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

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Abstract

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

Introduction

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

predominantly affects tissues expressing ACE2, such as the lungs, the cardiovascular system, the digestive tract and the biliary tree[12,13].

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

Bile acids modulate cholangiocyte ACE2

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

Bile acids control ACE2 levels via FXR

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

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

FXR regulates ACE2 in various cell types

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

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

FXR regulates viral infection *in vitro*

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

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

FXR regulates viral infection *in vivo*

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

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

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

FXR regulates infection in human organs

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

potentially improve donor organ function, enhance organ preservation and reduce reperfusion injury by perfusing grafts with warm oxygenated blood (packed red cells) or substitute perfusion solution prior to transplantation[29,30]. This setting ensured that the lungs remained in near physiological conditions during the experiment[31]. To assess the effect of UDCA, we surgically divided the right and the left lungs from the same donor and we connected them to 2 separate but identical ESNP circuits. This setting allowed us to administer UDCA on one lung (UDCA lung) and use the other lung as a matched control receiving carrier without UDCA (control lung) to facilitate comparison (Fig. 3a).

Immediately prior to UDCA administration we measured baseline ACE2 expression in both lungs (0 hours samples collected from lung parenchyma, airway and pulmonary vessels; n=4 independent samples from each part of the organ per lung, 24 samples in total; Fig 3b) and ACE2 activity in the circulating perfusate from each ESNP circuit (n=4 independent measurements per circuit; Fig. 3c). We then administered UDCA 'systemically' in the perfusate of the UDCA lung; UDCA was diluted in saline to 2000 ng/ml corresponding to the steady-state plasma concentration achieved in patients after multiple doses of oral UDCA[28]. At the same time the control lung received an equal volume of saline (carrier) (Fig 3a; 0 hours = UDCA/Carrier administration). We continued *ex situ* perfusion with UDCA for 6 hours. Repeat perfusate and tissue samples were collected at 6 hours, matching our pre-UDCA measurements (n=24 independent tissue samples and n=8 independent perfusate samples per timepoint). We observed that *ex vivo* treatment with UDCA reduced ACE2 expression in lung parenchyma, airway and pulmonary vessels and ACE2 activity in the perfusate, compared to the carrier control (Fig. 3b-c).

We then assessed the importance of this reduction in ACE2 on susceptibility to SARS-CoV-2 infection. We infected samples from the lung parenchyma, airway and vessels of each lung 6 hours after UDCA or carrier administration (n=4 parenchymal, n=4 bronchial, n=4

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

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

UDCA reduces ACE2 in humans

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

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

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

UDCA may improve COVID-19 outcome

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

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

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

Discussion

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

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

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

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

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

inflammation by modulating NFkB in multiple organs[44], including lungs[21], liver[17] and intestine[17]. Conversely, UDCA has been shown to reduce inflammation in multiple tissues, including lung, in an FXR-independent fashion[45]. Given the complex interplay between FXR, UDCA and inflammation[46], the balance of benefits for FXR activation in terms of SARS-CoV-2 infection and inflammation should be carefully considered. It is possible that FXR suppressors, beyond UDCA, which lack anti-inflammatory effects would be better suited for prophylaxis or early intervention and not indicated for severe disease with ongoing tissue inflammation[47]. Second, our study suggests that FXR activators used in clinical practice, such as obeticholic acid (OCA) may increase the risk of developing COVID-19 by upregulating ACE2 in healthy individuals. Conversely, in liver patients OCA may paradoxically prevent COVID-19 by reducing disease severity and ameliorating cholestasis, resulting in a net reduction of FXR activity. Therefore, further studies are needed to elucidate these points.

Our results identify UDCA as a particularly advantageous modulator of ACE2 levels, for use in COVID-19. We demonstrated that UDCA reduces SARS-CoV-2 infection *in vitro, in vivo* and *ex vivo;* and lowers ACE2 expression in the nasal epithelium of healthy volunteers. Although our animal data do not exclude that UDCA delays SARS-CoV-2 transmission beyond the duration of our experiments; our patient data illustrate that this does not change the net effect of reducing disease severity, which make it particularly attractive for investigation as pharmacological prophylaxis against SARS-CoV-2 infection. Compared to other agents, such as vaccines and monoclonals, it is easy to administer orally, easily stored, affordable and accessible to health systems world-wide for large scale production, as it is off patent. In addition, UDCA is well-tolerated, has limited drug- drug interactions, and a favourable safety profile enabling it to be administered for long periods of time. Of note, UDCA is already administered long-term for different clinical indications to vulnerable groups that would benefit from chemoprophylaxis, such as bone marrow and liver transplant patients,

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

Finally, we demonstrated that UDCA could reduce ACE2 levels and SARS-CoV-2 infection in machine perfused organs. This is one of the first studies testing the effect of a drug in a whole human organ perfused *ex situ*. This finding could prove important for organ transplantation, especially given concerns about peri-operative viral transmission[49]. Furthermore, although more data are required to definitively establish this approach, our work sets the stage for future studies using machine-perfused organs for pharmacological studies.

