Decoding the spatial chromatin organization and dynamic epigenetic landscapes of macrophage cells during differentiation and immune activation

The distinct and dynamic epigenetic codes of different cell types function as blueprints for precise spatial and temporal gene transcription in different developmental stages in response to different stimuli, such as immune cells during differentiation and immunological responses. DNA epigenetic codes are mainly embedded in various types of DNA and histone protein modifications, chromatin accessibility, and in the sophisticated organization of the three-dimensional (3D) genome. Epigenetic modifications in enhancers, which are often elegantly looped with target promoters in a topologically associating domain (TAD), can reprogram the transcription of cell-type-specific genes. While it was historically believed that TADs are conserved across cell types, emerging evidence suggests that TADs undergo dynamic changes during differentiation, especially at single-cell level. However, the mechanisms by which the four-dimensional (4D; 3D plus time dynamics) genome architecture and the integrated epigenetic codes orchestrate transcriptional programs during diverse differentiation and physiological processes, especially in immunological responses, are poorly characterized.

Macrophages are differentiated from monocytes and play a crucial role in the immunological defense against invading pathogens. During differentiation, the staining of the monocytes nucleus, which is originally a rounded shape, dynamically changes to a kidney bean shape or U shape, implying a dynamic 3D chromatin structure. The previous study has also demonstrated global changes in chromatin looping events during macrophage development. Upon infection, macrophages can be polarized into different cell subtypes, such as M1 and M2 macrophages, with distinct functional and phenotypic properties. M1 macrophages can be activated by lipopolysaccharide (LPS).
Embryonic stem cells and cranial neural crest cells have been instrumental in regulating gene expression. For instance, the simultaneous pre-polarization of embryonic stem cells and cranial neural crest cells has demonstrated that histone methylation and demethylation are instrumental in regulating the expression of cytokines, chemokines, and transcription factors, and subsequently modulating macrophage susceptibility loci.

Tuberculosis (TB) is a global infectious health threat even more lethal than HIV/AIDS, leading to approximately 1.3 million deaths and 10.4 million new cases worldwide annually. Upon Mycobacterium tuberculosis (M.tb) invasion into the lung, pulmonary monocytes differentiate into pulmonary macrophages and are activated for immune defense against M.tb infection. Extensive genome-wide association studies (GWAS) have identified many mycobacterial disease susceptibility loci, of which the majority are noncoding regulatory elements. However, it remains unknown which genes are the distal targets of these noncoding regulatory loci and how the SNPs influence the 3D genome.

In this work, we use the plastic THP-1 monocytes and the differentiated macrophages as a model system and delineate the comprehensive spatial chromatin organization and dynamic epigenetic landscapes of macrophages during differentiation and infection with M.tb. Furthermore, we propose a concept of TAD “Degree of Disorder” to measure the entropy of chromatin architecture inside immune-related TADs, which is correlated with chromatin accessibility, gene expression, and co-regulation during differentiation and activation. The integrated GWAS, Hi-C, eQTL, ChIP-Seq, and ATAC-Seq analysis identify the long-range target genes of mycobacterial disease-susceptible SNPs and identify LRRK2 as a potential drug target, whose inhibitor AdoCbl has an anti-tuberculosis effect both in vitro and in vivo. To verify the function of rs1873613, we introduce a single base pair mutation in THP-1, and verify that rs1873613 can enhance the enhancer activity, and thereby upregulate the expression of the LRRK2 gene.

### Results

#### Morphology, transcriptome, and epigenetic dynamics during macrophage differentiation and activation upon M.tb infection

To investigate the spatial chromatin organization and epigenetic dynamics of macrophages during differentiation and immune activation, we used THP-1 cells, a highly plastic human monocytic cell line that can be differentiated into macrophage-like cells and activated by infection, as a cell model. In our study, we named the THP-1 cells before differentiation, and after differentiation, and activated by M.tb H37Rv as Thp1-mono, Thp1-macro, and Thp1-M.tb, respectively. As shown in Fig. 1a, b, the nuclei of differentiated cells were transformed from ellipsoid to kidney bean shape or U shape upon phorbol 12-myristate-13-acetate (PMA) treatment, implying a possible 3D genome conformational change during this process. The distinct morphology alternation of this cell line during different lineages might be underlying a rapid reprogramming of gene expression profile and epigenetic codes during differentiation and activation.

Thus, we analyzed the transcriptomes of Thp1-mono, Thp1-macro, and Thp1-M.tb (Fig. 1c, d and Supplementary Fig. 1a–c), and identified the differentially expressed genes (Supplementary Data 1, 2). During differentiation from monocytes to macrophages, the expression levels of a group of genes related to development and differentiation, such as HOX, BCL11A, MYC, and NRG1, were significantly altered (Fig. 1c). After infection, the expression levels of immune-related chemokine and transcription factors, such as NFKB1, GBP1, CCL2, and IFIT2, were significantly increased (Supplementary Data 2). In addition, M1 macrophage marker genes such as CD80, CCR7, INHBA, and TNF-α were significantly upregulated (Fig. 1d and Supplementary Data 2), indicating that macrophages are activated and transformed to the M1 phenotype. Interestingly, the programmed death ligand 1 (PD-L1) gene, which can counteract activated T-cells, was dramatically upregulated after M.tb infection (Fig. 1d).

To delineate the dynamic epigenetic atlas of this cell during differentiation and activation, we systematically investigated the changes in chromatin states by the assay for transposase-accessible chromatin with sequencing (ATAC-Seq) and comprehensive histone chromatin immunoprecipitation and sequencing (ChIP-Seq). The modifications investigated included H3K27me3, H3K9me3, H3K4me3, H3K27ac, and H3K4me1. By combining these epigenetic modification data, we classified the chromatin states of Thp1-mono, Thp1-macro, and Thp1-M.tb cells into a 15-state model comprising 8 active states and 7 repressed states via the ChromHMM method (Fig. 1e). As shown in Fig. 1e, the chromatin regions enriched with H3K4me3 and H3K27ac showed relatively higher gene expression levels than unenriched regions. While the overall chromatin states of the whole genome were not dramatically altered, we observed dynamic changes in chromatin states in several chromosome regions, such as bivalent and genic enhancers (Fig. 1e).

To further investigate the dynamics of the enhancer and promoter epigenetic states, we comprehensively combined the data for H3K4me1, H3K4me3, H3K27ac, and H3K4me1 modification enrichment with ATAC-Seq peaks and defined the states of enhancers and promoters according to methods described in the previous studies. Supplementary Fig. 1d, e illustrates primed enhancers, poised enhancers, active enhancers, repressed promoters, bivalent promoters, and active promoters. The genes marked by active promoters had the highest expression levels in the RNA-Seq data, whereas those marked by repressed promoters had the lowest expression levels (Supplementary Fig. 1f). These results support the integrity of our histone modification and ATAC-Seq data, as well as our analysis of promoter and enhancer states.

Next, we analyzed the dynamics of enhancer and promoter states across Thp1-mono, Thp1-macro, and Thp1-M.tb cells during differentiation and infection (Fig. 1f and Supplementary Fig. 1g and Supplementary Data 3). Our data demonstrated that a dynamic transition in the promoter epigenetic state, especially the transition from a repressive state to active state and vice versa, could be associated with the gene expression level (Supplementary Fig. 1h). Upon M.tb infection, 424 promoters changed from the repressive state to the active state (Fig. 1f and Supplementary Data 3). Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analysis showed that the genes harboring these promoters were enriched in innate immune pathways such as ligand-dependent caspase activation (24.0%), NOTCH signaling (10.7%), and NF-kappa B signaling (9.3%) (Fig. 1g). These results suggest that M.tb infection can systematically turn on immune-defense gene expression by reprogramming the epigenetic state of promoters and enhancers and switching macrophages to the active M1 state. For example, the promoter region of TNFSF10, a typical death ligand involved in immune surveillance, was much more accessible and enriched with H3K4me3 modifications after M.tb infection. Accordingly, its transcription was also dramatically increased (Fig. 1h).

https://doi.org/10.1038/s41467-022-33558-5
Dynamic chromatin architectures of THP-1 cells during differentiation and immunological response

As the nuclei of THP-1 cells were transformed from ellipsoid to kidney-shaped after differentiation and activation, the 3D genome conformation might undergo a transformation during these processes. To test this hypothesis, we generated high-resolution (~5 Kb) genome-wide chromatin interaction maps using the in situ digestion-ligation-only (DLO) Hi-C method36,37, with ~1.5 billion sequencing reads for each library (Supplementary Table 1). Figure 2a shows a high-quality Hi-C heatmap with low noise, clearly displaying the TAD domains. With this high-resolution genome contact matrix, we identified the dynamics of the chromatin structure during macrophage differentiation and activation. For example, several strong chromatin interactions appeared around the MYC gene in the Hi-C contact matrix after differentiation, suggesting that reprogramming of the chromatin configuration and the epigenetic code in the MYC regulatory repressor region38 are involved in the regulation of MYC gene transcription (Fig. 2a and Supplementary Fig. 2a, b).

During THP-1 differentiation, 13.0% of TADs shifted at least one of their boundaries by >80 Kb, 13.8% fused into larger TADs, and 4.0% divided into small TADs. After M.tb infection, 1.4% fused into larger TADs, 16.2% separated into small TADs, and 10.0% shifted at least one of their boundaries (Fig. 2b). For example, before M.tb infection, both the GLS and STAT1 genes were located in the same TAD, while after immune activation, the STAT1 gene was relocated into a newly emerged independent TAD. The ATAC-Seq data indicated that the boundary region of this new TAD was significantly opened upon M.tb infection, implying that some regulatory proteins might bind in this region and thus possibly activate STAT1 expression (Fig. 2c).
KEGG analysis results demonstrated that the genes within these altered TAD boundaries during differentiation and activation were enriched in metabolic pathways and innate immune system pathways, respectively (Supplementary Fig. 2c, d). Collectively, these data suggested that the histone modification profile was remodeled at the TAD level during differentiation and activation, which might facilitate the regulation of immunological defense-related gene expression.

TAD “degree of disorder” of THP-1 cells during differentiation and immunological response

While most TAD boundaries remained intact (69.2%-72.4%), we found a portion of the chromatin interaction spot pattern inside TADs, especially the immune-related TADs (The TAD with immune genes located) were significantly altered, such as CC cytokine gene cluster (Fig. 2d) and HERC gene cluster (Supplementary Fig. 2e). Such highly organized interaction spots and randomly distributed spots might
represent different “degree of disorder” (DoD) of the chromatin architecture in the TADs (Fig. 2e). We developed an algorithm to quantify the DoD, as shown in Fig. 2f (more details are shown in Supplementary Fig. 2f–h and Methods). Briefly, we retained all the significant contact spots in the Hi-C matrix to filter the stochastic genome loci with random chromatin interactions. As the distance between these spots might reflect the similarity of the chromatin folding patterns, the overall mean distance between the spots was then calculated to represent the whole DoD value in the TAD (Fig. 2f and Supplementary Fig. 2f–h). As shown in Supplementary Fig. 2i, the DoD could indeed reflect highly organized chromatin folding patterns.

