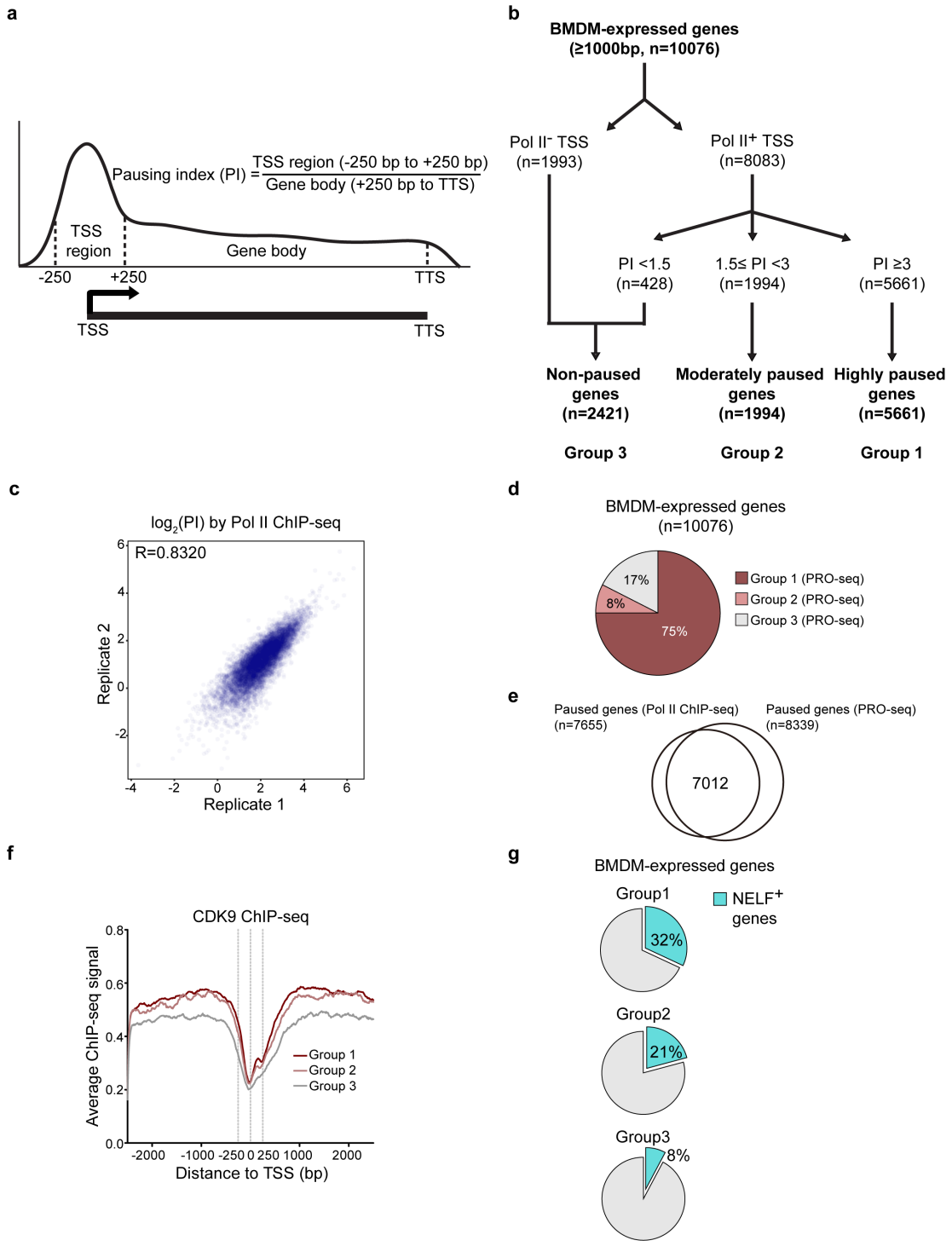
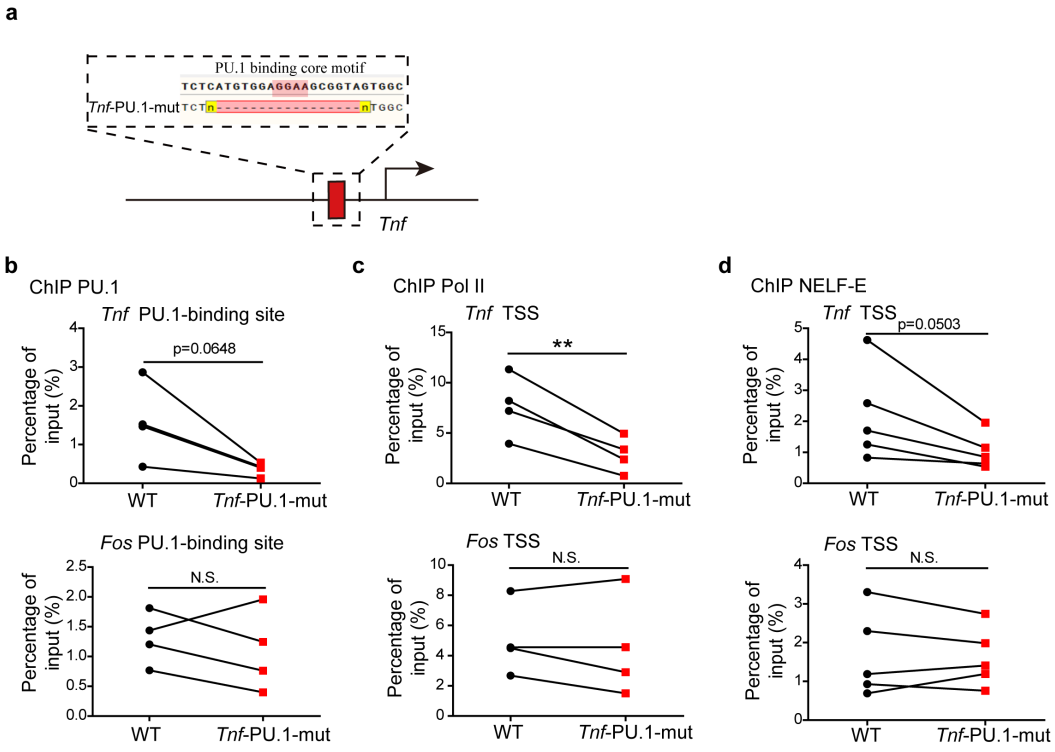
Supplementary information for

