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UNIVERSITY OF CALIFORNIA SAN DIEGO

Fibroblast-Expressed IL-17D in Skin Inflammation
and Cutaneous Wound Healing

A Dissertation submitted in partial satisfaction of the requirements
for the degree Doctor of Philosophy

in

Bioengineering

by

Lu Wang

Committee in charge:

Professor Jack Bui, Chair
Professor Geert Schmid-schoenbein, Co-Chair
Professor Prashant Mali
Professor Gerald Morris

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University of California San Diego

2026

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List of Abbreviations

AMP	antimicrobial peptide
APC	antigen-presenting cell
BMDM	bone marrow-derived macrophage
CCL	C-C motif chemokine ligand
CCL2	C-C motif chemokine ligand 2, also known as MCP-1
CCR	C-C motif chemokine receptor
CD	cluster of differentiation
CD93	cluster of differentiation 93
CTLA-8	cytotoxic T-lymphocyte-associated antigen 8
DAMP	danger-associated molecular pattern
DAZ	deleted in azoospermia
DAZAP2	DAZ-associated protein 2
DC	dendritic cell
DETC	dendritic epidermal T cell
DSS	dextran sulfate sodium
ECM	extracellular matrix
FBS	fetal bovine serum
GM-CSF	granulocyte-macrophage colony-stimulating factor
GO	Gene Ontology
GOBP	Gene Ontology Biological Process
Het	heterozygous
HSV13	Herpesvirus saimiri open reading frame 13
IFN	interferon
IgE	immunoglobulin E
IL	interleukin
IL-17	interleukin-17
IL-17A-F	interleukin-17 A through interleukin-17 F
IL-17D	interleukin-17 D

IL-17R	interleukin-17 receptor
IL-17RA-E	interleukin-17 receptor A through interleukin-17 receptor E
IL-23	interleukin-23
IL-25	interleukin-25, alternative name for IL-17E
ILC	innate lymphoid cell
ILC3	innate lymphoid cell, type 3
KGF	keratinocyte growth factor
KO	knockout
LC	Langerhans cell
MAPK	mitogen-activated protein kinase
3-MCA	3-methylcholanthrene
MCMV	Murine Cytomegalovirus
MCP-1	monocyte chemotactic protein-1
M-CSF	macrophage colony-stimulating factor
MFG-E8	milk fat globule-EGF factor 8
MHC	major histocompatibility complex
MyD88	myeloid differentiation primary response 88
NES	normalized enrichment score
NF- κ B	nuclear factor kappa B
NGF	nerve growth factor
NK cell	natural killer cell
NKT cell	natural killer T cell
ORF	open reading frame
PAMP	pathogen-associated molecular pattern
PBS	phosphate-buffered saline
PDGF	platelet-derived growth factor
rhIL-17D	recombinant human IL-17D
rmIL-17D	recombinant mouse IL-17D
SARS-CoV-2	severe acute respiratory syndrome coronavirus 2

scRNA-seq	single-cell RNA sequencing
SMURF2	SMAD-ubiquitin regulatory factor 2
SPF	specific pathogen-free
STAT5	signal transducer and activator of transcription 5
TAM pathway	Tyro3-Axl-Mertk pathway
TGF- β	transforming growth factor beta
Th2	T helper type 2
Th17	T helper type 17
Tc17	T cytotoxic type 17
TNF	tumor necrosis factor
TRAF	TNF receptor-associated factor
T _{RM}	resident memory T cell
T _{reg}	regulatory T cell
TSLP	thymic stromal lymphopoietin
T $\gamma\delta$ cell	gamma delta T cell
Umap	uniform manifold approximation and projection
WT	wild type

Acknowledgements

I would like to express my deepest gratitude to my advisor, Dr. Jack Bui, for his guidance, mentorship, and support throughout my doctoral training. His scientific insight and encouragement have shaped my development as a researcher and have been invaluable to the completion of this dissertation. I am also grateful to my dissertation committee members, Dr. Gerald Morris, Dr. Geert Schmid-schoenbein and Dr. Prashant Mali, for their time, thoughtful feedback, and guidance on this project. Their perspectives helped strengthen the experimental direction and interpretation of this work.

I am deeply thankful to the members of the Bui laboratory, past and present, for their technical assistance, scientific discussions, and friendship. I am especially grateful to Diane Dinh, Lance Nguyen, Taian Chen and Sophia Hidalgo for their contribution in the protocol development and data collection.

Results Section 2 contains unpublished material coauthored with Dinh, Diane. The dissertation author was the primary author of this section.

Results Section 3.1 contains unpublished material coauthored with Nguyen, Lance and Hidalgo, Sophia. The dissertation author was the primary author of this section.

Results Section 4 contains unpublished material coauthored with Chen, Taian. The dissertation author was the primary author of this section.

I would also like to acknowledge the Morris lab for the great scientific discussions we had in weekly joint lab meetings, the Klemke lab and the Gonias lab for sharing their equipment, the Meier lab for providing the HaCaT cell line.

I thank the friendly and helpful staff from the Animal Care Program (ACP) and the Center for Computational Biology and Bioinformatics (CCBB) at University of California, San Diego. I greatly appreciate the staff at the IGM Genomics Center, University of California, San Diego, for their technical support with single-cell RNA sequencing and bulk RNA sequencing. I also acknowledge the Department of Pathology community for providing a supportive academic environment.

This work was supported by the National Institutes of Health (NIH) under grant number R21 AI149023 to Dr. Jack Bui. This publication also includes data generated at the UC San Diego IGM Genomics Center utilizing an Illumina NovaSeq 6000 that was purchased with funding from a NIH SIG grant (S10 OD026929).

Finally, I am deeply grateful to my family, friends and my cat Rousong for their patience, encouragement and support. I also sincerely thank DaBoba and Yun Tea House for providing many small moments of comfort and motivation throughout graduate school.

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Abstract of the Dissertation

Fibroblast-Expressed IL-17D in Skin Inflammation and Cutaneous Wound Healing

By

Lu Wang

Doctor of Philosophy in Bioengineering

University of California San Diego, 2026

Professor Jack Bui, Chair

Professor Geert Schmid-schoenbein, Co-Chair

The interleukin-17 (IL-17) family comprises a group of cytokines involved in host defense against pathogens, autoimmune diseases and anti-tumor immunity, making IL-17 signaling pathways promising therapeutic targets. Among the six IL-17s found in human, Interleukin-17 D (IL-17D) is the least studied. Previous work from our lab has

demonstrated that IL-17D is regulated by the NRF2 pathway and contributes to tumor and virus surveillance through recruitment of natural killer cells. Compared to wild-type (WT) mice, IL-17D knockout (KO) mice exhibit reduced resistance to carcinogen-induced tumorigenesis, viral and bacterial infection, and experimental colitis.

As the function of IL-17D appears to be context specific, and a recent study demonstrated IL-17D may promote atopic dermatitis development through DDX5 inhibition in keratinocytes, this dissertation explored the roles of IL-17D in the skin biology. In mouse psoriasis-like and atopic dermatitis-like skin inflammation models, IL-17D KO and WT mice showed no significant differences in disease progression. Single-cell RNA-sequencing analyses revealed that IL-17D was mainly expressed by fibroblasts in both mouse and human skin. In scratch assays *in vitro*, IL-17D promoted migration of fibrosarcoma, macrophage and keratinocyte cell lines, as well as primary bone-marrow derived macrophages (BMDMs). *In vivo*, IL-17D modestly accelerated wound healing speed and changed wound morphology in a splinted excisional punch wound model. Together, these findings inferred a role of IL-17D in facilitating wound healing. Bulk RNA-sequencing analysis of BMDMs further suggested IL-17D can modulate antigen presentation- and phagocytosis-associated transcriptional programs, providing a potential mechanism by which IL-17D may influence macrophage function during tissue repair.

Introduction

1. IL-17 family cytokines in protective immunity and diseases

1.1. Overview of IL-17 family cytokines

Interleukins (ILs) are a group of cytokines mainly secreted by white blood cells that transfer signals such as growth, migration and differentiation between cells. Among them, the interleukin-17 (IL-17) family is highly involved in mucosal protection against pathogens, including fungi, bacteria and viruses. IL-17 cytokines are produced rapidly in early inflammation against pathogens. In barrier tissue such as skin, gut and airways, when sentinel immune cells – antigen-presenting cells (APCs) such as macrophages – encounter pathogens and stressed cells, these APCs get activated by pathogen-associated molecular patterns (PAMPs) and danger-associated molecular patterns (DAMPs). The APCs then relay the information to professional cytokine factory cells such as type 3 innate lymphoid cells (ILC3s), T helper type 17 (Th17) cells, gamma delta T ($T_{\gamma\delta}$) cells, which then produce pro-inflammatory cytokines including IL-17A and IL-17F. These pro-inflammatory cytokines not only alert local barrier epithelial cells to recruit innate immune cells, but also contribute in the activation of recruited immune cells so that they can kill the pathogen and clear up debris via phagocytosis [1, 2].

In human, IL-17 family comprise of 6 cytokines – IL-17A to IL-17F, and most of them signal through the IL-17 receptor (IL-17R) family members IL-17RA through IL-17RE. IL-17A was the first identified member of the IL-17 family in the 1990s, formally known as cytotoxic T-lymphocyte-associated antigen 8 (CTLA-8) [3, 4]. Other IL-17

family members were identified through subsequent expression sequence tags and large-scale sequencing of the genomes of several vertebrate species [5]. Most IL-17 cytokines form homodimers via disulfide bonds. The exception is that IL-17F, with 55% sequence homology to IL-17A, can form heterodimers with IL-17A and elicit similar but less potent downstream signaling and subsequent immune responses as IL-17A homodimers [6–10]. The IL-17F homodimers also elicit the same signaling pathway as IL-17A, which I will further discuss in later sections.

IL-17 cytokines are evolutionarily ancient and present in various organisms. Homologs to all 6 human IL-17s have been found in several fish species [11]. In Japanese medaka, knockout of IL-17A/F1 or its receptor can lead to intestinal antimicrobial cytokine signaling and alter gut microbiota [12, 13]. In Nile tilapia, effector T cells produce IL-17A in response to bacteria challenge, which in return modulates T cell activation via the nuclear factor kappa B (NF- κ B) pathway [14]. Similarly, Th17-type response was also demonstrated in zebrafish, teleost, carp and pufferfish [15–18]. Not only do IL-17s play a role in bridging innate and adaptive immunity in vertebrates, but they are also crucial in innate immunity in invertebrates that lack adaptive immune systems. The IL-17s found in invertebrates are less homologous to the 6 human cytokines in sequence and are often assigned different names. Here, these molecules are collectively referred as IL-17. In purple sea urchins challenged by bacteria, four IL-17s were upregulated in larvae but a different IL-17 was upregulated in adult animals [19]. Sea cucumber, pacific oyster, pearl oyster and thick shell mussel have also shown to have elevated IL-17 and inflammation during bacterial infection [20–23]. In the sea

cucumber study, silencing IL-17 led to reduced migration and proliferation of coelomocytes *in vitro* [20]. Recombinant pacific oyster IL-17 has also been shown to promote hemocytes proliferation *in vivo* [24]. In amphioxus, IL-17s are shown to induce expression of anti-bacterial proteins including the antimicrobial peptide (AMP), β -defensin, lysozyme-i and complement C3 [25]. Interestingly, IL-17 in nematodes can act as a neuromodulator and change oxygen sensing mechanism of the neurons, while regulating the worms' immunity and lifespan [26, 27].

Notably, IL-17-like sequences were also found in some viruses. The first discovery of IL-17 was granted by the fact that Herpesvirus Saimiri virus adopted IL-17 into their genome at open reading frame (ORF) 13, also noted as HVS13 and viral IL-17 (vIL-17) [3, 4]. HVS13 is 57% homologous to the mouse IL-17A, can activate IL-17RA and elicit IL-6 secretion in mouse fibroblasts [4]. The severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) were also known to have IL-17 mimicry sequence in its genome – ORF8. The structure of SARS-CoV-2 ORF8 is an Ig-like fold, which is topologically distinct from IL-17s [28]. However, it possesses the ability to bind to IL-17RA and IL-17RC, enhance their receptor dimerization and elicit downstream signaling through NF- κ B pathway [29, 30]. Since the interaction between IL-17A and IL-17RA is on one side of the IL-17A ligand, the ability of SARS-CoV-2 ORF8 interacting with IL-17RA may be a result of its surface features involving L84 [30, 31]. Meanwhile, SARS-CoV-2 ORF8 seems to have other binding sites on mammalian cells. ORF8 was able to activate mouse bone marrow-derived macrophages (BMDMs) and induce secretion of cytokines and chemokines via signaling protein myeloid differentiation primary response

88 (MyD88) and independent of IL-17RA [32]. In human patient monocytes, ORF8 glycoprotein is also able to induce the secretion of IL-1 β , IL-8 and C-C motif chemokine ligand (CCL) 2, which is far from the IL17-RA downstream responses [33]. It is also shown that ORF8 can bind to complement C3/C3b components and inhibit the alternative pathway of the complement system [34]. The possession of IL-17-like sequences is not essential for the infection, replication and pathogenicity of the two viruses [35, 36]. Other than activating the canonical IL-17RA/RC pathway, researchers hypothesize that the adoption of IL-17-mimicry sequences helps the SARS-Cov-2 virus modulate its host immune system via downregulation of major histocompatibility complexes (MHC)-I on immune cells and inhibition of Type I interferon (IFN) production [37, 38].

1.2. Structure and signaling of IL-17 cytokines and their receptors

IL-17 cytokines adopt an atypical cystine-knot structure, analogous to nerve growth factor (NGF) and platelet-derived growth factor (PDGF), but distinct from other immune cytokines [1, 39]. As of today, the structure and receptor binding of IL-17A, IL-17E (also known as IL-25) and IL-17F have been verified with X-ray crystallography. IL-17A and IL-17F dimers have been reported to have two interchain disulfides and two intramolecular disulfides, while the number of disulfide bonds have only be hypothesized for the other members of IL-17 family [39–41].

IL-17A is the most well-studied member of the cytokine family. Structure of the IL-17A homodimer and the binding geometry of IL-17A-IL-17RA was first elucidated [31, 42]. Yet it was later proved the downstream signaling of IL-17RA requires docking of IL-

17RC [31, 43, 44]. IL-17A, IL-17RA, IL-17RC together form a 2:2:2 hexameric signaling assembly and signal intracellularly via Act1 adaptor protein [45–47]. Act1 can bind several tumor necrosis factor (TNF) receptor-associated factor (TRAF) proteins. Upon recruited by the IL-17RA/RC complex, Act1 either bind TRAF6 and promote activation of the NF- κ B and mitogen-activated protein kinase (MAPK) pathways, or bind TRAF2/5 and mediate mRNA stabilization [5, 48, 49]. IL-17RD can also form complex with IL-17A and IL-17RA and prove essential for the canonical IL17RA/RC signaling in certain biological contexts, but the detailed mechanism remains unexplored [50, 51].

IL-17F was the first in the family to be structurally characterized in 2001 [39]. It was also structurally confirmed that IL-17F homodimers can bind IL-17RC only at a 1:2 ratio or bind to the IL-17RA/RC signaling complex [52]. The binding of IL-17F and IL-17RC may compete with the IL-17RA/RC signaling complex structurally, but the downstream signaling of IL-17F-IL-17RC has not been established. IL-17A/F heterodimers also signal through the IL-17RA/IL-17RC receptor complex and engage broadly similar downstream pathways to IL-17A and IL-17F homodimers [7–9, 53]. However, IL-17A, IL-17F, and IL-17A/F differ in receptor-binding properties and signaling potency, resulting in overlapping but non-identical inflammatory responses [7, 8, 54–56].

Although IL-17E is functionally different from IL-17A and IL-17F, it signals in a similar way: IL-17E, IL-17RB, IL-17RA form signaling complex at a 1:2:2 ratio and elicit downstream signaling via Act1 and TRAF6 [57–59]. Three features of IL-17E-IL-17RA/RB signaling that are distinct from IL-17A/F-IL-17RA/RC signaling has been

reported. Firstly, IL-17E can activate the NF- κ B and MAPK pathways without engaging TRAF6 [60]. Secondly, IL-17E can signal via TRAF4 and SMAD-ubiquitin regulatory factor 2 (SMURF2) to promote the degradation of DAZ-associated protein 2 (DAZAP2) [61]. Thirdly, IL-17E can activate the signal transducer and activator of transcription 5 (STAT5) pathway [62]. Together, these differences contribute to the functional differences between IL-17E and IL-17A/F.

The IL-17 of our interest, IL-17D, evolutionarily has the most conserved locus among its cytokine family. When fish IL-17s were identified, fish IL-17D showed the highest gene synteny as its human counterpart [63]. The crystal structure of IL-17D has not been fully characterized, but there are some known unique structural features. IL-17D is the largest in the IL-17 family. The monomer protein is 22 kDa by itself, and roughly 26 kDa after glycosylation. Hence the 53-kDa dimerized IL-17D is 1.5-fold as large as the 35-kDa IL-17A [64]. Moreover, IL-17D has not been reported to bind to any of the IL-17R family members. To date, the only known protein that binds to IL-17D is CD93, also known as complement protein 1 q subcomponent receptor C1qR1 or C1qRp [65]. The transmembrane version of CD93 can bind to extracellular signaling proteins such as multimerin-2 (MMRN2) and insulin-like growth factor-binding protein 7 (IGFBP7), and thereby regulate cell adhesion, migration, efferocytosis and angiogenesis [66, 67]. The only known biological process upstream of IL-17D is the nuclear factor erythroid 2-related factor 2 (NRF2) pathway activated by oxidative stress [68, 69].

The crystal structures of IL-17B and IL-17C have also not been validated, but they are reported to bind to IL-17RB and IL-17RE respectively and form complexes with IL-17RA [57, 70–72]. IL-17B appears to be an antagonist for IL-17E signaling [71, 73].

