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Supplemental Information

Cutaneous Immune Cell-Microbiota Interactions

Are Controlled by Epidermal JunB/AP-1

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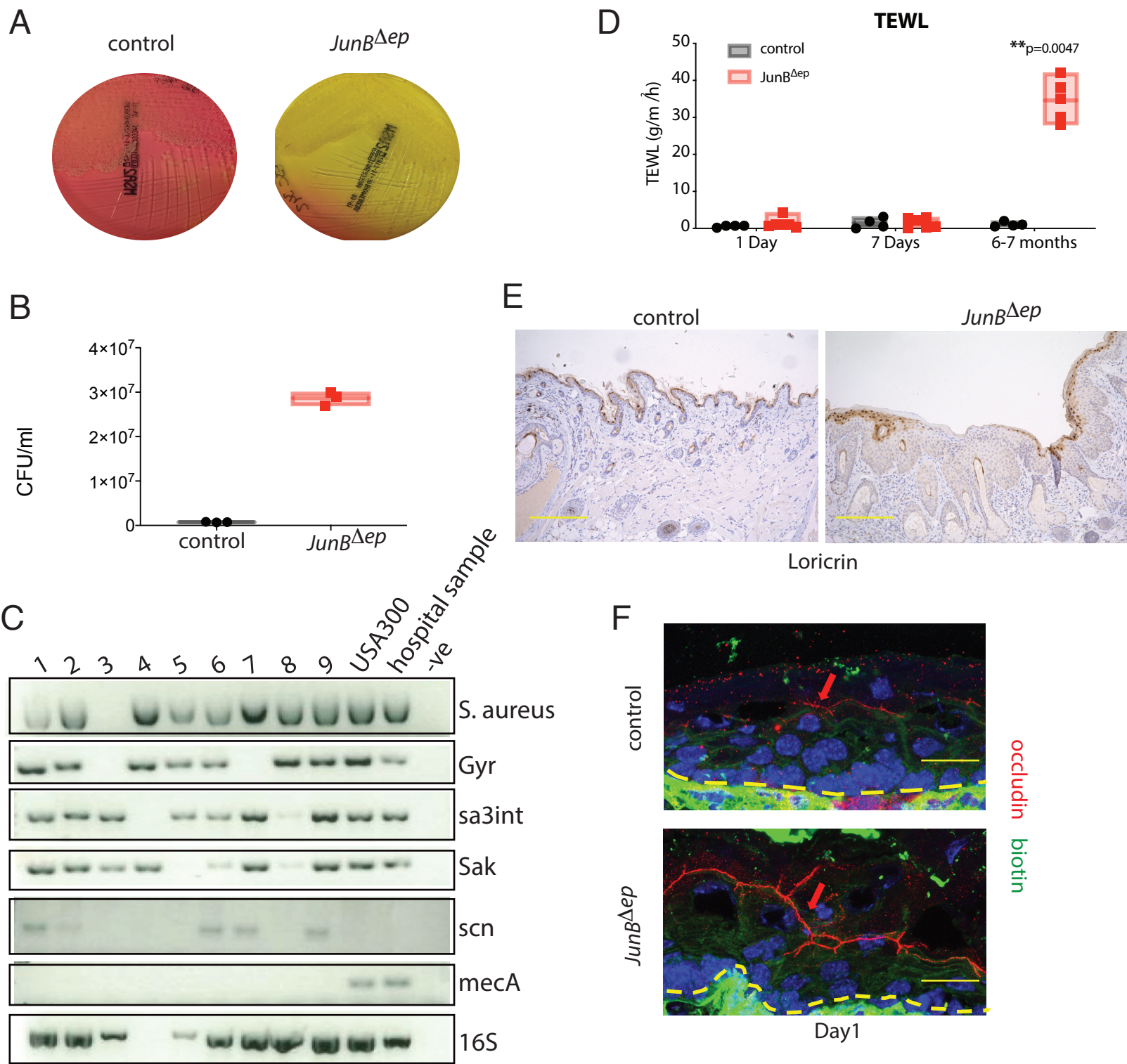


Figure S1. *JunB Δ ep* mice have human-derived *S. aureus* colonization and transient barrier defects. Related to Figure 1. A) Growth of bacteria on mannitol-salt agar plates isolated from control vs *JunB Δ ep* skin (n>10 per genotype). B) CFUs/ml of bacteria isolated control versus *JunB Δ ep* skin. C) PCR for *S. aureus*, *S. aureus* specific gyrase (*gyr*), human-specific immune invasion gene cluster including Staphylokinase (*sak*) and staphylococcal complement inhibitor (*scn*), a human-specific prophage integrase-gene *sa3int* and methicillin-resistance gene *mecA* with 16S as control from single colonies of bacteria grown on blood agar plates from *JunB Δ ep* skin. D) Trans-epidermal water loss measurements 1 and 7 days post-birth of control and *JunB Δ ep* mice, as well as 6-7 months old control and *JunB Δ ep* mice (n=4,6) (lesional skin). E) Loricrin staining at 6-7 months of age of the lesional skin of control and *JunB Δ ep* mice (n=5,6). Yellow scale bars indicate 100 μ m. F) Inside-out barrier assay using biotin diffusion in control and *JunB Δ ep* mice at 1-day post birth (n=4,5). Biotin-streptavidin is shown in green and occludin IF is shown in red. Dashed yellow line indicates the basement membrane separating epidermis-dermis. Red arrows show where the green biotin staining ends. Yellow scale bars indicate 50 μ m.

