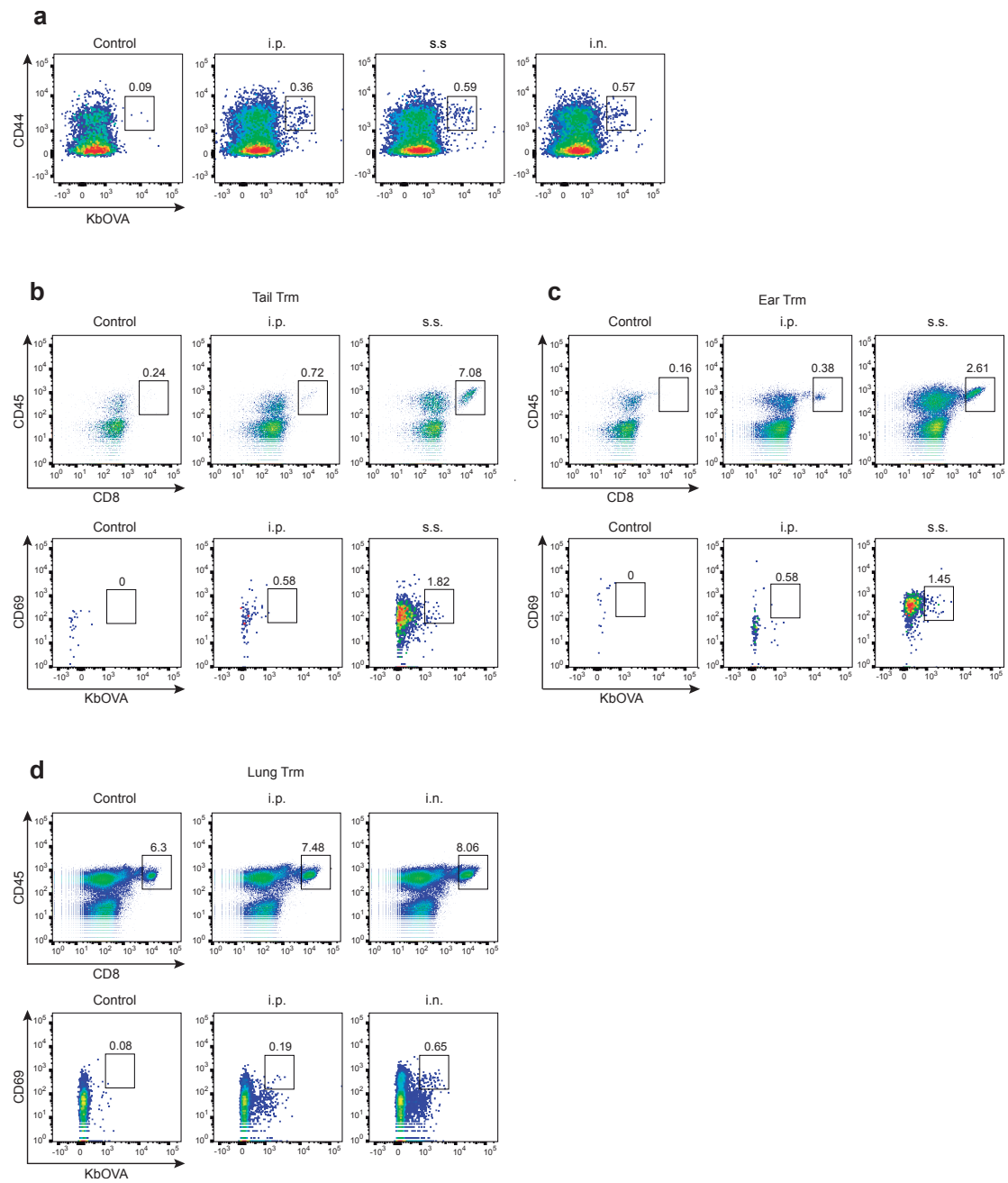
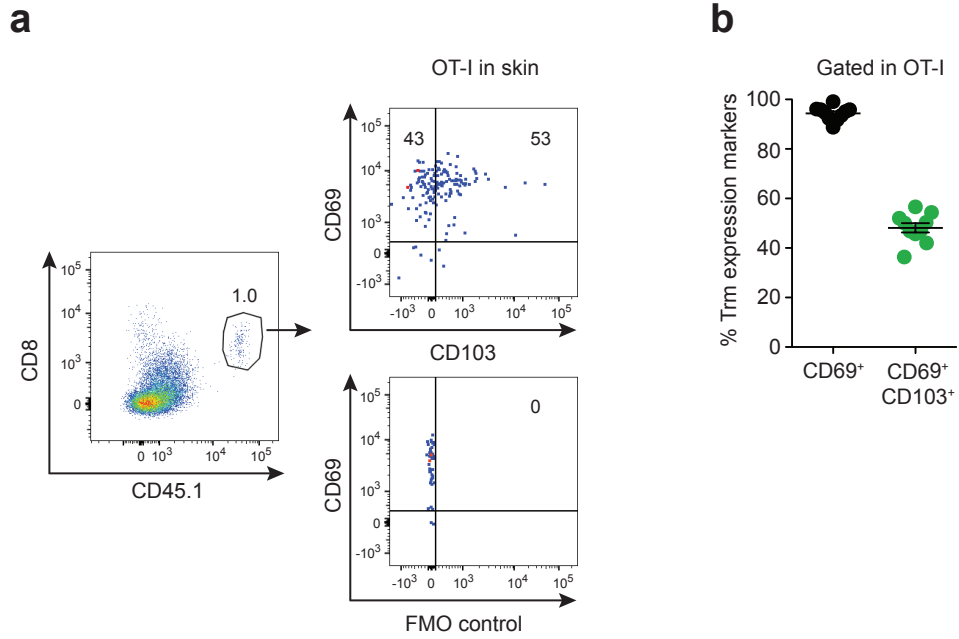


