Thymic stromal lymphopoietin controls hair growth

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Figure S1: Human IfTSLP is expressed in glandular structures in the dermis. Related to Figure 1.

A. Immunostaining IfTSLP in lesional and non-lesional tissue samples collected from atopic dermatitis patients. Scale = 100 μm.

B-C. Representative immunostaining of human skin of TSLP_{total} or long form TSLP (red) and CD34 or KRT77 (green) Data presented are from n=3 independent experiments using 3 different human donors. Scale bar = 100 μm.

D. qPCR showing ΔΔC_t value for gene expression of each TSLP isoform from epidermis or dermis in response to 24h wound ex vivo. Values are normalized to NW epidermis for this tissue sample. Data presented are from n=4 independent experiments using 2 different human donors in technical duplicate. Error bars represent ± SEM.

E. Schematic illustration representing cross section of human skin with sweat gland and hair follicle residing in dermis.
**Figure S2: TSLP is expressed in mouse skin after wounding.** Related to Figure 1

A. qPCR for Tslp from epidermis or dermis isolated from healthy, non-wounded mouse back skin; data are normalized to Tslp expression from whole skin.

B-C. Gene expression TSLP by qPCR (B) or ELISA (C) from skin and lymphoid organs. Statistics by one-way ANOVA where n=3 mice (thymus); n=3 mice (spleen); n=11 mice (telogen skin); n=31 mice (Anagen V) for qPCR and n=3 mice (thymus); n=4 mice (spleen); n=19 mice (telogen skin); n=13 mice (Anagen V) for ELISA.

D. Heatmap representation of gene expression of healing excisional wounds in balb/c mice (GSE23006). The normalized expression data has been z-score transformed for each gene across the time-course.

E-F. Immunostaining of skin samples collected 7d after wounding. Scale bar: 20 μm (E) and 100 μm (F).
**Figure S3: TSLP accelerates WIHG.** Related to Figure 2.

A. Photos of mouse skin during active hair cycling showing changes in skin pigment in C57BL/6 mice.

B. qPCR of gene expression of *Il7r* and *Crlf2* in whole skin in each hair follicle stage. Data presented are from n=2 experiments using n=27 mice (anagen I-IV); n=8 mice (anagen V); n=9 mice (anagen VI); n=3 mice (catagen); n=3 mice (telogen).

C. qPCR analysis of *Tslp* from wounded or non-wounded back skin (5dpw). Hair cycles of mice were synchronized, and wounds were induced during telogen or anagen (5 days after depilation) with timeline (right). Data presented are from n=1 experiment using n=3 mice per group.

D. Representative photographs of healing mouse wounds (4-mm full thickness wounds) after treatment with TSLP (100ng/wound) or vehicle.

E-F. Photographs of healing mouse wounds (E) and quantification (F) of total skin area that has entered anagen during WIHN assay (wound area=1.2 cm²) treated with mTslp (250ng total) or vehicle (0.1%BSA) on the day of wounding. Data presented are from n=2 experiments using n=4-6 mice per group.

G-H. Photographs (G) and quantification of skin in anagen (H) from mice after WIHN assay (wound area=1.2 cm²) treated with TSLPR neutralizing antibody (1ug total) or Rat IgG2A (1ug) on the day of wounding and 4 days after wounding. Photographs are representative of 4 mice per group across 1 experiment on 23DPW and 32DPW. nAb, neutralizing TSLPR antibody. IgG, Immunoglobulin. control.

I. Measurements of hair follicle at widest point (left) and epidermal thickness (right) from mouse skin 13 days after treatment with vehicle or TSLP. Data presented are from n=2 experiments using n=3 mice per group and 11-30 unique sections of tissue were analyzed.

Statistics determined using t-test where p<0.01 **, p<0.0001**** Error bars represent ± SEM.
Figure S4: TSLP promotes proliferation of transit amplifying cells. Related to Figure 3.

A. Gating strategy for flow cytometry to live, single cells for analysis.

B. Flow cytometry plots showing total EdU+ cells pregated on live, single cells.

C. Quantification of LGR5+ HFSC after 7d s.c. TSLP treatment by flow cytometry from Lgr5CreER transgenic mice. Data representative of n=4 mice across 2 experiments.

