Drug repurposing screens reveal cell-type-specific entry pathways and FDA-approved drugs active against SARS-Cov-2

Graphical abstract
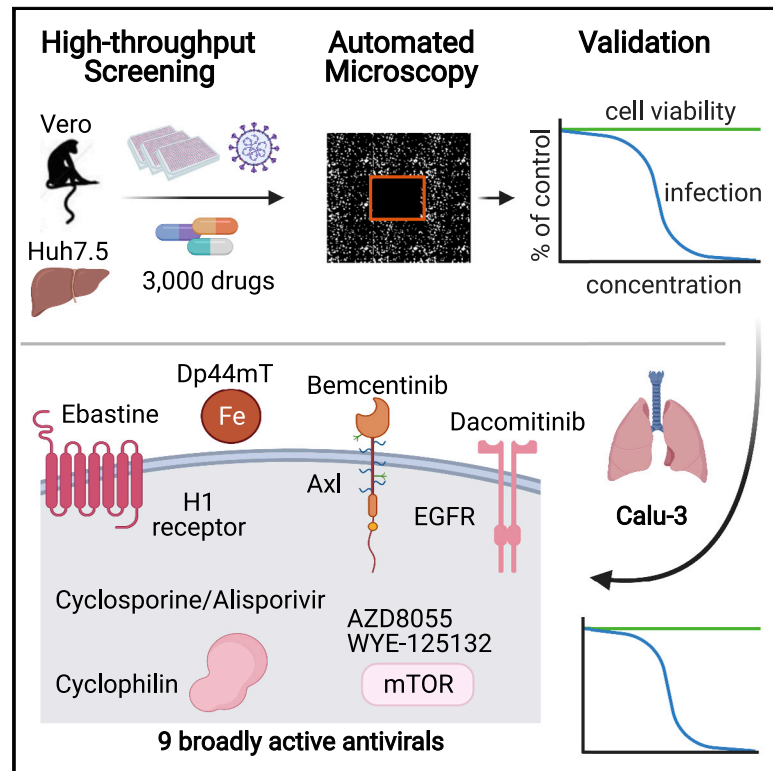
Authors

Mark Dittmar, Jae Seung Lee, Kanupriya Whig, ..., Holly Ramage, David C. Schultz, Sara Cherry

Correspondence

holly.ramage@jefferson.edu (H.R.), dschultz@upenn.edu (D.C.S.), cherrys@pennmedicine.upenn.edu (S.C.)

In brief

There is an urgent need for antivirals to treat the newly emerged SARS-CoV-2. Dittmar et al. find nine host-directed drugs are antiviral in respiratory cells, seven of which have been given to humans, and three are FDA approved. We show host targets that have the potential for rapid clinical implementation.

Highlights

- 3,000 compounds screened in two cell types against SARS-CoV-2

- Entry pathways are distinct in hepatocyte Huh7.5 and respiratory Calu-3 cells

- Only nine compounds that are active in Huh7.5 cells are active in Calu-3 cells

- Cyclosporin and cyclophilin inhibitors block SARS-CoV-2 infection in diverse cells

CellPress

Cell Reports

Resource

Drug repurposing screens reveal cell-type-specific entry pathways and FDA-approved drugs active against SARS-Cov-2

Mark Dittmar,[1,7] Jae Seung Lee,[1,7] Kanupriya Whig,[2,7] Elisha Segrist,[1] Minghua Li,[1] Brinda Kamalia,[2] Lauren Castellana,[1] Kasirajan Ayyanathan,[1] Fabian L. Cardenas-Diaz,[3] Edward E. Morrisey,[3] Rachel Truitt,[3] Wenli Yang,[3] Kellie Jurado,[4] Kirandeep Samby,[5] Holly Ramage,[6,*] David C. Schultz,[2,*] and Sara Cherry[1,2,4,8,*]

[1]Department of Pathology and Laboratory Medicine, University of Pennsylvania, Philadelphia, PA, USA
[2]Department of Biochemistry and Biophysics, University of Pennsylvania, Philadelphia, PA, USA
[3]Department of Medicine, University of Pennsylvania, Philadelphia, PA, USA
[4]Department of Microbiology, University of Pennsylvania, Philadelphia, PA, USA
[5]Medicines for Malaria Venture, Geneva, Switzerland
[6]Department of Microbiology, Thomas Jefferson University, Philadelphia, PA, USA
[7]These authors contributed equally
[8]Lead contact
*Correspondence: holly.ramage@jefferson.edu (H.R.), dschultz@upenn.edu (D.C.S.), cherrys@pennmedicine.upenn.edu (S.C.)
https://doi.org/10.1016/j.celrep.2021.108959

SUMMARY

There is an urgent need for antivirals to treat the newly emerged severe acute respiratory syndrome corona-virus 2 (SARS-CoV-2). To identify new candidates, we screen a repurposing library of ~3,000 drugs. Screening in Vero cells finds few antivirals, while screening in human Huh7.5 cells validates 23 diverse anti-viral drugs. Extending our studies to lung epithelial cells, we find that there are major differences in drug sensitivity and entry pathways used by SARS-CoV-2 in these cells. Entry in lung epithelial Calu-3 cells is pH independent and requires TMPRSS2, while entry in Vero and Huh7.5 cells requires low pH and triggering by acid-dependent endosomal proteases. Moreover, we find nine drugs are antiviral in respiratory cells, seven of which have been used in humans, and three are US Food and Drug Administration (FDA) approved, including cyclosporine. We find that the antiviral activity of cyclosporine is targeting Cyclophilin rather than calcineurin, revealing essential host targets that have the potential for rapid clinical implementation.

INTRODUCTION

Coronaviruses represent a large group of medically relevant viruses that were historically associated with the common cold. However, in recent years, members of the coronavirus family have emerged from animal reservoirs into humans and have caused novel diseases (Cui et al., 2019). First, severe acute respiratory syndrome coronavirus (SARS-CoV) emerged in China in 2003, followed by Middle East respiratory syndrome (MERS)-CoV in 2012 (de Wit et al., 2016; Weiss and Navas-Martin, 2005). Although SARS was in the end eradicated, MERS continues to cause infections in the Middle East. Beginning in December 2019 and continuing into January 2020, it became clear that a new respiratory virus was spreading in Wuhan, China. Rapid sequencing efforts revealed a coronavirus closely related to SARS, which was named SARS-CoV-2 (Wu et al., 2020). Unfortunately, this virus is highly infectious and has spread rapidly, creating a worldwide pandemic.

Identification of broadly acting SARS-CoV-2 antivirals is essential to clinically address SARS-CoV-2 infections. A potential route to candidate antivirals is through the deployment of drugs that show activity against related viruses. Previous studies found that the antiviral drug remdesivir, which was developed against the RNA-dependent RNA polymerase of Ebola virus, was also active against SARS-CoV-2 in vitro, with promising results in clinical trials (Beigel et al., 2020; Blanco-Melo et al., 2020; Warren et al., 2016). Chloroquine and its derivatives, including hydroxychloroquine, are approved for use in malaria, and many in vitro studies have found that these drugs are also active against coronaviruses, including SARS-CoV-2 (Liu et al., 2020; Wang et al., 2020). This led to early adoption of these agents to treat COVID-19 (the disease caused by SARS-CoV-2 infection); however, little efficacy of these agents has been demonstrated in subsequent clinical trials (Boulware et al., 2020). It remains unclear why these agents have not been more active in humans.

There are currently more than 3,000 US Food and Drug Administration (FDA)-approved drugs, as well as many others that have been tested in humans. We created an in-house library of 3,059 drugs, including ~1,000 FDA-approved drugs and ~2,100 drug-like molecules against defined molecular targets with validated pharmacological activity. In addition, we purchased drugs with reported anti-SARS-CoV-2 activity (e.g., remdesivir, lopinavir, azithromycin, etc.). Viruses encode unique proteins essential

for infection, and most approved antivirals target these virally encoded essential targets. This class of antivirals has been termed direct-acting antivirals. Viruses are also dependent on host cellular machineries for successful infection, and drugs that block these activities are host-targeted antivirals. Given our dearth of effective treatments, we developed a screening platform that would allow us to identify both direct-acting and host-targeted antivirals that can be potentially repurposed for use against SARS-CoV-2 (Ashburn and Thor, 2004).

We developed a specific and sensitive assay to quantify viral infection using a cell-based high-content approach. We began our studies in African green monkey (*Cercopithecus aethiops*) kidney epithelial cells (Vero) because they are routinely used to propagate SARS-CoV-2. They are robustly infected, and thus Vero cells are widely used as a model system to screen for antivirals (Harcourt et al., 2020; Jeon et al., 2020; Sheahan et al., 2020; Wang et al., 2020). We screened our in-house repurposing library, identifying only six drugs that were antiviral with low toxicity in the primary screen, of which five were validated in dose-response assays. Given how few candidates emerged, we reasoned that human cells might be a better model of infection and thus tested a panel of human cell lines to identify cells that are easy to grow and permissive to infection. We found that the human hepatocyte cell line Huh7.5 was readily infected with SARS-CoV-2. Screening in this human cell line, we identified three of the Vero hits and validated an additional 23 drugs that were active in dose-response experiments and showed a favorable selectivity index (SI) versus toxicity (Blight et al., 2002). These candidates targeted a wide variety of cellular activities, but few were active in Vero cells. However, one class, the chloroquines and their derivatives, was active in both cell types.

The entry pathway of SARS-CoV-2 has only begun to be elucidated, with much of what we know being inferred from studies of the related SARS-CoV-1 (Millet and Whittaker, 2018; Shang et al., 2020). The coronavirus glycoprotein, or Spike, requires proteolytic processing for entry (Belouzard et al., 2009; Millet and Whittaker, 2015; Shang et al., 2020). This processing can occur outside the cell or within the endolysosomal compartment (Millet and Whittaker, 2015; Shang et al., 2020). Both SARS-CoV-1 and -2 engage angiotensin-converting enzyme 2 (Ace2) as their plasma membrane receptor (Hofmann and Pöhlmann, 2004; Letko et al., 2020; Pairo-Castineira et al., 2021). Upon binding, the viruses, along with the receptor, are endocytosed into the cell into a low-pH endosomal compartment where there are proteases, including cathepsins, that can cleave Spike and allow for entry into the cytosol (de Duve et al., 1974; Ducharme and Farinotti, 1996; Yang and Shen, 2020). Because cathepsins require a low pH for activity, chloroquine and its derivatives that neutralize this low pH can effectively block viral entry (de Duve et al., 1974; Ducharme and Farinotti, 1996; Yang and Shen, 2020). Recent studies have also identified that a plasma membrane-associated serine protease, TMPRSS2, is active against Spike, cleaving the protein extracellularly and thereby bypassing the requirement for endosomal proteases (Glowacka et al., 2011; Hoffmann et al., 2020; Matsuyama et al., 2020). Whether SARS-CoV-2 enters through different routes in different cell types remains unclear.

Respiratory epithelial cells are the major cellular target for SARS-CoV-2 *in vivo* and have been used to explore the role of TMPRSS2 in infection. Perhaps surprisingly, although we found remdesivir was antiviral in respiratory Calu-3 cells, hydroxychloroquine was not. Because a panel of quinolines had no activity in Calu-3 cells, these data suggest that entry in these lung epithelial cells is independent of low-pH processing in the endosomal compartment. In contrast, the TMPRSS2 inhibitor camostat was highly active in Calu-3 cells but inactive in Vero and Huh7.5 cells. These data demonstrate distinct modes of entry in respiratory cells and are further supported by our studies using human induced pluripotent stem cell (iPSC)-derived respiratory cells (Letko et al., 2020). Further, these data suggest that there may be other fundamentally different cellular requirements in different cell types. We screened our 23 validated candidates from Huh7.5 cells in Calu-3 cells and found only 9 drugs showed favorable activity, including 3 FDA-approved drugs: cyclosporine, dacomitinib, and salinomycin. In additional studies, we found that cyclosporine analogs that target Cyclophilin A were active against SARS-CoV-2, but not compounds that target calcineurin. Identifying antivirals active in the respiratory tract is essential to move forward with clinical treatments for SARS-CoV-2.

RESULTS

Vero cells are permissive to infection and can be used for antiviral screening for direct-acting antivirals

SARS-CoV-2 is routinely propagated in Vero E6 cells (Harcourt et al., 2020; Hoffmann et al., 2020; Sheahan et al., 2020). When growing the virus in either Vero E6 or Vero CCL81 cells, two different strains of Vero cells from ATCC, we observed that SARS-CoV-2 (Isolate USA-WA1/2020) is cytopathic in Vero E6, but not in Vero CCL81 (data not shown) (Harcourt et al., 2020). Moreover, viral stocks propagated from either of these cells produced similar titers of virus (1×10^7 plaque-forming units [PFUs]/mL) suggesting that viral replication and cytotoxicity are separable. Therefore, we set out to develop a quantitative microscopy-based assay to measure the level of replication of SARS-CoV-2 more directly in infected cells. We chose Vero CCL81 to uncouple toxicity from infection and quantified infection 30 h postinfection (hpi) to focus our assay on inhibitors active within the first cycle of infection. We first validated that our antibodies could detect infection of SARS-CoV-2. We used an antibody to double-stranded RNA (dsRNA) and to SARS-CoV-2 Spike (Figure 1A) (Bonin et al., 2000; Tian et al., 2020).