1.3. Functions of IL-17 cytokines in protective immunity

Having introduced IL-17 family diversity, next I will briefly summarize how individual IL-17 cytokines contribute to protective immunity. IL-17A is the most studied member of the family and sometimes referred to as IL-17 in early papers. During bacteria and fungal infections, once APCs sense the pathogen and together with local tissue create a pro-inflammatory environment via cytokines like transforming growth factor-beta (TGF- β), IL-6, IL-1 β , IL-21, and IL-23, the recruited T cells – antigen-specific cells in the adaptive immune system originated from the thymus – differentiate to a specific state and produce cytokines including IL-17A, IL-17F, IL-22, granulocyte-macrophage colony-stimulating factor (GM-CSF) and TNFs. These cytokines then lead to production of antimicrobial peptides, recruitment of neutrophils that can phagocytose bacteria and repair of the tissue [1, 74, 75]. This whole process is named as type 17 or Th17 response. The type 17 response is mainly driven by Th17 cells, T $\gamma\delta$ cells, CD8⁺ T cytotoxic type 17 (Tc17) cells and ILC3 cells, and it is widely investigated in barrier tissues such as intestines, lungs and skin [1, 2, 76]. Recently a study also revealed memory effects of the type 17 immune response in lung after mucosal immunization. After mice received inhaled subunit vaccine for *K. pneumoniae*, their lung-resident CD4⁺ T helper cells differentiated to type 1 and type 17 phenotypes. These Th17 cells were critical for bacteria control after immunization, and IL-17R signaling in lung

fibroblasts were essential for vaccine potency [77]. During tissue injury, type 17 immune cells not only orchestrate early inflammatory responses, but they also promote tissue repair by inducing proliferation and migration of fibroblasts and epithelial cells and production of extracellular matrix (ECM) proteins [2, 78, 79]. IL-17F is often discussed as part of the type 17 responses, together as IL-17A, but it generally induces weaker inflammatory signals than IL-17A in experimental systems [1, 2].

Like IL-17A and IL-17F, IL-17C promote inflammatory responses, yet it is produced primarily by non-immune cells such as epithelial cells, smooth muscle cells and endocrine cells [80–83]. The secreted IL-17C can form a feedback autocrine loop and further push the source cells to a pro-inflammatory state [84, 85]. In gut and airway epithelium during bacterial infection, IL-17C elevate the expression of antimicrobial peptides, inflammatory cytokines and immune cell-recruiting chemokines in non-immune cells [80, 85, 86]. IL-17C and IL-17RE were shown to promote the closure of *S. aureus*-induced wounds in mice, but neither plays a major role in bacteria elimination [87]. During virus infection, cutaneous IL-17C help protect cutaneous sensory neurons by reducing apoptosis and promote neurite growth [81]. IL-17C has also been shown to regulate tight junctions in barrier tissues [88, 89].

IL-17E, despite having some similar pro-inflammatory functional readouts as IL-17A-C, primarily skews barrier tissue to a type 2 response, hence it's given a separate interleukin designation – IL-25 [2, 90]. For simplicity I will continue to name it IL-17E in this manuscript. Type 2 response defend the body against parasites, venoms and toxins. It is characterized by secretion of IL-4, IL-5, IL-9, and IL-13 cytokines, class

switch of B cells and production of immunoglobulin E (IgE), increase of barrier porosity, mucus secretion and tissue remodeling. Type 2 response is most established in allergic reactions and driven by T helper type 2 (Th2) cells, eosinophils, mast cells, basophils and macrophages [90]. IL-17E is involved early in type 2 responses. When epithelial cells sense disruption of barrier from cleaved tight junction proteins, they secrete alarmin proteins such as IL-17E, IL-33, and thymic stromal lymphopoietin (TSLP). These alarmins are sensed by various cell types and initiate tissue inflammation [2, 90]. IL-17B, proposed as the antagonist for IL-17E, does seem to have some anti-inflammatory functions [71, 91]. Yet some papers found it to be pro-inflammatory in a similar manner as IL-17E [92–94]. The mechanism of action for IL-17B is still unclear.

The host-protective attributes of IL-17s can also be problematic and contribute to autoimmunity, cancer, allergy and other inflammatory syndromes [1, 2, 5]. In later sections I will briefly present on IL-17s in skin pathogenesis.

1.4. Context-specific functions of IL-17D

Being the least-studied member of the IL-17s, IL-17D appears to have context-specific functions, as suggested in tumor models. In the mouse cell lines derived from 3-methylcholanthrene (3-MCA)-induced fibrosarcoma, overexpression of IL-17D in the tumor cell lines lead to production of CCL2, recruitment of natural killer (NK) cells and tumor rejection [68, 95]. In the mouse metastatic MC38 adenocarcinoma model, WNT11-overexpressing tumor cells showed upregulated IL-17D expression, which promoted macrophage polarizing to immunosuppressive CD206+ phenotype and facilitated metastasis [96].

IL-17D has also shown some pro-inflammatory protective roles in bacterial and viral infection models. IL-17D KO mice showed decreased survival under group A *Streptococcus* (GAS) infection and higher bacterial burden in the kidney [97]. In the murine cytomegalovirus (MCMV) infection model, IL-17D KO mice had decreased survival compared to the wild type (WT) mice. This antiviral protective role of IL-17D was mediated via recruitment of NK cells and neutrophils with CCL2 [69]. IL-17D KO mice also showed more severe scarring from Vaccinia virus-induced scarification [68].

In barrier tissues where other IL-17s have been extensively studied, IL-17D also exhibit context-dependent pro- or anti-inflammatory roles. In the dextran sulfate sodium (DSS)-induced mouse colitis model, IL-17D protects the mice from colitis by inducing IL-22 production in ILC3 [65]. This is similar to a canonical type 17 response. In fact IL-17A has been reported to have mixed roles in DSS-induced colitis – either protective or pathogenic [98]. In the mouse house dust mite-induced asthma model, epithelial cells produced IL-17D help control disease progression by restricting the type 2 immune response. Mechanistically IL-17D prevents the influx of monocytes by downregulating C-C motif chemokine receptor (CCR) 6 [99]. In the MC903-induced mouse atopic dermatitis model, IL-17D promotes skin inflammation and disease burden by inhibiting the expression of DEAD-box helicase 5 (DDX5) in keratinocytes [100].

2. Skin homeostasis and pathophysiology

2.1. Structure of human and mouse skin

Both human and mouse skin share the same layered organization. Other than appendages such as hair follicles, sebaceous glands, sweat glands, vasculature and

nerves, skin layers starting from the exterior to interior include epidermis, basement membrane, dermis, hypodermis/subcutaneous tissue. The epidermis is a stratified layer maintained by proliferative basal keratinocytes, which progressively differentiate through suprabasal layers to form the cornified barrier. The outermost layer of epithelium is stratum corneum, consisting layers of denucleated and dead keratinocytes. These keratinocytes undergo a specialized cell death program named cornification without activating typical apoptosis-related proteins [101]. During cornification, keratins inside the cells become densely packed, plasma membrane-associated lipids and proteins get crosslinked to form the cornified envelope, and intercellular spaces are filled with lipid lamellae [101, 102]. Hence a dense hydrophobic barrier layer is formed. Right under the sits the granular layer known as the stratum granulosum, sealing the live skin with tight junctions. The inner cell layers – the stratum spinosum – originates from keratinocyte proliferation in the stratum basale sitting on top of the basement membrane that marks the epidermis-dermis interface. Notably the epidermis is much thinner in mice than humans [103].

The dermis can be further separated into two layers: papillary and reticular. The boundary between the papillary and reticular dermis is less clearly defined, but the reticular dermis contains thicker, directionally oriented collagen bundles [104, 105]. Compared with human skin, mouse dorsal skin has a thinner epidermis and dermis, a higher density of hair follicles, and a panniculus carnosus muscle layer that contributes to wound contraction [104, 106].

Cells of the same cell type in the skin are often heterogeneous and functionally different based on their location. For example, fibroblasts, the major structural mesenchymal cells, are known to facilitate epithelial-mesenchymal signaling, promote angiogenesis and support hair follicle regeneration in the papillary dermis, while reticular fibroblasts are better known for their ability to produce and remodel ECM proteins [104, 107]. In mouse, papillary and reticular fibroblasts were shown to develop from distinct lineages in embryos [108]. Fibroblasts around skin appendages are usually specialized to support the specialized niches, as shown by hair-follicle associated dermal sheath fibroblasts. During hair cycling, dermal sheath form a contractile smooth-muscle-like layer that coordinates cell regression and hair follicle reorganization [109, 110].

2.2. Immune compartments in the skin at homeostasis

As the outermost barrier organ, skin is an immunologically active organ, allowing it to maintain tolerance to commensals while rapidly responding to injury, infection, and inflammation. Several different immune cell populations reside in the skin at homeostasis, while more variety of immune cells are recruited when the resident cells are activated. Notably, as the dermis houses lymphatics and blood vessels, it is more accessible by immune cells from the circulation than epidermis.

The epidermis is filled with keratinocytes, which play important roles in innate immunity. They express several different pattern recognition receptors and produce a variety of cytokines, including TSLP, TNFs, IL-33 and IL-1 family members, upon inflammation [111]. Immune cells including Langerhans cells (LCs), resident memory T

(T_{RM}) cells, regulatory T cells (T_{reg} cells), T_{γδ} cells reside between keratinocytes in the epidermis. LCs are skin-specific APCs in the same development lineage as tissue-resident macrophages [111]. They acquire a dendritic cell (DC)-like phenotype – once encountered with PAMPs or DAMPs, LCs increase their mobility, drain to the lymph nodes, and present antigens to lymphocytes inside lymph nodes. During inflammation, activated LCs elongate their dendrites outward beyond the tight junction and uptake external antigens [111, 112].

T_{γδ} cells are unique in T cells, as they are less found in draining secondary lymphoid tissues, and most of the times they are not activated by classical peptide–MHC complexes like other T cells. They recognize stress-associated, microbial, injury-associated signals and respond by releasing combination of cytokines such as keratinocyte growth factor (KGF), IL-13 and IL-17s [113, 114]. In mice, epidermal T_{γδ} cells are dominated by dendritic epidermal T cells (DETCs), which show dendritic morphology and are locally maintained after fetal thymic development [114]. Human counterparts of DETCs have not been well established. Even though human epidermal T_{γδ} cells are functionally similar to DETCs, they differ in the specific mechanisms [115].

In human epidermis, T_{RM} cells have a more prominent population than T_{γδ} cells. In both human and mice, epidermal CD8⁺ T_{RM} cells possess characteristics distinct from circulating T_{RM} cells: they adopt dendritic morphology similar to LCs and enhanced surveillance roles by patrolling the local tissue [116]. Even though human epidermal CD8⁺ T_{RM} cells occupy a similar surveillance niche as mouse DETCs, these T_{RM} cells are still adaptive memory T cells specialized for rapid responses to antigens they have

experienced. Interestingly, the skin of laboratory mice raised in specific pathogen-free (SPF) conditions harbors few epidermal CD8⁺ T_{RM} cells [117]. After viral infection, the DETCs in mouse epidermis appears to be displaced by CD8⁺ T_{RM} cells [118].

More types of immune cells are found in the dermis. The major resident immune populations are macrophages, DCs, mast cells, T cells and ILCs [111]. Myeloid cells are innate immune cells that develop through myelopoiesis rather than antigen-dependent maturation in secondary lymphoid organs. They bridge barrier defense and adaptive immunity by sensing tissue perturbation, producing inflammatory mediators, and shaping downstream lymphocyte responses. Macrophages and DCs are the major myeloid cells resident in the dermis.

Other than T cells previously mentioned in the epidermis, T_{reg} cells are particularly important in the dermis. T_{reg} cells are immune-suppressive T cells that limit excessive immune responses and maintain tolerance towards self-antigens. Their phenotype and functions are fine-tuned based on for suppressing excessive immune responses, regulating hair growth and follicle cycling, and maintaining immune tolerance [119, 120].

2.3. Immune responses at different stages of cutaneous wound healing

Many skin-resident immune cells sense tissue damage-associated signals and are hence heavily involved in cutaneous wound healing. The healing of skin tissue is characterized by three stages over the time course: inflammation, tissue formation and tissue remodeling [121, 122].

The inflammatory phase is dominated by rapid recruitment of innate immune cells. Neutrophils are among the first circulating leukocytes to enter the wound bed, where they limit microbial invasion and clear damaged tissue through phagocytosis, reactive oxygen species, proteases, antimicrobial peptides, and neutrophil extracellular traps. However, neutrophil responses must be tightly controlled, because excessive or prolonged neutrophil activity can amplify tissue damage and delay resolution.

Monocytes are subsequently recruited and differentiate into wound macrophages, which become central regulators of the transition from inflammation to repair. Early wound macrophages produce inflammatory cytokines such as TNF- α , IL-1 β , and IL-6, secrete chemokines that coordinate additional leukocyte recruitment, and phagocytose pathogens, debris, and apoptotic neutrophils. Experimental macrophage depletion studies have shown that macrophages are particularly important during early and middle stages of repair, where their loss impairs granulation tissue formation, angiogenesis, epithelialization, and wound closure [123–125].

As the wound enters the proliferative phase, the immune response shifts from pathogen defense and debris clearance toward inflammation resolution and tissue rebuilding. Macrophages adopt more reparative and pro-resolving states, a transition often simplified as a shift from M1-like to M2-like activity, although wound macrophage phenotypes are now recognized as heterogeneous and context-dependent.

Efferocytosis of apoptotic neutrophils helps drive this transition by promoting anti-inflammatory and reparative mediators, including IL-10, TGF- β and other factors that support angiogenesis, fibroblast activation, extracellular matrix deposition, and

keratinocyte migration [126, 127]. Adaptive and innate-like lymphocytes also contribute to this stage. Skin regulatory T cells help restrain excessive inflammation and promote barrier repair, while epidermal $\gamma\delta$ T cells, including murine dendritic epidermal T cells, can sense damaged keratinocytes and produce factors that support epithelial repair and antimicrobial barrier restoration [128, 129].

During remodeling, immune activity gradually declines as the wound matures from a provisional, highly cellular granulation tissue into a more organized scar. Macrophages continue to influence extracellular matrix turnover by regulating fibroblast activity, collagen deposition, matrix metalloproteinase expression, and vascular regression. At this stage, balanced immune resolution is essential: insufficient inflammation can impair microbial clearance and early repair, whereas persistent inflammatory signaling can maintain protease-rich, matrix-degrading conditions that contribute to chronic non-healing wounds. Similarly, prolonged pro-fibrotic signaling can promote excessive matrix accumulation and scarring. Thus, immune responses during wound healing are not simply inflammatory or anti-inflammatory; rather, their effects depend strongly on timing, magnitude, cellular source, and the local tissue context [130, 131].

Methods

1. Bioinformatics experiments

1.1. Skin single-cell RNA sequencing

A 2 × 2 cm section of dorsal skin was surgically excised from age-matched 6-8 month-old male C57BL/6 wildtype (WT), IL-17D KO and IL-17D heterozygous (Het) cagemate mice, and the attached fascia was gently removed with forceps. Then the skin piece was rinsed with phosphate-buffered saline (PBS; Omega Scientific), chopped and digested with 1 mg/mL Liberase TM (Roche, 5401119001) in PBS at 37 °C for 30 min. Then DNase (Roche 11284932001) was added to the digestion mixture at 50 U/mL for additional incubation at 37 °C for 15 min. The digested skin cell mixture was filtered through a 100- μ m strainer. Dead cells were removed with EasySep™ Dead Cell Removal Kit (STEMCELL, 17899). Live cells were diluted in PBS with 1% fetal bovine serum (FBS) and immediately processed with 10X Chromium platform using the GEM-X Single Cell 3' v4 Gene Expression kit (10x Genomics). Single-cell RNA sequencing (scRNA-seq) libraries were prepared according to the manufacturer's protocol (10x Genomics). Finally, libraries were sequenced on the Illumina NovaSeq 6000 sequencing system using pair-end sequencing according to 10x Genomics recommendations. Sample indexing was performed using the 10x Genomics Dual Index Plate TT Set A.

1.2. Bulk RNA sequencing

Cell RNA was extracted with the RNEasy Kit (Qiagen). Sequencing libraries were prepared by the UCSD IGM Genomics Center using Illumina Total RNA Prep with ribosomal RNA depletion, according to the manufacturer's instructions. Libraries were sequenced on the Illumina NovaSeq 6000 sequencing system.

1.3. Single-cell RNA sequencing data analyses

Sequences were aligned with Cell Ranger pipeline (v.9.0.0). Sequencing reads were mapped against the mouse GRCm39 reference genome.

Gene count matrices were analyzed with Seurat (v.5.4.0) pipeline in R (v.4.5.2). Cells were filtered based on gene number (200-6000) and mitochondrial transcript percentage (<20%). Data were normalized with NormalizeData and integrated, followed by principal component analysis, clustering and UMAP visualization.

Initial cell type identification for Seurat clusters was performed using SingleR (v.2.12.0) in R (v.4.5.2) with ImmGen dataset. Differentially expressed genes (DEGs) for each Seurat cluster or between two groups of cells were identified with FindMarkers function in Seurat package. Final Seurat cluster identities were assigned based on cluster-specific DEGs [110, 132–142].

Gene set enrichment analyses (GSEA) were performed with fgsea (v.1.36.2) in R (v.4.5.2). Hallmark, KEGG, Reactome and Biocarta gene sets were downloaded from the MSigDB GSEA Molecular Signatures Database (v2022.1). Gene Ontology Biological Process (GOBP) gene sets were obtained from the Bader Lab collection, version

May_01_2025. Enrichment results were evaluated based on normalized enrichment score and adjusted P value.

1.4. Bulk RNA sequencing data analyses

Sequences were aligned with STAR (v.2.7.11b) with the mouse GRCm39 reference genome. Count matrices were generated by featureCounts (v.2.0.6). Quality control for each experiment was performed with RNAseqQC (v.0.2.1) in R (v.4.5.2). DEG analyses were performed with DESeq2 (v.1.50.2) in R (v.4.5.2). GSEA was performed as described above for the single-cell RNA sequencing (scRNA-seq) analysis, with modifications appropriate for bulk RNA-seq data.

