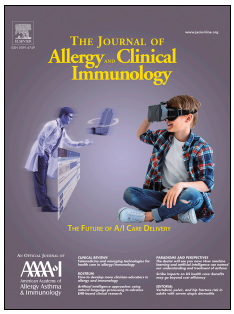
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Mild atopic dermatitis lacks systemic inflammation, and shows reduced non-lesional skin abnormalities

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Peripheral blood



Axes	Mild n=20	Moderate n=17	Severe n=24
Th2	∅	↑↑	↑↑↑
Th22	∅	↑↑	↑↑↑
Th1	∅	↑	↑↑
Th17	∅	↑	↑↑
CVD/ Athero	∅	↑↑	↑↑↑

Non-Lesional skin



Axes	Mild n=20	Moderate n=17	Severe n=24
Th2	↑	↑↑	↑↑↑
Th22	↑	↑↑	↑↑↑
Th1	∅	↑	↑↑
Th17	∅	↑	↑↑
Treg	↑	↑	↑
Barrier genes	∅	↓	↓↓

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Abbreviations: CVD/Athero: Cardiovascular disease/Atherosclerosis signaling; Th: T-helper; T-reg: T-regulatory

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Clinical Implication

Mild/limited AD is a localized skin disease and lacks the systemic immune activation seen in moderate-to-severe AD. Patients with mild/limited AD may benefit from Th2 targeting.

Capsule Summary

Most immune genes formed a severity-dependent expression gradient in lesional/non-lesional AD. Mild/limited AD lacked the systemic abnormalities characteristic of moderate-to-severe disease, with minimal immune activation in non-lesional skin and blood.

Key Words

Atopic dermatitis, mild, limited, severity, cytokines, qRT-PCR, OLINK, proteomics, systemic inflammation, immune tolerance

Abbreviations

AD: Atopic dermatitis

BSA: Body surface area

CAD: Coronary artery disease

CCL: C-C motif chemokine ligand

CXCL: C-X-C motif chemokine ligand

DC: Dendritic cell

FLG: Filaggrin

HR: Hazard ratio

IGA: Investigator Global Assessment

IL: Interleukin

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62 PCA: Principal component analysis

63 qRT-PCR: Quantitative real-time PCR

64 SCORAD: SCORing of atopic dermatitis

65 Th: T-helper

66 Treg: Regulatory T

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ABSTRACT

Background: Molecular studies in atopic dermatitis (AD) are largely restricted to patients with moderate-to-severe disease.

Objective: To evaluate skin and blood abnormalities in mild, moderate, and severe AD.

Methods: Skin and blood samples were obtained from 61 AD patients (20 mild/limited, 17 moderate, 24 severe) and 20 healthy subjects. Immune and barrier markers were measured in lesional, non-lesional, and healthy skin by qRT-PCR and immunohistochemistry, and in blood using the OLINK proteomic assay.

Results: Cellular markers of epidermal hyperplasia and T-cell/dendritic cell infiltration were increased in AD tissues of all severity patient groups versus controls, while downstream T_H2, T_H22, T_H1, T_H17-related mediators demonstrated incremental elevations with increasing disease severity, in both lesional and non-lesional skin. While T_H2 (IL13, CCL17, CCL26) and T_H22 (IL-22) cytokines were significantly elevated in both AD lesional and non-lesional skin of all patients regardless of severity, mild/limited AD patients showed increases in T_H1 (IFNG, CXCL9, CXCL10) and T_H17 (IL-17A, CCL20, CXCL1) markers in lesional, but not non-lesional skin. Treg-related mediators (IL-10, FOXP3) were comparably up-regulated in all groups, without displaying the severity-based gradient in other immune axes. Unsupervised clustering aligned samples along a severity spectrum, where non-lesional mild/limited AD skin clustered with healthy controls. Furthermore, while the blood profiles of moderate and severe AD patients showed gradual increases in $T_H1/T_H2/T_H17$-related and atherosclerosis/cardiovascular risk (CCL7, FGF21, IGFBP1) proteins, mild/limited AD lacked significant differences versus controls.

Conclusion: Mild/limited AD shows high T_H2/T_H22 activation primarily localized to skin lesions, and lacks the systemic inflammation of moderate and severe disease.

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INTRODUCTION

Atopic dermatitis (AD) is the most common inflammatory skin disease that affects up to 10% of adults, among which 20% are considered to have moderate-to-severe disease.[1, 2] Current insights into AD pathogenesis are largely limited to studies in moderate-to-severe patients, showing that the disease process involves epidermal barrier dysfunction[3, 4] and enhanced T-helper 2 (T_H2)/T_H22 immune response.[5-7] Patients with moderate-to-severe AD also harbor systemic immune abnormalities, as demonstrated by increased circulatory frequencies of T-cell/B-cell subsets,[8, 9] higher expression of pro-inflammatory cytokines and atherosclerosis and cardiovascular risk proteins,[10-13] and abnormalities in non-lesional skin.[14, 15] This new pathogenic understanding in moderate-to-severe AD has advanced the therapeutic development of targeted immunomodulators within this patient sub-group.[16-24]

While mild/limited AD, recognized as involving a limited body surface area (BSA), can persist throughout life without switching to moderate-to-severe disease affecting a larger BSA, these patients still experience impaired quality of life and intense pruritus,[25-27] with unsatisfactory options for long-term control.[28] Although mild disease accounts for the majority of AD cases,[1, 2] very few studies describing mild or limited (i.e. localized to skin folds) AD are available, inhibiting the development of targeted therapeutics in these patients. One microarray study reported increased inflammatory mediators (MMP12, CCL18, CXCL10, S100A9) in mild AD lesions, but did not make comparisons to moderate or severe AD.[29] A few studies identified positive correlations between disease severity and blood immune markers in AD cohorts that included patients with mild disease, but these studies did not specifically assess mild AD patients

by themselves.[8, 30-44] In psoriasis, molecular phenotyping revealed that mild psoriasis has greater expression of negative regulators compared to moderate and severe disease, which may be protective against progression to more severe disease.[45] Similarly, an evaluation of immune and barrier markers in AD patients across a severity spectrum could provide insight into important interactions within skin and blood that contribute to the disease phenotype, and offer therapeutic implications within each severity group.

Here, we present the first cellular and molecular profiling of lesional and non-lesional skin and proteomic analysis of blood from 20 AD patients with mild/limited disease, 17 with moderate disease, and 24 with severe disease, as compared to 20 healthy controls. In mild/limited AD, immune alterations in skin were primarily limited to lesional skin, and lacked systemic abnormalities that typify moderate-to-severe disease.

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METHODS

Study population

We enrolled 61 European American AD patients (20 mild/limited [10 female, 10 male, mean age 35.1], 17 moderate [5 female, 12 male, mean age 36.7], 24 severe [14 female, 10 male, mean age 38.2]), and 20 healthy control subjects (12 female, 8 male, mean age 35.3) (**Table 1**). There were no significant differences among these groups in age, gender, or race. All subjects provided informed written consent under local Institutional Review Board-approved protocols. Lesional/non-lesional skin biopsies (4.5mm punch) and peripheral blood samples were obtained from all subjects. Lesional biopsies were obtained from the upper extremities, particularly the antecubital fossae whenever possible. Non-lesional skin was defined as clinically unaffected skin that was taken in close proximity, but >10cm away from lesions. Inclusion criteria for AD patients include patients of any race between the ages of 18-90 who are not pregnant or lactating, with chronic AD that manifested in early childhood. Enrolled subjects had no history of other known inflammatory skin, autoimmune, or systemic disease (e.g. psoriasis, cardiovascular disease, coronary or carotid atherosclerosis, diabetes, inflammatory bowel disease, rheumatoid arthritis). Other exclusion criteria include history of immune compromise (e.g. HIV/AIDS, hepatitis B, opportunistic infections) or malignancy within the last 5 years, ongoing bacterial, viral, or fungal skin infections, the use of biologics within 12 weeks, systemic steroids, immunosuppressants, or phototherapy within 4 weeks, and topical steroids or calcineurin inhibitors on the entire affected extremity within 2 weeks of sample collection.

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The criteria we used for our limited/mild AD patients included SCORing Atopic Dermatitis (SCORAD) index<25, limited disease restricted to the flexural folds with BSA<5%, but with locally severe symptoms (Investigator Global Assessment [IGA])≥3. Patients with mild/limited AD never had >10% BSA involvement in their lifetime, and never used systemic therapies. Moderate and severe patients had more extensive disease with BSA>10%, and were differentiated by SCORAD (moderate 25≤SCORAD<50, severe SCORAD≥50).

Immunohistochemistry

Immunohistochemistry was performed on cryostat tissue sections by using purified mouse anti-human monoclonal antibodies (**Table E1**). Epidermal thickness and positive cells per mm of basement membrane were quantified using ImageJ software (version 1.42; National Institutes of Health, Bethesda, MD).

Quantitative gene expression analysis

RNA was extracted, followed by quantitative gene expression analyses with qRT-PCR, as previously described.[15, 46] Primers are listed in **Table E2**.

Serum proteomic analysis

Blood samples were centrifuged, and stored at -80^{o}C. Aliquots were analyzed with the ultra-sensitive proteomic OLINK Proseek® multiplex assay (a proximity extension assay using oligonucleotide-labeled antibody probe pairs)[47, 48] using the Inflammation and Cardiovascular disease (CVDII/III) panels, as previously described.[10]

Statistical analysis

Statistical analyses were performed using R software (www.R-project.org) and packages available through Bioconductor (www.bioconductor.org). qRT-PCR

expression values were normalized to human acid ribosomal protein, and imputed as 20% of the minimal value over the limit of detection. Data were log_2-transformed before analysis with a linear mixed-effects model, with severity and tissue (healthy, lesional, and non-lesional) as fixed factors and a random effect for patient ID. Fold-changes were estimated, and hypothesis testing was conducted with contrasts under the general framework for linear models using the "nlme" R package. Two sub-analyses were performed using linear mixed-effects model that were adjusted for age and gender, and for history of atopy (other than AD) (**Figs E1-E2**). Neither sub-analysis yielded significantly different results compared to the current model. Immunohistochemistry and cell count data were also evaluated using a linear mixed-effects model.

K-means unsupervised clustering was performed across principal components of the expression levels of all skin samples. ANOVA, in conjunction with the Tukey test, was used to identify markers that differentiated any two clusters (**Table E3**).

Blood proteomics data were scaled to adjust for batch using the "scale" function, and were evaluated using unpaired Student *t* tests. Skin and blood samples were associated with disease severity by Pearson coefficient correlation analysis.

The sample size of this study was determined from a power calculation from a published cross-sectional study of patients with AD,[49] in which 15 patients had greater than 90% power (at significance level 0.05) to detect differences versus controls in key markers of epidermal hyperplasia (K16), general inflammation (MMP12), Th2 (IL-13, CCL17/18/22/26), T_H1 (CXCL9/10), T_H17 (IL-36G, PI3, IL-20) and T_H17/T_H22 (IL-22, S100As).

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RESULTS

Mild/limited, moderate, and severe AD were defined by SCORAD<25, 25≤SCORAD<50, and SCORAD≥50, respectively. All mild AD patients had limited disease (BSA<5%) with locally severe lesions (IGA≥3), while all moderate and severe AD patients had more extensive disease (BSA>10%).

Epidermal hyperplasia and dendritic cell infiltration increase with AD severity

Immunohistochemistry was performed to assess epidermal hyperplasia and immune cell infiltration as a function of disease severity. Epidermal hyperplasia, as measured by epidermal thickness and KRT16 expression, $CD3^+$ T-cell infiltration, and DC-$LAMP^+$ mature dendritic cell (DC) infiltration were increased in the lesional skin of all AD sub-groups compared to both controls and non-lesional skin (**Fig 1**). All lesional samples stained positively for KRT16, while the prevalence of positive staining in non-lesional skin increased as a function of severity (mild/limited: 6/12 [50%], moderate: 8/15 [53%]. amd 1422 [64%]) (**Fig 1B**). KRT16 also showed more focal staining in mild/limited AD, while it was more evenly and diffusely distributed in moderate and severe AD. Additionally, by qRT-PCR, KRT16 expression was significantly higher in severe AD compared to both mild/limited and moderate AD (**Fig 1F**). DC-LAMP similarly displayed a clear upward trend with disease severity in both lesional and non-lesional skin (**Fig 1D, H**).

mRNA expression of immune in AD skin as a function of severity

Using qRT-PCR, we evaluated the mRNA expression of immune and barrier biomarkers in the lesional and non-lesional skin of AD patients with mild/limited, moderate, or severe disease, compared to healthy skin (**Figs 2-3, E3**). The general inflammation marker MMP12 was up-regulated versus controls and showed stepwise increases with disease severity in both lesional/non-lesional skin (**Fig 2**). The innate immunity cytokine IL-1B was only increased in severe AD lesions, but not in any other disease group. In general, the defining markers of polarized T-cell subsets (e.g. T_H2, T_H17, T_H22, T_H1) demonstrated an upward trajectory with increasing disease severity in both lesional and non-lesional tissues (**Figs 2-3, E3**). These genes were also consistently elevated in all AD skin lesions versus controls, but demonstrated severity-related differences within non-lesional skin. For example, most T_H2 (IL-13, CCL18, CCL17, CCL26) and T_H22 (IL-22) cytokines were up-regulated in non-lesional AD of all severity levels, although the T_H2 genes showed greater, more significant increases in moderate and/or severe patients compared to mild/limited patients (**Fig 2**). Only IL-31, a T_H2 cytokine involved in pruritus signaling along with OSM and OSMR, showed comparable expression levels in lesional AD of all severity categories (**Figs 2, E3**). Most S100As, which are modulated by both T_H22 and T_H17, were increased in non-lesional skin of all AD sub-groups (**Figs 2, E3**). In contrast to T_H2/T_H22, T_H17 (IL-17A, CCL20, CXCL1, STAT3) and T_H1 (IFNG, CXCL9/10, STAT1) genes were expressed in mild/limited non-lesional AD at comparable levels to controls, while they were significantly increased in the non-lesional skin of patients with more severe disease (except for IFNG) (**Figs 3, E3**). The T_H9 cytokine IL-9 also showed an upward trend with disease severity, and was highest in severe lesional AD (**Fig E3**).

mRNA expression of Treg, negative regulatory, and barrier genes in AD as a function of severity

Treg markers (IL-10, FOXP3, CTLA4) were also elevated in both lesional and non-lesional AD to similar degrees across all severity groups, without significant differences between these groups (**Fig 3**). Negative regulators (IL-34, IL-37) incrementally decreased with severity in both lesional and non-lesional AD, and were unchanged versus controls in mild/limited non-lesional AD (**Fig E3**). Similarly, terminal differentiation markers FLG and LOR progressively decreased with disease severity in all tissue types (**Fig E3**).

The mRNA expression levels of all immune and barrier markers are summarized in an unsupervised hierarchical clustering heatmap, illustrating that the non-lesional mild/limited AD phenotype most closely resembled healthy controls (**Fig 4**). As indicated by a yellow box, we identified a cluster of immune genes that trended upwards with severity in both non-lesional and lesional tissue, where gene expression in moderate non-lesional AD was comparable to mild/limited lesional AD, and severe non-lesional AD was comparable to moderate lesional AD. Similarly, a green box marked negative regulator and terminal differentiation genes that showed a down-trending expression gradient with severity in both lesional and non-lesional tissue.

Correlations between clinical severity and mRNA expression

Using Pearson coefficient correlation analysis, qRT-PCR expression levels of immune and barrier genes were correlated with SCORAD, a clinical measure of severity. In

lesional skin, significant positive correlations were observed between SCORAD and products related to general inflammation (MMP12), T_H2 (IL-13, IL-4R, IL-31, CCL17/18), T_H17 (IL-17A, CXCL1), T_H22 (IL-22, IL-32), and pruritus mediation (OSM) (r>0.3, P<0.05) (**Fig 5**). Both lesional and non-lesional skin also demonstrated strong positive correlations with SCORAD for the S100s (r>0.35, P<0.01), and negative correlations for the negative regulators IL-34/IL-37 and the terminal differentiation product LOR (r<-0.29, P<0.05) (**Figs 5, E4**).

Unsupervised clustering of lesional and non-lesional AD skin

We integrated healthy skin and lesional/non-lesional skin from mild/limited, moderate, and severe AD patients, using an unsupervised principal components analysis with k-means clustering (**Fig 6**). Based on mRNA expression measured by qRT-PCR, skin samples separated into four distinct clusters, ordered along a severity spectrum. From left to right, the four clusters primarily comprised of samples corresponding to: (1) healthy and mild/limited non-lesional AD; (2) moderate and severe non-lesional AD; (3) lesional AD of all severities; and (4) severe lesional AD. For each marker, the magnitude and significance of the difference between each pair of clusters is depicted in **Table E3**. Using this unbiased approach, we determined that a majority of these immune and barrier markers strongly distinguished each cluster from others, as demonstrated by the highly significant differences between cluster pairs.

Serum proteomics showed minimal systemic activation in mild/limited AD

We used the OLINK proteomic assay to evaluate 357 inflammatory and cardiovascular risk proteins in the blood of AD patients with mild, moderate, and severe disease, and healthy subjects. Serum proteins that were most dysregulated in any severity group versus controls are depicted in an unsupervised hierarchical heatmap (**Fig 7A**). In contrast to skin, we did not identify any proteins that were dysregulated in mild/limited AD blood, where in fact, some proteins were expressed at even lower levels than in controls. For example, T_H2 (CCL13, CCL17), T_H1 (CXCL10), and cardiovascular risk (IGFBP1) proteins were significantly increased in both moderate and severe, but not mild/limited AD versus controls. Other markers of general inflammation (MMP12), T_H1 (CXCL9), T_H17 (CCL20, KYNU, PI3), T-cell activation/migration (EZR, TNFRSF9, CCL19), atherosclerosis (CCL7, SELE, IL-16), cardiovascular risk (FGF21), and cell adhesion (CDH3) incrementally increased with disease severity, and were only significantly elevated versus controls in severe AD. We further correlated serum proteins with SCORAD, which showed strong positive correlations for markers of T_H2 (CCL13), atherosclerosis (IL-16, CCL7), cardiovascular risk (FGF21, IGFBP1), and cell adhesion (CDH3) (r>0.35, P<0.1) (**Fig 7B**).

