Negative elongation factor complex enables macrophage inflammatory responses by controlling anti-inflammatory gene expression

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Studies on macrophage gene expression have historically focused on events leading to RNA polymerase II recruitment and transcription initiation, whereas the contribution of post-initiation steps to macrophage activation remains poorly understood. Here, we report that widespread promoter-proximal RNA polymerase II pausing in resting macrophages is marked by co-localization of the negative elongation factor (NELF) complex and facilitated by PU.1. Upon inflammatory stimulation, over 60% of activated transcriptome is regulated by polymerase pause-release and a transient genome-wide NELF dissociation from chromatin, unexpectedly, independent of CDK9, a presumed NELF kinase. Genetic disruption of NELF in macrophages enhanced transcription of AP-1-encoding Fos and Jun and, consequently, AP-1 targets including Il10. Augmented expression of IL-10, a critical anti-inflammatory cytokine, in turn, attenuated production of pro-inflammatory mediators and, ultimately, macrophage-mediated inflammation in vivo. Together, these findings establish a previously unappreciated role of NELF in constraining transcription of inflammation inhibitors thereby enabling inflammatory macrophage activation.
Inflammation has evolved as a rapid response to environmental cues and contributes to a series of physiological and pathological processes including host defense, tissue damage, and metabolic alterations. The potentially harmful effects of inflammation necessitate the existence of precise regulatory mechanisms to control its magnitude and duration. Indeed, activation of macrophages, an essential component of inflammatory responses, is subject to exquisitely tight control at multiple levels. For example, during activation, macrophage transcriptome undergoes extensive reprogramming with hundreds of genes rapidly upregulated and downregulated hundreds or even thousands of fold, pointing at transcription as a critical node of regulatory circuitry in these cells. Macrophage activation is typically triggered by ligation of cell surface or endosomal receptors which initiates intracellular signaling, culminating in recruitment of sequence-specific transcription factors and RNA polymerase (Pol) II to the target gene loci. Historically, Pol II recruitment and transcription initiation were considered the major rate-limiting steps for gene activation. However, this view was later challenged by several studies reporting that in resting macrophages, transcription start sites (TSS) of many inflammatory genes such as Tnf are preloaded by Pol II raising the possibility that the rate-limiting steps to their activation occur post-transcription initiation.

Indeed, numerous recent studies conducted mainly in Drosophila and stem cells have described Pol II promoter–proximal pausing, pause-release and entry into productive elongation as equally susceptible to regulation. Specifically, after formation of the preinitiation complex (PIC), Pol II initiates transcription, synthesizes short (20–60 nt) nascent RNAs and then pauses. Further productive elongation requires signal-dependent pause-release to mobilize Pol II into the gene body regions. Given the importance of Pol II pausing, establishment of pause and its release are highly regulated by a plethora of positive and negative factors, including negative elongation factor (NELF), DRB sensitivity-inducing factor (DSIF), and positive transcription elongation factor-b (P-TEFb). In the canonical pause-release model derived from biochemical studies, the four-subunit NELF complex binds and retains Pol II within the promoter–proximal regions. Pause-release is believed to be triggered by signal-induced phosphorylation of NELF by the heterodimeric P-TEFb complex composed of cyclin-dependent kinase 9 (CDK9) and cyclin T1, which results in dismissal of NELF from promoters. In addition, P-TEFb phosphorylates DSIF converting it from pausing to elongation-promoting factor and serine 2 residues within the heptad repeats in Pol II C-terminal domain (also targeted by CDK12), which together is thought to facilitate Pol II entry into gene bodies and productive transcription elongation.

Post-initiation regulation of transcription is implicated in key biologic processes, including embryogenesis and development. The contribution of post-initiation mechanisms to immune cell function has not been widely appreciated although several pioneering studies have provided strong evidence for the existence of this type of regulation especially in cells such as macrophages that respond rapidly to environmental cues. Ligation of TLR4 followed by NF-κB recruitment leads to P-TEFb binding to numerous gene loci. In fact, studies by us and others have shown how P-TEFb loading and transcription elongation are targeted by negative regulators of inflammation including the glucocorticoid receptor and other transcription repressors, underscoring the physiological importance of immune gene regulation during early elongation. Nevertheless, these studies mainly focused on specific subsets of genes of interest, whereas the characteristics and a global impact of post-initiation control of transcription to macrophage activation remain to be thoroughly investigated.

Here, by employing genomic, pharmacological, and biochemical approaches, we comprehensively mapped the post-initiation transcriptional landscape during macrophage activation. We describe the surprisingly global and dynamic interactions of the “pausing factor” NELF with chromatin over the course of inflammatory activation of macrophages and the unexpected contribution of the lineage-determining transcription factor PU.1 to this process. Using genetic disruption of Nelfb in macrophages, we identify a functionally and transcriptionally diverse group of NELF-regulated genes that display aberrant responses to inflammatory signaling, and define a pathway linking paused genes under direct transcriptional control of NELF to their downstream effectors in the immune system. Finally, we describe the consequences of macrophage-specific NELF depletion in vivo thereby establishing a physiological role of NELF in mammalian inflammatory response.

**Results**

**Widespread Pol II promoter–proximal pausing in macrophages.** To comprehensively define the global Pol II pausing patterns as related to signal-induced transcription in murine primary bone marrow-derived macrophages (BMDM), we performed Pol II chromatin immunoprecipitation followed by high throughput sequencing (chromatin immunoprecipitation (ChIP)-seq) and precision nuclear run-on sequencing (PRO-seq). Out of 10,076 unique genes expressed in BMDM as defined by RNA-seq (referred to as “BMDM transcriptome” hereafter), an overwhelming majority of genes displayed features of promoter–proximal pausing as computationally defined by high pausing index (PI) calculated based on Pol II ChIP-seq signals in the TSS regions versus gene body regions (Fig. 1a, b, Supplementary Fig. 1a). Highly paused (PI ≥ 3, group 1) and moderately paused (1.5 ≤ PI < 3, group 2) genes made up 76% of the BMDM transcriptome (Fig. 1c, Supplementary Fig. 1b), whereas non-paused genes made up 24% (PI < 1.5, group 3). The global Pol II pausing pattern was highly reproducible across independent ChIP-seq data sets (Supplementary Fig. 1c). To examine whether paused Pol II was transcriptionally active, we employed PRO-seq which detects de novo transcripts and found enriched promoter-proximal short transcripts in resting BMDM (Fig. 1d, e). Interestingly, PRO-seq based quantification also revealed promoter-proximal pausing in approximately 83% of the transcriptome (Supplementary Fig. 1d), which largely overlapped with Pol II pausing defined by ChIP-seq (Supplementary Fig. 1e). Therefore, both Pol II occupancy and production of nascent transcripts in resting BMDM corroborate promoter-proximal pausing as a widespread phenomenon that affects approximately three-quarters of BMDM transcriptome, consistent with Pol II pausing occurrence reported in other species and cell types.

