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In systemic lupus erythematosus (lupus), environmental effects acting within a permissive genetic background lead to autoimmune dysregulation. Dysfunction of $CD4^+$ T cells contributes to pathology by providing help to autoreactive B and T cells, and $CD4^+$ T cell dysfunction coincides with altered DNA methylation and histone modifications of select gene loci. However, chromatin accessibility states of distinct T cell subsets and mechanisms driving heterogeneous chromatin states across patients remain poorly understood. We defined the transcriptome and epigenome of multiple $CD4^+$ T cell populations from lupus patients and healthy individuals. Most lupus patients, regardless of disease activity, had enhanced chromatin accessibility bearing hallmarks of inflammatory cytokine signals. Single cell approaches revealed that chromatin changes extended to naive $CD4^+$ T cells; uniformly affecting naive subpopulations. Transcriptional data and cellular and protein analyses suggested that the TNF family members, TNFα, LIGHT, and TWEAK, were linked to observed molecular changes and the altered lupus chromatin state. However, we identified a patient subgroup prescribed angiotensin receptor blockers (ARBs) which lacked TNF-linked lupus chromatin accessibility features. These data raise questions about the role of lupus-associated chromatin changes in naive $CD4^+$ T cell activation and differentiation and implicate ARBs in the regulation of disease-driven epigenetic states.

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Title: Angiotensin receptor blockers modulate the lupus CD4+ T cell epigenome characterized by TNF family-linked signaling

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Abstract

In systemic lupus erythematosus (lupus), environmental effects acting within a permissive genetic background lead to autoimmune dysregulation. Dysfunction of $CD4^+$ T cells contributes to pathology by providing help to autoreactive B and T cells, and $CD4^+$ T cell dysfunction coincides with altered DNA methylation and histone modifications of select gene loci. However, chromatin accessibility states of distinct T cell subsets and mechanisms driving heterogeneous chromatin states across patients remain poorly understood. We defined the transcriptome and epigenome of multiple $CD4^+$ T cell populations from lupus patients and healthy individuals. Most lupus patients, regardless of disease activity, had enhanced chromatin accessibility bearing hallmarks of inflammatory cytokine signals. Single cell approaches revealed that chromatin changes extended to naive $CD4^+$ T cells; uniformly affecting naive subpopulations. Transcriptional data and cellular and protein analyses suggested that the TNF family members, TNFα, LIGHT, and TWEAK, were linked to observed molecular changes and the altered lupus chromatin state. However, we identified a patient subgroup prescribed angiotensin receptor blockers (ARBs) which lacked TNF-linked lupus chromatin accessibility features. These data raise questions about the role of lupus-associated chromatin changes in naive $CD4^+$ T cell activation and differentiation and implicate ARBs in the regulation of disease-driven epigenetic states.

Keywords: SLE, Lupus, autoimmunity, epigenetics, chromatin accessibility, Tfh, Naive, CD4 T cell, TNFα, Angiotensin receptor

Introduction

Systemic lupus erythematosus (SLE; lupus) is a model systemic autoimmune disease; environmental effects acting within a permissive genetic background result in the breakdown of immune tolerance. Dysfunction of multiple cell types is associated with anti-nuclear autoantibody (ANA) production and tissue pathology (1). Antigen presenting cells are inappropriately activated, contributing to self-antigen presentation, cytokine-driven inflammation, and activation of adaptive immune cells (1, 2). The production of ANAs by B cell populations is a defining characteristic of disease (3); however, B cell-directed clinical therapies are imperfect and evidence suggests ANAs and tissue pathology are dependent on $CD4^+$ T cells (T_H) (4). For example, the HLA class II locus is the strongest human genetic susceptibility allele, consistent with T_H involvement in disease, and ANAs are high affinity, class-switched antibodies (Ab) that arise through germinal center (GC) responses dependent on Follicular T_H cells (Tfh) (5–8). Likewise, Th1, Th17, regulatory T cell (Treg), and $TCR\alpha\beta^+CD4^-CD8^-$ double negative (DN) SLE T cells have abnormal intrinsic signaling, altered frequencies, and invade tissues to contribute to pathology (7, 9–14).

Epigenetics has become an active area of study in lupus T cell biology as genetic susceptibility alone poorly explains changes in T_H subset frequencies and functions. Lupus T_H cells have hypomethylation of DNA surrounding costimulatory genes (*CD40L*, *CD9*) (15, 16) and interferon response genes (17, 18) as well as altered histone modifications at cytokine loci (*IL17*) (19). Lupus flare correlates with global changes in T cell DNA methylation (20). However, studies of T cell epigenetics in lupus have often relied on bulk T_H cells containing multiple subsets of naive, activated, and memory populations (15, 16, 19, 21–24). Limited studies have distinguished naive T_H cells from other populations (17, 18, 20) and prior naive T_H isolation methods do not remove effector memory subsets (25 and 26). Thus, epigenetic states of discrete T_H populations including Tfh and naive subsets remain incompletely described.

The accessibility of chromatin regions across the genome varies in different cell types and is influenced by activation, cell cycling, cytokine signaling, and other processes leading to transcriptional

responses (27). Direct measurement of chromatin accessibility by assay for transposase-accessible chromatin with sequencing (ATAC-seq) has emerged as a tractable measure of epigenetic cell state and transcriptional potential. Prior studies of T cell chromatin accessibility in lupus and have focused on peripheral blood mononuclear cells (PBMCs) without identifying individual T cell subsets (28), or Tregs (29), leaving unanswered questions about chromatin regulation in other T cell subsets.

We utilized ATAC-seq to profile the chromatin accessibility of multiple discrete T_H subsets—including naive T_H and Tfh – implicated in lupus. All populations of lupus T_H cells had a disease-specific chromatin signature. We confirmed that type I interferon (IFN) transcriptional responses are present, but variable, in lupus T_H cells; in parallel, the chromatin surrounding type I IFN-associated loci showed increased accessibility in lupus. However, T_H cells had more penetrant increased accessibility at gene loci involved in other cytokine signaling pathways and leukocyte activation, including enrichment of accessibility at loci containing common transcription factor motifs (NFκB, AP-1, IRF1). Although activation of type I IFN signaling has been associated with disease severity and autoantibody production, lupus-specific changes in T_H chromatin accessibility were more tightly associated with transcription of loci related to TNF family signaling. Transcriptional activation of TNF signaling in lupus T_H cells correlated with plasma levels of TNF family cytokines including TWEAK and with protein dysregulation of TNF family receptors including HVEM. Strikingly, lupus-associated epigenetic changes in T cells were absent in patients prescribed angiotensin receptor blocking (ARBs) drugs which have previously been shown to modulate NFκB-dependent pathways. Instead, patients prescribed ARBs displayed heightened type I IFN responses unassociated with disease severity and potentially linked to downregulation of the TNF pathway. Thus, we find that the disease-specific epigenetic signature in T_H cells in lupus is associated with TNF family members in addition to type I IFNs and may be modulated by ARBs.

RESULTS:

Lupus T_H cells retain open chromatin features that define T cell subsets.

We sorted 4 distinct T_H subsets to examine how the chromatin accessibility landscape is disrupted in lupus (Figure 1A). $CXCR5^+PD1^+$ circulating Tfh (cTfh) are heterogeneous cells that include recently activated T_H cells, memory Tfh, and effector Tfh (AcTfh) ($CD38^+ICOS^+$) (30). AcTfh are rare in circulation and more closely resemble GC Tfh transcriptionally and epigenetically (30). Changes in the biology of Tfh may contribute to pathology and ANA production in lupus. To ask if any changes observed were specific to Tfh differentiation, we also analyzed $CXCR3^+$ circulating Th1 cells and $CD45RA^+CD27^+$ naive T_H cells (Figure 1A).

ANA^+ lupus patients with low-to-moderate disease activity were recruited from outpatient clinics at the University of Pennsylvania. This cohort was largely female (92.3%), predominantly black (54%) and white (38%) self-reported race, possessed a median SLEDAI of 4 (range 0-12), a median age of 38 years, and were treated with hydroxychloroquine (77%), mycophenolate mofetil (15%), corticosteroids (46%) and other drugs (Table S1). Anonymous healthy control (HC) samples were collected from routine donors to the Human Immunology Core at the University of Pennsylvania. By flow cytometry, PBMCs in a subset of patients had non-significant increases in non-naive T_H cells ($CD45RA^+CD27^-$, $CD45RA^-CD27^-$, $CD45RA^-CD27^+$) (Figure 1B). There is no consensus on the dynamics of cTfh in lupus; in our cohort, cTfh cells were not expanded in lupus (Figure 1C) (29). There was also no significant difference in the percentage of circulating AcTfh between groups (Figure 1D).

T_H were enriched from cryopreserved PBMCs and 5000 cells of each subset were sorted for low-input transcriptional and ATAC analyses (Figure 1A) (31). ATAC-seq data were aligned to the genome and chromatin accessibility peaks were identified using standardized ENCODE pipeline methods (32, 33). Peaks were merged and filtered, and downstream analyses were performed using a consensus peak set containing 70,758 peaks integrated across all four cell subsets.

We first wanted to understand the depth of lupus epigenetic dysregulation. ATAC data were first applied to principal component analysis. Lupus T_H subsets and their healthy counterparts clustered together in PC1, PC2, and PC5 (Figure 1E-F). Representative accessibility tracks demonstrate comparable accessibility patterns in lupus and healthy cells at these loci (Figure S1A). PC1, which contains 40.9% of the variation, divides naive from activated T_H populations (Figure 1E). Chromatin peaks contributing to the clustering of non-naive cells along PC1 were annotated to genes and analyzed using pathway analysis: enrichment results point to processes of immune activation including "T cell activation" (Figure 1G). To ask if lupus T cell subsets maintain established epigenetic identities during disease, we extracted ATAC peaks enriched in naive T_H cells from published works and performed sample-wise peak set enrichment (34). Naive T_H of both lupus and healthy individuals were similarly enriched for the published naive chromatin peak set relative to activated cells (Figure 1H). We similarly examined Tfh identity utilizing published ATAC peaks enriched in GC Tfh populations (30). Lupus and HC AcTfh most closely resemble GC Tfh with cTfh also showing moderate enrichment for GC Tfh peaks (Figure 1I). These data suggest that epigenetic cellular identity is maintained in lupus. Interestingly, naive T_H and AcTfh populations in lupus are slightly more enriched for GC Tfh peaks compared to healthy cells (Figure 1I). Thus, although chromatin peaks defining separate subsets are maintained in lupus, disease-driven changes in accessibility are also apparent.

The lupus epigenome is characterized by enhanced chromatin accessibility in regions surrounding T cell activation and cytokine signaling genes.

Chromatin accessibility patterns separate T_H subsets and reiterate that T_H cells circulating in subjects with lupus are epigenetically similar to those in HCs (Figure 1E). However, additional principal components are lupus specific (Figure 2A). PC3 and PC4, representing 12% of total variation, separate lupus from HCs. All T_H subsets we examined were affected by lupus-driven PCs indicating a shared set of chromatin changes (Figure 2B-C). Using the combined data containing all T_H subsets and

controlling for cell subset-driven variation not altered by disease, we identified 12,625 chromatin peaks that were differentially accessible (DARs) between lupus and healthy T_H cells (FDR <0.025). We restricted subsequent analyses to DARs exceeding a log2 fold change of 0.58 (fold change ~ 1.5). This revealed a bias toward opening of chromatin regions in lupus with increased accessibility in 2683 regions in lupus T cells and only 246 regions of increased accessibility in HC T cells (Figure 2D & Data S1).

