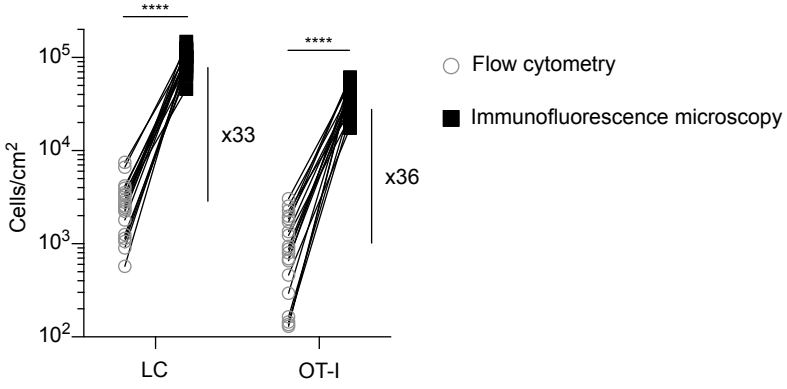
****
****
10^5
10^4
10^3
10^2
Cells/cm^2
x33
x36
LC
OT-I
Flow cytometry
Immunofluorescence microscopy

Figure S1. Related to Figure 1. Enumeration of epidermal cells by flow cytometry is an underestimate

Thy1.1$^+$ OT-I cells were adaptively transferred to naïve WT mice followed by VV-OVA skin infection (flank). At least 60 days post infection, LC and OT-I Trm cells were enumerated by either flow cytometry or immunofluorescence microscopy. LC are defined as live CD45$^+$MHCII$^+$ cells by flowcytometry and MHCII$^+$ cells by immunofluorescence microscopy. OT-I cells were identifies as live CD45$^+$Thy1.1$^+$ in flow cytometry and Thy1.1$^+$ by immunofluorescence microscopy. Data are representative from three separate experiments. ****$p < 0.0001$. Each symbol represents data from an individual animal and lines indicate data from the same animal.

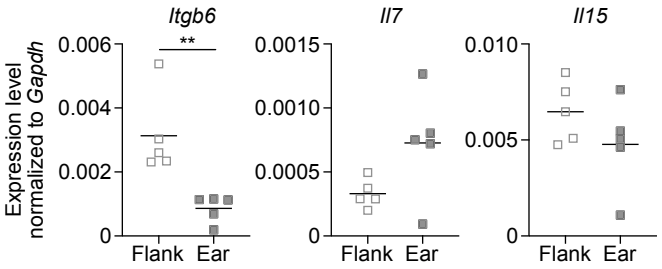
Itgb6
Il7
Il15
Expression level normalized to Gapdh
**
0.006
0.004
0.002
0
0.0015
0.0010
0.0005
0
0.010
0.005
0
Flank
Ear
Flank
Ear
Flank
Ear

Figure S2. Related to Figure 2. Gene expressions in flank and ear epidermis

Itgb6, *Il7*, and *Il15* mRNA relative expression normalized to *Gapdh* in epidermis isolated from flank or ear skin are shown. Data are representative from two separate experiments. **$p < 0.01$. Each symbol represents data from an individual animal.

