

1 **Bromodomain and Extraterminal Inhibition Blocks Inflammation-Induced Cardiac**  
2 **Dysfunction and SARS-CoV-2 Infection (Pre-Clinical)**

3 Richard J Mills<sup>1</sup>, Sean J Humphrey<sup>2</sup>, Patrick RJ Fortuna<sup>1</sup>, Mary Lor<sup>1</sup>, Simon R Foster<sup>1</sup>, Gregory A  
4 Quaife-Ryan<sup>1</sup>, Rebecca L. Johnston<sup>1</sup>, Troy Dumenil<sup>1</sup>, Cameron Bishop<sup>1</sup>, Rajeev Ruraraju<sup>3,4,5</sup>,  
5 Daniel J Rawle<sup>1</sup>, Thuy Le<sup>1</sup> Wei Zhao<sup>5</sup>, Leo Lee<sup>5</sup>, Charley Mackenzie-Kludas<sup>5</sup>, Neda R  
6 Mehdiabadi<sup>6</sup>, Christopher Halliday<sup>7</sup>, Dean Gilham<sup>7</sup>, Li Fu<sup>7</sup>, Stephen J. Nicholls<sup>8</sup>, Jan  
7 Johansson<sup>9</sup>, Michael Sweeney<sup>9</sup>, Norman C.W. Wong<sup>7</sup>, Ewelina Kulikowski<sup>7</sup>, Kamil A.  
8 Sokolowski<sup>10</sup>, Brian W. C. Tse<sup>10</sup>, Lynn Devilee<sup>1</sup>, Holly K Voges<sup>1</sup>, Liam T Reynolds<sup>1</sup>, Sophie  
9 Krumeich<sup>1</sup>, Ellen Mathieson<sup>1</sup>, Dad Abu-Bonsrah<sup>6,11</sup>, Kathy Karavendzas<sup>6</sup>, Brendan Griffen<sup>12</sup>,  
10 Drew Titmarsh<sup>12</sup>, David A Elliott<sup>6</sup>, James McMahon<sup>13,14</sup>, Andreas Suhrbier<sup>1,15</sup>, Kanta  
11 Subbarao<sup>3,5</sup>, Enzo R Porrello<sup>6,16</sup>, Mark J Smyth<sup>1</sup>, Christian R Engwerda<sup>1</sup>, Kelli PA MacDonald<sup>1</sup>,  
12 Tobias Bald<sup>1</sup>, David E James<sup>2</sup>, James E Hudson<sup>1,\*</sup>

13  
14 <sup>1</sup>QIMR Berghofer Medical Research Institute, Brisbane, QLD, Australia

15 <sup>2</sup> Charles Perkins Centre, School of Life and Environmental Science, The University of Sydney,  
16 Sydney, NSW, Australia

17 <sup>3</sup>The WHO Collaborating Centre for Reference and Research on Influenza, The Peter Doherty  
18 Institute for Infection and Immunity, Melbourne, VIC, Australia

19 <sup>4</sup>Department of Microbiology and Immunology, The University of Melbourne, Melbourne VIC,  
20 Australia

21 <sup>5</sup>The Peter Doherty Institute for Infection and Immunity, Melbourne, VIC, Australia

22 <sup>6</sup>Murdoch Children's Research Institute, The Royal Children's Hospital, Melbourne, VIC,  
23 Australia

24 <sup>7</sup>Resverlogix Corp., Calgary, Canada

25 <sup>8</sup>Victorian Heart Hospital, Monash University, Clayton, VIC, Australia

26 <sup>9</sup>Resverlogix Corp., San Francisco USA

27 <sup>10</sup>Preclinical Imaging Facility, Translational Research Institute, Brisbane, Australia

28 <sup>11</sup>Department of Paediatrics, The University of Melbourne, Melbourne, VIC, Australia

29 <sup>12</sup>Dynomics Inc., San Mateo, CA 94401, United States of America and Dynomics Pty Ltd,  
30 Brisbane, QLD, Australia

31 <sup>13</sup>Department of Infectious Diseases, The Alfred Hospital and Central Clinical School, Monash  
32 University, Melbourne, VIC, Australia

33 <sup>14</sup>Department of Infectious Diseases, Monash Medical Centre, Clayton, VIC, Australia

34 <sup>15</sup>GVN Center of Excellence, Australian Infectious Diseases Research Centre, Brisbane, QLD,  
35 Australia

36 <sup>16</sup>Department of Physiology, School of Biomedical Sciences, The University of Melbourne,  
37 Melbourne, VIC, Australia

38 <sup>17</sup>Faculty of Medicine and Health, The University of Sydney, Sydney, NSW, Australia

39 \* Corresponding author

40

41 Please address correspondence to:

42 Associate Professor James E. Hudson

43 QIMR Berghofer Medical Research Institute

44 Herston, Brisbane

45 QLD, 4006, Australia

46 Tel: +61 7 3362 0141

47 Email: [james.hudson@qimrberghofer.edu.au](mailto:james.hudson@qimrberghofer.edu.au)

48

49

1 **SUMMARY**

2 Cardiac injury and dysfunction occur in COVID-19 patients and increase the risk of mortality.  
3 Causes are ill defined, but could be direct cardiac infection and/or inflammation-induced  
4 dysfunction. To identify mechanisms and cardio-protective drugs, we use a state-of-the-art  
5 pipeline combining human cardiac organoids with phosphoproteomics and single nuclei RNA  
6 sequencing. We identify an inflammatory 'cytokine-storm', a cocktail of interferon gamma,  
7 interleukin 1 $\beta$  and poly(I:C), induced diastolic dysfunction. Bromodomain-containing protein 4 is  
8 activated along with a viral response that is consistent in both human cardiac organoids and  
9 hearts of SARS-CoV-2 infected K18-hACE2 mice. Bromodomain and extraterminal family  
10 inhibitors (BETi) recover dysfunction in hCO and completely prevent cardiac dysfunction and  
11 death in a mouse cytokine-storm model. Additionally, BETi decreases transcription of genes in  
12 the viral response, decreases ACE2 expression and reduces SARS-CoV-2 infection of  
13 cardiomyocytes. Together, BETi, including the FDA breakthrough designated drug apabetalone,  
14 are promising candidates to prevent COVID-19 mediated cardiac damage.

15 **Keywords:** Inflammation; COVID-19; organoids; heart; drug discovery

16

## 1 INTRODUCTION

2 SARS-CoV-2 infection leads to cardiac injury and dysfunction in 20-30% of hospitalized patients  
3 (Guo et al., 2020) and higher rates of mortality in patients with pre-existing cardiovascular  
4 disease (Shi et al., 2020; Wu and McGoogan, 2020). Inflammatory factors released as part of  
5 the 'cytokine storm' are thought to play a critical role in cardiac dysfunction in severe COVID-19  
6 patients (Chen et al., 2020). The cardiac sequelae reported in patients with COVID-19, include  
7 acute coronary syndromes, cardiomyopathy, acute pulmonary heart disease, arrhythmias and  
8 heart failure (Gupta et al., 2020). There have been multiple proposed aetiologies for these, yet  
9 clear mechanistic insight is lacking (Gupta et al., 2020). There is a severe inflammatory  
10 response in 5% of COVID-19 patients, associated with septic shock (Wu and McGoogan, 2020).  
11 This leads to a drop in blood pressure, and approximately 30% of hospitalized patients with  
12 COVID-19 require vasopressors to improve blood pressure (Goyal et al., 2020). Furthermore,  
13 68-78% have sustained cardiac dysfunction, primarily right ventricle dysfunction and left  
14 ventricular diastolic dysfunction (Puntmann et al., 2020; Szekely et al., 2020).

15 In severe disease, inflammation associated with a cytokine storm can cause cardiac dysfunction  
16 and pathology. COVID-19 induces a cytokine storm of similar magnitude to that induced by  
17 CAR-T cell-associated cytokine storms (Del Valle et al., 2020). Additionally, severe COVID-19 is  
18 associated with sepsis and bacterial products in the serum (Arunachalam et al., 2020), which  
19 are known drivers of cardiac pathology and dysfunction. In the absence of infection, well known  
20 inflammatory mediators such as TNF are associated with heart failure and have been  
21 demonstrated to induce systolic dysfunction (Feldman et al., 2000). Thus, inflammation is a  
22 likely mediator of cardiac injury and dysfunction in COVID-19 patients, which may result in  
23 further pathological consequences including inadequate organ perfusion and immune cell  
24 infiltration, further exacerbating disease. Thus, preventing cytokine-induced cardiac dysfunction  
25 may limit severe outcomes in COVID-19 patients. However, targeted treatment strategies,  
26 particularly in severe infections such as COVID-19, are currently lacking.

27 Several anti-inflammatory agents have shown clinical benefit for the acute management of  
28 COVID-19. Dexamethasone improved 28-day mortality in COVID-19 patients receiving invasive  
29 mechanical ventilation or oxygen at randomization (Horby et al., 2020). Additionally, Janus  
30 kinase (JAK)/signal transducer and activator of transcription (STAT) (ruxolitinib and baricitinib)  
31 and IL-6R inhibitors (tocilizumab and sarilumab) are currently in COVID-19 clinical trials.  
32 However, systemic immunosuppression may impede viral clearance thus potentially  
33 exacerbating disease (Mangalmurti and Hunter, 2020). To circumvent this, we aimed to identify  
34 cardiac-specific inflammatory targets that trigger cardiac dysfunction in response to the cytokine  
35 storm, reasoning that these might provide a safe and effective therapeutic option.

36 Here, we utilize multi-cellular human pluripotent stem cell (hPSC)-derived cardiac organoids  
37 (hCO) combined with phosphoproteomics and single nuclei RNA-sequencing (RNA-seq) to  
38 identify therapeutic targets and treatments for cardiac dysfunction. We recently adapted our  
39 hCO system (Mills et al., 2019; Mills et al., 2017) to include co-culture with endothelial cells that  
40 form enhanced branched endothelial structures surrounded by pericytes (**Figure S1**, Voges et  
41 al., In Preparation). This protocol results in a complex mixture of self-organising cells including  
42 epicardial, fibroblasts/pericytes, endothelial cells and cardiomyocytes. This was combined  
43 together with an optimized culture environment that reflects a maturation stage; mimicking the  
44 postnatal metabolic environment (Mills et al., 2019; Mills et al., 2017) followed by reversion to a  
45 more adult metabolic substrate provision (see **Methods**). This platform enabled rapid screening  
46 of cytokine combinations that recapitulate the COVID-19-induced cytokine storm (Mangalmurti

- 1 and Hunter, 2020) and cardiac dysfunction, with the subsequent application of -omic assays and
- 2 drug screening.

## 1 RESULTS

### 2 Cytokine-induced cardiac dysfunction

3 We began by examining the effects of a range of pro-inflammatory cytokines elevated in  
4 COVID-19 patients (Huang et al., 2020) on cardiac function in our hCO (Mills et al., 2017).  
5 Inflammatory molecules tested were likely candidates in COVID-19 including: TNF, IL-1 $\beta$ , IFN- $\gamma$ ,  
6 IL-6, IL-17A, and G-CSF, as well as pathogen-associated molecular patterns including poly(I:C)  
7 to mimic dsRNA, and lipopolysaccharide (LPS) to mimic TLR4 activation and septic responses.  
8 Using our RNA-seq data (Mills et al., 2017), we identified that the receptor genes *IL1R1*,  
9 *TNFRSF1A*, *TNFRSF1B*, *IFIH1*, *MYD88*, *IL6ST*, *IFNAR1*, *IL6R*, *TMEM173*, *IL17RA*, *IL17RB*,  
10 *IL17RC*, *IL17RD*, *IL17RE*, *IFNGR1*, *TLR3*, and *TLR4* were expressed at similar or higher  
11 abundance in our hCO compared to adult human heart (**Figure S2A**). In adult mouse hearts  
12 many of these are enriched in non-myocyte populations (Quaife-Ryan et al., 2017) (**Figure**  
13 **S2B**). We used single nuclei RNA sequencing (snRNA-seq) to assess cell specificity in our  
14 enhanced hCO (Voges et al., In Revision). Mapping to human heart snRNA-seq (Tucker et al.,  
15 2020) revealed the presence of pro-epicardial/epicardial cells, fibroblasts, activated  
16 fibroblasts/pericytes and cardiomyocytes (**Figure S2C,D**). Some cardiomyocytes were fetal-like,  
17 however there was a distinct sub-cluster that mapped adjacent to adult ventricular  
18 cardiomyocytes from human hearts (Gilsbach et al., 2018) (**Figure S2E**). The cytokine/pro-  
19 inflammatory receptors were expressed across different cell types, but were enriched and more  
20 highly expressed in epicardial cells and fibroblasts (**Figure S2F**) (Mills et al., 2017; Voges et al.,  
21 2017). We screened inflammatory factors in all pair-wise combinations in hCOs with multiple  
22 functional measurements including contractile force, rate, activation kinetics and relaxation  
23 kinetics (Mills et al., 2019; Mills et al., 2017) (**Figure 1A**). TNF caused a reduction in force, while  
24 IL-1 $\beta$ , IFN- $\gamma$ , poly(I:C) and LPS caused diastolic dysfunction characterized by a preserved  
25 contractile force but prolonged time from peak to 50% relaxation (**Figure S3**). A secondary full-  
26 factorial screen of TNF, IFN- $\gamma$ , IL-1 $\beta$ , and poly(I:C), once again revealed that TNF induced  
27 systolic dysfunction (**Figure 1B,D**) with a EC<sub>50</sub> of 1 ng/mL at 48 hours (**Figure S4A**). A  
28 combination of IL-1 $\beta$ , IFN- $\gamma$  and poly(I:C) induced diastolic dysfunction (**Figure 1C,E**), however  
29 also decreased the beating rate which may influence the kinetics of contraction (**Figure S5**,  
30 **Supplementary Video 1,2**). Changes in rate were not responsible for increased relaxation time,  
31 as hCO paced at 1 Hz retained the severe diastolic dysfunction phenotype (**Figure 1F**,  
32 **Supplementary Video 3,4**). Individually, IFN- $\gamma$  and IL-1 $\beta$  caused concentration-dependent  
33 diastolic dysfunction with an EC<sub>50</sub> of 0.8 ng/mL at 48 hours and 3 ng/mL at 24 hours,  
34 respectively, whereas poly(I:C) alone did not induce dysfunction (**Figure S4B to D**). These  
35 results were confirmed in an independent hPSC line, where the combination of IFN- $\gamma$ , IL-1 $\beta$  and  
36 poly(I:C) induced the most consistent, robust diastolic dysfunction (**Figure S6A to E**). Taken  
37 together, TNF induces systolic dysfunction consistent with previous *in vitro* (Vasudevan et al.,  
38 2013) and *in vivo* (Kubota et al., 1997) studies, and the combination of IFN- $\gamma$ , IL-1 $\beta$  and  
39 poly(I:C) induces severe diastolic dysfunction in hCO. The dominant factor identified that causes  
40 diastolic dysfunction, IFN- $\gamma$  (**Figure S6C**), is generally elevated in heart failure patients, but its  
41 role in heart failure is contradictory in animal models with both detrimental and beneficial effects  
42 reported (Levick and Goldspink, 2014).

### 43 Mechanisms driving cardiac cytokine storm-induced dysfunction

1 The most common types of cardiac dysfunction observed in hospitalized COVID-19 patients are  
2 right ventricular dysfunction or left ventricular diastolic dysfunction (Szekely et al., 2020).  
3 Therefore, we chose to interrogate the mechanism of diastolic dysfunction induced by IFN- $\gamma$ , IL-  
4 1 $\beta$  and poly(I:C), from here on referred to as ‘cardiac cytokine storm’ (CS). Protein  
5 phosphorylation is intimately linked with all biological functions (Needham et al., 2019), and thus  
6 reasoned that measuring the global phosphoproteome in hCO would provide an accurate  
7 fingerprint of the mechanistic targets driving dysfunction caused by CS. Leveraging the latest  
8 developments in our phosphoproteomics technology (Humphrey et al., 2015; Humphrey et al.,  
9 2018), we analysed CS-induced phosphorylation events in 20 pooled organoids, yielding ~100  
10  $\mu$ g of protein each. We identified over 7,000 phosphosites in each sample, accurately  
11 pinpointing 7,927 phosphorylation sites to a single amino acid residue on ~3,000 different  
12 phosphoproteins from single-run measurements (**Figure 2A, Figure S7**). Preliminary studies  
13 using TNF identified several known biological effects of this cytokine including decreased  
14 phosphorylation of protein kinase A and increased phosphorylation of beta-adrenergic receptor  
15 kinase 1 (also known as GRK2), supporting our approach (data not shown). Applying this  
16 technology to the CS treatment revealed 91 phosphosites that were consistently elevated  
17 across three biological replicates (**Figure 2B**). These sites were enriched for terms relating to  
18 proliferation, with transcription factors over-represented with 35 sites found on transcription  
19 factors or chromatin-binding proteins and 13 associated with the biological process term ‘cell  
20 proliferation’ (FDR < 0.05, Fisher’s exact test). Among these was phosphorylation of signal  
21 transducer and activator of transcription 1 (STAT1) S727 (median 13.9 fold), as well as two sites  
22 on BRD4 (Bromodomain-containing protein 4) S469 and S1083 (median 7.4 and 12.3 fold  
23 respectively) (**Figure 2B,C**). In light of the availability of specific small molecule inhibitors for  
24 each of these targets or their upstream regulators we focused on these proteins in subsequent  
25 functional assays. The cytokine receptor enrichment in non-myocytes (**Figure S2**) and broad  
26 expression of key mediators such as STAT1 and BRD4 (**Figure 3H** and **Figure 4F**) suggests a  
27 multi-cellular response mediates cardiac dysfunction.

28 We assessed activation of individual cell populations in hCOs using snRNA-seq of ~40 pooled  
29 hCOs per condition (**Figure 3A**) with mapping as described above (CTRL - **Figure S2C,D** and  
30 CS - **Figure S8A,B**). In the CS condition there was an increase in fibroblast and activated  
31 fibroblast number (**Figure 3B**). However, KEGG pathway analysis revealed a transcriptional  
32 response dominated by a viral response in both cardiomyocytes (CM1, CM2 and CM3 pooled)  
33 and fibroblasts (epicardial/fibroblast and pericyte/activated fibroblasts) (**Figure 3C,D**). There  
34 were fewer downregulated genes, predominantly in the fibroblasts and these were dominated by  
35 extracellular matrix (ECM) genes including *COL1A1*, *COL3A1* and *COL4A5* (**Figure 3E**). The  
36 top predicted mediators of the transcriptional response were STAT1 and general epigenetic  
37 activation by EP300 (**Figure 3F**). This is consistent with our phosphoproteomic data (**Figure 2**),  
38 given EP300 has been shown to share upto 78% of DNA binding regions with BRD4 in  
39 chromatin immunoprecipitation studies (Williams et al., 2020). These predicted target genes were  
40 also enriched for viral responses (**Figure 3F**). These analyses together revealed a robust viral  
41 response in the heart in multiple cell populations (**Figure 3G,H**), predicted to be mediated via  
42 STAT1 and epigenetic activation and BRD4.

