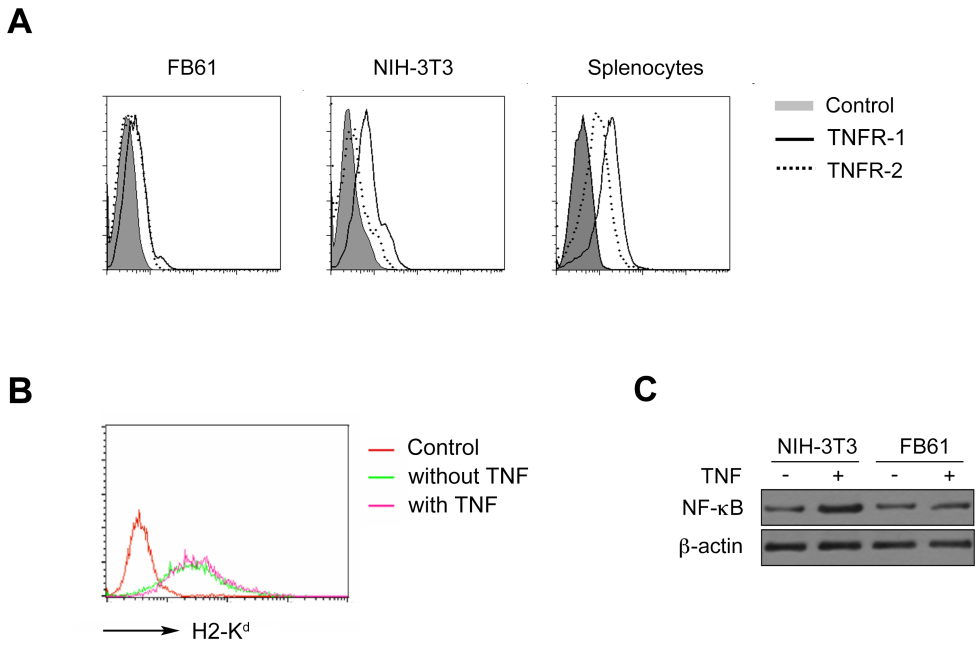

Figure S1
TNFR deficient tumor cells do not respond to TNF. (**A**) The TNFR-1 deficient FB61 cells, TNFR-1 competent NIH-3T3 cells and TNFR-competent splenocytes were stained for TNFR-1 (solid lines) and TNFR-2 (dotted lines). The corresponding cells without staining (shaded) served as negative controls. (**B**) FB61 cells were left untreated or stimulated with TNF (20 ng/ml) for 12 hours. H2-K^d was then stained for flow cytometry. Cells without staining were used as control. (**C**) FB61 cells and NIH-3T3 cells were stimulated with TNF (20 ng/ml) for 20 minutes. The NF-κB levels in nuclear extracts were determined by Western blot. The β-actin was used as internal control.

