SUPPLEMENTAL MATERIAL

Chong et al., http://dx.doi.org/10.1084/jem.20160800

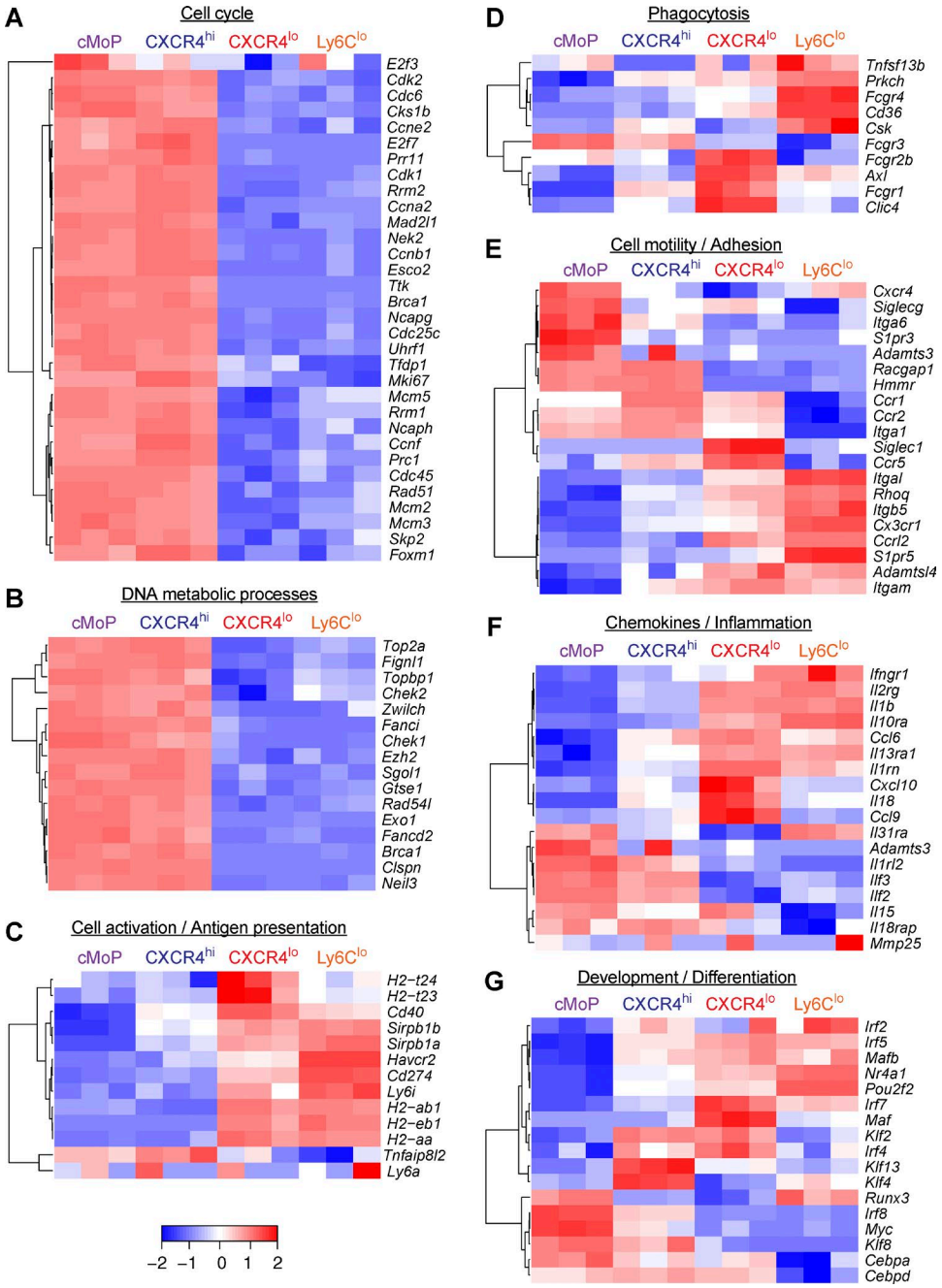


Figure S1. **Heatmaps of genes between cell subsets of the monocyte developmental pathway.** (A–G) NGS was performed on RNA extracted from sorted BM cMoPs, CXCR4^{hi} and CXCR4^{lo} Ly6C^{hi} monocytes and Ly6C^{lo} monocytes from three individual mice. Heat maps showing genes that are involved in cell cycle (A), DNA metabolic processes (B), cell activation and antigen presentation (C), phagocytosis (D), cell motility and adhesion (E), chemokines and inflammation (F) and development and differentiation (G).

