

## Accelerated Article Preview

# FXR inhibition may protect from SARS-CoV-2 infection by reducing ACE2

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1 **FXR inhibition may protect from SARS-CoV-2 infection by reducing ACE2**

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25

1 **Abstract**

2 Prevention of SARS-CoV-2 infection through the modulation of viral host receptors, such as  
3 ACE2<sup>1</sup>, could represent a new chemoprophylactic approach for COVID-19 complementing  
4 vaccination<sup>2,3</sup>. However, the mechanisms controlling ACE2 expression remain elusive. Here,  
5 we identify the farnesoid X receptor (FXR) as a direct regulator of ACE2 transcription in  
6 multiple COVID19-affected tissues, including the gastrointestinal and respiratory systems.  
7 We then use the over-the-counter compound z-guggulsterone (ZGG) and the off-patent drug  
8 ursodeoxycholic acid (UDCA) to reduce FXR signalling and downregulate ACE2 in human  
9 lung, cholangiocyte and intestinal organoids and in the corresponding tissues in mice and  
10 hamsters. We demonstrate that UDCA-mediated ACE2 downregulation reduces  
11 susceptibility to SARS-CoV-2 infection *in vitro*, *in vivo* and in human lungs and livers perfused  
12 *ex situ*. Furthermore, we illustrate that UDCA reduces ACE2 expression in the nasal  
13 epithelium in humans. Finally, we identify a correlation between UDCA treatment and positive  
14 clinical outcomes following SARS-CoV-2 infection using retrospective registry data, and  
15 confirm these findings in an independent validation cohort of liver transplant recipients. In  
16 conclusion, we identify a novel function of FXR in controlling ACE2 expression and provide  
17 evidence that modulation of this pathway could be beneficial for reducing SARS-CoV-2  
18 infection, paving the road for future clinical trials.

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## 1 **Introduction**

2 Since the beginning of the pandemic, the management of COVID-19 has improved  
3 significantly with the development of therapeutic agents, vaccines, and monoclonal  
4 antibodies<sup>4</sup>. Despite the transformational impact of vaccines in populations that can access  
5 them, significant global health challenges remain. New SARS-CoV-2 variants continue to  
6 emerge and are associated with high case rates and substantial global mortality. Treatment  
7 options, such as dexamethasone, remdesivir, molnupiravir and nirmatrelvir improve clinical  
8 outcome only in specific patient groups<sup>5,6</sup>; monoclonal antibodies, such as the REGN-COV2  
9 cocktail, show reduced neutralising efficacy against new variants<sup>7</sup>, while vaccines are  
10 restricted by variable efficacy<sup>8</sup>, the emergence of vaccine-resistant viral variants<sup>7</sup>, cost<sup>9</sup> and  
11 availability<sup>10</sup>. Finally, one of the biggest challenges remains prophylaxis in vulnerable and  
12 high-risk groups, such as immunocompromised patients who are not expected to mount an  
13 appropriate response to vaccines. The only prophylactic agents for these groups are  
14 monoclonal antibodies, which are hampered by the propensity of the viral spike to evolve to  
15 escape neutralisation<sup>7</sup>. Importantly, there are currently no other approved agents for  
16 pharmacological prophylaxis against COVID-19<sup>2</sup>. Therefore, there is a pressing unmet need  
17 for novel prophylactic agents that reduce the risk of severe disease<sup>3</sup>, are less prone to viral  
18 resistance and are compatible with healthcare systems in low- and middle-income countries.  
19 Viral host receptors represent logical therapeutic targets, because they are essential for  
20 SARS-CoV-2 cellular entry and infection<sup>1</sup>. Among these, the angiotensin converting  
21 enzyme 2 (ACE2), is particularly appealing<sup>1</sup>. ACE2 is a transmembrane carboxypeptidase  
22 with a broad substrate specificity, including angiotensin II, which acts as the main receptor  
23 for SARS-CoV-2. It directly binds the spike protein of different coronaviruses, with a high  
24 affinity for SARS-CoV-2, rendering it indispensable for viral entry<sup>11</sup>. Accordingly, COVID-19

1 predominantly affects tissues expressing ACE2, such as the lungs, the cardiovascular  
2 system, the digestive tract and the biliary tree<sup>12,13</sup>.

3 Modifying ACE2 expression could impede viral entry and protect against infection from  
4 SARS-CoV-2 and potentially other coronaviruses using the same receptor. Furthermore,  
5 because ACE2 is a host cell protein, its expression is not likely to be affected by mutations  
6 in the virus. Therefore, therapies modulating ACE2 expression may be effective against  
7 multiple SARS-CoV-2 variants with a higher genetic barrier to resistance. However, the  
8 mechanisms controlling ACE2 expression remain elusive. Here, we use human  
9 cholangiocyte organoids as a proof-of-principle system to demonstrate that the bile acid  
10 receptor farnesoid X receptor (FXR) controls ACE2 expression. We show that this  
11 mechanism applies in multiple SARS-CoV-2-affected tissues, including gastrointestinal and  
12 respiratory epithelia. Subsequently, we demonstrate that suppressing FXR signalling, with  
13 the approved drug ursodeoxycholic acid (UDCA) or the over-the-counter phytosteroid z-  
14 guggulsterone (ZGG), reduces ACE2 expression and SARS-CoV-2 infection *in vitro* and in  
15 an airborne transmission model in golden Syrian hamsters. We repeat our experiments in  
16 human lungs and livers perfused *ex situ* and demonstrate that UDCA administration at  
17 physiologically-relevant concentrations reduces ACE2 and viral infection in both organs *ex*  
18 *vivo*. We then demonstrate a reduction of ACE2 levels in the nasal epithelium of volunteers  
19 receiving clinically approved doses of UDCA. Finally, we interrogate an international registry  
20 cohort of patients with COVID-19 and chronic liver disease, identify a correlation between  
21 UDCA therapy and better clinical outcomes from COVID-19, and reproduce these results in  
22 a second independent cohort of liver transplant recipients.

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24 **Bile acids modulate cholangiocyte ACE2**

1 To explore the mechanisms controlling ACE2 expression, we used cholangiocyte organoids  
2 (COs), as proof-of-principle. Cholangiocytes are epithelial cells lining the lumen of the bile  
3 ducts and the gallbladder. We decided to focus on gallbladder cholangiocytes for multiple  
4 reasons. Cholangiocytes of the gallbladder express the highest ACE2 levels in the biliary  
5 tree<sup>12</sup> (Wilcoxon Rank-Sum test  $P < 4.5 \cdot 10^{-201}$ ) (Extended Data Fig. 1a-d), and one of the highest  
6 ACE2 levels in the body<sup>12</sup>. Thus, they can be infected by SARS-CoV-2 (Extended Data Fig.  
7 1e-f). Furthermore, they can be propagated as organoids<sup>14-16</sup> (Extended Data Fig. 1a-c) and  
8 maintain their gallbladder identity *in vitro* (Extended Data Fig. 1a-c) after addition of the bile  
9 acid chenodeoxycholic acid (CDCA) in their culture medium<sup>14</sup>. The resulting gallbladder  
10 cholangiocyte organoids (GCOs) express high levels of ACE2 (Extended Data Fig. 1c; 1g-i),  
11 retain their capacity to be infected by SARS-CoV-2 (Extended Data Fig. 2a-e), produce  
12 infective viral progeny (Extended Data Fig. 2c), and appropriately upregulate the expression  
13 of innate immune genes and antiviral response markers (Extended Data Fig. 2d). Importantly,  
14 in the absence of bile acids (CDCA) cholangiocyte organoids lose the expression of  
15 gallbladder markers, including ACE2 (Extended Data Fig. 1g-i), which demonstrates that  
16 CDCA is required for ACE2 expression. These results not only demonstrate that GCOs  
17 provide an appropriate platform to study the mechanisms controlling the expression of ACE2  
18 in human cells; they also identify CDCA as a key regulator of SARS-CoV2 receptor levels.

19

## 20 **Bile acids control ACE2 levels via FXR**

21 Since the bile acid CDCA is the most potent natural agonist of the bile acid receptor and  
22 transcription factor FXR<sup>17</sup>, we hypothesized that CDCA could control ACE2 expression acting  
23 through FXR. To test this hypothesis, we first confirmed that FXR is expressed in gallbladder  
24 cholangiocytes *in vivo* and in the corresponding GCOs *in vitro* (Extended Data Fig. 3a-c), and  
25 that it is activated by CDCA treatment, as evidenced by the upregulation of its downstream

1 target Small Heterodimer Partner (SHP) (Extended Data Fig. 3c). To confirm that FXR is  
2 essential for CDCA-induced ACE2 upregulation, we knocked-down FXR in cholangiocyte  
3 organoids using shRNAs, which prevented ACE2 and SHP upregulation following CDCA  
4 treatment (Extended Data Fig. 4a-c). To assess whether FXR could bind the ACE2 gene and  
5 potentially control its transcriptional activity, we analysed the ACE2 promoter region and  
6 identified the presence of the FXR responsive element (FXR RE) IR-1. Accordingly, we  
7 confirmed that activated FXR directly binds the ACE2 promoter using chromatin  
8 immunoprecipitation (ChIP-QPCR) (Fig. 1a) and showed the functional relevance of this  
9 binding using a luciferase reporter containing the ACE2 IR-1 region (Extended Data Fig. 4e-  
10 f). Importantly, site-specific mutagenesis on the IR-1 region reduced luciferase signal  
11 (Extended Data Fig. 4e-f), demonstrating the specificity of the FXR binding site on the ACE2  
12 promoter. Conversely, suppression of FXR signalling, using the FXR antagonist ZGG<sup>18</sup> or the  
13 clinically used drug UDCA<sup>17</sup>, reduced FXR activity as evidenced by decreased SHP levels  
14 (Extended Data Fig. 5a); decreased FXR presence on ACE2 promoter (Fig. 1a; Extended  
15 Data Fig. 4e-f); and downregulated the expression of ACE2 at the transcript and protein levels  
16 (Fig. 1c-d; Extended Data Fig. 5b-d). Considered together, these results demonstrate that  
17 FXR directly controls ACE2 expression in cholangiocytes (Fig. 1b).

18

### 19 **FXR regulates ACE2 in various cell types**

20 FXR is expressed in multiple cell types<sup>17,19-21</sup>, and it can be activated by bile acids, which are  
21 present not only in the gastrointestinal tract<sup>22</sup>, but also in the lungs<sup>20,21</sup> and in the systemic  
22 circulation<sup>22</sup>. Thus, ACE2 regulation through FXR may represent a general mechanism,  
23 extending beyond cholangiocytes. To explore this possibility, we repeated our experiments  
24 using primary organoids from key organs infected by SARS-CoV-2<sup>23</sup>, such as the lungs and  
25 the intestine. Importantly, the relevance of these platforms for studying SARS-CoV-2 infection

1 has already been demonstrated<sup>24,25</sup>. We first confirmed FXR expression in these tissues,  
2 both *in vivo* and *in vitro* (Extended Data Fig. 3a-c). Subsequently, we showed that treatment  
3 with physiological concentrations of CDCA (10  $\mu$ M)<sup>22</sup> resulted in FXR activation, evidenced  
4 by upregulation of the FXR downstream target SHP (Extended Data Fig. 3c) and increased  
5 ACE2 expression (Fig. 1c-d; Extended Data Fig. 5a-c). Conversely, suppression of FXR  
6 signalling by UDCA or ZGG reduced ACE2 and SHP levels in primary airway and intestinal  
7 organoids (Fig. 1c-d; Extended Data Fig. 5a-d). Importantly, CDCA, UDCA and ZGG  
8 exhibited no cytotoxic effects in the concentration range used for our experiments (Extended  
9 Data Fig. 5e-f). These results confirm that FXR participates in the regulation of ACE2  
10 expression in organoids derived from the respiratory, biliary and intestinal epithelium  
11 suggesting that FXR-mediated control of ACE2 expression may be relevant for several  
12 organs (Fig. 1b).

#### 14 **FXR regulates viral infection *in vitro***

15 Our results show that suppressing FXR signalling, with the clinically approved drug UDCA,  
16 used as first-line treatment in primary biliary cholangitis (PBC)<sup>26</sup> or the over-the-counter drug  
17 ZGG, reduces ACE2 expression in multiple cell types. To explore the relevance of this finding  
18 for COVID-19, we investigated whether FXR-mediated ACE2 downregulation could reduce  
19 susceptibility to SARS-CoV-2 infection *in vitro*. For this, we exposed gallbladder  
20 cholangiocyte, airway and intestinal organoids to physiological levels of CDCA, to simulate  
21 the baseline level of FXR activation present *in vivo*; and infected them with SARS-CoV-2  
22 isolated from a patient's nasopharyngeal swab<sup>45</sup> in the absence or presence of UDCA or  
23 ZGG (Fig. 1e-f). Suppression of FXR signalling with UDCA or ZGG reduced viral infection in  
24 all three types of organoids (Fig. 1e-f; Extended Data Fig 5a). We then explored whether the  
25 observed reduction in viral infection was a direct result of FXR-mediated ACE2

1 downregulation. First, we showed that knock-down of FXR using shRNAs decreases the  
2 expression of ACE2 and inhibits viral infection in cholangiocytes organoids independently of  
3 the presence of CDCA or UDCA/ZGG (Extended Data Fig. 4d). Accordingly, following  
4 knockdown, UDCA or ZGG treatment had no impact on viral infection (Extended Data Fig.  
5 4d). Next, to determine whether ACE2 modulation is the only mechanism by which UDCA  
6 and ZGG reduce SARS-CoV-2 infection, we treated HEK293T cells genetically engineered  
7 to over-express ACE2 independent of FXR<sup>27</sup> (Extended Data Fig. 6a-b) with UDCA or ZGG,  
8 then infected them with SARS-CoV-2. As expected, in the absence of ACE2 modulation,  
9 UDCA and ZGG did not affect viral replication (Extended Data Fig. 6c). Taken together, these  
10 results confirm that UDCA and ZGG reduce susceptibility to SARS-CoV-2 infection in multiple  
11 cell types *in vitro* via FXR-mediated ACE2 regulation.

### 13 **FXR regulates viral infection *in vivo***

14 To validate the relevance of these findings *in vivo*, we assessed the effect of UDCA on ACE2  
15 expression in FVB/N mice and Syrian Golden Hamsters. We compared ACE2 expression in  
16 the respiratory, biliary and intestinal epithelium of 4 mice treated with UDCA vs. 4 control  
17 animals not receiving UDCA (Extended Data Fig. 7a). We repeated the same experiment in  
18 Syrian Golden Hamsters receiving UDCA (n=3 hamsters) vs. control animals not receiving  
19 treatment (n=5 hamsters); and interrogated ACE2 levels in the nasal, respiratory, biliary and  
20 intestinal epithelium of these animals (Fig. 2a). Our results demonstrate that UDCA treatment  
21 reduces ACE2 expression in mice (Extended Data Fig. 7a-c) and hamsters (Fig. 2a-c;  
22 Extended Data Fig. 7d-e).

23 To explore whether UDCA-mediated ACE2 downregulation reduces SARS-CoV-2 infection  
24 *in vivo*, we used the well-established Syrian Golden Hamster model of infection. 9 animals  
25 were treated with UDCA for 7 days (UDCA group), to achieve plasma concentrations



1 comparable to UDCA levels in patient blood (Extended Data Fig 8a)<sup>28</sup>. Another 6 animals  
2 receiving only the vehicle were used as controls (control group). Neither group was directly  
3 infected with the virus (sentinel animals). On day 7 we inoculated n=5 independent, healthy  
4 hamsters with the SARS-CoV-2 delta variant (B.1.617.2) via the intranasal route (directly  
5 infected animals). Subsequently, each infected animal was co-housed with a group of n=3  
6 randomly selected sentinel (uninfected) hamsters from the UDCA or control group for a period  
7 of 4 days to interrogate SARS-CoV-2 transmission (Fig. 2a). Viral infection in sentinel animals  
8 was assessed with plaque assays from the animal lungs harvested at the end of the  
9 experiment (Extended Data Fig. 8b) and confirmed with daily swabs, and viral QPCR in tissue  
10 harvested from the lungs and nasal turbinates of the animals at the end of the experiment.  
11 Our data demonstrate that UDCA treatment prevented transmission of SARS-CoV-2 in n=6  
12 out of 9 sentinel animals (33% infected vs. 67% uninfected); while SARS-CoV-2 was  
13 transmitted in n=6 out of 6 (100%) sentinel hamsters receiving vehicle ( $P=0.027$ ; Fisher's  
14 exact test) (Fig. 2d-e) for the duration of the experiment. Both directly inoculated and control  
15 animals lost weight following viral infection, in contrast to UDCA animals which gained weight  
16 (Fig. 2f-g), suggesting a milder course of clinical disease in UDCA treated hamsters. In  
17 summary, our *in vivo* results confirm the chemoprophylactic potential of UDCA against  
18 COVID19.

19

## 20 **FXR regulates infection in human organs**

21 We then looked to validate these observations in whole human organs. We focused initially  
22 on the lung as one of the primary sites of SARS-CoV-2 infection. To conduct our experiments,  
23 we used a pair of human lungs, which was declined for transplantation, and performed *ex*  
24 *situ* normothermic perfusion (ESNP) with clinically appropriate mechanical ventilation to  
25 oxygenate the lungs *ex vivo* (Fig. 3a). ESNP was developed to objectively assess and

1 potentially improve donor organ function, enhance organ preservation and reduce  
2 reperfusion injury by perfusing grafts with warm oxygenated blood (packed red cells) or  
3 substitute perfusion solution prior to transplantation<sup>29,30</sup>. This setting ensured that the lungs  
4 remained in near physiological conditions during the experiment<sup>31</sup>. To assess the effect of  
5 UDCA, we surgically divided the right and the left lungs from the same donor and we  
6 connected them to 2 separate but identical ESNP circuits. This setting allowed us to  
7 administer UDCA on one lung (UDCA lung) and use the other lung as a matched control  
8 receiving carrier without UDCA (control lung) to facilitate comparison (Fig. 3a).  
9 Immediately prior to UDCA administration we measured baseline ACE2 expression in both  
10 lungs (0 hours samples collected from lung parenchyma, airway and pulmonary vessels; n=4  
11 independent samples from each part of the organ per lung, 24 samples in total; Fig 3b) and  
12 ACE2 activity in the circulating perfusate from each ESNP circuit (n=4 independent  
13 measurements per circuit; Fig. 3c). We then administered UDCA 'systemically' in the  
14 perfusate of the UDCA lung; UDCA was diluted in saline to 2000 ng/ml corresponding to the  
15 steady-state plasma concentration achieved in patients after multiple doses of oral UDCA<sup>28</sup>.  
16 At the same time the control lung received an equal volume of saline (carrier) (Fig 3a; 0 hours  
17 = UDCA/Carrier administration). We continued *ex situ* perfusion with UDCA for 6 hours.  
18 Repeat perfusate and tissue samples were collected at 6 hours, matching our pre-UDCA  
19 measurements (n=24 independent tissue samples and n=8 independent perfusate samples  
20 per timepoint). We observed that *ex vivo* treatment with UDCA reduced ACE2 expression in  
21 lung parenchyma, airway and pulmonary vessels and ACE2 activity in the perfusate,  
22 compared to the carrier control (Fig. 3b-c).  
23 We then assessed the importance of this reduction in ACE2 on susceptibility to SARS-CoV-  
24 2 infection. We infected samples from the lung parenchyma, airway and vessels of each lung  
25 6 hours after UDCA or carrier administration (n=4 parenchymal, n=4 bronchial, n=4

1 pulmonary vessel independent samples per lung; see methods) and observed that UDCA  
2 treatment reduced SARS-CoV-2 infection (Fig. 3d-e). These results validate that clinical  
3 doses of circulating UDCA can downregulate ACE2 levels and reduce SARS-CoV-2 infection  
4 in human lungs *ex vivo*.

