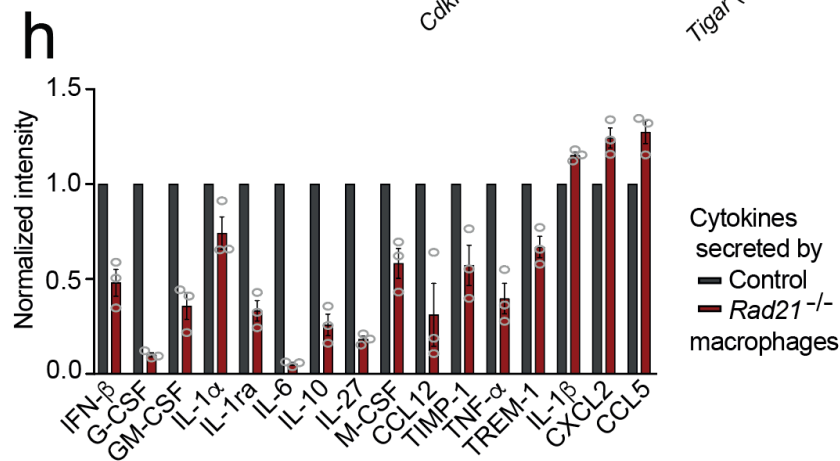
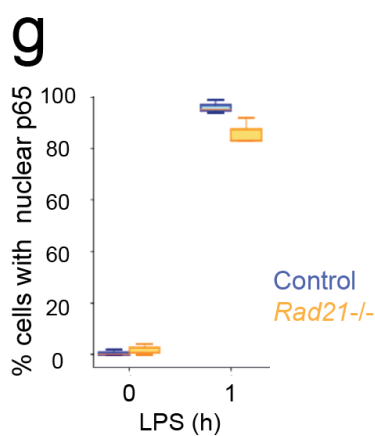
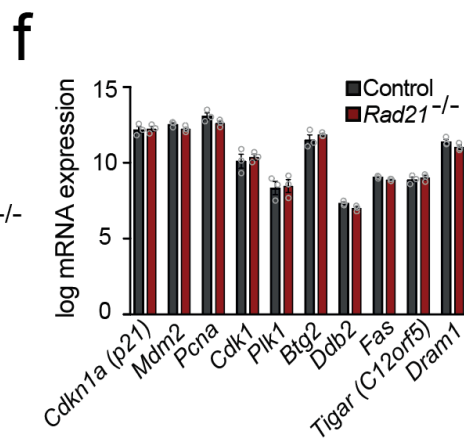
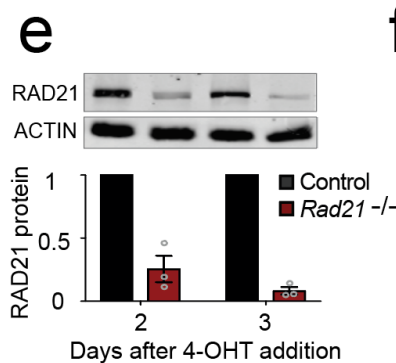
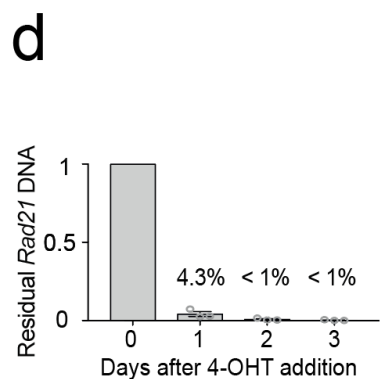
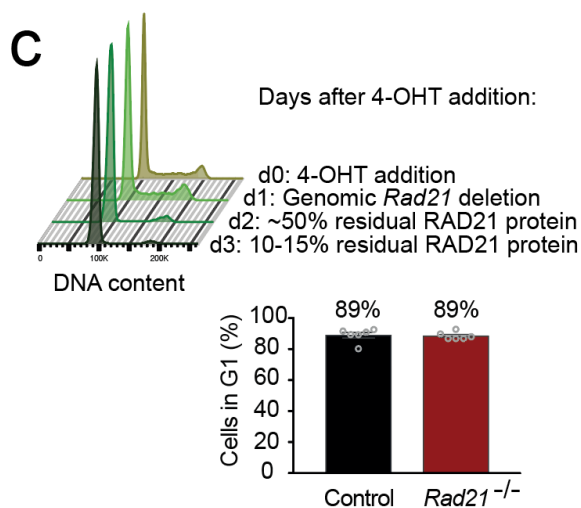
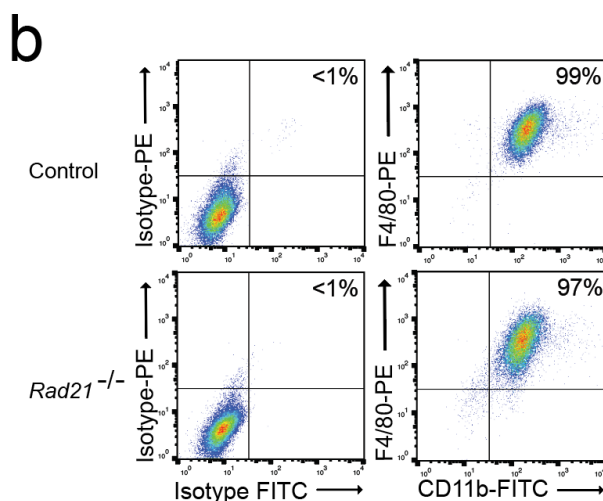
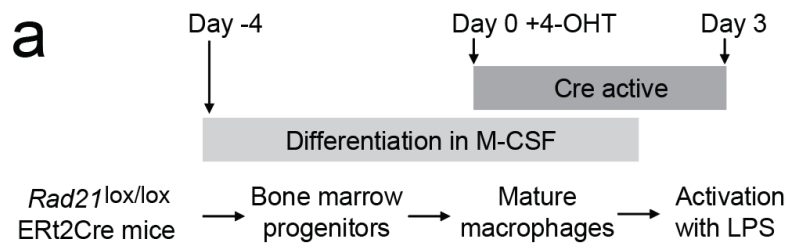


