Characterizing cellular heterogeneity in chromatin state with scCUT&Tag-pro

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Technologies that profile chromatin modifications at single-cell resolution offer enormous promise for functional genomic characterization, but the sparsity of the measurements and integrating multiple binding maps represent substantial challenges. Here we introduce single-cell (sc)CUT&Tag-pro, a multimodal assay for profiling protein–DNA interactions coupled with the abundance of surface proteins in single cells. In addition, we introduce single-cell ChromHMM, which integrates data from multiple experiments to infer and annotate chromatin states based on combinatorial histone modification patterns. We apply these tools to perform an integrated analysis across nine different molecular modalities in circulating human immune cells. We demonstrate how these two approaches can characterize dynamic changes in the function of individual genomic elements across both discrete cell states and continuous developmental trajectories, nominate associated motifs and regulators that establish chromatin states and identify extensive and cell-type-specific regulatory priming. Finally, we demonstrate how our integrated reference can serve as a scaffold to map and improve the interpretation of additional scCUT&Tag datasets.

Technologies that enable unsupervised transcriptomic profiling at single-cell resolution (i.e., single-cell RNA sequencing (scRNA-seq)) represent powerful tools not only for the discovery of cell types and states but also to reveal the structure and function of transcriptional regulatory networks1–6. In the same way, new methods for single-cell chromatin profiling, such as scATAC-seq, can lead to the identification and characterization of individual genomic regulatory regions and the exploration of their variation in a heterogeneous population7,8. Assays that profile chromatin accessibility exhibit a largely binarized phenotype, partitioning genomic regions into accessible or inaccessible elements in different tissue and cellular contexts. As a complement to accessibility measurements, genome-wide histone modification profiles offer an exciting opportunity to segment the genome into a more nuanced set of functional elements9–12. The 'histone code hypothesis' proposes that the combinatorial presence of multiple histone modifications marking a genomic region serves as a proxy for its regulatory function13,14.

Techniques like chromatin immunoprecipitation sequencing (ChIP-seq) and cleavage under targets and tagmentation (CUT&Tag) have been widely applied to measure the binding profiles of multiple transcription factors and histone modifications15,16. In addition, tailored computational tools such as ChromHMM17 and Segway18 can integrate the combinatorial binding patterns defined by each mark and assign individual genomic elements to a set of learned functional 'states'. Recently, multiple studies have demonstrated the ability to measure CUT&Tag profiles in single cells19,20. This represents an opportunity to not only identify regions that exhibit cell-type-specific accessibility but also highlight elements whose acquisition of activating, repressive or heterochromatic signatures varies within a heterogeneous population.

These advances lay out an exciting challenge for single-cell genomics: can the binding profiles of multiple histone modifications be used to infer the function of any individual genomic element within a single cell? Although the availability of single-cell CUT&Tag (scCUT&Tag) takes an important step toward this vision, two key challenges remain. First, single-cell chromatin data remain extremely sparse, particularly due to the inherent challenges associated with limited DNA input molecules. Second, scCUT&Tag experiments typically measure only a single histone modification profile in a single cell. Although informative, only the integrated combination of multiple histone modifications can be used to annotate functional state, but no current technology can robustly and simultaneously profile many histone modifications in the same cell, particularly when these marks may exhibit overlapping binding patterns. Recent pioneering advances enable paired measurements of cellular transcriptomes and individual CUT&Tag profiles using custom combinatorial indexing-based workflows21,22 and demonstrate how multimodal technologies can facilitate integrative analysis.

Here we present two techniques aimed to address these key challenges. We first introduce scCUT&Tag with cell surface proteins (scCUT&Tag-pro), a multimodal single-cell technology, that enables simultaneous profiling of an individual CUT&Tag profile with surface protein abundances and is compatible with the widely used 10x Genomics Chromium system. Second, we introduce a downstream analysis strategy for multimodal datasets: single-cell ChromHMM (scChromHMM). Our method first integrates data from multiple scCUT&Tag-pro experiments together into a common manifold, computationally generating coassay profiles for six histone modifications within individual cells. These measurements are used as input to a single-cell extension of the ChromHMM algorithm to return chromatin state annotations for each 200-bp genomic window at single-cell resolution.

We apply our tools and technologies to generate 64,876 scCUT&Tag-pro profiles (Supplementary Table 1) and combine this with existing datasets to create an integrated multimodal atlas of human peripheral blood mononuclear cells (PBMCs) that encompasses nine modalities spanning the central dogma. Together, these

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technologies and analytical tools allow us to explore heterogeneity in genome function at single-cell resolution, identify sequence elements whose presence accompanies changes in chromatin state and project new datasets onto our multimodal reference. We propose that these tools will help to facilitate the analysis and interpretation of chromatin state heterogeneity in single cells and lead to a better understanding of its role in establishing and regulating cellular identity.

Results

Simultaneous profiling of surface proteins and scCUT&Tag. The combinatorial pattern of post-translational modifications present on histone proteins correlates with the chromatin structure and regulatory potential of a genomic locus. Inspired by ASAP-seq, we reasoned that multimodal single-cell technologies could help address challenges in scCUT&Tag analysis by coupling robust single-cell measurements of one modality with more sparse measurements from another. For example, CITE-seq and ASAP-seq pair simultaneous measurements of highly expressed and well-characterized cell surface proteins with unsupervised but sparser transcriptomic measurements or chromatin accessibility profiles. We and others have recently demonstrated that the protein information in CITE-seq is highly informative in determining cellular state, whereas the paired RNA sequencing (RNA-seq) or assay for transposable-accessible chromatin sequencing (ATAC-seq) measurements enable the characterization of gene regulatory networks. ASAP-seq and integrated cellular indexing of chromatin landscape and epitope sequencing are enabled by recent optimizations in cell fixation and permeabilization that enable ATAC-seq to be performed on whole cells instead of individual nuclei.

We propose that a similar multimodal strategy could be applied for genome-wide profiling of protein–DNA interactions as well. We developed scCUT&Tag-pro, an assay that performs CUT&Tag assays on whole cells while simultaneously measuring surface protein levels (Fig. 1a and Methods). Inspired by ASAP-seq, our fixation and permeabilization conditions retain the cell membrane and associated proteins, enabling us to simultaneously measure cellular protein–DNA interactions in an unbiased manner.

We first evaluated the sensitivity and specificity of our CUT&Tag profiles. For example, in the H3K4me1 dataset, ADT expression patterns were measured simultaneously in each experiment (Supplementary Fig. 1d). For each experiment (Supplementary Table 1), we profiled a single histone modification pseudobulk profile was dependent on the number of included single cells but saturated between 500 and 1,000 cells (Fig. 1c). Overall, we found that unsupervised clustering of each scCUT&Tag dataset using a latent semantic indexing (LSI)-based dimension reduction workflow was capable of identifying major cell groups that define the human immune system (Fig. 1b) but did not recapitulate the high-resolution identification of cell states as observed in scRNA-seq or CITE-seq data.

