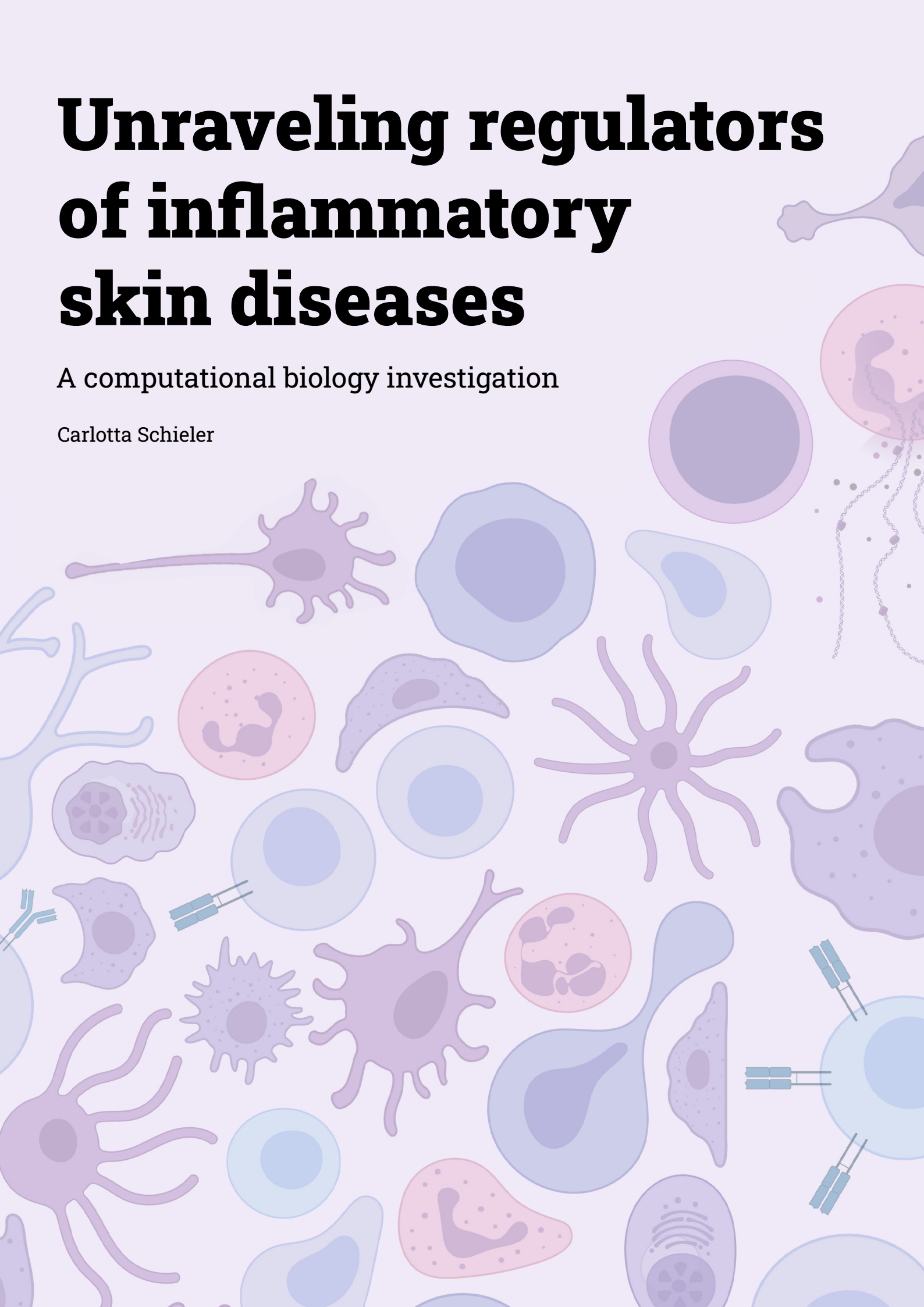# Unraveling regulators of inflammatory skin diseases

A computational biology investigation

Carlotta Schieler

Laymen Summary

Psoriasis is a chronic inflammatory skin disorder, which affects over 2-3% of adults worldwide. The disease is due to an overly activated immune system and results in dry, itchy, and scaly skin. Depending on the severity, it can hinder patients even in their everyday life. Although Psoriasis has been studied extensively and molecular mechanisms driving the disease have been uncovered, many underlying details remain unclear.

Therefore, in this study we try to bring light to these details and find drivers responsible for the development of Psoriasis. One of these drivers might be genes, which are only active in patients and not in healthy subjects. Therefore, we searched for publicly available datasets containing information about gene activity in psoriatic skin and healthy people. We then could compare if genes are activated or inactivated in patients and define these as differentially expressed genes (DEGs). We used this list of DEGs and looked if they are known to be involved in pathways regulating our body. Expectantly, we found that the activated genes commonly regulate the immune system.

Genes never work on their own, but they interact in a so-called gene-regulatory network. Thus, the activity of one gene might activate another gene and that affects even another one. We used an analysis technique revealing these underlying networks and highlight the genes, which are responsible for the activity of most other genes. Those genes are then considered regulators of Psoriasis.

Psoriasis is one of many inflammatory skin diseases. To evaluate if our found regulators are specific for Psoriasis or are just regulators found in inflammatory skin in general, we repeated all analysis with the disease Atopic Dermatitis. The itchy rash of Atopic Dermatitis is also a result of a disorder in the immune system. Even though the rashes of Psoriasis and Atopic Dermatitis can at times look very similar and therefore hard to diagnose, their underlying responsible molecular mechanism is different. Comparing our results of both diseases, we found a large overlap in the involved pathways as well as regulators. But also we were able to highlight distinct regulators for Psoriasis and Atopic Dermatitis. These regulators might help further studies understanding both diseases in more detail.

One additional point we studied, are the involved cell types in both diseases. Our skin contains many different cell types, not just different skin cells, but also cell types belonging to the immune system such as T-cells and B-cells. All our samples are taken by skin biopsies, and as result these samples contain a mixture of many different cell types. Thus our measurements contain the gene expression of all cells combined. Using a computational method called deconvolution, we can use these measurements to estimate how much of each cell type is present in such a biopsy sample. By this, we were able to show that more immune cells are present in samples from affected skin than in unaffected or healthy skin, indicating once again the involvement of the immune system.

Overall, our study highlighted the involvement of the immune system in both diseases. Further, we propose regulators for general inflammatory response as well as specific regulators for Psoriasis and Atopic Dermatitis.

Finding specific Regulators of Inflammatory Skin Disease

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Psoriasis is one of the most common chronic inflammatory disorder, but still until now many of the underlying mechanisms remain unclear. In this study we analyse large-scale public high-throughput sequencing data by performing differential expression analysis, pathway enrichment and integrating a gene random forest and gene set enrichment analysis to create a regulatory network. To distinguish markers of common skin inflammation from disease specific ones, we further integrate data of the skin inflammatory disease Atopic Dermatitis. We find that common differential expressed genes across all datasets are involved in immune system pathways, similar to the common genes of both diseases. In addition the disease-unique genes also show up in pro-inflammatory pathways, showing that both diseases are activated by different mechanism. Moreover, we find that Psoriasis and Atopic Dermatitis share many regulators, but we are able to distinguish unique regulators such as PRDM1, STAT3 and NR4A3 and create a gene regulatory network. Further, cellular deconvolution shows keratinization and infiltration of immune cells dominated by Monocytes and CD4$^+$ T-cells.

1 Introduction

Psoriasis is one of most common chronic inflammatory disorder of the skin as 2-3% of adults worldwide are estimated to be affected (Parisi et al., 2013). The disorder is characterized by altered keratinocyte differentiation, hyperproliferative epidermis and infiltrating inflammatory cells leading to extensive inflammation (Albanesi et al., 2018). Recently, it was shown that Psoriasis is also associated with other multiple co-morbid medical conditions such as cardiovascular disease, mental health conditions, metabolic syndrome or overt-diabetes (Amin et al., 2020; Davidovici et al., 2010).

There have been numerous efforts to characterize and understand the underlying molecular pathways of the disease. Current models of the pathogenesis describe the IL-23/IL-17-axis as the core mechanism inducing the disorder (Fotiadou et al., 2015), resulting in activation of infiltrating immune cells such as myeloid or conventional dendritic cells (mDC or cDC), neutrophils and T-cells leading to the proliferation of keratinocytes (Albanesi et al., 2018). But still until now many of the underlying mechanism remain unclear (Ayala-Fontánez, Soler, and McCormick, 2016).

One possibility of revealing those underlying mechanism is by using gene expression. Utilising differential gene expression analysis, common differential expressed genes of Psoriasis were already often annotated as for example seen in the study by Zeng et al., 2019. This large-scale study also created a co-expression network of lesional and non-lesional skin and further integrated knowledge such as druggability, genetic association and cell line-specific expression profiles. This analysis however is lacking an inference of regulators in Psoriasis. To create such a regulator-target network, different computational approaches

are attested. One method shown reliable is based on a random forest algorithm (Marbach et al., 2012). This splits the gene expression in different learning samples to find networks, and selects the networks which are most accurately able to predict the underlying data. The recently developed computational tool RegEnrich uses this approach to infer a regulator-target network and then further integrates differential expression analysis of regulators and their enrichment of downstream targets to identify key regulators between biological states (Tao, Radstake, and Pandit, 2021).

Apart from understanding the molecular pathways of Psoriasis, one can study the involvement of specific cell types. This can be investigated by obtaining scRNA-seq data. However, there is not yet a sufficient amount of scRNA-seq data of Psoriasis allowing for a large-scale study. But recently developed computational deconvolution tools enable analysing cell type fractions in bulk RNA-seq data. These tools are either build around reference-based, reference-free or marker-based algorithms. The reference-based method CIBERSORTx regularly outperforms other deconvolution tools in benchmarking studies (Jin and Liu, 2021; Sturm et al., 2019; Avila Cobos et al., 2020). It is an improved version of the CIBERSORT method and now allows scRNA-seq to act as the reference sample to infer cell type fractions in bulk RNA-seq data. However, so far it has not yet been sufficiently benchmarked on its performance in analysing skin samples. In contrast to that, xCell as a marker-based method, is an enrichment method, which has shown strong results in predicting the absence or presence of cell types (Sturm et al., 2019). Strictly speaking it is not a deconvolution method as it does not predict the abundance of cells, but can be used to find enriched cell types across samples. In order, to evaluate the expected keratinization and immune cell infiltration in Psoriasis, we applied both methods to all our bulk RNA-seq data.

Overall, this study aims to integrate large-scale public high-throughput transcriptomic data to find signatures and key regulators of Psoriasis, which can improve the understanding of the disease. In order to distinguish regulators of Psoriasis from basic markers of skin inflammation, we integrate data of the skin inflammatory disease Atopic Dermatitis as well.

First, we conduct differential gene expression analysis followed by pathway enrichment analysis and then integrating a gene random forest and gene set enrichment analysis to create a regulatory network using RegEnrich. Lastly, we perform deconvolution to estimate immune cell infiltration. We find that the common differential expressed genes across all datasets are involved in immune system pathways, similar to the common genes of both diseases. In addition, the disease-unique genes also show up in pro-inflammatory pathways, showing that both diseases are activated by different mechanisms. Additionally, we find that Psoriasis and Atopic Dermatitis share many regulators, but we can distinguish unique regulators such as PRDM1,

STAT3 and NR4A3. Further, deconvolution shows the infiltration of immune cells most predominately Monocytes and CD4$^+$ T-cells.

2 Methods

2.1 Data Acquisition

Nine RNA-seq datasets of Psoriasis (GSE107871, GSE41745, GSE67785, GSE83645, GSE47944, GSE63979, GSE117405, GSE54456, GSE74697) and one of Atopic Dermatitis (GSE65832) were obtained from the manually curated and publicly available database by Federico et al., 2020. These datasets were already pre-processed, harmonized and prepared according to the FAIR principles. Additional RNA-seq datasets were retrieved from NCBI GEO GSE121212, GSE140227 and GSE186063. The total number of obtained and analysed samples for Psoriasis is 254 lesional skin (L), 129 non-lesional skin (NL) and 186 skin samples from healthy control patients (HC) and 53 L and 53 NL samples for Atopic Dermatitis (table B.1.)

2.2 Differential Expression Analysis

Prior to differential expression analysis (DEA), genes with a median count of less than 3 across all samples were excluded from further analysis. DEA was carried out using the two-group comparison method Wald significance test of the DESeq2 R-package (version 1.34.0) (Love, Huber, and Anders, 2014). The used comparison was either LvsNL or LvsHC. Datasets with less than 6 samples or 50 DEGs (p-adjusted <= 0.1) were excluded from further analysis. To identify biological functions of the DEGs found in all datasets of each comparison, pathway enrichment analysis was carried out using the function gseGO (*ont="BP"*) of the R package clusterProfiler (version 4.2.0) (Yu et al., 2012) using the mean fold change across all datasets.

2.3 Regulator Enrichment and Network

The results from DEA were used to infer a regulator target network using a random forest approach and subsequent Gene Set Enrichment (GSEA). These methods were carried out with the RegEnrich R-package (version 1.4.0) (Tao, Radstake, and Pandit, 2021) integrating the differential expression of regulators and their targets and the enrichment analysis, providing a ranked list of regulators and their significance.

