

Figure S1 (refers to Fig 2). Higher frequency of activated $\gamma\delta$ T cells in the cervical lymph nodes of NIH mice compared to commercial vendors. Single-cell suspensions of cervical lymph node cells were stained for the $\gamma\delta$ TCR and activation markers, CD44 and CD62L. Each symbol represents an individual mouse, from 2 combined experiments. Significance was determined by ANOVA.

Corynebacterium mastitidis 16S ribosomal RNA gene, partial sequence

Sequence ID: [gb|AY834747.1](https://genbank.ncbi.nlm.nih.gov/GenBank/seq/aj01/aj010101.gb) Length: 1451 Number of Matches: 1

Range 1: 407 to 1400 [GenBank](#) [Graphics](#) ▼ Next Match ▲ Previous Match

Score	Expect	Identities	Gaps	Strand
1797 bits(973)	0.0	985/994(99%)	0/994(0%)	Plus/Minus
Query 50	TGGTTTNCGGGTGTTAGCAACTTTC	TTC	TGACGTGACGGGCGGTG	TGTACAAGGCCCGGAA 109
Sbjct 1400	TGGTTTTCGGGTGTTACCAACTTTC	CAT	GACGTGACGGGCGGTG	TGTACAAGGCCCGGAA 1341
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Sbjct 1340	CGTATTCACCGCAGCGTTGCTGATC	TGCGATTACTAGCGACTCCGACTTC	CATGGGGTTCGA 1281	
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Sbjct 1280	GTTGCAGACCCCAATCCGAAC	TAAGCCGACTTTACAAGGATTAGCTCC	ACCTCACGGTA 1221	
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Query 470	CGCATCTCTGCGCCGATCTGGTGT	ATGTCAAGCCAGGTAAGGTTCTTCGCG	TTCATCG 529	
Sbjct 980	CGCATCTCTGCGCCGATCTGGTGT	ATGTCAAGCCAGGTAAGGTTCTTCGCG	TTCATCG 921	
Query 530	AATTAATCCACATGCTCCGCGCTT	TGTGCGGGCCCCGTC AATTCCTTTG	AGTTT TAGCC 589	
Sbjct 920	AATTAATCCACATGCTCCGCGCTT	TGTGCGGGCCCCGTC AATTCCTTTG	AGTTT TAGCC 861	
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Sbjct 800	AAGAGGCTCACACCTAGCGCC	ACCCTTACAGCATGGACTACAGGGT	ATCTAATCCTG 741	
Query 710	TTGCGTACCCATGCTTTCGCTCCT	CAGCGTCAGTAACTGCCAGTAACTGC	CTTCGCCA 769	
Sbjct 740	TTGCGTACCCATGCTTTCGCTCCT	CAGCGTCAGTAACTGCCAGTAACTGC	CTTCGCCA 681	
Query 770	TCGGTGTTCCTCCTGATATCTG	CGCATTCCACCGCTACACCAGGAAT	TCCAGTTACCCT 829	
Sbjct 680	TCGGTGTTCCTCCTGATATCTG	CGCATTCCACCGCTACACCAGGAAT	TCCAGTTACCCT 621	
Query 830	ACAGCACTCAAGTTATGCCCGT	ATCGCCTGCACGCCGGAGTTAAGCC	CCGGAATTTAC 889	
Sbjct 620	ACAGCACTCAAGTTATGCCCGT	ATCGCCTGCACGCCGGAGTTAAGCC	CCGGAATTTAC 561	
Query 890	AGACGACGCGACAAACCACCT	TACGAGCTCTTACGCCAGTAAATCC	GACAAACGCTCGC 949	
Sbjct 560	AGACGACGCGACAAACCACCT	TACGAGCTCTTACGCCAGTAAATCC	GACAAACGCTCGC 501	
Query 950	ACCTTACGTATTACCGCGGCTG	CTGGCAGTAGTTAGCANNNGCTTCT	NCTCCACCTACC 1009	
Sbjct 500	ACCTTACGTATTACCGCGGCTG	CTGGCAGTAGTTAGCANNNGCTTCT	NCTCCACCTACC 441	
Query 1010	GTCACCCCAAAGGGCTTCGTCGG	TAGCGAAAGGA 1043		
Sbjct 440	GTCACCCCAAAGGGCTTCGTCGG	TAGCGAAAGGA 407		

Figure S2 (refers to Fig 2). *C. mastitidis* from mouse conjunctiva closely aligns with *C. mastitidis* isolated from human conjunctiva. *C. mastitidis* was isolated and expanded from C57BL/6 conjunctiva. It was then grown to a pure population and Genewiz (LLC) provided the sequence. The sequence is representative of at least 10 isolated colonies from pure populations.