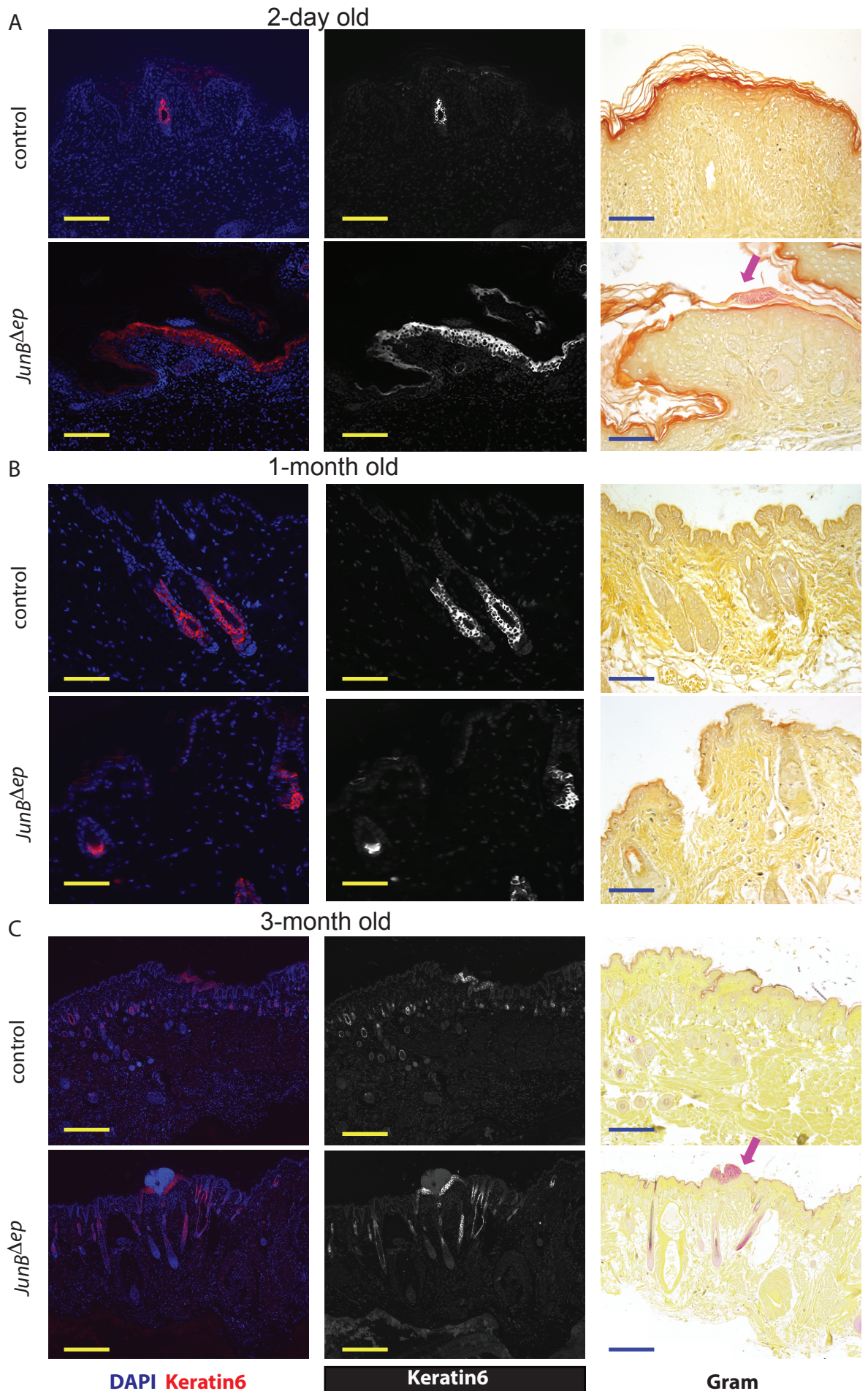


Figure S2. *JunB^{Δep}* mice do not have *S. aureus* colonization early in life. Related to Figure 1. Keratin6 and Gram staining in skin from control and *JunB^{Δep}* mice at 2 days (n=4,5), 1 month (n=4,4) and 3 months (n=7,8) post-birth. The yellow scale bars indicate 200μm in A, 100μm B and 500μm in C. The blue scale bars indicate 100μm in A, B and 500μm in C.

