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Evolution of pathologic T-cell subsets in atopic dermatitis from infancy to adulthood

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Evolution of polar T-cell subsets in healthy controls and atopic dermatitis patients from infancy to adulthood

Normal Cytokine Subset Development

IFN- γ
(CLA+>CLA-
CD4+>CD8+)

IL-13
(CLA+>CLA-
CD4+>CD8+)

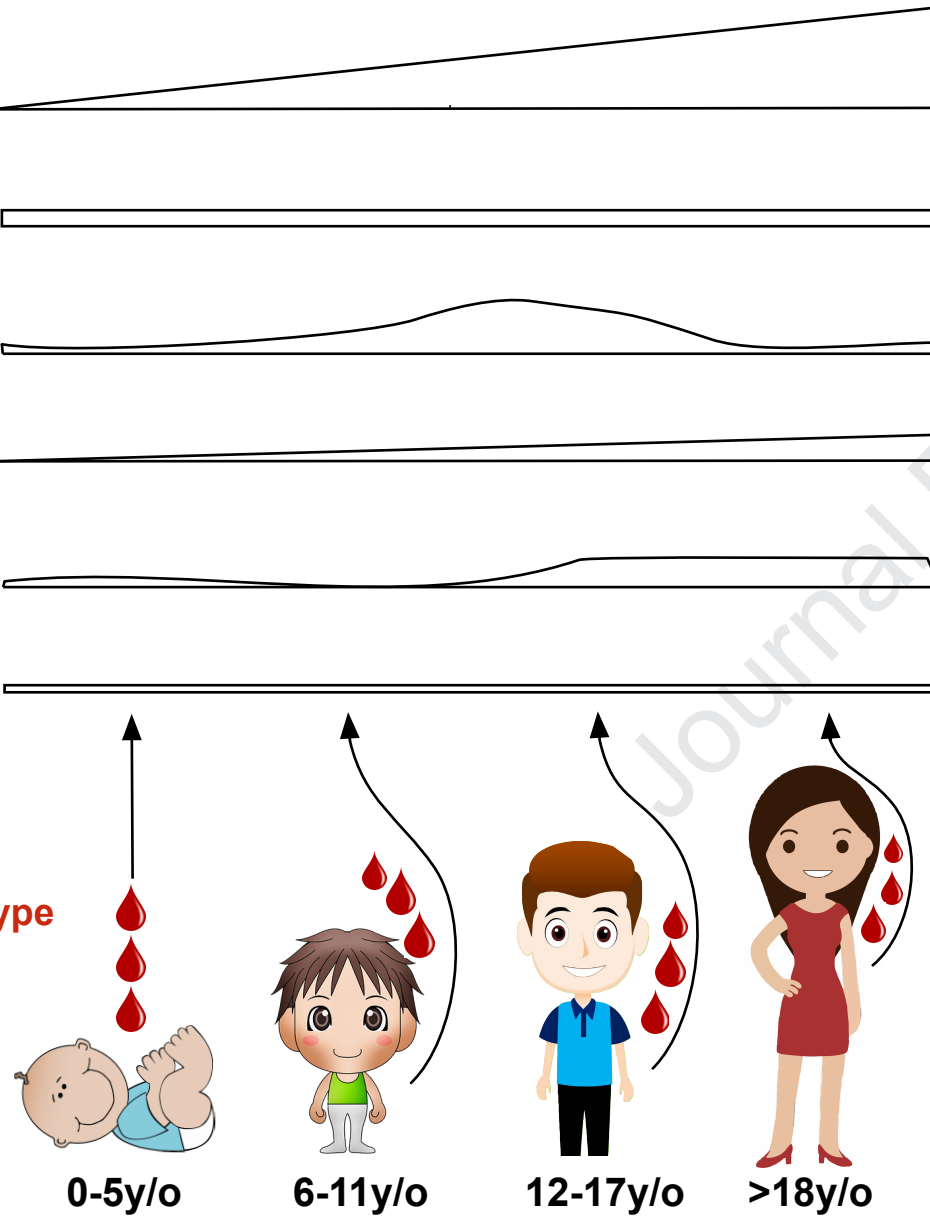
IL-9

IL-17
(CLA-)

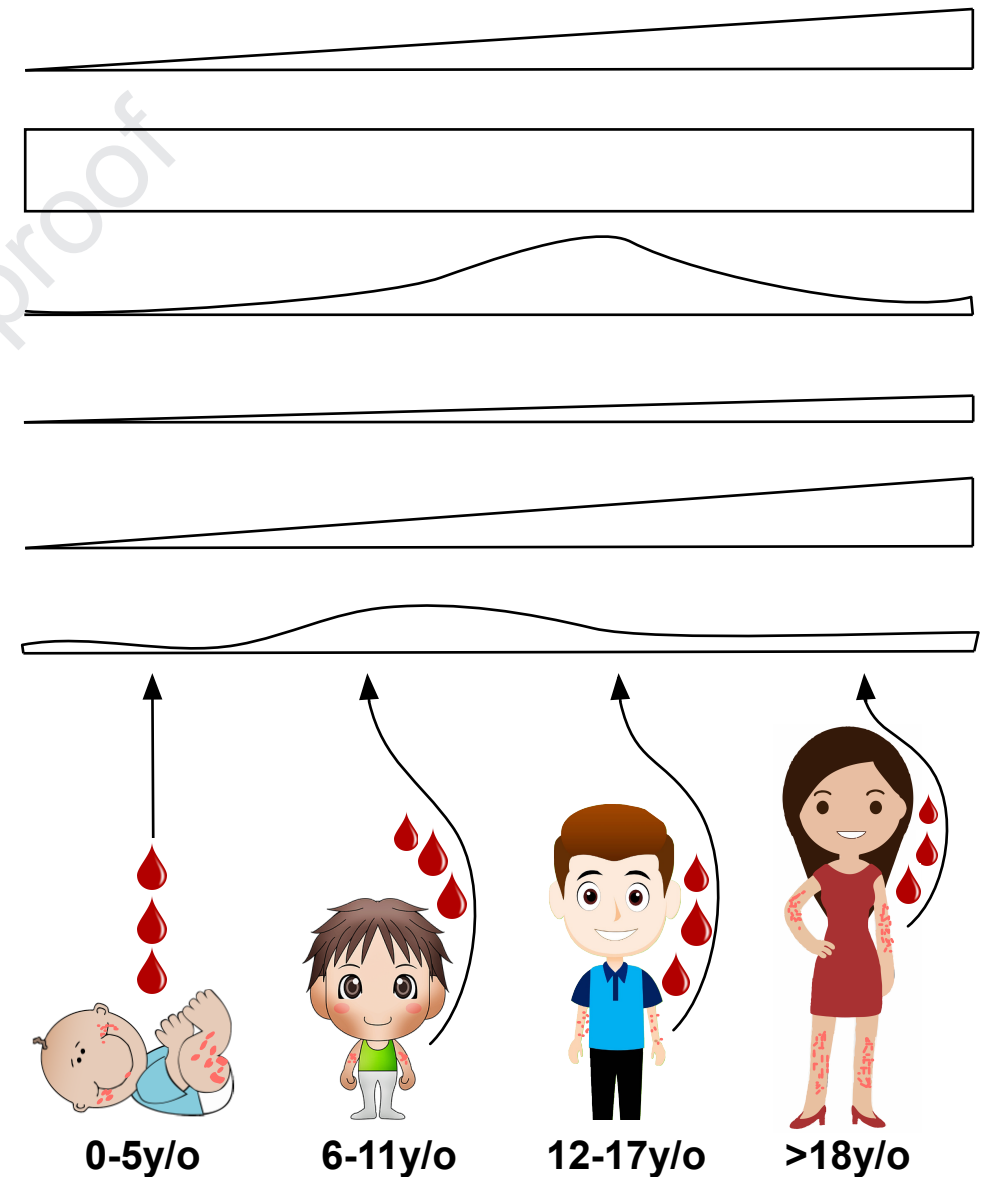
IL-22

Tregs
(CLA+)

**Blood
Phenotype**



AD Cytokine Subset Development



Abbreviations: AD, Atopic dermatitis; CLA, Cutaneous lymphocyte antigen; IFN- γ , Interferon γ ; IL, Interleukin; Tregs, T-regulatory cells

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2 **adulthood**

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46 **Keywords:** Atopic dermatitis, endotypes, T-cell, CLA, IL-13, IL-22, IFN- γ , ICOS,

47 HLA-DR

48

49 **Abstract**

50 **Background:** The circulating immune phenotype was defined in adults and
51 young children with early atopic dermatitis/AD, but chronologic changes in blood
52 of AD infants and children through adolescence have not been explored.

53 **Objective:** To compare immune activation and cytokine polarization in blood of
54 0-5y/o (n=39), 6-11y/o (n=26), 12-17y/o (n=21) and ≥ 18 y/o (n=43) patients with
55 AD vs. age-matched controls.

56 **Methods:** Flow cytometry was used to measure interferon- γ , interleukin (IL)-9,
57 IL-13, IL-17, and IL-22 cytokines in CD4⁺/CD8⁺ T-cells, with ICOS and HLA-DR
58 defining mid- and long-term T-cell activation, respectively, within skin-
59 homing/CLA⁺ vs. systemic/CLA⁻ T-cells. Unsupervised clustering differentiated
60 patients based on their blood biomarker frequencies.

61 **Results:** While CLA⁺ Th1 frequencies were significantly lower in infants with AD
62 vs. all older patients ($P < 0.01$), CLA⁺ Th2 T-cells were similarly expanded across
63 all AD age groups compared with controls ($P < 0.05$). After infancy, CLA⁻ Th2
64 frequencies were increased in AD in all age groups, suggesting systemic immune
65 activation with disease chronicity. IL-22 frequencies serially increased from
66 normal in infants to highly significant levels in adolescents and adults compared
67 to respective controls ($P < 0.01$). Unsupervised clustering aligned the AD profiles
68 along an age-related spectrum from infancy to adulthood (ICOS, IL-22 etc.).

69 **Conclusions:** The adult AD phenotype is achieved only in adulthood. Unique
70 cytokine signatures characterizing individual pediatric age endotypes may require
71 age-specific therapies. Future longitudinal studies, comparing the profile of

cleared vs. persistent pediatric AD, may define age-specific changes that predict AD clearance.

Capsule Summary: Decreased Th1/Th2 ratio characterizes the atopic dermatitis (AD) phenotype across all age groups, while chronic activation, IL-9, IL-22, and Treg changes characterize older patients. Even the adolescent AD profile differs from adult AD.

Clinical Implications: Diverse immune signatures in different pediatric and adult atopic dermatitis age groups argue for age-specific, rather than uniform, therapeutic interventions.

Abbreviations

95	
96	AD: Atopic dermatitis
97	ADQ: Atopic dermatitis quickscore
98	CD69: Cluster of Differentiation 69
99	CLA: Cutaneous Lymphocyte Antigen
100	EASI: Eczema area and severity index
101	HLA-DR: human leukocyte antigen DR
102	IFN- γ : Interferon gamma
103	IL: Interleukin
104	ICOS: Inducible co-stimulator molecule
105	ILC: Innate lymphoid cell
106	MHC: Major histocompatibility complex
107	PBMC: Peripheral Blood Mononuclear Cell
108	SCORAD: SCORing of Atopic Dermatitis
109	Tc1: Type 1 Cytotoxic T-cell
110	Tc17: Type 17 cytotoxic T-cell
111	Tc2: Type 2 cytotoxic T-cell
112	Tc22: Type 22 cytotoxic T-cell
113	TEWL: Transepidermal water loss
114	Tc: T cytotoxic
115	Th: T helper
116	Y/o: Years old
117	

Introduction

Infancy, childhood and adolescence are critical periods for immune system maturation.¹ Early abnormal immune development may cause immune-related disorders. Indeed, 85% of atopic dermatitis (AD) cases present before 5 years of age.² While young adults have a different AD phenotype from elderly patients,³ immune changes in AD between early childhood and adulthood are unknown.⁴

Blood studies that dissect developmental changes from infancy through adulthood are limited and primarily focused on T-helper (Th)1/Th2 subsets. Some studies with healthy controls showed expansion of Th1/Th2/T-cytotoxic (Tc)1 subsets with age,^{5, 6} while others reported no changes in Th2/Tc2 over time.⁷ Immature interferon (IFN)- γ response⁸ and low Th1/Tc1 frequencies⁹⁻¹¹ were seen in early stages of normal development, and abnormal Th1/Th2 ratios in cord blood and infants who develop AD were described.¹²⁻¹⁴ However, few studies compared pediatric and adult AD populations^{6, 15-17} and none directly compared consecutive age groups of AD with age-matched controls, which is critical in understanding normal vs. pathological development of acquired immunity.

The therapeutic arsenal available for 0-12 year old (y/o) AD patients is limited to topical agents and/or broad systemic immune suppressants.^{4, 18} Active development of targeted therapeutics is ongoing for adults and adolescents with AD and will eventually move to children, further necessitating the elucidation of pediatric endotypes at successive pre-adult age groups to introduce safe, effective, age-tailored targeted approaches.¹⁹

We compared T-cell memory subset activation and polarized CD4/CD8 subset frequencies within the skin-homing/cutaneous lymphocyte antigen/CLA⁺ and systemic/CLA⁻ compartments, in the blood of infants and toddlers (0-5y/o), young children (6-11y/o), adolescents (12-17y/o) and adults (≥18y/o) with moderate-to-severe AD. To differentiate pathologic from physiologic immune maturation, age-matched healthy controls were included. We found that decreased Th1/Th2 ratios were shared across all AD ages, but unique fingerprinting characterizes individual AD age groups, differentiating them from their age-matched peers. Our intracellular and T-cell blood biomarker data grouped the AD cohort, but not the controls, into three unique age phenotypes aligned along a spectrum.

Methods

Patient characteristics and blood samples

Blood was obtained (with signed Institutional Review Board-approved informed consent from parents and patients 12 years and older) from 39 infants and toddlers (0-5y/o; mean 23 months), 26 children (6-11y/o; mean 7.5yrs), 21 adolescents (12-17y/o; mean 14.9yrs) and 43 adults (≥18y/o; mean 43yrs) with moderate-to-severe AD, as well as healthy age-matched controls (24-30 in each group; demographic and laboratory data in Table E1). Sensitivity analyses on patient subsets who were matched for all demographic parameters yielded similar results to analysis including all subjects (Table E2A-B).

Disease severity was scored using SCORing of AD (SCORAD) for adults and SCORAD, Eczema Area and Severity Index (EASI), and Atopic Dermatitis Quickscore (ADQ) in those less than 18y/o. ADQ is a parent-administered tool, which assesses involvement and pruritus of 7 body parts and highly correlates with SCORAD.²⁰ In subjects <18y/o, eosinophil counts and skin-barrier assessment through transepidermal water loss (TEWL) of lesional (LS) and non-lesional (NL) arm skin (AquaFlux Model AF200; Biox, London, England)²¹ were measured when feasible. No patients received systemic immunosuppressive treatment within 4 weeks prior to this study, thus the data present baseline immune phenotyping of the studied populations. Concomitant allergic manifestations were recorded, and individuals were classified as either having general allergies (allergic rhinitis/asthma/allergic conjunctivitis/environmental), food allergies (FA), both, and neither. Controls had no personal history of AD. Only 1 child and 5 adolescent controls had history of non-cutaneous atopic manifestations, however sensitivity analysis excluding these subjects did not alter the results (Table E2C).

Isolation of Peripheral Blood Mononuclear Cells (PBMCs)

PBMCs were isolated from whole blood by Ficoll-Paque Plus (GE Healthcare, Sweden). Briefly, the blood was laid under Ficoll gradient; after spinning, PBMCs were collected at the interface between the plasma and the Ficoll gradient (see Methods in the Online Repository/OR).

Stimulation of blood cell populations for cytokine responses

Ex-vivo cell activation is required to detect cytokine production, as less than 1% of non-stimulated cells produce cytokines. Whole blood was incubated with PMA (25ng/ml) plus ionomycin (2µg/ml) in the presence of brefeldin A (10µg/ml) for 4 hours at 37°C to induce cytokine responses. After stimulation, red blood cells were lysed with FACS lysing solution to obtain leukocytes (see Methods in the OR).

Cell surface staining and intracellular staining on PBMCs and stimulated and non-stimulated CD4/CD8 T-cells

Peripheral blood mononuclear cells/PBMCs were stained with fluorochrome-labeled Abs to cell surface markers (CD3, CD8, CD4, CD45RO, CCR7, ICOS, HLA-DR, CLA, CCR4, CD25, CD127). Stimulated and non-stimulated blood cells were also stained for cell surface markers [CD3, CD4, and CLA (CD8⁺ T-cells were gated thorough CD3⁺CD4⁺ T subpopulation)] and permeabilized with FACS/perm to stain for cytokines including Interleukin (IL)-13, IL-22, IL-9, IFN-γ and IL-17 (see Methods in the OR).

Statistical analysis

Statistical analyses were performed with R software (www.R-projects.org). Means and medians were compared using the Welch t-test and Wilcoxon-Mann-Whitney test, respectively. Unsupervised hierarchical clustering of variables (T-cell subset frequencies, age, and clinical scores) was performed using the R package “hclust,” with a McQuitty agglomeration algorithm and Spearman coefficient as a similarity metric and presented as a heat map and a dendrogram. Individual scatter plots were constructed that display Spearman coefficients, 95%

confidence intervals, and p-values for AD and healthy samples. We performed k-means unsupervised clustering across principal components of the frequencies of all AD and separately among all healthy control subsets. We found that 3 clusters separated AD patients, but not controls, along a chronologic age spectrum. ANOVA, in conjunction with the Tukey test, was used to find markers that differentiated any two clusters. P-values and fold-changes (FCH) were designated as ***($p < 0.001$), **($p < 0.01$), *($p < 0.05$), and +($p < 0.1$).

We also performed a power calculation based on our previous flow cytometry study on moderate-to-severe adult AD patients.²² We determined that 39 AD patients would have greater than 90% power (at a significance level of 0.05) to detect differences versus controls in various inflammatory cell subsets ($CD4^+IL-22^+CLA^+$, $CD4^+IL-13^+CLA^-$, $CD8^+IL-17^+CLA^+$, $CD8^+$ Tcm HLA-DR $^+CLA^+$).

Results

Flow cytometry was used to measure frequencies of IFN- γ , IL-9, IL-13, IL-17A and IL-22-polarized T-cells, defining Th1/Tc1, Th9/Tc9, Th2/Tc2, Th17/Tc17 and Th22/Tc22 subsets in $CD4^+/CD8^+$ T-cells, respectively. Cell surface staining was used to assess expression of mid (inducible co-stimulator molecule/ICOS) and late (HLA-DR) activation markers in central (Tcm/CCR7 $^+CD45RO^+$) and effector (Tem/CCR7 $^-CD45RO^+$) memory T-cells in skin homing/cutaneous/ CLA^+ and systemic/ CLA^- compartments.

Patients and controls were divided into 4 consecutive age groups (infants and toddlers 0-5y/o, children 6-11y/o, adolescents 12-17y/o and adults ≥ 18 y/o). To display both healthy vs. pathologic developmental changes and immune abnormalities within each age group vs. controls, we present two types of comparison plots; both contain similar data but focus on either AD patients vs. controls for each age group or AD vs. control across all ages. To better represent the effect of values distribution, the comparison plots presented below contain both the mean (in black) and the median (in red) $\pm$ standard error, and their respective *p*-values. Results discuss the mean values, while median percentages for main comparisons discussed are presented in Table E3.

Skin-homing memory T-cell expansion and ICOS activation feature early

AD

Tcm and Tem T-cells are main components of the adaptive immune system, harboring distinct homing capacities.²³ While both express the skin homing marker CLA, only Tcm cells retain CCR7 positivity, which enables them to migrate into lymph nodes and function as an immunologic reserve.²⁴ After gating on CD3⁺ viable T-cells using flow cytometry, CD3⁺CD4⁺ and CD3⁺CD8⁺ were defined and analyzed separately. CCR7 and CD45RO were used to differentiate memory subsets within the CD4 and CD8 populations. CCR7⁺CD45RO⁻ defined naïve cells, CCR7⁺CD45RO⁺ central memory T-cells (Tcm), CCR7⁻CD45RO⁺ effector memory T-cells (Tem) and CCR7⁻CD45RO⁻ Effector/Temra/terminally differentiated T-cells (Fig. E1A-C). We then further defined activated ICOS/HLA-

DR activated Tcm/Tem memory subset, using CLA to segregate skin-homing (CLA⁺) versus systemic subsets (CLA⁻).

Normal development was characterized by a slight drop of CLA⁺ CD4⁺ Tem (but not Tcm) cells between infancy and childhood (infants 26.7% vs. children 19%, P=0.01; Figs. 1A-B, E1D-E), but this decrease was significantly more evident in AD (P<0.05; Figs. 1A-B, E1D-E). CD4⁺CLA⁺ Tem/Tcm cells were significantly higher in AD infants (Tcm: 20.4% vs. 13.4%, P=0.006; Tem: 38% vs. 26.7%, P=0.002; Fig. E1D-E) and children (Tcm: 18.8% vs. 11.3%, P=0.01; Tem: 27.7% vs. 19%, P=0.03; Fig. E1D-E) than controls, a difference that diminished with increasing age. T-cell memory subset fluctuations with age are presented in Figure E2. Due to increased proportions of effector and naïve cells, CD8⁺ Tcm/Tem subsets dropped between infancy and childhood exclusively in controls (Tcm: infants 12.8% vs. children 5.6%, P=0.008; Tem: infants 15.4% vs. children 5%, P<0.001; Fig. 1C-D), leading to higher frequencies in children with AD (P<0.01), but otherwise frequencies were overall similar between AD and controls over time (Fig. E1F-G). Significant CD4⁺/CD8⁺ effector cells increase with age characterized AD vs. controls, who showed decrease of this subset during the years (Fig. E2J, L).

CD4⁺ Tcm/Tem ICOS mid-activation continuously increased in both controls and AD, however it decreased significantly between adolescence and adulthood, exclusively in controls (Tcm CLA⁺: 24% vs. 9%, P=0.003; CLA⁻: 9.2% vs. 3.7%, P=0.001; Fig. 1E-H). Both skin-homing/CLA⁺ and systemic/CLA⁻ ICOS-activated CD4⁺ T-cell frequencies were significantly increased in infants and

adults with AD vs. respective controls ($P < 0.05$; Fig. 1E-H). Skin-homing $CD8^+$ Tcm/Tem ICOS activation increased gradually, most notably in AD patients (Fig 1I-L), with frequency differences uniquely seen in adults with AD vs. controls (Tcm: 18.4% vs. 12.5%, $P = 0.01$; Tem: 26% vs. 13.4%, $P < 0.001$; Fig. 1I-L).

The HLA-DR antigen, indicating chronic activation,²⁵ had similar (or even lower) expression in infants with and without AD ($P > 0.1$; Fig. E1H-K), but started to rise in AD children ($Tcm^+CD4^+CLA^+$ AD: 15.8% vs. 8.4%, $P = 0.02$; CLA^- : 7.2% vs. 2.8%, $P = 0.06$; Fig. E1H-I), reaching consistently high levels across $CD4^+/CD8^+/CLA^+/CLA^-$ Tcm/Tem cells in adults with AD compared to healthy individuals ($P < 0.05$; $CD8^+$ Tcm/Tem data not shown; Fig. E1H-K).

Decreased Th1/Th2 ratio characterizes AD across ages

Since T-cell activation leads to cytokine polarization we next studied different polar T-cell subsets. Representative flow cytometry plots and gating strategy are presented in Figure E3. Congruent with past publications,^{9, 10} IFN- γ levels increased with age in both control and AD subjects, with the lowest frequencies seen in infants (Fig. 2A-D). Nevertheless, in AD IFN- γ did not reach control levels, particularly within the skin homing compartment ($CD4^+CLA^+$: infants control 12% vs. AD 7.7%, $P = 0.04$; children control 19.5% vs. AD 13.6%, $P = 0.05$; adolescents control 18% vs. AD 13%, $P = 0.07$) until adulthood ($P = 0.2$; Figs. 2A-D, E4A-D). Even in adulthood, levels trended towards lower frequencies. Interestingly, the only population that showed lower systemic/ CLA^- $CD4^+/CD8^+$ IFN- γ was the 5-12y/o age group ($CD4^+$: control 20% vs. AD 14%, $P = 0.04$; $CD8^+$: control 39% vs. AD 28%, $P = 0.05$; Fig. E4B, D).