### 43 **Drugs for the prevention and treatment of cardiac dysfunction**

44 We next screened drugs that could potentially treat cardiac dysfunction caused by either TNF-  
45 induced systolic dysfunction or CS-driven diastolic dysfunction (**Figure 4A**). TNF is known to  
46 induce systolic dysfunction via GRK2 mediated repression of  $\beta$ -adrenergic receptor signaling  
47 (Vasudevan et al., 2013). The selective serotonin reuptake inhibitor, paroxetine hydrochloride,  
48 can inhibit GRK2 (Schumacher et al., 2015), but we found that it was toxic at effective *in vitro*



1 concentrations (Guo et al., 2017) (**Figure S9A**). GRK2 promotes clathrin-mediated endocytosis  
2 (Evron et al., 2012), and baricitinib was recently identified as a potential AP2-associated protein  
3 kinase 1 (AAK1)-mediated endocytosis inhibitor using machine learning (Richardson et al.,  
4 2020). Baricitinib prevented TNF-induced dysfunction in hCO (**Figure 4B** and **Figure S9A,B**).  
5 However, baricitinib was only protective against TNF-induced systolic dysfunction when co-  
6 administered with TNF and was not effective after 24 h TNF treatment (**Figure 4C**), possibly  
7 due to a reduction in cell surface receptor abundance. Additionally, hCO did not recover quickly  
8 from TNF-induced systolic dysfunction after the removal of TNF (**Figure 4C**) indicating that  
9 secondary remodeling events may have occurred.

10 A key signature of diastolic dysfunction under CS conditions was the elevated phosphorylation  
11 of transcriptional regulators. STAT1-S727 (**Figure 2C**) is associated with assembly into  
12 chromatin and is required for STAT1 transcriptional and biological activity in response to IFN- $\gamma$   
13 (Sadzak et al., 2008). The putative STAT1-S727 kinase is CDK8 (Bancerek et al., 2013), so we  
14 next tested two CDK8 inhibitors SEL120-34A (Rzymyski et al., 2017) and BI-1347 (Hofmann et  
15 al., 2020) previously shown to reduce STAT1-S727 phosphorylation. We also tested two  
16 inhibitors of the JAK/STAT pathway, baricitinib and ruxolitinib. However, none of these  
17 compounds, nor a broader spectrum CDK inhibitor flavopiridol, prevented the CS-induced  
18 diastolic dysfunction (**Figure S10**), noting that flavopiridol was toxic and reduced force and  
19 hence all kinetic parameters. Notably, SEL120-34A and BI-1347 specifically attenuated the rate  
20 and activation time defects under CS conditions (**Figure S10B,C,E,F**), which we validated in  
21 additional experiments (**Figure S11A-D**) and may still have clinical utility in this setting.

22 We observed elevated phosphorylation of the epigenetic regulator BRD4 and other epigenetic  
23 regulators in our CS treated hCO phosphoproteome, consistent with our snRNA-seq analysis.  
24 We have previously shown that bromodomain extraterminal inhibitors (BETi) reduce relaxation  
25 time in immature hCO (Mills et al., 2019), so we next evaluated three BETi available in an FDA  
26 compound library INCB054329 (Stubbs et al., 2019), JQ-1 (Filippakopoulos et al., 2010), and  
27 ABBV-744 (Faivre et al., 2020). Strikingly, INCB054329 prevented CS-induced diastolic  
28 dysfunction in a dose-dependent manner (**Figure 4D**, **Figure S12**) without affecting force or rate  
29 (**Figure S10A-G**, **Supplementary Video 5**). JQ-1 also showed improved diastolic function in  
30 one hPSC line at the highest concentration (**Figure S10H**), so an additional higher  
31 concentration for both JQ-1 and ABBV-744 were tested. JQ-1 protected hCO against CS-  
32 induced diastolic dysfunction, although INCB054329 was the most efficacious (**Figure S13A,B**).  
33 In contrast, ABBV-744 increased diastolic dysfunction in the hCO, potentially via its dual actions  
34 as an androgen receptor inhibitor, which is associated with prolonged QTc in patients  
35 undergoing androgen deprivation therapy (Gagliano-Jucá et al., 2018). To validate BRD4 as a  
36 target we used adeno associated virus 6 (AAV6) mediated delivery of shRNA and demonstrated  
37 that ~74% knockdown could also reduce diastolic dysfunction in CS treated hCO (**Figure S14**).

38 INCB054329-mediated BETi rescued dysfunctional hCO, and restored diastolic function  
39 following 24 h of CS conditions (**Figure 4E**). This is potentially because CS-induced diastolic  
40 dysfunction is reversible and is driven by the presence of the inflammatory mediators,  
41 demonstrated by partial hCO recovery 24 h after removing CS factors (**Figure 4E**). In patients,  
42 all inflammatory factors may be present simultaneously, and we found that INCB054329  
43 attenuated diastolic dysfunction with all four dysfunction inducing factors, TNF, IFN- $\gamma$ , IL-1 $\beta$ , and  
44 poly(I:C), present (**Figure S13C**). Taken together CS mediates diastolic dysfunction via BRD4-  
45 dependent mechanisms that can be blocked using BETi. As BRD4 is broadly expressed in our  
46 hCO (**Figure 4F**), it may also be responsible for the multi-cellular response observed.

## 1 **INCB054329 reduces the host response to SARS-CoV-2 infection in K18-hACE2 mouse** 2 **hearts**

3 We next assessed the response to SARS-CoV-2 infection *in vivo*. As mice are not susceptible to  
4 SARS-CoV-2 infection, we used a recently described K18-hACE2 model (Oladunni et al., 2020)  
5 to study the response and effects of BETi (**Figure 5A**). SARS-CoV-2 infected mice had severe  
6 lung pathology and substantial viral RNA reads in the lungs at 4-5 d.p.i. confirming successful  
7 lung infection (**Figure 5B,C**). RNA-seq of the lungs revealed increased expression of 419 genes  
8 (**Figure 5D**), strongly associated with a viral response (**Figure 5E, Table S1**). Concordant with  
9 our hCO data, Stat1 and Ep300 were top predicted transcriptional regulators (**Figure 5F, Table**  
10 **S2**). Interestingly, there was only negligible infection of the heart (**Figure 5C**) and no obvious  
11 pathology including necrosis, immune cell infiltration or fibrosis (**Figure 5G**). However, there  
12 was a substantial and robust upregulation of a viral response in the heart with ECM repression  
13 (**Figure 5H,I, Table S1**) including *Col1a1*, *Col3a1* and *Col4a2*. This was again enriched for  
14 Stat1 and Ep300 as top predicted transcriptional regulators (**Figure 5J, Table S2**), indicating a  
15 robust systemic response in the hearts of SARS-CoV-2 infected mice. This response could be  
16 partially blocked by INCB054329 treatment with repression of 91 genes that were enriched for  
17 the viral response (**Figure 5K-M, Table S1**). This response was more specific to the heart, as  
18 INCB054329 did not regulate any genes in the lungs (data not shown). The repression by  
19 INCB054329 was predicted to be primarily mediated via Ep300 rather than Stat1 (**Figure 5N,**  
20 **Table S2**). These results were further supported by Ingenuity Pathway Analysis of upstream  
21 regulators revealing strong activation signatures for IFNG, poly rI:rC-RNA and Stat1 in both  
22 lungs and hearts of K18-hACE2 SARS-CoV-2 infected mice, which INCB054329 strongly  
23 inhibited (**Table S3**). In addition to the viral response, one of the INCB054329 regulated genes  
24 was *Tnni3k* (logFC = -0.34, FDR = 0.0044) which has been shown to be a therapeutic target for  
25 cardiac dysfunction (Vagnozzi et al., 2013).

26 The CS induced response in the hCO (either fibroblasts or cardiomyocytes) and hearts of  
27 SARS-CoV-2 infected K18-hACE2 mice shared 32 regulated genes (**Figure 5O**) which were  
28 enriched for the viral response (**Figure 5P**). This indicated that CS mimics the paracrine  
29 response in the heart caused by SARS-CoV-2 infection and was further supported by Ingenuity  
30 Pathway Analysis, which predicted that the inflammatory networks were activated by IFN- $\gamma$   
31 (**Figure S15**).

32 Potentially important mediators and markers of the response were found by integrating the  
33 multiple datasets. The consistent transcriptional program in CS treated hCO (both fibroblasts  
34 and cardiomyocytes) and SARS-CoV2 infected K18-hACE2 mice, that were also down-  
35 regulated genes by INCB054329 treatment *in vivo* revealed 5 key targets. These comprise the  
36 key inflammatory genes *Nmi*, *Tap1*, *B2m*, *Stat1* and *Lgals3bp* (**Figure 5Q**). Of particular interest  
37 is LGALS3BP (galectin-3 binding protein) as it has been shown to be a top-predictor of COVID-  
38 19 severity in humans (Messner et al., 2020) and therefore interrogated its expression in our  
39 subsequent models.

## 40 **INCB054329 protects against inflammatory mediated dysfunction *in vivo* and by COVID-** 41 **19 serum**

42 The study into the efficacy of SARS-CoV2-related cytokine storm therapeutics on the heart is  
43 challenging, not least due to the inaccessibility of mouse echocardiography in biosafety level 3  
44 facilities, but also due to the rapid decrease in weight associated with severe lung/brain infection  
45 (**Figure 5R**) that requires euthanasia. Therefore, we tested the ability of INCB054329 to prevent  
46 LPS-induced cytokine storm effects *in vivo* (**Figure 6A**). LPS induced pro-inflammatory



1 cytokines TNF, IL-1 $\beta$  and IFN- $\gamma$ , which were elevated in the plasma (**Figure 6B**) along with  
2 *Lgals3bp* in the heart (**Figure 6C**). Treatment with INCB054329 blocked the LPS-induced pro-  
3 inflammatory cytokine production (**Figure 6B**) and *Lgals3bp* induction in the heart (**Figure 6C**).  
4 We observed a marked improvement in mortality, whereby all INCB054329-treated mice  
5 survived after 24 h of the LPS-challenge, compared with only 25% in the control group (**Figure**  
6 **6D**). To determine whether BETi could treat an established LPS-induced cytokine storm, we  
7 delayed injection of INCB054329 1.5 h after LPS injection and assessed cardiac function at 6 h  
8 (**Figure 6E**). INCB054329 fully prevented the decrease in cardiac function observed after LPS  
9 injection (**Figure 6F**). Together these findings demonstrate that BETi using INCB054329 has  
10 robust effects in preventing inflammatory-induced cardiac dysfunction *in vivo*.

11 We next assessed whether human serum from COVID-19 patients could induce dysfunction in  
12 hCO (**Figure 6G, Supplementary Spreadsheet 1,2**). We found that IFN- $\gamma$ , one of the key  
13 mediators of the cytokine response, was higher in patients with elevated BNP (> 0.3 ng/ml) but  
14 not CTNI (> 0.5 ng/ml) (**Figure 6H,I**). In accordance, IFN- $\gamma$  has been shown to correlate with  
15 COVID-19 disease progression and is elevated in patient serum in one of the most  
16 comprehensive profiling studies to date (Ren et al., 2021). We were able to demonstrate that  
17 hCO function was dictated by factors present in the human serum, as patients receiving  
18 noradrenaline as inotropic support elevated contractile force in our hCO (**Figure 6J**). Human  
19 COVID-19 patient serum with elevated BNP caused diastolic dysfunction in hCO (**Figure 6J**).  
20 INCB054329 prevented diastolic dysfunction caused by patient serum (**Figure 6K**). Induction of  
21 LGALS3BP was also prevented by treatment with multiple BETi (**Figure 6L**).

22 Collectively, these data indicate that BET inhibition with INCB054329 prevented cardiac  
23 dysfunction in multiple inflammatory models, as well as repressing the key COVID-19 severity  
24 marker LGALS3BP.

### 25 **INCB054329 decreases hACE2 expression and reduces SARS-CoV-2 in hPSC-cardiac** 26 **cells**

27 In our SARS-CoV-2 K-18 mouse infection studies INCB054329 treatment reduced endogenous  
28 *Ace2* in hearts *in vivo* (**Figure S17A**). Recently other investigators have also demonstrated that  
29 BETi reduced ACE2 *in vivo* and SARS-CoV-2 infection (Qiao et al., 2021). As SARS-CoV-2  
30 potentially infects human hearts and has been shown to infect human pluripotent stem cell-  
31 derived cardiac cells (hPSC-CM) (Sharma et al., 2020), we sought to determine whether BETi  
32 blocked infection (**Figure S16A**). We confirmed previous findings using 2D cultured hPSC-  
33 cardiac cell infection studies, where increasing the MOI increased the degree of cell death  
34 (**Figure S16B**). Infection with a low MOI (0.01) was sufficient for viral replication and cell death  
35 over 7 days (**Figure S16C**). A 3 day pre-incubation of INCB054329 was sufficient to reduce  
36 ACE2 expression ~4 fold (**Figure S16D**). Consequently, pre-treatment with INCB054329  
37 reduced SARS-CoV-2 N protein expression (**Figure S16E**) and intracellular viral RNA (**Figure**  
38 **S16F**). In addition to INCB054329, the widely used BETi JQ-1 reduced SARS-CoV-2 RNA  
39 (**Figure S16G**). Thus, BETi also has potential to block SARS-CoV-2 infection of cardiac cells in  
40 addition to preventing dysfunction.

### 41 **BETi for translation to the clinic**

42 We assessed the ability of all commercially available BETi compounds to prevent CS-induced  
43 diastolic dysfunction in hCO. We found that all compounds prevented dysfunction except for  
44 ABBV-744 (**Figure 7A**). As BETi with dual BD1 and BD2 activities display side-effects (Gilan et  
45 al., 2020), it is critical that we determine selectivity of the response. BD2-selective drugs such

1 as ABBV-744 and apabetalone have limited side effects. Apabetalone has been used for up to 3  
2 years in >1,700 humans at risk of cardiac disease with efficacy in preventing heart failure and  
3 favourable safety profile (Nicholls et al., 2021; Ray et al., 2020). ABBV-744 elevated diastolic  
4 dysfunction (**Figure 7A**), and we suspect its lack of efficacy was due to its on-target inhibition of  
5 the androgen receptor (AR). BD2-specific efficacy was confirmed using a novel BD2-specific  
6 molecule, RXV-2157 (**Figure 7B**) and we also confirmed efficacy with the BD-2 selective  
7 apabetalone (**Figure 7A,B**). Additionally, analysis of plasma from the ASSURE phase IIb clinical  
8 trial indicated that BD2-selective apabetalone reduced LGALS3BP in patients with  
9 cardiovascular disease (**Figure 7C**), which is a marker of COVID-19 severity (Messner et al.,  
10 2020).

11 We confirmed the BD2-specificity of blocking SARS-CoV-2 infection. The BD2-specific RXV-  
12 2157 and BD2-selective apabetalone molecules downregulated hACE2 (**Figure 7D**), which led  
13 to decreased surface expression (**Figure S16B**) and SARS-CoV-2 spike protein binding (**Figure**  
14 **S16C**). These compounds also reduced SARS-CoV-2 RNA (**Figure S17D, Figure 7E,F**) and  
15 viral titre, including a 2.6 fold decrease in viral titre with apabetalone (**Figure 7G**).

16 Together, this demonstrates that BD2 -selective BETi drugs are lead candidates for rapid  
17 clinical translation to prevent COVID-19 injury in the heart.

## 1 DISCUSSION

2 Cardiac dysfunction could be caused by systemic inflammation (Del Valle et al., 2020) or local  
3 cytokine production (Lindner et al., 2020). In this study we show that inflammatory mediators  
4 directly impacted cardiac function in hCO, a model free from the secondary effects and  
5 neurohormonal compensation present *in vivo*. We found that a combination of classical viral  
6 response cytokines IFN- $\gamma$  and IL-1 $\beta$ , combined with dsRNA mimics cytokine storm, resulting in  
7 severe diastolic dysfunction manifested by 20-50% increases in relaxation time without decline  
8 in systolic function. This is consistent with clinical data from heart failure with preserved ejection  
9 fraction (HFpEF) patients, where cardiomyocytes have increased time to 50% relaxation by  
10 ~13-18%, with similar overall values of 100-150 ms in both humans and hCO (Runte et al.,  
11 2017). It is also consistent with diastolic dysfunction reported in COVID-19 patients (Szekely et  
12 al., 2020). Therefore, our hCO model recapitulates key clinical features of diastolic dysfunction.

13 Our data highlight fibroblasts as key mediators in the inflammation-induced cardiac dysfunction.  
14 The receptors for inflammatory cytokines are more abundant in cardiac fibroblasts than other  
15 cardiac cell types both in hCO and *in vivo* (**Figure S2**), and CS elicited a more pronounced  
16 transcriptional response in the fibroblasts within hCO (**Figure 3C,D**). The interplay and intra-  
17 organ paracrine signalling between cardiac cell types is likely involved in this inflammation-  
18 induced cardiac dysfunction.

19 By performing high-sensitivity phosphoproteomics, RNA-seq and drug screening with our  
20 optimized hCO we identified a therapeutically tractable inflammation-BRD4 axis in inflammation-  
21 induced cardiac dysfunction. Our transcriptional profiling revealed a striking similarity between  
22 the inflammatory response in CS treated hCO and the *in vivo* response of SARS-CoV-2 infected  
23 K18-hACE2 mice, despite negligible cardiac infection in the heart. This is important, as systemic  
24 inflammatory signalling may be an important aspect of multi-organ dysfunction documented in  
25 COVID-19 patients and other inflammatory diseases.

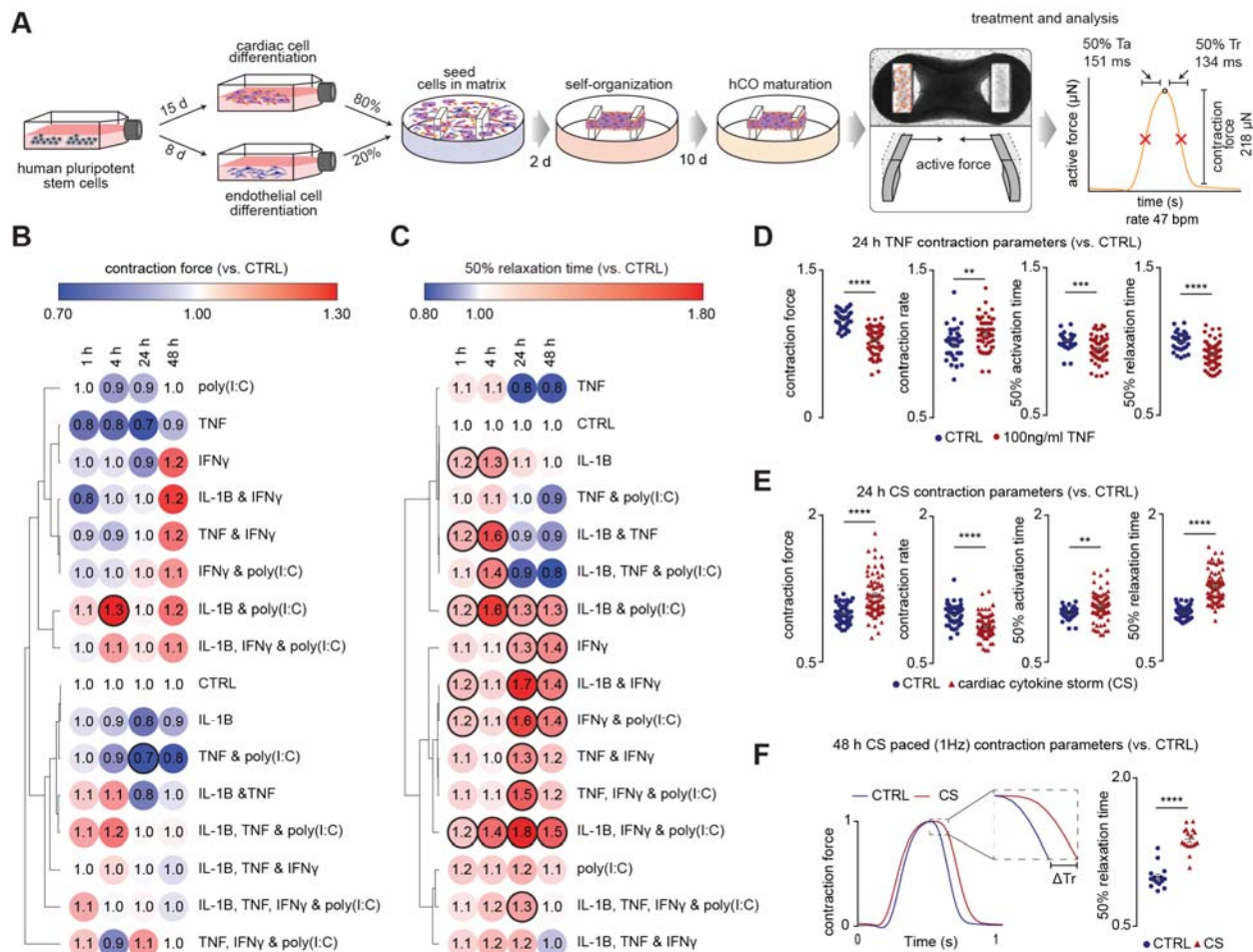
26 Our data establish BET inhibition as a viable therapeutic strategy to attenuate cytokine storm-  
27 induced cardiac dysfunction. Previously, BETi compounds have shown efficacy in small animal  
28 cytokine storm models (Nicodeme et al., 2010). There is also compelling evidence implicating  
29 bromodomains as key mediators in pathological pro-fibrotic signaling in heart failure (Duan et  
30 al., 2017; Stratton et al., 2019), in experimental models of pressure overload and myocardial  
31 infarction-induced HF (Anand et al., 2013) and in genetic cardiomyopathies (Antolic et al., 2020;  
32 Auguste et al., 2020). However, this study is instrumental in establishing BET inhibition as a  
33 therapeutic intervention to prevent cardiac dysfunction caused by inflammation.

