Supporting Information

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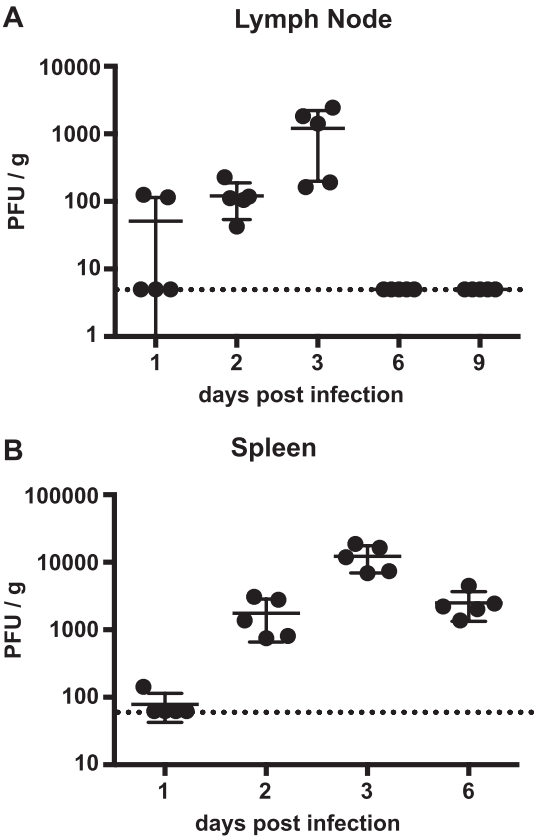

Fig. S1. Vaccinia virus (VV) replicates in both the draining lymph node (LN) and spleen following footpad infection. C57BL/6 mice were infected with 1.5×10^6 pfu of VV in the footpad, and at the indicated time points, organs were removed and viral titers were assessed in the draining LN (*A*) and spleen (*B*) by plaque assay. Dotted lines indicate the limit of detection by the assay. Symbols indicate individual mice, and lines indicate the means. $n = 5$ mice per group were analyzed for each time point.

