1   **Immunological and cardio-vascular pathologies associated with SARS-CoV-2 infection**

2   **in golden syrian hamster**

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Graphical Abstract
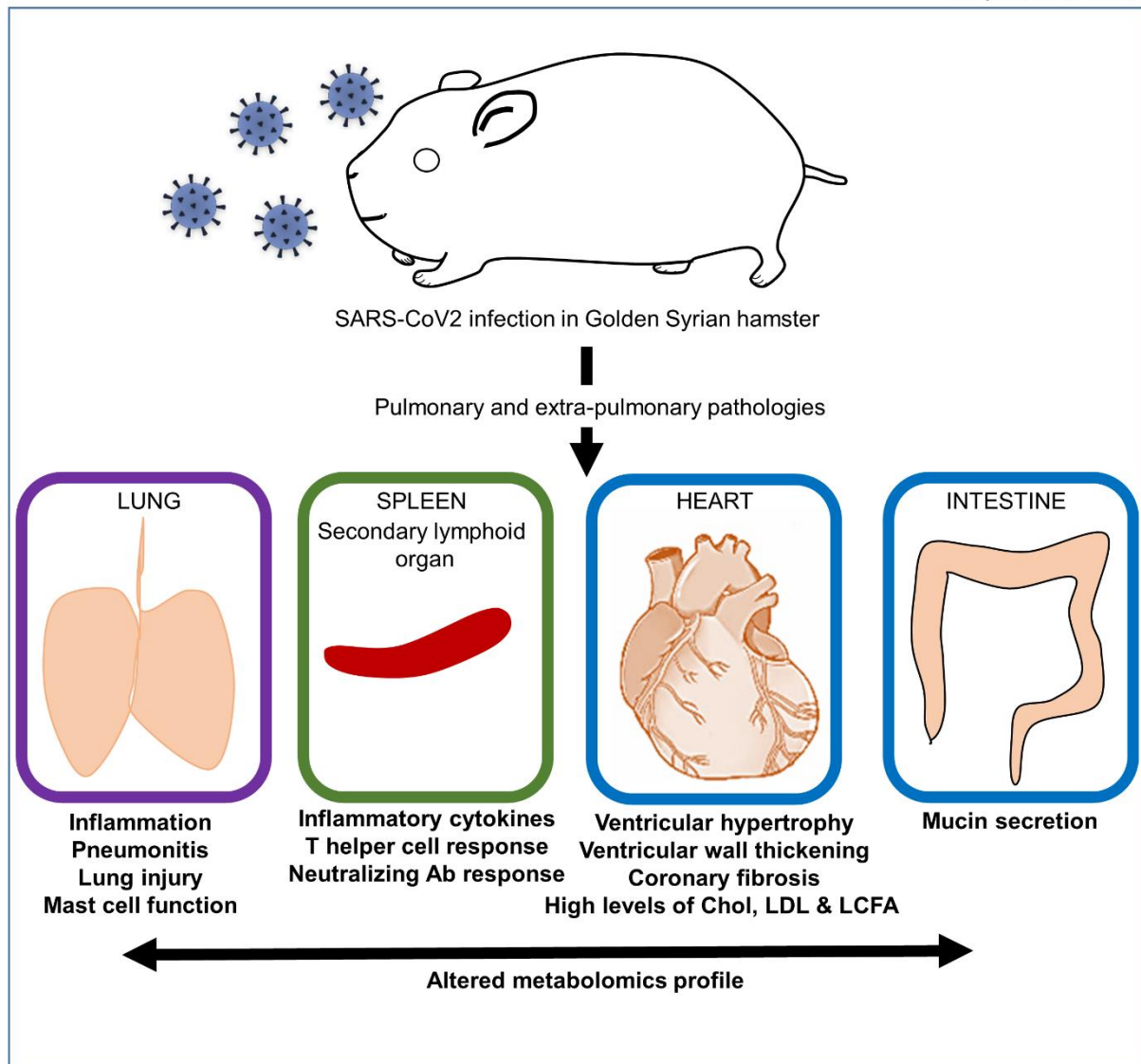
Summary

Severe acute respiratory syndrome coronavirus (SARS-CoV)-2 infection in golden Syrian hamster (GSH) causes lungs pathology and resembles to human corona virus disease (Covid-19). Extra-pulmonary pathologies and immunological parameters of SARS-CoV-2 infection remained undefined in GSH. Using *in silico* modelling, we identified the similarities between human and hamster angiotensin-converting enzyme-2 (ACE-2), neuropilin-1 (NRP-1) that bind to receptor-binding domain (RBD) and S1 fragment of spike protein of SARS-CoV-2. SARS-CoV-2 infection led to lung pathologies, and cardiovascular complications (CVC) marked by interstitial coronary fibrosis and acute inflammatory response. Serum lipidomic and

2

35   metabolomic profile of SARS-CoV-2-infected GSH revealed changes in serum triglycerides

36   (TG) and low-density lipoprotein (LDL), and alterations in metabolites that correlated with

37   Covid19. Together, we propose GSH as an animal model to study SARS-CoV-2 infection and

38   its therapy associated with pulmonary and extra-pulmonary pathologies.

39   **Keywords:** SARS-CoV2, Covid-19, hamster, cardio-vascular, immunology, lipidomics,

40   metabolomics, lung pathology.

Introduction

42   First reported in Wuhan, China in December 2019, SARS-CoV-2 has infected nearly 0.9 % of

43   the total world population with around 2.17 % mortality rate as of January 2021

44   (https://covid19.who.int/). Symptomatic COVID-19 is typically characterized by symptoms

45   ranging from mild to acute respiratory distress which is associated with cytokine storm (Chen

46   and Li, 2020; Verity et al., 2020). Other extra-pulmonary symptoms ranging from CVC,

47   inflammation, coagulopathies, multiple organ damage and neurological disorders have been

48   described in patients with mild to severe covid-19 infection (Chen and Li, 2020; Lamers et al.,

49   2020; Mao et al., 2020; Nishiga et al., 2020; Wu et al., 2020; Xiao et al., 2020; Xydakis et al.,

50   2020). The severity of covid-19 is governed by several host-virus factors such as dose of virus,

51   route of viral entry, gender, age and comorbidity (Chen and Li, 2020; Sungnak et al., 2020;

52   Wang et al., 2020). The RBD of spike (S) glycoprotein of SARS-CoV2 engages with

53   angiotensin-converting enzyme 2 (ACE2), a cellular receptor expressed on host cells, which

54   facilitates the viral entry into the host cell. Upon the engagement of ACE2 with SARS-CoV2,

55   cellular transmembrane protease 'serine 2' (TMPRSS2) mediates the priming of viral S protein

56   by cleaving at S1/S2 site induces the fusion of viral and host cellular membranes, thus

57   facilitating viral entry into the cells (Hoffmann et al., 2020). Lower levels of surface expression

58   of ACE2 in respiratory and olfactory epithelial cells, however indicates a possibility of

59   involvement of other co-receptors, which may be crucial for the infectivity of SARS-CoV2.

60   Neuropilin-1 (NRP-1), which is abundantly expressed on the surface of endothelial and

61   epithelial cells and binds to furin cleaved substrates, has been shown to facilitate SARS-CoV2

62   infectivity (Cantuti-Castelvetri et al., 2020; Daly et al., 2020). S1 fragment of the spike protein

63   cleaved by furin has been shown to directly bind to NRP1 expressed on the cell surface, and

64   thus promote the viral interaction with ACE2 (Cantuti-Castelvetri et al., 2020; Daly et al.,

65   2020).

66   Various non-human primates and small animal models have been described to study the

67   pathogenesis and transmission of SARS-CoV2 infection. SARS-CoV2 is inefficient in

68   infecting mice due to structural differences in mouse ACE2 as compared to human ACE2, thus

69   wild type mice are not the natural host to study SARS-CoV2 induced pathologies (Chan et al.,

70   2020). On the other hand, Syrian hamster, which was previously described as a model for

71   SARS-CoV infection, has recently gained much attention as a suitable model for studying

72   SARS-CoV2 infection (Chan et al., 2020; Sia et al., 2020). Remarkably, hamsters have been

73   shown to be infected through intranasal, oral and ophthalmic routes by SARS-CoV2 with

74   respectively descending lung viral load and pathologies (Imai et al., 2020). Moreover, airborne

75   transmission of SARS-CoV2 infection to uninfected animals and its reduction by the use of

76   surgical mask barrier between the cages corroborates with the clinical cases of SARS-CoV2

77   making Syrian hamster as a standard model for pre-clinical screening of effective therapeutics

78   and vaccine candidates. Despite several reports on hamsters describing the SARS-CoV2

79   pathology in the lung and pre-clinical evaluation of therapeutics against it, no study has focused

80   on cardiovascular complications associated with lipidomics and metabolomics changes and

81   related immunopathologies in SARS-CoV2 infected hamsters.

82   In the current study, we demonstrate using *in-silico* modelling, that host-virus interaction has

83   a striking similarity between humans and hamsters that involves TMPRSS2-mediated priming

4

84   of S protein, binding of S1 peptide with NRP-1 and subsequent binding of RBD to ACE2.

85   Intranasal infection of SARS-CoV2 in hamster resulted in high lung viral load with

86   significantly elevated lung injury on 2 and 4 dpi. Furthermore, our data shows aggressive

87   immune activation characterized by heightened expression of inflammatory cytokines on 2 and

88   4dpi. Strikingly, SARS-CoV2 infection in the hamster leads to significant interstitial coronary

89   fibrosis on day 7 and 14 dpi characterized by thickening of ventricular walls and

90   interventricular septum. These cardiovascular changes were associated with increased serum

91   triglycerides, low-density lipoprotein (LDL), cholesterol, high-density lipoprotein (HDL) on 4

92   dpi, and a marked increase in long chain fatty acids (LCFAs) on 7dpi. Finally, we found marked

93   changes in the metabolomics profile characterized by elevated N-acetylneuraminate and

94   allantoin which corroborated with the covid19 severe patients metabolomic profile. Taken

95   together, here we describe hamster as an excellent model to study cardiovascular complications

96   resulting from SARS-CoV2 infection and the underlying changes in lipidomics and

97   metabolomics serum profile which may serve as a suitable pre-clinical model to study not only

98   pulmonary damages associated with SARS-CoV2 infection but also extra-pulmonary

99   pathologies.

100   **Results**

101   ***In silico* interaction predicts similarities in the host-virus interaction between hamster**

102   **and human**

103   Interaction of SARS-CoV-2 RBD with host ACE2 receptor, TMPRSS protease and NRP-1 is

104   critical for virus entry into epithelial cells. However, the similarities of these interactions

105   between human (hu) and GSH (ha) remained unexplored. To compare residues of hu and ha

106   ACE2 that interact with RBD of SARS-CoV2, structure-guided sequence alignment was

107   performed with their respective sequences (**Figures S1A and 1A**). The overlay of crystal of

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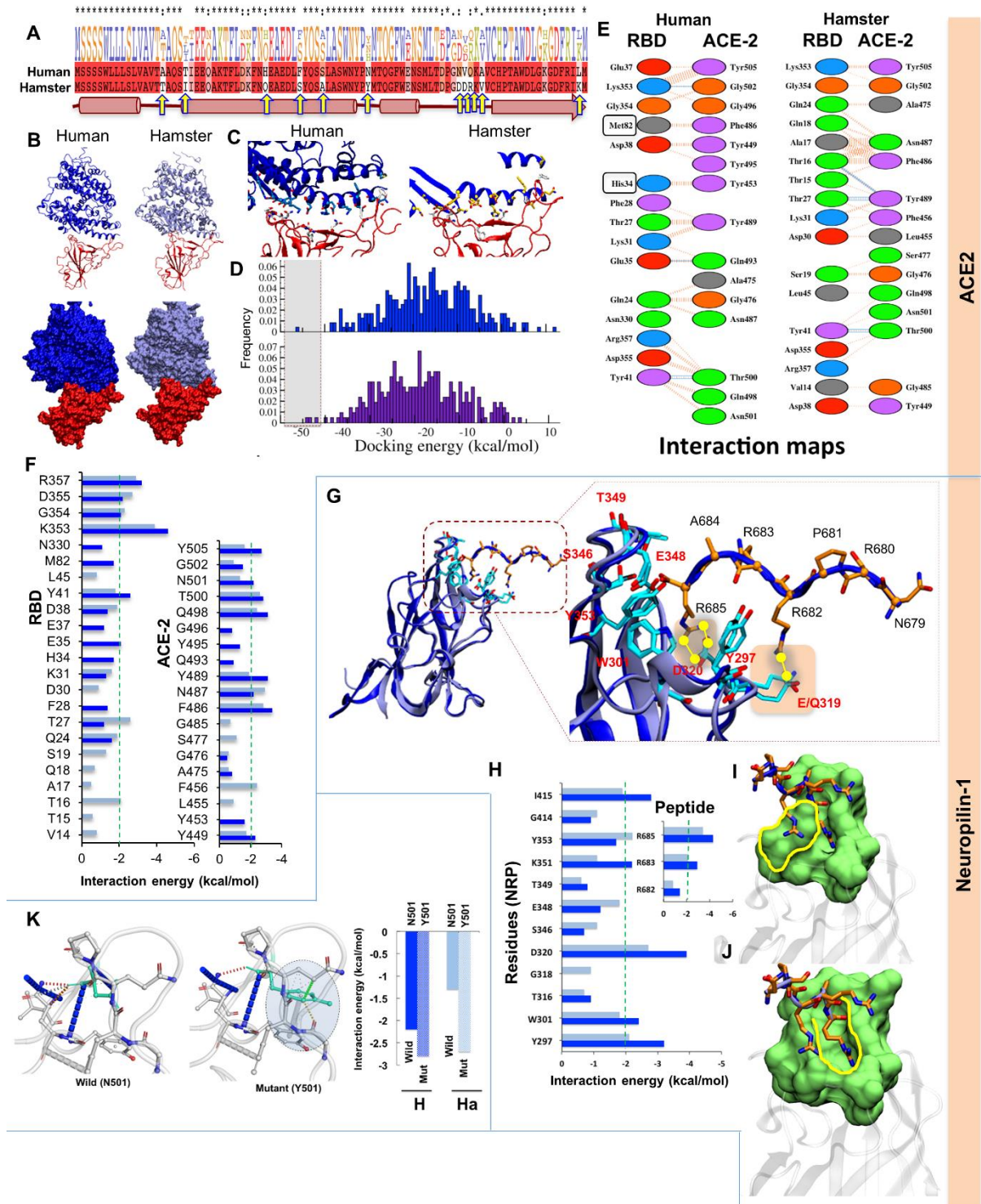
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109 huACE2 with modelled haACE2 was 1.2 Å, indicating the high structural resemblance. At

