METHODS

A549^{ACE2} cells

ACE expressing A549 cells were generated as previously described[1]. A549-ACE cells were grown in RPMI1640 media with 10% FBS and 1% Pen/Strep and maintained free of mycoplasma. Cells were infected at an MOI of 1 and fixed or lysed at 48 hours after infection.

SARS-CoV-2 infections

SARS-CoV-2 (USA-WA1/2020 strain) was obtained from BEI and propagated in Vero-E6 cells. The genome RNA was sequenced and found to be identical to GenBank: MN985325.1. Cells were infected with SARS-CoV-2 at MOI=1PFU/cell (A549^{ACE2} and iCM) or MOI=5PFU/cell (iAT2). Virus was added to cells for one hour at 37C, virus removed and replaced with medium. Cells were lysed at 48 hours post infection and RNA isolated. All infections and virus manipulations were conducted in a biosafety level 3 (BSL3) laboratory using appropriate protective equipment and protocols.

HEK cells

HEK293T cells were cultured in DMEM (with 4.5 g/L glucose, L-glutamine and sodium pyruvate), 10% fetal bovine serum (Sigma Aldrich F2442-500ML), and 1% Penicillin-Streptomycin (Gibco 15140122) and maintained free of mycoplasma. Calcium phosphate transfection was used to introduce GFP, Orf8, and Orf8 mutant plasmid DNA to HEK293T cells. Cells were washed 24 hours post-transfection with culture medium and fixed or pelleted and flash frozen 48 hours post-transfection. Cells were fixed using 4% PFA in PBS for 8 minutes. To pellet, cells were detached from the culture plate using TrypLE Express (Gibco 12605010) dissociation reagent, spun down for 5 minutes at 180xg, and flash frozen in liquid nitrogen.

Orf8 constructs

The Orf8 expression plasmid was obtained from Addgene, pLVX-EF1alpha-SARS-CoV-2-orf8-2xStrep-IRES-Puro (Addgene plasmid #141390). Orf8 deletion constructs were produced on the Orf8 backbone using Pfu Turbo HotStart DNA polymerase (Agilent 600322-51) and primers were created using the DNA-based primer design feature of the online PrimerX tool. Constructs were verified by Sanger sequencing.

iCM cells

The induced Pluripotent Stem Cell (iPSC) line Penn-123i-SV20 was generated using sendai virus vectors (Cytotune 2.0, Life technologies) in erythroblasts derived from patient-specific peripheral blood mononuclear cells (PBMCs) as previously described (Yang et. al 2012, 2015). This cell line has been deposited at WiCell Research Institute as part of a larger collection of iPSCs from the University of Pennsylvania (https://www.wicell.org/home/stem-cells/catalog-of-stem-cell-lines/collections/nhlbi-next-gen-rader.cmsx). iPSCs were maintained in StemMACS iPS Brew-XF (Miltenyi Biotec) on Geltrex (Life Technolgies) coated plates between passages 33 and 45. For cardiac differentiation (LaFlamme et. al 2007; Burridge et. al 2015; Palpant et. al 2017), iPSCs were dissociated into single cells using Accutase (Innovative Cell Technolgies) and plated at a density of 10^5 cells/cm^2 in iPS Brew with 2uM ROCK inhibitor Thiazovivin (Cayman Chemical). The following day, media was changed to iPS Brew supplemented with 1uM CHIR99021 (Cayman) for 24hr. Media was then switched to RPMI 1640 + B-27 supplement [minus insulin]

(both from Life Technolgies) supplemented with 100ng/mL Activin-A (StemCell Technologies) for 18hr. Cells were then switched to RPMI/ B-27 [minus] with 5ng/mL BMP-4 (Peprotech) and 1uM CHIR99021 for 48hrs. Wnt Inhibition was then induced by exchanging media to RMPI/ B-27 [minus] media with 1uM Xav939 (Millipore) for 48hr. Cells were then cultured for 72hr in RPMI/ B-27 [minus] to produce beating cardiomyocytes. Once beating cells were observed, the media was changed to RPMI/B-27 (Life Technologies) and cells were fed every 48hr. Once beating stabilized, cells were metabolically selected using RPMI (minus glucose) / B-27 supplement media for 72hr to enrich for cardiomyocytes. Cells were then re-plated onto 0.1% gelatin (Millipore) coated plates, subjected to another round of metabolic selection, and maintained in RMPI/B-27 media until being re-plated for experiments.

