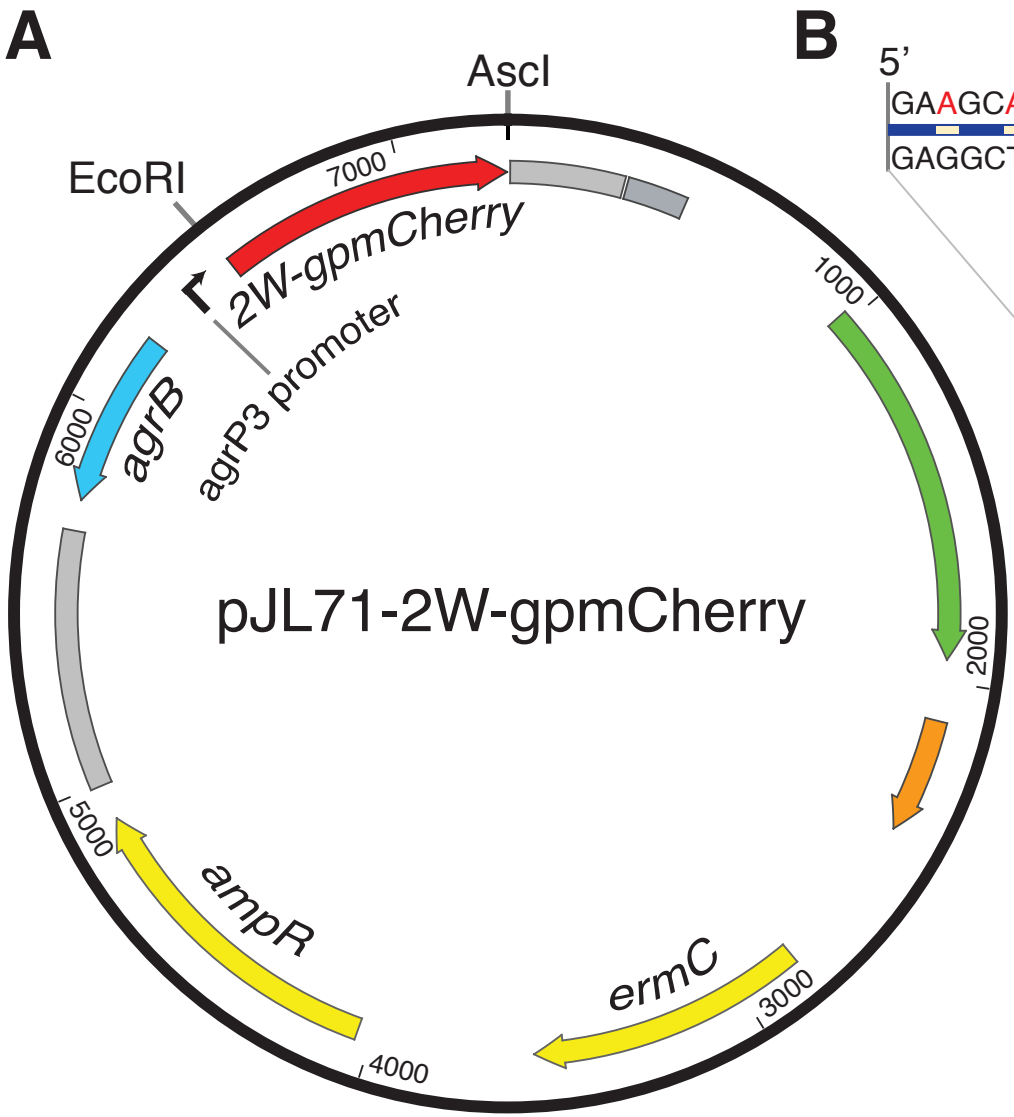
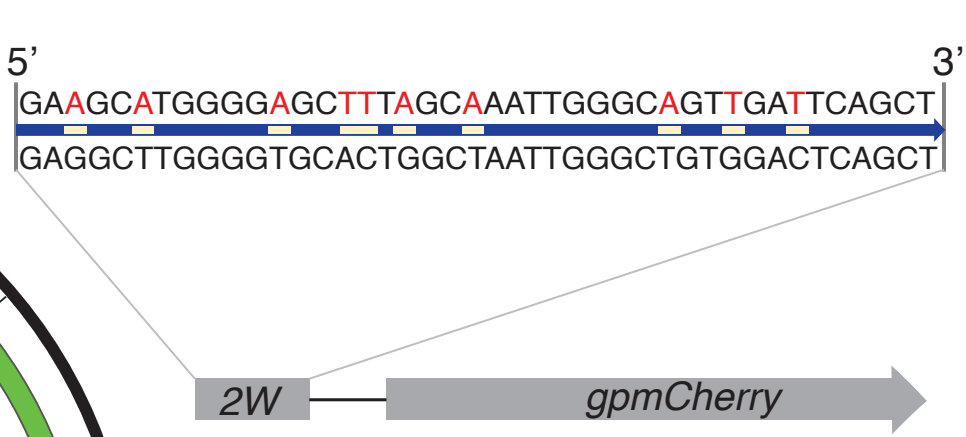
Figure S1 (related to Figure 1)