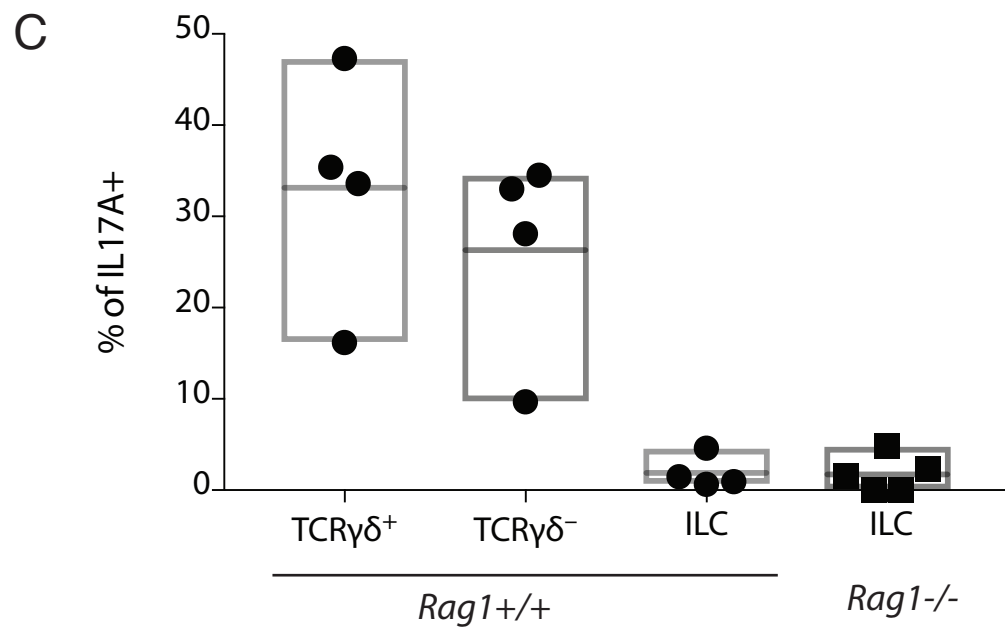
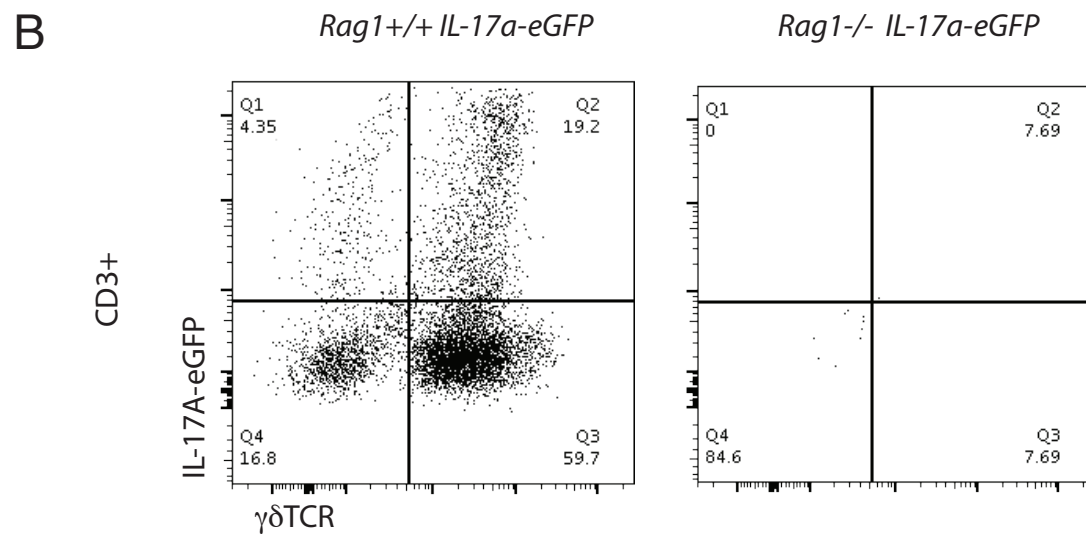
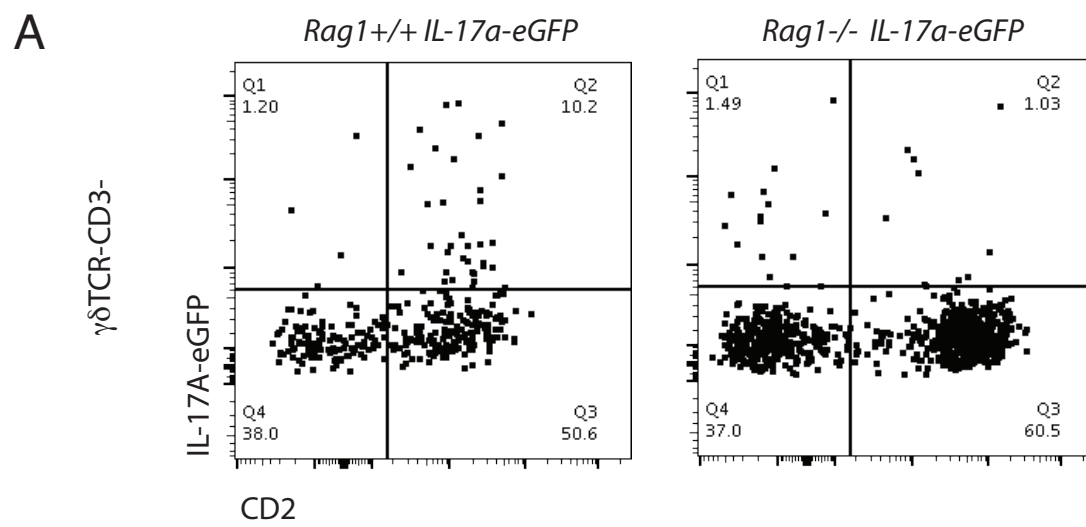