In controls, IL-13⁺CD4⁺CLA⁺ was lowest in infants, reaching a plateau in childhood. Conversely, in AD patients, levels were similarly elevated across all ages ($P>0.1$; Fig. 2E). Systemic/CLA⁻ Th2 was overall low in controls (Fig. 2F). Levels were significantly higher in AD than controls in children (2.8% vs. 0.6%, $P=0.005$; Fig. E4F), adolescents (1.5% vs. 0.7%, $P=0.006$; Fig. E4F) and adults (1.3% vs. 0.7%, $P=0.005$; Fig. E4F). While Tc2 cells were slightly higher in children with vs. without AD, differences in CD8⁺ subsets were more prominent in adulthood (Figs. 2G-H, E4G-H). Reflecting the Th1 and Th2 imbalances characterizing AD, the Th1/Th2 ratio was decreased in AD than controls in both CLA⁺/CLA⁻ subsets across the ages. Tc1/Tc2 was significantly lower in children and adults with AD vs. controls (Figs. 2I-L, E4I-L).

Both healthy controls and AD patients showed Th9 increases over time, peaking in adolescence and dropping in adulthood (Figs. 3A-B, E5A-B). No Th9 differences were observed between AD and controls, besides 5-12y/o children with AD that showed significantly increased CLA⁻ levels (0.76% vs. 0.3%, $P=0.04$; Fig. E5B). This AD age group also showed increased CLA⁺/CLA⁻ Tc9 levels (Figs. 3C-D, E5C-D). CLA⁺ Th17 cells were generally stable and similarly abundant across ages among control and AD (Figs. 3E, E5E), contrary to CLA⁻ Th17 cells which showed developmental expansion in both (Figs. 3F, E5F). Contrary to adolescents with AD that had lower CLA⁺ Tc17 than controls (1% vs. 4.7%, $P=0.04$), adults had significantly higher frequencies (2.1% vs. 1.4%, $P=0.05$; Figs. 3G-H, E5G-H). Systemic/CLA⁻ CD4⁺/CD8⁺ IL-22⁺ similarly increased with age in both controls and AD (Fig. 3J, 3L, E5J, E5L), while skin-

homing Th22/Tc22 cells increased with age, primarily in AD (Figs. 3I, 3K, E5I, E5K). Starting in childhood, skin-homing Th22/Tc22 were significantly higher in AD vs. controls, and incrementally increased with age (Th22: children 6.7% vs. 4%, $P=0.07$; adolescents 7.9% vs. 3.6%, $P=0.001$; adults 8.2% vs. 4.4%, $P<0.0001$; Figs. 3I, 3K, E5I, E5K). Polar T-cell subset development in controls and AD is summarized in Table E6 and illustrated in the graphical abstract.

The unsupervised hierarchical clustering heatmap in Figure 4 summarizes the above, displaying all polarized T-cell subsets for control and AD across age groups (red: positive/increase; blue: negative/decrease). Fold-changes (FCHs) of the mean frequencies of AD vs. controls for each age group are presented (stars and plus signs display significance). The green cluster includes subsets that were relatively low and stable among controls, but incrementally increased with age in AD. Most of these subsets were significantly increased in adults with AD vs. controls ($FCH>1.57$, $P<0.05$), while younger AD patients showed lower or no significance. The pink box shows increased IL-9 frequencies in childhood, which decline in adulthood, particularly in AD. The yellow cluster shows markers that increased in both controls and AD, therefore minimizing the differences between groups.

T-cell activation, clinical measures and IFN- γ are associated with age and AD chronicity

We also evaluated how clinical characteristics, including AD severity (SCORAD, EASI), patient age, disease duration, eosinophil counts, pruritus and TEWL relate to different polar T-cell subsets. Unsupervised hierarchical clustering of all T-cell

subset frequencies, clinical scores, AD duration and age was performed using Spearman correlations as a similarity metric, as displayed in the correlation heatmap and dendrogram in Figure 5 (red: positive correlation; blue: negative correlation, stars and plus signs display significance). Congruent with recent AD data,^{22, 26} which showed positive correlations between IL-13- and IL-22- and between IL-17- and IL-22-producing T-cells,^{22, 26, 27} these cytokines largely grouped together (turquoise rectangle). IFN- γ -producing T-cells clustered together with AD duration and patient age (yellow rectangles). A tight cluster gathering multiple clinical measures (green rectangles) was located adjacent to IL-9/IL-13-producing T-cells (purple rectangles). Significant correlations depicted in this heatmap are listed in Table E4, with selected individual scatter plots presented in Figures 6-7 and E6-E8.

SCORAD and EASI were positively correlated ($r=0.74$, $P<0.001$; Fig. 6A). SCORAD also correlated with skin-homing CD4⁺ cells ($r=0.24$, $P=0.0016$; Fig. 6B), pruritus ($r=0.54$, $P<0.0001$, Fig. 6C) and eosinophils ($r=0.25$, $P=0.001$; Fig. 6D). To evaluate normal vs. pathologic development with age, we comprehensively assessed age correlations in control and AD subjects, presenting both on the same scatter plots for clarity, when applicable. Age correlated positively with severity by SCORAD ($r=0.25$, $P=0.0016$; Fig. 6E) and EASI ($r=0.34$, $P<0.001$; Fig. 6F), and with all other clinical measures, including pruritus (Fig. 6G), eosinophils (Fig. 6H), and lesional TEWL (Fig. 6I-J).

While skin homing Tcm/Tem cells correlated negatively with age (red=AD, blue=controls; Fig 6K-L), the proportion of ICOS and HLA-DR-activated skin-

homing T-cells increased exclusively in AD (Fig. E6A-D). AD duration, which highly correlated with patients' age ($r=0.98$, $P<0.0001$; Fig. E6E), also demonstrated significant positive correlations with EASI and pruritus (Fig. E6F-H). Among the cytokine subsets (Fig. E6I-L), the most significant correlation was noted between AD duration and IFN- γ -producing cells ($r=0.58$, $P<0.0001$; Fig. E6L).

Overall IFN- γ increased with age in both AD and controls (Fig. E7A-D), however, although the Th1/Th2 (but not the Tc1/Tc2) ratio, and particularly its skin-homing component, increased with age in both groups, it remained significantly decreased in AD across all ages vs. controls (Fig. 7A-D). Conversely, differences between AD and controls were observed in skin-homing Th2/Tc2 throughout development, with controls never reaching AD levels (Fig. 7E-H). CLA⁺ Th22/Tc22 showed significantly higher developmental increases in AD (Fig. 7I, K), while systemic subsets generally overlapped (Fig. 7J, L). Similar negative trends of IL-9⁺ (Fig. E7E-H), and expansion of systemic Th17 cells were seen in controls and AD subjects (Fig. E7I-L).

Positive correlations between SCORAD and IL-13, IL-9 and IL-22-producing cells were recorded ($P<0.02$; Fig. E8A-C). EASI correlated with Th9, Th22 and pruritus ($P<0.03$; Fig. E8D-F), while pruritus was associated with Th22 and eosinophil counts ($P<0.032$; Fig. E8G-H). TEWL correlated with CD4⁺CLA⁺ cells, only in non-lesional skin (Fig. E8I-J). Th22 positively correlated with TEWL in lesional tissues (Fig. E8K-L), and AD severity correlated with TEWL in non-lesional skin (Fig. E8M-P).

Because Foxp3 staining requires cell permeabilization, surface markers were used for Treg identification. 90% of CD25⁺CD127⁻CCR4⁺ cells co-express Foxp3, therefore CD25⁺CD127⁻CCR4⁺ phenotype defined Tregs.²⁸ In analyzing T-regulatory cells (Tregs), both total Treg (CD25⁺CD127⁻) and skin-homing (CCR4⁺CLA⁺ fraction) Treg frequencies were captured. The only AD age group that showed significant increases of both total (Fig. E9A) and skin-homing (Fig E9B) Tregs vs. control was 5-12y/o (P<0.01). Developmentally, AD showed similar total Treg trends as controls, whereas discrepancies were more evident in skin-homing subsets (Fig. E9C-D).

Cytokine polarization and T-cell activation differentiate AD patients into separate age clusters along a spectrum

We integrated the T-cell and cytokine biomarkers to differentiate the entire AD cohort based on their blood phenotype. The principal components of all biomarker data for all subjects were analyzed using unsupervised k-means clustering, separately for AD patients and controls. As illustrated in Figure 8, in the AD cohort, the frequencies of different markers defined three meaningful clusters, aligning along a spectrum. While infants (pink ellipse) clustered on the far left, and adults (green ellipse) on the right, children and adolescents (blue ellipse) generally clustered together, between the infants and adults. The markers that best distinguished between each set of clusters appear in the boxes between the two cohorts and are summarized in Table E5. Th1/Th2 ratio, CD8⁺ activation and IFN-γ-producing T-cells differentiated adults from younger groups. Tregs, T-cell activation, and different cytokine subsets were able to differentiate

distinctive AD age groups. Applying the same model to the control population did not distinguish between age groups (Fig. 8).

Discussion

This is the first comprehensive study that compares systemic immune profiles of different AD age groups (0-5y/o, 6-11y/o, 12-17y/o and ≥ 18 y/o), with appropriate comparisons to controls. Since circulating CLA⁺ T-cells have been suggested as peripheral biomarkers in AD,²⁹ we utilized CLA to segregate between skin-homing and systemic immune changes that accompany healthy and AD development. Our data suggest unique endotypes in infants and toddlers, children, adolescents and adults with AD, possibly advocating for personalized, endotype-driven approaches rather than “one-size-fits-all” therapeutic strategies. In addition, the results start to define age-specific differences between pediatric AD patients and age-matched controls as a complement to our recent data showing differences between elderly vs. young AD adult endotypes.³ Our data suggest that even adolescents have a different profile from adults, possibly contributing to the somewhat lower response to the recently approved IL-4R α antagonist, dupilumab, in adolescents vs. adults.^{30, 31}

Immune responses at birth are immature¹⁰ with decreases of naïve T-cells in healthy children with increasing age, paralleled by increases in Tcm/Tem cells.³² Skin-homing Tcm/Tem cells are higher in infants and children with AD compared to controls and decrease with age, exclusively in AD. Elevated skin-homing memory cells cluster AD infants separately from other age groups,

suggesting early relevance. Excessive naïve-to-memory switch, which causes an influx of memory cells into the circulation in infancy and early childhood, may play a role in AD initiation, while exclusive decreases in the AD population over time may suggest increased migration of CLA⁺ Tcm/Tem cells to skin.

ICOS is involved in multiple adaptive immune responses, including induction of various T-cell polarizations,³³⁻³⁵ Treg regulation,³⁶ antibody responses (including IgE switching),³⁷ and innate lymphoid cell (ILC)2-mediated cytokine production.^{38, 39} ICOS has also been implicated in the pathogenesis of asthma.^{40, 41} We have previously shown that Tcm/Tem ICOS activation is higher in AD vs. control children.⁴² Subsequent data showed accelerated systemic/CLA⁻ ICOS T-cell activation in 0-3y/o AD infants.⁴³ Our present data support ICOS expansion in both healthy and AD development, particularly in CD4⁺ T-cells. However, positive correlations with age were exclusively seen in AD, and specifically in skin-homing memory subsets. Also, infants and adults with AD have higher CD4⁺CLA⁺/CLA⁻ ICOS activation than controls, a difference that is also significant for CD8⁺ subsets in children. The low CD8⁺ frequencies in infants, which increase with time,¹⁰ may explain the earlier activation of CD4⁺ subsets. Early involvement of ICOS in AD indicates its possible role in disease induction via either stimulation of T-cell subset polarization, Treg modulation, IgE induction, or ILC2s activation. Furthermore, targeting ICOS/ICOS-ligand in an asthma mouse model altered T-cell differentiation and ameliorated asthma.⁴⁴ Since AD often precedes asthma,⁴⁵ CLA⁻ ICOS expansion in AD infants may also be associated with non-cutaneous atopic manifestations. These data suggest the

potential for ICOS targeting as a preventive or therapeutic approach for AD and atopic associations, though this is speculative and further studies are needed.

The chronic T-cell activation MHC class II antigen, HLA-DR,⁴⁶ increases with age in healthy individuals.^{47, 48} HLA-DR activation has been shown in chronic adult AD.⁴² While it is not elevated in AD infants, its activation increases with age.

At birth, immune responses are Th2-biased, with low Th1/IFN- γ in both healthy newborns^{8, 9, 49, 50} and those who eventually develop AD.¹³ Our data shows that Th1 frequencies increase with age in both AD and control infants. However, and congruent with recent blood data,⁵¹ AD subjects display significantly lower IFN- γ frequencies than controls, particularly among CD4⁺CLA⁺ subsets. Conversely, CLA⁺Th2 cells are similarly increased across all AD age groups and are significantly higher than controls, even in infants. Systemic/CLA⁻ Th2 cells are significantly higher in AD starting in childhood, which implies systemic Th2 activation with greater chronicity. Consistent with the less dominant role of CD8 suggested in children with early AD⁴² and low CD8 abundance in infants,¹⁰ Tc2 elevations are mostly prominent in adult AD. CLA⁺ Th1/Th2 ratio correlation with age that is consistently decreased in AD compared to control, and the decreased CLA⁺/CLA⁻ Th1/Th2 ratio among all AD age groups best reflect the immune imbalance that accompanies AD from birth to adulthood.

Circulating Th9 cells have skin-homing predilection.^{52, 53} IL-9 is elevated in both skin and serum of children with AD, and was also correlated with disease severity.^{54, 55} While the mechanism by which IL-9 contributes to AD is unknown, IL-9 is linked with mast cell, eosinophil and ILC function and has been thought to

play a role in AD,⁵⁶ as well as in other atopic disorders.⁵⁷⁻⁶⁰ Accordingly, IL-9 subsets cluster closest to all AD clinical measures (eosinophils/pruritus/SCORAD/EASI/TEWL). Both normal and AD development are characterized by IL-9 increases with age, which drop in adulthood. We show considerably elevated systemic/CLA⁺ T-cells exclusively among the 5-12y/o AD group vs. controls, consistent with early allergy development in childhood with a subsequent decline in adulthood.^{61, 62} CLA⁺ systemic activation and cytokine polarization with age support the systemic nature of AD and need for systemic therapeutic approaches in moderate-to-severe disease, as well as the association of comorbidities and increased inflammatory and cardiovascular-associated proteins in more chronic AD.⁶³⁻⁶⁶

Black et al.⁶⁷ showed strong Th17 skewing early in life. While similar CLA⁺Th17 frequencies are seen in infants and older individuals regardless of AD, systemic/CLA⁺ Th17 cells are relatively low in infants of both groups and mature with time. IL-17 signatures characterize pediatric AD skin,⁵⁵ but these changes are not mirrored in the circulation. This discrepancy may be due to increased skin residence of Th17 cells, or by reciprocal peripheral regulation of Th17 and Th2 axes.^{68, 69}

Skin-homing Th22 is elevated in children, adolescents and adults with AD vs. controls. Congruent with past studies showing that IL-22 is correlated with AD severity,⁷⁰ we present positive correlations between IL-22-producing CD4⁺ cells, SCORAD, EASI and pruritus. CD8⁺ T-cells are major producers of IL-22 in adult AD skin.⁷⁰ Lower proportions of CD8⁺ vs. CD4⁺ in early childhood^{47, 71} and the

fact that IL-22 marks AD chronicity⁴² may explain why skin-homing Th22/Tc22 cell frequencies are highest in adolescents and adults. Elevated IL-22 levels, shown to negatively regulate IFN- γ ,⁷² may also contribute to the low Th1 frequencies in AD. Systemic/CLA⁺ Th22/Tc22 subset development was similar in AD and controls, potentially suggesting a greater pathogenic relevance of IL-22 in AD skin⁷³ and the impaired skin barrier.⁷⁴ Studies have shown positive correlations and cellular co-production of IL-13 and IL-22^{22, 75} and of IL-17 and IL-22.^{27, 76} Indeed, these markers cluster together in the correlation heatmap. IFN- γ clusters with patient age and disease duration, differentiating infants from children and adolescents, and adolescents from AD adults. The association of IFN- γ with disease chronicity agrees with previous chronic adult AD data, suggesting that IFN- γ possibly plays a role in inflammatory disorders chronicity, rather than in disease initiation.^{26, 42, 77} Expansion of CD8⁺ memory cells, increases in CLA⁺IL-22-producing cells, and intensification of ICOS⁺/HLA-DR⁺ activation with maturity may account for the positive correlations between age, disease duration and severity measures. Increasing severity with age was not seen in a recent study that followed AD infants only between 0-11 months.⁷⁸ TEWL increases with age in AD and correlates with IL-22 (which also increases with age), likely again reflecting the contribution of IL-22 to the barrier impairment in AD.

Besides maintaining immune tolerance,⁷⁹ Tregs influence activation of effector cells.^{80, 81} Only 5-12y/o children with AD show significantly higher total and skin homing Tregs than controls. This age group is characterized by other

unique features, including increased skin-homing Tcm/Tem cells, decreased systemic IFN- γ , and increased systemic IL-9 frequencies. Additionally, skin-homing IFN- γ is almost doubled from infancy to childhood AD, although still below age-matched controls. Multiple immunological changes occurring during these years may be involved in AD clearance, or development of non-cutaneous manifestations, commonly occurring during these years.^{82, 83}

Profiling AD across ages is imperative for targeted therapeutic development. Unlike psoriasis, in which targeted treatments lead to remarkable responses in most patients, AD responses to targeted therapeutics are much lower.⁸⁴ This disparity may be attributed to the multi-cytokine activation in AD, despite the shared Th2 activation, vs. the Th17-centered responses in psoriasis.⁸⁴ Additionally, AD has a highly varied endotype repertoire, with different immune polarizations.^{3, 85, 86} Despite common features, particularly elevated Th2 expression, AD is endotypically different across ages, and treatments should rather be tailored to the unique age endotype. While one could hypothesize that the immune changes merely reflect developmental phenomena that are age-related, the lack of clear clustering in controls implicates AD as the driver of the distinct, progressive age-related endotypic characteristics rather than age alone. The clustering model, based on the flow cytometry biomarkers splits the entire cohort into 3 separate age clusters only in AD patients. Expectedly, IFN- γ , IL-22, HLA-DR increased chronologically, distinguishing older subjects from infants with AD. Skin-homing Tcm/Tem cells and systemic ICOS activation, both higher in AD infants, separate infants from other groups. This

model demonstrates that a limited group of blood biomarkers can distinguish among various AD endotypes based on patient age, with a spectrum of age-dependent vs. only 'infantile' or 'adult' phenotypes. Because our data show that the 'adult' or 'stable' AD phenotype is only achieved in adulthood, it may be possible to intervene before adulthood and prevent the establishment of the adult AD phenotype.

This study has a few limitations. Despite inclusion of different age groups, this study was not longitudinal and thus did not follow the same cohort with time. Additionally, studies in blood do not allow one to measure T-cell subsets in skin, such as tissue-resident memory/Trm T-cells. Furthermore, the study characterized polyclonal T-cell responses, and not antigen-specific responses induced by culprit triggers. Finally, the pathogenicity of immune axes presented here cannot be further dissected without future targeted therapeutic studies to check mechanism.

AD was initially considered an early-onset pediatric disease with 75% "outgrowing" their disease by 10y/o.^{2, 83, 87, 88} More recent studies have established AD as a disorder that often persists into adulthood.^{89, 90} Comparing the profile of cleared vs. persistent pediatric AD, ideally through longitudinal studies, will better define age-specific characteristics that predict AD clearance.

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866 **Figure legends**

867 **Figure 1. A-D)** The frequency of CLA⁺ Tem (CD45RO⁺CCR7⁻) and Tcm
 868 (CD45RO⁺CCR7⁺) in CD4⁺/CD8⁺ T cells and **(E-L)** ICOS⁺ activation in CLA^{+/+}
 869 CD4⁺/CD8⁺ Tcm/Tem cells in healthy controls and AD patients across ages
 870 (ICOS+CLA+CD45RO+CCR7+/-). Bar plots represent means (black)/medians
 871 (red) ± SEMs. AD, Atopic dermatitis; CLA, Cutaneous lymphocyte antigen; SEM,
 872 Standard error of the mean/median; Tcm, central memory T-cells; Tem, effector
 873 memory T-cells. *P*-values are designated as ***<0.001, **<0.01, *<0.05, and
 874 ⁺<0.1.

875 **Figure 2. A-D)** IFN-γ⁺, **(E-H)** IL-13⁺, and **(I-L)** IFN-γ⁺/IL-13⁺ cytokine frequencies
 876 in CLA⁺, CLA⁻, CD4⁺ and CD8⁺ T-cells in healthy controls and AD patients across
 877 ages. The ratio between the % of IFN-γ⁺CLA⁺CD4⁺ (or CD8⁺) T cells and the %
 878 of their IL-13⁺ counterpart was calculated for each sample but was not multiplied
 879 by 100 and is therefore unitless. Bar plots represent means (black)/medians (red)
 880 ± SEMs. AD, Atopic dermatitis; CLA, Cutaneous lymphocyte antigen; SEM,
 881 Standard error of the mean/median. *P*-values are designated as ***<0.001,
 882 **<0.01, *<0.05, and ⁺<0.1.

883 **Figure 3. A-D)** IL-9⁺ and **(E-H)** IL-17⁺ and **(I-L)** IL-22⁺ frequencies in CLA⁺, CLA⁻,
 884 CD4⁺ and CD8⁺ T-cells in healthy controls and AD patients across ages. Bar
 885 plots represent means (black)/medians (red) ± SEMs. AD, Atopic dermatitis;
 886 CLA, Cutaneous lymphocyte antigen; SEM, Standard error of the mean/median.
 887 *P*-values are designated as ***<0.001, **<0.01, *<0.05, and ⁺<0.1.

Figure 4. Unsupervised hierarchical clustering heatmap displaying polarized T-cell subsets for control and AD across age groups (red: positive/increase; blue: negative/decrease). Fold-changes (FCHs) of the mean frequencies of AD vs. controls for each age group are listed on the right (stars and plus signs display significance). The green cluster includes subsets that were relatively low and stable among controls, but incrementally increased with age in AD. The pink box shows increased IL-9 frequencies in childhood, which decline in adulthood, particularly in AD. The yellow cluster shows markers that increased in both controls and AD.

Figure 5. Unsupervised hierarchical clustering of polarized cytokine subset frequencies (%) with AD clinical measures using Spearman correlation as a similarity metric. CD4⁺/CD8⁺ IL-13⁺, IL-17⁺ and IL-22⁺ subsets clustered together (turquoise box). IFN- γ ⁺-producing T-cell subsets grouped together with age and disease duration (yellow box). IL-9⁺ and some IL-13⁺ and IL-22⁺ T-cells (purple box) clustered adjacent to AD clinical measures (green box). The heatmap shows the positive (*red*) or negative (*blue*) correlations of all parameters, with color intensity reflecting the strength of the correlation (-1 to +1). Dendrograms are shown as a tree, representing the distance between variables. CLA, Cutaneous lymphocyte antigen. *P*-values are designated as ***<0.01, **<0.01, *<0.05, and +<0.1.

Figure 6. Spearman correlation scatter plots (linear regression [red=AD/blue=control line] with their CI [in gray]) for **(A-E)** SCORAD and **(F-L)** age versus clinical measures and Tem/Tcm subset frequencies (%). Dots colors

in plots A-D, F designate different AD patient ages from infancy to adulthood, as shown in Figure 1. CI, Confidence interval; CLA, Cutaneous lymphocyte antigen; EASI, Eczema area and severity index; LS, Lesional; NL, Non-lesional; SCORAD, SCORing of Atopic Dermatitis; Tcm, Central memory T-cells; Tem, Effector memory T-cells; TEWL, Transepidermal water loss.

