SUPPLEMENTAL MATERIAL

Chow et al., http://www.jem.org/cgi/content/full/jem.20101688/DC1

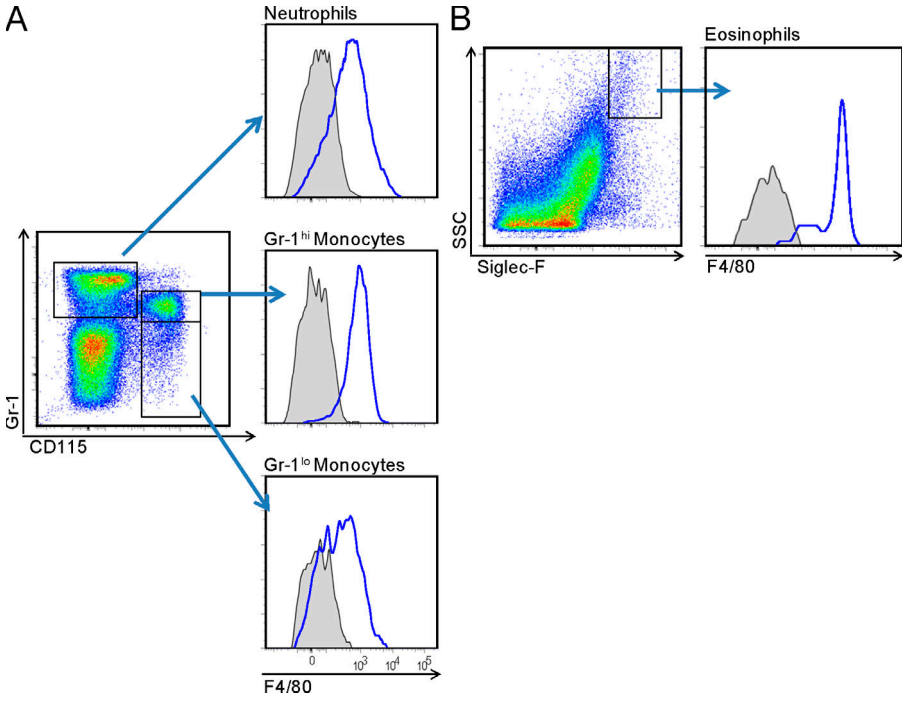

Figure S1. BM neutrophils, MOs, and eosinophils express F4/80. (A) Total DAPI$^-$ single cells were gated by Gr-1 and CD115 staining. Staining with F4/80 (blue line) and isotype control (gray shaded histogram) on Gr-1$^+$ CD115$^-$ neutrophils, Gr-1$^+$ CD115$^+$ Gr-1^{hi} MOs, and Gr-1$^-$ CD115$^+$ Gr-1^{lo} MOs is displayed. (B) Eosinophils were gated as SSC^{hi}Siglec-F$^+$ cells and expression of F4/80 (blue) and isotype control (gray) is displayed.

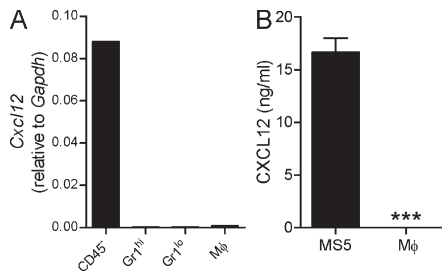

Figure S2. MΦ do not produce CXCL12. (A) CD45$^-$ cells, Gr-1^{hi} MOs, Gr-1^{lo} MOs, and MΦ were sorted from the BM, and expression of *Cxcl12* relative to *Gapdh* was assessed by Q-PCR. (B) Levels of CXCL12 in the supernatant of MS-5 culture in complete medium and in the supernatant from BMDM were measured by ELISA. Each experiment was performed once. ***, $P < 0.001$