110 interface a total of 17 and 16 residues of the RBD are in contact with 20 residues of hu and ha

111   ACE2, respectively **(Figures 1B-1E).** The notable features that was common for both hu/ha

112   ACE2 interfaces with RBD was the networks of hydrophilic interactions like hydrogen bonds

113   (H-bonds) and salt bridges between Lys31 and Lys353 of ACE2 with Glu35 and Asp38 of

114   RBD **(Figures 1E and 1F)**. Aromatic residues of RBD, Tyr449, Tyr453, Phe456, Phe486,

115   Tyr489, Tyr495 and Tyr505, contributing to binding affinity remained common for both hu/ha

116   ACE2 **(Figure 1F)**. Together, SARS-CoV2 RBD with hu/ha ACE2 interfaces shared a

117   substantial similarities in the buried surface area, the number of interacting residues and

118   hydrophilic interaction. hu/ha TMPRSS2 structurally looks very similar (**Figure S1B**). NRP-

119   1, which bind to S1 fragment of spike protein, facilitates SARS-CoV2 entry within the cells

120   (Cantuti-Castelvetri et al., 2020; Daly et al., 2020). Hu and ha NRP-1, the C-terminal R685 of

121   the CendR peptide, interacts identically with S1 fragment of spike protein residues, Y297,

122   W301, T316, D320, S346, T349 and Y353 (**Figures 1G, 1H and 1I**). The R682 and R685

123   sidechains S1 fragment of spike protein engages with NRP-1 sidechains of Y297 and Y353 via

124   stacked cation-π interactions (**Fig. 1I and J**). In huNRP-1, the residues interacting from C-

125   endR are R685 making H-bonds with residues Asp320, Ile415, Tyr353, Thr349 and Ser346 of

126   S1 fragment while in haNRP-1, the R685 remains stable but with less number of H-bonds with

127   residues Trp297, Gly318, Asp320, Tyr353 and Thr349. The other common interactions in

128   terms of hydrophobic contacts are with residues Thr316, Gly414, and Trp301. Glu348 and

129   Lys351 contributed only in huNRP-1 while Ser346 in haNRP-1. Among mutations at RBD,

130   the cause of stable Y501 over N501 was also compared in hu/ha, as Y501 gain additional

131   bonding with tyrosine residues (41@ACE2, 355@RBD) and H-bond with Arg353 (**Figure**

132   **1K**).

133   **SARS-CoV2 infection results in pulmonary pathologies characterized by inflammation**

134   **and lung injury**

7

Figure 2

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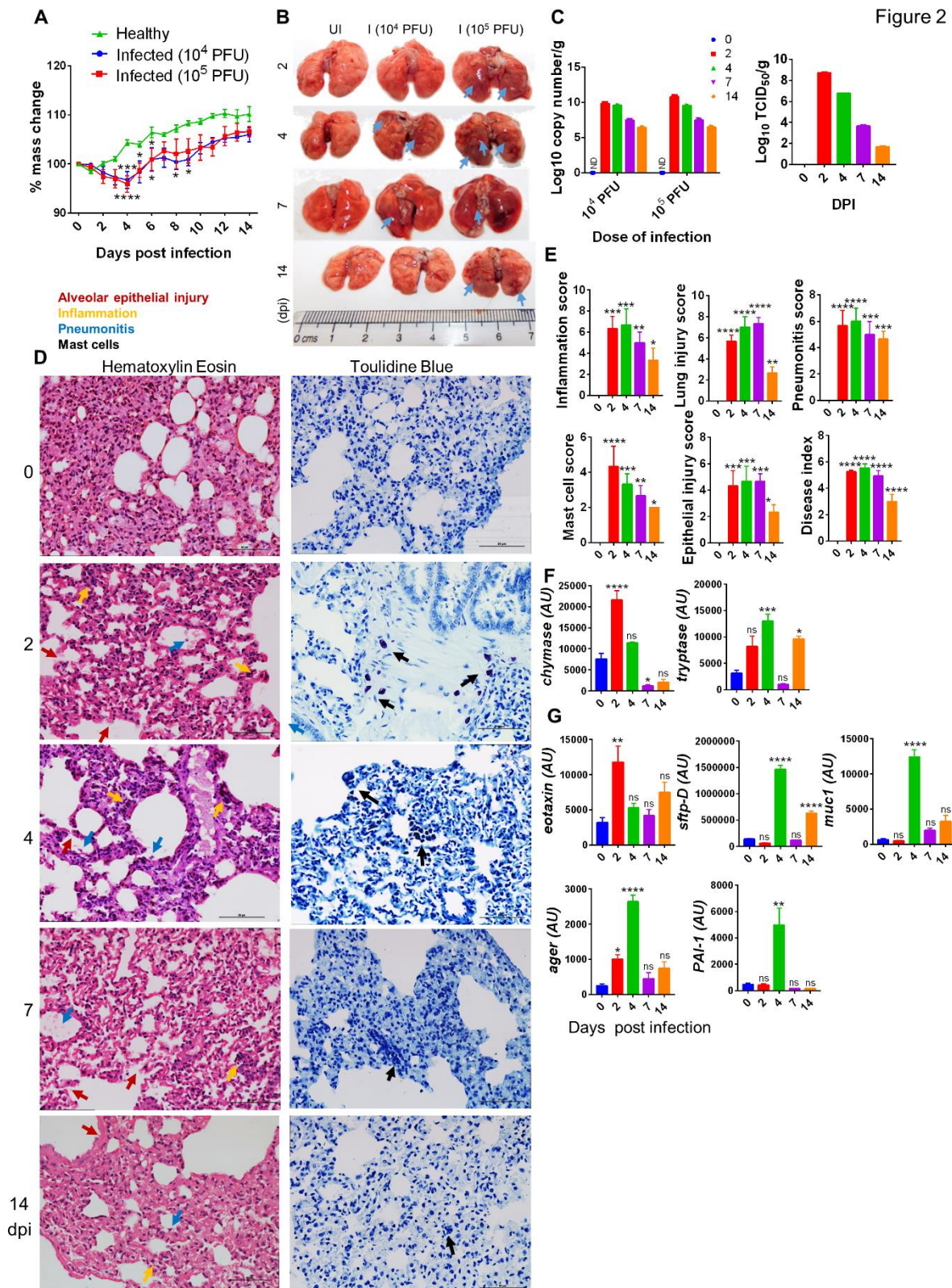
These *in silico* data provides a comparative molecular insight of SARS-CoV2 interaction and

entry in GSH showing similarities with human infection. Further, a decrease in body mass had

138   been reported in the initial phase of SARS-CoV2 infection. In line, intranasal SARS-CoV2

139   infection in GSH showed a gradual body mass loss peaking (~10% as compared to uninfected)

140   at 4 days post infection (dpi) with 10^4 and 10^5 plaque forming unit (PFU), in line with earlier

141   reports (**Figure 2A**) (Chan et al., 2020; Sia et al., 2020). This was accompanied by gross lungs

142   morphological changes characterized by pneumonitis regions with high viral load (~10.5 $\log_{10}$

143   copy number/g (lung mass) and $TCID_{50}$ at 2 dpi that gradually decreased at later time points

144   marking the recovery of the animals (**Figures 2B and 2C**). Covid-19 is marked by severity of

145   pneumonia, lung injury and influx of activated immune cells (Afrin et al., 2020; Chen and Li,

146   2020; Moore and June, 2020). In line with this, histological analysis revealed elevated disease

147   index score characterized by scores of inflammation, epithelial injury, lung injury and

148   pneumonitis with elevated infiltration of granulocytes and mast cells on 2, 4 and 7 dpi (**Figures**

149   **2D and 2E**). Consistently, mRNA expression of mast cells signature enzymes, chymase and

150   tryptase, was increased on 2 and 4 dpi corroborating mast cell enrichment (**Figure 2F**).

151   Furthermore, the expression of eotaxin, a CC chemokine and a potent chemoattractant for

152   airway eosinophils and mast cells that is particularly elevated in asthma and allergy conditions,

153   was upregulated at 2 dpi (**Figure 2G**). The expression of other lung injury markers like mucin

154   (muc-1: marker of respiratory infections), surfactant protein-D (sftp-D: acute lung injury

155   marker), advanced glycation end product (AGER: pro-inflammatory pattern recognition

156   receptor) and plasmonigen activator inhibitor-I (PAI-1: a key factor for lung fibrosis) was

157   upregulated at 4 dpi (**Figure 2G**). Collectively, these data indicated elevated lung pathologies

158   characterized by the upregulation of lung injury markers in SARS-CoV2 infection.

159   **SARS-CoV2 infection in hamster results in aggressive immune activation**

160   Most SARS-CoV2 infection studies in hamster have focused on pathological changes and viral

161   load in different organs. Moreover, Covid19 patients have shown an aggressive immune

162 activation leading to cytokine storm (Mathew et al., 2020; Moore and June, 2020; Verity et al.,


Figure 3

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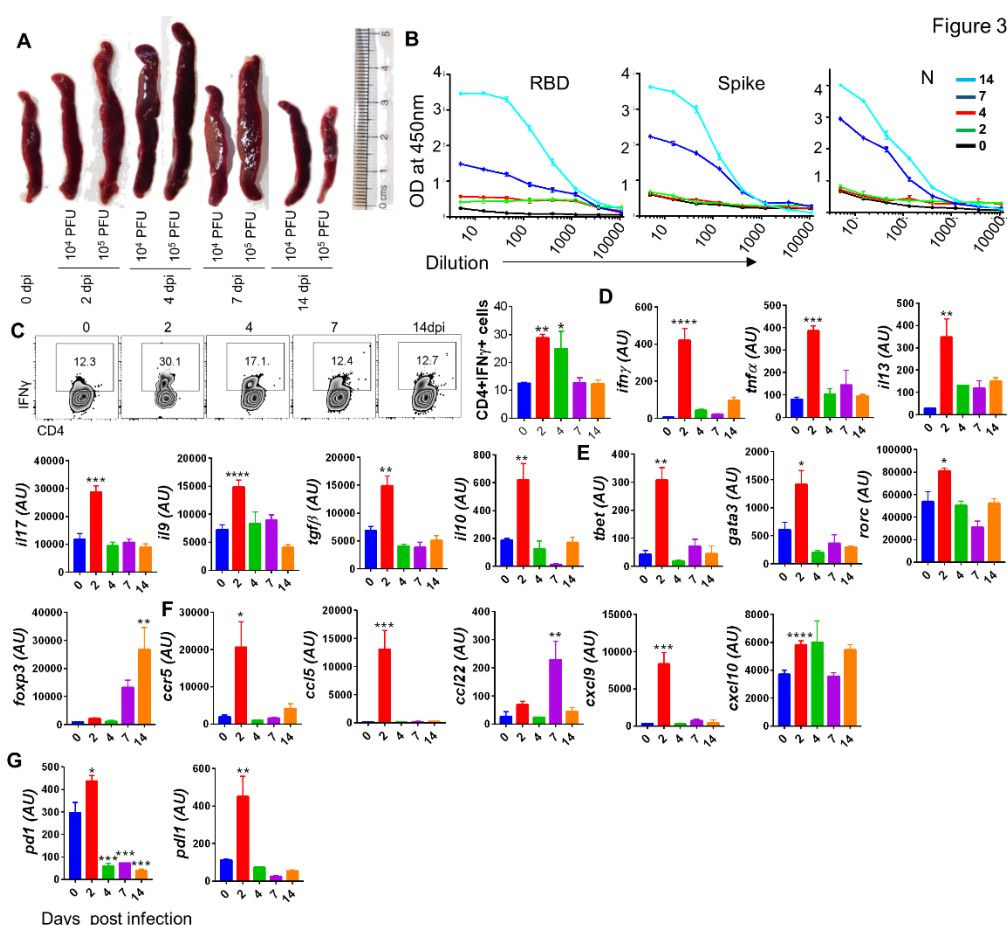
164 2020). Further, pathological changes of the lung and other organs have been attributed to the

165 acute inflammatory response caused by SARS-CoV2 infection. Consistently, profound

166 splenomegaly was observed in infected animals with ~60% increase in spleen size on 4 dpi for

167 10^5 PFU SARS-CoV2 infected hamsters as compared to the healthy control (**Figure 3A**),

168 indicating that splenomegaly could be an indicator of disease severity to define the efficacy of

169 therapeutics against SARS-CoV2 infection in GSH. To corroborate with the recovery response,

170 serum IgG titre for viral proteins, RBD, Spike and N protein was determined. As compared to

171 low dose, high dose of infection generate higher IgG titre against RBD (**Figure S2A**). The

172 RBD, Spike (prefusion S2P), and N protein ELISA detected the corresponding antibodies in

173 challenged animals as early as 7 dpi. The serum endpoint antibody titres against RBD, spike

174 and N protein were detected as early as 7 dpi with the titre 1:3610, 1:405 and 1:330

10

175 respectively, the titre of these antibodies was further enhanced to 1:8900, 1:550 and 1:660 at

176 14 dpi (**Figure 3B**), indicating for a steady recovery of infection. In addition, antigen-specific

177 frequency of $CD4^+IFN\gamma^+$ cells was elevated (~2.5 fold) without affecting the frequency of

178 $CD4^+$ T cells at 2 dpi as compared to uninfected GSH (**Figures 3C and S2B**). To define cellular

179 response particularly T helper (Th) cell response against SARS-CoV2 infection in GSH, we

180 performed mRNA expression of crucial cytokines, chemokines, transcription factors and

181 checkpoint inhibitors, and found an increased expression of Th1, Th2, Th9 and Th17 cells

182 signature cytokines at the peak of infection (i.e. 2 dpi) viz IFNγ, tumor necrosis factor (TNF)-

183 α; interleukin (IL)-13; IL-9, IL-17A along with their respective transcription factors, T-bet,

184 GATA3, RAR-related orphan receptor (ROR)-c (Grifoni et al., 2020; Mathew et al., 2020).

185 However, transcription factor forkhead box protein (Foxp)-3 was found to be elevated at 14

186 dpi suggesting activation of regulatory T (Tregs) cells at the senescence of infection.

