Article:
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Supplemental Materials

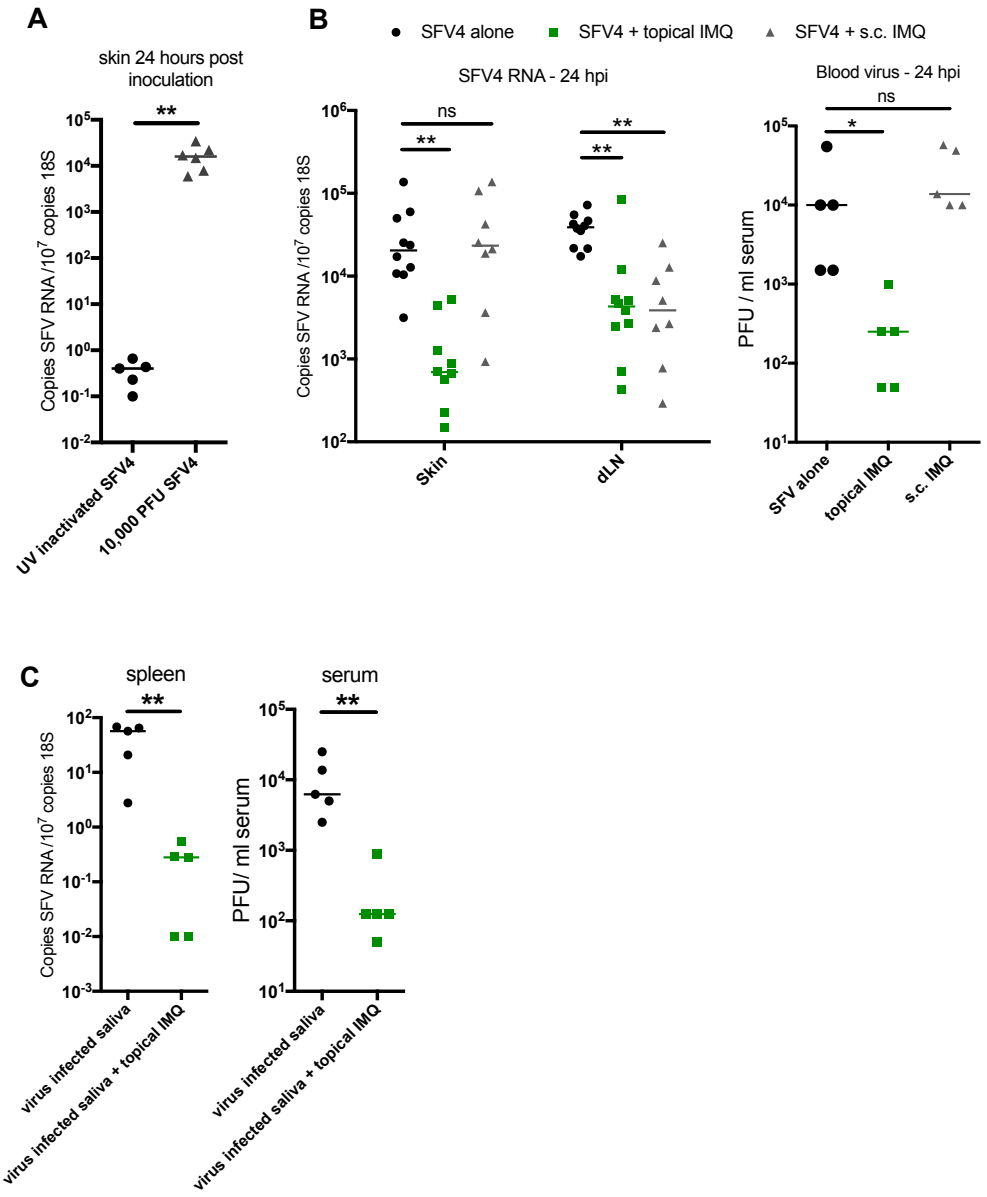

Fig. S1. Pre-exposure of skin to topical IMQ increased host resistance to infection with SFV4.
(A) Mice were inoculated with 10,000 PFU of SFV4 +/- UV inactivation, to define contribution of virus RNA inoculum to qPCR assay. Shown here is virus RNA copies at 24 hours post inoculation.
(B) Mice were treated with either 2 mg topical IMQ (5% IMQ), or 6 μg of IMQ (in PBS) injected subcutaneously (s.c.), 1h prior to infection with 10,000 PFU SFV4 at the same site. Copy number of SFV RNA (E1 gene) and host 18S was determined by qPCR at 24 hpi (n=8-10). Virus titers in the serum were quantified by plaque assay (n=5).
(C) Mice were inoculated with 10 000 PFU SFV4 with mosquito saliva derived from SFV-infected mosquitoes (saliva from 5 mosquitoes/injection of mice). To ensure saliva was derived from infected mosquitoes, only saliva from mosquitoes that exhibited high virus gene expression in their heads were used to inoculate mice (qPCR analysis was undertaken of each mosquito head post salivation). At 1hpi mice were treated with topical IMQ and virus titers at 24hpi determined by qPCR (spleen) and plaque assay (serum).
*p<0.05, **p<0.01, ***p<0.001, ns=not significant (Kruskal-Wallis test)

