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Short title: Vascular inflammation in atopic dermatitis patients

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Abstract

Background: In atopic dermatitis (AD), some studies have shown an association with increased cardiovascular disease in certain populations. However, other investigations found modest or no association. Despite conflicting results, molecular profiling studies in both AD skin and blood have demonstrated upregulation of atherosclerosis and cardiovascular risk-related markers. However, the underlying mechanisms connecting AD to vascular inflammation/atherosclerosis are unknown. In this study, we aim to determine factors associated with vascular inflammation/atherosclerosis in AD patients.

Methods: We used 18-FDG PET-CT to characterize vascular inflammation in AD patients and healthy subjects. In parallel, we assessed their skin and blood immune profiles to determine AD-related immune biomarkers associated with vascular inflammation. We also assessed levels of circulating microparticles, which are known to be associated withincreased cardiovascular risk.

Results: We found significant correlations between vascular inflammation and Th2-related products in skin and blood of AD patientsas well as atherosclerosis-related markers thatwere modulated by dupilumab. Circulating levels of endothelial microparticles were significantly higher in severe AD patients and tended to correlate with vascular inflammation assessed by PET-CT.

Conclusion: Vascular inflammation in AD is associated with enhanced Th2 response and clinical severity, which may explain cardiovascular comorbidities observed in select AD populations.Larger prospective studies are needed to further evaluate vascular inflammation and cardiovascular events and mortality in AD patients. Finally, as dupilumab treatment demonstrated significant modulation of atherosclerosis-related genes in AD patients compared to placebo, these data suggest that modulation of vascular inflammation with systemic treatment should be explored in patients with AD.

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Keywords: Atopic dermatitis, atherosclerosis, cardiovascular, microparticles, vascular inflammation

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Abbreviations:

18-FDG PET/CT: 18-fluorodeoxyglucose positron emission tomography/computed tomography

AD: Atopic Dermatitis

cDNA: Complementary DNA

EMPs: endothelial-derived microparticles

GEE: generalized estimating equations

GSVA: gene-set variation analysis

MMPs: metalloproteinases

MPs: Microparticles

PGF: Platelet Growth Factor

PMPs: Platelet-derived microparticles

qRT-PCR: Quantitative Real-Time Polymerase Chain Reaction

SCORAD*:* SCORing Atopic Dermatitis

TBR: Target-to-Background Ratio

BMI: Body mass index

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Introduction

Atopic dermatitis/AD is a prevalent inflammatory skin disease.[1]Recently, an increasing number of investigations have examined the link between cardiovascular risk factors as well as cardiovascular comorbiditiesand AD.[2-18] While preliminarystudies found AD to be positively associated with risk factors such as obesity and smoking, associations with cardiovascular disease/CVD (e.g. stroke, myocardial infarction) have been mixed.[3,5-15,17,18]Primarily in U.S. and Asian-based populations, positive associations have been found.[2,5,7,8,10,11]Others showedmodest or no relationship, or have suggested that increased CVD in AD may be related to greater cardiovascular risk factors associated with AD.[3,13,14,17,18]In a U.K. cohort, Silverwood et al recently found a moderately increased risk of non-fatal cardiovascular outcomes in AD patients, with a dose-response for more severe and active disease.[12]Furthermore, mortality datademonstrated a slightly increased risk of death in AD patients due to cardiovascular disease compared to subjects without AD.[16]

While the linkbetween AD and cardiovascular disease remains to be fully elucidated,[3] proteomic studies in the skin and blood of AD patientsrevealed upregulation of markers associated with atherosclerosis,[19-21]and one study identified significantly greater prognostic markers of atherosclerosis (E-selectin, MMPs, CCL19, CCL2, PGF, IL-6R, IL-8)in lesional and non-lesional skin of moderate-to-severe AD patients.[19]Significant increases ofmarkers related to atherosclerosis and angiogenesis havealso been observed in blood (e.g.CX3CL1/fractalkine, CCL8, M-CSF, and VEGF-A).[21] Furthermore, our group recently reported that prognostic markers of cardiovascular risk (myoglobin, GDF15, ST2, CCL7) incrementally increase with age in AD patients compared to healthy controls.[22]Taken together, thesestudiessuggestshared mechanisms between remote skin inflammation and the presence of vascular disease in AD. As suggested in other inflammatory disorders,[23,24] chronic inflammation may be primarily responsible for increased cardiovascular risk in AD.However, a comprehensive study linking the immune profileand cardiovascular risk in AD patients is lacking.

Aortic vascular inflammation is associated with increases in blood inflammatory biomarkers and is predictive of future cardiovascular events.[25,26]A recent study focused on evaluating vascular inflammation using 18-fluorodeoxyglucose positron emission tomography-magnetic resonance imaging(18-FDG PET-MRI)in moderate-to-severe AD

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patients comparedto healthycontrols, finding greater aortic and carotid inflammation in the AD cohort.[4] In the present study,we used18-FDG PET-CT to characterize the association between vascular inflammationand immune biomarkers ina cohort of relatively young moderate-to-severe AD patients with no known cardiovascular disease compared to healthy controls.We found significant correlations between vascular inflammation assessed by PET-CTand Th2-related products in the skin and blood of AD patients. Circulating levels of microparticles, which are derived from activated endothelial cells and shown to correlate with vascular inflammation and cardiovascular risk, were also significantly higher in AD patients. Levels of microparticles further demonstrated a trend towards correlation with vascular inflammation, as assessed by PET-CT. Finally, we demonstrated that dupilumab, but not placebo, was able to significantly modulate expression of atherosclerosis-related genes in AD patients.

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METHODS

Patients

Thirty patients (17 females, 13 males; mean age 32 years, age range 18-68; mean BMI25.1, range 20.0-34.7; mean SCORAD 51.8, range 37-79.5; mean EASI 21.8, range 15.3-48.5) with moderate-to-severe AD, and twenty-six healthy subjects matched for age, sex and coronary artery disease risk factors (16 females, 10 males; mean age 30 years, age range 18-59; mean BMI24.9, range 18.9-36.6), without known cardiovascular comorbidities, were recruited. Matched coronary artery disease risk factors include BMI, smoking status, dyslipidemia, hypertension, and CRP levels. The study was approved by a research ethics board and written informed consent of all patients was obtained. Healthy subjects had no atopic conditions. All patients had chronic AD since childhood. Patients with known cardiovascular disease, who had used an oral treatment for AD in the previous 4 weeks or a systemic treatment for AD in the previous 12 weeks were excluded. There were no significant differences in demographics parameters or coronary artery disease risk factors between groups(**Table 1**).

18FDG-PET-CT

All subjects underwent 18FDG-PET-CT to quantify vascular inflammationin the ascending aorta. Vascular inflammation was quantified using Target (atherosclerotic plaque) to background (blood) ratio (TBR) as previously described.[27] TBR scores are reported as mean (TBR_{Mean}), maximum (TBR_{Max}) and most-diseased segment (TBR_{MDS}).

Biopsy collection

4.5 mm punch biopsies were obtained from representative lesional and non-lesional AD skin (near the vicinity of, but > 10 cm away from lesions), and healthy skin. Biopsy samples were collected from the extremities, and only if not feasible, from the trunk, and always from skin that showed no evidence of infection.

Sample preparation

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Total RNA was extracted from skin biopsy samples using QiagenmiRNeasy Mini kit (Qiagen©, Hilden, Germany). RNA quality was assessed by TapeStation 4200 (Agilent Technologies).

Quantitative Real-Time Polymerase Chain Reaction (qRT-PCR)

Reverse transcription to complementary DNA (cDNA) from RNA was carried out using the High-Capacity cDNA Reverse Transcription Kit, and cDNA was amplified with TaqMan Gene Expression Master Mix (Applied Biosystems©, Foster City, California). TaqMan Low Density Array cards were used for qRT-PCR. Expression values (threshold cycle [Ct]) were normalized to housekeeping gene *hARP* and log2-transformed for analysis. Primers are listed in **Table S1**.

RNA-sequencing (RNA-Seq)

RNA-Seq was performed with the Illumina® HiSeq4000 (Illumina, San Diego, California; 100 cycles, single-read sequencing, 8 samples per lane), as previously described.[28-32] Sample quality was assessed using FastQC. RNA-sequencing data was profiled by Illumina HiSeq4000 to allow for more global analyses of genomic abnormalities associated with AD. Data was pre-processed using standard pipeline incorporating quality control metrics, such as FastQC and MultiQC, sequence alignment based on STAR RNA-sequencing aligner, and sequencing reads assignment to genomic features by featureCounts and *voom*-transformed. Fold-changes (FCHs) for comparisons of interest were estimated, and hypothesis testing was conducted using contrasts under the general framework for linear models in limma package. P-values from the moderated t-test were adjusted for multiple hypotheses using the Benjamini–Hochberg procedure, which controls False Discovery Rate/FDR. Differentially expressed genes/DEGs were defined by FCH>2.0 and FDR<0.05.

Serum analysis

SerumCCL11/eotaxin, CCL26/eotaxin3, CCL2/MCP-1, CCL3/MIP-1α, CCL4/MIP-1β, CXCL8/IL-8, CCL13/MCP-4, CCL22/MDC, CCL17/TARC, and CXCL10/IP-10were measured by using Meso Scale Discovery/MSD™ assays (Meso Scale Diagnostics©, Rockville, MD). Serum IL-13, IL-22 and IFN-γ were measured by Single Molecule

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Counting/SMC™ (EMD Millipore©, Darmstadt, Germany). Serum S100A8/S100A9 heterodimer concentrations were measured by Quantikine ELISA kits (R&D Systems, Minneapolis, Minn).

Flow cytometry analysis– Microparticles

Plasma samples were collected, centrifuged, and stored at −80 °C until processing. The technique used for microparticles isolation was adapted from previous reports.[33]Microparticles were defined as platelet-microparticles (PMPs, Annexin V+ CD41+) and endothelial-microparticles (EMPs, Annexin V+ CD41- CD31+).[34] EMPs expressing CD62E were considered activated EMPs.[35]

Statistical analysis

Analyses were performed using R-language (R-project.org) and Bioconductor Project packages (www.bioconductor.org), as described.[20,21] Protein and gene expression profiles were modeled by linear models using R's *lme* function, or *limma*framework[36] in the case of the RNA-sequencing data. For the RNA-sequencing data, p-values were adjusted for multiple hypotheses using the Benjamini-Hochberg procedure to produce false discovery rates/FDRs. Fold changes/FCHs>2.0 and FDR<0.05 were considered differentially expressed. Correlations between vascular imaging (TBR), mRNA, protein, and severity scores in skin and blood were evaluated by Pearson correlations. TBR measureswere compared between subjects with AD and matched healthy controls using the Student t-test. Mean expressions are displayed in a heatmap, where unsupervised clustering was performed using Euclidean distance and average agglomeration criteria. Pearson correlation coefficients were used to evaluate the relationship between immune markers and Aorta TBR measures. P<0.05 considered significant (***p<0.001, **p<0.01, *p<0.05, $^{+}$p<0.1). Additional methods can be found in the Supplementary Methods.

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RESULTS

We aimed to understand the relationship between vascular inflammation and inflammatory biomarkers in skin and blood from a cohort of 30 chronic AD patients with no known cardiovascular disease and 26 age-matched healthy controls (**Table 1**).No significant differences were found between groups for age, gender, body mass index, race/ethnicity, smoking status, hypertension, and presence of dyslipidemia. Levels of CRP, a biomarker for atherosclerosis that has prognostic value for predicting future cardiovascular events,[37] were also not significantly different between groups (**Table 1**).

Trend of greater vascular inflammation by 18-FDG PET-CT

We first assessed vascular inflammation using 18-FDG PET-CT in the aorta (**Fig S1**). While no significant differences were found in the TBR measures from the ascending aorta between AD patients versus healthy controls (TBR_{Max} [mean±standard deviation of 26 pairs] 1.92±0.16 in AD and 1.91±0.17 in healthy subjects, p=0.82;TBR_{Mean}[mean±standard deviation of all analyzed subjects] 1.48±0.14 in AD and 1.46±0.10 in healthy subjects), aTBR_{mean} value of > 1.6 is considered to be associated with greater cardiovascular risk,[38] and we observed Aorta TBR_{mean} values above 1.6 in several (n=5)AD patients (**Fig S1C**, highlighted red box).We also compared cardiovascular risk factors in AD patients with Aorta TBR_{mean}> 1.6 compared to those with TBR_{mean}< 1.6, finding no significant differences between the groups (**Table S2**).

Endothelial microparticles are upregulated in the blood of severe AD patients

We further quantified microparticles in AD patients. Microparticles are small vesicles which have been shown to correlate with cardiovascular risk and subclinical atherosclerosis.[39]We evaluated the main subsets of microparticles derived from platelets (PMPs)and endothelial cells (EMPs), that are released upon vascular inflammation, in the plasma of AD patients using flow cytometry (**Fig S2**). These microparticles have been shown to be released *in vitro* by endothelial cells following cytokine stimulation.[40]Overall, we observed greater, albeit non-significant, levelsof Annexin V+ microparticlesand PMPs in AD patients versushealthy controls (p=0.28 and 0.22 respectively)(**Fig1A**). We found significantly elevated EMPs (CD31+) and activated EMPs (CD31+CD62E+) in AD compared to controls (p<0.01) (**Fig 1A**). Upon stratifying patients by severity (above or

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below SCORAD 50), we further observed higher levels of microparticles ina subset of AD patients with severe disease, including significantly greaterEMPs and activated EMPs in severe AD patients compared to controls ($p<0.05$) and a trend towards significance for greaterAnnexin V+ microparticles and PMPs in the same subset, as compared to controls(**Fig 1B**).