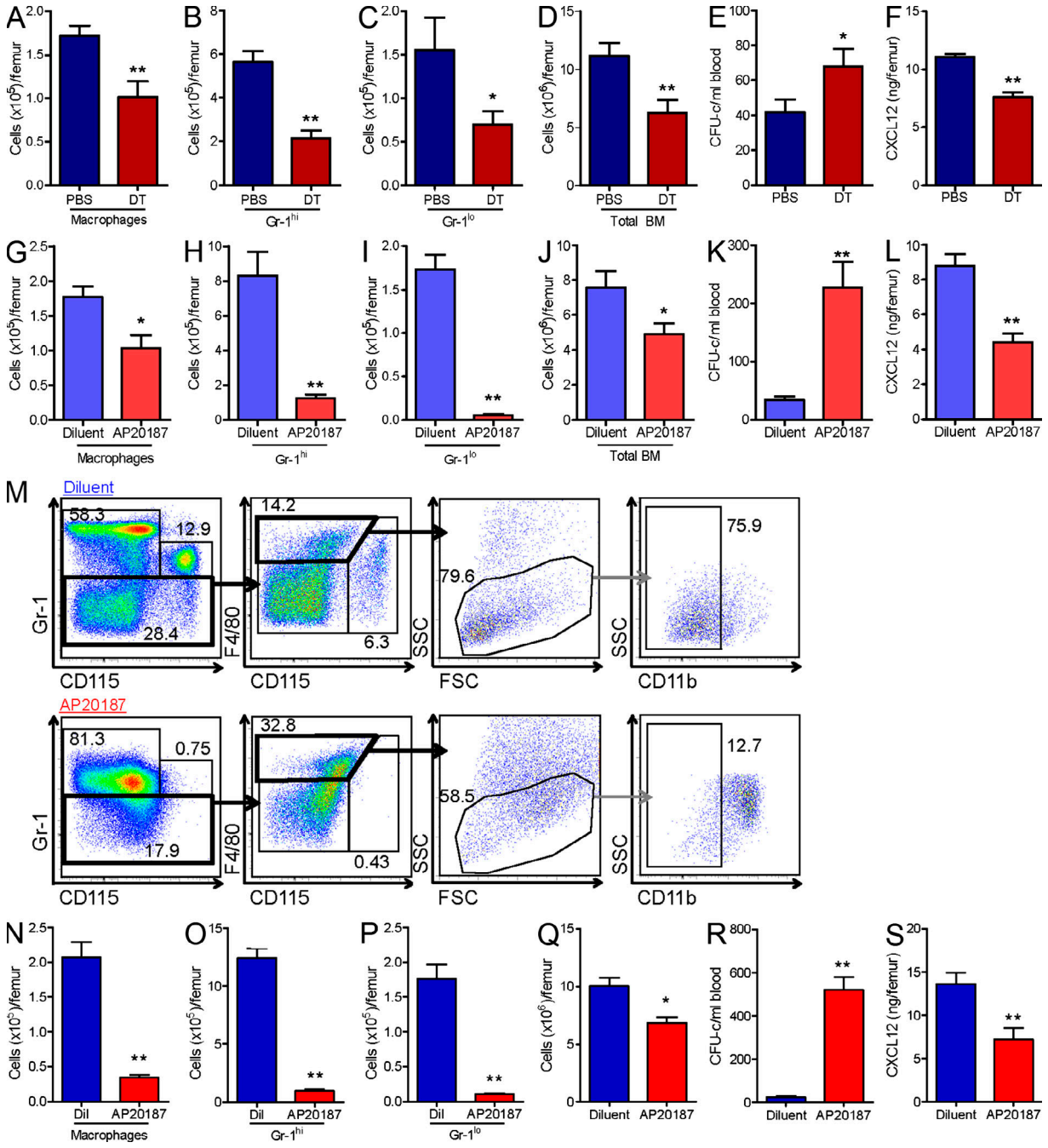

Figure S3. Three other models in which mononuclear phagocyte depletion is associated with robust HSC/progenitor mobilization and CXCL12 reduction. (A–F) CD11b-DTR BM chimeras treated with two doses of PBS (dark blue) or DT (dark red). Quantification of BM mononuclear phagocytes ($n = 9$; A–C), total nucleated cells ($n = 9$; D), CFU-C ($n = 15–16$; E), and BMEF CXCL12 levels ($n = 7$; F). Data are pooled from three independent experiments. (G–L) Mafia BM chimeras were injected with diluent (purple) or AP20187 (light red) and analyzed 24 h after the last injection. Quantification of mononuclear phagocytes ($n = 5$; G-I) and total nucleated cells per femur ($n = 5$; J), CFU-C per milliliter of blood ($n = 11–13$; K), and BMEF CXCL12 ($n = 5$; L). G–J and L show representative data from three independent experiments; K represents pooled data from three independent experiments. (M–S) Non-transplanted Mafia mice were injected with diluent (blue) or AP20187 (red) and analyzed 24 h after the final injection. (M) MΦ were gated by Gr-1^{lo} CD115^{int} F4/80^{+} SSC$^{int/lo}$ CD11b^{lo}. Gr-1^{lo} CD115^{int} F4/80^{+} SSC$^{int/lo}$ CD11b^{hi} gating demonstrated BM neutrophilia that leaked into the Gr-1^{lo}CD115^{int}F4/80^{+}SSC$^{int/lo}$ gate after treatment with