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SARS-CoV-2 infects and induces cytotoxic effects in human cardiomyocytes

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Abstract

Aims The coronavirus disease 2019 (COVID-19) is caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) and has emerged as global pandemic. SARS-CoV-2 infection can lead to elevated markers of cardiac injury associated with higher risk of mortality. It is unclear whether cardiac injury is caused by direct infection of cardiomyocytes or is mainly secondary to lung injury and inflammation. Here, we investigate whether cardiomyocytes are permissive for SARS-CoV-2 infection.

Methods and Results Two stains of SARS-CoV-2 infected human iPS-cardiomyocytes as demonstrated by detection of intracellular double strand viral RNA and viral spike glycoprotein protein expression. Increasing concentrations of virus RNA are detected in supernatants of infected cardiomyocytes, which induced infections in CaCo-2 cell lines documenting productive infections. SARS-COV-2 infection and induced cytotoxic and pro-apoptotic effects associated with abolished cardiomyocyte beating. RNA sequencing confirmed a transcriptional response to viral infection as demonstrated by the up-regulation of genes associated with pathways related to viral response and interferon signaling, apoptosis and reactive oxygen stress. SARS-CoV-2 infection and cardiotoxicity was confirmed in a 3D cardiosphere tissue models. Importantly, viral spike protein and viral particles were detected in living human heart slices after infection with SARS-CoV-2. Corona virus particles were further observed in cardiomyocytes of a patient with COVID-19. Infection of iPS-cardiomyocytes was dependent on cathepsins and ACE2, and was blocked by remdesivir.

Conclusions This study demonstrates that SARS-CoV-2 infects cardiomyocytes in vitro in an ACE2 and cathepsins-dependent manner. SARS-CoV-2 infection of cardiomyocytes is inhibited by the anti-viral drug remdesivir.

Translational Perspective Although this study cannot address whether cardiac injury and dysfunction in COVID-19 patients is caused by direct infection of cardiomyocytes, the demonstration of direct cardiotoxicity in cardiomyocytes, organ mimics, human heart slices and human hearts warrants the further monitoring of cardiotoxic effects in COVID-19 patients.

Introduction

The coronavirus disease 2019 (COVID-19) is caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) and has emerged as global pandemic. SARS-CoV-2 is an enveloped and single-stranded RNA virus type, which mainly invades alveolar epithelial cells and causes adult respiratory distress syndromes. COVID-19 is associated with myocardial injury, as assessed by increased troponin T and NT-proBNP levels accompanying increased cardiovascular symptoms in a significant number of SARS-CoV-2 infected patients[1–3]. Recent studies further demonstrate significantly reduced ejection fraction, higher left ventricular mass and raised native T1 and T2 assessed by magnetic resonance imaging in patients recovered from severe COVID-19[4]. Elevated levels of cardiac injury markers were associated with higher risk of in-hospital mortality in COVID-19 patients[5]. In patients showing clinical deterioration during COVID-19, left ventricular systolic dysfunction was noted in approximately 20 % of patients according to a most recent study[6]. In addition, patients with underlying cardiovascular disease represent a significant proportion of patients, who may suffer from severe courses after COVID-19 infections[7]. However, it is unclear whether elevated biomarkers of cardiac injury and long term effects on the cardiovascular system are directly caused by viral infection of cardiac tissue or are secondary to hypoxia and systemic inflammation during complicated COVID-19 courses. Earlier studies with cardiac tissue samples revealed mixed results. While one study did not find evidence for viral particles of the first SARS corona virus SARS-CoV[8], SARS-CoV RNA was identified in 35 % of autopsied human heart samples obtained from patients who succumbed to the SARS crisis during the Toronto SARS outbreak [9]. Other studies suggest that the Middle East respiratory syndrome-related coronavirus (MERS-CoV), which has similar pathogenicity as SARS-CoV-2, can cause acute myocarditis and heart failure[10]. Moreover, substantial amounts of viral SARS-CoV-2 RNA was detected in human hearts of COVID-19 patients[11–14] Although virus particles were identified in interstitial cells in myocardium of one patient[15], direct infection in cardiomyocytes of COVID-19 patients has not been described yet.

Single cell RNA sequencing and histological analyses demonstrated that human cardiomyocytes express the putative SARS-CoV-2 receptor angiotensin converting enzyme 2 (ACE2), particularly in patients with cardiovascular diseases[16,17] suggesting that cardiomyocytes could be targeted by SARS-CoV-2.

Therefore, we investigated whether SARS-CoV-2 infects human induced pluripotent stem cell-derived cardiomyocytes in culture and in two models of human cardiac tissue including human heart slices *in vitro*.

Methods

The use of all human cells and tissues was approved by the institutional ethics review boards and complies with the Declaration of Helsinki. All subjects gave informed written consent.

Cell Culture

hiPS-CM of two donors were obtained with an embryoid body-based protocol as described[18]. Cardiospheres were generated by adapting a previously described protocol[19] using hiPS cells. Living human heart slices (300 µm) were generated and cultured as described[9].

Viral infection

SARS-CoV-2-FFM1 and FFM2 were isolated and propagated in Caco-2 cells as described [20,21]. The viral stock was diluted to desired MOI in medium containing 1% fetal bovine serum and incubated with cells for 2 h. Then the infectious inoculum was removed and cells were supplemented with the respective culture medium[18]. Cardiospheres were cultured with 25µl of viral stock (1.10^7 TCID50/ml) and living human heart slices were incubated with 200µl of viral stock (1.10^7 TCID50/ml) for three to five days.

Quantification of SARS-CoV RNA in cell culture supernatants was performed as previously described[21]. For detection of viral titer, hiPS-CM were infected for 2 h, the infection medium was replaced, and supernatants were collected 48h post infection and used to infect confluent layers of CaCo-2 cells in 96-well plates. Cytopathogenic effects were assessed visually 48 h after infection. The infectious titer was determined as TCID50/ml.

For further details, see Online Data Supplemental "Expanded Methods".

