nature portfolio

Corresponding author(s): Giacomo Donati

Last updated by author(s): 2026 02 10

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	
☐	☒	The exact sample size (*n*) for each experimental group/condition, given as a discrete number and unit of measurement
☐	☒	A statement on whether measurements were taken from distinct samples or whether the same sample was measured repeatedly
☐	☒	The statistical test(s) used AND whether they are one- or two-sided *Only common tests should be described solely by name; describe more complex techniques in the Methods section.*
☐	☒	A description of all covariates tested
☐	☒	A description of any assumptions or corrections, such as tests of normality and adjustment for multiple comparisons
☐	☒	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)
☐	☒	For null hypothesis testing, the test statistic (e.g. *F*, *t*, *r*) with confidence intervals, effect sizes, degrees of freedom and *P* value noted *Give P values as exact values whenever suitable.*
☒	☐	For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings
☒	☐	For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes
☐	☒	Estimates of effect sizes (e.g. Cohen's *d*, Pearson's *r*), 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	Real Time qPCR: QuantStudio 6 Flex Real-Time PCR System version 1.3 (Thermo Fisher Scientific). Immunofluorecence: TCS SP8 Tandem Scanner Leica confocal microscope (leicaBiosystem) equipped with 20x dry and 40x or 63x immersion objectives (Zeiss). Chemiluminiscent signal detection: ChemiDco Touch Imaging System (BioRad). Flow cytometry: BD FACSVerse™ Universal Loader was used. Sequencing for ChIP-seq, seCLIP, RNA-seq, ReGenT-seq: Illumina NextSeq 500 or 1000 System.
Data analysis	A detailed description of all bioinformatics software, including software version and parameters, used for the analysis of ReGenT-seq, Viability RNAi screening, RNA-seq, ChIP-seq and seCLIP data has been provided in the Methods section. This includes: FastQC (v0.11.2), Trim Galore! (v0.5.0), STAR (v2.7.1a), Bowtie (v1.2.3), Bowtie2 (v2.3.5.1), SAMtools (v1.6), Picard MarkDuplicates (https://broadinstitute.github.io/picard), featureCounts (v1.6.1), edgeR (v3.32), MACS (v2.1.4), epic2 (v0.0.52), idr (v2.0.2), UMI-tools (v1.1.4), BEDTools (v2.29.2), deepTools (v3.3.1), CLIPper (https://github.com/YeoLab/clipper) and the companion post-processing tools available as Docker images from: https://github.com/YeoLab/merge_peak), HOMER (v4.11), ViennaRNA (v2.6.4), TEtranscripts (v2.2.3), RegTools (v1.0.0), rMATS (v4.1.2), R/Bioconductor. R software (v4.0.4) and Graphpad Prism version 8.2.1 were used for all other statistical analyses. A comprehensive list of R packages used has been provided in the Methods section. Imagej FIJI version 2.14.0 and Qupath (version 0.5.1; https://qupath.github.io/) were used for image analysis.

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

Omic data that support the findings of this study have been deposited in the Gene Expression Omnibus (GEO) under accession code GSE276632

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	For acute and chronic venous ulcers, pyoderma gangrenosum ulcers, and cutaneous human SCC samples, sex was not specifically analyzed in this study; therefore, both male and female participants were included. Primary vulvar keratinocytes were isolated from a Caucasian female.
Reporting on race, ethnicity, or other socially relevant groupings	No information on race, ethnicity, or social-related factors was collected.
Population characteristics	N/A
Recruitment	For acute and chronic venous ulcers, pyoderma gangrenosum ulcers, and cutaneous human SCC samples, tissue samples were obtained at Hokkaido University Hospital. Primary vulvar keratinocytes were isolated from tissue obtained at Ospedale Sant'Anna in Turin, from a Caucasian female.
Ethics oversight	Ethical approval for studies involving immortalized human keratinocytes was obtained from the Comitato Etico Territoriale (CET) Interaziendale, AOU Città della Salute e della Scienza di Torino (protocol #0002319). Studies involving acute and chronic venous ulcers, pyoderma gangrenosum ulcers, and cutaneous human squamous cell carcinoma (SCC) samples were approved by the Institutional Review Board of the Hokkaido University Graduate School of Medicine (approval IDs: 13-043, 14-063, and 15-029). All studies were conducted in accordance with the principles of the Declaration of Helsinki. Written informed consent was obtained from all participants.

