SUPPLEMENTAL MATERIAL

Auffray et al., http://www.jem.org/cgi/content/full/jem.20081385/DC1

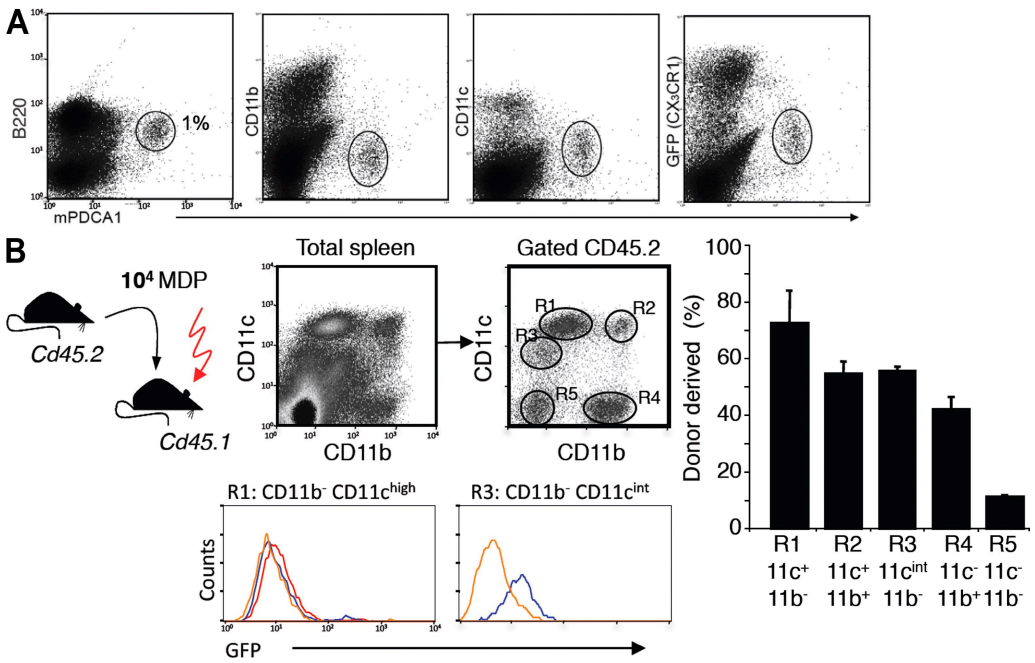


Figure S1. Expression of CX_3CR1 by PDCs. (A) PDCs express CX_3CR1. PDCA1$^+$ cells among $Cx_3cr1^{gfp/+}$ C57Bl/6 splenocytes express gfp/CX_3CR1 and intermediate levels of CD11c and B220. (B) Adoptive transfer of MDPs into irradiated host (900 rad). MDPs from Cd45.2 mice were adoptively transferred into Cd45.1 congenic recipients. Spleens of recipient mice were analysed at day 7 after transfer by flow cytometry using lineage marker and CD11b, CD11c, and CD8a$^+$ antibodies. The experiment was repeated five times with two to three mice per group and with similar results. Histograms represent the frequency of splenocytes derived from donor CD45.2 MDPs in an experiment with a high efficiency of MDP engraftment. Donor MDP appears to be very efficient at generating the population in R3 (CD11cint CD11b neg), which appears to be distinct from cDCs in R1 because they express lower CD11c and higher CX_3CR1. Error bars show SD.

