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ORIGINAL ARTICLE

Atopic Dermatitis, Urticaria and Skin Disease

Tape strips from early-onset pediatric atopic dermatitis highlight disease abnormalities in nonlesional skin

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Abstract

Background: Skin biopsies promote our understanding of atopic dermatitis/AD pathomechanisms in infants/toddlers with early-onset AD, but are not feasible in pediatric populations. Tape strips are an emerging, minimally invasive alternative, but global transcriptomic profiling in early pediatric AD is lacking. We aimed to provide global lesional and nonlesional skin profiles of infants/toddlers with recent-onset, moderate-to-severe AD using tape strips.

Methods: Sixteen tape strips were collected for RNA-seq profiling from 19 infants/toddlers (<5 years old; lesional and nonlesional) with early-onset moderate-to-severe AD (≤6 months) and 17 healthy controls.

Results: We identified 1829 differentially expressed genes/DEGs in lesional AD and 662 DEGs in nonlesional AD, vs healthy skin (fold-change ≥2, FDR <0.05), with 100% sample recovery. Both lesional and nonlesional skin showed significant dysregulations of Th2 (CCL17 and IL4R) and Th22/Th17 (IL36G, CCL20, and S100As)-related genes, largely lacking significant Th1-skewing. Significant down-regulation of terminal differentiation (FLG and FLG2), lipid synthesis/metabolism (ELOVL3 and FA2H), and tight junction (CLDN8) genes were primarily seen in lesional AD. Significant negative correlations were identified between Th2 measures and epidermal barrier gene-subsets and individual genes (FLG with IL-4R and CCL17; $r < -0.4$, $P < .05$). Significant correlations were also identified between clinical measures (body surface area/BSA, pruritus ADQ, and transepidermal water loss/TEWL) with immune and barrier mRNAs in lesional and/or nonlesional AD (FLG/FLG2 with TEWL; $r < -0.4$, $P < .05$).

Abbreviations: AD, atopic dermatitis; ADQ, pruritus atopic dermatitis quickscore; CLDN, claudin; DEG, differentially expressed gene; EASI, eczema area and severity index; ELOVL, elongation of very long chain fatty acids protein; FA2H, fatty acid 2-hydroxylase; FCH, fold-change; FDR, false discovery rate; FLG, filaggrin; GSVA, gene set variation analysis; IL, interleukin; LCE, late cornified envelope; OR, online repository; qRT-PCR, quantitative real-time polymerase chain reaction; RNA-seq, RNA-sequencing; SCORAD, scoring atopic dermatitis; STAT, signal transducer and activator of transcription; TEWL, transepidermal water loss; Th, T helper.

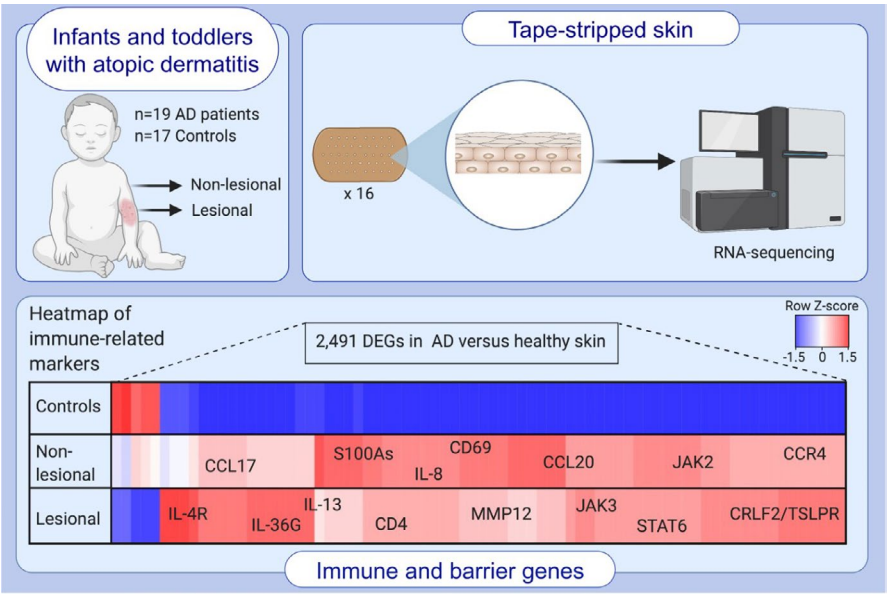
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Conclusion: RNA-seq profiling using tape strips in early-onset pediatric AD captures immune and barrier alterations in both lesional and nonlesional skin. Tape strips provide insight into disease pathomechanisms and cutaneous disease activity.

KEYWORDS

atopic dermatitis, biomarker, pediatric, skin barrier, tape strips



GRAPHICAL ABSTRACT

Tape strips are collected for RNA-seq profiling from 19 infants/toddlers (ages 0-5 years; lesional and nonlesional) with early-onset (≤6 months), moderate-to-severe AD and 17 healthy controls. We identify 2491 differentially expressed genes in AD vs healthy skin. We observe significant dysregulation of Th2 and Th22/Th17-related markers, largely lacking Th1 skewing. Significant decreases in barrier-related genes are seen primarily in lesional skin.

1 | INTRODUCTION

Atopic dermatitis/AD is the most common inflammatory skin disease in children, yet effective treatments in this population are lacking.[1] Until recently, comprehensive studies in skin of children at early disease initiation were lacking, inhibiting targeted therapeutic development for this age group. Studies using skin biopsies in infants and toddlers with early-onset moderate-to-severe AD determined the cutaneous phenotype of early-onset pediatric AD (within 6 months of disease initiation), showing differences from that of adults with long-standing disease.[2,3] Although Th2/Th22-dominance is shared by children and adults with AD, early-onset disease in infants and toddlers is also characterized by significant Th17 polarization and lacks the Th1-skewing that is characteristic of adults with chronic AD.[2-5] While in adults, whole-skin tissues have been instrumental in identifying biomarkers to track cutaneous disease severity and therapeutic response,[6-11] biopsies are largely not feasible in children. Furthermore, studies in blood are not able to capture the distinct immune and barrier profiles of lesional and non-lesional AD skin.[12-14]

Lately, therapeutics are actively being developed in infants and children[15-17] and several studies are following babies from birth for development of AD and associated comorbidities.[18-20] Hence, there is a great need to develop minimally invasive approaches that allow for serial sampling of both lesional and nonlesional skin of early-onset AD in infants and children for tracking disease activity in clinical trials and longitudinal studies.

Tape strips, capturing the stratum corneum and upper granular layer,[21,22] are emerging as an alternative to skin biopsies in both adults and children with AD.[21-46] Earlier proteomic studies in infants and children with AD evaluated a limited set of biomarkers in lesional and/or nonlesional skin.[23,33] We recently studied the mRNA profile in lesional and nonlesional tape-stripped skin of infants and toddlers (age 0-5 years; mean age 1.7 years) with early-onset AD (≤6 months of disease initiation), using a panel of 79 genes by quantitative real-time PCR/qRT-PCR.[46]

Only a few studies have performed global profiling of lesional and/or nonlesional tape-stripped AD skin using RNA-seq, including two studies on a cohort of adults with mild-to-severe AD[21,34] and one in children and adolescents.[24] The latter involved 4- to

17-year-olds with mild-to-severe AD, primarily analyzing nonlesional skin compared to skin from healthy controls, focusing on differences between children with and without food allergy.[24] This study showed higher Th2-skewing in the setting of concomitant food allergy and an overall sample recovery rate of 45%.[24]

We present the first global profiling of both lesional and nonlesional tape-stripped skin of infants and toddlers (age 0-5 years) with early-onset AD (≤6 months), as compared to age-appropriate controls. We obtained a sample recovery rate of 100%, capturing an immune and barrier phenotype that mirrors prior skin biopsy reports in similar populations.[2,3] Our results highlight vast abnormalities in nonlesional AD skin at early disease initiation, largely resembling lesional skin, and detect greater barrier disruption in comparison with biopsy studies.[2]

2 | METHODS

2.1 | Patient characteristics

Infants and toddlers were enrolled at the Ann and Robert H. Lurie Children's Hospital of Chicago after parents signed written informed consent, approved by the Lurie Institutional Review Board. The majority of AD patients and controls were European-American. Inclusion criteria: moderate-to-severe AD starting within the previous 6 months, age 0-5 years (Table S1).[46] Exclusion criteria: active skin or systemic infections, use of systemic immunosuppressants (within 4 weeks), topical steroids, antibiotics, or other immunomodulators (within 1 week) or moisturizers (within 12 hours) prior to collection. Control subjects did not have a personal or family history of atopy. Lesional (n = 19) and nonlesional (n = 11) tape-strip samples were obtained from AD children and 17 samples were attained from healthy tape-stripped skin of control children. Clinical disease measures included SCORing Atopic Dermatitis/SCORAD, Eczema Area and Severity Index/EASI, body surface area/BSA, and Pruritus Atopic Dermatitis Quickscore/ADQ. Transepidermal water loss/TEWL was evaluated as a barrier function measure prior to sampling after acclimation to ambient humidity, using the AquaFlux Model AF200 (Biox).

2.2 | Tape-strip collection, RNA extraction, and RNA-sequencing

Sixteen large D-Squame tape strips (CuDerm Corp) were serially collected from lesions on the extremities (antecubital or popliteal fossa), and from nonlesional skin in the same extremity, but far from lesions, in areas of completely normal-appearing skin, as described.[46] Skin samples had no signs of active infection. Healthy skin from control children of comparable age was collected from similar areas. The first two tape strips from each sample were discarded.[21,27,46]

RNA was extracted from pooled 3-16 tape strips using the miRNAeasy Mini Kit (Qiagen). RNA AmpliSeq libraries were constructed with the Ion AmpliSeq Transcriptome Human Gene Expression Kit using 5 ng RNA per sample. RNA-seq libraries were pooled and sequenced on the Ion S5 XL system sequencer with Ion 550 Chips, screening more than 20 000 genes per reaction.[24] Additional details can be found in the Appendix S1.

2.3 | Immunohistochemistry

Immunohistochemistry was performed on frozen lesional and nonlesional skin sections from infants and toddlers (age <5 years) with early-onset, moderate-to-severe AD and on healthy skin from controls of a similar age, using monoclonal antibodies, as previously described (Table S2).[46,47]

2.4 | Statistical analysis

RNA-seq alignment and quality control steps were performed, as described.[48] Fold-changes and hypothesis testing were obtained using the general framework for linear models in the *limma* package. *P*-values were adjusted for multiple hypotheses using the Bejamini-Hochberg procedure, controlling for false discovery rate/FDR. Genes with fold-change/FCH ≥2 and FDR <0.05 were considered differentially expressed. Spearman correlation coefficients were used to evaluate the relationship between mRNA expression in lesional and nonlesional AD skin and clinical measures (EASI, SCORAD, BSA, Pruritus ADQ), and the barrier measure, TEWL.

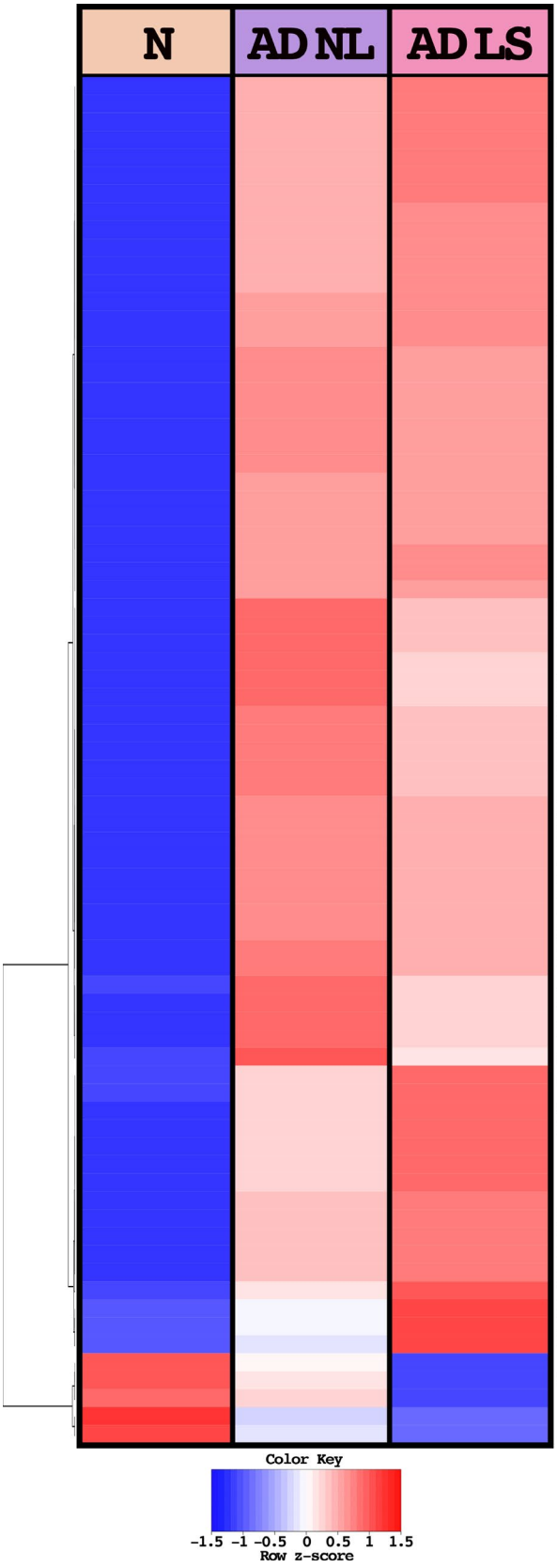
3 | RESULTS

We performed RNA-seq global profiling of tape strips obtained from lesional and nonlesional skin of 19 infants and children (age range 0-5 years, mean age 1.7 years) with early-onset moderate-to-severe AD (≤6 months since disease initiation) and healthy skin from 17 controls (mean age 1.7 years). Most AD (n = 14) and control (n = 15) children were <1-year-old and European-American (Table S1).

