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

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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 potently 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 inflammation1–3. Inflammatory signalling, in particular the type I interferon response, is known to be misregulated in the most severely affected patients with COVID-191–7. 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 transgene9,10, 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...
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 cancers. These proteins are known to be master transcriptional regulators and serve to bridge chromatin marks (mostly acetyl-lysines) to the transcriptional machinery. 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
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 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 screens20,21 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 sgRNAs. 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 surface, 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 TRAPR).

**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, with several small-molecule inhibitors in clinical trials, 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 (JQ1 and ABBV-744; the latter is currently in clinical trials NCT03360006 and NCT04454658) and three proteolysis targeting chimeraic (PROTAC) compounds that lead to the degradation of BRD2 (dBEt-64, ARV-7711 and BETd-260).

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**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 µM, ABBV-744 at 10 µM 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 µM, dBEt-6 at 20 nM, ABBV-744 at 0.01–10 µM) 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 BRD2\(^{32}\). 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 regulates ACE2 and SARS-CoV-2-induced host genes. (a) Heatmap showing expression of genes differentially expressed in cells treated with BET-domain inhibitors JQ1 and ABBV-744 or BRD2 CRISPRi knockdown. (b) Volcano plot showing genes differentially expressed in cells treated with BET-domain inhibitors JQ1 and ABBV-744 or BRD2 CRISPRi knockdown. (c) Downregulated genes in ABBV-744, JQ1 and BRD2 KD conditions. (d) Western blot showing expression of ACE2 in Calu-3 cells treated with BET-domain inhibitors JQ1 and ABBV-744 or BRD2 CRISPRi knockdown. (e) Western blot showing expression of ACE2 in Calu-3 cells treated with BET-domain inhibitors JQ1 and ABBV-744 or BRD2 CRISPRi knockdown. (f) Western blot showing expression of ACE2 in primary human bronchial epithelial cells treated with BET-domain inhibitors JQ1 and ABBV-744 or BRD2 CRISPRi knockdown.
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. CUT&RUN 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 ENRICHR 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 (Fig. 5c, d). 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. 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 model. We 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)
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.
a) Graphic illustrating the distribution of Muus, Basal cell, Ciliated cell, and Goblet cell in the Air/liquid interface.

b) Graph showing relative ACE2 mRNA levels:

- Untreated
- Mock
- 0.1 µM ABBV-744
- 0.3 µM ABBV-744

Mock SARS2 - 10^4 p.f.u.

- 0.1 µM ABBV-744
- 0.3 µM ABBV-744

Cd) Graph illustrating SARS-CoV-2 relative gene expression:

- Untreated
- Mock
- 0.1 µM ABBV-744
- 0.3 µM ABBV-744

Teer (cm^2)

- Mock SARS2 - 10^4 p.f.u.
- 0.1 µM ABBV-744
- 0.3 µM ABBV-744

- LDH mU ml^{-1}

- Mock SARS2 - 10^4 p.f.u.
- 0.1 µM ABBV-744
- 0.3 µM ABBV-744

e) Graph showing cytotoxicity:

- Mock SARS2 - 10^4 p.f.u.
- 0.1 µM ABBV-744
- 0.3 µM ABBV-744

f) Diagram indicating the experimental timeline:

- Day -1: ABBV-744 pre-treatment
- Day 0: SARS-CoV-2 infection
- Day 1: ABBV-744 +ABBV-744 (basal medium and apical wash)
- Day 2: +ABBV-744 (apical wash)
- Day 3: +ABBV-744 (basal medium replacement and apical wash)
- RNA extraction

- Day 4: TEER

- RNA-seq

- P = 0.0387
- P = 0.0125

h) Diagram showing the downregulated and upregulated interferon signalling genes with FDR = 0.01:

- FDR = 0.01
- Upregulated genes
- Downregulated genes
- Interferon signalling genes

i) Graph representing viral RNA (normalized counts):

- Vehicle
- ABBV-744

- log_{10} FC (ABBV-744 vs vehicle)
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. 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. 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 transcription of interferon signalling) with ruxolitinib has been shown to downregulate ACE2 levels in cell culture. 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 literature, 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 study 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 described 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. 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), 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. Given that ACE2 is known to promote recovery after lung injury and that SARS-CoV-2 manipulates the host interferon response, 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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Articles
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, A1360801), 1% pen/strep (Gibco, 15140-122), non-essential amino acids (NEAA; Sigma-Aldrich, M7145) and 1 mM sodium pyruvate (Sigma-Aldrich, S8636). These were treated in trypsin-EDTA 0.05% (Gibco, 11806026).