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DISCUSSION

The current understanding of AD pathogenesis, which includes T_H2-dominant immune activation and epidermal barrier dysfunction, is largely derived from studying moderate-to-severe patients.[3-7, 50] The natural history of mild/limited AD (for patients with long-standing disease) shows persistence throughout adulthood without switching to moderate-to-severe disease.[51] This suggests that mild/limited AD may be another distinct clinical and molecular endotype.[5, 49, 52, 53] Nevertheless, patients with mild/limited AD still suffer from intense pruritus and impaired quality of life,[25-27] not adequately controlled by topical therapeutics.[28] Although patients with mild/limited AD represent the bulk of the AD population and experience a large disease burden,[1, 25-27] molecular studies that compare mild/limited AD to more severe disease are unavailable.

This is the first characterization of molecular and cellular features in AD patients with mild/limited disease (i.e. limited in extent with BSA<5% and SCORAD<25, but with IGA≥3 in lesions). We evaluated the lesional/non-lesional skin and blood of mild/limited AD patients compared to controls and to patients with moderate (25≤SCORAD<50, BSA>10%) and severe (SCORAD≥50, BSA>10%) disease. By investigating similarities and differences among AD patients stratified by clinical severity, we are able to define common pathogenic features underlying all AD regardless of disease severity, as well as unique molecular differences that likely contribute to distinct clinical phenotypes. Overall, we found that immune and barrier abnormalities in mild/limited AD are restricted to focal skin lesions, while abnormalities in moderate and severe AD are more systemic, extending beyond clinically affected skin to non-lesional skin and blood.

In all AD sub-groups, regardless of disease severity, lesional and non-lesional skin biopsies were both characterized by increased expression of products related to T_H2 (IL-13, CCL17, CCL26) and T_H22 (IL-22, S100As) inflammation. These findings reiterate the pivotal pathogenic roles of T_H2 and T_H22 inflammation in all AD patients.[2-6, 43] At the same time, these T_H2/T_H22 markers were still expressed at the lowest levels in mild/limited AD, trended upwards with disease severity, and strongly correlated with SCORAD. T_H2/T_H22 correlations with clinical severity were also observed in previous studies, although many did not include patients with mild disease.[14, 54] The degree of T_H2/T_H22 activation may directly influence the severity of AD clinical phenotype. At the cellular level, DC-LAMP$^+$ DCs, which we recently showed to produce CCL17/CCL22,[55] also increased with disease severity, suggesting that their relative enrichment may directly contribute to greater T_H2-skewing in severe AD. The T_H2 cytokine IL-31 mediates pruritus through interactions between T-cells and cutaneous sensory neurons, along with OSM and OSMR.[56, 57] Consistent with prior reports,[58] these pruritus-related markers were elevated in all AD lesions and did not vary significantly with disease severity, unlike most other immune mediators evaluated in this study, suggesting that even patients with mild/limited disease experience significant pruritus.[1, 25-27]

In contrast to the T_H2/T_H22 axes that showed an upwards severity-based gradient, Treg markers (IL-10, FOXP3, CTLA4) were comparably increased in all AD sub-groups, and lacked significant differences among severity groups. Treg expansion in AD lesions is consistent with the concept of “parallel amplification,” where increased immune responses are necessarily accompanied by greater Treg-mediated counter-regulation.[59, 60] The consistent degree of Treg expression despite increasing T_H2 activity

with greater disease severity suggests that compared to moderate and severe AD, mild/limited AD may be preferentially skewed towards a state of immune tolerance, with greater Treg-mediated suppression of pathogenic T_H2 amplification. Moreover, Tregs have demonstrated considerable plasticity, with the ability to convert to T_H2 cells, and vice versa.[61] One possibility is that the mild/limited AD microenvironment may promote conversion from activated T-cells to Treg cells, although this hypothesis requires further testing. Similar to in psoriasis,[45] AD patients with mild/limited disease displayed the highest expression of negative regulators IL-34 and IL-37, with strong negative correlations to SCORAD, further highlighting that loss of immune tolerance may lead to a more severe and extensive clinical phenotype.

One major difference between mild/limited and moderate-to-severe AD is the lack of systemic inflammation in mild/limited disease. For example, unlike in moderate-to-severe disease where T_H1 (IFNG, CXCL9/10) and T_H17 (IL-17A, CCL20, CXCL1) cytokine increases were observed in both lesional and non-lesional skin, their up-regulation in mild/limited AD was restricted to focal skin lesions. Only the S100As were up-regulated in mild/limited non-lesional skin, likely reflecting concurrent IL-22 signaling. The relationship between disease severity and cutaneous immune responses was also elucidated by an unbiased clustering approach, where samples clustered along a gradient ordered by clinical severity. The fact that non-lesional mild/limited AD samples clustered with healthy controls further emphasizes the lack of immune activation in clinically unaffected skin in mild/limited compared to moderate-to-severe AD. In moderate-to-severe, but not mild AD, T_H1 activation may also represent the chronic

366 phase of AD,[62] where previous lesions resolved and are no longer clinically visible, but
367 may relapse in the future given the presence of residual activated immune markers.
368 The proteomic blood profile of mild/limited versus moderate and severe AD
369 further supports the notion that while moderate-to-severe AD is a systemic disease, the
370 inflammation in mild/limited AD is localized to skin lesions. Moderate and/or severe, but
371 not mild/limited AD, shows increases in markers of general inflammation (MMP12), T_H2
372 (CCL13, CCL17), T_H1 (CXCL9, CXCL10), and T_H17 (CCL20, KYNU). We also noted up-
373 regulation of proteins associated with increased atherosclerosis and cardiovascular risk
374 in moderate and/or severe, but not mild/limited AD blood. Some of these proteins,
375 including CCL7, IL-16, and E-selectin, have already been reported in moderate-to-
376 severe AD,[10, 13] while a few others (e.g. FGF21, IGFB1) have not yet been described in
377 adults with AD. High serum FGF21 may be predictive of the incidence of coronary artery
378 disease (CAD) (hazard ratio [HR]: 1.29; P<0.01) and metabolic syndrome (HR 1.70;
379 P<0.0001),[63] and may also elevate the risk of developing major cardiovascular events in
380 patients with CAD at baseline (HR 2.1, P=0.01).[64] IGFBP1 was shown to be expressed
381 by atherosclerotic plaques,[65] and correlate with the severity of coronary artery lesions in
382 unstable angina patients.[66] Given the increased expression of
383 atherosclerosis/cardiovascular risk proteins, moderate and severe AD patients may
384 have increased risk of future cardiovascular complications relative to individuals with
385 mild/limited AD or without AD, although the absolute risk may be low, and requires
386 investigation with much larger cohorts.
387 The unique molecular features of mild/limited AD also have implications for
388 therapeutic management. The current treatment framework is that mild/limited AD is

managed with topical agents like emollients, topical steroids, and topical calcineurin inhibitors, while systemic agents are reserved for moderate-to-severe AD. Even if these topical agents are initially effective, lesions may recur. Furthermore, continuous use of topical steroids can lead to higher systemic absorption, potentially with increased risk of systemic complications like diabetes.[67, 68] Additionally, AD patients with limited but locally severe disease still experience significant disease burden, pruritus, and relapse,[25-27] and may benefit from cytokine-targeting immunomodulators, with better safety profiles than traditional systemic treatments. Clinical trials of dupilumab, which targets T_H2 signaling, were highly successful in moderate-to-severe AD patients.[17] The genomic profile of mild/limited AD patients, which features prominent T_H2 activation without accompanying systemic inflammation, leads us to believe that T_H2 targeting may be beneficial for AD patients with mild/limited disease, and in fact lead to less relapse compared to moderate-to-severe patients who also have inflammation from other immune axes like T_H1/T_H17. Additionally, post- versus pre-treatment transcriptomic studies suggest that successful therapeutic targeting reverts lesional skin to a non-lesional phenotype.[16, 24] The fact that the non-lesional skin of moderate-to-severe AD patients is still highly inflammatory while the non-lesional skin of mild/limited AD patients is more similar to healthy individuals highlights that selective cytokine targeting in mild/limited AD patients may be beneficial in reverting the skin into a healthy phenotype.

This study has some limitations. As a cross-sectional descriptive study, we were unable to assess changes in key immune and barrier markers over time, and whether these changes corresponded to changes in clinical severity. Additionally, although the

current study is limited to European American adults, it would be interesting to extend these findings to other races and age groups (e.g. pediatric patients) in future studies. It would also be interesting to further explore how various co-morbidities (e.g. asthma) may affect the phenotype of each severity group, especially the expression of cardiovascular disease-related markers. Of note, the results of this study are unlikely to be influenced by disease duration, given that all patients who were included have long-standing AD since early childhood. Finally, while our study captures the profile of patients with limited but locally significant disease, it may not reflect the phenotype of patients with locally milder symptoms.

Overall, in this first study evaluating skin mRNA and cellular expression, and blood proteomic expression of key immune and barrier biomarkers in mild/limited AD compared to moderate and severe AD, we demonstrate that while mild/limited disease retains universal pathogenic features of AD, like T_H2/T_H22 activation, important cellular and molecular differences may distinguish AD patients by severity. In contrast to moderate and severe AD patients, who also have high levels of systemic inflammation, mild/limited AD patients present with focal lesional inflammation, potentially due to a relatively greater immune tolerance. While patients with limited but locally significant disease lack systemic inflammation, they often have high disease burden and recurrence.[1, 25-27, 67] These patients may potentially benefit from targeted T_H2/T_H22 systemic treatment, which may perhaps provide a more sustained therapeutic response.

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651 **Table 1.** Demographic Table

Characteristic	Parameter	Normal (n=20)	Mild AD (n=20)	Moderate AD (n=17)	Severe AD (n=24)	P-value
Age	**Mean ± SD**	35.3 ± 11.4	35.1 ± 16.1	36.7 ± 18.1	38.2 ± 14.1	0.860
	Median (range)	32 (18-58)	31 (18-72)	28.5 (21-68)	35 (24-60)	
Sex	**F**	12	10	5	14	0.227
	M	8	10	12	10	
SCORAD	**Mean ± SD**	--	15.4 ± 4.8	43.7 ± 3.7	69.0 ± 11.7	P<0.001
	Median (range)	--	16.0 (6.2-24.3)	44.5 (36.8-49.0)	66.0 (52.2-91.4)	

652 *AD: Atopic dermatitis; SCORAD: SCORing Atopic Dermatitis; SD: Standard deviation*

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FIGURE LEGENDS

FIG 1. Immunohistochemistry. A-D, Representative staining in lesional (LS) and non-lesional (NL) skin biopsies from atopic dermatitis patients with mild, moderate, and severe disease, and skin from healthy controls. E-H, Quantification of epidermal thickness, $KRT16^+$ keratinocytes, $CD3^+$ T-cells, and $DC\text{-}LAMP^+$ dendritic cells. Red bars represent means. *Black symbols*: significance of comparison to normal skin*; red symbols:* significance of comparison between lesional and non-lesional tape stripped skin; *colored symbols*: significance of comparison between respective groups. AD, Atopic dermatitis; LS, Lesional; Mod, Moderate; N, Normal; NL, Non-lesional; Sev, Severe. ***P<0.001, **P<0.01, *P<0.05, ^+P<0.1

FIG 2. General inflammation, innate immunity, T_H2, and T_H22 biomarkers. Fold-changes of immune mediators in lesional and non-lesional skin of patients with mild, moderate, and severe atopic dermatitis, as well as normal skin, as measured by qRT-PCR. Red bars represent means. *Black symbols*: significance of comparison to normal skin*; red symbols:* significance of comparison between lesional and non-lesional tape stripped skin; *colored symbols*: significance of comparison between respective groups. AD, Atopic dermatitis; LS, Lesional; Mod, Moderate; N, Normal; NL, Non-lesional; Sev, Severe. ***P<0.001, **P<0.01, *P<0.05, ^+P<0.1

FIG 3. T_H17, T_H1, and Treg biomarkers. Fold-changes of immune mediators in lesional and non-lesional skin of patients with mild, moderate, and severe atopic dermatitis, as well as normal skin, as measured by qRT-PCR. Red bars represent means. *Black symbols*: significance of comparison to normal skin*; red symbols:* significance of comparison between lesional and non-lesional tape stripped skin; *colored symbols*:

676 significance of comparison between respective groups. AD, Atopic dermatitis; LS,
677 Lesional; Mod, Moderate; N, Normal; NL, Non-lesional; Sev, Severe. ***P<0.001,
678 **P<0.01, *P<0.05, ^{+}P<0.1

679 **FIG 4.** Summary heatmap. Unsupervised hierarchical clustering heatmap of immune
680 and barrier mRNA expression levels, as measured by qRT-PCR, normalized across
681 rows. Table shows fold-changes for non-lesional versus normal, lesional versus normal,
682 and lesional versus non-lesional comparisons for mild, moderate, and severe atopic
683 dermatitis. The green box indicates a cluster of barrier genes that progressively
684 decrease with disease severity. The yellow box indicates a cluster of immune genes
685 that progressively increase with disease severity. AD, Atopic dermatitis; LS, Lesional; N,
686 Normal; NL, Non-lesional. ***P<0.001, **P<0.01, *P<0.05, ^{+}P<0.1

687 **FIG 5.** Correlations with clinical severity in lesional atopic dermatitis. Pearson
688 correlations of atopic dermatitis clinical disease severity scores, as measured by
689 SCORAD, with mRNA expression levels of immune and barrier biomarkers in lesional
690 skin. SCORAD, SCORing of atopic dermatitis.

691 **FIG 6.** K-means unsupervised clustering. Unsupervised clustering of all subjects across
692 all principal components of skin qRT-PCR expression levels using k-means analysis.
693 The first two principal components (PC1 and PC2) are shown on the x and y axes,
694 respectively. This analysis yielded four clusters, ordered from left to right, predominated
695 by: (1) normal and mild non-lesional atopic dermatitis (green ellipse), (2) moderate and
696 severe non-lesional AD (red ellipse), (3) mild, moderate, and severe lesional AD
697 (purple), (4) severe lesional AD (blue). AD, Atopic dermatitis; LS, Lesional; NL, Non-
698 lesional; PC, Principal component.

699 **FIG 7.** Serum biomarkers. A, Heatmap of differentially expressed proteins (fold-
700 change>1.5, P<0.05) in the blood of patients with mild, moderate, and severe atopic
701 dermatitis, and of normal individuals, normalized across rows, with a table of fold-
702 changes versus normal subjects. B, Pearson correlations atopic dermatitis clinical
703 disease severity scores, as measured by SCORAD, with protein levels in blood. AD,
704 Atopic dermatitis; N, Normal; SCORAD, SCORing of atopic dermatitis. ***P*<0.01,
705 ***P*<0.05

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Gene	Green-Red difference	Green-Red P-value	Blue-Red difference	Blue-Red P-value	Purple-Red difference	Purple-Red P-value	Blue-Green difference	Blue-Green P-value	Purple-Green difference	Purple-Green P-value	Purple-Blue difference	Purple-Blue P-value	ANOVA: P-value	ANOVA: FDR-adjusted P
S100A9	-5.17	0.00E+00	3.84	1.08E-13	3.11	3.05E-14	9.01	0.00E+00	8.27	0.00E+00	-0.74	3.27E-01	7.70E-48	3.54E-46
S100A8	-4.87	0.00E+00	3.95	9.24E-14	3.31	2.78E-14	8.82	0.00E+00	8.18	0.00E+00	-0.64	4.74E-01	2.55E-46	1.15E-44
S100A7	-5.50	0.00E+00	2.75	6.10E-08	2.55	2.40E-11	8.25	0.00E+00	8.05	0.00E+00	-0.20	9.67E-01	1.17E-44	5.15E-43
CCL22	-1.79	1.04E-13	3.21	0.00E+00	1.37	1.38E-11	5.00	0.00E+00	3.16	0.00E+00	-1.83	1.07E-11	5.92E-42	2.54E-40
KRT16	-2.41	2.29E-11	4.30	0.00E+00	2.72	2.78E-14	6.70	0.00E+00	5.12	0.00E+00	-1.58	1.23E-04	2.18E-40	9.14E-39
MMP12	-4.73	1.15E-14	4.54	3.51E-14	2.04	8.88E-07	9.26	0.00E+00	6.77	0.00E+00	-2.49	6.12E-06	2.26E-38	9.28E-37
OSM	-0.87	1.84E-04	3.46	0.00E+00	1.29	1.01E-10	4.33	0.00E+00	2.16	1.35E-14	-2.18	3.04E-14	1.79E-36	7.15E-35
IL13	-3.83	2.23E-14	3.75	6.76E-14	1.67	2.62E-06	7.58	0.00E+00	5.50	0.00E+00	-2.08	1.15E-05	2.26E-36	8.82E-35
CXCL1	-1.51	1.37E-07	3.65	0.00E+00	1.61	9.21E-11	5.15	0.00E+00	3.12	0.00E+00	-2.04	5.67E-10	5.17E-35	1.96E-33
CCL18	-4.18	2.38E-14	3.55	3.18E-11	1.87	1.69E-06	7.72	0.00E+00	6.05	0.00E+00	-1.67	2.06E-03	8.88E-35	3.29E-33
IL22	-9.29	0.00E+00	4.54	6.48E-06	2.30	3.56E-03	13.83	0.00E+00	11.59	0.00E+00	-2.24	5.34E-02	8.28E-34	2.98E-32
CCL17	-2.59	1.44E-12	4.02	1.49E-14	1.10	5.18E-04	6.61	0.00E+00	3.70	0.00E+00	-2.91	3.00E-12	1.50E-33	5.26E-32
IL32	-1.06	1.47E-08	2.27	0.00E+00	0.86	8.00E-08	3.33	0.00E+00	1.92	0.00E+00	-1.41	6.60E-11	1.71E-33	5.81E-32
S100A12	-3.20	1.20E-09	4.33	3.44E-12	3.69	3.22E-14	7.53	0.00E+00	6.89	0.00E+00	-0.64	6.27E-01	3.03E-33	9.99E-32
PI3	-3.38	5.94E-12	4.31	1.25E-13	2.57	7.91E-10	7.68	0.00E+00	5.95	0.00E+00	-1.73	2.87E-03	1.21E-32	3.89E-31
FOXP3	-1.07	1.66E-08	2.06	2.04E-14	0.95	6.05E-09	3.13	0.00E+00	2.01	0.00E+00	-1.11	2.26E-07	6.49E-32	2.01E-30
IL9	-3.47	2.00E-08	6.82	1.78E-14	2.49	3.06E-06	10.29	0.00E+00	5.96	8.88E-15	-4.33	1.06E-09	1.21E-30	3.64E-29
IL23A	-0.94	2.22E-05	2.45	1.74E-14	1.24	1.11E-10	3.39	0.00E+00	2.18	3.11E-15	-1.21	1.40E-06	3.58E-30	1.04E-28
OSMR	-0.52	7.52E-07	1.16	1.69E-14	0.40	1.11E-05	1.68	0.00E+00	0.92	2.46E-14	-0.76	2.56E-10	5.25E-29	1.47E-27
IL4R	-0.70	3.20E-05	1.76	2.53E-14	0.78	7.36E-08	2.47	0.00E+00	1.48	2.58E-14	-0.99	2.50E-07	1.94E-27	5.25E-26
IL31	-1.50	1.62E-02	5.82	2.76E-14	3.43	3.79E-12	7.32	0.00E+00	4.93	2.60E-14	-2.39	2.84E-04	2.01E-26	5.24E-25
CCL13	-1.26	3.17E-06	2.65	2.99E-14	1.06	5.20E-06	3.91	0.00E+00	2.32	2.93E-14	-1.59	2.10E-07	2.76E-26	6.89E-25
IL10	-0.89	6.07E-05	2.28	2.64E-14	0.89	2.42E-06	3.17	0.00E+00	1.78	3.02E-14	-1.38	3.38E-08	4.54E-26	1.09E-24

