SARS-CoV-2 protein encoded by ORF8 contains a histone mimic that disrupts chromatin regulation

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Abstract
SARS-CoV-2 emerged in China at the end of 2019 and caused the global pandemic of COVID-19, a disease with high morbidity and mortality. While our understanding of this novel virus is rapidly increasing, gaps remain in our understanding of how SARS-CoV-2 can effectively...
suppress host cell antiviral responses. Recent work on other viruses has demonstrated a novel mechanism through which viral proteins can mimic critical regions of human histone proteins.

Histone proteins are responsible for governing genome accessibility and their precise regulation is critical for a cell’s ability to control transcription and respond to viral threats. Here, we show that the protein encoded by ORF8 (Orf8) in SARS-CoV-2 functions as a histone mimic of two critical histone 3 sites containing an ARKS motif. Orf8 expression in cells disrupts multiple critical histone post-translational modifications (PTMs) while Orf8 lacking this histone mimic motif does not. Orf8 binds to numerous histone-associated proteins and to DNA, and is itself acetylated within the histone mimic site. Importantly, SARS-CoV-2 infection of multiple susceptible cell types causes the same global changes of histone post-translational modifications that are disrupted by Orf8 expression; these include induced pluripotent stem cell-derived alveolar type 2 cells (iAT2) and cardiomyocytes (iCM) and postmortem patient lung tissue. These findings demonstrate a novel function for the poorly understood SARS-CoV-2 ORF8 encoded protein and a mechanism through which SARS-CoV-2 disrupts host cell epigenetic regulation. Notably, this work provides a potential mechanism for emerging findings from human patients indicating that ORF8 deletion results in less severe illness and describes a potentially druggable pathway that may contribute to the virulence of SARS-CoV-2.

Main

SARS-CoV-2 has proven considerably more devastating and widespread than previous virus outbreaks. Recent findings indicate that several other highly virulent viruses disrupt host cell epigenetic regulation through mimicry of host cell proteins\(^1\)\(^{-3}\), particularly of histones\(^4\)\(^{-7}\). Histones function by wrapping DNA into complex structures and, in doing so, control access to the genome. Histone proteins are modified by a wide-range of post-translational modifications (PTMs) that are dynamically regulated to control gene expression\(^8\)\(^{-10}\). Histone mimicry allows viruses to disrupt
the host cell’s ability to regulate gene expression and respond to infection effectively. Thus far, only a few cases of such mimicry have been observed and validated, and no known cases of histone mimicry have been found within coronaviruses. Furthermore, there are few studies examining epigenetic changes associated with coronavirus infection and none yet published on SARS-CoV-2. However, recent work has demonstrated that SARS-CoV-2 infection induces low interferon expression indicating that it suppresses the innate antiviral host cell response.

While SARS-CoV-2 likely employs numerous mechanisms to dampen this response, we examined whether SARS-CoV-2 employs histone mimicry to disrupt histone regulation, to better understand how it evades host cell antiviral responses.

To investigate whether histone mimicry is utilized by the SARS-CoV-2 virus, we first performed a bioinformatic comparison of all SARS-CoV-2 viral proteins with all human histone proteins (Fig. S1a). Most SARS-CoV-2 proteins are highly similar to those in the coronavirus strain that caused the previous major SARS-CoV outbreak with the notable exception of proteins encoded by ORF3b and ORF8. Remarkably, we detected an identical match between a region of the protein encoded by ORF8 (henceforth called Orf8) and critical regions within the histone H3 amino terminal tail (Fig. 1a-b, S1a-b). Furthermore, Orf8 aligns to a longer stretch of amino acids (6 identical, sequential amino acids) than any previously described and validated case of histone mimicry (Fig. S1c). Based on a crystal structure of Orf8, this region of the protein falls in a disordered region that is potentially exposed to the cell in an Orf8 monomer. Most compelling is that the motif we detected contains the ‘ARKS’ sequence, which is found at two distinct sites in the histone H3 tail and is well-established as one of the most critical regulatory regions within H3. Both of these ARKS sites are modified with multiple crucial PTMs, including methylation and acetylation at H3 Lysine 9 (H3K9me3 and H3K9ac) and at H3 Lysine 27 (H3K27me3 and H3K27ac). Strikingly, this amino acid stretch is absent from the previous SARS-CoV virus Orf8-encoded protein (both before and after a deletion generated two distinct peptides, Orf8a and...
Orf8b\textsuperscript{22}) (Fig. S1d). Our proposed histone mimicry motif is also a considerably closer match than a previously proposed histone mimic in protein E of SARS-CoV-2 (Fig. S1e)\textsuperscript{23}. These findings indicate that Orf8 may act as a histone mimic to disrupt regulation of ARKS sites on histone H3, providing a novel mechanism through which this relatively poorly understood and highly divergent protein\textsuperscript{24–26} functions during infection.