With the weights of network edges between regulators and their target genes calculated by RegEnrich, we selected the regulator-target gene pairs based on two criteria: 1) weight > 0.01 and 2) co-occurrences regulator-target gene pairs in at least three datasets for Psoriasis, and at least two datasets for Atopic Dermatitis. The general inflammation network is generated

in the GSE121212 dataset which includes both Psoriasis and Atopic Dermatitis samples. Then the top three regulators and their target genes were selected. The mean of the weights was calculated for the final network edges. Cytoscape (Shannon et al., 2003) was used to visualize the network.

2.4 Estimation of immune cell infiltration

To estimate the infiltration of immune cells in affected skin, two different computational methods were applied. The deconvolution tool CIBERSORTx (Newman et al., 2019) was used to enumerate the proportions of distinct cell subpopulations from our acquired bulk RNA-seq datasets using the signature matrix DerM22 constructed for inflammatory skin conditions by Félix Garza et al., 2019 as reference. The analysis was carried out in a Docker environment. For comparison the enrichment method xCell (Aran, Hu, and Butte, 2017) using the R-package (version 1.1.0) was applied to calculate gene signature-based enrichment scores of specified cell types analogous to DerM22 (see table B.4 for used signature matrices) on the same data.

All used data is available at NCBI GEO. All code is available upon request.

3 Results

3.1 Psoriasis

Differential expression analysis and Pathway Enrichment

To find differential expressed genes (DEGs) we used the two-group comparison method Wald significance test from the DeSeq2 package (Love, Huber, and Anders, 2014).

We tested for the differences between lesional and non-affected or healthy skin. Table B.2 shows the number of found DEGs for each individual dataset after filtering for p-adjusted >0.1. We excluded datasets with less than 6 samples or 50 DEGs. Thus, 5 datasets (GSE67785, GSE83645, GSE63979, GSE83645, GSE121212) for the Lesional vs Non-Lesional (LvsNL) and 4 datasets (GSE63979, GSE117405, GSE54456, GSE121212) for the Lesional vs Healthy Control (LvsHC) design remained. Looking for similarities of the datasets in each design, a big overlap of DEGs namely 2121 and 3236 genes were found for both comparisons LvsNL and LvsHC respectively (fig.1A, A.1A). Looking at the overlapping genes with the highest median absolute fold change, we find many genes belonging to the *S100* (S100A74, S1000A12, S100A7), *small, proline-rich SPRR* (SPRR2A,SPPR2F, SPRR2A) and *SerpinB* (SERPINB3, SERPINB4) families as well as TMPRSS11D, LCE3A, PI3, CXCL8, TCN1 and TNIP3.

We used this list of commonly expressed genes and their mean fold change across the different datasets and carried out a gene set enrichment analysis with ClusterProfiler to find enriched pathways (fig.1D, A.1C). Focusing on the activated pathways, we see a large overlap (8 pathways) between both designs, which are strikingly all pathways directly involved or relating to the immune system such as *immune response, immune system process or response to external stimulus*. In contrast to that the suppressed pathways differ greatly. In the Lesional vs Healthy Control comparison, we mostly find back pathways concerning muscle involvement (fig. A.1C). Whereas in the design against non-affected skin, the suppressed pathways are very diverse ranging from *neurotransmitter transport* to *cell-substrate junction* (fig.1D). These findings suggest a large similarity between non-affected skin of patients and skin from healthy controls.

To study this further, we compared the 2121 and 3236 overlapping genes of LvsNL (fig.1A) and LvsHC (fig.A.1A) and found an overlap of 1425 genes (fig.A.1B). As expected the overlapping 1425 genes display a similar pathway involvement as before, mainly an activation of immune system pathways (fig.A.1D).

Next, we investigated the 696 exclusive NL genes to the 1811 exclusive HC genes. The NL exclusive genes again show many immune system pathways such as *inflammatory response, immune response, lymphocyte activation, alpha-beta T cell activation* (fig.A.1E), whereas the HC exclusive genes are involved in different pathways. The most activated pathways relate to DNA processing and the suppressed pathways include again muscle involvement (fig.A.1F).

In order to validate this, the two datasets (GSE63979, GSE121212) including both non-lesional and healthy control samples were used to repeat the above-described analysis, but now directly comparing NL against HC. DE analysis yielded a sharp decrease in the number of DEGs (fig.A.2A) and also in the size of the fold change.(fig.A.2B) Using the DEG for the pathway involvement study, reveal that those few DEGs are known to have a function in the immune system and skin development as pathways relating to these, are coming up in both datasets such as *immune system process, B cell activation and receptor signalling, immune response* (GSE121212, fig.A.2D), *keratinization and keratinocyte differentiation* (GSE63979, fig.A.2C).

Overall, this shows that healthy skin and non-affected skin is very similar, however subtle differences hinting towards epidermal dysregulation and inflammatory responses are present.

Regulator Scoring

In order to identify key regulators across both comparisons LvsNL and LvsHC, we used the R-package RegEnrich on each individual dataset. We calculated the median rank of the regulators in each design and used this median rank to sort the regulators. Again,
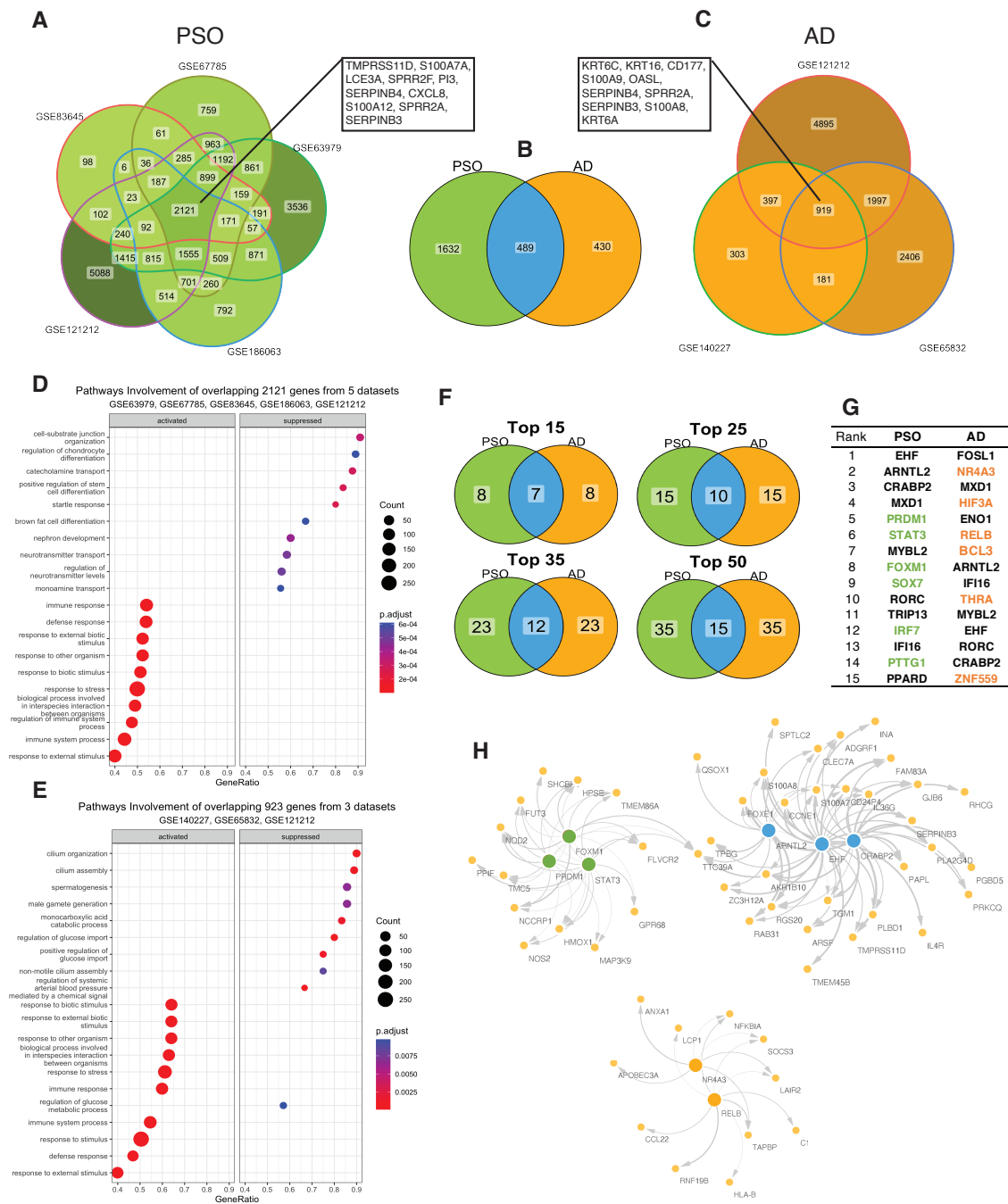
Figure 1: *A Overlapping of DEGs of each individual dataset of Psoriasis (PSO) finds 2121 common genes. Black box highlights genes with largest fold change from that list . C Analogous to A now with Atopic Dermatitis find 919 genes. B Overlapping the common genes from Psoriasis A and Atopic Dermatitis (AD) B reveal 489 common genes. Common DEGs were used for gene set enrichment analysis studying GO-annotated pathways in Psoriasis D and Atopic Dermatitis E. Revealing many immune related activated pathways. F Results from Regulator study using RegEnrich, showing overlap of Psoriasis and Atopic Dermatitis. G lists the top-15 of both diseases and highlights the unique regulators for Psoriasis (in green) and Atopic Dermatitis (in orange). These were used to create gene regulatory network in H by using the top three or two regulator-target pairs. Green dots depict regulators of Psoriasis, orange of Atopic Dermatitis and blue of shared regulators.*

we observe a big overlap between both designs as 30 out of top-50 regulators are the same (fig.A.1G).

To validate the findings of RegEnrich and to study the regulators in more details, we manually searched for downstream targets of the regulators using the manually curated database TRRUST (Han et al., 2015). If there were known downstream targets, we retrieved most of them in our list of differentially expressed genes. For instance this was the case for the very highly ranked STAT1, PRDM1 and EHF and their targets ACAT1, APP, BAX; CIITA, GCSAM, MKI67, MYC, PCNA; SPRR1B respectively. Whereas for MXD1 the downstream targets (ODC1, PTEN, TERT, SMAD) were often not found back.

Analogous to the study of Suárez-Fariñas et al., 2012 we also annotated our top-50 regulators whether they were already identified to be regulated by the cytokines IFN-γ, TNF-α, IL-17 and IL-22 as found by Chiricozzi et al., 2011 and Nograles et al., 2008. In table B.5 many of our regulators are found back in the IFN-γ regulated pathway, some in TNF-α and only CRABP2 is regulated by IL-17 and IFN-γ. Interestingly, CRABP2 is one of the few regulators, which rank differs greatly between both comparison studies. It is highly ranked as 6th in the LvsNL study, but is not differentially expressed in LvsHC and only ranked 148th.

3.2 Comparison to AD

To validate whether our findings are specific for Psoriasis or just common for inflammatory skin diseases, we obtained datasets for Atopic Dermatitis (AD) and repeated the same analysis. In total 107 samples were analysed analogous to Psoriasis. We found 919 common DEGs, which again included *S100* (S100A9, S100A8), *SerpinB* (SERPINB4, SERPINB3) and *SPR* (SPRR2A) family genes. In addition, *Keratin KRT* family genes (KRT6C, KRT16, KRT6A) are are detected to be highly expressed (fig.1C). These overlapping genes were once again used for the pathway enrichment analysis. Similar to Psoriasis, we also discover activated processes relating to the immune system (fig.1E).

Comparing lesional skin of Psoriasis and Atopic Dermatitis directly, many differential expressed genes are found (10294 genes, see fig. A.4A), however their overall fold change is decreased compared to LvsNL (fig.A.4B). But, we also observe an overlap of 489 genes of the common 2121 and 919 DEGs of Psoriasis and Atopic Dermatitis, so approximately half of the common DEGs of AD are also commonly found in Psoriasis (fig.1B).

Next, we studied once again the involved pathways of the 489 overlapping genes as well as the 1626 and 427 genes exclusive to PSO and AD respectively. Looking at the pathways of the overlapping genes, we find *epidermis-* and *epithelium development*, but besides to *response to bacterium* no pathway directly relating to the immune system (fig.A.3A). Interestingly, both exclusive gene sets show involved immune system pathways

such as *immune and defense response* in PSO (fig.A.3B) and *leukocyte and T cell activation and regulation of immune system process* in AD (fig.A.3C). This suggest that the genes responsible for the activation of the immune system are not the same for both diseases.

The results of the regulator comparison can be found in Figure 1F. Comparing the overlap of the highly ranked regulators in both diseases, we see a large overlap. 7 (EHF, ARNTL2, CRABP2, MXD1, MYBL2, RORC, IFI16) of the top-15 regulators are found in both diseases. But even when looking at the top-50 regulators 5 (PRDM1, STAT3, FOXM1, SOX7, PTTG1) of Psoriasis top-15 and 6 (NR4A3, HIF3A, RELB, BCL3, THRA, ZNF559) of Atopic Dermatitis are exclusively found in only the respective disease (fig.1G). The regulators NR4A3 and THRA of AD are found in the top-50 of Psoriasis LvsHC, however only ranked 39th and 24th respectively (tab.B.5).