To assess the role of factors associated with pausing and early elongation, we investigated the global occupancy of CDK9, the kinase subunit of P-TEFb, and the NELF–E subunit of the NELF complex in resting BMDM by ChIP-seq. P-TEFb plays a critical role in promoting elongation in a signal-dependent manner and, consistently, showed little occupancy in resting BMDM (Fig. 1f, Supplementary Fig. 1f). In contrast, the “pausing factor” NELF displayed striking TSS-centric distribution with 71% of NELF-E ChIP-seq peaks located near TSS (Fig. 1g, h). NELF-E occupancy strongly correlated with paused Pol II and vice versa: highly paused genes showed the highest NELF-E binding signals (Fig. 1i,j), and 32% of group 1 genes displayed NELF-E peaks in their TSS regions (hereafter, NELF+E genes) compared to only 8% of group 3 (Supplementary Fig. 1g). Reciprocally, 75% of NELF+E genes were highly paused genes (Fig. 1k), as exemplified by Tnf and Jun (Fig. 1l). Thus, in resting BMDM, the majority of transcriptome displayed features of promoter-proximal pausing...
revealed that 58% of genes expressed in BMDM exhibited PU.1.

PU.1 contributes to TSS-centric Pol II and NELF localization.

We next sought to identify factors that may contribute to establishing promoter–proximal pausing in macrophages. Motif analysis of DNA sequences in TSS regions of our three groups of genes revealed that ETS family transcription factor binding motifs were significantly enriched near TSS of highly paused genes (Fig. 2a; compare group 1 and 2-3). Out of 26 ETS family transcription factors, PU.1 is the lineage-determining factor and the highest expressed family member in macrophages (Fig. 2b). Interestingly, in addition to previously reported occupancy at gene enhancers29, our analysis of published PU.1 ChIP-seq data30 revealed that 58% of genes expressed in BMDM exhibited PU.1 occupancy near TSS (Fig. 2c). Moreover, TSS PU.1+ genes largely overlapped with the highly paused group 1 genes (Fig. 2d, e).

PU.1 ChIP-seq signals showed significant positive correlation with the degree of pausing (Fig. 2f, g), with representative genes, e.g., Tnf, demonstrating concomitant Pol II, NELF-E, and PU.1 occupancy near TSS (Fig. 2h).

To probe for the causal direct or indirect relationship between PU.1 and Pol II promoter–proximal pausing, PU.1 expression was manipulated in immortalized macrophages using the CRISPR–Cas9 technique. Diminishing PU.1 expression in fully differentiated macrophages (Fig. 2i) attenuated both Pol II and NELF-E binding near the Tnf TSS (Fig. 2j). To further corroborate a positive role for PU.1 in TSS-centric Pol II accumulation, we mutated a PU.1 binding site near the TSS in immortalized macrophages using the CRISPR–Cas9 technique (Supplementary Fig. 2a). ChIP experiments in the PU.1 binding site-mutated macrophages revealed...
Fig. 2 PU.1 contributes to establishment of promoter-proximal pausing in macrophages. a Motif enrichment analysis in TSS regions of BMDM-expressed genes. Top three most enriched transcription factor binding motifs in TSS region of each gene group are shown; x-axis is $-\log_{10}(p$ value) for each enriched motif. Binomial distribution was used for $p$ value calculation, and no adjustments were made for multiple comparisons. b Expression level (fragments per kilobase of transcript per million mapped reads [FPKM]) measured by RNA-seq in resting BMDM for each of the 26 mouse ETS family transcription factors. c The percentage of genes with PU.1+ TSS regions (PU.1+ genes) in BMDM-expressed genes. d Heat map of PU.1 ChIP-seq signals in resting BMDM around the TSS regions for BMDM-expressed genes. For each group, the rows were sorted as in Fig. 1a. e The overlap between PU.1+ BMDM-expressed genes and group 1 genes. f Average signals of PU.1 ChIP-seq in resting BMDM around TSS regions of group 1-3 genes, as indicated. g PU.1 ChIP-seq reads quantified in resting BMDM in $-150$ to $+50$ bp region (boxed in (f)) are shown as boxplots for group 1-3 genes, as indicated. The boxes and bars express data as in Fig. 1j. $P$ values (group 1/group 2 $p<2.2e^{-16}$, group 1/group 3 $p<2.2e^{-16}$, group 2/group 3 $p<2.2e^{-16}$) were calculated by two sided Mann–Whitney $U$ test. h Tracks of Pol II, NELF-E, and PU.1 ChIP-seq in resting BMDM are shown for Tnf, a representative group 1 gene. i Western blot for Pol.I and p38 (loading control) in WT and PU.1-KD iBMDM. Shown is one representative blot from three biological replicates. j The occupancy of Pol II and NELF-E in the TSS region of Tnf was assessed by ChIP-qPCR in WT and PU.1-KD iBMDM (Pol II: $n=6$, $P=0.0082$; NELF-E: $n=3$, $P=0.0277$). *$p<0.05$, **$p<0.01$ by two-sided paired Student’s $t$ test. $n$ represents biologically independent experiments. Source data are provided as a Source Data file.

impaired Pol II and NELF-E occupancy near the TSS (Supplementary Fig. 2b–d), providing additional genetic evidence for the contribution of a PU.1 cis-element in the assembly of the pausing machinery.

NELF dismissal is a key feature of macrophage activation. Widespread Pol II pausing in resting BMDM suggested that pause-release may mediate activation of macrophtage transcriptional activity by inflammatory signals. Following 1 h exposure of BMDM to LPS, a TLR4 agonist, activation of group 1 and 2 genes —approximately two-thirds of LPS-responsive genes—displayed a dramatic increase in both Pol II loading and elongation of paused Pol II into gene bodies indicative of pause-release (Fig. 3a, b, Supplementary Fig. 3a). In contrast, only 32% of LPS-induced macrophtage transcriptional activity were activated solely by de novo recruitment of Pol II to promoters (Fig. 3a, b, group 3 genes). Pause-release in response to LPS was highly reproducible (Supplementary Fig. 3b) and was mirrored by a significant decrease in PII of group 1 as well as group 2 genes in the LPS-activated compared to the resting BMDM (Fig. 3c, Supplementary Fig. 3c). To determine whether Pol II released into gene body regions was transcriptionally active, we assessed de novo transcription by PRO-seq. As expected, PRO-seq captured transcriptional pausing in over half (71%) of LPS-induced genes in resting BMDM (Supplementary Fig. 3d), as well as increasing transcriptional events across gene bodies and significantly attenuated PRO-seq-defined PI upon LPS treatment, further reinforcing the notion that pause-release acts as a hallmark of activation of group 1 genes (Fig. 3d–f). Representative genes from group 1 (Tnf) and group 3 (Il1b) along with their PI are shown in Fig. 3g.