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Control of inducible gene expression links cohesin to hematopoietic progenitor self-renewal and differentiation

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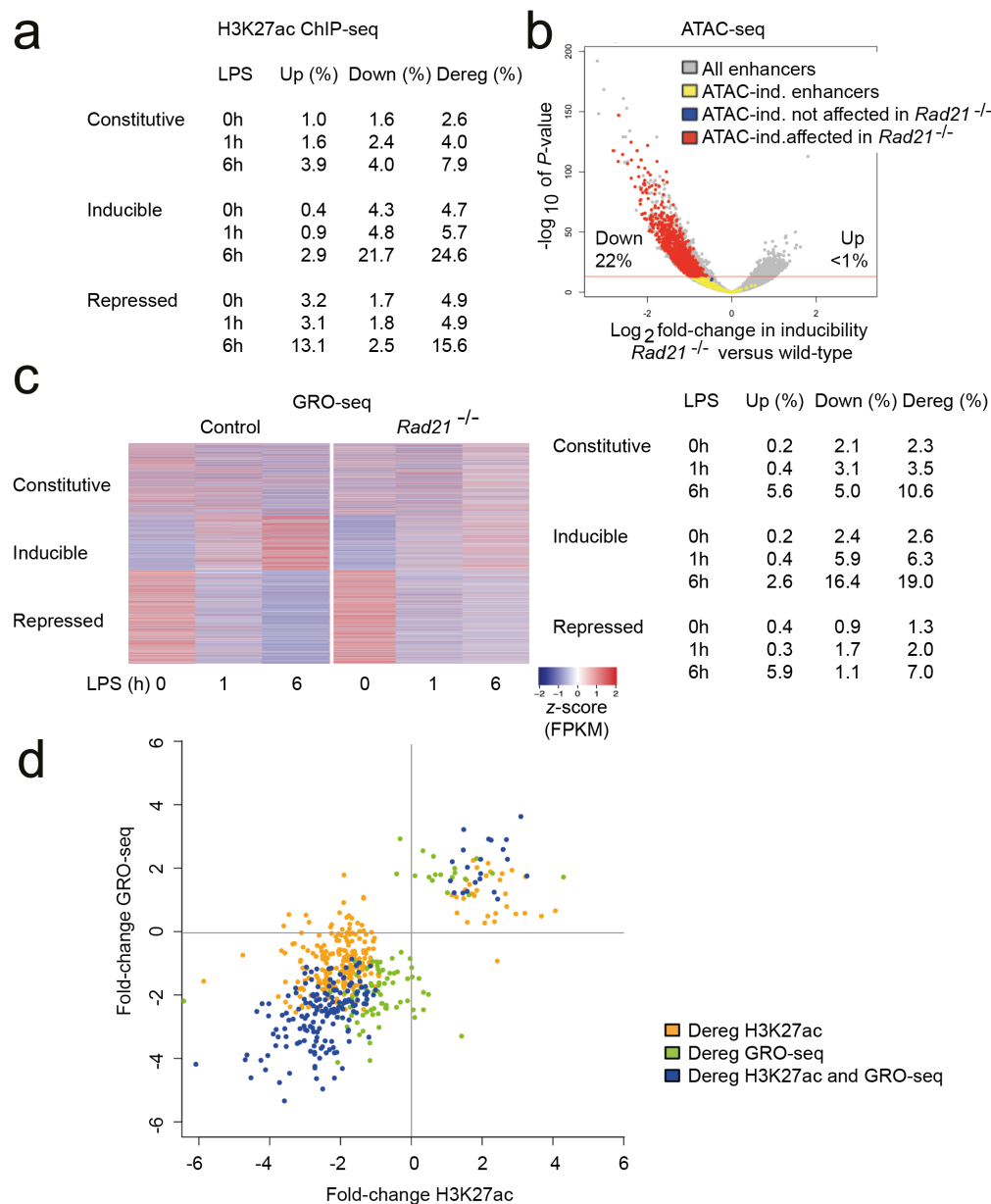
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Supplementary Figure 1