Nineteen lesional and 11 nonlesional tape-strip samples were obtained from AD children, and 17 samples were attained from healthy tape-stripped skin of control children. As some AD patients had very severe disease, it was difficult to obtain true normal-appearing skin for nonlesional samples from all children. We recovered RNA and performed RNA-seq in 100% of samples (19/19 and 11/11 of lesional and nonlesional AD, respectively, and 17/17 of controls).

3.1 | Tape-strips global transcriptomic profiling captures key immune mediators of atopic dermatitis

Using criteria of fold-change/FCH >2 and false discovery rate/FDR <0.05, we detected 1829 (1243 up- and 586 down-regulated) differentially expressed genes/DEGs in lesional AD and 662 DEGs (637



	NL vs N	LS vs N	LS vs NL
IL8 ------:	6.06**	8.44***	1.39
IL13 -----:	6.38	8.91*	1.40
IFNGR1 ---:	3.72	4.79**	1.29
GZMB -----:	6.48+	9.27**	1.43
IL7R -----:	4.87*	6.37**	1.31
ITGAM ----:	6.57*	9.15***	1.39
S100A12 --:	6.02*	8.07***	1.34
IL10 -----:	3.92	4.87+	1.24
CD83 -----:	2.46	2.78*	1.13
CXCL16 ---:	2.93+	3.37**	1.15
OASL -----:	4.79+	5.55*	1.16
STAT4 ----:	4.68	5.54*	1.18
CD4 ------:	9.83*	11.50***	1.17
CCRL2 ----:	5.34+	5.89*	1.10
CCL17 ----:	7.77*	9.08***	1.17
CD1A -----:	7.13*	6.51**	-1.09
ITGAX ----:	4.18*	3.92*	-1.07
IL4R -----:	6.58*	6.08**	-1.08
S100A9 ---:	5.57**	5.31***	-1.05
PDE2A ----:	4.79	4.57+	-1.05
CXCR4 ----:	5.06*	4.45**	-1.14
CXCR2 ----:	8.37*	7.24**	-1.16
CCL3 -----:	3.79+	3.78*	-1.00
IL36A ----:	10.70*	10.80**	1.01
CLEC10A --:	9.02*	8.88**	-1.02
STAT6 ----:	2.76	2.78*	1.01
STAT3 ----:	2.69	2.80+	1.04
JAK3 -----:	3.98	4.20*	1.06
IL3RA ----:	12.60**	13.30***	1.06
S100A8 ---:	5.81**	3.68**	-1.58
CSF1 -----:	4.03+	2.80+	-1.44
CTLA4 ----:	7.41+	4.43+	-1.67
IL36G ----:	4.53*	3.00*	-1.51
CCR4 -----:	8.23*	4.60+	-1.79
FCER1A ---:	12.80**	6.43*	-1.99
JAK2 -----:	5.40*	3.58+	-1.51
CRLF2 ----:	15.10**	8.66**	-1.74
TNFRSF9 --:	7.08+	4.73*	-1.50
CD1B -----:	17.40**	9.75**	-1.78
ITK ------:	8.08*	5.10*	-1.58
MMP12 ----:	20.60**	15.50***	-1.33
LAMP3 ----:	8.84**	7.24**	-1.22
CD207 ----:	24.60**	17.90***	-1.37
PDE4A ----:	9.04*	7.11**	-1.27
CD5 ------:	11.70*	9.08**	-1.29
CIITA ----:	7.42**	5.82***	-1.27
CD80 -----:	12.10**	8.95**	-1.35
CD86 -----:	16.90**	12.20***	-1.39
TNF ------:	12.10*	7.84*	-1.55
CCL20 ----:	11.20*	7.83**	-1.43
S100A7 ---:	5.58*	3.24*	-1.72
CCR7 -----:	11.40*	5.37*	-2.12
CD1C -----:	9.15*	4.78*	-1.91
CD1E -----:	7.44+	4.08+	-1.82
CCL22 ----:	3.70*	2.29+	-1.62
PDE4B ----:	3.91*	7.38***	1.89
TLR2 -----:	2.49	3.78*	1.52
CD69 -----:	8.97**	24.00***	2.68
CAMP -----:	5.12	10.50**	2.06
IL1RN ----:	2.87+	4.34**	1.51
IL1B -----:	4.26*	7.38***	1.73
IL1A -----:	3.93	6.89**	1.75
ITGB2 ----:	4.06*	6.22***	1.53
MX1 ------:	3.02	4.24**	1.40
VNN3 -----:	8.08*	14.90***	1.85
OSM ------:	4.69*	7.04***	1.50
CXCR1 ----:	4.45+	6.70**	1.51
TREM1 ----:	4.38+	11.60***	2.64
S100P ----:	1.71	2.96*	1.73
STAT1 ----:	1.85	3.48+	1.88
TNFSF10 --:	1.57	3.01*	1.91
IL18 -----:	-2.23	-6.49***	-2.91
CXCL12 ---:	-2.68	-11.50*	-4.30
IL34 -----:	-1.94	-8.18+	-4.22
IL37 -----:	-4.46	-8.03**	-1.80
CCL7 -----:	-4.92	-11.90*	-2.42

FIGURE 1 Summary heatmap of differentially expressed immune genes. Immune gene expression of multiple pathways in tape-stripped skin of atopic dermatitis/AD and controls using RNA-seq, based on criteria of fold change (|FCH|)>2 and false discovery rate (FDR) <0.05. Table shows FCHs in nonlesional vs normal (NL vs N), lesional vs normal (LS vs N), and lesional vs nonlesional (LS vs NL) tape-stripped AD skin. ***FDR < 0.001, **FDR < 0.01, *FDR < 0.05, $^{+}$FDR < 0.1

up- and 25 down-regulated) in nonlesional AD, compared to control skin, as depicted in the Venn diagram (Figure S1A) and heatmap (Figure S1B, Table S3). 601 DEGs (576 up-regulated, 25 down-regulated) were differentially modulated in both lesional and nonlesional vs control skin (Figure S1A, Table S3). While some differences were also noted between lesional and nonlesional AD, these did not attain statistical significance (Figure S1A, Table S3).

As whole-skin biopsy studies in early-onset pediatric AD demonstrated a distinct immune profile in this age group,[2,3,49] we evaluated a previously reported immune gene-subset,[4,6,11,48] as illustrated in a heatmap (Figure 1). Overall, there was significant dysregulation of inflammatory genes in both lesional and nonlesional tape-stripped AD vs control skin (Figure 1). Measures of general inflammation (MMP12 and PDE4B), and cellular infiltration, such as dendritic cells/DC (ITGAM/CD11b, ITGAX/CD11c, and FCER1A), Langerhans cells (CD207/Langerin), and T cells/T-cell activation and migration (CD4, CD5, ITK, and CCR7), significantly increased in lesional and nonlesional AD vs controls. Some markers demonstrated even greater changes in nonlesional AD (FCER1A, CD207/Langerin, CD1b, CCR7) than lesional skin (Figure 1, Table S3). Genes related to JAK/STAT signaling were also significantly up-regulated in either lesional (JAK3 and STAT6) or nonlesional (JAK2) AD vs controls (all FDR <0.05; Figure 1, Table S3).

We further observed significant dysregulation of measures of innate immunity (IL-1B and TNF) and major T-helper pathways in both AD tissues (Figure 1, Table S3). Many Th2 markers were significantly up-regulated in both AD tissues vs control skin (IL-4R, CCL17, CRLF2/TSLPR, and IL-7R), while few were significantly increased in either nonlesional (CCL22 and CCR4) or lesional (IL-13) skin (FDR <0.05; Figure 1, Table S3). Similar to our reports in skin biopsies,[2,3] we found highly up-regulated expression of Th17/Th22-related genes in lesional and/or nonlesional AD (IL-36A/G, CCL20, CAMP/LL37, VNN3, and S100As) (FDR <0.05; Figure 1, Table S3). Th1 measures exhibited smaller increases that did not attain significance for either lesional or nonlesional samples (CXCL9/CXCL10/CXCL11, STAT1, MX1, OASL). The negative regulators IL-34[50] and IL-37[6] were down-regulated in AD lesions (FDR <0.05 for IL-37; Figure 1, Table S3).

A sensitivity analysis showed no significant differences in AD children with (n = 4; one with asthma, three with food allergies) and without other atopic conditions (data not shown). Due to male predominance in the AD cohort, we also performed a sensitivity analysis of only male AD patients and controls, obtaining similar results (Figure S2-S3).

3.2 | Tape-strips capture epidermal barrier alterations at disease initiation

We next evaluated epidermal barrier alterations using a previously reported barrier gene-subset.[7,11,46,48] Predominantly in lesional AD tape-stripped skin, we noted vast and significant down-regulation of multiple barrier genes, including markers of terminal differentiation (FLG, FLG2, CDSN, LCE1B/LCE1C/LCE1E/LCE5A), lipid biosynthesis/metabolism (ALOXE3, FA2H, GAL, and ELOVL3), tight junction (GJB3 and CLDN8), and keratin (KRT77/KRT79) genes (FDR <0.05; Figure 2, Table S3). Barrier genes were also down-regulated in nonlesional AD vs normal skin, although most did not attain statistical significance (Figure 2, Figure S4, Table S3).

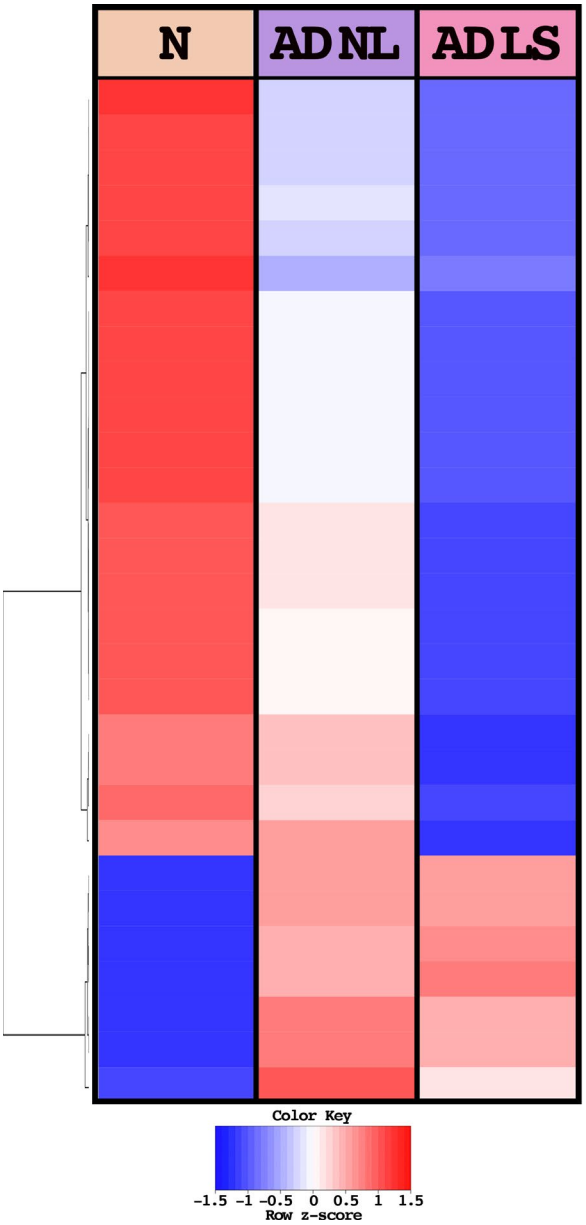
3.3 | Pathway-level comparisons show significant dysregulation of Th2/Th17/Th22 immune and barrier signatures

We performed gene-set-variation-analysis/GSVA to evaluate enrichment of previously published T-helper and epidermal terminal differentiation and lipid gene-sets (Figure 3A; Table S4).[51,52] While Th2- and Th17-pathways were significantly enriched in both lesional and nonlesional AD ($P < .05$), the Th22 pathway significantly increased in nonlesional AD ($P < .05$) and trended towards significance in lesional vs control skin ($P < .1$). No significant dysregulation was seen in the Th1 axis. Epidermal barrier and lipid genes were significantly down-regulated in lesional AD vs control skin ($P < .05$; Figure 3A).