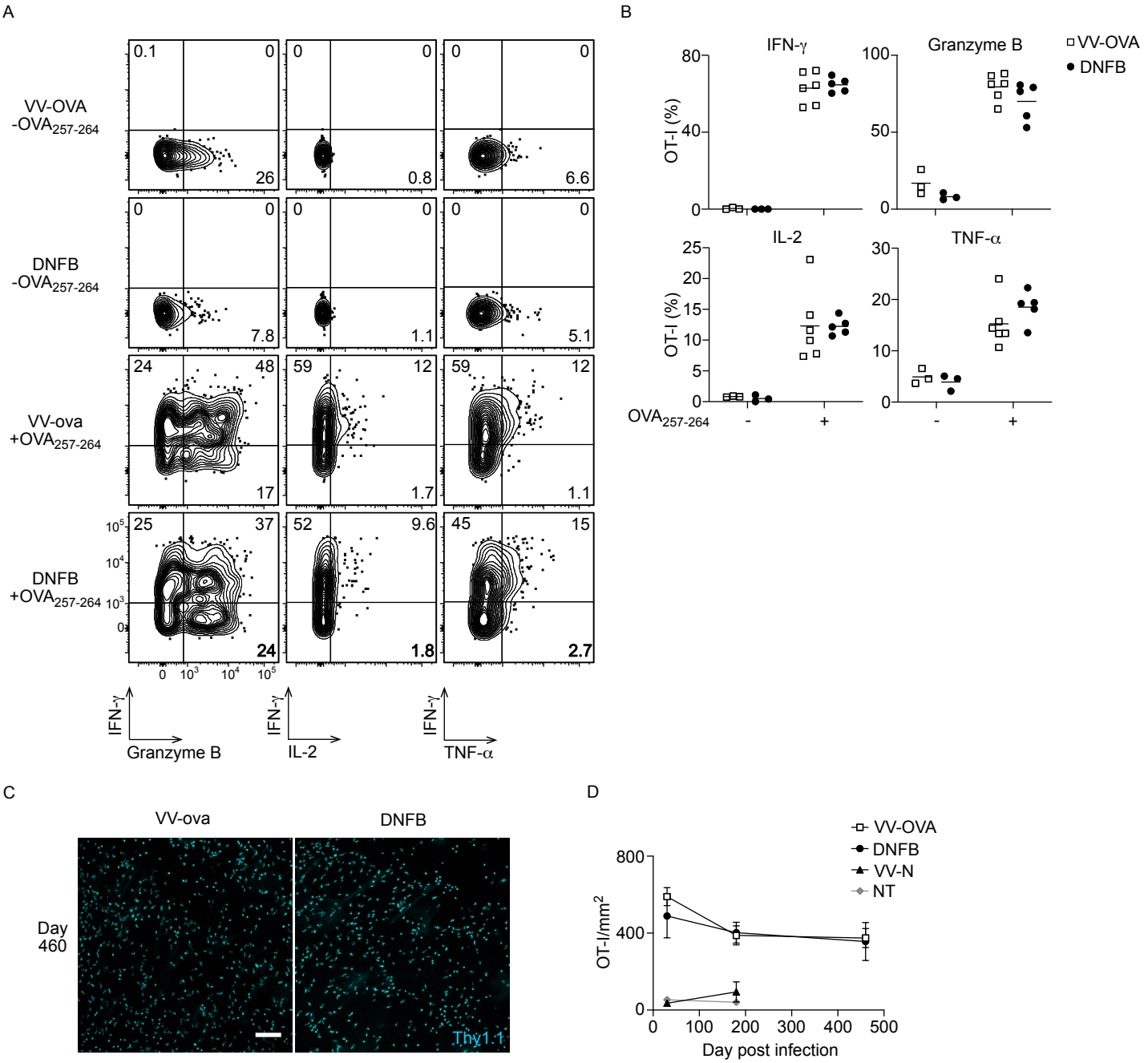
A
VV-OVA
-OVA257-264
DNFB
-OVA257-264
VV-ova
+OVA257-264
DNFB
+OVA257-264
0.1
0
26
0.8
6.6
7.8
1.1
5.1
24
48
17
59
12
1.7
1.1
25
37
24
52
9.6
1.8
45
15
2.7
IFN-γ
Granzyme B
IL-2
TNF-α
B
IFN-γ
Granzyme B
IL-2
TNF-α
OT-I (%)
VV-OVA
DNFB
OVA257-264
C
VV-ova
DNFB
Day
460
Thy1.1
D
VV-OVA
DNFB
VV-N
NT
OT-I/mm2
Day post infection

Figure S3. Related to Figure 4. Phenotype of antigen-specific Trm cells and bystander Trm cells

(A-B) Thy1.1$^+$ OT-I cells were adoptively transferred into naïve WT mice followed by skin VV-OVA infection on left flank skin. On day 5 post infection, the right flank was painted with 0.15% DNFB. Either left flank or right flank were epicutaneously challenged $OVA_{257-264}$ day 70 post infection. The mice were injected i.p. with brefeldin A 6 hour after the challenge and the epidermis were harvested for analyzing recall responses 12 hour after the challenge. The representative flow plots gated on live CD45$^+$Thy1.1$^+$ OT-I cells (A), and the frequency of OT-I cells (B). (C-D) Thy1.1$^+$ OT-I cells were adoptively transferred into naïve WT mice followed by skin VV-OVA infection on left flank skin. At the same day, some mice were also infected VV-N on the right flank. The mice not infected with VV-N were epicutaneously treated with DNFB on the right flank 5 days post infection. The epidermis from each site were harvested day 30, 180, 460 post infection. The representative immunofluorescence images of epidermal whole mount for Thy1.1$^+$ OT-I cells (C) and the quantification (D) are shown. Data are representative from two separate experiments. Scale bar represents 100 μm.

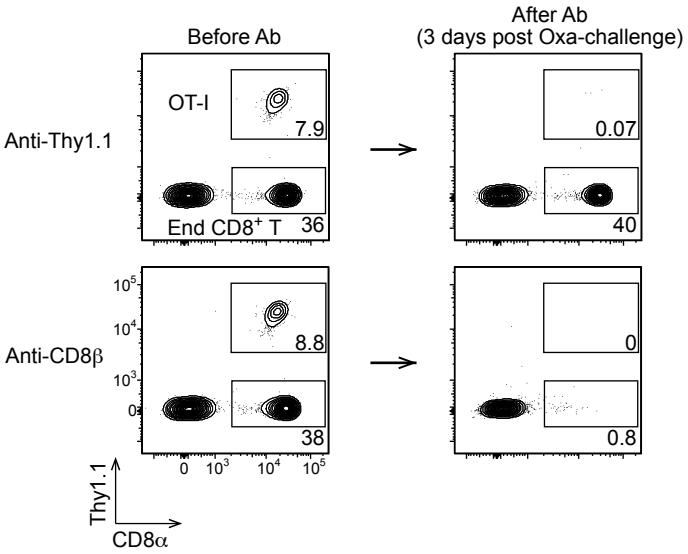
Before Ab
After Ab
(3 days post Oxa-challenge)
Anti-Thy1.1
OT-I
7.9
End CD8+ T
36
0.07
40
Anti-CD8β
8.8
38
0
0.8
Thy1.1
CD8α

