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Supplemental Information

Commensal Microbes and Hair Follicle

Morphogenesis Coordinately Drive

Treg Migration into Neonatal Skin

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Supplementary Figures

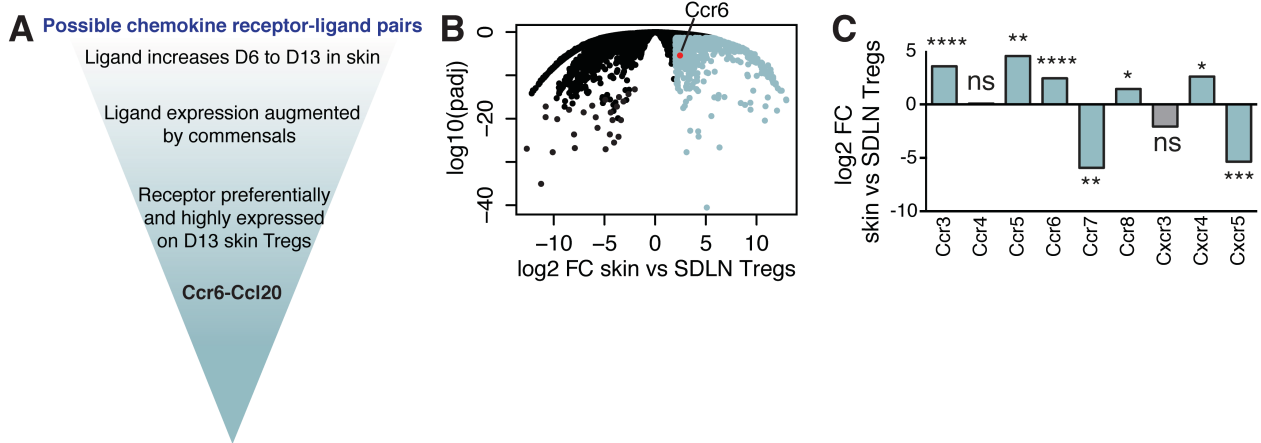


Figure S1 (Related to Figure 3) Relative expression of chemokine receptors on neonatal skin vs. LN Treg cells by RNA sequencing

(A) Design of discovery approach to narrow chemokine-receptor candidates mediating Treg cell migration to neonatal skin. **(B)** Volcano plot of \log_2 FC by $\log_{10}(\text{adjusted p-value})$ of RNA sequencing data from D13 skin and LN Treg cells. Data point for Ccr6 depicted in red. **(C)** \log_2 FC in D13 skin vs. LN Treg cells for receptors corresponding to chemokines increased in D13 skin. Blue = statistically significant difference.