Figure 7. Spearman correlation scatter plots (linear regression [red=AD/blue=control line] with their CI [in gray]) for CD4⁺/CD8⁺, CLA⁺/CLA⁻ (A-D) IFN- γ ⁺/IL-13⁺ producing T-cell ratio, (E-H) IL-13⁺, and (I-L) IL-22⁺ producing T-cell frequencies (%) versus age in AD patients (red dots/line) and controls (blue dots/line). CI, Confidence interval; CLA, Cutaneous lymphocyte antigen.

Figure 8. Unsupervised clustering of AD patients and healthy controls across all principal components of the blood flow cytometry marker frequencies (%) by k-means. In AD, the frequencies of different markers defined three meaningful age-clusters, aligning along a spectrum. While infants (pink ellipse) clustered on the far left, and adults (green ellipse) on the right, children and adolescents (blue ellipse) clustered together between the other age cohorts. The markers that best distinguished between clusters appear in the boxes between two cohorts (colors of markers parallel the colors of the relative age group). Arrows designate elevated frequencies of a given marker among the specific age group. In healthy controls, clusters did not clearly align patients along an age spectrum. CLA, Cutaneous lymphocyte antigen; ICOS, Inducible co-stimulator molecule; Tregs, T-regulatory cells.

Figure 1

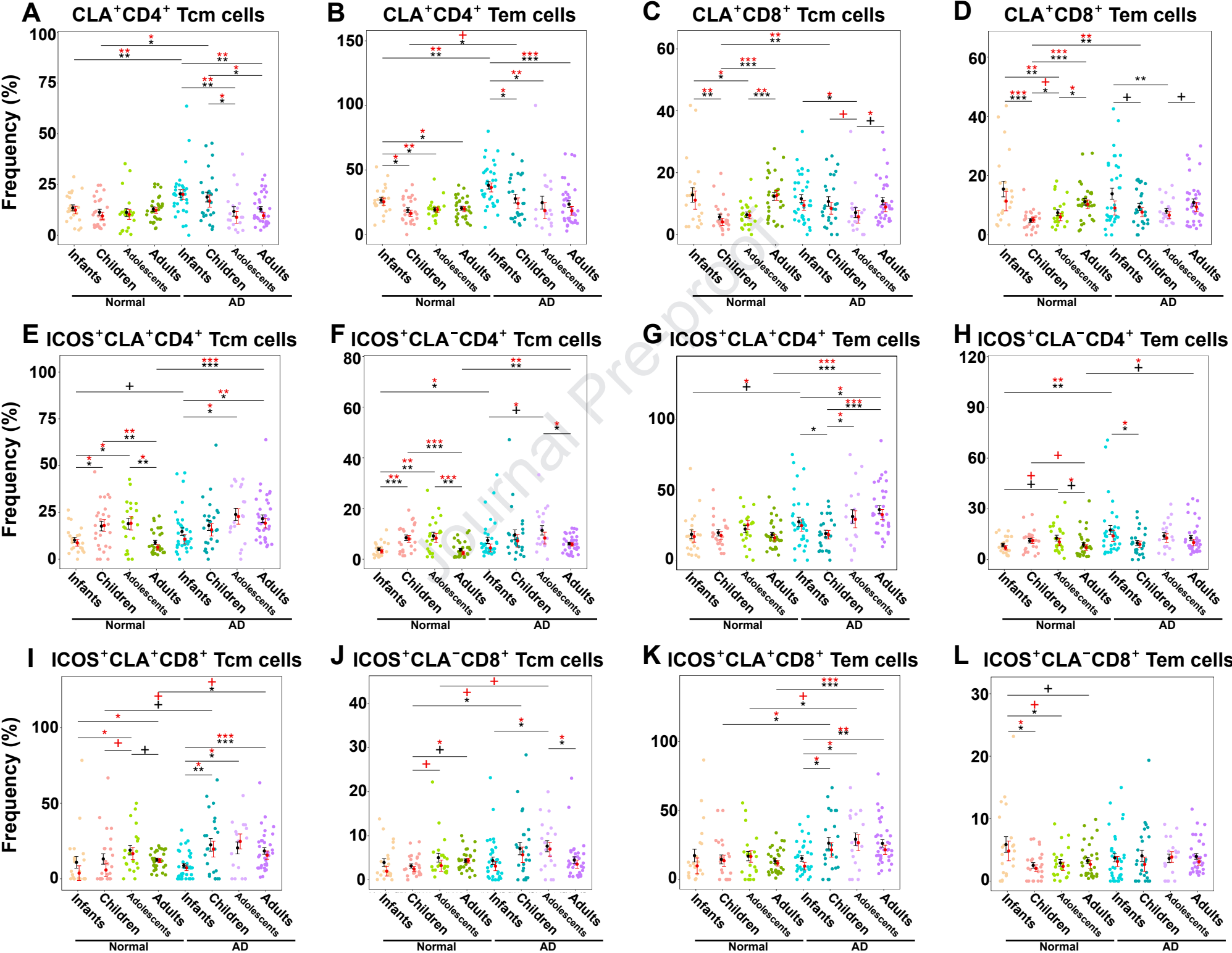


Figure 2

CD4⁺ T cells

CD8⁺ T cells

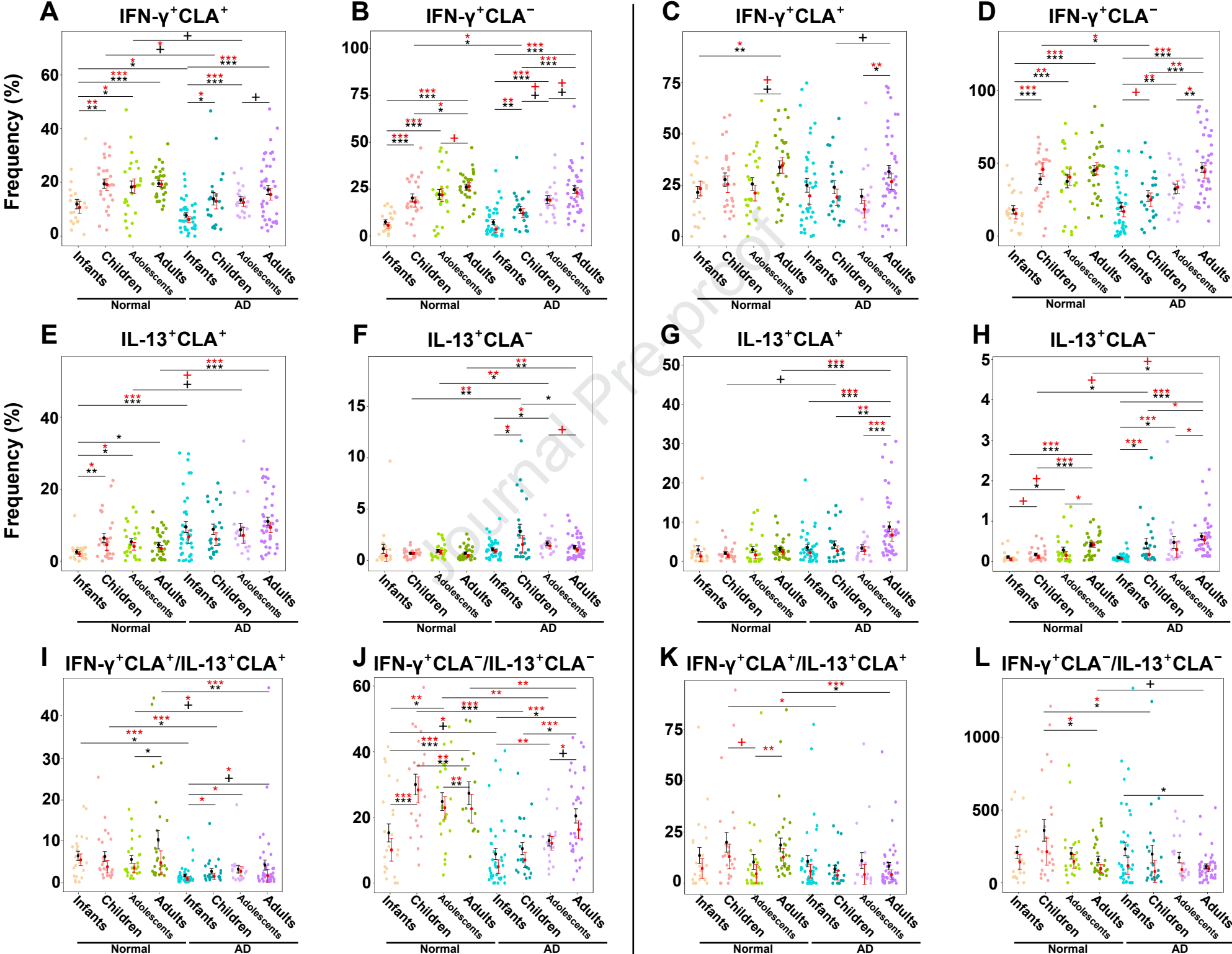


Figure 3

CD4⁺ T cells

CD8⁺ T cells

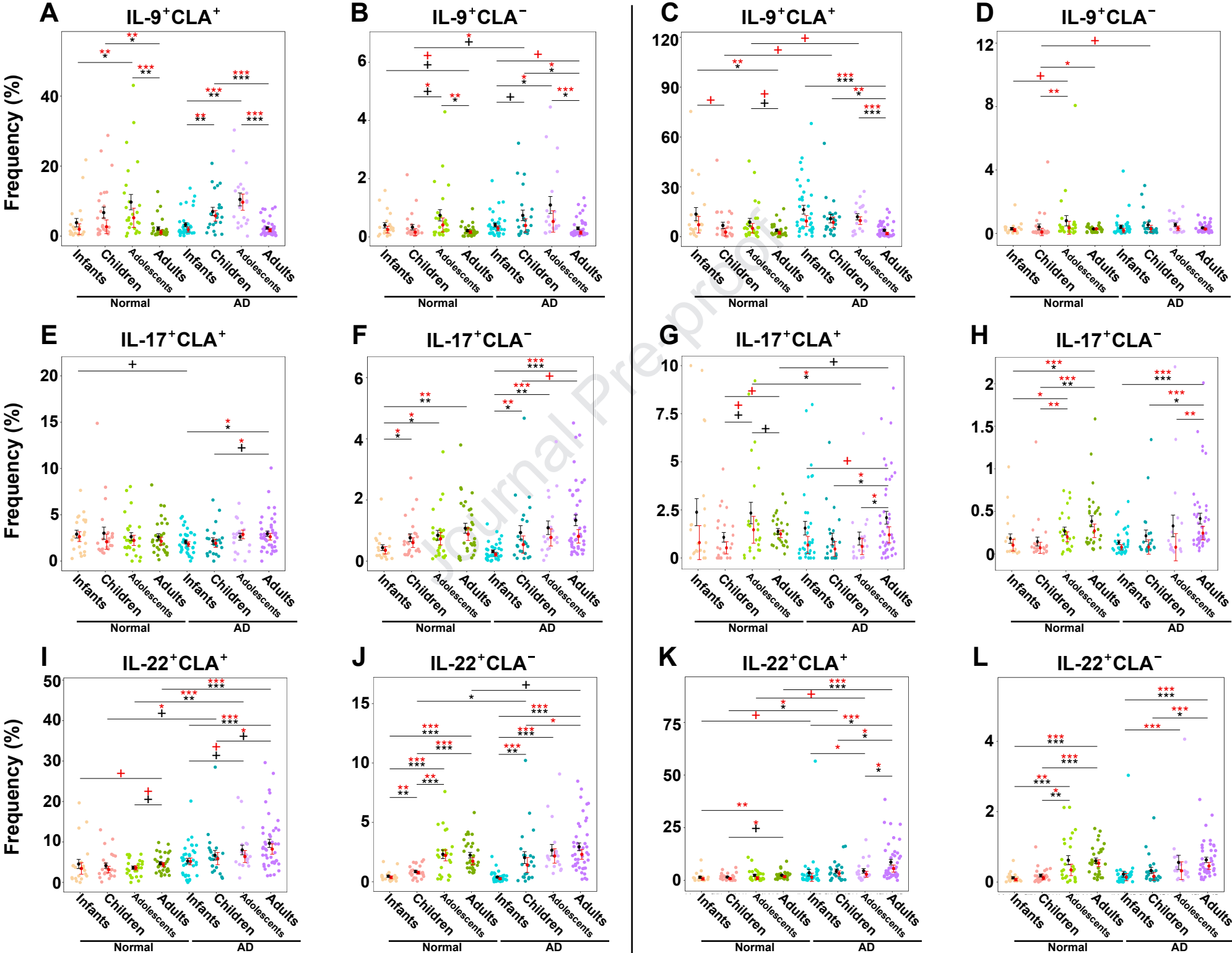
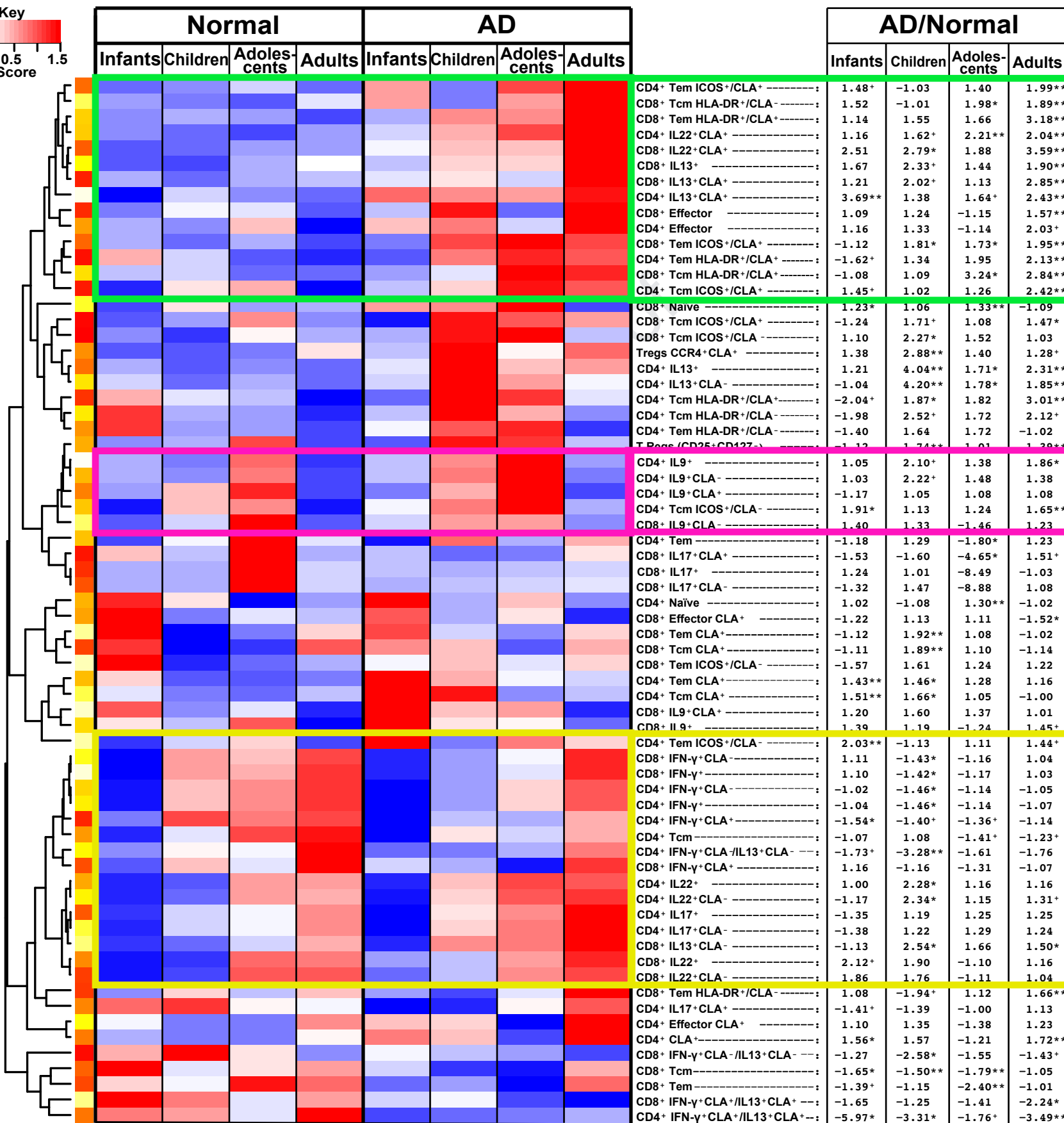
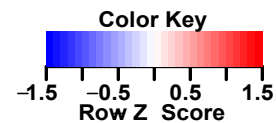


Figure 4



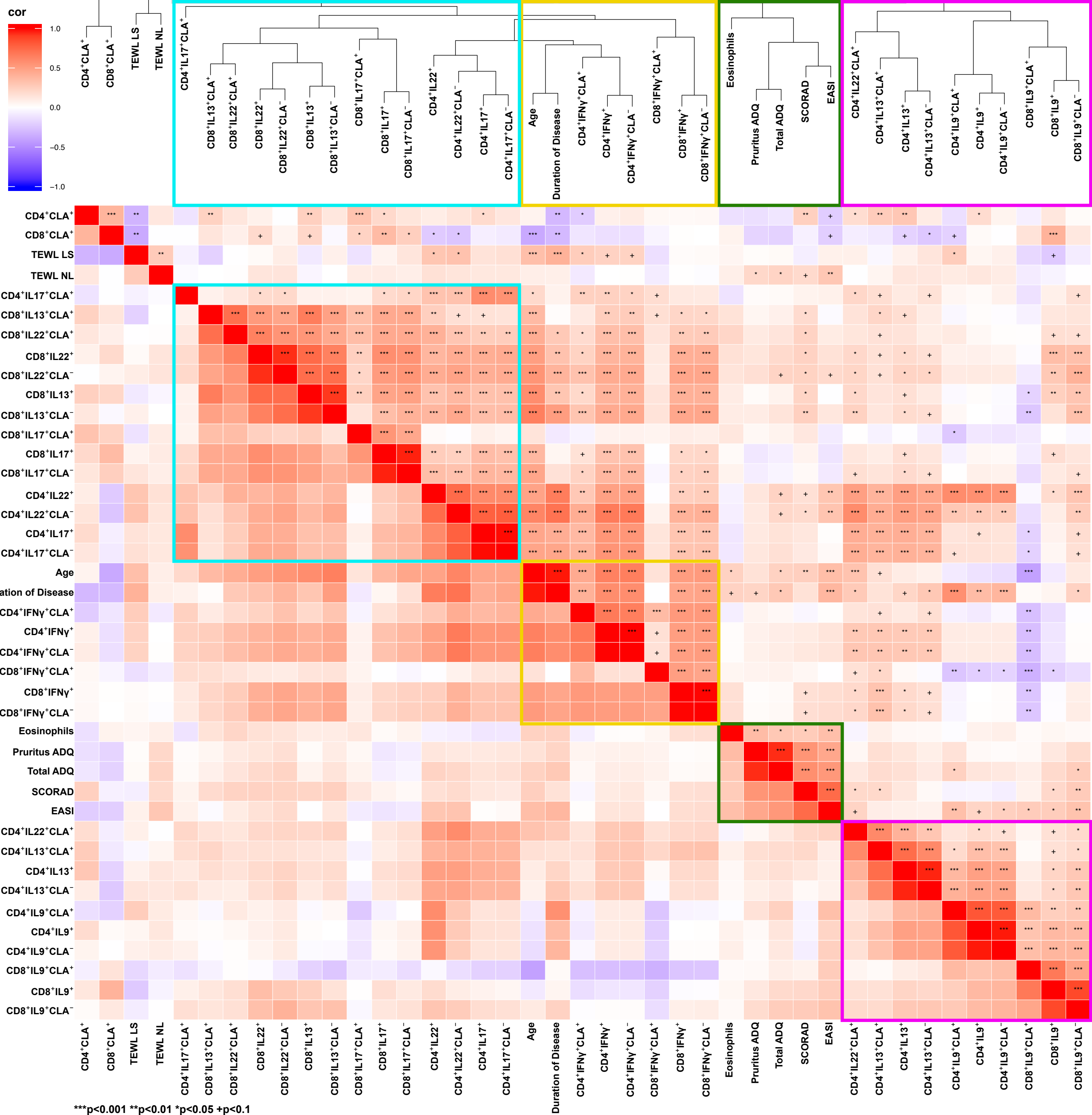
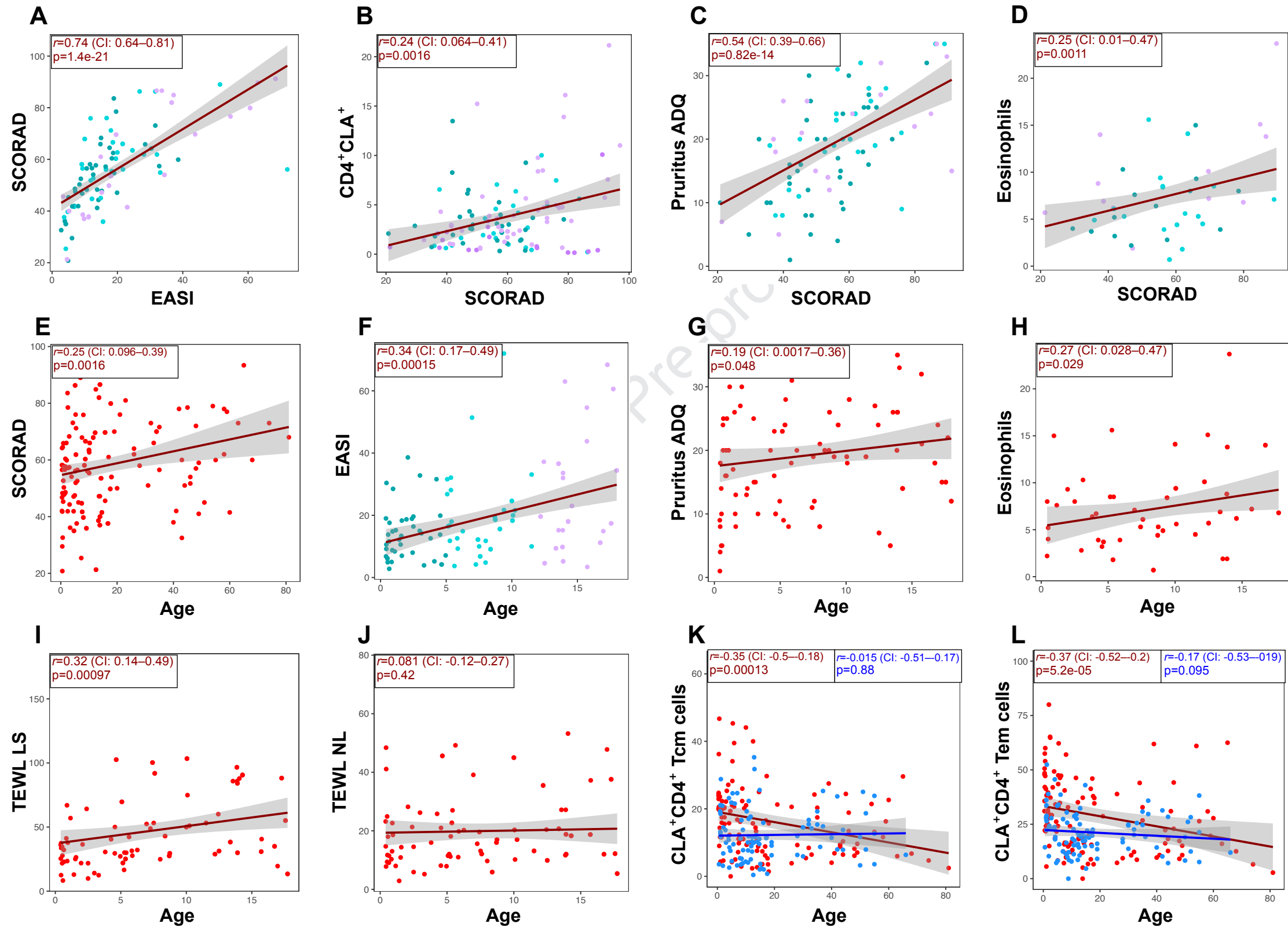
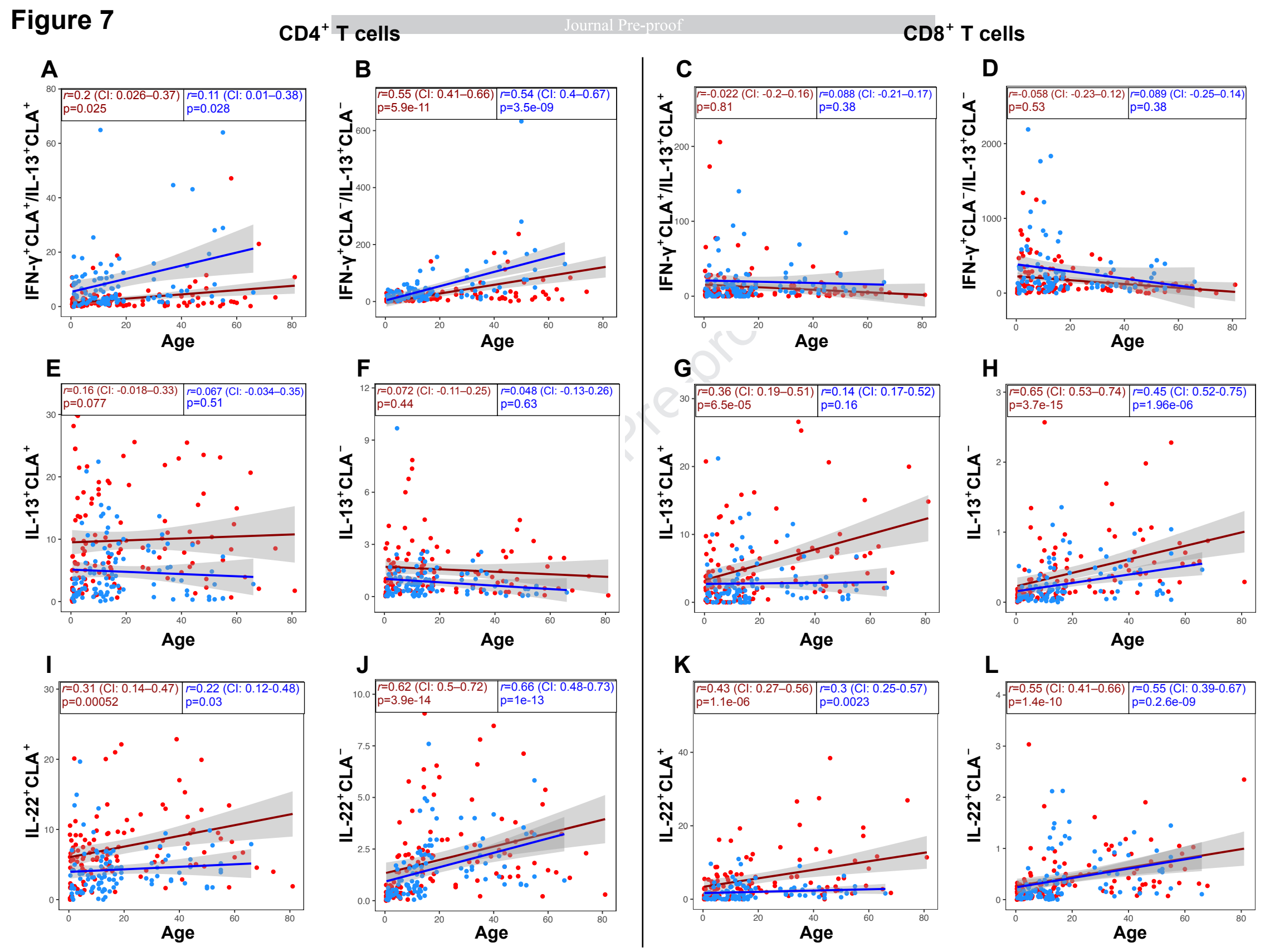