5 Importantly, FXR inhibitors are metabolized by the liver, and ultimately distributed to different  
6 tissues through the systemic circulation. To simulate this process, we repeated ESNP with 2  
7 human liver grafts (Extended Data Fig. 9a; see methods). One liver was perfused with UDCA  
8 (2000 ng/ml), while the other was perfused with carrier and served as a control. In keeping  
9 with our lung findings, we observed that 'systemic' UDCA treatment lowered ACE2 in the  
10 circulating perfusate (Extended Data Fig. 9b) and in gallbladder cholangiocytes (n=4  
11 independent samples from the grafts' gallbladder per timepoint) (Extended Data Fig. 9c-d)  
12 and reduced SARS-CoV-2 infection in gallbladder cholangiocytes (Extended Data Fig. 9e-f).  
13 These results confirm that suppression of FXR signalling via systemic administration of the  
14 approved drug UDCA can downregulate ACE2 in the circulating perfusate and tissue (lung  
15 parenchyma, bronchi, vessels, gallbladder epithelium) of machine-perfused organs and  
16 reduce SARS-CoV-2 infection *ex vivo*.

17

### 18 **UDCA reduces ACE2 in humans**

19 Our previous results encouraged us to assess the potential impact of UDCA on ACE2 levels  
20 in humans. Given the favourable safety profile, lack of side effects and limited cost of UDCA,  
21 we recruited 8 volunteers from the University Medical Centre Hamburg-Eppendorf and  
22 treated them with UDCA at the standard therapeutic dosage of 15 mg/kg/day<sup>26</sup> for 5 days  
23 (Supplementary Table S5). The volunteers' nasal epithelial cells were collected using  
24 nasopharyngeal swabs and ACE2 levels were measured at multiple timepoints before, during  
25 and after treatment with UDCA (see methods, Fig. 4a). Participants with non-detectable

1 cellular RNA in their nasopharyngeal swabs were excluded (n=2). Our results showed that in  
2 humans, UDCA reduces ACE2 levels in the nasal epithelium, which is a prime site of SARS-  
3 CoV-2 infection (Fig. 4b).

4 To validate our findings further, we took advantage of the drug's extensive use for cholestatic  
5 liver disorders (e.g., first-line treatment for the cholestatic autoimmune disorder primary  
6 biliary cholangitis, PBC). We interrogated a published serum proteomics dataset from the  
7 UK-PBC patient cohort<sup>32</sup>, comparing ACE2 levels in the serum of UDCA naïve patients  
8 (n=62) vs. patients receiving UDCA (n=308). We observed that UDCA correlates with lower  
9 serum ACE2 levels after linear regression for age, sex, body mass index (BMI), stage of liver  
10 disease (Child-Turcotte-Pugh class) and alkaline phosphatase (ALP) ( $P = 0.007$ ; Extended  
11 Data Fig. 10a-b; see methods), validating our previous findings (Supplementary Table S5;  
12 Supplementary File S1)..

#### 14 **UDCA may improve COVID-19 outcome**

15 Based on these observations, we decided to explore the potential impact of UDCA treatment  
16 on the outcome of COVID-19 in patients. For this, we interrogated the COVID-Hep/SECURE-  
17 Liver registries<sup>33,34</sup>. These registries comprise data of patients with chronic liver disease  
18 (n=1,096) who developed COVID-19, including patients with cholestatic liver disorders  
19 receiving UDCA (n=31) (Fig. 4c) (Supplementary Table S7 and S8). We observed that,  
20 accepting the potential for selection bias in case reporting, patients receiving UDCA had  
21 better outcomes compared to patients not receiving UDCA, including reduced hospitalisation,  
22 ICU admission and death (Fig. 4d), after propensity score matching (No UDCA:UDCA=5:1)  
23 for sex, age, diabetes, stage of liver disease (Child-Turcotte-Pugh class),  
24 immunosuppression, chronic pulmonary disease and non-alcoholic fatty liver disease  
25 (NAFLD) (Fig. 4d). We note that propensity score matching was not possible for alcohol-

1 related liver disease (ARLD), therefore these patients were excluded from the analysis (see  
2 methods). We then sought to replicate these results in a second independent patient cohort.  
3 For this, we interrogated liver transplant recipients in the Veterans Outcomes and Costs  
4 Associated with Liver disease (VOCAL) cohort who received at least two doses of a COVID-  
5 19 mRNA vaccine. Of 119 vaccinated participants that developed COVID-19, n=24 were  
6 receiving UDCA (Supplementary Table S9). These 24 participants on UDCA were matched  
7 with 72 who were not on UDCA (No UDCA:UDCA=3:1) for sex, age, ethnicity, BMI, location  
8 within the United States, diabetes, chronic pulmonary disease, the type of  
9 immunosuppression (calcineurin inhibitor therapy, with or without anti-metabolite therapy)  
10 and the dominant SARS-CoV-2 variant at time of infection (Fig. 4e). We observed that,  
11 accepting the potential for selection bias in case reporting, patients on UDCA were less likely  
12 to develop moderate, severe or critical COVID-19 ( $P=0.026$ ) (Fig. 4f) according to the  
13 National Institute of Health COVID-19 severity score<sup>35</sup>. Importantly, during the publication of  
14 this manuscript, we became aware of an independent study interrogating the association of  
15 exposure of UDCA and outcomes of COVID-19, among participants in the VOCAL cohort  
16 with cirrhosis. In this analysis, 1607 participants with cirrhosis and UDCA exposure were  
17 propensity score matched with 1607 participants with cirrhosis but without UDCA exposure.  
18 The authors found that on multivariable logistic regression, UDCA exposure was associated  
19 with a 46% reduced odds of developing COVID-19 (adjusted Odds ratio [aOR] 0.54, 95% CI  
20 0.41- 0.71,  $p<0.0001$ ). The association was observed across the spectrum of COVID-19  
21 against symptomatic illness (aOR 0.54, 95% CI 0.39-0.73,  $p<0.0001$ ), at least moderate  
22 COVID-19 (aOR 0.51, 95% CI 0.32-0.81,  $p=0.005$ ), and severe or critical COVID-19 (aOR  
23 0.48, 95% CI 0.25-0.94,  $p=0.03$ ). These results provide additional independent evidence  
24 reinforcing our study. Taken together, the findings from our exploratory and validation cohorts

1 support further investigation of the impact of UDCA on clinical outcomes of COVID-19 in a  
2 large prospective clinical trial.

3

#### 4 **Discussion**

5 Considered collectively, our findings demonstrate that FXR participates in the regulation of  
6 ACE2 expression in multiple tissues involved in SARS-CoV-2 replication. Suppression of  
7 FXR activity, using the clinically approved drug UDCA, downregulates ACE2 expression and  
8 reduces SARS-CoV-2 infection *in vitro*, *in vivo* and *ex vivo*. Furthermore, our clinical  
9 observations indicate that UDCA reduces ACE2 levels in the nasal epithelium of healthy  
10 individuals and suggest a potential correlation between UDCA and positive clinical outcomes  
11 in COVID-19 patients.

12 The finding that FXR regulates ACE2 is novel but not entirely surprising. The functions of  
13 ACE2 as a molecular chaperone for the amino acids transporter SLC6A19 and as a peptidase  
14 justify its presence in the GI tract and suggest a potential role in digestion. Accordingly, the  
15 upregulation in ACE2 expression by FXR, which is activated by bile, a digestive fluid, may  
16 reflect a mechanism to increase peptidase levels and amino acids absorption during  
17 digestion. Furthermore, in addition to its role in the GI system, FXR is expressed in multiple  
18 organs, including the lungs<sup>20,21</sup>, with a broad variety of functions, ranging from bile acid<sup>22</sup> and  
19 lipid metabolism<sup>36</sup>, to glucose homeostasis<sup>37</sup>, fibrosis<sup>38</sup> and inflammation<sup>39</sup>. Importantly, its  
20 natural ligands, such as bile acids and hormones<sup>40</sup> (e.g., androgens) are present in the  
21 systemic circulation<sup>22</sup>; and it is the therapeutic target of several approved drugs<sup>17</sup>. This broad  
22 expression and function explain how FXR could regulate ACE2 in multiple tissues beyond  
23 the biliary tree.

24 Our results illustrate the potential of ACE2 modulation as a novel host-directed treatment  
25 which might be efficacious as primary and secondary prophylaxis in COVID-19. These

1 findings are in keeping with existing studies illustrating the benefits of targeting the virus-host  
2 interaction for SARS-CoV-2 at the level of ACE2<sup>1</sup> or the spike protein<sup>7</sup>. Indeed, large  
3 Mendelian randomization analyses in over 7,554 patients hospitalised with COVID-19 and  
4 more than 1 million controls demonstrated that higher ACE2 levels strongly correlated with  
5 increased risk of COVID-19 hospitalisation, identifying ACE2 as a logical candidate for drug  
6 development in COVID19<sup>1</sup>. Additionally, the extensive clinical literature supporting  
7 Ronapreve and Evusheld, both dual combinations of monoclonal antibodies against the  
8 SARS-CoV-2 spike protein, demonstrates the utility of inhibiting the viral spike protein-ACE2  
9 interaction for prophylaxis and treatment in COVID19 for susceptible pre-Omicron  
10 variants<sup>41,42</sup>. However, targeting the viral spike protein with monoclonal antibodies is limited  
11 by diversity and evolution of the viral spike sequences, rendering many of these agents  
12 ineffective against new SARS-CoV-2 variants<sup>7</sup>. Conversely, targeting ACE2 is advantageous  
13 for multiple reasons. ACE2 modulation is a host-directed treatment, which does not target  
14 the virus. Such mechanisms may present a higher barrier to emergence of resistance  
15 although this has yet to be empirically demonstrated. Furthermore, since ACE2 is a critical  
16 mechanism for cell entry, the approach maybe more resilient as variants continue to  
17 emerge<sup>43</sup>. Finally, ACE2 is a common receptor for multiple coronaviruses, such as SARS-  
18 CoV and HCoV-NL63. Confirmation of the efficacy of this strategy may therefore provide a  
19 quickly deployable intervention in the event of future coronavirus outbreaks. Taken  
20 collectively with our own observations on the effects of ACE2 modulation for SARS-CoV-2  
21 infection, these points illustrate that ACE2 modulators warrant consideration as priority  
22 candidates for clinical evaluation in COVID-19 trials<sup>1</sup>.

23 Our finding that FXR signalling suppression through UDCA or ZGG reduces ACE2  
24 expression, and limits SARS-CoV-2 infection, identifies a new potential clinical application for  
25 FXR inhibitors, but also raises some points for consideration. First, FXR activation decreases

1 inflammation by modulating NFkB in multiple organs<sup>44</sup>, including lungs<sup>21</sup>, liver<sup>17</sup> and  
2 intestine<sup>17</sup>. Conversely, UDCA has been shown to reduce inflammation in multiple tissues,  
3 including lung, in an FXR-independent fashion<sup>45</sup>. Given the complex interplay between FXR,  
4 UDCA and inflammation<sup>46</sup>, the balance of benefits for FXR activation in terms of SARS-CoV-  
5 2 infection and inflammation should be carefully considered. It is possible that FXR  
6 suppressors, beyond UDCA, which lack anti-inflammatory effects would be better suited for  
7 prophylaxis or early intervention and not indicated for severe disease with ongoing tissue  
8 inflammation<sup>47</sup>. Second, our study suggests that FXR activators used in clinical practice, such  
9 as obeticholic acid (OCA) may increase the risk of developing COVID-19 by upregulating  
10 ACE2 in healthy individuals. Conversely, in liver patients OCA may paradoxically prevent  
11 COVID-19 by reducing disease severity and ameliorating cholestasis, resulting in a net  
12 reduction of FXR activity. Therefore, further studies are needed to elucidate these points.  
13 Our results identify UDCA as a particularly advantageous modulator of ACE2 levels, for use  
14 in COVID-19. We demonstrated that UDCA reduces SARS-CoV-2 infection *in vitro*, *in vivo*  
15 and *ex vivo*; and lowers ACE2 expression in the nasal epithelium of healthy volunteers.  
16 Although our animal data do not exclude that UDCA delays SARS-CoV-2 transmission  
17 beyond the duration of our experiments; our patient data illustrate that this does not change  
18 the net effect of reducing disease severity, which make it particularly attractive for  
19 investigation as pharmacological prophylaxis against SARS-CoV-2 infection. Compared to  
20 other agents, such as vaccines and monoclonals, it is easy to administer orally, easily stored,  
21 affordable and accessible to health systems world-wide for large scale production, as it is off  
22 patent. In addition, UDCA is well-tolerated, has limited drug- drug interactions, and a  
23 favourable safety profile enabling it to be administered for long periods of time. Of note,  
24 UDCA is already administered long-term for different clinical indications to vulnerable groups  
25 that would benefit from chemoprophylaxis, such as bone marrow and liver transplant patients,



1 for prevention of veno-occlusive disease<sup>48</sup> and treatment of cholangiopathy<sup>26</sup> respectively. It  
2 has excellent tolerability and minimal side effects in these patient groups<sup>26,48</sup> demonstrating  
3 the potential feasibility of using UDCA as pharmacological prophylaxis against COVID19 in  
4 vulnerable groups. Nevertheless, our study is not a clinical trial and therefore we cannot  
5 exclude the potential for confounding and selection biases. Consequently, it will be imperative  
6 to validate these results in prospective double blinded clinical trials and fully assess the  
7 impact of this drug on ACE2 levels and susceptibility to SARS-CoV-2 infection. For the  
8 absence of doubt, the authors do not support use of UDCA for COVID19 until appropriate  
9 policy informed by robust clinical evidence is available. The authors also do not condone the  
10 use of UDCA as a substitute for highly effective vaccinations in patients for which they are  
11 indicated.

12 Finally, we demonstrated that UDCA could reduce ACE2 levels and SARS-CoV-2 infection  
13 in machine perfused organs. This is one of the first studies testing the effect of a drug in a  
14 whole human organ perfused *ex situ*. This finding could prove important for organ  
15 transplantation, especially given concerns about peri-operative viral transmission<sup>49</sup>.  
16 Furthermore, although more data are required to definitively establish this approach, our work  
17 sets the stage for future studies using machine-perfused organs for pharmacological studies.  
18 In conclusion, these results validate CDCA-treated cholangiocytes organoids as a novel  
19 platform for disease modelling and drug testing against SARS-CoV-2 infection; identify FXR  
20 as a new therapeutic target in the management of COVID-19 and open up new avenues for  
21 the modulation of ACE2 through FXR for prevention of SARS-CoV-2 infection as well as other  
22 viruses using ACE2 for cell entry.

23

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## 10 **Figure Legends**

11 **Figure 1. FXR modulates ACE2 expression and SARS-CoV-2 infection.** (a) Chromatin

12 immunoprecipitation followed by QPCR (ChIP-QPCR) on cholangiocyte organoids showing

13 that the FXR agonist (CDCA) promotes binding of FXR on the ACE2 promoter which is

14 reduced by FXR inhibitors (UDCA/ZGG). OST $\alpha$  as positive control; ACE2 promoter adjoining

15 region as negative control; n=4 independent experiments; one-way ANOVA adjusted for

16 multiple comparisons; bars, standard deviation. (b) Schematic representation of the

17 suggested mechanism for FXR-mediated control of ACE2 expression and SARS-CoV-2

18 infection relative to panels (e-f). (c-d) QPCR (c) and immunofluorescence (d) showing ACE2

19 levels upon modulation of FXR activity in primary airway, biliary and intestinal organoids.

20 Housekeeping gene, PBGD; n=4 independent experiments; one-way ANOVA; centre line,

21 median; box, interquartile range (IQR); whiskers, range; bars, standard deviation. Yellow

22 scale bars 50  $\mu$ m; grey scale bars 25  $\mu$ m. (e) QPCR quantifying SARS-CoV-2 viral RNA 24

23 hours post infection (hpi) in primary organoids treated with physiological levels of bile acids

24 (CDCA) in the presence or absence of FXR inhibitors (UDCA/ZGG). Housekeeping gene,

25 GAPDH; n=4 independent experiments; one-way ANOVA adjusted for multiple comparisons;

1 centre line, median; box, interquartile range (IQR); whiskers, range; bars, standard deviation.  
2 (f) Immunofluorescence images demonstrating presence of SARS-CoV-2 spike protein 24  
3 hpi in organoids corresponding to (e). Scale bars 25  $\mu$ m. (CDCA, UDCA and ZGG  
4 concentration, 10  $\mu$ M).

5  
6 **Figure 2 FXR inhibition reduces ACE2 and SARS-CoV-2 infection *in vivo*.** (a) Schematic  
7 of the experiment performed in Syrian golden hamsters. Sentinel animals were not directly  
8 inoculated with virus. SARS-CoV-2 infection in sentinels was achieved through transmission  
9 from directly inoculated animals after co-housing. (b) QPCR showing that treatment with  
10 UDCA reduces ACE2 levels in hamster nasal turbinates and lungs. Housekeeping gene,  
11 GAPDH; n=5 vehicle/No UDCA group vs n=3 UDCA group; unpaired two-tailed t-test; centre  
12 line, median; box, interquartile range; whiskers, range; bars, standard deviation. (c)  
13 Immunofluorescence images showing ACE2 levels in nasal and respiratory epithelium of  
14 hamsters receiving UDCA vs. vehicle. N=3 hamsters/group. Scale bars 100  $\mu$ m. (d) QPCR  
15 showing SARS-CoV-2 RNA levels in swabs, nasal turbinates and lungs of directly inoculated  
16 hamsters and sentinel animals treated with UDCA/vehicle and co-housed with infected  
17 animals. Samples were collected after 4 days of co-housing. SARS-CoV-2 nucleocapsid RNA  
18 quantification relative to 18s. N=3 hamsters/group; n=9 UDCA animals vs n=6 vehicle  
19 animals; animals from each experiment are represented with different symbols; Kruskal-  
20 Wallis test adjusted for multiple comparisons. (e) Kaplan-Meier curve showing the  
21 percentage of animals with a PCR positive swab for SARS-CoV-2 over the course of the  
22 experiment outlined in panel (a). N=9 UDCA, n=6 vehicle, n=5 directly inoculated animals;  
23 Log-rank Mantel cox test comparing UDCA vs vehicle. (f) Percentage weight change from  
24 the start of the experiment outlined in panel (a). Bars, range. Day 0 corresponds to the start  
25 of co-housing. (g) Percentage weight change after SARS-CoV-2 infection in sentinel animals.

1 The time of infection was defined as the earliest day a sentinel animal developed a positive  
2 swab (day 3 for both UDCA/vehicle groups). N=3 independent experiments; n=9 UDCA, n=6  
3 vehicle, n=5 directly inoculated animals; unpaired two-tailed t-test; centre line, median; box,  
4 interquartile range; whiskers, range; bars, standard deviation.

5  
6 **Figure 3. FXR inhibition reduces ACE2 levels and SARS-CoV-2 infection in a human**  
7 **lung ex vivo.** (a) Schematic representation of the lung *ex situ* normothermic perfusion  
8 (ESNP) experiment performed; including type of samples harvested and timeline. 0h: 0 hours  
9 baseline sample collection and UDCA/carrier administration. The 0h samples were collected  
10 prior to UDCA administration. 6h: 6 hours after UDCA/carrier administration. For each  
11 timepoint, 4 independent tissue samples were obtained from the lung parenchyma (alveoli),  
12 the airways and the vessels for each lung and used for ACE2 measurement and viral infection  
13 (n=4 lung parenchyma, n=4 airway and n=4 pulmonary vessel samples per lung per  
14 timepoint). (b) QPCR demonstrating that UDCA treatment reduces ACE2 levels in human  
15 alveoli, airway and pulmonary vessels perfused *ex situ*. Housekeeping gene, GAPDH; n=4  
16 independent samples; unpaired two-tailed t-test; centre line, median; box, interquartile range  
17 (IQR); whiskers, range; error bars, standard deviation. (c) ACE2 enzymatic activity in the  
18 perfusate demonstrating that UDCA reduces ACE2. N=4 independent samples; unpaired  
19 two-tailed t-test; centre line, median; box, interquartile range (IQR); whiskers, range; bars,  
20 standard deviation. (d) QPCR showing that 6h of ESNP with UDCA reduces SARS-CoV-2  
21 infection in human alveoli, airway and pulmonary vessels *ex vivo*. Housekeeping gene,  
22 GAPDH. N=4 independent samples; unpaired two-tailed t-test. (e) Immunofluorescence  
23 staining for ACE2 and SARS-CoV-2 in human alveoli, airway and pulmonary vessels  
24 following ESNP with UDCA/carrier. N=4 independent samples. White scale bars 100  $\mu$ m,  
25 yellow scale bars 50  $\mu$ m. (UDCA, concentration, 2000 ng/ml). L, lumen.



