Supporting Information Appendix (Figures S1-S5, Tables S1 and S2 and Methods)

Supporting Information, Figures S1-S4

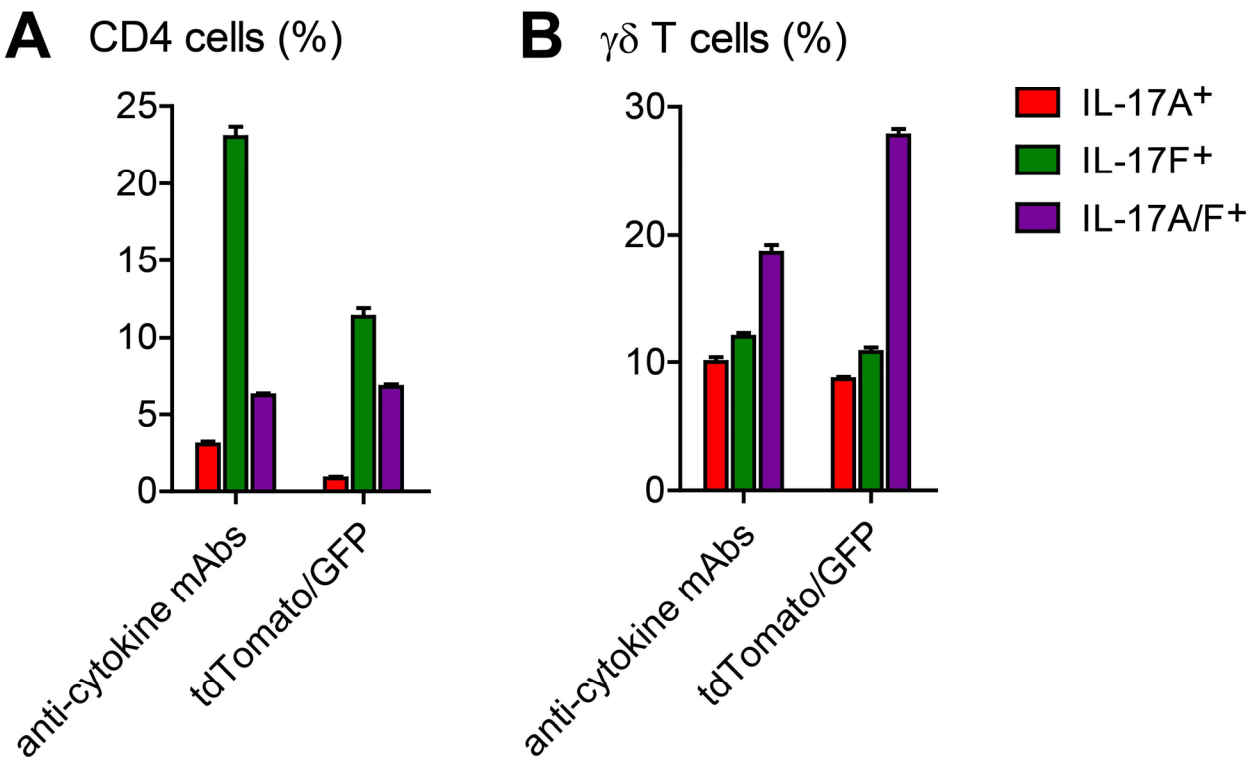

Figure S1. **In vitro validation of the IL-17A-tdTomato/IL-17F-GFP dual color reporter mouse strain.** The IL-17A-tdTomato/IL-17F-GFP dual color reporter mouse strain was validated in vitro by evaluating $CD4^+$ T cells and γδ T cells isolated from skin-draining lymph node cells after culturing the cells in Th17/IL-17 polarizing conditions for 3 days with the last 4 hours in the presence of brefeldin A and monensin (for anti-IL-17A and anti-IL-17F mAb labeling) or without brefeldin A and monensin (for tdTomato and GFP fluorescence quantification) and flow cytometry was performed (n= 6 replicates/group). (**A,B**) Mean percentage ± SEM of $IL\text{-}17A^+$, $IL\text{-}17F^+$ and $IL\text{-}17A/F^+$ $CD4^+$ T cells (Th17 cells) (**A**) and γδ T cells (**B**). In Th17 and γδ T cells, the percentage of $tdTomato^+$ and GFP^+ cells closely corresponded to the percentage of cells labeled with anti-IL-17A and anti-IL-17F mAbs.