Figure S3. ILC3s do not compensate for IL-17A production by T-cells in *Rag1*^{-/-} mice upon IMQ administration. Related to Figure 4.

A) Representative flow cytometry images of IL-17A expressing ILCs of control and *Rag1*^{-/-} mice upon 5-days of IMQ administration on the ears (n=4,5). B) Representative flow cytometry images of IL-17A expressing T-cells of control and *Rag1*^{-/-} mice 5-days of IMQ administration on the ears. C) Quantification of the distribution of IL-17A⁺ cells in *Rag1*^{+/+} compared to *Rag1*^{-/-} mice.

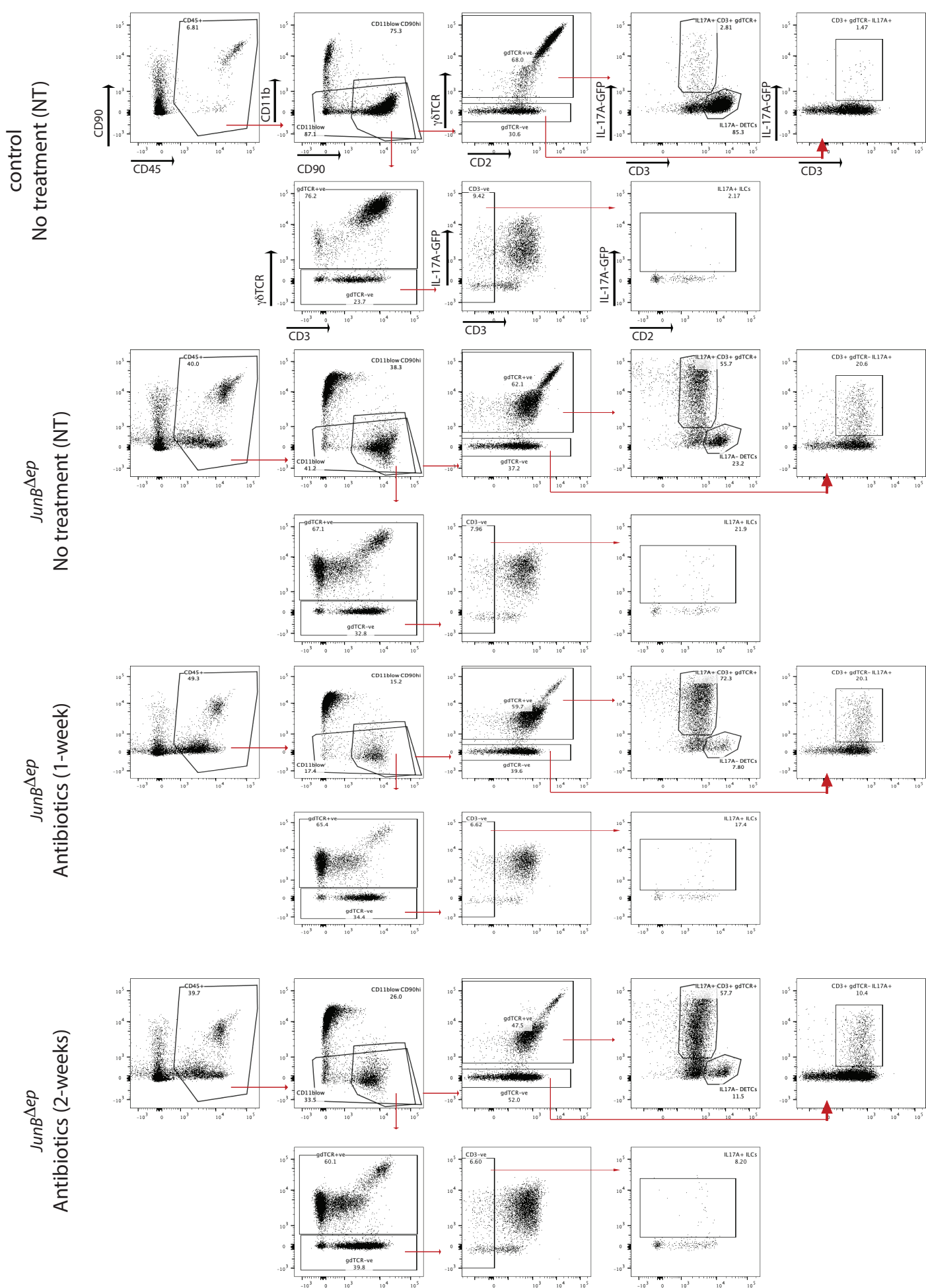


