8-25-2023

A Human STAT3 GOF Variant Alters T Cell Function to Drive Skin Inflammation

Kelsey Adele Toth
Washington University in St. Louis

WASHINGTON UNIVERSITY IN ST. LOUIS

Division of Biology and Biomedical Sciences
Immunology

Dissertation Examination Committee:
Megan Cooper, Chair
Anthony French
Chyi-song Hsieh
William McCoy
Gwendalyn Randolph

A Human STAT3 GOF Variant Alters T Cell Function to Drive Skin Inflammation

by
Kelsey Adele Toth

A dissertation presented to
Washington University in St. Louis
in partial fulfillment of the
requirements for the degree
of Doctor of Philosophy

August 2023
St. Louis, Missouri

Table of Contents

List of Figures

List of Tables

List of Abbreviations

AD – autosomal dominant
AR – autosomal recessive
BM – bone marrow
CITE-seq – cellular indexing of transcriptomes and epitopes
CLN – cervical lymph node
CMC – chronic mucocutaneous candidiasis
CNTF – ciliary neurotrophic factor
CRISPR – clustered regularly interspaced short palindromic repeats
DC – dendritic cell
DN – dominant negative
DNT – double-negative T cell;
Eff/TEM – effector/T effector memory
EGF – epidermal growth factor
Exh – exhausted
G-CSF – granulocyte colony-stimulating factor
GM-CSF – granulocyte-macrophage colony-stimuating factor
GOF – gain of function
Haplo – haploinsufficiency
IEI – inborn error of immunity
IFN – interferon
IFNAR – IFN-α/β receptor
Ig – immunoglobulin
IGF – insulin-like growth factor
IL – interleukin
IL-6R – IL-6 receptor
IMQ – imiquimod
Int – intermediate
ISG – IFN-stimulated gene
JAK – Janus kinase
JAKinib – JAK inhibitor
LIF – leukemia inhibitory factor
LOF – loss of function
MCP – monocyte chemoattractant protein
MIP – macrophage inflammatory protein
moDC – monocyte-derived dendritic cell
NK – natural killer
NOD – non-obese diabetic
NT – untreated
OSM – oncostatin M
PBMC – peripheral blood mononuclear cell
PDGF – platelet derived growth factor
PIAS – protein inhibitor of activated STAT
PJP – *Pneumocystis jiroveci* pneumonia
PMA – Phorbol 12-myristate 13-acetate
PTP – protein tyrosine phosphatases
pTreg – peripheral Treg
SCF – stem cell factor
SCID – severe combined immunodeficiency
seq – sequencing
SLE – systemic lupus erythematosus
SOCS – suppressor of cytokine signaling
T1D –type 1 diabetes
TB – Mycobacterium tuberculosis
TCM – T central memory
TCR – T cell receptor
Tfh – T follicular helper
TGF-β – transforming growth factor β
Th – T helper type
TLR – Toll-like receptor
TNF – tumor necrosis factor
Treg – regulatory T cell.
TYK – tyrosine kinase
UMAP – uniform manifold approximation and projection
WT – wild-type

Acknowledgments

Work on STAT3 GOF in the Cooper laboratory was supported by NIH/NIAID 1P01AI155393

An old teacher of mine liked to quote the poem by John Donne: 'No Man is an Island', and those words often came to mind during graduate school. This dissertation would have been impossible without the work of so many other people. I want all of you to know how grateful I am for your contributions, mentorship, and moral support.

I would first like to thank my mentor, Megan Cooper, for being incredibly patient with me while guiding the entire lab through a period that was difficult for just about everyone. You always knew how to keep me focused, and I always left our meetings feeling excited about my project. You did your best to shape me into a successful, independent immunologist, and I hope my future work will make you proud.

I also want to thank Erica Schmitt, who has been a role model throughout graduate school. I could only ever hope to emulate your tenacity and dedication to science. You always made time to share your expertise and listen to me rant about results I didn't understand. I will always appreciate your support.

My thesis committee has been instrumental in keeping this project (and me) on track. To Anthony French, Gwen Randolph, Chyi Hsieh, and almost-last-minute addition, Bill McCoy: thank you for teaching me to think critically about this project, and for not leaving Wash. U. before I finished my Ph.D. Your feedback has been invaluable, and it has been a privilege to learn under leaders in the field of immunology and rheumatology.

I also want to give special thanks to my first mentors in immunology: Andy Gelman, Dan Kreisel, Sasha Krupnick, and everyone from the thoracic surgery lab. I knew that there was no other place for me except in research after working with and learning from all of you.

Everyone in the Cooper Lab deserves my eternal gratitude. Thanks to Molly Keppel, Nermina Saucier, (also) Kelsey Trammel, and Katherine Owens for always knowing the answers to my (constant, often repeat) questions, genotyping my thousands of samples, and finding the reagent that was right under my nose. Thanks to Josh Tobin and Sammy Risma for being beacons of positivity. And to Maria Cîmpean, Hyogon (Stacey) Sohn, and Jahnavi Aluri, thank you for showing me what thoughtful, dedicated, independent immunologists look like. Thank you for sharing your plant collection, for running with me to get donuts in the breakroom, and for being role models of resilience in science and life in general. I can't express how grateful I am that we are friends.

I wouldn't have made it through graduate school without friends and family outside of the lab. To my brother in immunology, Alex Earhart, who knows pretty much everything: you have been there for me at all hours of the day, and I can't wait to see the brilliant work from your lab when you become a PI. To Amanda Lands-Ramrup, with her excellent taste in chill lo-fi beats, and Sarah Lands-Ramrup, with her constant encouragement: all I can say is thank you for being my best friends. And to everyone who has been a player at my D&D table: thank you for keeping me sane.

To my parents, Terry and Alice, who actually did the hard work to get me here: thank you for your unconditional support and love, for never doubting me when I said I was almost done with school, and for sacrificing years of your lives so that I could attend Wash. U. To my siblings, Terry and Jamie: you two are the reasons why I wanted to study immunology in the first place, so I hope you think my science project is cool.

Finally, to my partner Christian, who for the last 11 years has been holding my hand while I fumbled around in the dark: thanks for being my reason for waking up every day. I could write about you for a thousand pages and not have written enough.

Kelsey Adele Toth

Washington University in St. Louis

August 2023

Dedicated to my brother and sister.

ABSTRACT OF THE DISSERTATION

A Human STAT3 GOF Variant Alters T Cell Function to Drive Skin Inflammation

by

Kelsey Toth

Doctor of Philosophy in Biology and Biomedical Sciences

Immunology

Washington University in St. Louis, 2023

Professor Megan A. Cooper, Chair

Inborn errors of immunity (IEIs) are disorders caused by monogenic variants that lead to immune deficiency and dysregulation. Although rare, IEIs have shaped our understanding of how single genes function in the immune systems of both humans and mice. The identification of loss-of-function (LOF) and gain-of-function (GOF) variants in the highly conserved signal transducer and activator of transcription (STAT) family molecules have led to discoveries involving how STAT signaling affects immune cell development, differentiation, and the induction and regulation of immune responses. Germline GOF variants in *STAT3* cause early-onset multi-organ autoimmunity and immune dysregulation. Patients with STAT3 GOF syndrome have been treated successfully with inhibitors of upstream STAT3 signaling; however, the pleiotropic nature of *STAT3* expression makes the identification of mechanisms that initiate disease difficult. Furthermore, tissue sampling limited to the peripheral blood of patients creates additional challenges in understanding how GOF variants affect cell types in specific environments. Our group recently generated and described a mouse model of STAT3 GOF that displays T cell dysregulation that increases with age. We hypothesized that T cell dysregulation in STAT3 GOF

requires an inflammatory stimulus that would lead to autoimmune disease as seen in patients, and that this stimulus would activate tissue-specific inflammatory responses. In line with patient observations, we found that skin inflammation occurs spontaneously with age and is associated with increased local Th17 responses and neutrophil infiltration. A similar phenotype was elicited in younger mice with immune stimulation using the imiquimod (IMQ) model of skin inflammation, with increased IL-17A and IL-22 production by local Th17 cells that was cell-intrinsic. IMQ-induced skin inflammation was not dependent on $CD8^+$ T cells or γδ T cells, and $CD4^+$ T cells were sufficient to drive increased inflammation in STAT3 GOF mice. Single-cell analysis of T cells identified an increased $CD4^+$ T clonal response with upregulated *Il22* expression in expanded clones. IL-22 partially mediated the increased inflammation in STAT3 GOF. Concurrent treatment with IMQ and tofacitinib, a JAK inhibitor, resulted in decreased skin inflammation without affecting Th17 cytokine expression. In summary, this work suggests a role for Th17 in the development of autoimmunity in STAT3 GOF syndrome and presents a target for treatment that may not be affected by current therapies.

Chapter 1: Inborn errors of immunity of the JAK-STAT pathway

Parts of this chapter are adapted from a manuscript published in the Journal of Immunology:

KA Toth, EG Schmitt, and MA Cooper. Deficiencies and Dysregulation of STAT Pathways That Drive Inborn Errors of Immunity: Lessons from Patients and Mouse Models of Disease. *J. Immunol.*, 2023. https://doi.org/10.4049/jimmunol.2200905

Inborn errors of immunity (IEIs) are the result of single gene defects that alter development, function, and regulation of the human immune response. IEIs cause a broad clinical phenotype in patients, including susceptibility to infection, autoinflammation, immune dysregulation, atopy, bone marrow failure, and cancer susceptibility. Identifying the genetic causes of IEIs has improved with advances in diagnostics and genetic testing, and more than 485 distinct IEIs have been described (1). Although individually rare, the combined prevalence of IEIs is estimated to be between 1:1000 and 1:5000 worldwide (2). Identification and characterization of the molecular mechanisms of IEIs provide tremendous insight into genes critical for regulation of the human immune response. Investigation into the mechanisms of IEIs also provides a valuable tool for developing targeted therapies for patients, which can translate to treatment strategies for phenotypically similar diseases with shared signaling pathways (3-5).

Discovery of IEIs associated with altered function of six of the seven STAT proteins and several associated signaling pathways has led to a mechanistic understanding of how these signaling pathways dictate human immunity. STAT family proteins provide critical signals for multiple cellular functions within the innate and adaptive immune system (6). The seven STAT proteins are highly conserved across mammalian species and are expressed in nearly every tissue type. The highly conserved nature of the genes encoding STAT proteins is evident by the fact that loss-of-function (LOF) variants are significantly underrepresented in population databases, such as gnomAD (7). Although some STAT proteins share receptor associations and activate overlapping transcriptional programs, investigations of patients and relevant mouse models have provided evidence for the roles of individual STAT signaling in regulating the immune response (6, 8).

1.1 Overview of JAK/STAT signaling

STAT family proteins are transcription factors involved in many cellular processes, including regulation of immune cell responses, proliferation, differentiation, metabolism, and apoptosis (6). In immune cells, STATs can be activated by many cytokines and growth factors (9). Binding of a ligand to its surface transmembrane receptor causes a conformational change in associated intracellular JAK proteins, which recruit and phosphorylate STATs. Activated STATs form homodimers or heterodimers with other STATs and then translocate to the nucleus to modulate gene expression (6). STAT induction also activates negative feedback loops through the transcriptional upregulation of suppressor of cytokine signaling (SOCS) family proteins, protein inhibitor of activated STAT (PIAS) family proteins, and protein tyrosine phosphatases (PTPs). These negative regulators can inhibit STAT signaling through several mechanisms, including blocking STAT binding or JAK activity at the receptor, dephosphorylating JAKs or STATs, targeting JAK or STAT proteins for proteasome degradation, or blocking DNA binding of STAT dimers (10). STATs are strongly conserved in both humans and mice, with both species carrying genes for all seven known STAT proteins: STAT1, STAT2, STAT3, STAT4, STAT5A, STAT5B, and STAT6. Associated JAK proteins include JAK1, JAK2, JAK3, and TYK2 (6). Signaling pathways have been well characterized for each STAT, and multiple IEIs affecting these signaling pathways have been identified (**Figure 1.1**) (6, 8-11).

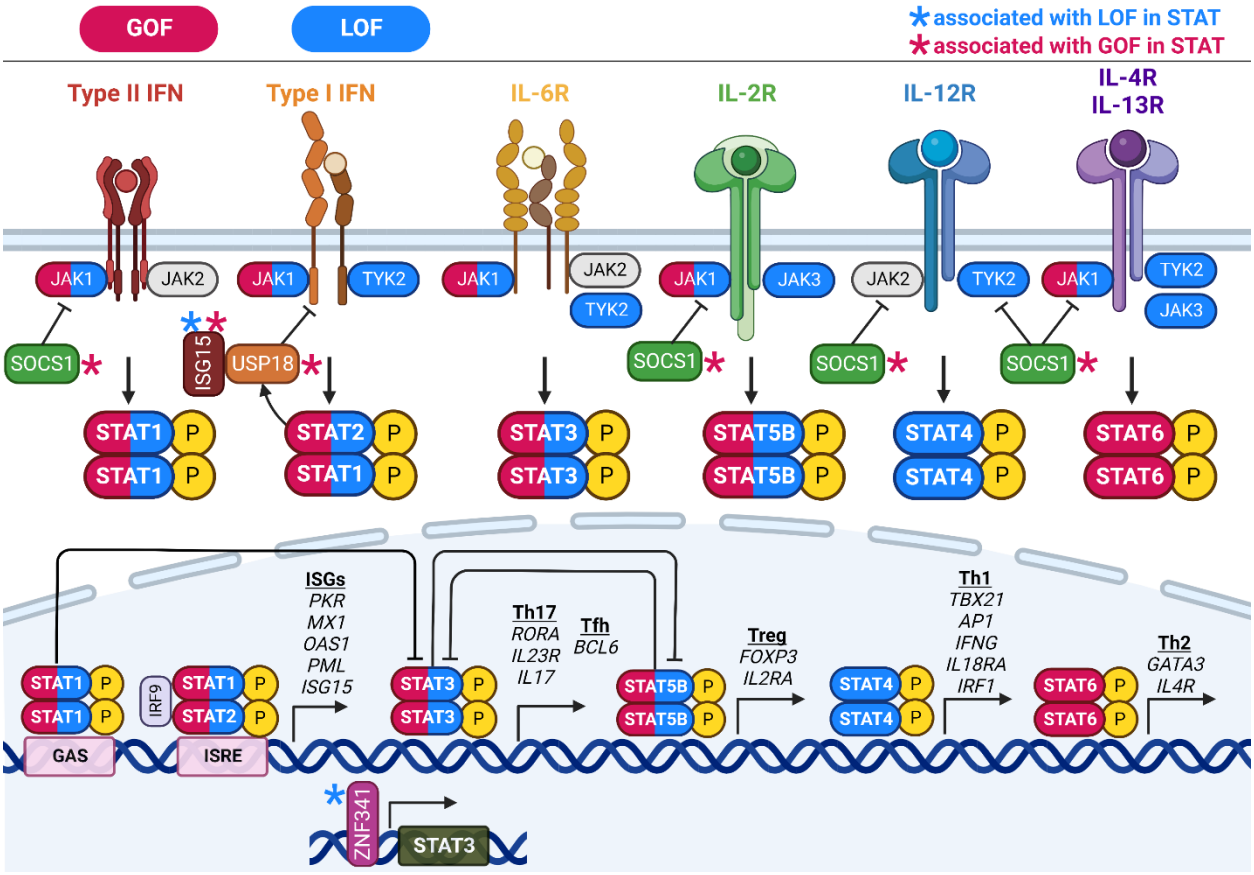

Figure 1.1. STAT signaling and inborn errors of immunity. Examples of classical cytokine signals activating STAT proteins relevant to IEI are shown. JAKs and STATs are denoted in red for gain-of-function (GOF) or blue for loss-of-function (LOF) in IEI. Defects in regulators, either loss- or gain- of regulatory function, are marked by asterisks in red if they result in gain of STAT signaling, or blue if they cause loss of STAT signaling. Type II IFN receptor (IFNGR) activates JAK1/JAK2, which phosphorylate STAT1, which binds to interferon-stimulated genes (ISGs). Type I IFN receptor (IFNAR) activates JAK1/TYK2, which subsequently phosphorylate STAT1 and STAT2. STAT1/STAT2 heterodimers associate with IRF9 and bind ISGs. IL-6R classically signals through JAK1/TYK2 or JAK2/TYK2 to activate STAT3, which forms homodimers and activates Th17-associated genes, with STAT1 and STAT5B competing for DNA binding sites. The IL-2R receptor signals through JAK1/JAK3 to activate STAT5B, which positively regulates Treg fate. IL-12R signaling through JAK2/TYK2 activates STAT4 and drives differentiation of the Th1 subset. IL-4R/IL-13R signal through JAK1/TYK2 or JAK1/JAK3 activates STAT6 and Th2 differentiation. Genetic variants in JAK/STAT regulators, USP18, ISG15, ZNF341, and SOCS1, all lead to altered STAT activity and IEI. USP18 interacts with STAT2 to inhibit type I IFN receptor assembly and signaling through IFNAR2, and loss of USP18 function leads to disease similar to STAT2 GOF. Intracellular ISG15 prevents degradation of USP18 in humans, and loss of ISG15 function leads to increased type I IFN/STAT2 signaling (STAT GOF), as well as impaired IFN-γ signaling due to the lack of free/secreted ISG15 (STAT LOF). ZNF341 positively regulates STAT3 expression, and LOF variants in ZNF341 lead to disease similar to that seen with STAT3 LOF. SOCS1 binds to and inhibits other substrate binding to JAK1, JAK2, and TYK2 in humans, and patients with SOCS1 haploinsufficiency have immune dysregulation with some features of STAT1 GOF and STAT5 GOF.

1.2 Disorders of STAT signaling associated with IEI

Genetic sequencing of patients with clinical phenotypes consistent with an IEI has led to rapid discovery of genes associated with disease, particularly during the last decade with the clinical availability of next-generation sequencing platforms such as exome sequencing. Monogenic germline or somatic genetic variants in genes encoding STAT proteins lead to IEIs or IEI "phenocopies" (1, 12). These genetic variants can lead to both LOF and GOF of the encoded STAT proteins. The formation of homodimers or heterodimers with these altered proteins can lead to different functional outcomes, including dominant-negative phenotypes when paired with wild-type protein. Studies of primary cells from patients with defects in STAT signaling, complemented by gene-edited cell lines and genetic mouse models, have provided the unique opportunity to understand their role in the immune system and relevance to human disease (**Tables 1.1 and 1.2**). However, STAT proteins are widely expressed, and their effects on nonimmune factors often lead to complex disease phenotypes.

Table 1.1. Immunologic and clinical phenotypes of IEIs due to monogenic defects in STAT genes

STAT (genetics)	Immunologic Changes in Patients	Clinical Disease		Mouse Model
		Infections	Other Clinical Features	
STAT1 LOF (AD)	↓ IFN-γ signaling	Mycobacterial, *Salmonella*	—	Susceptibility to infection (13, 14)
STAT1 LOF (AR)	↓ IFN-γ and IFN-α/β signaling	Mycobacterial, severe viral infections	—	Susceptibility to infection (13, 14)
STAT1 GOF (AD)	↓ Th17 response ↓ Marginal zone and switched memory B cells, plasma cells ↑ Th1 response ↑ Naive and transitional B cells ↑ Cytokine by monocytes and moDCs	CMC, bacterial infections, viral (herpesviruses), invasive fungal infection (*Candida* spp.)	Autoimmunity (hypothyroidism, diabetes, cytopenias, enteropathy); squamous cell carcinoma; cerebral aneurysm	Susceptibility to infection ↓ IFN-γ production ↓ Ag-specific $CD8^+$ T cells ↓ Th17 response (15, 16)
STAT2 LOF (AR)	↓ IFN-α–induced ISGs	Viral (vaccine-strain measles)	—	Susceptibility to infection (17)
STAT2 GOF (AR) (R148 with loss of regulation)	↑ IFN-α–induced ISGs ↑ Monocytes and DC CD169	—	Type I interferonopathy, early onset autoinflammation	—
STAT3 LOF (AD, DN)	↓ Th17 response ↓ Memory B cells and plasma cells ↓ IL-10 response ↑ IgE	CMC, skin and pulmonary bacterial infections, *S. aureus* susceptibility, PJP, aspergillosis (secondary)	Eczema, distinct facial features, retained primary teeth, connective tissue disorders, aneurysms, pneumatoceles	Susceptibility to infection ↓ Th17 response ↑ IgE production (18)
STAT3 GOF (AD)	Variable findings of: ↓ Peripheral Tregs ↓ DC subsets ↑ DNTs, Th17s	Bacterial (staph, strep) and viral infections (HSV), fungal (*Candida*)	Lymphoproliferation, autoimmune cytopenias, growth delay, and multiorgan autoimmunity, including endocrinopathies, enteropathy, interstitial lung disease, and others	Variant- and model-dependent: ↑ Effector $CD8^+$ T cells ↑ $NKG2D^+$ $CD8^+$ T cells ↑ Th17 polarization ↓ Induced Treg development (19-22)
STAT4 LOF (AD, DN)	↓ IL-12–induced IFN-γ ↓ Fungicidal activity	Paracoccidioidomycosis	—	Susceptibility to infection ↓ Th1 polarization ↓ Mature NK function (23, 24)
STAT4 GOF (AD)	↓ Neutrophils, $CD4^+$ T cells ↓ IgG, IgA	—	Disabling pansclerotic morphea, Skin ulcers	—
STAT5B LOF (AR)	↓ Tregs and naive T cells ↑ IgE ↑ Activated T cells	Viral infections (respiratory, skin, hemorrhagic varicella)	Eczema, autoimmunity, growth failure, lymphocytic interstitial pneumonitis	SCID Growth defects Perinatal lethality (25, 26)
STAT5B LOF (AD, DN)	↑ IgE	—	Growth failure, eczema	—
STAT5B GOF (somatic and germline)	Eosinophilia ↑ IgE	Recurrent viral respiratory infections	Atopic dermatitis, urticarial rash, diarrhea with failure to thrive	—
STAT6 GOF (AD)	Eosinophilia ↑ IgE ↑ Th2 response	*S. aureus*	Eosinophilic gastroenteritis, atopic dermatitis, allergy	Spontaneous skin disease ↑ IgE ↑ Th2 response (27)

Table 1.2. Immunologic and clinical phenotypes of IEIs in JAK or STAT regulators

STAT (genetics)	Immunologic Changes in Patients	Clinical Disease		Mouse Model
		Infections	Other Clinical Features	
JAK1 LOF (AR)	T cell lymphopenia	Mycobacteria, viruses	Urothelial carcinoma	↓ IFN-γ and IFN-α/β signaling Perinatal lethality (28)
JAK1 GOF (AD)	↓ IFN-γ production eosinophilia	Viral infections	Hepatosplenomegaly, thyroid disease, poor growth, eosinophilic enteritis, atopic dermatitis, allergy	Skin disease, autoimmunity (29, 30)
JAK3 LOF (AR)	SCID phenotype ↓ T and NK cells defect in B cell function	Recurrent, severe infections due to common viral pathogens; opportunistic infections; mucocutaneous candidiasis	Chronic diarrhea, failure to thrive	SCID phenotype (31)
TYK2 LOF (AR)	↓ Th1 differentiation ↓ IFN-γ production ↑ Th2 differentiation Variable serum IgE	Intracellular pathogens (mycobacteria including TB, Salmonella), viral infections	Atopy, eczema	Susceptibility to infection ↓ IL-12 and IFN-α/β signaling (32)
ISG15 LOF (AR)	↓ IFN-γ production	Mycobacterial infections	Type I interferonopathy, brain calcification, ulcerating skin lesions	Susceptibility to infection (33)
USP18 LOF (AR)	↑ IFN signaling	—	Type I interferonopathy	Hydrocephalus Hypersensitivity to type I IFN (34)
SOCS1 Haplo (AD)	↑ IFN-α/β and IFN-γ signaling	Recurring bacterial infections	Multisystemic autoimmunity (cytopenias, SLE, psoriasis, arthritis), lymphadenopathy, atopy	—
ZNF341 LOF (AR)	↓ Th17 response ↓ Memory B cell response ↑ IgE	CMC, bacterial skin infections (Candida), respiratory infections (S. aureus)	Phenocopies STAT3 LOF: eczema, facial dysmorphism, connective tissue disorders, pneumatoceles	—

1.2.1 STAT1 and STAT2

STAT1 and STAT2 signaling and roles in immune cell differentiation and function

STAT1 and STAT2 share signaling pathways downstream of type I, II, and III IFNs, which are important for the host response to infection (35). IFN-α, IFN-β, and IFN-λ binding to their respective receptors activate STAT1 and STAT2 heterodimers, whereas type II IFN signaling (IFN-γ) activates STAT1 homodimers (10). These STAT dimers form distinct transcription factor complexes that bind IFN-stimulated genes (ISGs), which inhibit viral

replication, degrade viral proteins, and provide signals to activate other immune cells (35). Whereas STAT2 signaling is limited to type I and type III IFNs, multiple cytokines and growth factors signal through STAT1, including, but not limited to, IL-6, IL-10, IL-12, IL-21, and IL-27 (36, 37). STAT1 signaling is important for a variety of immune cell functions. For example, STAT1 signaling drives IFN-γ– or IL-27–dependent activation of human proinflammatory macrophages (38, 39). STAT1 signaling through type I IFNs contributes to NK cell maturation and cytotoxicity (40). In adaptive immune cells, IFN-γ signaling through STAT1 amplifies and stabilizes Th1 function and identity and promotes class switching in B cells (41, 42). IL-27 can also inhibit the differentiation of Th17 through STAT1-dominant gene activation (43).

STAT1 LOF in humans and mouse models

Patients with STAT1 LOF variants can differ phenotypically depending on the mode of inheritance (36). Biallelic, autosomal recessive STAT1 LOF results in fatal mycobacterial and viral infections due to complete deficiency in STAT1 protein expression and defective downstream signaling of IFN-α/β, IFN-γ, IFN-λ, and IL-27 (44, 45). Patients with partial autosomal recessive STAT1 LOF have a similar but milder phenotype with viral and mycobacterial infections associated with impaired, but not completely abrogated, STAT1 protein or function (46). Monoallelic, autosomal dominant STAT1 LOF patients present with susceptibility to mycobacterial disease, but not severe viral infection (47). This is due to defects in signaling downstream of IFN-γ and IL-27, but not type I IFN, as mutant STAT1 can still form dimers with STAT2 (36, 45). Studies with primary patient cells have shown that STAT1 is important for maintaining Th1 cytokine production in $CD4^+$ T cells, despite the presence of other STATs that can also drive Th1 differentiation (48). Mice with complete deficiency of STAT1

(*Stat1*$^{-/-}$) are more broadly susceptible to bacterial and viral infection than are humans; however, they share a defect in responses to IFN-α/β and IFN-γ (13, 14, 36). STAT1 also drives optimal Th1 differentiation in mice (49, 50). Both studies of human STAT1 LOF and *Stat1*$^{-/-}$ mouse models have shown the necessary role of STAT1 signaling in the immune response to viral and mycobacterial infections and demonstrate a function that cannot be compensated for by other STAT proteins.

STAT1 GOF in humans and mouse models

STAT1 GOF is a heterogeneous disorder, with >90% of patients having chronic mucocutaneous candidiasis (CMC) (51-53). Patients are susceptible to bacterial and viral infections, mainly herpesvirus. Autoimmunity, particularly thyroid disease, is also a feature of disease in many patients (52). Multiple immune cells are impacted by STAT1 GOF. STAT1 phosphorylation was increased in patient bulk $CD3^+$ T cells, $CD4^+$ T cells, NK cells, and monocytes after IFN-α, IFN-β, IFN-γ, or IL-27 stimulation (52-54). Low memory B cell frequencies and low IgG2 and IgG4 in the serum were observed in a significant proportion of patients (52, 53). More than 80% of patients have decreased peripheral Th17 counts, demonstrating a mechanism for susceptibility to CMC, which has been shown in other IEIs to be associated with defects in Th17 immunity (52-54). STAT1 GOF shares some features with STAT3 LOF, including CMC, enhanced STAT1 activity, and defective Th17 responses (55). Studies of both diseases have demonstrated STAT1 inhibition of STAT3-mediated gene transcription and the requirement for a balance of STAT1 and STAT3 signaling for differentiation and function of Th17 cells (55-57). The mechanisms by which STAT1 GOF drives other clinical phenotypes are less clear. For example, defective type I IFN and IFN-γ

signaling with STAT1 LOF is thought to drive susceptibility to viral and mycobacterial disease; however, IFN-γ production by peripheral blood mononuclear cells (PBMCs) or $CD4^+$ T cells in STAT1 GOF patients has no clear pattern, with patients having shown normal, decreased, or even increased IFN-γ production *ex vivo* (54). Recent mouse models of STAT1 GOF have tried to elucidate mechanisms of disease. Qian et al. (15) modeled the p.R274W STAT1 GOF variant and examined susceptibility to herpesvirus infections. STAT1 GOF mice had impaired control of late-stage viral infection that was associated with decreased IFN-γ production in T cells and a cell-intrinsic defect in antigen-specific $CD8^+$ T cell responses. Another model of the p.R274Q variant was generated by Tamaura et al. (16) to study mechanisms leading to a Th17 defect. Spontaneous fungal disease was not observed, but infection with *Candida albicans* resulted in an impaired Th17 response in the small intestine and a skewing toward a Th1 phenotype in $CD4^+$ T cells. The responses observed in mice may point toward mechanisms of interest for future studies in humans.

STAT2 LOF in humans and mouse models

STAT2 LOF variants have been described in 12 patients as causing susceptibility to viral infection (58, 59). Unlike STAT1 LOF variants, which are associated with a broad range of viral and mycobacterial infections, patients with STAT2 LOF present with serious, often fatal, infections from live-attenuated vaccine strain viruses, including measles, mumps, rubella, and varicella zoster vaccines. Patients exhibit hyperinflammation with infection, in some cases including hemophagocytic lymphohistiocytosis, associated with a complete lack of response to type I IFNs (60, 61). Patients also have variable susceptibility to other naturally acquired viral infections, including influenza and enterovirus, with incomplete penetrance (58). Most studies of

STAT2 activity and cellular function in patient cells used dermal fibroblasts or transfected cell lines, with one study examining the loss of IFN-α–induced ISGs in bulk PBMCs (59-63). One recent study by Gothe et al. (64) made use of CRISPR/Cas9 technology to knock out STAT2 in induced pluripotent stem cell–derived macrophages to investigate hyperinflammation. In their study, STAT2 deficiency resulted in prolonged JAK1 activation after IFN-α stimulation. This was due to the loss of negative regulation by USP18, which is recruited to IFNAR2 by STAT2, thereby leading to transcriptional changes and cytokine expression similar to an IFN-γ response. Similar to humans with STAT2 LOF variants, *Stat2*$^{-/-}$ mice also have a defect in type I IFN responses and are susceptible to viral infection (17).

STAT2 GOF in humans

STAT2 GOF variants have been identified in two families thus far (65, 66). These patients presented with early-onset type I interferonopathy, characterized by uncontrolled production of type I IFN with neuroinflammation, cranial calcification, seizures, and autoinflammation. In one family, two affected siblings were identified with homozygous missense variants, p.R148W, leading to prolonged IFNAR stimulation in patient cells due to a disruption in the interaction of STAT2 with the negative regulator USP18. This led to enhanced type I IFN signaling and transcription of ISGs (65). A different homozygous variant in *STAT2*, p.R148Q, was identified in an affected infant. In this case, STAT2 was able to bind USP18, but USP18 interaction with IFNAR was impaired, leading to prolonged type I IFN signaling (66). Patients with these STAT2 GOF variants succumbed to disease; however, early intervention with a JAK inhibitor led to recovery of similar disease in USP18-deficient patients, suggesting the ability to provide targeted therapy with early recognition of disease (67).

1.2.2 STAT4 and STAT6

STAT4 and STAT6 signaling and role in immune cell differentiation and function

The most well-known roles of STAT4 and STAT6 in the immune response involve the polarization of Th1 and Th2 subsets, respectively. STAT1 and STAT4 drive Th1 differentiation by inducing *TBX21* (gene encoding T-bet) transcription following activation by IFN-γ and IL-12, respectively (68). T-bet inhibits induction of Th2 differentiation and function by inhibiting transcription of *GATA3* (68). STAT6 is activated by IL-4 and IL-13, and it drives GATA3 expression and commitment of naive $CD4^+$ T cells to the Th2 lineage (69). STAT6 signaling is also important in the formation of germinal centers, eosinophil activation, and helminth infection, and it is associated with airway hyperresponsiveness (70-72).

STAT4 LOF, STAT4 GOF, and STAT6 GOF in humans and mouse models

IEIs associated with STAT4 and STAT6 have recently been identified in humans (27, 73-77). A monoallelic LOF variant in *STAT4* was identified in a family with two members presenting with fungal infection due to paracoccidioidomycosis (73). Immune phenotyping did not demonstrate alterations of immune cell subsets, but it identified reduced IL-12–induced STAT4 phosphorylation and nuclear translocation and supported a dominant-negative role for the variant. Lymphocytes from patients produced less intracellular IFN-γ in response to IL-12 and had less fungicidal activity when infected with *Paracoccidioides brasiliensis in vitro*. Three STAT4 GOF variants have very recently been identified in four patients presenting with early-onset polyarthritis, and skin and mucosal lesions consistent with disabling pansclerotic morphea (77). These variants led to constitutive STAT4 phosphorylation, but without differences in Th1

skewing. Patient primary fibroblasts had impaired wound healing and IL-6 overexpression compared to healthy controls. Treatment with the JAK inhibitor, ruxolitinib, improved symptoms in two patients (77).

Autosomal dominant STAT6 GOF variants have recently been identified in individuals affected by severe atopic dermatitis, eosinophilic gastroenteritis, growth defects, and food allergy (27, 74-76). Eosinophilia has been noted in the skin and gastrointestinal lamina propria (27, 74-76). Patients had elevated serum IgE and peripheral blood Th2 frequency, consistent with the known role of STAT6 in inducing a Th2 response (27, 74-76). STAT6 LOF variants have not yet been reported in humans.

Mice deficient in STAT4 and STAT6 have been generated. *Stat4*$^{-/-}$ mice have different phenotypes dependent on genetic background. The earliest model on a mixed 129 and BALB/c background failed to develop Th1 responses or mature NK cell function in response to IL-12, and mice were susceptible to *Listeria monocytogenes* infection (23). Mice with *Stat4* deficiency on both the BALB/c and C57BL/6 backgrounds showed decreased Th1 responses during fungal infection, with those on the BALB/c background more susceptible to fungal infection (78). *Stat6*$^{-/-}$ mice are protected from airway hyperresponsiveness in response to allergen challenge, but they had impaired Th2 responses after IL-4 and IL-13 stimulation, as well as decreased serum IgE due to defects in class switching (70-72). A mouse model of STAT6 GOF was recently generated with the p.D419N variant, recapitulating major aspects of disease observed in humans (27). Homozygous STAT6 GOF mice were more prone to spontaneous skin inflammation with eosinophil infiltration than heterozygotes; however, both displayed increased

serum IgE and Th2 cytokine production, as well as atopic dermatitis–like disease when treated with the vitamin D3 analog MC903 (27).

1.2.3 STAT5A and STAT5B

STAT5 signaling and role in immune cell differentiation and function

There are two genes encoding STAT5: *STAT5A* and *STAT5B*. Although STAT5A and STAT5B are structurally similar, their expression, DNA-binding activity, and impact on gene transcription are distinct. In the immune response, STAT5B is important for promoting the identity of thymic and peripheral regulatory T cells (Tregs). IL-2 activates STAT5B, which drives *FOXP3* transcription and is required for Treg function, which cannot be compensated for by STAT5A (79, 80). STAT5B also antagonizes STAT3 activity by competing for binding to *BCL6*, *IL6RA*, and *IL17*, inhibiting the differentiation of Th17 and T follicular helper (Tfh) cells (81, 82). Furthermore, STAT5B is important in NK cell development due to its role in the IL-2 and IL-15 signaling pathways (40). STAT5B is also activated downstream of IL-5 in human eosinophils and promotes cell survival (83).

