Role of GPR109A in psoriasis

Leko, Lucija

Sveučilište u Zagrebu

Prehrambeno – biotehnološki fakultet

Molekularna Biotehnologija

Diplomski rad

Uloga GPR109A receptora u psorijazi

Lucija Leko
0058214683

Institut: Laboratorij za eksperimentalnu I molekularnu imunologiju i neurogenetiku, INEM – UMR 7355, CNRS, Orleans, France

Mentor: prof. dr. sc. Bernhard Ryffel, INEM, Orleans

Zagreb, lipanj 2023.

Université d'Orléans - Université de Zagreb

UFR Sciences et Techniques- Faculté de Nutrition et Biotechnologie -

Faculté des Sciences

MASTER SCIENCES DU VIVANT

Spécialité : Techniques Bio-Industrielles

INTERNSHIP REPORT

Role of GPR109A receptor in Psoriasis

By

Lucija Leko

(February 2023 – June 2023)

Internship institution and address: Immunologie Neurogénétique Expérimentales et Moléculaires, UMR7355 INEM, 45071 ORLEANS CEDEX 2, Group Immune response to infections and pollutants

Mentor/Supervisor: Bernhard Ryffel, scientist, M.D., Ph.D.

Zagreb, June 2023

TEMELJNA DOKUMENTACIJSKA KARTICA

Diplomski rad

Sveučilište u Zagrebu
Prehrambeno-biotehnološki fakultet
Diplomski sveučilišni studij Molekularna Biotehnologija

Znanstveno područje: Biotehnološke znanosti
Znanstveno polje: Biotehnologija

Uloga GPR109A receptora u psorijazi

Lucija Leko, 0058214683

Rad je izrađen: Laboratorij za eksperimentalnu i molekularnu imunologiju i neurogenetiku (INEM – UMR 7355, CNRS, Orléans France)
Mentor: *prof. dr. sc. Bernhard Ryffel,* INEM, Orléans

Sažetak: Psorijaza je kronična, upalna, kožna bolest karakterizirana hiperproliferacijom keratinocita i infiltracijom stanica imunološkog sustava u kožu. U ovom istraživanju, bioinformatička analiza je pokazala da je HCAR2, ljudski gen koji kodira za receptor kratkolančanih masnih kiselina GPR109A, izražen u keratinocitima i makrofagima u oštećenoj koži pacijenata s psorijazom. Imunohistokemijsko bojanje u mišjem imikvimod (IMQ) tretiranom modelu pokazalo je da je GPR109A prisutan u keratinocitima i infiltracijskim stanicama imunološkog sustava dermisa. Pokazali smo da je ekspresija gena Hcar2 izmjenjena u mišjem IMQ tretiranom modelu te da miševi koji nemaju GPR109A imaju pogoršan psorijatični fenotip. Razine upalnih medijatora poput MPO, LCN2 i IL-1β su povišene zajedno s ekspresijom Il-17a, što sve ukazuje na zaštitnu ulogu GPR109A u psorijazi. Daljnja istraživanja trebala bi se usredotočiti na upotrebu agonista GPR109A, poput butirata, kao potencijalne terapije u liječenju psorijaze.

Ključne riječi: psorijaza, imikvimod (IMQ) mišji model, GPR109A, *Hcar2*, upala kože
Rad sadrži: 30 stranica, 12 slika, 1 tablicu, 32 literaturna navoda
Jezik izvornika: engleski

Rad je u tiskanom i elektroničkom obliku (pdf format) pohranjen u knjižnici Prehrambeno-biotehnološkog fakulteta Sveučilišta u Zagrebu, Kačićeva 23, 10 000, Zagreb
Stručno povjerenstvo za obranu:

1. Prof. dr. sc. *Igor Stuparević*
2. Prof. dr. sc. *Višnja Besendorfer*
3. Izv. prof. dr. sc. *Kristina Radošević*
4. Prof. dr. sc. *Josef Hamacek*
5. Prof. dr. sc. *Aurelie Pelletier*
6. Prof. dr. sc. *Bernhard Ryffel*

Datum obrane: 3. srpnja 2023.

BASIC DOCUMENTATION CARD

Master Thesis

University of Zagreb
Faculty of Food Technology and Biotechnology
University graduate study Molecular Biotechnology

Scientific area: Biotechnical Sciences
Scientific field: Biotechnology

Role of GPR109A in Psoriasis

Lucija Leko, 0058214683

Thesis performed at: Laboratory of experimental and molecular immunology and neurogenetics (INEM – UMR 7355, CNRS, Orleans, France)
Supervisor: PhD *Bernhard Ryffel*, INEM, Orleans

Abstract: Psoriasis is a chronic, inflammatory skin disease characterized by keratinocyte hyperproliferation and immune cell infiltration. In this study, bioinformatic analysis showed that HCAR2, human gene encoding a short-chain fatty acid receptor GPR109A, was expressed in keratinocytes and macrophages in lesioned skin of psoriatic patients. In imiquimod (IMQ) induced mouse model immunostaining showed GPR109A to be located in keratinocytes and dermal inflammatory cells. We showed that Hcar2 expression was affected in IMQ-induced mouse model and that mice lacking GPR109A had an aggravated psoriasis-like phenotype. Levels of inflammatory mediators like MPO, LCN2 and IL-1β were elevated along with Il-17a expression, all indicating GPR109A has a protective role in psoriasis. Further research should focus on using GPR109A agonists, such as butyrate, as potential therapeutics in treatment of psoriasis.

Keywords: psoriasis, imiquimod (IMQ) mouse model, GPR109A, *Hcar2*, skin inflammation
Thesis contains: 30 pages, 12 figures, 1 table, 32 references
Original in: english

Thesis is in printed and electronic form deposited in the library of the Faculty of Food Technology and Biotechnology, University of Zagreb, Kačićeva 23, 10 000 Zagreb

Reviewers:

1. PhD. *Višnja Besendorfer,* Full professor
2. PhD *Igor Stuparević,* Full professor
3. PhD *Kristina Radošević*, Associated professor
4. PhD *Josef Hamacek,* Full professor
5. PhD *Aurelie Pelletier,* Full professor
6. PhD *Bernhard Ryffel,* Professor emeritus

Defence date: July 3rd 2023

PRESENTATION OF THE LABORATORY

The experimental part of this master thesis project took place at the Laboratory of Experimental and Molecular Immunology and Neurogenetics, UMR7355 INEM (fr. *Laboratoire d'Immunologie et Neurogénétique Expérimentales et Moléculaires*) located in Orléans. INEM is a research unit of the French National Centre for Scientific Research, CNRS (fr. *Le Centre national de la recherche scientifique*) associated with the University of Orléans in France. The laboratory is divided into two research groups: Immune responses to infections and pollutants and Neurogenetics and developmental neurotoxicity which together make up four different teams: Allergy, respiratory infection, and immunity; Inflammation, danger signals, infection, and lung pathologies; Neurogenetics; Developmental Neurotoxicity. The scientific objectives of the institute are to study molecular and cellular mechanisms involved in innate immune-inflammatory response to environmental challenges and infection of the lung and skin, as well as genetics of mental disabilities, and neurotoxicity processes during development. Artimmune is a spin-off contract research company of INEM that offers consulting and preclinical research services in immunology and inflammation with an emphasis on lung diseases, emanating from INEM since 2010. As a result of this partnership, a joint team ArtInem was formed in 2018; their focus is on the development of preclinical investigation models and identification of new therapeutic targets in pulmonary, cutaneous, intestinal, and neuroinflammation.

During my internship, experiments were done within the group ''Immune responses to infections and pollutants'' led by Dr. sc. Valérie Quesniaux. The focus of the group is on understanding the host's response to environmental exposures and infections at the molecular and cellular levels, the inflammatory pathways such as cGAS/STING and the role of inflammatory cytokines such as interleukin 1 (IL-1) family, IL-1-IL-17 axis, tumor necrosis factor α (TNF- α) family and other mediators in regulating the responses in mice and cells using inhibitors and genetically modified mice.

ABSTRACT

Psoriasis is a chronic, inflammatory skin disease characterized by keratinocyte hyperproliferation and immune cell infiltration accompanied by a disbalance in the levels of inflammatory mediators. In this study, bioinformatic analysis showed that *HCAR2*, human gene encoding a short-chain fatty acid receptor GPR109A, was expressed in keratinocytes and macrophages in lesioned skin of psoriatic patients. In imiquimod (IMQ) induced mouse model immunostaining showed GPR109A to be located in keratinocytes and dermal inflammatory cells. We showed that *Hcar2* expression was affected in IMQ-induced mouse model and that mice lacking GPR109A had an aggravated psoriasis-like phenotype with exacerbated epidermal hyperplasia and dermal inflammatory cell infiltration. Levels of inflammatory mediators like MPO, LCN2 and IL-1β were elevated along with *Il-17a* expression, all indicating GPR109A has a protective role in psoriasis. Further research should focus on using GPR109A agonists, such as butyrate, as potential therapeutics in treatment of psoriasis.

Keywords: psoriasis, imiquimod mouse model, GPR109A, *Hcar2*, skin inflammation

RÉSUMÉ

Le psoriasis est une maladie inflammatoire chronique de la peau caractérisée par une hyperprolifération des kératinocytes et une infiltration de cellules immunitaires accompagnée d'un déséquilibre des niveaux de médiateurs inflammatoires. Dans cette étude, l'analyse bioinformatique a montré que *HCAR2*, gène humain codant pour un récepteur d'acide gras à chaîne courte GPR109A, était exprimé dans les kératinocytes et les macrophages de la peau lésée de patients psoriasiques. Dans le modèle de souris induit par l'imiquimod (IMQ), l'immunomarquage a montré que GPR109A était localisé dans les kératinocytes et les cellules inflammatoires dermiques. Nous avons montré que l'expression de *Hcar2* était affectée dans le modèle de souris induit par l'IMQ et que les souris dépourvues de GPR109A présentaient un phénotype de psoriasis aggravé avec une hyperplasie épidermique exacerbée et une infiltration de cellules inflammatoires dermiques. Les niveaux de médiateurs inflammatoires tels que MPO, LCN2 et IL-1β étaient élevés, de même que l'expression de l'*IL-17a*, ce qui indique que le GPR109A joue un rôle protecteur dans le psoriasis. Des recherches supplémentaires devraient se concentrer sur l'utilisation d'agonistes de GPR109A, tels que le butyrate, en tant que traitements potentiels du psoriasis.

Mots clés : psoriasis, modèle murin imiquimod, GPR109A, *Hcar2*, inflammation cutanée

Firstly, I want to express my appreciation to Dr. sc. Valérie Quesniaux for accepting me into her group. I would like to thank my mentor, professor Bernhard Ryffel, for enabling me to acquire new knowledge and skills as a part of his team, and for all the kind words and help during my internship.

I would like to thank the whole INEM team for making me feel welcome and all their help. My sincere thanks go to Timna and Florence, for teaching me new skills and helping me every day. I am grateful to my Croatian colleagues, who shared this experience with me and made the last six months into memories I will always cherish. I would also like to thank my friends - Noor, Ana and Lucija for all your support.

My heartfelt thanks go to my aunts, uncles and grandparents for their support. And lastly, my deepest gratitude goes to my family – my mom, dad and brothers. Thank you for all the love and encouragement throughout the years, for always picking me up when I was feeling down and pushing me to go on.

