Original Research

Title: AI-guided discovery of the invariant host response to viral pandemics

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ABSTRACT (197 words)

We sought to define the host immune response, a.k.a, the "cytokine storm" that has been implicated in fatal COVID-19 using an AI-based approach. Over 45,000 transcriptomic datasets of viral pandemics were analyzed to extract a 166-gene signature using ACE2 as a 'seed' gene; ACE2 was rationalized because it encodes the receptor that facilitates the entry of SARS-CoV-2 (the virus that causes COVID-19) into host cells. Surprisingly, this 166-gene signature was conserved in all *vi*ral *p*andemics, including COVID-19, and a subset of 20-genes classified disease severity, inspiring the nomenclatures *ViP* and *severe-ViP* signatures, respectively. The *ViP* signatures pinpointed a paradoxical phenomenon wherein lung epithelial and myeloid cells mount an IL15 cytokine storm, and epithelial and NK cell senescence and apoptosis determines severity/fatality. Precise therapeutic goals were formulated and subsequently validated in high-dose SARS-CoV-2-challenged hamsters using neutralizing antibodies that abrogate SARS-CoV-2•ACE2 engagement or a directly acting antiviral agent, EIDD-2801. IL15/IL15RA were elevated in the lungs of patients with fatal disease, and plasma levels of the cytokine tracked with disease severity. Thus, the *ViP* signatures provide a quantitative and qualitative framework for titrating the immune response in viral pandemics and may serve as a powerful unbiased tool to rapidly assess disease severity and vet candidate drugs.

One Sentence Summary: The host immune response in COVID-19.

Key Words:

- Artificial Intelligence/Machine Learning
- Boolean Equivalent Clusters
- Angiotensin Converting Enzyme (ACE)-2
- Coronavirus COVID-19
- Immune response
- Lung alveoli
- Natural Killer (NK) cells
- Interleukin 15 (IL15)

PANEL: RESEARCH IN CONTEXT

Evidence before this study

The SARS-CoV-2 pandemic has inspired many groups to find innovative methodologies that can help us understand the host immune response to the virus; unchecked proportions of such immune response have been implicated in fatality. We searched GEO and ArrayExpress that provided many publicly available gene expression data that objectively measure the host immune response in diverse conditions. However, challenges remain in identifying a set of host response events that are common to every condition. There are no studies that provide a reproducible assessment of prognosticators of disease severity, the host response, and therapeutic goals. Consequently, therapeutic trials for COVID-19 have seen many more 'misses' than 'hits'. This work used multiple (> 45,000) gene expression datasets from GEO and ArrayExpress and analyzed them using an unbiased computational approach that relies upon fundamentals of gene expression patterns and mathematical precision when assessing them.

Added value of this study

This work identifies a signature that is surprisingly conserved in all viral pandemics, including Covid-19, inspiring the nomenclature ViP-signature. A subset of 20-genes classified disease severity in respiratory pandemics. The ViP signatures pinpointed the nature and source of the 'cytokine storm' mounted by the host. They also helped formulate precise therapeutic goals and rationalized the repurposing of FDA-approved drugs.

Implications of all the available evidence

The ViP signatures provide a quantitative and qualitative framework for assessing the immune response in viral pandemics when creating pre-clinical models; they serve as a powerful unbiased tool to rapidly assess disease severity and vet candidate drugs.

INTRODUCTION

As the rapidly unfolding COVID-19 pandemic claims its victims around the world, it has also inspired the scientific community to come up with solutions that have the potential to save lives. In the works are numerous investigational drugs at various phases of clinical trials, from rationalizing[1], to IRB approvals, recruitment and execution[2,3], all directed to meet an urgent and unmet need —i.e., ameliorate the severity of COVID-19 and reduce mortality.

Two obstacles make that task difficult—First, the pathophysiology of COVID-19 remains a mystery. The emerging reports generally agree that the disease has a very slow onset[4,5] and that those who succumb typically mount a 'cytokine storm'[4,6], i.e., an overzealous immune response. Despite being implicated as a cause of mortality and morbidity in COVID-19, we know virtually nothing about what constitutes (nature, extent) or contributes to (cell or origin) such an overzealous response. Consequently, treatment goals in COVID-19 have been formulated largely as a 'trial and error'-approach; this is reflected in the mixed results of the trials that have concluded[7]. Second, there is no established pre-clinical animal or human cell/organoid models for COVID-19; vetting the accuracy and/or the relevance of such models requires first an understanding of the host response in the disease.

We set out to define this aberrant host immune response in COVID-19 using machine learning tools that can look beyond interindividual variability to extract underlying gene expression patterns within multidimensional complex data. The approach was used across multiple cohorts of viral pandemics. The resultant pattern, i.e., signature, was subsequently exploited as a predictive model to navigate COVID-19. Findings not only pinpointed the precise nature of the cytokine storm, the culprit cell types and the organs, but also revealed disease pathophysiology, and helped formulate specific therapeutic goals for reducing disease severity. Key findings were validated in preclinical models of COVID-19 in Syrian hamsters and in the lungs and plasma of infected patients.

METHODS

Data Collection and Annotation: Publicly available microarray and RNA Seq databases were downloaded from the National Center for Biotechnology Information (NCBI) Gene Expression Omnibus (GEO) website[8-10]. A comprehensive catalog of these datasets is presented in **Table S1**. Gene expression summarization was performed by normalizing Affymetrix platforms by RMA (Robust Multichip Average)[11,12] and RNASeq platforms by computing TPM (Transcripts Per Millions)[13,14] values whenever normalized data were not available in GEO.

Statistics: Boolean analysis and other statistical approaches are covered in detail in *Supplementary Online M*aterials. Briefly, the StepMiner algorithm[15], BooleanNet statistics[16], and BECC (Boolean Equivalent Correlated Clusters)[17] are used to perform Boolean analyses. Gene signature is computed by using a scaled linear combination of gene expression values which is used to classify sample categories and the performance of the multi-class classification is measured by ROC AUC (Receiver Operating Characteristics Area Under The Curve) values. A color-coded bar plot is combined with a violin plot to visualize the gene signature-based classification and distribution of the gene signature score. Bubble plots of ROC-AUC values (radius of circles are based on the ROC-AUC) demonstrating the direction of gene regulation (Up, red; Down, blue) for the classification based on the 20 gene severe ViP signature and 166 gene ViP signature is visualized side by side.

Sample size estimation: Effect size (the magnitude of the difference between groups divided by the standard deviation) for IL15 measurement is estimated as 1/0.7 from GSE157103. In order to have an 80% power ($1-\beta = 0.8$) to detect a statistically significant difference ($\alpha = 0.05$) between high and low groups of patients, we need around 8 patients in each group.

Ethics statement: Animal studies were approved and performed in accordance with Scripps Research IACUC Protocol #20-0003 (PI: Tom Rogers, PMID: 32540903). Blood from COVID-19 donors was either obtained at a UC San Diego Health clinic under the approved IRB protocols of the University of California, San Diego (UCSD; 200236X) or recruited at the La Jolla Institute under IRB approved (LJI; VD-214). COVID-19 donors were California residents, who were either referred to the study by a health care provider or self-referred. The lung specimens from the

COVID 19 positive human subjects were collected using autopsy (study was IRB Exempt). All donations to this trial were obtained after telephone consent followed by written email confirmation with next of kin/power of attorney per California state law (no in-person visitation could be allowed into our COVID-19 ICU during the pandemic). The team member followed the CDC guidelines for COVID19 and the autopsy procedures[18,19].

Validation studies

Serum cytokine measurement: Blood from COVID-19 donors was either obtained at a UC San Diego Health clinic or recruited at the La Jolla Institute under active IRB protocols. Two independent methods were used: 1) Levels of IL15 cytokine was estimated using ELISA MAX Deluxe set (Biolegend Cat. No. 435104) or Meso Scale Discovery (MSD) according to the manufacturer's recommended protocol. 2)

Autopsy and biopsies of lungs: The detailed process of collecting and processing lung specimens from the COVID 19 positive human subjects is available in the supplementary methods.

Hamster study with anti-CoV-2 therapy: Lung samples from CoV-2-challenged 8-week old Syrian hamsters were generated exactly previously published study[20] with either anti-spike monoclonal antibody or anti-viral therapy (EIDD-2801). See Supplementary methods for details regarding IHC protocols.

Data deposition and materials sharing: All data is available in the main text or the supplementary materials. RNA Seq datasets from hamster studies have been deposited at NCBI GEO (GSE168095 and GSE157058).

RESULTS AND DISCUSSION

An ACE2-centric Study Design

To identify and validate an invariant (universal) gene signature of host response in COVID-19, we mined more than 45,000 publicly available datasets of viral pandemics across three species (human, mouse and rats) (***Step 1***; **Fig 1**). Three relatively widely accepted facts shaped our approach using Angiotensin-converting enzyme 2 (ACE2) as 'seed' gene in our computational studies: *(i)* ACE2 is the most well-known portal for SARS-CoV-2 entry into the host cell[21,22]; its expression in cell lines correlates with the expression of innate immune genes [23] and susceptibility to SARS-CoV spike protein-driven entry[24,25], and its depletion in mice abrogates SARS-CoV infection[26]; *(ii)* ACE2 is a potent negative regulator of the renin–angiotensin aldosterone system (RAAS)[27]; without such restraint, the RAAS contributes to exuberant inflammation in the setting of infections[28]; and finally, *(iii)* although the mechanism through which ACE2 suppresses inflammatory response remains poorly understood, accumulating evidence indicates that infections perturb ACE2 activity, allowing for uncontrolled inflammation[29-37].

As ***Step 2*** (**Fig 1**), we validated the signature in several human and mouse datasets of viral pandemics, and a subset of genes was identified and validated as indicators of disease severity. The signatures were then validated in SARS-CoV-2-infected cells and tissues and to explore the nature, extent and cell of origin of host response in mild and fatal COVID-19.

As ***Step 3*** (**Fig 1**), the gene signatures were prospectively used to navigate the uncharted territory of COVID-19 and pinpoint immunopathologic mechanisms, which revealed the nature (IL15), source (airway epithelium), intensity (quantitative measure) and consequence (NK cell senescence) of the cytokine storm and helped objectively formulate precise therapeutic goals to reduce the severity of COVID-19.

As ***Step 4*** (**Fig 1**), the gene signature and the mechanism of action (IL15/IL15RA) were validated in lung tissues from SARS-CoV-2 challenged golden hamster using RNASeq and IHC. In addition, precise therapeutic goal was validated in the SARS-CoV-2-challenged golden hamster model. The mechanism of action (IL15/IL15RA) was also validated by ELISA in plasma and IHC in lung tissues from UCSD COVID-19 cohort participants.

A Shared Host Response Signature in Respiratory Viral Pandemics

Because publicly available transcriptomic datasets from SARS-CoV-2-infected samples are still relatively few, any conclusion drawn from so few samples using any computational methodology is likely to lack robustness. We chose to use an informatics approach, i.e., Boolean Equivalent Correlated Clusters (BECC)[17], which can identify fundamental invariant (universal) gene expression relationships underlying any biological domain; in this case, we selected the biological domain of '*respiratory viral pandemics*'. BECC enables comparison of the normalized expression of two genes across all datasets by searching for two sparsely populated, diagonally opposite quadrants out of four possible quadrants (high-low and low-high), employing the BooleanNet algorithm[16]. There are six potential gene relationships assessed by BooleanNet: two symmetric (Equivalent and Opposite; **Fig 2A**) and four asymmetric[16]. Two genes are considered "Boolean Equivalent" if they are positively correlated with only high-high and low-low gene expression values. Two genes are considered "Boolean Opposite" if they are negatively correlated with only high-low and low-high gene expression values. Asymmetric Boolean implications result when there is only one sparsely populated quadrant. The BECC algorithm focuses exclusively on "Boolean Equivalent" relationships to identify potentially functionally related gene sets. Once identified, these invariant relationships have been shown to spur new fundamental discoveries[38,39], with translational potential[40], and most importantly, offer insights that aid the navigation of uncharted territories where nothing may be known[41,42].

We used GSE47963 [human airway epithelial (HAE) cultures with H1N1 and SARS-CoV infections; n = 438] as a 'test' dataset, which was comprised of human airway epithelial cell samples (HAE) infected *in vitro* with the causative agents of the 2009 'swine flu' (influenza A-H1N1; a triple recombination of human, avian, and swine influenza viruses[43-45]) and the 2002 Severe acute respiratory syndrome (SARS-CoV-1)[46] outbreaks (**Fig 2B**). These datasets were chosen now, and other datasets were prioritized later in the study, e.g., H5N1 (the causative agent of the avian flu in 2006-06[47]) and MERS-CoV (the causative agent of Middle East respiratory syndrome in 2012[48]) based upon the fact that they *all* contributed to outbreaks that are characterized by acute respiratory syndromes with high case-fatality rates[21].

ACE2 is used as a 'seed' to identify other genes that have 'Boolean Equivalent' and 'Boolean Opposite' relationships with ACE2. These genes were subsequently filtered using differential analysis on another dataset [GSE113211 (n = 118); **Fig 2B**] that profiled heterogeneous

immunophenotypes of children with viral bronchiolitis (confirmed positive for the virus in ~100% patients; of which 25 % were infected with Influenza/Para-Influenza and 14.8% with human CoV). Transcriptomes were analyzed in nasal mucosal scrapings (NMS) and PBMC samples taken during an acute visit (AV) and during a subsequent visit at convalescence (CV)[49]. 166 genes (**Table S2**; 1-1) retained the "Boolean Equivalent" relationship with ACE2 *and* their expression was downregulated during the convalescence visit. 26 genes (**Table S2;** 2-1) retained "Boolean Opposite" relationships with ACE2 *and* their expression was upregulated during the convalescence visit. All subsequent analyses were performed using the 166 –gene signature that had Boolean Equivalent relationship with ACE2 and that was down-regulated during a convalescent visit after acute viral bronchiolitis.

First, the 166-gene signature was evaluated in the test dataset-- it was used to rank order the samples and test for phenotype classification using a receiver operating characteristic curve [ROC curve; the area under this curve (AUC) represents degree or measure of separability] and displayed such classification using violin plots (**Fig 2C-D**). The signature classified the uninfected *vs*. infected samples with reasonable accuracy in the setting of SARS-CoV-1 infection (ROC-AUC = 0.81, **Fig 2C**). It also classified perfectly in the setting of H1N1 infection (ROC-AUC = 1.00, **Fig 2D**). Good classification was observed between samples from the acute visit (AV) and convalescence visit (CV) in children (test dataset; GSE113211; **Fig 2E-F**, *left*), as well as two independent adult cohorts (validation datasets that were generated in two prospective studies[50,51]; **Fig 2G-H**). All the patients in these cohorts were infected with respiratory viruses; in one cohort, ~45% were documented infections with pandemic Influenza strains H1N1 and H3N2 (GSE68310), whereas 100% of the patients in the other were victims of the H1N1 pandemic of 2009 (GSE21802). Regardless of the heterogeneity of these validation cohorts, the classification score using the 166-gene signature remained strong in both datasets (ROC-AUC = 0.83 - 0.96). Findings indicate that the viral pandemic signature was conserved among numerous respiratory viral pandemics, and for that reason, we christened it the '*Vi*ral *P*andemic' (*ViP*) Signature.

The *ViP* Signature Defines the 'Cytokine Storm' in Viral Pandemics

Reactome analyses on the 166 genes showed that the signature was largely enriched for genes within the immune system pathways, e.g., interferon and cytokine signaling, cellular processes that are critical for an innate immune response such as the ER-phagosome pathway and antigen

processing and presentation, and finally the adaptive immune system (**Fig 3A-C**). In other words, the signature reflected a typical host immune response that is expected during any viral infection. This is not surprising because an overzealous host immune response, i.e., a 'cytokine storm' is shared among all respiratory viral pandemics (Influenza, avian and swine flu)[52] and severe COVID-19 patients who succumb to the disease[6]. However, there were 3 surprising factors: (i) This signature and reactome profile emerged using ACE2 as a 'seed' gene, which is not the receptor for influenza strains to enter into host cells. (ii) It is also noteworthy that despite filtration through two unrelated datasets (**Fig 2B**), one *in vitro* and another *in vivo*, and the reduction in the number of genes in the ACE2-equivalent cluster during such iterative refinement, the pathways/processes represented in the 166-gene cluster (**Table S2**; 1-2) remained virtually unchanged. (iii) The only cytokine/receptor pair that emerged in this 166-gene cluster was interleukin-15 (IL15/IL15RA; **Fig 3A, C**), indicating that transcripts of this cytokine are invariably equivalent with ACE2 expression across all datasets analyzed. Findings are in keeping with the well-established role of IL15 in both the pathogenesis[53] and the severity [54] of virus-induced lung injury. They are also consistent with the fact that IL15-/- mice are protected from lethal influenza[55].

Next we tested this 166-gene signature in datasets of samples infected with viruses that have either caused pandemics in the past (SARS-CoV-1, MERS, Ebola, Zika, etc.) or continue to do so at present (Influenza A/B, HIV, HCV, etc). The signature perfectly classified uninfected and infected samples (ROC-AUC = 1.00; **Fig 3D**) in four humans (GSE56677, GSE45042, GSE17400, GSE30589) and two mouse SARS-CoV1 and MERS-CoV datasets (GSE19137, GSE52920). It also performed reasonably well in two other human and one mouse datasets (ROC-AUC ranging between 0.76-0.97; GSE37827, GSE33267, GSE50000; **Fig 3D**). Analysis of a time course of infection with SARS-CoV-1 (GSE33267; **Fig 3E**) revealed that classification of infected samples improved over time, beginning at 48 h and reaching perfection (ROC AUC = 1.00) at 60-72 h, which is consistent with epidemiologic findings in prior acute respiratory viral pandemics (SARS and MERS) have average incubation periods ranging ~2-7 days, which can sometimes last up to ~10-14 d. Among datasets with curated samples representing other viral outbreaks and/or pandemics that are neither respiratory nor acute, we found that classification scores for RNA viruses were significantly better compared to DNA viruses in *in vitro* systems (**Fig 3F** top, **Fig S1A**), especially for those that share clathrin-dependent endocytic methods to breach host cells (**Table S3**). However, the classification scores were indistinguishable between RNA and DNA

viruses in *in vivo* studies (**Fig 3F** bottom, **Fig S1A-B**). These results indicate that the 166-gene signature is shared among all viral infections, and not specific to respiratory viral pathogens.