Surface protein levels facilitate integration across experiments. We therefore examined the ADTs for 173 cell surface proteins, measured simultaneously in each experiment (Supplementary Fig. 1d). For example, in the H3K4me1 dataset, ADT expression patterns were fully concordant with cell types determined from chromatin-based clustering (i.e., uniform expression of CD3 in T cells, CD14 in monocytes and CD19 on B cells; Supplementary Fig. 1e). However, the protein modality revealed additional substructure, including mutual exclusivity of CD4 and CD8 in T cells, a division of monocytes into classical CD14+ and nonclassical CD16+ subgroups and canonical marker expression denoting mucosal-associated invariant T cells (Fig. 1f and Supplementary Fig. 1e). We used our recently introduced weighted nearest neighbor (WNN) analysis (Methods) to simultaneously cluster scCUT&Tag-pro based on a weighted combination of modalities (Fig. 1f and Supplementary Fig. 2a,b). For each experiment, we found that the protein modality substantially enhanced our ability to separate distinct cell types, echoing our previous findings in CITE-seq and ASAP-seq analysis (Supplementary Fig. 2c).
We compared the results of our single-cell analysis with FACS-sorted H3K4me1 ChiP-seq profiles from ENCODE\(^{35}\) (Methods). We observed that pseudobulk profiles generated from our WNN-derived scCUT&Tag-pro clusters clearly recapitulated the cell-type-specific ENCODE profiles (Fig. 1g). We also profiled biological replicates for one of the histone modifications (H3K4me1) and observed that these profiles were reproducible across replicates as well (Supplementary Fig. 2d). Moreover, downsampling our single-cell clusters to as low as 300 cells per profile still retained substantial cell type specificity in the ensuing pseudobulk tracks (Fig. 1g). Taken together, these analyses demonstrate that scCUT&Tag-pro datasets are comparable in sensitivity to existing technologies, exhibit low background, contain sufficient information to identify granular cell types and states and can be used to generate pseudobulk profiles that reflect high-quality bulk data when a sufficient number of cells are included.

As an alternative to fully unsupervised analysis, we found that single-cell protein measurements also facilitated high-resolution supervised annotation using reference datasets. For example, we recently introduced a carefully annotated reference dataset of 58 major and minor cell types and states in the circulating human immune system using a CITE-seq dataset of 161,764 cells and 228 surface proteins\(^{39}\). As our protein panel in this study largely overlaps with the reference dataset, we ‘mapped’ the cells from each scCUT&Tag-pro experiment onto our reference dataset (Methods) and repeated the procedure for our published ASAP-seq dataset\(^{39}\) as well. Performing query-to-reference mapping using shared protein markers enables joint visualization of all datasets (Fig. 2a, b) and also transfers a consistent set of annotations to each cell at multiple levels of resolution. This enabled us to partition each dataset into broad (level 1), granular (level 2) and fine-grained (level 3) classifications (Supplementary Fig. 3a). We also used this approach to infer a unified pseudotime trajectory (Methods) that modeled CD8\(^+\) T cell transitions from naive to effector states and confirmed the accuracy and resolution of our trajectory by comparing the developmental dynamics of key markers across experiments (Fig. 2c and Supplementary Fig. 3b).

Our reference-mapping workflow enabled us to explore the relationships between nine different modalities by harmonizing them into a common space and providing a consistent set of annotations (Fig. 2b). The modalities spanned the central dogma and ranged from measurements of chromatin accessibility, protein–DNA interactions (six targets), gene expression and protein abundances. Although the individual modalities were collected in separate experiments, each originated from a multimodal technology (ASAP-seq, scCUT&Tag-pro and CITE-seq) that was paired with a large and comprehensive panel of surface protein measurements to facilitate integration. For example, in Fig. 2d, we visualized data from each modality collected at the CD8A locus. As expected, we found robust gene and protein expression (as measured by CITE-seq), enriched chromatin accessibility (as measured by ASAP-seq) and the presence of activating histone modifications (as measured by scCUT&Tag-pro) in CD8\(^+\) T cells. In myeloid cell types, the locus was characterized by inaccessible chromatin and the presence of repressive histone modifications, which resulted in undetectable expression of CD8 RNA and protein.

scChromHMM characterizes chromatin state at single-cell resolution. We next aimed to use our dataset to explore heterogeneity in chromatin state within the human immune system. We first considered an analysis strategy exclusively based on tools that have been previously developed for bulk chromatin analysis and provide 25 pseudobulk CUT&Tag tracks (based on level 2 reference-derived annotations) as input. For example, ChromHMM\(^{14,17}\) trains a hidden Markov model (HMM) on the concatenated histone modification profiles for all cell types, and applies the Baum–Welch training algorithm\(^{36}\) to identify a set of possible hidden ‘chromatin states’. ChromHMM then applies the forward–backward algorithm\(^{36}\) individually on each pseudobulk track to calculate the posterior probability that each 200-bp genomic window in each cell type is assigned to a particular chromatin state.

After running the Baum–Welch step (Methods), ChromHMM obtained 12 states (Fig. 3a), which could be broadly grouped into active promoter (enriched for H3K4me3/H3K4me2/H3K27ac), active enhancer (enriched for H3K4me1/H3K27ac), repressed (enriched for H3K27me3) and heterochromatic (enriched for H3K9me3) states. We also observed more subtle heterogeneity in chromatin state within these broad categories, likely corresponding to strong/weak states as has been previously described\(^{37}\). We identified a very similar set of chromatin states when running the Baum–Welch step on pseudobulk tracks derived from level 3 annotations (Supplementary Fig. 4a, b). These results suggest that the Baum–Welch step of ChromHMM can be successfully run on pseudobulk tracks generated from scCUT&Tag-pro, returning a functionally interpretable set of chromatin states.

However, when attempting to associate these states with individual genomic elements in each cell type, we identified a limitation associated with the application of bulk methods to single-cell datasets. In particular, we found that in contrast to the initial Baum–Welch step for state identification, the final outputs of ChromHMM were highly dependent on the level of granularity used when generating pseudobulk profiles. For example, when we considered genomic windows assigned to enhancer states in CD8\(^+\) T cells (level 1), 16.4% were not annotated as enhancers in any of the more granular T cell subsets when we repeated the analysis using a higher-resolution subset of cell labels (level 2). Especially when analyzing developmental tissues, or systems characterized by continuous sources of heterogeneity, it may be detrimental to condition all downstream analyses on a fixed set of discrete cell type labels\(^{38}\).

