BRD2 inhibition blocks SARS-CoV-2 infection by reducing transcription of the host cell receptor ACE2

Avi J. Samelson^{1,2}, Quang Dinh Tran^{3,4}, Rémy Robinot⁵, Lucia Carrau⁶, Veronica V. Rezelj³, Alice Mac Kain^{3,4}, Merissa Chen^{1,2}, Gokul N. Ramadoss^{7,8}, Xiaoyan Guo^{1,2}, Shion A. Lim^{9,19}, Irene Lui⁹, James K. Nuñez^{10,11}, Sarah J. Rockwood⁷, Jianhui Wang¹², Na Liu¹², Jared Carlson-Stevermer¹³, Jennifer Oki¹³, Travis Maures¹³, Kevin Holden¹³, Jonathan S. Weissman^{10,11,14,15}, James A. Wells^{2,9,10}, Bruce R. Conklin^{7,10,16,17}, Benjamin R. TenOever⁶, Lisa A. Chakrabarti⁵, Marco Vignuzzi³, Ruilin Tian^{1,2,12} ✉ and Martin Kampmann^{1,2,18} ✉

SARS-CoV-2 infection of human cells is initiated by the binding of the viral Spike protein to its cell-surface receptor ACE2. We conducted a targeted CRISPRi screen to uncover druggable pathways controlling Spike protein binding to human cells. Here we show that the protein BRD2 is required for ACE2 transcription in human lung epithelial cells and cardiomyocytes, and BRD2 inhibitors currently evaluated in clinical trials potentially block endogenous ACE2 expression and SARS-CoV-2 infection of human cells, including those of human nasal epithelia. Moreover, pharmacological BRD2 inhibition with the drug ABBV-744 inhibited SARS-CoV-2 replication in Syrian hamsters. We also found that BRD2 controls transcription of several other genes induced upon SARS-CoV-2 infection, including the interferon response, which in turn regulates the antiviral response. Together, our results pinpoint BRD2 as a potent and essential regulator of the host response to SARS-CoV-2 infection and highlight the potential of BRD2 as a therapeutic target for COVID-19.

The ongoing COVID-19 pandemic is a public health emergency. As of September 2021, SARS-CoV-2, the novel coronavirus causing this disease, has infected over 200 million people worldwide, causing at least four and a half million deaths (<https://covid19.who.int>). New infections are still rapidly increasing, despite current vaccination programmes. The emergence of novel viral variants with the potential to partially overcome vaccine-elicited immunity highlights the need to elucidate the molecular mechanisms that underlie SARS-CoV-2 interactions with host cells to enable the development of therapeutics to treat and prevent COVID-19, complementing ongoing vaccination efforts.

SARS-CoV-2 entry into human cells is initiated by the interaction of the viral Spike protein with its receptor on the cell surface, angiotensin-converting enzyme 2 (ACE2). To uncover new therapeutic targets targeting this step of SARS-CoV-2 infection, we have conducted a focused CRISPR interference (CRISPRi)-based screen for modifiers of Spike binding to human cells. We expected that ACE2 and factors regulating ACE2 expression would be major hit genes in

this screen. A second motivation for identifying regulators of ACE2 was the fact that ACE2 affects inflammatory responses and is itself regulated in the context of inflammation^{1–3}. Inflammatory signalling, in particular the type I interferon response, is known to be misregulated in the most severely affected patients with COVID-19^{4–7}. Therefore, regulators of ACE2 expression would probably be relevant for COVID-19 in human patients, as suggested by clinical data⁸.

Previous CRISPR screens have been performed in cell-based models of SARS-CoV-2 infection that overexpressed an ACE2 transgene^{9,10}, represented cell types not primarily targeted by SARS-CoV-2¹¹ or were non-human cells¹². Although these studies elucidated major features of SARS-CoV-2 biology, we reasoned that the cell lines used would not have enabled the discovery of regulators of ACE2 expression in relevant human cell types.

We selected a lung epithelial cancer cell line, Calu-3, which endogenously expresses ACE2, to perform a targeted CRISPRi screen to find regulators of Spike protein binding. We found that the strongest hit genes are potent regulators of ACE2 levels. Knockdown

¹Institute for Neurodegenerative Diseases, University of California San Francisco, San Francisco, CA, USA. ²Chan-Zuckerberg Biohub, San Francisco, CA, USA. ³Viral Populations and Pathogenesis Unit, Institut Pasteur, Paris, France. ⁴École Doctorale BioSPC, Université de Paris, Sorbonne Paris Cité, Paris, France. ⁵Institut Pasteur, CIVIC Group, Virus and Immunity Unit, Université de Paris, Paris, France. ⁶Microbiology Department, NYU-Langone, New York, NY, USA. ⁷Gladstone Institutes, San Francisco, CA, USA. ⁸Biomedical Sciences PhD Program, University of California San Francisco, San Francisco, CA, USA. ⁹Department of Pharmaceutical Chemistry, University of California San Francisco, San Francisco, CA, USA. ¹⁰Department of Cellular and Molecular Pharmacology, University of California San Francisco, San Francisco, CA, USA. ¹¹Howard Hughes Medical Institute, University of California San Francisco, San Francisco, CA, USA. ¹²School of Medicine, Southern University of Science and Technology, Shenzhen, China. ¹³Synthego Corporation, Redwood City, CA, USA. ¹⁴Whitehead Institute for Biomedical Research, Cambridge, MA, USA. ¹⁵Department of Biology, Massachusetts Institute of Technology, Cambridge, USA. ¹⁶Department of Ophthalmology, University of California San Francisco, San Francisco, CA, USA. ¹⁷Department of Medicine, University of California San Francisco, San Francisco, CA, USA. ¹⁸Department of Biochemistry and Biophysics, University of California San Francisco, San Francisco, CA, USA. ¹⁹Present address: Department of Antibody Engineering, Genentech Inc., San Francisco, CA, USA. ✉e-mail: tianrl@sustech.edu.cn; martin.kampmann@ucsf.edu

Fig. 1 | CRISPRi screen reveals cellular factors controlling Spike protein binding. **a**, Human cell lines were incubated with different concentrations of recombinant SARS-CoV-2 Spike-RBD, and the amount of Spike-RBD bound per cell was quantified by flow cytometry. Only Calu-3 cells (black) display saturable binding, for which an EC_{50} value was fit. The error of EC_{50} is the 95% confidence interval. **b**, Spike-RBD binding is eliminated in Calu-3 cells with ACE2 knockout (KO, grey). **c**, Validation of CRISPRi activity of a Calu-3 CRISPRi line. Calu-3 cells stably expressing CRISPRi machinery were transduced with an sgRNA targeting CD81 (orange) or a non-targeting sgRNA (grey), and CD81 levels were determined by flow cytometry. The experiments in **a–c** were performed once. **d**, CRISPRi screen strategy. CRISPRi Calu-3 cells transduced with an sgRNA library targeting the 'druggable genome' were stained either with Spike-RBD or an anti-TFRC antibody. Cells were then FACS-sorted into bins (top and bottom 30%) based on Spike-RBD or anti-TFRC binding signal, and frequencies of cells expressing each sgRNA were determined for each bin by targeted next-generation sequencing. The CRISPRi screen was performed once. **e**, Rank-order plot of Spike-RBD hit genes. Genes selected for follow-up experiments are highlighted as red dots. **f**, Scatter plot of gene scores for the Spike-RBD screen (x axis) and the anti-TFRC screen (y axis). Genes selected for follow-up experiments are highlighted as red dots.

of these genes reduced or increased ACE2 levels transcriptionally, and prevented or enhanced, respectively, SARS-CoV-2 infection in cell culture.

We identified the transcriptional regulator bromodomain-containing protein 2 (BRD2) as a major node for host–SARS-CoV-2 interaction. BRD2 is part of the bromodomain and extra-terminal domain (BET) family of proteins that includes Brd3, Brd4 and BrdT. BETs are being explored as targets for a number of cancers¹³. These proteins are known to be master transcriptional regulators and serve to bridge chromatin marks (mostly acetyl-lysines) to the transcriptional machinery¹⁴. We found BRD2 inhibition to

downregulate ACE2 expression in Calu-3 cells, induced pluripotent stem cell (iPSC)-derived cardiomyocytes, primary human lung epithelial cells and reconstructed human nasal epithelia. Inhibition of BRD2 with small molecules, some of which are in phase I clinical trials, inhibited SARS-CoV-2 infection in primary human nasal epithelia and in Syrian hamsters. We propose BRD2 as a key regulator and potential therapeutic target for COVID-19.