Interestingly, the average gene expression levels within TADs were negatively correlated with their DoDs (Fig. 2g). Consistent with this result, TADs structure has been demonstrated to be related to gene transcriptional regulation\(^ {29}\). In addition, we found that the enhancer and active chromatin marks, such as H3K4me1, H3K27ac, and H3K4me3, were more enriched in the TADs with low DoD (Fig. 2h). In contrast, TADs with high DoD contains more transcription repression signals and heterochromatin signals (Supplementary Fig. 3a). Consistently, the A compartments, representing the transcriptionally active regions, have lower DoD than the B compartments (Fig. 2i).

Our data implied that the organization of TADs with low DoDs was more sophisticated than that of the TADs with higher DoDs. This characteristic might be mediated by the elegant cooperation with greater numbers of transcriptional regulatory factors within TADs, which may explain why the genes within the TADs with lower DoDs tend to be highly transcribed. Thus, it would be expected that the ATAC peaks in the TADs with lower DoDs should be more enriched than that of the higher ones. As shown in Supplementary Fig. 3b, the TAD DoDs was highly negatively correlated with ATAC signals within the TADs, suggesting the TAD DoD is likely associated with chromatin accessibility and the subsequent binding of transcriptional regulatory proteins.

In this scenario, the genes in highly organized TADs may be more likely to be synchronously co-regulated due to cooperation within the same transcriptional regulatory complex. To test this hypothesis, we defined the average gene co-regulation score (CRS, which means the genes synchronously upregulated or downregulated) (Supplementary Fig. 3c and Methods) within a TAD during differentiation and activation. Notably, we found that TADs with lower DoDs had overall higher CRSs (Supplementary Fig. 3d), supporting that highly organized chromatin tends to be more synchronously (or less randomly) co-regulated. As the degree of disorder is the indication of entropy, here we hypothesized that the TAD DoD might be associated with the entropy of the chromatin organization in a TAD: highly organized TADs with lower “TAD entropy” levels have overall higher gene transcription levels and more concerted co-regulation, whereas disordered TADs with higher TAD entropy levels generally exhibit lower transcription and more random co-regulation (Supplementary Fig. 3e).

Next, we analyzed the DoD dynamics of the TADs without boundary alterations during differentiation and activation. As shown in Supplementary Fig. 3f, 24.2% of TAD DoDs decreased, and 3.4% increased during cell differentiation, whereas 12.0% decreased and 5.4% increased during M.tb infection. Among the TADs with dynamic DoD, the overall DoD were gradually decreased when monocyte cell differentiated to macrophage cell and then to M1 cell type, which is in line with the plasticity of the cells (Supplementary Fig. 3g). Of note, our data demonstrated that the dynamic DoD, which represents the change of the chromatin organization inside the TAD, has a more profound influence on gene expression within the TAD than other types of boundary dynamics, including TAD shift, fusion, and division (Supplementary Fig. 3h). Importantly, the DoDs of immune function-related TADs were altered more extensively than those of non-immune function-related TADs upon M.tb infection (Fig. 2i).

Moreover, we observed that the number of upregulated genes was larger than that of the downregulated genes when DoD was decreased, while this phenomenon did not appear during DoD increasing (Fig. 2k). For example, the TAD DoDs of CC chemokine ligand family genes were significantly decreased upon M.tb infection. The expression of CCL1, CCL2, CCL7, and CCL8 was also synchronously upregulated (Fig. 2d). Consistent with our hypothesis, the chromatin in this TAD became more accessible after infection, which was in favor of the binding of transcriptional regulatory proteins such as NF-κB and API (Fig. 2d, marked by gray boxes). Together, the alteration of TAD DoDs was underlined the reorganization of chromatin physical architecture, especially in the immune function-related TADs, and could facilitate the coordinated transcription of anti-infection genes.

**Similar chromatin folding pattern in low DoD region in individual cells**

Since the bulk cell Hi-C contact matrix in the low DoD TADs is more orderly (Fig. 3d and Supplementary Fig. 2e), we speculated individual cell’s low DoD region tend to have the same or similar folding pattern and interact with a specific transcription complex, thereby forming aggregated interaction hotspots (Fig. 3a, b). To further investigate local chromatin architecture and TAD DoD at the single-cell level, we developed a single-cell-indexed DLO Hi-C (sciDLO Hi-C) to capture the chromatin conformation of individual cells based on DLO Hi-C\(^ {14,50}\) and two rounds of molecular barcoding to capture the chromatin conformation of individual cells (Fig. 3c). The advantage of this method is that only the 80 bp DNA fragments containing the proximity ligation junction were retained, which can greatly reduce the sequencing noise caused by multiple displacement amplification (Supplementary Fig. 4a–c) (see more detailed in Materials and Methods). By comparing with other single-cell Hi-C methods\(^ {39,40,41}\), we demonstrated that sciDLO Hi-C datasets contain the highest proportion of proximity ligation junction reads (Supplementary Fig. 4b).

By sciDLO Hi-C, we obtained 3D genome data of 409 Thp1-mono cells, 424 Thp1-macro cells, and 510 Thp1-M.tb cells (Supplementary Table 2). The territories between different chromosomes can be clearly distinguished based on the simulated 3D genome structure of the individual cells (Supplementary Fig. 4d, e). To investigate the heterogeneity of these three types of cells, we employed scHiCTools\(^ {12}\) to classify all the individual cells based on their 3D genome structures. As shown in Fig. 3d, three distinct clusters of cells were identified, representing Thp1-mono, Thp1-macro, and Thp1-M.tb, respectively. Among these, the Thp1-mono has obvious boundaries between the other two types of cells, whereas Thp1-macro and Thp1-M.tb are partially overlapped. This data reflected that PMA treatment could uniformity reprogram monocyte to a distinct cell-type macrophage, whereas macrophage activation caused M.tb infection was much more heterogeneous. Notably, compared to Thp1-macro and Thp1-mono cells, Thp1-M.tb were enriched with significantly more chromatin contacts around the immune genes (Fig. 3e). For example, the simulated chromatin structures of individual cells showed that the chromatin interaction between immune-related genes NOD2 and BRD7 were more contacted in Thp1-M.tb cells (Fig. 3f, g).

To explore the order and stochasticity of the genome organization of individual cells in immune-related TADs, we further analyzed chromatin contact patterns at single-cell level. We observed that while the individual chromatin contacts displayed a certain extent of heterogeneity, the chromatin structures in low DoD regions were uniformly folded. Take the innate immune-related STAT1 gene locus as an example (Fig. 3h–m). Upon infection, the expression of
STAT1 gene was highly upregulated, and the DoD value of STAT1 TAD was decreased (Fig. 3h, i). We further compared the STAT1 gene locus chromatin contact pattern between the Thp1-macro and Thp1-M.tb with single-cell Hi-C data (Fig. 3j–m). As shown in Fig. 3j, k, compared to the random chromatin contact pattern between STAT1 promoter and the potential enhancer in Thp1-macro (Fig. 3j and Supplementary Fig. 4f), the chromatin contacts of Thp1-M.tb in this region with relatively lower DoD values is much more consistent (Fig. 3k, m and Supplementary Fig. 4g). This data suggested that in the low DoD TADs, the individual cells tend to have similar chromatin contact pattern between different cis-regulatory elements, likely mediated by certain transcriptional regulatory proteins. Thus, they have an overall
higher gene expression level, compared with the stochastically organized TADs.

Remodeling of the chromatin configuration around GBP family genes orchestrates their coexpression upon M.tb infection

To further investigate local TAD DoD in detail, we explored the genome sites with dynamic DoD, such as the TAD with the guanylate-binding protein (GBP) gene family. The DoD value of this TAD was significantly decreased upon M.tb infection in the bulk cell DLO Hi-C data (Fig. 4a). The single-cell chromatin interaction matrix in this GBP region was merged as shown in Fig. 4b, in which each color represented the chromatin contact from the same individual cells. We calculated the DoD based on the merged chromatin interaction matrix from scDLO Hi-C data and found that the DoD was decreased after M.tb infection, which is consistent with the results from bulk cell Hi-C data.

By virtue of the merged single-cell contact matrix, we found that the chromatin contacts fluctuate considerably among individual cells, suggesting heterogeneous genome organizational patterns at the single-cell level. However, upon infection, this TAD has smaller DoD and more intrinsically organized local chromatin interaction patterns in comparison to the random pattern in the Thp1-macro cells with high DoD (Fig. 4b). For example, a series of genome loci in this TAD were sequentially interacted with GBP5 respectively among individual Thp1-M.tb cells, whereas the chromatin contact pattern of this location in Thp1-macro cells was much more random (Fig. 4c). As one Hi-C experiment can only capture chromatin interaction at single time-point, the merged interactions of GBP5 with other loci from the individual cells may reflect the chromatin interactions around GBP5 from different time-points. As they all looped with GBP5, it is likely that these genes are all assembled in a transcription factory.

Consistent with our observation that DoD is negatively correlated with gene co-regulation, Fig. 4a demonstrated that the expression levels of the GBP1-5 in the TAD were synchronously co-upregulated. The immunostaining assay showed that GBP family proteins assembled tightly around the surface of M.tb cells probably to prevent its spreading (Supplementary Fig. 5a). These observations may suggest that this relatively chaotic TAD with high DoD or entropy before the spreading became highly organized in response to M.tb infection. This may be coordinated by the opening of specific genome loci and the subsequent binding of corresponding proteins, which form new chromatin loops that link the genes into an active transcription factory. In this way, it could efficiently achieve synchronous co-transcription of these defense-related genes.

The transcription factory tends to form liquid–liquid phase separation (LLPS) condensates to efficiently activate gene transcription. It has been shown that super-enhancer-binding proteins MED1 and BRD4 undergo phase separation and can be used as phase separation marker proteins within the transcription factory. We then test whether GBP family gene region is relocated into LLPS zone in the process of immune activation. Based on previous BRD4 and MED1 ChIP-seq data, we found that GBP family genome regions were enriched with these two phase separation marker proteins (Fig. 4d). Our ATAC-seq and H3K4me3 ChIP-seq data indicate that the chromatin state around this region is activated and may bind with more regulatory proteins after infection (Fig. 4a). Furthermore, the integrated Hi-C chromatin loop and BRD4 and MED1 ChIP-seq analysis suggest that the BRD4 and MED1 occupied super-enhancers are spatially in close proximity to all the GBP family gene loci after M.tb infection (Fig. 4d).

To further confirm phase separation in GBP gene family region, we performed co-staining of BRD4 and MED1 by Immunofluorescence (Fig. 4e) with GBP family gene region (by fluorescence in situ hybridization). As shown in Fig. 4e and Supplementary Fig. 5b, the overlapping ratio of BRD4 and MED1 puncta and the DNA-FISH signal of GBP gene family region is significantly increased after infection, suggesting the GBP genes region tends to relocate into LLPS transcription factory zone to efficiently initiate this immune-defense gene expression. Together, our data implied that macrophages could dynamically adapt their 3D genome structure and coordinate with LLPS transcription factory to efficiently express this immune-defense gene to fulfill distinct physiological functions during immunological response.

