SUPPLEMENTAL MATERIAL

Supplemental Figures

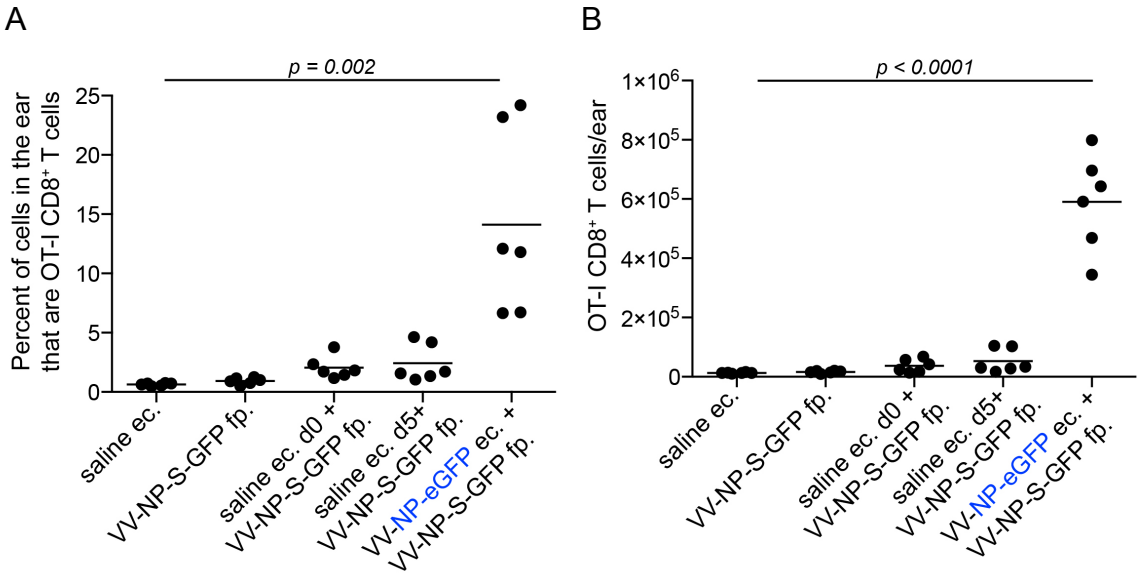


Figure S1. OT-I cells do not traffic to uninfected ears.
Mice received 2.5x10^5 wild-type OT-I CD8$^+$ cells prior to infection via the indicated routes: epicutaneous (ec.) or footpad subcutaneous (fp.). Five days post-infection single-cell suspensions of ears were generated by enzymatic digestion and analyzed via flow cytometry for the percentage of transferred OT-I CD8$^+$ T cells recovered as a function of total ear cells (**A**) or the total number of OT-I cells per ear (**B**). Very few transferred cells were present in uninfected ears that were mock ec. infected with sterile saline, or when T cells were activated via fp. inoculation without VV infection of the ear. Likewise, poking the ear on day 5 p.i. with a bifurcated needle (to create sterile inflammation) did not result in T cell immigration into the ear (saline ec. d5 + VV-NP-S-eGFP column). However, VV-infection with virus lacking cognate antigen for OT-I cells (VV-NP-eGFP) combined with concurrent infection fp. with VV-NP-S-eGFP (to activate T cells) resulted in T cell recruitment to the ear. NP-eGFP is colored blue to highlight the virus lacking SIINFEKL. Dots represent individual ears. Line shows mean. Statistics = unpaired two-tailed *t* test.