Yu et al. "Negative elongation factor complex enables macrophage inflammatory responses by controlling anti-inflammatory gene expression"

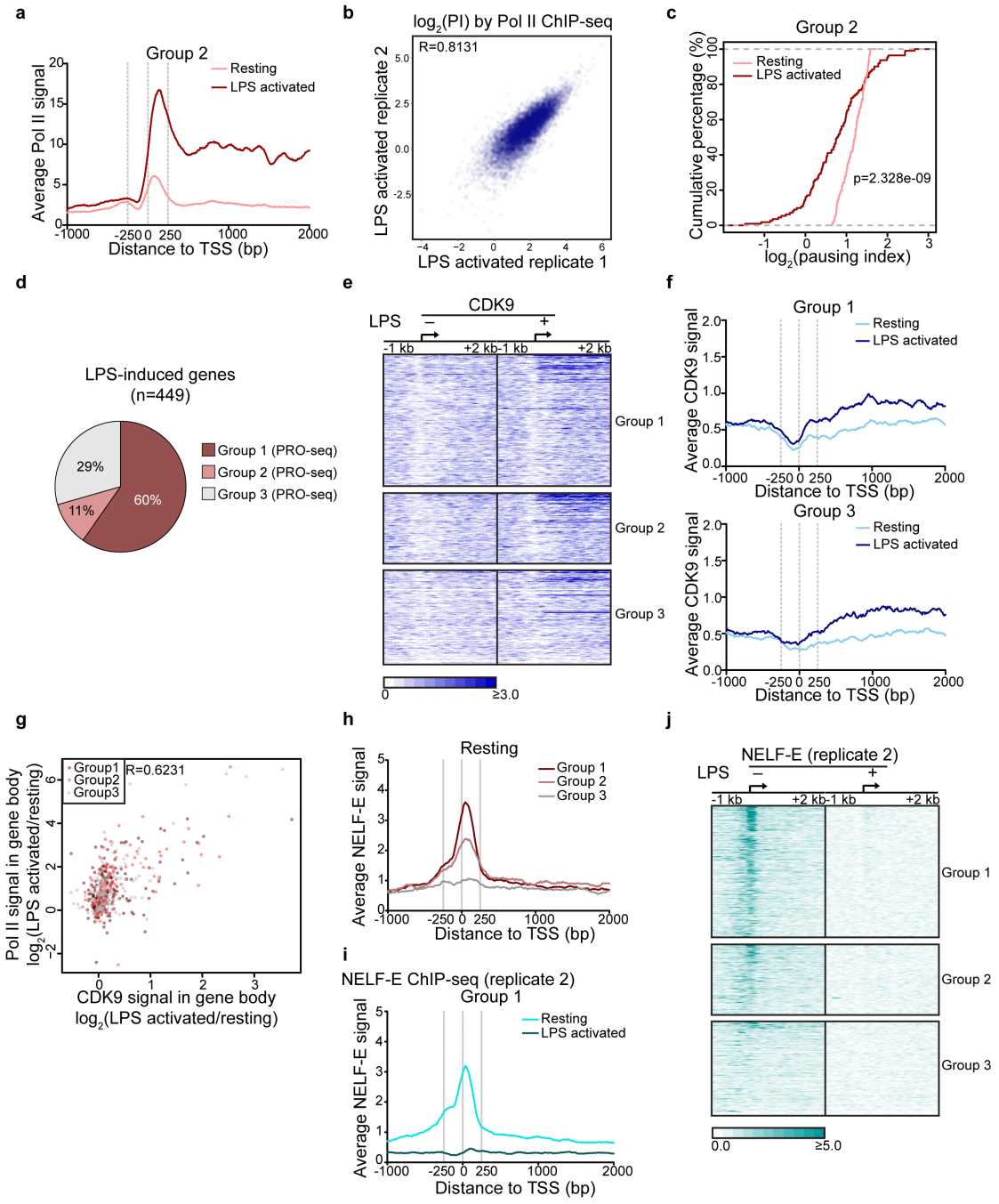


Supplementary Figure 1. BMDM-expressed genes classified by Pol II distribution patterns. (**a**) Calculation of Pol II pausing index (PI). For each gene, PI is defined as a ratio of Pol II ChIP-seq read density in the TSS region/gene body region. (**b**) A flow chart

