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Reporting Summary

Nature Research 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 Research 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: NA

Data analysis: Data analysis is described in the methods section as follows:

For analysis of histone PTM ChIP-sequencing, reads were demultiplexed using bcl2fastq2 (Illumina) with the options "--mask-short-adapter-reads 20 --minimum-trimmed-read-length 20 --no-lane-splitting --barcode-mismatches 0". Reads were trimmed using TRIMMOMATIC (Bolger et al., 2014) with the options "ILLUMINACLIP:[adapter.fa]:2:30:10 LEADING:5 TRAILING:5 SLIDINGWINDOW:4:15 MINLEN:15", and aligned to a hybrid hg38+C. floridanus (v7.5, RefSeq) genome assembly using bowtie2 v2.2.64 with the option "--sensitive-local". Alignments with a mapping quality below 5 (using samtools) and duplicated reads were removed peaks were called using macs25 v2.1.1.20160309 with the options "--call-summits –nomodel –B". Differential ChIP peaks were called using DiffBind6 using the options "bFullLibrarySize=FALSE, bSubControl=TRUE, bTagwise=FALSE" for dba.analyze(). For DiffBind testing the DESeq2 algorithm with blocking was used, and ChIP replicate was used as the blocking factor while testing for differences between Mock and infected samples. For ChIP signal tracks individual replicate tracks were produced for RPM and fold-enrichment over input control, merged, and averaged.

For Orf8 ChIP-sequencing analysis, alignments were performed with Bowtie2 (2.1.0)4 using the Hg38 genome using a ChIP-seq pipeline (https://github.com/shenlab-sinai/chip-seq_preprocess). Orf8 reads were mapped using NGS plot.

For ATAC-sequencing analysis, alignments were performed with Bowtie2 (2.1.0)4 using the Hg38 genome using a ChIP-seq pipeline (https://github.com/shenlab-sinai/chip-seq_preprocess). Reads were mapped using NGS plot.
To identify potential histone mimicry SARS-CoV-2 protein sequences were aligned to human histone protein sequences (H2A, H2B, H3.1,H3.2 H4, H2A.X, H2A.Z, macroH2A, and H3.3) using Multiple Sequence Comparison by Log-Expectation (MUSCLE) with default settings. SARS-CoV2 protein sequences were obtained from protein sequences published from the first Wuhan isolate.7

Images were analyzed using Image J software (version 2.0.0-rc-69/1.52p, build 269a0ad53f) Single z-plane images of HEK cells and human lung tissue, and summed z-stacks through A549 nuclei were used for PTM quantification. ROI of in-focus nuclei were semi-automatically defined using the DAPI channel and the analyze particles functionality with manual corrections. HEK histone PTMs were quantified in transfected and non-transfected neighboring cells using mean gray values. Strep-tagged Orf8 constructs (Streptactin-488) and GFP signal were used to define transfected cell and HEK histone PTMs levels of transfected cells were relativized to histone PTM levels in non-transfected neighbors. Histone PTMs were quantified in A549 and human lung tissue using integrated density values. dsRNA and SARS-CoV-2 nucleocapsid signal were used to define infected A549s and human lung cells, respectively.

For FACS, gating and sorting was conducted using BD FACS Software (1.2.0142). Post-Sort analysis was conducted with FlowJo (10.8.0).

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 Research 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 list of figures that have associated raw data
- A description of any restrictions on data availability

Data availability statement from methods section: All genome-wide sequencing data is available under accession number: GSE186628. All proteomics data are available via ProteomeXchange with identifier PXD034379.