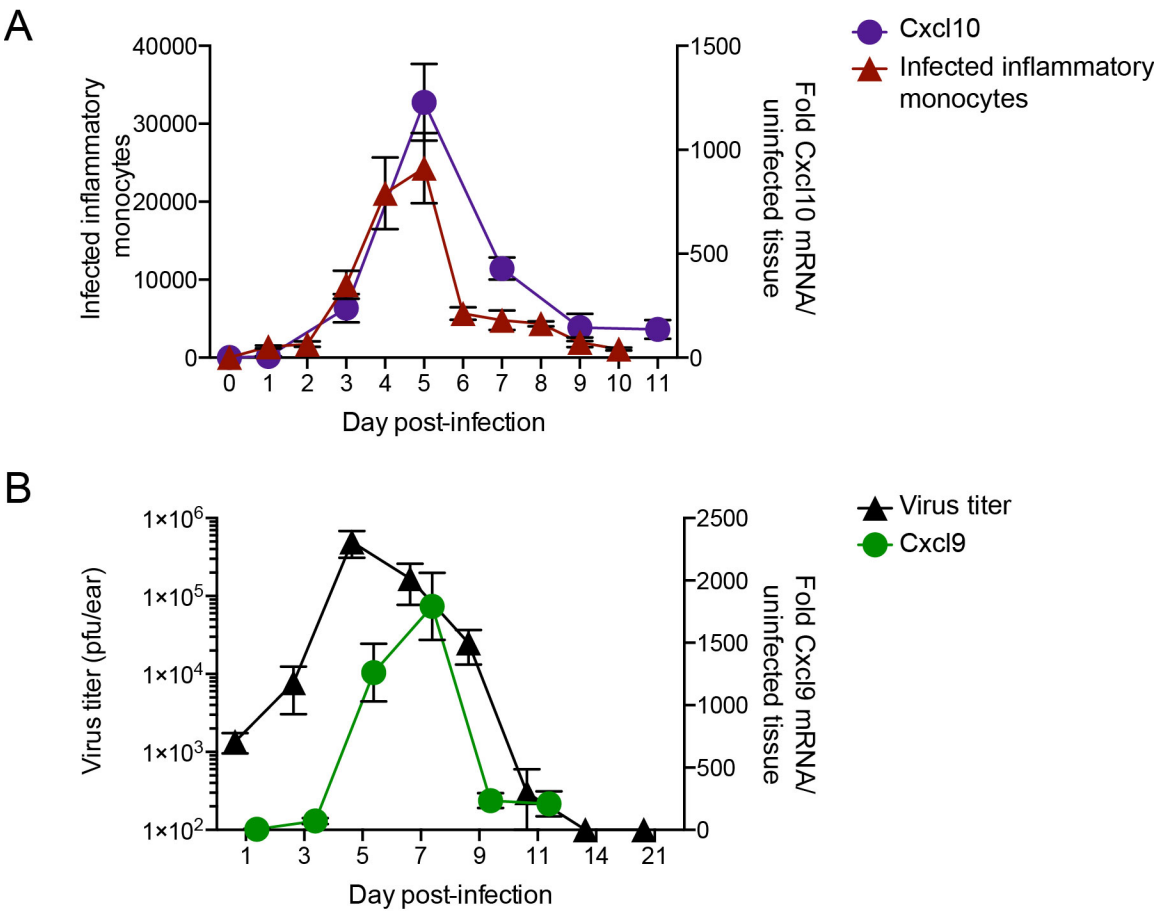


Figure S2. Cxcl9 and Cxcl10 mRNA expression peak on different days.
A. Graph showing the number of infected inflammatory monocytes determined by flow cytometry (red triangles, left y axis) recovered per ear per day in WT animals compared to the fold increase in *Cxcl*10 mRNA per day (blue circles, right y axis). Days are shown on the x axis. Both *Cxcl*10 mRNA levels and the number of infected inflammatory monocytes dropped dramatically after day 5 post-infection. **B**. Virus titer determined by plaque assay (black triangles, left y axis) shown in plaque forming units per ear compared to *Cxcl*9 mRNA fold increase over uninfected tissue (green circles, right y axis). *Cxcl*9 mRNA decreased between days 7 and 9, two days later than *Cxcl*10 mRNA.

