Supplemental Experimental Procedures

Mice. All experiments were performed on 6-12 week old animals. CD45.1^{+} and CD45.2^{+} C57BL/6 mice were bred in-house or purchased from Frederick Cancer Research Center (Frederick, Maryland). CD169-DTR transgenic mice were generated as described(Miyake et al., 2007). CD169-DTR heterozygous (CD169$^{DTR/+}$) mice were bred in-house by crossing CD169$^{DTR/DTR}$ with C57BL/6 mice. CD169$^{DTR/DTR}$ were crossed to *Ccr2*$^{-/-}$ mice and maintained at the Mount Sinai School of Medicine. Mx1-Cre [B6.Cg-Tg(Mx1-cre)1Cgn/J] and S100a4-Cre [BALB/c-Tg(S100a4-cre)1Egn/YunkJ] were purchased from Jackson Laboratory and bred in-house to R26^{Tomato} [B6.Cg-Gt(ROSA)26Sor$^{tm14(CAG-tdTomato)Hze/J}$] mice at Mount Sinai School of Medicine. Flk2-Switch mice were generated by crossing Flk2^{Cre} mice with R26$^{Tomato/GFP}$ [B6.129(Cg)-Gt(ROSA)26Sor$^{tm4(ACTB-tdTomato,-EGFP)Luo}$/J] mice and bred at the University of California Santa Cruz. B6.129S1-Csf-2rb2^{tm1Cgb} Csf-2rb^{tm1Clsc}/J (*Csf-2*$^{-/-}$) mice and CD11c-DTR mice were purchased from Jackson laboratories and bred at the Mount Sinai School of Medicine. Experimental procedures performed on the mice at each site were approved by the respective Institutional Animal Care and Use Committee of the Mount Sinai School of Medicine, Albert Einstein College of Medicine, Singapore Immunology Network and University of California at Santa Cruz.

Cell preparation. Nucleated single cell suspensions were enriched from peripheral blood, BM, peritoneum, spleen and lung. Peripheral blood was harvested under isoflurane anesthesia (Baxter). BM was harvested by flushing femurs with 1ml of PBS with a 21G needle (BD). Peritoneal cells were harvested by lavaging 10ml of PBS into the peritoneal cavity with a 19G needle (BD). Whole spleen and all or right lung lobes from a single mouse were digested for 45 minutes in an RPMI solution containing 0.4mg/ml Collagenase Type IV (Sigma) and 10% FCS. All cell suspensions were RBC lysed with 1x RBC Lysis solution (eBioscience, San Diego,

CA).

Flow cytometry. Fluorochrome-conjugated or biotinylated mAbs specific to mouse Gr-1 (Ly6C/G) (clone RB6-8C5), CD115 (clone AFS98), B220 (clone RA3-6A2), CD11b (clone M1/70), CD3 (clone 17A2), Ter119 (clone Ter119), CD45 (clone 30-F11), CD45.1 (clone A20), CD45.2 (clone 104), CD11c (clone N418), I-A/I-E (clone M5/114.15.2), Sca-1 (clone D7), Flk2 (clone A2F10), CD117 (clone 2B8), CD16/32 (clone 93), CD34 (clone RAM34), corresponding isotype controls, and secondary reagents (PerCP-efluor710-, PE-Cy7-, Alexa efluor450-conjugated Streptavidin) were purchased from eBioscience. Anti-F4/80 (clone CI:A3.1) was purchased from AbD Serotec (Raleigh, NC). Myeloid cells were gated as depicted in Fig. S1. Hematopoietic stem and progenitor cells were gated as follows: LT-HSC: Lin^- $Sca\text{-}1^+$ $CD117^+$ $Flk2^-$ $CD34^-$; MPP: Lin^- $Sca\text{-}1^+$ $CD117^+$ $Flk2^+$; CMP: Lin^- $Sca\text{-}1^-$ $CD117^+$ $CD34^+$ $CD16/32^{intermediate}$; GMP: Lin^- $Sca\text{-}1^-$ $CD117^+$ $CD34^+$ $CD16/32^{high}$; MDP: Lin^- $Flk2^+$ $CD115^+$ $CD117^{high}$.To measure BrdU incorporation in proliferating cells, animals were injected with 1mg BrdU i.p. 24 hour prior to harvest and samples were processed according to manufacturer's directions (BD Biosciences, San Jose, CA). APC-conjugated anti-BrdU (clone Bu20a) from eBiosciences or Biolegend (Biolegend, San Diego, CA) was used. Positive staining was gated in reference to cells from mice that that were not injected with BrdU. For Ki67 and BrdU double staining, cells were fixed and permeabilized with the transcription factor staining buffer set from eBiosciences, treated with 0.3 mg/ml DNaseI (Sigma-Aldrich, St Louis, MO) and stained with anti-Ki67 (clone SolA15) and anti-BrDU Abs. Multiparameter analysis was performed on an LSRII (BD) and analyzed with FlowJo software (Tree Star). $DAPI^+$ cells and doublets were excluded from all analyses. In BrdU analysis, dead cells were excluded using LIVE/DEAD Fixable Violet Dead Cell Stain (Invitrogen, Carlsbad, CA).