34 Clinical data from COVID-19 patients also point to additional cardiac pathologies. Microthrombi  
35 were reported in the hearts of 14 out of 40 patients (35%) that died from COVID-19, which was  
36 associated with areas of myocardial necrosis (Pellegrini et al., 2021). Consistent with these  
37 observations, we observed that “complement and coagulation cascades” were enriched in the  
38 lungs of K18-hACE2 mice with SARS-CoV-2 infection (**Figure 5E** and **Table S1**), and were  
39 likely related to the viral-induced inflammatory response. Intriguingly, we identified *serpine1*  
40 (also known as plasminogen activator inhibitor 1), was upregulated both in the lungs (3.9 fold)  
41 and in the heart (2.8 fold, both FDR < 0.05) of SARS-CoV-2 infected K18-hACE2 mice, which  
42 was abrogated in the heart upon treatment with the BETi INCB054329 (FDR < 0.05). *Serpine 1*  
43 is a key inhibitor of tissue-type plasminogen activator and urokinase-type plasminogen activator,  
44 and is required for fibrinolysis down-regulation and degradation of blood clots. In addition,  
45 arrhythmic events have been widely reported in COVID-19 patients (Nishiga et al., 2020).  
46 Indeed, we observed that arrhythmic events increased in hCO with CS, for which INCB054329  
47 also conferred protection (**Figure S18A-C**). These data suggest that BET inhibition may be

1 effective in attenuating multiple deleterious aspects of systemic inflammation on the heart that  
2 warrant further investigation.

3 We demonstrated that BETi are attractive therapeutic candidates, however the side effect  
4 profiles of some BETi may preclude their use in the clinic. Genetic ablation studies have shown  
5 that BRD4 plays an integral homeostatic role in cardiomyocytes, suggesting that the loss of BET  
6 proteins may have detrimental effects on mitochondrial energy production (Kim et al., 2020;  
7 Padmanabhan et al., 2020). Emerging evidence dissecting the roles of BD1 and BD2  
8 bromodomains in inflammatory disease models has indicated that BD2-selective inhibition  
9 preferentially blocks the induction of gene expression while minimally affecting established  
10 transcription programs (Gilan et al., 2020). More recently, BD2-selective drugs such as ABBV-  
11 744 and apabetalone have been developed to overcome these side-effect profiles. Whilst  
12 ABBV-744 was not effective in our hCO model (potentially due to its targeting of AR), we  
13 demonstrate that BD-2 selective compounds, RXV-2157 and apabetalone demonstrate efficacy.  
14 This underscores the need for careful BETi selection, despite broad ability to modulate critical  
15 target genes (**Figure 6L**) and utility for a variety of clinical conditions (Cochran et al., 2019).  
16 Importantly, BD2-selective BETi apabetalone reduced CS-induced diastolic dysfunction and  
17 downregulated ACE2 and reduced viral infection (**Figure S16 and Figure 7**). Taken together,  
18 the efficacy and known safety profile of apabetalone make it a prime candidate to protect  
19 against cardiac injury for inflammatory diseases such as COVID-19.

20 The overlap in risk factors for HFpEF and COVID-19 mortality suggests that our findings may  
21 also have broader implications. HFpEF risk factors including diabetes and obesity are also  
22 associated with chronic inflammation. Recent studies have shown that elevated inflammatory  
23 markers are associated with worsening heart function in HFpEF (Sanders-van Wijk et al., 2020),  
24 thus indicating that inflammation may drive dysfunction across multiple cardiac diseases and  
25 that BET inhibitors are putative therapeutic candidates.

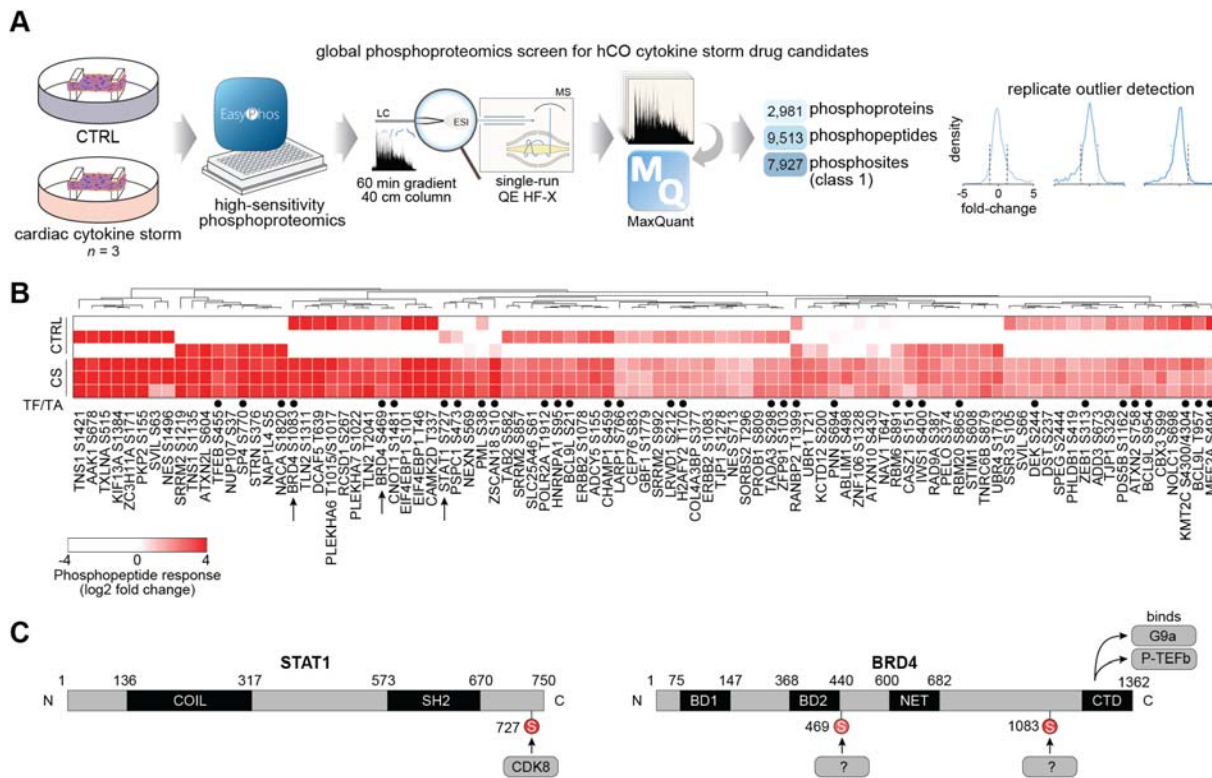


**Figure 1: Identification of pro-inflammatory factors driving cardiac dysfunction.**

- A) Schematic of experimental pipeline. Values for overall functional parameters are the mean of n = 1,100 hCO from 9 experiments.
- B) Impact of inflammatory modulators on force (systolic function). Bold outline indicates p < 0.05 using a one-way ANOVA with Dunnett's multiple comparisons test comparing each condition to CTRL at its' time point. n = 3-5 hCOs per condition from 1 experiment. hPSC cardiac cells- AA, Endothelial cells- RM3.5.
- C) Impact of inflammatory modulators on time to 50% relaxation (diastolic function). Bold outline indicates p < 0.05 using a one-way ANOVA with Dunnett's multiple comparisons test comparing each condition to CTRL at the respective time points. n = 3-5 hCOs per condition from 1 experiment. hPSC cardiac cells- AA, Endothelial cells- RM3.5.
- D) TNF causes systolic dysfunction. n = 37 and 63 hCOs for CTRL and TNF conditions, respectively from 6 experiments. hPSC cardiac cells- HES3, Endothelial cells- RM3.5 or CC. \*\* p < 0.01, \*\*\* p < 0.001, \*\*\*\* p < 0.0001, using Student's t-test.
- E) CS causes diastolic dysfunction n = 49 and 73 hCOs for CTRL and CS conditions, respectively from 6 experiments. hPSC cardiac cells- HES3, Endothelial cells- RM3.5 or CC. \*\* p < 0.01, \*\*\*\* p < 0.0001, using Student's t-test.
- F) Representative force curve of hCO under CTRL and CS conditions (1 Hz) 48 h after treatment. Relaxation of CTRL and CS under paced conditions (1 Hz) 48 h after treatment. n = 15 and 17 hCOs per condition, respectively, from 3 experiments. hPSC



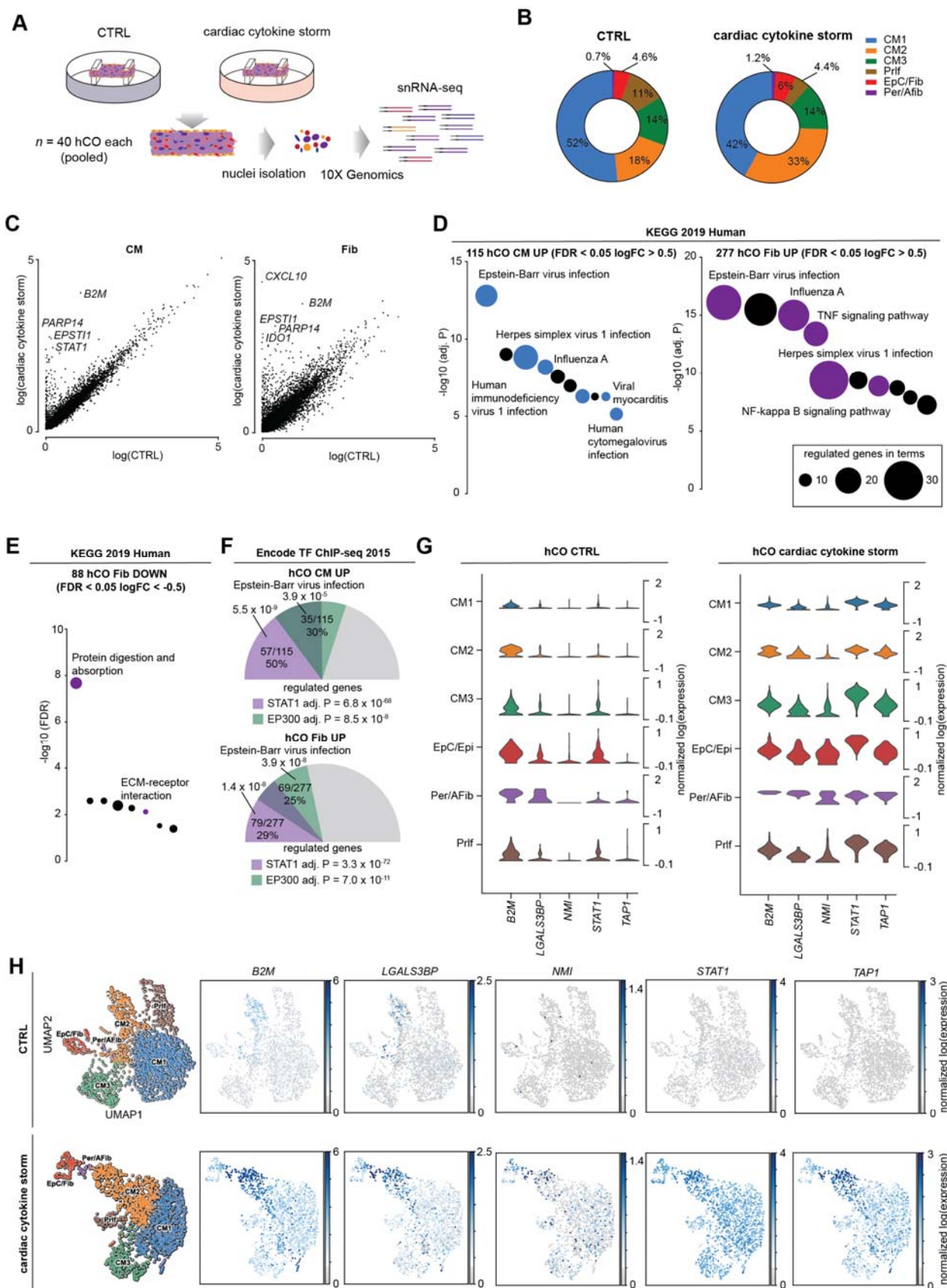
- 1 cardiac cells- HES3, Endothelial cells- RM3.5 or CC. \*\*\*\*  $p < 0.0001$ , using Student's t-
- 2 test.
- 3 CTRL – control. CS – cardiac cytokine storm conditions: IL-1 $\beta$ , IFN- $\gamma$  and poly(I:C).
- 4 Inflammatory screen in B-E repeated in an additional cell line in **Figure S6**. Ta – time from
- 5 50% activation to peak, Tr – time from peak to 50% relaxation.



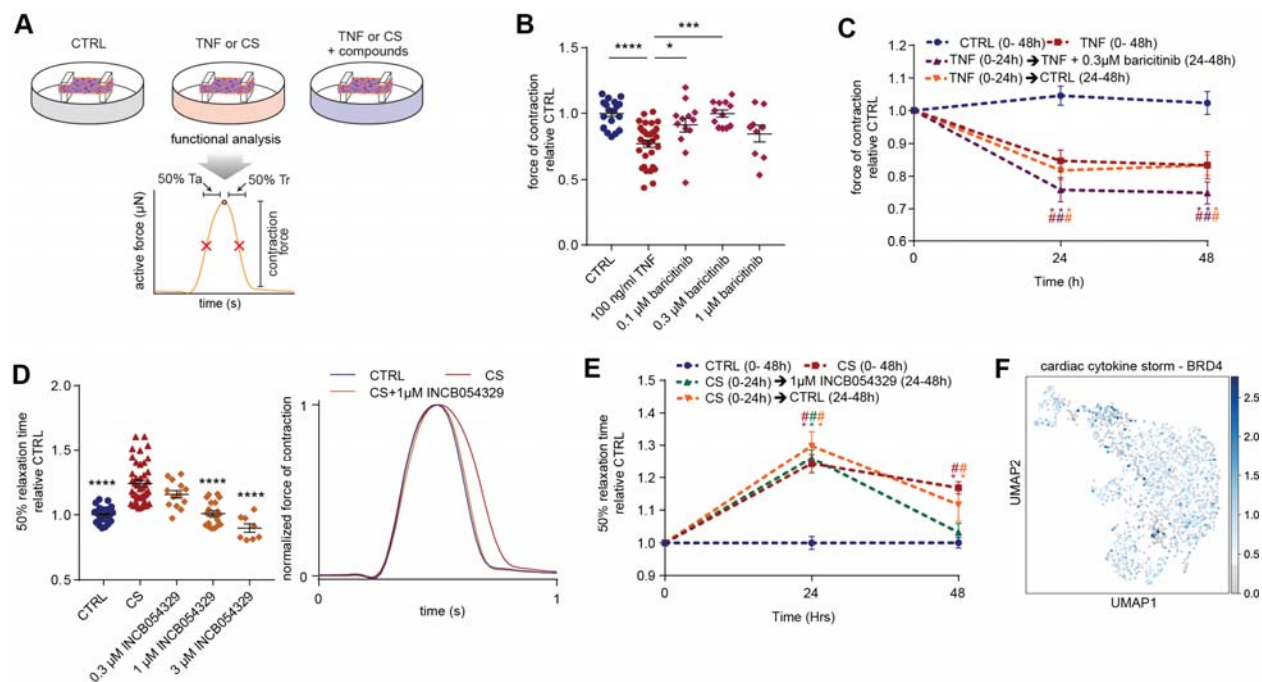
**Figure 2: Phosphoproteomics reveals signaling driving cardiac dysfunction.**

- 1 A) Schematic of the experiment and analysis.
- 2 B) Heat-map of enriched phosphopeptides in hCO following CS treatment (after 1 h). TF/TA
- 3 circles depict transcription factors and transcriptional activators.
- 4 C) Phosphorylation sites induced by CS on STAT1 and BRD4.

hPSC cardiac cells- AA, Endothelial cells- RM3.5.