NF-κB initiates systematic chromatin remodeling of its target genome regions during M.tb infection

During differentiation and M.tb infection, the overall DoD was gradually decreased, which is in line with the plasticity of these cells (Fig. 5a and Supplementary Fig. 6a). In these processes, the reduction of DoD was accompanied with the increase of chromatin loops, suggesting that more loop-mediated cis-element interaction occurs in low DoD region (Fig. 5b). Upon infection, about 1864 loops were strengthened. These chromatin loops related genes are listed in Supplementary Data 4. Notably, these genes are significantly enriched in immunity pathways, which is not observed in the genes located in the weakened loops (Supplementary Fig. 6b). The dynamic immunity-related enhancer-gene regulatory network in Chr12 during infection was shown in Supplementary Fig. 6c. The KEGG pathway analysis of strengthened loop anchor genes (Supplementary Data 4) showed that 6 of the top 10 enriched pathways were directly related to NF-κB signaling pathways (Supplementary Fig. 6d). Furthermore, a large number of NF-κB binding motifs were enriched around the transcription start site (TSS) of these strengthened loop anchor genes (Fig. 5c), suggesting that NF-κB participates in chromatin remodeling during M.tb infection. The immunofluorescence assay revealed that NF-κB (p65) was indeed translocated into the nucleus upon infection (Fig. 5d).

The chromatin accessibility and RNA expression analysis showed that, upon M.tb infection, the NF-κB target loci turned to be more open, and the expression level of target genes was significantly upregulated compared to the random genes (Fig. 5e). Moreover, the chromatin loops related to the NF-κB target genes were more strengthened compared to the random genes (Fig. 5f). We further
investigated the chromatin remodeling of the typical NF-κB target gene loci, such as IFITs, CCLs, GBPs, HERCs, NFKB1, and TNFSF10. Figure 5g demonstrated that upon infection, a greater number of loops were formed in these regions, and the corresponding DoD was also reduced. This data was further validated by ChIP-qPCR, showing that the NF-κB was significantly enriched in the loop anchor regions of IFIT3, CCL2, GBP4, and HERC2 upon infection (Supplementary Fig. 6e). This evidence suggests that, upon infection, NF-κB translocated into GBP gene clusters. e Colocalization between BRD4 and the GBP gene cluster by IF and DNA-FISH in Thp1-macro and Thp1-M.tb cells. IF, DNA-FISH, and merged channels (overlapping signal in white) are shown in separate images. The dashed line highlights the nuclear periphery, determined by DAPI staining. The rightmost column shows the area in the yellow box in greater detail. For each cell type, we counted 60 GBP gene loci. 18.3% (11/60) GBP loci were colocated with BRD4 in Thp1-macro cells and 65.0% (39/60) GBP loci were colocated with BRD4 in Thp1-M.tb cells. Each experiment was replicated three times.
IFIT genes can interact with each other and form a complex to perform antipathogenic functions\(^6\). Consistently, we observed synchronously upregulated expression of these three genes in the newly established loops (Fig. 5j). Notably, *IFT1B* and *IFT3*, which are not located in this spatially adjacent hub, were not upregulated. These data demonstrated a high-order chromatin structure mediated elegant spatial and temporal co-regulation of the NF-κB target genes transcription during immunoresponses (Fig. 5k).

Next, we collected monocytes from peripheral blood and induced them into hMDMs by macrophage colony-stimulating factor (M-CSF). After virulent tuberculosis strain H37Rv infection, the RNA-Seq and DLO Hi-C libraries of these hMDMs-M.tb cells were constructed. As shown in Supplementary Fig. 7a, the differentially expressed genes in Thp1-M.tb, such as *GBP1*, *GBP3*, *IFT3*, *CCL11*, and *PD-L1* (Supplementary Data 2), were also significantly upregulated in hMDMs-M.tb. The majority of the enriched KEGG pathways, such as NF-κB signaling
pathway, TNF signaling pathway, and Jak-STAT signaling pathway, are consistent between hMDMs-\(Mtb\) and THP-1-\(Mtb\) (Supplementary Fig. 7b, c).

Furthermore, we validated the TAD DoD dynamics of NF-\(\kappa B\) target loci after \(Mtb\) infection in hMDM cells, the TAD DoD value of \(H3K4me3\) dropped significantly (Supplementary Fig. 7d, \(P = 3.4E-13\)) compared to the control group, especially in \(GBP2\), \(GBP5\), \(NFKB1\), and \(TNFSF15\) gene loci (Supplementary Fig. 7e). Consistent with THP-1-derived macrophages (Fig. 4a and Fig. 5g), the DoD value of \(GBP\) gene locus decreased from 2.98 to 1.83 after \(Mtb\) infection in hMDMs (Supplementary Fig. 7f, g). These results suggest that the immune response of THP-1 infection model is highly similar to primary human macrophages.

**A remote NF-\(\kappa B\) enriched enhancer promotes the expression of \(PD-L1\) through a chromatin loop**

After engulfing \(Mtb\), macrophages can present \(Mtb\) antigens via major histocompatibility complex (MHC) molecules to T-cells to eliminate \(Mtb\) infection\(^{16}\). During this process, \(Mtb\) also evokes various strategies to escape immunological clearance. In this scenario, we observed the expression of immune checkpoint gene \(PD-L1\) was dramatically upregulated after \(Mtb\) infection (Fig. 1c). Through the Hi-C contact matrix, we identified a putative enhancer region highly enriched with H3K4me3 modification (Chr9: 4,760,070–4,769,779) contact with the \(PD-L1\) promoter through a Hi-C loop (Fig. 6a–c). Notably, the ATAC-seq data showed that these enhancer and promoter regions were more accessible, and enrichment of active chromatin modifications after \(Mtb\) infection (Fig. 6b, c), indicates that more regulatory proteins are enriched in the enhancer region. Transcription factor binding motif analysis revealed that both enhancer and promoter regions (Fig. 6b, c and Supplementary Fig. 8a, b) can be bound by NF-\(\kappa B\). Furthermore, by ChIP-qPCR, we proved that NF-\(\kappa B\) (p65) was significantly enriched in this enhancer region after infection (Fig. 6d).

Since NF-\(\kappa B\) can directly bind to \(PD-L1\) promoter and upregulate its gene transcription\(^{19}\), we speculate that this enhancer region can directly regulate the \(PD-L1\) gene expression via chromatin loop. To further confirm the function of \(PD-L1\) enhancer, we knocked out this enhancer region in the THP-1 cell line by the CRISPR/Cas9 system and validated its regulatory function (Fig. 6e and Supplementary Fig. 8c–f). As predicted, the qPCR and western blot data demonstrated that knockout of the \(PD-L1\) enhancer can indeed attenuate the upregulation of \(PD-L1\) expression at both mRNA and protein levels upon \(Mtb\) infection (Fig. 6f, g). Collectively, these results suggested a synchronous opening of the enhancer and promoter regions for transcription factor binding and activated \(PD-L1\) gene transcription during \(Mtb\) infection (Fig. 6h). This \(PD-L1\) enhancer has the potential to become anti-tuberculosis and even antitumor therapeutic target. It would be of great interest to further investigate how \(Mtb\) infection reprograms the epigenetic code in this enhancer locus and what is the physiological role of this modification in the pathogenesis of \(Mtb\).
Fig. 7 | Identification of long-range regulatory target genes of mycobacterial disease susceptibility loci. a Identification of long-range regulatory target gene of rs1873613. b The rs1873613 is located in the anchor of the dynamic LRRK2 chromatin loop. c Sanger sequencing result of the chr12: 40,552,317 loci in THP-1 control cell line. d Dimmer modification, chromatin state, and Hi-C loop information of rs1873613 and LRRK2 gene region from the UCSC Genome browser (http://genome.ucsc.edu). e Histone modification, chromatin state, and Hi-C loop information of LRRK2 and LRRK2 gene region from the UCSC Genome browser (http://genome.ucsc.edu). f ChIP-qPCR validation of the H3K4me3 enrichment in the LRRK2 enhancer region. The amount of immunoprecipitated DNA is represented as signal relative to the total amount of input chromatin (y-axis). Error bars show mean ± SD, n = 3 biologically independent repeats. P-values were calculated by two-sided Student’s t-test. h CFU assays of Mtb in THP-1 control and Thp1-rs1873613 cell lines under IFN-γ stimulation (20 ng/ml). Error bars show mean ± SD, n = 18 biologically independent samples were used for the control group and Thp1-rs1873613 group. P-values were calculated by unpaired one-sided t-test. i, j Immunofluorescence analysis and quantification of the colocalization of the Mtb (H37Ra-RFP) and Rab7 in BMDM treated with the LRRK2 inhibitor AdoCbl and PBS (control), respectively. k CFU assays of Mtb in BMDM treated with AdoCbl and PBS (control), respectively. Error bars show mean ± SD, n = 4 and n = 5 biologically independent samples were used for the control group and AdoCbl treatment group, respectively. P-values were calculated by unpaired one-sided t-test. l CFU assays of Mtb in C57BL/6 mouse lungs. Error bars show mean ± SD, n = 9 and n = 10 mice were used for the control group and AdoCbl treatment group, respectively. P-values were calculated by unpaired one-sided t-test.
Identification of long-range target genes of mycobacterial disease susceptibility loci via chromatin loop

To obtain a comprehensive map of mycobacterial disease susceptibility loci and their long-range regulatory gene targets via chromatin loop, we collected all reported susceptibility loci\(^{21,22}\) and performed integrated omics analysis of GWAS, eQTL, and Hi-C (Fig. 7a and Supplementary Fig. 9a and Supplementary Data 5). With DLO Hi-C data, we could systematically identify the target genes of these susceptibility loci through long-range chromatin interactions (Supplementary Fig. 9b). Of note, the chromatin interaction loops between the \(LRRK2\), \(NSL1\), and \(ASAPI\) and the corresponding susceptibility loci were significantly strengthened during \(M\text{.}tb\) infection (Fig. 7b and Supplementary Fig. 9c, d). Importantly, the target genes of susceptibility loci discovered by this integrated omics analysis, such as \(ASAPI\) and \(LRRK2\), have been reported to be involved in mycobacterial pathogenesis\(^{23,24}\), supporting the integrity of our multiomics analysis. Further, it would be of great importance to obtain GWAS raw data and perform a colocalization analysis of the GWAS and the eQTLs.

We further analyzed \(LRRK2\), which has a loop and eQTL correlation with the susceptibility SNP rs1873613. The DoD value of \(LRRK2\) locus was decreased after \(M\text{.}tb\) infection (Fig. 7b). Moreover, the simulated chromatin interaction based on single-cell Hi-C data revealed that rs1873613 and \(LRRK2\) tended to be adjacent in space upon infection (Supplementary Fig. 10a). Furthermore, this SNP is located right in the anchor of an \(LRRK2\) chromatin loop that was significantly strengthened after infection (Fig. 7b). This data suggests that this susceptibility SNP locus was dynamically reshuffled upon infection to spatially link with \(LRRK2\) to regulate its gene transcription.