We created an in-house library of 3,059 compounds, including ~1,000 FDA-approved drugs and another ~2,000 drug-like molecules against defined molecular targets with validated pharmacological activity. The library contains 678 known kinase inhibitors, 435 annotated cancer therapeutics, 190 epigenetic regulators, 411 anti-virals/infectives, and 596 G-protein-coupled receptors (GPCRs) and ion channel regulators. The remaining compounds fall into diverse target classes. We next optimized the dose and timing of infection by performing dose-response studies with known antivirals. Indeed, we found that hydroxychloroquine and remdesivir were active in Vero cells with IC_{50}s (concentration of a drug that is required for 50% inhibition) and CC50s (concetration of a drug that is required for 50% cell killing), demonstrating little cytotoxicity at the active doses (Figure 1B) (Wang et al., 2020).
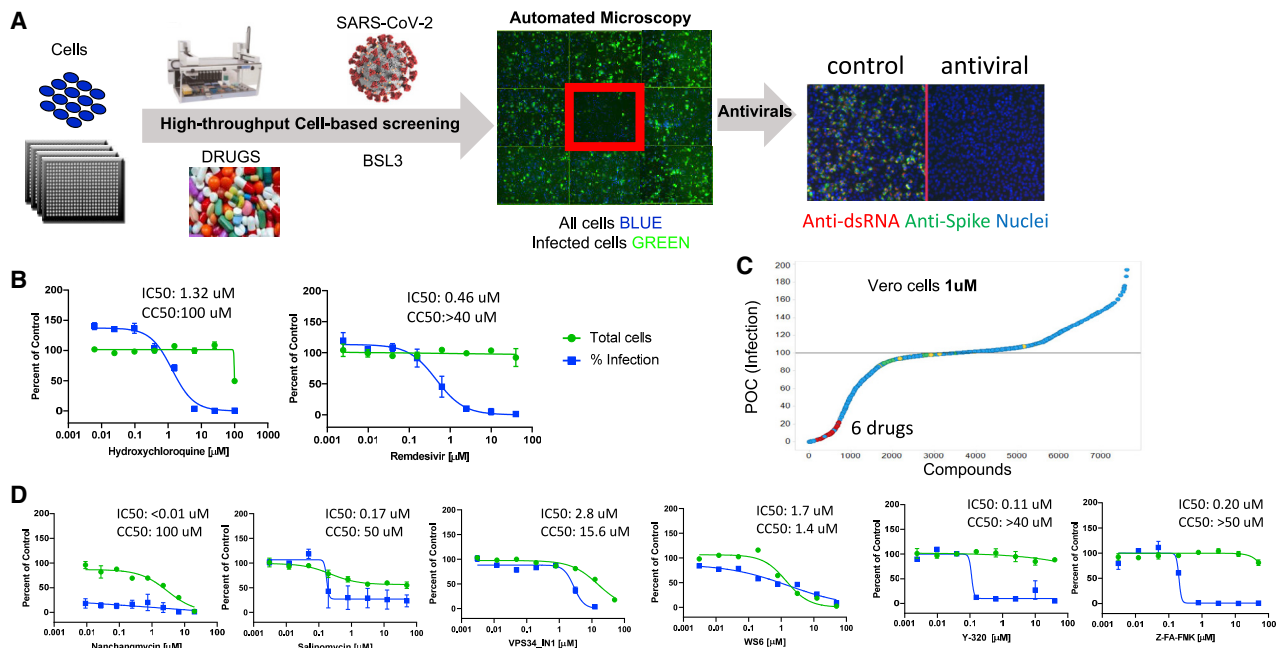
Figure 1. High-throughput screening in Vero cells to identify antivirals against SARS-CoV-2

(A) Schematic of the screening strategy. Vero cells were plated in 384-well plates, drugs were added, and the cells were infected with SARS-CoV-2 (MOI = 1). 30 hpi cells were stained for viral infection (dsRNA, Spike) and imaged using automated microscopy to define cell number and percent infection. Antivirals show little impact on cell number and block viral infection.

(B) Dose-response analysis of Vero cells treated with hydroxychloroquine or remdesivir. Each data point represents the average of two independent experiments ± SD.

(C) Percent of control (POC) for percentage of infection of the Vero drug screen performed at 1 μM. Six drugs had >60% reduction in infection with >80% cell viability.

(D) Dose-response analysis of six candidates identified in the screen. Each data point represents the average of ≥2 independent experiments ± SD.

Next, we validated the assay metrics and observed a Z' = 0.7 (Figure S1) (Zhang et al., 1999).

We used this assay pipeline to screen our in-house repurposing library in 384-well plates at a final concentration of 1 μM (Figure 1C) (Rausch et al., 2017). We quantified the percentage of infected cells, as well as the total cell number per well, to allow for exclusion of toxic compounds. We robustly identified the positive control remdesivir (Figure S1) (Wang et al., 2020). Using a threshold of <40% infection and >80% viability, as compared with the DMSO vehicle control, we identified only six drugs that had antiviral activity in our primary screen (Table S1). This included the natural product nanchangmycin, which we previously found in a drug repurposing screen against Zika virus (Rausch et al., 2017). Nanchangmycin was broadly active against viruses that enter cells through endocytosis, consistent with the role of endosomal acidification for SARS-CoV-2 entry in these cells (Rausch et al., 2017). We then re-purchased powders and validated the activity of these candidates in a dose-response assay where we observed antiviral activity for salinomycin, Y-320, Z-FA-FMK, and VPS34-IN1 in the absence of significant toxicity (Figure 1D; Figures S1 and S2).

Human hepatocyte Huh7.5 cells are permissive to infection and can be used to identify antivirals

Because Vero cells are derived from African green monkeys, we set out to identify a human cell line permissive to infection. To

this end, we infected a panel of human cell lines with SARS-CoV-2 and monitored infection by microscopy. We initially tested A549, Calu-1, Huh7, Huh7.5, HepG2, HaCaT, IMR90, NCI-H292, CFBE41o, and U2OS cells. We detected less than 1% infection of A549, Calu-1, Huh7, HepG2, HaCaT, IMR90, NCI-H292, CFBE41o, and U2OS cells (data not shown). Interestingly, although Huh7 cells were largely non-permissive, the derivative cell line Huh7.5 was permissive to SARS-CoV-2 (Figure 2A). Huh7.5 cells are defective in innate immune signaling (RIG-I) and are known to be more permissive to many viruses, including hepatitis C virus (HCV) (Blight et al., 2002). Remdesivir and hydroxychloroquine were active against SARS-CoV-2 in Huh7.5 cells with IC$_{50}$s that were more than 10-fold lower than those observed in Vero cells (Figure 2B).

We optimized our image-based assay in Huh7.5 cells using remdesivir and observed that Z' = 0.61 (Figures S2 and S4) (Zhang et al., 1999). We screened our repurposing library at 500 nM, quantifying both the percentage of infected cells and cell number to exclude toxic compounds (Figure 2C). We found that 33 drugs had antiviral activity in the absence of cytotoxicity (<40% infection, >80% viability, as compared with DMSO vehicle control) (Table S2). This included three of the six drugs identified in Vero cells: Z-FA-FMK, Y-320, and salinomycin. We also tested the other three drugs that emerged from the Vero screen and found that nanchangmycin was highly active but did not meet the criteria from the Huh7.5 screen due to toxicity, and WS6 had modest activity (Figures S2 and S3).
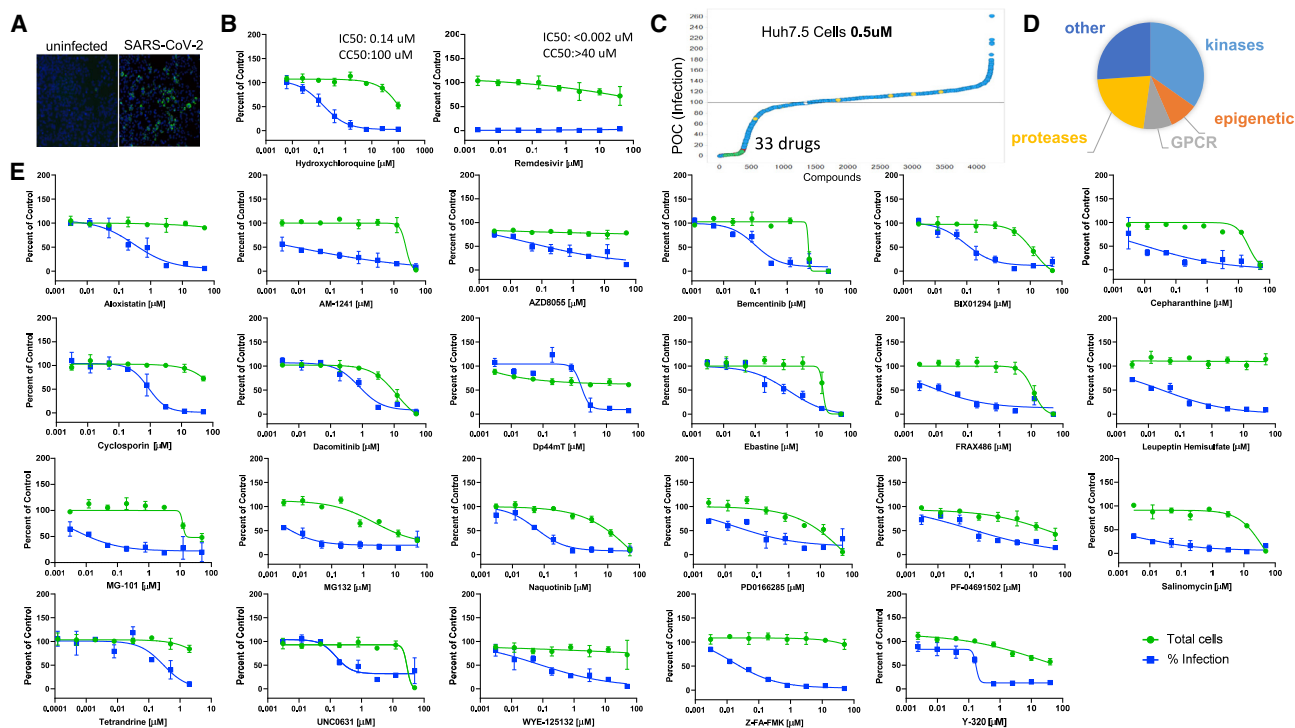
Figure 2. High-throughput screening in human Huh7.5 cells to identify antivirals against SARS-CoV-2

(A) Huh7.5 cells were infected with SARS-CoV-2 (MOI = 1) and 30 hpi processed for microscopy.

(B) Dose-response analysis of Huh7.5 cells treated with hydroxychloroquine or remdesivir. Data represent the average of three independent experiments ± SD.

(C) POC for percentage of infection of the Huh7.5 drug screen performed at 0.5 μM. 33 drugs had >60% reduction in infection with >80% cell viability.

(D) Distribution of 23 validated antivirals by drug target class.

(E) Dose-response analysis of the candidates with a selectivity index (SI) > 3 identified in the screen. Data represent the average of three independent experiments ± SD.

We repurchased powders for the 32 drugs and tested their activity in dose-response assays in Huh7.5 cells against SARS-CoV-2. The total cell number and the percent of infected cells were quantified. Remdesivir and hydroxychloroquine were used as positive controls, and the DMSO vehicle was included as a negative control (Wang et al., 2020). Of those tested, 23 drugs showed antiviral activity and fell into diverse classes (Figure 2D). Dose-response curves are shown for these 23 candidates, and the IC_{50}s and CC_{50}s were calculated (Figure 2E). The SI (ratio between antiviral and cytotoxicity potencies) was calculated, and the 23 candidates were antiviral with a SI >3 (Figure 2E; Figure S4; Table S3). Dose-responses curves for the other candidates that did not show a SI >3 are shown in Figures S2 and S5.

Direct-acting antivirals are likely to be active against the virus in multiple cell types, as was observed for remdesivir. In addition, host-directed antivirals that target key steps in the viral life cycle and are highly conserved and broadly expressed are also likely to emerge across cell types. One example is the endosomal acidification blocker hydroxychloroquine, which indeed scored as an antiviral in both cell types (de Duve et al., 1974; Ducharme and Farinotti, 1996; Liu et al., 2020). We next directly tested if the candidates identified in Huh7.5 cells were active in Vero cells. We found that five additional compounds were active in Vero

cells with a SI > 3, AZD8055, BIX01294, Ebastine, MG-132, and WYE-125132, albeit at higher concentrations (Figure S2). These were missed from the previous screen either due to low potency or toxicity at the screening concentration. Nevertheless, most of the antivirals that were validated in Huh7.5 cells were not active in Vero cells using this assay (Figure S6B).