Notably, the 166-gene host response signature was not specific for viral infections *per se*; it also performed well in classifying samples with bacterial infections, both *in vitro* and *in vivo*, and fungal infections *in vivo* (**Fig 3G**). These findings were not surprising because the prominent overrepresentation of interferon signaling that is captured within the signature (**Fig 3C**) is widely accepted as a shared fundamental aspect of host defense response during any infection[56]. Despite such apparent promiscuity, what is noteworthy is that the *ViP* signatures were relatively specific for infections/inflammation (**Fig S3**). The signature also implicated the epithelial and myeloid cells, but not ECs and fibroblasts contribute to host immune response because the classification scores were better for airway epithelial cells (AE) and dendritic cells (DC) compared to fibroblasts (FI) and microvascular endothelial cells (ME) (ROC-AUC: 0.66, 0.82 vs 0.43, 0.37; **Fig 3H**; *left*). These scores correlated well with ACE2 expression in these different cell types ($p < 0.001$; **Fig 3H**; *right*), raising the possibility that viral entry through the engagement of ACE2 and the induction of ACE2-equivalent host genes may be intertwined. That myeloid cells are major contributors to this signature was confirmed in five independent datasets; the 166-gene signature distinguished 'reactive' (M1-polarized) macrophages in them all (**Fig 3I**).

Together, these findings indicate that the ACE2-equivalent 166-gene signature is of broader relevance than just coronaviruses; the signature captures core fundamentals of host innate immune responses seen not just in respiratory viral pandemics, but viral, bacterial and even fungal infections. The airway epithelial cells and cells of myeloid lineages (DCs and macrophages) appear to be major contributors to the *ViP* signature.

A 20-gene Subset within the *ViP* Signature Detects Disease Severity

To determine what constitutes 'severe/fatal' disease, we rank-ordered the 166 genes within the *ViP* signature for their ability to classify Influenza A/B-infected adult patients by clinical severity[57,58] (n = 154; **Fig 4A**). Severe disease was defined as intubation and mechanical ventilation due to poor oxygenation and/or death. A set of top 20 genes (**Fig 4A; Table S2, 3-1; Table S4**) was sufficient to classify healthy controls from infected patients (ROC-AUC = 1.00) as well as distinguish mild from severe disease with reasonable accuracy (ROC-AUC = 0.95) in the test cohort (**Fig 4B**). Reactome pathway analyses revealed that compared to the *ViP* signature, the

'severity'-related 20-gene cluster enriches a completely different set of cellular processes, i.e., DNA damage (especially induction of genes that are critical for base excision repair; BER), stress-induced senescence, neutrophil degranulation and changes in cell cycle (**Fig 4C, Fig S2**). We validated this signature side-by-side with the 166-gene *ViP* signature in three human datasets that included samples from mild vs. severe disease during the avian (H7N9), IAV (H3N1 and others) and the swine (H1N1) flu viral pandemics (**Fig 4D,** *left*). Both the 166-gene *ViP* signature and the 20-gene severity signature performed similarly when it came to classifying control *vs*. mild disease, but the latter performed significantly better in classifying mild *vs*. severe disease, and did so consistently in both validation datasets (ROC-AUC ranging from 0.8-0.9; **Fig 4D,** *left*).

The severity signature performed well also in a large murine lung dataset in which mice were intranasally infected with non-lethal (NL, control), sub-lethal (SL, mild) and lethal (L, severe) doses of two different strains of H1N1 virus A; the Texas/36/91 (Tx91), which is non-lethal in C57Bl/6 mice and causes transient morbidity and compared against those infected with sublethal and lethal doses of the highly pathogenic Puerto Rico/8/34 (PR8), which causes ARDS and death in less than a week[59]. Harvested lungs were sorted into five different prospectively isolated cell subpopulations and analyzed by microarray (**Fig 4D,** *right*): alveolar macrophages, lymphocytes (BC, TC, NK), Ly6Chi mononuclear myeloid cells, neutrophils, CD45neg pulmonary epithelial cells. The 166-gene *ViP* signature distinguished the control *vs*. mild samples perfectly in all five cell types (ROC-AUC = 1.00; **Fig 4D,** *right*). The classification accuracy of the 20-gene severity signature, however, was most prominent in neutrophils (ROC-AUC = 1.00), followed by monocytes and macrophages (ROC-AUC = 0.9), and then epithelial cells (ROC-AUC = 0.8), but failed in lymphocytes. These findings suggest that the cells of the innate immune system are the primary contributors of disease severity.

We conclude that the 166-gene *ViP* signature that was initially built using *in vitro* infection datasets detects also the host immune response ('cytokine storm') in the complex *in vivo* systems where the response may be triggered by direct viral damage to the lung epithelium, but get propagated by feed-forward dysregulated immune response, both innate and adaptive. Surprisingly, this 166-gene *ViP* signature was not associated with disease severity; instead, severity-associated 20 genes that regulate stress and senescence-associated repression of protein expression and DNA damage (**Fig 4C**). DAVID GO analyses on the 20-gene signature indicated that 3 biological processes, e.g., transcriptional repression, apoptosis, and intermediates within the

type I IFN (IFNγ signaling) pathway (**Fig 4E**) indicative of cellular distress, senescence/aging and death are the determinants of severity/fatality.

The *ViP* Signatures are Induced in the Lung Epithelial and Immune cells in COVID-19

We next tested the ability of the *ViP* signatures to distinguish between SARS-CoV-2-infected samples and uninfected controls in 3 independent datasets, 2 of which were datasets generated from cells infected *in vitro* (**Fig 5A-C**) and one that was generated from lung samples from a fatal case of COVID-19 (**Fig 5D**). The signature perfectly classified infected from uninfected samples in them all (ROC-AUC 1.00; **Fig 5A-B-D**); of the 166 genes, both IL15 and IL15RA were notably elevated in infected samples (**Fig 5A**). The 20-gene signature performed reasonably well in distinguishing infected from uninfected A549 cells (ROC-AUC = 0.87; **Fig 5E**), and the healthy from the COVID-19 lung sample (ROC-AUC = 1.00; **Fig 5G**), but not in airway cells (bronchial; ROC-AUC = 0.57; **Fig 5F**). In fact, the 166-gene and 20-gene signatures perfectly classified infected vs. uninfected samples in all *in vitro* cellular models of CoV-2 infection, regardless of the tissue/organ (**Fig 5H***; left, middle*). The signatures performed nearly perfectly (ROC-AUC = 0.90 - 1.00; **Fig 5H**, *right*) across all lung cell types from COVID-19 infected patients analyzed by single cell sequencing.

We next tested the ability of these signatures to distinguish mild *vs*. fatal COVID-19 in single cell sequencing datasets from patient-derived lung samples (**Fig 5I**). The 166-gene signature was able to distinguish control *vs*. mild infection most effectively in macrophages, airway epithelium, CD4+ T cells and NK cells (**Fig 5I**, *lower panel, lower row*) and mild *vs*. severe disease in the epithelium and in NK cells (**Fig 5I**, *lower panel, upper row*). The 20-gene signature not only performed well in classifying control *vs*. mild infection in the same 4 cell types as above but also in B cells and CD8+ T cells (**Fig 5I**, *upper panel, lower row*). However, the 20-gene severity signature continued to perform most optimally in the epithelium (ROC-AUC = 1.00) and in NK cells (**Fig 5I**, *upper panel, upper row*). The signatures were rapidly induced also in monkeys challenged with SARS-CoV-2, and gradually suppressed during convalescence after 17 d (**Fig 5J**).

Because the ViP signature is comprised of IFN-signaling pathways and presumably IFN-stimulated genes (ISGs), we asked if the ViP/severe-ViP signatures offer any additional advantage beyond ISGs. Using Interferome v2.01 (http://www.interferome.org) we first confirmed that

155/166 genes in ViP signature and 18/20 genes in severe-ViP signatures were genes that are likely to be regulated by IFN signaling (**Fig 5K**). Surprisingly, despite such high degree of pathway overlap with ISGs, the severe-ViP signature (sViP) was able to prognosticate outcome (hospital-free days) in a cohort of patients with COVID-19 (**Fig 5L**; *left*). When compared head to head in an univariate analysis using Cox proportional hazards regression model, the prognostic effect of the severe-ViP signature emerged as superior to two different sets of previously published ISGs[60,61] in their ability to prognosticate hospital-free days (**Fig 5L**; *top right*). Three factors emerged as determinants of longer hospital stays: (i) the ICU admission status, (ii) need for mechanical ventilation and (iii) induction of the severe-ViP signature. A multivariate analysis using Cox proportional hazards regression model suggested that these three factors may be independent covariates of poor outcome (**Fig 5L**; *bottom right*).

Together, these findings show that the 166-gene *ViP* signature seen in other respiratory viral pandemics is conserved also in COVID-19. The cytokine storm (166-genes, which included IL15/IL15RA; **Table S2**) was induced in multiple cell types; however, the 20-gene *ViP* signature of disease severity and fatality was most prominently induced in two cell types: (i) the airway epithelial cells, known producers of IL15 after viral infections[62],[63] and (ii) the NK cells which are known targets of physiologic as well as overzealous IL15 response[64,65]. Findings also show that despite the ISG-like makeup of the ViP signatures, there are key components within the signature that is able to detect disease severity.

Viral infection and IL15 Induce, and Flu Vaccine Attenuates the *ViP* Signatures in NK cells

We next asked how the *ViP* signatures are impacted when NK cells are exposed to virus-infected epithelial cells. NK cells are known to lyse influenza virus-infected cells by direct cytotoxicity and antibody-dependent cellular cytotoxicity (ADCC); enhancing such NK cell function has been shown to control influenza virus infections [66]. Clearance of other viruses (HIV-1, other retroviruses, etc.) and cancer immunotherapies also leverage such NK cell-dependent ADCC[67,68]. We analyzed a transcriptomic dataset (GSE115203)[69] generated from co-culture studies of human PBMCs (3 donors) with influenza (H1N1 Puerto Rico/08/1934)-infected airway epithelial cells (A549) (**Fig 6A**; *top*). PBMCs (from co-culture), or NK cells FACS-sorted from the PBMC were then analyzed by RNA Seq, and the study had confirmed NK cell ADCC responses were durably induced in this assay *via* type I IFN release from PBMCs. We found that both the 166- and 20-

gene *ViP* signatures were induced in PBMCs and in NK cells sorted from the PBMCs (**Fig 6A**; *bottom left*), indicating that NK cells in these co-culture models were sufficient to capture the observed host immune response in patients with COVID-19.

To test the role of IL15 in the induction of *ViP* signatures, we leveraged three datasets—one that used recombinant IL15 (PBMCs; GSE77601), another that used anti-IL1Rβ mAb (mouse skin biopsies; GSE45551)[70], and a third study using the prototypic H3K27 demethylase inhibitor, GSK-J4; the latter was shown to inhibit NK cell effector cytokines in response to IL15 without impacting its cytotoxic killing activities (human, NK cells; GSE89484)[71]. Both *ViP* signatures were stimulated by IL15, but attenuated in the two other datasets where IL15's actions were blocked pharmacologically (**Fig 6A;** *right*). These findings indicate that IL15 could be necessary and sufficient to induce the *ViP* signatures.

Because two independent studies[72,73] recently showed that those vaccinated against influenza have lower odds of requiring intensive care, invasive ventilation and/or dying, we analyzed two transcriptomic datasets (GSE64655[74] and GSE133478[75]) in which PBMCs from subjects vaccinated with seasonal trivalent or quadrivalent influenza vaccine (TIV/QIV) were collected and analyzed for NK cell activation. The first study showed that the NK cells continued to demonstrate progressive attenuation of both the 166- and 20-gene signatures rapidly within 7 days (**Fig 6B**, *left*). The second study, in which the NK cell-enriched and depleted fractions collected pre- and post(30 d)-vaccination were tested for their response to re-stimulation with IL15 (low dose, 0.75 ng/ml, 18 h); such stimulation is known to enhance NK cell activity [76-78] and promote viral clearance [79-81]. Both ViP signatures were attenuated post-vaccination in NK cell-enriched fractions, but not in depleted fractions (**Fig 6B**, *right*). Because such post-vaccination attenuation happened in the setting of experimentally confirmed[75] enhancement of overall NK cell response, we conclude that attenuation of *ViP* signatures among recipients of TIV could continue to offer protection during re-challenge. Because such protection is seen in NK-cell enriched, but not depleted fractions, we conclude that the protection is mediated primarily *via* the preservation of functional NK cells.

An IL15-storm Originating in the Lung Alveoli Determines the Severity of COVID-19

We next analyzed the *ViP* signatures in transcriptomic datasets generated from multiple organs at autopsy. Both the 166- and 20-gene *ViP* signatures were predominantly enhanced in one organ,

the lungs (**Fig 6C-D**); and IL15/IL15RA were also elevated in the lungs (**Fig 6D**). These findings indicate that the 20- and 166-gene signatures go together and suggest a plausible cause and effect relationship. For instance, severity-related cellular events (such as epithelial and NK cell senescence) occur in the milieu of the organ that mounts the highest IL15-predominant cytokine response, i.e., lungs. We also found that IL15 and its receptor IL15RA were significantly increased in severe COVID-19 lungs (**Fig 6E**). These findings predict that an overzealous IL15-predominant cytokine response is the most consistent finding in the most severe cases of COVID-19 and that the lung epithelium is the likely source of such a storm.

These predictions were validated in a cohort of symptomatic COVID-19 patients who presented to the UC San Diego Medical Center with varying disease severity, ranging from mild to fatal (see **Table S5**). Plasma ELISA studies revealed that IL15 levels were significantly elevated during the acute compared to the convalescent visit (**Fig 6F**), and in whom the clinical presentation was moderate-to-severe compared to those with mild disease (**Fig 6G**). A sub-group analysis confirmed that while gender or age did not have a significant impact on plasma IL15 levels independently, the aged male (> 40 y) cohort had a significantly higher IL15 level than the young males (**Fig 6H**; *left*). No such pattern was noted among females. These findings are consistent with the fact that the gender gap in COVID-19-related deaths widens markedly with age[82]. Lungs collected during autopsies from patients who succumbed to COVID-19 (see **Table S6**) further confirmed that lung epithelial cells, especially the alveolar type II pneumocytes and alveolar immune cell infiltrates express high levels of IL15 and its receptor, IL15RA (**Fig 7A-B**).

Finally, in a cohort of patients with COVID-19, high levels of IL15 transcript carried a poor prognosis (lower probability of discharge from the hospital; **Fig 7C**). An univariate analysis using Cox proportional hazards regression model showed that the prognostic effect of high-IL15 was superior to ISGs[60,61] (**Fig 7D**; *left*), as we observed previously for ViP signatures (**Fig 5L**). A multivariate analysis using Cox proportional hazards regression model suggested that need for mechanical ventilation and IL15 induction may be independent covariates of poor outcome (**Fig 7D**; *right*). That the serum IL15 levels track disease severity was validated in a cohort of patients presenting to our institution with a diagnosis of COVID-19; critical/fatal disease was associated with significant elevation of the cytokine (**Fig 7E**).

Taken together, these findings support the following model of the immunopathogenesis of COVID-19 (**Fig 7C**): Airway epithelial cells and cells of the myeloid lineage and other immune

cells are the primary source of the 166-gene cytokine storm, of which, IL15 is a component. It is possible, that the primary target of IL15, i.e., NK cells, when exposed to this storm for a prolonged period undergo damage, stress-induced senescence and apoptosis. Our model is consistent with prior studies showing that the airway epithelial cells (especially bronchial) constitutively express the IL15 and IL15RA/B genes and that viral infections[63] and IFNγ induced the synthesis and secretion of IL15[63] and that prolonged and excessive stimulation with IL15 is known to induce NK cell exhaustion [64,65]. These findings are consistent with the emerging reports that NK cells are significantly exhausted and reduced in cases of severe COVID-19 infection[83,84] and that such reduction was seen as early as 3-6 days after the onset of symptoms[85]. We conclude that fatal COVID-19 is characterized by a paradoxical immune response, i.e., suppression of epithelial and NK cell functions (immunosuppression) in the setting of a cytokine storm (overzealous immune response).

The ViP Signatures Formulate Therapeutic Goals, Track Treatment Efficacy

Previously we showed that the attenuation of the *ViP* signature was 'associated' with the acquisition of natural convalescence in several respiratory viral pandemics (**Fig 2F-H**); we now asked if they could serve as a readout of therapeutic efficacy. We analyzed interventional studies in the setting of other viral infections that shared the *ViP* signature, i.e., HCV, HIV, Zika and Ebola (**Fig 3H**; **Fig S1**; **Table S3**). The 166-gene *ViP* signature classified HCV-infected liver biopsies treated or not with directly acting anti-viral agents (DAAs) (**Fig 8A-C**) and HIV-infected samples treated or not with anti-retroviral therapeutics (ART; ROC-AUC = 1.00; **Fig 8D**) with sufficient accuracy. In the case of Ebola, the *ViP* signature was somewhat effective in classifying crisis (i.e., acute) from convalescent PBMC samples (ROC AUC 0.64; **Fig S4A, top**), and previously described anti-Ebola therapeutic strategies (Topoisomerase depletion with siRNA[86] inhibited the signature in Ebola-infected alveolar epithelial cells (siTop; ROC AUC 1.00; **Fig S4A, bottom**)[86]. Finally, the *ViP* signature was accentuated in Zika infected human cortical neural progenitor cells (**Fig S4B**) and was effectively attenuated when these infected samples were treated with two investigational drugs that was found to be effective in inhibiting Zika infection. These findings imply that attenuation of the 166-gene *ViP* signature is a desirable therapeutic goal.