- 1 **Figure 3: snRNA-seq reveals CS activates viral responses in hCO**  
2 A) Schematic of experiment.  
3 B) Cell compositions identified in snRNA-seq.  
4 C) Differential normalized log<sub>2</sub> expression in cardiomyocytes and fibroblasts following CS  
5 treatment (all populations pooled for each cell type).  
6 D) Activation of viral responses in cardiomyocytes and fibroblasts revealed using KEGG  
7 pathway analysis of upregulated genes. Size represents number of genes regulated and  
8 the pathways of the coloured circles are highlighted by the text.  
9 E) Repression of ECM processes in fibroblasts revealed using KEGG pathway analysis of  
10 down-regulated genes. Size represents number of genes regulated and the pathways of  
11 the coloured circles are highlighted by the text.  
12 F) Analysis of the transcriptional response predicts STAT1 and EP300 as key mediators.  
13 Values presented are adjusted P values, number of genes regulated by the transcription  
14 factor/number of genes regulated, and % of genes regulated over the total. The size of  
15 the coloured slices represent the fraction of genes regulated (180° = 100%), and  
16 overlaps for each transcription factor are also depicted.  
17 G) Violin plots of key (see **Figure 5Q**) upregulated genes in CS treated hCO.  
18 H) UMAP of CTRL and CS treated hCO subpopulations and expression of key regulated  
19 genes.
- 20 hPSC cardiac cells- HES3, Endothelial cells- RM3.5. CM – cardiomyocyte, Prlf – proliferating,  
21 EpC – epicardial cells, Fib – fibroblasts, Per – pericytes, Afib – activated fibroblasts.
- 22



1

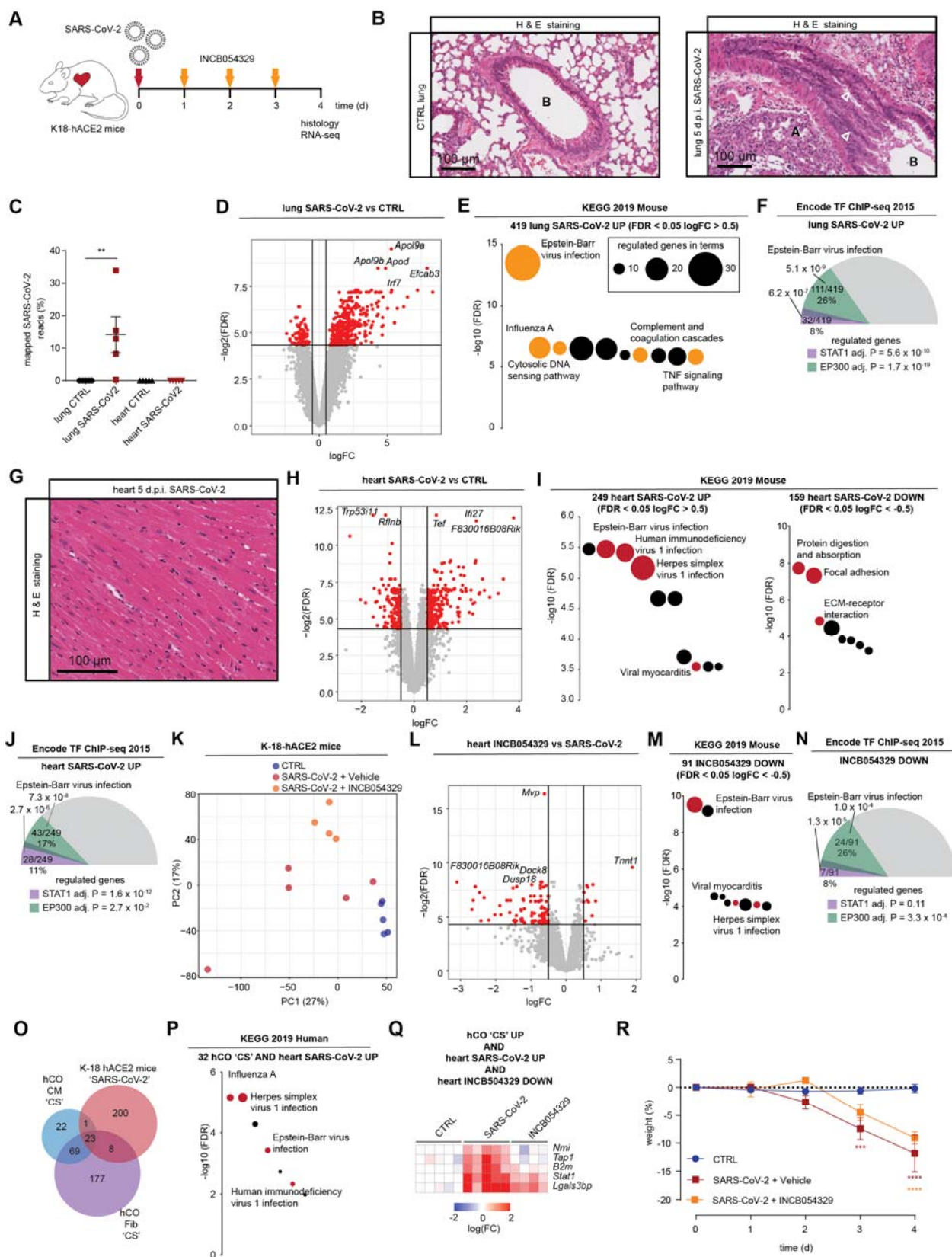
2 **Figure 4: Discovery of drugs that improve cardiac function**

- 3 A) Schematic of experiment.
- 4 B) Protection against systolic dysfunction (force of contraction) by baricitinib. n = 9-32 hCOs
- 5 per condition from 2-3 experiments. \*p<0.05, \*\*\* p<0.001, \*\*\*\* p<0.0001 using one-way
- 6 ANOVA with Dunnett's multiple comparisons test.
- 7 C) Assessment of hCO recovery from TNF and baricitinib treatment. n = 6-12 hCOs per
- 8 condition from 1-2 experiments. # p<0.05 compared to CTRL at the same time-point,
- 9 and \* p<0.05 compared to specific condition at 0 h with colour indicating comparison,
- 10 using two-way ANOVA with Dunnett's multiple comparisons test.
- 11 D) Protection against diastolic dysfunction (time to 50% relaxation time) by INCB054329. n
- 12 = 8-43 hCOs per condition from 2-4 experiments. \*\*\*\* p<0.0001, using one-way ANOVA
- 13 with Dunnett's multiple comparisons test compared to CS.
- 14 E) Assessment of hCO recovery from CS and INCB054329 treatment. n = 6-11 hCOs per
- 15 condition from 1-2 experiments. # p<0.05 compared to CTRL at the same time-point,
- 16 and \* p<0.05 compared to specific condition at 0 h with colour indicating comparison,
- 17 using two-way ANOVA with Dunnett's multiple comparisons test.
- 18 F) BRD4 is expressed in all cell-populations in hCO.

19 hPSC cardiac cells- HES3, Endothelial cells- RM3.5. Additional drug screen data including a

20 repeat in an additional cell line in **Supplementary Figures S9, S10, S12 and S13.**





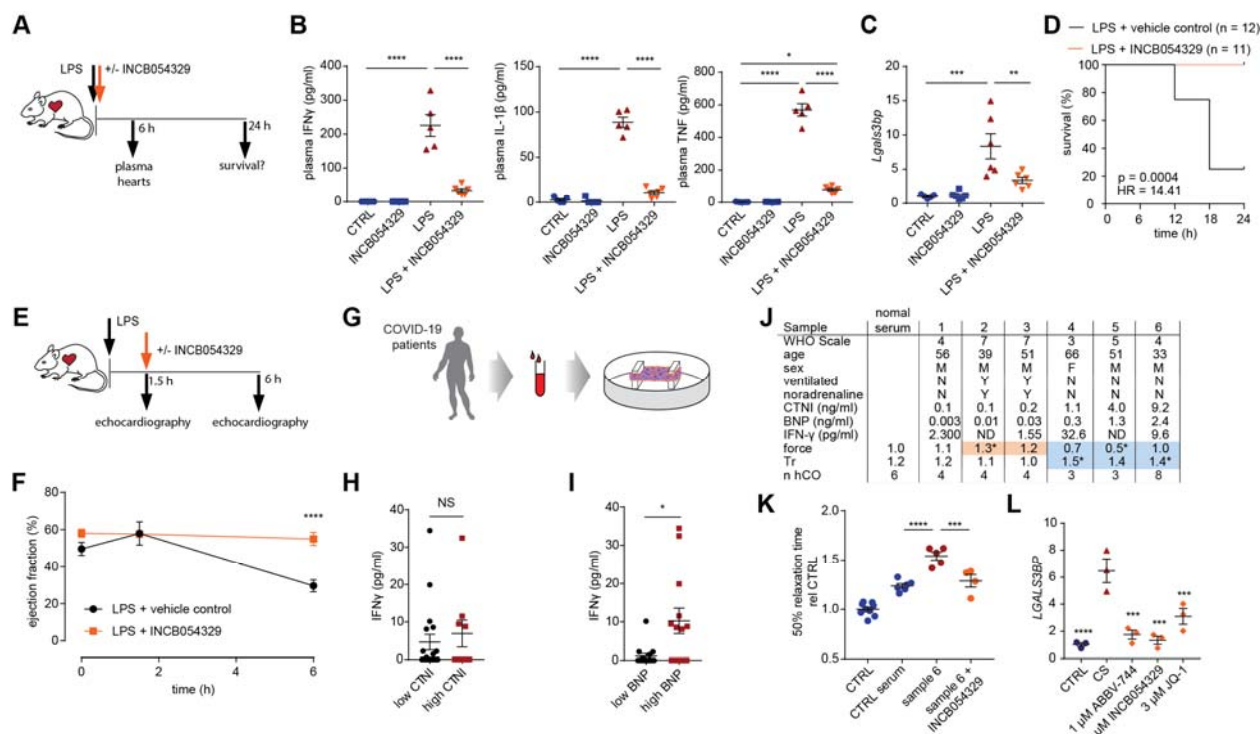
1 **Figure 5: SARS-CoV-2 activates viral responses in the heart despite negligible infection**  
2 **that is repressed by INCB054329.**

- 3 A) Schematic of the experiment.  
4 B) Lungs of SARS-CoV-2 infected K18-hACE2 mice 5 d.p.i. In comparison to the control  
5 there is sloughing of bronchial epithelium, and white arrowheads show (A) collapse of  
6 alveolar spaces and (B) bronchiolar lumen.  
7 C) Qualification of mapped reads in RNA-seq reveal severe lung infection with no/negligible  
8 heart infection. n = 5 mice per group.  
9 D) Volcano plot of lung RNA-seq reveals a robust upregulation ( $\log_{2}FC > 0.5$ ) of 419 genes  
10 and downregulation ( $\log_{2}FC < -0.5$ ) of 98 genes, both FDR < 0.05. n = 5 mice per group.  
11 E) Activation of viral responses in lungs revealed using KEGG pathway analysis of  
12 upregulated genes. Size represents number of genes regulated and the pathways of the  
13 coloured circles are highlighted by the text.  
14 F) Analysis of the transcriptional response predicts Stat1 and Ep300 as key mediators of  
15 infection in the lungs.  
16 G) Hearts of SARS-CoV-2 infected K18-hACE2 mice 5 d.p.i. The hearts appeared relatively  
17 normal without significant necrosis, fibrosis (Masson's Tri-chrome not shown) or immune  
18 infiltrates.  
19 H) Volcano plot of heart RNA-seq reveals a robust upregulation ( $\log_{2}FC > 0.5$ ) of 249 and  
20 downregulation ( $\log_{2}FC < -0.5$ ) of 159 genes, both FDR < 0.05. n = 5 mice per group.  
21 I) Activation of viral responses in hearts revealed using KEGG pathway analysis of  
22 upregulated genes. Repression of ECM in hearts revealed using KEGG pathway  
23 analysis of downregulated genes. Size represents number of genes regulated and the  
24 pathways of the coloured circles are highlighted by the text.  
25 J) Analysis of the transcriptional response predicts Stat1 and Ep300 as key mediators of  
26 the response in the heart.  
27 K) PCA plot of heart RNA-seq samples. n = 4-5.  
28 L) Volcano plot of heart RNA-seq reveals a robust upregulation ( $\log_{2}FC > 0.5$ ) of 11 genes  
29 and downregulation ( $\log_{2}FC < -0.5$ ) of 91 genes, both FDR < 0.05 in SARS-CoV-2  
30 infected K18-hACE2 mice treated with INCB054329. n = 4-5 mice per group.  
31 M) Repression of viral responses in hearts revealed using KEGG pathway analysis of down-  
32 regulated genes. Size represents number of genes regulated and the pathways of the  
33 coloured circles are highlighted by the text.  
34 N) Analysis of the transcriptional response predicts Ep300 as the key mediator of  
35 INCB054329 effects in the heart.  
36 O) Cross-analysis of the transcriptional responses in hCO with hearts of SARS-CoV-2  
37 infected K18-hACE2 mice.  
38 P) Co-regulated genes in panel (O) reveals a consistent activation of viral responses in  
39 both models using KEGG pathway analysis of upregulated genes. Size represents  
40 number of genes regulated and the pathways of the coloured circles are highlighted by  
41 the text.  
42 Q) Heat-map of genes induced by both CS in hCO and SARS-CoV-2 infected K18-hACE2  
43 mouse hearts that are also repressed with INCB054329 treatment.  
44 R) Severe weight loss by 4-5 d.p.i in SARS-CoV-2 infected K-18-hACE2 mice is due to  
45 severe lung infection and also brain infection and euthanasia is required.

46 d.p.i. – days post infection. Data presented as mean  $\pm$  standard error of the mean. \*\* p < 0.01,  
47 using Mann-Whitney \*\*\* p < 0.001 and \*\*\*\* p < 0.0001 using two-way ANOVA with Sidak's post  
48 hoc test compared to CTRL. For D, H, L – red dots are regulated as per the described cut-offs  
49 and grey dots are not. For F,J and N - Values presented are adjusted P values, number of

- 1 genes regulated by the transcription factor/number of genes regulated, and % of genes
- 2 regulated over the total. The size of the coloured slices represent the fraction of genes regulated
- 3 ( $180^\circ = 100\%$ ), and overlaps for each transcription factor are also depicted.
- 4

1



**Figure 6: INCB054329 prevents cardiac dysfunction in a mouse LPS-induced cytokine storm model and in response to COVID-19 patient serum.**

- A) Schematic for survival and expression experiments.
- B) INCB054329 blocks cytokine induction, measured 6 h after LPS using cytokine bead array assays. n = 5-6 mice. \*p<0.05, \*\*\*\* p<0.0001, using one-way ANOVA with Tukey's multiple comparisons test.
- C) INCB054329 blocks induction of *Lgals3bp* 6 h after LPS using qPCR. n = 5-6 mice. \*p<0.05, \*\*p<0.01, \*\*\* p<0.001, using one-way ANOVA with Tukey's multiple comparisons test.
- D) Kaplan-Meier curve of survival after LPS injection. n = 12 control and 11 INCB054329 treatment (67 mg/kg). P-value calculated using Gehan-Breslow-Wilcoxon test.
- E) Schematic for the cardiac function experiment.
- F) INCB054329 prevents the reduction in ejection fraction measured 6 h after LPS using echocardiography. n = 3-4 mice at 0 and 1.5 h and n= 8 at 6 h. \*\*\*\* p<0.0001, using two-way ANOVA with Sidak's multiple comparisons test.
- G) Schematic for the human COVID-19 serum experiments.
- H) IFN-γ was not higher in patients with elevated CTNI (> 0.5 ng/ml). n = 27.
- I) IFN-γ was higher in patients with elevated BNP (> 0.3 ng/ml). n = 27.
- J) Serum from COVID-19 patients with elevated BNP induce diastolic dysfunction. \* p< 0.05 using one-way ANOVA with Dunnett's multiple comparisons test compared to CTRL serum. Orange highlights hCO with elevated force of contraction. Blue highlights dysfunctional hCO.
- K) Diastolic dysfunction induced by COVID-19 patient 6 serum is prevented by 1 μM INCB054329. n = 4-9 hCO from 1 experiment. \*\*\* p< 0.001 and \*\*\*\* p < 0.0001 using one-way ANOVA with Dunnett's multiple comparisons test compared to CTRL serum.

2

3

4

5

6

7

8

9

10

11

12

13

14

15

16

17

18

19

20

21

22

23

24

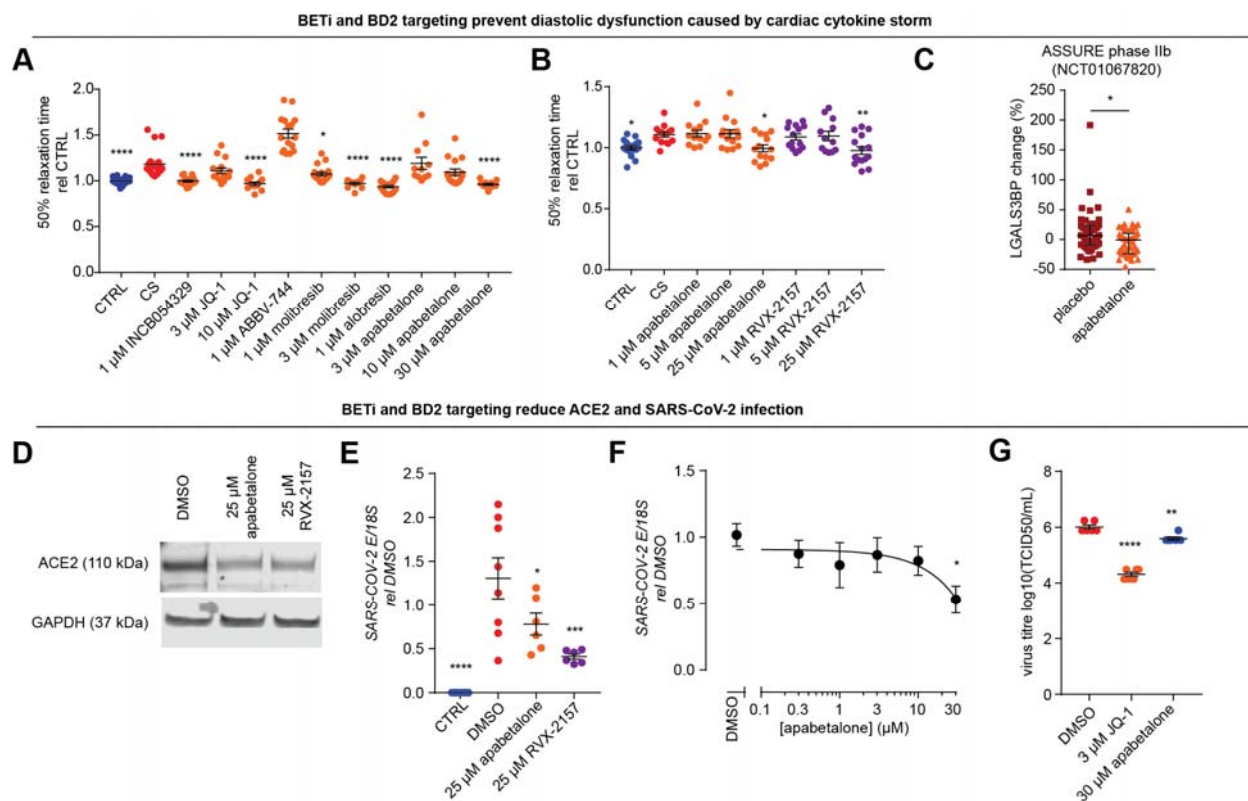
25

26

27

- 1 L) *LGALS3BP* is induced by CS and repressed by BETi in hCO. n = 3 each 2 x hCO
- 2 pooled. \* p < 0.05, \*\*\* p < 0.001, \*\*\*\* p < 0.0001 using one-way ANOVA with Dunnett's
- 3 multiple comparisons test compared to CS.
- 4 Data presented as mean ± standard error of the mean. hPSC cardiac cells- HES3, Endothelial
- 5 cells- RM3.5.





- 1
- 2 **Figure 7: BETi using BD2-selective molecules prevent CS induced diastolic dysfunction**
- 3 **in hCO and SARS-CoV-2 infection.**
- 4 A) All BETi (except ABBV-744) previously used in clinical trials prevent CS-induced
- 5 diastolic dysfunction. n = 12-19 hCOs per condition from 3 experiments. hPSC cardiac
- 6 cells- HES3 (no endothelial cells).
- 7 B) BETi specific for the BD2 domain (RVX-2157) or selective for the BD2 domain
- 8 (apabetalone) prevent CS-induced diastolic dysfunction. n = 12-18 hCOs per condition
- 9 from 3 experiments. hPSC cardiac cells- HES3 (no endothelial cells).
- 10 C) Apabetalone decreases serum LGALS3BP in the phase IIb ASSURE clinical trial. Data
- 11 are presented as a change from baseline. n = 47 placebo and 47 apabetalone.
- 12 D) BETi specific for the BD2 domain (RVX-2157) or selective for the BD2 domain
- 13 (apabetalone) decrease ACE2 expression after 3 days. hPSC cardiac cells – HES3.
- 14 E) Pre-treatment with BETi specific for the BD2 domain (RVX-2157) or selective for the
- 15 BD2 domain (apabetalone) for 3 days block SARS-CoV-2 infection. E-gene expression
- 16 in 2D cultured hPSC-CM 3 days after infection. n = 6-8 from 2 experiments (pooled from
- 17 hPSC cardiac cells- HES3 and AA).
- 18 F) Dose-response curve for apabetalone 3 day pre-treatment to block SARS-CoV-2
- 19 infection. E-gene expression in 2D cultured hPSC-CM 3 days after infection. n = 6 from 1
- 20 experiment. hPSC cardiac cells- HES3.
- 21 G) Apabetalone or JQ-1 3 day pre-treatment reduces SARS-CoV-2 titre in hPSC-CM 3 days
- 22 after infection. n = 6 from 1 experiment. hPSC cardiac cells- HES3.

