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Methods and Compositions for Inhibiting Viral Infection

Abstract

A method of treating or inhibiting a viral infection in a human subject, such as a SARS-CoV-2 infection, involves inhibiting in vivo the expression or activity of one or a combination of the subject's genes required for viral infection. Single genes or subsets of genes for inhibition of activity or expression are selected from certain identified genes. Methods of administration of certain known small molecules or other therapeutics which mimic loss of function of the identified genes are provided. Similar methods for conducting screens of host genes required for viral infection are shown.

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Background/Summary

CROSS-REFERENCE TO RELATED APPLICATIONS [0001] This application claims the benefit of the priorities of U.S. Provisional Patent Application No. 63/066,950 filed Aug. 18, 2020, and U.S. Provisional Patent Application No. 63/092,827, filed Oct. 16, 2020, which applications are incorporated herein by reference.

INCORPORATION-BY REFERENCE OF MATERIAL SUBMITTED IN ELECTRONIC FORM [0003] Applicant hereby incorporates by reference the Sequence Listing file labeled “NYGLIPP-118_US_ST25” (created on Jun. 2, 2023, 2,401 bytes).

BACKGROUND OF THE INVENTION

[0004] As of August 2020, SARS-CoV-2 (Severe Acute Respiratory Syndrome-Coronavirus-2), the virus that causes COVID-19, has infected more than 19 million people worldwide and led to the deaths of more than 700,000 people, according to the John Hopkins Research Center (Gardner, 2020). SARS-CoV-2 belongs to the family enveloped viruses known as Coronaviridae and was first reported in late 2019 in China. Over the past two decades, it is the third zoonotic virus to emerge: Compared to the other two coronaviruses, SARS-CoV (2002) and Middle East Respiratory Syndrome (MERS)-CoV (2012), SARS-CoV-2 shows an increased infectivity and lower case-fatality rate, contributing to its wide-spread transmission and resulting in a pandemic (Gates, 2020; Liu et al., 2020). Given that SARS-CoV-2 has already taken a major toll on human life and livelihoods worldwide, many research institutions, governmental organizations and pharmaceutical companies are working to identify antiviral drugs and develop new vaccines. Currently, there are at least 28 vaccines against SARS-CoV-2 in clinical trials and one FDA approved antiviral drug (remdesivir) that acts as an inhibitor of the SARS-CoV-2 viral RNA-dependent RNA polymerase (Beigel et al., 2020; Funk et al., 2020). A recent study identified small molecules that antagonize SARS-CoV-2 replication and infection by testing ~12,000 clinical-stage and FDA-approved inhibitors (Riva et al., 2020).

[0005] SARS-CoV-2 is an enveloped positive-sense RNA virus that relies on host factors for all stages of its lifecycle (Kim et al., 2020; Zhou et al., 2020). The viral envelope is coated by Spike protein heterotrimers that bind to angiotensin converting-enzyme 2 (ACE2) receptor, which is required for SARS-CoV-2 infection (Hoffmann et al., 2020a; Zhou et al., 2020). The Spike protein undergoes proteolytic cleavage that is catalyzed several host proteases, such as furin, TMPRSS2 and Cathepsin L, and can occur in the secretory pathway of the host cell or during viral entry in the target cell. Proteolytic cleavage is considered to be required for activation of Spike that in turn allows for viral-host membrane fusion and release of the viral RNA into the host cytoplasm

(Hoffmann et al., 2020b). Once in the cytoplasm, the virus utilizes the host and its own machinery to replicate its genetic material and assemble new viral particles. Recent proteomic studies have identified hundreds of host genes that directly bind to SARS-CoV-2 viral proteins and have mapped changes in the global protein phosphorylation landscape in response to viral infection, highlighting the interest in better understanding of host-virus genetic dependencies (Bouhaddou et al., 2020; Gordon et al., 2020).

[0006] A continuing need in the art exists for new and effective tools and methods for the treatment and prevention of viruses, including SARs-CoV-2.

SUMMARY OF THE INVENTION

[0007] To better understand host-virus genetic dependencies and find potential therapeutic targets, we performed a genome-scale CRISPR loss-of-function screen in human alveolar basal epithelial cells to identify genes whose loss confers resistance to SARS-CoV-2 viral infection. In the case of SARS-CoV-2, we identify genes whose loss confers resistance to viral infection of human alveolar basal epithelial cells. These gene targets (and inhibitors of these genes) provide new therapies in the battle against COVID-19.

[0008] We validate that these genes reduce SARS-CoV-2 infection using multiple orthogonal cell perturbations (CRISPR knock-out, RNA interference knock-down, and small-molecule inhibitors). For the top gene hits, we explore mechanisms of their antiviral activity using single-cell transcriptomics, flow cytometry and immunofluorescence. In one embodiment, we show that loss of RAB7A reduces viral entry by sequestering ACE2 receptors inside cells through altered endosomal trafficking. Top-ranked genes cluster into distinct pathways, including the vacuolar ATPase proton pump, Retromer, the Commander complex, and the Arp2/3 complex, with multiple genes in each pathway highly enriched in the CRISPR screen.

[0009] We demonstrate that genes required for SARS-CoV-2 infection in vitro also contribute to COVID-19 severity in human patients and that we can use the pooled CRISPR screen to pinpoint likely protein-coding genes responsible for noncoding variants associated with COVID-19 risk in human patients.

[0010] In one aspect, a method of treating or inhibiting a viral infection in a human subject comprises inhibiting in vivo the expression or activity of one or a combination of the subject's genes required for viral infection, wherein said gene or combination is selected from the genes identified in Tables I, II, III or IV. In one embodiment, such inhibition is temporary for the duration of the viral infection, if established, or to suppress susceptibility to viral infection for a short time period when the virus is present in the community.

[0011] In another embodiment, a method of treating, preventing or inhibiting a viral infection in a human subject comprises administering to a mammalian subject in need thereof one or more inhibitors of the activity or expression of said genes or combination of genes. In one embodiment, such inhibition is temporary for the duration of the viral infection, if established, or to suppress susceptibility to viral infection for a short time period when the virus is present in the community. Such inhibitors may be selected from one or a combination of the inhibitors identified in Tables III or IV. In other embodiment, the inhibitor is administered to the subject pre-infection or post-infection at a dosage effective to mimic a loss of function of its corresponding gene.

[0012] In one aspect, a method of treating or inhibiting a viral infection in a human subject comprises inhibiting in vivo the expression or activity of one or a combination of the subject's genes required for SARS-CoV-2 viral infection.

[0013] In one aspect, a method for identifying host target genes required for viral infection targeting lung cells comprising performing a genome-scale loss of function screen as described in the specification.

[0014] In another aspect, in concert with expression quantitative trait loci (eQTLs) and genome-wide association studies (GWAS), the pooled CRISPR screen can pinpoint protein-coding genes responsible for noncoding variants associated with COVID-19 risk in human patients and through

which noncoding variants associated with COVID-19 risk in human patients may function. This method provides a quantitative resource of the impact of the loss of each host gene on response to viral infection for every protein-coding gene in the human genome.

[0015] Still other aspects and advantages of these compositions and methods are described further in the following detailed description of the preferred embodiments thereof.

Description

BRIEF DESCRIPTION OF THE DRAWINGS

[0016] FIG. 1A-1D. A genome-scale CRISPR loss-of-function screen to identify genes that prevent SARS-CoV-2 infection of human alveolar epithelial cells. FIG. 1A is an overview of the genome-scale loss-of-function screen for host factors in human A549.sup.ACE2 cells requires for SARS-CoV-2 infection. Screen was performed by transducing human lung A549-ACE2 cells with CRISPR sequences from a GeCKOv2 genome-wide CRISPR Knockout library used to identify human host genes required for SARS-CoV-2 infection. Six different CRISPR sequences were used/gene to determine if knockout of individual genes produced a consistent result. After 10 days of cell growth, the cells were selected with puromycin and infected with SARS-CoV-2 at a low multiplicity of infection (MOI). Surviving cells were examined for guide RNAs vs. cells which were not infected (Control) by counting sgRNA counts. FIG. 1B shows the percent survival of human A549.sup.ACE2 cells transduced with the GeCKOv2 library with the indicated SARS-CoV-2 viral amount (MOI) at 6 days post-infection. FIG. 1C is a scatterplot of guide RNA read counts from A549.sup.ACE2 cells at 6 days post-infection with SARS-CoV-2 (MOI~0.01) versus cells prior to infection. Read counts are normalized log₂ reads. FIG. 1D is a volcano plot of median fold-change of guide RNAs for each gene and log₁₀ Robust Rank Aggregation (RRA) p-values. All genes with |fold-change|>4 and RRA p<10⁻³ are labeled. 54% of the top 50 ranked genes overlapped between the MOI 0.01 and MOI 0.3 screen.

[0017] FIG. 2 is a schematic of SARS-CoV-2 docking, entry, RNA genome release and transcription, and virion replication, assembly and release with top-ranked host genes from the CRISPR screen highlighted in red. All genes shown are ranked in the top 50 genes (top ~0.25% of library) in the low MOI CRISPR screen using Robust Rank Aggregation.

[0018] FIGS. 3A-3E demonstrate that enriched genes cluster into related pathways, are expressed broadly, interact directly with viral proteins, and are also involved in viral pathogenesis of pandemic flu and ZIKA. FIG. 3A is a classification of genes shown in FIG. 2 (top-ranked ~0.25% of the GeCKOv2 library) into specific complexes. FIG. 3B is a gene set enrichment analysis normalized enrichment scores for all significant (FDR q<0.1) Gene Ontology (GO) biological processes. FIG. 3C is the expression of top-ranked genes (same as in FIG. 3A) across the indicated human tissues from GTEx v8. Gene expression color scale is transcripts per million (TPM). FIG. 3D shows the RRA fold-change for the low MOI CRISPR screen for the high-confidence protein-protein interaction with the maximum fold-change for each viral gene from the Gordon et al. mass spectrometry dataset (2020). FIG. 3E is a clustering of top-ranked GO biological processes for CRISPR screens for ZIKA (Y. Li et al., 2019), H1N1 pandemic avian influenza IAV (B. Li et al., 2020), and SARS-CoV-2 (this study).

[0019] FIGS. 4A-4E show arrayed validation of genome-scale SARS-CoV-2 screen and identification of druggable gene targets. FIG. 4A is a quantification of SARS-CoV-2 infected A549.sup.ACE2 knockout lines immuno-stained with N protein as shown in A. Each gene was targeted with 3 different guide RNAs represented as diamond symbols (n=3 biological replicates, error bars indicate s.e.m.). The graph showing the SARS-Cov-2 CRISPR KO Screen arrayed validation, plotting % virus infected cells resulting from knocking out (inhibiting or suppressing expression of) the human genes expressed by lung cells. The genes identified at the left hand side

of the graph showed the greatest impact on infectivity when suppressed. (NT) refers to no perturbation/control. FIG. 4B shows the correlation of log 2 median fold change from the genome-scale CRISPR screen (low MOI) and percent of infected cells after individual (arrayed) gene perturbation shown in FIG. 4A. FIG. 4C shows druggable genes found in the Drug Gene Interaction database (DGIdb) among top 500 genes the genome-scale CRISPR screen ranked by RRA p-value. FIG. 4D shows the quantitative PCR (qPCR) of SARS-CoV-2 viral load present in A549.sup.ACE2 cells pretreated with 10 μ M for 2 hours of indicated small molecule inhibitors and then infected with SARS-CoV-2 at MOI of 0.1. The qPCR was performed on cells collected at 36 hours post-infection (hpi). Red bars indicate inhibitors that yield a greater than 100-fold reduction in viral load. Bars with hatch marks indicate an unreliable viral load measurement due to a large reduction in cell viability (see FIG. 4E). Inhibitors were maintained at the same concentration throughout the experiment (n=6 biological replicates, error bars indicate s.e.m.). FIG. 4E is a percent of A549.sup.ACE2 viable cells following inhibitor treatments at 10 μ M for 36 hpi determined using LIVE/DEAD stain and flow cytometry. Bars with red hatch marks indicate that inhibitor treatment had a large impact on viability (<90% viability). Significance testing for panels B, E, and F (data not shown).

[0020] FIG. 5A is a schematic of pooled CRISPR perturbations with Expanded CRISPR-compatible Cellular Indexing of Transcriptomes and Epitopes by sequencing (ECCITE-seq). FIG. 5B is a UMAP visualization of non-targeting and gene-perturbed cells after Linear Discriminant Analysis (LDA), revealing perturbation-specific clustering. FIG. 5C is a single-cell mRNA expression heatmap showing all differentially upregulated genes (adjusted p-value <0.01) for each target gene perturbation. Labeled genes were either a top-ranked gene from the genome-wide CRISPR screens (red) or among the top 5 differentially-expressed genes in any gene perturbation (black). FIG. 5D is a heatmap summarizing Gene Set Enrichment Analysis results for genes upregulated in any of the indicated target gene KO cells (all genes with p-value <0.01 and with a limit to the 300 most differentially-expressed genes; all enriched pathways with adjusted p-value <10⁻¹³).

[0021] FIG. 6A-6F demonstrate RAB7A loss results in a reduced cell surface expression and an increased endosomal accumulation of ACE2. FIG. 6A shows representative histograms of flow cytometry analysis to determine cell surface expression of ACE2 on A549 cell lines (A549 wild type (WT), A549.sup.ACE2, and ACE2 with Cas9 and non-targeting (NT) or RAB7A-targeting guide RNAs). The dashed line indicates the gate between the ACE2 negative and positive cells. FIG. 6B is a quantification of cell surface expression of ACE2 across indicated A549 cell lines. ACE2 expression level was normalized across all samples to the A549.sup.ACE2 cells targeted with non-targeting (NT) guides (n=2-3 gRNA-transduced lines per gene, error bars are s.e.m.). FIG. 6C shows the percent of cells with ACE2 accumulation in vesicles in NT and RAB7A-transduced A549.sup.ACE2 cells (n=2 biological replicates, error bars are s.e.m.). FIG. 6D shows representative histograms of flow cytometry analysis to determine cell surface expression in Calu-3 cells. The dashed line indicates the gate between the ACE2 negative and positive cells. FIG. 6E shows a fraction of ACE2⁺ cells (using gating shown in 6D). ACE2 expression level was normalized across all samples to the Calu-3 cells transduced with a non-targeting (NT) guide (n=3 biological replicates, error bars are s.e.m.). FIG. 6F shows the mean area of ACE2 foci in Caco-2 cells transduced with a NT or a RAB7A-targeting guide (n=4 biological replicates, 80-105 cells per replicate were scored, error bars are s.e.m.). Significance testing for 6B and 6E was performed with a one-way ANOVA (B: F=9.8, p <10⁻⁴; G: Calu-3: F=378, p<10⁻⁴, Caco-2: F=222, p<10⁻⁴) with false-discovery rate-corrected post-hoc tests. Significance testing for 6B and 6C and 6F was performed with an unpaired t-test. For all panels, * indicates p≤0.05, ** indicates p≤0.01, and *** indicates p≤0.001, and **** indicates p≤0.0001.

[0022] FIGS. 7A-7D demonstrate genome-wide loss-of-function CRISPR screen enriched gene identification. FIG. 7A is a scatterplot of guide RNA read counts from A549.sup.ACE2 cells at 3

days post-transduction with the GeCKOv2 library versus read counts from the library plasmid. Read counts are normalized log 2 reads. FIG. 7B is a RRA p-value distribution for all genes in the GeCKOv2 library. FIG. 7C is the overlap of top-ranked (top 500) genes between 3 different analysis methods (RRA, RIGER, and SBR). 142 genes are found by all 3 methods. FIG. 7D is the overlap of top 50 ranked genes between the MOI 0.01 and MOI 0.3 screen.

[0023] FIGS. 8A-8E show gene set enrichment and overlap of top-ranked genes with other viral infections. FIG. 8A-8D show four of the significant (FDR<0.1) top-ranked GO biological process terms and the fold-change ranks of their genes in the SARS-CoV-2 low MOI CRISPR screen. FIG. 8E shows normalized gene ranks of the top 50 genes from the SARS-CoV-2 low MOI CRISPR screen in CRISPR screens for ZIKA and H1N1 avian influenza (IAV).

[0024] FIGS. 9A-9E show orthogonal perturbations of enriched CRISPR screen genes with RNAi and small-molecule inhibitors. FIG. 9A shows immunofluorescence quantification of SARS-CoV-2 N protein at 36 hours post-infection (hpi) at MOI 0.1 in A549.sup.ACE2 cells pretreated with siRNAs for 48 hours (n=3 technical replicates, error bars represent s.e.m., NT indicates non-targeting control guide RNA). FIG. 9B shows the immunofluorescence quantification of SARSCoV-2 N protein at 36 hpi (MOI 0.1) in A549.sup.ACE2 cells pretreated for 2 hours with 10 μ M of the indicated inhibitors (n=3 biological replicates, error bars represent s.e.m.) FIG. 9C shows the immunofluorescence quantification of SARS-CoV-2 N protein at 36 hpi (MOI 0.1) in A549.sup.ACE2 cells pretreated with a combination of indicated inhibitors at 10 μ M each for 2 hours (n=3 biological replicates, error bars represent s.e.m.). FIGS. 10D and 10E show CD8+ T-cells, preincubated for 1 hour with the indicated inhibitor followed by CD3/CD28 stimulation, stained for TNF- α or INF γ , respectively (n=3 biological replicates, error bars represent s.e.m.).

[0025] FIG. 10 shows the quantitative PCR (qPCR) of SARS-CoV-2 viral load present in A549ACE2 cells (CRISPR-perturbed with either non-targeting or PIK3C3-targeting guide RNAs) pretreated for 2 hours with the indicated PIK3C3 molecule inhibitors at 10 μ M and then infected with SARS-CoV-2 at MOI of 0.1. The qPCR was performed at 36 hours post-infection (hpi). Inhibitors were maintained at the same concentration throughout the experiment (n=6 biological replicates, error bars indicate s.e.m.).

[0026] FIGS. 11A-11E show ECCITE-seq identifies common target gene signatures. FIG. 11A is a stacked violin plot of all genes targeted in ECCITE-seq (n=29; TMPRSS2 was not among the detected features). Single-cells are grouped by unique guide RNA target gene label of cells with a single detected guide RNA (n=4013 cells, median n=152 cells per target gene). Target gene expression is highlighted. FIG. 11B is a heatmap of Gene Set Enrichment Analysis results for genes downregulated in any of the indicated target gene perturbed cells (all genes with p-value <0.01 and with a limit to the 300 most differentially genes; all enriched pathways with adjusted p-value <0.01). FIG. 11C is a UMAP visualization of all DMSO and drug treated cells (n=3070 cells, median of n=604 cells per treatment) after running Linear Discriminant Analysis (LDA). Only enzastaurin clearly clusters away from DMSO control cells. FIGS. 11D-11E are heatmaps of Gene Set Enrichment Analysis results for genes upregulated (11D) and downregulated (11E) in any of the indicated treated cells to DMSO control cells (all genes with p-value <0.01 and with a limit to the 300 most differentially expressed genes; all enriched pathways with adjusted p-value <0.01).

[0027] FIGS. 12A-12B show flow cytometry for cell surface ACE2 expression and protein analysis of RAB7A protein after CRISPR targeting. FIGS. 12A and 12B show flow cytometry gating strategy to quantify cell surface expression of ACE2. FIG. 12A shows Live cells were first gated by the forward and side scatter area, then doublets were excluded by gating with the forward scatter area and width. Viable cells were selected by gating on side scatter area and LIVE/DEAD violet. FIG. 12B shows the gating strategy to determine ACE2+ cells. The gate was position such that <3% of A549 wild type and >85% of A549.sup.ACE2 cells were ACE2 positive. The same gating strategy was applied to all samples. Western blot on A549.sup.ACE2 cells perturbed with non-targeting (NT) or RAB7A-targeting guide RNAs and probed with a RAB7A antibody with GAPDH

was used as loading control was performed (data not shown).

[0028] FIGS. **13A-13B** demonstrate inflation of COVID-19 GWAS signal for eQTLs for CRISPR screen hit genes in lung and lead GWAS variant as an eQTL for the 8 genes in the 3p21.31 locus. FIGS. **13A** and **13B** are heatmaps showing the eQTL P-value of the lead variant 3:45867022:C:G from COVID-19 vs population GWAS (ANA_C2_V2) for the 8 genes in the 3p21.31 locus in 61 cell types and tissues from the eQTL Catalogue (**13A**) and 49 tissues from GTEx v8 (**13B**). A cell is colored in white, if there was no data for the variant-gene pair in the given eQTL dataset (e.g., the gene was not tested in eQTL mapping). eQTL datasets are clustered using the Euclidean distance and complete linkage method.

DETAILED DESCRIPTION

[0029] Given the current COVID-19 global pandemic, there is an urgent need to better understand the complex relationships between host and virus genetic dependencies. We report a genome-wide loss-of-function screen in human lung cells that identified host genes required for SARS-CoV-2 viral infection. We selected and validated 30 genes that were ranked among the top 200 genes. To support the ability of the screen to identify key dependencies, some of the well-known host genes involved in SARS-CoV-2 Spike protein binding and entry such as the ACE2 receptor and Cathepsin L were among the top-scoring genes (Hoffmann et al., 2020a). One of the validated genes (SIGMAR) encodes the Sigma-1 receptor that was recently identified to be modulated by drugs effective against SARS-CoV-2 in vitro (Gordon et al., 2020). Overall, the top-ranked genes clustered within several protein complexes including vacuolar ATPases, Retromer and endosome, Commander, ARP2/3, PI3K and others, highlighting both the critical importance of multiple genes within each pathway to viral pathogenesis and the diversity of molecular pathways involved in SARS-CoV-2 infection.

[0030] Using a “minipool” CRISPR library of perturbations targeting top-ranked genes from the genome-scale CRISPR screen and single-cell transcriptomics, we identified a group of 6 genes (RAB7A, PIK3C3, NPC1, CCDC22, ATP6V1A, and ATP6AP1) that had a similar transcriptional signature-upregulation of the cholesterol synthesis pathway. We have recently, in an independent study, identified that SARS-CoV-2 infection negatively downregulates the cholesterol synthesis pathway and that viral infection can be counteracted by drug treatments that upregulate the same pathway (Hoagland et al., 2020). It is possible that changes in lipid composition directly impacts SARS-CoV-2 virion maturation and infectivity, as has been previously shown for Hepatitis C and Influenza A (Aizaki et al., 2008; Bajimaya et al., 2017). Some of the 6 genes have previously been implicated in regulating low-density lipoprotein (LDL) cholesterol. For example, depletion of Rab7a leads to LDL accumulation in endosomes and NPC1 knockout cells show a reduction of cholesterol at the plasma membrane and an accumulation in the late endosome/lysosome compartments (Chang et al., 2005; Girard et al., 2014; Millard et al., 2000; Neufeld et al., 1996). An important future research direction will be to further understand the relationship between cholesterol synthesis pathways and SARS-CoV-2.

[0031] Furthermore, we screened a panel of the top-ranked genes and identified that RAB7A regulates cell surface expression of ACE2, likely by sequestering ACE2 in endosomal vesicles. Rab7a is involved in vesicular trafficking and its depletion has been shown to sequester other cell receptors in endosomes (Rush and Ceresa, 2013). Interestingly, RAB7A knock-out cell lines showed both altered cholesterol biosynthesis and sequestration of ACE2 receptor. Also, previous proteomics work showed that Rab7a has a strong interaction with viral protein nsp7 (Gordon et al., 2020). However, there is no NSP7 in the incoming virion, implying a post-entry/post-translational role for RAB7A. Thus, it is possible that loss of RAB7A blocks SARS-CoV-2 pathogenesis via multiple separate pathways, which is supported by the observation that it is the top-performing gene in our arrayed validation.

[0032] We also sought to use our CRISPR screen to help interpret recent human genetic studies to identify putative causal genes for COVID-19 (COVID-19 Host Genetics Initiative, 2020). A

pervasive problem in human genetic studies is that a majority of variation is in noncoding regions of the genome, making it challenging to understand mechanisms of action (ENCODE Project Consortium, 2012; Visel et al., 2009). Furthermore, this can make it challenging to identify causal variants and, even when identified, to map the genes through which these variants exert their biological effects (Sanjana et al., 2016). In our study, we first showed that eQTLs for top-ranked genes from the CRISPR screen are enriched for GWAS variants that correlate with COVID-19 severity. Then, we examined the locus with the strongest genome-wide signal for COVID-19 risk (3p21.31) and used our CRISPR screen results to identify 2 putative genes whose expression is modulated by these human genetic variants. Beyond COVID-19, this novel approach integrates colocalization analysis of eQTLs and GWAS variants with genome-scale loss-of-function CRISPR screens to bridge the divide between correlational and causal studies of human biology. This approach should prove useful for other human diseases and pathogens.

[0033] The methods and compositions described herein harnessed genome-scale loss-of-function to develop more refined therapeutic hypothesis and to identify certain drugs or compounds that may be useful in the inhibition of viral infection. In one embodiment, as shown herein, the methods and compositions identify PIK3C3 is a promising drug target. In the experiments described herein, four out of seven PIK3C3 inhibitors resulted in more than 100-fold reduction of SARS-CoV-2 viral load (SAR405, Compound-19, PIK-III, and Autophinib). Using a PIK3C3 polyclonal knockout A549ACE2 cell line, we found that among the top four PIK3C3 inhibitors, SAR405 may have some off-target effects (FIG. 10). In another embodiment, one of the drugs that shows a substantial reduction in SARS-CoV-2 viral load is tamoxifen. Tamoxifen is an FDA-approved drug given as prophylaxis to patients at risk of breast cancer and works via modulation of the estrogen receptor. Tamoxifen was included in our study as it targets protein kinase C as a secondary target (O'Brian et al., 1985). This mechanism is further supported by the observation that A549 cells have undetectable transcript levels of estrogen receptor 1 (Human Protein Atlas) (Uhlen et al., 2010). Considering that tamoxifen is typically given to patients for years as a cancer therapy and prophylactic (Marchant, 1976), it would be interesting to investigate if patients taking tamoxifen have a reduced risk of SARS-CoV-2 infection and/or display less severe symptoms post-infection.