We next sought to determine if the SARS-CoV-2 virus can induce the *ViP* signatures, and whether the signatures can track therapeutic response. We tested two therapeutic approaches. The

first approach was the use of N-hydroxycytidine, the parent of the prodrug MK-4482 (Molnupiravir, EIDD-2801) which has not only proven as a potent and selective oral antiviral nucleoside analogue in mice, guinea pigs, ferrets and human airway epithelium organoids[87-92], but also showing promise in Phase IIa trials in the treatment of COVID-19 patients (NCT04405570). We analyzed by RNA seq the lungs from SARS-CoV-2-challenged golden Syrian hamsters who were pre-treated either with this drug or vehicle control (see study protocol in **Fig 8E**). Both 166- and 20-gene ViP signatures were induced in the vehicle-treated arm, and effectively suppressed in the drug-treated arm to levels close to uninfected controls (GSE168095; **Fig 8F**). The second approach was the use of SARS-CoV-2-neutralizing antibodies whose design was inspired by monoclonal antibodies (mAbs) isolated from from convalescent donors[20]. A specific isotype of this antibody, which binds to the receptor-binding domain (RBD-A) of SARS-CoV-2 spike protein in a fashion that precludes binding to host ACE2, was demonstrated as effective in preventing infection and weight loss symptoms, in cell-based and *in vivo* hamster models of infection, respectively. We observed that SARS-CoV-2-challenged hamsters that were pre-treated with anti-CoV-2 antibody, but not the control Den3 antibody (see **Fig 8H** for study protocol) had 3 key findings: (i) they suppressed both the 166- and 20-*ViP* signatures that were otherwise induced in the infected lungs (GSE157058; **Fig 8I**); (ii) their lungs were protected from overwhelming immune cell infiltration and obliteration of alveolar space (**Fig 8J-K**); (iii) expression of IL15 and IL15 receptor was significantly reduced compared to what was observed in the infected lungs (**Fig 8J, L**).

These results validate the ACE-centric computational approach for identifying the *ViP* signatures, i.e., when ACE2•virus engagement was disrupted using antibodies, or reduced using directly acting anti-virals that prevent viral replication using Molnupiravir, the signatures were suppressed. The findings also indicate that the reversal of the signature and the IL15 storm could be used as a readout of therapeutic efficacy.

CONCLUSION

The major and unexpected finding in this work is that all viral pandemics (regardless of their acuity, causative virus, case fatality rates and clinical presentation) share a common fundamental host immune response. Summarized below are our three major findings.

First, we defined an invariant 166-gene host response --the so-called "cytokine storm"– that is surprisingly conserved among all viral pandemics. What was also unusual is that the signature emerged despite the rationalized use of ACE2 as a 'seed' gene to identify 'coronaviral infection-associated genes'. This suggests that while ACE2 may be the entry site for SARS-CoV-2, it is a prominently upregulated gene during host response to other viral infections. As a key regulator of the renin-angiotensin system (RAS), ACE2 expression is increased in the setting of multiple stressors, including non-CoV-2 infections. For example, IAV, H7N9 and rhinoviruses amplify the expression of ACE2 in the distal lung[93-95]. ACE2 activity is induced also in bacterial lung infections[29]. In fact, ACE2 protects against acute lung injury in several animal models of ARDS[96]. Thus, retrospectively, ACE2 is not as specific a 'seed' gene for SARS-CoV-2 as was assumed, and hence, it is not so surprising that the ACE2-equivalent ViP signature is more generalizable as a signature that is induced in respiratory infections. By comparing the *ViP* signatures head-to-head against ISGs, we presented evidence that *ViP* signatures, but not ISGs have prognostic significance. Regardless, our findings show that the ViP signatures capture the quality/nature of the shared 'invariant' host response, demonstrating their potential usefulness as diagnostic tools. The signatures also provide a 'quantitative' framework for assessing the variable degrees of such host response across patients/conditions, reflecting variable degrees of morbidity and mortality across the pandemics/outbreaks; these findings demonstrate their potential usefulness as prognostic tools. We conclude that the ViP signatures not just define the nature of the host immune response to viral pandemics, but also allows the tracking and quantification of such response.

Second, we define the precise nature of the cytokine storm and pinpoint the IL15 cytokine and its receptor, IL15RA as an invariant component. We demonstrate that systemic levels of IL15 tracks with disease severity among patients and that the levels are notably elevated in the aged male (the predisposed age group in COVID-19, as per reports worldwide). Using a combination of single cell RNA Seq and human lung histology, we also pinpoint the lung epithelial and myeloid cells as the key contributors to the *ViP* signature, and more specifically, IL15/IL15RA. These

findings were recently validated in another concurrent publication[97]-- multivariate analyses of soluble biomarkers identified that increased IL-15 is independently associated with mortality and that the levels of the cytokine was consistently high throughout the hospitalization in patients who died versus those who recovered.

Third, we found that a subset of 20-gene 'severe' *ViP* signature, indicative of stress-induced senescence, transcriptional repression, DNA damage and apoptosis is also shared among various viral pandemics. In patients with COVID-19, this signature was seen in lung epithelial and NK cells, which is intriguing because airway epithelial cells is a prominent source and the NK cells are a major target of IL15. Thus, the *ViP* signatures begin to paint a picture of 'paradoxical immunosuppression' at the heart of fatal COVID-19, in which, the observed NK cell exhaustion/depletion in severe COVID-19[83-85,98] could be a consequence of an overzealous IL15 storm, leading to their senescense and apoptosis.

In closing, given that the emerging pandemic is still largely a mystery to us in terms of how it picks its victims, the *ViP* signature we define here provides a computational framework for navigation in otherwise uncharted territory. While it is expected that the signature will be more effective and accurate when it is iteratively filtered using emerging COVID-19 datasets, we provide evidence for its usefulness now in formulating therapeutic strategies and rapidly screening for therapeutics. Because the *ViP* signature of host response is seen also in other viral pandemics tested, findings may also be relevant also in navigating management strategies in those pandemics.

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AUTHOR CONTRIBUTIONS

Role	Authors
Conceptualization; Funding acquisition; Supervision; Project administration	D.S., S.D., P.G.
Investigation; Methodology; Data curation, Formal Analysis • Computational modeling (D.S) • Data curation and analysis (D.S., P.G., S.D. S.T., M.B., G.D.K) • Hamster studies (C.T, M.R.F., S.K., A.C., V.C.) • Rapid Autopsy of Lungs (S.K, A.C., V.C.) • Plasma ELISA (G.D.K.) • Essential resources (animal and patient samples, tissues, etc) (T.F.R, N.B, D.R.B, J.D., L.C.A, S.C., J.D., S.I.R, V.P., S.A.R, D.M.S)	D.S., S.T, G.D.K., T.F.R, N.B, D.R.B, C.T, M.R.F., J.D., L.C.A, S.I.R, V.P, S.A.R, D.M.S, S.K, M.B., S.D., P.G.
Funding acquisition	D.S., S.D., P.G.
Software	D.S.
Visualization; Writing – original draft; Writing – review & editing	D.S., P.G., S.D.

Competing interests: The authors declare no competing interests.

Data and materials availability:

All data is available in the main text or the supplementary materials. RNA Seq datasets from hamster studies have been deposited at NCBI GEO (GSE168095 and GSE157058).

Supplementary Materials: Includes

- Detailed Materials and Methods
- Supplementary Text- **n/a**
- Figures **S1, S2**
- Tables **S1-6**
- External Databases **- None**
- References

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FIGURE LEGENDS

Figure 1. Study design.

(*From top to bottom*) *Step 1*: A database containing > 45,000 human, mouse and rat gene–expression data was mined to identify and validate an invariant signature for host response to viral pandemic (ViP) infection. ACE2, the portal for SARS-CoV-2 entry/uptake, was used as a 'seed' gene and Boolean Equivalent Correlated Clusters (BECC) was used as the computational method to identify gene clusters that share invariant relationships with ACE2. Once defined, these gene clusters (a.k.a., '*ViP signature*') were subsequently validated across multiple human and murine models of pandemic viral infection. *Step 2:* A subset of 20 genes from the ViP signature was selected that was strongly associated with severity of viral infection. These genes were validated in other cohorts to establish the 'Severe' ViP signature. Both 166- and 20-gene ViP signatures were validated on COVID-19 datasets. *Step 3*: Cross-validation studies in numerous other datasets helped- (i) define the nature (ii) and source of the cytokine storm in COVID-19, (iii) gain insights into the immunopathology of fatal disease, and (iv) set precise therapeutic goals. *Step 4*: Findings in step 3 were validated in hamsters and in a cohort of COVID-19 patients. A comprehensive catalog of the datasets analyzed in this work can be found in **Supplementary Table 1**.

Figure 2. Identification and validation of an invariant ACE2-centric signature of host response to viral infections.

(A) Computational approach to identify Boolean Equivalent and Opposite relationships. Number of samples in all four quadrants are used to compute two parameters (S, p). $S > 5$ and $p < 0.05$ is used to identify sparse quadrant. Equivalent relationships are discovered when top-left and bottom-right quadrants are sparse (*left*). Opposite relationships are discovered when top-right and bottom-left quadrants are sparse (*right*). **(B)** Schematic displaying the key computational steps and findings leading to the identification of the 166-gene host response signature using ACE2 as a 'seed' gene. See also **Table S2**. **(C)** Bar and violin plots displaying sample rank order (i.e., classification) of SARS-CoV-1-infected samples and distribution of the 166 gene-based signature in the test dataset (GSE47963, *in vitro* infections of human airway epithelial cells). ROC-AUC values of infected samples classifications are shown below each bar plot unless otherwise stated. **(D)** Analysis of H1N1-infected samples compared to uninfected controls using the 166-gene

signature like C. **(E)** Classification of patient samples used in datasets **F-H** based on their time of collection either during 'Acute Visit' (AV) in the setting of an acute respiratory viral infection and 'Convalescence Visit' (CV) after recovery. **(F)** Analysis of PBMC samples from children (GSE113211, left) and nasal mucosal scrapings (NMS, GSE113211, right). **(G)** Analysis of peripheral blood from adults (GSE68310). **(H)** Analysis of patient samples collected during the swine flu pandemic (GSE21802).

Figure 3. Validation of the ViP signatures in global pandemic viral infections.

(A) Heatmap of the 166-gene signature on test dataset (GSE47963, *in vitro* infections of human airway epithelial cells). Genes that are involved in cytokine signaling in immune system are highlighted in the left. **(B)** ReacFoam analysis on the 166-gene signature that visualizes genome-wide pathway analysis based on Voronoi tessellation. **(C)** Reactome pathway analysis of the 166 genes in the Vip signature. **(D)** ViP signature-based classification of CoV-infected samples (CoV) from uninfected controls (U) in diverse human and mouse datasets. **(E)** Time course of CoV infection shows that the ViP host-response signature is slowly induced in very late (48-72 h) in Calu-3 cells infected with SARS-CoV-1. **(F)** The accuracy (Y axis; ROC AUC) of the signature to classify viral infections differs between RNA viruses and DNA viruses (*X* axis) in *in vitro* system (top). However, they are indistinguishable in *in vivo* system (bottom). See also **Table S3** and **Fig S1**. **(G)** ViP signature-based classification of human and murine samples with fungal or bacterial infections in either *in vitro* or *in vivo* settings. **(H)** The signature captures host response to CoV infection in human primary lung alveolar epithelial cells (AE) and dendritic cells (DC) better than in Fibroblasts (FI) and Endothelial (ME) cells. The accuracy of classification (ROC-AUC) strongly correlates with ACE2 expression in these cells. **(I)** Classification of macrophage polarization states 'reactive' (M1 polarized), unstimulated M0 and tolerant M2-like samples using the 166-gene ViP signature across diverse datasets.

Figure 4. Identification of a 'severe ViP' signature.

(A) Heatmap of the 166 genes on a dataset (GSE101702) annotated with varying severity of infection (healthy controls, 52; mild, 63; severe, 44). Genes are ranked based on their strength of

association with severity (T-test between mild and severe). Genes that are involved in cytokine signaling in the immune system are highlighted on the left. Heatmap of top 20 selected genes ('severe ViP' signature) is shown on the right. **(B)** Bar and violin plots display sample rank order (i.e., classification) of patient samples and distribution of the 20-gene 'severe ViP' signature in the test dataset (GSE101702). ROC-AUC values of mild and severe cases are shown below the bar plot. **(C)** Reactome pathway analysis of 20 genes. **(D)** Bubble plots of ROC-AUC values (radius of circles are based on the ROC-AUC) demonstrating the direction of gene regulation (Up, red; Down, blue) for the classification based on the 20-gene severe ViP signature (top) and 166-gene ViP signature (bottom) in the test dataset (GSE101702), three more human datasets (H7N9, GSE114466; H1N1, GSE21802; IAV/H3N1 and others, GSE61821) and one mouse dataset (H1N1 Inf A, GSE42641). For each gene signature, ROC-AUC of controls vs Mild and Mild vs Severe are shown in top and bottom rows, respectively. In the mouse dataset (GSE42641) host response to lethal (L) and sublethal (SL) infection with H1N1 virus were assessed in five different lung cell types: Alv Mac, Lymphocytes, Monocytes, Neutrophil, Epithelial cells. Number of controls, mild and severe cases are shown at the top. **(E)** Summary of the 20-gene severe ViP signature and pathway analysis by DAVID GO (https://david.ncifcrf.gov/).

Figure 5. The ViP signatures define and measure the host immune response in COVID-19

(A) Heatmap of 166 genes in COVID-19 (GSE147507) dataset ranked by genes up-regulated in COVID-19 infected samples. Genes that are involved in cytokine signaling in the immune system are highlighted on the left. **(B-G)** Bar and violin plots displaying sample rank order (i.e., classification) and distribution of gene signature scores of COVID-19 (GSE147507) infected (CoV) and uninfected controls (C) in A549 (13 C, 6 CoV; **B, E**), normal human bronchial epithelial cells (NHBE, 7 C, 3 CoV; **C, F**), and patient lung autopsies (2 Normal, 1 CoV; **D, G**) based on 166-gene (**B, C, D**) and 20-gene ViP signatures (**E, F, G**). **(H)** Bubble plots of ROC-AUC values (radius of circles are based on the ROC-AUC) demonstrating the direction of gene regulation (Up, red; Down, blue) for the classification based on the 20 gene-severe ViP signature (top) and 166 gene ViP signature (bottom) in multiple independent datasets. **(I)** Bubble plots like panel H showing ROC-AUC of controls vs Mild and Mild vs Severe that are shown in the top and bottom rows, respectively, for each gene signature in the COVID-19 single-cell datasets (GSE145926).

Dataset is analyzed as a 'pseudo-bulk' of all cells or after selecting individual cell types using marker genes specifically expressed in these cell types. **(J)** ViP and severe ViP (sViP) signature-based classification of blood samples (GSE155363) before and up to 17 days after COVID-19 infections in 8 monkeys using ROC-AUC measurements. **(K)** Interferon stimulated genes (ISGs) are annotated in the ViP and sViP gene lists using Interferome v2.01 web application and displayed as Venn diagrams showing the number of genes regulated by one or more IFN types (Type I, II or III). **(L)** Hospital-free days analysis (45 days followup) of COVID-19 patients (GSE157103) limited to less than 70 years old using sViP signature (low and high group) is displayed as Kaplan-Meier estimates (left) of cumulative probability of discharge and its relationship with days in hospital. Cox-proportional hazard univariate analysis (right; *top*) of sViP (high vs low) is compared to ViP signature, Interferon Stimulated Gene-signatures (ISG1[61] and ISG2[60]), age, gender, ICU admission (icu) and mechanical ventilation (mv). Multivariate Cox-proportional hazard analysis (right; *bottom*) compares the variables that are significant in univariate settings, i.e., sViP, ICU admission (icu) and mechanical ventilation (mv).

Figure 6. ViP signatures reveal an interplay between IL15-storm and NK cell dysfunction in fatal COVID-19

(A) Bubble plots of ROC-AUC values (radius of circles are based on the ROC-AUC) demonstrating the direction of gene regulation (Up, red; Down, blue) for the classification based on the 20 gene severe ViP signature (top) and 166 gene ViP signature (bottom) in following datasets. RNASeq data (GSE115203) from PBMCs and sorted NK cells from PBMCs incubated with uninfected A549 cells for 12 hrs compared to infected A549 cells. PBMCs treated with IL15 compared to IL2 (GSE77601). RNASeq analysis of NK cells (GSE89484) treated with GSK-J4 compared to DMSO. Skin tissue in mice (GSE45551) is treated with anti-IL15RB antibody compared to PBS. **(B)** RNASeq data of NK cells isolated from two donors prior to vaccination compared (left) to days 1, 3, and 7 post-TIV vaccination like panel A. RNASeq data of NK enriched and NK depleted PBMCs from healthy donors compared to 30 day post-vaccination like panel A. **(C, D)** Heatmap of 20-gene (panel **C**) and 166-gene (panel **D**) ViP signatures in tissues collected during rapid autopsies on patients who succumbed to COVID-19. Genes are ranked according to the strength of differential expression (T-test) in lung tissue between normal and

infected tissue. **(E)** Box plots of IL15 and IL15RA in samples from varying severity of COVID-19. **(F-H)** Violin plots show levels of plasma IL15 in COVID-19 patients stratified by disease acquity (F), by clinical severity (G) and by gender and age (H). Welch's two sample unpaired t-test is performed to compute the p values. See also **Table S5** for patient metadata.

