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☐ Initial submission ☐ Revised version ☒ Final submission

Life Sciences Reporting Summary

Nature Research wishes to improve the reproducibility of the work that we publish. This form is intended for publication with all accepted life science papers and provides structure for consistency and transparency in reporting. Every life science submission will use this form; some list items might not apply to an individual manuscript, but all fields must be completed for clarity.

For further information on the points included in this form, see Reporting Life Sciences Research. For further information on Nature Research policies, including our data availability policy, see Authors & Referees and the Editorial Policy Checklist.

Experimental design

1. Sample size

Describe how sample size was determined.

Sample sizes were chosen based on prior research conducted in our laboratories to provide sufficient numbers of mice in each group to provide informative results and perform statistical testing.

2. Data exclusions

Describe any data exclusions.

Mice with gBT-I/Thy1.1 cell depletion efficiency <99% in the spleen were excluded from analysis in Supplementary Fig 3c only. This exclusion criteria was chosen to avoid accidental inclusion of contaminating circulating gBT-I cells (CD103-) in the analysis of CD103 expression levels on gBT-I TRM cells incorporating BrdU in the skin.

3. Replication

Describe whether the experimental findings were reliably reproduced.

Experiments were reliably reproduced and the number of experiments performed stated in methods and legends. All experiments were repeated successfully in this manuscript.

4. Randomization

Describe how samples/organisms/participants were allocated into experimental groups.

Mice of the same age/sex were randomly allocated to experimental groups.

5. Blinding

Describe whether the investigators were blinded to group allocation during data collection and/or analysis.

Blinding was not performed in this study. The experimental observations (viral titer, enumeration, cellular surface phenotypes) would be consistent irrespective of blinding.

Note: all studies involving animals and/or human research participants must disclose whether blinding and randomization were used.

6. Statistical parameters

For all figures and tables that use statistical methods, confirm that the following items are present in relevant figure legends (or in the Methods section if additional space is needed).

n/a	Confirmed
☐	☒ The exact sample size (*n*) for each experimental group/condition, given as a discrete number and unit of measurement (animals, litters, cultures, etc.)
☐	☒ A description of how samples were collected, noting whether measurements were taken from distinct samples or whether the same sample was measured repeatedly
☐	☒ A statement indicating how many times each experiment was replicated
☐	☒ The statistical test(s) used and whether they are one- or two-sided (note: only common tests should be described solely by name; more complex techniques should be described in the Methods section)
☐	☒ A description of any assumptions or corrections, such as an adjustment for multiple comparisons
☐	☒ The test results (e.g. *P* values) given as exact values whenever possible and with confidence intervals noted
☐	☒ A clear description of statistics including central tendency (e.g. median, mean) and variation (e.g. standard deviation, interquartile range)
☐	☒ Clearly defined error bars

See the web collection on statistics for biologists for further resources and guidance.

Software

Policy information about availability of computer code

7. Software

Describe the software used to analyze the data in this study.	Flow cytometry data was analysed using FlowJo 9.9.4 software (Treestar). Statistical testing was performed in and graphs were generated in Prism 7.0 (Graphpad). Intravital microscopy analysis and compilation was performed in Imparis (Bitplane), tracking analysis performed using Matlab (Mathworks), and movies compiled in After Effects (Adobe).

For manuscripts utilizing custom algorithms or software that are central to the paper but not yet described in the published literature, software must be made available to editors and reviewers upon request. We strongly encourage code deposition in a community repository (e.g. GitHub). *Nature Methods* guidance for providing algorithms and software for publication provides further information on this topic.

Materials and reagents

Policy information about availability of materials

8. Materials availability

Indicate whether there are restrictions on availability of unique materials or if these materials are only available for distribution by a for-profit company.	Any unique materials are available from the authors upon request.

9. Antibodies

Describe the antibodies used and how they were validated for use in the system under study (i.e. assay and species).	The clone and manufacturer of each antibody used in this study can be found in the Materials & Methods section of this paper. Antibodies were validated according to the manufacturers instructions.