[0034] Finally, many approaches for therapeutic discovery have focused on large-scale screens of compound libraries. Even when promising therapeutic candidates are identified, it can be challenging to understand the mechanisms responsible for reducing viral pathogenesis. Our forward-genetics approach allowed the inventors to first identify key host genes, which can then be targeted through a diversity of methods such as small-molecule inhibitors, blocking antibodies or gene knockdown. A key advantage of this approach is that the mechanism of action for any therapeutic is well-established from the outset. Taken together, this invention and the examples and figures herein identify essential host genes in SARS-CoV-2 viral pathogenesis and, through a broad range of analytic and experimental approaches, and validate their central role in infection. We identify mechanisms underlying top-ranked genes, including cholesterol synthesis and endosomal function, and use the high-throughput screen to further illuminate human genetic studies of COVID-19 risk and disease severity. In addition to providing new therapeutic targets to help end this pandemic, these methods and compositions provide a framework for harnessing massively-parallel genome editing to understand disease genetics and mechanisms.

[0035] In one aspect a method of treating, inhibiting, and/or preventing viral diseases comprising administering a composition that blocks or inhibits the expression, induction, activity, or signaling of one or more of one or a combination of the subject's genes required for viral infection. In one embodiment, the viral infection is caused by infection with the virus SARS-CoV-2. In certain embodiments, the methods involve administering to a mammalian subject (e.g., a human subject) in need thereof one or more inhibitors of the activity or expression of said genes or combination of such genes. The inhibitor can comprise a small molecule, a nucleic acid therapeutic, a peptide therapeutic, or another biologic therapeutic that mimics in the subject a loss of function of one or

more of the selected genes.

A. Subject

[0036] “Patient” or “subject” or “individual” as used herein means a mammalian animal, including a human, a veterinary or farm animal, a domestic animal or pet, and animals normally used for clinical research. In one embodiment, the subject of these methods and compositions is a human. In one embodiment, the subject has no viral infection but is in need of a prophylactic therapy. In another embodiment, the subject has a viral infection and has yet to be treated with any therapy. In another embodiment, the subject has a viral infection and is asymptomatic. In another embodiment, the subject has a viral infection, symptoms and is being treated with conventional methodologies, e.g., administration of currently available antivirals or anti-inflammatories, but is not responding to the treatment optimally or in a manner sufficient to achieve a sufficient therapeutic benefit. In another embodiment, the subject has a viral infection and is treated with conventional methodologies but is not achieving the desired therapeutically maximal response that been observed in other patients. In any of these embodiments, the viral infection is a SARS-CoV-2 infection.

B. Human Genes or Combinations of Human Genes Required for Viral Infection

[0037] In one embodiment, the gene or combination is selected from the human genes identified in Table I. Table I is a list of the top 1000 human genes (i.e., top 5% of the CRISPR screen of ~20,000 genes in the human genome) for both high MOI and low MOI SARS-CoV-2 virus screens as described herein. Each gene is identified with a ranking number as given by the RRA (Robust Rank Aggregation) algorithm and gene name for top 1000 genes in the low and high MOI screen. Inhibition of the expression or activity of these genes or their activity can retard or inhibit infection of the human cells by SARS-CoV-2. The genes are ranked by ability to prevent SARS-CoV-2 infection.

TABLE-US-00001 TABLE 1 Rank Top 1000 genes - low MOI Top 1000 genes - high MOI 1 VPS35 VPS35 2 VPS29 ATP6AP1 3 SNX27 SNX27 4 ATP6AP1 ATP6V0C 5 ATP6AP2 ATP6V1G1 6 ATP6V0C ATP6V0B 7 COMMD3 ACE2 8 COMMD3-BMI1 ATP6V1B2 9 ATP6V1H ATP6V0D1 10 VPS26A VPS29 11 ACTR3 SPEN 12 ACE2 ACTR3 13 ATP6V1G1 ATP6V1H 14 ATP6V1C1 ATP6AP2 15 ATP6V1B2 ATP6V1E1 16 SPEN TMEM199 17 CCDC22 ACTR2 18 CTSL1 RAB7A 19 RNF4 COMMD3 20 NUFIP1 PPID 21 UVRAG COMMD2 22 HDAC9 TOR1AIP1 23 COMMD2 ATP6V1A 24 HAL PIK3C3 25 KIAA1033 COMMD3-BMI1 26 ATP6V0B CTSL1 27 CTSL VPS26A 28 IL17D CCDC22 29 CCDC93 ARPC4-TTLL3 30 COMMD10 ATP6V1C1 31 PIK3R4 SLTM 32 SRSF1 POLE2 33 C11orf74 WDR81 34 ATP6V0D1 ARPC3 35 ACTR2 CTSL 36 COL11A1 C17orf50 37 SOSTDC1 C11orf96 38 USP33 LOC100289561 39 RAB7A UVRAG 40 COMMD4 C10orf131 41 PGBD4 CNOT4 42 SIAH1 CCZ1B 43 CHST10 ACP5 44 ARPC4 MRPS27 45 CCDC74A CHST14 46 ETV1 DPM3 47 SAMD15 ERMP1 48 FUNDC2 SFN 49 CCZ1B COMMD4 50 TNIP3 MDFI 51 AP1G2 RAET1G 52 ORAI2 ATL1 53 SLC38A8 KRTAP9-6 54 GEMIN2 CAPN7 55 CYB5R4 S1PR5 56 TMEM199 CCDC115 57 LRFN5 EIF4A3 58 ATPAF1 KIAA1033 59 PCDH17 KCNIP2 60 GNGT1 NAT8 61 STX7 ARPC2 62 SEMA6A GNB2L1 63 LLPH ODF3 64 C5orf55 ATAD3C 65 MYRIP SOCS1 66 KIAA0319 NRAP 67 NCAPG2 NECAP2 68 RNF187 SMARCB1 69 BCHE FBXO27 70 PPP1CB LIN52 71 RASA3 KPNA4 72 FAM211A LGALS1 73 SETD1B CEP104 74 ZNF639 HNRNPC 75 PODN NBPF4 76 REV1 NBPF6 77 AFF2 MCM3AP 78 CD1D LPCAT2 79 ARPC3 OGG1 80 GRID1 LOC388813 81 MBD6 PCID2 82 ZMYM6 ARID1A 83 TMX2 GOLPH3 84 DNM2 EPHX3 85 TGFBR2 NARS 86 SLC35B2 C3orf33 87 ZNF341 AK6 88 PPP3R1 ARPC4 89 NXN C1orf63 90 MAP3K3 GFER 91 RBM24 RNF125 92 ERI3 THBD 93 ARF5 TNKS 94 ZNF107 hsa-let-7i 95 ACTRT1 MBD6 96 MRPS23 CLDN14 97 BARD1 C1orf68 98 RAP1B UBC 99 WDR7 HIST1H3G 100 POLE OR2T11 101 OR10A6 WBSCR17 102 NUPR1 OR5D13 103 WDTC1 SEC22A 104 CERS1 SRD5A1 105 WDR91 KCTD3 106 SERPINB9 CCDC71L 107 LPPR5 PRMT7 108 OR5M1 CCDC93 109 SRSF6 STX7 110 ARSD BOC 111 OR5T1 SDPR 112

TMEM117 DEPDC1 113 HNRNP33 KIAA1683 114 MYF6 COG2 KLHL28 116 CAG
SF1 117 PGD CCDC77 118 FAP FBXO33 119 DPF3 THEM6 120 WNT2 PLXNB3 121 IL20RA
CATSPERB 122 OR13J1 KLC4 123 OR2T33 NPC1 124 SLC01A2 SAP30L 125 C10orf131
TBXAS1 126 TMEM33 MID2 127 APCDD1 SGOL1 128 STK19 SLC9A3R2 129 GZMK
C9orf171 130 BAX OR6Q1 131 SRFBP1 SRXN1 132 C17orf96 RPS19BP1 133 DNMT3B
SCGB2B2 134 TECR SLC29A4 135 MCF2L TMEM8C 136 BBS12 WFDC9 137 SH2D3A
NCOA5 138 DEFA4 TMEM82 139 NAA16 AGAP3 140 PSD4 SUS4 141 ADCY4 EPPK1 142
HAGHL CALCOCO2 143 ZNF878 IL37 144 WDR93 BECN1 145 DNAJB11 FAM166B 146
AGAP3 SLC37A1 147 C22orf23 OR6C1 148 CCL13 MRPS30 149 IKZF4 GLT8D1 150 GNB2L1
OTOS 151 ATP2C2 NELL1 152 CHKA ADSS 153 DNAJB8 ACAD10 154 LAMP5 TMED9 155
WBP2 POLR3A 156 HAND1 SLC35A2 157 LY96 ACSL3 158 DSTYK MRPL37 159 CKMT1B
VPS11 160 C14orf166B ADAD2 161 FUS SHMT1 162 BRPF1 ANKLE2 163 ABCB4 CENPV
164 NARS2 IPO7 165 AOX1 DSTYK 166 ATP6V1A FHL5 167 RGP1 SLC26A10 168 XAGE2
SLC39A10 169 SEPT9 AKT1S1 170 ACTB TMEM47 171 CMTM2 PRR19 172 SLC41A2 IL2
173 ZBTB40 CERS1 174 BCL2 HIGD1A 175 GBP1 COG3 176 SDR16C5 CDK5 177 SAPCD2
SRSF6 178 FAM167A EZH1 179 ARID1A MAST4 180 ATP5L2 ZNF655 181 LMLN ALX3 182
HIST1H2BJ FUT5 183 FAM90A1 HES7 184 C9orf156 RSRC1 185 MYB WDR91 186 NDUFB1
EFCAB1 187 CLEC16A COG8 188 KDM4E CASP5 189 ESYT1 GPR15 190 PLXNA1 FITM1
191 ZCCHC14 MX2 192 FOXH1 NKX6-3 193 EMILIN1 TPR 194 ABO VMA21 195 ZNF24
LARP7 196 IL1RAPL2 E2F3 197 ZBPB VPS54 198 AGPAT9 C1orf115 199 KRTAP15-1 C16orf3
200 SMAD4 PMFBP1 201 SEPT2 RPN2 202 ACADS SMC2 203 GTDC1 GRID1 204 PDCD2L
FANCG 205 ITGA4 KLHL8 206 DECR1 DEXI 207 FAIM OR10Q1 208 IL23A WDR7 209
FBXW2 PTK6 210 CPLX2 ACVR1C 211 NDUFB2 GALT 212 C20orf194 SERPING1 213 CDK3
SLC46A1 214 USP21 MRGPRX3 215 CENPI VSTM2B 216 ZPLD1 SMCR8 217 ANKRD49
MYH15 218 RAB11FIP1 TRIM40 219 LOXHD1 HACL1 220 EMC9 SSSCA1 221 ATIC KRT19
222 LGALS9C CEP97 223 CEP128 TMEM243 224 NSUN4 SNTN 225 CREBL2 CABP2 226
NRBF2 GPR33 227 CPT1B SYCP1 228 OR1C1 ARHGAP19 229 EIF3L OLFML2A 230 TOP1
NPAS1 231 NAA30 ZC3H7A 232 POU2AF1 TMEM57 233 PRR5L ZBTB48 234 CHRNG
AP2A2 235 VN1R2 ACOT6 236 CCNT1 RNF114 237 MDGA1 JUN 238 ATRIP UBE2R2 239
PLA2G12A TEX33 240 ORC6 LNPEP 241 VTI1A GMPPA 242 MAPK4 MMP12 243 WFDC13
SH3RF3 244 PRSS46 FGFR4 245 FGF7 HDAC9 246 C21orf58 ARL8B 247 NXPE3 OR2T4 248
TMC1 GAN 249 NME6 ATOH1 250 CTAGE4 BCL7C 251 PPP1R12A TANC2 252 NXF1 NVL
253 NUDT18 EMILIN3 254 ADPRH DMBX1 255 KRT18 SMAD5 256 ZNF442 ENY2 257
NTN5 DEPDC5 258 PSMB9 PDSS1 259 KCTD4 PTRF 260 USF2 KLK12 261 VAC14 DHX9
262 TRIQK C20orf166 263 CCDC74B BRK1 264 TMSB4Y ARHGAP35 265 C9orf170 C1orf52
266 PTPN14 DDX51 267 DNMT1 UBE4A 268 ZFYVE16 ZNF700 269 REC8 GNRH1 270
TSPAN4 COX8C 271 ETV6 C8B 272 PSMB4 PRMT6 273 TNFAIP8L1 INO80E 274 TSTD2
KCNG2 275 SLC30A1 SLC7A9 276 HIF3A SLC7A2 277 C16orf78 ZPLD1 278 SYCP1 VPS18
279 ITPK1 ZNF124 280 SMYD2 SLC6A9 281 KCTD19 WDR17 282 TBCE IQCH 283 RNF139
TMPRSS3 284 SHOC2 MAP7D3 285 PAQR4 TM9SF2 286 AQP6 TRIM58 287 EREG PSPH 288
CERCAM TCTEX1D4 289 CD3EAP EEF1B2 290 DNMBP OR4M2 291 CBLN4 EIF5B 292
HNRNPAB ZNF300 293 ACOX3 IL12RB2 294 FRY WFDC13 295 PHF19 TNS1 296 NAALAD2
C16orf62 297 VPS33A CPLX3 298 ZNF616 EIF3I 299 BAP1 C5orf34 300 NOVA1 TINAGL1
301 PCCA DSCAML1 302 TNPO2 SLC25A42 303 S100A4 C19orf24 304 PPIH GAMT 305
SPDL1 RAD54L 306 CAPZA2 FAM83G 307 STC2 ABCF1 308 WNT5A ZNF322 309 RAB17
TNFAIP8L2-SCNM1 310 IFNA17 SOX14 311 TMEM59 CIDEA 312 IQSEC1 SUB1 313 CNTN1
PSORS1C2 314 KLHL30 C8orf37 315 ZNF641 CPN2 316 CCDC91 TXK 317 ZNF521 GNPTAB
318 UNC93A MRPS24 319 GMEB1 DUS1L 320 PRRX2 ASPDH 321 MTERFD1 SRD5A3 322
TMEM221 F2R 323 COX6C MTRNR2L8 324 PRELID1 CCL20 325 SCGB1D1 MIXL1 326
TPBG ZDBF2 327 GAB3 ATP6V1D 328 UBE2V2 MAP3K11 329 LRRFIP1 MCM10 330 OAZ2

MFSD1 331 DMTF1 RARRES1 332 SEC62 UBE2V 333 PPP1R1 334 PPP1R1 SETBP1
335 PRAMEF1 ZNF529 336 C14orf169 C8orf44-SGK3 337 BCAR1 PTCDD1 338 POU5F2
PVRL3 339 LARGE SIGMAR1 340 SLC01B1 SLC37A2 34 KIAA1644 ABCB10 342 NCOA5
PAX7 343 FASLG DDN 344 SCAPER LRAT 345 IFNE GLTP 346 CHST12 NARFL 347 DPP6
FAM120B 348 ADAD1 CCDC64 349 ARHGAP28 LEPRE1 350 RGPD3 RTP1 351 ENY2 LDHB
352 ELF5 COPZ2 353 MRPS17 PROSER2 354 PSMC4 CATSPER4 355 SPANXN2 CCT4 356
CUTC PRKCA 357 GATA1 ARPP19 358 FCAMR SNRNP200 359 CPS1 GPR12 360 ANTXR2
SLC35B2 361 WDR27 COMMD10 362 PFKM LRRN2 363 FAM127C C10orf82 364 HRAS
IL12A 365 DRD1 PPAPDC1B 366 ZNF624 PGD 367 EPSTI1 ZNF626 368 CCDC138 KRTAP21-
3 369 PSMA3 SYNE1 370 CXCL5 C8orf48 371 H1FOO KLK14 372 CYBB PKNOX1 373
TMEM11 GLTPD1 374 WDR26 KIF5C 375 PRKCSH LIMA1 376 SPERT UBXN2B 377 SNX12
HSPBAP1 378 C16orf62 MRPL20 379 LGALS1 GLUD2 380 ZNF607 ACTG2 381 C1orf173
NME8 382 NBPF4 SGCZ 383 NBPF6 DIAPH2 384 CHAF1A GTPBP4 385 FRAT1 SLC02A1
386 CCNA1 AP4B1 387 PAQR9 CD81 388 MMP9 ZNF382 389 TMEM186 BCDIN3D 390
RAD18 ZNF331 391 PDE6A C21orf2 392 C17orf51 SH3BGRL 393 CXCL1 ATL2 394 DYRK2
ZNF414 395 NUDT10 POLR3C 396 GPR155 HBQ1 397 ZNF304 PLBD1 398 AFF4 PTCDD2 399
BCL10 BAX 400 CWC15 COG4 401 SNX22 ZNF641 402 AIM2 MORC1 403 ABCC11 C19orf53
404 RNF223 WHSC1 405 CCDC24 MRPS23 406 GPR113 TGFB2 407 TTC27 BAD 408 PHF20
MOB3B 409 CHCHD10 ATF5 410 WBP4 FCAMR 411 TRMT112 REG1A 412 UBQLN1 PHTF1
413 IFI16 CHD5 414 WDR81 ENTHD1 415 AGL POC1A 416 MAP3K10 PHF3 417 OSM
TMEM147 418 RPL39 C9orf89 419 C4BPA CCDC92 420 ZDHHC4 TNFRSF12A 421 GNAS
UNC5D 422 SOAT1 TPCN1 423 C12orf75 HIST1H3H 424 ANO6 HSD17B8 425 ZCCHC16
DSCR3 426 ZFH2 ALG14 427 CCDC151 TTL1 428 MYO7B GPR116 429 UNC5C SHCBP1L
430 GNPTAB TMEM105 431 STON1-GTF2A1L MYO7B 432 WDR48 PRSS33 433 MTRF1
MXRA5 434 CCDC130 COX17 435 HYAL2 EEF2 436 SYNC NUPR1 437 MYBL1 IQSEC1 438
ELMO1 UPK3BL 439 THTPA CLEC16A 440 HTN1 SLC6A16 441 ZC3H18 MYL6 442 GDAP2
PEX11G 443 RBPMS ARR3 444 BCORL1 ZNF273 445 DDX39A SPTBN5 446 TYW5 LCAT
447 LAMTOR5 RNF183 448 RNF141 NAGPA 449 KCNIP3 OR8K3 450 HTR1F SLC35A1 451
CLEC19A NUTF2 452 TMEM50A TCEANC 453 TUSC2 ABHD6 454 SLC25A15 MARK1 455
UVSSA SPOCK3 456 NR0B1 EREG 457 ADSS PLRG1 458 GALNT14 ZNF644 459 TEX101
POPDC3 460 ENOSF1 PRMT8 461 SAMD4A FUT2 462 GOLGB1 C1orf210 463 COL17A1
FAM162A 464 ZCCHC4 OPN1LW 465 VMA21 SPRR1B 466 SMPD3 BRDT 467 OFD1 NTMT1
468 SNAPC4 GPR82 469 TBC1D10C CLCN6 470 HECW2 GPATCH1 471 PLEKHA3 ACKR2
472 GPER ZSCAN30 473 DRC1 DDX18 474 TCHP PSD3 475 HLA-DRA MGAM 476 ISLR
ARRDC3 477 NOL12 KHDRBS2 478 SLC18A2 TMEM41B 479 RTN1 AAK1 480 DPP9
SLC16A14 481 IL26 ANKRD36 482 RGL1 RAB11FIP1 483 KIAA1432 LECT1 484 TMEM134
LRRC8D 485 CELA2A DSE 486 PPP1R15A FAM134A 487 EIF3G NR2F6 488 C3orf62 ACRV1
489 TGFB3 TNS4 490 PLEKHS1 CFHR1 491 PTF1A PRAMEF22 492 LTBP2 ROPN1L 493
IL1F10 EPOR 494 KIF3A OTUD7B 495 C3orf18 DCAF13 496 FAM78B B3GALT2 497 GMNC
KCNA4 498 ATP13A4 COG7 499 INSIG2 FAM21B 500 SPTAN1 SSH1 501 HYAL4 RTF1 502
ITGB1BP1 AIM2 503 SEMA7A C1QTNF7 504 SLC3A2 PLGRKT 505 RLN3 TRIM23 506
INHBB CXCR6 507 PDSS1 CER1 508 XPO6 CACNG2 509 EPHA5 CUL3 510 WDR31 HNF1A
511 MOB3B RASAL1 512 TIPARP OSGIN1 513 HSP90AB1 GUK1 514 ZNF175 WDR20 515
UBAP2 TSHZ2 516 MICALL2 RANBP9 517 SRSF3 CPB1 518 ZNF182 ST3GAL3 519 RBM20
TMEM179B 520 MKX SOCS7 521 BARX2 FDPS 522 PARK7 GSKIP 523 PUM1 ADARB1 524
TMEM5 DBF4B 525 GPR68 OR10AG1 526 VWA2 LAIR2 527 FAM73A COL3A1 528 CREBZF
GOLGB1 529 ASB16 FAP 530 USP9Y ALPP 531 TTI2 ZNF227 532 RELT ITPRIPL1 533 GFER
ALDH18A1 534 TMEM119 RPA1 535 STK17A C11orf21 536 TEX28 FBLN5 537 GFOD1
SLC16A3 538 RAB1A HMGB4 539 TGIF1 SPATA31A6 540 SKA3 TSC1 541 LOXL4 INHBC
542 KRT80 SNTA1 543 ICA1 PCSK9 544 FOXO3 VSIG1 545 VARS2 TP53BP2 546 BLOC1S1

ADCK1 547 LMO4 NIPSNAP3A 548 PHF3 CDC42EP1 549 HIST1H3B XPNPEP2 550 PRLHR
PSMC3IP 551 TSHZ1 MAMLD1 552 OR6C1 CTIF 553 ERCC1 RAB34 554 TRIM50 FCRLA
555 HEPH GPR158 556 SPRR4 MRI1 557 RNF20 NUMB 558 KIAA0930 RBKS 559 GLIPR1L2
PCNXL3 560 HDAC7 ACYP2 561 TFAM CCND1 562 CRLF1 PLOD1 563 C11orf16 ANKRD10
564 LEO1 OR6A2 565 ZFP36L2 B3GNT9 566 WDSUB1 NXF1 567 TRIM52 GALE 568
TNRC6A PLBD2 569 XKR9 PRKD2 570 TBC1D8 C19orf60 571 PPAT ZNF671 572 GP5 S1PR2
573 DUX2 DYRK3 574 MYOCD BPIFA2 575 RSPH10B2 ECM2 576 DNAAF3 NGRN 577
SLC9A7 H3F3C 578 ACVR1 CHEK2 579 HBS1L LSM6 580 PCP4 CPM 58 TGIF2LX UCK2
582 PDZRN4 AMPD2 583 CCDC126 ZNF253 584 KRTAP9-8 OR8B3 585 PTGER1 TROVE2
586 ADRA1D CCNI 587 PAQR5 EPB41 588 MAP7D3 GJA4 589 KRTCAP2 SWSAP1 590
C1orf63 SRSF3 59 FSD1L SPDYA 592 OR10G7 HOXD13 593 IFNL1 MS4A10 594 ZFAND2B
EYA3 595 POLR3A KIF2C 596 CAPG ETV1 597 STK31 GUSB 598 IQCA1 TOMM40L 599
B4GALNT1 ZMYND8 600 OR4A16 HP1BP3 601 GJB3 IL27RA 602 SAMD5 AGER 603
CTDSP2 NUP50 604 FNDC3B SNX14 605 EPPK1 TMEM55B 606 ACSF3 BCAN 607 CAPZA1
NAA11 608 KLHL3 MSANTD2 609 FBXL13 DACT3 610 ARL1 OTUD5 611 CLEC1B
FAM131A 612 LRRC8D STRA8 613 CYP19A1 DCAF8L1 614 CCDC92 NOX4 615 TTC4
OR5R1 616 FCHO1 TXLNA 617 SLC37A1 GSTO2 618 PSMD1 RHOJ 619 RAP1GDS1 PVALB
620 IFRD1 NEFL 621 TFAP4 CLASP2 622 RNASE1 CDKN1C 623 HELLS CNNM3 624
CXXC11 C1orf94 625 OR8G2 HOXD12 626 ACTG2 C22orf39 627 NDUFA8 FNDC1 628 PEX1
ELOVL1 629 GPR17 AREL1 630 PGLYRP1 MIA2 631 FAM135B TMEM86A 632 OR4D1 CLTA
633 ARHGAP31 USP28 634 CDR2 MILR1 635 KIAA0430 NFKBIL1 636 MORN1 GSTCD 637
TEX13B TNFSF18 638 SOCS7 ARHGAP22 639 C4orf17 EPS8L3 640 AP3M1 CCT8L2 641
SUGP2 RPL11 642 PTP4A3 KLHL9 643 RAD51B PNMA2 644 EPHB3 TRAPPC12 645 DTX4
FAM122B 646 NINL SPRED3 647 TRPC7 MPST 648 PRKCG PRKAA1 649 NFKBIL1 WDR67
650 PSMD9 PDCD1 651 OR6K3 PGPEP1 652 RPA1 CHMP4C 653 A2ML1 RNF139 654 SSPO
TUSC3 655 NCOA3 CYP11B2 656 CRISP3 GPX3 657 TAAR6 INPP4B 658 STT3B FOXI3 659
C7orf71 SPATA12 660 CENPV GPR182 661 KCNQ4 HPDL 662 CSF1R TTC21B 663 TGFBR3L
IKBKAP 664 TIE1 SGCG 665 SWSAP1 TTC25 666 BECN1 NUFIP1 667 NMUR1 ZBTB40 668
C1orf61 DCUN1D4 669 ZMYND10 C11orf83 670 OR8B4 OR2B11 671 FMNL2 IGFBP3 672
ZNF529 ANTXR2 673 ME2 PI3 674 HMG20B HTRA3 675 FOXRED1 EDARADD 676 DSE
QPRT 677 GABARAPL2 ZBTB8B 678 FREM3 ETV7 679 ACER3 SEC31B 680 SNX15
AMDHD2 681 FCHO2 HTR1E 682 RBFOX2 CPNE6 683 KIAA1109 SYNGAP1 684 ANKRD29
CCDC85A 685 NET1 ZBTB21 686 NUP205 FER 687 VPS36 FAM175A 688 APAF1 SLC25A33
689 NPAS3 ZNF101 690 CCDC169-SOHLH2 EPHX1 691 LSM6 A3GALT2 692 CBR4 DNAL4
693 ARPC4-TTLL3 KIF2A 694 IQCC ALG6 695 SDK1 PSEN1 696 SLC25A23 AATF 697
FBXL5 MORN3 698 RAB39B HHIPL2 699 CASP8 TMEM110-MUSTN1 700 TMEM161A
KLHL15 701 TF TRPC4 702 SGCB MAPK8IP2 703 LCE5A GHDC 704 LGI1 PPP1CB 705 NLK
WDR16 706 HECW1 BCORL1 707 VEZT MLKL 708 NANOS2 LIPC 709 MOS HIST1H2BH
710 MTPN TBCEL 711 TNKS2 WNT16 712 FAM104A ZNF248 713 SLC28A1 CDRT15 714
CEP68 CNTROB 715 OR51A4 KLKB1 716 PRKRIP1 RBM15 717 NDUFB6 TMC4 718
PPAPDC1A TRMT112 719 SMOX CACNA2D1 720 BCAS2 C2CD3 721 OR8K3 PARG 722
PLCXD3 HSDL1 723 DENND5B OR5B2 724 TP53BP2 KCNE1 725 HRH4 SLC25A31 726
OGG1 NDUFA3 727 RAB38 ANKRD54 728 TSPAN8 SLC1A4 729 CYB5R2 GAL3ST2 730
BRSK2 VPS41 731 CCDC107 TRAK1 732 TAF8 WDR18 733 HILPDA PSTPIP1 734 NDN
MEOX1 735 ZNF45 NIT2 736 IL27 TLE3 737 UPK1B TSPAN9 738 KMT2C RPS6KA4 739
MFSD8 MTRNR2L2 740 NRTN TIPARP 741 PARP9 EPHB4 742 NEURL1B NPAP1 743 ARNT
PRAF2 744 PRRC1 IL27 745 ST3GAL6 QPCTL 746 BHMT PCK1 747 TEAD2 POLR1D 748
ZNF567 AGTR1 749 PPID SNAP47 750 SKOR1 FAM196B 751 ARSF SON 752 ODF3L2
CYP4F12 753 HLA-F CS 754 TCEA1 MALL 755 C9orf171 GPD1L 756 RHOBTB2 ARAP2 757
C4orf51 DNAJC5 758 ZNF574 COX6C 759 HIF1A AADAT 760 LINGO2 HBE1 761 MIEN1