TABLE OF CONTENT:

ABREVIATIONS:

AMPs - antimicrobial peptides

BSA – bovine serum albumin

DCs – dendritic cells

H&E – hematoxylin and eosin

ILCs - innate lymphoid cells

IMQ – imiquimod

KO – knock out

K5 – keratin 5

K6 – keratin 6

K14 – keratin 14

K17 – keratin 17 (protein)

KRT17 – keratin 17 (gene)

LCN2 – lipocalin 2

LCs - Langerhans cells

mDCs - myeloid dendritic cells

MMF - monomethylfumarate

MPO – myeloperoxidase

PCA - principal component analysis

pDCs - plasmacytoid dendritic cells

PsA - psoriatic arthritis

RFP – red fluorescent protein

ROS - reactive oxygen species

SCFAs - short-chain fatty acids

scRNA-seq - Single cell RNA sequencing analysis

SFV – bovine fetal serum

TBS – Tris-buffer saline

TBST – Tris-buffer saline Tween

TCR – T cell receptor

TLR - toll-like receptor

Tregs – regulatory T cells

T-PER – tissue protein extraction reagent

UMAP - uniform manifold approximation and projection

WT – wild type

1. INTRODUCTION

1.1 Structure and function of skin

Skin is the largest human organ that serves as body's first line of defence against external factors like microorganisms, chemicals, and physical agents (Kabashima et al., 2019). It is comprised of multiple layers: epidermis, dermis and hypodermis (Figure 1). Epidermis is the surface layer; it does not have blood vessels and thus depends on dermis to provide nutrients. Keratinocytes are the primary cells of the epidermis, which is divided in 4 layers; the basal cell layer (stratum basale), the spinous or squamous cell layer (stratum spinosum), the granular cell layer (stratum granulosum), and the cornified or horny cell layer (stratum corneum) (Jiang et al., 2020). The epidermal structure limits entry and exit of water and enables animals' life outside of water. Langerhans cells (LCs) are situated among the keratinocytes. LCs are antigen presenting cells and subset of tissue-resident macrophages that develop dendritic cells (DC)-phenotype upon differentiation in the skin. Two subsets of T cells are also located in epidermis; γδ T cells are innate immune cells found in mice, but not human epidermis, and CD8+ resident memory T (T_{RM}) cells which are non-circulating memory T cells resulting from skin inflammation (Kabashima et al., 2019).

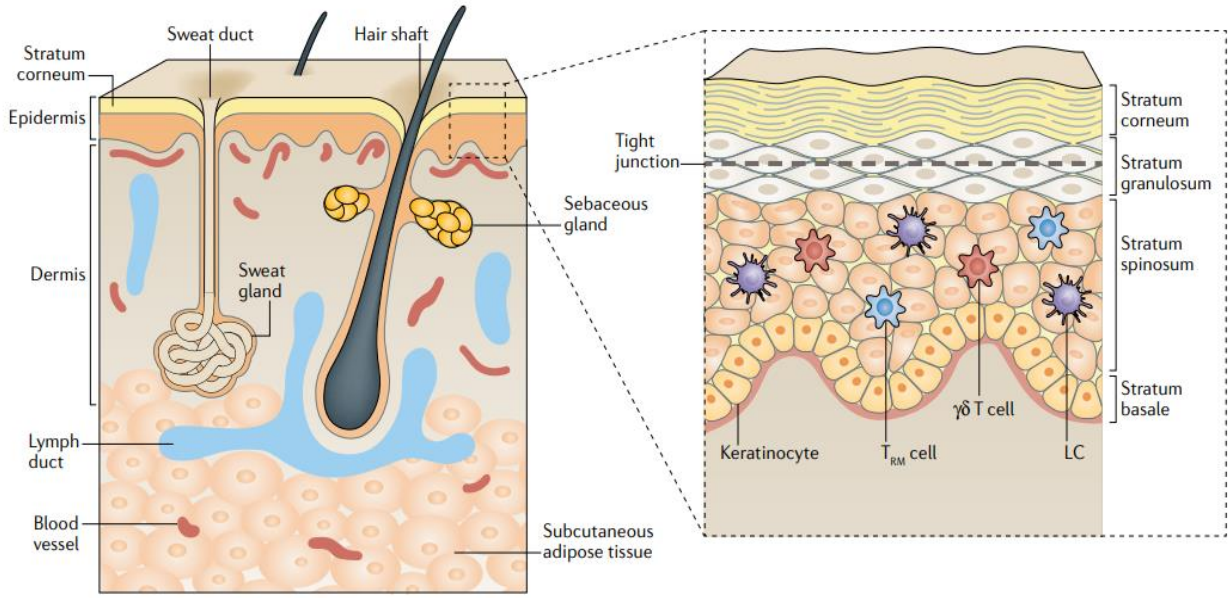

Figure 1. Structure of the skin (Kabashima et al., 2019)

Dermis is the intermediate layer of skin and consists of collagen, elastic tissue, other extracellular compounds, blood and lymphatic vessels, nerve endings, hair follicles and sweat glands. The primary cells are fibroblasts. It is the most prominent component of the skin responsible for flexibility and strength, while also helping thermal regulation and sensation in the skin (Jiang et al., 2020). Dermal DCs, macrophages, mast cells, γδ T cells and innate lymphoid cells (ILCs) can all be found in dermis. A few sentinel cells such as neutrophils, monocytes and αβ T cells will circulate dermis looking for pathogens. Upon inflammation, immune cells quickly accumulate (Kabashima et al., 2019). Subcutis, or hypodermis, is the innermost layer of skin, it stores fat and protects inner organs as a shock absorber (Jiang et al., 2020).

The barrier function of skin relies on keratinocytes and tight junctions, with dynamic communication between all cell types via mediators like cytokines, chemokines and growth factors enabling defence. Keratinocytes are major producers of inflammatory cytokines inducing keratinocyte proliferation and migration of leukocytes into skin, occurrences associated with diseases like psoriasis. They are also the main producers of antimicrobial peptides (AMPs), including defensins, cathelicidin (LL37), and psoriasin (S100A7). AMPs provide resistance to infections and have a vital role in skin homeostasis, as shown by diseases like atopic dermatitis and psoriasis where AMP levels are dysregulated (Jiang et al., 2020).

1.2 Psoriasis

Psoriasis is a chronic, inflammatory, autoimmune skin disease caused by environmental and genetic factors. Around 125 million people are affected globally, with bigger prevalence in western countries where psoriasis affects 2-4% of the population. Patient's quality of life is greatly impacted by the disease, making it a critical concern despite its low mortality rate (Zhou et al., 2022). Mean age of onset is 33 years, with the same percentage of occurrence in men and women. However, women are more likely to develop early onset psoriasis, which develops before the age of 40. Late onset psoriasis makes up only 25% of the cases and develops after the age of 40 (Griffiths et al., 2021). Patients experience multiple comorbidities including hyperlipidaemia, hypertension, coronary artery disease and type 2 diabetes. Up to 40% of patients develop inflammation of the joints resulting in psoriatic

arthritis (PsA) with skin manifestations preceding PsA up to 10 years (Rendon & Schäkel, 2019).

Clinical phenotypes of psoriasis can differ with the most common one being psoriasis vulgaris, which is recognised by chronic, well differentiated pink plaques covered in white scales on white skin and grey plaques on black skin. Plaques differ in thickness and size, with characteristic symmetry and ring-shaped appearance. Knees, elbows, back and scalp are common areas of occurrence. Other subtypes of the disease are guttate, erythrodermic and pustular psoriasis, all of them being much less common (Griffiths et al., 2021).

1.2.1. Pathophysiology of the disease

Psoriasis is characterized by infiltration of immune cells into the skin and increased epidermal thickness caused by abnormal keratinocyte activation. Keratinocyte derived factors, like antimicrobial peptides, have a crucial role in pathogenesis of psoriasis through activation of dendritic cells and macrophages (Silva de Malo et al., 2023). Recruited infiltrating macrophages are divided into two types; M1 macrophage produce cytokines like TNF-α, IL-1β and IL-23 while M2 macrophages produce anti-inflammatory IL-10 and TGF-β. Excessive activation of TLR 7/8 in psoriasis greatly affects M1/M2 ratio in favour of M1 macrophages (Kamata & Tada, 2022). Activated plasmacytoid dendritic cells (pDCs) promote maturation of myeloid dendritic cells (mDCs), as well as production of TNF-α, IL-12, and IL-23. This results in the activation of T helper cells (Th1 and Th17) which produce inflammatory cytokines. Keratinocytes, which play a role in the onset and maintenance of psoriasis, are activated by cytokines like IL-17, IL-21, IL-22 and start producing other cytokines, chemokines, and antimicrobial peptides. Numerous chemokines (like CXCL 1/2/3, CXCL 8, CXCL 9/10/11) recruit leukocytes like neutrophils and macrophages into the skin, along with other mediators, amplifying the inflammation (Zhou et al., 2022). Th17/Tregs balance is also impaired in the disease. Skin resident Tregs play a significant role in skin homeostasis with $Foxp3^+$ Tregs making up 20% to 40% of CD4+ T cells in skin. In psoriasis, Tregs are dysfunctional, failing to supress effector T cell responses and proliferation. Exposure to high levels of IL-6 leads to decreased Treg activity, along with microRNA (miR)-210, which inhibits $Foxp3^+$, being increased in CD4+ T cells resulting in decreased levels of suppressive cytokines like IL-10 and increased levels of pro-inflammatory cytokines like IL-17A. Studies also show

that a subset of Tregs in the skin can differentiate into IL-17A producing cells with $IL17A^{+}Foxp3^{+}CD4^{+}$ T cells present in skin (Pietraforte & Frasca, 2023). Psoriatic neutrophils also contribute to the pathogenesis, during degranulation proteases like myeloperoxidase (MPO), cathepsin G and proteinase 3 participate in generation of reactive oxygen species (ROS), proteolytic activation of inflammatory mediators and formation of autoantigens (Chiang et al., 2019). Psoriasis is accompanied by changes of expression in multiple gene pathways. For example, interferon signalling, TCR signalling and TLR signalling are upregulated while fatty acid metabolism is downregulated. Antimicrobial peptides were shown to be the most up-regulated pathway in multiple studies, enriched by genes like *LCN2* (lipocalin 2) and S100 family (Silva de Malo et al., 2023).

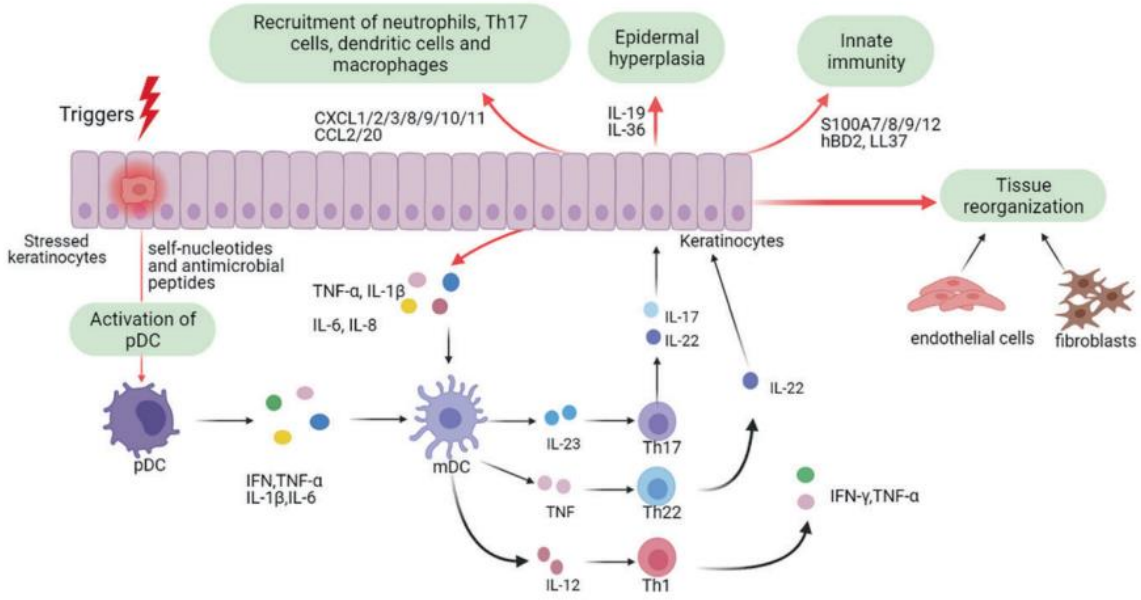

Figure 2. Pathogenesis of psoriasis (Zhou et al., 2022)

1.2.2 Diagnosis and treatment

Clinical diagnosis of psoriasis is based on the history, morphology, and distribution of skin lesions. Looking into family history of psoriasis, potential triggers, onset of symptoms and a thorough skin examination are performed. Heritability is the main risk factor. Genome-wide assessments have identified over 80 risk loci across the genome (Griffiths et al., 2021). Most significant is the HLA-C*06:02 allele, which is linked with early onset psoriasis and is present in over 60% of patients. It increases the risk for psoriasis by 9 to 23-fold (Rendon & Schäkel, 2019). The disease is triggered by environmental factors like stress, infection, alcohol

consumption, smoking, weight gain, obesity and in some cases even sunlight (Griffiths et al., 2021).