To determine whether Orf8 may act as a histone mimic, we examined whether Orf8 expression disrupts histone PTM regulation using an unbiased mass spectrometry approach. HEK cells were transfected with Orf8 containing a Strep tag or with a control GFP-expressing plasmid and transfected cells were isolated using fluorescence-activated cell sorting (FACS). Histones were purified using an acid-extraction method, and bottom-up unbiased mass spectrometry was performed to quantify all detected histone PTMs. Fitting with its potential role as a histone mimic, we found that numerous histone modifications were disrupted in response to Orf8 expression (Table S1). We focused on significantly disrupted histone PTMs with well-defined links to gene expression that contributed to at least 1% of the total peptide detected. Remarkably, we found numerous histone modifications associated with active gene expression were depleted in cells expressing Orf8 while histone modifications associated with chromatin compaction or transcriptional repression were increased in cells expressing Orf8 (Fig. 1c). In particular, modifications within the H3 ARKS motifs were highly disrupted. The peptide containing H3K9ac and H3K14ac, both well-established PTMs linked to active gene expression, was decreased by Orf8 expression. Conversely, the peptides containing H3K9 methyl modifications (H3K9me2 and H3K9me3), as well as peptides containing H3K27 methylation (H3K27me3 and H3.3K27me3, both on the canonical H3 and variant H3.3 histone) were robustly increased in response to Orf8. These data support a role for Orf8 as a putative histone mimic and demonstrate that it is capable of disrupting histone PTM regulation at numerous critical sites within histones.
Based on the pattern of histone PTM disruption observed, we hypothesized that Orf8 expression decreases chromatin accessibility. We transfected HEK cells with a control construct or a plasmid expressing Orf8, isolated transfected cells using FACS, and performed ATAC-sequencing (ATAC-seq) to assess changes in open and closed chromatin. Orf8 expression resulted in robust, global changes in chromatin accessibility, increasing chromatin compaction throughout the genome including at transcription start sites (TSS) and within genic regions (Fig. 1d, S2a). To define the role of the proposed Orf8 histone mimic site in chromatin regulation and to confirm mass spectrometry findings through an independent approach, we again transfected HEK cells with Orf8 and examined global changes in histone modifications. Here, we used immunofluorescent staining with antibodies to methylated or acetylated H3K9 and H3K27 to examine the modifications within the proposed histone mimic motif and that mass spectrometry data indicated are disrupted by Orf8. This approach allowed for co-staining of cells with a Strep-Tactin conjugated probe for visualization of Orf8 protein, ensuring that analyzed cells contained equivalent levels of Orf8 expression for comparisons of Orf8 and Orf8-deletion constructs. We found that cells expressing Orf8 exhibited increased H3K9me3 and H3K27me3 and decreased H3K9ac staining compared to control plasmid transfected cells (Fig. 1e-j). To determine whether these effects are due to the proposed histone mimic site within Orf8, we generated a deletion construct lacking the ARKSAP histone mimic site (Orf8-del). While Orf8-del was expressed at similar levels to Orf8 (Fig. S2b), it did not increase H3K9me3 or H3K27me3, and showed a trend toward decreasing the effect on H3K9ac (Fig. 1e-j). Thus, the ability of Orf8 to disrupt histone PTMs largely relies on the presence of the ARKSAP motif. Next, we examined another dominant form of Orf8 containing an acquired mutation (S84L) commonly found in strains SARS-CoV-2. This site lies outside the histone mimic region and thus is not expected to affect its ability to regulate histone PTMs. We found that Orf8-S84L also increased H3K9me3 and H3K27me3, while decreasing H3K9ac (Fig. S2c-e), indicating that, as predicated, this common mutation does not alter the potential histone mimicry. We did not detect significant global changes in H3K27ac using
these methods (Fig. S2f), potentially due to low H3K27ac basal levels and fitting with mass spectrometry results.