AP20187. (N–P) Quantification of absolute numbers of mononuclear phagocytes ($n = 5–6$) and CXCL12 levels in the BMEF by ELISA ($n = 5–6$) 1 d after the last injection of diluent (Dil) or AP20187 in nontransplanted Mafia mice. Data are pooled from two independent experiments and represented as mean± SEM. *, $P < 0.05$; **, $P < 0.01$.

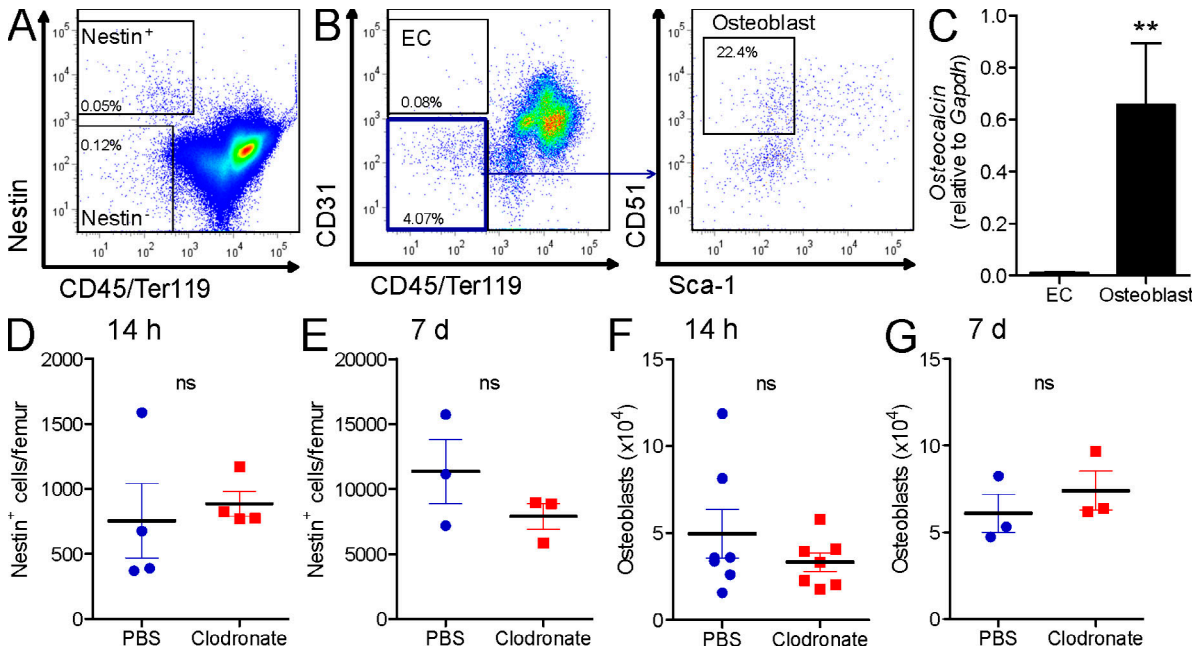

Figure S4. Sorting BM Nestin⁺ and Nestin⁻ fractions and bone endothelial cell and osteoblast fractions. (A and B) Sorting strategy for Nestin⁺ and Nestin⁻ fractions from the BM (A) and endothelial cells (EC) and osteoblasts from the bone (B). FACS plots were pregated on DAPI⁻ single cells. (C) Q-PCR of *Osteocalcin* expression relative to *Gapdh* in in vivo–sorted endothelial cells (EC) and osteoblasts. Representative data from three independent experiments are shown (n = 3–4). (D–G) Counts of Nestin⁺ cells in one femur (D and E) and osteoblasts digested from femurs, tibias, and humeri (F and G) 14 h (D and F) and 7 d (E and G) after treatment with PBS- (blue circles) or clodronate-encapsulated (red squares) liposomes. (D) Representative data from three independent experiments are shown (n = 3–4). (E and G) Experiments were performed once (n = 3). (F) Data pooled from two independent experiments (n = 7). **, P < 0.01; ns, not significant.