Lung epithelial cells show differences in drug sensitivities

We next focused on respiratory epithelial models because these are the most relevant to human SARS-CoV-2 infections. We found that a number of lung-derived epithelial cell lines were refractory to infection (e.g., A549, Calu-1, NCI-H292, CFBE41o). However, we found that Calu-3 cells, which have been shown to be permissive for many coronaviruses, including SARS-CoV-2, were readily infected (Figure 3A) (Hoffmann et al., 2020; Sheahan et al., 2020; Shen et al., 1994). We optimized assays using Calu-3 cells and tested their sensitivity to remdesivir and hydroxychloroquine. As expected, we found that the direct-acting antiviral remdesivir was active; however, hydroxychloroquine had little or no activity in Calu-3 cells (Figure 3B). Treatment with remdesivir not only revealed protection from viral infection (blue curve) but also showed increased cell number compared with DMSO control (green curve) likely because of increased
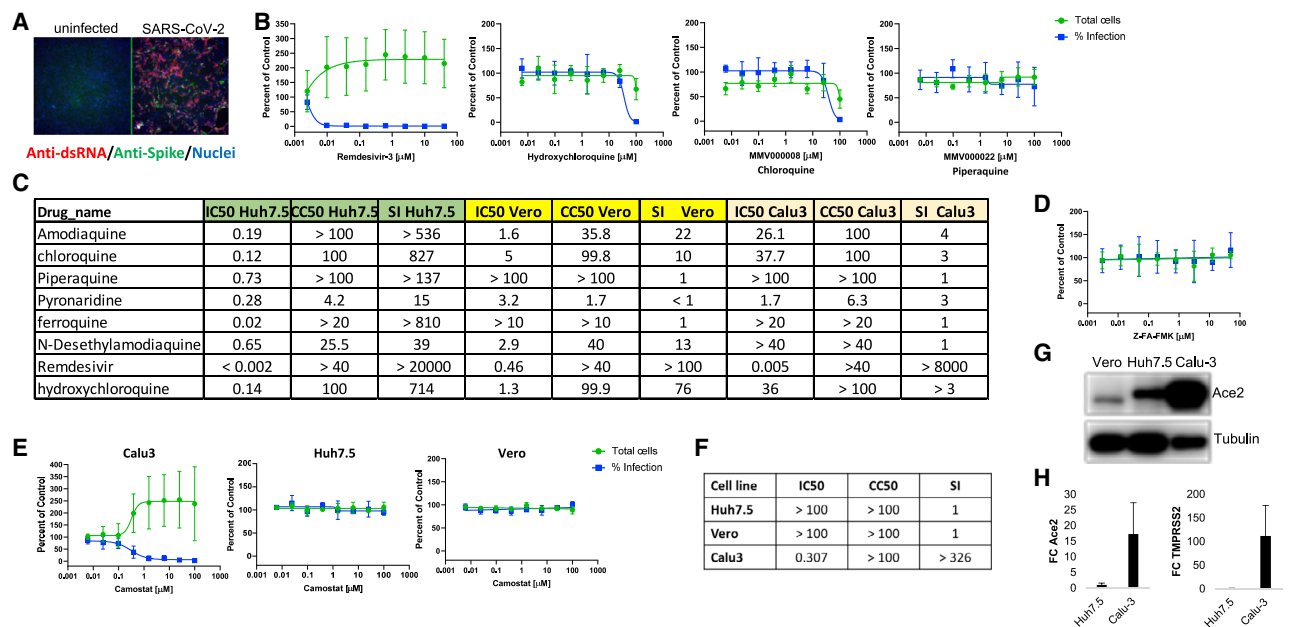
Figure 3. Cell-type-specific dependencies of entry inhibitors
(A) Calu-3 human lung epithelial cells were infected with SARS-CoV-2 (MOI = 0.5) and processed for microscopy 48 hpi.
(B) Dose-response analysis of Calu-3 cells treated with quinolines or remdesivir. Data represent the average of four independent experiments ± SD.
(C) IC$_{50}$, CC50, and SI for Vero, Huh7.5, and Calu-3 cells treated with a panel of quinolines or remdesivir. Data represent the average of four independent experiments ± SD.
(D) Dose-response analysis of Calu-3 cells treated with the cathepsin inhibitor Z-FA-FMK. Data represent the average of four independent experiments ± SD.
(E) Dose response analysis of Calu-3, Vero, and Huh7.5 cells treated with camostat. Data represent the average of ≥2 independent experiments ± SD.
(F) IC$_{50}$, CC50, and SI for camostat across cell types.
(G) Immunoblot of Vero, Huh7.5, and Calu-3 cells probed for Ace2 and tubulin as a loading control. Representative blot is shown.
(H) qRT-PCR of Ace2 or TMPRSS2 comparing Huh7.5 and Calu-3 cells. Data represent the mean ± SEM for ≥2 independent experiments.

cell growth or survival upon inhibition of viral infection. This led us to test the antiviral activity of a panel of chloroquine derivatives, and we found that none of these had activity against SARS-CoV-2 in Calu-3 cells (Figure 3B), although these compounds are active in both Vero cells and Huh7.5 cells (Figure 3C). Because chloroquine and its derivatives work by neutralizing the endosomal pH, this suggests that there are major differences in the requirement for endosomal acidification during infection of SARS-CoV-2 in the lung epithelial cell line Calu-3, as compared with the other cell lines tested.

Endosomal acidification is thought to be required for SARS-CoV-2 entry to maintain the low pH necessary for endosomal cysteine protease activity required for priming Spike for membrane fusion (Hoffmann et al., 2020). Consistent with the requirement for acidification in Vero and Huh7.5, the cathepsin inhibitor Z-FA-FMK emerged as an antiviral in both cell types (Figures 1D and 2E). We tested Z-FA-FMK in Calu-3 cells and found that it had no antiviral activity (Figure 3D), consistent with a lack of a requirement for endosomal acidification. We also tested the more specific cathepsin inhibitor SB 412515 and found that it was active in Huh7.5 and Vero cells, but not Calu-3 cells (Figure S7). Recent studies found the plasma membrane-associated serine protease, TMPRSS2, can prime the viral glycoprotein for entry in lung epithelial cells (Hoffmann et al., 2020). Therefore, we tested the role of TMPRSS2 by

treating cells with the known TMPRSS2 inhibitor camostat. We found that camostat was active in Calu-3 cells but had no activity in either Vero or Huh7.5 cells (Figures 3E and 3F) (Hoffmann et al., 2020). As we observed with remdesivir, camostat not only blocked infection, but treatment at antiviral doses allowed for cell growth as observed by the greater than 100% of cells remaining compared with vehicle control in Calu-3 cells. We also monitored the levels of Ace2 and TMPRSS2 in these cells. Immunoblot of Ace2 revealed expression in all three cell lines, with the highest level in Calu-3 cells (Figure 3G). We used qRT-PCR to compare the levels of Ace2 and TMPRSS2 in Huh7.5 and Calu-3 cells. We found that the RNA levels of Ace2 were ~15-fold higher in Calu-3 cells, and there was very little TMPRSS2 RNA detected in Huh7.5 cells (average CT = 33; Figure 3H). These data are consistent with the fact that Ace2 is required for infection, but that TMPRSS2 is not in every cell type.

We also tested the role of the main endosomal kinase phosphatidylinositol-3-phosphate/phosphatidylinositol 5-kinase (PIKfyve). Previous studies have shown that PIKfyve promotes internalization of diverse viruses, and it was recently shown to impact entry of coronaviruses, including SARS-CoV-2, in HeLa cells (Ou et al., 2020). Using the PIKfyve inhibitor apilimod, we found that PIKfyve promotes infection of SARS-CoV-2 in Huh7.5 and Vero cells, with modest activity in Calu-3 cells having an IC$_{50}$ 1,000-fold higher
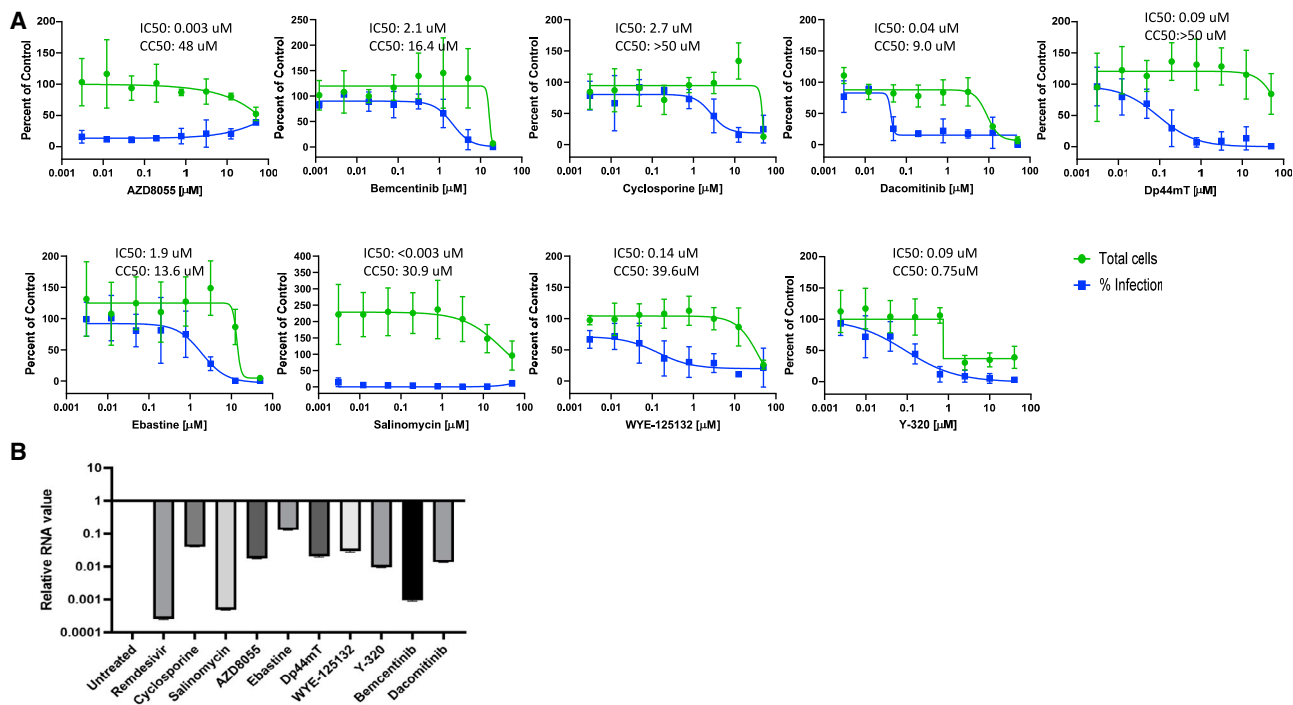
Figure 4. Validation of antiviral activity of nine candidates in Calu-3 cells

(A) Dose-response analysis of Huh7.5 candidate antivirals in Calu-3 cells with a SI > 3. Data represent the average of ≥4 independent experiments ± SD.

(B) qRT-PCR analysis of nine candidate antivirals in Calu-3 cells. Data represent the mean ± SEM for ≥2 independent experiments.

(Figure S3). These data suggest that the entry pathway used by SARS-CoV-2 shows cell-type specificity.

Nine candidates are antiviral against SARS-CoV-2 in lung epithelial cells

To determine which of the 23 antiviral candidates validated in Huh7.5 cells also had antiviral activity in Calu-3 cells, we performed dose-response studies. We found that nine drugs were active against SARS-CoV-2 in Calu-3 cells with a SI >3 (Figure 4A). In addition, we used a qRT-PCR assay to verify that treatment with these inhibitors blocked viral replication in Calu-3 cells. We found concordance with our microscopy-based assay, where each of these nine drugs attenuated infection as measured by qRT-PCR (Figure 4B).

These nine drugs include the following: two drugs with unclear targets (salinomycin, Y-320), kinase inhibitors (AZD8055, bemcentinib, dacomitinib, WYE-125132), the histamine receptor inhibitor (ebastine), an iron chelator Dp44mT, and the cyclophilin inhibitor cyclosporine. Because many kinase inhibitors were quite potent, this suggests an important role in intracellular signaling for infection. The other drugs tested in Calu-3 with a SI <3 are shown in Figure S4. The full table of candidates from the Huh7.5 screen with IC_{50}, CC50, and SI are shown in Figure S4.

Cyclosporine is antiviral likely through interactions with cyclophilins

Cyclosporine is an FDA-approved generic drug that is readily available and showed a micromolar IC_{50} with high selectivity in

both Huh7.5 and Calu-3 cells (Figures 3 and 4; Figure S4). Cyclosporine binds Cyclophilin A and prevents activation of the phosphatase calcineurin, which is required for the nuclear translocation of the nuclear factor of activated T cells (NFAT) (Friedman and Weissman, 1991; Hogan et al., 2003; Liu et al., 1991). Inhibition of this pathway in T cells is used as an immunosuppressant (Matsuda and Koyasu, 2000). Cyclosporins have been shown to have antiviral activity against a wide variety of viruses, including other coronaviruses (Bose et al., 2003; Carbajo-Lozoya et al., 2014; Castro et al., 2003; de Wilde et al., 2013; Liu et al., 2012a, 2012b; Luban, 2007; Ma-Lauer et al., 2020; Tian et al., 2010; Towers et al., 2003; von Brunn et al., 2015; von Hahn et al., 2012; Xu et al., 2010). The activity of cyclosporine against previously studied coronaviruses is Cyclophilin dependent and independent of calcineurin (Carbajo-Lozoya et al., 2014; von Brunn et al., 2015).