iAT2 cells

Generation of human-derived induced alveolar epithelial type II-like cells (iAT2) was performed as described[2]. To maintain a stable and pure culture of the iAT2 cell line, $SFTPC^{tdTomato+}$ cells were sorted and serially passage every 14 days. Cells were grown as an organoid format using 90% Matrigel with a cell density of 400 cells/ µl. Cells were fed using CK+DCI medium + Rock inhibitor for the first 48h after splitting and then change to K+DCI medium for 5 days followed by CK+DCI medium for 7 days. Every 14 days alveolosphere organoids were passaged, organoids were released from Matrigel using 2mg/ml dispase for 1h at 37°C, then the generation of single cells was reached using 0.05% Trypsin for 15min at 37°C. Cell quantification and viability were assessed using Trypan blue, finally, cells were passaged to new Matrigel drops let them polymerized for 30min at 37°C on a 5% CO2 incubator, after Matrigel solidified cells were fed according to plate format.

For the generation of 2D alveolar cells for virus infection, when alveolospheres organoids were passaged, cells were plated on pre-coated 1/30 matrigel plates at a cell density of 125000 cells/cm2 using CK+DCI medium + Rock inhibitor for the first 48h, and then the medium was changed to CK+DCI medium. 72 hours after cell plating, cells were infected using the SARS-CoV2 virus using MOI:5 for 48h.

Chromatin sequential salt extraction

Salt extractions were performed as described[3]. Briefly, a 2X RIPA solution was made (100 mM Tris pH 8.0, 2% NP-40, and 0.5% sodium deoxycholate) and mixed with varying concentrations of a 5 M NaCl solution to generate RIPA containing 0, 100, 200, 300, 400, and 500 mM NaCL. pelleted cells were resuspended in buffer A with protease inhibitors (0.3 M sucrose, 60 mM KCl, 60 mM Tris pH 8.0, 2 mM EDTA, and 0.5% NP-40) and rotated at 4 degrees C for 10 minutes. Nuclei were pelleted by centrifugation at 6000xG for 5 minutes at 4 degrees C. Supernatant was removed and saved and 200uL of RIPA with 0mM NaCl and protease inhibitors was added to the sample. Samples were mixed by pipetting 15 times and incubated on ice for 3 minutes before centrifuging for 3 minutes at 6500 xG at 4 degrees C. Supernatant was saved and RIPA steps were repeated for all NaCl concentrations. Samples were boiled and sonicated before analyzing by western blot.

Cell fractionation

Pelleted cells were briefly thawed on ice. Buffer 1 (15mM Tris-HCl (pH 7.5), 60 mM KCl, 15 mM NaCl, 5 mM MgCl2, 1 mM CaCl2, 0.25 M Sucrose with 1 mM PMSF, 1 mM DTT, and Complete Protease Inhibitor cocktail tablet added immediately before use) was added to the pellet at roughly 5 times the volume of the pellet and gently pipetted up and down to dissociate pellet. Samples were incubated on ice for 5 minutes and then an equal volume of buffer 1 with 0.4% NP-

40 was added to the sample. Samples were then mixed by inversion for 5 minutes at 4 degrees C. Samples were spun at 200xG for 10 minutes in a prechilled centrifuge to pellet nuclei. The supernatant (cytoplasmic fraction) was transferred to a new tube. Pellets were resuspended gently in 0.5mL buffer 1 to wash nuclei, then pelleted again and supernatant was discarded. Nuclear pellet solubilization buffer (150 mM NaCl, 50 mM Tris-HCl pH 8.0, 1% NP-40, 5 mM $MgCl_2$, with 1 mM PMSF, 1 mM DTT, and Benzonase enzyme at 250U/uL added shortly before use) was added to the pellet at half the volume of buffer 1 used. Samples were then incubated at room temperature in a thermoshaker until the pellet was fully dissolved. Benzonase enzyme was doubled in samples with undissolved material left after 20 minutes. Samples were then centrifuged at 13,000 RPM for 20 minutes at 4 degrees C. Supernatent (nuclei fraction) was collected. Sample concentrations were determined by BCA assay and samples were boiled in a western loading buffer for 10 minutes before analysis by western blot.