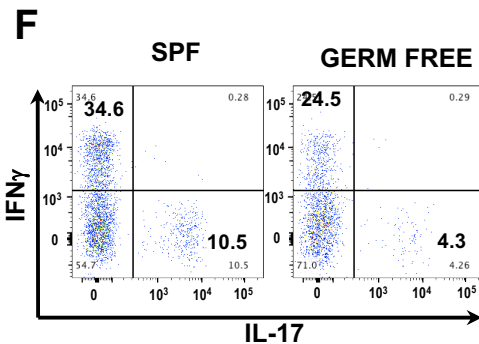
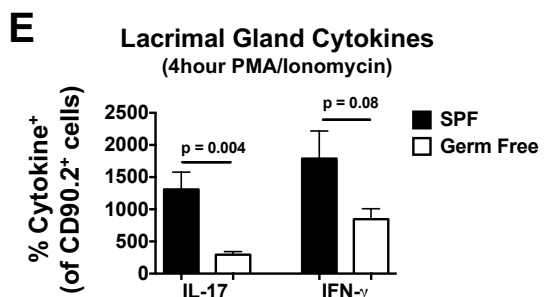
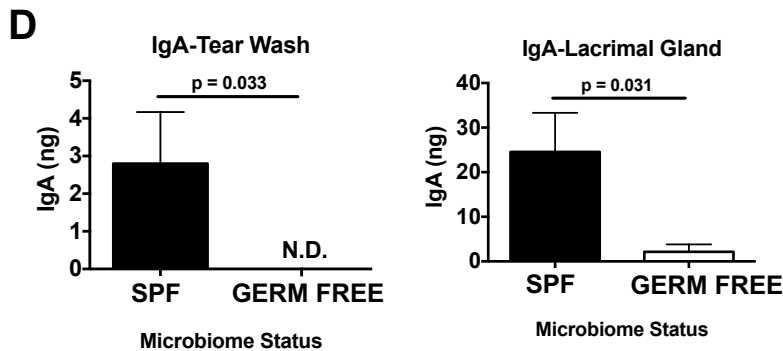
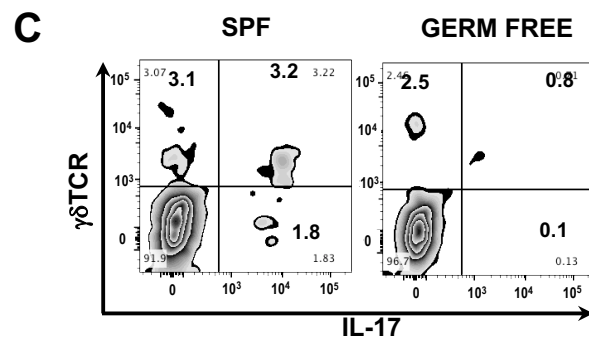
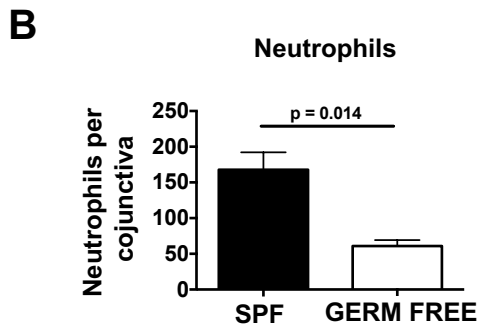
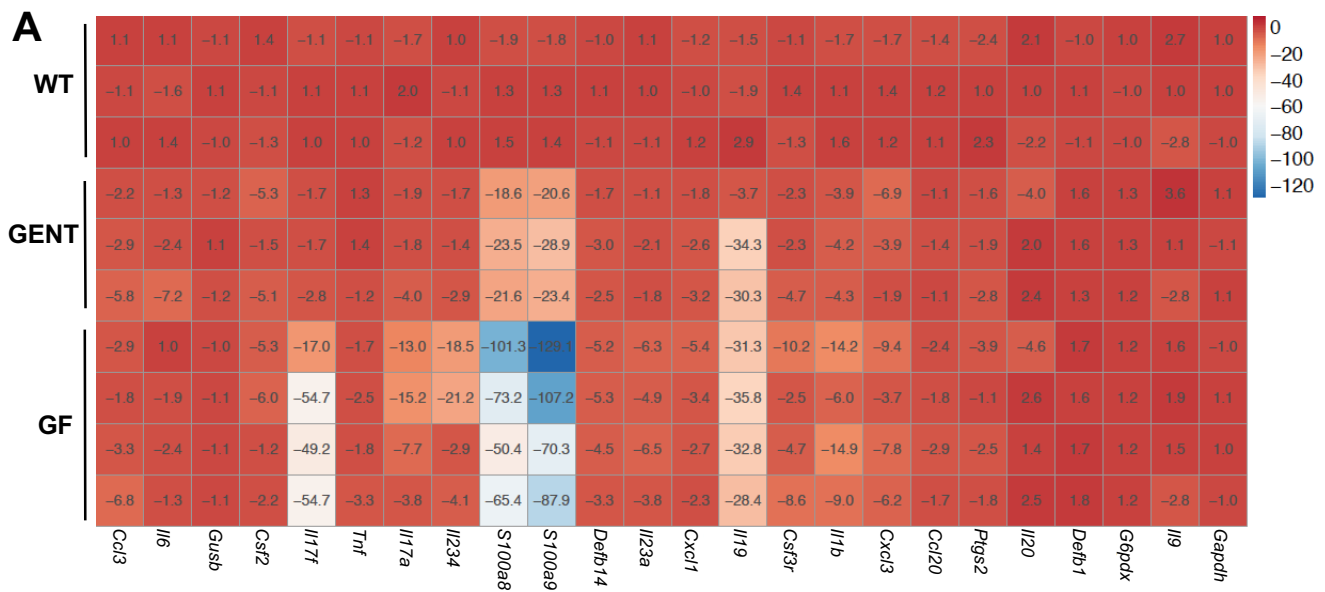