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**Figure 4. UDCA is associated with lower ACE2 levels and better clinical outcome in COVID-19 patients.** (a-b) Schematic representation of the study design. 6 healthy individuals received 15 mg/kg/day of UDCA for 5 days. ACE2 levels were measured via QPCR in nasal epithelial cells collected via nasopharyngeal swabs. Day 0 corresponds to samples collected immediately before starting UDCA treatment. Samples were collected daily during drug administration and again at day 22-23 and 24-28 to assess the washout of UDCA. (b) QPCR measurement of ACE2 levels in nasal epithelial cells collected with nasopharyngeal swabs. Each dot represents one individual measurement, lines connect dots from the same individual (n=6). Housekeeping gene, GAPDH; n=6 individuals; one-way ANOVA with Geisser-Greenhouse's correction. Please, refer to Supplementary Table S5 for participant characteristics. (c) Schematic overview of the analysis performed in the exploratory cohort corresponding to panel (d). (d) Propensity-score matched analyses showing major outcomes following SARS-CoV-2 infection in patients taking UDCA compared to non-UDCA controls. N=155 patients not on UDCA; n=31 patients on UDCA. Please, refer to Supplementary Table S6 and S7 for patient characteristics. Bars, 95% confidence interval. (e) Schematic overview of the analysis performed in the validation cohort corresponding to panel (f). (f) Propensity-score matched analyses showing disease severity following SARS-CoV-2 infection in patients taking UDCA compared to non-UDCA controls using the NIH COVID-19 severity score. Moderate +, moderate, severe or critical disease; severe +, severe or critical disease. N=72 patients not on UDCA; n=24 patients on UDCA. Please, refer to Supplementary Table S8 for patient characteristics. Bars, 95% confidence interval.

## 1 **Methods**

### 2 **Ethical approval**

3 All human samples were obtained from patients, deceased transplant organ donors or liver  
4 explants with informed consent for use in research, and ethical approval (Research Ethics  
5 Committee - REC 09/H0305/68; 14/NW/1146; 15/EE/0152; 15/WA/0131; 18/EE/0269; and  
6 Papworth Hospital Research Tissue Bank project number T02233). The mouse animal study  
7 was approved by the Animal Ethics Committee of the Medical University of Vienna and the  
8 Federal Ministry of Science, Research and Economy (BMWFW-66.009/0008-WF/3b/2015)  
9 and was performed according to the Animal Research: Reporting of In Vivo Experiments  
10 (ARRIVE) guidelines. The hamster animal study was approved by the University of Liverpool  
11 Animal Welfare and Ethical Review Board and performed under UK Home Office licences  
12 (PP9284915 and PP4715265) and it was completed at the University of Liverpool and  
13 conducted in accordance with the UK Home Office Animals Scientific Procedures Act (ASPA,  
14 1986). Human lungs and livers retrieved for transplantation but subsequently declined were  
15 used for *ex situ* normothermic perfusion experiments (National Research Ethics Committee  
16 (NREC) North East – Newcastle and North Tyneside 16/NE/0230, lung; NREC East of  
17 England – Cambridge East 14/EE/0137, liver). The study involving volunteers from the  
18 University Medical Centre Hamburg-Eppendorf was performed with informed consent and  
19 ethical approval (Ethik-Kommission der Ärztekammer Hamburg; Ref.No. 2021-300121-WF).  
20 The COVID-Hep.net and SECURE-Liver registries data were deemed not to constitute  
21 human research by Clinical Trials and Research Governance at the University of Oxford  
22 ([https://covid-hep.net/img/CTRG\\_COVID-Hep\\_20200402.pdf](https://covid-hep.net/img/CTRG_COVID-Hep_20200402.pdf)) and by the Institutional  
23 Review Board of University of North Carolina (<https://covidcirrhosis.web.unc.edu/faq/>)  
24 respectively. The study involving patients from the VOCAL cohort was performed with

1 informed consent and ethical approval from the Miami VA Institutional Review Board (Unique  
2 study approval ID 1477437-22).

3

#### 4 **10x single cell RNA sequencing, data analysis and availability**

5 We used our previously published single cell RNA sequencing (scRNAseq) dataset including  
6 primary cholangiocytes, cholangiocyte organoids originating from different regions of the  
7 biliary tree (intrahepatic ducts, common bile duct and gallbladder), and the same organoids  
8 following bile treatment. Tissue dissociation, cell isolation, 10X single cell library preparation  
9 and 10X data processing, normalisation and analysis was performed as previously  
10 described<sup>14</sup>. 10X raw data (fastq files) have been deposited in the repository ArrayExpress  
11 with the accession number E-MTAB-8495. Single cell RNA sequencing data were analysed  
12 using Anaconda-Navigator 1.9.12, Jupyter Notebook 6.0.3 and Rstudio (version 1.1.463).

13

#### 14 **Human tissue collection and processing**

15 Human primary tissue was obtained from biopsies, deceased transplant organ donors or liver  
16 explants after obtaining informed consent. Depending on the application, primary fresh tissue  
17 was embedded in OCT (Optimal Cutting Temperature) compound and stored at -80°C; or  
18 fixed in 10% formalin, dehydrated and embedded in paraffin. Sections from embedded tissue  
19 were cut at a thickness of 5-10 µm using a cryostat or a microtome and mounted on  
20 microscopy slides for further analysis.

21

## 1 **Bile sample collection and processing**

2 Human bile was collected during ERCP (Endoscopic Retrograde Cholangio-  
3 Pancreatography) or intraoperatively with informed consent from the patient. For viral RNA  
4 quantification samples were immediately lysed using an equal volume of RNA lysis buffer  
5 (Sigma) and stored at -20°C.

## 6 7 **Cell culture**

8 Primary cholangiocytes were isolated and cholangiocyte organoids were derived and  
9 cultured using our established methodology<sup>14,16,50</sup>. Cholangiocyte organoids obtained from  
10 intrahepatic ducts (IHD), common bile duct (CBD) and gallbladder (GB) tissue were used in  
11 this study. Cholangiocytes derived from any of the different regions of the biliary tree (IHD,  
12 CBD, GB) acquired a common gallbladder identity when treated with CDCA, as previously  
13 reported<sup>14</sup>. The experiments described were performed with cholangiocyte organoids derived  
14 from all the three regions of the biliary tree (IHD, CBD and GB) and provided congruent  
15 results. For consistency, the results shown correspond only to cholangiocyte organoids  
16 derived from the gallbladder (gallbladder cholangiocyte organoids – GCOs).

17 Human primary intestinal organoids, derived from terminal ileum biopsies were provided by  
18 Simon Buczacki's group. The organoids were derived following a modification of previously  
19 described protocols<sup>51</sup>, embedded in Matrigel and cultured in Intesticult (StemCell  
20 Technologies) supplemented with Penicillin-Streptomycin and Rho kinase inhibitor (Stratech  
21 Scientific).

22 Human primary airway organoids were provided by Joo-Hyeon Lee's lab. The organoids were  
23 derived and cultured as previously described<sup>24</sup>.

1 Vero E6 cells (ATCC™ CRL – 1586), HEK293 cells (ATCC™ CRL – 1573) and HEK293T  
2 cells (ATCC™ CRL – 3216) were grown on tissue culture plates or T25 flasks in 10% FBS  
3 DMEM supplemented with L-glutamine and Penicillin-streptomycin as previously described<sup>52</sup>.

4

### 5 **Biological materials availability**

6 Detailed protocols for the derivation of primary organoids have been previously reported<sup>24,50</sup>.

7 Cell lines are available from standard commercial sources ([https://www.lgcstandards-  
8 atcc.org](https://www.lgcstandards-atcc.org) - Vero E6 cells, ATCC™ CRL – 1586; HEK293 cells, ATCC™ CRL – 1573).

9

### 10 **Modulation of FXR activity**

11 Chenodeoxycholic acid (CDCA) and ursodeoxycholic acid (UDCA) were purchased from  
12 Sigma Aldrich (C9377-5G and U-5127-5G), while Z-Guggulsterone (ZGG) was purchased  
13 from Santa Cruz (sc-204414) and reconstituted following the manufacturer's instructions. To  
14 modulate FXR activity, organoids were incubated with a final concentration of 10  $\mu$ M CDCA;  
15 or 10  $\mu$ M CDCA in combination with 10  $\mu$ M of UDCA or ZGG.

16

### 17 **FXR knock-down**

18 FXR knock-down was performed in cholangiocyte organoids using commercially available  
19 lentiviral particles carrying shRNA gene silencer sequences against the human FXR (*NR1H4*)  
20 transcript (Santa Cruz; sc-38848-V). Commercially available lentiviral particles carrying  
21 control (scrambled) shRNA sequences (Santa Cruz; sc-108080) were used as control.  
22 Successfully transduced cholangiocyte organoids were selected with puromycin 24 hours  
23 after viral transduction. Quantification of FXR, ACE2 and SHP expression and SARS-CoV-2  
24 infection were performed 10 days after FXR knock-down.

25

## 1 **Chromatin immunoprecipitation**

2 Approximately  $6 \times 10^6$  cells were used for each chromatin immunoprecipitation (ChIP), and  
3 cells were incubated with fresh medium with 100  $\mu$ M of CDCA, UDCA or ZGG 2 h before  
4 collection. ChIP was performed using the True Micro ChiP kit (Diagenode C01010130)  
5 according to manufacturer's instructions. In brief, following pre-clearing, the lysate was  
6 incubated overnight with the FXR antibody (Santa Cruz sc-25309 X) (Supplementary Table  
7 S1) or non-immune IgG. ChIP was completed and immunoprecipitated DNA was purified  
8 using MicroChip DiaPure columns (Diagenode C03040001). Samples were analysed by  
9 QPCR using the  $\Delta\Delta C_t$  approach as previously described<sup>50</sup> (see Supplementary Table S3 for  
10 primer sequences). Primers flanking the FXR responsive element (FXR RE) on the well-  
11 known FXR target gene *OST $\alpha$* <sup>53</sup> were used as positive control, while primers flanking a site  
12 distant from the FXR RE on the ACE2 promoter were used and a negative control. The results  
13 were normalized to the enrichment observed with non-immune IgG ChIP controls.

## 15 **Luciferase reporter**

16 Two different fragments containing the FXRE IR-1 in the ACE2 gene and in the SHP gene  
17 were amplified using human genomic DNA as template and inserted onto pGL3-promoter  
18 luciferase vector. The ACE2 and SHP IR-1 mutants were generated using a site-directed  
19 mutagenesis approach (New England BioLabs E0554S). Sequences of primers used are  
20 reported in Supplementary Table S4. These gene reporter constructs were co-transfected  
21 with a commercially available FXR expression plasmid (OriGene, SC329876) into HEK293  
22 cells using TransIT-293 Transfection Reagent (MirusBio). 24 h after transfection, cells were  
23 treated with 50  $\mu$ M of CDCA, UDCA and ZGG in fresh media for 8 hours. Luciferase activity  
24 was determined with GLO-Luciferase Reporter Assay System (Promega, Madison, ONE-  
25 Glo™ Luciferase Assay System) and values were normalized to the empty pGL3 vector.

1

## 2 **Immunofluorescence, RNA extraction and QPCR**

3 Immunofluorescence, RNA extraction and quantitative real-time PCR (QPCR) were  
4 performed as previously described<sup>65,67,70,71</sup>. A complete list of the primary and secondary  
5 antibodies used is provided in Supplementary Table S1. A complete list of the primers used  
6 is provided in Supplementary Table S2.

7 All QPCR data were obtained using a QuantStudio 5 384 Well Block (Thermo Fisher). All  
8 QPCR data are presented as the median, interquartile range (IQR) and range (minimum to  
9 maximum) of four independent experiments unless otherwise stated. Values are relative to  
10 the housekeeping gene Hydroxymethylbilane Synthase (*HMBS*) or Glyceraldehyde-3-  
11 Phosphate Dehydrogenase (*GAPDH*). Statistical analysis is described in the relevant section.  
12 For comparative immunofluorescence images, the cells or sections being compared were  
13 stained simultaneously, using the same primary and secondary antibody master mix. All  
14 immunofluorescence images were acquired using a Zeiss LSM 700 or 710 confocal  
15 microscope using ZEN 2011 SP7 (Zeiss). The same laser power and exposure settings were  
16 used to acquire comparative images. Imagej 2.0.0-rc-69/1.53f software (Wayne Rasband,  
17 NIH, USA, <http://imagej.nih.gov/ij>) was used for image processing. Each  
18 immunofluorescence image is representative of at least 3 different experiments.

19

## 20 **Flow cytometry analyses**

21 Flow cytometry in organoids was performed as previously described<sup>50</sup>. In summary,  
22 organoids were collected using Cell Recovery Solution (Corning) for 20 min at 4 °C and were  
23 then centrifuged at 444g for 4 min and dissociated to single cells using StemPro Accutase  
24 (Invitrogen). Cells were subsequently fixed using 4% paraformaldehyde (PFA) for 20 min at  
25 4 °C. All flow cytometric analyses were performed on a BD LSR-II flow cytometer (BD

1 Biosciences) using BD FACS Diva 8.0.3 (BD Bioscience) and analysed using FlowJo  
2 v.10.4.2. The gating strategy is provided in Supplementary Figure S1.

3

#### 4 **Dose-response curves for ACE2**

5 Primary organoids were treated with 0.01  $\mu\text{M}$  – 1 mM of CDCA, UDCA or ZGG and ACE2  
6 expression was measured via QPCR. The inhibitory effect of UDCA and ZGG on FXR  
7 activation was assessed on cells treated with 10  $\mu\text{M}$  of CDCA. Data were analysed using the  
8 Sigmoidal, 4PL, X is log(concentration) function in GraphPad Prism.

9

#### 10 **Cytotoxicity and viability**

11 Primary organoids were treated with 0.1  $\mu\text{M}$  – 100  $\mu\text{M}$  of CDCA, UDCA or ZGG and the  
12 percentage of viable cells were counted using trypan blue and a Countess II cell counter  
13 (ThermoFisher). Cellular viability in primary organoids treated with 10  $\mu\text{M}$  of CDCA, UDCA  
14 or ZGG was measured using the resazurin-based assay PrestoBlue (Invitrogen, A13261)  
15 using SoftMax Pro 5.4.4 on a SpectraMax M2 (Molecular Devices)..

16

#### 17 **SARS-CoV-2 isolate**

18 The SARS-CoV-2 virus used in this study are the clinical isolate named "SARS-CoV-  
19 2/human/Liverpool/REMRQ0001/2020"<sup>52</sup> derived from a patient's naso-pharyngeal swab and  
20 isolated by Lance Turtle (University of Liverpool) and David Matthews and Andrew Davidson  
21 (University of Bristol) and the delta lineage (B.1.617.2) hCoV-19/England/SHEF-  
22 10E8F3B/2021 (G|SA|D accession number EP|\_|SL\_1731019) kindly provided by Professor  
23 Wendy Barclay, Imperial College London, London, UK through the Genotype to Phenotype  
24 National Virology Consortium (G2P-UK).

25



## 1 **SARS-CoV-2 infection**

2 All work with infectious SARS-CoV-2 was performed under containment level 3 (CL-3)  
3 conditions either at the Cambridge institute of Therapeutic Immunology and Infectious  
4 Diseases (CITIID) or at the Centre for Excellence and Long-acting Therapeutics (CELT).  
5 SARS-CoV-2 was gifted to the users of the CITIID CL-3 by Ian Goodfellow<sup>54,55</sup> and  
6 propagated on Vero E6 cells as previously described<sup>52</sup>. Viral titration was determined using  
7 the TCID50 method on Vero E6 cells<sup>52</sup>. For viral infection primary organoids were passaged  
8 and incubated with SARS-CoV-2 in suspension at a multiplicity of infection (MOI) of 1 for 2  
9 hours. Subsequently, the infected organoids were washed twice with 10 ml of culture media  
10 to remove the viral particles. Washed organoids were plated in 40 µl Matrigel domes, cultured  
11 in organoid medium and harvested at different timepoints.

12 To test whether SARS-CoV-2 produced by infected COs retained its infective capacity, the  
13 supernatant from infected COs was collected at 24 hours post infection and used to infect a  
14 fresh batch of SARS-CoV-2 naive organoids.

15

## 16 **Fixation of SARS-CoV-2 infected organoids/tissue**

17 Organoids for IF were cultured on coverslips, placed at the bottom of the wells of a 24-well  
18 plate. The culture medium was aspirated and replaced with 500 µl of 8% PFA for a minimum  
19 of 30 minutes. Following fixation, the coverslips were recovered, transferred to a clean plate,  
20 and fresh PBS was added. Primary tissue was fixed for a minimum of 4 hours with 8% PFA  
21 and then transferred to a clean plate with fresh PBS.

22

## 23 **Quantification of viral infection**

24 Organoids or primary tissue were infected in 24-well plates as described above. Total RNA  
25 samples were prepared by adding 500 µl of lysis buffer (25 mM Tris-HCL+ 4 M Guanidine

1 thiocyanate with 0.5%  $\beta$ -mercaptoethanol) to each well and transferring the lysate (1 ml) to a  
2 5 ml Eppendorf tube. Tubes were vortexed, and 100% analytical grade ethanol was added  
3 to a final concentration of 50%. After 10 minutes of incubation, 860  $\mu$ l of lysis buffer  
4 (containing MS2 bacteriophage as an internal extraction and amplification control) were  
5 added and thoroughly mixed. The RNA was then isolated using an RNA spin column as  
6 previously described<sup>56</sup>. Viral replication was quantified using QPCR for the expression of the  
7 viral RNA-dependent RNA polymerase (*RdRp*) gene with primers specific for a 222 bp long  
8 fragment from a conserved region of the gene. *GAPDH* was used as a housekeeping gene  
9 and MS2 was used as an internal reference as previously described<sup>56</sup>. Viral load was  
10 determined relative to *GAPDH*. The sequences of primers/probes used are provided in  
11 Supplementary Table S2.

12

### 13 **Transmission Electron Microscopy**

14 Infected organoids were fixed in 4% paraformaldehyde; 2.5% glutaraldehyde in 0.1M sodium  
15 cacodylate buffer overnight at 4°C, washed and stored in 0.1M sodium cacodylate buffer  
16 before processing. Samples were postfixed in 1% aqueous osmium tetroxide (TAAB, UK);  
17 1.5% potassium ferricyanide overnight at 4°C, washed thoroughly in dH<sub>2</sub>O and *en*  
18 *bloc* stained in 3% aqueous uranyl acetate (Agar Scientific, UK) for 24h at 4°C. Samples were  
19 dehydrated through an ethanol series, infiltrated with 1:1 propylene oxide:resin (TAAB, UK)  
20 and blocks of fresh resin polymerised at 60°C for 48h. Ultrathin sections of ~60nm were cut  
21 from blocks using an EM UC7 ultramicrotome (Leica Microsystems, UK) and mounted on  
22 copper grids coated with carbon and formvar (Agar Scientific, UK). Grids were post-stained  
23 in uranyl acetate and lead citrate, imaged using a HT7800 transmission electron microscope  
24 (Hitachi High Technologies, Japan) operating at 100kV and acquired using HT7800 TEM  
25 operating software version 01.21 (Hitachi).

1

## 2 **HEK293 cells stably expressing ACE2**

3 HEK293T cells stably expressing ACE2 were generated as previously described<sup>57</sup>. Briefly,  
4 HEK293T cells transduced with ACE2 under the control of the spleen focus-forming virus  
5 (SFFV) promoter were sorted for high cell-surface ACE2 expression and single-cell cloned.  
6 Following expansion, a clone with stable, homogeneously high expression of ACE2 was  
7 selected via FACS.