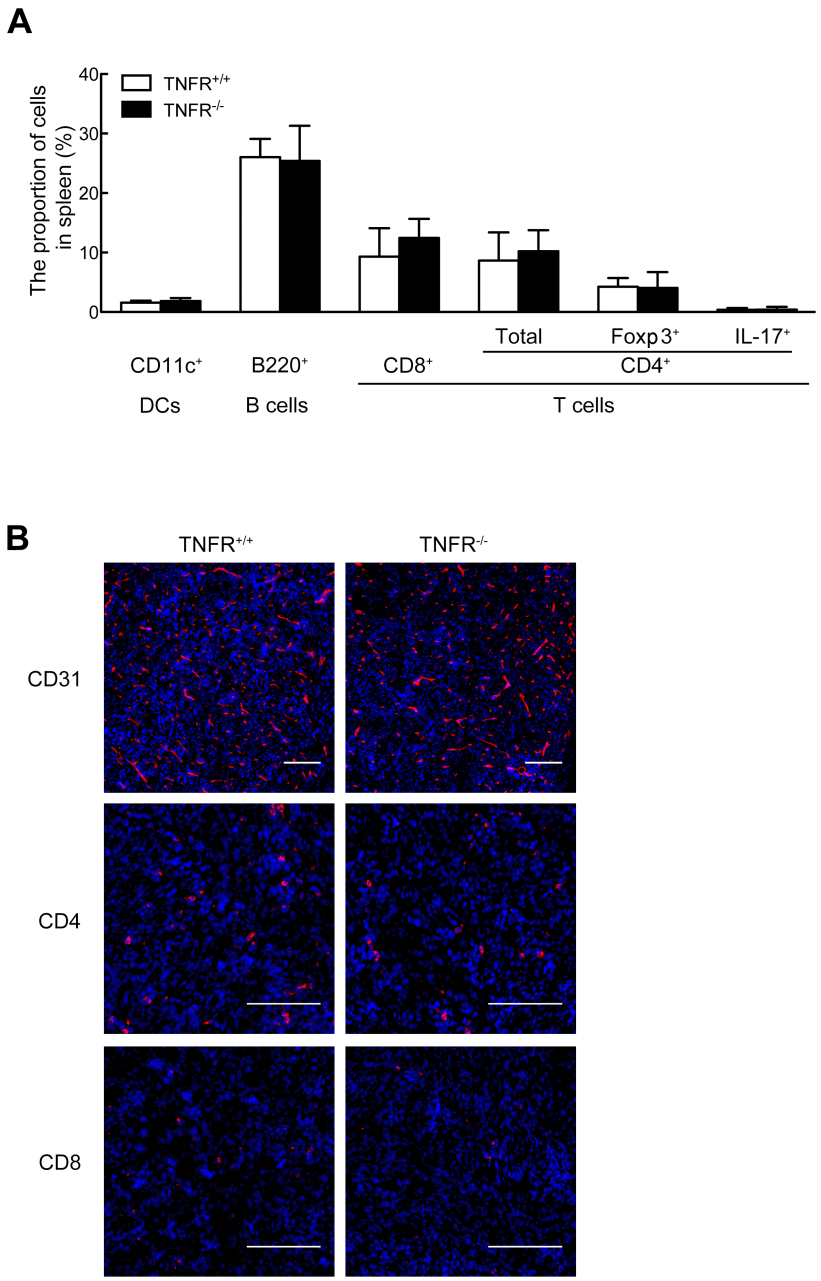

Figure S2

Comparison of possible immune regulatory cells in tumor-bearing TNFR$^{-/-}$ and TNFR$^{+/+}$ mice. (**A**) TNFR$^{+/+}$ and TNFR$^{-/-}$ mice were subcutaneously injected with 1 × 10^{6} FB61 cells. At day 8-10, spleen cells were prepared and stained for CD11c, B220, CD4, CD8, Foxp3 and IL-17 for flow cytometry analysis. Shown are percentages of indicated cells in the spleen of TNFR$^{+/+}$ and TNFR$^{-/-}$ mice (mean ± SEM). Each group contains 5-7 mice. (**B**) Immunohistochemistry analysis for CD31^{+} endothelial cells, CD4^{+} and CD8^{+} T cells in FB61 tumors isolated from TNFR$^{+/+}$ and TNFR$^{-/-}$ mice at day 8-10 after tumor cell inoculation. Nuclei were counterstained with DAPI. Images representative for at least 3 mice per group; original magnification is ×100 for CD31 staining and ×200 for CD4 or CD8 staining; bars indicate 300 μm.