10. Eukaryotic cell lines

a. State the source of each eukaryotic cell line used.	Vero cells (CSL, Australia) were used to grow HSV viruses . BHK-21 cells (ATCC) were used to grow VV viruses. MDCK (ATCC) and HEK293T cells (ATCC) were used to produce flu viruses.
b. Describe the method of cell line authentication used.	Cells were purchased from a commercial source.
c. Report whether the cell lines were tested for mycoplasma contamination.	Cell lines were tested for mycoplasma contamination and were not contaminated.
d. If any of the cell lines used are listed in the database of commonly misidentified cell lines maintained by ICLAC, provide a scientific rationale for their use.	No commonly misidentified cell lines were used.

Animals and human research participants

Policy information about studies involving animals; when reporting animal research, follow the ARRIVE guidelines

11. Description of research animals

Provide details on animals and/or animal-derived materials used in the study.	All mice used were female and aged between 6 and 12 weeks at the time of experimentation.

Policy information about studies involving human research participants

12. Description of human research participants

Describe the covariate-relevant population characteristics of the human research participants.	This study did not involve human research participants.

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Flow Cytometry Reporting Summary

Form fields will expand as needed. Please do not leave fields blank.

Data presentation

For all flow cytometry data, confirm that:

☒ 1. The axis labels state the marker and fluorochrome used (e.g. CD4-FITC).

☒ 2. The axis scales are clearly visible. Include numbers along axes only for bottom left plot of group (a 'group' is an analysis of identical markers).

☒ 3. All plots are contour plots with outliers or pseudocolor plots.

☒ 4. A numerical value for number of cells or percentage (with statistics) is provided.

Methodological details

5. Describe the sample preparation.	Refer to Methods p.15-16
6. Identify the instrument used for data collection.	BD LSR Fortessa II
7. Describe the software used to collect and analyze the flow cytometry data.	FlowJo 9.9.4 software (Treestar)
8. Describe the abundance of the relevant cell populations within post-sort fractions.	N/A
9. Describe the gating strategy used.	For all spleen and lymph node samples: FSCH lo/SSCH intermediate (lymphocytes) --> FSA/FSWlo (singlet gate) --> SSA/SSWlo (singlet gate #2) --> Live cells (negative for PI or LiveDead differentiator) --> +/- CD45.2+ (lymphocytes) where congenic marker expressed --> CD8+ (CD8+ T cells) --> Va2+ CD45.1+ (transgenic gBT-I or OT-I cells) or Va2+ Thy1.1+ (transgenic gBT-I or P14 cells). For all skin samples except AnnexinV analysis: FSCH lo/SSCH intermediate (lymphocytes) --> FSA/FSWlo (singlet gate) --> SSA/SSWlo (singlet gate #2) --> Live cells (negative for PI or LiveDead differentiator) --> +/- CD45.2+ (lymphocytes) where congenic marker expressed --> CD3 intermediate (T cells) ---> Va2+ CD45.1+ (transgenic gBT-I or OT-I cells) or Va2+ Thy1.1+ (transgenic gBT-I or P14 cells) or Va2+ GFP+ (OT-I/uGFP cells) --> CD103+ +/- CD69+ (skin TRM cells). CD69 and CD103 gating was determined by inspection of transgenic gBT-I cells in the spleen (CD103-CD69-) and/or comparison with endogenous Va2+ T cells in the skin. Annexin V analysis skin samples: FSCH lo/SSCH intermediate (lymphocytes) --> FSA/FSWlo (singlet gate) --> SSA/SSWlo (singlet gate #2) --> +/- CD45.2 + (lymphocytes) where congenic marker expressed --> CD3 intermediate (T cells) ---> Va2+ CD45.1+ (transgenic gBT-I cells) or Va2+ Thy1.1+ (transgenic gBT-I cells) --> CD103+CD69+ (skin TRM cells).

Tick this box to confirm that a figure exemplifying the gating strategy is provided in the Supplementary Information. ☒