Results

CRISPRi screen for Spike-RBD binding to cells. To identify cellular mechanisms controlling the binding of SARS-CoV-2 to human

Fig. 2 | Hit genes modulate ACE2 levels and SARS-CoV-2 infection. **a**, Western blotting for ACE2 and GAPDH in Calu-3 CRISPRi cells expressing no sgRNA or sgRNAs targeting different hit genes, or ACE2 knockout Calu-3 cells. The three lanes represent biological triplicates for each cell line. **b**, Quantification of ACE2 protein levels relative to GAPDH based on the data in **a**. Average normalized ACE2 intensity as a fraction of no sgRNA and the standard deviation for three biological replicates are shown. **c**, Relative amounts of ACE2 transcript levels measured by qPCR in Calu-3 CRISPRi cells expressing sgRNAs targeting different hit genes, compared to cells without sgRNA. Average relative gene expression of three technical replicates is shown from a single experiment. **d**, Calu-3 CRISPRi cells expressing different sgRNAs targeting hit genes were infected with SARS-CoV-2 and viral RNA in the supernatant was measured by RT-qPCR as a function of time post-infection. The average SARS-CoV-2 load and the standard deviation of three biological replicates are shown. **e**, Spike-RBD binding to Calu-3 cells was quantified by flow cytometry of Calu-3 cells expressing sgRNAs targeting individual hit genes after incubation with increasing concentrations of Spike-RBD. For genes for which data could be fitted with a binding curve, the EC_{50} was determined along with the 95% confidence intervals. Data points are average values from three biological replicates for each gene knockdown, with error bars representing the standard deviation, except for *ACE2* and *BRD2*, where only one experiment at each concentration was performed. **f**, Plaque assays in Calu-3 CRISPRi cells expressing different sgRNAs targeting hit genes were infected with SARS-CoV-2 as a function of time post-infection. Average SARS-CoV-2 titre and the standard deviation of six biological replicates are shown.

cells, we identified a cell line that would robustly bind the viral Spike protein (S). We measured binding of a previously described recombinant protein construct encompassing the SARS-CoV-2 Spike protein receptor-binding domain (RBD) with a C-terminal human immunoglobulin-G (IgG) Fc-domain fusion¹⁵, referred to hereafter as Spike-RBD, to several commonly used human cell lines (Fig. 1a and Extended Data Fig. 1a). Within this cell line panel, only Calu-3 cells displayed a binding curve consistent with specific binding of Spike-RBD, with a half-maximum effective concentration (EC_{50}) of 9.72 nM (95% CI: 4.37–22.42 nM). This value agrees with the dissociation constant of Spike-RBD–ACE2 binding determined in vitro (4.7 nM)¹⁶, within measurement error. Spike-RBD binding is dependent on ACE2 expression, as binding is abrogated in Calu-3 ACE2 knockout cells (Fig. 1b). Calu-3 cells are challenging to culture

compared to most cell lines—they proliferate slowly, and their adherence properties pose challenges for flow cytometry. Nevertheless, Calu-3 cells are a particularly attractive cell culture model for studying SARS-CoV-2 from the biological point of view, because they are derived from lung epithelia, which is selectively infected by SARS-CoV-2 in patients¹⁷, are known to support infection of SARS-CoV and SARS-CoV-2¹⁸ and have recently been reported to closely recapitulate gene-expression changes that occur in patients¹⁹.

We generated a polyclonal Calu-3 line constitutively expressing machinery to enable CRISPRi-based genetic screens^{20,21} and validated its CRISPRi activity (Fig. 1c and Extended Data Fig. 1b). CRISPRi uses a catalytically dead Cas9 (dCas9) fused to a transcriptional repressor, KRAB, to knock down genes at specific sites programmed by the loading of the dCas9-KRAB with a single-guide

RNA (sgRNA). Using this line, we then performed a focused CRISPRi screen for factors controlling Spike-RBD binding (Fig. 1d). To maximize our chances of identifying potential undiscovered therapeutic targets for COVID-19, we screened a sgRNA library targeting the ‘druggable genome’, comprising ~2,300 genes, with ~16,000 total sgRNAs including non-targeting control sgRNAs²². In parallel, we screened the same library using a fluorophore-conjugated antibody against the transferrin receptor (TFRC, also known as CD71) to control for factors that generally affect protein trafficking or protein binding to the cell surface (Fig. 1d–f). Owing to the limitations of the Spike-RBD binding assay and Calu-3 cells, this screen was conducted at a lower average representation (~100 sorted cells per sgRNA) than ideal, resulting in relatively high noise and therefore fewer hits crossing a false discovery rate cutoff of 0.1 than in typical CRISPR screens (Extended Data Fig. 1c and Methods).

Despite the increased noise, *ACE2*, as expected, was the strongest hit gene, knockdown of which decreased binding of Spike-RBD, while having no effect on TFRC levels (Fig. 1e,f). Conversely, *RAB7A*, which was recently reported to be essential for the trafficking of TFRC to the cell surface²³, was the strongest hit that decreased TFRC levels, with no effect on Spike-RBD binding (Fig. 1f). Generally, hits were not correlated between the two screens (Fig. 1f), demonstrating the specificity of each screen. Although the screens did not result in a large number of strong hits (Supplementary Table 1), we decided to validate the top 15 genes for which knockdown decreased Spike-RBD binding and the top five genes for which knockdown increased Spike-RBD binding. We cloned individual sgRNAs targeting each of these genes and evaluated their effect on Spike-RBD binding (Extended Data Fig. 2). Based on these experiments, we selected hits that robustly recapitulated their phenotypes from the primary screen for further characterization: two genes for which knockdown decreased Spike-RBD binding (*ACE2* and *BRD2*) and three genes for which knockdown increased Spike-RBD-binding (*CDC7*, *COMP* and *TRRAP*).

Hit genes modulate ACE2 levels and infection with SARS-CoV-2. Because Spike-RBD binding is dependent on ACE2 expression, we hypothesized that other hit genes might act by modulating ACE2 levels. Western blots for ACE2 levels in Calu-3 cell lines expressing sgRNAs against validated target genes (hereafter referred to as knockdown lines) indeed revealed marked changes in ACE2 protein levels. For hits associated with lower levels of Spike-RBD binding in the primary screen, we observed lower levels of ACE2 protein, and vice versa for those hits associated with higher levels of Spike-RBD binding (Fig. 2a,b). To distinguish whether hit genes affected ACE2 protein levels via transcriptional or post-transcriptional mechanisms, we performed quantitative polymerase chain reaction (qPCR) to measure *ACE2* transcript levels in these same

knockdown lines. For all tested genes, we observed changes in *ACE2* transcript levels that were concordant with the changes in ACE2 protein levels (Fig. 2c), indicating that they acted at the transcriptional level. Some genes, such as *COMP* and *TRAPP*, showed relatively modest effects on *ACE2* transcript levels, but quite large effects on ACE2 protein levels, suggesting that these hit genes additionally affect post-transcriptional regulation of ACE2 expression.

We next determined the effect of hit gene knockdown on susceptibility to SARS-CoV-2 infection. We infected cells expressing sgRNAs against hit genes with SARS-CoV-2 and measured virus replication 24, 48 and 72 h post-infection using qPCR with reverse transcription (RT–qPCR; Fig. 2d). Already at 24 h post-infection, viral genome copies diverged concordantly with changes in ACE2 levels and Spike-RBD binding: sgRNAs that lowered Spike-RBD binding reduced virus replication, and sgRNAs that increased Spike-RBD binding resulted in higher virus replication. *BRD2* knockdown abrogated viral replication in these cells to levels similar to *ACE2* knockdown, even at 72 h post-infection, and *COMP* knockdown supported an order of magnitude increase in viral titres. Focusing on these three hit genes—*ACE2*, *BRD2* and *COMP*—we then quantified how gene knockdown modulates Spike-RBD binding to cells (Fig. 2e). Knockdown of *ACE2* and *BRD2* abolished spike binding, while *COMP* decreased the EC₅₀ compared to wild type (WT) from 9.72 nM (95% CI: 4.37–22.42 nM) to 1.32 nM (95% CI: 0.59–3.20 nM), by almost a full order of magnitude (Fig. 2e). We also confirmed that gene knockdown decreased SARS-CoV-2 replication by performing plaque assays on WT, *BRD2* KD and *ACE2* knockdown cells (Fig. 2f).

BRD2 inhibitors prevent SARS-CoV-2 infection of human cells.

Given the stringent inhibition of SARS-CoV-2 infection achieved by *BRD2* knockdown, and the fact that *BRD2* is currently being evaluated as a therapeutic target in cancer^{13,24}, with several small-molecule inhibitors in clinical trials²⁵, we decided to focus on this hit gene.

We validated that CRISPRi knockdown of *BRD2* robustly reduced *BRD2* protein levels (Extended Data Fig. 3). Transgenic expression of full-length *BRD2* restored *ACE2* transcript levels (Fig. 3a), validating that the reduction in ACE2 expression triggered by CRISPRi targeting of *BRD2* was indeed due to *BRD2* knockdown. Transgenic expression of truncation mutants of *BRD2* did not rescue ACE2 expression (Fig. 3a), indicating that full-length *BRD2* is required for ACE2 expression.

To test the potential of *BRD2* as a therapeutic target for COVID-19, we treated cells with a panel of compounds targeting *BRD2*: two BET-domain inhibitors (JQ1²⁶ and ABBV-744²⁷; the latter is currently in clinical trials NCT03360006 and NCT04454658) and three proteolysis targeting chimaeric (PROTAC) compounds that lead to the degradation of *BRD2* (dBET-6²⁸, ARV-771²⁹ and BETd-260²⁹).