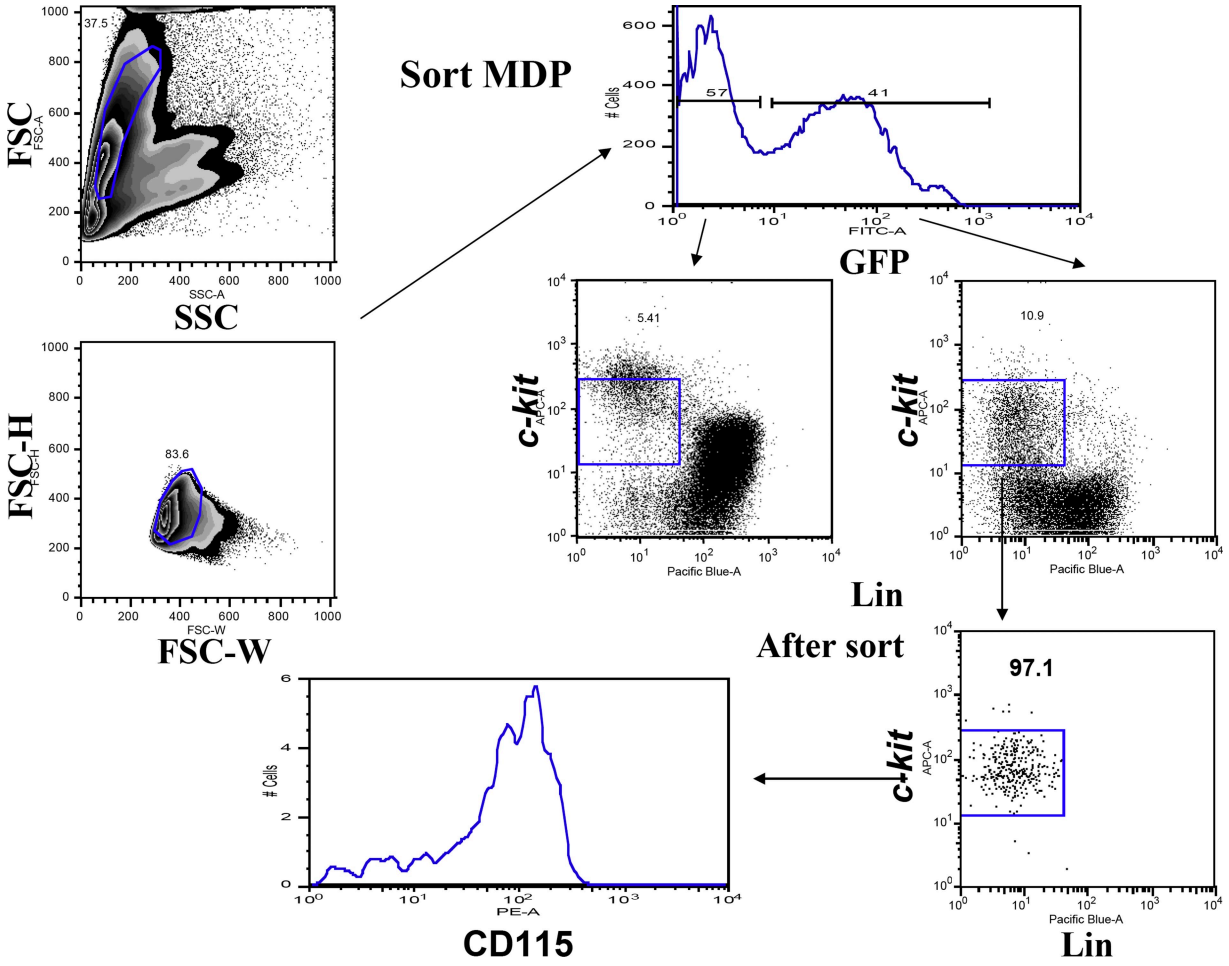


Figure S2. Sorting gates for MDPs and purity after sort.