STAT5B LOF in humans and mouse models

Biallelic LOF variants in *STAT5B* lead to a syndrome characterized by growth failure due to defects in growth hormone signaling, chronic lung disease, atopic dermatitis, skin infections, and autoimmune disease with near-absent Tregs with reduced function (79, 84). Studies of patient samples have demonstrated impaired NK cell development and function as well as dysregulated B cell differentiation (80, 85, 86). Heterozygous *STAT5B* dominant-negative variants have been identified in three families, with patients having growth failure and elevated

serum IgE, but normal peripheral blood Tregs (87). In the mouse, deficiency of both *Stat5a* and *Stat5b* leads to failure of lymphocyte development, with growth defects and perinatal lethality (25). *Stat5a*$^{-/-}$ mice have defective mammary gland development, whereas *Stat5b*$^{-/-}$ mice have defects in growth hormone signaling in the liver, suggesting that in the mouse STAT5A and STAT5B can compensate for each other in immune cell development (26, 88). Taken together, these investigations in patients and mouse models demonstrate the importance of STAT5B for human Treg development and function as well as nonredundant roles for STAT5A/B in human immune cell development and function that differ from the mouse.

STAT5 GOF in humans and mouse models

Although constitutive STAT5B activity due to somatic mutations has been observed in cancer and myeloproliferative disorders, GOF variants in *STAT5B* causing IEIs were recently identified in three patients with somatic heterozygous variants and two patients with germline variants. Patients had prominent symptoms of severe atopy including dermatitis with hypereosinophilia and food allergies, with additional clinical disease in patients with somatic variants including chronic diarrhea with failure to thrive and viral infections (89-91). Alterations in immune cell phenotype varied among patients, including one somatic patient with $CD4^+$ T cells skewed toward the Th2 subset and a reduction in Th17 and Tfh cells (89). In the family with a germline variant, they observed elevated effector memory T cells and Tregs, as well as increased TGF-β transcription in Tregs (91). Treatment with ruxolitinib, a JAK1/2 inhibitor, led to clinical improvement of disease in some patients, providing a targeted therapeutic approach (89-91).

1.2.4 JAK family proteins

Identification of patients with monogenic LOF or GOF in genes encoding JAKs highlights the importance of regulation of STAT signaling. In some cases, these diseases phenocopy those of the corresponding STATs; however, the versatility of JAK proteins with respect to ligands and signaling partners often leads to more broad immunologic consequences and clinical disease than single-gene defects in STATs. Human disease has been associated with JAK1, JAK3, and TYK2 (**Table 1.2**).

JAK1

JAK1 associates with common γ-chain receptors, gp130 receptors, common β-chain receptors, and class II cytokine receptors (10). One patient with two different homozygous missense variants in *JAK1* leading to hypomorphic protein function had susceptibility to mycobacterial infection, short stature, and bladder carcinoma (92). Lymphocytes from this patient showed global impairment as demonstrated by reduced STAT1, STAT3, STAT4, STAT5, and STAT6 phosphorylation following stimulation with JAK1-activating cytokines. Susceptibility to mycobacterial infection in this patient is similar to STAT1 deficiency and emphasizes the need for a functional JAK1/STAT1 signaling pathway for appropriate IFN-γ responses in this infection (92).

Heterozygous germline or somatic GOF variants in *JAK1* are associated with immune dysregulation characterized by growth defects, asthma, eosinophilia, atopic dermatitis, eosinophilic enteritis, and autoimmune thyroid disease (93, 94). These patients were responsive to treatment with JAK inhibitors. The overlapping clinical features of JAK1 GOF and STAT5B GOF suggest that this JAK may be most relevant for STAT5B signaling. The discovery of a

patient with a somatic JAK1 variant by Gruber et al. (94) is of particular interest, as it provided new insight into regulation of JAK1 and highlighted unique molecular mechanisms of disease. This patient had genetic mosaicism, an emerging mechanism of monogenic IEIs (12). However, although a portion of cells carried both mutant and wild-type alleles at the DNA level, careful single-cell transcriptome analysis of peripheral blood immune cells revealed monoallelic expression of *JAK1* in patient cells and a healthy individual (94). This suggests that for *JAK1*, and possibly other IEI genes, genetic mechanisms may be more complex than heterozygosity. Finally, analysis of the mechanism of JAK1 function in this patient demonstrated noncanonical neomorphic JAK1 signaling mediated by the mutant allele, and evaluating this in other STAT-related diseases will be important to understanding and differentiating canonical versus disease-associated signaling (94).

JAK3

JAK3 associates with common γ-chain receptors and its expression is limited to the lymphatic system, bone marrow, endothelial cells, and vascular smooth muscle cells (10). Patients with biallelic LOF JAK3 variants phenocopy X-linked SCID due to a lack T cells and NK cells, defects in B cell function, and IL-2Rγ signaling defects (95-97). This indicates that JAK3 is the critical mediator of signaling through this shared cytokine receptor over JAK1, given that JAK1 LOF patients do not develop SCID (95-97).

TYK2

TYK2 associates with class II cytokine receptors, gp130 receptors, IL-12R, IL-13R, and IL-23R (10). TYK2 GOF variants have not been found in IEIs, although somatic GOF mutations are associated with acute lymphoblastic leukemia (98). Patients with TYK2 LOF variants were

reported to have a disease phenotype of atopic dermatitis, intracellular bacterial infections including tuberculosis, and recurring viral infections (99, 100). Immune phenotyping showed increased serum IgE, loss of STAT1, STAT2, and STAT3 activation in response to IFN-α stimulation, an abrogated response to IL-12, and a skewing from Th1 toward Th2 differentiation (99, 100). Interestingly, homozygosity for a relatively common variant in *TYK2* is associated with susceptibility to tuberculosis (101), again supporting the importance of this signaling molecule for this infection. These findings indicate that in humans, TYK2 cannot be compensated for by other JAKs in type I IFN signaling.

1.2.5 Regulators of STAT activity

Among the regulators of STAT signaling, genetic variants in *USP18*, *ISG15*, *SOCS1*, and *ZNF341* are associated with IEIs (1) (**Table 1.2**). USP18 is recruited to IFNAR2 by STAT2, and it inhibits type I IFN receptor assembly and function, thus downregulating type I IFN responses (102). Deficiency of USP18 or the inability of USP18 to interact with intracellular ISG15 (which stabilizes USP18) leads to increased type I IFN signaling and a severe type I interferonopathy, similar to STAT2 GOF (67, 103). Similarly, patients with ISG15 deficiency can present with a type I interferonopathy due to unstable USP18 levels and excessive type I IFN signaling (104, 105). Alternatively, some patients with ISG15 deficiency present with susceptibility to mycobacteria due to a lack of free/secreted ISG15 and induction of IFN-γ (106). These studies in humans with ISG15 deficiency also revealed that in contrast to the mouse, ISG15 is redundant for antiviral immunity, as patients do not suffer from viral infections (33). SOCS1 negatively regulates multiple STAT signaling pathways by binding to and inhibiting the phosphorylation activity of JAK1, JAK2, and TYK2 in humans (107). *SOCS1* haploinsufficiency results in

multiorgan autoimmunity, including cytopenias, systemic lupus erythematosus, and/or psoriasis, with some patients having recurrent bacterial infections, lymphadenopathy, or atopy (108, 109). Patient cells exhibit elevated STAT1, STAT5, and STAT6 phosphorylation following cytokine stimulation, and Treg frequency and suppressor function, and switched memory B cells were decreased in some patients (108, 109). *SOCS1* haploinsufficiency in particular highlights the importance of regulation of the balance of STAT signaling for multiple immune cell types. Finally, ZNF341 activates *STAT3* transcription (110, 111). Patients with biallelic ZNF341 LOF variants have disease that phenocopies STAT3 LOF, presenting with atopic dermatitis, elevated serum IgE, recurrent *Candida* infections, and impaired Th17 responses (110, 111). Taken together, these disorders of STAT regulators emphasize the need for tightly controlled signaling cascades and further demonstrate the shared and unique features of IEIs impacting STAT signaling (**Table 1.2**).

1.3 Disorders of STAT3 signaling in IEI

1.3.1 Overview of STAT3 signaling

STAT3 is present in most cell types and is stimulated by many cytokines and growth factors that use gp130 type and other receptors (6, 112). A non-exhaustive list of STAT3-activating ligands includes IL-2, IL-6, IL-10, IL-21, IL-23, IL-22, IL-27, IL-5, IL-19, IL-11, IL-12, IFNg, TNFa, LIF, OSM, SCF, CNTF, MCP-1, CCL5, MIP-1a, EGF, PDGF, IGF-1, G-CSF, GM-CSF, and leptin (112-116). Like other STAT molecules, STAT3 is comprised of an N-terminus domain for stabilization upon DNA binding, a coiled-coil domain, a DNA-binding

domain, an SH2 domain for dimerization, a linker domain, and a C-terminal transcriptional activation domain. STAT3 can be phosphorylated at tyrosine (Tyr-705) and serine residues (Ser-724) in the C-terminal domain. While STAT3 relies on phosphorylation at these residues for canonical activation, unphosphorylated STAT3 can also activate gene transcription (117). STAT3 can form homodimers with other STAT3 molecules, or heterodimers with STAT1 and STAT5, which then translocate to the nucleus to transcribe genes involved with cell cycle progression, proliferation, differentiation, and survival (115, 118). Regulators of STAT3 activity include SOCS3 (which prevents phosphorylation of STAT3 by JAKs), phosphotyrosine phosphatases (which dephosphorylate STAT3 dimers in both the cytoplasm and nucleus), and PIAS3 (which inhibits DNA binding) (119). STAT3 has major functions in stromal cell proliferation, extracellular matrix remodeling and subsequent wound healing, and resistance to apoptosis (116, 120).

For example, IL-22, a cytokine involved in wound healing at mucosal barriers, activates STAT3 and the transcription of genes involved in cell proliferation in the intestine and skin (121). However, the overexpression of IL-22 can lead to disease through uncontrolled epithelial cell proliferation (122). Indeed, *STAT3* is a known oncogene due to its role in cell survival and proliferation, and somatic variants in *STAT3* that cause constitutive activity are associated with cancers, in particular large granular lymphocyte leukemia and aplastic anemia (116, 123).

1.3.2 STAT3 signaling in immune cells

STAT3 in adaptive immune cells

Due to its expression in multiple immune cell types, STAT3 is important in the induction and regulation of immune responses. In $CD4^+$ T cells, STAT3 is considered a master regulator of

Th17 polarization and function, connecting cytokines such as IL-6, IL-21, and IL-23 to transcription of genes including *RORA*, *RORC*, *IL23R*, and *IL17A*, which are important in the response to extracellular pathogens and mucosal barrier maintenance (124-127). STAT3 binding to *RORC* results in the transcription of RORγt, which is the transcription factor that defines Th17 lineage and also drives expression of effector cytokines including IL-17A, IL-17F, and IL-22 (128, 129). Other transcriptional targets of STAT3 in the context of Th17 differentiation include *Irf4*, *Batf*, *Maf*, *Ahr*, and *Il6ra* (130, 131). While Th17 have both pro-and anti-inflammatory roles, unregulated Th17 responses are also associated with autoimmunity, including inflammatory bowel disease, arthritis, psoriasis, and multiple sclerosis (132-134). The effector cytokines and receptors involved in Th17 function and stability have therefore been targets of therapies for these diseases, particularly IL-23R and IL-17 (134). Another $CD4^+$ T cell subset that requires STAT3 for identity and function are Tfh cells, which use STAT3 signaling to provide signals that promote class switching and somatic hypermutation in B cells, such as IL-21, CXCR5, ICOS, and PD-1 (68, 135). IL-21 and IL-6 signal through STAT3 to activate *BCL6*, which is the master transcription factor of Tfh lineage (136). Tregs require sustained FoxP3 expression for suppressor function and are integral in preventing the development of autoimmunity (137-139). STAT3 is known to compete with STAT5 for binding to *FOXP3* and inhibit Treg differentiation and function (127). However, STAT3 signaling through the IL-10 receptor in Tregs is required for control of Th17 responses and protection from spontaneous colitis in mice, as well as regulatory function through activation of IL-10 secretion (140).

$CD8^+$ T cells, which are important in host protection against infection and malignancy, require STAT3 for memory cell differentiation in both humans and mice (141-143). IL-10 and

IL-21 signaling through STAT3 activates transcription of Eomes, Blimp-1, and BCL6 for maturation of memory cells in mice (141). STAT3 also promotes the expression of genes involved in $CD8^+$ effector function and cytotoxicity, as well as differentiation into terminally exhausted cells, in the context of cancer and bacterial infection (144, 145). γδ T cells are a separate lineage from conventional αβ T cells that are primarily located in epithelial tissues such as the skin and intestine (146). Certain subsets of γδ T cells signal through STAT3 in response to inflammatory cytokines, such as IL-1β and IL-23 in the skin, to induce production of IL-17A, IL-17F, and IL-22 (147). B cells use IL-21 or IL-10 signaling through STAT3 for memory B cell and plasma cell differentiation, both of which depend on STAT3 signaling (148-150).

STAT3 in innate immune cells

STAT3 has both pro-inflammatory and regulatory roles in myeloid cells. STAT3 is important in mediating CXCR2-dependent chemotaxis in neutrophils (151). In neutrophil progenitors, G-CSF signals through STAT3 to activate emergency granulopoeisis (152). STAT3 signaling via IL-10R in both neutrophils and macrophages also prevents spontaneous colitis (153, 154). PD-L1 upregulation in monocytes in response to GM-CSF and IL-4 requires STAT3 and promotes immune tolerance (155). Dendritic cells require STAT3 signaling in response to Flt3L for development and maturation (156). Innate lymphoid cells (ILCs), which are important in mucosal immunity, rely on STAT3 for the development of the ILC3 subset and expression of IL-17 and IL-22 (157-159). In the context of cancer, STAT3 signaling can support and inhibit tumorigenesis through its effects on innate immune cells (160). For example, STAT3 inhibits of the cytotoxic capacity of NK cells, but also upregulates NKG2D expression (161, 162).

1.3.3 STAT3 LOF in humans and mouse models

The functions of STAT3 with respect to the human immune system are made apparent by genetic variants that cause loss of function. STAT3 LOF variants cause autosomal dominant hyper-IgE syndrome, an IEI characterized by high serum IgE, atopic dermatitis, CMC, connective tissue disorders, distinct facial features, and susceptibility to bacterial infection, including recurrent *Staphylococcus aureus* infections of the skin and lungs (163, 164). Circulating Th17 numbers and cytokine expression are characteristically decreased and thought to contribute to the prevalence of fungal infections (48, 165, 166). Decreased memory B cells and eosinophilia are also observed in many patients (149, 167). Studies of STAT3 LOF T cells *in vitro* have demonstrated the importance of STAT3 signaling in IL-10 production by $CD4^+$ T cells, memory T cell differentiation from naive cells, and in the differentiation and function of Th subsets Th17, Tfh, and Th9 (48, 56, 143, 168, 169). The similar phenotype of CMC and loss of Th17 responses in STAT3 LOF and STAT1 GOF highlight the antagonistic interaction between STAT1 and STAT3, where strong STAT1 activation can inhibit transcription of STAT3-dependent genes (55). The balance between STAT1 and STAT3 in regulating Th17 responses has also been demonstrated in mouse models (117, 170, 171). Mice completely deficient in STAT3 (*Stat3*$^{-/-}$) are embryonic lethal (172). However, a mouse model of a human STAT3 LOF, modeling a missense variant, phenocopied the elevated IgE and impaired Th17 responses seen in humans, specifically in response to bacterial infection (18, 173). These mouse models have been used to examine the potential efficacy of certain therapies in treating STAT3 LOF, including bone marrow transplantation and the effects of STAT3 LOF in nonimmune cells (18, 173).

1.3.4 STAT3 GOF in humans and mouse models

STAT3 GOF Syndrome

Heterozygous *STAT3* GOF variants that cause disease are rare and have been identified in 191 patients as of 2023 (174). 72 unique variants have been described in all domains of the STAT3 protein, and most variants are located in the DNA binding domain (174). As such, alterations in STAT3 activity are variable across individuals and include increased baseline phosphorylation, increased activity after cytokine stimulation, and delayed de-phosphorylation (114). Patients are around 2 years of age at the time of onset and have a broad array of clinical symptoms, the most common of which include lymphoproliferative disease (in the form of lymphadenopathy or splenomegaly), autoimmune cytopenias, and growth defects. Multi-organ autoimmunity is also common and affects the lungs, liver, joints, skin, intestine, and kidneys. Autoimmune endocrinopathy including type 1 diabetes, as well as recurrent infections, are also prevalent. Despite the association of activating somatic mutations in STAT3 with multiple types of cancer, malignancy was observed in only 6% of STAT3 GOF patients (174-178).

Immune cell changes in STAT3 GOF

The pleiotropic nature of STAT3 is made evident by the wide array of immune cell types affected by GOF variants, including monocytes, T cells, B cells, as well as nonimmune cells (174). Lymphocyte populations of the peripheral blood, including $CD4^+$ T cells, $CD8^+$ T cells, B cells, and NK cells, were variably decreased, with the exception of elevated double-negative T cells found in over 80% of patients assessed (174). Immunoglobulin levels were variably low, and over half of patients received immunoglobulin replacement therapy (174). Furthermore, while STAT3 can drive Th17 differentiation, only 27% of 41 patients examined showed

increased IL-17 cytokine production in the peripheral blood. Immunosuppressive treatment and limited tissue samples can make conclusions regarding whether Th17 cells are relevant to disease challenging (174, 177, 178), although reduction of elevated Th17 was correlated with improved symptoms in one patient that received IL-6R inhibitor treatment (177). Conversely, STAT3 is known to inhibit development of Tregs, and 39% of 69 STAT3 GOF patients examined exhibited decreased Tregs in their peripheral blood (174, 177, 178). In line with this, our lab recently demonstrated by single-cell RNA sequencing that Tregs from STAT3 GOF patients were transcriptionally similar to healthy donors, suggesting that Treg defects may not be driving disease (19). Additionally, we and others observed an expansion of effector $CD8^+$ T cells in some patients, suggesting that these cells may be important in driving disease (19, 20). Among myeloid populations, a study of APCs in a cohort of STAT3 GOF patients found that STAT3 activation decreases dendritic cell differentiation from hematopoietic progenitor cells, favoring CD14 surface expression, which may explain the increased frequency of $CD14^+$ monocytes and decreased frequency of dendritic cells in the peripheral blood (179).

Mouse models of STAT3 GOF

Recent mouse models of patient STAT3 GOF variants have been used to understand the mechanisms behind the variable clinical presentations in patients, including autoimmunity and Treg function (19-22). Mouse models demonstrate that there are not significant Treg functional defects, with mice having mild increases in Treg frequency at baseline. However, there is a selective defect in induced Treg generation *in vitro* and *in vivo* (19, 21, 22). We recently reported a STAT3 GOF mouse model on the C57BL/6 background with the p.G421R variant in the DNA-binding domain of STAT3, identical to a variant observed in patients (19). These mice had a

skewing of $CD4^+$ T cells to a Th1 phenotype in older adult mice and in a model of T cell–mediated colitis (19). Warshauer et al. generated a STAT3 GOF mouse model on the NOD background and identified accelerated autoimmune diabetes associated with an expansion of effector $CD8^+$ T cells in a transitional state that resisted terminal exhaustion (21). Two models from Masle-Farquhar et al. on the C57BL/6 background demonstrated expansion of a subset of effector $CD8^+$ T cells that expressed high levels of NKG2D (20). These $CD8^+$ T cells were also clonally expanded and drove lethal pathology in the p.K658N variant (20). Collectively, these models and transcriptional analysis of human samples suggest that Tregs may not drive disease in STAT3 GOF and suggest that $CD8^+$ T cells and altered $CD4^+$ polarization may be associated with disease, though this is likely dependent on other genetic and environmental risk factors.

Given our observation of T cell dysregulation in p.G412R variant STAT3 GOF mice, my doctoral dissertation explores how this affects T cell identity and function in inflamed tissue environments. We hypothesized that inflammatory stimuli would induce T cell dysregulation and subsequent tissue-specific autoimmune disease in STAT3 GOF. This dissertation work could aid in the identification of new targets for therapy.

Chapter 2: A human STAT3 gain-of-function variant drives local Th17 dysregulation and skin inflammation in a murine model

This chapter contains unpublished data.

2.1 Abstract

Germline gain-of-function (GOF) variants in *STAT3* lead to a syndrome of early-onset poly-autoimmunity and immune dysregulation. STAT3 is a pleiotropic transcription factor that affects the induction and regulation of immune responses; however, the cell types driving disease remain unclear. Using a mouse model of STAT3 GOF (p.G421R), we observed spontaneous and imiquimod (IMQ)-induced skin inflammation with increased local Th17 responses that were cell-intrinsic. $CD4^+$ T cells were sufficient to drive skin inflammation, and $CD8^+$ T cells and γδ T cells were not required. Single-cell analysis of $CD4^+$ T cells revealed upregulated *Il22* expression in expanded clones. Certain aspects of disease, including increased epidermal thickness, required the presence of STAT3 GOF in epithelial cells. JAK inhibitor treatment reduced inflammation without affecting Th17 cytokine production. Collectively, these data suggest a role for Th17 in the development of organ-specific immune dysregulation in STAT3 GOF.

2.2 Introduction

Signal transducer and activator of transcription 3 (STAT3) is a transcription factor involved in cell proliferation, differentiation, metabolism, apoptosis, and both regulation and induction of immune responses (116, 180, 181). Germline heterozygous gain-of-function (GOF) variants in *STAT3* cause an inborn error of immunity (IEI) characterized by immune dysregulation and early-onset multi-organ autoimmunity (175-177, 182). Patients with STAT3 GOF syndrome present with variable clinical manifestations; the most common include lymphoproliferation, autoimmune cytopenias, enteropathy, endocrinopathy, skin disease, interstitial lung disease, and

growth defects (174). The majority of GOF germline variants in *STAT3* lead to increased transcriptional activity of STAT3, but unlike somatic variants associated with cancer, they do not result in auto-phosphorylation (182). Thus, therapeutics that block upstream activation of STAT3 signaling, including JAK inhibitors and blockade of IL-6 signaling, are used to treat patients (182). However, the pleiotropic nature of *STAT3* creates a challenge in defining the mechanisms and major cell types that initiate immune dysregulation and organ-specific disease.

STAT3 is activated in multiple cell types by many different cytokines associated with pro- and anti-inflammatory responses, including IL-6, IL-10, IL-21, IL-23, and type I interferons (IFNs) (11, 123, 181, 183). In T cells, STAT3 is particularly important in the polarization and function of T-helper 17 cells (Th17), which maintain homeostasis with commensal organisms and eliminate specific pathogens at mucosal and barrier interfaces. IL-6, IL-23, and IL-21 signal through STAT3 in Th17 cells to induce expression of RORγt and RORα, IL-17A, IL-22, IL-23R, IL-10 (126, 127, 184). Conversely, STAT3 suppresses regulatory T cell (Treg) development by binding to the gene encoding FOXP3 (127). Enhanced Th17 and defective Treg responses are known drivers of autoimmune pathology in multiple human diseases, including rheumatoid arthritis, psoriasis, and Crohn's disease (183, 185). Given what is known about the role of STAT3 in T cell differentiation, patients with STAT3 GOF would be expected to have increased Th17 and decreased Tregs; however, less than one third of patients evaluated showed elevated Th17, and less than half of patients evaluated had lower Treg frequencies in the peripheral blood compared to healthy donors (174). Restoring elevated Th17 or decreased Tregs to normal frequencies has been associated with improved symptoms, as observed in two patients treated with tocilizumab, an IL-6 receptor antagonist (177, 186). Recent studies from our group and

others have suggested that Tregs from STAT3 GOF patients are similar to healthy controls, and that $CD8^+$ effector T cells are expanded in some patients and therefore might be mediating disease (19, 20). Understanding the cause of dysregulated T cell activity and how this drives the development and progression of disease in STAT3 GOF patients remains challenging due to prior immunosuppressive therapy and tissue sampling limited to the peripheral blood.

Previous studies of STAT3 GOF mouse models from our group and others have shown that STAT3 GOF leads to T cell dysregulation and lymphoproliferation, mirroring observations in patients. We previously observed an expansion of effector $CD4^+$ and $CD8^+$ T cells in older STAT3 GOF mice, as well as Th1 skewing in a colitis model that was associated with a defect in peripheral Treg (pTreg) generation, rather than impaired Treg function (19). Two other studies have highlighted a dysregulated $CD8^+$ T cell response that drives spontaneous disease as STAT3 GOF mice age or accelerates the development of type 1 diabetes (T1D) in STAT3 GOF mice on the NOD background (20, 21). The average age of onset in patients with STAT3 GOF is about 2 years, which combined with disease heterogeneity even among individuals with the same variant suggests that environmental factors significantly influence disease phenotype (174). We hypothesized that T cell dysregulation in STAT3 GOF requires an inflammatory stimulus that would lead to autoimmune disease as seen in patients, and that this stimulus would activate tissue-specific inflammatory responses. Here, we use a recently described STAT3 GOF mouse model that expresses a p.G421R variant in the DNA binding domain of the STAT3 protein (*Stat3*$^{p.G421R/+}$, referred to as 'STAT3 GOF') (19). This variant has been observed in patients with psoriasiform dermatitis and scleroderma (177, 186). We observed spontaneous skin disease in STAT3 GOF mice as they aged that was associated with an enhanced, local Th17 response. Skin

disease could also be induced in younger mice by the TLR7 agonist, imiquimod (IMQ), which resulted in a similar increased Th17 response in the skin and clonal expansion of $CD4^+$ T cells in the draining lymph nodes. Bone marrow chimeras showed that Th17 skewing is cell-intrinsic and suggest a role for STAT3 GOF in non-hematopoietic cells for enhanced skin disease. IMQ-induced disease could be ameliorated with JAK inhibitor therapy; however, Th17 cytokine expression was unaffected. Collectively, these data suggest the mechanisms for the development of autoimmunity and disease are organ-specific in patients with STAT3 GOF syndrome.

2.3 Results

2.3.1 Older adult STAT3 GOF (p.G421R) mice develop spontaneous skin inflammation

We recently generated a murine model of STAT3 GOF syndrome in which the p.G421R variant was introduced into C57BL/6 mice by CRISPR/Cas9 (19). In patients with STAT3 GOF syndrome, the p.G421R variant in the DNA binding domain does not confer constitutive STAT3 activation, but rather delayed STAT3 de-phosphorylation following stimulation (177). Heterozygous STAT3 GOF mice showed a similar delay in STAT3 de-phosphorylation (19). STAT3 GOF mice also developed splenomegaly and lymphadenopathy, with an expansion of $CD44^+$ $CD62L^-$ effector T cells by early adulthood that persisted as mice aged (19).

While young adult (7-9 weeks old) STAT3 GOF mice do not have spontaneous organ-specific disease beyond lymphoproliferation, we observed spontaneous ear swelling in older adult (>18 weeks old) STAT3 GOF mice compared to age-matched wild-type (WT) littermates

(**Figure 2.1A**). Dermatitis (measured by ear thickness) in STAT3 GOF mice appeared by 18 weeks of age and continued to increase over time (**Figure 2.1B**). Patients with STAT3 GOF have a relatively high frequency of skin disease (~60%); both psoriasiform dermatitis and scleroderma have been observed in patients with the p.G421R variant, as well as atopic dermatitis in other patients (174, 177, 186). Thus, this phenotype is relevant to clinical disease. Histopathologic analysis of ear sections revealed increased epidermal thickness, cellular infiltrate, and pathologic score in older adult STAT3 GOF mice compared to WT littermates (**Figure 2.1C & D**).

Analysis of immune cell populations in the skin revealed neutrophil and monocyte infiltration, with significant increases in $CD4^+$ and $CD8^+$ T cell frequency and number (**Figure 2.1E and Figure S2.1A**). We previously observed a spontaneous increase in $CD4^+$ $IFN\gamma^+$ cells in the spleens of STAT3 GOF mice by 20 weeks of age (19); however, this pattern was not observed in the skin (data not shown), suggesting tissue-specific differences in immune responses. Instead, the skin of STAT3 GOF mice had increased frequencies and numbers of $CD4^+$ T cells expressing Th17-related cytokines and transcription factors, including IL-17A, IL-22, and RORγt (**Figure 2.1F and Figure S2.1B**). In γδ T cells, which are major producers of IL-17A and IL-22 in mouse skin (187), there was an increase in the frequency and number of $IL\text{-}22^+$ cells, but not $IL\text{-}17A^+$ or $ROR\gamma t^+$ cells in older adult STAT3 GOF mice (**Figure 2.1G and Figure S2.1B**). As with our prior findings in mice >20 weeks of age, we observed lymphoproliferation in the cervical lymph nodes (CLN) and spleen (data not shown). There was no difference in the frequency of $IL\text{-}17A^+$ $CD4^+$ T cells or $IL\text{-}17A^+$ γδ T cells in the CLN or spleen of older STAT3 GOF mice (**Figure 2.1H & I and Figure S2.1C-F**), and total numbers were variably increased (**Figure S2.1C-F**). This is consistent with the lymphoproliferation

observed in this model (19). However, the frequency and number of IL-22$^+$ CD4$^+$ T cells was increased in both the CLN and spleen of older STAT3 GOF mice (**Figure 2.1H & I and Figure S2.1C & D**). The total number of IL-22$^+$ TCR$\gamma\delta^+$ cells was increased in the CLN, but not the spleen (**Figure S2.1E & F**). This suggests that the Th17 phenotype corresponds with organ-specific disease, and that there is both a local and peripheral IL-22 response driven by STAT3 GOF.

Having observed increased Th17 in older adult mice (>18 weeks) *in vivo*, we tested the ability of CD4$^+$ T cells from young adult STAT3 GOF mice (7-9 weeks) to polarize toward the Th17 subset *in vitro*. Both naïve and bulk splenic CD4$^+$ T cells from STAT3 GOF mice showed increased Th17 polarization *in vitro* with low levels of IL-6 compared to WT (**Figure S2.2A & B**). Bulk CD4$^+$ T cells cultured without IL-6 also produced significant IL-17A (**Figure S2.2B**), suggesting that TCR ligation and TGF-β signaling can induce Th17 polarization in STAT3 GOF CD4$^+$ T cells. Collectively, these findings demonstrate that STAT3 GOF supports Th17 skewing and leads to spontaneous skin disease with local infiltration of Th17 cells.

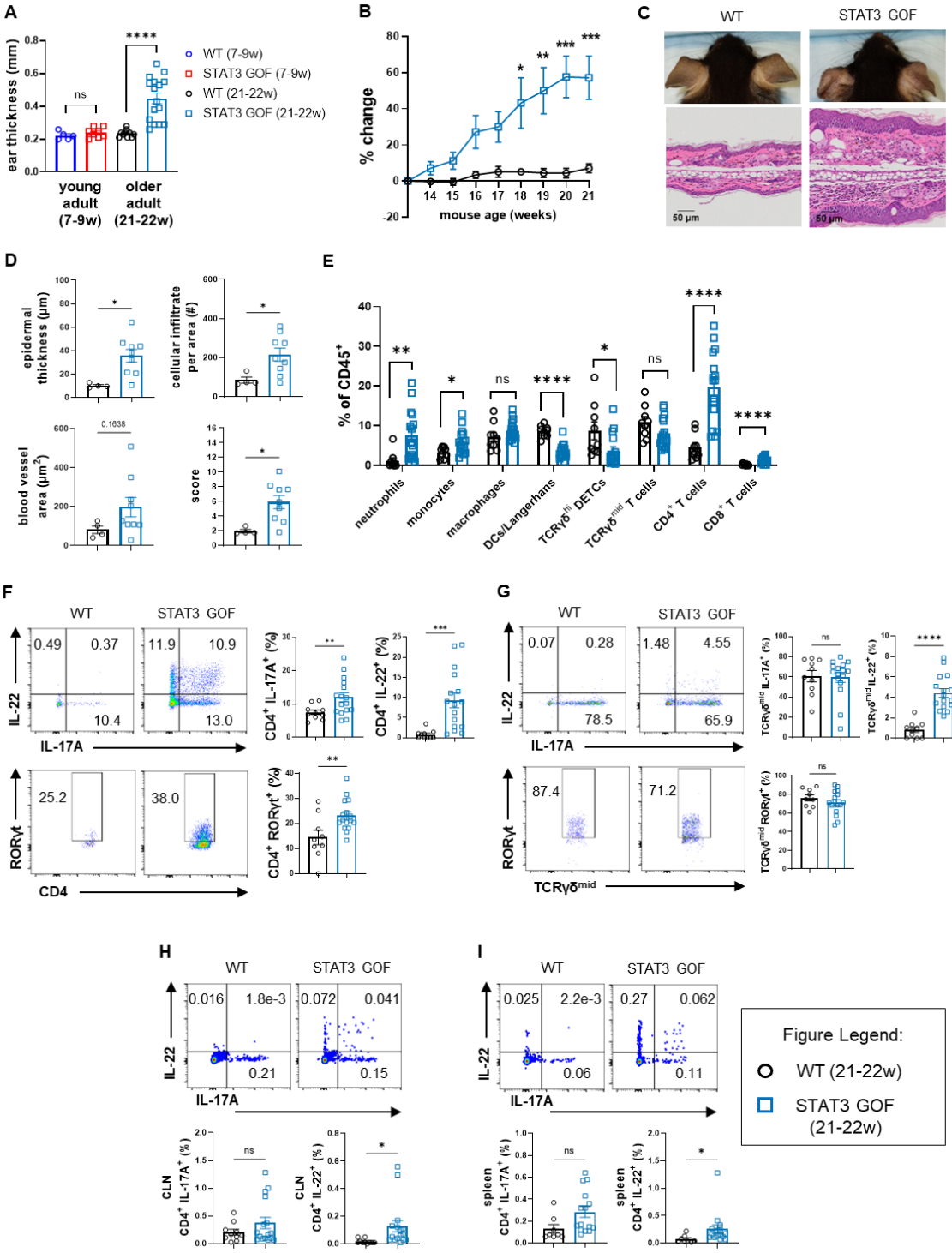

Figure 2.1: Older adult STAT3 GOF (p.G421R) mice develop spontaneous skin inflammation. (A) Ear thickness of young adult (7-9 weeks) and older adult (21-22 weeks) STAT3 GOF and WT littermates. (B) Percent

change in ear thickness of older adult STAT3 GOF and WT mice relative to baseline at 13 weeks of age. (C) Representative pictures of older adult WT and STAT3 GOF mice showing ear skin pathology (top) and H&E stained sections of ear tissue (bottom). (D) Histological measurements from H&E-stained sections of ear tissue. (E) Quantification of ear skin immune cell frequencies by flow cytometry. (F) Representative flow plot and quantification of IL-17A, RORγt, and IL-22 expression in $CD45.2^+$ $CD3^+$ $TCR\gamma\delta^-$ $CD8^-$ $CD4^+$ skin T cells and (G) $CD45.2^+$ $CD3^+$ $TCR\gamma\delta^{mid}$ skin T cells. Cytokine expression assessed after PMA/ionomycin stimulation. (H) Representative flow plot and quantification of IL-17A, and IL-22 expression in $CD45.2^+$ $CD3^+$ $TCR\gamma\delta^-$ $CD8^-$ $CD4^+$ CLN T cells and (I) spleen T cells. Cytokine expression assessed after PMA/ionomycin stimulation. Data are presented as mean ± SEM. Statistical significance determined by unpaired two-tailed or Welch's t-test (A, D-I), or 2-way repeated measures ANOVA with Šídák's multiple comparisons test (B), *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001. Data are representative of 4-16 mice (A-D), or 3 experiments with 10-16 mice (E-I).

2.3.2 Topical imiquimod elicits severe skin inflammation and a Th17 response in STAT3 GOF mice

Based on these findings, we hypothesize that disease in STAT3 GOF mice, and potentially in patients, is partly dictated by environmental factors that initiate local organ-specific inflammation. This hypothesis would explain why patients with the same genetic variant have different disease manifestations. To test this, we used the imiquimod (IMQ) model of psoriasiform dermatitis (188) to determine if an *in vivo* inflammatory challenge would lead to disease similar to the spontaneous skin inflammation seen in older adult STAT3 GOF mice in SPF housing.

