Supplemental Figures

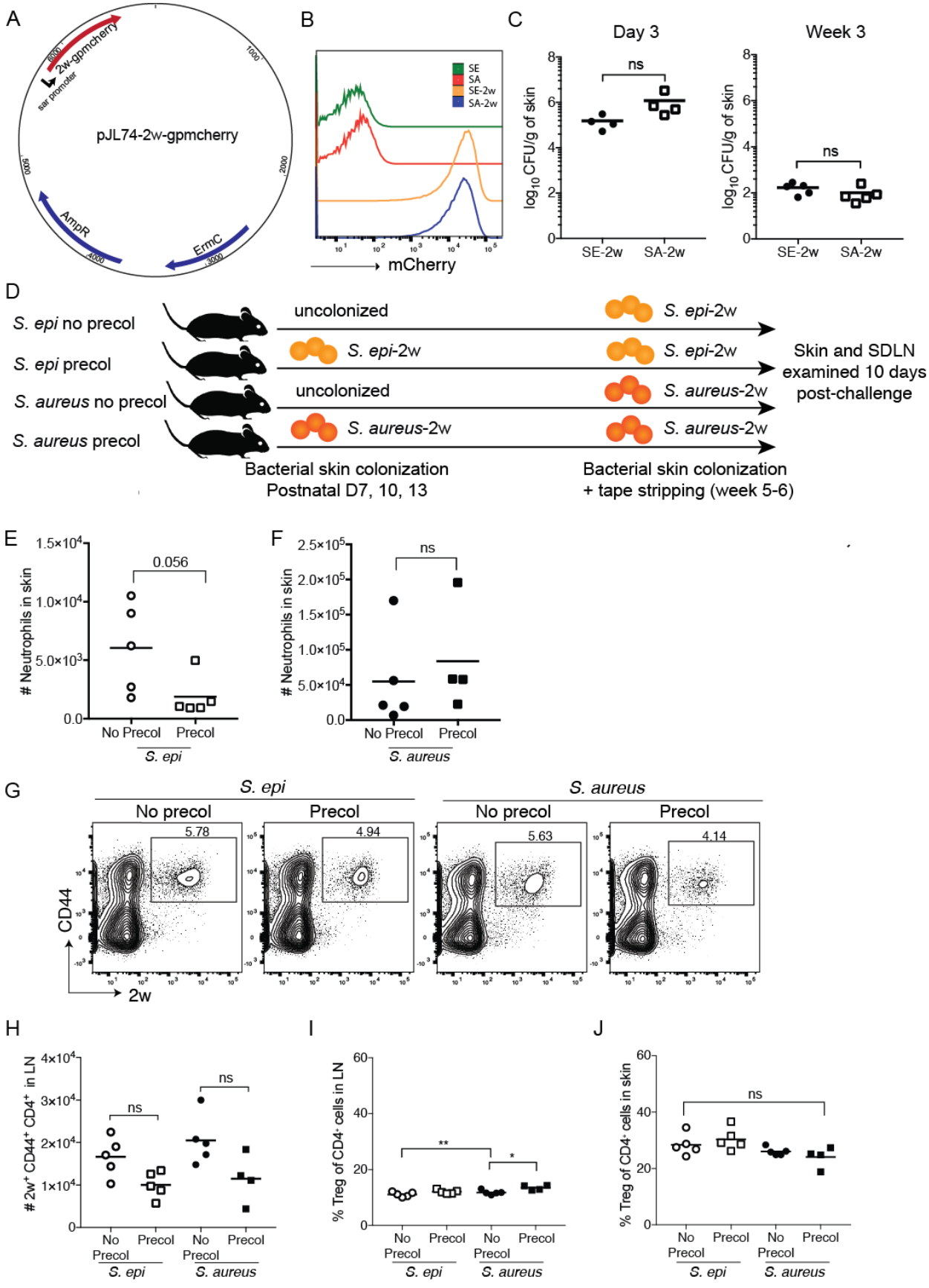

Figure S1: Construction and verification of antigen expression by *S. epi*-2w and *S. aureus*-2w (Related to Figure 1) (A) Modified pJL74-2W-gpmcherry plasmid construct. (B) Flow cytometric plot of mCherry expression of *S. epi*-2w vs. *S. aureus*-2w vs. parent strains. (C) CFU in the skin three days and three weeks post colonization. (D) Schematic depicting experimental procedures for data presented here and in Figure 1. Neonatal mice were not colonized (No precol) or colonized with either *S. epi*-2w or *S. aureus*-2w (Precol) every three days for one week and then challenged 3-4 weeks later with the same respective strain and superficial skin abrasion via gentle tape-stripping. (E) Absolute numbers of skin neutrophils in *S. epi* No precol vs. Precol mice. (F) Absolute numbers of skin neutrophils in *S. aureus* No precol vs. Precol mice. (G) Representative flow cytometry plots and (H) absolute numbers of $2w^{+}CD4^{+}CD44^{+}$ cells in SDLN of mice following challenge. (I) Percentage polyclonal Treg cells in SDLN and (J) skin of mice following challenge.