ATAC-sequencing

HEK cells were stained and sorted to isolate transfected cells using the same as described below. Sorted cells were resuspended in cold Lysis Buffer (10 uL per 10,000 cells; 10 mM Tris-Cl pH 7.5, 10 mM NaCl, 3 mM $MgCl_2$, 0.1% v/v NP-40, 0.1% v/v Tween-20, 0.01% v/v Digitonin), washed in Wash Buffer (10 mM Tris-Cl, pH 7.5, 10 mM NaCl, 3 mM $MgCl_2$, 0.1% v/v Tween-20), and Transposition was performed with Tagment DNA TDE1 (Illumina 15027865). Transposition reactions were cleaned with AMPure XP beads (Beckman A63880), and libraries were generated by PCR with NEBNext High Fidelity 2X PCR Master Mix (NEB M0541). Library size was confirmed on a BioAnalyzer prior to sequencing on the NextSeq 550 (40bp read length, paired end).

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.

ChIP-sequencing

For Orf8 ChIP-sequencing, 2 days after transfection, cells were fixed for 5 minutes with 1% PFA in PBS and then the reaction was quenched with 2.5M glycine. Cells were then washed twice and collected in PBS with protease and phosphatase inhibitors and then pelleted at 1200 rpm for 5 minutes. Cells were then rotated in lysis buffer 1 (50mM HEPES-KOH pH 7.5, 140mM NaCl, 1mM EDTA, 10% glycerol, 0.5% NP-40, 0.25% Triton x-100) for 10 minutes at 4°C and spun at 1350g for 5 minutes at 4°C to isolate nuclei. Supernatant was discarded and cells were resuspended in lysis buffer 2 (10mM Tris-HCl pH 8, 200mM NaCl, 1mM EDTA, 0.5mM EGTA) to lyse nuclei. Cells were rotated for 10 minutes at room temperature and were spun again at 1,350g for 5 minutes at 4°C. The supernatant was discarded and the pellet was resuspended in lysis buffer 3 (10mM Tris-HCl pH 8, 100mM NaCl, 1mM EDTA, 0.5mM EGTA 0.1% EDTA, 0.5% N-lauroylsarcosine). Lysates were sonicated on a Covaris sonicator for 40 minutes (200 cycles/burst). Triton was added to reach a final concentration of 1% and lysates were spun for 10 minutes at 20,000g at 4°C. Streptactin magnetic beads (MagStrep type 3 XT beads, iba #2-4090-002) were added to the lysates overnight rotating at 4°C. Beads were then washed with a low salt buffer (0.1%SDS, 1% triton, 2mM EDTA, 20mM TRIS pH 8, 150mM NaCl), a high salt buffer (0.1%SDS, 1% triton, 2mM EDTA, 20mM TRIS pH 8, 500mM NaCl), a Lithium Choride was buffer (150mM LiCl, 1% NP-40, 1% NaDOC, 1mM EDTA, 10mM TRIS pH 8) and then TE with 50mM NaCl. Chromatin was eluted from beads for 30 minutes shaking at room temperature 55uL BXT elution buffer (iba 2-1042-025) followed by the addition of 150uL elution buffer (50mM Tris-HCl pH8.0, 10mM EDTA, 1% SDS) for 30 minutes at 65°C. Samples were removed from beads and crosslinking was reversed by further incubating chromatin overnight at 65°C. RNA was digested with RNAase for 1 hour at 37°C and protein was digested with proteinase K for 30 minutes at

55°C. DNA was then purified with the Zymo PCR purification kit. The Illumina TruSeq ChIP purification kit was used to prepare samples for sequencing on an Illumina NextSeq 500 instrument (42bp read length, paired end).