We therefore devised an alternative approach, scChromHMM, where chromatin states are assigned at single-cell resolution. To do so, we first generated single-cell profiles with simultaneous measurements of six histone marks. Although it is not currently feasible to experimentally generate these data, we used our previously described anchoring workflow\(^{40}\) as a computational alternative. We recently demonstrated the use of this workflow to ‘transfer’ modalities across experiments\(^{38}\), for example, using a CITE-seq reference to accurately and robustly predict cell surface protein levels in an scRNA-seq dataset of human bone marrow. We applied a similar procedure in this study (Methods) to interpolate 20,000 single-cell profiles, each of which consisted of quantitative and genome-wide profiles for all six histone modifications and chromatin accessibility, alongside RNA and protein measurements. As in our previous studies, interpolated profiles represented a weighted average of ‘anchor’ cells, where the identification of anchors denoted a ‘matching’ of biological states across experiments based on shared protein expression patterns.

We assessed the accuracy of our interpolated profiles by comparing them to the original measurements that were obtained in separate experiments. For example, we computed the pseudobulk profile for H3K4me1 measurements based on our interpolated profiles, or the original measured values, and observed high quantitative concordance (Fig. 3b; \(R = 0.95\)). For each cell type, pseudobulk profiles of interpolated measurements clustered specifically with the pseudobulk profiles from the original measurements (Fig. 3c; additional histone marks are shown in Supplementary Fig. 5a, and cross-comparison across marks is shown in Supplementary Fig. 5b), demonstrating that our procedure retains cell-type-specific variation. Finally, we repeated the interpolation procedure to generate an independent set of 20,000 profiles and observed high reproducibility (\(R = 0.98\)) between runs (Supplementary Fig. 5c). Although our procedure cannot capture stochastic fluctuations, it does represent
**Fig. 2 | Protein measurements facilitate integrated analysis across modalities.**

**a.** Schematic workflow for integrated analysis. Datasets produced by scCUT&Tag-pro, ASAP-seq and CITE-seq are integrated together on the basis of a shared panel of cell surface protein measurements. **b.** Left: UMAP visualization of 230,597 total cells projected onto the reference dataset from Hao et al. Right: In addition to a harmonized visualization, cells from all experiments are annotated with a unified set of labels. **c.** We learned a unified pseudotime trajectory based on all experiments, representing the CD8 T cell transition from naive to effector states. We observe identical molecular dynamics for naive (CD55), memory (CD103) and effector (CD57) markers across all experiments, demonstrating that integrative analysis accurately identifies cells in matched biological states across experiments. **d.** Visualization of nine molecular modalities at the CD8A locus in B cell, CD4 T cell, CD8 T cell, dendritic cell, monocyte and NK cell groups. ASDC, AXL+ dendritic cell; cDC2, conventional dendritic cell 2; dnT, double-negative T cell; Eryth, erythrocyte; gdT, γδ T cell; HSPC, human stem and progenitor cell; TCM, central memory T cell; TEM, effector memory T cell; Treg, regulatory T cell.
**Fig. 3** | scChromHMM annotates chromatin states at single-cell resolution. 

**a.** Chromatin states returned by ChromHMM, which was run on 25 pseudobulk tracks for six histone marks. States are broadly grouped into five categories. 

**b.** Correlation comparing cell-type-specific pseudobulk profiles of H3K4me1 in CD14 monocytes generated from the original experiment or the interpolated values. Each point corresponds to a 200-bp genomic window (Methods). 

**c.** For each cell type, the interpolated and original profiles are highly correlated and clustered together. 

**d.** scChromHMM outputs at the PAX5 locus. Top: Pseudobulk profiles for six chromatin marks in three cell types. Yellow bar highlights a 200-bp genomic window near the TSS. Bottom: scChromHMM posterior probabilities representing the annotation for the highlighted window in each cell. The region is uniformly annotated with a promoter state in B cells, where PAX5 is transcriptionally active, and as a repressive state in other cell types. 

**e.** Metaplots exhibiting the enrichment of chromatin accessibility and histone modifications at functional regions identified by ChromHMM (left) and scChromHMM (right) in CD14 monocytes. K, kilobase.

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a form of data 'denoising' by averaging histone modification signals across cells in similar biological states and therefore alleviates the sparsity limitations associated with scUT&Tag measurements. We emphasize that the acquisition of protein measurements is essential for this strategy, as the protein modality enables the accurate identification of anchors across diverse datasets.

Having acquired interpolated chromatin profiles for six histone modifications at single-cell resolution, we next aimed to annotate the chromatin state of each 200-bp genomic window in each cell. To achieve this, we ran the forward−backward algorithm \(^1\)\(^2\)\(^3\)\(^4\) individually on each cell (Methods) using our interpolated profiles for six histone modifications and our previously calculated set of emission and transition probabilities as input. We note that data binarization is a requirement for this procedure and found that the frequency of our interpolated profiles across genomic windows exhibited clear evidence of bimodality, facilitating this process (Supplementary Fig. 6a). The output of scChromHMM represents, for each genomic window in each cell, the posterior probability distribution across 12 chromatin states. For example, in Fig. 3d, we visualize the posterior probabilities (promoter) of a genomic window for PAX5 expression in different cell types. 

Regions classified by scChromHMM as active promoters were enriched near the TSS of actively transcribed genes, active...
enhancer regions were distal to the TSS but enriched for accessible chromatin, and heterochromatic regions largely overlapped (76.3%) with a set of repetitive elements annotated by RepeatMasker
(Fig. 3e and Methods). We also found that putatively functional regions identified by scChromHMM exhibited increased accuracy and robustness compared to regions identified by ChromHMM run on pseudobulk profiles. Regions classified by scChromHMM consistently exhibited stronger enrichment for key histone
modifications (Fig. 3e) and RNA polymerase II (Supplementary Fig. 6b) compared to active promoters identified by ChromHMM. We conclude that scChromHMM enables chromatin state predictions at single-cell resolution and also improves the accuracy of state prediction.

Heterogeneity in repressive chromatin suggests regulatory priming. We used the output of scChromHMM to explore the dynamics of chromatin state changes across a trajectory of CD8 T cell maturation from naive to effector states. For example, we identified 14,585 genomic windows that acquired or lost a repressive chromatin state (Methods), indicating that extensive remodeling of repressive chromatin is associated with this cellular process, particularly at the sharp transition from naive to memory cells (Fig. 4a). To explore motifs that were associated with these transitions, we modified the chromVAR algorithm to identify motifs whose presence was correlated with an increased posterior probability across the learned chromatin states (Methods).

