Postmitotic differentiation of human monocytes requires cohesin-structured chromatin

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Cohesin is a major structural component of mammalian genomes and is required to maintain loop structures. While acute depletion in short-term culture models suggests a limited importance of cohesin for steady-state transcriptional circuits, long-term studies are hampered by essential functions of cohesin during replication. Here, we study genome architecture in a postmitotic differentiation setting, the differentiation of human blood monocytes (MO). We profile and compare epigenetic, transcriptome and 3D conformation landscapes during MO differentiation (either into dendritic cells or macrophages) across the genome and detect numerous architectural changes, ranging from higher level compartments down to chromatin loops. Changes in loop structures correlate with cohesin-binding, as well as epigenetic and transcriptional changes during differentiation. Functional studies show that the siRNA-mediated depletion of cohesin (and to a lesser extent also CTCF) markedly disturbs loop structures and dysregulates genes and enhancers that are primarily regulated during normal MO differentiation. In addition, gene activation programs in cohesin-depleted MO-derived macrophages are disturbed. Our findings implicate an essential function of cohesin in controlling long-term, differentiation- and activation-associated gene expression programs.

https://doi.org/10.1038/s41467-022-31892-2

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he ring-shaped protein complex cohesin and the CCCTC-binding factor CTCF comprise a core architectural unit of mammalian genomes. They colocalize at DNA loop anchors1,2 and form the boundaries of larger contact domains that spatially separate genes and regulatory elements from each other3–5. Current models suggest a dynamic, cyclic process of cohesin-dependent loop formation, which involves the NIPBL-mediated loading of cohesin to DNA, and the extrusion of loops (confined by CTCF-bound sites in convergent orientation), and the WAPL-mediated release of cohesin6. Cohesin-dependent DNA loop formation and CTCF-mediated insulation of contact domains are generally considered important for regulating the interplay of promoters and enhancers during gene transcription7,8.

While the functional importance of DNA loops and their anchors is well described for individual gene loci4,9–11, their relevance for global transcription control is less well established. Recent studies reported the rapid decay of contact domains as an immediate consequence of acute CTCF or cohesin removal but failed to observe major transcriptional changes12–14, questioning the importance of spatial genome organization for maintaining transcriptional output. However, it is unclear whether these observations, which were made under steady-state conditions, also reflect the relevance of 3-dimensional genome organization in the context of developmental, differentiation, or activated gene expression programs. It has been noted that genes with dynamic transcription (as observed in activation or differentiation processes) are associated with higher contact frequencies as compared to housekeeping genes15. This type of genes may also be more sensitive to cohesin-depletion16,17, highlighting the need for additional models to clarify the relationship between cohesin/CTCF function and transcription regulation.

However, due to the importance of cohesin for cell cycle progression18, studying the relevance of cohesin-dependent loop formation in the more complex settings of differentiation/development and over longer time periods remains difficult. One suitable model that allows the observation of gene-regulatory changes over several days in the absence of cell division is the differentiation of human peripheral blood monocytes (MO). These innate immune cells normally develop from myeloid progenitors in the bone-marrow before they enter the bloodstream as mature and patrolling effector cells19. They present a typical horseshoe-shaped nucleus, which rapidly reshapes upon differentiation into MO-derived cell types like macrophages (MAC) or dendritic cells (moDC). In vitro, this differentiation is accompanied by abundant changes in chromatin accessibility, transcription, or epigenetic landscapes, which all proceed in the absence of proliferation20,21. Notably, mutations in cohesin complex members, including RAD21, have been identified as early events in the pathogenesis of myeloid neoplasms22,23 and cohesin mutations or knockdown of cohesin subunits were shown to impair hematopoietic differentiation and enforce stem cell programs in hematopoietic progenitor cells16,24–26.

Here, we use this naturally post-proliferative primary cell system to study the function of CTCF and cohesin in differentiation. We generate high-resolution in situ Hi-C maps (>700x10^6 interactions per cell type) for human MO, MO-derived macrophages (MAC), and dendritic cells (moDC), and compare the effects of cohesin or CTCF depletion in each model. The present work provides evidence for abundant differentiation-associated changes in the genome organization of these cells and shows that the genes and enhancers affected by cohesin depletion are to a large extent also regulated during normal differentiation. We also show that the functional repertoire of MAC is altered when cohesin is depleted during differentiation. Hence, cohesin is required for the proper execution of differentiation- and activation-associated transcription programs.

### Results

**Genome-wide changes during MO differentiation.** To study the relationship between genome and transcription regulation, we analyzed two phenotypically distinct in vitro culture models for postmitotic human blood MO (Fig. 1a), including their differentiation into adherent, MO-derived macrophages (MAC, cultured for 7 days in the presence of 2% human AB-serum) or non-adherent MO-derived dendritic cells (moDC, cultured for 7 days in the presence of GM-CSF, IL-4 and 10% FCS). In MO and both differentiation endpoints, we initially mapped the global distribution of H3K27ac, a histone mark found at active regulatory elements (via ChIP-seq), accessible chromatin (via ATAC-seq), and transcription (via RNA-seq) for a total of three independent donors. As shown in Fig. 1b, the differentiation of MO into MAC or moDC induced abundant and consistent changes in chromatin accessibility, gene expression, and distribution of H3K27ac, both at the levels of individual peak regions or arrays of peak regions (super-enhancers). We observed characteristic gene expression changes, including the downregulation of known MO-specific genes (e.g. CD300E, NRA2 or VCAN), the upregulation of MAC-specific genes (e.g. VSIG4, CYP19A1 or CHITI) or moDC-specific genes (e.g. CD1A, ALOX15 or CCL13) during differentiation, as shown exemplary in Fig. 1c and in more detail in a heatmap of the top variable genes across the three cell types (Supplementary Fig. 1a). Gene set enrichment analysis across published data sets confirmed cell identities (Supplementary Fig. 1b, c) and cell type-specific gene expression patterns coincided with the cell type-specific distribution of H3K27ac and accessible chromatin (Fig. 1d, e and Supplementary Fig. 1d, peak positions and results of differential gene or peak analysis are provided in Supplementary Data File 1). Both, known and de novo-derived motif signatures at cell type-specific, accessible chromatin or active regulatory regions (Fig. 1f and Supplementary Fig. 1e, f, respectively) as well as their co-association networks were typical for the three cell types, e.g. with EGR2 being induced during differentiation of MAC and moDC20,27, STAT factors being active in moDC (as induced by GM-CSF and IL-4) and KLF signatures being lost in MAC20 (Fig. 1f, g). In addition, differentiation of MO significantly altered the ranking of super-enhancers at several cell type-specifically expressed loci, as shown in Supplementary Fig. 1g, h. This includes e.g., the CIITA transcription factor, which drives the antigen-presentation program in moDC28, CISH, which is required for moDC-mediated CTL activation29, the Natural resistance-associated macrophage protein 130 (encoded by SLCL1A1), which is involved in iron metabolism and host resistance to certain pathogens in MAC, or the ferrodoxin gene locus (FDX1), which is involved in the biosynthesis of steroids31, including Vitamin D (also see Supplementary Fig. 1g, h).

Having established that both differentiation endpoints showed typical features of MAC or moDC differentiation, we captured high resolution chromatin interactions in each of the three states (MO, MAC, moDC) using in situ Hi-C (between 760 x 10^6 and 930 x 10^6 interactions per cell type, quality metrics are shown in Supplementary Fig. 1i–k). In line with the differentiation-induced change in nuclear shape, we observed reproducible changes in interchromosomal interactions between MO and the two differentiated cell types, indicating global rearrangements of chromatin territories32 during differentiation, as also previously observed in plasma cell differentiation33. Figure 1h shows regional differences in ratios of normalized interchromosomal contacts across the genome between MO and MAC. Changes
Fig. 1 Epigenetic, transcriptional and architectural changes during MO differentiation. **a** Schematic of the experimental setup. Since cells served as controls for the siRNA-mediated knockdown of structural proteins, MO were either mock-treated or electroporated with a control siRNA (siCTRL) after isolation and before culture. **b** Multidimensional Scaling (MDS) plots of ATAC-seq, RNA-seq, and H3K27ac ChIP-seq data sets. H3K27ac data are shown either on the levels of individual peak regions (enhancers) or arrays of peaks (super-enhancers). Numbers of differentially accessible sites, expressed genes, or H3K27ac defined enhancers or super-enhancers are indicated. **c** Bar plots showing expression levels (mean normalized RPKM ± SE based on the RNA-seq data; n = 6 control samples from three donors) of typical cell type-associated genes. **d** Histograms of ChIP- and ATAC-seq coverage at differentially active regulatory regions (based on their H3K27ac deposition), centered on overlapping open chromatin. **e** Genome browser tracks for a moDC-specific example region (additional examples in Supplementary Fig. 1d). **f** Motif enrichment (hypergeometric test, Benjamini-Hochberg (BH) corrected) across open chromatin regions shown in (d). **g** Motif co-association networks for regions given in (d). The size of each node represents the motif enrichment (fraction of peaks) and co-associated TF motifs are indicated by coloring. Edge thickness indicates the frequency of motif co-association. **h** Comparative interchromosomal contact map of MO and MAC. Coloring indicates the enrichment of contacts in MO (red) or MAC (blue) across 2.5 Mb windows. **i** Clustering of Pearson correlation values for all significant interchromosomal contacts between individual donors and cell types. **j** In situ Hi-C contact map of MO (lower left) and MAC (upper right) across a 50 Mb interval of chromosome 1. The map represents the average of 3 donor replicates per condition. Corresponding presentations for moDC are given in Supplementary Fig. 11. **k** MDS plot of PC1 differences between cell types. Numbers of significantly different 50 K windows are indicated. **l** Source data are provided as a Source Data file.
were reproducible between donors, as indicated by the correlation matrix of significant interchromosomal contacts shown in Fig. 1i. Notably, interchromosomal contacts were more similar between moDC and MAC. An example map for intrachromosomal contacts is shown in Fig. 1j, indicating ample changes in chromatin compartments (indicated by the first eigenvector (PC1) values) between MO and MAC (corresponding MO/moDC and moDC/MAC comparisons are shown in Supplementary Fig. 1; similarities between individual samples are visualized in Fig. 1k). As also observed on the interchromosomal level, PC1 changes between moDC and MAC were less pronounced (but still significant), suggesting that most higher-order compartment changes occur early during differentiation along both routes.