- 1 TCID50 - Fifty-percent tissue culture infective dose. Data presented as mean  $\pm$  standard error of
- 2 the mean for all plots except for C which is median  $\pm$  interquartile range. \*  $p < 0.05$ , \*\*  $p < 0.01$ ,
- 3 \*\*\*  $p < 0.001$ , \*\*\*\*  $p < 0.0001$ , using one-way ANOVA with Dunnett's multiple comparisons test
- 4 (A,B – CS, E,F,G - DMSO) or Mann-Whitney (C).

## 1 REFERENCES

- 2 Anand, P., Brown, J.D., Lin, C.Y., Qi, J., Zhang, R., Artero, P.C., Alaiti, M.A., Bullard, J., Alazem, K.,  
3 Margulies, K.B., *et al.* (2013). BET bromodomains mediate transcriptional pause release in heart failure.  
4 *Cell* *154*, 569-582.
- 5 Antolic, A., Wakimoto, H., Jiao, Z., Gorham, J.M., DePalma, S.R., Lemieux, M.E., Conner, D.A., Lee, D.Y.,  
6 Qi, J., Seidman, J.G., *et al.* (2020). BET bromodomain proteins regulate transcriptional reprogramming in  
7 genetic dilated cardiomyopathy. *JCI Insight* *5*.
- 8 Arunachalam, P.S., Wimmers, F., Mok, C.K.P., Perera, R.A.P.M., Scott, M., Hagan, T., Sigal, N., Feng, Y.,  
9 Bristow, L., Tak-Yin Tsang, O., *et al.* (2020). Systems biological assessment of immunity to mild versus  
10 severe COVID-19 infection in humans. *Science* *369*, 1210-1220.
- 11 Auguste, G., Rouhi, L., Matkovich, S.J., Coarfa, C., Robertson, M.J., Czernuszewicz, G., Gurha, P., and  
12 Marian, A.J. (2020). BET bromodomain inhibition attenuates cardiac phenotype in myocyte-specific  
13 lamin A/C-deficient mice. *The Journal of clinical investigation* *130*, 4740-4758.
- 14 Bancerek, J., Poss, Z.C., Steinparzer, I., Sedlyarov, V., Pfaffenwimmer, T., Mikulic, I., Dölken, L., Strobl, B.,  
15 Müller, M., Taatjes, D.J., *et al.* (2013). CDK8 kinase phosphorylates transcription factor STAT1 to  
16 selectively regulate the interferon response. *Immunity* *38*, 250-262.
- 17 Chen, C., Li, H., Hang, W., and Wang, D.W. (2020). Cardiac injuries in coronavirus disease 2019 (COVID-  
18 19). *Journal of Molecular and Cellular Cardiology* *145*, 25-29.
- 19 Cochran, A.G., Conery, A.R., and Sims, R.J. (2019). Bromodomains: a new target class for drug  
20 development. *Nature Reviews Drug Discovery* *18*, 609-628.
- 21 Del Valle, D.M., Kim-Schulze, S., Huang, H.-H., Beckmann, N.D., Nirenberg, S., Wang, B., Lavin, Y., Swartz,  
22 T.H., Madduri, D., Stock, A., *et al.* (2020). An inflammatory cytokine signature predicts COVID-19 severity  
23 and survival. *Nature Medicine*.
- 24 Duan, Q., McMahon, S., Anand, P., Shah, H., Thomas, S., Salunga, H.T., Huang, Y., Zhang, R., Sahadevan,  
25 A., Lemieux, M.E., *et al.* (2017). BET bromodomain inhibition suppresses innate inflammatory and  
26 profibrotic transcriptional networks in heart failure. *Science translational medicine* *9*, eaah5084.
- 27 Evron, T., Daigle, T.L., and Caron, M.G. (2012). GRK2: multiple roles beyond G protein-coupled receptor  
28 desensitization. *Trends Pharmacol Sci* *33*, 154-164.
- 29 Faivre, E.J., McDaniel, K.F., Albert, D.H., Mantena, S.R., Plotnik, J.P., Wilcox, D., Zhang, L., Bui, M.H.,  
30 Sheppard, G.S., Wang, L., *et al.* (2020). Selective inhibition of the BD2 bromodomain of BET proteins in  
31 prostate cancer. *Nature* *578*, 306-310.
- 32 Feldman, A.M., Combes, A., Wagner, D., Kadakomi, T., Kubota, T., Li, Y.Y., and McTiernan, C. (2000). The  
33 role of tumor necrosis factor in the pathophysiology of heart failure. *Journal of the American College of*  
34 *Cardiology* *35*, 537-544.
- 35 Filippakopoulos, P., Qi, J., Picaud, S., Shen, Y., Smith, W.B., Fedorov, O., Morse, E.M., Keates, T.,  
36 Hickman, T.T., Felletar, I., *et al.* (2010). Selective inhibition of BET bromodomains. *Nature* *468*, 1067-  
37 1073.
- 38 Gagliano-Jucá, T., Trivison, T.G., Kantoff, P.W., Nguyen, P.L., Taplin, M.-E., Kibel, A.S., Huang, G., Bearup,  
39 R., Schram, H., Manley, R., *et al.* (2018). Androgen Deprivation Therapy Is Associated With Prolongation  
40 of QTc Interval in Men With Prostate Cancer. *J Endocr Soc* *2*, 485-496.
- 41 Gilan, O., Rioja, I., Knezevic, K., Bell, M.J., Yeung, M.M., Harker, N.R., Lam, E.Y.N., Chung, C.-w.,  
42 Bamborough, P., Petretich, M., *et al.* (2020). Selective targeting of BD1 and BD2 of the BET proteins in  
43 cancer and immuno-inflammation. *Science*, eaaz8455.
- 44 Gilsbach, R., Schwaderer, M., Preissl, S., Grüning, B.A., Kranzhöfer, D., Schneider, P., Nührenberg, T.G.,  
45 Mulero-Navarro, S., Weichenhan, D., Braun, C., *et al.* (2018). Distinct epigenetic programs regulate  
46 cardiac myocyte development and disease in the human heart in vivo. *Nature communications* *9*, 391.

- 1 Goyal, P., Choi, J.J., Pinheiro, L.C., Schenck, E.J., Chen, R., Jabri, A., Satlin, M.J., Campion, T.R., Nahid, M.,  
2 Ringel, J.B., *et al.* (2020). Clinical Characteristics of Covid-19 in New York City. *New England Journal of*  
3 *Medicine* **382**, 2372-2374.
- 4 Guo, S., Carter, R.L., Grisanti, L.A., Koch, W.J., and Tilley, D.G. (2017). Impact of paroxetine on proximal  
5  $\beta$ -adrenergic receptor signaling. *Cell Signal* **38**, 127-133.
- 6 Guo, T., Fan, Y., Chen, M., Wu, X., Zhang, L., He, T., Wang, H., Wan, J., Wang, X., and Lu, Z. (2020).  
7 Cardiovascular Implications of Fatal Outcomes of Patients With Coronavirus Disease 2019 (COVID-19).  
8 *JAMA Cardiology* **5**, 811-818.
- 9 Gupta, A., Madhavan, M.V., Sehgal, K., Nair, N., Mahajan, S., Sehrawat, T.S., Bikdeli, B., Ahluwalia, N.,  
10 Ausiello, J.C., Wan, E.Y., *et al.* (2020). Extrapulmonary manifestations of COVID-19. *Nature Medicine* **26**,  
11 1017-1032.
- 12 Hofmann, M.H., Mani, R., Engelhardt, H., Impagnatiello, M.A., Carotta, S., Kerenyi, M., Lorenzo-Herrero,  
13 S., Böttcher, J., Scharn, D., Arnhof, H., *et al.* (2020). Selective and Potent CDK8/19 Inhibitors Enhance NK-  
14 Cell Activity and Promote Tumor Surveillance. *Molecular Cancer Therapeutics* **19**, 1018-1030.
- 15 Horby, P., Lim, W.S., Emberson, J., Mafham, M., Bell, J., Linsell, L., Staplin, N., Brightling, C., Ustianowski,  
16 A., Elmahi, E., *et al.* (2020). Effect of Dexamethasone in Hospitalized Patients with COVID-19: Preliminary  
17 Report. medRxiv, 2020.2006.2022.20137273.
- 18 Huang, C., Wang, Y., Li, X., Ren, L., Zhao, J., Hu, Y., Zhang, L., Fan, G., Xu, J., Gu, X., *et al.* (2020). Clinical  
19 features of patients infected with 2019 novel coronavirus in Wuhan, China. *The Lancet* **395**, 497-506.
- 20 Humphrey, S.J., Azimifar, S.B., and Mann, M. (2015). High-throughput phosphoproteomics reveals in  
21 vivo insulin signaling dynamics. *Nature biotechnology* **33**, 990-995.
- 22 Humphrey, S.J., Karayel, O., James, D.E., and Mann, M. (2018). High-throughput and high-sensitivity  
23 phosphoproteomics with the EasyPhos platform. *Nature Protocols* **13**, 1897-1916.
- 24 Kim, S.Y., Zhang, X., Schiattarella, G.G., Altamirano, F., Ramos, T.A.R., French, K.M., Jiang, N., Szweda,  
25 P.A., Evers, B.M., May, H.I., *et al.* (2020). Epigenetic Reader BRD4 (Bromodomain-Containing Protein 4)  
26 Governs Nucleus-Encoded Mitochondrial Transcriptome to Regulate Cardiac Function. *Circulation* **142**,  
27 2356-2370.
- 28 Kubota, T., McTiernan, C.F., Frye, C.S., Slawson, S.E., Lemster, B.H., Koretsky, A.P., Demetris, A.J., and  
29 Feldman, A.M. (1997). Dilated Cardiomyopathy in Transgenic Mice With Cardiac-Specific Overexpression  
30 of Tumor Necrosis Factor- $\alpha$ . *Circulation research* **81**, 627-635.
- 31 Levick, S.P., and Goldspink, P.H. (2014). Could interferon-gamma be a therapeutic target for treating  
32 heart failure? *Heart failure reviews* **19**, 227-236.
- 33 Lindner, D., Fitzek, A., Bräuninger, H., Aleshcheva, G., Edler, C., Meissner, K., Scherschel, K., Kirchhof, P.,  
34 Escher, F., Schultheiss, H.-P., *et al.* (2020). Association of Cardiac Infection With SARS-CoV-2 in  
35 Confirmed COVID-19 Autopsy Cases. *JAMA Cardiology*.
- 36 Mangalmurti, N., and Hunter, C.A. (2020). Cytokine Storms: Understanding COVID-19. *Immunity* **53**, 19-  
37 25.
- 38 Messner, C.B., Demichev, V., Wendisch, D., Michalick, L., White, M., Freiwald, A., Textoris-Taube, K.,  
39 Vernardis, S.I., Egger, A.S., Kreidl, M., *et al.* (2020). Ultra-High-Throughput Clinical Proteomics Reveals  
40 Classifiers of COVID-19 Infection. *Cell systems* **11**, 11-24.e14.
- 41 Mills, R.J., Parker, B.L., Quaiife-Ryan, G.A., Voges, H.K., Needham, E.J., Bornot, A., Ding, M., Andersson,  
42 H., Polla, M., Elliott, D.A., *et al.* (2019). Drug Screening in Human PSC-Cardiac Organoids Identifies Pro-  
43 proliferative Compounds Acting via the Mevalonate Pathway. *Cell stem cell* **24**, 895-907.e896.
- 44 Mills, R.J., Titmarsh, D.M., Koenig, X., Parker, B.L., Ryall, J.G., Quaiife-Ryan, G.A., Voges, H.K., Hodson,  
45 M.P., Ferguson, C., Drowley, L., *et al.* (2017). Functional screening in human cardiac organoids reveals a  
46 metabolic mechanism for cardiomyocyte cell cycle arrest. *Proceedings of the National Academy of*  
47 *Sciences* **114**, E8372-E8381.

- 1 Needham, E.J., Parker, B.L., Burykin, T., James, D.E., and Humphrey, S.J. (2019). Illuminating the dark  
2 phosphoproteome. *Science Signaling* *12*, eaau8645.
- 3 Nicholls, S.J., Schwartz, G.G., Buhr, K.A., Ginsberg, H.N., Johansson, J.O., Kalantar-Zadeh, K., Kulikowski,  
4 E., Toth, P.P., Wong, N., Sweeney, M., *et al.* (2021). Apabetalone and hospitalization for heart failure in  
5 patients following an acute coronary syndrome: a prespecified analysis of the BETonMACE study.  
6 *Cardiovascular diabetology* *20*, 13.
- 7 Nicodeme, E., Jeffrey, K.L., Schaefer, U., Beinke, S., Dewell, S., Chung, C.W., Chandwani, R., Marazzi, I.,  
8 Wilson, P., Coste, H., *et al.* (2010). Suppression of inflammation by a synthetic histone mimic. *Nature*  
9 *468*, 1119-1123.
- 10 Nishiga, M., Wang, D.W., Han, Y., Lewis, D.B., and Wu, J.C. (2020). COVID-19 and cardiovascular disease:  
11 from basic mechanisms to clinical perspectives. *Nature Reviews Cardiology*.
- 12 Oladunni, F.S., Park, J.G., Pino, P.A., Gonzalez, O., Akhter, A., Allué-Guardia, A., Olmo-Fontánez, A.,  
13 Gautam, S., Garcia-Vilanova, A., Ye, C., *et al.* (2020). Lethality of SARS-CoV-2 infection in K18 human  
14 angiotensin-converting enzyme 2 transgenic mice. *Nature communications* *11*, 6122.
- 15 Padmanabhan, A., Alexanian, M., Linares-Saldana, R., González-Terán, B., Andreoletti, G., Huang, Y.,  
16 Connolly, A.J., Kim, W., Hsu, A., Duan, Q., *et al.* (2020). BRD4 (Bromodomain-Containing Protein 4)  
17 Interacts with GATA4 (GATA Binding Protein 4) to Govern Mitochondrial Homeostasis in Adult  
18 Cardiomyocytes. *Circulation* *142*, 2338-2355.
- 19 Pellegrini, D., Kawakami, R., Guagliumi, G., Sakamoto, A., Kawai, K., Gianatti, A., Nasr, A., Kutys, R., Guo,  
20 L., Cornelissen, A., *et al.* (2021). Microthrombi As A Major Cause of Cardiac Injury in COVID-19: A  
21 Pathologic Study. *Circulation*.
- 22 Puntmann, V.O., Carerj, M.L., Wieters, I., Fahim, M., Arendt, C., Hoffmann, J., Shchendrygina, A., Escher,  
23 F., Vasa-Nicotera, M., Zeiher, A.M., *et al.* (2020). Outcomes of Cardiovascular Magnetic Resonance  
24 Imaging in Patients Recently Recovered From Coronavirus Disease 2019 (COVID-19). *JAMA Cardiology*.
- 25 Qiao, Y., Wang, X.-M., Mannan, R., Pitchiaya, S., Zhang, Y., Wotring, J.W., Xiao, L., Robinson, D.R., Wu, Y.-  
26 M., Tien, J.C.-Y., *et al.* (2021). Targeting transcriptional regulation of SARS-CoV-2 entry factors ACE2 and  
27 TMPRSS2. *Proceedings of the National Academy of Sciences* *118*, e2021450118.
- 28 Quaife-Ryan, G.A., Sim, C.B., Ziemann, M., Kaspi, A., Rafehi, H., Ramialison, M., El-Osta, A., Hudson, J.E.,  
29 and Porrello, E.R. (2017). Multicellular Transcriptional Analysis of Mammalian Heart Regeneration.  
30 *Circulation* *136*, 1123-1139.
- 31 Ray, K.K., Nicholls, S.J., Buhr, K.A., Ginsberg, H.N., Johansson, J.O., Kalantar-Zadeh, K., Kulikowski, E.,  
32 Toth, P.P., Wong, N., Sweeney, M., *et al.* (2020). Effect of Apabetalone Added to Standard Therapy on  
33 Major Adverse Cardiovascular Events in Patients With Recent Acute Coronary Syndrome and Type 2  
34 Diabetes: A Randomized Clinical Trial. *Jama* *323*, 1565-1573.
- 35 Ren, X., Wen, W., Fan, X., Hou, W., Su, B., Cai, P., Li, J., Liu, Y., Tang, F., Zhang, F., *et al.* (2021). COVID-19  
36 immune features revealed by a large-scale single cell transcriptome atlas. *Cell*.
- 37 Richardson, P., Griffin, I., Tucker, C., Smith, D., Oechsle, O., Phelan, A., Rawling, M., Savory, E., and  
38 Stebbing, J. (2020). Baricitinib as potential treatment for 2019-nCoV acute respiratory disease. *Lancet*  
39 *395*, e30-e31.
- 40 Runte, K.E., Bell, S.P., Selby, D.E., Häußler, T.N., Ashikaga, T., LeWinter, M.M., Palmer, B.M., and Meyer,  
41 M. (2017). Relaxation and the Role of Calcium in Isolated Contracting Myocardium From Patients With  
42 Hypertensive Heart Disease and Heart Failure With Preserved Ejection Fraction. *Circ Heart Fail* *10*.
- 43 Rzymiski, T., Mikula, M., Żytkiewicz, E., Dreas, A., Wiklik, K., Gołas, A., Wójcik, K., Masiejczyk, M., Wróbel,  
44 A., Dolata, I., *et al.* (2017). SEL120-34A is a novel CDK8 inhibitor active in AML cells with high levels of  
45 serine phosphorylation of STAT1 and STAT5 transactivation domains. *Oncotarget* *8*, 33779-33795.
- 46 Sadzak, I., Schiff, M., Gattermeier, I., Glinitzer, R., Sauer, I., Saalmüller, A., Yang, E., Schaljo, B., and  
47 Kovarik, P. (2008). Recruitment of Stat1 to chromatin is required for interferon-induced serine



1 phosphorylation of Stat1 transactivation domain. Proceedings of the National Academy of Sciences of  
2 the United States of America *105*, 8944-8949.

3 Sanders-van Wijk, S., Tromp, J., Beussink-Nelson, L., Hage, C., Svedlund, S., Saraste, A., Swat, S.A.,  
4 Sanchez, C., Njoroge, J., Tan, R.S., *et al.* (2020). Proteomic Evaluation of the Comorbidity-Inflammation  
5 Paradigm in Heart Failure With Preserved Ejection Fraction: Results From the PROMIS-HFpEF Study.  
6 *Circulation* *142*, 2029-2044.

7 Schumacher, S.M., Gao, E., Zhu, W., Chen, X., Chuprun, J.K., Feldman, A.M., Tesmer, J.J.G., and Koch,  
8 W.J. (2015). Paroxetine-mediated GRK2 inhibition reverses cardiac dysfunction and remodeling after  
9 myocardial infarction. *Science translational medicine* *7*, 277ra231-277ra231.

10 Sharma, A., Garcia, G., Jr., Wang, Y., Plummer, J.T., Morizono, K., Arumugaswami, V., and Svendsen, C.N.  
11 (2020). Human iPSC-Derived Cardiomyocytes Are Susceptible to SARS-CoV-2 Infection. *Cell Rep Med* *1*,  
12 100052-100052.