IMQ is a TLR7 ligand that causes skin inflammation driven mostly by IL-6 and IL-23 signaling (188). These cytokines activate STAT3 and induce IL-17 and IL-22 production by Th17 and γδ T cells, which in turn mediate neutrophil recruitment and stimulate keratinocyte proliferation resulting in epidermal hyperplasia (189). Topical IMQ cream was applied daily to both ears of young adult WT and STAT3 GOF littermates for 7 consecutive days. Control mice were not treated (NT). By day 5, ear swelling was substantially greater in STAT3 GOF mice, with visible redness and scaling of the skin (**Figure 2.2A & B**). IMQ-treated STAT3 GOF mice

had more severe disease with significantly increased epidermal thickness and cellular infiltrate compared to IMQ-treated WT littermates (**Figure 2.2C**). Another model of STAT3 GOF (*Stat3*$^{p.T716M/+}$) generated in our laboratory, which carry the most common variant found in patients, also had increased ear swelling compared to WT littermates following IMQ treatment (**Figure S2.2C**) (174). This is consistent with a recent report of spontaneous skin disease in *Stat3*$^{p.T716M/T716M}$ mice from another group (20). We continued to use the *Stat3*p.$^{G421R/+}$ mice for all other studies.

Although total cellularity of the skin was not changed (**Figure S2.3A**), there was a greater influx of skin-infiltrating neutrophils and a decrease in the dendritic cell (DC)/Langerhans cell population in IMQ-treated STAT3 GOF mice (**Figure 2.2D**). Total cell number in the CLN was increased in IMQ-treated STAT3 GOF compared to WT (**Figure S2.3B**), but the frequencies and total cell numbers of CLN T and B cells were not significantly different between NT and IMQ-treated STAT3 GOF (**Figure S2.3C**).

STAT3 GOF mice were crossed with an IL-17 GFP reporter strain to test if there was a similar induction of Th17 cells following IMQ as observed in older adult STAT3 GOF mice. The frequency of *Il17a*-GFP$^+$ and total RORγt$^+$ CD4$^+$ T cells in the skin of STAT3 GOF mice was the same as WT at baseline, corresponding with a lack of spontaneous skin disease in young adults (**Figure 2.1A**). However, the frequency of *Il17a*-GFP$^+$ and total RORγt$^+$ CD4$^+$ T cells was significantly increased in IMQ-treated STAT3 GOF compared to WT (**Figure 2.2E**). WT mice, in contrast with STAT3 GOF, did not mount a significant skin Th17 response to IMQ (**Figure 2.2E**). Furthermore, while Treg frequency was increased in WT mice after treatment, this pattern was not observed in STAT3 GOF mice (**Figure 2.2E**), suggesting impaired differentiation or

recruitment of pTregs. This is similar to what we previously observed in a T cell transfer model of colitis (19). Skin $CD4^+$ T cells from STAT3 GOF mice also produced significantly more IL-22 following ex vivo PMA and ionomycin stimulation compared to WT (**Figure 2.2F**), again supporting a strong local Th17 response associated with skin disease. Consistent with prior studies of the IMQ model, γδ T cells in the skin produced both IL-17A and IL-22 with IMQ treatment (**Figure S2.3D & E)** (187, 190). However, there was no difference in the frequency of skin IL-$17A^+$, RORγt^+, or IL-22^+ γδ T cells between IMQ-treated STAT3 GOF and WT (**Figure S2.3D & E**). This finding of altered $CD4^+$ T cells in the skin, but not γδ T cells, suggests that the increased disease seen in STAT3 GOF mice is not due to increased cytokine production in γδ T cells.

Although $CD4^+$ IL-$17A^+$ frequency was not significantly different in the CLN, the frequency of $CD4^+$ IL-22^+ cells and numbers of both IL-$17A^+$ and IL-22^+ $CD4^+$ T cells were increased in IMQ-treated STAT3 GOF compared to WT (**Figure 2.2G & H)**. The frequencies of Tregs, $FoxP3^+$ RORγt^+, or total RORγt^+ $CD4^+$ T cells were not significantly different in the CLN, although the total numbers of these populations were increased in IMQ-treated STAT3 GOF compared to WT (**Figure S2.3F**). The total number of CLN IL-22^+ γδ T cells was increased in IMQ-treated STAT3 GOF compared to WT (**Figure S2.3G**), consistent with the increased cellularity (**Figure S2.3B**). In summary, IMQ elicited a severe local inflammatory response in STAT3 GOF mice, with increased ear swelling, neutrophil infiltration, and Th17 responses in the skin and draining lymph nodes. Together, these data support a role for local inflammation mediated by Th17 cells in STAT3 GOF mice.

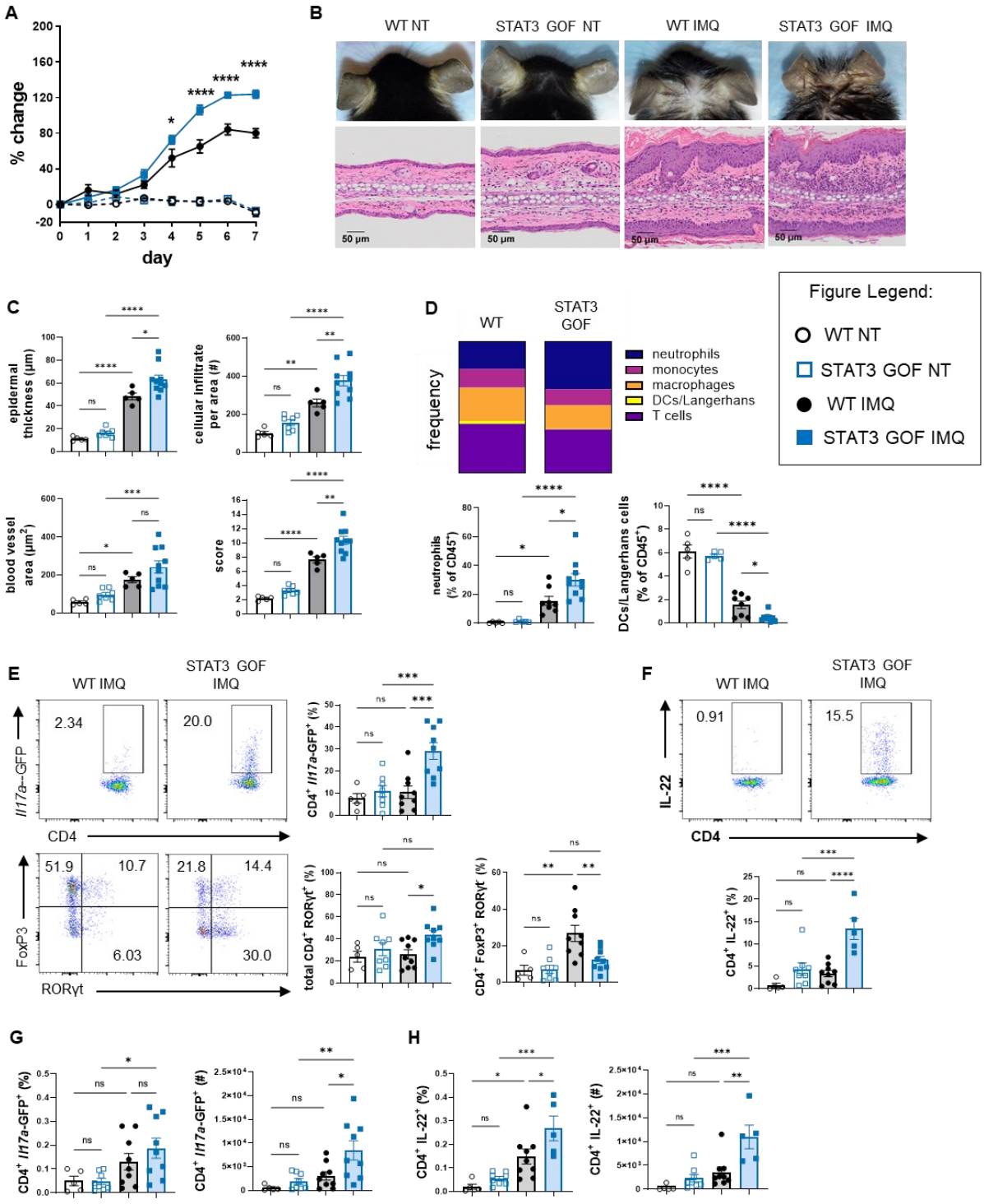

Figure 2.2: Topical imiquimod elicits severe skin inflammation and a Th17 response in young adult STAT3 GOF mice. (A) Percent change in ear thickness of young adult STAT3 GOF and WT littermates during IMQ treatment relative to baseline. (B) Representative pictures of NT and IMQ-treated WT and STAT3 GOF mice

showing ear skin pathology (top) and representative H&E-stained sections of ear tissue (bottom). (C) Histological measurements from H&E stained sections of ear tissue. (D) Frequency and quantification of indicated skin-infiltrating immune cells on day 7. (E) Representative flow plot and quantification of *Il17a*-GFP, RORγt, and FoxP3 expression in CD45.2$^+$ CD3$^+$ TCRγδ$^-$ CD8- CD4$^+$ skin T cells from IL-17 GFP mice treated with IMQ. (F) Representative flow plot and quantification of IL-22 expression in CD45.2$^+$ CD3$^+$ TCRγδ$^-$ CD8$^-$ CD4$^+$ skin T cells from IL-17 GFP mice treated with IMQ. IL-22 expression assessed after PMA/ionomycin stimulation. (G) Frequency and total number of *Il17a*-GFP$^+$ and (H) IL-22$^+$ CD45.2$^+$ CD3$^+$ TCRγδ$^-$ CD8$^-$ CD4$^+$ CLN T cells from IL-17 GFP mice treated with IMQ. IL-22 expression assessed after PMA/ionomycin stimulation. Data are presented as mean ± SEM. Data represent 4 experiments with 8-12 mice (A), 4 experiments with 5-10 mice (B-D), or 3 experiments with 5-9 mice (E-H). Statistical significance determined by 2-way repeated measures ANOVA with Šídák's multiple comparisons test (A) or 1-way ANOVA with Šídák's multiple comparisons test (C-H). *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001.

2.3.3 CD4$^+$ T cells are sufficient, and γδ T cells and CD8$^+$ T cells are not required to mediate IMQ-induced inflammation in STAT3 GOF mice

To test if adaptive immune cells were important for increased skin inflammation, STAT3 GOF mice were crossed to the *Rag-1*$^{-/-}$ background and treated with IMQ. *Rag*-deficient mice had milder ear swelling, and there was no significant difference in ear thickness between *Rag1*$^{-/-}$ WT and *Rag1*$^{-/-}$ STAT3 GOF mice (**Figure 2.3A**). Similarly, there were no differences in overall pathological score or individual measurements of epidermal thickness, cellular infiltrate count, or blood vessel area (**Figure 2.3B & C**). These data indicate that adaptive immune cells are required for IMQ-induced ear swelling and skin pathology in STAT3 GOF mice. To test if γδ T were required to drive disease, STAT3 GOF were crossed to mice lacking the *Tcrd* gene (*Tcrd*$^{-/-}$) and treated with IMQ. In the absence of γδ T cells, STAT3 GOF mice continued to have increased ear swelling compared to WT (**Figure 2.3D**). This was associated with an increase in skin neutrophil frequency (**Figure S2.4A**), indicating that γδ T cells are not required for IMQ-induced skin disease in STAT3 GOF.

Recent reports from mouse models of other STAT3 GOF variants have described dysregulated $CD8^+$ T cells in accelerated type I diabetes (21) and spontaneous skin pathology (20). To test if $CD8^+$ T cells were required, *Tcrd*$^{-/-}$ mice were depleted of $CD8^+$ cells prior to and during IMQ treatment. Again, ear swelling was worse in STAT3 GOF in the absence of both γδ T and $CD8^+$ T cells (**Figure 2.3E**). Although this was not associated with increased neutrophil infiltration into the skin (**Figure S2.4B**), the frequency of IL-$17A^+$ and IL-22^+ $CD4^+$ T cells in the skin was significantly increased in STAT3 GOF compared to WT (**Figure S2.4C**). This suggests that the altered cytokine profile in $CD4^+$ T cells can drive the differences in skin disease.

Given that Th17 cells accumulate in STAT3 GOF and other T cells are not required for IMQ-induced inflammation, we hypothesized that $CD4^+$ T cells were key mediators of disease in this model. To directly test if STAT3 GOF in $CD4^+$ T cells could drive skin inflammation, WT or STAT3 GOF $CD4^+$ T cells from IMQ-treated IL-17 GFP reporter mice were adoptively transferred into *Rag1*$^{-/-}$ hosts, which were treated with IMQ 24 hours later. STAT3 GOF $CD4^+$ T cells induced greater ear swelling than WT $CD4^+$ T cells (**Figure 2.3F**) and were associated with increased macrophages and decreased DCs/Langerhans cells in the skin compared to control *Rag1*$^{-/-}$ (**Figure S2.4D**). Transferred STAT3 GOF $CD4^+$ T cells had reduced expression of FoxP3 and increased expression of IL-22; however, *Il17a*-GFP expression was not significantly different (**Figure S2.4E**). Together, these data indicate that STAT3 GOF in $CD4^+$ T cells is pathogenic and that these cells are sufficient for skin inflammation.

2.3.4 STAT3 GOF bone marrow can exacerbate IMQ-induced inflammation in WT mice

STAT3 regulates signaling in hematopoietic and non-hematopoietic cells and is particularly important for proliferation in somatic cells (115, 116). In the skin, IL-22 activates STAT3 signaling in keratinocytes to inhibit differentiation and promote proliferation (191). While our model demonstrates that adaptive immune cells, but not γδ T or $CD8^+$ T cells, are required for skin inflammation, patients with STAT3 GOF syndrome have germline disease. It is therefore likely that organ-specific disease depends in part on STAT3 GOF in non-hematopoietic cells. To test if observed differences in skin inflammation were due to altered function of STAT3 GOF in hematopoietic cells, bone marrow (BM) chimeras were generated: WT mice that received bone marrow from STAT3 GOF mice (STAT3 GOF → WT) and vice versa (WT → STAT3 GOF). Mice were sub-lethally irradiated and engraftment was assessed in the peripheral blood at 12 weeks post-transplant. While STAT3 GOF → WT chimeras had near complete engraftment in all immune cell types tested in the peripheral blood, WT → STAT3 GOF chimeras unexpectedly had incomplete engraftment, particularly in the myeloid compartment (**Figure S2.5A**). Although some cell types of the skin are radioresistant, including dermal γδ T cells (192) and some types of dendritic cells (193), STAT3 GOF → WT chimeras also exhibited better engraftment of cell types in the skin compared to WT → STAT3 GOF chimeras (**Figure S2.5B**). All recipients were housed randomly and received equal doses of radiation at the same time, therefore these results demonstrate that WT → STAT3 GOF BM chimeras were of mixed nature (**Figure S2.5A & B**). This failure of WT BM to completely engraft in STAT3 GOF hosts

may provide some insight into the generally poor success rates of hematopoietic stem cell transplantation (HSCT) in patients with STAT3 GOF (174).

Spontaneous skin inflammation did not occur in STAT3 GOF → WT mice 12 weeks after transplant (**Figure S2.5C**). By contrast, WT → STAT3 GOF and STAT3 → STAT3 chimeras did develop spontaneous ear swelling compared to WT → WT (**Figure S2.5C**), suggesting that the combination of STAT3 GOF in hematopoietic and non-hematopoietic cells results in spontaneous disease. We focused IMQ studies on STAT3 GOF → WT chimeras due to the mixed nature of WT → STAT3 GOF chimeras. STAT3 GOF → WT BM chimeras were treated with IMQ at 12 weeks post-transplant. Ear thickness increased over the course of IMQ treatment in STAT3 GOF → WT chimeras compared to WT → WT chimeras (**Figure 2.3G**). This increase in ear thickness in STAT3 GOF → WT chimeras coincided with a higher pathological score driven by an increase in cellular infiltrate in the skin, but not epidermal thickness (**Figure S2.5D**). This suggests STAT3 GOF in hematopoietic cells affects recruitment of inflammatory immune cells, but the epidermal hyperplasia seen in older germline STAT3 GOF mice may be due to STAT3 GOF in the epidermal cells, lending support to our hypothesis that both hematopoietic and non-hematopoietic cells require STAT3 GOF for the full phenotype. Analysis of cytokine production in donor-derived T cells revealed an increase in the frequency of IL-17A$^+$ and IL-22$^+$ CD4$^+$ T cells in IMQ-treated STAT3 GOF → WT, demonstrating that the Th17 response is cell-intrinsic (**Figure 2.3H**). In summary, BM chimeras demonstrated that the Th17 response was intrinsic to STAT3 GOF-expressing T cells and was sufficient to drive IMQ-induced skin inflammation. Spontaneous skin disease and possibly changes in epidermal

thickness require the presence of STAT3 GOF in non-hematopoietic cells, suggesting that STAT3 GOF in epithelial cells is important for this disease phenotype.

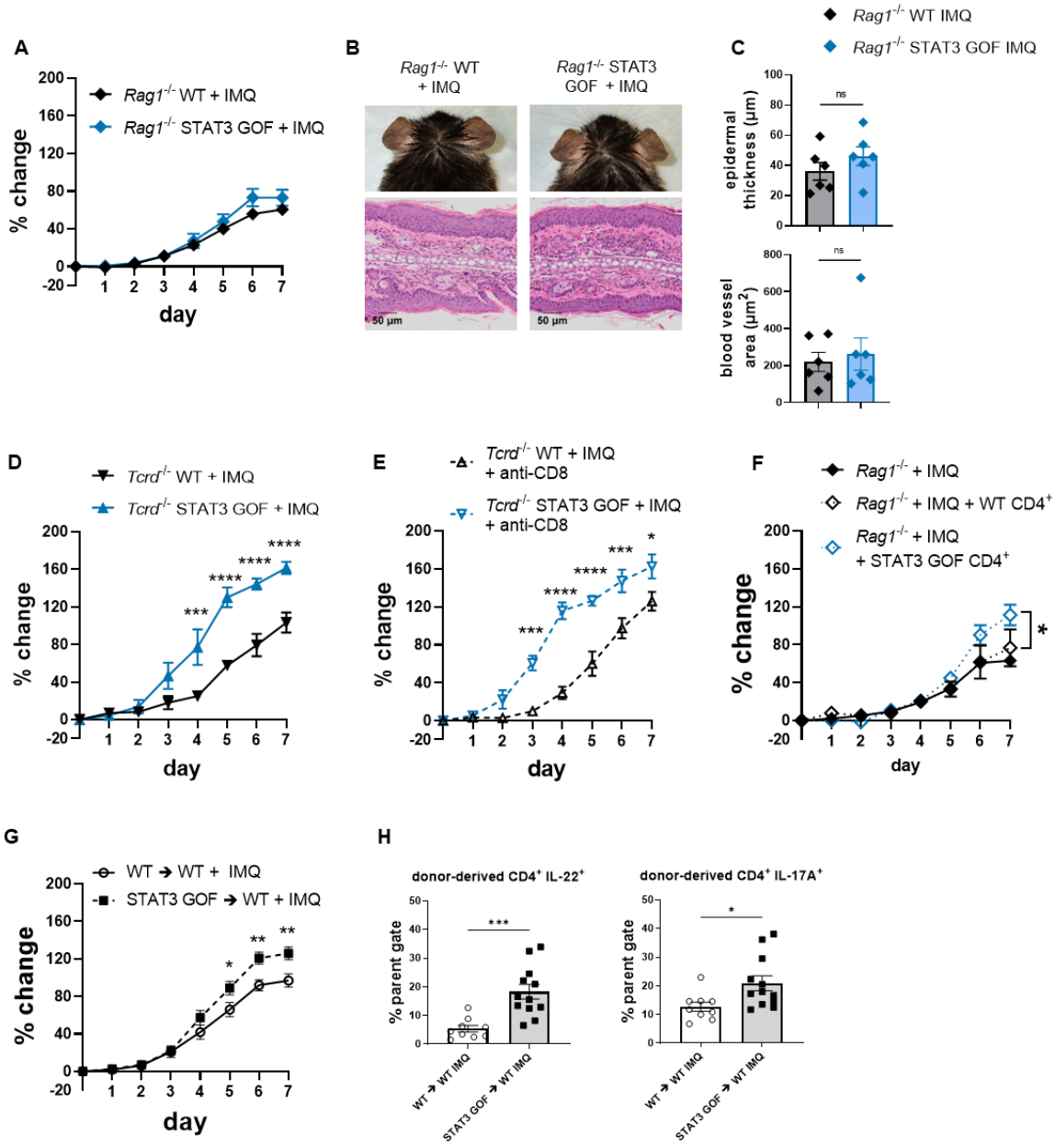

Figure 2.3: CD4$^+$ T cells are sufficient, and γδ T cells and CD8$^+$ T cells are not required to mediate IMQ-induced inflammation in STAT3 GOF mice. (A) Percent change in ear thickness of *Rag1*$^{-/-}$ mice during course of IMQ treatment relative to baseline. (B) Representative images of treated ears (top) and H&E stained sections of IMQ-treated *Rag1*$^{-/-}$ mice (bottom). (C) Histological measurements from H&E stained sections of ear tissue of IMQ-treated *Rag1*$^{-/-}$ mice. (D) Percent change in ear thickness of *Tcrd*$^{-/-}$ mice during course of IMQ treatment relative to

baseline. (E) *Tcrd*$^{-/-}$ were treated with 150 µg anti-CD8 antibody from d-1 to d7. IMQ treatment started on d0. Percent change in ear thickness during course of IMQ treatment relative to baseline. (F) 5x10^{6} WT or STAT3 GOF CD4^{+} T cells isolated from the CLN of IMQ-treated IL-17 GFP mice were adoptively transferred i.v. into *Rag1*$^{-/-}$ hosts. 24 hours after transfer, recipients were treated with IMQ. Percent change in ear thickness during course of IMQ treatment relative to baseline of *Rag1*$^{-/-}$ mice that received WT or STAT3 GOF CD4^{+} T cells. (G) Bone marrow from WT or STAT3 GOF (Ly5.1) was transplanted into irradiated WT (Ly5.2) recipients. On week 12-13 post-transplant, mice were treated with IMQ. Percent change in ear thickness during course of IMQ treatment relative to baseline. (H) Quantification of donor-derived CD4^{+} IL-22^{+} and IL-17A^{+} frequencies of skin tissue from the ear by flow cytometry. Data are presented as mean ± SEM. Statistical significance determined by 2-way repeated measures ANOVA with Šídák's multiple comparisons test (A, D-G) or unpaired two-tailed or Welch's t-test (C, H). Data represent 3 experiments with 5-7 mice (A, D, E), 3 experiments with 4-7 mice (F) or 4 experiments with 8-12 mice per group (G, H).

2.3.5 STAT3 GOF T cells show increased clonal expansion at baseline and after IMQ

We have shown that both older adult and IMQ-treated STAT3 GOF mice showed an enhanced Th17 response in the CLN as demonstrated by the increased frequency and number of IL-22^{+} CD4^{+} T cells and increased total numbers of IL-17A^{+} CD4^{+} T cells. To examine transcriptional changes caused by STAT3 GOF in T cells and to determine if STAT3 GOF drives increased clonal expansion, CITE-seq with TCR-seq was performed on enriched CD3^{+} cells from the CLN of young adult WT and STAT3 GOF littermates, with and without IMQ treatment (194). CITE-seq cell-surface protein markers included CD3e, TCRβ, CD4, CD8a, CD62L, and CD44 to identify naïve and effector T cell populations. A multimodal UMAP projection of 54 clusters across 135,567 cells from all conditions was generated using weighted nearest neighbor analysis of RNA and surface protein expression (**Figure 2.4A**) (195). Clustering remained similar between WT and STAT3 GOF and between NT and IMQ (**Figure 2.4B**). However, multiple clusters (2, 6, 10, 16, 24, 29, 39, 42, 45, and 52) were enriched for cells from STAT3 GOF samples (**Figure S2.6A**). Clusters of either single positive CD4 or CD8 T cells were selected based on antibody expression (which matched gene expression) then re-clustered and

manually annotated based on protein expression of CD44 and CD62L, and by gene signatures documented in literature (**Figure 2.4C, Figure S2.6B, and Table S2.1**). T cells were identified as naïve, effector/effector memory (Eff/TEM), central memory (TCM), exhausted (Exh), and Tregs ($CD4^+$ T cells only) or intermediate (Int, $CD8^+$ T cells only) (**Figure 2.4C & D, Figure S2.6B & C**). Frequencies of $CD4^+$ TCM and Exh clusters were increased in NT STAT3 GOF samples, and the TCM and Treg clusters increased after IMQ compared to WT (**Figure 2.4D**). The frequency of the $CD8^+$ TCM cluster was increased in NT STAT3 GOF compared to WT, suggesting that STAT3 GOF T cells have encountered antigen prior to treatment (**Figure S2.6C**).

Next, we examined the transcriptional differences in STAT3 GOF $CD4^+$ T cells by annotated cluster. At baseline, STAT3 target genes (*Socs3*, *Maf*, *Cxcr3*), and calcium binding genes (*S100a4*, *S100a6*, *S100a11*) were upregulated in the TCM cluster of STAT3 GOF $CD4^+$ T cells (**Figure 2.4E**), consistent with a baseline phenotype of increased STAT3 signaling in this model. Notably, T follicular helper cell (Tfh) genes such as *Il21* and *Cxcr5*, as well as exhaustion and inflammatory genes such as *Tigit*, *Tox2*, *S100a6*, and *S100a11* were upregulated in the STAT3 GOF NT Exh cluster (**Figure 2.4F**), suggesting that these cells have features of both Tfh and exhausted cells. In addition to ribosomal genes, several Th17-related genes were upregulated in the IMQ-treated STAT3 GOF Eff/TEM cluster, including *Il17a*, *Il17f*, *Il22*, *Ccr6*, and *Rora*, consistent with our observations by flow cytometry (**Figure 2.4G**). In the IMQ Treg cluster, upregulated genes in STAT3 GOF included targets of STAT3 (*Jun*, *Socs3*), *Ifngr1*, *Igfbp4*, and *Satb1*, which has been shown to alter pTreg differentiation (196). Upregulated genes in WT NT

Tregs included *Ikzf2*, *Il2ra*, and *Tgfb1* (**Figure 4H)**, suggesting altered stability of STAT3 GOF Tregs.

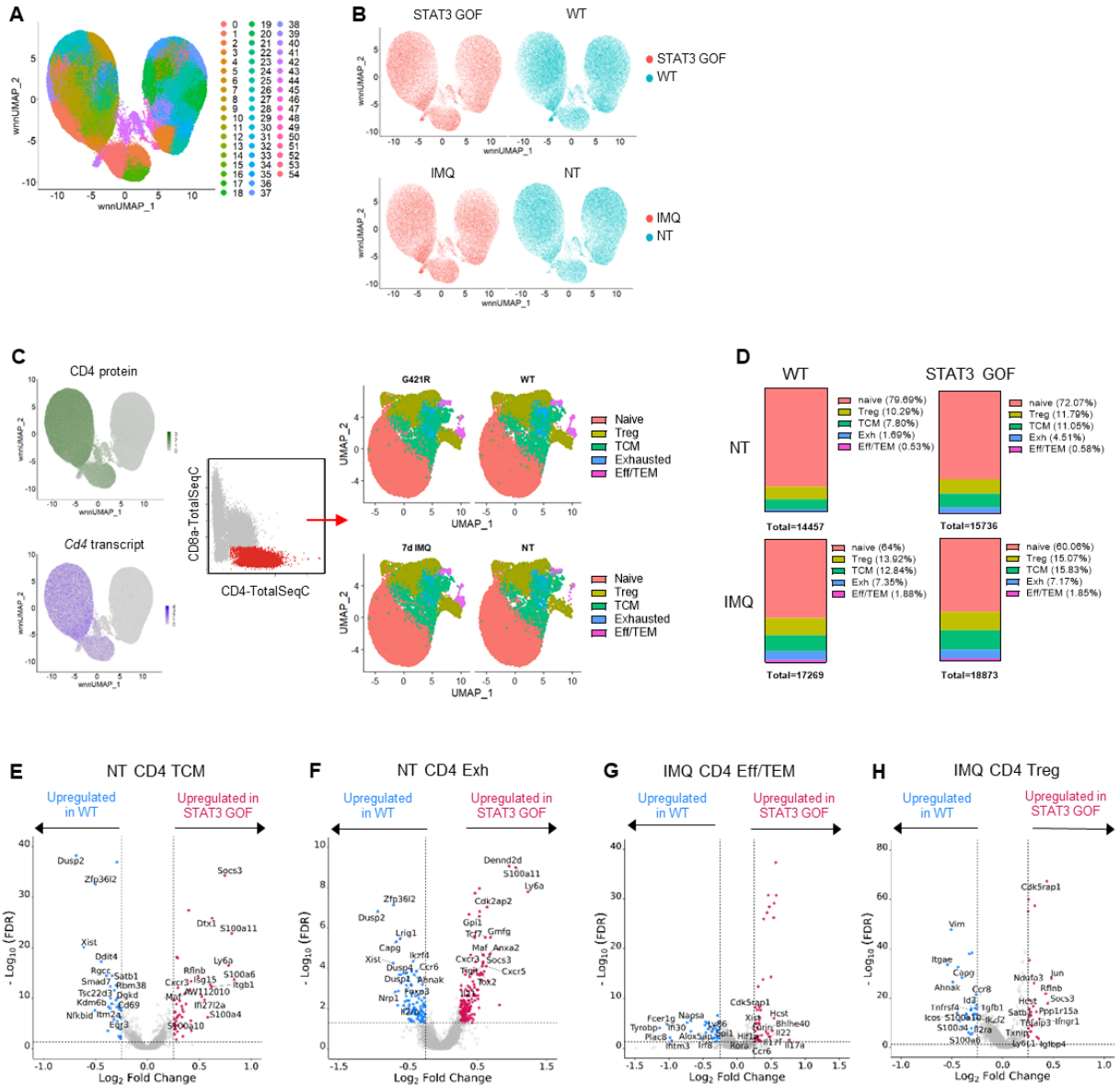

Figure 2.4: STAT3 GOF T cell single-cell RNA-Seq after IMQ treatment. T cells from the CLN of NT or IMQ-treated WT and STAT3 GOF littermates were isolated and labeled with Totalseq-C antibodies for CITE-seq. Single cell libraries were generated using 10X Genomics. The 5' GEX v2 libraries were dual indexed with VDJ and surface immune receptor sequencing. Cells were filtered based on mitochondrial content and feature count, then performed standard normalization and weighted nearest neighbor analysis in Seurat. (A) UMAP projection of $CD3^+$ T cells from CLN of untreated or IMQ-treated WT (n=2 per group) or STAT3 GOF (n=2 per group) mice used in scRNA-seq experiment showing unique clusters and (B) sample identity by genotype and treatment. (C) Cellselector function was used to separate single positive CD4 T cells, which were renormalized and reclustered based on CD44

and CD62L surface protein expression and canonical gene expression. (D) Frequencies of annotated $CD4^+$ clusters. (E-H) Volcano plots showing differential expression (adjusted $P < 0.05$, average log2 fold change > 0.25 or < -0.25) in cells identified as NT TCM, comparing WT and STAT3 GOF (E), cells identified as NT Exh, comparing WT and STAT3 GOF (F), cells identified as IMQ Eff/TEM, comparing WT and STAT3 GOF (G), and cells identified as IMQ Treg, comparing WT and STAT3 GOF (H). Ribosomal genes, genes ending in –Rik, and genes beginning with Gm- are not labeled in volcano plots.

Our data suggest $CD4^+$ T cells are important in disease, therefore we assessed T cell clonality and the transcriptional profile of expanded clones in STAT3 GOF CLN $CD4^+$ T cells compared to WT. At baseline, STAT3 GOF $CD4^+$ T cells showed an increased clonal response in the TCM, Exh, and to a minor extent, Treg clusters based on the presence of medium sized clones (defined as 5-20 clones) (**Figure 2.5A**). IMQ treatment and skin disease correlated with increased clonal expansion of Eff/TEM and TCM clusters in STAT3 GOF $CD4^+$ T cells compared to WT; however, clonal expansion was diminished in the Treg cluster of IMQ-treated STAT3 GOF $CD4^+$ T cells (**Figure 2.5A**), perhaps indicative of impaired pTreg generation or recruitment.

Expanded $CD4^+$ clones from NT STAT3 GOF mice showed upregulation of genes including *Socs3*, *Cxcr5*, *Maf*, *Junb*, as well as exhaustion-related genes such as *Tox* and *Tox2* (**Figure 2.5B**), providing additional evidence for a persistent stimulus in STAT3 GOF mice at baseline. Following IMQ treatment, expanded $CD4^+$ clones showed upregulation of *Socs3*, *Junb*, *Fos*, and *Il22*, while WT expanded $CD4^+$ clones showed upregulation of Treg-related genes including *Foxp3*, *Il2ra*, and *Ikzf2* (**Figure 2.5C**). This suggests that expanded effector $CD4^+$ T cell clones in STAT3 GOF are more likely to have a Th17 phenotype and are perhaps less likely to be Tregs. Surprisingly, STAT3 GOF expanded clones from the Treg cluster upregulated *Il22* (**Figure 2.5D**). We previously observed a defect in peripheral Treg induction in a model of T cell

transfer colitis (19). However, IMQ did not reduce Treg frequency in the CLN of STAT3 GOF mice (**Figure S2.3F**). Together, the diminished clonal expansion, Th17-like gene expression, and the observations of upregulated *Ikzf2*, *Il2ra*, and *Tgfb1* in all WT Tregs compared to STAT3 GOF, suggests that STAT3 GOF could alter Treg stability.

Two other studies of STAT3 GOF found increased clonal expansion of CD8+ T cells associated with accelerated diabetes development or lymphoproliferative disease (20, 21). Although $CD8^+$ T cells were not required for skin inflammation in our model, there was a similar increased $CD8^+$ clonal expansion at baseline in Eff/TEM, TCM, and Int clusters due to STAT3 GOF (**Figure S2.7A**). IMQ-treated STAT3 GOF $CD8^+$ T cells showed increased clonal expansion of TCM and Int clusters with the emergence of large sized clones (defined as 20-100 clones), but fewer Eff/TEM clones (**Figure S2.7A**). These data suggest that T cells in STAT3 GOF are more likely to be activated or antigen-experienced at baseline, and this could contribute to the increased clonal response observed following stimulation. In NT STAT3 GOF $CD8^+$ expanded clones, there was upregulation of *Ccl5*, *Ccr5*, *Ccl4*, *Ctla2a*, *Gzmk*, *Eomes*, *Nkg7*, and *Gzmb* (**Figure S2.7B**). Some of the transcripts upregulated in IMQ-treated STAT3 GOF $CD8^+$ expanded clones were similar and included *Ccl5*, *Ccl4*, *Ccr5*, *Gzmk*, *Nkg7*, *Ctla2a*, and *Tigit* (**Figure S2.7C**). As previously observed in the p.K392R variant on the NOD background (21), this indicates that STAT3 GOF drives expression of genes involved with cytotoxicity and chemotaxis in $CD8^+$ T cells at baseline and after stimulation with IMQ. Our experimental data suggests that this may be a bystander effect, as $CD8^+$ T cells are not required for disease.