We set out to perform initial structure-activity relationships (SARs) and to determine if this activity was through inhibition of Cyclophilin or of calcineurin. For these studies, we obtained a panel of cyclosporine analogs, including cyclosporin A, cyclosporin B, cyclosporin C, cyclosporin H, and isocyclosoporin A (Jeffery, 1991). We found that isocyclosporin A, cyclosporin A, cyclosporin B, and cyclosporin C were active with increasing IC_{50}s (Figures 5A–5D). None of these cyclosporin-related compounds are antiviral in Vero cells as measured by microscopy (Figure S5). PSC 833 is a non-immunosuppressant derivative of cyclosporine that does not inhibit calcineurin and has similar activity to cyclosporin C (Keller et al., 1992). TMN355 is a cyclophilin A inhibitor that is 27 times more potent than cyclosporin A in inhibiting
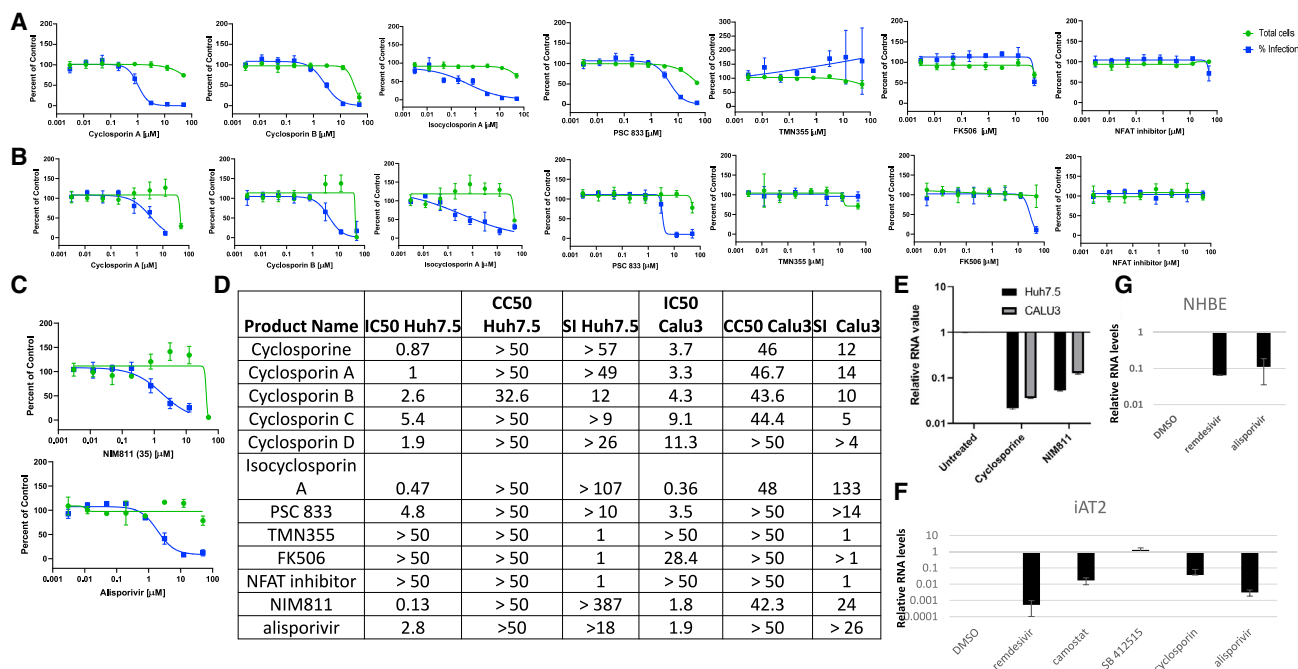
Figure 5. Cyclosporine is antiviral against SARS-CoV-2 independent of calcineurin

(A) Dose-response analysis of Huh7.5 cells treated with a panel of cyclosporins and related drugs. Data represent the average of ≥ 2 independent experiments ± SD.

(B) Dose-response analysis of Calu-3 cells treated with a panel of cyclosporins and related drugs. Data represent the average of five independent experiments ± SD.

(C) Dose-response analysis of Calu3 cells treated with cyclophilin-selective drugs NIM811 and alisporivir. Data represent the average of five independent experiments ± SD.

(D) Table of IC$_{50}$s, CC$_{50}$s, and SI of Huh7.5 cells and Calu-3 cells treated with the indicated drugs.

(E) qRT-PCR analysis of viral replication in Huh7.5 and Calu-3 cells treated with the indicated drugs.

(F) qRT-PCR analysis of viral replication in iAT2 cells treated with the indicated drugs.

(G) qRT-PCR of NHBE cells treated with the indicated drug.

For (E)–(G), data represent the mean ± SEM for ≥ 2 independent experiments.

the prolyl isomerase activity of Cyclophilin A (Yu et al., 2009). We found TMN355 to lack antiviral activity, suggesting that the enzymatic activity of Cyclophilin A is dispensable (Figures 5A–5D).

To further assess the mechanism by which cyclosporine is antiviral, we tested FK506, an inhibitor of calcineurin. FK506 binds the related immunophilin FKBP, rather than cyclophilin A, to block the phosphatase activity of calcineurin, and thus is also a potent immunosuppressant (Thomson et al., 1995). We found that FK506 has no activity against SARS-CoV-2 (Figures 5A–5D). Moreover, because one of the major targets of calcineurin is the activation of NFAT, we also tested whether an NFAT inhibitor impacted viral infection (Noguchi et al., 2004). We found that the NFAT inhibitor had no effect on infection (Figures 5A–5D). Altogether, we found that cyclosporins are potent antivirals against SARS-CoV-2 in lung epithelial Calu-3 cells, and that this activity is independent of calcineurin and NFAT.

There is also a class of cyclosporin analogs that block the activity of cyclophilins independent of calcineurin. We tested two, NIM811 and alisporivir. We found that both drugs were active in both Calu-3 and Huh7.5 cells (Figures 5C and 5D). Moreover, treatment of Calu-3 or Huh7.5 cells with cyclosporine or NIM811 leads to a reduction in viral replication as measured by qRT-PCR

(Figure 5E), further suggesting that the antiviral activity is Cyclophilin dependent and separable from calcineurin. Strikingly, the activity of this panel of drugs is similar in Calu-3 and Huh7.5 cells, further supporting that these antivirals are acting through the same target, Cyclophilin A, and through the same mechanism of action.

To further explore the relevance of cyclophilin inhibition as a target for antivirals, we tested two additional models of the respiratory tract. First, we explored alveolar type II epithelial (AT2) cells, which are a major target of SARS-CoV-2 infection in humans (Hou et al., 2020). We used iPSC-derived AT2 (iAT2) cells to model this infection. We differentiated the iPSC line SPC2 that expresses tdTomato from the endogenous locus of the surfactant protein-C (SFTPC), an AT2-specific cell marker, to confirm differentiation (Jacob et al., 2019). We treated cells with the indicated drugs and infected them with SARS-CoV-2 (MOI = 0.5). Three days later, we collected total RNA and performed qRT-PCR to monitor viral infection. We found that the SARS-CoV-2 polymerase inhibitor remdesivir was active and reduced infection by more than 1,000-fold (Figure 5F). In addition, as we observed in Calu-3 cells, treatment with the TMPRSS2 inhibitor camostat inhibited infection, while the

cathepsin inhibitor SB 412515 had no activity (Figure 5F). Moreover, cyclosporine and alisporivir were active in inhibiting SARS-CoV-2 infection in these respiratory cells (Figure 5F). Second, we tested primary normal human bronchial epithelial cells obtained from Lonza. We treated these cells with remdesivir or alisporivir and observed a significant reduction in viral replication as measured by qRT-PCR (Figure 5G). Altogether, these data suggest that cyclophilins are required for SARS-CoV-2 infection and thus present a target for antivirals.

DISCUSSION

The emergence of SARS-CoV-2 has led to devastating worldwide morbidity and mortality, creating an immediate need for new therapeutics and vaccines. Repurposing existing drugs can allow for rapid deployment of therapeutics that have already been tested in humans (Pandey et al., 2020). Remdesivir was developed against the Ebola virus RNA-dependent RNA polymerase and was also found to have robust activity against SARS-CoV-2 (Wang et al., 2020). Importantly, we found that remdesivir is active against SARS-CoV-2 across cell types. Chloroquine and hydroxychloroquine have been used for decades to treat malaria and have been shown to have *in vitro* antiviral activity against SARS-CoV-2 (Liu et al., 2020; Wang et al., 2020). However, we find that this antiviral activity is cell type specific. Lung epithelial cells are resistant to these drugs, and this may explain the lack of efficacy seen in many trials (Boulware et al., 2020).

To determine if there are additional drugs that are active against SARS-CoV-2 *in vitro*, we screened a repurposing library that includes ~1,000 FDA-approved drugs and ~1,000 additional drugs that have been tested in humans. Repurposing can be used to reveal new and similar pathways and targets, but also the time and monetary investment associated with repurposing is potentially less because these drugs often bypass phase 1 trials (Oprea et al., 2011; Pushpakom et al., 2019). Initial screens in Vero cells yielded few active drugs, leading us to pursue a screen in human Huh7.5 cells, a transformed hepatocyte line deficient in innate immune signaling. Using this model system, we identified 33 drugs and validated 23 with dose-response assays that showed antiviral activity and low toxicity. This includes many drugs that were previously shown to have activity against other coronaviruses (tetrandrine, cepharanthine, cyclosporine, aloxistatin, MG132, salinomycin) and SARS-CoV-2 (salinomycin, tetrandrine, cepharanthine, cyclosporine, ebastine) (Carbajo-Lozoya et al., 2014; de Wilde et al., 2011; Dyall et al., 2014; Fan et al., 2020; Jeon et al., 2020; Kim et al., 1995, 2019; Ma-Lauer et al., 2020; Peel and Scribner, 2013; Schneider et al., 2012; Yu and Lai, 2005).

The 23 drugs fall into distinct classes, and most have known targets. However, two drugs that were active across cell types, salinomycin and Y-320, do not have clear targets. Salinomycin is a polyether antibiotic and chemotherapy drug, which has been shown to be antiviral against many viruses, including coronaviruses (Dyall et al., 2014; Ianevski et al., 2020; Jeon et al., 2020; Mitani et al., 1975). Salinomycin was also identified in a Vero cell screen (Jeon et al., 2020). Mechanistically, some studies have suggested that salinomycin is an ionophore that

can attenuate viral entry by disrupting the acidification of the endosome (Jang et al., 2018). Other studies have implicated salinomycin in endoplasmic reticulum (ER) stress (Zhang et al., 2019). Studies in mice have shown antiviral activity against influenza (Jang et al., 2018). Salinomycin has also been characterized as an activator of autophagy, which may influence SARS-CoV-2 infection (Jiang et al., 2018; Verdoodt et al., 2012; Jangamreddy et al., 2013). Y-320 is a phenylpyrazoleanilide immunomodulatory agent that has been shown to inhibit IL-17 production by T cells and has activity in monkeys (Ushio et al., 2013). Interestingly, treatment with Y-320 is associated with decreased IL-6 production, a cytokine that is thought to be highly expressed in SARS-CoV-2 infection (Gubernatorova et al., 2020; Hong et al., 2020; Ushio et al., 2013). However, it is unclear how Y-320 could attenuate SARS-CoV-2 in non-immune cells.

Ebastine is a potent H1-histamine receptor antagonist used for allergic disorders outside of the United States, particularly in Asia (Van Cauwenberge et al., 2004). We found that ebastine is antiviral in all three cell types, although it is 10-fold less active in Vero cells (Jeon et al., 2020). Ebastine is orally available with few side effects, and there are clinical trials underway in China testing whether ebastine can impact COVID-19 outcomes (Lythgoe and Middleton, 2020). Because other H1-histamine receptor antagonists were not active, it is unclear why this particular agent is more effective at inhibiting SARS-CoV-2 infection. Interestingly, ebastine and its active metabolite, carebastine, are reported to inhibit expression of IL-6, whereas many other H1-histamine receptor antagonists do not (Nori et al., 2003; Okamoto et al., 2009).

We also identified six protease inhibitors as antiviral in Huh7.5 cells. Two cysteine protease inhibitors, Z-FA-FMK and MG-132, had activity in both Vero and Huh7.5 cells. None of the protease inhibitors were active in Calu-3 cells. This observation suggests that they are not targeting the viral proteases. Consistent with this, Z-FA-FMK is an inhibitor of cathepsins, which are required for SARS-CoV-2 entry in cells where endosomal proteases are required for Spike cleavage, and thus we observe no requirement in Calu-3 cells where TMPRSS2 is required for infection (Glowacka et al., 2011; Hoffmann et al., 2020; Matsuyama et al., 2020; Millet and Whittaker, 2015; Shang et al., 2020). This has important implications in diverse SARS-CoV-2 studies, where there may be cell-type-specific requirements for different steps in the replication cycle.

We also identified two inhibitors against the cellular histone methyltransferase G9a as antiviral in Huh7.5 cells. However, these drugs were not active in Calu-3 cells, suggesting that there are cell-type-specific requirements. AM1241 is a selective cannabinoid CB2 receptor agonist that we found was antiviral in Huh7.5 cells. GW842166X, another CB2 agonist that has a 10-fold higher IC_{50}, was not active. Moreover, dose-response studies found that AM1241 is not active in either Vero or Calu-3 cells.