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CCL26	-2.39	3.31E-14	1.64	1.40E-06	0.45	1.92E-01	4.03	0.00E+00	2.84	6.66E-15	-1.19	4.99E-04	2.64E-25	6.08E-24
CCL11	-2.34	1.70E-07	4.05	8.33E-14	1.30	1.22E-03	6.39	0.00E+00	3.64	3.00E-14	-2.75	7.86E-08	9.65E-25	2.12E-23
STAT3	-1.27	1.66E-10	1.09	1.89E-06	0.64	2.01E-04	2.36	1.60E-14	1.91	6.44E-15	-0.45	1.13E-01	5.60E-23	1.18E-21
CXCL2	-0.39	1.86E-01	2.35	2.09E-14	0.66	5.22E-04	2.73	2.89E-15	1.05	5.88E-07	-1.68	1.68E-11	9.69E-22	1.94E-20
IL37	1.11	1.53E-03	-2.29	4.23E-09	-1.82	3.86E-10	-3.40	3.38E-14	-2.92	2.54E-14	0.47	4.96E-01	3.11E-21	5.92E-20
CXCL10	-2.42	1.66E-07	3.51	9.89E-11	0.35	7.51E-01	5.93	0.00E+00	2.77	9.54E-10	-3.16	2.35E-09	3.68E-20	6.62E-19
STAT1	-0.85	2.56E-05	1.39	2.51E-09	0.66	1.62E-04	2.23	2.55E-14	1.51	8.37E-14	-0.73	2.39E-03	3.14E-19	5.34E-18
IFNG	-1.54	1.47E-05	2.71	7.92E-11	0.57	1.58E-01	4.25	1.57E-14	2.11	1.17E-09	-2.14	1.17E-07	1.10E-18	1.76E-17
IL5	-1.80	2.28E-03	3.97	1.31E-09	2.22	4.60E-06	5.77	3.24E-14	4.02	6.85E-13	-1.75	1.24E-02	4.10E-18	6.15E-17
CCL20	-2.44	6.08E-10	1.78	1.24E-04	0.58	2.21E-01	4.22	3.00E-14	3.02	5.24E-14	-1.20	1.48E-02	4.86E-18	6.80E-17
CXCL9	-2.21	4.14E-07	2.93	9.10E-09	-0.01	1.00E+00	5.14	1.95E-14	2.20	2.22E-07	-2.93	4.48E-09	1.22E-17	1.58E-16
IL6	-0.04	9.99E-01	3.13	3.97E-14	1.13	1.25E-04	3.17	2.30E-13	1.17	5.32E-04	-2.00	1.82E-07	6.49E-16	7.79E-15
CCL5	-0.38	3.33E-01	2.27	1.54E-13	0.29	4.47E-01	2.65	3.11E-14	0.67	1.44E-02	-1.98	2.33E-11	7.72E-16	8.49E-15
IL15	-0.13	8.43E-01	1.37	2.89E-11	0.43	8.15E-03	1.49	1.38E-11	0.56	1.71E-03	-0.94	2.61E-06	9.97E-13	9.97E-12
LOR	1.21	3.85E-04	-1.64	2.71E-05	-0.75	1.90E-02	-2.85	1.41E-11	-1.96	1.66E-09	0.89	4.49E-02	1.01E-12	9.97E-12
IL1B	-0.69	6.04E-02	2.17	1.68E-09	0.11	9.64E-01	2.86	4.55E-13	0.80	1.61E-02	-2.06	5.32E-09	1.18E-12	9.97E-12
IL4	0.81	5.95E-01	5.27	7.18E-10	3.36	9.15E-08	4.46	1.02E-06	2.56	4.81E-04	-1.90	5.23E-02	4.85E-12	3.39E-11
IL17A	-3.11	5.54E-05	2.49	1.01E-02	1.43	7.20E-02	5.59	4.61E-09	4.53	1.23E-09	-1.06	5.15E-01	5.84E-11	3.51E-10
FLG	1.04	9.22E-03	-1.13	1.75E-02	-0.11	9.77E-01	-2.17	2.37E-06	-1.15	2.17E-03	1.02	3.51E-02	5.90E-06	2.95E-05
IL2	-0.36	8.04E-01	1.86	7.30E-04	0.60	3.17E-01	2.22	1.30E-04	0.96	7.34E-02	-1.26	3.61E-02	1.07E-04	4.29E-04
JAK2	-0.01	1.00E+00	0.41	3.11E-02	-0.14	5.84E-01	0.42	4.31E-02	-0.13	7.29E-01	-0.55	1.28E-03	3.31E-03	9.92E-03
PPL	0.08	9.29E-01	0.31	2.24E-01	-0.01	1.00E+00	0.22	5.62E-01	-0.10	8.91E-01	-0.32	1.80E-01	2.01E-01	4.03E-01
IL31RA	0.04	9.99E-01	-0.30	7.34E-01	-0.03	9.99E-01	-0.33	7.03E-01	-0.07	9.92E-01	0.27	7.83E-01	7.18E-01	7.18E-01

Table E3. Pairwise comparisons between clusters by k-means unsupervised clustering based on cluster color.

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SUPPLEMENTARY TEXT

SUPPLEMENTARY FIGURE LEGENDS

FIG E1. Heatmap adjusted for age and gender. Heatmap of immune and barrier mRNA expression levels, as measured by qRT-PCR, normalized across rows and adjusted for age and gender with a linear mixed-effects model. Table shows fold changes for non-lesional versus normal, lesional versus normal, and lesional versus non-lesional comparisons for mild, moderate, and severe atopic dermatitis. AD, Atopic dermatitis; LS, Lesional; N, Normal; NL, Non-lesional. ***P<0.001, **P<0.01, *P<0.05, ^{+}P<0.1

FIG E2. Heatmap adjusted for history of atopy. Heatmap of immune and barrier mRNA expression levels, as measured by qRT-PCR, normalized across rows and adjusted for history of atopy (besides atopic dermatitis) with a linear mixed-effects model. Table shows fold changes for non-lesional versus normal, lesional versus normal, and lesional versus non-lesional comparisons for mild, moderate, and severe atopic dermatitis. AD, Atopic dermatitis; LS, Lesional; N, Normal; NL, Non-lesional. ***P<0.001, **P<0.01, *P<0.05, ^{+}P<0.1

FIG E3. Additional immune and barrier biomarkers. Fold-changes of immune and barrier genes from multiple pathways detected in lesional and non-lesional skin of patients with mild, moderate, and severe atopic dermatitis, as well as normal skin, as measured by qRT-PCR. Red bars represent means. *Black symbols*: significance of comparison to normal skin*; red symbols:* significance of comparison between lesional and non-lesional tape stripped skin; *colored symbols*: significance of comparison between respective groups. AD, Atopic

dermatitis; LS, Lesional; Mod, Moderate; N, Normal; NL, Non-lesional; Sev, Severe. ***P<0.001, **P<0.01, *P<0.05, ^{+}P<0.1

FIG E4. Correlations with clinical severity in non-lesional atopic dermatitis. Pearson correlations of atopic dermatitis clinical disease severity scores, as measured by SCORAD, with mRNA expression levels of immune and barrier biomarkers in non-lesional skin. SCORAD, SCORing of atopic dermatitis.

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Table E1. Antibodies used for immunohistochemistry.

Antibody	Vendor	Clone	Isotype	Dilution
KRT16	Santa Cruz Biotechnology	LL025	IgG1	1:500
CD3	BD Biosciences	SK7	IgG1	1:100
DC-LAMP	Beckman Coulter	104.G4	IgG1	1:50

Table E2. 49 immune and barrier primers used for qRT-PCR.

Gene	Assay ID	Gene	Assay ID
CCL5	Hs00174575_m1	IL-15	Hs01003716_m1
CCL11	Hs00237013_m1	IL-17A	Hs00174383_m1
CCL13	Hs00234646_m1	IL-22	Hs01574154_m1
CCL17	Hs00171074_m1	IL-23p19	Hs00372324_m1
CCL18	Hs00268113_m1	IL-31	Hs01098710_m1
CCL20	Hs00355476_m1	IL-31RA	Hs00371172_m1
CCL22	Hs01574247_m1	IL-32	Hs00992441_m1
CCL26	Hs00171146_m1	IL-34	Hs01050926_m1
CTLA4	Hs00175480_m1	IL-37	Hs00367201_m1
CXCL1	Hs00236937_m1	JAK2	Hs01078136__m1
CXCL2	Hs00601975_m1	KRT16	Hs00955082_g1
CXCL9	Hs00171065_m1	LOR	Hs01894962_s1
CXCL10	Hs00171042_m1	MMP12	Hs00159178_m1
FLG	Hs00856927_g1	OSM	Hs00171165_m1
FOXP3	Hs01085834_m1	OSMR	Hs00384276_m1
IFN-γ	Hs00989291_m1	PDL1 (CD274)	Hs00204257_m1
IL-1β	Hs01555410_m1	PI3	Hs00160066_m1
IL-2	Hs00174114_m1	PPL	Hs00160312_m1
IL-4	Hs00174122_m1	S100A7	Hs01923188_u1
IL-4R	Hs00965056_m1	S100A8	Hs00374264_g1
IL-5	Hs00174200_m1	S100A9	Hs00610058_m1
IL-6	Hs00174131_m1	S100A12	Hs00942835_g1
IL9	Hs00914237_m1	STAT1	Hs01013996_m1
IL-10	Hs00961622_m1	STAT3	Hs00374280_m1
IL-13	Hs00174379_m1		

qRT-PCR, quantitative real-time polymerase chain reaction

Table E3 (excel file). Pairwise comparisons between clusters by k-means unsupervised clustering based on cluster color.

Normals | Mild AD NL | Mild AD LS | Moderate AD NL | Moderate AD LS | Severe AD NL | Severe AD LS

A

H&E

B

KRT16 0/20 6/12 20/20 8/15 17/17 14/22 24/24

C

CD3

D

DC-LAMP

E

Epidermal Thickness

Thickness (μm)

F

KRT16

log_2(Normalized Expression)