D-E. Gating strategy for flow cytometry to CD34+ cells (D), α6 subsets and histogram showing EdU expression for each α6 subset (E).

F. Flow cytometry plots showing α6 and CD34 expression, gated on live cells.

G-L. Histogram (G-I) and quantification (J-L) of EdU expression for CD34+ α6Hi and CD34+ α6Lo cells isolated from mice treated with TSLP or vehicle control for 7 days (G, J) or wounded and treated with vehicle or TSLP for 7 days (H, K). Data presented are representative of n=4 mice across 2 experiments.
Figure S5: TSLPR expression is enriched in HF epithelial cells. Related to Figure 4.

A. Gating strategy for flow cytometry to live, single cells for analysis.

B. Flow plots for total live cells isolated from hair follicles during wound healing.

C. Quantification of TSLPR+ cells from HF or skin separated by CD45 antigen. Data presented are from n=2 independent experiments using n=2-4 mice per group in technical duplicate.

D. Flow cytometry for TSLPR+ cells between 0 and 7dpw isolated from skin after wounding. Data presented are from n=2 independent experiments using n=2-4 mice per group in technical duplicate.
**Figure S6: TSLPR expression in keratinocyte is essential for WHIG.** Related to Figure 5.

A. Gating strategy for flow cytometry to live, single, CD34+ cells for analysis in Figure 5.

B. Photographs of $Lgr5^{CreER}.Tslpr^{fl/fl}$ mice, $Lgr5^{CreER}.Tslpr^{fl/+}$ mice, and $Tslpr^{fl/fl}$ mice treated with 4-hydroxytamoxifen (4OHT) prior to excisional wound and TSLP treatment.

C-D. Photographs (C) and quantification WIHG (D) of $Lgr5^{CreER}.Tslpr^{fl/fl}$ mice, $Lgr5^{CreER}.Tslpr^{fl/+}$ mice treated with TSLP or vehicle on day 0 after wounding. All mice were treated with 4OHT prior to wound. Data are representative of n=4 mice across 3 experiments.

E. Timeline and immunostaining for Cyclin D1 (green) in wild-type mice treated with s.c. Vehicle or TSLP. Scale: 50 μm.

F. Timeline and immunostaining for Cyclin D1 (green) in $Lgr5^{CreER}.Tslpr^{fl/fl}$ or littermate control mice treated with 4OHT preceding s.c. injection with Vehicle or TSLP for 7 days. Scale: 20 μm.
**Table S1: Gene expression analysis comparing healing skin wounds to TSLP-treated skin.**
Upregulated genes collected during transition from inflammatory to proliferative phase of healing wounds in mouse or human skin that overlap with genes upregulated following subcutaneous TSLP treatment. Related to Figure 6.

| Genes         | Compare to mouse skin 24h wound vs control (GSE23006) | Compare to human day 6 vs day 1 wound (control) (GSE97615) | Overlapping Mouse and Human Genes |
|---------------|--------------------------------------------------------|-----------------------------------------------------------|----------------------------------|
| Ccl5          | CSF3                                                   | DSX6                                                     |
| Csf3          | IL1r1                                                  | STAT1                                                   |
| Il1rl1        | CXCL11                                                 | CXCL5                                                   |
| Cxcl11        | GRB14                                                  | TLR2                                                   |
| Gdap10        | IL6                                                    | PTGS2                                                   |
| Saa3          | CXCL2                                                  | CD274                                                   |
| Il6           | FABP4                                                  | MMP3                                                   |
| Il1rl1        | CXCL2                                                  | CXCL5                                                   |
| Cxcl2         | TNFRSF9                                                | IL6                                                    |
| Gbp2          | CXCL11                                                 | CXCL11                                                  |
| Gbp2b         | CD274                                                  | IL1RL1                                                  |
| Mmp3          | PTGS2                                                  | CSF3                                                   |
| Clec4d        | TLR2                                                   |                                                         |
| Cxcl1         | STEAP1                                                 |                                                         |
| Cd274         | GSMB                                                   |                                                         |
| Ddx6          | SLAMF7                                                 |                                                         |
| Cxcl1         | CTLA4                                                  |                                                         |
| Car4          | CXCL5                                                  |                                                         |
| Irgm2         | CXCL17                                                 |                                                         |
| Ptgds2        | CXCL9                                                  |                                                         |
| Tlr2          | STAT1                                                  |                                                         |
| Gbp3          | GBP6                                                   |                                                         |
| Ifit2         | DDX6                                                   |                                                         |
| Cxcl5         |                                                         |                                                         |
| Stat1         |                                                         |                                                         |