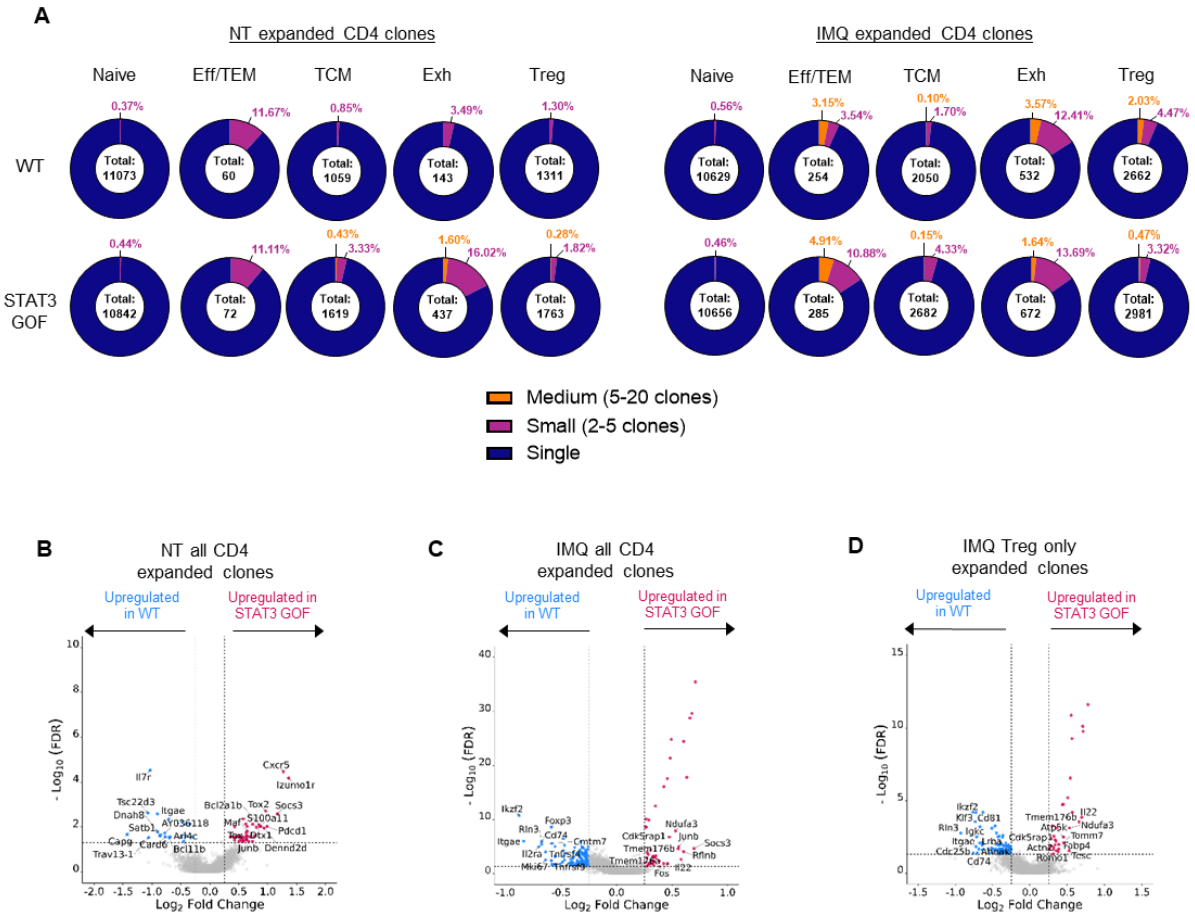

Figure 2.5: STAT3 GOF CD4$^+$ T cells show increased clonal expansion at baseline and after IMQ. (A) Clonotype frequency of the NT and IMQ treated CD4$^+$ T cells by annotated cluster generated with ScRepertoire. (B-D) Volcano plot showing differential expression (adjusted P < 0.05, average log2 fold change > 0.25 or < –0.25) in NT CD4$^+$ expanded clones, comparing WT and STAT3 GOF (B), IMQ CD4$^+$ expanded clones, comparing WT and STAT3 GOF, and IMQ Treg expanded clones, comparing WT and STAT3 GOF (D). Ribosomal genes, genes ending in –Rik, and genes beginning with Gm- are not labeled in volcano plots.

2.3.6 IL-22 signaling contributes to increased IMQ-induced inflammation in STAT3 GOF

Given the observation of expanded IL-22$^+$ CD4$^+$ T cells in STAT3 GOF mice, we hypothesized that IL-22 was important for driving enhanced disease induced by IMQ. Indeed, previous studies have demonstrated a requirement for IL-22 in the development of IMQ-induced skin inflammation (197). To test this hypothesis, STAT3 GOF mice were crossed to IL-22-

deficient (*Il22*$^{-/-}$) mice and treated with IMQ. In the absence of IL-22, ear swelling in both STAT3 GOF mice and WT mice was reduced, with *Il22*$^{-/-}$ STAT3 GOF mice having ear swelling similar to IL-22-sufficient WT mice from prior experiments (**Figure 2.2A data overlaid in Figure 2.6A for IL-22 sufficient WT and STAT3 GOF**). Although reduced overall, *Il22*$^{-/-}$ STAT3 GOF mice still had increased ear swelling compared to *Il22*$^{-/-}$ WT mice (**Figure 2.6A**), as well as increased epidermal thickness, cellular infiltrate count, and pathological score (**Figure 2.6B & C**). Although the frequency of skin-infiltrating neutrophils was not significantly different, there was a significant decrease in the frequency of DCs/Langerhans cells, as well as an increase in the total cellularity of the CLN in *Il22*$^{-/-}$ STAT3 GOF compared to WT (**Figure 2.6D & E**), which is similar to what we observed in older adult and IMQ-treated STAT3 GOF mice sufficient for *Il22*. The frequency of both IL-17A^{+} CD4^{+} T cells and γδ T cells was higher in *Il22*$^{-/-}$ STAT3 GOF compared to *Il22*$^{-/-}$ WT (**Figure 2.6F**). Additionally, the total number of IL-17A^{+} CD4^{+} T cells was increased in the CLN of *Il22*$^{-/-}$ STAT3 GOF (**Figure 2.6G**). Collectively, these data demonstrate that IL-22, which is enhanced in STAT3 GOF CD4^{+} T cells, partially mediates skin disease in STAT3 GOF and likely signals through non-hematopoietic cells to cause the full disease phenotype induced by IMQ.

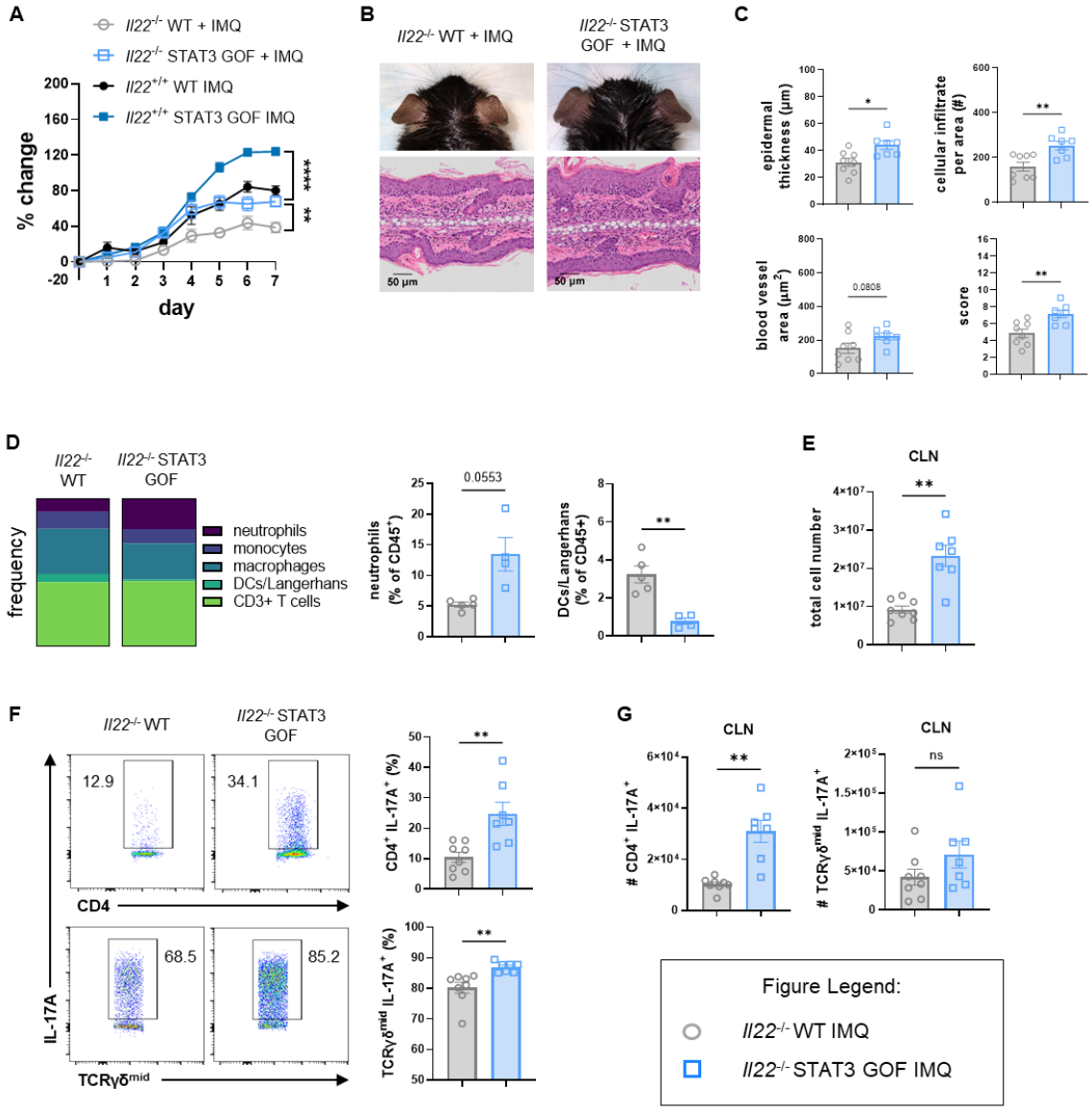

Figure 2.6: IL-22 signaling contributes to increased IMQ-induced inflammation in STAT3 GOF. (A) Percent change in ear thickness of *Il22*$^{-/-}$ WT and *Il22*$^{-/-}$ STAT3 GOF mice during course of IMQ treatment relative to baseline overlaid with data from Figure 2A (*Il22*$^{+/+}$). Day 7 statistics shown (top: *Il22*$^{+/+}$ STAT3 GOF vs *Il22*$^{-/-}$ STAT3 GOF; bottom: *Il22*$^{-/-}$ WT vs *Il22*$^{-/-}$ STAT3 GOF). (B) Representative images of treated ears and H&E stained sections of IMQ-treated *Il22*$^{-/-}$ WT and *Il22*$^{-/-}$ STAT3 GOF mice. (C) Histological measurements from H&E stained sections of ear tissue of IMQ-treated *Il22*$^{-/-}$ WT and *Il22*$^{-/-}$ STAT3 GOF mice. (D) Frequency and quantification of indicated cell populations in the skin measured by flow cytometry. (E) Number of total cells in CLN on day 7. (F) Representative flow plot and quantification of IL-17A expression in CD45.2^{+} CD3^{+} TCRγδ^{-} CD8^{-} CD4^{+} or CD45.2^{+} CD3^{+} TCRγδ^{mid} skin T cells. Cytokine expression assessed after PMA/ionomycin stimulation. (G) Total number of IL-17A expressing CD45.2^{+} CD3^{+} TCRγδ^{-} CD8^{-} CD4^{+} and CD45.2^{+} CD3^{+} TCRγδ^{mid} CLN T cells measured by flow cytometry. Cytokine expression assessed after PMA/ionomycin stimulation. Data are presented as mean ± SEM. Statistical significance determined by 2-way repeated measures ANOVA with Tukey's multiple comparisons test (A) or unpaired two-tailed or Welch's t-test (D-G). *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001. Data are representative of 3 experiments with 4-8 mice per group.

2.3.7 JAK inhibition prevents enhanced inflammation in STAT3 GOF caused by IMQ without affecting Th17 cytokine expression

JAK inhibitors (JAKinibs) have been used alone or in conjunction with IL-6R antagonists to treat STAT3 GOF patients, including individuals with the p.G421R variant (198). To determine if JAK signaling inhibition could reduce IMQ-induced inflammation in STAT3 GOF mice, young adult STAT3 GOF and WT mice were simultaneously treated with a JAKinib (oral tofacitinib) and IMQ for 7 days. Mice received either control chow or JAKinib-containing chow (1g JAKinib per kg chow) starting on day 0. Treatment with tofacitinib reduced ear thickness in STAT3 GOF mice to the level of IMQ-treated WT mice (**Figure 2.7A**). JAKinib treatment also reduced epidermal thickness, cellular infiltrate, and overall pathological score in STAT3 GOF mice compared to IMQ-only STAT3 GOF, and pathology was similar to that seen in WT mice (with or without JAKinib) (**Figure 2.7B & C**). These findings demonstrate that JAK inhibition can treat skin inflammation of STAT3 GOF mice in this model of disease.

Keratinocyte proliferation was measured by Ki-67 staining in the epidermis. Unexpectedly, JAKinib treatment resulted in increased percent Ki-67$^+$ cells in the epidermis for both WT and STAT3 GOF (**Figure 2.7D**). This contrasts with previous reports of tofacitinib reducing IMQ-induced epidermal cell proliferation by Ki-67 staining (199). Given the overall decrease in epidermal thickness with JAKinib treatment, increased turnover in epithelial cells may be due to processes involved with the resolution of inflammation or other proliferative mechanisms that do not rely on JAK1/3-STAT3 signaling. Neutrophil and monocyte infiltration in the skin was decreased in STAT3 GOF with JAKinib treatment, while DC/Langerhans cell frequencies were increased in both WT and STAT3 GOF (**Figure 2.7E**), indicating that immune

cell recruitment or retention in the skin may be altered by tofacitnib. Analysis of skin Th17 cells revealed no differences in IL-17A or IL-22 expression in STAT3 GOF following JAKinib treatment (**Figure 2.7F**). Together, these findings demonstrate that JAKinib treatment with tofacitinib can improve skin pathology, reduce cellular infiltration into the skin, and enhance keratinocyte proliferation without affecting IL-17A or IL-22 production by skin $CD4^+$ T cells. Collectively, these data provide strong supportive evidence for investigating local inflammatory responses and pathology in both immune cells and affected tissue in STAT3 GOF syndrome.

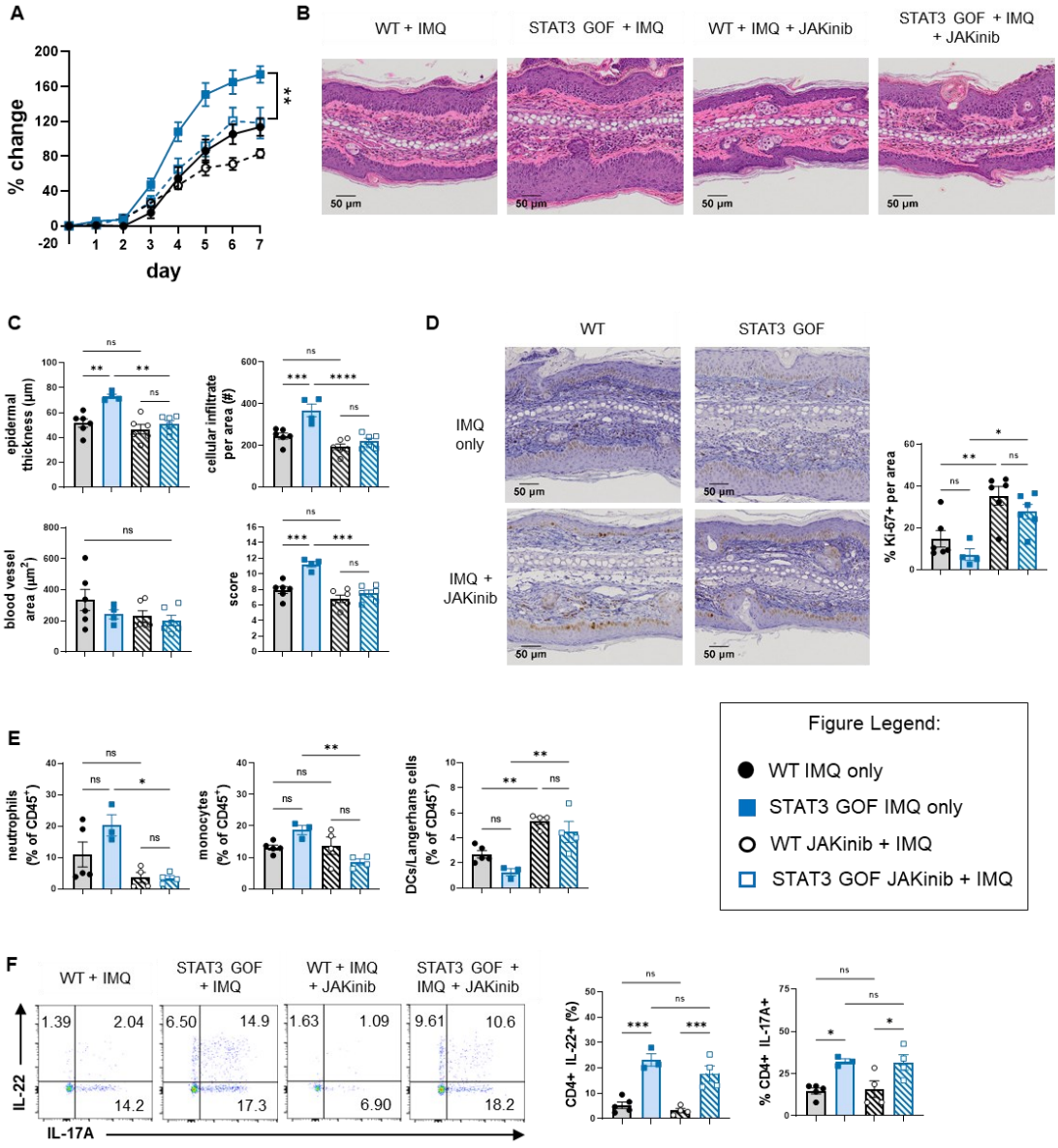

Figure 2.7: JAK inhibition prevents enhanced inflammation in STAT3 GOF caused by IMQ without affecting Th17 cytokine expression. (A) WT and STAT3 GOF littermates received tofacitinib delivered in chow formulation (Nutra-gel). Mice acclimated to Nutra-gel without drug for 3 days, then provided chow with 1g tofacitinib to 1 kg Nutra-gel (ad libitum access). IMQ treatment started on the same day. (A) Percent change in ear thickness during IMQ treatment relative to baseline. Day 7 statistics shown. (B) Representative images of H&E stained sections of IMQ only or IMQ + JAKinib treated ears. (C) Histological measurements from H&E stained sections of ear tissue. (D) Representative images of Ki-67 stained sections of IMQ only or IMQ + JAKinib treated ears with quantification. (E) Representative flow plot and quantification of indicated cell populations in the skin. (F) Representative flow plot and quantification of IL-17A and IL-22 expression in CD45.2^{+} CD3^{+} TCRγδ^{-} CD8^{-} CD4^{+} skin T cells. Cytokine expression assessed after PMA/ionomycin stimulation. Data are presented as mean ± SEM. Statistical significance determined by 2-way repeated measures ANOVA with Šídák's multiple comparisons test (A), or 1-way ANOVA with Šídák's multiple comparisons test (B-F). *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001. Data are representative of 2 experiments with 4-6 mice/group (A-D), or 3-5 mice/group (E-F).

2.4 Discussion

Here we demonstrate spontaneous and stimulus-driven skin disease in a mouse model harboring a pathogenic human STAT3 GOF variant (p.G421R) associated with a local, cell-intrinsic Th17 response that does not occur in WT mice. $CD4^+$ T cells are sufficient to drive increased ear swelling in STAT3 GOF mice following topical IMQ, and this response does not require γδ T or $CD8^+$ T cells. Single cell RNA sequencing revealed enhanced clonal $CD4^+$ T cell responses in STAT3 GOF, as well as increased expression of STAT3-regulated genes, including *Il22*, *Socs3*, *Junb*, and *Fos* in IMQ-treated expanded clones. IL-22-deficient STAT3 GOF mice had improved disease, but maintained increased infiltration of IL-$17A^+$ $CD4^+$ T cells. Treatment with a JAK inhibitor led to significant improvement of disease, associated with reduced neutrophil infiltration, increased keratinocyte proliferation, but no differences in Th17 cytokine expression.

Prior studies of peripheral blood cells from patients with STAT3 GOF have not demonstrated an overwhelming Th17 response (174). However, normalization of Th17 frequencies was associated with improvement of disease following tocilizumab treatment in two patients with the p.G421R variant, and in another with the p.P471R variant (177, 186, 200). This study demonstrates that in both spontaneous and IMQ-induced skin disease, there was a local (skin and CLN), but not systemic Th17 response. This finding suggests that analysis of peripheral blood immune cells in patients may not reflect the organ-specific inflammation at disease sites.

Unlike WT mice, skin inflammation in STAT3 GOF is associated with an increased Th17 response. We hypothesize that the enhanced production of Th17 cytokines can maintain STAT3 activation in immune cells and particularly through IL-22 signaling in epithelial cells. IL-22$^+$ CD4$^+$ T cells were increased in both the skin and peripheral tissues of older adult and IMQ-treated STAT3 GOF mice, and *Il22* transcript was upregulated in expanded CD4$^+$ T cell clones, including Tregs, after IMQ treatment. STAT3 transcriptional activity can drive IL-22 expression in CD4$^+$ T cells and γδ T cells through activating cytokines including IL-23 and IL-21 (122, 129, 159, 201, 202). In addition, the IL-22 receptor signals through STAT3 in keratinocytes and upregulates genes involved in keratinocyte proliferation and expression of CCL20 (191, 203-205). Consistent with these findings, the increased IL-22$^+$ CD4$^+$ T cell frequency in older adult and IMQ-treated STAT3 GOF mice coincided with increased epidermal thickness by histology; however, deletion of *Il22* did not completely prevent this. This is likely due to the expansion of IL-17A$^+$ CD4$^+$ or γδ T cells in the skin and cervical lymph nodes, as IL-17R signaling in keratinocytes can also drive proliferation and secretion of chemokines that recruit neutrophils to the skin during IMQ treatment (206). A previous study showed that the number of IL-17A$^+$ CD4$^+$ T cells was increased in IL-22$^{-/-}$ mice treated with IMQ, suggesting a compensatory mechanism that enhances IL-17A production in the absence of IL-22 (207). This compensation for absent IL-22 may be further exaggerated by STAT3 GOF, as we have observed increased IL-17A expression in skin γδ T cells with IL-22 deletion after IMQ, but not in IL-22-replete mice. Our findings of an enhanced local and systemic IL-22 response during skin inflammation in STAT3 GOF are also consistent with the observations of upregulated IL-22 expression in the skin and peripheral blood of patients with psoriasis (203, 205).

There is a diverse clinical phenotype in patients with STAT3 GOF, even among individuals with the same genetic variant (174). Similarly, mouse models of STAT3 GOF from our group and others have highlighted phenotypic differences likely due to different environmental and genetic factors, including allele expression of STAT3 GOF variants (19-21). In a model from Warshauer et. al., a heterozygous p.K392R variant (also in the DNA binding domain) on the NOD background led to accelerated diabetes driven by effector $CD8^+$ T cells that resisted terminal exhaustion and exhibited clonal expansion (21). In a separate study, a heterozygous p.K392R variant on the C57BL/6 background had progressive lymphoproliferation. This group observed increased Th17 differentiation *in vitro* and in a model of induced experimental autoimmune encephalomyelitis (EAE), but no difference in clinical disease (22). Masle-Farquhar et. al., reported two STAT3 GOF models on the C57BL/6 background with p.K658N, a variant associated primarily with somatic mutations in large granular lymphocytic (LGL) leukemia, or the p.T716M variant seen in STAT3 GOF monogenic disease. Both heterozygous and homozygous mice displayed significant lymphoproliferation mediated by dysregulated oligoclonal effector $CD8^+$ T cells resembling T-LGL cells (20). Transplantation of homozygous STAT3 GOF marrow into *Rag1*$^{-/-}$ recipients showed cell-intrinsic $CD8^+$ T cell expansion that was associated with fatal wasting disease, which was alleviated by $CD8^+$ T cell depletion (20). They also observed spontaneous skin inflammation in aged homozygous mice, although this was not explored in these models (20). Although epidermal $CD8^+$ T cells are increased in psoriatic lesions and can produce IL-17A and IL-22 (208, 209), our data clearly show that $CD8^+$ T cells are not required for increased skin inflammation with STAT3 GOF as there was no effect of $CD8^+$ depletion in *Tcrd*$^{-/-}$ mice. Similar

to the other studies, we did observe increased expression of cytotoxic genes in expanded $CD8^+$ clones both at baseline and after IMQ treatment (20, 21), suggesting baseline $CD8^+$ T cell dysregulation in our mouse model. Together, these observations in multiple models of STAT3 GOF highlight different genetic and cellular requirements for disease phenotypes.

We previously reported T cell dysregulation and lymphoproliferation in older adult STAT3 GOF mice with the p.G421R variant, including expansion of Tregs and Th1 cells, but no differences in Th17 ex vivo. Treg function was not altered by STAT3 GOF *in vitro*, suggesting that defects in Treg suppression are not driving disease in this variant; however, pTregs were decreased. In a T cell transfer model of colitis, there was an increased frequency of IL-$17A^+$ ex-Tregs from STAT3 GOF mice (19). Loss of FoxP3 expression and increased IL-17A expression in Tregs has been observed in autoimmune diseases in both humans and mice. In lesional skin (and PBMC) of patients with psoriasis, decreased FoxP3 expression and increased IL-17A expression in Tregs can be driven by STAT3-activating cytokines IL-23 and IL-21 ex-vivo (210, 211). In mice, FoxP3 instability has been observed in NOD mice (212). In our study, there were reduced numbers of Tregs at the site of skin inflammation and reduced clonal expansion of Tregs with IMQ treatment in STAT3 GOF mice. Furthermore, *Il22* was upregulated in the expanded Treg clones of IMQ-treated STAT3 GOF mice. These data suggest that STAT3 GOF may lead to dysregulated recruitment of pTregs to sites of inflammation, or loss of Treg stability that results in altered cytokine production.

This mouse model of skin inflammation suggests that the presence of STAT3 GOF in non-hematopoietic cells of the skin influences disease, highlighted by the lack of spontaneous disease in STAT3 GOF → WT BM chimeras, and no change in epidermal thickness despite

more severe IMQ-induced inflammation. This is consistent with the findings of high levels of STAT3 activity in keratinocytes from psoriatic lesions in patients (213, 214). In mice, overexpression of STAT3 in keratinocytes led to spontaneous and wound-induced skin inflammation resembling psoriasis (213). We unexpectedly found that STAT3 GOF bone marrow incompletely engrafts into WT recipients; however, this mixed chimera still developed spontaneous ear swelling as the mice aged. Finally, severe IMQ-induced disease could not be initiated in the absence of adaptive immune cells (*Rag1*$^{-/-}$). Together, these results indicate that the combination of STAT3 GOF in both CD4^{+} T cells and skin epithelial cells can drive the most significant disease. These findings contrast with a recent study demonstrating deletion of *Stat3* in keratinocytes diminished IMQ-induced skin inflammation, but deletion of *Stat3* in T cells had no effect (215). However, disease driven by STAT3 GOF is distinct from IMQ-induced inflammation in WT mice, as we have discovered that STAT3 GOF-driven disease is not dependent on γδ T cells and elicits a strong Th17 response. This could be due to the unique inflammatory environment caused by baseline lymphoproliferation in STAT3 GOF mice. Overall, these data demonstrate that STAT3 signaling in both hematopoietic and non-hematopoietic cells should be considered when designing therapies for patients.

STAT3 GOF patients present with early-onset poly-autoimmunity, ranging from enteropathy to skin disease (174). The current best therapies available include JAK inhibitors and IL-6R antagonists, which target multiple cell types (174). Our results show that JAK inhibition by tofacitinib, which targets JAK1 and JAK3, does not affect the enhanced Th17 response in STAT3 GOF despite reducing skin inflammation overall. Two major cytokine receptors involved in Th17 differentiation are IL-6R and IL-23R, which rely on JAK1/JAK2/TYK2 (160) and

JAK2/TYK2 (216), respectively. It is therefore likely that IL-6 or IL-23 signaling still occurs in T cells even in the presence of tofacitinib. Our data suggest that tofacitinib may have a greater effect on STAT3 GOF skin epithelial cells, as we have observed decreased epidermal thickness and increased proliferation by Ki-67 staining. The receptor for IL-22 is expressed mainly on non-hematopoietic cells and signals through JAK1/TYK2, therefore inhibition of this pathway by tofacitinib could explain the diminished epidermal cell proliferation and neutrophil recruitment (217, 218). In addition to JAK inhibitors, other therapies for STAT3 GOF include HSCT, although this has yielded mixed results and is likely confounded by the fact that transplantation tends to be performed later in disease when patients have significant comorbidities (174). Our finding of incomplete engraftment in WT → STAT3 GOF BM chimeras, despite all recipients receiving the same conditioning, may provide insight into the relatively low efficacy of HSCT in STAT3 GOF patients. Possible explanations include competitive advantage in the survival of STAT3 GOF hematopoietic cells or an alteration of radiosensitivity that is caused by STAT3 GOF (116, 219). Collectively, our data may highlight a new approach to HSCT that includes treatment with a JAK inhibitor.

STAT3 GOF is a complex monogenic disease that leads to lymphoproliferation and organ-specific autoimmunity in pediatric patients with a highly variable clinical phenotype. The findings here demonstrate local inflammatory responses mediated by $CD4^+$ Th17 cells and production of IL-22 can drive skin disease, which is most prominent when STAT3 GOF is also present in skin epithelial cells. While $CD8^+$ T cells have been implicated in other aspects of STAT3 GOF-related disease, this work highlights the need to investigate local immune responses in this and other immune dysregulation syndromes, and provides new insight into the

differences in the drivers of inflammation that likely depend upon the specific organ and cell type, genetic variables, and environment.

2.5 Materials and Methods

Mice

STAT3 GOF (p.G421R) mice were generated as previously described on a C57BL/6 background (19). p.T716M mice were generated on the C57BL/6 background using CRISPR-Cas9 technology by the Hope Center Transgenic Vectors Core at Washington University School of Medicine. *Il22*$^{-/-}$ mice were generously provided by the laboratory of Misty Good at University of North Carolina at Chapel Hill (220). *Rag1*$^{-/-}$ (JAX stock #002216), TCRδ deficient mice (*Tcrd*$^{-/-}$, JAX stock #002120), and IL-17 GFP reporter mice (*Il17*$^{atm1Bcgen/J}$, JAX stock #018472) all on the C57BL/6 background were purchased from Jackson Laboratories. C57BL/6 congenic for the Ly5.1 marker (B6.SJL-*Ptprc*^{a}*Pepc*^{b}/BoyCrCrl, strain #564) were purchased from Charles River. Male and female littermates aged 6-12 weeks were used in all experiments unless stated otherwise.

In vitro Th17 polarization

CD4^{+} T cells were enriched from the spleen and lymph nodes using the MojoSort™ Mouse CD4^{+} T Cell Isolation Kit (Biolegend). Naïve CD4^{+} T cells (CD3e^{+} CD25^{-} CD4^{+} CD44^{-} CD62L^{+}) were sorted on a BD FACS AriaFusion flow cytometer (BD Biosciences). 2x10^{5} sorted cells were cultured for 4 days on a 96-well flat bottom plate at 37ºC in complete RPMI 1640 medium supplemented with 10% FBS, 1% penicillin/streptomycin, and 50 μM beta-

mercaptoethanol. Cells were stimulated with 10 ug/mL plate-bound anti-CD3 (clone 145-2C11, BioXCell) and 2 ug/mL anti-CD28 (clone 37.51, BioXCell). For Th17 polarization, the culture medium included 5 ng/mL rmTGF-β1 (Cell Signaling Technology), 10 μg/mL anti-IFNγ (clone: XMG1.2, BioXCell), 10 μg/mL anti-IL-4 (clone: 11B11, BioXCell), with or without rmIL-6 (Peprotech).

Imiquimod-induced skin inflammation

To induce psoriasiform dermatitis in mice, 5% imiquimod (IMQ) cream (Taro) was applied to the dorsal and ventral sides of both ears for 7 consecutive days. Mice received approximately 5 mg IMQ daily. Ear thickness was measured daily with a 0.01mm dial thickness gauge (Peacock) by a single blinded scorer, calculated by the average of 3 separate measurements of the right ear.

$CD8^+$ T cell depletion

For $CD8^+$ T cell depletion experiments, mice were treated daily with 150 μg anti-CD8a (clone: 53-6.7, BioXCell) by intraperitoneal (i.p.) injection, starting 24 hours prior to the first IMQ treatment. $CD8^+$ T cell depletion was verified by flow cytometry.

Histology

For histological analysis of the mouse skin, ears were isolated at time of euthanasia and preserved overnight in 4% paraformaldehyde prior to paraffin embedding and hematoxylin and eosin staining (Digestive Diseases Research Cores Center, Washington University School of Medicine or Nationwide Histology, Missoula, MT). Whole slides were imaged with an Olympus BX61VS slide scanner microscope. Average epidermal thickness, blood vessel area, and cellular

infiltrate count were measured from 3 representative areas of each slide by a single blinded scorer using Qupath-0.3.1 software. Pathological score was determined by calculating the sum of the average epidermal thickness/10 + average blood vessel area/1000 + average cellular infiltrate count/100.

Skin tissue processing for flow cytometry

Dorsal and ventral halves of ears were separated, minced, and placed in 1.5 mL RPMI 1640 (Corning) supplemented with 1% Antibiotic-Antimycotic (Gibco), 1% MEM Nonessential Amino Acids (Corning), 1 mM sodium pyruvate (Corning), 25 mM HEPES Buffer (Corning), and 50 µM beta-mercaptoethanol. Ear tissue was then digested with 125 µg/mL LiberaseTL (Roche) and 100 µg/mL DNase I (Roche) on a rotating shaker for 90 minutes. Tissue was gently homogenized by passing through a syringe, then filtered through a 70 µm cell strainer to obtain single cell suspensions.

Flow Cytometry

Cells from the skin, cervical lymph nodes, whole blood, and spleen were isolated and stained as indicated. For surface staining, cells were washed with cell staining buffer (1X DPBS [Corning] + 2% FBS + 1 mM EDTA), treated with Fc block (clone 2.4G2), stained with surface antibodies for 30 minutes at 4°C, then washed again with cell staining buffer. For intranuclear staining, cells were fixed for 1 hour at room temperature with the eBioscience™ Foxp3 / Transcription Factor Staining Buffer kit and washed with permeabilization buffer (ThermoFisher Scientific). Cells were stained with intranuclear antibodies for 1 hour at room temperature, then washed with cell staining buffer. For intracellular staining, cells were stimulated with 5 ng/mL PMA (Sigma) and 0.5 µM ionomycin (Sigma) with GolgiPlug (BD) for 4 hours at 37°C + 5%

CO_2 prior to surface staining. Cells were fixed and permeabilized with the BD Cytofix/Cytoperm solution (BD Biosciences), washed with permeabilization buffer (ThermoFisher Scientific), then stained for intracellular antibodies for 30 minutes at 4°C. Dead cells were distinguished by Zombie Yellow (Biolegend). For GFP expression analysis, unstimulated cells were pre-fixed with 1% paraformaldehyde at 37ºC for 10 minutes before proceeding to fixation and permeabilization. Flow cytometry was performed on a LSRFortessa (BD Biosciences) or Cytek Aurora, and data were analyzed using FlowJo 10.7.1. The following fluorochrome-conjugated antibodies were used: anti-mouse CD3ε (145-2C11, Biolegend), anti-mouse CD62L (MEL-14, Biolegend), anti-mouse IL-22 (Poly5164, Biolegend), anti-mouse/human CD44 (IM7, Biolegend), anti-mouse FOXP3 (FJK-16s, Invitrogen), anti-mouse CD4 (GK1.5, Biolegend), anti-mouse CD45.2 (104, BD Biosciences), anti-mouse CD19 (6D5, Biolegend), anti-mouse CD8a (53-6.7, BD Biosciences), anti-mouse NK-1.1 (PK136, Biolegend), anti-mouse CD64 (X54-5/7.1, Biolegend), anti-mouse RORγt (Q31-378, BD Biosciences), anti-mouse IL-17A (TC11-18H10, BD Biosciences), anti-mouse/human CD11b (M1-70, Biolegend), anti-mouse CD11c (HL3, BD Biosciences), anti-mouse I-Ab (AF6-120.1, Biolegend), anti-mouse IFNγ (XMG1.2, BD Biosciences), anti-mouse γδ T-Cell Receptor (GL3, BD Biosciences), anti-mouse Ly-6G (1A8, Biolegend), anti-mouse CD196 (29-2L17, Biolegend), anti-mouse CD24 (M1/69, BD Biosciences), anti-mouse CD25 (PC61, BD Biosciences), anti-mouse IL-4 (11B11, BD Biosciences), anti-mouse GATA3 (L50-823, BD Biosciences), anti-mouse T-Bet (4B10, BD Biosciences).