Immgen database. Data from the Immunological Genome Project were utilized to identify genes expressed in tissue macrophages, but not in hematopoietic progenitors or monocytes. The extensive procedures for cell isolation, sorting, and RNA preparation are shown at http://www.immgen.org. The Candidate genes were

then cross-referenced to Jackson database list of available Cre lines: http://www.informatics.jax.org/recombinase.shtml.

Macrophage depletion. For depletion of lung macrophages, heterozygous CD169-DTR ($CD169^{DTR/+}$) were injected i.p. with 10μg/kg DT (Sigma) at time points indicated below. C57BL/6 mice injected with DT and $CD169^{DTR/+}$ mice not injected with DT did not demonstrate tissue macrophage depletion and were both utilized as control animals. $CD169^{DTR/+}$ animals injected with DT served as macrophage-depleted experimental mice. For $CD169^{DTR/+}$*Ccr2*$^{-/-}$ experiments, all mice were injected with one dose and assessed 2, 9, and 16 days after DT administration. For experiments with BM adoptive transfer, two doses of DT were administered on days 4 and 7 after BM transfer. For assessment of BrdU incorporation and lung cytokine expression, two doses of DT were administered at days -5 and -2 prior to harvest. For assessment of lung macrophage recovery after Csf-1R and Csf-2 blockade, two doses of DT were administered at days -12 and -9. Red pulp macrophages were depleted in CD11c-DTR mice upon intraperitoneal injection of 4 μg/kg DT. In some experiments, macrophages were depleted using intranasal and intravenous injection of 50 μl of liposomal clodronate. Cl_2MDP (or clodronate) was a gift from Roche Diagnostics (GmbH, Mannheim, Germany).

Quantitative real-time PCR (Q-PCR). For measurement of lung cytokine gene expression, half the lung mass was homogenized for 30 seconds with a tissue homogenizer in 1ml of PBS. 2 μl of this homogenized mix was lysed in buffer from the Dynabeads RNA Microkit (Invitrogen) in accordance with manufacturer's instructions. Conventional reverse transcription, using the Sprint PowerScript reverse transcriptase (Clontech) was performed in accordance with the manufacturer's instructions. Q-PCR was performed with SYBR GREEN on an ABI PRISM 7900HT Sequence Detection System (Applied Biosystems). The PCR protocol consisted of one cycle at 95°C (10 min) followed by 40 cycles of 95°C (15 s) and 60 C (1 min). Expression of glyceraldehyde-3-phosphate

dehydrogenase (Gapdh) was used as a standard. The average threshold cycle number (C_t) for each tested mRNA was used to quantify the relative expression of each gene: 2^[C_t(Gapdh)-C_t(gene)]. Primers used are listed below: Il4 (fwd) GGTCTCAACCCCCAGCTAGT, Il4 (rev) GCCGATGATCTCTCTCAAGTGAT, Il13 (fwd) CCTGGCTCTTGCTTGCCTT, Il13 (rev) GGTCTTGTGTGATGTTGCTCA, Csf1 (fwd) GAACACTGTAGCCACATGATTGG, Csf1 (rev) TTGACTGTCGATCAACTGCTG, Csf2 (fwd) TCGTCTCTAACGAGTTCTCCTT, Csf2 (rev) CCTGCTCGAATATCTTCAGGC, Flt3l (fwd) TGTGGCAGGGTCTAAGATGC, Flt3l (rev) CTTCTAGGGCTATGGGACTCC, Gapdh (fwd) TGTGTCCGTCGTGGATCTGA, Gapdh (rev) CCTGCTTCACCACCTTCTTGA.

Histopathologic analysis. Lungs were fixed in neutral 10% buffered formalin over night. Paraffin sections were stained with hematoxylin and eosin (H&E) or Periodic acid-Schiff (PAS) and examined by two lung pathology experts (M.B.B. and C.D.B). Pictures from tissue sections were acquired using a Nikon digital sight camera (Nikon, Tokyo, Japan) mounted to an Olympus BX45 microscope (Olympus, Tokyo, Japan). Magnification is based on multiplication of 10x ocular and 40x or 60x objectives.