Figure S4. Antibiotic treatment post development of skin inflammation does not provide therapeutic benefit. Related to Figure 6. Conventional flow plots (including gating strategy) of lymphoid cells in *JunB Δ ep* mice with or without 1 and 2-weeks of antibiotics compared to controls.

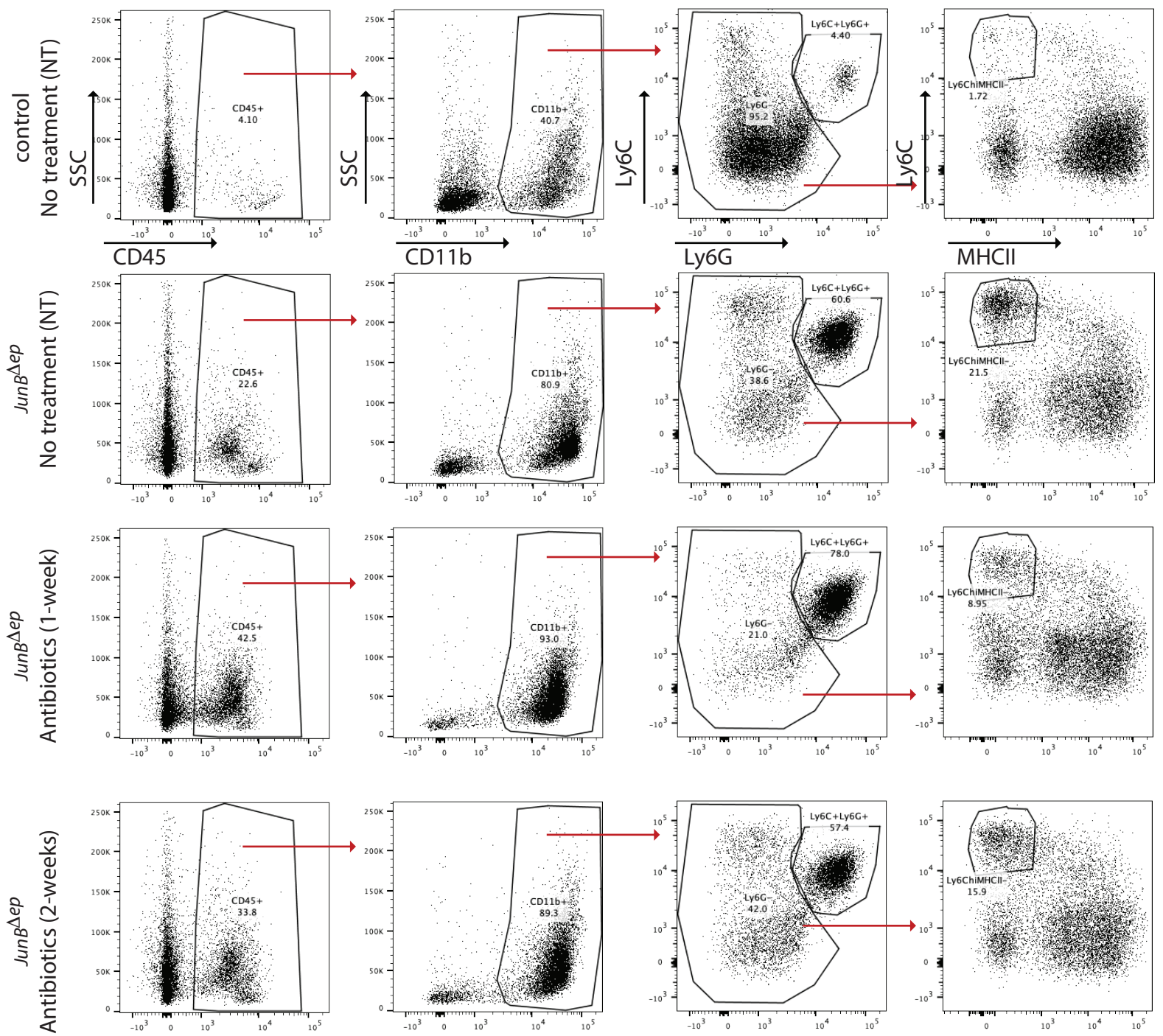


Figure S5. Antibiotic treatment post development of skin inflammation does not provide therapeutic benefit. Related to Figure 6. Conventional flow plots (including gating strategy) of myeloid cells in *JunB Δ ep* mice with or without 1 and 2-weeks of antibiotics compared to controls.