shows the scheme of BMDM-expressed genes classified according to Pol II distribution in resting BMDM. Genes with PI ≥ 3 are defined as highly paused (group 1), and genes with 1.5 ≤ PI < 3 as moderately paused (group 2). Genes with either PI < 1.5 or TSS region lacking apparent Pol II occupancy are defined as non-paused (group 3). (**c**) A scatter plot shows the correlation of PI between two biological replicates in resting BMDM. x- and y-axes indicate $\log_2$ normalized PI in replicates 1 and 2, respectively. Correlation coefficient (R) is shown. (**d**) Pie graph shows the percentage of all BMDM-expressed genes in each group based on PRO-seq defined PI. (**e**) The overlap between Pol II ChIP-seq defined and PRO-seq defined paused BMDM-expressed genes. (**f**) Average CDK9 ChIP-seq signals around TSS regions of group 1 (brown), group 2 (red) and group 3 (grey) genes in resting BMDM. (**g**) The percentage of $NELF^+$ genes in group 1, 2 and 3 of BMDM-expressed genes.

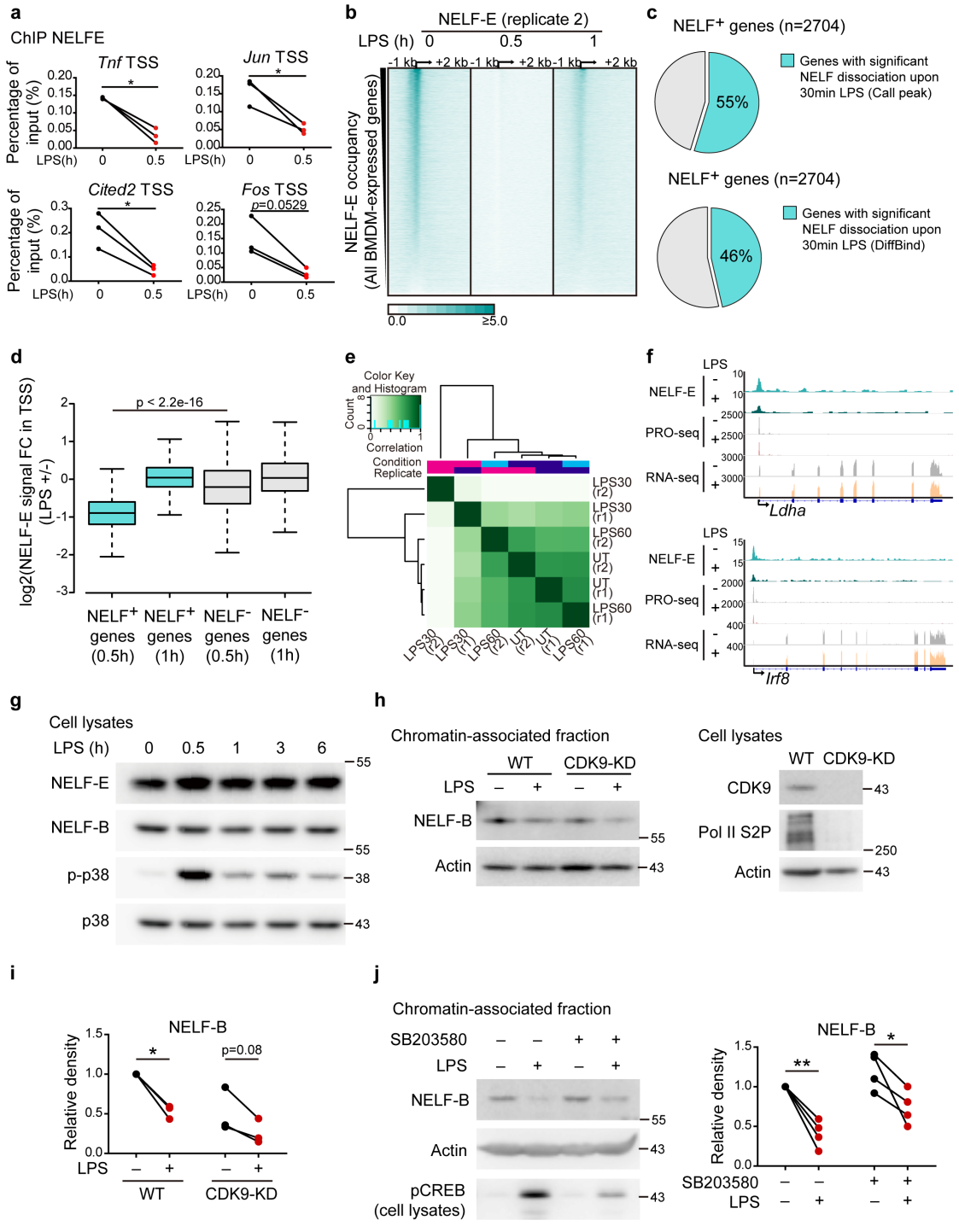


Supplementary Figure 2. Promoter-proximal PU.1 contributes to Pol II pausing. (**a**) PU.1 motif deletion in the *Tnf* promoter region (termed *Tnf*-PU.1-mut). (**b**) The occupancy of PU.1 (n=4) at the PU.1 binding site in the *Tnf* (top, *P*=0.0648) and *Fos* (bottom, *P*=0.4506) promoter region was assessed by ChIP-qPCR in WT and *Tnf*-PU.1-mut iBMDM. (**c**, **d**) The occupancy of Pol II (n=4) (**c**) and NELF-E (n=5) (**d**) in the TSS regions of *Tnf* (Pol II *P*=0.0082; NELF-E *P*=0.0503) and *Fos* (Pol II *P*=0.4388; NELF-E *P*=0.7478) was assessed by ChIP-qPCR in WT and *Tnf*-PU.1-mut iBMDM. ** $P<0.01$, 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.