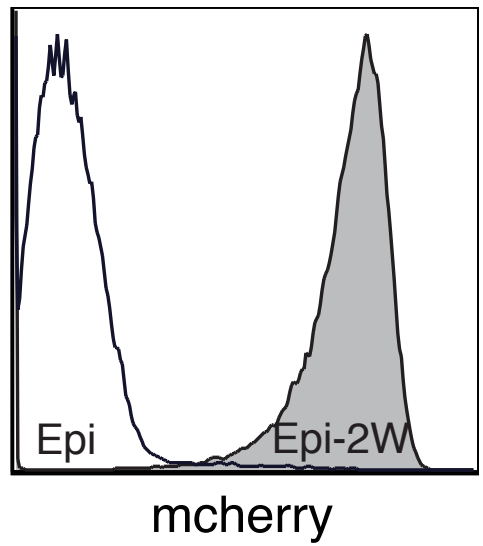


Figure S2 (related to Figures 2 & 3)

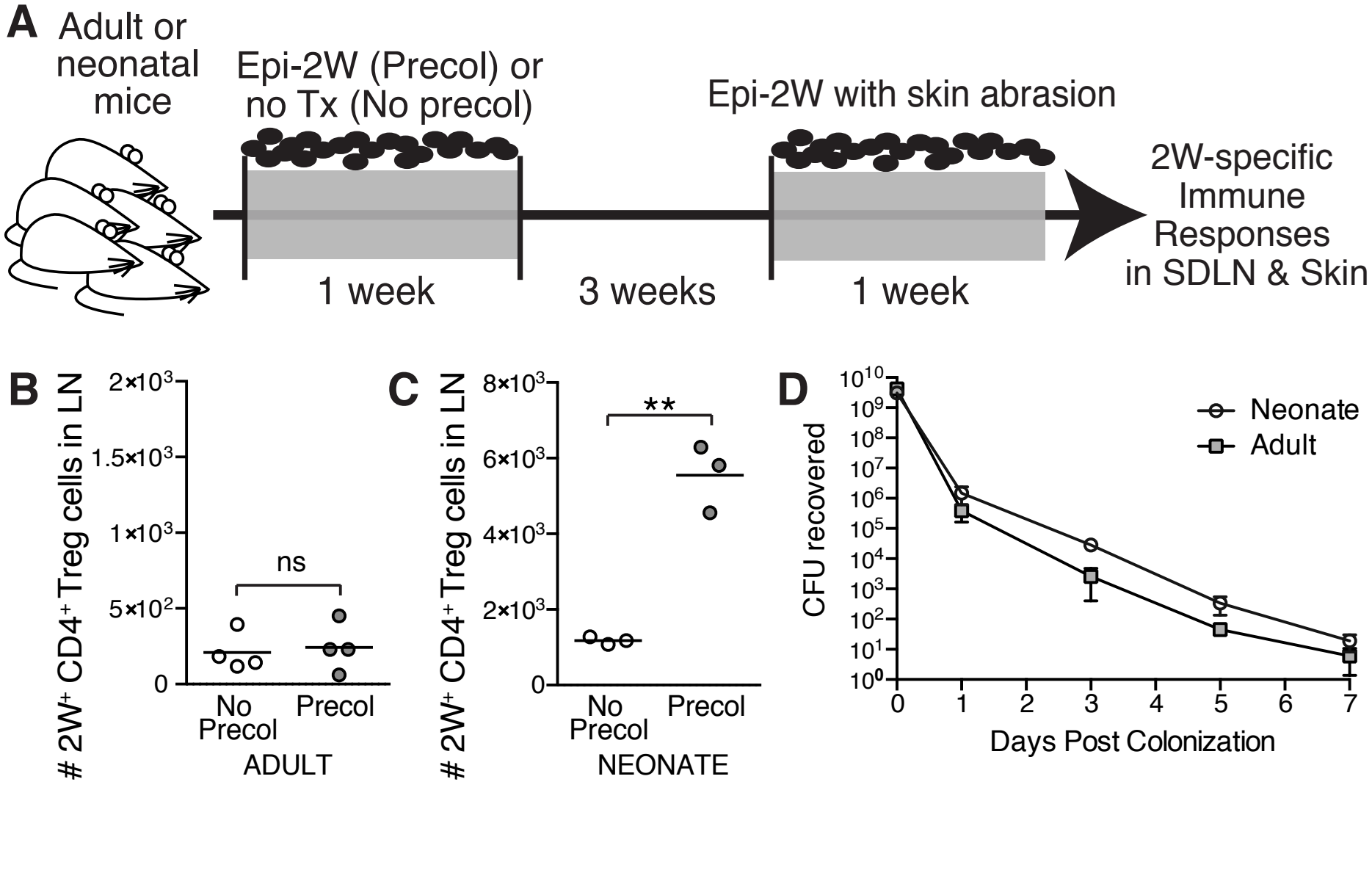


Figure S3 (related to Figure 4)