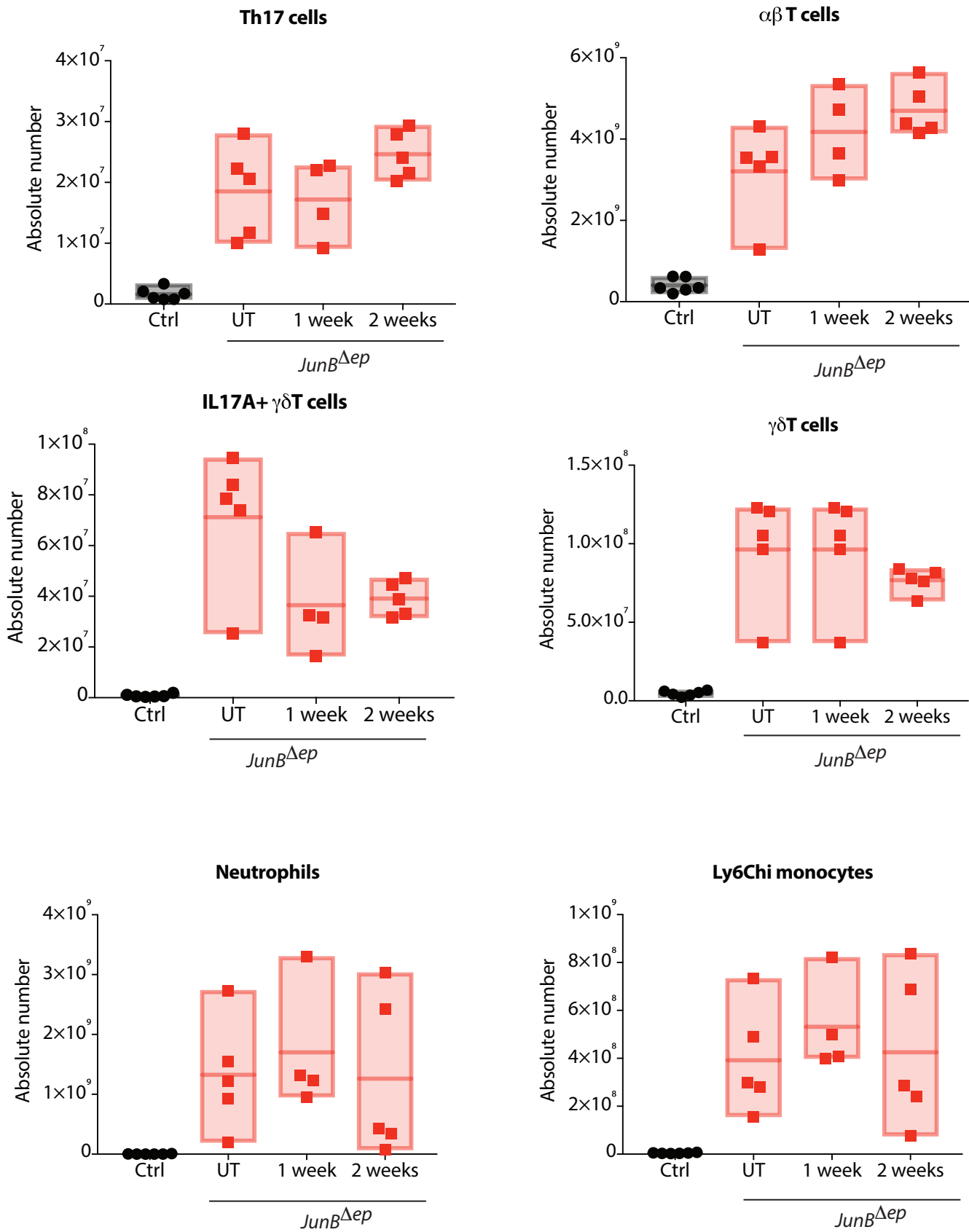


Figure S6. Increased number of $\gamma\delta$ T cells in lymph nodes upon 2-weeks of antibiotic treatment. Related to Figure 6. Number of infiltrating immune cells into the skin-draining lymph nodes of $JunB^{\Delta ep}$ mice with or without 1 and 2-weeks of antibiotics compared to controls as analysed by flow cytometry.