Our analyses revealed that genomic windows containing the motif bound by the transcription factor TBX21 (T-bet), which is a crucial regulator of T cell responses, exhibited highly dynamic chromatin states during T cell maturation (Fig. 4b). In particular, as cells transition from naive to memory and effector states, genomic windows containing TBX21 motifs acquired an increased probability of entering an active promoter or enhancer state. Conversely, genomic windows containing motifs bound by the critical T cell regulator LEF1 tended to exist in active chromatin states at the onset of the trajectory, but the same windows transitioned to acquire a repressive state as cells progressed through the trajectory (Fig. 4b). More generally, when aggregating cells into granular subgroups, we observed substantial cellular heterogeneity and cell type specificity for motif activity scores computed based on scChromHMM-determined probabilities (Supplementary Fig. 6c). This analysis nominated a suite of regulators for each cell type that were also differentially expressed at the transcriptional level (Supplementary Fig. 6c). Taken together, our results demonstrate the potential for integrated analyses of scCUT&Tag data to identify DNA sequence motifs that correlate with diverse chromatin states.

We next asked if cellular heterogeneity in repressive chromatin state was fully reflected in the cellular transcriptome. We first considered the scChromHMM-derived probabilities for the repressive state at genomic windows overlapping TSSs. We found that granular cellular states could be separated based on unsupervised clustering of these measurements (Methods), indicating that cellular identity is encoded not only in transcriptional output or immunophenotypes but also in genome-wide profiles of repressive chromatin state (Fig. 4c). To our surprise, we found that granular cellular identity remained encoded in repressive chromatin probabilities at TSSs, even after excluding the 3,000 most variable genes in the transcriptome (Fig. 4d). This suggested that even for genes whose expression did not vary across cell types, there may be substantial heterogeneity in chromatin state.

We therefore considered the set of loci where we observed cell-type-specific changes in repressive chromatin state (Methods).
and calculated their overlap with transcriptional changes as well. For example, we observed 1,597 TSS loci whose posterior probability (repressive chromatin state) and H3K27me3 signal was enriched or depleted in CD14 monocytes. Within the associated genes for these loci, we detected monocyt-specific transcriptional shifts in only 257 (16.1%) (Fig. 4e,f). When we did observe both transcriptional and chromatin-based shifts, they were highly concordant (i.e., the acquisition of a repressive state was associated with a decrease in gene expression). However, when we identified heterogeneity in repressive chromatin states in the absence of transcriptional variation, gene expression was either absent or at very low levels in all cell types (Fig. 4e). When considering their pseudo-bulk expression, the median expression of the set of 1,340 genes in PBMCs was below 0.5 transcripts per million. These genes are not informative when determining cellular state from scRNA-seq data, yet their repressive chromatin landscape exhibits clear patterns of cell type specificity (Fig. 4f).

We note that some genes exhibiting this pattern may in fact be differentially expressed across cell types but cannot be accurately quantified by scRNA-seq. To address this, we analyzed a publicly available transcriptome atlas dataset44, with high-quality bulk total RNA-seq profiles for sorted PBMC subsets. Of the 1,340 genes for which we detected monocyt-specific changes in repressive chromatin, but not transcription, 1,081 (81%) failed to show evidence of differential expression in bulk data as well (DESeq2 adjusted P > 0.01), demonstrating that our findings are not driven by artifacts driven by scRNA-seq.

Gene ontology analysis5 of these gene sets was enriched for DNA-binding transcription factors and transmembrane and channel proteins whose activity may regulate the activation of downstream signaling pathways and immune responses (Supplementary Fig. 6d). In a meta-analysis of these TSSs, monocytes exhibited enrichment for H3K27me3, weak enrichment of H3K4me3 and H3K4me2 and the absence of H3K27ac (Supplementary Fig. 6e). We therefore hypothesize that these chromatin shifts may represent a form of regulatory ‘poising’ that is not yet reflected in the current transcriptional output of each cell but may be relevant to its future behavior or potential. Our findings are consistent with previous studies of the epigenetic landscape in human T cells, which have shown that the promoters of many silent genes were enriched for active histone modifications and could be rapidly induced in response to environmental stimuli56. Moreover, studies in differentiating T cells have found that distinct and lineage-specific chromatin states can be present even for nonexpressed genes57,58.

Projecting scCUT&Tag datasets onto a multimodal atlas. Our analyses demonstrated the utility of projects data from diverse molecular modalities into a harmonized manifold, creating a multimodal atlas that spans CITE-seq, ASAP-seq and scCUT&Tag-pro experiments. However, it is not always possible to measure cell surface protein levels when performing scCUT&Tag, particularly when performing analysis of single nuclei. We therefore considered how to project unimodal scCUT&Tag profiles into our reference dataset.

We have recently released a framework, Azimuth59, which addresses a similar problem of projecting scRNA-seq datasets onto a CITE-seq-defined human PBMC reference. In Azimuth, we use a semi-supervised dimensionality reduction approach, supervised principal component analysis (PCA)56, which identifies the best transcriptomically defined vectors that separate CITE-seq defined cell types. We pursued a similar strategy to map a recently published H3K27me3 scCUT&Tag dataset of human PBMC nuclei60 onto our multimodal reference atlas. Using our scCUT&Tag-pro dataset as a reference, we calculated a modified LSI (Methods), which transforms the histone modification profiles into a low-dimensional space that retains separation of our multimodally defined cell types. We used this transformation to identify anchors and ‘map’ the query dataset into our reference dataset (Methods).

We observed that by performing reference mapping, we could substantially improve the interpretation of the query dataset, particularly the ability to resolve granular cell types. For example, unsupervised clustering of the query dataset49 was consistent with the previously published analysis and revealed broad clusters of immune subsets, including monocyte, B cell and T cell/NK cell subgroups (Fig. 5a). After reference mapping, monocytes could be segregated into CD14 and CD16+ subsets, T cell/NK cell subclusters could be further resolved into CD4 T, CD8 T and NK cell groups with additional heterogeneity for naive and effector states and B cells separated into different developmental stages (Fig. 5b). We found that we could classify (prediction score >0.5) 88% of cells at level 1 resolution and 73% of cells at level 2 resolution. We find high concordance when comparing patterns of cell-type-specific H3K27me3 binding compared in both the reference and query datasets (Fig. 5c–e), verifying the accuracy of our predictions. We note that although we transfer discrete annotations in this example, it is also possible to transfer continuous data, which would enable the prediction of one histone modification based on the measurement of another. Therefore, we propose that our multimodal atlas represents a broad scaffold for the circulating human immune system that can be used to map datasets from a wide variety of cutting-edge single-cell technologies.

Discussion

One of the key goals of multomic single-cell studies is to explore the relationships across different molecular modalities and how changes in one modality affect variation in another. Here, we pursue an integrative analysis where nine different molecular modalities are each measured in separate experiments. We subsequently integrate these data and derive what we colloquially refer to as single-cell ‘megaomic’ profiles, each representing an individual cell containing measured or interpolated measurements for many modalities. In addition, we introduce scChromHMM, which enables the exploration of heterogeneity in chromatin state across both discrete cell types and continuous trajectories.