2. *In vitro* experiments

2.1. Cell culture

Cell lines were cultured at 37°C with 5% CO₂ in RPMI 1640 medium supplemented with 10% FBS (Omega Scientific), 1% penicillin/streptomycin, 200 μ M sodium pyruvate, 1X non-essential amino acids (Gibco, 11140050), 200 μ M L-glutamine, 3.75‰ sodium bicarbonate and 5.5 μ M 2-mercaptoethanol. The D29M1 fibrosarcoma cell line was generated from primary mouse fibrosarcoma induced by 3-methylcholanthrene (3-MCA). The HaCaT human keratinocyte cell line was provided by the Meier lab at UC San Diego. The RAW 264.7 murine macrophage cell line was obtained from ATCC.

Primary BMDMs were derived from C57BL/6 WT mice. Femurs and tibias of mice were collected under sterile conditions, and bone marrow was flushed with PBS buffer.

Red blood cells were lysed by incubation with ammonium-chloride-potassium (ACK) lysing buffer (Gibco, A1049201) at room temperature for 5 min. Cells were passed through a 70- μ m cell strainer, centrifuged, and seeded in DMEM medium supplemented with 10% FBS, 1% penicillin/streptomycin and 25 ng/mL recombinant macrophage colony-stimulating factor (M-CSF; Gibco, 315-02). BMDMs were maintained at 37°C and 5% CO₂ and used between day 6 and 8 after seeding.

Primary fibroblasts were derived from C57BL/6 WT, IL-17D KO and IL-17D Het mice following a published protocol [143].

2.2. Scratch assay

For scratch assays, 400,000 cells were seeded per well in 6-well plates. A linear scratch was generated in each well with a P200 pipette tip. Cells were gently rinsed with PBS to remove detached cells and cultured in media containing 250 ng/mL recombinant mouse IL-17D (rmIL-17D) or recombinant human IL-17D (rhIL-17D). Recombinant IL-17Ds were synthesized by Trutino Biosciences. Scratch width was measured at five positions near marked regions using a Nikon Eclipse Ti microscope and NIS Elements AR software (v.3.2). Statistical analyses, including t-tests and one-way ANOVA, were performed with GraphPad Prism software (v.11.0.1).

3. *In vivo* experiments

3.1. Mice

Animals were housed in the UC San Diego Leichtag Vivarium. Mice were maintained at ambient temperature 20-22 °C on 12-h light/dark cycle. C57BL/6 WT mice

were obtained from Charles River Laboratories and bred in house. IL-17D KO mice were obtained from UC Davis Mutant Mouse Resource & Research Centers and bred in house. IL-17D Het mice were generated from crossing C57BL/6 WT and KO mice. At weaning, pups from multiple litters were combined by sex and genotype to generate mixed litter cagemates. Before treatments, mice were anesthetized intraperitoneally with 100 mg/kg ketamine mixed with 10 mg/kg xylazine in sterile Hank's balanced salt solution (HBSS)

3.2. Imiquimod-induced psoriasis model

Age- and sex-matched C57BL/6 WT, IL-17D KO and IL-17D Het mice 5–6 months old were anesthetized and treated topically on the ear with 25 μ g 5% imiquimod cream (Perrigo) or with petroleum gel control every day for four consecutive days. Cream and gel were weighed on an analytical balance before transferred and spread with a pipette tip. Disease burden was characterized by ear thickening, and ear thickness was measured daily with engineering calipers.

3.3. MC903-induced atopic dermatitis model

The mouse atopic dermatitis model was modified from published methods [144, 145]. Age- and sex-matched C57BL/6 WT and IL-17D KO mice 3–5 months old were anesthetized and treated topically on the ear with 0.5 μ g MC903 (Bio-Techne, 2700), also known as calcipotriol, dissolved in ethanol or with ethanol vehicle control. MC903 was prepared in ethanol at a concentration of 45 μ M and applied by topical drop-casting. Treatments were on a repeated schedule consisting of five consecutive days of

treatment followed by two days without treatment. Disease burden was characterized by ear thickening, and ear thickness was measured daily with engineering calipers.

3.4. Splinted wound punch model

The splinted wound punch model was adapted from a published method [146]. Age- and sex-matched C57BL/6 WT mice 5–6 months old were anesthetized intraperitoneally with ketamine and xylazine solution. Lidocaine in HBSS was administered subcutaneously at 2 mg/kg at the surgical site. The dorsal skin was shaved, treated with depilatory cream, and disinfected with ethanol wipes. Skin was lifted into a fold and wounded using a sterile 6-mm biopsy punch (Medline, MDS0033360B). Silicone splints, 12 mm in diameter, were previously cut from silicone sheets (Grace Bio-Labs, GBL664581), autoclaved, and sutured around each wound using sterile 6-0 polypropylene sutures according to predefined suture marks (Figure 1). Ethiqo XR (Fidelis Animal Health) was administered intraperitoneally at 3.25 mg/kg after surgery for analgesia.

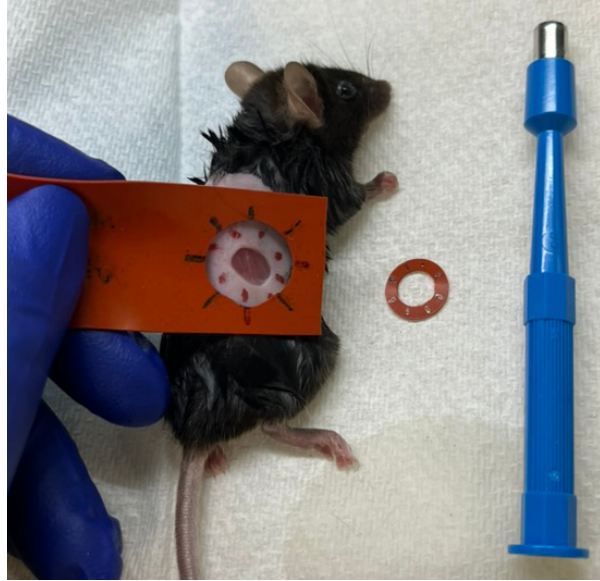


Figure 1. Splinted wound surgery. Left: suture marks around punched wound; middle: silicone splint with suture marks; right: sterile disposable biopsy punch.

Two wounds on each mouse were randomly assigned to treatment and control group. Wounds in the treatment group received 1 μ g rmlL-17D (Trutino Biosciences) by topical drop-casting once daily, while control wounds received an equivalent volume of vehicle. Wound lengths and widths were measured with digital calipers every 2–3 days. The size of each wound is approximated by elliptical area formula: $\pi \times \text{length} \times \text{width} / 4$. Percent wound closure is calculated relative to the original wound size at day 0. Statistical significance was analyzed via t-test using GraphPad Prism (v.11.0.1).

Results

1. IL-17D expression in skin from scRNA seq

1.1. IL-17D is primarily expressed by fibroblasts in homeostatic mouse skin

To investigate what cells produce IL-17D in the skin, scRNA-seq was performed on homeostatic skin from age- and sex- matched C57BL/6 WT, IL-17D KO and Het mice cagemates. 2 WT, 2 Het and 3 KO samples were collected and analyzed. After unsupervised clustering of all samples in Seurat pipeline, 23 clusters of cells were identified and visualized with uniform manifold approximation and projection (UMAP; Figure 2).

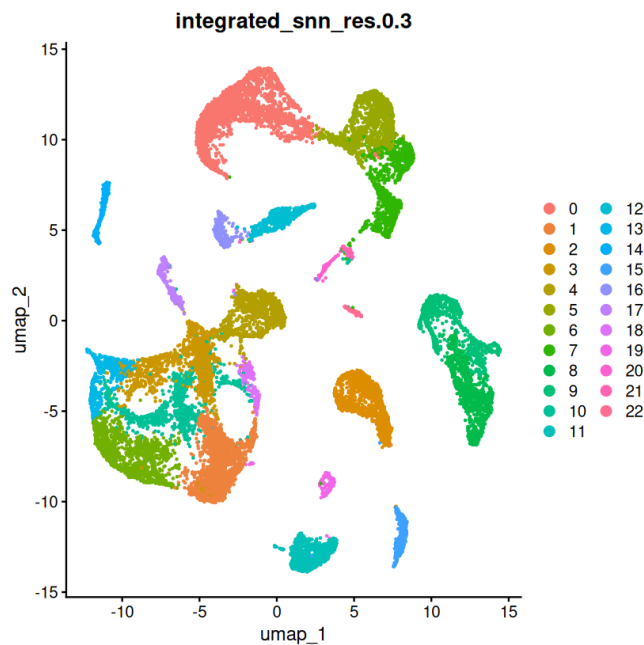


Figure 2. UMAP visualization of clusters identified from Seurat pipeline.

To get the cell identity for each cluster, first SingleR was used to match expression profile of each cluster to the ImmGen database, and initial cell identities for clusters were obtained for reference (Figure 3). Then using FindMarkers function in

Seurat package, a DEG list was generated for each cluster. Cluster 18 was identified to be mixed proliferating cells from upregulated genes such as Mki67, Top2a, Cenpf, Tpx2, Kif15. Cluster 21 was identified as erythroid cells from upregulated Alas2, Hba-a1, Hbb-bt, Gypa, Slc4a1. Hence these two clusters were removed for downstream analyses. Curated cell type identification was generated from the DEG lists (Figure 4), based on canonical cell type specific markers as plotted (Figure 5).

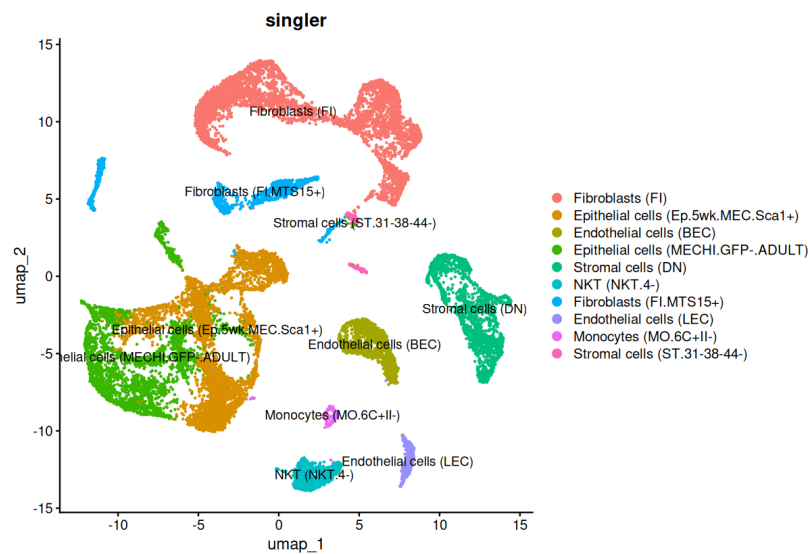


Figure 3. Initial cell type identity labels from SingleR using ImmGen database.

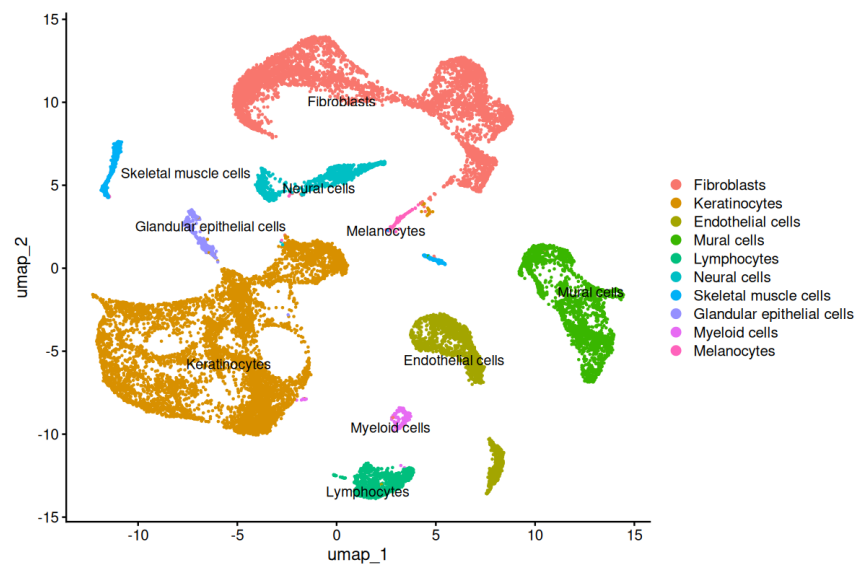


Figure 4. Curated cell type identity labels for clusters from DEG lists.

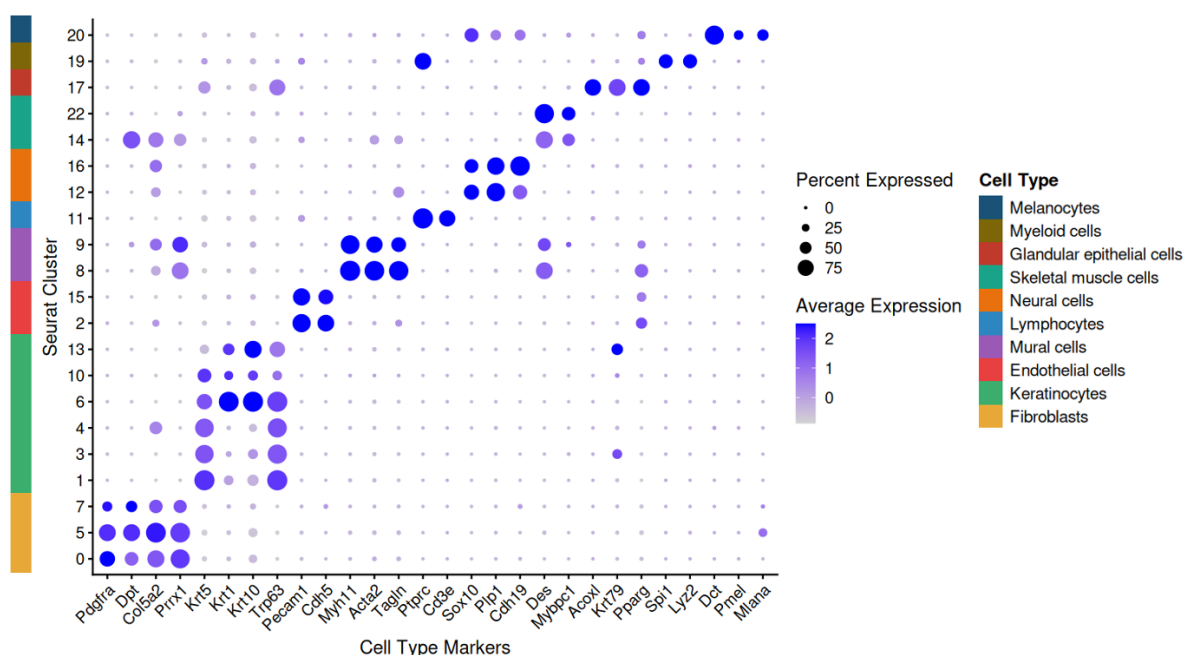


Figure 5. Expression of canonical cell type markers to assign identities to Seurat clusters.

All major skin compartments were identified in this experiment (Table 1). The presence of skeletal muscle cells probably comes from the *panniculus carnosus* muscle, which is a thin striated muscular layer underneath mouse skin. Notably this muscle lining is only present in sparse anatomical locations in the palm, abdomen, face and shoulders in human [147]. Comparing the cell proportions between WT, Het and KO samples, loss of IL-17D gene function did not change skin cell compartments drastically (Figure 6).

Expression of IL-17D in the skin was sparse, but concentrated in several subsets of cells, as shown in the UMAP visualization (Figure 7). When the expression of IL-17D was examined in each cell type and visualized with a violin plot, it was clear that these 709 cells expressing IL-17D mainly belonged to fibroblasts, keratinocytes and mural

cells (Figure 8). 67% of these IL-17D+ cells were fibroblasts. Fibroblasts also showed the highest percentage of IL-17D+ cells, even though a few mural cells had high IL-17D expression (Figure 9; Figure 10).

Table 1. Composition of cell types in mouse homeostatic skin from scRNA seq

Cell type	Number of cells	% Total
Fibroblasts	5147	26%
Keratinocytes	7409	37%
Endothelial cells	1836	9%
Mural cells	2184	11%
Lymphocytes	833	4%
Neural cells	1119	6%
Skeletal muscle cells	649	3%
Glandular epithelial cells	316	2%
Myeloid cells	172	1%
Melanocytes	163	1%
Total	19828	100%

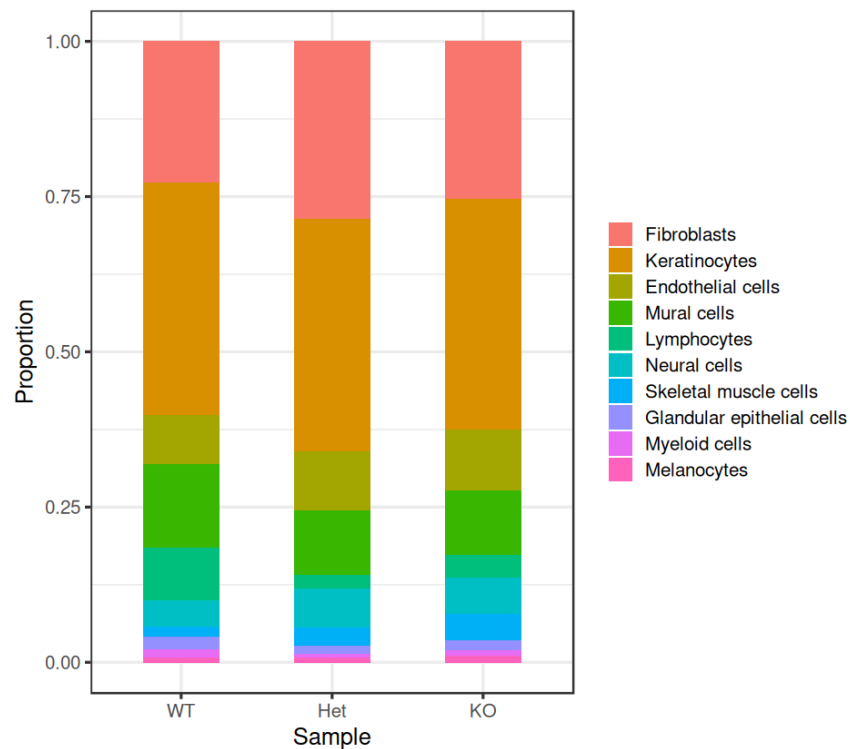


Figure 6. Proportion of annotated cell types in WT, IL-17D Het and IL-17D KO mice.