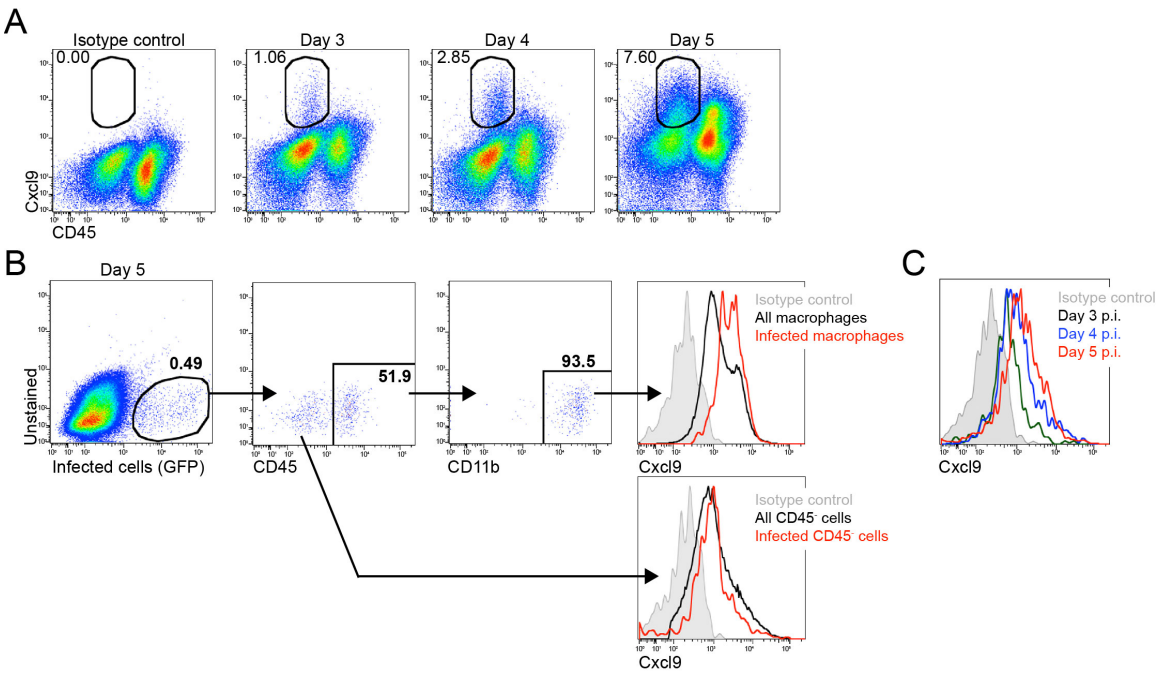
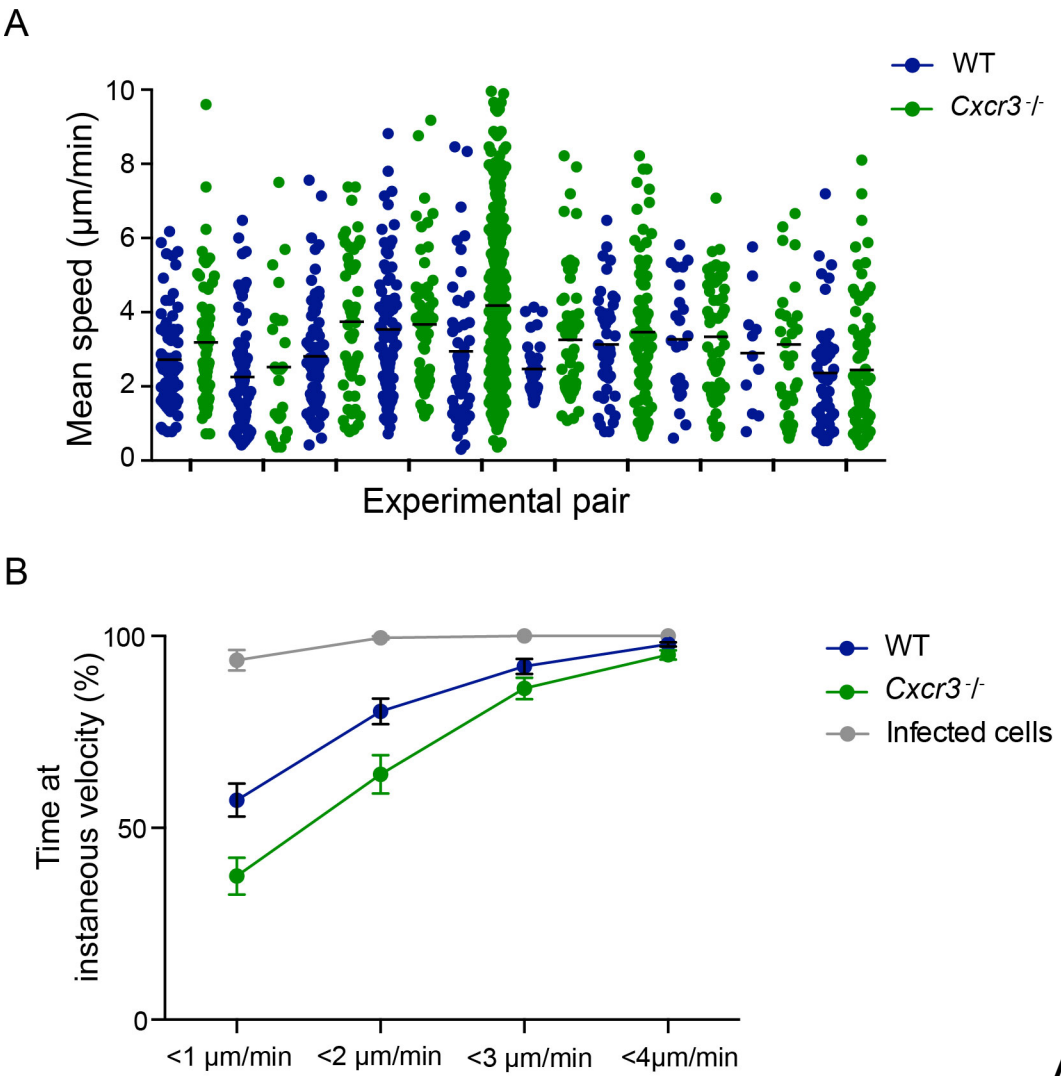