Figure 6



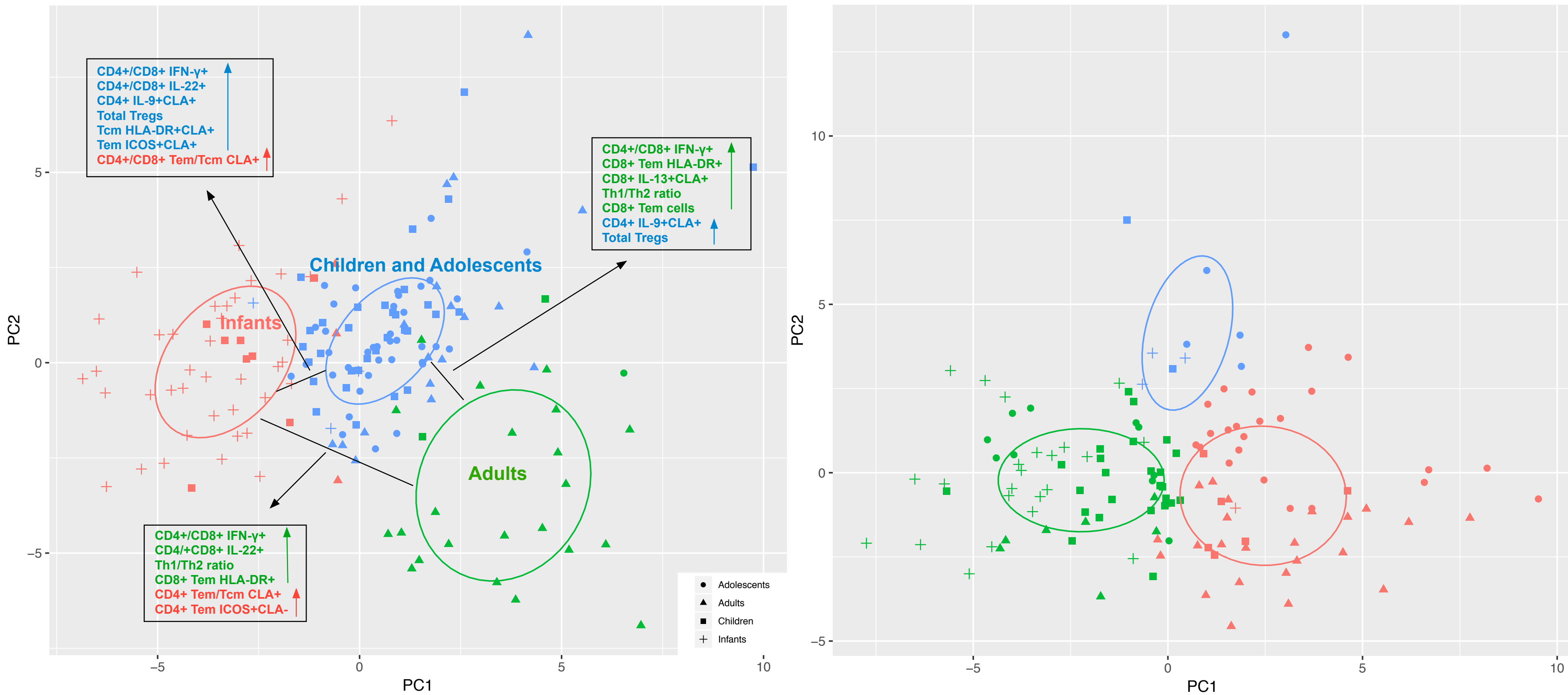


Table E1. Patient demographic and clinical severity scores

Characteristic	Parameter	Normal Infants (n=24)	Normal Children (n=27)	Normal Adolescents (n=28)	Normal Adults (n=30)	AD Infants (n=40)	AD Children (n=26)	AD Adolescents (n=23)	AD Adults (n=43)	P-Value b/w Normal and AD Infants	P-Value b/w Normal and AD Children	P-Value b/w Normal and AD Adolescents	P-Value b/w Normal and AD Adults
Age	Mean $\pm$ SD	2.45 $\pm$ 1.44	9.16 $\pm$ 2.12	14.36 $\pm$ 1.69	39.27 $\pm$ 11.97	1.93 $\pm$ 1.44	7.54 $\pm$ 1.94	15.06 $\pm$ 1.90	43.30 $\pm$ 15.09	0.1656	0.005724	0.1699	0.2263
	Median (range)	2.00 (0.56-4.93)	9.01 (5.05-11.87)	14.30 (12.13-17.46)	37 (19-66)	1.56 (0.41-4.96)	7.47 (5.08-11.51)	14.29 (12.19-17.99)	43 (18-81)				
Sex	Male	13	7	11	18	22	13	13	24	1	0.1275	0.3446	0.9082
	Female	11	20	17	12	18	13	10	19				
Race	White	18	18	16	24	19	9	16	34	0.03131	0.1289	0.1007	0.4775
	Hispanic	3	4	10	1	3	6	2	0				
	Black	3	3	1	2	8	6	2	6				
	Asian	0	2	1	3	10	5	3	3				
Eosinophils (%)	Mean $\pm$ SD	4.04 $\pm$ 4.05	2.25 $\pm$ 1.95	3.19 $\pm$ 2.38	NA	6.42 $\pm$ 3.44	6.71 $\pm$ 3.85	9.72 $\pm$ 5.90	NA	0.09849	1.03E-05	4.70E-05	
	Median (range)	2.15 (0.0-12.6)	1.9 (0.7-10.0)	2.4 (0.5-8.9)	NA	6.4 (2.2-15)	5.6 (0.7-15.6)	8.0 (1.9-23.7)	NA				
Disease Severity Scores													
SCORAD	Mean $\pm$ SD	NA	NA	NA	NA	52.48 $\pm$ 13.15	58.43 $\pm$ 14.91	58.95 $\pm$ 21.78	64.90 $\pm$ 15.64				
	Median (range)	NA	NA	NA	NA	52.5 (20.8-83.6)	57.3 (25.4-89.0)	53.5 (21.3-91.2)	65 (32.5-97)				
EASI	Mean $\pm$ SD	NA	NA	NA	NA	14.04 $\pm$ 8.64	17.76 $\pm$ 10.21	27.93 $\pm$ 20.56	NA				
	Median (range)	NA	NA	NA	NA	13.0 (2.8-38.6)	16.6 (3.7-51.4)	18.0 (4.6-68.4)	NA				
TEWL LS	Mean $\pm$ SD	NA	NA	NA	NA	36.24 $\pm$ 31.34	47.13 $\pm$ 24.78	58.30 $\pm$ 29.68	NA				
	Median (range)	NA	NA	NA	NA	28.6 (8.3-176.8)	42.7 (16.6-103.4)	57.6 (13.5-96.6)	NA				
TEWL NL	Mean $\pm$ SD	NA	NA	NA	NA	19.08 $\pm$ 15.27	19.02 $\pm$ 11.00	22.07 $\pm$ 13.66	NA				
	Median (range)	NA	NA	NA	NA	14.5 (2.9-76.7)	16.5 (5.9-49.2)	18.8 (5.4-53.2)	NA				

Pruritus ADQ	Mean $\pm$ SD	NA	NA	NA	NA	16.95 $\pm$ 7.51	21.96 $\pm$ 8.11	20.82 $\pm$ 8.73	NA				
	Median (range)	NA	NA	NA	NA	16.0 (1-32)	20.5 (7-35)	22.0 (5-35)	NA				
Total ADQ	Mean $\pm$ SD	NA	NA	NA	NA	33.65 $\pm$ 13.62	43.38 $\pm$ 13.36	42.75 $\pm$ 13.67	NA				
	Median (range)	NA	NA	NA	NA	34.0 (5-61)	46.5 (18-70)	44.4 (19-65)	NA				
Disease Duration	Mean $\pm$ SD	NA	NA	NA	NA	1.61 $\pm$ 1.38	6.33 $\pm$ 2.45	14.23 $\pm$ 2.45	NA				
	Median (range)	NA	NA	NA	NA	1.2 (0.2-4.7)	5.1 (2.0-10.5)	13.7 (9.6-17.7)	NA				
History of other atopic disorder (allergic rhinitis, asthma, allergic conjunctivitis, environmental)		0	1	5	0	18	16	13	7				
Food Allergies (FA)		0	0	0	0	1	3	3	2				
FA + other atopic disorder		0	0	0	0	1	6	4	10				

AD: Atopic dermatitis; SCORAD: Scoring of Atopic Dermatitis; SD: Standard deviation; NA: not available; EASI: Eczema Area and Severity Index; TEWL LS: Transepidermal Water Loss Lesional Skin; TEWL NL: Transepidermal Water Loss Non-Lesional Skin; Pruritus ADQ: Pruritus Atopic Dermatitis Quickscore; Total ADQ: Total Atopic Dermatitis Quickscore

Table E2A: Sensitivity analysis with race-matched infants

	N Infant (mean)	AD Infant (mean)	N Infant (median)	AD Infant (median)	P-value (means)- Infant AD vs. N	P-value (median)- Infant AD vs. N
CD4+IL13+CLA+	2.575	8.551	2.040	6.094	0.00284	0.00174
CD4+EM+CLA+	26.698	40.595	25.365	38.290	0.00296	0.00398
CD4+Tem ICOS+CLA-	8.604	18.119	7.009	15.642	0.00413	0.000722
CD4+CM+CLA+	13.4755	21.267	12.420	20.200	0.0163	0.0177
CD4+IFN γ +CLA-/IL13+CLA-	15.278	7.346	10.116	4.707	0.0187	0.03243
CD4+Tcm ICOS+CLA-	3.909	6.841	3.151	4.548	0.0222	0.0348
CD4+IFN γ +CLA+/IL13+CLA+	9.386	1.518	6.051	1.156	0.0239	0.000335
CD4+Tem HLADR+CLA+	10.207	5.309	8.512	4.078	0.0303	0.0225
CD4+Tcm HLADR+CLA+	11.102	4.954	6.438	4.326	0.0638	0.0868
CD4+IFN γ +CLA+	11.945	8.274	10.730	8.046	0.0952	0.146

Table E2B: Sensitivity analysis with age-matched children

	N Child (mean)	AD Child (mean)	N Child (median)	AD Child (median)	P-value (mean)- Child AD vs. N	P-value (median)- Child AD vs. N
CD4+IFN γ +CLA-/IL13+CLA-	32.881	9.934	35.858	6.515	1.11E-05	1.51E-05
Tregs (CCR4+CLA+)	7.598	21.365	5.030	18.20	0.00232	0.00113
CD8+Tem ICOS+CLA+	11.481	28.929	4.974	23.208	0.00572	0.00850
CD4+IL13+CLA-	0.647	2.925	0.672	2.105	0.00698	0.0105
CD8+Tem CLA+	4.826	9.588	5.066	7.690	0.00753	0.0163
Tregs (CD25+CD127-)	6.656	11.630	6.470	9.530	0.0121	0.0368
CD8+Tcm ICOS+CLA-	3.285	7.970	2.958	7.313	0.0144	0.0236
CD8+Tcm CLA+	5.812	11.146	4.025	8.480	0.0182	0.0169
CD4+Tcm HLADR+CLA+	9.344	17.661	7.158	16.245	0.0263	0.0458
CD4+IL22+CLA-	0.775	2.395	0.729	1.682	0.0353	0.0253

Table E2C: Sensitivity analysis using only adolescent controls with no atopic history

	N Adol (mean)	AD Adol (mean)	N Adol (median)	AD Adol (median)	P-value (mean)- Adol AD vs. N	P-value (median)- Adol AD vs. N
CD4+IL22+CLA+	4.137	8.021	4.441	6.400	0.00518	0.00203
CD8+Tem ICOS+CLA+	12.689	29.184	10.301	26.667	0.00580	0.0137
CD4+IFN γ +CLA+	20.417	13.482	18.937	12.608	0.0101	0.0136
CD4+IL13+CLA-	1.065	1.660	0.938	1.403	0.0424	0.0504
CD8+Tcm HLADR+CLA+	7.686	23.516	4.006	19.950	0.0428	0.0677
CD4+Tem ICOS+CLA+	19.775	31.052	18.330	29.024	0.0502	0.103
CD4+IFN γ +CLA+/IL13+CLA+	5.738	3.104	3.663	2.616	0.0634	0.0173
CD8+IFN γ +CLA-	42.573	32.263	42.495	33.569	0.0687	0.0736
CD4+Tem ICOS+CLA-	9.488	14.020	8.163	12.741	0.0688	0.138
CD4+Tem HLADR+CLA+	6.108	12.230	6.259	6.503	0.0978	0.557

*Sensitivity analyses for demographic parameters that were significantly different between AD and N within an age-group, with means, medians, and respective P-values for the top 10 most significant comparisons. These analyses were done for race-matched infants **(A)**, age-matched children **(B)**, and adolescent AD versus adolescent controls without atopic history **(C)**. Abbreviations AD: Atopic dermatitis; Adol: Adolescent; Child: Children; CLA: Cutaneous lymphocyte antigen; HLADR: Human leukocyte antigen-DR; ICOS: Inducible co-stimulator; IFN γ : Interferon-gamma; IL: Interleukin; N: Normal; Tcm: Central memory T; Tem: Effector memory T; Treg: Regulatory T*

Table E3A. Mean values for normal and atopic dermatitis infants, children, adolescents, and adult patients and respective p-values

									P- Values															
	N Infants	AD Infants	N Child	AD Child	N Adol	AD Adol	N Adults	AD Adults	Infants AD vs. N Infants	Child AD vs. N Child	Adol AD vs. N Adol	Adults AD vs. N Adults	N Infants vs. N Child	N Infants vs. N Adol	N Infants vs. N Adults	N Child vs. N Adol	N Child vs. N Adults	N Adol vs. N Adults	AD Infants vs. AD Child	AD Infants vs. AD Adol	AD Infants vs. AD Adults	AD Child vs. AD Adol	AD Child vs. AD Adults	AD Adol vs. AD Adults
CD4+IFNy+	7.648	7.365	20.242	13.973	22.037	18.974	25.870	24.200	0.871	0.040	0.371	0.501	8.17E-06	2.51E-05	1.12E-12	0.604	0.037	0.223	0.010	3.30E-05	4.59E-10	0.093	0.001	0.071
CD4+CLA+	2.383	3.726	2.066	3.398	2.035	1.610	2.934	5.053	0.044	0.107	0.469	0.009	0.641	0.488	0.311	0.960	0.189	0.057	0.679	0.005	0.127	0.028	0.076	1.58E-04
CD4+IFNy+CLA+	11.945	7.769	19.511	13.690	18.326	13.734	19.588	17.228	0.047	0.058	0.075	0.242	0.004	0.029	0.001	0.675	0.970	0.608	0.030	3.94E-04	7.63E-06	0.987	0.236	0.099
CD4+IFNy+CLA-	7.517	7.380	20.273	14.006	22.132	19.072	26.068	24.780	0.938	0.040	0.376	0.609	6.83E-06	2.30E-05	8.77E-13	0.594	0.034	0.217	0.010	3.37E-05	2.93E-10	0.090	3.96E-04	0.051
CD4+IL13+	1.168	1.411	0.757	3.067	1.024	1.804	0.831	1.916	0.625	0.004	0.010	4.95E-04	0.378	0.765	0.478	0.164	0.666	0.367	0.036	0.215	0.130	0.106	0.142	0.750
CD4+IL13+CLA+	2.575	9.492	6.428	9.114	5.338	8.483	4.548	11.055	9.20E-05	0.173	0.109	6.99E-06	0.009	0.010	0.033	0.481	0.203	0.479	0.858	0.659	0.409	0.780	0.300	0.215
CD4+IL13+CLA-	1.124	1.077	0.678	2.850	0.934	1.719	0.693	1.283	0.922	0.01	0.007	0.006	0.341	0.692	0.365	0.141	0.920	0.211	0.022	0.026	0.375	0.139	0.041	0.132
CD4+IFNy+CLA+IL13+CLA+	9.386	1.572	8.546	2.457	5.470	3.206	14.262	4.088	0.025	0.032	0.084	0.010	0.841	0.255	0.311	0.276	0.197	0.021	0.244	0.095	0.056	0.501	0.250	0.564
CD4+IFNy+CLA-IL13+CLA-	15.278	8.853	34.346	10.760	24.806	12.470	39.339	26.164	0.053	1.94E-06	0.141	0.137	1.48E-04	0.010	0.001	0.689	0.007	0.006	0.456	0.155	0.012	0.249	0.015	0.056
CD4+IL9+	0.491	0.514	0.406	0.881	0.906	1.194	0.250	0.464	0.877	0.054	0.474	0.036	0.591	0.104	0.071	0.044	0.160	0.006	0.121	0.061	0.685	0.437	0.085	0.047
CD4+IL9+CLA+	3.824	3.256	6.701	7.052	9.708	10.259	2.162	2.332	0.676	0.862	0.845	0.788	0.154	0.024	0.217	0.271	0.010	0.002	0.010	0.001	0.182	0.147	0.001	2.53E-04
CD4+IL9+CLA-	0.399	0.409	0.333	0.766	0.743	1.051	0.214	0.295	0.930	0.049	0.372	0.188	0.615	0.118	0.073	0.064	0.243	0.013	0.087	0.037	0.172	0.405	0.024	0.015
CD4+IL22+	0.593	0.594	0.935	2.169	2.340	2.654	2.314	2.675	0.995	0.031	0.594	0.437	0.049	8.11E-05	1.28E-07	0.001	6.13E-06	0.952	0.008	2.57E-04	5.45E-06	0.491	0.445	0.972
CD4+IL22+CLA+	4.552	5.261	4.139	6.750	3.627	7.910	4.746	9.663	0.595	0.073	0.002	3.78E-05	0.753	0.452	0.874	0.510	0.433	0.068	0.304	0.052	0.001	0.496	0.077	0.253
CD4+IL22+CLA-	0.467	0.399	0.870	2.063	2.313	2.595	2.239	2.943	0.562	0.037	0.635	0.085	0.006	3.72E-05	3.28E-08	0.001	5.10E-06	0.865	0.005	1.49E-04	1.08E-09	0.453	0.164	0.544
CD4+IL17+	0.510	0.377	0.805	0.973	0.883	1.087	1.120	1.403	0.270	0.529	0.452	0.241	0.083	0.067	0.003	0.712	0.137	0.317	0.020	0.004	7.54E-07	0.719	0.145	0.254
CD4+IL17+CLA+	2.906	2.062	3.012	2.166	2.633	2.608	2.605	2.953	0.083	0.265	0.964	0.441	0.892	0.657	0.585	0.632	0.585	0.960	0.804	0.193	0.013	0.392	0.096	0.455
CD4+IL17+CLA-	0.442	0.320	0.765	0.946	0.846	1.072	1.076	1.336	0.261	0.504	0.401	0.284	0.048	0.038	0.002	0.693	0.139	0.323	0.017	0.002	1.11E-06	0.693	0.192	0.342
CD8+IFNy+	18.328	20.179	38.883	28.485	37.309	30.301	44.737	46.063	0.647	0.052	0.217	0.782	6.51E-05	0.001	2.00E-07	0.785	0.245	0.186	0.081	0.029	1.37E-07	0.720	0.001	0.002
CD8+CLA+	2.744	3.770	1.684	1.939	1.924	1.979	N/A	N/A	0.452	0.752	0.950	N/A	3.39E-01	0.415	N/A	0.758	N/A	N/A	0.113	0.165	N/A	0.965	N/A	N/A
CD8+IFNy+CLA+	21.484	24.946	27.724	24.546	25.570	19.225	33.830	31.589	0.441	0.494	0.166	0.605	0.156	0.337	0.005	0.631	0.174	0.062	0.933	0.233	0.152	0.259	0.124	0.008
CD8+IFNy+CLA-	18.014	19.931	39.176	28.670	37.558	30.772	45.066	46.953	0.642	0.051	0.240	0.696	4.44E-05	0.001	1.51E-07	0.781	0.245	0.187	0.073	0.023	6.71E-08	0.684	0.001	0.002
CD8+IL13+	0.225	0.376	0.219	0.528	0.345	0.480	0.485	0.920	0.488	0.067	0.425	0.005	0.948	0.290	0.009	0.207	0.001	0.179	0.549	0.675	0.028	0.822	0.060	0.031
CD8+IL13+CLA+	2.936	3.544	2.091	4.429	2.989	3.223	3.094	8.822	0.627	0.041	0.839	7.15E-05	0.452	0.968	0.892	0.310	0.111	0.911	0.475	0.765	3.97E-04	0.364	0.008	3.78E-04