To understand the mechanism through which Orf8 disrupts host cell chromatin, we began by examining its intracellular localization. Notably, while Orf8 does not have a well-defined NLS, it is 15kD in size and thus is small enough to diffuse into the nucleus. We first transfected HEK cells with Strep-tagged Orf8 and using a cellular fractionation assay, detected Orf8 in both the cytoplasm and the nucleus (Fig. 2a). We performed immunofluorescence to confirm these findings through an independent approach. We found that Orf8 was present in the cytoplasm and was located at the periphery of the nucleus as well as in nuclear puncta (Fig. 2b). This expression pattern matches those described in a previous report\(^{27}\), although this study focused on a cytoplasmic role of Orf8. Given the observed expression pattern of Orf8, we next asked whether Orf8 is associated with Lamin. We found that Orf8 colocalized with LaminB1 and LaminA/C (Fig. 2c, Fig. S3a). Furthermore, Orf8 bound LaminB1, histone H3, and HP1\(\alpha\), a protein associated with both Lamin and histones (Fig. 2d). Similarly, reciprocal co-immunoprecipitation for LaminB1 and H3 confirmed Orf8 binding (Fig. 2d). These findings show nuclear localization of Orf8 and indicate association with chromatin.

We further examined the strength of the Orf8 association with chromatin, utilizing increasing salt concentrations to examine chromatin binding. We found that Orf8 dissociates from the chromatin fraction at salt concentrations between those at which Lamin dissociates and the peak at which histones dissociate while Orf8-del dissociates at lower salt concentrations (Fig. 2e). We next used ChIP-sequencing of Orf8 itself to determine whether and where Orf8 associates with genomic DNA. We discovered that Orf8 was enriched at transcription start sites and in genic regions within the human genome relative to input DNA (or compared to a control ChIP performed with cells that do not express Orf8) (Fig. S3b-c), although Orf8 binding does not show clearly defined peaks at...
specific genes as would be expected for an endogenous histone PTM (Fig. S3d). To confirm Orf8 association with open chromatin regions, we used ChIP-qPCR and observed greater Orf8 association with euchromatic compared heterochromatin genomic regions (Fig. S3e).

We next used targeted mass spectrometry to determine whether the proposed Orf8 histone mimic site is modified similarly to histones. Using a bottom-up approach, Orf8 was purified from cells, reduced, alkylated, and digested. Separation with liquid chromatography followed by parallel reaction monitoring mass spectrometry (LC-PRM-MS) then targeted 8 possible unmodified and modified forms of Orf8 commonly found on histones: phosphorylation on serine, then monomethylation, di-methylation, tri-methylation, and acetylation on lysine. Of these targets, unmodified and acetylated lysine were identified. The acetylated peptide contained the +42 Da mass shift and demonstrated almost complete coverage of all possible product ions from the N-terminus containing the acetyl-lysine (b ions) as well as from the C-terminus (y ions). High resolution mass spectrometry differentiated the precursor from the trimethylated peptide and matched all product ions within 10 ppm mass error (Fig. 2f, S4a). This demonstrates that Orf8 is acetylated at lysine 52, within the proposed histone mimic site, supporting a potential function of this region as a mimic of histone acetylation sites associated with active gene expression (H3K9ac and H3K27ac). This finding further suggests that this motif may be recognized and modified by host cell enzymes similarly to histone H3 and can act as a ‘sink’ for histone modifying enzymes.