In conclusion, these results validate CDCA-treated cholangiocytes organoids as a novel platform for disease modelling and drug testing against SARS-CoV-2 infection; identify FXR as a new therapeutic target in the management of COVID-19 and open up new avenues for the modulation of ACE2 through FXR for prevention of SARS-CoV-2 infection as well as other viruses using ACE2 for cell entry.

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Figure Legends

Figure 1. FXR modulates ACE2 expression and SARS-CoV-2 infection. (**a**) Chromatin immunoprecipitation followed by QPCR (ChIP-QPCR) on cholangiocyte organoids showing that the FXR agonist (CDCA) promotes binding of FXR on the ACE2 promoter which is reduced by FXR inhibitors (UDCA/ZGG). OSTα as positive control; ACE2 promoter adjoining region as negative control; n=4 independent experiments; one-way ANOVA adjusted for multiple comparisons; bars, standard deviation. (**b**) Schematic representation of the suggested mechanism for FXR-mediated control of ACE2 expression and SARS-CoV-2 infection relative to panels (e-f). (**c**-**d**) QPCR (**c**) and immunofluorescence (**d**) showing ACE2 levels upon modulation of FXR activity in primary airway, biliary and intestinal organoids. Housekeeping gene, PBGD; n=4 independent experiments; one-way ANOVA; centre line, median; box, interquartile range (IQR); whiskers, range; bars, standard deviation. Yellow scale bars 50 μm; grey scale bars 25 μm. (**e**) QPCR quantifying SARS-CoV-2 viral RNA 24 hours post infection (hpi) in primary organoids treated with physiological levels of bile acids (CDCA) in the presence or absence of FXR inhibitors (UDCA/ZGG). Housekeeping gene, GAPDH; n=4 independent experiments; one-way ANOVA adjusted for multiple comparisons;

centre line, median; box, interquartile range (IQR); whiskers, range; bars, standard deviation. (**f**) Immunofluorescence images demonstrating presence of SARS-CoV-2 spike protein 24 hpi in organoids corresponding to (e). Scale bars 25 μm. (CDCA, UDCA and ZGG concentration, 10 μM).

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

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

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

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 individulas; 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.

Methods

Ethical approval

All human samples were obtained from patients, deceased transplant organ donors or liver explants with informed consent for use in research, and ethical approval (Research Ethics Committee - REC 09/H0305/68; 14/NW/1146; 15/EE/0152; 15/WA/0131; 18/EE/0269; and Papworth Hospital Research Tissue Bank project number T02233). The mouse animal study was approved by the Animal Ethics Committee of the Medical University of Vienna and the Federal Ministry of Science, Research and Economy (BMWFW-66.009/0008-WF/3b/2015) and was performed according to the Animal Research: Reporting of In Vivo Experiments (ARRIVE) guidelines. The hamster animal study was approved by the University of Liverpool Animal Welfare and Ethical Review Board and performed under UK Home Office licences (PP9284915 and PP4715265) and it was completed at the University of Liverpool and conducted in accordance with the UK Home Office Animals Scientific Procedures Act (ASPA, 1986). Human lungs and livers retrieved for transplantation but subsequently declined were used for *ex situ* normothermic perfusion experiments (National Research Ethics Committee (NREC) North East – Newcastle and North Tyneside 1 16/NE/0230, lung; NREC East of England – Cambridge East 14/EE/0137, liver). The study involving volunteers from the University Medical Centre Hamburg-Eppendorf was performed with informed consent and ethical approval (Ethik-Kommission der Ärztekammer Hamburg; Ref.No. 2021-300121-WF). 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 (https://covid-hep.net/img/CTRG_COVID-Hep_20200402.pdf) and by the Institutional Review Board of University of North Carolina (https://covidcirrhosis.web.unc.edu/faq/) respectively. 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).