CD8+IL13+CLA-	0.114	0.101	0.179	0.467	0.287	0.461	0.419	0.630	0.694	0.046	0.291	0.028	0.151	0.032	4.73E-06	0.175	2.34E-04	0.139	0.012	0.023	6.47E-08	0.975	0.297	0.312
CD8+IFN γ +CLA+IL13+CLA+	13.535	10.690	19.937	7.252	10.347	10.440	18.629	8.298	0.449	0.766	0.488	0.010	0.718	0.500	0.630	0.579	0.825	0.666	0.865	0.479	0.219	0.549	0.400	0.610
CD8+IFN γ +CLA-IL13+CLA-	44.484	31.193	54.077	45.658	61.714	47.565	73.607	49.528	0.559	0.038	0.227	0.097	0.183	0.817	0.181	0.107	0.011	0.184	0.739	0.254	0.020	0.577	0.168	0.186
CD8+IL9+	0.732	1.020	0.626	0.624	0.895	0.849	0.363	0.528	0.324	0.996	0.906	0.075	0.743	0.669	0.069	0.524	0.334	0.120	0.183	0.573	0.041	0.433	0.650	0.159
CD8+IL9+CLA+	13.578	16.237	6.775	8.695	8.720	14.186	3.847	3.881	0.580	0.430	0.141	0.972	0.133	0.296	0.024	0.522	0.170	0.053	0.015	0.599	5.47E-05	0.092	0.004	0.002
CD8+IL9+CLA-	0.297	0.414	0.410	0.426	0.800	0.673	0.289	0.355	0.413	0.945	0.730	0.225	0.580	0.147	0.936	0.306	0.526	0.133	0.947	0.209	0.635	0.248	0.610	0.078
CD8+IL22+	0.160	0.341	0.206	0.395	0.670	0.593	0.649	0.753	0.096	0.152	0.764	0.372	0.472	0.002	3.75E-06	0.006	3.25E-05	0.901	0.725	0.283	0.002	0.416	0.017	0.481
CD8+IL22+CLA+	1.434	3.598	1.564	4.147	2.375	4.692	2.398	8.611	0.190	0.023	0.066	4.16E-05	0.824	0.214	0.112	0.227	0.088	0.972	0.768	0.565	0.017	0.714	0.010	0.025
CD8+IL22+CLA-	0.120	0.224	0.186	0.329	0.620	0.545	0.604	0.630	0.244	0.172	0.749	0.814	0.213	0.001	1.18E-06	0.004	2.45E-05	0.916	0.400	0.136	0.001	0.321	0.017	0.686
CD8+IL17+	0.237	0.294	0.219	0.229	2.883	0.325	0.422	0.410	0.636	0.940	0.326	0.884	0.893	0.309	0.063	0.307	0.128	0.344	0.581	0.839	0.295	0.491	0.038	0.531
CD8+IL17+CLA+	2.382	1.552	1.579	0.974	4.694	1.023	1.383	2.094	0.301	0.353	0.046	0.058	0.376	0.223	0.180	0.096	0.737	0.067	0.233	0.274	0.270	0.914	0.019	0.024
CD8+IL17+CLA-	0.180	0.137	0.146	0.222	2.925	0.315	0.384	0.416	0.481	0.392	0.336	0.729	0.656	0.312	0.019	0.306	0.005	0.349	0.266	0.165	1.50E-04	0.510	0.047	0.472
Tregs (CD25+CD127-)	6.403	5.697	7.037	12.212	11.527	11.642	5.177	7.197	0.573	0.003	0.950	4.43E-04	0.650	0.005	0.333	0.004	0.033	5.88E-05	1.75E-04	1.96E-04	0.007	0.770	0.002	0.003
Tregs (CCR4+CLA+)	8.118	11.242	8.272	23.786	9.468	13.293	13.998	17.906	0.230	4.88E-04	0.158	0.083	0.950	0.592	0.014	0.595	0.006	0.033	0.004	0.456	0.010	0.018	0.162	0.105
CD4+CM+CLA+	13.475	20.412	11.350	18.839	11.218	11.750	12.870	12.824	0.007	0.011	0.863	0.979	0.309	0.366	0.749	0.957	0.410	0.472	0.615	0.007	0.002	0.041	0.033	0.691
CD4+EM+CLA+	26.698	38.082	18.972	27.768	19.273	20.683	20.343	23.511	0.002	0.030	0.318	0.317	0.013	0.012	0.026	0.907	0.586	0.641	0.022	0.025	3.66E-04	0.608	0.330	0.843
CD4+Effector cells CLA+	6.932	7.607	5.538	7.478	5.662	4.117	8.248	10.135	0.682	0.350	0.365	0.462	0.322	0.439	0.322	0.945	0.077	0.140	0.954	0.045	0.357	0.097	0.363	0.021
CD4+Tcm ICOS+CLA+	10.221	14.789	17.700	18.018	18.971	23.955	8.953	21.656	0.072	0.931	0.260	1.57E-06	0.014	0.011	0.496	0.741	0.003	0.003	0.338	0.024	0.020	0.167	0.281	0.556
CD4+Tcm ICOS+CLA-	3.909	7.457	8.496	9.625	9.197	11.385	3.738	6.174	0.013	0.618	0.360	0.003	0.001	0.002	0.835	0.701	3.57E-04	0.002	0.357	0.087	0.352	0.518	0.103	0.013
CD4+Tem ICOS+CLA+	18.372	27.272	19.461	18.874	22.129	31.052	18.027	35.898	0.052	0.860	0.111	1.91E-06	0.779	0.356	0.924	0.438	0.622	0.194	0.040	0.516	0.048	0.031	2.65E-05	0.392
CD4+Tem ICOS+CLA-	8.604	17.477	11.149	9.824	12.679	14.020	8.851	12.783	0.003	0.516	0.617	0.062	0.139	0.052	0.886	0.489	0.234	0.091	0.016	0.305	0.133	0.105	0.179	0.630
CD4+Tcm HLADR+CLA+	11.102	5.452	8.450	15.817	7.852	14.303	2.862	8.602	0.088	0.020	0.125	3.52E-05	0.451	0.361	0.015	0.800	0.003	0.009	0.001	0.030	0.026	0.739	0.015	0.156
CD4+Tcm HLADR+CLA-	6.051	3.063	2.844	7.171	2.753	4.730	1.181	2.502	0.305	0.066	0.247	0.058	0.251	0.237	0.084	0.907	0.015	0.018	0.099	0.383	0.642	0.370	0.049	0.198
CD4+Tem HLADR+CLA+	10.207	6.294	8.139	10.876	6.277	12.230	5.419	11.540	0.072	0.455	0.103	0.001	0.517	0.066	0.023	0.500	0.319	0.467	0.107	0.106	0.005	0.751	0.824	0.853
CD4+Tem HLADR+CLA-	4.400	3.133	2.537	4.169	2.607	4.494	2.019	1.980	0.435	0.122	0.241	0.949	0.206	0.236	0.087	0.951	0.576	0.554	0.411	0.426	0.288	0.832	0.008	0.082
CD8+Tcm CLA+	12.795	11.574	5.653	10.673	6.408	7.069	12.344	10.814	0.654	0.008	0.724	0.386	0.009	0.017	0.868	0.557	9.13E-05	3.78E-04	0.657	0.037	0.670	0.115	0.943	0.072
CD8+Tem CLA+	15.463	13.867	4.904	9.409	7.466	8.036	11.186	10.925	0.623	0.003	0.683	0.877	0.001	0.009	0.147	0.040	2.13E-05	0.019	0.053	0.008	0.195	0.387	0.392	0.069
CD8+Effector cells CLA+	9.152	7.482	4.253	4.797	5.333	5.898	4.992	3.278	0.469	0.635	0.812	0.035	0.025	0.121	0.055	0.497	0.461	0.831	0.078	0.481	0.001	0.602	0.125	0.185

CD8+Tcm ICOS+CLA+	10.895	8.773	13.133	22.439	18.967	20.423	12.525	18.407	0.613	0.093	0.776	0.020	0.669	0.115	0.689	0.222	0.869	0.068	0.005	0.012	0.001	0.727	0.396	0.659
CD8+Tcm ICOS+CLA-	3.927	4.334	3.127	7.089	5.013	7.632	4.366	4.476	0.737	0.013	0.114	0.899	0.427	0.421	0.655	0.102	0.058	0.560	0.100	0.036	0.900	0.776	0.110	0.039
CD8+Tem ICOS+CLA+	17.315	15.412	14.538	26.308	16.905	29.184	13.409	26.175	0.711	0.033	0.039	1.55E-04	0.626	0.943	0.431	0.618	0.759	0.360	0.028	0.012	0.003	0.649	0.979	0.577
CD8+Tem ICOS+CLA-	5.846	3.734	2.508	4.036	2.920	3.632	3.248	3.950	0.146	0.140	0.414	0.298	0.019	0.041	0.066	0.553	0.266	0.650	0.788	0.915	0.785	0.725	0.933	0.701
CD8+Tcm HLADR+CLA+	9.883	9.115	10.513	11.498	7.252	18.053	6.880	19.539	0.786	0.823	0.034	5.64E-08	0.849	0.388	0.212	0.331	0.194	0.877	0.558	0.055	1.09E-04	0.128	0.052	0.574
CD8+Tcm HLADR+CLA-	4.954	3.978	4.440	4.381	3.830	7.602	5.921	11.174	0.508	0.958	0.046	1.94E-04	0.726	0.471	0.539	0.602	0.217	0.112	0.406	0.988	0.344	0.080	1.18E-06	0.061
CD8+Tem HLADR+CLA+	7.410	8.462	8.486	13.154	7.839	13.029	5.729	18.233	0.691	0.304	0.245	3.50E-08	0.738	0.889	0.423	0.853	0.307	0.402	0.262	0.275	2.06E-04	0.981	0.223	0.213
CD8+Tem HLADR+CLA-	2.979	3.216	4.474	2.306	3.474	3.904	4.577	7.613	0.819	0.055	0.780	0.004	0.225	0.615	0.141	0.363	0.930	0.235	0.310	0.663	3.26E-05	0.309	6.73E-07	0.029
CD4+Naïve cells	76.014	77.562	66.520	61.472	52.718	68.480	60.613	59.677	0.666	0.255	0.003	0.803	0.032	5.47E-05	2.59E-04	0.012	0.148	0.115	7.36E-05	0.007	1.81E-06	0.085	0.665	0.022
CD4+Tcm	11.930	11.100	18.075	19.438	24.907	17.675	26.410	21.455	0.715	0.601	0.059	0.068	0.016	0.001	1.60E-06	0.060	0.002	0.683	0.001	0.012	2.39E-05	0.540	0.455	0.176
CD4+Tem	6.514	5.500	9.947	12.796	15.585	8.660	9.414	11.536	0.457	0.253	0.011	0.163	0.101	0.002	0.077	0.057	0.793	0.023	0.001	0.012	6.77E-06	0.051	0.546	0.048
CD4+Effector cells	5.041	5.854	4.729	6.299	5.947	5.202	3.623	7.360	0.392	0.184	0.531	0.075	0.772	0.381	0.304	0.286	0.447	0.105	0.672	0.560	0.41	0.372	0.574	0.264
CD8+Naïve cells	51.973	64.082	60.900	64.812	55.577	73.750	56.350	51.734	0.031	0.394	0.001	0.189	0.106	0.568	0.386	0.338	0.257	0.880	0.874	0.025	0.003	0.041	0.003	3.26E-07
CD8+Tcm	25.817	15.653	16.197	10.792	17.599	9.835	19.878	18.906	0.026	0.010	0.006	0.626	0.024	0.068	0.146	0.612	0.078	0.391	0.070	0.035	0.237	0.616	1.04E-04	4.34E-05
CD8+Tem	11.849	8.531	10.927	9.493	15.175	6.317	13.680	13.604	0.088	0.457	0.002	0.965	0.646	0.245	0.352	0.123	0.122	0.577	0.599	0.194	0.003	0.101	0.034	1.36E-04
CD8+Effector cells	10.340	11.280	11.979	14.896	11.626	10.095	10.036	15.749	0.637	0.141	0.329	0.008	0.325	0.475	0.863	0.825	0.214	0.352	0.111	0.507	0.056	0.016	0.724	0.006

Table E3B. Median values for normal and atopic dermatitis infants, children, adolescents, and adult patients and respective p-values

									P- Values															
	N Infants	AD Infants	N Child	AD Child	N Adol	AD Adol	N Adults	AD Adults	Infants AD vs. N Infants	Child AD vs. N Child	Adol AD vs. N Adol	Adults AD vs. N Adults	N Infants vs. N Child	N Infants vs. N Adol	N Infants vs. N Adults	N Child vs. N Adol	N Child vs. N Adults	N Adol vs. N Adults	AD Infants vs. AD Child	AD Infants vs. AD Adol	AD Infants vs. AD Adults	AD Child vs. AD Adol	AD Child vs. AD Adults	AD Adol vs. AD Adults
CD4+IFN γ +	5.663	3.992	17.861	12.174	21.903	17.790	26.391	23.214	0.388	0.052	0.302	0.364	6.40E-06	6.41E-05	2.57E-10	0.500	0.029	0.103	0.003	3.38E-06	9.57E-11	0.088	0.001	0.097
CD4+CLA+	1.581	2.927	1.500	2.190	1.484	0.718	2.302	3.870	0.078	0.022	0.085	0.042	0.283	0.570	0.265	0.528	0.013	0.086	0.628	1.66E-04	0.214	0.006	0.127	4.12E-06

CD4+IFN γ +CLA+	10.730	6.255	19.172	12.946	18.538	12.720	19.349	15.540	0.042	0.011	0.113	0.136	0.002	0.026	8.30E-05	0.500	0.891	0.448	0.017	2.09E-04	2.63E-05	0.461	0.154	0.433
CD4+IFN γ +CLA-	5.645	3.714	18.088	12.198	21.919	17.845	26.398	23.216	0.379	0.054	0.324	0.400	4.28E-06	4.58E-05	2.05E-10	0.538	0.028	0.096	0.003	3.75E-06	5.58E-11	0.093	4.73E-04	0.066
CD4+IL13+	0.521	1.086	0.779	1.803	0.847	1.582	0.529	1.369	0.123	0.007	0.009	0.001	0.810	0.381	1.000	0.365	0.769	0.342	0.097	0.127	0.241	0.767	0.443	0.634
CD4+IL13+CLA+	2.040	6.758	4.588	6.114	4.115	6.701	3.455	9.400	0.001	0.123	0.108	2.39E-05	0.022	0.017	0.185	0.893	0.360	0.551	0.749	0.939	0.148	0.786	0.293	0.090
CD4+IL13+CLA-	0.464	0.891	0.673	1.459	0.777	1.504	0.437	1.023	0.289	0.013	0.005	0.009	0.810	0.418	0.769	0.345	0.497	0.180	0.058	0.014	0.399	1.000	0.161	0.060
CD4+IFN γ +CLA+IL13+CLA+	6.051	0.843	4.175	1.304	3.389	2.624	5.464	1.497	5.73E-05	4.08E-04	0.056	6.41E-06	0.901	0.433	0.467	0.644	0.263	0.107	0.039	0.007	0.043	0.415	0.994	0.350
CD4+IFN γ +CLA-IL13+CLA-	10.116	4.970	35.306	7.101	22.904	11.877	34.706	24.109	0.043	1.37E-06	0.001	0.001	3.09E-04	0.006	1.58E-06	0.213	0.006	0.001	0.129	0.008	2.40E-07	0.260	8.06E-05	0.006
CD4+IL9+	0.267	0.351	0.214	0.523	0.481	0.507	0.208	0.211	0.435	0.046	0.564	0.363	0.393	0.115	0.185	0.020	0.518	0.001	0.214	0.071	0.115	0.561	0.022	0.002
CD4+IL9+CLA+	1.961	1.865	2.652	5.394	5.230	8.935	1.101	1.656	0.652	0.346	0.315	0.485	0.118	0.008	0.367	0.247	0.003	6.44E-05	0.005	1.23E-05	0.140	0.228	1.70E-04	3.73E-07
CD4+IL9+CLA-	0.253	0.296	0.164	0.394	0.410	0.472	0.167	0.147	0.582	0.039	0.291	0.597	0.314	0.211	0.090	0.018	0.609	0.002	0.226	0.022	0.055	0.401	0.011	1.66E-04
CD4+IL22+	0.435	0.444	0.807	1.512	2.260	2.116	1.840	1.989	0.980	0.085	0.812	0.375	0.012	7.99E-05	6.31E-09	0.002	4.71E-06	0.847	4.39E-04	5.27E-10	4.14E-08	0.165	0.378	0.351
CD4+IL22+CLA+	3.442	5.174	3.170	5.982	3.606	6.283	4.395	8.241	0.137	0.035	2.46E-04	8.19E-05	0.662	0.651	0.062	0.939	0.145	0.089	0.304	0.054	0.001	0.345	0.043	0.383
CD4+IL22+CLA-	0.377	0.263	0.763	1.450	2.218	2.099	1.727	2.307	0.362	0.141	0.919	0.281	0.003	1.57E-05	4.00E-10	0.002	1.53E-06	0.913	1.26E-04	5.31E-12	3.73E-14	0.165	0.034	0.477
CD4+IL17+	0.385	0.271	0.651	0.582	0.780	0.762	0.996	0.936	0.426	0.844	0.434	0.349	0.049	8.96E-02	0.007	0.939	0.286	0.317	0.011	8.70E-05	4.30E-08	0.282	0.045	0.312
CD4+IL17+CLA+	2.638	1.825	2.091	1.865	2.235	2.711	2.141	2.628	0.140	0.351	0.591	0.230	0.675	0.440	0.537	0.869	0.894	0.819	0.889	0.160	0.023	0.211	0.042	0.701
CD4+IL17+CLA-	0.355	0.220	0.605	0.512	0.731	0.751	0.884	0.811	0.260	0.776	0.462	0.318	0.021	0.037	0.008	0.923	0.334	0.351	0.005	2.16E-05	2.25E-09	0.271	0.055	0.593
CD8+IFN γ +	15.667	16.053	45.807	26.526	40.483	29.900	46.182	41.604	0.967	0.060	0.251	0.907	1.36E-04	0.003	1.72E-06	0.773	0.425	0.229	0.060	0.025	4.76E-07	0.670	0.003	0.016
CD8+CLA+	0.855	2.059	0.719	0.927	1.417	0.753	N/A	N/A	0.097	0.166	0.833	N/A	1.13E-01	0.406	N/A	0.271	N/A	N/A	0.034	0.002	N/A	0.560	N/A	N/A
CD8+IFN γ +CLA+	23.358	19.786	25.432	19.330	21.156	13.050	34.618	26.705	0.791	0.526	0.081	0.506	0.229	0.434	0.012	0.764	0.154	0.052	0.640	0.514	0.116	0.181	0.161	0.006
CD8+IFN γ +CLA-	15.342	16.881	45.703	26.485	40.481	31.094	46.282	44.073	0.915	0.060	0.261	0.960	7.16E-05	0.002	1.34E-06	0.818	0.415	0.224	0.056	0.021	2.00E-07	0.633	0.002	0.010
CD8+IL13+	0.103	0.141	0.140	0.208	0.183	0.270	0.467	0.692	0.226	0.044	0.261	0.007	0.431	0.303	4.63E-04	0.525	4.67E-04	0.030	0.042	0.034	6.71E-12	0.928	0.001	0.001
CD8+IL13+CLA+	1.310	2.247	1.438	3.121	1.638	2.421	2.460	6.702	0.237	0.164	0.449	4.44E-05	0.843	0.774	0.123	0.712	0.114	0.134	0.655	0.824	1.16E-04	0.583	0.010	3.90E-04
CD8+IL13+CLA-	0.060	0.078	0.122	0.184	0.158	0.264	0.419	0.536	0.426	0.057	0.169	0.096	0.070	0.074	4.46E-06	0.577	3.51E-04	0.023	2.14E-04	4.82E-05	2.48E-15	0.867	0.025	0.024
CD8+IFN γ +CLA+IL13+CLA+	7.190	5.842	13.043	4.218	4.559	3.723	12.751	4.242	0.465	0.037	0.982	9.33E-05	0.363	0.395	0.129	0.055	0.729	0.007	0.786	0.700	0.802	1.000	0.954	0.924
CD8+IFN γ +CLA-IL13+CLA-	38.959	29.056	56.026	41.155	59.395	45.281	77.830	48.033	0.512	0.021	0.224	0.113	0.132	0.804	0.685	0.147	0.017	0.240	0.745	0.743	0.212	0.908	0.607	0.474
CD8+IL9+	0.297	0.451	0.111	0.306	0.471	0.534	0.360	0.369	0.388	0.054	0.777	0.419	0.085	0.431	0.682	0.003	0.054	0.115	0.312	0.953	0.202	0.271	0.994	0.357
CD8+IL9+CLA+	7.202	9.416	2.744	7.499	5.078	12.186	2.299	1.883	0.233	0.118	0.036	0.578	0.074	0.346	0.004	0.347	0.664	0.052	0.185	0.859	4.98E-06	0.173	0.002	2.10E-05
CD8+IL9+CLA-	0.216	0.181	0.091	0.297	0.361	0.394	0.291	0.278	0.640	0.129	0.743	0.467	0.245	0.094	0.282	0.002	0.028	0.240	0.928	0.150	0.301	0.181	0.492	0.252