Cepharanthine and tetrandrine are both bis-benzylisoquinoline alkaloids produced as natural products from herbal plants (Weber and Opatz, 2019). Tetrandrine, a traditional Chinese medicine and calcium channel blocker, has been shown to antagonize calmodulin. It has anti-tumor and anti-inflammatory effects and can effectively inhibit fibroblasts, thereby inhibiting pulmonary fibrosis (Huang et al., 2019; Qian and Huang, 1989). Multiple studies have suggested that tetrandrine has antiviral

activity, including against dengue virus and herpes simplex 1 virus (Hu et al., 1997; Liou et al., 2008). Tetrandrine has also been shown to inhibit entry of Ebola virus into host cells *in vitro* and showed therapeutic efficacy against Ebola in preliminary studies on mice (Sakurai et al., 2015). Currently, there is an ongoing clinical trial using tetrandrine in COVID-19 patients to improve pulmonary function (Lythgoe and Middleton, 2020). Cepharanthine is reported to have anti-inflammatory and immunoregulatory properties and is used to treat a variety of acute and chronic conditions outside of the United States (Bailly, 2019). Both cepharanthine and tetrandrine were previously shown to have antiviral activity against the human coronavirus OC43 and in recent studies on SARS-CoV-2 in Vero cell screens (Fan et al., 2020; Jeon et al., 2020; Kim et al., 2019). Although both of these molecules were antiviral in our Huh7.5 screen, neither were active in Calu-3 cells. This may suggest that they are modulating endosomal entry pathways.

We identified few metabolic regulators. Dp44mT is a potent iron chelator that we found to be antiviral against SARS-CoV-2 in Huh 7.5 and Calu-3 cells (Yu et al., 2009). A clinical trial with the iron chelator deferoxamine is underway (ClinicalTrials.gov: NCT04333550). The mechanism by which iron chelators control infection remains unknown.

We screened ∼650 kinase inhibitors and identified several that are antiviral against SARS-CoV-2. FRAX486 is a p21-activated kinase (PAK) inhibitor that is antiviral in Huh7.5 cells but only modestly impacted infection of Calu-3 cells (Dolan et al., 2013). Other PAK inhibitors were not identified in our screens. PAK is required for entry by many viruses (Van den Broeke et al., 2010). PD0166285 is a potent Wee1 and Chk1 inhibitor that is antiviral in Huh7.5 cells but shows strong toxicity in Calu-3 cells (Wang et al., 2001).

We also found three mammalian target of rapamycin (mTOR) inhibitors, AZD8055, PF-04691502, and WYE-125132, are antiviral against SARS-CoV-2 in Huh-7 and Calu-3 cells. These are highly potent ATP competitive mTOR inhibitors that target both TORC1 and TORC2. In our library, none of the rapamycin analogs that selectively inhibit mTORC1 were active. We also identified two potent selective and irreversible inhibitors of epidermal growth factor receptor (EGFR), dacomitinib and naquotinib. Importantly, dacomitinib is a potent antiviral in Calu-3 cells. For many viruses, EGFR activation promotes viral entry, which may also be the case for SARS-CoV-2 (Diao et al., 2012; Eierhoff et al., 2010; Hu et al., 2018; Iwamoto et al., 2019; Zheng et al., 2014).

Bemcentinib is a first-in-class Axl inhibitor that we found inhibits SARS-CoV-2 infection of Huh7.5 cells and Calu-3 cells (Holland et al., 2010). Axl can be used as an attachment factor for the entry for many viruses, including Ebola and Zika viruses (Meertens et al., 2017; Shimojima et al., 2007). Although not published, news releases suggest that bemcentinib has demonstrated promise in preclinical data against early infection with the SARS-CoV-2. A fast-tracked clinical trial is underway in the United Kingdom (Mckee, 2020).

Cyclosporine is a commonly used immunosuppressant that binds Cyclophilin A and inhibits the calcium-dependent phosphatase calcineurin, which is required for the nuclear translocation of the NFAT (Friedman and Weissman, 1991; Hogan et al., 2003; Liu et al., 1991, 2012b; Matsuda and Koyasu, 2000). Inhibition of this pathway in T cells is used as an immunosuppressant. We found that cyclosporine and some derivatives are active in both Huh7.5 and Calu-3 cells but have no activity in Vero cells. A recent screen in Vero cells did find activity with cyclosporine against SARS-CoV-2 (Jeon et al., 2020). Cyclophilin A is a ubiquitously expressed peptidyl-prolyl *cis-trans* isomerase (Galat, 1993). Cyclophilin A and other Cyclophilins have chaperone-like activity and take part in protein-folding processes (Göthel and Marahiel, 1999). Cyclophilin A has been shown to be an important cellular factor that facilitated many diverse viral infections. This includes human immunodeficiency virus type 1 (HIV-1), influenza virus, HCV, hepatitis B virus (HBV), vesicular stomatitis virus (VSV), vaccinia virus (VV), SARS-CoV, and rotavirus (RV) (Bose et al., 2003; Castro et al., 2003; Liu et al., 2012a, 2012b; Luban, 2007; Tian et al., 2010; Towers et al., 2003; Xu et al., 2010; Yang et al., 2008; Zhou et al., 2012). The coronaviruses HCoV-229E, HCoV-NL63, feline infectious peritonitis coronavirus (FPIV), mouse hepatitis virus (MHV), avian infectious bronchitis, and SARS-CoV have been found to be attenuated by cyclosporin A (de Wilde et al., 2011; Ma-Lauer et al., 2020; Tanaka et al., 2017). Cyclosporine and its non-immunosuppressive derivatives can inhibit replication of a number of viruses, including some coronaviruses. In most cases, the responsible cyclophilin is CypA (Peel and Scribner, 2013; Zhou et al., 2012), but CypA and CypB were found to be required for feline coronavirus (FCoV) replication (Tanaka et al., 2017). For human coronaviruses HCoV-NL63 and HCoV-229E, cyclophilin A is required for infection in CaCo-2 cells (Carbajo-Lozoya et al., 2014) and Huh-7.5 cells, respectively (von Brunn et al., 2015; von Hahn et al., 2012). It is generally thought that the activity of cyclosporine against coronaviruses is Cyclophilin dependent and independent of calcineurin.

We found that a number of cyclosporins were antiviral with similar potencies, including cyclosporine, cyclosporin A, cyclosporin B, and the metabolic breakdown product of cyclosporin A, isoscyclosporin A. To further address the role of calcineurin, we tested a non-immunosuppressant derivative of cyclosporine that does not inhibit calcineurin and has a similar activity to cyclosporin C. FK506, a calcineurin inhibitor independent of cyclophilin A, has no activity. Moreover, an NFAT inhibitor is not active. Altogether, these data suggest that cyclosporins are potent antivirals against SARS-CoV-2 in lung epithelial Calu-3 cells. Importantly, we found that the cyclophilin inhibitors that do not target calcineurin NIM811 and alisporivir have antiviral activity in both Calu-3 and Huh7.5 cells. However, the enzymatic activity of Cyclophilin A is likely dispensable because TMN355 was inactive. Strikingly, the activities of all of these drugs are similar in the two cell lines, suggesting that the activity is cell-type independent, inhibiting the same target using the same mechanism of action. To further support a dependence on cyclophilin in the human respiratory tract, we tested two additional models: iPSC-derived AT2 cells and primary human bronchial epithelial cells. We found that inhibition of cyclophilin was protective in both models. Altogether, our data suggest that alisporivir and cyclosporine would block SARS-CoV-2 in diverse infected tissues *in vivo*.

To move forward clinically, there are two approaches. First, one could consider Cyclophilin inhibitors that do not have

immunosuppressive activity, such as alisporivir, or others that have been used in clinical trials (Naoumov, 2014; Pawlotsky, 2020; Peel and Scribner, 2013). This would potentially block viral infection in the absence of immune inhibition. Another possibility is to use Cyclophilin inhibitors that also target calcineurin (e.g., cyclosporine). This is of interest because one of the major complications of COVID-19 is the hyper-inflammatory response and cytokine storm associated with increased immune activation. To prevent hyper-activation, there has been interest in treating COVID-19 patients with immunosuppressants (Moore and June, 2020). There are ongoing trials for a variety of agents, including anti-IL-6 and JAK inhibitors, and two clinical trials using sirolimus, the FDA-approved mTOR inhibitor, which selectively inhibits mTORC1. We find no antiviral activity of sirolimus or other rapamycin derivatives. In contrast, cyclosporin A is an approved immunosuppressant that we found is also antiviral at concentrations at least close to those achieved in vivo (Faulds et al., 1993). Therefore, it may be useful to implement clinical trials using cyclosporin A as an immunosuppressant because it would potentially ameliorate symptoms by two mechanisms (Willicombe et al., 2020). Excitingly, prospective trials using cyclosporin A have recently begun (ClinicalTrials.gov: NCT04412785). A retrospective observational study recently found that in hospitalized patients, among the prescribed therapies, including lopinavir/ritonavir and hydroxychloroquine, only cyclosporine was significantly associated with a decrease in mortality (Guisado-Vasco et al., 2020). Perhaps the combined activity of immunosuppression and antiviral activity led to these promising results.

There have been a large number of screens posted in the literature that suggest antiviral activity of several existing drugs (e.g., azithromycin, favipiravir, lopinavir, ribavirin, ritonavir, tetracycline, etc.). These drugs and most antiviral screens with SARS-CoV-2 have been performed in Vero cells, with toxicity as readouts. Medicines for Malaria Venture (MMV) has compiled a list of drugs with reported antiviral activity against SARS-CoV-2 (https://www.mmv.org/mmv-open/covid-box). We tested >60 of the 80 compounds and find that in addition to the quinolines and drugs found in our screen, there are few additional compounds that show activity at less than 2 μM and SI >3. Although it is possible that some of these drugs are false negatives in our screens, it is likely that many of these candidates do not have antiviral activity when either measuring viral antigen production or when looking in different cell types. It is very important that newly identified candidate antivirals be tested for their impact on viral replication more directly. Moreover, given the striking differences in sensitivities across cell types, it is important to validate the activity of any new antivirals in respiratory epithelial cells.

Altogether, these studies highlight the roles of cellular genes in viral infection and cell-type differences, and our discovery of nine broadly active antivirals suggests new avenues for therapeutic interventions. We found nine drugs with antiviral activity in lung epithelial cells. Seven of these drugs have been used in humans, three of these are FDA approved in the United States (cyclosporine, dacomitinib, and salinomycin), and ebastine is approved outside of the United States. Although clinical trials are underway with some of these candidates, additional trials will be needed to determine the efficacy of these antivirals in COVID-19 patients, to inform future treatment strategies.

Limitations of study
We have identified a number of drugs that are active against SARS-CoV-2 in cell culture models. Future studies will be needed to determine if these drugs are also active in vivo during SARS-CoV-2 infection.

STAR★METHODS

Detailed methods are provided in the online version of this paper and include the following:

- KEY RESOURCES TABLE
- RESOURCE AVAILABILITY
  - Lead contact
  - Materials availability
  - Data and code availability
- EXPERIMENTAL MODEL AND SUBJECT DETAILS
- METHOD DETAILS
  - Infections
  - RT-qPCR
- QUANTIFICATION AND STATISTICAL ANALYSIS

AUTHOR CONTRIBUTIONS

D.C.S. and S.C. conceived and oversaw the study. H.R., D.C.S., and S.C. wrote the manuscript. M.D. J.S.L., K.W., E.S., M.L., B.K., L.C., and K.A. performed experiments and analyzed data. F.L.C., E.E.M., R.T., W.Y., K.J., K.S., and H.R. contributed critical expertise, cells, and/or reagents.

DECLARATION OF INTERESTS

The authors declare no competing interests.

REFERENCES

Ashburn, T.T., and Thor, K.B. (2004). Drug repositioning: identifying and developing new uses for existing drugs. Nat. Rev. Drug Discov. *3*, 673–683.

Bailly, C. (2019). Cepharanthine: An update of its mode of action, pharmacological properties and medical applications. Phytomedicine *62*, 152956.

Beigel, J.H., Tomashek, K.M., Dodd, L.E., Mehta, A.K., Zingman, B.S., Kalil, A.C., Hohmann, E., Chu, H.Y., Luetkemeyer, A., Kline, S., et al. (2020). Remdesivir for the Treatment of Covid-19 - Preliminary Report. Reply. N. Engl. J. Med. *383*, 994.

Belouzard, S., Chu, V.C., and Whittaker, G.R. (2009). Activation of the SARS coronavirus spike protein via sequential proteolytic cleavage at two distinct sites. Proc. Natl. Acad. Sci. USA *106*, 5871–5876.

Blanco-Melo, D., Nilsson-Payant, B.E., Liu, W.-C., Uhl, S., Hoagland, D., Møller, R., Jordan, T.X., Oishi, K., Panis, M., Sachs, D., et al. (2020). Imbalanced Host Response to SARS-CoV-2 Drives Development of COVID-19. Cell *181*, 1036–1045.e9.

Blight, K.J., McKeating, J.A., and Rice, C.M. (2002). Highly permissive cell lines for subgenomic and genomic hepatitis C virus RNA replication. J. Virol. *76*, 13001–13014.

Bonin, M., Oberstrass, J., Lukacs, N., Ewert, K., Oesterschulze, E., Kassing, R., and Nellen, W. (2000). Determination of preferential binding sites for anti-dsRNA antibodies on double-stranded RNA by scanning force microscopy. RNA *6*, 563–570.

Bose, S., Mathur, M., Bates, P., Joshi, N., and Banerjee, A.K. (2003). Requirement for cyclophilin A for the replication of vesicular stomatitis virus New Jersey serotype. J. Gen. Virol. *84*, 1687–1699.