Next, we sought to identify key factors that mediated differential regulation of paused vs. non-paused genes. P-TEFb is a positive regulator of transcription elongation, implicated in gene activation. Measuring global CDK9 occupancy in LPS-activated BMDM by ChIP-seq (Supplementary Fig. 3e) revealed similar CDK9 recruitment patterns in group 1 and group 3 genes (Supplementary Fig. 3f). Indeed, enhanced CDK9 recruitment in LPS-activated BMDM correlated with increased Pol II traveling into gene body regions regardless of the gene pausing status (Supplementary Fig. 3g). Because CDK9 recruitment was not a feature unique to paused genes, we next assessed the behavior of the pausing factor NELF during macrophage activation. Among LPS-activated genes, NELF-E occupancy correlated well with the pausing status as illustrated by prevalent NELF positivity in group 1 genes and scarcity of NELF in group 3.
**Fig. 3** Macrophase activation is associated with Pol II pause-release. **a** Heat map of Pol II ChIP-seq signals in resting and LPS-activated (1 h) BMDM around the TSS regions of group 1–3 LPS-inducible genes. For each group, the rows were sorted by decreasing Pol II ChIP-seq signal in the TSS region in resting BMDM. **b** Average Pol II ChIP-seq signals in resting and LPS-activated BMDM around TSS of group 1 (top) and group 3 (bottom) LPS-inducible genes. **c** The empirical cumulative distribution function (ECDF) plot of Pol II PI distribution of group 1 LPS-inducible genes in resting and LPS-activated BMDM. **d** Average PRO-seq signals (sense strand) in resting and LPS-activated BMDM around TSS of group 1–3 LPS-inducible genes that were classified by PRO-seq defined PI. For each group, the rows were sorted by decreasing PRO-seq signals (sense strand) in TSS regions. **e** The empirical cumulative distribution function (ECDF) plot of PRO-seq-defined PI of group 1 LPS-inducible genes in resting and LPS-activated (0.5 h) BMDM. **f** Tracks of Pol II ChIP-seq, NELF-E ChIP-seq, and PRO-seq data sets, as indicated, for representative group 1 (Tnf) and group 3 (Iihb) genes in resting (left) and LPS-activated (right) BMDM. **g** The percentage of NELF-E genes in group 1–3 of LPS-inducible genes. **h** Heat map of NELF-E ChIP-seq signals in resting and LPS-activated (0.5 h) BMDM around the TSS regions of group 1–3 LPS-inducible genes. For each group, the rows were sorted as in (a). **i** Average NELF-E ChIP-seq signals in resting and LPS-activated BMDM around TSS of group 1 (top) and group 3 (bottom) LPS-inducible genes.

genes (Fig. 3h, Supplementary Fig. 3h). Interestingly, across multiple NELF-E ChIP-seq data sets, NELF occupancy dramatically declined within 0.5 h of LPS stimulation (Fig. 3i, j, Supplementary Fig. 3i, j). These results indicate that Pol II pause-release and NELF dissociation were hallmark events in inflammatory activation of macrophage transcriptome.

NELF dissociation from chromatin is global yet transient. Given a dramatic NELF loss from promoters in response to LPS (Supplementary Fig. 4a), we wondered whether such dismissal was restricted to LPS-inducible genes or occurred at a global scale across genome. Interestingly, NELF-E ChIP-seq revealed a highly dynamic pattern of NELF-E occupancy, in which NELF complex was globally dismissed from thousands of NELF+ genes 0.5 h post LPS stimulation (Fig. 4a, b, Supplementary Fig. 4b, c; approximately half of NELF+ genes), and reloaded to the TSS regions by 1 h approaching levels seen in resting BMDM (Fig. 4a, b, Supplementary Fig. 4b, d, e). Indeed, NELF dissociation was not restricted to LPS-inducible genes as illustrated by striking loss of NELF despite lack of transcriptional response from two representative paused genes Ldha and Irf8 (Supplementary Fig. 4f).

ChIP-seq data was corroborated biochemically, whereby the abundance of chromatin-associated NELF (assessed by immunoblotting for NELF-E and NELF-B subunits) decreased dramatically after 0.5 h of LPS exposure and was fully restored by 1 h.
Fig. 4 NELF is globally and transiently released from chromatin upon macrophage activation. a, b Heat map (a) and average signals (b) of NELF-E ChIP-seq around TSS of all BMDM-expressed genes (n = 10076 genes) for BMDM treated with LPS as indicated. The rows in a were sorted by decreasing NELF-E occupancy in TSS regions in resting BMDM. c Immunoblotting of chromatin-associated NELF-E, NELF-B, and actin in BMDM treated as indicated (n = 4). NELF-E and NELF-B bands were quantified by densitometry, normalized to internal control (actin), and expressed relative to untreated (0 h) sample (−1) (bottom) (NELF-E LPS 0/0.5 h P = 0.0063, LPS 0.5/1 h P = 0.0138; NELF-B LPS 0/0.5 h P = 0.005, and LPS 0.5/1 h P = 0.0314). d Immunoblotting of chromatin-associated NELF-E, p65, Pol II S2P, and actin in mock (PBS) and flavopiridol (300 nM)-pretreated (0.5 h) BMDM followed by LPS (0.5 h) (n = 3). NELF-E bands in (d, e, f) were quantified and normalized as in (c) (bottom) (NELF-E LPS 0/0.5 h P = 0.0077, flavopiridol pretreated LPS 0/0.5 h P = 0.0161). e Immunoblotting of chromatin-associated NELF-B, p65, and actin in WT and MyD88 KO BMDM with or without Pam3CSK4 stimulation (10 ng/ml for 0.5 h) (n = 3). f Immunoblotting of chromatin-associated NELF-B and actin in mock (DMSO) and Bay 11-7082 (5 μM)-pretreated (0.5 h) BMDM followed by LPS (0.5 h). p-p65 blot is control for Bay 11-7082 activity (n = 3). Statistics of NELF-E bands (bottom) (LPS 0/0.5 h P = 0.0016; Bay 11-7082 pretreated LPS 0/0.5 h P = 0.1042). g NELF-E occupancy in Tnf, Jun, Cited2, Fos TSS regions was assessed by ChIP-qPCR in mock (DMSO) and Bay 11-7082 (5 μM)-pretreated (0.5 h) BMDM followed by LPS (0.5 h). Heat map (a) and average signals (b) (LPS 0 h/0.5 h, 0.5 h/1 h) (NELF-E WT/MYD88 KO = 0.0322, LPS 0 h/0.5 h P = 0.0081, LPS 0.5 h/1 h P = 0.008; MYD88 KO/MYD88 KO + Pam3CSK4, P = 0.23). h Bar graph for fold change in NELF-E occupancy in Tnf, Jun, Cited2, Fos TSS regions was assessed by ChIP-qPCR in mock (DMSO) and Bay 11-7082 (5 μM)-pretreated (0.5 h) BMDM followed by LPS (0.5 h). n represents biologically independent experiments. Source data are provided as a Source Data file.