The International Psoriasis Counsil differentiates two groups of patients: eligible for topical therapy and eligible for systemic therapy. To be suitable for systemic therapy patient need to have more than 10% of body surface area affected, develop psoriasis at special sites (scalp, face, palms) or be non-responsive to topical therapy. There are several types of therapy. Topical treatments include corticosteroids, vitamin D, analogues, calcineurin inhibitors such as cyclosporine A, keratolytics and combinations of topical agents. Main drawback is poor adherence to treatment (Griffiths et al., 2021). Oral drugs like methotrexate, cyclosporine A, fumaric acid esters and retinoids are used for systemic treatment. These drugs function as immunomodulators and require close clinical monitoring. New possibilities for treatment of psoriasis come from biologics, engineered recombinant monoclonal antibodies and receptor fusion proteins. Biologics are administrated subcutaneously and target specific pathways, in psoriasis that is the IL-23/Th17 axis and TNF-α-signalling (Rendon & Schäkel, 2019). There are currently eleven biologics in use: four anti- TNF-α, three anti-IL-17, four anti-IL-23. Although they are highly effective, not all patients respond in the same way (Griffiths et al., 2021)

1.3 GPR109A

The GPR109A/HCAR2/NIACR1 receptor is a transmembrane G protein-coupled receptor that binds to nicotinic acid (niacin) and short-chain fatty acids (SCFAs), such as butyrate. Encoding gene is referred to as *HCAR2*. The receptor is implicated in a variety of diseases, including multiple sclerosis, Parkinson's disease and colon cancer (Yang et al., 2023). It also has a potential role in atopic dermatitis and psoriasis (Krejner et al., 2018; Schwarz et al., 2017). GPR109A is expressed in white and brown adipose tissue, keratinocytes and various immune cells including monocytes, macrophages, neutrophils and dendritic cells including Langerhans cells (Hanson et al., 2012). Monomethylfumarate (MMF), the active metabolite of Fumaderm (Biogen), a drug used to treat moderate-to-severe psoriasis, is another potent GPR109A agonist, which suggests that GPR109A may be a potential target for Fumaderm (Tang et al., 2008).

Gut-skin axis is a newer concept referring to the interactive relationship between the microbiomes of skin and gut, with increasing evidence that the axis has a significant role in skin homeostasis and diseases. Gut microbiota generates SCFAs during fibre fermentation, those include acetate, propionate, butyrate and isobutyrate. SCFAs activate cells through G-protein-coupled receptors GPR41, GPR43 and GPR109A (Xiao et al., 2023). GPR109A activated antigen presenting cells of the colon exert anti-inflammatory effect resulting in expansion of Tregs and IL-10 producing T cells. This is supported by GPR109A knockout mice being more susceptible to colitis and colon cancer (Singh et al., 2014). Commensals also have a role in regulating inflammation in areas other than gut, including respiratory and urogenital tract, the oral cavity, and the skin (Belkaid & Naik, 2013). Since skin commensals also produce SCFAs it is possible they have a similar role in the skin. Topically applied sodium butyrate suppressed allergic contact dermatitis in mice with the effect being associated with induction of Treg cells (Schwarz et al., 2017). Those discoveries make GPR109A an interesting target in the skin for further research.

1.4 Objectives

Psoriasis is a chronic skin disease that greatly impacts patients' quality of life. Understanding the dysfunction of immune system that occurs in patients is a key to discovering novel therapies. While a lot has been discovered in pathophysiology of the disease, potential therapeutic targets are still being researched. Recently, the focus of the team led by dr. sc. Bernhard Ryffel, where I performed my internship, has been researching the role of cytosolic DNA receptor STING and AIM2 inflammasome in the onset of psoriasis. While results for both research targets have shown promise, the main objective of this study was to evaluate if GPR109A receptor plays a role in the inflammatory pathway of imiquimod (IMQ) induced psoriasis in mice and if it has potential to be a therapeutic target.

The specific objectives of the research were:

(i) Evaluate kinetics of GPR109A and selected cytokines expression in experimental IMQ induced psoriasis using qPCR and interrogate human public data bases.

(ii) Confirm the involvement of GPR109A receptor in the disease using GPR109A deficient mice by investigating clinical signs of psoriasis, inflammatory cell infiltration of the skin and mediators in comparison with wild-type mice.

(iii) Analyse the cellular GPR109A localization in the skin by immunofluorescence.

2. MATERIALS AND METHODS

2.1 Single cell RNA sequencing analysis (scRNA-seq)

We re-analyzed single-cell transcriptomic data (GSE150672) from Human Psoriatic skin and controls skin (Hughes et al., 2020). The dataset was downloaded and the RDS file was imported into R (R Core Team, 2023) environment version v4.2.3 and Seurat v4.1.1 (Hao et al., 2021) by filtering genes expressed in at least 200 cells and cells expressing at least 3 genes. For the pre-processing step, outlier cells were filtered out based on three metrics (nCount_RNA < 40000, nFeature_RNA >= 3 & nFeature_RNA < 4000 and mitochondrial percentage expression <40). The top 3,000 variable genes were then identified using the 'vst' method using the *FindVariableFeatures* function. Percent of mitochondrial genes was regressed out in the scaling step, and Principal Component Analysis (PCA) was performed using the top 3,000 variable genes and the top 30 PCs were selected for dimension reduction by Uniform Manifold Approximation and Projection (UMAP). Clusters were identified using the authors annotation. Then, differential gene expression analysis was performed using *FindAllMarkers* function in Seurat with default parameters to obtain a list of significant gene markers for each cluster of cells. Visualization of genes illustrating expression levels was performed using R/Seurat commands (DimPlot, FeaturePlot and DotPlot) using ggplot2 (Wickham, 2016) and scCustomize (Marsh, 2023) R packages.

2.2 Mouse model and treatments

Imiquimod (IMQ) induced model of skin inflammation is a widely used murine model for preclinical studies of psoriasis proposed by van der Fits et al. in 2009. IMQ is daily topically applied to the back and/or ear skin of mice inducing localized skin and systemic inflammation through activation of toll-like receptor (TLR) 7/8. The inducible inflammatory events mirror human psoriasis, including psoriasis-like histologic features, activation of proinflammatory pathways central to human psoriasis and recruitment of similar cellular infiltrates (Hawkes et al., 2017).

C57BL/6J wild type (BL6/WT), GPR109A-mRFP and GRP109A knock out (KO) mice were breed and housed at UPS44-TAAM (CNRS, Orleans, France) temperature-controlled animal facility and were given free access to food and water. To induce psoriasis-like inflammation, mice were treated with 20 mg of IMQ cream (Aldara 5%) on each ear from day 0 to day 5 of the experiment. Control groups were treated with 20 mg of Vaseline on each ear. Every day of treatment body weight and ear thickness were measured. Ear appearance was examined to determine the inflammation score by two parameters: erythema and scaling (Table 1). On day 6, mice were euthanized by CO_2 inhalation. Necropsy was performed in which ears were taken for one of the following analyses: histology, immunofluorescence, ELISA, PCR.

To analyse the kinetics of *Hcar2* (encoding gene for GPR109A) expression, 7 groups of C57BL/6J wild type (WT) mice were treated with IMQ different number of days, from 0 to 6, after which ear samples were taken for extraction of RNA.

Table 1. Correlation between inflammation score values and erythema and scaling of ear skin

Score	Erythema	Scaling
0	No redness	No peeling skin
1	Mild redness	Mild peeling skin
2	Moderate redness	Moderate peeling skin
3	Severe redness	Severe peeling skin
4	Very severe redness	Very severe peeling skin

2.3 Histology, Hematoxylin and Eosin staining (H&E)

Dissected ears were stored in 10% formalin at 4°C before being embedded in paraffin. Tissue blocks were used to prepare 3 µm thick tissue sections with microtome (Leica Biosystems, USA), which were then used for H&E staining. Slides were deparaffinized in xylene for 10 minutes and then submerged in 100%, 95% and 75% ethanol baths for one minute in each. Samples were then stained in hematoxylin for 2 minutes to stain nuclei and in eosin for 4 minutes to stain cytoplasm. After each staining, slides were washed with tap water for 5 minutes to remove stain residue and rinsed with deionized water for another minute. Finally,

dehydration was performed by submerging the slides in 75%, 95% and 100% ethanol baths for one minute each and then in xylene for another minute. Samples were mounted with a mounting medium (Eukitt®) and scanned by NanoZoomer NDP scan 1.0.9. Acquired histology photos were analysed in NDP view 2.0 software (Hamamatsu Photonics K.K., Japan).

2.4 Immunofluorescence

Dissected ears were stored in 10% formalin at 4°C before frozen embedding in OCT embedding matrix. Frozen blocks were used to prepare 10 µm thick tissue sections with cryostat (Leica Biosystems, USA). Slides were used to analyse GPR109A localization with immunofluorescence. Anti-RFP antibody (1:100, AbcamTM) was used as a primary antibody for staining of ear skin tissue of GPR109A-mRFP mice. Anti-rabbit IgG Alexa Flour 532 (1:500, Invitrogen) was used as a secondary antibody. Tissue was blocked by washing in TBST Wash buffer two times for five minutes and incubating with block solution (TBS-1% BSA buffer, 10% SFV) for an hour in a stain tray. Antibody staining was performed by removing block solution from slides, covering the tissue with antibody diluted in block solution and incubating over night at 4°C in a stain tray. Next day, slides were washed in TBST wash buffer three times for five minutes, secondary antibody was applied, and slides were incubated for 60 minutes at room temperature. Slides were again washed in TBST wash buffer three times. To mount the slides, excess liquid was removed from slides and tissue was covered with DAPI solution for 10 minutes. Slides were again washed twice in TBST wash buffer and once in deionized waters. Coverslips were placed using mounting medium and slides were left to dry and stored in the cold room. Slides were scanned with laser scanning confocal microscope (Zeiss) and pictures were analysed in ImageJ program (Schneider et al., 2012).

2.5 RNA extraction

After necropsy, ear samples were quickly frozen on dry ice. RNA extraction was then performed using RNeasy Plus Mini Kit (Qiagen). To perform tissue homogenization, ear samples were transferred to new Precellys® (Bertin Technologies) 2 mL tubes with 600 µL of RLT buffer (to which was added β-mercaptoethanol). Tissue was cut into smaller pieces with scissors and 6 beads (2.8 mm diameter) were added to tubes. Homogenization was then performed using Precellys® Evolution Touch homogenizer (Bertin Technologies) at 6500 rpm,

3x30 sec with 15 sec breaks in between cycles. Lysate was centrifuged at 10000 rpm for 3 minutes and supernatant was transferred. RNA extraction was then performed according to the kit manufacturer's instructions.

2.6 Reverse Transcription

RNA concentration was determined using Nanodrop One (Thermo Scientific). Reverse Transcription was performed after determining the concentration of extracted RNA using GoScriptTM Reverse Transcription System kit (Promega), according to the manufacturer's instructions. The reaction was carried out in Veriti Thermal Cycler, 96-well (Applied Biosystems).