Cohesin depletion in mature, postmitotic macrophages.

- a) *Rad21* deletion in mature macrophages.
- b) Flow cytometric analysis of the macrophage markers CD11b and F4/80 on control and *Rad21*^{-/-} macrophages 3d after 4-OHT. Representative of 3 biological replicates.
- c) DNA content of macrophages at the indicated times after 4-OHT, and % cells in G1 3d after 4-OHT. Mean ± SEM of 6 biological replicates.
- d) qPCR amplification of the floxed *Rad21* exons normalized to genomic sites on chromosome 2 and 3. Mean ± SEM of 3 biological replicates.
- e) Immunoblot of RAD21 protein normalized to Actin at the indicated times after 4-OHT. Mean ± SEM of 3 biological replicates.
- f) p53 target gene expression in control and *Rad21*-deleted macrophages quantified by RNA-seq. Mean ± SEM of 3 biological replicates.
- g) Enumeration of cells with nuclear-localized NF-κB p65 (>50%) at 1 h post-LPS treatment. Mean ± SEM of 3 biological replicates. Mean ± SEM of 113 to 340 cells per genotype in each of 3 biological replicates. Box plots show the median and lower and upper quartiles, whiskers show the maximum and minimum data points up to 1.5 times the interquartile range.
- h) Quantification of cytokines in media conditioned by LPS-pulsed control and *Rad21*-deleted macrophages determined by ELISA (for IFN-β) or cytokine array (all other cytokines). Mean ± SE of 3 biological replicates. The secretion of 26 cytokines increased in control macrophages 8 h after LPS ($P < 0.05$, fold-change > 2). Compared to wild-type, secretion by *Rad21*-deficient macrophages was significantly increased for 3, and significantly decreased for 13 cytokines ($P < 0.05$, two-sided *t*-test).