To characterize the underlying regulator relationships, we used the weights of network edges between regulators and their target genes calculated by RegEnrich to infer a network. For this we selected the top three regulator-target gene pairs based on weights and co-occurrences regulator-target gene pairs in at least three datasets for Psoriasis and two for Atopic Dermatitis. In Figure 1H we see the visual representation of these networks. Again, the network representing shared networks is quite large (in green) and even shares one target TTC39A together with the PSO network (in red). Both AD unique regulators (NR4A3 and RELB in blue) share 4 of 14 targets in their network.

3.3 Immune cell infiltration

Next, we investigated both diseases on infiltration of immune cells. For this we utilized the deconvolution tool CIBERSORTx and the enrichment method xCell. CIBERSORTx deconvolutes bulk RNA-seq data by comparing it against signatures of cell types. We used the previously for skin inflammatory disease developed DerM22 signature (Félix Garza et al., 2019). xCell already provides signatures of 64 cell types. For comparing both tools, we used the corresponding cell types from DerM22 in xCell. Both used signatures can be found in Table B.4. Figure 2 shows the deconvolution results of CIBERSORTx. For this the abundance of cell types were averaged across all datasets.

Comparing Psoriasis and Atopic Dermatits lesions to their non-lesional counterpart and healthy subjects, we find an increase in the fractions of keratinocytes (PSO: L 49.8%, NL 36.1%; AD: L 44.6%, NL 35.1%; HC:31.4%) and the sum of all immune cells (PSO: L12.5%, NL 10.5%; AD: L 14.8%, NL 12.2%; HC:10.4%). Adipose stem cells (PSO: L 15.0%, NL 26.5%; AD: L 19.1%, NL 26.8%; HC:33.5%) and adipocytes (PSO: L 16.8%, NL 20.9%; AD: L 14.8%, NL 15.6%; HC:17.4%) on the other hand decrease in lesional samples. Overall Psoriasis always shows a larger change than Atopic Dermatitis (fig.2A). The increase of immune cells is
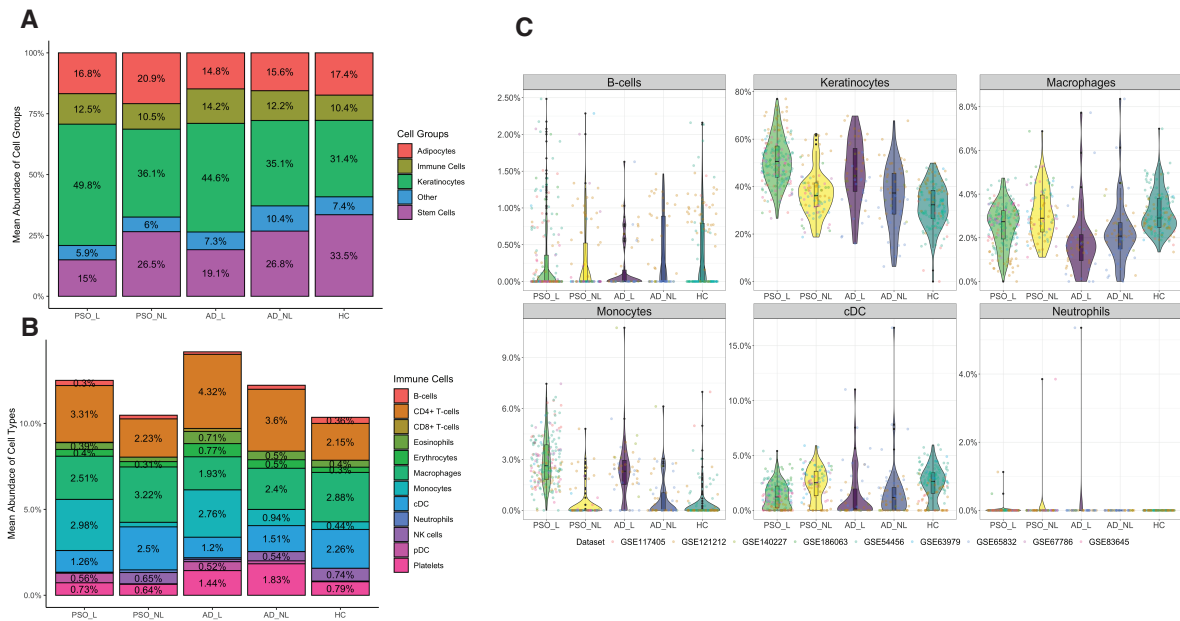
Figure 2: *Deconvolution Results of CIBERSORTx of healthy subjects and patients with Psoriasis and Atopic Dermatitis using signature matrix DerLM22 as reference. A Comparing the average cellular abundance of across different cell group types. B Detailed depiction of immune cells from A. C Violin plots including boxplot and individual samples of each dataset showing abundance of sixselected cell types For all analysed cell types see figure A.6. Defined cell groups: Adipocytes: Adipocytes, Preadipocytes; Immune cells: Platelets, NK cells, Neutrophils, cDC, Erythrocytes, Eosinophils, pDC, B-cells, CD8+ T-cells, CD4+ T-cells, Monocytes, Macrophages; Keratinocytes: Keratinoytes; Stem cell: Adipose Stem Cell; other: Chondrocytes, Endothelial cells, Fibroblasts, MSC, Osteoblast, Smooth muscle.*

mostly attributed to an increase of monocytes (PSO: L 2.98%, NL 0.26%; AD: L 2.76%, NL 0.94%; HC:0.44%), CD4$^+$ (PSO: L 3.31%, NL 2.23%; AD: L 4.32%, NL 3.6%; HC:2.15%) and pDCs (PSO: L 0.56%, NL 0.03%; AD: L 0.52%, NL 0.17%; HC:0.04%) (fig.2B). On the other hand, we also find an abundance decrease of NK cells (PSO: L 0.05%, NL 0.65%; AD: L 0.14%, NL 0.54%; HC:0.74%), macrophages (PSO: L 2.51%, NL 3.22%; AD: L 1.93%, NL 2.40%; HC:2.88%) and cDCs (PSO: L 1.26%, NL 2.50%; AD: L 1.20%, NL 1.51%; HC:2.26%) and overall find almost no presence of neutrophils (PSO: L 0.11%, NL 0.15%; AD: L 0.0%, NL 0.0%; HC:0.0%) . To validate if these findings are similar across all datasets we looked at the distribution of the individual datasets in Figure 2C (full figure in fig.A.6). We find that all datasets behave similarly and rarely cluster together.

In contrast, the enrichment method xCell cannot be used to derive the abundance of cell types. But it provides enrichment scores, therefore it can compare enrichment of cells between samples. Looking at the results we find keratinocytes and immune cells to be more enriched in lesional samples than in non-lesional ones (fig.A.5A). The enrichment of adipocytes decreases as well. However, looking at the performance of the individual datasets and not at the mean enrichment, we observe that xCell is only able to predict keratinocytes in one out of nine datasets (fig.A.5B). The category "*other cell types*" including Chondrocytes, Endothelial cells, Fibroblasts, MSC, Osteoblast and Smooth muscle

makes up by far the largest enriched category, and decreases in lesional skin of Psoriasis, but increases in lesional skin of Atopic Dermatitis. It must be noted that xCell provides no signature for adipose stem cells and thus cannot be tested on that. Overall, we can observe a larger cluster effect of datasets in xCell compared to CIBERSORTx. There seems to be more variation in the results between the datasets than between the cell types and many enrichment scores are close to zero (fig.A.7).

4 Discussion

4.1 Non affected and healthy skin exhibit subtle differences

From all applied analysis techniques, we can observe a large overlap between the design comparing lesional against non-lesional samples as well as against healthy control samples. Further, comparing NL directly against HC in differential expression analysis, we find almost no significant differences. This might indicate that skin tissues of patients from unaffected areas are similar to the ones of healthy people and both comparison studies can be used to investigate underlying mechanisms of the disease. However, the subtle differences we find indicate that non-lesional skin of patients already show slight indications towards an inflammatory state compared to skin from healthy controls. These findings are similar to previous stud-

ies (Tsoi et al., 2019), and suggest that non-lesional skin indicate the ability to manifest towards lesional skin. However, how much of these results are due to patient bias, cannot be stated. The differences between non-lesional and healthy skin can also seem higher as it might stem from the fact that they have been obtained from different subjects, whereas lesional and non-lesional skin is taken from the same person.

4.2 Psoriasis and Atopic Dermatitis are both inflammatory diseases induced by different pathways

Taking the differentially expressed genes found in each dataset for each design and looking in which pathway these genes are normally involved in, revealed that in both designs commonly activated pathways are involved in the functioning of the immune system. As Psoriasis is an immune-mediated disease, in which due to an immune response we see an increase of keratinocyte proliferation, it is expected that the common DEGs across the datasets are found to be involved in the up-regulation of the responsible pathways. On the other hand, the suppressed pathways are more diverse over the two different designs. Looking at the pairwise-comparison of affected skin against healthy patient skin, we find that most pathways are involved in muscle contraction. That result might come from improper skin biopsies deeper than the epidermis leading to inclusion of muscle tissue. Pathway enrichment of Atopic Dermatitis also revealed immune system pathways. That is also the case then including only genes, which are exclusively expressed in either disease, implying that both diseases are induced by different inflammatory pathways.

4.3 Regulators of disease

Our analysis shows known regulators for Psoriasis

One goal of this study was to identify key regulators of Psoriasis. For this we utilized the novel regulator enrichment tool RegEnrich. Again, we observe a big overlap between both designs and the results most often align with the experimentally proven interactions annotated in TRRUST. Further we have seen that many of our determined key regulators were previously found in a study to find genes affected by IFN-γ cytokine regulation (Nograles et al., 2008) as well as some by TNF-α (Chiricozzi et al., 2011) (Table B.5), which are key mediators of inflammation in Psoriasis.

In a previous integrative study of 3 microarray Psoriasis datasets, comparing lesional with non-lesional samples, researchers scanned for transcription factors (TFs) associated with their found overlapping set of differentially expressed genes (Zeng et al., 2019). Most of their identified TFs can also be found in our list of important regulators. We further identify STAT1, STAT3

and EHF in both of our comparisons, as well as FOSL1 in LvsNL. They identify *Forkhead box C1* (FOXC1) to be negatively correlated with their list of DEGs. This TF is not present in our list of regulators, but additional *Forkhead box family* members FOXM1, FOXE1 and FOXP1 are observed in both designs. This might indicate involvement of this family in the development of Psoriasis. Another study further confirms this finding as they not only detect STAT1 as being specifically upregulated in IFN-γ pathways, but additionally see FOXE1 yielding synergistic effects between IL-17 and TNF-α on keratinocytes (Suárez-Fariñas et al., 2012).

So overall, our findings agree with previous Psoriasis studies indicating the credibility of our applied methods. Thus, we further aimed to integrate Atopic Dermatitis to reveal more disease specific characteristics.

PSO and AD share many regulators of disease

Psoriasis and Atopic Dermatitis are both skin inflammatory diseases and therefore are expected to share responsible regulators. Approximately half of the top-15 regulators are shared between both diseases. These 7 regulators (EHF, ARNTL2, CRABP2, MXD1, MYBL2, RORC, IFI16) have already been associated or annotated with inflammatory states. Looking beyond the top-15 the overlap becomes less, but we still find many regulators active in both diseases. One example is PPARD, ranked 15th and 27th in PSO and AD respectively. The PPAR family and in particular α, β and γ is involved in skin homeostasis and injury repair (Michalik and Wahli, 2007). The activation of the *nuclear hormone receptor peroxisome proliferator activator β and δ* (PPARB, PPARD) has been shown to induce a Psoriasis-like skin disease *in vivo* (Romanowska et al., 2010). Our results align as we identified PPARD as one of our key regulators together with the γ *coactivator 1-β* (PPARGC1B). Further, one of the related pathways of PPARGC1B is the regulation of lipid metabolism by PPARA, which was found enriched in Suárez-Fariñas et al., 2012. Interestingly, treating Psoriasis plaques with agonists of the PPAR family had clinical benefits only when taken orally instead of applying it topically on the affected skin (Varani et al., 2006).

Unique regulators highlight differences of PSO and AD

We find 6 regulators in the top-15 which are exclusively found in each disease, even when considering the top-50 ranked regulators of both disorders.