Results

Expression of receptor and co-receptor

We first addressed if human induced pluripotent stem cell-derived cardiomyocytes (hiPS-CM) showed the expression of the SARS-CoV-2 receptors ACE2 and the serine proteases TMPRSS2 and cathepsins, which mediate priming of the viral S-protein[22]. ACE2 was well expressed on mRNA level in hiPS-CM but not in undifferentiated iPS cells (**Figure 1a**, **Online Supplement Figure Ia-d**). The cathepsins CTSB and CTSL were highly expressed, whereas TMPRSS2 was detected only at very low levels by RNA sequencing (**Figure 1a**). Quantitative RT-PCR confirmed the expression of ACE2, but TMPRSS2 was below the detection level (**Figure 1b/c**). ACE2 protein expression was confirmed by immunostainings using two different antibodies in iPS-CM (**Figure 1d**, **Online Supplement Figure Ib/d**). Interestingly, ACE2 expression in cardiomyocytes was lower and ACE2 was localized more to cytoplasmic and perinuclear regions as compared to other TMPRSS2-positive cells such as the human CaCo-2 cell line, which is known to be highly permissive for SARS-CoV and SARS-CoV-2 infection[21–23] (**Figure 1b, Online Supplement Figure Ie**). However, membrane staining was occasionally detected in control and SARS-CoV-2 infected iPS-CM (**Online Supplement Figure 1d, f**; indicated by arrows). These data document that human cardiomyocytes possess receptor and activators described thus far necessary for effective SARS-CoV-2 infection, but show a lower expression and distinct localization of ACE2.

hiPS-CM are infected by SARS-CoV-2

To test if hiPS-CM are directly targeted and are permissive for SARS-CoV-2 infection, hiPS-CM were infected with isolates of SARS-CoV-2[20] (**Figure 2a**). SARS-CoV-2 infected hiPS-CM showed increased intracellular double stranded virus RNA as demonstrated by immunostaining (**Figure 2b, Online Supplement Figure IIa**). Viral RNA in supernatants was further assessed by PCR and was dose- and time-dependently increased after infection with SARS-CoV-2 (**Figure 2c-e**). Consistently, the expression of the viral spike glycoprotein protein was detected in a time- and dose dependent manner after infection with different strains of the virus (FFM1 and FFM2[20]) (**Figure 2f-h, Online Supplement Figure IIc-d)**. Control experiments confirmed spike protein expression in α-sarcomeric actinin-expressing cardiomyocytes (**Figure 2i, Online Supplement Figure If**). The supernatant of hiPC-CM contained fully infectious virus, as demonstrated by titration in CaCo-2 cells (**Figure 2j**) indicating that the virus undergoes full replicatory cycles in hiPS-CM. Interestingly, the frequency of beating was significantly augmented at 24h to 48h post infection, but was

abolished at later time points (**Figure 2k**). SARS-CoV-2 infection reduced cell counts (**Figure 2l)** and augmented apoptosis in hiPS-CM (**Figure 2m**). Profound cytopathogenic effects were visible at later time points (96 h) (**Figure 2h**). However, cytotoxicity in cardiomyocytes was detected at later time points compared to CaCo-2 cells, which quickly round up and showed severe cytotoxicity at 24 h (**Online Suppl. Figure IIe,f**). RNA sequencing demonstrated that infected hiPS-CM showed a strong transcriptional response to viral infection including interferon activation (**Figure 2n**) and signatures of apoptosis and oxidative stress (**Figure 2o**).

SARS-CoV-2 infects cardiomyocytes in three dimensional cardiac tissue

Next, we determined if SARS-CoV-2 infects cardiomyocytes in a three dimensional tissue environment using human cardiospheres generated by hiPS-cells, which are generated by a modified previously published protocol[19] (**Figure 3a-b**). SARS-CoV-2 time-dependently affected beating frequency of cardiospheres with a profound inhibition at 5 days post infection (**Figure 3c**). At 5 days post infection, cardiospheres showed a reduced size (**Figure 3d**) consistent with the induction of cell death. SARS-CoV-2 infection was further documented by spike protein staining (**Figure 3d, Online Supplement Figure III**).

Finally, we addressed whether SARS-CoV-2 infects human heart tissue by using living human cardiac tissue slices, which were obtained from explanted hearts[24] (**Figure 4a-f**). Here, increased spike protein expression was shown in four different samples derived from three explants (**Figure 4c-e**). Infection was associated with morphological signs of tissue injury such as areas with loss of α-saromeric actinin signal and disorganized structure compared to homogenous mock controls (**Figure 4c**). Spike protein expression was detected in α-sarcomeric actinin positive cardiomyocytes (**Figure 4d**). The virus was further identified in infected human heart tissue in cardiomyocytes by electron microscopy (**Figure 4f, Online Supplement Figure IV**). Of note, stages of the entire replicatory cycle were detected (**Figure 4f**, right panel). Finally, we detected SARS-CoV-2 in an endomyocardial biopsy of a patient with COVID-19. This 27-year-old patient was diagnosed with COVID-19 and suffered a complicated course of COVID-19 with severe lung injury and reduced right and left ventricular ejection fraction (see **Online Supplement Method** for more details). Virus particles were detected in cardiomyocytes associated with visible cytotoxic effects such as focal loss of myofibrils (**Figure 4g**).

Cathepsin and RNA-dependent RNA polymerase inhibitors prevent iPS-CM infection

Having demonstrated that SARS-CoV-2 can infect human cardiomyocytes, we tested strategies to interfere with viral infection. First, we determined if interfering with ACE2, which was shown to block virus infection of organoids [25], also is effective in cardiomyocytes. Indeed, recombinant ACE2 or neutralizing antibodies blocked spike protein expression (**Figure 5a**). Since cardiomyocytes essentially lack TMPRSS2 but express cathepsins, we additionally tested the effect of the protease inhibitor N-Acetyl-L-leucyl-L-leucyl-L-methional (ALLM), which preferentially blocks cathepsins[26]. Indeed, inhibition of cathepsins reduced spike protein expression (**Figure 5b**). Moreover, the viral RNA-dependent RNA polymerase inhibitor remdesivir inhibited spike protein expression (**Figure 5c**).