Fig. 3 | BRD2 inhibitors potently reduce ACE2 levels and SARS-CoV-2 infection. **a**, Transgenic constructs expressed in Calu-3 cells (left), and transcript levels of *ACE2* relative to *ACTB* in Calu-3 cells transduced with enhanced green fluorescent protein–*BRD2* truncations in a *BRD2* knockdown background (right). Average relative *ACE2* gene expression compared to non-transduced cells and the standard deviation of $n = 3$ biological replicates are shown. aa, amino acid. **b**, Transcript levels of *ACE2* relative to *ACTB* in Calu-3 cells treated with *BRD2* inhibitors (JQ1 at 10 μ M, ABBV-744 at 10 μ M and dBET-6 at 200 nM) were quantified at 24 h (dark grey bars) and 72 h (light grey bars) post-treatment. Average *ACE2* mRNA levels relative to vehicle-treated of three technical replicates are shown from a single experiment. **c**, Transcript levels of *ACE2* relative to *ACTB* in primary human bronchial epithelial cells treated (NHBE) with *BRD2* inhibitors (JQ1 at 10 μ M, dBET-6 at 20 nM, ABBV-744 at 0.01–10 μ M) were quantified at 72 h post-treatment. Average *ACE2* mRNA levels relative to vehicle and the standard deviation of $n = 3$ biological replicates are shown, except for vehicle-treated, for which $n = 6$ replicates are shown. **d**, Transcript levels of *ACE2* relative to 18S ribosomal RNA (rRNA) in human iPSC-derived cardiomyocytes treated with the indicated concentrations of *BRD2* inhibitors were quantified at 72 h post-treatment. Average *ACE2* mRNA levels relative to vehicle-treated and the standard deviation of $n = 3$ biological replicates for each condition are shown, except for the vehicle-treated sample, for which $n = 6$ biological replicates are shown. **e**, SARS-CoV-2 viral RNA in supernatant measured by RT–qPCR at 24 h post-infection of Calu-3 cells. Infection took place 72 h after treatment with the indicated concentrations of *BRD2* inhibitors. Measurements are the average of $n = 5$ biological replicates. Error bars show the standard deviation. **f**, Plaque assays in Calu-3 CRISPRi cells treated with increasing concentrations of the BET inhibitors JQ1 or ABBV-744 infected with SARS-CoV-2 as a function of time post-infection. Average viral titre and the standard deviation of six biological replicates are shown, except for vehicle and untreated, for which three biological replicates are shown.

After only 24 h of treatment with these drugs, *ACE2* messenger RNA (mRNA) levels measured by qPCR decreased roughly twofold (Fig. 3b). This effect was magnified after treatment for 72 h, when almost no *ACE2* mRNA was detectable for any of the BRD2-targeting compounds tested, phenocopying *BRD2* knockdown (Fig. 3b). Similarly, we found that BET inhibitors led to substantial decreases in *ACE2* mRNA levels in primary human bronchial epithelial cells (Fig. 3c) and human iPSC-derived cardiomyocytes (Fig. 3d)—two non-transformed cell types that are susceptible to SARS-CoV-2 infection^{30,31}. Importantly, BET inhibitors were non-toxic to Calu-3

cells, primary human bronchial epithelial cells and cardiomyocytes at effective concentrations (Extended Data Fig. 4).

Because pharmacological inhibition of BRD2 phenocopied *BRD2* knockdown, we hypothesized that these same compounds might prevent infection of cells exposed to SARS-CoV-2. To test this, we treated Calu-3 cells for 72 h with the BET inhibitors JQ1 and ABBV-744, and measured SARS-CoV-2 replication at 48 h post-infection. Strikingly, we found that treated cells displayed 100-fold decreased viral replication versus untreated cells (Fig. 3e), a similar effect size to *BRD2* or *ACE2* knockdown (Fig. 2d,e).

BRD2 regulates ACE2 and SARS-CoV-2-induced host genes.

We next asked whether BRD2 controls transcription of additional genes beyond ACE2. We performed RNA-sequencing (RNA-seq) of Calu-3 cells after treatment with the BET-domain inhibitors JQ1 and ABBV-744 as well as BRD2 CRISPRi knockdown (Supplementary Table 2). We also included CRISPRi knockdown of two other validated hit genes from our screen, COMP and ACE2, as well as overexpression of the viral protein E, which has been reported to interact

with BRD2³². RNA-seq of BRD2 knockdown and BET-domain inhibitor treated cells recapitulated downregulation of ACE2 (Fig. 4a). TMPRSS2, the gene encoding a protease important for viral entry in many cell types, was not a differentially expressed gene in any condition (Supplementary Table 2). Surprisingly, BRD2 knockdown or pharmacological inhibition also resulted in marked downregulation of genes involved in the type I interferon response, while ACE2 knockdown slightly increased expression of those same genes

Fig. 4 | BRD2 controls genes induced by interferon and SARS-CoV-2 infection. **a**, Differentially expressed genes (DEGs) from RNA-sequencing of Calu-3 cells under different treatment conditions compared to control cells: 72-h treatment with 10 μ M JQ1 or 10 μ M ABBV-744, BRD2 knockdown (KD), SARS-CoV-2 protein E overexpression (OE), COMP KD and ACE2 KD. The quasi-likelihood method glmQLFTest was implemented in edgeR. *P* values were adjusted for multiple comparisons using the Benjamini–Hochberg method. The heatmap shows the log₂-fold change (log₂FC) relative to untreated controls for each condition (columns) for genes (rows) among the top 50 DEGs (by *P* values) in at least one condition. ACE2 was not among these genes and is shown as a separate row. Inset: a cluster of genes downregulated upon both BRD2 inhibition and BRD2 knockdown. Among these, genes associated with the Gene Ontology (GO) term ‘Cellular Response to Type I interferon’ are marked by black circles. **b**, Significantly enriched (FDR < 0.05) GO biological process terms for the genes shown in the inset in **a**. **c**, Enrichment analysis for genes in the inset in **a** reveals COVID-19-related gene sets. Genes associated with a gene set are marked in blue. **d**, Calu-3 cells expressing sgRNAs knocking down genes essential for interferon signalling assayed for ACE2 gene expression relative to ACTB by qPCR. Average ACE2 mRNA level relative to non-targeting control (NTC) sgRNA and the standard deviation of *n* = 3 biological replicates are shown for each condition. **e**, WT (dark grey) or BRD2 KD (light grey) Calu-3 cells were treated with IFN- β , and transcript levels of ACE2 relative to ACTB were quantified at 72 h post-treatment by qPCR. Average ACE2 levels relative to vehicle-treated and the standard deviation of *n* = 3 biological replicates are shown for each condition. **f**, Primary human bronchial epithelial cells (left) and human iPSC-derived cardiomyocytes (right) were treated with the indicated concentrations of IFN- β , and transcript levels of ACE2 relative to ACTB (for Calu-3 and primary human bronchial epithelial cells) or 18S rRNA (cardiomyocytes) were quantified at 72 h post-treatment by qPCR. Average ACE2 mRNA levels relative to WT and the standard deviation of *n* = 3 biological replicates are shown for each condition.

(Fig. 4a,b). Furthermore, the genes downregulated by both BRD2 knockdown and inhibition were strongly enriched in genes induced by SARS-CoV-2 infection in patient and cultured cells (Fig. 4c).

These findings are compatible with two distinct mechanisms: BRD2 could independently regulate ACE2 and SARS-CoV-2-induced interferon response genes, or BRD2 could mediate the response to interferon, which in turn regulates ACE2 transcription. ACE2 expression has been reported to be induced by interferons in some studies^{1,2}. Other studies, however, suggest that interferon suppresses ACE2 expression³.

In Calu-3 cells, disruption of basal interferon signalling, via knockdown of the genes essential for interferon signal transduction—*IRF9*, *STAT1* or *IFNAR1*—abrogated ACE2 expression (Fig. 4d and Extended Data Fig. 5). Conversely, treatment with exogenous interferon- β (IFN- β) stimulates ACE2 expression in a concentration-dependent manner. Upon BRD2 knockdown, however, this concentration-dependent increase in ACE2 expression is inhibited (Fig. 4e). Thus, BRD2 is required for interferon-induced ACE2 expression. Treatment with IFN- β similarly strongly increased ACE2 mRNA levels in primary human bronchial epithelial cells, but reduced ACE2 mRNA levels in human iPSC-derived cardiomyocytes (Fig. 4f), suggesting that the effect of interferons on ACE2 can be context-dependent.

To test whether BRD2 is a direct transcriptional regulator of ACE2, we performed CUT&RUN³³ to comprehensively map genomic loci bound by BRD2 in Calu-3 cells (Supplementary Table 3). CUT&RUN is similar to chromatin immunoprecipitation sequencing (ChIP-seq), as it measures the occupancy of factors bound to DNA, but has the advantage of higher sensitivity and a lower requirement for cell numbers³³. Genes adjacent to BRD2-bound sites detected in our experiment showed a highly significant overlap with BRD2-bound sites previously mapped by ChIP-seq in NCI-H23³⁴ cells, another lung epithelium-derived

cancer cell line (Fig. 5a). To further validate our CUT&RUN analysis, we performed binding and expression target analysis³⁵ (BETA) to uncover direct BRD2 targets that were differentially expressed upon BRD2 knockdown, and identified several interferon response genes as direct BRD2 targets that were downregulated (Fig. 5b). We verified that a previously described³⁴ BRD2 binding site upstream of *PVT1* was also detected in our experiment (Fig. 5c). We also mapped a BRD2 binding site upstream of the several interferon-stimulated genes (ISGs), including *IRF9*, *STAT1* and *MX1* (Fig. 5d). Although there was some signal in the WT background at the ACE2 locus that is decreased in BRD2 knockdown cells, there were no peaks as determined by the peak calling algorithm (Fig. 5e), suggesting that BRD2 is not a direct transcriptional regulator of ACE2 expression.

We also performed CUT&RUN for histone H2A.Z, which was previously reported to modulate the magnitude of ISG expression and thus connect BRD2 activity to interferon stimulation³⁶. We found decreased H2A.Z occupancy at ISGs in BRD2 knockdown cells (Fig. 5d), recapitulating the role of BRD2 as a potential chaperone of H2A.Z. These results support a model in which BRD2 controls the transcription of key interferon response genes, which can in turn induce ACE2 transcription in some cell types (Fig. 5f). Alternatively, ACE2 expression may be controlled by other genes that are expressed in a BRD2-dependent, interferon-stimulated manner (Fig. 5f).