Substantial recent progress has been made in simultaneous profiling of multiple modalities in single cells22,23,31,32, but currently, it is not feasible to simultaneously profile the genome-wide binding patterns of six different histone modifications in the same cell, particularly when there is a partial overlap in their localization. Additionally, future extensions of CUT&Tag enabling simultaneous measurements of RNA and protein levels are likely to result in decreased per-modality data quality, as has been previously described36,29. Our approach therefore represents a feasible and broadly applicable alternative, and we demonstrate that our megaomic profiles are highly quantitative, sensitive and robust. However, interpolated modality predictions cannot capture stochastic technical or biological variation or be used to detect associations between multiple histone modifications within the same cell.

The successful integration of CITE-seq, ASAP-seq and scCUT&Tag-pro datasets originates from a shared panel of cell surface protein levels that are collected in each experiment. We emphasize that cell surface protein levels represent one of multiple potential modalities that can be used for megomics integration. For example, two recent pioneering approaches (Paired-Tag22 and CoTECH23) introduced combinatorial indexing-based approaches for simultaneous scCUT&Tag and scRNA-seq in single cells, enabling the integration of additional multomic technologies (i.e., Paired-Seq) via transcriptomic measurements. Our scCUT&Tag-pro technology represents a complementary solution that is compatible with the 10x Genomics Chromium system, and will be particularly valuable for profiling immune cell types that are well defined by their surface protein landscape.
We found substantial heterogeneity in chromatin state even at genes that did not exhibit transcriptional variation. In particular, we observed cell-type-specific repression, as demarcated by the presence of H3K27me3, even for genes that were expressed at similarly low levels across all cell types. Although it is possible that this heterogeneity in repressive chromatin state has minimal functional consequence, cellular variation in chromatin state may also underlie future cell type-specific transcriptional responses. In addition to previous findings in human immune cells, Mellis et al. found that cell-type specificity was encoded not only in steady-state transcriptional output but also in future responses to environmental perturbations. Similarly, Shim et al. found that genes with heterogeneous H3K27me3 landscapes across distinct organ systems were enriched for key developmental, regulatory and morphological functions. We propose that scUTag&Tag-pro represents a sensitive technique for identifying loci exhibiting heterogeneous repressive chromatin within an organ system as well. Future experiments profiling chromatin state in the bone marrow will help to elucidate the developmental dynamics that establish this heterogeneity.

Moving forward, we expect that integrated analyses across multiple modalities will not only help to understand the molecular state of single cells but also reveal new insights on fundamental questions in gene regulation. For example, the activation of gene expression is correlated with changes in chromatin accessibility, the acquisition and loss of histone modifications, the binding of transcription factors and RNA polymerase and the formation of new DNA contacts. However, the regulatory relationships and temporal ordering between these steps remains poorly understood. Motivated by the ability of RNA velocity analysis to use known temporal relationships between unspliced and spliced reads to infer the direction of developmental trajectories, we envision that single-cell multimodal analysis will help to order the coordinated molecular events that collectively establish cellular heterogeneity.

**Online content**

Any methods, additional references, Nature Research reporting summaries, source data, extended data, supplementary information, acknowledgements, peer review information; details of author contributions and competing interests; and statements of data and code availability are available at https://doi.org/10.1038/s41587-022-01250-0.

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Methods

Protocols. A step-by-step online protocol for scCUT&Tag-pro is available on the protocols.io platform (https://www.protocols.io/private/57E45A03D5311ECA43BDA5A8A9FEEAC02).

PBMC acquisition and processing. Cryopreserved healthy donor PBMCs were purchased from AskCells. After thawing into DMEM with 10% FBS, the cells were spun down at 4 °C for 5 min at 400 × g and washed twice with PBS with 2% BSA. After centrifugation, the pellet is resuspended in staining buffer (2% BSA and 0.01% Tween in PBS).

scCUT&Tag antibodies. The antibodies used were H3K4me1 (1:100, Abcam, ab8895), H3K4me2 (1:100, Abcam, ab23236), H3K4me3 (1:100, Abcam, ab212324), H3K27ac (1:100, Abcam, ab177178), H3K27me3 (1:100, Cell Signaling Technology, 9733), H3K36me3 (1:100, Abcam, ab8898), Phospho-Rpb1 CTD (Ser/Thr) (1:50, Cell Signaling, 13546) and guinea pig anti-rabbit (1:100, of our own). Full details are available in Table 2.

scCUT&Tag-pro experimental workflow. Surface protein antibody staining. Five million thawed PBMCs were resuspended in 200 µl staining buffer (2% BSA and 0.01% Tween in PBS) and incubated for 15 min with 10 µl Fc receptor block (BioLegend, TruStain FcX) on ice. The samples were then evenly distributed into five tubes, and 2 µl washing antibody was added to each tube. Cells were then washed three times with washing buffer and resuspended to a final concentration of 1 million PBMCs. The mixture was used for each staining reaction. After cellular lysis, the panel of oligo-conjugated antibodies (one test is sufficient for two million cells) was added to the cells to incubate for 30 min on ice. After staining, cells were washed three times with 1 ml staining buffer and resuspended in 100 µl staining buffer.

Fixation and permeabilization. A total of 1.25 µl 16% methanol-free formaldehyde (Thermo Fisher Scientific, P28906) was added for fixation (final concentration, 0.1%) at room temperature for 5 min. The cross-linking reaction was stopped by addition of 12 µl 1.25M glycine solution. Subsequently, cells were washed twice with PBS. The permeabilization was performed by adding isotypic lysis buffer (20 mM Tris-HCl, pH 7.4, 150 mM NaCl, 5 mM MgCl2, 0.1% NP-40, 0.1% Tween-20, 1% BSA and 1X protease inhibitors) on ice for 7 min. Subsequently, 1 ml cold wash buffer (20 mM HEPES, pH 7.6, 150 mM NaCl, 0.5 mM spermidine and 1X protease inhibitors) was added, and cells were centrifuged at 800 × g for 5 min at 4 °C.

