nature immunology

SUPPLEMENTARY INFORMATION

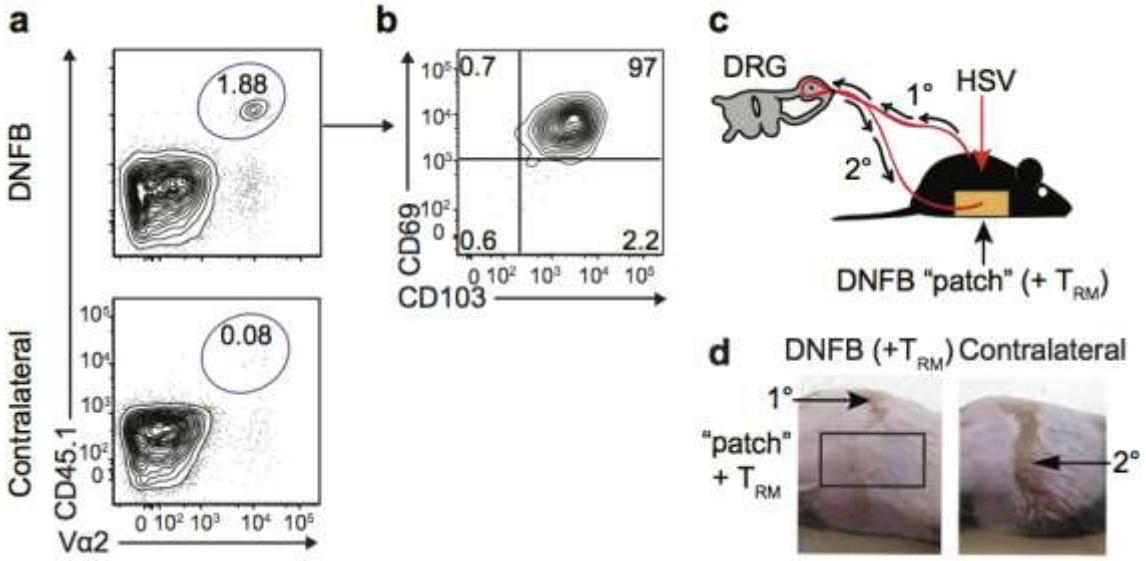
https://doi.org/10.1038/s41590-017-0027-5

In the format provided by the authors and unedited.

Local proliferation maintains a stable pool of tissue-resident memory T cells after antiviral recall responses

Simone L. Park[1], Ali Zaid[1], Jyh Liang Hor[1], Susan N. Christo[1], Julia E. Prier[1], Brooke Davies[1], Yannick O. Alexandre[1], Julia L. Gregory[1], Tiffany A. Russell[2], Thomas Gebhardt[1], Francis R. Carbone[1], David C. Tscharke[2], William R. Heath[1,3], Scott N. Mueller[1,3]* and Laura K. Mackay[1,3]*