Th2/Th22 and atherosclerosis-related products are upregulated in AD skin and blood

To determine the immune factors that contribute to vascular inflammation in AD patients, we next analyzed their skin and blood inflammatory signatures using RNAseq, qPCR and protein measurements, obtaining results confirmatory ofprior studies.[21,29,41]For global molecular profiling of the AD lesional and non-lesionalskin transcriptome, we performed RNA-Seq using Illumina HiSeq4000. Criteria of fold-change/FCH>2.0 and false discovery rate/FDR<0.05 defined differentially expressed genes/DEGs. For AD lesional versus normal skin comparisons, we identified 3,733 DEGs (1,563 up and 2,170 down). For AD non-lesional versus normal skin comparisons, we found 660 DEGs (408 up and 252 down) (**Table S3; Fig S3**). A principal component analysis/PCA based on RNA-Seq data separated lesional, non-lesional and normal skin (**Fig S4A**). Significant dysregulation was evident in both lesional and non-lesional AD skin, consistent with prior studies (**Table S3, Fig S3**).[19-21]As many of the top dysregulated genes included immune genes, we next evaluated a curated immune gene subset,[32,42,43] as illustrated in **FigS4B**. We found significant dysregulation in both lesional and non-lesional AD skin compared to controlsfor markers related to T-cell activation (CD69, ICOS), Th2 (IL-4R, IL-13, CCL13, CCL17, CCL18) and Th17/Th22 (IL-22, PI3, S100A7) pathways (FDR<0.05) (**Fig S4B**).[44,45]

In addition to immune genes, multiple atherosclerosis/cardiovascular risk genes also known be associated with AD, were dysregulated in both lesional and non-lesional skin (S100A8/S100A9/S100A12, IL-36A, IL-36G) or in lesional skin only (IL-6) (**Fig S4B**). IL-37, which is protective against atherosclerosis,[46] was significantly downregulated in lesional skin (**Fig S4B**). We thus further evaluated a curated subset of atherosclerosis markers[19-21]and found 42% of these genes (28 of 66) were differentially expressed in AD compared to normal skin (**Fig 2A**).Lesional and non-lesional AD skin showed significant

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upregulation of markers related to inflammatory cytokines (IL-36G/A/RN, IL-8), monocyte recruitment (CCL2), tissue remodeling (MMP1) and Th22 (S100A8), as compared to normal skin ($p<0.05$) (**Fig 2A**). A few markers related to inflammation and vascular adhesion (IL-6, ICAM1, SELE) were only significantly upregulated in lesional AD skin versus controls ($p<0.01$) (**Fig 2A**).

Gene-set variation analysis/GSVAwas then performed to evaluate enrichment of previously published pathways.[44,45,47] The atherosclerosis signaling pathway was significantly enriched in lesional AD skin versus controls ($p<0.01$) (**Fig2B**). There was also significantly higher upregulation of the meta-analysis derived AD transcriptome/MADAD, and pathways related to T-cell activation, Th1, Th2, and Th17 in AD lesional and non-lesional skin compared to controls ($p<0.05$) (**Fig S5**).

To further confirm and expand RNAseq data, we used qRT-PCR to evaluate a panel of immune genes,including those that are often below detection on RNAseq.[29,48] Similar to RNAseq data and confirmatory of prior studies,[29,41] we observed strong up-regulation of T-cell migration (CCL19/CCL21), Th2- (IL-13, IL-10, IL-31, CCL13/CCL17/CCL26), Th22-related products (IL-22, S100A7/8/9/12), markers of tissue-remodeling (MMP1/12) in lesional and/or non-lesional AD compared to normal skin ($p<0.05$) (**Fig S6**).Th22 marker IL-32 and tissue remodeling marker MMP13 were significantly increased in AD lesional versus non-lesional skin ($p<0.05$) (**Fig S6**). Furthermore, upregulation of Th1 markerIFNγ, Th17- related products(IL-17A, CCL20, PI3), and measures of epidermal hyperplasia (K16)were shown in AD lesional and/or non-lesional versus normal skin ($p<0.1$) (**Fig S6**).

To assess circulatory abnormalities in AD blood, we evaluated a panel of 14 inflammatory proteins in the serum of our cohort (**Fig S7,Table S4**). AD patients presented with significant, or trending toward significant,Th2 (IL-13, CCL13/CCL17/CCL22/CCL26), Th1 (CXCL10),Th22 (IL-22, S100A8/A8)upregulationin serum compared to healthy subjects, as previously reported ($p<0.1$) (**Fig S7**).[21]Other Th2 (CCL11) and Th1- (IFNγ, CCL2/CCL3/CCL4) related markers were detected but did not demonstrate statisticallysignificant differences in the serum of AD patients versus healthy controls, while innate immunity marker IL-8/CXCL8 was undetectable(**Table S4**).

Vascular inflammation correlated with Th2 biomarkers in AD skin and blood

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We performed Pearson correlation coefficient analysis in order to understand how various skin and blood biomarkers correlated with vascular inflammation, as measured by Aorta $TBR_{Max/Mean/MDS}$ (18-FDG PET-CT) and microparticle levels. Unsupervised hierarchical clustering of significant qRT-PCR markers, RNAseq pathways, serum biomarkers, Aorta TBR measures, microparticlelevels and clinical severity (SCORAD, EASI) is displayed in a correlation heatmap in **Fig3**.

Microparticles (PMPs, EMPs, Activated EMPs) and CCL26 (Th2 chemokine) serum levels tightly clustered together (blue rectangle; **Fig 3, Table S5**). A significant correlation was identified between activated EMPs and serum levels of CCL26 ($r=0.53$, $p<0.05$). Additionally, microparticles, particularly PMPs, tended to correlate with Aorta TBR_{MDS} and TBR_{Max}($r>0.4$, $p<0.1$).

In contrast, Ao TBR values, SCORAD, EASI,the atherosclerosis signaling pathway, markers related to T-cell migration (CCL19), Th2 (IL-13, CCL17), Th22 (S100A8/9, IL-22, IL-32),and tissue remodeling marker MMP12, all largely clustered together (green rectangle; **Fig 3, Table S5**).Significant (or trending toward significant) correlations in this cluster were found between Ao TBR measures andmarkers related to Th2 (CCL17), Th22 (IL-22, S100A8, S100A9, IL-32), tissue remodeling (MMP12), T-cell migration (CCL19) ($r=[0.4-0.59]$; $p<0.1$) (**Fig 3**, **Table S45**). Positive correlations were also found between AoTBR_{Max} and pathways related to T-cell activation, Th1, Th2,and the MADADgene-sets ($r=[0.4-0.6]$; $p<0.1$) (**Fig 3,Table S5**). Individual genes in these pathways, that have also been associated with atherosclerosis, including IL-1RN (innate immunity), IL-15 (T-cell activation), CXCL16 (Th1-related), CCR4, CCL11, CCL17, CCL22 (Th2-related),[21,49-53]also significantly positively correlated with Aorta TBR_{MDS} ($r=[0.51-0.68]$; $p<0.05$) (**Fig S8**). IL-37 was negatively correlated with Aorta TBR_{MDS}($r=-0.55$; $p<0.05$) (**FigS8**).

In addition, while the individual serum levels of the Th2 cytokine, IL-13,trended toward positive correlations with Aorta TBR measures ($p=0.12$), and the levels of the Th1 marker,IFNγ,trended toward negative correlations with Aorta TBR measures ($p=0.19$) (**Fig4**), the ratio of these Th2/Th1 products (as measured by IL-13/IFNγ) correlated more significantly with Aorta TBR measures ($r=0.34-0.42$; $p<0.1$) (**Figs 3-4, Table S5**).

We further evaluated for differences in vascular and inflammatory markers in AD patients with Aorta $TBR_{mean}> 1.6$ compared to $TBR_{mean}< 1.6$. We found significantly higher expression of markers of innate immunity (IL-1a, IL-1RN), Th2 (CCL17), as well as

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Th17/Th22-related markers (IL-36A, S100A8/A9) in the TBR_{mean}> 1.6 group as compared to the TBR_{mean}< 1.6 group (**Table S6**).

Dupilumab progressively modulates atherosclerosis-related markers

To understand whether selective Th2-targeting treatment is able to modulate vascular inflammation-related genes, we analyzed the atherosclerosis signaling pathway genes in a previously reported cohort treated with dupilumab, an IL-4Rα antagonist, for 16 weeks.[43]Dupilumab significantly modulated expression of multiple atherosclerosis-related genes (23/63), from baseline through Week 16, whereas this modulation was not seen in the placebo group (**Fig 5A**).These genes included markers such as IL-1RN, IL-36G, IL-36A, S100A8, MMP9, CCL2, SELE, IL-37 and APOC1. GSVA also showedsignificant enrichment of atherosclerosis-related genes from baseline through Week 16 in the dupilumab-treated group only ($p<0.001$) (**Fig 5B**). In addition, percent improvement of atherosclerosis-related genes significantly correlated with percent improvement of clinical severity, as measured by SCORAD ($r=0.59$; $p<0.05$) (**Fig 5C**), further suggesting that clinical severity and atherosclerosis pathogenesis are linked in AD patients.

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DISCUSSION

To our knowledge, this is the first study to investigate the association between active markers of cardiovascular disease and the immune profile of AD patients. We report that vascular inflammation in AD patients is positively correlated with the expression of several lesional skin biomarkers including those related to Th2 and Th22 pathways. While there was no statistical difference in vascular inflammation between the AD and control groups, the number of subjects with an$AoTBR_{Mean}$higher than 1.6 was numerically higher in AD patients. Additionally, a subset of severe patients presented with elevated concentrations of circulating endothelial microparticles compared to controls.Of note, both EMPs and vascular inflammationhave been correlated with coronary artery disease development in prior studies.[26,54-56] Among the top upregulated genes in lesional and non-lesional AD skin included multiple atherosclerosis-associated genes, such as Th22-related (IL-22, IL-32, S100A8/A9), tissue remodeling (MMP1, MMP12), T-cell migration (CCL19), inflammatory cytokines (IL-6, IL-8, IL-36G/A/RN), monocyte migration (CCL2) and vascular adhesion (ICAM-1, SELE) pathways.[57-64]IL-22, IL-32 and IL-6 have been demonstrated to be involved in the inflammatory cascade leading to atherosclerosis plaque formation,[57-60] through increased production of monocyte chemoattractants (CCL2), endothelial cell adhesion markers ICAM-1,[58,60] SELE[61]or MMPs, which are implicated in vascular tissue remodeling.[62] S100A8/9 are released by macrophages and endothelial cells during vascular inflammation or tissue damage and were shown to be upregulated in atherosclerotic plaques.[63]The lymphoid organizing chemokine, CCL19, has also been reported to be upregulated in atherosclerotic plaques.[64] Furthermore, many of these skin markers were upregulated in the blood of AD patients compared to healthy subjects (IL-22, S100A8/A9). Dysregulation of these biomarkers in both the serum and non-lesional AD skin further suggests skin-blood interaction with chronic systemic inflammation in AD, which could lead to atherosclerosis development. Our data is in accordance with the hypothesis that in AD, proteins originate from lesional skin and then progressively leak into non-lesional skin and blood, with the greatest levels found in elderly AD patients,[22] thus adding to the literature that have suggested AD to bea risk factor for cardiovascular outcomes in certain populations.[2,5,7,8,10-12,17]

Significant (or trending toward significant) correlations were found between vascular inflammation and key-AD skin biomarkers, such as Th2- (CCL17, CCL22) and

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Th22- (IL-22, S100A8/A9 and IL-32) related markers. Analysis of the blood signature in our cohort further revealed that Th2, but not Th1pathwaytrended toward positive correlation with vascular inflammation. Therefore, atherosclerosis, which is classically considered to be a Th1-dominant disease, might instead be mediated by the Th2 immune response in AD patients.[65] However, the role of the Th2 immune response in atherosclerosis has been controversial.[66]While some studies reporta protective effect,[67,68]others have demonstrated a pro-atherogenic impact.[69-71]Of note, increasedexpression of CCL17 and CCL22 has been found in atherosclerotic lesions,with one study suggesting the role of CCL17 in driving atherosclerosis via restraining regulatory T-cell homeostasis.[52,72]Conversely, an antibody targeting CCL17 reduced atheroprogression in a murine model.[52]Another study using an asthma allergic mouse model with accelerated atherosclerosis-plaque formation reported that targeting the Th2 pathway and reducing IL-4 and IL-13 levels could improve vascular lesions.[70]Thisobservation isin favor of the hypothesis that a Th2 immune response drives atherosclerosis development in Th2-skewed inflammatory disorders.

To our knowledge, this study was also the first to assess microparticles in AD. Microparticle levels, particularly from endothelial cells and platelets, are elevated in patients with cardiovascular disease, metabolic syndrome, and other inflammatory diseases, such as psoriasis.[73] Moreover, plasma levels of microparticles have been shown to be predictive of atherosclerosis and coronary artery disease.[74] EMP release is directly related to endothelial dysfunction and can be triggered upon two major pathways: apoptosis or endothelial cells activation.[75] When the endothelium becomes functionally activated, CD62E+ EMPs levels are increased.[75] In our AD cohort, we found increased EMPs, particularly CD62E+ EMPs, favoring vascular inflammation as the cause of their release. We found that the Th2 chemokine CCL26 was significantly correlated with vascular inflammation and microparticle concentration in our study. CCL26 is expressed by endothelial cells or monocytes upon IL-4 stimulation[76] and in one report, it was suggested that CCL26 could trigger atherosclerosis pathogenesis.[77]Moreover, EMPs are biomarkers of vascular inflammation, and they can also exert an autocrine action on the endothelium, further perpetuating vascular inflammation.[78,79]

While the link between AD and cardiovascular disease remains to be clarified,[3]increased cardiovascular and atherosclerosis risk-related proteinshave been

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detectedin both the skin and blood of AD patients compared to controls.[19-22,80]This raises the question of whether AD treatment can modulate these markers. In the present study, we expand on these data, showing that dupilumab treatment significantly modulated atherosclerosis-related genes. S100A8/A9 skin levels were shown to normalize in AD skin following dupilumab,[43] as did levels of other Th2- (CCL17/CCL26) and Th22-related markers in AD skin that were correlated with vascular inflammation in our study. The concomitant improvement of clinical severity and atherosclerosis-related gene dysregulation suggests that in AD patients, atherosclerosis/vascular inflammation is linked to clinical severity and that treatment of patients with severe disease could help prevent the development of vascular inflammation. However, asAorta TBR and endothelial microparticles were not directlyevaluatedin these patients, these results suggest that future AD studiesare needed to evaluate the effects of systemic treatment on vascular inflammation.

In the study of atherosclerosis, there has been increasing evidence demonstrating the role of the IL-1 cytokine family, including IL-1α, IL-1β, and IL-1RN,on atherogenesis and mechanisms of cardiovascular disease.[81,82]Aproof-of-concept study showed that treating patients with a history of prior myocardial infarction with canakinumab, an IL-1β antagonist, resulted in significantly lower recurrence of cardiovascular events.[82]In AD, tape-strip molecular profiling studies have shown significantly greater expression ofIL-1 family of cytokines in lesional and/or non-lesional skin compared to controls,[83-85]with one study identifyinghigher expression of IL-1 cytokines in uninvolved AD skin in patients with *FLG* gene mutations compared to those without.[85]In our analysis, we observed IL-1RN to be significantly correlated with Aorta TBR_{MDS}. Additionally, IL-1a and IL-1RN were more significantly expressed in AD patients with $TBR_{mean} > 1.6$ as compared to $TBR_{mean} < 1.6$. Finally, expression of IL-1RN significantly decreased with dupilumab treatment.Thus, given the role of the IL-1 gene family in atherogenesis and cardiovascular disease, as well as its significant modulation in AD, additional studies are warranted to assess the role of IL-1 on vascular inflammation in AD. Of note, in a mouse model of severe dermatitis, persistent IL-1 secretion from the skin was associated with cardiovascular disease,[86] suggesting that IL-1 may represent a link between skin and vascular disease in AD.[87]

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IL-37, another member of IL-1 cytokine family and downregulated in AD lesions, is a negative immune regulator with anti-inflammatory properties.[81,88-90]In our data, IL-37 negatively correlated with Aorta TBR_{MDS}, in line withmouse studies that have shownIL-37 to significantly attenuate arterial plaque formation, suggesting a protective role of IL-37 in atherosclerosis.[90-92]Additional studies are needed tounderstand the precise role of IL-37 on the mechanisms underlying cardiovascular disease.