CD8+IL22+	0.100	0.192	0.128	0.184	0.357	0.332	0.580	0.668	0.123	0.067	0.937	0.406	0.444	0.005	8.05E-07	0.013	6.32E-06	0.360	0.370	0.005	1.77E-07	0.104	1.83E-04	0.015
CD8+IL22+CLA+	0.674	1.438	0.835	2.976	0.982	2.916	1.732	5.576	0.061	0.039	0.039	1.59E-05	0.459	0.216	0.003	0.425	0.043	0.261	0.227	0.029	4.70E-06	0.548	0.026	0.067
CD8+IL22+CLA-	0.068	0.140	0.114	0.160	0.345	0.318	0.536	0.457	0.224	0.166	0.830	0.905	0.219	0.001	1.35E-07	0.013	7.66E-06	0.486	0.162	2.79E-04	1.44E-08	0.093	0.001	0.085
CD8+IL17+	0.107	0.089	0.081	0.114	0.264	0.083	0.297	0.301	0.987	0.081	0.162	0.914	0.431	0.054	0.003	0.006	1.34E-05	0.455	0.475	0.899	5.81E-05	0.651	0.001	0.003
CD8+IL17+CLA+	0.799	0.674	0.538	0.364	1.661	0.606	1.251	1.204	0.508	0.481	0.033	0.962	0.369	0.290	0.454	0.057	0.099	0.522	0.233	0.786	0.093	0.431	0.009	0.041
CD8+IL17+CLA-	0.103	0.078	0.072	0.084	0.211	0.079	0.272	0.245	0.967	0.166	0.191	0.898	0.810	0.025	0.001	0.006	1.02E-05	0.351	0.274	0.355	8.64E-07	0.887	0.001	0.003
Tregs (CD25+CD127-)	5.150	5.420	6.630	9.530	10.900	11.200	5.260	6.970	0.561	0.017	0.782	0.001	0.135	2.45E-04	0.818	0.005	0.015	8.76E-07	7.92E-05	1.13E-05	0.004	0.918	0.010	0.002
Tregs (CCR4+CLA+)	5.820	7.630	6.230	18.200	7.925	11.900	11.850	15.300	0.156	3.99E-04	0.094	0.144	0.912	0.193	0.001	0.367	0.002	0.002	0.004	0.287	0.008	0.077	0.476	0.117
CD4+CM+CLA+	12.420	20.090	9.514	16.810	10.289	8.830	12.297	9.650	0.007	0.023	0.990	0.779	0.325	0.103	0.876	0.759	0.256	0.181	0.479	0.001	0.002	0.019	0.042	0.342
CD4+EM+CLA+	25.365	36.470	16.490	24.100	19.290	18.250	19.660	18.330	0.005	0.092	0.852	0.850	0.031	0.003	0.038	0.776	0.621	0.666	0.018	0.002	1.78E-04	0.335	0.283	0.910
CD4+Effector cells CLA+	7.651	5.440	4.320	4.045	3.202	3.450	7.387	4.999	0.378	0.497	0.647	0.318	0.116	0.102	0.629	0.974	0.033	0.021	0.928	0.124	0.466	0.188	0.368	0.030
CD4+Tcm ICOS+CLA+	8.761	10.720	18.023	15.758	19.187	22.861	6.401	19.259	0.169	1.000	0.261	1.68E-07	0.037	0.033	0.392	0.775	0.005	0.013	0.248	0.026	0.003	0.145	0.164	0.440
CD4+Tcm ICOS+CLA-	3.151	4.463	8.001	7.264	8.328	8.355	2.454	5.857	0.039	0.788	0.358	0.002	0.002	0.005	0.550	0.992	1.95E-04	0.001	0.268	0.018	0.637	0.258	0.283	0.015
CD4+Tem ICOS+CLA+	16.725	24.570	17.600	18.115	25.072	29.024	15.782	32.419	0.042	0.974	0.189	1.38E-06	0.463	0.164	0.804	0.283	0.613	0.111	0.109	0.420	0.020	0.042	9.36E-05	0.340
CD4+Tem ICOS+CLA-	7.010	14.018	11.264	8.993	10.428	12.741	7.049	10.065	0.003	0.302	0.669	0.024	0.194	0.094	0.666	0.536	0.087	0.044	0.017	0.679	0.149	0.107	0.182	0.429
CD4+Tcm HLA-DR+CLA+	6.438	4.326	6.473	15.789	6.279	9.549	1.862	6.813	0.086	0.058	0.264	3.98E-09	1.000	0.620	1.67E-05	0.724	2.13E-04	0.029	0.003	0.152	0.019	0.461	0.044	0.538
CD4+Tcm HLA-DR+CLA-	2.061	0.911	2.262	4.716	1.996	2.209	0.520	1.190	0.250	0.071	0.881	1.09E-04	0.709	0.769	0.034	0.841	4.24E-05	1.43E-05	0.001	0.303	0.223	0.242	0.005	0.873
CD4+Tem HLA-DR+CLA+	8.512	4.757	3.968	7.464	6.259	6.503	3.799	9.215	0.059	0.243	0.579	1.23E-04	0.148	0.157	0.060	0.657	0.945	0.408	0.162	0.330	0.001	0.991	0.352	0.365
CD4+Tem HLA-DR+CLA-	1.529	1.468	1.360	2.354	1.271	2.606	0.944	1.510	0.507	0.038	0.360	0.102	0.285	0.565	0.162	0.716	0.965	0.265	0.036	0.278	0.824	0.530	0.028	0.256
CD8+Tcm CLA+	11.109	9.430	4.050	8.480	6.160	5.735	12.660	8.890	0.942	0.009	0.659	0.324	0.010	0.025	0.526	0.296	1.33E-04	0.001	0.653	0.016	0.700	0.071	0.863	0.012
CD8+Tem CLA+	11.382	9.080	5.102	7.690	6.023	6.645	10.313	9.412	0.531	0.009	0.560	0.554	5.98E-05	0.009	0.419	0.079	5.54E-05	0.019	0.159	0.115	0.605	0.602	0.610	0.256
CD8+Effector cells CLA+	6.460	5.543	4.110	3.606	4.030	3.030	3.621	3.260	0.566	0.846	0.762	0.183	0.023	0.023	0.041	0.831	0.435	0.713	0.225	0.214	0.021	0.599	0.376	0.924
CD8+Tcm ICOS+CLA+	3.735	7.295	5.822	19.633	16.667	24.721	12.136	15.659	0.558	0.091	0.921	0.088	0.628	0.014	0.023	0.080	0.219	0.255	0.013	0.029	0.001	0.782	0.751	0.686
CD8+Tcm ICOS+CLA-	1.961	3.105	2.459	5.675	3.218	6.940	4.356	3.548	1.000	0.055	0.068	0.484	0.965	0.174	0.161	0.090	0.043	0.876	0.145	0.027	0.474	0.575	0.242	0.029
CD8+Tem ICOS+CLA+	9.841	9.695	13.601	21.448	16.689	26.667	11.837	21.531	0.597	0.040	0.052	3.46E-04	0.805	0.664	0.624	0.456	0.584	0.831	0.049	0.019	0.003	0.667	0.818	0.544
CD8+Tem ICOS+CLA-	4.818	3.143	2.042	2.679	2.364	3.850	2.686	3.106	0.141	0.435	0.496	0.140	0.036	0.087	0.148	0.529	0.298	0.748	0.869	0.806	0.250	0.943	0.337	0.786
CD8+Tcm HLA-DR+CLA+	9.502	6.457	6.354	7.056	4.270	18.549	5.924	18.648	0.749	0.972	0.042	2.29E-09	0.938	0.432	0.663	0.560	0.827	0.388	0.865	0.049	1.39E-05	0.111	0.001	0.958
CD8+Tcm HLA-DR+CLA-	2.673	2.016	3.448	2.393	2.903	5.044	4.121	11.376	0.664	0.691	0.048	1.39E-04	0.462	0.953	0.205	0.332	0.396	0.101	0.352	0.032	1.96E-06	0.089	4.47E-06	0.017

CD8+Tem HLADR+CLA+	4.361	6.255	4.416	4.751	5.331	9.719	4.596	15.959	0.728	0.438	0.361	3.06E-10	0.990	0.981	0.650	0.990	0.649	0.802	0.597	0.430	2.39E-05	0.989	0.016	0.024
CD8+Tem HLADR+CLA-	1.786	2.302	3.203	1.638	2.874	1.806	3.300	6.633	0.842	0.046	0.383	0.001	0.139	0.194	0.027	0.741	0.642	0.382	0.376	0.826	2.31E-06	0.559	7.00E-07	0.002
CD4+Naïve cells	80.750	79.750	66.200	63.000	58.100	69.250	61.750	63.700	0.706	0.240	0.005	1.000	0.038	3.37E-05	1.49E-04	0.015	0.144	0.176	5.04E-05	0.006	7.57E-06	0.150	0.719	0.056
CD4+Tcm	9.370	8.510	17.400	15.700	22.950	16.550	25.500	17.900	0.460	0.869	0.072	0.049	0.007	2.41E-04	3.65E-06	0.049	0.004	0.442	3.95E-04	0.006	3.84E-05	0.723	0.349	0.248
CD4+Tem	4.560	4.290	8.950	10.100	12.350	8.345	7.860	10.500	0.633	0.162	0.016	0.141	0.159	3.44E-04	0.027	0.030	0.767	0.013	2.39E-04	0.007	2.27E-05	0.205	0.958	0.121
CD4+Effector cells	4.400	4.718	2.940	4.970	5.260	4.600	1.195	2.490	0.500	0.125	0.365	0.014	0.314	0.286	3.90E-04	0.150	0.008	2.93E-04	0.597	0.494	0.041	0.309	0.058	0.217
CD8+Naïve cells	53.350	69.800	61.500	65.200	61.250	78.500	57.850	53.300	0.026	0.497	0.003	0.317	0.130	0.496	0.550	0.624	0.257	0.511	0.824	0.058	0.002	0.044	0.005	9.57E-06
CD8+Tcm	22.600	10.200	15.800	10.600	15.200	10.030	19.700	17.200	0.026	0.015	0.007	0.532	0.110	0.213	0.566	0.915	0.086	0.090	0.200	0.144	0.013	0.689	0.001	3.02E-04
CD8+Tem	9.875	6.160	9.460	7.660	11.900	4.655	13.400	11.400	0.046	0.237	0.002	0.818	0.806	0.330	0.265	0.220	0.106	0.846	0.640	0.197	0.002	0.098	0.015	1.10E-04
CD8+Effector cells	9.095	8.285	11.800	15.100	11.250	11.050	7.325	14.100	0.801	0.181	0.450	0.018	0.130	0.311	0.585	0.765	0.056	0.185	0.046	0.410	0.034	0.024	0.952	0.056

Mean expression of all T-cell subset frequencies in healthy and AD individuals, as well as P-values (measured by Welch's t-test) for all AD versus healthy comparisons and comparisons within the healthy group and within the AD group **(A)**. Median expression of all T-cell subset frequencies in healthy and AD individuals, as well as P-values (measured by Wilcoxon-Mann-Whitney test) for all AD versus healthy comparisons and comparisons within the healthy group and within the AD group **(B)**. Abbreviations AD: Atopic dermatitis; Adol: Adolescent; Child: Children; CLA: Cutaneous lymphocyte antigen; HLADR: Human leukocyte antigen-DR; ICOS: Inducible co-stimulator; IFN γ : Interferon-gamma; IL: Interleukin; N: Normal; Tcm: Central memory T; Tem: Effector memory T; Treg: Regulatory T

Table E4. Selected Spearman correlations and their P-values in atopic dermatitis patients

AD Age		
Marker	r	P-value
Duration of Disease	0.980	1.12E-56
CD4+IFN γ +CLA-	0.647	1.39E-15
CD8+IL13+CLA-	0.646	3.67E-15
CD4+IFN γ +	0.638	4.76E-15
CD8+IL13+	0.581	6.66E-12
CD8+IL22+CLA-	0.546	1.36E-10
CD8+IL22+	0.501	8.84E-09
CD8+IL17+CLA-	0.462	1.06E-07
CD4+IFN γ +CLA+	0.439	5.45E-07
CD8+IL22+CLA+	0.428	1.11E-06
CD8+IL9+CLA+	-0.405	5.03E-06
CD8+IL17+	0.372	3.15E-05
CD8+IL13+CLA+	0.358	6.46E-05
EASI	0.340	1.52E-04
CD4+IL22+CLA+	0.312	5.16E-04
CD8+CLA+	-0.370	9.35E-04
SCORAD	0.250	1.38E-03
Total ADQ	0.237	1.14E-02
Eosinophils	0.238	3.48E-02
CD4+IL13+CLA+	0.163	7.74E-02
Pruritus ADQ	0.155	1.02E-01
CD8+IFN γ +CLA+	0.150	1.03E-01
CD4+IL9+CLA-	-0.145	1.15E-01
CD8+IL17+CLA+	0.143	1.21E-01
CD4+IL9+	-0.122	1.85E-01
CD8+IL9+CLA-	0.111	2.30E-01
CD4+IL13+	0.098	2.86E-01
CD8+IL9+	-0.093	3.17E-01
CD4+IL9+CLA+	-0.088	3.41E-01
CD4+IL13+CLA-	0.072	4.37E-01
CD4+CLA+	0.061	5.07E-01
CD8+IFN γ +CLA-IL13+CLA-	-0.058	5.26E-01
CD8+IFN γ +CLA+IL13+CLA+	-0.022	8.09E-01

EASI		
Marker	r	P-value
CD4+IL9+CLA+	0.331	3.44E-03
CD8+IL9+CLA-	0.316	5.45E-03
CD8+IL9+CLA+	0.265	2.05E-02
CD8+IL9+	0.260	2.35E-02
CD4+IL9+CLA-	0.245	3.30E-02
CD4+IL22+CLA+	0.224	5.18E-02
CD4+IL9+	0.221	5.52E-02
CD4+CLA+	-0.213	6.50E-02
CD8+CLA+	-0.209	6.94E-02
CD8+IFN γ +CLA-IL13+CLA-	0.112	3.24E-01
CD4+IL13+CLA+	0.093	4.24E-01
CD4+IL13+	0.081	4.88E-01
CD4+IL13+CLA-	0.071	5.41E-01
CD8+IFN γ +CLA+IL13+CLA+	0.019	8.67E-01
CD8+IFN γ +CLA+	-0.002	9.86E-01

SCORAD		
Marker	r	P-value
EASI	0.738	1.42E-21
CD8+IL9+CLA-	0.271	3.32E-03
CD4+CLA+	0.246	7.53E-03
CD8+IL9+	0.203	2.89E-02
CD4+IL22+CLA+	0.201	2.95E-02
CD4+IL13+CLA+	0.200	3.17E-02
CD4+IL13+	0.126	1.75E-01
CD4+IL9+CLA-	-0.071	4.45E-01
CD8+IL9+CLA+	0.070	4.56E-01
CD8+CLA+	-0.063	5.93E-01
CD4+IL9+CLA+	-0.050	5.97E-01
CD4+IL9+	-0.046	6.19E-01
CD8+IFN γ +CLA+	0.044	6.41E-01
CD4+IL13+CLA-	0.029	7.54E-01
CD8+IFN γ +CLA+IL13+CLA+	-0.017	8.58E-01
CD8+IFN γ +CLA-IL13+CLA-	0.013	8.86E-01

Total ADQ		
Marker	r	P-value
SCORAD	0.576	2.54E-11
EASI	0.532	1.59E-09
CD8+IL9+CLA-	0.261	2.68E-02
CD4+IL9+CLA+	0.240	4.26E-02
CD8+CLA+	-0.192	1.05E-01
CD8+IL9+	0.192	1.07E-01
CD4+IL13+CLA+	0.182	1.26E-01
CD4+IL13+	0.162	1.74E-01
CD4+IL9+	0.136	2.56E-01
CD4+IL9+CLA-	0.121	3.12E-01
CD4+IL22+CLA+	0.120	3.15E-01
CD4+IL13+CLA-	0.113	3.44E-01
CD4+CLA+	-0.107	3.69E-01
CD8+IFN γ +CLA+IL13+CLA+	-0.095	4.20E-01
CD8+IL9+CLA+	0.088	4.64E-01
CD8+IFN γ +CLA+	0.070	5.59E-01
CD8+IFN γ +CLA-IL13+CLA-	-0.039	7.39E-01

Pruritus ADQ		
Marker	r	P-value
Total ADQ	0.945	9.72E-56
SCORAD	0.547	3.80E-10
EASI	0.456	4.45E-07
CD8+IL9+CLA-	0.185	1.20E-01
CD8+CLA+	-0.185	1.21E-01
CD4+IL13+CLA+	0.170	1.54E-01
CD4+IL9+CLA+	0.151	2.06E-01
CD4+CLA+	-0.138	2.49E-01
CD4+IL13+	0.133	2.65E-01
CD8+IL9+	0.121	3.11E-01
CD8+IFN γ +CLA+IL13+CLA+	-0.108	3.62E-01
CD4+IL13+CLA-	0.087	4.68E-01
CD8+IFN γ +CLA+	0.062	6.07E-01
CD4+IL22+CLA+	0.044	7.17E-01
CD4+IL9+	0.032	7.88E-01
CD8+IL9+CLA+	0.031	7.97E-01
CD4+IL9+CLA-	0.021	8.62E-01
CD8+IFN γ +CLA-IL13+CLA-	-0.002	9.89E-01

Eosinophil Count		
Marker	r	P-value
Pruritus ADQ	0.350	2.42E-03
EASI	0.335	2.76E-03
Total ADQ	0.288	1.35E-02
SCORAD	0.267	1.88E-02
CD4+CLA+	-0.133	3.46E-01
CD4+IL9+CLA-	0.086	5.46E-01
CD4+IL13+CLA+	0.080	5.74E-01
CD4+IL9+	0.070	6.21E-01
CD8+CLA+	0.063	6.56E-01
CD8+IFN γ +CLA+	0.048	7.36E-01
CD4+IL22+CLA+	-0.048	7.37E-01
CD8+IFN γ +CLA+IL13+CLA+	0.046	7.43E-01
CD8+IL9+	0.044	7.56E-01
CD4+IL13+	0.036	8.00E-01
CD8+IL9+CLA-	0.032	8.22E-01
CD4+IL13+CLA-	0.030	8.30E-01
CD8+IL9+CLA+	0.025	8.62E-01
CD8+IFN γ +CLA-IL13+CLA-	0.020	8.85E-01
CD4+IL9+CLA+	-0.012	9.33E-01

TEWL NL		
Marker	r	P-value
EASI	0.294	2.71E-03

TEWL LS		
Marker	r	P-value
Age	0.333	5.89E-04

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Significant Spearman correlations ($P < 0.05$), with accompanying correlation coefficient (r) and P -value between various T-cell subsets and age or clinical scores (e.g. EASI, SCORAD, pruritus ADQ, TEWL NL, TEWL LS, eosinophil count). Abbreviations ADQ: Atopic dermatitis Quickscore; Adol: Adolescent; Child: Children; CLA: Cutaneous lymphocyte antigen; EASI: Eczema Area and Severity Index; HLADR: Human leukocyte antigen-DR; IFN γ : Interferon-gamma; IL: Interleukin; LS: Lesional; N: Normal; NL: Non-lesional; SCORAD: SCORing Atopic Dermatitis; Tcm: Central memory T; Tem: Effector memory T; TEWL: Transepidermal water loss; Treg: Regulatory T

Duration of Disease		
Marker	r	P-value
CD4+IFN γ +	0.584	2.45E-08
CD4+IFN γ +CLA-	0.583	2.74E-08
CD4+IL9+CLA+	0.570	6.39E-08
CD8+IL13+CLA-	0.507	2.50E-06
CD4+IFN γ +CLA+	0.406	2.51E-04
CD8+IL22+CLA-	0.392	4.28E-04
CD4+IL9+CLA-	0.381	6.34E-04
EASI	0.376	6.46E-04
CD4+IL9+	0.350	1.81E-03
CD8+IL22+	0.317	4.96E-03
CD4+CLA+	-0.304	7.22E-03
CD8+IL13+	0.299	8.15E-03
CD8+CLA+	-0.299	8.34E-03
CD4+IL13+CLA-	0.289	1.09E-02
CD8+IL22+CLA+	0.281	1.35E-02
Total ADQ	0.284	1.41E-02
CD4+IL22+CLA+	0.276	1.50E-02
CD8+IL9+CLA-	0.235	3.95E-02
Pruritus ADQ	0.222	5.68E-02
CD4+IL13+	0.217	5.84E-02
Eosinophils	0.234	9.18E-02
CD8+IL17+CLA-	0.155	1.77E-01
SCORAD	0.153	1.80E-01
CD4+IL13+CLA+	0.149	1.96E-01
CD8+IFN γ +CLA-IL13+CLA-	0.143	2.05E-01
CD8+IL17+CLA+	-0.093	4.26E-01
CD8+IL9+	0.055	6.35E-01
CD8+IL17+	0.049	6.75E-01
CD8+IFN γ +CLA+IL13+CLA+	0.039	7.30E-01
CD8+IL9+CLA+	0.028	8.07E-01
CD8+IL13+CLA+	0.016	8.89E-01
CD8+IFN γ +CLA+	-0.006	9.61E-01

Table E5: Pairwise comparisons between age group associated clusters

	Adults vs. Infants	Children and Adolescents vs. Infants	Children and Adolescents vs. Adults	P-values			P-value ANOVA	FDR ANOVA
				Adults vs. Infants	Children and Adolescents vs. Infants	Children and Adolescents vs. Adults		
CD4+IFN γ +	23.985	11.339	-12.646	2.82E-14	1.03E-08	6.47E-08	2.56E-19	1.74E-17
CD4+IFN γ +CLA-	24.326	11.726	-12.600	2.88E-14	7.95E-09	1.44E-07	4.46E-19	2.99E-17
CD4+IFN γ +CLA+	16.491	5.763	-10.728	1.30E-13	1.00E-03	1.85E-07	2.49E-13	1.64E-11
CD8+IFN γ +CLA-	37.265	13.395	-23.870	2.22E-13	8.51E-04	4.17E-07	5.10E-13	3.31E-11
CD8+IFN γ +	36.183	12.373	-23.810	3.67E-13	1.86E-03	2.93E-07	9.04E-13	5.78E-11
CD4+IL22+CLA-	2.477	2.543	0.067	3.26E-07	3.52E-10	0.987	5.75E-11	3.62E-09
CD4+IL22+	1.010	2.831	1.821	7.52E-02	3.82E-11	2.49E-04	6.00E-11	3.72E-09
CD4+IL9+CLA+	-0.975	5.543	6.518	6.81E-01	2.10E-07	2.57E-07	2.51E-09	1.53E-07
CD8+IL13+CLA-	0.701	0.334	-0.367	5.93E-08	1.44E-03	4.61E-03	8.43E-08	5.06E-06
CD4+Naïve cells	-22.633	-8.536	14.097	5.04E-08	1.05E-02	3.47E-04	1.12E-07	6.60E-06
CD8+IL22+CLA-	0.780	0.311	-0.469	7.63E-08	1.11E-02	1.01E-03	1.63E-07	9.45E-06
CD4+IL17+CLA-	1.167	0.843	-0.324	2.34E-06	3.36E-05	0.300	3.66E-07	2.09E-05
CD4+IL17+	1.174	0.816	-0.358	1.75E-06	5.30E-05	0.228	3.79E-07	2.12E-05
CD8+Tem HLADR+CLA-	5.887	0.329	-5.558	1.62E-06	9.26E-01	2.96E-06	5.29E-07	2.91E-05
CD4+IL13+	0.068	1.791	1.723	0.987	6.20E-06	2.73E-04	1.50E-06	8.11E-05
CD4+IL9+	-0.135	0.734	0.869	0.782	6.58E-05	6.80E-05	3.48E-06	1.84E-04
CD4+IL9+CLA-	-0.092	0.603	0.695	0.848	7.50E-05	1.24E-04	5.57E-06	2.90E-04
CD4+Tcm	12.321	7.113	-5.208	1.07E-05	1.25E-03	8.09E-02	7.32E-06	3.73E-04
CD4+IL13+CLA-	0.141	1.539	1.398	0.934	1.85E-05	1.47E-03	7.95E-06	3.97E-04
CD8+IL22+	0.767	0.292	-0.474	4.99E-06	0.047	5.16E-03	9.67E-06	4.74E-04
CD4+Tem CLA+	-21.405	-12.280	9.124	2.02E-05	0.002	0.090	1.47E-05	0.001
CD4+Tem	8.138	2.779	-5.358	8.69E-06	0.081	0.003	1.70E-05	0.001
CD4+IFN γ +CLA+IL13+CLA-	73.154	5.044	-68.110	6.60E-05	0.924	1.73E-04	3.91E-05	0.002
CD4+IL22+CLA+	2.017	4.986	2.969	0.295	4.58E-05	0.065	7.44E-05	0.003
Tregs CD25+CD127+	1.378	4.627	3.249	0.561	7.94E-05	0.028	7.69E-05	0.003
CD8+Tcm ICOS+CLA+	6.025	13.250	7.225	0.257	7.12E-05	0.123	1.20E-04	0.005
CD8+IL17+CLA-	0.395	0.152	-0.243	7.12E-05	0.102	0.018	1.28E-04	0.005
CD8+Naïve cells	-14.301	3.940	18.241	4.73E-03	0.490	9.87E-05	1.67E-04	0.007
CD4+IFN γ +CLA+IL13+CLA+	5.002	0.293	-4.709	3.34E-04	0.955	6.48E-04	2.05E-04	0.008
CD4+Tcm CLA+	-10.615	-6.947	3.668	5.60E-04	0.004	0.345	2.68E-04	0.010
CD8+IFN γ +CLA+	13.375	-4.151	-17.526	0.010	0.496	3.61E-04	5.62E-04	0.021
CD8+Tem CLA+	-4.363	-6.058	-1.696	0.105	0.001	0.683	0.002	0.059
CD4+Tcm HLADR+CLA+	2.583	7.579	4.996	0.629	0.002	0.148	0.003	0.101
CD8+IL13+CLA+	5.232	1.302	-3.930	0.002	0.546	0.026	0.003	0.118
CD8+Effector cells	6.634	-0.497	-7.131	0.012	0.958	0.004	0.004	0.135