Discussion

Together, SARS-CoV-2 can infect human cardiomyocytes in culture as well as in two different models of cardiac tissue. Infection was documented by various read outs including intracellular viral double strand RNA and spike protein expression, as well as extracellular viral RNA. The virus was further detected by electron microscopy in cells of the infected human heart slices and in an endocardial biopsy of a COVID-19 patient. Importantly, functional virus could be isolated in supernatants of infected cardiomyocytes documenting that SARS-CoV-2 undergoes a full replicatory cycle. Of note, viral infection was confirmed with cells of two different hiPS-donors and two viral strains and our in vitro data are consistent with a recent online publication [27].

The expression of the SARS-CoV-2 receptor ACE2 in cardiomyocytes was confirmed on mRNA and protein levels, but its localization was distinct from CaCo-2 cells. Although ACE2 could be detected at the cell membrane in some iPS-CM, it was preferentially detected in the cytoplasm and the perinuclear region (**Figure 1d, Online Supplement Figure I**). The preferential intracellular localization of ACE2 observed in cultured iPS-CM was similar to the staining pattern of human heart samples[16] and may suggest that ACE2 is shuttling between the plasma membrane and intracellular compartments. Despite the relatively low membrane ACE2 expression, ACE2 was functionally required for virus infection. Thus, recombinant ACE2 and neutralizing antibodies against ACE2 blocked viral spike protein expression after SARS-CoV-2 infection.

Whereas the receptor ACE2 was well expressed on mRNA and protein level, the previously described protease activator TMPRSS2 was very lowly expressed in hiPS-CM, suggesting that activation of the S-protein may be mediated by other cysteine proteases such as cathepsin L and B, which also can mediate viral activation[22]. Indeed, the protease inhibitor ALLM,

which preferentially blocks cathepsin L and B [26], reduced viral spike protein expression. Viral infection was associated with cytotoxic effects and inhibition of beating of cardiomyocytes in our in vitro cultures and cardiospheres suggesting a potential detrimental effect of SARS-CoV-2 infection on the human heart. Interestingly, cardiomyocytes are less susceptible to SARS-CoV-2 infection and cytotoxicity compared to $TMPRSS2^{+}$ CaCo-2 cells, which showed a faster and more severe cytotoxic response. These findings, if translatable to a clinical setting, may suggest that cardiomyocyte infection may only occur under conditions of a high local virus concentration and longer time exposure. Viral infection may be facilitated in patients with cardiovascular disease, due to the described augmentation of ACE2 expression and cardiovascular disease-associated vascular inflammation allowing infiltration of virus-loaded immune cells.

SARS-CoV-2 elicits a typical transcriptional response to viral infection including the activation of interferon pathways. Since we recently demonstrated that SARS-CoV-2 infection deregulates pathways involved in ER stress and protein homeostasis [21], one may hypothesize that viral infection may induce ER stress leading to prolonged unfolded protein response and subsequent alteration in calcium homeostasis and cardiomyocyte cell death [28].

There is compelling evidence that patients suffering from COVID-19 show profound elevations of cardiac injury biomarkers and deteriorated right and left ventricular cardiac function, however, whether cardiac injury is directly caused by cardiomyocyte infection is unclear. Viral RNA has been detected at significant levels in cardiac tissue[11–14] and cardiomyocyte infection may occur during conditions of vascular leakage and tissue inflammation[29]. Our data showing virus particles in cardiomyocytes of a COVID-19 patient, further supports that viral infection can indeed occur. However, whether a direct infection is the main cause leading to the high incidence of cardiac involvement observed after COVID-19[4] is unclear. While viral RNA meanwhile has been detected in various studies[11–14], only few cases reported the detection of the virus particle by electron microscopy in the heart but mainly in non-parenchymal cells[15]. It is also debated whether COVID-19 induces myocarditis. While some studies document infiltration of inflammatory cells[4,14], others did not find elevated inflammatory cytokines in cardiac tissue in autopsies of patients who died of COVID-19[13]. Therefore, the incidence and consequences of SARS-CoV-2 infection of cardiomyocytes in human hearts deserves further studies. Our in vitro data may suggest that infected cardiomyocytes may undergo apoptosis, a transient process, which may lead to secondary replacement fibrosis but not necessarily inflammation. The profound induction of interferon pathways observed in our RNA sequencing analysis may further imply an anti-viral

response in infected cardiomyocytes. However, theses in vitro experiments by no means can mimic the complex effects of the virus on cellular and systemic levels. Thus, it will be important to study the presence of corona virus particles and tissue morphology not only post mortem but in cardiac biopsies at the first time of increased troponin release, during the further progression and after recovery in order to gain insights into a potential relation of acute infection with cardiac injury.

Finally, the used models can serve as experimental tools for further testing the effects of therapeutic strategies. Interfering with ACE2 by neutralizing antibodies or recombinant ACE2, which was previously shown to block infection of organoids[25], or inhibition of the RNA polymerase with remdesivir efficiently inhibited viral spike protein expression. Our findings showing that cathepsins and likely not TMPRSS2 are involved in infection of cardiomyocytes may support the testing of Cathepsin L inhibitors as recently suggested by Liu et al [30].

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Disclosures section

The authors have nothing to disclose

Data availability

The data and analytic methods will be made upon request to other researchers for purposes of reproducing the results or replicating the procedure. The study materials will only be made available if sufficient material can be provided to other researchers for purposes of reproducing the results or replicating the procedure.