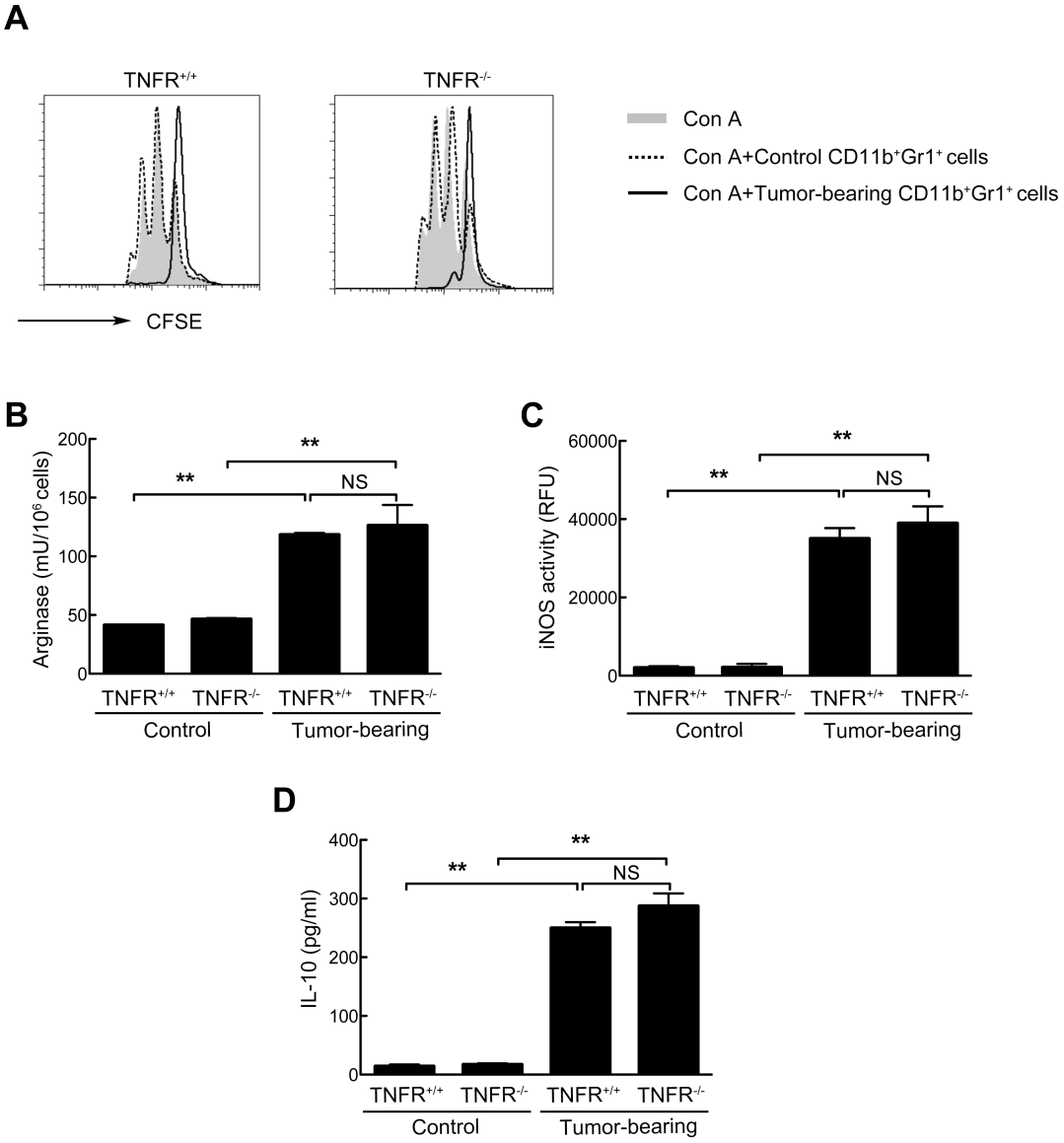

Figure S3
$CD11b^+Gr1^+$ cells from tumor-bearing mice have T-cell suppressive activities. Splenic $CD11b^+Gr1^+$ cells were isolated from tumor-free (as control) or FB61 tumor-bearing $TNFR^{+/+}$ and $TNFR^{-/-}$ mice. (**A**) The CFSE-labeled $TNFR^{+/+}$ splenocytes were stimulated with Con A in the absence (shaded) or presence of $CD11b^+Gr1^+$ cells, which were isolated from either tumor-bearing (solid lines) or control mice (dotted lines) as indicated. After 72 hours, cells were collected and stained for CD4. The T-cell proliferation was determined by CFSE dilutions in gated $CD4^+$ T cells. (**B**) The activity of ariginase and (**C**) iNOS in isolated $TNFR^{+/+}$ and $TNFR^{-/-}$ $CD11b^+Gr1^+$ cells were measured as described in the Methods. (**D**) $CD11b^+Gr1^+$ cells were stimulated with LPS and IFNγ. The IL-10 content in the culture supernatant was measured by a CBA kit. Data are shown as mean $\pm$ SEM. NS, not significant. $^{**}P < 0.01$.