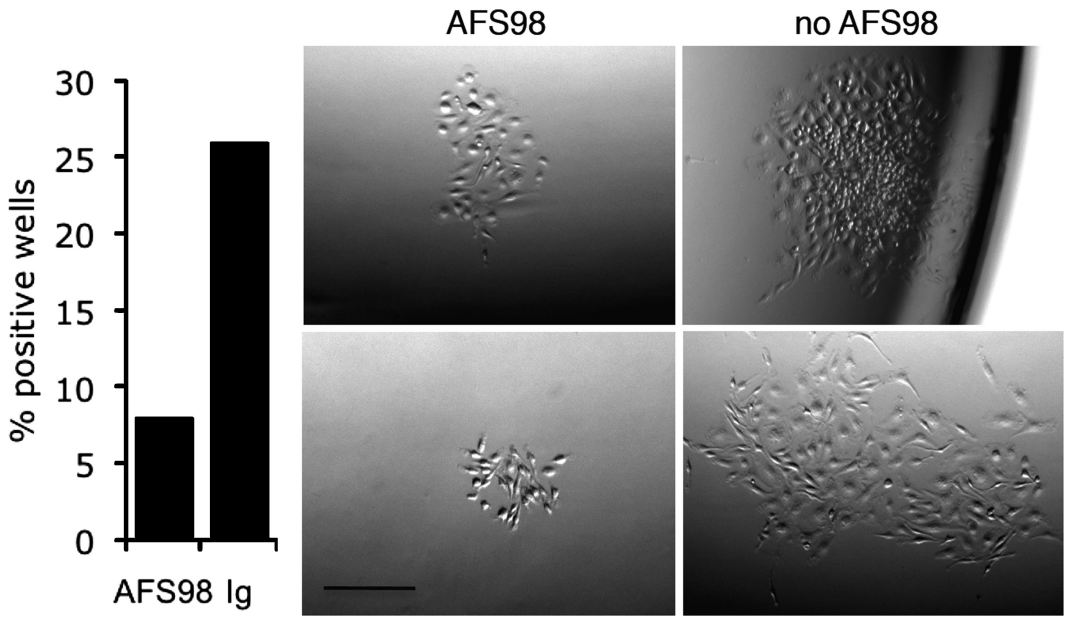


Figure S3. Effects of the AFS98 anti-CD115 antibody on the response of MDP to M-CSF in vitro. BM cells from *Cx$_3$cr1$^{gfp/+}$* mice were labeled with IL7Ra, CD117, a lineage cocktail (CD11c, CD11b, NK1.1, CD3, Ter119, CD19, and Gr1), antibodies, or with the same cocktail plus PE-conjugated anti-CD115 antibody (AFS98) at a 1/100 dilution. MDPs defined as lineage$^-$ CX$_3$CR1$^+$ CD117^{int-} were sorted as single cells with a FACSAria into 96-well plates and cultured in the presence of 50 ng/ml CSF-1 as previously described (Fogg, D.K., C. Sibon, C. Miled, S. Jung, P. Aucouturier, D.R. Littman, A. Cumano, and F. Geissmann. 2006. *Science*. 311:83–87). Positive wells were counted at day 7 (left) and photographed. Representative bright-field pictures of clones grown from single MDPs sorted with or without AFS98 in the antibody cocktail are displayed on the right. AFS98 was only added to the antibody cocktail for cell sorting, and no AFS98 was added during culture. Bar, 100 µm.