Regions of accessible chromatin can prime transcription, recruit chromatin modifying complexes, or act as enhancers of distal genes. We found that approximately 45% of all chromatin regions in the data are located within 3Kb of a gene promoter but only 25% of the 2683 lupus DARs are located in gene promoter regions (Figure S2A) (35). In contrast, there is a relative enrichment of intronic regions making up nearly 50% of DARs (Figure S2A). Interested in how T_H chromatin accessibility might relate to lupus susceptibility SNPs, we identified linked (r2 >0.5) proxy SNPs using published lupus SNP data and determined whether they overlapped with open chromatin in T_H cells (8, 36). Among 6,103 proxy SNPs, 198 overlapped regions of open chromatin in the combined T_H dataset: roughly half of these occur in gene promoter regions (Figure S2B) (Data S2). Higher-powered future analyses are needed to understand potential causal relationships between these susceptibility loci and accessibility and links between altered accessibility and 3D chromatin architecture.

To identify active transcriptional pathways involved in the observed differential accessibility of lupus T_H we utilized gene sets and pathways defined in Gene Ontology Biological Processes (37) and the MSigDB Hallmark gene sets (38). DARs showing increased accessibility in lupus were enriched for programs including “Lymphocyte activation”, “T cell activation” and multiple Hallmark cytokine-signaling pathways including those of IFNγ, TNFα, and IFNα (Figure 2E). IFNγ and IFNα response pathways were enriched in multiple lupus T_H subsets including naive T_H and Tfh populations (Figure 2F-G). Fewer Th1 samples were isolated and analyzed and they were not significantly enriched for these pathways. Genes involved in the TNFα signaling pathway were enriched amongst lupus DARs in all subsets

except Th1-like cells, which have high baseline enrichment in HCs (Figure 2H). We next asked if DARs were localized over known DNA-binding motifs of transcription factors (TF) associated with regulating these pathways. Motifs binding AP-1 family and NFκB family transcription factors appear among the most highly enriched, as might be expected downstream of cytokines such as TNFα (Figure 2I). IRF3 and IRF1 motifs, downstream interferon signaling, were also enriched in this dataset (Data S3). Motif enrichment analysis limited to lupus DARs located in gene promoters identified nearly identical motif enrichments (Figure S2C).

All T_H subsets from lupus subjects cluster together in PC3 and PC4 (Figure 2B) and therefore there is an epigenetic signature specific to lupus and independent of cell subset (Figure 2E-G). However, we also asked if there were disease-linked chromatin changes specific to particular T_H subsets. We performed pairwise comparisons of lupus and healthy naive, cTfh, and AcTfh. For each comparison, DARs (FDR <0.025) separating lupus and healthy cells were identified and we examined whether DARs with increased accessibility in lupus overlapped across evaluated T cell subsets. We observed increased accessibility in 1141, 958, and 1539 DARs in naive, AcTfh, and cTfh lupus cells, respectively. More than 50% of DARs identified in each subset were also identified in at least one other subset indicating substantial overlap (Figure S2D). In contrast, there were 493, 329, and 709 chromatin peaks in naive, AcTfh, and cTfh respectively which were identified as differentially accessible (FDR <0.025) in only one lupus T_H subset (Figure S2D). Lupus DARs specific to AcTfh and cTfh did not show significant (FDR <0.05) pathway enrichments. Naive T_H specific DARs (n = 493) were significantly enriched for Hallmark Inflammatory Response and Hallmark TNFα Signaling gene sets (Data S4). Many subset-specific lupus DARs in the different T cell subsets surround the same gene loci. For example, different peaks in the ICOS locus show increased accessibility in lupus AcTfh, naive, and cTfh cells (Figure S2E). Cell subset specific accessibility, transcriptomes, and transcription factor activity might regulate lupus responses, but these responses activate similar gene networks and pathways across different cell subsets.

Combined transcriptional and chromatin accessibility data highlight cytokine driven dysregulation in lupus.

Lupus T_H cells are inappropriately activated by self-antigen, immune complexes, and cell-cell interactions. Transcriptional studies have highlighted type I IFN responses in lupus T_H and PBMCs (39–41). We demonstrated enhanced chromatin accessibility surrounding genes involved in lymphocyte activation, TNFα signaling, and IFNα and IFNγ responses in lupus T_H cells (Figure 2). We utilized RNA-seq transcriptional profiling of the same samples to ask if epigenetic changes correlated with transcriptional activity. There was separation of distinct T_H subsets in PCA (Figure 3A). Similar to epigenetic data, lupus-specific transcription was present in all subsets (Figure 3B). After integration of the samples, more than 700 genes were differentially expressed between lupus and HCs (padj <0.05, FC>1.5) (Figure 3C). Many genes contained in the Hallmark IFNα response and Hallmark TNFα signaling pathways including *IFI27*, *IFI44*, *RELB*, *KLF6,* and *CD83*, had significantly higher expression in lupus: transcriptional changes in the naive compartment were verified by qPCR (Figure S3A). Gene set enrichment analysis identified enrichment of these pathways and the IFNγ response pathway in most cell populations (Figure 3D). GSVA was used to measure enrichment of these pathways in individual lupus subjects and cell subsets and IFNγ, TNFα, and IFNα pathways were transcriptionally enriched in lupus naive, AcTfh, and cTfh cell subsets (Figure 3E-F, Figure S3B). The enrichment of IFNγ and TNFα pathways was not significant in Th1 cells.

We leveraged these data containing matched chromatin accessibility and transcriptional profiles to define which transcription factors drive the lupus phenotype in each T_H subset. Taiji integrates chromatin accessibility at TF motifs and expression of transcription factors with expression of target genes to generate a page rank score; a quantitative measure of TF activity (42). We used Taiji to identify differentially active transcription factors in lupus and healthy T_H cells and point to potential drivers of dysregulation (Figure 3G). TFs with the highest activity (page rank score > 0.002) were selected and

then among those TFs, TFs with the greatest fold change in activity between lupus and healthy conditions were examined. The results highlight interferon activity: IRF8 and IRF1 appear differentially active in naive and Th1 cells (Figure 3G). However, a much larger set of transcription factors contained in the TNFα signaling gene set appears among the active transcription factors in lupus cells; these include FOSL1, KLF6, RELB, BHLHE40, EGR3, and SMAD3 (Figure 3G). Visualized differently, the networks of regulated gene targets of RELB, KLF6, and IRF1 in naive T_H are much larger than those of healthy T_H (Figure 3H, Figure S3C-D). Thus, there is a robust difference in regulatory activity of these TFs in disease and these data corroborate our findings to implicate aberrant cytokine stimulation across multiple T_H populations. These data also suggest that IFNα, a canonical lupus-associated cytokine, does not act alone to alter cell profiles.

Single cell multi-ome analysis of naive T_H cells demonstrates ubiquitous dysregulation in lupus.

We have shown that lupus naive T_H cells retain the characteristics of naive T_H cells and cluster with healthy naive cells (Figure 1E). However, these cells also have lupus-specific epigenetic and transcriptional signatures linked to activation and cytokine signaling. Expansion of a novel $CD45RA^+CD27^+$ antigen-experienced population among lupus naïve T cells or shifts in the frequencies of naive T_H subpopulations could explain this result and potentially indicate that quiescent true naïve T cells are unaltered in disease. Alternatively, genetic susceptibility to lupus or direct influence of circulating signals, including cytokines, may lead to chromatin opening preceding T cell activation and affecting naïve resting populations.

To examine these alternative hypotheses, we generated single cell multi-ome chromatin accessibility and nuclear RNA data from sorted $CD45RA^+CD27^+CD4^+$ T cells in three healthy and six lupus subjects. We identified 237 DARs; 233 were more accessible in lupus mirroring the enhanced accessibility found in bulk ATAC-seq results. The 233 lupus DARs demonstrated significant enrichment of expected pathways, including “cytokine production” and “tumor necrosis factor

production" (Figure 4A). We next directly compared the bulk and single-cell data sets by asking whether the DARs identified in bulk ATAC analyses were enriched in single cell chromatin profiles of lupus subjects. The peak set containing bulk ATAC-defined lupus DARs (Figure 2C) was highly enriched relative to HCs in all but one lupus sample (Figure 4B). Nuclear RNA data also demonstrated increased expression of many transcripts identified in bulk RNA-seq assays including *IFI44*, *KLF6*, *REL*, and *NFKB1*: genes contained in IFNα and TNFα-related gene sets (Data S5).

Single cell nuclear RNA and ATAC data of sorted naive T_H cells were independently integrated and merged and cells were clustered (43, 44). Six primary clusters containing unique chromatin accessibility peaks or gene expression patterns were identified (Figure 4C); one additional cluster (Cluster 7) was present at very low frequency in two subjects (one healthy and one lupus). These clusters fit into established naïve subpopulations in the literature. Cluster 2 had unique upregulation of *IL2RA* expression (Figure S4A). Previous studies have described IL2RA^{high} naive subpopulations as effector precursors or Treg-like naive cells (45, 46). Supporting this, chromatin surrounding *FOXP3* was more accessible in Cluster 2 and Cluster 5 and DARs defining Cluster 2 were significantly enriched for the Hallmark IL2-STAT5 signaling pathway which may relate to high expression of *IL2RA* (not shown). Cells in Cluster 5 have open chromatin around the *IFNG* locus and increased expression of *CCL5* and *GZMA* and are, therefore, similar to previously described stem cell memory-like CD45RA$^+$CD27$^+$CD4$^+$ T cell populations (Figure S4B-D) (46, 47). The remaining four clusters have been labelled "true naive" populations in published analyses and are more tightly clustered along UMAP projections (46). Among the four "true naive" clusters, cluster 3 exhibits the most significant differences including increased *SOX4* expression (Figure S4E-F). Previous work has suggested that *SOX4* expressing naive T_H subpopulations are enriched in young individuals and associated with CXCL13 expression upon activation (46, 48). Thus, single cell data recapitulate the lupus chromatin phenotype and prior studies of the naïve T_H compartment.

We asked whether specific clusters contributed more to the lupus phenotype. DARs defining Cluster 5 were enriched for multiple TNF-related gene sets and JAK-STAT signaling (Figure 4D). Potential overabundance of this cluster might explain the lupus naïve T_H phenotype. However, there was no consistent or significant lupus-associated differences in naive cluster frequencies (Figure 4E); thus, altered dynamics within subpopulations did not explain the lupus epigenetic phenotype in naïve T_H cells. To more accurately place where the chromatin changes identified in bulk ATAC sequencing occurred across the naïve clusters, we used cluster-wise pseudo-bulk analyses to generate a lupus chromatin enrichment score for each sample. Lupus cells of every cluster were enriched for the lupus chromatin signature relative to healthy cells in the same cluster (Figure 4F). Similarly, multiple lupus DEGs have increased expression in lupus naive T_H cells across all clusters (Figure S4G). These data suggest lupus is not characterized by shifting frequencies of naive subpopulations nor is the lupus signature restricted to one aberrant naive T_H cluster. Rather, differential chromatin accessibility in lupus results from widespread changes to accessibility within all subpopulations of $CD45RA^+CD27^+CD4^+$ T cells, including “true naive” T_H cells.