Supplementary Figure 3. NELF dismissal during Pol II pause-release is a key feature of macrophage activation. (**a**) Average Pol II ChIP-seq signals around TSS of group 2 (moderately paused) LPS-inducible genes in resting and LPS-activated (1 h) BMDM. (**b**) The correlation of PI between two biological replicates of Pol II ChIP-seq in LPS-activated

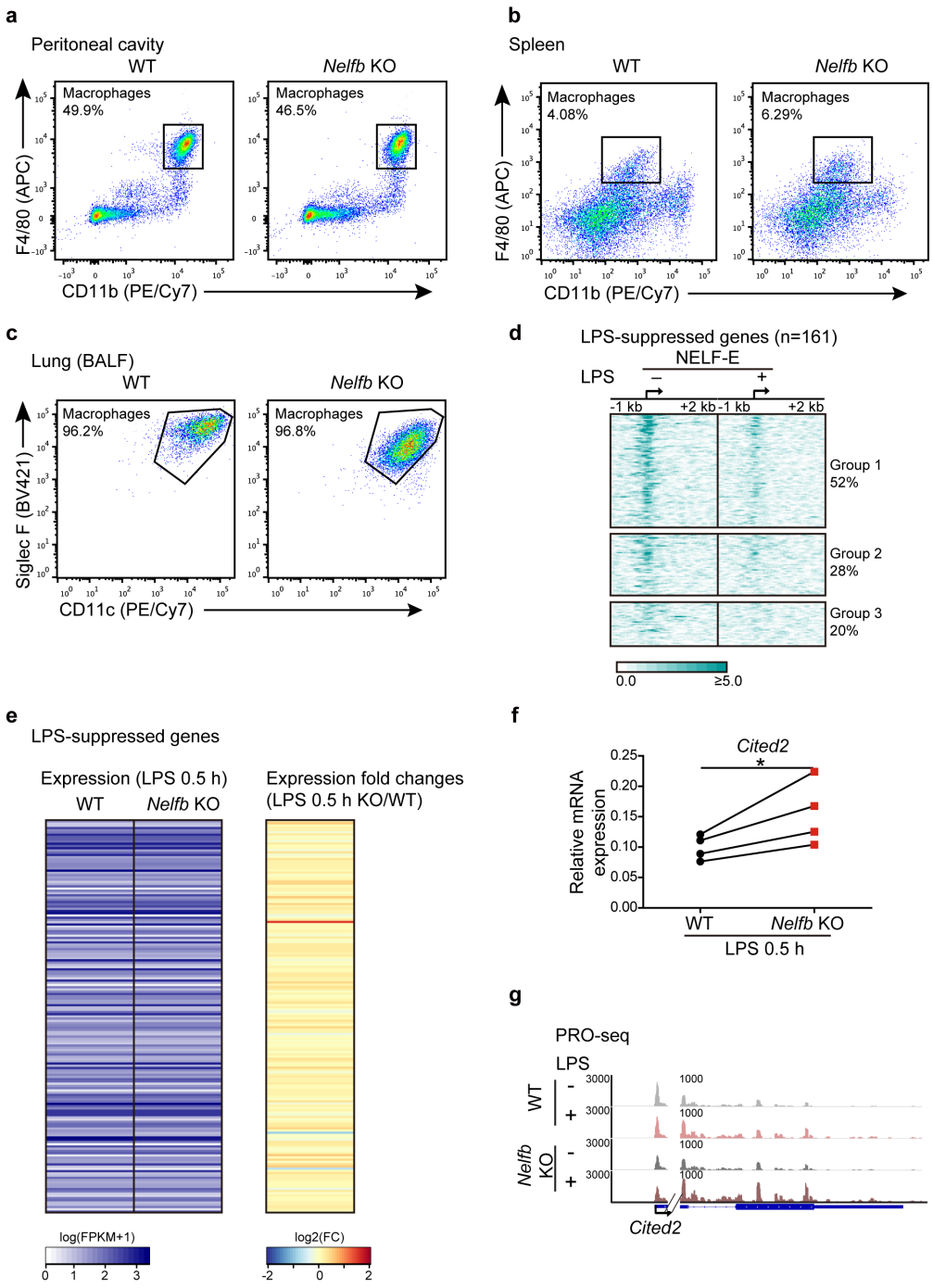
BMDM. x- and y-axes indicate $\log_2$ normalized PI in two replicates. Correlation coefficient (R) is shown. (**c**) The ECDF plot of Pol II PI distribution of group 2 LPS-inducible genes in resting and LPS-activated (1 h) BMDM. (**d**) The percentage of LPS-induced genes in groups 1, 2, 3 re-defined by PRO-seq. (**e**) Heat map of CDK9 ChIP-seq signals around the TSS of LPS-inducible genes in resting and LPS-activated (1 h) BMDM. For each of the three groups, the rows were sorted by the decreasing Pol II ChIP-seq signal in TSS region in resting BMDM. (**f**) Average CDK9 ChIP-seq signals around TSS regions of group 1 (top) and group 3 (bottom) genes in resting and LPS-activated (1 h) BMDM. (**g**) The correlation between CDK9 ChIP-seq signals (fold changes LPS activated/resting; x-axis) and Pol II ChIP-seq signals (fold changes LPS activated/resting; y-axis) in the bodies of genes in each group. Correlation coefficient (R) is shown. (**h**) Average NELF-E ChIP-seq (replicate 1) signals around TSS regions of group 1, 2 and 3 LPS-inducible genes, as indicated, in resting BMDM. (**i**) Average NELF-E ChIP-seq (replicate 2) signals around TSS regions of group 1 genes in resting and LPS-activated (0.5 h) BMDM. (**j**) Heat map of NELF-E (replicate 2) ChIP-seq signals around the TSS of LPS-inducible genes in resting and LPS-activated (0.5 h) BMDM. For each of the three groups, the rows were sorted by the decreasing Pol II ChIP-seq signal in TSS region in resting BMDM.