To further explore the function of SNP rs1873613, we introduced a “T” to “C” single base pair mutation in chr12:40552317 using the CRISPR/Cas9 system in \(M\text{.}tb\)-infected THP-1 cell lines and named the mutated cell line as Thp1-rs1873613 (Fig. 7c, d). Through the ENCODE ChromHMM chromatin state information from UCSC browser, we found that rs1873613 is located in a strong enhancer upstream of \(LRRK2\). This enhancer interacts with \(LRRK2\) promoter through a long-range chromatin loop (Fig. 7e). Therefore, we speculated that rs1873613 would affect the activity of the enhancer, thereby regulating the expression of \(LRRK2\) gene.

Since \(LRRK2\) is an IFN-\(\gamma\) target gene, we stimulated THP-1 control and Thp1-rs1873613 with IFN-\(\gamma\), respectively, and then evaluated the enhancer activity by ChIP-qPCR. As shown in Fig. 7f, under IFN-\(\gamma\) stimulation, the Thp1-rs1873613 had significantly more (\(P = 0.0071\)) active chromatin mark (H3K4me3) enriched in the enhancer region compared to the THP-1 control. This data indicates that the enhancer region of Thp1-rs1873613 is more sensitive to IFN-\(\gamma\) and has stronger enhancer activity than that of the THP-1 control. Moreover, rs1873613 significantly promoted the inductive effect of IFN-\(\gamma\) on \(LRRK2\) expression (Fig. 7g).

Consistently, the eQTL analysis data also showed that a T:C mutation can indeed increase the expression of \(LRRK2\) in the lungs (Supplementary Fig. 10b). Since the expression of \(LRRK2\) is positively correlated with the intracellular survival of \(M\text{.}tb\)\(^{16}\), we therefore investigated the intracellular proliferation of \(M\text{.}tb\) in THP-1 control and Thp1-rs1873613 cell lines. As shown in Fig. 7h, the bacterial load of Thp1-rs1873613 was indeed significantly higher than that of the THP-1 control group. Taken together, we verified that rs1873613 can enhance the \(LRRK2\) enhancer activity and upregulate the expression of \(LRRK2\), further supporting the correlation of this SNP to mycobacterial disease susceptibility. In the future, it would be of great importance to perform more in vivo experiments to further evaluate the causal mechanisms of rs1873613 and other SNPs in mycobacterial disease susceptibility.

It has been demonstrated that \(LRRK2\) promoted the proliferation of \(M\text{.}tb\) by inhibiting phagosome maturation\(^{16}\), suggesting \(LRRK2\) might be a TB drug target. Thus, we investigated the anti-\(M\text{.}tb\) effect of AdoCbl, an inhibitor of LRRK2\(^{26}\). As shown in Fig. 7i–k, AdoCbl can indeed promote the maturation of phagosomes in TB-infected bone-marrow-derived macrophages (BMDM) and decrease the count of colony-forming units of intracellular \(M\text{.}tb\). Furthermore, the in vivo experiment showed that AdoCbl could significantly inhibit the proliferation of \(M\text{.}tb\) in the lungs (Fig. 7j and Supplementary Fig. 10c). This result was further supported by hematoxylin-eosin staining of tissue sections, showing that AdoCbl significantly inhibited the initiation of lung and spleen lesions (Supplementary Fig. 10d). These data revealed that the TB susceptibility SNP rs1873613 in the dynamic loop anchor of \(LRRK2\) enhancer could promote the \(LRRK2\) enhancer activity, thereby upregulating the expression of the \(LRRK2\). \(LRRK2\) protein subsequently represses the clearance of \(M\text{.}tb\) by inhibiting phagosome maturation. We demonstrated that this process can be attenuated by a potential TB drug candidate, AdoCbl (Supplementary Fig. 10e).

Collectively, we delineated the 3D genome landscape of THP-1 cells during differentiation and infection at single-cell resolution. Our data showed that the immunological enhancer-promoter loops, especially the NF-\(\kappa\)B target regions, were reorganized to orchestrate synchronous defense gene transcription for \(M\text{.}tb\) clearance. It provides a comprehensive resource for epigenetic regulation of immunocytes and for \(M\text{.}tb\) infection studies, such as anti-\(M\text{.}tb\) drug screening and the TB pathogenesis mechanisms of the patients with susceptibility SNPs. Importantly, we proposed TAD DoD to measure the genome organizational patterns, which are correlated with the chromatin epigenetic states, chromatin structure variability in individual cells, and expression and co-regulation of the genes within the TAD, supporting that the order and stochasticity of genome architecture are related to its function. These data shed insights into the dynamic genome organizational patterns at single-cell level and illustrated how the spatial organization of chromatin coordinates gene transcriptional programs during differentiation or in response to different stimuli, such as immunological response.

Methods

Cell culture

THP-1 cells (ATCC, TIB-202) were grown in RPMI-1640 containing 10% FBS. To differentiate the monocytes from macrophages, cells were treated with PMA (phorbol 12-myristate 13-acetate, sigma, Catalog code: P8139) for 48 h at a final concentration 40 ng/ml, and then washed with prewarmed PBS and incubated with fresh culture medium for another 24 h.

Human peripheral blood total monocyte was isolated using MagniSort\textsuperscript{TM} Human pan-Monocyte Enrichment Kit (Invitrogen, Catalog code: S804-6837-74). To differentiate the human pan-monocyte to human monocyte-derived macrophages (hMDMs), cells were treated with human M-CSF (macrophage colony-stimulating factor, Chamat Biotechnology, Catalog code: CML16-20MP) for 10 days at a final concentration 50 ng/ml, and then washed with prewarmed PBS and incubated with fresh culture medium for another 24 h.

Mycobacterium tuberculosis infection

For \(M\text{.}tb\) infection experiment, H37Rv or H37Ra culture (3000 × g, RT, 10 min), washed twice with RPMI-1640, resuspended in 1 ml THP-1 culture medium, and dispersed using BD insulin syringes (BD, Catalog code: 328421). THP-1-derived macrophages (Thp1-macro) or human monocyte-derived macrophages (hMDMs) were infected with H37Rv or H37Ra at MOI (multiplicity of infection) 20. Four hours later the infected cells were washed twice with prewarmed PBS and incubated with a fresh culture medium for another 8 h.

RNA-seq library preparation

RNA was extracted using the RNAiso Plus (Takara, Catalog code: 9109) according to the manufacturer’s protocol. Sequencing libraries were

https://doi.org/10.1038/s41467-022-33558-5
prepared using the VAHTS Stranded mRNA-Seq Library Prep Kit (Vazyme, Catalog code: NR602-02) according to the manufacturer's protocol.

ChIP-Seq library preparation

4 × 10^6 cells were used per immunoprecipitation. Antibody of H3K4me1 (Abcam, Catalog code: ab8585, 1:100 dilution), H3K4me3 (Abcam, Catalog code: ab8588, 1:100 dilution), H3K9me3 (Abcam, Catalog code: ab8589, 1:100 dilution), H3K27ac (Abcam, Catalog code: ab4729, 1:100 dilution), H3K27me3 (Millipore, 07-449, 1:100 dilution), and NF-xB p65 (CST, Catalog code: 8242, 1:100 dilution) were used in this study. All antibody dilutions were 1:100. The ChIP DNA was obtained using SimpleChIP® Enzymatic Chromatin IP Kit (CST, Catalog code: #9003). After immunoprecipitation, ChIP-Seq library was constructed using NEBNext® Ultra™ DNA Library Prep Kit (NEB, Catalog code: E7370S). In addition, the ChIP DNA was also used as the template for ChIP-qPCR.

ATAC-seq library preparation

1 × 10^6 cells were centrifuged at 800 × g for 5 min and then washed once using 500 μl of cold 1× PBS and centrifuged at 800 × g for another 5 min. Cells were lysed using cold lysis buffer (10 mM Tris-HCl (pH 8.0 at 25°C), 10 mM NaCl, 0.3% Igepal CA-630). After lysis, nuclei were spun down at 800 × g for 4°C for 10 min. The pellet was resuspended in the transposase reaction mix (10 μl 5× TTBL buffer (Vazyme, Catalog code: TD501-02), 5 μl TTE Mix V50 buffer (Vazyme, Catalog code: TD501-02), and 35 μl ddH2O water). Tagmentation was carried out for 10 min at 55°C. Immediately following transposition, the DNA was purified using a Qiagen MinElute PCR Purification Kit (Qiagen, Catalog code: 28004) and eluted with 10 μl elution buffer. The ATAC-Seq library was amplified by the primers in TruePrepTM Index Kit V2 for Illumina® (Vazyme, Catalog code: TD202).

In situ DLO Hi-C experiment

Five million cells were double crosslinked with 1.5 mM EGS (Sigma, Catalog code: E3257) and 1% formaldehyde (Sigma, Catalog code: F8775) and lysed in lysis buffer (10 mM Tris-HCl (pH 8.0 at 25°C), 10 mM NaCl, 0.3% Igepal CA-630, 0.5% SDS, and complete protease inhibitor (Roche)), incubated at 60°C for 5 min, and placed on ice immediately. After incubation, the nuclei were pelleted by centrifugation at 1800 × g for 5 min and washed once with ice-cold PBS. A total of 310 μl of ddH2O, 20 μl of 20% Triton X-100, 40 μl of 10× NEBuffer 2.1, and 30 μl of MseI (NEB, Catalog code: R0525L, 5 units/μl) was then added to the nuclei and incubated for 6 h at 37°C with rotation at 13 r.p.m. After restriction enzyme digestion, 50 μl of MseI half linkers (600 ng/μl), 5 μl of 100 mM ATP, 20 μl of T4 DNA ligase (Thermo, Catalog code: EL0011, 5 units/μl), and 25 μl of ddH2O were added to the 400 μl of digested chromatin and mixed thoroughly. The mixture was then incubated at 25°C for 1 h with rotation at 15 r.p.m. After half-linker ligation, the nuclei were centrifuged at 4°C for 5 min at 1000 r.p.m. and washed twice with 1 ml of ice-cold PBS. The linker-ligated nuclei were gently resuspended in 200 μl of 1× T4 DNA ligation buffer (Thermo) containing 0.5 units/μl T4 polynucleotide kinase (NEB) and incubated at 37°C for 30 min. The 200 μl reaction complexes were added to 300 μl of 1× T4 DNA ligation buffer (Thermo) containing 0.5 units/μl T4 DNA ligase (Thermo, Catalog code: EL0011). Ligation was performed at 20°C for 2 h with rotation at 15 r.p.m. The nuclei were centrifuged at 4°C for 5 min at 1800 × g and resuspended in 400 μl of ddH2O. Protein digestion was performed by adding 25 μl of 10 mg/ml proteinase K (Sigma, Catalog code: 39450-01-6), 50 μl of 10% SDS, and 25 μl of 5 M NaCl, and the tubes were incubated for 2 h at 65°C. After incubation, an equal volume of phenol:chloroform:isoamyl alcohol (25:24:1) was added to the sample, shaken vigorously, and then centrifuged for 10 min at 14,000 × g. Next, the supernatant was transferred to a new tube. This process was repeated twice. DNA was precipitated at room temperature with 5 μl of Dr. GenTLE Precipitation Carrier (Takara, Catalog code: 9094), 50 μl of 3 M sodium acetate (pH 5.2), and 555 μl of isopropanol. The precipitated DNA was washed once with 80% ethanol and dissolved in 160 μl of ddH2O. A total of 20 μl of 10× CutSmart buffer, 10 μl of SAM (NEB, Catalog code: B9003S), and 10 μl of MseI (NEB, Catalog code: R0637L, 2 units/μl) were added to the 160 μl DNA sample, and digestion was performed at 37°C for 1 h. The digested DNA sample was subjected to electrophoresis in native PAGE gels. The specific 80 bp DLO Hi-C DNA fragments were excised and transferred to a 0.6 ml tube with a pierced bottom. This tube was then placed into a 1.5 ml tube, and the gel slices were shredded by centrifugation at 14,000 × g for 10 min. A total of 400 μl of TE buffer was added to the 1.5 ml tube (to ensure that the shredded gel was fully immersed in buffer), and the mixture was incubated for 20 min at 80°C, followed by a 2 h incubation at 37°C with rotation at 15 r.p.m. Next, the shredded gel, along with the buffer, was transferred into the filter cup of a 2 ml Spin-X tube filter (Costar, Catalog code: S160). After centrifugation, the eluate was transferred into a new 2 ml tube. A total of 4 μl of Dr. GenTLE Precipitation Carrier (Takara, Catalog code: 9094), 40 μl of 3 M sodium acetate (pH 5.2), and an equal volume of isopropanol were added to precipitate the DNA. The precipitated DNA was washed once with 80% ethanol and dissolved in 40 μl of ddH2O. Next, 1.5 μl of T4 DNA ligase1 (500 ng/μl), 1.5 μl of PE-adaptor2 (500 ng/μl), 5 μl of 10× T4 DNA ligase buffer, and 5 μl of ddH2O DNA ligase (Thermo, Catalog code: EL0012) were added to the 40 μl of DLO Hi-C DNA fragments and incubated at 16°C for approximately 30 min. 90 μl of AMPure XP beads (Beckman, Catalog code: A63880) was added to the ligation mixes and washed twice with 80% ethanol to remove excess Illumina sequencing adaptors. Next, 45 μl of ddH2O was used to wash DNA from the beads. The eluted DNA was repaired using PreCR Repair Mix (NEB, Catalog code: M0309S) for 20 min at 37°C in a final volume of 50 μl. 510 μl of repaired DNA was used as a template and amplified for fewer than 13 cycles. The PCR product is the final DLO Hi-C sequencing library.