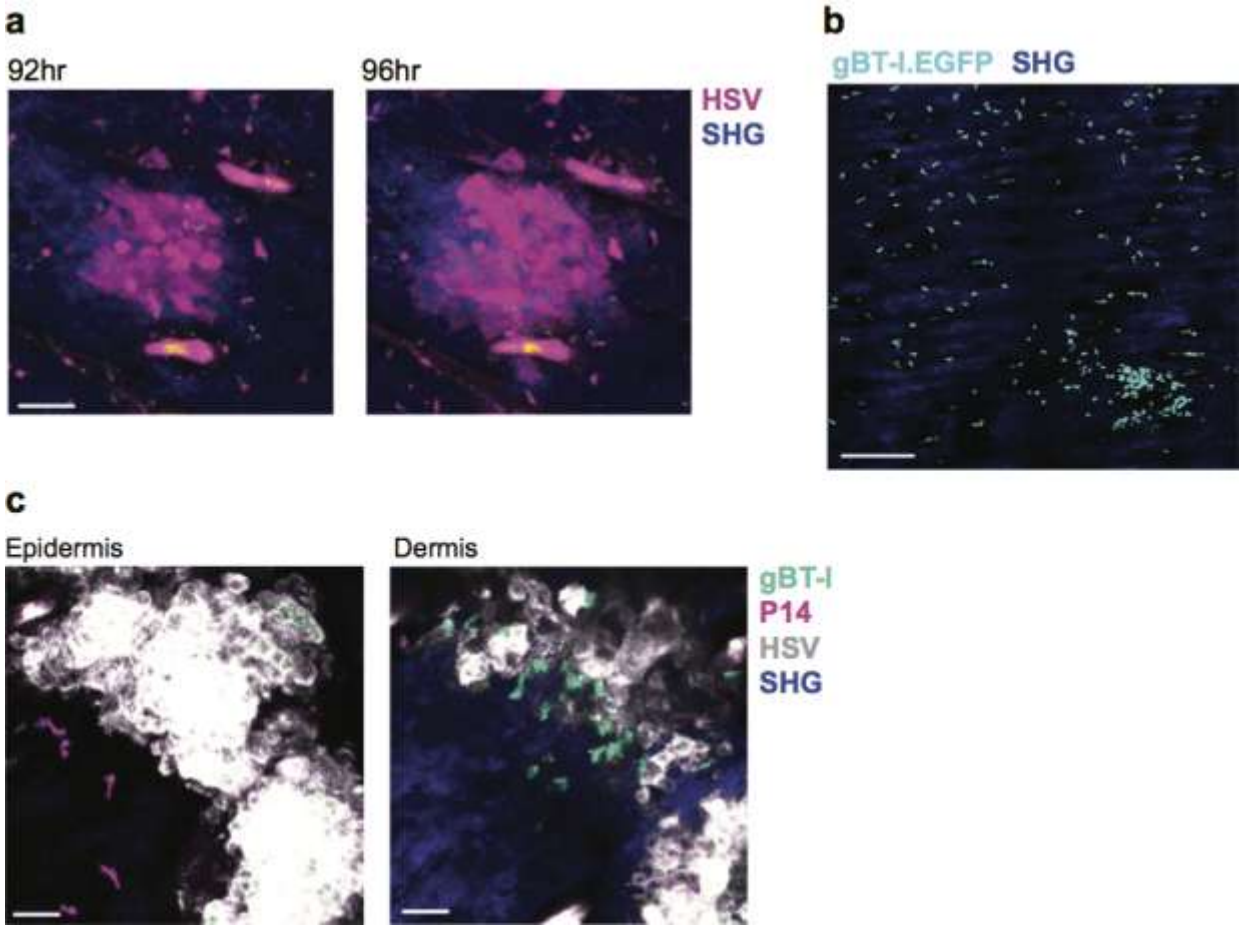
[1]Department of Microbiology and Immunology, The University of Melbourne and The Peter Doherty Institute for Infection and Immunity, Melbourne, VIC, Australia. [2]John Curtin School of Medical Research, The Australian National University, Canberra, ACT, Australia. [3]The Australian Research Council Centre of Excellence in Advanced Molecular Imaging, The University of Melbourne, Melbourne, VIC, Australia. Simone L. Park and Ali Zaid contributed equally to this work. Scott N. Mueller and Laura K. Mackay jointly supervised this work. *e-mail: smue@unimelb.edu.au; lkmackay@unimelb.edu.au



Supplementary Figure 1

Skin T_{RM} cells mediate local antiviral protection.