We next confirmed that the integrity of the TLR pathway leading to NF-κB activation was required for NELF dissociation. Because TLR4 activates MyD88 and, to lesser extent, TRIF adapters, we used Pam3CSK4, a TLR1/2 agonist to activate specifically MyD88. As expected, Pam3CSK4 induced NELF dismissal from chromatin in BMDM, and MyD88 deletion abrogated this release (Fig. 4e). Furthermore, Bay 11-7082, an IκB-kinase (IKK) inhibitor—but not SB203580, an inhibitor of p38 MAPK less relevant to NF-κB activation—also prevented LPS-induced NELF chromatin dismissal (Fig. 4f). The reversal of NELF release by IKK inhibition was apparent at individual target genes, e.g., Tnf, Jun, Cited2, Fos TSS regions as demonstrated by ChIP-quantitative polymerase chain reaction (qPCR) (Fig. 4g). Thus, activation of macrophage transcriptome by LPS led to a transient MyD88-IKK-dependent yet CDK9-independent NELF dismissal from chromatin.
NELF regulates early inducible transcription activation. To investigate the role of NELF in macrophage function, we took a genetic approach by generating mice with myeloid cell-specific deletion of the Nelfb gene (Nelfb<sup>−/−</sup>, Lyz2Cre, referred to as Nelfb KO hereinafter<sup>22</sup>). Consistent with previous reports in other cell types<sup>33</sup>, deletion of Nelfb led to destabilization of the NELF complex and degradation of other subunits including NELF-E (Fig. 5a), confirming it to be an informative loss-of-function model for studying the role of the NELF complex in macrophages. Depletion of NELF in myeloid cells resulted in no gross abnormalities of animals or apparent alterations of macrophage populations in vivo under homeostatic condition (Supplementary Fig. 5a–c). Moreover, RNA-seq and PRO-seq showed that NELF deficiency did not significantly or systematically alter transcriptionomes of resting BMDM with <2% of all expressed genes affected (Fig. 5b, c). Thus, NELF was largely dispensable for maintaining baseline macrophage gene expression, in line with grossly normal phenotypes of Nelfb KO mice.

Next, we examined the role of NELF during macrophage activation by exposing Nelfb KO BMDM to LPS. Strikingly, 49 of 83 genes induced by 0.5 h of LPS stimulation (59%) were superinduced in Nelfb KO compared to WT controls pointing at a profound role of NELF in early inducible transcription (Fig. 5d).

Interestingly, among 161 genes whose expression was suppressed by LPS, NELF-E ChIP-seq revealed similar LPS-induced NELF dismissal from group 1 and 2 genes (Supplementary Fig. 5d) as seen for LPS-induced genes. However, NELF deficiency did not profoundly alter expression levels of LPS-downregulated genes (Supplementary Fig. 5e). Intriguingly, among genes superinduced by LPS in Nelfb KO, NELF<sup>+</sup> and NELF<sup>−</sup> genes were almost evenly represented (Fig. 5d–f), suggesting both direct and indirect mechanisms for their regulation. Among paused NELF<sup>−</sup> LPS-super-induced genes, we noted a striking overrepresentation of inflammation inhibitors (highlighted in red in Fig. 5e). For example, Cbp/p300-interacting transactivator (Cited) 2 blocks NF-kB interaction with histone acetyltransferase p300<sup>34</sup>. We confirmed upregulated steady state mRNA as well as de novo transcripts of a representative NELF<sup>+</sup> anti-inflammatory gene Cited2 in Nelfb KO by qPCR and PRO-seq, respectively (Supplementary Fig. 5f, g). Conversely, the Il10 gene encoding a
**Fig. 6 NELF controls IL-10 expression by constraining the transcription of AP-1.**

**a** RT-qPCR analysis of Il10 mRNA in WT and Nelfb KO BMDM stimulated with LPS for indicated time. Representative (left) and cumulative (LPS 0.5 h, n = 7, P = 0.0002) (right) data are shown. **b** IL-10 protein ELISA in WT and Nelfb KO BMDM stimulated with LPS for indicated time. Representative (left) and cumulative (LPS 6 h, n = 8, P = 0.0066) (right) data are shown. **c** NELF-E ChIP-seq tracks Jun and Fos in resting (−) and LPS-activated (+) (0.5 h) BMDM. **d** PRO-seq (sense strand) tracks for Jun and Fos in WT and Nelfb KO BMDM cultured with (+) or without (−) LPS for 0.5 h. **e** Cumulative RT-qPCR data showing expression level of Jun (n = 4, P = 0.0301) and Fos (n = 6, P = 0.0039) in WT and Nelfb KO BMDM treated with LPS for 0.5 h. **f** Immunoblot analysis of c-Fos, c-Jun, and p38 in whole cell lysates of WT and Nelfb KO BMDM treated with LPS for indicated time (left: a representative result of five replicates). For each replicate, c-Fos and c-Jun bands were quantified by densitometry at 60 min time point, normalized to internal control (p38) and expressed relative to WT (=1) (c-Jun P = 0.0041; c-Fos P = 0.0144). Mean ± SD. **g** WT and Nelfb KO BMDM were pretreated with SP600125 (50 μM) or SB203580 (20 μM) for 0.5 h, where indicated, and IL10 expression following 0.5 h of LPS co-incubation was assessed by RT-qPCR (n = 6). Relative Il10 expression was normalized to the levels in WT cells that were set as 1. **h** Out of 29, 20 NELF– genes super-induced in Nelfb KO are potential (P < 0.001) AP-1 targets. *P < 0.05, **P < 0.01, ***P < 0.001. N.S. P > 0.05 by two-sided paired Student’s t test. n represents biologically independent experiments. Source data are provided as a Source Data file.