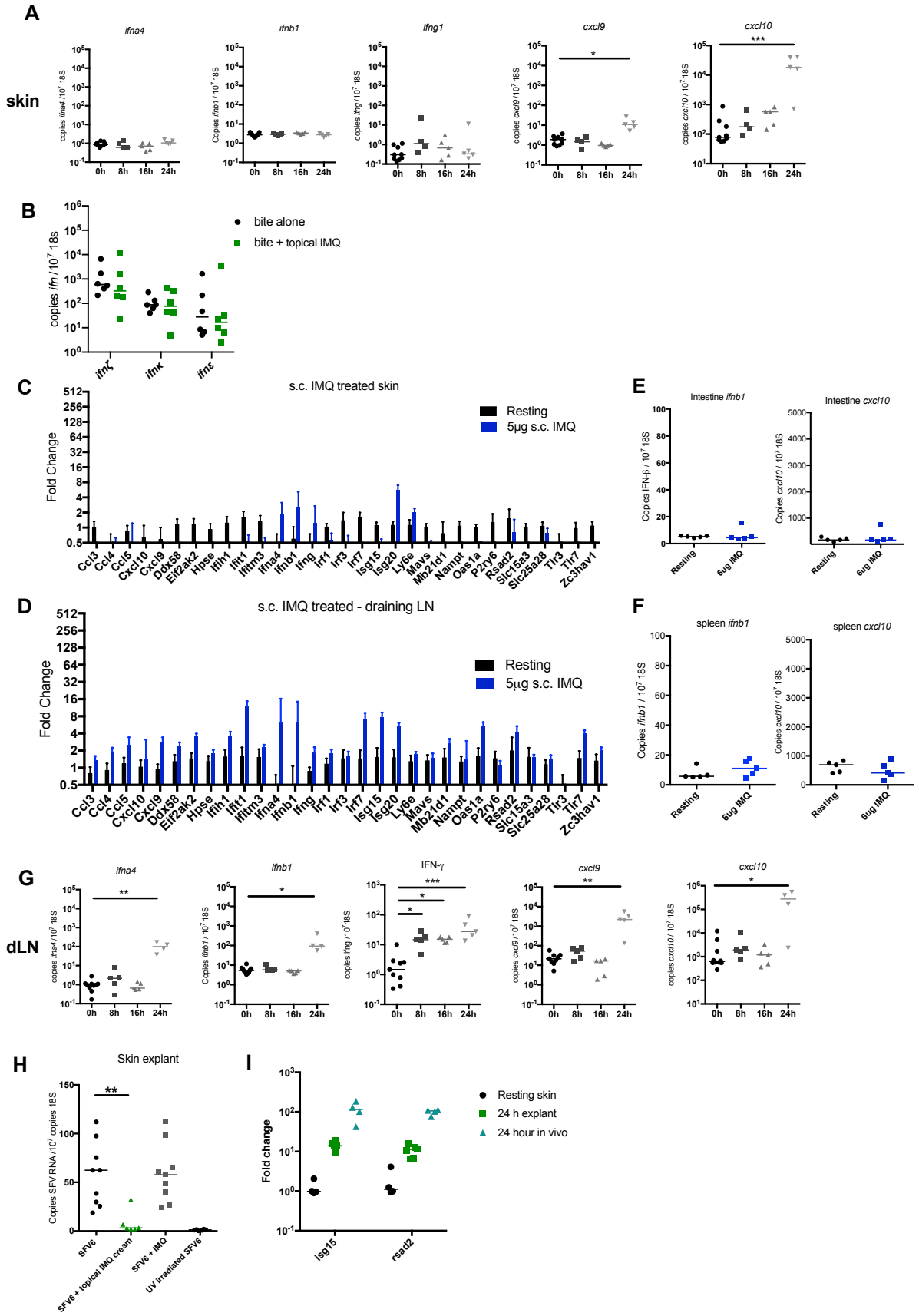

Fig. S2. Type I IFN and ISG expression in skin and draining LN following IMQ application.