Tagmentation. Permeabilized cells were directly resuspended with 150 µl antibody buffer (20 mM HEPES, pH 7.6, 150 mM NaCl, 2 mM EDTA, 0.5 mM spermine, 1% BSA and 1X protease inhibitors) with primary antibody (e.g., anti-H3K4me1) and incubated overnight on a rotator at 4 °C. The next day, cells were washed once with 150 µl wash buffer and centrifuged for 5 min at 800 × g. After removing the supernatant, the cells were resuspended in 150 µl wash buffer with secondary antibody and incubated for 1 h at room temperature on a rotator. The cells were washed twice with 150 µl wash buffer to remove excess remaining antibodies. The cells were then resuspended in 150 µl high-salt wash buffer (20 mM HEPES, pH 7.6, 300 mM NaCl, 0.5 mM spermidine and 1X protease inhibitors) with 7.5 µl PAG-Tn5 (EpiCypher, 15-1017) and incubated for 1 h on rotator at room temperature. The cells were then washed twice with high-salt wash buffer and resuspended in 100 µl tagmentation buffer (20 mM HEPES, pH 7.6, 300 mM NaCl, 0.5 mM spermidine and 1X protease inhibitors). The samples were placed in a PCR machine and incubated for 1 h at 37 °C. When tagmentation was done, the reaction was stopped by adding 4 µl 0.5X EDTA. Tagmentation steps were performed in 0.2-ml tubes to minimize cell loss.

Single-cell encapsulation, PCR and library construction. After tagmentation, cells were centrifuged for 5 min at 1,000 g, and the supernatant was discarded. Cells were resuspended with 30 µl 1X Diluted Nuclei Buffer (10X Genomics), counted and diluted to a concentration based on the targeted cell number. Seven microliters of oligo-conjugated antibodies (one test is sufficient for two million cells) was added for incubation on ice for 15 min. The cells were then spun down at 4 °C for 5 min at 400 × g.

Sequencing. The final libraries (CUT&Tag library, ADT library and HTO library) were sequenced on NextSeq 550 with the following recipe: i5:16bp, i7: 8bp, read1: 34bp, read2: 34bp.

Reference mapping. We integrated each of our scCUT&Tag-pro datasets with a previously published reference CITE-seq dataset. In previous work, we applied supervised PCA (sPCA) to identify a linear projection of the scRNA-seq measurements in the CITE-seq dataset that maximally retained the information captured in both modalities. This transformation can be applied to new scRNA-seq query datasets to identify anchors with the CITE-seq reference. These anchors were used for annotation and visualization of the query cells. Our framework for query-reference mapping works well for single cell ATAC-seq experiments as well as a part of our Atlas of immune cells, which is accessible at https://www.nih DOWNLOADED FROM nature.com/naturebiotechnology. 1.25D (ADT modality) and 2.30D (scCUT&Tag modality).

Interpolating multiple histone modification profiles in single cells. To perform an integrated analysis of chromatin state at single-cell resolution, we first generated a set of single-cell profiles that comprised interpolated, genome-wide and quantitative measurements of six histone modifications. In previous work, we demonstrated how to use anchors between reference and query datasets to transfer normalized quantitative information (with default parameters) to annotate query cells at multiple levels of resolution and visualize these cells in 2D UMAP. After all datasets were assembled, we used monocle 3 with default parameters to identify an integrated developmental trajectory that orders cells from all experiments.
We applied a similar workflow for this study (schematized in Fig. 2a). First, we considered a CITE-seq dataset1, where each cell contained transcriptomic measurements alongside quantifications for a panel of 228 surface proteins. Although the original dataset contained 161,764 cells, interpolating genome-wide profiles for multiple modalities at this scale created memory and storage constraints, and we therefore selected a random subset of 20,000 cells for downstream analysis. In Supplementary Fig. 5c, we demonstrate that we obtained highly concordant results when repeating the procedure on a second random downsampling of 20,000 cells.

We then found anchors between each scCUT&Tag-pro dataset and the 20,000 CITE-seq cells. Specifically, the ‘anchors’ in this procedure represent pairwise correspondences of cells: a CITE-seq cell that lacks the histone modification profile of interest and an scCUT&Tag-pro cell in a matched biological state, but where the histone modification was directly measured. Anchors were determined using the FindTransferAnchors function, and we used the same protein sPCA transformation described in ‘Reference mapping’. We used these anchors to interpolate (i.e., ‘impute’) the number of fragments for each histone modification, in each 200-bp genomic window, at single-cell resolution (see Stuart et al.1, ‘Feature Imputation’). For example, in each of the 20,000 CITE-seq cells, the interpolated levels of H3K4me1 represent a weighted average of the anchor-forming cells in the H3K4me1 scCUT&Tag-pro. We repeated this procedure for each of the six modifications, as well as the ASAP-seq dataset28.

The model assumes that each 200-bp genomic window (‘genomic interval’) in ChromHMM12. For clarity, we reintroduce the key components of the model here. The model prioritizes anchors that are most similar to the query cell, enabling us to accurately access the accessibility of peaks that share the same DNA sequence motif while carefully controlling for technical biases. Here, we aim to detect associations between transcription factors and chromatin accessibility by examining deviations (i.e., gains/losses) in the accessibility of peaks that share the same DNA sequence motif while carefully controlling for technical biases. Here, we aim to detect associations between transcription factors and the acquisition of distinct chromatin states. We therefore ran the chromVAR algorithm40, but instead of passing single-cell chromatin accessibility profiles as input, we used our single-cell posterior state probabilities. We repeat this process separately for promoter, enhancer and repressor states. Using the promoter state as an example, chromVAR calculates, for each motif and each cell, the difference between the observed promoter posterior probabilities for peaks containing the motif and the expected posterior probability (based on an average across all cells). chromVAR next performs a similar calculation for a matched background peak set to calculate bias-corrected deviation scores (Fig. 1b and Supplementary Fig. 6c).

Assigning chromatin states at single-cell resolution. To integrate information from multiple histone modifications, we used the multivariate HMM introduced in ChromHMM17. For cells, we reintroduce the key components of the model here. The model assumes that each 200-bp genomic window (‘genomic interval’) can be represented based on one of K hidden states, where each state is defined by the combinatorial presence or absence of multiple histone modifications. The full likelihood of the model is:

\[
\mathcal{v}(a, b, p) = \frac{1}{a!} \sum_{a,b,p} a! b! p! \prod_{t \in 1..n} b_t \prod_{m \in 1..M} \prod_{k \in 1..K} p_{m,k}^{a_{m,k}} (1 - p_{m,k})^{1 - a_{m,k}},
\]

where \(v(a, b, p)\) represents the observed data, such that \(a_{m,k}\) is 1 if mark \(m\) is present at genomic interval \(k\) and 0 otherwise. \(p_{m,k}\) represents the probability that mark \(m\) is present at a genomic interval in state \((m, k)\) (‘emission probability’), \(b_t\) represents the probability of the transition from state \(i\) to state \(k\) (‘transition probability’) and \(a_{m,k}\) represents the ‘hidden state’ associated with each at genomic interval \(c_i\). The first step of the procedure is to learn a set of possible chromatin states and their associated emission and transition probabilities. We used ChromHMM’s variant of the Baum–Welch algorithm, as implemented in the LearnModel command, setting the number of states to 12 and the maximum number of iterations to 1,000. As input to ChromHMM17, we provided the histone modification profiles for all cell types, where cell type identity was determined after reference mapping (level 2 resolution). The 12 learned states and associated emission probabilities are shown in Fig. 3a. After learning a set of chromatin states, we next inferred the path of hidden states (hidden state sequence) through each chromosome at single-cell resolution. To do so, we extended the ChromHMM method to calculate the posterior distribution of state assignments for each 200-bp genomic interval in each cell. We refer to the following procedure as scChromHMM:

(1) The input to scChromHMM is the set of interpolated histone modifications in single cells, as described above.