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	Sample size calculation was not performed. A minimum of n = 3 was used for each experiment.
Data exclusions	We present all data obtained, including negative results.
Replication	All key experiments were independently repeated between two and four times. The exact number of replicates for each experiment is indicated in the corresponding figure legends.
Randomization	Randomization was not applicable, as all cell lines and biological samples were analyzed in the same manner.
Blinding	Investigators were not blinded to cell line identities during analysis, as knowledge of sample identity was necessary to set up the analysis. In some cases, key experiments were performed independently by two different operators.

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
☐	☒ Antibodies
☐	☒ Eukaryotic cell lines
☒	☐ Palaeontology and archaeology
☐	☒ Animals and other organisms
☒	☐ Clinical data
☒	☐ Dual use research of concern
☒	☐ Plants

Methods

n/a	Involved in the study
☐	☒ ChIP-seq
☐	☒ Flow cytometry
☒	☐ MRI-based neuroimaging

Antibodies

Antibodies used

Vinculin Sigma-Aldrich SAB4200080
pFAK (y925) Cell Signaling3284S
P4hb Abcam 2792
Lamp1 Sigma-Aldrich L1418
HP1y Millipore 05-960 (clone 42s2)
HP1y Cell Signaling 2619
H3K27Ac3 Abcam ab177178 (clone EP16602)
Tom20 Santa Cruz sc-11415 (clone FL-145)
WGA Thermo Fisher Scientific 11261
Alexa-488 conjugated Phalloidin Thermo Fisher Scientific A12379
Alexa-568 conjugated Phalloidin Thermo Fisher Scientific A12380
Lamin B1 Abcam ab65986
Actin beta Sigma-Aldrich A5441
Gapdh Santa Cruz sc-25778 (clone FL-355)

Validation

Test of each antibody was performed following the manufacturer's instructions. A range of concentrations was first tested to determine the working range, and the appropriate concentration for each antibody was then selected. The concentrations used for the different experiments performed in this study are reported in Supplementary Table 8.

Eukaryotic cell lines

Policy information about cell lines and Sex and Gender in Research

Cell line source(s)

EPCs were isolated from the dorsal skin of adult female mice, as described in the Methods section.
SCC cells were isolated from a murine SCC tumor, as described in the Methods section.
Primary vulvar epidermal cells were isolated from a patient undergoing surgery, as described in the Methods section.
The HaCaT cell line was kindly provided by Valentina Proserpio (University of Turin).
Lenti-X 293T and Retro-X 293T cell lines were kindly provided by Salvatore Oliviero (University of Turin).

Authentication

The cell lines Lenti-X 293T, Retro-X 293T and HaCaT used in this study were not authenticated. EPs were authenticated based on the expression of molecular markers. Primary vulvar epidermal cells were authenticated by sequencing.

Mycoplasma contamination

Cells were routinely tested for mycoplasma contamination, and all cells used were mycoplasma-negative.

Commonly misidentified lines
(See ICLAC register)

The cells used in this study are not listed in the ICLAC database.

Animals and other research organisms

Policy information about studies involving animals; ARRIVE guidelines recommended for reporting animal research, and Sex and Gender in Research

Laboratory animals

(CBAxC57BL/6)F1 mice were purchased from Charles River. Mice were housed under controlled environmental conditions, with a 12 h light/12 h dark cycle. Ambient temperature was maintained at 20–24 °C.

Wild animals

No wild animals were used in this study.

Reporting on sex

Sex was not specifically analyzed in this study; therefore, both male and female mice, and cells derived from them, were included.

Field-collected samples

This study did not involve field-collected samples.