CD8+Tem ICOS+CLA+	5.485	12.108	6.622	0.453	0.003	0.280	0.004	0.143
CD8+Tcm HLADR+CLA+	10.335	10.257	-0.079	0.033	0.007	1.000	0.005	0.145
CD8+Tem	5.247	-0.345	-5.592	0.017	0.970	0.006	0.006	0.198
CD4+IL17+CLA+	1.288	0.507	-0.781	0.004	0.272	0.113	0.007	0.198
CD8+Effector cells CLA+	-4.570	-2.774	1.796	0.010	0.055	0.440	0.008	0.226
CD4+IL13+CLA+	-0.035	4.409	4.444	1.000	0.015	0.049	0.008	0.229
CD8+IL17+CLA+	0.731	-0.664	-1.395	0.286	0.220	0.010	0.012	0.321
CD8+IL9+CLA+	-8.800	-2.116	6.684	0.009	0.649	0.055	0.012	0.321
CD8+IL13+	0.709	0.244	-0.464	0.009	0.405	0.115	0.013	0.328
CD4+Tem ICOS+CLA-	-8.238	-4.029	4.209	0.019	0.201	0.308	0.023	0.542
CD8+Tcm CLA+	-2.498	-4.210	-1.713	0.420	0.019	0.638	0.026	0.597
CD8+Tem HLADR+CLA+	7.009	7.170	0.161	0.123	0.031	0.999	0.027	0.597
CD4+Tcm ICOS+CLA-	-1.975	2.717	4.693	0.571	0.177	0.034	0.028	0.597
CD4+Tcm ICOS+CLA+	4.248	7.190	2.942	0.434	0.023	0.639	0.031	0.617
CD8+Tcm ICOS+CLA-	-1.177	2.186	3.363	0.702	0.136	0.047	0.031	0.617
CD4+Tem HLADR+CLA+	1.993	5.482	3.489	0.753	0.034	0.387	0.040	0.716
CD8+IL22+CLA+	4.546	1.976	-2.571	0.062	0.450	0.383	0.076	1.00
CD8+Tcm	2.806	-2.710	-5.517	0.558	0.407	0.087	0.090	1.00
CD4+Effector cells	2.203	-1.360	-3.563	0.406	0.564	0.077	0.092	1.00
CD8+IL9+CLA-	0.036	0.240	0.203	0.965	0.107	0.313	0.101	1.00
CD8+IL17+	0.226	0.031	-0.194	0.123	0.938	0.194	0.125	1.00
CD4+Tem HLADR+CLA-	-1.824	0.474	2.298	0.297	0.873	0.123	0.144	1.00
CD8+IFN γ +CLA+IL13+CLA-	-101.376	-49.219	52.157	0.167	0.526	0.609	0.184	1.00
CD8+IL9+	-0.238	0.178	0.415	0.592	0.640	0.190	0.213	1.00
CD8+CLA+	3.071	-1.244	-4.315	0.519	0.494	0.283	0.227	1.00
CD8+IFN γ +CLA+IL13+CLA+	-8.738	-7.932	0.807	0.386	0.310	0.992	0.257	1.00
CD8+Tcm HLADR+CLA+	3.841	0.516	-0.885	0.943	-4.726	0.335	0.364	1.00
Tregs CCR4+CLA+	3.026	0.678	3.157	0.502	0.131	0.999	0.499	1.00
CD4+Effector cells CLA+	-2.420	0.647	-0.254	0.992	2.167	0.683	0.642	1.00
CD4+Tcm HLADR+CLA+	-0.908	0.887	-0.975	0.797	-0.067	0.999	0.798	1.00
CD4+CLA+	-0.589	0.801	-0.168	0.974	0.421	0.887	0.816	1.00
CD4+Tem ICOS+CLA+	2.145	0.902	0.733	0.980	-1.412	0.952	0.910	1.00
CD8+Tem ICOS+CLA-	0.128	0.990	-0.225	0.951	-0.353	0.922	0.914	1.00

P-values, as measured by Welch's t-test, that compare the three clusters that were obtained by k-means unsupervised clustering of all AD patients, as well as both raw and FDR-adjusted ANOVA score among all clusters. Abbreviations ANOVA: Analysis of variance; CLA: Cutaneous lymphocyte antigen; FDR: False discovery rate; HLADR: Human leukocyte antigen-DR; ICOS: Inducible co-stimulator; IFN γ : Interferon-gamma; IL: Interleukin; Tcm: Central memory T; Tem: Effector memory T; Treg: Regulatory T

Table E6. Evolution of pathologic T-cell subsets in atopic dermatitis from infancy to adulthood

	Healthy controls				Atopic Dermatitis			
	0-5y/o	6-11y/o	12-17y/o	≥ 18y/o	0-5y/o	6-11y/o	12-17y/o	≥18y/o
IFN-γ CLA+>CLA- CD4+>CD8+	---	↑↑↑	↑↑↑	↑↑↑↑	↓	↑↑	↑↑	↑↑↑
IL-13 CLA+>CLA- CD4+>CD8+	---	↑	↑	↑	↑↑↑↑	↑↑↑↑	↑↑↑↑	↑↑↑↑
IL-9	---	↑	↑↑	↓	↓	↑↑ CLA->CLA+	↑↑↑ CLA->CLA+	↓
IL-17	---	↑ CLA-	↑↑ CLA-	↑↑ CLA-	---	↑ CLA-	↑↑ CLA-	↑↑ CLA-
IL-22	---	---	↑ CLA-	↑ CLA-	---	↑	↑↑ CLA+	↑↑↑ CLA+
Tregs (CLA+)	---	---	---	---	---	↑↑	↑	↑

Arrows show developmental changes in healthy controls and AD across ages, as well as AD vs. control changes in the AD groups. CLA, Cutaneus Lymphocyte Antigen; Tregs, T-regulatory cells

Figure E1

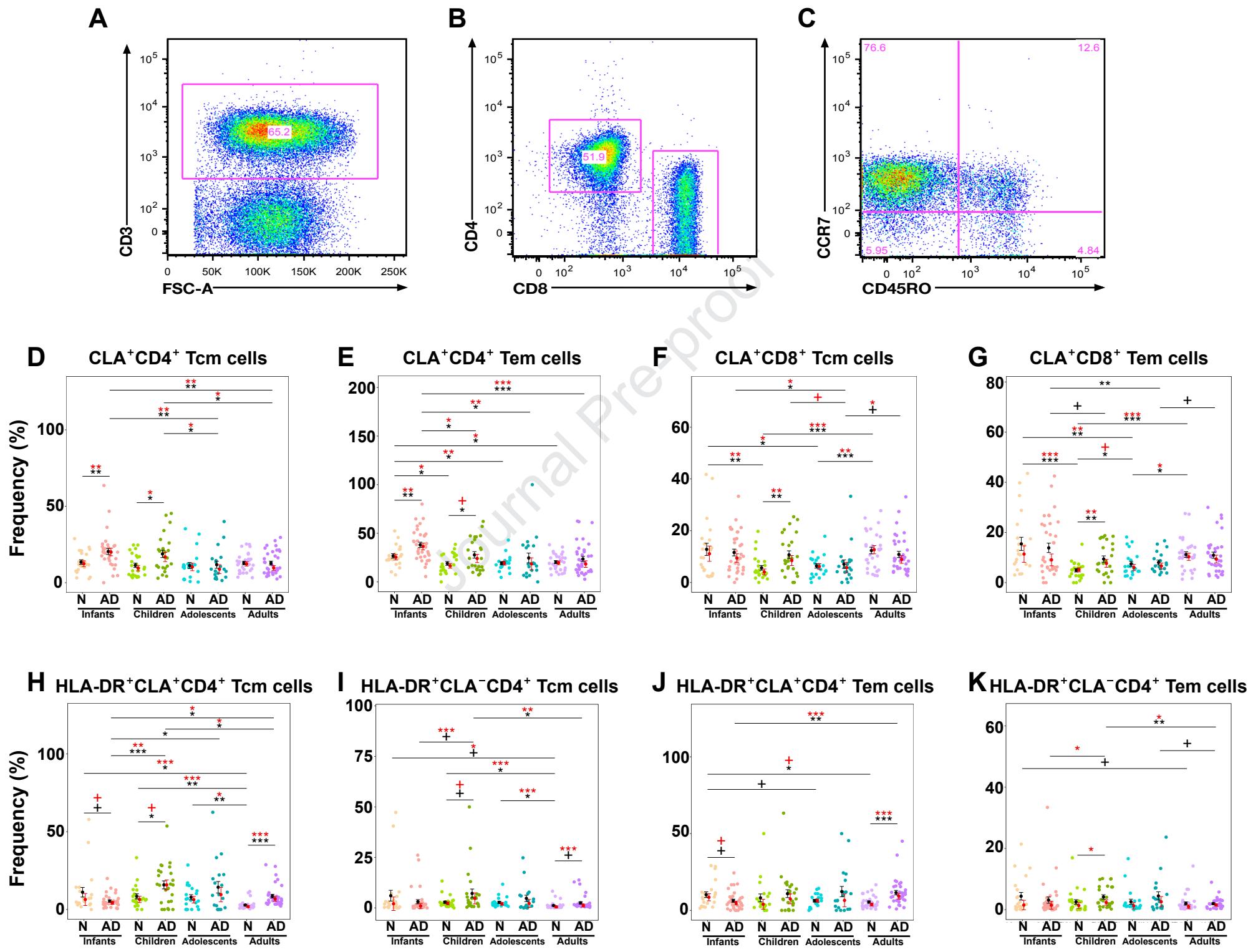


Figure E2

CD4⁺ T cells

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CD8⁺ T cells

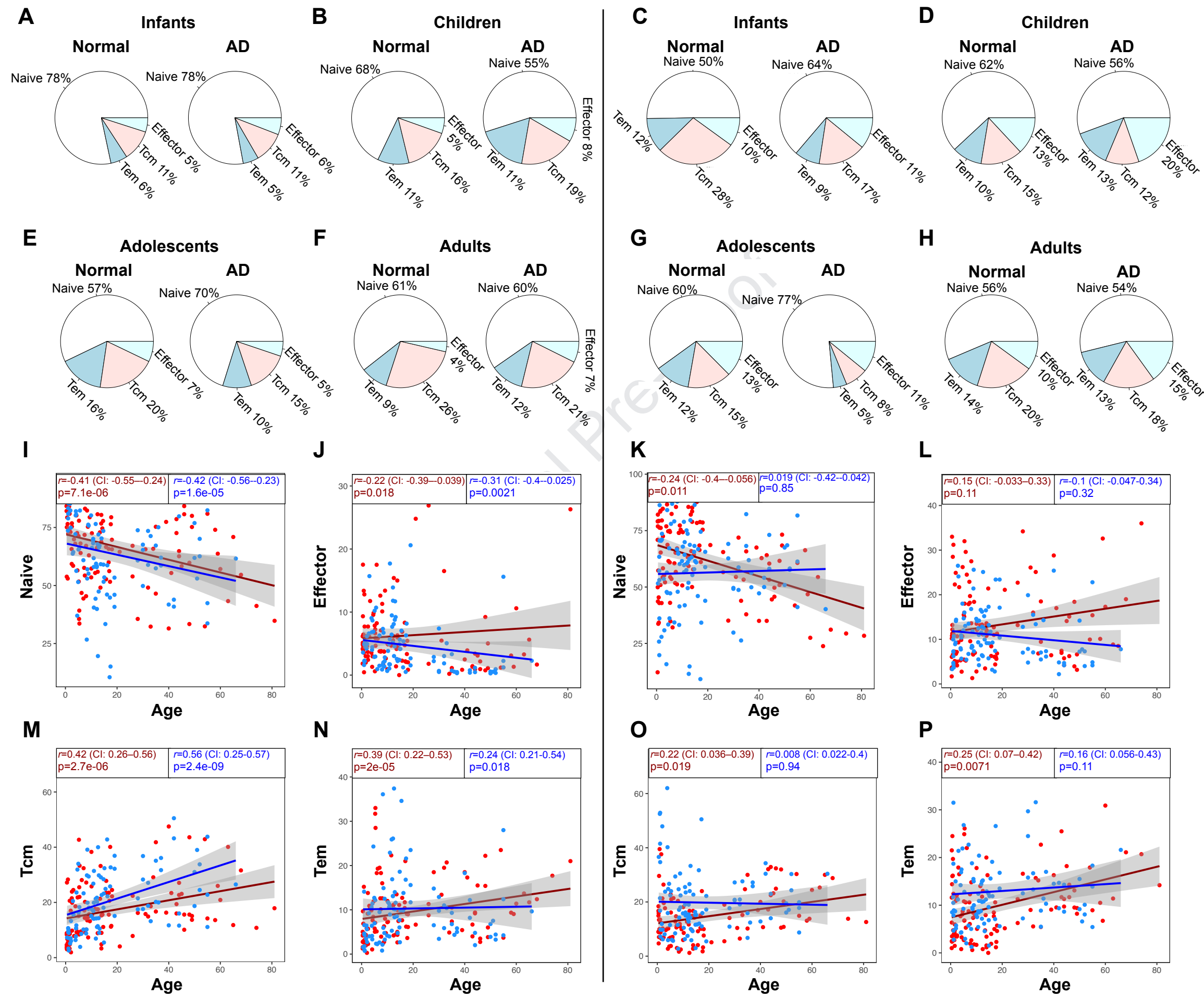


Figure E3

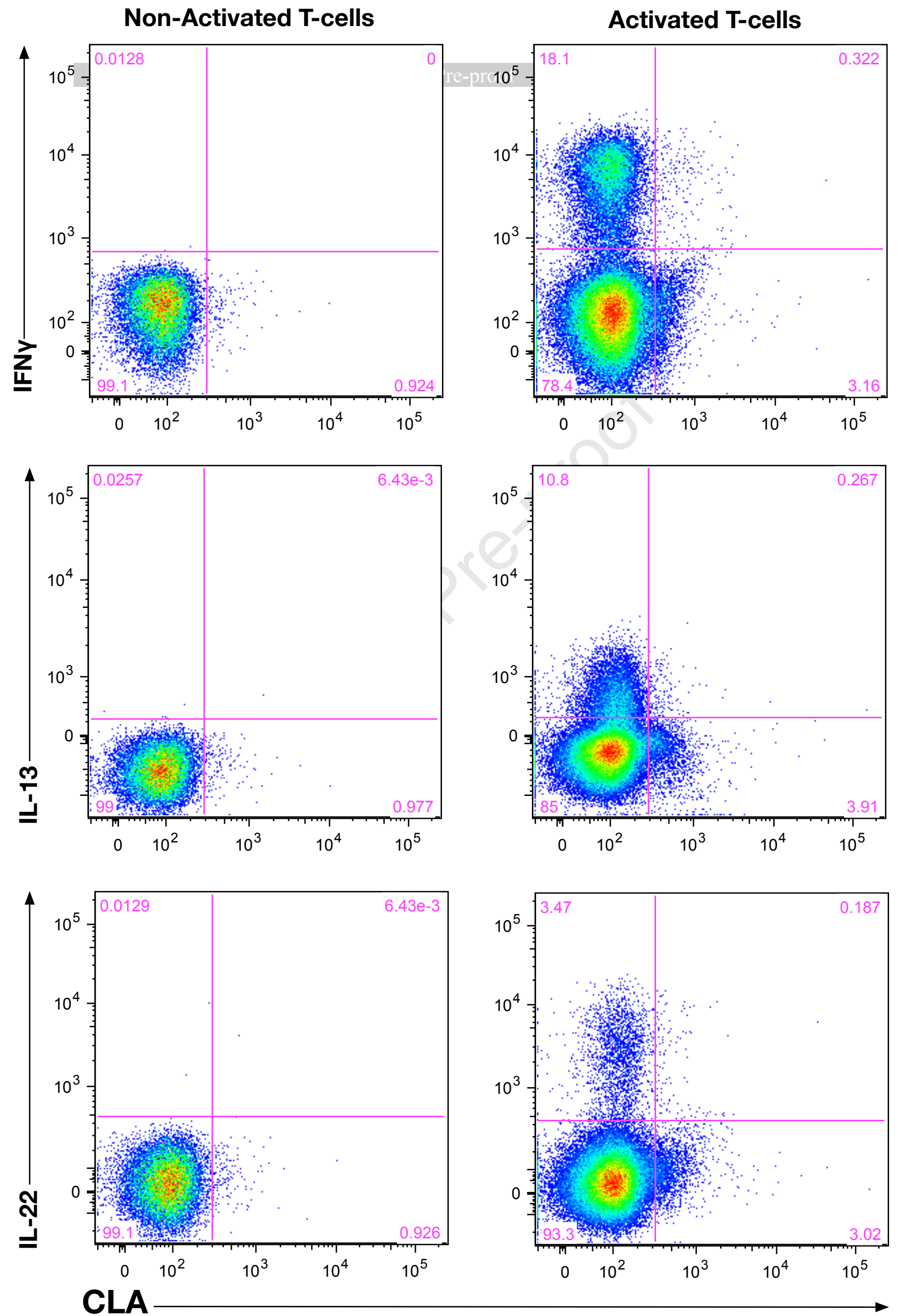
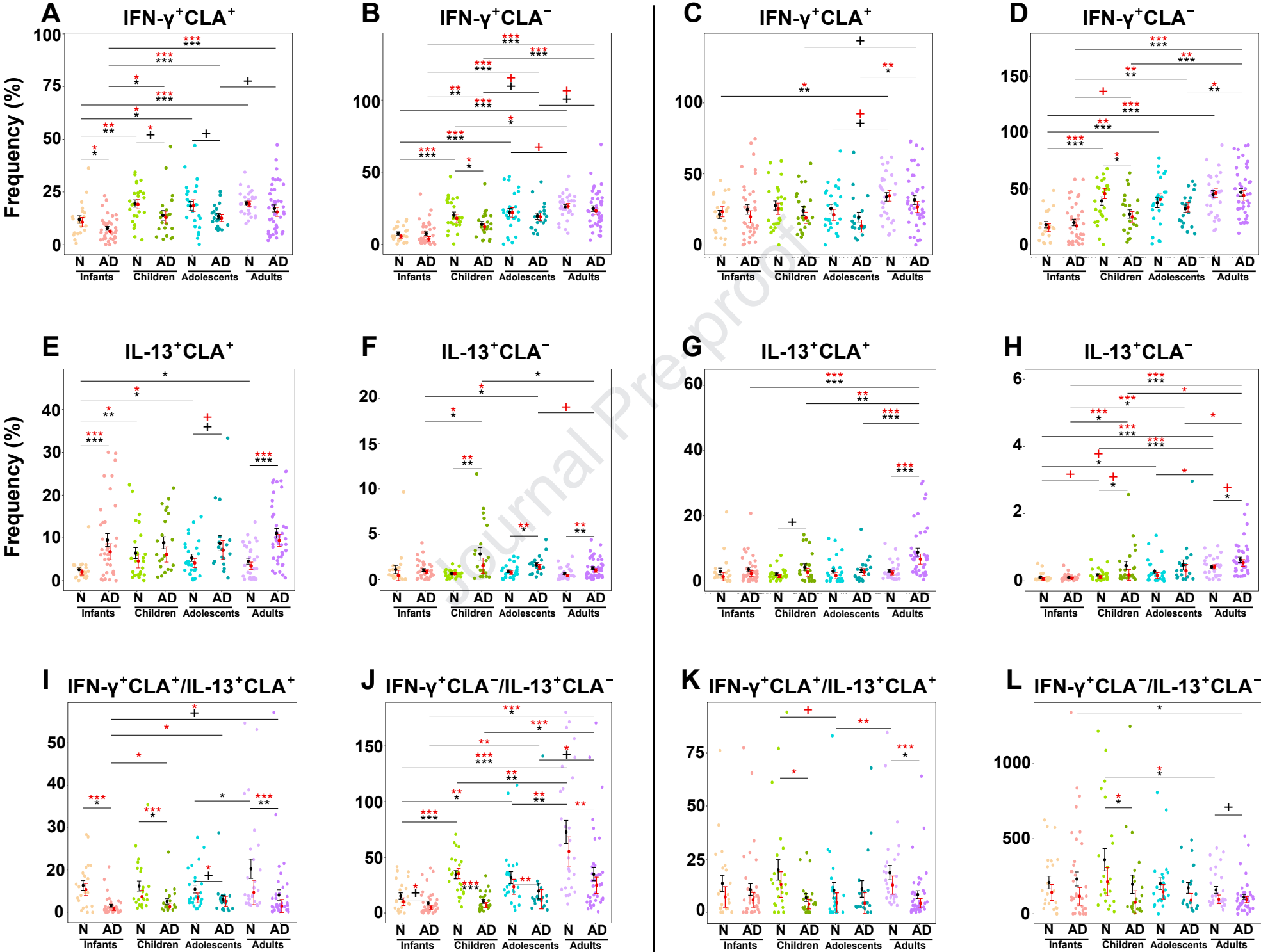


