Supplementary Materials List:

Materials & Methods
Fig. S1. Gating strategy and qPCR validation of REX3 mice.
Fig. S2. Gating strategy and qPCR validation for identifying epidermal cell types.
Fig. S3. Validation of the STAT-1 flox K5-Cre mouse strain tested in the vitiligo model.
Table S1. Surface markers used to identify epidermal cell types.
Table S2. Keratinocyte-cell ratios in the epidermis.
Supplementary References

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Materials & Methods

Determining cell ratios in human skin. Discarded skin from panniculectomy surgeries at the University of Massachusetts Medical School was treated with 50U/mL Dispase II in PBS for 1h at 37°C or overnight at 4°C (patient consent for experiments was not required because human tissue left over from surgery is considered discarded material, and is IRB exempt). The epidermis was removed as one sheet with a razor blade and was mechanically dissociated with a syringe plunger and 70µm mesh filter paper. Samples were stained in 1% FBS-PBS with LiveDead Blue (Invitrogen, 1:1000) and the following antibodies from Biolegend per the manufacturer's instructions: Fc block, CD45 AF700, CD49f PerCP-Cy5.5, cKit APC, CD31 AF488, Langerin PE,

CD11c PE-Cy7 and CD3 Pacific Blue. Samples were fixed in 2% PFA and run on an LSR II flow cytometer. Data was analyzed with FlowJo software version X.

Cell sorting. To confirm the gating strategy, epidermal cell populations were stained as samples used for flow cytometry and sorted with a FACSAria II (BD Biosciences). Sorted cells were further processed for RNA extraction (Qiagen kit) to confirm enrichment of cell-specific transcripts.

qPCR. RNA was isolated from sorted cells or mouse ear tissue using RNeasy kits (Qiagen). cDNA was prepared using iScript kits (Biorad), and qPCR was performed with SYBR green kits in an iCycler iQ according to the manufacturer's recommendations (Biorad). Mouse primers used were follows: K6 forward 5-GGAGGCTGTGTCCTCTCG-3, K6 5as reverse TAGAAAAAGTTACTTTTTATAAATCTG-3; K8 forward 5-TGCAGAACATGAGCATTC-3, K8 reverse 5-CAGAGGATTAGGGCTGAT-3 (Peters et al., 2001); CD68 forward 5-TGGACAGCTTACCTTTGGATTCA-3, CD68 reverse 5-TGTATTCCACCGCCATGTAGTC-3 (Kothapalli et al., 2007); CD3E forward 5-AAGTCGAGGACAGTGGCTACTAC-3, CD3E reverse 5-CATCAGCAAGCCCAGAGTGATACA-3 (Nakamura et al., 2007); CD11c forward 5-CTGGATAGCCTTTCTTCTGCTG-3, CD11c reverse 5-GCACACTGTGTCCGAACTC-3 (Shaul et al., 2010); Langerin forward 5-ACGCACCCCAAAGACCTGGTACAG-3, Langerin reverse 5-AGACACCCTGATATTGGCACAGTG-3 (Takahara et al., 2002); CXCL9 forward 5-AATGCACGATGCTCCTGCA-3, CXCL9 reverse 5-AGGTCTTTGAGGGATTTGTAGTGG-3; CXCL10 forward 5-GCCGTCATTTTCTGCCTCA-3, CXCL10 reverse 5-CGTCCTTGCGAGAGGGATC-3 (Groom al., 2012); β-actin forward 5et

GGCTGTATTCCCCTCCATCG-3, β -actin reverse 5- CCAGTTGGTAACAATGCCATGT-3

(Rashighi et al., 2014).

Supplementary Figures & Legends:

Figure S1



Fig. S1. Gating strategy and qPCR validation of REX3 mice. A. Gating strategy for determining chemokine expression for all samples included single, live cells. **B**. Variation of chemokine expression in epidermis and **C**. lymph nodes of naïve mice taken down at each time point was not significant. **D**. CXCL9 and CXCL10 expression in the skin of REX3 vitiligo mice were confirmed via qPCR.

Figure S2

Gating validation



Fig. S2. Gating strategy and qPCR validation for identifying epidermal cell types. Sample flow cytometry plots for determining epidermal cell types are shown. These gates were also used to sort cells and perform qPCR for cell specific transcripts as shown. Data were normalized to β -actin expression and are represented as % of max expression.



Fig. S3. Validation of the STAT-1 flox K5-Cre mouse strain tested in the vitiligo model. A. CXCL9 and B. CXCL10 are not produced by tail epidermis from STAT1-flox +/+ K5-cre+ when stimulated in vitro with IFN- γ and TNF- α (EC₅₀, 24h, qPCR normalized to actin, representative experiment run in duplicate).

Supplementary Tables:

Cell Type	Markers
T cells	CD45+CD3+
γδ T cells (mouse)	CD45+CD3 ^{hi}
Keratinocytes (basal)	CD45-CD49f+
Langerhans cells	CD45+CD11c+ and/or Langerin+

Table S1. Surface markers used to identify epidermal cell types.

Table S2. Keratinocyte-cell ratios in the epidermis.

	Human abdomen (n=3-4)	Krt14-Kitl* Mouse tail (n=8- 9)
# Keratinocytes per T cell	13.3 <u>+</u> 6.1	22.1 <u>+</u> 10.6
# Keratinocytes per Langerhans cell	30.7 <u>+</u> 14.3	43.8 <u>+</u> 35.3**
# Keratinocytes per melanocyte	28.7 <u>+</u> 22.7*	5.3 <u>+</u> 0.4

*high variability may be due to donor site sampling; see also Cichorek et al 2013 (Cichorek *et al.*, 2013)

**high variability may be due to mouse skin/hair cycling; see also Paus et al 1998 (Paus *et al.*, 1998)

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