Our study has limitations, including a younger aged cohort, limited sample size, lack of standardized protocols across published studies to assess vascular inflammation through 18-FDG PET-CT and circulating microparticles, and the inability to prove direct causality. Both modalities arealso only surrogate markers of vascular inflammation.However,observing similar results with two techniques strengthens our data. Finally, we did not assess for coronary atherosclerotic plaques with coronary computed tomography angiography/CCTA, which has prognostic value and is a measure of prospective cardiovascular outcomes.[93-95]CCTAhas been utilized in several psoriasis studies[96-98] andsuggested to be a more sensitive measure for evaluating cardiovascular changes associated with anti-inflammatory treatment.[99]Future studies in AD can consider leveraging multimodality imaging techniques to further characterize vascular inflammation in these patients.

Vascular inflammation in AD may be a mild phenomenon that worsens in persistent and severe patients. Thus, observing differences in young subjects can be difficult, particularly in a limited cohort, and might explain why some of our results only trended towards statistical significance. However, the presence of patients witha range of TBR_{mean}values [1.2-1.8], with 5 patients having TBR_{mean}> 1.6,allowed us to identify significant correlations between immune biomarkers and TBR measures.We further tested if these 5 patients had any differences in clinical cardiovascular risk factors (smoking, BMI, hypertension, dyslipidemia, and CRP), but found no significant differences in AD patients withTBR_{mean}>1.6 compared to TBR_{mean}<1.6. In contrast, vascular inflammation-related markers (IL-1a, IL-1RN, IL-36A, S100A8/S9) were significantlyincreased in the TBR_{mean}>1.6 group compared to TBR_{mean}<1.6, suggesting that the higher TBR mean in AD is associated with the systemic inflammation of this disease.In addition, as our AD cohort was of younger age and not otherwise at increased risk for cardiovascular disease, these data suggest that vascular inflammation in AD

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patients might beassociatedwith atype-2 driven response, which may alsoexplain cardiovascular events observed in select AD populations.[2,5,7,8,10-12,17]However, therelationship between cardiovascular disease and AD is likely complex and remains to be clearlyelucidated.[3]Additionally, the higher estimates of cardiovascular disease in select populations has been suggested to be driven by differences in behavioral, environmental, and cardiovascular risk factors.[14,17]Larger prospective studies are thus needed to further evaluate vascular inflammation as well as cardiovascular events and mortality in AD patients. Lastly, dupilumab treatment demonstrated significant modulation of atherosclerosis-related genes in AD patients compared to placebo, suggesting that future studies areneeded to investigatethe effects of systemic treatment on vascular inflammation in AD patients.

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Table 1. Baseline patient demographics

Characteristics	Healthy Controls	Atopic Dermatitis	P-value
Sample size (n)	26	30	
Age (mean. SD)	30 (13.6)	32 (16)	0.69
Gender			0.79
M	10	13	
F	16	17	
Body Mass Index (mean. SD)	24.9 (4.4)	25.1 (3.9)	0.83
Race/ethnicity			0.18
African American	3	2	
Asian	0	4	
Caucasian	23	24	
SCORAD (mean. SD)	na	51.8 (9.13)	
EASI (mean. SD)	na	21.8 (8.02)	
BSA (mean. SD)	na	38.0 (19.20)	
Active smoking			1
No	22	25	
Yes	4	5	
Dyslipidemia			0.67
No	24	26	
Yes	2	4	
Hypertension			
No	25	27	0.61
Yes	1	3	
CRP (mean. SD)	1.5 (1.29)	3.12 (3.49)	0.97

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FIGURE LEGENDS

Fig 1. Concentration of serum microparticles from atopic dermatitis patients and healthy subjects. The microparticles (MPs) isolated from the serum of 29 subjects with Atopic Dermatitis (AD) and 14 healthy controls (HC) were analyzed by flow cytometry. **A.** Scatter plots depict concentrations (MPs/μL) of Annexin V+ MPs, platelet- (PMPs), endothelial- (EMPs) and activated endothelial-derived MPs. **B.** Results after stratifying patients by clinical severity (*moderate*: SCORAD<50; *severe*: SCORAD>50) are also presented. Mean concentrations (red bar) +/- SD (boxplots) are also shown. Number of samples used for this analysis is shown below the graphs (n). *Black symbols*: significance of comparison to healthy controls. $^{**}P<0.01$, $^{*}P<0.05$, $^{+}P<0.1$

Fig 2. Atherosclerosis signaling pathway. **A.** Summary heatmap of differentially expressed atherosclerosis signaling genes in atopic dermatitis (AD) skin and healthy controls using RNA-seq with criteria of fold-change (|FCH|)>2 and false discovery rate (FDR)<0.05. Table shows FCHs in AD lesional and non-lesional versus normal (LS vs N, NL vs N) and lesional versus non-lesional (LS vs NL) comparisons. **B.** Mean z-score of atherosclerosis-related gene-set variation analysis pathway in lesional (LS) and non-lesional (NL) AD skin and healthy control (HC) skin. Number of samples used for this analysis is shown below the graphs (n). *Black symbols*: significance of comparison to healthy controls*; red symbols:* significance of comparison between lesional and non-lesional AD skin. $^{**}P<0.01$, $^{*}P<0.05$, $^{+}P<0.1$

Fig 3. Correlation heatmap. Heatmap of Pearson correlations between skin and blood biomarkers, Aorta TBR measures, microparticle levels, RNA-seq pathways, and SCORing Atopic Dermatitis (SCORAD). $^{***}P<0.001$, $^{**}P<0.01$, $^{*}P<0.05$, $^{+}P<0.1$. Red denotes positive and blue denotes negative correlations. Colored boxes denote correlation clusters with microparticles and serum levels of CCL26 (blue box) and AD-related skin and blood biomarkers, RNA-seq pathways, and Aorta TBR measures (green box).

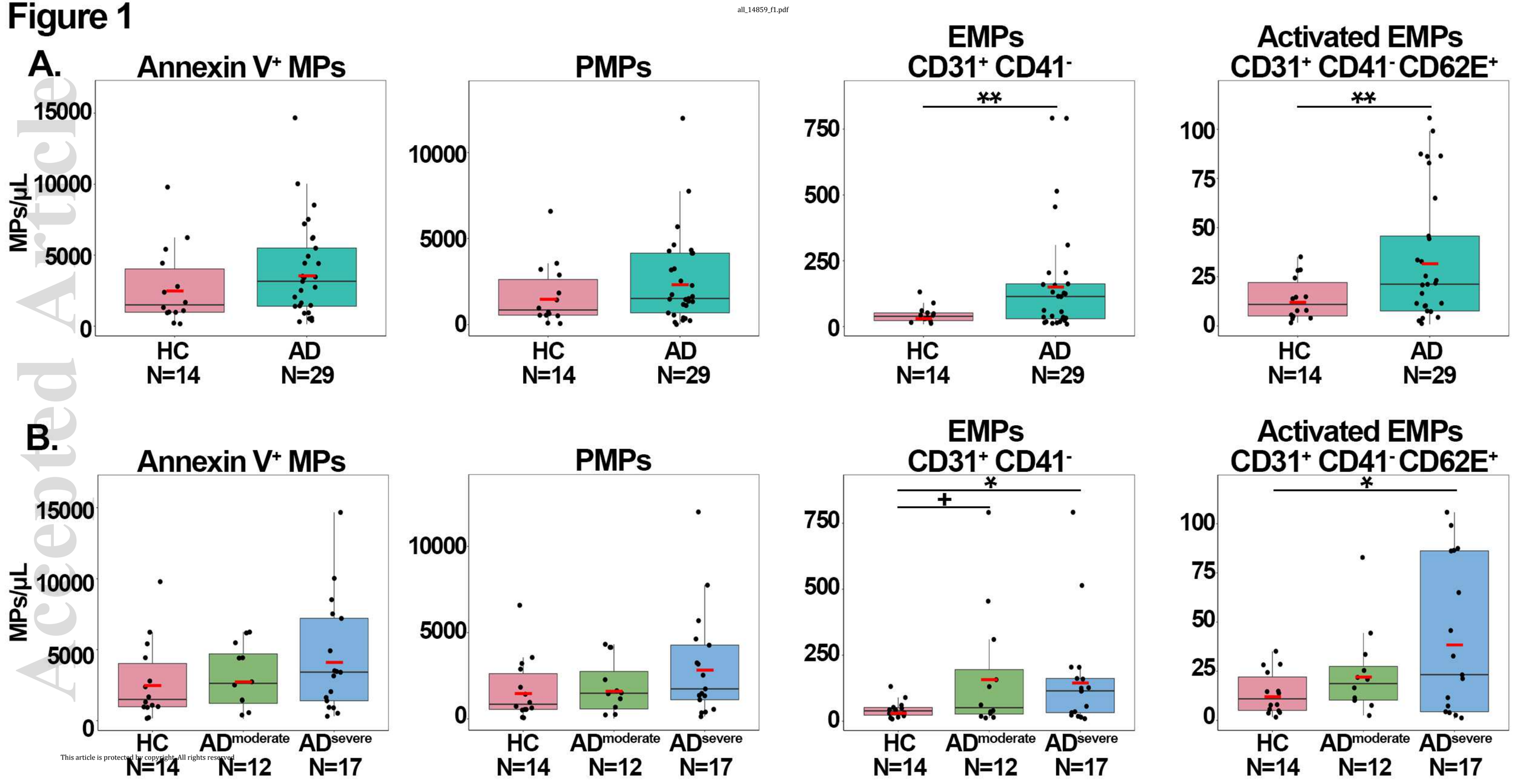
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Fig 4 A-C.Correlation scatterplots. Pearson correlation plots of serum IL-13, IFNγ, and the ratio of IL-13/IFNγ with Aorta TBR_{max}, TBR_{MDS}, and TBR_{mean}. Red dots indicate each AD patient and the red line represents the linear regression with their confidence intervals (gray-shaded areas). Blue dots indicate each control and the blue line represents the linear regression with their confidence intervals (gray-shaded areas). *r,* Pearson correlation coefficient with associated p-value. **P*<0.05.

Fig 5. Dupilumabanalysis. **A.** Summary heatmap depicting changes in atherosclerosis-related signaling genes in dupilumab-treated or placebo-treated groups in lesional and non-lesional AD skin at Weeks 0, 4, and 16. Table shows FCHs in dupilumab-treated or placebo-treated groups at Weeks 4 and 16 versus baseline, and FCHs in dupilumab versus placebo-treated groups at Weeks 4 and 16. ****P*<0.001, ***P*<0.01, **P*<0.05, ^{+}P<0.1 **B.** Overall percent improvement of atherosclerosis-related signaling genes in dupilumab- and placebo-treated groups at Weeks 4 and 16. **C.**Pearson correlation scatterplot of percent improvement in SCORing atopic dermatitis (SCORAD) and percent improvement in atherosclerosis. Red dots denote AD patients and the linear regression is represented as a blue line with its confidence interval (gray-shaded area). *r,*Pearson correlation coefficient with associated p-value. *P<0.05.