Correlation of transcriptional and architectural changes. We next tested whether MO differentiation was also associated with changes on the levels of chromatin loops and topology-associated domains (TADs). In total, we detected 2.6 K, 3.6 K, and 3.1 K TADs in MO, moDC, and MAC, respectively, which were frequently associated with co-binding of RAD21 and CTCF (Fig. 2a, positions of TADs and loops, as well as RAD21 and CTCF peak positions are provided in Supplementary Data File 2) at their boundaries (15k bp wide). Insulation scores slightly increased during differentiation (Fig. 2b), in line with previous observations in neuronal differentiation. MO differentiation coincided with a differentiation-associated increase in TAD boundary insulation, as previously suggested. Cohesin-independent boundaries that were associated with H3K27ac or chromatin accessibility were enriched for typical cell type-specific enhancer (PU.1, CEBP, AP1) or general promoter motifs (NRF1, NFY; shown in Supplementary Fig. 2b), suggesting that regulatory elements contribute to TAD boundaries independent of CTCF or cohesin. Comparisons between cell types revealed the strengthening or de novo appearance of TADs during MO differentiation (Fig. 2c, results of differential TAD score analyzes are provided in Supplementary Data File 2), while fewer TADs appeared weakened. TAD boundaries with higher scores in MO or MAC showed accordant changes in insulation scores (Fig. 2d, similar plots for other comparisons are shown in Supplementary Fig. 2c, d).

To globally assess the relationships between architectural changes and gene expression, epigenetic features or transcription factor binding, we adopted gene set testing and asked whether features in a given feature list (e.g. genes or peaks overlapping differential loops or TADs) tend to be differentially expressed or active. Corresponding results for genes associated with TADs either strengthened or weakened during MO to MAC differentiation are presented in the right panel of Fig. 2e. The ranks of TAD-associated genes clearly indicate that TADs strengthened in MAC tend to contain genes that are upregulated in MAC, whereas TADs weakened in MAC tend to contain genes that are downregulated in MAC. Concordant tendencies were also detected in gene set enrichment analyzes of TADs that were different between MO and moDC, or moDC an MAC (adjusted...
enrichment $P$ values are given in the right panel of Fig. 2e). Similar analyzes addressing H3K27ac (both on the level of individual peak regions or super-enhancers) showed that H3K27ac changed in the same direction as TAD scores (Supplementary Fig. 2e, f). Hence, changes in TADs generally correlated with concordant transcriptional or regulatory activity changes, which has also been noted in other systems.

On the level of loops, we detected 11.1 K, 13.1 K, and 15.1 K domains in MO, moDC, and MAC, respectively, which were mostly associated with co-binding of RAD21 and CTCF at their anchor (Fig. 3a), and CTCF motifs were mostly in the preferred sense/antisense orientation (Supplementary Fig. 3a). We also plotted the same distributions across RAD21 and CTCF bound regions that were not associated with anchors of loop domains (Fig. 3b). The comparison of insulation scores between enhancers ranked by their activity, or subdivided by their association with loop anchors, RAD21, or CTCF showed that sites out-side of loop anchors also acted as insulators, suggesting that the in-situ Hi-C approach likely only detected a subset of loop domains. It also showed that insulation at loop anchors generally increased during
MO differentiation, even more pronounced as observed in TADs (Supplementary Fig. 3b). CTCF- and cohesin-independent enhancers at loop anchors showed specific motif signatures with YY1 being the top enriched motif (Supplementary Fig. 3c). This is in line with previous studies implicating this transcription factor YY1 being the top enriched motif (Supplementary Fig. 3c). This is also observed in other comparisons (Fig. 4d, e). Interestingly, loop anchors enriched in MO or MAC showed divergent distributions of insulation scores and directionality indices. While MAC-enriched loop anchors showed typical features of induced structural loops (increased insulation and directionality around the loop anchor), MO-enriched loop anchors showed similar insulation scores in both MO and MAC and only slight difference in directionality between MO and MAC, suggesting that the latter may not represent major structural changes (Fig. 3d). Similarly, differences were observed in moDC (Supplementary Fig. 3c, d).

To study the relationship between gene expression and architectural changes, we tested for the enrichment of genes associated with cell type-specifically strengthened loop domains in cell type-specifically expressed genes. As shown in Fig. 3g, genes associated with strengthened loop domains are also more highly expressed in the same cell type. The same type of relationship was also observed for H3K27ac-marked enhancer candidate regions (Fig. 3h) and super-enhancers (Fig. 3i). Hence, architectural changes during MO differentiation are frequently co-associated with corresponding epigenetic and transcriptional changes. Interestingly, loop domains that were strengthened or established during MO differentiation were enriched in non-coding genes (and depleted in coding genes, see Fig. 3j). However, this did not result in the detectable enrichment of lincRNA expression in MAC (or moDC) (Supplementary Fig. 3g), suggesting that de novo loop formation during MO differentiation is frequently initiated in chromosomal regions that are comparably poor in coding genes. In line with this, we observed a number of loops that were not associated with transcriptional changes but correlated with the appearance of novel regulatory elements (gain of accessibility/H3K27ac, e.g. the loop in the PKHD1L1 locus in Supplementary Fig. 3d marked with an asterisk and examples shown in Supplementary Fig. 3h). While the functional relevance of these loop domains is unclear, their generation suggests that structural changes during differentiation are induced by the generation of novel, differentiation-induced cohesin-loading sites.

Properties of loop anchors of differentiation-associated loop domains. We further studied the properties of loop anchors and correlated differential connectivity with signals for two major boundary factors, the cohesin complex component RAD21, and the transcription factor CTCF. Overlaps between cell type-enriched loop anchors and RAD21 or CTCF binding were strong in MAC or moDC and less pronounced in MO where strengthened loops more frequently lacked evidence for CTCF binding (Fig. 4a and Supplementary Fig. 4a, b). Motif enrichment analyses across CTCF-independent anchor sites (which were often co-marked by H3K27ac) identified motifs corresponding to TFs that were previously also identified in enhancers in MO and MO-derived cells (like PU.1, AP1, and C/EBP, etc., see Fig. 4a, b and Supplementary Fig. 4a–c for other comparisons).

To test whether signals for RAD21 corresponded with altered loop strength, we performed peak set enrichment analyzes. As shown for MO/MAC in Fig. 4c and in summary for all comparisons in Fig. 4d, the strengthening of loops during MO differentiation significantly correlated with higher signals of RAD21 peaks within corresponding loop anchors in the differentiated cell type. The weakening of differential loops during MO differentiation also showed a trend towards higher signals of RAD21 peaks in MO within corresponding anchors (summarized in Fig. 4d). Since we noted a substantial fraction of RAD21 sites not detected as loop anchors in Hi-C, we also analyzed motif signatures across differential cohesin signals (Supplementary Fig. 4d–f). Here, we also observed an enrichment of enhancer-associated TFs in MO, while in MAC, differential RAD21 sites were dominated by CTCF. ChIP-seq coverage for CTCF was less dynamic during MO differentiation and changes were less pronounced compared to RAD21 (Fig. 4a). However, we still observed a similar enrichment of anchor overlapping CTCF peaks in differentially bound peaks (Fig. 4e, f). Interestingly, the percentage of CTCF-bound sites and CTCF motif scores were both lower in MO-specific loop anchors compared to CTCF-bound sites in MAC-specific loop anchors, suggesting that lower affinity CTCF sites are lost during differentiation, while higher affinity CTCF sites are engaged in loop formation in MAC (Supplementary Fig. 4g). Overall, these findings suggest that MO-derived cells lose regulatory loops but gain CTCF-dependent structural loops.

