Supplementary Figure Titles and Legends

Supplemental Figure 1, related to Figure 2. Most DCs are not infected after ec. inoculation of rVV. Single-cell suspensions of ears were analyzed by flow cytometry at the indicated days post-infection (d.p.i). Cells were processed through 70 μm nylon filters to exclude keratinocytes, then gated on $CD45^+$ cells. VV-infected cells were identified by eGFP-expression. **A**) Pseudocolored dot plots from ears 5 d.p.i. showing CD11c expression after gating on all the $CD45^+$ cells in the ear (left panel) and on only infected $CD45^+$ cells (right panel). **B**) Infected cell counts at the indicated d.p.i. N=6 ears/timepoint. Error bars= SEM.

Supplemental Figure 2, related to Figure 4. Spatial separation of OT-I CD8+ T cells and keratinocytic lesions. A) Frozen tissue section 5 d.p.i. with VV-NP-S-eGFP (green). CD8 staining=white. Nuclei=blue (DAPI). **B)** Maximum intensity projection (MIP) of multiphoton microscopy (MPM) images 5 d.p.i. of a LysM-eGFP mouse (green macrophages, monocytes, and neutrophils) infected with VV-NP-S-BFP (pseudocolored magenta). OT-I $CD8^+$ T cells=red. Collagen=blue.

Supplemental Figure 3, related to Figure 6. Keratinocytic lesion size is not significantly altered by $CD8^+$ T cell depletion. Mice were infected ec. with VV-NP-S-eGFP (n=4/group) and depleted of $CD8^+$ T cells via antibody administration every other day. Seven d.p.i., lesion diameter was measured microscopically based on fluorescence using a 10x objective. Dots represent individual lesions. Statistics=student's t test.

Movie Titles and Legends

Movie S1, related to Figure 2. Motility of rVV-infected cells. Scene 1) Identification of moderately mobile rVV-infected cells in the dermis after epicutaneous (ec.) infection. Maximum intensity projections of MPM images acquired at the indicated timepoints post e.c. infection with VV-NP-S-eGFP (green infected cells (virions not labeled)). Scene 2) Mobile cells outlie major keratinocytic lesions. Maximum intensity projections of MPM images acquired at 4D post e.c. infection with rVV-NP-S-eGFP (green). A large lesion can be seen in the lower portion of the image. Dragontail tracks (showing only the last 20 min. of cellular migration) are indicated for outlying cells. Right panel) higher magnification images shows movement of mobile infected cells. Boxes on grid=50 µm. Scene 3) Mobility of a rVV-infected (BFP^+) $eGFP^+$ (from a LysM-eGFP mouse) cell. Maximum intensity projections of MPM images acquired in a LysM-eGFP mouse (green myelomonocytic cells including macrophages, monocytes, and neutrophils) infected e.c. with rVV-BFP-ub-SIINFEKL (pseudocolored magenta). Collagen of the dermis is visualized by second harmonic generation (blue). Colocalization of the green (eGFP) and magenta (rVV) channels is shown in white. Left panel=cell migration track of an infected $eGFP^+$ cell over a 20 min. imaging period. Large ticks on grid=5 µm. In all images, collagen of the dermis is visualized by second harmonic generation (blue), time=min, and scalebars=µm.

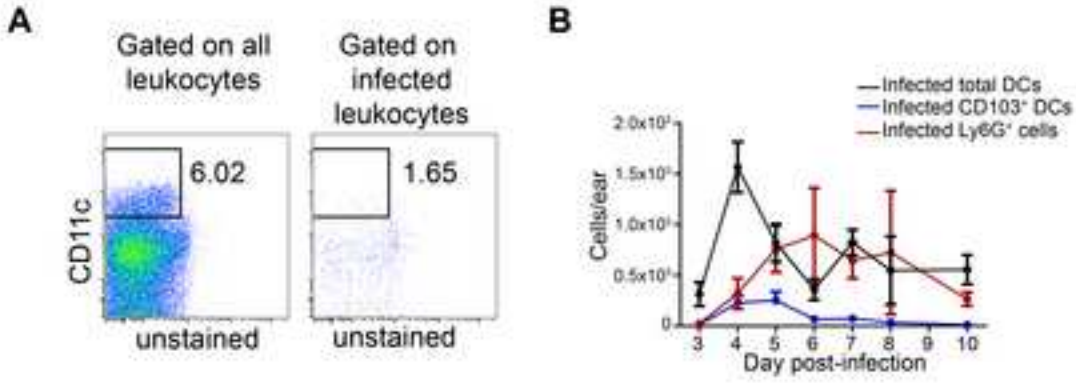
Movie S2, related to Figure 3. $eGFP^+$ (from a LysM-eGFP mouse) cell immigration to the site of rVV infection. Maximum intensity projections of MPM images acquired in a LysM-eGFP mouse (green myelomonocytic cells including macrophages, monocytes, and neutrophils) ear infected with rVV-NP-S-BFP (pseudocolored magenta), and autofluorescent hairs appear as magenta. Collagen of the dermis is visualized by second harmonic generation (blue). Scalebars=µm. Time=min.

