Supplementary Figure Legends

Supplementary Figure 1. MVA skin scarification induced smaller pox lesions that healed significantly faster compared to VACV skin scarification in immunocompetent mice. C57BL/6 mice were immunized with 1.8 X 10^6 pfu MVA or VACV by skin scarification. Photographs of pox lesion were taken on day 4, 7, 14 and 28 post-immunization.

Supplementary Figure 2. Delivery of MVA via s.s. generates stronger cellular responses compared to i.d., s.c., and i.m. infection routes. C57BL/6 mice were immunized with 1.8 X 10^6 pfu MVA via indicated routes. Activated T cells in draining lymph nodes (a) and spleen (b) were isolated at 7 days post infection, and T cell response against VACV was measured based on IFN-γ secretion. Symbols represent individual mice (n = 5 mice/group). *p < 0.05, **p < 0.01.

Supplementary Figure 3. Delivery of MVA via s.s. generates T cells that are qualitatively distinct from those generated from i.d., s.c., i.m.. a-b. Venn diagram analysis of genes up-regulated (a) or down-regulated (b) in pairwise comparisons between T cells activated via MVA s.s., i.d., s.c., i.m. (day 5) relative to that of T_N. c-d. Fold change analysis of genes shared among s.s., i.d., s.c. and i.m. activated T cells (day 5) relative to that of T_N. c, 146 shared up-regulated genes, d, 41 shared down-regulated genes. e. qRT-PCR analysis of cell homing molecule gene expression in s.s., i.d., s.c. and i.m. activated T cells (day 5) relative to that of T_N. Graphs show the mean ± s. d. (n=5). ns = not significant, *p < 0.05, **p < 0.01.

Supplementary Figure 4. Phenotyping of tissue-resident memory T cell surface marker on lung CD8^+ T_{RM} cells generated by MVA infection via skin scarification, intra-tracheal administration or intra-peritoneal injection. Flow cytometric analysis of T cell proliferation and homing receptor expression on OT-I cells residing in lung at 45 days post MVA infection.
Naïve OT-I Thy1.1+ cells were transferred into Thy1.2+ recipient mice one day before mice were infected with $1.8 \times 10^6$ pfu MVA-Ova by s.s., i.t. or i.p.. At 45 days after infection, proliferation and tissue-homing receptor expression of OT-I $T_{RM}$ cells isolated from lung tissue were analyzed by flow cytometry. Data are representative of three independent experiments (n = 5 mice per group). ESL, E-selectin ligand.

**Supplementary Figure 5.** Skin $T_{RM}$ cells generated by MVA infection via skin scarification, intra-tracheal administration or intra-peritoneal injection. Flow cytometric analysis and quantification of skin $T_{RM}$ cells at day 45 post $1.8 \times 10^6$ pfu MVA infection via indicated routes. Data are representative of three independent experiments (n = 5 mice per group). Graphs show the mean ± s. d. (n=5). **p < 0.01.

**Supplementary Figure 6.** Gating strategy for the analysis of memory OT-I cell populations.

**Supplementary Table 1.** List of abbreviation.
Supplementary Figure 1
Supplementary Figure 2

(a) 

(b)
Supplementary Figure 3
Supplementary Figure 4
**Skin Scarification**

**Intra-tracheal**

**Intra-peritoneal**

Supplementary Figure 5
Supplementary Figure 6
| Abbreviation | Definition                      |
|--------------|--------------------------------|
| MVA          | Modified Ankara Virus           |
| VACV         | Vaccinia Virus                  |
| OVA          | Ovalbumin                       |
| WR-VACV      | Western-reserve Vaccinia Virus  |
| s.s.         | skin scarification              |
| i.d.         | intradermal                     |
| s.c.         | subcutaneous                    |
| i.m.         | intramuscular                   |
| i.t.         | intratracheal                   |
| i.p.         | intraperitoneal                 |
| Teff         | Effector T cells                |
| TRM          | Tissue resident memory T cells  |
| TCM          | Central Memory T cells          |
| TEM          | Effector Memory T cells         |
| LN           | lymph nodes                     |
| PCA          | Principle Component Analysis    |
| WT           | wide-type                       |
| BW           | Body Weight                     |
| qRT-PCR      | Quantitative real time PCR       |

**Supplementary Table 1**