Author Contribution

Denisa Bojkova, designed and performed viral infection experiments and performed statistical analysis

Julian U. G. Wagner, designed and performed cell culture and subsequent analysis and performed statistical analysis

Mariana Shumliakivska, performed cell culture studies and subsequent analysis

Galip S. Aslan, performed cell culture studies and subsequent analysis

Umber Saleem and Arne Hansen, provided iPS-CM

Guillermo Luxán supervised and performed histological analayis

Stefan Günther performed and analysed RNA sequencing study

Minh Duc Pham, Jaya Krishnan developed and provided human iPS derived cardiospheres

Patrick N. Harter, Utz H. Ermel, and Achilleas S. Frangakis performed eletron microscropy studies of cell culture material

Hendrik Milting provided human heart for generation of human heart slices

Andreas M. Zeiher provided conceptual support and designed the studies, contributed to manuscript writing

Karin Klingel performed electron microscopy of human case

Jindrich Cinatl designed and supervised virus generation and infection experiments, provided conceptual support and contributed to manuscript writing

Andreas Dendorfer provided human heart slices

Thomas Eschenhagen provided iPS-CM and provided conceptual input for study design

Carsten Tschöpe provided the case report

Sandra Ciesek designed and supervised all virus infection experiment and provided conceptual support

Stefanie Dimmeler designed the experiments and wrote the daft of the manuscript

All authors have made contributions to and corrected the manuscript.

Supplemental Materials

Expanded Methods

Online-only Figures I – IV

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Figure Legends

Figure 1: Expression of SARS-CoV-2 receptors and co-activators in hiPS-derived cardiomyocytes. a, Expression in RNA sequencing data of hiPS-CM (n=3, biological replicates). b/c, Confirmation by qRT-PCR (n=3, biological replicates), **c** shows a representative gel. **d**, ACE2 protein expression was detected by antibody (Abcam) in hiPS-CMs. Cells were counterstained by α–sarcomeric actinin and DAPI. A representative experiment out 6 biological replicates of two different iPS-CM donors is shown.

Figure 2: SARS-CoV-2 infects iPS-derived cardiomyocytes. a, Design of experiment. **b**, Immunostaining of double-stranded RNA (dsRNA) in hiPS-CMs after infection with SARS-CoV-2 FFM1 for 48 h. **c,d**, SARS-CoV-2 RNA was measured by qRT-PCR in the supernatant of infected hiPS-CM (d: 48 h, e: MOI 1). N=3 biological replicates in c/d. **e-h**, Spike glyocoprotein was measured by immunohistochemistry after infection with isolate SARS-CoV-2 FFM1 (f-g,i) or SARS-CoV-2 FFM2 (g). Panel e shows a dose-response with n=9 biological replicates. **i**, Counterstainings with α-sarcomeric actinin confirmed expression of spike protein in hiPS-CM (48h). **j,** Infectious virus in supernatants from infected hiPS-CM was determined by titration in Caco-2 cells 48 h post infection. n=3 biological replicates. **k,** Beating rate of hiPS-CMs. n=4 biological replicates. **l**, Number of cells (DAPI+ nuclei) after 48h (MOI 0.01) or 96h (MOI 1) of infection (V) vs. mock (M) control. n=3 biological replicates. **m**, Quantification of cleaved caspase-3+ area after 48 h of infection (V) vs. mock (M). n=3 biological replicates. **n,o**, RNA sequencing of mock or SARS-CoV-2 infected hiPC-CM (MOI 1, 48 h), n=3 biological replicates. **n**, GO term analysis (Metascape) of top up-regulated genes (>5 fold, FDR<0.2). n=3 biological replicates. **o**, Top10 enriched terms in PANTHER database for DEGs, FDR<0.2. Data are mean±SEM analyzed by using two-sided, unpaired t-test (k,l), ordinary one-way ANOVA with post hoc Tukey's (e, m), Dunn's (f) comparison or Kruskal-Wallis test with Dunn's comparison (d, k). MOI: multiplicity of infection, **p<0.01, ***p<0.001, ****p<0.0001.

Figure 3: SARS-CoV-2 infects iPS-derived human cardiospheres. **a**, Study design of cardiosphere infection, **b**, hiPS-derived cardiosphere (light microscope image). **c**, Beating frequency of cardiospheres after 3 and 5 days post infection (dpi). N=12 (3 dpi); N=6 (5 dpi), all biological replicates. **d,** Expression of spike glycoprotein at 3 and 5 days post infection (dpi). Data were statistically assessed using one-way ANOVA with post hoc Tukey's test. n.s. = not significant, *p<0.05, ****p<0.0001.

Figure 4: SARS-CoV-2 infects living human cardiac tissue slices. **a-c**, Infection of human cardiac tissue slices of three explants. **b**, Scheme of experiment. **c-e**, Spike protein expression in infected human heart slices (n=4 samples of three different explants, biological replicates). Quantification of individual images of each explant is shown in **e**. Data were statistically assessed with unpaired T-test with Welch's correction. *p<0.05. Representative images are shown in **c**, higher magnification of a representative cardiomyocyte expressing spike protein is shown in **d**. **f**, Electron microscopy of infected human cardiac tissue slices. Arrow heads indicate putative virus particles. **g**, Electron microscopy of a human endomyocardial biopsy taken from a COVID-19 patient. Arrow heads indicate putative virus particles.

Figure 5: Cardiomyocyte infection is inhibited by ACE2, cathepsin and RNA polymerase inhibition. a, Effect of recombinant ACE2 (5 μg/ml) or neutralizing antibodies (80 μg/ml) on SARS-CoV-2 infection. Spike protein was quantified after 48 h of infection (MOI=1). n=9 (virus), n=7 (virus+rhACE2), n=6 (Isotope control) und n=7 (virus + ACE2 AB), all biological replicates. **b**, Effect of the protease inhibitor N-Acetyl-L-leucyl-L-leucyl-L-methional (ALLM; 1 μM; added during and post infection) on spike protein expression after infection with SARS-CoV-2 (MOI=1) at 48h. n=3 biological replicates. **c**, Effect of remdesivir (1 μM, added post-infection). Since ALLM and remdesivir were solved in DMSO, virus infected cells were also treated with DMSO in panels b and c. n=3 biological replicates. Data were statistically assessed using one-way ANOVA with post hoc Dunnett's (a, b) or post hoc Holm-Sidak's test (c). n.s. = not significant, *p<0.05, ***p<0.001, ****p<0.0001.