Single cell multi-ome analysis permits the direct association of chromatin accessibility with transcription (49). We performed peak-gene linkage analysis, identifying correlations between chromatin accessibility and gene expression for individual genes at the single cell level. We examined peak-gene linkages for the 663 DEGs identified in nuclear RNA data and identified 637 peak-gene associations unique to lupus cells while 255 peak-gene associations were unique to healthy cells (Data S6). Unique peak-gene linkages of lupus cells demonstrate an association of altered chromatin with differential gene expression at the single cell level (Data S6). An example of this is the *IFI44* locus which has many peak-gene linkages in lupus cells (Figure 4G) and not in healthy cells (Figure S4H).

Collectively, we find that disease-specific cellular heterogeneity does not drive the lupus signatures of naive T_H cells. Existing heterogeneity among flow-cytometry defined $CD45RA^+CD27^+$ T_H cells is maintained in lupus. Instead, lupus chromatin and transcriptional changes are widespread

across naive subpopulations and cell states, consistent with a ubiquitous feature including environmental stimuli like cytokines or genetic polymorphisms affecting the naive T_H pool broadly. Further, these data suggest a direct association between lupus-associated changes in accessibility and gene expression in naive T_H cells.

Lupus T_H cells appear in two epigenetically distinct groups defined by transcriptional activation of TNFα signaling pathway genes.

Single cell data reinforced the observation that naive T_H in lupus patients are epigenetically and transcriptionally altered. A small number of subjects with lupus did not have the disease-specific epigenetic signature but segregated with healthy subjects (Figure 2F-H & Figure 4B). To probe this variability, we added naïve T_H from additional subjects. The expanded cohort had the expected increased accessibility of cytokine-driven pathways (Figure S5A). In this larger cross-sectional analysis, four lupus samples clustered with healthy samples in PCA (Figure 5A). This effect became more apparent when using GSVA to assign a single value to the cumulative enrichment of lupus DARs in each sample (Figure 5B). Lupus patients separate into two groups: Group 1 patients have modified chromatin accessibility of naive T_H cells with the lupus signature while the epigenetic landscape of Group 2 lupus patients overlaps that of HCs (Figure 5B). Applying the same enrichment tests to cTfh, AcTfh, and Th1, we found that Group 2 lupus patients with "healthy" naive T_H chromatin also lacked lupus-specific chromatin dysregulation in activated populations (Figure S5B-D). The separation of lupus patients into distinct groups explains variability in lupus patients seen at the single cell level (Figure 4B). Thus, the dysregulation of chromatin in T_H cells affects different cell subsets similarly, but is found only within a subgroup of patients.

Clinical disease features and molecular profiles of lupus patients have previously been used to identify subgroups within lupus patients (40, 41). We asked if the two distinct lupus epigenetic subgroups correlated with molecular features. We compared the naive T_H transcriptional profiles of the

two lupus groups and identified 518 DEGs between the two groups including 448 genes which have elevated expression in patients with altered chromatin accessibility (Group 1) (Figure 5C) and 70 genes with higher expression in Group 2 lupus individuals (Data S7). Our previous results demonstrated that lupus T_H cells are enriched for cytokine signaling pathways including TNFα signaling, IFNα response, and IFNγ response gene sets and we focused on these genes. We identified robust transcriptional enrichment of the TNFα signaling pathway in Group 1 lupus patients and not in either Group 2 lupus patients or HCs (Figure 5D). Type I IFN responses, however, showed overlap between samples in the two different lupus groups and variability among individuals (Figure 5E) and the IFNγ response did not show bias between the lupus groups (Figure S5E). We next asked if peak-gene linkages in the single cell multi-ome dataset reflected the utilization of TNF-related genes. Among the 637 peak-gene linkages unique to lupus, 78 ATAC peaks were linked to 29 different genes in the TNFα signaling pathway, including *TNFAIP3* (Figure S5F-G; Data S6). These data corroborate TNF family cytokines as regulators of the lupus chromatin landscape and the existence of two epigenetically distinct lupus T cell phenotypes.

Increased expression of many TNFα signaling genes, including *RELB,* accompanied the TNF-related enrichment in Group 1 lupus subjects. We examined gene expression of TNF family receptors in lupus groups and controls. *TNFRSF1A* (TNFR1) expression is significantly higher in Group 2 lupus subjects compared to Group 1 and *TNFRSF1B* (TNFR2) follows a similar insignificant trend (Figure 5F-G). Dysregulation of these receptors might indicate differential receptor utilization or feedback signaling. Other TNF family receptors including TNFRSF12A (TWEAKR) and TNFRSF14 (HVEM) showed heterogeneous expression patterns but were not significantly altered between lupus groups (Figure S5H-I). The expression of the TNF family ligands for these receptors have previously been reported to change during lupus, including increased TNFα concentrations in some patients (50, 51). TWEAK, the ligand of TNFRSF12A, has previously been reported to be involved in lupus pathology (52) and LIGHT (*TNFSF14*), a ligand of HVEM (*TNFRSF14*), is commonly dysregulated in murine lupus (53, 54).

We used flow cytometry of a distinct cohort of lupus patients to ask if TNF family receptors and HCs are dysregulated in lupus T_H. TNFR1, TNFR2, and TWEAKR and high expression of HVEM were detected (Figure S6A) in naive T_H cells. Naive T_H from lupus and healthy subjects had similar MFI and percent positive for both TNFR1 and TNFR2 (Figure 5H, S6B-C). Non-naive T_H cells didn't show significant change in TNFR1 expression in lupus patients (Figure S6D-E). In contrast, the MFI of TNFR2 in non-naive T_H cells and cTfh was significantly increased in lupus patients (Figure 5I and Figure S6F-G). TWEAKR expression was not different in lupus T cells (Figure S6H-I). The frequency of $HVEM^+$ cells was reduced in both naive and non-naïve lupus T_H (Figure 5J-K). HVEM expression was also reduced in $CD8^+$ T cells and $CD14^+$ monocytes from subjects with lupus (Figure S6J-K). BTLA, the inhibitory binding partner of HVEM, was maintained at normal levels in lupus T cells (not shown). In subjects with concordant samples, we verified that dysregulated expression of HVEM correlates with the presence of the Group 1 lupus epigenetic signature (Figure S6L-M). In these Group 1 lupus individuals, there is reduced CD28 expression on T_H cells, an effect that has been linked to TNFα signaling (Figure S6N-P) (55, 56). Thus, dysregulated expression of both HVEM and TNFR2 and the association of the lupus epigenetic signature with transcriptional activation of TNFα signaling suggests a complex environment in which multiple cytokines, potentially TNFα or LIGHT, impact the molecular biology of T_H cells.

Prescriptions for angiotensin receptor blockers (ARB) correlate with naive T_H cell epigenetic and transcriptional states in lupus.

We found that randomly selected lupus patients separate into two groups with distinct T_H chromatin accessibility. However, questions remained regarding the longevity of chromatin states among lupus patients and upstream mechanisms linked to changes in signaling and subsequent chromatin accessibility. To test whether the absence of disease-associated chromatin in Group 2 was stable, we recalled one subject at a second time point. The two samples from this Group 2 lupus patient

clustered together indicating large scale maintenance of chromatin architecture over a two-year period (Figure 6A & Figure S7A-B).

We then looked further into the clinical backgrounds of the lupus patients to identify potential upstream corollaries of the epigenetic footprint. Neither age, race, gender, DMARD usage at the time of accrual, the presence or absence of nephritis, nor the SLEDAI, distinguished Group 1 and Group 2 (Table S2). Additionally, subjects with a diagnosis of Mixed Connective Tissue Disease or Sjogren's were present in both groups (Data S8). One Group 2 subject had a history of cancer treatment and chemotherapy might have altered the epigenetic profile (Data S8). Instead, we found differences in therapies: three Group 2 lupus subjects were prescribed Losartan, an ARB, and two were treated with Rituximab. ARBs are commonly used in lupus patients to treat hypertension and reduce proteinuria to protect renal function (57). ARBs have been reported to reduce TNFα concentrations in those taking them (58) and influence NFκB-dependent signaling (59). Given this, we hypothesized that ARBs were a potential upstream signal associated with T cell chromatin states in our data.

We expanded the cohort again, including subjects in our repository that were receiving ARBs and/or Rituximab at the time of sample collection, patients prescribed neither, and additional HCs. Hierarchical clustering of ATAC data again identified several lupus patients whose epigenetic profiles intermixed with healthy individuals (Figure 6A). Using previously defined lupus DARs, we performed GSVA enrichment analysis as before. Two additional subjects fell within Group 2 lacking the lupus-associated epigenetic signature (Figure 6B & Figure S7A). This second cohort of non-randomly selected patients and controls showed similar variation of enrichment and HCs lacked lupus enrichment as expected (Figure S7B). There was no relationship between age, gender, race, or the SLEDAI and presence of the lupus epigenetic signature (Figure S7C & Data S8). Instead, we confirmed that having a prescription for an ARB correlated with decreased enrichment for lupus associated chromatin changes (Figure 6C) and accounted for almost all the subjects in Group 2. This does not seem to be due to alterations in the renin-angiotensin pathway as prescription of angiotensin converting enzyme

(ACE) inhibitors had no effect on patient groupings (Figure S7D). Several patients prescribed Rituximab lacked the lupus epigenetic signature (Figure S7E). However, these patients were uniformly prescribed ARBs, suggesting that ARB use is the dominant factor influencing epigenetic state (Figure S7E). ARB use does not correlate with the SLEDAI in this cross-sectional study (Figure S7F).

In this extended cohort, the transcriptomes of Group 1 and Group 2 subjects remain distinguished by differences in TNFα signaling transcriptional enrichment (Figure 6D) and no significant differences in either the IFNα or the IFNγ response gene sets (Figure 6E & Figure S7G). In parallel with the epigenetic landscape, lupus patients prescribed ARBs lacked TNFα signaling enrichment. Analyses of transcriptional networks in autoimmunity have suggested that TNFα and type I IFNs oppose each other molecularly. It is thus interesting that patients prescribed ARBs lack TNFα signaling enrichment and have greater IFNα response enrichment and the opposite is true of patients not prescribed ARBs (Figure 6F-G).

TNF family cytokines are altered in lupus patients on ARBs and correlate with epigenetic changes in naive T_H cells.

Our data clearly identified an association between TNFα signaling and an altered T cell epigenetic landscape (Figure 5D & Figure 6D). We further demonstrated an association between the use of ARBs and the lupus T cell epigenetic state (Figure 6C). It is possible that direct inhibition of angiotensin II receptor type 1 (AT1) on T cells by ARB therapy alters molecular state, but low to undetectable levels of *AGTR1* expression in naive T cells suggests that this is not the case (Figure S8A). Instead, changes in cytokine abundance in patients on ARBs might mediate epigenetic and transcriptional effects indirectly. To test this, we profiled select plasma cytokines (Figure 7). There is significant variability amongst lupus patients; however, lupus patients segregate in hierarchical clustering of cytokines with a trend toward increasing proinflammatory profiles (Figure 7A). Several

lupus patients have increased levels of IFNα2, IFNγ, and IL-1β, as have been previously described (Figure 7A) (41, 60).