www.pnas.org/cgi/doi/10.1073/pnas.1818256116

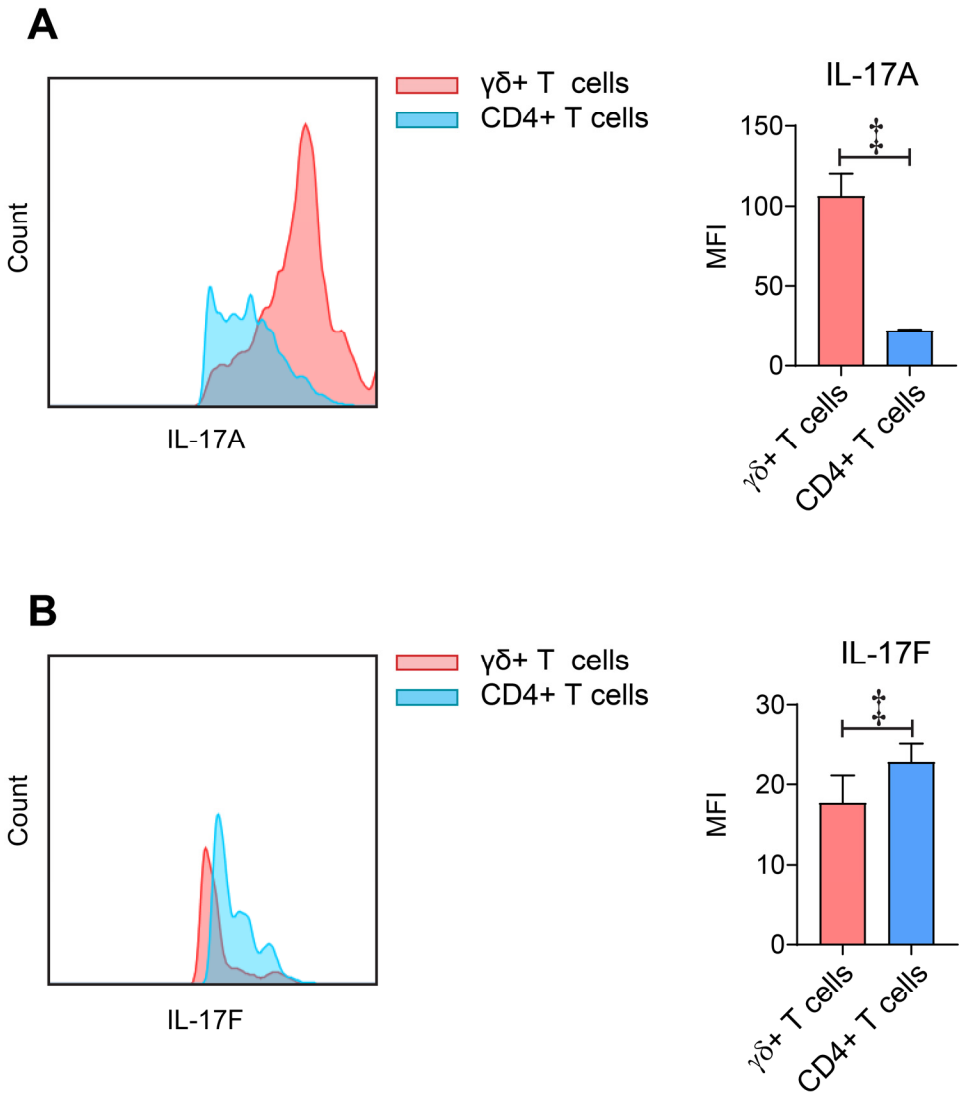

Figure S2. Differential expression levels of IL-17A and IL-17F in γδ T cells versus CD4$^+$ T cells. Skin draining lymph nodes were harvested from wt C57BL/6 mice (n=10 mice/group) on day 7 after *S. aureus* skin infection and flow cytometry was performed. (**A**) Mean fluorescence intensity (MFI) ± SEM of IL-17A-expressing γδ T cells and CD4$^+$ T cells. (**B**) MFI ± SEM of IL-17F-expressing γδ T cells and CD4$^+$ T cells. ‡P <0.001, as measured by a 2-tailed Student's t-test. The MFI of IL-17A-expressing γδ T cells was 5-fold higher than IL-17A-expressing CD4$^+$ T cells whereas the MFI of IL-17F-expressing CD4$^+$ T cells was slightly higher than IL-17F-expressing γδ T cells.