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 histone PTM ChIP-sequencing, 4-10M cells were resuspended in 1mL of lysis buffer 1 (50mM HEPES-KOH pH 7.5, 140mM NaCl, 1mM EDTA, 10% glycerol, 0.5% NP-40, 0.25% Triton X-100) and rotated at 4C for 10min, followed by centrifugation removal of supernatant. Cells were then re-suspended in 1mL lysis buffer 2 (10mM Tris-HCl pH 8.0, 200mM NaCl, 1mM EDTA, 0.5mM EGTA), rotated for 10min at 4C, followed by centrifugation removal of supernatant. Cells were then resuspended in 1mL of lysis buffer 3 (10mM Tris-HCl pH 8.0, 100mM NaCl, 1mM EDTA, 0.5mM EGTA, 0.1% Na-Deoxycholate, 0.5% N-lauroylsarcosine) and rotated again for 10min at 4C. This was followed by sonication with a Covaris S220 sonicator for 35 minutes (peak incident power: 140; duty factor: 5 %; cycles/burst: 200). This was followed by addition of 110uL Triton X-100, and centrifugation for 15minutes at maximum speed (20kg) at 4C to clear lysate. Lysate chromatin concentration was then equalized according to DNA content (as measured with a qubit flourometer). Following this, 5% of equivalently-treated chromatin from *C. floridanus* pupae were added to all samples according to chromatin concentration, and 50uL of lysate saved as input shearing control. 250uL of equalized lysate were then added to washed, antibody-conjugated protein A/G Dynabeads (2ug antibody conjugated to 15uL/15uL A/G dynabeads, resuspended in 50uL per IP) and IPs were rotated overnight at 4C in a final volume of 300uL. The following day, IPs were washed 5x in RIPA wash buffer (50mM HEPES-KOH pH 7.5, 500mM LiCl, 1mM EDTA, 1% NP-40, 0.7% Na-Deoxycholate) and once in TE pH 8.0. Washes were followed by two elutions into 75 µl of elution buffer (50 mM Tris-HCl pH 8.0; 10 mM EDTA; 1% SDS) at 65˚C for 45 min with shaking (1,100 RPM). DNA was purified via phenol:chloroform:isoamyl alcohol (25:24:1) followed by ethanol precipitation. Pelleted DNA was resuspended in 25 µl TE. Libraries for sequencing were prepared using the NEBNext® Ultra™ II DNA Library Prep Kit for Illumina® (NEB E7645), as described by the manufacturer but using half volumes of all reagents and starting material. For PCR amplification optimal number of PCR cycles was determined using a qPCR side-reaction using 10% of adapter-ligated, size-selected DNA. 7-10 cycles of PCR were used for hPTM libraries and 5 cycles were used for Input controls.

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 v2.2.6[4] with the option "--sensitive-local". Alignments with a mapping quality below 5 (using samtools) and duplicated reads were removed peaks were called using macs2[5] v2.1.1.20160309 with the options "--call-summits –nomodel –B". Differential ChIP peaks were called using DiffBind[6] 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.

ChIP results were confirmed by qPCR using the following primers sets:

Heterochromatin:
Ch3 forward: AAATGCTAAGAGGGTGTGGG
Ch3 reverse: GAGAGTTGCCAGGAACAGAG
Euchromatin
Nanog enhancer forward: TGTTGAACCATATTCCTGAT
Nanog enhancer reverse: TCTACCAGTCTCACCAAG

RNA-sequencing

RNA was extracted using a Qiagen RNA purification kit. Samples were prepared for sequencing using the Illumina TruSeq purification kit and sequenced on an Illumina NextSeq 500 instrument (75bp read length, single read). For RNA sequencing analysis, alignments were performed using Salmon 0.12.0. DESeq2 was used to calculate differential gene expression. DAVID was used to find enriched GO terms compared to a background list of all genes expressed over a basemean of 5. To compare differentially expressed genes with interferon response genes, interferome.org (v2.01) was used to generate a list of all A549 interferon response genes. Library size was confirmed on a BioAnalyzer prior to sequencing on the NextSeq 550 using single-end, 75 cycles).

Immunoprecipitation

Anti–Strep tag affinity purification for Lamin complex proteins: Protein and binding partners were purified with affinity strep tag purification. For Orf8 PTM analysis and mass spec binding partner analysis, whole cell lysates were prepared as described below. Frozen cell pellets were thawed briefy and suspended in lysis buffer [immunoprecipitation (IP) buffer (50 mM tris-HCl, pH 7.5 at 4°C; 150 mM NaCl, 1 mM EDTA, 10mM sodium butyrate) supplemented with 0.5% Nonidet P 40 Substitute (NP-40; Fluka Analytical) and cOmplete mini EDTA-free protease and PhosSTOP phosphatase inhibitor cocktails (Roche)]. Samples were incubated on a tube rotator for 30 min at 4°C. Debris was pelleted by centrifugation at 13,000 × *g*, at 4°C for 15 min. Lysates were then incubated with streptactin magnetic beads (40ul; MagStrep type 3 XT beads, iba #2-4090-002) for 2 hours rotating at 4°C. Beads were washed three times with 1 ml wash buffer (IP buffer supplemented with 0.05% NP-40) and then once with 1 ml IP buffer. Strep-tagged Orf8 complexes were eluted from beads in Buffer BXT (IBA Lifesciences; Cat. # 2-1042-025) shaking at 1100 rpm for 30 min.