Figure S3 (refers to Fig 3). Germ free mice have a poorly developed ocular immune response. (A) Nanostring assessment of genes known to be regulated by IL-17 signaling. Each row represents bulk mRNA from a single mouse from two experiments. **(B-F)** Conjunctival tissue from WT or Germ Free (GF) mice was harvested, treated with collagenase, dispersed into single-cell suspensions and **(B)** assessed for neutrophil numbers directly *ex vivo* or **(C)** stimulated with PMA and ionomycin in the presence of brefeldin A for 4 hours. After stimulation intracellular IL-17A was assessed by flow cytometry. **(D)** Ocular surfaces were washed with 10 μ l of PBS or lacrimal glands were homogenized in 500 μ l of PBS and IgA concentration was assessed using ELISA. **(E & F)** Lacrimal gland cells were stimulated with PMA and ionomycin in the presence of brefeldin A for 4 hours and IL-17 and IFN γ was assessed using flow cytometry. Bars represent the mean **(D)** concentration of IgA or **(E)** number of cytokine producing cells \pm SEM from a pool of two experiments (n=6).

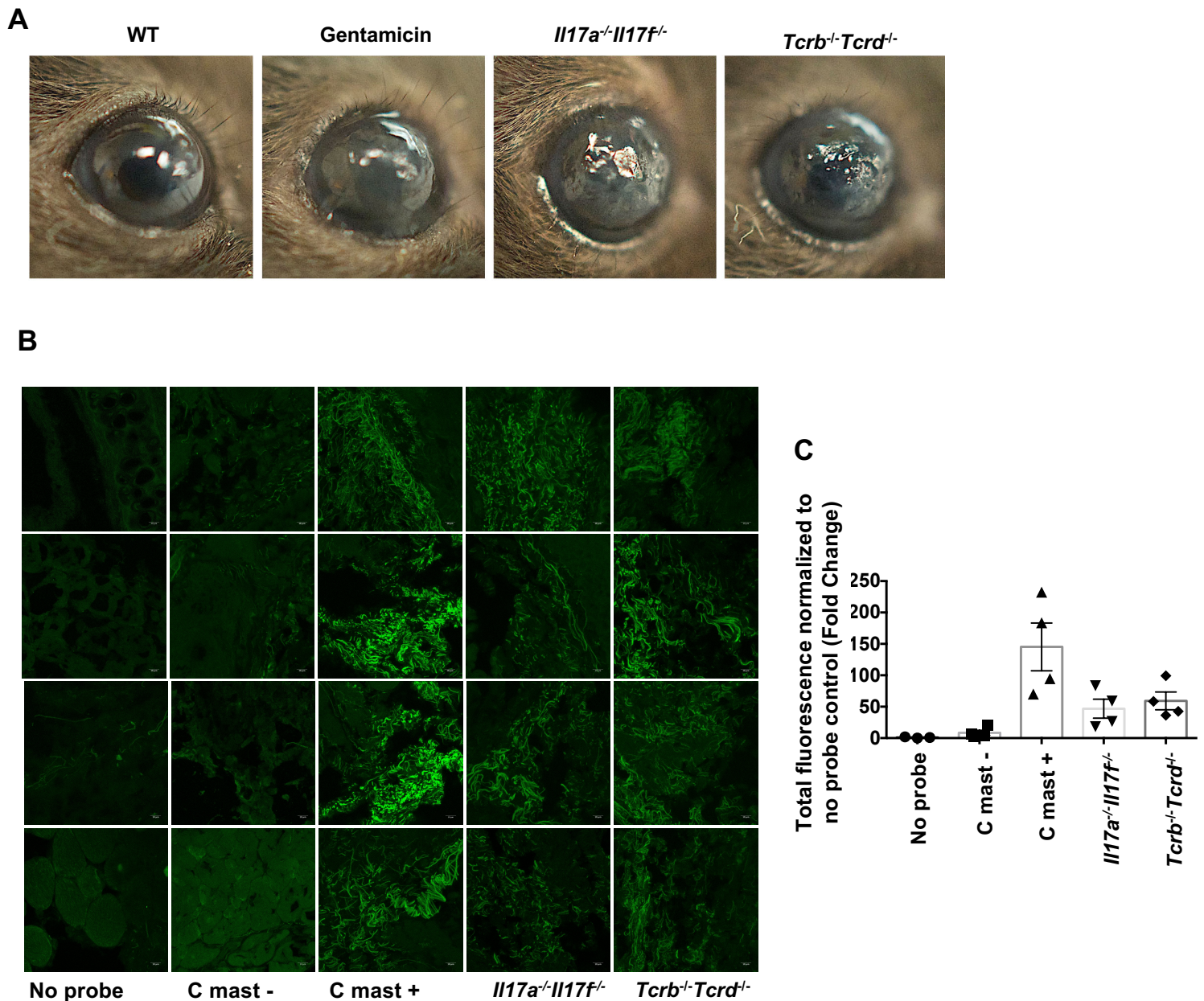


Figure S4 (refers to Figure 4). Immunodeficient mice are more susceptible to ocular infection with *C. albicans*. (A) WT mice were topically treated with PBS or gentamicin ophthalmic gel daily for 6 days. After 6 days, WT groups, *Il17a^{-/-}Il17f^{-/-}*, and *Tcrb^{-/-}Tcrd^{-/-}* mice were ocularly infected with 5×10^5 CFU of *Candida albicans*. Briefly, mice were anesthetized and the ocular surface was gently dabbed with gauze. *C. albicans* (strain SC5314) was then applied in 5 μ l of PBS and remained on the surface for 30 minutes until mice awoke. Fifteen hours after infection, mice were sacrificed. Images represent ocular pathology at the end point. (B & C) Frozen sections of whole eyes (with eyelids) were stained with fluorescent probes against *Corynebacterium spp.* Data are from 4 individual mice. Background from images in (B) was subtracted and despeckled using FIJI software. Images were converted to binary mode to allow region of interest generation and filament detection in the max image projection. Total fluorescence was then normalized to the 'no probe' control in (C). Each sample represents an individual mouse.