G

CD3

log_2Counts

H

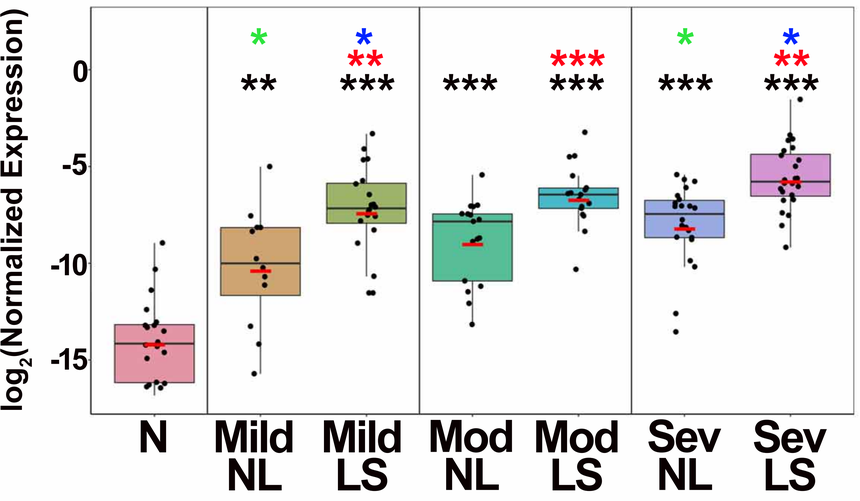
DC-LAMP

log_2Counts

N | Mild AD NL | Mild AD LS | Mod AD NL | Mod AD LS | Sev AD NL | Sev AD LS

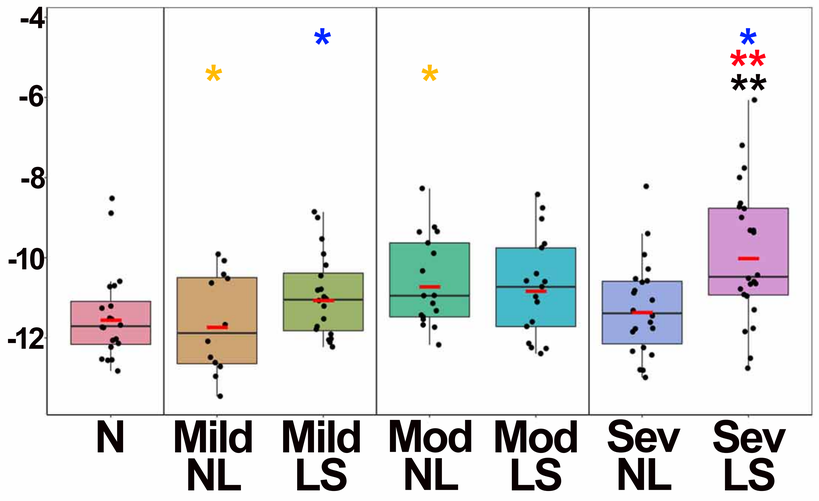
General Inflammation

MMP12



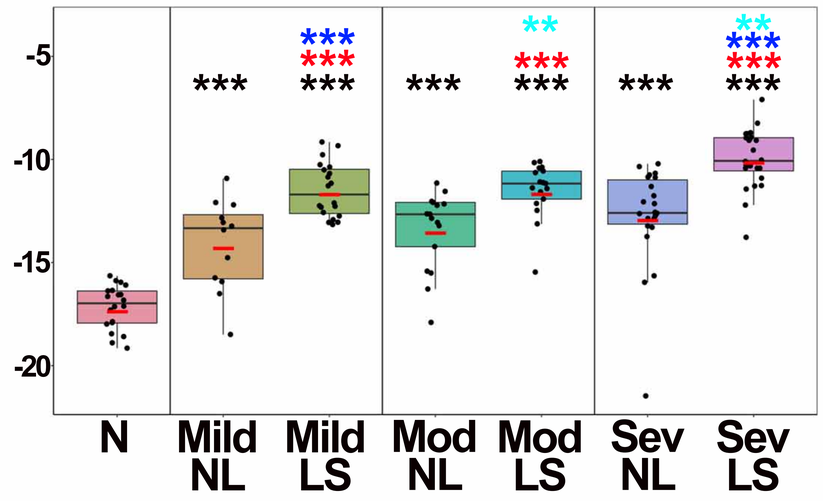
Innate Immunity

IL-1B



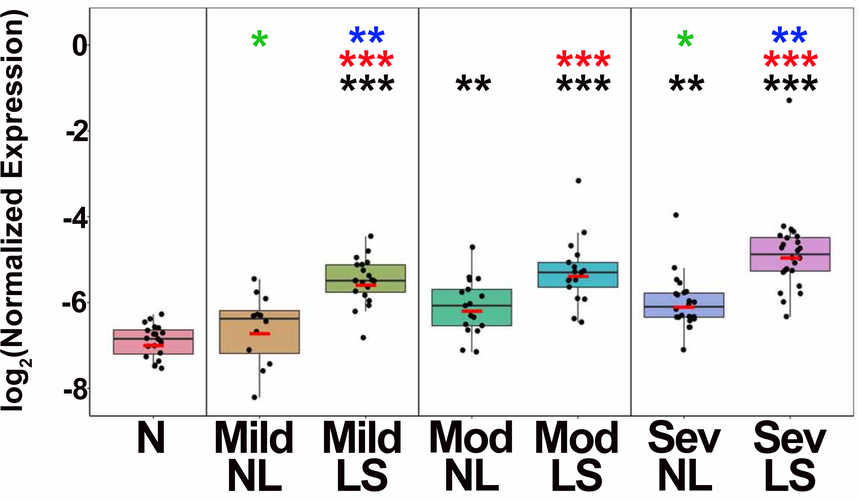
Th2

IL-13

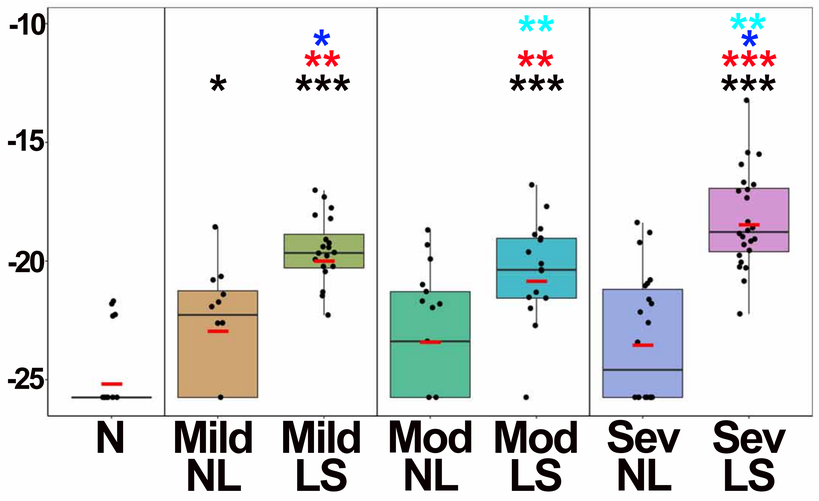


Th2

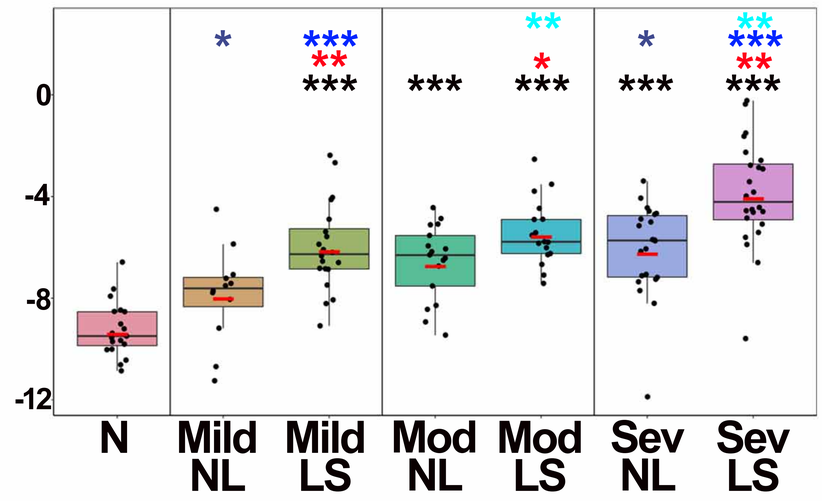
IL-4R



IL-31

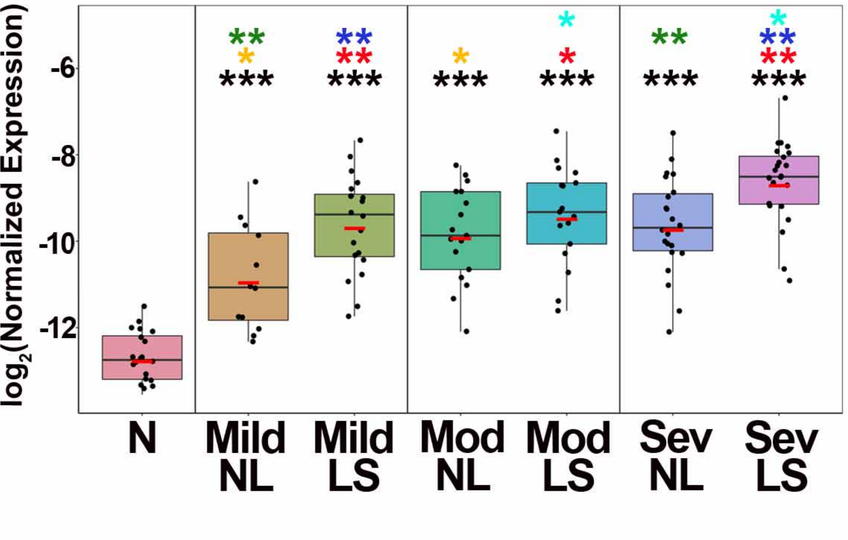


CCL17



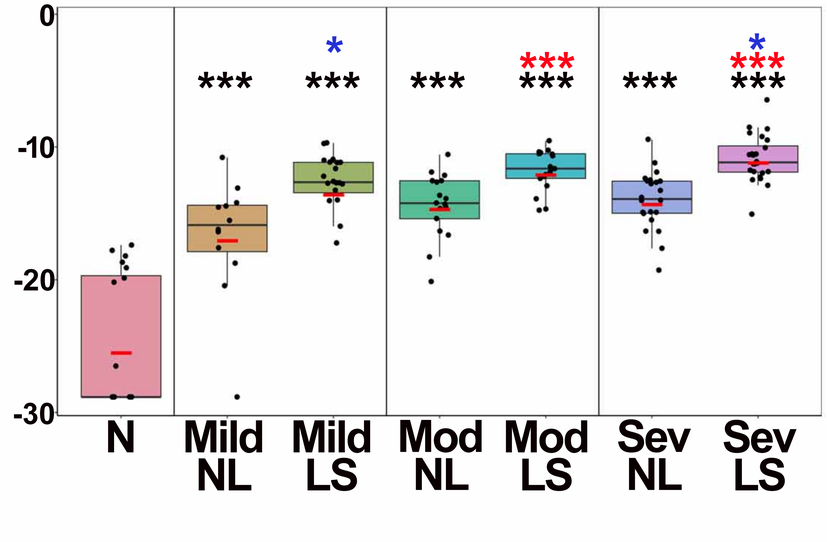
Th2

CCL26



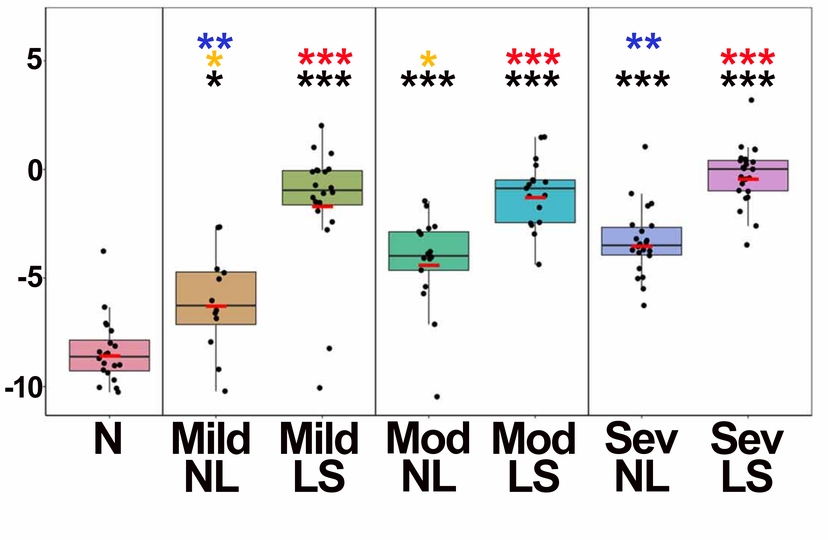
Th22

IL-22



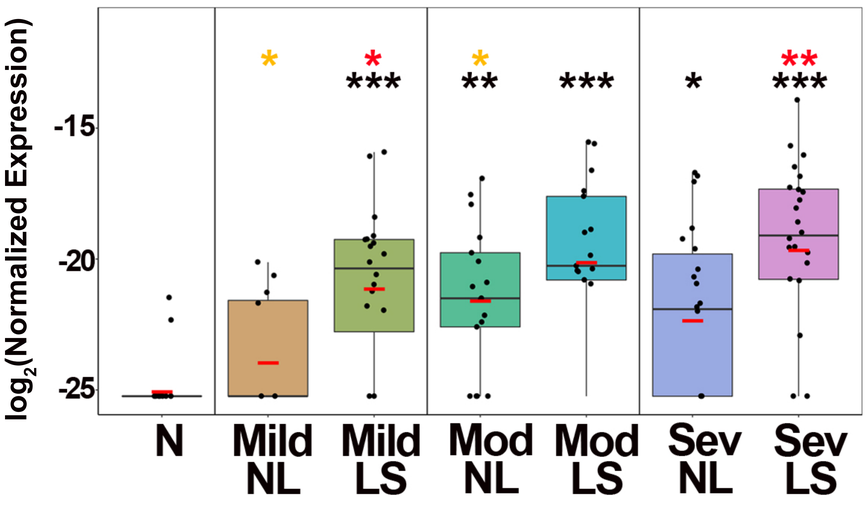
Th17/Th22

S100A8

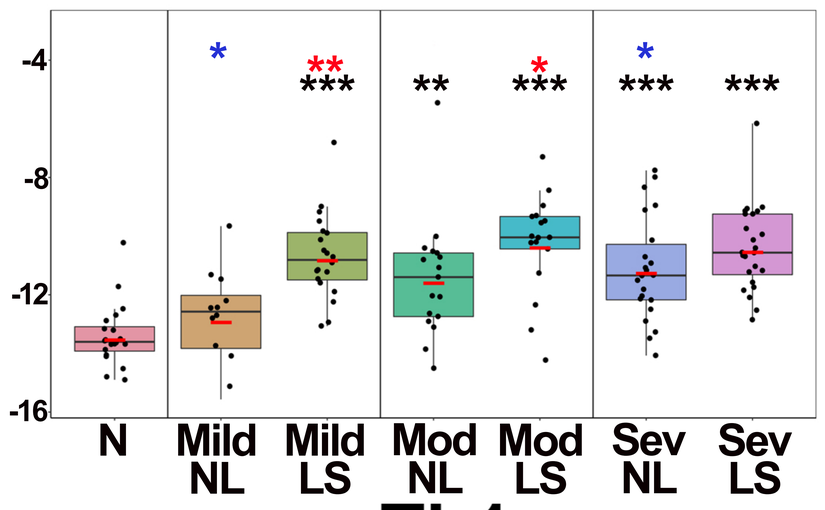


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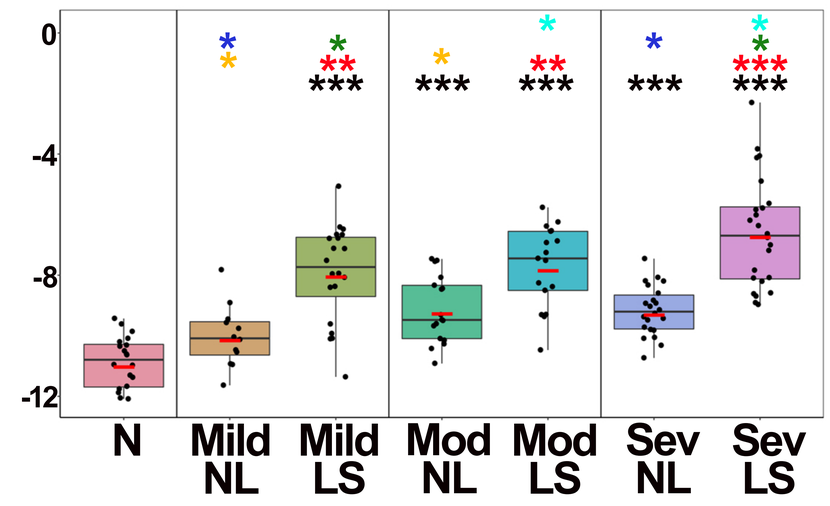
IL-17A