Fig S2

(A,B) Mosquito-bitten mouse skin was treated with a single topical application of IMQ 1-hour post bite. Copy number of *ifna4*, *ifnb1*, *ifng*, *cxcl9* and *cxcl10* transcripts (A) and atypical type I IFNs (B) in the skin were determined by qPCR (n=5-10).

(C-F) Mice were injected with 6 µg of IMQ s.c. (C) Fold change in gene expression of ISG and IFN in skin (C) and dLN (D) was determined by qPCR at 24h post treatment (n=4). (E,F) Copy number of host *ifnb1*, *cxcl10*, and 18S in the small intestine (E) and spleen (F) were determined by qPCR at 24h post treatment (n=5).

(G) Mosquito-bitten mouse skin was treated with a single topical application of IMQ 1-hour post bite. Copy number of *ifna4*, *ifnb1*, *ifng*, *cxcl9* and *cxcl10* transcripts in the dLN were determined by qPCR (n=5-10)

(H) 6mm skin explants from mice were infected with 100,000 PFU SFV6 and treated with a single application of topical IMQ or 6µg of IMQ *ex vivo*. SFV RNA and host 18S were determined by qPCR at 24hpi (n=6-9). As a control, to demonstrate quantity of residual virus RNA from the inoculum, explants were also infected with UV inactivated virus (derived from same 100,000 PFU inoculum).

(I) Fold induction of prototypic ISGs *isg15* and *rsad2* at 24 hours post topical application of IMQ in skin explants in vitro and skin applied with topical IMQ *in vivo*.

*p<0.05, **p<0.01, ***p<0.001, ns=not significant (Kruskal-Wallis test)