Since Th2 cytokines inhibit synthesis of barrier products, including terminal differentiation proteins and lipids,[53-58] we used Spearman correlations to evaluate correlations between the Th2- and barrier-related gene-subsets across all our skin samples. We found a significant negative correlation between these signatures ($r = -0.42$, $P = .0035$) (Figure 3B). We further performed Spearman correlations between individual up- or down-regulated AD biomarkers within these respective pathways, as presented in a correlation heatmap (Figure S5), obtaining clusters of positive and negative correlations by unsupervised clustering. Markers of cellular infiltrates and immune genes largely positively correlated and clustered together, including cellular- (CD4, ITGAX/CD11c, CD1A/CD1B/CD1C, CD207/Langerin), innate immunity- (IL-1A/B, IL-8/CXCL8), Th2- (IL-4R, IL-13, and CCL17), and Th17/Th22-(IL-36A/G, CCL20, CAMP/LL37, and S100As) related markers ($r > 0.4$, $P < .05$) (Table S5, Figure S5). Epidermal differentiation, lipid, and tight junction genes also largely clustered together, correlating with each other (FLG, FLG2, LCE1B/LCE1C/LCE1D/LCE1E, FA2H, ELOVL3, and CLDN8) ($r > 0.4$, $P < .05$) (Table S5, Figure S5). In line with the correlation by pathway, we noted significant negative correlations between various epidermal barrier (FLG, CLDN8, and LCE1C) and lipid genes (ELOVL3) with Th2 markers (IL-4R and CCL17/TARC) ($r < -0.4$, $P < .5$) (Table S5, Figure S5).

3.4 | Comparisons between tape-strips and whole-skin biopsies portray shared and unique signatures

We compared the relative (fold-change) differences in lesional and nonlesional AD vs controls in tape strips with whole-tissue data obtained from our previously published AD study in a cohort of



	NL vs N	LS vs N	LS vs NL
LCE1B:	−2.38	−3.42*	−1.44
ALOXE3:	−2.15	−3.10*	−1.44
FA2H:	−7.68+	−20.00***	−2.61
FLG2:	−3.75	−7.62**	−2.03
CDSN:	−3.45	−6.61*	−1.91
LCE1C:	−2.72	−3.37*	−1.24
KRT77:	−4.90	−21.80***	−4.44
GAL:	−5.27	−23.90***	−4.54
FLG:	−3.90	−14.60**	−3.74
KRT79:	−4.66	−17.70***	−3.80
LCE1E:	−2.56	−5.39*	−2.11
NR2F2:	−5.37	−20.00**	−3.73
SPTLC3:	−1.91	−4.90*	−2.56
LCE1D:	−2.08	−6.53+	−3.14
ELOVL3:	−2.32	−8.30*	−3.57
GJB3:	−2.47	−6.90*	−2.79
CLDN8:	−3.96	−19.90**	−5.02
LCE5A:	−3.21	−11.40*	−3.56
SCEL:	−1.56	−7.35*	−4.70
CDH11:	−1.74	−10.60*	−6.07
PSORS1C2:	−1.58	−4.30+	−2.72
PNPLA3:	−1.07	−3.95+	−3.71
ELOVL5:	4.16*	4.18**	1.01
ANXA5:	3.08	3.08+	1.00
SOAT1:	3.48+	4.16**	1.20
PPARG:	3.74	4.88*	1.30
ANXA6:	7.78**	5.79**	−1.34
HMGCS1:	3.51+	2.91*	−1.21
SPTLC1:	4.40*	2.48	−1.77

FIGURE 2 Summary heatmap of differentially expressed epidermal barrier-related genes. Barrier gene expression in tape-stripped skin of atopic dermatitis/AD and healthy skin using RNA-seq, based on criteria of fold change (|FCH|) >2 and false discovery rate (FDR) <0.05. Table shows FCHs in nonlesional vs normal (NL vs N), lesional vs normal (LS vs N), lesional vs nonlesional (LS vs NL) tape-stripped AD skin. ***FDR <0.001, **FDR <0.01, *FDR <0.05, $^{+}$FDR <0.1

similar age (mean age of 1.3 years)[2] using scatterplots (Figure 4A-B). Overall, both modalities detected significant dysregulation in lesional skin, while nonlesional skin showed greater inflammation in tape-strips vs biopsies. Comparing both lesional and nonlesional skin (vs controls), we found a largely concordant Th2-skewing (IL-4R, IL-13, CCL17/TARC, and CCL22), although some Th2 markers (CCL26 and CCL7) showed higher expression in biopsies (Figure 4, Table S6). Many Th17/Th22-related markers displayed similar expression in tape-stripped and biopsied nonlesional (IL-22, IL-36G, PI3, and S100A7/8) or lesional (CCL20, S100A8, and PI3) skin; however, a few Th17/Th22 markers demonstrated greater lesional up-regulations in skin biopsies (IL-19, IL-26, IL-22, and S100A9/12) (Figure 4, Table S6). In contrast, greater down-regulation of several barrier-related genes (FLG, CLDN8, FA2H, CDSN) was captured by tape strips in both lesional and nonlesional skin. Negative regulators IL-34 and IL-37 were similarly or better captured by tape strips in AD tissues (Figure 4).

3.5 | Immunohistochemistry validates the presence of cellular infiltrates in the outer epidermis

Using immunohistochemistry, we validated the expression of dendritic cell/DC and Langerhans cell/LC markers (CD1a and CD11c) in lesional, nonlesional, and control skin from our similarly aged biopsy

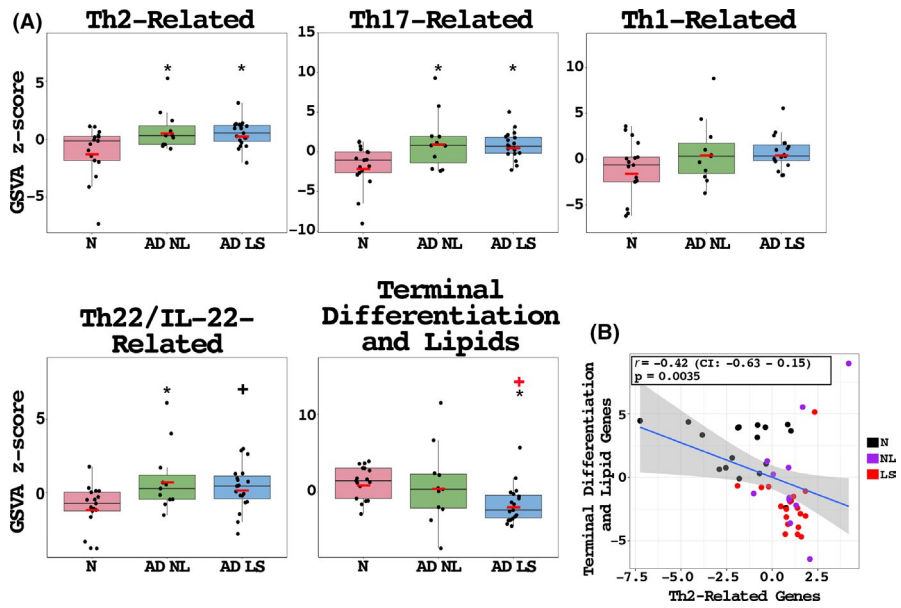


FIGURE 3 T-helper and epidermal terminal differentiation and lipid gene-sets. (A) Mean z-scores of T-helper and barrier-related gene-set variation analysis pathways in normal, nonlesional (NL), and lesional (LS) tape-stripped skin of atopic dermatitis/AD and healthy controls. *Black symbols*: Significance of comparison to normal (N); *red symbols*: Significance of comparison between lesional and nonlesional tape-stripped skin. $^{***}P < .001$, $^{**}P < .01$, $^{*}P < .05$, $^{+}P < .1$ (B) Spearman correlation scatterplot between significantly modulated Th2- and barrier-related genes in tape strips. Black circles denote healthy skin, purple circles denote nonlesional AD, and red circles denote lesional AD skin samples

cohort (Figure S6).[2,3] Overall, positive immunostaining for these cellular markers was noted already in the superficial layers of the lesional and nonlesional AD epidermis, highlighted by the 40X inserts (Figure S6). Foci of $CD1a^+$ staining (marks both DCs and LCs) (Figure S6A-C) and $CD11c^+$ myeloid DCs were observed in the upper epidermis (Figure S6D-F).

3.6 | Molecular alterations correlate with atopic dermatitis clinical severity

We evaluated correlations between expression of immune and barrier markers in lesional and nonlesional AD skin with clinical severity (EASI, SCORAD, BSA, Pruritus ADQ) and barrier function (TEWL) measures (Table 1). EASI correlated with the IL-17/Th17-related marker IL-36G ($r > 0.4$, $P = .073$), while SCORAD negatively correlated with barrier genes in lesional and nonlesional AD (SOAT1, FADS2, and CLDN1) ($r < -0.4$, $P < .1$) (Table 1). We observed significant, or trending toward significant, positive correlations of BSA with lesional expression of T-cell/T-cell activation (ICOS), Th2-(IL-13RA1 and IL-4R) and Th17-(IL-17A/F, CXCL1, IL-36G, and STAT3) related products ($r > 0.4$, $P < .1$) (Table 1). In nonlesional skin, BSA positively correlated with AHR (aryl hydrocarbon receptor), involved in Th22-differentiation[59,60] and IL-7R, a component of the thymic stromal lymphopoietin receptor[61] ($r > 0.4$, $P < .1$), and significantly negatively correlated with the lipid metabolism measure DGAT2 ($r < -0.4$, $P < .05$) (Table 1).

Significant, or trending toward significant, negative correlations with TEWL were found in lesional and/or nonlesional skin, including markers of terminal differentiation (FLG, FLG2, LCEs, and CDSN) and the negative immune regulator IL-34 ($r < -0.4$, $P < .1$) (Table 1). TEWL also significantly correlated with the inflammatory marker MMP12 in nonlesional AD ($r > 0.4$, $P < .05$) (Table 1). Pruritus ADQ showed significant positive correlations with lesional and/or nonlesional inflammatory measures (CD4 and MMP12) ($r > 0.4$ $P < .05$), and negative correlations with barrier-related genes (ELOVL3, FA2H, AWAT1/DGA2, and CLDN8) and IL-34 ($r < -0.4$, $P < .1$) (Table 1). All correlations are provided in Table S7.

4 | DISCUSSION

To our knowledge, we present the first tape-strip global transcriptomic profiling study in infants and toddlers ages 0-5 years (mean age, 1.7 years, mostly within the first year of life) with moderate-to-severe, early-onset (≤6 months of disease initiation) AD. Biopsies cause pain and scarring and are infeasible in studies in young children. Given the rapid progress of exploration of novel targeted treatments, including current clinical trials of dupilumab, in children with AD as young as 6 months of age,[15,16] minimally invasive alternatives to biopsies to track therapeutic responses in clinical trials and longitudinal studies are needed.[18,19,62]

Tape-strip studies in the pediatric population have been limited thus far, including proteomic studies that evaluated a panel of proteins in nonlesional skin of infants (<1 year) in one study[33] and lesional and nonlesional AD skin from children (<12 years) in another report.[23] Using qRT-PCR, our group also recently evaluated a panel of 79 AD-related biomarkers.[46] A prior RNA-seq transcriptomic