Supplementary Fig. 4h–k, show genome tracks covering example loci, including KLF4 and STK17B, which loosen regulatory loops and down-regulate the expression of both genes during MO differentiation, as well as CYP19A1 and BTG2, where differentiation-induced loop structures correlate with either up- or down-regulation of genes contained within, suggesting that structural loops may not only foster, but also suppress gene regulation, e.g. by isolating genes from regulatory elements outside of the loop. Notably, the relationships between differentiation-associated TAD/loop changes and gene/enhancer activity changes were never uniform. As obvious from the bar code plots shown in Figs. 2e, 3g, 4i, many genes or cis-elements were insensitive to changes in TAD or loop strength. This is in line with the mild effects on gene transcription observed after acute cohesin depletion.

Collectively, the analysis of architectural changes suggest that MO differentiation is associated with rearrangements in higher-order chromosomal territories (correlating with nuclear shape changes). We also observe differentiation-associated changes in TADs and loops that frequently parallel concordant changes in gene expression or enhancer/promoter activity. We also detect fewer CTCF-independent regulatory loops and a large gain of primarily CTCF-dependent structural loops during differentiation (schematically summarized in Fig. 4k).

Architectural effects of cohesin or CTCF knockdown. Our observations in the MO differentiation model suggested that alterations in genome structure correlated well with gene expression changes. To further explore the relationship between both types of changes and to determine, which types of genes required cohesin or CTCF during MO differentiation, we performed functional studies using siRNA-mediated knockdown of RAD21 and CTCF. The experimental setup is schematically depicted in Fig. 5a. To enable transfection of MO, we used specifically designed siRNAs with backbone-modifications that do not activate innate immune cells like human MO. As exemplary shown for MAC, protein levels of CTCF and RAD21 gradually decreased over the 7 day-culture period, reaching knockdown levels of 70–80% for CTCF and 80–90% for RAD21 at day 7 (Supplementary Fig. 5a). On transcript level the knockdown of...
Fig. 4 Differentiation-associated shift from regulatory to structural loops. a Genomic distance distribution of RAD21, CTCF, H3K27ac ChIP-seq, and ATAC-seq coverage at peak-centered loop anchors (divided into four groups, as indicated, and sorted by H3K27ac coverage) that were either strengthened or weakened in MAC compared to MO. Loop anchor size is indicated in the schematic in the top left corner, where the arch indicates the loop domain. Venn diagrams on the left show the overlap between cell-type-specific loop anchors, RAD21, CTCF, and H3K27ac peaks for each set. Top de novo-derived motifs for CTCF-independent loop anchors are given on the right along with the significance of motif enrichment (hypergeometric test, BH adjusted) across open chromatin at CTCF-independent differential loop anchors or CTCF peak areas at CTCF-overlapping domains weakened in MAC (133) and strengthened in MAC (61).

b Balloon plot showing motif enrichment along with color-coded P-values (hypergeometric test, BH adjusted) across open chromatin at CTCF-independent differential loop anchors or CTCF peak areas at CTCF-overlapping domains weakened in MAC (133) and strengthened in MAC (61).

c Summary of P-values (two-sided rotation tests, BH correction for paired tests) for pairwise comparisons between MO, MAC, and moDC indicating that loop formation correlates with cell-type-specific RAD21 signals. d Peak set enrichment of loop anchor-associated CTCF peaks (equivalent to c, d). e, f Peak set enrichment of loop anchor-associated H3K27ac peaks (equivalent to c, d).

i Gene expression

j Summary of P-values (two-sided rotation tests, BH correction for paired tests) for pairwise comparisons between MO, MAC, and moDC indicating that loop formation correlates with cell-type-specific RAD21 signals. d Peak set enrichment of loop anchor-associated CTCF peaks (equivalent to c, d). e, f Peak set enrichment of loop anchor-associated H3K27ac peaks (equivalent to c, d).

k Schematic summary of the observed architectural changes during MO differentiation. Architectural changes were concordant with changes in gene expression or the activity of regulatory elements, both within domains (loops and TADs), as well as at loop anchors. (a, b, d, f, h, j) Source data are provided as a Source Data file.
Fig. 5 Cohesin knockdown affects 3D genome architecture during MO differentiation. a Schematic of the experimental setup. MO were either mock-treated or electroporated with control siRNA (siCTRL) or specific siRNAs (targeting CTCF or RAD21) and cultured in MAC or moDC culture conditions. b Genomic distance distributions of RAD21 and CTCF ChIP-seq coverage at significantly reduced RAD21 peaks upon RAD21 knockdown (left panel) or significantly reduced CTCF peaks upon CTCF knockdown in MAC. c Tracks representing the first eigenvector values (PC1, 50kb resolution) for the indicated cell types. d Effect of the RAD21 or CTCF knockdown on indicated features. Colored bars represent the number of detectable features as percentage of the same feature in control MAC. Numbers represent feature counts in control MAC. e MDS plots comparing TAD and loop score data sets of the indicated conditions. Numbers of significantly different domains are indicated. f Histogram of insulation scores (Ins) and directionality indices (DI) across all loop anchors for siRNA-treated or control MAC. g Distribution of loop sizes for the top 500 domains gained or lost upon RAD21 or CTCF knockdown in MAC. Solid bars of boxes represent the interquartile ranges (25–75%) with an intersection at the median; whiskers represent max/min values; P values: Mann–Whitney U-test, two-sided. (f, g) Source data are provided as a Source Data file.

Both factors was sustained during MO differentiation (RNA levels 7 days after siRNA transfection: MAC RAD21 11%, CTCF 43%, moDC RAD21 18%, CTCF 46%). Knockdown of both factors was also reflected by the reduced genome-wide binding of CTCF and RAD21 as measured by ChIP-sequencing (Fig. 5b and Supplementary Fig. 5b). Since we did not have internal controls for normalization, the effects may actually be stronger (normalization tends to attenuate differences in knock-out settings). However, it is clear that even after 7 days of knockdown, both CTCF and RAD21 were still detectable. Binding patterns suggested that some binding sites were more resistant to degradation/turnover than others, a phenomenon that has also been observed in other systems.

To study the effects of CTCF and RAD21 knockdown on genome architecture, we collected additional epigenetic and transcriptome data (via ATAC-, ChIP-, and RNA-sequencing, peak positions and results of differential gene or peak analysis are provided in Supplementary Data File 3, 4) and captured chromatin interactions in both differentiated states (MAC, moDC) using in situ Hi-C (quality metrics are shown in Supplementary Fig. 5c, d).

As already observed in previous studies in other systems, CTCF and RAD21 knockdown had no significant impact on compartmentalization as measured by the eigenvector values (PC1) (Fig. 5c and Supplementary Fig. 5e). Also, the changes on interchromosomal interactions observed between MO and the two differentiated cell types were not substantially altered in knockdown cells (Supplementary Fig. 5i), suggesting that global rearrangements of chromatin territories that are associated with nuclear shape-changes proceed either independent of CTCF or RAD21 or early during differentiation before the knockdown made an impact. However, we detected abundant architectural changes on the level of local spatial chromatin organization as summarized in Fig. 5d (Supplementary Fig. 5g for moDC, positions of TADs and loops, as well as RAD21 and CTCF peak positions are provided in Supplementary Data File 4) for feature counts and in Fig. 5e for significantly different TAD and loop scores. We observed a global reduction of TADs and loops, which coincided with a loss of insulation at TAD boundaries and loop anchors, particularly in RAD21 knockdown cells (Fig. 5f and Supplementary Fig. 5h). When we compared chromatin loops that were lost upon cohesin or CTCF depletion with those that remained (appearing stronger in RAD21 or CTCF KD samples), we noted significant differences in loop sizes. Upon cohesin depletion, cells tended to lose smaller loop domains while strengthened loops appeared larger (Fig. 5g and Supplementary Fig. 5i). To some extent this was also observed in CTCF-depleted cells, in line with the predicted increase of loop sizes upon CTCF depletion.

An exemplary interaction map (LPL locus on chromosome 8) for MAC and corresponding tracks for epigenome and TF data highlighting these differences is shown in Fig. 6a (for moDC the CCR1 locus is shown in Supplementary Fig. 6a). Changes in loop anchor strength correlated with RAD21 and CTCF binding data upon knockdown — loop anchors of weakened domains were also enriched for weaker ChIP-seq signals of the corresponding factor and loop anchors in strengthened domains remained stable (Fig. 6b, d and Supplementary Fig. 6b, d). Likewise, the signal strength of RAD21 and CTCF peaks flanking strengthened or weakened loop anchors correlated with their signal in rankings between knockdown and control cells (Fig. 6c, e and Supplementary Fig. 6c, e).
suggesting a direct link between the level of CTCF/cohesin occupancy at anchors of loop domains and their strength. The above data clearly suggested different degrees of susceptibility towards CTCF/cohesin loss across loop domains. The comparison between CTCF motif scores (which often correlate with sequence binding affinity) at CTCF binding sites revealed significant differences between anchors of strengthened and weakened loop domains, indicating that differences in binding affinity may contribute to the observed gradient of knock-down susceptibility (Fig. 6f and Supplementary Fig. 6f).