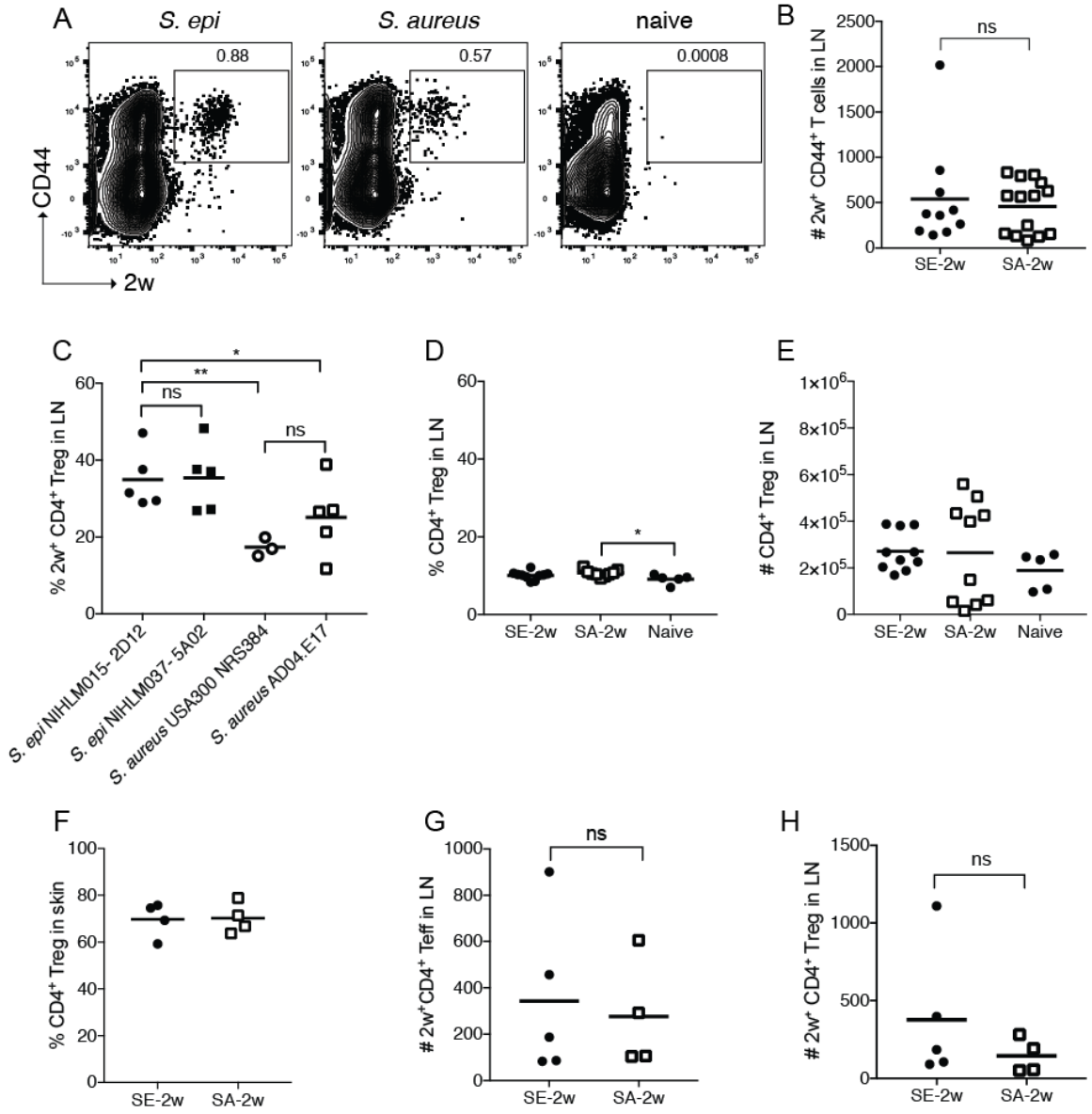

Figure S2: Neonatal skin colonization does not impact polyclonal Treg populations in LN or skin. (Related to Figure 2) Neonatal mice were colonized on postnatal day 7, 10 and 13 or left uncolonized (Naïve) and the primary 2w response in the SDLN was assessed at weaning age. (A) Representative flow cytometry plots and (B) absolute numbers of $2w^+CD4^+CD44^+$ cells in SDLN following neonatal colonization. (C) Percentage 2w-specific Tregs in SDLN of mice at weaning age following neonatal colonization with 2w-expressing clinical *S. epi* and *S. aureus* isolates. (D) Percentage and (E) absolute numbers of polyclonal Tregs in the SDLN of mice following neonatal colonization with either *S. epi*-2w, *S. aureus*-2w or no bacteria as in A and B. (F) Percentage of polyclonal Tregs in skin on day 16 following neonatal colonization. (G) Absolute numbers of 2w-specific Teff in SDLN. (H) Absolute numbers of 2w-specific Treg in SDLN.