For Psoriasis this includes PRDM1, STAT3, FOXM1, SOX7 and PTTG1. SOX7 is already considered as one of the hallmark genes of Psoriasis as it was one of the 13 DEGs upregulated in all 216 patients in one large scale study (Swindell et al., 2014). PRDM1 or Blimp-1 was previously highlighted as key IL-13 induced factor that drove the inflammatory function of

Th17 (Jain et al., 2016). The same study also found a co-localization with beyond others STAT3. STAT3 was further described in another study as the activator of transcription together with RORC for the differentiation of Th17 cells (Guntermann et al., 2017). In addition, there are also multiple studies describing FOXM1 and PTTG1 involvement in Psoriasis and the TNF-α pathway (Zhou et al., 2021; Ishitsuka et al., 2013)

Compared to Psoriasis the exclusive regulators in Atopic Dermatitis are NR4A3, HIF3A, RELB, BCL3, THRA and ZNF559. Literature concerning AD is much sparser as not many RNA-seq studies yet exist, which study the transcriptome on high throughput level. Even though our found regulators are not yet specifically annotated in transciptomic Atopic Dermatitis data, they have shown to be involved in IL-4 and IL-13 regulation, and are considered the core mechansim of AD and targeting them during treatment has showed promising clinical applications (Chiricozzi et al., 2020). NR4A4 was found to be essential for *in-vitro* GM-CSF+IL-4 Bone Marrow Derived Dendritic Cells (BMDC) and the differentiation of monocytes into DCs (Boulet et al., 2019). In another study, NR4A4 deficiency led to up-regulation of Th2 cytokine genes in T reg cells (CD4$^+$CD25^{hi}) (Sekiya et al., 2015). The other found core regulator RELB (fig.1H) is a member of the *NFκB/Rel* family of transcription factors. Previous studies have shown their involvement in inflammatory responses (Grossmann et al., 1999), and skin of mice lacking RELB exhibited skin lesions similar to Atopic Dermatitis (Barton, HogenEsch, and Weih, 2000). Another regulator, *B-cell leukemia-3* (BCL3) was found to be weakly induced in human keratinocytes cultures upon IL-4/IL-13 treatment and its mRNA expression was elevated in lesional Atopic Dermatitis skin measured by qPCR (Büchau et al., 2009).

We suggest looking for these regulators in future studies especially high-throughput transcriptome studies and evaluate their significance during disease progression.

Top scorer ARNTL2

The *Aryl hydrocarbon receptor nuclear translocator-like 2* (ARNTL2) is in both disorders a very highly ranked regulator (2nd in PSO, 8th in AD). This transcription factor belongs to the PAS (PER, ARNT, SIM) superfamily, known to be involved in the adaptation to environmental stress such as specific pollutants or low atmospheric and cellular oxygen levels (Taylor and Zhulin, 1999). It is studied that another member of the PAS superfamily, *aryl hydrocarbon receptor* (AhR), is involved in the regulation of the immune response. And more specifically among others, also in Th17 cells and in the pathway of Il-22 production (Quintana et al., 2008; Rutz et al., 2011), a central mechanism of Psoriasis.

ARNTL2 (or Bmal2) is known to dimerize with a *hypoxia-inducible factors HIF1α,* but also functions as the β subunit of the circadian CLOCK protein (Graham and Presnell, 2017). So far ARNTL2 has been studied in normal tissue and several tumour types (Hogenesch et al., 2000; Mazzoccoli et al., 2012), and together with other core clock genes it was found to be up-regulated in inflammatory bowel disease (Palmieri et al., 2015). Further, it was shown that its upregulation and interaction with CLOCK, followed an induction of the secretomes Smoc2 , driving the establishing of lethal metastatic lesions in Adenocarcinoma (Brady et al., 2016). Even though the fact that ARNTL2 is commonly found in differential expression analysis of Psoriasis (Li et al., 2014; Jabbari et al., 2014; Suárez-Fariñas et al., 2012), it has not yet been further investigated whether it has a significant role in the disease progression of Psoriasis. However, the loss of one of its paralogs Arnt, had shown the triggering of dermal angiogenesis, blood vessel dilation and clotting defects resulting in skin showing tumour stroma and psoriatic lesions (Wondimu et al., 2012). In addition, further studying of the ahR/ARNT system revealed different roles in skin homeostasis and its influence on chloracne and hyperpigmentation (Furue et al., 2014).

Due to the role of this family in the adaptation to environmental stress, and showing the involvement in skin disorders as well as the involvement of specifically ARNTL2 in cancer lesions, further characterization of this ARNTL-2 dependent pathway might reveal mechanism of Psoriasis disease progression or initiation.

The odd one CRABP2

As mentioned before, the comparison of LvsNL and LvsHC result in very similar findings. However, one regulator which performed drastically different is the Cellular Retinoic Acid Binding Protein 2 (CRBABP2). It is one of the most highly ranked regulators when comparing affected skin to unaffected skin in both diseases, but does no longer seem important when compared to skin of healthy controls. Further it is the only regulator to be found affected by IL-17 cytokine regulation in our list of regulators according to the list of Chiricozzi et al., 2011 and Nograles et al., 2008, which however might not be a complete list. In the study by Chiricozzi et al., 2011 there was also seen quite a discrepancy as the fold change was more than 8-fold higher in the IL-17 mediated pathway than in the IFN-γ one. Studies suggest that CRABP2 is important for skin growth and development (Collins and Watt, 2008), its expression is reduced during the aging process (Bielli et al., 2019) and it promotes the survival of Malignant Peripheral Nerve Sheath Tumor Cells (Fischer-Huchzermeyer et al., 2017). But so far to our knowledge it has not yet been specifically studied whether it plays an important role in immunological skin diseases.

4.4 Deconvolution

The currently most reliable deconvolution tools are mostly based on algorithms needing a reference to infer cell type-proportions. Marker-based and reference-free methods mostly perform less accurate in benchmarking studies compared to reference-based methods (Jin and Liu, 2021). But the reliability of reference-based methods is limited by the origin and quality of that reference gene expression profile (Schelker et al., 2017). Therefore, we used the reference-based method CIBERSORTx with the signature matrix DerM22, which includes mRNA expression signatures of skin-specific and immune cell types (Félix Garza et al., 2019). Despite being tested on its validation, the provider of this signature matrix also notes two fundamental limitations: the fidelity of the reference profile and their application of cross-platform data. Both limitations are being addressed, but that still remains reason for caution during evaluation of the results of our deconvolution analysis.

xCell is a maker-gene based approaches, which performs a statistical test for enrichment of the marker genes (Aran, Hu, and Butte, 2017). Thus, strictly speaking it is not a deconvolution tool, but an enrichment method, which evaluates every cell type independently. Therefore, it should be used for comparing across samples, but not across cell types. The user can select between 64 immune and non-immune cell types to be included in the analysis. As DerM22 is derived from skin tissue, we selected the same cell types in xCell, excluding adipose stem cells as there was no good fit to that cell type. One limitation of xCell is that the results are dependent on all samples provided in a single run. Thus different results are obtained, when samples are run together with other samples. Moreover, it does not perform well with only few input samples and fails to detect signals in non-heterogeneous samples. However, it is very robust against background-predictions and is suggested for determining the presence or absence of a cell type (Sturm et al., 2019).

The results of our deconvolution with CIBERSORTx perform expectedly very similar to the ones of Félix Garza et al., 2019, who provide the signature reference we use. These results agree with the current knowledge of the mechanism of Psoriasis as we see an increase of keratinocytes and immune cells. The observed increase of $CD4^+$ T-cells and pDCs fit the described pathogenesis in literature. However, our results do not show the commonly described macrophage and neutrophil infiltration (Rendon and Schäkel, 2019). Similar, to Félix Garza et al., 2019 we also only find an unexpected low fraction of $CD8^+$ T-cells. They hypothesized that this might be due to discrepancy in sample acquisition as they are located in the borderline of the epidermis and dermis. Our results with different datasets however might indicate a bias in the underlying signature matrix.

xCell on the other hand shows that $CD8^+$ T-cells

are enriched in lesional samples (fig.A.7). But overall, the results of xCell exhibit a bias towards the source dataset. The results of each individual dataset are clustering together with a higher degree than mixing with each other. One concerning result is that xCell detects the presence of keratinocytes in only one out of nine datasets.

Running a very simple benchmark experiment showed problems of both tools with accurately predicting the presence or absence of cell types. However, this benchmark simulation itself exhibits many limitations due to lack of time, but nevertheless it also displays the need for better testing and reveal limitations of computational deconvolution tools. There are many variables, and changing even only one of them can lead to drastically different results. Overall, the findings are very dependent on the input reference data and should thereby always be seen with caution. As most deconvolution tools rely on an input of scRNA-seq and the costs of this technique are decreasing, the generation of more accurate scRNA-seq might be more beneficial than trying to improve the computational tools.

5 Conclusion

In conclusion, in this study on large-scale datasets we combined differential expression, gene regulatory network inference and enrichment analysis to reveal regulators and further could show an immune cell infiltration in both Psoriasis and Atopic Dermatitis. We have seen that our found genes, pathways, and regulators are consistent with the current knowledge about Psoriasis and Atopic Dermatitis, reflecting the credibility of our applied methods. To asses the clinical relevance of our found regulators, further analytical and laboratory studies will be necessary.

Bibliography

Albanesi, Cristina et al. (2018). "The Interplay Between Keratinocytes and Immune Cells in the Pathogenesis of Psoriasis." eng. In: *Frontiers in immunology* 9, p. 1549. ISSN: 1664-3224 (Print). DOI: 10.3389/fimmu.2018.01549.

Amin, Mina et al. (2020). "Psoriasis and Co-morbidity." eng. In: *Acta dermato-venereologica* 100.3, adv00033. ISSN: 1651-2057 (Electronic). DOI: 10.2340/00015555-3387.

Aran, Dvir, Zicheng Hu, and Atul J Butte (2017). "xCell: digitally portraying the tissue cellular heterogeneity landscape". In: *Genome Biology* 18.1, p. 220. ISSN: 1474-760X. DOI: 10.1186/s13059-017-1349-1. URL: https://doi.org/10.1186/s13059-017-1349-1.

Avila Cobos, Francisco et al. (2020). "Benchmarking of cell type deconvolution pipelines for transcriptomics data". In: *Nature Communications* 11.1, p. 5650. ISSN: 2041-1723. DOI: 10.1038/s41467-020-19015-1. URL: https://doi.org/10.1038/s41467-020-19015-1.

Ayala-Fontánez, Nilmarie, David C Soler, and Thomas S McCormick (2016). "Current knowledge on psoriasis and autoimmune diseases". eng. In: *Psoriasis (Auckland, N.Z.)* 6, pp. 7–32. ISSN: 2230-326X. DOI: 10.2147/PTT.S64950. URL: https://pubmed.ncbi.nlm.nih.gov/29387591https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5683130/.

Barton, D, H HogenEsch, and F Weih (2000). "Mice lacking the transcription factor RelB develop T cell-dependent skin lesions similar to human atopic dermatitis." eng. In: *European journal of immunology* 30.8, pp. 2323–2332. ISSN: 0014-2980 (Print). DOI: 10.1002/1521-4141(2000)30:8<2323::AID-IMMU2323>3.0.CO;2-H.

Bielli, Alessandra et al. (2019). "Cellular retinoic acid binding protein-II expression and its potential role in skin aging". eng. In: *Aging* 11.6, pp. 1619–1632. ISSN: 1945-4589. DOI: 10.18632/aging.101813. URL: https://pubmed.ncbi.nlm.nih.gov/30888968https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6461173/.

Boulet, Salix et al. (2019). "The orphan nuclear receptor NR4A3 controls the differentiation of monocyte-derived dendritic cells following microbial stimulation". In: *Proceedings of the National Academy of Sciences* 116.30, 15150 LP –15159. DOI: 10.1073/pnas.1821296116. URL: http://www.pnas.org/content/116/30/15150.abstract.

Brady, Jennifer J et al. (2016). "An Arntl2-Driven Secretome Enables Lung Adenocarcinoma Metastatic Self-Sufficiency." eng. In: *Cancer cell* 29.5, pp. 697–710. ISSN: 1878-3686 (Electronic). DOI: 10.1016/j.ccell.2016.03.003.

Büchau, Amanda S et al. (2009). "Bcl-3 Acts as an Innate Immune Modulator by Controlling Antimicrobial Responses in Keratinocytes". In: *Journal of Investigative Dermatology* 129.9, pp. 2148–2155. ISSN: 0022-202X. DOI: 10.1038/jid.2009.49. URL: https://doi.org/10.1038/jid.2009.49.

Chiricozzi, Andrea et al. (2011). "Integrative Responses to IL-17 and TNF-α in Human Keratinocytes Account for Key Inflammatory Pathogenic Circuits in Psoriasis". In: *Journal of Investigative Dermatology* 131.3, pp. 677–687. ISSN: 0022-202X. DOI: 10.1038/jid.2010.340. URL: https://doi.org/10.1038/jid.2010.340.

Chiricozzi, Andrea et al. (2020). "Targeting IL-4 for the Treatment of Atopic Dermatitis". eng. In: *ImmunoTargets and therapy* 9, pp. 151–156. ISSN: 2253-1556. DOI: 10.2147/ITT.S260370. URL: https://pubmed.ncbi.nlm.nih.gov/33062619https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7532907/.

Collins, Charlotte A and Fiona M Watt (2008). "Dynamic regulation of retinoic acid-binding proteins in developing, adult and neoplastic skin reveals roles for β-catenin and Notch signalling". In: *Developmental Biology* 324.1, pp. 55–67. ISSN: 0012-1606. DOI: https://doi.org/10.1016/j.ydbio.2008.08.034. URL: https://www.sciencedirect.com/science/article/pii/S0012160608011615.