10x single cell RNA sequencing, data analysis and availability

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

Human tissue collection and processing

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

Bile sample collection and processing

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

Cell culture

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

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

Human primary airway organoids were provided by Joo-Hyeon Lee's lab. The organoids were derived and cultured as previously described[24].

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

Biological materials availability

Detailed protocols for the derivation of primary organoids have been previously reported[24,50]. Cell lines are available from standard commercial sources (https://www.lgcstandards-atcc.org - Vero E6 cells, ATCC™ CRL – 1586; HEK293 cells, ATCC™ CRL – 1573).

Modulation of FXR activity

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

FXR knock-down

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

Chromatin immunoprecipitation

Approximately 6 × 10^6 cells were used for each chromatin immunoprecipitation (ChIP), and cells were incubated with fresh medium with 100 μM of CDCA, UDCA or ZGG 2 h before collection. ChIP was performed using the True Micro ChiP kit (Diagenode C01010130) according to manufacturer's instructions. In brief, following pre-clearing, the lysate was incubated overnight with the FXR antibody (Santa Cruz sc-25309 X) (Supplementary Table S1) or non-immune IgG. ChIP was completed and immunoprecipitated DNA was purified using MicroChip DiaPure columns (Diagenode C03040001). Samples were analysed by QPCR using the ΔΔ*C*t approach as previously described[50] (see Supplementary Table S3 for primer sequences). Primers flanking the FXR responsive element (FXR RE) on the well-known FXR target gene *OST*α[53] were used as positive control, while primers flanking a site distant from the FXR RE on the ACE2 promoter were used and a negative control. The results were normalized to the enrichment observed with non-immune IgG ChIP controls.

Luciferase reporter

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

Immunofluorescence, RNA extraction and QPCR

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

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

Flow cytometry analyses

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

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

Dose-response curves for ACE2

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

Cytotoxicity and viability

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

SARS-CoV-2 isolate

The SARS-CoV-2 virus used in this study are the clinical isolate named "SARS-CoV-2/human/Liverpool/REMRQ0001/2020"[52] derived from a patient's naso-pharyngeal swab and isolated by Lance Turtle (University of Liverpool) and David Matthews and Andrew Davidson (University of Bristol) and the delta lineage (B.1.617.2) hCoV-19/England/SHEF-10E8F3B/2021 (G|SA|D accession number EP|_|SL_1731019) kindly provided by Professor Wendy Barclay, Imperial College London, London, UK through the Genotype to Phenotype National Virology Consortium (G2P-UK).

SARS-CoV-2 infection

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

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

Fixation of SARS-CoV-2 infected organoids/tissue

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

Quantification of viral infection

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

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

Transmission Electron Microscopy

Infected organoids were fixed in 4% paraformaldehyde; 2.5% glutaraldehyde in 0.1M sodium cacodylate buffer overnight at 4°C, washed and stored in 0.1M sodium cacodylate buffer before processing. Samples were postfixed in 1% aqueous osmium tetroxide (TAAB, UK); 1.5% potassium ferricyanide overnight at 4°C, washed thoroughly in dH_2O and *en bloc* stained in 3% aqueous uranyl acetate (Agar Scientific, UK) for 24h at 4°C. Samples were dehydrated through an ethanol series, infiltrated with 1:1 propylene oxide:resin (TAAB, UK) and blocks of fresh resin polymerised at 60°C for 48h. Ultrathin sections of ~60nm were cut from blocks using an EM UC7 ultramicrotome (Leica Microsystems, UK) and mounted on copper grids coated with carbon and formvar (Agar Scientific, UK). Grids were post-stained in uranyl acetate and lead citrate, imaged using a HT7800 transmission electron microscope (Hitachi High Technologies, Japan) operating at 100kV and acquired using HT7800 TEM operating software version 01.21 (Hitachi).

HEK293 cells stably expressing ACE2

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

Luciferase reporter for SARS-CoV-2 replication

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

Animal study

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

Hamsters: the experiments were 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. Golden Syrian Hamsters were purchased from Janvier Labs, France. Animals were housed in a 12 hours/12 hours dark/light cycle, with a humidity of 45-65% and temperature of 20-24°C. Age matched male animals were used weighing between 80 – 100g. Animals were assigned randomly to treatment and control groups. Animals in the treatment groups received a daily oral regimen of UDCA (416 mg/kg) by oral gavage while animals in the control group received vehicle only. The animals were fed ad libitum and treatment continued for 7 days to achieve similar blood concentration of UDCA observed in patients taking UDCA (Extended Data Fig. 9a)[28].