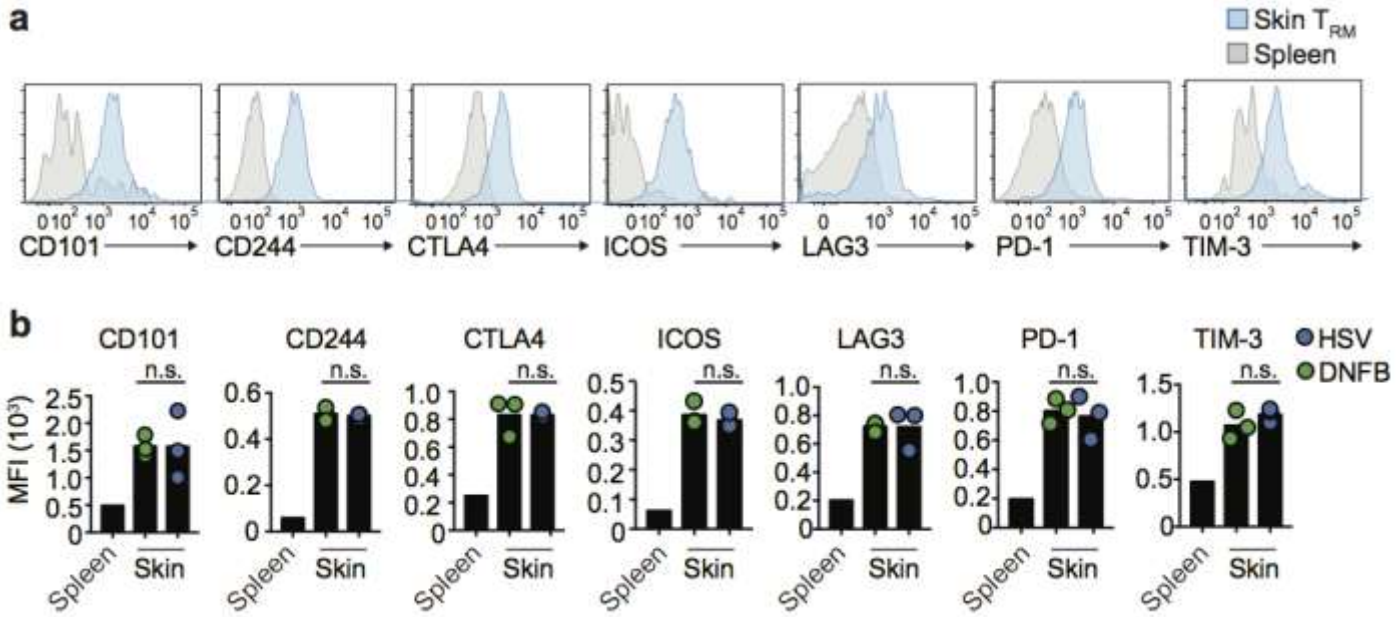
Frequency **(a)** and CD69 and CD103 expression **(b)** of CD45.1^{+} gBT-I T cells isolated from DNFB-treated skin (upper panel) or non-treated contralateral flank skin (lower panel). Data are representative of 2 experiments with n = 5 group. (**c**) Schematic depicting "patch" protection model. Mice were seeded with effector gBT-I cells and treated with DNFB on the lower left skin flank to lodge T_{RM} cells. >30d post DNFB treatment, mice were infected with HSV on the upper left skin flank so that infection could be initiated in the absence of T_{RM}, leading to primary (1°) infection of the upper skin region and the dorsal root ganglion (DRG). Viral emergence from the DRG results in secondary (2°) lesion formation, which is interrupted in DNFB-treated skin patch containing T_{RM} cells. (**d**) Photographs depicting 1° and 2° lesion formation in DNFB-treated skin possessing a "patch" of T_{RM} cells on the lower left flank (DNFB + T_{RM}, left panel) or on the non-treated contralateral flank (right panel) 6d after HSV infection. Data are representative of 2 experiments with n = 5 group.



Supplementary Figure 2

T_{RM} cell behavior after virus challenge.