Boulware, D.R., Pullen, M.F., Bangdiwala, A.S., Pastick, K.A., Lofgren, S.M., Okafor, E.C., Skipper, C.P., Nascene, A.A., Nicol, M.R., Abassi, M., et al. (2020). A Randomized Trial of Hydroxychloroquine as Postexposure Prophylaxis for Covid-19. N. Engl. J. Med. *383*, 517–525.

Carbajo-Lozoya, J., Ma-Lauer, Y., Malešević, M., Theuerkorn, M., Kahlert, V., Prell, E., von Brunn, B., Muth, D., Baumert, T.F., Drosten, C., et al. (2014). Human coronavirus NL63 replication is cyclophilin A-dependent and inhibited by non-immunosuppressive cyclosporine A-derivatives including Alisporivir. Virus Res. *184*, 44–53.

Castro, A.P., Carvalho, T.M., Moussatché, N., and Damaso, C.R. (2003). Redistribution of cyclophilin A to viral factories during vaccinia virus infection and its incorporation into mature particles. J. Virol. *77*, 9052–9068.

Cui, J., Li, F., and Shi, Z.L. (2019). Origin and evolution of pathogenic coronaviruses. Nat. Rev. Microbiol. *17*, 181–192.

de Duve, C., de Barsy, T., Poole, B., Trouet, A., Tulkens, P., and Van Hoof, F. (1974). Commentary. Lysosomotropic agents. Biochem. Pharmacol. *23*, 2495–2531.

de Wilde, A.H., Zevenhoven-Dobbe, J.C., van der Meer, Y., Thiel, V., Narayanan, K., Makino, S., Snijder, E.J., and van Hemert, M.J. (2011). Cyclosporin A inhibits the replication of diverse coronaviruses. J. Gen. Virol. *92*, 2542–2548.

de Wilde, A.H., Raj, V.S., Oudshoorn, D., Bestebroer, T.M., van Nieuwkoop, S., Limpens, R.W.A.L., Posthuma, C.C., van der Meer, Y., Bárcena, M., Haagmans, B.L., et al. (2013). MERS-coronavirus replication induces severe in vitro cytopathology and is strongly inhibited by cyclosporin A or interferon-α treatment. J. Gen. Virol. *94*, 1749–1760.

de Wit, E., van Doremalen, N., Falzarano, D., and Munster, V.J. (2016). SARS and MERS: recent insights into emerging coronaviruses. Nat. Rev. Microbiol. *14*, 523–534.

Diao, J., Pantua, H., Ngu, H., Komuves, L., Diehl, L., Schaefer, G., and Kapadia, S.B. (2012). Hepatitis C virus induces epidermal growth factor receptor activation via CD81 binding for viral internalization and entry. J. Virol. *86*, 10935–10949.

Dolan, B.M., Duron, S.G., Campbell, D.A., Vollrath, B., Shankaranarayana Rao, B.S., Ko, H.Y., Lin, G.G., Govindarajan, A., Choi, S.Y., and Tonegawa, S. (2013). Rescue of fragile X syndrome phenotypes in Fmr1 KO mice by the small-molecule PAK inhibitor FRAX486. Proc. Natl. Acad. Sci. USA *110*, 5671–5676.

Ducharme, J., and Farinotti, R. (1996). Clinical pharmacokinetics and metabolism of chloroquine. Focus on recent advancements. Clin. Pharmacokinet. *31*, 257–274.

Dyall, J., Coleman, C.M., Hart, B.J., Venkataraman, T., Holbrook, M.R., Kindrachuk, J., Johnson, R.F., Olinger, G.G., Jr., Jahrling, P.B., Laidlaw, M., et al. (2014). Repurposing of clinically developed drugs for treatment of Middle East respiratory syndrome coronavirus infection. Antimicrob. Agents Chemother. *58*, 4885–4893.

Eierhoff, T., Hrincius, E.R., Rescher, U., Ludwig, S., and Ehrhardt, C. (2010). The epidermal growth factor receptor (EGFR) promotes uptake of influenza A viruses (IAV) into host cells. PLoS Pathog. *6*, e1001099.

Fan, H.H., Wang, L.Q., Liu, W.L., An, X.P., Liu, Z.D., He, X.Q., Song, L.H., and Tong, Y.G. (2020). Repurposing of clinically approved drugs for treatment of coronavirus disease 2019 in a 2019-novel coronavirus-related coronavirus model. Chin. Med. J. (Engl.) *133*, 1051–1056.

Faulds, D., Goa, K.L., and Benfield, P. (1993). Cyclosporin. A review of its pharmacodynamic and pharmacokinetic properties, and therapeutic use in immunoregulatory disorders. Drugs *45*, 953–1040.

Friedman, J., and Weissman, I. (1991). Two cytoplasmic candidates for immunophilin action are revealed by affinity for a new cyclophilin: one in the presence and one in the absence of CsA. Cell *66*, 799–806.

Galat, A. (1993). Peptidylproline cis-trans-isomerases: immunophilins. Eur. J. Biochem. *216*, 689–707.

Glowacka, I., Bertram, S., Müller, M.A., Allen, P., Soilleux, E., Pfefferle, S., Steffen, I., Tsegaye, T.S., He, Y., Gnirss, K., et al. (2011). Evidence that TMPRSS2 activates the severe acute respiratory syndrome coronavirus spike protein for membrane fusion and reduces viral control by the humoral immune response. J. Virol. *85*, 4122–4134.

Göthel, S.F., and Marahiel, M.A. (1999). Peptidyl-prolyl cis-trans isomerases, a superfamily of ubiquitous folding catalysts. Cell. Mol. Life Sci. *55*, 423–436.

Gubernatorova, E.O., Gorshkova, E.A., Polinova, A.I., and Drutskaya, M.S. (2020). IL-6: Relevance for immunopathology of SARS-CoV-2. Cytokine Growth Factor Rev. *53*, 13–24.

Guisado-Vasco, P., Valderas-Ortega, S., Carralón-González, M.M., Roda-Santacruz, A., González-Cortijo, L., Sotres-Fernández, G., Martí-Ballesteros, E.M., Luque-Pinilla, J.M., Almagro-Casado, E., La Coma-Lanuza, F.J., et al. (2020). Clinical characteristics and outcomes among hospitalized adults with severe COVID-19 admitted to a tertiary medical center and receiving antiviral, antimalarials, glucocorticoids, or immunomodulation with tocilizumab or cyclosporine: A retrospective observational study (COQUIMA cohort). EClinicalMedicine *28*, 100591.

Harcourt, J., Tamin, A., Lu, X., Kamili, S., Sakthivel, S.K., Murray, J., Queen, K., Tao, Y., Paden, C.R., Zhang, J., et al. (2020). Severe Acute Respiratory Syndrome Coronavirus 2 from Patient with Coronavirus Disease, United States. Emerg. Infect. Dis. *26*, 1266–1273.

Hoffmann, M., Kleine-Weber, H., Schroeder, S., Krüger, N., Herrler, T., Erichsen, S., Schiergens, T.S., Herrler, G., Wu, N.H., Nitsche, A., et al. (2020). SARS-CoV-2 Cell Entry Depends on ACE2 and TMPRSS2 and Is Blocked by a Clinically Proven Protease Inhibitor. Cell *181*, 271–280.e8.

Hofmann, H., and Pöhlmann, S. (2004). Cellular entry of the SARS coronavirus. Trends Microbiol. *12*, 466–472.

Hogan, P.G., Chen, L., Nardone, J., and Rao, A. (2003). Transcriptional regulation by calcium, calcineurin, and NFAT. Genes Dev. *17*, 2205–2232.

Holland, S.J., Pan, A., Franci, C., Hu, Y., Chang, B., Li, W., Duan, M., Torneros, A., Yu, J., Heckrodt, T.J., et al. (2010). R428, a selective small molecule inhibitor of Axl kinase, blocks tumor spread and prolongs survival in models of metastatic breast cancer. Cancer Res. *70*, 1544–1554.

Hong, J., Jing, S., Zhang, Y., Chen, R., Owusu-Ansah, K.G., Chen, B., Xie, H., Zhou, L., Zheng, S., and Jiang, D. (2020). Y-320, a novel immune-modulator, sensitizes multidrug-resistant tumors to chemotherapy. Am. J. Transl. Res. *12*, 551–562.

Hou, Y.J., Okuda, K., Edwards, C.E., Martinez, D.R., Asakura, T., Dinnon, K.H., 3rd, Kato, T., Lee, R.E., Yount, B.L., Mascenik, T.M., et al. (2020). SARS-CoV-2 Reverse Genetics Reveals a Variable Infection Gradient in the Respiratory Tract. Cell 182, 429–446.e14.

Hu, S., Dutt, J., Zhao, T., and Foster, C.S. (1997). Tetrandrine potently inhibits herpes simplex virus type-1-induced keratitis in BALB/c mice. Ocul. Immunol. Inflamm. 5, 173–180.

Hu, W., Zhang, S., Shen, Y., and Yang, Q. (2018). Epidermal growth factor receptor is a co-factor for transmissible gastroenteritis virus entry. Virology 521, 33–43.

Huang, T., Xu, S., Deo, R., Ma, A., Li, H., Ma, K., and Gan, X. (2019). Targeting the Ca²⁺/Calmodulin-dependent protein kinase II by Tetrandrine in human liver cancer cells. Biochem. Biophys. Res. Commun. 508, 1227–1232.

Ianevski, A., Yao, R., Fenstad, M.H., Biza, S., Zusinaite, E., Reisberg, T., Lysvand, H., Løseth, K., Landsem, V.M., Malmring, J.F., et al. (2020). Potential antiviral options against SARS-CoV-2 infection. Viruses 12, 642.

Iwamoto, M., Saso, W., Sugiyama, R., Ishii, K., Ohki, M., Nagamori, S., Suzuki, R., Aizaki, H., Ryo, A., Yun, J.H., et al. (2019). Epidermal growth factor receptor is a host-entry cofactor triggering hepatitis B virus internalization. Proc. Natl. Acad. Sci. USA 116, 8487–8492.

Jacob, A., Vedaie, M., Roberts, D.A., Thomas, D.C., Villacorta-Martin, C., Alysandratos, K.D., Hawkins, F., and Kotton, D.N. (2019). Derivation of self-renewing lung alveolar epithelial type II cells from human pluripotent stem cells. Nat. Protoc. 14, 3303–3332.

Jang, Y., Shin, J.S., Yoon, Y.S., Go, Y.Y., Lee, H.W., Kwon, O.S., Park, S., Park, M.S., and Kim, M. (2018). Salinomycin Inhibits Influenza Virus Infection by Disrupting Endosomal Acidification and Viral Matrix Protein 2 Function. J. Virol. 92, e01441-18.

Jangamreddy, J.R., Ghavami, S., Grabarek, J., Kratz, G., Wiechec, E., Fredriksson, B.A., Rao Pariti, R.K., Cieślar-Pobuda, A., Panigrahi, S., and Łos, M.J. (2013). Salinomycin induces activation of autophagy, mitophagy and affects mitochondrial polarity: differences between primary and cancer cells. Biochim. Biophys. Acta 1833, 2057–2069.

Jeffery, J.R. (1991). Cyclosporine analogues. Clin. Biochem. 24, 15–21.

Jeon, S., Ko, M., Lee, J., Choi, I., Byun, S.Y., Park, S., Shum, D., and Kim, S. (2020). Identification of antiviral drug candidates against SARS-CoV-2 from FDA-approved drugs. Antimicrob. Agents Chemother. 64, e00819-20.

Jiang, J., Li, H., Qaed, E., Zhang, J., Song, Y., Wu, R., Bu, X., Wang, Q., and Tang, Z. (2018). Salinomycin, as an autophagy modulator—a new avenue to anticancer: a review. J. Exp. Clin. Cancer Res. 37, 26.

Keller, R.P., Altermatt, H.J., Nooter, K., Poschmann, G., Laissue, J.A., Bollinger, P., and Hiestand, P.C. (1992). SDZ PSC 833, a non-immunosuppressive cyclosporine: its potency in overcoming P-glycoprotein-mediated multidrug resistance of murine leukemia. Int. J. Cancer 50, 593–597.

Kim, J.C., Spence, R.A., Currier, P.F., Lu, X., and Denison, M.R. (1995). Coronavirus protein processing and RNA synthesis is inhibited by the cysteine proteinase inhibitor E64d. Virology 208, 1–8.

Kim, D.E., Min, J.S., Jang, M.S., Lee, J.Y., Shin, Y.S., Song, J.H., Kim, H.R., Kim, S., Jin, Y.H., and Kwon, S. (2019). Natural Bis-Benzylisoquinoline Alkaloids-Tetrandrine, Fangchinoline, and Cepharanthine, Inhibit Human Coronavirus OC43 Infection of MRC-5 Human Lung Cells. Biomolecules 9, 696.

Larionov, A., Krause, A., and Miller, W. (2005). A standard curve based method for relative real time PCR data processing. BMC Bioinformatics 6, 62.

Letko, M., Marzi, A., and Munster, V. (2020). Functional assessment of cell entry and receptor usage for SARS-CoV-2 and other lineage B betacoronaviruses. Nat. Microbiol. 5, 562–569.

Liou, J.T., Chen, Z.Y., Ho, L.J., Yang, S.P., Chang, D.M., Liang, C.C., and Lai, J.H. (2008). Differential effects of triptolide and tetrandrine on activation of COX-2, NF-kappaB, and AP-1 and virus production in dengue virus-infected human lung cells. Eur. J. Pharmacol. 589, 288–298.