Movie S3, related to Figure 4. Behavior of Ag-specific T cells toward VV-infected keratinocytes or outlying infected monocytes. Scene 1) Most Ag-specific T cells do not enter keratinocytic lesions**.** MPM images acquired on day 6 post-infection of ears of

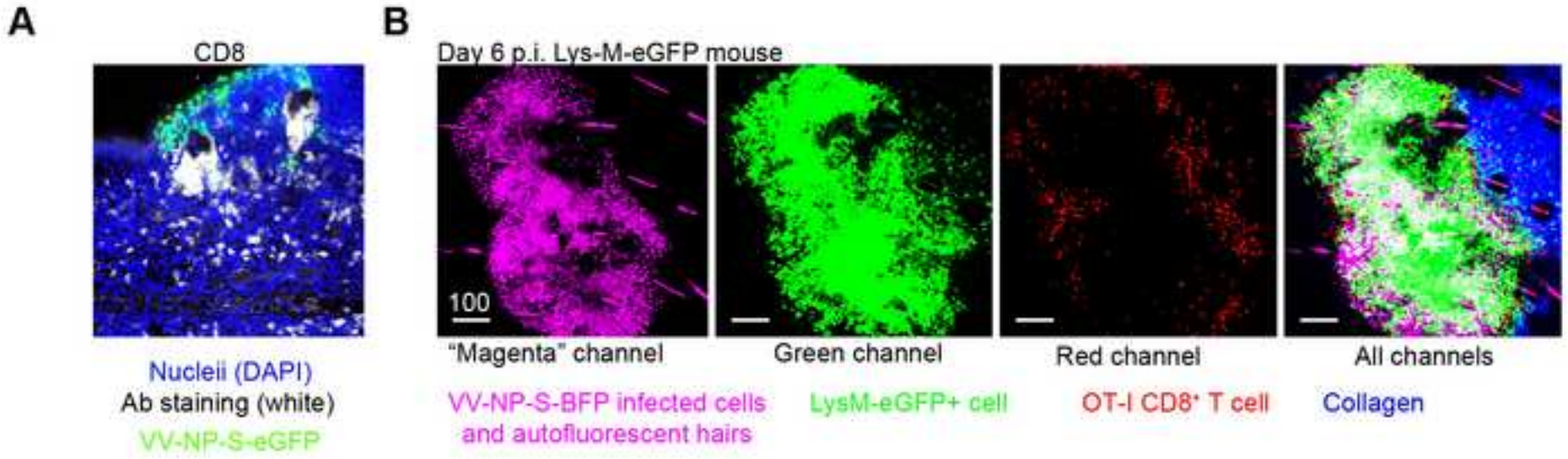
mice infected with VV-NP-S-eGFP. Prior to infection, mice were given 2.0 x 10^5 dsRed OT-I $CD8^+$ T cells (red). Image shows a rotation of the 3D MIP. Spike-like projections are hairs. Scene 2) Ag-specific T cells accumulate at the periphery of viral lesions. MPM images acquired 5 d.p.i. of a LysM-eGFP mouse (green myelomonocytic cells including macrophages, monocytes, and neutrophils) ear infected with rVV-NP-S-BFP (pseudocolored magenta). Prior to infection, mice were given 2.0 x 10^5 dsRed OT-I $CD8^+$ T cells (red). Note that T cells largely fail to traffic into keratinocytic lesion, while green myelomonocytes do. Right panel=no eGFP signal for clarity. Scene 3) T cell killing a rVV-infected cell. Mice were given 2.0 x 10^5 dsRed OT-I $CD8^+$ T cells (red) prior to infection with VV-NP-S-eGFP (green). Shown are MPM images acquired on 5 d.p.i. (left panel). The large black "empty" area in the lower left corner is a hair follicle. Right panel) Spot calculation of target cell as it breaks into small pieces (white spot) and of a control, non-target cell (yellow spot). Scene 4) Most infected cells are reduced to fragments after $CD8^+$ T cell influx. Mice were given 2.0 x 10^5 dsRed OT-I $CD8^+$ T cells (red) prior to infection with VV-NP-S-eGFP (green). Late on day 5 p.i., numerous T cells have entered a rVV-infected area, and most green cells are now < 5 µm in diameter. Shown are MPM images acquired 5 d.p.i. All images are maximum intensity projections, and in all images collagen of the dermis is visualized by second harmonic generation (blue), scalebar=µms, and time=min.

Movie S4, related to Figure 6. AT38 treatment results in relocation of OT-I $CD8^+$ T cells into VV keratinocytic lesions. Mice were given 2.0 x 10^5 dsRed OT-I $CD8^+$ T cells (red) prior to infection with VV-NP-S-eGFP (green). Collagen (dermis)=blue. On day 4 p.i., mice were given AT38 or vehicle alone (cntrl). Top panels show maximum intensity projections of MPM images in all channels; bottom panels show only the red (T cell) channel with the lesion border in dashed white lines. Scalebar=50 µm. Time=min

Supplemental Figure S1



Supplemental Figure S2



Supplemental Figure S3