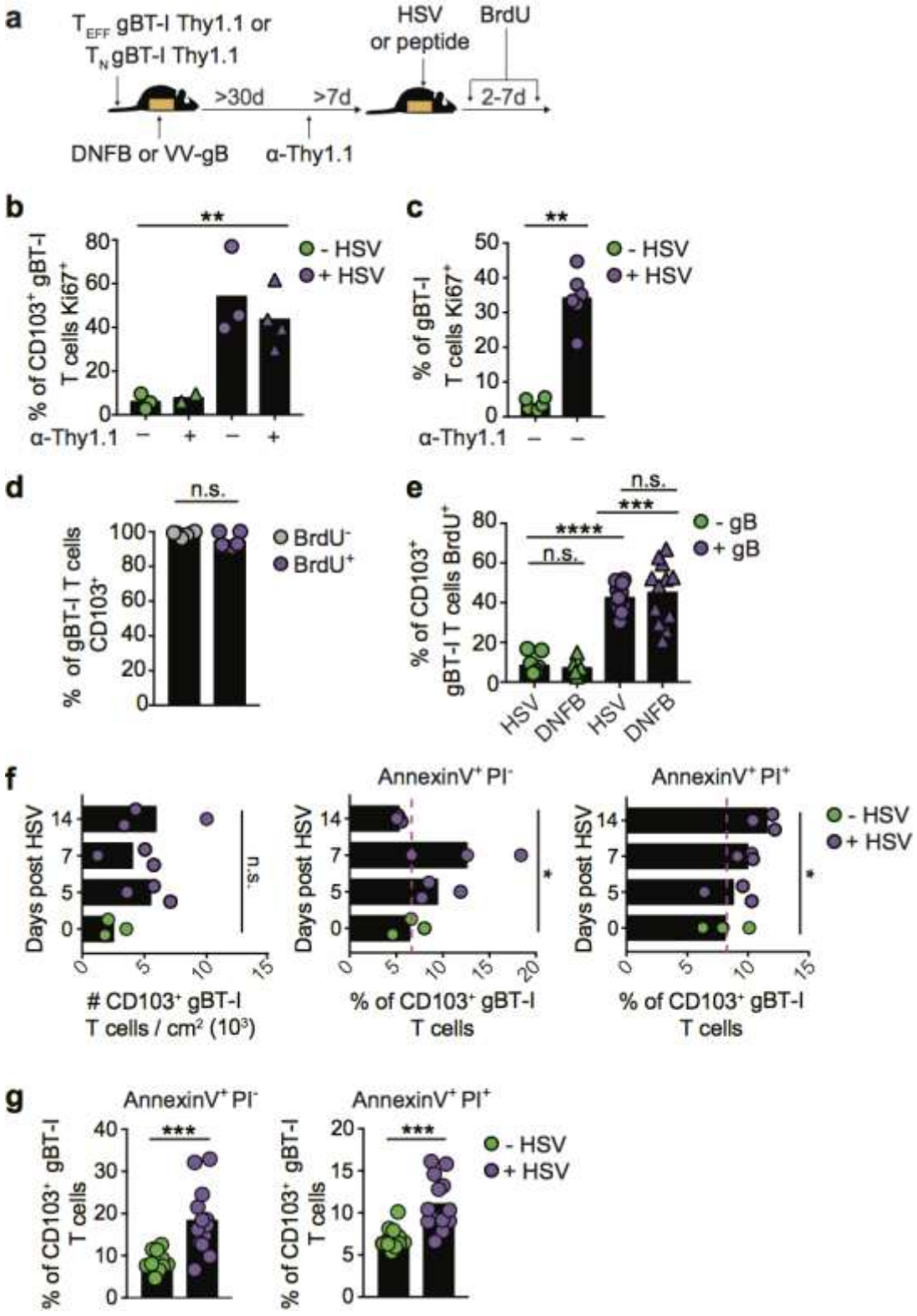
(a) IV-2PM images of HSV-mCherry^{+} cells in the same region of skin 92 or 96h after HSV infection on the upper flank. Scale bar; 50μm. Data are representative of 4 experiments. **(b)** IV-2PM image of EGFP^{+} gBT-I T cells in a region of skin 94h after infection. Scale bar; 200μm. Data are representative of 4 experiments. **(c)** IV-2PM images showing responding EGFP^{+} gBT-I T cells amongst HSV-infected cells at the dermal-epidermal border (right panel), and DsRed^{+} P14 T_{RM} cells in the epidermis (left panel) from the same region of skin 84h after infection. Data are representative of 2 experiments. Scale bar; 40μm.



Supplementary Figure 3

Expression of inhibitory receptors by skin T_{RM} cells.

(a) Surface expression of inhibitory markers on DNFB-lodged skin Thy1.1$^+$ gBT-I T_{RM} cells (blue) compared to splenic Thy1.1$^+$ gBT-I T cells (grey) >30d post DNFB treatment. **(b)** Mean fluorescence intensity (MFI) of inhibitory markers on Thy1.1$^+$ gBT-I T_{RM} cells in HSV-infected or DNFB-treated skin compared to splenic Thy1.1$^+$ gBT-I T cells >30d post-HSV or DNFB treatment. n.s.; not significant, two-tailed Mann Whitney U-test. Data are representative of 2 experiments with n=3 mice per group. Bars represent the mean.