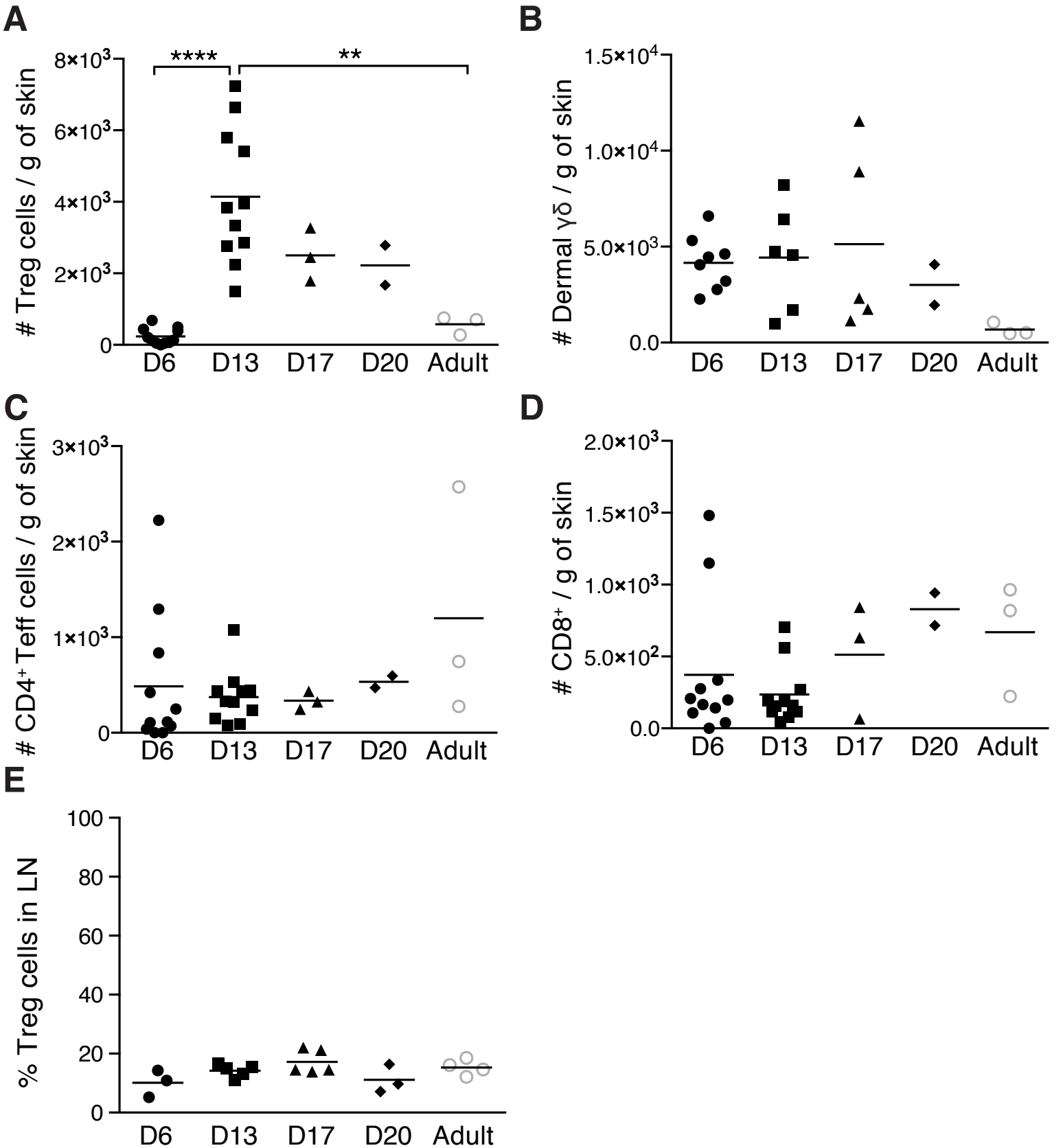