**Table S2: Primers used for qPCR.**

| Species and Gene | Forward (5'-3') | Reverse (3'-5') |
|------------------|-----------------|-----------------|
| Mouse Tslp       | TTTGCCCGGAGAAAGAGAGAG | TTTGGACTTCTTGTGGCATTTC |
| Human TSLPtotal  | TTTGGAATGCGAGCCCTTCAG | AGGTGTAGAAATTTGAGGAGAG |
| Human fTSLP      | GACTGGCAATGAGCGGAAA | TCTTCCCCACGAGGAGTAA |
| Human sTSLP      | CGTAAACTTTGCGGCCTATGA | ACTTGGGTCTTGGTCCCCTCA |
| Human FLG        | GTTGGTTAAAGATGAAGGGATTTCGCG | GCTTGACCAACTTGAATACCAT |
| Mouse Crlf2      | CTACATGACCCCTGACCTTG | GGCACAGGATTTGAGTGTG |
| Mouse Itfi    | GCCGTATGTCAACATGCTCTG | AGCATCCAGACCTTCCATCTC |
| Human GAPDH      | ATGGGAAAGTTGAGGGTCGGGA | CAGCGTCAAAGGAGGAGGAGG |
| Mouse Gapdh      | GCACAGTCAAGGCCGAGAAT | GCCTTCTCCATGGTGGTGAAG |
| Human DDX6       | AGCCCGAGGAATCAACAATAAG | ACTGAGGTAAGAAAGGAGAGGA |

**Table S3: Primers used for Genotyping.**

| Primers used for mouse Genotyping | Forward (5'-3') | Reverse (3'-5') |
|----------------------------------|-----------------|-----------------|
| Mouse Tslpr<sup>Fox</sup>         | GGAAGGAACATGACGATGAGG | GAAACCCGAAGTCATAGCAG |
| Mouse Lgr5<sup>CreERT2</sup>     | CCTACTGCAAGACTTACCAG | GCATTGGGTGAAATGAGCAG |
### Table S4: STAR Methods reagent and resource table

| REAGENT or RESOURCE | SOURCE | IDENTIFIER |
|---------------------|--------|------------|
| **Antibodies**      |        |            |
| Mouse TSLPR-APC (Clone 22H9) | Biolegend | 151805 |
| Mouse TSLPR-BV421(Clone 22H9) | Biolegend | 151807 |
| Mouse CD45-Pacific Blue (Clone 30-F11) | Biolegend | 103126 |
| Mouse CD3-PE (Clone 17A2) | Tonbo | 50-0032-U100 |
| Mouse TSLP (Clone 17A2) | Biolegend | 515202 |
| Mouse CD34-PE (Clone Mec14.7) | Biolegend | 119307 |
| Mouse CD49f-AF647 (GoH3) | Biolegend | 313610 |
| antiEGFP-AF488 (pAb) | Invitrogen | A-21311 |
| Mouse CD31-APC (Clone 390) | Biolegend | 102410 |
| Mouse Thy1.2-PerCP-Cy5.5 (Clone 53-21) | Biolegend | 140322 |
| Mouse Ki67 (Clone SP6) | Invitrogen | MA5-14520 |
| Mouse TSLP nAb (Clone 152614) | Novus Bio | MAB555100 |
| Human TSLP | Abcam | Ab47943 |
| Human DDX6 | Invitrogen | PA5-18478 |
| Human Ki67-Pacific Blue (Clone KI-67) | Biolegend | 350512 |
| Cyclin D1 | Cell Signaling Technology | 55506 |
| Human KRT15 | invitrogen | Cat #MA5-11344 |
| Human hfTSLP | Show Vendor | N/A |
| Anti Rabbit IgG-AF555 | Invitrogen | A10520 |

| **Biological Samples** |        |            |
|------------------------|--------|------------|
| Human wound punch biopsy | Duke University Health System | N/A |

