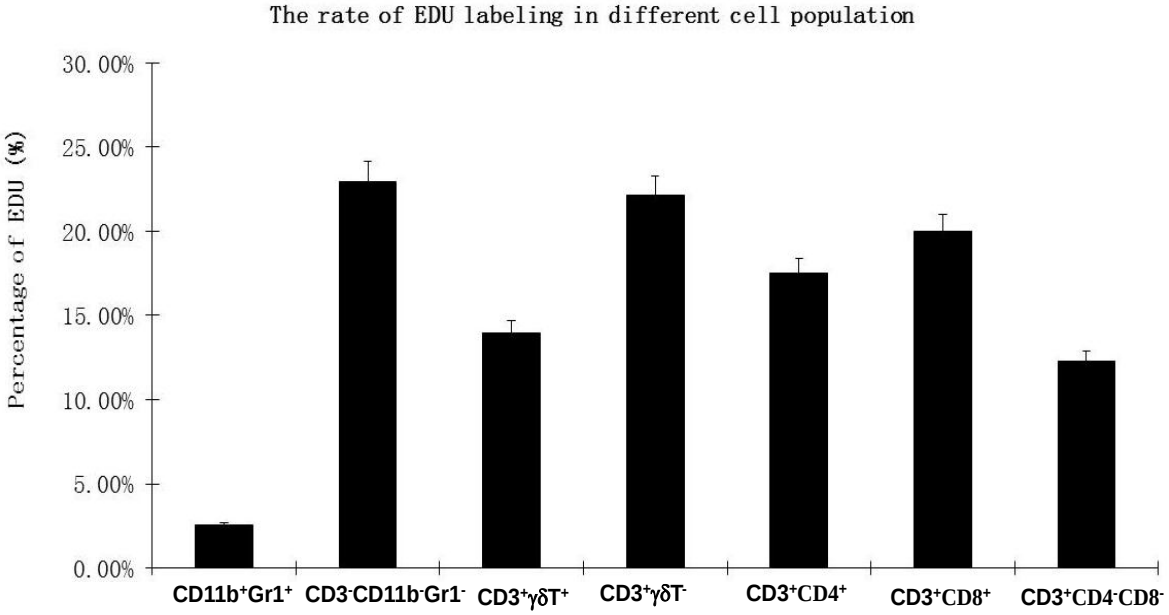




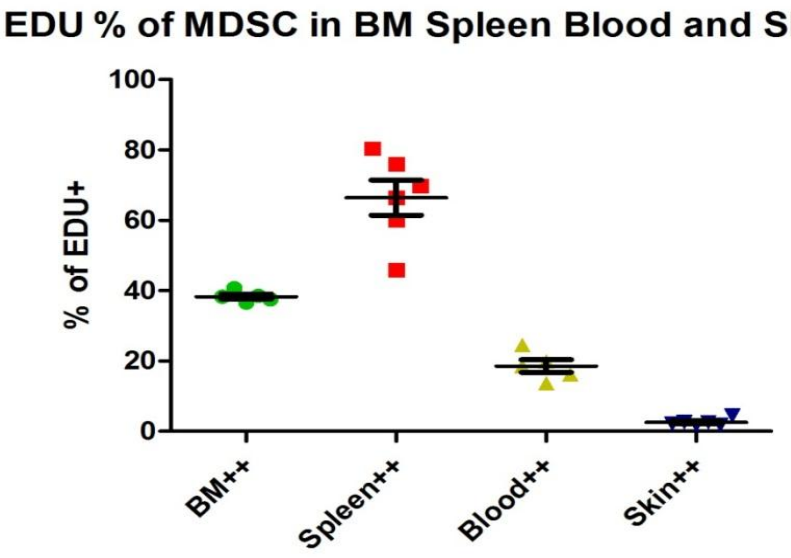


Supplementary Figure 8- Cell proliferation in different cell population. EDU labeling was used to measure DNA synthesis, a marker for cell proliferation. For this study, EDU was injected (i.p. injection of 50µl of 1mg/ml EDU) into mice the day before the experiment. Different cell populations were sorted after specific antibody labeling. **A** shows EDU positivity (%) in different cell populations in skin tumor tissues ($CD11b^+Gr1^+$; $CD11b^-Gr1^-CD3^-$; $CD3^+\gamma\delta T^+$; $CD3^+\gamma\delta T^-$; $CD3^+CD4^+$; $CD3^+CD8^+$; $CD3^+CD4^-CD8^-$). **B** shows the percentage of EDU positive cells in CD11b+Gr1+ cells from bone marrow, spleen, blood and skin.