Figure 1

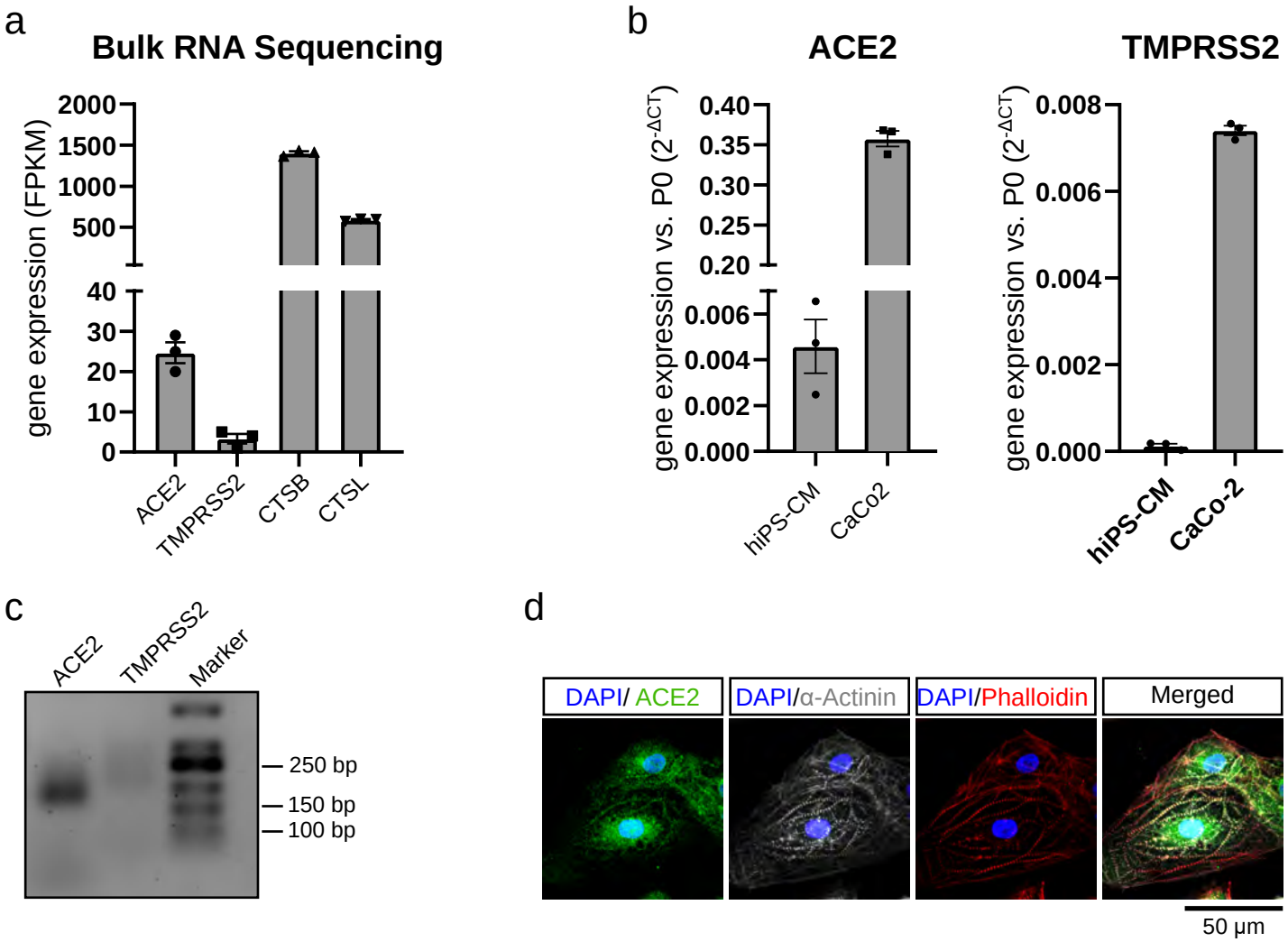


Figure 2

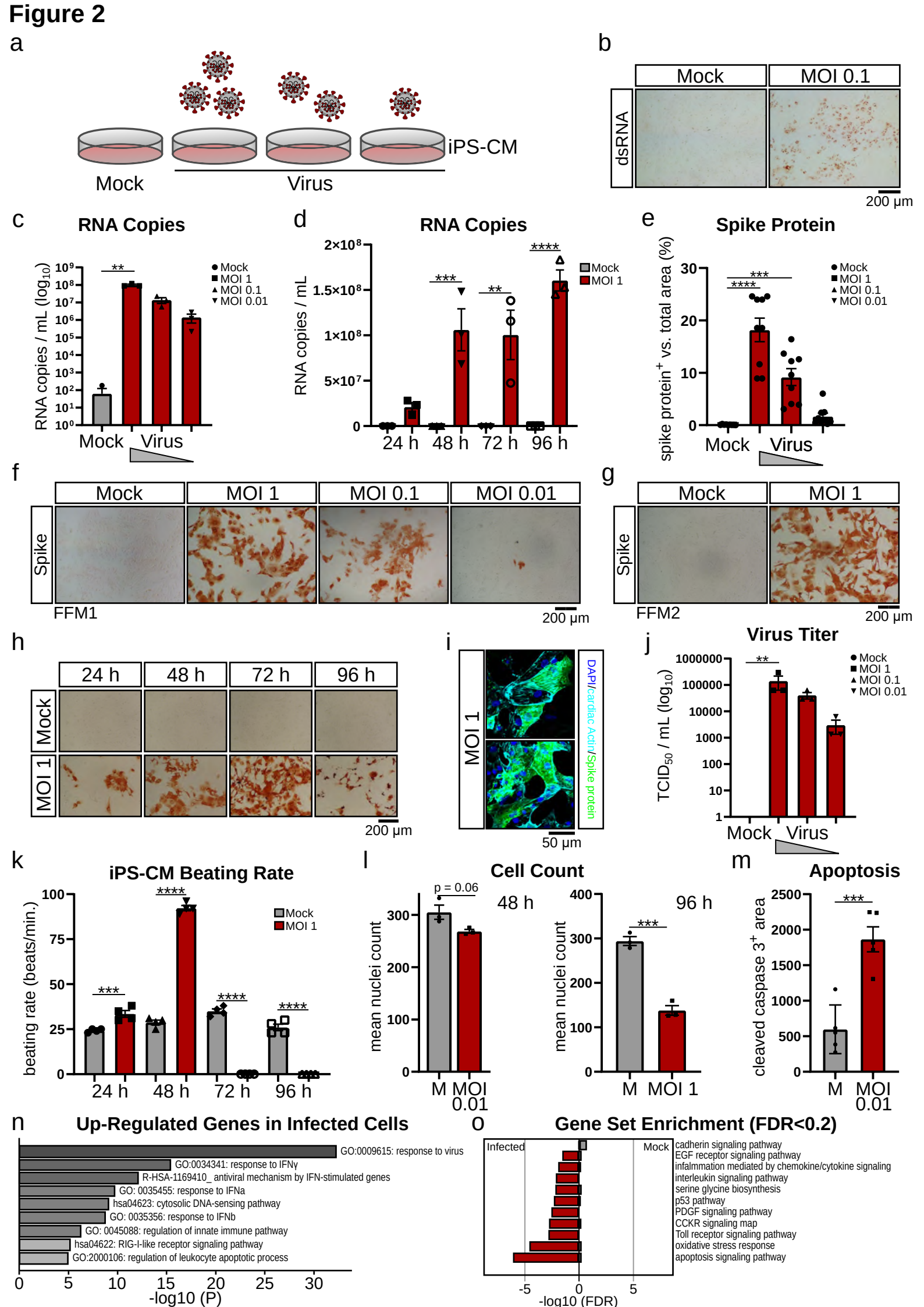

a
Mock
Virus
iPS-CM
b
Mock
MOI 0.1
dsRNA
200 µm
c
RNA Copies
RNA copies / mL (log10)
Mock
MOI 1
MOI 0.1
MOI 0.01
Mock
Virus
d
RNA Copies
RNA copies / mL
Mock
MOI 1
24 h
48 h
72 h
96 h
e
Spike Protein
spike protein+ vs. total area (%)
Mock
MOI 1
MOI 0.1
MOI 0.01
Mock
Virus
f
Mock
MOI 1
MOI 0.1
MOI 0.01
Spike
FFM1
200 µm
g
Mock
MOI 1
Spike
FFM2
200 µm
h
24 h
48 h
72 h
96 h
Mock
MOI 1
200 µm
i
MOI 1
DAPI/cardiac Actin/Spike protein
50 µm
j
Virus Titer
TCID50 / mL (log10)
Mock
MOI 1
MOI 0.1
MOI 0.01
Mock
Virus
k
iPS-CM Beating Rate
beating rate (beats/min.)
Mock
MOI 1
24 h
48 h
72 h
96 h
l
Cell Count
p = 0.06
48 h
96 h
mean nuclei count
M
MOI 0.01
MOI 1
m
Apoptosis
cleaved caspase 3+ area
M
MOI 0.01
n
Up-Regulated Genes in Infected Cells
GO:0009615: response to virus
GO:0034341: response to IFNγ
R-HSA-1169410_ antiviral mechanism by IFN-stimulated genes
GO: 0035455: response to IFNa
hsa04623: cytosolic DNA-sensing pathway
GO: 0035356: response to IFNb
GO: 0045088: regulation of innate immune pathway
hsa04622: RIG-I-like receptor signaling pathway
GO:2000106: regulation of leukocyte apoptotic process
-log10 (P)
o
Gene Set Enrichment (FDR<0.2)
Infected
Mock
cadherin signaling pathway
EGF receptor signaling pathway
infalmmation mediated by chemokine/cytokine signaling
interleukin signaling pathway
serine glycine biosynthesis
p53 pathway
PDGF signaling pathway
CCKR signaling map
Toll receptor signaling pathway
oxidative stress response
apoptosis signaling pathway
-log10 (FDR)