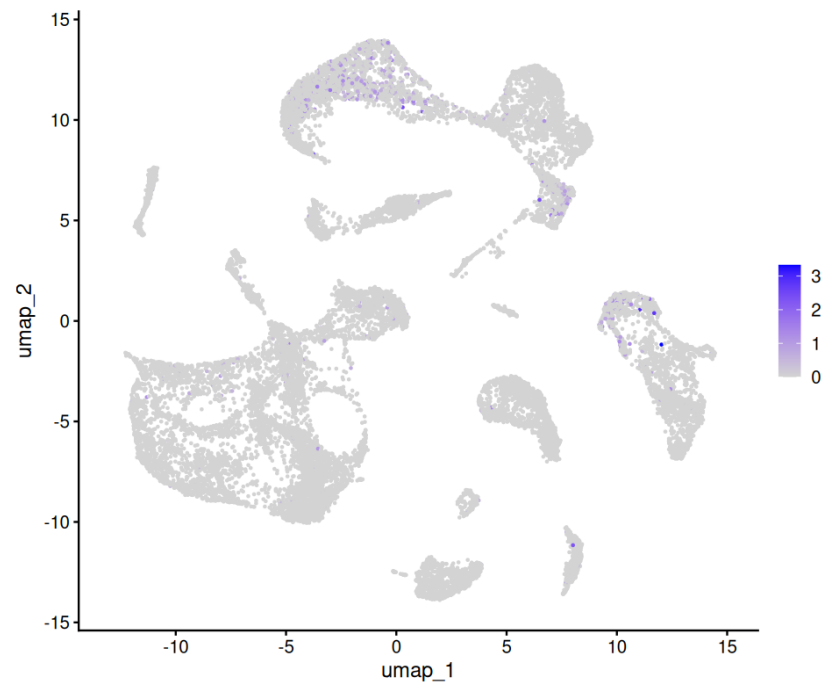


Figure 7. UMAP visualization of IL-17D expression in mouse skin.

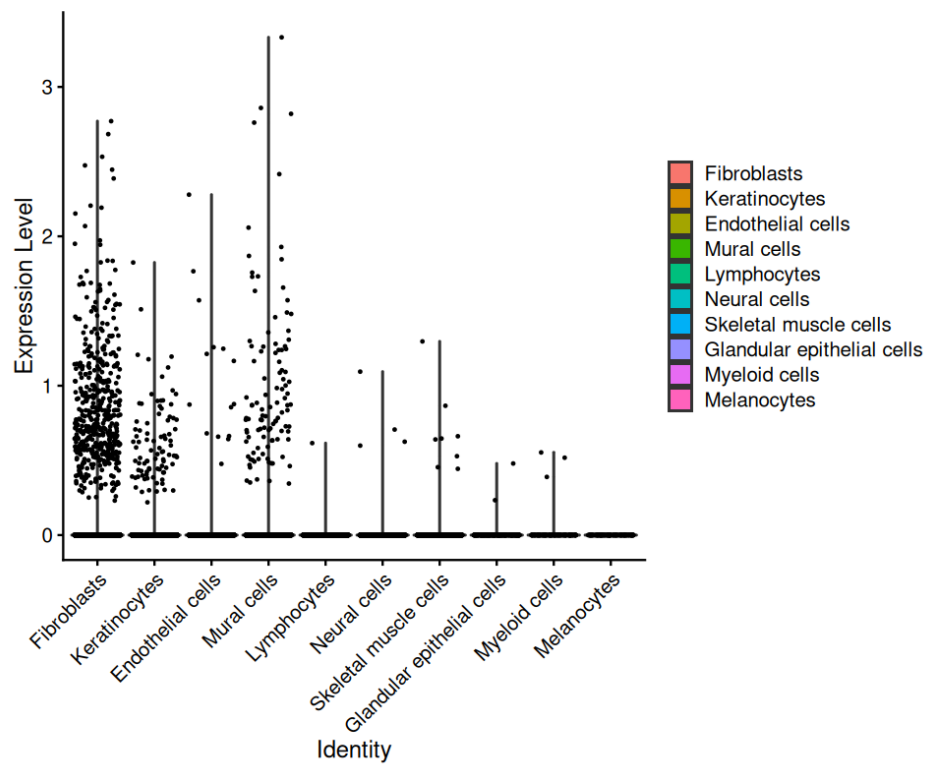


Figure 8. Violin plot showing IL-17D expression in annotated cell types.

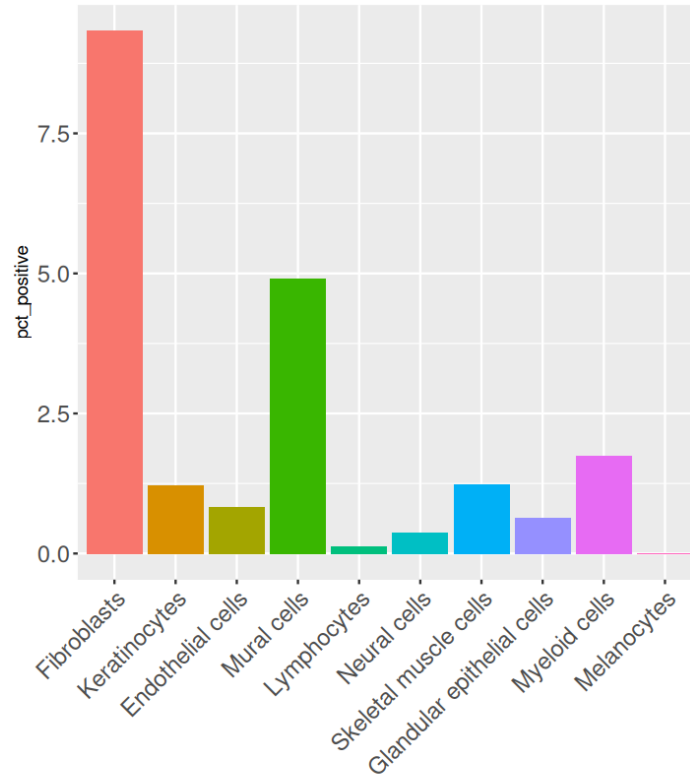


Figure 9. Percentage of IL-17D+ cells in annotated cell types.

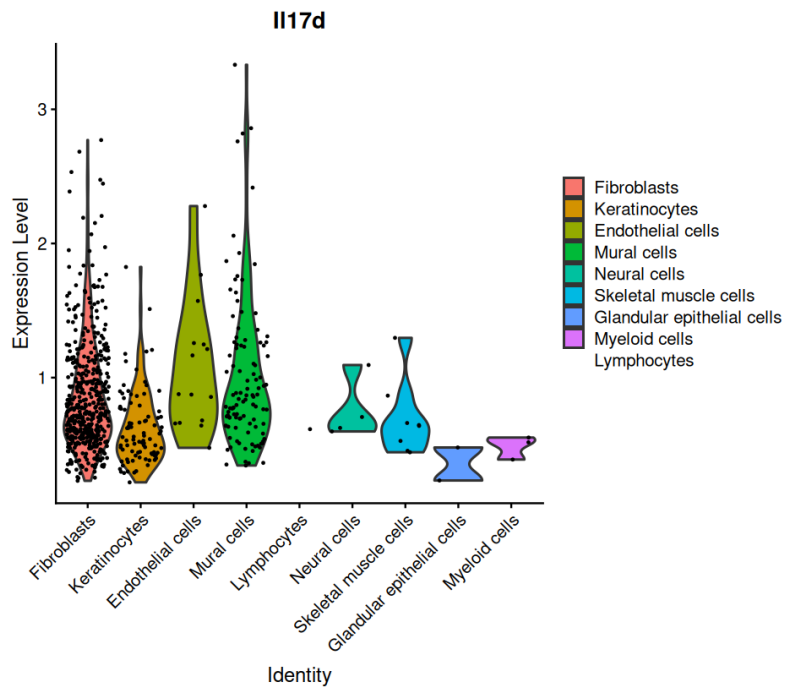


Figure 10. Violin plot showing IL-17D expression in IL-17D+ cells among annotated cell types.

In this dataset, the only published receptor for IL-17D – CD93, was predominantly expressed in endothelial cells (Figure 11).

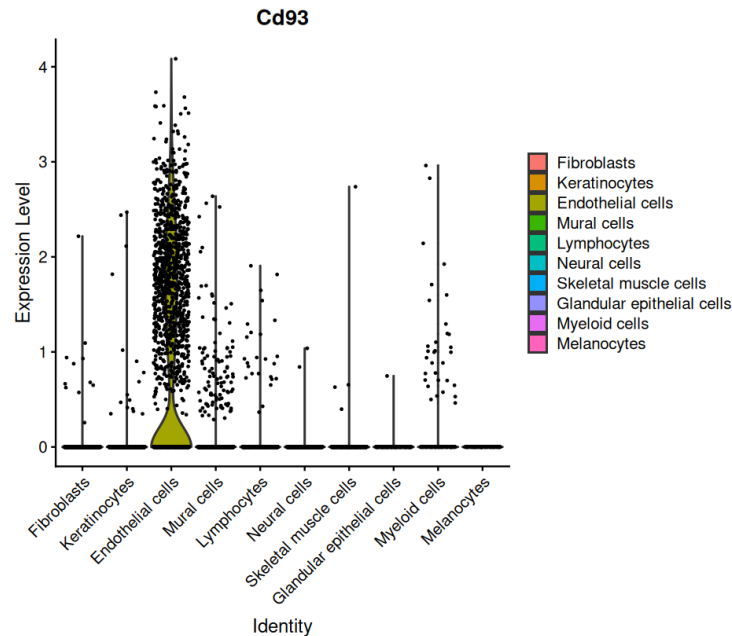


Figure 11. Violin plot showing CD93 expression in annotated cell types.

1.2. IL-17D is expressed by distinct fibroblast subsets at homeostasis

Fibroblasts were subsetted from the scRNA seq dataset and re-clustered with Seurat to further investigate the IL-17D+ fibroblasts. 9 fibroblast clusters were initially identified and DEG lists were exported for each cluster and compared to published literature on fibroblast subsets. Two clusters were both identified as Lef1+ dermal papilla fibroblasts [132]. One of them appeared to be contaminated with markers associated with non-fibroblast lineages; hence these two clusters were combined. The curated 8 clusters showed distinct expression profiles of published fibroblast subsets (Table 2; Figure 12; Figure 13) [110, 132–142].

Table 2. Composition of fibroblast sub-clusters in mouse homeostatic skin from scRNA seq

Cluster	Curated Label	Number of cells	% Total
0	Lef1+ dermal papilla fibroblasts	1422	32%
1	Tnn+ interstitial fibroblasts	891	20%
2	Cgref1+ matrix-producing fibroblasts	605	13%
3	Cdh4+ papillary fibroblasts	471	10%
4	Dcn+ reticular fibroblasts	458	10%
5	Hmcn+ reticular fibroblasts	366	8%
6	Tnmd+ reticular fibroblasts	222	5%
7	Cdh19+ Schwann-like fibroblasts	72	2%
Total		4507	100%

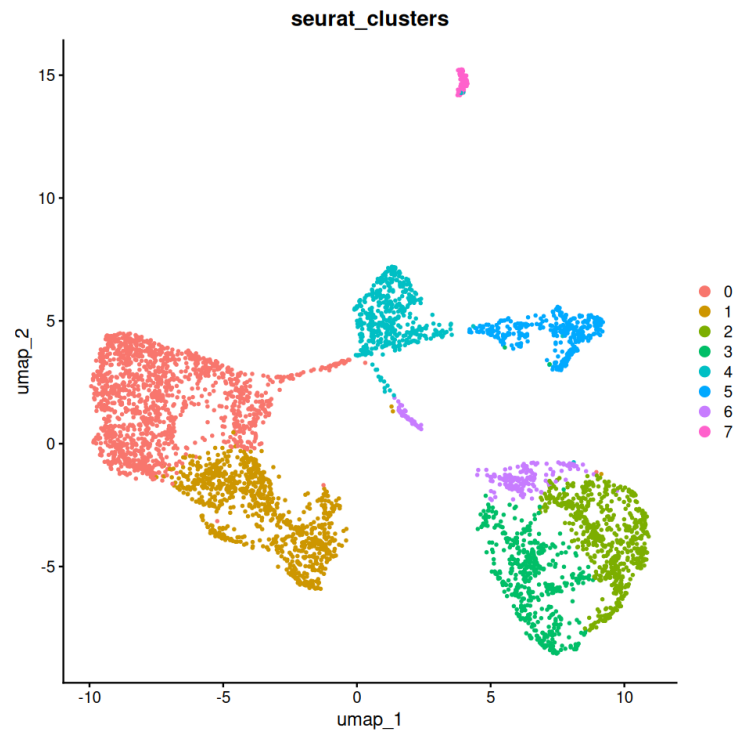


Figure 12. UMAP visualization of fibroblast subclusters from Seurat.

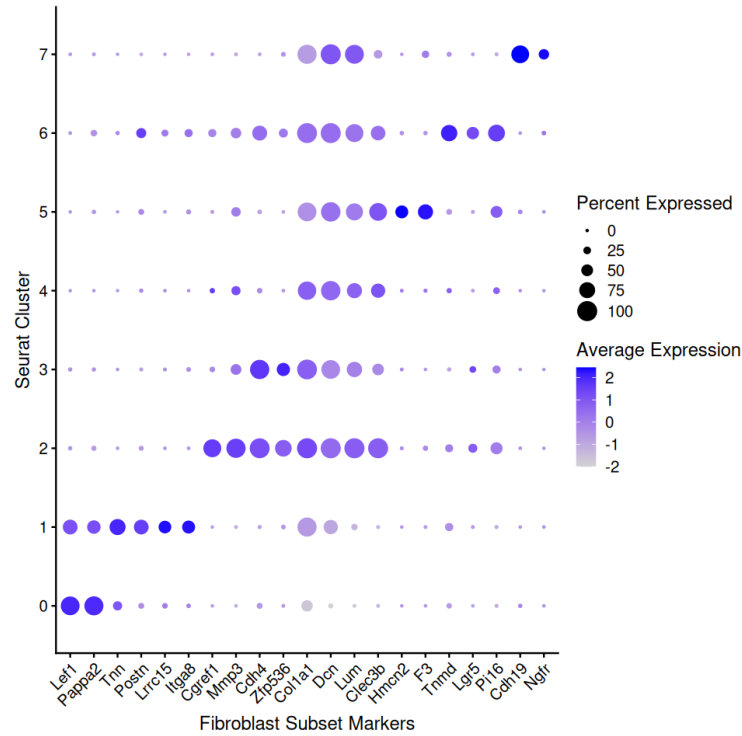


Figure 13. Expression of published fibroblast subset markers in fibroblast Seurat clusters.

Several features are noteworthy in these clusters. Cluster 1 contains some cells with strong activated myofibroblast feature, marked by genes such as Postn and Lrrc15 [135, 148, 149]. Clusters 4 did not exhibit distinct marker genes, but it was separated from other clusters by the absence or low expression of specific marker genes. Cluster 7 matched with recently identified Schwann-like fibroblasts, while lacking the canonical Schwann cell markers Sox10 and Plp1 [110, 142, 150].

IL-17D expression in these fibroblasts was sparse but enriched across several subsets (Figure 14; Figure 15). Clusters 0, 1, 5 and 7 showed higher percentage of IL-17D+ cells, over 10% (Figure 16). Even though IL-17D+ cells in other clusters also had expression level above 1, the numbers of IL-17D+ cells in those clusters were not

enough for DEG analyses (Figure 17). Comparing the cell proportions between WT, Het and KO samples, WT samples had less cluster 2 and 3 fibroblasts (Figure 18).

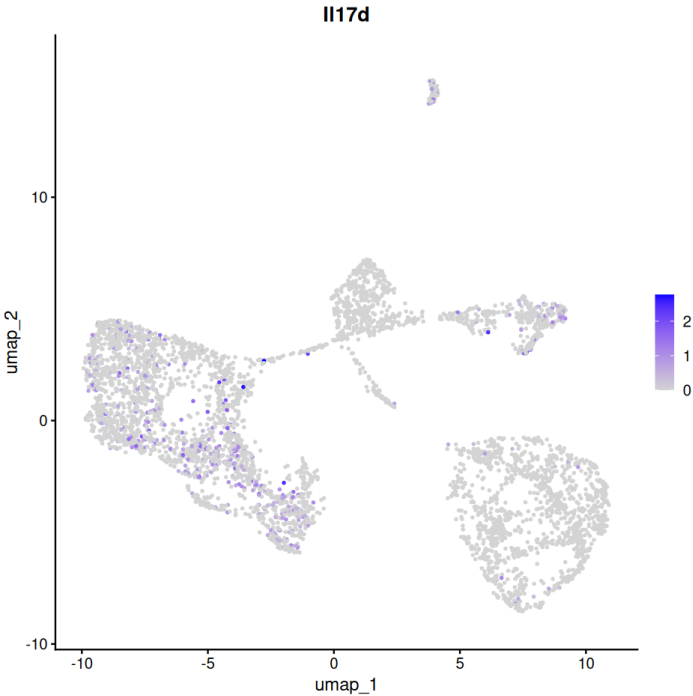


Figure 14. UMAP visualization of IL-17D expression in mouse skin fibroblasts.