187 Interestingly, Tregs cytokines IL-10 and transforming growth factor (TGF)-β sets in much

188 early at 2 dpi which maybe to counterbalance aggressive inflammatory reaction (**Figures 3D**

189 **and 3E**). We also found boosted expression of IL-6 and inducible nitric oxide synthase (inos)

190 at 2 dpi, indicative of cytokine storm and oxidative environment (**Figure S2C**). Further, we

191 found elevated expression of C-C chemokine receptor (CCR5) and C-C motif chemokine

192 ligand (CCL)-5, at 2 dpi, which regulates T cell function and chemotaxis. CCL-22, which is

193 essential for Treg-DCs cross talk, was elevated at 7 dpi. The expression of CXCL9 and CXCL-

194 10, which are crucial for effector T cell trafficking and activation, and have been described as

195 a biomarker for CVC, was upregulated at 2 dpi (**Figure 3F**). PD-1 and its ligand, PDL-1, were

196 also found to be upregulated at the onset of infection, suggesting induction of checkpoints to

197 suppress aggressive immune activation (**Figure 3G**). These data suggests that SARS-CoV2

198 infection in GSH induces an acute immune activation, chemotaxis and expansion of immune

199 cell population at the peak that resembles to a pattern of cytokine storm reported in Covid19

200 (Afrin et al., 2020; Moore and June, 2020).

Defining cardiovascular pathologies in SARS-CoV2 infected hamsters

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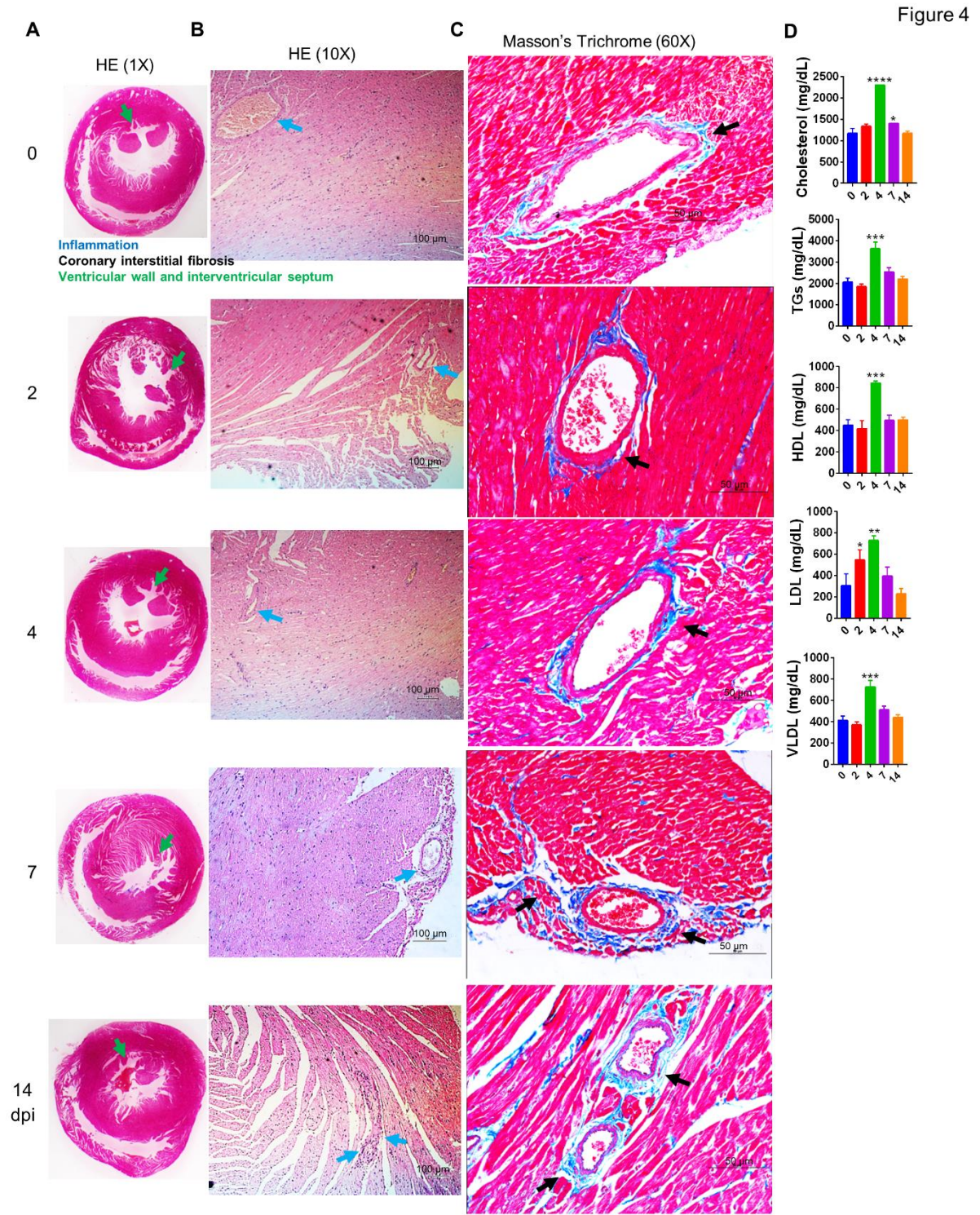
203 Myocardial injury occurs in around 25% of hospitilized covid-19 patients that includes

204 thromboembolic diseases and cases of arrhythmia (Giustino et al., 2020). Interplay between

205 host-virus interaction through ACE2-RBD and its impact on renin-angiotensin system (RAS)

206 and host immunological response are in central to the development of CVC (Giustino et al.,

207 2020). However, lack of suitable SARS-CoV2 animal model has been a limitation for studying

208 cardiovascular related complications arising. Acute inflammatory response, CXCL9/10

209 upregulation and oxidative environment made us rationale that SARS-CoV2 infection in GSH

210 may lead to CVC. Indeed, 7 dpi heart showed marked ventricular hypertrophy (**Figure S3A)**

211 associated with reduced ventricular space characterized by thickening of ventricular walls and

212 interventricular septum at 7 and 14 dpi (**Figure 4A**). The increased ventricular walls thickening

213 at 7 and 14 dpi was marked by increased inflammation surrounding the coronary artery and

214 elevated interstitial coronary fibrosis (**Figure 4B and 4C**). Interestingly, increased fibrosis is

215 an established pathophysiological stage in majority of CVC (Kong et al., 2014; Travers et al.,

216 2016). Further, since CVC are often linked with perturbed serum lipid profile, we reasoned that

217 cardio-vascular pathologies of SARS-CoV2 in hamsters maybe related to changes in

218 circulating lipid molecules. Indeed, serum lipid profile showed elevated cholesterol, TGs,

219 HDL, LDL and VLDL levels at 4 dpi (**Figure 4D**). Together, our results provide evidence for

220 CVC arising due to SARS-CoV2 infection in hamsters.

221 **Long chain fatty acid accumulation in SARS-CoV2 infection hamsters maybe responsible**

222 **for CVC**

223 Since our preliminary data revealed perturbations in lipid profile of SARS-CoV2 infected

224 hamster, we set out to do a detailed characterization of modulation in the abundance of lipid

225 moieties at different time points of infection through ultra-performance liquid chromatography-

226 tandem mass spectrometry (UPLC-MS/MS) to identify the potential lipid biomarkers

227 associated with cardio-vascular pathologies. Our LC-MS/MS data identified 831 lipid

228 molecules from the hamster serum samples **(Figure 5A)**. These 831 lipid molecules were 11

Figure 5

229

acylcarnitine (AcCar), 15 cholesterol ester (CE), 50 ceramide (CE), 2 Co-enzyme Q (CoQ), 31

diglycerides (DG), 13 ditetradecanoyl-sn-glycero-3-phosphoethanolamine (DMPE), 10

232   hexocylceramide (Hex-Cer), 31 lysophosphatidylcholine (LPC), 19

233   lysophosphatidylethanolamine (LPE), 7 lysophosphatidylinositol (LPI), 105 oxidized (Ox),

234   107 phosphatidylcholine (PC), 41 phosphatidylethanolamine (PE), 7 phosphatidylglycerol

235   (PG), 38 phosphatidylinositol (PI), 141 ether bond containing lipid (Plasmenyl), 4

236   phosphatidylserine (PS), 56 sphingomyelin (SM), 5 saturated lipids (SO), 135 triglycerides

237   (TG) (**Figure 5B**). Interestingly, 7 dpi showed the most distinct PCA distribution plot that

238   corroborated well with the severe 7 dpi cardio-vascular pathology of SARS-CoV2 infected

239   hamsters (unpublished data). Next in order to identify the uniquely perturbed lipid molecules

240   on 2, 4, 7 and 14 dpi hamster serum samples, we carried out t-test analysis compared to

241   uninfected (0 dpi) control. Resulting volcano plots for 2, 4, 7 and 14 dpi (top to bottom)

242   indicates log2 fold change of lipid molecules which were differentially regulated as compared

243   to the uninfected control (**Figure 5C**). Importantly, 275 lipids were differentially regulated as

244   identified by *t*-test analysis (described in methods section) of which 65 lipids molecules were

245   up-regulated while 208 were downregulated as identified by log2 fold change by volcano plots

246   (**Figure 5D and Table S1**). Further, heatmap for log2 fold change values for differentially

247   regulated lipids and for normalized relative abundance showed distinct clusters of lipids at

248   different time points (**Figures 5E and S3B**). Our lipidomics data show that unsaturated TGs

249   (4-11 double bonds (db)), plasmanly-PE (1-6 db) and plasmanyl-TGs (1-3 db) were uniquely

250   upregulated during infection while saturated TGs (1-3 db), DGs, oxidized lipids, PC, PE and

251   PI were down regulated. However, both saturated TGs and oxidized lipids, implicated in CVC,

252   were downregulated upon infection. Interestingly, upregulated TGs were mainly long chain

253   fatty acid (LCFAs) TGs while majority of downregulated TGs were medium chain fatty acid

254   (MCFAs) TGs that we reasoned as mediators of CVC in hamsters as increased LCFAs and

255   decreased MCFAs are associated with increased risks of heart diseases (Labarthe et al., 2008).