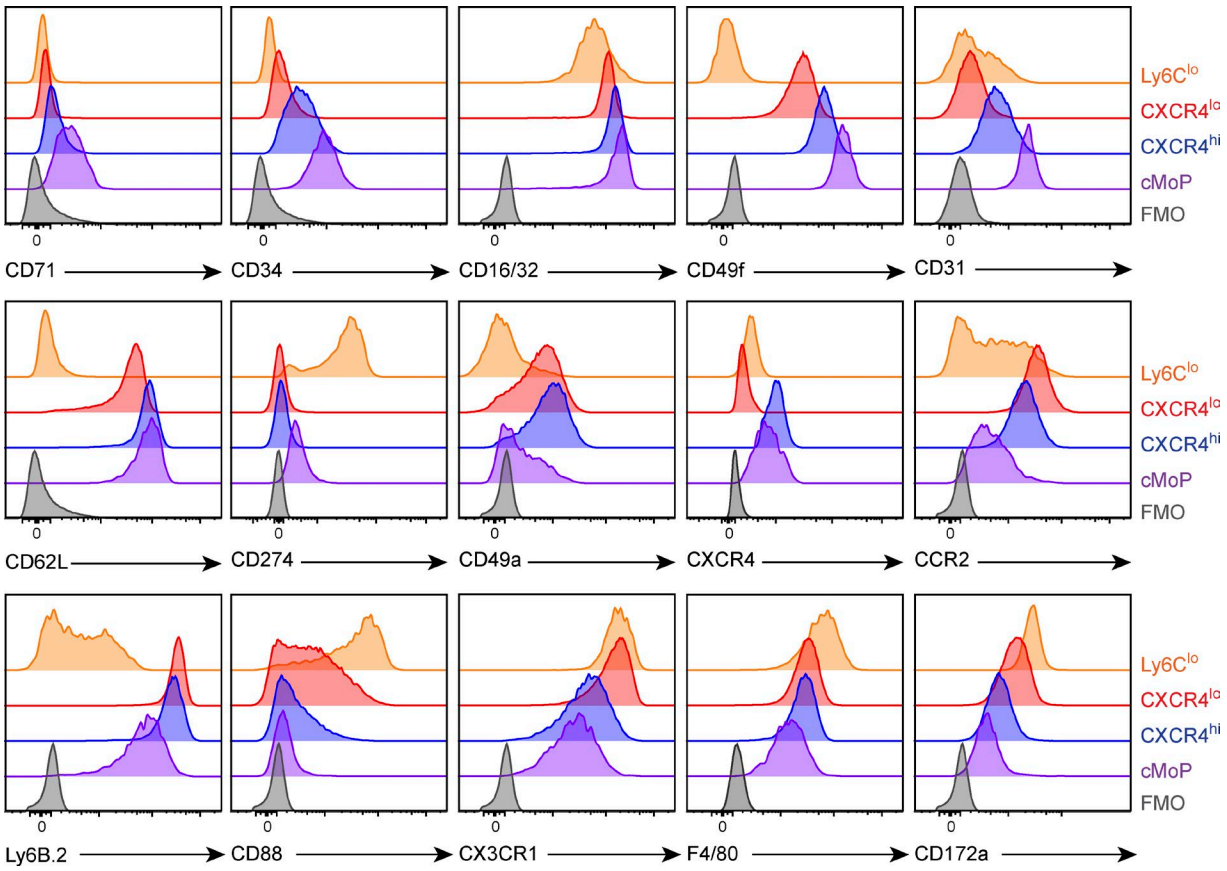


Figure S2. **Cell surface marker expression for distinguishing monocyte subsets.** Flow cytometry was performed on BM cMoPs, CXCR4^{hi} and CXCR4^{lo} Ly6C^{hi} monocytes and Ly6C^{lo} monocytes for the analysis of indicated surface markers. Results are representative of one out of two independent experiments.

