Supplementary Notes

From subsection: **An atlas of immune cells in resting and stimulated states**

We observed high technical reproducibility (ATAC and RNA mean Pearson's R value = 0.89 and 0.80, respectively) and biological reproducibility (ATAC and RNA, mean Pearson's R value = 0.85 and 0.77, respectively) across replicates. We further confirmed the quality of our data by analyzing the enrichment of ATAC-seq reads mapping to transcription start sites (TSSs), and the expression of cell type-specific genes (Supplementary Table 1). As expected, we observed strong enrichment of reads at TSSs genome-wide and at promoters of cell type-specific genes such as *CD8A* in CD8$^+$ T cells (Fig. 1d, Supplementary Fig. 2a, b).

From subsection: **Identifying immune memory-associated accessible regions**

Memory CD4$^+$ T effectors had 2,275 peaks that exhibited increased accessibility compared to naive CD4$^+$ T effector cells and only 130 peaks showed significantly decreased accessibility. In contrast to the increase in accessibility during maturation observed in lymphocytes, immature NK cells transitioning to memory NK cells gained accessibility at 1,211 sites while losing 2,526 accessible sites.

Characterizing peaks that correspond to lineage-specific and shared memory components in more detail, we observed signatures characteristic of enhancers in multiple blood-related cell types (Supplementary Fig. 3). Specifically, candidate binding sites of TFs with known roles in regulating memory T cell formation, such as RUNX3[1], BCL6[2,3] and NFKB1[4], showed strong enrichment in peaks that exhibit increased accessibility in T and B memory cells.

From subsection: **Stimulation leads to large-scale chromatin changes**

Connecting chromatin changes to gene expression, we found significant correlation between promoter accessibility (defined as a 5kb window around the start of a gene) and gene expression in resting state samples (R = 0.4) and stimulated samples (R = 0.37) (Supplementary Fig. 8b). Furthermore, we observed significant increases in accessibility in promoter regions of genes with the largest increases in expression upon stimulation (Supplementary Fig. 8c). Overall, these results illustrate the global chromatin and transcriptional changes of immune cells upon stimulation.

We next sought to identify transcription factors that may drive cell type and stimulation-responsive elements. We investigated variation in accessibility at position weight matrix (PWM)-predicted TF binding regions across cell types and conditions (Supplementary Fig. 5d). For example, the SPI1 motif is most enriched in B cells, DCs, and monocytes, consistent with gene expression data (Supplementary Fig. 5e). The corresponding TF for the SPI1 motif is integral to both myeloid and lymphoid B cell development[5].

In contrast, we found that the BATF motif (or perhaps another TF from the AP-1 family, which can have similar PWMs) was consistently enriched in accessibility regions across stimulated samples compared to their corresponding unstimulated state (Supplementary Fig. 5d). This suggests a shared effect of BATF and/or related transcription factors on chromatin regulation in stimulated samples across cell lineages, which was previously identified in stimulated CD4$^+$ T cells[6]. Additionally, the putative activity of BATF correlates with upregulation of *BATF* expression (Supplementary Fig. 5f) and the expression of several classes of previously identified BATF-target genes (Supplementary Fig. 5g)[7]. Thus, our analysis identified large-scale genome-wide changes in chromatin accessibility and gene expression upon stimulation in B and T cells putatively attributable to specific sets of TFs.

From section: **Discussion**

Interestingly, rs6927172 was not detected as an eQTL in GTEx v7, likely because tissues represent cell mixtures with generally low proportions of immune cells and even lower proportions in activated states. However, Wu et al. demonstrated that the disruption of an 11bp region harboring rs6927172 significantly decreased gene expression of *TNFAIP3* in stimulated HEK293 T cells[8], suggesting this variant drives *TNFAIP3* expression.