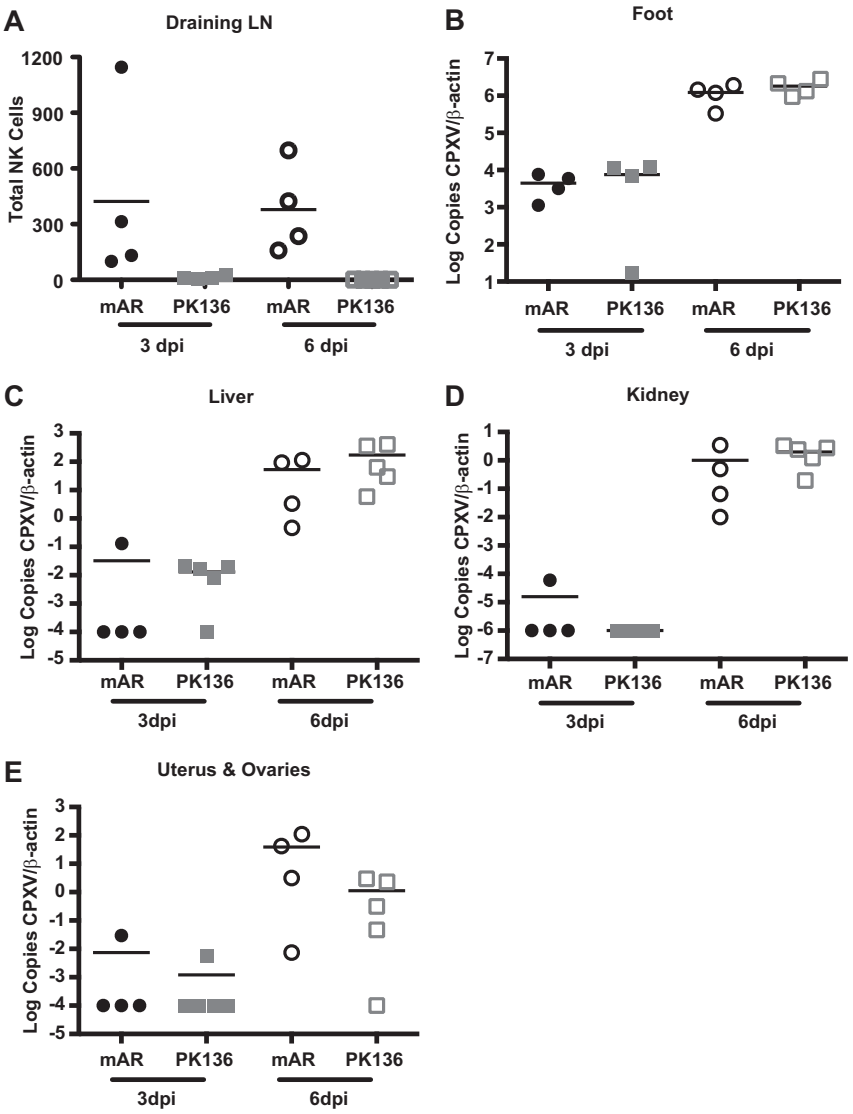

Fig. S2. Natural killer (NK) cells limit viral replication and spread following footpad infection. C57BL/6 mice were treated i.p. with 200 μg of control antibody (mAR) or NK cell-depleting antibody (anti-NK1.1, PK136) 2 d before infection and 4 d postinfection (dpi). (*A*) Depletion was assessed in the nondraining lymph node (LN) by staining lymphocytes for $NK1.1^{+}NKp46^{+}CD3^{-}CD19^{-}$ cells. At the indicated time points, organs were removed and viral titers were assessed by quantitative PCR in the foot (*B*), liver (*C*), kidney (*D*), and uterus and ovaries (*E*). Cowpox virus (CPXV) copy number was normalized to β-actin copy numbers and then multiplied by 1,000. Symbols indicate individual mice, and lines indicate the means. $n = 4–5$ mice per group were analyzed for each time point. Data are representative of three independent experiments.

