Cell Host & Microbe, Volume *28*

Supplemental Information

During *Aspergillus* Infection, Monocyte-Derived DCs, Neutrophils, and Plasmacytoid DCs Enhance Innate Immune Defense through CXCR3-Dependent Crosstalk

Yahui Guo, Shinji Kasahara, Anupam Jhingran, Nicholas L. Tosini, Bing Zhai, Mariano A. Aufiero, Kathleen A.M. Mills, Mergim Gjonbalaj, Vanessa Espinosa, Amariliz Rivera, Andrew D. Luster, and Tobias M. Hohl

SUPPLEMENTAL INFORMATION

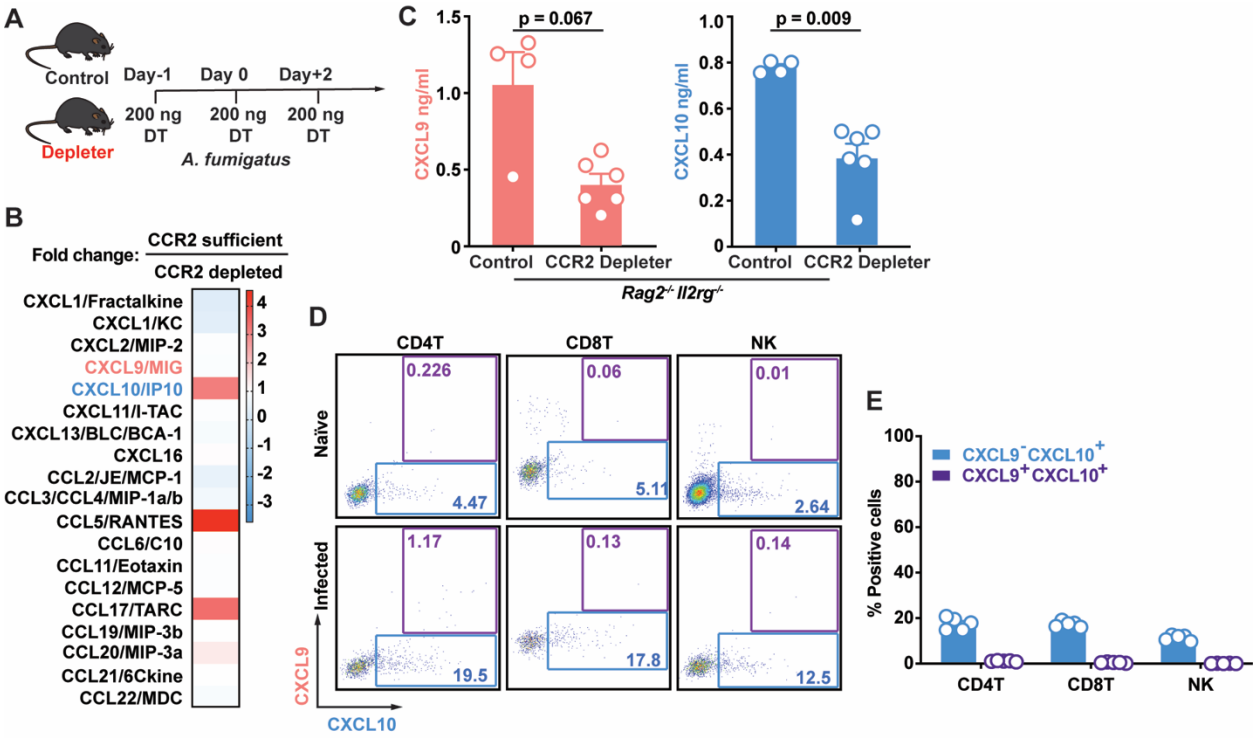

Figure S1. CCR2^{+} myeloid cells regulate CXCL9 and CXCL10 production during *A. fumigatus* infection. Related to Figure 1.

(A) Experimental Scheme: Diphtheria toxin (DT) was administered intraperitoneally (i.p.) as indicated to ablate DTR^{+} cells in mouse strains that express the CCR2-DTR transgene (Depleter) or non-transgenic littermates that not do express the CCR2-DTR transgene (Control).

(B) Fold change in lung chemokine levels in CCR2-sufficient (CCR2-DTR$^{-/-}$ *Rag2*$^{-/-}$*Il2rg*$^{-/-}$) versus CCR2-depleted (CCR2-DTR$^{+/-}$ *Rag2*$^{-/-}$*Il2rg*$^{-/-}$) mice that lack lymphoid lineage cells 36 h pi with 3 × 10^{7} CEA10 conidia, as measured by proteome profiler array (n = 2 per group, data pooled from 2 experiments).

