



Fig.S1. Single-cell suspension of popliteal lymph node for IFA. For the experiment to prepare for lymph node single cell suspension, adult female Balb/c mice (n=4) were inoculated with either 1×10^5 or 1×10^6 pfu of rMP12-GFP at footpad, and popliteal lymph nodes were collected at 1 dpi. Collected lymph nodes were incubated in RPMI1640 containing 0.8 mg/ml collagenase/ dispase (Roche) and 0.2 mg/ml collagenase P (Roche) at 37°C for 20 min. The suspension, after several pipetting steps, was transferred into PBS with 2% FBS with minimum contamination of the debris of the lymph node. The remaining lymph node tissue was further digested with RPMI containing collagenases/dispase at 37°C for 10 min, and the cell suspension was collected into the same tube. Cells were collected after centrifugation and resuspended into PBS. The single-cell suspension was spotted onto 12-well glass slides and dried for 1 hour. Cells were fixed with 4% paraformaldehyde in PBS at room temperature for 10 min and used immediately for IFA. Blocking was performed with PBS containing normal mouse sera, and subsequently, primary antibodies were incubated at 37°C for 1 hour; i.e., biotin-conjugated anti-Langerin/CD207 rat IgG2a antibody (clone 929F3.01, Dendritics), biotin-conjugated anti-mouse CD11c antibody (clone N418, BioLegend), or a mixture of biotin rat IgG2a κ isotype control (clone RTK2758, BioLegend) and biotin rat IgG2b κ isotype control (clone RTK4530, BioLegend). Slides were incubated with streptavidin-DyLight™ 594 (Cat#405222, BioLegend) at 37°C for 1 hour and soaked into PBS containing 0.5% Triton X-100 for permeablization. Then, slides were stained with anti-RVFV N rabbit polyclonal antibody and subsequently with FITC-conjugated donkey anti-rabbit IgG (Poly4064, BioLegend).