PTP5J2D1 762 DYSJL1 763 NEXK1 COX5B 764 GOLGA2 SLC10A5 765
HIST3H2A CASQ2 766 TSPAN16 TRA2B 767 NANOS3 BHLHE41 768 TMEM9B ZNF792 769
F7 ENAH 770 PASK HUS1 771 OR8J1 IL17D 772 TNFSF12 PRKRIR 773 HOXB3 MAFB 774
FAM83C KCTD8 775 F13A1 COX6B1 776 OMA1 MAX 777 FCN1 LIMS2 778 SURF4 CD7 779
FABP3 CCDC68 780 ZSCAN30 SIRPG 781 KLK10 NODAL 782 OR5W2 WDR47 783 ERGIC1
CCL13 784 PSMC3 IFNE 785 COPRS GIMAP1 786 ATL1 HIPK3 787 ERF LYRM2 788 ATG5
TRUB1 789 MEF2BNB LAMC3 790 CAPN5 TECR 791 C11orf49 CCIN 792 LIPT1 DIDO1 793
MAP4K3 ERAP2 794 BSND SNRPC 795 CUZD1 C2orf57 796 CXorf23 CAMK2D 797 PSME3
C20orf85 798 C12orf56 PIWIL3 799 UBE2R2 LMAN1L 800 SYT3 SOX12 801 PDAP1 NDC1
802 ASGR2 OR10Z1 803 RABL2B LIN28B 804 HS2ST1 STARD13 805 AP4S1 EMC10 806
HAP1 AMOTL2 807 SLC26A8 WISP3 808 BFSP1 KIAA0100 809 PSD2 SMIM10 810 GLIS3
RNF34 811 VIL1 RTN4R 812 CALCOCO2 PRR9 813 HMBS HTR1A 814 OCEL1 LGALS7B
815 ARHGAP17 VSIG2 816 RHCE SLC27A2 817 HRASLS MTHFD1L 818 DCX LDOC1 819
G6PC TAF1L 820 ZNF503 KLHL35 821 FOXD4 REV1 822 ANK2 SPSB4 823 GNRH1 IRX3
824 GPR153 HOXA3 825 SAE1 PTGES 826 KXD1 CT62 827 NOA1 ZNF195 828 LAIR1 EBF2
829 PSG9 CCDC102B 830 MRI1 NKAPL 831 FFAR4 PDF 832 FRA10AC1 VTI1B 833 ALX4
LIG3 834 PRKRIR OVOL3 835 IL37 PPP3CB 836 ANXA8 ENSA 837 PGM5 KCNJ11 838
NME8 SPEF2 839 COX10 PRKX 840 LRCH1 SPACA3 841 GOLGA7B DERL1 842 MRPL47
LRRC20 843 YTHDF1 SLC26A1 844 NTRK1 RBM18 845 SLC25A51 UFM1 846 RCBTB2
CHADL 847 KRT16 ADAM10 848 SOX21 FAM46D 849 CD164 CD8B 850 CD3G ARHGEF18
851 MPL SFTPA1 852 TMPRSS3 SCARF2 853 TPGS2 SLC25A5 854 MYH9 DOK7 855
C10orf2 LRFN3 856 RAB39A MFAP3L 857 MPPED1 TMEM117 858 KCNS3 ALOX15 859
TARS BFSP1 860 TPP2 ADCK2 861 SGMS2 POU3F2 862 KCNE2 BPIFB2 863 MRPS34
NYAP2 864 MTRNR2L8 TST 865 NF2 TSGA10 866 PTPN6 RAB3D 867 GMEB2 TNNT3 868
C3 SCEL 869 FZR1 CSNK2A3 870 OR3A2 GAB4 871 DDX55 CD96 872 CLK4 ALDH16A1
873 CHGB TPD52L1 874 THSD7B USP35 875 MFSD9 ARL15 876 PCK1 TAS2R14 877
FAM71C TRHDE 878 METTL3 DOCK6 879 TAF11 OR56A5 880 MDK NKD1 881 DYDC2
RNASE1 882 FAM21A ARFRP1 883 HORMAD2 LATS2 884 C10orf82 RABL2B 885 CHCHD7
FZD8 886 GBP4 ECI1 887 OGN ANKRD50 888 SRPK2 USP50 889 FDX1 DCST1 890 CTNNB1
NUDCD3 891 PARPBP GDF1 892 C1orf233 VPS13D 893 TRIM31 TIGD5 894 CEP63
ITGB1BP1 895 POGLUT1 RRAGA 896 KLHL5 TIGD1 897 AGBL3 KPNA7 898 RNF39 GPR17
899 ZNF749 COX4I1 900 COQ3 PP1AL4F 901 PLEK TUBB2B 902 TRIP11 FMN2 903 DEXI
SMIM12 904 CCDC168 HS2ST1 905 MRPS26 NPY4R 906 ANKRD33B REEP4 907 KIAA1191
CDKN2D 908 TUT1 SLC38A8 909 ETV5 ANG 910 BCL2L2 ZBED1_X 911 CD97 ZBED1_Y
912 GAL3ST4 CA5B 913 EPOR ENTPD4 914 OR9I1 OR2C1 915 PMCH CHPF 916 ITGAE
TRIM33 917 BTBD18 STEAP1 918 CIDEC NUAKE2 919 GBE1 LAMP5 920 ACADM OR2T35
921 CLIP3 POLD1 922 HEY2 COL9A2 923 HTRA2 RSPH1 924 ROCK2 FOLR3 925 EHD3
BRWD1 926 PECR BCL2L13 927 PTPDC1 MOB3C 928 PLEKHG6 LRRC15 929 C2orf44 GH1
930 RRAD TNFSF12 931 OR10J5 CDT1 932 IL17RA ZNF556 933 BAD SH3BP1 934 STK33
RTN3 935 HES3 STEAP2 936 PLXDC2 NF2 937 SNAP25 TMEM63C 938 MBL2 KIAA1191
939 CCNB1 KRTAP4-2 940 FMR1 DFFA 941 ALG9 CSF3R 942 STX5 PCNT 943
LOC100507003 NFATC2 944 SF1 FGF22 945 ACAT1 C12orf23 946 TKTL2 C10orf95 947
HOMEZ PPP1R35 948 ANKZF1 EYA2 949 PPP1R3C KIAA1467 950 CNTNAP2 CPA3 951
LPIN1 HEBP1 952 DDX59 EPHA3 953 KATNBL1 TSPAN11 954 PDHA2 C4orf46 955 FAM98C
GBE1 956 CCDC51 CORO2A 957 FBXO46 NRN1L 958 GPR56 PRAMEF15 959 TRIM6-
TRIM34 PRAMEF9 960 SRPX2 RRAGB 961 SMAGP MAN2B1 962 SLC12A1 SNX12 963
TRIP10 CHCHD10 964 LSM2 GCOM1 965 RERGL MYZAP 966 MMADHC ME2 967 USP40
TRIP11 968 FAM149A WAC 969 WDR49 RASL10B 970 SIGLEC5 CDC25A 971 OR51V1
AMBN 972 NEU4 HIST1H2AM 973 CYP2J2 ZNF345 974 C14orf182 RPTOR 975 FDX1L
TNNC2 976 NDUFC2 TIMM9 977 TMEM167B ACBD5 978 TUSC5 FAM96A 979 C7orf73

TMEM185A 980 GAG1 FAM21C 981 PRR21 AHNK2 982 CACNG6 SPHK2 983 ZNF74
 POGZ 984 PTPRF GPR52 985 LOC389895 RGS13 986 TRIM72 FRMD8 987 ITPRIPL1 MED29
 988 SSX1 NCBP1 989 TCEANC PTK2B 990 S100A9 ABHD8 991 DHX57 LAPTM5 992
 MPZL2 KRTAP4-4 993 CBX8 GABRQ 994 OR10W1 RPS6KL1 995 NKX2-5 CD320 996
 LOC100130357 MARVELD2 997 ZNF354C SCAF8 998 LOC286238 RGS2 999 ACAP3 ARX
 1000 PINK1 SEMA6A

[0038] In one embodiment, the gene or combination is selected from the genes identified in Table II. Table II is a list of the 69 (low MOI SARS-CoV-2 screen) or 73 (high MOI SARS-CoV-2 screen) genes that were identified among the top 500 genes ranked in the CRISPR screens described herein. These genes were among the top 500 in each screen for which drugs that inhibit their expression or activity are useful in retarding or inhibiting infection of the human cells by SARS-CoV-2. Among these are 10 genes that overlapped in both screens. The genes are ranked by ability to prevent SARS-CoV-2 infection as given in the ranked lists.

TABLE-US-00002 TABLE 2 Low MOI High MOI Rank Druggable Genes Druggable Genes
 Common 1 ACE2 ACE2 ACE2 2 ACTR2 HDAC9 ACTR2 3 PPID CTSL ATP6V1A 4 ATP6V1A
 PIK3R4 CTSL 5 PIK3C3 ACTR2 ARID1A 6 CTSL COL11A1 ADSS 7 ACP5 SIAH1 HDAC9 8
 S1PR5 ETV1 PGD 9 SOCS1 BCHE BAX 10 SMARCB1 TGFB2 EREG 11 OGG1 MAP3K3 12
 ARID1A POLE 13 GFER CA6 14 THBD PGD 15 SRD5A1 FAP 16 PRMT7 SLCO1A2 17
 TBXAS1 BAX 18 SLC29A4 DEFA4 19 ADSS CHKA 20 IPO7 LY96 21 AKT1S1 FUS 22 IL2
 BRPF1 23 CDK5 ABCB4 24 EZH1 AOX1 25 CASP5 ATP6V1A 26 E2F3 ACTB 27 PTK6 BCL2
 28 ACVR1C ARID1A 29 SERPING1 MYB 30 KRT19 NDUFV1 31 JUN KDM4E 32 LNPEP
 SMAD4 33 MMP12 ITGA4 34 FGFR4 IL23A 35 HDAC9 NDUFB2 36 GNRH1 CDK3 37
 PRMT6 ATIC 38 KCNG2 CPT1B 39 SLC6A9 TOP1 40 IL12RB2 CHRNG 41 TXK KRT18 42
 SRD5A3 PSMB9 43 F2R PSMB4 44 MAP3K11 SMYD2 45 SETBP1 AQP6 46 SIGMAR1 EREG
 47 CATSPER4 BAP1 48 PRKCA CNTN1 49 IL12A TPBG 50 PGD SLCO1B1 51 KLK14 FASLG
 52 SLCO2A1 PSMC4 53 BAX GATA1 54 TGFB2 CPS1 55 BAD HRAS 56 REG1A DRD1 57
 TNFRSF12A PSMA3 58 TPCN1 MMP9 59 EEF2 PDE6A 60 LCAT DYRK2 61 ABHD6
 MAP3K10 62 MARK1 OSM 63 EREG GNAS 64 PRMT8 SOAT1 65 BRDT HYAL2 66 CLCN6
 BCORL1 67 MGAM HTR1F 68 EPOR ADSS 69 KCNA4 ENOSF1 70 HLA-DRA 71 SLC18A2
 72 DPP9 73 TGFB2

[0039] In one embodiment, the gene or combination is selected from the genes identified in Table III. Table III is a list of the top 69 genes identified in the low MOI SARS-CoV-2 screen and the corresponding drugs known to inhibit expression or activity of these genes. These genes are not ranked in the same order as for Table I or Table II. The drugs are listed as inhibiting the corresponding gene (see, e.g., the Drug Gene Interaction database: www.dgidb.org/). Previous reports have established that these small-molecule drugs can inhibit (“mimic loss-of-function”) of the corresponding gene in the list. That is, the drug copies loss-of-function/knock-out of the gene without the need for other means of knocking out gene expression or activity, such as CRISPR, genome editing, etc.

TABLE-US-00003 TABLE III Rank Gene Inhibitor 1 ABHD6 WWL70 | WWL123 2 ACE2
 COMPOUND 28 [PMID: 18324760] | XNT [PMID: 18391097] | MLN-4760 | GL1001 | ABZ-
 SER-PRO-TYR(NO2)—OH | LISINOPRIL 3 ACP5 STREPTOZOTOCIN | G-CSF |
 ANALGESICS | TPA | M-CSF 4 ACTR2 ETOPOSIDE | MCP-1 5 ACVR1C
 GROWTH/DIFFERENTIATION FACTOR-1 | GROWTH/DIFFERENTIATION FACTOR-10 |
 ACTIVIN B GROWTH/DIFFERENTIATION FACTOR-9 | GROWTH/DIFFERENTIATION
 FACTOR-7 | SB-431542 | GROWTH/DIFFERENTIATION FACTOR-3 | ACTIVIN A | INHIBIN
 A | ACTIVIN AB 6 ADSS ALANOSINE 7 AKT1S1 OSI-027 | AZD8055 | MLN0128 8 ARID1A
 DASATINIB | Trastuzumab | GSK126 9 ATP6V1A CRUENTAREN 10 BAD AT-101 11 BAX
 PREDNISOLONE | METHOTREXATE | NITROGEN MUSTARD | DOXORUBICIN | CA4P |
 PHENYLEPHRINE | DHEA | PHENOBARBITAL | BICALUTAMIDE | DHT | MELATONIN |

SULFORAPHANE | MEDROXYPROGESTERONE | ETOPOSIDE | COBALT | C5A | ADM | FLUDARABINE | TESTOSTERONE UNDECANOATE | NFX | QUERCETIN | M-CSF | CARVEDILOL | INTERLEUKIN-1 | VINORELBINE | SALINE | ERBITUX | OXALIPLATIN | ESTRAMUSTINE | HUMAN CHORIONIC GONADOTROPIN | IL-4 | PMSG | NICOTINE | NEUROPROTECTANTS | TNP-470 | VERAPAMIL | RETINOL | ANTHRACYCLINE | 5-FLUOROURACIL | BORTEZOMIB | ERYTHROMYCIN | INHIBINS | PROCARBAZINE | RAPAMYCIN | BETA-LAPACHONE | HYPERBARIC OXYGEN | CIPROFLOXACIN | BCG | PRAVASTATIN | ATENOLOL | INTERFERON ALFA-2B | OXYTOCIN | ANTI-INFLAMMATORY DRUGS | LEUCOVORIN | DOCETAXEL | NALTREXONE | CAMPTOTHECIN | PROTEIN PHOSPHATASE INHIBITOR | ANDROSTENEDIONE | RETINOIC ACID | MITOXANTRONE | XK469 | CYCLOPHOSPHAMIDE | DACARBAZINE | FLT3 LIGAND | CISPLATIN | VIOXX | LEVODOPA 12 BRDT XD14 | (+)-JQ1 13 CASP5 M826 | EMRICASAN | Z-WEHD-FMK 14 CATSPER4 PGF1 $\hat{1}$ \pm | PGE1 | PGE2 | PROGESTERONE | MIBEFRADIL | NNC55-0396 15 CDK5 PURVALANOL B | BS-194 | AT-7519 | ALVOCIDIB | DINACICLIB | Roniciclib | ALOISINE A | RGB-286638 | DINACICLIB | PURVALANOL A | CDK1/5 INHIBITOR | AZD- 5438 | SCH727965 | AT-7519 | CDK2 INHIBITOR IV | 1-AZAKENPAULLONE | GSK-3 INHIBITOR IX | SELICICLIB | AMINOPURVALANOL A | DINACICLIB | TG02 | DINACICLIB | ALVOCIDIB | RGB-286638 | PHA-793887 16 CLCN6 DIDS 17 CTSL HORMONES | LHVS | KGP94 18 E2F3 LYMPHOTOXIN- $\hat{1}$ \pm | TUMOUR NECROSIS FACTOR MEMBRANE FORM 19 EEF2 SS1(DSFV)-PE38 | DENILEUKIN DIFTITOX | VB4-845 20 EPOR METHOXY POLYETHYLENE GLYCOL-EPOETIN BETA | DARBEPOETIN ALFA | HEMATIDE | HEMATIDE | DARBEPOETIN ALFA | PEGINESATIDE | DARBEPOETIN ALFA | DARBEPOETIN ALFA | ERYTHROPOIETIN | EPOETIN ALFA | CAPSAICIN | EPOETIN ALFA | ERYTHROPOIESIS- STIMULATING AGENT | PEGINESATIDE ACETATE | LU AA24493 | METHOXY POLYETHYLENE GLYCOL-EPOETIN BETA | PEGINESATIDE | EPOETIN ALFA | ERYTHROPOIETIN | EPOETIN ALFA 21 EREG PANITUMUMAB | CETUXIMAB | HUMAN CHORIONIC GONADOTROPIN 22 EZH1 GSK343 23 F2R RIGOSERTIB SODIUM | TYROSINE KINASE INHIBITOR | MORPHINE | TFLLR-NH2 | ETHANOL | ATOPAXAR | VORAPAXAR SULFATE | WORTMANNIN | TRANSFORMING GROWTH FACTOR | [3H]HATRAP | LEPIRUDIN | VORAPAXAR | PROSTAGLANDIN E1 | THALIDOMIDE | THROMBIN | BLEOMYCIN | RWJ-56110 | F16357 | KINASE INHIBITOR | GLUCOCORTICOID | VORAPAXAR | RUSALATIDE | ASPIRIN | DALTEPARIN | ATROPINE | ANTI- VEGF | ARGATROBAN | SCH-530348 | ATOPAXAR 24 FGFR4 BGJ398 | DOVITINIB | AZD4547 | CVBT-141H | CVBT-141B | ENMD-2076 | PALIFERMIN | PALIFERMIN | BRIVANIB | ARQ- 087 | FP-1039 | LY-2874455 | FGF/VEGF RECEPTOR TYROSINE KINASE INHIBITOR, PD173074 | BGJ-398 | INFIGRATINIB | ACTB1003 | LY2874455 | FGF401 | ERDAFITINIB | FGF-6 | XL- 999 | CP-459632 | BAY-1163877 | BRIVANIB ALANINATE | ISIS- FGFR4 | NINTEDANIB | DOVITINIB | ENMD-981693 | TRAFTERMIN | NINTEDANIB ESYLATE | NINTEDANIB | AZD- 4547 | BLU9931 | ORANTINIB 25 GFER ETHANOL 26 GNRH1 DDS | LITHIUM | ABARELIX | DDC | GOSERELIN | GNRH PHARMACCINE | AMINOGLUTETHIMIDE | DIETHYLDITHIOCARBAMATE | CONJUGATED ESTROGENS | 6-OHDA | RESERPINE | VITAMIN E | LEUPROLIDE | OZARELIX | TNF-ALPHA | RALOXIFENE | CAPTOPRIL | ANTIDEPRESSANT | BUTYRATE | BESTATIN | NORELIN 27 HDAC9 SB939 | PCI-24781 | TMP269 | ROMIDEPSIN | CUDC-101 | VORINOSTAT | GIVINOSTAT | CUDC-101 | BELINOSTAT | BELINOSTAT | PRACINOSTAT | AR-42 | PANOBINOSTAT | PANOBINOSTAT | PANOBINOSTAT | PIVANEX | SCRIPTAID | BELINOSTAT | MGCD-0103 | DACINOSTAT | BELINOSTAT | VORINOSTAT | TRICHOSTATIN A | ROMIDEPSIN | VORINOSTAT | GIVINOSTAT | CUDC-101 | GIVINOSTAT | QUISINOSTAT | ROMIDEPSIN | ENTINOSTAT | VALPROIC ACID | PANOBINOSTAT | RESMINOSTAT | VORINOSTAT | VALPROIC ACID |