Upon stimulation, Coornaert et al.[9] found that *TNFAIP3* expression first decreases and then reappears, suggesting that the initial removal of A20 (encoded by *TNFAIP3)* is essential for optimal NFKB1 activation[9]. While the mechanism that leads to the opening of the region containing rs6927172 and the suppression of A20 is unclear, we propose a model in which A20 is first down-regulated by other factors, allowing activation of NFKB1 (Supplementary Fig. 14). Next, NFKB1 binds to the region containing rs6927162, resulting in the reappearance of A20 expression. Such a hypothesis is supported by studies focused on the regulation of NFKB1[10]. Thus, rs6927172 likely prevents the return of A20 by disrupting the binding of NFKB1, which subsequently results in inappropriate NFKB1 signaling.

Additional Methods

Data collection

Fetal sample processing

Human thymus was obtained from 18- to 22-gestational-week specimens under the guidelines of the Committee on Human Research (UCSF IRB)–approved protocols from the Department of Obstetrics, Gynecology and Reproductive Science, San Francisco General Hospital. Fetal samples were obtained after legal, elective termination of pregnancy with written informed consent for fetal tissue donation to biomedical research. Consent for tissue donation was obtained by clinical staff after the decision to pursue termination was reached by patients. Personal Health Information and Medical Record Identifiers/access is at no point available to researchers, and no such information is associated with tissue samples at any point. Tissue was washed and cut into small pieces using scissors. Thymocytes were extracted by mashing tissue pieces gently using the back of a sterile syringe. To extract TECs, remaining tissue pieces were digested for 30 min at 37 °C using medium containing 100 µg ml^{-1} DNase I (Roche, Switzerland) and 100 µg ml^{-1} Liberase TM (Sigma-Aldrich, MO, USA) in RPMI. Fragments were triturated through a 5-ml pipette every 6 min to mechanically aid digestion. At 30 min, tubes were spun briefly to pellet undigested fragments and the supernatant was discarded. Fresh digestion medium was added to remaining fragments and the digestion was repeated using a glass Pasteur pipette for trituration. Supernatant from this second round of digestion was also discarded. A third round of enzymatic digestion was performed using digestion medium supplemented with trypsin-EDTA for a final concentration of 0.05%. Remaining thymic fragments were digested for another 30 min or until a single cell suspension was obtained. The cells were moved to cold MACS buffer (0.5% BSA, 2 mM EDTA in PBS) to stop the enzymatic digestion. Following digestion, TECs were enriched by density centrifugation over a three-layer Percoll gradient with specific gravities of 1.115, 1.065 and 1.0. Stromal cells isolated from the Percoll-light fraction (between the 1.065 and 1.0 layers) were washed in MACS buffer. Samples were sorted on FACS Aria flow cytometer (BD Biosciences, CA, USA) up to >95% purity. Sorted cells were washed once in PBS, cryopreserved in Bambanker freezing media (LYMPHOTEC Inc, Japan) for ATAC experiments and in TriReagent (Sigma-Aldrich, MO, USA) for RNA experiments. Cells frozen in Bambanker freezing media were stored in liquid nitrogen until ready to use. Cells frozen in TriReagent were stored at -80°C until further use.

NFKB1 ChIP-seq from heterozygous donors

Isolated CD4^{+} T cells were stimulated for 24 hours with anti-human CD3/CD28 dynabeads (Thermo Fisher Scientific, MA, USA) at a 1:1 cell to bead ratio and 50 unit/ml of human IL-2 (UCSF Pharmacy). The cells were harvested, fixed with 1% formaldehyde (Thermo Fisher Scientific, MA, USA) for 10 min, washed twice with cold PBS and frozen at -80C. The chromatin was sonicated to generate fragments of 200-500bp in length, followed by incubation with rabbit polyclonal p50 (#3035, Cell Signaling, Danvers, MA, USA) and p65 (ab16502, Abcam, Cambridge, UK) antibodies overnight. Precipitated chromatin was washed, de-crosslinked and DNA extraction was carried out using phenol-chloroform (Sigma). ChIP DNA was prepared for high throughput sequencing using Accel-NGS 2S Plus DNA library kit (Swift Biosciences) as per manufacturer's protocol. DNA libraries were sequenced on an Illumina Hiseq4000 with a paired-end 75bp run (CAT, UCSF).