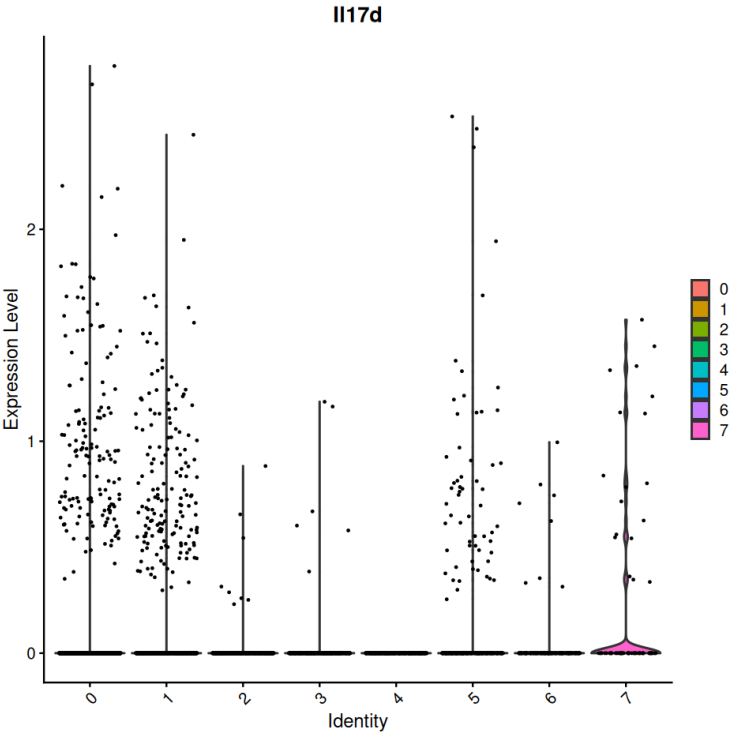


Figure 15. Violin plot showing IL-17D expression in fibroblast subclusters.

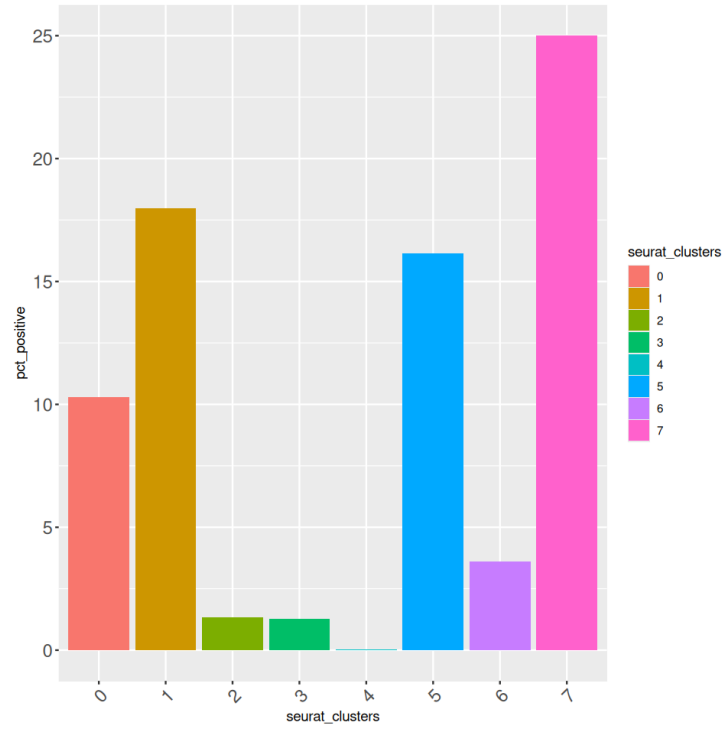


Figure 16. Percentage of IL-17D+ cells in fibroblast subclusters.

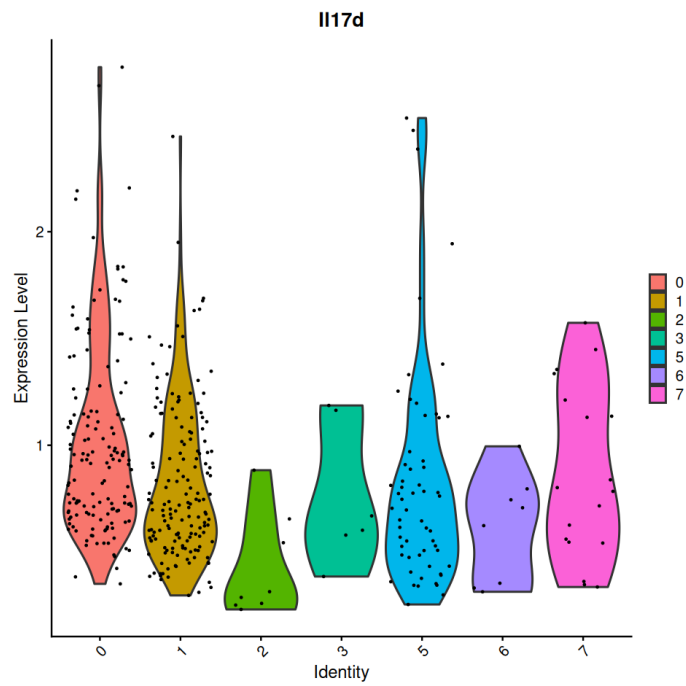


Figure 17. Violin plot showing IL-17D expression in IL-17D+ cells in fibroblast subclusters.

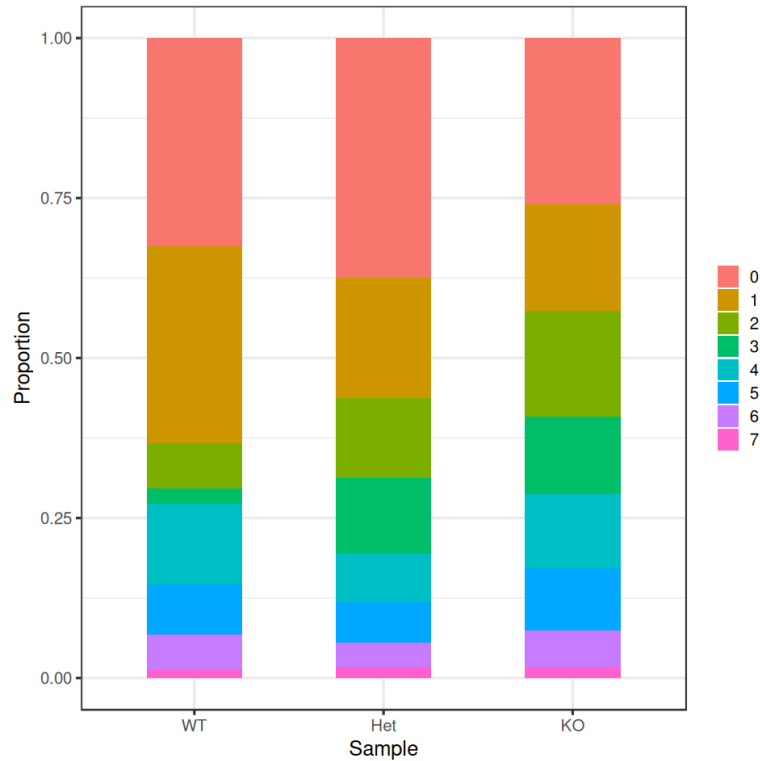


Figure 18. Proportion of skin fibroblast subclusters in WT, IL-17D Het and IL-17D KO mice.

Since Cluster 1 showed higher markers for the pro-inflammatory myofibroblast, the log-transformed expression of myofibroblast markers and Il17d for each cell was plotted in jitter plots and examined using Spearman's correlation (Figure 19, Figure 20). Spearman's correlation coefficients from both analyses of Postn vs Il17d and Lrrc15 vs Il17d had absolute values less than 0.2, indicating negligible correlation between expression of myofibroblast markers and Il17d. The bands from values less than 1.5 on both x and y axes very likely resulted from low discrete raw counts (between 1 and 5) of mRNA, instead of real correlation. Meanwhile there are many cells on x- and y- axes, reflecting the cells only positive for Il17d or myofibroblast markers. Correlation between myofibroblast markers Lrrc15 and Postn had a much larger Spearman's correlation coefficient of 0.32, and a lot more cells with higher expression contributed to this result

(Figure 21). Still there were more cells positive for only one of the two markers, which confirmed there were only a small number of potential myofibroblasts in cluster 1. This was predictable for homeostatic tissue. Within the 391 Postn+ Lrrc15+ potential myofibroblast population, the Spearman's correlation coefficients still showed absolute values less than 0.2 (Figure 22; Figure 23). Together, there's no evident relationship between IL-17D expression and myofibroblast phenotype.

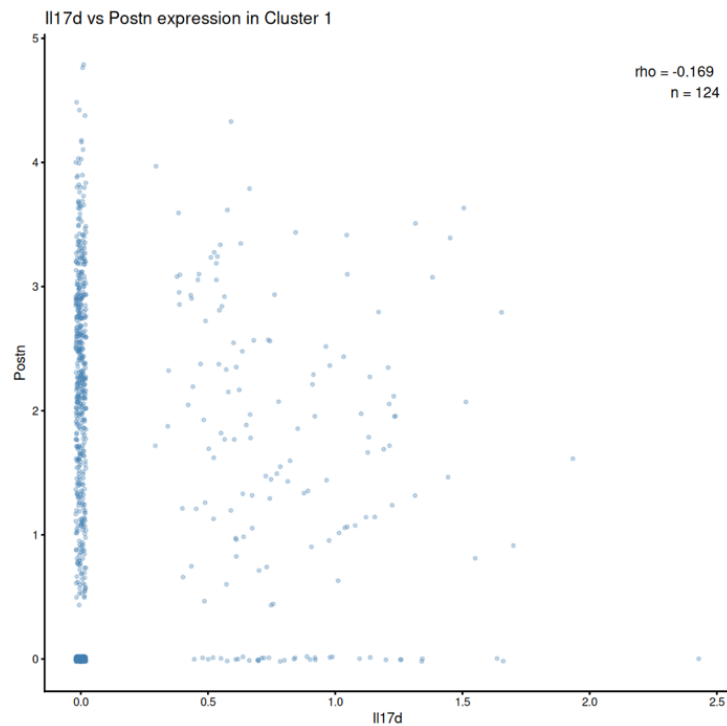


Figure 19. Jitter plot showing expression level of myofibroblast marker Postn vs Il17d in fibroblast Cluster 1.

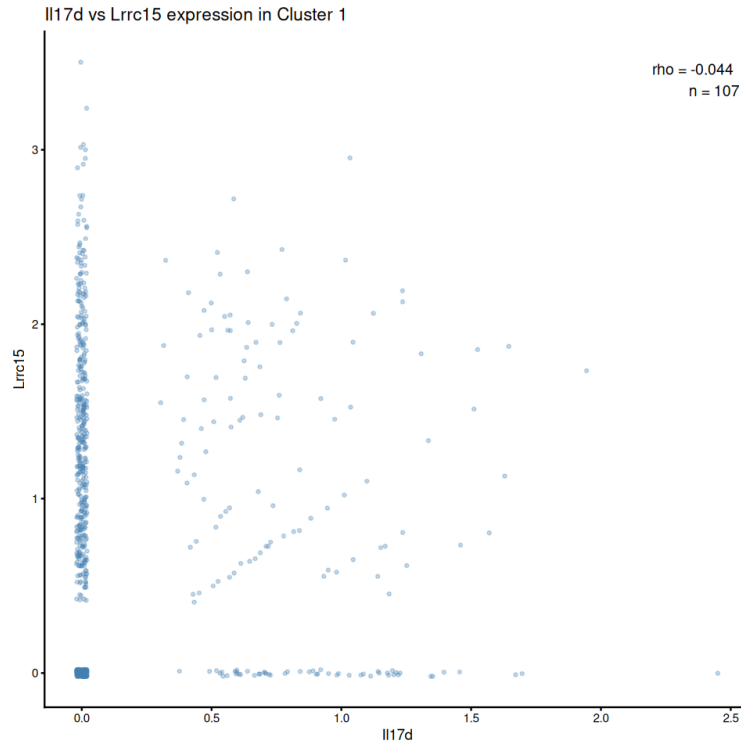


Figure 20. Jitter plot showing expression level of myofibroblast marker Lrrc15 vs Il17d in fibroblast Cluster 1.

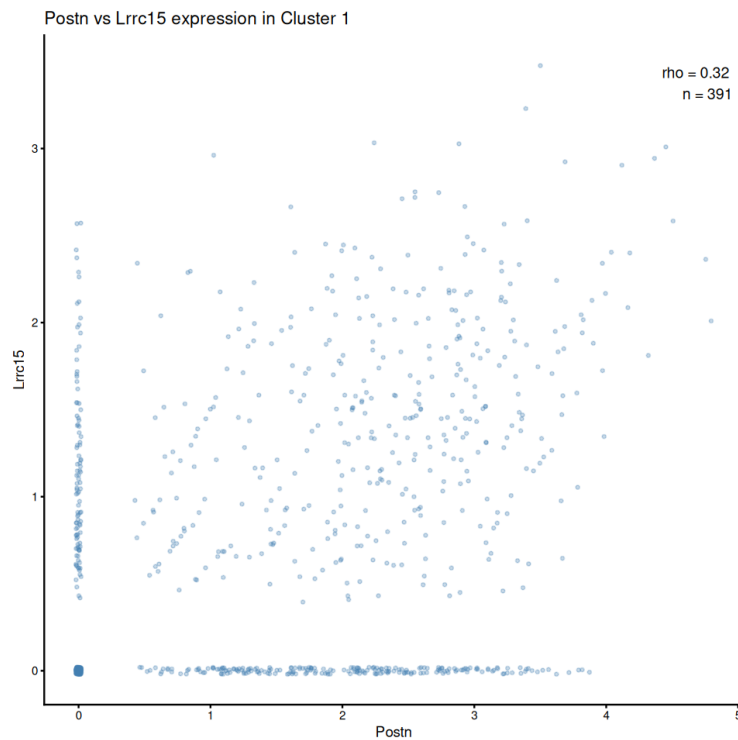


Figure 21. Jitter plot showing expression level of myofibroblast markers Lrrc15 vs Postn in fibroblast Cluster 1.

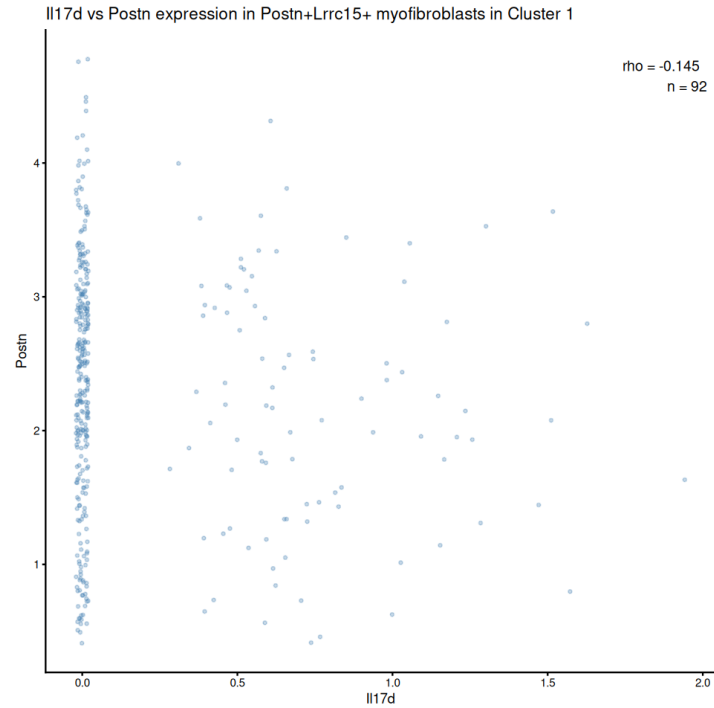


Figure 22. Jitter plot showing expression level of myofibroblast marker Postn vs Il17d in Postn+Lrrc15+ myofibroblasts in Cluster 1.

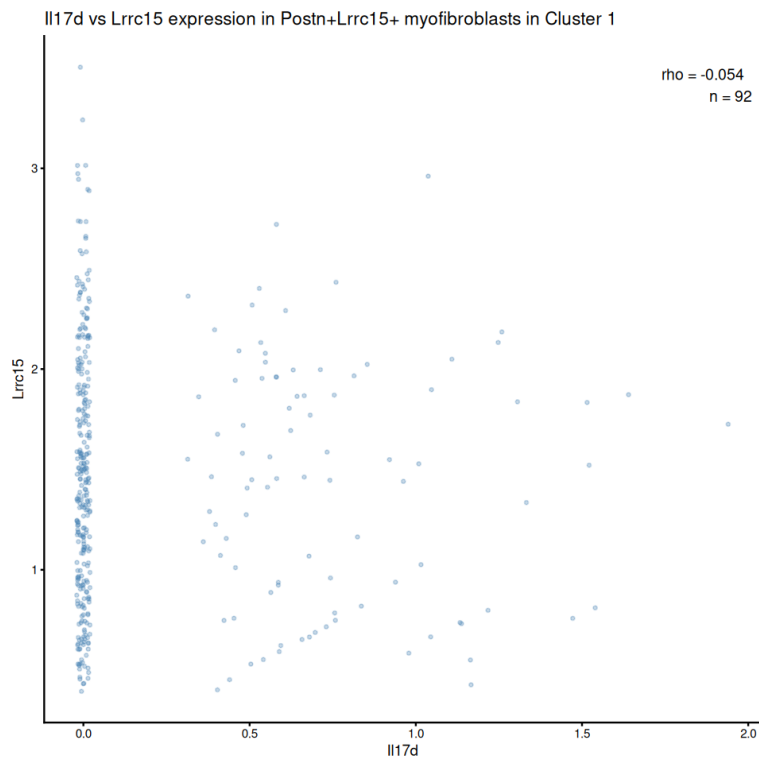


Figure 23. Jitter plot showing expression level of myofibroblast marker Lrrc15+ vs Il17d in Postn+Lrrc15+ myofibroblasts in Cluster 1.