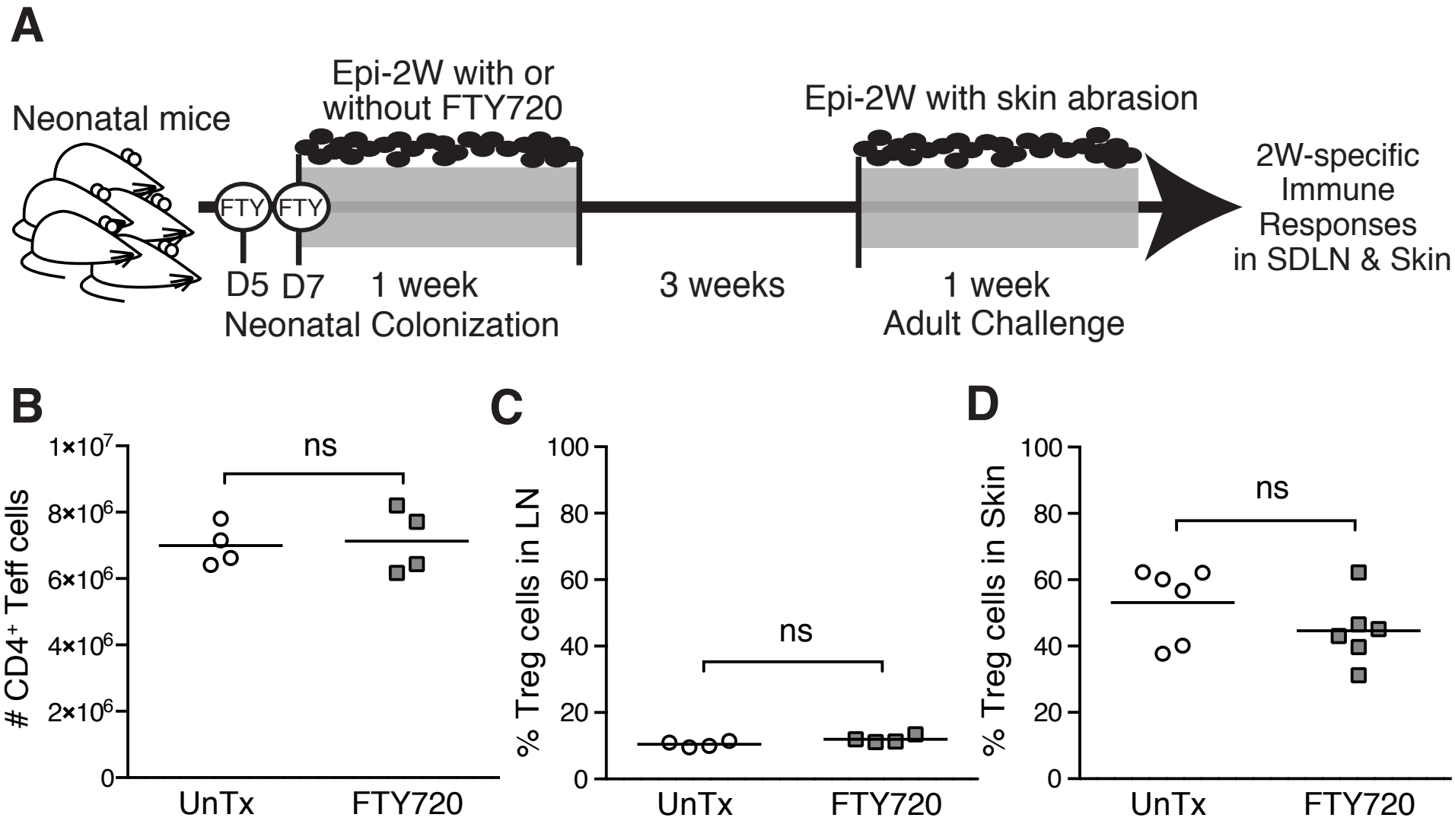