CCL20

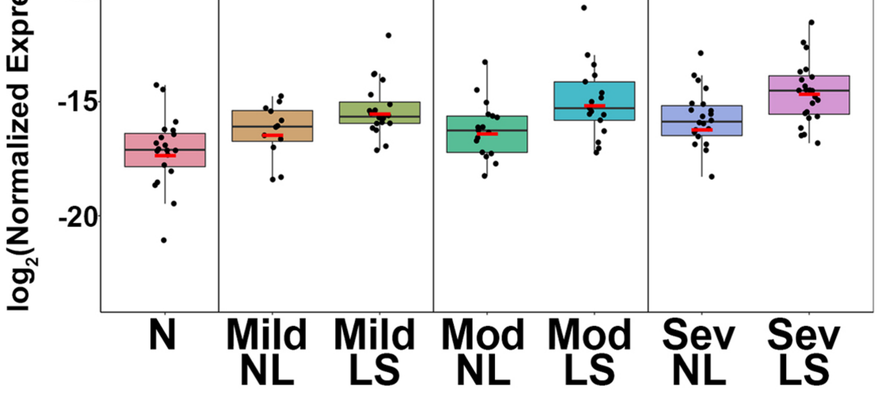


CXCL1

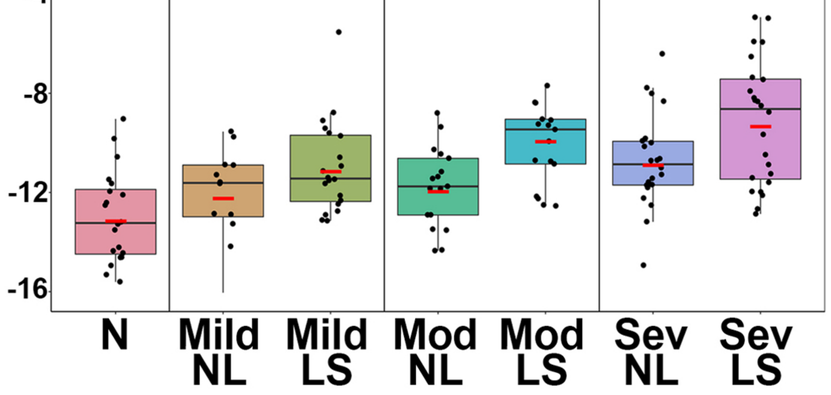


Th1

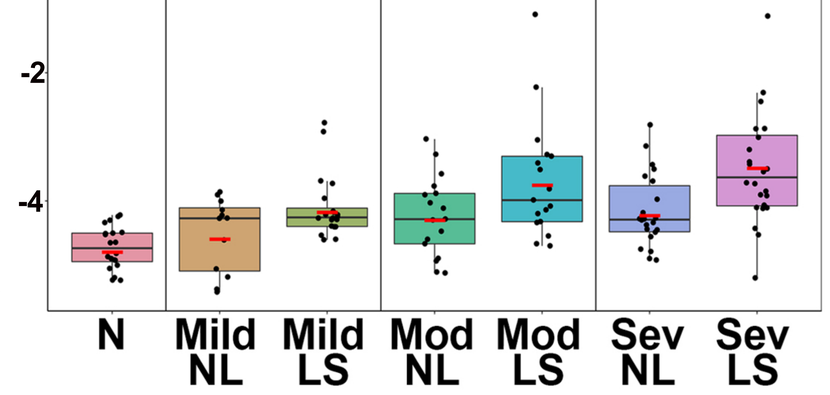
IFNγ



CXCL10

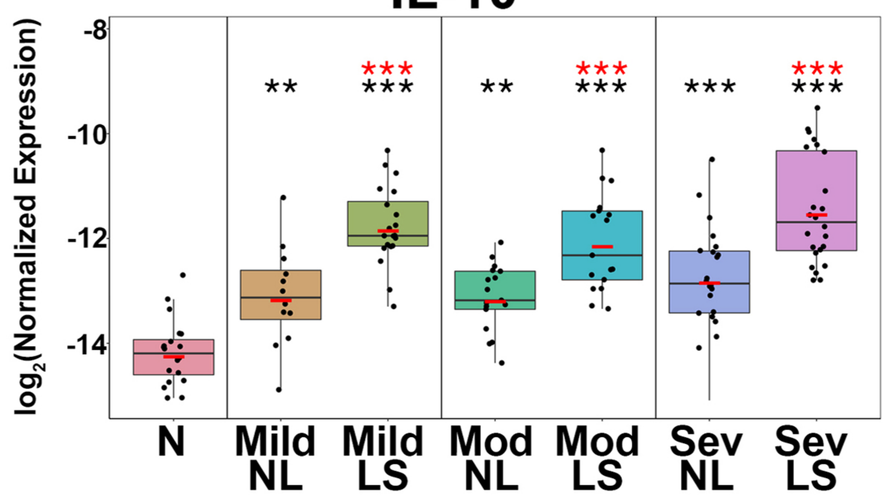


STAT1

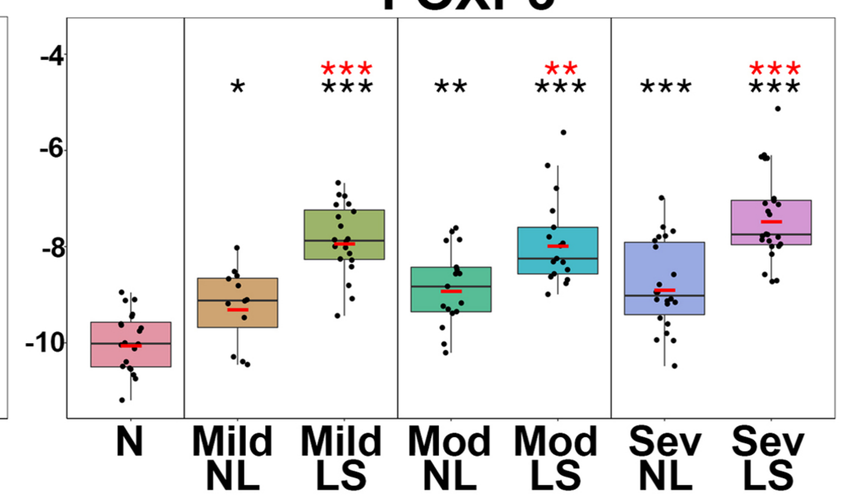


Regulatory T Cells

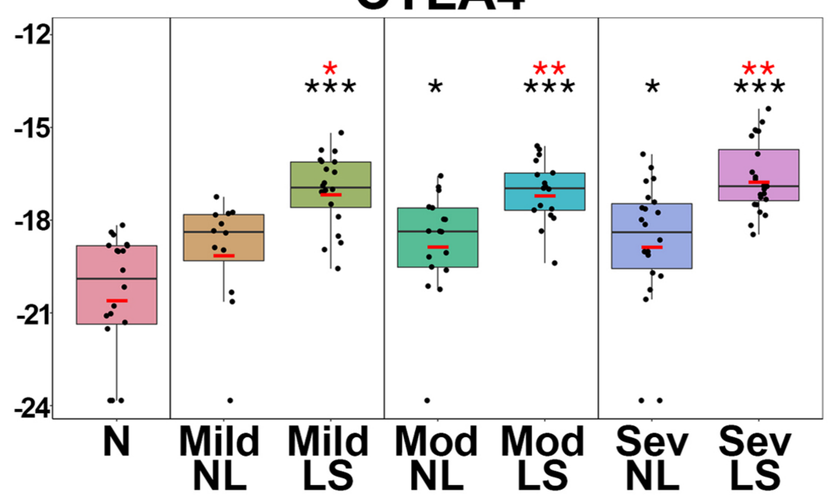
IL-10

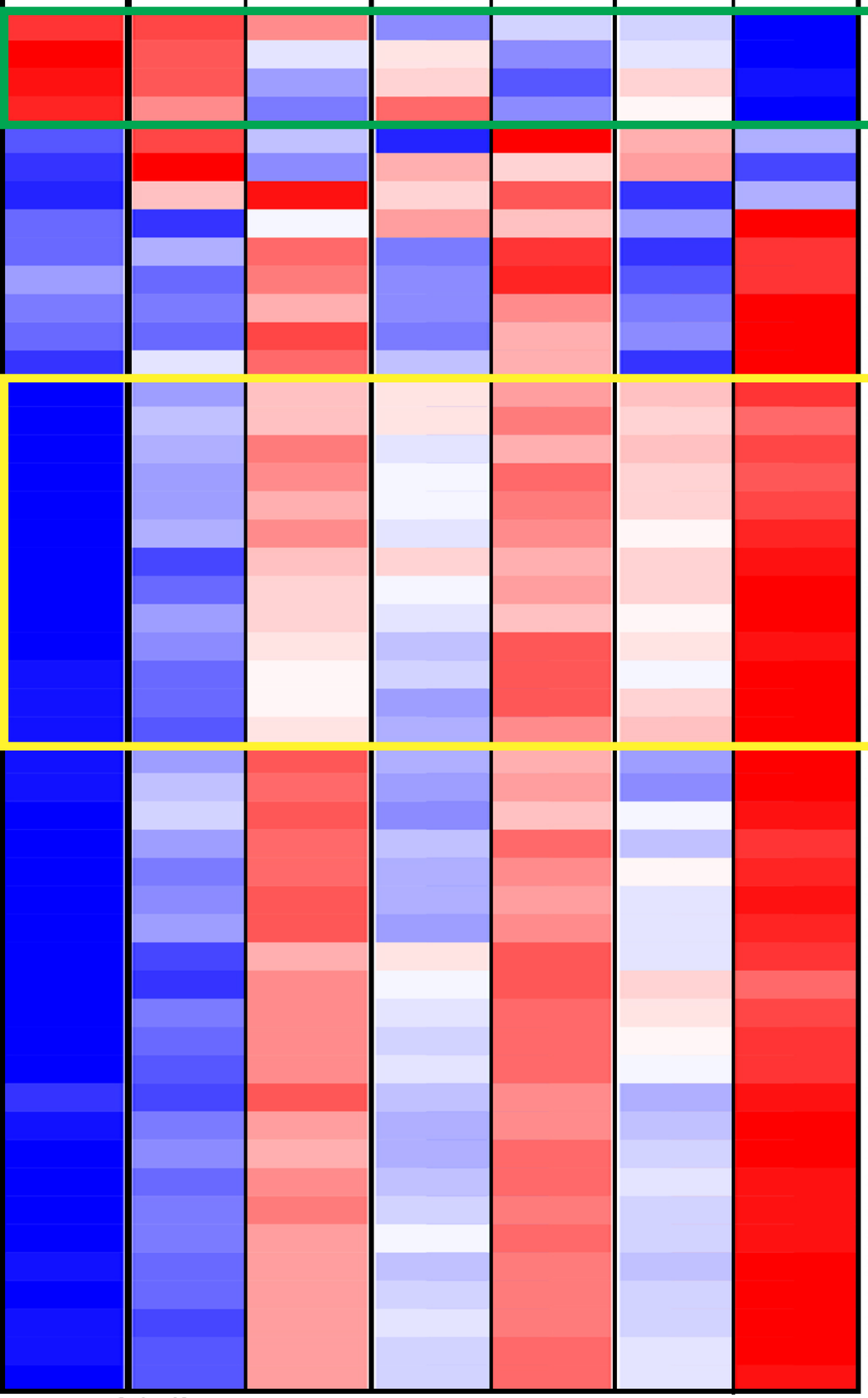


FOXP3



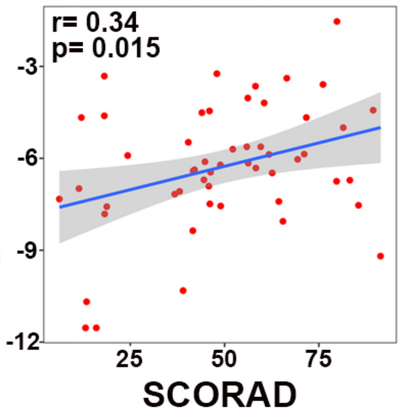
CTLA4

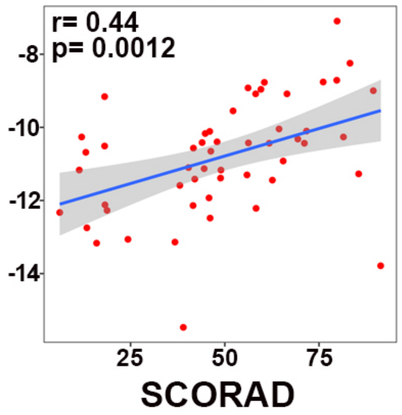


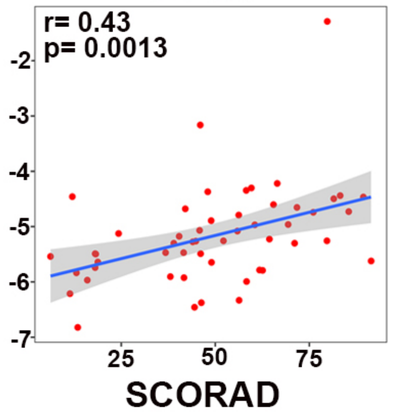


	Normal	Mild AD NL	Mild AD LS	Moderate AD NL	Moderate AD LS	Severe AD NL	Severe AD LS
FLG ------:	-1.03	-1.29	-1.26	-2.40*	-2.00*	1.20	-1.98+	-3.72***	-1.88*
LOR ------:	-1.41	-2.70***	-1.91*	-2.33*	-3.73***	-1.61**	-2.79***	-6.57***	-2.36**
IL37 -----:	-1.35	-5.37***	-3.97***	-2.55**	-7.92***	-3.11***	-2.69***	-11.60***	-4.31***
IL34 -----:	-1.34	-2.58***	-1.92**	-1.25	-2.47***	-1.97***	-1.77***	-3.70***	-2.09***
JAK2 -----:	1.09	1.03	-1.06	-1.01	1.11	1.13	1.06	1.02	-1.04
IL31RA ---:	1.90+	1.13	-1.68+	1.44	1.39	-1.04	1.48	1.04	-1.42
PPL ------:	1.10	1.16	1.05	1.09	1.13	1.04	1.01	1.05	1.04
IL1B -----:	-1.13	1.41	1.59	1.79	1.65	-1.08	1.15	2.91**	2.53**
IL15 -----:	1.11	1.56**	1.40+	1.02	1.67**	1.63**	-1.08	1.69**	1.83***
IL2 ------:	-1.19	2.12*	2.52+	-1.07	2.94*	3.14**	-1.26	2.67**	3.38**
CCL5 -----:	1.02	1.65*	1.62+	1.08	1.82*	1.69**	1.02	2.89***	2.83***
IL6 ------:	1.03	3.08***	3.00**	1.06	2.09*	1.98*	1.14	4.06***	3.55***
IL4 ------:	2.79	9.28***	3.33+	2.27	5.79*	2.55	-1.02	18.30***	18.70***
CCL26 ----:	3.57***	8.47***	2.37**	7.19***	9.80***	1.36*	8.23***	16.80***	2.04**
IL22 -----:	370.00***	3850.00***	10.40	1800.00***	10900.00***	6.04***	2260.00***	20400.00***	9.01***
CCL18 ----:	17.40***	130.00***	7.48***	29.20***	79.40***	2.72***	63.00***	257.00***	4.07**
S100A7 ---:	15.40***	165.00***	10.70***	39.20***	251.00***	6.40***	73.80***	316.00***	4.29***
MMP12 ----:	14.70**	110.00***	7.48**	36.40***	178.00***	4.89***	63.70***	342.00***	5.37**
IL13 -----:	8.73***	51.20***	5.86***	14.10***	51.60***	3.67***	21.30***	148.00***	6.95***
STAT3 ----:	1.28	2.29***	1.80**	2.16***	2.36***	1.09	2.23***	3.55***	1.59**
CCL17 ----:	2.64+	9.47***	3.59**	6.36***	14.20***	2.23*	8.91***	40.20***	4.51**
IL9 ------:	7.05*	32.30***	4.58*	16.90***	44.90***	2.66+	22.40***	504.00***	22.50***
CXCL9 ----:	2.20	3.88**	1.77	2.77+	7.19***	2.59**	3.87*	9.38***	2.42+
STAT1 ----:	1.15	1.54***	1.34+	1.41+	2.06***	1.46*	1.48*	2.48***	1.67**
CXCL10 ---:	1.68	4.00**	2.38+	2.29	9.24***	4.04***	4.73**	14.10***	2.98*
CCL11 ----:	1.65	6.26***	3.79*	3.28*	12.70***	3.87***	8.46***	42.10***	4.97**
CXCL2 ----:	1.46	2.92***	2.01*	1.54+	2.37***	1.54*	1.45	3.69***	2.55***
IL31 -----:	4.75*	36.40***	7.66**	3.40+	20.20***	5.94**	3.11+	105.00***	33.70***
IL5 ------:	5.82**	31.00***	5.33***	3.52+	14.40***	4.09*	7.97***	49.50***	6.21***
CTLA4 ----:	2.76+	10.80***	3.90*	3.34*	10.50***	3.14**	3.31*	14.30***	4.31**
PI3 ------:	4.49*	65.80***	14.70***	6.42**	43.40***	6.77***	16.30***	121.00***	7.43***
CCL13 ----:	2.03*	7.29***	3.60***	2.42**	4.97***	2.05***	2.94***	9.36***	3.18***
IL10 -----:	2.09**	5.28***	2.53***	2.07**	4.29***	2.07***	2.65***	6.53***	2.46***
IL17A ----:	2.15	15.20***	7.07*	11.00**	30.60***	2.77	6.57*	42.30***	6.44**
CCL20 ----:	1.51	6.54***	4.33**	3.83**	8.82***	2.30*	4.82***	7.93***	1.65
S100A9 ---:	8.68**	158.00***	18.20***	30.70***	214.00***	6.97***	49.70***	374.00***	7.52***
S100A8 ---:	5.45*	118.00***	21.70***	18.10***	157.00***	8.70***	33.30***	282.00***	8.46***
S100A12 --:	3.47+	64.90***	18.70***	13.10***	93.80***	7.18***	15.30***	167.00***	10.90***
IL23A ----:	1.09	4.30***	3.96***	1.77*	3.44***	1.95**	1.61+	5.64***	3.50***
OSM ------:	1.64	4.42***	2.69**	1.96*	4.75***	2.42***	2.19*	10.40***	4.76***
IFNG -----:	1.70	3.52***	2.07+	1.92	4.55***	2.36**	2.18	6.46***	2.97*
KRT16 ----:	3.02*	31.60***	10.50***	5.96***	37.80***	6.33***	8.15***	86.80***	10.70***
FOXP3 ----:	1.68*	4.35***	2.59***	2.20**	4.21***	1.92**	2.23***	5.97***	2.67***
CCL22 ----:	2.15*	7.13***	3.31**	3.99***	9.42***	2.36**	3.32***	15.60***	4.68***
PDL1 -----:	1.50	3.93***	2.62**	2.06***	4.47***	2.17***	2.11**	7.37***	3.49***
CXCL1 ----:	1.89	7.81***	4.13**	3.37***	9.05***	2.68**	3.29***	19.40***	5.91***
IL32 -----:	1.24	3.13***	2.51***	2.08**	3.49***	1.68**	1.96**	5.41***	2.76***
IL4R -----:	1.21	2.65***	2.19***	1.74**	3.06***	1.76***	1.86**	4.12***	2.22***
OSMR -----:	1.20	1.94***	1.62**	1.51**	2.13***	1.41**	1.52***	2.46***	1.62***

