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

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

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

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SUMMARY

There is an urgent need for antivirals to treat the newly emerged severe acute respiratory syndrome coronavirus 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 antiviral 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
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., 2008; 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 (Głowacka 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, daocmitinib, 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 x 10⁷ plaque-forming units [PFUs]/mL) suggesting that viral replication and cytopathicity 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 IC₅₀ₐ₅ (concentration of a drug that is required for 50% inhibition) and CC₅₀ₐ₅ (concentration of a drug that is required for 50% cell killing), demonstrating little cytotoxicity at the active doses (Figure 1B) (Wang et al., 2020).
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 \( \mu M \) (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, CFBE410, and U2OS cells. We detected less than 1% infection of A549, Calu-1, Huh7, HepG2, HaCaT, IMR90, NCI-H292, CFBE410, 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 IC50s 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).
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 IC50s and CC50s 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...
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 coronavirus, 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 IC50 1,000-fold higher.
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 IC50, CC50, and SI are shown in Figure S4.

Cyclosporine is antiviral likely through interactions with cyclophilins

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 isocyclosporin A (Jeffery, 1991). We found that isocyclosporin A, cyclosporin A, cyclosporin B, and cyclosporin C were active with increasing IC50s (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
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 model 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, cepharehantane, cyclosporine, alokistatin, MG132, salinomycin) and SARS-CoV-2 (salinomycin, tetrandrine, cepharehantane, 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; Ivanovsk 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 (Jang et al., 2018; Verdoordt 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 (Giowacka et al., 2011; Hoffmann et al., 2020; Matsuyma 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 cannabimoid CB2 receptor agonist that we found was antiviral in Huh7.5 cells. GW842166X, another CB2 agonist that has a 10-fold higher IC50, was not active. Moreover, dose-response studies found that AM1241 is not active in either Vero or Calu-3 cells.

Cepharehantane and tetrandrine are both bis-benzyloisquino-line 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 (Doilan 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 naqotoninib. 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).

Cytophosphoinositol 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 (Goethe 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 Calu-Co-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 cycloporsins were antiviral with similar potencies, including cyclosporine, cyclosporin A, cyclosporin B, and the metabolic breakdown product of cyclosporin A, isocyclosporin 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 cycloporsins 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 (Willcombe 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, tacrolimus, 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 a 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:

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SUPPLEMENTAL INFORMATION
Supplemental information can be found online at https://doi.org/10.1016/j.celrep.2021.108959.

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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.

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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: CCTCTGCTCCCTTCTCGTGA | Winkler et al., 2021 | N/A |
| 18S F: AACCCGTTGAAACCCATT | Rausch et al., 2017 | N/A |
| 18S R: CCATCCAATCGTAGTAGCG | 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.
Vero E6 cells (ATCC 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% CO2.Calu-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 (corning) 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/ampotericin-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 1x10⁷ pfu/mL and 1.5x10⁶ TCID50/mL (6). This seed stock was sequence verified and amplified in Vero

### 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 (10μM) 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 = (%InfectionSample / Average %InfectionDMSO)*100] and Z-score [Z = (%InfectionSample - Average %InfectionDMSO / Standard Deviation %InfectionDMSO) 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 = % InfectionSample / Avg % InfectionDMSO Contrast]. A non-linear regression curve fit analysis (GraphPad Prism 8) was performed on the aggregated average POC Infection and cell viability from ≥ 2 independent experimental replicates versus the log10 transformed concentration values to calculate IC50 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 IC50 values (SI = CC50/IC50).

### 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: 10μM
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 Research) 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.