(C) Lung CXCL9 and CXCL10 levels in CCR2-DTR$^{+/-}$ *Rag2*$^{-/-}$*Il2rg*$^{-/-}$ and littermates (CCR2-DTR$^{-/-}$ *Rag2*$^{-/-}$*Il2rg*$^{-/-}$) (n = 4-6 per group) at 48 h pi with 3×10^7 CEA10 conidia.

(D) Representative plots of RFP (CXCL9) and BFP (CXCL10) expression in indicated lung leukocytes isolated from Rex3 Tg → C57BL6.SJL BM chimeric mice at baseline (naïve, top row) and 48 h pi with 3×10^7 CEA10 conidia (infected, bottom row). The blue and purple gates indicate the frequency of BFP$^+$ (CXCL9$^+$) and BFP$^+$RFP$^+$ (CXCL9$^+$ CXCL10$^+$) cells, respectively.

(E) The graphs indicate the frequency of CXCL9$^-$ CXCL10$^+$ and CXCL9$^+$ CXCL10$^+$ CD4$^+$ T cells, CD8$^+$ T cells and NK cells at 48 h pi.

(C, E) Data are representative of two independent experiments. Dots represent individual mice and data are presented as mean ± SEM, (C) Statistical analysis: Mann-Whitney test.

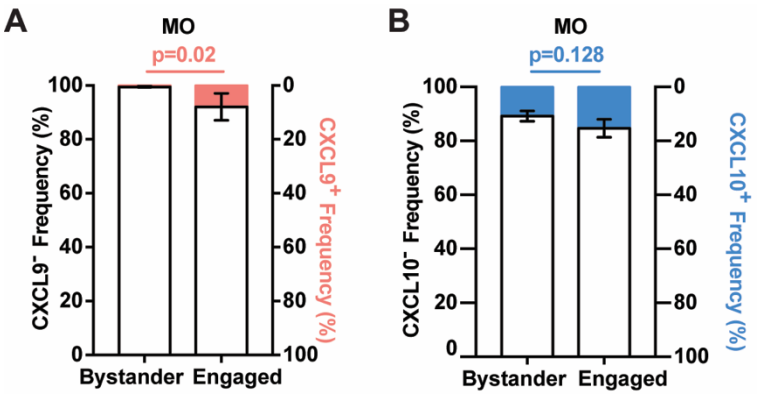

Figure S2. Monotye CXCL9 and CXCL10 expression during *A. fumigatus* infection. Related to Figure 2.

(A) Proportion of RFP^+ ($CXCL9^+$; pink bar) and RFP^- ($CXCL9^-$; white bar); and (B) BFP^+ ($CXCL10^+$; blue bar) and BFP^- ($CXCL10^-$; white bar) expression in indicated bystander and fungus-engaged leukocytes isolated infected Rex3 Tg → C57BL6.SJL BM chimeric mice (n=7) with 3×10^7 AF633-labeled CEA10 conidia.

(A and B) Data are presented as mean ± SEM. Statistical analysis: Mann-Whitney test.

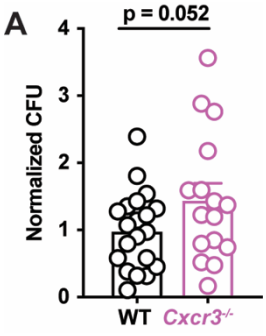

Figure S3. CXCR3 is critical for anti-*Aspergillus* defense. Related to Figure 3.

(A) Normalized Lung CFUs in C57BL6 (WT) and *Cxcr3*$^{-/-}$ mice 72 h pi with 3 × 10^7 CEA10 conidia. Dots represent individual mice and data were pooled from 2 independent experiments. Data are presented as mean ± SEM. Statistical analysis: Mann-Whitney test.