Figure 7. **Lung alveolar cells contribute to the IL15 storm in fatal COVID-19**

(**A**) Normal lung tissue obtained during surgical resection (*left*) or lung tissue obtained during autopsy studies on COVID-19 patients (*right*) were stained for IL15 and IL15RA. Representative images are shown. Mag = 10X. (**B**) Violin plots display the intensity of staining for IL15RA (top) and IL15 (bottom), as determined by IHC profiler. (**C**) Hospital-free days analysis (45 days followup) of COVID-19 patients (GSE157103) limited to males less than 70 years old using the abundance of IL15 transcripts (intermediate and high groups) is displayed as Kaplan-Meier estimates (left) of cumulative probability of discharge and its relationship with days in hospital. (**D**) Cox-proportional hazard univariate analysis (right; *top*) of sViP (high vs low) is compared to ViP signature, Interferon Stimulated Gene-signatures (ISG1, PMID:15619625; ISG2, PMID:21478870), age, gender, ICU admission (icu) and mechanical ventilation (mv). Multivariate Cox-proportional hazard analysis (right; *bottom*) compares the variables that are significant in univariate settings, i.e., sViP, ICU admission (icu) and mechanical ventilation (mv). (**E**) *Top*: Schematic displays the workflow for patient blood collection and assessment of IL15 levels by mesoscale. *Bottom*: Bar (top) and violin (bottom) plots for the levels of IL15 cytokine (score = Z score of the log reduced mesoscale concentration data). ROC AUC numbers indicate the strength of classification between patients with critical/fatal disease course vs. those with non-critical infection. (**F**) Summary of IL15 signaling and the hypothetical role of NK cells in the severity of COVID-19 infections.

Figure 8. Validation of ViP signature-guided therapeutic goals

(A-C) The 166-gene ViP signature-was used to classify liver biopsies from HCV-infected patients treated or not with directly acting anti-viral agents. ROC-AUC values are shown below each bar plot unless otherwise stated. **(D)** 166-gene ViP signature-based classification of blood samples

from HIV-infected patients treated with anti-retroviral therapy (ART). **(E)** The compound EIDD-2801 (MK-4482; 500 mg/kg) or vehicle (*Veh*) was administered at indicated doses to Golden Syrian hamsters 4 h prior to intranasal infection with SARS-CoV-2. Hamsters were were sacrificed on day 5 and lungs we analyzed by RNA sequencing. **(F)** Bar (top) and violin (bottom) plots using the ViP (left) or sViP (right) signature-based classification of lung samples from hamsters in E and uninfected controls. **(G)** Schematic showing the experimental design for validating the ViP signatures as useful tools to assess therapeutic efficacy. *Uninf*, uninfected; *Den3 and Anti-CoV-2* indicate SARS-CoV-2 challenged groups that received either a control mAb or the clone CC12.2 of anti-CoV-2 IgG, respectively. (**H**) Bar (*top*) and violin (*bottom*) plots display the 166- and 20-gene ViP signatures in the uninfected and the SARS-CoV-2 challenged groups, treated with control or anti-CoV-2 IgG. (**I-K**) Lungs harvested from the 3 groups of hamsters were analyzed by H&E and IHC. Representative images are shown in I. Mag = 10X. Bar graphs in J display the abundance of cellularity and infiltrates in the lungs of the 3 groups, as determined by ImageJ. Violin plots in K display the intensity of staining for IL15RA (*top*) and IL15 (*bottom*), as determined by IHC profiler.

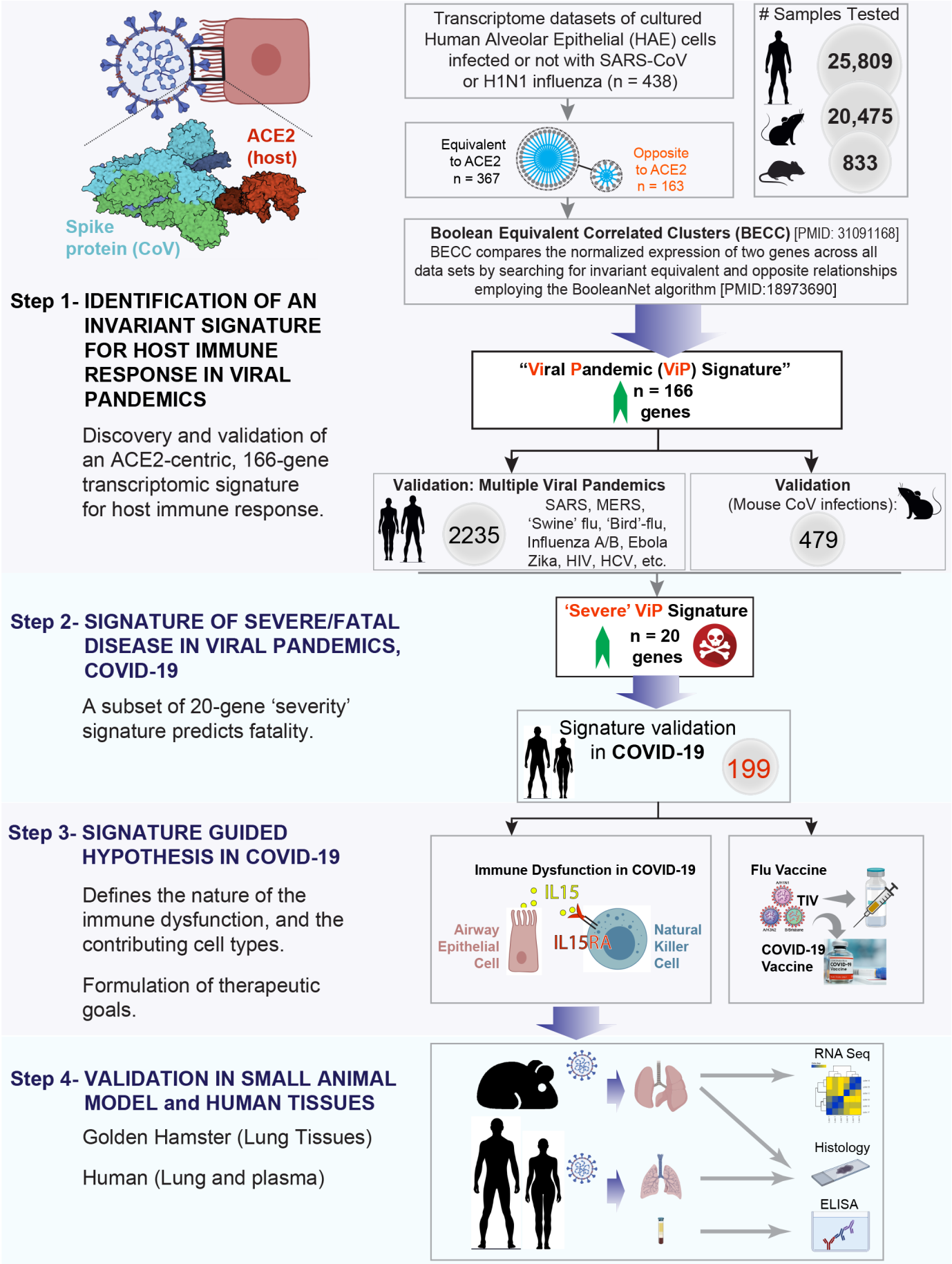

ACE2 (host)
Spike protein (CoV)
Transcriptome datasets of cultured Human Alveolar Epithelial (HAE) cells infected or not with SARS-CoV or H1N1 influenza (n = 438)
Samples Tested
25,809
20,475
833
Equivalent to ACE2 n = 367
Opposite to ACE2 n = 163
Boolean Equivalent Correlated Clusters (BECC) [PMID: 31091168]
BECC compares the normalized expression of two genes across all data sets by searching for invariant equivalent and opposite relationships employing the BooleanNet algorithm [PMID:18973690]
Step 1- IDENTIFICATION OF AN INVARIANT SIGNATURE FOR HOST IMMUNE RESPONSE IN VIRAL PANDEMICS
Discovery and validation of an ACE2-centric, 166-gene transcriptomic signature for host immune response.
"Viral Pandemic (ViP) Signature"
n = 166 genes
Validation: Multiple Viral Pandemics
2235
SARS, MERS, 'Swine' flu, 'Bird'-flu, Influenza A/B, Ebola Zika, HIV, HCV, etc.
Validation (Mouse CoV infections):
479
Step 2- SIGNATURE OF SEVERE/FATAL DISEASE IN VIRAL PANDEMICS, COVID-19
A subset of 20-gene 'severity' signature predicts fatality.
'Severe' ViP Signature
n = 20 genes
Signature validation in COVID-19
199
Step 3- SIGNATURE GUIDED HYPOTHESIS IN COVID-19
Defines the nature of the immune dysfunction, and the contributing cell types.
Formulation of therapeutic goals.
Immune Dysfunction in COVID-19
IL15
IL15RA
Airway Epithelial Cell
Natural Killer Cell
Flu Vaccine
TIV
COVID-19 Vaccine
Step 4- VALIDATION IN SMALL ANIMAL MODEL and HUMAN TISSUES
Golden Hamster (Lung Tissues)
Human (Lung and plasma)
RNA Seq
Histology
ELISA

Figure 1

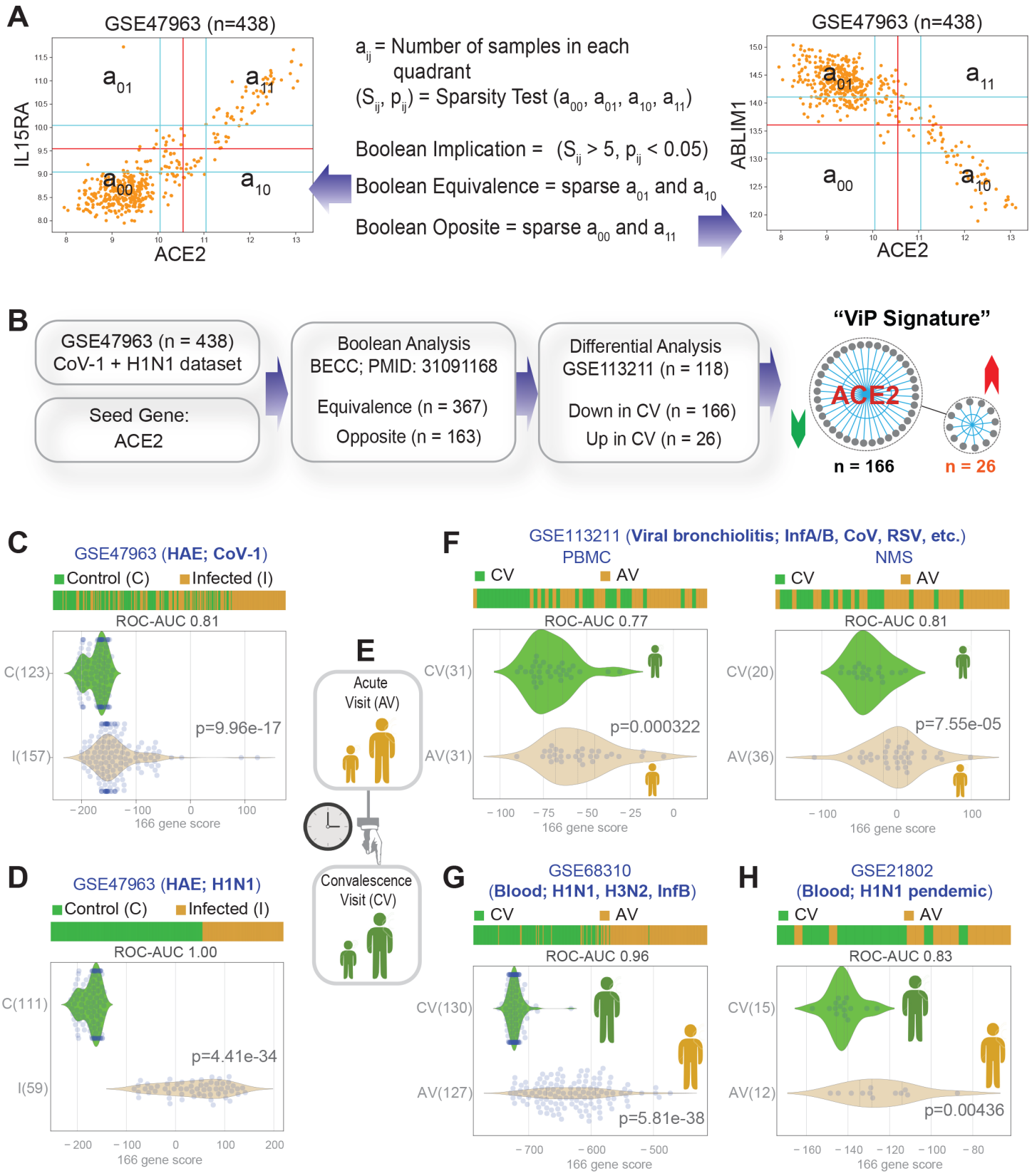

A
GSE47963 (n=438)
IL15RA
ACE2
a_{01}
a_{11}
a_{00}
a_{10}
a_{ij} = Number of samples in each quadrant
(S_{ij}, p_{ij}) = Sparsity Test $(a_{00}, a_{01}, a_{10}, a_{11})$
Boolean Implication = $(S_{ij} > 5, p_{ij} < 0.05)$
Boolean Equivalence = sparse a_{01} and a_{10}
Boolean Oposite = sparse a_{00} and a_{11}
GSE47963 (n=438)
ABLIM1
ACE2
B
GSE47963 (n = 438)
CoV-1 + H1N1 dataset
Seed Gene:
ACE2
Boolean Analysis
BECC; PMID: 31091168
Equivalence (n = 367)
Opposite (n = 163)
Differential Analysis
GSE113211 (n = 118)
Down in CV (n = 166)
Up in CV (n = 26)
"ViP Signature"
ACE2
n = 166
n = 26
C
GSE47963 (HAE; CoV-1)
Control (C)
Infected (I)
ROC-AUC 0.81
C(123)
I(157)
p=9.96e-17
166 gene score
D
GSE47963 (HAE; H1N1)
Control (C)
Infected (I)
ROC-AUC 1.00
C(111)
I(59)
p=4.41e-34
166 gene score
E
Acute
Visit (AV)
Convalescence
Visit (CV)
F
GSE113211 (Viral bronchiolitis; InfA/B, CoV, RSV, etc.)
PBMC
NMS
CV
AV
ROC-AUC 0.77
CV(31)
AV(31)
p=0.000322
166 gene score
ROC-AUC 0.81
CV(20)
AV(36)
p=7.55e-05
166 gene score
G
GSE68310
(Blood; H1N1, H3N2, InfB)
CV
AV
ROC-AUC 0.96
CV(130)
AV(127)
p=5.81e-38
166 gene score
H
GSE21802
(Blood; H1N1 pendemic)
CV
AV
ROC-AUC 0.83
CV(15)
AV(12)
p=0.00436
166 gene score