Liu, J., Farmer, J.D., Jr., Lane, W.S., Friedman, J., Weissman, I., and Schreiber, S.L. (1991). Calcineurin is a common target of cyclophilin-cyclosporin A and FKBP-FK506 complexes. Cell 66, 807–815.

Liu, X., Zhao, Z., Li, Z., Xu, C., Sun, L., Chen, J., and Liu, W. (2012a). Cyclosporin A inhibits the influenza virus replication through cyclophilin A-dependent and -independent pathways. PLoS ONE 7, e37277.

Liu, X., Zhao, Z., Xu, C., Sun, L., Chen, J., Zhang, L., and Liu, W. (2012b). Cyclophilin A restricts influenza A virus replication through degradation of the M1 protein. PLoS ONE 7, e31063.

Liu, J., Cao, R., Xu, M., Wang, X., Zhang, H., Hu, H., Li, Y., Hu, Z., Zhong, W., and Wang, M. (2020). Hydroxychloroquine, a less toxic derivative of chloroquine, is effective in inhibiting SARS-CoV-2 infection in vitro. Cell Discov. 6, 16.

Luban, J. (2007). Cyclophilin A, TRIM5, and resistance to human immunodeficiency virus type 1 infection. J. Virol. 81, 1054–1061.

Lythgoe, M.P., and Middleton, P. (2020). Ongoing Clinical Trials for the Management of the COVID-19 Pandemic. Trends Pharmacol. Sci. 41, 363–382.

Ma-Lauer, Y., Zheng, Y., Malešević, M., von Brunn, B., Fischer, G., and von Brunn, A. (2020). Influences of cyclosporin A and non-immunosuppressive derivatives on cellular cyclophilins and viral nucleocapsid protein during human coronavirus 229E replication. Antiviral Res. 173, 104620.

Matsuda, S., and Koyasu, S. (2000). Mechanisms of action of cyclosporine. Immunopharmacology 47, 119–125.

Matsuyama, S., Nao, N., Shirato, K., Kawase, M., Saito, S., Takayama, I., Nagata, N., Sekizuka, T., Katoh, H., Kato, F., et al. (2020). Enhanced isolation of SARS-CoV-2 by TMPRSS2-expressing cells. Proc. Natl. Acad. Sci. USA 117, 7001–7003.

Mckee, S. (2020). First patient dosed in bemcentinib COVID-19 trial. Pharmatimes Online, June 3, 2020. http://www.pharmatimes.com/news/first_patient_dosed_in_bemcentinib_covid-19_trial_1341390.

Meertens, L., Labeau, A., Dejarnac, O., Cipriani, S., Sinigaglia, L., Bonnet-Madin, L., Le Charpentier, T., Hafirassou, M.L., Zamborlini, A., Cao-Lormeau, V.M., et al. (2017). Axl Mediates ZIKA Virus Entry in Human Glial Cells and Modulates Innate Immune Responses. Cell Rep. 18, 324–333.

Millet, J.K., and Whittaker, G.R. (2015). Host cell proteases: Critical determinants of coronavirus tropism and pathogenesis. Virus Res. 202, 120–134.

Millet, J.K., and Whittaker, G.R. (2018). Physiological and molecular triggers for SARS-CoV membrane fusion and entry into host cells. Virology 517, 3–8.

Mitani, M., Yamanishi, T., and Miyazaki, Y. (1975). Salinomycin: a new monovalent cation ionophore. Biochem. Biophys. Res. Commun. 66, 1231–1236.

Moore, J.B., and June, C.H. (2020). Cytokine release syndrome in severe COVID-19. Science 368, 473–474.

Naoumov, N.V. (2014). Cyclophilin inhibition as potential therapy for liver diseases. J. Hepatol. 61, 1166–1174.

Noguchi, H., Matsushita, M., Okitsu, T., Moriwaki, A., Tomizawa, K., Kang, S., Li, S.T., Kobayashi, N., Matsumoto, S., Tanaka, K., et al. (2004). A new cell-permeable peptide allows successful allogeneic islet transplantation in mice. Nat. Med. 10, 305–309.

Nori, M., Iwata, S., Munakata, Y., Kobayashi, H., Kobayashi, S., Umezawa, Y., Hosono, O., Kawasaki, H., Dang, N.H., Tanaka, H., et al. (2003). Ebastine inhibits T cell migration, production of Th2-type cytokines and proinflammatory cytokines. Clin. Exp. Allergy 33, 1544–1554.

Okamoto, T., Iwata, S., Ohnuma, K., Dang, N.H., and Morimoto, C. (2009). Histamine H1-receptor antagonists with immunomodulating activities: potential use for modulating T helper type 1 (Th1)/Th2 cytokine imbalance and inflammatory responses in allergic diseases. Clin. Exp. Immunol. 157, 27–34.

Oprea, T.I., Bauman, J.E., Bologa, C.G., Buranda, T., Chigaev, A., Edwards, B.S., Jarvik, J.W., Gresham, H.D., Haynes, M.K., Hjelle, B., et al. (2011). Drug Repurposing from an Academic Perspective. Drug Discov. Today Ther. Strateg. 8, 61–69.

Ou, X., Liu, Y., Lei, X., Li, P., Mi, D., Ren, L., Guo, L., Guo, R., Chen, T., Hu, J., et al. (2020). Characterization of spike glycoprotein of SARS-CoV-2 on virus

entry and its immune cross-reactivity with SARS-CoV. Nat. Commun. *11*, 1620.

Pairo-Castineira, E., Clohisey, S., Klaric, L., Bretherick, A.D., Rawlik, K., Pasko, D., Walker, S., Parkinson, N., Fourman, M.H., Russell, C.D., et al. (2021). Genetic mechanisms of critical illness in Covid-19. Nature *591*, 92–98.

Pandey, A., Nikam, A.N., Shreya, A.B., Mutalik, S.P., Gopalan, D., Kulkarni, S., Padya, B.S., Fernandes, G., Mutalik, S., and Prassl, R. (2020). Potential therapeutic targets for combating SARS-CoV-2: Drug repurposing, clinical trials and recent advancements. Life Sci. *256*, 117883.

Pawlotsky, J.M. (2020). COVID-19 pandemic: Time to revive the cyclophilin inhibitor alisporivir. Clin. Infect. Dis. *71*, 2191–2194.

Peel, M., and Scribner, A. (2013). Optimization of cyclophilin inhibitors for use in antiviral therapy. In Successful Strategies for the Discovery of Antiviral Drugs, M.C. Desai and N.A. Meanwell, eds. (The Royal Society of Chemistry), pp. 384–418.

Pushpakom, S., Iorio, F., Eyers, P.A., Escott, K.J., Hopper, S., Wells, A., Doig, A., Guilliams, T., Latimer, J., McNamee, C., et al. (2019). Drug repurposing: progress, challenges and recommendations. Nat. Rev. Drug Discov. *18*, 41–58.

Qian, Y.M., and Huang, Y.H. (1989). [Effects of tetrandrine on rabbit platelet aggregation, thromboxane A2 generation and calmodulin activity]. Zhongguo Yao Li Xue Bao *10*, 61–65.

Rausch, K., Hackett, B.A., Weinbren, N.L., Reeder, S.M., Sadovsky, Y., Hunter, C.A., Schultz, D.C., Coyne, C.B., and Cherry, S. (2017). Screening Bioactives Reveals Nanchangmycin as a Broad Spectrum Antiviral Active against Zika Virus. Cell Rep. *18*, 804–815.

Sakurai, Y., Kolokoltsov, A.A., Chen, C.C., Tidwell, M.W., Bauta, W.E., Klugbauer, N., Grimm, C., Wahl-Schott, C., Biel, M., and Davey, R.A. (2015). Ebola virus. Two-pore channels control Ebola virus host cell entry and are drug targets for disease treatment. Science *347*, 995–998.

Schneider, M., Ackermann, K., Stuart, M., Wex, C., Protzer, U., Schätzl, H.M., and Gilch, S. (2012). Severe acute respiratory syndrome coronavirus replication is severely impaired by MG132 due to proteasome-independent inhibition of M-calpain. J. Virol. *86*, 10112–10122.

Shang, J., Wan, Y., Luo, C., Ye, G., Geng, Q., Auerbach, A., and Li, F. (2020). Cell entry mechanisms of SARS-CoV-2. Proc. Natl. Acad. Sci. USA *117*, 11727–11734.

Sheahan, T.P., Sims, A.C., Zhou, S., Graham, R.L., Pruijssers, A.J., Agostini, M.L., Leist, S.R., Schäfer, A., Dinnon, K.H., 3rd, Stevens, L.J., et al. (2020). An orally bioavailable broad-spectrum antiviral inhibits SARS-CoV-2 in human airway epithelial cell cultures and multiple coronaviruses in mice. Sci. Transl. Med. *12*, eabb5883.

Shen, B.Q., Finkbeiner, W.E., Wine, J.J., Mrsny, R.J., and Widdicombe, J.H. (1994). Calu-3: a human airway epithelial cell line that shows cAMP-dependent Cl- secretion. Am. J. Physiol. *266*, L493–L501.

Shimojima, M., Ikeda, Y., and Kawaoka, Y. (2007). The mechanism of Axl-mediated Ebola virus infection. J. Infect. Dis. *196* (*Suppl 2*), S259–S263.

Tanaka, Y., Sato, Y., and Sasaki, T. (2017). Feline coronavirus replication is affected by both cyclophilin A and cyclophilin B. J. Gen. Virol. *98*, 190–200.

Thomson, A.W., Bonham, C.A., and Zeevi, A. (1995). Mode of action of tacrolimus (FK506): molecular and cellular mechanisms. Ther. Drug Monit. *17*, 584–591.

Tian, X., Zhao, C., Zhu, H., She, W., Zhang, J., Liu, J., Li, L., Zheng, S., Wen, Y.M., and Xie, Y. (2010). Hepatitis B virus (HBV) surface antigen interacts with and promotes cyclophilin a secretion: possible link to pathogenesis of HBV infection. J. Virol. *84*, 3373–3381.

Tian, X., Li, C., Huang, A., Xia, S., Lu, S., Shi, Z., Lu, L., Jiang, S., Yang, Z., Wu, Y., and Ying, T. (2020). Potent binding of 2019 novel coronavirus spike protein by a SARS coronavirus-specific human monoclonal antibody. Emerg. Microbes Infect. *9*, 382–385.

Towers, G.J., Hatziioannou, T., Cowan, S., Goff, S.P., Luban, J., and Bieniasz, P.D. (2003). Cyclophilin A modulates the sensitivity of HIV-1 to host restriction factors. Nat. Med. *9*, 1138–1143.

Ushio, H., Ishibuchi, S., Oshita, K., Seki, N., Kataoka, H., Sugahara, K., Adachi, K., and Chiba, K. (2013). A new phenylpyrazoleanilide, y-320, inhibits interleukin 17 production and ameliorates collagen-induced arthritis in mice and cynomolgus monkeys. Pharmaceuticals (Basel) *7*, 1–17.

Van Cauwenberge, P., De Belder, T., and Sys, L. (2004). A review of the second-generation antihistamine ebastine for the treatment of allergic disorders. Expert Opin. Pharmacother. *5*, 1807–1813.

Van den Broeke, C., Radu, M., Chernoff, J., and Favoreel, H.W. (2010). An emerging role for p21-activated kinases (Paks) in viral infections. Trends Cell Biol. *20*, 160–169.

Verdoodt, B., Vogt, M., Schmitz, I., Liffers, S.T., Tannapfel, A., and Mirmohammadsadegh, A. (2012). Salinomycin induces autophagy in colon and breast cancer cells with concomitant generation of reactive oxygen species. PLoS ONE *7*, e44132.

von Brunn, A., Ciesek, S., von Brunn, B., and Carbajo-Lozoya, J. (2015). Genetic deficiency and polymorphisms of cyclophilin A reveal its essential role for Human Coronavirus 229E replication. Curr. Opin. Virol. *14*, 56–61.

von Hahn, T., Schiene-Fischer, C., Van, N.D., Pfaender, S., Karavul, B., Steinmann, E., Potthoff, A., Strassburg, C., Hamdi, N., Abdelaziz, A.I., et al. (2012). Hepatocytes that express variants of cyclophilin A are resistant to HCV infection and replication. Gastroenterology *143*, 439, 47.e1.

Wang, Y., Li, J., Booher, R.N., Kraker, A., Lawrence, T., Leopold, W.R., and Sun, Y. (2001). Radiosensitization of p53 mutant cells by PD0166285, a novel G(2) checkpoint abrogator. Cancer Res. *61*, 8211–8217.

Wang, M., Cao, R., Zhang, L., Yang, X., Liu, J., Xu, M., Shi, Z., Hu, Z., Zhong, W., and Xiao, G. (2020). Remdesivir and chloroquine effectively inhibit the recently emerged novel coronavirus (2019-nCoV) in vitro. Cell Res. *30*, 269–271.

Warren, T.K., Jordan, R., Lo, M.K., Ray, A.S., Mackman, R.L., Soloveva, V., Siegel, D., Perron, M., Bannister, R., Hui, H.C., et al. (2016). Therapeutic efficacy of the small molecule GS-5734 against Ebola virus in rhesus monkeys. Nature *531*, 381–385.

Weber, C., and Opatz, T. (2019). Bisbenzylisoquinoline Alkaloids. Alkaloids Chem. Biol. *81*, 1–114.