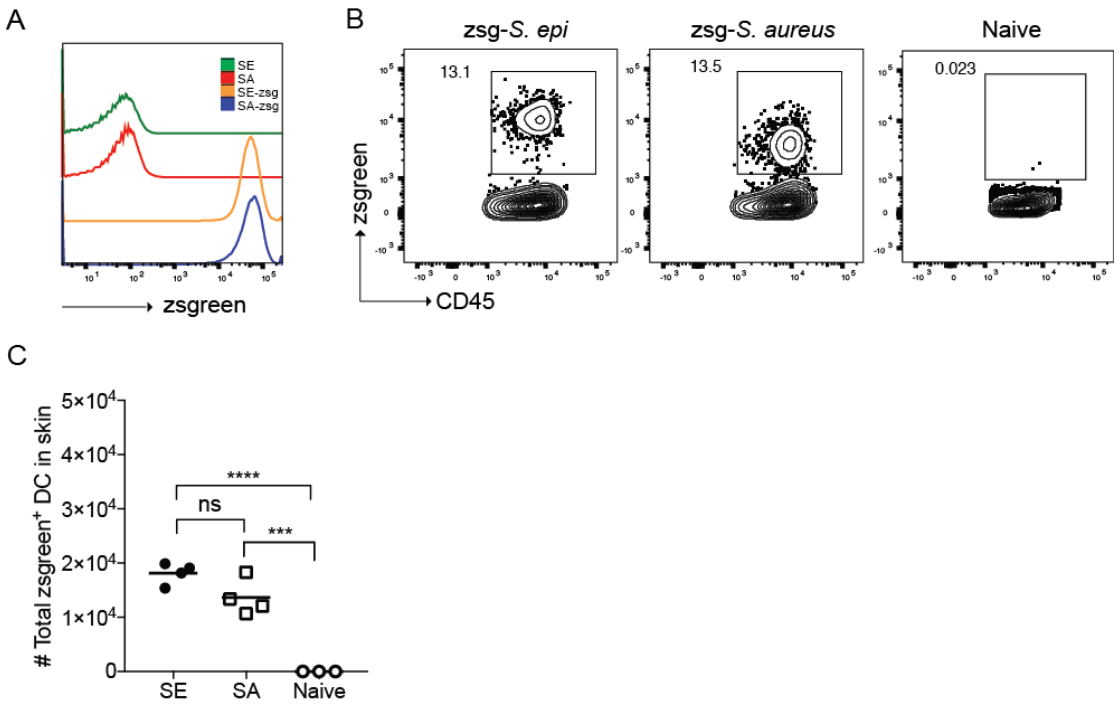

Figure S3: Equivalent dendritic cell uptake of *S. epi* and *S. aureus* following neonatal colonization (Related to Figure 3) (A) Representative histogram of zsgreen intensity of zsg-*S. epi* vs. zsg-*S. aureus* vs. parent strains as measured by flow cytometry. Neonatal mice were either uncolonized or colonized with zsg-*S. epi* or zsg-*S. aureus* on postnatal day 9 and skin was harvested 18 hours later. (B) Representative flow cytometry plots and (C) absolute numbers of zsgreen^{+} DCs in skin of mice following neonatal colonization. Plots gated on live CD45^{+}CD11c^{+}MHCII^{hi} cells.

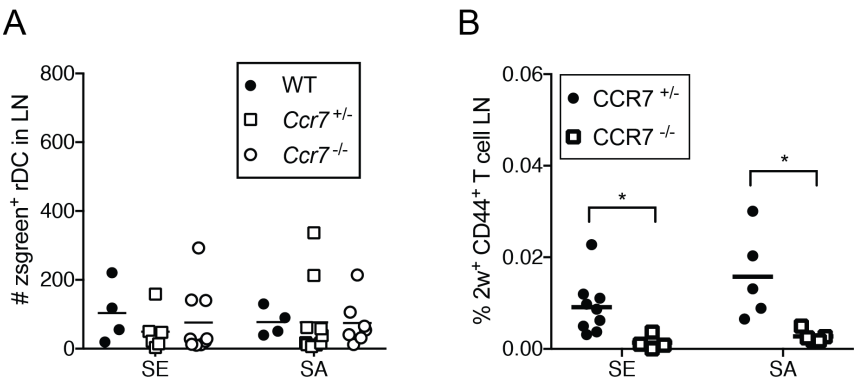

Figure S4: Levels of *S. epi* and *S. aureus* zsgreen signal in LN-resident DCs and percentage of bacteria-specific CD4⁺ T cells in mice with and without CCR7 (Related to Figure 4) Neonatal WT, *Ccr7*$^{+/-}$ and *Ccr7*$^{-/-}$ mice were colonized with *zsg-S. epi* or *zsg-S. aureus* on postnatal day 9 and SDLN were harvested 18 hours later. (A) Absolute numbers of zsgreen⁺ resident DCs (rDCs) in the SDLN. (B) Percentage 2w⁺CD4⁺CD44⁺ cells of all CD4⁺ in SDLN of *Ccr7*$^{+/-}$ and *Ccr7*$^{-/-}$ mice following neonatal colonization with *S. epi*-2w or *S. aureus*-2w. Data are combined from 4 experiments.