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Figure 1

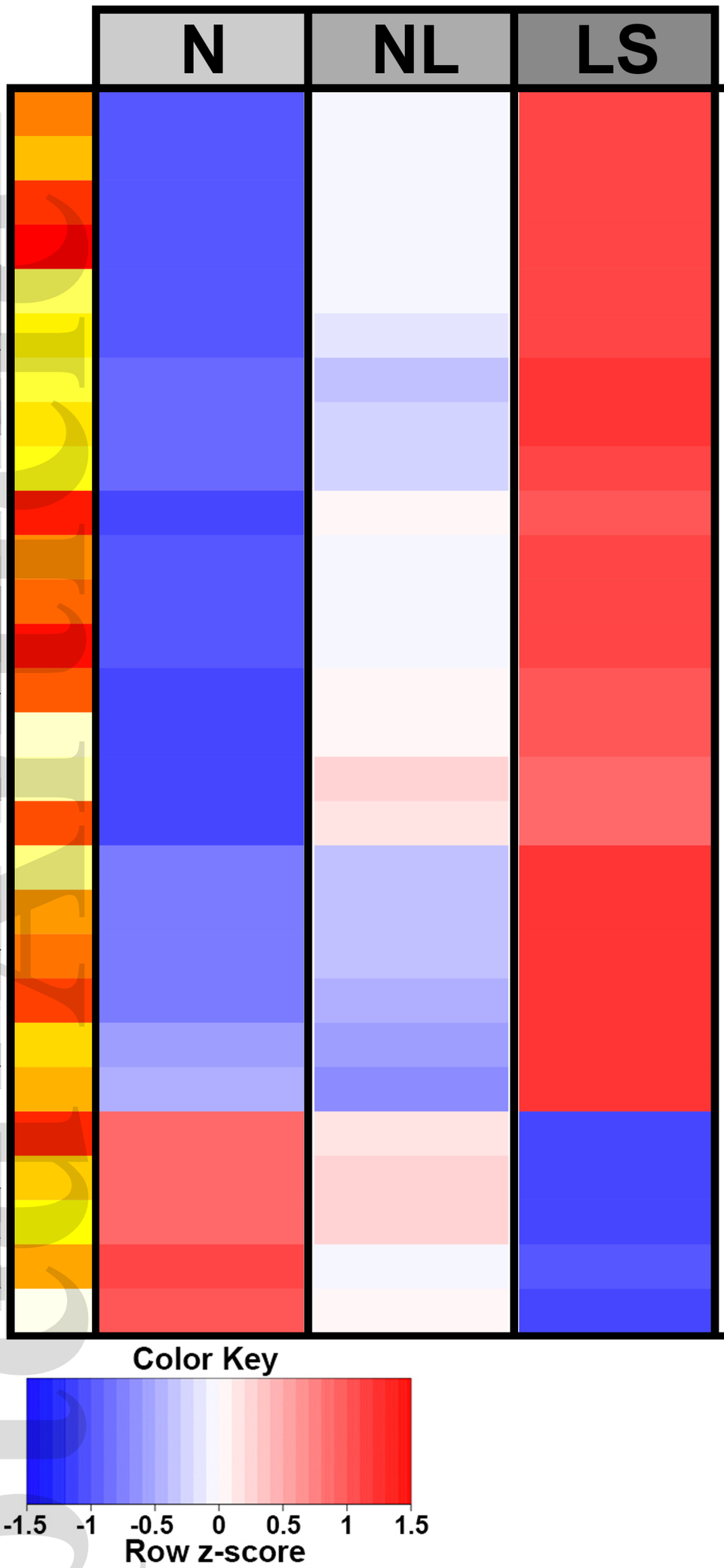


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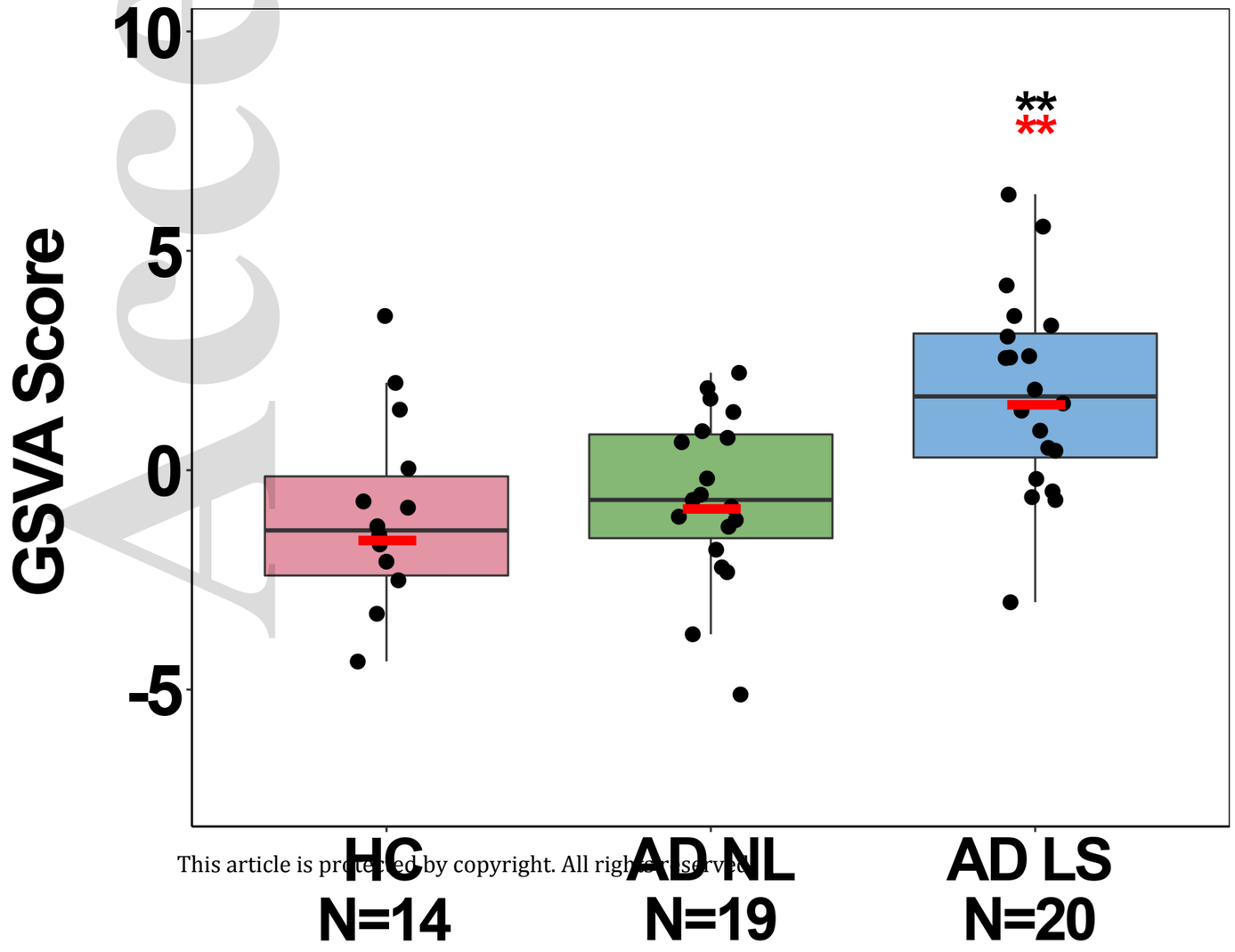
Figure 2

A.



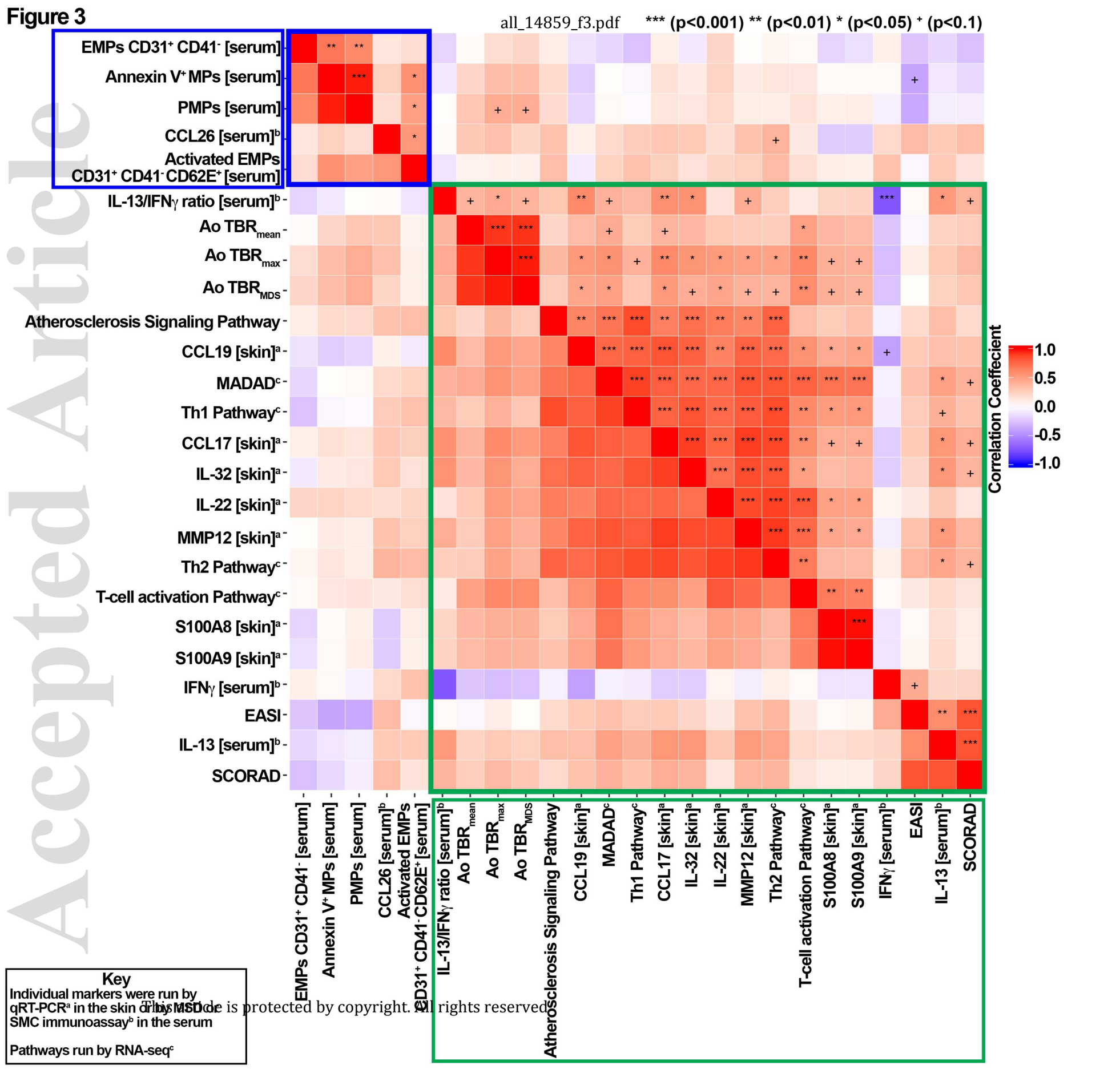
	NL vs N	LS vs N	LS vs NL
IL36RN -----:	1.53*	2.54**	1.66**
IL36G ------:	2.19**	5.56**	2.54**
PLA2G2A ----:	3.24*	14.40**	4.46**
IL36A ------:	5.42*	49.30**	9.10**
MMP1 -------:	7.29*	85.30**	11.70**
CCL2 -------:	1.94+	5.28**	2.72**
CD36 -------:	1.24	2.36**	1.90**
SELE -------:	1.78+	7.07**	3.98**
LYZ --------:	1.28	2.07**	1.62*
S100A8 -----:	18.80**	165.00**	8.80**
TNFRSF12A --:	1.65	2.82**	1.71*
APOL1 ------:	1.74*	3.14**	1.80**
IL8 --------:	4.12*	17.60**	4.27**
CXCR4 ------:	1.87*	3.28**	1.76**
IFNG -------:	1.52	2.20*	1.45
PLA2G2F ----:	1.94**	2.76**	1.42*
PLA2G3 -----:	1.73*	2.37**	1.37+
ICAM1 ------:	1.15	2.10**	1.83**
PLA2G4D ----:	1.21	2.62**	2.16**
IL6 --------:	1.48	7.58**	5.12**
SERPINA1 ---:	1.14	2.55**	2.23**
SELP -------:	-1.01	2.02**	2.03**
MMP9 -------:	-1.09	2.12**	2.31**
F3 ---------:	-1.62+	-3.84**	-2.37**
IL37 -------:	-1.96	-6.98**	-3.56**
APOC1 ------:	-1.57	-4.09**	-2.61*
APOE -------:	-2.00*	-3.88**	-1.94**
PNPLA3 -----:	-1.91*	-4.20**	-2.20**