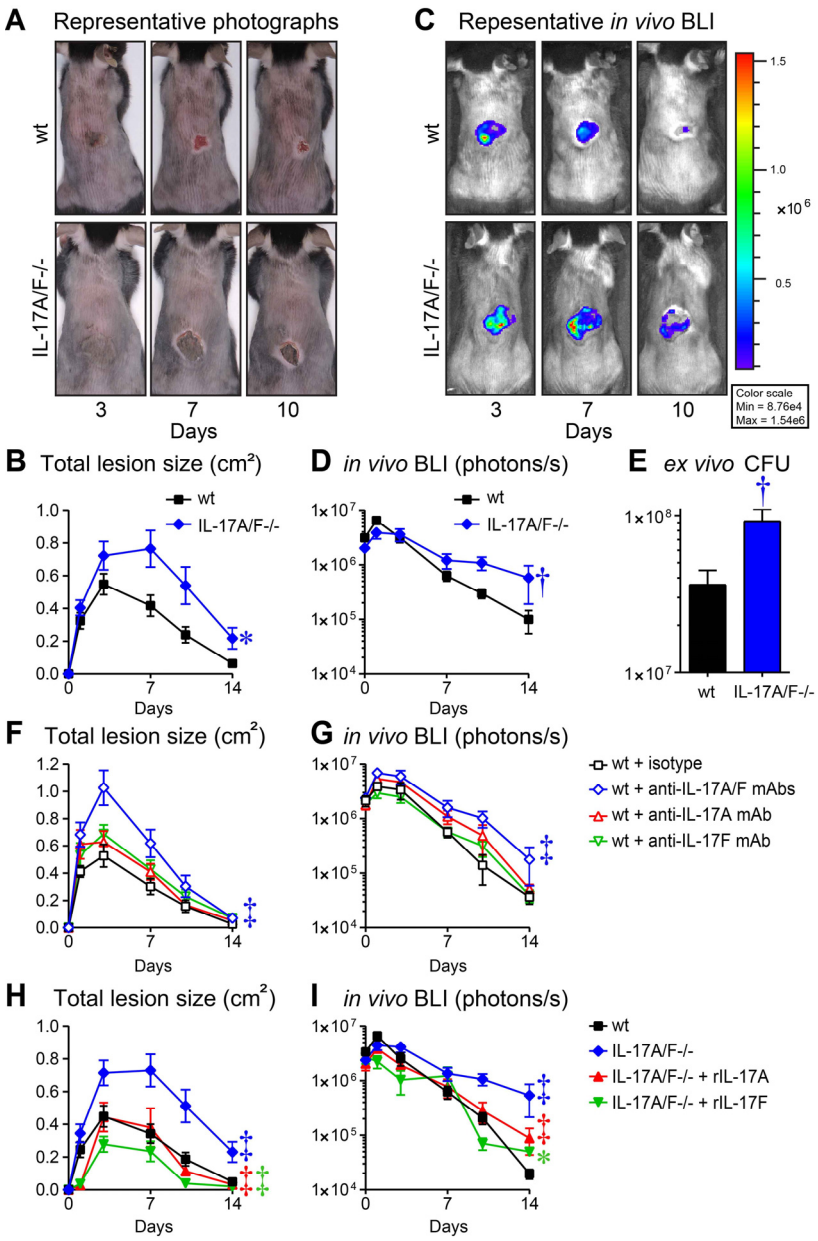

Figure S3. IL-17A/F-deficient mice have impaired host defense against a *S. aureus* skin infection. *S. aureus* skin infection was performed on IL-17A/F$^{-/-}$ and wt mice (n=5-10 mice/group). (**A**) Representative photographs of the skin lesions. (**B**) Mean total lesion size (cm^2) ± SEM. (**C**) Representative in vivo bioluminescence imaging (BLI) signals. (**D**) Mean in vivo bioluminescence imaging (BLI) signals (total flux [photons/s]) ± SEM. (**E**) Mean ex vivo CFU recovered on day 7 ± SEM. (**F,G**) Wt mice were treated systemically (i.p.) with anti-IL-17A, anti-IL-17F or a combination of anti-IL-17A and anti-IL-17F neutralizing mAbs and mean total lesion size (cm^2) ± SEM (**F**) and mean total flux (photons/s) ± SEM (**G**) were measured. (**H,I**) IL-17A/F$^{-/-}$ mice were treated with recombinant IL-17A (rIL-17A) or rIL-17F i.d. along with the bacterial inoculum and mean total lesion size (cm^2) ± SEM (**H**) and mean total flux (photons/s) ± SEM (**I**) were measured. *$P < 0.05$; †$P < 0.01$, ‡$P < 0.001$, as measured by 2-way ANOVA (**B–D**,**F–I**) or 2-tailed Student's t test (**E**). Data are a compilation of at least 2 independent experiments.