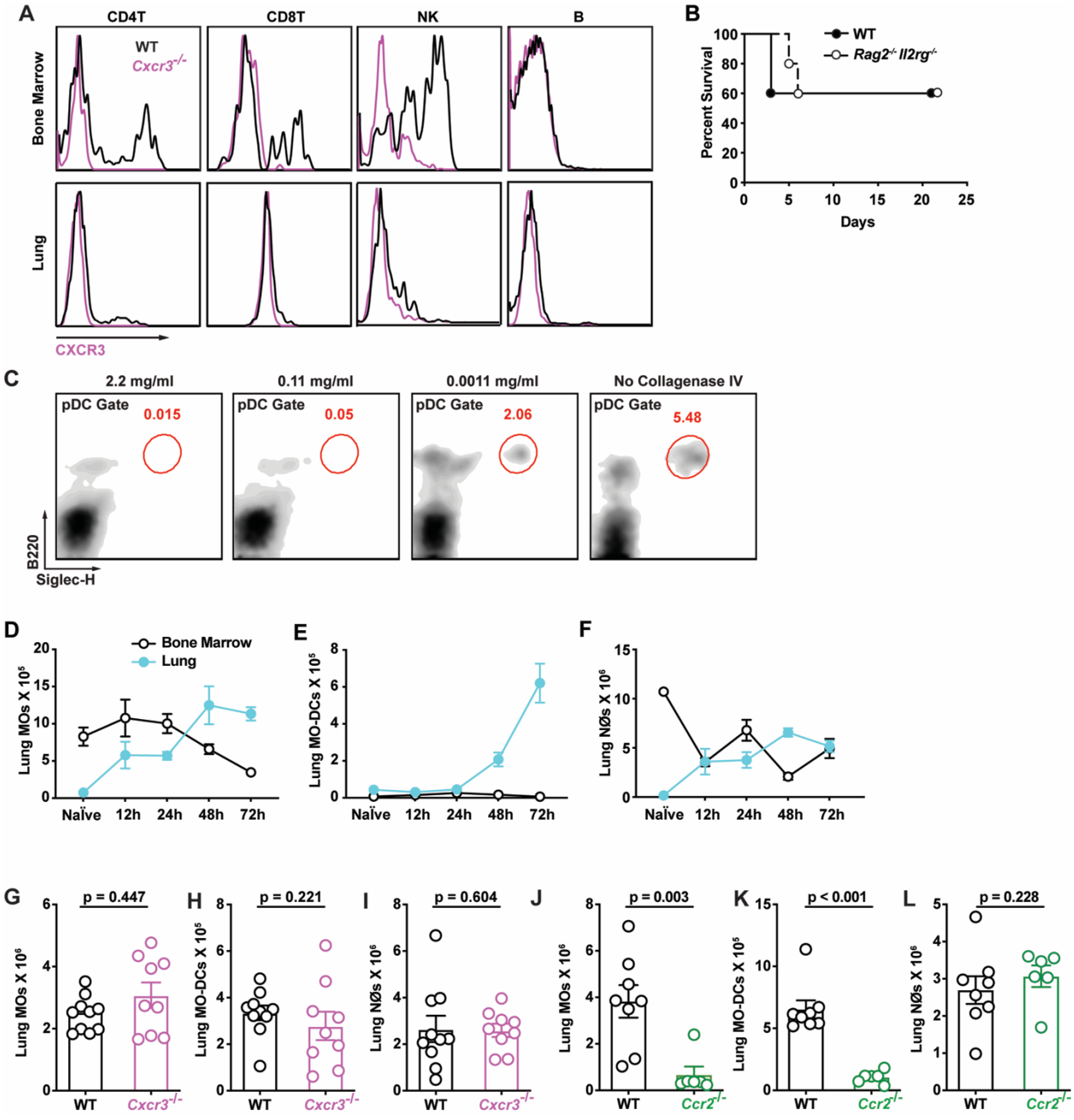

Figure S4. CXCR3 expression on lung leukocytes and pDC identification in lung digests. Related to Figure 4.

(A) Representative CXCR3 surface expression in the indicated bone marrow (top row) and lung (bottom row) leukocytes that were isolated from C57BL6 (WT, black lines; *Cxcr3*$^{+/+}$) or *Cxcr3*$^{-/-}$ mice (purple lines).

(B) Kaplan-Meier survival of C57BL6 (n = 5 per group) and *Rag2*$^{-/-}$*Il2rg*$^{-/-}$ (n = 5 per group) mice challenged with 4-8 × 10^7 CEA10 conidia.

(C) Representative flow cytometry plots of B220$^+$Siglec-H$^+$ lung pDCs with the indicated concentration of type IV collagenase included in lung preparations to obtain single cells for flow cytometric analysis.

(D-F) Lung (blue filled dots) and bone marrow (open black dots) (D) monocyte, (E) Mo-DC, and (F) neutrophil numbers at baseline and indicated times pi with 3 × 10^7 CEA10 conidia (n = 5 per group).