Because our data suggested that ARB use correlated with changes in transcriptional TNFα signaling and to epigenetic state, we focused on the effects of ARBs on cytokine profiles. Subjects prescribed ARBs had increased levels of IL-15, IL-2, IL-17A, IL-12p40, IL-10, and sIL-1R1 (Figure 7B-E & Figure S8B-C). Lupus nephritis with proteinuria is a frequent indication for ARB prescription and has long been associated with overproduction of IL-17 and abundant Th17 cells (61). In contrast, ARB treatment of mice promotes expansion of Tregs which produce IL-10 (62, 63). IFNα2 is similarly increased among several patients on ARBs (Figure 7F) potentially reflecting transcriptional data showing enrichment for IFNα responses in patients prescribed ARBs (Figure 6G).

TNFα concentrations did not differ in lupus patients separated by ARB prescription (Figure 7G). In contrast, other TNF family members, including TRAIL and TWEAK were altered in association with ARB prescription (Figure 7H-I). TRAIL was elevated in patients prescribed ARBs while TWEAK was the only cytokine we measured that was decreased in lupus subjects prescribed ARBs. TWEAK is also significantly higher in Group 1 lupus patients compared to HCs while Group 2 lupus patients do not have altered TWEAK levels (Figure S8D). TWEAK is likewise strongly correlated to the transcriptional activation of TNFα signaling in naive T_H cells of lupus patients (Figure 7K). Levels of LIGHT also significantly correlated with T cell TNFα signaling enrichment across lupus patients (Figure 7J). These strong correlations between epigenetic changes and TNF family member cytokine abundance implicate them in the development of the lupus epigenetic landscape and its regulation by ARBs.

DISCUSSION

Here, we provide a detailed analysis of chromatin accessibility in naive and Tfh lineage T_H cells in lupus and HCs. Naive and effector T_H cells in most lupus patients have enhanced chromatin accessibility with parallel transcriptional changes surrounding cytokine signaling pathways genes, including the TNF signaling family. Single cell multi-ome analysis showed that changes to accessibility in lupus naive T_H cells are ubiquitous in all subpopulations and not driven by heterogeneity of the compartment. The chromatin landscape of a subset of lupus patients prescribed ARBs resembles that of HCs. The use of ARBs is also linked to altered cytokine profiles, including reduced TWEAK and TWEAK levels directly correlate with T cell TNFα signaling enrichment. These findings have important implications for how we consider disease-associated cytokine production, treat and study different lupus patient subgroups, evaluate naive T_H responses, and use ARBs in lupus treatment.

Dysregulation of T_H functions and altered frequencies of cell subsets in lupus might be the result of disease-driven T_H cells with abnormal phenotypes and lost cellular identity. For example, others have linked $CXCR5^+CD4^+$ circulating T cell populations to autoantibody production and disease activity (12, 13, 64). However, our data suggest that the chromatin accessibility specific to T_H subsets is maintained in lupus and that cellular identity is not widely affected. Nonetheless, lupus T_H demonstrated robust changes to their chromatin accessibility profiles compared to healthy cells which might have an influence on their function. In these subjects, cTfh and AcTfh maintain their subset-driven chromatin accessibility but lupus AcTfh more closely resemble GC Tfh than their healthy counterparts. Inappropriate activation of Tfh and altered function in lupus may be linked to cytokine-associated signaling and changes to the epigenome defined here.

Questions of marker fidelity and cell identity are particularly challenging in naive T_H cells. Because many studies have previously used only CD45RA positivity when evaluating lupus naive T_H populations (17, 18, 20), it is possible that memory T_H cells with reactivated CD45RA expression

might contaminate isolated cells. Our restrictive gating using CD45RA$^+$CD27$^+$CD4$^+$ naive cells in bulk accessibility assays resulted in chromatin accessibility profiles which maintained published naive chromatin accessibility signatures, but were nonetheless affected by disease-associated changes. Single cell multi-ome ATAC and nuclear RNA sequencing of CD45RA$^+$CD27$^+$CD4$^+$ T cells supported this finding, and we identified several previously defined subpopulations within naive T_H cells. In lupus, the distribution and cell architecture of these subpopulations were maintained and, instead, we demonstrated that the lupus epigenetic signature penetrated all naive subpopulations including true naive T_H cells. It would be of great interest to examine whether these changes to naive and effector T_H biology influence the many observed functional deficiencies and alterations of lupus T_H cells during activation and differentiation.

Lupus is heterogeneous with significant variability in transcriptional phenotypes, inflammatory signatures, cytokine expression, and disease activity. These differences have been used to group lupus patients into meaningful categories which inform how we think of the disease (40, 41, 65–67). Our characterization utilized the epigenetic landscape. We identified two groups of lupus patients, independent of disease activity and demographics, distinguished by the presence (Group 1) or absence (Group 2) of lupus-specific T_H chromatin accessibility. This separation allowed for extended clinical analysis and better understanding of the potential mechanisms influencing chromatin changes.

Transcriptionally, TNFα signaling was highly enriched in lupus T_H populations along with other cytokine pathways compared to healthy cells. However, when we directly compared the epigenetically distinct groups of lupus patients, only Group 1 lupus patients with disease-altered chromatin were enriched for TNFα signaling. Clinical review linked reduced TNFα signaling enrichment and absence of the lupus chromatin state in Group 2 patients to ARB prescription. ARBs are used to treat hypertension and have a beneficial effect on reducing proteinuria and improving renal and cardiovascular outcomes in lupus (68), but they are not generally used as disease modifying agents.

Angiotensin receptors are highly expressed on endothelial cells and ARB effects on T cells may be indirect. However, multiple immunologic effects of angiotensin inhibition have been documented including changes in cytokine and chemokine production and T_H differentiation. Hypertensive effects of angiotensin depend on TNFα and angiotensin inhibition reduced NFκB signaling and TNFα production (58, 59, 69, 70). In keeping with these observations, lupus patients prescribed ARBs were less likely to have the lupus-driven chromatin accessibility signature and had reduced transcriptional enrichment for the TNFα signaling pathway. Patients prescribed ARBs also had significantly higher plasma IL-10, IL-17, and other cytokines. These changes in cytokine profiles may relate to disease characteristics prior to ARB prescription or may be a direct effect of ARB. Longitudinal study of lupus patients initiating ARB therapy would distinguish between these possibilities and indicate whether ARB usage influences cytokines in lupus to alter chromatin and transcriptional states and clinical outcomes.

The heterogeneity in TNFα signaling, potentially linked to ARB usage, in epigenetically distinct patients is noteworthy given the complex relationship between TNFα and lupus. TNFα levels are increased in lupus patient plasma (71) and elevated levels of sTNFR1 and sTNFR2 predate lupus nephritis flares (72). TNFα blockade in autoimmune diseases like rheumatoid arthritis is associated with the unmasking of a type I IFN signature and the development of drug-induced lupus (73, 74). Studies of both plasmacytoid DCs and macrophages suggest that the two cytokines oppose each other (75, 76). This complex interplay between TNFα and type I IFNs may explain why absence of the epigenetic signature in Group 2 subjects is not associated with clear differences in disease activity. Heightened type I IFN activity may predate ARB prescription or could be exacerbated by TNF inhibition in patients prescribed ARBs. It is, however, difficult to interpret these results in a cross-sectional study as we do not know the subjects' SLEDAI or IFN levels prior to ARB prescription. It will be important to design assays of the epigenetic state in individual lupus patients before and after ARB

prescriptions and to consider a trial of ARBs in lupus patients without nephritis, the most common indication for the drugs.

The identified TNF transcriptional enrichment in lupus stems from cytokine signaling through classical NFκB-dependent pathways but may not be directly mediated by TNFα itself. Multiple TNF family cytokines signal through TRAFs to activate canonical and/or non-canonical NFκB signaling cascades (77). LIGHT leads to the activation of NFκB and AKT signaling associated with T cell proliferation, survival, and effectors responses and has been linked to inflammation (78, 79). TWEAK is capable of activating both canonical and non-canonical NFκB signaling and has previously been linked to the development of lupus nephritis (52, 80, 81). Other TNF cytokines and receptors not specifically evaluated cannot be discounted. At a single timepoint, TNFα was not differentially abundant in the plasma of the two groups. Instead, patients prescribed ARBs had reduced plasma TWEAK which significantly correlated with naive T_H cell TNFα signaling enrichment and epigenetic state. T cell signaling through the TWEAK receptor (Fn14; TWEAKR) is not well understood and mouse T cells may not express TWEAKR (82). We found that only a small percentage of human T cells express TWEAKR, perhaps arguing against a direct signaling mechanism. LIGHT plasma levels were also increased in our lupus cohort and lupus patients have reduced HVEM expression on T cell surfaces compared to healthy individuals. Similar to TWEAK, LIGHT levels are correlated to the TNFα transcriptional signature and thus implicated in TNFα signaling responses in lupus subgroups.

Our data resolve the chromatin accessibility landscape of T_H cells in lupus and demonstrate that they acquire disease-associated changes to cytokine-related loci. The ubiquitous nature of chromatin accessibility changes in affected individuals, including in naive T_H cells, might suggest a common signaling mechanism such as a circulating cytokine. Based on our data, we can postulate that TNF family cytokines act on T_H cells in some lupus patients to alter transcriptional and epigenetic features. Introduction of ARBs may indirectly inhibit this TNF phenotype through modulation of TNF family cytokine levels. TNFα, TWEAK, and LIGHT should all be studied as putative effectors in this

process. The stratification of lupus patients into two distinct groups based on the presence of disease-associated chromatin accessibility changes and the use of ARBs provides a unique avenue for study and potential for ARBs beyond their current clinical indication. Finally, the association of TNF signaling with chromatin changes in T_H cells in lupus supports further study of TNF family cytokines and how TNF-related chromatin accessibility changes might influence the function of lupus T cells and contribute to, or limit, disease.

Methods:

Human sample processing

Lupus patients were recruited from outpatient visits at the University of Pennsylvania. Venous blood was collected in K2EDTA tubes. PBMCs and plasma samples were isolated using Ficoll-Pague or Lymphoprep reagents and density gradient centrifugation. HCs were similarly processed. Additional control PBMCs were obtained from the Human Immunology Core at the University of Pennsylvania where PBMCs were isolated from whole blood or leukapheresis products by similar methods. All PBMCs were cryopreserved in media containing 10% DMSO.

Sex as a biological variable

Because lupus is a disease which disproportionately affects females, most subjects included in this study are female.

Flow cytometry and cell sorting

For ATAC and RNA-seq studies, cryopreserved PBMCs were enriched for T_H by negative selection (Stem Cell Technologies #100-0696). T_H were stained (Table S3) and sorted using a BD FACS Aria II to isolate naive T_H cells ($CD4^+CD45RA^+CD27^+$), circulating Tfh ($CXCR5^+PD1^+CD38^-ICOS^-$), activated circulating Tfh ($CXCR5^+PD1^+CD38^+ICOS^+$), and Th1 cells ($CXCR5^-PD1^-CXCR3^+$).