Supplementary Figure 2

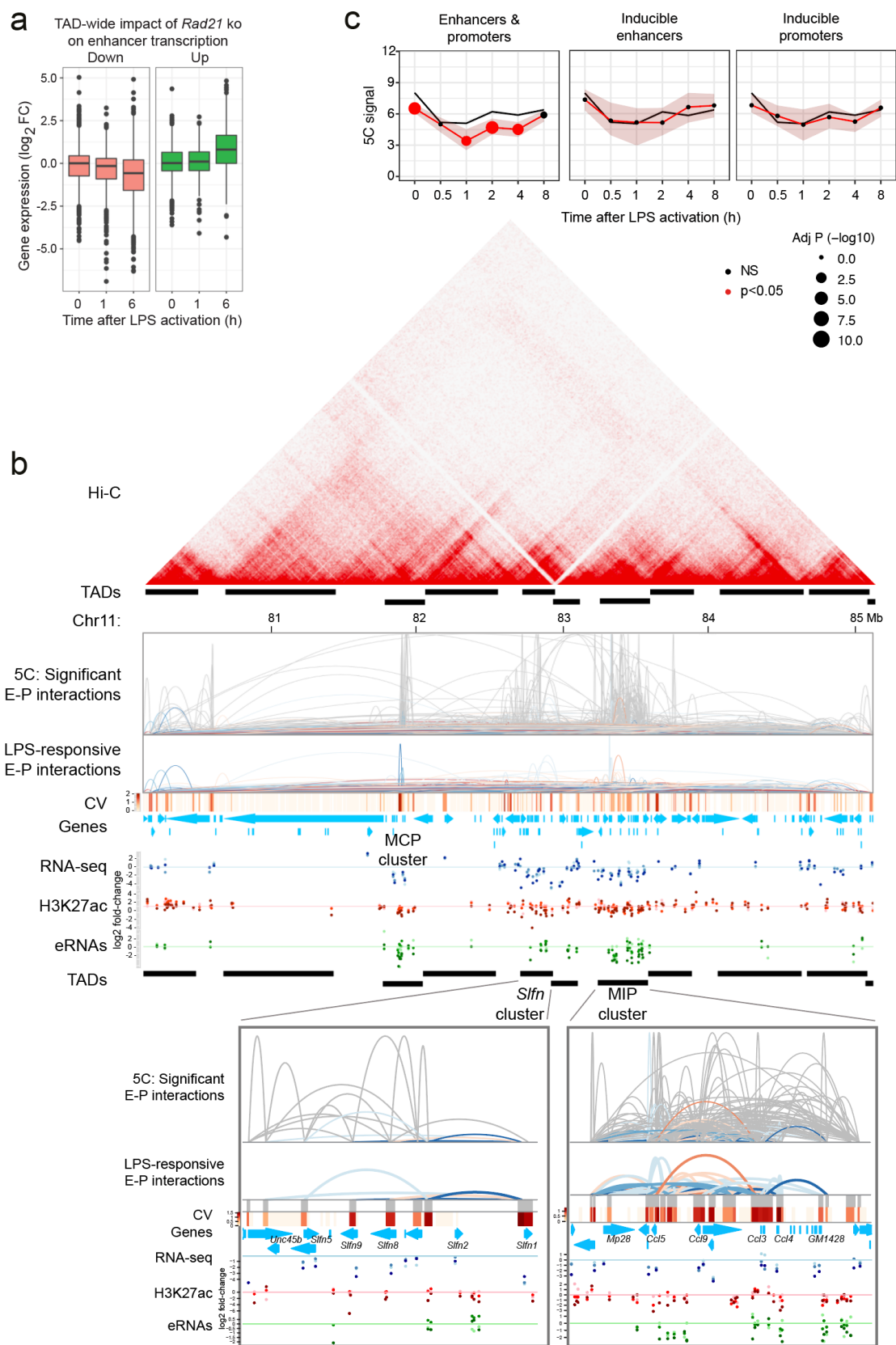
Supporting data for restricted enhancer dynamics in cohesin-deficient macrophages.

a) Frequency of deregulated constitutive, inducible and repressed enhancers²² by H3K27ac ChIP-seq (adj. $P < 0.05$) in *Rad21*-deleted macrophages. H3K27ac ChIP-seq reads were normalized to H3 ChIP-seq and analysed by DESeq2. Based on 2 biological replicates per genotype and condition.

b) Volcano plot of chromatin accessibility at constitutive, inducible and repressed enhancers as determined by ATAC-seq 6 h after LPS. Based on 2 biological replicates per genotype and condition.

c) Heatmap of eRNA transcription as determined by GRO-seq (left) and frequency of deregulation (DESeq2 adj. $P < 0.05$) in *Rad21*-deleted macrophages (right) at intergenic enhancers²² that are constitutively active ($n = 3775$), inducible ($n = 2893$) or repressed ($n = 4914$). Based on 2 biological replicates per genotype and condition. z-scores were determined based on FPKM.