ABBV-744 reduces infection in primary cells and in vivo. We then tested whether ABBV-744, a bromodomain inhibitor currently in clinical trials, could reduce SARS-CoV-2 infection and infection-associated phenotypes in more physiological models.

First, we investigated a human nasal epithelial model³⁷. We treated reconstituted nasal epithelia maintained in air/liquid interface conditions with 100 nM and 300 nM ABBV-744 (above the reported half-maximum inhibitory concentration (IC₅₀) of 4–18 nM)

Fig. 5 | BRD2 directly regulates transcription of interferon-induced genes. **a**, Genes associated with BRD2 CUT&RUN peaks within 10 kb of a transcription start site determined in this study in Calu-3 cells overlap significantly with published BRD2 ChIP-seq peaks from the indicated datasets (*P* < 0.0001, two-sided Fisher’s exact test). **b**, BETA was performed to identify direct BRD2 targets that were differentially expressed upon BRD2 knockdown using the one-tailed Kolmogorov–Smirnov test. Many interferon response genes were identified as direct BRD2 targets. Direct BRD2 targets that were downregulated upon BRD2 knockdown were analysed by ENRICH for enriched Reactome pathways. Pathways with adjusted *P* values less than 0.05 are displayed. **c–e**, CUT&RUN experiments were conducted to map BRD2, H2A.Z and H3K4me3 genomic localization in WT (blue traces) and BRD2 knockdown (red) Calu-3 cells. Each trace represents an independent biological replicate. **c**, Known BRD2 regulatory sites are recapitulated. Raw signal tracks for WT and BRD2 knockdown cells are shown at the known BRD2 locus *PVT1*. BRD2 ChIP-seq tracks from human lung cells are shown. BRD2 peaks were called over IgG using SEACR at FDR < 0.05. **d**, Identified BRD2 and histone H2A.Z occupancy and peak calling at ISGs in BRD2 knockdown and WT Calu-3 cells. Raw signal tracks for BRD2, histone H2A.Z and H3K4me3 are shown. BRD2 peaks were called over IgG using SEACR at FDR < 0.05. BRD2 ChIP-seq tracks from human lung cells are also shown. **e**, Raw BRD2 signal tracks and peak calling as for **c**, at the ACE2 locus. **f**, Proposed model for BRD2 control of ACE2 expression.

and performed SARS-CoV-2 or mock infections (Fig. 6a). First, we found that ABBV-744 treatment reduced *ACE2* levels in these conditions (Fig. 6b). Apical supernatants did not show significant changes in viral RNA concentrations at two or four days post-infection (Extended Data Fig. 6a). Intracellular viral RNA concentrations, however, were significantly decreased in the

ABBV-744 conditions (Fig. 6c). Furthermore, epithelial barrier integrity, as measured by transepithelial electrical resistance (Fig. 6d) and cytotoxicity (Fig. 6e), were rescued in infected cells treated with ABBV-744. Thus, ABBV-744 partially inhibited SARS-CoV-2 replication and fully rescued epithelial barrier integrity in a primary human nasal epithelial model.

Fig. 6 | BRD2 inhibitors prevent cytotoxicity and reduce SARS-CoV-2 infection in human primary nasal epithelia and inhibit SARS-CoV-2 infection in Syrian hamsters. **a**, Design for reconstructed human nasal epithelia experiments. **b**, *ACE2* transcript levels relative to the average of *GAPDH*, *TFRC*, *RPL13* and *ACTB* as a function of ABBV-744 concentration and/or SARS-CoV-2 infection (right). The average *ACE2* mRNA level relative to mock infection and vehicle-treated of $n = 3$ biological replicates, with error bars representing the standard deviation, are shown. **c**, Intracellular SARS-CoV-2 gene expression relative to the average of *GAPDH*, *TFRC*, *RPL13* and *ACTB* as a function of ABBV-744 concentration and/or SARS-CoV-2 infection (right). The average of $n = 3$ biological replicates and error bars representing the standard deviation are shown. *P* values were determined using Student's unpaired two-tailed *t*-test. SARS-CoV-2 gene expression relative to mock infection and vehicle-treated are shown. **d**, Transepithelial electrical resistance (TEER), evaluated as a function of ABBV-744 concentration and/or SARS-CoV-2 infection. The average of $n = 4$ biological replicates and error bars representing the standard deviation are shown. *P* values were determined using Student's unpaired two-tailed *t*-test. **e**, Cytotoxicity, as measured by LDH release, evaluated as a function of ABBV-744 concentration and/or SARS-CoV-2 infection. The average of $n = 4$ biological replicates and error bars representing the standard deviation are shown. *P* values were determined using Student's unpaired two-tailed *t*-test. **f**, Experimental design for the Syrian hamster experiments. **g**, Volcano plot showing differentially expressed genes for Syrian hamster lungs infected or not with SARS-CoV-2. A subset of ISGs for which BRD2 peaks were identified by CUT&RUN are labelled. **h**, Volcano plot showing differentially expressed genes for Syrian hamster lungs infected with SARS-CoV-2 and treated with vehicle or ABBV-744 at 100 nM. A subset of ISGs for which BRD2 peaks were identified by CUT&RUN are labelled. **i**, Normalized viral RNA counts for Syrian hamsters infected with SARS-CoV-2 and treated with vehicle or ABBV-744 at 100 nM. The average of $n = 8$ biological replicates and error bars representing the standard deviation are shown.

Next, we tested whether ABBV-744 could reduce SARS-CoV-2 infection in golden Syrian hamsters. Syrian hamsters provide a physiologically relevant model for SARS-CoV-2 infection, with high viral replication and signs of lung involvement^{38–41}. In our hands, hamsters did not show substantial weight loss following SARS-CoV-2 infection (Extended Data Fig. 6b). After 24 h of treatment with ABBV-744 or vehicle, hamsters were infected with SARS-CoV-2 (Fig. 6f) and treated daily with ABBV-744 or vehicle. Three days post-infection, the lungs of hamsters were harvested and subjected to RNA-seq. Infected, but untreated, hamsters showed marked upregulation of a number of genes including ISGs when compared to uninfected controls (Fig. 6g). By contrast, infected hamsters treated with ABBV-744 showed a downregulation of ISG (Fig. 6h) levels relative to vehicle-treated infected hamsters, confirming ABBV-744 activity. Remarkably, viral RNA counts were reduced by about five orders of magnitude in the ABBV-744-treated hamsters versus those treated with vehicle controls (Fig. 6i). Thus, BRD2 inhibition can dramatically decrease SARS-CoV-2 infection in Syrian hamsters.

Discussion

We have demonstrated that BRD2 is necessary for *ACE2* expression in a number of different SARS-CoV-2-relevant systems. We also found that treatment with ABBV-744, a bromodomain inhibitor, can reduce SARS-CoV-2 viral RNA concentrations in primary human nasal epithelial cells and Syrian hamsters. These findings suggest that pharmacological BRD2 inhibitors may be of therapeutic benefit to prevent or reduce the impact of SARS-CoV-2 infection.

Our data suggest that BRD2 is an indirect regulator of *ACE2* transcription and thus viral entry in COVID-19-relevant cell types. This is consistent with two other recent studies that show that BET-domain inhibitors can prevent SARS-CoV-2 infection in culture and reduce *ACE2* gene expression^{42,43}. Our data suggest that BRD2 is the most likely target of the BET-domain inhibitors used in these studies, as opposed to other BET-domain containing proteins, such as BRD4.

We also show that BRD2 is required for interferon-mediated stimulation of *ACE2* expression, as both exogenous interferon stimulation and basal interferon stimulation of *ACE2* expression are blocked upon BRD2 knockdown or pharmacological inhibition (Fig. 6i). Indeed, inhibition of the Janus kinase (required for transduction of interferon signalling) with ruxolitinib has been shown to downregulate *ACE2* levels in cell culture⁴⁴. This does not, however, preclude a more direct, and interferon-independent, regulatory mode.

Our data also show that BRD2 activity is essential for the transcription of ISGs in cell culture and in Syrian hamsters. Based on

our findings and the previous literature³⁶, BRD2 regulation of ISG transcription is probably mediated by a reduction in histone H2A.Z occupancy at these promoters. Intriguingly, these BRD2-dependent effects might be cell-type specific, as one study⁴⁵ has shown that BET inhibition, but not the BET inhibitor ABBV-744, can block cardiac dysfunction due to inflammation caused by SARS-CoV-2 infection. Either way, our data indicate that BRD2 could be a key regulator of the host response to SARS-CoV-2 infection.

The previously described³² interaction between the SARS-CoV-2 E protein and BRD2 might have evolved to manipulate gene expression during infection, including the expression of *ACE2*. In isolation, however, protein E overexpression in Calu-3 cells did not recapitulate expression changes resulting from BRD2 knockdown or inhibition (Fig. 4a). These data suggest that there is no direct effect of protein E on BRD2 function, or that other viral or host factors expressed during SARS-CoV-2 infection are required to modulate BRD2 function. Further studies are needed to define the function of the protein E–BRD2 interaction.

Several previous CRISPR screens aiming to uncover strategies to inhibit SARS-CoV-2 infection were carried out in cell lines in which an *ACE2* transgene was overexpressed^{9,10}. Those screens therefore failed to uncover BRD2 as a regulator of endogenous *ACE2* expression. BRD2 did show a phenotype, however, in a CRISPR screen carried out in Vero-E6 cells (which express *ACE2* endogenously)¹², although it was not further characterized in that study. These differences highlight the importance of conducting CRISPR-based screens in disease-relevant cell types.