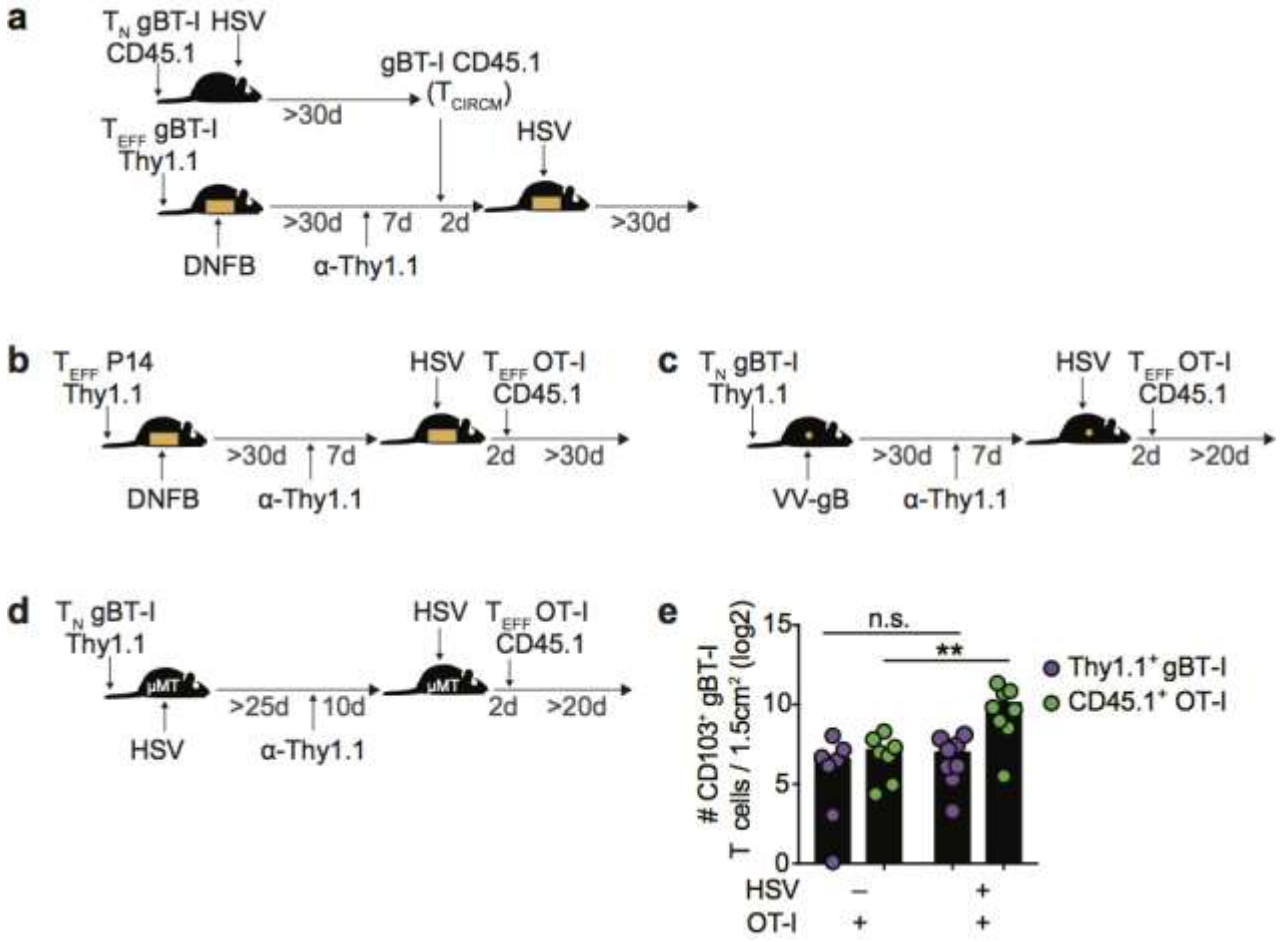


Supplementary Figure 4

Skin T_{RM} cells undergo local proliferation and cell death after rechallenge.