Based on this observation of acetylation of OrfK52, we predicted that there may be depletion of histone acetylation in proximity of Orf8. We therefore examined the localization of histone modifications in cells expressing Orf8 and found that H3K9ac is depleted in regions surrounding Orf8, while regions of enriched H3K9me3 typically appear at or in close proximity to Orf8 puncta (Fig. S4b). This localization pattern is consistent with a role for Orf8 as a histone mimic that disrupts host cell chromatin regulation causing both local and global changes in histone PTMs.
Finally, we used mass spectrometry to identify additional binding partners beyond Lamin-associated complexes (Table S2). Top hits included the HAT complex protein MORF4L, several zinc finger proteins, and the transcription factor SP2 which we confirmed by co-immunoprecipitation (Fig. S4c). Together, these results support a model in which Orf8 associates with chromatin and is recognized and modified by histone acetyltransferase enzymes. It then acts as a sink for host cell chromatin regulators and transcription factors, and causes both localized changes in critical histone PTMs at neighboring regions within the nucleus and global disruptions of chromatin regulation. These data define a role for Orf8 in disruption of host cell histone PTMs through a novel case of histone mimicry of the ARKS motifs in H3.

Next, we examined Orf8 and histone PTM regulation in the physiological context of viral infection and in a cell type relevant to the COVID-19 disease state using the A549 lung derived cell line expressing the ACE receptor (A549ACE). We obtained a highly specific antiserum for Orf8 (Fig. S5a-b) and stained A549ACE cells infected with SARS-CoV-2 for Orf8 and with J2 antibody for dsRNA, an intermediate in the replication of SARS-CoV-2 RNA, to identify infected cells. We detected Orf8 in both the cytoplasm and nuclear periphery of infected cells where it colocalized with LaminA/C (Fig. 3a, S5b), similar to the patterns observed in cells transfected with Orf8 (Fig. 2a-c). The requirement for viral inactivation through methods such as fixation prevented subsequent biochemical analysis of virally expressed Orf8. However, we confirmed that in A549ACE cells exogenously expressing Orf8, sequential salt extractions showed similar Orf8 chromatin association as in HEK cells (Fig S5c) and similar Orf8 localization (Fig. S5d) as in HEK cells. To determine whether similar chromatin disruptions occur in the context of viral infection as we detected in response to Orf8 expression, we infected A549ACE cells with SARS-CoV-2 or performed a mock infection with an MOI of 1 and fixed and stained cells for H3K9 and H3K27 modifications 48 hours after infection. SARS-CoV-2 infection increased H3K9me3 and H3K27me3 and decreased H3K9ac (Fig. 3b-g), replicating the effects of Orf8 expression.
Together, these data indicate that both Orf8 expression and SARS-CoV-2 infection result in global changes in histone regulation and chromatin accessibility, providing a novel mechanism through which SARS-CoV-2 can disrupt host cell function.

New data from COVID-19 human patients, recently published in *The Lancet*, found that a 382-nucleotide deletion variation in SARS-CoV-2 that blocks expression of the ORF8 gene (Fig. 4a) is associated with a milder infection in COVID-19 patients. Furthermore, Orf8 expression has been shown to block type 1 interferon and NF-kB responsive promoters and to inhibit induction of interferon-stimulated genes during viral infection. To determine whether disruption of chromatin and transcriptional regulation could contribute to the lack of a robust interferon response, we performed RNA-sequencing to determine how gene expression is disrupted by SARS-CoV-2 infection in A549 cells. We found that, despite widespread differential gene expression (Fig. S6a-b), interferon viral response genes were only mildly induced by infection as measured by gene ontology analysis, overlap with a defined set of A549 interferon response genes, or examination of specific response genes (Fig. S6b-e). These data support recent findings indicating SARS-CoV-2 results in an imbalanced host response with a limited interferon response.