8

## 9 **Luciferase reporter for SARS-CoV-2 replication**

10 A luciferase reporter for SARS-CoV-2 protease activity during viral replication was generated  
11 as previously described<sup>57</sup>. In brief, HEK293T reporter cells stably expressing ACE2, Renilla  
12 luciferase (Rluc) and SARS-CoV-2 Papain-like protease-activatable circularly permuted  
13 firefly luciferase (FFluc) were seeded in flat-bottomed 96-well plates. The following morning,  
14 cells were treated with the indicated doses of CDCA, UDCA and ZGG, and infected with  
15 SARS-CoV-2 at a MOI of 0.01. The SARS-CoV-2 RdRp inhibitor remdesivir and a neutralising  
16 antibody cocktail blocking the interaction between SARS-CoV-2 spike and ACE2 (REGN-  
17 COV2) were included as positive controls. After 24 hours, cells were lysed in Dual-Glo  
18 Luciferase Buffer (Promega, E2920) diluted 1:1 with PBS and 1% NP-40. Lysates were then  
19 transferred to opaque 96-well plates, and viral replication quantitated as the ratio of  
20 FFluc/Rluc activity measured using the Dual-Glo kit (Promega) according to the  
21 manufacturer's instructions. FFluc/Rluc ratios were expressed as fraction of maximum, then  
22 analysed using the Sigmoidal, 4PL, X is log(concentration) function in GraphPad Prism.

23

## 24 **Animal study**

1 *Mice*: the experiments were performed in accordance with the Animal Research: Reporting  
2 of In Vivo Experiments (ARRIVE) guidelines and approved by the Animal Ethics Committee  
3 of the Medical University of Vienna and the Federal Ministry of Science, Research and  
4 Economy (BMFWF-66.009/0008-WF/3b/2015). Friend Virus B NIH (FVB/N) mice were bred  
5 in house. Animals were housed in a 12 hours/12 hours dark/light cycle, with a humidity of 45-  
6 65% and temperature of 20-24°C. Chow was obtained from SAFE—Scientific Animal Food  
7 & Engineering (product number A04). Age matched female animals were used. Animals were  
8 assigned randomly to treatment and control groups. Animals in the treatment group received  
9 chow supplemented with 1% w/w UDCA and 1%w/w cholic acid (CA), while animals in the  
10 control group received chow supplemented with 1% w/w cholic acid (CA)<sup>58</sup>. Cholic acid was  
11 used to activate FXR and study the impact of UDCA on FXR activation<sup>19</sup>. The animals were  
12 fed ad libitum for 7 days. Data were analysed blinded to the identity of the experimental  
13 groups.

14 *Hamsters*: the experiments were performed in accordance with the UK Home Office Animals  
15 Scientific Procedures Act (ASPA, 1986). Additionally, all studies were approved by the  
16 University of Liverpool Animal Welfare and Ethical Review Board and performed under UK  
17 Home Office licences PP9284915 and PP4715265. Golden Syrian Hamsters were purchased  
18 from Janvier Labs, France. Animals were housed in a 12 hours/12 hours dark/light cycle, with  
19 a humidity of 45-65% and temperature of 20-24°C. Age matched male animals were used  
20 weighing between 80 – 100g. Animals were assigned randomly to treatment and control  
21 groups. Animals in the treatment groups received a daily oral regimen of UDCA (416 mg/kg)  
22 by oral gavage while animals in the control group received vehicle only. The animals were  
23 fed ad libitum and treatment continued for 7 days to achieve similar blood concentration of  
24 UDCA observed in patients taking UDCA (Extended Data Fig. 9a)<sup>28</sup>.

1 For testing UDCA intervention against SARS-CoV-2 infection, one hamster was directly  
2 inoculated by the intranasal route with  $1 \times 10^2$  PFU/hamster in 100 $\mu$ l of PBS. Each infected  
3 hamster was placed on one side of a transmission cage. The cage was divided with an  
4 aerated barrier allowing the infected hamster to be co-housed with previously treated  
5 uninfected animals housed on the other side permitting to study viral infection by aerosol  
6 transmission. Daily swabs were collected from all animals to monitor the infection via QPCR  
7 for the viral N gene. On day 4 post infection the hamsters were euthanised and lungs and  
8 nasal turbinates were collected for quantification of viral infection. The experiment was  
9 repeated n=3 times for a total of n=9 UDCA animals and n=6 vehicle animals. Data were  
10 analysed blinded to the identity of the experimental groups.

11

#### 12 **Quantification of UDCA concentration**

13 UDCA was quantified from hamster plasma using a LCMS assay that was validated using  
14 FDA industry guidelines. Quantification was achieved via LC-MS/MS (6500+ QTRAP,  
15 SCIEX) operating in negative mode. UDCA was detected using MRM where the following  
16 ions were monitored for quantification: UDCA (m/z 391 > 391 and internal standard  
17 mefloquine 379.1>320.1). A stock solution of 1 mg/ml UDCA was prepared in methanol and  
18 stored at 4°C until use. A standard curve was prepared in plasma by serial dilution from 40000  
19 ng/mL to 312.5 ng/mL and an additional blank solution was also used. Chromatographic  
20 separation was achieved using a multi-step gradient with a Acquity BEH C18 column (2.1mm  
21 x 100mm 1.7 $\mu$ m; Waters, Wilmslow, UK) using mobile phases A (100% water, 0.1% formic  
22 acid and 5mM ammonium formate) and B (90% acetonitrile 10% methanol, 0.1% formic acid  
23 and 5mM ammonium formate). Chromatography was conducted over 3.5 minutes. At the  
24 start of each run, mobile phase A was 80% until 0.5 minutes when mobile phase B was  
25 increased to 47% over 0.5 minutes. Mobile phase B was then increased over 1 minute to

1 51%. Mobile phase B was then increased to 100% at 2.5 minutes which was held until 3  
2 minutes. Mobile phase B was reduced to 20% and held till 3.5 minutes. Samples were  
3 extracted from hamster plasma via protein precipitation. Briefly, 100µl of standard, QC, blank  
4 plasma, or study sample were treated with 400µl of ACN. Samples were then vortexed  
5 followed by centrifugation at 3500rpm for 5 minutes. 400µl of supernatant was transferred to  
6 fresh glass vials and evaporated under a steady stream of nitrogen. Samples were  
7 reconstituted in 50:50 water methanol and analysed. Inter- and intra- assay variance was  
8 assessed by 3 levels of independent quality controls. Coefficient of variation of accuracy and  
9 precision were <15% in all assays.

### 11 ***Ex situ* normothermic perfusion (ESNP) of human lungs**

12 For the *ex situ* perfusion of a single pair of human lungs, two bespoke ESNP circuits  
13 (Medtronic, Ireland) were used. In brief, this circuit facilitates pressure monitored perfusion  
14 with normothermic perfusion solution consisting of bovine serum albumin (BSA) (70 g/l),  
15 dextran 40 (5 g/l), modified Krebs Henseleit buffer (9.2 g/l), sodium bicarbonate solution (28  
16 ml/l), calcium chloride (25 ml/l) and heparin (3750 units/l). The pair of human lungs used,  
17 were perfused following the physiological principles previously described<sup>59</sup>.

### 18 *Lung ESNP experimental setup*

19 The experiment shown in Fig. 3 was performed on a pair of lungs declined for clinical lung  
20 transplantation due to the donor's past medical history. The left and the right lungs were  
21 divided at the carina and common pulmonary artery bifurcation. For each isolated lung, the  
22 pulmonary artery and the pulmonary vein were cannulated and an endobronchial tube was  
23 inserted into the main bronchus. The cannulae of each lung were connected to an entirely  
24 independent ESNP circuit (control and experimental lung circuits). The endobronchial tube  
25 of each lung was connected to an independent mechanical ventilator (Drägerwerk AG & Co.

1 KGaA, Germany). Mechanical ventilation was performed using room air with a positive end  
2 expiratory pressure of 5 mmHg, target tidal volume of 130 ml at 5 bpm for the left lung and  
3 140 ml at 6 bpm for the right lung.

#### 4 *Timing and duration of ESNP*

5 Perfusion started simultaneously for both lungs. After 30 minutes of stable perfusion,  
6 UDCA/carrier was administered and this time point was defined as 0 hours (0h). ESNP was  
7 performed for 6 hours for both lungs after administration of UDCA/carrier. The end of  
8 experiment timepoint is defined as 6 hours (6h).

#### 9 *Experimental timepoints and sample collection*

10 Baseline tissue and perfusate samples were collected simultaneously from both lungs before  
11 UDCA/carrier administration. We defined these samples as 0h samples. Corresponding  
12 samples were collected simultaneously from each lung at the end of the experiment at 6h.  
13 We defined those as 6h samples.

14 The following samples were collected from each lung per timepoint: n=4 independent lung  
15 biopsies, n=4 surgically excised independent samples from the pulmonary artery, n=4  
16 surgically excised independent samples from the main bronchus, and n=4 independent  
17 samples from the perfusate in each circuit. The independent tissue samples refer to different  
18 locations of the organ. Biopsies were surgically excised and the lung parenchymal samples  
19 from 0h were taken from peripheral areas separated from the remaining lung by staples  
20 (Medtronic, Ireland) to seal the defect. 6h samples were taken using the same approach.  
21 Please also refer to the results section 'UDCA limits SARS-CoV-2 infection in human organs  
22 *ex vivo*'.

#### 23 24 ***Ex situ* normothermic perfusion (ESNP) of donor livers**

1 The OrganOx metra normothermic liver perfusion device was used for *ex situ* perfusion of  
2 human livers as previously described<sup>14,29,31</sup>. The machine, which is clinically used for  
3 preservation of livers for transplantation enables prolonged automated organ preservation by  
4 perfusing it with ABO-blood group-matched normothermic oxygenated blood. The perfusion  
5 device incorporates online blood gas measurement, as well as software-controlled algorithms  
6 to maintain pH, PO<sub>2</sub> and PCO<sub>2</sub> (within physiological limits), temperature, mean arterial  
7 pressure and inferior vena cava pressure within physiological normal limits.

### 8 *Experimental setup*

9 2 donor livers not used for transplantation were maintained with ESNP. In brief, the hepatic  
10 artery, portal vein, inferior vena cava and bile duct were cannulated, connected to the device  
11 and perfusion commenced. One liver was randomly chosen to receive a solution of UDCA  
12 dissolved in 0.9% NaCl (experimental liver), while the other liver (control) was chosen to  
13 receive the same volume of carrier (0.9% NaCl). UDCA was resuspended in 0.9% (w/v) NaCl  
14 solution and injected in the blood circuit to achieve a final concentration of 2000 ng/ml, which  
15 is the steady state concentration of UDCA detected in serum after multiple doses of UDCA<sup>28</sup>.

### 16 *Timing and duration of ESNP*

17 The time of UDCA/carrier administration following the start of ESNP was defined as 0 hours  
18 (0h). ESNP was performed for 12 hours for both livers after administration of UDCA/carrier.  
19 The end of experiment timepoint is defined as 12 hours (12h).

### 20 *Experimental timepoints and sample collection*

21 Baseline tissue and perfusate samples were collected from each liver before UDCA/carrier  
22 administration. We defined these samples as 0h samples. Corresponding samples were  
23 collected from each liver at the end of the experiment at 12h. We defined those as 12h  
24 samples.



1 The following samples were collected from each liver per timepoint: n=4 independent  
2 surgically excised gallbladder samples, n=4 independent liver biopsies, and n=4 independent  
3 samples from the circulating perfusate. Independent gallbladder samples and liver biopsies  
4 were obtained from different locations of the organ. Gallbladder samples were surgically  
5 excised and the gallbladder wall was sutured to close the defect.

6

### 7 ***Ex vivo* SARS-CoV-2 infection of human tissue**

8 The infection of human tissue maintained *ex vivo* with SARS-CoV-2 occurred in a CL-3 facility  
9 following the ESNP experiment. 4 independent samples from the lung parenchyma, bronchi  
10 and the vasculature (pulmonary artery) of each lung were collected per timepoint, i.e. at the  
11 start of the ESNP experiment (0h) and at the end (6 hours) of the lung perfusion. 4  
12 independent samples from each ESNP liver gallbladder were collected per timepoint, i.e. at  
13 the start of the ESNP experiment (0h) and at the end (12 hours) of the liver perfusion. Sample  
14 collection was performed as described in the section '*Ex situ* normothermic perfusion (ESNP)  
15 of donor livers'.

16 The freshly obtained lung, bronchial, vascular and gallbladder tissues were processed into  
17 small rectangular pieces of 0.5x0.5 cm and were rinsed with University of Wisconsin solution  
18 (lung, bronchial and vascular tissues) or William's E medium with supplements as previously  
19 described<sup>50</sup> (gallbladder tissue). Washed specimens were placed in wells of a 24-well plate  
20 (one specimen per well) and infected with SARS-CoV-2. An inoculum of  $1.2 \times 10^5$  PFU/ml at  
21 500  $\mu$ l per well was used. After two hours, the inoculum was removed, and the specimens  
22 were washed three times with PBS. The infected human tissue was then cultured in 500  $\mu$ l  
23 of advanced DMEM with supplements<sup>60</sup> (lung, bronchial and vascular tissues) or William's E  
24 medium with supplements (gallbladder tissue). Supernatant and tissue were harvested for  
25 QPCR and immunofluorescence at 2 and 24 hours post infection.

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## **Blood sample collection and processing**

Blood samples were collected from patients as part of the UK-PBC Nested Cohort study after obtaining informed consent, anonymised and analysed by a blinded researcher. To obtain serum from full blood, the samples were spun at 4°C at 1000 g for 10 minutes to allow for serum separation and serum was collected as the supernatant. All blood samples were collected after fasting.

## **ACE2 enzymatic activity**

ACE2 enzymatic activity was performed on serum samples and tissue lysates using the ACE2 activity fluorometric kit (abcam ab273297) following manufacturer's instructions using SoftMax Pro 5.4.4 on a SpectraMax M2 (Molecular Devices).

## **ACE2 measurement in nasal epithelial cells of volunteers**

### *Recruitment*

Following approval by local ethics committee (Ethik-Kommission der Ärztekammer Hamburg; Ref.No. 2021-300121-WF), the study was advertised in the University Medical Centre Hamburg-Eppendorf amongst clinicians regularly prescribing UDCA, and thus familiar with the drug and its possible side-effects. 8 clinicians who volunteered to participate in the study were recruited following informed consent. The characteristics of the volunteers are provided in Supplementary Table S5.

### *Study design and exclusion criteria*

UDCA was self-administered at the clinically approved dose of 15mg/kg/day in a single morning dose for 5 days. Nasal epithelium samples for ACE2 measurement were collected each morning prior to UDCA administration using the Citoswab nasopharyngeal swab

1 collection kit (Corona Smear). Day 0 samples were collected before the first UDCA dose.  
2 Daily morning samples were collected during UDCA treatment (days 1-5). Following drug  
3 washout, repeat nasopharyngeal swabs were collected between days 22 and 28. Volunteers  
4 providing samples with no detectable RNA were excluded from the study.

#### 5 *ACE2 measurement*

6 RNA was extracted from the Citoswab nasopharyngeal swab collection kit (Corona Smear)  
7 using the RNeasy micro kit (Qiagen) according to the manufacturer's instructions. ACE2  
8 mRNA levels were measured using QPCR (see Immunofluorescence, RNA extraction and  
9 QPCR section). The results are shown as fold change over the housekeeping gene GAPDH.  
10

10

#### 11 **Serum proteome analysis in patients with primary biliary cholangitis (PBC)**

12 Serum proteomic analysis was performed in the UK-PBC patient cohort as described in  
13 Barron-Millar, *et al.*<sup>32</sup>. Blood samples were obtained with informed consent and appropriate  
14 ethical approval (UK-PBC tissue bioresource, 14/NW/1146). Serum samples were assayed  
15 using the Olink proteomics™ platform ([www.olink.com](http://www.olink.com)).  
16

16

#### 17 **Patient data**

18 Data for patients with chronic liver disease were collected as described elsewhere<sup>34,61</sup>.  
19 Briefly, collated results from two open online reporting registries (COVID-Hep.net and  
20 SECURE-Liver) were examined. Reports were asked to report cases of laboratory confirmed  
21 COVID-19 in patients with chronic liver disease at the end of the disease course. Anonymous  
22 clinical and demographic data were collected, filtered to remove duplicate entries, those with  
23 incomplete records, those with prior liver transplantation, those not over 18 years of age,  
24 those over 90 years of age, and those without laboratory confirmed infection.

1 Data for patients with liver transplant were collected as described elsewhere<sup>35</sup>. Briefly, data  
2 from patients with a liver transplant who were alive on the 1<sup>st</sup> of March 2020 were examined.  
3 Participants who had no COVID-19 infection, were unvaccinated or developed COVID-19  
4 within 30 days from their first UDCA prescription were excluded. The resulting study sample  
5 included n=24 patients on UDCA and 95 who were not on UDCA and was used for the  
6 analysis.

### 8 **Statistical analyses**

9 Statistical analyses were performed using Microsoft Excel v 16.19, Rstudio (version 1.1.463),  
10 GraphPad Prism 9 or Stata 15.1 (StataCorp, College Station, TX, USA). The normal  
11 distribution of our values was evaluated using the Shapiro-Wilk test where appropriate. For  
12 comparison between two groups, a two-tailed Student's t-test or the non-parametric Mann-  
13 Whitney test were used depending on the normality of our distribution. To compare matched  
14 samples from the same individual a two-tailed paired Student's t-test was used (the normal  
15 distribution of our data was confirmed using the Shapiro-Wilk test). Variance between  
16 samples was tested using the Brown-Forsythe test. For comparing multiple groups to a  
17 reference group one-way ANOVA followed by Dunnett's test was used between groups with  
18 equal variance. Immunofluorescence images are representative of 4 independent  
19 experiments. Data are represented in box plots and elements are defined as follow: center  
20 line, median; box, interquartile range (IQR); whiskers, range; error bars, standard deviation.  
21 Serum proteomic analysis was performed using Rstudio (version 1.1.463). The correlation  
22 between ACE2 levels and UDCA administration was interrogated using multiple linear  
23 regression analysis with the *lm* function in R. ACE2 expression data was defined as the  
24 independent variable, while UDCA administration, sex, age, body mass index (BMI), stage of

1 chronic liver disease according to the Child-Turcotte-Pugh class and alkaline phosphatase,  
2 were defined as dependent variables.

3 COVID-Hep and SECURE-Liver data (exploratory cohort): for propensity score-matched  
4 analyses, 1:5 matched samples (using the nearest neighbour approach) were constructed  
5 with hospitalisation, physician-reported requirement for intensive care, intensive care  
6 admission, mechanical ventilation, and death as the outcome variables. Covariables used  
7 were age, sex and categorical stage of chronic liver disease according to the *Child-Turcotte-*  
8 *Pugh* class, diabetes, chronic pulmonary disease (COPD/asthma), immunosuppressive  
9 therapy, increased BMI (BMI>25), and the presence of non-alcoholic fatty liver disease, due  
10 to its association with increased COVID19 risk. Importantly, alcohol-related liver disease  
11 (ARLD) is also associated with increased COVID19 risk; however, patients could not be  
12 propensity scored matched between the 2 cohorts, as there were no patients with ARLD  
13 receiving UDCA. Consequently, patients with ARLD were excluded.

14 VOCAL data (validation cohort): for propensity score-matched analyses, 1:3 matched  
15 samples (using a Greedy matching algorithm) were constructed with moderate/severe/critical  
16 COVID-19 and severe/critical COVID-19 according to the NIH severity scale as the outcome  
17 variables. Covariables used were age, sex, ethnicity, location within the United States,  
18 diabetes, BMI, COPD, type of immunosuppressive therapy (calcineurin Inhibitor with or  
19 without anti-metabolite therapy) and dominant SARS-CoV-2 variant.

20 Propensity score matching was performed using the *teffects* function in Stata. The average  
21 treatment effect on the treated (ATET) was calculated with robust Abadie-Imbens standard  
22 errors and derived 95% confidence intervals are presented.

23

1 **Data availability:** Single-cell RNA sequencing data are available on ArrayExpress.  
2 Accession number: E-MTAB-8495. Source data for in vivo experiments and relative to  
3 Extended Data Figure 10 are provided.  
4

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12

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15 interests to disclose.