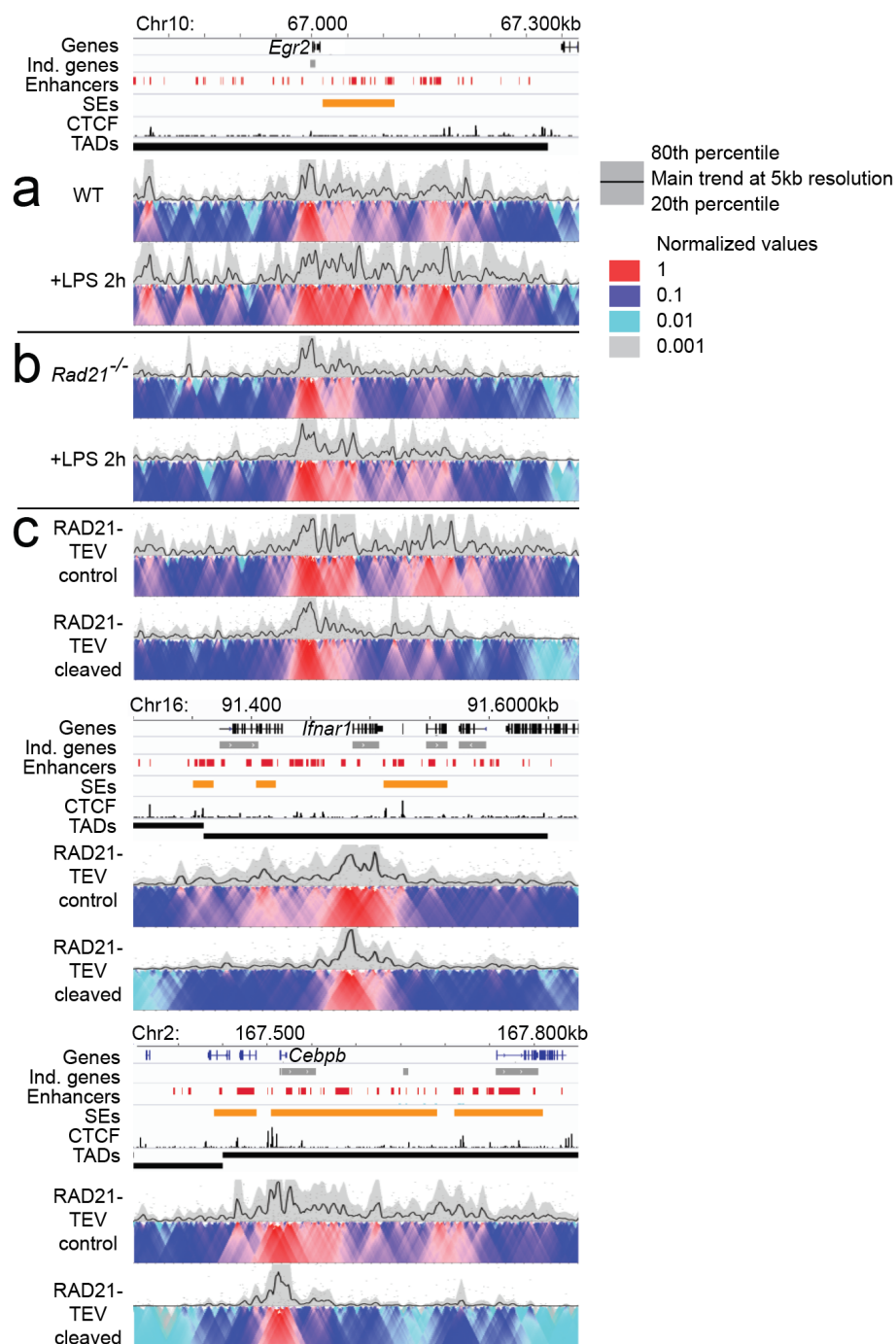
d) Correlation between changes in H3K27ac and eRNA transcription (GRO-seq) at intergenic inducible enhancers²² 6 h after LPS stimulation of *Rad21*-deleted versus control macrophages. Of 1461 intergenic inducible enhancers included in the DESeq2 analysis, the number of deregulated ($P < 0.05$) enhancers was 431 for H3K27ac, 283 for eRNA transcription (GRO-seq), and 185 for both H3K27ac and eRNA transcription. H3K27ac and eRNA transcription were significantly correlated ($P < 10e-16$, Spearman correlation = 0.52).



Supplementary Figure 3

Deregulated genes and enhancers at the genomic level.