There is a growing literature about the relationship between COVID-19 disease severity, *ACE2* expression and interferon regulation^{1–6}. Given that *ACE2* is known to promote recovery after lung injury and that SARS-CoV-2 manipulates the host interferon response^{46–48}, the misregulation of these two pathways may play a major role in enhancing the severity of COVID-19. Our data suggest that BRD2 is central to this regulatory network and therefore pharmacological targeting of BRD2 may be a promising therapeutic strategy for the treatment of COVID-19: BRD2 inhibition could both block viral entry, through *ACE2* downregulation, and act as an ‘emergency brake’ for misregulated patient immune responses to COVID-19, via downregulation of ISGs.

Online content

Any methods, additional references, Nature Research reporting summaries, source data, extended data, supplementary information, acknowledgements, peer review information; details of author contributions and competing interests; and statements of data and code availability are available at <https://doi.org/10.1038/s41556-021-00821-8>.

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Methods

Cell culture. Calu-3 cells were cultured in RPMI 1640 (Life Technologies, 22400-105) with 10% fetal bovine serum (FBS; VWR, 89510-186), 1% pen/strep (Life Technologies, 15140122) and 5 mM glutamine (Life Technologies, 25030081) at 37°C and 5% CO₂. Cells were split by treatment with TrypLE (Life Technologies, 12604013) for 15 min, quenching with media and spinning down at 200g for 5 min.

At Institut Pasteur, where virus infections were carried out, Calu-3 cells were cultured in minimum essential medium (MEM; Gibco, 11095-080) with 20% FBS (Gibco, A3160801), 1% pen/strep (Gibco, 15140-122), 1% non-essential amino acids (NEAA; Sigma-Aldrich, M7145) and 1 mM sodium pyruvate (Sigma-Aldrich, S8636). These were split in trypsin-EDTA 0.05% (Gibco, 11580626).

HEK293 cell culture and the production of lentivirus were performed as previously described⁴⁹.

A vial of short tandem repeat (STR) authenticated Caco-2 cells was obtained from the UCSF Cell and Genome Engineering Core (CGEC). Caco-2 cells were cultured in Eagle's minimum essential medium (EMEM; ATCC, 30-2003) with 20% FBS (VWR, 89510-186), 1% pen/strep (Life Technologies, 15140122) and 5 mM glutamine (Life Technologies, 25030081) at 37°C and 5% CO₂.

A vial of A549 cells was obtained from D. Ruggero's laboratory as a gift. A549 cells were cultured in Dulbecco's modified Eagle medium (DMEM; Thermo Fisher Scientific, 10313-039) with 10% FBS (VWR, 89510-186), 1% pen/strep (Life Technologies, 15140122) and 5 mM glutamine (Life Technologies, 25030081) at 37°C and 5% CO₂.

Human iPSC-derived cardiomyocytes were generated and cultured, as previously described⁵⁰, from AICS90 iPSCs (Allen Institute Cell catalogue). Drugs were added on day 69 of differentiation, and cardiomyocytes were collected for analysis on day 72.

Normal human bronchial epithelia (Mattek NHBE-CRY) were cultured following the supplier's instructions.

Generation of the Calu-3 ACE2 knockout line. The polyclonal ACE2 knockout Calu-3 cell line was generated using the Gene KO kit V2 from Synthego, using three sgRNAs targeting ACE2 with the following protospacer sequences: sRNA1, 5'-GACAUUCUCUUCAGUAAU-3'; sRNA2, 5'-AAACUUGUCCAAAUGUCU-3'; sRNA3, 5'-UUACAGCAACAAGGUGAGA-3'. The sgRNAs were designed according to Synthego's multiguide gene knockout kit⁴⁰. Briefly, two or three sgRNAs are bioinformatically designed to work in a cooperative manner to generate small, knockout-causing fragment deletions in early exons. These fragment deletions are larger than standard indels generated from single guides. The genomic repair patterns from a multiguide approach are highly predictable on the basis of the guide spacing and design constraints to limit off-targets, resulting in a higher-probability protein knockout phenotype.

The ribonucleoprotein complex with a ratio of 4.5:1 of sgRNA and Cas9 was delivered following the protocol of the SE Cell Line 4D-NucleofectorTM X Kit (Lonza, V4XC-1012), using the nucleofection program DS-130 on the Lonza 4D X unit. At 72 h post-transfection, genomic DNA was extracted to serve as the template for PCR amplification of the region that covers the sites targeted by the sgRNAs with the following two primers: ACE2-F, 5'-CTGGGACTCCAAATCA GGA-3'; ACE2-R, 5'-CGCCCAACCAAGTTCAAAG-3'. Sanger sequencing reactions using the sequencing primer ACE2-seq 5'-CAAAATCAGGGATATGG AGGCAACATC-3' were then performed, and the knockout efficiency was determined to be 80% with ICE software from Synthego⁵¹ (v2, <https://ice.synthego.com/#/>).

Generation of the Calu-3 CRISPRi line. The parental Calu-3 line was obtained from the UCSF Cell and Genome Engineering Core. Calu-3 cells were cultured at 37°C with 5% CO₂ in EMEM medium containing 10% FBS, 100 U ml⁻¹ streptomycin, 100 µg ml⁻¹ penicillin and 2 mM glutamine. To generate the CRISPRi lines, ~3 × 10⁶ cells were seeded into medium containing lentiviral particles packaging dCas9-BFP-KRAB under a UCOE (universal chromatin opening element)-SFFV (spleen focus-forming virus) promoter⁵². Five days post-infection, blue fluorescent protein (BFP)-positive cells were sorted using BD Fusion. To validate the CRISPRi line, Calu-3-CRISPRi cells were transduced with lentiviral particles expressing non-targeting sgRNA (protospacer 5'-GCTCCAGTCGGC ACCACAG-3') or CD81-targeting sgRNA (protospacer 5'-GGCTGGCAGGATG CGCGG-3'). CD81 expression was measured seven days post-transduction by dislodging cells with TrypLE and live cells were stained with Allophycocyanin (APC)-conjugated anti-human CD81 antibody (Biolegend, 349509). CD81 expression was assessed on a BD LSRII system with >90% of Calu-3 CRISPRi cells with CD81 knocked down compared to a non-targeting sgRNA control.

Spike-RBD binding assay. Recombinant biotinylated SARS-CoV-2 spike Spike-receptor-binding domain with a C-terminal human IgG Fc domain fusion (referred to as Spike-RBD) was prepared as previously described⁴⁸. Calu-3 cells were grown in 96-well flat-bottom plates until >50% confluence. Medium was aspirated and cells were washed once with phosphate-buffered saline (PBS). Cells were then treated with TrypLE to release them from the plate, RPMI 1640 medium was added to dilute TrypLE, and cells were pelleted by centrifugation at 200g for 5 min. From

this point on, all steps were carried out on ice. Cells were incubated in 3% bovine serum albumin (BSA; Sigma-Aldrich, A7030) in Dulbecco's phosphate-buffered saline (DPBS; Sigma-Aldrich, D8537) for 15 min to block, then washed twice in 3% BSA in DPBS by centrifugation at 200g for 5 min in V-bottom plates, followed by resuspension. Spike-RBD was diluted in 3% BSA to the appropriate concentrations and incubated with cells for 30 min on ice. Cells were then washed twice with 3% BSA in DPBS and incubated with anti-strep PE-Cy7 (Thermo Fisher, SA1012) at 5 µg ml⁻¹. Cells were washed twice and subjected to flow cytometry on a FACSCelesta in HTS mode. Cells were gated to exclude doublets and the median PE-Cy7 signal was calculated for each sample. The gating strategy is shown in Supplementary Fig. 2. BD FACSDiva (version 8.01.1) was used to collect flow cytometry data and perform fluorescence activated cell sorting (FACS). EC₅₀ values and their 95% confidence intervals were calculated by fitting the RBD binding data into a Sigmoidal 4PL model in Prism 6.

CRISPRi screen. Calu-3 cells were infected with the H1 CRISPRi sgRNA library²² as described in ref. ⁴⁹ and selected using treatment with 1 µg ml⁻¹ puromycin for 3 days. After selection, cells were stained with 10 nM Spike-RBD as described above or for TFRC as previously described⁴⁹ and subjected to FACS, where cells were sorted into the top 30% and bottom 30% based on high and low expression of TFRC or Spike-RBD. Because of the viability and stickiness known for Calu-3 cells, coverage was lower than optimal, at 200-fold over the library diversity. Sorted populations were spun down at 200g for 5 min, and genomic DNA was isolated as described in ref. ⁴⁹. sgRNA cassettes were amplified by PCR and sequencing and analysis was performed as described in ref. ⁴⁹ but with FDR of 0.1 rather than 0.05 or 0.01, due to noise.

Validation of screening hits. Individual sgRNAs were selected based on phenotypes in the primary screens and cloned into a lentiviral expression vector as described⁴⁹. Protospacer sequences of these sgRNAs are provided in cells expressing sgRNAs were selected using treatment with 1 µg ml⁻¹ puromycin for 3–7 days.

Drug treatments. Drugs (ABV-744, Selleckchem, S8723; JQ1, Sigma-Aldrich, SML1524; dBET-6, Selleckchem, S8762) were dissolved in dimethyl sulfoxide (DMSO) or water according to the manufacturers' instructions. Cells were treated with drugs for 72 h with medium changes performed every 24 h with medium containing fresh drug.