| **Chemicals, Peptides, and Recombinant Proteins** |        |            |
|---------------------------------------------------|--------|------------|
| Ghost Dye™ Violet 510 | Tonbo | 13-0870-T100 |
| Recombinant mouse TSLP | R & D Biosystems | 555-TSB-010 |
| Recombinant human TSLP | Biolegend | 582404 |
| Recombinant human hfTSLP | This paper | NA |
| Recombinant human sfTSLP | This paper | NA |
| iScript cDNA Synthesis kit | Biorad | Cat# 1708891 |
| Triton X-100 | Sigma-Aldrich | T8787 |
| ProlongGold Antifade reagent | Thermo Fisher Scientific | P36930 |
| qPCRBIO SyGreen Blue Mix Hi-ROX | Genesee Scientific | 17-507DB |
| Epilife Calcium Free Media | Gibco | MEPICF |
| Human Keratinocyte Growth Supplement (HKGS) | Gibco | S0015 |
| TRIzol | Thermo Fisher Scientific | 15596026 |
| DNase I | VWR | IC19006210 |
| Collagenase II | Life Technologies | 17101015 |
| Hoechst 33342 | Invitrogen | H3570 |
| 0.5% Trypsin-EDTA (10x), no phenol red | Gibco | 1540054 |
| Click-iT™ Plus EdU Cell Proliferation Kit for Imaging, Alexa Fluor™ 647 dye | Invitrogen | C10640 |
| (Z)-4-Hydroxytamoxifen | Sigma     | H7904  |
|-------------------------|-----------|--------|

**Critical Commercial Assays**

| Test                        | Supplier   | Code   |
|-----------------------------|------------|--------|
| TSLP ELISA kit (human)      | Biolegend  | 434204 |
| TSLP ELSIA kit (mouse)      | Biolegend  | 434104 |

**Experimental Models: Cell Lines**

| Cell Line                                | Supplier | Code   |
|------------------------------------------|----------|--------|
| Normal human epidermal keratinocytes (HEKa) | Gibco    | C0055C |

**Experimental Models: Organisms/Strains**

| Organism                                | Supplier       | Stock No     |
|-----------------------------------------|----------------|--------------|
| C57BL/6J mice                           | Jackson Labs   | Stock No: 00064 |
| Lgr5-eGFP-IRES-creERT2                  | Blanche Capel, Duke University | Jax Stock No: 008875 |
| Tslpr<sup>flox/flox</sup>               | Steven Ziegler, Benaroya Institute | N/A |


Experimental Methods

1. Animal study

Female and male C57BL/6/J (6-12 weeks of age; the Jackson Laboratory, Bar Harbor, ME), were used in our studies. Mice were maintained under specific pathogen-free conditions and were sustained under regulated conditions with food and water ad libitum in the pathogen-free facility at Duke University. All mice were in the same hair cycle while in vivo experiments were performed. The protocol was approved by the Institutional of Duke University Animal Care and Use Committee under protocol A156-17-06. Lgr5<sup>CreER</sup>.Tslpr<sup>fl/fl</sup> mice were used to study the role of TSLPR ablation in HFSCs during developmentally programmed hair growth and growth after wounding. Briefly, Lgr5<sup>CreER</sup> mice (kindly provided by Blanche Capel, Duke University; available from Jackson Labs, Stock #008875) (Barker et al., 2007) were crossed to Tslpr<sup>fl/fl</sup> mice (kindly provided by Stephen Ziegler, Benaroya Institute). Genotypes were validated using PCR and all experimental cohorts were co-housed; animal experiments were controlled using littermates lacking Lgr5<sup>CreER</sup> or at least one copy of Tslpr<sup>fl</sup>. To study the role of TSLPR in WIHG in adult mice, animals were carefully shaved and treated with 50ug 4-Hydroxytamoxifen (4OHT) (Sigma # H7904) dissolved in 100% ethanol at p46, p48, and p50. 4OHT was delivered dropwise directly to the back skin in the center of the back. Mice were then wounded at p54 in second telogen.

In vivo skin injury: 6-12-week old male and female mice were used in experiments where 4-mm diameter punch biopsy wounds or 12-mm diameter wounds were induced on the back skin of anesthetized mice. All experimental groups were in an equivalent telogen stage of the hair cycle unless otherwise stated. 4-6 full-thickness wounds were prepared paravertebrally on the back of each mouse using a punch biopsy instrument for 4mm wounds. Only one wound in the center of the back was used for 12-mm diameter excisional wounds.