To examine site-specific histone PTM regulation at interferon response genes and to determine whether the global chromatin disruptions that we observed in A549 cell lines occur in cell types that are highly relevant to COVID-19 disease, we analyzed chromatin regulation of lung host cells by infecting human induced pluripotent stem cell-derived lung alveolar type 2 (iAT2) pulmonary cells. This is a highly disease-relevant cell type similar to the lung cells infected in human patients that express all of the major markers of primary human AT2 cells. We then used ChIP-sequencing with ChIP-RX normalization (Fig. S7a) to allow for detection for global changes in histone PTMs. Strikingly, we found that infected iAT2s showed globally increased H3K9me3 and
H3K27me3 and decreased H3K9ac (Fig. 4b), again matching the effects of Orf8 expression. In addition to global changes, increased H3K9me3 and H3K27me3 and decreased H3K9ac were found at interferon-stimulated genes such as IFITM2, ADAR and FOSL2 (Fig. 4c), indicating a potential chromatin-based mechanism that dampens induction of key response genes during viral infection. In addition, we carried out ChIP-sequencing experiments in human induced pluripotent stem cell-derived cardiomyocytes (iCMs) to determine if similar effects are observed in a second cell type that is sensitive to SARS-CoV-2 infection. We again observed the same global changes in histone PTMs found in response to Orf8 expression and in response to infection in A549 ACE cells and iAT2 cells (Fig. S7b).

Lastly, we obtained postmortem lung tissue samples from three COVID-19 patients and matched controls. We stained tissue for SARS-CoV-2 Nucleocapsid protein to identify infected cells and for H3K9me3 to measure histone PTM changes. We found that in all patient samples, infected cells showed increased H3K9me3 staining compared to neighboring cells within the same tissue as well as compared to control tissue (Fig. 4d-e, S7c). While limited sample availability limits the conclusions that can be drawn from this assay, this finding indicates that histone PTMs are also disrupted in patients with severe COVID-19 disease. Furthermore, this result supports data obtained from exogenous Orf8 expression and infection of A549 ACE, iAT2, and iCM cells showing dramatic changes in histone PTM regulation. Together, our data examining the specific role of the Orf8 protein in disrupting chromatin regulation and the equivalent widespread chromatin disruptions in response to SARS-CoV-2 infection, provide a mechanism that explains differential disease progression and severity in patients with a deletion of the Orf8 coding region in the SARS-CoV-2 genome.

The work described here identifies a novel case of histone mimicry in the SARS-CoV-2 virus and defines a mechanism through which SARS-CoV-2 acts to disrupt host cell chromatin regulation.
We found that the protein encoded by the SARS-CoV-2 ORF8 gene contains an ARKS motif and that Orf8 expression disrupts histone PTM regulation. Orf8 is associated with chromatin-associated proteins, histones, DNA and the nuclear lamina both in the context of exogenous expression and virally infected cells. Furthermore, Orf8 is itself acetylated within the histone mimic motif similarly to histones. SARS-CoV-2 infection results in identical global histone PTM disruptions as Orf8 expression in numerous cell types including A549^{ACE}, iAT2s, iCMs, and COVID-19 human patient lung tissue. Finally, this work provides a molecular basis for the recent discovery that patients infected with a form of SARS-CoV-2 containing a deletion in the gene encoding Orf8 have less severe illness and better outcomes\textsuperscript{28}.

Ultimately, these findings explain the function of the previously poorly understood and highly divergent SARS-CoV-2 protein Orf8. In addition, given that many epigenetic pathways and histone modifying enzymes are druggable, in many cases with therapeutics already approved for use in humans, this work suggests potential avenues for the development of treatments that target epigenetic pathways. Finally, these data have critical implications for our understanding of COVID-19 pathogenesis in patients and emerging viral strains carrying deletions and mutations in the ORF8 gene.

**Author contributions**

J. Kee designed, performed, and analyzed the majority of the experiments. S. Thudium generated cells, samples, and DNA constructs. K. Palozola performed ATAC-seq and generated samples for histone PTM analysis. K. Gladstad and Z. Zhang performed and analyzed ChIP-sequencing experiments. J. Cesare performed and analyzed mass spectrometry experiments with guidance from B.A. Garcia. R. Truitt generated iCM cells with guidance from W. Yang. F.L. Cardenas, generated iAT2 cells with guidance from E. Morrisey. D.N. Kotton and K.D. Alysandratos provided stem cell lines. Y. Li performed SARS-CoV-2 viral infections. S.R. Weiss provided input and
expertise and lead viral work. S.L. Berger provided input and expertise and lead ChIP-sequencing studies. E. Korb lead the project and wrote the manuscript.