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	A samples size of 3 biological replicates was used for all experiments where ever possible for sequencing and imaging experiments. In the case of RNA-sequencing and analysis with DESeq2, 3 replicates is established as sufficient to provide adequate power. For imaging experiments, 3 replicates ensured sufficient power, reproducibility, and that one replicate would not cause an inaccurate outcome. For viral data, the numbers of replicates were determined from many years of past history by the Weiss lab that provides statistically robust datasets for expected effect sizes.
Data exclusions	No data exclusions were used, expect in the case where immunocytochemistry staining failed in a necessary control or test sample and the data set could therefore not be used.
Replication	3 biological replicates were used for all experiments where ever possible and all replications confirmed findings with only expected variability in effect size found between replicates.
Randomization	Allocation of samples was random between manipulations.
Blinding	Imaging and analysis were performed with experimenter blinded to experimental condition where ever possible. For some instances, such as for patient tissue imaging, analysis required targeted selection, imaging, and analysis of infected cells compared to uninfected cells. This required the experimenter was aware of cell infection status while imaging. However, in these cases, the measurement of interest (such as a histone modification stain) was not viewed prior to choosing fields to avoid biasing selection to ensure the experimenter was still blinded to the measurement of interest. For all other sample and analysis processing where the experimenter was not blinded, the output was fully quantitative and thus unlikely to be biased by knowledge of samples or it was not possible such as for western blots where samples had to be loaded in a specific order.

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
☒	☐ Human research participants
☒	☐ Clinical data
☒	☐ Dual use research of concern

Methods

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

Antibodies

Antibodies used: A full antibody list is shown in Supplemental Table 6.

Validation: All antibodies used are well validated and widely used antibodies with the exception of the SARS-CoV-2 Orf8 antibody. This we validated using 1) Cells expressing Orf8 compared to control cells not expression Orf8 and 2) mock infected verses SARS-CoV-2 infected cells. All other antibodies were validated on the manufacturer's website for human cells for assays used with information included on the website including relevant citations. All antibodies used were cited in previously published studies.

Eukaryotic cell lines

Policy information about cell lines

Cell line source(s): The lab of Susan Weiss generated A549-ACE cells. HEK293T and Vero E6 cells were obtained from ATCC.

Authentication: Full authentication of A549-ACE cells and iAT2 cells cells are described in:
Li, Y. et al. SARS-CoV-2 induces double-stranded RNA-mediated innate immune responses in respiratory epithelial derived cells and cardiomyocytes. New HEK293T cells and Vero E6 cells were obtained at the onset of this project to ensure pure lines were used for this study.

Mycoplasma contamination: All cell lines used were confirmed to be negative for mycoplasma and are retested twice annually.

Commonly misidentified lines
(See ICLAC register): No misidentified cell lines were used in this study.

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.

All genome-wide sequencing data is available under accession number GSE186628 at
https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE186628

Files in database submission

GSM5657935 Mock_n1 A549 ATAC-Seq
GSM5657936 Mock_n2 A549 ATAC-Seq
GSM5657937 WT_n1 A549 ATAC-Seq
GSM5657938 WT_n2 A549 ATAC-Seq
GSM5657939 Del_n1 A549 ATAC-Seq
GSM5657940 Del_n2 A549 ATAC-Seq
GSM5657941 24h Mock n1 A549-ACE cells RNA-Seq
GSM5657942 24h Mock n2 A549-ACE cells RNA-Seq
GSM5657943 24h Mock n3 A549-ACE cells RNA-Seq
GSM5657944 24h SARS-CoV-2 WT n1 A549-ACE cells RNA-Seq
GSM5657945 24h SARS-CoV-2 WT n2 A549-ACE cells RNA-Seq
GSM5657946 24h SARS-CoV-2 WT n3 A549-ACE cells RNA-Seq
GSM5657947 24h SARS-CoV-2 Del n1 A549-ACE cells RNA-Seq
GSM5657948 24h SARS-CoV-2 Del n2 A549-ACE cells RNA-Seq
GSM5657949 24h SARS-CoV-2 Del n3 A549-ACE cells RNA-Seq
GSM5657950 48h Mock n1 A549-ACE cells RNA-Seq
GSM5657951 48h Mock n2 A549-ACE cells RNA-Seq
GSM5657952 48h Mock n3 A549-ACE cells RNA-Seq
GSM5657953 48h SARS-CoV-2 WT n1 A549-ACE cells RNA-Seq
GSM5657954 48h SARS-CoV-2 WT n2 A549-ACE cells RNA-Seq
GSM5657955 48h SARS-CoV-2 WT n3 A549-ACE cells RNA-Seq
GSM5657956 48h SARS-CoV-2 Del n1 A549-ACE cells RNA-Seq