B. Atherosclerosis Signaling Pathway



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Figure 3

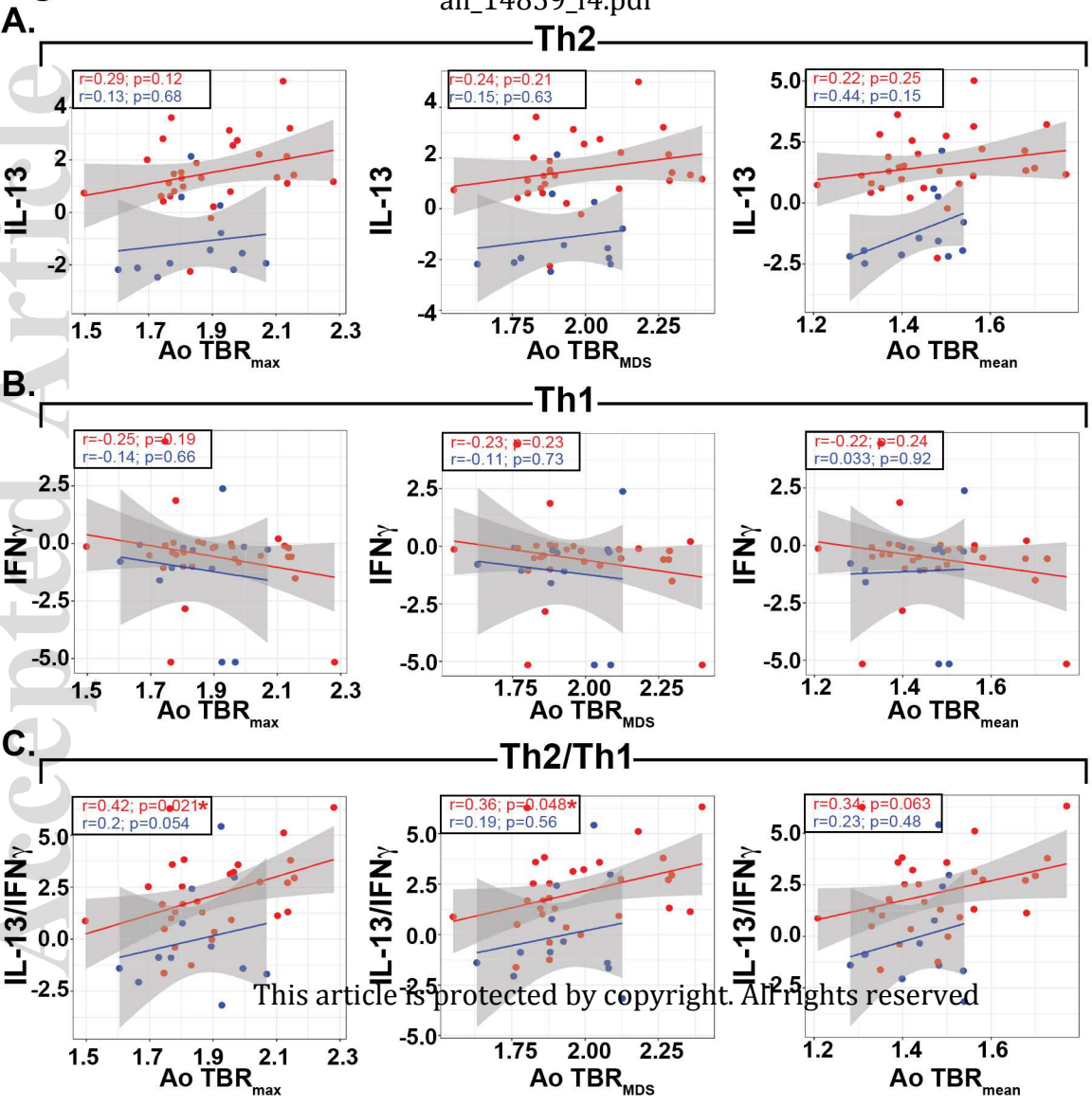


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Figure 4

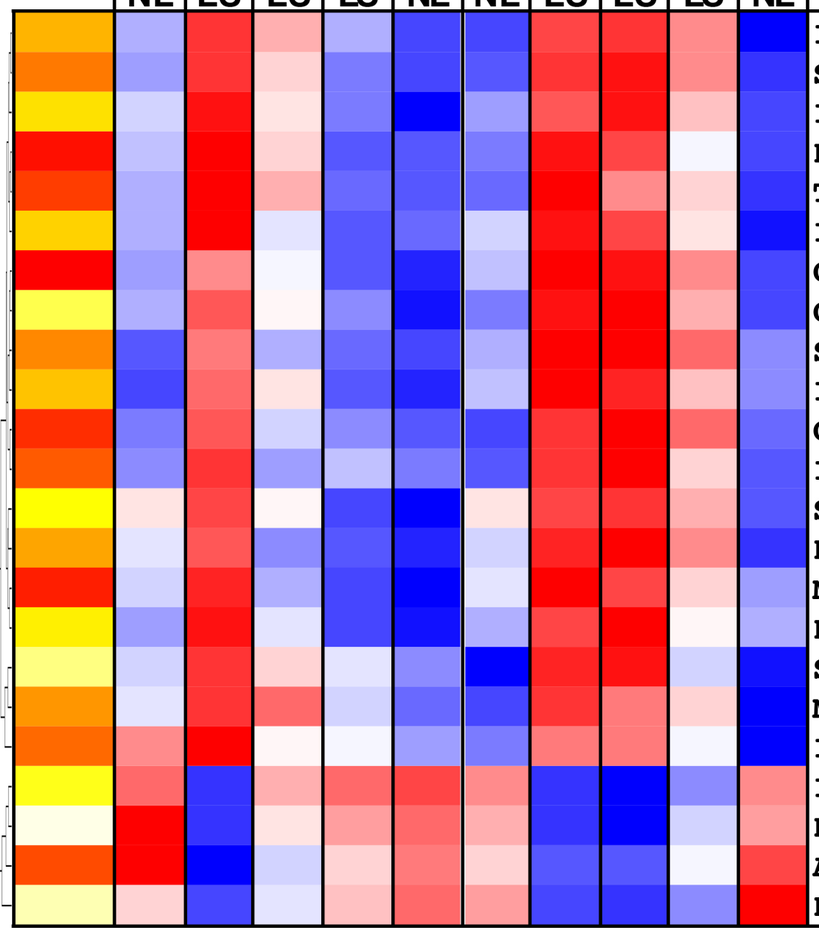
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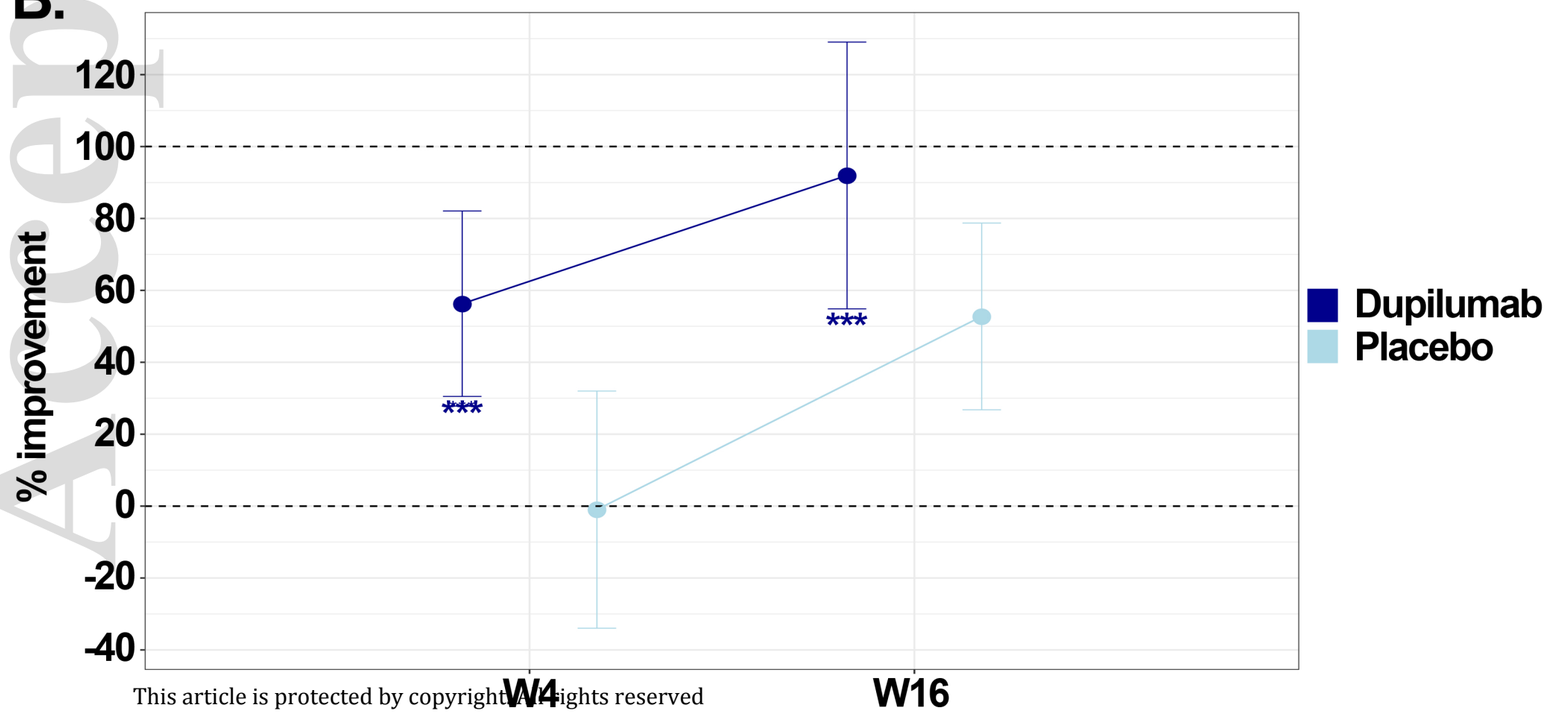
Figure 5

A.

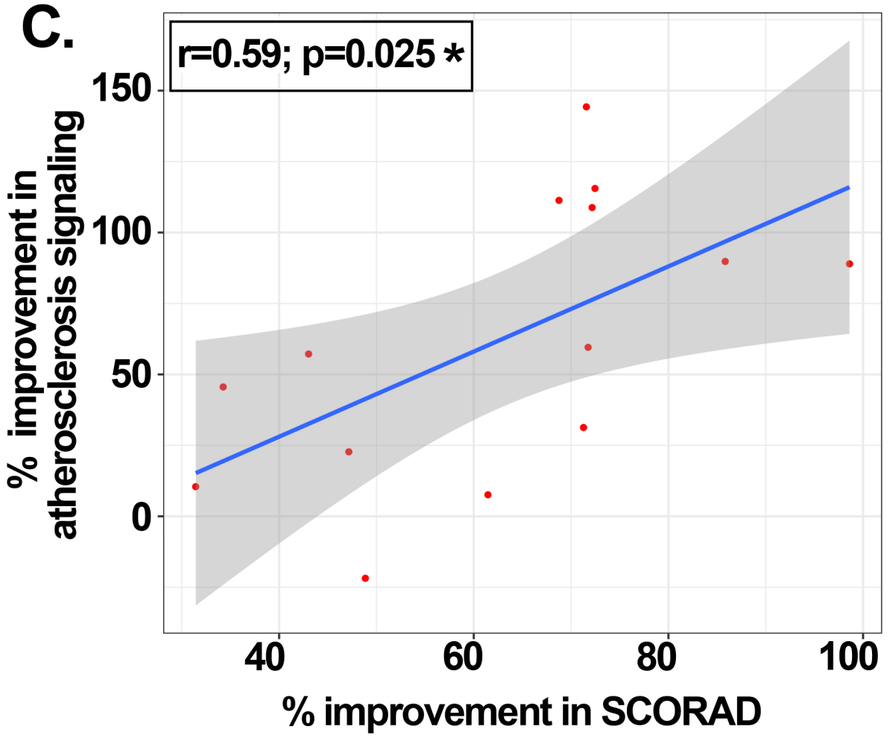


	Dupilumab W4 vs Baseline	Dupilumab W16 vs Baseline	Placebo W4 vs Baseline	Placebo W16 vs Baseline	Dupilumab vs Placebo W4	Dupilumab vs Placebo W16
ITGB2 ------:	-1.28+	-1.78***	1.01	-1.18	-1.30	-1.51+
SELPLG -----:	-1.45*	-2.10***	1.08	-1.20	-1.56*	-1.75*
IL36G ------:	-1.70***	-2.64***	1.23	-1.32	-2.09**	-1.99*
PLA2G3 -----:	-1.45**	-2.05***	-1.09	-1.49**	-1.33+	-1.37+
TNFRSF12A --:	-1.41*	-2.07***	-1.28+	-1.47*	-1.10	-1.41
IL6 --------:	-1.86*	-2.56**	-1.14	-1.60	-1.63	-1.61
CCL2 -------:	-1.31*	-1.83***	-1.05	-1.39+	-1.25	-1.32
CXCR4 ------:	-1.46*	-2.01***	1.08	-1.45	-1.58+	-1.39
SELE -------:	-1.74**	-2.11**	1.00	-1.33	-1.74+	-1.59
ICAM1 ------:	-1.34+	-2.04***	-1.15	-1.62*	-1.17	-1.26
CCR2 -------:	-1.60**	-1.87***	1.13	-1.16	-1.80*	-1.61+
IL36A ------:	-1.83**	-1.73*	1.28	-1.34	-2.34**	-1.29
S100A8 -----:	-1.48**	-2.28***	1.03	-1.25	-1.53*	-1.83**
PLA2G2A ----:	-2.49***	-2.93***	1.14	-1.39	-2.83**	-2.11+
MMP1 -------:	-10.20***	-22.90***	-1.61	-4.84**	-6.32**	-4.74*
PLA2G4D ----:	-2.18***	-3.35***	1.22	-1.66*	-2.65***	-2.02*
SERPINA1 ---:	-1.75*	-2.22**	1.08	-2.48**	-1.88+	1.12
MMP9 -------:	-1.19	-2.13**	-1.27	-1.63	1.06	-1.31
IL1RN ------:	-1.82**	-1.91**	-1.00	-1.38	-1.82*	-1.39
IL37 -------:	3.26***	4.28***	-1.28	1.42	4.18***	3.02*
PNPLA3 -----:	1.64*	1.94**	-1.13	1.47	1.86*	1.32
APOC1 ------:	1.72*	2.04**	-1.02	1.39	1.75	1.47
PLA2R1 -----:	1.31	1.55*	-1.02	1.16	1.34	1.33

B.



C.



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SUPPLEMENTARY TEXT

Supplementary Methods

Flow cytometry analysis – Microparticles

Recovered plasma was centrifuged for 15 min at 5000g at 4°C. Microparticles were labeled using FITC-conjugated Annexin V buffer (BD Biosciences, San Jose, CA), and counted and isolated with Megamix beads (Biocytex, Marseille, France). Microparticles were then stained with the following antibodies: CD41a-Pacific Blue, CD45-V500, CD106-BV605, CD54-BV711, CD14-QDot800, CD62E-PE, CD40L-PE Cf594, CD31-PECy7, CD146-APC, CD16-APC AF750. Samples were processed with LSRII and analyzed with FlowJo V10.

Serum immunoassays

Serum samples were stored at −80 °C after centrifugation. Electrochemiluminescence immunoassay using Meso Scale Discovery/MSDTM was used in a multiplex format to quantify the concentrations of ten chemokines (chemokine panel 1: CCL11/eotaxin, CCL26/eotaxin3, CCL2/MCP-1, CCL3/MIP1α, CCL4/MIP-1β, CXCL8/IL-8, CCL13/MCP-4, CCL22/MDC, CCL17/TARC, and CXCL10/IP-10)(Meso Scale Diagnostics©, Rockville, MD). We used Single Molecule Counting/SMCTM (EMD Millipore©, Darmstadt,Germany) to quantify human IL-13 (cat#03-0059-01), IL-22 (cat#03-0059-01), and IFN-γ (cat# 03-0049-00), as described. Quantikine ELISA kits (R&D Systems, Minneapolis, Minn) were used to quantify human S100A8/S100A9 Heterodimer concentrations (R&D Systems, cat#DS8900).

Table S1. Primers for qRT-PCR

Gene	Assay ID
MMP1	Hs00233958_m1
MMP12	Hs00899662_m1
MMP13	Hs00233992_m1
KRT16	Hs00955082_g1
IL10	Hs00961622_m1
IL13	Hs00174379_m1
IL17A	Hs00174383_m1
IL22	Hs01574154_m1
IL31	Hs01098710_m1
IL32	Hs00992441_m1
IFNG	Hs00989291_m1
CCL13	Hs00234646_m1
CCL17	Hs00171074_m1
CCL19	Hs00171149_m1
CCL20	Hs01011368_m1
CCL21	Hs00989654_g1
CCL26	Hs00171146_m1
S100A7	Hs00161488_m1
S100A8	Hs00374264_g1
S100A9	Hs00610058_m1
S100A12	Hs00194525_m1
PI3	Hs00160066_m1

Table S2. Cardiovascular risk factors in AD by Aorta TBR_{mean}

	Aorta TBR_{mean} <1.6 *(n=25)*	**Aorta TBR_{mean} ≥ 1.6** *(n=5)*	**P-value**
CVD risk factor			
BMI, mean (sd)	25.3 (3.8)	24.2 (4.7)	0.66
Smoking	4	1	1
Dyslipidemia	3	1	0.5
Hypertension	3	0	1
CRP, mean (sd)	3.3 (3.8)	2.3 (1.5)	0.3

Abbreviations: AD Atopic dermatitis; CVD cardiovascular disease

Table S6. Significantly increased vascular and inflammatory markers in AD patients with Aorta $TBR_{mean} \geq 1.6$ compared to Aorta $TBR_{mean} < 1.6$

Markers in AD lesions	Fold-change*	P-value
IL36A	5.33	0.003
CCL17	4.37	0.023
PI3	3.94	0.032
K16	3.36	0.0001
S100A8	2.77	0.007
IL1A	2.70	0.011
TNFRSF12A	2.65	0.011
S100A9	2.55	0.020
PLA2G3	1.57	0.029
NFKB1	1.52	0.009
PDGFC	1.43	0.036
IL1RN	1.42	0.007

Abbreviations: AD Atopic dermatitis

**Fold-change comparing Aorta $TBR_{mean} \geq 1.6$ versus Aorta $TBR_{mean} < 1.6$*

Supplementary Figure Legends

Fig S1. Vascular inflammation of thoracic Aorta (Ao) assessed by 18FDG PET/CT in atopic dermatitis (AD) patients and healthy controls (HC). Target-to-Background Ratio (TBR) scores are reported as **A.** most-diseased segment (TBR_{MDS}), **B.** maximum (TBR_{Max}), and **C.** mean (TBR_{Mean}). Red bars indicate the mean values. Number of samples used for this analysis is shown below the graphs (n). Patients with a TBR_{mean} value $\geq$ 1.6 are highlighted (dotted red box).