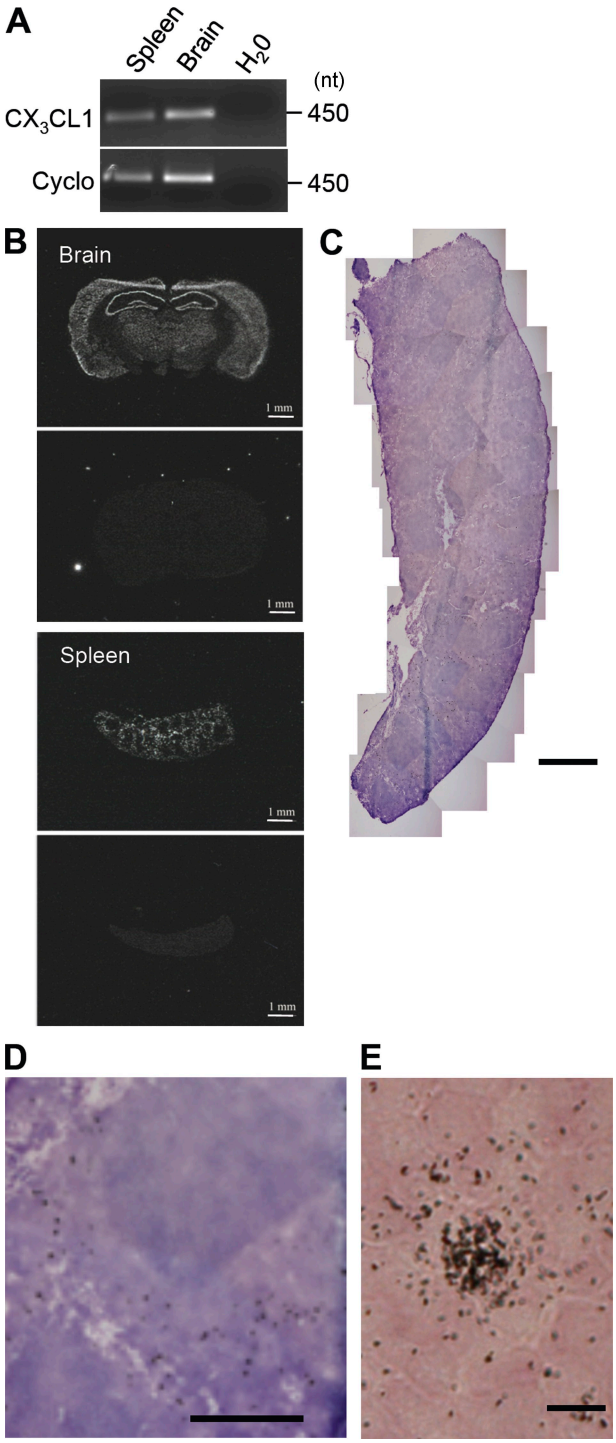


Figure S4. Fractalkine/CX_3CL1 expression and recruitment of monocytes to the spleen. (A) Mouse fractalkine/CX_3CL1 transcripts are detected in the spleen and brain of C57/Bl6 mice. Ubiquitously expressed cyclophilin (cyclo) is shown as a reference. (B) Expression of CX_3CL1 is detected in autoradiograms from brain tissue sections (positive control), and spleen tissue sections from WT mice, hybridized with antisense (top) and sense (negative control; bottom) ^{35}S-labeled probes are also detected. Bar, 1 mm. (C) Bright-field micrographs from spleen tissue section hybridized with the Cx_3cl1 antisense probe. CX_3CL1 transcript-positive cells were detected around B cell follicles, in the marginal zone/T cell area. Bar, 500 µm. (D) Magnification of C, making it possible to see the perifollicular locations of positive cells. Bar, 200 µm. (E) magnification of C to distinguish the numerous silver grain in the positive cells. Bar, 10 µm .

Cell Cycle (Data brut)	Bone marrow	Spleen n=6
% G1	58.8	95.33 ±1.12
% S	19.5	0.99 ±0.22
% G2/M	17.3	1.85 ±036

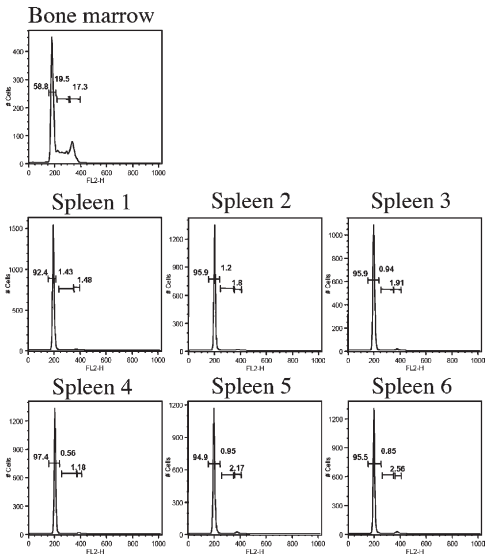


Figure S5. Analysis of the proliferation of monocytes in the spleen and BM. BALB/c mice were infected with 3×10^3 bacteria. 48 h later, spleen and leg bones were harvested. BM was flushed with RPMI 5% FCS and spleen were treated with collagenase/DNase. After lysis of red blood cells, cell suspensions were separately enriched for CD11b (MACs) and stained for CD11b, Ly6c, DX5, CD3, CD19, and Ly6G surface markers. Monocytes ($CD11b^+$ $Ly6C^+$ $DX5^-$ $CD3^-$ $CD19^-$ $Ly6G^-$) were sorted and splenic monocytes from individual mice were treated independently for cell cycle, whereas monocytes from BM were pooled. Cell cycle is defined as the following: cells were washed in PBS and fixed in 70% EtOH at 4°C for 30 minutes. Samples were then stained with 50 µg/ml PI in PBS complemented with 50 µg/ml RNase at 37°C for 30 min. Samples were then analyzed by flow cytometry.

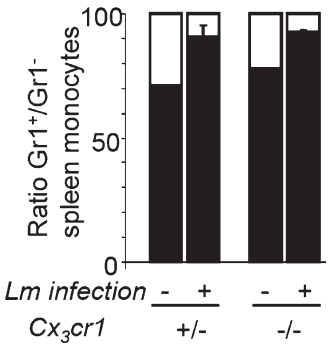


Figure S6. Ratio of $Gr1^+$ and $Gr1^-$ monocytes in the spleens of infected and control mice. BALB/c $Cx_3cr1^{+/-}$ and $Cx_3cr1^{-/-}$ were infected with 3×10^5 bacteria or received PBS as control. Bar graphs show the frequency (mean ± SEM) of spleen monocytes subsets in infected and controls mice. Error bars represent mean and SD (n = 4–6 mice per group).