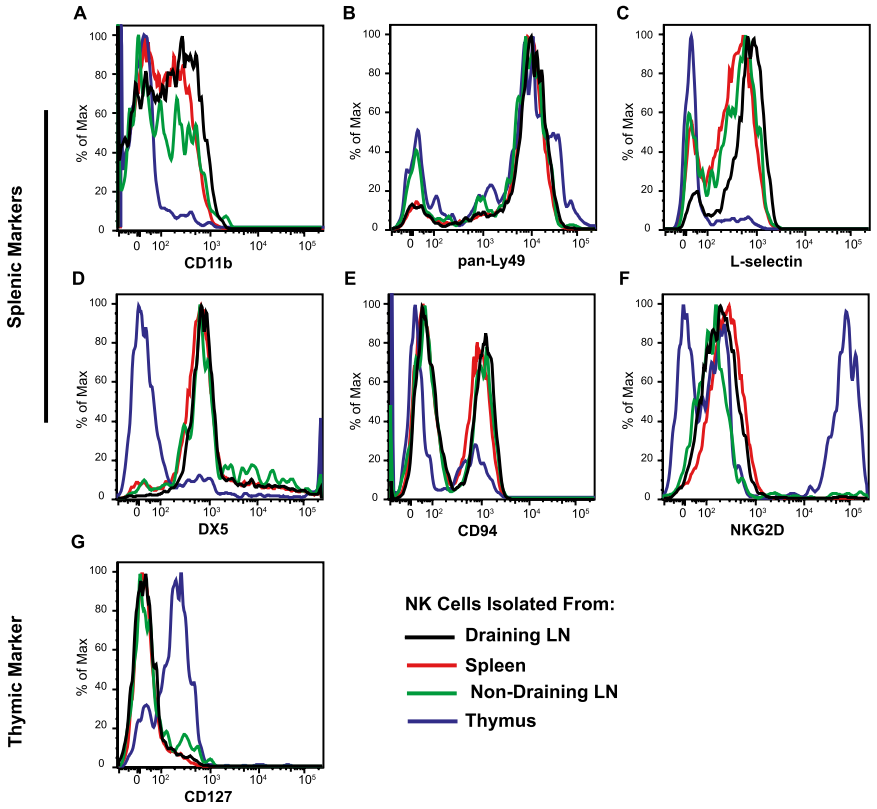

Fig. S3. Natural killer (NK) cells isolated from the draining lymph node (LN) phenocopy NK cells isolated from the spleen. C57BL/6 mice were infected as before and at 1, 2, and 3 d postinfection (dpi). LNs were isolated, stained, and analyzed by flow cytometry. Following identification of NK cells (NK1.1$^+$CD3$^-$CD19$^-$), surface expression of CD11b (*A*), pan-Ly49 (*B*), L-selectin (*C*), DX5 (*D*), CD94 (*E*), NKG2D (*F*), or CD127 (*G*) was analyzed. Histograms shown are from 1 dpi but are representative of all time points observed. Representative results from two independent experiments are shown.

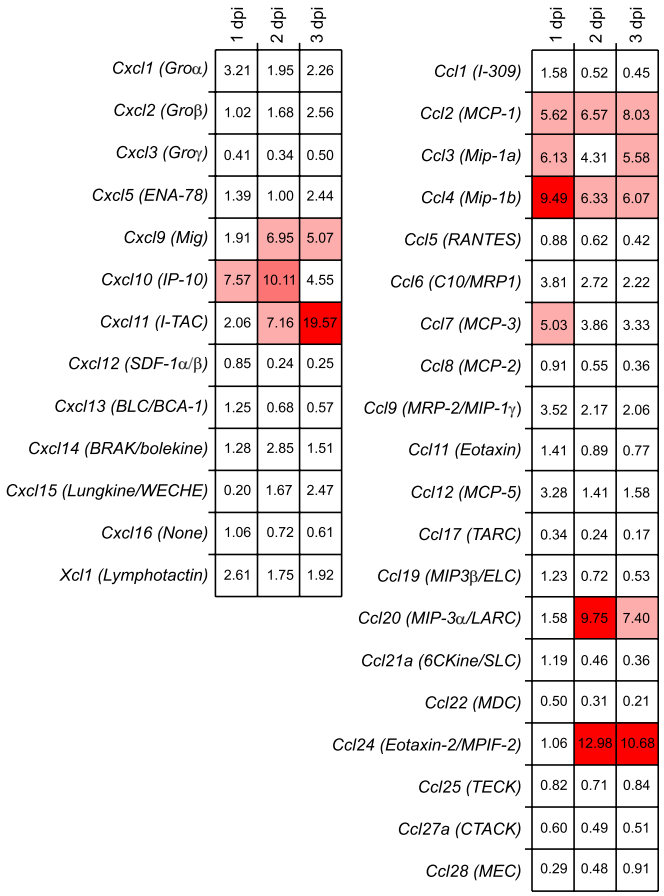

Fig. S4. Chemokine expression following cowpox virus (CPXV) footpad infection infection. RNA transcript levels were assessed in C57BL/6 mice infected with 1.5×10^6 pfu of CPXV in the footpad at the indicated time points. Lymph nodes (LNs) from three independently infected mice were isolated. Lysates from either the nondraining or draining LN were generated and run in triplicate, and chemokine transcripts were normalized to the control *Hprt1* transcript for each sample. The fold change located in each square depicts the average transcript level in the draining LN relative to the nondraining LN.