Figure S4 (related to Figure 6)



Supplemental Figure Legends

Figure S1: Construct and verification of antigen expression by Epi-2W, Related to Figure 1 and Experimental Procedures

(A) Modified pJL71-2W-gpmCherry plasmid construct. **(B)** 2W sequence optimized for *Staphylococcus* (top) versus eukaryotic sequence (bottom) was inserted 5' to gpmCherry creating a 2W-gpmCherry fusion protein. **(C)** Flow cytometric plot of mCherry expression of Epi-2W versus wild-type *S. epidermidis* (Epi).

Figure S2: Absolute numbers of 2W-Treg cells in LN and dynamics of Epi-2W skin colonization in neonatal and adult mice, Related to Figures 2 & 3 (**A)** Schematic depicting experimental procedures for data presented here and in Figures 2 & 3. Adult or neonatal mice were not colonized (No precol) or colonized with Epi-2W (Precol) every three days for one week and then challenged 3-4 weeks later with Epi-2W and superficial skin abrasion. **(B)** Absolute numbers of $CD44^{+}CD4^{+}FoxP3^{+}2W^{+}$ cells in SDLN of adult **(C)** and neonatal mice following challenge. **(D)** Serial Epi-2W CFU counts from back skin of adult and 7-day-old neonatal mice colonized once with Epi-2W on day 0.