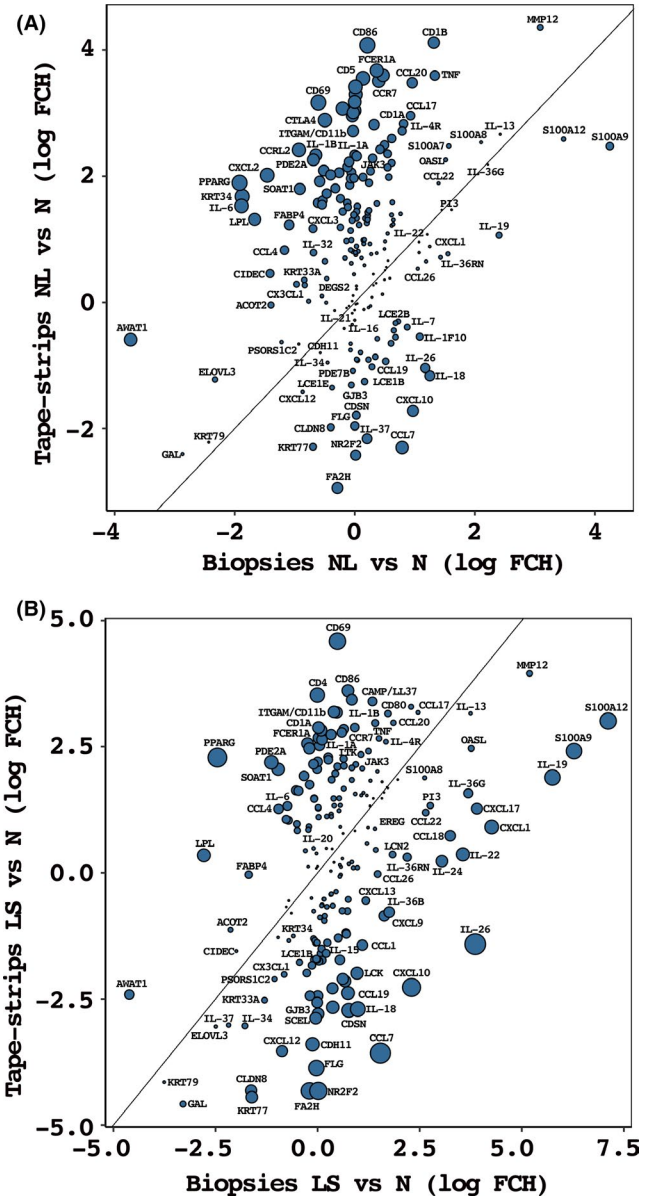


FIGURE 4 Scatterplots comparing relative log2 fold-change (FCH) differences in tape-stripped vs biopsied samples. Nonlesional (A) and lesional (B) atopic dermatitis/AD vs healthy skin in tape-strip data (y-axis) compared with whole-tissue data reported in a previously published AD study in a cohort of similar age (x-axis). Sizes of the circles represent the absolute differences in log2 FCH between expression of AD vs controls in tape-strips and whole-skin tissue data

study in children (ages 4-17 years) with mild-to-severe AD with and without food allergies focused on nonlesional skin, and identified a higher Th2 signature in children with AD and concomitant food-allergies, as compared to those without food allergies.[24] Two other tape-strip RNA-seq studies using a cohort of mild-to-severe adult AD patients have also been reported, primarily evaluating differences between nonlesional AD and normal skin.[21,34] These adult studies identified an inflammatory group with higher Th2 inflammation, but the tape-strip data largely did not replicate the vast differentiation abnormalities seen in skin biopsies.[21,34] These previous RNA-seq studies reported sample recovery rates ranging between 18% and 89% across healthy, nonlesional and/or lesional skin samples,[21,24,34] with a total sample recovery rate of 45% across a study of children and adolescents.[24]

In the present RNA-seq profiling study, we achieved 100% recovery rates in both lesional and nonlesional AD, but also in control samples and were able to show that tape-strips capture the characteristic immune and barrier signatures in skin lesions as identified in biopsy studies within the same pediatric age-group.[2,3] Moreover, our data potentially capture even greater inflammatory and epidermal barrier dysregulations in tape-stripped nonlesional AD skin at early disease initiation, as compared to reports in whole-skin biopsies.[2,3] As such, tape strips may provide novel insights into disease mechanisms in nonlesional AD skin, representing a sub-clinical, yet molecularly altered tissue.

Our global profiling in tape strips shows that the superficial epidermis of both lesional and nonlesional AD skin is characterized by increases in cellular markers of T/B-cells (CD4 and CD5) and dendritic cells (ITGAM/CD11b, ITGAX/CD11c, FCER1A, LAMP3/DC-LAMP, and CD1b). In fact, some measures, such as the T-cell migration marker CCR7, CD207/Langerin, and FCεRIA (expressed by inflammatory epidermal dendritic cells[63] as well as mast cells, basophils, and eosinophils), showed even greater increases in nonlesional AD skin, illustrating the significant inflammatory burden already present in nonlesional skin of infants with early-onset moderate-to-severe AD.

mRNA profiling in skin biopsies from infants with early-onset pediatric AD showed strong Th2 and Th22/Th17-polarization, and lack of the characteristic Th1-skewing seen in adults with longstanding AD.[2-5] Likewise, this tape-strip study captured significant up-regulation of key Th2 mediators in lesional and nonlesional AD tissues, such as IL-4R (target of the FDA-approved biologic dupilumab), IL-13 (target of emerging biologics), CCL17/TARC, and genes related to the OX40-TSLP axis (CRLF2/TSLPR, IL-7R), which perpetuates Th2 activation.[64,65] Also, tape-stripped lesional and nonlesional AD skin demonstrated significant up-regulation of Th17/Th22 axis products (IL-36A/G, CCL20, VNN3, CAMP/LL37, and S100As), largely lacking Th1-skewing.

Epidermal barrier attenuation of terminal differentiation, tight junction, and lipid-related products are also a hallmark of chronic adult AD skin.[6-8,50,53] However, previous reports from infants and toddlers with AD observed smaller and/or nonsignificant down-regulation of terminal differentiation mediators (FLG, PSORS1C2, and late cornified envelope [LCE] proteins LCE1B/LCE1E/LCE2B) in skin biopsies of 0 to 5-year-old children, and differential lipid and tight-junction alterations compared to adults with AD.[2,3] Using tape stripping, we identified significant down-regulation of numerous terminal differentiation products (FLG, FLG2, CDSN, SCEL, and LCEs), as well as lipid (FA2H, ELOVL3, and GAL) and tight junction (CLDN8) abnormalities in AD lesions, with a trend for decreases in nonlesional skin as well. Furthermore, upon comparing relative fold-change differences in expression data between tape-strips and previously described biopsies[2] from lesional and nonlesional skin, as compared to normal skin, it is evident that tape strips in both lesional and nonlesional AD skin detected enhanced down-regulation of barrier mediators,

TABLE 1 Spearman correlations of cutaneous AD biomarkers with clinical measures

Correlations of AD biomarkers in lesional skin with clinical measures

BSA			TEWL			Pruritus ADQ			SCORAD		
Marker	*r*	*P* value	Marker	*r*	*P* value	Marker	*r*	*P* value	Marker	*r*	*P* value
A.											
IL17F	0.700	.003	FLG2	−0.879	.002	AWAT1/DGA2	−0.698	.012	FADS2	−0.525	.044
IL13RA1	0.553	.029	LCE1D	−0.818	.007	CDH19	−0.656	.020	SOAT1	−0.499	.058
CXCL1	0.544	.032	ACER1	−0.758	.016	FA2H	−0.642	.024	SGPP1	−0.493	.062
CXCL17	0.538	.034	LCE5A	−0.721	.024	ELOVL3	−0.604	.038	ORMDL3	−0.477	.072
PDE7B	0.535	.035	LCE1E	−0.709	.028	FABP4	−0.572	.052			
IL36G	0.529	.037	CDSN	−0.697	.031	PPARG	−0.561	.058	**EASI**		
IL20	0.521	.041	LCE2C	−0.685	.035	CDH11	−0.551	.063	**Marker**	***r***	***P* value**
IFNGR2	0.521	.041	ANXA9	−0.673	.039	CLDN8	−0.551	.063	IL36G	0.479	.073
ICOS	0.515	.044	LCE2D	−0.661	.044	FABP7	−0.491	.1			
CXCR2	0.512	.045	IL34	−0.661	.044	IL34	−0.488	.1			
IL17A	0.494	.054	LCE2A	−0.648	.049	CD4	0.642	.024			
FASLG	0.482	.061	FLG	−0.612	.066	CIITA	0.618	.032			
GZMB	0.482	.061	CDH11	−0.600	.073						
STAT3	0.479	.062	LCE1C	−0.588	.080						
TLR1	0.468	.070	PSORS1C2	−0.588	.080						
IL17C	0.468	.070	LCE2B	−0.564	.096						
IL4R	0.456	.078	TGFB1	0.612	.066						
VNN3	0.441	.089	ITGAX/ CD11c	0.588	.080						
IL9	0.438	.091	CD69	0.588	.080						
IL2RA	0.438	.091	PDE4B	0.552	.1						
IL19	0.426	.1									

Correlations of AD biomarkers in nonlesional skin with clinical measures

BSA			TEWL			Pruritus ADQ			SCORAD		
Marker	*r*	*P* value	Marker	*r*	*P* value	Marker	*r*	*P* value	Marker	*r*	*P* value
B											
DGAT2	−0.638	.047	FLG	−0.685	.035	CRLF2/TSLPR	−0.738	.046	SOAT1	−0.709	.028
FFAR2	−0.565	.089	FABP4	−0.636	.054	MMP12	0.810	.022	CLDN1	−0.576	.088
AHR	0.590	.073	SCEL	−0.624	.060	PI3	0.667	.083			
IL7R	0.559	.093	ANXA9	−0.624	.060						
ICOS	0.547	.1	IL34	−0.612	.066						
			FLG2	−0.600	.073						
			ELOVL3	−0.600	.073						
			ACOT2	−0.600	.073						
			MMP12	0.648	.049						
			CD1A	0.576	.088						

Note: Correlation coefficient |r|> 0.4 and $P < .1$.

Abbreviations: AD, atopic dermatitis; BSA, body surface area; EASI, eczema area and severity index; Pruritus ADQ, Atopic dermatitis quickscore; SCORAD, scoring atopic dermatitis; TEWL, transepidermal water loss.

including several terminal differentiation markers (FLG, CDSN), compared to biopsied skin. The lower expression of differentiation markers in tape-strips vs whole-skin biopsies from early-onset AD in infants may be attributed to a dilutional effect in skin biopsies and possibly suggests that tape strips are better positioned to capture barrier abnormalities.

Our correlation analyses identified a significant negative correlation between Th2 and barrier gene-subsets. We also noted significant individual correlations between mRNA expression in lesional and/or nonlesional skin and some clinical measures, particularly with BSA and TEWL, but less so with EASI, SCORAD, and Pruritus ADQ severity scores. Among significant correlations with BSA were the lesional expression of Th2- (IL-4R and IL-13RA1) and Th17/Th22-related (IL-17A/F, IL-36G, and VNN3) markers, congruent with a recent report showing BSA as an objective measure of clinical severity.[66] TEWL, an objective functional measure of the barrier, also showed significant positive correlations with inflammatory markers and negative correlations with barrier components, including terminal differentiation markers (FLG, FLG2, and PSORS1C2) and lipid biosynthesis/metabolism genes (ELOVL3 and FABP4). These results further suggest that superficial sampling of the epidermis via tape stripping could provide novel insights into barrier function.

There were a few limitations of this study, including a relatively smaller sample size, the relatively laborious process of tape stripping and variations in depth of tape-strip sampling in AD vs control skin. We had fewer nonlesional samples, due to some AD patients having very severe disease, making it difficult to obtain true normal-appearing skin for nonlesional samples from all children. Moreover, as these were young AD patients, it was difficult to perform more invasive procedures, such as blood draws and skin biopsies, that could have allowed for additional analysis and comparisons of gene expression differences within the same cohort. Future protein expression studies are needed to further define these abnormalities. Evaluation of a lesion score (eg, Total Sign Score) may have also aided in the identification of additional biomarkers. While we had more males in the AD cohort vs controls, a sensitivity analysis in only males revealed similar results. Finally, while differences between lesional vs nonlesional AD skin did not meet statistical significance, highlighting the highly abnormal nonlesional AD phenotype, variations between patients may not be fully captured in our assessment.

In sum, we achieved 100% sample recovery rates in our tape-strip study and captured the characteristic inflammatory milieu of Th2/Th22/Th17 polarization, with no Th1-skewing in early-onset pediatric AD skin. Studies in blood from pediatric moderate-to-severe AD infants and toddlers within 6 months of disease initiation have already found significant Th2 and Th17 up-regulation in the periphery.[13,14] The increased systemic inflammation already at early AD initiation in infants that is significantly correlated with nonlesional skin expression,[13,14] together with the significant immune dysregulation that extends to uninvolved tape-stripped skin, may suggest the potential value for early systemic intervention, to appropriately treat all disease compartments in severe AD early in life.

Given that tape strips provide a noninvasive alternative to skin biopsies, this approach may be able to track both systemic and local skin immune responses in future clinical trials and longitudinal studies in infants and children. Tape strips may be perhaps even better positioned than whole-skin biopsies to uncover novel insights into barrier alterations in AD.

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AUTHOR CONTRIBUTION

ABP contributed to the study design, data analysis, data interpretation, and writing the manuscript. YRY contributed to data interpretation and

writing the manuscript. JW contributed to data analysis, data interpretation and writing the manuscript. EDD and AD contributed to data interpretation and edited the manuscript. RL, MMF, TC, and SMR contributed to sample collection and edited the manuscript. NZ contributed to the experiments. JGK edited and improved the manuscript. ASP recruited the patients and edited the manuscript. EGY contributed to the study and experimental design, data interpretation and writing the manuscript.