Single-cell DLO Hi-C experiment

Nuclei preparation. Five million cells were crosslinked with 1% formaldehyde (Sigma, Catalog code: F8775) for 10 min, and lysed in lysis buffer (10 mM Tris-HCl (pH 8.0 at 25°C), 10 mM NaCl, 0.3% Igepal CA-630, 0.5% SDS, and complete protease inhibitor (Roche)) at 60°C for 5 min, and placed on ice immediately. After incubation, the nuclei were pelleted by centrifugation at 1800 × g for 5 min and washed once with nuclei wash buffer (PBS, which contain 0.5% Triton X-100, 0.05% Tween 20, and 0.05% CA-630).

MseI digestion. The digestion buffer (a total of 310 μl of ddH2O, 20 μl of 20% Triton X-100, 40 μl of 10× NEBuffer 2.1, and 30 μl of MseI (NEB, Catalog code: R0525L, 10 units/μl) was then added to the nuclei pellet and digested for 3 h at 37°C in theromixer (Eppendorf) with rotation at 1000 r.p.m.

Indexed half-linker ligation. After digestion, 560 μl 2× T4 DNA ligase buffer were added to the nuclei, divided into 96-well plates, and adjusted to the volume of 10 μl/tube. Next, 1 μl barcoded half linkers (50 μM/μl) (Supplementary Data 6) were added to each tube and mixed well. After incubation at room temperature for 5 min, 1 μl T4 DNA ligase (Thermo, Catalog code: EL001I, 5 units/μl) was added to each tube. The ligation reaction was performed at 20°C for 30 min, and then incubated for 10 min at 4°C. The sample in 96-well plates was transferred to 1.5 ml DNA LoBind Tube (Eppendorf, Catalog code: B14809M), centrifuged at 1800 × g for 5 min at 4°C, and washed 4 times with nuclei wash buffer.

Fragment-end phosphorylation and in situ proximity ligation. The linker-ligated nuclei were gently resuspended in 200 μl of 1× T4 DNA
ligation buffer (Thermo) containing 0.5 units/µl T4 polynucleotide kinase (NEB, Catalog code: M0200L) and then incubated at 37 °C for 30 min. The 200 µl reaction complexes were added to 300 µl of 1× T4 DNA ligation buffer (Thermo) containing 0.5 units/µl T4 DNA ligase (Thermo, Catalog code: EL0011). Ligation was performed at 20 °C for 2 h with rotation at 15 r.p.m. The nuclei were centrifuged at 4 °C for 5 min at 1800 × g and resuspended in nuclei wash buffer.

Single-cell selection and multiple displacement amplification. The nuclei were stained by DAPI (Thermo, Catalog code: D1306) and diluted by nuclei wash buffer. The density of the nucleus in liquid was carefully checked under the fluorescence microscope, and adjusted to 30 nuclei/µl. Next, about 1 µl nuclei were digested with proteinase K to release the DNA, the reaction system is as follows: 830 nuclei/µl of 5 min at 1800 × g; 2 h with rotation at 15 r.p.m. The nuclei were centrifuged at 4 °C for 5 min and resuspended in nuclei wash buffer.

Multiple displacement amplification recycle and sequencing library construction. After multiple displacement amplification (MDA) reaction, 1 µl 10% SDS and 79 µl ddH₂O were added to the sample, and the DNA was purified by 200 µl VAHTS DNA Clean Beads (Vazyme, Catalog code: N411-01). The following steps of MmeI digestion, 30 bp contact DNA fragment recovery are the same as in situ DLO Hi-C protocol described above. For sequencing library construction, we replaced the previous Illumina sequencing adapter with a homemade MGI-2000 platform sequencing adapter with the second round of indexes (Supplementary Fig. 4c and Supplementary Data 6). Previous similar methods have shown that due to the number of barcodes (96 × 14) being far more than the number of nuclei (30 × 14), therefore most of the single nuclei are labeled by a unique barcode.

Immunofluorescence combined with DNA-FISH
Cells grown on coated glass were fixed in 4% formaldehyde solution (Sigma, Catalog code: 47608-25OM-L-F) at room temperature for 5 min followed by PBS buffer washing for three times. Next, cells were permeabilized with 0.4% SDS in PBS for 5 min at RT. Following three washes in PBS for 5 min, cells were blocked in blocking buffer (1× PBS / 5% normal serum / 0.3% Triton X-100) for 60 min. After blocking, antibodies (anti-BRD4, Abcam, Catalog code: ab128874, 1:250 dilution; anti-MEDI, Abcam, Catalog code: ab66496, 1:250 dilution) in antibody dilution buffer (1× PBS / 1% BSA / 0.3% Triton X-100) at 2 µg/ml final concentration were incubated with the slides overnight at 4 °C. Slides were then washed three times with PBS and recognized by secondary antibodies (Goat anti-Rabbit IgG Alexa Fluor 488, Life Technologies, Catalog code: A11008, 1:1000 dilution) in the dark for 30 min.

After immunofluorescence, cells were placed in prewarmed PBS and incubated at 60 °C for 20 min, then incubated in 70% ethanol, 85% ethanol, and then 100% ethanol for 1 min at RT. After alcohol dehydration, the cells were heated on a hot plate at 82 °C for 10 min in 80% formamide (Sigma, Catalog code: 47671-1L-F) and 2× SSC for DNA denaturation. Next, cells were incubated for 12 h in hybridization solution with 2 µM Alexa535-dUTP labeled GFP DNA-FISH probes (Ch1: 89.448,890-89.739,345, Spatial FISH Co. Ltd.) in the presence of 50% formamide, 8% dextran sulfate sodium salt (Sigma), and 2× SSC. After hybridization, the cells were washed for three times with 30% formamide and three times with 2× SSC. Next, the slides were stained with DAPI (Thermo, Catalog code: D1306) and observed under a super-resolution microscope (Nikon, N-SIM).

CFU assays
Bone-marrow-derived macrophage cells (BMDM) were seeded into six-well plate (1 × 10⁶/well) and infected with H37Ra at MOI (multiplicity of infection) 20. Four hours later, the infected cells were washed twice with prewarmed PBS and incubated with a fresh culture medium. Then the cells were treated with AdoCbl (Sigma, Catalog code: C0884) at a final concentration of 200 µM. The PBS solution was used as a negative control. Three days later, the cells were lysed with 0.1% triton X-100 and plated for bacterial burden enumeration using a serial dilution method on plates of Middlebrook 7H11 agar containing OADC enrichment (BD, Catalog code: 211886) and BBL MGIT PENTA antibiotics (BD, Catalog code: 245114). CFUs were counted after 3–4 weeks of incubation at 37 °C.

Immunofluorescence
For immunofluorescence of GBP1-5, Rab7, and NF-kB, monocyte-derived macrophages (Thp1-macro) were infected with RFP-H37Ra at MOI (multiplicity of infection) 20. The infected cells were washed twice with prewarmed PBS 4 h and incubated with a fresh culture medium for another 8 h. Next, the cells were crosslinked with 4% formaldehyde for 15 min at room temperature and rinsed three times with 1× PBS. The specimen was blocked in blocking buffer (1× PBS / 5% normal serum / 0.3% Triton X-100) for 60 min. After blocking, respective antibodies (GBP1-5, Santa Cruz, Catalog code: sc-166942 AF488, 1:250 dilution; Rab7, Santa Cruz, Catalog code: sc-376362 AF488, 1:250 dilution; NF-kB p65, Santa Cruz, Catalog code: sc-8008 AF546, 1:250 dilution) in antibody dilution buffer (1× PBS / 1% BSA / 0.3% Triton X-100) at 2 µg/ml final concentration were incubated with the slides overnight at 4 °C. Slides were washed three times with PBS and stained with DAPI (Life Technologies) and observed under the fluorescence microscope.

Genome editing in THP-1 cells by CRISPR/Cas9 system
In order to introduce the “T” to “C” mutation at the genome site chr12:40552317 (rs1873613) into THP-1 cells, we designed a sgRNA (sequence: “CTCGTGGACTATTGTATCT” to target chr12:40552317, “C” to “T”) in the donor plasmid. In the homologous fragment, to avoid the cleavage of the homologous recombination arms of the DNA donor by Cas9 upon transfection, we also mutated the PAM sequence of the sgRNA target (chr12:40552317, “C” to “T”) in the donor plasmid. In the homologous fragment, we simultaneously mutated the chr12: 40552327 site (“C“ to “T”) and PAM sequence of the sgRNA (chr12: 40552332, “C” to “T”). Then, we ligated the homologous DNA fragment, sgRNA cassette, GFP fluorescent protein gene, and puromycin-resistance gene into PUC19 plasmid. The structure of the PUC19-sgRNA-GFP-Puro-rs1873613 plasmid is illustrated in Supplementary Fig. II.