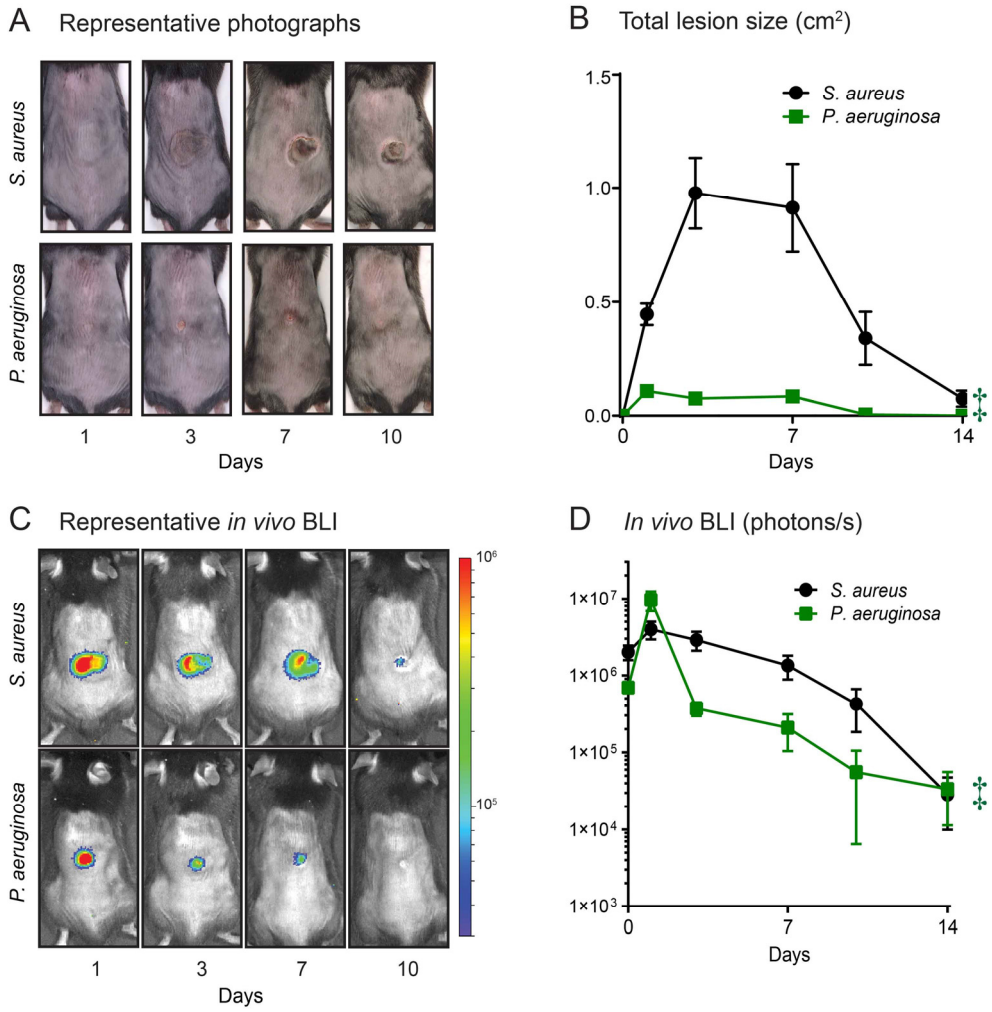

Figure S4. Mouse models of *S. aureus* skin infection and *P. aeruginosa* skin infection. *S. aureus* or *P. aeruginosa* skin infection was performed on wt mice (n=6 mice/group). (**A**) Representative photographs of the skin lesions. (**B**) Mean total lesion size (cm^2) ± SEM. (**C**) Representative in vivo bioluminescence imaging (BLI) signals. (**D**) Mean in vivo BLI signals (total flux [photons/s]) ± SEM. ‡$P < 0.001$, as measured by 2-way ANOVA (days 3-14).

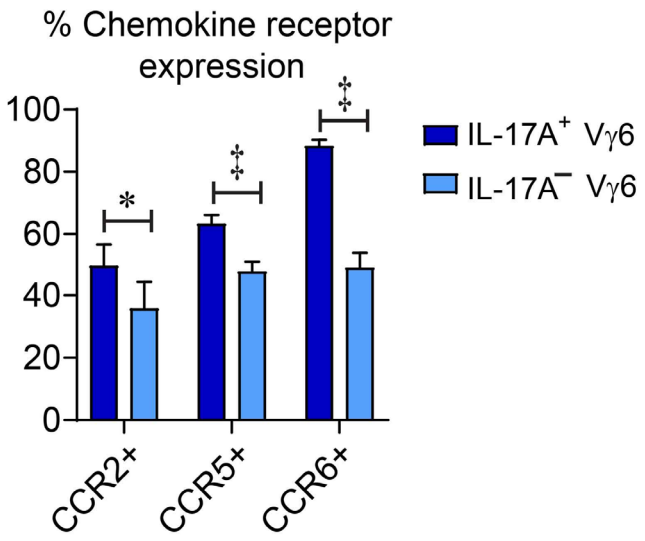

Figure S5. An increased percentage of Vγ6$^+$ IL-17A$^+$ T cells expressed chemokine receptors than Vγ6$^+$ IL-17A$^-$ T cells. Skin draining lymph node specimens were harvested from wt mice on day 28 after the *S. aureus* skin infection and flow cytometry was performed to evaluate the mean percentage ± SEM of Vγ6$^+$ IL-17A$^+$ T cells verses Vγ6$^+$ IL-17A$^-$ T cells expressing CCR2, CCR5 and CCR6 (n=5 mice/group). **P* <0.05, ‡*P*<0.001, as calculated by a 2-tailed Student's t-test.