Collection of publicly available data

Progenitor RNA-seq and ATAC-seq data from GEO

ATAC-seq and RNA-seq of hematopoiesis progenitors and several differentiated cell types were downloaded[11], and processed through the respective ATAC-seq and RNA-seq pipeline described below. Only data from healthy controls was included throughout this study.

ATAC-seq data from ENCODE

To serve as a negative control for GWAS enrichment analyses, we collected data from ATAC-seq samples from tissues with low proportions of immune cells. We used the ENCODE data portal to download all available raw fastq ATAC-seq files from the calf muscle and breast epithelium human tissues. All data were processed with the same ATAC-seq data processing pipeline described below.

Obtaining GWAS summary statistics

We downloaded the full set of GWAS summary statistics of 13 complex traits from 9 autoimmune traits and 4 primarily non-autoimmune and thus negative control traits (Sunburn, Alzheimer's disease, Type 2 diabetes, and Schizophrenia) that have been previously aggregated[12]. For analyses that relied on fine-mapped disease-associated variants, we downloaded the list of PIC variants. These represent individual GWAS regions that have been fine-mapped with a previously described statistical method [13].

Obtaining Blood eQTL summary statistics

From the GTEx data portal we downloaded v7 eQTL estimates for all SNP-gene pairs tested with whole blood gene expression.

Data analysis

ChIP-seq

We aligned ChIP-seq reads using bowtie2 version 2.2.9 with default parameters and a maximum paired-end insert distance of 2kbp. The bowtie2 index was constructed with the default parameters for the hg19 reference genome. We filtered out reads that mapped to chrM and used samtools version 1.4 to filter out reads with MAPQ < 30 and with the flags '-F 1804' and '-f 2'. Additionally, duplicate reads were discarded using picard version 1.134 (http://broadinstitute.github.io/picard/). TF-bound peaks were identified with MACS2 version 2.1.1 under default parameters and '--nomodel --nolambda --keep-dup all --call-summits'. Additionally, the appropriate input-DNA background was set with the '--control' parameter. A consensus set of peaks was defined by merging overlapping (1bp or more) peaks identified in at least two samples across all samples. We then used the 'get_count' function from the nucleoATAC python package to count the number of fragments within the consensus peak set across all samples[14]. We used samtools version 1.4 'mpileup' to count reads aligning to rs6927172.

Exploratory analysis

We used tSNE, PCA, and k-means clustering to explore trends in gene expression and chromatin accessibility variation. As input to these methods, we used the read count matrices corrected for sample quality (with TSS enrichment as a proxy) and batch effects (with donor as a proxy). After using trimmed mean of M-values (TMM) to estimate scaling factors[15] and applying the voom transformation[16], we used the 'removeBatchEffect' function from limma to regress out batch and sample quality effects [17]. Aside from the removal of these effects, normalized counts are equivalent to the addition of a 0.5 pseudocount to the fragment count and a log2 transformation of the fragment counts per million (CPM). For analyses that included previously published samples, we did not remove batch effects, because these batches do not have overlapping cell types. However, batch appeared to have a minimal effect on sample clusters and these analyses were exploratory in nature. The package Rtsne version 0.13 (https://github.com/jkrijthe/Rtsne) was used for tSNE analysis with default parameters, unless there were too few samples in which case the perplexity was set to 10. Additionally, we performed k-means clustering (with k set to the total number of cell-type by condition pairs) and then estimated the accuracy of ATAC-seq and RNA-seq unsupervised clustering by computing the HA-adjusted RAND index[18] considering the known cell-type/condition pairs as the ground truth clustering. We repeated the clustering 100 times to estimate the average HA-adjusted RAND index. When comparing HA-adjusted RAND index values between the RNA-seq and ATAC-seq samples, we used the intersection of samples.