Figure 3

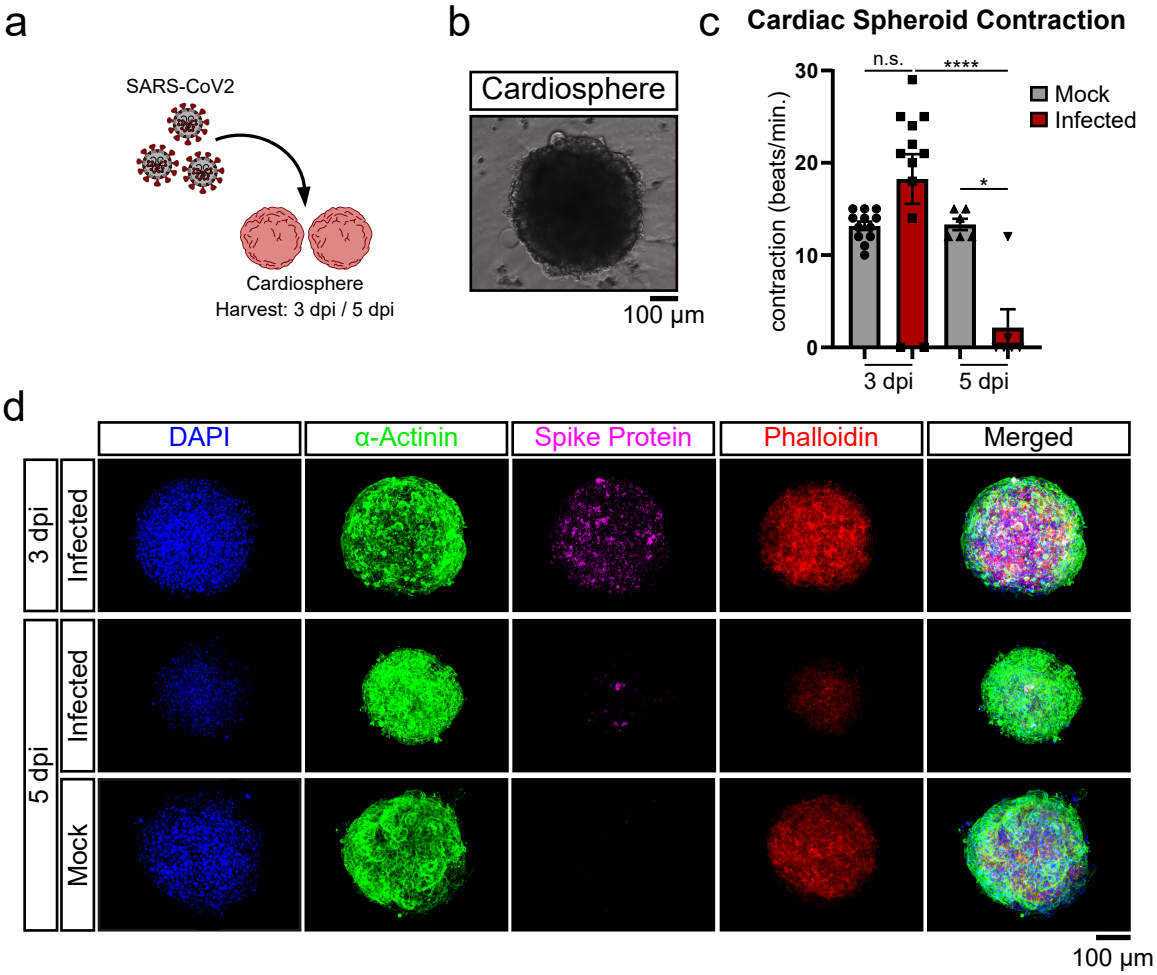

a
SARS-CoV2
Cardiosphere
Harvest: 3 dpi / 5 dpi
b
Cardiosphere
100 µm
c
Cardiac Spheroid Contraction
n.s.
****
*
Mock
Infected
contraction (beats/min.)
30
20
10
0
3 dpi
5 dpi
d
DAPI
α-Actinin
Spike Protein
Phalloidin
Merged
3 dpi
Infected
5 dpi
Infected
Mock
100 µm