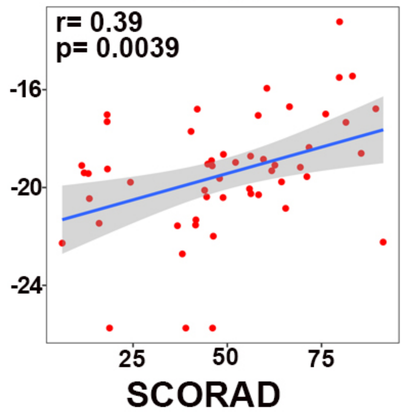
General Inflammation

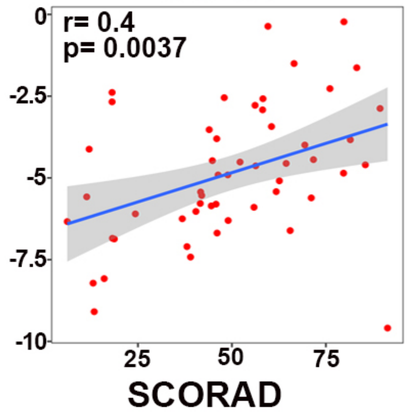
MMP12


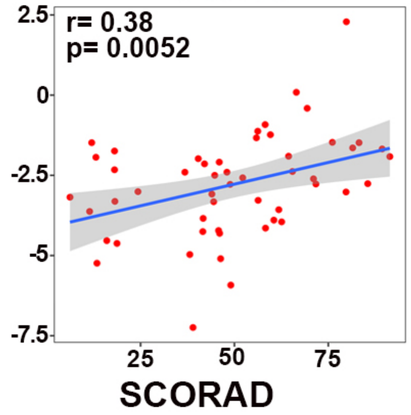
IL-13


IL-4R


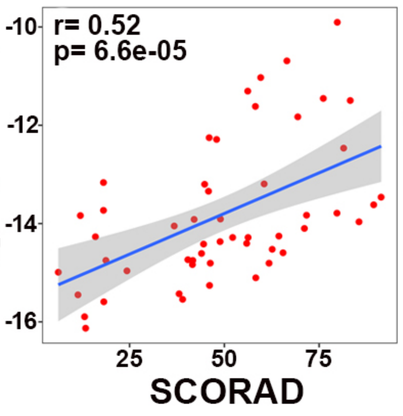
Th2

IL-31


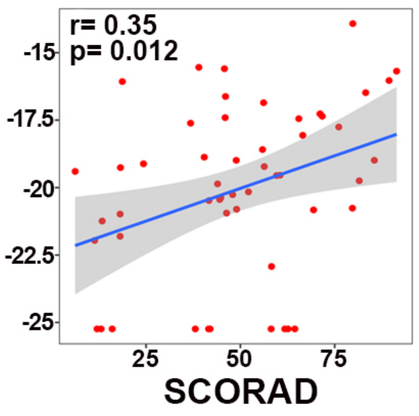
CCL17


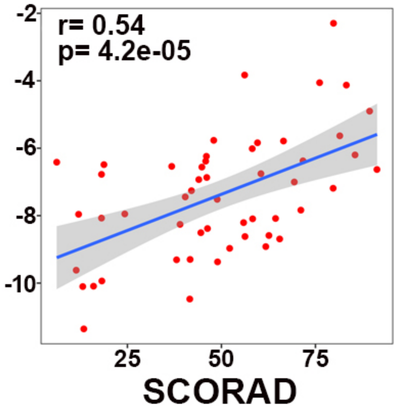
CCL18


Th2

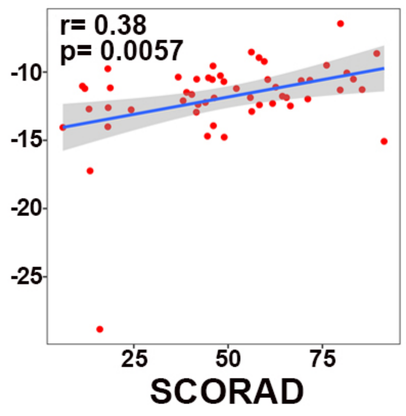
OSM


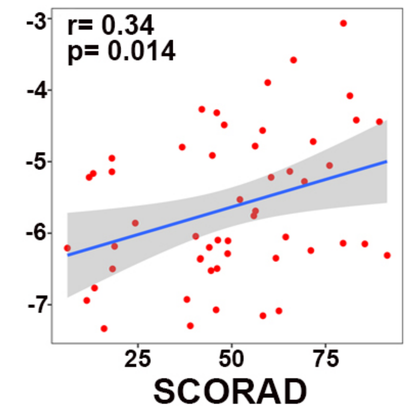
Th17

IL-17A


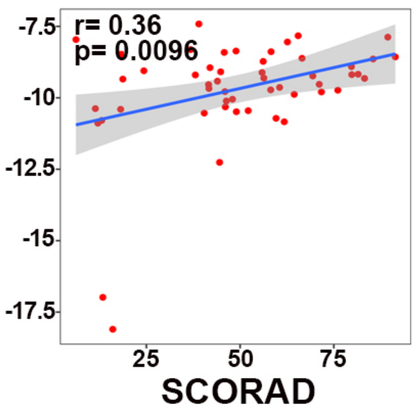
CXCL1


Th22

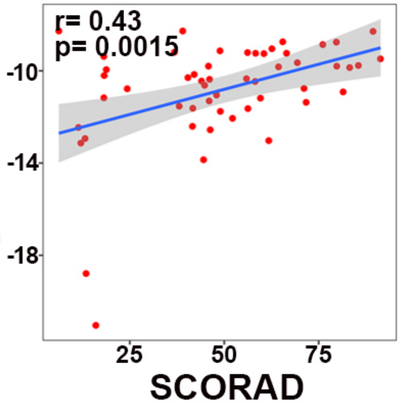
IL-22


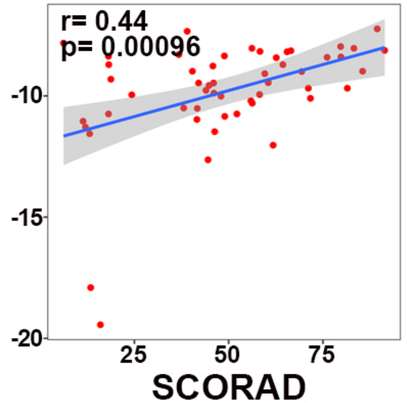
IL-32


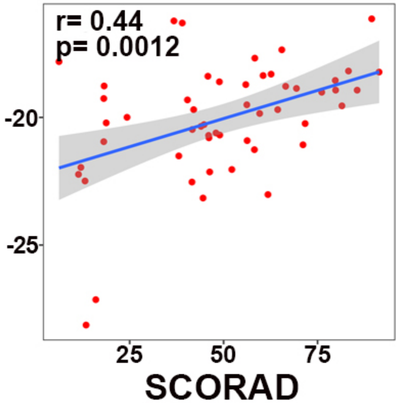
Th17/Th22

S100A7


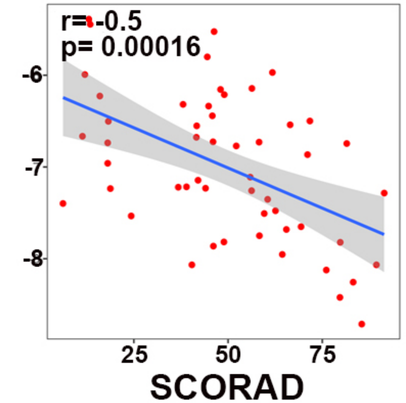
Th17/Th22

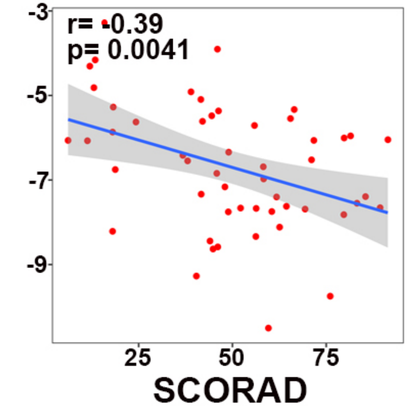
S100A8


S100A9


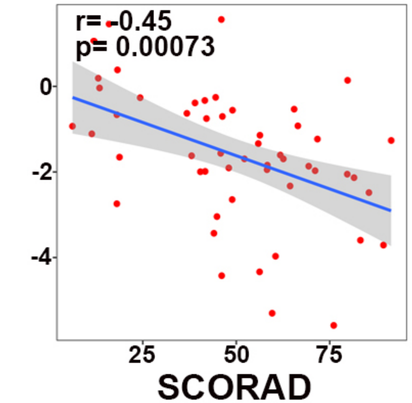
S100A12


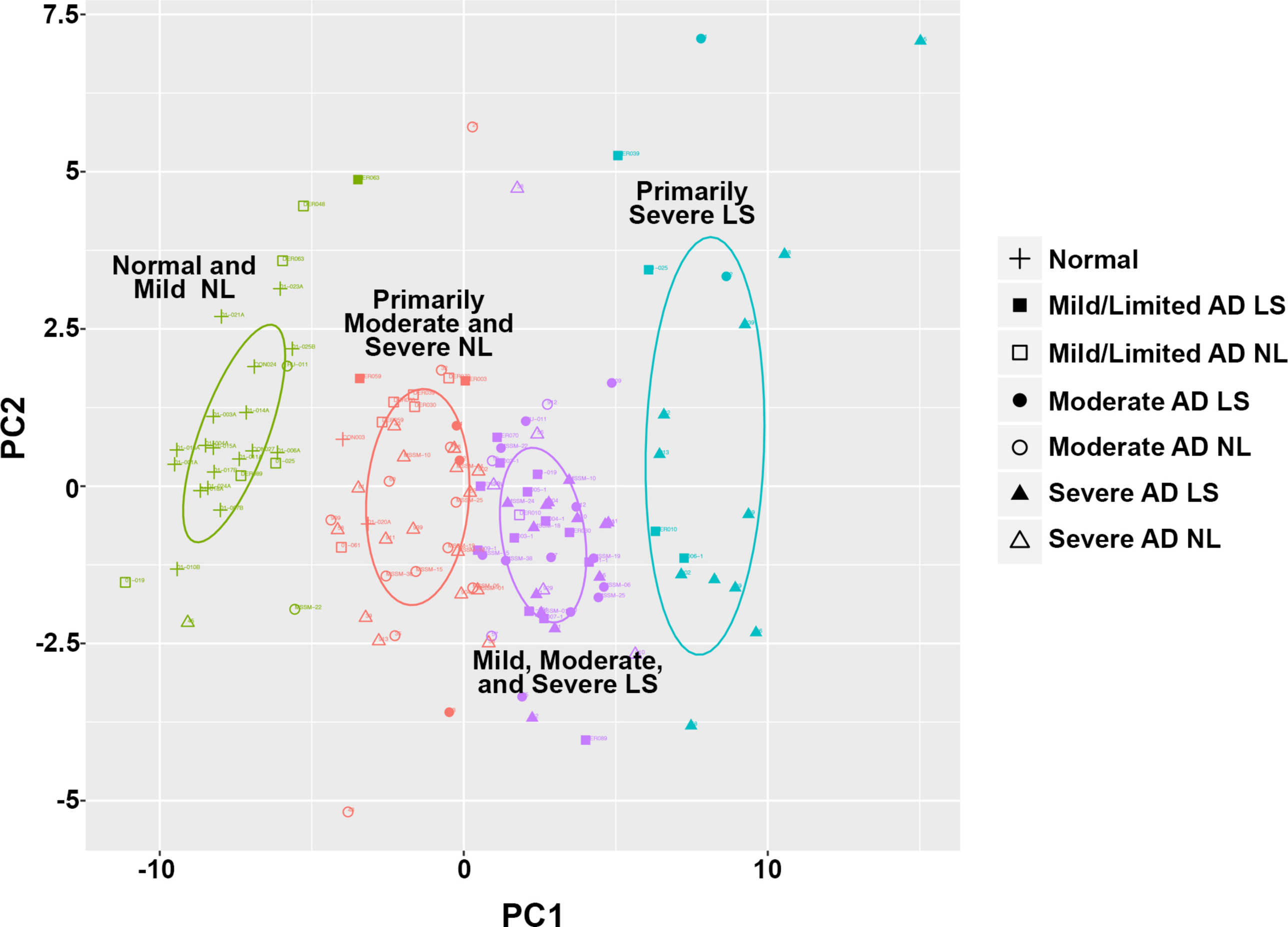
Negative Regulators

IL-34


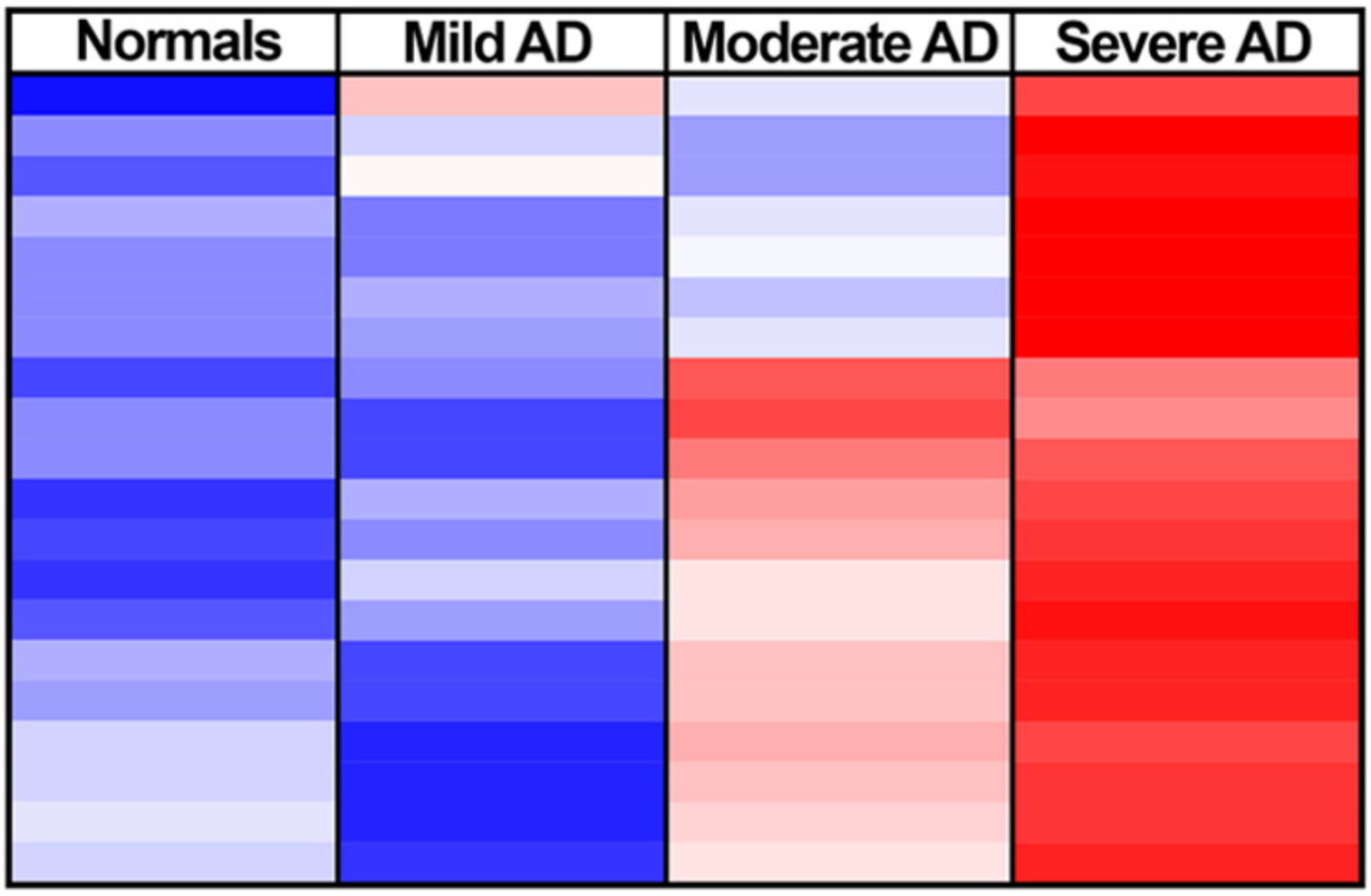
IL-37


Terminal Differentiation

LOR


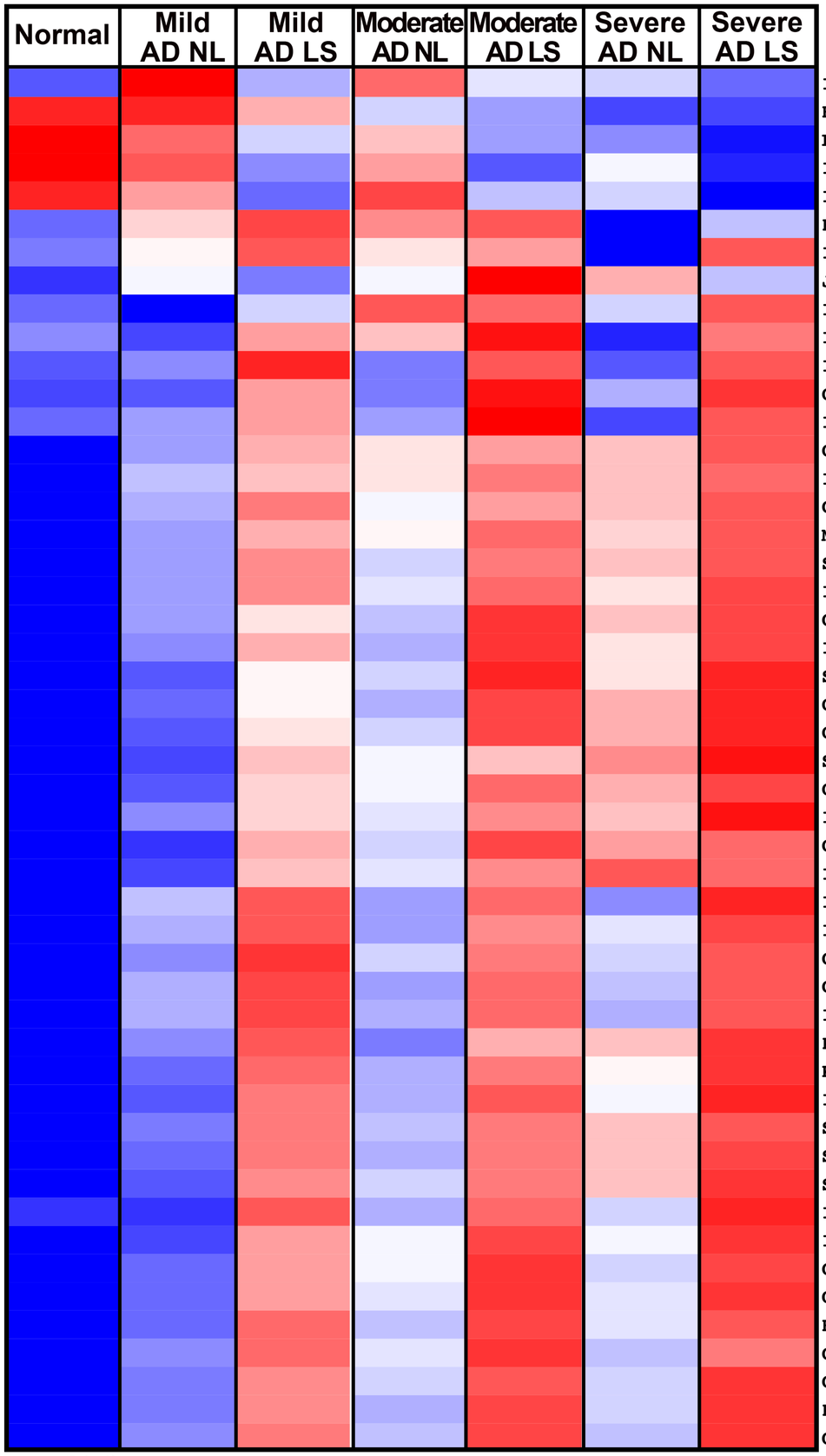

Normal and
Mild NL
Primarily
Moderate and
Severe NL
Primarily
Severe LS
Mild, Moderate,
and Severe LS
Normal
Mild/Limited AD LS
Mild/Limited AD NL
Moderate AD LS
Moderate AD NL
Severe AD LS
Severe AD NL
7.5
5
2.5
0
-2.5
-5
-10
0
10
PC2
PC1