Fig S2. Gating strategy used for flow cytometric analysis of platelet- (PMPs) and endothelial-derived microparticles (EMPs). Representative example of the gating strategy used to identify microparticles isolated from the serum of atopic dermatitis (AD) patients and healthy controls (HC). Microparticles were identified using size beads and *via* SSC-H/Annexin V. Debris were removed *via* SSC-H/FSC-H. Whole microparticles were further gated using Annexin V expression. Microparticles were defined as PMPs (Annexin V+ CD41+), EMPs (Annexin V+ CD41- CD31+) and activated EMPs (Annexin V+ CD41- CD31+ CD62E+).

Fig S3. Summary heatmap of all differentially expressed genes in normal (N), and lesional (LS) and non-lesional (NL) AD skin using RNA-seq with criteria of fold-change (|FCH|)>2 and false discovery rate (FDR)<0.05.

Fig S4. A. Principal component analysis plot of RNA-seq transcriptomic data. Green circle depicts cluster of AD lesional genes, pink circle depicts AD non-lesional genes, and blue circle depicts controls. **B.** Summary heatmap of immune genes in normal (N), and AD non-lesional (NL) and lesional (LS) skin using RNA-seq with criteria of fold change (|FCH|)>2 and false discovery rate (FDR)<0.05. Table shows FCHs in non-lesional versus normal (NL vs N), lesional versus normal (LS vs N), and lesional vs lesional (LS vs LS) skin. **FDR<0.01, *FDR<0.05, $^{+}$FDR<0.1.

Fig S5. Immune gene signatures. Mean z-scores of immune-related gene-set variation analysis pathways in lesional (LS) and non-lesional (NL) atopic dermatitis (AD) skin as well as healthy control skin (HC). Number of samples used for this analysis is shown below the graphs (n). *Black symbols*: significance of comparison to healthy controls*; red symbols:* significance of comparison between lesional and non-lesional AD skin. ***P<0.001, **P<0.01, *P<0.05, ^{+}P<0.1

Fig S6. Immune and epidermal hyperplasia markers. Fold-changes of immune and barrier markers detected in lesional (LS) and non-lesional (NL) atopic dermatitis (AD) skin and healthy control (HC) skin, as measured by quantitative real-time PCR. Number of samples used for this analysis is shown below the graphs (n). *Black symbols*: significance of comparison to healthy controls*; red symbols:* significance of comparison between lesional and non-lesional AD skin. ***P<0.001, **P<0.01, *P<0.05, ^{+}P<0.1

Fig S7. Immune markers in serum. Concentrations of immune markers in atopic dermatitis (AD) patients and healthy controls (HC). *Black symbols*: significance of comparison to healthy controls. ***P<0.001, **P<0.01, *P<0.05, ^{+}P<0.1

Fig S8. Correlation scatterplots. Pearson correlation plots of atherosclerosis-related skin markers of various immune gene pathways and Aorta TBR_{MDS}. Red dots indicate each AD patient and the red line represents the linear regression with their confidence intervals (gray-shaded areas). Blue dots indicate each control and the blue line represents the linear regression with their confidence intervals (gray-shaded areas). *r,* Pearson correlation coefficient with associated p-value. *P<0.05.

Figure S1

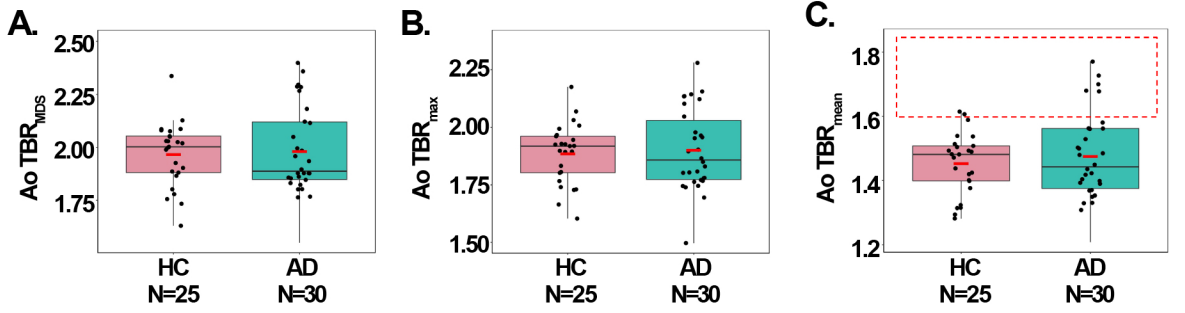

A.
Ao TBR$_{MDS}$
2.50
2.25
2.00
1.75
HC
N=25
AD
N=30
B.
Ao TBR$_{max}$
2.25
2.00
1.75
1.50
HC
N=25
AD
N=30
C.
Ao TBR$_{mean}$
1.8
1.6
1.4
1.2
HC
N=25
AD
N=30

Figure S2

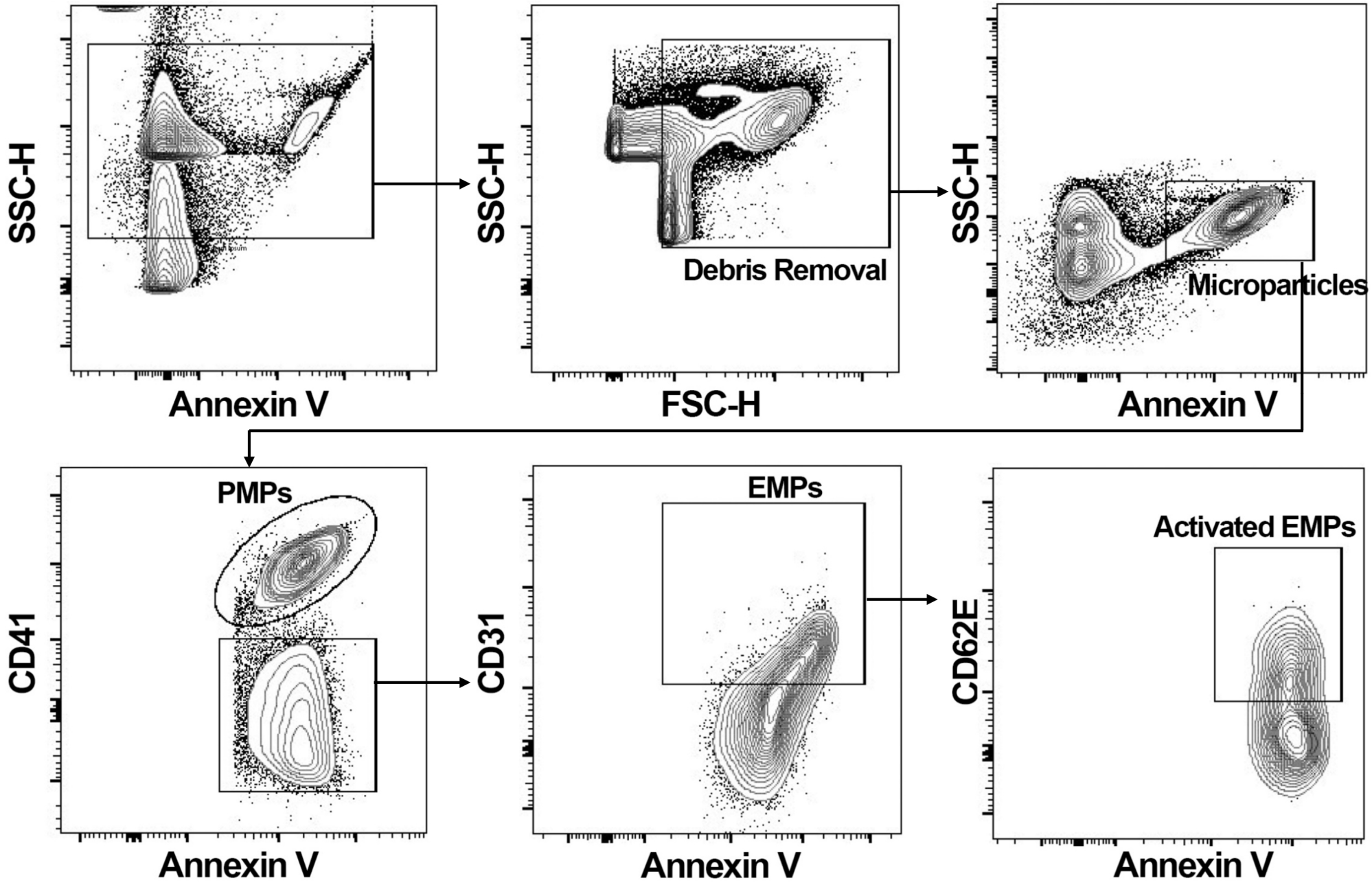

SSC-H
Annexin V
SSC-H
FSC-H
Debris Removal
SSC-H
Annexin V
Microparticles
PMPs
CD41
Annexin V
CD31
EMPs
Annexin V
CD62E
Activated EMPs
Annexin V

Figure S3

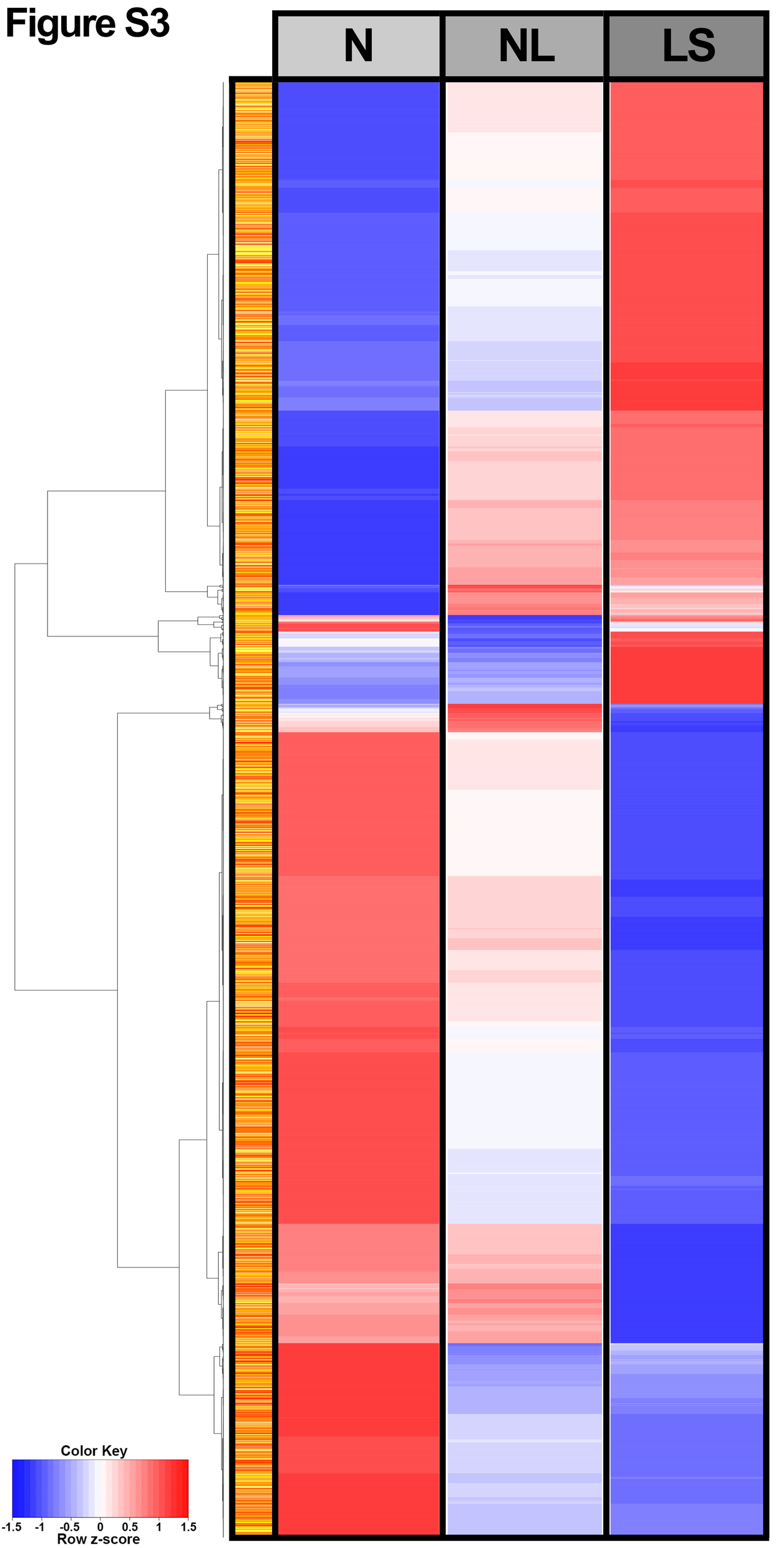
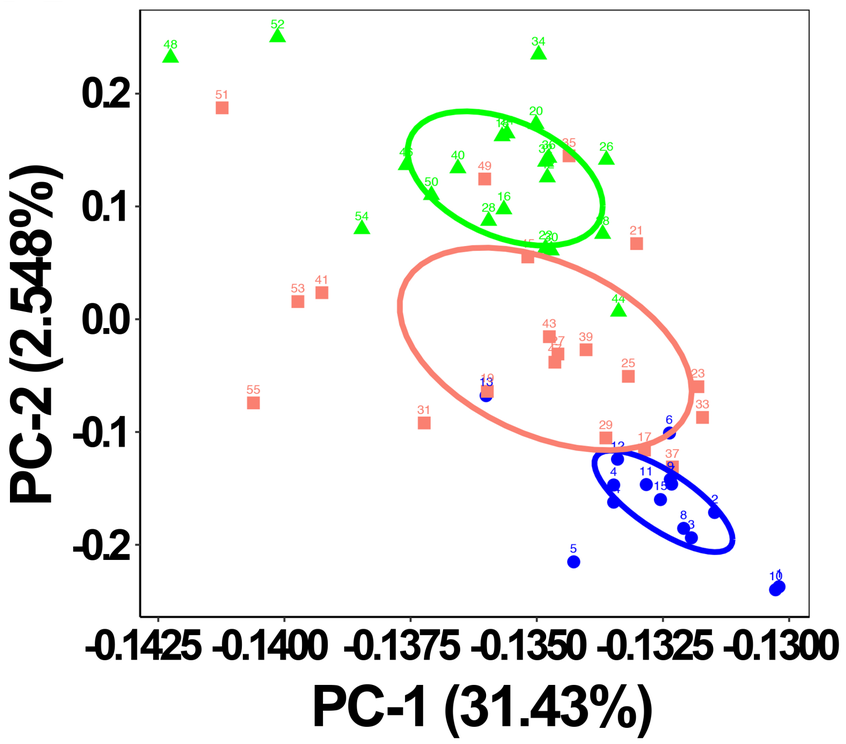
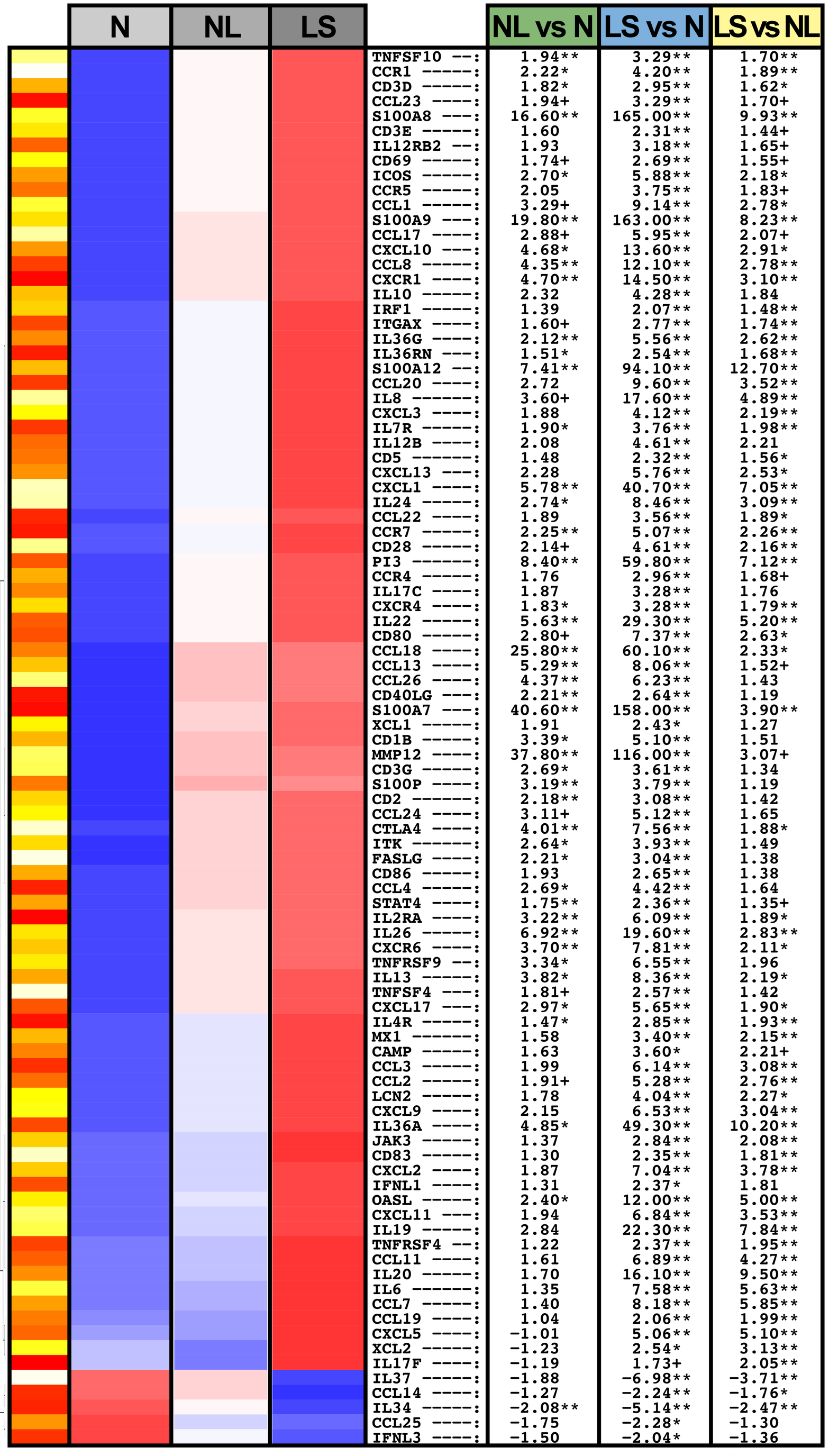


