Supplementary information, Data S1 Methods

Flow Cytometry

Cells were stained with indicated antibody at 4 °C for 30 min and analyzed by flow cytometry (BD Biosciences).

RNA isolation and Real-time PCR

Total RNA was extracted with Trizol according to the manufacturer's guidelines (Invitrogen). Any remaining DNA was removed with the DNA-free kit (Ambion) and was re-purified with the RNAeasy kit (Qiagen). Taqman real-time gene expression assays were run on an ABI StepOnePlus system according to manufacturer's protocol (Applied Biosystems). Gene expression was normalized to that of GAPDH or actin. The following MGB (minor groove binder) assays from Applied Biosystems and IDT were used for gene-expression analysis: human GAPDH, Hs02786624_g1; human TNFα, Hs01113624_g1; human IL-12p40, Hs00233688_m1; human IL-6, Hs99999032_m1; human CXCL11, Hs00171138_m1; human IL-10, Hs00961620_g1, human CCL-17, Hs01128674_g1; human CCL18, Hs00268113_m1; human CCL24, Hs00171082_m1; mouse TNFα (58740533); mouse IL-10 (59929059); mouse actin (79286383).

Western blot analysis

Cells were collected and lysed in M2 buffer (20 mM Tris at pH 7, 0.5% NP-40, 250 mM NaCl, 3 mM EDTA, 3 mM EGTA, 2 mM DTT, 0.5 mM PMSF, 20 mM β-glycerol phosphate, 1 mM sodium vanadate, and 1 mg/ml leupeptin). Cell lysates were

separated by SDS-PAGE and analyzed by immunoblot. The proteins were visualized by enhanced chemiluminescence (ECL, Peirce).

Electroporation

Monocytes were transfected with Amaxa Nucleofector II and the kits supplied (VPA-1008) according to the manufacturer's instructions (Lonza). The siRNAs targeting human Rac1 (pool L-003560-00-00-0005) and pool siRNA control (D001206-13-20) from Thermo-Dharmacon were used. After electroporation, monocytes were cultured for 24 hrs in the in RPMI-1640 medium supplemented with 10% (vol/vol) FBS and 2 mM glutamine and then treated with GM-CSF or M-CSF for 6 days. Further polarization was achieved by treating cells with LPS/IFNγ (M1) or IL-4 (M2).

Isolation of Mouse Primary Monocytes

Bone marrow derived monocytes were isolated mouse femurs and tibias and cultured for 2 hrs. The attached monocyte enriched cells were cultured in RPMI-1640 medium supplemented with 10% (vol/vol) FBS and 2 mM glutamine, with penicillin (100 U/ml) and streptomycin (100 µg/ml). For differentiation, cells were cultured for 6 days in the presence of recombinant mouse M-CSF (40 ng/ml). For M1 polarization, bone marrow derived monocytes treated for 6 days with M-CSF were then treated with LPS (100 ng/ml) and IFNγ (20 ng/ml) for 24 hrs. For M2 polarization, bone marrow derived monocytes treated for 6 days with M-CSF were then treated with IL-4 (25 ng/ml) plus IL-10 (10 ng/ml) for 24 hrs.

Immunohistochemical Analyses

Paraffin-embedded slides were deparaffinized and antigens were unmasked by autoclaving at 121°C for 10 min in Sodium Citrate (pH 6.0) buffer. Slides were incubated with primary antibody (anti-F4/80) in 4 °C overnight. Signals were detected with VECTASTIN ABC Elite kit (Vector Laboratories) and DAB Substrate Kit (Vector Laboratories). Quantitative analysis of $F4/80^{+}$ cells was performed by counting cells in ten high-power fields (20×) per two tissue sections from 6 to 10 mice per group.

Evaluation of Lung Tumors

For determining tumor multiplicity and maximal sizes, whole lungs were inflated with and fixed in 4% paraformaldehyde for 24 hrs. Lungs were paraffin-embedded and serial sections at 400 microns were histologically examined with hematoxylin and eosin (H&E) stain. For quantitation of lung tumor, tumor numbers of 5 serial sections per lung were counted and totaled.

Breast tumor and metastatic burden analysis

Primary tumor burden of *MMTV-PyMT* mice was determined by caliper measurements on live mice at day 100. Metastatic disease was assessed by examining serial sections of formalin-fixed paraffin-embedded lungs. Entire lungs were sectioned and the number of metastatic foci (>5 cells) counted on 5 sections taken every 400 microns following staining with H&E. 13-15 lungs were analyzed for each cohort indicated.