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Figure 1. Orf8 histone mimicry and localization. (a,b) Orf8 contains an ARKS motif that matches the histone H3 tail regions surrounding the critical sites H3K9 (a) and H3K27 (b). (c) Mass spectrometry analysis of histone PTMs in control (GFP) or Orf8 expressing HEK cells isolated by FACS. Z-score and fold changes are shown for modifications that are significantly changed in response to Orf8 expression, were detected over a minimal threshold of 1% of the total
peptide abundance, and have well-established functions. Full results and raw data are shown in Table S1. (d) ATAC-sequencing of 2 independent replicates of HEK cells expressing GFP or Orf8 isolated by FACS. Reads per million mapped surrounding the transcription start site (TSS) of all genes are averaged. (e-j) HEK cells transfected with GFP or Strep-Orf8 show that Orf8 expression increases H3K9me3 (e-f) and H3K27me3 (g-h) while decreasing H3K9ac (i-j). Conversely, Orf8 with a deletion of the histone mimic site ARKSAP (Orf8-del) does not affect these histone PTMs. N = 614 (GFP), 497 (Orf8), 170 (Orf8-del) cells for H3K9me3; 616, 550, 154 cells for H3K27me3; 666, 568, 170 cells for H3K9ac compiled from 3 independent transfections. ***, p<0.001, 1-way ANOVA with post-hoc 2-sided t-test and Bonferroni correction. Scale bars = 5µM.
Figure 2. Mechanism of Orf8 function. (a). Subcellular fractionation of HEK cells transfected with Strep-Orf8 indicates Orf8 is present in the cytoplasm and nucleus. (b) Staining of HEK cells transfected with Strep-Orf8 shows Orf8 is expressed in the cytoplasm and at the nuclear periphery as well as in nuclear puncta. (c) Orf8 colocalizes with Lamin at the nuclear periphery and within nuclear puncta. Images shown include rotation of z-stacks (right and bottom panel for each stain).
to demonstrate colocalization throughout the nucleus. (d) Orf8 co-immunoprecipitates with Lamin complex-associated proteins including LaminB, HP1, and H3. Streptactin-conjugated beads were used for Orf8 IPs. '-' indicates cells that are not expressing Orf8 for negative control IPs performed in parallel. (e) Sequential salt extraction of HEK cells expressing Orf8 indicates Orf8 is present in chromatin fractions and dissociates from chromatin between peaks of dissociation for LaminB and histone protein H3. ‘Cyto’ indicates cytoplasmic fraction. (f) Targeted mass spectrometry analysis of trypsin-digested Orf8 shows Orf8 is acetylated at lysine 52, at the site of the proposed histone mimic in Orf8. The intact 2+ charged peptide or precursor at 879.9508++ m/z was isolated and fragmented resulting in the MS/MS spectra shown. After fragmentation, the MS/MS spectra show unfragmented precursor (green) with matching product ions (b ions in blue, y ions in red) within 10ppm mass error. Each fragment’s intensity is given relative to the highest ion in the MS/MS spectra across the m/z range. The color, letter, and number of each fragment indicates the sequence that fragment contains within the larger peptide (top). Y fragments (red) indicate C-terminus matched fragments. B fragments (blue) indicate N terminus matched fragments. Scale bars = 5µM.
Figure 3. SARS-CoV-2 infection affects histone PTMs. (a) Orf8 and LaminA/C staining of
SARS-CoV-2 infected ACE\textsuperscript{A549} cells at MOI=1, 48 hours post infection. (b) H3K9me3 staining of
ACE\textsuperscript{A549} cells 48 hours after SARS-CoV-2 or mock infection at MOI=1. (c) Quantification of H3K9me3. N = 475 (Mock), 158 (SARS-CoV-2), 265 (Uninfected neighbor) cells per condition from 3 independent infections. (d) H3K27me3 staining of ACE\textsuperscript{A549} cells 48 hours after SARS-CoV-2 or mock infection at MOI=1. (e) Quantification of H3K27me3. N= 455 (Mock), 133 (SARS-CoV-2), 250 (Uninfected neighbor) cells per condition from 3 independent infections. (f) H3K9ac staining of ACE\textsuperscript{A549} cells 48 hours after SARS-CoV-2 or mock infection at MOI=1. (g) Quantification of H3K9ac. N= 385 (Mock), 177 (SARS-CoV-2), 268 (Uninfected neighbor) cells per condition from 3 independent infections. White arrows indicate infected cells. Grey arrows indicate uninfected neighbors. ***, p<0.001, 1-way ANOVA with post-hoc 2-sided t-test and Bonferroni correction. Scale bars = 5\textmu M.
**Figure 4.** Genome-wide sequencing demonstrates global changes in histone PTMs in response to SARS-CoV-2 infection. (a) Model of the SARS-CoV-2 genome indicating the site of deletion that decreases disease severity in COVID-19 patients. (b) ChIP-sequencing of iPSC derived iAT2 cells fixed by 4% PFA 48 hours after SARS-CoV-2 or mock infection at MOI=5. ChIP-RX normalization shows globally increased H3K9me3 and H3K27me3 and decreased H3K9ac. N = at least 3 infections per ChIP fixed by 4% PFA 48 hours after SARS-CoV-2 or mock infection at MOI=5. (c) ChIP-sequencing gene tracks for interferon response genes with...
limited responses to SARS-CoV-2 infection. (d) Postmortem COVID-19 patient lung tissue
stained for H3K9me3 and Nucleocapsid protein to identify SARS-CoV-2 infected cells. (e)
Quantification of H3K9me3 in infected cells compared to neighboring cells from the same tissue
slice. N = 12 SARS-CoV-2 infected cells and 131 uninfected neighboring cells from 3 COVID
patient samples and 60 cells from 3 control patients. 1-way ANOVA with post-hoc 2-sided t-test
and Bonferroni correction. Scale bar = 10µM.
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Orf8 histone mimicry and localization. (a,b) Orf8 contains an ARKS motif that matches the histone H3 tail regions surrounding the critical sites H3K9 (a) and H3K27 (b). (c) Mass spectrometry analysis of histone PTMs in control (GFP) or Orf8 expressing HEK cells isolated by FACS. Z-score and fold changes are
shown for modifications that are significantly changed in response to Orf8 expression, were detected over a minimal threshold of 1% of the total peptide abundance, and have well-established functions. Full results and raw 300 data are shown in Table S1. (d) ATAC-sequencing of 2 independent replicates of HEK cells expressing GFP or Orf8 isolated by FACS. Reads per million mapped surrounding the transcription start site (TSS) of all genes are averaged. (e-j) HEK cells transfected with GFP or Strep-Orf8 show that Orf8 expression increases H3K9me3 (e-f) and H3K27me3 (g-h) while decreasing H3K9ac (i-j). Conversely, Orf8 with a deletion of the histone mimic site ARKSAP (Orf8-del) does not affect these histone PTMs. N = 614 (GFP), 497 (Orf8), 170 (Orf8-del) cells for H3K9me3; 616, 550, 154 cells for H3K27me3; 666, 568, 170 cells for H3K9ac compiled from 3 independent transfections. ***, p<0.001, 1-way ANOVA with post-hoc 2-sided t-test and Bonferroni correction. Scale bars = 5μM.
Figure 2