To explore potential IL-17D expression along a developmental trajectory, Monocle3-based trajectory analysis was performed to infer potential transcriptional relationships among fibroblast subclusters. Pseudotime ordering suggested putative transcription continuum including clusters 0, 1, 4, 5 and 6 (Figure 24). However, IL-17D expression did not correlate with any of the inferred trajectories shown as black lines on the UMAP illustration (Figure 25).

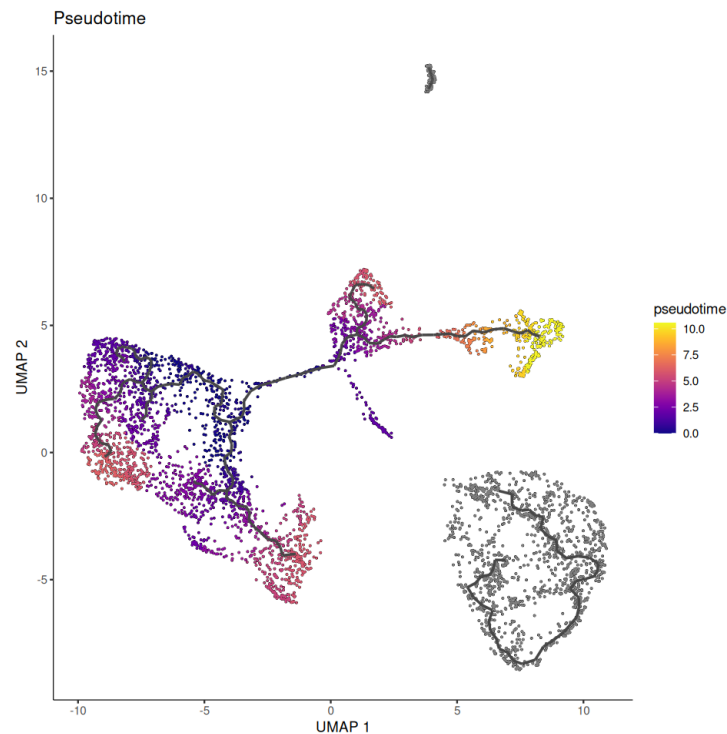


Figure 24. Pseudotime ordering of fibroblast subclutlers inferred by Monocle3.

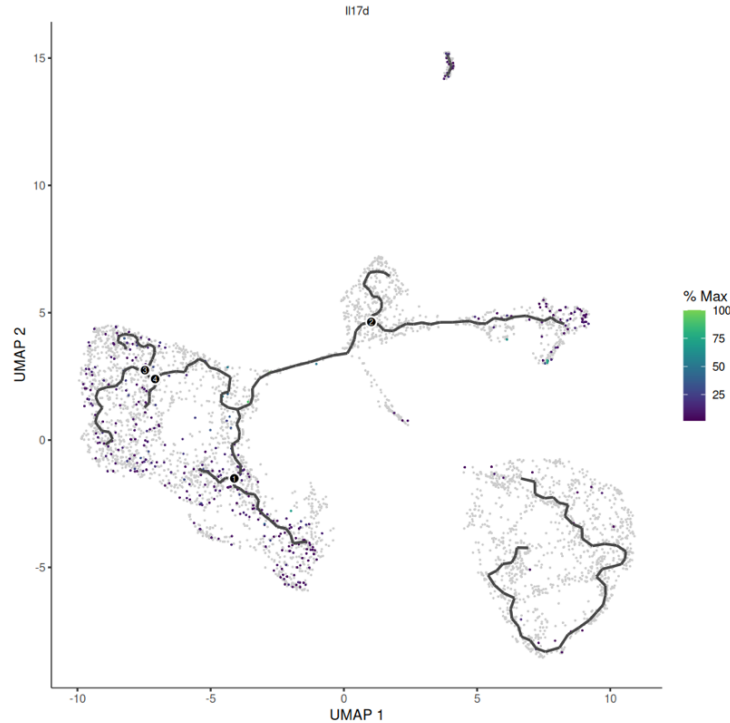


Figure 25. Monocle-based trajectories and IL-17D expression in fibroblast subclusters.

1.3. IL-17D+ Cdh19+ Schwann-like fibroblasts exhibit enrichment of NF- κ B signaling pathway

To understand the biology of IL-17D+ fibroblasts, DEG and GSEA was performed on fibroblast subclusters 0, 1, 5 and 7. The only results with statistical significance were in cluster 7 Cdh19+ Schwann-like fibroblasts. Hallmark pathway "TNF- α signaling via NF- κ B" and Gene Ontology (GO) pathway "Cellular response to corticotropin-releasing hormone (CRH)" showed normalized enrichment score (NES) above 1.5 (Figure 26). 52 genes were enriched in the Hallmark TNF- α signaling via NF- κ B pathway (Figure 27). The small GO cellular response to CRH pathway comprise of 6 genes. Its enrichment in IL-17D+ fibroblasts from subcluster 7 was led by 3 genes: Nr4a1, Nr4a2, and Nr4a3, indicating an early cellular stress response [151]. These 3 genes are also part of the

Hallmark TNF- α signaling via NF- κ B pathway. DEG results of IL-17D+ fibroblasts from subcluster 7 was statistically insignificant.

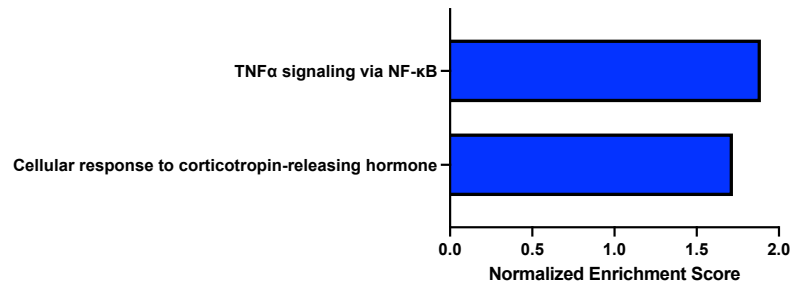


Figure 26. Pathways significantly enriched in IL-17D+ fibroblasts in subcluster 7 from GSEA.



Figure 27. GSEA enrichment plot showing enrichment of Hallmark TNF- α signaling via NF- κ B pathway in IL-17D+ fibroblasts from subcluster 7.

1.4. Skin lymphocyte composition is largely preserved upon IL-17D loss of function

Lymphocytes were also subsetting from the mouse homeostatic skin scRNA seq dataset and re-clustered with Seurat. 10 clusters were identified (Figure 28). Cell type of each cluster was identified with SingleR using ImmGen database (Figure 9). Comparing the cell proportions between WT, Het and KO samples, WT samples had slightly more

NK1.1- natural killer T (NKT) cells and less T_{reg} cells, but generally the compositions were preserved across the genotypes (Figure 30).

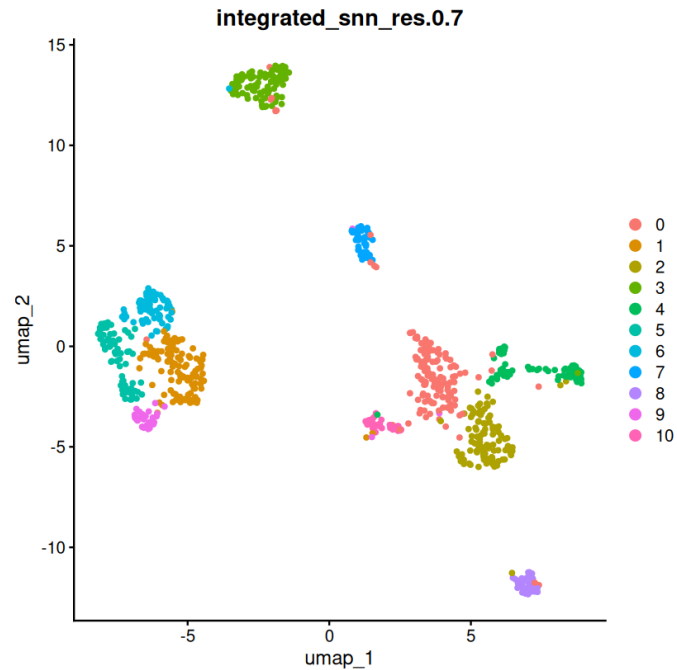


Figure 28. UMAP visualization of lymphocyte subclusters from Seurat.

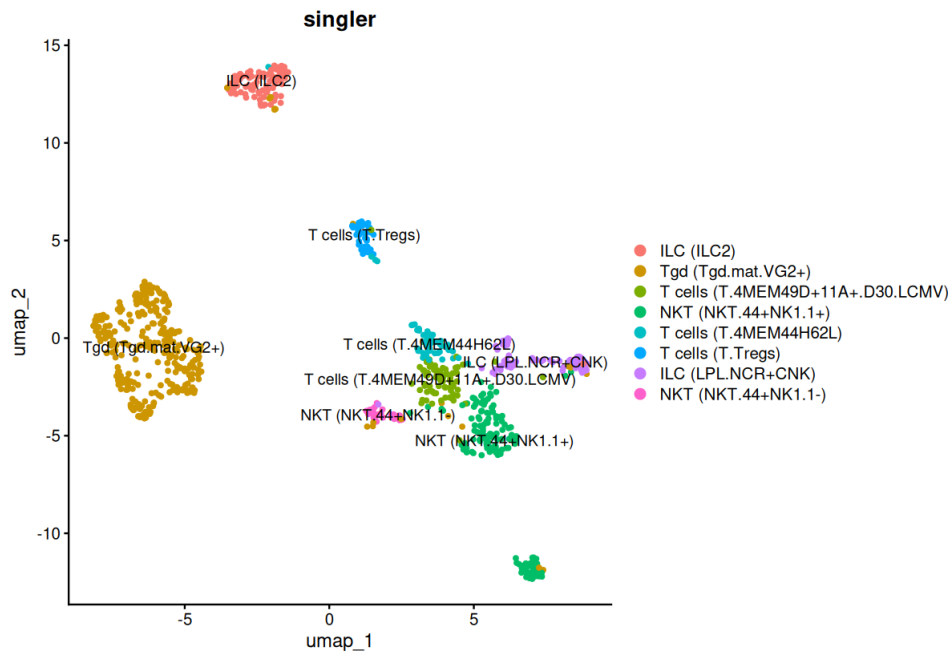


Figure 29. Lymphocyte subcluster cell type labels from SingleR using ImmGen database.

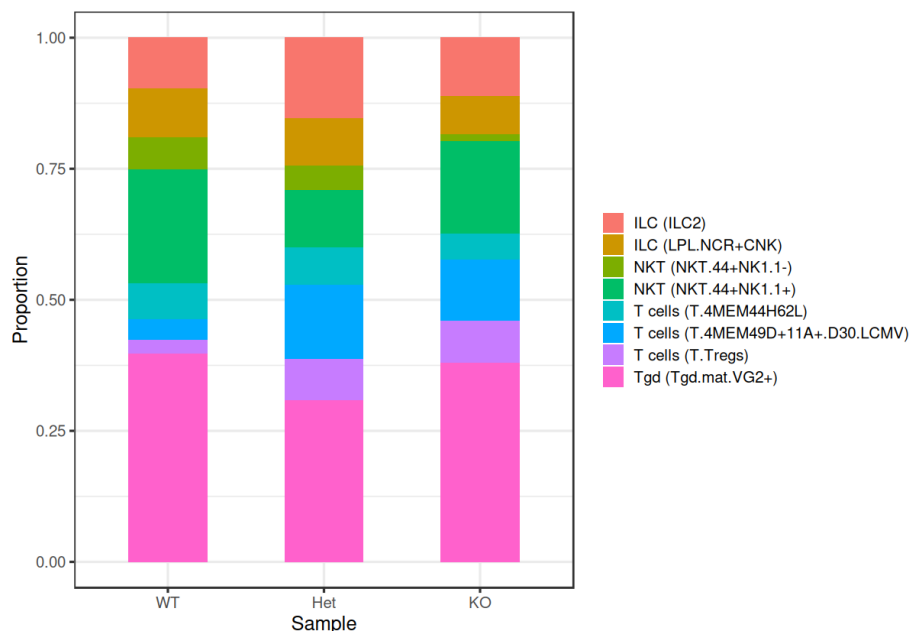


Figure 30. Proportion of skin lymphocyte subsets in WT, IL-17D Het and IL-17D KO mice.

GSEA analyses revealed significant enrichment of G2M checkpoint and E2F targets pathways in IL-17D+ cells from several lymphocyte subpopulations, indicating proliferation of IL-17D+ cells. However no other biological processes were identified from DEG and GSEA analyses.

1.5. IL-17D is also primarily expressed in fibroblasts in human skin

Published scRNA seq datasets were re-analyzed to investigate the expression of IL-17D in human skin. From a published scRNA seq dataset of skin samples from 3 healthy human donors and 3 patients with keloids [152], 22 clusters of cells were identified (Figure 31) and labeled based on DEG markers outlined in the publication (Figure 32). In these clusters, expression of IL-17D was predominantly in fibroblasts (Figure 33; Figure 34). No substantial difference in IL-17D expression was observed between samples from healthy donors and keloids (Figure 35).

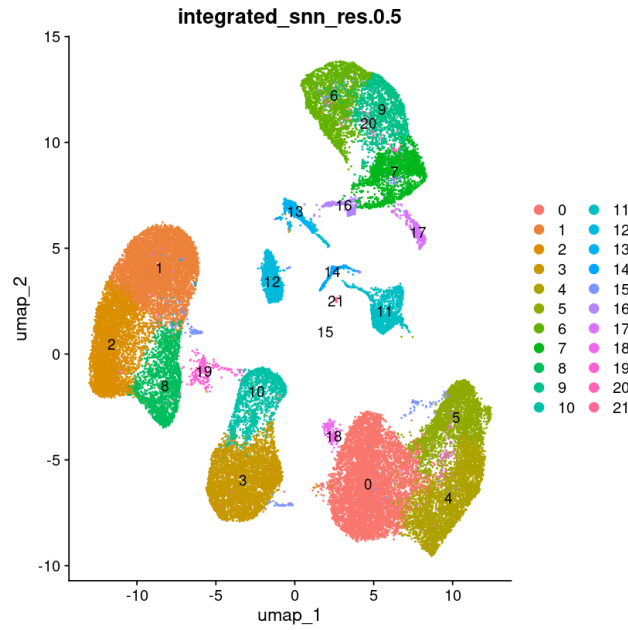


Figure 31. UMAP visualization of cells in human keloids and healthy skin from Seurat.

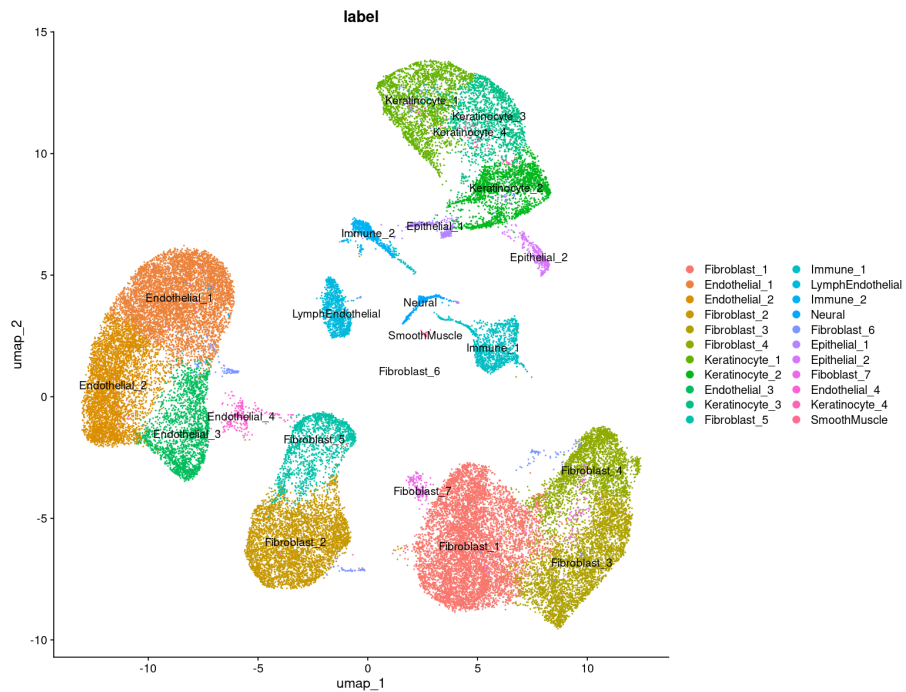


Figure 32. Curated cell type identity labels for clusters from DEG markers in the original literature.

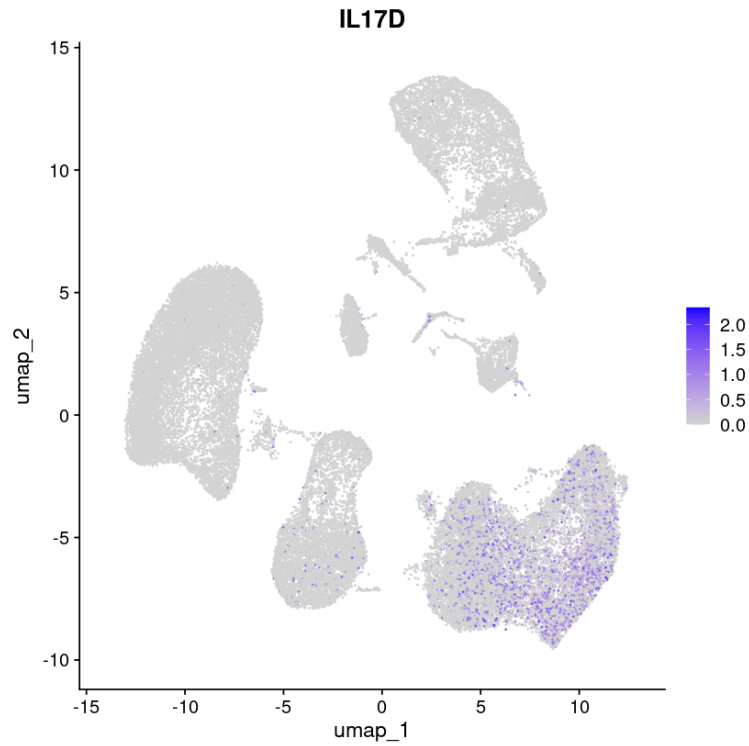


Figure 33. UMAP visualization of IL-17D expression in human keloids and healthy skin.

