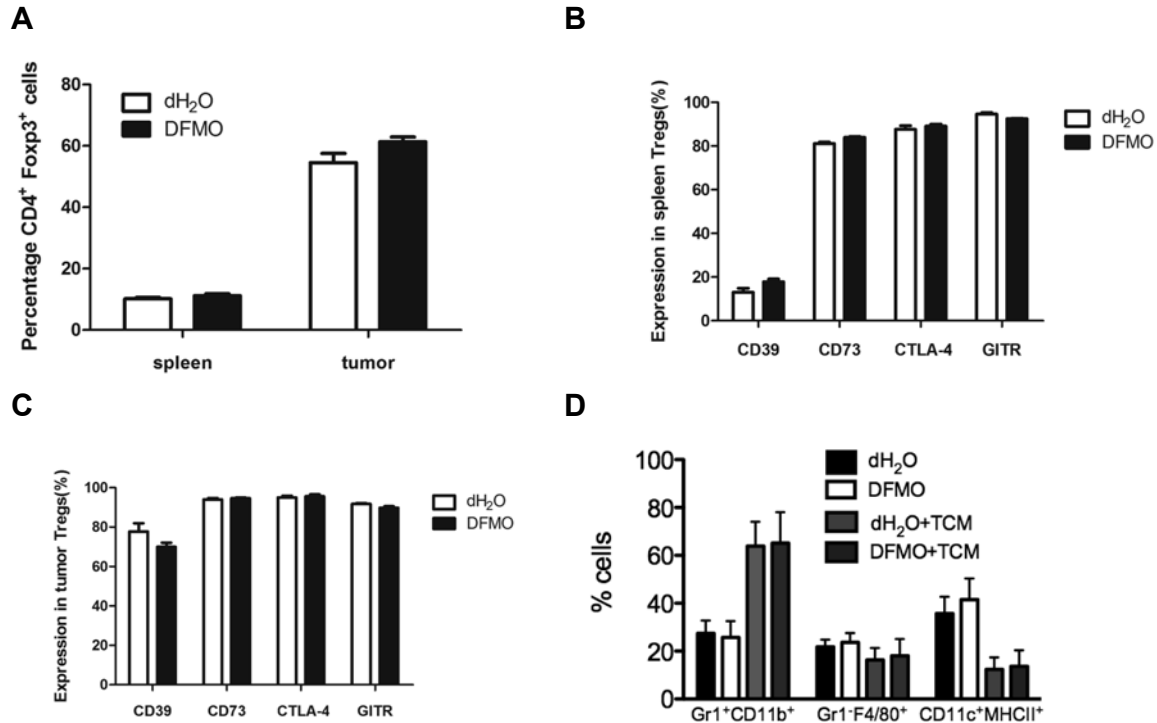
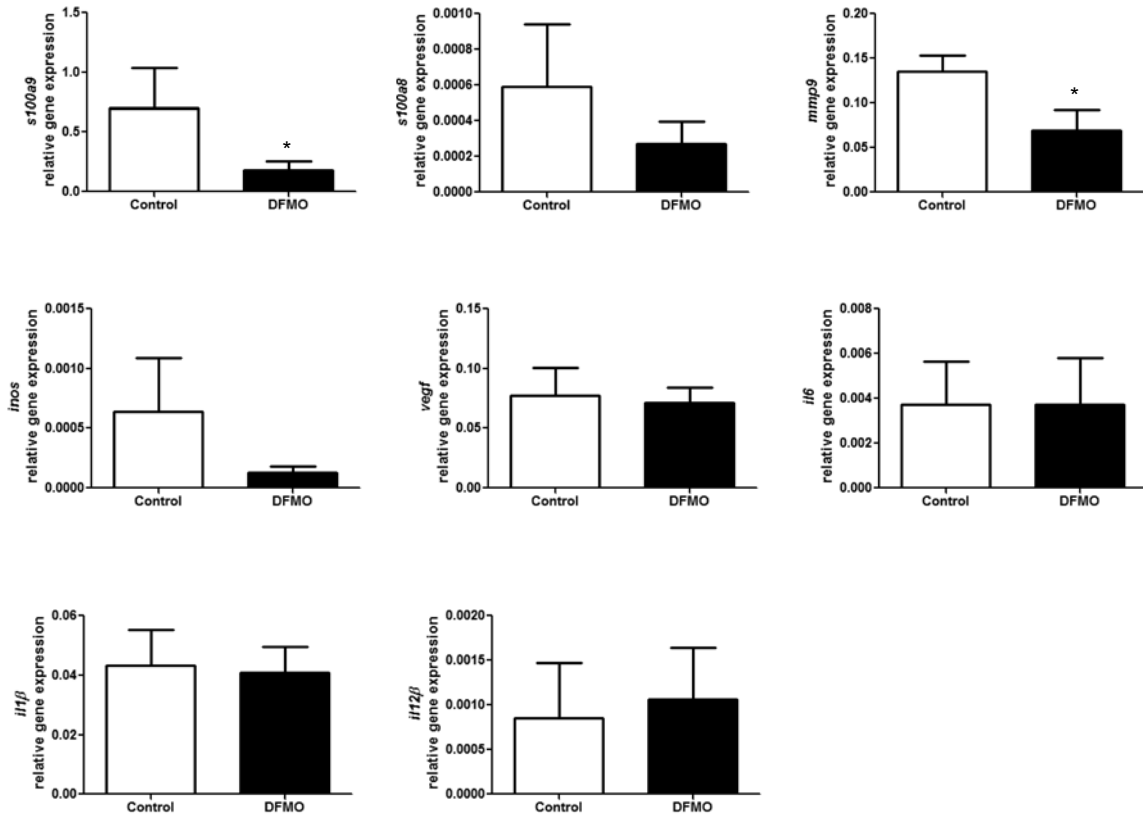