- a) Domain-wide impact of *Rad21* deletion on enhancer activity and gene expression. Of 2754 called TADs³, 1830 contained deregulated genes, 689 contained GROseq-deregulated enhancers, and 1451 contained H3K27ac-deregulated enhancers. TADs that contained deregulated genes were enriched for deregulated enhancers (odds ratio = 5.02, $P = 4.47\text{e-}43$ for GRO-seq and odds ratio = 7.23, $P = 2.70\text{e-}111$ for H3K27ac). The figure shows log₂ fold-changes in gene expression (RNA-seq, *Rad21*-deleted versus wild-type macrophages) for TADs with decreased (left) or increased (right) eRNA transcription of intergenic enhancers²² in *Rad21*-deleted macrophages at the indicated times after LPS stimulation. Based on 2 biological replicates per genotype and condition.
- b) Top, Hi-C map³ of the ~5Mb region on mouse chr11:80.141.160 to 85.160.410 with TAD annotation. Upper middle, 5C chromatin contacts wild-type macrophages between restriction fragments containing at least one annotated enhancer or promoter (E-P interactions), and chromatin contact changes in response to LPS-stimulation (LPS-responsive E-P interactions). Grey loops: Contacts that change < 2-fold over six time points from 0 to 8 h LPS. Red: > 2-fold induced contacts. Blue: > 2-fold decreased contacts. LPS-responsive chromatin contacts are shown below. Lower middle, the coefficient of variation (CV) is an indicator whether interactions change in response to LPS. Log₂ fold-changes in transcripts (RNA-seq), H3K27ac (ChIP-seq), and eRNAs (GRO-seq) are shown for *Rad21*^{-/-} versus wild-type macrophages. Light, medium and dark colors represent fold-change at 0, 1 and 6 h of LPS treatment for H3K27ac and eRNAs, and 0, 2 and 8 h for transcripts. MCP (the ~260kb MCP/monocyte chemotactic protein cluster containing *Ccl1*, *Ccl2*, *Ccl7*, *Ccl11* and *Ccl8*) and MIP (the ~330kb MIP/macrophage inflammatory protein cluster containing *Ccl5*, *Ccl3*, *Ccl15*, *Ccl4*, *Ccl6*, and *Ccl9*) mark domains enriched for downregulated enhancers. Insets show significant E-P interactions and LPS-responsive E-P interactions for two TADs rich in inducible genes, the *Sfrr* gene cluster and the MIP cluster. Based on one time series of 6 5C experiments.
- c) LPS-induced 5C interactions involving (from left to right) enhancers and promoters, inducible enhancers, and promoters of inducible genes in wild-type macrophages. The normalized strength of each set of interactions was compared at each time point to the normalized strength of all other enhancer-promoter interactions (black line) using a Wilcoxon rank sum test. Red symbols indicate adjusted $P < 0.05$. Shaded areas represent 95% confidence intervals.