AD-RTS-IL-12 | USTEKINUMAB | VIRULIZIN | DC-RTS-IL-12 | BRIAKINUMAB | CID 446414 | DNA IL-12 | HUMANIZED SMART ANTI-IL-12 ANTIBODY | Ustekinumab 29 IL12RB2 INCELL-1001 + AD-1001 | AS1409 | DENILEUKIN DIFTITOX 30 IL2 GARLIC EXTRACT | PHYTOCHEMICALS | HALOFUGINONE | DIETHYLSTILBESTROL DIPHOSPHATE | MACROLIDE ANTIBIOTIC | TRICHOSTATIN A | ALPHA-METHYL-P- TYROSINE | BROMOCRIPTINE | CSA | QUININE | CEFEPIME | CAROTENOIDS | RCD4 | HUMAN CHORIONIC GONADOTROPIN | PRAVASTATIN | PROCHLORPERAZINE | OFLOXACIN | DHEA | PENTAMIDINE ISETHIONATE | AMIKACIN SULFATE | TICARCILLIN | METOCLOPRAMIDE | LAK | PEFLOXACIN | GLYBURIDE | PROTEASOME INHIBITOR | CALCITRIOL | ROXITHROMYCIN | CLOZAPINE | BECLOMETHASONE | AZIRIDINE | RTGF-BETA | ITRACONAZOLE | IMIPENEM | DIDEMNIN B | BACILLUS CALMETTE-GUERIN | CELL DIFFERENTIATING AGENT | MONOCLONAL ANTIBODY 17-1A | MARIJUANA | PARACETAMOL | PROTEIN KINASE C INHIBITOR | PYRIMETHAMINE | 4-HYDROPEROXYCYCLOPHOSPHAMIDE | CGP41251 | INTERFERON BETA-1B | MTX | CYCLOOXYGENASE INHIBITOR | GARLIC | SIGNAL TRANSDUCTION INHIBITORS | NG-MONOMETHYL-L-ARGININE | MISTLETOE LECTIN | GENTAMICIN | TROPISETRON 31 IPO7 MKC-1 32 JUN ALPHA-DIFLUOROMETHYLOARNITHINE | DFMO | ARSENIC TRIOXIDE | T-5224 | T-5224 33 KCNA4 VERAPAMIL | NERISPIRDINE | DALFAMPRIDINE | TEDISAMIL | GUANIDINE HYDROCHLORIDE 34 KCNG2 TEDISAMIL | DALFAMPRIDINE | NERISPIRDINE | GUANIDINE HYDROCHLORIDE 35 KLK14 COMPOUND 4D [PMID: 25489658] | COMPOUND 3 [PMID: 23849879] 36 KRT19 PD-98059 | DEXAMETHASONE 37 LCAT STREPTOZOTOCIN | TESTOSTERONE PROPIONATE | TESTOSTERONE | PREDNISOLONE 38 LNPEP COMPOUND 17 [PMID: 23916253] | INHIBITOR 19 [PMID: 21476495] | TOSEDOSTAT 39 MAP3K11 URM-099 | CEP-1374 | CEP-1347 | COMPOUND 8 [PMID: 24044867] 40 MARK1 7-HYDROXYSTAUROSPORINE 41 MGAM MIGLITOL | MIGLITOL | ACARBOSE | VOGLIBOSE | MIGLITOL | VOGLIBOSE | VOGLIBOSE | ACARBOSE | ACARBOSE | MIGLITOL 42 MMP12 AZD6605 | COMPOUND 20 [PMID: 22153340] | COMPOUND 5 [PMID: 24900526] | V85546 | ILOMASTAT | AZD1236 | COMPOUND 1 [PMID: 24900526] | AZD1236 | NEOVASTAT | RXP470.1 43 OGG1 CAFFEINE | SELENIUM 44 PGD PENICILLAMINE | PHENOBARBITAL 45 PIK3C3 PKI-587 | PWT33597 | XL-765 | SF1126 | SAR405 | GDC-0980 | PX-866 | GDC-0941 | COMPOUND 82 [PMID: 21332118] | BKM120 | BEZ235 | SF1126 | XL147 | PF-4691502 | VPS34-IN-1 | COMPOUND 28 [PMID: 24387221] | GSK2636771 | PI-103 | SOPHORETIN | BYL719 | TORIN 2 | BAY80-6946 | PIK-III | BGJ398 46 PPID SCY-635 47 PRKCA G-6983 | 7-HYDROXYSTAUROSPORINE | SOPHORETIN | G-6976 | BALANOL | MIDOSTAURIN | BRYOSTATIN-1 | SOTRASTAUIN | SOTRASTAUIN | SOTRASTAUIN ACETATE | CEP-2563 | RUBOXISTAURIN | ENZASTAUIN | BRYOSTATIN | INGENOL MEBUTATE | AFFINITAC | ENZASTAUIN | MIDOSTAURIN | (7S)-HYDROXYL- STAUROSPORINE | INGENOL MEBUTATE | GSK-690693 48 PRMT6 EPZ020411 | MS023 | MS049 | C21 49 PRMT7 DS-437 50 PRMT8 MS023 | MS049 51 PTK6 COMPOUND 19A [PMID: 21855335] | VANDETANIB | TILFRINIB 52 REGIA LINOMIDE | INSULIN 53 S1PR5 Siponimod | FINGOLIMOD HYDROCHLORIDE | AMISELIMOD HYDROCHLORIDE 54 SERPING1 C1-INH | RECOMBINANT HUMAN C1 ESTERASE INHIBITOR 55 SETBP1 N/A 56 SIGMAR1 E-5842 | (RS)-PPCC | RIMCAZOLE | ANAVEX 1007 | SSR- 125047 | DEXTROMETHORPHAN | [3H]PENTAZOCINE | MS- 377 | ANAVEX 2-73 | (+)-SKF 10.047 | NE-100 | E-6276 | SIGMA ANTAGONIST | ANAVEX 2-73 | OPC-14523 | DEXTROMETHORPHAN HYDROBROMIDE MONOHYDRATE | PRX-00023 | PRE-084 | RIMCAZOLE | BD-1047 | OPC-14523 | SA4503 | PENTAZOCINE LACTATE | DEXTROMETHORPHAN | DEXTROMETHORPHAN | (-)-PENTAZOCINE | SR-31742A | NALTREXONE | DEXTROMETHORPHAN POLISTIREX | DEXTROMETHORPHAN | NE-100

| PENTAZOCINE | HYDROCHLORIDE | DEUTEROTHROMBOPHAN | ANAVEX 1-41 57 SLC29A4 CIMETIDINE | DIPYRIDAMOLE | RHODAMINE123 | DECYNIIUM 22 | QUININE | FLUOXETINE | QUINIDINE | VERAPAMIL | DESIPRAMINE 58 SLC6A9 [3H](R)-NPTS | SSR-103800 | BITOPERTIN | GSK931145 | ORG- 24461 | ALX-5407 | ORGANON | [3H]SB-733993 | [3H]NFPS | PF- 03463275 | BITOPERTIN | ORGANON-2 | [35S]ACPPB | (R)-NFPS | [3H]N-METHYL-SSR504734 | LY2365109 | SSR-504734 | N-METHYL-SSR504734 | GSK1018921 | ORGANON-3 | R1678 | SSR-103800 | GLIATECH | [3H]GSK931145 59 SLCO2A1 [3H]PGE2 60 SMARCB1 radiotherapy | EPZ-6438 | Alvocidib | Tazemetostat | DZNeP | Vorinostat | PANOBINOSTAT | Fenretinide | Palbociclib | CFI- 400945 61 SOCS1 INSULIN | IL-2 62 SRD5A1 LEVONORGESTREL | DUTASTERIDE | FINASTERIDE | FINASTERIDE | DUTASTERIDE | DUTASTERIDE 63 SRD5A3 DUTASTERIDE 64 TBXAS1 NV-52 | NM-702 | COMPOUND 7P [PMID: 7861416] | NV-52 | PMA | COMPOUND 10A [PMID: 1447738] 65 TGFB2 LERDELIMUMAB | TRABEDERSEN | BELAGENPUMATUCEL- L | FRESOLIMUMAB | AP12009 | FRESOLIMUMAB | LERDELIMUMAB 66 THBD SIMVASTATIN | CILOSTAZOL | LEVOTHYROXINE | GINKGO BILOBA | CURCUMIN | PROSTAGLANDIN E1 67 TNFRSF12A ENAVATUZUMAB | TWEAK 68 TPCN1 VERAPAMIL | PHOSPHATIDYL (3,5) INOSITOL BISPHOSPHATE | CD2+ 69 TXK COMPOUND 31 [PMID: 24915291] | ACALABRUTINIB | IBRUTINIB | COMPOUND 38 [PMID: 24915291] | PRN694 | COMPOUND 23 [PMID: 17600705] | BOSUTINIB | COMPOUND 7 [PMID: 22464456]

[0040] In one embodiment, the gene or combination is selected from the genes identified in Table IV. Table IV is a list of the top 73 genes identified in the high MOI SARS-CoV-2 screen and the corresponding drugs known to inhibit expression or activity of these genes. These genes are not ranked in the same order as for Table I or Table II. The drugs are listed as inhibiting the corresponding gene (see, e.g., the Drug Gene Interaction database: www.dgidb.org/). Previous reports have established that these small-molecule drugs can inhibit (“mimic loss-of-function”) of the corresponding gene in the list. That is, the drug copies loss-of-function/knock-out of the gene without the need for other means of knocking out gene expression or activity, such as CRISPR, genome editing, etc.

TABLE-US-00004 Rank Gene Inhibitor Drug 1 ABCB4 ORTATAXEL | AMYLIN | VINBLASTINE | AMYLIN | TARIQUIDAR | TYR0 $\hat{\pm}$ - CGRP (HUMAN) | VINCRISTINE | PRAMLINTIDE | CYCLOSPORINE | MITOMYCIN-C | $\hat{\pm}$ - CGRP | ADRENOMEDULLIN | LY335979 | ADRENOMEDULLIN 2/INTERMEDIN | CALCITONIN | VALSPODAR | CURCUMIN I | [125I]BH-AMY (RAT, MOUSE) | BENZQUINAMIDE | CBT-1 2 ACE2 COMPOUND 28 [PMID: 18324760] | XNT [PMID: 18391097] | MLN-4760 | GL1001 | ABZ-SER-PRO-TYR(NO2)—OH | LISINOPRIL 3 ACTB ETHINYLESTRADIOL | ENVIRONMENTAL ESTROGEN | CYCLOPHOSPHAMIDE 4 ACTR2 ETOPOSIDE | MCP-1 5 ADSS ALANOSINE 6 AOX1 MENADIONE | ISOVANILLIN 7 AQP6 HG2+ 8 ARID1A DASATINIB | Trastuzumab | GSK126 9 ATIC FOLIC ACID | METHOTREXATE 10 ATP6V1A CRUENTAREN 11 BAP1 TRICHOSTATIN A (TSA) | EVEROLIMUS | LBH-589 | VALPROIC ACID | VORINOSTAT (SAHA) | SUNITINIB 12 BAX PREDNISOLONE | METHOTREXATE | NITROGEN MUSTARD | DOXORUBICIN | CA4P | PHENYLEPHRINE | DHEA | PHENOBARBITAL | BICALUTAMIDE | DHT | MELATONIN | SULFORAPHANE | MEDROXYPROGESTERONE | ETOPOSIDE | COBALT | C5A | ADM | FLUDARABINE | TESTOSTERONE UNDECANOATE | NFX | QUERCETIN | M-CSF | CARVEDILOL | INTERLEUKIN-1 | VINOELBINE | SALINE | ERBITUX | OXALIPLATIN | ESTRAMUSTINE | HUMAN CHORIONIC GONADOTROPIN | IL-4 | PMSG | NICOTINE | NEUROPROTECTANTS | TNP-470 | VERAPAMIL | RETINOL | ANTHRACYCLINE | 5-FLUOROURACIL | BORTEZOMIB | ERYTHROMYCIN | INHIBINS | PROCARBAZINE | RAPAMYCIN | BETA-LAPACHONE | HYPERBARIC OXYGEN | CIPROFLOXACIN | BCG | PRAVASTATIN | ATENOLOL | INTERFERON ALFA-2B | OXYTOCIN | ANTI-

INFLAMMATORY DRUGS | LEUCOVORIN | DOCETAXEL | NALTREXONE | CAMPTOTHECIN | PROTEIN PHOSPHATASE INHIBITOR | ANDROSTENEDIONE | RETINOIC ACID | MITOXANTRONE | XK469 | CYCLOPHOSPHAMIDE | DACARBAZINE | FLT3 LIGAND | CISPLATIN | VIOXX | LEVODOPA 13 BCHE IMMUNOTOXIN | RIVASTIGMINE | ECHOTHIOPHATE IODIDE | ISOFLUROPHATE | PHYSOSTIGMINE | RIVASTIGMINE | ISOFLUROPHATE | DEMECARIUM BROMIDE | ECHOTHIOPHATE IODIDE | RIVASTIGMINE | HEXAFLURONIUM BROMIDE | HEXAFLURONIUM BROMIDE | RIVASTIGMINE TARTRATE | TACRINE HYDROCHLORIDE | PHENOTHIAZINE | ECHOTHIOPHATE IODIDE | TRIAMCINOLONE | IRINOTECAN | BAMBUTEROL | DONEPEZIL | RIVASTIGMINE | ANESTHETICS | HEXAFLURONIUM BROMIDE | TACRINE | Succinylcholine | PYRIDOSTIGMINE | DEMECARIUM BROMIDE | BUPIVACAINE | OPIOIDS 14 BCL2 HYDROQUINONE | PENTOXIFYLLINE | ENDOTHELIN RECEPTOR ANTAGONIST | VENETOCLAX | FAS LIGAND | SELENIUM | ERYTHROMYCIN | DES | TAZAROTENE | DOLASTATIN 10 | MICELLAR PACLITAXEL | ISIS 3521 | OBATOCLAX | ABT-263 | ABT-737 | CILENGITIDE | 5- FLUORODEOXYURIDINE | CISPLATIN | OBLIMERSON | ZD1694 | URSODEOXYCHOLIC ACID | CHEMOSENSITIZER | PACLITAXEL | VALPROIC ACID | PHENYL BUTYRATE | ORTATAXEL | ANG1005 | MITOXANTRONE | EGFR TYROSINE KINASE INHIBITOR | ALPHA-TOCOPHEROL | INTERLEUKINS | CISPLATIN | OBATOCLAX | HYPOXANTHINE | NSAIDS | NAVITOCCLAX, ABT-263 | HEN EGG LYSOZYME | DDTc | NAVITOCCLAX | LIPOSOMAL DOXORUBICIN | AT-101 | VENETOCLAX | AT101 | AT-101 | CARBOPLATIN | PEG-DOCETAXEL | GAMMA-INTERFERON | ATRA | IFN | TESETAXEL | OBLIMERSEN | PMSG | BORTEZOMIB | BEAUVERICIN | TGFβ1 | SAPONIN | NAVITOCCLAX | MCI-186 | DOCETAXEL | DOXORUBICIN | STREPTOZOTOCIN | C5A | ESTRAMUSTINE | NAVITOCCLAX | PACLITAXEL | EPIRUBICIN | 5-AMINOLAEVULINIC ACID | OBLIMERSEN | CA4P | ISOTRETINOIN | ETOPOSIDE | OBATOCLAX MESYLATE | MILATAXEL | NDGA | OXALIPLATIN | VENETOCLAX | EMD 121974 | OXALIPLATIN | PROTEASE INHIBITOR | PACLITAXEL | HYPERBARIC OXYGEN | SPC- 2996 | OBLIMERSEN | METHYLPREDNISOLONE | ORAL CONTRACEPTIVE | EPISIALIN | TENIPOSIDE | TRAIL | 13- CIS-RETINOIC ACID | RIBAVIRIN 15 BCORL1 N/A 16 BRPF1 PFI-4 | COMPOUND 2 [PMID: 25408830] | COMPOUND 3 [PMID: 25408830] | COMPOUND 34 [PMID: 25974391] | NI-57 | COMPOUND 1 [PMID: 25408830] | OF-1 17 CA6 SAR566658 | ETHOXZOLAMIDE 18 CDK3 RGB-286638 | TG02 | ALVOCIDIB | N/A 19 CHKA TCD-717 | DEXAMETHASONE 20 CHRNG ROCURONIUM BROMIDE | RAPACURONIUM BROMIDE | VECURONIUM BROMIDE | GALLAMINE TRIETHIODIDE | ATRACURIUM BESYLATE | DOXACURIUM CHLORIDE | METOCURINE IODIDE | TUBOCURARINE CHLORIDE | MIVACURIUM CHLORIDE | PIPECURONIUM BROMIDE | SUCCINYLCHOLINE CHLORIDE | PANCURONIUM BROMIDE | DECAMETHONIUM BROMIDE | CISATRACURIUM BESYLATE 21 CNTN1 S-ALLYLGLYCINE 22 COL11A1 OCRIPLASMIN | COLLAGENASE CLOSTRIDIUM HISTOLYTICUM 23 CPS1 CARGLUMIC ACID | CARGLUMIC ACID 24 CPT1B L-CARNITINE | PERHEXILINE 25 CTSL HORMONES | LHVS | KGP94 26 DEFA4 CYTOKINES 27 DPP9 1G244 | COMPOUND 24DD [PMID: 20684603] 28 DRD1 METHYLERGONOVINE MALEATE | CABERGOLINE | FENOLDOPAM | PROCHLORPERAZINE | LISURIDE | OLANZAPINE | FLUPENTHIXOL | QUETIAPINE | LOXAPINE | PERGOLIDE | ETILEVODOPA | PERGOLIDE | SCH-23390 | FENOLDOPAM | FLUPENTHIXOL | OLANZAPINE | PROMAZINE | (+)-SKF-82526 | PERGOLIDE MESYLATE | THIORIDAZINE | APOMORPHINE | ILOPERIDONE | HALOPERIDOL | ZUCLOPENTHIXOL | [3H]SCH-23390 | MINAPRINE | PERPHENAZINE | CARPHENAZINE | CARPHENAZINE | PROPIOMAZINE | ACETOPHENAZINE | FENOLDOPAM MESYLATE | PERPHENAZINE | BSF-78438 | PROCHLORPERAZINE | N- PROPYLNORAPOMORPHINE | THIORIDAZINE |

PROMAZINE | CLOZAPINE | AMOXAPINE | SKF- 76783 | LEVODOPA | THIORIDAZINE | 5- HYDROXYTRYPTAMINE | (+)-ADTN | NORADRENALINE | FLUPHENAZINE | A77636 | PIMOZIDE | LU AE04621 | FLUPENTHIXOL | ASENAPINE | THIETHYLPERAZINE | MELEVODOPA | METHYLERGONOVINE | KETANSERIN | FENOLDOPAM | CLOZAPINE | XP21279 | CHLORPROTHIXENE | SPIPERONE | PROPIOMAZINE | ACETOPHENAZINE | ZUCLOPENTHIXOL | LU 02-750 | FLUPHENAZINE | DOPAMINE | CHLORPROMAZINE | SKF- 38393 | BROMOCRIPTINE | ECOPIPAM | (-)-SKF-82526 | PERICIAZINE | METHYLERGONOVINE | COCAINE | TRIFLUPROMAZINE | [125I]SCH23982 | LEVODOPA | SKF- 83566 | FENOLDOPAM | FLUPHENAZINE | TRIFLUPROMAZINE | ROTIGOTINE | MINAPRINE | ECOPIPAM | LISURIDE | THIETHYLPERAZINE | ROTIGOTINE | SKF-83959 | ERGOLOID MESYLATES | PERGOLIDE | CLOZAPINE | METHYLERGONOVINE | CHLORPROTHIXENE | D1 AGONIST D2 ANTAGONIST | MESORIDAZINE | (+)-BUTACLAMOL | APOMORPHINE | PERGOLIDE | LISURIDE 29 DYRK2 COMPOUND 5B [PMID: 24900464] | COMPOUND 3B [PMID: 23454515] | LEUCETTINE L41 30 ENOSF1 3TC | AZT | ANTIVIRAL 31 EREG PANITUMUMAB | CETUXIMAB | HUMAN CHORIONIC GONADOTROPIN 32 ETV1 TRAMETINIB 33 FAP ARI-3099 34 FASLG ANGIOTENSIN CONVERTING ENZYME INHIBITOR | APG101 | TUMOR NECROSIS FACTOR-BETA 35 FUS ATRA 36 GATA1 Daunorubicin | Cytarabine | N/A 37 GNAS CISPLATIN | 5-FLUOROURACIL 38 HDAC9 SB939 | PCI-24781 | TMP269 | ROMIDEPSIN | CUDC-101 | VORINOSTAT | GIVINOSTAT | CUDC-101 | BELINOSTAT | BELINOSTAT | PRACINOSTAT | AR-42 | PANOBINOSTAT | PANOBINOSTAT | PANOBINOSTAT | PIVANEX | SCRIPTAID | BELINOSTAT | MGCD-0103 | DACINOSTAT | BELINOSTAT | VORINOSTAT | TRICHOSTATIN A | ROMIDEPSIN | VORINOSTAT | GIVINOSTAT | CUDC-101 | GIVINOSTAT | QUISINOSTAT | ROMIDEPSIN | ENTINOSTAT | VALPROIC ACID | PANOBINOSTAT | RESMINOSTAT | VORINOSTAT | VALPROIC ACID | SODIUM PHENYLBUTYRATE 39 HLA-DRA NIVOLUMAB | PEMBROLIZUMAB | ATEZOLIZUMAB 40 HRAS Pz-1 | Sirolimus | Selumetinib | PD0325901 | AZD8055 | EGFR INHIBITOR | BRYOSTATIN-1 | rigosertib | VIRAL VECTOR | LONAFARNIB | REOLYSIN | Everolimus | LAK | ADENOVIRAL VECTOR | STZ | HYDROXYUREA | Dasatinib | AZD4547 | DOCETAXEL | Binimetinib | L-744,832 | BKM120 | EVEROLIMUS | NS1 | MK2206 | IL-6 | AZ8010 | Panitumumabm | CI-1040 | FTI-277 | BINIMETINIB (MEK162) | Trametinib | Tipifarnib | AZD8055 | E6201 | GEMCITABINE | ANTISENSE OLIGONUCLEOTIDES | ANTINEOPLASTIC | DES | STREPTOZOTOCIN | DIMETHYL SULFOXIDE | ANTHRACYCLINES | BYL719 | INSULIN | PLX7904 | NITROGLYCERIN | ERYTHROPOIETIN | MEK162 | HEXAMETHYLENE BISACETAMIDE | BEZ235 | cabozantinib | ISIS-2503 | BEVACIZUMAB | ANTIBIOTIC | SELUMETINIB (AZD6244) | PD173074 | VITAMIN E | MNU | PD-98059 | SF1126 | Metformin | Cetuximab | DIETHYLSTILBESTROL | SCH772984 41 HTR1F 5-CT | ALMOTRIPTAN MALATE | Î±-METHYL-5-HT | GR 127935 | RIZATRIPTAN | LY344864 | DIPROPYL-5-CT | NARATRIPTAN | METHIOHEPIN | SUMATRIPTAN | DOI | TRYPTAMINE | 5-HYDROXYTRYPTAMINE | 5-BODMT | 1- NAPHTHYLPIPERAZINE | DONITRIPTAN | 5-MEO-DMT | LASMIDITAN | 2-METHYL-5-HT | ZOLMITRIPTAN | DIHYDROERGOTAMINE | NARATRIPTAN | OLANZAPINE | LASMIDITAN | LASMIDITAN | BRL-15572 | BRL-54443 | RISPERIDONE | SERTINDOLE | XANOMELINE | METERGOLINE | METHYLERGONOVINE | ZOLMITRIPTAN | 8-OH-DPAT | QUETIAPINE | 5-MEOT | TFMPP | ELETRIPTAN HYDROBROMIDE | NAN 190 | ZOLMITRIPTAN | ELETRIPTAN | [3H]LY334370 | SUMATRIPTAN | LY334370 | METHYSERGIDE | NARATRIPTAN | YOHIMBINE | ERGOTAMINE | SUMATRIPTAN | CLOZAPINE 42 HYAL2 EPINEPHRINE | HORMONES | DEXAMETHASONE 43 IL23A BRIAKINUMAB | BRIAKINUMAB | CNTO 1275 | USTEKINUMAB | Ustekinumab | USTEKINUMAB | USTEKINUMAB | TILDRAKIZUMAB |

USTEKINUMAB | GUSELUMAB 44 ITGA4 VEDOLIZUMAB | VEDOLIZUMAB |
ATL/TV1102 | NATALIZUMAB | FIRATEGRAST | CDP323 | ATL1102 | VEDOLIZUMAB |
NATALIZUMAB | FIRATEGRAST | GSK683699 | SB-683699 | R1295 | FIRATEGRAST |
NATALIZUMAB | R1295 | NATALIZUMAB 45 KDM4E ML324 | COMPOUND 6A [PMID:
18942826] 46 KRT18 ANTIVIRAL | MITOMYCIN-C | IL-6 | RIBAVIRIN 47 LY96 L6H21 |
ERITORAN TETRASODIUM | NEOCEPTIN-3 48 MAP3K10 CEP-1374 | URM-099 | CEP-
1347 49 MAP3K3 COMPOUND 5N [PMID: 20483621] 50 MMP9 ANDECALIXIMAB |
MARIMASTAT | PG-116800 | NEOVASTAT | COMPOUND 29E [PMID: 23631440] | CGS-
27023A | PRINOMASTAT | ANDECALIXIMAB | S-3304 | BEVACIZUMAB | SB-3CT |
ILOMASTAT | COL-3 | AZD6605 | AZD1236 | SL422 | TST10088 | MARIMASTAT | AE-941 |
COMPOUND 1A [PMID: 15055993] 51 MYB RETINOID | 12-O-
TETRADECANOYLPHORBOL-13- ACETATE | LR3001 | 5-FLUOROURACIL |
DOXORUBICIN | G4460 | TAXOL 52 NDUFB2 NV-128 | ME-344 | METFORMIN
HYDROCHLORIDE 53 NDUFV1 NV-128 | ME-344 | METFORMIN HYDROCHLORIDE 54
OSM IL-10 | TNF-ALPHA 55 PDE6A COMPOUND 53 [PMID: 19631533] | PENTOXIFYLLINE
| DIPYRIDAMOLE 56 PGD PENICILLAMINE | PHENOBARBITAL 57 PIK3R4 PI-103 |
BYL719 | BYL719 | BUPARLISIB HYDROCHLORIDE | PX-866 | GDC-0941 | PKI-587 |
BEZ235 | GSK2126458 | SF1126 | PF-4691502 | PWT33597 | PKI-587 | GDC-0980 | XL- 765 |
BAY80-6946 | BAY80-6946 | PX-866 | BGJ398 | GSK2636771 | XL147 | BKM120 |
SOPHORETIN | DACTOLISIB 58 POLE FLUDARABINE PHOSPHATE | PEMBROLIZUMAB |
GEMCITABINE HYDROCHLORIDE | CLOFARABINE | CYTARABINE 59 PSMA3
CARFILZOMIB | BORTEZOMIB | OPROZOMIB | IXAZOMIB CITRATE | MARIZOMIB |
BORTEZOMIB | CARFILZOMIB 60 PSMB4 OPROZOMIB | CARFILZOMIB | BORTEZOMIB |
IXAZOMIB CITRATE | MARIZOMIB | BORTEZOMIB | CARFILZOMIB 61 PSMB9
CARFILZOMIB | OPROZOMIB | BORTEZOMIB | BORTEZOMIB | MARIZOMIB | IXAZOMIB
CITRATE | CARFILZOMIB 62 PSMC4 CARFILZOMIB | OPROZOMIB | BORTEZOMIB |
IXAZOMIB CITRATE | CARFILZOMIB | BORTEZOMIB 63 SIAH1 OMIGAPIL 64 SLC18A2
LISDEXAMFETAMINE DIMESYLATE | [3H]TBZOH | TETRABENAZINE | VALBENAZINE |
DEXTROAMPHETAMINE | RESERPINE | TETRABENAZINE | BENZPHETAMINE
HYDROCHLORIDE | LOBELINE | RESERPINE | [125I]IODOVINYL-TBZ | ALSEROXYLON |
DEXTROAMPHETAMINE SULFATE | TETRABENAZINE | RESERPINE |
DEUTETRABENAZINE | RESERPINE | RESCINNAMINE | KETANSERIN |
METHAMPHETAMINE | DEXTROAMPHETAMINE | DESERPIDINE | TETRABENAZINE |
DEXTROAMPHETAMINE ADIPATE | NBI-98854 | ALSEROXYLON |
DEXTROAMPHETAMINE SACCHARATE | RESERPINE | ALSEROXYLON | [11C]DTBZ |
NRP104 | AMPHETAMINE | [125I]7-AZIDO-8- IODOKETANSERINE | TETRABENAZINE 65
SLCO1A2 TESTOSTERONE | [3H]ESTRONE-3-SULPHATE | RIFAMYCIN SV | HORMONES |
RIFAMPICIN | NARINGIN | [3H]BSP | [3H]DPDPE 66 SLCO1B1 RIFAMYCIN SV |
CLOFIBRATE | GEMFIBROZIL | GEMFIBROZIL | FENOFIBRATE | ESTRONE-3-SULPHATE
| GLYCYRRHIZIN | METHOTREXATE | TROGLITAZONE | DOCETAXEL | RIFAMPICIN |
INDOCY ANINE GREEN | PRAVASTATIN | Rosuvastatin | [3H]ESTRADIOL-17^β.sup.2-
GLUCURONIDE | PRAVASTATIN | [3H]ESTRONE-3- SULPHATE | CYCLOSPORIN A |
ATORVASTATIN 67 SMAD4 Sapanisertib | CETUXIMAB | Gemcitabine | 5- FLUOROURACIL |
Cisplatin | Crizotinib | N/A | Paclitaxel | Carboplatin | SD-093 | TGF | Irinotecan | Imatinib |
Alectinib 68 SMYD2 LLY-507 | AZ505 | BAY-598 69 SOAT1 NIFEDIPINE | 6-
HYDROXYDOPAMINE | DEXAMETHASONE | TESTOSTERONE | EMZETIBE |
ATORVASTATIN | URSODEOXYCHOLIC ACID | LOVASTATIN 70 TGFB2 COMPOUND
15B [PMID: 16539403] | COMPOUND 13A [PMID: 23639540] | TG-C | LY2109761 |
COMPOUND 13D [PMID: 23639540] 71 TGFB3 CYTOKINES | DEXAMETHASONE |
RANTES | BFGF | GM- CSF | TG-C | TGF-BETA 2 | RETINOIC ACID 72 TOP1 TOPOTECAN |

LIPOTECAN | IRINOTECAN | IRINOTECAN | BECATECARIN | CAFFEINE |
 ETIRINOTECAN PEGOL | IRINOTECAN HYDROCHLORIDE HYDRATE | (S)-DRF-1042 |
 CAMPTOTHECIN | IRINOTECAN | ARQ-501 | IRINOTECAN SUCROSOATE | LE-SN38 |
 IRINOTECAN | PEG- CAMPTOTHECIN | IRINOTECAN | PEG-IRINOTECAN | TOPOTECAN
 | TOPOTECAN | LURTOTECAN | TOPOISOMERASE INHIBITORS | NKTR-102 |
 RUBITECAN | KARENITECIN | IRINOTECAN | NEW AGENTS | CISPLATIN |
 CYCLOPHOSPHAMIDE | C-1311 | TOPOTECAN | CAMPTOTHECIN | PEG-SN38 | N/A 73
 TPBG NAPTUMOMAB ESTAFENATOX | ANATUMOMAB MAFENATOX

[0041] The following Table V identifies certain available inhibitors, target genes and notes the SelleckChem or MedChem Express vendor numbers for the inhibitors.