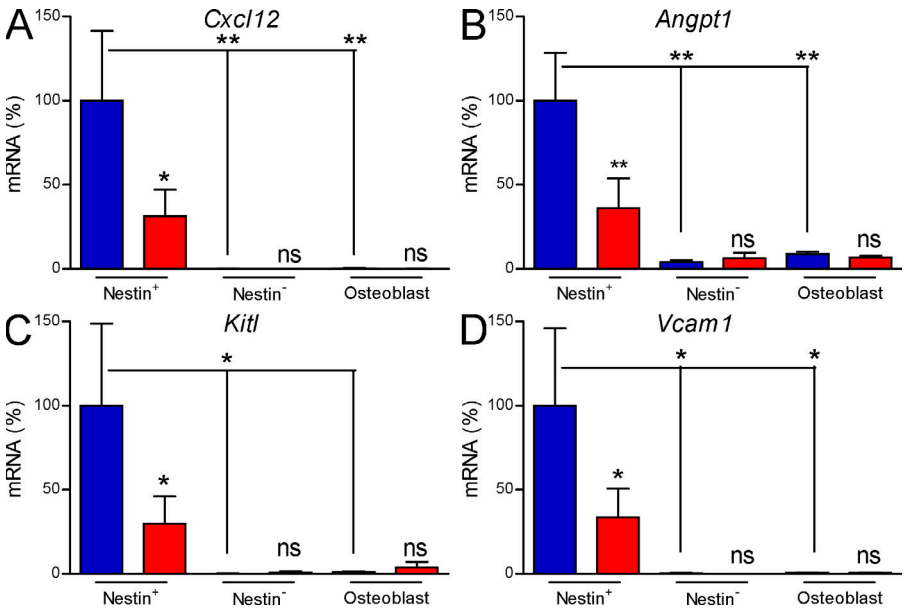

Figure S5. Retention gene expression is reduced in Nestin⁺ cells 7 d after mononuclear phagocyte treatment. (A–D) Relative expression of *Cxcl12* (A), *Angpt1* (B), *Kitl* (C), and *Vcam1* (D) in Nestin⁺ and Nestin⁻ fractions sorted from the BM and osteoblasts sorted from the bone 7 d after treatment with PBS- (blue bars) or clodronate-encapsulated (red bars) liposomes. Data were analyzed by one-way ANOVA/Newman-Keuls test. n = 3. *, P < 0.05; **, P < 0.01; ns, not significant

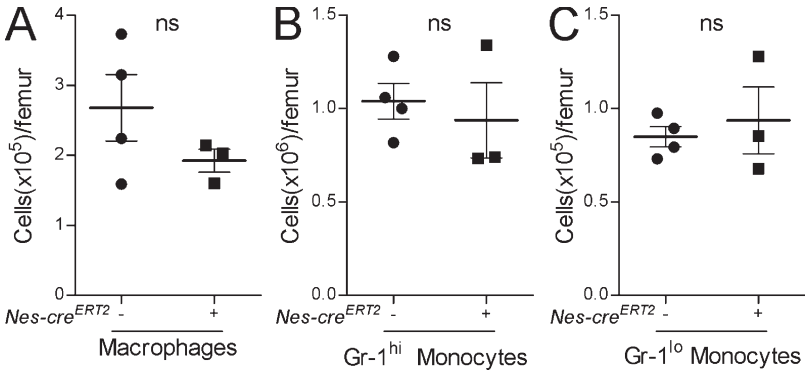

Figure S6. Mononuclear phagocytes are not reduced 7 d after depletion of Nestin⁺ cells. Tamoxifen-treated *Nes-Cre^{ERT2}/iDTR* (+) and control *iDTR* (−) animals were analyzed 7 d after administration of DT for numbers of MΦ (A), Gr-1^{hi} MOs (B), and Gr-1^{lo} MOs (C). ns, not significant.