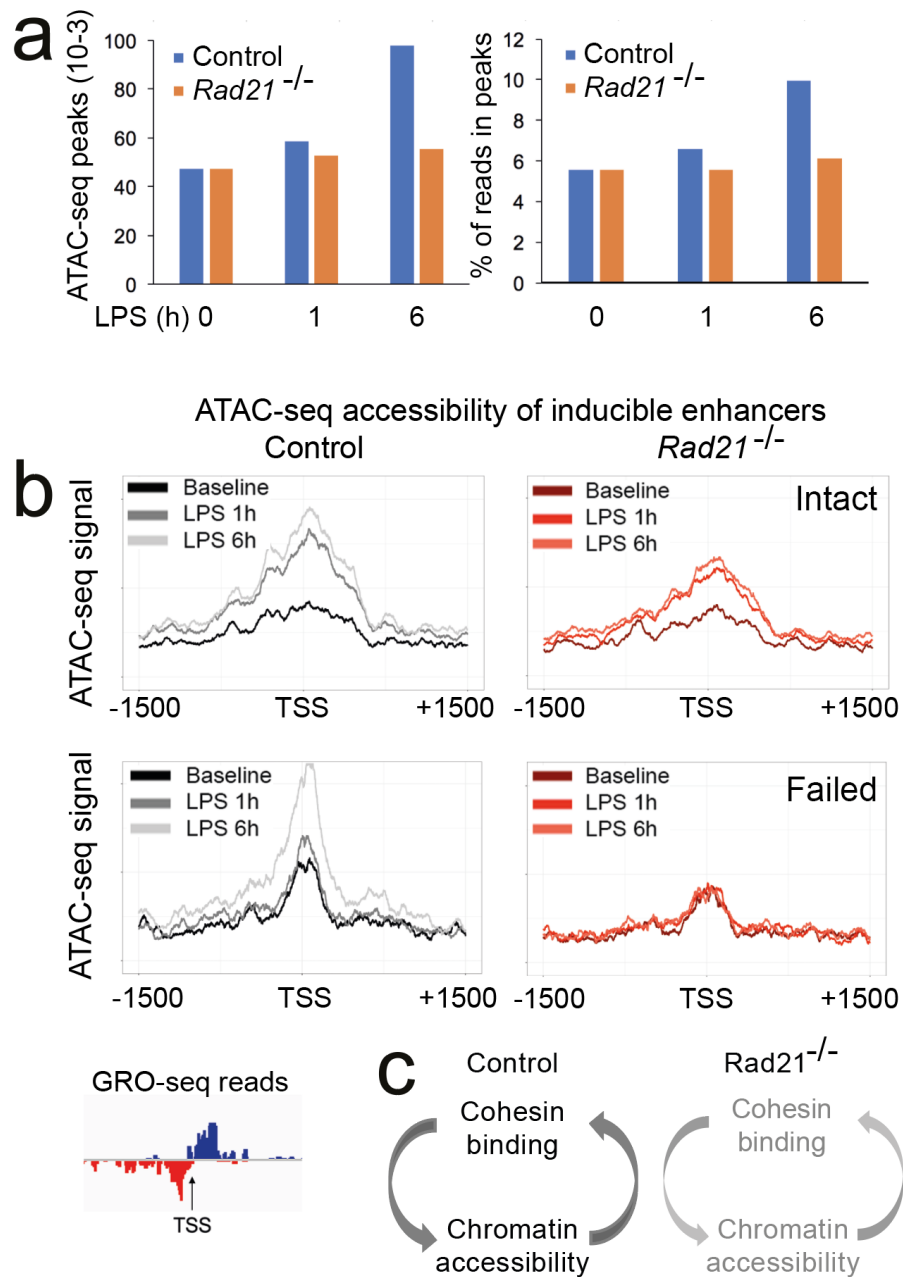
Supplementary Figure 4

4C analysis of chromatin contacts.

a) Top: Chromosomal coordinates, genes, inducible genes, enhancers²², super-enhancers (SEs), CTCF ChIP-seq and TADs. Below: Main trend at 5kb resolution (mean with 20th to 80th percentile and normalized values in sliding windows sized 2-50kb (colors). Chromatin interactions in wild-type macrophages before and 2 h after LPS, suggesting re-configured interactions of the *Egr2* promoter with the downstream super-enhancer. Two merged biological replicates.

b) Interactions of the *Egr2* promoter with downstream super-enhancer in wild-type and *Rad21*^{-/-} macrophages before and after LPS (2 h). Two merged biological replicates.

c) Interactions of the *Egr2*, *Ifnar1*, and *Cebpb* promoters before and 8 h after TEV induction in RAD-TEV macrophages.