For testing UDCA intervention against SARS-CoV-2 infection, one hamster was directly inoculated by the intranasal route with $1x10^2$ PFU/hamster in 100µl of PBS. Each infected hamster was placed on one side of a transmission cage. The cage was divided with an aerated barrier allowing the infected hamster to be co-housed with previously treated uninfected animals housed on the other side permitting to study viral infection by aerosol transmission. Daily swabs were collected from all animals to monitor the infection via QPCR for the viral N gene. On day 4 post infection the hamsters were euthanised and lungs and nasal turbinates were collected for quantification of viral infection. The experiment was repeated n=3 times for a total of n=9 UDCA animals and n=6 vehicle animals. Data were analysed blinded to the identity of the experimental groups.

Quantification of UDCA concentration

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

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

Ex situ normothermic perfusion (ESNP) of human lungs

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

Lung ESNP experimental setup

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

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

Timing and duration of ESNP

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

Experimental timepoints and sample collection

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

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

Ex situ normothermic perfusion (ESNP) of donor livers

The OrganOx metra normothermic liver perfusion device was used for *ex situ* perfusion of human livers as previously described[14,29,31]. The machine, which is clinically used for preservation of livers for transplantation enables prolonged automated organ preservation by perfusing it with ABO-blood group-matched normothermic oxygenated blood. The perfusion device incorporates online blood gas measurement, as well as software-controlled algorithms to maintain pH, PO2 and PCO2 (within physiological limits), temperature, mean arterial pressure and inferior vena cava pressure within physiological normal limits.

Experimental setup

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

Timing and duration of ESNP

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

Experimental timepoints and sample collection

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

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

Ex vivo SARS-CoV-2 infection of human tissue

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

The freshly obtained lung, bronchial, vascular and gallbladder tissues were processed into small rectangular pieces of 0.5x0.5 cm and were rinsed with University of Wisconsin solution (lung, bronchial and vascular tissues) or William's E medium with supplements as previously described[50] (gallbladder tissue). Washed specimens were placed in wells of a 24-well plate (one specimen per well) and infected with SARS-CoV-2. An inoculum of $1.2x10^5$ PFU/ml at 500 µl per well was used. After two hours, the inoculum was removed, and the specimens were washed three times with PBS. The infected human tissue was then cultured in 500 µl of advanced DMEM with supplements[60] (lung, bronchial and vascular tissues) or William's E medium with supplements (gallbladder tissue). Supernatant and tissue were harvested for QPCR and immunofluorescence at 2 and 24 hours post infection.

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

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

ACE2 measurement

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

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

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

Patient data

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

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

Statistical analyses

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

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

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

VOCAL data (validation cohort): for propensity score-matched analyses, 1:3 matched samples (using a Greedy matching algorithm) were constructed with moderate/severe/critical COVID-19 and severe/critical COVID-19 according to the NIH severity scale as the outcome variables. Covariables used were 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.

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

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

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Extended Data Figure legends

Extended Data Fig. 1. Expression of SARS-CoV-2 entry genes in cholangiocytes. (**a**) Schematic illustration of different primary human cholangiocyte populations corresponding to different areas of the biliary tree and cholangiocyte organoids (COs) derived from different areas of the biliary tree grown in absence or presence of the bile acids. (**b**) UMAP plot

illustrating different cholangiocyte populations from (a) analysed by scRNAseq. (**c**) UMAP plots showing that viral entry related genes are predominantly expressed in extrahepatic cholangiocytes and COs treated with bile acids. (**d-e**) Immunofluorescence illustrating that ACE2 is expressed in extrahepatic cholangiocytes (**d**) and that SARS-CoV-2 infects gallbladder cholangiocytes of COVID-19+ patient but not intrahepatic cholangiocytes (**e**). N=4 independent samples. Scale bars 50 μm. (**f**) QPCR confirming detection of SARS-CoV-2 RNA in bile of COVID-19+ patients. Housekeeping gene, HMBS; n=4; two-tailed Mann-Whitney test; centre line, median; box, interquartile range (IQR); whiskers, range; bars, standard deviation. (**g**) Violin plot of scRNAseq data from (b) showing that cholangiocyte organoids upregulate ACE2 when treated with bile acids regardless of their region of origin. (**h**) QPCR validating that upon treatment with the bile acid CDCA cholangiocyte organoids assume a gallbladder identity expressing the gallbladder marker SOX17 and upregulating ACE2 at levels comparable to primary gallbladder. Housekeeping gene, HMBS; n=4 independent experiments; one-way ANOVA adjusted for multiple comparisons; ns, non-significant; centre line, median; box, interquartile range (IQR); whiskers, range; bars, standard deviation. (**i**) Immunofluorescence showing that CDCA induces ACE2 expression in gallbladder cholangiocyte organoids (GCOs). N=4 independent experiments. Scale bars 50 μm.