TABLE-US-00005 TABLE V Available SelleckChem/MedChem Express Target Gene inhibitors
 Vendor Code HDAC9 Vorinostat S1047 Belinostat S1085 Panobinostat S1030 Pracinostat S1515
 BRD 4354 BRD4354 TMP195 S8502 Cathepsin L Odanacatib S1115 MG-101 (ALLN) S7386 SID
 26681509 SID2668 PRKCA Tamoxifen S1238 Sotrastaurin S2791 Staurosporine S1421
 Enzastaurin S1055 Serabelisib (MLN1117) S8581 PIK3C3 Idelalisib S2226 (VPS34) Buparlisib
 S2247 SAR405 S7682 VVPS34 inhibitor 1 S8456 (Compound 19) Vps34-PIK-III S7683
 Autophinib S8596 CALR Gentamicin S4030 DRD2 Quetiapine S5741 Olanzapine S2493 MMP12
 Ilomastat S7157 Doxycycline Hyclate S4163 MAPK3 PD0325901(Mirdametinib) S1036
 Binimetinib (MEK162) S7007 BRPF1 GSK6853 GSK6853 control Remdesivir S8932

[0042] In one embodiment, the gene is PIK3C3. In another embodiment, the gene or gene combination is selected from RAB7A, ACE2, ACTR2, ACTR3, ARPC3, and ARPC4. In another embodiment, the gene or combination comprises one or more of CTSL, ATP6AP1, ATP6AP2, ATP6V0B ATP6V0C, ATP6V0D1, ATP6V1A, ATP6V1B2, ATP6V1C1, ATP6V1E1, ATP6V1G1, ATP6V1H, TMEM199, and TOR1AIP1. In another embodiment, the gene or combination comprises one or more of VPS26A, VPS29, VPS35, and SNX27. In another embodiment, the gene or combination comprises one or more of COMMD2, COMMD3, COMMD3-BMI1, and COMMD4. In still a further embodiment, the gene or combination comprises one or more of PIK3C3/VPS34, WDR81, and ACP5.

[0043] In yet another embodiment, the gene or combination comprises one or more of DPM3, ERMP1, PPID, CHST14, SLTM and SPEN. The various gene combinations can also include one or more of PIK3C3, Cathepsin L, PRKCA, MMP12, BRPF1, DRD2, MAPK3, CALR and HCAC9. Still other combinations or selections of genes are shown in FIG. 1D, in FIG. 2, in FIG. 3C, in FIG. 4A or 4D, in FIG. 5C, in FIG. 6B, in FIG. 8E. In still another embodiment, the genes include one or more of the 69 genes identified in Table III. In yet another embodiment, the genes to be inhibited are one or more of the 73 genes identified in Table IV. Still other combination of genes can be inhibited in the practice of the methods, therapeutic regimens or compositions described herein.

[0044] By the term “druggable gene” as used herein is meant a human gene for which an inhibitor of expression, activity or signalling is known and identified. For example, see the 69 low MOI druggable genes and inhibitors in Table III and the 73 high MOI druggable genes and corresponding inhibitors in Table IV. In one embodiment as mentioned above, the druggable gene is PIK3C3.

C. Inhibitor, Blocker, Antagonist

[0045] By the general terms “blocker”, “inhibitor” or “antagonist” is meant an agent that inhibits, either partially or fully, the activity or production of a target molecule, e.g., as used herein, specifically one or more of one or a combination of the human subject's genes required for viral infection. In one embodiment, the viral infection is caused by SARS-CoV-2. In particular, these terms refer to a composition or compound or agent capable of decreasing levels of gene expression, mRNA levels, protein levels or protein activity of the target molecule. Illustrative forms of antagonists include, for example, proteins, polypeptides, peptides (such as cyclic peptides), antibodies or antibody fragments, peptide mimetics, nucleic acid molecules, antisense molecules,

ribozymes, aptamers, RNAi molecules, and small organic molecules. Illustrative non-limiting mechanisms of antagonist inhibition include repression of ligand synthesis and/or stability (e.g., using, antisense, ribozymes or RNAi compositions targeting the ligand gene/nucleic acid), blocking of binding of the ligand to its cognate receptor (e.g., using anti-ligand aptamers, antibodies or a soluble, decoy cognate receptor), repression of receptor synthesis and/or stability (e.g., using, antisense, ribozymes or RNAi compositions targeting the ligand receptor gene/nucleic acid), blocking of the binding of the receptor to its cognate receptor (e.g., using receptor antibodies) and blocking of the activation of the receptor by its cognate ligand (e.g., using receptor tyrosine kinase inhibitors). In addition, the blocker or inhibitor may directly or indirectly inhibit the target molecule.

[0046] In one embodiment, the inhibitor(s) are selected from one or a combination of the inhibitors identified in Table III or Table IV. In another embodiment, the inhibitor or a combination of inhibitors is identified in FIG. 4D or 4E. In another embodiment, the inhibitor useful in the methods is one or a combination a combination of Remdesivir, PIK-III, Compound 19, SAR405, Autophinib, ALLN, Tamoxifen and Ilomastat. The selected inhibitor is administered to the subject pre-infection or post-infection at a dosage effective to mimic a loss of function of its corresponding gene.

[0047] i. Salts—The compositions described herein also includes salts of the specific compounds described in Tables III or IV. As used herein, “salts” refers to derivatives of the disclosed compounds wherein the parent compound is modified by converting an existing acid or base moiety to its salt form. Examples of salts include, but are not limited to, mineral acid (such as HCl, HBr, H.sub.2SO.sub.4) or organic acid (such as acetic acid, benzoic acid, trifluoroacetic acid) salts of basic residues such as amines; alkali (such as Li, Na, K, Mg, Ca) or organic (such as trialkyl ammonium) salts of acidic residues such as carboxylic acids; and the like. The salts of compounds described or referenced herein can be synthesized from the parent compound which contains a basic or acidic moiety by conventional chemical methods. Generally, such salts can be prepared by reacting the free acid or base forms of these compounds with a stoichiometric amount of the appropriate base or acid in water or in an organic solvent, or in a mixture of the two; generally, nonaqueous media like ether, ethyl acetate, ethanol, isopropanol, or acetonitrile (ACN) are preferred.

[0048] The “pharmaceutically acceptable salts” of compounds described herein or incorporated by reference include a subset of the “salts” described above which are, conventional non-toxic salts of the parent compound formed, for example, from non-toxic inorganic or organic acids. Lists of suitable salts are found in Remington's Pharmaceutical Sciences, 17th ed., Mack Publishing Company, Easton, Pa., 1985, p. 1418 and Journal of Pharmaceutical Science, 66, 2 (1977), each of which is incorporated herein by reference in its entirety.

[0049] The phrase “pharmaceutically acceptable” is employed herein to refer to those compounds, materials, compositions, and/or dosage forms which are, within the scope of sound medical judgment, suitable for use in contact with the tissues of human beings and animals without excessive toxicity, irritation, allergic response, or other problem or complication, commensurate with a reasonable benefit/risk ratio.

[0050] ii. Prodrug—By the term “prodrug” is meant a compound or molecule or agent that, after administration, is metabolized (i.e., converted within the body) into the parent pharmacologically active molecule or compound, e.g., inhibitor of one or more of the gene identified herein. Prodrugs are substantially, if not completely, in a pharmacologically inactive form that is converted or metabolized to an active form (i.e., drug)—such as within the body or cells, typically by the action of, for example, endogenous enzymes or other chemicals and/or conditions. Instead of administering an active molecule directly, a corresponding prodrug is used to improve how the composition/active molecule is absorbed, distributed, metabolized, and excreted. Prodrugs are often designed to improve bioavailability or how selectively the drug interacts with cells or processes

that are not its intended target. This reduces adverse or unintended undesirable or severe side effects of the active molecule or drug.

[0051] iii. Biosimilar—A “biosimilar” is a biological product, generally a large and complex molecule, produced from living organisms, and monitored to ensure consistent quality that is highly similar to a reference product, e.g., an already FDA-approved biological drug. A biosimilar that receives FDA approval must have no clinically meaningful differences from the reference drug in purity, safety, molecular structure and bioactivity, or potency.

[0052] iv. Antibody and Fragments—By the term “antibody” or “antibody molecule” is any immunoglobulin, including antibodies and fragments thereof, that binds to a specific antigen. As used herein, antibody or antibody molecule contemplates intact immunoglobulin molecules, immunologically active portions of an immunoglobulin molecule, and fusions of immunologically active portions of an immunoglobulin molecule.

[0053] The antibody may be a naturally occurring antibody or may be a synthetic or modified antibody (e.g., a recombinantly generated antibody; a chimeric antibody; a bispecific antibody; a humanized antibody; a camelid antibody; and the like). The antibody may comprise at least one purification tag. In a particular embodiment, the framework antibody is an antibody fragment. The term “antibody fragment” includes a portion of an antibody that is an antigen binding fragment or single chains thereof. An antibody fragment can be a synthetically or genetically engineered polypeptide. Examples of binding fragments encompassed within the term “antigen-binding portion” of an antibody include (i) a Fab fragment, a monovalent fragment consisting of the VL, VH, CL and CHI domains; (ii) a F(ab')₂ fragment, a bivalent fragment comprising two Fab fragments linked by a disulfide bridge at the hinge region; (iii) a Fd fragment consisting of the VH and CHI domains; (iv) a Fv fragment consisting of the VL and VH domains of a single arm of an antibody, (v) a dAb fragment, which consists of a VH domain; and (vi) an isolated complementarity determining region (CDR). Furthermore, although the two domains of the Fv fragment, VL and VH, are coded for by separate genes, they can be joined, using recombinant methods, by a synthetic linker that enables them to be made as a single protein chain in which the VL and VH regions pair to form monovalent molecules (known as single chain Fv (scFv)). Such single chain antibodies are also intended to be encompassed within the term “antigen-binding fragment” of an antibody. These antibody fragments are obtained using conventional techniques known to those in the art, and the fragments can be screened for utility in the same manner as whole antibodies. Antibody fragments include, without limitation, immunoglobulin fragments including, without limitation: single domain (Dab; e.g., single variable light or heavy chain domain), Fab, Fab', F(ab')₂, and F(v); and fusions (e.g., via a linker) of these immunoglobulin fragments including, without limitation: scFv, scFv₂, scFv-Fc, minibody, diabody, triabody, and tetraabody. The antibody may also be a protein (e.g., a fusion protein) comprising at least one antibody or antibody fragment.

[0054] The antibodies useful in the methods are preferably “immunologically specific”, which refers to proteins/polypeptides, particularly antibodies, that bind to one or more epitopes of a protein or compound of interest, but which do not substantially recognize and bind other molecules in a sample containing a mixed population of antigenic biological molecules.

[0055] The antibodies useful in inhibiting the genes or gene products of the human genes identified herein may be further modified. For example, the antibodies may be humanized. In a particular embodiment, the antibodies (or a portion thereof) are inserted into the backbone of an antibody or antibody fragment construct. For example, the variable light domain and/or variable heavy domain of the antibodies of the instant invention may be inserted into another antibody construct. Methods for recombinantly producing antibodies are well-known in the art. Indeed, commercial vectors for certain antibody and antibody fragment constructs are available.

[0056] The antibodies of the instant invention may also be conjugated/linked to other components. For example, the antibodies may be operably linked (e.g., covalently linked, optionally, through a linker) to at least one cell penetrating peptide, detectable agent, imaging agent, or contrast agent.

The antibodies of the instant invention may also comprise at least one purification tag (e.g., a His-tag). In a particular embodiment, the antibody is conjugated to a cell penetrating peptide.

[0057] V. Aptamer—The term “aptamer” refers to a peptide or nucleic acid that has an inhibitory effect on a target. Inhibition of the target by the aptamer can occur by binding of the target, by catalytically altering the target, by reacting with the target in a way which modifies the target or the functional activity of the target, by ionically or covalently attaching to the target as in a suicide inhibitor or by facilitating the reaction between the target and another molecule. Aptamers can be peptides, ribonucleotides, deoxyribonucleotides, other nucleic acids or a mixture of the different types of nucleic acids. Aptamers can comprise one or more modified amino acid, bases, sugars, polyethylene glycol spacers or phosphate backbone units as described in further detail herein.

[0058] vi. RNA and DNA—The terms “RNA interference,” “RNAi,” “miRNA,” and “siRNA” refer to any method by which expression of a gene or gene product is decreased by introducing into a target cell one or more double-stranded RNAs, which are homologous to a gene of interest (particularly to the messenger RNA of the gene of interest). Gene therapy, i.e., the manipulation of RNA or DNA using recombinant technology and/or treating or preventing viral disease by introducing modified RNA or modified DNA into cells via a number of widely known and experimental vectors, recombinant viruses and CRISPR technologies, may also be employed in delivering, via modified RNA or modified DNA, effective inhibition of the pathways and gene products of the host genes identified herein as necessary to viral infection to accomplish the outcomes described herein with the combination therapies described. Such genetic manipulation can also employ gene editing techniques such as CRISPR (Clustered Regularly Interspaced Short Palindromic Repeats) and TALEN (transcription activator-like effector genome modification), among others. See, for example, the textbook National Academies of Sciences, Engineering, and Medicine. 2017. Human Genome Editing: Science, Ethics, and Governance. Washington, DC: The National Academies Press. <https://doi.org/10.17226/24623>, incorporated by reference herein for details of such methods.

[0059] vii. Small Molecule—The term “small molecule” when applied to a pharmaceutical generally refers to a non-biologic, organic compound that affects a biologic process which has a relatively low molecular weight, below approximately 900 daltons. Small molecule drugs have an easily identifiable structure, that can be replicated synthetically with high confidence. In one embodiment a small molecule has a molecular weight below 550 daltons to increase the probability that the molecule is compatible with the human digestive system's intracellular absorption ability. Small molecule drugs are normally administered orally, as tablets. The term small molecule drug is used to contrast them with biologic drugs, which are relatively large molecules, such as peptides, proteins and recombinant protein fusions, frequently produced using a living organism. Among the compositions identified in Tables III or IV are a host of suitable small molecules.

D. Effective Amounts, Routes

[0060] A “therapeutically effective amount” of a compound or a pharmaceutical composition refers to an amount effective to prevent, inhibit, treat, or lessen the symptoms of a particular viral disorder or disease. For example, “therapeutically effective amount” may refer to an amount sufficient to reduce the typical symptoms of fever, cough, inflammation, loss of smell, loss of breath and cytokine “storm” effects of SARS-CoV-2 infection, among other symptoms. Other viral diseases that may be treated similarly may be related ZIKA or MERS or other coronavirus infections.

[0061] A “pharmaceutically acceptable excipient or carrier” refers to, without limitation, a diluent, adjuvant, excipient, auxiliary agent or vehicle with which an active agent of the present invention is administered. Pharmaceutically acceptable carriers are those approved by a regulatory agency of the Federal or a state government or listed in the U.S. Pharmacopeia or other generally recognized pharmacopeia for use in animals, and more particularly in humans, can be sterile liquids, such as water and oils, including those of petroleum, animal, vegetable or synthetic origin, such as peanut

oil, soybean oil, mineral oil, sesame oil and the like. Water or aqueous saline solutions and aqueous dextrose and glycerol solutions are preferably employed as carriers, particularly for injectable solutions. Suitable pharmaceutical carriers are described in "Remington's Pharmaceutical Sciences" by E. W. Martin (Mack Publishing Co., Easton, PA); Gennaro, A. R., Remington: The Science and Practice of Pharmacy, (Lippincott, Williams and Wilkins); Liberman, et al., Eds., Pharmaceutical Dosage Forms, Marcel Decker, New York, N.Y.; and Kibbe, et al., Eds., Handbook of Pharmaceutical Excipients, American Pharmaceutical Association, Washington.

[0062] As used herein, the term "treatment" refers to any method used that imparts a benefit to the subject, i.e., which can alleviate, delay onset, reduce severity or incidence, or yield prophylaxis of one or more symptoms or aspects of the viral disease, disorder, or condition. For the purposes of the present invention, treatment can be administered before, during, and/or after the onset of symptoms. In certain embodiments, treatment occurs after the subject has received conventional therapy. In some embodiments, the term "treating" includes abrogating, substantially inhibiting, slowing, or reversing the progression of the viral disease or symptoms thereof, substantially ameliorating clinical or aesthetical symptoms of a condition, or substantially preventing the appearance of clinical or aesthetical symptoms of a condition, or decreasing the severity and/or frequency one or more symptoms resulting from the disease.

[0063] As used herein, the term "prevent" refers to the prophylactic treatment of a subject who is at risk of contracting the viral disease resulting in a decrease in the probability that the subject will develop the disease.

[0064] The term "therapeutic regimen" as used herein refers to the specific order, timing, duration, routes and intervals between administration of one or more therapeutic agents. In one embodiment a therapeutic regimen is subject-specific. In another embodiment, a therapeutic regimen is virus-specific or symptom-specific. In another embodiment, the therapeutic regimen changes as the subject responds to the therapy. In another embodiment, the therapeutic regimen is fixed until certain therapeutic milestones are met. In one embodiment, the therapeutic regimen involves one or more doses of an inhibitor of one such gene, coupled with administration of one or more doses of an inhibitor of another gene required for viral infection. In one embodiment, the one or more doses of a first inhibitor is administered simultaneously or sequentially with one or more doses of a second or additional inhibitor (the term additional meaning any number of different inhibitors of a different gene. In another embodiment, the therapeutic regimen involving the combination of a first inhibitor and a second or additional inhibitor involves administering the first inhibitor less frequently than that inhibitor would be administered if it was administered as a sole therapeutic agent. In another embodiment, the therapeutic regimen involving the combination of inhibitors of the activity or expression of different host genes required for infection involves administering all combined inhibitors less frequently than the inhibitors would be administered if they were administered as sole therapeutic agents.

[0065] By "therapeutic effect" or "treatment benefit" as used herein is meant an improvement or diminution in severity of a symptom of the viral disease, for example, an increase in ease of breathing, a decrease in fever or inflammation, etc, including an improvement or diminution in severity of treatment side effect, or reduction the dosage amount or repetition or duration of administration of a therapeutic composition or agent, e.g., less frequent administration of a particular inhibitor. In one embodiment, the therapeutic benefit of the two or more inhibitors of one or more host genes is enhanced relative to the administration of any inhibitor alone. In another embodiment, the therapeutic benefit is a synergistic therapeutic effect or treatment benefit, e.g., a benefit more than would be expected by the treatment benefits of each inhibitor of Table III or Table IV additively. In another embodiment, the administration of multiple inhibitors produces improved tolerability of one or more inhibitors. In another embodiment, the administration of multiple inhibitors of one or more of the genes or gene products described herein permits use of a dosage amount of at least one inhibitor that is lower than the dose approved for single agent use. In

a further embodiment, the administration of multiple inhibitors of multiple of the genes or gene products or activity described herein enhances the duration of the therapeutic effect or treatment benefit achieved with any inhibitor administered alone.

[0066] An “effective amount” is meant the amount of the inhibitors, e.g., those specified in Tables III or IV, taken alone, or in combination or with other conventional anti-viral medicaments, sufficient to provide a therapeutic benefit or therapeutic effect after a suitable course of administration.

[0067] It should be understood that the “effective amount” for an inhibitor varies depending upon the inhibitor/antagonist selected for use in the method. Regarding doses, it should be understood that “small molecule” drugs are typically dosed in fixed dosages rather than on a mg/kg basis. With an injectable a physician or nurse can inject a calculated amount by filling a syringe from a vial with this amount. In contrast, tablets come in fixed dosage forms. Some dose ranging studies with small molecules use mg/kg, but other dosages can be used by one of skill in the art, based on the teachings of this specification. In one embodiment an effective amount for the selected inhibitor(s) includes without limitation about 0.001 to about 25 mg/kg subject body weight. Such a range includes 0.001, at least 0.01, at least 0.1, at least 1, at least 10, at least 20, or at least 25 mg/kg body weight. In another embodiment, the range of effective amount is 1 to 5 mg/kg body weight. In another embodiment, the range of effective amount is 1 to 10 mg/kg body weight. In another embodiment, the range of effective amount is 1 to 20 mg/kg body weight. Still other doses falling within these ranges are expected to be useful. Where the inhibitor is a protein, e.g., antibody, antibody fragment or recombinant protein or peptide, the effective amount can be about 0.01 to 25 mg antibody/injection. In one embodiment, the effective amount is 0.01 to 10 mg antibody/injection. In another embodiment, the effective amount is 0.01 to 1 mg antibody/injection. In another embodiment, the effective amount is 0.01 to 0.10 mg antibody/injection. In another embodiment, the effective amount is 0.2, 0.5, 0.8, 1.0, 1.2, 1.4, 1.6, 1.8, 2.0, 2.2, 2.4, 2.6, 2.8, 3.0 up to more than mg antibody/injection. Still other doses falling within these ranges are expected to be useful.

[0068] In another embodiment of the combination methods of this invention, the effective amount is a “low dose” of inhibitor. Such a low dose is defined as a dose lower than that used if the inhibitor were to be administered alone or for another condition. In another embodiment, the low dose is less than 1 mg/kg subject body weight. In one embodiment, the low dose is less than 0.1 mg/kg subject body weight. In one embodiment, the low dose is less than 0.01 mg/kg subject body weight. In one embodiment, the low dose is less than 0.001 mg/kg subject body weight. In one embodiment, the low dose is between 0.001 and 0.0001 mg/kg subject body weight. In one embodiment, the low dose is a normal dose administered less frequently than a monotherapy dose, e.g., less than once a day. A low dose for the inhibitor in one embodiment, can be from 0.01 to 25 mg antibody/injection, with the injections administered at extended intervals.

[0069] Low doses can also include regimens of administration where the dosages are administered over extended periods of time in comparison to known anti-viral doses and regimens. In one embodiment, a dosage of one or more inhibitors can be administered daily, weekly, bi-weekly, monthly, or for as long as the virus persists in the subject. Further when in a vaccine composition, effective dosages can be administered once every 6 months or once/year, as needed.

[0070] In one embodiment, the dose and dosage regimen of the selected inhibitor(s) that is suitable for administration to a particular patient may be determined by a physician considering the patient's age, sex, weight, general medical condition, and whether the inhibitor is administered to prevent viral infection or to treat viral infection and its symptoms. The physician may also consider the route of administration of the agent, the pharmaceutical carrier with which the agents may be combined, and the agents' biological activity.

[0071] By “administration” or “routes of administration” include any known route of administration that is suitable to the selected inhibitor or inhibitors, and that can deliver an effective

amount to the subject. Routes of administration useful in the methods described herein include one or more of oral, parenteral, intravenous, intra-nasal, sublingual, intraocular injection, intravitreal injection, via a depot formulation or device, by inhalation, or any other route suitable to the selected inhibitor.

F. Pharmaceutical Compositions

[0072] Pharmaceutical compositions may include one or more of the inhibitors identified herein contained in a single composition comprising at least one carrier (e.g., pharmaceutically acceptable carrier). Alternatively, multiple inhibitors may be administered separately (e.g., administered in separate compositions comprising at least one carrier (e.g., pharmaceutically acceptable carrier)). The pharmaceutical preparations containing the inhibitors may be conveniently formulated for administration with an acceptable medium such as water, buffered saline, ethanol, polyol (for example, glycerol, propylene glycol, liquid polyethylene glycol and the like), dimethyl sulfoxide (DMSO), oils, detergents, suspending agents or suitable mixtures thereof. The concentration of the agents in the chosen medium may be varied and the medium may be chosen based on the desired route of administration of the pharmaceutical preparation. Except insofar as any conventional media or agent is incompatible with the inhibitors to be administered, its use in the pharmaceutical preparation is contemplated.

[0073] In a particular embodiment, multiple inhibitors may be administered sequentially and/or concurrently. For example, an inhibitor of one host gene may be administered before, after, and/or at the same time as an inhibitor of another host gene. When the inhibitor compositions are not administered at the same time, the compositions should be administered close enough in time such that the two compositions are capable of acting synergistically, additively or in a manner to achieve a treatment benefit in the subject.