The distribution of insulation scores and directionality indices across anchors of differential loops (Fig. 6g and Supplementary Fig. 6g) suggest that the RAD21 knockdown likely had a much stronger impact than anticipated from the analysis of differential RAD21 peaks or loops, since insulation clearly also dropped at anchors of loop domains that appear strengthened. Hence, while we call differential loops weaker or stronger based on the comparative analysis, in reality (at least for the RAD21 knockdown) they would be better categorized as less or more resistant, respectively. In a direct comparison of differential loop domains, we observed little overlap between anchors of loops that are strengthened upon CTCF or RAD21 knockdown, while anchors of weakened loops overlapped substantially (Fig. 6h and Supplementary Fig. 6h), suggesting some level of redundancy, as expected by the pivotal functions of both CTCF and cohesin in defining loop domains.

**Fig. 6 Properties of loop domain boundaries altered upon cohesin depletion.**

**a** Comparative in situ Hi-C interaction map and corresponding genome browser tracks for the LPL locus on Chr.8. Arrows indicate differential loops. **b, d** Genomic distance distribution across peak-centered loop anchors either strengthened and weakened by RAD21 (in **b**) or CTCF knockdown (in **d**) in MAC, as described in Fig. 4a. **c, e** Peak set enrichment of loop anchor-associated RAD21 (**c**) or CTCF (**e**) peaks. Enrichment of loop anchor-overlapping peaks that were altered by RAD21 or CTCF knockdown is plotted across all peaks ranked by their signal in siRNA-treated versus control MAC. **f** Distribution of CTCF motif scores across the indicated peak sets. Solid bars of boxes represent the interquartile ranges (25–75%) with an intersection at the median; whiskers represent max/min values; P values: Mann–Whitney U-test, two-sided; dotted line: detection threshold. **g** Histograms of insulation scores and directionality indices across differential loop anchor regions. **h** Venn diagrams, P values, and odd ratios for overlaps between loop anchors that are affected by RAD21 or CTCF knockdown in MAC (Fisher’s exact test, two-sided). (b, d, f–h) Source data are provided as a Source Data file.
These analyzes showed that knockdown of RAD21 and CTCF clearly affected chromatin organization during MO differentiation on the levels of TADs and loops, although at different degrees. 3D genome architecture in MO-derived MAC and moDC is most affected upon cohesin depletion with loops on sub-TAD levels losing connectivity.

**Effects of CTCF knockdown on transcriptional landscapes.** We next asked, whether these architectural changes would also affect transcriptional programs. Figure 7a illustrates differences in chromatin accessibility, gene expression and H3K27ac deposition between control MAC and knockdown cells, which were abundant and significant (corresponding data for moDC are shown in Supplementary Fig. 7a). Super-enhancers were only marginally affected (no significantly different SE in comparisons of CTCF KD samples and only few in RAD21 KD). Effects of the CTCF knockdown on MO differentiation were generally more moderate compared to the cohesin knockdown. As indicated by the de novo motif analysis shown in Fig. 7b (Supplementary Fig. 7b for moDC), chromatin accessibility changes in the CTCF knockdown were dominated by CTCF sites, suggesting that the activities of other transcription factors driving MO differentiation were not drastically altered. Accessible sites (as well as H3K27ac regions) lost upon CTCF knockdown, were significantly enriched at loop anchors, but motif composition was not different between
loop anchor-associated and non-associated sites (data shown in Supplementary Tables 3 and 4).

Gene expression profiles indicated the aberrant expression of “non-lineage” genes in MAC upon CTCF-depletion. Prominent examples are marked in gray in the volcano plot in Fig. 7c (corresponding data for moDC are shown in Supplementary Fig. 7c). The observed dysregulation is most likely explained by the weakening of CTCF boundaries, which increases aberrant contacts of these “non-lineage” genes (examples are shown in Fig. 7d). It is known that CTCF can have boundary-independent functions at gene promoters\(^13,14\), hence we tested how genes with CTCF binding at their proximal promoters would be affected by CTCF knockdown. As shown in Fig. 7e (and Supplementary Fig. 7d for moDC), these genes tended to be downregulated upon CTCF knockdown, suggesting that boundary-independent functions of CTCF contribute to the altered transcriptional profiles observed after CTCF knockdown. As shown Fig. 7f (Supplementary Fig. 7e for moDC), the expression of housekeeping genes (as defined by the HRT Atlas\(^45\)) was least affected by the CTCF knockdown and loop domains containing them tended to remain stable upon knockdown. Interestingly, depletion of CTCF mainly affected genes that were normally induced during MO differentiation (Fig. 7g and Supplementary Fig. 7f for moDC). Pathways were only significantly enriched in genes upregulated during knockdown, likely reflecting the aberrant gene induction in these cells (Fig. 7h and Supplementary Fig. 7g for moDC). The complex relationships between CTCF knockdown and associated changes in chromatin interaction profiles and transcription suggest locus-specific contributions of CTCF both as insulator or transcription factor, which may entail divergent effects on transcription upon CTCF knockdown. While the effects of CTCF knockdown were moderate, they were clearly significant. It is likely that changes would increase with a more rapid and complete CTCF depletion, which has so far not been feasible in human primary MO.

Effects of cohesin knockdown on transcriptional landscapes. Notably, the composition of differential accessible chromatin regions was different in RAD21 and CTCF knockdown MAC. Cohesin depletion clearly affected sites that normally gain accessibility during MO differentiation into MAC (Fig. 8a). Accessibility changes were less pronounced in moDC, which may relate to quality issues in moDC ATAC-seq data (Supplementary Fig. 8a, for QC data see Supplementary Table 10). In regions that gained accessibility, we observed an enrichment of NFκB (REL) motifs (Fig. 8a), while regions that were lost upon RAD21 knockdown (as shown in Fig. 8a, b) resembled the MAC-specific signature observed during normal MO differentiation (Supplementary Fig. 1e), except for CTCF. The latter was particularly enriched in loop anchor-associated, accessible regions that were lost upon RAD21 knockdown (compared to corresponding regions not associated with loops, see Supplementary Table 3). Contrasting the CTCF knockdown results, in which CTCF-dependent accessible regions were enriched in loop anchors, cohesin-dependent accessible regions were under-represented at loop anchors (data shown in Supplementary Table 3). Hence, accessibility changes during RAD21 knockdown occur mainly in regulatory elements that were not directly involved in loops that are detected with in situ Hi-C.

During normal differentiation, we had observed several regions for which the appearance of de novo loops coincided with accessibility changes (see Supplementary Fig. 3h), suggesting the creation of novel cohesin-loading sites. Interestingly, while these loops were not generated (as expected) during RAD21 knockdown, differentiation-associated chromatin accessibility changes were still observed (Fig. 8c).

In line with the altered motif signatures in differentially accessible chromatin, RAD21 knockdown in MAC clearly affected gene expression programs during MO differentiation (as indicated by purple and red gene symbols in the volcano plot shown in Fig. 8d). Genes that were downregulated in MAC upon RAD21 knockdown were strongly enriched for genes upregulated during MO differentiation (purple bars and curve in Fig. 8e), while upregulated genes in MAC upon RAD21 knockdown were enriched for genes generally regulated during differentiation (both up and down, green bars and curve in Fig. 8e) as well as some “non-lineage” genes (gray gene symbols in Fig. 8d). Concordantly, the expression of housekeeping genes was least affected by the RAD21 knockdown and loop domains containing them tended to remain stable upon knockdown (Fig. 8f). In line with this, RAD21-strengthened loops tended to be enriched for unaffected genes, while weakened loops tended to be enriched for RAD21 sensitive genes (Fig. 8g) and RAD21 sensitive TADs were significantly enriched for genes downregulated upon RAD21 knockdown (Fig. 8h). Pathway enrichment identified several MAC-relevant pathways (e.g., ECM-receptor interaction, protein digestion or steroid biosynthesis) as being enriched genes affected in RAD21 knockdown (Fig. 8i). Similar observations were also made in moDC (see Supplementary Fig. 8b–g). Hence, the observed transcriptional changes suggested that cohesin depletion significantly affected differentiation-associated regulation.

Changes in transcription programs in MAC were associated with corresponding changes in the activity of gene regulatory elements (as detected by H3K27ac ChIP-seq). Regions with decreased H3K27ac upon RAD21 knockdown showed a significant positive correlation with increased H3K27ac during MO differentiation (Fig. 8j, purple bars and curve), while regions with increasing H3K27ac upon RAD21 knockdown showed a significant negative correlation with decreasing H3K27ac during MO differentiation (Fig. 8j, green bars and curve), further supporting the requirement for cohesin for the normal MO differentiation program (results for moDC are shown in Supplementary Fig. 8h). To find out whether these changes in the enhancer landscapes were associated with characteristic transcription factor signatures, we performed de novo and known motif searches across accessible regions in differential H3K27ac regions. Here, we split regions based on their activity in MO. De novo motif signatures were partially overlapping between weakened and strengthened loops, with ETS (PU.1), CEBP and AP1 motifs being enriched (over genomic background) in all types of differential enhancers (Fig. 8k). In direct comparisons (Fig. 8l), we observed the enrichment of NFκB (REL) and AP1 motifs in strengthened enhancers (in line with signatures observed in differentially accessible sites), while weakened enhancers were enriched in CTCF motifs (Fig. 8l). The latter is in line with a significant overlap of weakened H3K27ac regions with loop anchors (data shown in Supplementary Table 4).