Cryopreserved PBMCs from lupus patients and HCs were thawed, washed, and stained (Table S4) for separate flow cytometry phenotyping.

RNA-seq

Cells were processed for RNA-seq following manufacturer protocols (Takara SMART-Seq® v4 Ultra® Low Input RNA Kit). Libraries were sequenced with the Center for Spatial and Functional Genomics at

the Children's Hospital of Philadelphia: NovaSeq 6000 paired-end sequencing. FASTQ files were pseudo-aligned to hg38 with Kallisto (83) and count data were imported into R for filtering, quality assessment and statistics. Differentially expressed genes (FDR <0.05) were determined utilizing DESeq2 (84). Sample-wise GSVA enrichment of selected gene sets was calculated using GSVA (85).

Quantitative PCR

Cryopreserved naïve T_H were thawed and RNA was isolated (Qiagen RNEasy Mini kit). cDNA was prepared (Thermo Fisher #4368814). TaqMan® PCR probes for KLF6 (Thermo Fisher Assay Hs00154550_m1), IFI44 (Thermo Fisher Assay Hs00197427_m1), IFI27 (Thermo Fisher Assay Hs01086373_g1), RELB (Thermo Fisher Assay Hs00232399_m1), CD83 (Thermo Fisher Assay Hs00188486_m1), and SDHA (Thermo Fisher Assay Hs00417200_m1) were used for quantification. Reported 2^-ΔΔCt values were calculated using SDHA as a control gene.

ATAC-seq

Cells were processed for ATAC-seq according to standard protocols (31). Libraries were sequenced as with the RNA-seq: NovaSeq 6000 system paired-end sequencing. ATAC data were analyzed and processed using the encode ATAC-seq pipeline (https://github.com/ENCODE-DCC/atac-seq-pipeline). FASTQ files were aligned to hg38 using Bowtie2. Duplicates and blacklist regions were filtered and open chromatin peaks were called with MACS2 (FDR 0.01). A combined peak list was made containing all T_H samples using bedtools. Normalization and DAR analysis were performed using DESeq2. Genomic and gene annotations were performed using ChIPSeeker and enrichment analyses were done with ChipEnrich and contained gene sets belonging to the GO: Biological Processes and Hallmark gene set collections (35, 37, 86, 87). Sample-wise enrichment of chromatin data for selected gene sets or selected chromatin region sets was performed utilizing GSVA (85). Transcription factor motif

analyses were performed using HOMER (88). Taiji transcription factor analysis and network analysis was performed as described (42).

Published chromatin accessibility data: Chromatin accessibility peaks enriched in GC Tfh relative to other T cells were obtained from publicly available data (GSE130794)(30). To identify T_H chromatin accessibility peaks which define naive T_H cells, published data of human T_H cells were analyzed (GSE179613 and GSE179593) using PCA (34).

Plasma Analysis

Magnetic bead-based Milliplex assays were performed with the help of the Human Immunology Core at the University of Pennsylvania. Plasma analytes concentrations were detected on a FLEXMAP 3D instrument running Luminex® xPONENT® 4.2; Bio-Plex Manager™ Software 6.1. Analytes measured included immune targeted assays (HCYTMAG-60K-PX38 and HSCRMAG-32K-PX14 kits) and researcher-selected analytes LIGHT (HCVD1MAG-67K-01), TWEAK (HCMBMAG-22k-01), and TRAIL (HCYP2MAG-62K-01).

SLE GWAS Analysis

Published GWAS-defined lupus SNPs were linked to proxy-SNPs with a linkage disequilibrium r^2 cutoff of 0.5 as described (8, 36).

10X Single Cell Sequencing

Cryopreserved PBMCs were thawed prior to negative selection (Stem Cell Technologies #100-0696). At least 100,000 naive T_H cells ($CD45RA^+CD27^+$) were sorted from 3 healthy subjects and 6 lupus patients. All procedures were done according to 10X Genomics protocols (10X Genomics #1000285, 10X Genomics #2000264, 10X Genomics #2000261). Libraries were quantified and assessed for

quality by TapeStation (Agilent 2200 TapeStation system) before sequencing; 10X libraries were sequenced on a NextSeq2000 platform. Chromium procedures and sequencing were performed in collaboration with the Center for Host Microbial Interactions at the University of Pennsylvania School of Veterinary Medicine.

Preprocessing of 10X Multi-ome ATAC and GEX data were performed using Cell Ranger (10X Genomics) and human assembly hg38. Data were loaded into R and filtered for enhanced quality control (43). RNA count data and ATAC count data were independently normalized and integrated then merged. Normalization and subsequent dimensionality reduction by Weighted Nearest Neighbor analysis was done using Signac (44). Annotation and functional analyses of identified chromatin regions, including sample-wise enrichment analyses, were performed as previously described for bulk sequenced populations.

Statistics

DESeq2 was used to identify DEGs and DARs from bulk isolate sequencing data. FDR less than 0.05 were used throughout the study and ATAC data were restricted to FDR <0.025 & absolute Log2 fold change greater than 0.58 for further analyses (Figure 2). Enrichment scores of selected gene sets and peak sets were calculated as above using the normalized difference in empirical cumulative distribution functions (CDFs) of gene/region ranks inside and outside the gene set or chromatin region set (85). Where applicable, an unpaired, two-sided T test, with Welch's correction, was applied for direct hypothesis testing. Multiple one-way ANOVAs with multiple comparisons correction (Tukey) were performed as needed. Data are graphed with mean ± standard deviation overlayed. For correlative analyses, a linear correlation along a scatterplot was drawn and Spearman correlation coefficients are reported (Figure 7).

Study Approval

All human subject research was performed after informed consent in accordance with the institutional review boards at the University of Pennsylvania.

Data and Materials availability

RNA-seq, ATAC-seq, and 10X multi-ome sequences will be available for download at the Sequence Read Archive (BioProject PRJNA1169288). Raw data for graphs is found in the Isolated Values Sheet.

Author Contributions

TML conceived the original idea, supervised research, acquired funding, and contributed edits to the manuscript. APH and TML designed the study with guidance from AW and DB. AK, SS, JD, and TML acquired patient samples. SS and JD performed clinical evaluation and SLEDAI of recruited subjects. JK performed secondary clinical review and blinded analyses. JB consulted on statistical applications. APH performed the research and data analysis and created visualizations. APH drafted the first version of the manuscript.

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Figure Legends

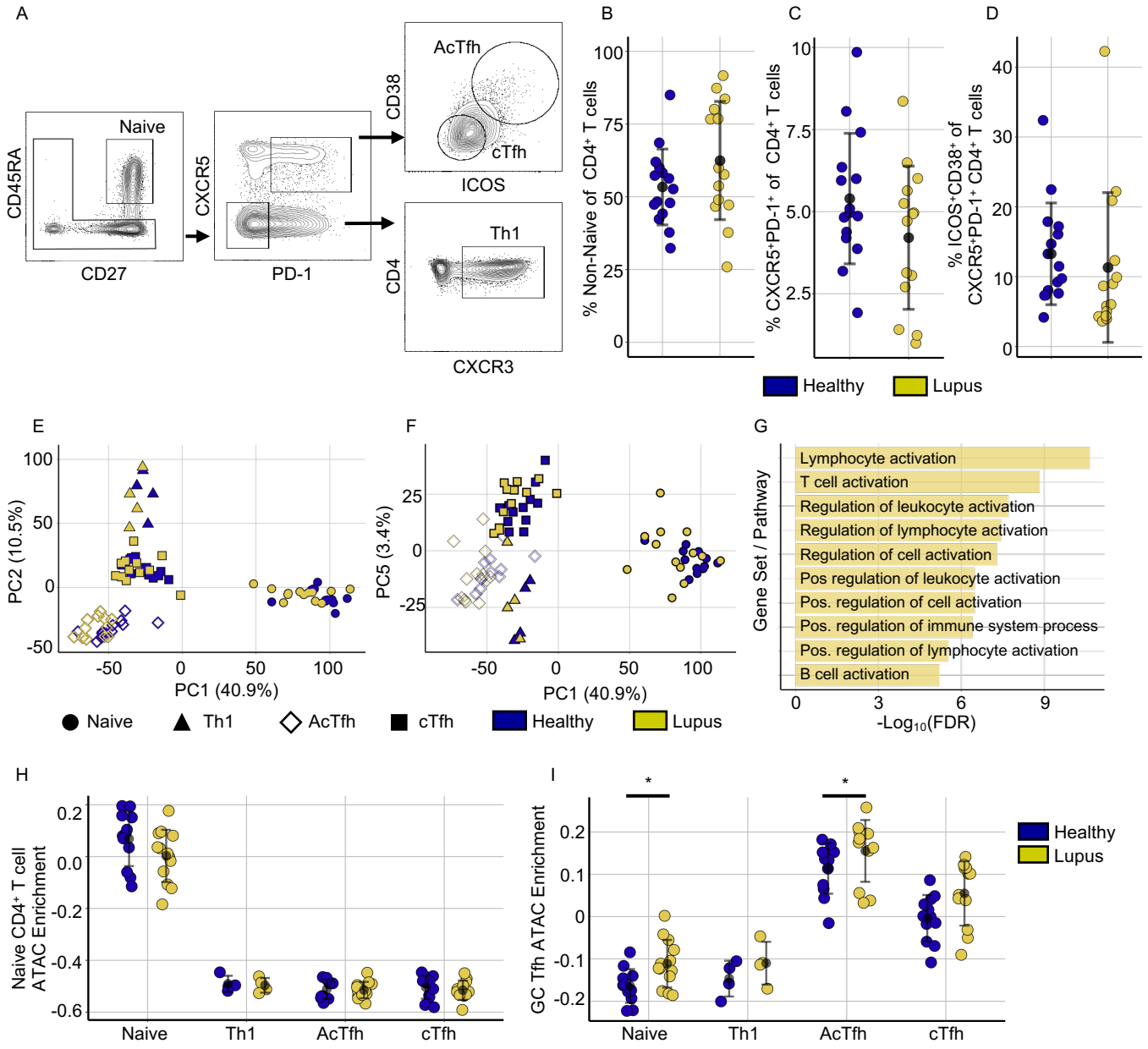

Figure 1: Lupus T_H cells retain open chromatin features that define T cell subsets. **A**) Representative gating strategy to purify CD45RA$^+$CD27$^+$ naive T_H, CXCR5$^+$PD1$^+$ICOS$^+$CD38$^+$ AcTfh, CXCR5$^+$PD1$^+$ICOS$^-$CD38$^-$ cTfh, and CXCR5$^-$PD1$^-$CXCR3$^+$ Th1 cells. **B**) Frequency of non-naive T_H cells among CD4$^+$ T cells in lupus (n=14) and healthy individuals (n=15) by flow cytometry. **C**) Frequency of CXCR5$^+$PD1$^+$ T cells among CD4$^+$ T cells in lupus (n=14) and healthy individuals (n=15). **D**) Frequency of AcTfh cells among CD4$^+$ T cells in lupus (n=14) and healthy individuals (n=15). **E**) Principal component analysis (PC1 x PC2) of ATAC data for sorted T_H populations. **F**) PCA (PC1 x PC5) of ATAC data for sorted T_H populations. Colors distinguish lupus or healthy samples and shapes distinguish T_H subset. **G**) Ten most significant ChipEnrich pathway

enrichment results for peaks defining non-naive $CD4^+$ T cells in PC1. **H**) Sample-wise peak-set variation analysis of ATAC data across lupus and healthy T_H populations against published chromatin peaks enriched in naive T_H cells. **I**) Sample-wise peak-set variation analysis of published chromatin peaks enriched in GC Tfh cells across lupus and healthy T_H populations. Error is reported as SD. ATAC data represent 25 naive T_H cell samples (13 Lupus, 12 Healthy), 8 Th1 samples (4 Lupus, 4 Healthy), 24 cTfh samples (12 Lupus, 12 Healthy), and 24 AcTfh samples (12 Lupus, 12 Healthy). *P < 0.05; **P < 0.01, ***P <0.001 unpaired 2-tailed t tests (B-D, H-I).