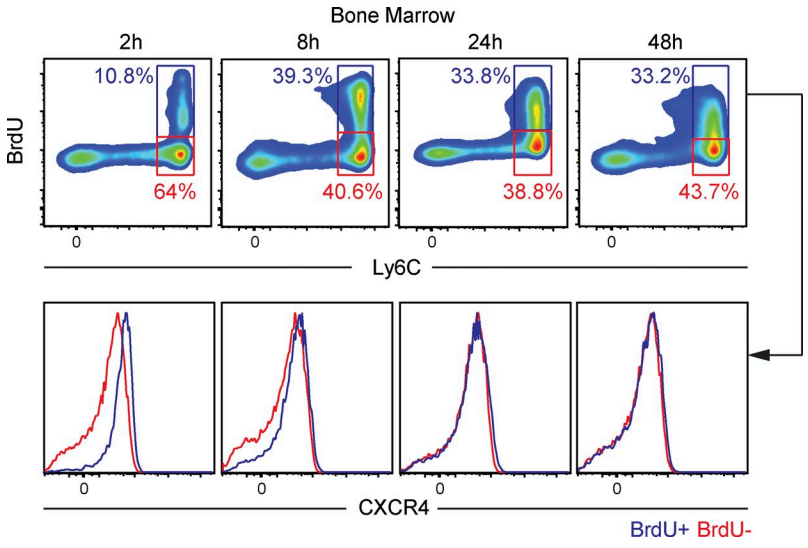


Figure S3. **Gating strategy for the analysis of CXCR4 expression on BM Ly6C^{hi} monocytes.** Representative flow cytometry plots showing progressive BrdU incorporation into BM Ly6C^{hi} monocytes (*top panels*) and subsequent gating for CXCR4 expression (*bottom panels*) of BrdU^{+} cells (*red box*), BrdU^{-} cells (*blue box*; n = 4–5 mice per group). Results representative of one out of two independent experiments.

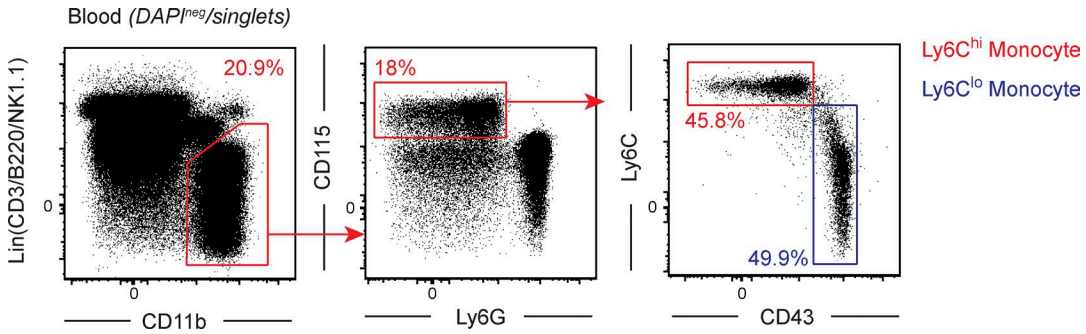
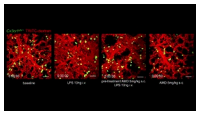
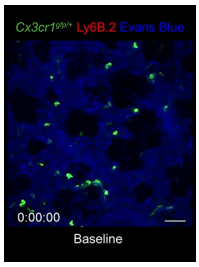


Figure S4. **Gating strategy of blood monocytes in mice.** Gating strategy for blood monocytes (pregated on DAPI^{neg}/singlets). Monocytes are defined as Lin(CD3/B220/NK1.1)$^{-}$CD115^{+}Ly6G^{-}CD11b^{+} and Ly6C^{hi}CD43^{-} or Ly6C^{lo}CD43^{+} according to the subset. Flow cytometry plots are representative of one out of at least three independent experiments.



Video 1. **Monocyte margination in the pulmonary vasculature.** Time-lapse image sequence of maximum intensity projection displaying the behavior of GFP^{+} cells in the lungs (alveolar space) of *Cx3cr1*$^{gfp/+}$ mice. TRITC-dextran (250 µg) was administered i.v. to delineate the lung vasculature. Shown are the motility patterns of GFP^{+} monocytes at baseline and after treatment with LPS (10 ng), AMD3100 (5 mg/kg), or LPS and AMD3100 together. For mice treated with LPS, monocytes are observed to reduce their speed and increase duration of adherence in the lung endothelium. This phenomenon was abolished when mice were pretreated with AMD3100. Videos are representative of 3 mice per treatment. Elapsed time is shown as hours:minutes:seconds. Scale bar: 50 µm.



Video 2. **Visualization of Ly6C^{hi} monocytes in *Cx3cr1*$^{gfp/+}$ mice with an anti–Ly6B.2 antibody.** Time-lapse image sequence of maximum intensity projection displaying the behavior of GFP^{+} cells in the lungs (alveolar space) of *Cx3cr1*$^{gfp/+}$ mice. Evans blue (50 µg) was administered i.v. to delineate the lung vasculature. 5 min after the beginning of imaging, Ly6B.2-PE (4 µg) was injected i.v., and allowed the visualization of Ly6C^{hi} monocytes (Ly6B.2^{+}CX$_3$CR1^{+}), Ly6C^{lo} monocytes (Ly6B.2^{-}CX$_3$CR1^{+}) and neutrophils (Ly6B.2^{+}CX$_3$CR1^{-}). Video is representative of 3 mice. Elapsed time is shown as hours:minutes:seconds. Scale bar: 40 µm.