File Name: Supplementary Information
Description: Supplementary Figures

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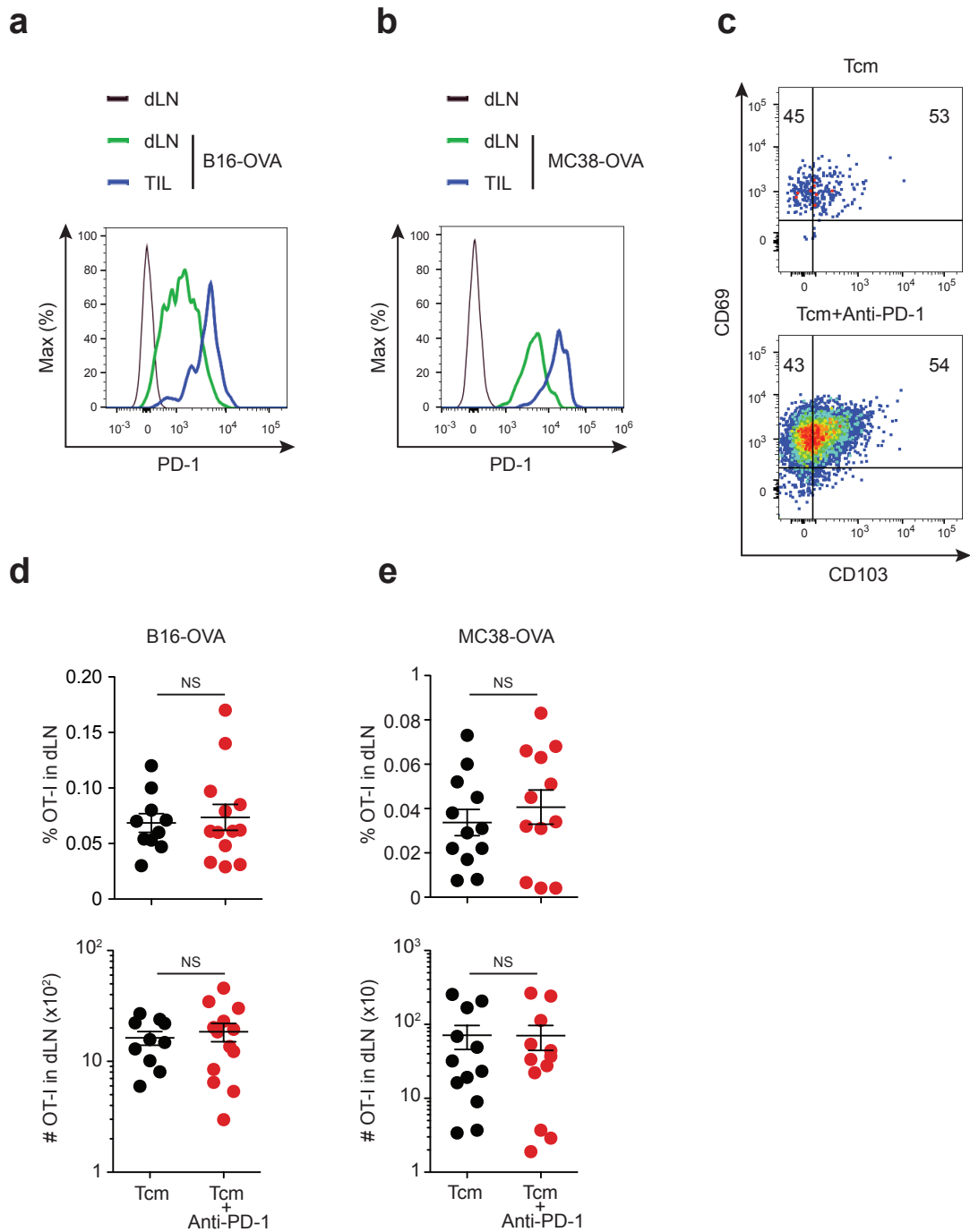