2.7 qPCR

qPCR was used to amplify *Il17*, *Krt17* and *Hcar2* genes. *Gapdh* housekeeping gene was used to normalize data. Reaction was performed using GoTaq qPCR Master Mix kit (Promega), according to the manufacturer's instructions. Primers used in the reaction were ordered from Qiagen. The reaction was carried out in AriaMx Real-Time PCR System (Agilent Technologies).

2.8 Protein extraction and protein dosage

Protein extraction was performed with tissue protein extraction reagent – T-PER (Thermo Scientific™) and Halt™ Protease and Phosphatase Inhibitor Cocktail (1/100, Thermo Scientific™) using Precellys® Evolution Touch homogenizer (Bertin Technologies). Ear samples, which were previously stored at -20°C, were cut into tiny pieces with scissors in 2 mL Precellys tubes filled with 500 µL of extraction medium and 3 beads (2.8 mm diameter). Tissue was homogenized at 6500 rpm, 3x30 sec. Obtained samples were centrifuged at 10000 rpm, 4°C for 10 minutes. Supernatant was transferred to a plate and stored at –20°C. Protein concentration was analysed following the instruction received in Pierce BCA Protein Assay Kit (Thermo ScientificTM). Absorbance was measured at 562 nm with CLARIOStar Microplate reader (BMG Labtech).

2.9 ELISA dosage

Obtained proteins were used to measure the levels of cytokines IL-1β and IL-10 as well as proteins myeloperoxidase (MPO) and lipocalin 2 (LCN2). Levels were analysed by ELISA assay kits (DuoSet®, R&D Systems) according to the manufacturer's instructions. Absorbance was measured at 450 nm by ELX800TM Absorbance Microplate reader (BioTek).

2.10 Statistical analysis

All the data were analysed by GraphPad Prism 9 (GraphPad Software Inc., San Diego, CA, USA). Ordinary One-Way ANOVA and 2Way ANOVA tests was performed, and all the values are expressed in the form of mean ± SEM. Statistical significance was defined at a p-value *** < 0.001, ** < 0.01, and * < 0.05. Statistical differences between the experimental groups are represented by a star on the top of the column.

3. RESULTS

3.1 Analysis of *GPR109A/HCAR2* gene in the scRNA-Seq of lesioned and non-lesioned skin of psoriatic patients

In order to investigate the role of GPR109A (encoding gene *HCAR2*) signaling for the pathophysiology of psoriasis, initially we assessed a previously published database containing single-cell transcriptome of non-lesioned and lesioned skin from psoriasis patients and re-analysed these data (Hughes et al., 2020).

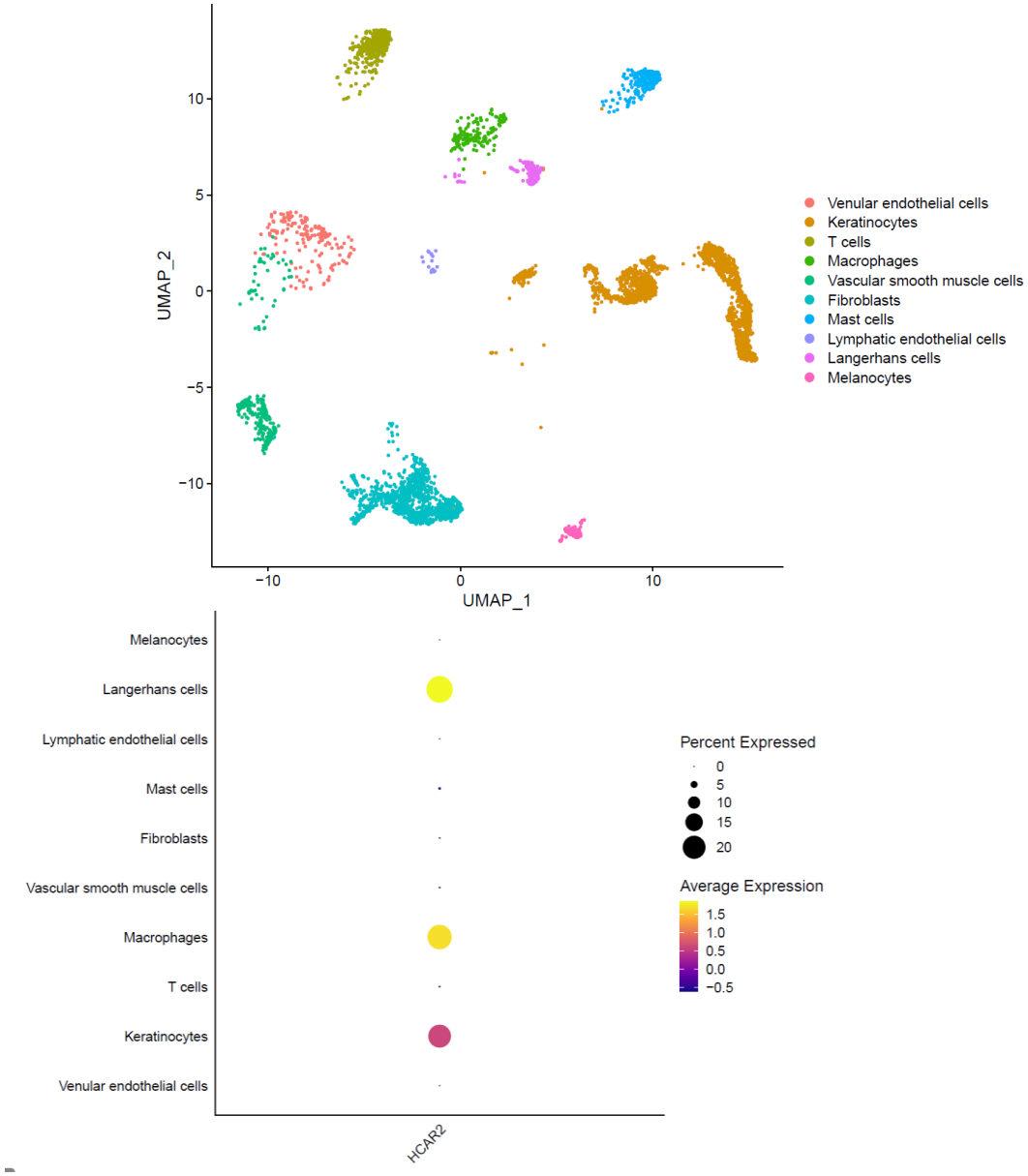

Figure 3. Bioinformatic analysis of *HCAR2* expression in non-lesioned skin of psoriatic patients

Our re-analysis (Figure 3) found that, among the different clusters of cells, the expression of *HCAR2* in non-lesioned skin was detected mainly in the Langerhans cells and macrophages populations. However, in the lesioned skin of psoriasis patients *HCAR2* expression is predominantly in keratinocytes and to a lesser extend in macrophages (Figure 4).

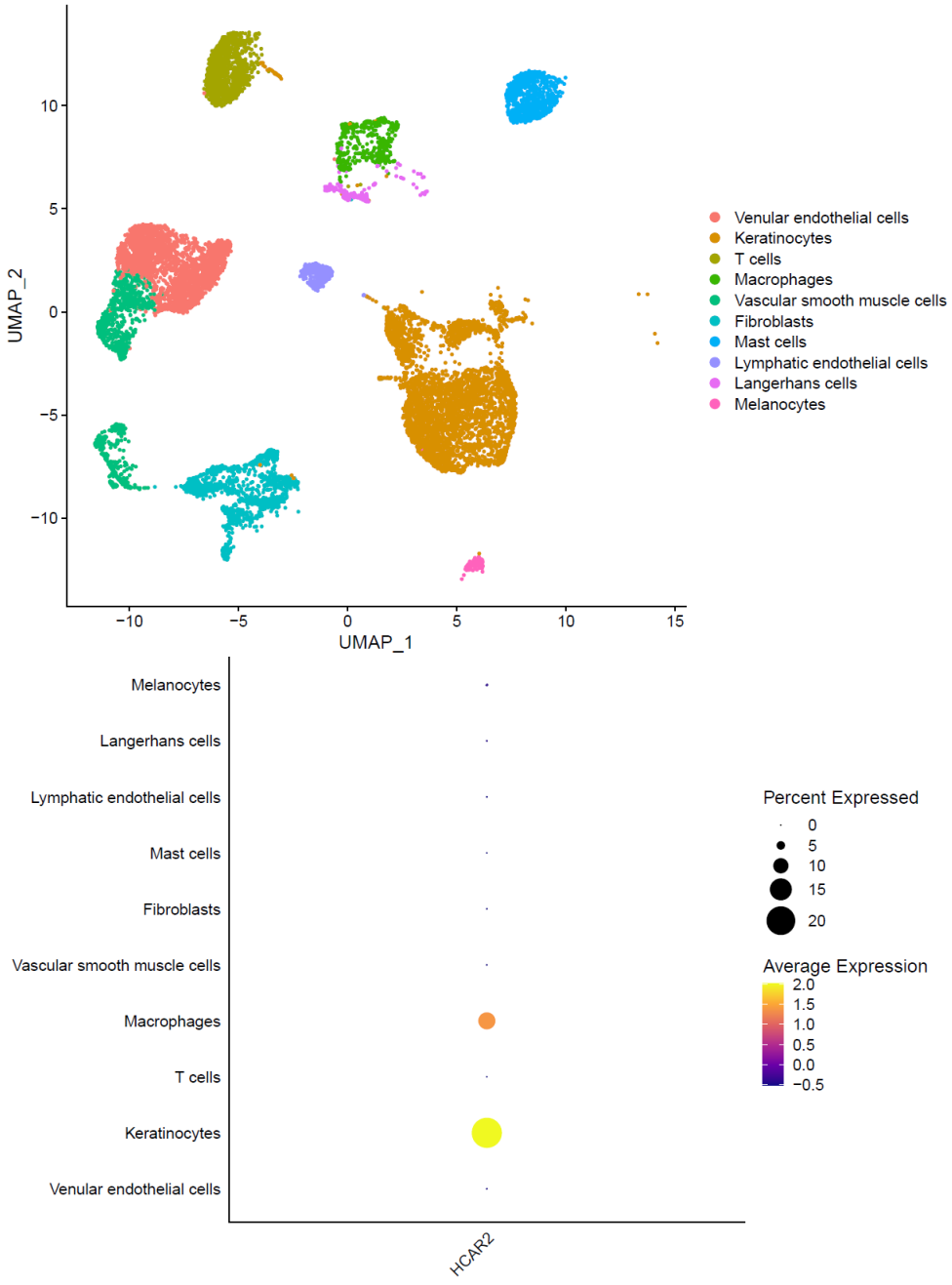

Figure 4. Bioinformatic analysis of *GPR109A/HCAR2* expression in lesioned skin of psoriatic patients

3.2 Kinetics of GPR109A receptor expression during 6-day IMQ treatment

mRNA levels of *Hcar2* gene were measured with qPCR to show GPR109A kinetics. Results show that expression of *Hcar2* is affected by IMQ treatment. *Hcar2* expression is upregulated from the second day of IMQ administration, with the expression being the highest on day 5. Day 6 shows the start of lowering of upregulation (Figure 5).