Supporting Information, Tables S1 and S2

V name	3' V-REGION	N	5' J-REGION	J name
*TRGV6*01*	tgtgcatgctgggata		...gctcaggttttcacaaggtattt	*TRGJ1*01*
*TRGV5*01*	tgtgcctgctgggat..		..agctcaggttttcacaaggtattt	*TRGJ1*01*

Table S1. Analysis of *TRG* CDR3 V-J junction. *TRGV6* and *TRGV5* nucleotide (nt) sequences (underlining = nucleotide differences) of 3' V-region and 5' J-region from the top *TRGV6* and the *TRGV5* nt sequences in Figure 5A. Germline residues not depicted are shown as periods (*e.g.*, "…") (each individual period = a single unincorporated germline nt).

V name	3' V-REGION	N	P	D-REGION	P	5' J-REGION	J name	D name
*TRDV4*01*	tgtgggtcagatatc			…ggaggga …….	G	ctcctgggacacc cgacagatgtttttt	TRDJ2*01	TRDD2*01

Table S2. Analysis of *TRD* CDR3 V-D-J junction. *TRDV4* nucleotide (nt) sequence of 3' V-region and 5' J-region from the top *TRDV4* nt sequence in Figure 5A. Germline residues not depicted are shown as periods (*e.g.*, "…") (each individual period = a single unincorporated germline nt).