Extended Data Fig. 2. CDCA-treated GCOs can be infected by SARS-CoV-2. (**a**) Schematic representation of the methodology used to infect GCOs with SARS-CoV-2 and test the capacity of SARS-CoV-2 virions produced in GCOs to infect new (uninfected) cells. (**b**) Immunofluorescence validating SARS-CoV-2 infection in GCOs. N=4 independent experiments. Scale bars 50 μm. (**c**) QPCR confirming infection of CDCA-treated GCOs with SARS-CoV-2 propagated in VERO E6 cells (top panel) and with SARS-CoV-2 propagated in

GCOs treated with CDCA (bottom panel), illustrating that SARS-CoV-2 produced in GCOs+CDCA retains its infectious capacity. Scale bars 50 μm; housekeeping gene, GAPDH; n=4 independent experiments; Kruskal-Wallis test adjusted for multiple comparisons; centre line, median; box, interquartile range (IQR); whiskers, range; bars, standard deviation. (**d**) QPCR showing up-regulation of innate immune and anti-viral response genes in GCOs+CDCA following SARS-CoV-2 infection. Housekeeping gene, GAPDH; n=4, 2 biological and 2 technical replicates; two-tailed Mann-Whitney test (IL-1β, IL-6, IFNα) and two-tailed unpaired t-test (TNFα, IFNλ). (CDCA concentration, 10 μM); centre line, median; box, interquartile range (IQR); whiskers, range; bars, standard deviation. (**e**) Transmission electron micrograph of uninfected cholangiocyte organoids (left panel) and cholangiocyte organoids infected with SARS-CoV-2 in the absence (central panel) or presence of CDCA (right panel) showing key morphological features of viral infection and cell death, such as production of viral particles, formation of pathologic vacuoles (Vc) and swollen mitochondria (Mt). N=3 independent experiments. Scale bars 5 μm.

Extended Data Fig. 3. FXR is present and active in tissues affected by COVID-19 and their corresponding CDCA-treated organoids. (**a-b**) Immunofluorescence images confirming expression of FXR in primary human gallbladder, lungs and intestinal tissue (**a**) and in corresponding primary organoids treated with physiological levels of bile acids (CDCA, 10 μM) (**b**). N=4 independent experiments. White scale bars 100 μm; grey scale bar 25 μm. (**c**) QPCR analysis validating expression of FXR and its downstream effector SHP in primary tissue and corresponding organoids in presence or absence of physiological levels of bile acids (CDCA). CDCA treatment increases FXR and SHP expression in organoids to levels that are closer to primary tissue. Housekeeping gene, HMBS; n=4 independent experiments; one-way ANOVA adjusted for multiple comparisons; centre line, median; box, interquartile

range (IQR); whiskers, range; bars, standard deviation. GCOs, gallbladder cholangiocyte organoids; AOs, airway organoids; IOs, intestinal organoids.

Extended Data Fig. 4. UDCA and ZGG require FXR to reduce ACE2 and SARS-CoV-2 infection. (**a-b**) Immunofluorescence (**a**) and QPCR (**b**) showing downregulation of FXR expression following FXR knock-down (KD) in cholangiocyte organoids. Scale bars 25 μm. Housekeeping gene HMBS. One-way ANOVA adjusted for multiple comparisons; n=4 independent experiments; centre line, median; box, interquartile range; whiskers, range; bars, standard deviation. (**c**) QPCR on FXR KD cholangiocyte organoids showing no change in the expression of ACE2 and the FXR downstream effector SHP following treatment with CDCA, UDCA or ZGG, demonstrating that FXR is indispensable for regulating ACE2 and SHP through these compounds. ACE2 and SHP expression in wild-type organoids shown in Fig. 1c and ED Fig. 5a. Housekeeping gene, HMBS; n=4; one-way ANOVA adjusted for multiple comparisons; ns, non-significant; centre line, median; box, interquartile range; whiskers, range; bars, standard deviation. (**d**) QPCR quantifying SARS-CoV-2 RNA 24 hours post infection in FXR KD cholangiocyte organoids treated with CDCA, UDCA or ZGG. SARS-CoV-2 infection in the presence of FXR is shown in Fig. 1e. Housekeeping gene, GAPDH; n=4 independent experiments; one-way ANOVA adjusted for multiple comparisons; centre line, median; box, interquartile range; whiskers, range; bars, standard deviation. (CDCA, UDCA and ZGG concentration, 10 μM). (**e**) Schematic illustrating the luciferase reporter construct containing the FXR responsive element IR-1 identified in Fig. 1a and the mutagenesis strategy employed in panel (f). (**f**) Luciferase reporter assay in HEK293 cells showing the transcriptional activity associated with the IR-1 located in the ACE2 promoter upon treatment with CDCA, UDCA or ZGG. Site-directed mutagenesis on IR-1 abolishes FXR binding/transactivation confirming the specificity of FXR binding on the ACE2 IR-1. IR-1

