

1 **Heat inactivated modified vaccinia virus Ankara boosts Th1 cellular and humoral**
2 **immunity as vaccine adjuvant**

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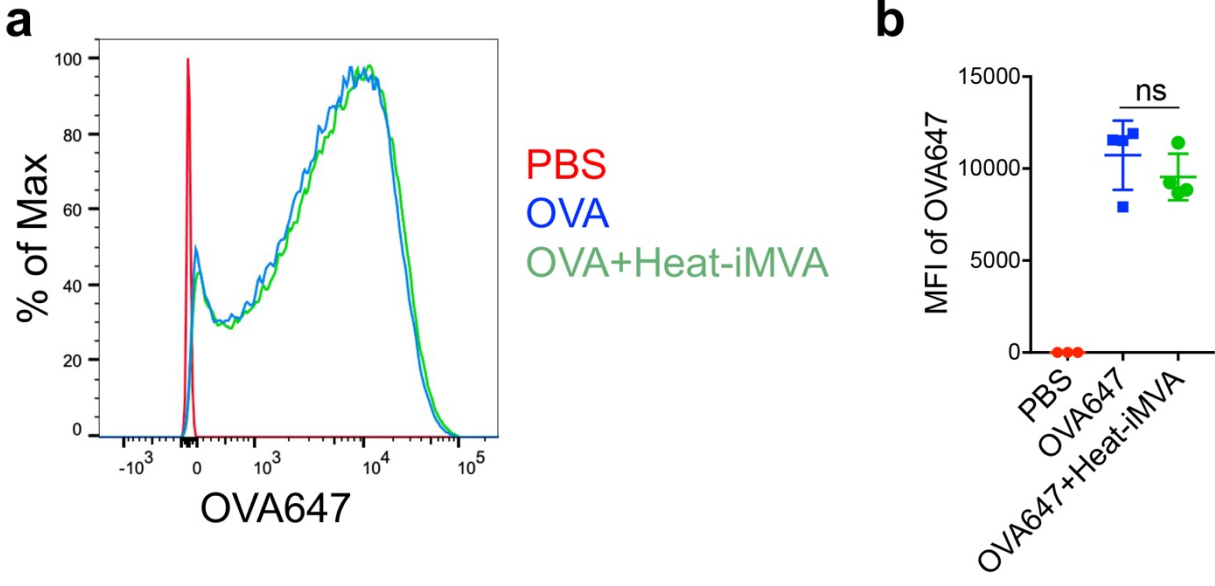
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19 Short title: heat-inactivated MVA as a vaccine adjuvant

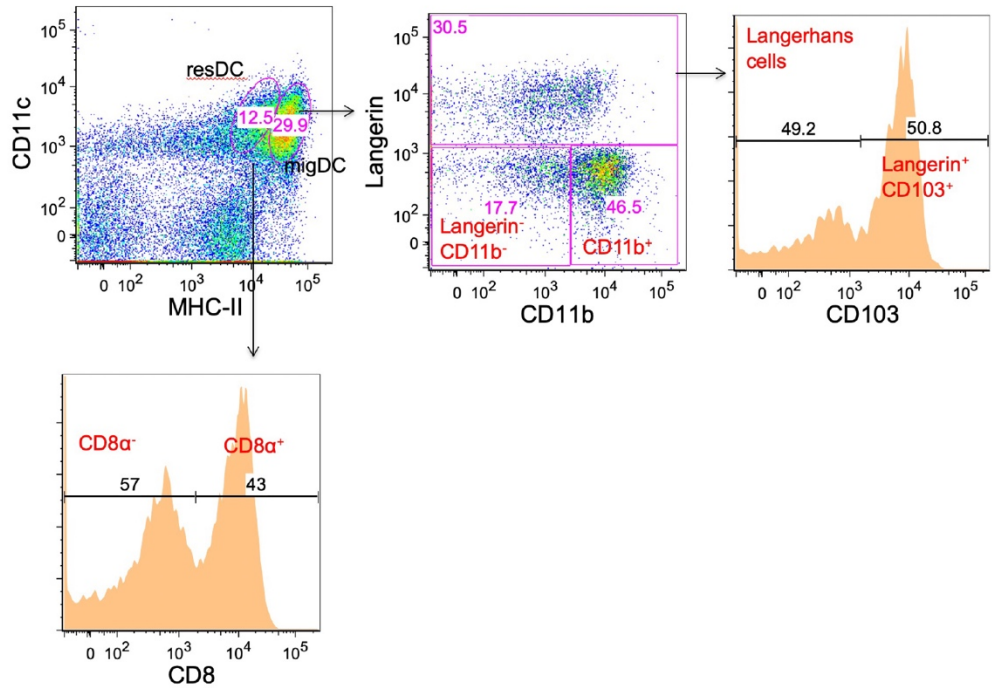
20 This file contains:

- 21 - Supplementary Figure 1
- 22 - Supplementary Figure 2
- 23 - Supplementary Figure 3



24 **Supplementary Figure 1. Heat-iMVA infection of BMDCs does not enhance antigen uptake**
25 **in vitro.** (a-b) BMDC were infected with Heat-iMVA (MOI of 1) for 1 h and then incubated
26 with OVA-647 (0.5 mg/ml) for 1 h. The fluorescence intensities of phagocytosed OVA-647 in
27 BMDC were measured by flow cytometry.

28 **Supplementary Figure 2. Time-resolved transcriptome profiling of WT or STING^{Gt/Gt}**
29 **BMDCs infected with either live MVA or heat-iMVA. (a)** A heat map of a one-way
30 hierarchical clustering analysis of the top 200 genes ranked by Z-score of log₂RPKM, indicating
31 genes that exhibited the most statistically significant changes in gene expression over the course
32 of the experiment. Several clusters of genes with similar gene expression changes were observed
33 (indicated as a1-2, b1-3, and c). **(b)** A heat map of a subset of genes from panel A, showing IFN-
34 regulated genes and genes involved in inflammation. **(c)** A heat map of a one-way hierarchical
35 cluster analysis of MVA and Heat-iMVA transcriptome, using log₂ RPKM, illustrating the
36 temporal pattern of viral gene expression changes. Light gray: 2 h. Medium gray: 4 h. Black: 6 h.



37 **Supplementary Figure 3. Gating strategy of dendritic cell populations in skin LN.** Skin LN
 38 were harvested and digested into single cell suspension. Cells were stained with a cocktail of
 39 antibodies to distinguish DC subsets. Within single cells, dead cells, CD119⁺, DX5⁺, TER119⁺
 40 and CD3ε⁺ cells were excluded for analysis. The CD11c^{hi} MHC II^{int} population represents
 41 lymphoid resident DCs, and can be further divided into CD8⁺ and CD8⁻ DCs. CD11c^{int/hi} MHC
 42 II^{hi} cells were further analyzed for the expression of Langerin and CD11b to define skin
 43 migratory DC subsets. Langerin⁻ cells were divided into CD11b⁺ DCs and CD11b⁻ DCs.
 44 Langerin⁺ cells were further analyzed for the expression of CD103 and divided into two groups:
 45 CD103⁺ DCs and langerhans cells.