(2) As ChromHMM, we use multiple chromatin marks as an independent set of Bernoulli random variables, we must binarize the interpolated values present in each cell. As shown in Supplementary Fig. 6a, the distribution of abundances for each histone modification exhibited clear bimodality. We assigned bimodality thresholds via manual inspection (red dashed line), which were congruent with the results of Hartigan’s dip test. Binarization thresholds were set independently for each mark but kept constant across single cells. After thresholding, each genomic interval in each cell is represented by the presence or absence of six histone modifications.

(3) We ran the forward–backward algorithm (a dynamic programming algorithm to learn the posterior probabilities of hidden state variables in an HMM) on each cell independently. The output of this procedure is a posterior probability distribution over all 12 states for each genomic interval in each cell.

Identification of scChromHMM and ChromHMM results. In addition to scChromHMM, we also annotated genomic intervals using the standard ChromHMM workflow17. Although this approach does not return state predictions in individual cells, it does return posterior probability distributions for each cell type (level 2 resolution). We therefore compared the predictions from ChromHMM17 with the predictions from scChromHMM after grouping by cell type. In Fig. 3e, we perform a meta-analysis across all regions assigned to promoter, enhancer, repressor and heterochromatic states and explore the level of enrichment for each modification in a 10-kb window centered on these regions. We combined the posterior probabilities for functionally related states (promoters, states 1 and 2; enhancers, states 3 and 4; and repressor, states 9 and 10).

We compare results on CD14 monocytes, as this was the most abundant cell type in our dataset. For ChromHMM17, we selected all regions with >75% probability posterior probability in CD14 monocytes for meta-analysis, and for scChromHMM, we selected all regions where the majority of CD14 monocytes had >75% posterior probability. In Supplementary Fig. 6b, we also compare average polymerase II fragment abundance as measured by scCUT&Tag-pro on promoter regions as identified by either ChromHMM or scChromHMM.

Using chromVAR to identify associations between motifs and functional states. The chromVAR package identifies associations between transcription factors and chromatin accessibility by examining deviations (i.e., gains/losses) in the accessibility of peaks that share the same DNA sequence motif while carefully controlling for technical biases. Here, we aim to detect associations between transcription factors and the acquisition of distinct chromatin states. We therefore ran the chromVAR algorithm, but instead of passing single-cell chromatin accessibility profiles as input, we used our single-cell posterior state probabilities. We run this process separately for promoter, enhancer and repressor states. Using the promoter state as an example, chromVAR calculates, for each motif and each cell, the difference between the observed promoter posterior probabilities for peaks containing the motif and the expected posterior probability (based on an average across all cells). chromVAR next performs a similar calculation for a matched background peak set to calculate bias-corrected deviation scores (Fig. 1b and Supplementary Fig. 6c).

Characterizing regulatory priming based on repressive chromatin state. In Fig. 4, we analyze cells based on their posterior probability (repressive state) at 19,306 TSSs. Based on a previous TSS definition, we considered all genomic intervals that overlapped with a window starting 2,500 bp upstream of the TSS and extending 500 bp downstream. In each cell, we quantified the level of repression at each TSS as the average of the repressive state posterior probability for each of these windows. We generated a repressive score matrix that represents the level of repression at each cell (rows) at each TSS (columns). We applied TF-IDF normalization to this matrix followed by SVD and used singular vectors 2:30 as input to UMAP visualization in Fig. 4c.

In Fig. 4d, we visualize cell–cell correlations based on the repressive score matrix. In the left heatmap, we use all 19,306 TSSs as input to LSI, and use dimensions 2:30 to calculate a correlation matrix. In the right heatmap, we perform the same analysis procedure, but exclude the top 3,000 TSSs that were identified as highly variable based on gene expression levels, as determined by SCTransform. In both heatmaps, cells are grouped based on their predicted annotation, but the order of cells within an individual group is random.

In Fig. 6c, we consider the overlap between heterogeneity in repressive chromatin state and transcription factor binding. Using the MonoPoly command in Seurat (default parameters), which performs a Wilcoxon sum-rank test, we compared CD14 monocytes with all other cells. We performed tests for scChromHMM-derived posterior probabilities (repressive state), H3K27me3 levels...
Comparative analysis to detect differentially expressed genes in the bulk RNA-seq dataset. In Fig. 4e, we show gene loci where we observed gene loci where we observed shifts in repressive chromatin when comparing CD14 monocytes to remaining cell types, but we did not detect transcriptional differences between these cell types in scRNA-seq data. We further validate the lack in transcriptional shifts of the 1,340 loci using bulk RNA-seq data. We quantify bulk RNA-seq-sorted PBMC dataset using salmon 1.4.0 (ref. 50) for four major cell types: CD14 monocytes (three replicates), B cells (two replicates), CD8 T cells (two replicates) and natural killer cells (three replicates). We performed differential expression analysis on the bulk RNA-seq data comparing the TPM estimates of the CD14 monocytes with other cell types using DESeq2 (ref. 50) 1.30.1. Of the 1,340 loci, 1,081 (81%) did not show evidence of differential expression in the bulk RNA-seq dataset (DESeq2 (ref. 50) adjusted P < 0.01). Of the remaining 257 loci that showed transcriptional differences in scRNA-seq data, 74% showed evidence (adjusted P < 0.01) in bulk data as well.

Mapping scCUT&Tag datasets to a multimodal reference. In Seurat v4 (ref. 50), we used scPCA to integrate multimodal (CITE-seq) and unimodal (scRNA-seq) datasets. The scPCA reduction aims to project transcriptomic measurements into a low-dimensional space that most closely resembles the WNN graph. We constructed a WNN graph that identifies, for each cell, a set of neighbor cells in the most similar set of cell types using a weighted combination of RNA and protein modalities. This graph represents a linear kernel, L, that can be used to supervise the dimension reduction process for transcriptomic measurements.

As described in Barshan et al.51, there is a closed form solution to scPCA based on a data matrix X, kernel L and centering matrix H. The projection matrix U is represented by the eigenvectors of matrix XLHLX. We apply the resulting projection matrix (U=data) to new scRNA-seq datasets to identify anchors between query and reference datasets. We can repeat this process using the protein modality to calculate (L=Urna).