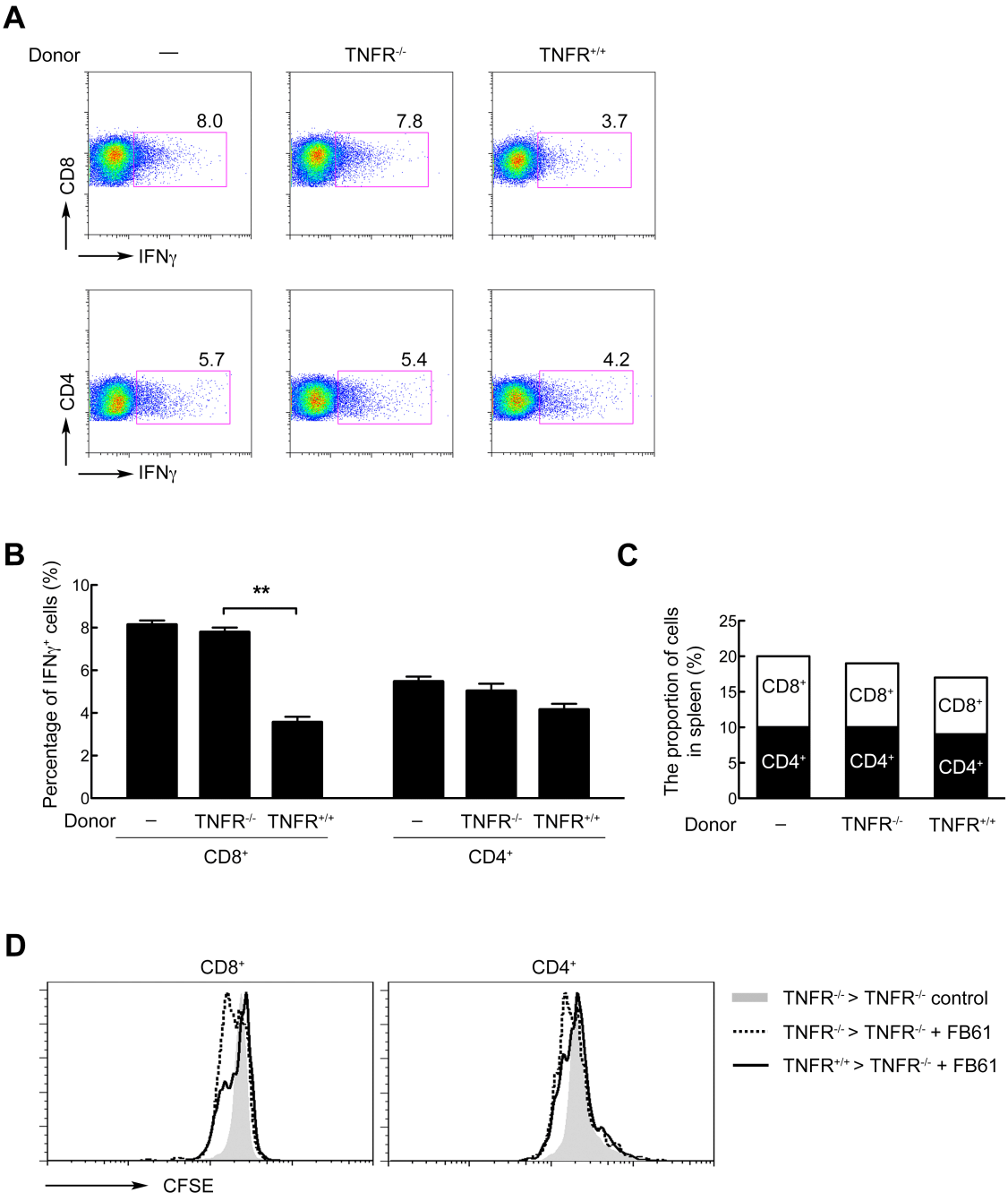

Figure S4

Adoptive transfer of TNFR-competent MDSCs suppresses anti-tumor T cell responses in TNFR-deficient mice. (**A**) The $TNFR^{-/-}$ mice were left untreated (–) or transferred with $TNFR^{-/-}$ or $TNFR^{+/+}$ MDSCs, and then injected with FB61 cells as described in **Figure 3**. At day 7 after tumor cell inoculation, spleen cells were isolated, stimulated with PMA/ionomycin and stained for intracellular IFNγ. Numbers in the representative dot plots indicate the $IFN\gamma^+$ cell percentages in gated $CD8^+$ or $CD4^+$ cells. Each group contains 3-4 mice. (**B**) The percentages of $IFN\gamma^+$ cells in **A** are presented as the mean ± SEM. $^{**}P < 0.01$. (**C**) The average proportion of total $CD4^+$ (closed) or $CD8^+$ (open) cells in splenocytes of the two groups of mice in **A**. (**D**) Tumor-specific proliferation of $CD8^+$ T cells is inhibited in $TNFR^{-/-}$ mice that received $TNFR^{+/+}$ MDSCs. The splenocytes isolated from $TNFR^{-/-}$ > $TNFR^{-/-}$ (dashed) or $TNFR^{+/+}$ > $TNFR^{-/-}$ mice (solid lines) at day 7 were labeled with CFSE, and cultured in the presence of FB61 cell lysate. As control, $TNFR^{-/-}$ > $TNFR^{-/-}$ splenocytes were cultured in the absence of FB61 cell lysate (shaded). After 72 hours, cells were collected and stained. The T-cell proliferation was determined by CFSE dilutions of gated $CD8^+$ or $CD4^+$ T cells.