256   In addition to CVC, SARS-CoV2 can infect human gastro-intestinal tract cells expressing

257 ACE2 and cause associated pathologies (*15*). Moreover, severe SARS-CoV2 infection is also

258 known to cause multiple organ failure affecting kidney, liver, brain (*16*). To evaluate extra-

259 pulmonary pathophysiological of infected GSH, we carried out detailed histological analysis

260 of these major organs. Results show a significantly increase in mucin expression at 2, 4 and 7

261 dpi hamsters indicative of onset of inflammation and gastro-intestinal injury (**Figure S4A).**

262 However, there were no gross changes observed in brain, liver and kidney of infected as

263 compared to uninfected hamsters at 2 dpi (**Figure S4B).**

Metabolomics changes in SARS-CoV2 infection in hamsters and identification of potential metabolic biomarkers for disease pathophysiology

266 Emerging literature has suggested that the metabolomic changes are associated with Covid-19

267 patients (Shen et al., 2020). Attempts were made to identify signature metabolite that could

268 help in predicting the disease severity and progression. In order, to find a clinical correlation

269 of metabolomics signatures associated with SARS-CoV2 infection in hamster, we carried out

270 detailed metabolomics analysis for 2, 4, 7 and 14 dpi infected serum samples and compared it

271 with uninfected (0 dpi) control. A schematic flow for the metabolomics study is shown in

272 (**Figure 6A).** Corroborating with highest viral load and immune response, 2 dpi showed distinct

273 PCA distribution (**Figure S5A**). *t-test* analysis identified 82 differentially regulated metabolites

274 out of 334, of which 61 metabolites were upregulated and 22 were downregulated (described

275 in method section) (**Figures 6B and 6C).** The volcano plots indicating log2 fold change in

276 metabolites abundance vs uninfected control was exploited to plot heatmap showing unique

277 clusters (**Figures 6D, 6E and Table S2**). The relative abundance of individual serum samples

278 have been depicted as heatmap (**Figure S5B**). Collectively riboflavin pathway, arginine and

279 proline metabolism were significantly impacted by these differentially regulated metabolites

280 (**Figure 6F).** Next, correlation analysis with *Bo Shen et al 2020* metabolomics profile of sever

281 and non-severe covid19 patients depicted as (**Figure 6G)** (Shen et al., 2020) revealed an

Figure 6

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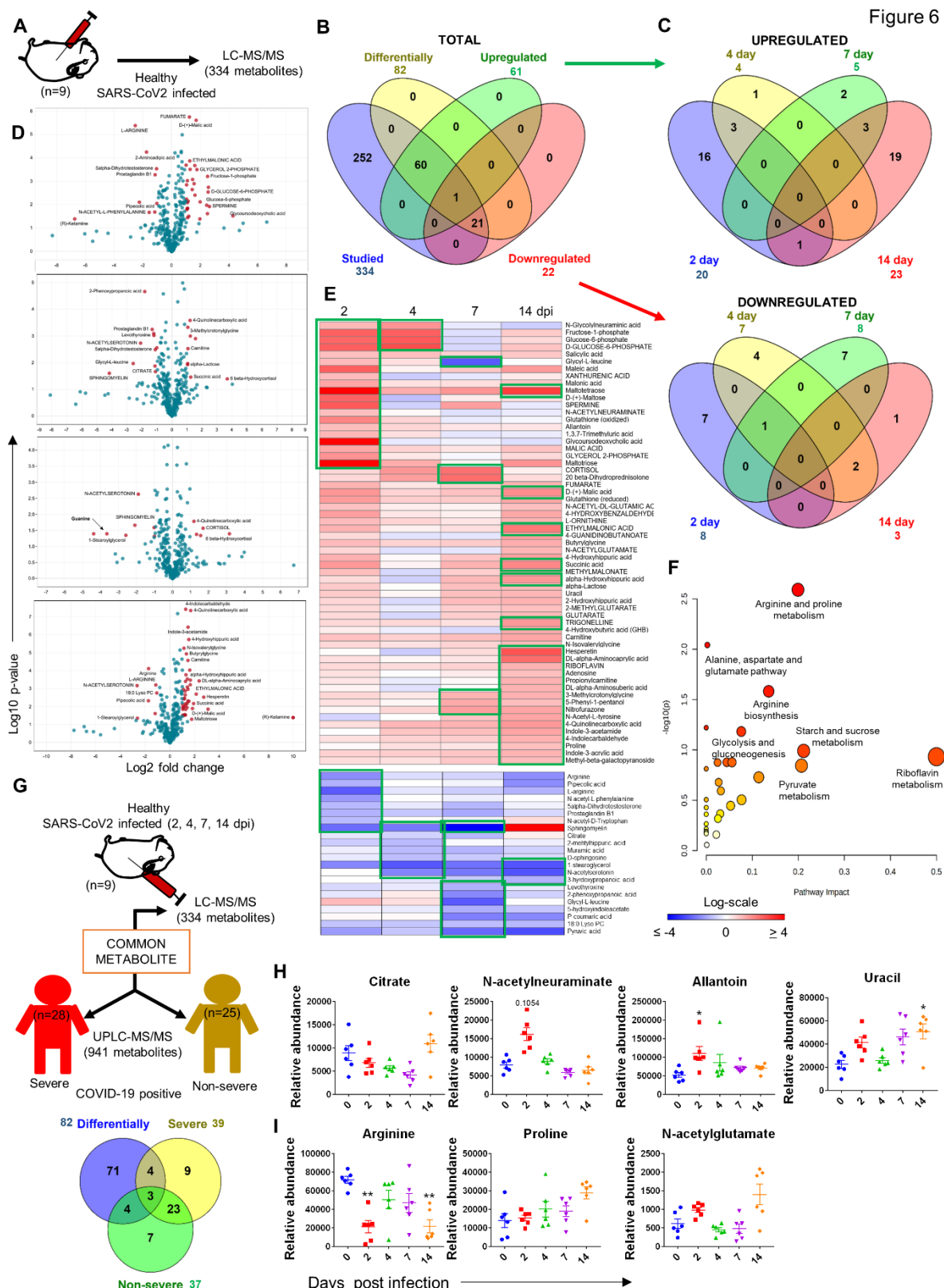
overlap of 21 metabolites with non-covid 19 metabolites, 37 with non-severe, 37 with severe

and 5 with severe vs non-severe metabolites shown as a Venn diagram (**Figures S5C and**

285   **S5D).** Importantly, 4 metabolites were common between severe covid19 patients and our

286   differentially regulated metabolites viz Citrate, N-acetylneuraminate, allantoin and uracil,

287   while 3 metabolites, Arginine, proline and N-acetylglutamate, were common between severe,

288   non-severe and our differentially regulated metabolites (**Figures 6G, 6H and 6I).** Remarkably,

289   N-acetlyneuraminate and Allantoin were highly upregulated at 2 dpi in our study and

290   corroborated well with severe disease profile in covid19 patients (**Figure 6H**). Moreover, both

291   N-acetylneuraminate and allantoin have been previously described as CVD biomarkers (Zhang

292   Lei et al., 2018). Together, based on the previously published study we identify and propose

293   biomarkers for disease severity in hamsters and also identify mediators for extra-pulmonary

294   manifestations.

295   **Discussion**

296   Pulmonary as well as extra-pulmonary clinical and histopathological changes associated with

297   SARS-CoV2 infection in human had been described by several groups (Moore and June, 2020;

298   Nishiga et al., 2020; Wang et al., 2020). Golden Syrian hamster as a pre-clinical SARS-CoV2

299   infection model was recently shown to mimic viral entry and replication naturally similar to

300   human and therefore is often favoured over transgenic mouse model (Chan et al., 2020; Imai

301   et al., 2020; Lee et al., 2020; Sia et al., 2020). The infection in hamster causes lung pathologies

302   which resembles that of the human lung marked by pneumonitis, inflammation and alveolar

303   epithelial injury (Chan et al., 2020; Lee et al., 2020; Sia et al., 2020). This is not a surprising

304   observation as hACE2 and haACE2 receptors had previously been shown to share major

305   sequence homology. Such similarities between hACE2 and haACE2 strongly point to

306   interaction with SARS-CoV2 receptor binding domain (RBD) structures with a similar trend

307   of binding affinity. Our *in silico* data on haACE2 extends the previous knowledge providing

308   greater insight into the interaction established between haACE2 and RBD protein. Moreover,

309   the interacting residues and changes in interaction energy is strikingly similar between hamster

310   and human and as a result the furin cleaved S subunit could enter the interacting groove of

311   haNRP-1 and prime it for the interaction with ACE2 in a manner similar to human. Importantly,

312   the host-virus interaction of SARS-CoV2 infection in hamster was found to be similar to human

313   infection for the recently reported SARS-CoV2 mutant. Since, *in silico* data was suggestive of

314   similarity in SARS-CoV2 interaction in hamsters and humans, which corroborates well with

315   intranasal infection and subsequent lung viral load in hamsters as reported by previous studies.

316   Since, increased viral load in the lungs is reflective of lung pathologies and have been shown

317   to induce inflammation and injuries in the clinical cohorts, we reasoned that consistent

318   pathophysiological changes maybe occurring in the hamsters as well. In line, we observed

319   profound pneumonitis, inflammation and alveolar epithelial injury upon histological

320   assessments of the lung. Our data show that lung pathologies long persists after the virus

321   clearance from the lung since we observed high pathological score on 7 dpi. This corroborates

322   with the clinical cases in which it has been observed that lung pathophysiology is perturbed

323   long after the recovery of infected individuals. We also found significant upregulation of the

324   expression of lung injury markers on 2 or 4 dpi corroborating the lung pathologies.

325   Viremia is an important pathological characteristic of SARS-CoV2 infection in humans, further

326   SARS-CoV2 is known to shed viral proteins which could be circulating in the serum long after

327   the virus is cleared off. The host responds to these pathogenic factors by producing IgG

328   antibodies for its effective neutralization. Therefore, we also evaluated the antibody titre for

329   viral proteins viz RBD, Spike and N protein in the sera of the infected hamsters. We found that

330   IgG response to SARS-CoV2 was most profound on 7 and 14 dpi which corroborates well with

331   the notion of antibody priming and expansion. An important arm of the host response against

332   SARS-CoV2 depends on immune recognition of the invading pathogen and its activation.

333   Interestingly, we also observed profound splenomegaly in infected animals on 2 and 4 dpi

334   pointing at an active and immune cells stimulation and proliferation. Further, numerous clinical

335   studies have shown that SARS-CoV2 infection is characterized by cytokine storm which is

336   responsible for lung pathologies and respiratory distress in severe cases. Important mediators

337   of inflammatory response such as IFNγ, IL6, IL17A, etc have been shown to be produced in

338   robust amount and drive the escalated immune regulation. Our data show dramatic increase in

339   Th1 cell response mediated by IFNγ for *in vitro* stimulated 2 dpi splenocytes. qPCR studies

340   revealed a similar activation pattern for several inflammatory cytokines and chemokines along

341   with immuno-regulatory transcription factors indicating that the immune activation sets in

342   quite early during infection and maybe playing a role in pathological damages associated with

343   lung and other organs during infection.

344   SARS-CoV2 can affect cardiovascular system which results in inflammation, endothelial

345   activation and microvascular thrombosis. The cardiovascular complications have particular

346   been reported in co-morbidity patients and may lead to life threatening condition. Since, there

347   is a lack of knowledge about the cardio-vascular complication arising from SARS-CoV2

348   infection in hamster we carried out a detailed histological analysis to monitor the changes

349   occurring in and around cardiomyocytes of the heart muscle. Interstingly, we found profound

350   thickening of the ventricular and interventricular septum with marked depression in ventricular

351   space capacity on 7 and 14 dpi heart samples. These changes were accompanied by deposition

352   of fibrous mass around coronary artery and inflammation which is believed to be one of the

353   major causes of cardio-vascular complication and arrythemia. Observation of cardio-vascular

354   complication on 7 and 14 dpi was a remarkable finding which hinted strongly at extra-

355   pulmonary damages caused by SARS-CoV2. It also pointed at soluble mediators of host or the

356   pathogen which could lead to cardio-vascular complication long after the virus is cleared off

357   from the lungs. One such important mediators of cardio-vascular diseases are serum lipid

358   molecules. This prompted us to carry out lipidomics profiling of the serum of the infected and

359   uninfected hamster samples. We found global changes in the lipid profile associated with

360 infection but quite remarkably 7 and 14 dpi samples produced the largest clusters of altered

361 lipid molecules which was in perfect agreement with out heart histology data. Interestingly,

362 SARS-CoV2 infected hamster serum samples showed an increase in LCFAs accumulation as

363 compared to MCFAs which have been described as biomarkers of CVC and heart failure.

364 In addition to lack of data on the extra-pulmonary changes associated with SARS-CoV2

365 infection in hamster, no studies have been done to evaluate the metabolomics changes

366 occurring in the hamster infected with SARS-CoV2 infection. Therefore we carried out both

367 reverse phase and HILIC phase LC-MS/MS analysis for metabolomics changes in infected

368 hamsters. Moreover, we also observed striking similarities between the metabolomics profile

369 of hamsters compared with the clinical database. When compared to the metabolomic profile

370 of severe and non severe Covid19 patients, 7 metabolites were found to be common in

371 hamsters. These metabolites were citrate, N-acetylneuraminate, allantoin, uracil. Proline,

372 arginine and N-acetylglumate out of which N-acetylneuraminate and allantoin showed positive

373 correlation with severe covid19 metabolomic profile with sharp peak in abundance at 2dpi.

374 Both allantoin and N-acetylneuraminate increased abundance have been shown to be

375 biomarkers for increased heart risk. Together, our data provides first insight into the key

376 metabolites which could be used as the biomarkers for the efficient and rapid evaluation of

377 SARS-CoV2 disease severity.

378 As of now hamster model for SARS-CoV2 has been described as a suitable model to study

379 lung pathologies and clinical parameter such as body wight changes. However, a robust

380 hamster animal model which mimics the histopathological changes in extra pulmonary regions

381 upon SARS-CoV2 infection has not been evaluated. Therefore, in the current study we provide

382 a detailed insight into the cardio-vascular and immunopathological changes associated with

383 SARS-CoV2 describing the lipidomic and metabolomics changes in the infected hamsters and

384   provide the first proof-of-concept (PoC) study for a robust pre-clinical model for pulmonary

385   and extra-pulmonary pathologies associated with SARS-CoV2 infection.