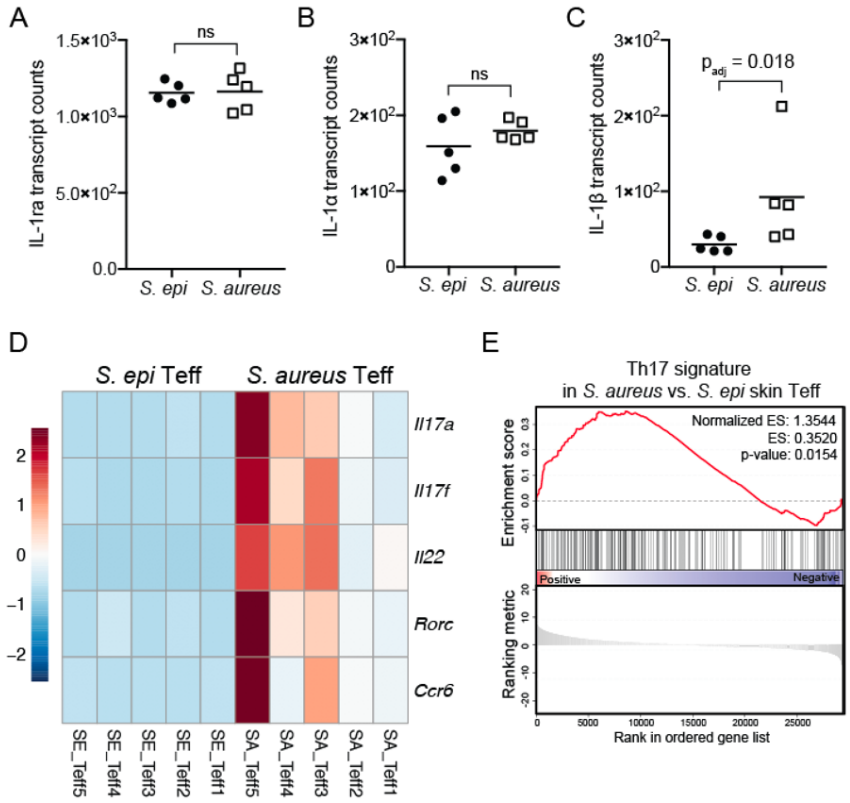

Figure S5: Increased IL-1β and Th17 signature in *S. aureus*-colonized neonatal skin (Related to Figure 5) Pups were colonized every two days starting at birth with *S. epi* or *S. aureus* and skin was harvested at day 21 for whole tissue RNAseq. (A) IL-1ra, (B) IL-1α and (C) IL-1β transcript counts from whole skin of *S. epi* vs. *S. aureus* colonized pups. Tregs and $CD4^+$ Teffs were also sorted from the skin of these mice and subjected to bulk RNA sequencing. (D) Heatmap of expression of Th17-associated genes in skin $CD4^+$ Teff from *S. epi* versus S. *aureus* colonized mice. (E) Gene set enrichment analysis for Th17 gene signature in *S. aureus* vs. *S. epi* $CD4^+$ Teff.

