Figure S1. Expression of S100A9 and A8 in myeloid cells in tumor-bearing mice. (A and B) HPCs were enriched from bone marrow of naive, tumor-free mice. Cells were cultured with 20 ng/ml GM-CSF and 10 ng/ml IL-4 for 5 d, followed by a 48-h incubation with GM-CSF and IL-4 or with GM-CSF, IL-4, and 5 ng/ml TNF (G+I+T). RNA or whole-cell lysates were prepared immediately after isolation of cells from bone marrow (day 0) or after indicated times in culture (1, 3, 5, or 7 d). (A) RT-PCR and Southern blot were performed. (B) Protein levels were detected by Western blotting. Three experiments yielded similar results.
Figure S5. Cytotoxic activity of splenocytes from S100A9KO mice. Splenocytes were isolated from wild-type (WT) or S100A9−/− (KO) mice 12 d after injection of EL-4 cells, restimulated in vitro with irradiated EL-4 cells for 6 d, and used as effectors in a standard 6-h chromium release assay. EL-4 cells, B16 cells, and peritoneal macrophages obtained from naive C57BL/6 mice were used as targets. Experiments were performed in duplicate using three mice per group. Mean ± SD are shown.

Figure S4. Tumor growth in S100A9KO mice. C3 (0.5 × 10⁶) sarcoma cells were injected into wild-type (WT) or S100A9KO mice, and tumor size was measured using calipers. Each group included four mice, and tumor size for each mouse is shown.

Figure S2. TCM affects differentiation of DCs and MDSCs in vitro. HPC enriched from bone marrow of naive BALB/c mice were cultured with GM-CSF and IL-4 for 5 d in the presence of 25% (vol/vol) conditioned media from NIH 3T3 fibroblasts (FCM) or CT26 colon cancer cells (CT26). Cells were labeled with APC-conjugated anti-CD11c antibody and PE-conjugated anti–IAa antibody (top) or PE-conjugated anti–Gr-1 antibody and PerCP-conjugated anti-CD11b (bottom) and analyzed by flow cytometry.

Figure S3. Differentiation of myeloid cells in S100A9-deficient mice. (A) Cell phenotypes were evaluated in spleens of S100A9+/+ wild-type and S100A9−/− KO mice by flow cytometry. Each group included four mice. Mean ± SD are shown. (B) HPCs were enriched from bone marrow of wild-type and S100A9KO mice and cultured for 7 d with GM-CSF. LPS was added during the last 24 h of culture. Cell phenotype was evaluated using multicolor flow cytometry. Each group included three mice. Mean ± SEM are shown.

Figure S5. Cytotoxic activity of splenocytes from S100A9KO mice. Splenocytes were isolated from wild-type (WT) or S100A9−/− (KO) mice 12 d after injection of EL-4 cells, restimulated in vitro with irradiated EL-4 cells for 6 d, and used as effectors in a standard 6-h chromium release assay. EL-4 cells, B16 cells, and peritoneal macrophages obtained from naive C57BL/6 mice were used as targets. Experiments were performed in duplicate using three mice per group. Mean ± SD are shown.
Figure S6. Immunohistochemical evaluation of tumors from S100A9KO mice. EL-4 tumors were established in S100A9−/− mice and their wild-type littermates. (A) Frozen sections were stained with goat anti–mouse S100A9 antibody (R&D Systems), followed by staining with anti–goat IgG conjugated with biotin and ABC kit (Vector Laboratories) and counterstained with hematoxylin. (B) Frozen sections were stained with rat anti–mouse Gr-1 antibody (BD Biosciences), followed by staining with anti–rat IgG conjugated with biotin and ABC kit and counterstained with hematoxylin. Tumors from wild-type, but not from KO, mice showed presence of brown S100A9+ and Gr-1+ cells. (C) Frozen sections were stained with rat anti–mouse CD8 antibody, followed by staining with anti–rat IgG conjugated with biotin and ABC kit and counterstained with hematoxylin. (D) Frozen sections were stained with rat anti–mouse CD4 antibody, followed by staining with anti–rat IgG conjugated with biotin and ABC kit and counterstained with hematoxylin. Tumors from KO mice, but not from wild-type, mice showed presence of brown CD8+ and CD4+ cells. (E) Tumor tissues were fixed in formalin and embedded in paraffin. Sections were stained with hematoxylin-eosin. Bar, 100 µm.
Figure S7. Expression of S100A9 and S100A8 in transfected ES cells. (A) Expression of S100A9 and A8 was evaluated at different time points during DC differentiation from ES cells using RT-PCR and Southern blot. Lane 1, ES cells; lanes 2–6, embryoid bodies on days 1, 3, 5, 8, and 12 d of development, respectively; lane 7, cells generated from embryoid bodies after 35-d culture with GM-CSF and IL-3.

Figure S8. Phenotype of myeloid cell colonies. Cells were generated from ES cells transfected with empty vector ES-R1 or with S100A8/9 (MRP8/14 ES-R1) as described in Materials and methods, and then used for analysis of colony formation in semisolid medium supporting growth of myeloid colonies (StemCell Technologies). Bar, 500 µm.
Figure S9. Colony formation by spleen and bone marrow cells from S100A9Tg mice. Spleen and bone marrow cells (BM) were isolated from wild-type FVB/N (WT) and S100A9Tg (Tg) mice and used for analysis of colony formation in semisolid medium supporting growth of myeloid colonies. Each experiment was performed in duplicates. For illustration purposes, plates were stained with MTT dye (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide; Sigma-Aldrich). Bar, 500 µm.