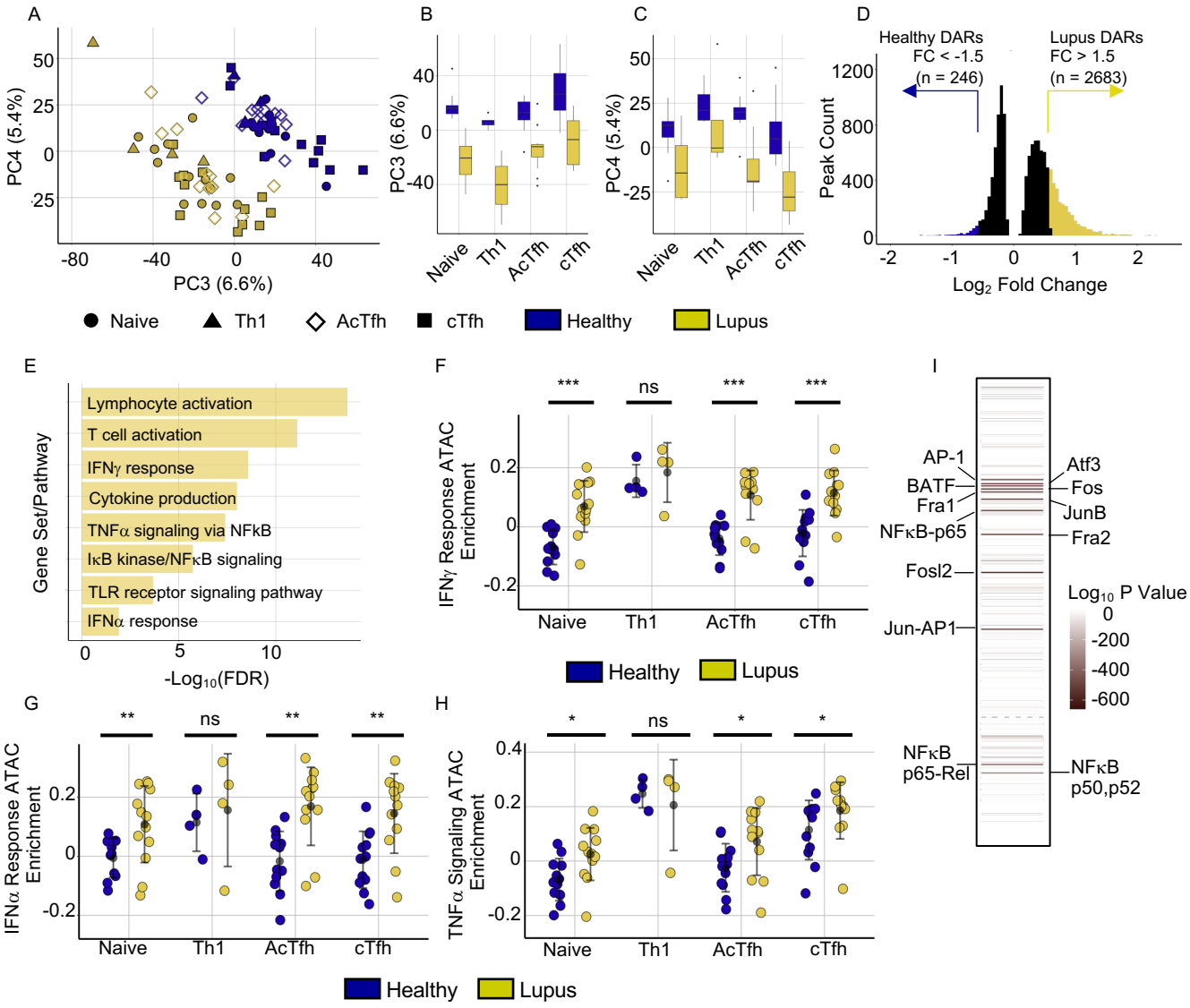

Figure 2: The lupus epigenome is characterized by enhanced chromatin accessibility surrounding T cell activation and cytokine signaling genes. A) PCA plot (PC3 x PC4) of ATAC data for sorted T_H populations. Colors distinguish lupus or healthy samples and shapes distinguish T_H subsets. **B-C**) PCA loadings separated by disease and cell type for PC3 (B) and PC4 (C). **D**) Quantitation of differentially accessible regions (DARs) between lupus and healthy combined T_H cells defined as padj <0.025 (n=12,625) and depicted in black. Regions more accessible in lupus samples (n=2683) (padj <0.025 & FC > 1.5) in yellow. Regions more accessible in HCs (n=246) (padj <0.025 & FC > 1.5) in blue. **E**) Pathway enrichment analysis of lupus DARs (padj <0.025 & FC > 1.5) (n = 2683 regions) among GO:Biological Process and MSigDB Hallmark

gene sets. **F-H**) Sample-wise peak-set variation enrichment scores for Hallmark IFNγ response gene loci (F), Hallmark IFNα response gene loci (G), and Hallmark TNFα signaling gene loci (H). **I**) HOMER TF motif analysis results and top transcription factor motifs enriched in lupus DARs (n = 2683). Error is reported as SD. ATAC data represent 25 naive T_H samples (13 Lupus, 12 Healthy), 8 Th1 samples (4 Lupus, 4 Healthy), 24 cTfh samples (12 Lupus, 12 Healthy), and 24 AcTfh samples (12 Lupus, 12 Healthy). *P < 0.05; **P < 0.01, ***P <0.001 unpaired 2-tailed t tests (A-I).

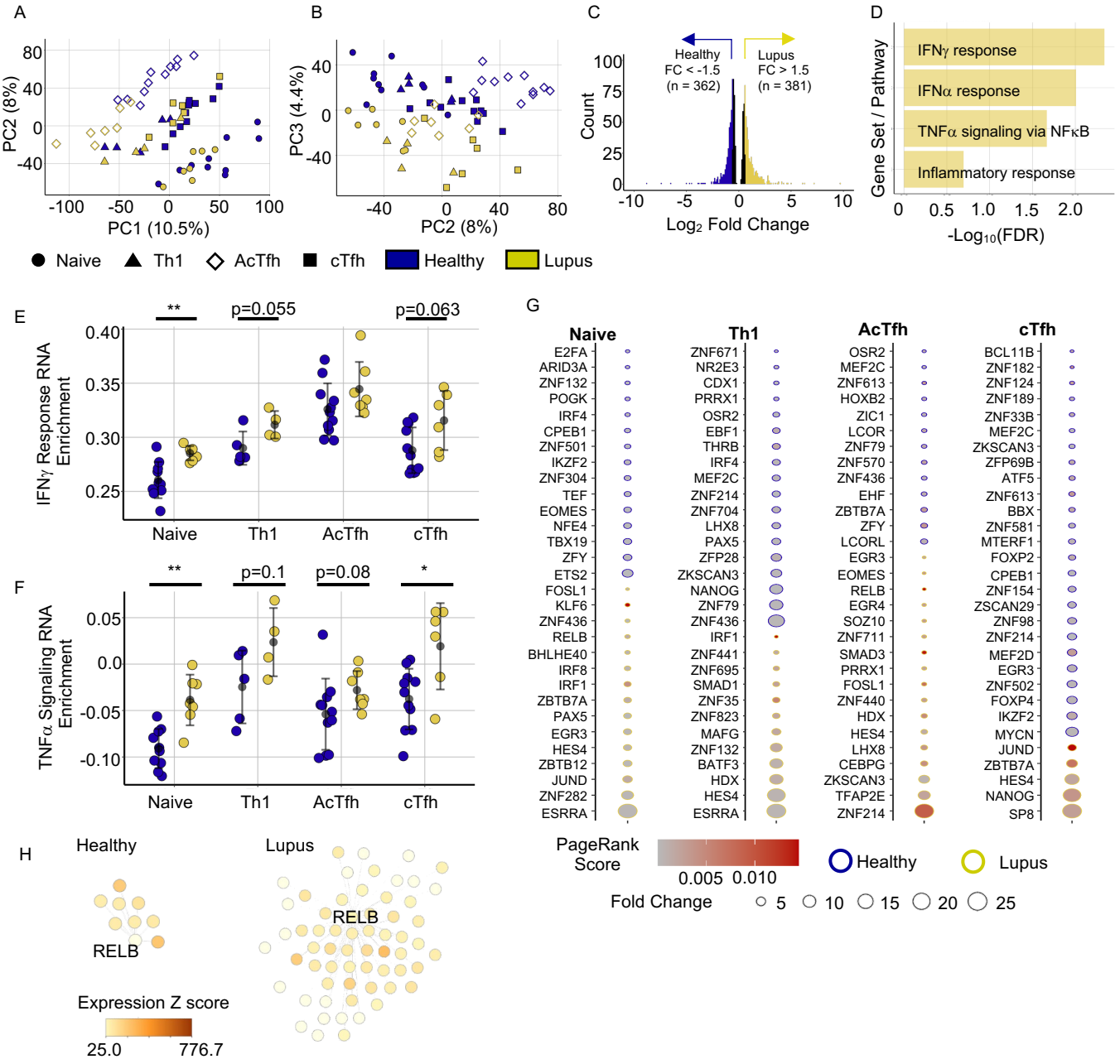

Figure 3: Transcriptional and chromatin accessibility data highlight cytokine driven dysregulation in lupus. A-B) PCA (PC1 x PC2 (A) and PC2 x PC3 (B)) of RNA-seq data for T_H subsets. Colors indicate lupus or healthy samples and shapes indicate T_H subsets. **C**) RNA-seq defined differentially expressed genes (DEGs) between lupus and healthy samples (naive, AcTfh, cTfh, Th1) defined as padj <0.05 (black)(n=1,132). Genes with higher expression in lupus samples (n=381) (padj <0.05 & FC > 1.5) in yellow. Genes with higher expression in healthy samples (n=362) (padj <0.05 & FC > 1.5) in blue. **D**) GSEA pathway results for lupus DEGs (n = 381) among GO:Biological Process and MSigDB Hallmark gene sets. **E-F**) GSVA enrichment of Hallmark IFNγ response gene set (E) and Hallmark TNFα signaling via NFκB gene set (F) in T_H populations of lupus subjects and HCs. **G**) Differentially active transcription factors (TFs) in lupus and healthy T_H subsets

with a TAIJI page rank score > 0.0002 in either lupus or healthy populations. The thirty TFs with the highest fold change (indicated by circle size) between lupus and healthy conditions for each cell type are displayed; greater TF page rank activity score in healthy subjects (blue) or lupus subjects (yellow). **H**) Taiji-defined RELB TF gene regulatory networks among lupus or healthy naive T_H cells (edge weight cutoff = 100). Node color saturation is proportional to node expression. Error is reported as SD. RNA-seq data represent 17 naive T_H samples (7 Lupus, 10 Healthy), 9 Th1 samples (4 Lupus, 5 Healthy), 17 cTfh samples (6 Lupus, 11 Healthy), and 18 AcTfh samples (7 Lupus, 11 Healthy). *P < 0.05; **P < 0.01, ***P <0.001 paired 2-tailed t tests (A-H).