Correlation between promoter accessibility and gene expression

For each sample, we counted the number of filtered ATAC-seq reads that aligned to 5kb promoter regions based on annotations of unique protein coding genes from gencode v25. Once again, we used trimmed mean of M-values (TMM) to estimate scaling factors and applied the voom transformation to compute log2(CPM) counts following the addition of a 0.5 pseudocount. We merged samples from the same cell type and condition across donors by averaging the log2(CPM) accessibility values for each promoter region. Finally, we quantile normalized accessibility values to a standard normal distribution along with the processed gene expression values. We reported Pearson's R correlation values to assess the relationship between promoter accessibility and gene expression. Values presented in Supplementary Fig. 8 are from all samples, however we report condition and cell type-specific correlation values in Supplementary Table 1.

Enrichment analysis of differentially accessible regions

We used Fisher's exact tests implemented in the LOLA tool[19] to quantify enrichment of sets of differentially accessible peaks in different lineages in comparison to a universe set of all peak regions that were shared between comparisons. As a catalogue for potential enrichment we considered a collection comprising peaks from the CODEX database[20], ENCODE TFBS, ENCODE chromatin state segmentations and candidate binding sites for motifs in the JASPAR database[21] determined by the motifmatchr R package (https://github.com/GreenleafLab/motifmatchr). The rank represents the rank of each dataset for a given peak set. Max rank means, the largest (i.e. worst) rank among the following scores from a Fisher's exact test: odd-ratio, p-value, support. The white squares represent non-significant enrichments (q-value >= 0.01) or enrichments that could not be computed, because there was no overlap.

Enrichment analysis of differentially expressed genes

For each subset we identified significantly differentially expressed genes with a *q*-value less than 0.01 and absolute log2FC greater than 1. We used g:Profiler to identify pathways that were significantly enriched for stimulation-associated genes[22] with an ordered query based on a ranking of differential expression q-values and Bonferroni p-value correction. Top enriched pathways per cell subset are listed in Supplementary Table 1 and Supplementary Fig. 8 displays a heatmap of these results.

Variance decomposition

We were interested in decomposing the total variance of chromatin accessibility across samples into variance components attributable to specific factors. Therefore, we fitted a random effects model:

$$a_{ij} = \kappa_{i,s(j)} + \beta_{i,l(j)} + \gamma_{i,c(j)} + \zeta_{i,s(j),l(j)} + \eta_{i,s(j),c(j)} + \delta_{i,d(j)} + \lambda_i t_j + \epsilon_{ij},$$

where chromatin accessibility (a) at peak i for a sample j is a function of the effects of the stimulation condition (κ), lineage (β), cell type (γ), lineage/stimulation interaction (ζ), cell/stimulation interaction (η), donor (δ), TSS enrichment (t) of ATAC-seq reads (λ), and the residual error (ϵ). For notational convenience, we define a function for each feature in the model that looks up sample-specific information, i.e., $s(j)$ represents the stimulation condition associated with sample j. We represented accessibility with the log2(CPM) ATAC-seq read counts (with the addition of a pseudocount of 0.5) at consensus peaks across samples, which were normalized for read depth with TMM normalization. Additionally, we scaled accessibility at each peak across samples to have mean=0 and variance=1. We included the effects of d and t (as a proxy for sample quality), to control for their effects, since the other parameters are our primary interest. Across all peaks, we modelled the distribution of effects:

$$(\kappa, \beta, \gamma, \zeta, \eta, \delta, \lambda, \epsilon) \sim MVN\left(0, diag(\sigma_s^2, \sigma_l^2, \sigma_c^2, \sigma_{sl}^2, \sigma_{sc}^2, \sigma_d^2, \sigma_t^2, \sigma_\epsilon^2)\right).$$