Figure S3. Both non-hematopoietic skin cells and inflammatory monocytes produce CXCL9 post-infection in WT mice.

A. Non-hematopoetic cells are infected with VV and produce CXCL9. Pseudocolored flow cytometric dot plots of intracellular staining for CXCL9 (Y axis) versus CD45 (denoting hematopoietic cells, x axis). Day post-infection is shown above each plot for CXCL9 staining. Isotype control staining was performed on day 5 post-infection to control for inflammation-induced autofluorescence. The percentage of $CD45^-$ $CXCL9^+$ cells is shown at the top left. **B**. Flow dot plots and histograms showing CXCL9 production by infected inflammatory monocytes on day 5 p.i. Approximately 0.5 percent of cells recovered were GFP^+ virus-infected cells in WT mice. Fifty-percent of GFP^+ cells were $CD45^+$. A majority of these cells (>93%) expressed the inflammatory monocyte (and macrophage) marker CD11b. Gating on these cells revealed that infected monocytes/macrophages (red lines in the overlay on right) express high levels of CXCL9. **C**. Flow cytometric histograms from mice 3-5 days post-infection showing expression of CXCL9 in infected inflammatory monocytes. Although samples were stained and run on the same day and in the same experiment (they were infected on different days), infected inflammatory monocytes expressed the highest levels of CXCL9 on day 5 post-infection. Isotype control staining is shown in grey.



A.

Figure S4. ***The movement of WT or Cxcr3 -/- OT-I T cells differs in the same microscopic field.***

Graph showing the speed of WT (blue dots) or *Cxcr3* $^{-/-}$ (green dots) T cells moving in the skin in an area of VV-infected cells. Each experimental pair shows cellular mobility in the same microscopic field. Images were acquired using multiphoton microscopy and speeds calculated using Imaris as described in the Materials and Methods. Line = mean. **B**. Graph showing the percent of WT (blue) or *Cxcr3* $^{-/-}$ (green) OT-I T cells that were interacting with virus-infected cells during a representative 1 hr. imaging session and that possessed an instantaneous velocity less than the indicated speed. Error bars= SEM. Not all virus-infected cells were immobile, but most moved at slow speeds (grey lines).

Supplemental Movie Legends

Movie S1. OT-I T cells locate infected cells lacking cognate antigen expression within infected skin. Mice were transferred with 2.5 X 10^5 wild-type (WT) OT-I $CD8^+$ T cells (red) prior to infection with VV lacking (left panel, NP-eGFP) or expressing (right panel, VV-NP-S-eGFP) SIINFEKL. Three-dimensional reconstructions of multiphoton microscopic (MPM) images were taken in the skin 5 days post-infection (p.i.). The dermal collagen was visualized in blue. This movie shows that although contact times differed between the two experimental settings, T cells were able to locate and contact infected cells lacking cognate antigen. Scale bar= microns. Time = minutes.