Interferon treatments. IFN-β (R&D Systems, 8499-IF) was dissolved according to the manufacturer's instructions. Cells were treated with IFN-β for 72 h, with medium changes performed every 24 h with medium containing fresh IFN-β.

Quantitative PCR. qPCR was performed and analysed as described in ref. ⁴⁹. Primers: ACE2 forward, GGTCTTCTGTACCCGATTT; ACE2 reverse, CATC CACCTCCACTTCTCTAAC; ACTB forward, ACCTTCTACAATGAGCTGCG; ACTB reverse, CCTGGATAGCAACGTACATGG; IRF9 forward, GCCTACAA GGTGTATCAGTTG; IRF9 reverse, TGCTGTCGCTTTGATGGTACT; IFNAR1 forward, AACAGGAGCGATGAGTCTGTC; IFNAR1 reverse, TGCAGAAATGG TGAAATGAGTCA; STAT1 forward, CAGCTTGACTCAAATTCCTGGA; STAT1 reverse, TGAAGATTACGCTTGCTTTTCCT.

Western blotting. Cells from one confluent well of a six-well plate were lysed in RIPA buffer plus cComplete EDTA-free protease inhibitor tablets (Roche, 11873580001) and spun for 10 min at 21,000g at 4°C. The pellet was removed and a bicinchoninic acid (BCA) assay (Thermo Fisher, 23225) was performed on the remaining supernatant. Lysate volumes with equivalent protein content were diluted with sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) loading dye and subjected to gel electrophoresis on 4–12% BisTris SDS-PAGE gels (Life Technologies, NP0322). Gels were then transferred and blocked in 5% non-fat dry milk (NFD) for 1 h at room temperature. Antibodies in fresh 5% NFD were added (mouse monoclonal GAPDH 1:10,000; goat polyclonal ACE2, R&D Tech AF933, 1:200; rabbit monoclonal BRD2, Abcam 197865, 1:5,000) and incubated at 4°C for at least 16 h. Membranes were washed four times with TBS + 0.1% Tween-20 and incubated with secondary antibodies (1:10,000 donkey anti-goat-800 (LI-COR, 926-32214), donkey anti-mouse-680 (LI-COR, 926-68072) and horse radish peroxidase (HRP) donkey anti-rabbit (CST, 7074P2)). Membranes were visualized using a LI-COR or Femto HRP kit (Thermo Fisher, 34094). Uncropped images of western blots are provided as Supplementary Fig. 1.

Virus. The SARS-CoV-2 strain used (BetaCoV/France/IDF0372/2020 strain) was propagated once in Vero-E6 cells and was a gift from the National Reference Centre for Respiratory Viruses at Institut Pasteur, Paris, originally supplied through the European Virus Archive goes global platform.

Cytotoxicity measurements of Calu-3 cells. Thirty-thousand Calu-3 cells per well were seeded into Greiner 96-well white-bottom plates and incubated for 48 h at 37°C and 5% CO₂. The cells were then treated with identical drug concentrations as in the infection assays, for five days, by refreshing the medium with 100 µl per

well fresh drug-containing medium every 24 h. Cell viability was then assayed by adding 100 µl per well of CellTiter-Glo 2.0 (Promega) and incubated for 10 min at room temperature. Luminescence was recorded with an Infinite 200 Pro plate reader (Tecan) using an integration time of 1 s.

Virus infection assays. Thirty-thousand Calu-3 cells per well were seeded into 96-well plates and incubated for 48 h at 37 °C and 5% CO₂. At the time of infection, the medium was replaced with virus inoculum (multiplicity of infection of 0.1 plaque-forming units (p.f.u.) per cell) and incubated for 1 h at 37 °C and 5% CO₂. Following the 1-h adsorption period, the inoculum was removed, replaced with fresh medium, and the cells incubated at 37 °C and 5% CO₂. At 24 h, 48 h and 72 h post-infection, the cell-culture supernatant was collected, and the viral load was assessed by RT-qPCR as described previously³². Briefly, the cell-culture supernatant was collected, heat-inactivated at 95 °C for 5 min, and used for RT-qPCR analysis. SARS-CoV-2-specific primers targeting the N gene region—5'-TAATCAGACAAGGAAGTATTA-3' (forward) and 5'-CGAAGGTGTGACTTCCATG-3' (reverse)—were used with the Luna Universal One-Step RT-qPCR Kit (New England Biolabs) in an Applied Biosystems QuantStudio 6 thermocycler or an Applied Biosystems StepOnePlus system, with the following cycling conditions: 55 °C for 10 min, 95 °C for 1 min, and 40 cycles of 95 °C for 10 s, followed by 60 °C for 1 min. The number of viral genomes is expressed as p.f.u. equivalents per ml, and was calculated by performing a standard curve with RNA derived from a viral stock with a known viral titre.

Plaque assays. Viruses were quantified by plaque-forming assays. For this, Vero-E6 cells were seeded in 24-well plates at a concentration of 1×10^5 cells per well. The following day, tenfold serial dilutions of individual virus samples in serum-free DMEM medium were added to infect the cells at 37 °C for 1 h. After the adsorption time, a solid agarose overlay (DMEM, 10% (vol/vol) PBS and 0.8% agarose) was added. The cells were incubated for the further three days before fixation with 4% formalin and visualization using crystal violet solution.

CUT&RUN. CUT&RUN was performed with one million Calu-3 cells. Cells were removed from the plate by treatment with Versene (Life Technologies 15040066) for 20 min and resuspended in fresh medium. They were then spun down and washed twice with DPBS before proceeding with the CUTANA CUT&RUN kit (EpiCypher, 14-0050). The experiment was performed with the included IgG and H3K4Me control antibodies and the BRD2 antibody (Abcam 197865) as well as *Escherichia coli* spike-in DNA according to the kit protocol.

QuantSeq analysis. Raw sequencing reads from QuantSeq were trimmed using Trimmomatic⁵³ (v0.39, PMID 24695404) and mapped to the human reference transcriptome (GRCh38, GENCODE Release 36) using Salmon⁵⁴ (v1.3.0) to obtain transcript abundance counts. Gene-level count estimates were obtained using tximport⁵⁴ (v1.18.0) with default settings. Subsequently, differential gene-expression analyses were performed using the glmQLFTest method implemented in the edgeR package⁵⁴ (v3.28.1). Cluster⁵⁵ (v3.0) was used for hierarchical clustering and Java TreeView⁵⁶ (v1.1.6r4) for visualization.

CUT&RUN analysis. CUT&RUN analysis was performed as previously described⁵⁷. Briefly, paired-end reads were mapped to the human genome GRCh38 using Bowtie2 (v2.3.4.1) with the options --end-to-end --very-sensitive --no-unal --no-mixed --no-discordant --phred33 -I 10 -X 1000. Sparse enrichment analysis for CUT&RUN (SEACR⁵⁸, <https://seacr.fredhutch.org/>) was used for peak calling. H3K4me3 and BRD2 peaks were normalized to IgG control. Published BRD2 ChIP-seq data in human lung cells were obtained from ChIP-Atlas (<https://chip-atlas.org/>). The Integrative Genomics Viewer (IGV, igv.org) was used for visualization.

SARS-CoV-2 infection of reconstructed human nasal epithelia. MucilAir (<https://www.epithelix.com/products/mucilair>), corresponding to reconstructed human nasal epithelium cultures differentiated in vitro for at least four weeks, was purchased from Epithelix. Epithelix obtains donor consent and this study does not involve any human participants. The cultures were generated from pooled nasal tissues obtained from 14 human adult donors. Cultures were maintained in air/liquid interface (ALI) conditions in transwells with 700 µl of MucilAir medium (Epithelix) in the basal compartment, and kept at 37 °C under a 5% CO₂ atmosphere. SARS-CoV-2 infection was performed as previously described³⁷. Briefly, the apical side of ALI cultures was washed for 20 min at 37 °C in Mucilair medium (+/- drug) to remove mucus. Cells were then incubated with 10^4 p.f.u. of the isolate BetaCoV/France/IDF00372/2020 (EVAg collection, Ref-SKU 014V-03890; provided by S. Van der Werf). The viral input was diluted in DMEM medium (+/- drug) to a final volume 100 µl, and left on the apical side for 4 h at 37 °C. Control wells were mock-treated with DMEM (Gibco) for the same duration. Viral inputs were removed by washing twice with 200 µl of PBS (5 min at 37 °C) and once with 200 µl of Mucilair medium (20 min at 37 °C). The basal medium was replaced every 2–3 days. Apical supernatants were collected every 2–3 days by adding 200 µl of Mucilair medium on the apical side, with an incubation of 20 min at 37 °C before collection.

For ABBV-774 treatment, cultures were pretreated for four days with 100 nM or 300 nM of the drug. For this pretreatment, ABBV-774 was added to an apical wash at day -4 and to the basal compartment from day -4 to day 0. The drug was then added on the apical side during viral adsorption at day 0, and then every 2–3 days to both the apical wash and the basal compartment throughout the infection.

Transepithelial electrical resistance measurements. The apical side of transwell cultures was washed for 20 min at 37 °C in Mucilair medium. The transwells were then transferred in a new 24-well plate and DMEM was added to both the apical (200 µl) and basal (700 µl) sides. The transepithelial electrical resistance was then measured using an Evom3 ohm meter (World Precision Instruments).

Lactate dehydrogenase cytotoxicity assay. Diluted culture supernatants (1:25) were pretreated with Triton-X100 1% for 2 h at room temperature for viral inactivation. Lactate dehydrogenase (LDH) dosage was performed using the LDH-Glo cytotoxicity assay kit (Promega) following the manufacturer's instructions. Luminescence was measured using an EnSpire luminometer (Perkin Elmer).