[0074] Selection of a suitable pharmaceutical preparation depends upon the method of administration chosen. For example, inhibitor compositions may be administered parenterally by intravenous injection into the blood stream, or by subcutaneous, intramuscular or intraperitoneal injection. Pharmaceutical preparations for parenteral injection are known in the art. If parenteral injection is selected as a method for administering the antibodies, steps must be taken to ensure that sufficient amounts of the molecules reach their target cells to exert a biological effect. The lipophilicity of the agents, or the pharmaceutical preparation in which they are delivered, may be increased so that the molecules can better arrive at their target locations.

[0075] Pharmaceutical compositions containing the inhibitors/antagonists described herein as the active ingredient in intimate admixture with a pharmaceutical carrier can be prepared according to conventional pharmaceutical compounding techniques. The carrier may take a wide variety of forms depending on the form of preparation desired for administration. In preparing the agent in oral dosage form, any of the usual pharmaceutical media may be employed, such as, for example, water, glycols, oils, alcohols, flavoring agents, preservatives, coloring agents and the like in the case of oral liquid preparations (such as, for example, suspensions, elixirs and solutions); or carriers such as starches, sugars, diluents, granulating agents, lubricants, binders, disintegrating agents and the like in the case of oral solid preparations (such as, for example, powders, capsules and tablets). Because of their ease in administration, tablets and capsules represent the most advantageous oral dosage unit form in which case solid pharmaceutical carriers are obviously employed. If desired, tablets may be sugar-coated or enteric coated by standard techniques. For parenteral compositions, the carrier will usually comprise sterile water, though other ingredients, for example, to aid solubility or for preservative purposes, may be included. Injectable suspensions may also be prepared, in which case appropriate liquid carriers, suspending agents and the like may be employed.

[0076] A pharmaceutical preparation of the invention may be formulated in dosage unit form for ease of administration and uniformity of dosage. Dosage unit form, as used herein, refers to a physically discrete unit of the pharmaceutical preparation appropriate for the patient undergoing

treatment. Each dosage should contain a quantity of active ingredient calculated to produce the desired effect in association with the selected pharmaceutical carrier. Procedures for determining the appropriate dosage unit are well known to those skilled in the art. Dosage units may be proportionately increased or decreased based on the weight of the patient. Appropriate concentrations for alleviation of a particular pathological condition may be determined by dosage concentration curve calculations, as known in the art.

[0077] In accordance with the present invention, the appropriate dosage unit for the administration of the compositions of the invention may be determined by evaluating the toxicity of the active therapeutic inhibitor in animal models. Various concentrations of the above-mentioned inhibitors including those in combination may be administered to a mouse model of the viral disease, and the minimal and maximal dosages may be determined based on the results of significant reduction of fever, shortness of breath, and side effects as a result of the treatment. Appropriate dosage unit may also be determined by assessing the efficacy of the inhibitor compositions in combination with other standard drugs for treatment or prophylaxis of viral diseases. The dosage units of the inhibitors may be determined individually or in combination.

[0078] The compositions comprising the inhibitors of the instant invention may be administered at appropriate intervals, for example, at least twice a day or more until the pathological symptoms are reduced or alleviated, after which the dosage may be reduced to a maintenance level. The appropriate interval in a particular case would normally depend on the condition of the patient.

[0079] The present invention also includes pharmaceutical kits useful, for example, in the treatment or prevention of viral infection or symptoms referred to herein which include one or more containers containing one or more selected inhibitors in therapeutically effective amounts or for administration according to a desired therapeutic regimen. Such kits can further include, if desired, one or more of various conventional pharmaceutical kit components, such as, for example, containers with one or more pharmaceutically acceptable carriers, additional containers, etc., as will be readily apparent to those skilled in the art. Instructions, either as inserts or as labels, indicating quantities of the components to be administered, guidelines for administration, and/or guidelines for mixing the components, can also be included in the kit. Components that permit these efficacy studies can also be included in the kits.

[0080] In the descriptions of the compositions and methods discussed herein, the various components can be defined by use of technical and scientific terms having the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs and by reference to published texts. Such texts provide one skilled in the art with a general guide to many of the terms used in the present application. The definitions contained in this specification are provided for clarity in describing the components and compositions herein and are not intended to limit the claimed invention.

[0081] Throughout this specification, the words “comprise”, “comprises”, and “comprising” are to be interpreted inclusively rather than exclusively. The words “consist”, “consisting”, and its variants, are to be interpreted exclusively, rather than inclusively. It should be understood that while various embodiments in the specification are presented using “comprising” language, under various circumstances, a related embodiment is also described using “consisting of” or “consisting essentially of” language.

[0082] The term “a” or “an”, refers to one or more, for example, “a gene,” is understood to represent one or more genes. As such, the terms “a” (or “an”), “one or more,” and “at least one” are used interchangeably herein.

[0083] As used herein, the term “about” means a variability of 10% from the reference given, unless otherwise specified.

EXAMPLES

[0084] The following examples should be construed to encompass any and all variations that become evident as a result of the teaching provided herein.

Example 1: Materials and Methods

Human Cell Culture

[0085] Human alveolar basal epithelial adenocarcinoma cells (A549, ATCC CCL-185), human hepatocellular carcinoma (Huh7.5, a kind gift from C. Rice), human colorectal carcinoma (Caco-2, ATCC HTB-37), and human embryonic kidney cells HEK293FT (Thermo) were maintained at 37° C. and 5% CO₂ in D10 media, which consists of DMEM (Caisson Labs) with 10% Serum Plus II Medium Supplement (Sigma-Aldrich). Lung adenocarcinoma (Calu-3, ATCC HTB-55) and monkey kidney epithelial cells (Vero E6, ATCC CRL-1586) were maintained in EMEM (ATCC) media with 10% Serum Plus II Medium Supplement (Sigma-Aldrich).

Viral Strains

[0086] SARS-related coronavirus 2 (SARS-CoV-2), isolate USA-WA1/2020 (NR-52281), was produced as previously described (Blanco-Melo et al., 2020; Daniloski et al., 2020). SARS-CoV-2 was grown in Vero E6 cells in DMEM supplemented with 2% FBS, 4.5 g/L D-glucose, 4 mM L-glutamine, 10 mM non-essential amino acids, 1 mM sodium pyruvate and 10 mM HEPES. Plaque assays were used to determine infectious titers of SARS-CoV-2 by infection of Vero E6 cells in Minimum Essential Media supplemented with 2% FBS, 4 mM L-glutamine, 0.2% BSA, 10 mM HEPES and 0.12% NaHCO₃ and 0.7% agar.

[0087] A549 overexpressing human ACE2 (A549.sup.ACE2) cells (a gift from B. Rosenberg, Icahn School of Medicine at Mount Sinai) were previously described (Bouhaddou et al., 2020). To generate ACE2 expressing cells, the human ACE2 coding sequence was amplified and cloned into the BamHI site of the lentiviral vector pHR-PGK (Addgene 79120). We generated lentiviral particles (as described below) and transduced 5×10⁴ A549 cells plated in a 12-well plate in the presence of polybrene (8 µg/ml). We confirmed hACE2 expression by western blot analysis (Thermo MA5-32307).

Lentiviral Production, Transduction and SARS-CoV-2 Infection

[0088] The Human GeCKOv2 A and B libraries (Addgene, 1000000048) were used for genome-scale CRISPR knockout screens (Sanjana et al., 2014). We mixed equal amount of the A and B library plasmids to target each gene with 6 guide RNAs. Briefly, 225 cm² flasks of 80% confluent HEK293FT cells (Thermo) were transfected with 25 µg GeCKOv2 plasmid, 14 µg pMD2.G and 20 µg psPAX2 mixed in 2.5 mL OptiMEM (Thermo) and 175 µL Polyethylenimine (1 mg/ml) (Polysciences). After 6 hours, media was changed to D10 media with 1% bovine serum albumin (Sigma) added to improve virus stability. After 60 hours, viral supernatants were harvested and centrifuged at 3,000 rpm at 4° C. for 10 min to pellet cell debris and filtered using 45 µm PVDF filters (CellTreat). The supernatant was then ultracentrifuged for 2 hours at 100,000 g (Sorvall Lynx 6000) and the pellet resuspended overnight at 4° C. in PBS with 1% BSA.

[0089] Following lentiviral titrations, about 330 million A549.sup.ACE2 expressing cells were transduced at MOI of 0.5. Cells were selected with 2 µg/µL puromycin (Thermo) for 12 days to ensure proper selection. Throughout the experiment the representation was monitored such that each guide RNA is represented by at least 1000 cells (~125 M cells). Roughly 1000× representation of cells expressing GeCKOv2 library were infected with SARS-CoV-2 isolate USA-WA1/2020 (NR-52281) at either MOI of 0.01 (low MOI) and 0.3 (high MOI). Surviving cells were collected on day 6 post-infection for genomic DNA isolation.

Genomic DNA Isolation, Guide RNA Amplification and Quantification

[0090] We used a two-step PCR protocol (PCR1 and PCR2) to amplify the guide RNA cassette for Illumina sequencing. Briefly, genomic DNA (gDNA) was extracted using a previously described protocol (Chen et al., 2015). For the first PCR reaction, we used all gDNA available for each sample. We performed the PCR1 using Taq-B polymerase (Enzymatics) and used multiple reactions where each reaction contained up to 10 µg gDNA per 100 µL PCR reaction. PCR1 products for each sample were pooled and used for amplification with barcoded PCR2 primers. For each sample, we performed 12 PCR2 reactions (using 5 µL of the pooled PCR1 product per PCR2

reaction) with Q5 polymerase (NEB). PCR2 products were pooled and then normalized within each biological sample before combining uniquely-barcoded separate biological samples. The pooled product was then gel-purified from a 2% E-gel EX (Life Technologies) using the QiaQuick gel extraction kit (Qiagen). The purified, pooled library was then quantified with TapeStation 4200 (Agilent Technologies). PCR products were run on a 2% agarose gel and the correct size band was extracted. Sequencing was performed on the NextSeq 550 instrument using the HighOutput Mode v2 with 75 bp paired-end reads (Illumina). For PCR1 the following primer set was used: 5' GAGGGCCTATTTCCCATGATTC 3' (SEQ ID NO: 1) and 5' GTTGCAGAAAAGAACGTTACCG 3' (SEQ ID NO: 2). For PCR2 the following primers were used: 5' AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACACGACGCTCTTCC GATCT (N.sub.1-9) (BC.sub.8) TCTTGTGGAAGGACGAAACACCG 3' (SEQ ID NO: 3) and 5' CAAGCAGAAGACGGCATACGAGAT (BC.sub.8) GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT (N.sub.1-9) TCTACTATTCTTTCCCTGCACTGT 3', (SEQ ID NO: 4) where N is a stagger of 1 to 9 nucleotides and BC is a barcode of 6 nucleotides. All PCR1 and PCR2 primer sequences, including full barcodes, are listed on the GeCKO website (<http://genome-engineering.org/gecko/>).

[0091] Sequencing reads were demultiplexed upon sequencing based on Illumina i7 barcodes present in PCR2 reverse primers using Illumina BaseSpace. We performed adaptor trimming by treating the hU6 promoter sequence as a 5' adapter, using cutadapt v1.13 [-e 0.2-O 5-m 20-g TCTTGTGGAAGGACGAAACACCG] (SEQ ID NO: 5). Processed guide RNA sequences were aligned to the GeCKOv2 reference allowing for up to 1 mismatch using bowtie v1.1.2 [-a-best-strata-v 1-norc] with alignment rates of 81% to 86%.

Computational Analyses of Genome-Scale CRISPR Screens

[0092] Guide RNA counts were processed using the MaGeCK pipeline with an output of RRA p-values and gene ranks (Chen et al., 2018). We separately ranked the gene using the RIGER (weighted-sum) and second-best rank methods, as previously described (Chen et al., 2015). Gene Set Enrichment Analyses were performed using the fgsea package with Gene Ontology for biological processes (c5.bp.v7.1.symbols) (Korotkevich et al., 2019). GTEx v8 tissue specific enrichment was performed using the Multi Gene Query function available on the GTEx website: www.gtexportal.org/home/multiGeneQueryPage (accessed Aug. 1, 2020) (Aguet et al., 2019). GO enrichments for SARS-CoV-2, ZIKA, and IAV CRISPR screens were performed using GOrilla to find all significant enrichments (FDR p-value <10⁻³) (Eden et al., 2009). FDR p-values were log₁₀-transformed and normalized to create the heatmap shown in FIG. 3E.

Generation of Gene-Perturbed A549.SUP.ACE2 .Lines and SARS-CoV-2 Infection

[0093] For each gene of interest, 3 guides were designed using GUIDES software and were subsequently cloned into an all-in-one vector with an optimized Cas9 scaffold (pCC_01, Addgene 139086) containing Cas9, a guide RNA cassette and a puro resistant cassette (Legut et al., 2020; Meier et al., 2017). Following sequence confirmation by Sanger sequencing, lentivirus was produced individually for each plasmid and the target cells were transduced in presence of polybrene (10 µg/mL, Santa Cruz). Gene-perturbed A549.sup.ACE2 cells were selected for at least 10 days with 2 µg/mL (A549ACE2, Calu-3), 3 µg/mL (Caco-2) or 5 µg/mL (Huh7.5ACE2) puromycin (Thermo). To determine the SARS-2 infection susceptibility of the A549.sup.ACE2 gene-perturbed lines, 10,000 cells were plated per well of 96-well plates. After 24 hours, the cells were infected with SARS-CoV-2 at MOI of 0.1. At 36 hours post-infection, the cells were either fixed and processed for immunofluorescence or cellular RNA was harvested for qRT-PCR analyses. All infections with SARS-CoV-2 were performed with 3 biological replicates.

Immunofluorescence of Nucleocapsid (N) Protein

[0094] Cells were fixed with 5% formaldehyde and immunostained for nucleocapsid (N) protein and visualized with a secondary antibody labeled with AlexaFluor-568 (Thermo). SARS-CoV-2 nucleocapsid (N) antibody (clone 1C7C7) was obtained from the Center for Therapeutic Antibody

Discovery at the Icahn School of Medicine at Mount Sinai. Nuclei were stained with DAPI. Full wells were imaged and quantified for SARS-CoV-2 infected cells using a Celigo imaging cytometer (Nexcelom Biosciences). All infections with SARS-CoV-2 were performed with 3 biological replicates. Representative images from the top gene knockout hits were acquired using the EVOS M5000 Imaging System (Thermo).

Identification of Druggable Genes and Drug Treatments

[0095] To identify druggable genes among the top CRISPR screen hits, we cross-referenced the top 500 genes from the RRA analysis with the data about drugs and their gene targets obtained from Drug Gene Interaction database (DGIdb, retrieved on Jun. 3, 2020) as well as manual literature search (Cotto et al., 2018). Inhibitors that target the genes of interest (and remdesivir) was obtained from 2 companies: SelleckChem and MedChemExpress. The catalog number and vendor information is available in the Key Resource section.

[0096] To test drug efficacy in reducing SARS-CoV-2 infection, 10,000 A549.sup.ACE2 cells were seeded per well of a 96-well plate. Cells were treated with inhibitors at 10 μ M for two hours before infection and inhibitors were maintained throughout the course of infection. Cells were infected with SARS-CoV-2 at MOI of 0.1 for 36 hours and the cells were collected for analysis via qRT-PCR or processed for immunofluorescence (N protein and quantified by Celigo). For FIG. 4D, the remdesivir data was collected in an independent experiment.

Cell Viability Assays

[0097] Cell viability following drug treatments was performed the same way as described above. Thirty-six hours post drug treatment the cells were collected and stained with LIVE-DEAD Violet (Thermo). Cell acquisition was performed using a Sony SH800S cell sorter with a 100 μ m sorting chip. We used the following gating strategy: 1) We excluded the cell debris based on the forward and reverse scatter; 2) Doublets were excluded by plotting FSC height vs FCS area, 3) Dead cells were quantified using live-dead violet stain. For all samples, we recorded at least 5,000 cells that pass the gating criteria described above. Flow cytometry analyses were performed using FlowJo v10. Cell viability for A549-ACE2 treated with DMSO or 10 μ M amlodipine was performed 24 hours post treatment using Trypan Blue (Thermo Fisher) and automated cell counting (Nexcelom AutoT4).

Quantitative Reverse-Transcription PCR (qRT-PCR) of Viral RNA

[0098] RNA was extracted from cells grown in 96-well plates by using the RNeasy 96 Kit (Qiagen) per the manufacturer's instructions. RNA was reverse-transcribed and PCR amplified using KAPA SYBR FAST One-step Universal qRT-PCR Kit (Roche). SARS-CoV-2 replication was assessed by using primers specific to the N mRNA (Forward 5'-CTCTTG TAGATCTGTTCTCTAAACGAAC-3' (SEQ ID NO: 6); Reverse 5'-GGTCCACCAAACGTAATGCG-3' (SEQ ID NO: 7)). SARS-CoV-2 N mRNA levels were normalized to beta tubulin (Forward 5'-GCCTGGACCACAAGTTTGAC-3' (SEQ ID NO: 8); Reverse 5'-TGAAATTCTGGGAGCATGAC-3' (SEQ ID NO: 9)). Reactions were ran and analyzed on a Lightcycler 480 II Instrument (Roche). Relative quantification was calculated by comparing the cycle threshold (C.sub.t) values using $\Delta\Delta C_{sub.t}$.

siRNA Transfections and SARS-CoV-2 Infection

[0099] All of the siRNAs were ordered from Dharmacon and their catalog number can be found in the Key Resource (data not shown). To knockdown individual genes, 10,000 A549.sup.ACE2 cells were seeded in 96-well plates and transfected with siRNAs using Lipofectamine RNAiMAX (Thermo) following the manufacturer's protocol. Forty-eight hours later, the cells were infected with SARS-CoV-2 at an MOI of 0.1 for 36 hours. Cells were fixed with 5% formaldehyde, stained with nucleocapsid protein (clone 1C7C7, ISMMS), and visualized with AlexaFluor-568 conjugated secondary antibody (Thermo). Nuclei were stained with DAPI, and full wells were imaged with a Celigo imaging cytometer (Nexcelom Biosciences).

Minipool CRISPR Library and Lentiviral Production

[0100] To generate the minipool, we combined equimolar amounts the same guide RNA vectors

(cloned in pCC1) that we used for arrayed validation (see above Generation of gene-perturbed A549.sup.ACE2 lines and SARS-CoV-2 infection). In total, we combined 3 guide RNAs x 30 genes plus 9 non-targeting guide RNAs. The non-targeting guide RNA plasmids were added at half the molar ratio of the other plasmids. Lentivirus was produced via transfection of the minipool with appropriate packaging plasmids (psPAX2: Addgene 12260; pMD2.G: Addgene 12259) using polyethylenimine (PEI) reagent in HEK293FT. The target A549.sup.ACE2 cells were transduced in presence of polybrene (10 µg/mL, Santa Cruz) at very low MOI to ensure entry of a single virus per cell. The survival was 2.4% after 2 days of selection with 2 µg/mL puromycin (Thermo). On day 10 post-transduction, the cells were collected and processed for ECCITE-seq.

Expanded CRISPR-Compatible Cellular Indexing of Transcriptomes and Epitopes (ECCITE-Seq) [0101] We divided the ECCITE-seq pool into several sub-pools and performed cell-hashing. Cell hashing was performed as described in a previously published protocol (Mimitou et al., 2019). Hashed cells were pooled. For each pool, we resuspended 1 million cells in 100 µl staining buffer (2% BSA, 0.01% Tween in PBS). We then added 10 µl Human TruStain FcX Fc Receptor Blocking Solution (BioLegend) and incubated on ice for 10 minutes. We then added hashing antibodies (BioLegend), incubated on ice for 30 minutes, and washed cells 3 times with staining buffer. In total, we ran four lanes of a Chromium Single Cell Immune Profiling Solution v1.0 5' kit (10× Genomics) targeting recovery of 12,000 cells per lane (superloading) in experiment 1 and 20,000 cells in experiment 2. Gene expression (mRNA), hashtags (Hashtag-derived oligos, HTOs) and guide RNA (Guide-derived oligos, GDOs) libraries were constructed (Smibert et al., 2019). Each replicate was sequenced on two NextSeq550 75-cycle high-output runs (Illumina). Sequencing reads from the mRNA library were mapped to the hg38 reference genome (Ensembl v97) using Cellranger (v3.0.1, 10× Genomics). To generate count matrices for HTO and GDO libraries, the CITE-seq-count package was used (<https://github.com/Hoohm/CITE-seq-Count> v1.4.2). Count matrices were then used as input into the Seurat R package (v3.2) for downstream analyses (Stuart et al., 2019). Cell viability was determined to be ~95%, and then cells were profiled using Chromium Single Cell 5' Solution (10× Genomics).

ECCITE-Seq Library Construction and Sequencing

[0102] For the ECCITE-seq experiment, we ran two lanes of a 10× Genomics 5' kit (Chromium Single Cell Immune Profiling Solution v1.0, #1000014, #1000020, #1000151) aiming for recovery of 12,000 cells per lane (superloading). Gene expression (mRNA), hashtags (Hashtag-derived oligos, HTOs), protein (Antibody-derived oligos, ADTs) and guide RNA (Guide-derived oligos, GDOs) libraries were constructed by following 10× Genomics and ECCITE-seq protocols. All libraries were sequenced together on two NextSeq550 75-cycle high-output runs (Illumina). Sequencing reads from the mRNA library were mapped to the hg38 reference genome (Ensembl v97) using Cellranger (v3.0.1, 10× Genomics). To generate count matrices for HTO and GDO libraries, the CITE-seq-count package was used (<https://github.com/Hoohm/CITE-seq-Count> v1.4.2). Count matrices were then used as input into the Seurat R package (v3.2) for downstream analyses (Stuart et al., 2019).

Data Pre-Processing in Seurat

[0103] Cells with low quality metrics, high mitochondrial gene content (>17.5%) and low number of genes detected (≤1800) were removed. The median number of detected genes was 3309. RNA counts were log-normalized using the standard Seurat workflow. HTO counts were normalized using the centered log-ratio transformation approach, with margin-2 (normalizing across cells). To identify cell doublets and assign experimental conditions to cells, we used the HTOseqDemux function (Stoeckius et al., 2018). HTOseqDemux-defined cell doublets and negatives were removed from any downstream analyses.

[0104] Cellular guide RNA identity for cells in the ECCITE-seq pool was assigned based on GDO unique molecular identifier (UMI) counts. We considered a guide RNA detected with ≥16 µMI counts (the median UMI counts per guide RNA was 503 counts, 93% of cells with ≥1 sgRNA). We

observed that 64% of the cells had >1 detected guide RNA (29% 1 guide RNA, 27% 2 guide RNAs, 17% 3 guide RNAs), which is surprising given a MOI <0.05 and an experimentally determined 2.4% survival rate upon selection (single integration probability of ~98% assuming Poisson statistics). To maximize cell recovery, we retained cells with 1-3 guide RNAs per cell (n=10,265) for downstream analysis but with several stringent filtering steps that we describe in detail.

[0105] First, we collapsed the guide RNA information down to target gene level (3 guide RNA per target gene) in the following way: 1) For all cells (with 1-3 guide RNA), we assigned a unique target gene if all detected guide RNAs targeted the same gene, or if the additional guide RNA detected was a non-targeting (NT) guide RNA. 2) For remaining cells, we assigned 2 or 3 target genes. Next, we removed all cells with 3 guide RNAs targeting 3 separate genes. We also removed cells if the combination of 2 unique target genes contained guide RNAs for 2 genes that were classified as ‘perturbed’ (see below) to avoid mixing of transcriptomic perturbation signatures. Similarly, we removed cells with a combination of guide RNAs for 2 unique target genes, if cells with guide RNAs for both genes were classified as ‘non-perturbed’ to avoid introduction of genetic interaction effects for gene KOs that do not lead to an RNA perturbation signature on their own. Lastly, we only retained cells with 2 unique target genes, if cells with guide RNAs for one of the 2 target genes could be classified as ‘perturbed’, while the other retained a non-perturbed (‘escaping’) classification. For this last case, we tested and verified that the second target gene does not contribute to the observed RNA perturbation signature. Based on this selection process, we assigned the target gene label based on 1) the uniqueness of the target gene by guide RNA labels, or 2) based on the driving target gene (1 target gene classified as ‘perturbed’, while the other was classified ‘non-perturbed’, see below). This filtering process yielded 6,522 cells total with the majority (4,715 cells) containing a single best guide RNA or unique target gene.

RNA-Based Clustering of Single Cells and ECCITE-Seq Data

[0106] ECCITE-seq single-cell clustering was done in a multistep process. First, we classified cells based on their perturbation status to discriminate between ‘perturbed’ cells (cells detectable with guide RNA-dependent perturbation signature/gene expression changes) or ‘escaping’ (non-perturbed) cells that can occur if the Cas9 introduced mutation was inconsequential or if loss of the target gene did not lead to transcriptomic changes. We classified cells to be perturbed or non-perturbed relative to cells with non-targeting (NT) guide RNAs using mixscape with default parameters (Papalexi et al., 2020).

[0107] In brief, we calculated the local perturbation signature to remove confounding sources of variation (e.g. cell cycle) by subtracting the average expression of the k=20 nearest NT neighbors from each cell's original RNA profile using the first 40 principal components. The perturbation signatures were centered but not scaled using the ScaleData function. Next, we used RunMixscape, which determines for each target gene class, all of the differentially expressed genes (DEGs) relative to NT cells on the original RNA counts. Then RunMixscape calculates the perturbation score of every cell by using the DEG perturbation signatures and projected cells into a single dimension. Lastly, a Gaussian mixture model is fitted to the distribution of perturbation scores of NT and each target gene class separately, with the assumption that non-perturbed (‘escaping’) cells cannot be discriminated from NT cells, while ‘perturbed’ cells group separately. In this way, all cells targeting a gene for which no DEGs could be identified (min.de.genes=5 with log fc.threshold=0.25) were classified as non-perturbed. For all other target genes with DEGs, all cells that had a similar perturbation score with NT cells were classified as non-perturbed.