A

JAX mice (no *C. mast*)
 JAX mice + *C. mast*

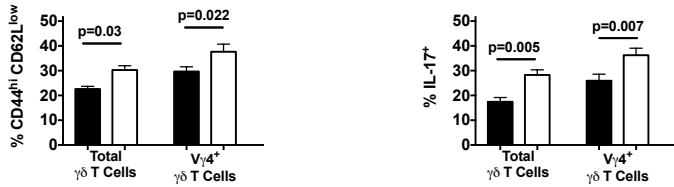
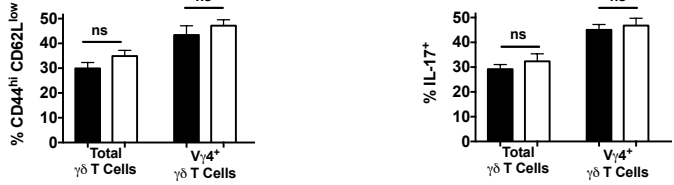
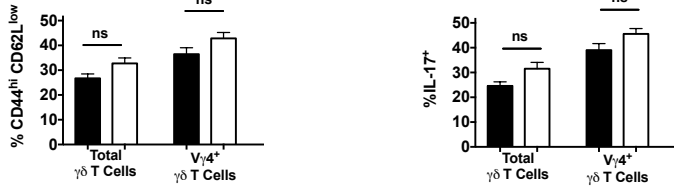
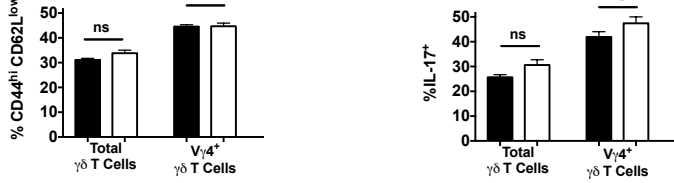
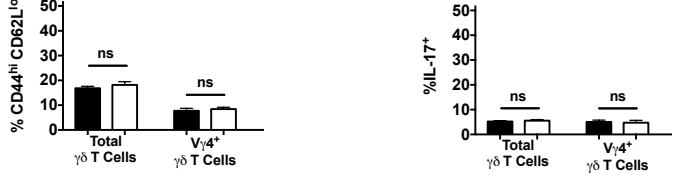
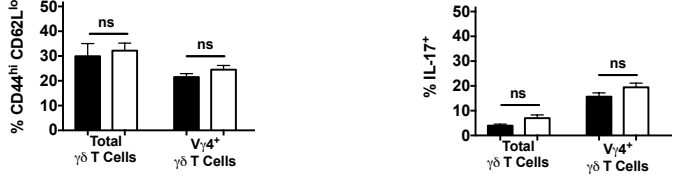
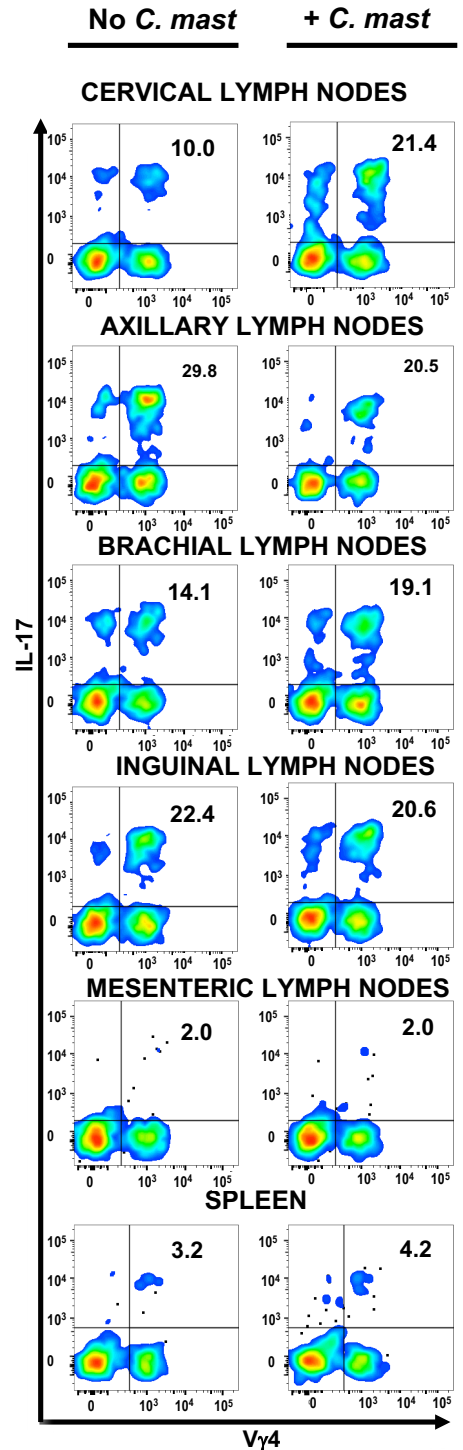
CERVICAL LYMPH NODES**AXILLARY LYMPH NODES****BRACHIAL LYMPH NODES****INGUINAL LYMPH NODES****MESENTERIC LYMPH NODES****SPLEEN****B**