Bone Marrow chimeras

Bone marrow chimeras were generated by transplanting whole bone marrow cells from 7-12 week old CD45.2^{+} donors into CD45.1^{+} recipients that were irradiated with 2 doses of 550 cGy. 2x10^{6} cells were injected retro-orbitally into lethally irradiated recipients. Recipients received 0.5 mg/mL sulfamethoxazole and 0.1 mg/mL trimethoprim in drinking water *ad libitum* for 2 weeks post-transplant. Engraftment was assessed in the peripheral blood at 6, 8, 10, and 12 weeks post-transplant. IMQ experiments were started 12 weeks post-transplant.

Single Cell RNA sequencing and TCR sequencing

Bulk T cells were isolated from the cervical lymph nodes of 2 untreated and 2 IMQ-treated WT and STAT3 GOF littermates (1 male and 1 female per group) using the EasySep Mouse T cell Isolation Kit (StemCell) following the manufacturer's instructions. 1 x10^{6} purified T cells were labeled with anti-mouse CD3e (C0182), TCRβ (C0120), CD4 (C0001), CD8a (C0002), CD44 (C0073), and CD62L (C0112) Totalseq-C antibodies (Biolegend) for CITE-seq (194). The 5' GEX v2 libraries were generated and dual indexed with VDJ and surface immune receptor sequencing following the manufacturer's instructions, loading 30,000 cells per well (10X Genomics). Libraries were sequenced and processed by the Genome Technology Access Center at the McDonnell Genome Institute at Washington University School of Medicine following the manufacturer's instructions (10X Genomics). Raw data was filtered, aligned, and aggregated using Cellranger v.6.0.0 and feature-barcode matrix analysis was performed in Rstudio with the Seurat v4 R package. A total of 135825 cells were filtered based on mitochondrial content and feature count. Cells with >20% mitochondrial DNA were filtered from analysis, leaving a total of 135567 cells. Normalization, scaling, and dimensionality reduction were performed using default parameters, with resolution at 0.8 and 30 dimensions for

the reduction. For CITE-seq, RNA and protein data were integrated using weighted nearest neighbor analysis (195). The CellSelector function was used to separate single-positive CD4 and CD8 populations based on surface protein expression, and the $CD4^+$ and $CD8^+$ populations were renormalized using the same procedure. Non-T cell contaminants were removed from analysis. Clusters were manually annotated based on CD44 and CD62L surface protein expression and by expression of canonical genes found in literature. Differential gene expression was calculated using the 'FindMarkers' function and the default Wilcoxon rank-sum test. Seurat, EnhancedVolcano, ggplot2, and dittoseq R packages were used for data visualization. T cell clonotypes were assigned and clonotype dynamics were analyzed using the ScRepertoire R package (221). Clonotypes were assigned using the 'cloneCall=strict' function, which integrates the VDJC genes of the TCR and the nucleotide sequence of the CDR3 region to define clonotype.

Adoptive transfer

IL-17 GFP WT reporter mice and IL-17 GFP STAT3 GOF mice were treated with IMQ for 7 consecutive days. On day 7, cervical lymph nodes and spleen were isolated and $CD4^+$ T cells purified using the MojoSort™ Mouse $CD4^+$ T Cell Negative Isolation Kit (Biolegend). Purified $CD4^+$ T cells were >95% pure by flow cytometry. $5x10^6$ cells were injected into the tail veins of sex-matched *Rag1*$^{-/-}$ mice. 24 hours after transfer, *Rag1*$^{-/-}$ recipients were treated with IMQ for 7 consecutive days.

Oral tofacitinib treatment

Tofacitinib citrate (MedChemExpress) was delivered in chow formulation by combining Nutra-gel dry mix kit (Bio-serv) with tofacitinib at a ratio of 1g tofacitinib to 1kg Nutra-gel. Briefly, Nutra-gel was prepared according to manufacturer's instruction. Tofacitinib powder was added to the heated water and dry powder mixture, then mixed in a blender for 20-30 seconds. Nutra-gel with and without tofacitinib was stored at 4°C until use for up to 7 days. Mice were given Nutra-gel chow without drug for 3 days to acclimate to the diet before starting tofacitinib treatment. Mice received fresh Nutra-gel daily (with or without tofacitinib) with *ad libitum* access.

Statistics

Prism 9 was used to calculate all statistics. A 2-tailed unpaired t test or Welch's t test was used for comparisons of 2 groups. For comparisons of 4 groups, samples were analyzed by one-way ANOVA with Šídák's multiple comparison test. Percent change in ear thickness was analyzed by two-way repeated measures ANOVA with Šídák's multiple comparisons test. For comparisons of 3 or more groups with 2 independent variables, two-way ANOVA with Šídák's multiple comparisons test was used.

2.6 Supplementary Figures

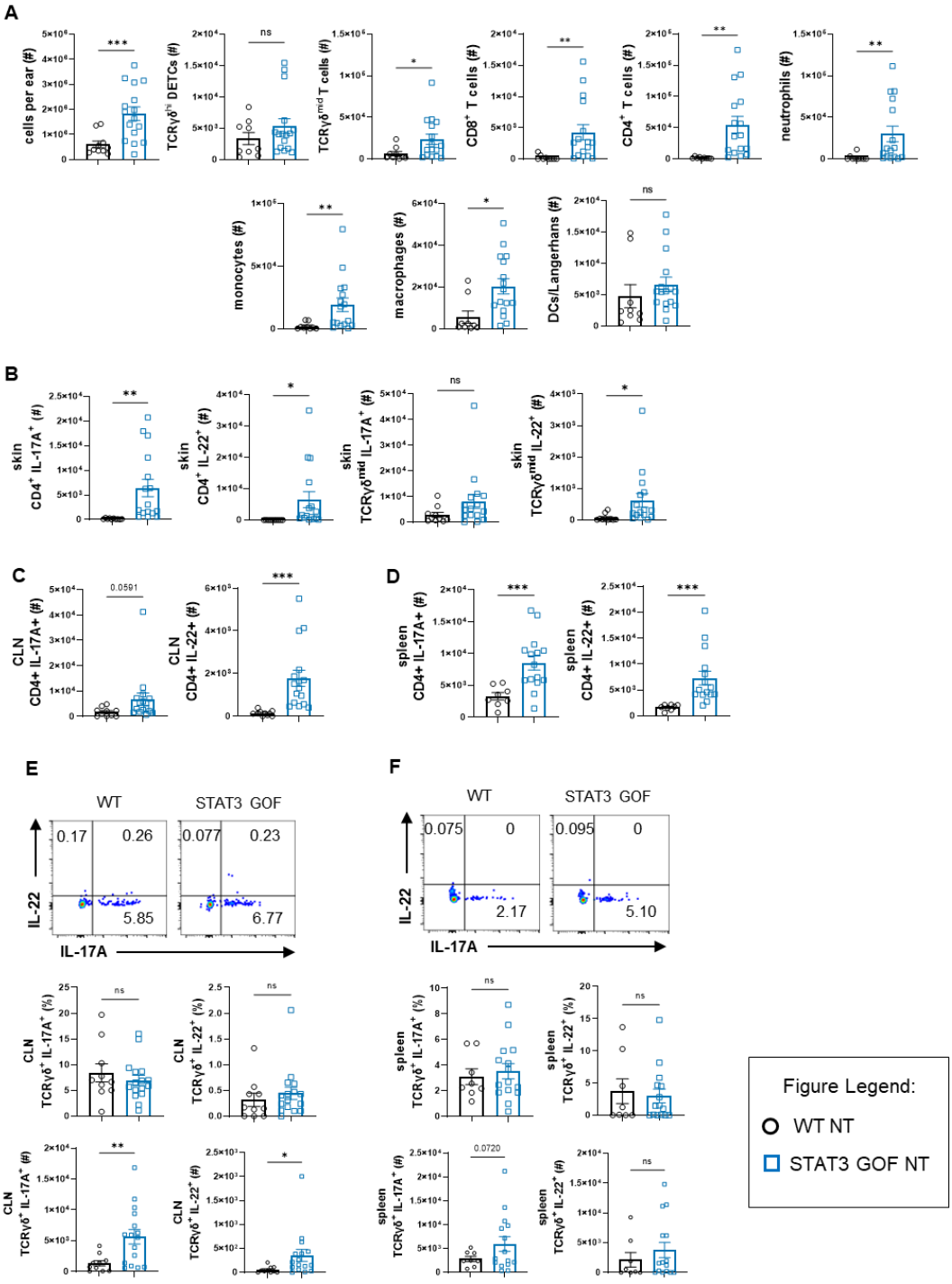

Figure S2.1: Older adult STAT3 GOF (p.G421R) mice develop spontaneous skin inflammation. (A) Absolute cell numbers of cells per ear and of skin immune cells of older adult WT and STAT3 GOF mice. (B) Absolute cell numbers of CD45.2$^+$ CD3$^+$ TCRγδ$^-$ CD8$^-$ CD4$^+$ IL-17A$^+$ and IL-22$^+$ skin, (C) CLN, and (D) spleen T cells of older

adult WT and STAT3 GOF mice. Cytokine expression assessed after PMA/ionomycin stimulation. (E) Representative flow plots, quantification, and absolute cell number of IL-17A, and IL-22 expression in CD45.2^{+} CD3^{+} TCRγδ^{+} T cells in the CLN and (F) spleen. Cytokine expression assessed after PMA/ionomycin stimulation. Data are presented as mean ± SEM. Statistical significance determined by unpaired two-tailed or Welch's t-test, *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001. Data represent 3 experiments with 10-16 mice.

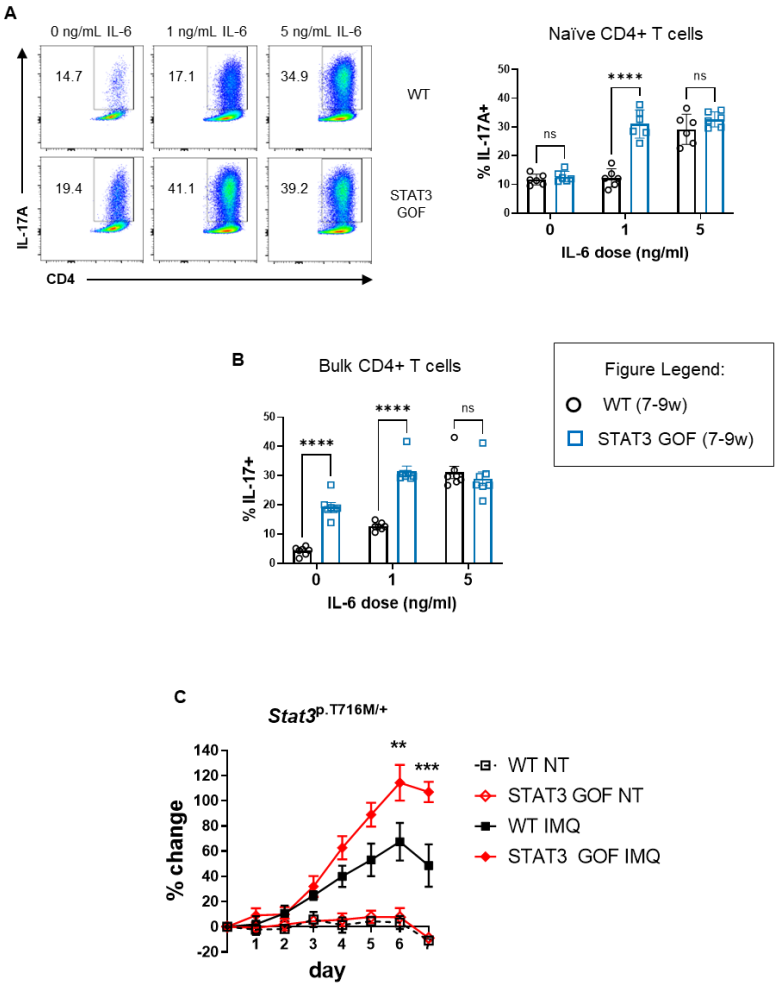

Figure S2.2: Th17 polarization of naïve and bulk CD4^{+} T cells (p.G421R) and percent change in ear thickness during IMQ treatment (p.T716M). (A) Representative flow plot and quantification of IL-17A following Th17 polarization of naïve CD4^{+} T cells with varying concentrations of IL-6. (B) Quantification of Th17 polarization of bulk splenic CD4^{+} T cells with varying concentrations of IL-6. (C) Adult WT and *Stat3*$^{p.T716M/+}$ littermates were treated with topical 5% imiquimod cream on both ears for 7 consecutive days. Percent change in ear thickness during course of IMQ treatment relative to baseline. Data are presented as mean ± SEM. Statistical significance determined by two-way ANOVA with Šídák's multiple comparisons test (A-B), or 2-way repeated measures ANOVA with Šídák's multiple comparisons test (C), *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001. Data represent 2 experiments with 6 mice/group (A), 2 experiments with 4 mice/group (B), or 2 experiments with 4-7 mice/group.

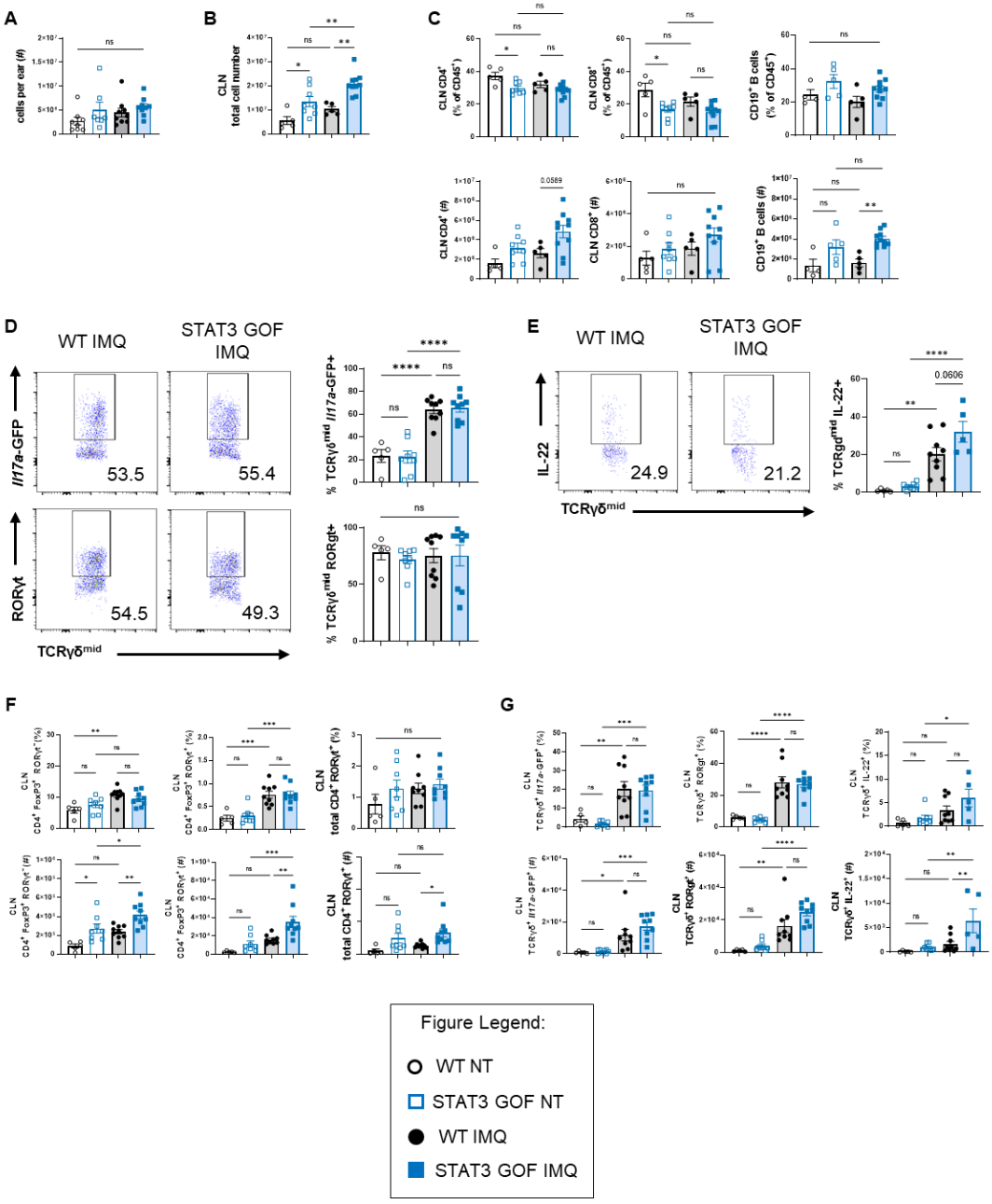

Figure S2.3: Topical imiquimod elicits severe skin inflammation and a Th17 response in young adult STAT3 GOF mice. (A) Total number of cells per ear. (B) Total number of CLN cells. (C) Frequency and total number of lymphocyte populations in CLN. (D) Representative flow plots and frequency of CD45.2$^+$ CD3$^+$ TCRγδ^{mid} *Il17a*-GFP$^+$, RORγt$^+$, and (E) IL-22$^+$ populations in skin. IL-22 expression assessed after PMA/ionomycin stimulation. (F) Frequency and total number of CD45.2$^+$ CD3$^+$ TCRγδ$^-$ CD4$^+$ FoxP3$^+$ RORγt$^-$, FoxP3$^+$ RORγt$^+$, and total RORγt$^+$ populations in CLN. (G) Frequency and number of CD45.2$^+$ CD3$^+$ TCRγδ$^+$ RORγt$^+$, *Il17a*-GFP$^+$, or IL-22$^+$ populations in CLN. IL-22 expression assessed after PMA/ionomycin stimulation. Data represent 4 experiments with 8-12 mice (A), 4 experiments with 5-10 mice (B-C), or 3 experiments with 5-9 mice (E-G) Statistical significance determined by 1-way ANOVA with Šídák's multiple comparisons test. *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001.

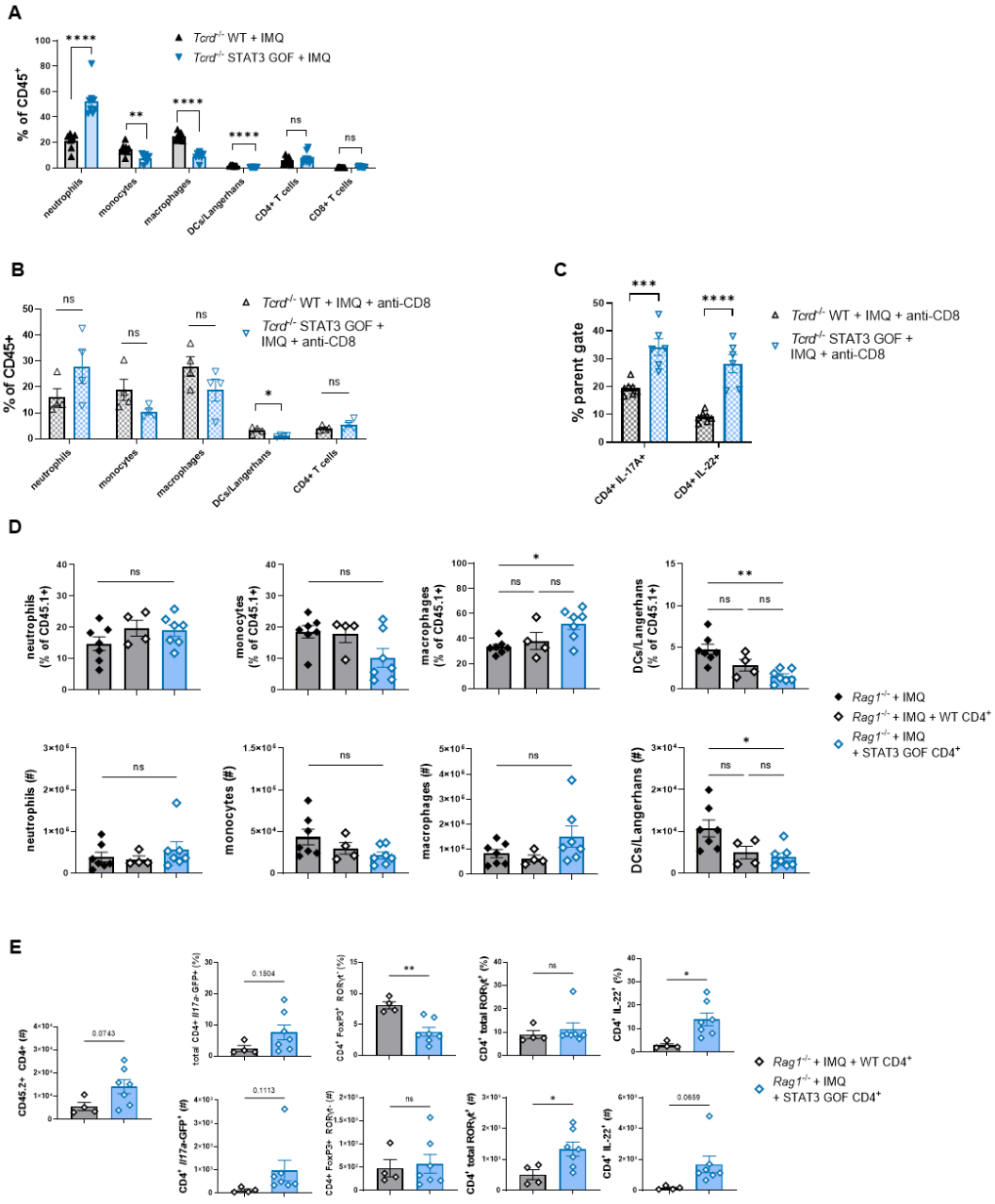

Figure S2.4: CD4$^+$ T cells are sufficient, and γδ T cells and CD8$^+$ T cells are not required to mediate IMQ-induced inflammation in STAT3 GOF mice. (A) Quantification of skin immune cell frequencies of *Tcrd*$^{-/-}$ mice treated with IMQ. (B) Quantification of skin immune cell frequencies of *Tcrd*$^{-/-}$ mice treated with IMQ + anti-CD8. (C) Quantification of Th17 cytokine frequencies in CD45.2$^+$ CD3$^+$ TCRγδ$^-$ CD8$^-$ CD4$^+$ T cells of *Tcrd*$^{-/-}$ mice treated with IMQ + anti-CD8. (D) Quantification of skin immune cell frequencies of *Rag1*$^{-/-}$ control or adoptive transfer recipients treated with IMQ. (E) Quantification of total adoptively transferred CD45.2$^+$ CD4$^+$ T cells and transcription factor/cytokine frequencies of in transferred CD4$^+$ T cells found in skin. Data are presented as mean ± SEM. Statistical significance determined by unpaired two-tailed or Welch's t-test (A-C, E) or 1-way ANOVA with Šídák's multiple comparisons test. Data represent 3 experiments with 5-7 mice.

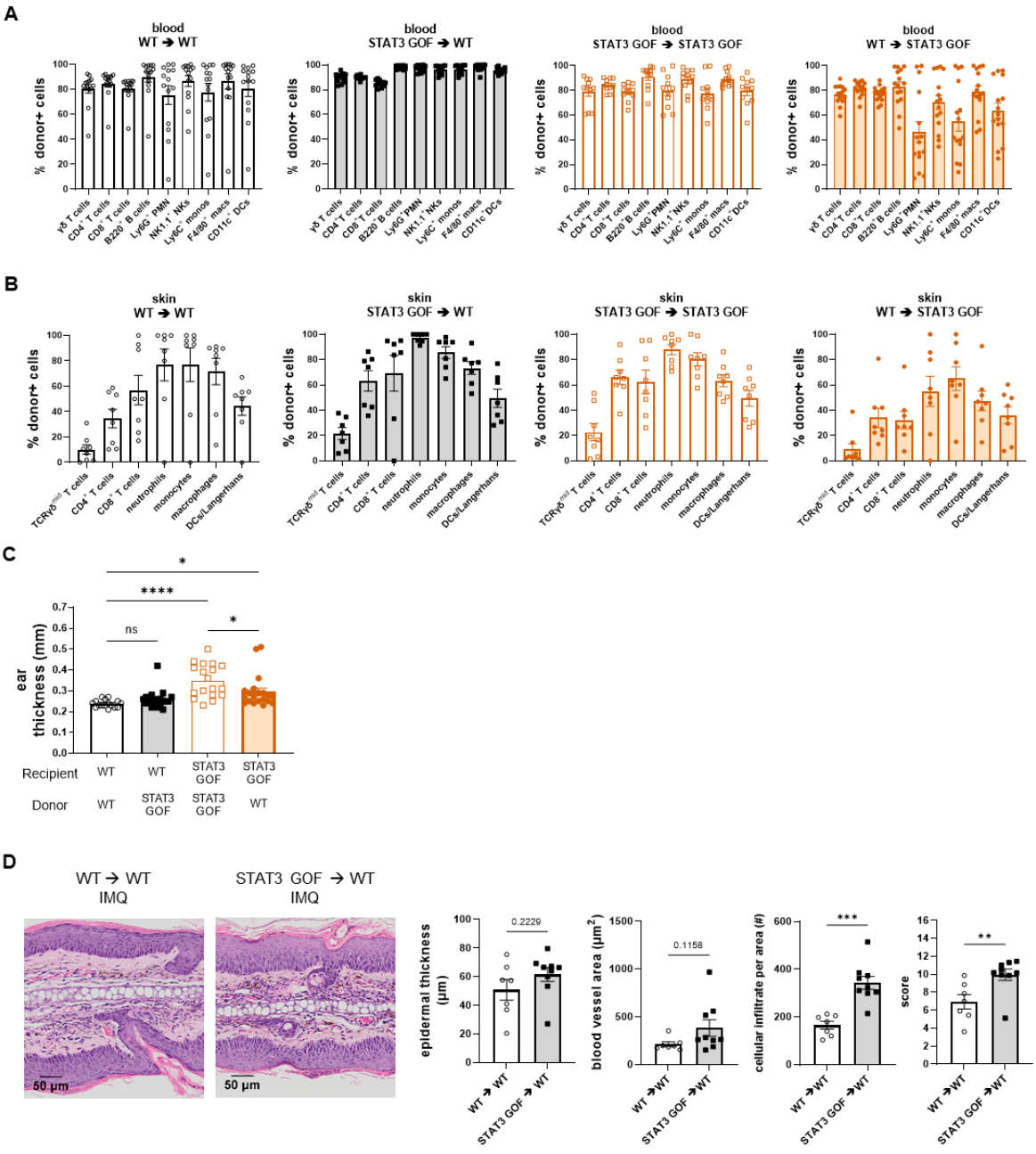

Figure S2.5: STAT3 GOF bone marrow can exacerbate IMQ-induced inflammation in WT mice. Bone marrow from WT or STAT3 GOF (Ly5.1) was transplanted into irradiated WT (Ly5.2) recipients. Mice were allowed to reconstitute for 12 weeks post transplant. (A) Peripheral blood and (B) skin were analyzed for engraftment at 12 weeks post transplant in untreated mice. (C) Ear thickness of untreated bone marrow chimeras 12 weeks post transplant. (D) Histological measurements from H&E stained sections of ear tissue of IMQ-treated WT → WT or STAT3 GOF → WT chimeras. Data are presented as mean ± SEM. Statistical significance determined by 1-way ANOVA with Šídák's multiple comparisons test (C) or unpaired two-tailed or Welch's t-test (D). Data represent 4 experiments with 8-12 mice per group.

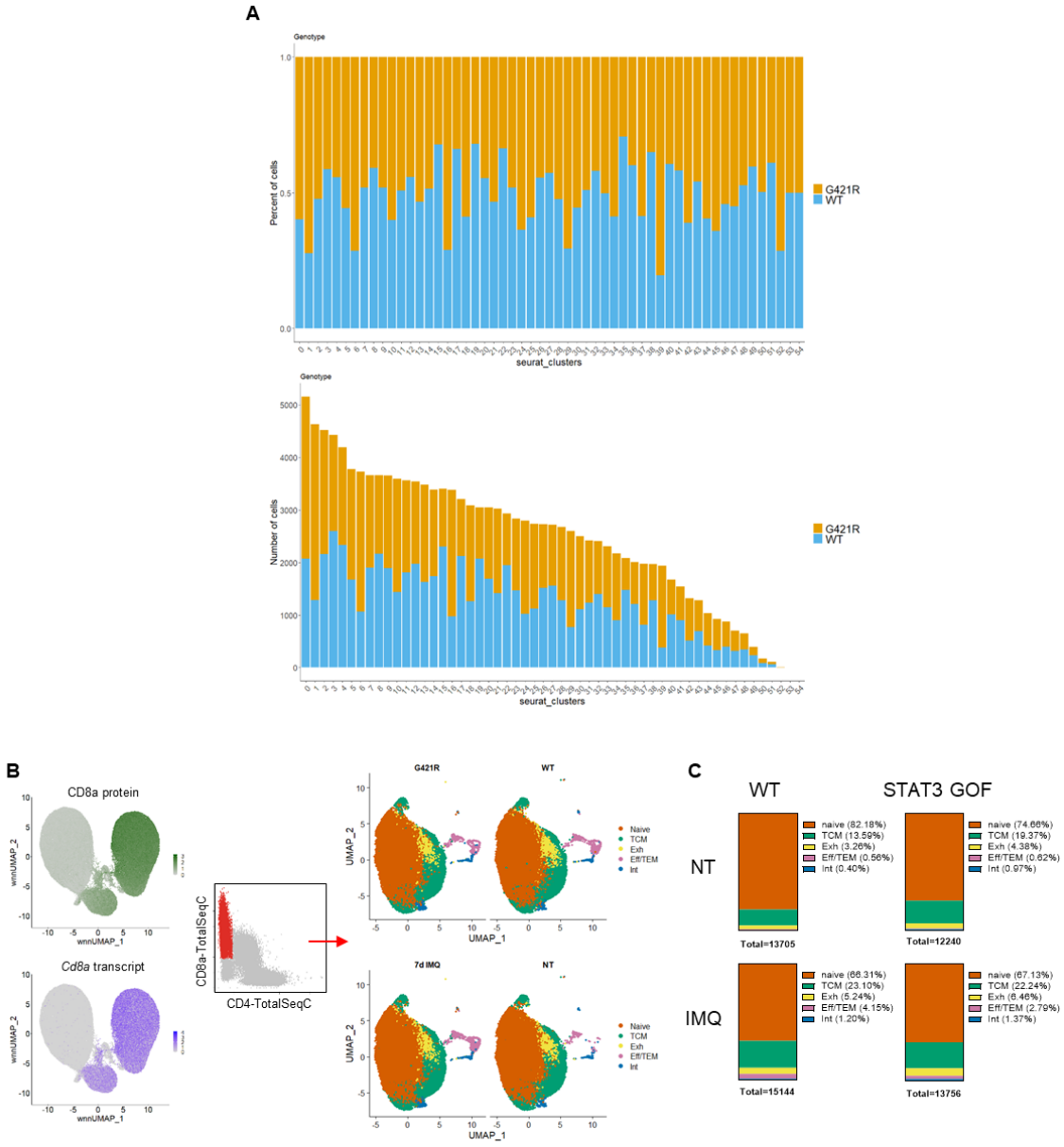

Figure S2.6: STAT3 GOF T cell single-cell RNA-Seq after IMQ treatment. (A) Percent (top) and number (bottom) of WT or STAT3 GOF cells in each cluster. (B) Cellselector function was used to separate single positive CD8 T cells, which were renormalized and reclustered based on CD44 and CD62L surface protein expression and canonical gene expression. (C) $CD8^{+}$ T cell cluster frequencies based on genotype and treatment.

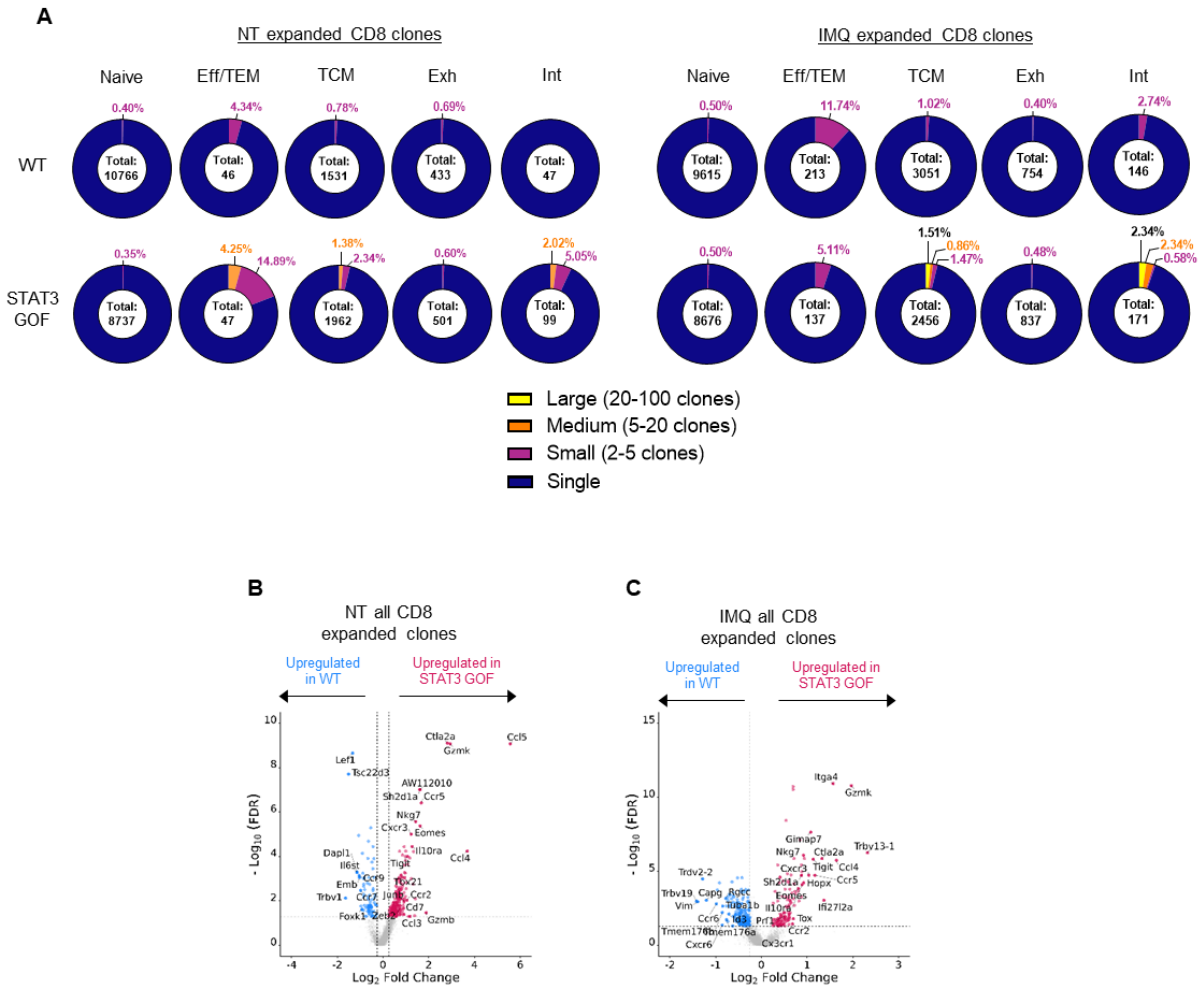

Figure S2.7: STAT3 GOF CD8$^+$ T cells show increased clonal expansion at baseline and after IMQ. (A) Clonotype frequency of the NT and IMQ treated CD8$^+$ T cells by annotated cluster generated with ScRepertoire. (B) Volcano plots showing differential expression (adjusted P < 0.05, average log2 fold change > 0.25 or < –0.25) in NT CD8$^+$ expanded clones and (C) IMQ CD8$^+$ expanded clones, comparing WT and STAT3 GOF. Ribosomal genes, genes ending in –Rik, and genes beginning with Gm- are not labeled on volcano plots.