Weiss, S.R., and Navas-Martin, S. (2005). Coronavirus pathogenesis and the emerging pathogen severe acute respiratory syndrome coronavirus. Microbiol. Mol. Biol. Rev. *69*, 635–664.

Willicombe, M., Thomas, D., and McAdoo, S. (2020). COVID-19 and Calcineurin Inhibitors: Should They Get Left Out in the Storm? J. Am. Soc. Nephrol. *31*, 1145–1146.

Winkler, E.S., Gilchuk, P., Yu, J., Bailey, A.L., Chen, R.E., Chong, Z., Zost, S.J., Jang, H., Huang, Y., Allen, J.D., et al. (2021). Human neutralizing antibodies against SARS-CoV-2 require intact Fc effector functions for optimal therapeutic protection. Cell. https://doi.org/10.1016/j.cell.2021.02.026.

Wu, F., Zhao, S., Yu, B., Chen, Y.M., Wang, W., Song, Z.G., Hu, Y., Tao, Z.W., Tian, J.H., Pei, Y.Y., et al. (2020). A new coronavirus associated with human respiratory disease in China. Nature *579*, 265–269.

Xu, C., Meng, S., Liu, X., Sun, L., and Liu, W. (2010). Chicken cyclophilin A is an inhibitory factor to influenza virus replication. Virol. J. *7*, 372.

Yang, N., and Shen, H.M. (2020). Targeting the Endocytic Pathway and Autophagy Process as a Novel Therapeutic Strategy in COVID-19. Int. J. Biol. Sci. *16*, 1724–1731.

Yang, F., Robotham, J.M., Nelson, H.B., Irsigler, A., Kenworthy, R., and Tang, H. (2008). Cyclophilin A is an essential cofactor for hepatitis C virus infection and the principal mediator of cyclosporine resistance in vitro. J. Virol. *82*, 5269–5278.

Yu, G.Y., and Lai, M.M. (2005). The ubiquitin-proteasome system facilitates the transfer of murine coronavirus from endosome to cytoplasm during virus entry. J. Virol. *79*, 644–648.

Yu, Y., Kalinowski, D.S., Kovacevic, Z., Siafakas, A.R., Jansson, P.J., Stefani, C., Lovejoy, D.B., Sharpe, P.C., Bernhardt, P.V., and Richardson, D.R. (2009). Thiosemicarbazones from the old to new: iron chelators that are more than just ribonucleotide reductase inhibitors. J. Med. Chem. *52*, 5271–5294.

Zhang, J.H., Chung, T.D., and Oldenburg, K.R. (1999). A Simple Statistical Parameter for Use in Evaluation and Validation of High Throughput Screening Assays. J. Biomol. Screen. *4*, 67–73.

Zhang, Y., Li, F., Liu, L., Jiang, H., Hu, H., Du, X., Ge, X., Cao, J., and Wang, Y. (2019). Salinomycin triggers endoplasmic reticulum stress through ATP2A3 upregulation in PC-3 cells. BMC Cancer *19*, 381.

Zheng, K., Kitazato, K., and Wang, Y. (2014). Viruses exploit the function of epidermal growth factor receptor. Rev. Med. Virol. *24*, 274–286.

Zhou, D., Mei, Q., Li, J., and He, H. (2012). Cyclophilin A and viral infections. Biochem. Biophys. Res. Commun. *424*, 647–650.

STAR★METHODS

KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Antibodies		
Mouse anti-dsRNA J2	Absolute	Ab01299
Human anti-SARS-CoV-2 Spike (Clone CR3022)	Absolute	Ab01680
Ace2	R&D Systems	Cat#AF933
Tubulin	Sigma Aldrich	Cat#T6199-200UL
Bacterial and virus strains		
SARS-CoV-2 (WA1)	BEI	NR-52281
Chemicals, peptides, and recombinant proteins		
Chemical library	Selleckchem, MedChemExpress, MedKoo	N/A
M MLV Reverse Transcriptase	Invitrogen	Cat#28025013
Quantitative Synthetic RNA from SARS-Related Coronavirus 2	BEI	NR-52358
SYBR green master mix	Applied Biosystems	Cat#4368708
Trizol	Invitrogen	Cat#15596018
Hoechst 33342	Sigma Aldrich	Cat#B2261-25MG
Random Primers	Invitrogen	Cat#48190011
Critical commercial assays		
Zymo RNA clean and concentrator 25	Zymo Research	Cat#R1018
Experimental models: Cell lines		
Cercopithecus aethiops: Vero E6	ATCC	ATCC CRL-1586
Cercopithecus aethiops: Vero CCL81	ATCC	ATCC CCL81
Human: Huh7.5	C. Rice, Rockefeller	N/A
Human: Calu-3	ATCC	ATCC HTB-55
Human: SPC2 iPSC clone SPC2-ST-B2	Boston University	N/A
Human: NHBE	Lonza	Cat#CC-2540
Oligonucleotides		
SARS2 F: ATGCTGCAATCGTGCTACAA	Winkler et al., 2021	N/A
SARS2 R: CCTCTGCTCCCTTCTGCGTA	Winkler et al., 2021	N/A
18S F: AACCCGTTGAACCCCATT	Rausch et al., 2017	N/A
18S R: CCATCCAATCGGTAGTAGCG	Rausch et al., 2017	N/A
Software and algorithms		
PRISM 8.4.3	GraphPad Software	N/A
MetaXpress 5.3.3	Molecular Devices	N/A
Spotfire	PerkinElmer	N/A
QuantStudio 6 1.3	Applied Biosystems	N/A

RESOURCE AVAILABILITY

Lead contact

Further information and requests for resources and reagents should be directed to and will be fulfilled by the Lead Contact, Sara Cherry (cherrys@pennmedicine.upenn.edu).

Materials availability

This study did not generate new unique reagents.

Data and code availability

This study did not generate new code.

EXPERIMENTAL MODEL AND SUBJECT DETAILS

Vero E6 cells (ATTC CRL-1586), Vero CCL81 (ATCC, CCL81) and Huh 7.5 (C. Rice, Rockefeller) were cultured in DMEM, supplemented with 10% (v/v) fetal bovine serum, 1% (v/v) penicillin/streptomycin, 1% (v/v) L-Glutamax and were maintained at 37°C and 5% CO2Calu-3 cells (ATCC HTB-55) were obtained from ATCC and cultured in MEM, supplemented with 10% (v/v) fetal bovine serum, 1% (v/v) non-essential amino acids, 1% (v/v) penicillin/streptomycin, 1% (v/v) L-glutamine, and were maintained at 37°C and 5% CO2. Alveolar organoids and 2D cultures of iPSC (SPC2 iPSC line, clone SPC2-ST-B2, Boston University) derived alveolar epithelial type 2 cells(iAT2) were differentiated and maintained as alveolospheres embedded in 3D Matrigel in CK+DCI media, as previously described (Jacob et al., 2019). iAT2 were passaged every two weeks by dissociation into single cells via the sequential application of dispase (2mg/ml, Thermo Fisher Scientific, 17105-04) for 1h at 37°C and 0.25% trypsin (Invitrogen, 25300054) for 8 min at 37°C and re-plated as 50 μL drops of Growth Factor Reduced Matrigel (Corning, 356231) in CK+DCI media supplemented with ROCK inhibitor for the first 48h, as previously described (ref). This line expresses tdTomato from the endogenous locus of surfactant protein-C (SFTPC), an AT2 cell specific marker (ref). The cells were > 50% positive upon generation of 2D alveolar cells for viral infection. To generate this monolayer, alveolospheres were dispersed into single cells, then plated on plates precoated with 3% Matrigel (coring) using CK+DCI media with ROCK inhibitor and then the medium was changed to CK+DCI media at day 3.Normal human bronchial epithelial Cell (NHBE, Lonza CC-2540) were cultured in Bronchial Epithelial Basal Medium (BEBM, Lonza CC-3171) supplemented with bovine pituitary extract, hydrocortisone, hEGF, epinephrine, transferrin, insulin, retinoic acid, triiodothyronine and gentamicin/amphotericin-B, according to manufacturers recommendations. Cells were maintained for a maximum of three passages.

SARS-CoV-2 was obtained from BEI (USA WA1/2020 strain). Stocks were prepared by infection of Vero E6 cells in 2% serum plus 10mM HEPES for five days, freeze-thawed, and clarified by centrifugation (PO). Titer of the stock was determined by plaque assay using Vero E6 cells and were 1×10^7 pfu/mL and 1.5×10^6 TCID50/mL (6). This seed stock was sequence verified and amplified in Vero CCL81 (P1) at 1.5×10^6 TCID50/mL and used for all experiments. All work with infectious virus was performed in a Biosafety Level 3 laboratory and approved by the Institutional Biosafety Committee and Environmental Health and Safety.

METHOD DETAILS

Infections

Cells were plated in 384 well plates (20μL/well) 3,000 cells per well for Vero, 3,000 cells per well Huh7.5, 7,500 cells per well Calu-3. The next day, 50nL of drugs were added. The positive control remdesivir (10uM) and the negative control DMSO were spotted on each plate. One hour later cells were infected with SARS-CoV-2 (Vero, MOI = 1; Huh7.5 MOI = 1; Calu-3 MOI = 0.5) Cells were fixed (30hpi Vero and Huh7,5, 48hpi Calu-3) in 4% formaldehyde/PBS for 15min at room temperature and then washed three times with PBS. Cells were blocked (2% BSA/PBST) for 60 minutes and incubated in primary antibody (anti-dsRNA J2) overnight at 4°C. Cells were washed 3x in PBST and incubated in secondary antibody (anti-mouse alexa 488) and Hoescht 33342 for 1h at room temperature. Cells were washed 3x in PBST and imaged at 10X using ImagXpress Micro capturing four sites per well. The total number of cells and the number of infected cells were measured using cell scoring module (MetaXpress 5.3.3), and the percentage of infected cells was calculated. The aggregated percentage of infection of the DMSO (n = 32) and remdesivir control wells (n = 16) on each assay plate were used to calculate z'-factors, as a measure of assay performance and data quality. Sample well infection was normalized to aggregated DMSO plate control wells and expressed as Percentage of Control [POC = (%Infection$_{sample}$/ Average %Infection$_{DMSO}$)*100] and Z-score [Z = (%Infection$_{sample}$ - Average %Infection$_{DMSO}$) / Standard Deviation %Infection$_{DMSO}$]in Spotfire (PerkinElmer). Z-score is analogous to the standard deviation. Candidate hits were selected as compounds with POC < 40% and viability > 80%, compared to DMSO vehicle control.

Candidate drugs were repurchased as powders from Selleckchem, MedChemExpress, and MedKoo and suspended in DMSO. Drugs were arrayed in 8-pt dose-response in 384 well plates. Infections were performed using the screening conditions. DMSO (n = 32) and 10 μM remdesivir (n = 16) were included on each validation plate as controls for normalization. Infection at each drug concentration was normalized to aggregated DMSO plate control wells and expressed as percentage-of-control (POC = % Infection $_{sample}$/Avg % Infection $_{DMSO\ cont}$).

A non-linear regression curve fit analysis (GraphPad Prism 8) was performed on on the aggregated average POC Infection and cell viability from $\geq$ 2 independent experimental replicates versus the log10 transformed concentration values to calculate IC$_{50}$ values for Infection and CC50 values for cell viability for each drug/cell line combination. Error bars represent the standard deviation of replicate data for each drug concentration tested in independent experiments.Selectivity index (SI) was calculated as a ratio of drug's CC50 and IC$_{50}$ values (SI = CC50/IC$_{50}$).

RT-qPCR

Huh7.5 (750,000 cells/well), Calu-3 cells (750,000 cells/well), NHBE (1,000,000/well), iAT2 (1,250,000 cells/well) were plated in 6 well plates. The next day for Huh7.5, Calu-3 and NHBE or 3 days later for iAT2 cells drugs were added. The final concentrations: 10uM

Remdesivir, 10uM Salinomycin, 10uM Bemcentinib, 10uM NIM811, 10 uM alisporivir, 5uM cyclosporine, 5uM ebastine, 5uM Dacomitinib, 2uM AZD8055, 2uM Dp44mT, 2uM WYE125132, 2uM Y-320. One hour later cells were infected with SARS-CoV-2 (MOI = 0.5). Total RNA was purified using Trizol (Invitrogen) followed by RNA Clean and Concentrate kit (Zymo Researc) 24 hpi for Huh7.5, 48 hpi for Calu-3 and 72hpi for iAT2 or NHBE cells. For cDNA synthesis, reverse transcription was performed with random hexamers and Moloney murine leukemia virus (M-MLV) reverse transcriptase (Invitrogen). Synthesized RNA was used as a standard (BEI). Gene specific primers to SARS-CoV-2 (Wuhan v1, NSP14) and SYBR green master mix (Applied Biosystems) were used to amplify viral RNA and 18S rRNA primers were used to amplify cellular RNA using the QuantStudio 6 Flex RT-PCR system (Applied Biosystems). Relative quantities of viral and cellular RNA were calculated using the standard curve method (Larionov et al., 2005). Viral RNA was normalized to 18S RNA for each sample (Wuhan V1/18S).

QUANTIFICATION AND STATISTICAL ANALYSIS

Statistical significance of was assessed either using the two-tailed Student t test or z-scores as described. Details of all statistical analysis can be found in the legends of both the main and supplemental figures, including the statistical tests used, the value of n.