NELF binding yet, markedly transcriptionally super-induced by LPS in NELF-deficient BMDM (Fig. 5g, h), consistent with secondary effects of NELF on Il10, thus underscoring a multifaceted nature of the inhibitory impact of NELF on inflammatory transcriptome of macrophages.

**NELF controls an AP-1-dependent circuit to target IL-10.**

Given the importance of IL-10 in immune regulation, we focused on the Il10 gene as a model for understanding the indirect effects of NELF on macrophage transcriptome. First, we confirmed the RNA-seq and PRO-seq results by RT-qPCR in Nelfb KO BMDM, which showed heightened Il10 transcript induction peaking by 1 h of LPS exposure (Fig. 6a, Supplementary Fig. 6a). Second, gene expression data was corroborated by ELISA that revealed overproduction of IL-10 protein in Nelfb KO BMDM (Fig. 6b). Given that Il10 is a NELF+ gene, we hypothesized that NELF inhibited IL-10 production by targeting factor(s) that promoted Il10 expression. Among NELF-repressed genes (Fig. 5e), Jun and Fos encoding the subunits of the heterodimeric AP-1 transcription factor complex, are both direct NELF targets with prominent LPD-sensitive NELF-E occupancy around their TSS regions (Fig. 6c). NELF deletion in BMDM resulted in enhanced de novo transcription of Jun in Nelfb KO (Fig. 6g). Conversely, NELF deficiency did not alter TLR-induced activation of canonical NF-κB and MAPK signaling events (Supplementary Fig. 6c). To assess whether attenuation of AP-1 transcription by NELF was potentially affecting other NELF– genes undergoing super-induction by LPS in Nelfb KO (Fig. 5f), we analyzed them for consensus transcription factor binding motifs within −1000 bp to +100 bp relative to TSS36. Interestingly, 20 of these genes (n = 29) were in fact AP-1 targets (Fig. 6h) and LPS increased Fos and Jun occupancy at Il10, Il10,
and Tnfaip3 gene promoters (Supplementary Fig. 6d, e), consistent with the notion that NELF regulated a subset of activated macrophage transcriptome by controlling AP-1. Among such indirect targets of NELF downstream of AP-1 were a number of genes encoding both anti- and pro-inflammatory mediators including Tnfaip3 and Il6 (Supplementary Fig. 6f). Taken together, NELF controlled expression of key immune regulators such as IL-10 by targeting an AP-1-dependent transcriptional circuit.

NELF positively regulates macrophage-mediated inflammation. As our results implied that NELF controlled the expression of important immune mediators including IL-10, we sought to assess the general contribution of NELF to inflammatory cytokine production by macrophages in vitro and in vivo. Corroborating a positive role of NELF in inflammation, its deficiency attenuated the production of IL-6, TNF, and IL-12p40 in response to LPS in cultured BMDM (Fig. 7a). Moreover, blocking the biological activities of IL-10 in Nelfb KO BMDM with anti-IL-10R antibody completely rescues IL-6 production (Fig. 7b) suggesting that reduced cytokine expression in Nelfb KO BMDM is at least in part due to augmented levels of IL-10.

To investigate the physiological role of NELF in controlling inflammatory cytokine balance in vivo, we employed the acute sterile peritonitis model in which recruitment of inflammatory macrophages is driven by IL-637. By examining peritoneal cell populations in this model (Supplementary Fig. 7a), we found that NELF deficiency in the myeloid lineage dramatically compromised infiltration of F4/80+CD11b+ macrophages into the peritoneal cavity (Fig. 7c) without affecting total number of peritoneal cells or the fraction of other peritoneal myeloid cell populations such as monocytes and neutrophils (Fig. 7d, Supplementary Fig. 7b, c). Moreover, peritoneal cells in Nelfb KO mice contained less Il6 mRNA compared to WT controls (Fig. 7e), suggesting that reduced IL-6 production in this model might be responsible for compromised macrophage mobilization in Nelfb KO mice. To test this possibility, Nelfb KO mice were administrated exogenous IL-6, which partially rescued the impaired macrophage recruitment phenotype (Fig. 7f). Collectively, these results demonstrate how by facilitating IL-6 production during inflammatory challenge, NELF serves as a positive regulator of macrophage-driven inflammation in vivo.

Discussion

Despite overwhelming evidence for the post-initiation checkpoint in the regulation of transcription in Drosophila and mammalian cells, relatively little is known about the role of pause-release...
mechanisms in the immune system. Here, we show that over 60% of inflammatory macrophage transcriptome is regulated primarily through Pol II proximal–promoter pausing and release, pointing at the ubiquity of post-initiation control of macrophage activation, analogous to previously described observations during certain processes such as organism development. Importantly, our data revealed unexpected modes of regulation and function of the NELF complex during macrophage activation that could not be predicted based on the existing knowledge obtained from cells of non-immune lineages. In resting macrophages, NELF is broadly associated with paused Pol II (Supplementary Fig. 7d), and its deletion did not lead to broad transcription derepression or “bursts” in the absence of activation signals resulting in CD9 recruitment. In response to TLR signaling, the NELF complex is rapidly and globally evicted from chromatin in a stimulus-dependent yet CD9-independent manner, releasing the “brake” on paused genes. Notably, as NELF dismissal in macrophages occurs at the global level and is not restricted to LPS-inducible genes, we envision that it likely poises genes in a nonspecific manner for subsequent activation, licensing them for subsequent signal-specific actions by P-TEFB, DSIF, and other regulators. Functionally, NELF promoted macrophage inflammatory gene transcription, in part, by attenuating AP-1-dependent expression of a key anti-inflammatory cytokine IL-10 (Supplementary Fig. 6) and, in part, by directly constraining the expression of NELF+ inflammation inhibitors. Thus, NELF behaves as a multifunctional regulator of macrophage transcriptome to modulate the outcomes of macrophage-mediated inflammatory responses.