Next, we transfected the PUC19-sgRNA-GFP-Puro-rs1873613 plasmid into Thp1-cas9 cell line (THP-1 cell line with stably expressing Cas9) using Nucleic Acid Transfection Enhancer (NATE, InvivoGen, Catalog code: lyec-nate) and PolyJet (SigmaGen, Catalog code: SL100688). After 36 h of puromycin (final concentration: 2.5 µg/ml) selection, single cells were seeded into 96-well plates. During colony expansion, genotyping was carried by PCR and Sanger sequencing to screen single-cell clones which carried rs1873613 (Fig. 7c, d). We named the homoygote containing “T” to “C” mutation at chr12: 40552317 sites (rs1873613) as Thp1-rs1873613. Two days after IFN-γ stimulation (final concentration 20 ng/ml), the enrichment of H3K4me3 in LRRK2 enhancer region and LRRK2 mRNA expression levels were investigated by ChIP-qPCR and RT-qPCR.

Mouse models
All wild-type female C57BL/6 mice used in this study were purchased from Beijing Vital River Laboratory Animal Technology and all the experiments in this study were approved by the Scientific Ethic Committee of Huazhong Agricultural University (NO. HZAUMO-2019-019)
and maintained at the Laboratory Animal Centre of Huazhong Agriculture University under specific pathogen-free (SPF) conditions with 12-h light/dark cycles. Room temperature was maintained at 25 °C. The humidity level was controlled between 40 and 60%. Based on the principles of laboratory animal welfare and ethics, this study optimized the design of the project and strictly plans the number of animals required. A total of 28 6-week-old female C57BL/6 mice (20 ± 2 g) were planned. Among them, four were used for bone narrow macrophage isolation experiments, and the remaining 24 were used for Mycobacterium tuberculosis H37Ra infection test and AdoCbl drug treatment experiment.

**Animal experiment**

For *M. tb* infection, 6-week-old female mice (20 ± 2 g) were infected by intravenous injection with a dose of 5 × 10⁶ cfu/mL of 200 μL *M. tb* H37Ra suspension. All animals were randomly distributed into two groups of 12 each. Then mice received a gavage feeding of AdoCbl (Sigma, Cat# C0884) at a daily dose of 0.5 mg/kg-bw, and an equal volume of sterile PBS used as a negative control. These mice were given the therapy for 1-day post-*M. tb* infection. Mice were euthanized after 15 days of drug treatment. Lungs and spleen were taken out for histopathological observation and CFU analysis. A part of the left lung was fixed in 4% paraformaldehyde for a minimum time of 48 h, and tissues were paraffin-embedded to make a section for hematoxylin and eosin (H&E) staining. Homogenates of lungs and spleen were plated for bacterial burden enumeration using a serial dilution method on plates with the Burrows-Wheeler Aligner-MEM60 (version: 0.7.17-r1188) was applied to map the reads to the human reference genome (hg19). Mapped reads were kept for further processing. TopHat55 (version: v2.1.1) was used to trim the paired-end reads, and Trimmomatic54 (version: 0.33) was used to filter out the low-quality bases and adapter sequences. Clean reads longer than 36 bp at both ends were kept for further processing. TopHat2 (version: v2.1.1) with bowtie256 (version: 2.3.5.1) was used to align the paired-end RNA-Seq reads to the human genome assembly (hg19). The reads with a mapping quality higher than 30 were considered uniquely mapped reads. Peaks were called by MACS263 (version: 1.7). The reads with a mapping quality higher than 30 were used: 1, 2, 4 differences in the hamming distance were allowed for the comparison between “read barcode VS library barcode”, “barcode1 VS barcode2 (within same reads)”, “barcode R1 VS barcode R2 (barcode within R1 and R2)”.

**RNA-seq analysis**

FastQC (version: v0.11.8) was used to assess the quality of RNA-Seq reads, and Trimmmomatic44 was used to trim the ends of reads. Clean reads were aligned to the human genome assembly (hg19) with the Burrows-Wheeler Aligner-MEM (version: 0.7.17-r1188). For each sample, SAMtools1 (version: 1.7) was used to sort the mapped reads by position. After marking the duplication reads by Picard toolkit (version: 1.119), uniquely mapped reads and non-redundant sequences were kept for further analysis by SAMtools with the parameter F: 1024, q 20. For counting the uniquely mapped reads in the PETs, the parameter “–fq1 [R1-fq] –fq2 [R2-fq]” of Juicer Tools (version: 1.9.9) was used with default resolutions. The HiCUPs algorithm of Juicer Tools (version: 1.9.9) was used to generate loop lists at resolutions of 5 kb, 10 kb and 25 kb.

**ChIP-seq analysis**

The reads filtering steps were the same as “RNA-Seq analysis”. Trimmmed reads were aligned to the human genome assembly (hg19) with the Burrows-Wheeler Aligner-MEM (version: 0.7.17-r1188). For each sample, SAMtools1 (version: 1.7) was used to sort the mapped reads by position. After marking the duplication reads by Picard toolkit (version: 1.119), uniquely mapped reads and non-redundant sequences were kept for further analysis by SAMtools with the parameter F: 1024, q 20. F-Seq2 (within same reads) and barcode2 (within two ends) were used by the pre command of Juicer Tools (version: 1.9.9) of Juicer Tools. The reference genome was divided into fragments according to the restriction enzyme sites, and the uniquely mapped sequence pairs were aligned to the restriction enzyme fragments. If two ends from a paired-read were mapped to the same restriction enzyme fragment, the paired-read was considered a self-ligation product. If two ends from a paired-read were mapped to two adjacent restriction enzyme fragments, the paired-read was considered a religation product. Both the self-ligation and religation reads were excluded for further analysis. If multiple sequences had both ends aligned to the same positions, only one sequence was retained for further analysis, since such reads probably resulted from PCR amplification.

**Annotation of chromatin states of enhancer and promoter**

Promoter regions were defined as the genomic regions 1 kb in front of the transcription start sites (TSS) of RefSeq genes. Repressed promoters were defined as promoter regions without H3K4me3 peaks. Bivalent promoters were defined as the promoter regions enriched with H3K4me3 and H3K27me3 peaks. Active promoters were defined as promoter regions enriched with H3K4me3 peaks but without H3K27me3 peaks.

For each cell type, the open non-promoter regions with ATAC-Seq peaks were initially selected as the candidate regions of enhancers. As previously described34,35, the candidate regions with H3K4me1 peaks were defined as enhancers. The active mark H3K27ac and inactive mark H3K27me3 were combined to define the different states of enhancers. An enhancer with H3K27ac peaks defined as a poised enhancer. If there were H3K27ac peaks located in the enhancer region, the enhancer was defined as an active enhancer. The enhancers with neither H3K27ac nor H3K27me3 peaks were considered a poised state.

**In situ DLO Hi-C analysis**

As the read1 sequences in the 2 × 150 bp paired-end reads in the in situ DLO Hi-C library contain all the chromatin interaction pair information, only read1 sequences were retained for further analysis. Linker filtering was conducted with DLO Hi-C Tool (version: 0.3.9)4. Reads with mapping scores of greater than 32 were retained for subsequent analysis. Via the linker sequence, the sequences with interaction pair information were extracted from the raw reads.

To increase the alignment rate, the restriction endonuclease recognition site was complemented at the end of the sequence. Burrows-Wheeler Aligner-ALN (version: 0.7.17-r1188) was used to align the interaction sequences to the human reference genome (hg19) with the parameter -n 0. Only the uniquely mapped reads with mapping quality (MAPQ) scores of ≥20 were retained and paired for further analysis.

The reference genome was divided into fragments according to the restriction enzyme sites, and the uniquely mapped sequence pairs were aligned to the restriction enzyme fragments. If two ends from a paired-read were mapped to the same restriction enzyme fragment, the paired-read was considered a self-ligation product. If two ends from a paired-read were mapped to two adjacent restriction enzyme fragments, the paired-read was considered a religation product. Both the self-ligation and religation reads were excluded for further analysis. If multiple sequences had both ends aligned to the same positions, only one sequence was retained for further analysis, since such reads probably resulted from PCR amplification.

To identify chromatin loops, interaction matrices were converted to hic files by the pre command of Juicer Tools (version: 1.9.9) with default resolutions. The HiCUPs algorithm of Juicer Tools (version: 1.9.9) was used to generate loop lists at resolutions of 5 kb, 10 kb and 25 kb.

**Single-cell DLO Hi-C data analysis**

The processing of the scDLO Hi-C sequencing data is basically as same as the bulk DLO Hi-C data processing9, with modifications in the PETs extraction step to recognize the barcodes and an additional step to split the final valid reads according to the barcodes. These two additional steps were implemented in the scDLO Hi-C tools4 (https://github.com/GangCaoLab/sciDLO, version 0.0.1).

In PETs extraction step, the parameter “–fq1 [R1_file] –fq2 [R2_file] –linker ATAC-Seq linker” was used. For the cell split step, the default parameters were used: 1, 2, 4 differences in the hamming distance were allowed for the comparison between “read barcode VS library barcode”, “barcode1 VS barcode2 (within same reads)”, “barcode R1 VS barcode R2 (barcode within R1 and R2)”.

**In situ DLO Hi-C analysis**

As the reads sequences in the 2 × 150 bp paired-end reads in the in situ DLO Hi-C library contain all the chromatin interaction pair information, only read1 sequences were retained for further analysis. Linker filtering was conducted with DLO Hi-C Tool (version: 0.3.9)4. Reads with mapping scores of greater than 32 were retained for subsequent analysis. Via the linker sequence, the sequences with interaction pair information were extracted from the raw reads.

To increase the alignment rate, the restriction endonuclease recognition site was complemented at the end of the sequence. Burrows-Wheeler Aligner-ALN (version: 0.7.17-r1188) was used to align the interaction sequences to the human reference genome (hg19) with the parameter -n 0. Only the uniquely mapped reads with mapping quality (MAPQ) scores of ≥20 were retained and paired for further analysis.

The reference genome was divided into fragments according to the restriction enzyme sites, and the uniquely mapped sequence pairs were aligned to the restriction enzyme fragments. If two ends from a paired-read were mapped to the same restriction enzyme fragment, the paired-read was considered a self-ligation product. If two ends from a paired-read were mapped to two adjacent restriction enzyme fragments, the paired-read was considered a religation product. Both the self-ligation and religation reads were excluded for further analysis. If multiple sequences had both ends aligned to the same positions, only one sequence was retained for further analysis, since such reads probably resulted from PCR amplification.

To identify chromatin loops, interaction matrices were converted to hic files by the pre command of Juicer Tools (version: 1.9.9) with default resolutions. The HiCUPs algorithm of Juicer Tools (version: 1.9.9) was used to generate loop lists at resolutions of 5 kb, 10 kb and 25 kb.
Single-cell embedding using the scHiCtools package\(^{42}\) (version: 0.0.3), all cells matrix was generated with 1 Mb resolution. For cell embedding using the “InnerProduct” to calculate the similarity between all cells, and using MDS method to mapping all cell’s contact matrix to a 2-dimensional vector. Thp-Mtb cells are classified into “immune highly activated” and “immune lowly activated” by the threshold of the mean value of all Thp-Mtb cell’s “separation score”, which is equal to the mean distance to the closest 4 Thp1-macro cells in the 2-dimensional space.