GSM5657957 48h SARS-CoV-2 Del n2 A549-ACE cells RNA-Seq
GSM5657958 48h SARS-CoV-2 Del n3 A549-ACE cells RNA-Seq
GSM5657959 GFP_n1 HEK cells ATAC-Seq
GSM5657960 GFP_n2 HEK cells ATAC-Seq
GSM5657961 Orf8_n1 HEK cells ATAC-Seq
GSM5657962 Orf8_n2 HEK cells ATAC-Seq
GSM5657963 ARKSAP_n1 HEK cells ATAC-Seq
GSM5657964 ARKSAP_n2 HEK cells ATAC-Seq
GSM5657965 GFP CUT&TAG
GSM5657966 Orf8 CUT&TAG
GSM5657967 ARKSAP CUT&TAG
GSM5657989 24h Mock n1 iAT2 cells RNA-Seq
GSM5657990 24h Mock n2 iAT2 cells RNA-Seq
GSM5657991 24h Mock n3 iAT2 cells RNA-Seq
GSM5657992 24h SARS-CoV-2 WT n1 iAT2 cells RNA-Seq
GSM5657993 24h SARS-CoV-2 WT n2 iAT2 cells RNA-Seq
GSM5657994 24h SARS-CoV-2 WT n3 iAT2 cells RNA-Seq
GSM5657995 24h SARS-CoV-2 Del n1 iAT2 cells RNA-Seq
GSM5657996 24h SARS-CoV-2 Del n2 iAT2 cells RNA-Seq
GSM5657997 24h SARS-CoV-2 Del n3 iAT2 cells RNA-Seq
GSM5657998 48h Mock n1 iAT2 cells RNA-Seq
GSM5657999 48h Mock n2 iAT2 cells RNA-Seq
GSM5658000 48h Mock n3 iAT2 cells RNA-Seq
GSM5658001 48h SARS-CoV-2 WT n1 iAT2 cells RNA-Seq
GSM5658002 48h SARS-CoV-2 WT n2 iAT2 cells RNA-Seq
GSM5658003 48h SARS-CoV-2 WT n3 iAT2 cells RNA-Seq
GSM5658004 48h SARS-CoV-2 Del n1 iAT2 cells RNA-Seq
GSM5658005 48h SARS-CoV-2 Del n2 iAT2 cells RNA-Seq
GSM5658006 48h SARS-CoV-2 Del n3 iAT2 cells RNA-Seq
GSM5658830 A549 cells with Mock infection - Input, replicate r1
GSM5658831 A549 cells with Mock infection - Input, replicate r2
GSM5658832 A549 cells with Mock infection - Input, replicate r3
GSM5658833 A549 cells with CoV2 infection - Input, replicate r1
GSM5658834 A549 cells with CoV2 infection - Input, replicate r2
GSM5658835 A549 cells with CoV2 infection - Input, replicate r3
GSM5658836 A549 cells with CoV2-Orf8 deletion infection infection - Input, replicate r1
GSM5658837 A549 cells with CoV2-Orf8 deletion infection infection - Input, replicate r2
GSM5658838 A549 cells with CoV2-Orf8 deletion infection infection - Input, replicate r3
GSM5658839 A549 cells with Mock infection - K27m3, replicate r1
GSM5658840 A549 cells with Mock infection - K27m3, replicate r2
GSM5658841 A549 cells with Mock infection - K27m3, replicate r3
GSM5658842 A549 cells with CoV2 infection - K27m3, replicate r1
GSM5658843 A549 cells with CoV2 infection - K27m3, replicate r2