Figure S3: Lymphocyte subsets in skin and SDLN by age, Related to Figure 4

Absolute number of **(A)** Treg cells **(B)** dermal γδ T cells **(C)** $CD4^{+}FoxP3^{neg}$ T cells (Teff) **(D)** and $CD8^{+}$ T cells per gram of skin in 6-day-old (D6), 13-day-old (D13), 17-day-old (D17) and 6-8 week old (Adult) mice. **(E)** Percentage of Treg cells of $CD4^{+}$ T cells in LN by age. Data representative of two independent experiments with ≥3 mice per group.

Figure S4: Profile of polyclonal $CD4^{+}$ population in the SDLN and skin following treatment with FTY720 during neonatal Epi-2W precolonization, Related to Figure 6

(A) Schematic depicting experimental procedures for data presented here and in Figure 6. Neonatal mice were colonized for one week with Epi-2W beginning on D7 and FTY720 or saline (UnTx) was administered on postnatal D5 and D7. Mice were then challenged 3-4 weeks later with Epi-2W and superficial skin abrasion. **(B)** Absolute number of $CD4^{+}FoxP3^{neg}$ T cells (Teff) in LN and **(C)** percentage of $CD4^{+}$ Treg cells in LN and **(D)** skin 10 days after Epi-2W challenge. Data representative of three independent experiments.

Supplemental Experimental Procedures

Tissue processing for flow cytometry

To isolate immune populations from skin, mice were shaved following euthanasia and trunk skin was removed. Skin was manually defatted using forceps to bluntly separate subcutaneous layers. Skin was weighed to later calculate absolute cells numbers per gram of tissue. Skin was then minced with scissors and digested for 40 minutes at 37° with 2.0 mg/ml collagenase type 11, 500 µg/ml hyaluronidase and 100 µg/ml DNase (all from Sigma). The digested tissue was then mashed over a 100 µM filter and washed with culture media to obtain a single cell suspension. For isolation of T cells from the intestinal lamina propria, the small and large intestine were first cleaned of mesentery, fat, peyers patches and fecal contents and cut into <1 cm pieces. The tissue was then incubated with 1 mM DTT followed by two consecutive incubations with 30 mM EDTA and 10 mM HEPES to remove epithelial cells. Remaining tissue was digested with 100 U/ml type 8 collagenase and 150 µg/ml DNase I (both from Sigma) for 1 hour at 37°C followed by dispersal over 70 µm filters. Leukocytes were enriched by density centrifugation over a 40/80% Percoll gradient.

Tetramer staining of skin

Single cell suspensions from skin were incubated for one hour in the dark at room temperature in 2x volume blocking solution with the phycoerythrin-conjugated 2W tetramer at a final concentration of 10nM. Cells were then washed with culture media prior to staining in blocking solution with a surface antibody cocktail including Dump markers (CD11b, CD11c, B220, CD49b and F4/80) to allow exclusion during data analysis of non-T cell populations that might bind the tetramer non-specifically. Cells were then fixed and permeabilized for intracellular FoxP3 staining as described elsewhere. No enrichment for tetramer-positive cells was performed for skin samples prior to analysis.

Antibodies for flow cytometry

Fluorophore-conjugated antibodies specific for mouse cell surface antigens and intracellular transcription factors were purchased from eBiosciences, BD Biosciences or Biolegend. The following antibodies and clones were used: anti-B220 (RA3-6B2), anti-CD3 (145-2C11), anti-CD4 (GK1.5, RM4-5), anti-CD8a, anti-CD11b (M1/70), anti-CD11c (HL3), ant-CD44 (IM7), anti-CD45 (30-F11), anti-CD49b (DX5) anti-CTLA4 (UC10-4F10-11), anti-F4/80 (BM8), anti-FoxP3 (FJK-16s), anti-ICOS (C398.4A), anti-Ly6G (1A8), anti-MHC Class II (M5/114.15.2), Live/Dead Ghost Dye (Tonbo).