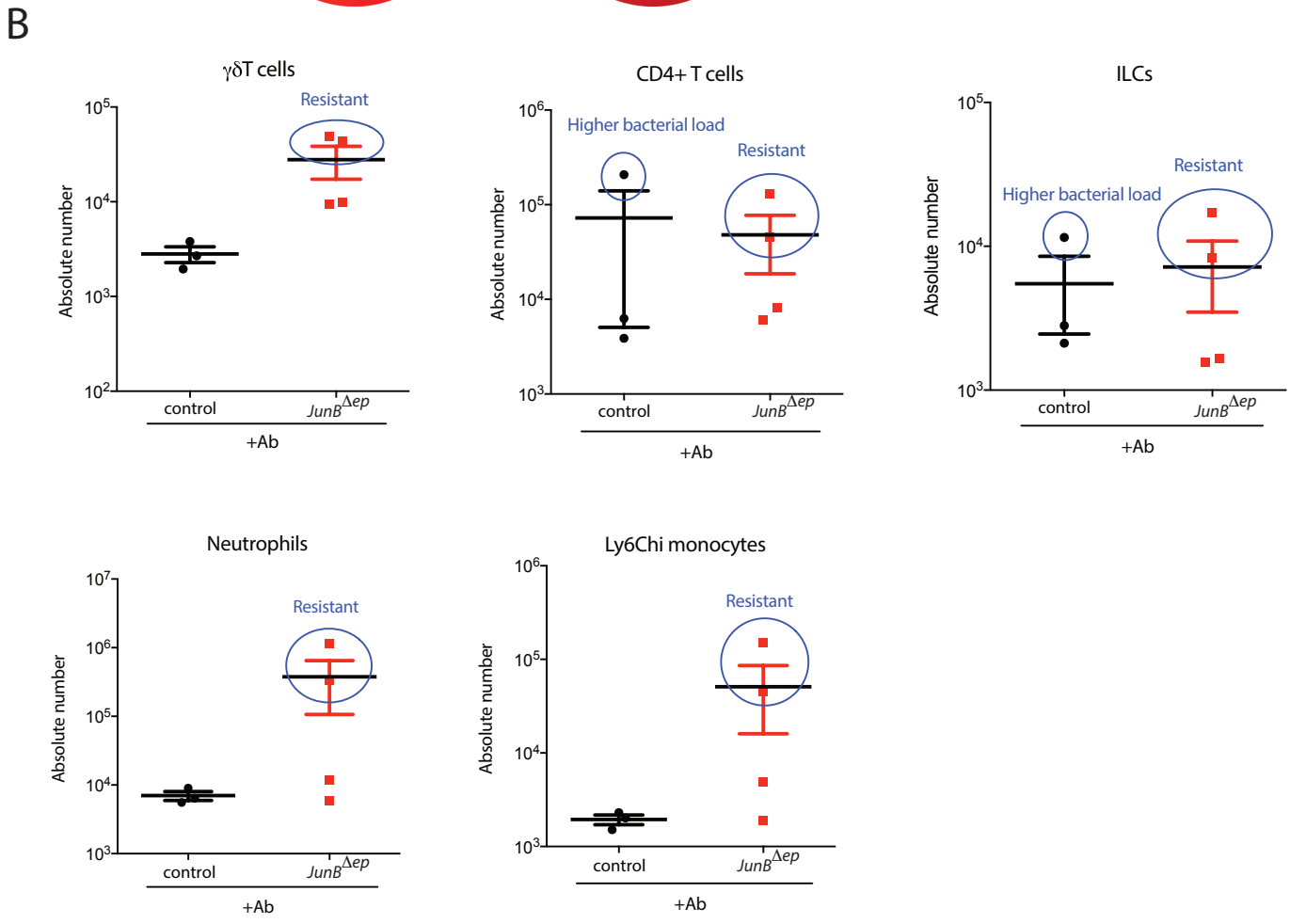
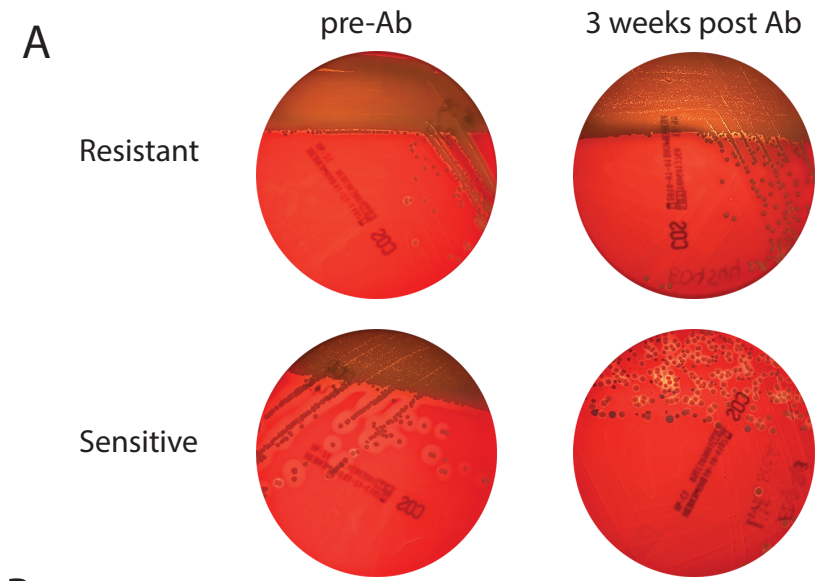