Anti–Strep tag affinity purification for for binding partner confirmation (SP2): Cells were then rotated in lysis buffer 1 (50mM HEPES-KOH pH 7.5, 140mM NaCl, 10mM sodium butyrate, 1mM EDTA, 10% glycerol, 0.5% NP-40, 0.25% Triton x-10) supplemented with 0.5% Nonidet P 40 Substitute (NP-40; Fluka Analytical) and cOmplete mini EDTA-free protease and PhosSTOP phosphatase inhibitor cocktails (Roche)) for 10 minutes at 4°C and spun at 1350g for 5 minutes at 4°C to isolate nuclei. Supernatant was discarded and cells were resuspended in lysis buffer 2 (10mM Tris-HCl pH 8, 200mM NaCl, 10mM sodium butyrate, 1mM EDTA, 0.5mM EGTA) to lyse nuclei. Cells were rotated for 10 minutes at room temperature and were spun again at 1,350g for 5 minutes at 4°C. The supernatant was discarded and chromatin pellet was resuspended in lysis buffer 3 (10mM Tris-HCl pH 8, 100mM NaCl, 10mM sodium butyrate, 1mM EDTA, 0.5mM EGTA 0.1% EDTA, 0.5% N-lauroylsarcosine). Lysates were sonicated using a tip sonicator with 3, 5

second bursts, at 70% power with chilling on ice between bursts. After sonication, lysates were brought to concentration of 1% Triton X-10 to disrupt lamina protein interactions. Debris was pelleted by centrifugation at 16000 x g, at 4°C and supernatant was incubated with streptactin magnetic beads (40ul; MagStrep type 3 XT beads, iba #2-4090-002) for 2 hours rotating at 4°C. Beads were washed three times with 1 ml wash buffer (IP buffer supplemented with 0.05% NP-40) and then once with 1 ml IP buffer. Strep-tagged Orf8 complexes were eluted from beads in Buffer BXT (IBA Lifesciences; Cat. # 2-1042-025) shaking at 1100 rpm for 30 min.

Reverse Immunoprecipitation: Chromatin pellet lysate was yielded as described above for Lamin complex protein immunoprecipitation. Lysates were combined with antibody conjugated protein A Dynabeads (15 ug antibody conjugated to 100ul Dynabeads) and rotated overnight at 4°C. The following day, beads were washed three times with 1 ml wash buffer (IP buffer supplemented with 0.05% NP-40) and then once with 1 ml IP buffer. Chromatin protein complexes were eluted from beads in elution buffer (50mM Tris-HCl pH8.0, 10mM EDTA, 1% SDS) for 30 minutes shaking at 65°C.

Immunocytochemistry

Fluorescent Immunocytochemistry of HEK cells and A549-ACE2 cells: Cells were fixed in 4% paraformaldehyde for 10 minutes, washed with PBS. Fixed cells were permeabilized using 0.5% Triton-X in PBS for 20 minutes. The cells were blocked in blocking solution (PBS, 3% BSA, 2% serum, 0.1% Triton-X) for at least 1 hour and stained with designated primary antibody overnight at 4°C. The following day cells coverslips were washed with PBS incubated with secondary antibodies for 1 hour at room temperature. For detection of strep-tagged Orf8, streptactin DY-488 (IBA Lifesciences; Cat. # 2-1562-050; 1:500) was added to secondary antibody solution. Nuclei were stained with DAPI (1:1000 in PBS) for 10 minutes and washed in PBS. Coverslips were mounted onto microscope slides using ProLong Gold antifade reagent (ThermoFisher).

Immunohistological staining of patient lung tissue: Formalin-fixed paraffin-embedded slides were obtained from Penn's Pathology Clinical Service Center. Slides were deparaffinized and rehydrated as follows: 10 min. Xylene x2, 10 min. 100% Ethanol x2, 5 min. 95% Ethanol, 5 min. 70% Ethanol, 5 min. 50% Ethanol, then running distilled water. Then, slides were processed using heat-induced epitope retrieval (HIER). Slides were incubated in hot sodium citrate buffer (10mM Sodium Citrate, 0.05% Tween-20, pH 6.0), placed in a pressure cooker, and heated in a water bath for 25 minutes with high pressure settings. Slides were cooled at room temperature and washed in TBS x2. Membranes were permeabilized TBS 0.4% Triton-X 100 for 20 min. Slides incubated in blocking solution (TBS; 10% goat serum; 1% BSA; 0.025% Triton-X 100) for 2 hours. Slides were incubated in mouse primary antibody solution of anti SARS-CoV-2 nucleocapsid and rabbit anti-H3K9me3 antibody solution overnight at 4°C. The following day, slides were washed with TBS and incubated in secondary antibody solution. Nuclei were stained with DAPI (5ug/ml) in TBS for 10 min and washed with TBS. Coverslips were mounted with ProLong Gold antifade reagent (ThermoFisher).