16

### 17 **Extended Data Figure legends**

18 **Extended Data Fig. 1. Expression of SARS-CoV-2 entry genes in cholangiocytes. (a)**

19 Schematic illustration of different primary human cholangiocyte populations corresponding to  
20 different areas of the biliary tree and cholangiocyte organoids (COs) derived from different  
21 areas of the biliary tree grown in absence or presence of the bile acids. **(b)** UMAP plot  
22 illustrating different cholangiocyte populations from (a) analysed by scRNAseq. **(c)** UMAP  
23 plots showing that viral entry related genes are predominantly expressed in extrahepatic  
24 cholangiocytes and COs treated with bile acids. **(d-e)** Immunofluorescence illustrating that  
25 ACE2 is expressed in extrahepatic cholangiocytes **(d)** and that SARS-CoV-2 infects

1 gallbladder cholangiocytes of COVID-19+ patient but not intrahepatic cholangiocytes (e). N=4  
2 independent samples. Scale bars 50  $\mu$ m. (f) QPCR confirming detection of SARS-CoV-2  
3 RNA in bile of COVID-19+ patients. Housekeeping gene, HMBS; n=4; two-tailed Mann-  
4 Whitney test; centre line, median; box, interquartile range (IQR); whiskers, range; bars,  
5 standard deviation. (g) Violin plot of scRNAseq data from (b) showing that cholangiocyte  
6 organoids upregulate ACE2 when treated with bile acids regardless of their region of origin.  
7 (h) QPCR validating that upon treatment with the bile acid CDCA cholangiocyte organoids  
8 assume a gallbladder identity expressing the gallbladder marker SOX17 and upregulating  
9 ACE2 at levels comparable to primary gallbladder. Housekeeping gene, HMBS; n=4  
10 independent experiments; one-way ANOVA adjusted for multiple comparisons; ns, non-  
11 significant; centre line, median; box, interquartile range (IQR); whiskers, range; bars,  
12 standard deviation. (i) Immunofluorescence showing that CDCA induces ACE2 expression  
13 in gallbladder cholangiocyte organoids (GCOs). N=4 independent experiments. Scale bars  
14 50  $\mu$ m.

15  
16 **Extended Data Fig. 2. CDCA-treated GCOs can be infected by SARS-CoV-2.** (a)  
17 Schematic representation of the methodology used to infect GCOs with SARS-CoV-2 and  
18 test the capacity of SARS-CoV-2 virions produced in GCOs to infect new (uninfected) cells.  
19 (b) Immunofluorescence validating SARS-CoV-2 infection in GCOs. N=4 independent  
20 experiments. Scale bars 50  $\mu$ m. (c) QPCR confirming infection of CDCA-treated GCOs with  
21 SARS-CoV-2 propagated in VERO E6 cells (top panel) and with SARS-CoV-2 propagated in  
22 GCOs treated with CDCA (bottom panel), illustrating that SARS-CoV-2 produced in  
23 GCOs+CDCA retains its infectious capacity. Scale bars 50  $\mu$ m; housekeeping gene, GAPDH;  
24 n=4 independent experiments; Kruskal-Wallis test adjusted for multiple comparisons; centre  
25 line, median; box, interquartile range (IQR); whiskers, range; bars, standard deviation. (d)

1 QPCR showing up-regulation of innate immune and anti-viral response genes in  
2 GCOs+CDCA following SARS-CoV-2 infection. Housekeeping gene, GAPDH; n=4, 2  
3 biological and 2 technical replicates; two-tailed Mann-Whitney test (IL-1 $\beta$ , IL-6, IFN $\alpha$ ) and  
4 two-tailed unpaired t-test (TNF $\alpha$ , IFN $\lambda$ ). (CDCA concentration, 10  $\mu$ M); centre line, median;  
5 box, interquartile range (IQR); whiskers, range; bars, standard deviation. (e) Transmission  
6 electron micrograph of uninfected cholangiocyte organoids (left panel) and cholangiocyte  
7 organoids infected with SARS-CoV-2 in the absence (central panel) or presence of CDCA  
8 (right panel) showing key morphological features of viral infection and cell death, such as  
9 production of viral particles, formation of pathologic vacuoles (Vc) and swollen mitochondria  
10 (Mt). N=3 independent experiments. Scale bars 5  $\mu$ m.

11

12 **Extended Data Fig. 3. FXR is present and active in tissues affected by COVID-19 and**  
13 **their corresponding CDCA-treated organoids. (a-b)** Immunofluorescence images  
14 confirming expression of FXR in primary human gallbladder, lungs and intestinal tissue (a)  
15 and in corresponding primary organoids treated with physiological levels of bile acids (CDCA,  
16 10  $\mu$ M) (b). N=4 independent experiments. White scale bars 100  $\mu$ m; grey scale bar 25  $\mu$ m.  
17 (c) QPCR analysis validating expression of FXR and its downstream effector SHP in primary  
18 tissue and corresponding organoids in presence or absence of physiological levels of bile  
19 acids (CDCA). CDCA treatment increases FXR and SHP expression in organoids to levels  
20 that are closer to primary tissue. Housekeeping gene, HMBS; n=4 independent experiments;  
21 one-way ANOVA adjusted for multiple comparisons; centre line, median; box, interquartile  
22 range (IQR); whiskers, range; bars, standard deviation. GCOs, gallbladder cholangiocyte  
23 organoids; AOs, airway organoids; IOs, intestinal organoids.

24

25 **Extended Data Fig. 4. UDCA and ZGG require FXR to reduce ACE2 and SARS-CoV-2**

1 **infection. (a-b)** Immunofluorescence **(a)** and QPCR **(b)** showing downregulation of FXR  
2 expression following FXR knock-down (KD) in cholangiocyte organoids. Scale bars 25  $\mu$ m.  
3 Housekeeping gene HMBS. One-way ANOVA adjusted for multiple comparisons; n=4  
4 independent experiments; centre line, median; box, interquartile range; whiskers, range;  
5 bars, standard deviation. **(c)** QPCR on FXR KD cholangiocyte organoids showing no change  
6 in the expression of ACE2 and the FXR downstream effector SHP following treatment with  
7 CDCA, UDCA or ZGG, demonstrating that FXR is indispensable for regulating ACE2 and  
8 SHP through these compounds. ACE2 and SHP expression in wild-type organoids shown in  
9 Fig. 1c and ED Fig. 5a. Housekeeping gene, HMBS; n=4; one-way ANOVA adjusted for  
10 multiple comparisons; ns, non-significant; centre line, median; box, interquartile range;  
11 whiskers, range; bars, standard deviation. **(d)** QPCR quantifying SARS-CoV-2 RNA 24 hours  
12 post infection in FXR KD cholangiocyte organoids treated with CDCA, UDCA or ZGG. SARS-  
13 CoV-2 infection in the presence of FXR is shown in Fig. 1e. Housekeeping gene, GAPDH;  
14 n=4 independent experiments; one-way ANOVA adjusted for multiple comparisons; centre  
15 line, median; box, interquartile range; whiskers, range; bars, standard deviation. (CDCA,  
16 UDCA and ZGG concentration, 10  $\mu$ M). **(e)** Schematic illustrating the luciferase reporter  
17 construct containing the FXR responsive element IR-1 identified in Fig. 1a and the  
18 mutagenesis strategy employed in panel (f). **(f)** Luciferase reporter assay in HEK293 cells  
19 showing the transcriptional activity associated with the IR-1 located in the ACE2 promoter  
20 upon treatment with CDCA, UDCA or ZGG. Site-directed mutagenesis on IR-1 abolishes FXR  
21 binding/transactivation confirming the specificity of FXR binding on the ACE2 IR-1. IR-1  
22 located in the SHP promoter used as positive control. N=3 independent experiments; one-  
23 way ANOVA adjusted for multiple comparisons; bars, standard deviations. (CDCA, UDCA  
24 and ZGG concentration, 50  $\mu$ M).

25

1 **Extended Data Fig. 5. FXR modulation in biliary, airway and intestinal.** (a) QPCR  
2 analysis in primary airway, biliary and intestinal organoids demonstrating that CDCA  
3 activates FXR, while UDCA and ZGG inhibit it, as evidenced by corresponding changes in  
4 the expression of the FXR downstream target SHP. Housekeeping gene, HMBS; n=4  
5 independent experiments; one-way ANOVA adjusted for multiple comparisons; centre line,  
6 median; box, interquartile range (IQR); whiskers, range; bars, standard deviation. (b)  
7 Immunofluorescence showing ACE2 expression levels in primary organoids in absence of  
8 bile acids. The panel is complementary to Fig. 1d showing the modulation of ACE2 following  
9 FXR activation (CDCA) and inhibition (UDCA/ZGG). N=4 independent experiments. Yellow  
10 scale bars 50  $\mu\text{m}$ ; grey scale bar 25  $\mu\text{m}$ . (c) Flow cytometry histograms showing changes in  
11 ACE2 levels upon modulation of FXR activity in primary airway, biliary and intestinal  
12 organoids. n=3 independent experiments. (CDCA, UDCA and ZGG concentration, 10  $\mu\text{M}$ ).  
13 (d) Dose-response curves showing the effect of 0.01  $\mu\text{M}$  – 1 mM of CDCA, UDCA and ZGG  
14 on the expression of ACE2 in primary airway, biliary and intestinal organoids (n=3  
15 independent experiments). Response defined as percentage of the maximal ACE2  
16 expression level for each condition via QPCR. Bars, SEM. (e) Percentage of non-viable cells  
17 following treatment of airway, biliary and intestinal organoids with CDCA, UDCA and ZGG at  
18 a range of 0.1  $\mu\text{M}$  – 100  $\mu\text{M}$  showing that these compounds cause minimal cell death within  
19 the tested range. N=3 independent experiments. Bars, range. (f) Resazurin assay  
20 (PrestoBlue) showing that treatment with 10  $\mu\text{M}$  of CDCA, UDCA or ZGG does not affect  
21 cellular viability. N=4 independent experiments; one-way ANOVA adjusted for multiple  
22 comparisons; ns, non-significant; centre line, median; box, interquartile range (IQR);  
23 whiskers, range; bars, standard deviation.

24  
25 **Extended Data Fig. 6. ACE2 downregulation is required for the UDCA/ZGG-mediated**

1 **reduction in SARS-CoV-2 infection. (a-b)** QPCR analysis **(a)** and immunofluorescence **(b)**  
2 illustrating ACE2 and FXR expression in wild-type HEK293 cells and HEK293T cells stably  
3 expressing ACE2. Primary human airway tissue used as positive control. Housekeeping  
4 gene, HMBS; n=4; one-way ANOVA adjusted for multiple comparisons; centre line, median;  
5 box, interquartile range (IQR); whiskers, range; bars, standard deviation. Scale bars 100  $\mu$ m.  
6 **(c)** SARS-CoV-2 infection of HEK293T cells genetically engineered to stably express ACE2.  
7 Cells were treated with the indicated doses of CDCA, UDCA or ZGG, infected with SARS-  
8 CoV-2 at an MOI of 0.01, and analysed after 24 h. The SARS-CoV-2 RdRp inhibitor  
9 remdesivir and a neutralising antibody cocktail blocking the interaction between SARS-CoV-  
10 2 spike and ACE2 (REGN-COV2) were included as positive controls. N=3; one-way ANOVA  
11 adjusted for multiple comparisons; mean values  $\pm$  SEM;

12  
13 **Extended Data Figure 7. FXR inhibition reduces ACE2 levels *in vivo*.** **(a)** Schematic  
14 representation of the experiment performed. **(b)** QPCR showing that treatment with UDCA in  
15 FVB/N mice reduces ACE2 levels in lung, gallbladder and intestinal tissue. Housekeeping  
16 gene, GAPDH; n=4 animals per group (UDCA vs. no UDCA control group, see methods);  
17 unpaired two-tailed t-test; centre line, median; box, interquartile range (IQR); whiskers, range;  
18 bars, standard deviation. **(c)** Immunofluorescence images showing ACE2 levels upon  
19 treatment with UDCA in respiratory, biliary and intestinal epithelium in FVB/N mice. N=4 mice  
20 per group. White scale bars 100  $\mu$ m; yellow scale bars 50  $\mu$ m. **(d)** QPCR showing that  
21 treatment with UDCA in Syrian golden hamsters reduces ACE2 levels in the gallbladder and  
22 intestinal tissue. Housekeeping gene, GAPDH; n=5 vehicle/No UDCA group vs n=3 UDCA  
23 group; unpaired two-tailed t-test; centre line, median; box, interquartile range (IQR); whiskers,  
24 range; bars, standard deviation. **(e)** Immunofluorescence images showing ACE2 levels upon  
25 treatment with UDCA in biliary and intestinal epithelium in Syrian golden hamsters. N=3

1 hamsters per group. White scale bars 100  $\mu\text{m}$ .

2

3 **Extended Data Figure 8. UDCA plasma concentration *in vivo*.** (a) UDCA concentration in  
4 the plasma of hamsters over 7 days of treatment with 416 mg/kg/day of UDCA. N=6  
5 animals/group; line, median. (b) Viral titre showing levels of infectious virus as measured by  
6 plaque assay in lungs in directly inoculated hamsters and sentinel animals treated with UDCA  
7 or vehicle and co-housed with infected animals. Samples were collected after 4 days of co-  
8 housing. N=6 UDCA vs n=3 vehicle animals; Kruskal-Wallis test with Dunn's correction for  
9 multiple comparisons. Animals from each experiment are represented with different symbols;  
10 line, median.

11

12 **Extended Data Figure. 9. FXR inhibition reduces SARS-CoV-2 infection in human**  
13 **organs *ex vivo*.** (a) Photograph and schematic of the liver *ex situ* normothermic perfusion  
14 (ESNP) experiment performed; including type of samples harvested and timeline. 0h: 0 hours  
15 baseline sample collection and UDCA/carrier administration. The 0h samples were collected  
16 immediately prior to UDCA administration. 12h: 12 hours after UDCA/carrier administration.  
17 For each timepoint, 4 independent tissue samples were obtained from the grafts' gallbladder  
18 and used for ACE2 measurement and viral infection (n=4 gallbladder tissue samples per  
19 timepoint). (b) ACE2 enzymatic activity measurement showing that ESNP with UDCA  
20 reduces ACE2 activity in the circulating perfusate, compared to carrier only control. N=4  
21 independent samples; unpaired two-tailed t-test; centre line, median; box, interquartile range  
22 (IQR); whiskers, range; bars, standard deviation. (c) Immunofluorescence images showing  
23 ACE2 expression in gallbladder cholangiocytes and cells of the vasculature (smooth muscle  
24 and endothelial cells) in human livers before and after ESNP with UDCA/carrier. N=4  
25 independent samples. White scale bars 100  $\mu\text{m}$ ; yellow scale bars 50  $\mu\text{m}$ , grey scale bars 25



1  $\mu\text{m}$ . (d) QPCR demonstrating that ESNP with UDCA reduces ACE2 levels in gallbladder  
2 cholangiocytes, compared to carrier only control. Housekeeping gene, HMBS; n=4; unpaired  
3 two-tailed t-test; centre line, median; box, interquartile range (IQR); whiskers, range; bars,  
4 standard deviation. (e-f) QPCR (e) and immunofluorescence (f) showing that UDCA reduces  
5 SARS-CoV-2 infection in human gallbladder *ex vivo*. Housekeeping gene, GAPDH; n=4; one-  
6 way ANOVA adjusted for multiple comparisons; centre line, median; box, interquartile range  
7 (IQR); whiskers, range; bars, standard deviation. Scale bars 25  $\mu\text{m}$ . (UDCA, concentration,  
8 2000 ng/ml).

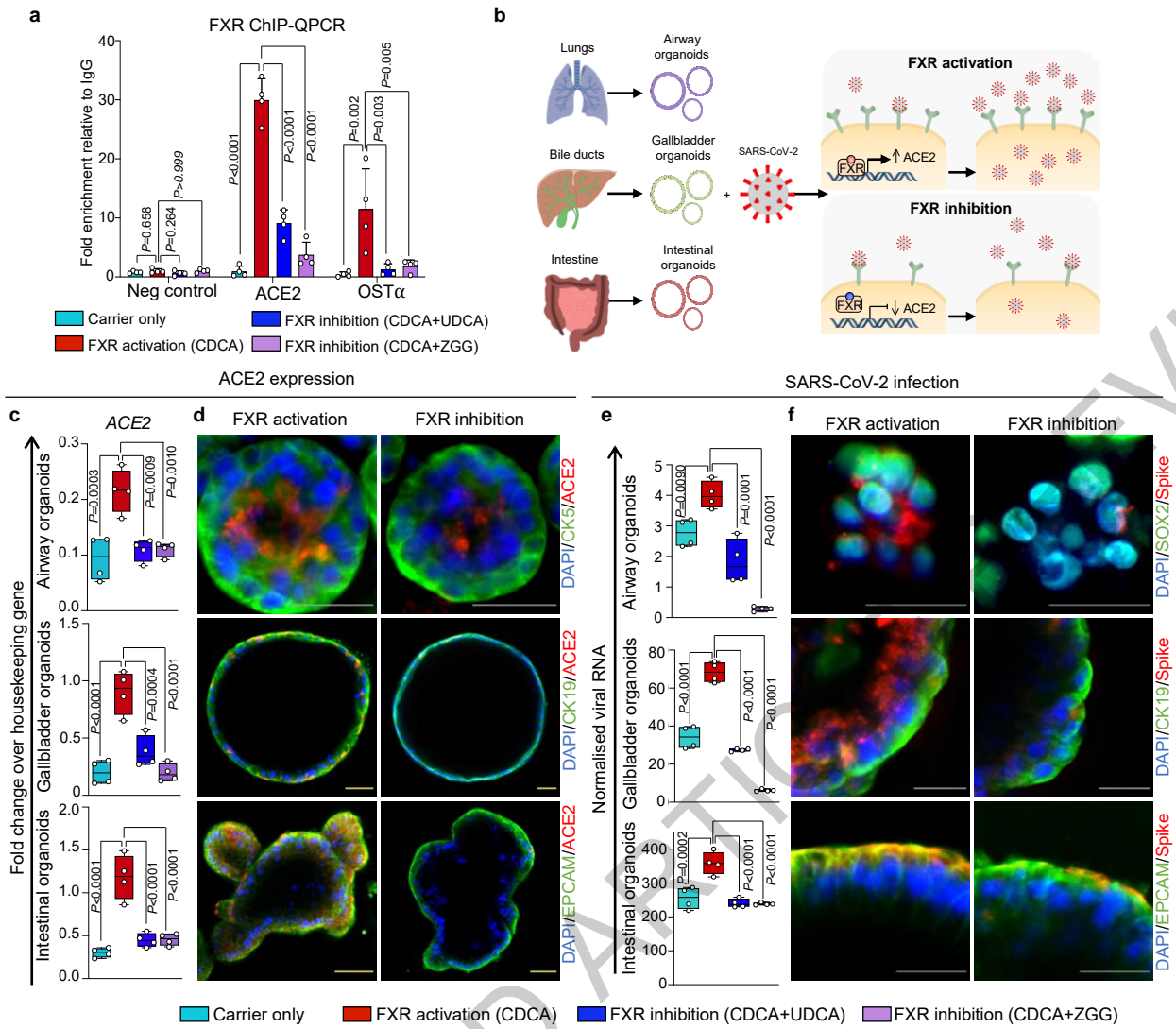
9  
10 **Extended Data Fig. 10. UDCA is associated with lower ACE2 levels in patients with**  
11 **PBC.** (a) Schematic illustrating the patient cohorts compared in (b). (b) Multiple linear  
12 regression analysis of serum ACE2 demonstrates that UDCA correlates with lower ACE2  
13 levels in patients with primary biliary cholangitis (PBC) receiving UDCA (n=308) vs. PBC  
14 patients naïve to treatment (n=62). Values plotted are  $\beta$  coefficients. (BMI, body mass index;  
15 ALP, alkaline phosphatase). Bars, 95% confidence interval.

