

Reporting Summary

Nature Portfolio wishes to improve the reproducibility of the work that we publish. This form provides structure for consistency and transparency in reporting. For further information on Nature Portfolio policies, see our [Editorial Policies](#) and the [Editorial Policy Checklist](#).

Statistics

For all statistical analyses, confirm that the following items are present in the figure legend, table legend, main text, or Methods section.

n/a	Confirmed
<input type="checkbox"/>	<input checked="" type="checkbox"/> The exact sample size (<i>n</i>) for each experimental group/condition, given as a discrete number and unit of measurement
<input type="checkbox"/>	<input checked="" type="checkbox"/> A statement on whether measurements were taken from distinct samples or whether the same sample was measured repeatedly
<input type="checkbox"/>	<input checked="" type="checkbox"/> The statistical test(s) used AND whether they are one- or two-sided <i>Only common tests should be described solely by name; describe more complex techniques in the Methods section.</i>
<input type="checkbox"/>	<input checked="" type="checkbox"/> A description of all covariates tested
<input type="checkbox"/>	<input checked="" type="checkbox"/> A description of any assumptions or corrections, such as tests of normality and adjustment for multiple comparisons
<input type="checkbox"/>	<input checked="" type="checkbox"/> A full description of the statistical parameters including central tendency (e.g. means) or other basic estimates (e.g. regression coefficient) AND variation (e.g. standard deviation) or associated estimates of uncertainty (e.g. confidence intervals)
<input type="checkbox"/>	<input checked="" type="checkbox"/> For null hypothesis testing, the test statistic (e.g. <i>F</i> , <i>t</i> , <i>r</i>) with confidence intervals, effect sizes, degrees of freedom and <i>P</i> value noted <i>Give P values as exact values whenever suitable.</i>
<input checked="" type="checkbox"/>	<input type="checkbox"/> For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings
<input checked="" type="checkbox"/>	<input type="checkbox"/> For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes
<input type="checkbox"/>	<input checked="" type="checkbox"/> Estimates of effect sizes (e.g. Cohen's <i>d</i> , Pearson's <i>r</i>), indicating how they were calculated

Our web collection on [statistics for biologists](#) contains articles on many of the points above.

Software and code

Policy information about [availability of computer code](#)

Data collection	For data collection no specific software was used except for the software running the instruments used for the single cell sequencing and fluorescence microscopy imaging that are listed in the Methods section.
Data analysis	Custom analysis software tools are available on GitHub at https://github.com/RippeLab/RWireX and https://github.com/RippeLab/ACDC-microscopy . The published data analysis software used in our study is listed in Supplementary Table 8. Additional data analysis scripts can be downloaded from the Zenodo repository https://www.doi.org/10.5281/zenodo.13221210 .

For manuscripts utilizing custom algorithms or software that are central to the research but not yet described in published literature, software must be made available to editors and reviewers. We strongly encourage code deposition in a community repository (e.g. GitHub). See the Nature Portfolio [guidelines for submitting code & software](#) for further information.

Data

Policy information about [availability of data](#)

All manuscripts must include a [data availability statement](#). This statement should provide the following information, where applicable:

- Accession codes, unique identifiers, or web links for publicly available datasets
- A description of any restrictions on data availability
- For clinical datasets or third party data, please ensure that the statement adheres to our [policy](#)

The requested information is provided in the data availability section of the manuscript and in Supplementary Table 7 of the study.

Research involving human participants, their data, or biological material

Policy information about studies with [human participants or human data](#). See also policy information about [sex, gender \(identity/presentation\), and sexual orientation](#) and [race, ethnicity and racism](#).

Reporting on sex and gender	n/a
Reporting on race, ethnicity, or other socially relevant groupings	n/a
Population characteristics	n/a
Recruitment	n/a
Ethics oversight	n/a

Note that full information on the approval of the study protocol must also be provided in the manuscript.

Field-specific reporting

Please select the one below that is the best fit for your research. If you are not sure, read the appropriate sections before making your selection.