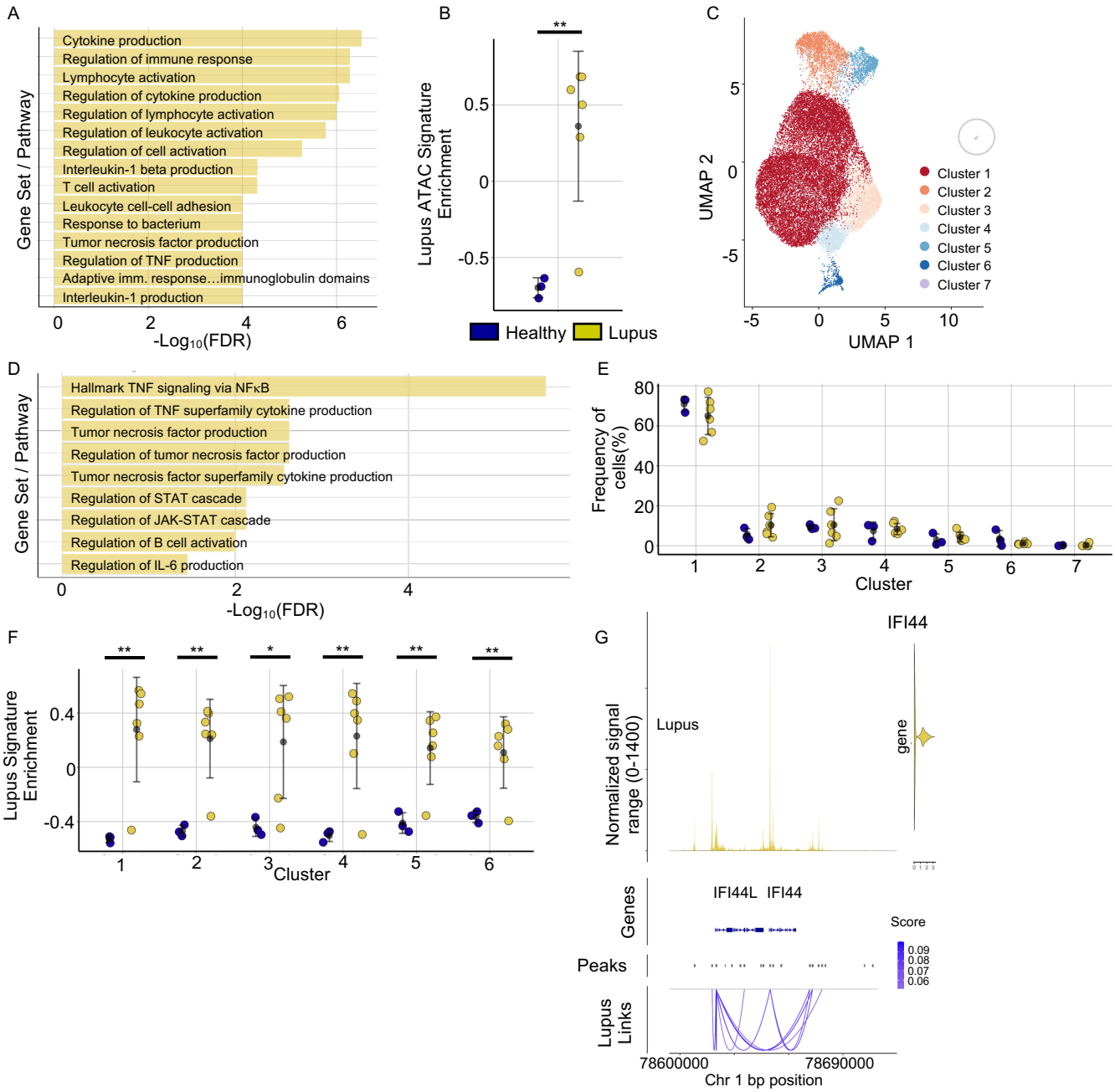

Figure 4: Single cell multi-ome analysis of naive T_H cells demonstrates ubiquitous dysregulation in lupus. A) Pathway enrichment analysis results for 10X single cell ATAC (scATAC) defined DARs of greater accessibility in lupus naive T_H cells. Pathways used in enrichment include GO:Biological Process and Hallmark gene sets. **B**) GSVA enrichment score for lupus-associated DARs (Figure 2C, n= 2683 regions) amongst integrated scATAC data sets of lupus subjects and healthy controls. **C**) UMAP dimensional reduction of multi-omic single cell clusters from naive T_H cells. Single cell nuclear RNA and scATAC data from naive T_H of lupus and healthy individuals were independently integrated and then combined for cluster analysis and UMAP visualization. **D**) Pathway enrichment analysis results for scATAC defined DARs of cluster 5 in naive T_H cells. **E**) Frequency of cells among naive T_H clusters for individual lupus and healthy subjects. **F**) Enrichment of

signature lupus-associated DARs (Figure 2C, n= 2683) among naive T_H clusters. **G**) Peak-gene linkage analysis results depicting chromatin accessibility track (top), gene expression (top right), and peak-gene linkages (bottom) along the *IFI44* locus in lupus naive T_H cells. Error is reported as SD. Single cell multi-ome data include cell from 6 lupus and 3 healthy subjects (A-G). *P < 0.05; **P < 0.01, ***P <0.001 paired 2-tailed t tests (B, E-F)**.**

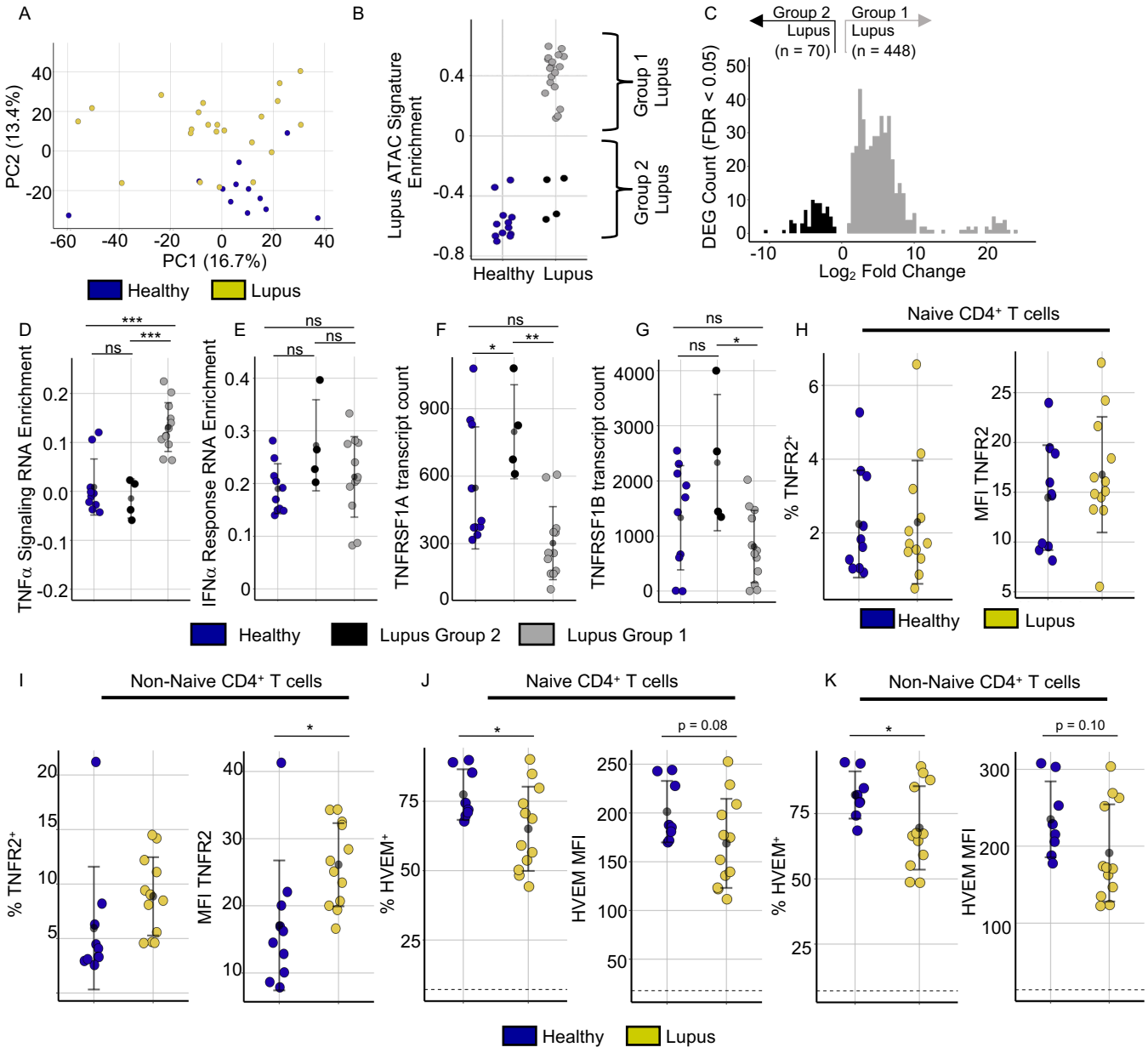

Figure 5: Lupus naive T_H cells with open chromatin have a TNF signature. A) PCA (PC1 x PC2) of ATAC data for naive T_H cells from lupus and healthy individuals. Colors indicate lupus or healthy states. **B)** Enrichment of signature lupus-associated DARs (Figure 2C, n= 2683) among naive T_H samples. Brackets highlight lupus samples grouped into either "Group 1" or "Group 2" based on lupus enrichment score. **C)** DEGs from RNA-seq data (padj <0.05) between Group 1 and Group 2 lupus naive T_H cells (Figure 5B). Genes more highly expressed in Group 1 (grey) or more highly expressed in Group 2 (black) are indicated. **D-E)** RNA-seq data GSVA of Hallmark TNFα signaling via NFκB **(D)** and Hallmark IFNα response gene sets **(E)** in naive T_H cells in Group 1 lupus subjects, Group 2 lupus subjects, and healthy controls. **F-G)** *TNFRSF1A* **(F)** and *TNFRSF1B* **(G)** gene transcript counts in Group 1 lupus subjects, Group 2 lupus subjects, and healthy

controls. **H-K)** Flow cytometry of lupus patients and healthy control PBMCs. **H-I)** Frequency of $TNFR2^+$ (right) and TNFR2 MFI (left) amongst naïve (H) and non-naïve (I) T_H in lupus patients (n=12) and healthy controls (n=10). **J-K)** Frequency of $HVEM^+$ (right) and HVEM MFI amongst naïve (J) and non-naïve (K) T_H in lupus patients (n=12) and healthy controls (n=8). Error is reported as SD. ATAC data represent 34 naive T_H samples (22 Lupus, 12 Healthy) **(A-B)**. RNA-seq data represent 25 samples (16 Lupus, 10 Healthy) **(C-G)**. 10X single cell multi-ome data include cell from 6 lupus and 3 healthy subjects (I). *P < 0.05; **P < 0.01, ***P <0.001 multiple one-way ANOVA with Tukey multiple comparisons correction (D-G). *P < 0.05; **P < 0.01, ***P <0.001 2-tailed t tests **(H-K)**.