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REFERENCES

1. Perkin MR, Strachan DP, Williams HC, Kennedy CT, Golding J, Team AS. Natural history of atopic dermatitis and its relationship to serum total immunoglobulin E in a population-based birth cohort study. *Pediatr Allergy Immunol*. 2004;15(3):221-229.
2. Brunner PM, Israel A, Zhang N, et al. Early-onset pediatric atopic dermatitis is characterized by TH2/TH17/TH22-centered inflammation and lipid alterations. *J Allergy Clin Immunol*. 2018;141(6):2094-2106.
3. Esaki H, Brunner PM, Renert-Yuval Y, et al. Early-onset pediatric atopic dermatitis is TH2 but also TH17 polarized in skin. *J Allergy Clin Immunol*. 2016;138(6):1639-1651.
4. He H, Li R, Choi S, et al. Increased cardiovascular and atherosclerosis markers in blood of older patients with atopic dermatitis. *Ann Allergy Asthma Immunol*. 2020;124(1):70-78.
5. Zhou L, Leonard A, Pavel AB, et al. Age-specific changes in the molecular phenotype of patients with moderate-to-severe atopic dermatitis. *J Allergy Clin Immunol*. 2019;144(1):144-156.
6. Suarez-Farinas M, Ungar B, Correa da Rosa J, et al. RNA sequencing atopic dermatitis transcriptome profiling provides insights into novel disease mechanisms with potential therapeutic implications. *J Allergy Clin Immunol*. 2015;135(5):1218-1227.
7. Suarez-Farinas M, Tintle SJ, Shemer A, et al. Nonlesional atopic dermatitis skin is characterized by broad terminal differentiation defects and variable immune abnormalities. *J Allergy Clin Immunol*. 2011;127(4):954.
8. Guttman-Yassky E, Suarez-Farinas M, Chiricozzi A, et al. Broad defects in epidermal cornification in atopic dermatitis identified through genomic analysis. *J Allergy Clin Immunol*. 2009;124(6):1235.
9. Gittler JK, Shemer A, Suarez-Farinas M, et al. Progressive activation of T(H)2/T(H)22 cytokines and selective epidermal proteins characterizes acute and chronic atopic dermatitis. *J Allergy Clin Immunol*. 2012;130(6):1344-1354.
10. Suarez-Farinas M, Dhingra N, Gittler J, et al. Intrinsic atopic dermatitis shows similar TH2 and higher TH17 immune activation compared with extrinsic atopic dermatitis. *J Allergy Clin Immunol*. 2013;132(2):361-370.
11. Guttman-Yassky E, Bissonnette R, Ungar B, et al. Dupilumab progressively improves systemic and cutaneous abnormalities in patients with atopic dermatitis. *J Allergy Clin Immunol*. 2019;143(1):155-172.
12. Czarnowicki T, He H, Canter T, et al. Evolution of pathologic T-cell subsets in patients with atopic dermatitis from infancy to adulthood. *J Allergy Clin Immunol*. 2020;145(1):215-228.
13. Brunner PM, He H, Pavel AB, et al. The blood proteomic signature of early-onset pediatric atopic dermatitis shows systemic inflammation and is distinct from adult long-standing disease. *J Am Acad Dermatol*. 2019;81(2):510-519.
14. Brunner PM, Israel A, Leonard A, et al. Distinct transcriptomic profiles of early-onset atopic dermatitis in blood and skin of pediatric patients. *Ann Allergy Asthma Immunol*. 2019;122(3):318.
15. Study to investigate the efficacy and safety of dupilumab administered with topical corticosteroids (TCS) in participants ≥6 to <12 years with severe atopic dermatitis (AD). ClinicalTrials.gov. https://clinicaltrials.gov/ct2/show/NCT03345914. Updated November 22, 2019. Accessed March 12, 2020.
16. Safety, pharmacokinetics and efficacy of dupilumab in patients ≥6 months to <6 years with severe atopic dermatitis (liberty AD PRESCHOOL). ClinicalTrials.gov. https://clinicaltrials.gov/ct2/show/NCT03346434. Updated June 14, 2019. Accessed March 12, 2020.
17. Simpson EL, Paller AS, Siegfried EC, et al. Efficacy and safety of dupilumab in adolescents with uncontrolled moderate to severe atopic dermatitis: a phase 3 randomized clinical trial. *JAMA Dermatology*. 2020;156(1):44-56.
18. Paternoster L, Savenije OEM, Heron J, et al. Identification of atopic dermatitis subgroups in children from 2 longitudinal birth cohorts. *J Allergy Clin Immunol*. 2018;141(3):964-971.
19. Koseki R, Morii W, Noguchi E, et al. Effect of filaggrin loss-of-function mutations on atopic dermatitis in young age: a longitudinal birth cohort study. *J Hum Genet*. 2019;64(9):911-917.
20. Tran MM, Lefebvre DL, Dharma C, et al. Predicting the atopic march: results from the Canadian healthy infant longitudinal development study. *J Allergy Clin Immunol*. 2018;141(2):601.
21. Dyjack N, Goleva E, Rios C, et al. Minimally invasive skin tape strip RNA sequencing identifies novel characteristics of the type 2-high atopic dermatitis disease endotype. *J Allergy Clin Immunol*. 2018;141(4):1298-1309.
22. Kim BE, Goleva E, Kim PS, et al. Side-by-Side comparison of skin biopsies and skin tape stripping highlights abnormal stratum corneum in atopic dermatitis. *J Invest Dermatol*. 2019;139(11):2387.
23. Hulshof L, Hack DP, Hasnoe QCJ, et al. A minimally invasive tool to study immune response and skin barrier in children with atopic dermatitis. *Br J Dermatol*. 2019;180(3):621-630.
24. Leung DYM, Calatroni A, Zaramela LS, et al. The nonlesional skin surface distinguishes atopic dermatitis with food allergy as a unique endotype. *Sci Transl Med*. 2019;11(480).
25. Koppes SA, Brans R, Ljubojevic Hadzavdic S, Frings-Dresen MH, Rustemeyer T, Kezic S. Stratum corneum tape stripping: monitoring of inflammatory mediators in atopic dermatitis patients using topical therapy. *Int Arch Allergy Immunol*. 2016;170(3):187-193.
26. Voegeli R, Rawlings AV, Breternitz M, Doppler S, Schreier T, Fluhr JW. Increased stratum corneum serine protease activity in acute eczematous atopic skin. *Br J Dermatol*. 2009;161(1):70-77.
27. Clausen ML, Slotved HC, Krogfelt KA, Agner T. Measurements of AMPs in stratum corneum of atopic dermatitis and healthy skin-tape stripping technique. *Sci Rep*. 2018;8(1):1666.
28. Amarbayasgalan T, Takahashi H, Dekio I, Morita E. Interleukin-8 content in the stratum corneum as an indicator of the severity of inflammation in the lesions of atopic dermatitis. *Int Arch Allergy Immunol*. 2013;160(1):63-74.
29. Angelova-Fischer I, Mannheimer AC, Hinder A, et al. Distinct barrier integrity phenotypes in filaggrin-related atopic eczema following sequential tape stripping and lipid profiling. *Exp Dermatol*. 2011;20(4):351-356.
30. Morita E, Takahashi H, Niihara H, et al. Stratum corneum TARC level is a new indicator of lesional skin inflammation in atopic dermatitis. *Allergy*. 2010;65(9):1166-1172.
31. Sano Y, Masuda K, Tamagawa-Mineoka R, et al. Thymic stromal lymphopoietin expression is increased in the horny layer of patients with atopic dermatitis. *Clin Exp Immunol*. 2013;171(3):330-337.
32. Yamaguchi J, Aihara M, Kobayashi Y, Kambara T, Ikezawa Z. Quantitative analysis of nerve growth factor (NGF) in the atopic dermatitis and psoriasis horny layer and effect of treatment on NGF in atopic dermatitis. *J Dermatol Sci*. 2009;53(1):48-54.

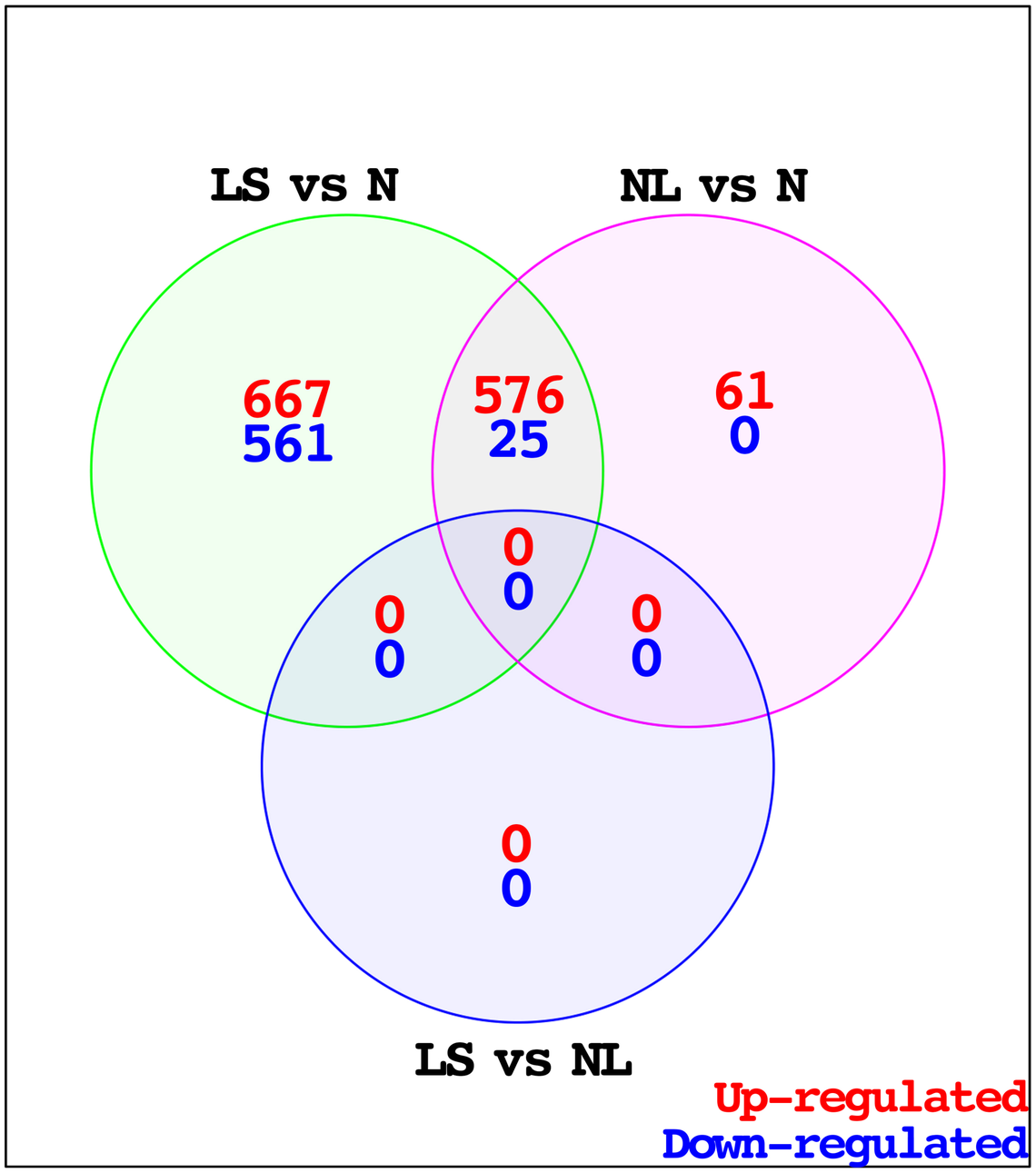
33. McAleer MA, Jakasa I, Hurault G, et al. Systemic and stratum corneum biomarkers of severity in infant atopic dermatitis include markers of innate and T helper cell-related immunity and angiogenesis. *Br J Dermatol*. 2019;180(3):586-596.
34. Berdyshev E, Goleva E, Bronova I, et al. Lipid abnormalities in atopic skin are driven by type 2 cytokines. *JCI insight*. 2018;3(4).
35. Berekméri A, Latzko A, Alase A, et al. Detection of IL-36γ through noninvasive tape stripping reliably discriminates psoriasis from atopic eczema. *J Allergy Clin Immunol*. 2018;142(3):988.
36. Tham EH, Dyjack N, Kim BE, et al. Olfactory receptors expression in the skin of atopic dermatitis patients. *J Allergy Clin Immunol*. 2018;141(2):AB135.
37. Clausen ML, Jungersted JM, Andersen PS, Slotved HC, Krogfelt KA, Agner T. Human beta-defensin-2 as a marker for disease severity and skin barrier properties in atopic dermatitis. *Br J Dermatol*. 2013;169(3):587-593.
38. Dapic I, Kobetic R, Brkljacic L, Kezic S. Jakasa I. Quantification of free fatty acids in human stratum corneum using tandem mass spectrometry and surrogate analyte approach. *Biomed Chromatogr*. 2018;32(2).
39. Winget JM, Finlay D, Mills KJ, et al. Quantitative proteomic analysis of stratum corneum dysfunction in adult chronic atopic dermatitis. *J Invest Dermatol*. 2016;136(8):1732-1735.
40. Riethmuller C, McAleer MA, Koppes SA, et al. Filaggrin breakdown products determine corneocyte conformation in patients with atopic dermatitis. *J Allergy Clin Immunol*. 2015;136(6):1573.
41. Sakabe J-I, Kamiya K, Yamaguchi H, et al. Proteome analysis of stratum corneum from atopic dermatitis patients by hybrid quadrupole-orbitrap mass spectrometer. *J Allergy Clin Immunol*. 2014;134(4):957.
42. Janssens M, van Smeden J, Gooris GS, et al. Increase in short-chain ceramides correlates with an altered lipid organization and decreased barrier function in atopic eczema patients. *J Lipid Res*. 2012;53(12):2755-2766.
43. Kezic S, O'Regan GM, Yau N, et al. Levels of filaggrin degradation products are influenced by both filaggrin genotype and atopic dermatitis severity. *Allergy*. 2011;66(7):934-940.
44. Broccardo CJ, Mahaffey S, Schwarz J, et al. Comparative proteomic profiling of patients with atopic dermatitis based on history of eczema herpeticum infection and *Staphylococcus aureus* colonization. *J Allergy Clin Immunol*. 2011;127(1):186.
45. Broccardo CJ, Mahaffey SB, Strand M, Reisdorph NA, Leung DYM. Peeling off the layers: skin taping and a novel proteomics approach to study atopic dermatitis. *J Allergy Clin Immunol*. 2009;124(5):1113.
46. Guttman-Yassky E, Diaz A, Pavel AB, et al. Use of tape strips to detect immune and barrier abnormalities in the skin of children with early-onset atopic dermatitis. *JAMA Dermatology*. 2019;155(12):1358-1370.
47. Del Duca E, Pavel AB, Dubin C, et al. Major differences in expression of inflammatory pathways in skin from different body sites of healthy individuals. *J Invest Dermatol*. 2019;139(10):2228.
48. Sanyal RD, Pavel AB, Glickman J, et al. Atopic dermatitis in African American patients is TH2/TH22-skewed with TH1/TH17 attenuation. *Ann Allergy Asthma Immunol*. 2019;122(1):99.
49. Czarnowicki T, He H, Krueger JG, Guttman-Yassky E. Atopic dermatitis endotypes and implications for targeted therapeutics. *J Allergy Clin Immunol*. 2019;143(1):1-11.
50. Esaki H, Ewald DA, Ungar B, et al. Identification of novel immune and barrier genes in atopic dermatitis by means of laser capture microdissection. *J Allergy Clin Immunol*. 2015;135(1):153-163.
51. Dhingra N, Shemer A, Correa da Rosa J, et al. Molecular profiling of contact dermatitis skin identifies allergen-dependent differences in immune response. *J Allergy Clin Immunol*. 2014;134(2):362-372.
52. Guttman-Yassky E, Ungar B, Noda S, et al. Extensive alopecia areata is reversed by IL-12/IL-23p40 cytokine antagonism. *J Allergy Clin Immunol*. 2016;137(1):301-304.
53. Kim BE, Leung DY. Significance of skin barrier dysfunction in atopic dermatitis. *Allergy Asthma Immunol Res*. 2018;10(3):207-215.
54. Howell MD, Kim BE, Gao P, et al. Cytokine modulation of atopic dermatitis filaggrin skin expression. *J Allergy Clin Immunol*. 2009;124(3 Suppl 2):R7-R12.
55. Brunner PM, Leung DYM, Guttman-Yassky E. Immunologic, microbial, and epithelial interactions in atopic dermatitis. *Ann Allergy Asthma Immunol*. 2018;120(1):34-41.
56. Danso MO, van Drongelen V, Mulder A, et al. TNF-alpha and Th2 cytokines induce atopic dermatitis-like features on epidermal differentiation proteins and stratum corneum lipids in human skin equivalents. *J Invest Dermatol*. 2014;134(7):1941-1950.
57. Kim BE, Bin L, Ye Y-M, Ramamoorthy P, Leung DYM. IL-25 enhances HSV-1 replication by inhibiting filaggrin expression, and acts synergistically with Th2 cytokines to enhance HSV-1 replication. *J Invest Dermatol*. 2013;133(12):2678-2685.
58. Gutowska-Owsiak D, Schaupp AL, Salimi M, Taylor S, Ogg GS. Interleukin-22 downregulates filaggrin expression and affects expression of profilaggrin processing enzymes. *Br J Dermatol*. 2011;165(3):492-498.
59. Trifari S, Kaplan CD, Tran EH, Crellin NK, Spits H. Identification of a human helper T cell population that has abundant production of interleukin 22 and is distinct from TH-17, TH1 and TH2 cells. *Nat Immunol*. 2009;10(8):864-871.
60. Eyerich K, Eyerich S. Th22 cells in allergic disease. *Allergo J Int*. 2015;24(1):1-7.
61. Zhong J, Sharma J, Raju R, et al. TSLP signaling pathway map: a platform for analysis of TSLP-mediated signaling. *Database (Oxford)*. 2014;2014:bau007.
62. Wan J, Mitra N, Hoffstad OJ, Yan AC, Margolis DJ. Longitudinal atopic dermatitis control and persistence vary with timing of disease onset in children: a cohort study. *J Am Acad Dermatol*. 2019;81(6):1292-1299.
63. Wollenberg A, Kraft S, Hanau D, Bieber T. Immunomorphological and ultrastructural characterization of langerhans cells and a novel, inflammatory dendritic epidermal cell (IDEC) population in lesional skin of atopic eczema. *J Invest Dermatol*. 1996;106(3):446-453.
64. Liu YJ. Thymic stromal lymphopoietin and OX40 ligand pathway in the initiation of dendritic cell-mediated allergic inflammation. *J Allergy Clin Immunol*. 2007;120(2):238.
65. Wu J, Guttman-Yassky E. Efficacy of biologics in atopic dermatitis. *Expert Opin Biol Ther*. 2020;20(5):525-538.
66. Suh TP, Ramachandran D, Patel V, et al. Product of investigator global assessment and body surface area (IGAxBSA): a practice-friendly alternative to the eczema area and severity index to assess atopic dermatitis severity in children. *J Am Acad Dermatol*. 2020;82(5):1187-1194.