Figure 2

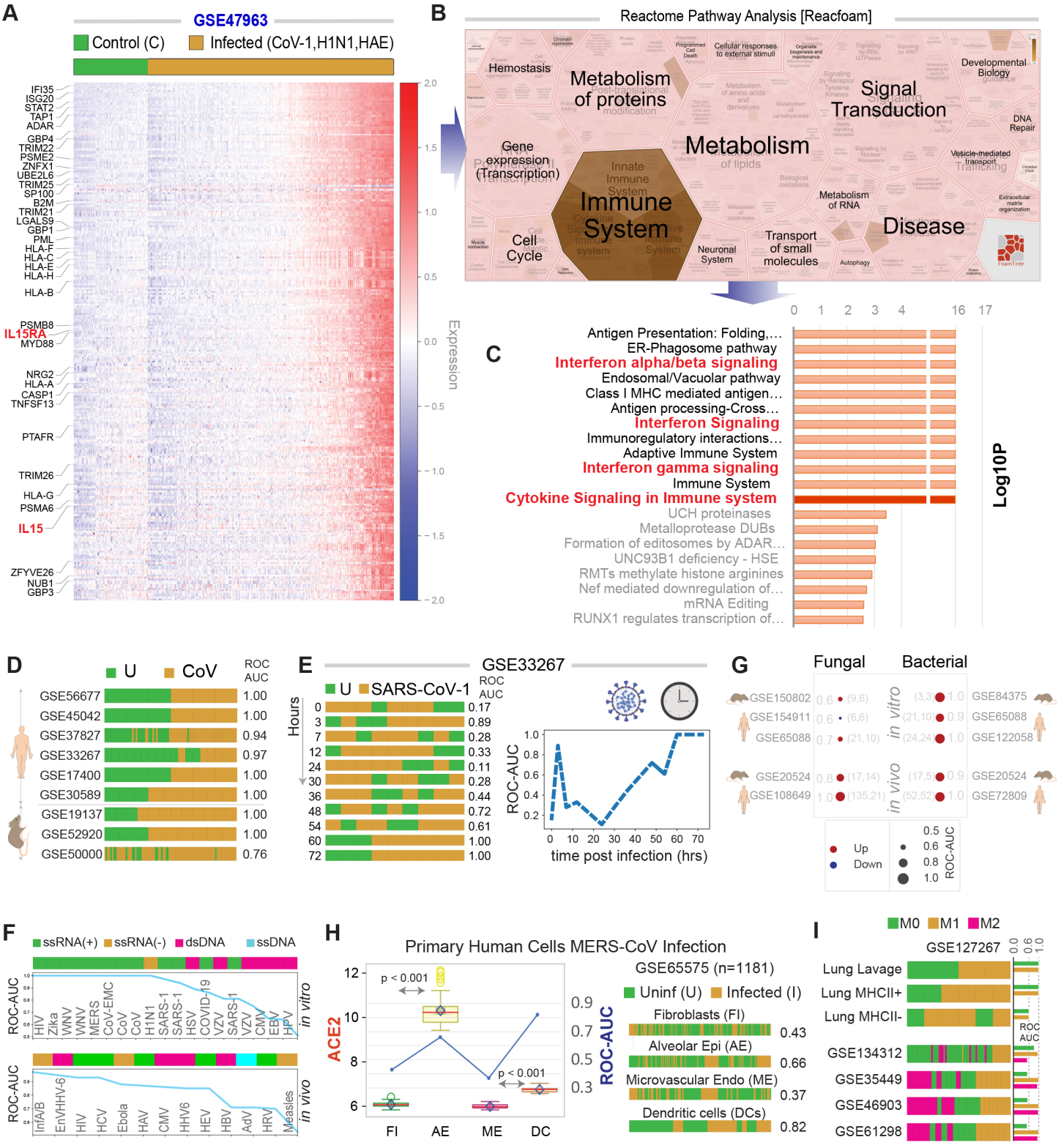

A
GSE47963
Control (C)
Infected (CoV-1,H1N1,HAE)
IFI35
ISG20
STAT2
TAP1
ADAR
GBP4
TRIM22
PSME2
ZNFX1
UBE2L6
TRIM25
SP100
B2M
TRIM21
LGALS9
GBP1
PML
HLA-F
HLA-C
HLA-E
HLA-H
HLA-B
PSMB8
IL15RA
MYD88
NRG2
HLA-A
CASP1
TNFSF13
PTAFR
TRIM26
HLA-G
PSMA6
IL15
ZFYVE26
NUB1
GBP3
Expression
B
Reactome Pathway Analysis [Reacfoam]
Hemostasis
Metabolism of proteins
Signal Transduction
Developmental Biology
DNA Repair
Gene expression (Transcription)
Metabolism
Vesicle-mediated transport
Innate Immune System
Immune System
Metabolism of RNA
Extracellular matrix organization
Cell Cycle
Disease
Neuronal System
Transport of small molecules
Autophagy
C
Antigen Presentation: Folding,…
ER-Phagosome pathway
Interferon alpha/beta signaling
Endosomal/Vacuolar pathway
Class I MHC mediated antigen…
Antigen processing-Cross…
Interferon Signaling
Immunoregulatory interactions…
Adaptive Immune System
Interferon gamma signaling
Immune System
Cytokine Signaling in Immune system
UCH proteinases
Metalloprotease DUBs
Formation of editosomes by ADAR…
UNC93B1 deficiency - HSE
RMTs methylate histone arginines
Nef mediated downregulation of…
mRNA Editing
RUNX1 regulates transcription of…
Log10P
D
U
CoV
ROC AUC
GSE56677 1.00
GSE45042 1.00
GSE37827 0.94
GSE33267 0.97
GSE17400 1.00
GSE30589 1.00
GSE19137 1.00
GSE52920 1.00
GSE50000 0.76
E
GSE33267
U
SARS-CoV-1
Hours
0 0.17
3 0.89
7 0.28
12 0.33
24 0.11
30 0.28
36 0.44
48 0.72
54 0.61
60 1.00
72 1.00
ROC-AUC
time post infection (hrs)
G
Fungal
Bacterial
GSE150802
GSE154911
GSE65088
GSE20524
GSE108649
in vitro
in vivo
GSE84375
GSE65088
GSE122058
GSE20524
GSE72809
Up
Down
ROC-AUC
F
ssRNA(+)
ssRNA(-)
dsDNA
ssDNA
ROC-AUC
HIV
Zika
WNV
WNV
MERS
CoV-EMC
CoV
CoV
H1N1
SARS-1
SARS-1
HSV
COVID-19
VZV
SARS-1
VZV
CMV
EBV
HPV
in vitro
InfA/B
EnVHHV-6
HIV
HCV
Ebola
HAV
CMV
HHV6
HEV
HBV
AdV
HRV
Measles
in vivo
H
Primary Human Cells MERS-CoV Infection
ACE2
p < 0.001
p < 0.001
FI
AE
ME
DC
GSE65575 (n=1181)
Uninf (U)
Infected (I)
ROC-AUC
Fibroblasts (FI) 0.43
Alveolar Epi (AE) 0.66
Microvascular Endo (ME) 0.37
Dendritic cells (DCs) 0.82
I
M0
M1
M2
GSE127267
Lung Lavage
Lung MHCII+
Lung MHCII-
GSE134312
GSE35449
GSE46903
GSE61298
ROC AUC

Figure 3

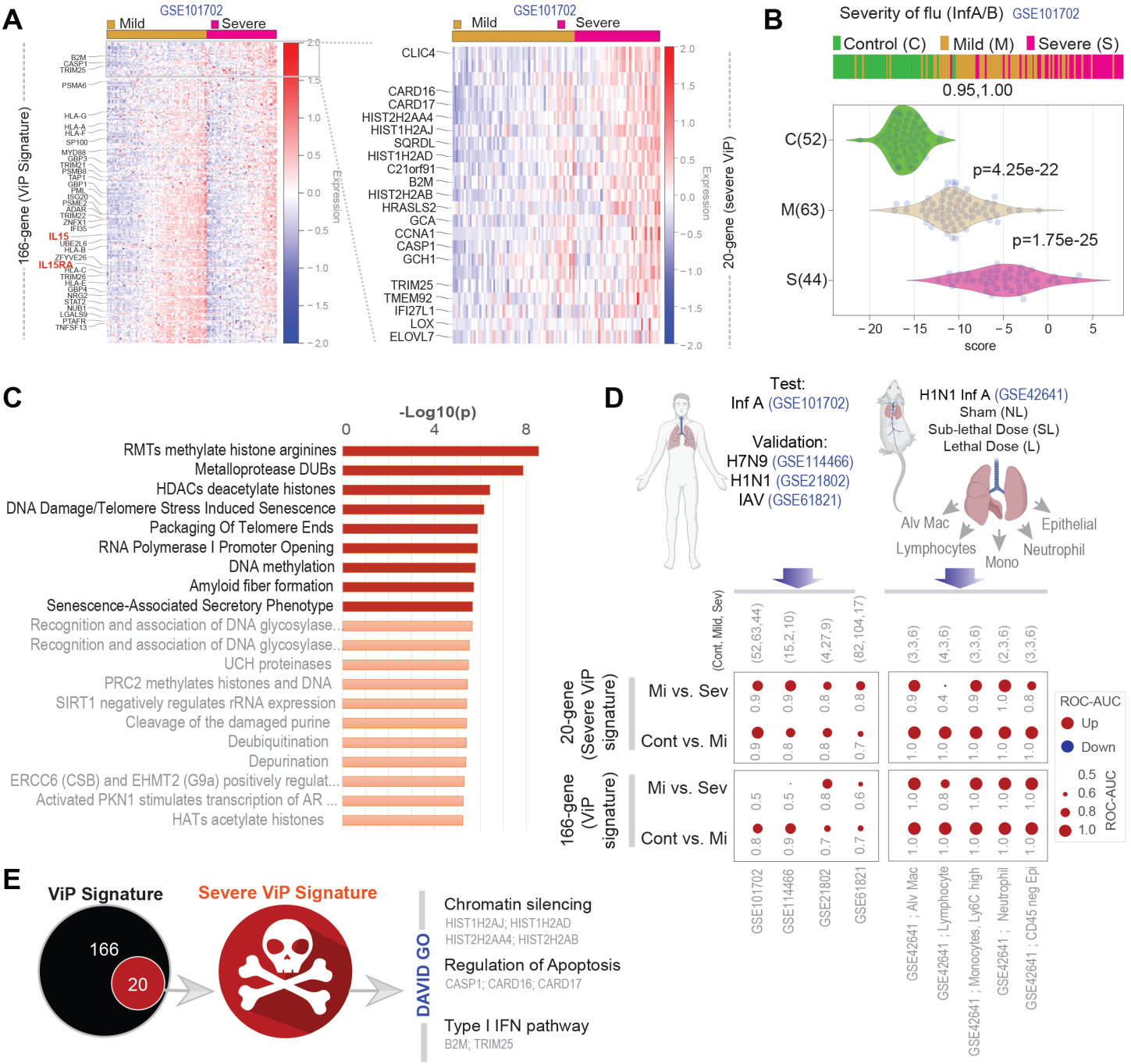

A
GSE101702
Mild
Severe
166-gene (ViP Signature)
B2M
CASP1
TRIM25
PSMA6
HLA-G
HLA-A
HLA-F
SP100
MYD88
GBP3
TRIM21
PSMB8
TAP1
GBP1
PML
ISG20
PSMC2
ADAR
TRIM22
ZNFX1
IFI35
IL15
UBE2L6
HLA-B
ZCYVE26
IL15RA
HLA-C
TRIM26
HLA-E
GBP4
NRG2
STAT2
NUB1
LGALS9
PTAFR
TNFSF13
Expression
20-gene (severe ViP)
CLIC4
CARD16
CARD17
HIST2H2AA4
HIST1H2AJ
SQRDL
HIST1H2AD
C21orf91
B2M
HIST2H2AB
HRASLS2
GCA
CCNA1
CASP1
GCH1
TRIM25
TMEM92
IFI27L1
LOX
ELOVL7
B
Severity of flu (InfA/B) GSE101702
Control (C)
Mild (M)
Severe (S)
0.95,1.00
C(52)
M(63)
S(44)
p=4.25e-22
p=1.75e-25
score
C
-Log10(p)
RMTs methylate histone arginines
Metalloprotease DUBs
HDACs deacetylate histones
DNA Damage/Telomere Stress Induced Senescence
Packaging Of Telomere Ends
RNA Polymerase I Promoter Opening
DNA methylation
Amyloid fiber formation
Senescence-Associated Secretory Phenotype
Recognition and association of DNA glycosylase...
Recognition and association of DNA glycosylase...
UCH proteinases
PRC2 methylates histones and DNA
SIRT1 negatively regulates rRNA expression
Cleavage of the damaged purine
Deubiquitination
Depurination
ERCC6 (CSB) and EHMT2 (G9a) positively regulat...
Activated PKN1 stimulates transcription of AR ...
HATs acetylate histones
D
Test:
Inf A (GSE101702)
Validation:
H7N9 (GSE114466)
H1N1 (GSE21802)
IAV (GSE61821)
H1N1 Inf A (GSE42641)
Sham (NL)
Sub-lethal Dose (SL)
Lethal Dose (L)
Alv Mac
Epithelial
Lymphocytes
Mono
Neutrophil
20-gene (Severe ViP signature)
166-gene (ViP signature)
Mi vs. Sev
Cont vs. Mi
(Cont, Mild, Sev)
(52,63,44)
(15,2,10)
(4,27,9)
(82,104,17)
(3,3,6)
(4,3,6)
(3,3,6)
(2,3,6)
(3,3,6)
ROC-AUC
Up
Down
GSE101702
GSE114466
GSE21802
GSE61821
GSE42641 ; Alv Mac
GSE42641 ; Lymphocyte
GSE42641 ; Monocytes, Ly6C high
GSE42641 ; Neutrophil
GSE42641 ; CD45 neg Epi
E
ViP Signature
Severe ViP Signature
166
20
DAVID GO
Chromatin silencing
HIST1H2AJ; HIST1H2AD
HIST2H2AA4; HIST2H2AB
Regulation of Apoptosis
CASP1; CARD16; CARD17
Type I IFN pathway
B2M; TRIM25

Figure 4

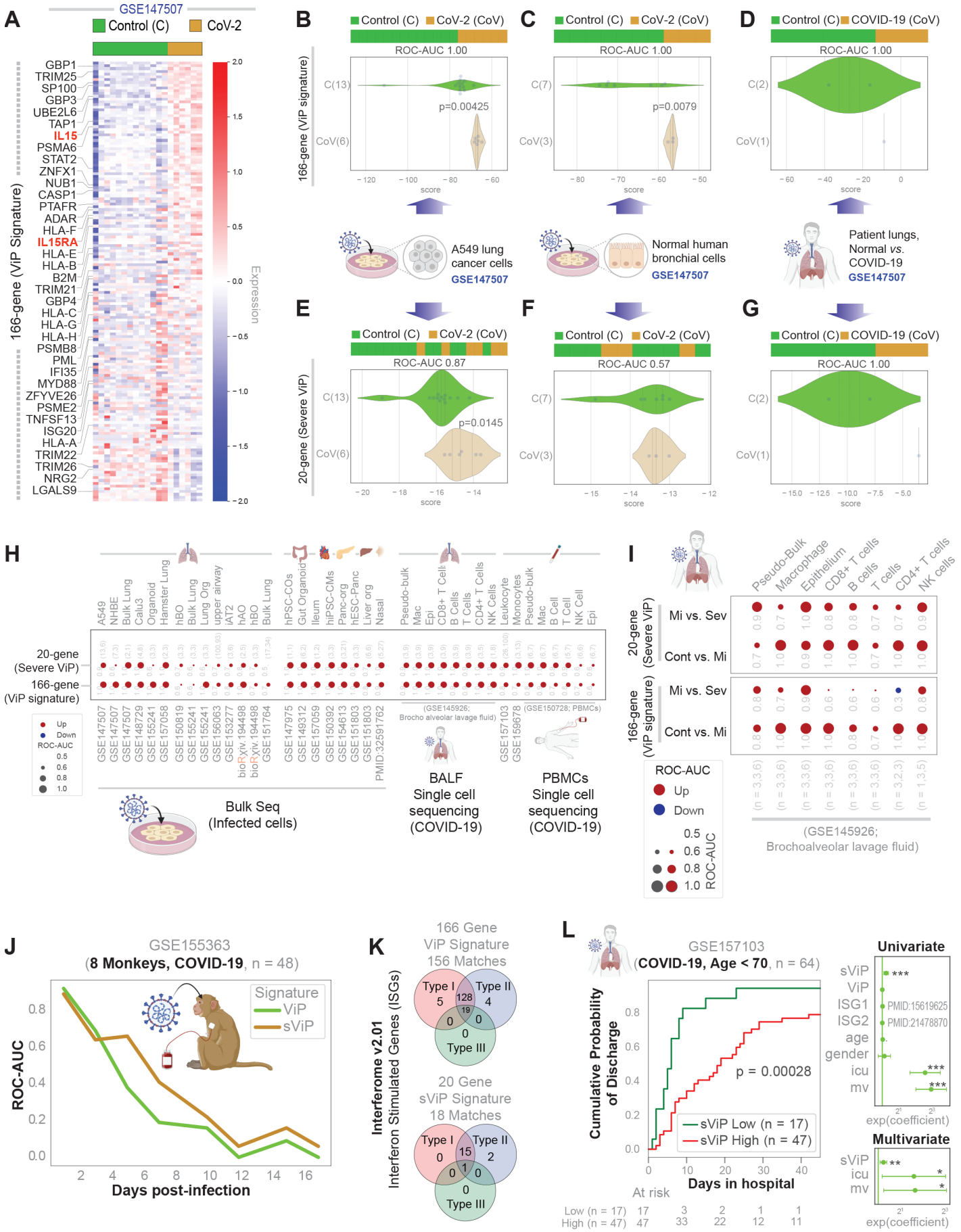

A
GSE147507
Control (C)
CoV-2
166-gene (ViP Signature)
GBP1
TRIM25
SP100
GBP3
UBE2L6
TAP1
IL15
PSMA6
STAT2
ZNFX1
NUB1
CASP1
PTAFR
ADAR
HLA-F
IL15RA
HLA-E
HLA-B
B2M
TRIM21
GBP4
HLA-C
HLA-G
HLA-H
PSMB8
PML
IFI35
MYD88
ZFYVE26
PSME2
TNFSF13
ISG20
HLA-A
TRIM22
TRIM26
NRG2
LGALS9
Expression
B
Control (C)
CoV-2 (CoV)
ROC-AUC 1.00
166-gene (ViP signature)
C(13)
CoV(6)
p=0.00425
score
A549 lung cancer cells
GSE147507
C
Control (C)
CoV-2 (CoV)
ROC-AUC 1.00
C(7)
CoV(3)
p=0.0079
score
Normal human bronchial cells
GSE147507
D
Control (C)
COVID-19 (CoV)
ROC-AUC 1.00
C(2)
CoV(1)
score
Patient lungs, Normal vs. COVID-19
GSE147507
E
Control (C)
CoV-2 (CoV)
ROC-AUC 0.87
20-gene (Severe ViP)
C(13)
CoV(6)
p=0.0145
score
F
Control (C)
CoV-2 (CoV)
ROC-AUC 0.57
C(7)
CoV(3)
score
G
Control (C)
COVID-19 (CoV)
ROC-AUC 1.00
C(2)
CoV(1)
score
H
A549
NHBE
Bulk Lung
Calu3
Organoid
Hamster Lung
hBO
Bulk Lung
Lung Org
upper airway
iAT2
hAO
hBO
Bulk Lung
hPSC-COs
Gut Organoid
Ileum
hiPSC-CMs
Panc-org
hESC-Panc
Liver org
Nasal
Pseudo-bulk
Mac
Epi
CD8+ T Cell
B Cells
T Cells
CD4+ T Cells
NK Cells
Leukocyte
Monocytes
Pseudo-bulk
Mac
B Cell
T Cell
NK Cell
Epi
20-gene (Severe ViP)
166-gene (ViP signature)
Up
Down
ROC-AUC
GSE147507
GSE147507
GSE147507
GSE148729
GSE155241
GSE157058
GSE150819
GSE155241
GSE155241
GSE156063
GSE153277
bioRxiv.194498
bioRxiv.194498
GSE151764
GSE147975
GSE149312
GSE157059
GSE150392
GSE154613
GSE151803
GSE151803
PMID:32591762
(GSE145926; Brocho alveolar lavage fluid)
GSE157103
GSE159678
(GSE150728; PBMCs)
Bulk Seq (Infected cells)
BALF Single cell sequencing (COVID-19)
PBMCs Single cell sequencing (COVID-19)
I
Pseudo-Bulk
Macrophage
Epithelium
CD8+ T cells
B cells
T cells
CD4+ T cells
NK cells
20-gene (Severe ViP)
Mi vs. Sev
Cont vs. Mi
166-gene (ViP signature)
Mi vs. Sev
Cont vs. Mi
ROC-AUC
Up
Down
(GSE145926; Brochoalveolar lavage fluid)
J
GSE155363
(8 Monkeys, COVID-19, n = 48)
Signature
ViP
sViP
ROC-AUC
Days post-infection
K
166 Gene ViP Signature 156 Matches
Type I
Type II
Type III
20 Gene sViP Signature 18 Matches
Interferome v2.01
Interferon Stimulated Genes (ISGs)
L
GSE157103
(COVID-19, Age < 70, n = 64)
Cumulative Probability of Discharge
p = 0.00028
sViP Low (n = 17)
sViP High (n = 47)
Days in hospital
At risk
Low (n = 17)
High (n = 47)
Univariate
sViP
ViP
ISG1
ISG2
age
gender
icu
mv
PMID:15619625
PMID:21478870
exp(coefficient)
Multivariate
sViP
icu
mv
exp(coefficient)