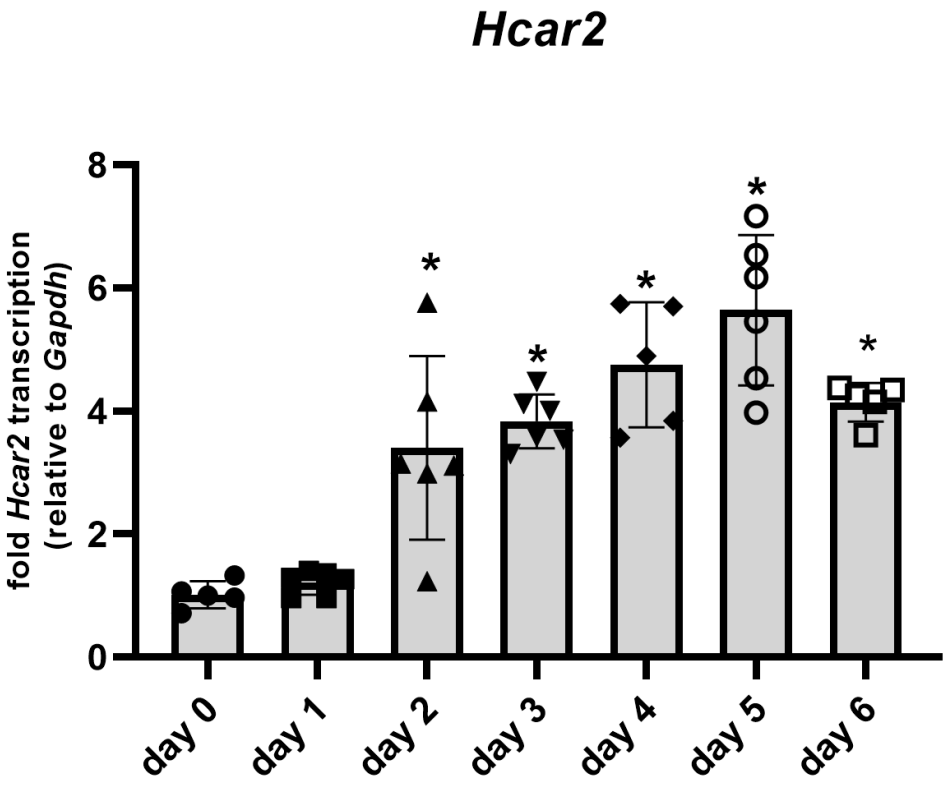

Figure 5. Kinetics of *Hcar2* expression in skin: qPRC results for mRNA levels in ear skin of *Hcar2* during six days of IMQ treatment of WT mice. Gene expression was normalized to *Gapdh* according to the delta cycle threshold method. n= 6 mice/group. Data are represented as mean ± SEM. *** $p < 0.001$, ** $p < 0.01$, and * $p < 0.05$. IMQ= imiquimod, WT= wild-type.

3.3 The genetic absence of GPR109A increases the inflammation in the IMQ-induced psoriasis-like mouse model

3.3.1. Ear inflammation and histological analysis

To examine whether psoriasis-like inflammation phenotype differs in GPR109 KO mice after 6 days of IMQ treatment topically on the ear skin, ear thickness measurement and inflammation score determination were performed daily. Inflammation score was determined as a cumulative score of erythema and scaling.

Ear thickening was induced in both WT and GPR109 KO mice treated with IMQ. However, GPR109 KO mice show a significant increase of the inflammation score and increase in thickness of the ear after six days of treatment. Mice were treated with Vaseline as a control group and showed consistent thickness during the entire treatment period (Figure 6.)

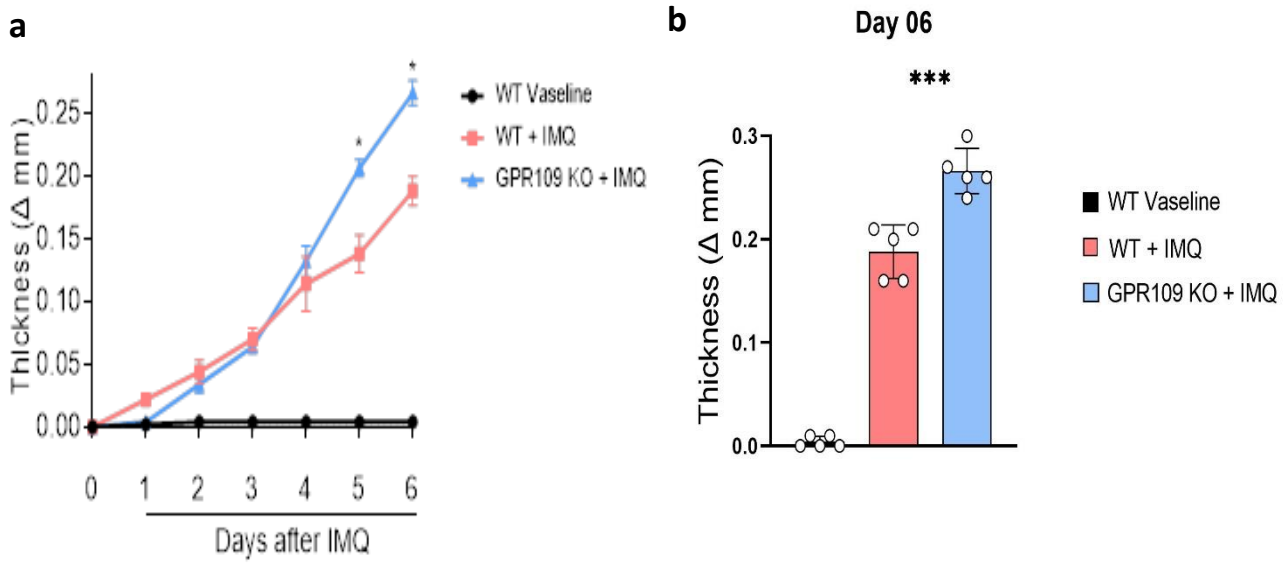

Figure 6. Difference of ear thickness over six days of treatment (a) and on last day (b) of IMQ/Vaseline treatment of BL6/WT and GPR109 KO mice. The study has been repeated at least 3 times with comparable results. n= 5 mice/group. Data are represented as mean ± SEM. *** $p < 0.001$, ** $p < 0.01$, and * $p < 0.05$. IMQ= imiquimod, WT= wild-type, KO= knock-out.

Inflammation score is the highest for GPR109KO mice, with the most visible redness and scaling of the skin. Vaseline control group did not develop any changes in phenotype, while scaling and redness was visible in WT mice treated with IMQ, but to a lesser extent than GPR109KO mice (Figure 7).

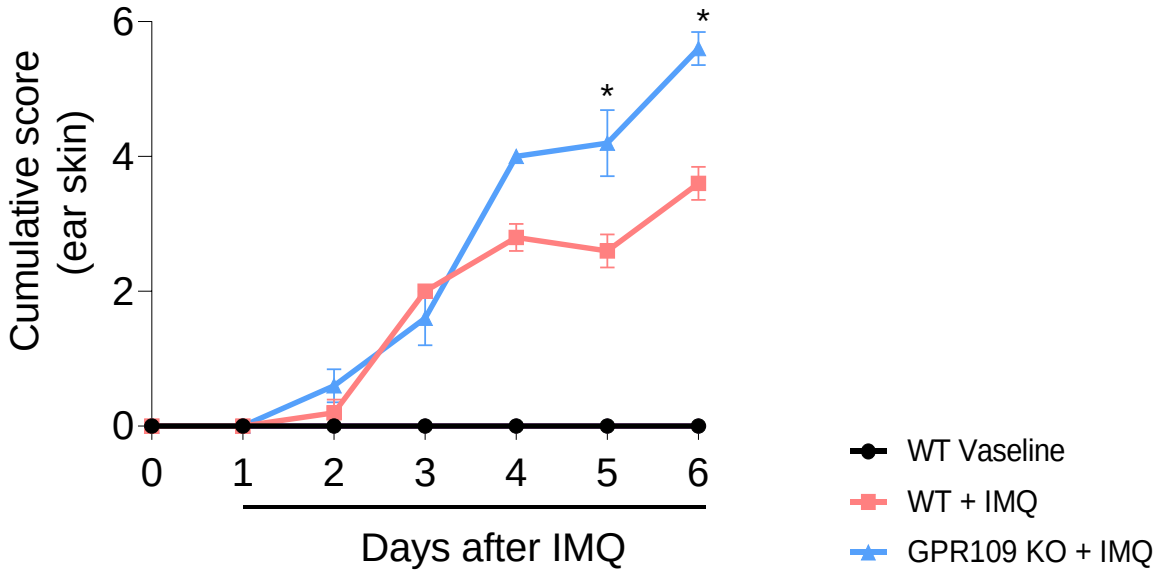

Figure 7. Inflammation score values of ear skin (erythema and scaling) during six days of IMQ/Vaseline treatment of WT and GPR109KO mice. n= 5 mice/group. Data are represented as mean ± SEM. *** $p < 0.001$, ** $p < 0.01$, and * $p < 0.05$. IMQ= imiquimod, WT= wild-type, KO= knock-out.

Next step was performing H&E staining of ear tissue sections. Staining was done to analyse the phenotype of ear skin and examine inflammation and cellular infiltration characteristic of psoriasis. Epidermal hyperplasia and hyperkeratosis are visible in both IMQ treated mice groups, with GPR109AKO having an exacerbated reaction. There is a visible dermal inflammatory cell infiltration in both WT and GPR109AKO mice treated with IMQ, which is significantly stronger in GPR109AKO mice. As expected, there is no hyperplasia or dermal inflammatory cell infiltration in control group treated with Vaseline (Figure 8).

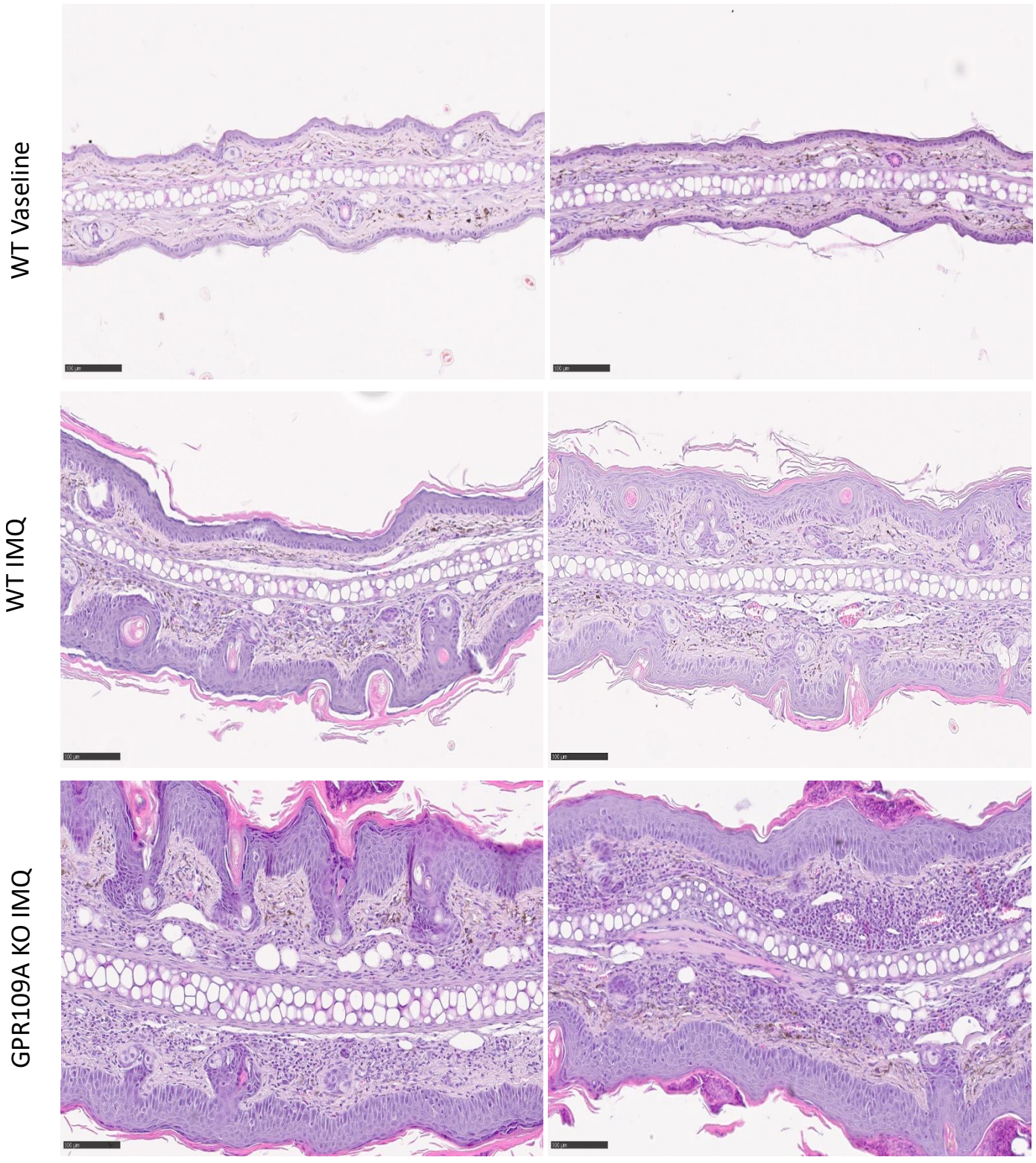

Figure 8. H&E staining of ear skin of WT and GPR109AKO mice, after six days of IMQ/Vaseline treatment. Pictures show sections from two repeated experiments to show consistency. n= 5 mice/group. Magnification 10x. Scale bar 250 µm. IMQ= imiquimod, WT= wild-type, KO= knock-out.