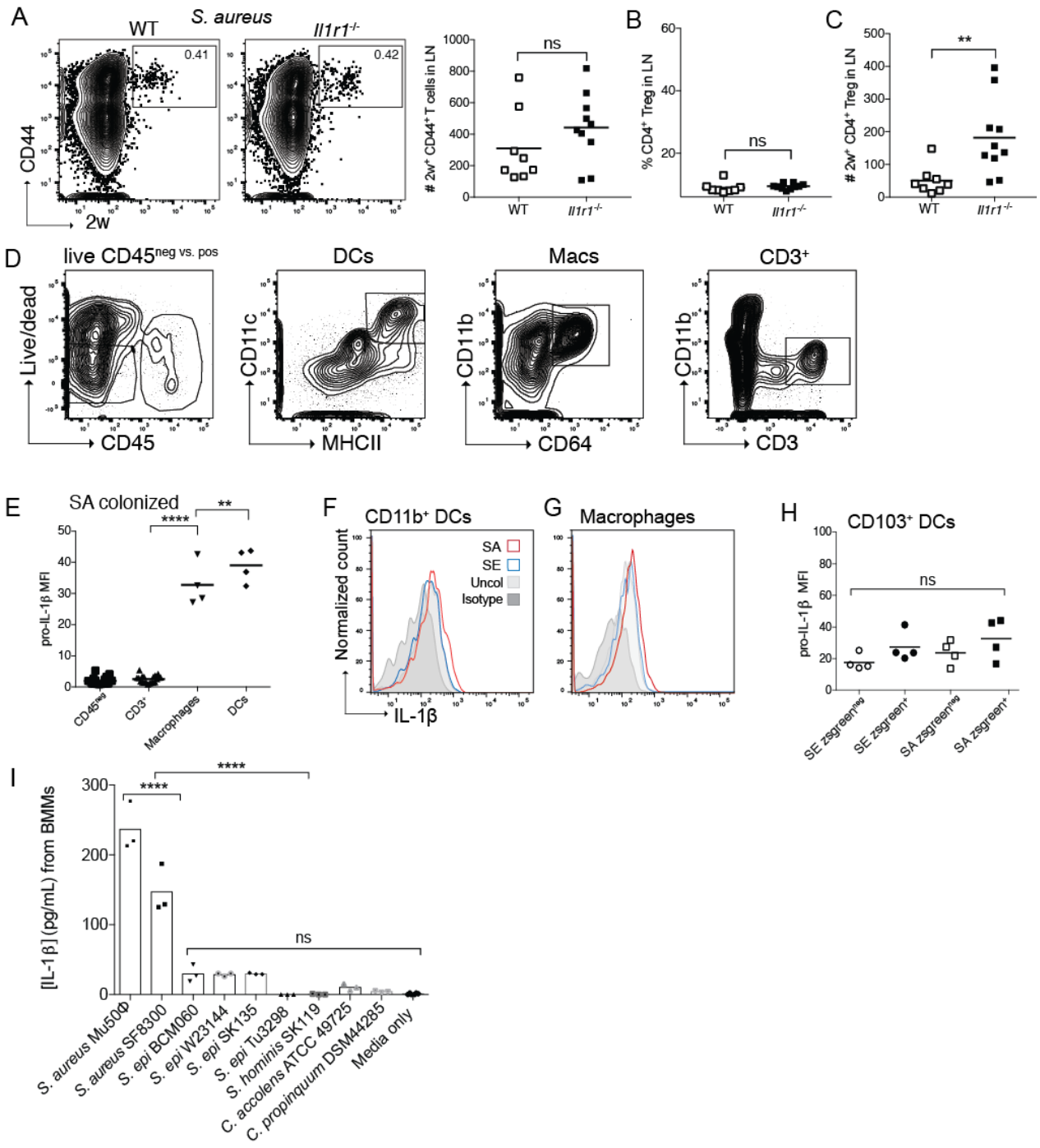

Figure S6: Role of IL-1R1 and levels of (pro-)IL-1β following exposure to *S. aureus* versus *S. epi* and other skin bacteria (Related to Figure 6) Neonatal WT and *Il1r1*$^{-/-}$ mice were colonized on postnatal day 7, 10 and 13 and the primary 2w response in the skin-draining LN assessed at weaning age. (A) Representative flow cytometry plots and absolute numbers of 2w^{+}CD4^{+}CD44^{+} cells in LN of WT and *Il1r1*$^{-/-}$ mice following neonatal colonization. (B) Percentage polyclonal Tregs and (C) absolute number of 2w-specific CD4^{+} Treg in LN of WT and *Il1r1*$^{-/-}$ mice following neonatal colonization. Flow cytometry was used

to measure levels of pro-IL-1β in skin cells of day 10 neonates, following overnight bacterial colonization. (D) Representative flow plots demonstrating gating strategy to delineate live $CD45^{pos}$ vs. $CD45^{neg}$, dendritic cells, macrophages and $CD3^+$ populations in the skin of mice. DC, Mac and $CD3^+$ plots plots depict gating directly from live-$CD45^+$ population. DCs were further subdivided based on $CD11b^+$ or $CD103^+$ expression. (E) pro-IL-1β MFI in $CD45^{neg}$, $CD3^+$, Macrophage and DC skin populations following overnight colonization with *S. aureus*. Representative histograms of pro-IL-1β expression in skin (F) $CD11b^+$ DCs and (G) macrophages. (H) pro-IL-1β MFI in $zsgreen^+$ and $zsgreen^{neg}$ $CD103^+$ DCs in *S. epi* versus *S. aureus*-colonized mice. (I) Levels of IL-1β by ELISA in media of BMMs exposed for 2 hours to culture supernatants of various skin bacteria.