Microscopy

Cells were imaged on an upright Leica DM 6000, TCS SP8 laser scanning confocal microscope with 405 nm, 488, 552, and 638 nm lasers. The microscope uses 2 HyD detectors and 3 PMT detectors. Objectives used were a 63x HC PL APO CS2 oil objective with a NA of 1.40. Type F immersion liquid (Leica) was used for oil objectives. Images were 175.91 x 171.91 miocrons, 1024 by 1024 pixels, and 16-bits per pixel. For PTM quantification, HEK cells and human lung tissue were imaged at a single z-plane and A549 cells were imaged with a z-stack through the nucleus.

Image analysis

Images were analyzed using Image J software. 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.

Protein alignment

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]

Fluorescence activated cell sorting

Frozen cell pellets were gently resuspended in 1 mL FACS buffer (Ca^{2+}/Mg^{2+}-free PBS, 2% BSA), pelleted at 500 x g at 4˚C for 5 minutes, and supernatant was removed. The cells were then gently resuspended in 1 mL FACS buffer with a 1:500 dilution of anti-streptactin antibody and rotated at 4˚C 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. An Aria cells sorter was used to sort positive cells.

Histone extraction

Transfected cells were isolated by FACS as described above. Sorted cells were pelleted, resuspended in 1 mL cold H2SO4, and rotated overnight at 4˚C. Following the overnight incubation, cells were pelleted at max speed and the supernatant was transferred to a fresh tube. TCA was added to 25% by volume and the cells were left on ice at 4˚C overnight. Cells were again pelleted at max speed and the supernatant was discarded. Pre-chilled acetone was then used to gently wash the pellet twice. Following the second wash, the tubes were left to air dry before being resuspending in water. Samples were then broken up by alternating 10 minutes of sonication and 30 minutes shaking at 50˚C until pellets were fully dissolved.

Mass spectrometry

Histone PTM analysis by quantitative mass spectrometry:

Purification of histones was validated by SDS-PAGE followed by Coomassie staining demonstrating sufficient enrichment. A BCA (Thermo Fisher) was performed for protein estimation using the manufacturer's instructions and 20µg of histone were used for chemical derivatization and digestion as described before[8]. Briefly, unmodified lysines were derivatized twice with a 1:3 ratio of acetonitrile and proprionic anhydride. Histones were then digested with trypsin in a 1:20

enzyme:protein ratio at 37°C overnight. Digested histones with newly formed N-termini were derivatized twice as done before. Finally, histones were desalted as described above.

Dried histone peptides were reconstituted in 0.1% formic acid. A synthetic library of 93 heavy labeled and derivatized peptides containing commonly measured histone PTMs [5] was spiked into the endogenous samples to a final concentration of approximately 100ng/µl of endogenous peptides and 10fm/µl of each heavy labeled synthetic analyte. For each analysis, 1µl of sample was injected on column for data-independent analysis (DIA) analyzed on a Q-Exactive HF (Thermo Scientific) attached to an Ulimate 3000 nano UPLC system and Nanospray Flex Ion Source (Thermo Scientific). Using the same column and buffer conditions as described above, peptides were separated on a 63-minute gradient at 400nL/min starting at 4% buffer B and increasing to 32% buffer B over 58 minutes, and then increasing to 98% buffer B over 5 minutes. The column was then washed at 98% buffer B over 5 minutes and equilibrated to 3% buffer B. Data independent acquisition was performed with the following settings. A full MS1 scan from 300 to 950 m/z was acquired with a resolution of 60,000, ACG target of 3e6, and maximum injection time of 55ms. Then a series of 25 MS2 scans were acquired across the same mass range with sequential isolation windows of 24 m/z with a collision energy of 28, a resolution of 30,000, AGC target of 1e6, and maximum injection time of 55ms. Data analysis and manual inspection using the synthetic library as a reference was performed with Skyline (MacCoss Lab). Ratios were generated using R Studio and statistics carried out in excel.