(G-H) Lung (G) monocyte, (H) Mo-DC, and (I) neutrophil numbers in C57BL6 (WT; open black dots) and *Cxcr3*$^{-/-}$ mice (open purple dots) at 72 h pi (n = 10 per group).

(J-L) Lung (J) monocyte, (K) Mo-DC, and (L) neutrophil numbers in C57BL6 (WT; open black dots) and *Ccr2*$^{-/-}$ mice (open green bars) at 72 h pi (n = 10 per group).

(D-L) Data are representative of two independent experiments. Dots represent individual mice and data are presented as mean ± SEM. Statistical analysis: Mann-Whitney test.

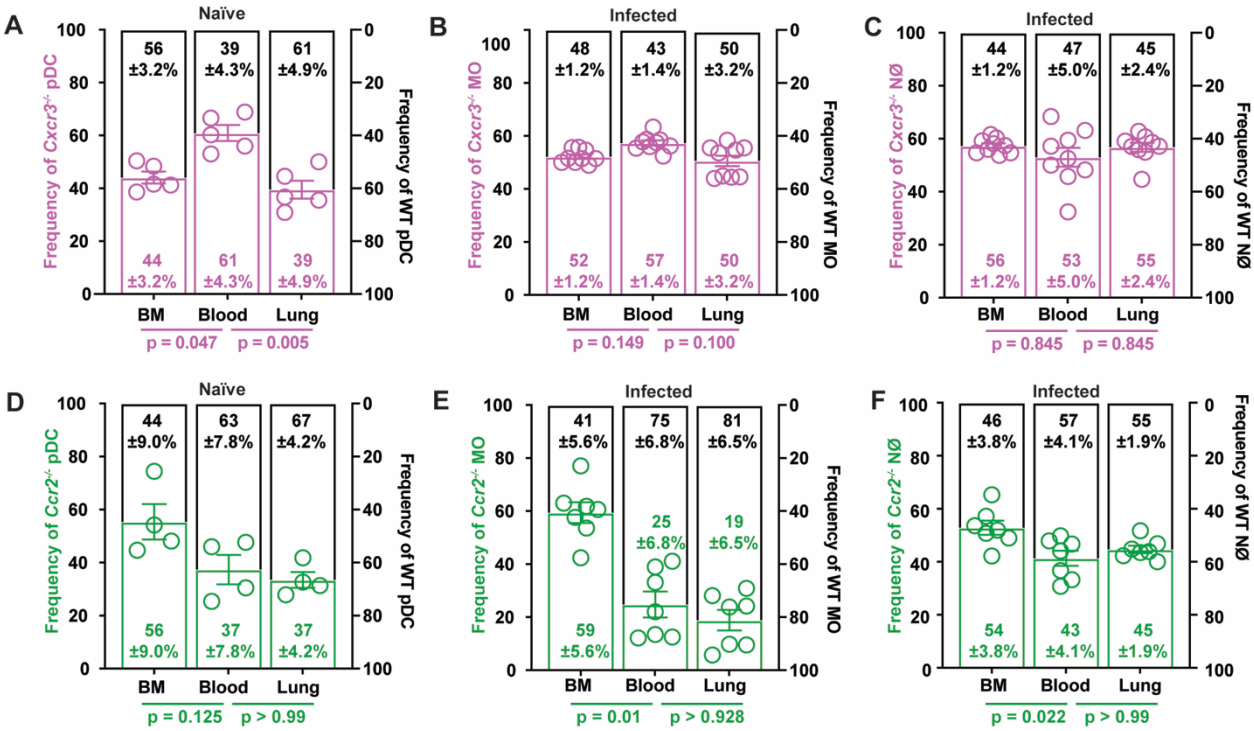

Figure S5. CXCR3 does not regulate the trafficking of lung monocytes, Mo-DCs, and neutrophils. Related to Figure 5.

(A) Relative frequencies of *Cxcr3*$^{-/-}$ (open purple bars) and *Cxcr3*$^{+/+}$ (open black bars) pDCs in the BM, blood, and lung of mixed BM chimeric (1:1 mix of CD45.1^{+} *Cxcr3*$^{+/+}$ and CD45.2^{+} *Cxcr3*$^{-/-}$ BM cells → CD45.1^{+}CD45.2^{+}) mice at baseline.