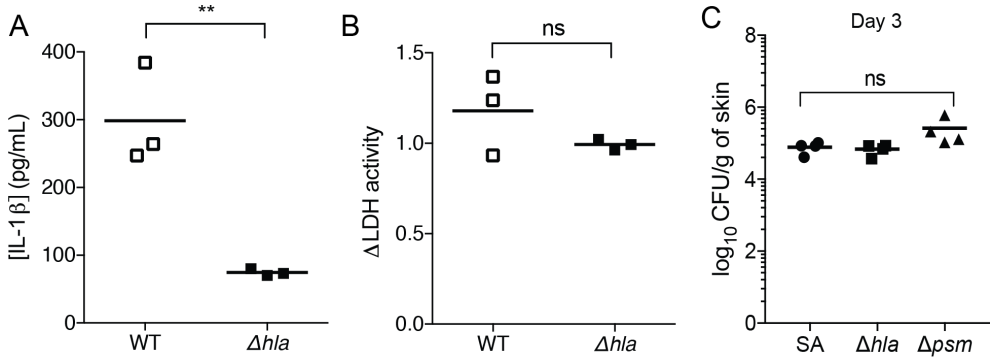

Figure S7: Alpha toxin deficiency reduces *S. aureus*-induced IL-1β release from BMM's (Related to Figure 7) Bone-marrow derived myeloid cells (BMMs) were exposed *in* vitro to sterile WT or *Δhla S. aureus* supernatant for 2 hours. (A) Levels of released IL-1β in BMM media as measured by ELISA. (B) LDH levels in BMM media. (C) CFU in the skin of neonatal mice three days post colonization with WT, *Δhla* or *Δpsm S. aureus*.

Supplemental Table 1. Oligonucleotides used for *S. aureus* allelic replacement mutagenesis using a standardized protocol based on pKOR1. (Related to STAR Methods)

Oligo ID	Nucleotide sequence 5' to 3'
For deletion of genes encoding psmβ1 and psmβ2	
psmβ-X1	GAGTTCCACAAAGCACAATACAC
psmβ-X2	GAATCCAAATAATTTACCTAGTAAACCCACGGTATCTTTAATTGCGTTAAATAAACCTTC
psmβ-X3	GAAGGTTTATTTAACGCAATTAAAGATACCGTGGGTTTACTAGGTAAATTATTTGGATTC
psmβ-X4	CCATTGGCAGAAATGATTCTAAGG
psmβ-X5	GGGG ACAAGTTTGTACAAAAAAGCAGGCT CAATATGTCATTGAAAAACGCTGAACTAC
psmβ-X6	GGGG ACCACTTTGTACAAGAAAGCTGGGT TCATGGATTTACAGATGTGGAAGC
psmβ-S1	CGTTTTGGTGACGCATCATAC
psmβ-S2	CACGATGCCCATCAACTTCC
For introduction of nonsense mutation into start codon of hld	
hld-X1	CTGTACTTAATACCACTAATTATAGCTG
hld-X2	CACCGATTGTTGAAATGATATCTTGTGCAATTGAAATCACTCCTTCC
hld-X3	GGAAGGAGTGATTTCAATTGCACAAGATATCATTTCAACAATCGGTG
hld-X4	CCCAACTTATCTGTTATGATTTACG
hld-X5	GGGG ACAAGTTTGTACAAAAAAGCAGGCT CCACCTACTATCACACTCTC
hld-X6	GGGG ACCACTTTGTACAAGAAAGCTGGGT GATATCCTGCTCGTAGTGGTGC
hld-S1	CCAAAAAGAAGAAGGTGCATGTGC
hld-S2	CATTACAAAAAAGGCCGCGAG