(**a**) Experimental schematic. Mice were seeded with effector Thy1.1$^+$ gBT-I (T_{EFF}) cells and treated with DNFB, or were seeded with naïve Thy1.1$^+$ gBT-I (T_N) cells and infected with VV-gB. >30d after treatment or infection mice were administered Thy1.1 antibody to

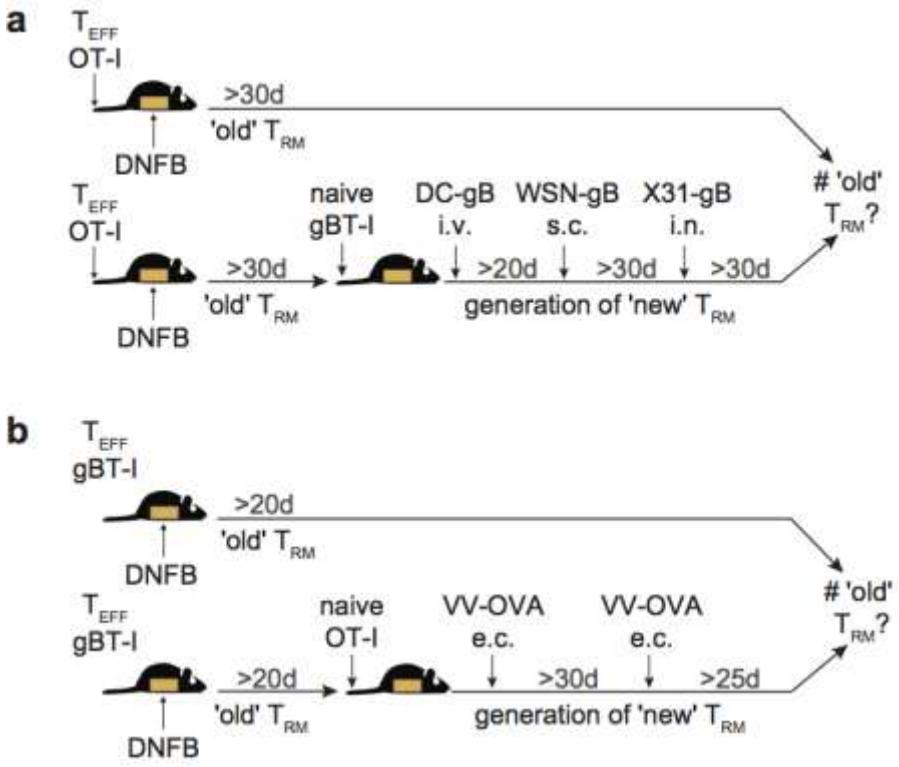
deplete Thy1.1$^+$ gBT-I T_{CIRCM} cells. Mice were then challenged with HSV infection or transcutaneous peptide application and treated daily with BrdU i.p. post-challenge. (**b, c**) Ki67 expression by (**b**) DNFB-lodged skin Thy1.1$^+$ gBT-I T_{RM} cells or (**c**) splenic Thy1.1$^+$ gBT-I T_{CIRCM} cells 7d after HSV challenge. Data are representative of 2 experiments with n=2, 3 or 6 in **b** or n=5 or 6 mice per group in **c**. **p=0.0072, Kruskal Wallis test with Dunn's multiple comparisons in **b** and **p=0.0043, two-tailed Mann Whitney U-test in **c**. (**d**) Proportion of BrdU$^+$ or BrdU$^-$ DNFB-induced skin Thy1.1$^+$ gBT-I cells expressing CD103 in Thy1.1 antibody treated mice 7d post-HSV challenge. Data are pooled from 3 experiments, n=6 mice. n.s.; non-significant, p=0.2835, two-tailed Mann Whitney U-test. (**e**) Mice were seeded with either Thy1.1$^+$ gBT-I T_N cells and infected with HSV or Thy1.1$^+$ gBT-I T_{EFF} cells and treated with DNFB. >30d later mice were treated with Thy1.1 antibody. Shown is proportion of CD103$^+$ Thy1.1$^+$ gBT-I T_{RM} cells in DNFB-treated or previously HSV infected skin incorporating BrdU 2d following topical application of gB peptide. Data are pooled from 3 experiments with n=3 or 4 mice per group. n.s.; p=0.5137 and p=0.6070, ***p=0.0001, ****p<0.0001, two-tailed Mann Whitney U-test. **(f)** Number of DNFB-lodged Thy1.1$^+$ gBT-I T_{RM} cells in DNFB treated skin (left panel) of Thy1.1 antibody treated mice, and AnnexinV and PI expression by these cells at the indicated time points following HSV infection above the DNFB patch. Data are representative of 1 or 3 experiments with n =3 or 4 mice per time point per experiment. *p=0.0435 (middle panel) or 0.0291 (right panel), Kruskal Wallis test. **(g)** AnnexinV and PI staining of DNFB-lodged Thy1.1$^+$ gBT-I skin T_{RM} cells 7d post-HSV infection above the DNFB patch or at steady state. Data are pooled from 3 experiments with n=11 or 13 mice per group. ***p=0.0002, two-tailed Mann Whitney U-test. Bars represent the mean.