Table S2.1: scRNA-seq annotated cluster signatures

T cell	Cluster Annotation	Surface Antibody Expression	Canonical Genes Expressed
CD4	Naïve	CD62L hi, CD44 low	*Ccr7, Tcf7, Lef1, Sell, Igfbp4, Il7r, S1pr1, Klf2*
CD4	Eff/TEM	CD62L low, CD44 hi	*Id2, Tbx21, Prdm1, Zeb2, Stat4, Igals1, S100a4, Itgb1, Itgb7, Il18r1, Klrg1, Cx3cr1, S1pr5, Tnf, Ifng, Il17a, Il17f, Il22, Il13, Il4, Gata3, Bcl6*
CD4	TCM	CD62L mid/hi, CD44 low/mid	*Ccr7, Sell, Il7r, Tcf7, Cxcr3, Bcl2, Il2rb, Cd27, Bach2, Eomes, Id3, Bcl6, Foxo1, Zeb1, S1pr1, Klf2*
CD4	Exh	CD44 mid/hi	*Pdcd1, Havcr2, Eomes, Tox, Tigit, Cd39, Ctla4, Lag3, Cd200, Hif1a, Tbc1d4*
CD4	Treg	CD44 mid/hi	*Foxp3, Il2ra, Il2rb*
CD8	Naïve	CD62L hi, CD44 low	*Tcf7, Lef1, Ccr7, Igfbp4, Nsg2, Slfn5, Cnr2, Cxcr6, Klf2, S1pr4, S1pr1, Cd27, Il7ra, Bcl2*
CD8	Eff/TEM	CD62L low, CD44 hi	*Klrg1, Prdm1, Eomes, Tbx21, Ctla4, Ifng, Il2, Hif2a, Prf1, Bhlhe40, Il12rb2, Id2, Zeb2, Klre1, S1pr6, Cxcr3, Cx3cr1, Itgam, Il2ra, Itgal, Ly6c1, Il2rb, Il15ra, Ccr5, Tnf, Gzm*
CD8	TCM	CD62L mid/hi, CD44 low/mid	*Ccr7, Il7ra, Tcf1, Il2rb, Cxcr3, Cd27, Eomes, Bcl6, Stat3, Id3, Nsg2, Slfn5, Cnr2, Cxcr6, Klf2, S1pr4, S1pr1, Bcl2, Foxo3, Fasl, Itgal, Ly6c1, Il15ra, Lef1*
CD8	Exh	CD44 mid/hi	*Pdcd1, Ctla4, Tox, Tigit, Eomes, Lag3, Cd39, Fasl*
CD8	Int	CD62L hi/low, CD44 hi/low	Memory and effector genes

Chapter 3: Conclusions and Future Directions

STAT3 GOF syndrome is an IEI characterized by early-onset autoimmune disease and lymphoproliferation. Our knowledge of how STAT3 GOF alters the immune system comes mostly from the peripheral blood, but the effects of STAT3 GOF in specific tissues has not been fully investigated. We and others have used mouse models of STAT3 GOF to study the mechanisms behind the development and progression of autoimmunity in patients, allowing for the study of local immune responses. The goal of my dissertation is to identify the cell types that drive skin disease in STAT3 GOF, explore how STAT3 GOF changes functionality of these cells, and determine if these altered functions could be targeted for therapy. This chapter discusses how my results fit in with our current understanding of STAT3 GOF syndrome, the implications for future treatments, and the questions that still remain.

3.1 Tissue-specific T cell dysregulation in STAT3 GOF

Immune dysregulation in patients with STAT3 GOF syndrome is commonly associated with lymphoproliferation; however, patients present with different clinical manifestations in multiple tissues. This suggests that aberrant responses from lymphocytes, including T cells, play a role in the development of multi-organ autoimmunity (182). The role of STAT3 in the development of Th17 and the inhibition of Treg differentiation would suggest that dysregulation of these two T cell subsets can drive disease. However, not all patients with STAT3 GOF syndrome displayed aberrant Th17 or dysfunctional Treg responses in the peripheral blood as would be expected (174). We hypothesized that T cell dysregulation in STAT3 GOF would lead to aberrant tissue-specific responses that lead to autoimmunity.

We observed that STAT3 GOF mice with the p.G421R variant developed spontaneous skin inflammation as they aged. Analysis of T cell subsets in the skin revealed increased expression of Th17 markers including IL-17A, RORγt, and IL-22 in $CD4^+$ T cells of older adult STAT3 GOF mice compared to WT. Increased IL-22^+, but not IL-$17A^+$ $CD4^+$ T cell frequencies were also observed in the draining lymph nodes, and the spleen. A similar phenotype could be induced by IMQ, in which case an increased Th17 response occurred in local STAT3 GOF tissues, but not WT. Furthermore, IMQ-treated WT mice had increased Treg induction into the skin, which did not occur in STAT3 GOF mice. We found that STAT3 GOF $CD4^+$ T cells were sufficient to induce severe skin inflammation with IMQ treatment, and that γδ T and $CD8^+$ T cells were not required. Within the draining lymph nodes of IMQ treated mice, we found upregulation of Th17 genes (*Il17a*, *Il17f*, *Il22*, *Ccr6*, *Rora*) in STAT3 GOF effector $CD4^+$ T cells and in contrast, upregulation of Treg-related genes (*Ikzf2*, *Il2ra*, *Tgfb1*) in WT Tregs by single cell RNA sequencing. Among expanded T cell clones, there was upregulation of *Il22* and downregulation of *Foxp3*, *Il2ra*, and *Ikzf2* in STAT3 GOF. From these data, we conclude that T cell dysregulation in STAT3 GOF causes a pathogenic local Th17 response that is distinct from systemic responses and potentially alters Treg stability.

This conclusion is supported by other studies of STAT3 GOF mouse models that show T cell dysregulation in specific tissues. Our lab previously observed tissue-specific T cell dysregulation in the p.G421R variant, in which lymphoproliferation in older adult STAT3 GOF mice was unexpectedly associated with a Th1 phenotype in the spleen at baseline and in an adoptive transfer model of colitis (19). Two separate studies of the p.K392R variant mice showed Th1-skewing in the spleen as well (21, 22). However, analysis of inflamed tissues

showed alternative T cell responses. The p.K392R variant on the NOD background showed expansion of effector $CD8^+$ T cells infiltrating the pancreatic islets (21). Induction of experimental autoimmune encephalomyelitis (EAE) in p.K392R variant on the C57BL/6 background resulted in an expansion of the Th17 subset and a reduction of Tregs in the central nervous system (22). Our results contrast with previous reports of skin inflammation and T cell dysregulation in two other mouse models of STAT3 GOF, in which $CD8^+$ T cells were found to be the mediators of spontaneous lethal disease in mice homozygous for the p.K658N variant (20). However, the cellular phenotype within the skin was not examined in these mice, or in homozygotes of the p.T716M variant, which also developed spontaneous skin disease. To our present knowledge, ours is the first in-depth study of skin disease in STAT3 GOF. Taken together, our findings support the conclusion that the nature of T cell dysregulation in STAT3 GOF, even within the same variant, greatly depends on the tissue environment.

There are still questions regarding T cell dysregulation in STAT3 GOF that need to be addressed. While we observed spontaneous skin inflammation in STAT3 GOF mice as they aged, we did not determine the mechanisms required to initiate this response. Based on the results described in this dissertation, I hypothesize that $CD4^+$ T cell dysregulation, specifically of IL-22-secreting Th17, caused by STAT3 GOF initiates spontaneous skin disease. Monitoring *Rag1*$^{-/-}$ or *Il22*$^{-/-}$ STAT3 GOF mice for spontaneous skin inflammation would be useful initial experiments to support this hypothesis. A major caveat in designing experiments to test the requirement of Th17 for the initiation of spontaneous disease is that the plasticity and multiple subsets of $CD4^+$ T cells or shared effector cytokine profile with γδ T cells and innate lymphoid cells (ILCs) creates a challenge in targeting this subset for genetic deletion or depletion.

Additionally, we have yet to investigate STAT3 GOF pTreg dysregulation in the context of skin disease. Experiments with fate-mapping transgenic mouse models may be useful in helping us understand if STAT3 GOF can induce the loss of Treg identity and adoption of a Th17 phenotype, and if this affects Treg suppressor function in different inflammatory environments. Finally, we have not examined dysregulation of Tfh cells, which also require STAT3 signaling for identity and function. Future studies of STAT3 GOF in Tfh may shed light on the development of autoimmune cytopenias in patients through the induction of autoantibodies and autoreactive B cells (174).

3.2 STAT3 GOF in hematopoietic and non-hematopoietic cells

We have demonstrated that STAT3 GOF causes T cell dysregulation that results in increased IL-22 production in $CD4^+$ T cells. However, IL-22 signaling mostly occurs through non-hematopoietic cells (205). In the skin, IL-22 signaling through its receptor in keratinocytes activates STAT3 and the transcription of genes involved with proliferation and chemokine expression (122, 222). IL-22 signaling is also important in mediating skin inflammation in the IMQ model, driving epidermal hyperplasia and neutrophil recruitment (197). I hypothesize that STAT3 GOF T cell dysregulation in the context of skin disease drives the activation of STAT3 within non-hematopoietic cells, leading to the observed phenotype of increased epidermal thickness and neutrophil infiltration into the skin.

In our study, we found that STAT3 GOF in hematopoietic cells is sufficient to drive increased inflammation with IMQ treatment at 12 weeks post-transplant, and that the increased Th17 response is T cell-intrinsic. In these chimeras, we observed increased ear swelling and cell infiltration into the skin; however, this increase in IL-17A and IL-22 expression in STAT3 GOF $CD4^+$ T cells was not sufficient to cause increased epidermal thickness or spontaneous disease. This indicates that the response to IMQ in the epidermis could be due to STAT3 GOF activity in those cells. Indeed, STAT3 GOF → STAT3 GOF and WT → STAT3 GOF chimeras developed spontaneous ear swelling. However, WT → STAT3 GOF chimeras had on average incomplete engraftment of myeloid populations in the blood and most radio-sensitive cell types of the skin at 12 weeks post-transplant. Although the WT → STAT3 GOF chimera was mixed in nature, we can infer that STAT3 GOF in non-hematopoietic cells also plays a role in the signaling cascades that lead to spontaneous skin disease.

Other mouse models of STAT3 GOF have shown that pathogenic T cell defects are cell-intrinsic; however, the responses appear to be tissue-specific. A study from our lab showed that homozygous STAT3 GOF (p.G421R) bone marrow could induce lymphoproliferation and a Th1 response in the spleen of WT recipients, but spontaneous disease was not observed at 12 weeks post-transplant, unlike germline p.G421R homozygous mice, which died around the time of weaning (19). Homozygous STAT3 GOF (p.K658N or p.T716M) bone marrow induced spontaneous disease marked by lymphoproliferation, hair loss, and wasting disease in WT recipients on the *Rag1*$^{-/-}$ background (20). In this case, disease was prevented by $CD8^+$ T cell depletion (20). Finally, heterozygous STAT3 GOF (p. K392R) bone marrow accelerates the development of diabetes in WT recipients on the NOD background (21). These findings and the

results within this dissertation support our conclusion that STAT3 GOF causes tissue-specific, T cell-intrinsic defects, but cannot rule out a role for STAT3 GOF in non-hematopoietic cells responding to signals from dysregulated T cells in the development of spontaneous disease.

Additional studies are needed to understand how STAT3 GOF in specific cell types, both hematopoietic and non-hematopoietic, affects spontaneous skin inflammation. An important study that we were unable to perform is analyzing the effect of STAT3 GOF in specific cell types. These experiments would require an inducible transgenic model, which we were unable to acquire during this thesis project. While we demonstrated that STAT3 GOF in hematopoietic cells is sufficient to drive increased skin inflammation, our bone marrow chimeras were limited by the presence of radio-resistant cell types in the skin. Therefore, an inducible transgenic system (for example, expressing the STAT3 GOF variant in keratinocytes only) would be superior in determining which cell types are necessary for initiating skin disease. Furthermore, future studies should be directed toward understanding why WT bone marrow failed to engraft into STAT3 GOF recipients. Understanding the cause of incomplete engraftment would be extremely important for patients, as HCST has had limited success in treating STAT3 GOF syndrome. Generation of an intentional 1:1 mixed bone marrow chimera, combining WT and STAT3 GOF donor cells to transplant into a WT recipient, would help us determine if STAT3 GOF confers a competitive advantage in hematopoietic cells.

3.3 Cellular targets of JAK signaling inhibition

STAT3 signaling canonically requires phosphorylation by the JAK proteins associated with a given cytokine or growth factor receptor (6). JAK inhibitor therapy has successfully treated the symptoms of patients with STAT3 GOF syndrome (198). We have concluded that T cell dysregulation is a major driver of disease in STAT3 GOF, and that T cell responses are organ-specific. We have also concluded that STAT3 GOF in both hematopoietic and non-hematopoietic cells is important in driving skin inflammation. Therefore, it is important to understand what cells are affected by JAKinib treatment. To our present knowledge, the results described in this dissertation are the first analysis of the effects of JAK inhibition on immune cells in STAT3 GOF mice.

To determine if JAK inhibition could ameliorate IMQ-induced ear swelling in STAT3 GOF mice, we simultaneously treated STAT3 GOF and WT littermates with oral tofacitinib (a JAK1/3 inhibitor) in chow formulation and induced skin inflammation with topical IMQ. JAKinib treatment reduced ear swelling in both WT and STAT3 GOF mice, and this was associated with a reduction in epidermal thickness and neutrophil skin infiltration. We were surprised to find that JAK inhibition had no effect on IL-22 or IL-17A production by $CD4^+$ T cells in the skin, despite having shown that STAT3 GOF T cells are sufficient to drive increased skin inflammation and that the presence of IL-22 plays a large role in the differences in skin disease observed between WT and STAT3 GOF. These data led us to conclude that STAT3 signaling in Th17 is likely continuing through pathways that are not targeted by tofacitinib. Our

findings support the use of combination therapy that targets multiple cell types and pathways for patients with STAT3 GOF syndrome.

Additional studies are needed to determine if other JAK inhibitors can ameliorate T cell dysregulation, and in doing so, prevent other aspects of disease. Treatment with ruxolitinib, a JAK1/2 inhibitor also used to successfully treat STAT3 GOF syndrome, may be able to ameliorate Th17 dysfunction through the inhibition of IL-6R and IL-23R signaling (160, 216). Furthermore, long-term treatment of STAT3 GOF mice with tofacitinib would allow us to study the development of spontaneous disease, and whether uncontrolled Th17 dysregulation can still cause disease despite the likely inhibition of IL-22 signaling pathways in the skin.

3.4 Concluding Remarks

Although they are rare, IEI provide the opportunity to understand how a single gene influences the entire immune system. When the products of such genes are as widespread and pleiotropic as STAT proteins, we face the additional challenge of dissecting the interplay between immune and non-immune cells in multiple tissues. Mouse models of IEI allow us to take patient observations and explore their effects across the body without relying on a single tissue source or the confounding variables introduced by concurrent immunosuppressive treatment. Moreover, the effects of successful drug therapies on certain cell types and tissues can be studied more easily in mouse models, allowing for the fine-tuning of treatment regimens for patients. Collectively, my doctoral dissertation research provides evidence for organ-specific T

cell dysregulation and highlights a need for studies of local immune responses in the development of targeted therapies for autoimmunity in IEI.

References

1. Tangye, S. G., W. Al-Herz, A. Bousfiha, C. Cunningham-Rundles, J. L. Franco, S. M. Holland, C. Klein, T. Morio, E. Oksenhendler, C. Picard, A. Puel, J. Puck, M. R. J. Seppänen, R. Somech, H. C. Su, K. E. Sullivan, T. R. Torgerson, and I. Meyts. 2022. Human Inborn Errors of Immunity: 2022 Update on the Classification from the International Union of Immunological Societies Expert Committee. *J Clin Immunol* 42: 1473-1507.
2. Schmitt, E. G., and M. A. Cooper. 2021. Genetics of Pediatric Immune-Mediated Diseases and Human Immunity. *Annu Rev Immunol* 39: 227-249.
3. Amaya-Uribe, L., M. Rojas, G. Azizi, J. M. Anaya, and M. E. Gershwin. 2019. Primary immunodeficiency and autoimmunity: A comprehensive review. *J Autoimmun* 99: 52-72.
4. Choi, J., R. Fernandez, H. T. Maecker, and M. J. Butte. 2017. Systems approach to uncover signaling networks in primary immunodeficiency diseases. *J Allergy Clin Immunol* 140: 881-884.e888.
5. Ma, C. S., and S. G. Tangye. 2019. Flow Cytometric-Based Analysis of Defects in Lymphocyte Differentiation and Function Due to Inborn Errors of Immunity. *Front Immunol* 10: 2108.
6. Philips, R. L., Y. Wang, H. Cheon, Y. Kanno, M. Gadina, V. Sartorelli, C. M. Horvath, J. E. Darnell, Jr., G. R. Stark, and J. J. O'Shea. 2022. The JAK-STAT pathway at 30: Much learned, much more to do. *Cell* 185: 3857-3876.
7. Karczewski, K. J., L. C. Francioli, G. Tiao, B. B. Cummings, J. Alföldi, Q. Wang, R. L. Collins, K. M. Laricchia, A. Ganna, D. P. Birnbaum, L. D. Gauthier, H. Brand, M. Solomonson, N. A. Watts, D. Rhodes, M. Singer-Berk, E. M. England, E. G. Seaby, J. A. Kosmicki, R. K. Walters, K. Tashman, Y. Farjoun, E. Banks, T. Poterba, A. Wang, C. Seed, N. Whiffin, J. X. Chong, K. E. Samocha, E. Pierce-Hoffman, Z. Zappala, A. H. O'Donnell-Luria, E. V. Minikel, B. Weisburd, M. Lek, J. S. Ware, C. Vittal, I. M. Armean, L. Bergelson, K. Cibulskis, K. M. Connolly, M. Covarrubias, S. Donnelly, S. Ferriera, S. Gabriel, J. Gentry, N. Gupta, T. Jeandet, D. Kaplan, C. Llanwarne, R. Munshi, S. Novod, N. Petrillo, D. Roazen, V. Ruano-Rubio, A. Saltzman, M. Schleicher, J. Soto, K. Tibbetts, C. Tolonen, G. Wade, M. E. Talkowski, B. M. Neale, M. J. Daly, and D. G. MacArthur. 2020. The mutational constraint spectrum quantified from variation in 141,456 humans. *Nature* 581: 434-443.
8. Villarino, A. V., Y. Kanno, and J. J. O'Shea. 2017. Mechanisms and consequences of Jak-STAT signaling in the immune system. *Nat Immunol* 18: 374-384.
9. Luo, Y., M. Alexander, M. Gadina, J. J. O'Shea, F. Meylan, and D. M. Schwartz. 2021. JAK-STAT signaling in human disease: From genetic syndromes to clinical inhibition. *J Allergy Clin Immunol* 148: 911-925.
10. Hu, X., J. Li, M. Fu, X. Zhao, and W. Wang. 2021. The JAK/STAT signaling pathway: from bench to clinic. *Signal Transduct Target Ther* 6: 402.
11. O'Shea, J. J., and R. Plenge. 2012. JAK and STAT signaling molecules in immunoregulation and immune-mediated disease. *Immunity* 36: 542-550.
12. Aluri, J., and M. A. Cooper. 2021. Genetic Mosaicism as a Cause of Inborn Errors of Immunity. *J Clin Immunol* 41: 718-728.

13. Meraz, M. A., J. M. White, K. C. Sheehan, E. A. Bach, S. J. Rodig, A. S. Dighe, D. H. Kaplan, J. K. Riley, A. C. Greenlund, D. Campbell, K. Carver-Moore, R. N. DuBois, R. Clark, M. Aguet, and R. D. Schreiber. 1996. Targeted disruption of the Stat1 gene in mice reveals unexpected physiologic specificity in the JAK-STAT signaling pathway. *Cell* 84: 431-442.
14. Durbin, J. E., R. Hackenmiller, M. C. Simon, and D. E. Levy. 1996. Targeted disruption of the mouse Stat1 gene results in compromised innate immunity to viral disease. *Cell* 84: 443-450.
15. Qian, W., C. A. Miner, H. Ingle, D. J. Platt, M. T. Baldridge, and J. J. Miner. 2019. A Human STAT1 Gain-of-Function Mutation Impairs CD8(+) T Cell Responses against Gammaherpesvirus 68. *J Virol* 93.
16. Tamaura, M., N. Satoh-Takayama, M. Tsumura, T. Sasaki, S. Goda, T. Kageyama, S. Hayakawa, S. Kimura, T. Asano, M. Nakayama, H. Koseki, O. Ohara, S. Okada, H. Ohno, and M. Kobayashi. 2020. Human gain-of-function STAT1 mutation disturbs IL-17 immunity in mice. *Int Immunol* 32: 259-272.
17. Park, C., S. Li, E. Cha, and C. Schindler. 2000. Immune response in Stat2 knockout mice. *Immunity* 13: 795-804.
18. Steward-Tharp, S. M., A. Laurence, Y. Kanno, A. Kotlyar, A. V. Villarino, G. Sciume, S. Kuchen, W. Resch, E. A. Wohlfert, K. Jiang, K. Hirahara, G. Vahedi, H. W. Sun, L. Feigenbaum, J. D. Milner, S. M. Holland, R. Casellas, F. Powrie, and J. J. O'Shea. 2014. A mouse model of HIES reveals pro- and anti-inflammatory functions of STAT3. *Blood* 123: 2978-2987.
19. Schmitt, E. G., K. A. Toth, S. I. Risma, A. Kolicheski, N. Saucier, R. J. F. Berríos, Z. J. Greenberg, J. W. Leiding, J. J. Bleesing, A. Thatayatikom, L. G. Schuettpelz, J. R. Edwards, T. P. Vogel, and M. A. Cooper. 2022. A human STAT3 gain-of-function variant confers T cell dysregulation without predominant Treg dysfunction in mice. *JCI Insight* 7.
20. Masle-Farquhar, E., K. J. L. Jackson, T. J. Peters, G. Al-Eryani, M. Singh, K. J. Payne, G. Rao, D. T. Avery, G. Apps, J. Kingham, C. J. Jara, K. Skvortsova, A. Swarbrick, C. S. Ma, D. Suan, G. Uzel, I. Chua, J. W. Leiding, K. Heiskanen, K. Preece, L. Kainulainen, M. O'Sullivan, M. A. Cooper, M. R. J. Seppänen, S. Mustjoki, S. Brothers, T. P. Vogel, R. Brink, S. G. Tangye, J. H. Reed, and C. C. Goodnow. 2022. STAT3 gain-of-function mutations connect leukemia with autoimmune disease by pathological NKG2D(hi) CD8(+) T cell dysregulation and accumulation. *Immunity* 55: 2386-2404.e2388.
21. Warshauer, J. T., J. A. Belk, A. Y. Chan, J. Wang, A. R. Gupta, Q. Shi, N. Skartsis, Y. Peng, J. D. Phipps, D. Acenas, J. A. Smith, S. J. Tamaki, Q. Tang, J. M. Gardner, A. T. Satpathy, and M. S. Anderson. 2021. A human mutation in STAT3 promotes type 1 diabetes through a defect in CD8+ T cell tolerance. *J Exp Med* 218.
22. Woods, J., S. E. Pemberton, A. D. Largent, K. Chiang, D. Liggitt, M. Oukka, D. J. Rawlings, and S. W. Jackson. 2022. Cutting Edge: Systemic Autoimmunity in Murine STAT3 Gain-of-Function Syndrome Is Characterized by Effector T Cell Expansion in the Absence of Overt Regulatory T Cell Dysfunction. *J Immunol* 209: 1033-1038.
23. Kaplan, M. H., Y. L. Sun, T. Hoey, and M. J. Grusby. 1996. Impaired IL-12 responses and enhanced development of Th2 cells in Stat4-deficient mice. *Nature* 382: 174-177.
24. Powell, D. A., A. P. Hsu, L. F. Shubitz, C. D. Butkiewicz, H. Moale, H. T. Trinh, T. Doetschman, T. G. Georgieva, D. M. Reinartz, J. E. Wilson, M. J. Orbach, S. M.

Holland, J. N. Galgiani, and J. A. Frelinger. 2022. Mouse Model of a Human STAT4 Point Mutation That Predisposes to Disseminated Coccidiomycosis. *Immunohorizons* 6: 130-143.
25. Yao, Z., Y. Cui, W. T. Watford, J. H. Bream, K. Yamaoka, B. D. Hissong, D. Li, S. K. Durum, Q. Jiang, A. Bhandoola, L. Hennighausen, and J. J. O'Shea. 2006. Stat5a/b are essential for normal lymphoid development and differentiation. *Proc Natl Acad Sci U S A* 103: 1000-1005.
26. Udy, G. B., R. P. Towers, R. G. Snell, R. J. Wilkins, S. H. Park, P. A. Ram, D. J. Waxman, and H. W. Davey. 1997. Requirement of STAT5b for sexual dimorphism of body growth rates and liver gene expression. *Proc Natl Acad Sci U S A* 94: 7239-7244.
27. Takeuchi, I., K. Yanagi, S. Takada, T. Uchiyama, A. Igarashi, K. Motomura, Y. Hayashi, N. Nagano, R. Matsuoka, H. Sugiyama, T. Yoshioka, H. Saito, T. Kawai, Y. Miyaji, Y. Inuzuka, Y. Matsubara, Y. Ohya, T. Shimizu, K. Matsumoto, K. Arai, I. Nomura, T. Kaname, and H. Morita. 2023. STAT6 gain-of-function variant exacerbates multiple allergic symptoms. *J Allergy Clin Immunol* 151: 1402-1409.e1406.
28. Rodig, S. J., M. A. Meraz, J. M. White, P. A. Lampe, J. K. Riley, C. D. Arthur, K. L. King, K. C. Sheehan, L. Yin, D. Pennica, E. M. Johnson, Jr., and R. D. Schreiber. 1998. Disruption of the Jak1 gene demonstrates obligatory and nonredundant roles of the Jaks in cytokine-induced biologic responses. *Cell* 93: 373-383.
29. Yasuda, T., T. Fukada, K. Nishida, M. Nakayama, M. Matsuda, I. Miura, T. Dainichi, S. Fukuda, K. Kabashima, S. Nakaoka, B. H. Bin, M. Kubo, H. Ohno, T. Hasegawa, O. Ohara, H. Koseki, S. Wakana, and H. Yoshida. 2016. Hyperactivation of JAK1 tyrosine kinase induces stepwise, progressive pruritic dermatitis. *J Clin Invest* 126: 2064-2076.
30. Sabrautzki, S., E. Janas, B. Lorenz-Depiereux, J. Calzada-Wack, J. A. Aguilar-Pimentel, B. Rathkolb, T. Adler, C. Cohrs, W. Hans, S. Diener, H. Fuchs, V. Gailus-Durner, D. H. Busch, H. Höfler, M. Ollert, T. M. Strom, E. Wolf, F. Neff, and M. Hrabě de Angelis. 2013. An ENU mutagenesis-derived mouse model with a dominant Jak1 mutation resembling phenotypes of systemic autoimmune disease. *Am J Pathol* 183: 352-368.
31. Park, S. Y., K. Saijo, T. Takahashi, M. Osawa, H. Arase, N. Hirayama, K. Miyake, H. Nakauchi, T. Shirasawa, and T. Saito. 1995. Developmental defects of lymphoid cells in Jak3 kinase-deficient mice. *Immunity* 3: 771-782.
32. Karaghiosoff, M., H. Neubauer, C. Lassnig, P. Kovarik, H. Schindler, H. Pircher, B. McCoy, C. Bogdan, T. Decker, G. Brem, K. Pfeffer, and M. Müller. 2000. Partial impairment of cytokine responses in Tyk2-deficient mice. *Immunity* 13: 549-560.
33. Osiak, A., O. Utermöhlen, S. Niendorf, I. Horak, and K. P. Knobeloch. 2005. ISG15, an interferon-stimulated ubiquitin-like protein, is not essential for STAT1 signaling and responses against vesicular stomatitis and lymphocytic choriomeningitis virus. *Mol Cell Biol* 25: 6338-6345.
34. Ritchie, K. J., M. P. Malakhov, C. J. Hetherington, L. Zhou, M. T. Little, O. A. Malakhova, J. C. Sipe, S. H. Orkin, and D. E. Zhang. 2002. Dysregulation of protein modification by ISG15 results in brain cell injury. *Genes Dev* 16: 2207-2212.
35. Schneider, W. M., M. D. Chevillotte, and C. M. Rice. 2014. Interferon-stimulated genes: a complex web of host defenses. *Annu Rev Immunol* 32: 513-545.
36. Boisson-Dupuis, S., X. F. Kong, S. Okada, S. Cypowyj, A. Puel, L. Abel, and J. L. Casanova. 2012. Inborn errors of human STAT1: allelic heterogeneity governs the diversity of immunological and infectious phenotypes. *Curr Opin Immunol* 24: 364-378.

37. Duodu, P., G. Sosa, J. Canar, O. Chhugani, and A. M. Gamero. 2022. Exposing the Two Contrasting Faces of STAT2 in Inflammation. *J Interferon Cytokine Res* 42: 467-481.
38. Hu, X., C. Herrero, W. P. Li, T. T. Antoniv, E. Falck-Pedersen, A. E. Koch, J. M. Woods, G. K. Haines, and L. B. Ivashkiv. 2002. Sensitization of IFN-gamma Jak-STAT signaling during macrophage activation. *Nat Immunol* 3: 859-866.
39. Kalliolias, G. D., and L. B. Ivashkiv. 2008. IL-27 activates human monocytes via STAT1 and suppresses IL-10 production but the inflammatory functions of IL-27 are abrogated by TLRs and p38. *J Immunol* 180: 6325-6333.
40. Vargas-Hernández, A., and L. R. Forbes. 2019. JAK/STAT proteins and their biological impact on NK cell development and function. *Mol Immunol* 115: 21-30.
41. Hu, X., and L. B. Ivashkiv. 2009. Cross-regulation of signaling pathways by interferon-gamma: implications for immune responses and autoimmune diseases. *Immunity* 31: 539-550.
42. Jenner, R. G., M. J. Townsend, I. Jackson, K. Sun, R. D. Bouwman, R. A. Young, L. H. Glimcher, and G. M. Lord. 2009. The transcription factors T-bet and GATA-3 control alternative pathways of T-cell differentiation through a shared set of target genes. *Proc Natl Acad Sci U S A* 106: 17876-17881.
43. Liu, H., and C. Rohowsky-Kochan. 2011. Interleukin-27-mediated suppression of human Th17 cells is associated with activation of STAT1 and suppressor of cytokine signaling protein 1. *J Interferon Cytokine Res* 31: 459-469.
44. Dupuis, S., E. Jouanguy, S. Al-Hajjar, C. Fieschi, I. Z. Al-Mohsen, S. Al-Jumaah, K. Yang, A. Chapgier, C. Eidenschenk, P. Eid, A. Al Ghonaium, H. Tufenkeji, H. Frayha, S. Al-Gazlan, H. Al-Rayes, R. D. Schreiber, I. Gresser, and J. L. Casanova. 2003. Impaired response to interferon-alpha/beta and lethal viral disease in human STAT1 deficiency. *Nat Genet* 33: 388-391.
45. Chapgier, A., S. Boisson-Dupuis, E. Jouanguy, G. Vogt, J. Feinberg, A. Prochnicka-Chalufour, A. Casrouge, K. Yang, C. Soudais, C. Fieschi, O. F. Santos, J. Bustamante, C. Picard, L. de Beaucoudrey, J. F. Emile, P. D. Arkwright, R. D. Schreiber, C. Rolinck-Werninghaus, A. Rösen-Wolff, K. Magdorf, J. Roesler, and J. L. Casanova. 2006. Novel STAT1 alleles in otherwise healthy patients with mycobacterial disease. *PLoS Genet* 2: e131.
46. Chapgier, A., X. F. Kong, S. Boisson-Dupuis, E. Jouanguy, D. Averbuch, J. Feinberg, S. Y. Zhang, J. Bustamante, G. Vogt, J. Lejeune, E. Mayola, L. de Beaucoudrey, L. Abel, D. Engelhard, and J. L. Casanova. 2009. A partial form of recessive STAT1 deficiency in humans. *J Clin Invest* 119: 1502-1514.
47. Dupuis, S., C. Dargemont, C. Fieschi, N. Thomassin, S. Rosenzweig, J. Harris, S. M. Holland, R. D. Schreiber, and J. L. Casanova. 2001. Impairment of mycobacterial but not viral immunity by a germline human STAT1 mutation. *Science* 293: 300-303.
48. Ma, C. S., N. Wong, G. Rao, A. Nguyen, D. T. Avery, K. Payne, J. Torpy, P. O'Young, E. Deenick, J. Bustamante, A. Puel, S. Okada, M. Kobayashi, R. Martinez-Barricarte, M. Elliott, S. Sebnem Kilic, J. El Baghdadi, Y. Minegishi, A. Bousfiha, N. Robertson, S. Hambleton, P. D. Arkwright, M. French, A. K. Blincoe, P. Hsu, D. E. Campbell, M. O. Stormon, M. Wong, S. Adelstein, D. A. Fulcher, M. C. Cook, P. Stepensky, K. Boztug, R. Beier, A. Ikincioğullari, J. B. Ziegler, P. Gray, C. Picard, S. Boisson-Dupuis, T. G. Phan, B. Grimbacher, K. Warnatz, S. M. Holland, G. Uzel, J. L. Casanova, and S. G. Tangye. 2016. Unique and shared signaling pathways cooperate to regulate the

differentiation of human CD4+ T cells into distinct effector subsets. *J Exp Med* 213: 1589-1608.
49. Afkarian, M., J. R. Sedy, J. Yang, N. G. Jacobson, N. Cereb, S. Y. Yang, T. L. Murphy, and K. M. Murphy. 2002. T-bet is a STAT1-induced regulator of IL-12R expression in naïve CD4+ T cells. *Nat Immunol* 3: 549-557.
50. Lucas, S., N. Ghilardi, J. Li, and F. J. de Sauvage. 2003. IL-27 regulates IL-12 responsiveness of naive CD4+ T cells through Stat1-dependent and -independent mechanisms. *Proc Natl Acad Sci U S A* 100: 15047-15052.
51. van de Veerdonk, F. L., T. S. Plantinga, A. Hoischen, S. P. Smeekens, L. A. Joosten, C. Gilissen, P. Arts, D. C. Rosentul, A. J. Carmichael, C. A. Smits-van der Graaf, B. J. Kullberg, J. W. van der Meer, D. Lilic, J. A. Veltman, and M. G. Netea. 2011. STAT1 mutations in autosomal dominant chronic mucocutaneous candidiasis. *N Engl J Med* 365: 54-61.
52. Toubiana, J., S. Okada, J. Hiller, M. Oleastro, M. Lagos Gomez, J. C. Aldave Becerra, M. Ouachée-Chardin, F. Fouyssac, K. M. Girisha, A. Etzioni, J. Van Montfrans, Y. Camcioglu, L. A. Kerns, B. Belohradsky, S. Blanche, A. Bousfiha, C. Rodriguez-Gallego, I. Meyts, K. Kisand, J. Reichenbach, E. D. Renner, S. Rosenzweig, B. Grimbacher, F. L. van de Veerdonk, C. Traidl-Hoffmann, C. Picard, L. Marodi, T. Morio, M. Kobayashi, D. Lilic, J. D. Milner, S. Holland, J. L. Casanova, and A. Puel. 2016. Heterozygous STAT1 gain-of-function mutations underlie an unexpectedly broad clinical phenotype. *Blood* 127: 3154-3164.
53. Zhang, W., X. Chen, G. Gao, S. Xing, L. Zhou, X. Tang, X. Zhao, and Y. An. 2021. Clinical Relevance of Gain- and Loss-of-Function Germline Mutations in STAT1: A Systematic Review. *Front Immunol* 12: 654406.
54. Okada, S., T. Asano, K. Moriya, S. Boisson-Dupuis, M. Kobayashi, J. L. Casanova, and A. Puel. 2020. Human STAT1 Gain-of-Function Heterozygous Mutations: Chronic Mucocutaneous Candidiasis and Type I Interferonopathy. *J Clin Immunol* 40: 1065-1081.
55. Zhang, Y., C. A. Ma, M. G. Lawrence, T. J. Break, M. P. O'Connell, J. J. Lyons, D. B. López, J. S. Barber, Y. Zhao, D. L. Barber, A. F. Freeman, S. M. Holland, M. S. Lionakis, and J. D. Milner. 2017. PD-L1 up-regulation restrains Th17 cell differentiation in STAT3 loss- and STAT1 gain-of-function patients. *J Exp Med* 214: 2523-2533.
56. Ma, C. S., N. Wong, G. Rao, D. T. Avery, J. Torpy, T. Hambridge, J. Bustamante, S. Okada, J. L. Stoddard, E. K. Deenick, S. J. Pelham, K. Payne, S. Boisson-Dupuis, A. Puel, M. Kobayashi, P. D. Arkwright, S. S. Kilic, J. El Baghdadi, S. Nonoyama, Y. Minegishi, S. A. Mahdaviani, D. Mansouri, A. Bousfiha, A. K. Blincoe, M. A. French, P. Hsu, D. E. Campbell, M. O. Stormon, M. Wong, S. Adelstein, J. M. Smart, D. A. Fulcher, M. C. Cook, T. G. Phan, P. Stepensky, K. Boztug, A. Kansu, A. İkincioğullari, U. Baumann, R. Beier, T. Roscioli, J. B. Ziegler, P. Gray, C. Picard, B. Grimbacher, K. Warnatz, S. M. Holland, J. L. Casanova, G. Uzel, and S. G. Tangye. 2015. Monogenic mutations differentially affect the quantity and quality of T follicular helper cells in patients with human primary immunodeficiencies. *J Allergy Clin Immunol* 136: 993-1006.e1001.
57. Zheng, J., F. L. van de Veerdonk, K. L. Crossland, S. P. Smeekens, C. M. Chan, T. Al Shehri, M. Abinun, A. R. Gennery, J. Mann, D. W. Lendrem, M. G. Netea, A. D. Rowan, and D. Lilic. 2015. Gain-of-function STAT1 mutations impair STAT3 activity in patients with chronic mucocutaneous candidiasis (CMC). *Eur J Immunol* 45: 2834-2846.