Figure 5

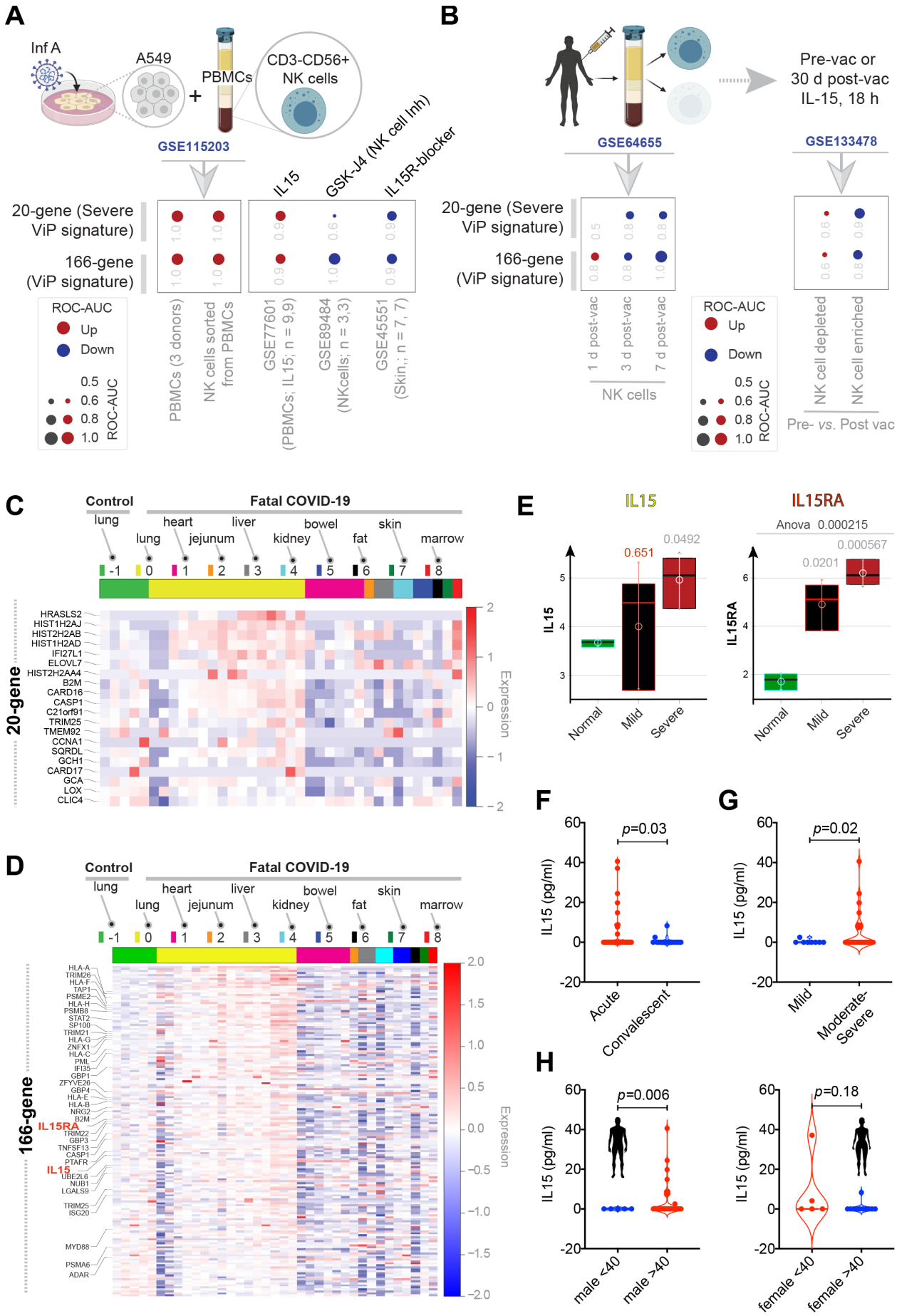
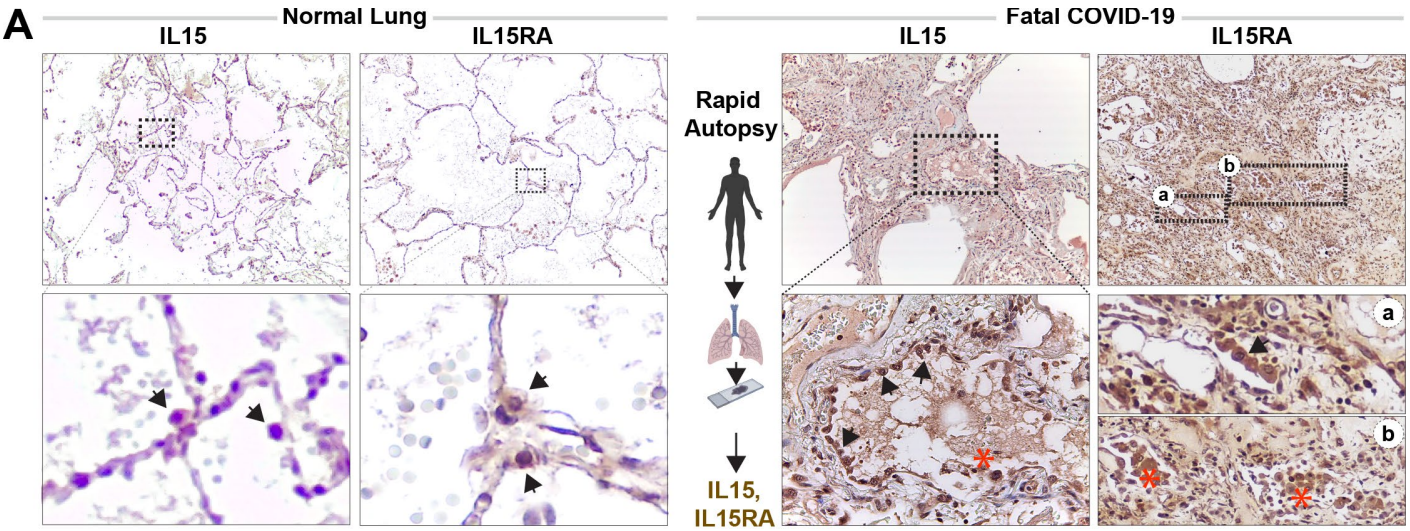
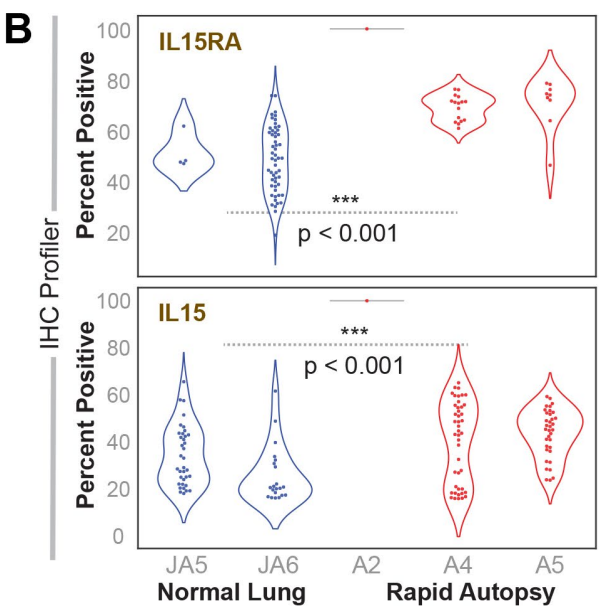
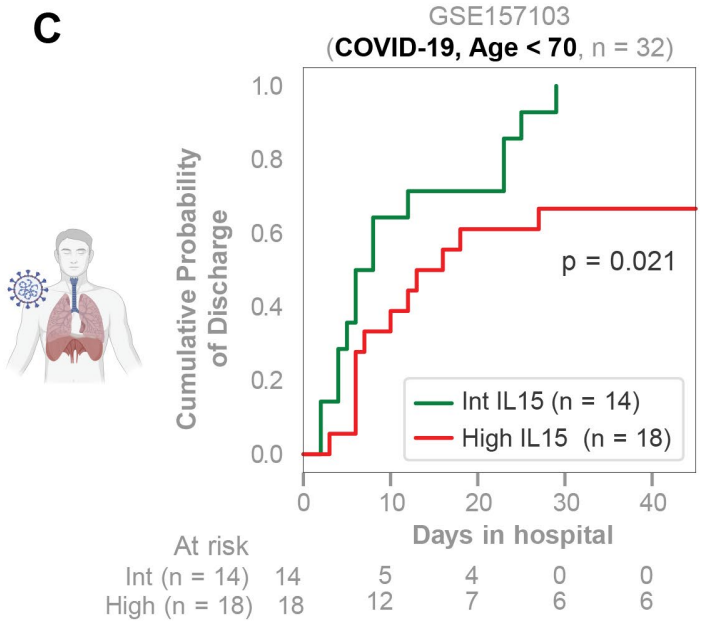
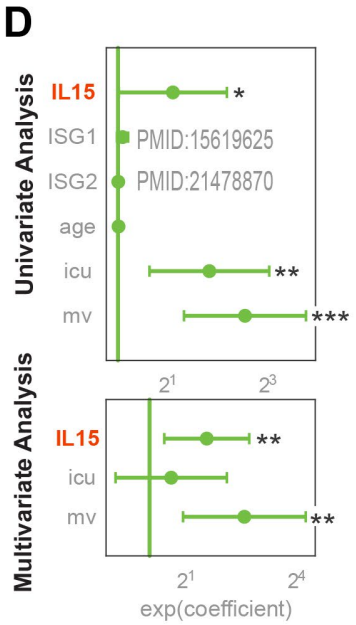

A
Inf A
A549
PBMCs
CD3-CD56+ NK cells
GSE115203
IL15
GSK-J4 (NK cell Inh)
IL15R-blocker
20-gene (Severe ViP signature)
166-gene (ViP signature)
ROC-AUC
Up
Down
0.5
0.6
0.8
1.0
ROC-AUC
PBMCs (3 donors)
NK cells sorted from PBMCs
GSE77601 (PBMCs; IL15; n = 9,9)
GSE89484 (NKcells; n = 3,3)
GSE45551 (Skin.; n = 7, 7)
B
Pre-vac or 30 d post-vac IL-15, 18 h
GSE64655
GSE133478
1 d post-vac
3 d post-vac
7 d post-vac
NK cells
NK cell depleted
NK cell enriched
Pre- vs. Post vac
C
Control
Fatal COVID-19
lung
heart
liver
bowel
skin
jejunum
kidney
fat
marrow
20-gene
HRASLS2
HIST1H2AJ
HIST2H2AB
HIST1H2AD
IFI27L1
ELOVL7
HIST2H2AA4
B2M
CARD16
CASP1
C21orf91
TRIM25
TMEM92
CCNA1
SQRDL
GCH1
CARD17
GCA
LOX
CLIC4
Expression
D
166-gene
IL15RA
IL15
Expression
E
IL15
IL15RA
Anova 0.000215
0.651
0.0492
0.0201
0.000567
Normal
Mild
Severe
F
p=0.03
IL15 (pg/ml)
Acute
Convalescent
G
p=0.02
Mild
Moderate-Severe
H
p=0.006
male <40
male >40
p=0.18
female <40
female >40

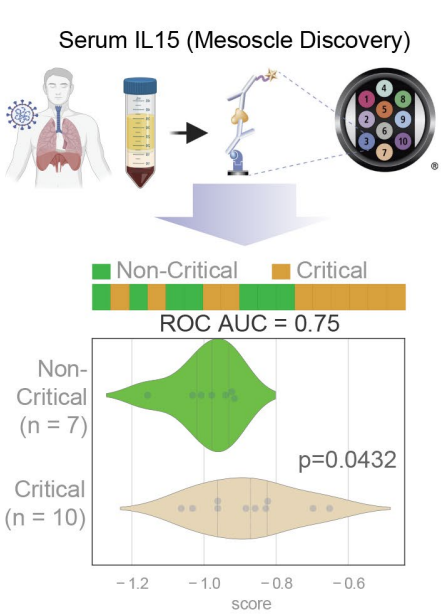
Figure 6


A
Normal Lung
IL15
IL15RA
Fatal COVID-19
IL15
IL15RA
Rapid Autopsy
IL15, IL15RA
a
b


B
IHC Profiler
IL15RA
IL15
Percent Positive
p < 0.001
***
JA5
JA6
A2
A4
A5
Normal Lung
Rapid Autopsy


C
GSE157103
(COVID-19, Age < 70, n = 32)
Cumulative Probability of Discharge
p = 0.021
Int IL15 (n = 14)
High IL15 (n = 18)
Days in hospital
At risk
Int (n = 14) 14 5 4 0 0
High (n = 18) 18 12 7 6 6


D
Univariate Analysis
IL15
ISG1
ISG2
age
icu
mv
PMID:15619625
PMID:21478870
Multivariate Analysis
IL15
icu
mv
exp(coefficient)


E
Serum IL15 (Mesoscle Discovery)
Non-Critical
Critical
ROC AUC = 0.75
Non-Critical (n = 7)
Critical (n = 10)
p=0.0432
score

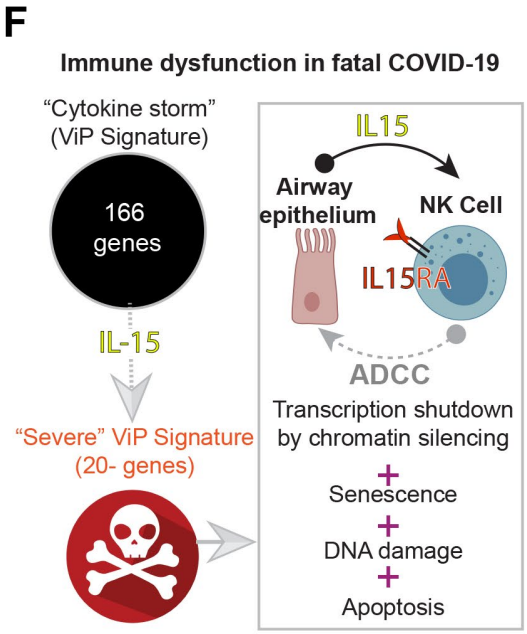

F
Immune dysfunction in fatal COVID-19
"Cytokine storm" (ViP Signature)
166 genes
IL-15
"Severe" ViP Signature (20- genes)
IL15
Airway epithelium
NK Cell
IL15RA
ADCC
Transcription shutdown by chromatin silencing
+
Senescence
+
DNA damage
+
Apoptosis