Mechanism of Orf8 function. (a) Subcellular fractionation of HEK cells transfected with Strep-Orf8 indicates Orf8 is present in the cytoplasm and nucleus. (b) Staining of HEK cells transfected with Strep-Orf8 shows Orf8 is expressed in the cytoplasm and at the nuclear periphery as well as in nuclear puncta. (c) Orf8 colocalizes with Lamin at the nuclear periphery and within nuclear puncta. Images shown include rotation of z-stacks (right and bottom panel for each stain) to demonstrate colocalization throughout the nucleus. (d) Orf8 co-immunoprecipitates with Lamin complex-associated proteins including LaminB, HP1,
and H3. Streptactin-conjugated beads were used for Orf8 IPs. '-' indicates cells that are not expressing Orf8 for negative control IPs performed in parallel. (e) Sequential salt extraction of HEK cells expressing Orf8 indicates Orf8 is present in chromatin fractions and dissociates from chromatin between peaks of dissociation for LaminB and histone protein H3. 'Cyto' indicates cytoplasmic fraction. (f) Targeted mass spectrometry analysis of trypsin-digested Orf8 shows Orf8 is acetylated at lysine 52, at the site of the proposed histone mimic in Orf8. The intact 2+ charged peptide or precursor at 879.9508++ m/z was isolated and fragmented resulting in the MS/MS spectra shown. After fragmentation, the MS/MS spectra show unfragmented precursor (green) with matching product ions (b ions in blue, y ions in red) within 10ppm mass error. Each fragment’s intensity is given relative to the highest ion in the MS/MS spectra across the m/z range. The color, letter, and number of each fragment indicates the sequence that fragment contains within the larger peptide (top). Y fragments (red) indicate C terminus matched fragments. B fragments (blue) indicate N terminus matched fragments. Scale bars = 5μM.
Figure 3