Davidovici, Batya B et al. (2010). "Psoriasis and systemic inflammatory diseases: potential mechanistic links between skin disease and co-morbid conditions." eng. In: *The Journal of investigative dermatology* 130.7, pp. 1785–1796. ISSN: 1523-1747 (Electronic). DOI: 10.1038/jid.2010.103.

Federico, Antonio et al. (2020). "Manually curated and harmonised transcriptomics datasets of psoriasis and atopic dermatitis patients". In: *Scientific Data* 7.1, p. 343. ISSN: 2052-4463. DOI: 10.1038/s41597-020-00696-8. URL: https://doi.org/10.1038/s41597-020-00696-8.

Félix Garza, Zandra C et al. (2019). "Characterization of disease-specific cellular abundance profiles of chronic inflammatory skin conditions from deconvolution of biopsy samples". In: *BMC Medical Genomics* 12.1, p. 121. ISSN: 1755-8794. DOI: 10.1186/s12920-019-0567-7. URL: https://doi.org/10.1186/s12920-019-0567-7.

Fischer-Huchzermeyer, Susan et al. (2017). "The Cellular Retinoic Acid Binding Protein 2 Promotes Survival of Malignant Peripheral Nerve Sheath Tumor Cells". In: *The American Journal of Pathology* 187.7, pp. 1623–1632. ISSN: 0002-9440. DOI: https://doi.org/10.1016/j.ajpath.2017.02.021. URL: https://www.sciencedirect.com/science/article/pii/S0002944017302316.

Fotiadou, Christina et al. (2015). "IL-17A, IL-22, and IL-23 as Markers of Psoriasis Activity: A Cross-sectional, Hospital-based Study". In: *Journal of Cutaneous Medicine and Surgery* 19.6, pp. 555–560. ISSN: 1203-4754. DOI: 10.1177/1203475415584503. URL: https://doi.org/10.1177/1203475415584503.

Furue, Masutaka et al. (2014). "Role of AhR/ARNT system in skin homeostasis". In: *Archives of Dermatological Research* 306.9, pp. 769–779. ISSN: 1432-069X. DOI: 10.1007/s00403-014-1481-7. URL: https://doi.org/10.1007/s00403-014-1481-7.

Graham, Allie and Jason Presnell (2017). "Hypoxia Inducible Factor (HIF) transcription factor family expansion, diversification, divergence and selection in eukaryotes". In: *PLOS ONE* 12, e0179545. DOI: 10.1371/journal.pone.0179545.

Grossmann, Mathis et al. (1999). "New insights into the roles of ReL/NF-κB transcription factors in immune function, hemopoiesis and human disease". In: *The International Journal of Biochemistry & Cell Biology* 31.10, pp. 1209–1219. ISSN: 1357-2725. DOI: https://doi.org/10.1016/S1357-2725(99)00068-0. URL: https://www.sciencedirect.com/science/article/pii/S1357272599000680.

Guntermann, Christine et al. (2017). "Retinoic-acid-orphan-receptor-C inhibition suppresses Th17 cells and induces thymic aberrations". In: *JCI Insight* 2.5. ISSN: 2379-3708. DOI: 10.1172/jci.insight.91127. URL: https://doi.org/10.1172/jci.insight.91127.

Han, Heonjong et al. (2015). "TRRUST: a reference database of human transcriptional regulatory interactions". In: *Scientific Reports* 5.1, p. 11432. ISSN: 2045-2322. DOI: 10.1038/srep11432. URL: https://doi.org/10.1038/srep11432.

Hogenesch, John B et al. (2000). "The Basic Helix-Loop-Helix-PAS Protein MOP9 Is a Brain-Specific Heterodimeric Partner of Circadian and Hypoxia Factors". In: *The Journal of Neuroscience* 20.13, RC83 LP –RC83. DOI: 10.1523/JNEUROSCI.20-13-j0002.2000. URL: http://www.jneurosci.org/content/20/13/RC83.abstract.

Ishitsuka, Yosuke et al. (2013). "Pituitary tumor transforming gene 1 induces tumor necrosis factor-α production from keratinocytes: implication for involvement in the pathophysiology of psoriasis." eng. In: *The Journal of investigative dermatology* 133.11, pp. 2566–2575. ISSN: 1523-1747 (Electronic). DOI: 10.1038/jid.2013.189.

Jabbari, Ali et al. (2014). "Dominant Th1 and minimal Th17 skewing in discoid lupus revealed by transcriptomic comparison with psoriasis." eng. In: *The Journal of investigative dermatology* 134.1, pp. 87–95. ISSN: 1523-1747 (Electronic). DOI: 10.1038/jid.2013.269.

Jain, Renu et al. (2016). "Interleukin-23-Induced Transcription Factor Blimp-1 Promotes Pathogenicity of T Helper 17 Cells". In: *Immunity* 44.1, pp. 131–142. ISSN: 10747613. DOI: 10.1016/j.immuni.2015.11.009. URL: https://linkinghub.elsevier.com/retrieve/pii/S1074761315004616.

Jin, Haijing and Zhandong Liu (2021). "A benchmark for RNA-seq deconvolution analysis under dynamic testing environments". In: *Genome Biology* 22.1, p. 102. ISSN: 1474-760X. DOI: 10.1186/s13059-021-02290-6. URL: https://doi.org/10.1186/s13059-021-02290-6.

Li, Bingshan et al. (2014). "Transcriptome analysis of psoriasis in a large case-control sample: RNA-seq provides insights into disease mechanisms". eng. In: *The Journal of investigative dermatology* 134.7, pp. 1828–1838. ISSN: 1523-1747. DOI: 10.1038/jid.2014.28. URL: https://pubmed.ncbi.nlm.nih.gov/24441097https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4057954/.

Love, Michael I, Wolfgang Huber, and Simon Anders (2014). "Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2". In: *Genome Biology* 15.12, p. 550. ISSN: 1474-760X. DOI: 10.1186/s13059-014-0550-8. URL: https://doi.org/10.1186/s13059-014-0550-8.

Marbach, Daniel et al. (2012). "Wisdom of crowds for robust gene network inference". eng. In: *Nature methods* 9.8, pp. 796–804. ISSN: 1548-7105. DOI: 10.1038/nmeth.2016. URL: https://pubmed.ncbi.nlm.nih.gov/22796662https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3512113/.

Mazzoccoli, Gianluigi et al. (2012). "Association study of a polymorphism in clock gene PERIOD3 and risk of inflammatory bowel disease." eng. In: *Chronobiology international* 29.8, pp. 994–1003. ISSN: 1525-6073 (Electronic). DOI: 10.3109/07420528.2012.705935.

Michalik, Liliane and Walter Wahli (2007). "Peroxisome proliferator-activated receptors (PPARs) in skin health, repair and disease". In: *Biochimica et Biophysica Acta (BBA) - Molecular and Cell Biology of Lipids* 1771.8, pp. 991–998. ISSN: 1388-1981. DOI: https://doi.org/10.1016/j.bbalip.2007.02.004. URL: https://www.sciencedirect.com/science/article/pii/S1388198107000339.

Newman, Aaron M et al. (2019). "Determining cell type abundance and expression from bulk tissues with digital cytometry". In: *Nature Biotechnology* 37.7, pp. 773–782. ISSN: 1546-1696. DOI: 10.1038/s41587-019-0114-2. URL: https://doi.org/10.1038/s41587-019-0114-2.

Nograles, K E et al. (2008). "Th17 cytokines interleukin (IL)-17 and IL-22 modulate distinct inflammatory and keratinocyte-response pathways." eng. In: *The British journal of dermatology* 159.5, pp. 1092–1102. ISSN: 1365-2133 (Electronic). DOI: 10.1111/j.1365-2133.2008.08769.x.

Palmieri, Orazio et al. (2015). "Systematic analysis of circadian genes using genome-wide cDNA microarrays in the inflammatory bowel disease transcriptome". In: *Chronobiology International* 32.7, pp. 903–916. ISSN: 0742-0528. DOI: 10.3109/07420528.2015.1050726. URL: https://doi.org/10.3109/07420528.2015.1050726.

Parisi, Rosa et al. (2013). "Global Epidemiology of Psoriasis: A Systematic Review of Incidence and Prevalence". In: *Journal of Investigative Dermatology* 133.2, pp. 377–385. ISSN: 0022-202X. DOI: https://doi.org/10.1038/jid.2012.339. URL: https://www.sciencedirect.com/science/article/pii/S0022202X1536098X.

Quintana, Francisco J et al. (2008). "Control of T(reg) and T(H)17 cell differentiation by the aryl hydrocarbon receptor." eng. In: *Nature* 453.7191, pp. 65–71. ISSN: 1476-4687 (Electronic). DOI: 10.1038/nature06880.

Rendon, Adriana and Knut Schäkel (2019). *Psoriasis Pathogenesis and Treatment*. DOI: 10.3390/ijms20061475.

Romanowska, Malgorzata et al. (2010). "Activation of PPARbeta/delta causes a psoriasis-like skin disease in vivo." eng. In: *PloS one* 5.3, e9701. ISSN: 1932-6203 (Electronic). DOI: 10.1371/journal.pone.0009701.

Rutz, Sascha et al. (2011). "Transcription factor c-Maf mediates the TGF-β-dependent suppression of IL-

22 production in T(H)17 cells." eng. In: *Nature immunology* 12.12, pp. 1238–1245. ISSN: 1529-2916 (Electronic). DOI: 10.1038/ni.2134.

Schelker, Max et al. (2017). "Estimation of immune cell content in tumour tissue using single-cell RNA-seq data". In: *Nature Communications* 8.1, p. 2032. ISSN: 2041-1723. DOI: 10.1038/s41467-017-02289-3. URL: https://doi.org/10.1038/s41467-017-02289-3.

Sekiya, Takashi et al. (2015). "Suppression of Th2 and Tfh immune reactions by Nr4a receptors in mature T reg cells". eng. In: *The Journal of experimental medicine* 212.10, pp. 1623–1640. ISSN: 1540-9538. DOI: 10.1084/jem.20142088. URL: https://pubmed.ncbi.nlm.nih.gov/26304965https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4577835/.

Shannon, Paul et al. (2003). "Cytoscape: a software environment for integrated models of biomolecular interaction networks." eng. In: *Genome research* 13.11, pp. 2498–2504. ISSN: 1088-9051 (Print). DOI: 10.1101/gr.1239303.

Sturm, Gregor et al. (2019). "Comprehensive evaluation of transcriptome-based cell-type quantification methods for immuno-oncology". eng. In: *Bioinformatics (Oxford, England)* 35.14, pp. i436–i445. ISSN: 1367-4811. DOI: 10.1093/bioinformatics/btz363. URL: https://pubmed.ncbi.nlm.nih.gov/31510660https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6612828/.

Suárez-Fariñas, Mayte et al. (2012). "Expanding the psoriasis disease profile: interrogation of the skin and serum of patients with moderate-to-severe psoriasis". eng. In: *The Journal of investigative dermatology* 132.11, pp. 2552–2564. ISSN: 1523-1747. DOI: 10.1038/jid.2012.184. URL: https://pubmed.ncbi.nlm.nih.gov/22763790https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3472561/.

Swindell, William R et al. (2014). "Cellular dissection of psoriasis for transcriptome analyses and the post-GWAS era". In: *BMC Medical Genomics* 7.1, p. 27. ISSN: 1755-8794. DOI: 10.1186/1755-8794-7-27. URL: https://doi.org/10.1186/1755-8794-7-27.

Tao, Weiyang, Timothy R D J Radstake, and Aridaman Pandit (2021). "RegEnrich: An R package for gene regulator enrichment analysis reveals key role of ETS transcription factor family in interferon signaling". In: *bioRxiv*, p. 2021.01.24.428029. DOI: 10.1101/2021.01.24.428029. URL: http://biorxiv.org/content/early/2021/01/26/2021.01.24.428029.abstract.

Taylor, B L and I B Zhulin (1999). "PAS domains: internal sensors of oxygen, redox potential, and light." eng. In: *Microbiology and molecular biology reviews : MMBR* 63.2, pp. 479–506. ISSN: 1092-2172 (Print). DOI: 10.1128/MMBR.63.2.479-506.1999.

Tsoi, Lam C et al. (2019). "Atopic Dermatitis Is an IL-13-Dominant Disease with Greater Molecular Heterogeneity Compared to Psoriasis". eng. In: *The Journal of investigative dermatology* 139.7, pp. 1480–1489. ISSN: 1523-1747. DOI: 10.1016/j.jid.2018.12.018. URL: https://pubmed.ncbi.nlm.nih.gov/30641038https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6711380/.

Varani, James et al. (2006). "Thiazolidinediones: potential as therapeutics for psoriasis and perhaps other hyperproliferative skin disease". In: *Expert Opinion on Investigational Drugs* 15.11, pp. 1453–1468. ISSN: 1354-3784. DOI: 10.1517/13543784.15.11.1453. URL: https://doi.org/10.1517/13543784.15.11.1453.