Supporting Information, Methods

Preparation of *S. aureus* for skin inoculation

S. aureus bioluminescent strain was streaked onto tryptic soy agar (TSA) plates (tryptic soy broth [TSB] plus 1.5% bacto agar [BD Biosciences) and grown overnight at 37°C. Single colonies were cultured in TSB at 37°C in a shaking incubator (240 rpm) for 18 hours, followed by a 2-hour subculture of a 1:50 dilution of the overnight culture. The bacteria were pelleted, resuspended in PBS, and washed 3 times. The absorbance (A_{600}) was measured to estimate the CFU for inoculation, which was verified after overnight culture on TSA plates.

Preparation of *P. aeruginosa* for skin inoculation

P. aeruginosa Xen41 was streaked onto plates containing Luria-Bertani (LB) broth plus agar (1.5%) (Becton Dickinson). Single colonies were cultured overnight in LB broth at 37°C with shaking at 240 rpm, followed by a 2.5-hour subculture at 1:50 dilution of the overnight culture to obtain mid-logarithmic phase bacteria. The bacteria were pelleted, resuspended in PBS, and washed 3 times. The absorbance (A_{600}) was measured to estimate the CFU for inoculation, which was verified after overnight culture on LB plus agar plates.

Validation of IL-17A/F dual color reporter mice

The reporter mouse strain was validated by evaluating $CD4^+$ T cells and γδ T cells isolated from skin-draining lymph node cells after *in vitro* Th17/IL-17 polarizing conditions. Briefly, $CD3^+$ T cells were isolated from naïve IL-17A-tdTomato/IL-17F-GFP dual color reporter mice skin-draining lymph nodes using Pan T cell isolation kit (Miltenyi Biotec). Naïve $CD3^+$ T cells (1×10^5) in 96 well plates were cultured in complete RPMI 1640 containing extra L-Glutamine and 2-mercaptoethanol ± CD3/CD28 Dynabeads (Gibco) and Th17/IL-17 polarizing conditions were performed with inclusion of murine IL-6 (mIL-6) (50 ng/mL, BioLegend), human TGF-β1 (1 ng/mL, BioLegend), mIL-23 (5 ng/mL, BioLegend), anti-mouse IL-4 (10 μg/mL, BioLegend), and anti-mouse IFN-γ (10 μg/mL, BioLegend). On day 3, cells were washed once and then restimulated in complete RPMI with PMA (20 ng/mL) and ionomycin (1 μg/mL) for 4 hours with brefeldin A and monensin (for anti-IL-17A and anti-IL-17F mAb labeling) or without brefeldin A and monensin (for tdTomato and GFP fluorescence quantification). After harvesting, flow cytometry demonstrated that tdTomato and GFP were expressed within Th17 cells and γδ T cells and the expression corresponded to the endogenous IL-17A and IL-17F protein levels, as determined using specific mAbs and conventional intracellular flow cytometry (Fig. S1).

Mouse model of *P. aeruginosa* skin infection and lesion size and in vivo BLI quantification

Mice were anesthetized (2% isoflurane) and the dorsal backs were shaved and injected i.d. with a sub-lethal inoculum of 5×10^5 CFU/100 µL PBS of P. aeruginosa strain Xen41 using a 29 gauge insulin syringe. Total lesion size (cm^2) and in vivo BLI were performed as described above for the *S. aureus* skin infection model.