Figure S7. Characterization of the immune cells in mice resistant to antibiotics. Related to Figure 6.
 A) Representative images of blood agar plates of swabs derived from $JunB^{\Delta ep}$ mice skin sensitive or resistant to 3-weeks of antibiotic treatment post-lesion development.
 B) Number of immune cell infiltrates into the skin of $JunB^{\Delta ep}$ mice skin sensitive or resistant to 3-weeks of antibiotic treatment post-lesion development

Name	F (5'-3')	R (5'-3')
Duox1	GATTCTCCTGGTTGGGACA	CCTGCAGAACCTCTCATGA
Il13ra2	AGCGAATGGAGTGAAGAGGA	GCTCAATGTGGGTTTCAGGTT
Stac2	CAAGCTCCAGCGATTTAAGCG	AGCCTCACTGGTTTGAGCG
Tslp	ACGGATGGGGCTAACTTACAA	AGTCCTCGATTTGCTCGAACT
Aqp3	AACCCTGCTGTGACCTTTG	GCTGCTGTGCCTATGAACTG
Il4ra	TGGGCTGTGATTTTGCTTTTGG	GTGCTGGGGTGGGAATCTGGTC
Nod2	CAGGTCTCCGAGAGGGTACTG	GCTACGGATGAGCCAAATGAAG
Osm	TGCCCCGCCAATATCCTC	GTGTGGGCTCAGGTATCTCCAG
Slc4a11	CAGGACTCCGGTGAATACTTCT	GATGCTCTCGCCAGACACAA
Mmp13	CTTCTTCTTGTTGAGCTGGACTC	CTGTGGAGGTCACTGTAGACT
Serpinb3a	CAGATGATGAAACAAAACATCG	AGACCTTGAGTGCTGCTCATA
il4	GGTCTCAACCCCAAGCTAGT	GCCGATGATCTCTCTCAAGTGAT
il1b	GCAACTGTTCTGAACTCAACT	ATCTTTTGGGGTCCGTCAACT
lcn2	TGGCCCTGAGTGTGATGTG	CTCTTGAGCTCATAGATGGTGC
S100a8	AAATCACCATGCCCTCTACAAG	CCCCTTTTATCACCATCGCAA
mdefb14	TCCAGGGGACGCATTCTCA	ACCGCTATTAGAACATCGACCTA
il36a (il1f6)	GCAGCATCACCTTCGCTTAGA	CAGATATTGGCATGGGAGCAAG
il36b (il1f8)	AGAGTATTCAAATGTGGGAACCG	GACCCATACCATCTGTTGTGAG
il36g (il1f9)	TCCTGACTTTGGGGAGGTTTT	TCACGCTGACTGGGGTACT
cxcl1	CTGGGATTCACCTCAAGAACATC	CAGGGTCAAGGCAAGCCTC
cxcl2	CCAACCACCAGGCTACAGG	GCGTCACACTCAAGCTCTG
ccl2	TTAAAAACCTGGATCGGAACCAA	GCATTAGCTTCAGATTTACGGGT
S100a9	ATACTCTAGGAAGGAAGGACACC	TCCATGATGTCATTTATGAGGGC
S.aureus	AATCTTTGTGCGGTACACGATATTCTTCACG	CGTAATGAGATTTTCAGTAGATAATAACA
Gyr	AGTACATCGTCGTATACTATATGG	ATCACGTAACAGTTCAAGTGTG
sa3int	GAAAAACAAACGGTGCTAT	TTATTGACTCTACAGGCTGA
Sak	AAGGCGATGACGCGAGTTAT	GCGCTTGGATCTAATTCAAC
scn	AGCACAAGCTTGCCAACATCG	TTAATATTTACTTTTTAGTGC
mecA	AAAATCGATGGTAAAGGTTGGC	AGTTCTGCAGTACCGGATTTGC
16S	9AACTCTGTTATTAGGGAAGAACA	CCACCTTCTCCGTTTTGTCACC

Table S1. Sequences of Oligonucleotides. Related to STAR Methods.