(B and C) Relative frequencies of *Cxcr3*$^{-/-}$ (open purple bars) and *Cxcr3*$^{+/+}$ (open black bars) (B) monocytes and (C) neutrophils in the BM, blood, and lung of mixed BM chimeric (1:1 mix of CD45.1^{+}*Cxcr3*$^{+/+}$ and CD45.2^{+}*Cxcr3*$^{-/-}$ BM cells → CD45.1^{+}CD45.2^{+}) mice 72 h pi.(D) Relative frequencies of *Ccr2*$^{-/-}$ (open green bars) and *Ccr2*$^{+/+}$ (open black bars) pDCs in the BM, blood, and lung of mixed BM chimeric (1:1 mix of CD45.1^{+}*Cxcr3*$^{+/+}$ and CD45.2^{+}*Cxcr3*$^{-/-}$ BM cells → CD45.1^{+}CD45.2^{+}) mice at baseline.

(E-F) Relative frequencies of *Ccr2*$^{-/-}$ (open green bars) and *Ccr2*$^{+/+}$ (open black bars) (E) monocytes and (F) neutrophils in the BM, blood, and lung of mixed BM chimeric (1:1 mix of CD45.1^{+}*Ccr2*$^{+/+}$ and CD45.2^{+}*Ccr2*$^{-/-}$ BM cells $\rightarrow$ CD45.1^{+}CD45.2^{+}) mice 72 h pi.

(A-F) Data were pooled from 2 or 3 independent experiments and presented as mean ± SEM, Statistical analysis: Mann-Whitney test.

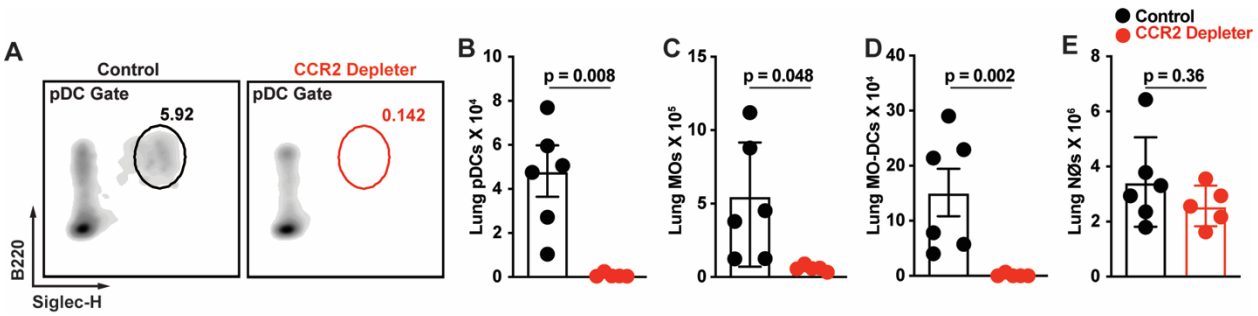

Figure S6. pDCs are depleted in CCR2 Depleter mice. Related to Figure 6.

(A) Representative flow cytometry plots of lung $B220^{+}Siglec\text{-}H^{+}$ pDC, (B) lung pDC, (C) lung monocyte, (D) lung Mo-DC, and (E) lung neutrophil numbers in DT-treated CCR2 Depleter mice (CCR2-$DTR^{+/-}$; red symbols) and non-Tg littermate controls (CCR2-$DTR^{-/-}$; black symbols) at 72 h pi with 3×10^{7} CEA10 conidia.

(B-E) Data were presented as mean ± SEM. Statistical analysis: Mann-Whitney test.

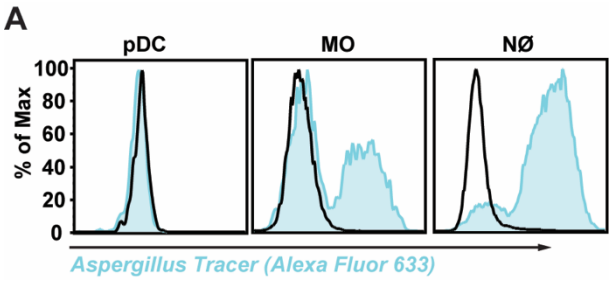

Figure S7. pDCs do not bind to or engulf *A. fumigatus* conidia. Related to Figure 7.

(A) AF633 fluorescence intensity in indicated BM leukocytes co-cultured for 24 h with FLARE (blue line) or AF633-unlabeled conidia (MOI = 5).