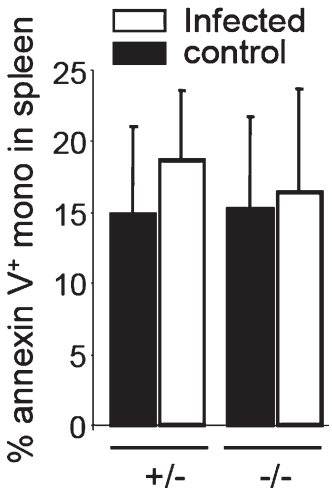


Figure S7. Frequency of annexin-V–positive cells in the spleens of infected and control mice. BALB/c $Cx_3cr1^{+/-}$ and $Cx_3cr1^{-/-}$ were infected with 3×10^5 bacteria or received PBS as control. Bar graphs show annexin-V binding by spleen monocytes after 24 h by flow cytometry in individual mice. Error bars represent mean and SD (n = 4–6 mice per group).

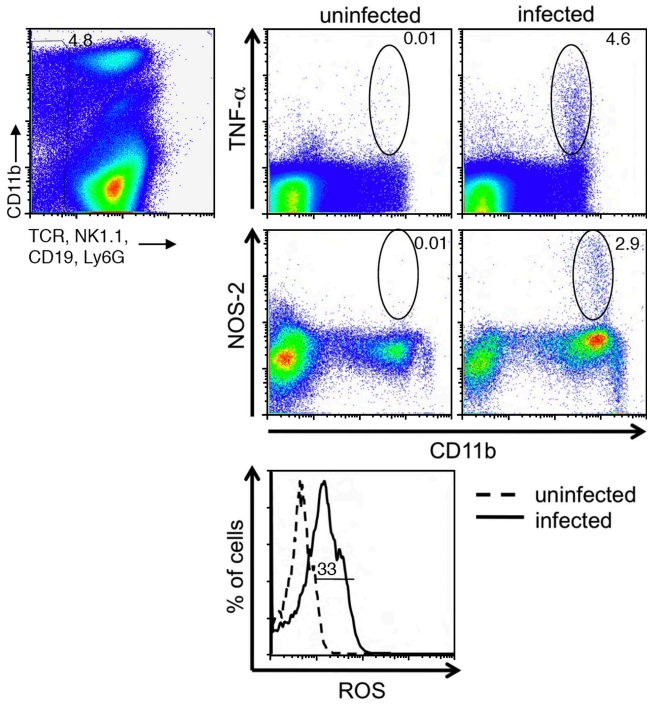
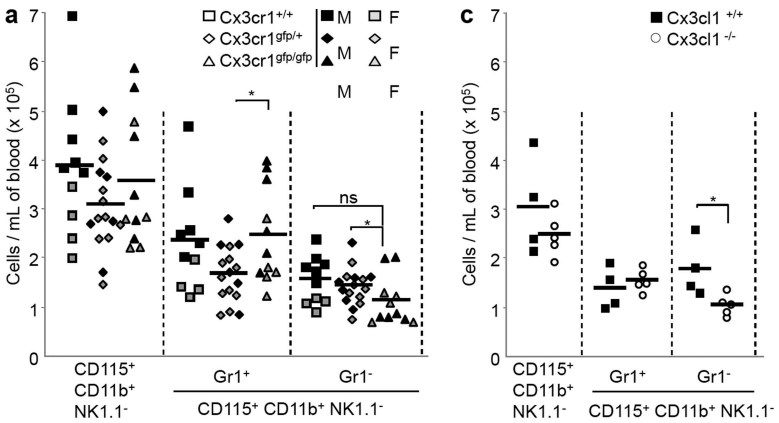


Figure S8. Production of TNF-α, iNOS, and ROI by lineage$^-$ CD11c^{low} CD11b$^+$ Gr1$^+$/Ly6c$^+$ spleen cells from uninfected C57/Bl6 mice and C57/Bl6 mice infected with 7×10^3 bacteria for 48 h.



b

CD115+ CD11b+	Cx3cr1gfp/gfp	Cx3cr1+/+	Cx3cr1gfp/+
Cx3cr1gfp/gfp			
Cx3cr1+/+	0.5262		
Cx3cr1gfp/+	0.4897	0.1023	