#### 18 **Additional references**

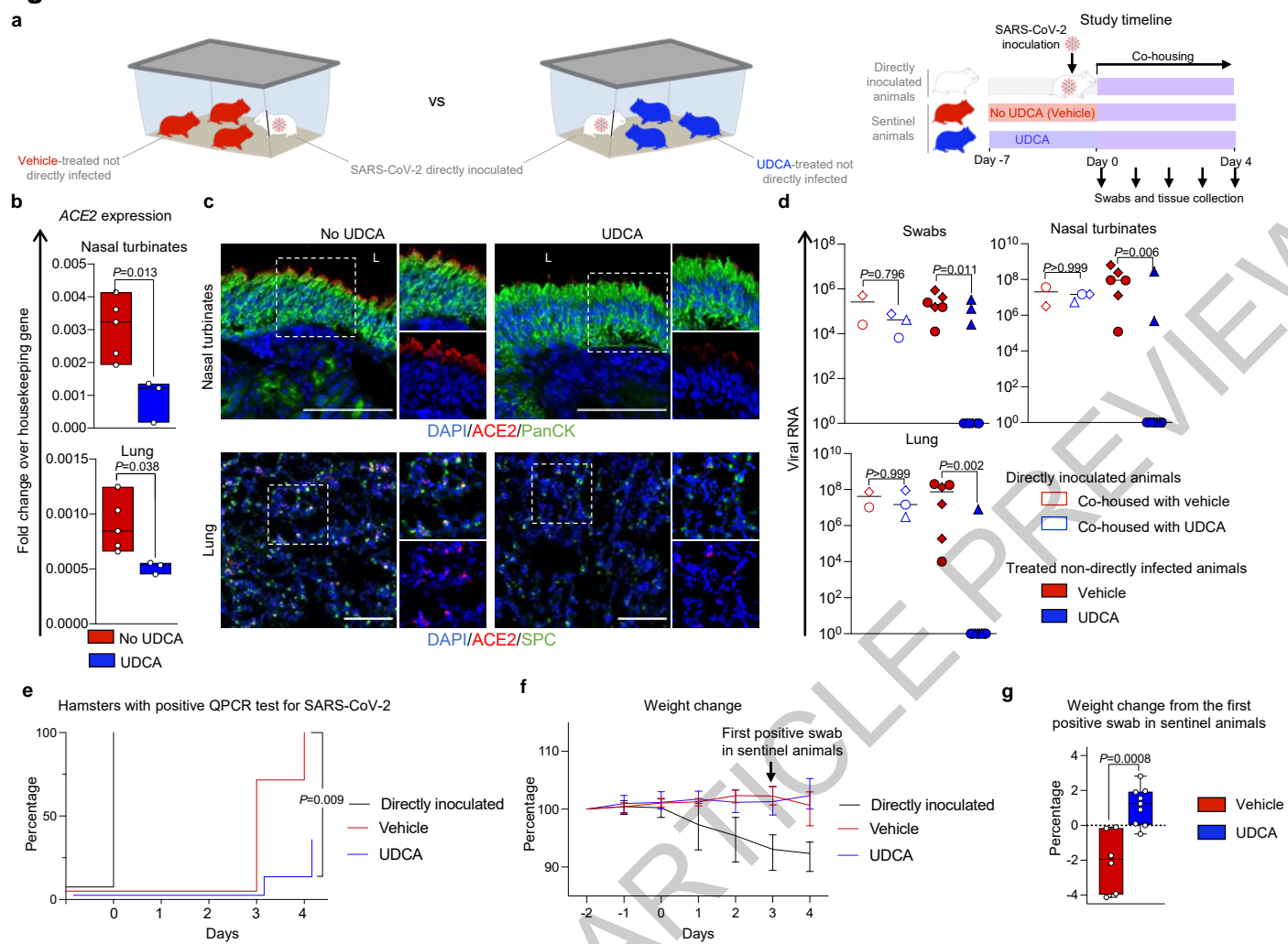
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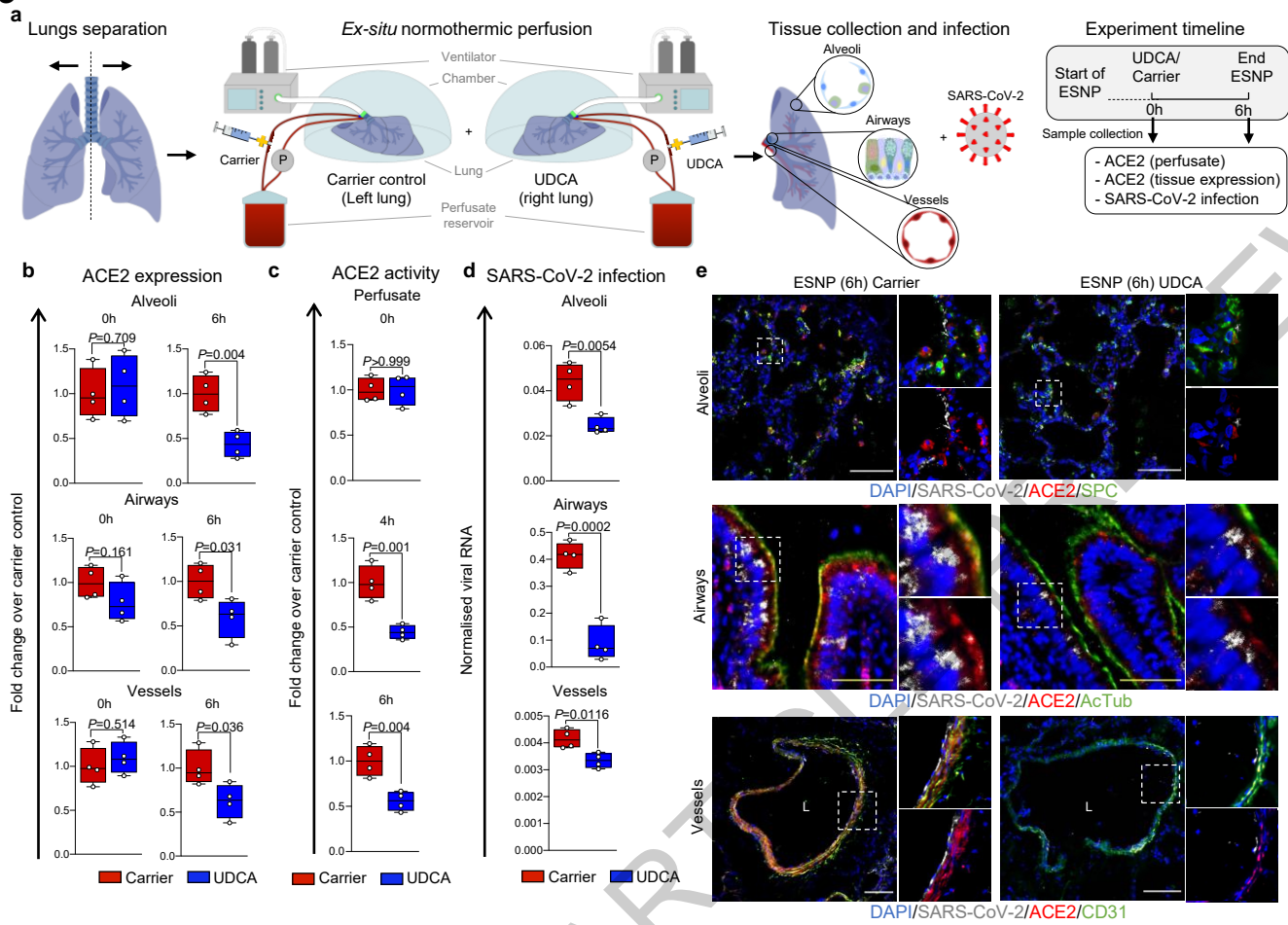
# Figure 1. FXR modulates ACE2 expression and SARS-CoV-2 infection



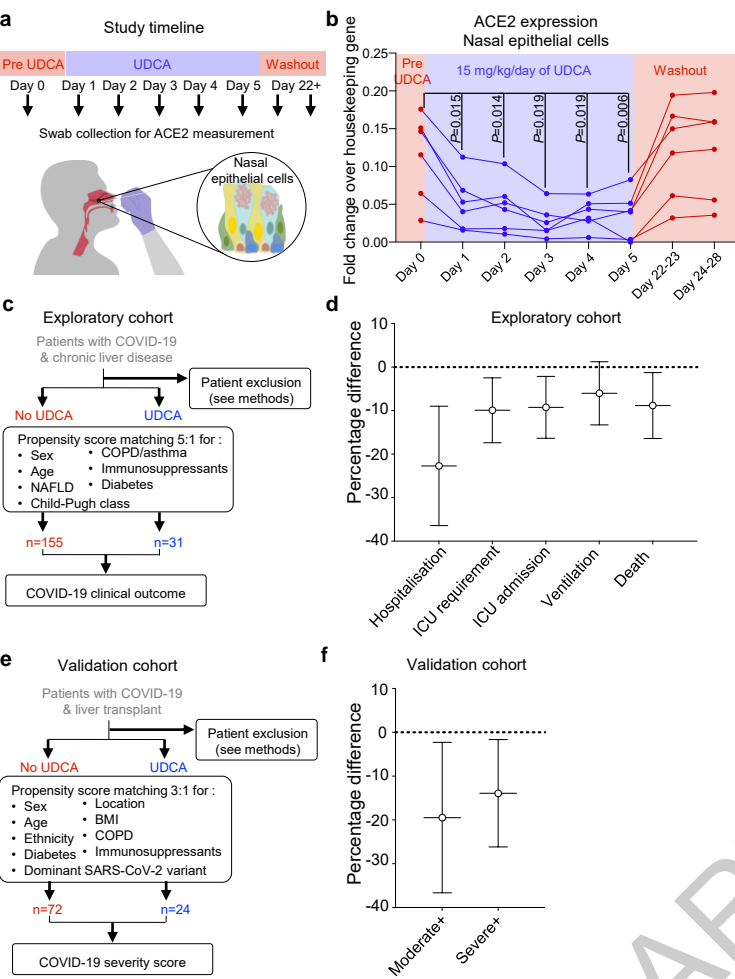
**Figure 2. FXR inhibition reduces ACE2 and SARS-CoV-2 infection *in vivo*.**



# Figure 3. FXR inhibition reduces ACE2 levels and SARS-CoV-2 infection in a human lung *ex vivo*



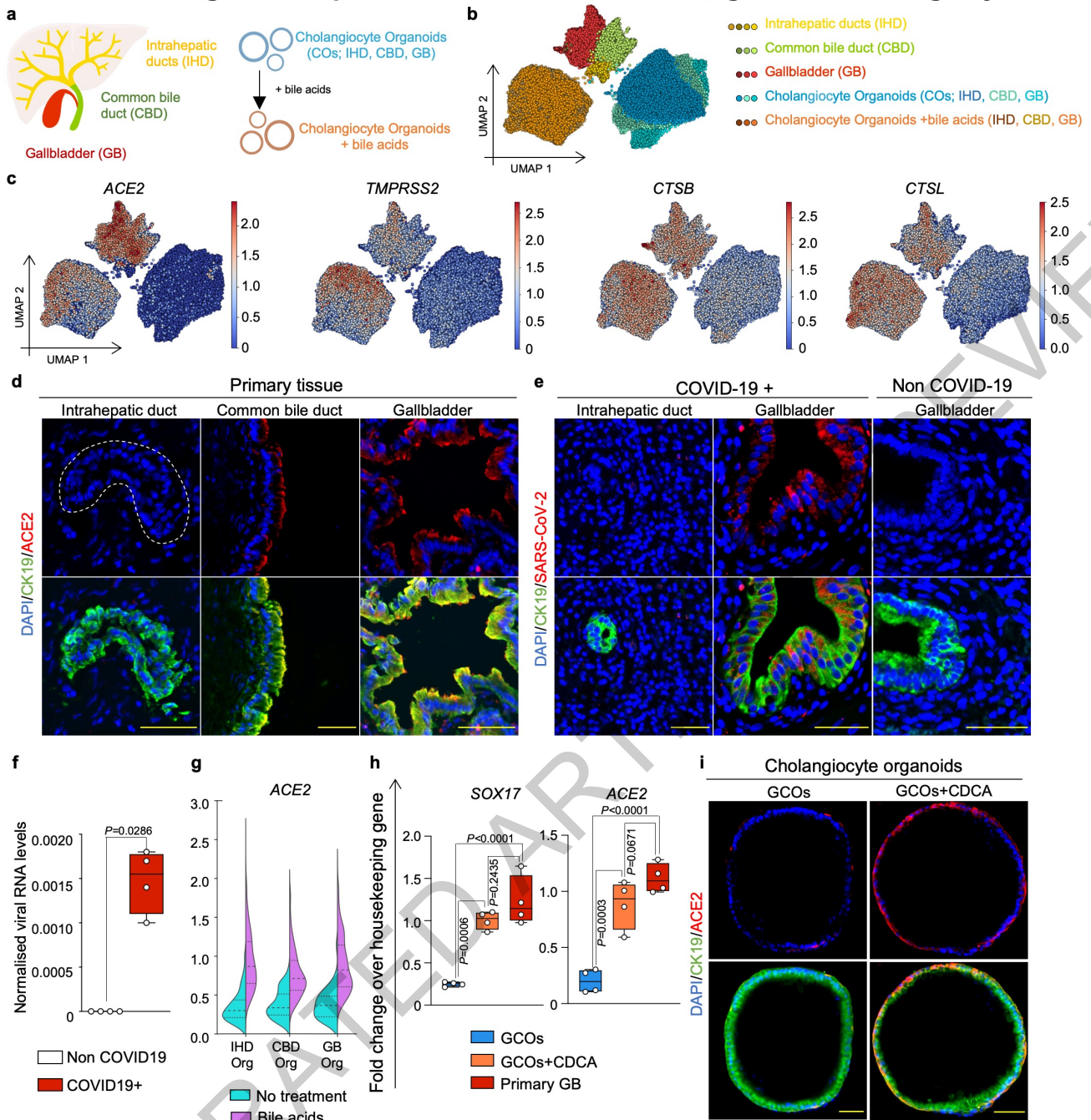
**Figure 4. UDCA is associated with lower ACE2 levels and better clinical outcome in COVID-19 patients**



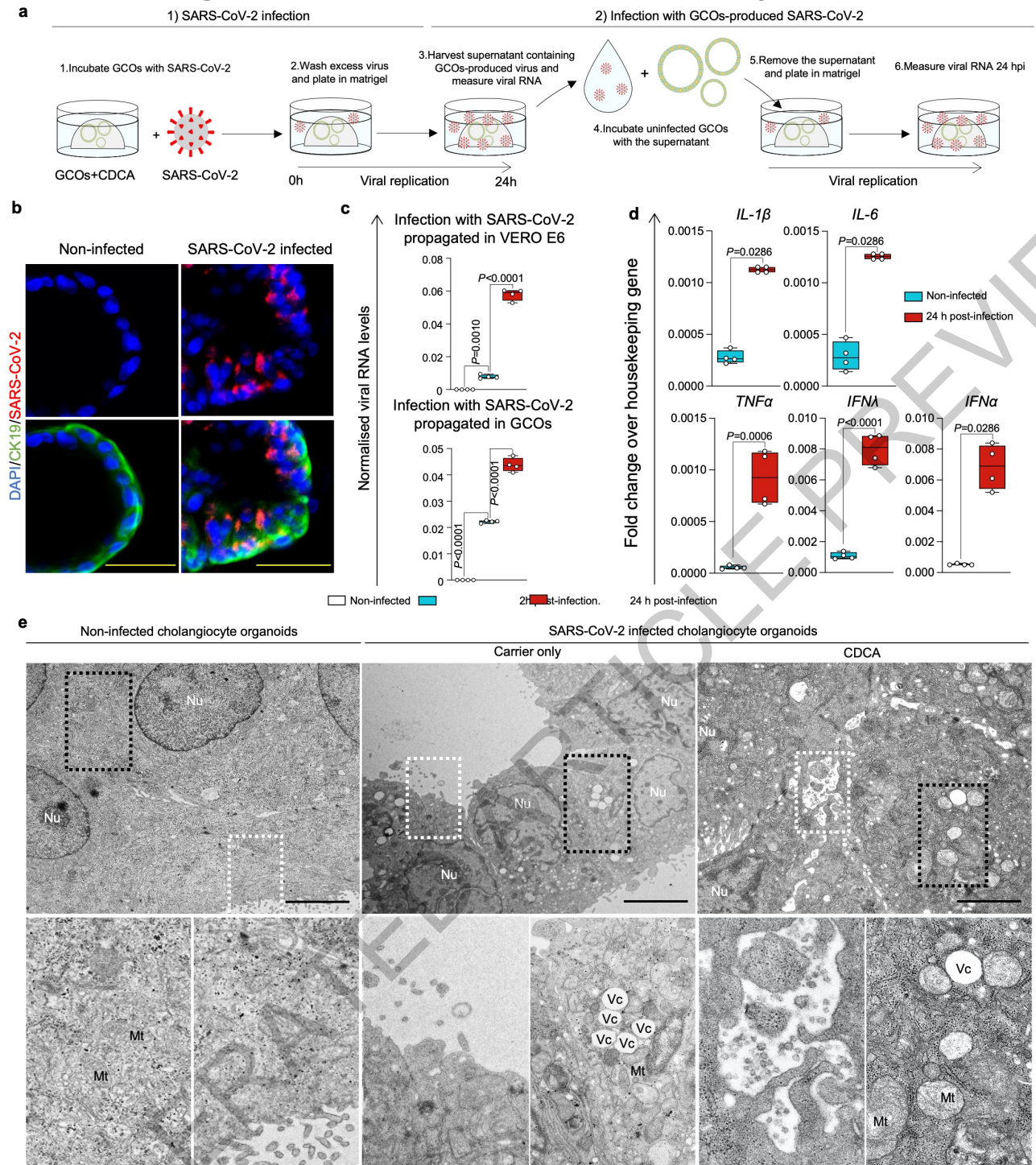
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# Extended Data Figure 1. Expression of SARS-CoV-2 entry genes in cholangiocytes



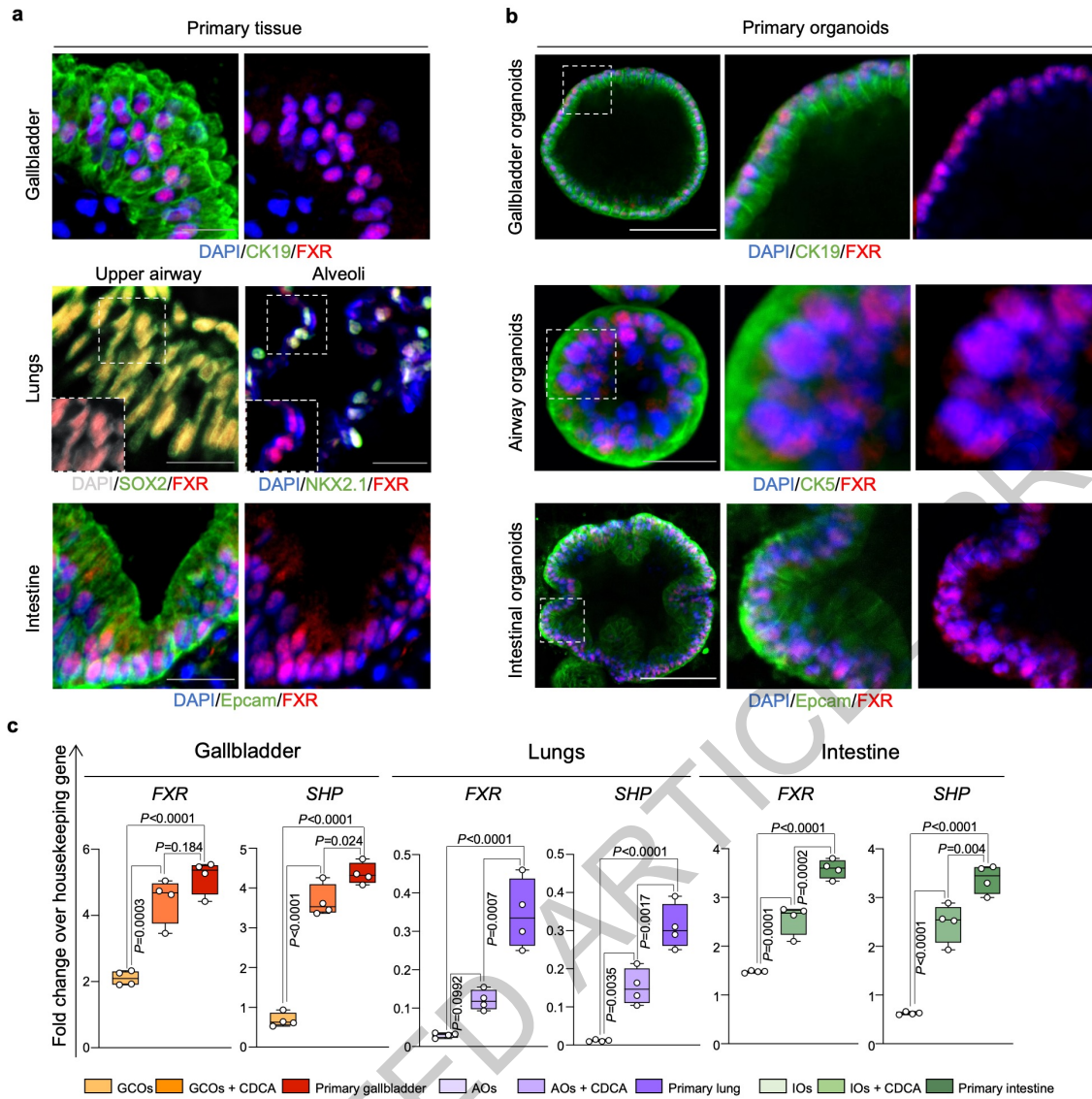
# Extended Data Figure 2. CDCA-treated GCOs can be infected by SARS-CoV-2