58. Duncan, C. J. A., and S. Hambleton. 2021. Human Disease Phenotypes Associated with Loss and Gain of Function Mutations in STAT2: Viral Susceptibility and Type I Interferonopathy. *J Clin Immunol* 41: 1446-1456.
59. Freij, B. J., A. T. Hanrath, R. Chen, S. Hambleton, and C. J. A. Duncan. 2020. Life-Threatening Influenza, Hemophagocytic Lymphohistiocytosis and Probable Vaccine-Strain Varicella in a Novel Case of Homozygous STAT2 Deficiency. *Front Immunol* 11: 624415.
60. Hambleton, S., S. Goodbourn, D. F. Young, P. Dickinson, S. M. Mohamad, M. Valappil, N. McGovern, A. J. Cant, S. J. Hackett, P. Ghazal, N. V. Morgan, and R. E. Randall. 2013. STAT2 deficiency and susceptibility to viral illness in humans. *Proc Natl Acad Sci U S A* 110: 3053-3058.
61. Moens, L., L. Van Eyck, D. Jochmans, T. Mitera, G. Frans, X. Bossuyt, P. Matthys, J. Neyts, M. Ciancanelli, S. Y. Zhang, R. Gijsbers, J. L. Casanova, S. Boisson-Dupuis, I. Meyts, and A. Liston. 2017. A novel kindred with inherited STAT2 deficiency and severe viral illness. *J Allergy Clin Immunol* 139: 1995-1997.e1999.
62. Shahni, R., C. M. Cale, G. Anderson, L. D. Osellame, S. Hambleton, T. S. Jacques, Y. Wedatilake, J. W. Taanman, E. Chan, W. Qasim, V. Plagnol, A. Chalasani, M. R. Duchen, K. C. Gilmour, and S. Rahman. 2015. Signal transducer and activator of transcription 2 deficiency is a novel disorder of mitochondrial fission. *Brain* 138: 2834-2846.
63. Alosaimi, M. F., M. C. Maciag, C. D. Platt, R. S. Geha, J. Chou, and L. M. Bartnikas. 2019. A novel variant in STAT2 presenting with hemophagocytic lymphohistiocytosis. *J Allergy Clin Immunol* 144: 611-613.e613.
64. Gothe, F., J. Stremenova Spegarova, C. F. Hatton, H. Griffin, T. Sargent, S. A. Cowley, W. James, A. Roppelt, A. Shcherbina, F. Hauck, H. T. Reyburn, C. J. A. Duncan, and S. Hambleton. 2022. Aberrant inflammatory responses to type I interferon in STAT2 or IRF9 deficiency. *J Allergy Clin Immunol* 150: 955-964.e916.
65. Duncan, C. J. A., B. J. Thompson, R. Chen, G. I. Rice, F. Gothe, D. F. Young, S. C. Lovell, V. G. Shuttleworth, V. Brocklebank, B. Corner, A. J. Skelton, V. Bondet, J. Coxhead, D. Duffy, C. Fourrage, J. H. Livingston, J. Pavaine, E. Cheesman, S. Bitetti, A. Grainger, M. Acres, B. A. Innes, A. Mikulasova, R. Sun, R. Hussain, R. Wright, R. Wynn, M. Zarhrate, L. A. H. Zeef, K. Wood, S. M. Hughes, C. L. Harris, K. R. Engelhardt, Y. J. Crow, R. E. Randall, D. Kavanagh, S. Hambleton, and T. A. Briggs. 2019. Severe type I interferonopathy and unrestrained interferon signaling due to a homozygous germline mutation in STAT2. *Sci Immunol* 4.
66. Gruber, C., M. Martin-Fernandez, F. Ailal, X. Qiu, J. Taft, J. Altman, J. Rosain, S. Buta, A. Bousfiha, J. L. Casanova, J. Bustamante, and D. Bogunovic. 2020. Homozygous STAT2 gain-of-function mutation by loss of USP18 activity in a patient with type I interferonopathy. *J Exp Med* 217.
67. Alsohime, F., M. Martin-Fernandez, M. H. Temsah, M. Alabdulhafid, T. Le Voyer, M. Alghamdi, X. Qiu, N. Alotaibi, A. Alkahtani, S. Buta, E. Jouanguy, A. Al-Eyadhy, C. Gruber, G. M. Hasan, F. A. Bashiri, R. Halwani, H. H. Hassan, S. Al-Muhsen, N. Alkhamis, Z. Alsum, J. L. Casanova, J. Bustamante, D. Bogunovic, and A. A. Alangari. 2020. JAK Inhibitor Therapy in a Child with Inherited USP18 Deficiency. *N Engl J Med* 382: 256-265.

68. Saravia, J., N. M. Chapman, and H. Chi. 2019. Helper T cell differentiation. *Cell Mol Immunol* 16: 634-643.
69. Ansel, K. M., I. Djuretic, B. Tanasa, and A. Rao. 2006. Regulation of Th2 differentiation and Il4 locus accessibility. *Annu Rev Immunol* 24: 607-656.
70. Akira, S. 1999. Functional roles of STAT family proteins: lessons from knockout mice. *Stem Cells* 17: 138-146.
71. Hebenstreit, D., G. Wirnsberger, J. Horejs-Hoeck, and A. Duschl. 2006. Signaling mechanisms, interaction partners, and target genes of STAT6. *Cytokine Growth Factor Rev* 17: 173-188.
72. Wang, W., L. Wang, and B. Zha. 2021. The roles of STAT6 in regulating B cell fate, activation, and function. *Immunol Lett* 233: 87-91.
73. Schimke, L. F., J. Hibbard, R. Martinez-Barricarte, T. A. Khan, R. de Souza Cavalcante, E. Borges de Oliveira Junior, T. Takahashi França, A. Iqbal, G. Yamamoto, C. Arslanian, C. Feriotti, T. A. Costa, J. Bustamante, S. Boisson-Dupuis, J. L. Casanova, J. A. Marzagao Barbuto, M. Zatz, R. Poncio Mendes, V. L. Garcia Calich, H. D. Ochs, T. R. Torgerson, O. Cabral-Marques, and A. Condino-Neto. 2017. Paracoccidioidomycosis Associated With a Heterozygous STAT4 Mutation and Impaired IFN-γ Immunity. *J Infect Dis* 216: 1623-1634.
74. Suratannon, N., C. Ittiwut, W. A. Dik, R. Ittiwut, K. Meesilpavikkai, N. Israsena, P. Ingrungruanglert, V. Dalm, P. L. A. van Daele, A. Sanpavat, N. Chaijitraruch, B. Schrijver, S. Buranapraditkun, T. Porntaveetus, S. M. A. Swagemakers, I. J. H, T. Palaga, K. Suphapeetiporn, P. J. van der Spek, N. Hirankarn, P. Chatchatee, P. Martin van Hagen, and V. Shotelersuk. 2023. A germline STAT6 gain-of-function variant is associated with early-onset allergies. *J Allergy Clin Immunol* 151: 565-571.e569.
75. Baris, S., M. Benamar, Q. Chen, M. C. Catak, M. Martínez-Blanco, M. Wang, J. Fong, M. J. Massaad, A. P. Sefer, A. Kara, R. Babayeva, S. B. Eltan, A. D. Yucelten, E. Bozkurtlar, L. Cinel, E. Karakoc-Aydiner, Y. Zheng, H. Wu, A. Ozen, K. Schmitz-Abe, and T. A. Chatila. 2023. Severe allergic dysregulation due to a gain of function mutation in the transcription factor STAT6. *J Allergy Clin Immunol*.
76. Sharma, M., D. Leung, M. Momenilandi, L. C. W. Jones, L. Pacillo, A. E. James, J. R. Murrell, S. Delafontaine, J. Maimaris, M. Vaseghi-Shanjani, K. L. Del Bel, H. Y. Lu, G. T. Chua, S. Di Cesare, O. Fornes, Z. Liu, G. Di Matteo, M. P. Fu, D. Amodio, I. Y. S. Tam, G. S. W. Chan, A. A. Sharma, J. Dalmann, R. van der Lee, G. Blanchard-Rohner, S. Lin, Q. Philippot, P. A. Richmond, J. J. Lee, A. Matthews, M. Seear, A. K. Turvey, R. L. Philips, T. F. Brown-Whitehorn, C. J. Gray, K. Izumi, J. R. Treat, K. H. Wood, J. Lack, A. Khleborodova, J. E. Niemela, X. Yang, R. Liang, L. Kui, C. S. M. Wong, G. W. K. Poon, A. Hoischen, C. I. van der Made, J. Yang, K. W. Chan, J. S. D. Rosa Duque, P. P. W. Lee, M. H. K. Ho, B. H. Y. Chung, H. T. M. Le, W. Yang, P. Rohani, A. Fouladvand, H. Rokni-Zadeh, M. Changi-Ashtiani, M. Miryounesi, A. Puel, M. Shahrooei, A. Finocchi, P. Rossi, B. Rivalta, C. Cifaldi, A. Novelli, C. Passarelli, S. Arasi, D. Bullens, K. Sauer, T. Claeys, C. M. Biggs, E. C. Morris, S. D. Rosenzweig, J. J. O'Shea, W. W. Wasserman, H. M. Bedford, C. D. M. van Karnebeek, P. Palma, S. O. Burns, I. Meyts, J. L. Casanova, J. J. Lyons, N. Parvaneh, A. T. V. Nguyen, C. Cancrini, J. Heimall, H. Ahmed, M. L. McKinnon, Y. L. Lau, V. Béziat, and S. E. Turvey. 2023. Human germline heterozygous gain-of-function STAT6 variants cause severe allergic disease. *J Exp Med* 220.

77. Baghdassarian, H., S. A. Blackstone, O. S. Clay, R. Philips, B. Matthiasardottir, M. Nehrebecky, V. K. Hua, R. McVicar, Y. Liu, S. M. Tucker, D. Randazzo, N. Deuitch, S. Rosenzweig, A. Mark, R. Sasik, K. M. Fisch, P. Pimpale Chavan, E. Eren, N. R. Watts, C. A. Ma, M. Gadina, D. M. Schwartz, A. Sanyal, G. Werner, D. R. Murdock, N. Horita, S. Chowdhury, D. Dimmock, K. Jepsen, E. F. Remmers, R. Goldbach-Mansky, W. A. Gahl, J. J. O'Shea, J. D. Milner, N. E. Lewis, J. Chang, D. L. Kastner, K. Torok, H. Oda, C. D. Putnam, and L. Broderick. 2023. Variant STAT4 and Response to Ruxolitinib in an Autoinflammatory Syndrome. *N Engl J Med* 388: 2241-2252.
78. Myers, R. C., C. W. Dunaway, M. P. Nelson, J. L. Trevor, A. Morris, and C. Steele. 2013. STAT4-dependent and -independent Th2 responses correlate with protective immunity against lung infection with Pneumocystis murina. *J Immunol* 190: 6287-6294.
79. Kanai, T., J. Jenks, and K. C. Nadeau. 2012. The STAT5b Pathway Defect and Autoimmunity. *Front Immunol* 3: 234.
80. Jenks, J. A., S. Seki, T. Kanai, J. Huang, A. A. Morgan, R. C. Scalco, R. Nath, R. Bucayu, J. M. Wit, W. Al-Herz, D. Ramadan, A. A. Jorge, R. Bacchetta, V. Hwa, R. Rosenfeld, and K. C. Nadeau. 2013. Differentiating the roles of STAT5B and STAT5A in human CD4+ T cells. *Clin Immunol* 148: 227-236.
81. Jones, D. M., K. A. Read, and K. J. Oestreich. 2020. Dynamic Roles for IL-2-STAT5 Signaling in Effector and Regulatory CD4(+) T Cell Populations. *J Immunol* 205: 1721-1730.
82. Owen, D. L., and M. A. Farrar. 2017. STAT5 and CD4 (+) T Cell Immunity. *F1000Res* 6: 32.
83. Stout, B. A., M. E. Bates, L. Y. Liu, N. N. Farrington, and P. J. Bertics. 2004. IL-5 and granulocyte-macrophage colony-stimulating factor activate STAT3 and STAT5 and promote Pim-1 and cyclin D3 protein expression in human eosinophils. *J Immunol* 173: 6409-6417.
84. Kofoed, E. M., V. Hwa, B. Little, K. A. Woods, C. K. Buckway, J. Tsubaki, K. L. Pratt, L. Bezrodnik, H. Jasper, A. Tepper, J. J. Heinrich, and R. G. Rosenfeld. 2003. Growth hormone insensitivity associated with a STAT5b mutation. *N Engl J Med* 349: 1139-1147.
85. Pelham, S. J., M. S. Caldirola, D. T. Avery, J. Mackie, G. Rao, F. Gothe, T. J. Peters, A. Guerin, D. Neumann, D. Vokurkova, V. Hwa, W. Zhang, S. C. Lyu, I. Chang, M. Manohar, K. C. Nadeau, M. I. Gaillard, L. Bezrodnik, V. Iotova, N. W. Zwirner, M. Gutierrez, W. Al-Herz, C. C. Goodnow, A. Vargas-Hernández, L. R. Forbes Satter, S. Hambleton, E. K. Deenick, C. S. Ma, and S. G. Tangye. 2022. STAT5B restrains human B-cell differentiation to maintain humoral immune homeostasis. *J Allergy Clin Immunol* 150: 931-946.
86. Vargas-Hernández, A., A. Witalisz-Siepracka, M. Prchal-Murphy, K. Klein, S. Mahapatra, W. Al-Herz, E. M. Mace, A. F. Carisey, J. S. Orange, V. Sexl, and L. R. Forbes. 2020. Human signal transducer and activator of transcription 5b (STAT5b) mutation causes dysregulated human natural killer cell maturation and impaired lytic function. *J Allergy Clin Immunol* 145: 345-357.e349.
87. Klammt, J., D. Neumann, E. F. Gevers, S. F. Andrew, I. D. Schwartz, D. Rockstroh, R. Colombo, M. A. Sanchez, D. Vokurkova, J. Kowalczyk, L. A. Metherell, R. G. Rosenfeld, R. Pfäffle, M. T. Dattani, A. Dauber, and V. Hwa. 2018. Dominant-negative

STAT5B mutations cause growth hormone insensitivity with short stature and mild immune dysregulation. *Nat Commun* 9: 2105.
88. Liu, X., G. W. Robinson, K. U. Wagner, L. Garrett, A. Wynshaw-Boris, and L. Hennighausen. 1997. Stat5a is mandatory for adult mammary gland development and lactogenesis. *Genes Dev* 11: 179-186.
89. Ma, C. A., L. Xi, B. Cauff, A. DeZure, A. F. Freeman, S. Hambleton, G. Kleiner, T. R. Leahy, M. O'Sullivan, M. Makiya, G. O'Regan, S. Pittaluga, J. Niemela, J. Stoddard, S. D. Rosenzweig, M. Raffeld, A. D. Klion, and J. D. Milner. 2017. Somatic STAT5b gain-of-function mutations in early onset nonclonal eosinophilia, urticaria, dermatitis, and diarrhea. *Blood* 129: 650-653.
90. Eisenberg, R., M. D. Gans, T. R. Leahy, F. Gothe, C. Perry, M. Raffeld, L. Xi, S. Blackstone, C. Ma, S. Hambleton, and J. D. Milner. 2021. JAK inhibition in early-onset somatic, nonclonal STAT5B gain-of-function disease. *J Allergy Clin Immunol Pract* 9: 1008-1010.e1002.
91. Kasap, N., K. Aslan, L. T. Karakurt, H. Bozkurt, H. Canatan, O. Cavkaytar, A. Eken, and M. Arga. 2022. A novel gain-of-function mutation in STAT5B is associated with treatment-resistant severe atopic dermatitis. *Clin Exp Allergy* 52: 907-910.
92. Eletto, D., S. O. Burns, I. Angulo, V. Plagnol, K. C. Gilmour, F. Henriquez, J. Curtis, M. Gaspar, K. Nowak, V. Daza-Cajigal, D. Kumararatne, R. Doffinger, A. J. Thrasher, and S. Nejentsev. 2016. Biallelic JAK1 mutations in immunodeficient patient with mycobacterial infection. *Nat Commun* 7: 13992.
93. Del Bel, K. L., R. J. Ragotte, A. Saferali, S. Lee, S. M. Vercauteren, S. A. Mostafavi, R. A. Schreiber, J. S. Prendiville, M. S. Phang, J. Halparin, N. Au, J. M. Dean, J. J. Priatel, E. Jewels, A. K. Junker, P. C. Rogers, M. Seear, M. L. McKinnon, and S. E. Turvey. 2017. JAK1 gain-of-function causes an autosomal dominant immune dysregulatory and hypereosinophilic syndrome. *J Allergy Clin Immunol* 139: 2016-2020.e2015.
94. Gruber, C. N., J. J. A. Calis, S. Buta, G. Evrony, J. C. Martin, S. A. Uhl, R. Caron, L. Jarchin, D. Dunkin, R. Phelps, B. D. Webb, J. M. Saland, M. Merad, J. S. Orange, E. M. Mace, B. R. Rosenberg, B. D. Gelb, and D. Bogunovic. 2020. Complex Autoinflammatory Syndrome Unveils Fundamental Principles of JAK1 Kinase Transcriptional and Biochemical Function. *Immunity* 53: 672-684.e611.
95. Russell, S. M., N. Tayebi, H. Nakajima, M. C. Riedy, J. L. Roberts, M. J. Aman, T. S. Migone, M. Noguchi, M. L. Markert, R. H. Buckley, J. J. O'Shea, and W. J. Leonard. 1995. Mutation of Jak3 in a patient with SCID: essential role of Jak3 in lymphoid development. *Science* 270: 797-800.
96. Macchi, P., A. Villa, S. Giliani, M. G. Sacco, A. Frattini, F. Porta, A. G. Ugazio, J. A. Johnston, F. Candotti, J. J. O'Shea, and et al. 1995. Mutations of Jak-3 gene in patients with autosomal severe combined immune deficiency (SCID). *Nature* 377: 65-68.
97. Frucht, D. M., M. Gadina, G. J. Jagadeesh, I. Aksentijevich, K. Takada, J. J. Bleesing, J. Nelson, L. M. Muul, G. Perham, G. Morgan, E. J. Gerritsen, R. F. Schumacher, P. Mella, P. A. Veys, T. A. Fleisher, E. R. Kaminski, L. D. Notarangelo, J. J. O'Shea, and F. Candotti. 2001. Unexpected and variable phenotypes in a family with JAK3 deficiency. *Genes Immun* 2: 422-432.
98. Waanders, E., B. Scheijen, M. C. Jongmans, H. Venselaar, S. V. van Reijmersdal, A. H. van Dijk, A. Pastorczak, R. D. Weren, C. E. van der Schoot, M. van de Vorst, E. Sonneveld, N. Hoogerbrugge, V. H. van der Velden, B. Gruhn, P. M. Hoogerbrugge, J. J.

van Dongen, A. Geurts van Kessel, F. N. van Leeuwen, and R. P. Kuiper. 2017. Germline activating TYK2 mutations in pediatric patients with two primary acute lymphoblastic leukemia occurrences. *Leukemia* 31: 821-828.
99. Wu, P., S. Chen, B. Wu, J. Chen, and G. Lv. 2020. A TYK2 Gene Mutation c.2395G>A Leads to TYK2 Deficiency: A Case Report and Literature Review. *Front Pediatr* 8: 253.
100. Minegishi, Y., M. Saito, T. Morio, K. Watanabe, K. Agematsu, S. Tsuchiya, H. Takada, T. Hara, N. Kawamura, T. Ariga, H. Kaneko, N. Kondo, I. Tsuge, A. Yachie, Y. Sakiyama, T. Iwata, F. Bessho, T. Ohishi, K. Joh, K. Imai, K. Kogawa, M. Shinohara, M. Fujieda, H. Wakiguchi, S. Pasic, M. Abinun, H. D. Ochs, E. D. Renner, A. Jansson, B. H. Belohradsky, A. Metin, N. Shimizu, S. Mizutani, T. Miyawaki, S. Nonoyama, and H. Karasuyama. 2006. Human tyrosine kinase 2 deficiency reveals its requisite roles in multiple cytokine signals involved in innate and acquired immunity. *Immunity* 25: 745-755.
101. Kerner, G., N. Ramirez-Alejo, Y. Seeleuthner, R. Yang, M. Ogishi, A. Cobat, E. Patin, L. Quintana-Murci, S. Boisson-Dupuis, J. L. Casanova, and L. Abel. 2019. Homozygosity for TYK2 P1104A underlies tuberculosis in about 1% of patients in a cohort of European ancestry. *Proc Natl Acad Sci U S A* 116: 10430-10434.
102. Arimoto, K. I., S. Löchte, S. A. Stoner, C. Burkart, Y. Zhang, S. Miyauchi, S. Wilmes, J. B. Fan, J. J. Heinisch, Z. Li, M. Yan, S. Pellegrini, F. Colland, J. Piehler, and D. E. Zhang. 2017. STAT2 is an essential adaptor in USP18-mediated suppression of type I interferon signaling. *Nat Struct Mol Biol* 24: 279-289.
103. Meuwissen, M. E., R. Schot, S. Buta, G. Oudesluijs, S. Tinschert, S. D. Speer, Z. Li, L. van Unen, D. Heijsman, T. Goldmann, M. H. Lequin, J. M. Kros, W. Stam, M. Hermann, R. Willemsen, R. W. Brouwer, I. W. F. Van, M. Martin-Fernandez, I. de Coo, J. Dudink, F. A. de Vries, A. Bertoli Avella, M. Prinz, Y. J. Crow, F. W. Verheijen, S. Pellegrini, D. Bogunovic, and G. M. Mancini. 2016. Human USP18 deficiency underlies type 1 interferonopathy leading to severe pseudo-TORCH syndrome. *J Exp Med* 213: 1163-1174.
104. Zhang, X., D. Bogunovic, B. Payelle-Brogard, V. Francois-Newton, S. D. Speer, C. Yuan, S. Volpi, Z. Li, O. Sanal, D. Mansouri, I. Tezcan, G. I. Rice, C. Chen, N. Mansouri, S. A. Mahdaviani, Y. Itan, B. Boisson, S. Okada, L. Zeng, X. Wang, H. Jiang, W. Liu, T. Han, D. Liu, T. Ma, B. Wang, M. Liu, J. Y. Liu, Q. K. Wang, D. Yalnizoglu, L. Radoshevich, G. Uzé, P. Gros, F. Rozenberg, S. Y. Zhang, E. Jouanguy, J. Bustamante, A. García-Sastre, L. Abel, P. Lebon, L. D. Notarangelo, Y. J. Crow, S. Boisson-Dupuis, J. L. Casanova, and S. Pellegrini. 2015. Human intracellular ISG15 prevents interferon-α/β over-amplification and auto-inflammation. *Nature* 517: 89-93.
105. Martin-Fernandez, M., M. Bravo García-Morato, C. Gruber, S. Murias Loza, M. N. H. Malik, F. Alsohime, A. Alakeel, R. Valdez, S. Buta, G. Buda, M. A. Marti, M. Larralde, B. Boisson, M. Feito Rodriguez, X. Qiu, M. Chrabieh, M. Al Ayed, S. Al Muhsen, J. V. Desai, E. M. N. Ferre, S. D. Rosenzweig, B. Amador-Borrero, L. Y. Bravo-Gallego, R. Olmer, S. Merkert, M. Bret, A. K. Sood, A. Al-Rabiaah, M. H. Temsah, R. Halwani, M. Hernandez, F. Pessler, J. L. Casanova, J. Bustamante, M. S. Lionakis, and D. Bogunovic. 2020. Systemic Type I IFN Inflammation in Human ISG15 Deficiency Leads to Necrotizing Skin Lesions. *Cell Rep* 31: 107633.
106. Bogunovic, D., M. Byun, L. A. Durfee, A. Abhyankar, O. Sanal, D. Mansouri, S. Salem, I. Radovanovic, A. V. Grant, P. Adimi, N. Mansouri, S. Okada, V. L. Bryant, X. F. Kong,

A. Kreins, M. M. Velez, B. Boisson, S. Khalilzadeh, U. Ozcelik, I. A. Darazam, J. W. Schoggins, C. M. Rice, S. Al-Muhsen, M. Behr, G. Vogt, A. Puel, J. Bustamante, P. Gros, J. M. Huibregtse, L. Abel, S. Boisson-Dupuis, and J. L. Casanova. 2012. Mycobacterial disease and impaired IFN-γ immunity in humans with inherited ISG15 deficiency. *Science* 337: 1684-1688.

107. Liau, N. P. D., A. Laktyushin, I. S. Lucet, J. M. Murphy, S. Yao, E. Whitlock, K. Callaghan, N. A. Nicola, N. J. Kershaw, and J. J. Babon. 2018. The molecular basis of JAK/STAT inhibition by SOCS1. *Nat Commun* 9: 1558.
108. Hadjadj, J., C. N. Castro, M. Tusseau, M. C. Stolzenberg, F. Mazerolles, N. Aladjidi, M. Armstrong, H. Ashrafian, I. Cutcutache, G. Ebetsberger-Dachs, K. S. Elliott, I. Durieu, N. Fabien, M. Fusaro, M. Heeg, Y. Schmitt, M. Bras, J. C. Knight, J. C. Lega, G. Lesca, A. L. Mathieu, M. Moreews, B. Moreira, A. Nosbaum, M. Page, C. Picard, T. Ronan Leahy, I. Rouvet, E. Ryan, D. Sanlaville, K. Schwarz, A. Skelton, J. F. Viallard, S. Viel, M. Villard, I. Callebaut, C. Picard, T. Walzer, S. Ehl, A. Fischer, B. Neven, A. Belot, and F. Rieux-Laucat. 2020. Early-onset autoimmunity associated with SOCS1 haploinsufficiency. *Nat Commun* 11: 5341.
109. Lee, P. Y., C. D. Platt, S. Weeks, R. F. Grace, G. Maher, K. Gauthier, S. Devana, S. Vitali, A. G. Randolph, D. R. McDonald, R. S. Geha, and J. Chou. 2020. Immune dysregulation and multisystem inflammatory syndrome in children (MIS-C) in individuals with haploinsufficiency of SOCS1. *J Allergy Clin Immunol* 146: 1194-1200.e1191.
110. Frey-Jakobs, S., J. M. Hartberger, M. Fliegauf, C. Bossen, M. L. Wehmeyer, J. C. Neubauer, A. Bulashevska, M. Proietti, P. Fröbel, C. Nöltner, L. Yang, J. Rojas-Restrepo, N. Langer, S. Winzer, K. R. Engelhardt, C. Glocker, D. Pfeifer, A. Klein, A. A. Schäffer, I. Lagovsky, I. Lachover-Roth, V. Béziat, A. Puel, J. L. Casanova, B. Fleckenstein, S. Weidinger, S. S. Kilic, B. Z. Garty, A. Etzioni, and B. Grimbacher. 2018. ZNF341 controls STAT3 expression and thereby immunocompetence. *Sci Immunol* 3.
111. Béziat, V., J. Li, J. X. Lin, C. S. Ma, P. Li, A. Bousfiha, I. Pellier, S. Zoghi, S. Baris, S. Keles, P. Gray, N. Du, Y. Wang, Y. Zerbib, R. Lévy, T. Leclercq, F. About, A. I. Lim, G. Rao, K. Payne, S. J. Pelham, D. T. Avery, E. K. Deenick, B. Pillay, J. Chou, R. Guery, A. Belkadi, A. Guérin, M. Migaud, V. Rattina, F. Ailal, I. Benhsaien, M. Bouaziz, T. Habib, D. Chaussabel, N. Marr, J. El-Benna, B. Grimbacher, O. Wargon, J. Bustamante, B. Boisson, I. Müller-Fleckenstein, B. Fleckenstein, M. O. Chandesris, M. Titeux, S. Fraitag, M. A. Alyanakian, M. Leruez-Ville, C. Picard, I. Meyts, J. P. Di Santo, A. Hovnanian, A. Somer, A. Ozen, N. Rezaei, T. A. Chatila, L. Abel, W. J. Leonard, S. G. Tangye, A. Puel, and J. L. Casanova. 2018. A recessive form of hyper-IgE syndrome by disruption of ZNF341-dependent STAT3 transcription and activity. *Sci Immunol* 3.
112. Mackie, J., C. S. Ma, S. G. Tangye, and A. Guerin. 2023. The ups and downs of STAT3 function: too much, too little and human immune dysregulation. *Clin Exp Immunol* 212: 107-116.
113. Akira, S. 2000. Roles of STAT3 defined by tissue-specific gene targeting. *Oncogene* 19: 2607-2611.
114. Jägle, S., M. Heeg, S. Grün, A. Rensing-Ehl, M. E. Maccari, C. Klemann, N. Jones, K. Lehmberg, C. Bettoni, K. Warnatz, B. Grimbacher, A. Biebl, U. Schauer, R. Hague, O. Neth, A. Mauracher, J. Pachlopnik Schmid, A. Fabre, L. Kostyuchenko, M. Führer, M. R. Lorenz, K. Schwarz, J. Rohr, and S. Ehl. 2020. Distinct molecular response patterns of

activating STAT3 mutations associate with penetrance of lymphoproliferation and autoimmunity. *Clin Immunol* 210: 108316.
115. Gharibi, T., Z. Babaloo, A. Hosseini, M. Abdollahpour-Alitappeh, V. Hashemi, F. Marofi, K. Nejati, and B. Baradaran. 2020. Targeting STAT3 in cancer and autoimmune diseases. *Eur J Pharmacol* 878: 173107.
116. Yu, H., D. Pardoll, and R. Jove. 2009. STATs in cancer inflammation and immunity: a leading role for STAT3. *Nat Rev Cancer* 9: 798-809.
117. Yang, X. O., A. D. Panopoulos, R. Nurieva, S. H. Chang, D. Wang, S. S. Watowich, and C. Dong. 2007. STAT3 regulates cytokine-mediated generation of inflammatory helper T cells. *J Biol Chem* 282: 9358-9363.
118. Delgoffe, G. M., and D. A. Vignali. 2013. STAT heterodimers in immunity: A mixed message or a unique signal? *Jakstat* 2: e23060.
119. Faletti, L., S. Ehl, and M. Heeg. 2021. Germline STAT3 gain-of-function mutations in primary immunodeficiency: Impact on the cellular and clinical phenotype. *Biomed J* 44: 412-421.
120. Huynh, J., A. Chand, D. Gough, and M. Ernst. 2019. Therapeutically exploiting STAT3 activity in cancer - using tissue repair as a road map. *Nat Rev Cancer* 19: 82-96.
121. Pickert, G., C. Neufert, M. Leppkes, Y. Zheng, N. Wittkopf, M. Warntjen, H. A. Lehr, S. Hirth, B. Weigmann, S. Wirtz, W. Ouyang, M. F. Neurath, and C. Becker. 2009. STAT3 links IL-22 signaling in intestinal epithelial cells to mucosal wound healing. *J Exp Med* 206: 1465-1472.
122. Zheng, Y., D. M. Danilenko, P. Valdez, I. Kasman, J. Eastham-Anderson, J. Wu, and W. Ouyang. 2007. Interleukin-22, a T(H)17 cytokine, mediates IL-23-induced dermal inflammation and acanthosis. *Nature* 445: 648-651.
123. O'Shea, J. J., D. M. Schwartz, A. V. Villarino, M. Gadina, I. B. McInnes, and A. Laurence. 2015. The JAK-STAT pathway: impact on human disease and therapeutic intervention. *Annu Rev Med* 66: 311-328.
124. Zhou, L., Ivanov, II, R. Spolski, R. Min, K. Shenderov, T. Egawa, D. E. Levy, W. J. Leonard, and D. R. Littman. 2007. IL-6 programs T(H)-17 cell differentiation by promoting sequential engagement of the IL-21 and IL-23 pathways. *Nat Immunol* 8: 967-974.
125. McGeachy, M. J., Y. Chen, C. M. Tato, A. Laurence, B. Joyce-Shaikh, W. M. Blumenschein, T. K. McClanahan, J. J. O'Shea, and D. J. Cua. 2009. The interleukin 23 receptor is essential for the terminal differentiation of interleukin 17-producing effector T helper cells in vivo. *Nat Immunol* 10: 314-324.
126. Hillmer, E. J., H. Zhang, H. S. Li, and S. S. Watowich. 2016. STAT3 signaling in immunity. *Cytokine Growth Factor Rev* 31: 1-15.
127. Kane, A., E. K. Deenick, C. S. Ma, M. C. Cook, G. Uzel, and S. G. Tangye. 2014. STAT3 is a central regulator of lymphocyte differentiation and function. *Curr Opin Immunol* 28: 49-57.
128. Yasuda, K., Y. Takeuchi, and K. Hirota. 2019. The pathogenicity of Th17 cells in autoimmune diseases. *Semin Immunopathol* 41: 283-297.
129. Yeste, A., I. D. Mascanfroni, M. Nadeau, E. J. Burns, A. M. Tukpah, A. Santiago, C. Wu, B. Patel, D. Kumar, and F. J. Quintana. 2014. IL-21 induces IL-22 production in CD4+ T cells. *Nat Commun* 5: 3753.