We used a maximum likelihood approach to jointly estimate the σ^2 parameters for each factor. To obtain robust estimates we found it beneficial to pool peaks. We found pooling 100 peaks represented a good compromise between computational cost and statistical robustness. To assess uncertainty of variance estimates, we repeated the analysis on 100 sets of 100 randomly selected peaks with replacement. The total biological variance explained (TBVE) by the factors of interest is,

$$TBVE = \sigma_s^2 + \sigma_l^2 + \sigma_c^2 + \sigma_{sl}^2 + \sigma_{sc}^2.$$

Therefore, the proportion of biological variance explained (PBVE) contributed by a factor is the variance estimate σ^2 for that factor divided by $TBVE$. We listed the median value across all bootstrap replicates. For results reported we limited our analysis to cell types from the four donors with the most cell samples collected and excluded cell types with fewer than three biological replicates.

Visualizing TF ATAC-seq footprints

We aggregated ATAC-seq insertion counts around candidate binding sites for motifs in the JASPAR database[21] determined by the motifmatchr R package (https://github.com/GreenleafLab/motifmatchr) using transcription factor footprinting methods previously described[23].

Boxplot visualizations

Unless otherwise mentioned, all boxplot visualizations represent the median, two hinges (25th and 75th percentile) and whiskers. Whiskers show a line from the hinge to 1.5 * the difference between the first and third quartile. Points that extend beyond the whiskers are displayed individually.

TF position weight matrix (PWM) motif analyses

For determining PWMs enriched in open chromatin regions we used chromVAR version 1.0.1 with default parameters on read counts within consensus peaks of samples merged by donor[24]. Following identification of condition-associated TFs with chromVAR we wanted to examine the effect of a few of these TFs on allele-specific chromatin accessibility. We used the PWM of a TF of interest to predict the binding affinity of a 41 bp genomic region centered on the heterozygous site. The binding affinity or match score was computed using the 'motifmatchr' R package (https://github.com/GreenleafLab/motifmatchr), which is a wrapper for the MOODS motif matching suite[25]. The relative binding score was determined by subtracting the binding affinity match score of the alternative allele from that of the reference allele. As a threshold for presence or absence of motif matching we used a p value cutoff of 3×10^{-3}. In this way we grouped heterozygous sites into three groups: predicted TF affinity for the reference (relative match > 1), alternative (relative match < -1) or no preference (absolute value of the relative match < 0.01).

Peak clustering

To test whether disease heritability was enriched within stimulation-specific chromatin accessible from B and T cell lineages we used a supervised peak clustering approach. First, we scaled the matrix of ATAC-seq read counts per sample (indicated by j) across all consensus peaks (indicated by i) to values between 0 and 1 with

$$x'_{i,j} = \frac{x_{i,j} - \min(x_{i,\cdot})}{\max(x_{i,\cdot}) - \min(x_{i,\cdot})},$$

where x' represents the scaled matrix. Peaks in a sample that were in the top decile were automatically set to 1 to represent the fully accessible state. Per peak we computed the median scaled accessibility across samples from the same broad cell type and condition.

Our goal was to identify peaks that express a specific accessibility profile. We defined a profile of interest with a vector of length equal to the number of merged lineage and condition samples with values of either 0 or 1 corresponding to closed or open chromatin accessibility. We consider 11 profiles of interest (Supplementary Fig. 10d). To identify peaks with a similar profile we computed the average Euclidean distance between each of the ideal accessibility profiles and each peak. Peaks more similar to an ideal peak profile should have smaller distances to the peak profile. Additionally, when computing the distance, we incorporated a weight per sample to influence the importance of matching accessibility in different merged samples. This was important to find peaks that were accessible in resting samples (weight of 1), while allowing for the possibility that the peak was accessible in the same lineage but stimulated samples (weight of 0).

To determine a distance cutoff of a peak cluster, we permuted the peak accessibility values for each sample and computed a null distribution of distances for each lineage and peak cluster type. We used a peak distance threshold resulting in fewer than 5% false positives. Finally, peaks passing this peak distance threshold were assigned to a single profile of interest based on the minimum distance, thus forming disjoint sets of accessible regions.

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