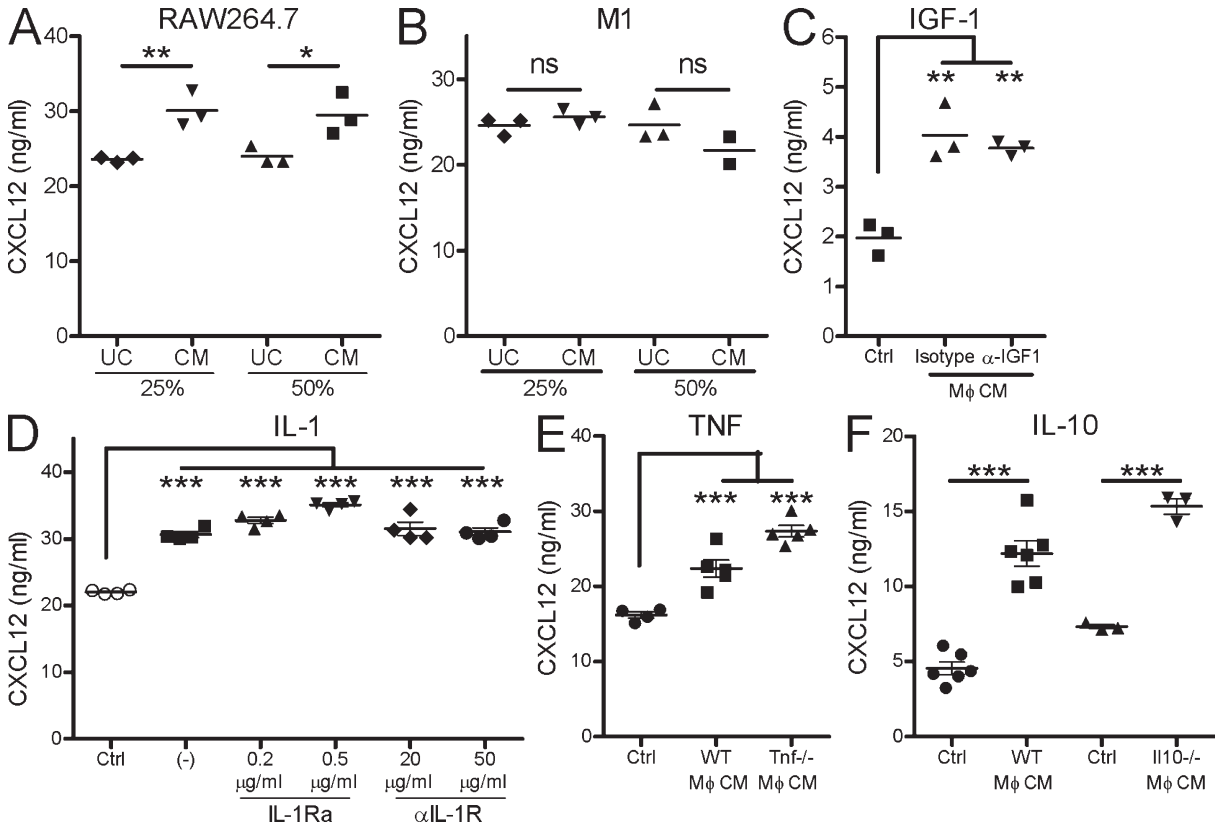

Figure S7. Soluble factor from MΦ, but not myeloblast, cell line enhances stromal CXCL12 production. 200,000 RAW264.7 MΦ (A) and M1 myeloblast (B) cells were cultured in 300 µl of their respective media. After 3 d of culture, the supernatant or fresh control medium was mixed with complete medium at either 25 or 50% concentration to make conditioned medium (CM) or unconditioned medium (UC), respectively. CM or UC was transferred onto 5,000 MS-5 stromal cells plated 24 h prior. CXCL12 levels were determined by ELISA. (C–F) 5,000 MS-5 cells were cultured in complete medium (Ctrl) or media conditioned by MΦ (MΦ CM) that was supplemented with blocking antibody to IGF-1 (C), IL-1 receptor antagonist (D), and blocking antibody to IL-1R (D). In E and F, the ability of MΦ CM derived from TNF- (E) and IL-10–deficient (F) animals to up-regulate CXCL12 production from MS-5 cells were compared with MΦ CM derived from wild-type animals. All experiments were performed 1–2 times. Data were analyzed by one-way ANOVA/Newman-Keuls test. *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$; ns, not significant.