Interestingly, promoter–proximal pausing was highly correlated with PU.1 occupancy around the TSS regions. PU.1 was previously implicated as a pioneer factor at macrophage-specific enhancers that helps establish and maintain open chromatin environment. Indeed, PU.1 is essential for macrophage lineage specification and for the expression of a wide array of both constitutively expressed and inducible genes. Previous global analysis of PU.1 distribution by ChIP-seq demonstrated that approximately 80% of DNA-associated PU.1 is enriched at distal regulatory elements with the remaining 20% occupying TSS regions yet, functions of TSS-associated PU.1 remain poorly understood. We propose that PU.1 may aide in retaining paused Pol II near promoters yet detailed mechanisms underlying PU.1’s action in this context await further investigation. ETS family members have been shown to function in the vicinity of core promoter elements helping to recruit basal transcriptional machinery, hence, it is plausible that PU.1 directly or indirectly interacts with PIC components to maintain the paused status of Pol II.

In recent years, components of basal machinery involved in Pol II pausing and early elongation have been studied extensively using in vitro biochemical approaches, in invertebrates such as fission yeast and in Drosophila. In higher organisms including mammals, the immune system demands both rapid and exquisitely accurate transcriptional responses to internal and environmental triggers, which makes it ideal for investigating the mechanism underlying transcriptional control. Indeed, it is well-appreciated that rapid activation of macrophage transcriptome upon infectious challenges is essential for eliciting adequate innate immunity, however, how much of it occurs during early elongation checkpoint remains unclear. What dictates stable Pol II pausing in macrophages? What are the behaviors and functions of key pausing factors during macrophage activation and how they compare to their canonical functions derived from biochemical studies? For example, in vitro, co-incubation of NELF and CD9 abolishes NELF binding to pausing complex implicating CD9-mediated phosphorylation in NELF release. Whether this model uniformly applies to different cellular contexts is unclear, and there has been a precedent of CD9-independent regulation of NELF. Our data on NELF dismissal during acute macrophage activation call into question the role of CD9 in this process. Indeed, both pharmacological and genetic CDK9 loss-of-function experiments point to CD9-independent LPS-induced NELF release. Nevertheless, analysis of TLR4 signaling via MyD88 and IKKs effectively abolished inducible NELF dissociation, linking canonical TLR signaling to pause-release. Thus, our study in macrophages illustrates that the mechanisms of post-initiation transcriptional control in different cell types might be tailored to specific signaling wiring and functional needs of a given cell.

We have previously shown that post-initiation steps of the transcription cycles including pause-release and productive elongation are targeted by the well-known inhibitors of macrophage-driven inflammation such as GR, a finding with clear therapeutic implications. However, the coherent picture of the regulatory principles underlying transcription elongation in macrophages has been lacking and, consequently, the specific impact of NELF on an acute inflammatory response is debated. A critical role in pausing implies that NELF represses “leaky” Pol II read-through; hence, NELF+ genes in a NELF KO would lose this checkpoint and display higher basal expression or augmented transcriptional response to an inducer. An equally plausible scenario, however, is that NELF “concentrates” transcriptionally active initiated Pol II to elicit a rapid and potent response to a stimulus; if so, loss of NELF may attenuate gene induction. Here, our comprehensive genomic and genetic analyses of NELF actions in macrophages revealed that the complex functional footprint of NELF in inflammation derives not only from its biochemical activities, but from the diversity of its targets. Indeed, NELF affects genes on each side of the inflammatory spectrum and, critically, transcription factors such as AP-1, that further propagate both pro- and anti-inflammatory networks. We report that the ultimate pro-inflammatory function of NELF is a compound of its direct inhibitory effects on NELF anti-inflammatory mediators as well as indirect “brakes” on the production of the AP-1 target, a broad-spectrum immuno-modulatory cytokine IL-10. These mechanistically and temporally distinct constraints on inflammation inhibitors override those that NELF imposes on paused pro-inflammatory genes. Consequently, NELF deficiency over the course of inflammatory stimulus leads to an accumulation of IL-10 that remodels the inflammatory transcriptome broadly dampening inflammatory gene expression in cultured macrophages and yielding a hypo-inflammatory phenotype in vivo. Interestingly, similarly dominant effects of IL-10 in genetic systems when both pro- and anti-inflammatory arms are affected have been documented for Fos deficiency. Basal transcription factors and chromatin regulators are increasingly viewed as therapeutic targets in a wide range of diseases ranging from autoimmunity to cancer. Identification of NELF as a key permissive factor in inflammation needs to be considered when evaluating pause-release machinery in future drug design efforts.

Methods

Mice. The laboratory animal facility has been accredited by AAALAC (Association for Assessment and Accreditation of Laboratory Animal Care International) and the IACUC (Institutional Animal Care and Use Committee) of Tsinghua University approved all animal protocols used in this study. All mice were housed in isolated ventilated cages (maxima six mice per cage) barrier facility at Tsinghua University. The mice were maintained on a 12/12-h light/dark cycle, 22–26 °C, 40–70% humidity with sterile pellet food and water ad libitum. Nelfb+/- mice were generated. Mice with myeloid-specific deletion of Nelfb (Nelfb+/-, Lys2Cre) were generated by crossing Nelfb+/- mice to Lys2Cre mice. Mice were purchased from the Jackson Laboratory. Age and gender matched mice were used for experiments.

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Cell culture and reagents. Murine BMMDM were obtained and maintained in DMEM supplemented with 10% fetal bovine serum (FBS) and 10% L929 cell supernatant as a conditioned medium to provide an adequate source of growth factor. Cell culture grade (Esherichia coli 0111:B4) and P3AMCSK4 were purchased from InvivoGen and used at a concentration of 10 ng/ml. SP600125 and SB203580 were purchased from Selleck, Bay 11-7082 was obtained from Sigma-Aldrich and flavopiridol was purchased from Santa Cruz Biotechnology. All inhibitors were added 0.5 h prior to LPS addition and were present throughout LPS exposure.

Chromatin immunoprecipitation (ChIP) assay and Chip-seq. For Pol II, NELF-E, CDK9, Fox, Jun, PU.1 ChIP assays, cells were used as indicated and left untreated or stimulated with LPS (10 ng/ml) as indicated in Figure legends. 10–25 × 10⁶ cells per condition were fixed in 1% methanol-free formaldehyde (Thermo Scientific). Antibodies against NELF-E (10705-1-AP) was from ProteintechTM. Antibody against NELF-B (ab167401) was from Abcam. All the other antibodies used. After purification, immunoprecipitated DNA was analyzed by RT-qPCR (primer sequences are listed in Supplementary Data 1) and relative occupancies were normalized to input DNA. For Pol II and CDK9 ChIP-seq, 10 ng of DNA was ligated to adapters and 100–300 bp DNA fragments were purified to prepare libraries at Weill Cornell Epigenomics Core. For NELF-E ChIP-seq, libraries were prepared using the NEBNext Ultra II Library Prep Kit for Illumina (NEB), size selected (150–250 bp) and PCR-amplified for 15 cycles. The libraries were sequenced at the Weill Cornell Epigenomics Core using HiSeq2500.