Supplementary Figure 4. NELF is globally and transiently dismissed from chromatin upon macrophage activation. (**a**) The occupancy of NELF-E (n=3) in the TSS region of *Tnf* (*P*=0.016), *Jun* (*P*=0.0434), *Cited2* (*P*=0.0325), *Fos* (*P*=0.0529) was assessed by ChIP-

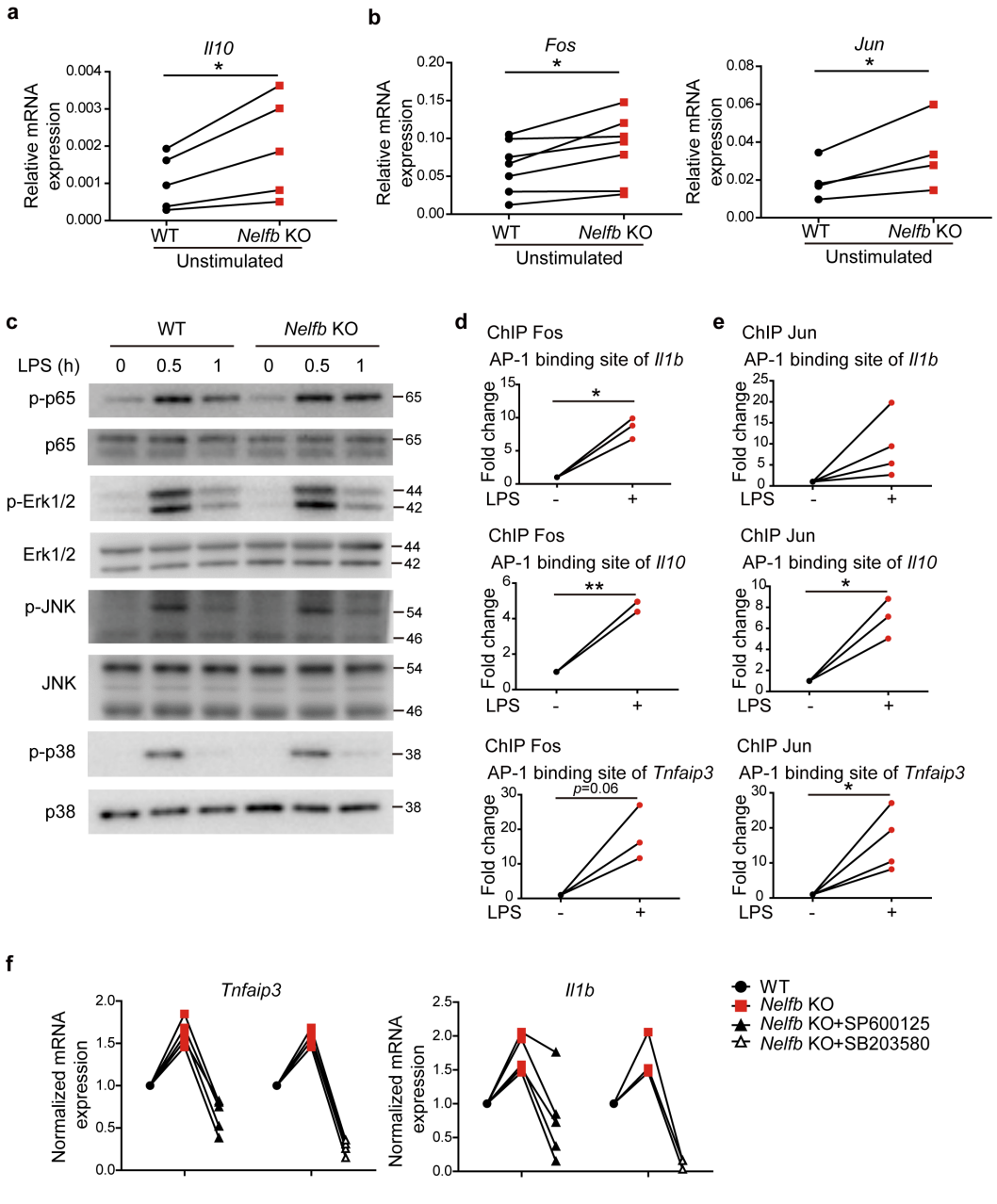
qPCR in resting and LPS-activated (0.5 h) BMDM. (**b**) Heat map of NELF-E ChIP-seq (replicate 2) signals around the TSS regions of all BMDM-expressed genes in resting and LPS-activated, as indicated, BMDM. Each row indicates one gene, and the rows were sorted by the decreasing NELF-E ChIP-seq signal near TSS in resting BMDM. (**c**) Percentage of $NELF^+$ genes with significant NELF dissociation upon LPS stimulation as defined by peak calling (top) and DiffBind (bottom). (**d**) NELF-E ChIP-seq signal changes upon 0.5 h (0.5 h vs. 0 h) and 1 h (1 h vs. 0 h) LPS stimulation in TSS regions of $NELF^+$ and $NELF^-$ genes shown as boxplot. Boxes outline the data from 1^{st} quartile to 3^{rd} quartile, and bars indicate the boundary whose distribution is not exceeded to 1.5 fold of interquartile range extended from both 1^{st} quartile and 3^{rd} quartile. p-value ($NELF^+$ genes 0.5 h/$NELF^-$ genes 0.5 h $P < 2.2e^{-16}$) was calculated by two-sided Mann-Whitney U test. (**e**) Correlation heat map shows the similarity of NELF-E ChIP-seq signal in TSS regions of all unique genes among samples from two replicates upon 0.5 h and 1 h LPS stimulation. (**f**) Tracks of NELF-E ChIP-seq, PRO-seq (sense strand) and RNA-seq for representative LPS non-regulated $NELF^+$ paused genes (*Ldha* and *Irf8*) in resting (-) and LPS-activated (0.5 h, +) BMDM. (**g**) NELF-E, NELF-B, p-p38 and p38 were detected by western blotting in total cell lysates of BMDM treated with LPS for indicated time. Shown is one representative blot from three biological replicates. (**h**) Chromatin-associated NELF-B and actin were detected by western blotting in WT and CDK9-KD iBMDM with or without LPS stimulation (left). CDK9, Pol II S2P and actin were visualized in total cell lysates (right). Shown is one representative blot from three biological replicates. (**i**) For each replicate from (**h**), NELF-B bands were quantified by densitometry, normalized to internal control (actin) and expressed relative to untreated WT sample (=1) (WT LPS 0 h/WT LPS 0.5 h P=0.011; CDK9-KD LPS 0 h/CDK9-KD LPS 0.5 h P=0.0814). (**j**) Chromatin-associated NELF-B and actin were detected by western blotting in mock (DMSO) and SB203580 (20 μM) –pretreated (0.5 h) BMDM; pCREB blot from total cell lysates is used as a control for SB203580 activity. Shown is one representative result from four biological

replicates (left). For each replicate, NELF-B protein level was quantified by densitometry, normalized to internal control (actin) and expressed relative to untreated sample (=1) (right) (LPS 0 h/0.5 h P=0.0064; SB203580 pretreated LPS 0 h/LPS 0.5 h P=0.0463). * P<0.05, ** P<0.01, 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.