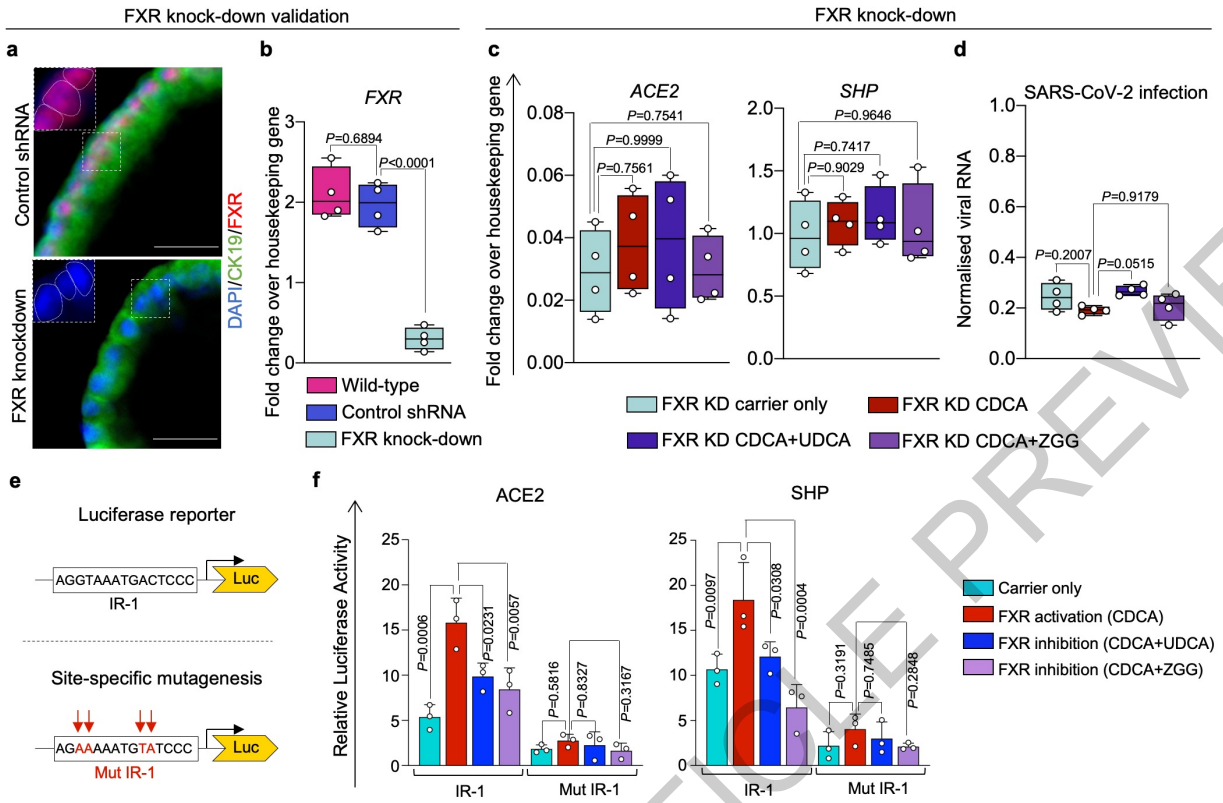
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### Extended Data Figure 3. FXR is present and active in tissues affected by COVID-19 and their corresponding CDCA-treated organoids

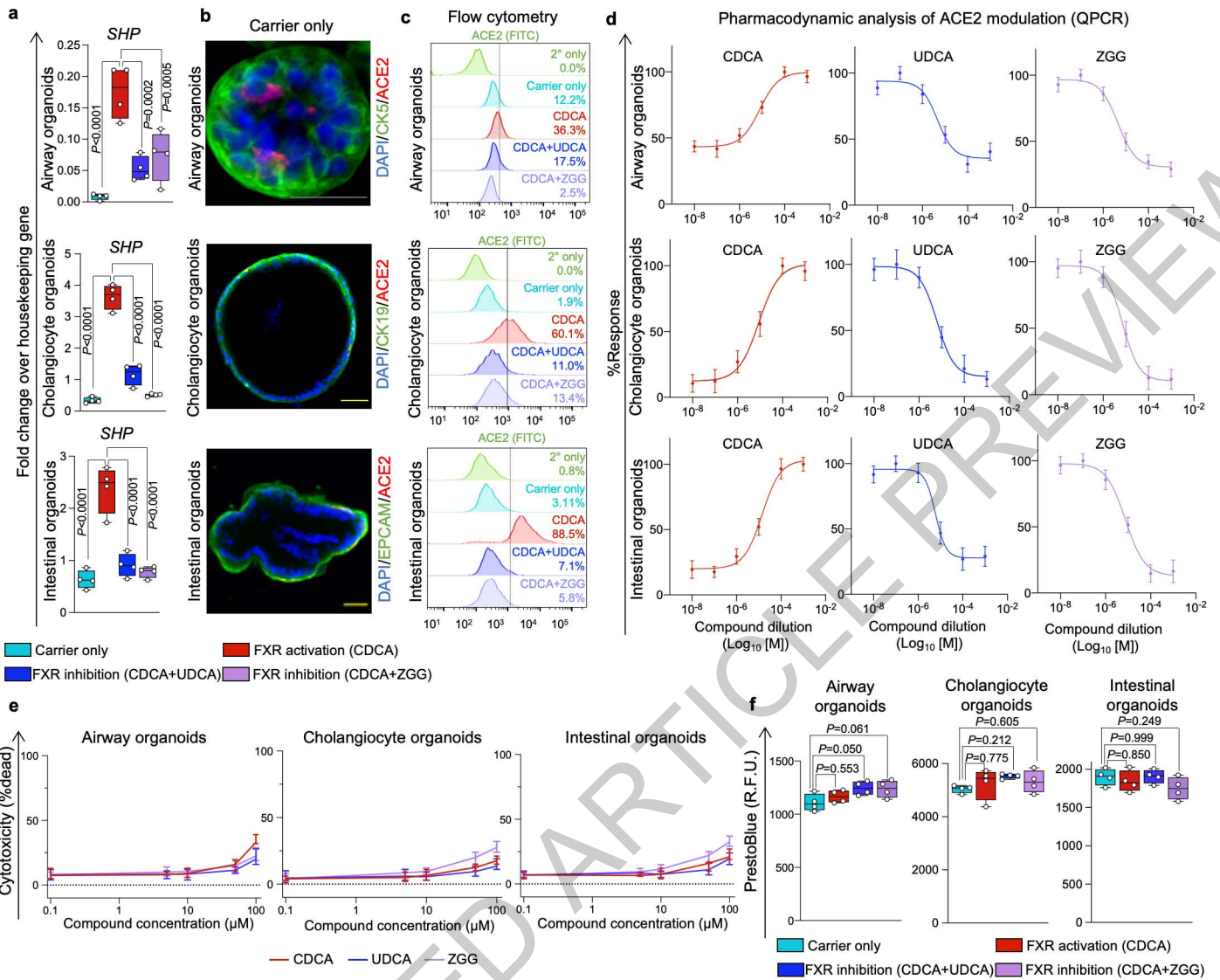


# Extended Data Figure 4. FXR is indispensable for prevention of SARS-CoV-2 infection via UDCA and ZGG

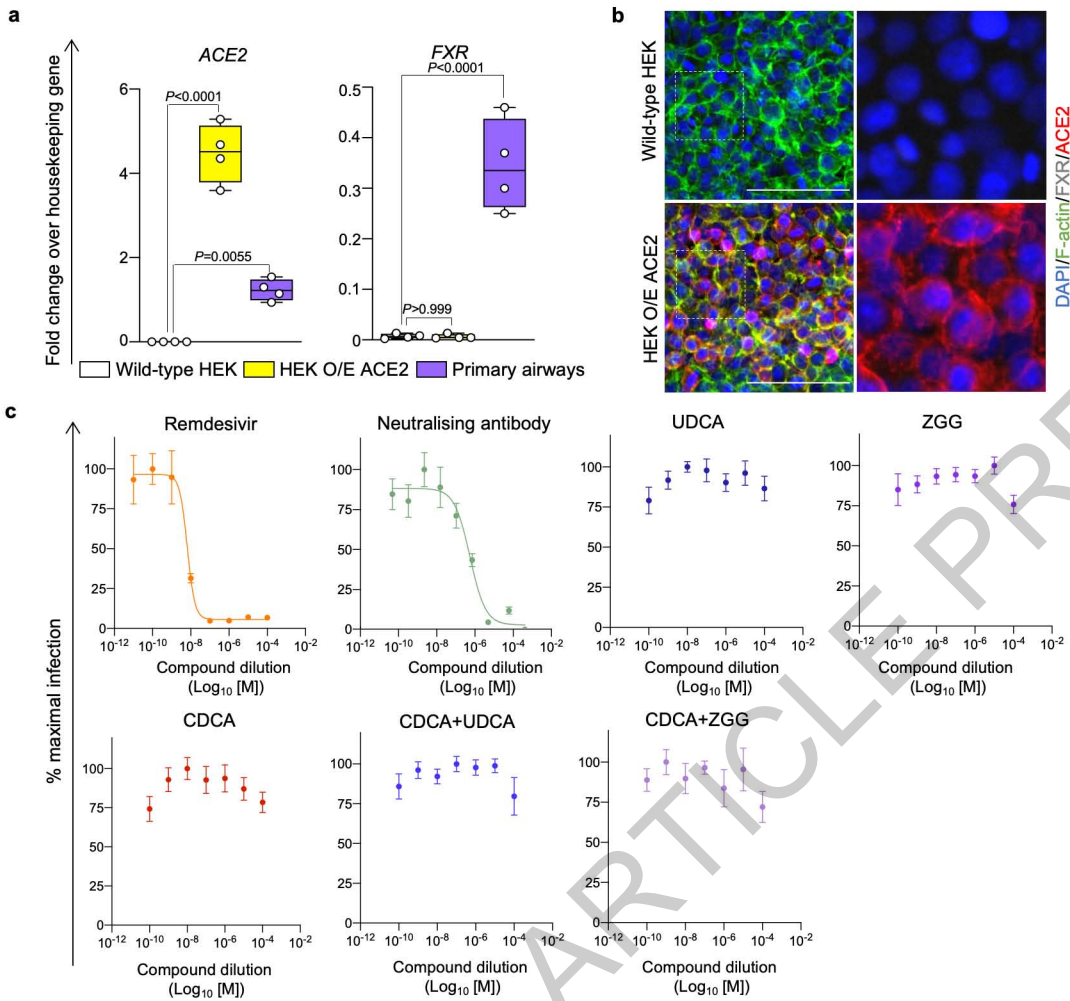


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# Extended Data Fig 5. Effects of FXR modulation in biliary, airway and intestinal organoids



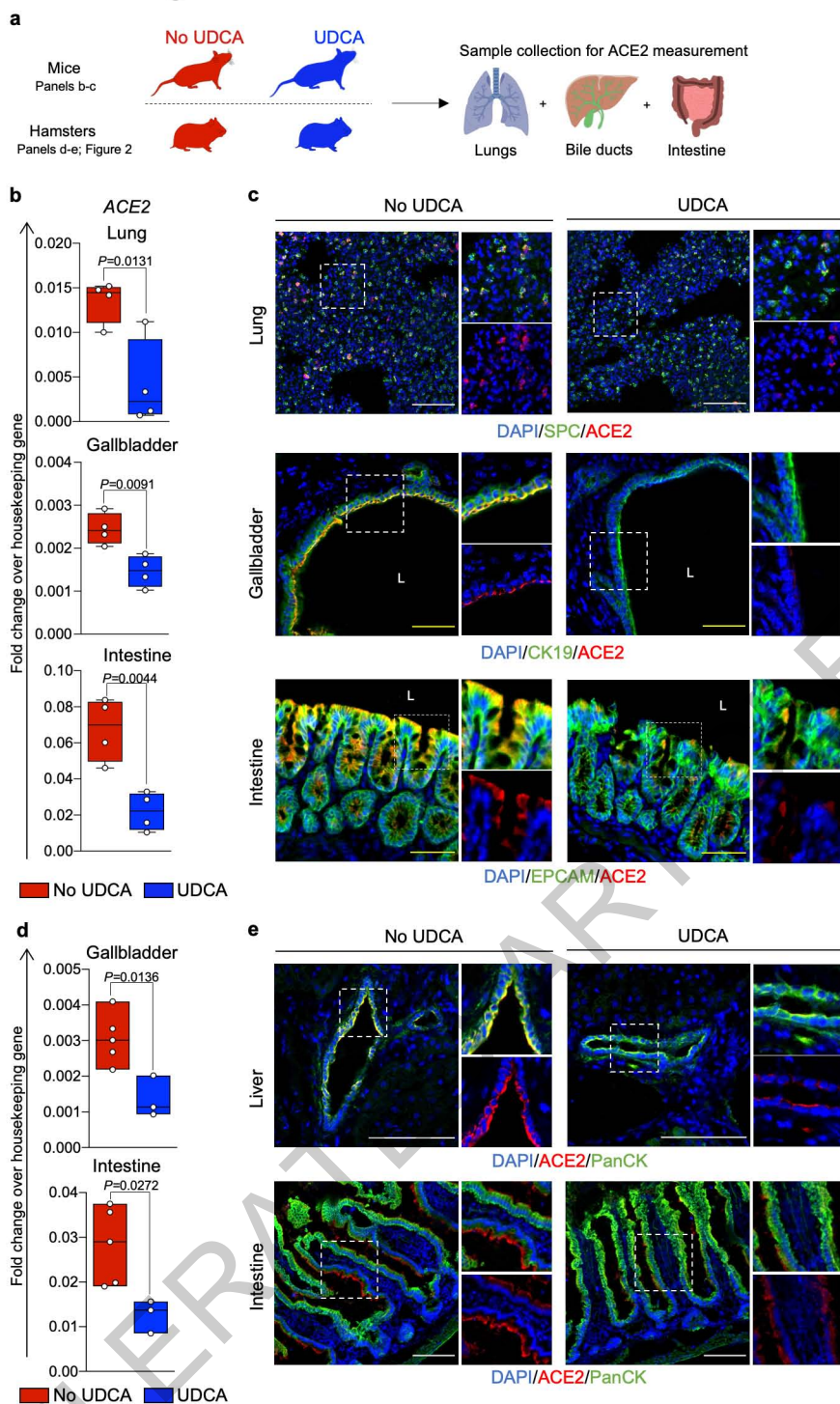
# Extended Data Figure 6. ACE2 downregulation is required for the UDCA/ZGG-mediated reduction in SARS-CoV-2 infection



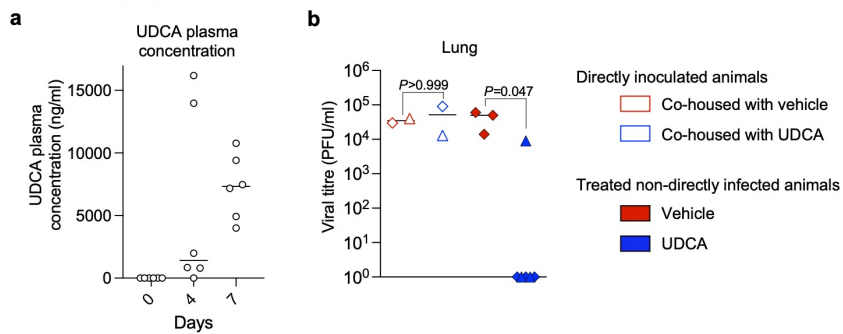
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## Extended Data Figure 7. FXR inhibition reduces ACE2 *in vivo*



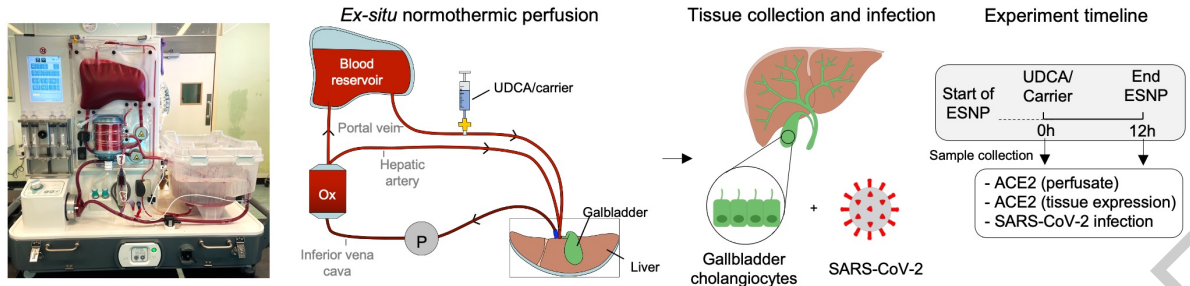
# Extended Data Figure 8. UDCA treatment prevents SARS-CoV-2 infection in Golden Syrian Hamsters



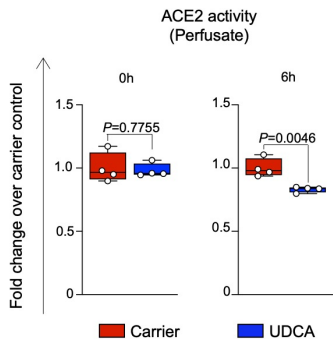
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# Extended Data Figure 9. FXR inhibition reduces SARS-CoV-2 infection in a human liver ex vivo