Wondimu, Assefa et al. (2012). "Loss of Arnt (Hif1β) in mouse epidermis triggers dermal angiogenesis, blood vessel dilation and clotting defects." eng. In: *Laboratory investigation; a journal of technical methods and pathology* 92.1, pp. 110–124. ISSN: 1530-0307 (Electronic). DOI: 10.1038/labinvest.2011.134.

Yu, Guangchuang et al. (2012). "clusterProfiler: an R package for comparing biological themes among gene clusters". eng. In: *Omics : a journal of integrative biology* 16.5, pp. 284–287. ISSN: 1557-8100. DOI: 10.1089/omi.2011.0118. URL: https://pubmed.ncbi.nlm.nih.gov/22455463https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3339379/.

Zeng, Fanfan et al. (2019). "Integrated analysis of gene expression profiles identifies transcription factors potentially involved in psoriasis pathogenesis." eng. In: *Journal of cellular biochemistry* 120.8, pp. 12582–12594. ISSN: 1097-4644 (Electronic). DOI: 10.1002/jcb.28525.

Zhou, Mi et al. (2021). "FOXM1 regulates the proliferation, apoptosis and inflammatory response of keratinocytes through the NF-κB signaling pathway." eng. In: *Human & experimental toxicology* 40.7, pp. 1130–1140. ISSN: 1477-0903 (Electronic). DOI: 10.1177/0960327120984225.

Appendix

A Figures
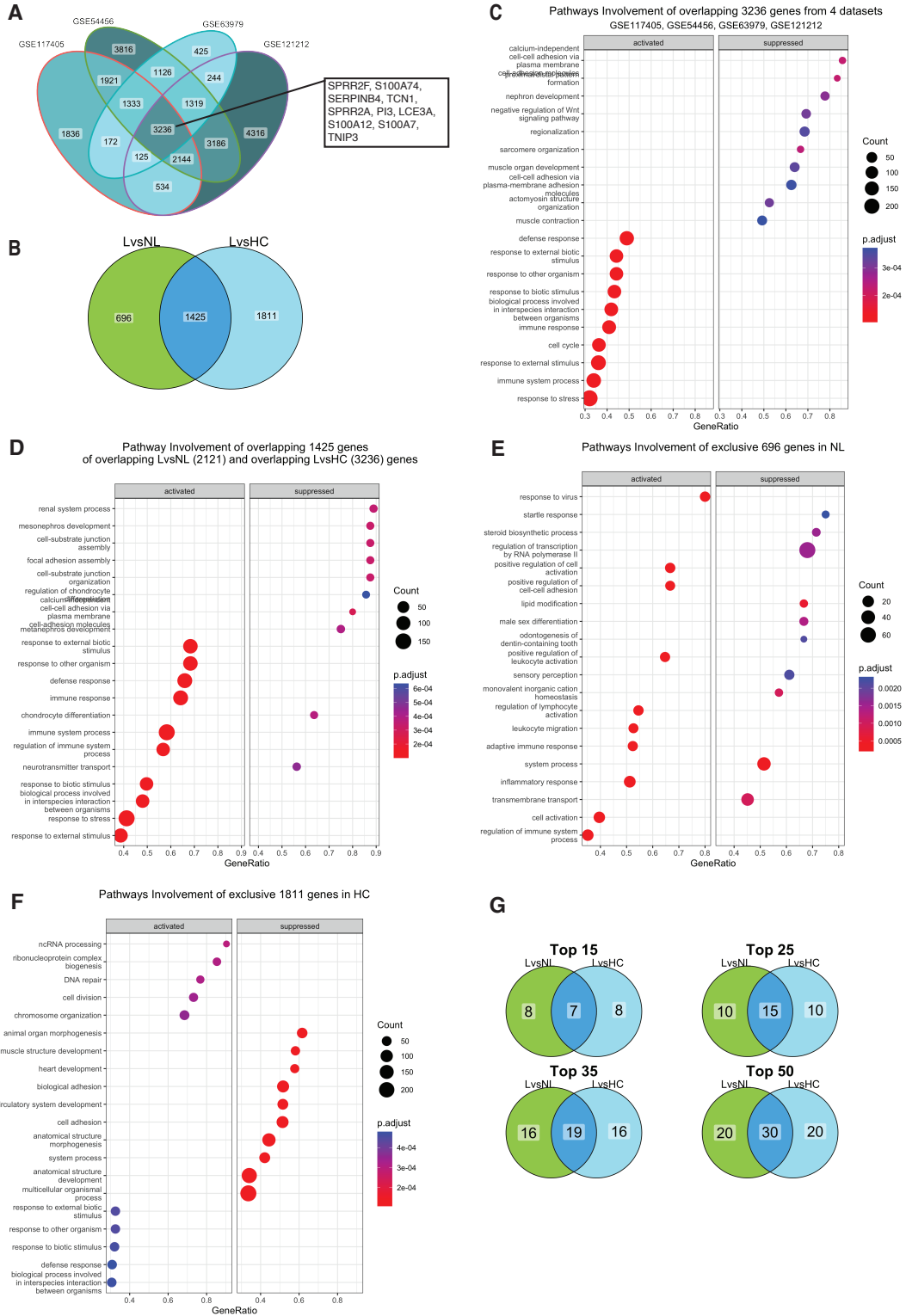
Figure A.1: *Comparison Non-Lesional and Healthy Skin. **A** Showing overlap of 3236 DEGs of each dataset with design LvsHC. and their involved pathways in **C**. **B** Overlap of overlapping 2121 DEGs of LvsNL (1 A) and 3236 DEGs LvsHC from **A**. Involved pathways of theses 1425 overlapping genes **D**, 696 exlcusive NL genes **E** and 1811 exclusive HC genes **F** from **B**. **G** shows common top-regulators of LvsNL and LvsHC after RegEnrich analysis. Full list can be found in table B.5.*

A

|	Dataset	Non-zero counts	LFC >0 (up)	LFC < 0 (down)	Outliers	Low counts
NLvsHC	GSE63979	24079	1820, 7.6%	2156, 9%	0, 0%	934, 3.9%
	GSE121212	24535	948, 3.9%	1031, 4.2%	0, 0%	3330, 14%
LvsNL	GSE63979	23624	5950, 25%	8741, 37%	0, 0%	0, 0%
	GSE121212	23725	7218, 30%	8974, 38%	0, 0%	0, 0%
LvsHC	GSE63979	21746	3820, 18%	4162, 19%	0, 0%	0, 0%
	GSE121212	23715	8166, 34%	6938, 29%	0,0%	0,0%

B

C

D
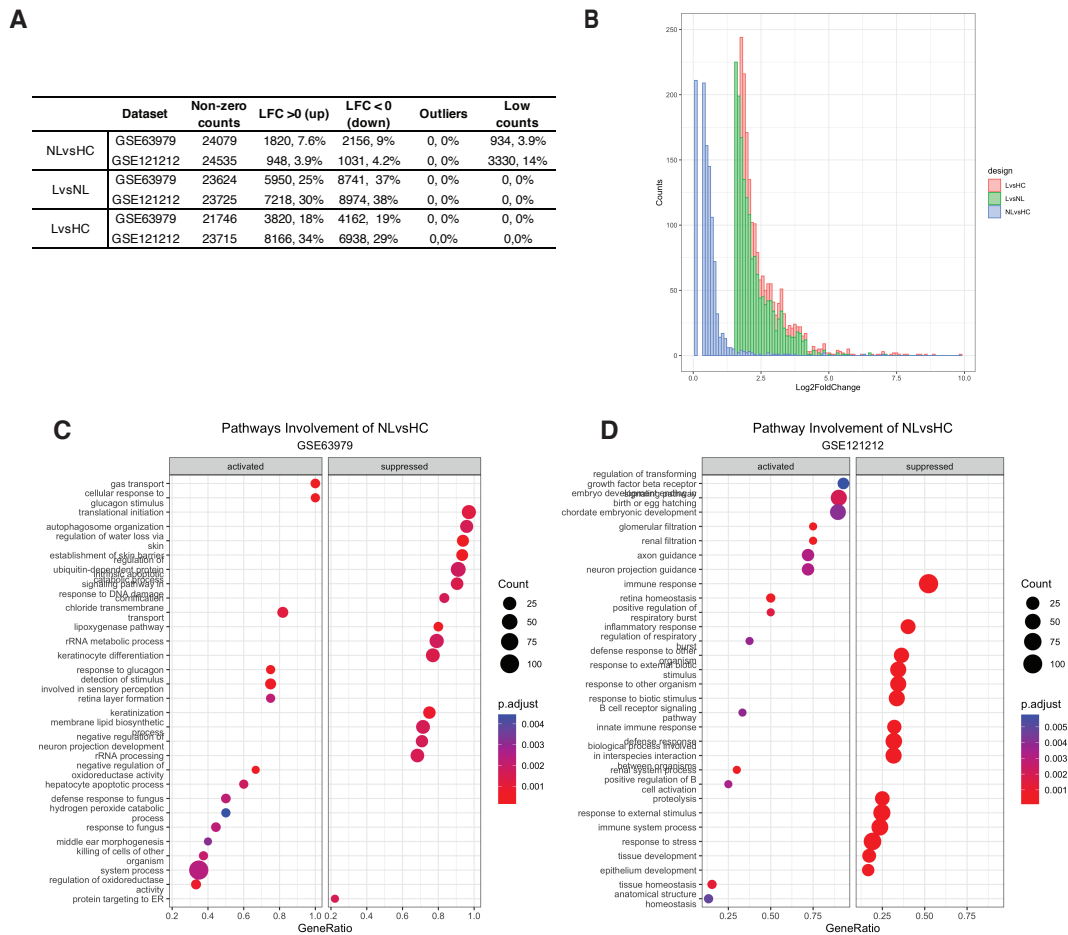
Figure A.2: *Comparison of Non-Lesional directly against healthy skin. **A** Results of differential expression analysis of GSE63979 and GSE121212 using DeSeq2. Three different comparison studies were run: non-lesional skin versus healthy control (NLvsHC), lesional versus non-lesional (LvsNL) and lesional versus heahlty control (LvsHC). Number of differential expressed genes (LFC up or down) drops significantly in NLvsHC design. **B** shows that the size of LogFC also decreases sharply compared to LvsNL or LvsHC. Involved Pathways from DE analysis with two-group comparison NL versus HC for dataset GSE63979 **C** and GSE121212 **D**.*
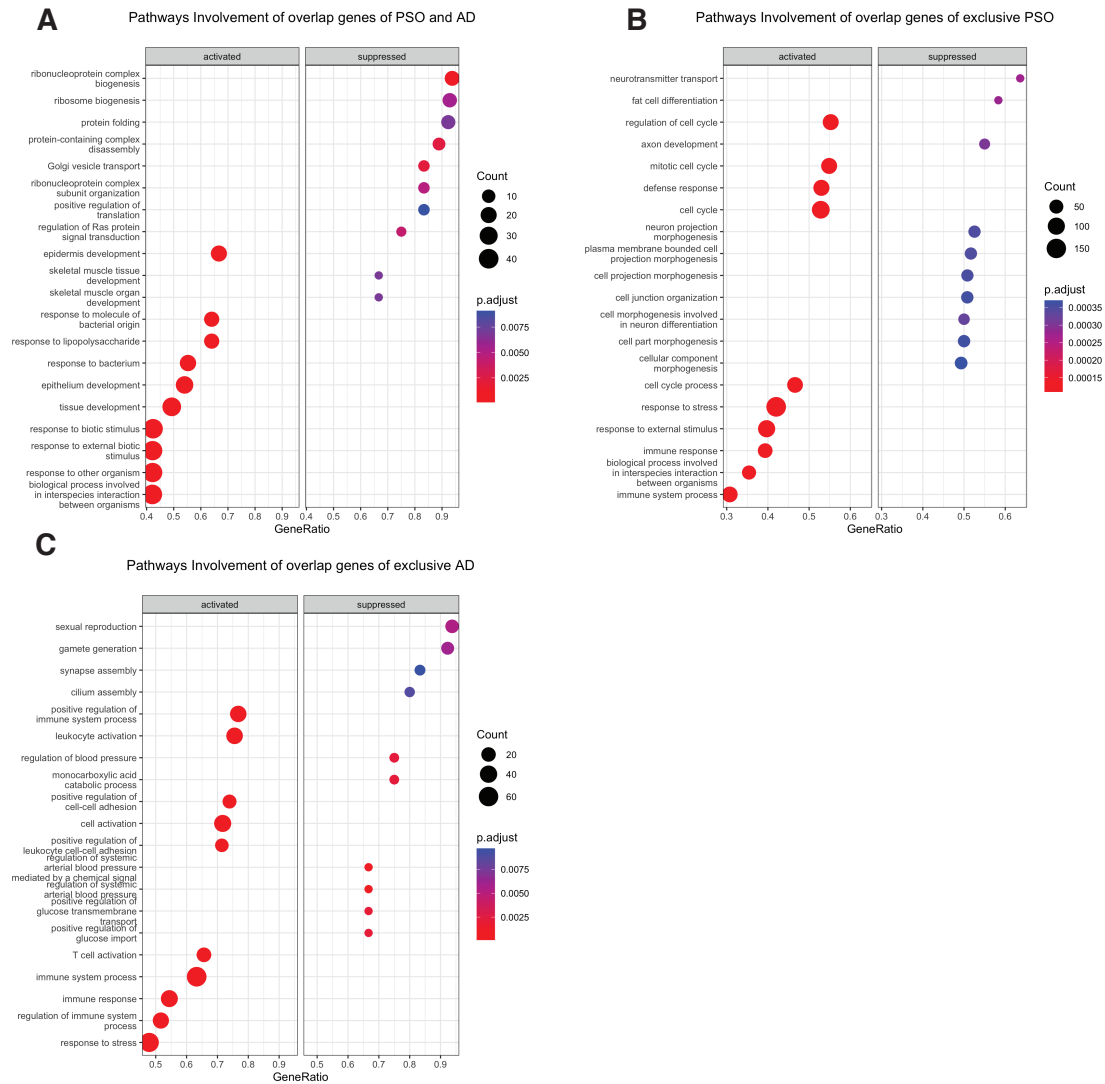
Figure A.3: *Comparison of pathway involvement Psoriasis and Atopic Dermatitis.* **A** *showing the pathway involvement of the common 489 genes of the overlapping genes of Psoriasis and Atopic Dermatits (fig.1B in blue). Besides response to bacterim no immune system related pathways, but skin development pathways are found such as epithelium- and epidermis development.* **B** *showing the pathway involvement of the common 1632 genes in Psoriasis, but not found in Atopic Dermatits (fig. 1B in green).* **C** *showing the pathway involvement of the 430 common genes in Atopic Dermatitis but not found in Psoriasis(fig. 1B in orange). Both* **B** *and* **C** *show involvement of immune system pathways.*