Viral RNA quantification. Apical supernatants were stored at -80 °C until thawing and were diluted fourfold in PBS for quantification in a 96-well PCR plate. Supernatants were then inactivated for 20 min at 80 °C. One microlitre of supernatant was added directly to 4 µl of PCR reaction mix for SARS-CoV-2 RNA quantification. PCR was carried out in a final volume of 5 µl per reaction in 384-well plates using the Luna Universal Probe One-Step RT-qPCR kit (New England Biolabs) with SARS-CoV-2 N-specific primers (forward, 5'-TAA TCA GAC AAG GAA CTG ATT A-3'; reverse, 5'-CGA AGG TGT GAC TTC CAT G-3') on a QuantStudio 6 Flex thermocycler (Applied Biosystems). Standard curves were established in parallel using purified SARS-CoV-2 viral RNA.

Tissue RNA quantification. ACE2 and SARS-CoV-2 expression levels were quantified in epithelial cells by real-time qPCR. The epithelial cultures were washed in ice-cold PBS and then lysed in 150 µl of Trizol reagent (Thermo Fisher Scientific) added to the apical side of the insert for 5 min. RNA was purified using the Direct-zol miniprep kit (ZR2080, Zymo Research). Transcripts of genes of interest (ACE2, SARS-CoV-2 N gene) were amplified in a final volume of 5 µl per reaction in 384-well plates using the Luna Universal Probe One-Step RT-qPCR kit (New England Biolabs) on a QuantStudio 6 Flex thermocycler. The RT-qPCR results were normalized to the mean expression of four reference genes (*GAPDH*, *TFR*, *ALAS1* and *RLP13*) to compute relative gene expression, as described previously³⁷. The ACE2 primers used were ACE2-For 5'-TGG GAC TCT GCC ATT TAC TTA C-3' and ACE2-Rev 5'-CCA GAG CCT CTC ATT GTA GTC T-3'.

In vivo infections. All animal infections were conducted at the Icahn School of Medicine at Mount Sinai, in the biosecurity level 3 (BSL-3) facility of the Global Health and Emerging Pathogen Institute approved by the Institutional Animal Care and Use Committee at Icahn School of Medicine at Mount Sinai under protocol number IACUC#20-0743. Six- to eight-week-old male golden Syrian hamsters (*Mesocricetus auratus*) were purchased from Jackson Laboratories, housed in pairs and fed ad libitum. On the day of infection, animals were anaesthetized by administration of 100 µl of a ketamine HCl/xylazine (4:1) mix by intraperitoneal injection and infected intranasally with 1,000 p.f.u. of SARS-CoV-2 USA-WA1/2020 diluted in 100 µl of PBS. At the indicated time points, animals were treated with 1 ml of ABBV-774 by oral gavage. The drug was prepared fresh daily to a final concentration of 20 mg kg⁻¹ in 0.5% hydroxypropyl methylcellulose/0.5% Tween 80 in water. On day 3 after infection, animals received 100 µl of a mix of pentobarbital/PBS (1:4) intraperitoneally and, once anaesthetized they were cervically dislocated and lung lobes collected in 1 ml of Trizol reagent. Tissues were homogenized in a tissue lyser for two cycles of 40 s, spun down for 5 min at 8,000g, and the supernatants were stored at -80 °C for plaque assay or RNA extraction.

RNA extraction. RNA extraction was performed following the instructions from the manufacturer of TRIzol reagent (Invitrogen). Briefly, a 1/5 volume of chloroform was added to the lung supernatants in TRIzol, phases were separated by centrifugation and RNA was precipitated by overnight incubation with isopropanol at -20 °C. The RNA pellet was washed with ethanol 70% and resuspended in RNase-free water. RNA was quantified by NanoDrop and resuspended to a final concentration of 100 ng µl⁻¹ in water.

RNA-seq. One microgram of RNA was used as the starting material for library preparation. The kit employed was a TruSeq RNA Library Prep kit v2 (Illumina) over polyadenylated RNA and the manufacturer's instructions were followed. The sequencing was performed on an Illumina NextSeq 500 instrument. The raw reads obtained from the run were aligned against the Syrian golden hamster genome (MesAur1.0) in the BaseSpace platform by Illumina, with the tool 'RNA-Seq Alignment'.

Statistics and reproducibility. All experiments were carried out using standard cell biological/biochemical techniques with pre-validated reagents including

cell lines. Accordingly, the minimum standard of at least three replicates per experiment was used for all experiments, with the following exceptions. In Fig. 1a,b we report the dose responses of different cell types to Spike protein where each dose response has data points for seven doses. These experiments were performed once. In Extended Data Fig. 1 we report a dose response with data points for two doses. These experiments were performed once. In Extended Data Fig. 2 we report measured dose responses where each dose response was performed in duplicate and with data points for seven to eight doses. Experiments that were performed with $n = 1$ are reported in Figs. 1c,e,f, 2c and 3b. No statistical method was used to predetermine sample size. No data were excluded from analyses. Experiments were not randomized. Investigators were not blinded to allocation during experiments and outcome assessment.

Reporting Summary. Further information on research design is available in the Nature Research Reporting Summary linked to this Article.

Data availability

Sequencing data are available from the NCBI Gene Expression Omnibus (GEO) with the following accession numbers: GSE165025 (RNA-sequencing data associated with Fig. 4), GSE182993 (CUT&RUN data associated with Fig. 5) and GSE182994 (RNA-sequencing data associated with Fig. 6f–h). Previously published BRD2 ChIP-seq data that were re-analysed here are available under accession codes GSE113714 and GSE104481. All other data supporting the findings of this study are available from the corresponding author on reasonable request. Source data are provided with this paper.

Code availability

Analysis of the CRISPRi screen results was carried out using custom code (MAGECK-iNC) developed in the Kampmann laboratory. This has been described previously⁴⁹ and is freely available at <https://kampmannlab.ucsf.edu/mageck-inc>.

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Author contributions

R.T., A.J.S. and M.K. conceptualized the overall project, analysed the results and prepared the manuscript, with input from all co-authors. V.V.R., A.M.K. and Q.D.T. performed and analysed live-virus experiments in Calu-3 cells, with guidance from M.V. R.R. performed and analysed human nasal epithelia experiments with guidance from L.A.C. L.C. performed and analysed the Syrian hamster experiments, with guidance from B.R.T. G.N.R. and S.J.R. performed and analysed the experiments with cardiomyocytes, with guidance from B.R.C. R.T., A.J.S., M.C. and X.G. performed and analysed all other experiments, with guidance from M.K. J.W. performed and analysed basal interferon signalling knockdown experiments in Calu-3 cells, with guidance from R.T. N.L. analysed the QuantSeq data, with guidance from R.T. S.A.L., I.L. and J.A.W. generated Spike-RBD. J.K.N. and J.S.W. generated the Calu-3 CRISPRi cell line. J.C.-S., J.O., T.M. and K.H. designed and provided sgRNAs to generate the ACE2 knockout cell line.

Competing interests

J.C.-S., J.O., T.M. and K.H. are employees and shareholders of Synthego Corporation. All other authors declare no competing interests.

Additional information

Extended data is available for this paper at <https://doi.org/10.1038/s41556-021-00821-8>.

Supplementary information The online version contains supplementary material available at <https://doi.org/10.1038/s41556-021-00821-8>.

Correspondence and requests for materials should be addressed to Ruilin Tian or Martin Kampmann.

Peer review information *Nature Cell Biology* thanks Andrew Bowie, Ke Lan and the other, anonymous, reviewers for their contribution to the peer review of this work.

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Extended Data Fig. 1 | Calu-3 cells bind Spike-RBD specifically and were engineered to express CRISPRi machinery enabling CRISPRi screening.

a, Spike-RBD binding in different cell types at 20 nM and 200 nM Spike-RBD was quantified by flow cytometry. **b**, Expression of CRISPRi machinery (dCas9-BFP-KRAB) in the CRISPRi Calu-3 line indicated by the expression of BFP by flow cytometry. **c**, Enrichment of sgRNAs targeting specific genes (coloured dots) or non-targeting control sgRNAs plotted against the negative log of the P-value with a FDR of 0.1 shown (dashed lines).

Extended Data Fig. 2 | Individual sgRNA re-test of screening hits. a-i, Spike-RBD signal measured by flow-cytometry as a function of Spike-RBD concentration. Blue lines represent cells expressing the sgRNA targeting the gene of interest, black lines represent un-transduced control cells in the same well. Average of two technical replicates are shown.

Extended Data Fig. 3 | BRD2 is effectively knocked down by CRISPRi. Western blot for BRD2 and the loading control GAPDH in CRISPRi Calu-3 cells expressing no sgRNA or sgRNAs targeting ACE2 or BRD2. Three lanes represent samples from three independent wells.

Extended Data Fig. 4 | Non-toxic concentration range of BRD2 inhibitors. **a**, Calu-3 cells were treated with vehicle or the indicated concentrations of JQ1 or ABBV-744 for 5 days. Cell viability was then assayed with CellTiter-Glo 2.0 to calculate viability. Error bars represent the standard deviation of four biological replicates. Treatments are relative to untreated cells. **b**, Human iPSC-derived cardiomyocytes were treated for 72 hours with vehicle or the indicated concentrations of JQ1 or ABBV-744, and the percentage of dead cells was quantified as the ratio of propidium iodide-positive cells (dead cells) over Hoechst-positive cells (all cells). Error bars represent the standard deviation of three biological replicates (six biological replicates for the vehicle condition). **c**, Primary human bronchial epithelial (NHBE) cells were treated with ABBV-744 at the indicated concentrations for 72 hours and toxicity was assessed using CellTiter-Glo 2.0. Error bars represent the standard deviation of four biological replicates. P-values determined using Mann-Whitney two-tailed test. Treatments are relative to vehicle cells.