Figure S4. Related to Figure 6 and 7. Depletion of circulating CD8$^+$ T cells

Thy1.1$^+$ OT-I cells were adoptively transferred into naïve WT mice followed by skin VV-OVA infection on left flank. On day 5 post infection, the right flank was painted with 0.15% DNFB. More than 35 days after DNFB treatment, mice were sensitized at abdomen with oxazolone twice with 5 days interval. The mice were treated with titrated anti-Thy1.1 or anti-CD8b mAbs and then both flanks were challenged by oxazolone at day 5 after the second sensitization. Blood samples were collected one day before mAbs treatment and 3 days after oxazolone challenge. Representative flow plots gated on live CD3$^+$TCRβ^+ T cells in blood are shown.

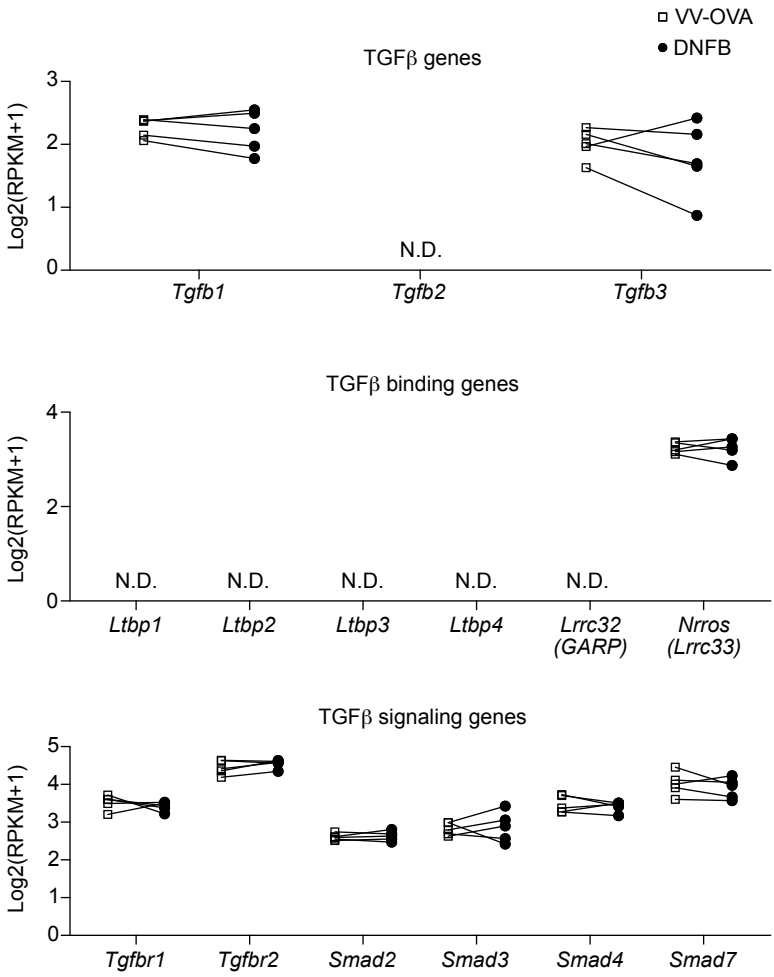
VV-OVA
DNFB
TGFβ genes
Log2(RPKM+1)
3
2
1
0
N.D.
Tgfb1
Tgfb2
Tgfb3
TGFβ binding genes
Log2(RPKM+1)
4
2
0
N.D.
N.D.
N.D.
N.D.
N.D.
Ltbp1
Ltbp2
Ltbp3
Ltbp4
Lrrc32 (GARP)
Nrros (Lrrc33)
TGFβ signaling genes
Log2(RPKM+1)
5
4
3
2
1
0
Tgfbr1
Tgfbr2
Smad2
Smad3
Smad4
Smad7

Figure S5. Related to Figure 7. TGFβ-related gene expression in antigen-specific Trm and bystander Trm

Thy1.1^{+} OT-I cells were adoptively transferred into naïve WT mice followed by skin VV-OVA infection on left flank. On day 5 post infection, the right flank was painted with 0.15% DNFB. At day 57-70 after infection, liveCD45^{+}Thy1.2^{-} Thy1.1^{+}OT-I Trm from each flank from the same animals were FACS sorted and analyzed by RNA-seq. Mice from 2 individual animals were pooled for each repeat. N.D. is not detected if RPKM of all samples were less than 4.