☒ Life sciences ☐ Behavioural & social sciences ☐ Ecological, evolutionary & environmental sciences

For a reference copy of the document with all sections, see nature.com/documents/nr-reporting-summary-flat.pdf

Life sciences study design

All studies must disclose on these points even when the disclosure is negative.

Sample size	No sample size calculation was performed, and time points for TNF α treatment of 30 min (early response) and 240 min (late response) were chosen. This study design followed that of previous publications on the proinflammatory TNF α response (Papantonis 2012, EMBO J; Diermeier 2014, Genome Biol; Kolovos 2016, Genome Res; Weiterer 2020, EMBO J). By comparing three independent replicates of each time point for the scATAC-seq and scRNA-seq analysis (corroborated by bulk RNA-seq), we demonstrated the reproducibility of our findings as described in the Supplementary Materials.
Data exclusions	<p>scRNA-seq: Cells were filtered using a minimal threshold of 100 detected genes, a maximal threshold of 5 percent mitochondrial counts, and a minimal threshold of 5,000 UMI counts. Outliers were removed per sample by filtering out cells with more UMI counts than the mean plus twice the standard deviation and outside of plus/minus three times the standard deviation of mitochondrial counts. Cells in cell cycle stages G2M and S were removed.</p> <p>snATAC-seq: Cells were filtered using a minimal threshold of $10^{4.5}$ for the number of unique fragments and a TSS ratio above 7. Cell doublets were removed with Amulet in scDbtFinder using a 5th percentile cutoff for significant q-values. Additionally, outliers were removed by filtering out cells with blacklist ratios above the mean plus twice the standard deviation. Cells in G2M and S phase were removed.</p> <p>snRNA-seq: Cells were filtered using a minimal threshold of 100 detected exoncounted genes, a maximal threshold of 5 percent mitochondrial counts, and filtering out cells with exonic UMI counts above/below the mean plus/minus thrice the standard deviation per sample. Cells in G2M and S phase were removed.</p> <p>Multiome snRNA-/snATAC-seq: For RNA data, high-quality cells were selected using a minimal threshold of 5,000 UMI counts and minimal and maximal thresholds of 5 and 40 percent mitochondrial counts. Outliers were removed per sample by filtering out cells with UMI counts above the mean plus twice the standard deviation. Cells in G2M and S phase were removed. For ATAC data, high-quality cells were selected using minimal thresholds of $10^{3.5}$ unique fragments and a TSS enrichment score of 7. Cell doublets were removed using Amulet in scDbtFinder. Additionally, outliers were removed by filtering out cells with unique fragments above 30,000 and blacklist ratios above the mean plus twice the standard deviation. A mixed cluster composed of 86 cells from all conditions was excluded. Finally, high-quality cells from both ATAC and RNA were selected.</p> <p>Imaging data: Cell nuclei at the image borders or cells that displayed overexposure in individual channels were removed. For the co-expression analysis, only nuclear masks containing a minimum of one and a maximum of two co-expression masks were selected. We further filtered out nuclei from the analysis if the area of the subcellular co-expression masks and sum of intensities represented outliers from the overall population. Minimum fluorescence intensity thresholds for CXCL1, 2, 3, and 8 were defined from the minimum bimodal intensity distribution and adjusted based on visual inspection of each channel in the co-expression mask.</p>

Replication	The scATAC-seq and scRNA-seq data acquisition were conducted for three independent replicates (starting from a new aliquot of HUVEC cells) for all three time points. Fluorescence microscopy imaging experiments were also conducted in multiple replicates for quantification. The number of replicates for each gene used in the padFISH experiments is stated in the Source Data and in the relative figure legends.
Randomization	n/a
Blinding	Investigators were not blinded during data collection and analysis since the same researchers were conducting the data acquisition and the data analysis.

Reporting for specific materials, systems and methods

We require information from authors about some types of materials, experimental systems and methods used in many studies. Here, indicate whether each material, system or method listed is relevant to your study. If you are not sure if a list item applies to your research, read the appropriate section before selecting a response.