SUPPORTING INFORMATION

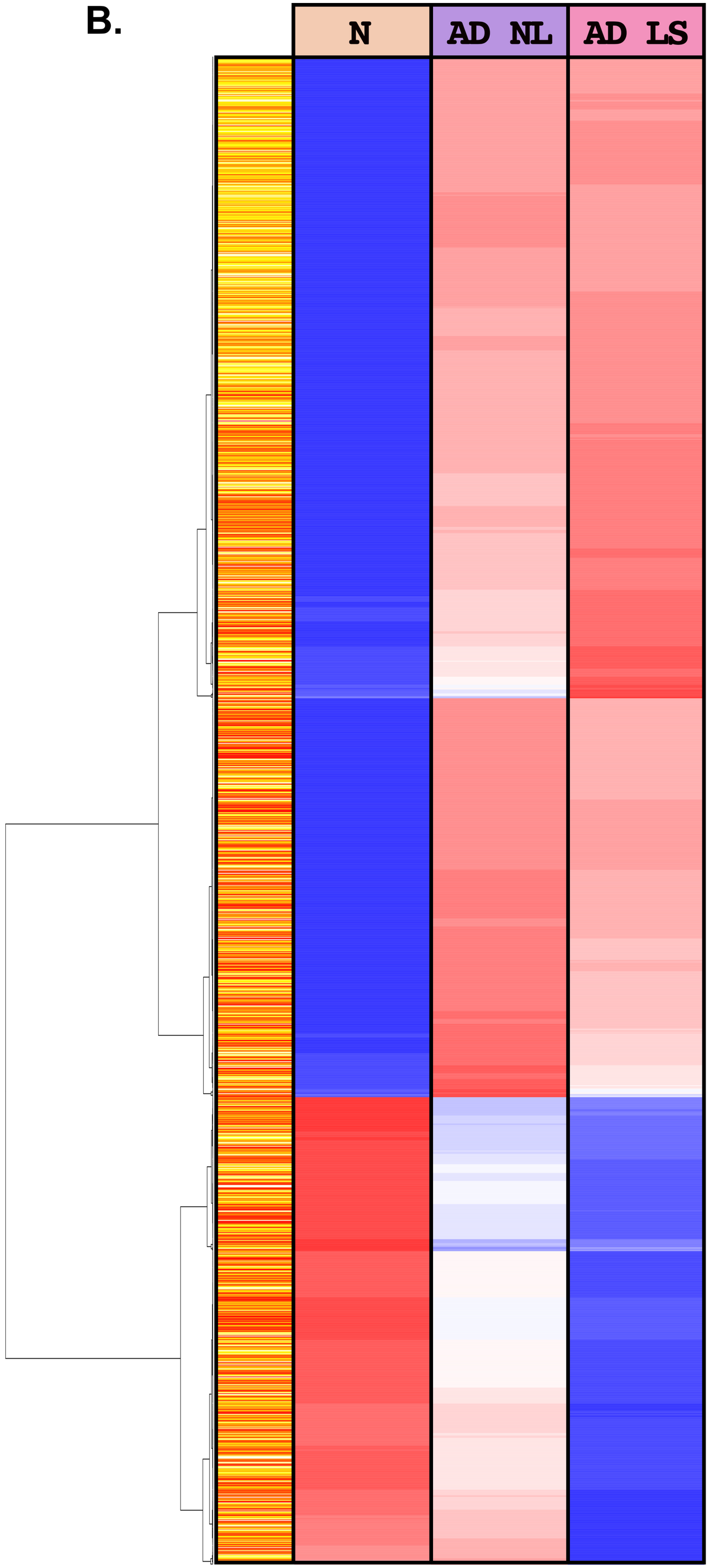
Additional supporting information may be found online in the Supporting Information section.

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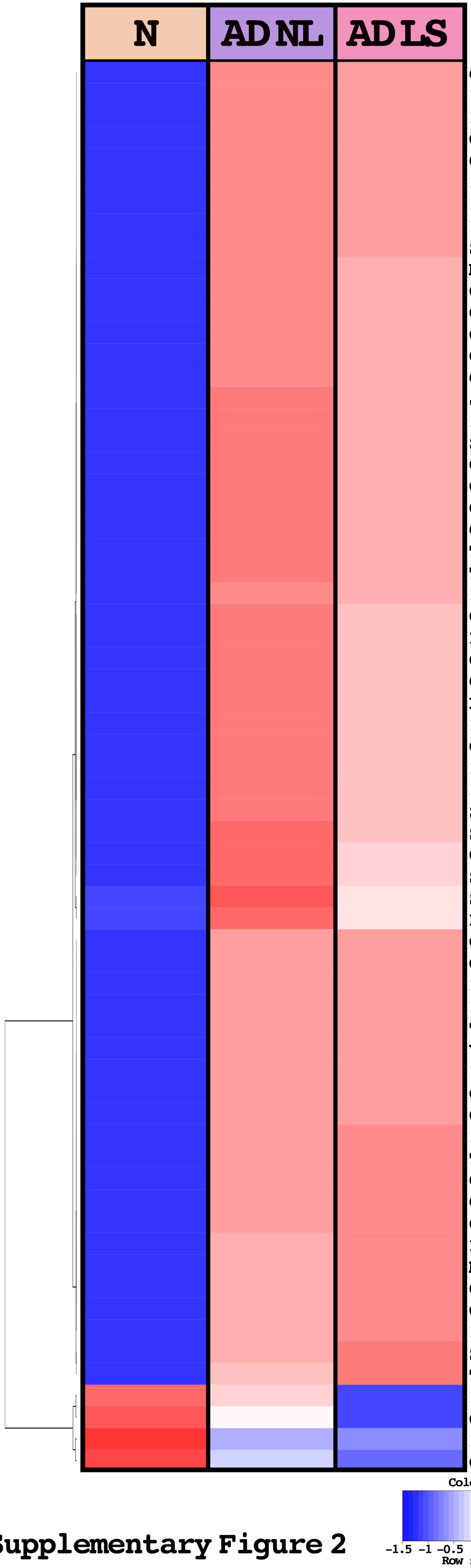
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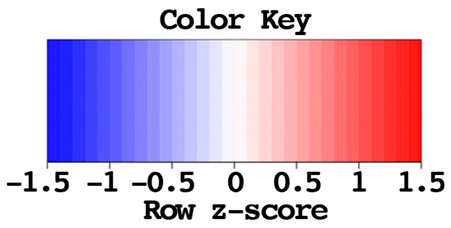
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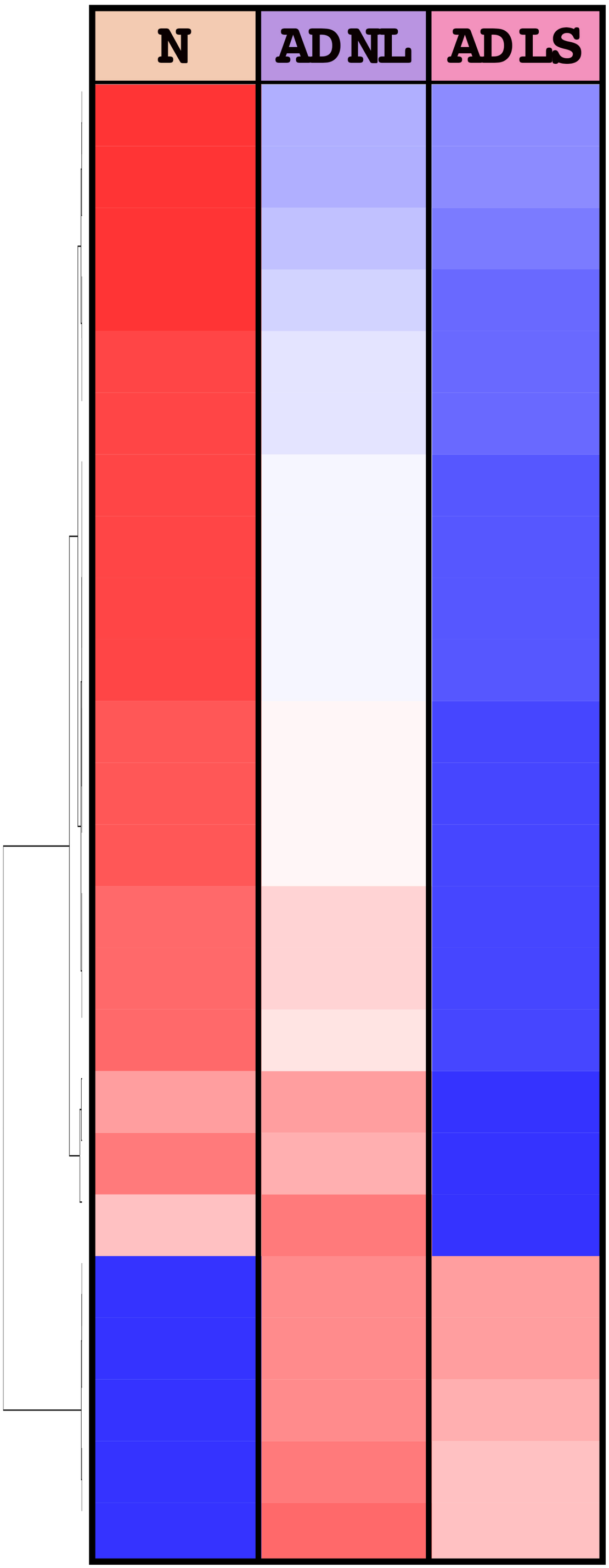
Supplementary Figure 1