Figure E4

CD4⁺ T cells

CD8⁺ T cells



Journal Pre-proof

CD8⁺ T cells

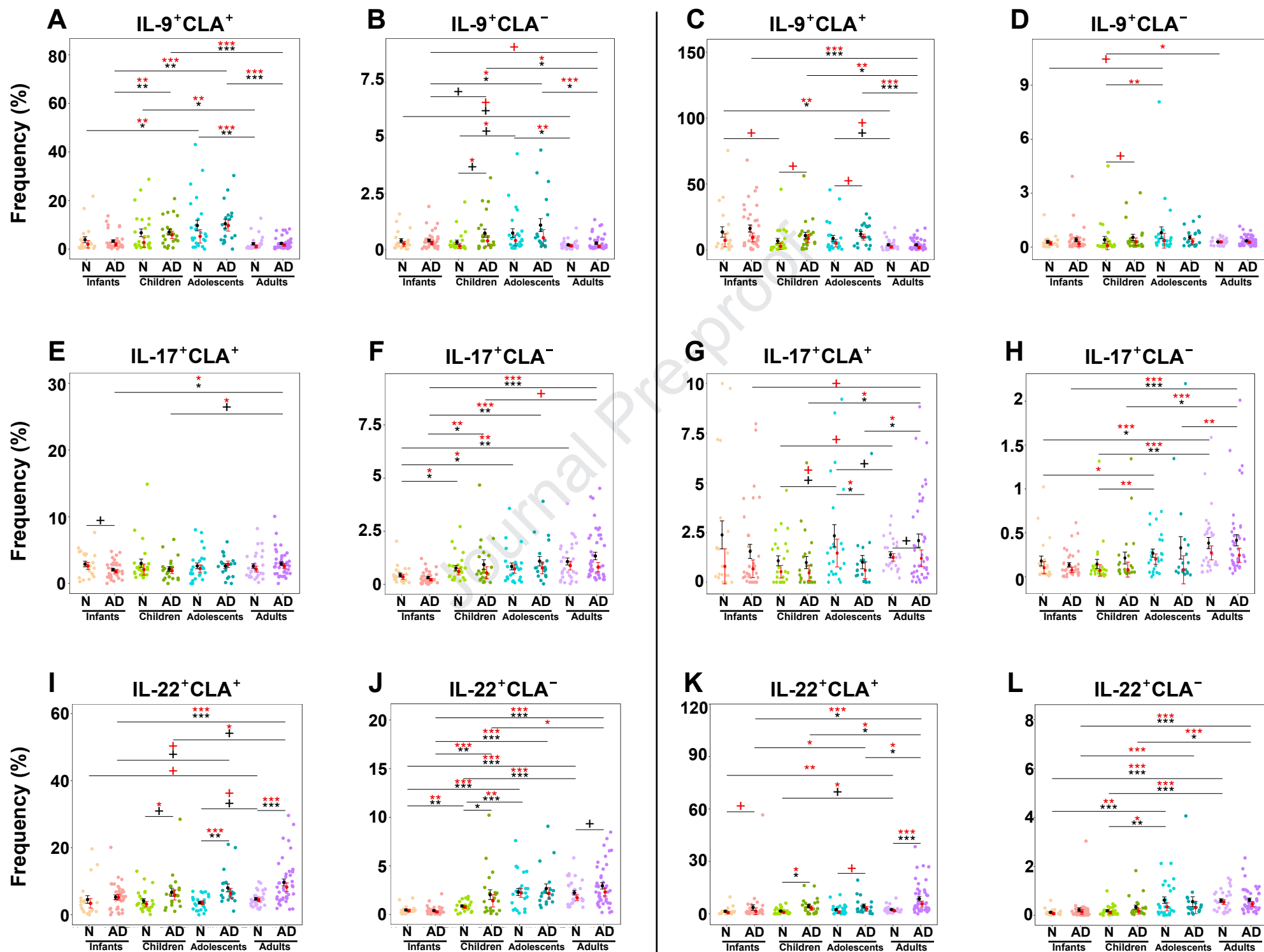


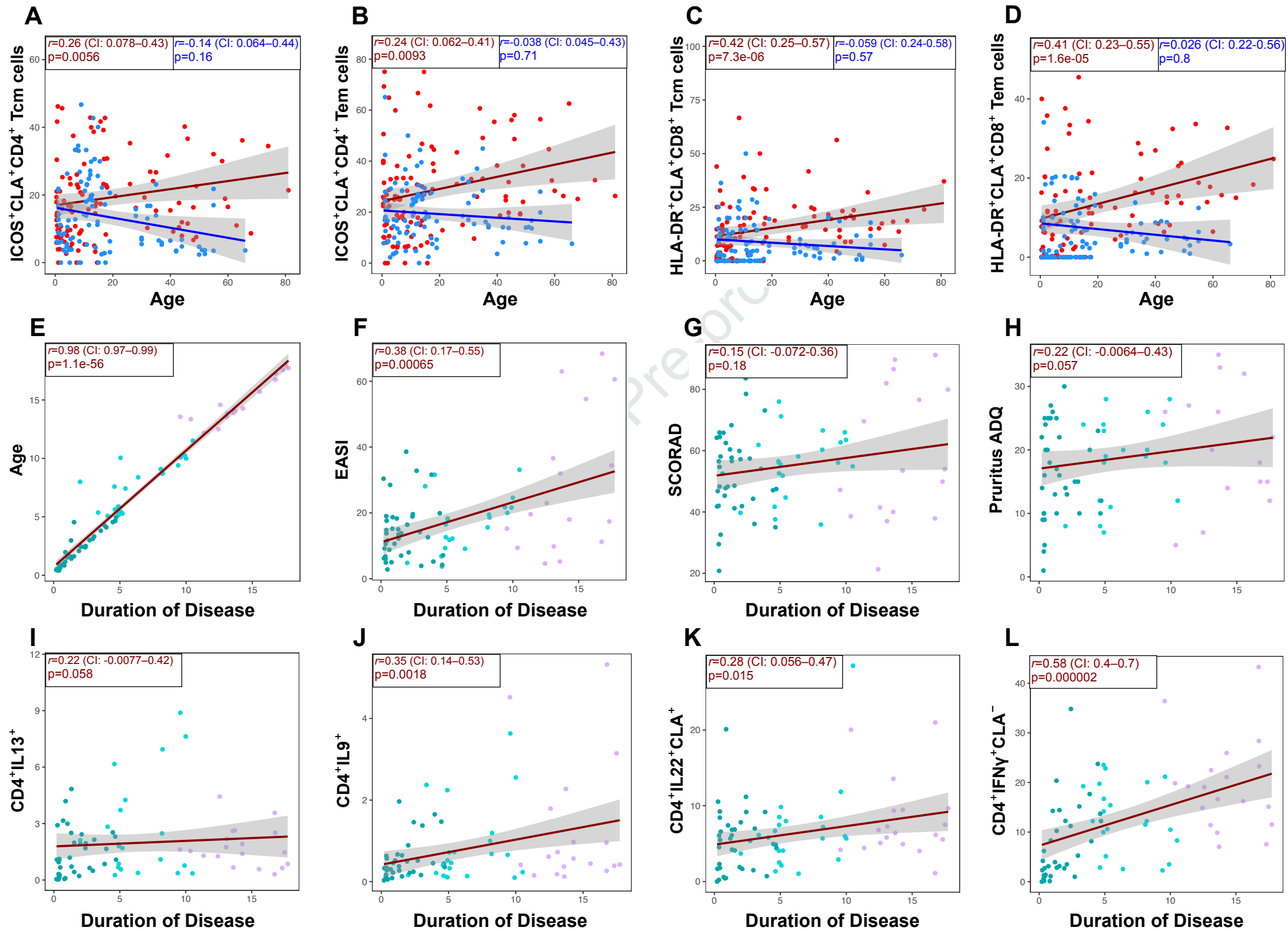
Figure E6

Figure E7

CD4⁺ T cells

Journal Pre-proof

CD8⁺ T cells

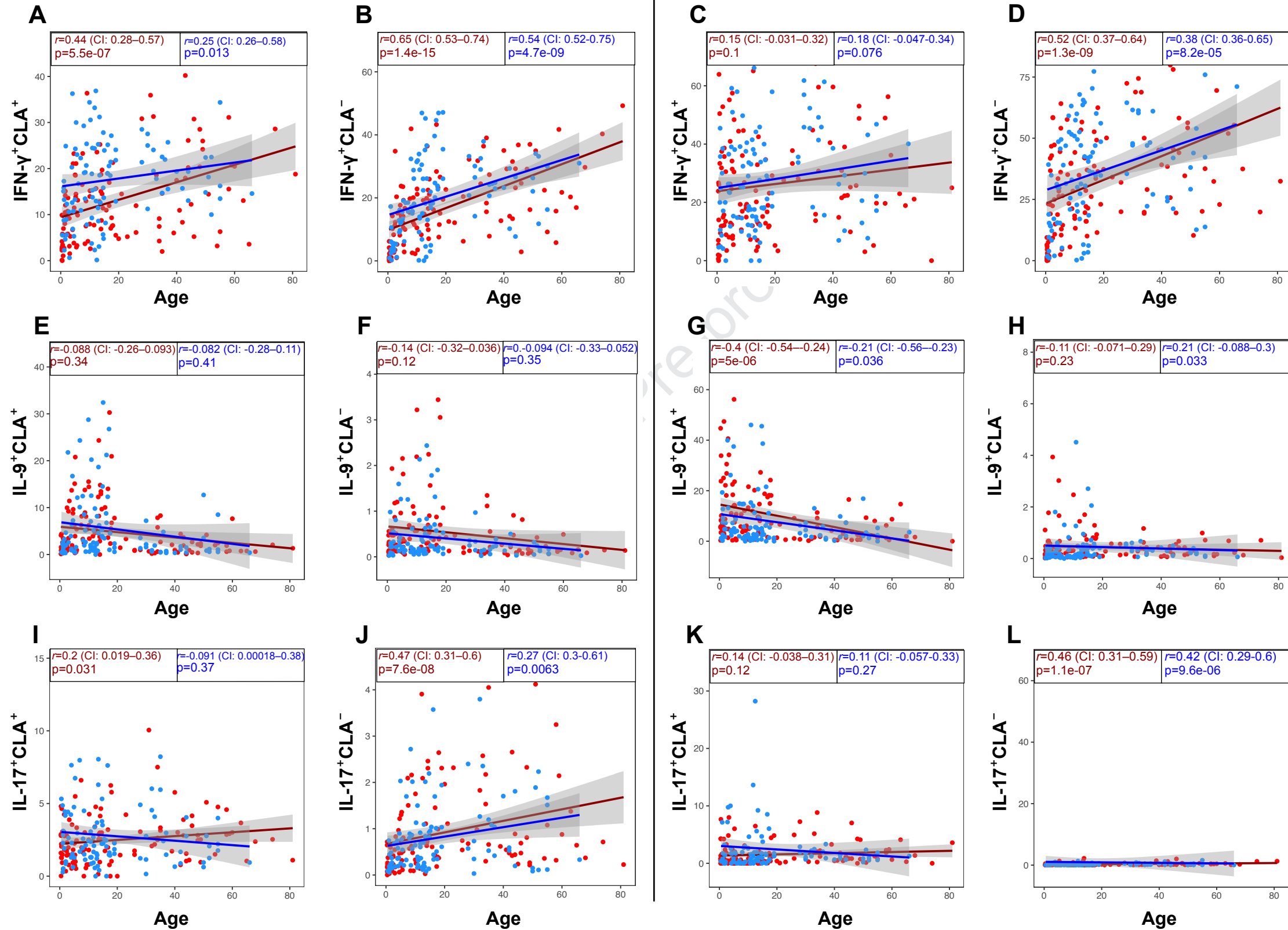
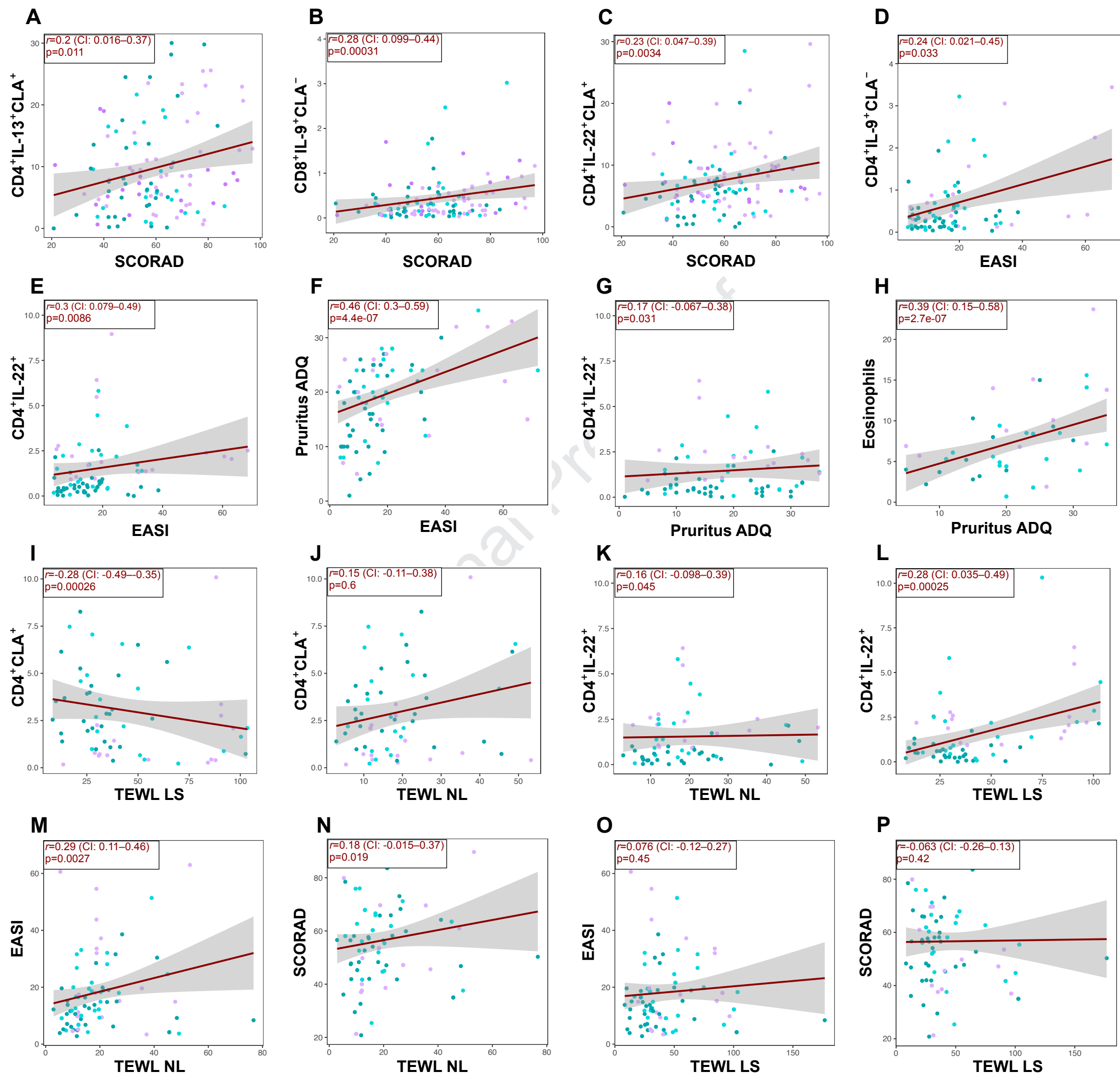
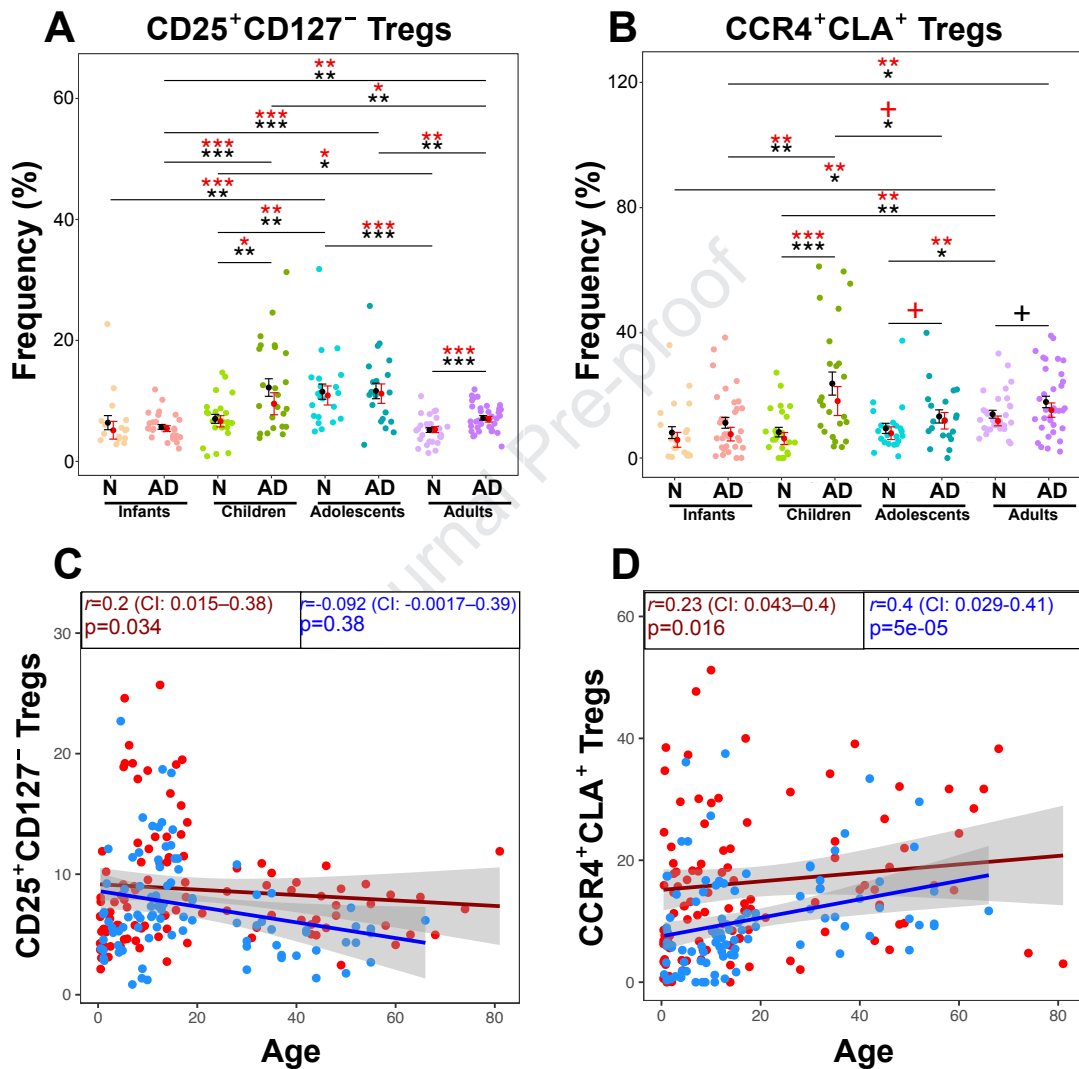


Figure E8





1 **Supplementary Materials and Methods**

2 **Isolation of Peripheral blood mononuclear cells and multiparameter flow** 3 **cytometry blood analyses**

4 Peripheral blood mononuclear cells (PBMCs) were obtained by gradient
5 centrifugation with Ficoll-Paque Plus (Amersham Biosciences, Pittsburgh, PA).
6 The blood was laid under the Ficoll gradient; after spinning, the PBMCs were
7 collected at the interface between the plasma and the Ficoll gradient and washed
8 with PBS before staining for flow cytometry analysis. For T-cell surface staining,
9 PBMCs were washed and incubated for 30 minutes in ice with fluorochrome-
10 conjugated monoclonal antibodies (mAbs) to cell surface molecules (CD3-Pe AF
11 610, CD8-V-500, CD4-Qdot800, CD45RO-PeCy7, CLA-FITC, ICOS-
12 PerCpCy5.5, CCR7-Alexa Fluor 700, HLA-DR-APC-H7, CCR4-BV 421, CD25-
13 PE, CD127-BV 650, Live/dead-Blue Dye). Then, the cells were fixed in ice with
14 4% paraformaldehyde (BD Biosciences, San Jose, CA) for 20 minutes. For
15 intracellular cytokine staining, whole blood was activated with 25 ng/mL of
16 phorbol 12-myristate 13-acetate (PMA) and 2 µg/mL of ionomycin in the
17 presence of 10 µg/mL brefeldin A (protein transporter inhibitor) for 4 hours at
18 37°C. All reagents were purchased from Sigma Aldrich (St. Louis, MO). Non-
19 activated controls were only treated with brefeldin A. After this incubation, EDTA
20 (2 mM; Invitrogen, Grand Island, NY) was added for 15 minutes at room
21 temperature (RT) to stop activation. The blood was then incubated with FACS
22 lysing solution for 10 minutes at RT and washed with PBS twice. Then, the cells
23 were incubated for 20 minutes with fluorochrome-conjugated mAbs to cell

surface molecules (CD3-Pe AF 610, CD4-Qdot800, CLA-FITC). Subsequently, the cells were permeabilized (Perm/Wash; BD Biosciences, San Jose, CA) and incubated for 20 min with fluorochrome conjugated mAbs (IL-13-PerCpCy5.5, IL-22-PeCy7, IL-9-Pe, IFN- γ - Alexa Fluor 700 and IL-17-APC efluor 780) in 5% mouse serum (eBioscience, San Diego, CA). All incubations for cell surface and intracellular staining were done at RT. Appropriate FMO-isotype controls were used. Following incubations, cells were washed and subsequently acquired with a BD LSR II flow cytometer (BD Biosciences) and analyzed by flow-Jo software (Treestar, Ashland, OR).

Supplementary Figure legends

Figure E1. After gating on CD3⁺ viable T-cells using flow cytometry, CD3⁺CD4⁺ and CD3⁺CD8⁺ were defined and analyzed separately. CCR7 and CD45RO were used to differentiate memory subsets within the CD4 and CD8 populations. CCR7⁺CD45RO⁻ defined naïve cells, CCR7⁺CD45RO⁺ central memory T-cells (Tcm), CCR7⁻CD45RO⁺ effector memory T-cells (Tem) and CCR7⁻CD45RO⁻ Effector/Temra/terminally differentiated T-cells **(A-C)**. We then further defined activated ICOS/HLA-DR activated Tcm/Tem memory subset, using CLA to segregate skin-homing (CLA⁺) versus systemic (CLA⁻) subsets, for each memory population. Gating strategy for **(A)** CD3 vs. FSC-A, **(B)** CD4 vs. CD8, and **(C)** CCR7 vs. CD45RO cells. **(D-G)** CD4⁺/CD8⁺ Tem (CD45RO⁺CCR7⁻) and Tcm (CD45RO⁺CCR7⁺) subset frequencies and **(H-K)** HLA-DR⁺CLA^{+/+} CD45RO⁺CCR7^{+/+} in AD and their respective controls across ages. Bar plots represent means (black)/medians (red) ± SEMs. AD, Atopic dermatitis; CLA, Cutaneous lymphocyte antigen; CCR, C-C Motif Chemokine Receptor 7; FSC-A, Forward Scatter-Area; SEM, Standard error of the mean/median; Tcm, central memory T-cells; Tem, effector memory T-cells. *P*-values are designated as ***<0.001, **<0.01, *<0.05, and +<0.1.

Figure E2. (A-H) Pie charts showing the distribution of CD4⁺/CD8⁺ naïve, Tcm, Tem, and effector T-cells in infants, children, adolescents and adults. **(I-P)** Spearman correlation scatter plots (linear regression [red=AD/blue=control line] with their CI [in gray]) for frequencies (%) of naïve/effector/T_{CM}/T_{EM} T-cells in the CD4⁺ **(I-J, M-N)** and CD8⁺ **(K-L, O-P)** compartments versus age in AD patients

(red dots/line) and controls (blue dots/line). AD, Atopic dermatitis; CI, Confidence interval; r , Spearman correlation coefficient with CI and associated P-value; Tcm, central memory T-cells; Tem, effector memory T-cells.

Figure E3. Gating strategy of IFN- γ , IL-13, and IL-22 versus CLA positivity in CD4⁺ and CD8⁺ non-activated and PMA/ionomycin activated T-cells in a representative patient with AD. CLA, Cutaneous lymphocyte antigen.

Figure E4. A-D) IFN- γ ⁺, **(E-H)** IL-13⁺, and **(I-L)** IFN- γ ⁺/IL-13⁺ cytokine frequencies in CLA⁺, CLA⁻, CD4⁺ and CD8⁺ T-cells in AD and their respective controls across ages. The ratio between the % of IFN- γ ⁺CLA⁺CD4⁺ (or CD8⁺) T cells and the % of their IL-13⁺ counterpart was calculated for each sample but was not multiplied by 100 and is therefore unitless. Bar plots represent means (black)/medians (red) $\pm$ SEMs. AD, Atopic dermatitis; CLA, Cutaneous lymphocyte antigen; SEM, Standard error of the mean/median. *P*-values are designated as ***<0.001, **<0.01, *<0.05, and ⁺<0.1.

Figure E5. A-D) IL-9⁺, **(E-H)** IL-17⁺ and **(I-L)** IL-22⁺ frequencies in CLA⁺, CLA⁻, CD4⁺ and CD8⁺ T-cells in AD and their respective controls across ages. Bar plots represent means (black)/medians (red) $\pm$ SEMs. AD, Atopic dermatitis; CLA, Cutaneous lymphocyte antigen; SEM, Standard error of the mean/median. *P*-values are designated as ***<0.001, **<0.01, *<0.05, and ⁺<0.1.

Figure E6. Spearman correlation scatter plots (linear regression [red=AD/blue=control line] with their CI [in gray]) for **(A-D)** frequencies (%) of ICOS⁺ and HLA-DR⁺ activated CLA⁺/CD4⁺/CD8⁺ Tem (CD45RO⁺CCR7⁻) and Tcm (CD45RO⁺CCR7⁺) T-cells versus age and **(E-L)** disease duration versus

clinical scores and various cytokine-producing T-cell subsets in AD patients (red dots/line) and control subjects (blue dots/line). CI, Confidence interval; CLA, Cutaneous lymphocyte antigen; EASI, Eczema area and severity index; r, Spearman correlation coefficient with CI and associated P-value; SCORAD, SCORing of Atopic Dermatitis.

Figure E7. Spearman correlation scatter plots (linear regression [red=AD/blue=control line] with their CI [in gray]) for frequencies (%) of CD4⁺/CD8⁺, CLA⁺/CLA⁻ (A-D) IFN- γ ⁺, (E-H) IL-9⁺, and (I-L) IL-17⁺ producing T-cells versus age in AD patients (red dots/line) and controls (blue dots/line). CI, Confidence interval; CLA, Cutaneous lymphocyte antigen; r, Spearman correlation coefficient with CI and associated P-value.

Figure E8. Spearman correlation scatter plots (linear regression [red=AD/blue=control line] with their CI [in gray]) for (A-C) SCORAD, (D-F) EASI, (G-H) Pruritus, and (I-P) TEWL versus clinical measures and frequencies (%) of various T-cell subsets. Dots colors represent different AD patient ages from infancy to adulthood as shown in Figure 1. CI, Confidence interval; CLA, Cutaneous lymphocyte antigen; EASI, Eczema area and severity index; LS, Lesional; NL, Non-lesional; SCORAD, SCORing of Atopic Dermatitis; TEWL, Transepidermal water loss.

Figure E9. A) Total and **B)** skin-homing Treg frequencies in AD and their respective controls across ages and **(C-D)** Spearman correlation scatter plots (linear regression [blue=control/red=AD lines] with their CI [in gray]) for Treg frequency (%) versus age. Bar plots represent means (black)/medians (red) $\pm$

103 SEMs. r , Spearman correlation coefficient with CI and associated P -value. AD,
104 Atopic dermatitis; CI, Confidence interval; CLA, Cutaneous lymphocyte antigen;
105 SEM, Standard error of the mean/median; Tregs, T-regulatory cells. P -values are
106 designated as *** <0.001 , ** <0.01 , * <0.05 , and + <0.1 .