

Supplementary Materials

Antibodies and reagents. The following fluorochrome-conjugated antibodies were used for flow cytometry: anti-mouse CD11b-FITC (M1/70), CD11c-APC (N418), IFN γ -APC (XMG1.2), CXCR3-PE (CXCR3-173), CD8-PE (53-6.7), CD25-PE (PC61.5), CD40L-PE (MR1), B7.1-PE (16-10A1), B7.2-PE (GL1), IA/IE-PE (M5/114.15.2), CD40-PE (1C10), F4/80-PE (CI:A3-1), CD14-PE (Sa14-2), IL4R α -PE (mIL4R-M1), Gr1-PE (RB6-8C5), IL2-PE (JES6-5H4), TNF α -PE (MP6-XT22), Ly6C-PE/Cy7 (HK1.4), PD1-PE (RMP1-30), PDL1-PE (MIH5), Ly6G-APC/Cy7 (1A8), CD4-APC/Cy7 (RM4-5) and control IgG mAbs were purchased from Biolegend. Anti-mouse/rat CCR2-APC (475301) was purchased from R&D systems. Thy1.1-perCP (OX-7) and Ki67-FITC staining set were purchased from BD. Foxp3-APC staining kit was purchased from eBiosciences. For in vivo depletion antibodies, Thy1.1-specific mAb (HIS51) was purchased from eBiosciences. CCR2-specific mAb (MC21) was provided by Professor M. Mack (University of Regensburg, Regensburg, Germany). Ly6G-specific mAb (clone 1A8) was purchased from Bio X cell. CCR2-specific inhibitor CCX872 was provided by ChemoCentryx Inc. (Mountain View, California, USA). Chloromethylfluorescein diacetate succinimidyl ester (CFSE) was purchased from Invitrogen. Cyclophosphamide (CTX) was purchased from Baxter. Gemcitabine (Gem), 5-fluorouracil (5-FU), doxorubicin (Dox) and melphalan (Mel) were purchased from Sigma.

Cell preparation and flow cytometry. Peripheral blood was obtained via tail vein bleeding. Spleen and tumor samples were gently dissociated into single-cell suspension. Red blood cells were removed by ACK lysing buffer. After washing with PBS, cells were stained for surface markers, acquired data using a LSRII (BD Biosciences) and analyzed with FlowJo software (Treestar Inc.) or BD FACSDiva software (BD Biosciences). To quantify the numbers of each subset of myeloid cells in tumor tissue, tumor masses were weighed before being processed into single-cell suspension. For blood and tumor samples, cells were enumerated using BD

Liquid Counting Beads (BD Biosciences) following manufacturer's instruction. For isolation of different subset of myeloid cells, cells were stained with CD11b and Ly6C Abs and subjected to cell sorting using a FACS Aria (BD Biosciences). The purity of the sorted cells was normally greater than 98%. Negative isolation kits from STEMCELL Technologies Inc. were used to isolate CD4+ and CD8+ T cells following manufacturer's instruction.