Supplementary Figure 1. Generation of Trm cells after different routes of rVACV-OVA infection (a) Representative FACS dot-plots of endogenous OVA-specific circulating memory CD8⁺ T cells in the dLN 30 d after i.p. (5×10^4 p.f.u.), s.s. (2×10^6 p.f.u.) or i.n. (5×10^4 p.f.u.) infection with rVACV-OVA. (b-d) Representative FACS dot-plots showing endogenous CD45⁺CD8⁺ cells (top panels), and OVA-specific CD69⁺ Trm cells (bottom panels) infiltrating the tail skin (b) or the ear (c) 30 d after s.s. in the tail, and in the lung (d) 30 d after i.n. infection with rVACV-OVA.



Supplementary Figure 2. Tcm cells give rise to Trm cells upon MC38-OVA inoculation

(a) Representative FACS dot-plots for OT-I identification (left) and Trm expression markers (right).
 (b) Frequency of Trm expression markers in OT-I cells in the skin of mice that rejected MC38-OVA tumors upon Tcm transfer (see Fig. 5a). Pool of two independent experiments represented as individual data and mean \pm s.e.m. (n=5/group)



Supplementary Figure 3. PD-1 expression and treatment with anti-PD-1 after Tcm transfer

(a, b) Mice were inoculated with B16-OVA i.d. (a) or MC38-OVA s.c. (b) and OT-I CD45.1⁺ Tcm cells transferred i.v after 7 or 15 d respectively. At day 7 after inoculation of Tcm cells, PD-1 expression in transferred OT-I within the tumor or in the tumor dLN was analyzed. (c) Representative FACS dot-plots of tumor infiltrating OT-I cells in 15 mg of B16-OVA tumor. (d, e) Mice were inoculated with B16-OVA i.d. (d) or MC38-OVA s.c. (e), transferred with OT-I Tcm cells and treated with anti-PD1 as indicated in Fig. 6c. Frequency (top) and numbers (bottom) of OT-I cells in dLN are shown. (a, b) Representative histograms from three independent experiments (n=2-3/control group, 3-4/tumor groups). (d, e) Pool of three independent experiments represented as individual points and mean \pm s.e.m. (n=3-5/group). NS, not significant by two-tailed unpaired Student's t test (d, e top) and two-tailed nonparametric Mann-Whitney test (e bottom).