GSM5658844 A549 cells with CoV2 infection - K27m3, replicate r3
GSM5658845 A549 cells with CoV2-Orf8 deletion infection infection - K27m3, replicate r1
GSM5658846 A549 cells with CoV2-Orf8 deletion infection infection - K27m3, replicate r2
GSM5658847 A549 cells with CoV2-Orf8 deletion infection infection - K27m3, replicate r3
GSM5658848 A549 cells with Mock infection - K9ac, replicate r1
GSM5658849 A549 cells with Mock infection - K9ac, replicate r2
GSM5658850 A549 cells with Mock infection - K9ac, replicate r3
GSM5658851 A549 cells with CoV2 infection - K9ac, replicate r1
GSM5658852 A549 cells with CoV2 infection - K9ac, replicate r2
GSM5658853 A549 cells with CoV2 infection - K9ac, replicate r3
GSM5658854 A549 cells with CoV2-Orf8 deletion infection infection - K9ac, replicate r1
GSM5658855 A549 cells with CoV2-Orf8 deletion infection infection - K9ac, replicate r2
GSM5658856 A549 cells with CoV2-Orf8 deletion infection infection - K9ac, replicate r3
GSM5658857 A549 cells with Mock infection - K9m3, replicate r1
GSM5658858 A549 cells with Mock infection - K9m3, replicate r2
GSM5658859 A549 cells with Mock infection - K9m3, replicate r3
GSM5658860 A549 cells with CoV2 infection - K9m3, replicate r1
GSM5658861 A549 cells with CoV2 infection - K9m3, replicate r2
GSM5658862 A549 cells with CoV2 infection - K9m3, replicate r3
GSM5658863 A549 cells with CoV2-Orf8 deletion infection infection - K9m3, replicate r1
GSM5658864 A549 cells with CoV2-Orf8 deletion infection infection - K9m3, replicate r2
GSM5658865 A549 cells with CoV2-Orf8 deletion infection infection - K9m3, replicate r3
GSM5664216 HEK293T GFP n1 RNA-seq
GSM5664217 HEK293T GFP n2 RNA-seq
GSM5664218 HEK293T Orf8 n1 RNA-seq
GSM5664219 HEK293T Orf8 n2 RNA-seq
GSM5664220 HEK293T Orf8 n3 RNA-seq
GSM5664221 HEK293T Orf8dARKSAP n1 RNA-seq
GSM5664222 HEK293T Orf8dARKSAP n2 RNA-seq
GSM6211041 A549 cells with ARKSAP deletion, CoV2 infection, Input control, replicate r1
GSM6211042 A549 cells with ARKSAP deletion, CoV2 infection, Input control, replicate r2
GSM6211043 A549 cells with ARKSAP deletion, CoV2 infection, Input control, replicate r3
GSM6211044 A549 cells with Mock infection, Input control, replicate r1