Gr1+	Cx3cr1gfp/gfp	Cx3cr1+/+	Cx3cr1gfp/+
Cx3cr1gfp/gfp			
Cx3cr1+/+	0.7782		
Cx3cr1gfp/+	0.0484	0.0578	

Gr1-	Cx3cr1gfp/gfp	Cx3cr1+/+	Cx3cr1gfp/+
Cx3cr1gfp/gfp			
Cx3cr1+/+	0.0671		
Cx3cr1gfp/+	0.0382	0.5271	

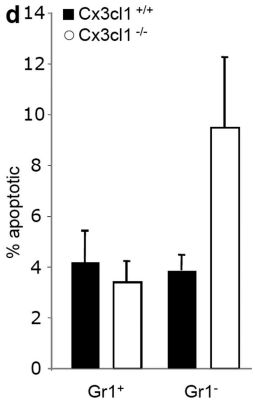


Figure S9. Monocyte survival in CX_3CR1-deficient mice and CX_3CL1-deficient mice. (a) Total blood monocytes ($CD115^+$ $CD11b^+$ $NK1.1^-$) and subsets ($Gr1^+/Ly6c^+$ and $Gr1^+/Ly6c^-$ monocytes) were enumerated in blood from *$Cx_3cr1^{+/+}$* (squares), *$Cx_3cr1^{gfp/+}$* (diamonds), and *$Cx_3cr1^{gfp/gfp}$* (triangles) 6–8-wk-old littermates. Each symbol corresponds to an individual mouse. Numbers for female mice are gray and for male mice are black. (b) The table represents p-values from the Wilcoxon test for the monocytes counts displayed in a. (c) Total blood monocytes ($CD115^+$ $CD11b^+$ $NK1.1^-$) and subsets ($Gr1^+/Ly6c^+$ and $Gr1^+/Ly6c^-$ monocytes) were enumerated in blood from *$Cx_3cl1^{+/+}$* (filled squares) and *$Cx_3cl1^{-/-}$* (open circles) mice. Horizontal lines represent the mean of results from individual mice. (d) Annexin-V staining of $Ly6c^+$ monocytes and $Ly6c^-$ monocytes in *$Cx_3cl1^{+/+}$* and *$Cx_3cL1^{-/-}$* mice. Asterisks indicate significative differences between groups ($P < 0.05$ using the Wilcoxon test). Error bars show SD.