Figure 7

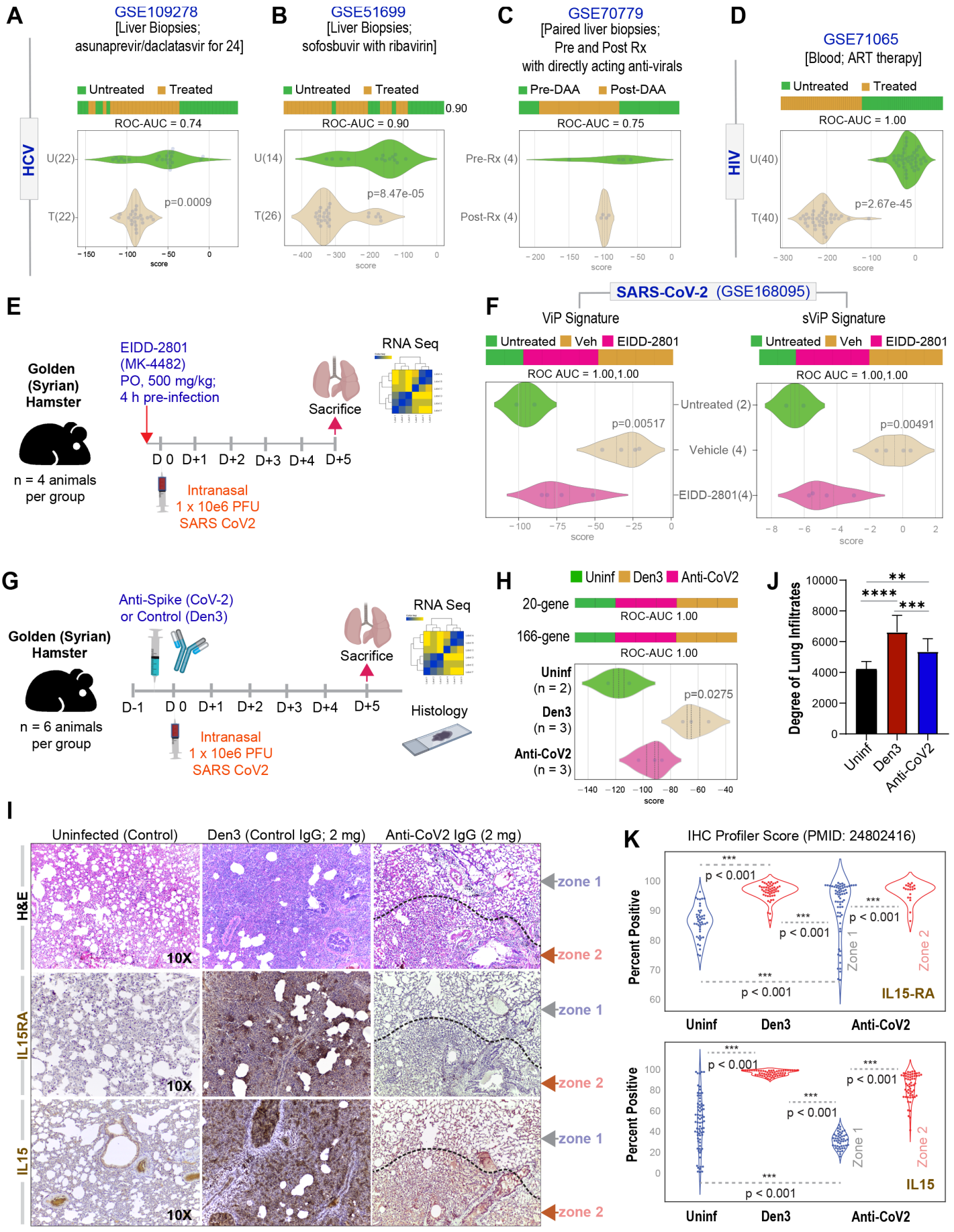

A
GSE109278
[Liver Biopsies;
asunaprevir/daclatasvir for 24]
Untreated
Treated
ROC-AUC = 0.74
HCV
U(22)
T(22)
p=0.0009
score
B
GSE51699
[Liver Biopsies;
sofosbuvir with ribavirin]
Untreated
Treated
0.90
ROC-AUC = 0.90
U(14)
T(26)
p=8.47e-05
score
C
GSE70779
[Paired liver biopsies;
Pre and Post Rx
with directly acting anti-virals
Pre-DAA
Post-DAA
ROC-AUC = 0.75
Pre-Rx (4)
Post-Rx (4)
score
D
GSE71065
[Blood; ART therapy]
Untreated
Treated
ROC-AUC = 1.00
HIV
U(40)
T(40)
p=2.67e-45
score
E
Golden
(Syrian)
Hamster
n = 4 animals
per group
EIDD-2801
(MK-4482)
PO, 500 mg/kg;
4 h pre-infection
D 0
D+1
D+2
D+3
D+4
D+5
Sacrifice
RNA Seq
Intranasal
1 x 10e6 PFU
SARS CoV2
F
SARS-CoV-2 (GSE168095)
ViP Signature
sViP Signature
Untreated
Veh
EIDD-2801
ROC AUC = 1.00,1.00
Untreated (2)
Vehicle (4)
EIDD-2801(4)
p=0.00517
p=0.00491
score
G
Golden (Syrian)
Hamster
n = 6 animals
per group
Anti-Spike (CoV-2)
or Control (Den3)
D-1
D 0
D+1
D+2
D+3
D+4
D+5
Sacrifice
RNA Seq
Histology
Intranasal
1 x 10e6 PFU
SARS CoV2
H
Uninf
Den3
Anti-CoV2
20-gene
166-gene
ROC-AUC 1.00
Uninf
(n = 2)
Den3
(n = 3)
Anti-CoV2
(n = 3)
p=0.0275
score
J
Degree of Lung Infiltrates
Uninf
Den3
Anti-CoV2
I
Uninfected (Control)
Den3 (Control IgG; 2 mg)
Anti-CoV2 IgG (2 mg)
H&E
IL15RA
IL15
10X
zone 1
zone 2
K
IHC Profiler Score (PMID: 24802416)
Percent Positive
p < 0.001
Zone 1
Zone 2
IL15-RA
IL15
Uninf
Den3
Anti-CoV2

Figure 8

Table 1

Multiple Viral Pandemics Validation

Human

GSEID	Number of Sample	Dataset
GSE47963	438	Mitchell 2013 flu
GSE113211	118	Jones 2019 flu tpm
GSE68310	880	Zhai 2015 flu
GSE21802	40	Bermejo-Martin 2010 H1N1
GSE56677	33	Selinger 2014 cov
GSE45042	32	Josset 2013 cov
GSE37827	87	Katze 2012 cov
GSE33267	99	Sims 2013 cov
GSE17400	27	Yoshikawa 2010 cov
GSE30589	33	DeDiego 2011 cov
GSE125817	163	Johnson 2020 mo-DC HIV
GSE146423	6	Liao 2020 zika
GSE138841	8	van Tol 2020 wnv
GSE136342	35	Suthar 2019 wnv
GSE40396	65	Hu 2013 infection
GSE140713	57	Shen 2020 hiv
GSE147507	69	Melo 2020 SARS-CoV-2 Hs
GSE137965	45	Scott 2019 hpv
Total	2235	

Mouse

GSEID	Number of
GSE19137	21
GSE52920	9
GSE50000	86
GSE130629	363
Total	479

Dataset
Kelvin 2014 cov Mm
Jimenez Guardeno 2014 cov Mm
Katze 2014 cov Mm
Price 2020 ebola

GSEID	Number of Sample	Dataset
GSE10006	87	Carolan 2008 hae
GSE101702	159	Tang 2019 IAV
GSE101931	38	Hubal 2017 AA T2D
GSE104423	29	Bondar 2017 HF processed
GSE104674	48	Stenvers 2019 obese circadian
GSE107480	15	Li 2020 heart
GSE108369	8	Ferretti 2018 Ibuprofen
GSE109278	44	Alao 2018 hcv
GSE113211	118	Jones 2019 flu tpm
GSE114466	42	Guan 2018 flu
GSE1145	17	Schinke 2004 heart 1
GSE116023	8	Takeda 2018 quin
GSE116436	7623	Monks 2018 NCI-60 Drug Response
GSE11784	171	Tilley 2011 hae
GSE117911	18	Hosmillo 2020 intestine
GSE119291	44	Readhead 2018 drugs 1
GSE120596	66	Theken 2019 ibuprofen
GSE123342	192	Vanhaverbeke 2019 blood chronic heart
GSE124534	81	Matualatupauw 2019 MetS
GSE128844	12	Bargiela 2019 hcq
GSE129534	16	Prince 2019 race
GSE133054	23	Ren 2020 heart hs
GSE135188	36	Quast 2019 smoking
GSE136342	35	Suthar 2019 wnv
GSE13896	70	Shaykhiev 2009 Mac COPD
GSE2125 GSE13896	115	WS Woodruff + Shaykhiev 2009 Alv Mac
GSE13933	87	Turetz 2009 hae
GSE139540	15	Deutschman 2019 vitk
GSE141910	366	Morley 2019 heart
GSE145412	32	Paczkowska-Abdulsalam 2020 MetS
GSE145994	8464	Auerbach 2020 drugs
GSE147507	69	Melo 2020 SARS-CoV-2 Hs
GSE148729	96	Wyler 2020 CoV-2 1
GSE148818	24	Pennycuick 2020 Y+O tracheal
GSE149312	22	Lamers 2020 cov
GSE15323	9	Yu 2011 virus
GSE1541	20	dos Santos 2004 lung
GSE17400	27	Yoshikawa 2010 cov
GSE17905	157	Wang 2010 hae 2
GSE18385	161	Wang 2010 hae 1
GSE19667	121	Strulovici Barel 2010 hae
GSE20250	127	Butler 2011 hae
GSE20257	135	Shaykhiev 2011 HAE
GSE2125	45	Woodruff 2005 mac
GSE21802	40	Bermejo-Martin 2010 H1N1
GSE22309	110	Wu 2007 Muscle diabetes

GSE27128	6	Eckle 2013 lung
GSE27876	19	Yoon 2011 asthma
GSE29819	38	Gaertner 2012 heart
GSE30589	33	DeDiego 2011 cov
GSE33267	99	Sims 2013 cov
GSE3442	5	Cuadras 2002 caco-2 rotavirus 1
GSE36930	54	Tome Carneiro 2013 wine
GSE37827	87	Katze 2012 cov
GSE39291	126	Deferme 2013 vitk
GSE39740	6	Medigeshi 2020 caco-2 JEV
GSE41649	8	Chamberland 2009 Asthma
GSE42955	29	Molina Navarro 2013 heart
GSE43696	108	Voraphani 2014 asthma
GSE45042	32	Josset 2013 cov
GSE46909	128	Shao 2013 drugs
GSE47460	153	Peng 2016 lung COPD I
GSE48060	52	Suresh 2014 Chronic cardiology outcome
GSE5060	54	Carolan 2006 hae 1
GSE51699	40	Meissner 2014 hcv 1
GSE51952	105	Van den Hof 2014 drugs
GSE5258	564	CMAP 2006 drug
GSE52601	24	Akat 2014 heart
GSE5406	210	Hannenhalli 2006 heart
GSE56677	33	Selinger 2014 cov
GSE57345 GSE57338	313	Liu 2015 heart
GSE58162	6	Pavuluri 2014 aspirin
GSE58837	31	Cardoso 2016 drugs
GSE59867	436	Maciejak 2015 Heart failure
GSE60408	864	Woo 2015 drugs
GSE63127	230	Tilley 2016 hae
GSE63950	9	Wickramasinghe 2015 hmo
GSE69039	18	Jung 2016 obesity
GSE69683	498	Bigler 2017 asthma
GSE70779	18	Meissner 2016 Cir
GSE71065	80	Jacobson 2016 hcq
GSE71634	40	Clarelli 2017 ifnb
GSE71994	40	Silva 2018 htn
GSE74144	36	Rouet 2018 blood htn
GSE74235	15	Smyth 2016 blood
GSE75360	21	Dluzen 2016 race
GSE76583	16	Guo 2016 aspirin
GSE76886	359	Wood 2016 mpa
GSE77239	18	Costarelli 2017 omp
GSE77343	197	Casey 2016 heart blood
GSE7832	20	Tilley 2009 hae
GSE80132	27	Rialdi 2016 Topoisomerase 1
GSE84453	128	Esser 2018 blood htn

GSE85871	212	Lv 2017 drugs
GSE89334	6	Chen 2017 zika
GSE9008	4	Whyte 2007 wine
GSE93101	33	Yu 2019 Acute heart outcome
GSE93840	54	Bell 2017 cpz
GSE95588	78	Kuo 2019 mac
GSE97287	19	Xie 2018 scls
GSE97460	688	Bush 2017 drugs
GSE98895	40	D'Amore 2018 MetS
Total	25809	

GSEID	Number of Sample	Dataset
GSE104530	9	Nelson 2018 htn mm
GSE120042	27	Omura 2018 heart Mm
GSE19137	21	Kelvin 2014 cov Mm
GSE35182	24	Coronado 2012 heart Mm
GSE42641	255	Brandes 2013 flu mm
GSE50000	86	Katze 2014 cov Mm
GSE52920	9	Jimenez Guardeno 2014 cov Mm
GSE53607	30	Omura 2014 heart Mm
GSE61555	34	Jabbari 2015 baricitinib mm
GSE84568	332	Moodley 2016 jak Mm
GSE89712	6	Li 2016 heart mm
Total	833	

GSEID	Number of Sample	Dataset
GSE119135	866	Davis 2019 drugs Rat
GSE144219	743	Podtelezhnikov 2020 drugs Rat
GSE144548	18	Peng 2020 heart rat
GSE40348	300	De Abrew 2015 drugs Rat
GSE57822	5587	Gusenleitner 2014 drugs Rat
GSE59927	12921	Auerbach 2014 drug Rat
GSE63403	40	Abdel Aziz 2016 omp rat
Total	20475	

Table 2

Table S2:

1-1: List of genes that are equivalent to ACE2 that are elevated in acute infection and reduced in convalescent SARS-CoV infection (ViP signature) [n = 166]

ABCD1	ACE2	ADAMTSL3	ADAR	ADPRHL2	APOBEC3B	APOL4	APOL6
AZI2	B2M	BCL2L14	BLZF1	BRIP1	C19orf66	C21orf91	C3orf38
C6orf62	CARD16	CARD17	CASP1	CCNA1	CD274	CHMP5	CLIC4
CNP	COL16A1	CSAG1	CSAG2	CXCL16	CYP21A2	CYP2J2	DCLRE1C
DHX58	DTX3L	ELOVL7	ETV7	FAM26F	FANCA	FAS	FBXO6
FGD6	FRMD3	GBP1	GBP3	GBP4	GCA	GCH1	GLRX
GMPR	GPT2	GTPBP1	HDX	HESX1	HIST1H2AD	HIST1H2AJ	HIST2H2AA4
HIST2H2AB	HLA-A	HLA-B	HLA-C	HLA-E	HLA-F	HLA-G	HLA-H
HRASLS2	HSH2D	HSPB9	HTR2B	IDO1	IFI16	IFI27L1	IFI35
IFNE	IGFBP4	IL15	IL15RA	IL4I1	ISG20	KCNE4	KLHDC7B
LAP3	LGALS3BP	LGALS9	LGALS9C	LMO2	LOX	LPAR6	LYSMD2
MASTL	MMAA	MOV10	MUC13	MYD88	MYH7	N4BP1	NAT8
NCOA7	NMI	NRG2	NUB1	NUPR1	OPTN	PANX1	PARP12
PARP14	PARP9	PDCD1LG2	PHF11	PLSCR1	PML	PNPT1	PPP2R2A
PSMA6	PSMB8	PSME2	PTAFR	PTPRR	RBCK1	RBMS2	RNF114
RNF19B	RTP4	S1PR2	SAMD9	SCARB2	SCO2	SECTM1	SHISA5
SLC15A3	SLC16A1	SLC25A28	SLC8A2	SP100	SPATS2L	SQRDL	STARD5
STAT2	TAP1	TDRD7	TLR2	TLR3	TMEM140	TMEM62	TMEM92
TNFSF10	TNFSF13	TOR1B	TRAFD1	TREX1	TRIM21	TRIM22	TRIM25
TRIM26	TRIM69	TYMP	UBA6	UBE2L6	UNC93B1	VAMP5	WARS
WDFY1	ZBP1	ZFYVE26	ZNF618	ZNF620	ZNFX1		

1-2: Reactome analysis ACE2 equivalent genes that are elevated in acute infection and reduced in convalescent SARS-CoV infection [n = 166]

Name	pValue	FDR
ER-Phagosome pathway	1.1102230246251565e-16	5.773159728050814e-15
Antigen Presentation: Folding, assembly and peptide loading of class I MHC	1.1102230246251565e-16	5.773159728050814e-15
Interferon alpha/beta signaling	1.1102230246251565e-16	5.773159728050814e-15
Endosomal/Vacuolar pathway	1.1102230246251565e-16	5.773159728050814e-15
Class I MHC mediated antigen processing & presentation	1.1102230246251565e-16	5.773159728050814e-15
Antigen processing-Cross presentation	1.1102230246251565e-16	5.773159728050814e-15
Interferon Signaling	1.1102230246251565e-16	5.773159728050814e-15
Immunoregulatory interactions between a Lymphoid and a non-Lymphoid cell	1.1102230246251565e-16	5.773159728050814e-15
Adaptive Immune System	1.1102230246251565e-16	5.773159728050814e-15
Immune System	1.1102230246251565e-16	5.773159728050814e-15
Interferon gamma signaling	1.1102230246251565e-16	5.773159728050814e-15
Cytokine Signaling in Immune system	1.1102230246251565e-16	5.773159728050814e-15
Formation of editosomes by ADAR proteins	0.0007332592542007577	0.03111717347600229
UNC93B1 deficiency - HSE	0.0007332592542007577	0.03111717347600229
UCH proteinases	0.0007589554506342022	0.03111717347600229
Nef mediated downregulation of MHC class I complex cell surface expression	0.0013748418863652745	0.053618833568245705
mRNA Editing	0.0017596398496316779	0.0633470345867404
mRNA Editing: A to I Conversion	0.002858899412564564	0.09434368061463061
C6 deamination of adenosine	0.002858899412564564	0.09434368061463061
Metalloprotease DUBs	0.0037503329544381625	0.10925191811698953
Antigen processing: Ubiquitination & Proteasome degradation	0.0038640552673664397	0.10925191811698953

DAP12 interactions	0.004046367337666279	0.10925191811698953
RMTs methylate histone arginines	0.004046367337666279	0.10925191811698953
Cytosolic sensors of pathogen-associated DNA	0.004271400865153652	0.11105642249399494
Diseases of Immune System	0.004638186457844418	0.11131647498826602
Diseases associated with the TLR signaling cascade	0.004638186457844418	0.11131647498826602

Table S2:

2-1: List of genes that are opposite to ACE2 that are reduced in acute infection and elevated in convalescent SARS-CoV infection [n = 26]