Cytokine treatment: When used, recombinant murine Tslp (R&D Systems, 555-TS-010) was delivered directly to wound site in a total volume of 5μL. p50 mice in second telogen were anesthetized, shaved, and injected at tail base using 31g needle and allowed to diffuse across mouse back. Either 100μL 0.01% BSA in 1x DPBS or 250ng TSLP in 100μL total PBS was delivered; experimenters were blinded to animal groups.

Hair cycle synchronization and analysis: Hair follicle synchronization and analysis of stages were based on criteria outlined in Müller-Röver et al. (Müller-Röver et al., 2001). Briefly, hair was gently depilated in defined areas on dorsal skin of mouse. Mice were shaved with electric clippers when in telogen. Entry to anagen was determined by darkening of the skin and re-appearance of hair. Cycle entry into catagen was determined by pigmentation in the skin. Hair cycle progression was completed using ImageJ to quantify skin darkening (coupled to melanogenesis). Mice were checked every 2-4 days. Hair cycle phenotypes are consistently observed in both male and female mice. Mice were monitored for hair growth with were taken every 2-4 days after stimulation. Telogen' denotes telogen following a complete hair cycle.

Quantification of total skin darkening and measurements of area of anagen skin was completed using ImageJ Software. When harvesting skin for subsequent analysis, scissors were used to bluntly dissect away the epidermis, dermis, and minimal subcutaneous fat from the underlying muscle. Subcutaneous fat was mechanically removed.

2. Flow cytometry

To produce skin cell suspensions from whole skin, skin pieces were processed as described in (Yang et al., 2017). Skin samples collected during anagen (where stated), skin pieces were floated first on 2% Collagenase Type II for 1-2h on ice prior to floating on Trypsin GNK overnight at 4C. Epidermis was gently peeled away from dermis following incubation step. Air interface was maintained with epidermis during each step. Dermal tissue minced using surgical scissors and incubated at 37C for 1h in collagenase type II (2 mg/ml; Thermo Fisher Scientific). To produce skin cell suspensions from hair follicles, hair was gently depilated from intact skin surrounding wounds and submerged in 0.1% Trypsin GNK solution and incubated on ice for 1h with agitation to encourage physical dissociation. Solution was inactivated with equal volume keratinocyte medium containing 5% FCS and filtered through 30um filter. Epidermal cell
suspensions were prepared from dorsal skin and stained for CD45, eGFP (LGR5), CD34, and integrin α6 (ITGA6). Bulge HFSCs were defined as CD45 LGR5⁺CD34⁺. Suprabasal bulge cells were identified as ITGA6lo and basal bulge cells were identified as ITGA6hi. LGR5 eGFP signal amplified using anti-EGFP-AF488 (Invitrogen A-21311). Flow cytometry was performed on Canto II (BD Biosciences) and data were analyzed using FlowJo software (Treestar). Staining antibodies are listed in reagent table; Live dead staining (GhostViolet, Tonbo) was performed according to manufacturer instructions. All samples were blocked using anti CD16/32 Antibody (Biolegend) and staining was performed in FACS buffer for 40 minutes on ice.

3. Cell culture

Adult human keratinocytes (Gibco) maintained for up to 6 passages in T-75 flasks. Cells were grown in 37°C serum-free EpiLife cell culture medium (Gibco) with human keratinocyte growth supplement containing 0.05 mM Ca2+. Cells were passaged by dissociating adherent cells 0.05% Trypsin EDTA, equal volume of 1x defined trypsin inhibitor, and resuspended in complete keratinocyte medium. Media was replaced 16-24h after passaging into well plates for experiments containing recombinant human TSLP (Biolegend) at a concentration of 100 ng/mL.

4. EdU proliferation

EdU was reconstituted in sterile PBS at 10 mM concentration Thermo Fisher Scientific (MA, USA, Catalog # A10044), and stored at ~20 °C. EdU was delivered using intraperitoneal injection 3h prior to tissue collection at 20 ug/g (eg 20g mouse received 400ug EdU in 200uL PBS). Copper-catalyst based Click reaction performed according to manufacturer instructions (Invitrogen C10640, C10635) and analyzed using flow cytometry.