386   **Acknowledgments**

398

399   **Author Contributions**

400   Conceived, designed and supervised the study: AA; Designed and performed the experiments:

401   AA, ZAR, RD, SS; ABSL3 procedures: ZAR, RD, SS, AKP; *in silico* study: SA; qPCR primer

402   designing and analysis: ZAR; FACS: ZAR; Histology and analysis: ZAR, MRT;

403   Metabolomics: YK, ZAR, SKG; Lipidomics: YK, ZAR, SKG; ELISA: TS; Viral load: GPM;

404   Virus stock and TCID50: SSamal; Omnics analysis: AKY, SAgarwal, ZAR, RD, SS;

405   Contributed reagents/materials/analysis tools: AA; Wrote the manuscript: ZAR, AA.

406   **Declaration of Interests**

407   The authors declare no conflict of interest.

408   **Figure legends**

409   **Figure titles and legends**

410   **Figure 1. Computational analysis deciphering the structural and interaction level**

411   **insights of key host proteins, ACE2 (A to F), NRP-1 (G to J) and N501Y (K) involved in**

412   **SARS-CoV-2 infection. (A)** the sequence alignment of human and hamster ACE2 sequences.

413   Along with secondary structure the residual variations are highlighted in yellow arrows

414   indicating the differences among sequences. **(B)** crystal structure of human ACE2. The hACE2

415   (in blue), modelled haACE2 (in ice-blue), and RBD is in red. The surface view of h/ha ACE2

416   with RBD. **(C)** The residue level interaction map, ACE2, is rendered in cartoon and colored

417   for h(blue) and for hamster (ice-blue). The amino acid residues are coloured as per atom-type

418   such as O:red, N:blue, C:cyan/yellow/white. **(D)** The molecular docking data of ACE2 and

419   RBD interaction (in Histogram). The selective docked pose is shown in a dotted rectangular

420   box. **(E and F)** the interaction energies calculated at h/ha interfaces. The interacting residues

421   are aromatic:purple, polar:green, acidic:red and aliphatic:grey. The types of interaction below

422   <-3.0 Å distance are shown by blue lines and hydrophobic interactions are in orange dotted

423   lines. The bar graphs are the residue-wise interaction energies (in kcal/mol) for both RBD and

424   ACE2. The key residues (<-2.0 kcal/mol) are highlighted via green dotted lines. **(G)** The

425   overlay of h/ha NRP-1 along with CendR peptide, rendered in cartoon. The zoom-out view of

426   interacting interfaces of h/ha NRP-1 loop residues and CendR peptide residues. Peptide

427   residues are rendered in licorice and color-coded in atom-wise. The hydrogen bond forming

428   residues are shown by yellow lines. **(H)** the bar graph showing the residue-wise interaction

429   energy. **(I and J)** the binding groove of NRP-1 showing how nicely CendR peptides fit in h/ha.

430 **(K)** The N501 and Y501 interaction patterns in hACE2-RBD. The bar graph showing the

431 difference of 501 in h/ha in wild and mutant states.

432 **Figure 2. Pulmonary pathologies of SARS-CoV2 infected hamsters.** (A) % body mass

433 change. (B) Gross morphology of lungs showing pneumonitis region. (C) Lung viral load

434 expressed as Log10 copy number/g or log10 TCID50/g. (D) Microscopic images of HE and

435 TB stained lungs (E) histological scores (F & G) lung qPCR showing mean relative mRNA

436 expression with standard error mean (SEM). Alveolar epithelial injury (red), inflammation

437 (blue), pneumonitis (yellow) and mast cells (black). *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$,

438 ****$P < 0.0001$ (one-way anova).

439 **Figure 3. Immunological response against SARS-CoV2 infection in hamsters.** (A) Changes

440 in spleen length of SARS-CoV2 infected animals at different dpi as compared to uninfected

441 animals (B) Serum IgG titre against SARS-CoV2 viral proteins (C) FACS for IFNγ secretion

442 showing representative dot plots with % age frequency values and a bar graph showing mean

443 $\pm$ SEM. Relative mRNA expression of (D) cytokines, (E) transcription factors, (F) chemokines

444 and (G) checkpoint inhibitors in the spleen of infected vs uninfected hamsters. *$P < 0.05$, **P

445 < 0.01, ***$P < 0.001$, ****$P < 0.0001$ (one-way anova).

446 **Figure 4. Cardiovascular complications in the heart of SARS-CoV2 infected hamsters.** (A

447 & B) heart HE stain captured at 1X and 10X showing ventricular walls and interstitial septum

448 (green arrow) and inflammation around coronary artery (blue arrow) (B) heart MT stains

449 showing interstitial coronary fibrosis (black arrow). (C) serum gross lipid profile. *$P < 0.05$,

450 **$P < 0.01$, ***$P < 0.001$, ****$P < 0.0001$ (one-way anova).

451 **Figure 5. Serum lipid profile of SARS-CoV2 infected hamster.** (A) Scheme for lipidomics

452 analysis (B) classification of detected lipid in this study (C) volcano plot for 2, 4, 7 and 14 dpi

453 (D) Venn diagram for differentially regulated lipids (E & F) log2 fold change heatmap.

Figure 6. Serum metabolomics profile of SARS-CoV2 infected hamster. (A) Schematic representation for metabolomics. (B & C) Venn diagrams for differentially regulated. (D) volcano plot for 2, 4, 7 and 14 dpi. (E) heatmap for log2 fold change (F) pathways impacted. (G) Scheme and Venn diagram for correlation with Bo Shen et al 2020. (H) dot plot for relative abundance of metabolites common with severe profile (I) or severe vs non-severe profile. *P < 0.05 (one-way anova).

Star Methods

Animal Ethics and biosafety statement

6-8 weeks old female golden Syrian hamsters were acclimatized in biosafety level-2 (BSL-2) for one week and then infected in Animal BSL3 (ABSL-3) institutional facility. The animals were maintained under 12 h light and dark cycle and fed standard pellet diet and water ad libitum. All the experimental protocols involving the handling of virus culture and animal infection were approved by RCGM, institutional biosafety and IAEC animal ethics committee.

Virus preparation and determination of viral titers

SARS-Related Coronavirus 2, Isolate USA-WA1/2020 virus was used as challenge strain, which was grown and titrated in Vero E6 cell line grown in Dulbecco's Modified Eagle Medium (DMEM) complete media containing 4.5 g/L D-glucose, 100,000 U/L Penicillin-Streptomycin, 100 mg/L sodium pyruvate, 25mM HEPES and 2% FBS. The virus stocks were plaque purified and amplified at THSTI Infectious Disease Research Facility (Biosafety level 3 facility) as described previously (Harcourt et al., 2020; Mendoza et al., 2020).

SARS-CoV2 infection in golden Syrian hamster

Infection in golden Syrian hamsters were carried out as previously described (Chan et al., 2020; Sia et al., 2020). Briefly anesthetized through ketamine (150mg/kg) and xylazine (10mg/kg)

25

477   intraperitoneal injection and thereafter infection was established intranasally with 10^4 PFU

478   (100µl) or 10^5 PFU (100µl) of live SARS-CoV2 or with DMEM mock control inside ABSL3

479   facility.

Clinical parameters of SARS-CoV2 infection

481   All infected animals were housed for 14 days and their body mass was monitored as previously

482   described (Chan et al., 2020; Sia et al., 2020). 9 animals from each group were sacrificed on 2,

483   4, 7 and 14 days post infection (dpi) and their blood serum along with body organs such as

484   lungs, intestine, liver, spleen and kidney were collected. Serum samples were stored at -80 °C

485   until further use. Lung and spleen samples of infected and uninfected animals were compared

486   for any gross morphological changes. Lungs samples were homogenized in 2 ml DMEM media

487   and used for viral load and TCID50 determination. A section of lung, intestine along with other

488   organs were fixed in 10% formalin solution and used for histological studies. Spleen were

489   strained through 40 µm cell strainer with the help of syringe plunger and used for qPCR and

490   immunophenotyping studies.

Viral load

492   Homogenized lung samples of 2, 4, 7, 14 dpi along with uninfected controls were centrifuged

493   for 10 min at 4°C and their supernatant was collected. 100 µl of supernatant from each sample

494   was then mixed with 900 µl of Trizol reagent (Invitrogen) and RNA isolation was carried out

495   as per manufacturer's protocol. Copy number estimation of SARS-CoV-2 RNA has been

496   described previously (Anantharaj et al., 2020). Briefly, 200 ng of RNA was used as template

497   for reverse transcription polymerase chain reaction (RT-PCR). CDC-approved commercial kit

498   was used for of SARS-CoV-2 N gene: 5′-GACCCCAAAATCAGCGAAAT-3′ (Forward), 5′-

499   TCTGGTTACTGCCAGTTGAATCTG-3′   (Reverse)   and   5′-FAM-

500   ACCCCGCATTACGTTTGGTGGACC-BHQ1 -3′ (Probe) detection and sub-genomic RNA

501 copy numbers were estimated by $\Delta\Delta Ct$ method. Hypoxanthine-guanine

502 phosphoribosyltransferase (HGPRT) gene was used as an endogenous control for

503 normalization through quantitative RT-PCR. The region of N gene of SARS-CoV-2 starting

504 from 28287 – 29230 was cloned into pGEM®-T-Easy vector (Promega). This clone was

505 linearized using SacII enzyme and *in vitro* transcribed using the SP6 RNA polymerase

506 (Promega). The transcript was purified and used as a template for generating standard curve to

507 estimate the copy number of SARS-CoV-2 N RNA (Anantharaj et al., 2020).

508 **TCID50**

509 For TCID50 determination, 50 µl of homogenized lung supernatant samples were incubated

510 with confluent Vero-E6 cells in 96-well plates as described previously (Chan et al., 2020).

511 Briefly, serial dilutions of 10-folds from each sample were added to the wells containing Vero-

512 E6 cells monolayer in DMEM media in quadruplicate. After 4 days of incubation, TCID50 was

513 determined through Reed and Münch endpoint method with one TCID50 equivalent to the

514 amount of virus required to cause cytopathic effect in 50% of inoculated wells. TCID50 values

515 were expressed as TCID50/ gram of lung mass. For determination of virus titers, tissue samples

516 (lungs) were homogenized to a final 10% (w/v) suspension in DMEM medium with gentamicin

517 (Invitrogen, USA). The tissue samples from 2 to 14 dpi were used to infect Vero E6 cell

518 monolayers in 48-plates as described previously. Virus titers were expressed as $TCID_{50}$ per

519 gram of tissue

520 **ELISA**

521 The binding-antibody response to SARS CoV-2 post infection was measured using ELISA

522 based platform as described earlier (Shrivastava et al., 2018). The antibody response was

523 measured against Spike, RBD and N protein post virus challenge at different time point.

524 Soluble spike prefusion trimeric protein (S2P) (Wrapp et al., 2020), in house RBD (Ha et al.,

525   2020) and N proteins were coated on Maxisorp plates (Nunc) at different protein concentrations

526   (Spike S2P trimer; 1 µg/ml, RBD; 2µg/ml and N protein at 0.5ug/ml) in 1X

527   carbonate/bicarbonate buffer, pH 9.6 overnight at 4 °C. The following day, the plates were

528   blocked using 250 µl of PBS containing 5 % skimmed milk (blocking buffer). Three fold

529   serially diluted sera (with 1:5 as starting dilution) in dilution buffer (1:5 times dilution of

530   blocking buffer) were added to wells of the plates. The plates were incubated at room

531   temperature (RT) for 1 h and then washed three times with washing buffer (PBS + 0.05 %

532   tween 20), the ELISA plates with N protein coating were washed additionally once with high

533   salt PBST (Phosphate buffer with 500 mM NaCl and 0.05 % Tween 20) and incubated with

534   biotinylated anti-hamster IgG antibody (Sigma) for another 1 h and washed subsequently with

535   the washing buffer and incubated further with Avidin-HRP (Sigma) for 45 min at RT. Post

536   incubation plates were washed four times and 100 µl of TMB substrate (Thermo Fisher

537   Scientific) was added to the washed wells. The reaction was stopped by adding 100 µl of 1 N

538   H_2SO_4 and the plates were read at 450 nm on a 96-well microtiter plate reader. Sera end point

539   titers were calculated as the reciprocal of serum dilution giving OD 450 nm readings higher

540   than lowest dilution of the placebo or control arm + two times standard deviations

541   *In silico* **molecular modelling and protein-protein docking**

542   In the absence of crystal structure of hamster ACE2 structures, the crystal structure of human

543   ACE2 and RBD, pdb-ids 6m17, 6VW1 were considered as templates to generate the robust

544   model of hamster through homology modelling (Srivastava et al., 2018). The robustness of

545   each model was evaluated through ERRAT, PROSA and Ramachandran plot (allowed and

546   disallowed %), followed by structure optimization through energy minimization to allow

547   conformational relaxation of the protein structure (Ha et al., 2020). The same was carried out

548   for neurolipin-1 protein of hamster as human neurolipin crystal structure (pdb-id 6JJQ) is

549   reported. The most stable proteins were picked to perform the protein-protein (for RBD-ACE2)

550 and protein-peptide (NRP-1 and CendR peptide of spike protein) docking studies through

551 different algorithms. Rigid docking through PyDock allows some steric clashes while flexible

552 docking by SwarmDock was done on relaxed structures of proteins to generate candidate

553 solutions with at least one near native structure (Mattapally et al., 2018). Clustering of docked

554 poses was conducted to generate the most likely complex of ACE2-RBD and NRP1-Cend

555 peptide based on number of conformers and lowest binding energy (from -50 to -40 kcal/mol).