ABCC5	ABLIM1	AHNAK	C17orf89	CDCA7L	CEP68	EIF3L	EIF4B
EPHA4	FAM172A	FITM2	GDF11	GLS	GSK3B	KLHL36	NDUFA10
NRBP2	OIP5-AS1	PCBD2	RPL15	RPL27A	RPL3	SLC16A7	SLC35E2
VIPR1	VPS13A						

2-2: Reactome analysis for genes opposite to ACE2 that are reduced in acute infection and elevated in convalescent SARS-CoV infection [n = 26]

Name	pValue	FDR
GTP hydrolysis and joining of the 60S ribosomal subunit	3.514097013379569e-9	1.9431648823342584e-7
L13a-mediated translational silencing of Ceruloplasmin expression	3.514097013379569e-9	1.9431648823342584e-7
Eukaryotic Translation Initiation	6.072390257294558e-9	1.9431648823342584e-7
Cap-dependent Translation Initiation	6.072390257294558e-9	1.9431648823342584e-7
Formation of a pool of free 40S subunits	6.240078331831711e-8	0.00000156001958295792 78
Peptide chain elongation	0.0000013850692115457974	0.00002829669938364532
Nonsense Mediated Decay (NMD) independent of the Exon Junction Complex (EJC)	0.0000016858595985880243	0.00002829669938364532
Eukaryotic Translation Elongation	0.0000017685437114778324	0.00002829669938364532
Eukaryotic Translation Termination	0.00000213179667318819 1	0.000029845153424634674
Selenocysteine synthesis	0.0000029067716003083888	0.00003164524306820127
Viral mRNA Translation	0.0000030334945886334452	0.00003164524306820127
Response of EIF2AK4 (GCN2) to amino acid deficiency	0.0000031645243068201268	0.00003164524306820127
SRP-dependent cotranslational protein targeting to membrane	0.0000037338339450299074	0.000033604505505269167
Translation	0.0000037640053116572147	0.00003387604780491493
Nonsense-Mediated Decay (NMD)	0.00000455543210642339 6	0.00003644345685138717
Nonsense Mediated Decay (NMD) enhanced by the Exon Junction Complex (EJC)	0.00000455543210642339 6	0.00003644345685138717
Axon guidance	0.0000137764598248102 39	0.00009643521877367167
Nervous system development	0.0000211198763962448908	0.00014261250385816382
Influenza Viral RNA Transcription and Replication	0.0000237687506430273 03	0.00014261250385816382
Selenoamino acid metabolism	0.0000279001574078341 36	0.00016740094444700482
Regulation of expression of SLITs and ROBOs	0.0000293946552372137 4	0.0001712730921954453
Major pathway of rRNA processing in the nucleolus and cytosol	0.0000342546184390890 6	0.0001712730921954453
Influenza Infection	0.0000437189805841953 2	0.0002185949029209766
rRNA processing in the nucleus and cytosol	0.0000526564158199738 2	0.0002632820790998691
Signaling by ROBO receptors	0.0000955248302904054 8	0.0004641491015906496
rRNA processing	0.0001160372753976624	0.0004641491015906496
Metabolism of amino acids and derivatives	0.0017249564294193886	0.006899825717677555
Cellular responses to stress	0.002127603149398083	0.008510412597592332
Developmental Biology	0.002376902588972607	0.009507610355890428
Cellular responses to external stimuli	0.0024219649238000907	0.009687859695200363

Table S2:
3-1: 20 gene severe ViP signature [n = 20]

CLIC4	CARD16	CARD17	HIST2H2AA4	HIST1H2AJ	SQRDL	HIST1H2AD	C21orf91
B2M	HIST2H2AB	HRASLS2	GCA	CCNA1	CASP1	GCH1	TRIM25
TMEM92	IFI27L1	LOX	ELOVL7				

3-2: Reactome analysis for severe ViP signature genes [n = 20]

Name	pValue	FDR
RMTs methylate histone arginines	2.92E-09	6.66E-07
Metalloprotease DUBs	1.37E-08	1.567E-06
HDACs deacetylate histones	3.86E-07	2.934E-05
DNA Damage/Telomere Stress Induced Senescence	6.93E-07	3.947E-05
Packaging Of Telomere Ends	1.32E-06	4.987E-05
RNA Polymerase I Promoter Opening	1.32E-06	4.987E-05
DNA methylation	1.654E-06	4.987E-05
Amyloid fiber formation	1.969E-06	4.987E-05
Senescence-Associated Secretory Phenotype (SASP)	2.196E-06	4.987E-05
Recognition and association of DNA glycosylase with site containing an affected purine	2.267E-06	4.987E-05
Recognition and association of DNA glycosylase with site containing an affected pyrimidine	3.034E-06	5.638E-05
UCH proteinases	3.317E-06	5.638E-05
PRC2 methylates histones and DNA	3.642E-06	5.638E-05
SIRT1 negatively regulates rRNA expression	3.978E-06	5.638E-05
Cleavage of the damaged purine	3.978E-06	5.638E-05
Deubiquitination	4.068E-06	5.638E-05
Depurination	4.337E-06	5.638E-05
ERCC6 (CSB) and EHMT2 (G9a) positively regulate rRNA expression	5.124E-06	6.149E-05
Activated PKN1 stimulates transcription of AR (androgen receptor) regulated genes KLK2 and KLK3	5.556E-06	6.372E-05
HATs acetylate histones	5.793E-06	6.372E-05
Cleavage of the damaged pyrimidine	6.498E-06	6.498E-05
Depyrimidination	6.498E-06	6.498E-05
Ub-specific processing proteases	7.814E-06	6.986E-05
Nucleosome assembly	8.127E-06	6.986E-05
Deposition of new CENPA-containing nucleosomes at the centromere	8.127E-06	6.986E-05
Condensation of Prophase Chromosomes	8.732E-06	6.986E-05
Meiotic recombination	1.075E-05	8.596E-05
B-WICH complex positively regulates rRNA expression	1.394E-05	9.464E-05
Meiotic synapsis	1.394E-05	9.464E-05
Base-Excision Repair, AP Site Formation	1.394E-05	9.464E-05

Table S3. Virus Infection Datasets Used in this Study

Virus	Type (RNA/DNA)	Portal of Entry (CME-dependent or independent)	GSE ID	In vitro/in vivo	Cell type	ROC AUC
HIV	ss RNA (+)	CME-dependent	GSE125817	in vitro	human monocyte derived dendritic cells (moDCs)	1
Zika	ss RNA (+)	CME-dependent	GSE146423	in vitro	A549 cells (Adenocarcinomic human alveolar basal epithelial cells)	1
WNV	ssRNA (+)	CME-dependent	GSE138841	in vitro	A549 cells (Adenocarcinomic human alveolar basal epithelial cells)	1
WNV	ssRNA (+)	CME-dependent	GSE136342	in vitro	Human monocyte derived DCs moDCs	1
CoV (MERS)	ss RNA (+)	CME-dependent	GSE56677	In vitro	Calu-3 2B4 cells; Human Coronavirus EMC 2012 (HCoV-EMC)	1
CoV (HCoV-EMC)	ss RNA (+)	CME-dependent	GSE45042	In vitro	Calu-3 2B4 cells were infected with Human Coronavirus EMC 2012 (HCoV-EMC)	1
CoV	ss RNA (+)	CME-dependent	GSE17400	In vitro	Calu3 cells with SARS-CoV1 and Dhori virus (DHOV), a member of the Orthomyxoviridae family within the Thogotovirus genus	1
CoV	ss RNA (+)	CME-dependent	GSE30589	In vitro	Vero E6 cells SARS-CoV-1	1
H1N1	ss RNA (-)	CME-dependent	GSE47963	In vitro	HAE cultures with H1N1 Inf virus	1
Inf A/B	ss RNA (-)	CME-dependent	GSE68310	in vivo (human)	Peripheral blood cells	0.97
CoV (SARS-CoV-1)	ss RNA (+)	CME-dependent	GSE33267	In vitro	Calu-3 cells were infected with either icSARS CoV or the icSARS deltaORF6	0.97
EnV HHV-6	ds DNA	CME-independent	GSE40396	in vivo (human)	Peripheral blood cells	0.95
CoV (SARS-CoV-1)	ss RNA (+)	CME-dependent	GSE37827	In vitro	Calu-3 cells were infected with either icSARS CoV or the cSARS Bat SRBD	0.94
HIV	ss RNA (+)	CME-dependent	GSE140713	in vivo (human)	PBMCs	0.93
HCV	ss RNA (+)	CME-dependent	GSE40184	in vivo (human)	PBMCs	0.93
HSV	ds DNA	CME-independent	GSE46042	in vitro	Pluripotent stem cell derived neuronallineages	0.89
Ebola	ss RNA (-)	CME-dependent (and Macropinocytosis)	GSE130629	in vivo (mouse)	Liver and spleen	0.88
HAV	ss RNA (+)	CME-dependent	GSE40396	in vivo (human)	Peripheral blood cells	0.87
CMV	ds DNA	CME-independent	GSE81246	in vivo (human)	PBMCs	0.86
SARS-CoV-2	ss RNA (+)	CME-dependent	GSE147507	In vitro	Primary human lung epithelium (NHBE) and transformed lung alveolar (A549) cells were mock treated or infected with SARS-CoV-2 (USA-WA1/2020)	0.86
HHV6	ds DNA	CME-independent	GSE40396	in vivo (human)	Peripheral blood cells	0.85
HEV	ss RNA (+)	CME-dependent	GSE36539	in vivo (human)	Peripheral blood cells	0.85
VZV	ds DNA	CME-independent	GSE136586	in vitro	Primary keratinocytes	0.81
SARS-CoV1	ss RNA (+)	CME-dependent	GSE47963	In vitro	HAE cultures with SARS-CoV1	0.81
VZV	ds DNA	CME-independent	GSE54385	in vitro	human dermal fibroblasts and neuron	0.75
HBV	ds DNA	CME-dependent	GSE135501	in vivo (human)	Peripheral blood cells	0.71
AdV	ss DNA	CME-dependent	GSE40396	in vivo (human)		0.71
HRV	ss RNA (+)	CME-dependent	GSE40396	in vivo (human)		0.7
CMV	ds DNA	CME-independent	GSE99454	in vitro	MRC-5 and ARPE-19 cells	0.65
EBV	ds DNA	CME-dependent	GSE135644	in vitro	AGS and AGS-EBV cells	0.64
Measles	ss RNA (-)	CME-independent (Caveolin-dependent)	GSE5808	in vivo	PBMC	0.53
HPV	ds DNA	CME-independent	GSE137965	in vitro	Primary keratinocytes	0.51

Table 4

Table S4. Table of 20 genes that define "severity" within the 166-gene ViP signature

Gene Name	Host responses/infection	Targeted pathway	Drug/biologics-FDA approved
CLIC4	Chloride intracellular channels 4 is required for the replication of Chikungunya virus (PMID: 31483794)	-	Ribavirin PMID: 31483794
CARD16	CARD 16 increases after the influenza A virus (PMID: 29299535)	Therapeutics on NLRP3 NLRP3 inhibitor—	Canakinumab Anakinra rilonacept
CARD17	CARD 17 increases after Chikungunya virus (CHIKV) infection (PMID: 31211814) & after the influenza A virus (PMID: 29299535)	Therapeutics on NLRP3 NLRP3 inhibitor—	Canakinumab Anakinra rilonacept
HIST2H2AA4	Histone family	pan-histone deacetylase (HDAC) inhibitor SAHA	SAHA
HIST1H2AJ	Histone family	pan-histone deacetylase (HDAC) inhibitor SAHA	SAHA
SQRDL	Sulfide quinone reductase		
HIST1H2AD	Histone family	pan-histone deacetylase (HDAC) inhibitor SAHA	SAHA
C21orf91	Cold Sore Susceptibility Gene 1, influences pathogenesis of herpes virus (PMID: 27081513)	-	
B2M	b2 microglobulin mRNA levels are transiently increased after infection with Neurovirulent Sindbis Virus, suggesting that transcription of these mRNAs are coordinately regulated in neurons. (PMCID: PMC112110		
HIST2H2AB	Histone family	pan-histone deacetylase (HDAC) inhibitor SAHA	SAHA
HRASLS2	Phospholipase A And Acyltransferase 2		
GCA	Grancalcin/GCA heterodimerization of TLR9 is important for TLR9-mediated downstream signaling during fine tune processes against viral infection (PMID: 26648480)		
CCNA1	Cyclin A1		
CASP1	Caspase 1		
GCH1	GTP cyclohydrolase; Neopterin is a biomarker for viral infection It is produced as a by-product in tetrahydrobiopterin (BH4) de novo synthesis and mirrors the activity of the rate limiting enzyme in the BH4 synthesis cascade		
TRIM25	tripartite motif containing 25 (TRIM25) is an E3 ubiquitin ligase and activates RIG1. It is involved in innate immune responses regulates intracellular signaling and/or RNA virus replication. PMID: 29018447		
TMEM92	Transmembrane Protein 92		
IFI27L1	Interferon Alpha Inducible Protein 27 Like 1		
LOX	Lipoxygenase increased in lung cells during RSV infection. It also gives higher CCL3 and CCL5 (PMID: 28579398)	Maraviroc prevent the function of CCR5 receptor and it will block CCL5 binding (PMID: 22637726)	Maraviroc
ELOVL7	host fatty acid elongase 7; required for efficient virus release and virion infectivity (PMID: 25732827)		

Table 5

Table S5. Demographics of the UCSD COVID-19 cohort participants for plasma.

UCSD ID	Acute/ Convalescent	Days post symptom onset	Age	Gender	Peak Disease Severity
CoV3	Acute	37	59	M	Critical
CoV23	Acute	18	72	F	Moderate
CoV36	Acute	17	43	F	Critical
CoV37	Acute	4	25	F	Mild
CoV38	Acute	4	32	M	Mild
CoV41	Acute	7	83	M	Mild
CoV40	Acute	10	83	M	Severe to Critical (NRB in ICU but never intubated)
CoV42	Acute	6	58	M	Mild to Moderate
CoV64	Acute	13	62	M	Moderate
CoV65	Acute	28	42	M	Critical
CoV66	Acute	6	53	F	Moderate
Cov77	Acute	9	81	M	Mod-Severe
Cov78	Acute	10	42	M	Mod-Severe
CoV81	Acute	22	37	F	Critical
CoV82	Acute	8	53	M	Critical
CoV92	Acute	10	84	M	Critical
CoV93	Acute	4	41	M	Critical
CoV97	Acute	13	65	M	Critical
CoV98	Acute	5	86	M	Critical
CoV99	Acute	22	77	F	Critical
CoV14	Convalescent	24	32	M	Mild
CoV21	Convalescent	27	42	M	Mild
CoV22	Convalescent	34	51	M	Mild
CoV25	Convalescent	21	40	F	Mild
CoV26	Convalescent	30	43	F	Mild
CoV29	Convalescent	30	55	F	Mild
CoV30	Convalescent	27	40	M	Mild to Moderate
CoV31	Convalescent	33	64	M	Mild
Cov7	Convalescent	25	44	F	mild
Cov8	Convalescent	22	45	M	mild
Cov9	Convalescent	25	43	F	mild
Cov12	Convalescent	25	39	F	mild
Cov18	Convalescent	23	48	F	mild
Cov62	Convalescent	50	55	M	mild
Cov63	Convalescent	39	68	M	mild to moderate
Cov64	Convalescent	56	44	F	mild
Cov201	Acute	12	73	M	moderate
Cov204	Acute	13	54	M	moderate
Cov205	Acute	9	37	M	moderate
Cov206	Acute	10	77	F	moderate
Cov207	Acute	8	49	M	moderate
Cov210	Acute	5	48	M	moderate
Cov211	Acute	8	39	F	moderate
Cov202	Acute	10	37	M	severe
Cov106	Convalescent	74	59	M	Moderate
Cov103	Convalescent	79	50	M	mild to moderate
Cov208A	Acute	7	84	M	Fatal
Cov213A	Acute	NA	37	M	Asymptomatic
Cov215	Acute	9	68	M	Severe
Cov218	Acute	5	53	M	Severe
CoV220	Acute	N/A	55	M	Asymptomatic
CoV221	Acute	5	68	M	moderate to severe
CoV222	Acute	5	68	M	moderate
CoV223	Acute	7	57	M	moderate to severe

Table S6. Demographics of the UCSD COVID-19 cohort participants for lung tissue.

Sample Name	Age	Gender	Days spent hospitalized COVID + until expired	Smoking history	Drugs administered during hospitalization	Subsequent infection?	Reason for surgery (NON SARS-CoV-2)	Histology (NON SARS-CoV-2)
Autopsy 2	64	Male	35 days	Smoked 1.5-2 Packs per day (PPD) until 1988 when they quit. Smoked cannabis daily	Lasix (furosemide), Amiodarone HCL, stress dose steroids, vancomycin/zosyn, IV ceftriaxone, norepinephrine and vasopressin, digoxin, vecuronium	MSSA from being intubated		
Autopsy 3	NA	NA	NA	NA	NA	NA		
Autopsy 4	90	Female	3	NA	vancomycin/zosyn, low dose of norepinephrine	none		
Autopsy 5	57	Male	28	NA	Cefepime, Hydroxychloroquine, vancomycin, Norepinephrine, vasopressin, esmolol, Unasyn, bivalirudin, pressors (levo, vaso, phenylephrine), linezolid ,Tocilizumab v. Placebo (information was not given what group he belonged to)	E.feacalis and klebsillea PNA from tracheal aspirate		
JA 5 (NON SARS-CoV-2)	46	Female	NA	Non-smoker	NA	NA	Left Lower Lobe Nodule	Invasive Adenocarcinoma
JA 6 (NON SARS-CoV-2)	70	Male	NA	Non-smoker	NA	NA	Left Lower Lobe Nodule	Granulomatous-like inflammation and scar