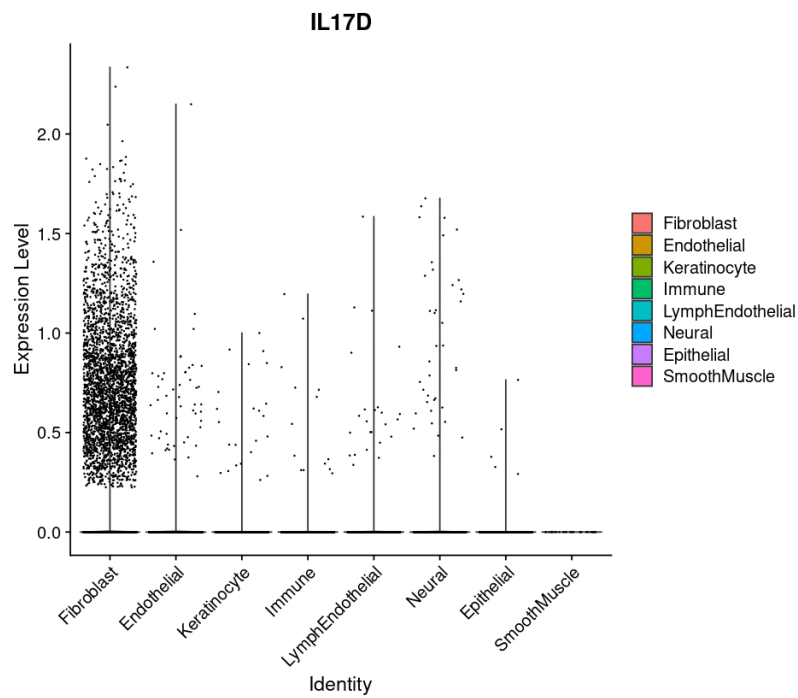


Figure 34. Violin plot showing IL-17D expression in different cell types in human keloids and healthy skin.

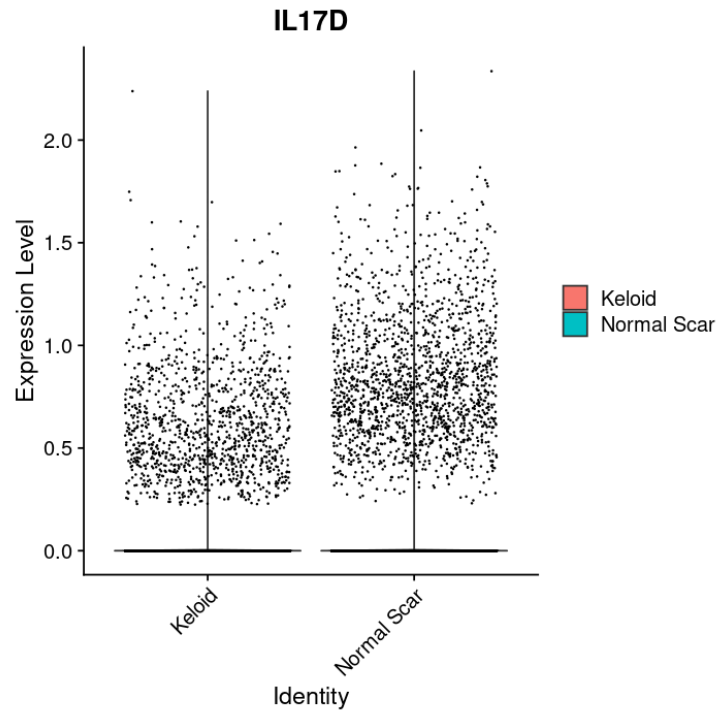


Figure 35. Violin plot showing IL-17D expression in human keloids versus healthy skin.

In another published scRNA seq dataset, 9 fibroblast subsets were identified in samples from healthy donors and patients from 23 skin diseases including psoriasis, atopic dermatitis, acne, alopecia areata, hidradenitis suppurativa, prurigo nodularis, granulomatous, infectious and lupus/interface diseases, scarring and fibrotic disorders, and 5 cutaneous tumors. [110]. Among the nine fibroblast subsets, five clusters were disease-associated, whereas three clusters — F1, F2, and F2/3 — were enriched in healthy donor samples. IL-17D expression in this fibroblast dataset clearly mapped with the density of cell in healthy donors in Figure 3d of the original paper, with highest expression in the F2 universal cluster (Figure 36).

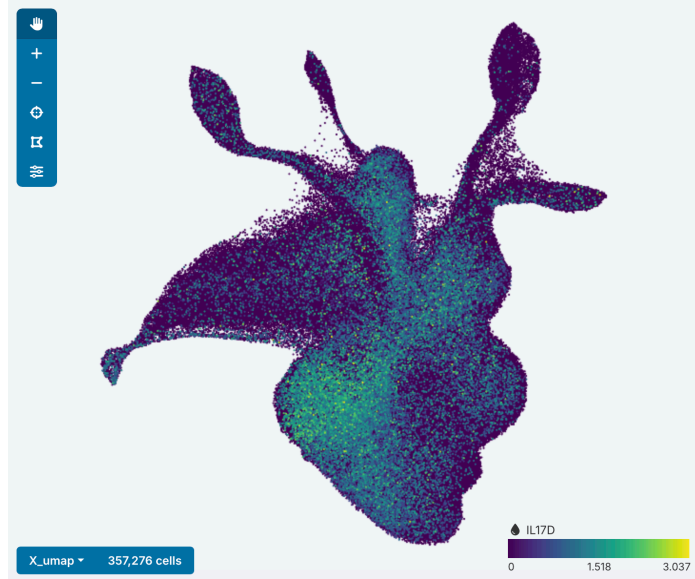


Figure 36. UMAP visualization of IL-17D expression in human skin fibroblasts from healthy donors and patients.

2. IL-17D has limited role in skin inflammation models *in vivo*

To explore the role of IL-17D in skin inflammation, two widely adopted *in vivo* mouse models were used: Imiquimod-induced psoriasis-like model and MC903-induced atopic dermatitis-like model. In both models no difference was observed in the disease progression, measured by ear thickening, between WT and IL-17D KO mice (Figure 37; Figure 38), suggesting limited roles of IL-17D in skin inflammatory diseases, especially psoriasis and atopic dermatitis.

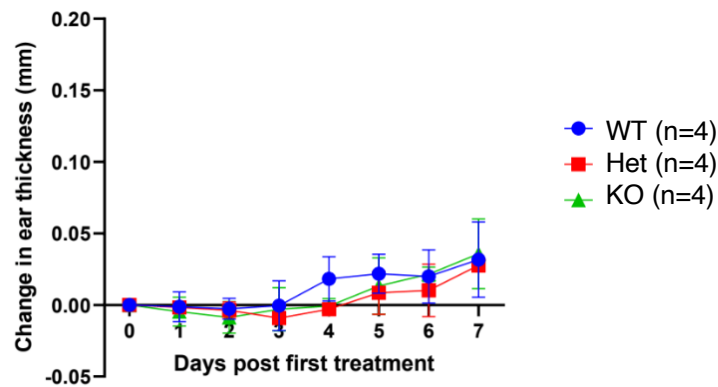


Figure 37. Ear thickness change in Imiquimod-treated psoriasis model.

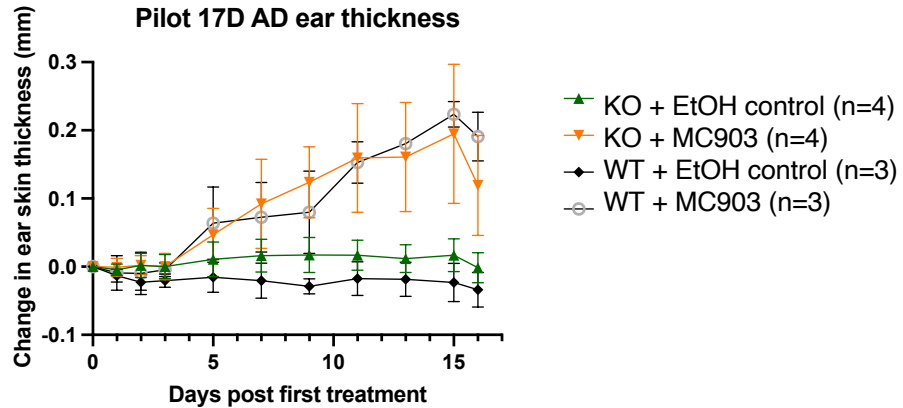


Figure 38. Ear thickness change in MC903-induced atopic dermatitis model.

Acknowledgement

Section 2 contains unpublished material coauthored with Dinh, Diane. The dissertation author was the primary author of this section.

3. IL-17D may promotes wound healing *in vitro* and *in vivo*

3.1. IL-17D promotes cell migration in scratch assays *in vitro*

To investigate the role of IL-17D in wound healing, scratch assays were performed on cell lines treated with rmIL-17D *in vitro*. After cells reach >60% confluence in culture, a scratch was created on the culture with a pipette tip. The gap width of scratch was measured under the microscope and normalized with respect to initial gap width at time 0. Upon treated with recombinant IL-17D, D29M1 fibrosarcoma cell line, RAW267.4 macrophage cell line, HaCaT keratinocyte cell line and primary BMDM from WT mice all exhibited faster gap closure in scratch assay (Figure 39-Figure 42), indicating enhanced migration and/or proliferation of the cells. Heat-inactivated rmIL-17D was used as a negative control in the scratch assay with D29M1 cell line. B16-OVA

melanoma cell line did not respond to rmlL-17D-Fc treatment in the scratch assay (Figure 43).

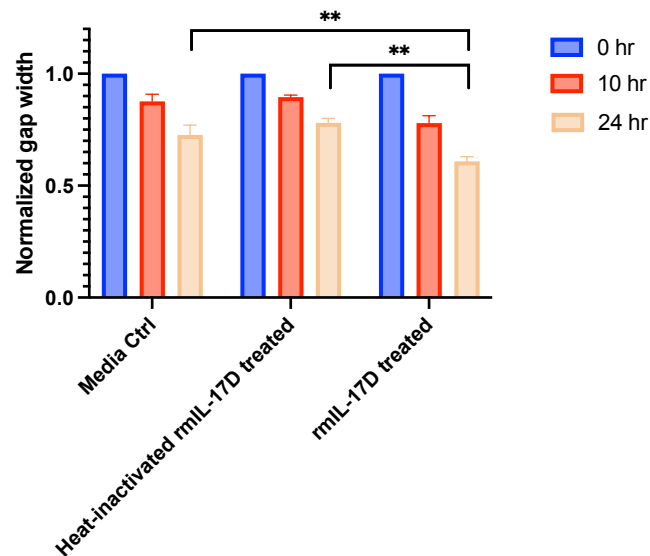


Figure 39. Gap closure of D29M1 fibrosarcoma cell line treated with rmlL-17D in scratch assay, representative of three experiments.

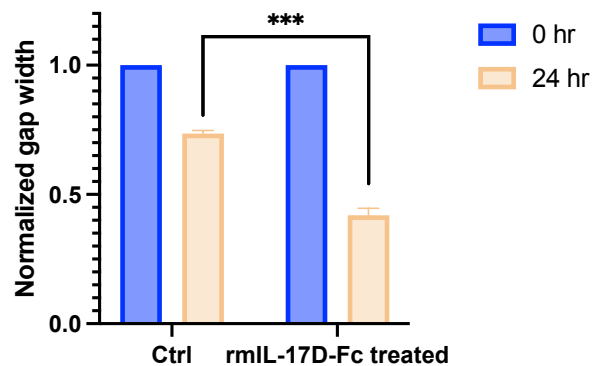


Figure 40. Gap closure of RAW264.7 macrophage cell line treated with rmlL-17D-Fc in scratch assay, representative of two experiments.

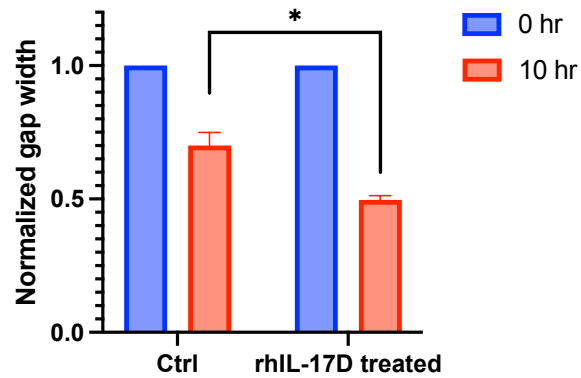


Figure 41. Gap closure of HaCaT human keratinocyte cell line treated with rhIL-17D in scratch assay.

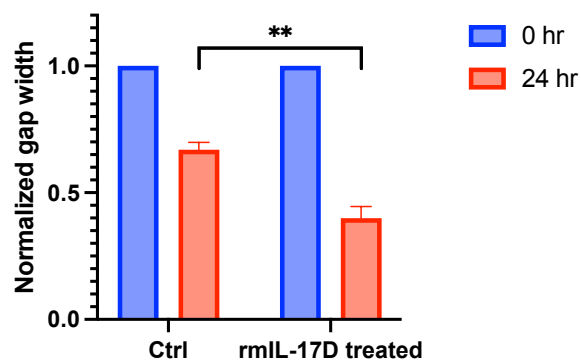


Figure 42. Gap closure of primary BMDM treated with rmIL-17D in scratch assay, representative of two experiments.

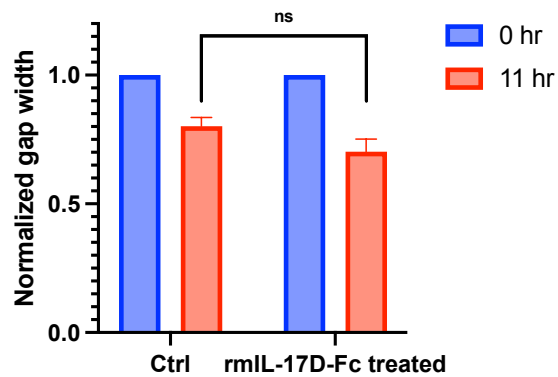


Figure 43. Gap closure of B16-OVA melanoma cell line treated with rmIL-17D-Fc in scratch assay.

Meanwhile, no changes in proliferation were observed for D29M1 cell line treated with rmIL-17D (Figure 44), indicating its scratch assay results mainly from migration.

Further investigation on the effects of rmIL-17D on macrophage and keratinocyte proliferation is ongoing.

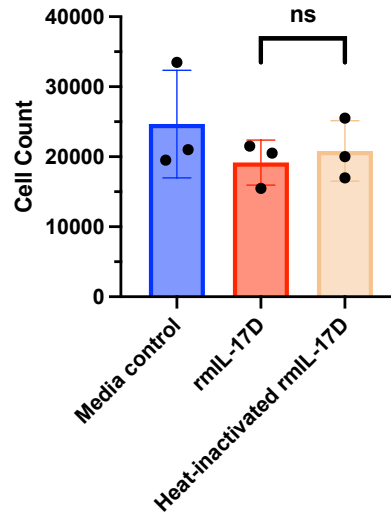


Figure 44. Proliferation of D29M1 fibrosarcoma cell line after treated with rmIL-17D for 24 h, representative of two experiments.

Acknowledgement

Section 3.1 contains unpublished material coauthored with Nguyen, Lance and Hidalgo, Sophia. The dissertation author was the primary author of this section.

3.2. IL-17D may promotes wound healing *in vivo*

Splinted excisional wound punch experiments were performed on mice to explore the role of IL-17D in wound healing *in vivo*, following an established protocol [146]. Wounds were treated daily with either 1 ug rmIL-17D or HBSS buffer. The rmIL-17D-treated group showed faster wound healing on day 4 post-surgery, but no statistical significance was observed (Figure 45). However, clear advance of wound edge towards the center of the wound and re-epithelialization were visually observed in the rmIL-17D treated group (Figure 46). The silicone splints started detaching from the animals on

day 5 post-surgery; therefore, further optimization of the protocol is underway to capture the complete time course of wound healing.

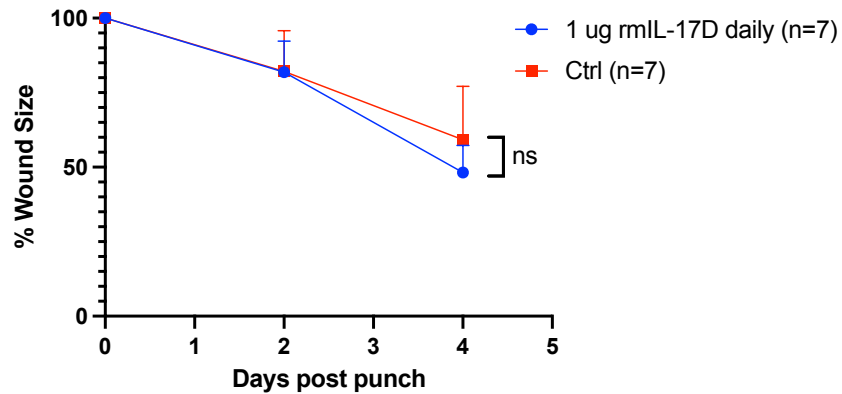


Figure 45. Percentage wound closure of splinted excisional wounds treated with rmIL-17D and HBSS control on WT mice, representative of two experiments.

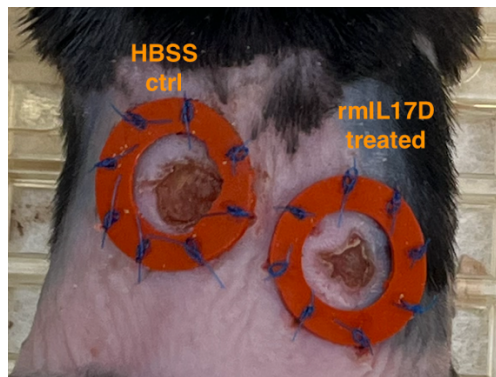


Figure 46. Appearance of splinted wounds on day 4 post-surgery.