Similar trends were observed in moDC (Supplementary Fig. 8a–j), except for motif signatures. At enhancers strengthened in moDC upon RAD21 knockdown, we observed the enrichment of ETS:IRF (EIRE) and EGR motifs, while weakened enhancers were enriched in CTCF, KLF and AP1 motifs (Supplementary Fig. 8j). Previous work in mouse macrophages demonstrated distinct transcriptional functions of AP1 family members\(^46\). The difference in the distribution of AP1 motifs in strengthened and weakened anchors between MAC and moDC likely points to differing activities of individual AP1 family members in these cell types.

Taken together, our analyzes of genome organization, as well as transcriptional and enhancer landscapes showed that the reduction of cohesin has profound effects on genome structure and transcription programs associated with differentiation.
Fig. 8 Loss of cohesin profoundly affects MO differentiation-dependent transcriptional programs. a Distribution of ATAC-seq signals across differentially accessible sites in control MAC, RAD21 siRNA-treated cells as well as freshly isolated MO. Top de novo-derived motifs for each cluster are given (as in Fig. 7b). Signature motifs that are associated with MO differentiation are highlighted. b Comparative enrichment of known TF motifs across differentially accessible chromatin regions of the indicated comparisons. c Comparative in situ Hi-C interaction maps and corresponding genome browser tracks for example regions of differentiation-associated loops. Arrows mark differential loops that were induced upon MO differentiation. d Volcano plot of genes differentially expressed in control versus RAD21 knockdown MAC, as described in Fig. 7c. e Enrichment of genes that were differentially expressed upon RAD21 KD across all genes ranked by their signal in MO versus MAC. f Analysis of housekeeping genes. Top panel: Gene set enrichment of all genes ranked by their signal in siRNA-treated versus control MAC. Bottom panel: loop set enrichment for domains containing housekeeping genes across all loop domains ranked by their score in siRNA-treated versus control MAC (ES, enrichment score; NES, normalized enrichment score; P not determined due to unbalanced gene-level statistics). g Enrichment of genes in altered loop domains across all genes ranked by their signal in siRNA-treated versus control MAC. h Gene set enrichment of genes in weakened TADs across all genes ranked by their signal in siRNA-treated versus control MAC. i KEGG pathway analysis of genes affected by RAD21 loss in moDC. j Enrichment of H3K27ac-marked regions affected by RAD21 KD across all enhancers ranked by their signal intensity in MO versus MAC. k Genomic distance distributions of H3K27ac and ATAC signals at differential H3K27ac peaks, centered on overlapping open chromatin. Top de novo-derived motifs are presented as in (a). l Motif enrichment across open chromatin associated with H3K27ac peak regions as indicated. P-values were determined using hypergeometric tests in (a, b, i, k, l) or two-sided rotation tests in (e, g, h, j) and adjusted for multiple testing (BH correction) except in (a, h, k). (a, b, i, k, l) Source data are provided as a Source Data file.
Cohesin depletion strongly reduces contact frequencies and the insulation of contact domains which leads to the dysregulation of enhancers and genes that are significantly associated with the normal differentiation process.

Innate activation programs are altered in cohesin-depleted MAC. A hallmark function of MO and MAC is their ability to induce innate inflammatory responses upon pathogen encounter, which are primarily regulated through NFKB-dependent pathways. To investigate whether cohesin depletion during the differentiation of primary human MO would affect the functionality of differentiated MO-derived MAC, we compared the immediate response of control MAC with RAD21 knockdown MAC, both exposed to bacterial lipopolysaccharide (LPS) for 4 h (Fig. 9a). Control MAC and of RAD21 knockdown MAC induced overlapping transcriptional programs (as demonstrated by GSEA in Supplementary Fig. 9a, b), which were, however, quantitatively different. The scatter plot in Fig. 9b depicts LPS-induced changes in control MAC contingent on the differences observed between LPS-stimulated MAC and cohesin-depleted MAC (RAD21 KD). As also seen in the heatmap in Fig. 9c, some genes were consistently less induced in cohesin-depleted MAC, including IL12B and IL10 (examples are given in Fig. 9d, top panel), while the larger fraction of induced genes was more induced in cohesin-depleted MAC (Fig. 9d, bottom panel), including IFNBJ1. Other prominent inflammatory cytokine genes like IL6 and TNF, however, were not significantly altered (Supplementary Fig. 9c). This contrasts with previous observations in mouse bone-marrow derived macrophages (BMM), where the Rad21/- BMM were generally less responsive towards LPS stimulation.16 To allow the side-by-side comparison of altered LPS-responses between macrophages of both species, we re-analyzed the published mouse data and compared gene expression data of both species across orthologous genes. Corresponding heatmaps (Supplementary Fig. 9d, e; either based on the differential human gene set shown in Fig. 9c, or on the original clustering shown in Fig. 2 of Cuartero et al.) as well as gene expression profiles of individual target genes (Supplementary Fig. 9f) highlight the different effects of cohesin loss on LPS response between both species. Hence, innate inflammatory responses are not blunted in cohesin-depleted human MO-derived MAC, as described for mouse BMM, but significantly altered and even partly enhanced.

Discussion

This study provides a comprehensive analysis of cohesin and CTCF functions in differentiating MO. We show that normal postmitotic differentiation of MO entails significant alterations in nuclear organization, including widespread changes in chromatin looping. Both acquired and lost loops were enriched in genes and enhancers that were also regulated during MO differentiation, suggesting that conformational changes are linked to transcriptional changes during MO differentiation. To further evaluate their relationship, we depleted the CCCTC-binding factor CTCF and the cohesin complex component RAD21 during MO differentiation. While the depletion of CTCF had a minor impact on enhancer and transcription landscapes, the knockdown of RAD21 had a more pronounced impact: cohesin depletion resulted in loop dispersion, as well as an altered enhancer and gene activation, which was associated with altered transcription factor signatures. Strikingly, dysregulated genes and enhancers were strongly enriched for those regulated during differentiation. In addition, innate activation programs were altered in cohesin-depleted MO-derived MAC, suggesting that cohesin is required for functional MO differentiation and activation.

The changes during human MO differentiation resemble those observed in other developmental or trans-differentiation models, where architectural changes generally coincided with transcriptional changes. Studies in various differentiation models, including ESC-derived cells, or T cells had identified specific transcription factors that were enriched at cell stage-specific loop anchors. In a leukemia model of monocyte-macrophage differentiation (PMA-treatment of the THP-1 cell line) for example, it was shown that dynamic looping events were preferentially connecting regulatory elements that were enriched in AP1 binding sites in the differentiated state. In our primary cells, CTCF-independent loops were generally enriched for motifs...
corresponding to TFs that were also found in cell-type-specific enhancer regions (including PU.1, AP1, etc.), suggesting that it is not a single factor that drives the formation of dynamic regulatory loops. This is also in line with promoter-capture Hi-C data generated from 17 blood cell types, which found both H3K27ac and H3K4me1 enriched at promoter-interacting sites\(^\text{55}\). In contrast to the THP-1 model, loops that were gained during post-mitotic differentiation into MAC or moDC were to the large extent CTCF-anchored. Some of the observed differences may be attributed to the very high Hi-C sequencing coverage in the published THP-1 study (>five billion reads per cell state), which likely improved the detection of regulatory loops. However, the predominance of structural loops in macrophages may point to biological rather than technical differences between the primary and the cell line model of MAC differentiation. Notably, MO are already postmitotic and functional effector cells and their differentiation covers a much smaller developmental scale compared to stem cell-based models. This is mirrored by the lack of marked A/B compartment switches, which are more common in stem cell differentiation models\(^\text{34,53}\). Interestingly, we observed the appearance of contact loops in regions with low or no transcriptional activity. Many of these de novo loops disappeared upon cohesin-depletion and were characterized by the co-appearance of chromatin accessibility. This suggests that the newly remodelled sites may serve to load cohesin before it extrudes the novel chromatin loops. Remodeling has indeed been functionally associated with cohesin. Mutations in cohesin components or several subunits of the SWI/SNF chromatin-remodeling complex are associated with neurodevelopmental disorders in humans that present overlapping clinical phenotypes\(^\text{54}\). More recent work in neutrophils showed that both ATP-dependent chromatin remodelers BRG1 and BRM were required for the Calcium-induced recruitment of the cohesin loading factor NIPBL to promoter-distal elements\(^\text{55}\). Given that the majority of chromatin loops acquired during MO differentiation were structural and confined by a pair of convergent CTCF sites, it is conceivable that they were created via novel loading sites that became available during MO differentiation. In such a scenario, cohesin loading would follow the action of transcription factors that recruit remodeling complexes and other cofactors like NIPBL to rewire loop architecture during differentiation.