[0108] We first applied mixscape to all cells with a sgRNAs targeting a unique target gene (n=4,715 cells). We identified perturbed cells for 11 out of 30 target genes. We then added cells with sgRNA combination of a perturbed target gene and a non-perturbed target gene as described above (n=6,522 cells) and repeated the classification. Again, we identified perturbed cells for 11 out of 30 target genes. We removed all non-perturbed (‘escaping’) cells. Downstream analyses

were conducted using this set of $n=2,023$ perturbed cells and $n=352$ NT cells. For visualization, we performed linear discriminate analysis (LDA) using the mixscape-derived class labels and performed UMAP dimensionality reduction using all ($n=11$) component shown in FIG. 5B.

Differential Expression and Gene Set Enrichment Analyses

[0109] We used FindMarkers in Seurat to find differentially expressed genes between non-targeting cells and cells that belonged to a targeted gene class. We used all genes with adjusted p-value <0.01 and minimal log.sub.eFC threshold >0.1 filter as input into the heatmap in FIG. 5C. The same FindMarkers analysis (but using the non-adjusted p-value <0.01 with a maximum of the 300 most significant DEGs) was used as input into the EnrichR package to run pathway analysis using the human WikiPathways database (v. 2019) (Kuleshov et al., 2016; Slenter et al., 2018). FIG. 5D shows-log.sub.10 transformed p-values for the union of all enriched pathways with an adjusted p-value <0.01 across the 11 target genes with perturbation signatures.

Flow Cytometry of ACE2 Cell Surface Expression

[0110] Cells were harvested, counted and about 100,000 cells were washed with Dulbecco's phosphate-buffered saline (PBS, Caisson Labs) and then stained with LIVE/DEAD Violet stain (Thermo, 34864). Following a wash with $1\times$ PBS, all subsequent washes and antibody dilutions were performed using $1\times$ PBS supplemented with 2% FBS. Wild-type or Cas9-perturbed A549.sup.ACE2, Caco-2 and Calu-3 cells were stained for 30 min on ice with $0.25\text{ }\mu\text{g}$ of anti-ACE2 antibody (R&D Systems, AF933) in $\sim 50\text{ }\mu\text{L}$ residual volume. Following two washes with $1\times$ PBS with 2% FBS, samples were stained on ice for 20 min with $7.5\text{ }\mu\text{L}$ of anti-goat-APC secondary antibody (R&D Systems, F0108). Cell acquisition and sorting was performed using a Sony SH800S cell sorter with a $100\text{ }\mu\text{m}$ sorting chip. We used the following gating strategy: 1) We excluded the cell debris based on the forward and reverse scatter; 2) Doublets were excluded by plotting FSC height vs FCS area, 3) Dead cells were excluded by live-dead stain. We recorded at least 5,000 cells for A549ACE2 and at least 3,000 cells for Calu-3 and Caco-2 that pass the gating criteria described above. Gates to determine ACE2-APC⁺ cells were set based on control A549 wild type cells or only secondary antibody stained Calu-3 and Caco-2, where the percent of ACE2 positive cells was set as $<5\%$ (background level). Flow cytometry analyses were performed using FlowJo v10.

Immunofluorescence of ACE2 and Endo/Lysosomal Markers

[0111] A549.sup.ACE2 Cas9-transduced (specific gene or non-targeting) cell lines were seeded on poly-D-lysine-coated coverslips (Electron Microscopy Sciences) 24 hours before they were fixed with 4% formaldehyde (Sigma) diluted in $1\times$ PBS (Caisson Labs) (Daniloski et al., 2020). Following 3 washes with $1\times$ PBS, cells were blocked with $1\times$ PBS with 2% BSA for 30 min at room temperature. Cells were stained with mouse anti-2A antibody diluted at 1:250 (clone 3H4, Millipore Sigma, MABS2005) to recognize ACE2-2A, and rabbit anti-EEA1 diluted at 1:100 (Thermo, MA5-14794). Cell were incubated with primary antibodies for 3 hours at room temperature in a moisturized chamber. Coverslips were then washed 5 times with $1\times$ PBS with 2% BSA and then incubated with Alexa-conjugated secondary antibodies (Jackson ImmunoResearch) diluted at 1:1000 for 45 min at room temperature. Coverslips were then washed 5 times with $1\times$ PBS with 2% BSA, and DAPI (Sigma) was added in the fourth wash at a concentration of $0.1\text{ }\mu\text{g/mL}$. To stain the lysosomes, LysoTracker DeepRed (Thermo) was diluted to 70 nM in culture media and was added to actively growing cells on coverslips and were incubated for 1 hour at 37°C .

[0112] Following incubation with LysoTracker, cells were processed for immunofluorescence as described above. Confocal images were acquired on a Zeiss LSM 780 with a $20\times/0.8$ Plan-APOCHROMAT objective or a $63\times/1.4$ Plan-APOCHROMAT objective (Zeiss). Epifluorescence images were acquired using the same system; both fitted with a Zeiss Axiocam 506 mono (Zeiss). Images were processed using Zen Black 2012 (Zeiss) and FIJI 2.1.0; Java 1.8.0-202. Linear histogram adjustments were applied uniformly within experiments for clarity of presentation.

[0113] ACE2 localization was determined on images taken on a Zeiss LSM 780 with a 20×/0.8 Plan-APOCHROMAT objective using Zen Black (Zeiss). The A549-ACE2 polyclonal cell line had both ACE2 positive and negative cells. Only ACE2 positive cells were then manually scored if they had a distinct accumulation of cytoplasmic vesicles-like hollow structures, compared to the non-targeting (NT) control. In the NT control, the ACE2 staining was seen on the plasma membrane and diffuse in the cytoplasm. The scoring was performed on 2 biological replicates (that is, two separate non-targeting controls and two separate Rab7A knockout lines engineered using 4 separate Cas9 guide RNAs). We scored ~430 cells per replicate.

Western Blots

[0114] A549.sup.ACE2 or Huh7.5.sup.ACE2 cells were collected, washed with 1×PBS and lysed with TNE buffer (10 mM Tris-HCl, pH 7.4, 150 mM NaCl, 1 mM EDTA, 1% Nonidet P-40) in presence of protease inhibitor cocktail (Bimake B14001) for 1 hour on ice. Cells lysates were spun for 10 min at 10,000 g, and protein concentration was determined with the BCA assay (Thermo). Equal amounts of cell lysates (20 µg) were denatured in Tris-Glycine SDS Sample buffer (Thermo LC2676) and loaded on a Novex 4-20% Tris-Glycine gel (Thermo XP04205BOX). PageRuler pre-stained protein ladder (Thermo 26616) was used to determine the protein size. The gel was run in 1× Tris-Glycine-SDS buffer (IBI Scientific IBI01160) for about 120 min at 120V. Proteins were transferred on a nitrocellulose membrane (BioRad 1620112) in presence of prechilled 1× Tris-Glycine transfer buffer (FisherSci LC3675) supplemented with 20% methanol for 100 min at 100V. Immunoblots were blocked with 5% skim milk dissolved in 1×PBS with 1% Tween-20 (PBST) and incubated overnight at 4° C. separately with the following primary antibodies: rabbit anti-RAB7A (0.1 µg/mL, NovusBio, NBP1-87174), rabbit anti-GAPDH (0.1 µg/mL, Cell Signaling, 2118S). Following the primary antibody, the blots were incubated with IRDye 680RD donkey anti-rabbit (0.2 µg/mL, LI-COR 926-68073) or with IRDye 800CW donkey anti-mouse (0.2 µg/mL, LI-COR 926-32212). The blots were imaged using Odyssey CLx (LI-COR).

T Cell Isolation and Activation

[0115] Human primary T cells were sourced from a de-identified healthy donor's LeukoPaks (New York Blood Center). First, peripheral blood mononuclear cells (PBMCs) were isolated via gradient centrifugation using Lymphoprep (StemCell Technologies 07811). CD8⁺ T cells were isolated from PBMCs by positive magnetic selection with the EasySep Human CD8 Positive Selection Kit (StemCell Technologies 17853). CD4⁺ T cells were isolated from PBMCs by negative magnetic selection with the EasySep Human CD4 T cell Isolation Kit (StemCell Technologies 17952). Isolated T cells were immediately plated in ImmunoCult-XF T Cell Expansion Medium (StemCell Technologies 10981) supplemented with 10 ng µL.sup.-1 recombinant human IL-2 (StemCell Technologies 78036) at 1×10⁶ cells mL.sup.-1. T cells were then activated with the ImmunoCult Human CD3/CD28 T Cell Activator (StemCell Technologies 10971) and expanded for 14 days. At days 3, 5 and 7 post-activation, T cells were diluted to 2.5×10⁵ cells mL.sup.-1 with fresh ImmunoCult-XF T Cell Expansion Medium supplemented with IL2. At days 9, 11 and 13 post-activation, T cells were maintained at a density of 2×10⁶ cells mL.sup.-1, and half of the culture media was replaced with fresh media supplemented with IL2. At day 14 post activation, T cells were washed off to remove IL2 and rested overnight in ImmunoCult-XF T Cell Expansion Medium prior to the activation assay.

[0116] T cell activation was measured by intracellular cytokine staining following anti-CD3/CD28 antibody stimulation. First, CD4⁺ and CD8⁺ T cells were pre-incubated for 1 hour at 37° C. with selected inhibitors at 10 µM concentration, or with vehicle control (DMSO). Each inhibitor was tested in triplicate. Following pre-incubation, T cells were activated with the ImmunoCult Human CD3/CD28 T Cell Activator, according to manufacturer's recommendations. 5 µg mL.sup.-1 brefeldin A (BioLegend 420601) and 2 µM monensin (BioLegend 420701) were added alongside the T Cell Activator to accumulate the cytokines inside the cell. After 4 h of activation at 37° C., CD4⁺ and CD8⁺ T cells were combined for staining, washed with PBS and stained with

LIVE/DEAD Fixable Dead Cell Stain Kit (Thermo Fisher Scientific L34955) for 5 minutes at room temperature in the dark. Then, FITC anti-human CD8a (clone RPA-T8, BioLegend 301014) was added followed by 20 min incubation on ice. The cells were then washed with PBS, fixed and permeabilized using Fixation Buffer (BioLegend 420801). Fixation was performed for 20 min on ice. Then, the cells were washed with Intracellular Staining Permeabilization Wash Buffer (BioLegend 421002) and stained with PE/Cy7 anti-human TNF- α (clone MAb11, BioLegend 502930) and PE anti-human IFN- γ (clone 4S.B3, BioLegend 502508) antibodies. The staining was performed for 20 min on ice. Afterwards, the cells were washed and resuspended in the Intracellular Staining Permeabilization Wash Buffer.

[0117] Acquisition was performed using Sony SH800S Cell Sorter with a 100 μ m sorting chip. Cells were sequentially gated to 1) include leukocytes based on forward and side scatters, 2) exclude doublets, 3) exclude dead cells and 4) include CD8⁺ cells. At least 10,000 viable CD8⁺ events were collected per sample.

Inflation of COVID-19 GWAS Signal for Variants that are Cis-eQTLs

[0118] To estimate the importance of CRISPR screen hit genes in susceptibility to COVID-19 in humans, we tested for inflation of COVID-19 GWAS signal for variants that are eQTLs in GTEx v8 lung for highly-ranked genes enriched in the CRISPR screens ($n=588$ genes found in the top 500 genes by at least 2 analysis methods of RRA, RIGER, and second-best ranks for at least one screen MOI) (Aguet et al., 2019). We used summary statistics of the six COVID-19 GWAS generated by the COVID-19 Human Genetics Initiative (round 3 meta-analyses, released Jul. 2, 2020): 1) ANA_A2_V2-very severe respiratory confirmed COVID vs. population, 2) ANA_B1_V2-hospitalized COVID vs. not hospitalized COVID, 3) ANA_B2_V2-hospitalized COVID vs. population, 4) ANA_C1_V2-COVID vs. lab/self-reported negative, 5) ANA_C2_V2-COVID vs. population, 6) ANA_D1_V2-predicted COVID from self-reported symptoms vs. predicted or self-reported non-COVID (COVID-19 Host Genetics Initiative, 2020). We measured inflation using the lambda inflation statistic relative to the chi-square quantile function of 0.1, $\lambda_{\text{sub}.0.1}$, i.e. estimating the inflation among 10% of the most significant tests. We calculated $\lambda_{\text{sub}.0.1}$ for two sets of eQTLs: 1) all lead eQTLs in lung (14,113 genes with significant eQTLs in lung at FDR 5%, serving as a background set), 2) all lead eQTLs in lung for CRISPR hit genes (274 out of 588 top-ranked genes have eQTLs in lung at FDR <0.05).

[0119] To test the significance of $\lambda_{\text{sub}.0.1}$, we used a permutation-based test. We selected n lead eQTLs from the background set $k=10,000$ times, where n is the number of lead eQTLs for CRISPR hit genes tested in a given COVID-19 GWAS and calculated $\lambda_{\text{sub}.0.1}$ on the permuted data. We calculated permutation p-values as the proportion of permuted $\lambda_{\text{sub}.0.1}$ as extreme as or more extreme than the observed $\lambda_{\text{sub}.0.1}$.

Prioritization of Genes in the 3p21.31 Locus Based on eQTL Data

[0120] We focused on eight genes, LIMD1, SACM1L, SLC6A20, LZTFL1, CCR9, FYCO1, CXCR6, and XCR1, in the 3p21.31 locus that has been shown to robustly associate with COVID-19 susceptibility (COVID-19 Host Genetics Initiative, 2020; Ellinghaus et al., 2020). We gathered summary statistics for 110 eQTL data sets from the eQTL Catalogue (61 data sets, mostly in immune cell types with and without treatment) (Kerimov et al., 2020) and GTEx v8 (49 tissues) (Aguet et al., 2019). We first prioritized the genes based on the p-value in eQTL studies for the lead GWAS variant 3:45867022:C:G from the COVID vs population GWAS (ANA_C2_V2, largest COVID-19 GWAS to date) generated by the COVID-19 HGI. Since observing low eQTL p-value for the lead GWAS variant does not necessarily translate into shared genetic causality, we further performed colocalization analysis.

Colocalization Analysis of COVID-19 GWAS and Cis-eQTLs in the 3p21.31 Locus

[0121] To assess evidence for shared causal variant of a cis-eQTL and a COVID-19 GWAS, we used the Bayesian statistical test for colocalization, coloc (Giambartolomei et al., 2014), assuming one causal variant per trait. We only included cis-eQTLs for genes for colocalization test, if there

was a cis-eQTL with a nominal p-value $<10^{-4}$ within 100 kb of the lead ANA_C2_V2 COVID-19 GWAS variant. Coloc was run on a 1 Mb region centered on the lead GWAS variant (± 500 kb from the variant) with prior probabilities set to $p_{\text{sub.1}}=10^{-4}$, $p_{\text{sub.2}}=10^{-4}$, $p_3=5 \times 10^{-6}$. We defined suggestive support for colocalization between the ANA_C2_V2 COVID-19 GWAS and cis-eQTL signal if posterior probability for one shared causal variant (PP_4) >0.25 .

[0122] Allelic heterogeneity of gene expression in cis is widespread (Aguet et al., 2019), and it violates the assumption of one causal variant per trait. Thus, we also used the development version of coloc (github.com/chriswallace/coloc/tree/condmask) (Wallace, 2020) in a wider 2 Mb region centered at the lead ANA_C2_V2 COVID-19 GWAS variant. The enhanced version of coloc allows conditioning and masking to overcome the single causal variant assumption. For eQTL datasets from the eQTL Catalogue, we used method=mask with LD data from the 1000 Genomes Project (1000 Genomes Project Consortium et al., 2015) CEU population that matches the genetic ancestry of the population in majority of the studies in the eQTL Catalogue. For eQTL data sets from the GTEx Project, we used method=cond with LD calculated from the individuals that had gene expression data in the given tissue in GTEx. We used the mode=iterative and allowed for a maximum of three variants to condition/mask. We set the r^2 threshold to 0.01 to call two signals independent when masking, and we set the p-value threshold to 10^{-4} to call the secondary signal significant. We used method=single for the ANA_C2_V2 COVID-19 GWAS.

[0123] As a result, the maximum PP_4 (posterior probability for shared causal variants) estimates with conditioning/masking were similar to PP_4 estimates from the standard coloc run, suggesting no additional colocalization events with secondary independent cis-eQTLs.

Regional Association Plots for the 3p21.31 Locus

[0124] Immunofluorescence co-stained for ACE2, EEA1 and Lysotracker in A549ACE2 cells with a CRISPR guide RNA targeting RAB7A revealed that ACE2 shows a distinct colocalization with EEA1 (an early endosome marker) and a less frequent colocalization with Lysotracker (a lysosomal marker) (data not shown). Data points in the locuszoom-like regional association plots as shown in FIG. 6C are colored by the LD between the lead ANA_C2_V2 COVID-19 GWAS variant 3:45867022:C:G and other variants in the region. For plotting data for COVID-19 GWAS and eQTLs from the eQTL Catalogue, we used genotype data from the 1000 Genomes Project, and calculated a weighted average r^2 value based on the counts of global ancestral populations in the analysis (if multiple global ancestry populations were studied). Note that for European populations, we only included non-Finnish populations in the 1000 Genomes Project. For plotting data for eQTLs from the GTEx project, we used genotype data available via dbGaP, accession phs000424.v8, and calculated r^2 using the specific individuals that had expression data from the given tissue.

Quantification and Statistical Analysis

[0125] General data analysis—In all cases, we have mentioned specific software tools used in the respective section of the Methods. All other data analysis was performed in GraphPad Prism 8 and RStudio.

[0126] Data representation—In all boxplots, boxes indicate the median and interquartile ranges, with whiskers indicating either 1.5 times the interquartile range, or the most extreme data point outside the 1.5-fold interquartile. All transfection experiments show the mean of three replicate experiments, with error bars representing the standard error of mean.

Results—a High-Throughput Screen to Identify Genes Required for SARS-CoV-2 Infection

[0127] To identify key genes required for SARS-CoV-2 infection, we performed a genome-scale loss-of-function targeting 19,050 genes in the human genome using the GeCKOv2 CRISPR-Cas9 library (Sanjana et al., 2014). The GeCKOv2 library contains 122,411 CRISPR single-guide RNAs (6 guide RNAs per gene) and has previously been used in several previous CRISPR screens, including for drug resistance, immunotherapy, synthetic lethality, mitochondrial disease, and

therapeutic discovery for muscular dystrophy (Erb et al., 2017; Jain et al., 2016; Lek et al., 2020; Patel et al., 2017; Shalem et al., 2014). First, we transduced a human alveolar basal epithelial cell line (A549) that constitutively expresses ACE2 (referred to as A549ACE2) with an all-in-one lentiviral vector containing Cas9, guide RNAs from the GeCKOv2 human library, and a puromycin resistance gene. The transduction was performed at a low multiplicity of infection (MOI ~0.2) to ensure that most cells would receive only one guide RNA construct (FIG. 1A). We then selected with puromycin so that only library-transduced cells remained. We also measured the survival rate after puromycin selection was complete (3 days) to ensure high coverage of the 122,411 guide RNAs (~1,000 cells per guide RNA). After puromycin selection was complete, we cultured the cells for 9 days to ensure protein depletion after CRISPR gene targeting.

[0128] Next, we infected the GeCKOv2 pool of A549ACE2 cells with SARS-CoV-2 virus (Isolate USA-WA1/2020 NR-52281) at either a high (0.3) or a low (0.01) MOI. We verified that SARS-CoV-2 infects A549ACE2 cells by staining for the nucleocapsid (N) protein at 24 hours post-infection (data not shown) and, at day 6 post-infection, we measured cell survival for both the high and low MOI conditions (FIG. 1B). As expected, the higher MOI infection resulted in fewer surviving cells at day 6 post-infection. Next, we extracted genomic DNA and, via amplicon sequencing, we quantified guide RNA abundance in each biological condition (FIG. 1A). To confirm that library representation was properly maintained, we computed the correlation between the guide RNA representation in the plasmid library and after puromycin selection ($r=0.84$) (FIG. 7A). In contrast, after SARS-CoV-2 infection, there was a much greater degree of guide RNA dropout, as expected given that SARS-CoV-2 rapidly kills A549ACE2 cells without CRISPR perturbations (FIGS. 1C, D).

[0129] Using robust-rank aggregation (RRA) on the guide relative enrichments, we computed gene-level scores to identify genes where loss-of-function mutations led to enrichment within the pool (FIG. 1D) (Kolde et al., 2012). We identified approximately 1,000 genes with significant RRA enrichment ($p<0.05$) (FIG. 7B). We also used two other previously published methods to compute gene enrichments (RIGER weighted-sum and second-best rank) and found a high degree of overlap between enriched genes identified by all three methods (FIG. 7C) (Chen et al., 2015; Luo et al., 2008). We also found a high degree of shared genes across both the low and high SARS-CoV-2 MOI conditions: When examining the top 50 most enriched genes, we found that 27 of them were shared between the low and high MOI conditions (FIG. 7D), suggesting that several host genes involved in viral pathogenesis function independently of viral dose.

Results—Enriched Genes are Involved in Multiple Aspects of the Viral Lifecycle and are Broadly Expressed

[0130] Upon close examination of the most enriched genes, we found genes involved in key aspects of viral entry and replication (FIG. 2). For example, the well-established entry receptor angiotensin-converting enzyme 2 (ACE2) receptor was ranked as the 12th most-enriched gene in the low MOI screen and 7th in the high MOI screen (See the list in Table I) (Hoffmann et al., 2020a; Zhou et al., 2020). Among the top 50 enriched genes, we identified several sets of related genes that function together in complexes, giving us further confidence in the genome-scale screen (FIGS. 2 and 3A). We found genes essential for initial attachment and endocytosis (ACE2, RAB7A and 4 members of the ARP2/3 complex: ACTR2, ACTR3, ARPC3, and ARPC4), Spike protein cleavage and viral membrane fusion (CTSL and 13 members of the vacuolar-ATPase proton pump: ATP6AP1, ATP6AP2, ATP6V0B, ATP6V0C, ATP6V0D1, ATP6V1A, ATP6V1B2, ATP6V1C1, ATP6V1E1, ATP6V1G1, ATP6V1H, TMEM199, and TOR1AIP1), endosome recycling (4 members of the endosomal protein sorting Retromer complex: VPS26A, VPS29, VPS35, and SNX27; 4 members of the endosomal trafficking Commander complex: COMMD2, COMMD3, COMMD3-BMI1, and COMMD4; and 3 members of the PI3K pathway: PIK3C3/VPS34, WDR81, and ACP5), ER-Golgi trafficking (DPM3, ERMP1, PPID, and CHST14), and transcriptional modulators (SLTM and SPEN). A consistent theme among the enriched complexes is endosome

function and regulation (V-ATPase proton pump, Retromer, Commander, Class 3 PI3Ks) (Banerjee and Kane, 2020; Mallam and Marcotte, 2017; McNally and Cullen, 2018). Gene Set Enrichment Analysis on the full ranked list of genes identified significantly-enriched Gene Ontology (GO) categories for endosome processing, transport and acidification and categories related to cytokinesis and virion attachment (FDR $q < 0.1$) (FIGS. 3B and 8A-8D; and data not shown) (Subramanian et al., 2005).

[0131] Although we performed our CRISPR screen in human lung cells, we explored whether the expression of host genes whose loss reduces SARS-CoV-2 infection were lung-specific or more broadly expressed. To answer this question, we took the top-ranked genes and examined their expression across 12 tissues using 4,790 RNA-sequencing datasets from the Genotype-Tissue Expression (GTEx) v8 database (FIG. 3C) (Aguet et al., 2019). Virtually all of the top gene hits were broadly expressed across all tissues, implying that these mechanisms may function independent of cell or tissue type. Among the top-ranked genes, only ACE2 showed tissue-specific expression with a particular enrichment in testis, small intestine, kidney and heart (FIG. 3C).

Results—Enriched Genes Directly Interact with Viral Proteins and are Also Essential for Other Viral Pathogens

[0132] Recently, Krogan and colleagues performed an in-depth study of SARS-CoV-2 protein-protein interaction networks by over-expressing affinity-tagged versions of each protein encoded in the viral genome followed by tandem mass spectrometry after pulldown (Gordon et al., 2020). Their study identified 332 high-confidence SARS-CoV-2-human protein-protein interactions (PPIs). We found that many of the highly-ranked genes from our loss-of-function screen had direct PPI with different viral proteins (FIG. 3D, and data not shown). For example, two highly-ranked subunits of the vacuolar-ATPase proton pump, ATP6AP1 and ATP6V1A, interact with SARS-CoV-2 non-structural protein 6 (nsp6) and membrane (M) protein, respectively. ATP6AP1 was ranked 4th in the low MOI CRISPR screen and 2nd in the high MOI CRISPR screen (See the list of genes in Table I) and its role in viral pathogenesis is further reinforced by its strong PPI interaction (Mass spectrometry Interaction Statistics [MIST] score=0.99) (Verschueren et al., 2015). Another key endocytosis protein, RAB7A, is ranked in the top 50 genes in both CRISPR screens and interacts strongly with non-structural protein 7 (nsp7) (MIST score=0.97). We also compared the top-ranked genes with another proteomic study that used proximity labeling in A549 cells over-expressing BioID-tagged viral proteins and found that 22 out of the top 50 low MOI CRISPR screen genes had direct interactions with viral genes—a significant enrichment over randomly chosen genes ($p = 2 \times 10^{-4}$) (Samavarchi-Tehrani et al., 2020).

[0133] Since similar loss-of-function CRISPR screens have been performed to identify host genes required for other viral pathogens, we next sought to understand whether the hits identified in our SARS-CoV-2 screen were shared with those identified in prior screens for ZIKA and pandemic H1N1 avian influenza (IAV) (Y. Li et al. 2019, B. Li et al., 2020). We examined whether top-ranked hits from the ZIKA and IAV screens shared similar genes and similar functional categories. Overall, there was greater similarity between GO categories of enriched genes for SARS-CoV-2 and ZIKA (FIG. 3E) and data not shown. However, when examining the top 50 genes from the SARS-CoV-2 screen, we found several genes that were highly enriched in all 3 viral pathogen screens (FIG. 8E). This group included subunits of the vacuolar-ATPase proton pump, a well-known family of genes essential for acidification and endosomal processing (Banerjee and Kane, 2020).