Materials & experimental systems

n/a	Involved in the study
<input type="checkbox"/>	<input checked="" type="checkbox"/> Antibodies
<input type="checkbox"/>	<input checked="" type="checkbox"/> Eukaryotic cell lines
<input checked="" type="checkbox"/>	<input type="checkbox"/> Palaeontology and archaeology
<input checked="" type="checkbox"/>	<input type="checkbox"/> Animals and other organisms
<input checked="" type="checkbox"/>	<input type="checkbox"/> Clinical data
<input checked="" type="checkbox"/>	<input type="checkbox"/> Dual use research of concern
<input checked="" type="checkbox"/>	<input type="checkbox"/> Plants

Methods

n/a	Involved in the study
<input type="checkbox"/>	<input checked="" type="checkbox"/> ChIP-seq
<input checked="" type="checkbox"/>	<input type="checkbox"/> Flow cytometry
<input checked="" type="checkbox"/>	<input type="checkbox"/> MRI-based neuroimaging

Antibodies

Antibodies used	Primary antibody: NF-κB p65 (D14E12) XP Rabbit mAb (Cell Signaling Technologies, cat. 8242, Lot. 20) was used at 1:500 dilution from stock. Secondary antibody: Goat anti-Rabbit IgG (H+L) labeled with Alexa Fluor 647 (Invitrogen, cat. A21244, lot. 2836809) diluted 1:1000 from stock. For ChIP-seq antibodies, see below.
Validation	The anti-NFκB primary antibody was validated by the manufacturer (https://www.cellsignal.com/products/primary-antibodies/nf-kb-p65-d14e12-xp-rabbit-mab/8242). Data Sheet (https://www.cellsignal.com/products/8242/datasheet?images=1&protocol=0&size=A4) and Certificate of Analysis (https://media.cellsignal.com/coa/8242/20/8242-lot-20-coa.pdf) are available on the manufacturer's website.

Eukaryotic cell lines

Policy information about [cell lines and Sex and Gender in Research](#)

Cell line source(s)	Primary human umbilical endothelial cells (HUVECs) pooled from donated human tissue were acquired from Lonza (catalogue number, 00191027; lot, 18TL232828; sex, male/female mixed).
Authentication	The company Lonza has provided a certificate of analysis for lot 18TL232828 used in our experiments. The following tests have been carried out: virus testing, microbial testing, cell performance testing.
Mycoplasma contamination	Mycoplasma test were negative according to the certificate of analysis. In addition, we performed mycoplasma tests on the aliquots from the same batch used in our experiments and also obtained negative results.
Commonly misidentified lines (See ICLAC register)	n/a

Plants

Seed stocks	n/a
Novel plant genotypes	n/a
Authentication	n/a

Data deposition

- ☒ Confirm that both raw and final processed data have been deposited in a public database such as [GEO](#).
- ☒ Confirm that you have deposited or provided access to graph files (e.g. BED files) for the called peaks.

Data access links

May remain private before publication.

Gene Expression Omnibus (GEO, <https://www.ncbi.nlm.nih.gov/geo/>); accession number GSE273430. ChIP-seq as well as other sequencing data from our study can be accessed at this GEO repository with reviewer access token chmxuiikvvixbgp.

Files in database submission

BigWig, bed

Genome browser session

(e.g. [UCSC](#))

n/a

Methodology

Replicates

2 biological replicates for H3K27ac ChIP-seq using antibodies from different companies

Sequencing depth

25-30 Mio reads were sequenced per sample.

Antibodies

Anibodies targeting acetylated lysine 27 of histone H3 (H3K27ac) were from Abcam, catalogue number ab4729, and from Diagenode, catalogue number, C15210016 (H3K27ac ChIP-seq grade antibody). The IgG controls antibody was from Active Motif, catalogue number 53017.

Peak calling parameters

Peak calling was performed as previously described (Stadhouders et al. 2015, <https://github.com/supatt-lab/rChIPSeqTools>). Peaks with ≥ 20 read counts at each peak summit and $FDR \leq 0.001$ (using nonspecific, IgG, ChIP-seq data as background) were selected.

Data quality

Only reads with alignment quality ≥ 10 were used.

Software

Raw read files were aligned to the human reference genome (hg38) using Bowtie2. The ShortRead package was used to convert these into genome-wide coverage files.