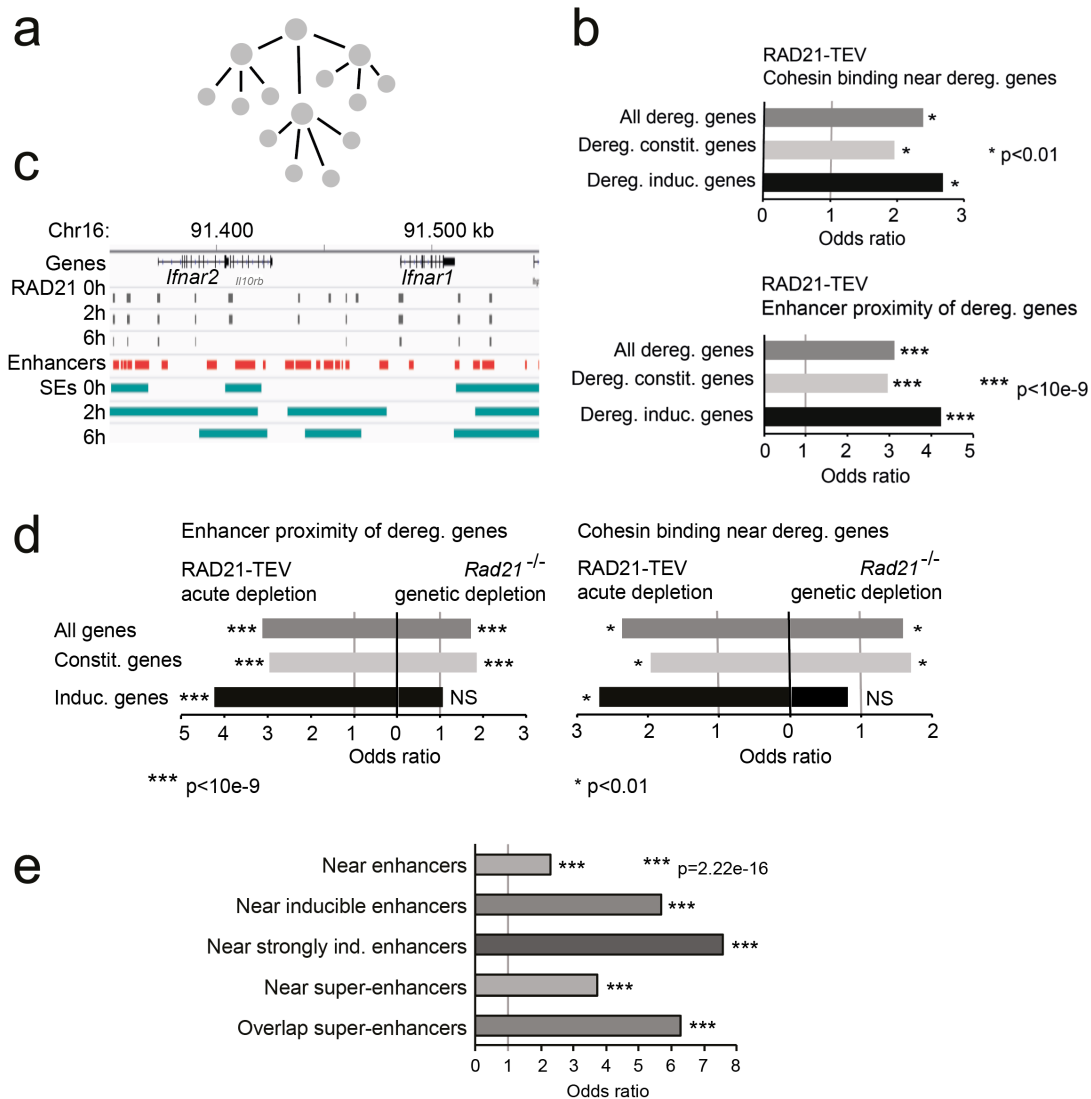
Supplementary Figure 5

Enhancer accessibility in cohesin-depleted macrophages.

a) The number of ATAC-seq peaks (left) and the percentage of reads in peaks (right) for control and *Rad21*^{-/-} macrophages before and after LPS induction. Peaks were called based on 2 merged biological replicates.

b) Inducible enhancers were classified into maintained (top) or failed (bottom) based on DESeq2 analysis of H3K27ac ChIP-seq replicates (adj. $P < 0.05$). Transcription start sites within inducible enhancers were identified by analysis of GRO-seq data (inset). ATAC-seq profiles centered on enhancer TSSs are shown for control (grey) and *Rad21*^{-/-} macrophages (orange) before and after 1 and 6 h of LPS stimulation. Based on 2 independent ATAC-seq experiments per genotype and condition.

c) Model for the relationship between cohesin binding and chromatin accessibility based on⁴⁷⁻⁵⁰ where cohesin binding promotes chromatin accessibility, which in turn facilitates cohesin binding.



Supplementary Figure 6

Vulnerability of inducible gene expression.

a) Model to illustrate the hierarchical organization of inducible gene expression. Nodes (circles) represent genes and their products, and lines (edges) indicate regulatory relationships.

b) Deregulated constitutive and inducible genes after acute degradation of RAD21-TEV are enriched for cohesin binding (RAD21 peaks at baseline within 10kb of gene body, top) and for location within 40 kb of macrophage super-enhancers (bottom). Odds ratios were determined by two-sided Fisher's exact test. Meta-analysis of 3 RNA-seq experiments and 2 RAD21 ChIP-seq experiments per condition.

c) Genes involved in the regulation of inducible gene expression are deregulated by cohesin cleavage in RAD21-TEV macrophages. In addition to genes and TADs, tracks show RAD21 ChIP-seq peaks, enhancers, and super-enhancers (SEs) mapped at the indicated times after LPS in wild-type macrophages. Deregulated genes are shown in black. Meta-analysis of 3 RNA-seq experiments and 2 RAD21 ChIP-seq experiments per condition.

d) Features of immediate cohesin targets are diluted as more inducible genes become deregulated with time after cohesin depletion. Odds ratios and *P*-values (Fisher's exact test) for RAD21 binding (ChIP-seq peaks within 10kb) and enhancer proximity (super-enhancer within 40kb) of deregulated genes in RAD21-TEV and *Rad21*^{-/-} macrophages at baseline (not LPS stimulated).

e) Odds ratios and *P*-values (Fisher's exact test) for the genomic enrichment of inducible genes²³ within 40kb of ('near') enhancers²², inducible enhancers, LPS-induced H3K27ac signal of log₂ FC >1.5 ('strongly inducible') enhancers, or super-enhancers.