Results—Validation of Enriched Genes Using CRISPR Knock-Out, RNA Interference and Small Molecule Inhibitors

[0134] To test the ability of top-ranked genes to block SARS-CoV-2 viral infection, we picked 30 genes ranked among the top 200 genes in our RRA analysis for independent validation. Each gene was targeted with 3 guide RNAs distinct from the guides present in the GeCKOv2 genome-wide CRISPR library. The guides were synthesized individually and cloned into the pCC1 lentiviral

vector, a modified version of lentiCRISPRv2 with the F+E optimized guide RNA scaffold (Legut et al., 2020).

[0135] Cas9-perturbed A549.sup.ACE2 lines were infected with SARS-CoV-2 at an MOI of 0.1 and the percentage of infected cells was determined by immunofluorescence against the SARS-CoV-2 N protein at 36 hours post-infection (hpi). For all of the Cas9-perturbed cell lines we observed a reduced percentage of infected cells with an up to 10-fold reduction in SARS-CoV-2 infection, compared to the cell lines with non-targeting sgRNAs. Immunofluorescence images of A549ACE2 knockout lines infected with SARS-CoV-2 at MOI of 0.1 and fixed 36 hours post-infection showed SARS-CoV-2 N protein and DAPI (data not shown). Among the genes where loss provided the greatest protection against SARS-2 infection were vesicular trafficking genes like RAB7A, CCDC22 and VPS35, and other genes with well-established roles such as the ACE2 receptor and the protease cathepsin L (CTSL) (FIG. 4A). As an orthogonal gene perturbation method, we also validated a more extensive list of genes via siRNA knockdown for 48 hours, followed by infection with SARS-CoV-2 at MOI of 0.1 (See Table I). Quantification of N-protein immunofluorescent images revealed a substantial reduction of the percent infected cells (FIG. 10A). We found a significant correlation between the percent infection in the arrayed validation and the median fold-change (log 2) from the genome-wide CRISPR screen ($rs=-0.6$, $p=5\times 10^{-4}$) (FIG. 4B).

[0136] For these genes, we performed a full multi-step viral replication growth curve and found that the CRISPR perturbations decrease viral load across all time points (5, 10, 24, and 48 h) compared to the non-targeting control (data not shown). To test if the identified hits block viral infection in other cell lines, for 8 genes we generated polyclonal CRISPR knockouts in a human liver cell line (Huh7.5.sup.ACE2), which were then infected with SARS-CoV-2 at an MOI of 0.1. Compared to non-targeting guide RNA controls, we found reduced infection with all 8 genes tested in the Huh7.5ACE2 cells ().

[0137] As an orthogonal gene perturbation method, we also validated a more extensive list of top-ranked genes via siRNA knockdown for 48 h, followed by infection with SARS-CoV-2 at MOI of 0.1. Quantification of N-protein immunofluorescent images revealed a substantial reduction of the percent infected cells (data not shown).

[0138] By cross-referencing the top 500 genes from the CRISPR screen with the Drug Gene Interaction database (DGIdb), we identified a set of 69 druggable genes (FIG. 4C; see list of genes in Tables II and III) (Cotto et al., 2018). Based on this list and a manual literature search, we selected 9 genes that were a primary or a secondary target of one of the 26 small molecule inhibitors (FIG. 4D). Among the 26 inhibitors, 9 are FDA approved and 7 are in Phase 2 or Phase 3 clinical trials for diverse ailments. We pretreated A549.sup.ACE2 cells for 2 hours with 10 μ M of each inhibitor and then infected with SARS-CoV-2 and analyzed the cells at 36 hpi. As a positive control, we also included remdesivir, which inhibits the viral RNA polymerase and is the only currently approved treatment for COVID-19 in the United States (Beigel et al., 2020). We assessed the efficacy of each inhibitor on blocking viral infection using immunofluorescence and quantitative PCR (qPCR).

[0139] Seven of the 26 inhibitors that we tested (PIK-III, Compound-19, SAR405, autophinib, ALLN, tamoxifen and ilomastat) resulted in >100-fold reduction of viral load as measured by qPCR (FIG. 4D). Among the best performing inhibitors, 4 of them target the same gene VPS34 and 2 inhibitors (autophinib and ALLN) reduce the viral load more than 1000-fold. Similar results were obtained by immunofluorescent imaging of SARS-CoV-2 N protein (FIG. 10B). By testing the top four PIK3C3 inhibitors in combination with CRISPR targeting of PIK3C3, we found that Compound-19, PIK-III and autophinib were specific while SAR405 resulted in greater viral inhibition in PIK3C3 CRISPR-perturbed cells, suggesting some potential off-target activity for SAR405 (FIG. 10, and data not shown).

[0140] Combinations of the top performing inhibitors overall showed an additive effect and further

protected the cells from SARS-2 infection, with the best protection observed by combining tamoxifen and SAR405 (FIG. 10C). Next, we measured cell viability at 36 hours post inhibitor treatment using flow cytometry. We observed more than 50% reduction of A549.sup.ACE2 cell viability with two pan-HDAC inhibitors, panobinostat and pracinostat (FIG. 4F). To explore the effects of the inhibitors on other important cell types during viral infection, we tested the effect of select inhibitors on primary human CD8⁺ T-cell activation. Human primary CD8⁺ T-cell gating strategy was as follows. Cells were first gated by the forward and side scatter area and then doublets were excluded by gating through the forward scatter area and width. Live CD8⁺ cells were selected by gating on LIVE-DEAD violet and CD8⁺ FITC. The non-stimulated or CD3/CD28 stimulated viable CD8⁺ T cells were gated on TNF- α PE/Cy7 and IFN γ PE to determine the percentage of TNF- α and IFN γ positive cells. (data not shown). Among the tested inhibitors, only idelalisib resulted in a significant reduction in release of cytokines tumor necrosis factor (TNF) and interferon-gamma (IFN γ), consistent with previous reports (FIG. 9D, 9E) (Martinelli et al., 2018). Other inhibitors with higher activity in blocking SARS-CoV-2 infection (e.g. tamoxifen) had no effect on T-cell activation and cytokine secretion (FIG. 9C, 10D-10E).

Results—Single-Cell Sequencing Identifies Cholesterol Biosynthesis as a Common Mechanism Underlying Multiple Enriched Genes from the CRISPR Screen

[0141] Next, we sought to understand the mechanisms underlying how individual genes identified in our loss-of-function screen prevent SARS-CoV-2 infection and if host gene loss alters cell transcriptional programs. For this, we utilized the Expanded CRISPR-compatible Cellular Indexing of Transcriptomes and Epitopes by sequencing (ECCITE-seq) method to couple pooled CRISPR perturbations of our top hit genes with a single-cell transcriptomic and proteomic readout (Mimitou et al., 2019) (FIG. 5A; and data not shown). Importantly, cells were infected at low MOI to maximize the fraction of cells that express a single guide RNA, and therefore can be assigned a specific gene perturbation. For this, we pooled all 90 individual guide RNA plasmids (3 per target gene) used to validate our genome-scale screen and 9 non-targeting (NT) sgRNAs (FIG. 4A).

[0142] Using ECCITE-seq, we identified a median of 152 single cells per target gene with a single guide RNA. We observed specific reduction of target gene expression in cells grouped by target gene, indicating nonsense-mediated decay of transcripts with frameshift indel mutations after CRISPR modification (FIG. 11A). This effect was more pronounced for genes with higher expression. To classify cells for each target gene as either ‘perturbed’ (different from cells with NT guide RNAs) or ‘escaping’ (similar to cells with NT sgRNAs), we used a recently described Gaussian mixture model framework (Papalexi et al., 2020). Using this framework, we classified cells for 11 of the 30 target genes more than 5 differentially expressed “perturbed” genes with a minimal log fold change (FIG. 5B), implying that loss of these genes results in a detectable transcriptomic shift. It is likely that loss of the other 19 genes results in more subtle changes in only a few genes; however, even for ‘non-perturbed’ genes with sufficient basal expression, we detected clear changes in the CRISPR target gene (FIG. 11A). We repeated the ECCITE-seq experiment focusing on the 11 genes with detectable transcriptomic shifts upon target gene perturbation. Combining both replicate experiments, we obtained 18,853 cells that expressed only one guide RNA with a median of 1,388 cells per target gene.

[0143] We found that loss of 6 of the ‘perturbed’ genes—ATP6AP1, ATP6V1A, CCDC22, NPC1, PIK3C3 and RAB7A, which are part of the endosomal entry pathway—yielded similar gene expression signatures among upregulated differentially expressed genes (FIGS. 5C, 5D and 11B). These 6 target gene perturbations all led to upregulation of pathways affecting lipid and cholesterol homeostasis (FIG. 5D). Recently, we performed a large survey of >20,000 potential pharmacological treatments for COVID-19 and, for compounds effective at preventing viral infection, we identified induction of the cholesterol biosynthesis pathway as a potential mechanism of viral inhibition (Hoagland et al., 2020). Loss-of-function mutations in these 6 genes may function through a similar mechanism (induction of cholesterol synthesis) that combats the virus-

mediated suppression of cholesterol synthesis. Among the significant differentially-expressed genes, we also found 61 genes from the enriched CRISPR screen genes (n=20 genes upregulated; n=41 genes down-regulated). For example, NPC1, ATP6V1F and ATP6V1E1 are upregulated in most of the 6 endosomal entry pathway gene-perturbed cells (FIG. 5C), suggesting compensatory upregulation of related genes to mitigate target gene loss.

[0144] To understand how these changes impact lipid production, we measured cholesterol levels in cells after CRISPR perturbation and found that loss of these genes increases cholesterol by between 10% and 50%, depending on the perturbation (FIG. 5D). To show that increases in cholesterol leads to increased SARS-CoV-2 resistance, we treated A549.sup.ACE2 cells with amlodipine, a calcium-channel antagonist that increases intracellular cholesterol (Mori et al., 1988; Ranganathan et al., 1982). We verified that amlodipine increases cholesterol levels in A549.sup.ACE2 cells (data not shown) and found that pre-treatment with amlodipine results in reduced SARS-CoV-2 viral infection, as measured by qPCR for nucleocapsid RNA, plaque formation, and number of viral RNA reads from RNA-sequencing, with only a modest impact on cell viability (data not shown). RNA-sequencing of cells treated with amlodipine shows a similar differential gene expression profile as seen in our ECCITE-seq with CRISPR perturbations with the most significant upregulated pathway as cholesterol biosynthesis (data not shown).

Results—RAB7A Knockout Results in a Reduced Cell Surface Expression and an Increased Endosomal Accumulation of ACE2

[0145] Next, we sought to determine if any of the top-ranked genes regulate cell surface expression of ACE2, as surface ACE2 is required for SARS-CoV-2 infection (Hoffmann et al., 2020a). To measure the cell surface expression of ACE2, we performed flow cytometry on A549 wild-type cells and a panel of A549.sup.ACE2 CRISPR-perturbed cells. ACE2 expression was detected in A549.sup.ACE2, but not in A549 wild type cells, validating the antibody specificity (FIGS. 6A and 12A-12C). Flow cytometry analysis of the A549.sup.ACE2 CRISPR-perturbed lines revealed that RAB7A knock-out cells have a significantly reduced cell surface expression of ACE2 compared to cells transduced with a non-targeting guide RNA (FIG. 6B). Rab7a is a small GTPase that is involved in regulating cellular processes such as vesicular transport and membrane trafficking (Guerra and Bucci, 2016). We hypothesized that the observed reduction of ACE2 at the cell surface in RAB7A knock-out cells may be due to impaired vesicular trafficking and accumulation of ACE2 in the cytoplasm.

[0146] Immunofluorescence staining of ACE2 on the A549.sup.ACE2 polyclonal cell line was targeted with a non-targeting (NT) or a RAB7A-targeting guide. In NT cells, ACE2 localizes at the cell membrane and in the cytoplasm, while in RAB7A-targeted cells, ACE2 shows a distinct pattern of localization to vesicles. ACE2 is detected in most cells, and RAB7A knockout leads to revealed an increased accumulation of ACE2 in the cytoplasm and in vesicle-like hollow structures reminiscent of endo-lysosomes (data not shown). We detected these accumulations in about 35% of RAB7A knockout cells with ACE2 staining, compared to the control where ACE2 was primarily localized at the plasma membrane and small cytoplasmic puncta (FIG. 6C). Finally, we investigated which cellular compartments accumulate ACE2 in RAB7A knockout cells. Co-immunofluorescence images showed that in RAB7A knockout cells ACE2-containing vesicles often colocalize with EEA1, an early endosomal marker and less frequently with LysoTracker, a lysosome marker (data not shown). Efficient Rab7a protein depletion across the RAB7A CRISPR-perturbed lines used was confirmed by western blot (FIG. 12C).

[0147] Since ACE2 was overexpressed in our A549.sup.ACE2 cells, we considered whether Rab7A loss would lead to similar cytoplasmic sequestration in cells with endogenous ACE2 expression. Flow cytometry analysis in Caco-2 colon and Calu-3 lung cells revealed that RAB7A knock-out cells have significantly reduced cell surface expression of ACE2 compared to cells transduced with a non-targeting guide RNA (FIG. 6D, 6E). Immunofluorescence staining of ACE2 on Caco-2 cells transduced with a NT or a RAB7A-targeting guide showed that in NT cells, ACE2 localizes at the

cell membrane and in the cytoplasm, while in RAB7A-targeted cells, ACE2 shows a distinct pattern of localization to vesicles. We also found that RAB7A knock-out in Caco-2 cells results in larger cytoplasmic ACE2 puncta compared to the control cells, further supporting cytoplasmic accumulation of ACE2 in the absence of Rab7A (FIG. 6F).

Results—Genes Required for SARS-CoV-2 Infection In Vitro Contribute to COVID-19 Risk in Human Patients

[0148] Quantile-quantile (Q-Q) plots showed the expected and observed p-value distribution of COVID-19 hospitalized versus not hospitalized genome-wide association study (GWAS) for three sets of variants: all variants tested in the GWAS, variants that are lead eQTLs in GTEx Lung, and variants that are lead eQTLs in GTEx Lung for the top-ranked genes enriched in at least one CRISPR screen (data not shown). Inflation estimate $\lambda_{\text{sub}.0.1}$ measures the inflation of test statistics relative to the $\chi^2_{\text{sup}.2}$ quantile function of 0.1. i.e., 10% of the most significant tests. A histogram of the permuted $\lambda_{\text{sub}.0.1}$ estimate to test the significance of the inflation of the signal for variants that are eQTLs for CRISPR screen hit genes (n=243 variants) in Lung in the COVID-19 hospitalized versus not hospitalized GWAS was performed. The observed $\lambda_{\text{sub}.0.1}$ value was 2.14 (data not shown). Prioritization of genes in the 3p21.31 locus associated with COVID-19 versus population GWAS was studied. Genes in the locus were ranked according to the second-best guide RNA score in the low MOI and high MOI pooled CRISPR screens. eQTL p-values for the lead GWAS variant 3:45867022:C:G was shown in different cell types and tissues from the eQTL Catalogue and GTEx v8, 110 eQTL data sets in total. Nine cell types/tissues were highlighted, where the eQTL p-value for the lead GWAS variant is $<10^{-4}$ for at least one gene in the region. Suggestive support for colocalization between the GWAS and eQTL signal (posterior probability for one shared causal variant (PP4) >0.25 was shown. The transcripts of the eight protein-coding genes (LIMD1, SACM1L, SLC6A20, LZTFL1, CCR9, FYCO1, CXCR6 and XCR1) in the locus were obtained. Ranks and eQTL p-values were aligned to match the start of the. The regional association of the COVID-19 versus population GWAS and cis-eQTLs for the eight genes from the associated locus in the cell type/tissue where the lead GWAS variant 3:45867022:C:G has the lowest eQTL p-value was shown. All above data was shown in the provisional application but not shown here.

[0149] Lastly, we sought to determine to what extent top-ranked genes from the CRISPR screen mediate COVID-19 risk. Genetic variants have been shown to often exert their effect on complex traits or disease via cis-regulation of gene expression (Gamazon et al., 2018). Thus, we hypothesized that genetic regulatory variants for genes pinpointed by the CRISPR screen would show an increased signal for genetic association for COVID-19 in the human population. To this end, we analyzed if expression quantitative trait loci (eQTLs) from lung tissue for these genes would be enriched for overall association signal in data from the COVID-19 Host Genetic Initiative (HGI) with six COVID-19-related genome-wide association studies (GWAS) (Aguet et al., 2019; COVID-19 Host Genetics Initiative, 2020). The Q-Q plots compared the expected and observed P-value distribution of COVID-19 GWAS P-value ($-\log_{10}$) for all the variants tested in GWAS, variants that are lead eQTLs in GTEx Lung, variants that are lead eQTLs in GTEx Lung for the top-ranked genes enriched in at least one CRISPR screen for ANA_A2_V2 (very severe respiratory confirmed COVID-19 vs. population), ANA_B2_V2 (hospitalized COVID-19 vs. population), ANA_C1_V2 (COVID-19 vs. lab/self reported negative), ANA_C2_V2 (COVID-19 vs. population), and ANA_D1_V2 (predicted COVID-19 from self-reported symptoms vs. predicted or self-reported non-COVID-19) GWAS, respectively. Inflation estimate $\lambda_{0.1}$ measures the inflation of test statistics relative to the chi-square quantile function of 0.1. i.e., 10% of the most significant tests (data not shown) The histogram of the permuted $\lambda_{0.1}$ estimate was observed to test the significance of the inflation of variants that are eQTLs for CRISPR screen hit genes in GTEx Lung in the given COVID-19 GWAS (data not shown). We observed inflation of GWAS association signal for variants that are cis-eQTLs for top-ranked CRISPR genes in the hospitalized versus not

hospitalized COVID-19 GWAS, compared to all lung eQTLs ($\lambda_{\text{sub.0.1}}=2.14$, permutation p-value=0.03,). We did not see a significant signal for the other five GWASs, which could be explained by the low power of these early GWAS data (data not shown). Nevertheless, our results suggest that genes that are required for SARS-CoV-2 infection in vitro contribute to COVID-19 risk in humans.

[0150] The only individual locus with a robust human genetic association to COVID-19 susceptibility is 3p21.31, but the functional mechanisms of this association have been unclear and the locus includes multiple protein-coding genes (for example, LIMD1, SACM1L, SLC6A20, LZTFL1, CCR9, FYCO1, CXCR6, and XCR1) (COVID-19 Host Genetics Initiative, 2020; Ellinghaus et al., 2020). To elucidate which genes might mediate the genetic association, we first observed that the SLC6A20 gene and the CXCR6 gene have a relatively high rank in our CRISPR screen (data not shown). Next, we analyzed if the COVID-19 ANA_C2_V2 GWAS lead variant 3:45867022:C:G affects the expression of any of these genes. Using data from the eQTL Catalogue and GTEx v8 (Aguet et al., 2019; Kerimov et al., 2020), we analyzed all eQTL associations for this variant, and performed colocalization analysis to assess if the eQTL and GWAS signal share the same causal variant. CXCR6 and SLC6A20 stood out, with the eQTL data indicating that the COVID-19-associating variant affects the expression of these two genes (FIGS. 13A, 13B) (Giambartolomei et al., 2014; Wallace, 2020). The eQTL affecting CXCR6 is active in memory T follicular helper (Tfh) CD4^{sup.} T cells and has a suggestive colocalization with the GWAS (data not shown) (Schmiedel et al., 2018). The colocalization signal is very strong for the cis-eQTL for SLC6A20 in four tissues from GTEx. Colocalization analysis of COVID-19 GWAS and eQTLs was performed in different cell types and tissues. Posterior probability (y-axis) for the five different hypothesis H0 (no association), H1 (association in GWAS), H2 (association in eQTL), H3 (association both in GWAS and eQTL, two independent SNPs), H4 (association both in GWAS and eQTL, one shared SNP) from coloc is shown for each of the eight genes (LIMD1, SACM1L, SLC6A20, LZTFL1, CCR9, FYCO1, CXCR6, and XCR1) used in the colocalization analysis, with results from the eQTL Catalogue and GTEx v8. We used the ANA-C2-V2 (COVID-19 vs. population) GWAS. For eQTL studies using microarray technology with multiple probes mapping to the same gene, we chose the probe which resulted in the lowest eQTL p-value for the lead COVID-19 GWAS variant to represent the gene. While we observe suggestive signal for colocalization with eQTLs for CXCR6 in Tfh memory cells (Schmiedel et al. 2018), most posterior support is attributed to H1 indicating insufficient power. Also, there is suggestive signal for colocalization with eQTLs for LZTFL1 in Testis in GTEx, but the eQTL signal is not statistically significant (FDR >0.05) and lead eQTL variant has GWAS p-value=0.4011. (data not shown). Other genes in the region, such as LZTFL1, FYCO1, and XCR1, also have regulatory variants affecting their expression, but two distinct variants likely drive the GWAS and eQTL signals (data not shown). Given that both the CRISPR screen and eQTL data support the causal role of SLC6A20 and CXCR6, it is possible that the COVID-19 GWAS association in the 3p21.31 locus is driven by pleiotropic effects of the same variant on multiple genes in different cell types.

[0151] Each and every patent, patent application, and publication, including websites cited throughout specification, are incorporated herein by reference. Similarly, the SEQ ID NOs which are referenced herein and which appear in the appended Sequence Listing are incorporated by reference. While the invention has been described with reference to particular embodiments, it will be appreciated that modifications can be made without departing from the spirit of the invention. Such modifications are intended to fall within the scope of the appended claims.

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Claims

1. A method of treating or inhibiting a viral infection in a human subject comprising inhibiting in vivo the expression or activity of one or a combination of the subject's genes required for viral infection by administering to a mammalian subject in need thereof one or more inhibitors of the activity or expression of said gene or combination of genes.
2. The method according to claim 1, wherein the viral infection is COVID-19, wherein the gene or combination of genes comprises one or more of PIK3C3, PRKCA, Cathepsin L, MMP12 and ACE2, and wherein: (i) the inhibitor of PIK3C3 is one or more of Autophinib, SAR405, Compound 19, or PIK-III; (ii) the inhibitor of PRKCA is Tamoxifen; (iii) the inhibitor of Cathepsin L is ALLN, CAS No. 110044-82-1; (iv) the inhibitor of MMP12 is Ilomastat; and (v) the inhibitor of ACE2 is one or more of Compound 28 (PMID: 18324760), XNT (PMID: 18391097), MLN-4760, GL11001, and ABZ-Ser-Pro-Tyr (NO.sub.2)—OH, and Lisinopril.
3. The method according to claim 1, wherein said gene or combination of genes is selected from the genes identified in Tables I, II, III or IV.
4. The method according to claim 1, wherein said inhibitor is selected from one or a combination of the inhibitors identified in Table III or Table IV.
5. The method according to claim 1, wherein said inhibitor is selected from one or a combination of the inhibitors identified in FIG. 4D or 4E.
6. The method according to claim 1, wherein said inhibitor is one or a combination of Remdesivir, PIK-III, Compound 19, SAR405, Autophinib, ALLN, Tamoxifen and Ilomastat.
7. The method according to claim 1, wherein the selected inhibitor is administered to the subject pre-infection or post-infection at a dosage effective to mimic a loss of function of its corresponding gene.
8. The method according to claim 1, wherein said gene is RAB7A, ACE2, ACTR2, ACTR3, ARPC3, and ARPC4.
9. The method according to claim 1, wherein said gene or combination comprises one or more of CTSL, ATP6AP1, ATP6AP2, ATP6V0B, ATP6V0C, ATP6V0D1, ATP6V1A, ATP6V1B2, ATP6V1C1, ATP6V1E1, ATP6V1G1, ATP6V1H, TMEM199, and TOR1AIP1.
10. The method according to claim 1, wherein said gene or combination comprises one or more of VPS26A, VPS29, VPS35, and SNX27.
11. The method according to claim 1, wherein said gene or combination comprises one or more of COMMD2, COMMD3, COMMD3-BMI1, and COMMD4.
12. The method according to claim 1, wherein said gene or combination comprises one or more of PIK3C3/VPS34, WDR81, and ACP5.
13. The method according to claim 1, wherein said gene or combination comprises one or more of DPM3, ERMP1, PPID, CHST14, SLTM and SPEN.
14. The method according to claim 1, wherein said gene or combination comprises one or more of PIK3C3, Cathepsin L, PRKCA, MMP12, BRPF1, DRD2, MAPK3, CALR and HCAC9.
15. The method according to claim 1, wherein the genes are one or more of genes or subsets of genes identified in FIG. 1D, FIG. 2, FIG. 3C, FIG. 4A, FIG. 4D, FIG. 5C, FIG. 6B, or FIG. 8E.
- 16.-21. (canceled)
22. The method according to claim 1, wherein the genes are one or more of the 69 genes identified in Table III.
23. The method according to claim 1, wherein the genes are one or more of the 73 genes identified in Table IV.
24. The method according to claim 1, wherein the viral infection is caused by infection with the virus SARS-CoV-2.
25. The method according to claim 1, wherein the inhibitor comprises a small molecule, a nucleic

acid therapeutic, a peptide therapeutic, or another biologic therapeutic that mimics in the subject a loss of function of one or more of the selected genes.

26. A method for identifying host target genes required for viral infection targeting lung cells comprising performing a genome-scale loss of function screen by a) transducing a selected cell line with a lentiviral vector containing Cas9, guide RNA from the GeCKOv2 human library, and an antibiotic resistance gene at a low MOI to cause CRISPR gene targeting; b) culturing the cells to ensure protein depletion after CRISPR gene targeting using said guide RNA; c) infecting pooled cells of (b) with selected virus; d) extracting genomic DNA and, via amplicon sequencing, quantifying guide abundance in each biological condition; e) identifying genes where loss-of-function mutations led to enrichment within the pools.