SARS-CoV-2 infection affects histone PTMs. (a) Orf8 and LaminA/C staining of SARS-CoV-2 infected ACEA549 cells at MOI=1, 48 hours post infection. (b) H3K9me3 staining of ACEA549 cells 48 hours after SARS-CoV-2 or mock infection at MOI=1. (c) Quantification of H3K9me3. N = 475 (Mock), 158 (SARS-CoV-2), 265 (Uninfected neighbor) cells per condition from 3 independent infections. (d) H3K27me3 staining of ACEA549 cells 48 hours after SARS-CoV-2 or mock infection at MOI=1. (e) Quantification of H3K27me3.

Figure 3 cont.

(f) H3K9ac staining of ACEA549 cells 48 hours after SARS-CoV-2 or mock infection at MOI=1. (g) Quantification of H3K9ac.
H3K27me3. N= 455 (Mock), 133 (SARS-CoV-2), 250 (Uninfected neighbor) cells per condition from 3 independent infections. (f) H3K9ac staining of ACEA549 cells 48 hours after SARS-CoV-2 or mock infection at MOI=1. (g) Quantification of H3K9ac. N= 385 (Mock), 177 (SARS-CoV-2), 268 (Uninfected neighbor) cells per condition from 3 independent infections. White arrows indicate infected cells. Grey arrows indicate uninfected neighbors. ***, p<0.001, 1-way ANOVA with post-hoc 2-sided t-test and Bonferroni correction. Scale bars = 5μM.

Figure 4

Genome-wide sequencing demonstrates global changes in histone PTMs in response to SARS-CoV-2 infection. (a) Model of the SARS-CoV-2 genome indicating the site of deletion that decreases disease severity in COVID-19 patients. (b) ChIP-sequencing of iPSC derived iAT2 cells fixed by 4% PFA 48 hours
after SARS-CoV-2 or mock infection at MOI=5. ChIP-RX normalization shows globally increased H3K9me3 and H3K27me3 and decreased H3K9ac. N = at least 3 infections per ChIP fixed by 4% PFA 48 hours after SARS-CoV-2 or mock infection at MOI=5. (c) ChIP-sequencing gene tracks for interferon response genes with limited responses to SARS-CoV-2 infection. (d) Postmortem COVID-19 358 patient lung tissue stained for H3K9me3 and Nucleocapsid protein to identify SARS-CoV-2 infected cells. (e) Quantification of H3K9me3 in infected cells compared to neighboring cells from the same tissue slice. N = 12 SARS-CoV-2 infected cells and 131 uninfected neighboring cells from 3 COVID patient samples and 60 cells from 3 control patients. 1-way ANOVA with post-hoc 2-sided t-test and Bonferroni correction. Scale bar = 10μM.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- SupplementalFigures.pdf
- TableS1HistonePTMs.xlsx
- TableS2Orf8bindingpartnerIP.xlsx
- NATUREemailattachmentSMC16092415661.pdf