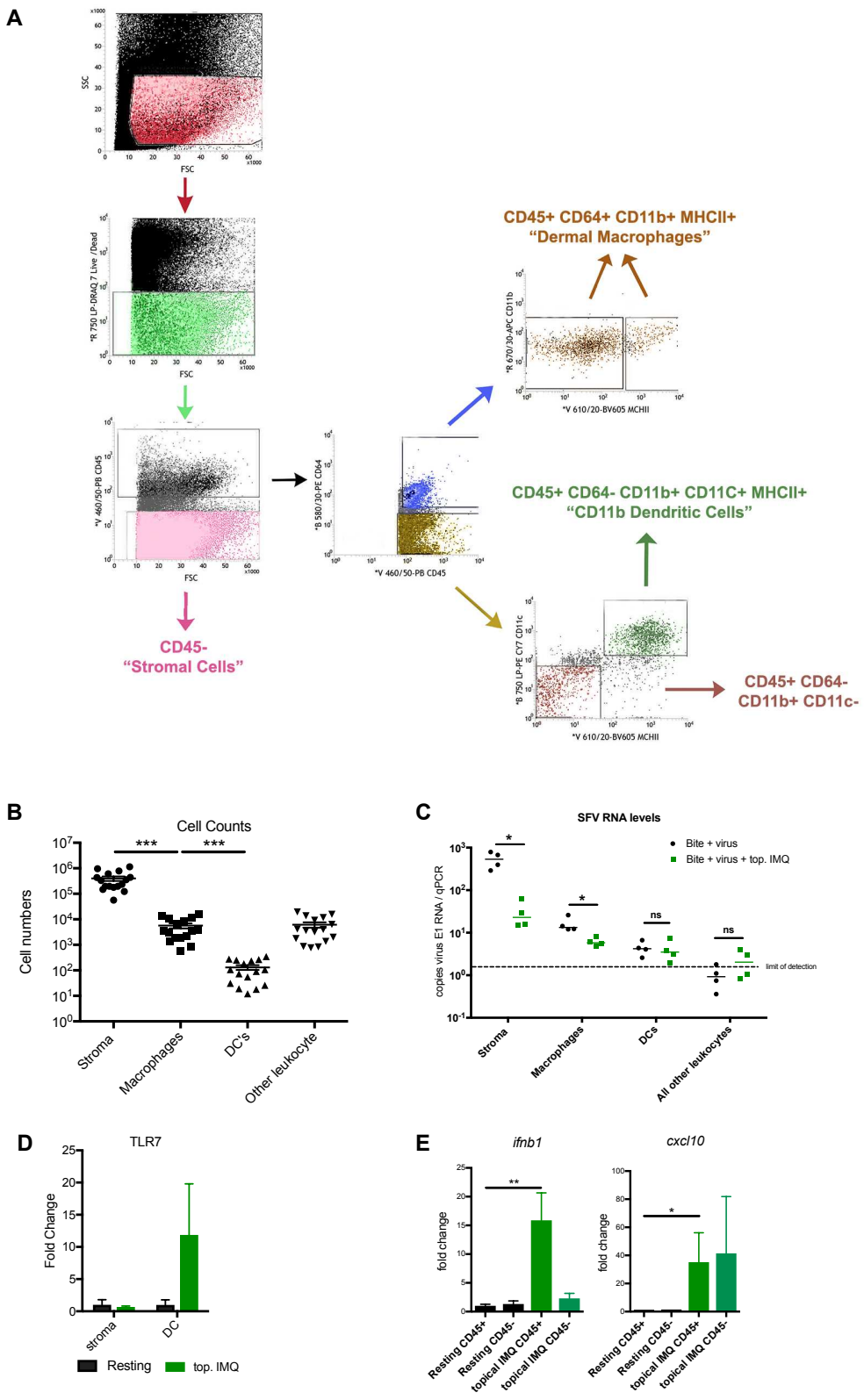

Fig. S3. Gene expression analysis of skin inoculation site-derived FACS isolated cells

(A-B) Gating strategy of flow cytometry sort. Individual mice were mosquito bitten and infected with SFV6. At 24 hpi, skin from each mouse was separately digested enzymatically in presence of transcriptional inhibitors, cells stained for lineage markers and FACS sorted at 4°C. Therefore, each data point in Fig 5 represents cells derived from an individual mouse. Cells sorted into "stroma"

(CD45-), "macrophage" (CD45+ CD64+ CD11b+, MHC$^{low\ to\ high}$), dermal dendritic cell "DCs" (CD45+ CD64- CD11c^{hi} CD11b+ MHC-II^{hi}) and "all other leukocyte" compartments, which are the bulk combination of all other remaining CD45+ve cells. Sorting strategy was based on a previously published protocol *(37)*. CD11b was uniformly high in both CD64+ cells and cells in the DC gate, therefore voltage was lowered in this channel (CD11b-APC) to increase efficiency of sorting and was not used as basis for sorting strategy.
(B) Representative cell counts of a FACS sort (n=16).
(C,D) Mice were infected with SFV6 s.c. in the presence of a mosquito bite, then treated with topical IMQ from 1h post infection. At 24 hpi, skin was digested and cells FACS isolated. Copy number of SFV RNA (C) and *tlr7* (D) was determined by qPCR (n=4).
(E) Mice were bitten with mosquitoes and 1 hour later treated with topical IMQ. At 24h post treatment skin was digested and cells sorted into "stroma" (CD45-) and "leukocytes" (CD45+). Copy number of host *ifnb1*, *cxcl10*, and 18S were determined by qPCR (n=3). *p<0.05, **p<0.01, ***p<0.001, ns=not significant (Mann-Whitney test).