GSM6211045 A549 cells with Mock infection, Input control, replicate r2
GSM6211046 A549 cells with Mock infection, Input control, replicate r3
GSM6211047 A549 cells with CoV2 infection, Input control, replicate r1
GSM6211048 A549 cells with CoV2 infection, Input control, replicate r3
GSM6211049 A549 cells with Orf8 deletion, CoV2 infection, Input control, replicate r1
GSM6211050 A549 cells with Orf8 deletion, CoV2 infection, Input control, replicate r2
GSM6211051 A549 cells with Orf8 deletion, CoV2 infection, Input control, replicate r3
GSM6211052 A549 cells with ARKSAP deletion, CoV2 infection, H3K9ac IP, replicate r1
GSM6211053 A549 cells with ARKSAP deletion, CoV2 infection, H3K9ac IP, replicate r2
GSM6211054 A549 cells with ARKSAP deletion, CoV2 infection, H3K9ac IP, replicate r3
GSM6211055 A549 cells with Mock infection, H3K9ac IP, replicate r1
GSM6211056 A549 cells with Mock infection, H3K9ac IP, replicate r2
GSM6211057 A549 cells with Mock infection, H3K9ac IP, replicate r3
GSM6211058 A549 cells with CoV2 infection, H3K9ac IP, replicate r1
GSM6211059 A549 cells with CoV2 infection, H3K9ac IP, replicate r3
GSM6211060 A549 cells with Orf8 deletion, CoV2 infection, H3K9ac IP, replicate r1
GSM6211061 A549 cells with Orf8 deletion, CoV2 infection, H3K9ac IP, replicate r2
GSM6211062 A549 cells with Orf8 deletion, CoV2 infection, H3K9ac IP, replicate r3
GSM6215565 Mock_n1 (Set 2)
GSM6215566 Mock_n2 (Set 2)
GSM6215567 Mock_n3 (Set 2)
GSM6215568 WT_n1 (Set 2)
GSM6215569 WT_n2 (Set 2)
GSM6215570 dOrf8_n1 (Set 2)
GSM6215571 dOrf8_n2 (Set 2)
GSM6215572 dOrf8_n3 (Set 2)
GSM6215573 dARKSAP_n1 (Set 2)
GSM6215574 dARKSAP_n2 (Set 2)
GSM6215575 dARKSAP_n3 (Set 2)
GSM6215576 Mock_n1 (Set 3)
GSM6215577 Mock_n2 (Set 3)
GSM6215578 Mock_n3 (Set 3)
GSM6215579 WT_n1 (Set 3)
GSM6215580 WT_n2 (Set 3)
GSM6215581 WT_n3 (Set 3)
GSM6215582 dOrf8_n1 (Set 3)
GSM6215583 dOrf8_n3 (Set 3)
GSM6215584 dARKSAP_n1 (Set 3)
GSM6215585 dARKSAP_n2 (Set 3)
GSM6215586 dARKSAP_n3 (Set 3)
GSM6215587 24h Mock n1
GSM6215588 24h Mock n2
GSM6215589 24h Mock n3
GSM6215590 24h SARS-CoV-2 WT n1
GSM6215591 24h SARS-CoV-2 WT n2
GSM6215592 24h SARS-CoV-2 WT n3
GSM6215593 24h SARS-CoV-2 DelOrf8 n1
GSM6215594 24h SARS-CoV-2 DelOrf8 n2
GSM6215595 24h SARS-CoV-2 DelOrf8 n3
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GSM6215597 24h SARS-CoV-2 DelARKSAP n2
GSM6215598 24h SARS-CoV-2 DelARKSAP n3
GSM6215599 48h Mock n1
GSM6215600 48h Mock n2
GSM6215601 48h Mock n3
GSM6215602 48h SARS-CoV-2 WT n1
GSM6215603 48h SARS-CoV-2 WT n2
GSM6215604 48h SARS-CoV-2 WT n3
GSM6215605 48h SARS-CoV-2 DelOrf8 n1
GSM6215606 48h SARS-CoV-2 DelOrf8 n2
GSM6215607 48h SARS-CoV-2 DelOrf8 n3
GSM6215608 48h SARS-CoV-2 DelARKSAP n1
GSM6215609 48h SARS-CoV-2 DelARKSAP n2
GSM6215610 48h SARS-CoV-2 DelARKSAP n3