To calculate local DoD from single-cell data, the bulk cell DoD calculation pipeline was used with a few modifications including: (1) merged single-cell data as input; (2) the “significant interaction points” in the first step were replaced by the single-cell long-range (>20 kb) contacts; (3) the mean distance was divided by 5 kb, to scale at the same level as the bulk cell DoD value; (4) sampling the same number of the contacts in the compared regions of different samples when DoD comparison between samples was performed.

**Simulation of chromosome three-dimensional structure of single cells**

The chromatin was simulated to generate the chromatin 3D structure using software “nuc_dynamic” (version: 1.3.0)\(^{72}\). For global chromatin simulation, the software was run with the parameter: “-s 8.0 4.0 2.0 1.0 0.4 0.2 0.1”. For local region structure simulation, the parameter “-s 0.1 0.05 0.01 0.005” was used. And the results were saved as Protein Data Bank (PDB) file format and visualized with PyMOL software (version: 2.3.0).

**Hi-C loop analysis**

The Thp1-mono and Thp1-macro contact matrices were randomly downsampled to 351,514,529 intrachromosomal contacts (the same number of intrachromosomal contacts as the Thp1-Mtb matrix). The HiCCUPS algorithm was employed to call loops from the normalized Thp1-mono and Thp1-macro libraries. To find the differential interaction loops between two samples, the loops were divided into overlapping and nonoverlapping loops. For differential analysis of overlapping loops, the surrounding 5 × 5 window was compared between the two matrices by Wilcoxon tests, as previously described. For nonoverlapping loops, the fold enrichment values of the peak overall local neighborhoods were also calculated in the sample without the loop, as previously described. If all calculated fold enrichment values of the pixels in the sample without the loop were lower than 1.3, the peak in the sample with the loop was considered a differential peak.

**TAD boundary calling**

TAD boundaries were called at a resolution of 40 kb, as previously described. The interaction frequencies within 2 Mb downstream and 2 Mb upstream were compared with the default parameters of DomainCaller (version: 0.1.0). The directionality index (DI) was used to quantify the bias in each bin. Domains were inferred from hidden Markov model (HMM) state calls.

If the distance between two TAD boundaries in two samples was within 80 kb, these TAD boundaries were considered conserved TAD boundaries in the two samples. If the distance between two TAD boundaries was greater than 80 kb, the two TADs were considered shifted TADs. If one TAD in the previous cell state corresponded to multiple smaller TADs in the sample of the subsequent cell state, the TAD was considered a separated TAD. If multiple small TADs in the previous cell state corresponded to a large TAD in the subsequent cell state, this TAD was considered a fused TAD.

The Hi-C datasets were applied to distinguish A and B compartments. If the values in the first eigenvector were higher than 0, the corresponding bins were marked as A compartments. If the values in the first eigenvector were smaller than 0, the corresponding bins were marked as B compartments.

**TAD “Degree of Disorder” (DoD) and Co-Regulation Score (CRS) calculation**

MDkNN\(^{33}\) pipeline (https://github.com/GangCaoLab/MDkNN, version 0.0.1) was used for TAD DoD calculation. The significant chromatin interactions were first identified based on a Poisson process model. The expected value of the contact matrix was calculated by considering both distance-dependent decay and the local interaction background, as described in the previous studies. The window parameters \(p\) and \(\omega\) of the donut filter were set to 4 and 7, respectively, according to the previous studies. After allocating all significant interactions in the TAD contact matrix \(M_i\), the \(k\) nearest neighbors \(P_k\) with \(k = 3\) for each significant interaction \(p_i = (x_i, y_i)\), \(i \in [1, n]\) were calculated. The mean distance within the contact matrix \(\langle MD_i\rangle\) between the significant point \(p_i\) was measured as the mean distance to its \(k\) nearest neighbors, as shown in Supplementary Fig. 2f:

\[
MD_i = \frac{\sum dist(p_i, p_j)}{k}
\]

where \(dist(p_i, p_j) = \sqrt{(x_i - x_j)^2 + (y_j - y_j)^2}\) is the Euclidean distance in the matrix heatmap between \(p_i\) and \(p_j\). The KDTree data structure was used to accelerate the nearest neighbors searching process. The mean value of all local MDs, \(DoD = \sum MD_i/n\), in a TAD was defined as the TAD DoD, which represented the “disorder state” of the chromatin interactions within the TAD.

Since the two-sample Kolmogorov–Smirnov (KS) test is sensitive to differences in both the location and the shape of the distribution, it was used for statistical comparisons between the TAD DoD values of two biological samples. Here, the D statistic was calculated as follows:

\[
D_{n,m} = \sup_x |F_{1,n}(x) - F_{2,m}(x)|
\]

where \(sup\) is the supremum function and \(F_{1,n}\) and \(F_{2,m}\) are the cumulative distribution functions of the MD of different samples. For balancing the effect of sequencing depth, all sample’s contact matrices are reconstructed using the same number of valid reads.

The consistency of the overall transcriptional change direction (upregulation or downregulation) of the genes within a TAD during differentiation and activation was defined as the TAD gene co-regulation score (CRS). The regulation direction score (DS) of a TAD \(t\) was defined as follows:

\[
DS_t = \sum_{i=1}^{n} sgn(\log_{2}(\text{FoldChange}_i))
\]

where \(sgn\) is the sign function, and \(i\) is for gene \(i\) inside the specific TAD. The TAD CRS was defined as the absolute value of the DS: CRS\(t = |ds_t|\).

**Collection of GWAS-associated risk SNPs**

In this study, we collected SNPs from: (1) previous TB-related GWAS studies\(^{20,22,26}\) (Supplementary Data 5-subTable); (2) GWAS catalog (https://www.ebi.ac.uk/gwas); (3) UK Biobank GWAS datasets (http://www.nealelab.is/uk-biobank). For the SNPs from previous TB-related GWAS studies, we kept SNPs with \(P\) values ≤0.0001. After the data merge, all the SNPs were overlapped with our DLO Hi-C loops. Only the SNPs located in the loop anchor regions were kept (Supplementary Data 5-subTable2). The \(P\) values for meta-analyses were not adjusted because the raw GWAS data of several previous GWAS analyses were not available.
Integrated analysis of GWAS, eQTL, and Hi-C data
Significant eQTLs associated with risk SNPs were obtained from the GTEx portal (http://www.gtexportal.org), which includes gene expression data for 43 different tissues. Chromatin interaction loops with risk SNPs overlapped with significant eQTL gene regions within relevant tissues were selected for further investigation and are listed in Supplementary Data S-1. The R package ggplot2 was employed to demonstrate the interactions between SNPs and significant gene regions with Hi-C loops.

Reporting summary
Further information on research design is available in the Nature Research Reporting Summary linked to this article.

Data availability
The data that support this study are available from the corresponding authors upon reasonable request. All sequencing data generated in this study have been deposited in the Gene Expression Omnibus (GEO) under accession “GSE208046”. The ChIP-seq data of NF-kB (GM15510 cell line) are download from ENCODE Data Coordination Center (ENCODE DCC) (http://hgdownload.cse.ucsc.edu/goldenPath/hg19.encodeDCC/wgEncodeSydhTbfs). The ChIP-seq data of MEDI and BRD4 are download from GEO under accession “GSE208046” (https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE160670). The GWAS SNP was collected from the GWAS catalog (https://www.ensembl.org) and UK Biobank GWAS datasets (http://www.nealelab.is/uk-biobank). Source data are provided with this paper.

Code availability
Source code of “MDkNN” pipeline used for TAD DoD calculation can be found on GitHub: https://github.com/GangCaoLab/MDkNN. Source code of “sciDLO Hi-C tools” pipeline used for single-cell DLO Hi-C analysis can be found on GitHub: https://github.com/GangCaoLab/sciDLO.

References
1. Kanherkar, R. R., Bhatia-Dey, N. & Csoka, A. B. Epigenetics across the human lifespan. Front. Cell Dev. Biol. 2, 49 (2014).
2. Zhang, Q. & Gao, X. Epigenetic regulation of the innate immune response to infection. Nat. Rev. Immunol. 19, 417–432 (2019).
3. Calo, E. & Wysoczka, J. Modification of enhancer chromatin: what, how, and why? Mol. Cell 49, 825–837 (2013).
4. Buenrostro, J. D., Giresi, P. G., Zaba, L. C., Chang, H. Y. & Greenleaf, W. J. Transposition of native chromatin for fast and sensitive epigenomic profiling of open chromatin, DNA-binding proteins and nucleosome position. Nat. Methods 10, 1213–1218 (2013).
5. Mourad, R. & Cuvier, O. Predicting the spatial organization of chromosomes using epigenetic data. Genome Biol. 16, 182 (2015).
6. Heintzman, N. D. et al. Histone modifications at human enhancers reflect global cell-type-specific gene expression. Nature 459, 108–112 (2009).
7. Nora, E. P. et al. Spatial partitioning of the regulatory landscape of the X-inactivation centre. Nature 459, 381–385 (2012).
8. Bonev, B. et al. Multiscale 3D genome re-rewiring during mouse neural development. Cell 171, 557–572.e24 (2017).
9. Ulanov, S. V. et al. Order and stochasticity in the folding of individual Drosophila genomes. Nat. Commun. 12, 1–17 (2021).
10. Garg, S. K., Vitvitsky, V., Gendelman, H. E. & Banerjee, R. Monocyte differentiation, activation, and mycobacterial killing are linked to transsulfuration-dependent redox metabolism. J. Biol. Chem. 281, 38712–38720 (2006).
11. Skinner, B. M. & Johnson, E. E. Nuclear morphologies: their diversity and functional relevance. Chromosoma 126, 195–212 (2017).
12. Phanstiel, D. H. et al. Static and dynamic DNA loops form AP-1-bound activation hubs during macrophage development. Mol. Cell 67, 1037–1048.e6 (2017).
13. Chávez-Galán, L., Olleros, M. L., Vesin, D. & Garcia, I. Much more than M1 and M2 macrophages, there are also CD169+ and TCR+ macrophages. Front. Immunol. 6, 263 (2015).
14. Shapouri-Moghaddam, A. et al. Macrophage plasticity, polarization, and function in health and disease. J. Cell. Physiol. 233, 6425–6440 (2018).
15. Kumar, D., Cinghu, S., Oldfield, A. J., Yang, P. & Jothi, R. Decoding the function of bivalent chromatin in development and cancer. Genome Res. 31, 2170–2184 (2021).
16. Minoux, M. et al. Gene bivalency at polycomb domains regulates cranial neural crest positional identity. Science 355, eaaw2913 (2017).
17. Ivashkiv, L. B. Epigenetic regulation of macrophage polarization and function. Trends Immunol. 34, 216–223 (2013).
18. Natoli, G., Ghisletti, S. & Barozzi, I. The genomic landscapes of inflammation. Genes Dev. 25, 101–106 (2011).
19. World Health Organization. Global Tuberculosis Report 2018. (World Health Organization, Geneva, 2018).
20. Shi, C. & Pamer, E. G. Monocyte recruitment during infection and inflammation. Nat. Rev. Immunol. 11, 762–774 (2011).
21. Thye, T. et al. Genome-wide association analyses identifies a susceptibility locus for tuberculosis on chromosome 18q11.2. Nat. Genet. 42, 739–741 (2010).
22. Thye, T. et al. Common variants at 11p13 are associated with susceptibility to tuberculosis. Nat. Genet. 44, 257–259 (2012).
23. Curtis, J. et al. Susceptibility to tuberculosis is associated with variants in the ASAPI gene encoding a regulator of dendritic cell migration. Nat. Genet. 47, 523–527 (2015).
24. Sobota, R. S. et al. A locus at 9q33.3 confers resistance to tuberculosis in highly susceptible individuals. Am. J. Hum. Genet. 98, 514–524 (2016).
25. Chimusa, E. R. et al. Genome-wide association study of ancestry-specific TB risk in the South African Coloured population. Hum. Mol. Genet. 23, 796–809 (2014).
26. Zhang, F.-R. et al. Genomewide association study of leprosy. N. Engl. J. Med. 361, 2608–2618 (2009).
27. Qi, H. et al. Discovery of susceptibility loci associated with tuberculosis in Han Chinese. Hum. Mol. Genet. 26, 4752–4767 (2017).
28. Zheng, R. et al. Genome-wide association study identifies two risk loci for tuberculosis in Han Chinese. Nat. Commun. 9, 4072 (2018).
29. Heinz, S. et al. Transcription elongation can affect genome 3D structure. Cell 174, 1522–1536.e22 (2018).
30. Smale, S. T. Transcriptional regulation in the immune system: a status report. Trends Immunol. 35, 190–194 (2014).
31. Raggi, F. et al. Regulation of human macrophage M1–M2 polarization balance by hypoxia and the triggering receptor expressed on myeloid cells-1. Front. Immunol. 8, 1097 (2017).
32. Labonte, A. C., Tosello-Trampont, A.-C. & Hahn, Y. S. The role of transcriptional regulation in the immune system: a status report. Trends Immunol. 35, 190–194 (2014).
37. Zhang, Z. et al. Robust capturing chromosome conformation using the DLO Hi-C 2.0 method. J. Genet. Genomics 47, 655–658 (2020).