A

	Dataset	Non-zero counts	LFC >0 (up)	LFC < 0 (down)	Outliers	Low counts
PSO NLvsHC	GSE121212	24535	948, 3.9%	1031, 4.2%	0, 0%	3330, 14%
PSO LvsNL	GSE121212	23725	7218, 30%	8974, 38%	0, 0%	0, 0%
PSO LvsHC	GSE121212	23715	8166, 34%	6938, 29%	0,0%	0,0%
PSO L vs AD L	GSE121212	23875	6511, 27%	3783, 16%	0,0%	0,0%

B

C
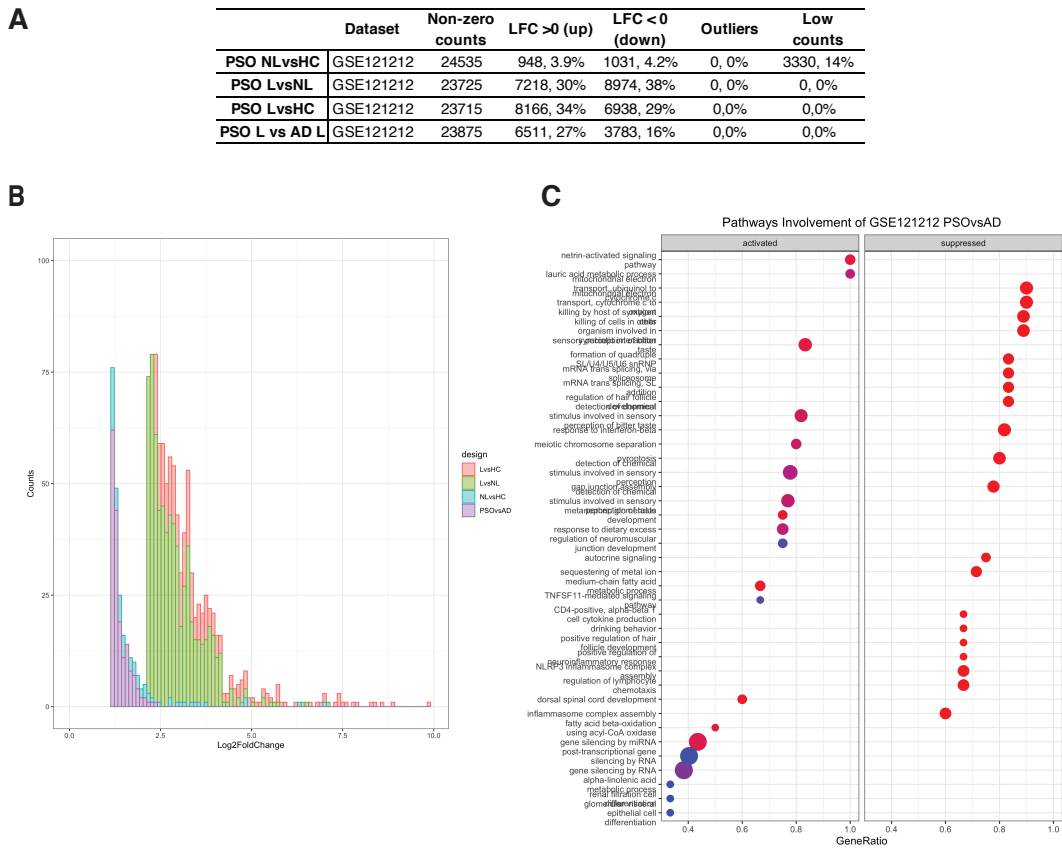
Figure A.4: *Comparison of Lesional skin of Psoriasis and Atopic Dermatits **A** Results of differential analysis of GSE121212 using DeSeq2. Psoriasis and Atopic Dermatitis show many DEGs. For comparison of the number of DEGs also the results of other DE analysis is shown. **B** The magnitude of LogFC is lower compared to the comparison against non-affected and healthy skin. Similar to the level of the NLvsHC comparison. **C** Involved Pathways from DE analysis show some immune system and skin development related pathways. Overall, very diverse set of pathways.*

A

B

C
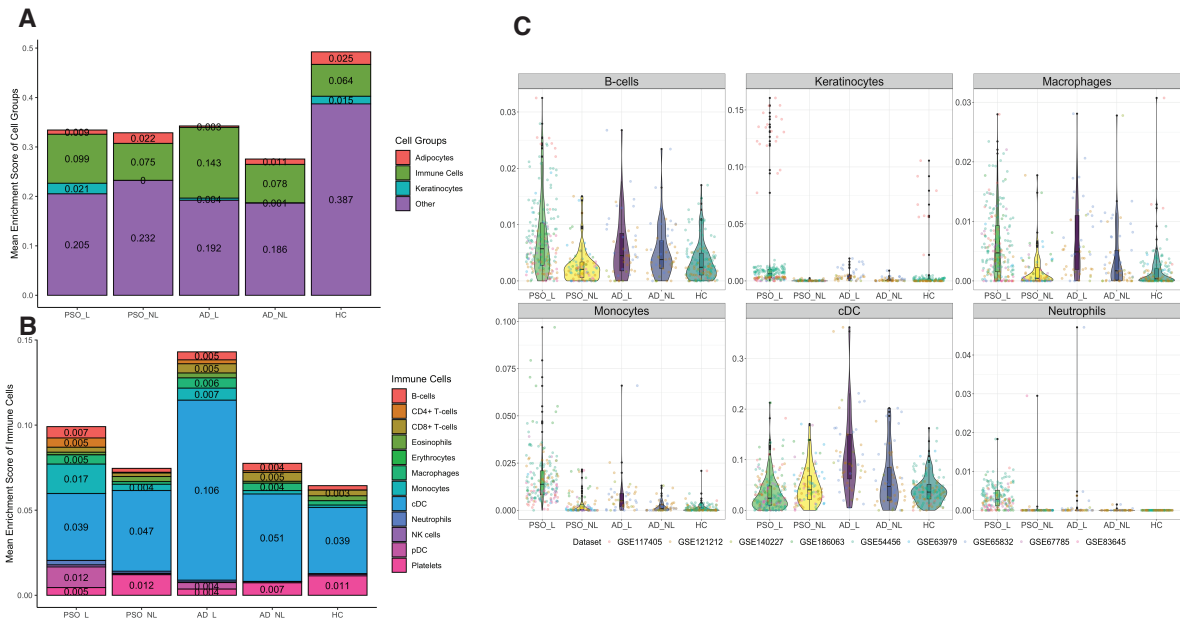
Figure A.5: *Enrichment Results of xCell of healthy subjects and patients with Psoriasis and Atopic Dermatitis. Cell types were specified as can be seen in table B.4. **A** Comparing the enrichment results of different cell group types. **B** Detailed depiction of immune cells from **A**. **C** Violin plots including boxplot and individual samples of each dataset showing abundance of selected cell types. Defined cell groups: Adipocytes: Adipocytes, Preadipocytes ; Immunecells: Platelets, NK cells, Neutrophils, cDC, Erythrocytes, Eosinophils, pDC, B-cells, CD8+ T-cells, CD4+ T-cells, Monocytes, Macrophages; Keratinocytes: Keratinoytes; other: Chondrocytes, Endothelial cells, Fibroblasts, MSC, Osteoblast, Smooth muscle*
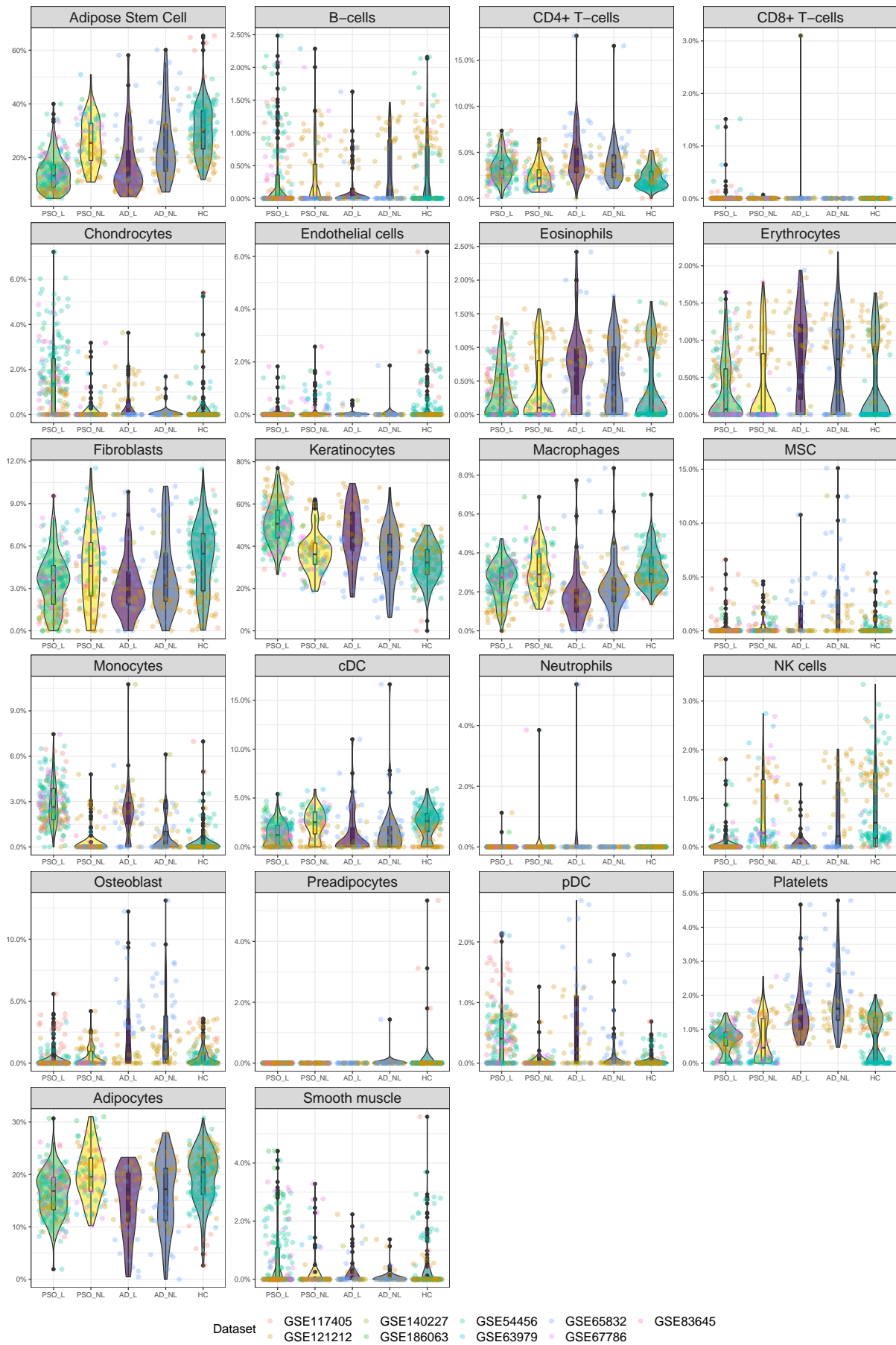
Figure A.6: *Violin plots including boxplots and individual samples of each dataset showing abundance of all analysed cell types in CIBERSORTx.*
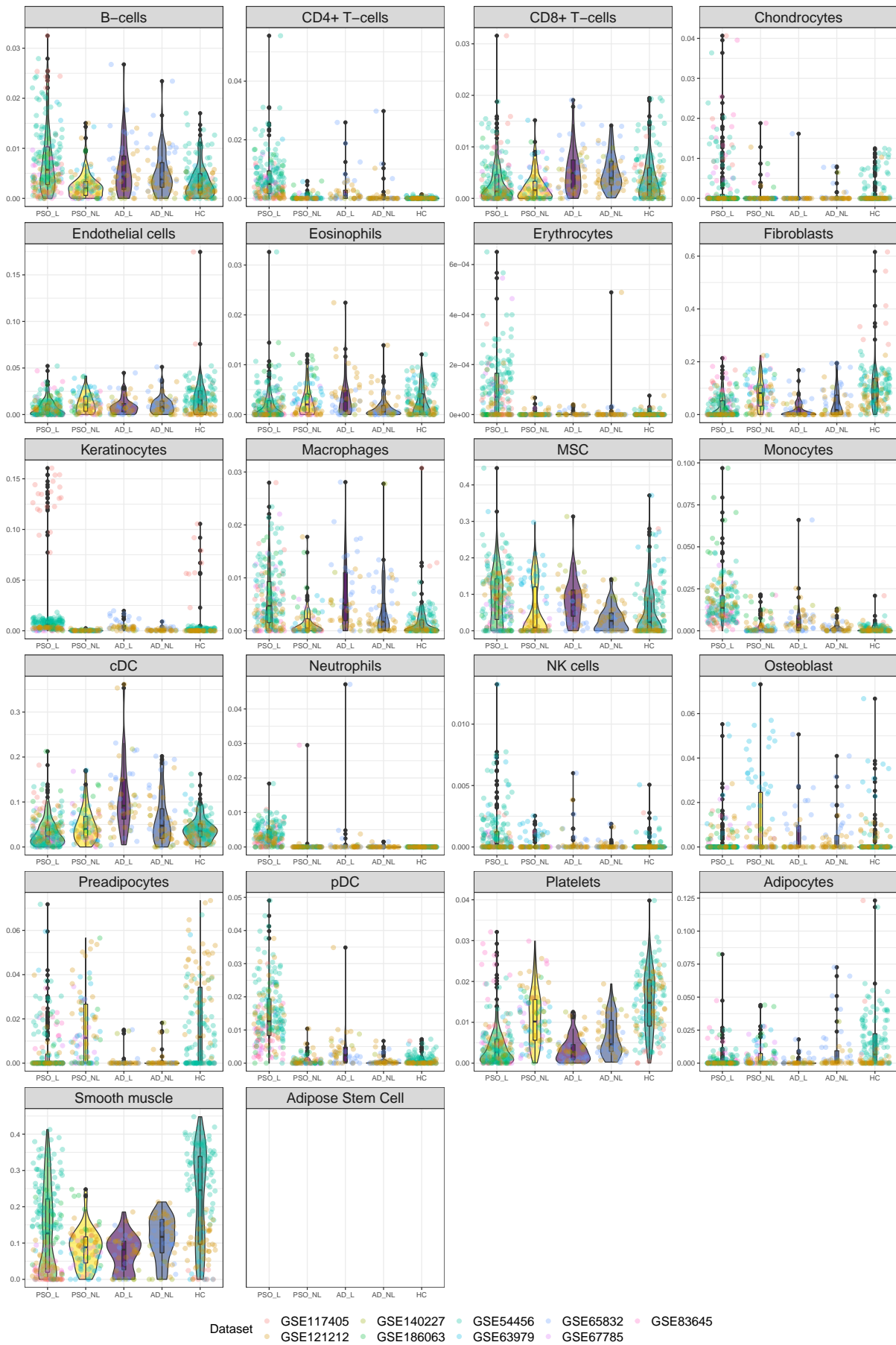
Figure A.7: *Violin plots including boxplots and individual samples of each dataset showing abundance of all analysed cell types in xCell.*