The observed mild phenotype of CTCF-depletion during MO differentiation is in line with a recent study demonstrating minor transcriptional effects of Auxin-mediated CTCF depletion during CEBPA-induced transdifferentiation of B-cells into macrophages\(^\text{56}\), suggesting that CTCF (and its function as an insulator) may be dispensable for MO differentiation. However, given the incomplete removal of CTCF via the siRNA approach and the presence of (at least partially) knockdown-resistant CTCF sites\(^\text{38}\), we cannot rule out that complete depletion of CTCF (although difficult to achieve in primary MO) may have a stronger impact in the primary differentiation model. It should generally be noted that the knockdown approach used in the present study entails the typical siRNA-inherent limitations. While we can effectively reduce mRNA levels, we are unable to control the availability of the targeted proteins, which is entirely dependent on their degradation rate. Hence, the effects of knockdown will gradually increase with protein turnover and will be less evident early after culture, where protein levels for CTCF and RAD21 are less affected. In addition, we observe a mixture of cohesin or CTCF sites that are either sensitive or (at least partially) resistant to knockdown, a phenomenon that has also been observed in earlier studies in other systems\(^\text{37,38}\). Consequently, early events during MO differentiation likely proceed normally. This may include the early changes in nuclear shape (and the corresponding territorial changes), which were not different between control and knockdown cells, as well as early changes in cohesin-mediated looping. Given the relatively long culture (7 days) it is also likely that some of the effects observed on the level of chromatin accessibility or transcription regulation are secondary. A further limitation in our study is the lack of internal controls (e.g. RNA spike-in controls, or barcoded control histones for H3K27ac ChiP assay) which could have improved data normalization. Despite these limitations, this work clearly shows that sufficient cohesin levels are required for normal MO differentiation. As previously noted\(^\text{11}\), the observed sensitivity towards cohesin loss was limited to a subset of genes expressed in MO and MO-derived cells. Our analyses further suggest that genes affected by cohesin depletion are enriched for dynamically regulated genes and concurrently depleted for housekeeping genes, which is in line with the observation that housekeeping genes are generally less connected to distal enhancers compared to regulated, dosage-dependent genes\(^\text{12}\).

Along the same lines, we also show that cohesin-depleted MAC display a strongly altered innate immune activation profile. Here, genes induced by the inflammatory stimulus may be affected on various levels: 1) Their response may be affected by the altered chromatin conformation and interaction landscapes in MAC that were differentiated in the presence or absence of cohesin. 2) Their response may also be altered due to secondary alterations in signaling networks required to maintain the normal response. 3) Their response may be directly affected by cohesin depletion, which may prevent the formation of LPS-induced loops that are necessary for a normal response. Interaction landscapes can rapidly change during macrophage stimulation, as exemplified by conformation changes observed after 2 h of IFN\(\beta\) stimulation in mouse macrophages\(^\text{37}\) or 2 h of LPS treatment of THP1 cells\(^\text{58}\), NFkB and AP-1, the main TF driving the early responses to pathogen-associated molecular patterns like LPS, frequently bind promoter-distal sites\(^\text{59}\) and may require cohesin to properly interact with their target promoters during activation. Interestingly, in our human post-proliferative MO differentiation model, the changes include both the up- and down-regulation of inflammatory response genes. This is in contrast with previous observations in mouse MAC\(^\text{16}\), where cohesin depletion generally blunted inflammatory gene activation. One possible explanation for this difference may be the higher level of IFN\(\beta\)1 expression in cohesin-depleted human MAC, while the counterpart in mice appeared deficient in IFN signals and IFN\(\beta\) treatment was able to partially restore the inflammatory response in cohesin-depleted mouse MAC\(^\text{16}\). Some of the differences may also be attributed to species divergent LPS responses, as previously described\(^\text{60}\).

In conclusion, we demonstrate that the siRNA-mediated depletion of cohesin interferes with normal human MO differentiation as well as innate immune functions of human MO-derived macrophages. The expression of housekeeping genes appears to be less affected by cohesin depletion, while the expression of dynamically regulated genes (differentiation-associated or activation-induced) was more dependent on cohesin. Our work also implies that MO and their progeny derived from cohesin-mutated myeloid precursors may have defects both in differentiation and activation programs, which may contribute to disease pathologies.

**Methods**

**Cells.** Collection of blood cells from healthy donors was performed in compliance with the Helsinki Declaration. All donors signed an informed consent. Blood sampling, the leukapheresis procedure, and subsequent purification of peripheral blood monocytes were approved by the ethical committee of the University of Regensburg (reference number 12-101-0260). Donors received an expense allowance, as approved by the ethical committee. Blood MO were separated by leukapheresis of male healthy donors followed by density gradient centrifugation over

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NATURE COMMUNICATIONS | https://doi.org/10.1038/s41467-022-31892-2 | www.nature.com/naturecommunications
Mix (81.1 µl 20, 10 mM Tris pH 8, 1 mM EDTA). Beads were resuspended in 100 µl 1x B&W buffer (2x B&W buffer: 10 mM Tris/HCl, pH 7.5, 1 mM MgCl2, 0.1% Triton X-100). For LPS stimulation, 2 × 106 mock electroporated cells were washed twice with 150 µl 0.5 M EDTA, beads were collected on a magnet and the supernatant was discarded. Beads were resuspended on ice in 48 µl ice-cold ligation mix (22.5 µl water, 25 µl 2x T4 DNA ligase buffer (Enzymatics), 0.5 µl 10% Tween 20, and 0.8 µl 25 µl Bioext Nextra DNA sequencing adapters were added per 20 µl reaction (0.1 µl of the bead suspension in a 50 µl reaction with NEB-Nextra CTX PCR master mix (NEB) and 0.5 µM each Hi-C forward and reverse primers (Euromics; Hi-c fwd: AATGATACGGGCGACCACGAC, Hi-C rev: CCAACGAGAGCGGGATACG). Libraries were collected on a magnet and purification of the amplified DNA was carried out with magnetic beads (Agencourt AMPure XP) in a 1:1.6 ratio. Purified samples were eluted in 20 µl of EB. Quality of the generated Hi-C libraries was analyzed using the High Sensitivity D1000 ScreenTape Kit (Agilent) and concentration was determined using the Qubit High Sensitivity dsDNA Kit (Thermo Fisher Scientific). Libraries were sequenced 42 base pair-end on an Illumina NextSeq500. Sequencing libraries are listed in Supplementary Table 6.

In situ Hi-C

In situ Hi-C was essentially performed as described with slight modifications. For robustness, three biological replicates of three independent donors were used for each condition. Cells were pelleted by centrifugation and crosslinked in 1% formaldehyde in PBS for 10 min at room temperature. The reaction was quenched by adding 1/20th volume of 2x PBS and, fixed cells were washed twice with ice-cold 0.5% BSA in PBS. Aliquots of 2 M cells each were snap-frozen and stored at –80 °C. After thawing cell pellets on ice, nuclei were isolated by resuspending the cell pellet in 200 µl wash buffer 1 (50 mM Tris/150 mM NaCl, 20 mM EDTA, 0.5% SDS, 1x protease inhibitor cocktail (Roche)). Nuclei were incubated at 62 °C for 7 min in a PCR cycler and spun down at 2500 × g for 5 min at room temperature. Most of the supernatant was discarded, leaving the nuclei in 10 µl of liquid. Samples were resuspended in 245 µl reaction buffer (25 µl 10× Triton X-100, 25 µl Dpn II buffer (NEB), 195 µl water) and rotated at 37 °C for 15 min.

ChIP-seq library preparation

Chromatin immunoprecipitation (ChIP) was performed in biological replicates of three independent donors as described previously with slight modifications. Chromatin for all ChIP-seq experiments of siRNA transfected cells was harvested 7 days after transfections, while chromatin from cells that were differentiated after purification with NEB-Nextra CTX PCR master mix (NEB) was harvested 5 days after transfection. Chromatin was sheared using the Qubit dsDNA HS Kit (Thermo Fisher Scientific). Libraries were sequenced 42 base pair-end on the Illumina NextSeq500. Illumina sequencing libraries are listed in Supplementary Table 6 (H3K27ac, 8 (RAD21), and 9 (CTCF). Immunoblotting

For immunoblotting of whole-cell extracts, 2 × 106 cells were lysed in 120 µl 2x SDS-Lysis Buffer (20% Glycerin, 4% SDS, 10% 2-mercaptoethanol, 0.107 M Tris pH 6.8, 0.02% bromophenol blue) and heated at 95 °C for 5 min. SDS-PAGE was performed using a 10% polyacrylamide gel (Biorad #4561035) at 120 V for 1 h and protein was semi-dry blotted on a PVDF membrane at 11 V for 1 h. Blots were blocked for 1 h at RT with 5% milk powder and antibody incubation was conducted overnight at 4 °C using anti-CTCF (CST, #2899), 1:500 anti-RAD21 (Abcam, ab992) at 1:1000 dilution, following five washing steps, goat-anti-rabbit HRP conjugated secondary antibody (Dako P0448) was added in a 1:2500 dilution and incubated for 1 h at RT. After washing, bands were detected using ECL reagent (Amerham) and Fusion Pulse Detection System (Vilber). Blots were stripped using 1:10 diluted Reblot Plus mild solution (Merck). Actin was stained with anti-actin (Sigma-Aldrich, A2066) antibody in 1:2500 dilution for 30 min at RT and detection was performed as described above. Densitometric analyses were conducted in ImageJ V.1.51.