Figure 4

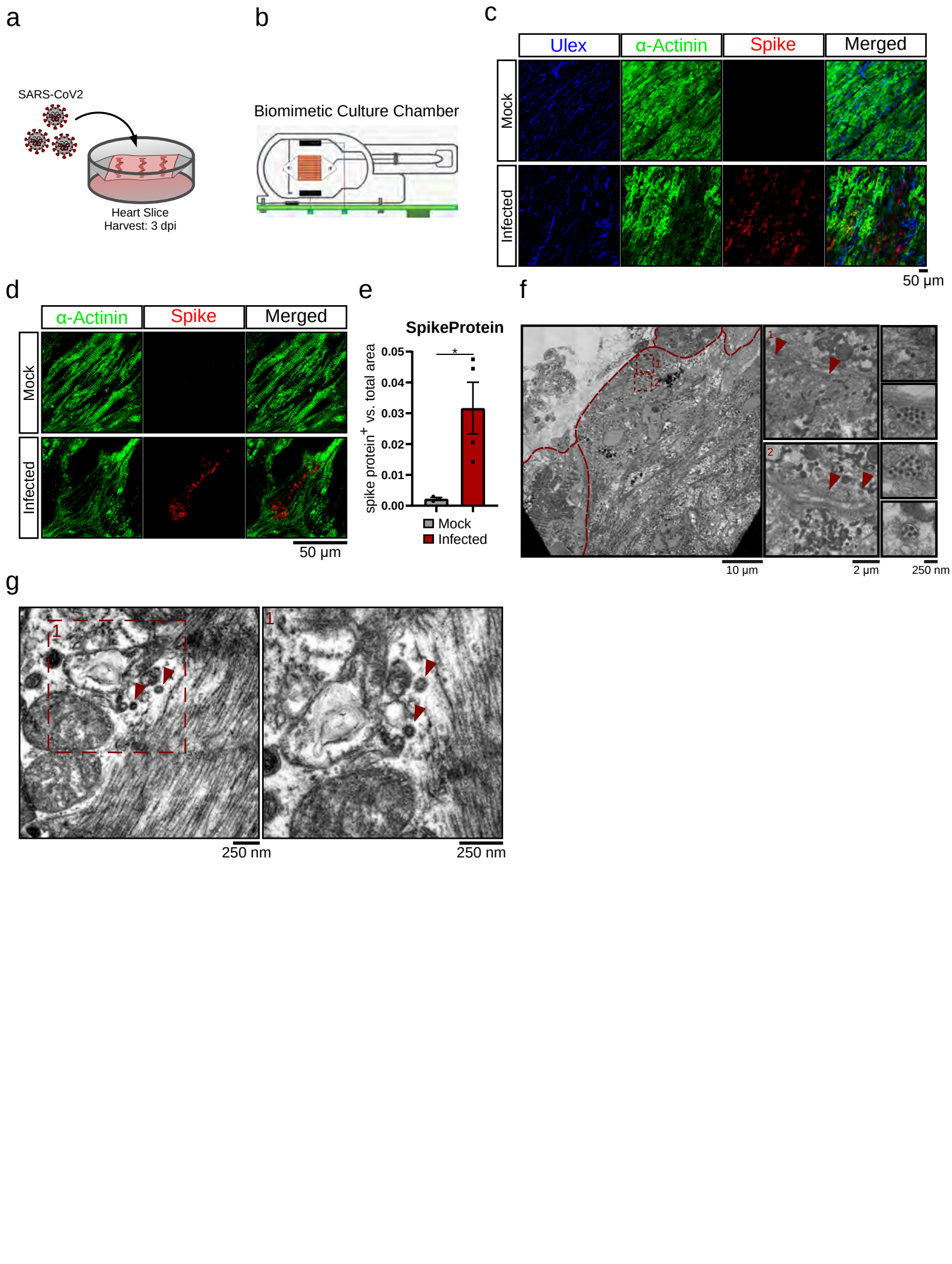
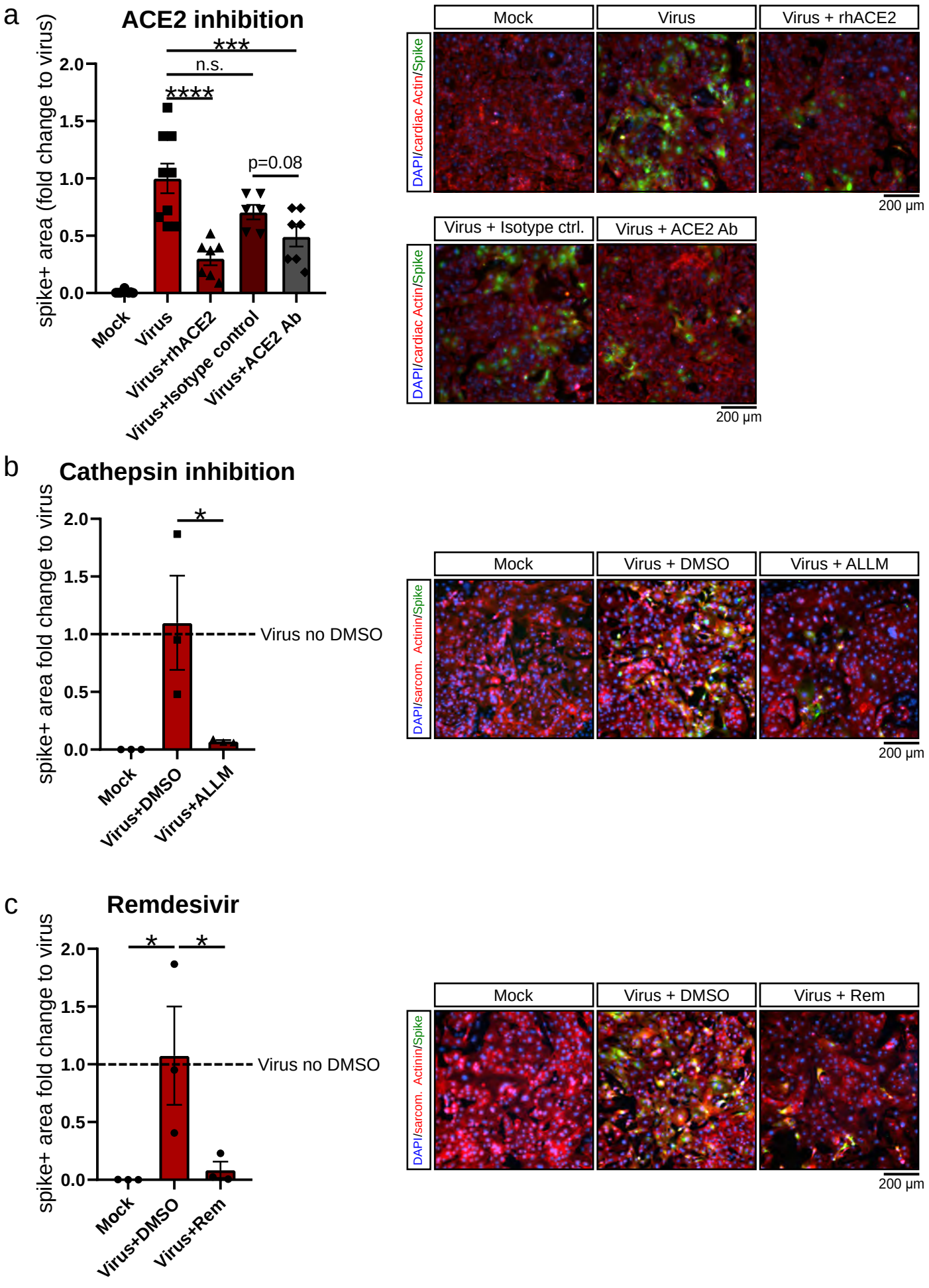

a
SARS-CoV2
Heart Slice
Harvest: 3 dpi
b
Biomimetic Culture Chamber
c
Ulex
α-Actinin
Spike
Merged
Mock
Infected
50 µm
d
α-Actinin
Spike
Merged
Mock
Infected
50 µm
e
SpikeProtein
spike protein+ vs. total area
0.05
0.04
0.03
0.02
0.01
0.00
*
Mock
Infected
f
1
2
10 µm
2 µm
250 nm
g
1
1
250 nm
250 nm

Figure 5


a
ACE2 inhibition
spike+ area (fold change to virus)
***
n.s.
****
p=0.08
Mock
Virus
Virus+rhACE2
Virus+Isotype control
Virus+ACE2 Ab
Mock
Virus
Virus + rhACE2
DAPI/cardiac Actin/Spike
200 µm
Virus + Isotype ctrl.
Virus + ACE2 Ab
DAPI/cardiac Actin/Spike
200 µm
b
Cathepsin inhibition
spike+ area fold change to virus
Virus no DMSO
Mock
Virus+DMSO
Virus+ALLM
Mock
Virus + DMSO
Virus + ALLM
DAPI/sarcom. Actinin/Spike
200 µm
c
Remdesivir
spike+ area fold change to virus
Virus no DMSO
Mock
Virus+DMSO
Virus+Rem
Mock
Virus + DMSO
Virus + Rem
DAPI/sarcom. Actinin/Spike
200 µm

Graphical Abstract