5. Skin explant preparation and culture.

Normal skin samples were obtained from otherwise discarded tissue of male and female patients age ~40-70 years undergoing abdominoplasty at Duke University Medical Center under protocol Pro00079799 and used in an anonymized fashion. All human samples for this study were obtained according to the protocols approved by the Institutional Review Board at Duke University. Tissue was prepared as described (Suwanpradid et al., 2017). Tissue was processed by removing adipose tissue layer and cut 1cm by 1cm by 0.5cm (depth). Tissue was placed on surgifoam to maintain epidermal-air interface in 0.5mL DMEM Dulbecco’s modified Eagle’s medium (DMEM) (Gibco #11054001) containing 2% FBS in 12-well tissue culture dish. To wound tissues, surgical scissors were held with blades perpendicular to tissue and 20 repeated incisions were made to tissue. Unless stated otherwise, all SOC samples were cultured for 24 hours. To separate epidermis and dermis, skin samples were floated on Trypsin GNK (0.3% trypsin/0.1% glucose, 14.8 mM NaCl, 5.3 mM KCl) (Sigma Aldrich, St. Louis, MO), for 15 minutes at 37 °C. Epidermal and dermal fractions were subsequently minced for trizol-RNA extraction.

6. Immunofluorescent staining and microscopy

Murine back and human skin wounds were excised and placed into OCT cassettes and stored at -80°C for sectioning. Antibodies used can be found in the Key Resources Table. OCT sections were cut at 9 μM were prepared as following:Human skin sections and cell culture slides were fixed using 4% PFA for 10 minutes. Tissues/cells were permeabilized with 0.1% Triton for 10 minutes. Mouse skin sections were fixed and permeabilized using Acetone at -20°C for 15 minutes. All samples were subsequently blocked for a minimum of 1h. Tissue sections were stained with a primary antibody overnight at 4°C. The following day, sections were washed stained with secondary Ab at 1:800 dilution at room temperature in the dark for 3h. After washing and adding a Hoechst stain at 1:6000 dilution, sections were mounted using Gold Anti-Fade reagent and a cover slip and imaged using the Olympus IX73 or Zeiss 780 upright confocal microscope.

7. RNA isolation, cDNA synthesis, and quantitative RT-PCR (qPCR)

Total RNA was isolated from trizol using TRIZOL reagent per the manufacturer’s instructions (Invitrogen), followed by treatment with DNase I. RNA was quantified using Nanodrop 1000 or cDNA was generated
using cDNA Synthesis Kit (Bio-Rad, Hercules, CA). Amplification was detected using Fast SYBR Green Master Mix (Applied Biosystems) or sybrgreen blue (PCR biosystems) and 10-20ng cDNA used per qPCR reaction. qPCR was performed with primers as indicated in resource table. GAPDH expression was used as internal control (see primer sequences). Fold changes calculated for gene expression in human samples were normalized to donor-matched non-wounded control. In vitro and in vivo experiments were normalized to untreated or unwounded controls as indicated. Data are represented as fold change or using ddCt method as indicated. Where necessary, data were Log2 transformed to best represent the observed proportional changes.

8. Statistical analyses

Group sizes were determined based on the results of preliminary experiments without pre-determination of sample size. Preliminary experiments were performed to determine requirements for sample size, considering resources available and ethical, reductionist animal use. Mice were randomly assigned to groups. Mouse studies were not performed in a blinded fashion. Statistical analysis using the two-tailed unpaired Student’s t test, under the untested assumption of normality, was calculated using Prism software (GraphPad) unless specified otherwise. A p-value of <0.05 was considered statistically significant. Where p<0.01 **, p<0.001***, p<0.0001 ****. Error bars represent ± SEM.

9. Protein quantification and ELISA

Total protein was extracted from mouse tissue with Pierce RIPA Buffer (ThermoScientific) with proteinase inhibitor cocktail (EDTA-free, Roche). Protein concentration was determined using DC protein assay. TSLP mouse ELISA kit was used to determine the amount of TSLP according to manufacturer instructions.