Figure S5 (refers to Fig 5). Colonization with *C. mast* induces immunity only in local draining lymph nodes. Mice from JAX Laboratories were given PBS or were inoculated with 1×10^8 CFU of *C. mast* once every three days, totaling three inoculations. After 3 weeks, cells from noted lymph nodes or spleen were stained for $\gamma\delta$ TCR and activation markers (CD44 and CD62L) or were stimulated for 4 hours with PMA and ionomycin in the presence of brefeldin A and were stained for $\gamma\delta$ TCRs and intracellular IL-17. (A) Bars represent the mean (left) CD44^{hi}CD62L^{low} % or (right) IL-17% of $\gamma\delta$ T cells \pm SEM. (B) Flow plots represent IL-17 production in V γ 4⁺ $\gamma\delta$ T cells after stimulation. Data are pooled from two independent experiments ($n = 6$, JAX & 8 JAX + *C. mast*). Statistical significance was determined using ANOVA.

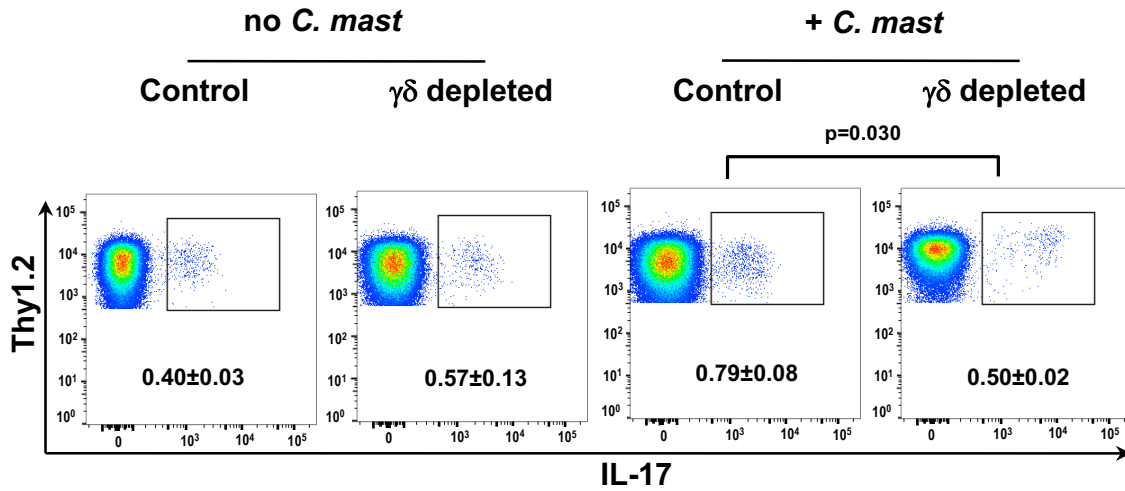


Figure S6 (refers to Fig 7). $\gamma\delta$ TCR (UC7-13D5) depletion antibody reduces IL-17 in the draining lymph nodes. Mice from JAX Laboratories were given PBS or were inoculated with 1×10^8 CFU of *C. mast.* once every three days, totaling three inoculations. After 3 weeks, mice were depleted of $\gamma\delta$ T cells using a 500 μg i.p. injection (UC7-13D5). After 4 days, mice were sacrificed and single-cell suspension of eye-draining lymph node cells were stimulated with PMA and ionomycin in the presence of brefeldin A for 4 hours. After stimulation, cells were stained for intracellular IL-17. The CD90.2⁺ population is depicted and flow plots are representative of two experiments (n=3 per group). Numbers represent the mean frequency of IL-17⁺ cells \pm SEM.