13 Shi, S., Qin, M., Shen, B., Cai, Y., Liu, T., Yang, F., Gong, W., Liu, X., Liang, J., Zhao, Q., *et al.* (2020).  
14 Association of Cardiac Injury With Mortality in Hospitalized Patients With COVID-19 in Wuhan, China.  
15 *JAMA Cardiology* *5*, 802-810.

16 Stratton, M.S., Bagchi, R.A., Felisbino, M.B., Hirsch, R.A., Smith, H.E., Riching, A.S., Enyart, B.Y., Koch,  
17 K.A., Cavasin, M.A., Alexanian, M., *et al.* (2019). Dynamic Chromatin Targeting of BRD4 Stimulates  
18 Cardiac Fibroblast Activation. *Circulation research* *125*, 662-677.

19 Stubbs, M.C., Burn, T.C., Sparks, R., Maduskuie, T., Diamond, S., Rupal, M., Wen, X., Volgina, A.,  
20 Zolotarjova, N., Waeltz, P., *et al.* (2019). The Novel Bromodomain and Extraterminal Domain Inhibitor  
21 INCB054329 Induces Vulnerabilities in Myeloma Cells That Inform Rational Combination Strategies.  
22 *Clinical Cancer Research* *25*, 300-311.

23 Szekely, Y., Lichter, Y., Taieb, P., Banai, A., Hochstadt, A., Merdler, I., Oz, A.G., Rothschild, E., Baruch, G.,  
24 Peri, Y., *et al.* (2020). The Spectrum of Cardiac Manifestations in Coronavirus Disease 2019 (COVID-19) -  
25 a Systematic Echocardiographic Study. *Circulation* *0*.

26 Tucker, N.R., Chaffin, M., Fleming, S.J., Hall, A.W., Parsons, V.A., Jr, K.C.B., Akkad, A.-D., Herndon, C.N.,  
27 Arduini, A., Papangeli, I., *et al.* (2020). Transcriptional and Cellular Diversity of the Human Heart.  
28 *Circulation* *0*.

29 Vagnozzi, R.J., Gatto, G.J., Jr., Kallander, L.S., Hoffman, N.E., Mallilankaraman, K., Ballard, V.L.T.,  
30 Lawhorn, B.G., Stoy, P., Philp, J., Graves, A.P., *et al.* (2013). Inhibition of the cardiomyocyte-specific  
31 kinase TNNI3K limits oxidative stress, injury, and adverse remodeling in the ischemic heart. *Science*  
32 *translational medicine* *5*, 207ra141-207ra141.

33 Vasudevan, N.T., Mohan, M.L., Gupta, M.K., Martelli, E.E., Hussain, A.K., Qin, Y., Chandrasekharan, U.M.,  
34 Young, D., Feldman, A.M., Sen, S., *et al.* (2013). G $\beta\gamma$ -independent recruitment of G-protein coupled  
35 receptor kinase 2 drives tumor necrosis factor  $\alpha$ -induced cardiac  $\beta$ -adrenergic receptor dysfunction.  
36 *Circulation* *128*, 377-387.

37 Voges, H.K., Mills, R.J., Elliott, D.A., Parton, R.G., Porrello, E.R., and Hudson, J.E. (2017). Development of  
38 a human cardiac organoid injury model reveals innate regenerative potential. *Development (Cambridge,*  
39 *England)* *144*, 1118-1127.

40 Williams, L.M., McCann, F.E., Cabrita, M.A., Layton, T., Cribbs, A., Knezevic, B., Fang, H., Knight, J., Zhang,  
41 M., Fischer, R., *et al.* (2020). Identifying collagen VI as a target of fibrotic diseases regulated by  
42 CREBBP/EP300. *Proceedings of the National Academy of Sciences of the United States of America* *117*,  
43 20753-20763.

44 Wu, Z., and McGoogan, J.M. (2020). Characteristics of and Important Lessons From the Coronavirus  
45 Disease 2019 (COVID-19) Outbreak in China: Summary of a Report of 72,314 Cases From the Chinese  
46 Center for Disease Control and Prevention. *JAMA* *323*, 1239-1242.

47

## 1 ACKNOWLEDGEMENTS

2 We thank Clive Berghofer and Lyn Brazil (and others) for their generous philanthropic donations  
3 to help set up SARS-CoV-2 research at QIMR Berghofer MRI. We used the Australian National  
4 Fabrication Facility Queensland Node for the fabrication of the Heart-Dyno molds. We thank Dr I  
5 Anraku for his assistance in managing the PC3 (BSL3) facility at QIMR Berghofer MRI. We  
6 thank Dr Alyssa Pye and Mr Fredrick Moore (Queensland Health, Brisbane) for providing the  
7 SARS-CoV-2 virus. We thank Grace Chojnowski and Michael Rist for FACS at QIMR Berghofer.  
8 Microscopy was aided by Tam Nguyen and Nigel Waterhouse at QIMR Berghofer. Prof Edouard  
9 Stanley for provision of the RM3.5 iPSC line (Murdoch Children's Research Institute,  
10 Melbourne, Australia). We thank Nadine Shultz and Paul Collins for the sequencing and also  
11 Scott Wood, Pamela Mukhopadhyay, John Pearson, Nic Waddell and Ross Koufariotis for  
12 bioinformatics assistance. We thank Compounds Australia ([www.compoundsaustralia.com](http://www.compoundsaustralia.com)) for  
13 providing access to compounds, however all experiments herein used compounds sourced from  
14 MedChem Express or Selleckchem. We thank the Translational Research Institute for providing  
15 core facilities that enabled this research, particularly Preclinical Imaging and Biological  
16 Resources Facility.

17 We acknowledge grant and fellowship support from the National Health and Medical Research  
18 Council of Australia (J.E.H., M.J.S., C.R.E., T.B.), Heart Foundation of Australia (J.E.H.), QIMR  
19 Berghofer Medical Research Institute (J.E.H.), The Stafford Fox Foundation (E.R.P.), the Royal  
20 Children's Hospital Foundation (E.R.P.), Australian Research Council Strategic Initiative in Stem  
21 Cell Science (Stem Cells Australia) (E.R.P. and J.E.H.) and the Medical Research Future Fund  
22 (MRFF9200008) (J.E.H., T.B., M.J.S., K.P.A.MD., C.R.E., E.R.P.). M.J.S. is supported by  
23 Health and Medical Research Council of Australia Program (APP1132519) and Investigator  
24 (APP1173958) grants. A.S. is also supported by Investigator grant (APP1173880). Queensland  
25 Health supported this research project. The Murdoch Children's Research Institute is supported  
26 by the Victorian Government's Operational Infrastructure Support Program. This project  
27 received support from Dynomics Inc. J.E.H. is supported by a Snow Medical Fellowship.

## 28 AUTHOR CONTRIBUTIONS

29 R.J.M., S.J.H., G.A.Q-R., S.K., M.L., L.T.R., R.R., K.A.S., B.W.C.T., D.J.R., T.L., S.R.F., W.Z.,  
30 L.L., C.M-K., D.A-B., K.K., T.B., J.E.H., performed experiments, L.D., H.K.V.,L.T.R., E.M.,  
31 developed cardiac organoid cultures, R.J.M., S.J.H., P.R.J.F., G.A.Q-R., M.L., N.R.M., D.G.,  
32 L.F., E.K., R.L.J., T.D., C.B., B.G., D.T., R.R., K.S., E.R.P., T.B., J.E.H., analysed data, J.M.,  
33 C.H., D.G., L.F., S.J.N., J.J., M.S., N.C.W.W., E.K., provided clinical samples, R.J.M., S.J.H.,  
34 M.J.S., C.R.E., K.P.A.MD., D.G., E.K., T.B., D.A.E, K.S., A.S., D.E.J, J.E.H, designed the  
35 project, R.J.M., S.J.H., M.J.S., C.R.E., K.P.A.MD., D.G., L.F., E.K., R.R., K.S., E.R.P., A.S.,  
36 T.B., D.E.J, J.E.H, interpreted data, R.J.M., S.J.H., D.E.J, J.E.H. wrote the manuscript, all  
37 authors edited the manuscript.

## 38 DECLARATION OF INTERESTS

39 R.J.M., J.E.H., G.A.Q.-R., D.M.T. and E.R.P. are listed as co-inventors on pending patents held  
40 by The University of Queensland and QIMR Berghofer Medical Research Institute that relate to  
41 cardiac organoid maturation and putative cardiac regeneration therapeutics. J.E.H. is a co-  
42 inventor on licensed patents held by the University of Goettingen. R.J.M, E.R.P., D.M.T., B.G.  
43 and J.E.H. are co-founders, scientific advisors and stockholders in Dynomics Inc. D.M.T. and  
44 B.G. are employees of Dynomics Inc. /Dynomics Pty Ltd. C.H., D.G., L.F., J.J., M.S., N.C.W.W.  
45 and E.K. are employed by Resverlogix. S.J.N. has received honoraria and research support

- 1 from Resverlogix. QIMR Berghofer Medical Research Institute has filed a patent on the use of
- 2 BETi.

## 1 **MODELS**

### 2 **Mice**

3 Mouse work was undertaken in accordance with the Australian Code for Care and Use of  
4 Animals for Scientific Purposes, as outlined by the National Health and Medical Research  
5 Council of Australia. Animal work was approved by the QIMR Berghofer Medical Research  
6 Institute and University of Queensland Animal Ethics Committees.

7 For LPS experiments wild-type (WT) C57BL/6 were purchased from Walter and Eliza Hall  
8 Institute for Medical Research, the Australian Research Centre in Western Australia or bred in  
9 house at QIMR Berghofer Medical Research Institute. Mice used in this study were older than 6  
10 weeks and were sex-matched. The number of mice in each group of treatment for each  
11 experiment is indicated in the figure legends. No mice were excluded based on pre-established  
12 criteria and randomization was applied immediately prior to treatment in therapy experiments.

13 For SARS-CoV-2 infection studies, heterozygous K18-hACE2 C57BL/6J mice (strain B6.Cg-  
14 Tg(K18-ACE2)2PrImn/J) were purchased from The Jackson Laboratory, USA, and bred in  
15 house at QIMR Berghofer Medical Research Institute and genotyped using standard PCR as  
16 per Jackson Labs genotyping protocol.

### 17 **Cell lines**

18 Ethical approval for the use of human embryonic stem cells (hESCs) was obtained from QIMR  
19 Berghofer's Ethics Committee and was carried out in accordance with the National Health and  
20 Medical Research Council of Australia (NHMRC) regulations. hESCs utilized were female HES3  
21 (WiCell), and male RM3.5 iPSC line (generated by Edouard Stanley (Murdoch Children's  
22 Research Institute, Melbourne, Australia)). The following cell lines were obtained from the CIRM  
23 hPSC Repository funded by the California Institute of Regenerative Medicine: CW30382A  
24 (male, designated AA) and CW30318C (female, designated CC) which were both obtained from  
25 FujiFilm. hPSC lines were maintained in mTeSR-1 (Stem Cell Technologies)/Matrigel (Millipore)  
26 and passaged using TrypLE (ThermoFisher Scientific) or ReLeSR (Stem Cell Technologies).  
27 Quality control was performed with Karyotyping and mycoplasma testing.

### 28 **Human COVID-19 plasma and serum**

29 Plasma samples were obtained from individuals with PCR confirmed COVID-19 infection in the  
30 community or hospital as a part of the COVID-19 Biobank (Alfred Human Research and Ethics  
31 Committee - Project 182/20). Individuals consented to provide additional blood that was  
32 processed within 24 h of collection for plasma and peripheral blood mononuclear cells. Whole  
33 blood was centrifuged at 1000 x g for 10 min at 22°C-24°C then plasma removed within 5 mm of  
34 the buffy coat. Plasma aliquots were transferred to 2 ml cryovials for long term storage at -  
35 80°C. Plasma was thawed and immediately use for ELISA for CTNI, IFN- $\gamma$  and BNP. Calcium  
36 was added to 10 mM to normalize calcium levels of citrated plasma and clot. The supernatant  
37 was removed (serum) and used for the hCO experiments (50% serum/50% WM). No viral RNA  
38 was detected in hCO treated with human COVID-19 serum.

### 39 **Human ASSURE trial plasma**

40 The design and rationale of the ASSURE trial (ClinicalTrials.gov identifier NCT01067820) is  
41 described in. Patients with established cardiovascular disease received 200 mg apabetalone  
42 daily for 26 weeks on top of standard of care, which included statins. Baseline and end of study  
43 EDTA plasma samples were analysed using SOMAScan™.

### 44 **SARS-CoV-2 stock production and titration at QIMR Berghofer**

1 SARS-CoV-2 infection studies at QIMR Berghofer were conducted in a dedicated PC3 (BSL3)  
2 suite, with safety approval from the QIMR Safety Committee (P3600). The SARS-CoV-2 virus  
3 was isolated from a patient and was a kind gift from Queensland Health Forensic & Scientific  
4 Services, Queensland Department of Health; the isolate, hCoV-19/Australia/QLD02/2020; has  
5 been sequenced as is available at GISAID (<https://www.gisaid.org/>). Virus stock was generated  
6 by infecting Vero E6 cells (C1008, ECACC, Wiltshire, England; Sigma Aldridge, St. Louis, MO,  
7 USA) and after 3 days culture supernatant was clarified by centrifugation at 3000 x g for 15 min  
8 at 4°C, and was aliquoted and stored at -80°C. Virus titre was determined using standard TCID<sub>50</sub>  
9 assay by infecting Vero E6 cells with 10-fold serial dilutions of virus stock and measuring  
10 cytopathic effect with titre calculation by the method of Spearman and Karber. Virus was  
11 determined to be mycoplasma free (La Linn et al., 1995) and fetal calf serum used for culture  
12 determined to be endotoxin free (Johnson et al., 2005).

### 13 **SARS-CoV-2 stock production at The Doherty Institute**

14 SARS-CoV-2 isolate CoV/Australia/VIC01/2020, provided by the Victorian Infectious Diseases  
15 Reference Laboratory (VIDRL) was amplified in Vero cells and stock vials were stored at -80°C.  
16 The amplified virus was sequenced to confirm that there were no mutations resulting from  
17 passage in Vero cells. All work with infectious virus was performed inside a biosafety cabinet, in  
18 a biosafety containment level 3 facility, and personnel wore powered air-purifying respirators  
19 (3M TR-315A VERSAFLO Cat# RPPKTR315A, from Safetyquip) or P2 masks. Vero cells were  
20 obtained from VIDRL and maintained in Minimum Essential Medium (MEM, Media Preparation  
21 Unit, Peter Doherty Institute) with 5% FBS, Penicillin-Streptomycin, GlutaMax (and 7.5ml  
22 HEPES (all ThermoFisher Scientific).

## 23 **METHODS**

### 24 **Cardiac differentiation**

25 Cardiac differentiation was performed as previously described (Hudson et al., 2012; Mills et al.,  
26 2017; Voges et al., 2017). hPSCs were seeded on Matrigel-coated flasks at  $2 \times 10^4$  cells/cm<sup>2</sup>  
27 and cultured in mTeSR-1 for 4 days. To induce cardiac mesoderm, hPSCs were cultured in  
28 RPMI B27-medium (RPMI 1640 GlutaMAX+ 2% B27 supplement without insulin, 200 µM L-  
29 ascorbic acid 2-phosphate sesquimagnesium salt hydrate (Sigma) and 1%  
30 Penicillin/Streptomycin (ThermoFisher Scientific), supplemented with 5 ng/ml BMP-4 (RnD  
31 Systems), 9 ng/ml Activin A (RnD Systems), 5 ng/ml FGF-2 (RnD Systems) and 1 µM  
32 CHIR99021 (Stem Cell Technologies). Mesoderm induction required daily medium exchanges  
33 for 3 days. This was followed by cardiac specification using RPMI B27- containing 5 µM IWP-4  
34 (Stem Cell Technologies) for another 3 days, and then further 7 days using 5 µM IWP-4 RPMI  
35 B27+ (RPMI1640 Glutamax + 2% B27 supplement with insulin, 200 µM L-ascorbic acid 2-  
36 phosphate sesquimagnesium salt hydrate and 1% Penicillin/Streptomycin) with media change  
37 every 2-3 days. For the final 2 days of differentiation, hPSCs were cultured in RPMI B27+.  
38 Harvest of differentiated cardiac cells involved enzymatic digestion, firstly in 0.2% collagenase  
39 type I (Sigma) containing 20% fetal bovine serum (FBS) in PBS (with Ca<sup>2+</sup> and Mg<sup>2+</sup>) at 37°C for  
40 1 h, and secondly in 0.25% trypsin-EDTA at 37°C for 10 minutes. Cells were filtered through a  
41 100 µm mesh cell strainer (BD Biosciences), centrifuged at 300 x g for 3 min, and resuspended  
42 in α-MEM Glutamax, 10% FBS, 200 µM L-ascorbic acid 2-phosphate sesquimagnesium salt  
43 hydrate and 1% Penicillin/Streptomycin. Previous flow cytometry analysis indicated that  
44 differentiated cardiac cells were ~70% α-actinin<sup>+</sup>/CTNT<sup>+</sup> cardiomyocytes, ~30% CD90 stromal  
45 cells (Voges et al., 2017).

### 46 **Endothelial differentiation**

1 Endothelial cell differentiation was performed following a protocol modified from (Orlova et al.,  
2 2014). hPSCs were seeded onto Matrigel-coated T-25 or T-75 tissue culture flasks at the  
3 density  $5 \times 10^3$  cells/cm<sup>2</sup> and cultured in mTeSR-1 for 3 days. Mesoderm was induced with  
4 RPMI B27- (RPMI 1640 GlutaMAX+ 2% B27 supplement without insulin, 200  $\mu$ M L-ascorbic  
5 acid 2-phosphate sesquimagnesium salt hydrate (Sigma) and 1% Penicillin/Streptomycin  
6 (ThermoFisher Scientific), and the small molecules 25 ng/ml Activin A (R&D systems), 30 ng/ml  
7 Bone morphogenic protein-4 (BMP4) (R&D systems), 1.5  $\mu$ M CHIR99021 (Stemgent), and 50  
8 ng/ml Vascular Endothelial Growth Factor type A (VEGF-A) (Peprotech) for 3 days (no media  
9 changes). Endothelial cell fate was further specified with RPMI B27- medium supplemented with  
10 50 ng/ml VEGF-A and 10  $\mu$ M SB431542 with media changes every 2 to 3 days until day 8.

### 11 **FACS sorting endothelial cells**

12 Endothelial cells were harvested on day 8 of differentiation using TrypLE (ThermoFisher  
13 Scientific). Single cells were separated using a 100  $\mu$ m strainer and labelled with CD31 antibody  
14 (1:200, M082329-2, DAKO) at 4°C for 45 min followed by 30 min staining with a goat anti-  
15 mouse secondary antibody conjugated to AlexaFluor 488 or 555 (1:400, A-11001 and A-21422,  
16 ThermoFisher Scientific). Cells were analysed using Becton Dickinson FACSAria II, gated on  
17 forward and side scatter. Single cells were identified and sorted based on CD31+ expression.  
18 CD31+ endothelial cells were expanded in EGM-2 (Lonza) in Matrigel flasks and cryopreserved.

