# Supplementary Figure S1: Isolation and identification of BMDMs.



#### Flow Cytometry analysis of BMDM formation.

BMDMs were isolated and differentiated according to previous established protocols (doi:10.1007/978-1-0716-2128-8\_8.). The cells were first gated on FSC and SSC to remove debris and conjugates. Mature BMDMs were identified as CD11b+F4/80+ populations.

## Supplementary Figure S2: Subconjunctival injection and ocular HSV-1 infection.



### A. Subconjunctival injection.

- 1. A 33G needle was filled with the sterile solution intended for injection.
- 2. The eyelid of the anesthetized mouse was gently pulled down to expose the conjunctival sac. The needle was held at a slight angle and inserted into the subconjunctival space, located between the sclera and conjunctiva, near the edge of the eye.
- 3. Light pressure was applied on the injection site for several minutes to prevent leakage and ensure proper administration of the solution.

(Arrow) A crescent swell of the conjunctival sac can be observed after successful injection.



### B. Corneal scratching and HSV-1 infection.

- 1. The eyelid of the anesthetized mouse was gently pulled down to expose the cornea. The tip of a 33G needle was used to scratch the corneal epithelium, making 3-5 horizontal and 3-5 vertical scratches. (indicated by arrows)
- 2. A volume of 5ul of HSV strain Mckrae ( $1 \times 10^{6}$  PFU/ml) was applied to the freshly scratched cornea.
- 3. The eyelid was gently massaged to enhance the absorption of the virus into the cornea.

## Supplementary Figure S3: supplementary qRT-PCR results.



#### A. NLRP12 mRNA expression in macrophage cell lines.

BMDMs, THP-1 cells and RAW264.7 cells were infected with HSV-1. The mRNA expression of NLRP12 was detected at specified time points using qRT-PCR.



#### B. HSV gB and gD mRNA expression.

At 24h post-infection, cells from the LV-Ctrl group and LV-NLRP12 group were collected for qRT-PCR analysis. The mRNA expression of HSV-gB and HSV\_gD was presented.

A. The cells in the single cell suspensions of the dLNs is predominantly leukocytes (>98%).



Supplementary Figure S4: Gating strategy for flow cytometry analysis of dLNs.

**B.** Gating strategy for Macrophages.



C. Gating strategy for DCs.



D. Gating strategy for T cells.