located in the SHP promoter used as positive control. N=3 independent experiments; one-way ANOVA adjusted for multiple comparisons; bars, standard deviations. (CDCA, UDCA and ZGG concentration, 50 μM).

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

comparisons; ns, non-significant; centre line, median; box, interquartile range (IQR); whiskers, range; bars, standard deviation.

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

Extended Data Figure 7. FXR inhibition reduces ACE2 levels *in vivo*. (**a**) Schematic representation of the experiment performed. (**b**) QPCR showing that treatment with UDCA in FVB/N mice reduces ACE2 levels in lung, gallbladder and intestinal tissue. Housekeeping gene, GAPDH; n=4 animals per group (UDCA vs. no UDCA control group, see methods); unpaired two-tailed t-test; centre line, median; box, interquartile range (IQR); whiskers, range; bars, standard deviation. (**c**) Immunofluorescence images showing ACE2 levels upon treatment with UDCA in respiratory, biliary and intestinal epithelium in FVB/N mice. N=4 mice per group. White scale bars 100 μm; yellow scale bars 50 μm. (**d**) QPCR showing that treatment with UDCA in Syrian golden hamsters reduces ACE2 levels in the gallbladder and

intestinal tissue. Housekeeping gene, GAPDH; n=5 vehicle/No UDCA group vs n=3 UDCA group; unpaired two-tailed t-test; centre line, median; box, interquartile range (IQR); whiskers, range; bars, standard deviation. (**e**) Immunofluorescence images showing ACE2 levels upon treatment with UDCA in biliary and intestinal epithelium in Syrian golden hamsters. N=3 hamsters per group. White scale bars 100 μm.

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

Extended Data Figure. 9. FXR inhibition reduces SARS-CoV-2 infection in human organs *ex vivo*. (**a**) Photograph and schematic of the liver *ex situ* normothermic perfusion (ESNP) experiment performed; including type of samples harvested and timeline. 0h: 0 hours baseline sample collection and UDCA/carrier administration. The 0h samples were collected immediately prior to UDCA administration. 12h: 12 hours after UDCA/carrier administration. For each timepoint, 4 independent tissue samples were obtained from the grafts' gallbladder and used for ACE2 measurement and viral infection (n=4 gallbladder tissue samples per timepoint). (**b**) ACE2 enzymatic activity measurement showing that ESNP with UDCA reduces ACE2 activity in the circulating perfusate, compared to carrier only control. N=4 independent samples; unpaired two-tailed t-test; centre line, median; box, interquartile range

(IQR); whiskers, range; bars, standard deviation. (**c**) Immunofluorescence images showing ACE2 expression in gallbladder cholangiocytes and cells of the vasculature (smooth muscle and endothelial cells) in human livers before and after ESNP with UDCA/carrier. N=4 independent samples. White scale bars 100 μm; yellow scale bars 50 μm, grey scale bars 25 μm. (**d**) QPCR demonstrating that ESNP with UDCA reduces ACE2 levels in gallbladder cholangiocytes, compared to carrier only control. Housekeeping gene, HMBS; n=4; unpaired two-tailed t-test; centre line, median; box, interquartile range (IQR); whiskers, range; bars, standard deviation. (**e-f**) QPCR (**e**) and immunofluorescence (**f**) showing that UDCA reduces SARS-CoV-2 infection in human gallbladder *ex vivo.* Housekeeping gene, GAPDH; n=4; one-way ANOVA adjusted for multiple comparisons; centre line, median; box, interquartile range (IQR); whiskers, range; bars, standard deviation. Scale bars 25 μm. (UDCA, concentration, 2000 ng/ml).

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

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