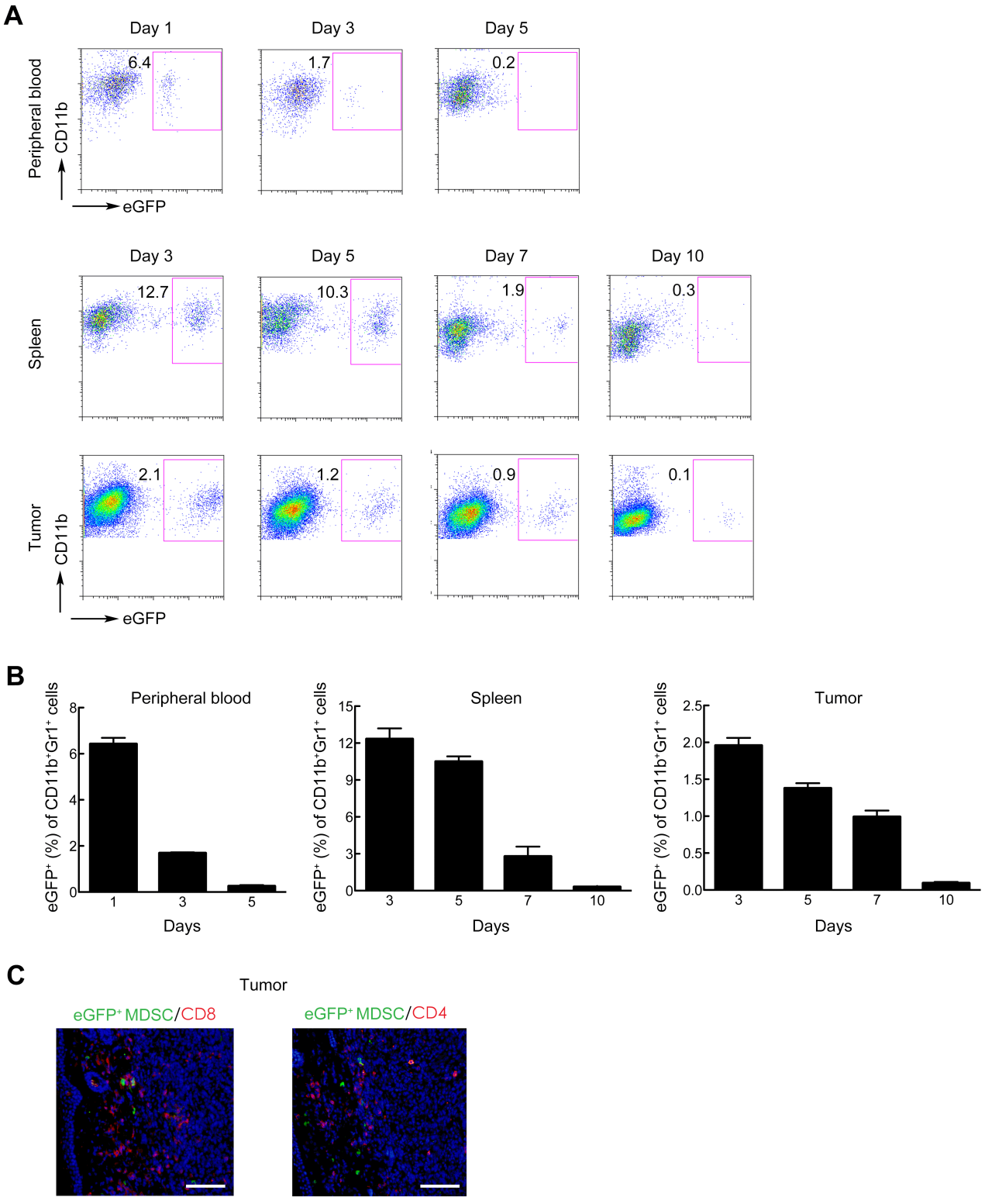

Figure S5

Adoptively transferred MDSCs accumulate in spleens and tumors of the recipient mice. (**A**) The $CD11b^+Gr1^+$ cells were isolated from MCA205 tumor-bearing eGFP-transgenic mice as described in the Methods. Syngeneic C57BL/6 mice were intravenously injected with 5×10^6 of the isolated cells, and 12 hours later subcutaneously inoculated with 1×10^6 MCA205 tumor cells. At different days after tumor cell inoculation, total cells from the peripheral blood, spleen and tumor tissues were stained and analyzed by flow cytometry. $CD11b^+Gr1^+$ cells were gated and the numbers indicate the percentages of eGFP positive cells in total gated cells at each indicated time point. (**B**) Percentages of $eGFP^+$ cells in total $CD11b^+Gr1^+$ cells in peripheral blood, spleen or tumor tissues of the recipient mice are presented as the mean ± SEM. Each group contains 3-5 mice. (**C**) The transferred MDSCs are detected in the vicinity