Trypsin & chymotrypsin digestion of Orf8 for identification of Orf8 modifications:

The gel band containing Orf8 was destained with 50 mM Ammonium bicarbonate with 50% acetonitrile (ACN). The band was then reduced in 10 mM dithiothreitol in 50 mM ammonium bicarbonate for 30 min at 55°C. Next, the band was alkylated with 100 mM iodoacetamide in 50 mM ammonium bicarbonate at RT for 30 min in the dark. The protein was then digested by incubation with chymotrypsin or trypsin in approximately 1:20 enzyme:protein ratio at 37°C overnight. Following digestion, the supernatant was collected. To extract additional peptides from the gel, 150 µL of 50% ACN and 1% trifluoroacetic acid (TFA) was added and incubated with constant shaking for 30min. The supernatant was collected and 100 µL of ACN was added and incubated with constant shaking for 10min. The final supernatant was collected. All three supernatants were combined and dried. The dried samples were reconstituted by 0.1% TFA and desalted with the C18 micro spin column (Harvard Apparatus). The column was prepared with 200 µL of 100% ACN and equilibrated with 200 µL of loading buffer 0.1% TFA. Peptides were loaded onto the column, washed with a loading buffer, and eluted with 200 µL of 70% acetonitrile in 0.1% formic acid (FA). All steps for loading, washing, and elution were carried out with benchtop centrifugation (300 x g for 2 min). The eluted peptides were dried in a centrifugal vacuum concentrator.

Orf8 vs control IP for identification of binding partners:

Orf8 immunoprecipitation elutants were reduced and alkylated as described above. Proteins were then digested and desalted with mini S-Trap (Protifi LLC) using the manufacturer's instructions. Briefly, 25 µL of elutant was combined with 25 µL of 10% sodium dodecyl sulfate (SDS) to a final SDS concentration of 5% SDS after alkylation. Samples were then acidified with phosphoric acid and precipitated by adding 90% methanol (MeOH) in 100 mM triethylammonium bicarbonate (TEAB) in a 6:1 (volume:volume) ratio. Protein was then added to the trap with benchtop centrifugation (4,000 x g for 1 min), washed, and digested with trypsin in a 1:10 enzyme:protein ratio at 37°C overnight. Following digestion, peptides were eluted from the trap with 40 µL of 100 mM TEAB, 40 µL 0f 0.2% FA, and 40 µL of 50% ACN in 0.2% FA. Combined elutant volumes were then dried.

Chymotrypsin LC-MS/MS and LC-PRM/MS analysis:

Dried peptides were reconstituted with 0.1% FA, and 2 µg of each sample was injected. Chymotrypsin digested Orf8 samples were analyzed on a Q-Exactive (Thermo Scientific) coupled to an Easy nLC 1000 UHPLC system and Nanospray Flex Ion Source (Thermo Scientific). The LC was equipped with a 75 µm x 20 cm column packed in house using Reprosil-Pur C18 AQ (2.4 µm; Dr. Maisch GmbH, Germany). Using aqueous solution of 0.1% FA as buffer A and organic solution of 80% ACN 0.1% FA as buffer B, peptides were separated on a 85 minute gradient at 400nL/min starting at 3% buffer B and increasing to 32% buffer B over 79 minutes, then increasing to 50% buffer B over 5 minutes, and finally increasing to 90% buffer B over 1 minute. The column was then washed at 90% buffer B over 5 minutes and equilibrated to 3% buffer B. Data dependent acquisition was performed with dynamic exclusion of 40 seconds. A full MS1 scan from 350 to 1200 m/z was acquired with a resolution of 70,000, ACG target of 1e6, and maximum injection time of 50ms. Then, a series of MS2 scans were acquired for the top 15 precursors with a charge state of 2-7, a collision energy of 28, and an isolation window of 2.0 m/z. Each MS2 scan was acquired with a resolution of 17,500, AGC target of 2e5, and maximum injection time of 50ms. A database search was performed using the human SwisProt sequence and Orf8 sequence with Proteome Discoverer 2.4 (Thermo Scientific) with the following search criteria: carboxyamidomethylation at cysteine residues as a fixed modification; oxidation at methionine, acetylation at lysine, mono-, di-, and tri-methylation at lysine residues as variable modifications; two maximum allowed missed cleavage; 10 ppm precursor MS tolerance; a 0.2 Da MS/MS. An unscheduled parallel reaction monitoring method[9] was developed to identify 45 possible modified and unmodified peptide targets of Orf8. Peptides were separated with the same LC gradient conditions. A full MS1 scan from 300 to 900 m/z was acquired with a resolution of 70,000, ACG target of 1e6, and maximum injection time of 50ms. Then, a series of MS2 scans were acquired with a loop count of 23 precursors, a collision energy of 28, and an isolation window of 1.2 m/z. Each MS2 scan was acquired with a resolution of 17,500, AGC target of 1e6, and maximum injection time of 100ms. Data analysis and manual inspection was performed with Skyline[10] (MacCoss Lab) and IPSA[11].