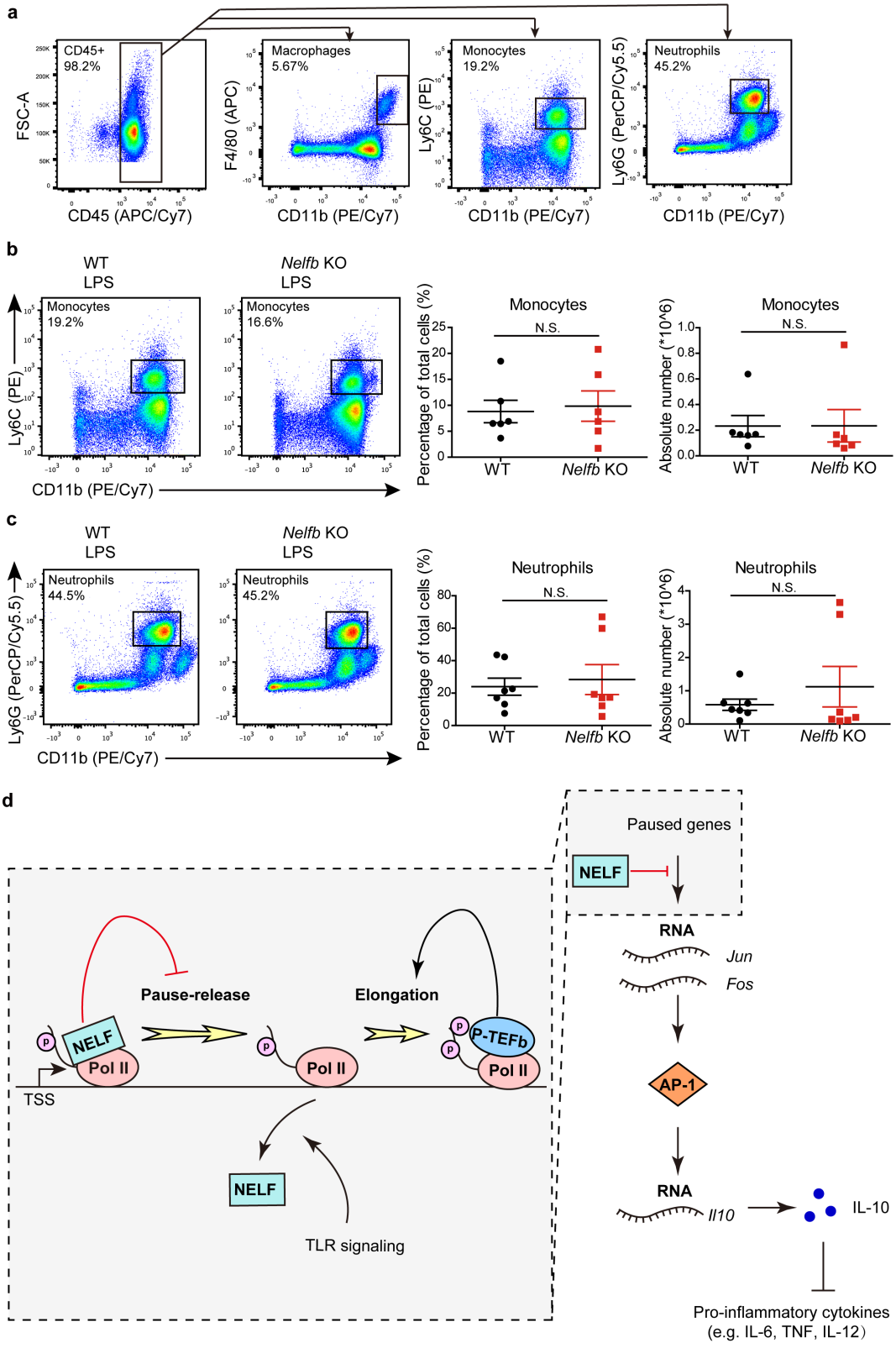
Supplementary Figure 5. NELF is involved in the transcription of LPS-suppressed gene. (**a**, **b**, **c**) FACS analysis of macrophage populations in peritoneal exudates (**a**: $CD45^{+}CD11b^{+}F4/80^{+}$), spleen (**b**: $CD45^{+}CD11b^{+}F4/80^{+}$) and lung (broncho-alveolar

lavage fluid, BALF) (**c**: $CD45^+CD11c^+$Siglec F^+) in WT and *Nelfb* KO mice. (**d**) Heat map of NELF-E ChIP-seq signals in resting and LPS-activated (0.5 h, +) BMDM around the TSS regions of group 1, 2 and 3 LPS-suppressed genes (n=161). For each group, the rows were sorted by decreasing NELF-E ChIP-seq signal in TSS regions in resting state. (**e**) Heat maps show the RNA-seq expression level (FPKM+1) in WT and *Nelfb* KO (left) and fold changes (*Nelfb* KO/WT) (right) of LPS-suppressed genes in LPS-treated (0.5 h) BMDM. (**f**) Cumulative RT-qPCR data showing expression level of *Cited2* in WT and *Nelfb* KO BMDM treated with LPS for 0.5 h (n=4 biologically independent experiments, P=0.045). * P<0.05 by two-sided paired Student's t test. (**g**) PRO-seq (sense strand) tracks for *Cited2* in WT and *Nelfb* KO BMDM cultured with (+) or without (-) LPS for 0.5 h. Source data are provided as a Source Data file.