of T cells in the outer rim of tumors. The syngeneic $TNFR^{-/-}$ mice were transferred with $eGFP^+$ MDSCs and inoculated with MCA205 tumor cells as described in **A**. At the day 7 after tumor cell inoculation, the $eGFP^+$ MDSCs (green) and $CD4^+$ or $CD8^+$ cells (red) in tumors were visualized by immunofluorescence staining. Nuclei were counterstained with DAPI (blue). Images are representative for at least 3 mice per group; original magnification is ×100; bars indicate 100 μm.

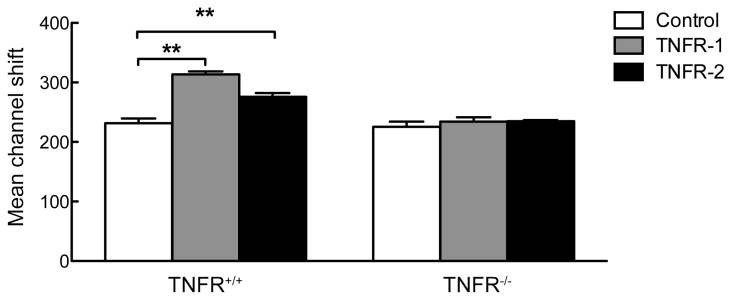

Figure S6
The expression of TNFR-1 and TNFR-2 on MDSCs. $TNFR^{+/+}$ and $TNFR^{-/-}$ mice were subcutaneously injected with 1×10^6 FB61 cells. At day 8-10, spleen cells were prepared and stained with anti-CD11b, anti-Gr1, anti-TNFR-1 or anti-TNFR-2 or isotype control mAbs (white) for flow cytometry analysis. Shown is the expression of TNFR-1 (grey) or TNFR-2 (black) as mean channel shift on gated $CD11b^+Gr1^+$ cells (mean ± SEM). $^{**}P < 0.01$.