B Tables

	Dataset	Samples	LvsNL	LvsHC
Psoriasis	GSE107871	8 L, 8 NL, 8 HC	8 L vs 8 NL	
	GSE41745	3 L , 3 NL	3 L vs 3 NL	
	GSE67785	14 L, 14 NL	14 L vs 14 NL	
	GSE83645	20 L, 5 NL	20 L vs 5 NL	
	GSE47944	32 L, 32 NL, 20 HC	8 L vs 8 NL	
	GSE186063	13 L, 13 NL	13 L vs 13 NL	
	GSE63979	7 L, 27 NL, 8 HC	7 L vs 27 NL	7 L vs 8 HC
	GSE121212	28 L, 27 NL, 38 HC	28 L vs 27 NL	28 L vs 38 HC
	GSE117405	19 L, 9 HC		19 L vs 9 HC
	GSE54456	92 L, 82 HC		92 L vs 82 HC
	GSE74697	18 L, 18 HC		18 L vs 18 HC
AD	**GSE65832**	20 L, 20 NL	20L vs 20 NL	
	GSE140227	6 L, 6 NL	6Lvs6NL	
	GSE121212	27 L, 27 NL	27Lvs27NL	

Table B.1: *Overview of the analyzed datasets for Psoriasis and Atopic Dermatitis (AD). Datasets depicted not in bold were excluded after differential expression analysis. Exclusion criteria: less than 6 samples or 50 DEGs (see table B.2)*

		Dataset	Non-zero counts	LFC >0 (up)	LFC < 0 (down)	Outliers	Low counts
Psoriasis	LvsNL	GSE107871	19200	12, 0.062%	11, 0.036%	0.0%	1489, 7.8%
		GSE41745	23456	745, 3.2%	336, 1.4%	20, 0.085%	3184, 14%
		GSE67785	21313	5371, 25%	5349, 25%	0, 0%	0, 0%
		GSE83645	16260	2551, 16%	2177, 13%	23, 0.14%	0, 0%
		GSE47944	24992	8, 0.032%	13, 0.052%	0, 0%	2908, 12%
		GSE186063	21224	4620, 22%	4096, 19%	0, 0%	0, 0%
		GSE63979	23624	5950, 25%	8741, 37%	0, 0%	0, 0%
		GSE121212	23725	7218, 30%	8974, 38%	0, 0%	0, 0%
	LvsHC	**GSE63979**	21746	3820, 18%	4162, 19%	0, 0%	0, 0%
		GSE121212	23715	8166, 34%	6938, 29%	0,0%	0,0%
		GSE117405	25285	5604, 22%	5712, 23%	0.0%	0, 0%
		GSE54456	22484	8009, 36%	10080, 45%	0, 0%	3184, 14%
		GSE74697	35060	1, 0.0029%	12, 0.034%	0, 0%	9517, 27%
AD	LvsNL	**GSE65832**	25950	3696, 14%	2678, 10%	0.0%	504, 1.9%
		GSE140227	28698	1332, 4.6%	638, 2.2%	0.44%	3893, 14%
		GSE121212	23974	4205, 18%	4003, 17%	4, 0.017%	0, 0%

Table B.2: *Results of differential expression analysis using DeSeq2. Statistics based on p-adjusted>0.1 . Datasets with less than 6 samples or 50 sDEGs were excluded from further analysis. Datasets highlighted in bold remained for further analysis. LFC: LogFoldChange*

	Dataset	Non-zero counts	LFC >0 (up)	LFC < 0 (down)	Outliers	Low counts
NLvsHC	GSE63979	24079	1820, 7.6%	2156, 9%	0, 0%	934, 3.9%
	GSE121212	24535	948, 3.9%	1031, 4.2%	0, 0%	3330, 14%
LvsNL	GSE63979	23624	5950, 25%	8741, 37%	0, 0%	0, 0%
	GSE121212	23725	7218, 30%	8974, 38%	0, 0%	0, 0%
LvsHC	GSE63979	21746	3820, 18%	4162, 19%	0, 0%	0, 0%
	GSE121212	23715	8166, 34%	6938, 29%	0,0%	0,0%

Table B.3: *Results of differential expression analysis of GSE63979 and GSE121212 using DeSeq2. Three different comparison studies were run: non-lesional skin versus healthy control (NLvsHC), lesional versus non-lesional (LvsNL) and lesional versus heahlty control (LvsHC). Number of differential expressed genes (LFC up or down) drops significantly in NLvsHC design. LFC: LogFoldChange*

DerM22	xCell
adipose.stem.cells	
B.cells	B-cells
CD4..T.cells	CD4+ T-cells
CD8..T.cells	CD8+ T-cells
chondrocytes	Chondrocytes
endothelial.cells	Endothelial cells
eosinophils	Eosinophils
erythroblasts	Erythrocytes
fibroblasts	Fibroblasts
keratinocytes	Keratinocytes
macrophages	Macrophages
mesenchymal.stromal.cells	MSC
monocytes	Monocytes
myleoid.dendritic.cells	cDC
neutrophils	Neutrophils
NK.cells	NK cells
osteoblasts	Osteoblast
pc.adipocytes	Preadipocytes
plasmacytoid.dendritic.cells	pDC
platelets	Platelets
sc.adipocytes	Adipocytes
smooth.muscle.cells	Smooth muscle

Table B.4: *Cell types of signature matrix DerM22 from Félix Garza et al., 2019 (left column). Right column shows chosen corresponding cell types from xCell n=64 signature matrix. No good corresponding cell type fro adipose stem cells from DerM22.*

	LvsNL				LvsHC		
	Gene symbol	Median Rank	Cytokine regulation		Gene symbol	Median Rank	Cytokine regulation
1	EHF	3.8		1	ARNTL2	4.1	IFN-γ
2	ARNTL2	7.8	IFN-γ	2	EHF	4.5	
3	CRABP2	9.8	IFN-γ, IL-17	3	FOXE1	9.5	
4	MXD1	19.0		4	STAT1	10.6	IFN-γ
5	PRDM1	23.0	IFN-γ	5	MXD1	12.2	
6	STAT3	24.0	IFN-γ	6	PTTG1	16.1	
7	MYBL2	24.2		7	SOX7	17.4	
8	FOXM1	25.2		8	PITX1	19.8	
9	SOX7	25.8		9	NMI	21.9	IFN-γ
10	RORC	30.'8		10	IFI16	22.5	IFN-γ
11	TRIP13	32.8	IFN-γ	11	PRDM1	27.8	IFN-γ
12	IRF7	32.8	IFN-γ, TNF-α	12	EZH2	34.6	
13	IFI16	33.0	IFN-γ	13	NFE2L3	36.4	IFN-γ, TNF-α
14	PTTG1	37.5		14	TSC22D3	43.6	
15	PPARD	40.0	IFN-γ	15	FOXP3	44.4	
16	NFKBIZ	42.0		16	TRIM22	44.5	IFN-γ, TNF-α
17	EZH2	43.8		17	FOXM1	46.1	
18	HDAC1	44.2		18	PPARD	51.0	IFN-γ
19	NMI	47.5	IFN-γ	19	CBX7	54.2	
20	MED8	48.5	IFN-γ	20	ELF3	56.6	IFN-γ, TNF-α
21	FOSL1	48.5		21	GATA3	61.4	
22	IRF8	50.5		22	PPARGC1B	62.1	
23	TRIM22	51.5	IFN-γ, TNF-α	23	TRIP13	62.5	IFN-γ
24	FOXE1	51.8		24	THRA	66.9	
25	STAT1	52.5	IFN-γ	25	KLF15	69.1	
26	XPC	57.5	IFN-γ	26	HDAC1	73.5	
27	NAA15	60.5		27	POU6F1	75.1	
28	PPARA	75.2		28	MED8	75.2	IFN-γ
29	SUB1	76.5	IFN-γ	29	MYBL2	76.0	
30	TLE1	77.0		30	BRIP1	79.5	
31	ZNF471	77.8		31	ELL2	80.4	
32	RUVBL1	77.8		32	STAT3	80.4	IFN-γ
33	NRBF2	78.2	IFN-γ	33	MTF1	80.5	IFN-γ
34	TFDP1	80.0		34	NPAS1	80.8	
35	MCM6	80.2	IFN-γ	35	TLE2	81.0	
36	ENO1	80.5		36	IKZF3	81.4	
37	FOXP3	82.8		37	ZBTB16	81.9	
38	ID4	84.0		38	ASCC3	81.9	
39	TRIM21	94.2	IFN-γ	39	NR4A3	86.2	
40	E2F7	96.2	TNF-α	40	NRBF2	88.1	IFN-γ
41	MCM4	101.5	IFN-γ	41	SP110	88.6	IFN-γ
42	MTF1	101.8	IFN-γ	42	E2F7	93.0	TNF-α
43	HNRNPAB	108.0		43	EAF1	101.4	
44	SLC2A4RG	108.5		44	FOSL1	102.1	
45	NELFCD	113.8		45	TGFB1I1	103.5	IFN-γ, TNF-α
46	ILF2	114.2		46	TLE1	104.1	
47	SOX5	114.5		47	MCM6	107.8	IFN-γ
48	DBX2	114.5		48	RORC	108.4	
49	POU6F1	114.5		49	ILF2	109.5	
50	ASCC3	114.8		50	BRCA2	112.8	IFN-γ

Table B.5: *Top-50 Regulators of Lesional vs Non-Lesional (left) and Lesional vs Healthy Control (right) comparison after RegEnrich analysis. Cytokine regulation if induced by IL-17, IFN-gamma and TNF-alpha in keratinocytes (Nograles et al., 2008; Chiricozzi et al., 2011).*