130. Durant, L., W. T. Watford, H. L. Ramos, A. Laurence, G. Vahedi, L. Wei, H. Takahashi, H. W. Sun, Y. Kanno, F. Powrie, and J. J. O'Shea. 2010. Diverse targets of the transcription factor STAT3 contribute to T cell pathogenicity and homeostasis. *Immunity* 32: 605-615.
131. Ciofani, M., A. Madar, C. Galan, M. Sellars, K. Mace, F. Pauli, A. Agarwal, W. Huang, C. N. Parkhurst, M. Muratet, K. M. Newberry, S. Meadows, A. Greenfield, Y. Yang, P. Jain, F. K. Kirigin, C. Birchmeier, E. F. Wagner, K. M. Murphy, R. M. Myers, R. Bonneau, and D. R. Littman. 2012. A validated regulatory network for Th17 cell specification. *Cell* 151: 289-303.
132. Harris, T. J., J. F. Grosso, H. R. Yen, H. Xin, M. Kortylewski, E. Albesiano, E. L. Hipkiss, D. Getnet, M. V. Goldberg, C. H. Maris, F. Housseau, H. Yu, D. M. Pardoll, and C. G. Drake. 2007. Cutting edge: An in vivo requirement for STAT3 signaling in TH17 development and TH17-dependent autoimmunity. *J Immunol* 179: 4313-4317.
133. Liu, X., Y. S. Lee, C. R. Yu, and C. E. Egwuagu. 2008. Loss of STAT3 in CD4+ T cells prevents development of experimental autoimmune diseases. *J Immunol* 180: 6070-6076.
134. Schnell, A., D. R. Littman, and V. K. Kuchroo. 2023. T(H)17 cell heterogeneity and its role in tissue inflammation. *Nat Immunol* 24: 19-29.
135. Nurieva, R. I., Y. Chung, D. Hwang, X. O. Yang, H. S. Kang, L. Ma, Y. H. Wang, S. S. Watowich, A. M. Jetten, Q. Tian, and C. Dong. 2008. Generation of T follicular helper cells is mediated by interleukin-21 but independent of T helper 1, 2, or 17 cell lineages. *Immunity* 29: 138-149.
136. Hart, A. P., and T. M. Laufer. 2022. A review of signaling and transcriptional control in T follicular helper cell differentiation. *J Leukoc Biol* 111: 173-195.
137. Kim, J. M., J. P. Rasmussen, and A. Y. Rudensky. 2007. Regulatory T cells prevent catastrophic autoimmunity throughout the lifespan of mice. *Nat Immunol* 8: 191-197.
138. Wan, Y. Y., and R. A. Flavell. 2007. Regulatory T-cell functions are subverted and converted owing to attenuated Foxp3 expression. *Nature* 445: 766-770.
139. Williams, L. M., and A. Y. Rudensky. 2007. Maintenance of the Foxp3-dependent developmental program in mature regulatory T cells requires continued expression of Foxp3. *Nat Immunol* 8: 277-284.
140. Chaudhry, A., R. M. Samstein, P. Treuting, Y. Liang, M. C. Pils, J. M. Heinrich, R. S. Jack, F. T. Wunderlich, J. C. Brüning, W. Müller, and A. Y. Rudensky. 2011. Interleukin-10 signaling in regulatory T cells is required for suppression of Th17 cell-mediated inflammation. *Immunity* 34: 566-578.
141. Cui, W., Y. Liu, J. S. Weinstein, J. Craft, and S. M. Kaech. 2011. An interleukin-21-interleukin-10-STAT3 pathway is critical for functional maturation of memory CD8+ T cells. *Immunity* 35: 792-805.
142. Ives, M. L., C. S. Ma, U. Palendira, A. Chan, J. Bustamante, S. Boisson-Dupuis, P. D. Arkwright, D. Engelhard, D. Averbuch, K. Magdorf, J. Roesler, J. Peake, M. Wong, S. Adelstein, S. Choo, J. M. Smart, M. A. French, D. A. Fulcher, M. C. Cook, C. Picard, A. Durandy, M. Tsumura, M. Kobayashi, G. Uzel, J. L. Casanova, S. G. Tangye, and E. K. Deenick. 2013. Signal transducer and activator of transcription 3 (STAT3) mutations underlying autosomal dominant hyper-IgE syndrome impair human CD8(+) T-cell memory formation and function. *J Allergy Clin Immunol* 132: 400-411.e409.
143. Siegel, A. M., J. Heimall, A. F. Freeman, A. P. Hsu, E. Brittain, J. M. Brenchley, D. C. Douek, G. H. Fahle, J. I. Cohen, S. M. Holland, and J. D. Milner. 2011. A critical role for

STAT3 transcription factor signaling in the development and maintenance of human T cell memory. *Immunity* 35: 806-818.
144. Xin, G., D. M. Schauder, B. Lainez, J. S. Weinstein, Z. Dai, Y. Chen, E. Esplugues, R. Wen, D. Wang, I. A. Parish, A. J. Zajac, J. Craft, and W. Cui. 2015. A Critical Role of IL-21-Induced BATF in Sustaining CD8-T-Cell-Mediated Chronic Viral Control. *Cell Rep* 13: 1118-1124.
145. Sun, Q., X. Zhao, R. Li, D. Liu, B. Pan, B. Xie, X. Chi, D. Cai, P. Wei, W. Xu, K. Wei, Z. Zhao, Y. Fu, L. Ni, and C. Dong. 2023. STAT3 regulates CD8+ T cell differentiation and functions in cancer and acute infection. *J Exp Med* 220.
146. Nielsen, M. M., D. A. Witherden, and W. L. Havran. 2017. γδ T cells in homeostasis and host defence of epithelial barrier tissues. *Nat Rev Immunol* 17: 733-745.
147. Agerholm, R., J. Rizk, M. T. Viñals, and V. Bekiaris. 2019. STAT3 but not STAT4 is critical for γδT17 cell responses and skin inflammation. *EMBO Rep* 20: e48647.
148. Deenick, E. K., D. T. Avery, A. Chan, L. J. Berglund, M. L. Ives, L. Moens, J. L. Stoddard, J. Bustamante, S. Boisson-Dupuis, M. Tsumura, M. Kobayashi, P. D. Arkwright, D. Averbuch, D. Engelhard, J. Roesler, J. Peake, M. Wong, S. Adelstein, S. Choo, J. M. Smart, M. A. French, D. A. Fulcher, M. C. Cook, C. Picard, A. Durandy, C. Klein, S. M. Holland, G. Uzel, J. L. Casanova, C. S. Ma, and S. G. Tangye. 2013. Naive and memory human B cells have distinct requirements for STAT3 activation to differentiate into antibody-secreting plasma cells. *J Exp Med* 210: 2739-2753.
149. Avery, D. T., E. K. Deenick, C. S. Ma, S. Suryani, N. Simpson, G. Y. Chew, T. D. Chan, U. Palendira, J. Bustamante, S. Boisson-Dupuis, S. Choo, K. E. Bleasel, J. Peake, C. King, M. A. French, D. Engelhard, S. Al-Hajjar, S. Al-Muhsen, K. Magdorf, J. Roesler, P. D. Arkwright, P. Hissaria, D. S. Riminton, M. Wong, R. Brink, D. A. Fulcher, J. L. Casanova, M. C. Cook, and S. G. Tangye. 2010. B cell-intrinsic signaling through IL-21 receptor and STAT3 is required for establishing long-lived antibody responses in humans. *J Exp Med* 207: 155-171.
150. Fornek, J. L., L. T. Tygrett, T. J. Waldschmidt, V. Poli, R. C. Rickert, and G. S. Kansas. 2006. Critical role for Stat3 in T-dependent terminal differentiation of IgG B cells. *Blood* 107: 1085-1091.
151. Panopoulos, A. D., L. Zhang, J. W. Snow, D. M. Jones, A. M. Smith, K. C. El Kasmi, F. Liu, M. A. Goldsmith, D. C. Link, P. J. Murray, and S. S. Watowich. 2006. STAT3 governs distinct pathways in emergency granulopoiesis and mature neutrophils. *Blood* 108: 3682-3690.
152. Zhang, H., H. Nguyen-Jackson, A. D. Panopoulos, H. S. Li, P. J. Murray, and S. S. Watowich. 2010. STAT3 controls myeloid progenitor growth during emergency granulopoiesis. *Blood* 116: 2462-2471.
153. Takeda, K., B. E. Clausen, T. Kaisho, T. Tsujimura, N. Terada, I. Förster, and S. Akira. 1999. Enhanced Th1 activity and development of chronic enterocolitis in mice devoid of Stat3 in macrophages and neutrophils. *Immunity* 10: 39-49.
154. Zigmond, E., B. Bernshtein, G. Friedlander, C. R. Walker, S. Yona, K. W. Kim, O. Brenner, R. Krauthgamer, C. Varol, W. Müller, and S. Jung. 2014. Macrophage-restricted interleukin-10 receptor deficiency, but not IL-10 deficiency, causes severe spontaneous colitis. *Immunity* 40: 720-733.

155. Wölfle, S. J., J. Strebovsky, H. Bartz, A. Sähr, C. Arnold, C. Kaiser, A. H. Dalpke, and K. Heeg. 2011. PD-L1 expression on tolerogenic APCs is controlled by STAT-3. *Eur J Immunol* 41: 413-424.
156. Laouar, Y., T. Welte, X. Y. Fu, and R. A. Flavell. 2003. STAT3 is required for Flt3L-dependent dendritic cell differentiation. *Immunity* 19: 903-912.
157. Takatori, H., Y. Kanno, W. T. Watford, C. M. Tato, G. Weiss, Ivanov, II, D. R. Littman, and J. J. O'Shea. 2009. Lymphoid tissue inducer-like cells are an innate source of IL-17 and IL-22. *J Exp Med* 206: 35-41.
158. Rankin, L. C., M. J. Girard-Madoux, C. Seillet, L. A. Mielke, Y. Kerdiles, A. Fenis, E. Wieduwild, T. Putoczki, S. Mondot, O. Lantz, D. Demon, A. T. Papenfuss, G. K. Smyth, M. Lamkanfi, S. Carotta, J. C. Renauld, W. Shi, S. Carpentier, T. Soos, C. Arendt, S. Ugolini, N. D. Huntington, G. T. Belz, and E. Vivier. 2016. Complementarity and redundancy of IL-22-producing innate lymphoid cells. *Nat Immunol* 17: 179-186.
159. Guo, X., J. Qiu, T. Tu, X. Yang, L. Deng, R. A. Anders, L. Zhou, and Y. X. Fu. 2014. Induction of innate lymphoid cell-derived interleukin-22 by the transcription factor STAT3 mediates protection against intestinal infection. *Immunity* 40: 25-39.
160. Johnson, D. E., R. A. O'Keefe, and J. R. Grandis. 2018. Targeting the IL-6/JAK/STAT3 signalling axis in cancer. *Nat Rev Clin Oncol* 15: 234-248.
161. Gotthardt, D., E. M. Putz, E. Straka, P. Kudweis, M. Biaggio, V. Poli, B. Strobl, M. Müller, and V. Sexl. 2014. Loss of STAT3 in murine NK cells enhances NK cell-dependent tumor surveillance. *Blood* 124: 2370-2379.
162. Zhu, S., P. V. Phatarpekar, C. J. Denman, V. V. Senyukov, S. S. Somanchi, H. T. Nguyen-Jackson, E. M. Mace, A. F. Freeman, S. S. Watowich, J. S. Orange, S. M. Holland, and D. A. Lee. 2014. Transcription of the activating receptor NKG2D in natural killer cells is regulated by STAT3 tyrosine phosphorylation. *Blood* 124: 403-411.
163. Minegishi, Y., M. Saito, S. Tsuchiya, I. Tsuge, H. Takada, T. Hara, N. Kawamura, T. Ariga, S. Pasic, O. Stojkovic, A. Metin, and H. Karasuyama. 2007. Dominant-negative mutations in the DNA-binding domain of STAT3 cause hyper-IgE syndrome. *Nature* 448: 1058-1062.
164. Holland, S. M., F. R. DeLeo, H. Z. Elloumi, A. P. Hsu, G. Uzel, N. Brodsky, A. F. Freeman, A. Demidowich, J. Davis, M. L. Turner, V. L. Anderson, D. N. Darnell, P. A. Welch, D. B. Kuhns, D. M. Frucht, H. L. Malech, J. I. Gallin, S. D. Kobayashi, A. R. Whitney, J. M. Voyich, J. M. Musser, C. Woellner, A. A. Schäffer, J. M. Puck, and B. Grimbacher. 2007. STAT3 mutations in the hyper-IgE syndrome. *N Engl J Med* 357: 1608-1619.
165. Ma, C. S., G. Y. Chew, N. Simpson, A. Priyadarshi, M. Wong, B. Grimbacher, D. A. Fulcher, S. G. Tangye, and M. C. Cook. 2008. Deficiency of Th17 cells in hyper IgE syndrome due to mutations in STAT3. *J Exp Med* 205: 1551-1557.
166. Milner, J. D., J. M. Brenchley, A. Laurence, A. F. Freeman, B. J. Hill, K. M. Elias, Y. Kanno, C. Spalding, H. Z. Elloumi, M. L. Paulson, J. Davis, A. Hsu, A. I. Asher, J. O'Shea, S. M. Holland, W. E. Paul, and D. C. Douek. 2008. Impaired T(H)17 cell differentiation in subjects with autosomal dominant hyper-IgE syndrome. *Nature* 452: 773-776.
167. Tsilifis, C., A. F. Freeman, and A. R. Gennery. 2021. STAT3 Hyper-IgE Syndrome-an Update and Unanswered Questions. *J Clin Immunol* 41: 864-880.

168. Ma, C. S., D. T. Avery, A. Chan, M. Batten, J. Bustamante, S. Boisson-Dupuis, P. D. Arkwright, A. Y. Kreins, D. Averbuch, D. Engelhard, K. Magdorf, S. S. Kilic, Y. Minegishi, S. Nonoyama, M. A. French, S. Choo, J. M. Smart, J. Peake, M. Wong, P. Gray, M. C. Cook, D. A. Fulcher, J. L. Casanova, E. K. Deenick, and S. G. Tangye. 2012. Functional STAT3 deficiency compromises the generation of human T follicular helper cells. *Blood* 119: 3997-4008.
169. Zhang, Y., A. M. Siegel, G. Sun, T. Dimaggio, A. F. Freeman, and J. D. Milner. 2019. Human T(H)9 differentiation is dependent on signal transducer and activator of transcription (STAT) 3 to restrain STAT1-mediated inhibition. *J Allergy Clin Immunol* 143: 1108-1118.e1104.
170. Kimura, A., T. Naka, and T. Kishimoto. 2007. IL-6-dependent and -independent pathways in the development of interleukin 17-producing T helper cells. *Proc Natl Acad Sci U S A* 104: 12099-12104.
171. Wan, C. K., A. B. Andraski, R. Spolski, P. Li, M. Kazemian, J. Oh, L. Samsel, P. A. Swanson, 2nd, D. B. McGavern, E. P. Sampaio, A. F. Freeman, J. D. Milner, S. M. Holland, and W. J. Leonard. 2015. Opposing roles of STAT1 and STAT3 in IL-21 function in CD4+ T cells. *Proc Natl Acad Sci U S A* 112: 9394-9399.
172. Takeda, K., K. Noguchi, W. Shi, T. Tanaka, M. Matsumoto, N. Yoshida, T. Kishimoto, and S. Akira. 1997. Targeted disruption of the mouse Stat3 gene leads to early embryonic lethality. *Proc Natl Acad Sci U S A* 94: 3801-3804.
173. Myles, I. A., E. D. Anderson, N. J. Earland, K. A. Zarember, I. Sastalla, K. W. Williams, P. Gough, I. N. Moore, S. Ganesan, C. J. Fowler, A. Laurence, M. Garofalo, D. B. Kuhns, M. D. Kieh, A. Saleem, P. A. Welch, D. A. Darnell, J. I. Gallin, A. F. Freeman, S. M. Holland, and S. K. Datta. 2018. TNF overproduction impairs epithelial staphylococcal response in hyper IgE syndrome. *J Clin Invest* 128: 3595-3604.
174. Leiding, J. W., T. P. Vogel, V. G. J. Santarlas, R. Mhaskar, M. R. Smith, A. Carisey, A. Vargas-Hernández, M. Silva-Carmona, M. Heeg, A. Rensing-Ehl, B. Neven, J. Hadjadj, S. Hambleton, T. Ronan Leahy, K. Meesilpavikai, C. Cunningham-Rundles, C. M. Dutmer, S. O. Sharapova, M. Taskinen, I. Chua, R. Hague, C. Klemann, L. Kostyuchenko, T. Morio, A. Thatayatikom, A. Ozen, A. Scherbina, C. S. Bauer, S. E. Flanagan, E. Gambineri, L. Giovannini-Chami, J. Heimall, K. E. Sullivan, E. Allenspach, N. Romberg, S. G. Deane, B. T. Prince, M. J. Rose, J. Bohnsack, T. Mousallem, R. Jesudas, M. M. D. Santos Vilela, M. O'Sullivan, J. Pachlopnik Schmid, Š. Průhová, A. Klocperk, M. Rees, H. Su, S. Bahna, S. Baris, L. M. Bartnikas, A. Chang Berger, T. A. Briggs, S. Brothers, V. Bundy, A. Y. Chan, S. Chandrakasan, M. Christiansen, T. Cole, M. C. Cook, M. M. Desai, U. Fischer, D. A. Fulcher, S. Gallo, A. Gauthier, A. R. Gennery, J. Gonçalo Marques, F. Gottrand, B. Grimbacher, E. Grunebaum, E. Haapaniemi, S. Hämäläinen, K. Heiskanen, T. Heiskanen-Kosma, H. M. Hoffman, L. I. Gonzalez-Granado, A. L. Guerrerio, L. Kainulainen, A. Kumar, M. G. Lawrence, C. Levin, T. Martelius, O. Neth, P. Olbrich, A. Palma, N. C. Patel, T. Pozos, K. Preece, S. O. Lugo Reyes, M. A. Russell, Y. Schejter, C. Seroogy, J. Sinclair, E. Skevofilax, D. Suan, D. Suez, P. Szabolcs, H. Velasco, K. Warnatz, K. Walkovich, A. Worth, M. R. J. Seppänen, T. R. Torgerson, G. Sogkas, S. Ehl, S. G. Tangye, M. A. Cooper, J. D. Milner, and L. R. Forbes Satter. 2023. Monogenic early-onset lymphoproliferation and autoimmunity: Natural history of STAT3 gain-of-function syndrome. *J Allergy Clin Immunol* 151: 1081-1095.

175. Flanagan, S. E., E. Haapaniemi, M. A. Russell, R. Caswell, H. L. Allen, E. De Franco, T. J. McDonald, H. Rajala, A. Ramelius, J. Barton, K. Heiskanen, T. Heiskanen-Kosma, M. Kajosaari, N. P. Murphy, T. Milenkovic, M. Seppänen, Å. Lernmark, S. Mustjoki, T. Otonkoski, J. Kere, N. G. Morgan, S. Ellard, and A. T. Hattersley. 2014. Activating germline mutations in STAT3 cause early-onset multi-organ autoimmune disease. *Nat Genet* 46: 812-814.
176. Haapaniemi, E. M., M. Kaustio, H. L. Rajala, A. J. van Adrichem, L. Kainulainen, V. Glumoff, R. Doffinger, H. Kuusanmäki, T. Heiskanen-Kosma, L. Trotta, S. Chiang, P. Kulmala, S. Eldfors, R. Katainen, S. Siitonen, M. L. Karjalainen-Lindsberg, P. E. Kovanen, T. Otonkoski, K. Porkka, K. Heiskanen, A. Hänninen, Y. T. Bryceson, R. Uusitalo-Seppälä, J. Saarela, M. Seppänen, S. Mustjoki, and J. Kere. 2015. Autoimmunity, hypogammaglobulinemia, lymphoproliferation, and mycobacterial disease in patients with activating mutations in STAT3. *Blood* 125: 639-648.
177. Milner, J. D., T. P. Vogel, L. Forbes, C. A. Ma, A. Stray-Pedersen, J. E. Niemela, J. J. Lyons, K. R. Engelhardt, Y. Zhang, N. Topcagic, E. D. Roberson, H. Matthews, J. W. Verbsky, T. Dasu, A. Vargas-Hernandez, N. Varghese, K. L. McClain, L. B. Karam, K. Nahmod, G. Makedonas, E. M. Mace, H. S. Sorte, G. Perminow, V. K. Rao, M. P. O'Connell, S. Price, H. C. Su, M. Butrick, J. McElwee, J. D. Hughes, J. Willet, D. Swan, Y. Xu, M. Santibanez-Koref, V. Slowik, D. L. Dinwiddie, C. E. Ciaccio, C. J. Saunders, S. Septer, S. F. Kingsmore, A. J. White, A. J. Cant, S. Hambleton, and M. A. Cooper. 2015. Early-onset lymphoproliferation and autoimmunity caused by germline STAT3 gain-of-function mutations. *Blood* 125: 591-599.
178. Fabre, A., S. Marchal, V. Barlogis, B. Mari, P. Barbry, P. S. Rohrlich, L. R. Forbes, T. P. Vogel, and L. Giovannini-Chami. 2019. Clinical Aspects of STAT3 Gain-of-Function Germline Mutations: A Systematic Review. *J Allergy Clin Immunol Pract* 7: 1958-1969.e1959.
179. Korenfeld, D., K. Roussak, S. Dinkel, T. P. Vogel, H. Pollack, J. Levy, J. W. Leiding, J. Milner, M. Cooper, and E. Klechevsky. 2021. STAT3 Gain-of-Function Mutations Underlie Deficiency in Human Nonclassical CD16(+) Monocytes and CD141(+) Dendritic Cells. *J Immunol* 207: 2423-2432.
180. O'Shea, J. J., M. Gadina, and R. D. Schreiber. 2002. Cytokine signaling in 2002: new surprises in the Jak/Stat pathway. *Cell* 109 Suppl: S121-131.
181. Levy, D. E., and C. K. Lee. 2002. What does Stat3 do? *J Clin Invest* 109: 1143-1148.
182. Vogel, T. P., J. W. Leiding, M. A. Cooper, and L. R. Forbes Satter. 2022. STAT3 gain-of-function syndrome. *Front Pediatr* 10: 770077.
183. Gaffen, S. L., R. Jain, A. V. Garg, and D. J. Cua. 2014. The IL-23-IL-17 immune axis: from mechanisms to therapeutic testing. *Nat Rev Immunol* 14: 585-600.
184. Vogel, T. P., J. D. Milner, and M. A. Cooper. 2015. The Ying and Yang of STAT3 in Human Disease. *J Clin Immunol* 35: 615-623.
185. Sakaguchi, S., N. Mikami, J. B. Wing, A. Tanaka, K. Ichiyama, and N. Ohkura. 2020. Regulatory T Cells and Human Disease. *Annu Rev Immunol* 38: 541-566.
186. Khoury, T., V. Molho-Pessach, Y. Ramot, A. R. Ayman, O. Elpeleg, N. Berkman, A. Zlotogorski, and Y. Ilan. 2017. Tocilizumab Promotes Regulatory T-cell Alleviation in STAT3 Gain-of-function-associated Multi-organ Autoimmune Syndrome. *Clin Ther* 39: 444-449.

187. Cai, Y., X. Shen, C. Ding, C. Qi, K. Li, X. Li, V. R. Jala, H. G. Zhang, T. Wang, J. Zheng, and J. Yan. 2011. Pivotal role of dermal IL-17-producing γδ T cells in skin inflammation. *Immunity* 35: 596-610.
188. van der Fits, L., S. Mourits, J. S. Voerman, M. Kant, L. Boon, J. D. Laman, F. Cornelissen, A. M. Mus, E. Florencia, E. P. Prens, and E. Lubberts. 2009. Imiquimod-induced psoriasis-like skin inflammation in mice is mediated via the IL-23/IL-17 axis. *J Immunol* 182: 5836-5845.
189. Flutter, B., and F. O. Nestle. 2013. TLRs to cytokines: mechanistic insights from the imiquimod mouse model of psoriasis. *Eur J Immunol* 43: 3138-3146.
190. Pantelyushin, S., S. Haak, B. Ingold, P. Kulig, F. L. Heppner, A. A. Navarini, and B. Becher. 2012. Rorγt+ innate lymphocytes and γδ T cells initiate psoriasiform plaque formation in mice. *J Clin Invest* 122: 2252-2256.
191. Eyerich, K., V. Dimartino, and A. Cavani. 2017. IL-17 and IL-22 in immunity: Driving protection and pathology. *Eur J Immunol* 47: 607-614.
192. Sumaria, N., B. Roediger, L. G. Ng, J. Qin, R. Pinto, L. L. Cavanagh, E. Shklovskaya, B. Fazekas de St Groth, J. A. Triccas, and W. Weninger. 2011. Cutaneous immunosurveillance by self-renewing dermal gammadelta T cells. *J Exp Med* 208: 505-518.
193. Bogunovic, M., F. Ginhoux, A. Wagers, M. Loubeau, L. M. Isola, L. Lubrano, V. Najfeld, R. G. Phelps, C. Grosskreutz, E. Scigliano, P. S. Frenette, and M. Merad. 2006. Identification of a radio-resistant and cycling dermal dendritic cell population in mice and men. *J Exp Med* 203: 2627-2638.
194. Stoeckius, M., C. Hafemeister, W. Stephenson, B. Houck-Loomis, P. K. Chattopadhyay, H. Swerdlow, R. Satija, and P. Smibert. 2017. Simultaneous epitope and transcriptome measurement in single cells. *Nat Methods* 14: 865-868.
195. Hao, Y., S. Hao, E. Andersen-Nissen, W. M. Mauck, 3rd, S. Zheng, A. Butler, M. J. Lee, A. J. Wilk, C. Darby, M. Zager, P. Hoffman, M. Stoeckius, E. Papalexi, E. P. Mimitou, J. Jain, A. Srivastava, T. Stuart, L. M. Fleming, B. Yeung, A. J. Rogers, J. M. McElrath, C. A. Blish, R. Gottardo, P. Smibert, and R. Satija. 2021. Integrated analysis of multimodal single-cell data. *Cell* 184: 3573-3587.e3529.
196. Kitagawa, Y., N. Ohkura, Y. Kidani, A. Vandenbon, K. Hirota, R. Kawakami, K. Yasuda, D. Motooka, S. Nakamura, M. Kondo, I. Taniuchi, T. Kohwi-Shigematsu, and S. Sakaguchi. 2017. Guidance of regulatory T cell development by Satb1-dependent super-enhancer establishment. *Nat Immunol* 18: 173-183.
197. Van Belle, A. B., M. de Heusch, M. M. Lemaire, E. Hendrickx, G. Warnier, K. Dunussi-Joannopoulos, L. A. Fouser, J. C. Renauld, and L. Dumoutier. 2012. IL-22 is required for imiquimod-induced psoriasiform skin inflammation in mice. *J Immunol* 188: 462-469.
198. Forbes, L. R., T. P. Vogel, M. A. Cooper, J. Castro-Wagner, E. Schussler, K. G. Weinacht, A. S. Plant, H. C. Su, E. J. Allenspach, M. Slatter, M. Abinun, D. Lilic, C. Cunningham-Rundles, O. Eckstein, P. Olbrich, R. P. Guillerman, N. C. Patel, Y. Y. Demirdag, C. Zerbe, A. F. Freeman, S. M. Holland, P. Szabolcs, A. Gennery, T. R. Torgerson, J. D. Milner, and J. W. Leiding. 2018. Jakinibs for the treatment of immune dysregulation in patients with gain-of-function signal transducer and activator of transcription 1 (STAT1) or STAT3 mutations. *J Allergy Clin Immunol* 142: 1665-1669.
199. Morelli, M., C. Scarponi, L. Mercurio, F. Facchiano, S. Pallotta, S. Madonna, G. Girolomoni, and C. Albanesi. 2018. Selective Immunomodulation of Inflammatory

Pathways in Keratinocytes by the Janus Kinase (JAK) Inhibitor Tofacitinib: Implications for the Employment of JAK-Targeting Drugs in Psoriasis. *J Immunol Res* 2018: 7897263.
200. Wienke, J., W. Janssen, R. Scholman, H. Spits, M. van Gijn, M. Boes, J. van Montfrans, N. Moes, and S. de Roock. 2015. A novel human STAT3 mutation presents with autoimmunity involving Th17 hyperactivation. *Oncotarget* 6: 20037-20042.
201. Sutton, C. E., S. J. Lalor, C. M. Sweeney, C. F. Brereton, E. C. Lavelle, and K. H. Mills. 2009. Interleukin-1 and IL-23 induce innate IL-17 production from gammadelta T cells, amplifying Th17 responses and autoimmunity. *Immunity* 31: 331-341.
202. Liang, S. C., X. Y. Tan, D. P. Luxenberg, R. Karim, K. Dunussi-Joannopoulos, M. Collins, and L. A. Fouser. 2006. Interleukin (IL)-22 and IL-17 are coexpressed by Th17 cells and cooperatively enhance expression of antimicrobial peptides. *J Exp Med* 203: 2271-2279.
203. Wolk, K., E. Witte, E. Wallace, W. D. Döcke, S. Kunz, K. Asadullah, H. D. Volk, W. Sterry, and R. Sabat. 2006. IL-22 regulates the expression of genes responsible for antimicrobial defense, cellular differentiation, and mobility in keratinocytes: a potential role in psoriasis. *Eur J Immunol* 36: 1309-1323.
204. Harper, E. G., C. Guo, H. Rizzo, J. V. Lillis, S. E. Kurtz, I. Skorcheva, D. Purdy, E. Fitch, M. Iordanov, and A. Blauvelt. 2009. Th17 cytokines stimulate CCL20 expression in keratinocytes in vitro and in vivo: implications for psoriasis pathogenesis. *J Invest Dermatol* 129: 2175-2183.
205. Wolk, K., S. Kunz, E. Witte, M. Friedrich, K. Asadullah, and R. Sabat. 2004. IL-22 increases the innate immunity of tissues. *Immunity* 21: 241-254.
206. Moos, S., A. N. Mohebiany, A. Waisman, and F. C. Kurschus. 2019. Imiquimod-Induced Psoriasis in Mice Depends on the IL-17 Signaling of Keratinocytes. *J Invest Dermatol* 139: 1110-1117.
207. Tortola, L., E. Rosenwald, B. Abel, H. Blumberg, M. Schäfer, A. J. Coyle, J. C. Renauld, S. Werner, J. Kisielow, and M. Kopf. 2012. Psoriasiform dermatitis is driven by IL-36-mediated DC-keratinocyte crosstalk. *J Clin Invest* 122: 3965-3976.
208. Szabo, S. K., C. Hammerberg, Y. Yoshida, Z. Bata-Csorgo, and K. D. Cooper. 1998. Identification and quantitation of interferon-gamma producing T cells in psoriatic lesions: localization to both CD4+ and CD8+ subsets. *J Invest Dermatol* 111: 1072-1078.
209. Res, P. C., G. Piskin, O. J. de Boer, C. M. van der Loos, P. Teeling, J. D. Bos, and M. B. Teunissen. 2010. Overrepresentation of IL-17A and IL-22 producing CD8 T cells in lesional skin suggests their involvement in the pathogenesis of psoriasis. *PLoS One* 5: e14108.
210. Bovenschen, H. J., P. C. van de Kerkhof, P. E. van Erp, R. Woestenenk, I. Joosten, and H. J. Koenen. 2011. Foxp3+ regulatory T cells of psoriasis patients easily differentiate into IL-17A-producing cells and are found in lesional skin. *J Invest Dermatol* 131: 1853-1860.
211. Shi, Y., Z. Chen, Z. Zhao, Y. Yu, H. Fan, X. Xu, X. Bu, and J. Gu. 2019. IL-21 Induces an Imbalance of Th17/Treg Cells in Moderate-to-Severe Plaque Psoriasis Patients. *Front Immunol* 10: 1865.
212. Zhou, X., S. L. Bailey-Bucktrout, L. T. Jeker, C. Penaranda, M. Martínez-Llordella, M. Ashby, M. Nakayama, W. Rosenthal, and J. A. Bluestone. 2009. Instability of the transcription factor Foxp3 leads to the generation of pathogenic memory T cells in vivo. *Nat Immunol* 10: 1000-1007.

213. Sano, S., K. S. Chan, S. Carbajal, J. Clifford, M. Peavey, K. Kiguchi, S. Itami, B. J. Nickoloff, and J. DiGiovanni. 2005. Stat3 links activated keratinocytes and immunocytes required for development of psoriasis in a novel transgenic mouse model. *Nat Med* 11: 43-49.
214. Miyoshi, K., M. Takaishi, K. Nakajima, M. Ikeda, T. Kanda, M. Tarutani, T. Iiyama, N. Asao, J. DiGiovanni, and S. Sano. 2011. Stat3 as a therapeutic target for the treatment of psoriasis: a clinical feasibility study with STA-21, a Stat3 inhibitor. *J Invest Dermatol* 131: 108-117.
215. Ravipati, A., S. Nolan, M. Alphonse, D. Dikeman, C. Youn, Y. Wang, N. Orlando, G. Patrick, S. Lee, R. V. Ortines, H. Liu, R. J. Miller, C. A. Dillen, M. Marchitto, S. S. Cai, L. S. Miller, and N. K. Archer. 2022. IL-6R/Signal Transducer and Activator of Transcription 3 Signaling in Keratinocytes rather than in T Cells Induces Psoriasis-Like Dermatitis in Mice. *J Invest Dermatol* 142: 1126-1135.e1124.
216. Parham, C., M. Chirica, J. Timans, E. Vaisberg, M. Travis, J. Cheung, S. Pflanz, R. Zhang, K. P. Singh, F. Vega, W. To, J. Wagner, A. M. O'Farrell, T. McClanahan, S. Zurawski, C. Hannum, D. Gorman, D. M. Rennick, R. A. Kastelein, R. de Waal Malefyt, and K. W. Moore. 2002. A receptor for the heterodimeric cytokine IL-23 is composed of IL-12Rbeta1 and a novel cytokine receptor subunit, IL-23R. *J Immunol* 168: 5699-5708.
217. Lejeune, D., L. Dumoutier, S. Constantinescu, W. Kruijer, J. J. Schuringa, and J. C. Renauld. 2002. Interleukin-22 (IL-22) activates the JAK/STAT, ERK, JNK, and p38 MAP kinase pathways in a rat hepatoma cell line. Pathways that are shared with and distinct from IL-10. *J Biol Chem* 277: 33676-33682.
218. Dudakov, J. A., A. M. Hanash, and M. R. van den Brink. 2015. Interleukin-22: immunobiology and pathology. *Annu Rev Immunol* 33: 747-785.
219. Di Maggio, F. M., L. Minafra, G. I. Forte, F. P. Cammarata, D. Lio, C. Messa, M. C. Gilardi, and V. Bravatà. 2015. Portrait of inflammatory response to ionizing radiation treatment. *J Inflamm (Lond)* 12: 14.
220. Mihi, B., Q. Gong, L. S. Nolan, S. E. Gale, M. Goree, E. Hu, W. E. Lanik, J. M. Rimer, V. Liu, O. B. Parks, A. N. Lewis, P. Agrawal, M. L. Laury, P. Kumar, E. Huang, S. S. Bidani, C. J. Luke, J. K. Kolls, and M. Good. 2021. Interleukin-22 signaling attenuates necrotizing enterocolitis by promoting epithelial cell regeneration. *Cell Rep Med* 2: 100320.
221. Borcherding, N., N. L. Bormann, and G. Kraus. 2020. scRepertoire: An R-based toolkit for single-cell immune receptor analysis. *F1000Res* 9: 47.
222. Boniface, K., F. X. Bernard, M. Garcia, A. L. Gurney, J. C. Lecron, and F. Morel. 2005. IL-22 inhibits epidermal differentiation and induces proinflammatory gene expression and migration of human keratinocytes. *J Immunol* 174: 3695-3702.