RNA-seq. BMDM RNA was isolated using Qiagen RNA-easy kit. Total RNA was polyA enriched and Illumina-compatible sequencing libraries were prepared with TrueSeq mRNA-Seq sample preparation kit (Illumina). Quality control of RNA and libraries was performed with the BioAnalyzer 2100. Pair-end sequencing was performed at the Weill Cornell Epigenomics Core using HiSeq2500.

Preparation of chromatin-associated fraction. Chromatin-associated fraction was prepared using stepwise fractionation protocol37. Briefly, cells were rinsed twice with ice-cold PBS, scraped into microcentrifuge tubes, and pelleted at 1500g for 4 min. Cells in the supernatant of the first centrifugation were used to prepare the chromatin supernatant fraction. Cells in the pellets were resuspended in 2× packed cell volume (PCV) of ice-cold buffer A (10 mM HEPES pH 7.9, 1.5 mM MgCl₂, 10 mM KCl, 1 mM DTT, 0.5 mM PMSF, 1× protease inhibitor cocktail) for 1 min and centrifuged at 250g for 4°C for 2 min. The cell pellets were resuspended in equal volume of Buffer A (with 0.5% NP-40) for 1 min to break down the cell membrane and nuclei pelleted at 2500g, 4°C for 2 min. The nuclei were resuspended in equal volume of low-salt buffer (10 mM HEPES pH 7.9, 1.5 mM MgCl₂, 75 mM KCl, 0.5% NP-40, 1 mM DTT, 0.5 mM PMSF, 1× protease inhibitor cocktail) on ice for 10 min followed by centrifugation at 5000g, 4°C for 2 min. The low-salt extracted nuclei (containing chromatin-associated factors) were boiled with 5.6× CV of 1× SDS-loading buffer to generate chromatin-associated fraction for immunoblotting. Antibodies for NELF-B (Abcam, ab167401), NELF-E (Proteintech, 10705-1-AP), Pol II S2P (Abcam, ab5095), NF-kB p65 (C2Z84, Cell Signaling Technology, 4764) were used to detect chromatin-associated proteins. Actin levels were detected as an anti-actin antibody (ABclonal, AC026) served as loading control.

Reverse transcription and qPCR. RNA was extracted from whole cell lysates with a Total RNA Purification Kit (GeneMark) and reverse-transcribed to cDNA with Moloney Murine Leukemia Virus Reverse Transcriptase. qPCR was performed in triplicate with an ABI StepOnePlus thermal cycle. Threshold cycle numbers were normalized to triplicate samples amplified with primers specific for glyceraldehyde-3-phosphate dehydrogenase (G3pdh). Primer sequences are listed in the Supplementary Data 1.

Flow cytometry. After removing red blood cells with ACK lysis buffer, cells were stained with the following fluorescence-conjugated antibodies for 30 min at 4°C: CDS4R-APC/Cy7 (Biolegend, clone, dilution 1:400, Cat 103116), CD11b-PE/Cy7 (Biolegend, clone M1/70, dilution 1:400, Cat 102161), F4/80-APC (eBioscience, clone BM8, dilution 1:400, Cat 17-4801-82), Ly6G-PerCP/Cy5.5 (BD, clone 1A8, dilution 1:400, Cat 560602), Ly6C-PE (BD, clone AL-21, dilution 1:200, Cat 560592), CD11c-PE/Cy7 (eBioscience, clone N418, dilution 1:400, Cat 25-0114-82), and Siglec F- BV421 (Biolegend, clone E50-2440, dilution 1:800, Cat 562006). The 30-min staining was analyzed within 6 h post-injection, mice were sacrificed by cervical dislocation, and cell populations were analyzed on FACS Fortessa flow cytometer (BD Biosciences). Further analysis was implemented using Flowjo software. Cell populations were defined as follows: macrophages in peritoneal cavities and spleen: CDS4R+CD11b+F4/80⁺; macrophages in bronchio-alveolar lavage fluid (BALF): CD45⁺CD11c⁺SiglecF⁺; monocytes: CD45⁺CD11b⁻Ly6G⁻; neutrophils: CD45⁺CD11b⁺Ly6G⁺.

Sequence data alignment, visualization, and quantification. Pol II ChIP-seq, CDK9 ChIP-seq, NELF-E ChIP-seq and PRO-seq data (the reverse reads from paired-end data) were collected. Adapter sequences were trimmed from the ends of reads by Cutadapt (V1.14), and the reads that failed to pass the quality control (Q > 20) were discarded. PU.1 ChIP-seq data set was downloaded from NCBI GEO Datasets under the GEO accession: GSE38279 (GSM490294). SRA files were converted to fastq files using fastq-dump included in SRA toolkit. ChIP-seq and PRO-seq reads in fastq files were aligned to mouse genome (UCSC mm10) using Bowtie (version 1.1.2)38 to generate alignment files of uniquely mapped reads with maximum allowed mismatch of 2 (m = 1 - n = 2) for each ChIP-seq data set, and PRO-seq reads aligned with short seed length (k = 10). ChIP-seq reads aligned to genome were extended to 150 bp from their 3’end for further analysis. ChIP-seq and PRO-seq alignment files were visualized as bedgraph files with normalized read counts (per 10 million reads) at 1 bp resolution, which were generated by using Homer (v4.7.2)39. Bedgraph files were loaded to IGV (Integrative Genomics Viewer, v2.3), and individual gene tracks were obtained as snapshots from IGV.
As PRO-seq reads are complementary to in vitro nuclear run-on products, the beginning of PRO-seq reads reflects the actual transcription-active site. Therefore, we showed that PRO-seq leads to 1 bp false of reads counts, and used the reads aligned to anti-sense strand as the transcription-active signals in sense strand for each gene. To determine ChIP-seq and PRO-seq signals around TSS, we first counted ChIP-seq extended reads (frag-length 150) and PRO-seq shortened reads (frag-length 1 -strand) every 10 bp from TSS to both upstream 1 kb and downstream 1 kb regions for each gene by using annotaTeaks.pl program in HOMER. The output counting matrixes were next used to generate signal heat map around TSS regions by Cluster Treeview 1.1.6. The average signals around TSS regions were calculated as the average reads count per bin (10 bp) per gene.