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

A vial of short tandem repeat (STR) authenticated Caco-2 cells was obtained from the UCSC Cell and Genomics 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, 13031-039) 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 described, 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’-GACAUUCUCUUCAGUAAUAU-3’; sRNA2, 5’-AAACUUGGCGAAAGAUGUCU-3’; sRNA3, 5’-UUACAGCAAAACGCGAGA3’. The sgRNAs were designed according to Synthego’s multiguide gene knockout kit50. Briefly, two or three sgRNAs are pre-packaged dCas9-BFP-KRAB under a UCOE (universal chromatin opening enhancer) and a bicistronic G418 selection cassette. The parental Calu-3 line was obtained from the UCSF Cell and Genome Engineering Core (CGEC). Caco-2 cells were cultured in RPMI 1640 medium (Life Technologies, 22400-1187), 10% FBS (VWR., 89510-186), 1% pen/strep (Life Technologies, 15140122) and 5 mM glutamine (Life Technologies, 25030081) at 37°C and 5% CO2. 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 CRISPRi line. The parental Calu-3 line was obtained from the UCSC Cell and Genome Engineering Core. Calu-3 cells were cultured at 37°C with 5% CO2 in EMEM medium containing 10% FBS, 100 μM 1,10-phenanthroline, 100 μg/ml streptomycin, and 1 mM glutamine. To generate the CRISPRi line, ~3 x 104 cells were seeded into medium containing lentiviral particles packaged dCas9-BFP-KRAB under a UCOE (universal chromatin opening element)-SFFV (spleen focus-forming virus) promoter51. Five days post-infection, blue fluorescent protein (BFP)-positive cells were sorted by flow cytometry. 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. 47. sgRNA cassettes were amplified by PCR and sequencing analysis was performed as described in ref. 46 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 described in ref. 44. Proteasome spacers of these sgRNAs are provided in cells expressing sgRNAs were selected using treatment with 1 μg/ml puromycin for 3–7 days.

Drug treatments. Drugs (ABBV-744, Selleckchem, S8723; J1, Sigma-Aldrich, SML1524; IBET-6, Selleckchem, S8762) were dissolved in dimethyl sulfoxide (DMSO) or water according to the manufacturers’ instructions. Cells were treated with drugs for 72h with medium changes performed every 24h 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 72h, with medium changes performed every 24h with medium containing fresh IFN-β.

Quantitative PCR. qPCR was performed and analysed as described in ref. 48, Primers: ACE2 forward, GGTTCTCTTGCACGGATT; ACE2 reverse, CATC CACGGCAACCCTCCTAAAC; ACTB forward, ACCTGAGCAACCTATGAGC; ACTB reverse, CCTGGATAGCAACGTACATGG; RFP forward, GCCCTACAAGGATGTACAG; RFP reverse, TGGTGC GCCGTGTTGATGCT; IFNAR1 forward, AACAAGGCGGATGACTGCT; IFNAR1 reverse, TGGCAAATGG GTAGAATGCTA; STAT1 forward, GACCTTGACTCAATTCTGCA; STAT1 reverse, TGAAGATTCCGTGTTCCTTTCCT.

Western blotting. Cells from one confluent well of a six-well plate were lyzed in RIPA buffer plus Complete EDTA-free protease inhibitor tablets (Roche, 11837580010) and spun for 10 min at 21,000g at 4°C. The pellet was removed and a bicinonic 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 (NFDM) for 1h at room temperature. Antibodies in fresh 5% NFDM 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 16h. Membranes were washed four times with 0.1% Tween-20 and incubated with secondary antibodies (1:10,000 donkey anti-goat, 1:5,000 donkey anti-mouse) (LI-COR, 926-32214, donkey anti-rabbit 6807-0682) and horse radish peroxidase (HRP) donkey anti-rabbit (CST, 70742). Membranes were visualized using a LI-COR or Femto HRP kit (Thermo Fisher, 40949).

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 48h 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 100ul per
well fresh drug-containing medium every 24 h. Cell viability was then assessed 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 well) and incubated for 1 h at 37°C and 5% CO2. Following the 1-h adsorption period, the inoculum was replaced, washed 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 previously. Briefly, the cell culture supernatant was collected, heat-inactivated at 55°C for 5 min, and used for RT-qPCR analysis. SARS-CoV-2 RdRp primers targeting the N gene region—5′-TATACGACAGAGAATGCTA-T3′ (forward) and 5′-CGAAGGTGTGACTCTCATG-3′ (reverse)—were used with the Luna Universal One-Step RT–qPCR Kit (New England Biolabs) on a QuantStudio 6 Flex thermocycler (Applied Biosystems). Standard curves were established in parallel using purified SARS-CoV-2 viral RNA.

**QuantSeq analysis.** Raw sequencing reads from QuantSeq were trimmed using Trimmomatic (v0.39, PMID 24699405) and mapped to the human reference transcriptome (GRCh38, GENCODE Release 36) using Salamon (v1.3.0) to obtain transcript abundance counts. Gene-level count estimates were obtained by using tximport (v1.18.0) with default settings. Subsequently, differential gene-expression analyses were performed using the glmQLFTest method implemented in the edgeR package (v3.28.1). Cluster (v3.1.0) was used for hierarchical clustering and Java TreeView (v1.1.64r) for visualization.