556 Post-docking analysis followed by energy minimization of complexes showed that both the

557 protein structures displayed an initial structural rearrangement that was followed by

558 convergence indicating structural fluctuation at interface site specially at residual level i.e

559 conformational changes and chi angle variation. The overall structural fluctuation of hACE2

560 (human ACE2) was observed to be less than that of haACE2 (hamster ACE2). This is expected

561 as the protein structure (template) of the former has high resolution and covers more space,

562 while the latter is the model. An inventory of structural and energetic features of the complexes

563 was obtained by analysing the hydrogen bonds (cut-off of 3.5Å for the donor-acceptor distance

564 and 150° for the donor-hydrogen-acceptor angle) and hydrophobic contacts (non-polar atoms

565 separated by a distance of at-most 4.0 Å). π-π interactions were considered to be formed when

566 the short inter-atomic carbon-carbon distance (SICD) was smaller than 4.8 Å (Kanwal et al.,

567 2016). In an effort to dissect these interactions from the docking simulations, total interaction

568 energy between ACE2 and RBD was calculated, within the framework of Amber-force field

569 description.

570 **qPCR**

571 RNA was isolated from lung homogenate and spleen samples using Trizol-choloroform as

572 previously described (Rizvi et al., 2018). Thereafter, RNA was quantitated by NanoDrop and

573 1 µg of total RNA was then reverse-transcribed to cDNA using the iScript cDNA synthesis kit

574 (Biorad; #1708891) (Roche). Diluted cDNAs (1:5) was used for qPCR by using KAPA

29

575 SYBR® FAST qPCR Master Mix (5X) Universal Kit (KK4600) on Fast 7500 Dx real-time

576 PCR system (Applied Biosystems) and the results were analyzed with SDS2.1 software. The

577 relative expression of each gene was expressed as fold change and was calculated by

578 subtracting the cycling threshold (Ct) value of hypoxantine-guanine phosphoribosyltransferase

579 (HGPRT-endogenous control gene) from the Ct value of target gene (ΔCT). Fold change was

580 then calculated according to the previously described formula POWER(2,-ΔCT)*10,000

581 (Malik et al., 2017). The list of the primers are provided as follows.

Gene	Forward	Reverse
HGPRT	GATAGATCCACTCCCATAACTG	TACCTTCAACAATCAAGACATTC
Rorc	GGGAAATGTGGGAACGCTGT	AAACGCAAGGCACTGAGGAA
tryptase beta-2	TCGCCACTGTATCCCCTGAA	CTAGGCACCCTTGACTTTGC
chymase	ATGAACCACCCTCGGACACT	AGAAGGGGGCTTTGCATTCC
muc1	CGGAAGAACTATGGGCAGCT	GCCACTACTGGGTTGGTGTAAG
Foxo1	AGGATAAGGGCGACAGCAAC	GTCCCCGGCTCTTAGCAAAT
Sftpd	TGAGCATGACAGACGTGGAC	GGCTTAGAACTCGCAGACGA
Eotaxin	ATGTGCTCTCAGGTCATCGC	TCCTCAGTTGTCCCCATCCT
Ager	ATTCCTGACGGCAAAGGGAC	ACTGTGTTCGAGGGACTGTG
PAI-1	CCGTGGAACCAGAACGAGAT	ACCAGAATGAGGCGTGTCAG
IFNy	TGTTGCTCTGCCTCACTCAGG	AAGACGAGGTCCCCTCCATTC
TNFa	AGAATCCGGGCAGGTCTACT	TATCCCGGCAGCTTGTGTTT
IL13	AAATGGCGGGTTCTGTGC	AATATCCTCTGGGTCTTGTAGATGG
IL17A	ATGTCCAAACACTGAGGCCAA	GCGAAGTGGATCTGTTGAGGT
IL9	CTCTGCCCTGCTCTTTGGTT	CGAGGGTGGGTCATTCTTCA
TGFb	ACGGAGAAGAACTGCTGTG	GGTTGTGTTGGTTGTAGAGG
IL10	GGTTGCCAAACCTTATCAGAA ATG	TTCACCTGTTCCACAGCCTTG
T-bet	ACAAGGGGGCTTCCAACAAT	CAGCTGAGTGATCTCGGCAT
GATA3	GAAGGCAGGGAGTGTGTGAA	GTC TGA CAG TTC GCA CAG GA
FOXP3	GGTCTTCGAGGAGCCAGAAGA	GCCTTGCCC TTC TCA TCC A
CCR5	TGT GAC ATC CGT TCC CCC T	GGC AGG GTG CTG ACA TAC TA
CCL5	CTACGCTCCTTCATC TGC CTC	CCT TCG GGT GAC AAA AAC GAC
CCL22	CGT GGC TCT CAT CCT TCT TGC	CAG ATG CTG TCT TCC ACG TTG G
CXCL9	TGG GTA TCA TCC TCC TGG AC	AAT GAG GAC CTG GAG CAA AC
CXCL10	TGGAAATTATTCCTGCAAGTCA	GTG ATC GGC TTC TCT CTG GT
PD1	CTGAAAAGGGTTAAG CCA GC	GCC TCC AGG ATT CTC TCT GTT
PDL1	TGATCATCCCAGACCCGCTC	CTC CTC GAA CTG CGT ATC GT
IL6	GGACAATGACTATGTGTTGTTAGAA	AGGCAAATTTCCCAATTGTATCCAG
iNOS	TGAGCCACTGAGTTCTCCTAAGG	TCCTATTTCAACTCCAAGATGTTCTG

582

583 **Histology**

584   Excised tissues of animal organs were fixed in 10% formaline solution and processed for

585   paraffin embedding. The paraffin blocks were cut into 3-µm-thick sections and then mounted

586   on silane-coated glass slides. One section from each organ samples was stained with

587   hematoxylin and eosin. Lung, heart and colon samples were stained with taulidine blue,

588   masson's trichrome and mucicarmine stains respectively. Each stained section was analysed

589   and captured at 10X and 60X magnification. Heart images were also captured at 1X

590   magnification. Blind assessment and scoring of each section for each samples was performed

591   by professional histologist.

Immunophenotyping of splenocytes

593   0.5 million RBC lysed splenocytes were used for intracellular cytokine staining by re-

594   stimulation with PMA (phorbol 12-myristate13-acetate; 50 ng/ml; Sigma-Aldrich), ionomycin

595   (1.0 µg/ml; Sigma-Aldrich) and monensin (#554724; GolgiStop, BD Biosciences) for 6 h or

596   with RBD protein (50 µg) for 72 h. Cell surface staining with anti-mouse CD4 (GK1.5) PerCp

597   (Biolegend) was carried out for 20 min in dark at RT. Intracelluar anti-mouse IFN-γ (XMG1.2)

598   (BioLegend) staining was then carried out after fixing the cells in Cytofix solution and

599   permeabilization with 1X Perm/Wash Buffer using kit (BD Biosciences; #554714) for 20 min

600   in dark at RT. Cells were then washed and acquired on FACS Canto II and were analysed with

601   FlowJo software (Tree star) as previously described (Malik et al., 2017).

Lipid extraction and lipidomics

603   Lipid extraction from serum sample was performed as previously described method with some

604   modifications (Schwaiger et al., 2018). 0.3 ml methanol was added to 100 µl of serum samples

605   and mixed for 30 sec followed by addition of 1.25 ml methyl-tert-butyl ether (MTBE). The

606   mixture was incubated at RT for 1 h on a shaker followed by addition of 0.3 ml of MS grade

607   water was for the phase separation. 10 min after incubation at RT, samples were spin down at

608   400 rpm at 10 ℃ for 5 min. Organic upper phase was collected and dried in a speed vac and

609   stored at -80 ℃ till further use. Moreover, lipids extracted were dissolved in 100 µl of 65:30:5

610   (acetonitrile: 2-propanol: water v/v/v). An acquity HSS T3 (2.1 mm X 100 mm X 1.8 Um,

611   WATERS) was then utilized for carrying out lipid separation by exploiting ultra-performance

612   liquid chromatography (UPLC). Solvent A and B were water/ acetonitrile (2:3 v/v) and 2-

613   propanol/acetonitrile (9:1, v/v) respectively, at 0.3ml/min flow rate and 40 ℃ column

614   temperature. 18 min total run time was utilized with the following gradient setup, 0 to 12 min

615   solvent B ramped from 30 to 97% and a hold for another 3 min. From 15.2 to 18 min, B was at

616   30%. Acquisition of data was carried out on a high-resolution mass spectrometer, orbitrap

617   fusion (Thermo Scientific) equipped with a heated electrospray ionization source. Auxiliary

618   gas and ESI sheath gas were 20 and 60 respectively. Negative and positive spray voltage was

619   3000 volts. For full MS run, 120k resolution was used with automatic gain control (AGC)

620   targeted of 200000 and mass ranges between 250-1200. Resolution was kept at 30K with AGC

621   target 50000for MSMS. Collision energy for fragmentation used was 27+/-3.

622   **Lipid data analysis**

623   Lipidmatch flow was utilized with default settings for peak picking (using mzmine), lipid

624   annotation, blank filtration and combining positive and negative data (Koelmel et al., 2017).

625   Thereafter, data analysis was carried out as follows: the data was normalized by sum, perato

626   scaled, and log-transformed for analysis in metaboanalyst.

627   **Metabolomics analysis LC-MS/MS reverse phase and HILIC**

628   Metabolites were extracted from 100 µl of serum samples by using 100% methanol. Thereafter,

629   mixture were vortexed for 1 min and kept on ice for protein precipitation. After centrifugation

630   (10000 rpm for 10 min at 4 ℃) supernatant was collected and distributed in two tubes for polar

631   and nonpolar metabolite analysis. Collected supernatants were then dried using a speed vacuum

632   at for 20 to 25 min at RT and stored at -80 °C till further analysis. For reverse phase metabolites

633   were dissolved in 15% methanol in water (v/v) and for polar metabolite analysis samples were

634   dissolved in 50% acetonitrile in water (v/v).

635   **Measurement of metabolites**

636   Orbitrap Fusion mass spectrometer (Thermo Scientific) coupled with heated electrospray ion

637   source was used for data acquisition. Data acquisition methods have been followed as per

638   published protocols (Kumar et al., 2020; Naz et al., 2017) with minor modifications. Briefly

639   for MS1 mode, mass resolution was kept at 120,000 and for MS2 acquisition, mass resolution

640   was 30,000. Mass range of data acquisition was 60–900 da. Extracted metabolites were

641   separated on UPLC ultimate 3,000. Data were acquired on reverse phase and HILIC column

642   and positive and negative ionization mode both. Reverse phase column was HSS T3 and HILIC

643   column was XBridge BEH Amide (Waters Corporation). For polar compound separation,

644   solvent A was 20 mM ammonium acetate in the water of PH 9.0 and mobile phase B was 100%

645   acetonitrile. Te elution gradient starts from 85% B to 10% B over 14 min with flow rate of

646   0.35 ml/min. For reverse phase, Solvent A was water and B was methanol with 0.1% formic

647   acids added in both. Te elution gradient starts with 1% B to 95% B over 10 min with flow rate

648   0.3 ml/min. sample injection volume was 5ul. Pool quality control (QC) sample was run after

649   every five samples to monitor signal variation and drif in mass error. Data matrices have been

650   provided in Supplementary Table 1 and representative spectra of one of the samples acquired

651   in positive and negative modes of HILIC and RP have been provided in Supplementary Fig. 3.

652   Data processing. All LC/MS acquired data has been processed using the Progenesis QI for

653   metabolomics (Water Corporation) software using the default setting. Te untargeted workflow

654   of Progenesis QI was used to perform retention time alignment, feature detection,

655   deconvolution, and elemental composition prediction. Metascope plug of Progenesis QI has

656   been used for the in-house library with accurate mass, fragmentation pattern and retention time

657   for database search. We have also used online available spectral library for further confirmation

658   of identification. Cut-off for retention time match was 0.5 min and spectral similarity was more

659   than 30% fragmentation match in Progenesis QI. Peaks that had a coefcient of variation (CV)

660   less than 30% in pool QC samples were kept for the further analysis of data. Additionally,

661   manual verifcation of each detected feature has been done for the selection of right peaks.

662   **Lipidomics and metabolomics data analysis**

663   For both metabolomics and lipidomics data, the data was cleaned up to remove zero intensity

664   values. The average calculations were done with the remaining available intensity values. The

665   fold change was calculated as the ratio of average intensity values of cases divided by the

666   average intensity of controls as-

667

$$FC = \frac{\frac{(\sum_{i=1}^{m} I_{ki})}{m}}{\frac{(\sum_{j=1}^{n} I_j)}{n}}$$

668   Where,

669   *FC = fold change*

670   *m = number of available non-zero intensity values for case*

671   *n = number of available non-zero intensity values for control*

672   I_{ki} = *intensity of ith case sample for condition k, where k $\in$ {2D, 4D, 7D, 14D post infection}*

673   I_j = *intensity of jth control sample*

674   A two-tailed t-test considering similar means of case and control population was used to

675   calculate the p-values. The FC was transformed to log_2FC and a threshold was set to ±2 FC (±1

676   at log_2FC). The significance threshold for differentially expression was set using simultaneous

677   threshold filters of the log_2FC of ±1 as well as a p-value threshold of ≤0.05. This was followed

678   for both the metabolomics and the lipidomics data. The significantly expressed analytes

679   (metabolites and the lipids) are also represented on the volcano plots. For the volcano plots,

680   the p-values were transformed into $-\log_{10}$ P-values for plotting. The significant values as

681   described above were highlighted and labeled on the graph using Tableau (version 2020.3).