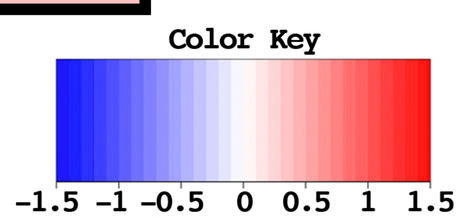
	NL vs N	LS vs N	LS vs NL
CD1A -----:	20.60***	17.50***	-1.18
ITGAM ----:	14.60**	12.60***	-1.16
IFNGR1 ---:	12.60**	10.90**	-1.16
OASL -----:	2.46	2.32	-1.06
CD207 ----:	35.30***	27.80***	-1.27
IL36A ----:	7.84*	7.40**	-1.06
IL13 -----:	12.20*	11.40*	-1.07
IL7R -----:	13.20***	12.10***	-1.09
S100A12 --:	8.76**	7.91**	-1.11
MMP12 ----:	66.30***	43.70***	-1.52
CAMP -----:	11.20*	8.79*	-1.28
CCL3 -----:	24.30***	17.80***	-1.37
CXCR2 ----:	18.90***	13.70***	-1.38
CD86 -----:	118.00***	70.10***	-1.69
CD5 ------:	22.80***	17.30***	-1.32
JAK3 -----:	4.01	3.28	-1.22
IL1A -----:	8.59*	6.29*	-1.37
S100A9 ---:	8.91***	6.46**	-1.38
CXCL16 ---:	3.91+	3.18+	-1.23
CRLF2 ----:	77.70***	40.40***	-1.92
CIITA ----:	28.30***	16.60***	-1.71
CD80 -----:	12.60**	8.37**	-1.51
TNF ------:	32.70***	18.30***	-1.79
TNFRSF9 --:	9.13+	6.25+	-1.46
IL4R -----:	8.00*	6.12*	-1.31
CD1B -----:	8.26**	4.96*	-1.66
PDE4A ----:	12.90**	6.86*	-1.88
CCR7 -----:	30.40***	13.70***	-2.22
CCL20 ----:	23.40**	11.20**	-2.09
LAMP3 ----:	8.21**	5.17*	-1.59
ITK ------:	7.72	4.92	-1.57
CCRL2 ----:	14.40**	8.27*	-1.74
ITGAX ----:	5.49*	4.00+	-1.37
IL36G ----:	9.23**	6.14**	-1.50
STAT6 ----:	5.50*	3.96+	-1.39
S100A8 ---:	10.30***	5.54**	-1.87
CD1C -----:	14.20**	6.94*	-2.04
STAT4 ----:	7.87	4.48	-1.76
S100A7 ---:	6.63*	3.04	-2.18
FCER1A ---:	9.09*	4.08	-2.23
CD83 -----:	5.74*	5.59**	-1.03
OSM ------:	9.43**	9.16***	-1.03
IL8 ------:	24.70***	23.30***	-1.06
IL3RA ----:	36.60***	34.20***	-1.07
TLR2 -----:	3.66	3.62	-1.01
VNN3 -----:	9.39*	9.29**	-1.01
IL1RN ----:	9.89***	10.40***	1.05
CXCR4 ----:	12.80***	13.50***	1.06
CD4 ------:	15.70***	15.90***	1.02
IL1B -----:	11.50***	13.60***	1.18
TREM1 ----:	11.00***	13.10***	1.19
CXCR1 ----:	5.17+	5.99*	1.16
CCL17 ----:	14.70***	16.50***	1.12
CLEC10A --:	16.80***	19.00***	1.13
PDE4B ----:	10.70***	14.60***	1.37
MX1 ------:	2.90	3.33	1.15
CD69 -----:	77.70***	141.00***	1.81
GZMB -----:	8.49*	11.50**	1.35
ITGB2 ----:	13.00***	17.80***	1.36
S100P ----:	3.20	4.10*	1.28
TNFSF10 --:	2.27	2.84	1.25
IL18 -----:	-1.77	-5.51*	-3.11
CXCL12 ---:	-7.03	-62.20*	-8.86
IL37 -----:	-4.51	-5.67	-1.26
CCL7 -----:	-20.00	-91.40**	-4.57



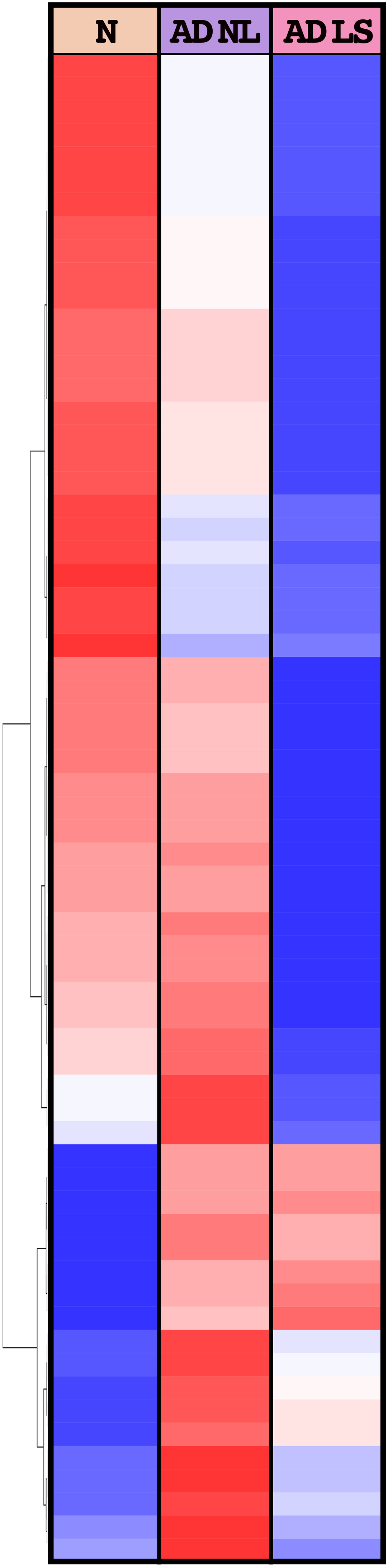
Supplementary Figure 2



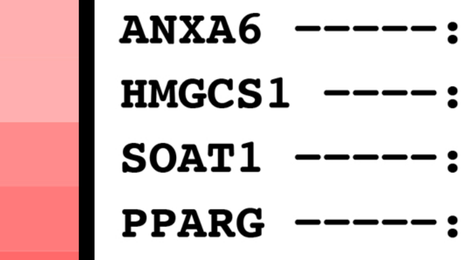
	NL vs N	LS vs N	LS vs NL
LCE1B ---:	-1.62	-1.72	-1.06
LCE1C ---:	-2.18	-2.37	-1.09
CDSN ----:	-6.03	-9.86+	-1.63
FLG2 ----:	-2.70	-4.22*	-1.56
LCE1E ---:	-3.52	-6.95	-1.97
FA2H ----:	-5.45	-14.10	-2.59
ALOXE3 --:	-1.56	-2.41	-1.54
FLG -----:	-4.68	-18.50	-3.96
LCE5A ---:	-4.97	-21.00+	-4.22
ELOVL3 --:	-5.08	-18.90	-3.73
KRT79 ---:	-4.17	-24.40**	-5.86
GAL -----:	-3.93	-19.00+	-4.82
GJB3 ----:	-2.03	-4.32	-2.12
SPTLC3 --:	-1.61	-4.45	-2.76
CLDN8 ---:	-3.04	-34.70+	-11.40
NR2F2 ---:	-3.01	-23.80	-7.89
KRT77 ---:	-1.03	-6.41	-6.22
CDH11 ---:	-1.77	-33.40+	-18.90
SCEL ----:	1.45	-3.99	-5.79
SOAT1 ---:	7.35**	6.55**	-1.12
PPARG ---:	3.68	3.50	-1.05
ELOVL5 --:	17.20***	11.70***	-1.48
ANXA6 ---:	6.13*	4.02+	-1.52
HMGCS1 --:	7.26*	4.35+	-1.67