A

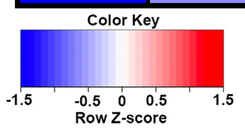


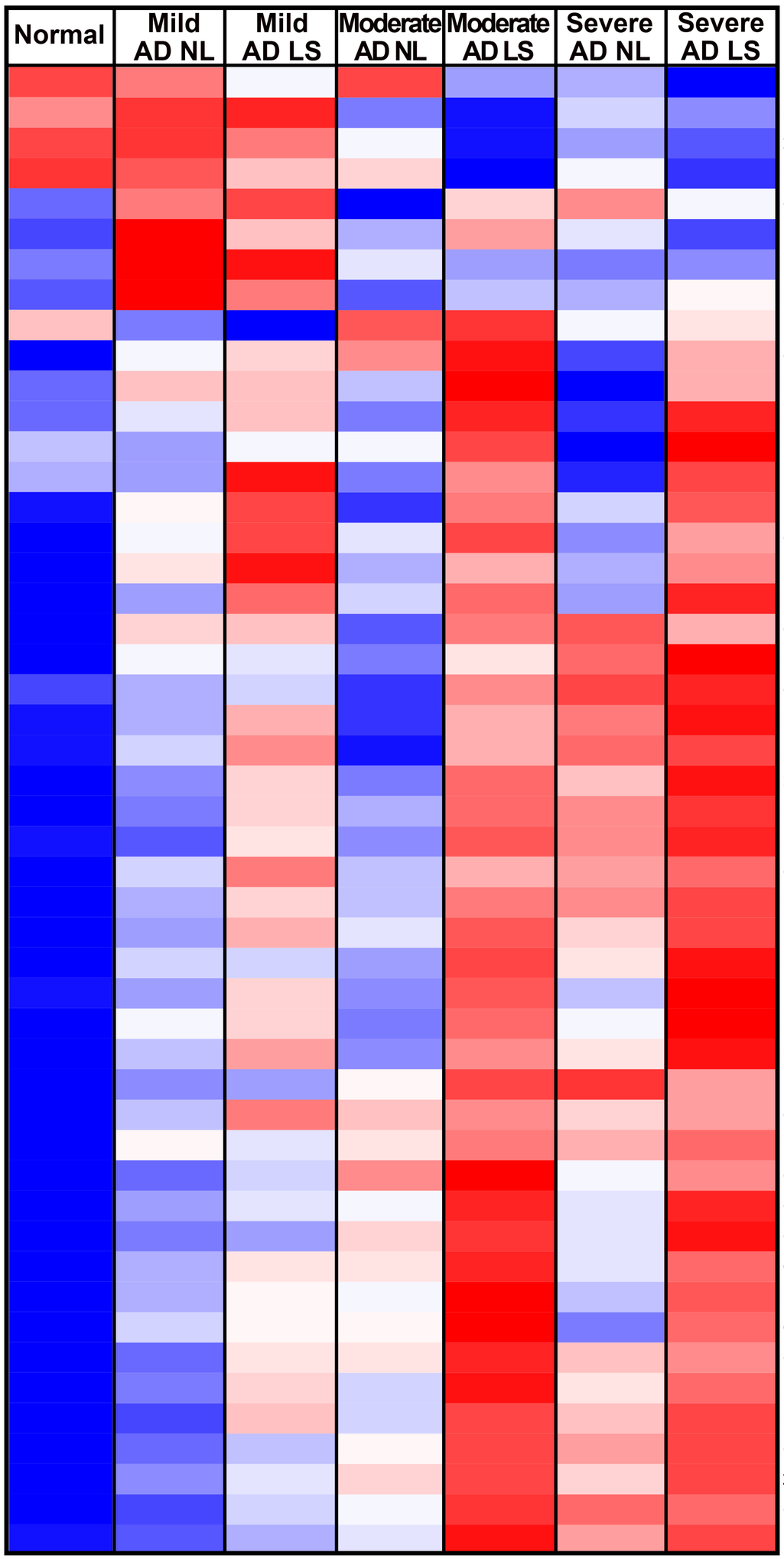
	Mild vs N	Moderate vs N	Severe vs N
CXCL9	1.35	1.23	1.54*
SELE	1.08	1.01	1.53*
CCL20	1.30	1.11	1.78**
TNFRSF9	-1.07	1.06	1.51*
SIRT2	-1.03	1.13	1.54*
IL-1RN	1.06	1.08	1.64**
PI3	1.03	1.11	1.58**
CCL17	1.17	2.13**	2.01**
IGFBP1	-1.12	1.58*	1.43*
PRSS27	-1.11	1.47	1.55**
CXCL10	1.21	1.53*	1.74**
CCL13	1.17	1.74**	2.28**
EZR	1.35	1.54	2.29**
CCL7	1.10	1.37	1.94**
MMP12	-1.21	1.33	1.78**
CDH3	-1.20	1.31	1.75**
CCL19	-1.36	1.22	1.42*
FGF21	-1.34	1.22	1.55*
KYNU	-1.45	1.14	1.55**
IL-16	-1.36	1.16	1.63**

B



	Mild NL vs N	Mild LS vs N	Mild LS vs NL	Moderate NL vs N	Moderate LS vs N	Moderate LS vs NL	Severe NL vs N	Severe LS vs N	Severe LS vs NL
IL31RA ---:	1.89+	1.11	-1.70+	1.51	1.21	-1.25	1.14	-1.01	-1.15
FLG ------:	-1.02	-1.33	-1.30	-1.77	-1.90*	-1.08	-2.27*	-2.24**	1.01
LOR ------:	-1.49	-2.86***	-1.92*	-2.02*	-3.49***	-1.72*	-3.52***	-5.27***	-1.50
IL37 -----:	-1.47	-5.79***	-3.93**	-2.12*	-7.46***	-3.52***	-3.48***	-9.22***	-2.65**
IL34 -----:	-1.39	-2.62***	-1.89**	-1.08	-1.98***	-1.84***	-1.94**	-3.25***	-1.68***
PPL ------:	1.09	1.15	1.06	1.10	1.13	1.03	-1.05	1.05	1.10
IL4 ------:	2.70	8.76**	3.24+	3.11	5.41	1.74	-4.32	8.78**	38.00**
JAK2 -----:	1.09	1.03	-1.06	1.08	1.26	1.17	1.14	1.07	-1.06
IL1B -----:	-1.29	1.27	1.65	2.02	1.93	-1.05	1.19	1.89	1.59
IL2 ------:	-1.27	2.06+	2.62+	1.82	3.24***	1.78**	-1.39	2.36+	3.29*
IL6 ------:	1.19	3.47***	2.91**	1.05	2.84**	2.70**	-1.06	2.80**	2.96**
CCL5 -----:	1.04	1.74*	1.67+	1.10	2.28*	2.07**	1.24	2.15**	1.73
IL15 -----:	1.10	1.57**	1.43*	1.14	2.14***	1.88***	-1.06	1.82***	1.94***
CCL26 ----:	3.38**	8.20***	2.43**	6.46***	8.67***	1.34	7.54***	12.20***	1.62
IL22 -----:	362.00**	4030.00***	11.10	1940.00***	14300.00***	7.41***	3750.00***	18000.00***	4.79*
CCL18 ----:	13.30***	104.00***	7.76***	25.70***	71.50***	2.78**	48.60***	134.00***	2.76+
MMP12 ----:	13.60**	104.00***	7.62**	44.80***	217.00***	4.83*	75.20***	247.00***	3.29
S100A7 ---:	14.50***	157.00***	10.80***	27.90***	215.00***	7.71**	93.20***	304.00***	3.26**
IL13 -----:	6.95***	43.40***	6.24***	13.60***	58.40***	4.28***	23.20***	82.40***	3.55*
CXCL9 ----:	2.72+	4.91***	1.80	3.30+	10.90***	3.30*	5.09**	9.03***	1.78
IFNG -----:	1.88	4.02***	2.14*	2.24	6.42***	2.86**	3.27*	5.95***	1.82
STAT1 ----:	1.15	1.57***	1.37*	1.43	2.22***	1.56*	1.61**	2.26***	1.40*
CXCL10 ---:	1.97	4.92**	2.49+	2.78	12.80***	4.60**	6.44**	14.20***	2.20
CCL11 ----:	1.87	7.16***	3.83*	4.37*	21.20***	4.86***	10.80**	29.10***	2.70*
STAT3 ----:	1.29	2.38***	1.85**	2.02**	2.41***	1.19	2.89**	3.90***	1.35
CCL17 ----:	2.46	9.03***	3.67*	6.39***	16.20***	2.54+	10.50***	19.60***	1.86
IL9 ------:	6.44+	30.50***	4.73+	15.20***	56.00***	3.70*	41.20***	201.00***	4.87
CCL20 ----:	1.42	6.58***	4.62**	3.54**	11.40***	3.23**	7.04***	10.00***	1.43
IL17A ----:	1.99	14.10***	7.10*	6.61*	20.50***	3.09	35.80***	32.90***	-1.09
IL31 -----:	5.00*	38.80***	7.76**	3.91+	32.20***	8.23**	3.18	65.40***	20.60***
IL5 ------:	5.19**	28.90***	5.57***	4.92*	22.10***	4.48*	7.46*	32.20***	4.32*
CCL13 ----:	2.01*	7.48***	3.72***	2.77**	5.82***	2.10**	2.79**	6.67***	2.40*
CXCL2 ----:	1.67+	3.35***	2.01**	1.57	2.96***	1.89**	1.66*	3.04***	1.83**
IL10 -----:	2.16**	5.56***	2.57***	2.09**	4.80***	2.29***	2.09*	5.13***	2.45**
PI3 ------:	4.59*	67.80***	14.80***	3.85+	30.80***	8.01***	25.20***	97.90***	3.89*
KRT16 ----:	2.89*	30.60***	10.60***	4.65**	28.20***	6.08***	10.30***	50.80***	4.94**
IL4R -----:	1.24	2.73***	2.20***	1.54*	2.98***	1.94***	1.96**	3.50***	1.79**
S100A9 ---:	7.77**	145.00***	18.70***	18.30***	153.00***	8.32***	65.70***	242.00***	3.68**
S100A8 ---:	4.72+	106.00***	22.40***	11.30**	113.00***	9.96***	47.90***	200.00***	4.18***
S100A12 --:	3.87+	70.80***	18.30***	12.40***	86.20***	6.96***	37.50***	170.00***	4.54**
IL23A ----:	1.06	4.35***	4.10***	1.72	4.33***	2.51**	1.95*	5.38***	2.76**
IL32 -----:	1.24	3.17***	2.56***	2.25**	4.27***	1.89**	2.34*	4.60***	1.97*
CCL22 ----:	1.98+	6.83***	3.45**	4.19***	11.40***	2.72**	3.52**	10.30***	2.92**
OSMR -----:	1.28+	2.07***	1.61**	1.62**	2.50***	1.54**	1.63**	2.55***	1.56**
FOXP3 ----:	1.57+	4.13***	2.64***	2.12**	4.67***	2.20**	2.34**	4.43***	1.90**
CTLA4 ----:	2.53	9.91***	3.91**	3.90*	13.00***	3.34***	3.52+	9.56***	2.71
CXCL1 ----:	2.17+	8.82***	4.07**	3.43**	11.10***	3.23**	3.44**	13.60***	3.94**
PDL1 -----:	1.62+	4.29***	2.64***	2.07**	5.52***	2.67***	2.27**	6.08***	2.68**
OSM ------:	1.87*	5.00***	2.67**	2.20*	6.42***	2.92***	2.15*	6.59***	3.07**

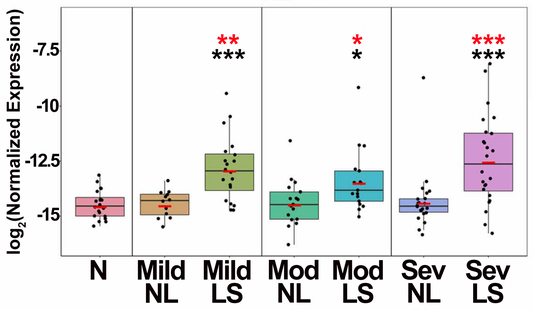




	Mild NL vs N	Mild LS vs N	Mild LS vs NL	Moderate NL vs N	Moderate LS vs N	Moderate LS vs NL	Severe NL vs N	Severe LS vs N	Severe LS vs NL
IL34 -----:	-1.13	-1.64**	-1.45**	-1.00	-2.06***	-2.05*	-1.92	-3.31***	-1.72*
FLG ------:	1.29	1.38	1.07	-2.34	-3.34*	-1.43**	-1.77	-2.17*	-1.22
LOR ------:	1.17	-1.23	-1.45	-2.48	-6.92***	-2.79*	-3.56*	-4.81***	-1.35
IL37 -----:	-1.18	-2.42**	-2.05	-2.48+	-16.60***	-6.70**	-3.67*	-12.30***	-3.35*
CCL5 -----:	1.75	1.93	1.10	-1.39	1.47	2.04	1.69	1.32	-1.28
JAK2 -----:	1.58*	1.24	-1.28	1.09	1.30+	1.19	1.15	-1.00	-1.15
PPL ------:	1.64	1.57+	-1.04	1.12	1.04	-1.08	-1.01	1.00	1.02
IL31RA ---:	3.42	2.09	-1.64	-1.01	1.32	1.33	1.26	1.57	1.24
IL1B -----:	-1.34	-1.71	-1.28	1.21	1.28	1.05	-1.08	-1.05	1.03
CXCL2 ----:	1.92	2.26**	1.18	2.69+	3.54**	1.31*	1.26	2.32**	1.83
IL6 ------:	1.45	1.47	1.01	1.18	2.16*	1.83	-1.30	1.43	1.86+
IL15 -----:	1.25	1.48*	1.18	1.01	2.01***	1.99*	-1.14	1.94***	2.22*
IL2 ------:	-1.10	1.16	1.28	1.16	1.96	1.70+	-1.71	2.38	4.06
IL4 ------:	-1.10	5.07	5.57	-1.35	2.69	3.63	-2.01	3.51	7.06
IL5 ------:	6.32	21.60***	3.42	1.32	14.60**	11.10	4.02	19.30**	4.82
CCL13 ----:	3.04+	6.47***	2.13	2.89+	6.35***	2.20*	2.13	4.46***	2.09
IL10 -----:	2.98*	6.16***	2.07	2.06	3.58***	1.74+	2.01+	3.97***	1.98
IL31 -----:	3.72	20.60***	5.53	5.01	22.90***	4.58	3.20	33.50***	10.40+
CXCL9 ----:	3.27	3.62+	1.11	1.66	4.78+	2.88	4.82	3.56+	-1.36
STAT3 ----:	1.83+	1.78*	-1.03	1.42	2.05**	1.44	2.72+	3.46***	1.27
CXCL10 ---:	1.81	2.35	1.30	-1.05	5.65+	5.96+	7.84	8.74*	1.11
PI3 ------:	3.12	10.90**	3.49	1.36	11.40**	8.39*	16.60*	42.30***	2.55
IFNG -----:	1.87	2.92	1.57	1.04	2.65	2.55	3.29	3.62+	1.10
KRT16 ----:	3.14	10.30***	3.28*	2.55	21.20***	8.28*	11.70*	44.10***	3.77
S100A9 ---:	5.02	30.30**	6.04+	9.40+	91.30***	9.71+	59.70*	164.00***	2.74
S100A8 ---:	2.48	16.40**	6.62	4.27	72.20***	16.90+	44.50*	126.00***	2.83+
CCL18 ----:	13.80*	58.20***	4.23+	12.00+	40.00***	3.34**	46.70*	73.50***	1.57+
S100A7 ---:	15.30+	59.20***	3.86	19.70*	163.00***	8.26+	122.00**	238.00***	1.95
IL13 -----:	6.85*	29.70***	4.33	13.50*	63.20***	4.68+	24.30**	72.80***	2.99
STAT1 ----:	1.40	1.39*	-1.01	1.28	1.90**	1.49	1.51	2.01***	1.34
IL23A ----:	1.45	2.16+	1.49	1.40	3.03*	2.16	1.57	3.80**	2.42
IL4R -----:	1.85+	2.05***	1.11	1.44	2.63***	1.83**	1.82+	3.19***	1.75
FOXP3 ----:	1.83	2.66**	1.45+	1.58	2.89**	1.83*	2.22	3.87***	1.74+
IL17A ----:	2.77	3.07	1.11	5.31	14.60***	2.76	15.80+	8.84*	-1.79
CCL26 ----:	4.78*	13.20***	2.76*	9.05*	12.90***	1.43	8.43*	10.60***	1.26
IL22 -----:	1460.00+	674.00*	-2.17	1880.00+	15900.00***	8.48+	4790.00*	18400.00***	3.84
CCL11 ----:	2.32	4.88	2.10	14.20+	38.60***	2.72+	5.65	13.20**	2.33
CCL22 ----:	2.20	2.87*	1.30	3.27+	8.34***	2.55*	2.87+	8.21***	2.86*
CXCL1 ----:	1.80	2.13+	1.19	4.28+	9.05***	2.12+	2.78	9.25***	3.33+
OSMR -----:	1.55+	1.85***	1.19+	1.85*	2.61***	1.41*	1.68*	2.27***	1.35
PDL1 -----:	1.94*	2.62***	1.35	2.46*	5.50***	2.23*	2.00+	4.01***	2.01
OSM ------:	2.22	2.70**	1.22	2.73	7.00***	2.57+	1.61	4.22***	2.62
CTLA4 ----:	2.09	4.84	2.31	4.59	11.60*	2.52+	5.31	7.05+	1.33
IL32 -----:	1.44	2.32**	1.61	1.87	3.73***	1.99*	2.13	2.97**	1.39
S100A12 --:	2.35	24.70**	10.50*	8.21	77.00***	9.37*	20.00*	66.20***	3.32
CCL17 ----:	2.27	3.69*	1.63	6.21+	18.00***	2.90**	9.94*	17.00***	1.71
MMP12 ----:	7.21	18.30*	2.54	40.90+	176.00***	4.30+	44.10*	194.00***	4.40
CCL20 ----:	1.37	2.70+	1.98*	3.32	10.00***	3.02	7.81+	7.82**	1.00
IL9 ------:	2.00	4.64*	2.32	8.56*	127.00***	14.80*	26.70*	68.80***	2.58