682   **Volcano plot** $-\log_{10}$P value vs $\log_2$ fold change was plotted on the Volcano plot. Tableau

683   (version 2020.3) was used to highlight and annotate some of the significant

684   hits(metabolites/lipids). The points above or below ± 1 $\log_2$ fold change (i.e. ± 2 fold change)

685   and 1.33 on the $-\log_{10}$Pvalue scale (i.e. ≤ 0.05 p-value) were selected for annotation and were

686   colored red for visual distinction. For the heat map construction, we used $\log_2$ fold changes for

687   both the metabolites as well as lipids heat maps. Gene-E software

688   (https://software.broadinstitute.org/GENE-E/) was used to create the heat maps. For the analyte

689   (metabolite/lipid) with a zero values in control but a positive intensity reading value in cases,

690   we considered them as overexpressed vs control in that particular condition, and used a

691   maximum $\log_2$ FC imputed manually. The value chosen for such cases was the maximum value

692   from the data. For setting the color scale, we used blue color to represent all the values below

693   -4 $\log_2$ FC (1/16 FC) and all the values above +4 $\log_2$ FC were colored red (+16 FC). **For**

694   **whole data heat map** Clustvis was used to generate the heat map for the complete dataset for

695   both metabolites as well as lipids. **PCA plots** raw values of the metabolites and lipids were

696   taken for all 9 samples. The web-based tool ClustVis was used to create the sample PCA plots

697   to check the clustering of biological samples (ClustVis: a web tool for visualizing clustering of

698   multivariate data using Principal Component Analysis and heatmap) (Metsalu and Vilo, 2015).

699   **Comparison against the human data** The data from the article (Shen et al., 2020) was

700   downloaded and manually compared with the hamster metabolites data obtained from our

701   study. The metabolites common to each category were than compared against each other to

702   identify metabolites unique to each category as classified by the authors.

703   **Statistical analysis**

704 All the results were analysed and plotted by using Graph pad prism 7.0 software. Body mass,

705 lung mass, gene expression, FACS, ELISA, $TCID_{50}$ and qPCR studies were compared and

706 analyzed by using one-way ANOVA or student t-test for n = 6 hamsters samples per group.

707 Metabolomics and lipidomics analysis was carried out with n=9 serum samples per group. P-

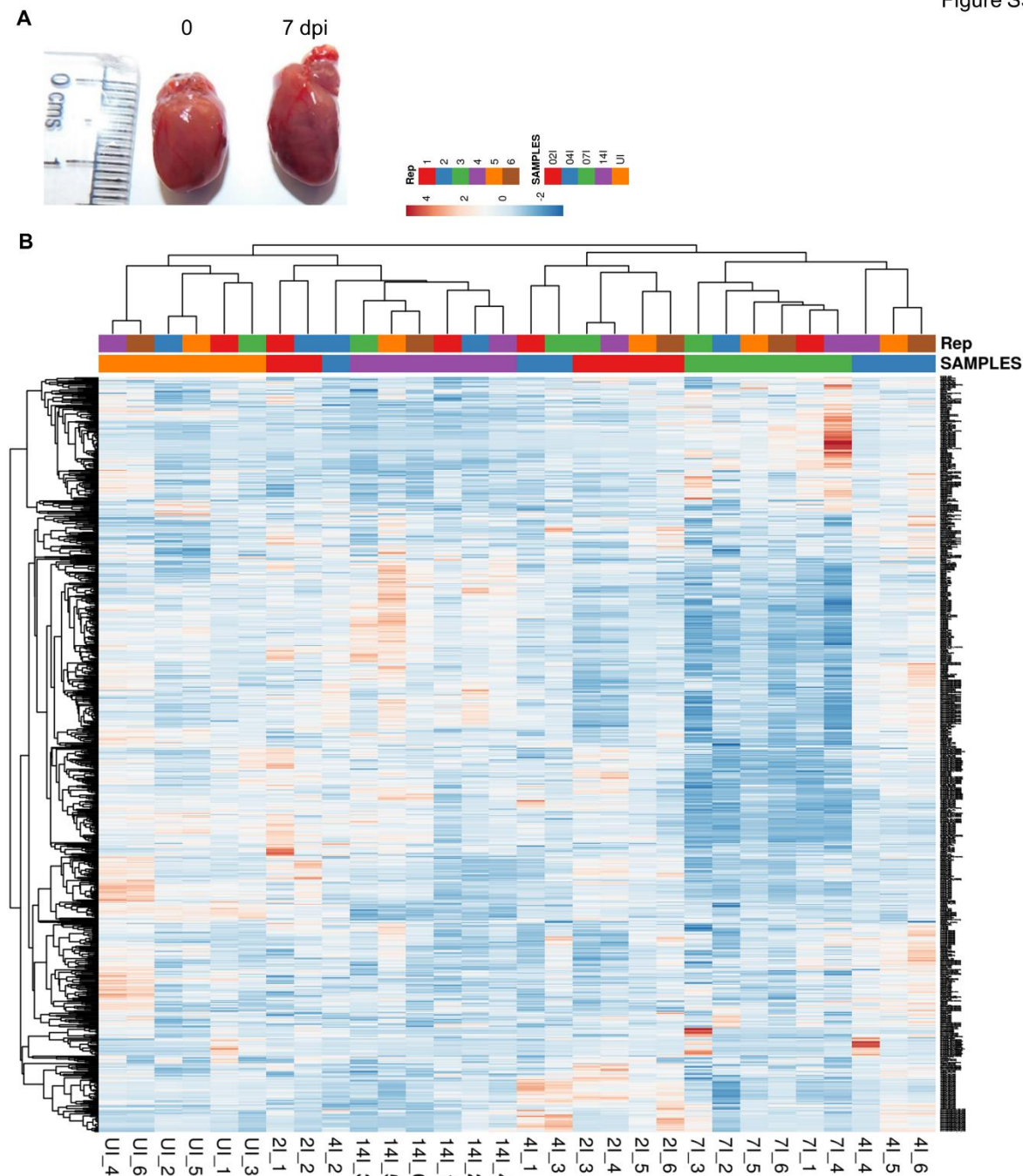708 value of less than 0.05 and was considered as statistically significant.

709 **Supplementary figures**

710
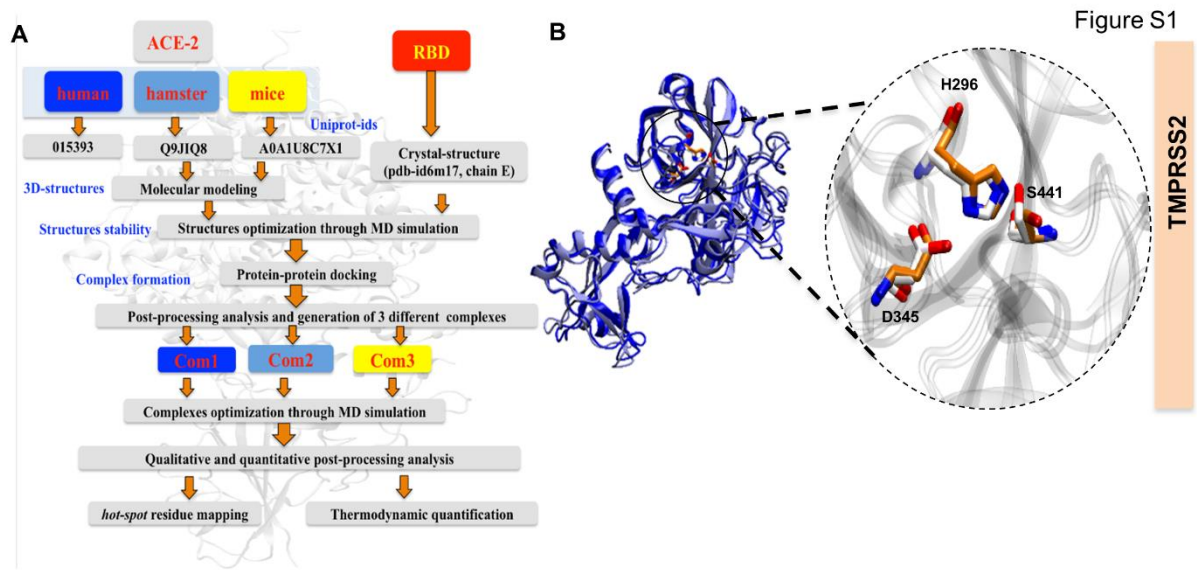
711 **Supplementary figure 1. *In silico* analysis.** (A) Scheme for h/ha ACE2-RBD interaction (B)

712 catalytic triad for hamster TMPRSS2.

Figure S2

713
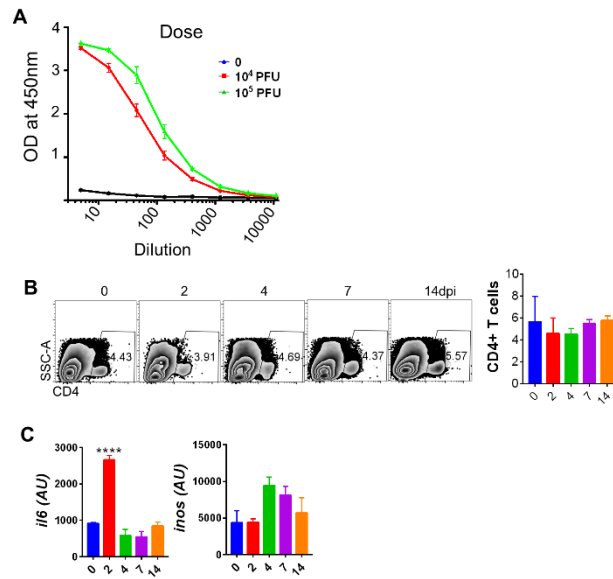
Supplementary figure 2. Immunological response. (A) antibody titre for different dose of infection (B) FACS dot plot and bar graph showing mean $\pm$ SEM % frequency of CD4+ cells. (C) bar graph showing mean value $\pm$ SEM of mRNA expression of IL6 and iNOS genes in the splenocytes samples. ****$P < 0.0001$ (one-way anova).