Figure S4

A.



B.



	NL vs N	LS vs N	LS vs NL
TNFSF10 --:	1.94**	3.29**	1.70**
CCR1 -----:	2.22*	4.20**	1.89**
CD3D -----:	1.82*	2.95**	1.62*
CCL23 ----:	1.94+	3.29**	1.70+
S100A8 ---:	16.60**	165.00**	9.93**
CD3E -----:	1.60	2.31**	1.44+
IL12RB2 --:	1.93	3.18**	1.65+
CD69 -----:	1.74+	2.69**	1.55+
ICOS -----:	2.70*	5.88**	2.18*
CCR5 -----:	2.05	3.75**	1.83+
CCL1 -----:	3.29+	9.14**	2.78*
S100A9 ---:	19.80**	163.00**	8.23**
CCL17 ----:	2.88+	5.95**	2.07+
CXCL10 ---:	4.68*	13.60**	2.91*
CCL8 -----:	4.35**	12.10**	2.78**
CXCR1 ----:	4.70**	14.50**	3.10**
IL10 -----:	2.32	4.28**	1.84
IRF1 -----:	1.39	2.07**	1.48**
ITGAX ----:	1.60+	2.77**	1.74**
IL36G ----:	2.12**	5.56**	2.62**
IL36RN ---:	1.51*	2.54**	1.68**
S100A12 --:	7.41**	94.10**	12.70**
CCL20 ----:	2.72	9.60**	3.52**
IL8 ------:	3.60+	17.60**	4.89**
CXCL3 ----:	1.88	4.12**	2.19**
IL7R -----:	1.90*	3.76**	1.98**
IL12B ----:	2.08	4.61**	2.21
CD5 ------:	1.48	2.32**	1.56*
CXCL13 ---:	2.28	5.76**	2.53*
CXCL1 ----:	5.78**	40.70**	7.05**
IL24 -----:	2.74*	8.46**	3.09**
CCL22 ----:	1.89	3.56**	1.89*
CCR7 -----:	2.25**	5.07**	2.26**
CD28 -----:	2.14+	4.61**	2.16**
PI3 ------:	8.40**	59.80**	7.12**
CCR4 -----:	1.76	2.96**	1.68+
IL17C ----:	1.87	3.28**	1.76
CXCR4 ----:	1.83*	3.28**	1.79**
IL22 -----:	5.63**	29.30**	5.20**
CD80 -----:	2.80+	7.37**	2.63*
CCL18 ----:	25.80**	60.10**	2.33*
CCL13 ----:	5.29**	8.06**	1.52+
CCL26 ----:	4.37**	6.23**	1.43
CD40LG ---:	2.21**	2.64**	1.19
S100A7 ---:	40.60**	158.00**	3.90**
XCL1 -----:	1.91	2.43*	1.27
CD1B -----:	3.39*	5.10**	1.51
MMP12 ----:	37.80**	116.00**	3.07+
CD3G -----:	2.69*	3.61**	1.34
S100P ----:	3.19**	3.79**	1.19
CD2 ------:	2.18**	3.08**	1.42
CCL24 ----:	3.11+	5.12**	1.65
CTLA4 ----:	4.01**	7.56**	1.88*
ITK ------:	2.64*	3.93**	1.49
FASLG ----:	2.21*	3.04**	1.38
CD86 -----:	1.93	2.65**	1.38
CCL4 -----:	2.69*	4.42**	1.64
STAT4 ----:	1.75**	2.36**	1.35+
IL2RA ----:	3.22**	6.09**	1.89*
IL26 -----:	6.92**	19.60**	2.83**
CXCR6 ----:	3.70**	7.81**	2.11*
TNFRSF9 --:	3.34*	6.55**	1.96
IL13 -----:	3.82*	8.36**	2.19*
TNFSF4 ---:	1.81+	2.57**	1.42
CXCL17 ---:	2.97*	5.65**	1.90*
IL4R -----:	1.47*	2.85**	1.93**
MX1 ------:	1.58	3.40**	2.15**
CAMP -----:	1.63	3.60*	2.21+
CCL3 -----:	1.99	6.14**	3.08**
CCL2 -----:	1.91+	5.28**	2.76**
LCN2 -----:	1.78	4.04**	2.27*
CXCL9 ----:	2.15	6.53**	3.04**
IL36A ----:	4.85*	49.30**	10.20**
JAK3 -----:	1.37	2.84**	2.08**
CD83 -----:	1.30	2.35**	1.81**
CXCL2 ----:	1.87	7.04**	3.78**
IFNL1 ----:	1.31	2.37*	1.81
OASL -----:	2.40*	12.00**	5.00**
CXCL11 ---:	1.94	6.84**	3.53**
IL19 -----:	2.84	22.30**	7.84**
TNFRSF4 --:	1.22	2.37**	1.95**
CCL11 ----:	1.61	6.89**	4.27**
IL20 -----:	1.70	16.10**	9.50**
IL6 ------:	1.35	7.58**	5.63**
CCL7 -----:	1.40	8.18**	5.85**
CCL19 ----:	1.04	2.06**	1.99**
CXCL5 ----:	-1.01	5.06**	5.10**
XCL2 -----:	-1.23	2.54*	3.13**
IL17F ----:	-1.19	1.73+	2.05**
IL37 -----:	-1.88	-6.98**	-3.71**
CCL14 ----:	-1.27	-2.24**	-1.76*
IL34 -----:	-2.08**	-5.14**	-2.47**
CCL25 ----:	-1.75	-2.28*	-1.30
IFNL3 ----:	-1.50	-2.04*	-1.36

Figure S5

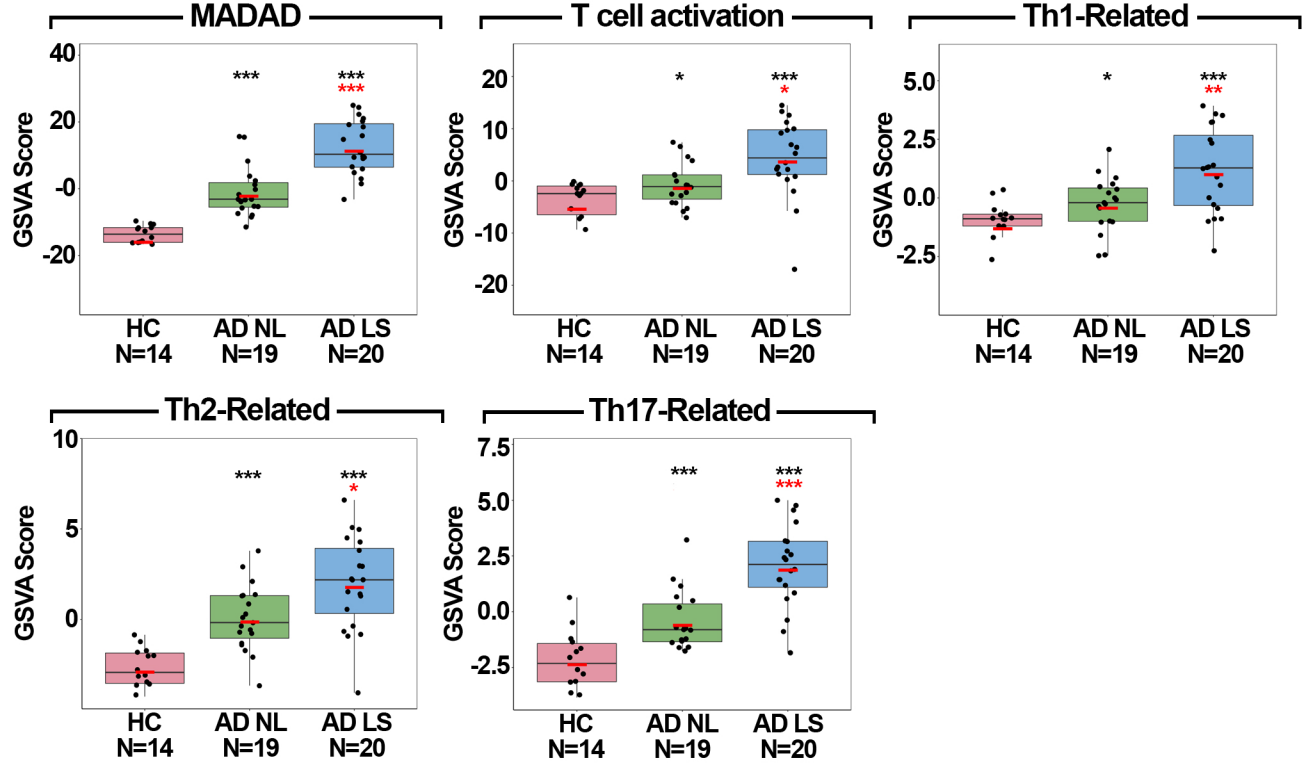

MADAD
T cell activation
Th1-Related
Th2-Related
Th17-Related
GSVA Score
HC N=14
AD NL N=19
AD LS N=20

Figure S6

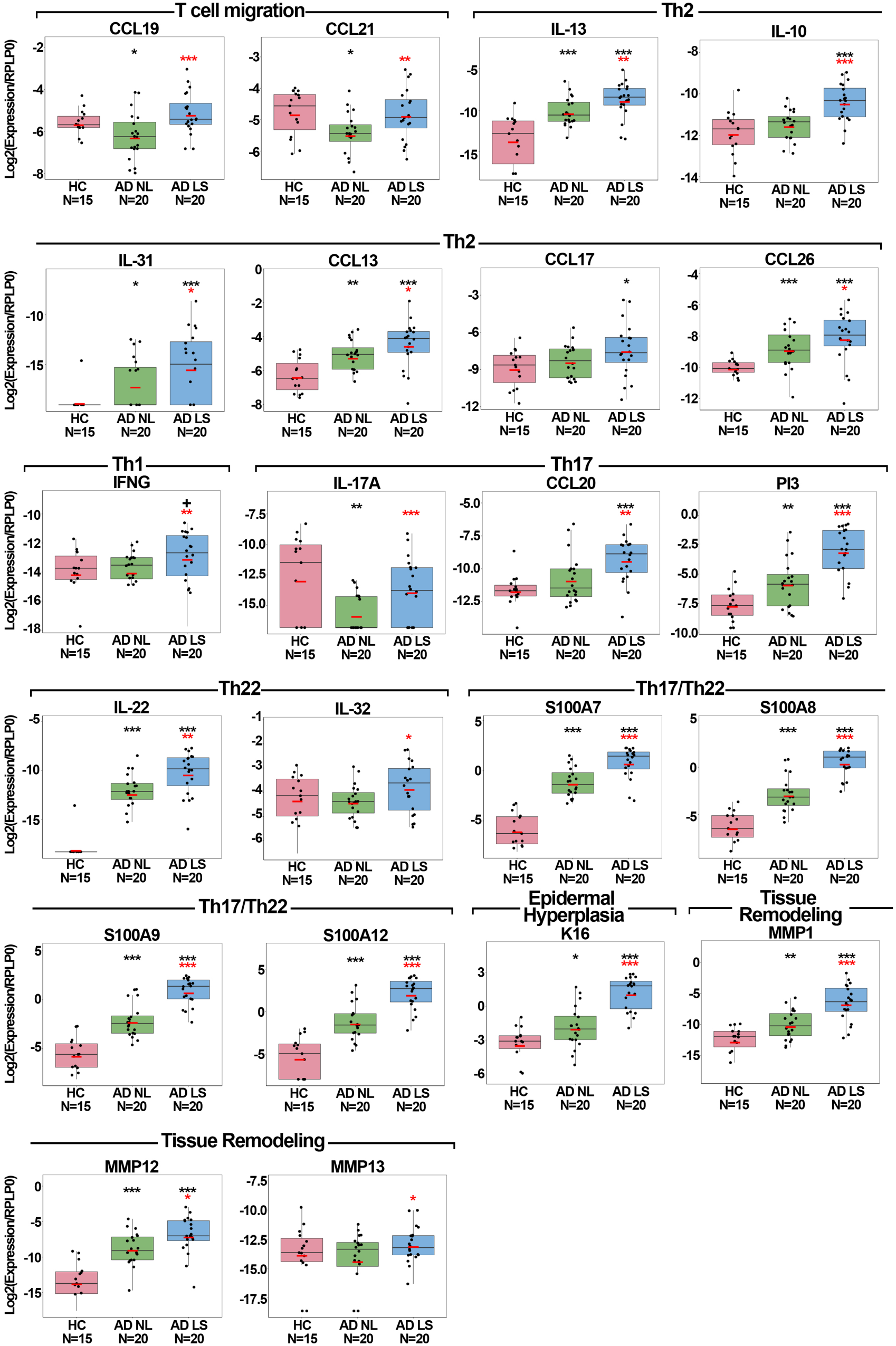

T cell migration
CCL19
CCL21
Th2
IL-13
IL-10
Th2
IL-31
CCL13
CCL17
CCL26
Th1
IFNG
Th17
IL-17A
CCL20
PI3
Th22
IL-22
IL-32
Th17/Th22
S100A7
S100A8
Th17/Th22
S100A9
S100A12
Epidermal Hyperplasia
K16
Tissue Remodeling
MMP1
Tissue Remodeling
MMP12
MMP13
Log2(Expression/RPLP0)
HC N=15
AD NL N=20
AD LS N=20