### 19 **hCO fabrication**

20 hCO culture inserts were fabricated using SU-8 photolithography and PDMS molding (Mills et  
21 al., 2017). Differentiated cells were mixed at ratio of 20% endothelial cells and 80%  
22 cardiomyocytes/fibroblasts to form hCO. Acid-solubilized bovine collagen 1 (Devro) was salt  
23 balanced using 10x DMEM (ThermoFisher Scientific) and pH neutralized using 0.1M NaOH  
24 before combining with Matrigel and then the cell suspension on ice. Each hCO contained  $5 \times$   
25  $10^4$  cells, a final concentration of 2.6 mg/ml collagen I and 9% Matrigel. 3.5  $\mu$ L of suspension  
26 was pipetted into the hCO culture insert and incubated at 37°C with 5% CO<sub>2</sub> for 45 min in order  
27 to gel. After gelling,  $\alpha$ -MEM GlutaMAX (ThermoFisher Scientific), 10% fetal bovine serum (FBS),  
28 200  $\mu$ M L-ascorbic acid 2-phosphate sesquimagnesium salt hydrate (Sigma) and 1%  
29 Penicillin/Streptomycin (ThermoFisher Scientific) was added. hCO were subsequently cultured  
30 in maturation media (MM) (Mills et al., 2017) with medium changes every 2 to 3 days for 5 days  
31 (7 days old hCO). To better approximate adult metabolic provisions a 'weaning medium' (WM)  
32 was utilized. hCO were cultured in WM containing 4% B27 – insulin, 5.5 mM glucose, 1 nM  
33 insulin, 200  $\mu$ M L-ascorbic acid 2-phosphate sesquimagnesium salt hydrate, 1% P/S, 1%  
34 GlutaMAX (100x), 33  $\mu$ g/mL aprotinin and 10  $\mu$ M palmitate (conjugated to bovine serum  
35 albumin in B27) in DMEM without glucose, glutamine and phenol red (ThermoFisher Scientific)  
36 with media changes every 2-3 days.

### 37 **Force analysis of hCO**

38 The elasticity of the Heart Dyno poles enables the contractile properties to be determined via  
39 tracking pole deflection, which directly correlates with force (Mills et al., 2017). Videos of 10  
40 seconds were made of each hCO with the Nikon ANDOR WD Revolution Spinning Disk  
41 microscope (magnification 4x). While imaging, hCO were incubated at 37°C, 5% CO<sub>2</sub> to prevent  
42 changes in contractile behaviour. For pacing, hCOs were electrically stimulated at 1 Hz with 5  
43 ms square pulses with 20 mA current using a Panlab/Harvard Apparatus Digital Stimulator.  
44 Videos were then analysed with a custom written Matlab program (Mills et al., 2017). This  
45 facilitated the analysis of the contractile properties of the organoids and the production of time-  
46 force graphs (Mills et al., 2017). Moreover, data was obtained regarding additional important  
47 functional parameters including the contraction rate and the activation and relaxation time of the  
48 organoids.



## 1 **Immunostaining of hCO (endothelial)**

2 hCO were fixed with 1% paraformaldehyde (Sigma) for 1 h. Cells were stained with primary  
3 antibodies CD31 (1:200, M082329-2, DAKO), NG2 (1:200, 14-6504-82, ThermoFisher  
4 Scientific) and cardiac troponin T (1:400, ab45932, Abcam) in 5% FBS and 0.25% TritonX-100  
5 Blocking Buffer at 4°C overnight on a rocker. Cells were washed twice for 1 h with Blocking  
6 Buffer and labelled with secondary antibodies goat anti-mouse IgG<sub>1</sub> AlexaFluor 488 (1:400, A-  
7 21121), goat anti-mouse IgG<sub>2a</sub> AlexaFluor 555 (1:400, A-21137) and goat anti-rabbit IgG  
8 AlexaFluor 633 (1:400, A-21070) and Hoechst33324 (all ThermoFisher Scientific) at 4°C  
9 overnight on a rocker. Cells were again washed with Blocking Buffer twice for 1 h and mounted  
10 on microscope slides using ProLong Glass (ThermoFisher Scientific).

## 11 **Phosphoproteomics**

12 Phosphoproteomics experiments were performed with biological triplicates. Phosphopeptides  
13 were enriched from 20 pooled hCO, yielding approximately 100 µg of total protein per condition.  
14 The high-sensitivity EasyPhos workflow was employed as previously described (Humphrey et  
15 al., 2018). Briefly, pooled organoids were lysed in SDC buffer (4% Sodium deoxycholate, 100  
16 mM Tris pH 8.5) and immediately heated for 5 min at 95°C. Lysates were cooled on ice, and  
17 sonicated with a tip-probe sonicator (50% output power, 30 seconds). An aliquot of lysate was  
18 taken and diluted 1:5 in 8 M Urea from which protein concentration was determined by BCA  
19 assay (Thermo Fisher Scientific). Aliquots corresponding to 100 µg of protein were  
20 subsequently diluted in SDC buffer into a 96-well deep-well plate, reduced and alkylated at 45°C  
21 for 5 min by the addition of 10 mM Tris (2-carboxyethyl)phosphine (TCEP)/40 mM 2-  
22 Chloroacetamide (CAA) pH 8, and digested by the addition of 1:100 Lys-C and Trypsin  
23 overnight at 37°C with agitation (1,500 rpm). After digestion phosphopeptides were enriched in  
24 parallel according to the EasyPhos workflow as described (Humphrey et al., 2018). Eluted  
25 phosphopeptides were dried in a SpeedVac concentrator (Eppendorf) and resuspended in MS  
26 loading buffer (0.3% TFA/2% acetonitrile) prior to LC-MS/MS measurement.

## 27 **LC-MS/MS Measurement**

28 Phosphopeptides were loaded onto a 40 cm column fabricated in-house with 75 µM inner  
29 diameter fused silica packed with 1.9 µM C18 ReproSil particles (Dr. Maisch GmbH). A column  
30 oven (Sonation) was used to maintain column temperature at 60°C, and a U3000 HPLC system  
31 (Dionex, Thermo Fisher Scientific) was connected to a Q Exactive HF X benchtop Orbitrap  
32 mass spectrometer (Thermo Fisher Scientific) with a NanoSpray Flex ion source (Thermo  
33 Fisher Scientific). For all samples, peptides were separated using a binary buffer system of  
34 0.1% (v/v) formic acid (buffer A) and 80% (v/v) acetonitrile/0.1% (v/v) formic acid (buffer B).  
35 Peptides were eluted at a flow rate of 400 nl/min and separated with a gradient of 3 – 19%  
36 buffer B over 40 minutes, followed by 19 – 41% buffer B over 20 minutes, and peptides were  
37 analysed with a full scan (350 – 1,400 m/z; R=60,000 at 200 m/z) at a target of 3e6 ions,  
38 followed by up to ten data-dependent MS2 scans using HCD (target 1e5; max. IT 50 ms;  
39 isolation window 1.6 m/z; NCE 27%; min. AGC target 2e4), detected in the Orbitrap mass  
40 analyser (R=15,000 at 200 m/z). Dynamic exclusion (30 s) and Apex trigger (2 to 4 s) were  
41 enabled.

## 42 **MS data processing**

43 RAW MS data was processed in the MaxQuant software environment (Cox and Mann, 2008)  
44 (version 1.6.0.9), searching against the Human UniProt Reference database (December 2019  
45 release), using default settings with the addition of 'Phospho(STY)' as a variable modification  
46 and 'Match between runs' switched on for all analyses. Data analysis was performed using the  
47 Perseus software package (Tyanova and Cox, 2018).

## 1 **Single nuclei RNA-sequencing of hCO**

2 Pooled hCO (~40) were homogenized in 4 mL lysis buffer (300 mM sucrose, 10 mM Tris-HCl  
3 (pH = 8), 5 mM CaCl<sub>2</sub>, 5 mM magnesium acetate, 2 mM EDTA, 0.5 mM EGTA, 1 mM DTT)(all  
4 Sigma-Aldrich) with 30 strokes of a dounce tissue grinder (Wheaton). Large pieces of hCO were  
5 allowed to settle and homogenate was passed through pre-wetted 40 µm cell strainers (Becton  
6 Dickinson). Remaining hCO material in the douncer was resuspended in 4 ml and the douncing  
7 and filtering steps were repeated twice. All steps of the homogenization were performed on ice.  
8 The filtered homogenate was centrifuged at 1500 x g for 5 min at 4°C. Nuclei pellets were then  
9 re-suspended in PBS. A fraction of resuspended nuclei were then stained with Hoechst33324  
10 nuclear stain (1:500 dilution) and counted on a haemocytometer under fluorescent microscope.

11 The nuclei were then re-centrifuged (1500 x g for 5 min at 4°C) and resuspended at a density to  
12 load ~5,000 nuclei per sample. Cells were loaded into the Chromium Controller (10X Genomics)  
13 for gel bead emulsion (GEM) formation. Library preparation was conducted according to the  
14 manufacturer's recommended protocol using the Chromium Next GEM Single Cell 3' GEM,  
15 Library & Gel Bead Kit v3.1. Libraries were sequenced on the NextSeq 500/550 v2 (Illumina)  
16 with 150 bp reads and were sequenced to ~250,000 reads per cell.

17 Raw fastq reads for each sample were processed using CellRanger v3.1.0. Default options  
18 were used with CellRanger and a custom made pre mRNA reference using GRCh38 v3.0.0 was  
19 used to map the reads and for count quantification with the CellRanger counts tool. Following  
20 this, the counts were then aggregated together to create a single matrix that contained all the  
21 samples. Reads from the single nuclei sequencing data were aligned to a human pre-mRNA  
22 GRCh38 reference genome. All pre-processing and filtering steps of the datasets were  
23 subsequently carried out via the Python package Scanpy  
24 (<https://scanpy.readthedocs.io/en/stable/>) (Wolf et al., 2018). Briefly, there was an initial filtering  
25 step for genes that are expressed in 3 or more cells and cells with at least 200 detected genes,  
26 subsequently removing cells displaying high expression of mitochondrial genes using a cut-off  
27 of 4%. We then filtered out cells that had a count depth with a threshold of under 2,000 and  
28 higher than 40,000 to remove debris and potential doublets. Gene expression was subsequently  
29 normalized for each cell by total expression, scaled by 10,000 and log scaled. Highly variable  
30 genes were then identified for clustering. Leiden clustering with an initial resolution of 0.2 was  
31 performed to identify clusters within the data. A published human cardiac snRNA-seq dataset  
32 was then used to identify overlap of marker gene sets for main human heart cell types (e.g.  
33 cardiomyocytes, fibroblasts, epicardium, pericytes, etc) with the clusters of our dataset, and  
34 were labelled accordingly (Tucker et al., 2020). Further refined clustering was carried out on  
35 specific clusters that showed overlap of more than one of the various heart cell types.  
36 Visualization of the datasets was primarily carried out using nonlinear dimensionality reduction  
37 UMAP plots (Becht et al., 2019). In the snRNA-seq we note the lower than expected percentage  
38 of non-myocytes and the loss of endothelial cells (Mills et al., 2017; Voges et al., 2017),  
39 indicating the protocol requires further optimization for hCO samples.

## 40 **hCO comparison to bulk nuclei RNA sequencing data for PCA**

41 Nuclear RNA-seq dataset generated from sorted cardiomyocyte nuclei at two stages (fetal and  
42 adult) was obtained from BioProject ID: PRJNA353755 (Gilsbach et al., 2018). RNA-seq dataset  
43 was mapped to the human genome (hg38) using STAR aligner (version 2.7.3a). Annotations  
44 and genome files (hg38) were obtained from *Ensembl (release 102)*. Uniquely mapped reads  
45 were counted across genes with a program in Bioconductor R (Huber et al., 2015) package,  
46 featureCounts (version 2.0.1) (Liao et al., 2014). Subsequent analyses of the count data were  
47 performed in the R statistical programming language with the Bioconductor packages edgeR  
48 (Robinson et al., 2010) and the annotation package org.Hs.eg.db. In this dataset, only genes

1 with > 0.5 counts per million (CPM) in at least 4 samples were retained for statistical analysis.  
2 Additionally, ribosomal and mitochondrial genes as well as pseudogenes, and genes with no  
3 annotation (Entrez Gene identification) were removed before normalization and statistical  
4 analysis.

5 Principal component analysis (PCA) performed using the intersection of the 25% most highly  
6 variable genes of the snRNA-seq dataset and genes expressed in the bulk samples. Each of the  
7 two datasets were log transformed and scaled separately before running the dimensionality  
8 reduction method.

### 9 **Pro-inflammatory stimulation of hCO**

10 Cytokines (human) and factors were added individually and in combinations in WM: 100 ng/ml  
11 TNF, 10 ng/ml IL-1 $\beta$ , 100 ng/ml IFN- $\gamma$ , 100 ng/ml IL-6, 100 ng/ml IL-17A, 100 ng/ml G-CSF  
12 (Amgen), 10  $\mu$ g/ml poly(I:C) (HMW, Invivogen) or 1  $\mu$ g/ml LPS (from *Escherichia coli* strain  
13 0127:B8, Sigma) (all Peprotech unless noted). Additional concentrations were performed for the  
14 dose-response curves for TNF, IL-1 $\beta$ , IFN- $\gamma$  and poly(I:C) as indicated. The function of hCO was  
15 determined before addition of these factors as a baseline (time 0 h) and any changes  
16 normalized to the original baseline and to the control. The medium was not exchanged unless  
17 noted.

### 18 **Drug screening**

19 Compounds were sources from MedChem Express (unless noted) and dissolved at 10 mM in  
20 DMSO and vehicle controls used. A larger batch of INCB054329 was sourced from  
21 Selleckchem. The following compounds were used at 2 or 3 doses previously shown to have *in*  
22 *vitro* efficacy as per the reference papers for: JQ-1 (Selleckchem), INCB054329, ABBV-744,  
23 ruxolitinib, baricitinib, flavopiridol, SEL120-34A, BI-1347, and paroxetine hydrochloride.  
24 Additional compounds tested were molibresib, alobresib, and apabetalone. For some  
25 experiments apabetalone and RVX-2157 were sent blinded by Resverogix. Compounds were  
26 given at the time of pro-inflammatory factor addition, except for experiments with baricitnib and  
27 INCB054329 where recovery of function was also assessed by addition 24 h following addition  
28 of inflammatory factors.

### 29 **Linear regression of cytokine storm responses**

30 To determine factor effects, second order OLS linear regression was performed across all  
31 relevant samples using binary predictors (cytokine presence/absence) with force,  
32 relaxation/activation times as the outcome variables. p-values were determined using two-tailed  
33 t-tests. Normality (Shapiro-Wilks), heteroskedasticity (Breusch-Pagan), linearity (Harvey-Collier),  
34 multicollinearity (condition no.) and skewness/kurtosis (Jarque-Bera) were all checked with the  
35 respective tests. The coefficient of determination, R<sup>2</sup> (adjusted), was used to determine  
36 goodness of fit.

### 37 **SARS-CoV-2 K18-hACE2 mouse infection model**

38 Female K18-hACE2 mice were lightly anesthetized using isoflurane and 50  $\mu$ l of SARS-CoV-2  
39 at 5 x 10<sup>4</sup> TCID<sub>50</sub> per mouse was administered via intranasal inoculation (i.n.). On 1, 2 and 3  
40 dpi, INCB054329 or placebo control was administered via oral gavage. Mice were randomized  
41 and received either vehicle 30% (m/v) Kolliphor 15 HS (Sigma) in PBS or 2 mg per 30 g mouse  
42 body weight of INCB054329 at 20 mg/ml in the Kolliphor solution (66.7 mg/kg). At 4 or 5 d.p.i.,  
43 mice were euthanized by cervical dislocation and heart and lung tissue was fixed in 10%  
44 formalin for histology. At 4 d.p.i. lungs or hearts were homogenized in TRIzol for RNA extraction  
45 and stored at -80°C.

### 46 **Bulk RNA-seq from SARS-CoV-2 K18-hACE2 mouse infection model**

1 Illumina Stranded Total RNA Prep with Ribo-Zero Plus kits were used to prepare total RNA for  
2 sequencing. Libraries were sequenced on the NextSeq 500/550 v2 (Illumina) with 150 bp reads  
3 and were sequenced to ~60,000,000 reads per sample. Sequence reads were trimmed for  
4 adapter sequences using Cutadapt version 1.9 (Martin, 2011) and aligned using STAR version  
5 2.5.2a (Dobin et al., 2013) to the Mus Musculus GRCm38 assembly with the gene, transcript,  
6 and exon features of Ensembl (release 102) gene model, and the SARS-CoV-2 Ensembl  
7 genome assembly ASM985889v3. Quality control metrics were computed using RNA-SeQC  
8 version 1.1.8 (DeLuca et al., 2012) and expression was estimated using RSEM version 1.2.30  
9 (Li and Dewey, 2011). Protein-coding genes with > 5 CPM in  $\geq 5$  samples were kept for further  
10 analysis. Trimmed mean of M-values (TMM) normalization and differential expression analysis  
11 were performed using the R package edgeR (Robinson et al., 2010). The glmQLFit() function  
12 was used to fit a quasi-likelihood negative binomial generalised log-linear model to the read  
13 counts for each gene. Using the glmQLFTest() function, we conducted gene-wise empirical  
14 Bayes quasi-likelihood F-tests for a given contrast. Differentially expressed genes (DEGs) were  
15 determined using absolute log<sub>2</sub> fold change (logFC) > 0.5 and a false discovery rate (FDR) <  
16 0.05. The function prcomp() was used for principal component analysis (PCA). To estimate  
17 SARS-CoV-2 replication levels, sequence reads were aligned to SARS-CoV-2 only, and  
18 samtools (Li et al., 2009) version 1.9 was used to estimate the mapping rate of the reads to the  
19 viral genes.

## 20 **Comparison of different RNA-seq data**

21 KEGG and Encode TF analyses was performed using Enrichr (Kuleshov et al., 2016). BioMart  
22 Ensembl (release 102) was used to obtain the mouse to human orthologues used in  
23 comparisons. Networks were generated through the use of Ingenuity Pathway Analysis (IPA) on  
24 DEGs (QIAGEN Inc., <https://www.qiagenbioinformatics.com/products/ingenuity-pathway-analysis>).  
25 Up-Stream Regulators enriched in differentially expressed genes (no logFC cutoff,  
26 FDR < 0.05) in direct and indirect interactions were investigated by performing Core Analysis  
27 using (QIAGEN).

## 28 **Quantitative RT-PCR**

29 RNA was extracted using QIAGEN RNAeasy Micro Kits (Qiagen) or Trizol. cDNA synthesis  
30 using Superscript III (ThermoFisher Scientific) was carried out as per manufacturer's  
31 instructions. Final primer concentration of 200-250 nM was used and gene expression was  
32 assessed over 40 cycles on an Applied Biosciences Quant Studio 5. *18S* (for viral infection  
33 studies) or *HPRT1* or *hprt* (for gene expression) were used as internal controls.

## 34 **Mouse LPS cytokine storm model**

35 LPS (from *Escherichia coli* strain 0127:B8, Sigma) suspended in PBS was injected  
36 intraperitoneally into mice at 0.6 mg per 30 g mouse body weight for inflammatory cytokine,  
37 gene expression and survival studies and 1 mg was used for cardiac function studies. After the  
38 injection of LPS mice were randomized and received either vehicle 30% (m/v) Kolliphor 15 HS  
39 (Sigma) in PBS or 2 mg per 30 g mouse body weight of INCB054329 at 20 mg/ml in the  
40 Kolliphor solution (66.7 mg/kg). Treatment groups were blinded. For the experiments, mice were  
41 closely monitored and checked hourly for signs of sepsis.

## 42 **Mouse LPS plasma cytokine assays**

43 Serum cytokine levels (Ilf $\eta$ - $\gamma$ , Il-1 $\beta$  and Tnf) were determined with a CBA Flex Set Multiplex  
44 Cytokine Bead Array (BD Biosciences).