38. Fulco, C. P. et al. Systematic mapping of functional enhancer-promoter connections with CRISPR interference. Science 354, 769–773 (2016).

39. Wang, X.-T., Dong, P.-F., Zhang, H.-Y. & Peng, C. Structural heterogeneity and functional diversity of topologically associating domains in mammalian genomes. Nucleic Acids Res. 43, 7237–7246 (2015).

40. Flyamer, I. M. et al. Single-nucleus Hi-C reveals unique chromatin reorganization at oocyte-to-zygote transition. Nature 544, 110–114 (2017).

41. Tan, L., Xing, D., Chang, C.-H., Li, H. & Xie, X. S. Three-dimensional genome structures of single diploid human cells. Science 361, 924–928 (2018).

42. Xu, F. et al. NF-κB-dependent IFIT3 induction by HBx promotes Hepatitis B virus replication. Front. Microbiol. 10, 2382 (2019).

43. Schaffner, A. et al. Vitamin B12 modulates Parkinson LRRK2 kinase activity through allosteric regulation and confers neuroprotection. Cell Res. 31, 313–329 (2019).

44. Cusanovich, D. A. et al. Massive multiplex single-cell profiling of chromatin accessibility by combinatorial cellular indexing. Science 348, 910–914 (2015).

45. Boyles, A. P., Guinney J., Crawford, G. E. & Furay, T. S. F-Seq: a feature density estimator for high-throughput sequence tags. Bioinformatics 24, 2537–2538 (2008).

46. Xu, W. scHiDO Hi-C tools—decoding the spatial chromatin organization and dynamic epigenetic landscapes of macrophage cells during differentiation and immune activation. Zenodo https://zenodo.org/record/7022431#.YzmbExZ8yPo (2022).

47. Stevens, T. J. et al. 3D structures of individual mammalian genomes studied by single-cell Hi-C. Nature 544, 59–64 (2017).

48. Yang, G. et al. REV-erbo dynamically modulates chromatin looping to control circadian gene transcription. Science 359, 1274–1277 (2018).

49. Schaffner, A. et al. 3D map of the human genome at kilobase resolution reveals principles of chromatin looping. Cell 159, 1665–1680 (2014).

50. Yang, G. et al. Topological domains in mammalian genomes identified by analysis of chromatin interactions. Nature 485, 376–380 (2012).

51. Ramani, V. et al. Model-based analysis of ChIP-Seq (MACS). Nature Biotechnol. 22, 75–79 (2004).

52. Ramani, V. et al. scHiCTools: a computational toolbox for analyzing single-cell Hi-C data. bioRxiv https://www.biorxiv.org/content/10.1101/769513v2 (2020).

53. Ramani, V. et al. Regulation of PD-L1 expression by NF-κB in cancer. Front. Immunol. 11, 584626 (2020).

54. Ramani, V. et al. LRRK2 is a negative regulator of Mycobacterium tuberculosis phagosomal maturation in macrophages. EMBO J. 37, e98694 (2018).

55. Ramani, V. et al. Vitamin B12 modulates Parkinson's disease LRRK2 kinase activity through allosteric regulation and confers neuroprotection. Cell Rep. 29, 313–329 (2019).

56. Cusanovich, D. A. et al. Multiplex single-cell profiling of chromatin accessibility by combinatorial cellular indexing. Science 348, 910–914 (2015).

57. Cusanovich, D. A. et al. Massively multiplex single-cell Hi-C. Nat. Methods 14, 263–266 (2017).

58. Bolger, A. M., Lohse, M. & Usadel, B. Trimmomatic: a flexible trimmer for illumina sequence data. Bioinformatics 30, 2141–2142 (2014).

59. Trapnell, C., Pachter, L. & Salzberg, S. L. TopHat: discovering splice junctions with RNA-Seq. Bioinformatics 25, 1105–1111 (2009).

60. Clamp, M. et al. Ensembl 2002: accommodating comparative genomics. Nucleic Acids Res. 31, 38–42 (2003).

61. Li, H. et al. The sequence alignment/map format and SAMtools. Bioinformatics 25, 2078–2079 (2009).

62. Boyle, A. P., Guinney J., Crawford, G. E. & Furay, T. S. F-Seq: a feature density estimator for high-throughput sequence tags. Bioinformatics 24, 2537–2538 (2008).

63. Zhang, Y. et al. Model-based analysis of ChIP-Seq (MACS). Genome Biol. 9, R127 (2008).

64. Hong, P. et al. The DLO Hi-C tool for digestion-ligation-only Hi-C chromosome conformation capture data analysis. Genes 11, 289 (2020).

65. Durand, N. C. et al. scHiDO Hi-C tools—decoding the spatial chromatin organization and dynamic epigenetic landscapes of macrophage cells during differentiation and immune activation. Zenodo https://zenodo.org/record/7022431#.YzmbExZ8yPo (2022).

66. Schaffner, A. et al. 3D structures of individual mammalian genomes studied by single-cell Hi-C. Nature 544, 59–64 (2017).

67. Yang, G. et al. REV-erbo dynamically modulates chromatin looping to control circadian gene transcription. Science 359, 1274–1277 (2018).

68. Yang, G. et al. 3D map of the human genome at kilobase resolution reveals principles of chromatin looping. Cell 159, 1665–1680 (2014).

69. Yang, G. et al. Topological domains in mammalian genomes identified by analysis of chromatin interactions. Nature 485, 376–380 (2012).

70. Yang, G. et al. scHiCTools: a computational toolbox for analyzing single-cell Hi-C data. bioRxiv https://www.biorxiv.org/content/10.1101/769513v2 (2020).

71. Yang, G. et al. LRRK2 is a negative regulator of Mycobacterium tuberculosis phagosomal maturation in macrophages. EMBO J. 37, e98694 (2018).

72. Yang, G. et al. Vitamin B12 modulates Parkinson’s disease LRRK2 kinase activity through allosteric regulation and confers neuroprotection. Cell Rep. 29, 313–329 (2019).

73. Cusanovich, D. A. et al. Multiplex single-cell profiling of chromatin accessibility by combinatorial cellular indexing. Science 348, 910–914 (2015).

74. Cusanovich, D. A. et al. Massively multiplex single-cell Hi-C. Nat. Methods 14, 263–266 (2017).

75. Bolger, A. M., Lohse, M. & Usadel, B. Trimmomatic: a flexible trimmer for illumina sequence data. Bioinformatics 30, 2141–2142 (2014).

76. Trapnell, C., Pachter, L. & Salzberg, S. L. TopHat: discovering splice junctions with RNA-Seq. Bioinformatics 25, 1105–1111 (2009).

77. Langmead, B. & Salzberg, S. L. Fast gapped-read alignment with Bowtie 2. Nat. Methods 9, 357 (2012).

78. Clamp, M. et al. Ensembl 2002: accommodating comparative genomics. Nucleic Acids Res. 31, 38–42 (2003).

79. Anders, S., Pyl, P. T. & Huber, W. HTPseq—a Python framework to work with high-throughput sequencing data. Bioinformatics 31, 166–169 (2015).

80. Love, M. I., Huber, W. & Anders, S. Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. Genome Biol. 15, 550 (2014).

81. Li, H., Durbin, R. Fast and accurate long-read alignment with Burrows-Wheeler transform. Bioinformatics 26, 589–595 (2010).
experiments. W.X., P.H., D.L., C. Wu, C. Wang, J.M., and X.H. performed data analysis and interpretation. X. Cheng, X. Cao, and A.R. conducted virulent tuberculosis strain H37Rv infection experiment. D.L., G.C., G.L., W.X., P.H., and C. Wu wrote the manuscript, with input from all other authors. R.Z., Z.T., and Z.F. revised the manuscript. G.C. and G.L. supervise the project.

**Competing interests**
The authors declare no competing interests.

**Additional information**

**Supplementary information** The online version contains supplementary material available at https://doi.org/10.1038/s41467-022-33558-5.

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**Peer review information** Nature Communications thanks Minna Kaikkonen, and the other, anonymous, reviewer(s) for their contribution to the peer review of this work. Peer reviewer reports are available.

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**Da Lin, Weize Xu, Ping Hong, Chengchao Wu, Zhihui Zhang, Siheng Zhang, Lingyu Xing, Bing Yang, Wei Zhou, Qin Xiao, Jinyue Wang, Cong Wang, Yu He, Xi Chen, Xiaojian Cao, Jiangwei Man, Aikebaier Reheman, Xiaofeng Wu, Xingjie Hao, Zhe Hu, Chunli Chen, Zimeng Cao, Rong Yin, Zhen F. Fu, Rong Zhou, Zhaowei Teng, Guoliang Li & Gang Cao**

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