Extended Data Fig. 5 | Validation of knockdown of interferon regulators by CRISPRi. a-c, Calu-3 cells expressing sgRNAs knocking down genes essential for interferon signal transduction assayed for transcript levels of sgRNA targets relative to *ACTB* by qPCR. mRNA levels are fraction of control sgRNA. Error is the standard deviation of three biological replicates.

Extended Data Fig. 6 | Viral replication in apical supernatants of reconstructed human nasal epithelia cultures and bodyweight of hamsters throughout the course of SARS-CoV-2 infection. a-c, Calu-3 cells expressing sgRNAs knocking down genes essential for interferon signal transduction assayed for transcript levels of sgRNA targets relative to *ACTB* by qPCR. mRNA levels are fraction of control sgRNA. Error is the standard deviation of three biological replicates. **A**, Apical supernatants of either infected or mock-infected nasal epithelia treated with ABBV-744 at the indicated concentrations or not treated (NT) were isolated and assayed for SARS-CoV-2 N RNA content. Average of four biological quadruplicates are shown with error bars representing the standard deviation. **b**, Hamsters were weighed over the course of SARS-CoV-2 infection and weights were plotted as a percent of bodyweight on the day of infection. Inset, zoom in on body weight percent between 85 and 110 percent.

Reporting Summary

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- ☐ ☒ The exact sample size (n) for each experimental group/condition, given as a discrete number and unit of measurement
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- ☐ ☒ The statistical test(s) used AND whether they are one- or two-sided
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- ☒ ☐ A description of all covariates tested
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Software and code

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Data collection BD FACSDiva (version 8.01.1) was used to collect flow cytometry data and perform FACS.

Data analysis Prism 6 was used for all plotting and data analysis other than the RNA-seq, CRISPRi screen and CUT&RUN data analysis. Flow data was analyzed in FlowJo(v10.4.0). CUT&RUN analysis was performed as previously described. Briefly, paired-end reads were mapped to the human genome GRCh38 using Bowtie2 (v2.3.4.1) with options: --end-to-end --very-sensitive --no-unal --no-mixed --no-discordant --phred33 -I 10 -X 1000. Sparse Enrichment Analysis for CUT&RUN (SEACR, v1.3, <https://seacr.fredhutch.org/>) was used for peak calling. H3K4me3 and BRD2 peaks were normalized to IgG control. Published BRD2 ChIP-seq data in human lung cells was obtained from ChIP-Atlas (<https://chip-atlas.org/>). The Integrative Genomics Viewer (IGV, v2.9.4, igv.org) was used for visualization. Raw sequencing reads from QuantSeq were trimmed using Trimmomatic43 (v0.39) and mapped to the human reference transcriptome (GRCh38, GENCODE Release 36) using Salmon44 (v1.3.0) to obtain transcript abundance counts. Gene-level count estimates were obtained using tximport45 (v1.18.0) with default settings. Subsequently, differential gene-expression analyses were performed using the glmQLFTest method implemented in the edgeR package46(v3.28.1). Cluster47 (v3.0) was used for hierarchical clustering and Java TreeView48 (v1.1.6r4) for visualization. CRISPRi screen data analysis was performed with custom scripts as described in Tian, Ruilin et al. "CRISPR Interference-Based Platform for Multimodal Genetic Screens in Human iPSC-Derived Neurons." Neuron vol. 104,2 (2019): 239-255.e12. doi:10.1016/j.neuron.2019.07.014 using custom scripts, which are freely available at: <https://kampmannlab.ucsf.edu/mageck-inc>. The ICE software from Synthego (v2, <https://ice.synthego.com/#/>) was used to determine CRISPR knockout efficiency. Binding and Expression Target Analysis (BETA, v1.0.7, <http://cistrome.org/BETA/>) was performed to identify direct BRD2 targets that were differentially expressed upon BRD2 knockdown.

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- Accession codes, unique identifiers, or web links for publicly available datasets
- A list of figures that have associated raw data
- A description of any restrictions on data availability

Sequencing data are provided available on NCBI Gene Expression Omnibus (GEO) with the following accession numbers: GSE165025 (RNA sequencing data associated with Fig. 4), GSE182993 (CUT&RUN data associated with Fig. 5), and GSE182994 (RNA sequencing data associated with Fig. 6f-h). Previously published BRD2 ChIP-seq data that were re-analysed here are available under accession code GSE113714 and GSE104481. Source data are provided with this study. All other data supporting the findings of this study are available from the corresponding author on reasonable request.

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Life sciences study design

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Sample size	No sample size calculation was performed. As per standard practice in molecular and cell biology, we generally chose to do at least three replicates for each experiment.
Data exclusions	No data was excluded from analysis
Replication	All attempts at replication were successful. All experimental details necessary to replicate the results in other labs are provided in the Methods section. Experiments were replicated in at least technical triplicate. Most experiments were performed in at least biological triplicate.
Randomization	Randomization does not apply to cell-based experiments, in which large numbers of cells from a given source were partitioned among experimental conditions. Hamsters were randomly assigned to treatment and control groups.
Blinding	This is not relevant to our study, since no subjective rating of data was involved. All results involved equipment-based quantitative measurements rather than human-based evaluation or classification.

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We require information from authors about some types of materials, experimental systems and methods used in many studies. Here, indicate whether each material, system or method listed is relevant to your study. If you are not sure if a list item applies to your research, read the appropriate section before selecting a response.

Materials & experimental systems

n/a	Involved in the study
☐	☒ Antibodies
☐	☒ Eukaryotic cell lines
☒	☐ Palaeontology and archaeology
☐	☒ Animals and other organisms
☒	☐ Human research participants
☒	☐ Clinical data
☒	☐ Dual use research of concern

Methods

n/a	Involved in the study
☒	☐ ChIP-seq
☐	☒ Flow cytometry
☒	☐ MRI-based neuroimaging

Antibodies

Antibodies used	Mouse monoclonal GAPDH (6C5) [Santa Cruz Biotech sc-32233]; Goat polyclonal ACE2 [R&D Tech AF933]; Rabbit monoclonal BRD2 (EPR7642) [abcam 139690]; Donkey Anti-Goat-800 [LICOR 926-32214]; LICOR Donkey Anti-Mouse-680 [LICOR 926-68072]; HRP Donkey anti-rabbit [CST 7074P2] multiple lots of each antibody were used.
Validation	Goat ACE2 [R&D Tech AF933]: in-house validation using a ACE2 knockdown and knockout line. We validated knockdown by qPCR and knockout by digital PCR.

Western blots of those same lines, when compared to WT, showed the absence of a band at the ACE2 molecular weight. 25 citations validation by manufacturer: correct size
 Rabbit monoclonal BRD2 [abcam 139690]:
 validation by manufacturer: knockout validated, <https://www.abcam.com/hrp-brd2-antibody-epr7642-ab198536.html?productWallTab=ShowAll>
 Mouse GAPDH
 validation by manufacturer: 3095 citations, correct size. <https://www.scbt.com/p/gapdh-antibody-6c5>

Eukaryotic cell lines

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Cell line source(s)	Calu3 cells were obtained from the UCSF Cell and Genome Core. A vial of STR authenticated Caco-2 cells was obtained from the UCSF Cell and Genome Engineering Core (CGEC) iPSCs (Allen Institute Cell Catalog: AICS90) HEK293T (ATCC: CRL-3216) NHEBs (Mattek NHBE-CRY) A vial of A549 cells (ATCC: CCL-185) was obtained from Davide Ruggero's lab as a gift.
Authentication	Cells were not authenticated for this study
Mycoplasma contamination	All the cell lines used in this study are not contaminated with mycoplasma. These cell lines were subject to mycoplasma detection once every 3 months using the UNiversal Mycoplasma Detection Kit (ATCC 30-1012KTM)
Commonly misidentified lines (See ICLAC register)	No commonly misidentified lines were used

Animals and other organisms

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

Laboratory animals	Mesocricetus auratus, syrian golden hamster, male, 6-8 weeks old
Wild animals	The study did not involve wild animals
Field-collected samples	The study did not involve samples collected from the field
Ethics oversight	All experimental procedures and protocols were approved by the Institutional Animal Care and Use Committee at Icahn School of Medicine at Mount Sinai under protocol number IACUC#20-0743

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

Flow Cytometry

Plots

Confirm that:

- ☒ The axis labels state the marker and fluorochrome used (e.g. CD4-FITC).
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- ☒ All plots are contour plots with outliers or pseudocolor plots.
- ☒ A numerical value for number of cells or percentage (with statistics) is provided.

Methodology

Sample preparation	Calu3 cells were trypsinized with TrypLE and resuspended in RPMI1640 for flow cytometry
Instrument	BD LFSRFortessa X14
Software	BD FACSDive (Version 8.0.1.1) and FlowJo (Version 10.0.7)
Cell population abundance	Cell populations are differentiated by fluorescent markers. All populations have sufficient events.
Gating strategy	Cells were gated based on FSCA/SSCA to differentiate live from dead cells. This population was then gated based on FSC-W x FSC-A to gate single cells. Gating strategy is shown in supplemental figure 2.

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