Here, we apply a conceptually similar procedure to the integration of multimodal (HJK27me3 scCUT&Tag-pro, from this study) and unimodal datasets (HJK27me3 scCUT&Tag, from Wu et al.51). For unsupervised analysis of the scCUT&Tag dataset, we downloaded fragment files from Gene Expression Omnibus with accession code GSE157910. We filtered cells with less than 250 fragments or more than 5,000 fragments, and we repeated the LSI-based analysis workflow to perform unsupervised analysis (Fig. 5a).

For each scCUT&Tag-pro dataset, the reference mapping procedure described above used the protein measurements to map each cell into a reference-defined space. We constructed a k-nearest neighbor graph (k = 30) in this space. This defined a kernel matrix L, where Lij = 1 if cells i, j are neighbors and 0 otherwise. Our goal was to compute a SVD of the matrix XL, where X is the TF-IDF normalized peak barcode matrix representing the quantified scCUT&Tag profiles in the multimodal experiment. Unlike scRNA data, the large number of features (peaks) in the scCUT&Tag profiles made it computationally expensive to perform SVD. We circumvented this problem by performing eigendecomposition of the matrix L^T(X^TX)L, where the dimensionality is determined by the number of cells but returns the same result. The result was a modified LSI that represents a low-dimensional version of the chromatin profiles but has been supervised by the protein measurements. We used the FindTransfer/Anchors (reduction = ‘lsiproject’, reference.reduction = ‘LSI’, dmins = 1.30, kanchor = 20) to identify anchors between our scCUT&Tag-pro dataset and the unimodal query. We used these anchors to annotate cell types in the query dataset and assign prediction scores.

Lastly, we also used these anchors to interpolate protein values for 173 surface proteins in the scCUT&Tag query dataset. We then applied the previously computed U=data transformation to map these cells onto the original CITE-seq reference52, and we filtered cells with prediction score <0.5. We performed this step for visualization purposes only, as it allowed us to view the query scCUT&Tag dataset in the same UMAP visualization as Fig. 2b, which represents all datasets in this study (Fig. 2b).
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Our web collection on statistics for biologists contains articles on many of the points above.

**Software and code**

Policy information about availability of computer code

**Data collection**

The libraries were sequenced on Nextseq550 and demultiplexed using bcftools with the following parameters:

```
bam2fastq --use-bases-mask=Y34,18,Y16,Y34 \
   --create-fastaq-for-index-reads \ 
   --min-trimmed-read-length=8 \ 
   --mask-short-adapter-reads=8 \ 
   --barcode-mismatches=1 \ 
   --ignore-missing-positions \ 
   --ignore-missing-controls \ 
   --ignore-missing-filter \ 
   --ignore-missing-bcq \ 
   -r 6 -w 6 \ 
   -R $fastq \ 
   --output-dir=$output \ 
   --output-dir=$output \ 
   --sample-sheet=$sheet
```

**Data analysis**

Custom code: https://github.com/satijalab/scchromhmm

Softwares used in the study:
- R packages:
  - Seurat v4
  - Signac v1.3.0
  - chromVAR v1.14.0
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Data generated for this manuscript are deposited in the Gene Expression Omnibus with accession code: GSE195725. The processed datasets are available as open-access downloads at: https://zenodo.org/record/5504061.

Publicly available datasets used in Figure 2, 3, 4 and Supplementary Figure 3:
PBM C ASAP-seq: https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSM4732109
PBM C CITE-seq: https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE164378

Publicly available datasets used in Figure 1D:
PBM C ASAP-seq: https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSM4732123

Publicly available datasets used in Figure 1G:
CD4 T cells H3K4me1: https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSM1220567
CD8 T cells H3K4me1: https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSM1220569
B cells H3K4me1: https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSM1027296
Monocytes H3K4me1: https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSM110793

Publicly available datasets used in Supplementary Figure 1C and Figure 5:
PBM C K27me3: https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSM5034344
PBM C K27ac: https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSM5034344

Publicly available datasets used in DE analysis with the bulk total RNA-seq:
http://ftp.sph.emory.edu/mRNA_CS001/DeSeq.R
https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE138734

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Life sciences study design

All studies must disclose on these points even when the disclosure is negative.

Sample size
We profiled 9,575, 10,386, 8,304, and 8,232 cells for H3K4me2, H3K4me3, H3K9me3 and H3K27me3 histone modification marks respectively on a human PBM C sample, while also generating 17,770 and 15,609 cells each with two replicates for the H3K4me1 and H3K27ac histone modification marks.

Data exclusions
Cells of low data quality were excluded from the analysis (Methods). We filter the fragment file to keep the cellular barcodes (C3k) that were present in the filtered ADT and HTO libraries (10 protein and 10 hashing antibody counts), and also contained at least 100 fragments. Cells that pass these thresholds are used as input to the HTOdemux() function in Seurat for demultiplexing and doublet removal.

Replication
Two replicates for each of the H3K4me1 and H3K27ac histone modification marks were generated and the global profiles of each histone mark are reproducible (Supplementary Figure).

Randomization
Randomization is not relevant for this study because there was a single wild type experimental group.

Blinding
Blinding was not relevant for this study because the objective was not to compare two conditions and the data was generated from a single wild type condition.
Reporting for specific materials, systems and methods

We require information from authors about some types of materials, experimental systems and methods used in many studies. Here, indicate whether each material, system or method listed is relevant to your study. If you are not sure if a list item applies to your research, read the appropriate section before selecting a response.

| Materials & experimental systems | Methods |
|---------------------------------|---------|
| n/a | n/a |
| ☐ | ☐ |
| ☒ | ☒ |
| ☐ | ☐ |
| ☒ | ☐ |
| ☐ | ☐ |
| ☒ | ☒ |
| ☐ | ☒ |
| ☒ | ☒ |

Antibodies

Antibodies used

The antibodies used were H3K4me1 (1:100, Abcam, ab8895), H3K4me2 (1:100, Abcam, ab32356), H3K4me3 (1:100, Abcam, ab213224), H3K27ac (1:100, Abcam, ab177178), H3K27me3 (1:100, Cell Signaling Technology, 9733), H3K9me3 (1:100, Abcam, ab8898), Phospho-Rb1 CTD (Ser2/Ser5) (1:50, Cell Signaling, 13546) and guinea pig anti-rabbit (1:100, Novus Biologicals, NB1-72768). TotalSeq-A conjugated antibodies and panels were obtained from BioLegend (399907, see Supplementary Table 2 for a list of antibodies, clones and barcodes). Fragment Goat Anti-Mouse IgG (Jackson ImmunoResearch, 115-007-003)

Validation

All antibodies are commercially available and validated by the vendor.