Supplementary figure 3. Morphological changes in heart and lipid relative abundance heatmap. (A) Heart hypertrophy on 7 dpi (B) heatmap for normalized relative abundance.
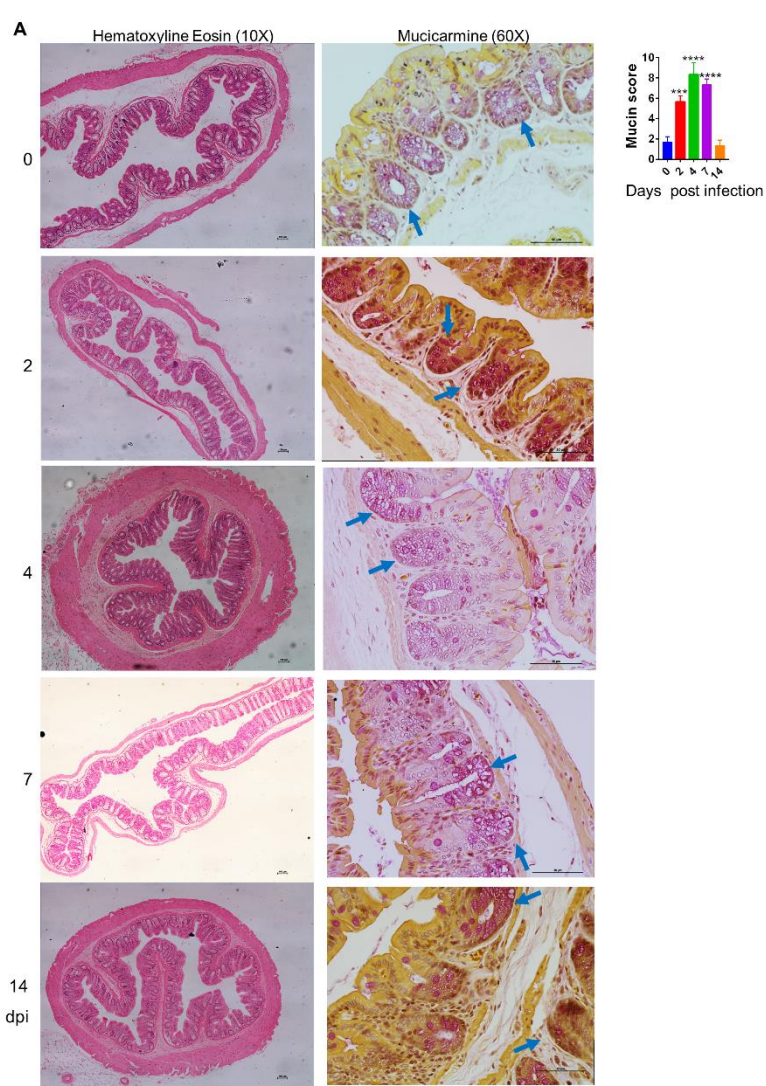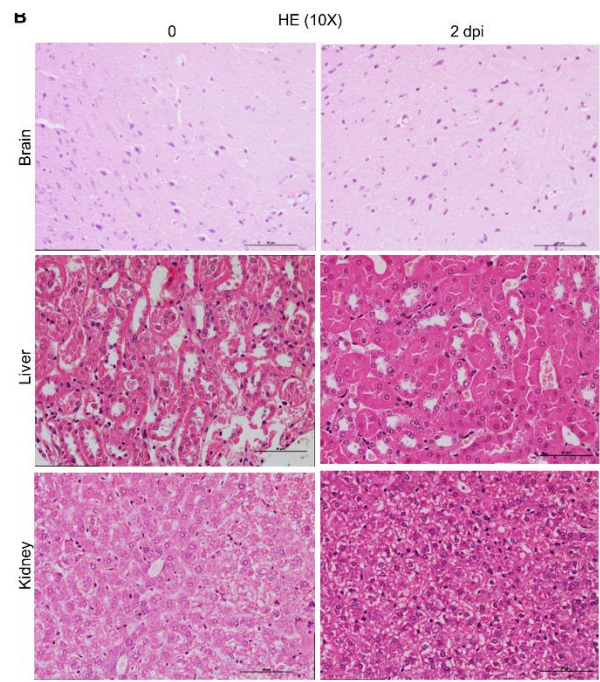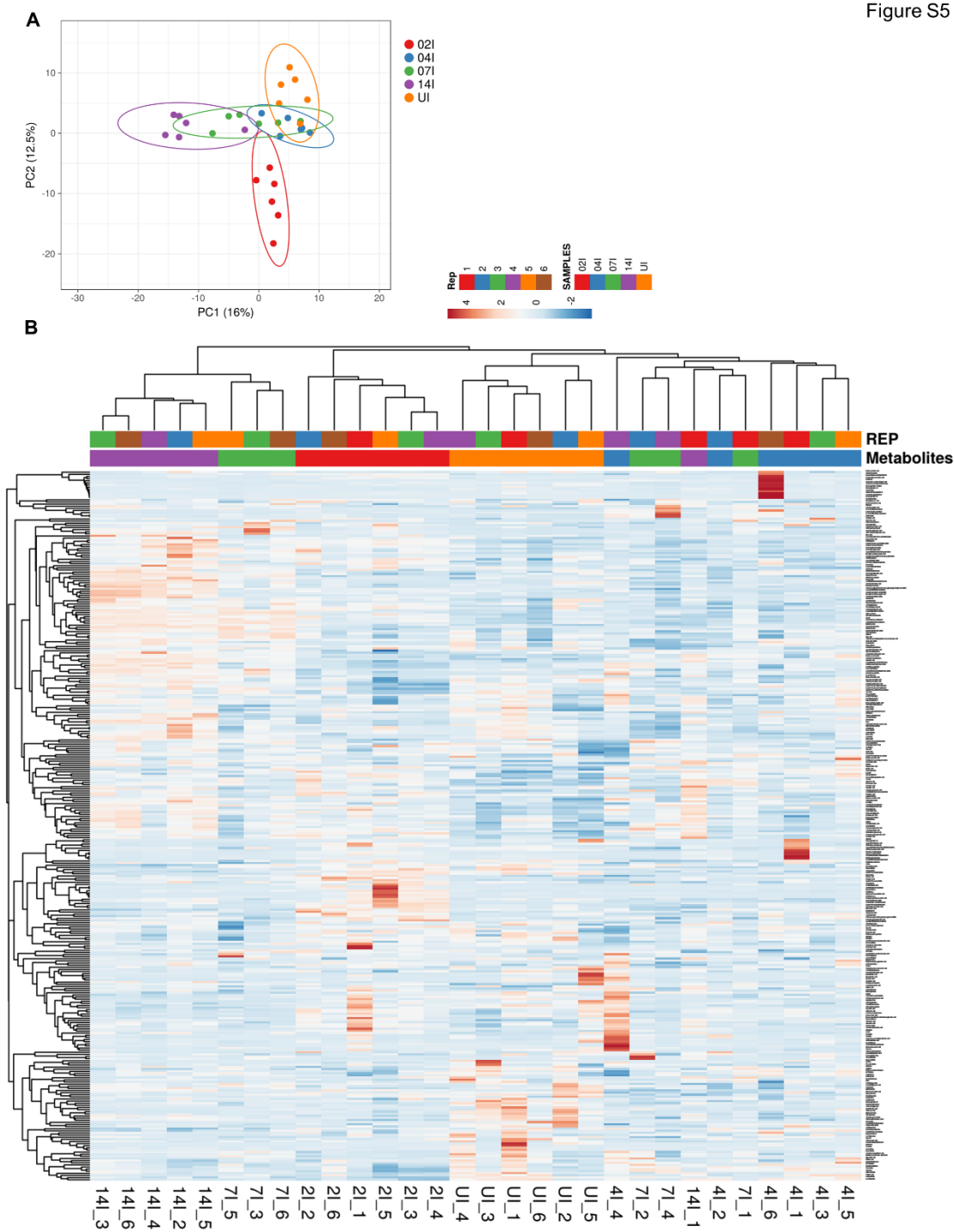
Figure S4

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723   **Supplementary figure 4. Extra-pulmonary changes** (A) H & E stain (B) mucicarmine stain

724   for the presence of mucin (blue arrow) of colon on 2, 4, 7 and 14 dpi (C) H & E stain for brain,

725   liver and kidenty on 2 dpi. ***P < 0.001, ****P < 0.0001 (one-way anova).
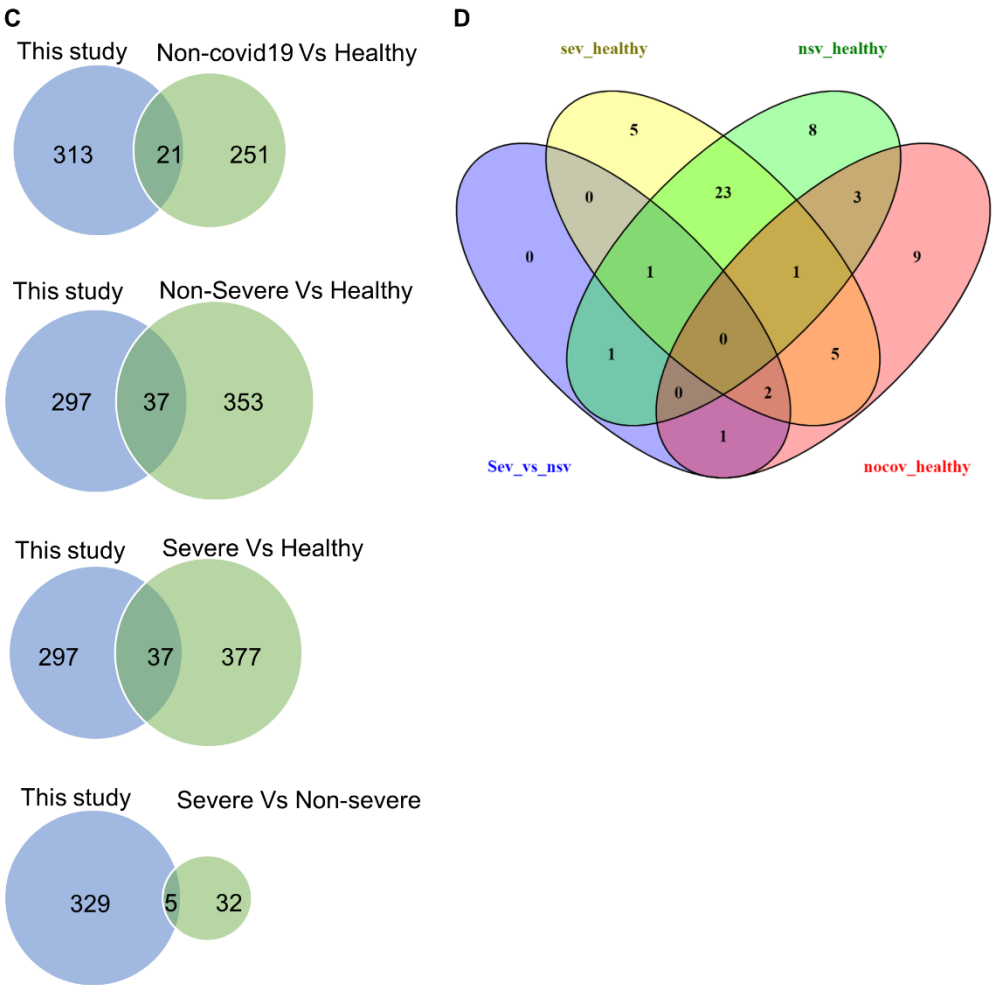
Figure S5

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Supplementary figure 5. Metabolomics. (A) PCA plot for metabolomics (B) heatmap showing normalized relative abundance (C & D) Venn diagram showing correlation of metabolites with Bo Shen et. al. metabolomics profile in covid19 patients.

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Figure 1

Figure 2

Figure 3

Figure 4

A HE (1X) **B** HE (10X) **C** Masson's Trichrome (60X) **D**

Inflammation
Coronary interstitial fibrosis
Ventricular wall and interventricular septum

0

2

4

7

14
dpi

100 µm

50 µm

Figure 5

Figure 6

A Healthy SARS-CoV2 infected → LC-MS/MS (334 metabolites) (n=9)

B TOTAL
Studied 334, Differentially 82, Upregulated 61, Downregulated 22

C UPREGULATED
4 day 4, 7 day 5, 2 day 20, 14 day 23

DOWNREGULATED
4 day 7, 7 day 8, 2 day 8, 14 day 3

D Volcano plots: Log10 p-value vs Log2 fold change

E Heatmap 2, 4, 7, 14 dpi (metabolites listed)

F Pathway analysis: -log10(p) vs Pathway Impact
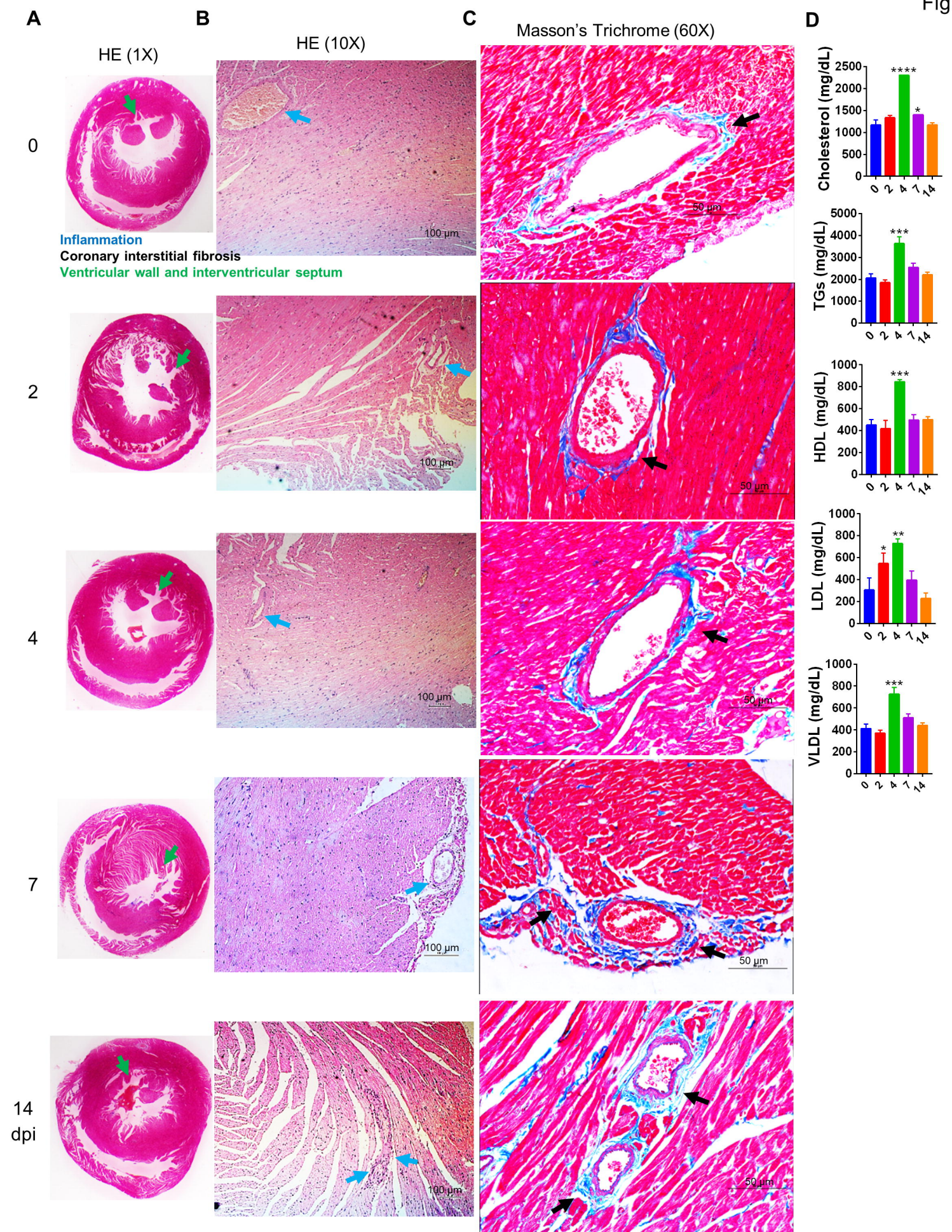Arginine and proline metabolism; Alanine, aspartate and glutamate pathway; Arginine biosynthesis; Glycolysis and gluconeogenesis; Starch and sucrose metabolism; Pyruvate metabolism; Riboflavin metabolism
Log-scale ≤ -4, 0, ≥ 4

G Healthy SARS-CoV2 infected (2, 4, 7, 14 dpi), (n=9), LC-MS/MS (334 metabolites), COMMON METABOLITE, Severe (n=28), Non-severe (n=25), UPLC-MS/MS (941 metabolites), COVID-19 positive
82 Differentially, Severe 39: 71, 4, 9, 4, 3, 23, 7, Non-severe 37

H Citrate, N-acetylneuraminate (0.1054), Allantoin (*), Uracil (*)

I Arginine (**, **), Proline, N-acetylglutamate
Days post infection →