## 45 **Cardiac function in vivo**



1 Cardiac function was assessed using a Vevo 2100 ultrasound system fitted with a MS550D  
2 transducer, which has a 40 MHz center frequency (Fujifilm Visualsonics). Depilated mice were  
3 anaesthetized by isoflurane inhalation (1.5% at 1 L oxygen / min) delivered via a nose cone,  
4 kept warm on a heated stage, with respiration and heart rate monitored on ECG pads. B Mode  
5 images of the left ventricle were obtained from three short-axis views (proximal, mid and distal  
6 positions) and one parasternal long-axis view. Cardiac function parameters were calculated  
7 using the Simpson's tool in VevoLab analysis software v3.2.6 (Fujifilm Visualsonics). Briefly, the  
8 endocardial areas were traced from all three short-axis views, and the length of the ventricle  
9 was determined, in systole and diastole. These measurements were used to calculate ejection  
10 fraction.

## 11 **ELISA**

12 Human CNTI ELISA (RayBiotech), IFN- $\gamma$  (RnD Systems) and BNP ELISA (Abcam) was used as  
13 per manufacturer's instructions.

## 14 **Immunoblotting**

15 Protein from 2D hPSC-cardiac cell cultures was extracted using RIPA lysis buffer supplemented  
16 with protease inhibitor cocktail (Roche). Protein lysate concentration was estimated using a  
17 BCA assay (ThermoFisher Scientific). 15  $\mu$ g protein was resolved on 4-12% Bis-Tris  
18 polyacrylamide gel (Invitrogen) at 200V for 20 min and then transferred at 20 V for 1 h onto  
19 polyvinylidene difluoride (PVDF) membrane as per manufacturer's recommendations. After 1 h  
20 blocking using a 1:1 mix of LI-COR Odyssey Blocking Buffer (LI-COR Biotechnology) and PBS,  
21 membranes were incubated overnight on a platform shaker with primary antibodies for ACE2  
22 (1:200, R&D Systems, AF933) and GAPDH (1:1000, Cell Signaling Technologies, 97166S).  
23 Membranes were washed 5 times 3 minutes in PBS with 0.5% Tween, prior to incubation with  
24 IRDye® secondary antibodies (1:10000 for IRDye® 800CW Goat anti-Mouse IgG Secondary  
25 Antibody, 926-32210, and 680RD Donkey anti-Goat IgG Secondary Antibody, LI-COR  
26 Biotechnology, 925-68074) for 1 h at room temperature. Membranes were washed thoroughly (5  
27 x 3 min in PBS + 0.5% Tween) and were then imaged on a LI-COR Odyssey® CLx.  
28 Densitometry was performed using ImageStudio Lite (version 4).

## 29 **Flow Cytometry for ACE2 and SARS-CoV-2 spike binding assays**

30 hPSC-cardiac cells were differentiated as above, then plated at 100,000 per  $\text{cm}^2$  on gelatin  
31 coated plates and cultured for 5 days prior to infection experiments. For 2D cultured hPSC-CM,  
32 cells were pre-treated with MM with or without the indicated compounds or DMSO as a vehicle  
33 control for 3 days prior to assays. Cells were washed 2 x with PBS and detached using 0.25%  
34 Trypsin/EDTA (ThermoFisher Scientific) for ~10-15 min at 37°C. This was then neutralized with  
35 equivolume 3% bovine serum albumin (Sigma) in PBS (Binding Buffer). Cells were then  
36 centrifuged at 300 x g for 3 min and the supernatant removed. Cells were then incubated under  
37 different conditions.

38 For ACE2 assays the following was used for control, 1:200 Goat IgG Alexa Fluor 647-  
39 conjugated antibody, and assay 1:200 anti-human ACE2 AlexFluor 647 conjugated antibody  
40 and 1:200 anti-human CD90 (all RnD Systems) and were incubated for 60 min at 4°C in Binding  
41 Buffer. The cells were then washed in Binding Buffer, centrifuged at 300 x g for 3 min and  
42 supernatant removed. Both conditions were then incubated with 1:400 goat anti-mouse IgG  
43 secondary antibody conjugated to Alexa Fluor 555 (ThermoFisher Scientific) in Binding Buffer  
44 for 45 min at 4°C. The cells were then washed in Binding Buffer, centrifuged at 300 x g for 3 min  
45 and supernatant removed.

46 For SARS-CoV-2 binding assays the following was used for control, Binding Buffer only, and  
47 assay 1  $\mu$ g rSARS-CoV-2 Spike RB (per ~100,000 cells) and 1:200 anti-human CD90 (all RnD

1 Systems) and were incubated for 60 min at 4°C in binding buffer. The cells were then washed in  
2 Binding Buffer, centrifuged at 300 x g for 3 min and supernatant removed. Both conditions were  
3 then incubated with 1:400 F(ab')<sub>2</sub>-goat anti-human IgG Fc secondary antibody conjugated to  
4 Alexa Fluor 488 and 1:400 goat anti-mouse IgG secondary antibody conjugated to Alexa Fluor  
5 555 (both ThermoFisher Scientific) in Binding Buffer for 45 min at 4°C. The cells were then  
6 washed in binding buffer, centrifuged at 300 x g for 3 min and supernatant removed.

7 For flow cytometry cells were resuspended in 300 µl Binding Buffer, put through a 100 µm cell  
8 strainer to remove any clumps. Cells were assessed on a BD LSRFortessa Flow Cytometer,  
9 gated on FSC-A/SSC-A and then remove doublets using FSC-W/H, and CD90 assessed using  
10 YG586/15, ACE2 using R670/14 and SARS-CoV-2 spike protein B530/30. Negative controls  
11 were used to draw gates.

## 12 **hPSC-CM SARS-CoV-2 infection at QIMR Berghofer**

### 13 hPSC-cardiac cell infection

14 hPSC-cardiac cells were differentiated as above, then plated at 100,000 per cm<sup>2</sup> on gelatin  
15 coated plates and cultured for 5 days prior to infection experiments. For 2D cultured hPSC-CM,  
16 cells were pre-treated with 1 ml of MM with or without the indicated compounds or DMSO as a  
17 vehicle control for 3 days prior to infection. Media was removed before infecting cells with  
18 SARS-CoV-2 at MOI 0.01 for 1 h at 37°C. Cells were washed 3 x with MM and replaced with 1  
19 ml of MM with compounds or DMSO. For intracellular RNA cells were washed 3x with PBS  
20 before harvesting RNA in Trizol. For supernatant experiments 500 µl of supernatants were  
21 harvested each day and replaced with 500 µl of media. Supernatants were frozen at -80°C until  
22 they were titred. Titration was performed in Vero cell monolayers in 96 well plates: inoculated  
23 serial 10-fold dilutions in quadruplicate and incubated for 4 days. Cytopathic effect recorded and  
24 virus titre in log<sub>10</sub> TCID<sub>50</sub>/ml recorded.

### 25 Imaging

26 For immunostaining cells or hCO were fixed in 4% paraformaldehyde and stained. Cells were  
27 stained with primary antibodies Nucleocapsid protein SARS-CoV-2 (1:200, 40143-MM05, Sino  
28 Biological) and cardiac troponin T (1:400, ab45932, Abcam) in Blocking Buffer at 4°C for 2 h for  
29 2D or overnight for hCO on a rocker. Cells were washed twice with Blocking Buffer and labelled  
30 with secondary antibodies goat anti-mouse IgG AlexaFluor 488 (1:400, A-11001) and goat anti-  
31 rabbit IgG AlexaFluor 555 (1:400, A-21428) and Hoechst3332 (all ThermoFisher Scientific) at  
32 4°C for 2 h for 2D or overnight for hCO on a rocker. Cells were again washed with Blocking  
33 Buffer twice and then put into PBS and were imaged using a Leica Thunder microscope.

### 34 Quantitative RT-PCR

35 Performed as described above.

## 36 **hPSC-CM SARS-CoV-2 infection experiments at The Peter Doherty Institute**

### 37 hPSC-CM infection

38 The human embryonic stem cell line HES3 NKX2-5<sup>eGFP/w</sup> was used for viral infection studies in  
39 2D monolayer cultures (Elliott et al., 2011). Cardiac cells were differentiated as previously  
40 described (Anderson et al., 2018), frozen at day 10 following differentiation and stored at -80°C.  
41 Cardiac cells were subsequently thawed in RPMI+B27 media (RPMI 1640 supplemented with  
42 2% B-27 Supplement minus vitamin A, 1% GlutaMAX and 1% Penicillin/Streptomycin- All from  
43 ThermoFisher Scientific) with Rock inhibitor (Selleck Chemicals) for 24 h. Cardiac cells were  
44 then maintained in RPMI + B27 media for an additional 2 days, enriched for cardiomyocytes  
45 with lactate purification media - DMEM, no glucose, no glutamine, no phenol red supplemented  
46 with 1% GlutaMAX and 1% Penicillin/Streptomycin (Thermo Fisher Scientific) and 5 mM Sodium



1 L-Lactate (Sigma Aldrich) for 2 days, and subsequently cultured in MM from day 15 to day 23  
2 post differentiation prior to viral infection.

3 The media was removed from cultures of cardiac myocytes and Vero cells in 24 well plates.  
4 Vero cell plates were washed with 1ml of serum free media per well. Media was removed, wells  
5 were inoculated with  $10^4$  TCID<sub>50</sub> of virus (MOI = 0.01) in 100  $\mu$ l serum free media and incubated  
6 for 1 h at room temperature. The inoculum for Vero cells incorporated TPCK-treated Trypsin  
7 (Worthington Biochemical Corporation) at 1  $\mu$ g/ml. Following virus adsorption, the inoculum was  
8 removed and replaced with 500  $\mu$ l media. After ~10 minutes, this was removed and stored at -  
9 80°C as the day 0 sample. 500  $\mu$ l per well of media was replenished and plates were incubated  
10 at 37°C in 5% CO<sub>2</sub>. Each day from day 1 through 6 post-infection, 500  $\mu$ l of culture supernatant  
11 was harvested and replenished with 500  $\mu$ l of media. Supernatants were stored at -80°C till they  
12 were titrated.

### 13 Virus titration

14 The amount of infectious virus present in the samples was assayed in Vero cell monolayers in  
15 96 well plates. Samples inoculated into 4 wells were diluted serially in 10-fold dilutions in serum  
16 free media containing 1  $\mu$ g/ml TCPK-treated Trypsin and incubated for at 37°C in 5% CO<sub>2</sub>.  
17 Cytopathic effect was scored on day 4 post-infection and virus titre expressed in log<sub>10</sub>  
18 TCID<sub>50</sub>/ml. The lower limit of detection was 1.7 log<sub>10</sub> TCID<sub>50</sub>/ml.

### 19 **SOMAscan™ Proteomic Analysis**

20 SOMAscan™ proteomic technology uses Somamers as an affinity reagent (Somalogic Inc.).  
21 Plasma samples from 47 patients from each group that received apabetalone or placebo in the  
22 ASSURE trial were analysed and LGALS3BP quantified (Nicholls et al., 2016).

### 23 **Data reproducibility and statistical analysis**

24 hCO force experiments were performed on quality controlled hCO (proper formation around the  
25 poles, non-arrhythmic, no broken arms, no necking (Mills et al., 2017) across multiple  
26 experiments with multiple cell line combinations to ensure reproducibility. Automated force  
27 analysis removes the requirement for blinding of hCO experiments. Personnel performing the  
28 animal experiments and analyses were blinded to the conditions or treatments. Statistics were  
29 performed using GraphPad Prism v8 unless noted.

### 30 **REFERENCES**

31 Anderson, D.J., Kaplan, D.I., Bell, K.M., Koutsis, K., Haynes, J.M., Mills, R.J., Phelan, D.G.,  
32 Qian, E.L., Leitoguinho, A.R., Arasaratnam, D., *et al.* (2018). NKX2-5 regulates human  
33 cardiomyogenesis via a HEY2 dependent transcriptional network. *Nature communications* 9,  
34 1373.  
35 Becht, E., McInnes, L., Healy, J., Dutertre, C.-A., Kwok, I.W.H., Ng, L.G., Ginhoux, F., and  
36 Newell, E.W. (2019). Dimensionality reduction for visualizing single-cell data using UMAP.  
37 *Nature biotechnology* 37, 38-44.  
38 Cox, J., and Mann, M. (2008). MaxQuant enables high peptide identification rates, individualized  
39 p.p.b.-range mass accuracies and proteome-wide protein quantification. *Nature biotechnology*  
40 26, 1367-1372.  
41 DeLuca, D.S., Levin, J.Z., Sivachenko, A., Fennell, T., Nazaire, M.D., Williams, C., Reich, M.,  
42 Winckler, W., and Getz, G. (2012). RNA-SeQC: RNA-seq metrics for quality control and process  
43 optimization. *Bioinformatics (Oxford, England)* 28, 1530-1532.  
44 Deutsch, E.W., Csordas, A., Sun, Z., Jarnuczak, A., Perez-Riverol, Y., Ternent, T., Campbell,  
45 D.S., Bernal-Llinares, M., Okuda, S., Kawano, S., *et al.* (2017). The ProteomeXchange

- 1 consortium in 2017: supporting the cultural change in proteomics public data deposition. *Nucleic*  
2 *Acids Res* **45**, D1100-d1106.
- 3 Dobin, A., Davis, C.A., Schlesinger, F., Drenkow, J., Zaleski, C., Jha, S., Batut, P., Chaisson,  
4 M., and Gingeras, T.R. (2013). STAR: ultrafast universal RNA-seq aligner. *Bioinformatics*  
5 (Oxford, England) **29**, 15-21.
- 6 Elliott, D.A., Braam, S.R., Koutsis, K., Ng, E.S., Jenny, R., Lagerqvist, E.L., Biben, C.,  
7 Hatzistavrou, T., Hirst, C.E., Yu, Q.C., *et al.* (2011). NKX2-5(eGFP/w) hESCs for isolation of  
8 human cardiac progenitors and cardiomyocytes. *Nature methods* **8**, 1037-1040.
- 9 Gilsbach, R., Schwaderer, M., Preissl, S., Grüning, B.A., Kranzhöfer, D., Schneider, P.,  
10 Nührenberg, T.G., Mulero-Navarro, S., Weichenhan, D., Braun, C., *et al.* (2018). Distinct  
11 epigenetic programs regulate cardiac myocyte development and disease in the human heart in  
12 vivo. *Nature communications* **9**, 391.
- 13 Huber, W., Carey, V.J., Gentleman, R., Anders, S., Carlson, M., Carvalho, B.S., Bravo, H.C.,  
14 Davis, S., Gatto, L., Girke, T., *et al.* (2015). Orchestrating high-throughput genomic analysis with  
15 Bioconductor. *Nature methods* **12**, 115-121.
- 16 Hudson, J., Titmarsh, D., Hidalgo, A., Wolvetang, E., and Cooper-White, J. (2012). Primitive  
17 cardiac cells from human embryonic stem cells. *Stem cells and development* **21**, 1513-1523.
- 18 Humphrey, S.J., Karayel, O., James, D.E., and Mann, M. (2018). High-throughput and high-  
19 sensitivity phosphoproteomics with the EasyPhos platform. *Nature Protocols* **13**, 1897-1916.
- 20 Johnson, B.J., Le, T.T., Dobbin, C.A., Banovic, T., Howard, C.B., Flores Fde, M., Vanags, D.,  
21 Naylor, D.J., Hill, G.R., and Suhrbier, A. (2005). Heat shock protein 10 inhibits  
22 lipopolysaccharide-induced inflammatory mediator production. *The Journal of biological*  
23 *chemistry* **280**, 4037-4047.
- 24 Kuleshov, M.V., Jones, M.R., Rouillard, A.D., Fernandez, N.F., Duan, Q., Wang, Z., Koplev, S.,  
25 Jenkins, S.L., Jagodnik, K.M., Lachmann, A., *et al.* (2016). Enrichr: a comprehensive gene set  
26 enrichment analysis web server 2016 update. *Nucleic acids research* **44**, W90-W97.
- 27 La Linn, M., Bellett, A.J., Parsons, P.G., and Suhrbier, A. (1995). Complete removal of  
28 mycoplasma from viral preparations using solvent extraction. *J Virol Methods* **52**, 51-54.
- 29 Li, B., and Dewey, C.N. (2011). RSEM: accurate transcript quantification from RNA-Seq data  
30 with or without a reference genome. *BMC Bioinformatics* **12**, 323.
- 31 Li, H., Handsaker, B., Wysoker, A., Fennell, T., Ruan, J., Homer, N., Marth, G., Abecasis, G.,  
32 and Durbin, R. (2009). The Sequence Alignment/Map format and SAMtools. *Bioinformatics*  
33 (Oxford, England) **25**, 2078-2079.
- 34 Liao, Y., Smyth, G.K., and Shi, W. (2014). featureCounts: an efficient general purpose program  
35 for assigning sequence reads to genomic features. *Bioinformatics (Oxford, England)* **30**, 923-  
36 930.
- 37 Martin, M. (2011). Cutadapt removes adapter sequences from high-throughput sequencing  
38 reads. *2011* **17**, 3.
- 39 Mills, R.J., Titmarsh, D.M., Koenig, X., Parker, B.L., Ryall, J.G., Quaife-Ryan, G.A., Voges, H.K.,  
40 Hodson, M.P., Ferguson, C., Drowley, L., *et al.* (2017). Functional screening in human cardiac  
41 organoids reveals a metabolic mechanism for cardiomyocyte cell cycle arrest. *Proceedings of*  
42 *the National Academy of Sciences* **114**, E8372-E8381.
- 43 Nicholls, S.J., Puri, R., Wolski, K., Ballantyne, C.M., Barter, P.J., Brewer, H.B., Kastelein, J.J.,  
44 Hu, B., Uno, K., Kataoka, Y., *et al.* (2016). Effect of the BET Protein Inhibitor, RVX-208, on  
45 Progression of Coronary Atherosclerosis: Results of the Phase 2b, Randomized, Double-Blind,  
46 Multicenter, ASSURE Trial. *American journal of cardiovascular drugs : drugs, devices, and other*  
47 *interventions* **16**, 55-65.
- 48 Orlova, V.V., van den Hil, F.E., Petrus-Reurer, S., Drabsch, Y., Ten Dijke, P., and Mummery,  
49 C.L. (2014). Generation, expansion and functional analysis of endothelial cells and pericytes  
50 derived from human pluripotent stem cells. *Nature protocols* **9**, 1514-1531.

- 1 Robinson, M.D., McCarthy, D.J., and Smyth, G.K. (2010). edgeR: a Bioconductor package for  
2 differential expression analysis of digital gene expression data. *Bioinformatics* (Oxford, England)  
3 26, 139-140.
- 4 Tucker, N.R., Chaffin, M., Fleming, S.J., Hall, A.W., Parsons, V.A., Jr, K.C.B., Akkad, A.-D.,  
5 Herndon, C.N., Arduini, A., Papangeli, I., *et al.* (2020). Transcriptional and Cellular Diversity of  
6 the Human Heart. *Circulation* 0.
- 7 Tyanova, S., and Cox, J. (2018). Perseus: A Bioinformatics Platform for Integrative Analysis of  
8 Proteomics Data in Cancer Research. *Methods in molecular biology* (Clifton, NJ) 1711, 133-  
9 148.
- 10 Voges, H.K., Mills, R.J., Elliott, D.A., Parton, R.G., Porrello, E.R., and Hudson, J.E. (2017).  
11 Development of a human cardiac organoid injury model reveals innate regenerative potential.  
12 *Development* (Cambridge, England) 144, 1118-1127.
- 13 Wolf, F.A., Angerer, P., and Theis, F.J. (2018). SCANPY: large-scale single-cell gene  
14 expression data analysis. *Genome biology* 19, 15.

15

16