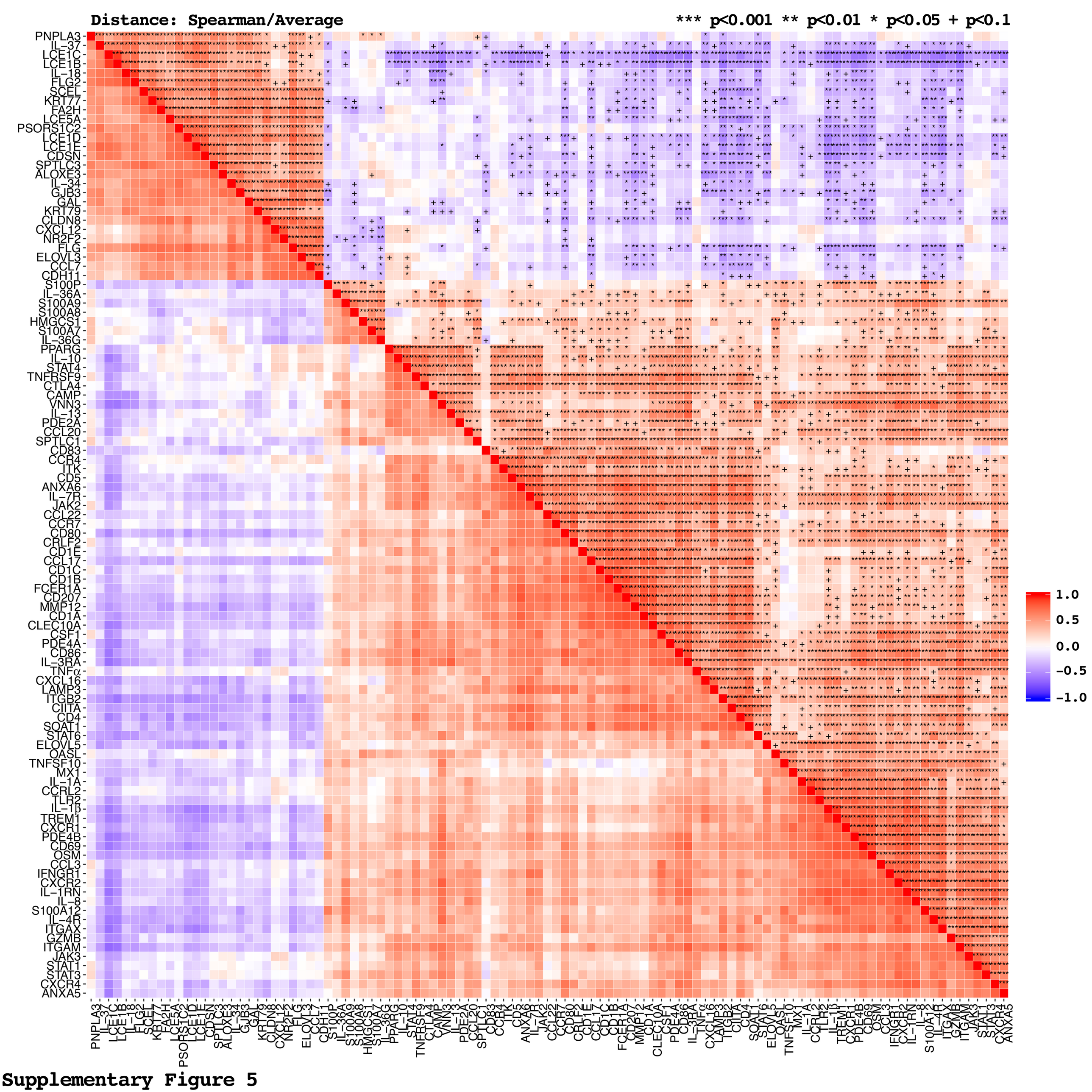
Supplementary Figure 3



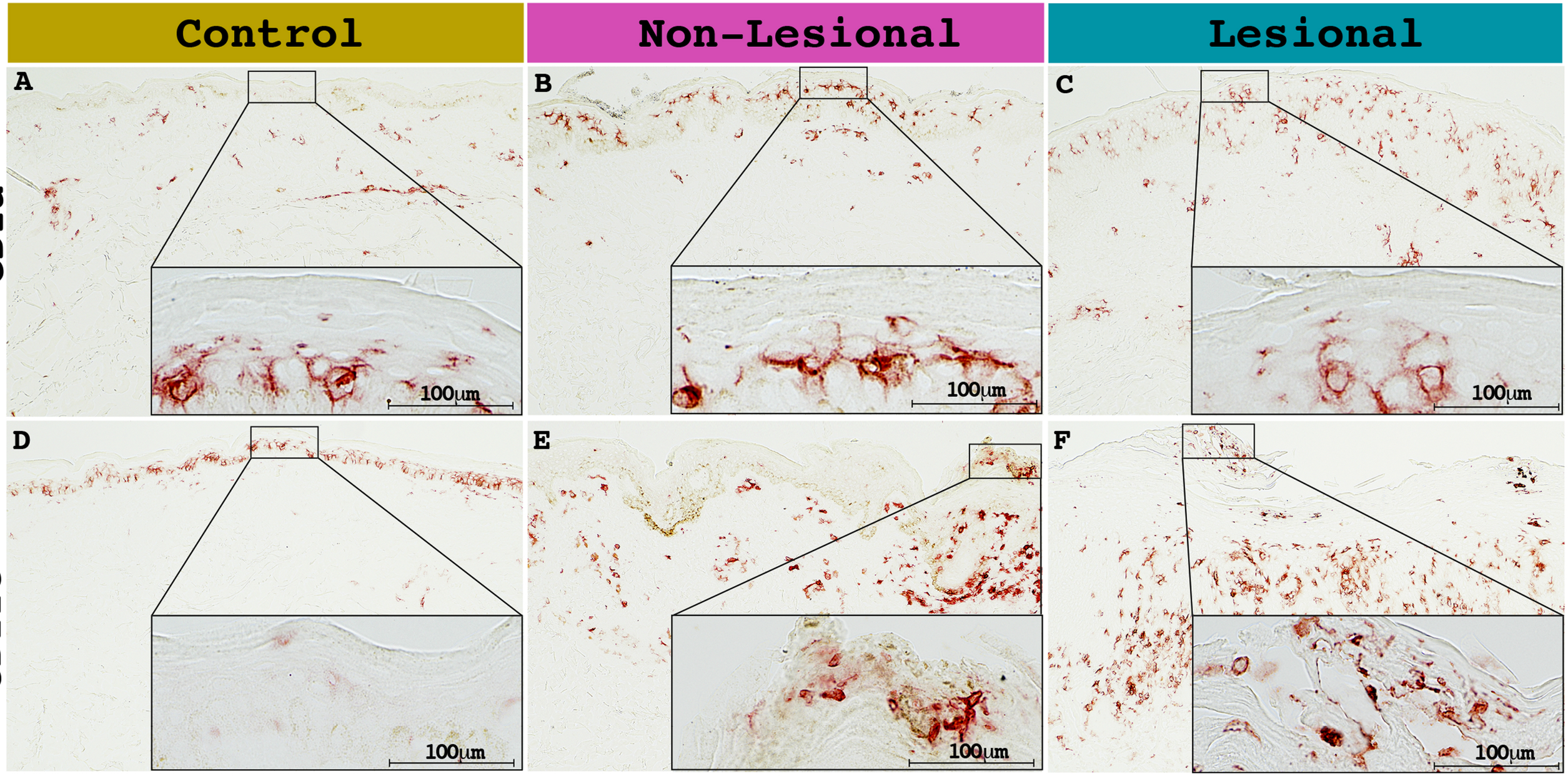
	NL vs N	LS vs N	LS vs NL
KRT77 -----:	-4.90	-21.80***	-4.44
GAL -------:	-5.27	-23.90***	-4.54
DHCR7 -----:	-1.55	-2.37	-1.54
FLG -------:	-3.90	-14.60**	-3.74
KRT79 -----:	-4.66	-17.70***	-3.80
LCE1E -----:	-2.56	-5.39*	-2.11
NR2F2 -----:	-5.37	-20.00**	-3.73
LCE2A -----:	-1.28	-1.71	-1.33
CLDN8 -----:	-3.96	-19.90**	-5.02
GJB3 ------:	-2.47	-6.90*	-2.79
LCE5A -----:	-3.21	-11.40*	-3.56
PPL -------:	-1.21	-1.80	-1.49
LCE2C -----:	-1.21	-1.78	-1.48
LCE2B -----:	-1.23	-1.94	-1.57
PSORS1C2 --:	-1.58	-4.30+	-2.72
SPTLC3 ----:	-1.91	-4.90*	-2.56
LCE1D -----:	-2.08	-6.53+	-3.14
ELOVL3 ----:	-2.32	-8.30*	-3.57
CDH19 -----:	-1.87	-5.39	-2.88
FLG2 ------:	-3.75	-7.62**	-2.03
CDSN ------:	-3.45	-6.61*	-1.91
LCE2D -----:	-1.69	-2.31	-1.37
LCE1B -----:	-2.38	-3.42*	-1.44
ALOXE3 ----:	-2.15	-3.10*	-1.44
FA2H ------:	-7.68+	-20.00***	-2.61
LCE1C -----:	-2.72	-3.37*	-1.24
LCE1F -----:	-1.11	-2.03	-1.84
CLDN7 -----:	-1.09	-1.84	-1.68
SCEL ------:	-1.56	-7.35*	-4.70
CDH11 -----:	-1.74	-10.60*	-6.07
AWAT1 -----:	-1.50	-5.32	-3.54
FADS2 -----:	-1.04	-3.29	-3.15
ACOT2 -----:	-1.03	-2.19	-2.12
PNPLA3 ----:	-1.07	-3.95+	-3.71
CGNL1 -----:	1.02	-1.47	-1.49
GPAM ------:	1.00	-2.44	-2.44
CLDN11 ----:	1.02	-2.61	-2.65
ANXA9 -----:	1.06	-1.35	-1.43
ACER1 -----:	1.07	-1.74	-1.87
KRT33A ----:	1.22	-5.73	-7.02
EVPL ------:	1.09	-1.33	-1.46
CIDEC -----:	1.38	-2.94	-4.05
FADS1 -----:	1.28	-1.60	-2.06
FABP7 -----:	1.70	-3.03	-5.14
KRT23 -----:	1.71	-1.59	-2.73
KRT34 -----:	3.21	-2.54	-8.15
GJB5 ------:	1.90	-1.41	-2.69
ELOVL5 ----:	4.16*	4.18**	1.01
ANXA5 -----:	3.08	3.08+	1.00
CLDN1 -----:	1.99	2.06	1.03
ANXA6 -----:	7.78**	5.79**	-1.34
HMGCS1 ----:	3.51+	2.91*	-1.21
SOAT1 -----:	3.48+	4.16**	1.20
PPARG -----:	3.74	4.88*	1.30
DGAT2 -----:	1.73	2.08	1.20
CERS3 -----:	2.64	1.49	-1.77
LPIN1 -----:	1.76	1.30	-1.35
ORMDL3 ----:	2.98	1.78	-1.67
SPTLC1 ----:	4.40*	2.48	-1.77
KRT2 ------:	2.51	1.82	-1.38
LPL -------:	2.49	1.27	-1.96
FAR2 ------:	3.15	1.36	-2.32
DEGS2 -----:	1.30	1.09	-1.20
CLN8 ------:	2.29	1.09	-2.10
FABP4 -----:	2.34	-1.02	-2.40



Supplementary Figure 4



Supplementary Figure 5



Supplementary Figure 6

Supplementary Table 1. Demographics and clinical severity scores

	AD patients (n=19)	Controls (n=17)	P Value
Age (y), mean (SD)	1.7 (1.75)	1.7 (1.6)	0.91
Female sex, n (%)	5 (26.3%)	12 (75%)	0.02
Clinical severity scores, mean (SD)			
SCORAD	56.5 (17.7)	N/A	
EASI	21.0 (12.5)	N/A	
TEWL, lesional, g/h/m^2	68.4 (50.9)	N/A	
TEWL, non-lesional, g/h/m^2	25.1 (14.4)	N/A	
Pruritus ADQ	17.7 (8.2)	N/A	
Body Surface Area (%)	33.5 (18.0)	N/A	
Patient history			
History of Atopy (%)	4 (21.1)	0 (0)	

Supplementary Table 2. Antibodies used for immunohistochemistry

Antigen	Manufacturer	Clone	Isotype	Dilution
CD11c	BD Biosciences	B-ly6	Mouse IgG1	1:100
CD1a	Abcam	C1A/771	Mouse IgG1	1:2000

Supplementary Table 2. Antibodies used for immunohistochemistry

Antigen	Manufacturer	Clone	Isotype	Dilution
CD11c	BD Biosciences	B-ly6	Mouse IgG1	1:100
CD1a	Abcam	C1A/771	Mouse IgG1	1:2000

Appendix S1

SUPPLEMENTARY TEXT

Supplementary Methods

Each large D-Squame (D102) tape-strip was applied to the antecubital or popliteal fossa for lesional skin. Additionally, normal-appearing skin that is far away from lesions, but in the same extremity, was sampled for non-lesional skin. With gloves, tape-strips were applied with pressure with the Cu-derm pressurizer device (D500). The pressure instrument has a plunger mechanism to ensure even and consistent pressure is applied to each tape-strip. After pressure is applied and released, tape-strips are moved by gloved hands and stuck onto the D-Squame Standard storage cards (D120). Using a pen, small marks were made on the skin after placement of the first tape-strip to ensure consistent placement of each subsequent tape-strip. Cards were immediately stored on dry ice in biohazard specimen bags and then transferred to a -80C freezer until time for shipment. The tapes were pooled together and thoroughly washed with QIAzol for RNA. RNA was extracted from pooled 3-16 tape-strips using the miRNAeasy Mini Kit (Qiagen, Hilden, Germany).

Supplementary Figure Legends

Figure S1

A. Venn diagram depicting unique and common up-regulated (red) and down-regulated (blue) differentially expressed genes/DEGs in lesional and non-lesional atopic dermatitis/AD skin compared to normal skin, as well as in lesional versus non-lesional AD skin. ***B.*** Heatmap of all DEGs by RNA-seq in normal (N), non-lesional (NL), and lesional (LS) AD skin.

Figure S2

Summary heatmap of immune genes in male AD patients and controls. Immune gene expression of multiple pathways in tape-stripped skin of atopic dermatitis/AD and controls using RNA-seq, based on 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 non-lesional (LS vs NL) tape-stripped AD skin.

***FDR<0.001, **FDR<0.01, *FDR<0.05, $^{+}$FDR<0.1

Figure S3

Summary heatmap of epidermal barrier-related genes in male AD patients and controls. Barrier gene expression in tape-stripped skin of atopic dermatitis/AD and controls using RNA-seq, based on 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), lesional vs non-lesional (LS vs NL) tape-stripped AD skin. ***FDR<0.001, **FDR<0.01, *FDR<0.05, $^{+}$FDR<0.1

Figure S4

Summary heatmap of barrier-related genes in tape-stripped skin of atopic dermatitis/AD and controls using RNA-seq. Table shows FCHs in non-lesional versus normal (NL vs N), lesional versus normal (LS vs N), lesional vs non-lesional (LS vs NL) tape-stripped AD skin. ***FDR<0.001, **FDR<0.01, *FDR<0.05, +FDR<0.1

Figure S5

Spearman correlation matrix heatmap consisting of individual up- or down-regulated AD biomarkers of immune and barrier gene-subsets across normal, non-lesional, and lesional AD skin. Blue shades illustrate negative correlations and red shades illustrate positive correlations. Clusters of positive and negative correlations were obtained by unsupervised clustering. +P<0.1; *P<0.05; **P<0.01; ***P<0.001

Figure S6

Representative micrographs of dendritic cells (CD11c^{+}) and Langerhans cells (CD1a^{+}). CD1a^{+} (A-C) and CD11c^{+} (D-F) cells were stained in healthy skin (A, D) and non-lesional (B, E), and lesional (C, F) atopic dermatitis/AD skin and viewed at 10x magnification. 40X magnification inserts illustrate CD1a^{+} or CD11c^{+} cellular infiltrates in the outer epidermal layers. Bar = 100μm.