Movie S2. *Cxcr3* $^{-/-}$ OT-I $CD8^+$ T cells enter and patrol the inflamed tissue. Mice were transferred with 2.5 X 10^5 *Cxcr3* $^{-/-}$ OT-I $CD8^+$ T cells (red) prior to infection with VV-NP-S-eGFP (green, not present in this microscopic field). Three-dimensional reconstructions of multiphoton microscopic (MPM) images were taken in the skin 5 days post-infection (p.i.). The dermal collagen was visualized in blue. This movie shows the mobility of *Cxcr3* $^{-/-}$ OT-I $CD8^+$ T cells in areas of the dermis that lack virus-infected cells. Scale bar= 50 microns. Time = minutes.

Movie S3. C*xcr3* $^{-/-}$ OT-I $CD8^+$ T cells accumulate at the borders of keratinocytic foci of viral replication. Mice received 2.5 X 10^5 *Cxcr3* $^{-/-}$ OT-I $CD8^+$ T cells (red) prior to infection with VV-NP-S-eGFP (green). This 3D reconstruction of MPM images was taken 5 days p.i. Dermal collagen was visualized in blue. This movie shows mobile *Cxcr3* $^{-/-}$ OT-I $CD8^+$ T cells around a keratinocytic lesion. Scale bar= 50 microns. Time = minutes.

Movie S4. Both wild-type and C*xcr3* $^{-/-}$ OT-I CD8^{+} T cells locate non-keratinocytic virus-infected cells. Mice received 2.5 X 10^5 wild-type (WT) (red, left panel) or *Cxcr3* $^{-/-}$ (red, right panel) OT-I CD8^{+} T cells prior to epicutaneous infection with VV-NP-S-eGFP (green). These 3D reconstructions of MPM images were taken 4.5 days p.i. Dermal collagen was visualized in blue. This movie shows that both WT and C *Cxcr3* $^{-/-}$ OT-I CD8^{+} T cells were able to locate infected inflammatory monocytes (previously extensively characterized by flow cytometry and immunohistochemistry and distinguished from infected keratinocytes by their mobility and morphology). Scale bar = microns. Time = minutes.

Movie S5. C*xcr3* $^{-/-}$ OT-I CD8^{+} T cells occasionally "chased" virus-infected cells. MPM MIP movie showing a CXCR3$^{-/-}$ (red) OT-I CD8^{+} T cell actively following a VV-infected cell (green) on day 5 post-infeciton. Dermal collagen was visualized in blue. Scale bar = microns. Time = minutes.

Movie S6. A larger percentage of wild-type OT-I T cells entered highly infected areas compared to C*xcr3* $^{-/-}$ OT-I CD8^{+} T cells. Mice received WT (cyan) and *Cxcr3* $^{-/-}$ (red) OT-I CD8^{+} T cells prior to epicutaneous infection with VV-NP-S-eGFP (green). These 3D reconstructions of MPM images were taken 5 days p.i. Dermal collagen was visualized in blue. An area of heavy virus infection is shaded in the left panel. A higher magnification of the boxed area is shown on the left. This movie shows that both WT and *Cxcr3* $^{-/-}$ OT-I CD8^{+} T cells were able to locate infected inflammatory monocytes (green), but more WT T cells penetrated the field of infected cells. Scale bar = microns. Time = minutes.

Movie S7. C*xcr3* $^{-/-}$ OT-I CD8^{+} T cells had higher mobility in areas of infection. Mice received WT (cyan) and *Cxcr3* $^{-/-}$ (red) OT-I CD8^{+} T cells prior to epicutaneous infection with VV-NP-S-eGFP (green). These 3D reconstructions of MPM images were taken 5 days p.i. Dermal collagen was visualized in blue. This video montage shows *Cxcr3* $^{-/-}$ (red) OT-I CD8^{+} T cells rapidly moving between infected cells. Scale bar = microns. Time = minutes.