Ethics oversight	Animal experiments were performed in accordance with procedures approved by the Institutional Animal Care and Use Committee of the Università degli Studi di Torino and the Italian Ministry of Health (authorization number 427/2023-PR).

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

Plants

Seed stocks	N/A
Novel plant genotypes	N/A
Authentication	N/A

ChIP-seq

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.*	https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE276621
Files in database submission	For each replicate IP/Input pair, the following files have been deposited: - Raw FASTQ files - BigWig files of IP vs. Input–normalized signal tracks - BED files from diffuse peak calling using epic2 (FDR < 1×10^{-4})
Genome browser session (e.g. UCSC)	N/A

Methodology

Replicates	N = 2 independent replicates were performed. Pearson's correlation coefficient = 0.94 (p < 2.2e-16).
Sequencing depth	Read length = 61+61bp paired-end. Sequencing depth (number of reads per sample): - CBX3 IP Replicate 1: 38885160 (total), 27285608 (uniquely mapped), 26254870 (quality-filtered, deduplicated) - Input Replicate 1: 40375224 (total), 29526588 (uniquely mapped), 28869199 (quality-filtered, deduplicated) - CBX3 IP Replicate 2: 44167775 (total), 30975210 (uniquely mapped), 29250883 (quality-filtered, deduplicated) - Input Replicate 2: 40837052 (total), 29636517 (uniquely mapped), 28720190 (quality-filtered, deduplicated)
Antibodies	CBX3 antibody (Sigma-Aldrich, clone 42s2, 05-690)
Peak calling parameters	Following quality controls (performed with FastQC v0.11.2), paired-end sequencing reads were trimmed using Trim Galore! v0.5.0 and aligned to the mouse reference genome (mm10/GRCm38) using Bowtie v2.3.5.1. Duplicated alignments (identified by Picard MarkDuplicates, https://broadinstitute.github.io/picard) and low-quality alignments/multi-mapping reads were excluded using SAMtools v1.6. Immunoprecipitation and corresponding control (Input DNA) datasets were treated identically. Input-normalized ChIP-seq fold-enrichment signal tracks were obtained using MACS v2.1.4 (command: callpeak -g mm --nomodel -f BAMPE -q 0.05 --SPMR -B and bdgcmp -m FE). For Cbx3 ChIP-seq, braod peak calling was performed using epic2 (command: --genome mm10 --false-discovery-rate-cutoff 0.05 --guess-bampe).
Data quality	Number of diffuse peaks: - Replicate 1 = 14566 - Replicate 2 = 19935 - Reproducible peaks across replicates (FDR < 1e-4, log2FC > 0.5) = 5816
Software	FastQC (v0.11.2), Trim Galore! (v0.5.0), Bowtie2 (v2.3.5.1), SAMtools (v1.6), Picard MarkDuplicates, (https://broadinstitute.github.io/picard), MACS (v2.1.4), epic2 (v0.0.52).

Flow Cytometry

Plots

Confirm that:

☒ The axis labels state the marker and fluorochrome used (e.g. CD4-FITC).

☒ The axis scales are clearly visible. Include numbers along axes only for bottom left plot of group (a 'group' is an analysis of identical markers).

☐ All plots are contour plots with outliers or pseudocolor plots.

☒ A numerical value for number of cells or percentage (with statistics) is provided.

Methodology

Sample preparation	Cells were washed with 1X PBX, detached using trypsin, and resuspended in 1X PBS.
Instrument	BD FACSVerse™ Universal Loader (BD Biosciences)
Software	BD FACSDiva software (BD Biosciences) and FlowJo software
Cell population abundance	At least 1,000 events per sample have been acquired.
Gating strategy	CCells were initially gated using FSC-A and SSC-A plots to generate the P1 population. Singlets were then gated out of P1 using FSC-W and FSC-H plots, generating the P2 population. Cells were counted over a 30-second acquisition period. For GFP-positive cells, a gate was set on the P1 population based on a GFP-negative control, using GFP versus SSC-A plots to identify positive events.

☐ Tick this box to confirm that a figure exemplifying the gating strategy is provided in the Supplementary Information.