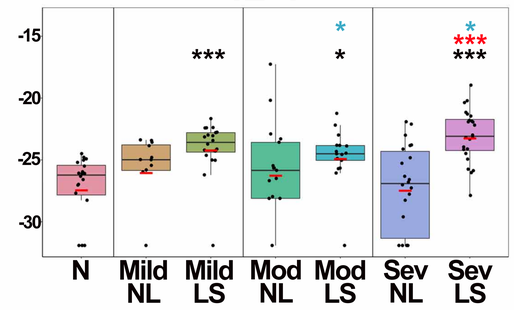
Innate Immunity

IL-6

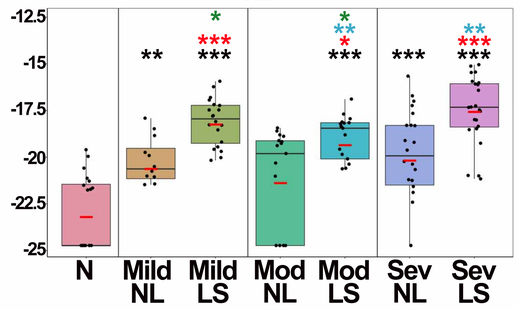


Th2

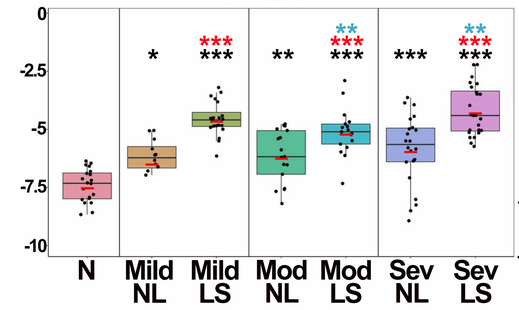
IL-4



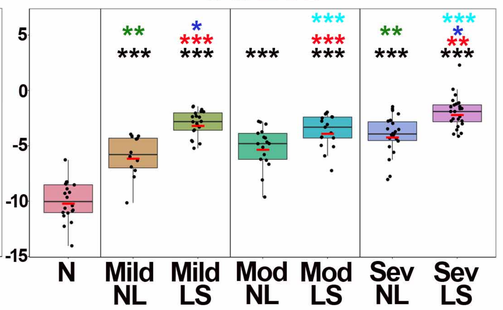
IL-5



CCL13

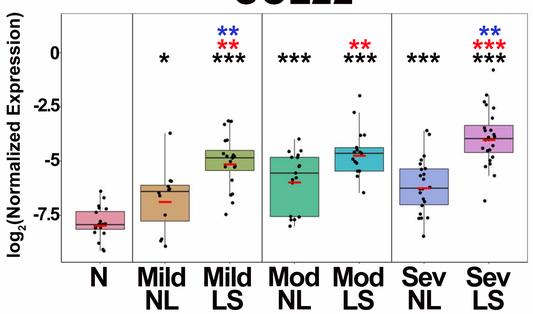


CCL18



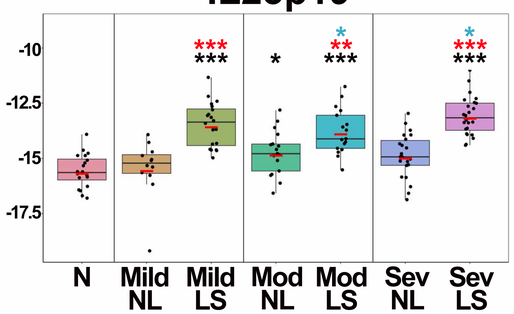
Th2

CCL22

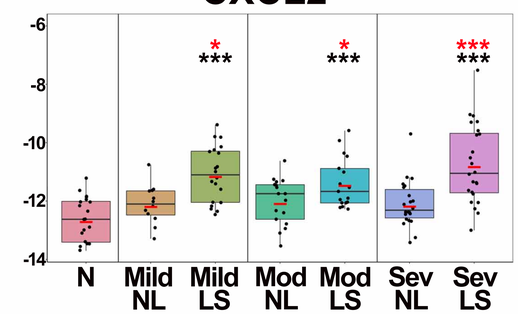


Th17

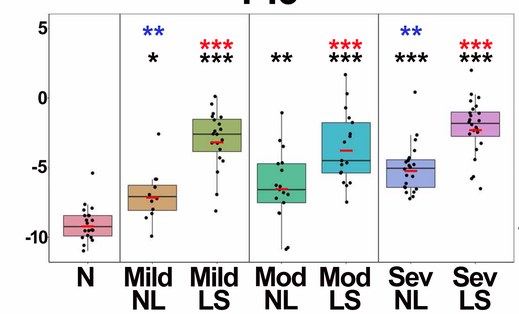
IL23p19



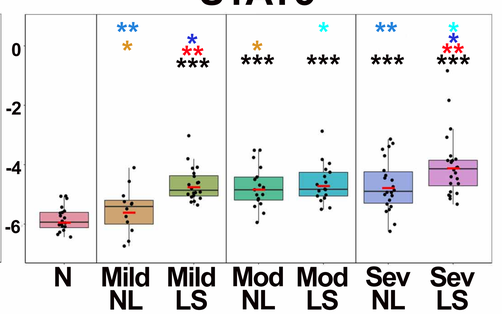
CXCL2



PI3

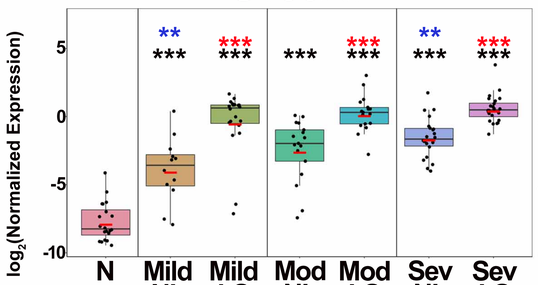


STAT3

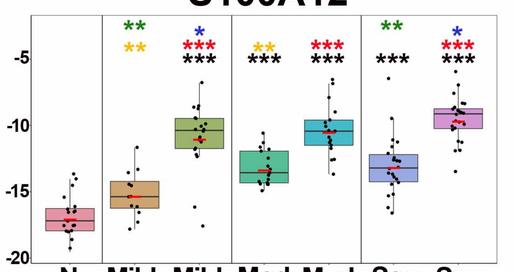


Th17/Th22

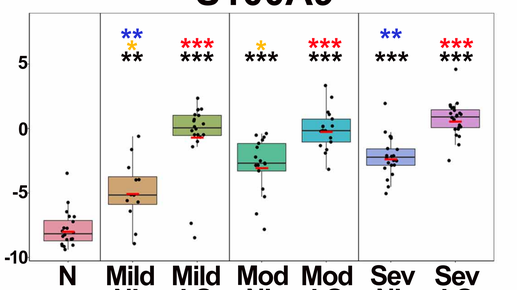
S100A7



S100A12

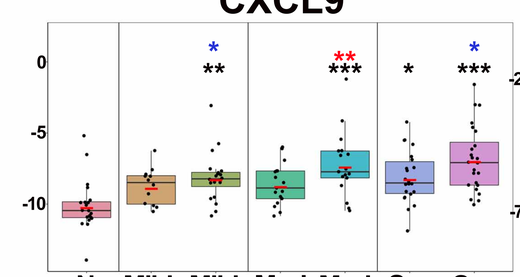


S100A9

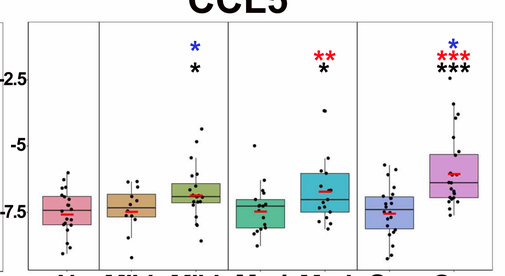


Th1

CXCL9

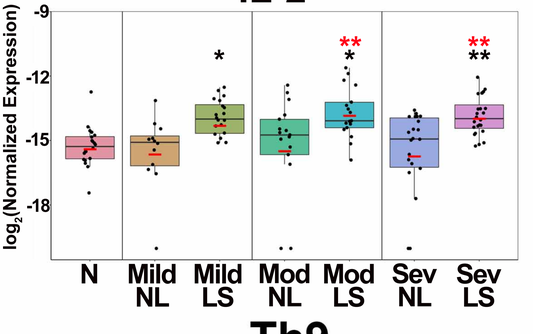


CCL5

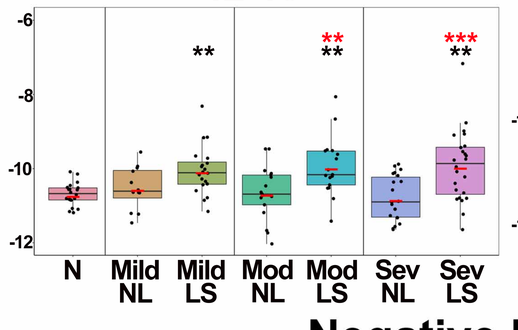


Th1/NK/T-Cell Activation

IL-2

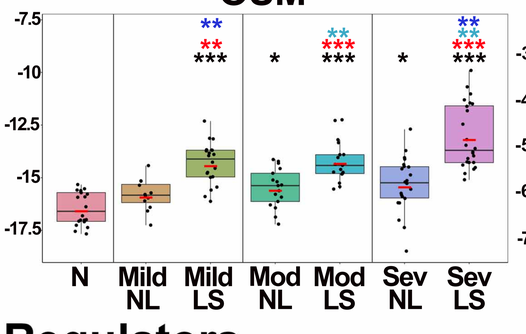


IL-15

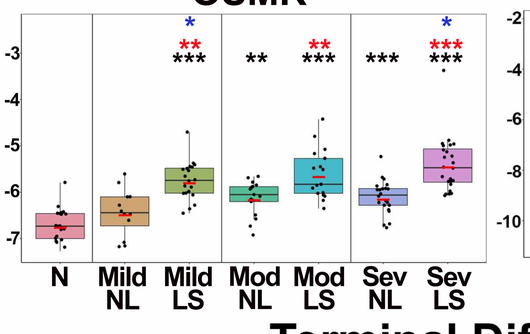


Pruritus Markers

OSM

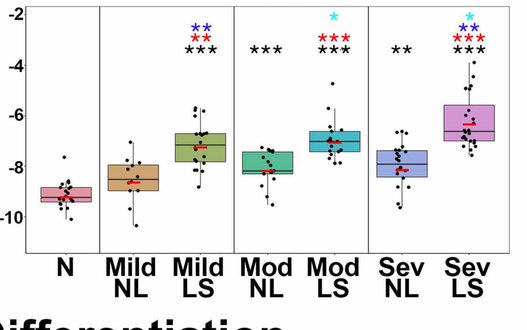


OSMR



Regulatory T Cells

PDL1



Th9

IL-9

Negative Regulators

IL-34

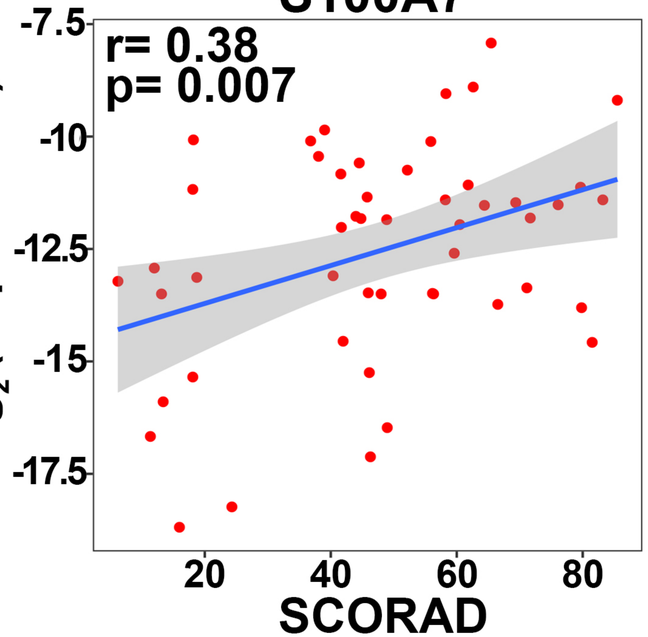
IL-37

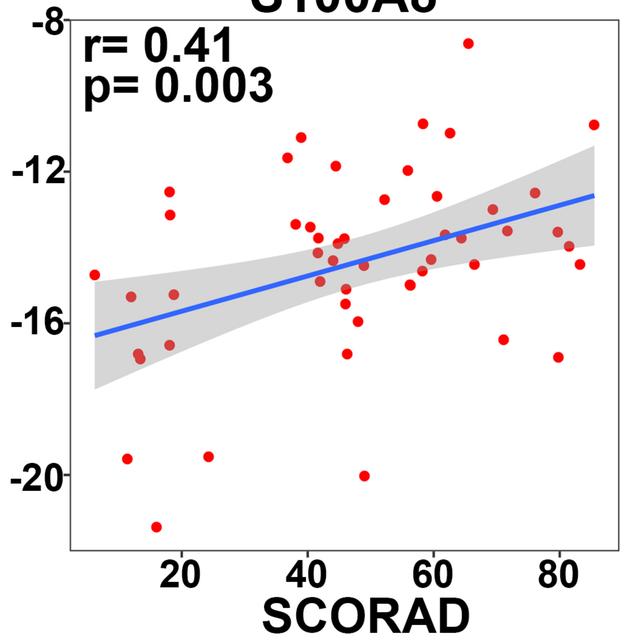
Terminal Differentiation

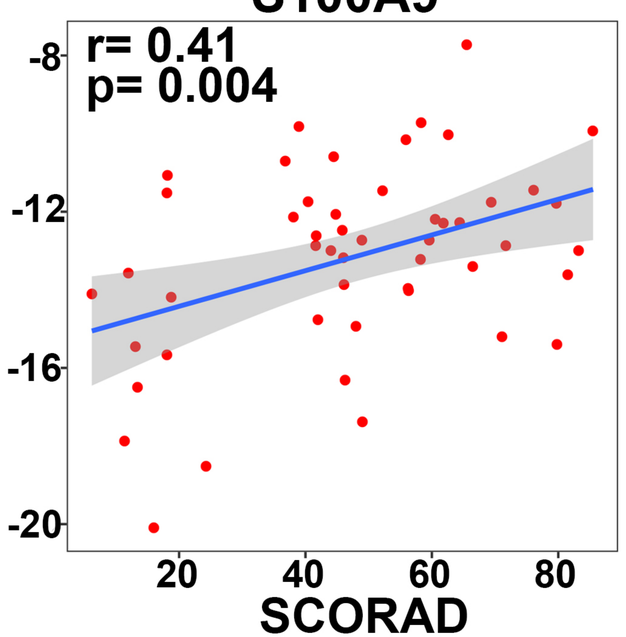
FLG

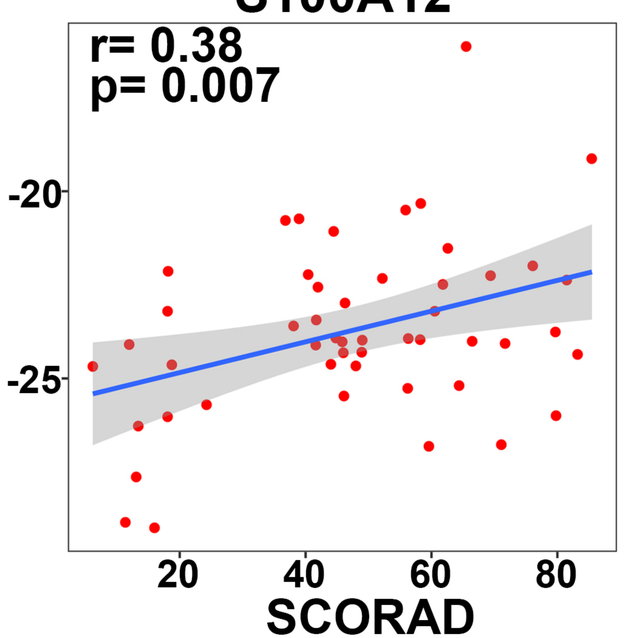
LOR

Th17/Th22

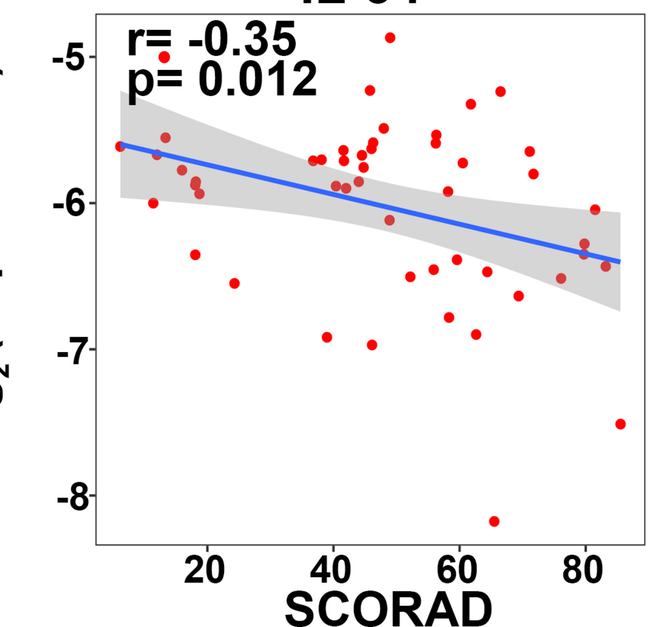
S100A7


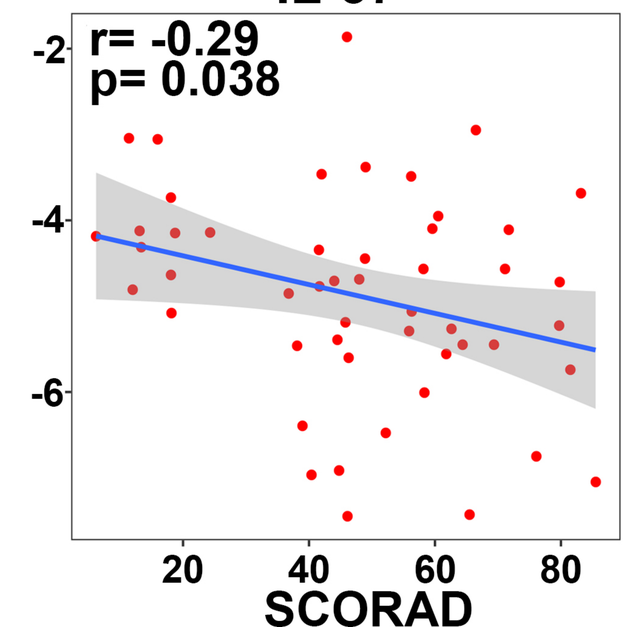
S100A8


S100A9


S100A12


Negative Regulators

IL-34


IL-37


Terminal Differentiation

LOR