3.3.2 mRNA levels of genes involved in psoriasis

mRNA levels of two genes, *Krt17* and *Il-17a*, were measured with qPCR to compare expression of psoriasis significant genes between knock-out and wild type mice. *Il-17* transcripts are upregulated in GPR109KO mice compared to WT groups (Figure 9a) while *Krt17* expression is increased in IMQ-treated mice but doesn't differ between WT and KO mice (Figure 9b).

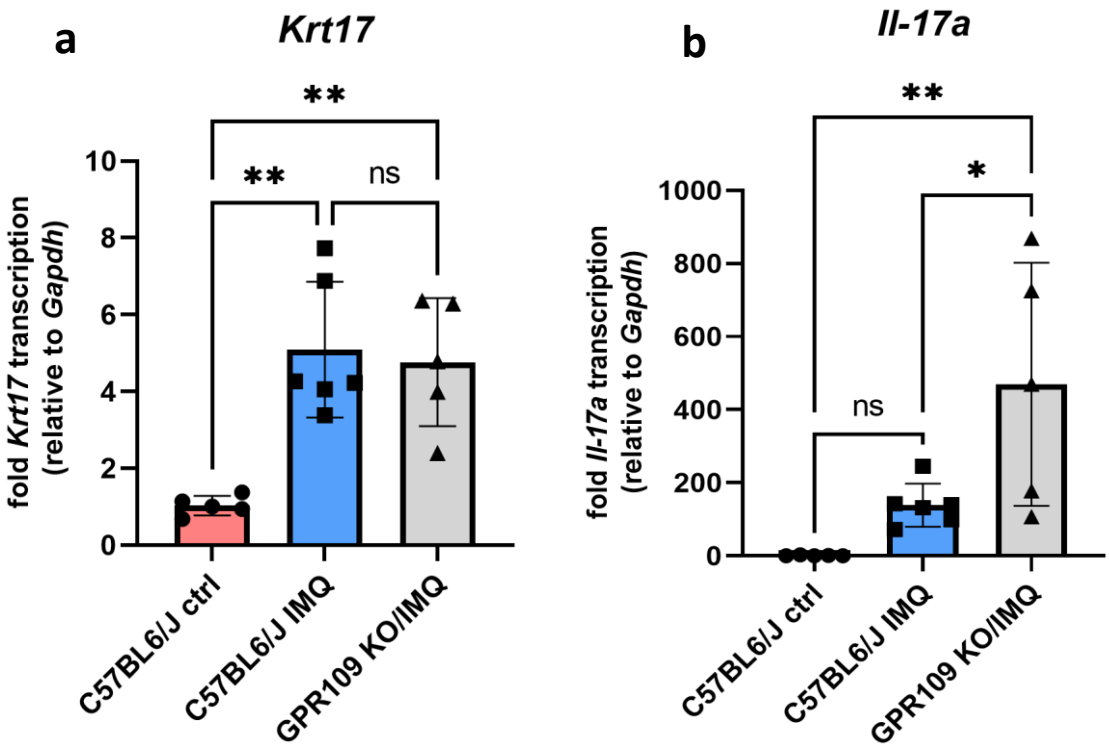

Figure 9. qPRC results for mRNA levels in ear skin of genes *Krt17* (a) and *Il-17a* (b) after six days of IMQ treatment of WT and GPR109KO mice. Gene expression was normalized to *Gapdh* according to the delta cycle threshold method. n= 5 mice/group. Data are represented as mean ± SEM. *** $p < 0.001$, ** $p < 0.01$, and * $p < 0.05$. IMQ= imiquimod, WT= wild-type, KO= knock-out.

3.3.3. Production of inflammatory mediators

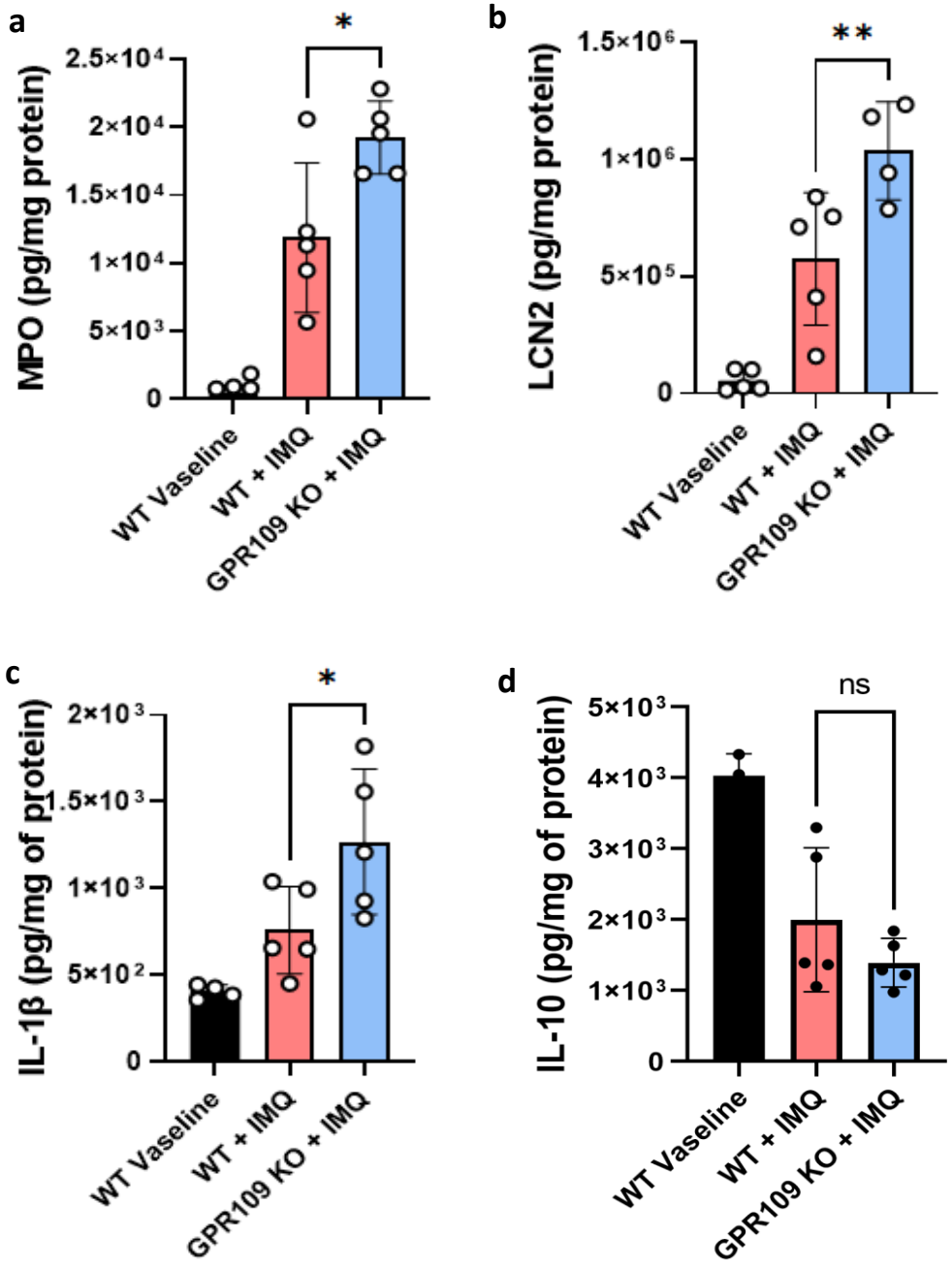

Figure 10. MPO (a), LCN2 (b), IL-1β (c), IL-10 (d) concentrations after six days of IMQ treatment of BL6/WT and GPR109 KO mice. Concentrations were standardized with concentration of total extracted proteins. The study has been repeated at least 2 times with comparable results. n= 5 mice/group. Data are represented as mean ± SEM. *** $p < 0.001$, ** $p < 0.01$, and * $p < 0.05$. IMQ= imiquimod, WT= wild-type, KO= knock-out.

Levels of two inflammatory mediators, MPO and LCN2, were measured by ELISA to assess inflammation (Figure 10a, 10b). Levels of cytokines IL-1β and IL-10 were also assessed by ELISA as examples of cytokines significant in psoriasis onset and maintenance (Figure 10c, 10d). Levels of MPO, LCN2, and IL-1β are increased in IMQ treated groups compared to control, with GPR109KO group levels being higher than WT levels. Levels of IL-10, an anti-inflammatory cytokine, are lower in GPR109KO group than in WT group.

3.4 Localization of GPR109A receptor in mouse ear skin using immunofluorescence

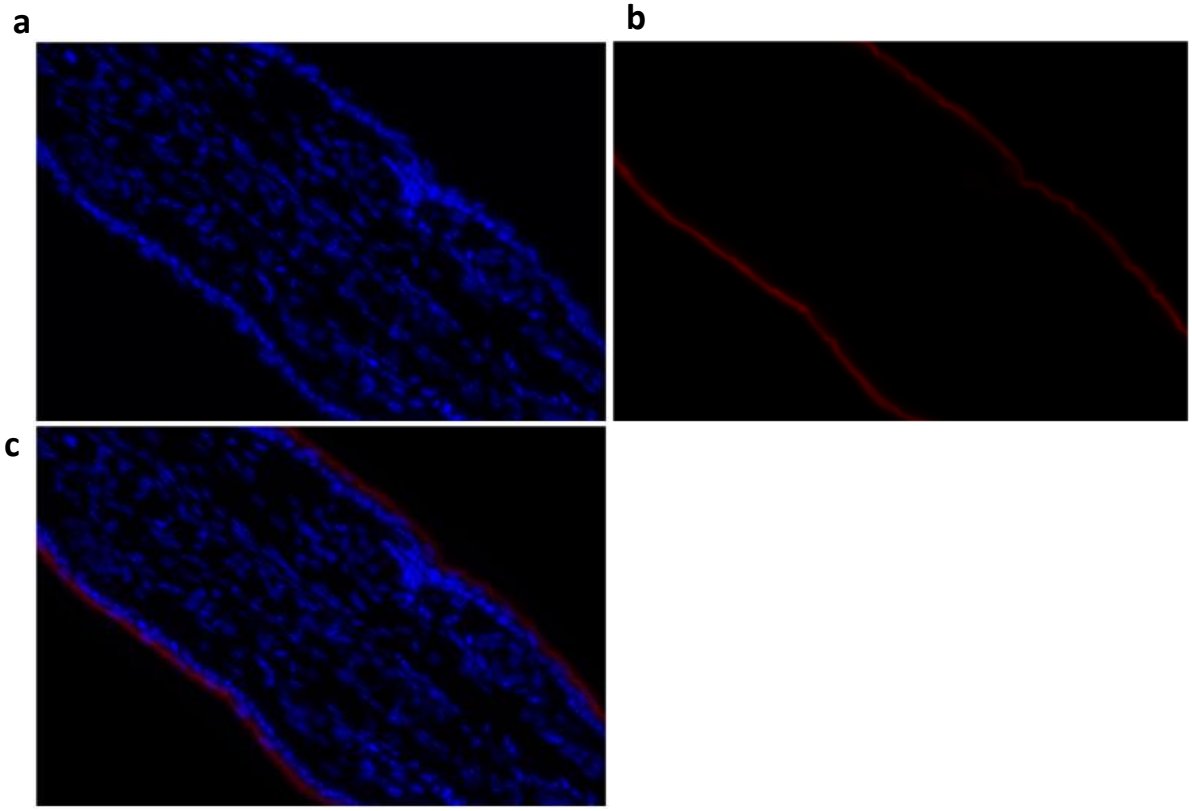

Figure 11. Frozen ear sections of GPR109A-mRFP mice treated with Vaseline. (a) Nuclear staining was performed with DAPI (blue). (b) Expression of GPR109A using anti-RFP antibody (red). (c) Merged pictures a and b. Magnification 20x. Photos show one representative experiment. RFP= red fluorescent protein.