Figure S7

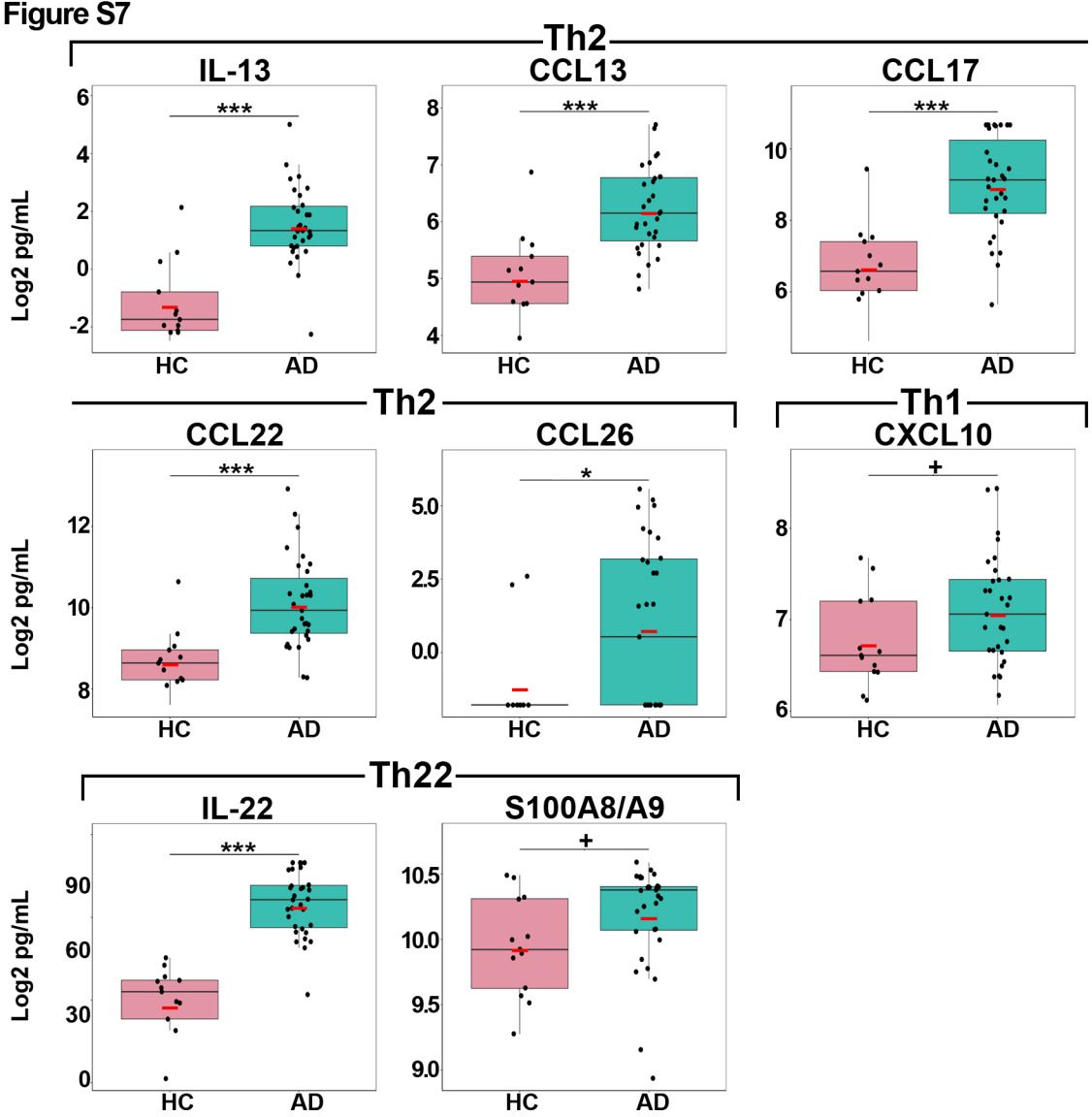
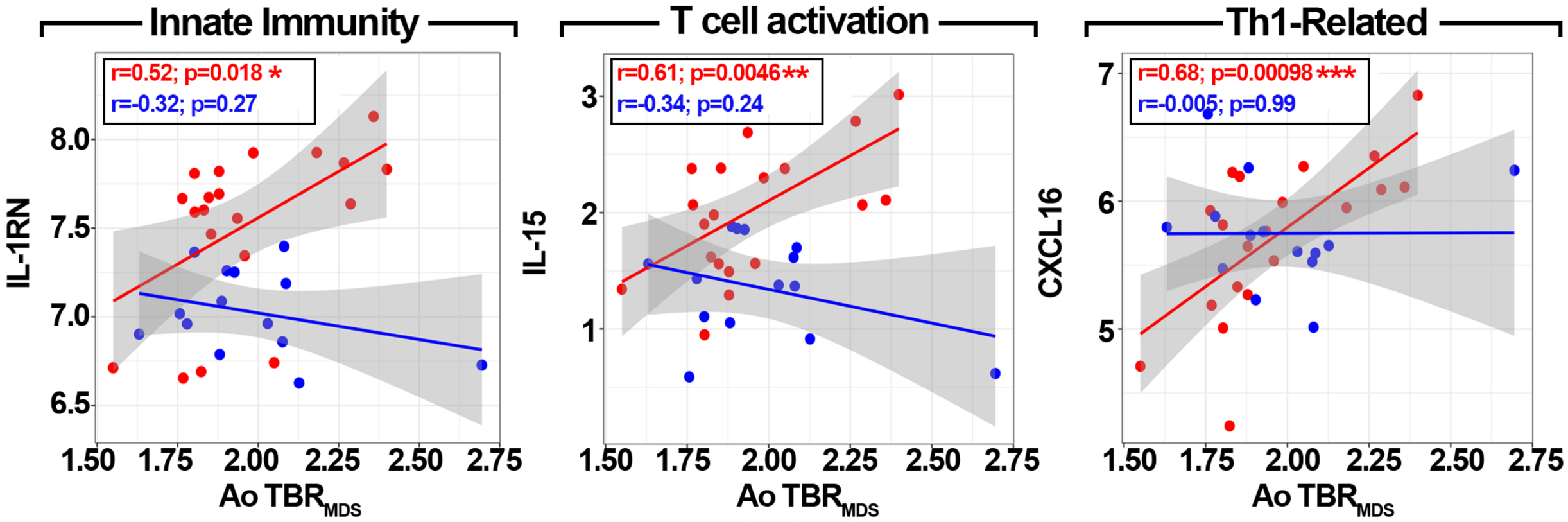
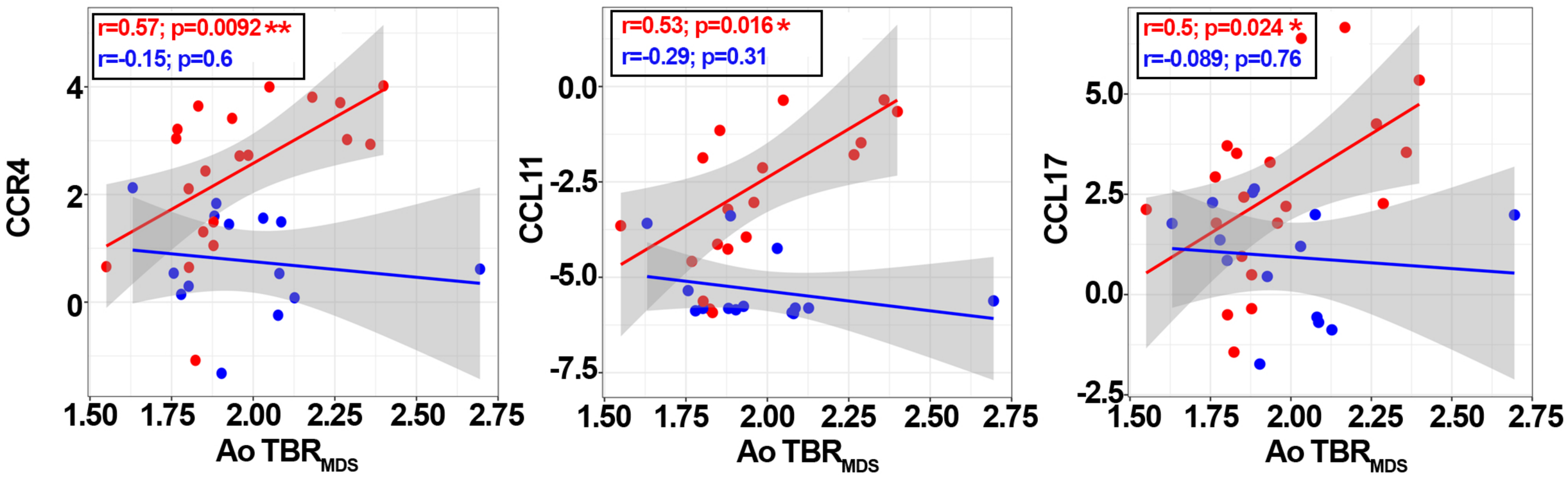
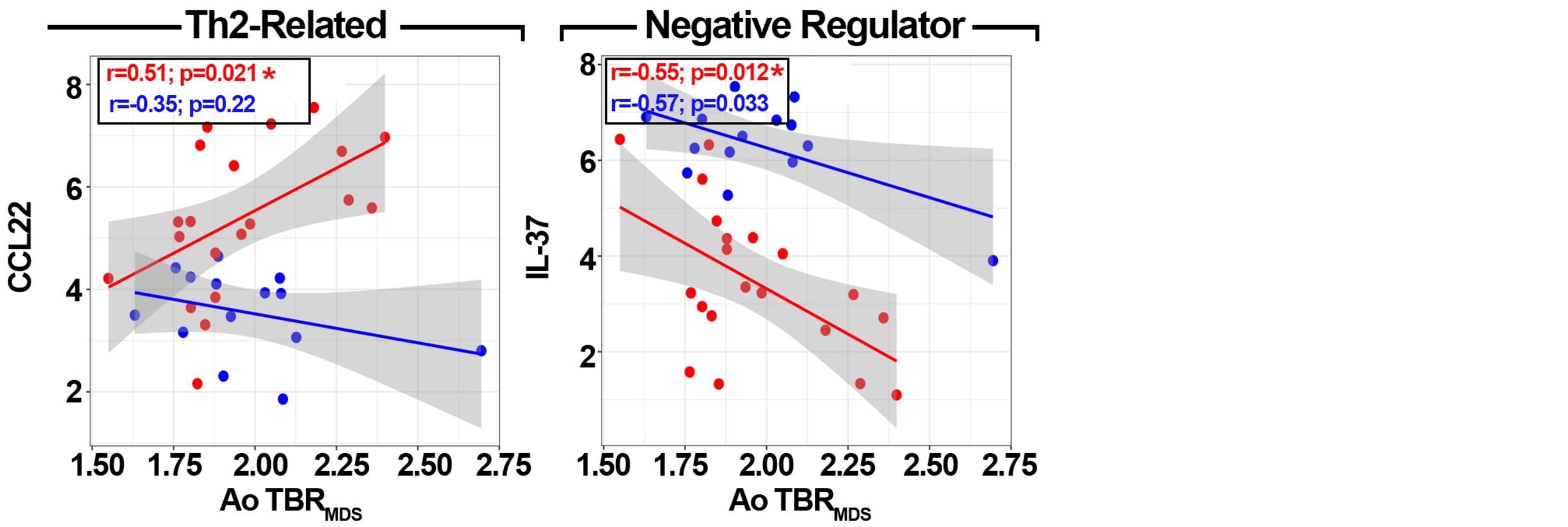

Th2
IL-13
CCL13
CCL17
Log2 pg/mL
***
HC
AD
Th2
Th1
CCL22
CCL26
CXCL10
*
+
Th22
IL-22
S100A8/A9

Figure S8


Innate Immunity
r=0.52; p=0.018 *
r=-0.32; p=0.27
IL-1RN
T cell activation
r=0.61; p=0.0046**
r=-0.34; p=0.24
IL-15
Th1-Related
r=0.68; p=0.00098***
r=-0.005; p=0.99
CXCL16
Ao TBR_MDS


Th2-Related
r=0.57; p=0.0092**
r=-0.15; p=0.6
CCR4
r=0.53; p=0.016*
r=-0.29; p=0.31
CCL11
r=0.5; p=0.024 *
r=-0.089; p=0.76
CCL17
Ao TBR_MDS


Th2-Related
r=0.51; p=0.021 *
r=-0.35; p=0.22
CCL22
Negative Regulator
r=-0.55; p=0.012*
r=-0.57; p=0.033
IL-37
Ao TBR_MDS