**QuantSeq analysis.** CUT&RUN analysis was performed as previously described. Briefly, paired-end reads were mapped to the genome GRCh38 using Bowtie2 (v2.3.2) with the options ‘-no-mixed --no-discordant –phred33 -I 10 -X 100’. Sparse enrichment analysis for CUT&RUN (SEACR, https://seacr.fredhutch.org/) was used for peak calling. H3K4me and BRD2 peaks were normalized to IgG control. Published BRD2 ChIP-seq data in human lung cells were obtained from ChipAtlas (https://chip-atlas.org/). The Integrative Genomics Viewer (IGV, gvg.org) was used for visualization.

**SARS-CoV-2 infection of reconstructed human nasal epithelium.** MuclAir ([https://www.epithelix.com/products/muclair](https://www.epithelix.com/products/muclair)), 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 prepared 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 MuclAir medium (Epithelix) in the basal compartment, and kept at 37°C under a 5% CO2 atmosphere. SARS-CoV-2 infection was performed as previously described. Briefly, the apical side of ALI cultures was washed for 20 min at 37°C in MuclAir medium (+/− drug) to remove mucus. Cells were then incubated with 100 µl of the isolate BetaCoV/France/IDF00372/2020 (EpiCypher, 14-0050). The experiment was performed with the included IgG and H3K4Me control antibodies and the BRD2 antibody (Abcam 197865) as well as Esherichia coli spike-in DNA according to the kit protocol.

**Tissue RNA quantification.** ACE2 and SARS-CoV-2 expression levels were quantified in epithelial cells by real-time qPCR. The epithelial cultures were cultured 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, TP53, ALAS1 and RPL13) to compute relative gene expression, as described previously. The ACE2 primers used were ACE2-For 5′-TGG GAC TTC GCC ATT TAC CTA C-3′ and ACE2-Rev 5′-CCA GAG CCT CTC ATT A-3′. The primers used were ACE2-For 5′-TGG GAC TTC GCC ATT TAC CTA 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 Ichnan School of Medicine at Mount Sinai, in the biosafety level 3 (BSL-3) facility of the Global Health and Emerging Pathogen Institute approved by the Institutional Animal Care and Use Committee at Ichnan 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-744 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 lungs 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 of the manufacturer of TRIzol reagent (Invitrogen). 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 ml−1 in water.

**RNA-seq.** One microgram of RNA was used as the starting material for library preparation. The kit employed was a TrueSeq RNA Library Prep kit v2 (Illumina) over polyadenylated RNA and the manufacturer’s instructions were followed. The sequencing was performed on an Illumina NextSeq 5000 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,c, 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 (MAcroCK-iNG) developed in the Kampmann laboratory. This has been described previously and is freely available at https://kampmannlab.ucsf.edu/imageck-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. performed and 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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Statistics

For all statistical analyses, confirm that the following items are present in the figure legend, table legend, main text, or Methods section.

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Our web collection on statistics for biologists contains articles on many of the points above.

Software and code

Policy information about availability of computer code

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-liol-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, iga.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 (v3.0.0) was used for hierarchical clustering and Java TreeView48 (v1.1.6r4) for visualization.

CRISPR screen data analysis was performed with custom scripts as described in Tian, Rulin 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/mageek-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.

For manuscripts utilizing custom algorithms or software that are central to the research but not yet described in published literature, software must be made available to editors and reviewers. We strongly encourage code deposition in a community repository (e.g. GitHub). See the Nature Research guidelines for submitting code & software for further information.
Data

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All manuscripts must include a data availability statement. This statement should provide the following information, where applicable:

- 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 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.

Field-specific reporting

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Life sciences study design

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

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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Materials & experimental systems

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- [ ] Antibodies
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Methods

- [ ] Involved in the study
- [ ] ChIP-seq
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Antibodies

- Antibodies used: Mouse monoclonal GAPDH (6CS) [Santa Cruz Biotech sc-32233]; Goat polyclonal ACE2 [R&D Tech AP933]; Rabbit monoclonal BRD2 (EPA7642) [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 AP933]; in-house validation using a ACE2 knockdown and knockout line. We validated knockdown by qPCR and knockout by digital PCR.
**Eukaryotic cell lines**

**Policy information about cell lines**

| 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 AS49 cells (ATCC: CCL-185) was obtained from Davide Ruggiero’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**

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

**Methodology**

| Sample preparation | Calu3 cells were trypsinized with TrypLE and resuspended in RPMI1640 for flow cytometry |
| Instrument | BD LSRSFortessa X14 |
| Software | BD FACSDiva (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.