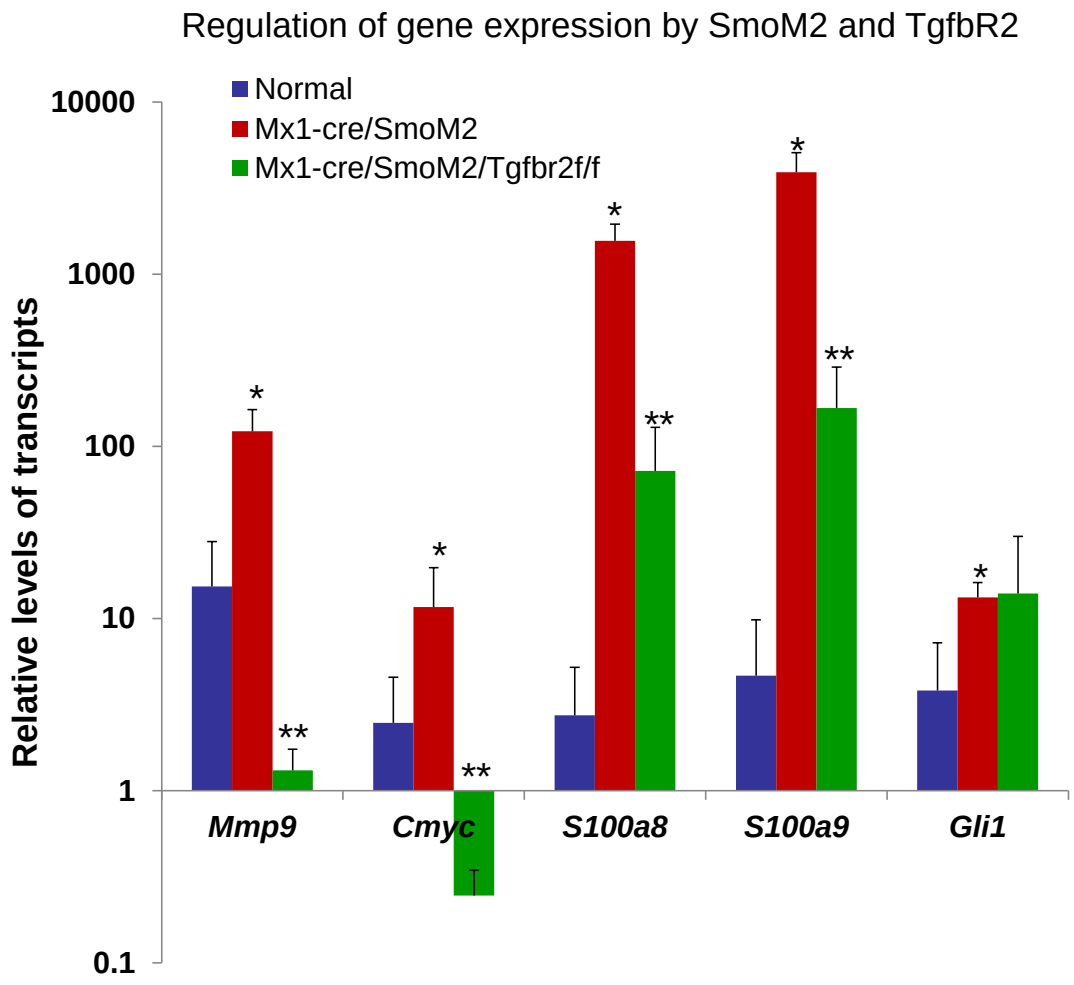
A



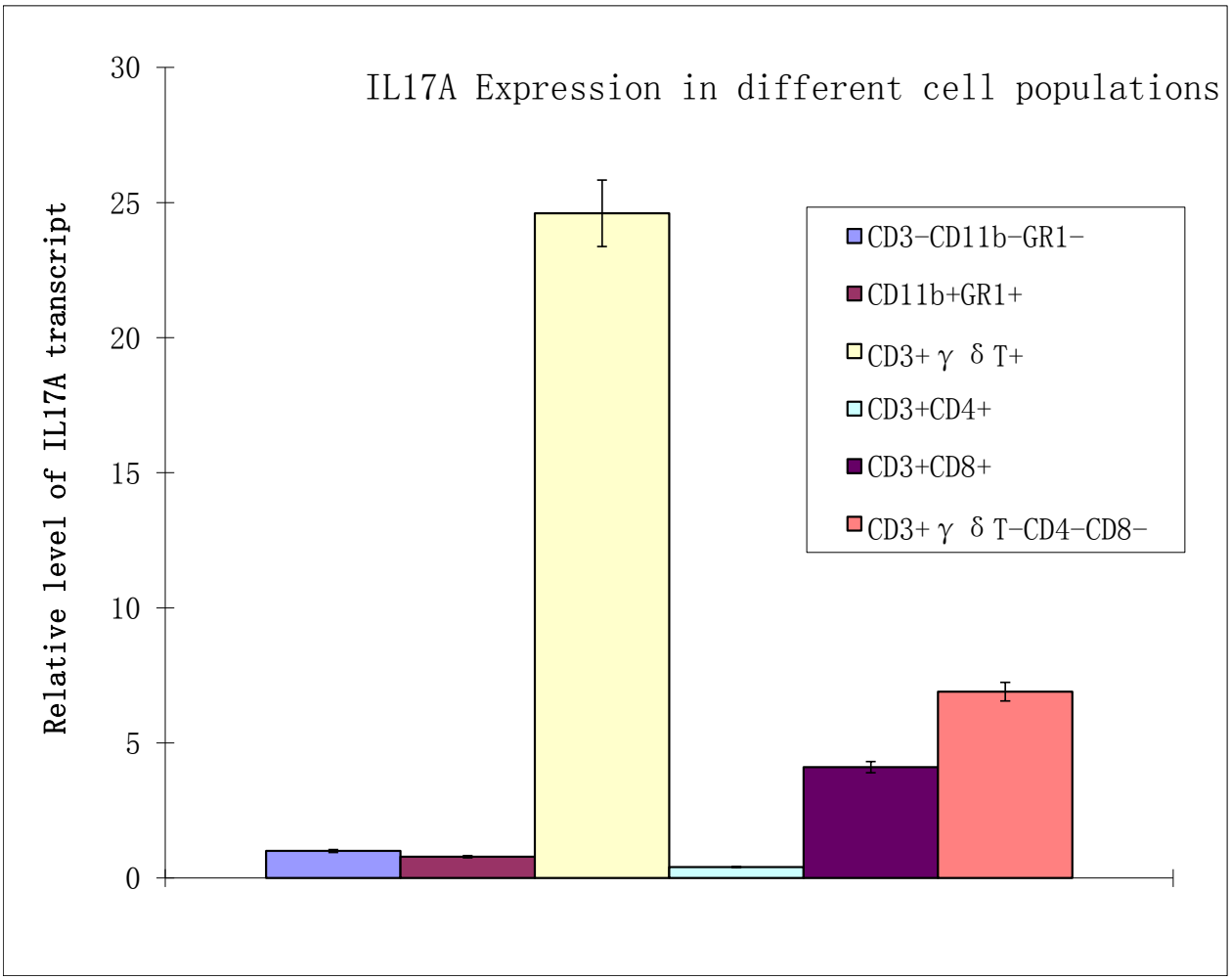
B



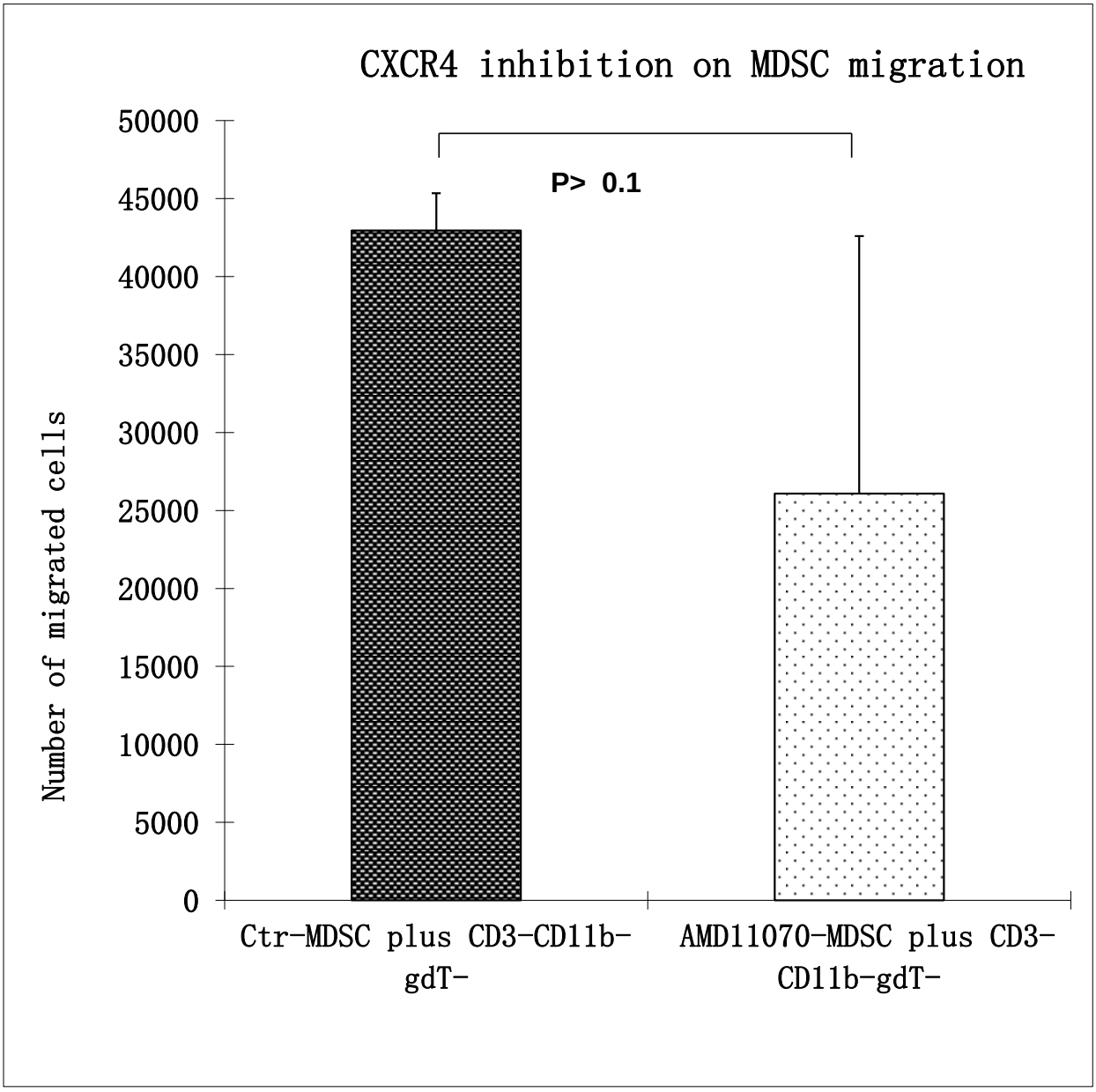
Supplementary Figure 9- Expression of genes in the skin tissues of Mx1-cre/R26-SmoM2 and Mx1-cre/R26-SmoM2/TgfβR2$^{f/f}$ mice. Total RNAs were extracted from mouse skin tissues. Real-time PCR (taqman) was performed to detect expression of several genes: MMP9, c-MYC, S100A8, S100A9 and Gli1. Significant difference between normal and tumorous skin tissues was indicated as * whereas significant reduction between Mx1-cre/R26-SmoM2 and Mx1-cre/R26-SmoM2/TgfβR2$^{f/f}$ mice was indicated as ** (p values< 0.05 by Student's *t* test).



Supplementary Figure 10- Relative level of IL-17A expression in different sorted cell populations in skin tumor-bearing mice (K14-creER/R26-SmoM2^{YFP}). CD3-CD11b-Gr1- cells contain