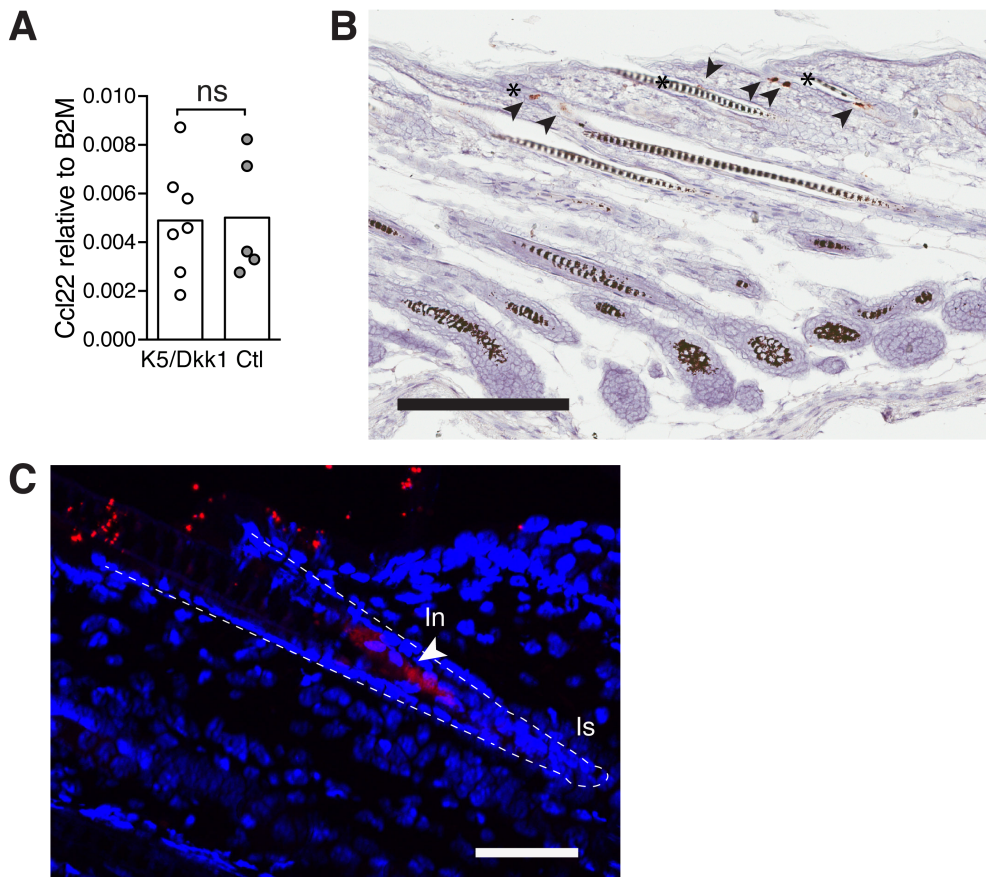


Figure S2 (Related to Figure 4) Ccl20 but not Ccl22 is expressed in the infundibulum of neonatal hair follicles

(A) Fold change expression of Ccl22 vs. beta-2-microglobulin mRNA transcripts in skin from D13 K5/Dkk1 vs. Ctl littermates. **(B)** In situ hybridization for Ccl20 mRNA transcript in skin of D13 SPF mice. Arrows = visualization of Ccl20 mRNA signal. * = hair follicle infundibulum. Scale bar = 200 μ m. **(C)** Neonatal mouse skin colonized with mCherry-expressing *S. epidermidis* (red), DAPI counterstain (blue), dashed line designates outline of hair follicle, arrowhead = mCherry signal in hair follicle, In = hair follicle infundibulum, Is = hair follicle isthmus, Scale bar = 50 μ m.

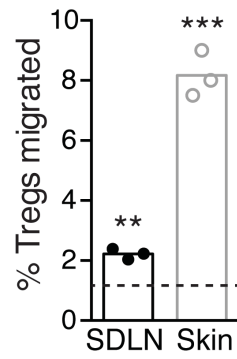


Figure S3 (Related to Figure 6) Ccl20 induces migration of Treg cells from neonatal skin but not SDLN

Skin-draining lymph nodes (SDLN) and CD4⁺ enriched skin lymphocytes were isolated from neonatal mice and incubated in a transwell system with Ccl20 (100ng/ml) or no chemokine. Percentage of Treg cells migrating to Ccl20 after 3 hours is depicted. P-values are in comparison to control wells with no chemokine, which for both populations yielded 1% of Tregs in the lower transwell. This control threshold is depicted by a dashed line. Each data point depicts a separate transwell. Data representative of two independent experiments.

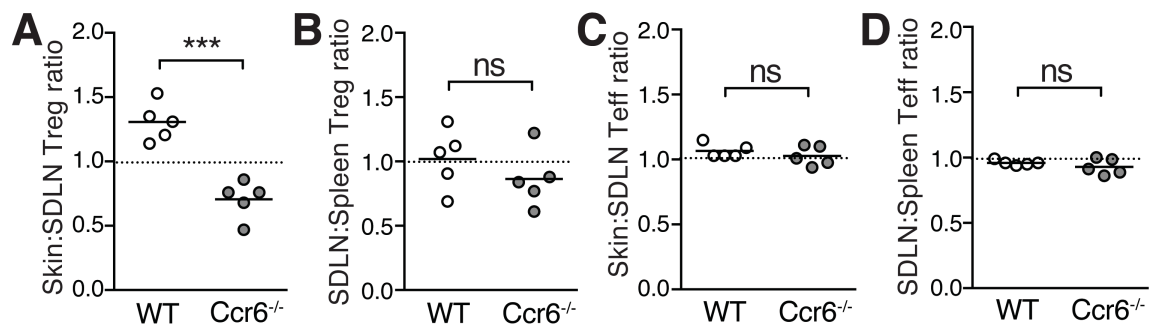


Figure S4 (Related to Figure 7) Ccr6 directs Treg cell migration into neonatal skin but not lymphoid organs

(A) Skin:SDLN ratio for wt and Ccr6^{-/-} Treg cells (i.e. % wt Treg cells in skin divided by % wt Treg cells in SDLN), **(B)** SDLN:Spleen ratio for wt and Ccr6^{-/-} Treg cells, **(C)** Skin:SDLN ratio for wt and Ccr6^{-/-} CD4⁺ Teff cells, and **(D)** SDLN:Spleen ratio for wt and Ccr6^{-/-} CD4⁺ Teff cells.