10. Microarray and RNA Sequencing analysis

The mouse microarray data from Christmann et al. (GSE34297) and the skin samples from Chen et al. (GSE23006) were each normalized independently by the robust multichip average (RMA) approach to eliminate systematic differences across the arrays using the affy (Gautier et al., 2004) bioconductor (Huber et al., 2015) package from the R statistical programming environment. Differential expression between skin treated with subcutaneous TSLP or vehicle was carried out using the limma (Ritchie et al., 2015) package.

The RNA-sequencing dataset from Iglesias-Bartolome et al. (GSE97615) contained samples from human axillary skin wounds at baseline (Day 1, unwounded), two days after full-thickness 3-mm punch biopsy wounding (Day 3), and five days after wounding (Day 6) (Iglesias-Bartolome et al., 2018). The raw data was downloaded and processed using the fastp toolkit (Chen et al., 2018) to trim low-quality bases and Illumina sequencing adapters from the 3’ end of the reads. Only reads that were 20nt or longer after trimming were kept for further analysis. Reads were mapped to the GRCh38v93 version of the human genome and transcriptome (Kersey et al., 2012) using the STAR RNA-seq alignment tool (Dobin et al., 2013). Reads were kept for subsequent analysis if they mapped to a single genomic location. Gene counts were compiled using the featureCounts tool (Liao et al., 2014). Only genes that had at least 10 reads in any given library were used in subsequent analysis. This resulted in a set of 12 samples and 23,378 genes. Normalization and differential expression between Day 6 and Day 1, as well as Day 3 and Day 1, was carried out using the DESeq2(Love et al., 2014) bioconductor (Huber et al., 2015) package with the R statistical programming environment. The p-values were corrected for multiple hypothesis testing by the Benjamini-Hochberg method. A gene was considered downregulated if it had an adjusted p-value <= 0.05 and a negative fold-change in either the Day 3-vs-Day 1 analysis or the Day 6-vs-Day 1 analysis. Likewise, a gene was considered upregulated if it was significant in either comparison along with a negative fold-change.

11. Data availability

We used the publicly available microarray datasets from Christmann et al. (GSE34297), Chen, et al (GSE23006) and the RNA-seq data set from Iglesias-Bartolome et al. (GSE97615).

12. iFTSLP pAb design overview (AbClonal):
Synthesized and subcloned target TSLP gene (130 amino acids) to bacterial expression vector and expressed protein for use as antigen. Additional peptide expression for positive purification using specific fragment (DFTNCDFEKIAAYLSTISKDLITYMSGTKSTENVTSCSNRPHELQTEIQSMTFNPTAGCASLAKE) And negative purification of sfTSLP non-specific peptide. The antibody was designed to only target the specific fragment using cross purification.

**Protein expression and purification**

1. Supernatant
2. Supernatant 2 (2M Urea dissolved inclusion bodies)
3. Inclusion body diluted in 2 folds (BM Urea dissolved inclusion bodies)
4. Inclusion body diluted in 10 folds (BM Urea dissolved inclusion bodies)
5. pET-28a-SUMO Empty Induced expression
6. 0.4mg/ml BSA
7. Marker

pET-28a-SUMO-TSLP (29-159aa) was expressed as inclusion bodies. The inclusion body protein was 6 mg/mL concentration, so it was used for immunization.

![ELISA Image]
Antisera were purified by the specific fragment (peptide 1) and the non-specific fragment (peptide 2).

Antibody concentrations
E9417(P) Conc.: 0.58mg/mL; E9417(C) Conc.: 0.59mg/mL
E9418(P) Conc.: 0.34mg/mL; E9418(C) Conc.: 0.32mg/mL
E9419(P) Conc.: 0.90mg/mL; E9419(C) Conc.: 0.27mg/mL
E9420(P) Conc.: 0.33mg/mL; E9420(C) Conc.: 0.18mg/mL

Sensitivity:

Note: Lanes one through four were loaded with 10ng, 5ng, 1ng, and 500pg of antigen, respectively. The antibodies are in a 1:1000 dilution ratio.

Analysis:
- Bands of 34KD were observed for E9417(C), E9418(C), and E9419(C), consistent with the expected molecular weight.
- 10ng of Ag can be detected by antibodies E9417(C), E9418(C)
- 5ng of Ag can be detected by antibodies E9419(C)

E9419(C) used for immunostaining as described (1:400 dilution)
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