Immunofluorescence staining was performed to asses GPR109 cellular localization in ear skin. Results shows that upon IMQ treatment, GPR109A is found in keratinocytes in epidermis as well as immune cells infiltrating the dermis (Figure 12a,12d,12e). In control group treated with Vaseline, no GPR109A signal was found (Figure 11b,11c).

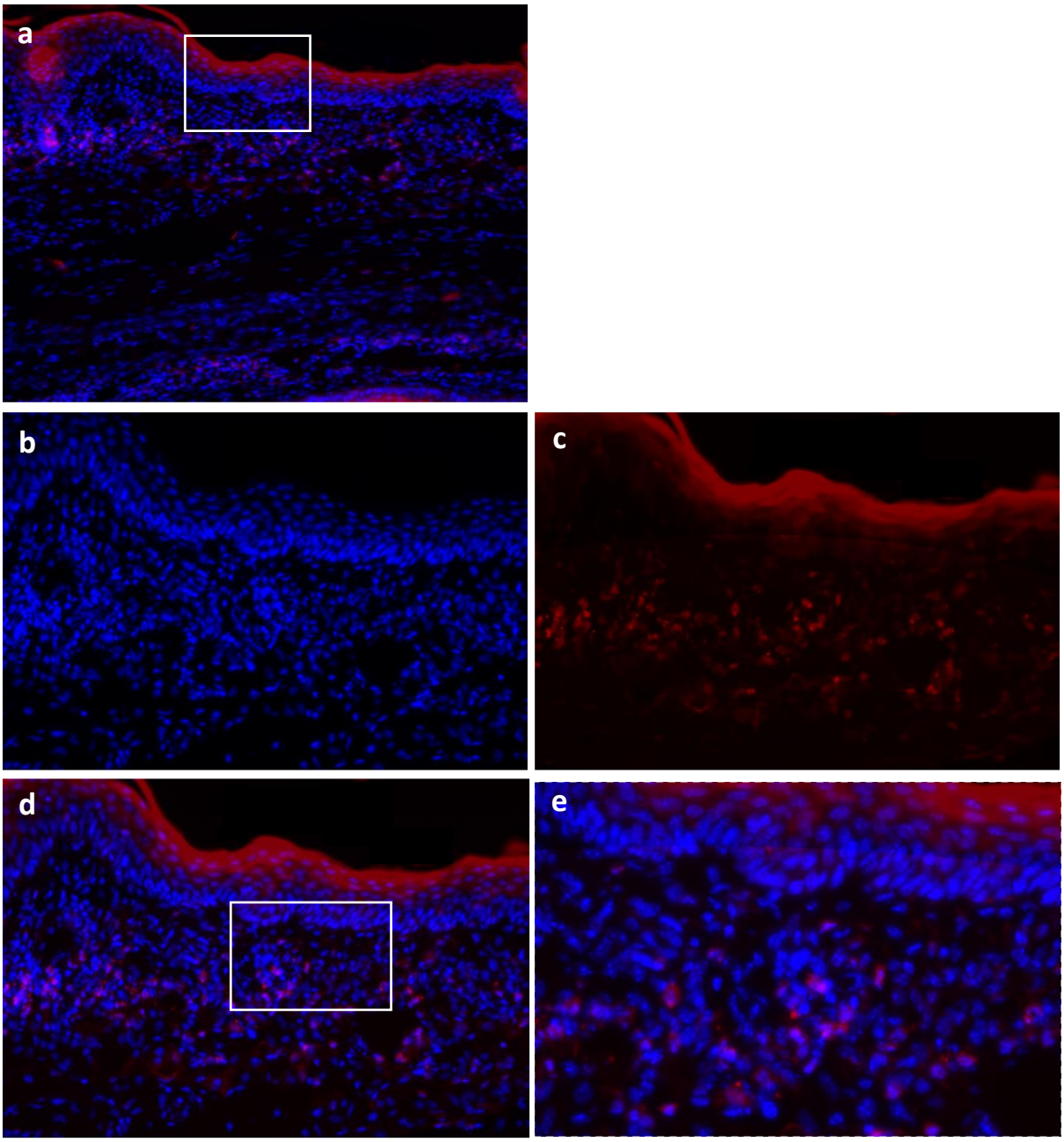

Figure 12. Frozen ear sections of GPR109A-mRFP mice treated with IMQ. (a) Merged picture of nuclear and GPR109A staining. White square marks zoomed in area shown in pictures b, c and d. Magnification 20x (b) Nuclear staining performed with DAPI (blue). (c) Expression of GPR109A using anti-RFP antibody (red). (d) Merged pictures b and c. White square marks zoomed in area shown in picture e. (e) 2x zoom of ear section for better visualization of colocalization of nuclear and GPR109A staining. Photos show one representative experiment. IMQ=imiquimod. RFP= red fluorescent protein.

4. DISCUSSION

Psoriasis is an inflammatory skin disease characterized by abnormal keratinocyte proliferation and immune cell infiltration in dermis. Fumaderm™ is a drug approved for psoriasis treatment. Monomethylfumarate (MMF), the drugs active metabolite, is a potent and selective GPR109A agonist, which poses the question of a potential role GPR109A might have in the disease (Tang et al., 2008).

To assess the potential role of GPR109A in psoriasis, we first turned to bioinformatics. Re-analysis of single-cell transcriptomic data from human psoriatic skin and controls skin identified clusters of cells prevalent in skin, including keratinocytes, T cells, and macrophages. *GPR109A/HCAR2* expression was then analyzed. In lesioned skin, gene was found to be downregulated in all the cells expressing *HCAR2*, except for keratinocytes where it was upregulated. Krejner at el. (Krejner et al., 2018) researched GPR109A in skin biopsy samples from human patients with psoriasis and showed sodium butyrate activated expression of *HCAR2.* They suggested that IMQ-mouse model would be an interesting continuation of the study.

Ever since van der Fits et al. (van der Fits et al., 2009) showed that topical application of IMQ is an easy and effective way of inducing psoriasis-like disease in mice, IMQ-mouse model has been regularly used for psoriasis research. IMQ application results in the influx of various immune cells in skin, as well as hyperplasia of epidermis. We treated BL6/WT mice with IMQ to assess the kinetics of GPR109A receptor expression (gene *Hcar2*), and then both BL6/WT and GPR109 KO mice to assess the effect that removal of GPR109A receptor has on psoriasis.

Expression of *Hcar2* in WT mice is shown to be upregulated which indicated that the receptor really is affected in the IMQ-induced psoriasis model, as well as shown that the 6 days application model is a good model to use to analyse GPR109A, as the last day of treatment shows the reduction of upregulation. After this, we turn our focus to GPR109 KO mice, to assess what effect the lack of receptor is going to have on psoriasis.

During IMQ treatment, just by measuring ear thickness and observing redness and scaling of ears, it was visible that GPR109KO mice have an aggravated psoriasis-like symptoms compared to wild type mice. This was further confirmed by H&E staining of ear skin.

Epidermal hyperplasia and dermal cell infiltration characteristic to psoriasis is amplified in GPR109KO mice. Exacerbated psoriasis-like phenotype in GPR109KO mice indicates that GPR109A receptor could have a protective role in the disease.

Psoriasis is accompanied by a disbalance in the levels of cytokines and other inflammatory proteins as well as keratinocyte hyperproliferation. Wanting to see the impact GPR109A has on these occurrences, analysis was done at both mRNA and protein level. Each epidermal layer of keratinocytes expresses different keratins. In psoriatic skin K10-K1 pair is downregulated, while K14-K5 and K17-K6 are upregulated making K6 and K17 proliferative markers of keratinocytes in psoriasis (Lin et al., 2022). Interestingly, *Krt17* was not differently expressed in KO mice compared to WT treated with IMQ, indicating that GPR109A does not have an effect on *Krt17*, and amplified keratinocyte hyperproliferation in KO mice is not a result of *Krt17* upregulation. IL-23/IL-17 axis is thought to be the predominant pathway in psoriasis pathogenesis. *Il-17a* is upregulated in psoriatic skin (Kutwin et al., 2021). IL-17A acts directly on keratinocytes and results in production of cytokines, chemokines and antimicrobial peptides promoting a positive feedback loop (Mosca et al., 2021). Expression of *Il-17a* is strongly upregulated in KO mice compared to WT groups, showing that GPR109A affects important cytokine's level.

Myeloperoxidase (MPO) and lipocalin-2 (LCN2) are important components of granules released by neutrophils which infiltrate the dermis in significant levels. MPO is the most abundant enzyme found in neutrophilic granules. It is cytolytic and affects cells and proteins by breaking down hydrogen peroxide and generating hypochlorous acid. (Neu et al., 2021). Lipocalin-2 plays a role in the innate immune system by inducing infiltration, migration, and adhesion of inflammatory cells like neutrophils into skin lesions. Keratinocytes are the second main source of LCN2 and it was shown that LCN2 participates in keratinocyte proliferation (Ren & Xia, 2022). Our results show an increase in both MPO and LCN2 levels in KO mice compared to wild type controls which goes in hand with intensified cell infiltration and hyperkeratosis in GPR109KO mice.

IL-1 cytokine family has also been implicated in psoriasis pathogenesis with genetic studies showing that polymorphism in the *IL-1B* gene can be used to differentiate early and late onset psoriasis patients. IL-1β is known to be critical in IL-17 T cell differentiation and activation. Studies have shown that IL-1β expression levels are significantly elevated in

psoriatic lesioned skin (Cai et al., 2019) which is in support of our results. IL-10, on the other hand, is an anti-inflammatory cytokine which acts as an immunoregulator that inhibits the synthesis of other cytokines. It is produced by monocytes, stimulated T lymphocytes (Th2 and Tregs), macrophages and keratinocytes (Kutwin et al., 2021). Different studies have shown decreased *IL-10* expression in psoriatic skin (Krejner et al., 2018; Kutwin et al., 2021) which aligns with our results.

Immunofluorescence analysis of cellular GPR109A localization had no signal in Vaseline control group, while in psoriasis-like mouse model, GPR109A is present in both keratinocytes and infiltrating immune cells in dermis. To confirm which infiltrating cells express GPR109A, it would be necessary to perform another experiment, this time staining different types of infiltrating cells (like neutrophils and macrophages) along with GPR109A. A problem in the experiment was the strong autofluorescence of keratins. Keratins are the main structural proteins of keratinocytes and are also one of the main fluorophores of the skin (Giovannacci et al., 2019). Localization of both keratins and GPR109-RFP in keratinocytes gave a very intense signal in epidermis, making it impossible to see cellular localization of the receptor. Anti-RFP antibody was used to try and minimize the strong RFP signal, which resulted in visible GPR109A localization in infiltrating immune cells.

5. CONCLUSION

In conclusion, GPR109A is found localized in keratinocytes and infiltrating immune cells in dermis in the IMQ-induced mouse model. ELISA and qPCR results indicate that GPR109A receptor plays a role in balancing the levels of inflammatory mediators as GPR109KO mice showed amplified expression of inflammatory proteins and reduced expression of anti-inflammatory IL-10. Those results, together with the fact that GPR109KO mice have an exacerbated psoriasis-like phenotype indicate GPR109A has a protective role in regulation of skin inflammation.