Genome browser session
(e.g. UCSC)

NA

Methodology

Replicates

Replicates for each ChIP-seq experiment are described in the Figure Legend that corresponds to that ChIP experiment.

Sequencing depth

This information is described in detail in the methods section. In short, all RNA-seq experiments were single-end reads. All ChIP-seq and ATAC-seq experiments were paired-end. Read lengths are provided in methods for each experiment.

Antibodies	A full antibody table with all required information is included in Supplemental Table 6.
Peak calling parameters	Peaks were called using macs250 v2.1.1.20160309 with the options "--call-summits –nomodel –B". Differential ChIP peaks were called using DiffBind51 using the options "bFullLibrarySize=FALSE, bSubControl=TRUE, bTagwise=FALSE" for dba.analyze(). For DiffBind testing the DESeq2 algorithm with blocking was used, and ChIP replicate was used as the blocking factor while testing for differences between Mock and infected samples.
Data quality	Library size and purity was confirmed on a BioAnalyzer prior to sequencing. For DiffBind testing the DESeq2 algorithm with blocking was used, and ChIP replicate was used as the blocking factor while testing for differences between Mock and infected samples.
Software	For analysis of histone PTM ChIP-sequencing, reads were demultiplexed using bcl2fastq2 (Illumina) with the options "--mask-short-adapter-reads 20 --minimum-trimmed-read-length 20 --no-lane-splitting --barcode-mismatches 0". Reads were trimmed using TRIMMOMATIC (Bolger et al., 2014) with the options "ILLUMINACLIP:[adapter.fa]:2:30:10 LEADING:5 TRAILING:5 SLIDINGWINDOW:4:15 MINLEN:15", and aligned to a hybrid hg38+C. floridanus (v7.5, RefSeq) genome assembly using bowtie2 v2.2.64 with the option "--sensitive-local". Alignments with a mapping quality below 5 (using samtools) and duplicated reads were removed peaks were called using macs25 v2.1.1.20160309 with the options "--call-summits –nomodel –B". Differential ChIP peaks were called using DiffBind6 using the options "bFullLibrarySize=FALSE, bSubControl=TRUE, bTagwise=FALSE" for dba.analyze(). For DiffBind testing the DESeq2 algorithm with blocking was used, and ChIP replicate was used as the blocking factor while testing for differences between Mock and infected samples. For ChIP signal tracks individual replicate tracks were produced for RPM and fold-enrichment over input control, merged, and averaged. In order to account for potential global differences in hPTM abundance that would otherwise be missed by more standard quantile normalization-type approaches, high-quality de-duplicated read counts were produced for both human-mapping and C. floridanus-mapping reads, resulting in proportions of reads mapping to exogenous genome for each hPTM. Input controls were also treated in this way to account for potential differences in initial spike-in addition between samples. For each hPTM, the proportion of spike-in reads were normalized by the appropriate input-control value. Because spike-ins should be inversely proportional to target chromatin concentration, a ratio of CoV/Mock values was produced for each hPTM X replicate, and for CoV2 samples resulting signal values were divided by this ratio. This resulted in per-bp signal values adjusted by the degree of global difference in a given hPTM's level between sample types. For ATAC-seq and Orf8 ChIP-sequencing analysis, alignments were performed with Bowtie2 (2.1.0)4 using the Hg38 genome using a ChIP-seq pipeline (https://github.com/shenlab-sinai/chip-seq_preprocess). Orf8 reads were mapped using NGS plot.

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	HEK cell pellets were gently resuspended in 1 mL FACS buffer (Ca2+/Mg2+-free PBS, 2% BSA), pelleted at 500 x g at 4℃ for 5 minutes, and supernatant was removed. Cells transfected with Orf8, and/or mutant Orf8 construct and non-transfected control cells were then gently resuspended in 1 mL FACS buffer with a 1:500 dilution of streptactin-DY488 and rotated at 4℃ for 1 hour, protected from light. Cells were then washed twice in 1 mL FACS buffer resuspended in 1 mL FACS buffer and filtered through a 35 uM mesh into FACS tubes. Cells were collected in FACS buffer and pelleted for subsequent experiments.
Instrument	BD Influx (modular cell sorter) equipped with 488 nm laser and 530/40 nm detector
Software	Gating and sorting was conducted using BD FACS Software (1.2.0142). Post-Sort analysis was conducted with FlowJo (10.8.0).
Cell population abundance	Non-transfected control cells were stained with Strep-488 solution in parallel with cells transfected with Orf8 and Orf8 mutant constructs. 488+ gating was set such that < 1% of parent population (P2) was considered 488+ in control cells.
Gating strategy	Cells were first gated on FSC (Forward Scatter) vs. Trigger-Pulse Width to exclude doublets and cell debris. Cells were then gated of FSC vs SSC (Side scatter) to further exclude cell debris. Cells were then gated on 488 signal, where threshold was set by non-transfected control cells grown. Streptactin-488 positives cells were collected in FACS buffer and pelleted for subsequent experiments. An example of gating strategy can be found in Supplementary Figure 2.

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