Supplementary Figure 5

The stability of pre-existing skin T_{RM} cell populations after viral challenge.

(a) Experimental schematic related to Fig. 5a-c. Mice received Thy1.1$^+$ gBT-I T_{EFF} cells and were treated with DNFB. After >30d Thy1.1$^+$ gBT-I T_{CIRCM} cells were depleted using Thy1.1 antibody. A separate cohort received CD45.1$^+$ gBT-I T_N cells and was infected with HSV. >30d post-infection, splenic CD45.1$^+$ T_{CIRCM} cells from these donors were transferred into mice containing DNFB-lodged Thy1.1$^+$ gBT-I T_{RM} cells and these recipients were then infected with HSV above DNFB-treated skin, which was analysed >30d post-infection. (**b**) Experimental schematic related to Fig. 5d-f. Mice received Thy1.1$^+$ P14 T_{EFF} cells and were treated with DNFB. After >30d Thy1.1$^+$ P14 T_{CIRCM} cells were depleted using Thy1.1 antibody. >7d later mice were infected with HSV above DNFB-treated skin and transferred CD45.1$^+$ OT-I T_{EFF} cells. DNFB-treated skin was analyzed >30d post-HSV infection. (**c**) Experimental schematic related to Fig. 5g-i. Mice received Thy1.1$^+$ gBT-I (T_N) cells and were infected with VV-gB on the lower skin flank e.c. >30d post-infection Thy1.1$^+$ gBT-I T_{CIRCM} cells were depleted using Thy1.1 antibody. >7d later mice were challenged with HSV above VV-challenged skin and CD45.1$^+$ OT-I T_{EFF} cells were transferred 2d later. VV-challenged skin was analysed >20d post HSV-infection. **(d)** Experimental schematic related to Supplementary Fig. S5e. μMT mice received Thy1.1$^+$ gBT-I (T_N) cells and were infected with HSV on the lower left flank. >25d post-infection, mice were treated with Thy1.1 antibody to remove Thy1.1$^+$ gBT-I T_{CIRCM} cells. 10d post-antibody treatment mice were challenged with HSV on the upper left flank and transferred CD45.1$^+$ OT-I T_{EFF} cells i.v. 2d post-infection. **(e)** Number of Thy1.1$^+$ gBT-I and CD45.1$^+$ OT-I T_{RM} cells in HSV-challenged skin >20d post-HSV infection. Data are pooled from 2 experiments with n = 7 or 8 mice per group. **p=0.0059, two-tailed Mann Whitney U-test. Bars represent the mean.



Supplementary Figure 6

Multiple challenges generate de novo T_{RM} cells without displacing pre-existing T_{RM} cells in skin.

(**a**) Experimental schematic related to Fig. 6b. Mice were transferred EGFP$^+$ OT-I T_{EFF} cells i.v. and treated with DNFB to establish a population of ('old') T_{RM} cells. One cohort of mice was left untreated whereas a second cohort was adoptively transferred CD45.1$^+$ gBT-I T_N cells followed by i.v. injection of gB-pulsed dendritic cells (DC gB). >20d following priming mice were boosted with WSN-gB s.c. and left to rest another >30d before boosting with X31-gB i.n to generate 'new' CD45.1$^+$ gBT-I skin T_{RM} cells. DNFB-treated skin was analysed >30d following final immunization. (**b**) Experimental schematic related to Fig. 6c. Mice were transferred CD45.1$^+$ gBT-I T_{EFF} cells and treated with DNFB to establish a population of ('old') T_{RM} cells. Mice were transferred EGFP$^+$ OT-I T_N cells then infected with VV-OVA e.c. contralateral to DNFB-treated skin. >30d following primary VV-OVA infection mice were re-challenged with VV-OVA e.c. at an unrelated site to generate 'new' T_{RM} cells. DNFB-treated skin was analysed >25d following secondary VV-OVA challenge.