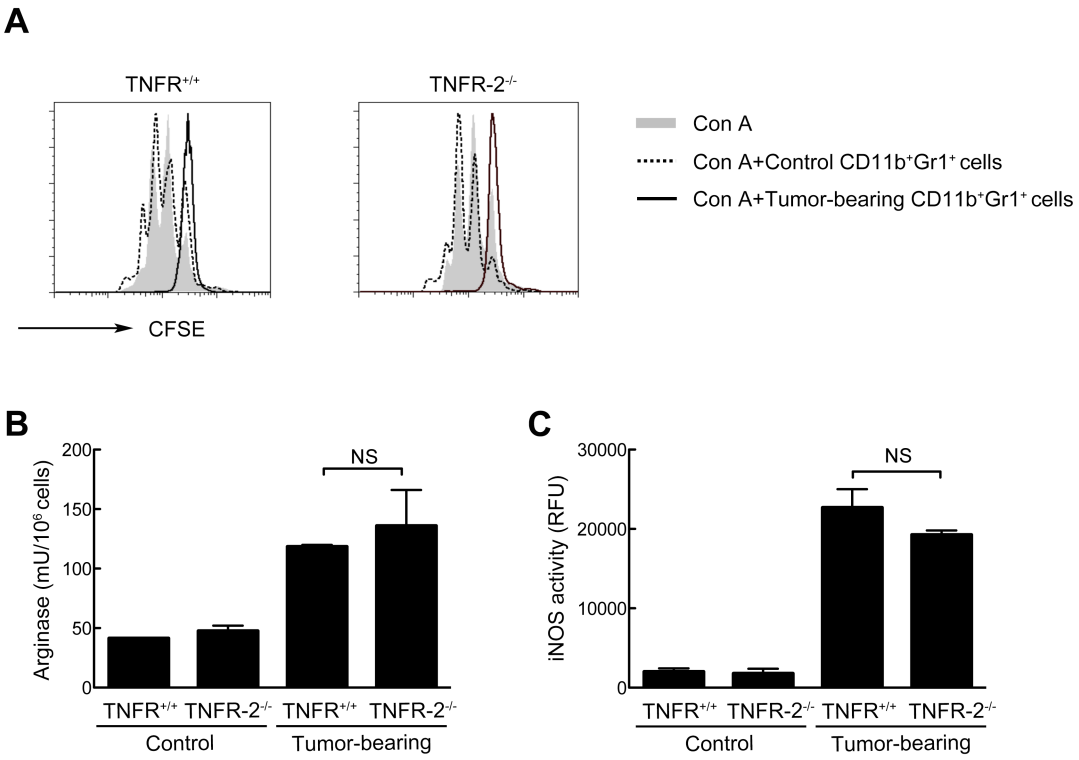

Figure S7

TNFR-2 deficiency does not affect the function of MDSCs. Splenic $CD11b^+Gr1^+$ cells were isolated from tumor-free (as control) or FB61 tumor-bearing $TNFR^{+/+}$ and $TNFR\text{-}2^{-/-}$ mice. (**A**) The CFSE-labeled $TNFR^{+/+}$ splenocytes were stimulated with Con A in the absence (shaded) or presence of $CD11b^+Gr1^+$ cells, which were isolated from either tumor-bearing (solid lines) or control mice (dotted lines) as indicated. After 72 hours, cells were collected and stained for CD4. The T-cell proliferation was determined by CFSE dilutions in gated $CD4^+$ T cells. (**B**) The activity of ariginase and (**C**) iNOS in isolated $TNFR^{+/+}$ and $TNFR\text{-}2^{-/-}$ $CD11b^+Gr1^+$ cells were measured as described in the Methods. Data are shown as mean ± SEM. NS, not significant.

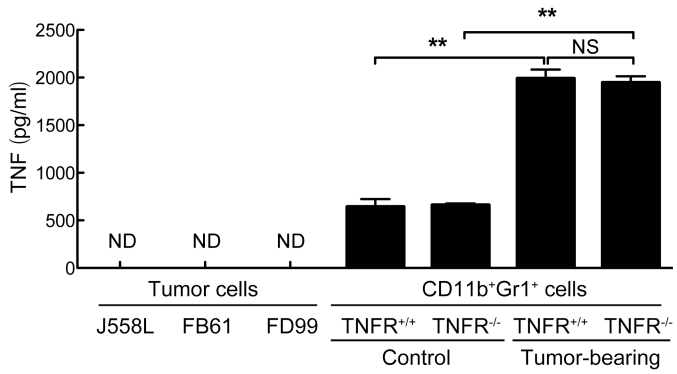

Figure S8

The host CD11b^{+}Gr1^{+} cells, but not tumor cells secrete TNF. The tumor J558L, FB61 and FD99 cells were cultured at 2 × 10^{4} cells in 200 μl medium for 48 hours. CD11b^{+}Gr1^{+} cells from mice as indicated were cultured at 2 × 10^{5} cells in 200 μl medium, stimulated with LPS and IFNγ for 16 hours. The TNF concentrations in the culture supernatant were determined by a CBA kit. ND, not detectable. NS, not significant. ***P* < 0.01.