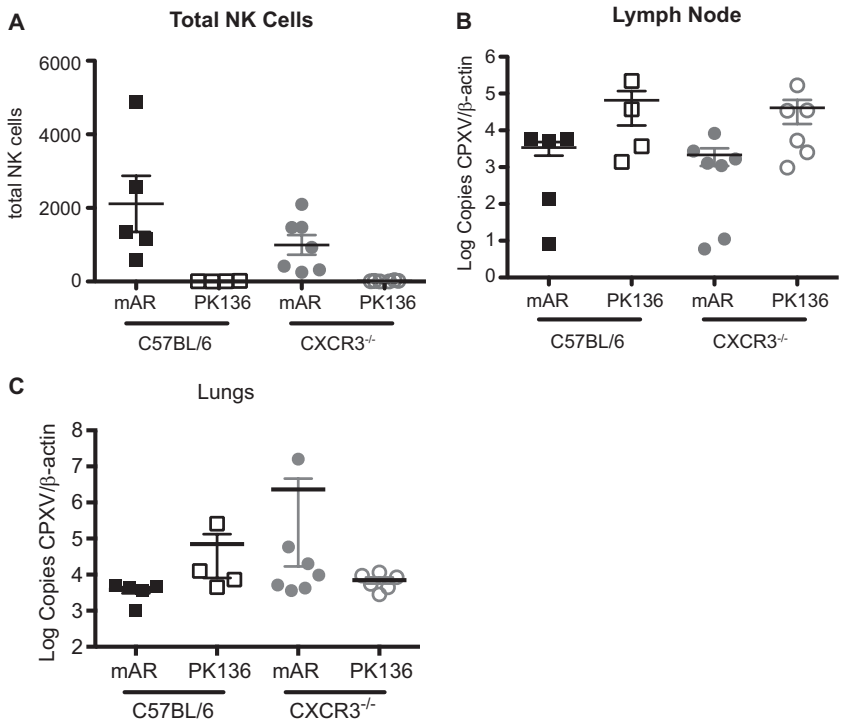

Fig. S5. CXCR3 deficiency does not lead to increased viral spread. CXCR3$^{-/-}$ or C57BL/6 mice were treated i.p. with 200 μg of control antibody (mAR) or NK-depleting (anti-NK1.1, PK136) antibody 2 d before infection. At 3d postinfection, lymph nodes and lungs were isolated and total NK cell number (*A*) and viral titers (*B* and *C*) were assessed as before. Symbols indicate individual mice, and lines indicate the means and SEM. $n = 4–7$ mice per group were analyzed for each time point. CPXV, cowpox virus.

Table S1. Primer and probe sequences for quantitative PCR

Gene	Forward	Reverse	Probe
CPXV	5′-CGGCTAAGAGTTGCACATCCA-3′	5′-TCTGCTCCATTTAGTACCGATTCTAG-3′	5′-AGGACGTAGAATGATCTTGTA-3′
β-Actin	5′-AGCTCATTGTAGAAGGTGTGG-3′	5′-GGTGGGAATGGGTCAGAAG-3′	5′-TTCAGGGTCAGGATACCTCTCTTGCT-3′
Chemokine receptors			
CCL19	5′-CGCATCATCCGAAGACTGAAG-3′	5′-TTTACTCAAGACACAGGGCTC-3′	5′-CCAAGAACAAAGGCAACAGCACCA-3′
CCL21	5′-GGGAACCTCTAAGTCTGGAAAG-3′	5′-TTGAGGGCTGTGTCTGTTC-3′	5′-AAGGAAAGGGCTCCAAGGGCT-3′
CXCL9	5′-CAAATCCCTCAAAGACCTCAAAC-3′	5′-GATCTCCGTTCTTCAGTGTAGC-3′	5′-CCCCAAGCCCCAATTGCAACAA-3′
CXCL10	5′-TCATCCCTGCGAGCCTAT-3′	5′-CTTGATGGTCTTAGATTCCGGAT-3′	5′-CCCACGTGTTGAGATCATTGCCAC-3′

CPXV, cowpox virus.