Fig. S4

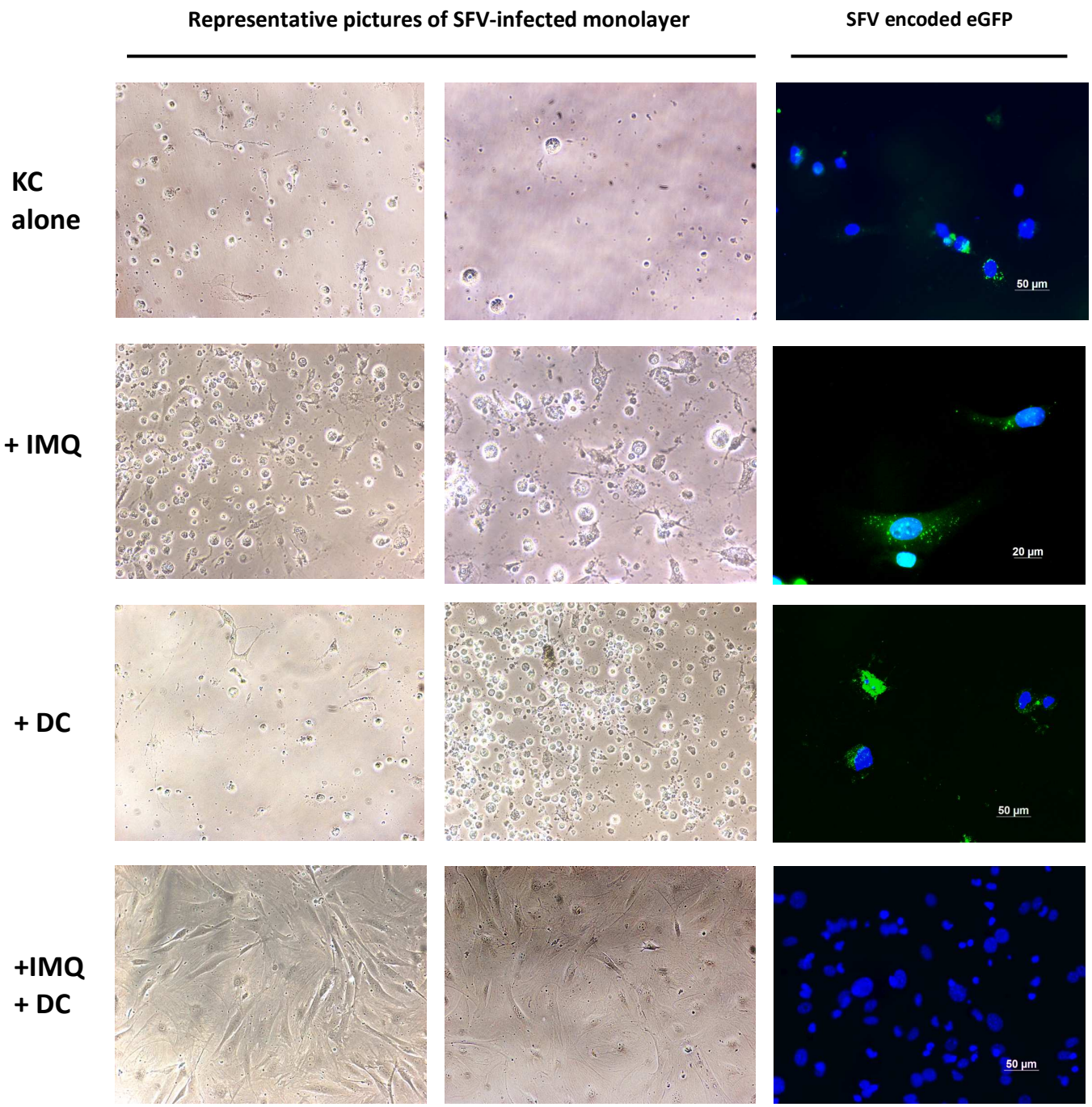

Fig. S4. IMQ-mediated protection against virus infection in keratinocytes is dependent on help from leukocytes.

Monolayers of primary mouse keratinocytes were infected *in vitro* with SFV4(Xho)-EGFP (green) at an MOI of 0.1 in the presence or absence of bone marrow-derived DCs, then treated 1 hour later with 0.2 µg/ml IMQ. Cells were stained for DAPI (blue).

Shown are representative images taken by brightfield and epifluorescence microscopy.

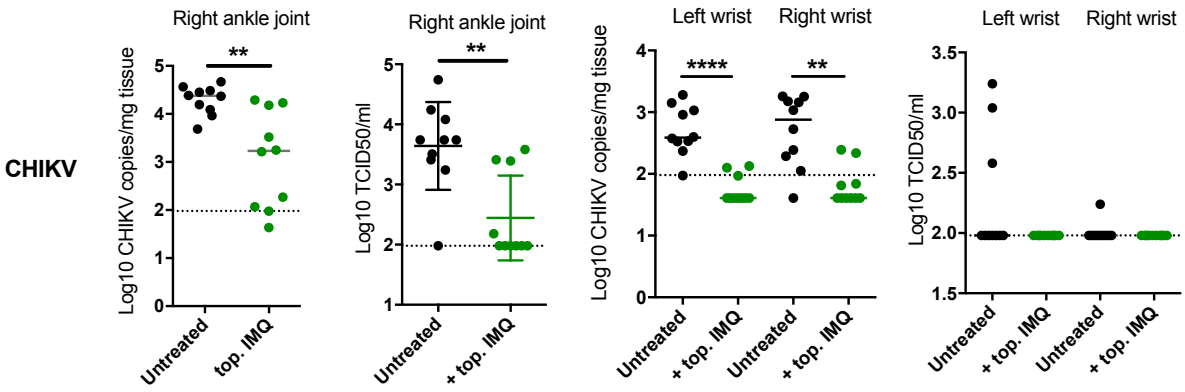

Fig. S5. Topical IMQ prevents systemic dissemination of CHIKV to joint tissue remote from inoculation site.

Mice were co-inoculated with 10^6 PFU of CHIKV (s.c.) alone and treated with topical IMQ at 1hpi and 5 hpi. Dissemination of virus to distal joints were assessed at 5 days post infection in the right ankle joint and both wrist joints. Quantities of CHIKV RNA were determined by qPCR and infectious virus titers quantified by TCID50 (n=10).