keratinocytes and fibroblasts. CD11b^{+}Gr1^{+} cells are MDSC population in mice. The γδT cells are indicated as CD3^{+}γδT^{+}. T cells include CD3^{+}CD4^{+}, CD3^{+}CD8^{+} and CD3^{+}CD4^{-}CD8^{-}.



Supplementary Figure 11- The effect of CXCR4 inhibitor AMD3100 on MDSC migration towards skin cells. We showed that $CD3^{-}CD11b^{-}Gr1^{-}$ cells, which include keratinocytes and fibroblasts, are more effective in inducing migration of $CD11b^{+}Gr1^{+}$ cells (Figure 7A). When CXCR4 antagonist AMD3100 (1.25μM) was used to incubate with $CD11b^{+}Gr1^{+}$ cells before placing onto the top chamber, migration of $CD11b^{+}Gr1^{+}$ cells was not significantly reduced ($P>0.05$).



Supplementary Figure 12- The proposed model for MDSC migration during SmoM2-induced skin cancer development. Based on our results, we proposed a model for MDSC recruitment at the tumor site during hedgehog signaling-mediated skin tumor development. In this model, activation of hedgehog signaling in keratinocytes (via SmoM2 expression) results in elevated TGFβ expression (particularly TGFβ2). As a result, TGFβ signaling is activated in many cell types, including keratinocytes, fibroblasts, $CD11b^{+}Gr1^{+}$ cells and T cells. Since $CD11b^{+}Gr1^{+}$ cells are not present in skin tissues before hedgehog signaling activation, secreted cytokine TGFβ2 needs to travel to the peripheral blood (may be facilitated by tumor exosomes). As a result of TGFβ signaling activation, CCL2 expression is increased in the tumor microenvironment (TME) whereas CCR2 expression is increased in MDSC cells. Circulating MDSC cells are now migrated towards CCL2 enriched TME and stay there to help create the immune suppressive TME.