ATAC-seq

ATAC-seq was carried out as described before. For robustness, three biological replicates comprising three independent donors were used for each condition. Briefly, cells were harvested after 7 days of culture and treated in culture medium with DMSO (Sigma) at a final concentration of 200 µM for 30 min at 37 °C prior to transfection. After DMSO 1 treatment, cells were washed twice with ice-cold PBS and cell viability and the corresponding cell count was assessed. For each ATAC reaction 5 × 106 cells were aliquoted into a new tube and washed five times with 50 × 0.5 M EDTA, beads were collected on a magnet and the supernatant was discarded. The cell suspension was resuspended in 100 µl Blunting Mix (81.1 µl water, 0.5% 10% Tween 20, 10 µl 100× T4 DNA ligase buffer (Enzymatics), 4 µl 10 mM DNTP, 2 µl (6 U) T4 DNA polymerase (Enzymatics), 0.4 µl (2 U) Klenow fragment (Enzymatics), 2 µl (20 µl) T4 polynucleotide kinase (Enzymatics)) and incubated for 30 min at 37 °C in a PCR cycler. Reactions were stopped by adding 2.5 µl 5x EDTA. Beads were collected on a 1.5 ml 1× B&K containing 0.1% Triton X-100, and once with 180 µl TET. Beads were resuspended in 50 µl a T-sail Mix (41.3 µl water, 0.5% 10% Tween 20, 5 µl NEBuffer 2, 0.2 µl 100 mM DAP, 3 µl (15 U) Exo-Kleno (Enzymatics) and incubated for 7 days at 37 °C in a PCR cycler. Reactions were stopped by adding 1.5 µl 0.5 M EDTA, beads collected on a magnet and the supernatant was discarded. Beads were resuspended on ice in 48 µl ice-cold ligation mix (22.5 µl water, 25 µl 2x T4 DNA ligation buffer (Enzymatics), 0.5 µl 10% Tween 20, and 0.8 µl 25 µl Bioext Nextra DNA sequencing adapters were added per 20 µl reaction (0.1 µl of the bead suspension in a 50 µl reaction with NEB-Nextra CTX PCR master mix (NEB) and 0.5 µM each Hi-C forward and reverse primers (Euromics; Hi-c fwd: AATGATACGGGCGACCACGAC, Hi-C rev: CCAACGAGAGCGGGATACG). Libraries were collected on a magnet and purification of the amplified DNA was carried out with magnetic beads (Agencourt AMPure XP) in a 1:1.6 ratio. Purified samples were eluted in 20 µl of EB. Quality of the generated Hi-C libraries was analyzed using the High Sensitivity D1000 ScreenTape Kit (Agilent) and concentration was determined using the Qubit High Sensitivity dsDNA Kit (Thermo Fisher Scientific). Libraries were sequenced 42 base pair-end on an Illumina NextSeq500. Sequencing libraries are listed in Supplementary Table 6.
cells. Lysis was washed out with 1 ml of ATAC-BSB buffer containing 0.1% Tween-20. Nuclei were pelleted at 500 x g for 10 min at 4 °C in a fixed angle centrifuge. The supernatant was discarded carefully, and the cell pellet was resuspended in 50 µl of transposition mixture (25 µl 2x TD buffer, 2.5 µl transposase (100 nM final; Illumina), 16.5 µl PBS, 0.5 µl 1% digitonin, 0.5 µl 10% Tween-20, 5 µl H2O) by pipetting up and down 6 times. The reaction was incubated at 37 °C for 30 min with mixing (1000 rpm), before the DNA was purified using the Monarch PCR & DNA Cleanup Kit (NEB) according to the manufacturer's instructions. Purified DNA was eluted in 20 µl of EB, and 10 µl of the purified sample was objected to a 10 cycle PCR amplification using Nextera i7- and i5-index primers (Illumina). Purification and size selection of the amplified DNA was carried out with magnetic beads (Agencourt AMPure XP). For purification the ratio of i5 to i7 beads was set to 1:1.8, whereas for size selection ratio was set to 1:0.55. Purified samples were eluted in 15 µl of EB. The quality and concentration of the generated ATAC-libraries were analyzed using the High Sensitivity D1000 ScreenTape Kit (Agilent) and concentrations were assessed with the Qubit dsDNA Assay Kit (Life Technologies). The quality of dsDNA libraries was analyzed using the High Sensitivity D1000 ScreenTape Kit (Agilent) according to the manufacturer’s instructions. The concentration and quality of the purified RNA was analyzed using the RNA ScreenTape Kit (Agilent). Generation of dsDNA libraries for Illumina sequencing from total cellular RNA was carried out using the TruSeq Stranded Total RNA Kit (Illumina) or the Scriptquant Total RNA Kit (Agilent) according to the manufacturer’s instructions. The quality of dsDNA libraries was analyzed using the High Sensitivity D1000 ScreenTape Kit (Agilent) and concentrations were assessed with the Qubit dsDNA HS Kit (Thermo Fisher Scientific). Sequencing was performed using the Illumina NextSeq550 sequencer and libraries are listed in Supplementary Tables 11 and 12. Accession numbers of published CAGE-seq data for human MO, moDC, and MAC are listed in supplementary Table 13 and additional published human RNA-seq data for human MO, moDC, and MAC are listed in Supplementary Table 14. Published and reanalyzed RNA-seq data for mouse bone marrow-derived macrophages (wildtype and Rad21-deficient, LPS stimulated and unstimulated) are listed in Supplementary Table 15.

RNA-seq library preparation. Total cellular RNA was isolated from MO, MAC, and moDC (using TruSeq Total RNA LPS, or sirNA-treated) using the RNeasy Mini Kit (Qiagen) according to the manufacturer’s instructions. The concentration and quality of the purified RNA was analyzed using the RNA ScreenTape Kit (Agilent). Generation of dsDNA libraries for Illumina sequencing from total cellular RNA was carried out using the TruSeq Stranded Total RNA Kit (Illumina) or the Scriptquant Total RNA Kit (Agilent) according to the manufacturer’s instructions. The quality of dsDNA libraries was analyzed using the High Sensitivity D1000 ScreenTape Kit (Agilent) and concentrations were assessed with the Qubit dsDNA HS Kit (Thermo Fisher Scientific). Sequencing was performed using the Illumina NextSeq550 sequencer and libraries are listed in Supplementary Tables 11 and 12. Accession numbers of published CAGE-seq data for human MO, moDC, and MAC are listed in Supplementary Table 13 and additional published human RNA-seq data for human MO, moDC, and MAC are listed in Supplementary Table 14. Published and reanalyzed RNA-seq data for mouse bone marrow-derived macrophages (wildtype and Rad21-deficient, LPS stimulated and unstimulated) are listed in Supplementary Table 15.