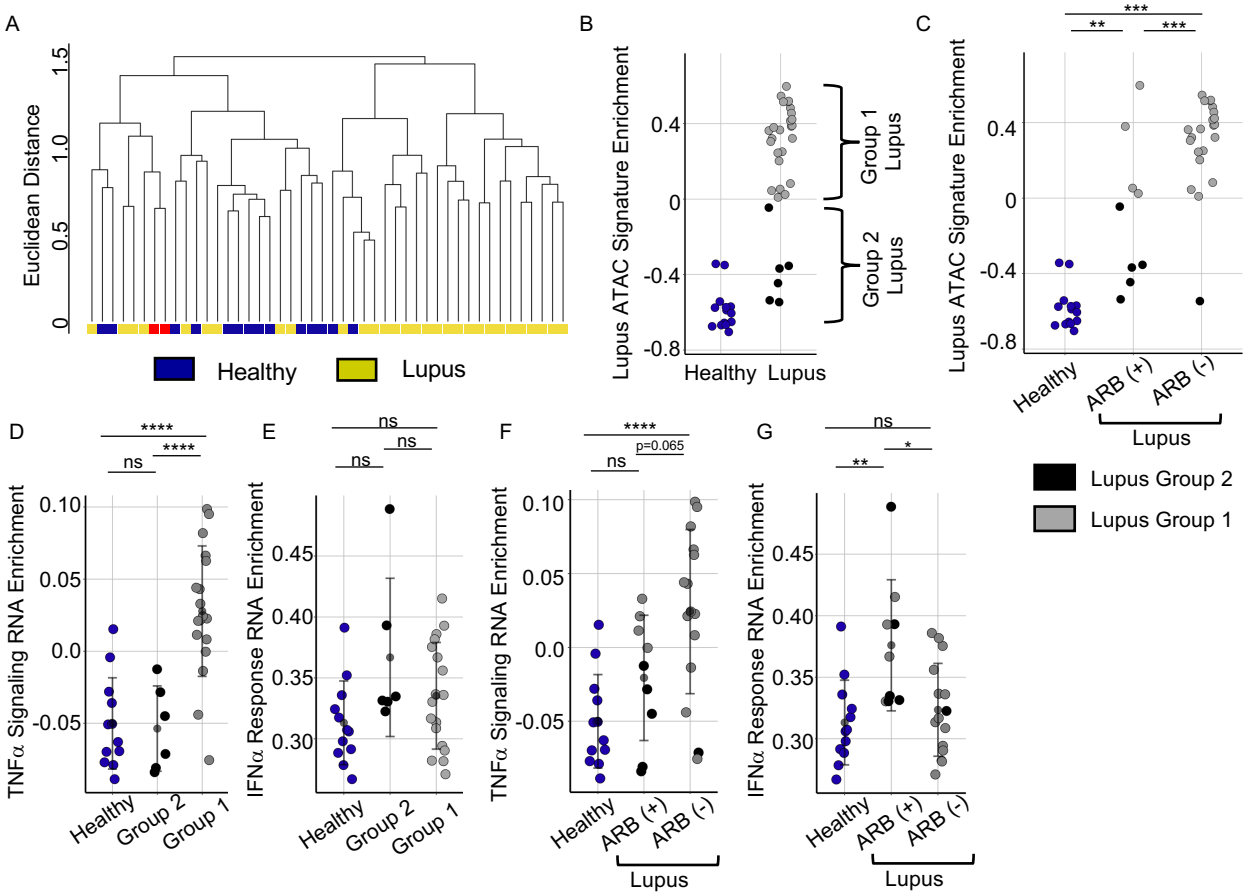

Figure 6: ARB prescription is associated with epigenetic and transcriptional changes in lupus T cells. A) Dendrogram representing hierarchically clustered ATAC-seq profiles of lupus and healthy samples. A single Group 2 lupus subject appears twice and was tracked over more than 2 years and resampled (red). **B)** Enrichment of signature lupus-associated DARs (Figure 2C, n= 2683) among naive T_H samples in this expanded dataset. Brackets highlight lupus samples grouped into either "Group 1" (grey) or "Group 2" (black) based on lupus enrichment score. **C)** Enrichment of signature lupus-associated DARs (Figure 2C, n= 2683) in naive T_H cells graphed in healthy individuals and lupus individuals prescribed (ARB+) and not prescribed (ARB-) angiotensin receptor blocking drugs. **D-E)** RNA-seq data GSVA of Hallmark TNFα signaling via NFκB **(D)** and Hallmark IFNα response gene sets **(E)** in naive T_H cells in Group 1 (grey) lupus subjects, Group 2 (black) lupus subjects, and healthy controls (blue). **F-G)** RNA-seq data GSVA of Hallmark TNFα signaling via NFκB **(F)** and Hallmark IFNα response gene sets **(G)** in naive T_H in healthy controls, ARB+ lupus patients, and ARB- lupus patients. Error is reported as SD. ATAC data represent 46 naive T_H samples (31 Lupus patients (+ 1 duplicated second timepoint sample **(A)**), 14 Healthy) **(B-C)**. RNA-seq data represent 36 samples (24 Lupus, 12 Healthy) **(D-G)**. Across the 24 lupus patients presented, 9 are prescribed ARBs and 15 are not **(F-G)**. *P < 0.05; **P < 0.01, ***P <0.001 multiple one-way ANOVA with Tukey multiple comparisons correction **(C-G)**.

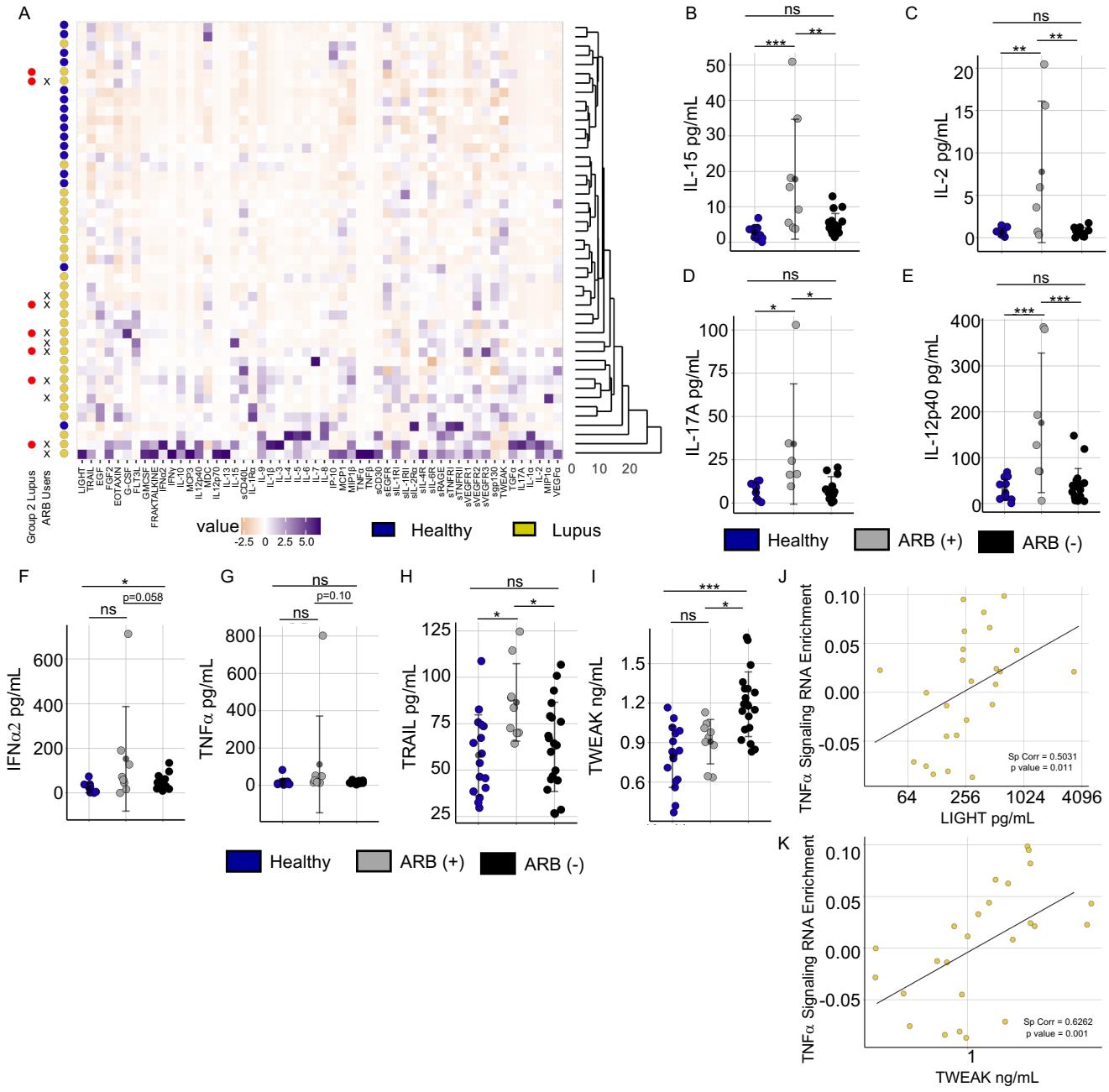

Figure 7: TNF family cytokines correlate with ARB use and epigenetic dysregulation of lupus T_H cells. A) Heatmap of plasma analytes (x axis) in hierarchically clustered lupus and healthy controls. Group 2 sorted lupus subjects and ARB-prescribed lupus patients are noted. **B-I)** Plasma concentrations of selected analytes in healthy controls (n = 16), lupus patients prescribed ARBs (n= 9), and lupus patients not prescribed ARBs (n=21). Analytes shown are IL-15 **(B)**, IL-2 **(C)**, IL-17A **(D)**, and IL-12p40 **(E)**, IFNα2 **(F)**, TNFα **(G)**, TRAIL **(H)**, TWEAK **(I)**. **J)** Scatterplot showing linear regression line and spearman correlation coefficient measuring the relationship between plasma LIGHT concentrations (x axis) and RNA-seq GSVA enrichment scores Hallmark TNFα signaling via NFκB in naive T_H for concordant lupus samples (n=25). **K)** Scatterplot showing linear regression line and spearman correlation coefficient measuring the relationship between plasma

TWEAK concentrations (x axis) and RNA-seq GSVA enrichment scores Hallmark TNFα signaling via NFκB in naive T_H for concordant lupus samples (n=25). Error is reported as SD. *P < 0.05; **P < 0.01, ***P <0.001 multiple one-way ANOVA with Tukey multiple comparisons correction **(B-I)**.