Supplementary Figure 6. NELF deficiency does not affect TLR-induced activation of canonical signaling pathways. (**a**, **b**) Cumulative RT-qPCR data showing expression level of *Il10* (n=5, *P*=0.0286), *Fos* (n=7, *P*=0.0203) and *Jun* (n=4, *P*=0.0497) in WT and *Nelfb* KO resting BMDM. (**c**) Immunoblot analysis of p-p65, p65, p-Erk1/2, Erk1/2, p-JNK, JNK, p-p38 and p38 in whole cell lysates of WT and *Nelfb* KO BMDM treated with

LPS for indicated time. Shown is one representative blot from three biological replicates. (**d**, **e**) Occupancies of Fos (*Il1b* n=3, P=0.0147; *Il10* n=3, *P*=0.0025; *Tnfaip3* n=4, *P*=0.0633) (**d**) and Jun (*Il1b* n=4, *P*=0.1146; *Il10* n=3, *P*=0.0316; *Tnfaip3* n=4, *P*=0.0389) (**e**) at the AP-1 binding site of *Il1b, Il10* and *Tnfaip3* were assessed by ChIP-qPCR in resting and LPS-activated (1 h) BMDM. (**f**) WT and *Nelfb* KO BMDM were pretreated with SP600125 (50 μM) or SB203580 (20 μM), as indicated, for 0.5 h, and *Tnfaip3* (left) and *Il1b* (right) expression following 0.5 h of LPS co-incubation was measured by RT-qPCR (SP600125-*Tnfaip3*: n=5; SP600125-*Il1b*: n=5; SB203580-*Tnfaip3*: n=4; SB203580-*Il1b*: n=3). Relative mRNA expression was normalized to the levels in WT cells that were set as 1. * $P<0.05$, ** $P<0.01$, *** $P<0.001$, 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.


a
CD45+
98.2%
FSC-A
CD45 (APC/Cy7)
Macrophages
5.67%
F4/80 (APC)
CD11b (PE/Cy7)
Monocytes
19.2%
Ly6C (PE)
CD11b (PE/Cy7)
Neutrophils
45.2%
Ly6G (PerCP/Cy5.5)
CD11b (PE/Cy7)
b
WT
LPS
Nelfb KO
LPS
Monocytes
19.2%
Monocytes
16.6%
Ly6C (PE)
CD11b (PE/Cy7)
Monocytes
N.S.
Percentage of total cells (%)
WT
Nelfb KO
Monocytes
N.S.
Absolute number (*10^6)
WT
Nelfb KO
c
WT
LPS
Nelfb KO
LPS
Neutrophils
44.5%
Neutrophils
45.2%
Ly6G (PerCP/Cy5.5)
CD11b (PE/Cy7)
Neutrophils
N.S.
Percentage of total cells (%)
WT
Nelfb KO
Neutrophils
N.S.
Absolute number (*10^6)
WT
Nelfb KO
d
Paused genes
NELF
RNA
Jun
Fos
Pause-release
Elongation
NELF
Pol II
Pol II
P-TEFb
Pol II
TSS
AP-1
NELF
RNA
Il10
IL-10
TLR signaling
Pro-inflammatory cytokines
(e.g. IL-6, TNF, IL-12)

Supplementary Figure 7. The role of NELF in peritonitis model *in vivo* and working model. (**a**) Gating strategy for FACS analysis of peritoneal cells. $CD45^+$ cells were gated from the total peritoneal cells, and macrophages ($F4/80^+CD11b^+$), monocytes ($CD11b^+Ly6C^+$) and neutrophils ($CD11b^+Ly6G^+$) were subsequently gated from $CD45^+$ cells. (**b**, **c**) FACS analysis of the monocyte (**b**: $CD45^+CD11b^+Ly6C^+$) and neutrophil (**c**: $CD45^+CD11b^+ Ly6G^+$) populations in peritoneal exudates of WT and *Nelfb* KO mice 9 h after an intraperitoneal injection of LPS (100 ng/mouse). Representative FACS distribution (left) and cumulative (monocytes: n=6; neutrophils: n=7 biologically independent animals) percentage (middle) as well as absolute cell numbers (right) are shown. N.S. $P>0.05$ by two-sided unpaired Student's *t* test. Error bars indicate SEM. (**d**) Working model: NELF-mediated post-initiation control of the pro- and anti-inflammatory regulation network. Left: Promoter-proximal Pol II pausing landscape in resting macrophages is broadly marked by NELF occupancy. LPS stimulation leads to CDK9-independent NELF dismissal from TSS regions and pause-release. P-TEFb is recruited to phosphorylate Pol II leading to transcription elongation in gene body regions. Right: NELF enforces the transcriptional pause on its direct targets, *Jun* and *Fos*, which, in turn, constrains *Il10* transcription thereby serving a permissive role in pro-inflammatory cytokine production and macrophage-mediated inflammation. Source data are provided as a Source Data file.