Histology and immunofluorescence microscopy

10-mm skin punch biopsies were fixed in formalin (10%) and embedded in paraffin and 4 µm sections were cut and stained with hematoxylin and eosin (H&E) and Gram stain. For immunofluorescence labeling, paraffin sections were deparaffinized by heat-mediated antigen-retrieval in Trilogy buffer (Cell Marque), blocked for 1 hour at room temperature blocking buffer (PBS with 10% goat serum), and then incubated at 4° C overnight with 10 µg/mL rabbit anti-LTA antibody [AstraZeneca (77)], 5 µg/mL rabbit anti-RFP antibody (Abcam), or 10 µg/mL chicken anti-GFP antibody (Abcam) in blocking buffer. Sections were then incubated for 1 hour at room temperature with 2 µg/mL AlexaFluor-488 goat anti-rabbit IgG (ThermoFisher) or 2 µg/mL AlexaFluor-488 goat anti-chicken IgY (ThermoFisher) diluted in blocking buffer and subsequently mounted in Vectashield with DAPI (Vector Labs). Total lesion width (H&E stained sections) and bacterial band width (Gram stained sections) were measured using Image J (http://imagej.nih.gov/ij/).

Flow cytometry

Cells from skin or lymph node specimens were incubated with TruStain fcX (Biolegend) to

block Fc receptor binding, surface markers were labeled with mAbs against CD4 (GK1.5), TCRγδ (GL3), CD45 (30-F11), Ly6G (1A8), Ly6C (AL-21), CD11b (M1/70.15.11.5), CD3 (145-2C11), F4/80 (RE126) (all Miltenyi Biotec) as well as CCR2 (SA203G11), CCR5 (REA354), CCR6 (29-2L17) (all BioLegend) and cell viability was assessed with propidium iodide (Miltenyi Biotec). For intracellular flow cytometry of cells from wt C57BL/6 mice, 1×10^6 cells per well were incubated with RPMI supplemented with 10% FBS, 2 mM glutamine, 100 U/mL penicillin, 100 µg/mL streptomycin, and cell stimulation cocktail plus protein transport inhibitors (eBioscience), containing PMA, ionomycin, brefeldin A and monensin. Cells were harvested, washed and stained with Viability Fixable Dye (Miltenyi Biotec) and labeled with surface markers and anti-IL-17A (TC11-18H10, BD Biosciences), anti-IL-17F (REA666, Miltenyi Biotec), anti-IL-22 (IL22JOP) (eBioscience), anti-TNF (REA636) or anti-IFNγ (AN.18.17.24) (both Miltenyi Biotec). To identify other cellular sources of IL-17A and IL-17F, live cells were first labeled with anti-CD45 (30-F11, Miltenyi Biotec) mAb, then $CD8^+$ T cells were labeled with anti-CD8α (53-6.7, Miltenyi Biotec) mAb, ILC3s were labeled with anti-CD127 (A7R34, Miltenyi Biotec) mAb, anti-RORγt (B2D, eBiosciences) mAb, and hematopoietic lineage mAb cocktail (eBiosciences), NK cells were labeled with anti-NK1.1 (PK136, BioLegend) mAb, and myeloid cells were labeled with an anti-CD11b mAb (M1/70.15.11.5, Miltenyi Biotec). Then intracellular IL-17A and IL-17F were labeled. To identify the $Vγ6^+$ cells, live cells were first labeled with PE-Cy7 anti-CD3 mAb and PerCP anti-TCRγδ mAb followed by labeling for $Vγ5^+$ cells using FITC anti-Vγ5 mAb (F536) and other γδ T cell subsets including $Vγ1^+$, $Vγ2^+$ cells with PE anti-Vγ1.1/1.2 mAb (4B2.9, BioLegend), $Vγ4^+$ cells with Biotin anti-Vγ4 mAb (UC3), and $Vγ7^+$ cells with Biotin anti-Vγ7 mAb (GL1.7). Biotinylated Vγ4 and Vγ7 antibodies were then labeled with PE anti-Streptavidin (3A20.2,

BioLegend). In this approach, Vγ5$^+$ cells were labeled with FITC, Vγ1$^+$, Vγ2$^+$, Vγ4$^+$ and Vγ7$^+$ cells were labeled with PE, and Vγ6$^+$ cells were the unlabeled negative γδ T cell population (Fig. 6A). Cell acquisition was performed on a MACSQuant flow cytometer (Miltenyi Biotec) and data were analyzed using MACSQuantify software (Miltenyi Biotec) and FlowJo software (Treestar).