Trypsin Orf8 LC-MS/MS and LC-PRM/MS analysis and IP LC-MS/MS analysis:

Dried peptides were reconstituted with 0.1% FA, and 2 µg of each sample was injected. Data dependent acquisition runs were analyzed on a Q-Exactive HF or HF-X (Thermo Scientific) attached to an Ulimate 3000 nano UPLC system and Nanospray Flex Ion Source (Thermo Scientific). Using the same column and buffer conditions as described above, peptides were separated on a 112 minute gradient at 400nL/min starting at 5% buffer B, increasing to 35% buffer B over 104 minutes, and then increasing to 60% buffer B over 8 minutes. The column was then washed at 95% buffer B for 5 minutes and equilibrated to 5% buffer B. Data dependent acquisition was performed with dynamic exclusion of 45 seconds. A full MS1 scan from 380 to 1200 m/z was acquired with a resolution of 120,000, ACG target of 3e6, and maximum injection time of 32ms. Then, a series of MS2 scans were acquired for the top 20 precursors with a charge state of 2-5, a collision energy of 28, and an isolation window of 1.2 m/z. Each MS2 scan was acquired with a resolution of 30,000, AGC target of 1e6, and maximum injection time of 32ms (HF) or 55ms (HF-X). A database search was performed using the human SwisProt sequence and Orf8 sequence with Proteome Discoverer 2.3 (Thermo Scientific) with the following search criteria: carboxyamidomethylation at cysteine residues as a fixed modification; oxidation at methionine, acetylation, mono-, di-, and tri-methylation at lysine residues as variable modifications; two maximum allowed missed cleavage; 10 ppm precursor MS1 tolerance; a 0.2 Da MS2 tolerance. An unscheduled parallel reaction monitoring method[9] was developed to identify 16 possible modified and unmodified peptide targets of Orf8. Peptides were separated with the same LC gradient conditions. A full MS1 scan from 350 to 950 m/z was acquired with a resolution of 120,000, ACG target of 3e6, and maximum injection time of 100ms. Then, a series of MS2 scans were acquired with a loop count of 16 precursors, a collision energy of 28, and an isolation window

of 1.2 m/z. Each MS2 scan was acquired with a resolution of 30,000, AGC target of 1e6, and maximum injection time of 100ms. Data analysis and manual inspection was performed with Skyline[10] (MacCoss Lab) and IPSA[11].

Antibodies

Target	Species	Source	Cat #	Dilution	Assay used
H3K9me3	Rb	Active Motif	39161	500	HEK/A549 ICC, IHC
H3K9ac	Ms	Active Motif	61251	500	HEK ICC
H3K27me3	Rb	Active Motif	39155	500	HEK/A549 ICC
H3K27ac	Ms	Active Motif	39134	500	HEK ICC
Lamin-B1	Rb	Abcam	Ab16048	500	HEK ICC, IP, WB
Lamin A/C	Ms	Santa Cruz	sc-376248	200	HEK ICC
H3K9ac	Rb	Active Motif	39917	500	A549 ICC
Orf8_ab1	Rb	Bioworld	NCP0036	250	A549 ICC
Orf8_ab2	Rb	Bioworld	NCP0010	250	A549 ICC
dsRNA	Ms	Jena Bioscience	RNT-SCI-10010200	200	A549 ICC
HP1alpha	Rb	Abcam	Ab109028	5000	IP, WB
H3	Rb	Abcam	Ab1791	10,000	IP, WB
SP2	Rb	ThermoFisher	PA5-35984	1000	WB
Streptactin DY-488	NA	IBA Lifesciences	2-1562-050	500	HEK, A549 ICC, FACS
Streptactin HRP	NA	IBA Lifesciences	2-1502-001	5000	WB
SARS-CoV-2 nucleocapsid	Rb	ThermoFisher	MA1-7403	15	IHC
Anti-mouse AF488	Gt	ThermoFisher	A-11001	500	ICC, IHC
Anti-rabbit AF647	Gt	ThermoFisher	A-21244	500	ICC, IHC
Anti-mouse AF647	Dk	ThermoFisher	A-21235	500	HEK ICC
Anti-rabbit AF568	Gt	ThermoFisher	A10042	500	HEK/A549 ICC

ICC = immunocytochemistry, WB = Western blot, IHC = immunohistochemistry

Data analysis and availability

Box and whisker plots show center line median, box limits for upper and lower quartiles, whiskers for 1.5x interquartile range, and points are outliers. ANOVA testing and plots were generated with R. Bonforonni corrections were applied for multiple comparisons. Fiji was used for image analysis. 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. All genome-wide sequencing data is available under accession numbers: GSE163664, GSE163670, GSE163676, and GSE163774.

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