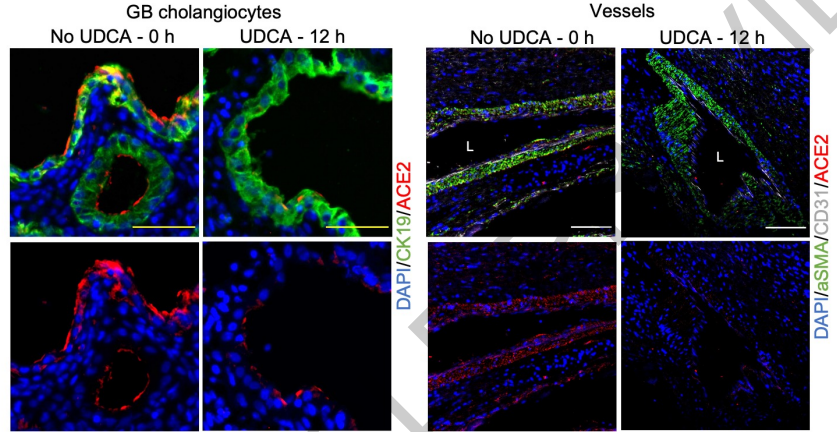
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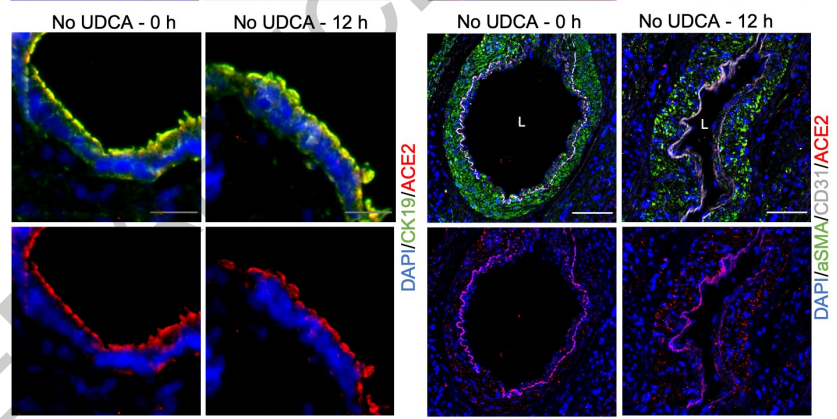
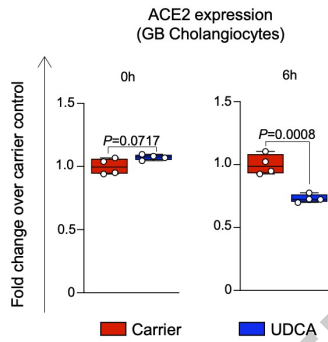
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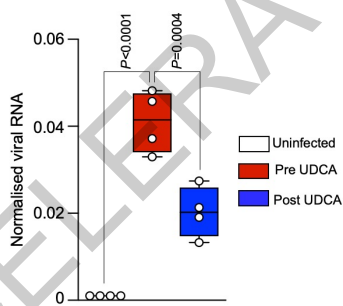
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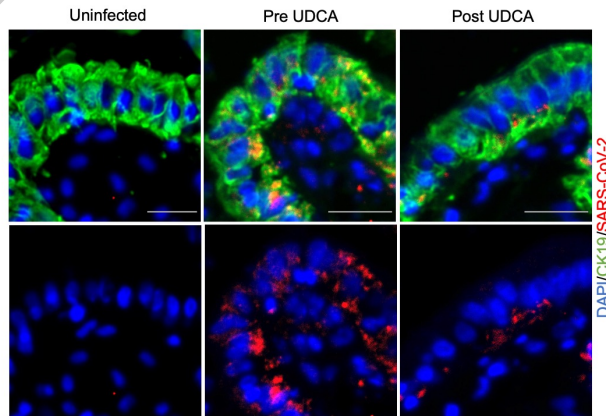
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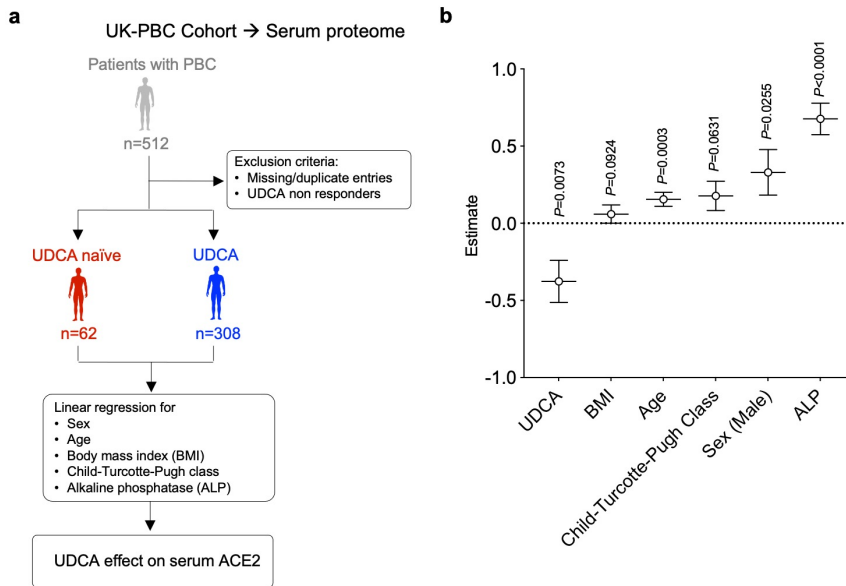
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# Extended Data Figure 10. UDCA is associated with lower ACE2 levels in patients with PBC



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### Software and code

Policy information about [availability of computer code](#)

**Data collection** BD FACS Diva 8.0.3 (BD Bioscience; for analyses on LSR-II); ZEN 2011 SP7 (Zeiss) on a Zeiss LSM 700 or 710 confocal microscope; QuantStudio 5 384 Well Block (Thermo Fisher); SoftMax Pro 5.4.4 on SpectraMax M2 (Molecular Devices); HT7800 TEM operating software version 01.21 (Hitachi) on a HT7800 transmission electron microscope (Hitachi High Technologies, Japan).

**Data analysis** Microsoft Excel v 16.19; GraphPad Prism 9; Stata 15.1 (StataCorp, College Station, TX, USA); FlowJo v 10; ImageJ 2.0.0-rc-69/1.53f; Anaconda-Navigator 1.9.12; Jupyter Notebook 6.0.3; Rstudio (version 1.1.463).

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Single-cell RNA sequencing data are available on ArrayExpress. Accession number: E-MTAB-8495. Source data are provided with this paper.

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## Life sciences study design

All studies must disclose on these points even when the disclosure is negative.

Sample size

No statistical analyses were performed to predetermine sample size. The samples size for each experiment is specified in the corresponding figure legend. At least 4 biological replicates were used for in vitro experiments. At least 4 mice and 3 hamsters per group were used for in vivo experiments. For ESNP experiments 4 independent samples from the same organ before and after UDCA treatment were used for each tissue. For animal experiments, group sizes were estimated based on previous study variance and cage capacity. For in vitro and ex vivo experiments, sample size of n=4 was chosen as the minimum number of samples required to adequately provide standard deviation, median and range. For human prospective study, the maximum number of individuals that could be recruited during the study period was used. For retrospective clinical studies, the maximum number of individuals in our cohort meeting the study criteria was used.

Data exclusions

In the COVID-Hep.net and SECURE-Liver registries data were filtered to remove duplicate entries, those with incomplete records, those with prior liver transplantation, those younger than 18 years of age and older than 90 years of age, and those without laboratory confirmed infection. Due to the absence of patients with Alcohol Related Liver Disease (ARLD) in the UDCA group, patients with ARLD were excluded from the analysis in both groups. In the UK-PBC proteomic study data were filtered to remove duplicate entries, those with incomplete records and those classified as non-responders to treatment according to PARIS2 criteria. For the study involving participants from the University Medical Centre Hamburg-Eppendorf, two individuals were excluded because of undetectable RNA levels in nasopharyngeal swabs. No further data were excluded from the analyses presented in this manuscript. In the VOCAL cohort of liver transplant recipients, participants who had no COVID-19 infection, were unvaccinated or developed COVID-19 within 30 days from their first UDCA prescription were excluded.

Replication

All experiments were repeated and validated as stated in the respective figure legends.

Randomization

No formal randomization method was used to assign primary organoids to study groups. For in vitro experiments primary organoids were plated in 24-well plates and different wells of the same plates were randomly allocated to one of the 4 experimental groups (Carrier only; CDCA; CDCA+UDCA; CDCA+ZGG). For in vivo experiments, animals were randomly allocated to the treatment or control group. For analysis of the UK-PBC proteomic cohort covariables such as age, sex, BMI, stage of liver disease (Child-Turcotte-Pugh class) and ALP were controlled for via multiple linear regression. For analysis of the COVID-Hep.net and SECURE-Liver registries patient cohort covariables such as age, sex, diabetes, NAFLD and stage of liver disease (Child-Turcotte-Pugh class) were controlled for via propensity score matching analyses. For the VOCAL cohort of liver transplant recipients covariable such as age, sex, ethnicity, location within the United States, diabetes, BMI, COPD, type of immunosuppressive therapy (calcineurin inhibitor with or without anti-metabolite therapy) and dominant SARS-CoV-2 variant were controlled for via propensity score matching analyses.

Blinding

Blinding was performed for all experiments. If blinding was not possible at the time of the experiment, specifically in directly inoculated hamster samples and organs perfused ex situ, samples were collected and anonymised and sample processing was subsequently performed by a blinded researcher.

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n/a	Involved in the study
<input type="checkbox"/>	<input checked="" type="checkbox"/> Antibodies
<input type="checkbox"/>	<input checked="" type="checkbox"/> Eukaryotic cell lines
<input checked="" type="checkbox"/>	<input type="checkbox"/> Palaeontology and archaeology
<input type="checkbox"/>	<input checked="" type="checkbox"/> Animals and other organisms
<input type="checkbox"/>	<input checked="" type="checkbox"/> Human research participants
<input checked="" type="checkbox"/>	<input type="checkbox"/> Clinical data
<input checked="" type="checkbox"/>	<input type="checkbox"/> Dual use research of concern

### Methods

n/a	Involved in the study
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<input type="checkbox"/>	<input checked="" type="checkbox"/> Flow cytometry
<input checked="" type="checkbox"/>	<input type="checkbox"/> MRI-based neuroimaging

## Antibodies

Antibodies used

A full list of the antibodies used can be found in Supplementary Table S1.

## Validation

Anti-ACE2; R&D; AF933; dilution 1:50 / 1:100; [https://resources.rndsystems.com/pdfs/datasheets/af933.pdf?v=20221029&\\_ga=2.68548246.663064109.1667061858-1717995244.1667061858](https://resources.rndsystems.com/pdfs/datasheets/af933.pdf?v=20221029&_ga=2.68548246.663064109.1667061858-1717995244.1667061858).

Anti-ACE2; abcam; ab15348; dilution 1:500; <https://www.abcam.com/ace2-antibody-ab15348.html>.

Anti-ACE2; abcam; ab108209; dilution 1:500 / 1:100; <https://www.abcam.com/ace2-antibody-epr4436-ab108209.html>.

Anti-EPCAM; R&D; MAB9601; dilution 1:50 / 1:100; [https://resources.rndsystems.com/pdfs/datasheets/mab9601.pdf?v=20221029&\\_ga=2.232101272.663064109.1667061858-1717995244.1667061858](https://resources.rndsystems.com/pdfs/datasheets/mab9601.pdf?v=20221029&_ga=2.232101272.663064109.1667061858-1717995244.1667061858).

Anti-EPCAM; R&D; AF960; dilution 1:100; [https://resources.rndsystems.com/pdfs/datasheets/af960.pdf?v=20221029&\\_ga=2.232101272.663064109.1667061858-1717995244.1667061858](https://resources.rndsystems.com/pdfs/datasheets/af960.pdf?v=20221029&_ga=2.232101272.663064109.1667061858-1717995244.1667061858).

Anti-Cytokeratin 19; abcam; ab7754; dilution 1:100; <https://www.abcam.com/cytokeratin-19-antibody-a53-ba2-cytoskeleton-marker-ab7754.html>.

Anti-Cytokeratin 19; abcam; ab52625; dilution 1:100; <https://www.abcam.com/cytokeratin-19-antibody-ep1580y-cytoskeleton-marker-ab52625.html>.

Anti-SOX2; abcam; ab15830; dilution 1:100; <https://www.abcam.com/sox2-antibody-ab15830.html>.

Anti-SOX2; R&D; AF2018; dilution 1:100; [https://resources.rndsystems.com/pdfs/datasheets/af2018.pdf?v=20221029&\\_ga=2.31979961.663064109.1667061858-1717995244.1667061858](https://resources.rndsystems.com/pdfs/datasheets/af2018.pdf?v=20221029&_ga=2.31979961.663064109.1667061858-1717995244.1667061858).

Anti-NKX2.1; abcam; ab72876; dilution 1:100; <https://www.abcam.com/tnfr1-antibody-8g7g31-ab72876.html>.

Anti-Cytokeratin 5; Thermo Fisher; MA5-17057; dilution 1:100; <https://www.thermofisher.com/antibody/product/Cytokeratin-5-Antibody-clone-2C2-Monoclonal/MA5-17057>.

Anti-Surfactant protein C; Merck Millipore; AB3786; dilution 1:300; [https://www.merckmillipore.com/GB/en/product/Anti-Prosurfactant-Protein-C-proSP-C-Antibody,MM\\_NF-AB3786](https://www.merckmillipore.com/GB/en/product/Anti-Prosurfactant-Protein-C-proSP-C-Antibody,MM_NF-AB3786).

Anti-Acetylated alpha tubulin; Sigma; T7451; dilution 1:500; <https://www.sigmaaldrich.com/GB/en/product/sigma/t7451>.

Anti-CD31; Novus biological; NB100-2284; dilution 1:100; [https://www.novusbio.com/products/cd31-pecam-1-antibody\\_nb100-2284](https://www.novusbio.com/products/cd31-pecam-1-antibody_nb100-2284).

Anti-CD31; abcam; ab119339; dilution 1:100; <https://www.abcam.com/cd31-antibody-hec7-ab119339.html>.

Anti-Alpha smooth muscle actin; abcam; ab124964; dilution 1:100; <https://www.abcam.com/alpha-smooth-muscle-actin-antibody-epr5368-ab124964.html>.

Anti-SARS-CoV spike glycoprotein; abcam; ab273433; dilution 1:100; <https://www.abcam.com/sars-spike-glycoprotein-antibody-1a9-ab273433.html>.

Anti-SARS-CoV-2 nucleocapsid; Sino Biological; 40143-R019; dilution 1:100; <https://www.sinobiological.com/antibodies/cov-nucleocapsid-40143-r019>.

Anti-SOX17; R&D; AF1924; dilution 1:100; [https://resources.rndsystems.com/pdfs/datasheets/af1924.pdf?v=20221029&\\_ga=2.206978708.663064109.1667061858-1717995244.1667061858](https://resources.rndsystems.com/pdfs/datasheets/af1924.pdf?v=20221029&_ga=2.206978708.663064109.1667061858-1717995244.1667061858).

Anti-FXR; Novus biological; NBP2-16550; dilution 1:100; [https://www.novusbio.com/products/foxr-nr1h4-antibody\\_nbp2-16550](https://www.novusbio.com/products/foxr-nr1h4-antibody_nbp2-16550).

Anti-FXR; Santa Cruz; sc-25309 X; dilution 1:100; <https://datasheets.scbt.com/sc-25309.pdf>.

Anti-Actin; abcam; ab208080; dilution 1:100; <https://www.abcam.com/alexa-fluor-555-actin-antibody-epr16769-ab208080.html>.

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Policy information about [cell lines](#)

Cell line source(s)

Vero E6 cells (ATCC™ CRL – 1586) were kindly donated by Gordon Dougan's laboratory. HEK293 cells (ATCC™ CRL – 1573) and HEK293T cells (ATCC™ CRL – 3216) were kindly donated by Nicholas J Mathenson's laboratory. Primary human tissues were used to derive organoids. All human tissues were obtained with full ethical approval (REC reference numbers: 12/EE/0253, NRES Committee East of England, Cambridge Central and 15/EE/0152 NRES Committee East of England, Cambridge South) and informed consent from the patients or the donors' families.

Authentication

None of the cell lines used were authenticated

Mycoplasma contamination

All line tested negative for mycoplasma.

Commonly misidentified lines  
(See [ICLAC](#) register)

No commonly misidentified cell lines were used in the study.

## Animals and other organisms

Policy information about [studies involving animals](#); [ARRIVE guidelines](#) recommended for reporting animal research

Laboratory animals

FVB/N female mice aged between 9 and 12 weeks were used. Golden Syrian Hamsters were purchased from Janvier Labs (France). Age matched male hamsters weighing between 80 – 100g were used. All animals were housed in a 12 hours/12 hours dark/light cycle, with a humidity of 45-65% and temperature of 20-24°C.

Wild animals

No wild animals were used in the study.

Field-collected samples

No field collected samples were used in the study.

Ethics oversight

The mouse study was approved by the Animal Ethics Committee of the Medical University of Vienna and the Federal Ministry of Science, Research and Economy (BMWFV-66.009/0008-WF/3b/2015) and was performed according to the Animal Research: Reporting of In Vivo Experiments (ARRIVE) guidelines.  
The hamster study was performed in accordance with the UK Home Office Animals Scientific Procedures Act (ASPA, 1986). Additionally, all studies were approved by the University of Liverpool Animal Welfare and Ethical Review Board and performed under UK Home Office licences PP9284915 and PP4715265.

Note that full information on the approval of the study protocol must also be provided in the manuscript.

## Human research participants

Policy information about [studies involving human research participants](#)

Population characteristics	Population characteristics of the human research participants are listed in Supplementary Table S5, Supplementary Table S6, Supplementary Table S7 and Supplementary Table S8.
Recruitment	Existing samples or datasets were used (e.g., data from COVID-Hep.net and SECURE-Liver registries, serum samples from the UK-PBC Cohort study, data from the VOCAL cohort). For the study involving volunteers from the University Medical Centre Hamburg-Eppendorf, following approval by local ethics committee (Ethik-Kommission der Ärztekammer Hamburg; Ref.No. 2021-300121-WF), the study was advertised in the University Medical Centre Hamburg-Eppendorf amongst clinicians regularly prescribing UDCA, and thus familiar with the drug and its possible side-effects. 8 clinicians who volunteered to participate in the study were recruited following informed consent. We appreciate the potential for selection and confounding bias in any study which is not a clinical trial, and this limitation is clearly stated in the results and discussion sections.
Ethics oversight	The COVID-Hep.net and SECURE-Liver registries data were deemed not to constitute human research by Clinical Trials and Research Governance at the University of Oxford ( <a href="https://covid-hep.net/img/CTRG_COVID-Hep_20200402.pdf">https://covid-hep.net/img/CTRG_COVID-Hep_20200402.pdf</a> ) and by the Institutional Review Board of University of North Carolina ( <a href="https://covidcirrhosis.web.unc.edu/faq/">https://covidcirrhosis.web.unc.edu/faq/</a> ) respectively. The study involving volunteers from the University Medical Centre Hamburg-Eppendorf was performed with informed consent and ethical approval from the Ethik-Kommission der Ärztekammer Hamburg (Ref.No. 2021-300121-WF). The study involving patients with PBC from the UK-PBC Nested Cohort was performed with informed consent and ethical approval from the National Research Ethics Committee (NREC) North West (14/NW/1146). The study involving patients from the VOCAL cohort was performed with informed consent and ethical approval from the Miami VA Institutional Review Board (Unique study approval ID 1477437-22).

Note that full information on the approval of the study protocol must also be provided in the manuscript.

## Flow Cytometry

### Plots

Confirm that:

- The axis labels state the marker and fluorochrome used (e.g. CD4-FITC).
- The axis scales are clearly visible. Include numbers along axes only for bottom left plot of group (a 'group' is an analysis of identical markers).
- All plots are contour plots with outliers or pseudocolor plots.
- A numerical value for number of cells or percentage (with statistics) is provided.

### Methodology

Sample preparation	Cholangiocyte organoids obtained from intrahepatic ducts, common bile duct and gallbladder human tissues obtained from biopsies, deceased transplant organ donors or liver explants after obtaining informed consent were used. Organoids were harvested from Matrigel using Cell Recovery Solution for 20 minutes in ice. Organoids were treated with StemPro Accutase for 5 minutes at 37°C to dissociate cell clumps into single cells; fixed in 4% PFA for 20 minutes at 4°C; blocked with 10% donkey serum (Gibco) + 0.1% Triton-X in PBS (Gibco) for 30 minutes; stained with primary antibodies from abcam (See supplementary table S1) in 1% donkey serum (Gibco) + 0.1% Triton X in PBS (Gibco) for 1 hour at room temperature; primary antibody was subsequently washed in PBS (Gibco) for 5 minutes for three times; organoids were then stained with secondary antibodies (See supplementary table S1) for 1 hour at room temperature and filtered through a 40-µm filter and analysed. A detailed description of the protocol can be found in Methods section "Flow cytometry analyses".
Instrument	BD LSR-II from BD Biosciences.
Software	FlowJo version 10.
Cell population abundance	No post-sort fraction was collected. For each experiment at least 10.000 events were captured.
Gating strategy	Initial cell populations were gated using FSC and SSC to remove cell debris and large cell clumps. Subsequently, FSC-W and FSC-A were used to gate only single cells and remove doublets. This population was then used in fluorescent histograms. Samples stained for the secondary antibody only were used to set the gates. The gating strategy employed to analyse flow cytometry data can be found in Supplementary Figure S1.

- Tick this box to confirm that a figure exemplifying the gating strategy is provided in the Supplementary Information.