Unupregulated and downregulated genes in PRO-seq were defined as normalized PRO-seq reads (RPKM + 1) fold changes (Nefb KO/WT) ≥ 1.4 for up-regulated genes and fold changes (Nefb KO/WT) ≤ 0.6 for down-regulated genes. Counting PRO-seq reads for each gene was implemented using annotaTeaks.pl program in HOMER.

Peaks of Pu.1 ChIP-seq and NELF-E ChIP-seq were called by findPeaks program in HOMER (FDR < 0.001).

RNA-seq data analysis. For coverage of mapped RNA-seq reads in transcripts, the expression level of each gene transcript was calculated as the average fragments count of three biological replicates, which was subsequently normalized as the fragments count per kilobase of transcript per million mapped reads (FPKM). BMDM-expressed genes were defined as genes with FPKM ≥ 1 in resting BMDM. Both expression level (FPKM) and differential gene expression between experimental conditions was identified using Cuffdiff program in Cufflinks 2.2.4. Genes with p-value < 0.05 and (FPKM + 1) fold changes ≥ 1.5 between WT resting and LPS treated (1 h) BMDM were regarded as LPS-inducible (n = 449). Unupregulated and downregulated genes in Nefb KO BMDM were identified as p-value < 0.05, (FPKM + 1) fold changes ≥ 1.4 for up-regulated genes and (FPKM + 1) fold changes ≤ 0.6 for downregulated genes. LPS-induced (n = 83) and LPS-suppressed (n = 161) genes at p-value < 0.05 were identified as LPS-inducible and LPS-suppressed genes, respectively. Genes with p-value < 0.05 and (FPKM + 1) fold changes ≥ 1.4 for induced genes and fold change (LPS 0.5 h/ LPS 0 h) ≥ 1.4 for induced genes and fold change (LPS 0.5 h/ h) ≤ 0.6 for suppressed genes. Superinduced and “more-suppressed” genes in Nefb KO BMDM were defined as LPS-induced or LPS-suppressed (0.5 h) genes in the WT, which were further upregulated or downregulated in Nefb KO BMDM.

PI calculation and gene categorization. Refseq gene annotation for mm10 was obtained from UCSC table browser, and genes from mitochondrial and random chromosomes were filtered out. 24030 total unique genes were defined as the total longest variant for each gene with unique gene symbol. To precisely calculate Pol II PI for genes with sufficient gene body length, we chose unique genes longer than 1 kb for further Pol II distribution pattern analysis in Pol II ChIP-seq replicate 1. We defined TSS region for each gene as +250 bp to +250 bp relative to TSS, and the gene body was defined as the +250 bp from TSS to TTS (transcription termination site defined by the UCSC annotation, which means cleavage and polyadenylation site). Using the findPeaks program in HOMER, we set 500 bp window to search Pol II enriched region in Pol II ChIP-seq replicate 1 data in resting BMDM, and obtained the threshold value for statistical analysis. Genes with statistical value > 0.001 and in enriched TSS regions (FDR < 0.001) as 38. Therefore, in 10076 BMDM-expressed genes in 1 kb from TSS to both upstream 1 kb and downstream 1 kb regions for each gene by using annotaTeaks.pl program in HOMER. The output counting matrixes were next used to generate signal heat map around TSS regions by Cluster Treeview 1.1.6. The average signals around TSS regions were calculated as the average reads count per bin (10 bp) per gene.

Unupregulated and downregulated genes in PRO-seq were defined as normalized PRO-seq reads (RPKM + 1) fold changes (Nefb KO/WT) ≥ 1.4 for up-regulated genes and fold changes (Nefb KO/WT) ≤ 0.6 for down-regulated genes. Counting PRO-seq reads for each gene was implemented using annotaTeaks.pl program in HOMER.

Peaks of Pu.1 ChIP-seq and NELF-E ChIP-seq were called by findPeaks program in HOMER (FDR < 0.001).

Assessing NELF occupancy at TSS regions. We used two methods to classify NELF occupancy at TSS regions. (1) We used DiffBind (v2.120.0) to assess differential NELF occupancy at TSS regions. BED files for TSS regions of all genes were used as peak region input, and sorted BAM files for two replicates of NELF ChIP-seq were used as input for reads counting. We used EdgeR mode in DiffBind to identify differential binding, and considered fold change (LPS 0.5 h/0h) of NELF-E ChIP-seq reads count less than 0.5 and p value < 0.05 as threshold for significant NELF dismissal in TSS regions. (2) Peak calling in ChIP-seq is often utilized to identify differential occupancy. We used findPeaks program in HOMER to call NELF-E ChIP-seq peaks, with LPS 0.5 h condition as background and LPS 0 h condition as enrichment, to identify genes with TSS regions showing significant NELF dismissal upon LPS stimulation.

Motif enrichment analysis. To identify enriched known transcription regulatory elements in target TSS regions, we used findMotifsGenome.pl program in HOMER to find enriched motifs. Sequences from TSS regions of these genes were used as inputs and randomly extracted sequences from the genome were used as background.

Statistics. Statistical analysis was performed by using two-tailed Student’s t test or other model where indicated. p < 0.05 was taken as statistically significant unless otherwise indicated. Statistical analyses were performed using GraphPad Prism 7 and R 3.3.0.

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

Data availability. All genomic data described herein are deposited in Gene Expression Omnibus under accession numbers GSE122292, GSE123557, GSE123300, GSE103795, and GSE123370. The source data underlying Figs. 2i, j, 4c–g, 5a, 6a, b, 6–g, 7-a and Supplementary Figs. 2b–d, 4a, 4b–h, 5f, 6a, 6d–f, and 7a, b are provided as a Source Data file. Other data that support the findings of this study are available from the corresponding author upon reasonable request.

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Author contributions
L.Y. and B.Z. designed the research, performed the experiments, analyzed the data, and wrote the paper. M.A.S. performed NELEF-Chip-seq and RNA-seq experiments. D.D. performed NELEF-Chip-seq and Bay11-7082 Chip-qPCR experiments. Y.S. and M.C. performed Pol II ChIP-seq experiments. D.A.R. and B.T. performed CDK9 ChIP-seq experiments. Z.G. generated CDK9-KD iBMDD. X.Z. generated PU.1-Tnf-mUT iBMDD. R.L. generated NELEF mice. Y.C. performed computational genome-wide data analysis and processing. I.R. and X.H. conceptualized the project, designed the research, supervised the experiments, and wrote the paper.

Competing interests
The authors declare no competing interests.

Additional information
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