GPR109A could potentially be a therapeutic target for psoriasis, with activation of receptor leading to calming of disease. That hypothesis is supported by published works. Singh et. al researched GPR109A in gut and showed that GPR109A was essential in butyrate-mediated IL-18 production in colonic epithelium, as well as GPR109A signalling promoting anti-inflammatory properties in colonic dendritic cells and macrophages thus enabling them to induce Treg differentiation (Singh et al., 2014). Schwarz et al. topically treated mice with sodium butyrate which resulted in reduction of IMQ-induced inflammation and induction of *Il-10* and *Foxp3* transcripts (Schwarz et al., 2021). It would be necessary to perform further research into activation of GPR109A with its agonists, such as sodium butyrate, and see if GPR109A activation can induce Treg differentiation in skin.

6.BIBLIOGRAPHY

Belkaid, Y., & Naik, S. (2013). Compartmentalized and systemic control of tissue immunity by commensals. *Nature Immunology*, *14*(7), 646–653. https://doi.org/10.1038/ni.2604

Cai, Y., Xue, F., Quan, C., Qu, M., Liu, N., Zhang, Y., Fleming, C., Hu, X., Zhang, H. ge, Weichselbaum, R., Fu, Y. xin, Tieri, D., Rouchka, E. C., Zheng, J., & Yan, J. (2019). A Critical Role of the IL-1β–IL-1R Signaling Pathway in Skin Inflammation and Psoriasis Pathogenesis. *Journal of Investigative Dermatology*, *139*(1), 146–156. https://doi.org/10.1016/j.jid.2018.07.025

Chiang, C. C., Cheng, W. J., Korinek, M., Lin, C. Y., & Hwang, T. L. (2019). Neutrophils in Psoriasis. *Frontiers in Immunology*, *10*(October). https://doi.org/10.3389/fimmu.2019.02376

Giovannacci, I., Magnoni, C., Vescovi, P., Painelli, A., Tarentini, E., & Meleti, M. (2019). Which are the main fluorophores in skin and oral mucosa? A review with emphasis on clinical applications of tissue autofluorescence. *Archives of Oral Biology*, *105*(March), 89–98. https://doi.org/10.1016/j.archoralbio.2019.07.001

Griffiths, C. E. M., Armstrong, A. W., Gudjonsson, J. E., & Barker, J. N. W. N. (2021). Psoriasis. *The Lancet*, *397*(10281), 1301–1315. https://doi.org/10.1016/S0140-6736(20)32549-6

Hanson, J., Gille, A., & Offermanns, S. (2012). Role of HCA2 (GPR109A) in nicotinic acid and fumaric acid ester-induced effects on the skin. *Pharmacology and Therapeutics*, *136*(1), 1–7. https://doi.org/10.1016/j.pharmthera.2012.06.003

Hao, Y., Hao, S., Andersen-Nissen, E., Mauck, W. M., Zheng, S., Butler, A., Lee, M. J., Wilk, A. J., Darby, C., Zager, M., Hoffman, P., Stoeckius, M., Papalexi, E., Mimitou, E. P., Jain, J., Srivastava, A., Stuart, T., Fleming, L. M., Yeung, B., … Satija, R. (2021). Integrated analysis of multimodal single-cell data. *Cell*, *184*(13), 3573-3587.e29. https://doi.org/10.1016/j.cell.2021.04.048

Hawkes, J. E., Gudjonsson, J. E., & Ward, N. L. (2017). The Snowballing Literature on Imiquimod-Induced Skin Inflammation in Mice: A Critical Appraisal. *Journal of Investigative Dermatology*, *137*(3), 546–549. https://doi.org/10.1016/j.jid.2016.10.024

Hughes, T. K., Wadsworth, M. H., Gierahn, T. M., Do, T., Weiss, D., Andrade, P. R., Ma, F., de

Andrade Silva, B. J., Shao, S., Tsoi, L. C., Ordovas-Montanes, J., Gudjonsson, J. E., Modlin, R. L., Love, J. C., & Shalek, A. K. (2020). Second-Strand Synthesis-Based Massively Parallel scRNA-Seq Reveals Cellular States and Molecular Features of Human Inflammatory Skin Pathologies. *Immunity*, *53*(4), 878-894.e7. https://doi.org/10.1016/j.immuni.2020.09.015

Jiang, Y., Tsoi, L. C., Billi, A. C., Ward, N. L., Harms, P. W., Zeng, C., Maverakis, E., Michelle Kahlenberg, J., & Gudjonsson, J. E. (2020). Cytokinocytes: The diverse contribution of keratinocytes to immune responses in skin. *JCI Insight*, *5*(20), 1–15. https://doi.org/10.1172/jci.insight.142067

Kabashima, K., Honda, T., Ginhoux, F., & Egawa, G. (2019). The immunological anatomy of the skin. *Nature Reviews Immunology*, *19*(1), 19–30. https://doi.org/10.1038/s41577-018-0084-5

Kamata, M., & Tada, Y. (2022). Dendritic Cells and Macrophages in the Pathogenesis of Psoriasis. *Frontiers in Immunology*, *13*(June), 1–10. https://doi.org/10.3389/fimmu.2022.941071

Krejner, A., Bruhs, A., Mrowietz, U., Wehkamp, U., Schwarz, T., & Schwarz, A. (2018). Decreased expression of G-protein-coupled receptors GPR43 and GPR109a in psoriatic skin can be restored by topical application of sodium butyrate. *Archives of Dermatological Research*, *310*(9), 751–758. https://doi.org/10.1007/s00403-018-1865-1

Kutwin, M., Migdalska-Sęk, M., Brzeziańska-Lasota, E., Zelga, P., & Woźniacka, A. (2021). An analysis of il-10, il-17a, il-17ra, il-23a and il-23r expression and their correlation with clinical course in patients with psoriasis. *Journal of Clinical Medicine*, *10*(24), 1–17. https://doi.org/10.3390/jcm10245834

Lin, Y., Zhang, W., Li, B., & Wang, G. (2022). Keratin 17 in psoriasis: Current understanding and future perspectives. *Seminars in Cell and Developmental Biology*, *128*(June), 112–119. https://doi.org/10.1016/j.semcdb.2021.06.018

Marsh, S. (2023). *scCustomize: Custom Visualizations & Functions for Streamlined Analyses of Single Cell Sequencing* (R package version 1.1.1). https://cran.r-project.org/package=scCustomize

Mosca, M., Hong, J., Hadeler, E., Hakimi, M., Liao, W., & Bhutani, T. (2021). The Role of IL-17 Cytokines in Psoriasis. *ImmunoTargets and Therapy*, *Volume 10*(October), 409–418. https://doi.org/10.2147/itt.s240891

Neu, S. D., Strzepa, A., Martin, D., Sorci-Thomas, M. G., Pritchard, K. A., & Dittel, B. N. (2021). Myeloperoxidase inhibition ameliorates plaque psoriasis in mice. *Antioxidants*, *10*(9), 1–15. https://doi.org/10.3390/antiox10091338

Pietraforte, I., & Frasca, L. (2023). Autoreactive T-Cells in Psoriasis: Are They Spoiled Tregs and Can Therapies Restore Their Functions? *International Journal of Molecular Sciences*, *24*(5). https://doi.org/10.3390/ijms24054348

R Core Team. (2023). *R: A Language and Environment for Statistical Computing*. R Foundation for Statistical Computing. https://www.r-project.org

Ren, K., & Xia, Y. (2022). Lipocalin 2 Participates in the Epidermal Differentiation and Inflammatory Processes of Psoriasis. *Journal of Inflammation Research*, *15*, 2157–2166. https://doi.org/10.2147/JIR.S358492

Rendon, A., & Schäkel, K. (2019). Psoriasis pathogenesis and treatment. *International Journal of Molecular Sciences*, *20*(6), 1–28. https://doi.org/10.3390/ijms20061475

Schwarz, A., Bruhs, A., & Schwarz, T. (2017). The Short-Chain Fatty Acid Sodium Butyrate Functions as a Regulator of the Skin Immune System. *Journal of Investigative Dermatology*, *137*(4), 855–864. https://doi.org/10.1016/j.jid.2016.11.014

Schwarz, A., Philippsen, R., & Schwarz, T. (2021). Induction of Regulatory T Cells and Correction of Cytokine Disbalance by Short-Chain Fatty Acids: Implications for Psoriasis Therapy. *Journal of Investigative Dermatology*, *141*(1), 95-104.e2. https://doi.org/10.1016/j.jid.2020.04.031

Silva de Malo, B. M., Veras, F. P., Zwicky, P., Lima, D., Ingelfinger, F., Varela Martins, T., da Silva Prado, D., Schärli, S., Publio, G., Hiroki, C. H., Melo, P. H., Saraiva, A., Norbiato, T., Lima, L., Ryffel, B., Vogl, T., Roth, J., Waisman, A., Nakaya, H. I., … Alves-Filho, J. C. (2023). S100A9 Drives the Chronification of Psoriasiform Inflammation by Inducing IL-23/Type 3 Immunity. *Journal of Investigative Dermatology*, *S0022-202X(23)01825-0*. https://doi.org/10.1016/j.jid.2023.02.026

Singh, N., Gurav, A., Sivaprakasam, S., Brady, E., Padia, R., Shi, H., Thangaraju, M., Prasad, P. D., Manicassamy, S., Munn, D. H., Lee, J. R., Offermanns, S., & Ganapathy, V. (2014). Activation of Gpr109a, receptor for niacin and the commensal metabolite butyrate, suppresses colonic inflammation and carcinogenesis. *Immunity*, *40*(1), 128–139. https://doi.org/10.1016/j.immuni.2013.12.007

Tang, H., Lu, J. Y. L., Zheng, X., Yang, Y., & Reagan, J. D. (2008). The psoriasis drug monomethylfumarate is a potent nicotinic acid receptor agonist. *Biochemical and Biophysical Research Communications*, *375*(4), 562–565. https://doi.org/10.1016/j.bbrc.2008.08.041

van der Fits, L., Mourits, S., Voerman, J. S. A., Kant, M., Boon, L., Laman, J. D., Cornelissen, F., Mus, A.-M., Florencia, E., Prens, E. P., & Lubberts, E. (2009). Imiquimod-Induced Psoriasis-Like Skin Inflammation in Mice Is Mediated via the IL-23/IL-17 Axis. *The Journal of Immunology*, *182*(9), 5836–5845. https://doi.org/10.4049/jimmunol.0802999

Wickham, H. (2016). *ggplot2: Elegant Graphics for Data Analysis* (2nd ed.). Springer-Verlag. https://doi.org/10.1007/978-3-319-24277-4

Xiao, X., Hu, X., Yao, J., Cao, W., Zou, Z., Wang, L., Qin, H., Zhong, D., Li, Y., Xue, P., Jin, R., Li, Y., Shi, Y., & Li, J. (2023). The role of short-chain fatty acids in inflammatory skin diseases. *Frontiers in Microbiology*, *13*(February), 1–11. https://doi.org/10.3389/fmicb.2022.1083432

Yang, Y., Kang, H. J., Gao, R., Wang, J., Han, G. W., DiBerto, J. F., Wu, L., Tong, J., Qu, L., Wu, Y., Pileski, R., Li, X., Zhang, X. C., Zhao, S., Kenakin, T., Wang, Q., Stevens, R. C., Peng, W., Roth, B. L., … Liu, Z. J. (2023). Structural insights into the human niacin receptor HCA2-Gi signalling complex. *Nature Communications*, *14*(1), 1692. https://doi.org/10.1038/s41467-023-37177-6

Zhou, X., Chen, Y., Cui, L., Shi, Y., & Guo, C. (2022). Advances in the pathogenesis of psoriasis: from keratinocyte perspective. *Cell Death and Disease*, *13*(1). https://doi.org/10.1038/s41419-022-04523-3