RNA Extraction and mRNA Quantification for Gene Expression Arrays

Total RNA from homogenized (Pro200 Series homogenizer [Pro Scientific]) 10-mm skin punch biopsies was extracted by using TRIzol reagent (Invitrogen) and Direct-zol™ RNA kit (Zymo Research), according to the manufacturer's recommendations. cDNA was generated utilizing a High-Capacity cDNA Reverse Transcription Kit with RNase Inhibitor (ThermoFisher). Quantitative real-time PCR (Q-PCR) was performed using a pre-formatted TaqMan mouse immune gene expression array, as well as a custom TaqMan mouse host defense peptide gene expression array (Applied Biosystems). Gene expression was normalized by the house keeping genes GAPDH, Gusb and Hprt and the ΔΔCT method was utilized by software to calculate the relative expression (BioRad).

Analysis of public RNA-seq datasets

RNA-seq FASTQ files of mouse lymph nodes on days 0 and 28 following a *S. aureus* skin infection (SRA SRP126124) and on days 0 and 7 from *S. aureus*-infected mouse skin (SRA SRP040121) were downloaded from the NCBI SRA database, and information about the samples was provided in these respective publications (25, 52). Sequencing reads were mapped to the UCSC mm10 mouse reference genome by STAR. Gene expression level normalization using

DESeq2 Bioconductor R package. MiXCR software was used to extract TCR CDR3 sequences from RNA-seq data. Analysis were performed with "-p rna-seq" option recommended for analysis RNA-seq data. The IMGT/Junction Analysis tool was used to analyze in detail the CDR3 V-D-J and V-J junctions.

CDR3 definition

The TCR CDR3 region was defined as the a.a. residues starting with the C at position 104 and ending with the F at position 118 based on IMGT nomenclature (http://www.imgt.org/) and numbering system. Likewise, gene names of V and J regions are designated according to IMGT nomenclature for T cell receptors of mice. Data visualization and TCR repertoire comparison were done in R (version 3.1.2).

RNA isolation for RNA-sequencing

On day 28, mouse lymph nodes from naïve mice, *S. aureus*-infected mice and *P. aeruginosa*-infected mice were stabilized by addition of RNAlater (Ambion). Homogenization, total RNA extraction, RNA quantification and RNA integrity was accessed as previously described (25). Samples with RIN (RNA integrity number) ≥ 8 were used for this study.

RNA-Sequencing and analysis

Construction of indexed libraries and assessment of quantity and quality of the libraries (average size 400 bp) was performed as previously described (25). Libraries molar concentration were validated by qPCR for library pooling. RNA-seq was performed on the Illumina HiSeq 4000 platform using PE150 chemistry (Illumina). RNA-seq data were deposited in the NCBI Sequence

Read Archive (SRA) (accession number: SRP194263) (https://www.ncbi.nlm.nih.gov/sra?term=SRP194263) (86). RNA-seq analysis was performed for the prior RNA-seq datasets available from the NCBI SRA database, as described above.

Amplification of TCR complementarity-determining region 3 (CDR3)

For targeted CDR3 sequencing, we synthesized cDNA with anchor sequence incorporation, according to our previously described methods (25).

TCR library preparation

All libraries from purified TCR amplicons were prepared using the Nextera XT Index kit (Illumina Inc.) according to the manufacturer's instructions for the "16S Metagenomic Sequencing Library Preparation" protocol with minor modifications, as we previously described (25). The quality of the libraries were also assessed by Agilent 2100 Bioanalyzer and the average library size was 600-700 bp.

TCR library sequencing and analysis. Libraries were pooled to a final pool concentration of 4 nM including a 10% PhiX Control v3 (Illumina) spike-in. Sequencing was performed on an Illumina MiSeq® sequencer using the 600-cycle MiSeq Reagent Kit v3 (Illumina) with paired-end reads. Raw sequencing reads were processed for FASTQ conversion and demultiplexing using MiSeq Reporter. MiXCR software were used to extract TCR CDR3 sequences from sequencing data. All gene names used are according to IMGT nomenclature and data analyses was performed as described in the analysis of public RNA-seq datasets, above.