4. IL-17D alters macrophage antigen presentation- and phagocytosis-related transcriptional programs *in vitro*

Bulk RNA seq analyses was performed on cell lines to understand the mechanism of IL-17D in scratch assays *in vitro*. DEG and GSEA analyses on D29M1 fibrosarcoma cells treated with rmIL-17D did not yield statistically significant results. The genetically modified D29M1-17D cell line showed enrichment in protein synthesis- and

cell survival-associated pathways compared to the parental cell line, but no coherent biologically meaningful program was identified.

For macrophages, GSEA analyses on neither RAW 264.7 macrophage cell line nor BMDM treated with rmlL-17D had statistically significant outputs. To identify the genes regulated by IL-17D in BMDM, heatmaps illustrating expression profiles of individual replicates were generated for two experiments: experiment 1 of BMDMs treated with 250 ng/mL rmlL-17D for 24 h and experiment 2 of BMDMs treated with either Het or IL-17D KO fibroblast *in vitro* culture supernatants for 6 h. No clear polarization towards M1 or M2 phenotypes were observed in both experiments, but genes associated with antigen presentation was downregulated after rmlL-17D treatment in experiment 1 (Figure 47). KO fibroblasts showed lowest expression of some antigen presentation genes in experiment 2, indicating the effect of rmlL-17D on BMDM antigen presentation may be masked by other secretory factors from fibroblasts.

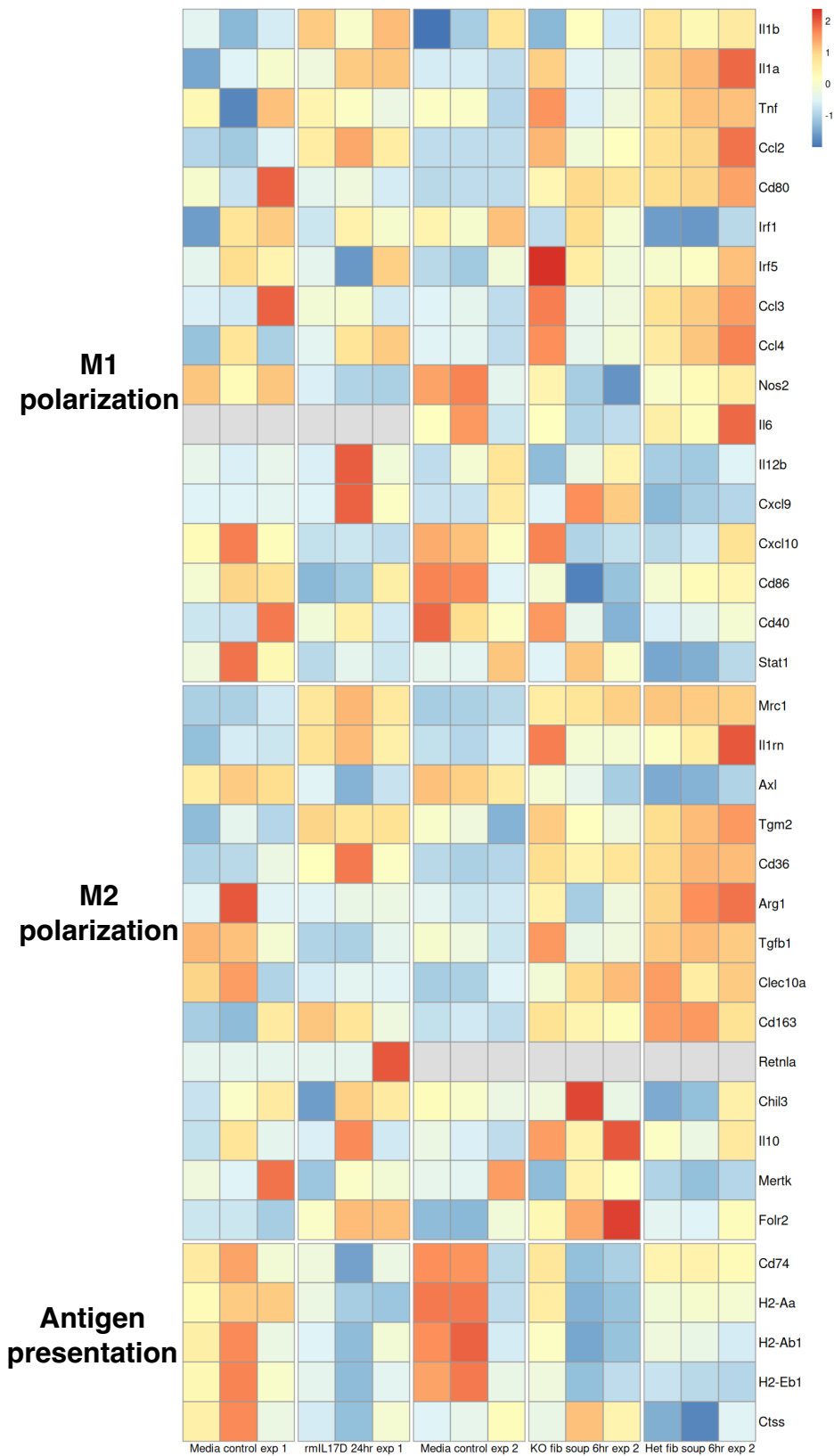


Figure 47. Expression of M1/M2 polarization- and antigen presentation-associated genes in BMDM.

Since Axl was downregulated by IL-17D in both experiments (Figure 47), other genes associated with phagocytosis were also explored. DEG analysis showed upregulation of Soc3, Mrc1, Itgam, Msr1 and Lrp1 in BMDMs treated with rIL-17D versus untreated controls (Figure 48), while no significant changes in phagocytosis-associated genes were observed in experiment 2 (Figure 49). Combining both experiments, more genes in Tyro3-Axl-Mertk (TAM), milk fat globule-EGF factor 8 (MFG-E8), Lrp1 and complement, scavenger receptor, Fc receptor and intracellular engulfing machinery pathways appeared to be regulated by IL-17D (Figure 50).

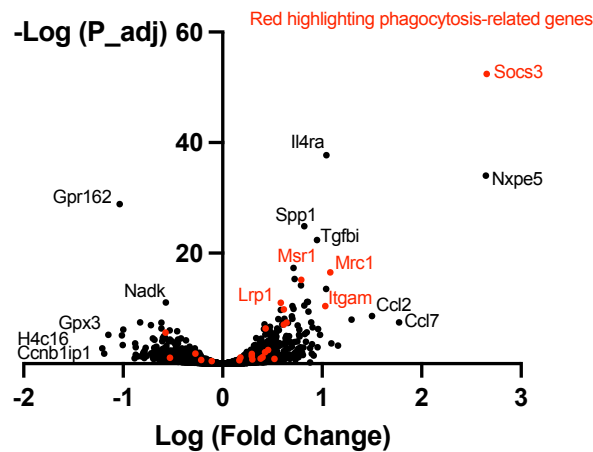


Figure 48. Volcano plot of DEGs in BMDMs treated with rIL-17D versus untreated controls.

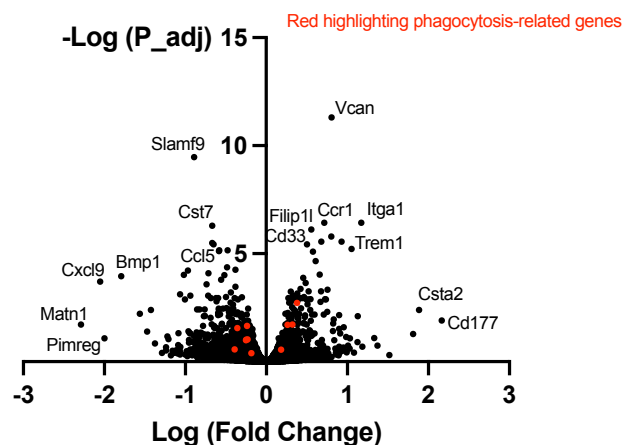


Figure 49. Volcano plot of DEGs in BMDMs treated with Het versus IL-17D KO fibroblast culture supernatant.

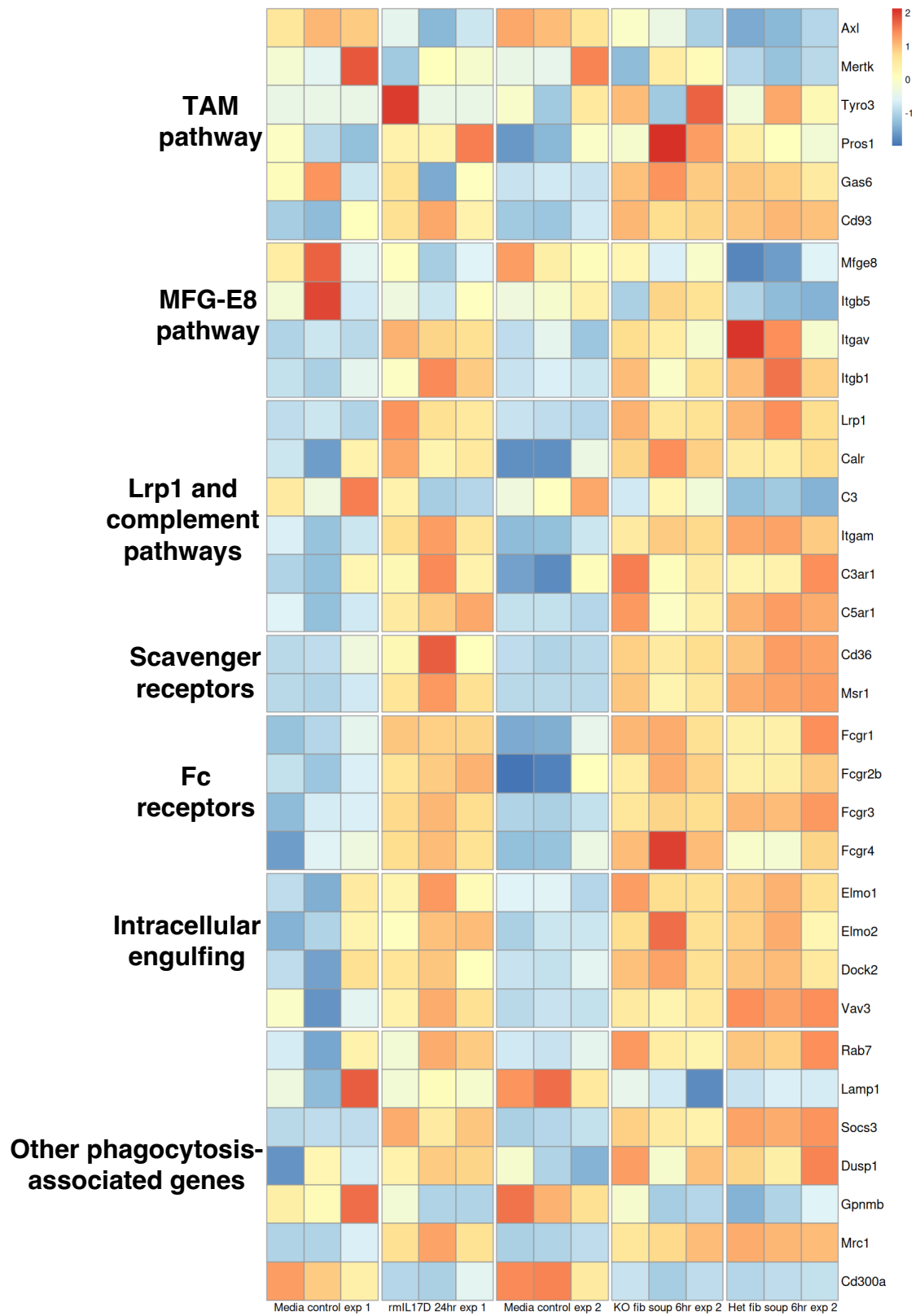


Figure 50. Expression of phagocytosis-associated genes in BMDM.

Acknowledgement

Section 4 contains unpublished material coauthored with Chen, Taian. The dissertation author was the primary author of this section.

Discussion

1. Cellular sources and niches of IL-17D in skin

Our single-cell analyses suggest that IL-17D is not broadly produced by immune cells in skin, but is instead enriched in dermal fibroblast populations, supporting the idea that IL-17D may function as a stromal cytokine within the skin microenvironment. This observation matches with previous studies that identified non-immune cells to be the major source of IL-17D.

Among mouse skin fibroblasts, expression of IL-17D is not restricted to one fibroblast subset. Lef1+ dermal papilla fibroblasts, Tnn+ interstitial fibroblasts, Bnc3+ reticular fibroblasts and Cdh19+ Schwann-like fibroblasts all show IL-17D expression at homeostasis. Cdh19+ Schwann-like fibroblasts may represent a specialized niche for IL-17D expression in the skin as they presented upregulated TNF- α signaling pathway in IL-17D+ cells. These cells in our study exhibit expression profiles similar to Schwann cells, but they express pan-fibroblast markers Pdgfra and lack Schwann cell marker Sox10. Meanwhile recent publications highlighted similar Cdh19+/Ngfr+ fibroblast subpopulation [110, 142]. Hence, we named this IL-17D+ cell cluster Schwann-like fibroblasts. Expression of IL-17D was not picked up in nerve cells and Schwann cells in our study. However as peripheral glial fibroblast is recognized in tissue repair and IL-17B has been identified in injured peripheral nerves [153, 154], IL-17D may also be involved in nerve-tissue crosstalk, especially during wound healing.

2. Limited roles of IL-17D in skin inflammation mouse models

Even though other IL-17s has been well studied in skin inflammation, our data suggested that IL-17D does not play a significant role in psoriasis and atopic dermatitis. This is in contrast to the study where IL-17D was identified to promote MC903-induced atopic dermatitis in mouse [100]. This may come from the fact that our studies were on IL-17D KO mice to study loss-of-function. The natural level of IL-17D may be too low to have any significant effect. Larger dose of IL-17D may well have a different effect, as shown in the study on DDX5.

Our re-analysis of published data revealed IL-17D was higher in fibroblasts found in healthy human skin compared to diseased lesions, suggesting IL-17D may be involved in maintaining skin inflammation to the desired level for protective immunity. This is in line with the fact that IL-17B is an antagonist for IL-17E, suggesting cytokines in IL-17 family can have contrast roles in inflammation.

3. Potential mechanisms of IL-17D-mediated tissue repair

As rmIL-17D promotes migration of several skin cell types in scratch assays *in vitro*, and alters wound morphology in splinted wounds *in vivo*, we propose a potential role of IL-17D in mediating tissue repair, especially in the skin. Because both macrophage cell line RAW264.7 and primary BMDMs responded to rmIL-17D in scratch assay, we hypothesize IL-17D mediates skin wound healing by altering macrophage functions. In fact, studies have shown that macrophage efferocytosis is important in wound healing [126, 127, 155]. More functional studies are needed to consolidate our hypothesis.

Another interesting link between IL-17D and wound healing in this study came from the expression of IL-17D in Schwann-like fibroblasts. Emerging evidence are indicating that peripheral glial repair niches coordinate macrophage recruitment, fibroblast proliferation, and myofibroblast transition during early wound healing [153, 154, 156–158]. Thus, IL-17D expression by Cdh19+/Ngfr+ fibroblasts may reflect a broader neuro-stromal repair program, although direct spatial and functional validation will be required.

4. Limitations and future directions

The strongest evidence of IL-17D expression in fibroblasts in this work – scRNA and bulk sequencing results – are at transcriptional level. There's lack of protein-level evidence. This partly results from limited commercially available IL-17D antibodies. We will confirm the presence of IL-17D in sorted skin fibroblasts with the only functional commercial IL-17D antibody applicable to ELISA and Western blot. Meanwhile we are generating an inducible IL-17D reporter mice to better capture the expression of this cytokine. Also, the sequencing results do not fully reflect the special organization of cells. We have started analyzing spatial transcriptomics data to address this limitation.

The evidence of IL-17D acting on macrophages in this study is only *in vitro*. More functional studies and *in vivo* studies are needed. We are currently exploring the effect of IL-17D on macrophages with *in vitro* efferocytosis assays. We also plan to analyze infiltrated immune cells in the splinted wound punch model and hypothesize to see a difference in macrophage populations.

The cellular mechanism of IL-17D was not elucidated. Migration- and matrix-associated pathways were not significantly altered in pathway analysis in bulk RNA sequencing of cells responding to rmlIL-17D in scratch assays. We may need further perturbation in our models, such as CD93 inhibition, to elucidate the molecular mechanism.

As discussed in the backgrounds section, there are many differences between mouse and human skin physiology. Our study with mouse cell lines and *in vivo* models may not reflect what can happen in human skin. Human HaCaT cell line did respond to rhIL-17D in scratch assay. More functional evidence of IL-17D in human system is needed. This may be achieved with studies on human organoids or human skin grafts.

5. Conclusions

In summary, we identified that at homeostasis IL-17D is mainly expressed in skin fibroblasts in mouse and human. Several mouse skin fibroblast subpopulations expressed IL-17D. In the Cdh19⁺ Schwann-like fibroblasts, IL-17D⁺ cells are associated with TNF- α signaling.

IL-17D does not play a significant role in psoriasis and atopic dermatitis. Meanwhile, IL-17D expression is higher in fibroblast found in healthy human skin compared to diseased lesions, suggesting a role of maintaining protective tissue inflammation.

Exogenous recombinant IL-17D promotes gap closure in scratch assays on D29M1, RAW264.7, HaCaT cell lines *in vitro*, but not B16-OVA melanoma cell line. This

effect is mainly through cell migration. We have some data suggesting exogenous recombinant IL-17D may promote wound healing in splinted excisional punch model *in vivo*, but closer investigation is required. The effect of IL-17D on wound healing may result from altering macrophage functions in antigen-presentation and phagocytosis.

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