Hi-C analysis. The Hi-C data was primarily performed using the pipeline implemented in the HOMER package (v4.11) as described in Materials and methods. Samples and total read coverage are summarized in Supplementary Table 6. Raw sequencing reads were trimmed using homotools with options “trim –3 GATC -matchStart min 20 min 20” before individual alignment to the reference chromosomes of the human genome (GRCh38.p10, release 27, www.gencodegenes.org) using bowtie2. Mapped reads were paired and collected in HOMER style TagDirectories. TagDirectories for further analysis, filtered TagDirectories were generated using options “–tbp 1 -restrictionSite GATC -both -genomic hg38 -removePEbg -removeSelfLigation -removeSpikes 10000 5”. Quality measures including the fractions of unique read pairs after filtering of local interactions, fractions of interchromosomal read pairs, total contacts after filtering and fraction of interactions with an expected distance determined by HOMER were plotted in Supplementary Figs. 1-k, 5-c, d. For downstream analyses, filtered TagDirectories of control cell populations (mock, siCTRL) or siRNA treatments (RAD21 or CTFCF) were combined for each donor. For the initial detection of inter-chromosomal interactions, we merged donor tagTagDirectories. The analyzeHiC program of HOMER was used to detect significant inter-chromosomal interactions of each cell type at a resolution of 2.5 Mb. Log ratios of inter-chromosomal interaction matrices of MO and MAC (as shown in Fig. 1b) were calculated donor-wise in R, averaged across donors and plotted using the image function in R. Pearson correlations across merged significant interactions of individual cell types and donors were calculated and correlation matrices shown in Fig. 1a and Supplementary Figure 5f. The cell types were clustered and plotted using the heatmap.2 function in R. The multidimensional scaling (MDS) plot of PC1 data shown in Fig. 1k was generated using the plotMDS function in edgeR. HOMER’s findTADsAndLoops.pl program and parameters “-find -res 2500 -window 50000 -genome hg38” and filtered TagDirectories. PC1 tracks as shown in Figs. 1, 3, and Supplementary Figure 11. 5e represent averages of three biological replicates. The transposition patterns for chromosome 3 shown as osmograms in Fig. 5b, 6a and Supplementary Figures 11, 3d, 6a were generated donor-wise using the batch-MakeHiCMatrix.pl program and parameter “-split” or in Figs. 7d and 8c using parameters “-split -rotate -frac .5”, averaged across donors and plotted using the image function in R. The multidimensional scaling (MDS) plot of PC1 data shown in Fig. 1k was generated using the plotMDS function in edgeR. HOMER’s findTADsAndLoops.pl program and parameters “-find -res 2500 -window 50000” and “-find -res 3000 -window 15000” were used to initially identify TADs and Loops for merged data sets of individual cell types. TADs and Loops were merged using HOMER’s merge2Bed.pl function and then scored for interactions in replicate donor samples using findTADsAndLoops.pl and the option “-score”. For comparisons of siRNA treated samples, we also calculated scores using the parameter “-normTotal 5000000000” to normalize for total interactions. Size factors for normalization in DeSeq2 (v2.13.0) were then calculated by dividing total scores from scoring without interaction normalization by total scores derived from interaction normalized scoring. MDS plots of TAD scores in Figs. 3, 5, 8, and 16 were paired and collected in HOMER style TagDirectories for further analysis. Filtered TagDirectories were analyzed using the high sensitivity D1000 screenTape kit (Agilent) and concentrations were assessed with the qubit dsDNA HS kit (Thermo Fisher Scientific). Sequencing was performed using the illumina nextseq550 sequencer and libraries are listed in Supplementary Tables 11 and 12. Accession numbers of published CAGE-seq data for human MO, moDC, and MAC are listed in supplementary Table 13 and additional published human RNA-seq data for human MO, moDC, and MAC are listed in Supplementary Table 14. Published and reanalyzed RNA-seq data for mouse bone marrow-derived macrophages (wildtype and Rad21-deficient, LPS stimulated and unstimulated) are listed in Supplementary Table 15.

ChIP-seq analysis. Reads (single-end) were aligned to the human genome (GRCh38/p10) using bowtie2 in very sensitive mode. Lower quality alignments were removed by filtering reads with mapQ <= 10. Only reads mapping to single unique genomic locations were selected into 500000000 tracks as shown in Figs. 2c, 3e, 5e. Total cellular RNA was isolated from MO, MAC, and moDC using the TruSeq Stranded Total RNA Kit (Illumina) or the Scriptquant Total RNA Kit (Agilent) according to the manufacturer’s instructions. The quality of dsDNA libraries was analyzed using the High Sensitivity D1000 ScreenTape Kit (Agilent) and concentrations were assessed with the Qubit dsDNA HS Kit (Thermo Fisher Scientific). Sequencing was performed using the Illumina NextSeq550 sequencer and libraries are listed in Supplementary Tables 11 and 12. Accession numbers of published CAGE-seq data for human MO, moDC, and MAC are listed in Supplementary Table 13 and additional published human RNA-seq data for human MO, moDC, and MAC are listed in Supplementary Table 14. Published and reanalyzed RNA-seq data for mouse bone marrow-derived macrophages (wildtype and Rad21-deficient, LPS stimulated and unstimulated) are listed in Supplementary Table 15.

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Ranger Tomita is a PhD student at the University of Cambridge. He is interested in the field of synthetic biology and is working on developing new tools for genome editing. He is currently working on a project that aims to create a new type of CRISPR-Cas9 system that can be used to edit DNA in a more efficient and precise manner. He is also interested in the application of synthetic biology in the field of medicine, particularly in the context of personalized medicine.
heatmap.2 function of the gplots package in R. Gene set enrichment analyzes using individual gene sets were performed using the function fry of the limma package (v3.46.0) in R. Corresponding bar code representations (as shown in Supplementary Fig. 1b, c) were plotted using the barcodeplot function of the limma package (v3.46.0), and bar plots of adjusted enrichment P values were plotted using standard functions in R (Figs. 2e, 3g, 4i).

Gene set enrichment analyzes using gene sets in the ImmuneSigDB subset of the MSigDB (retrieved as described in Supplementary Note 5) were performed using the function camera of the limma package (v3.46.0) in R. Corresponding bar code representations (as shown in Supplementary Fig. 1b, c) were plotted using the barcodeplot function of the limma package (v3.46.0). For the presentation of expression levels of selected genes as shown in Fig. 1c, Figs. 9d, and Supplementary Figs. 2a, 9c, f, normalized and batch-corrected expression data were corrected for transcript length and plotted using the ggplot2 (v3.3.3) package in R. Statistically significant enriched KEGG pathways were identified using the kegg function in limma (v3.46.0) and balloon plots of significance levels shown in Figs. 7h, 8i and Supplementary Figs. 7g, 8g were generated using the ggplot2 (v3.3.3) package in R. The scatter plot shown in Fig. 9b was also generated using the ggplot2 (v3.3.3) package in R. For comparisons of human and mouse expression data (as presented in Supplementary Fig. 9d, e), orthogonal gene pairs were identified using the getLDS function of the biomartR (v2.46.1) package, and data was scaled independently for each species.

Generation of read coverage tracks. HOMER was used to generate sequencing-depth normalized bedGraph/bigWig files of ChIP-seq and ATAC-seq data (using standard parameters for ChIP and a fixed fragment length of 65 bp for ATAC). For visualization purposes and equivalent to the normalization of read count data, ATAC-seq and H3K27ac ChIP-seq tracks from individual donors were scaled based on the total number of reads (5000 to 40,000) for each track. The normalized bedGraph files were averaged across all samples and normalized BigWigs files from replicate data sets were averaged using the program bigWigMerge and dividing the count data by the number of samples. Sequencing-depth normalized bedGraph/bigWig files of RAD21 and CTCF ChIP-seq runs were averaged as above (without prior scaling), except for the knockdown data sets, which were merged on tagDirectory level before the generation of sequencing-depth normalized bedGraph/bigWig files. BedGraph files were converted to BigWig using the program bedGraphToBigWig. Tracks and loop data for selected regions as shown in Figs. 1e, j, 3c, d, 6a, 7d, 9c and Supplementary Figs. 1d, 1h, 4h–k, and 6a were visualized using the pyGenomeTracks software (v3.5). Figures were assembled and formatted in Adobe Illustrator (v2.5.21).

Data availability

The raw sequencing data generated in this study are deposited with EGA (study accession number is EGAS00000105508). The human sequencing data are available under restricted access and can be obtained by qualified researchers. Processed data files (bigwig tracks and peak files for ATAC and ChIP, interactions for HiC data and read count tables for RNA-seq data) are deposited with ArrayExpress (accession numbers: E-MTAB-10845, E-MTAB-10846, E-MTAB-10848, E-MTAB-10849). [https://www.ebi.ac.uk/arrayexpress/experiments/E-MTAB-XXXX/, etc.]. The source data underlying Figs. 1c, f, g, i, k, 2b, d, e, 3f–i, 4a, b, d, f, h, 5f, 6b, d, f–h, 7b, e, f, h, 8a, b, i, k, 1b–d and Supplementary Figs. 1a–c, e, f, i, k, 2a–f, 3a–c, e, g–a, 4a–g, 5a, c, f, h, i, 6b, d, f–h, 7b, 8g, 9c–f are provided as a Source Data file. Source data are provided with this paper.

Received: 15 June 2021; Accepted: 6 July 2022; Published online: 25 July 2022

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1. Wendt, K. S. et al. Cohesin mediates transcriptional insu... NATURE COMMUNICATIONS | https://doi.org/10.1038/s41467-022-31892-2 | www.nature.com/naturecommunications

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Acknowledgements

We thank Sven Heinz (UCSD, San Diego) for sharing protocols. Hi-C-, ChIP-, ATAC- and RNA-sequencing were conducted at the NGS Core of the Leibniz Institute for Immunotherapy (LIT, University Regensburg and University Medical Center Regensburg, Germany). This study was funded by a grant of the Else Kröner-Fresenius-Stiftung to M.R. (2014_A223), and a SPP2202 Priority Program grant to C.G. by the Deutsche Forschungsgemeinschaft (GE 3202/1-1).

Author contributions

M.R. designed the study with contributions by J.M., C.G., and A.F.; J.M. performed most experiments with contributions from A.F., K.Ma., K.Me., M.N., J.R., H.S., U.A., and C.G.; R.M. contributed to data acquisition; M.R. analyzed sequencing data with help from J.M.; M.R. wrote the original draft with contributions from all authors.

Funding

Open Access funding enabled and organized by Projekt DEAL.

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/s41467-022-31892-2.