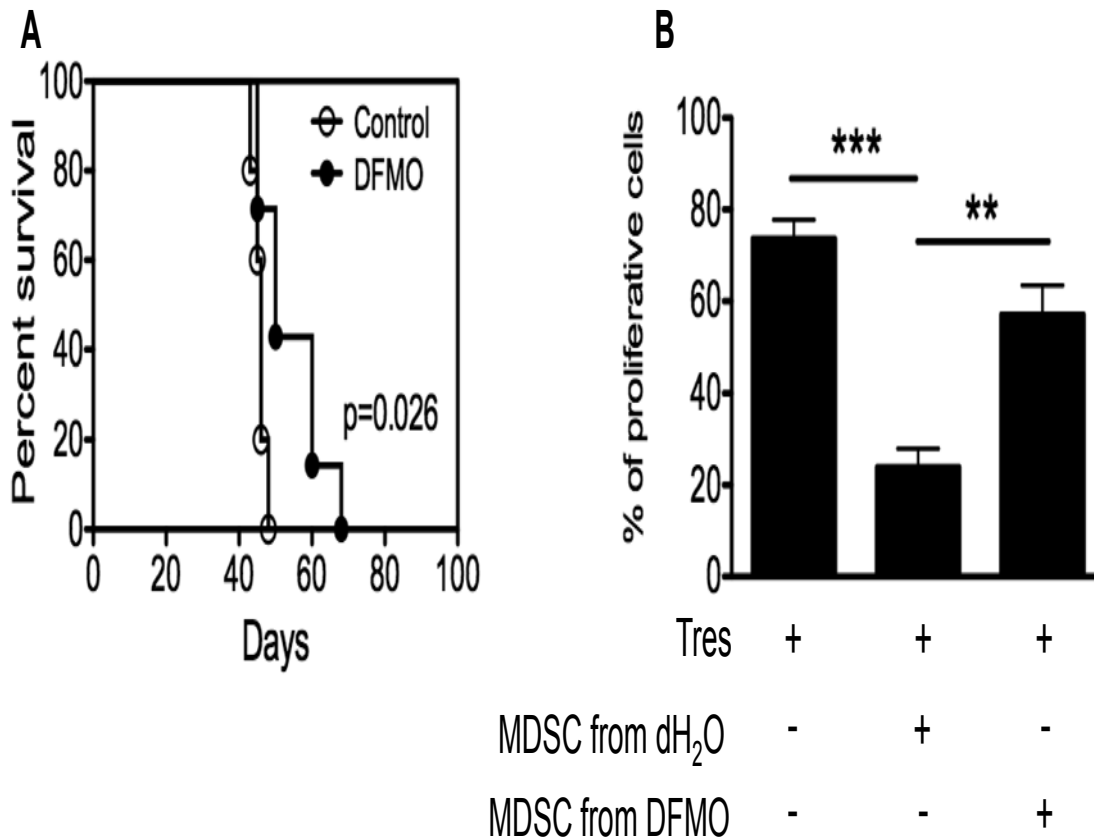
Supplemental Figure 1. *In vitro* growth inhibition of melanoma cells by DFMO. Murine B16F10, human A375, Skemel 5 and Skemel 28 melanoma cells were incubated for 3-5 days in culture with DFMO at the indicated concentrations. Cell growth was determined by the MTT assay from triplicate wells. Data shown are mean \pm SD.



Supplemental Figure 2. The effect of DFMO on tumor-associated CD4⁺Foxp3⁺ regulatory T cells (Tregs) and MDSC differentiation. (A) Percent CD4⁺Foxp3⁺ T cells in spleen and tumor infiltrates. Percent CD39⁺, CD73⁺, CTLA-4⁺ and GITR⁺ cells among Tregs in spleen **(B)** and tumor infiltrates **(C)**. Cells were collected from B16F10-bearing DFMO treated or control mice 14 days after tumor inoculation (3 mice per group). **(D)** BM cells were cultured with GM-CSF and IL-4 for 5 days in complete culture medium or in the TCM in the presence of DFMO or dH₂O as controls. The cell phenotypes were examined by flow cytometry. Data (mean ± SEM) are representative of 2 independent experiments.



Supplemental Figure 3. Altered gene expression in MDSCs following DFMO treatment. Bone marrow cells were cultured with GM-CSF and IL-6 in the presence or absence (as control) of DFMO at 10 mM for 4 d, and Gr1⁺CD11b⁺ MDSCs were selected using anti-CD11b magnetic beads. Expression levels of a series of indicated genes associated with MDSC activity were measured by real-time quantitative RT-PCR. GAPDH expression was measured for normalization. One representative of two experiments is shown.



Supplemental Figure 4. DFMO treatment increases the survival of ID8-bearing mice. (A) ID8-OVA cells (10^7) were injected i.p. into C57BL/6 mice (8 mice per group). DFMO was administered as a 1% solution in drinking dH₂O to mice starting 7 days after tumor injection. Mice fed with dH₂O without DFMO were used as controls. Mice survival was monitored every 3 days. (B) Suppressive activity of MDSCs as shown by quantification of eFluor450-labeled CD4⁺ T responder cells (Tres) cocultured with the indicated Gr1⁺CD11b⁺ MDSCs from DFMO-treated tumor-bearing mice versus control mice. The ratio of T cell/MDSC was 2:1. **, $p < 0.01$; ***, $p < 0.001$ (3 mice per group).