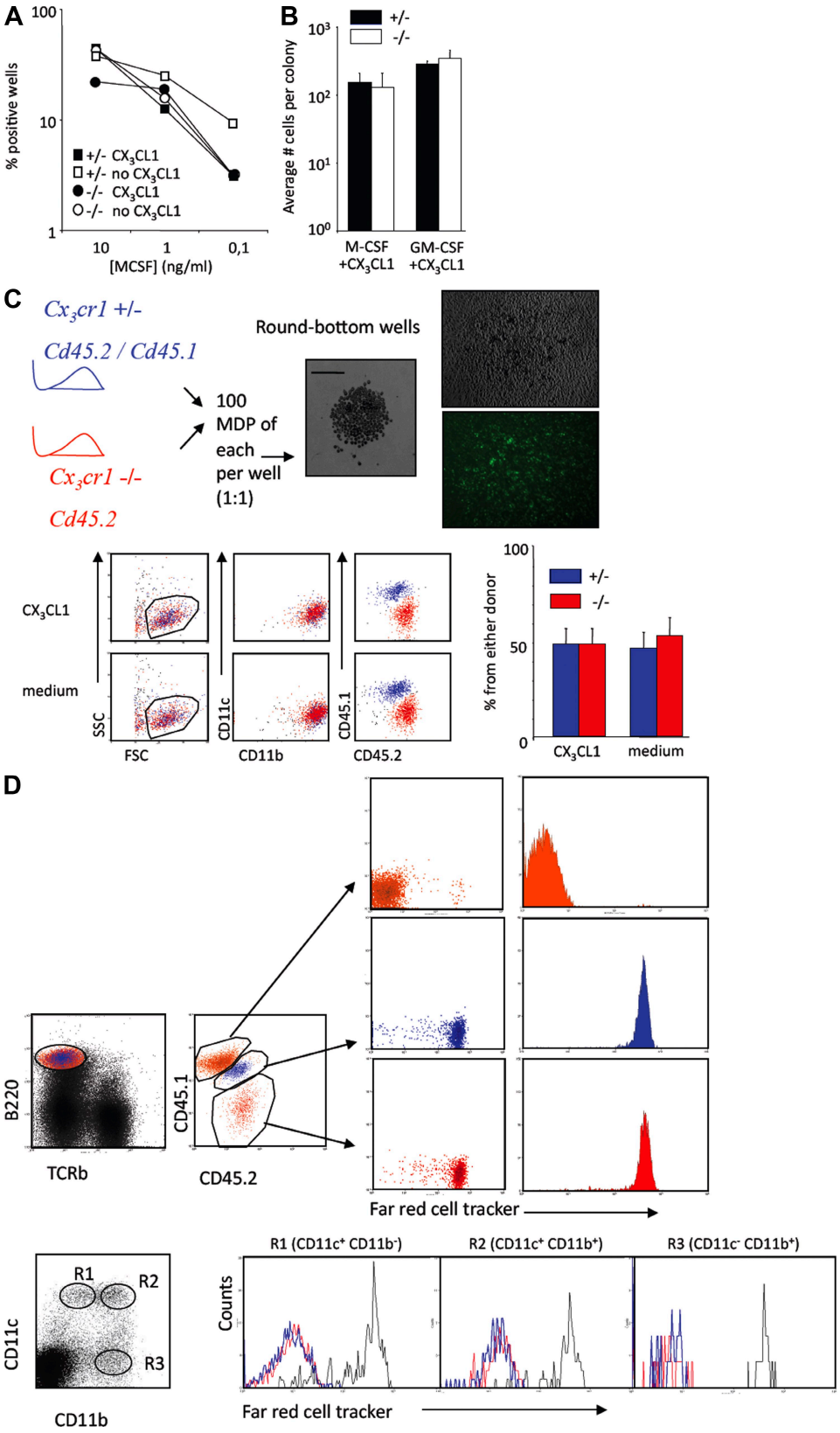


Figure S10. CX_3CR1 and CX_3CL1 are dispensable for MDP cloning, survival, and proliferation in vitro and in vivo. (a) In vitro cloning efficiency of MDPs. MDP from *$Cx_3cr1^{+/-}$* and *$Cx_3cr1^{-/-}$* mice were purified by flow cytometry as previously described (Fogg, D.K., C. Sibon, C. Miled, S. Jung, P. Aucouturier, D.R. Littman, A. Cumano, and F. Geissmann. 2006. *Science.* 311:83–87) and cloned in a 96-well plate coated with recombinant CX_3CL1-fractalkine or BSA alone and in the presence of increasing concentration of M-CSF. Cloning efficiency was measured as previously described (Fogg, D.K., C. Sibon, C. Miled, S. Jung, P. Aucouturier, D.R. Littman, A. Cumano, and F. Geissmann. 2006. *Science.* 311:83–87), and wells containing a colony of at least 200 cells after 3–10 d of culture were considered positive. The experiment was performed three times with similar results. (b) Proliferation of MDP. Bar graphs represent the mean size of colonies obtained from single clones described in a and for similar experiments in which M-CSF was replaced wth GM-CSF. Results are the mean and SD from three experiments. (c) In vitro competition experiment. Equal numbers (10^2) of MDP from *$Cx_3cr1^{+/-}$ Cd45.1/2* mice (blue) and *$Cx_3cr1^{-/-}$Cd45.2/2* mice (red) were mixed and seeded in round-bottom 96-well plates coated with recombinant CX_3CL1 or BSA alone and cultivated in culture medium with 10 ng/ml M-CSF. The percentages of CD45.1/2 and CD45.2/2 cells were determined after 5 d of culture. At least 10 wells were studied for each experimental condition. The bar graph represents the mean and SD of a representative experiment out of four experiments performed. Bar, 100 µm. (d) In vivo proliferation of MDPs and MDP-derived cells. 10^4 MDPs from *$Cx_3cr1^{+/-}$ Cd45.1/2* mice (blue), 10^4 MDPs from *$Cx_3cr1^{-/-}$ Cd45.2/2* mice (red), and 10^6 $CD19^+$ B cells (as a control; black) were labeled with Cell Tracker 633 (Bodipy 630/650 MeBr) and adoptively transferred into an irradiated (1,000 rad) *Cd45.1* congenic recipient. 6 d later, donor MDP-derived cells from both *Cd45.1/2* and *Cd45.2/2* mice were no longer labeled, whereas B cells retained the dye.