JAK inhibitors dampen activation of interferon-activated transcriptomes and the SARS-CoV-2 receptor ACE2 in human renal proximal tubules

Highlights
We provide transcriptomic and epigenetic data sets for human renal proximal tubules

Cytokine stimulation induces distinct genetic pathways in the kidney

Short isoform of ACE2, dACE2, is expressed in renal proximal tubules

Type I interferons increase dACE2, but not full ACE2 expression
JAK inhibitors dampen activation of interferon-activated transcriptomes and the SARS-CoV-2 receptor ACE2 in human renal proximal tubules

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SUMMARY
SARS-CoV-2 infections initiate cytokine storms and activate genetic programs leading to progressive hyperinflammation in multiple organs of patients with COVID-19. While it is known that COVID-19 impacts kidney function, leading to increased mortality, cytokine response of renal epithelium has not been studied in detail. Here, we report on the genetic programs activated in human primary proximal tubule (HPPT) cells by interferons and their suppression by ruxolitinib, a Janus kinase (JAK) inhibitor used in COVID-19 treatment. Integration of our data with those from patients with acute kidney injury and COVID-19, as well as other tissues, permitted the identification of kidney-specific interferon responses. Additionally, we investigated the regulation of the recently discovered isoform (dACE2) of the angiotensin-converting enzyme 2 (ACE2), the SARS-CoV-2 receptor. Using ChIP-seq, we identified candidate interferon-activated enhancers controlling the ACE2 locus, including the intronic dACE2 promoter. Taken together, our study provides an in-depth understanding of genetic programs activated in kidney cells.

INTRODUCTION
A form of acute respiratory distress syndrome (ARDS) caused by SARS-CoV-2 is a major contributor to the death toll of COVID-19 (Gibson et al., 2020). ARDS is closely linked to cytokine storm, an unrestrained release of proinflammatory cytokines and chemokines (Kim et al., 2021). This, in turn, may result in multi-organ failure (Mokhtari et al., 2020) and coagulopathies (Vinayagam and Sattu, 2020), affecting, amongst others, the kidney (Ahmadian et al., 2021). Acute kidney injury (AKI), potentially resulting from cytokine storm (Chong and Saha, 2021), is a known complication of COVID-19, and it has also been proposed that decline in renal function in hospitalized patients is caused by the virus itself (Lynch and Tang, 2020). Even before the SARS-CoV-2 pandemic, AKI was a significant medical and socioeconomic burden, with estimated one in three intensive care patients suffering from decline in kidney function (Hoste et al., 2018).

In addition to other mechanisms, SARS-CoV-2 was shown to able to infect kidney epithelium, directly contributing to organ damage (Braun et al., 2020; Peng et al., 2020; Su et al., 2020; Sun et al., 2020). It is known that its infectivity depends on the receptor, angiotensin-converting enzyme 2 (ACE2) (Hoffmann et al., 2020). Physiologically, ACE2 serves as an element of renin-angiotensin-aldosterone system and bradykinin system (Donoghue et al., 2000; Tipnis et al., 2000). In SARS-CoV-2 infection, the viral spike protein binds ACE2 and facilitates viral entry into cells. ACE2 expression has been detected in the kidney (Sungnak et al., 2020) and proximal tubules via single-cell transcriptome analysis (Chen et al., 2020; He et al., 2020). However, transcriptional regulation of ACE2 and its expression pattern in the kidney are poorly understood. Human studies indicate that changes in ACE2 expression are linked to type 2 diabetic nephropathy (Mizunri et al., 2008), IgA nephropathy (Mizunri et al., 2011), hypertension (Koka et al., 2008), and nephrosclerosis (Wang et al., 2010). Usually, decrease in ACE2 is associated with disease, which may dysregulate ACE/ACE2 ratio; however, both ACE and ACE2 may be regulated by independent pathways (Mizunri and Ohashi, 2015).

Recently, a new isoform of ACE2, deltaACE2 (dACE2), was identified in several cell types (Blume et al., 2020; Fignani et al., 2020; Lee et al., 2021; Ng et al., 2020; Onabajo et al., 2020). Contrary to earlier reports (Ziegler et al., 2020), where ACE2 was suggested to be an interferon-stimulated gene (ISG), its new isoform dACE2

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expression appears to be significantly regulated by cytokine or viral stimulation. In fact, in some cells, such as pancreatic β-cells, dACE2 may be the prevalent isoform even at the baseline (Fignani et al., 2020). Usually, decrease in ACE2 expression is linked with disease progression; however, it is unknown whether dACE2 has a specific effect, as methods used to this date assessed ACE2 without discerning between isoforms. Additionally, increased ACE2 levels were found in several animal models of kidney disease, and contribution of dACE2 to these changes remains to be assessed (Moon et al., 2008).

Here, for the first time, we show global transcriptional regulation in cytokine-stimulated human primary proximal tubule (HPPT) cells. We assess overlaps between responses to IFNα, IFNβ, and IFNγ, and we compare IFNβ-stimulated genetic programs to available AKI and COVID-19 data sets to investigate shared pathways in renal response to injury. We show interferon-inducible genetic pathways unique for the kidney and shared with other human tissues. We assess interferon stimulated gene downregulation by JAK inhibitor ruxolitinib and, finally, describe in detail the regulatory landscape of the ACE2 locus in renal proximal tubule cells.

These findings provide in-depth understanding of interferon-mediated immune responses in the kidney, especially in the context of ACE2 activation observed in SARS-CoV-2 infection, and may serve as a basis for better understanding of the commonalities and differences between cytokine stimulation of various tissues.

Figure 1. Comparison of signaling pathways induced by IFNα, IFNβ, and IFNγ stimulation of HPPT cells
(A) Venn diagram of unique and common genes induced by each cytokine. (B–D) GSEA results of p values and FDR in top 10 significantly represented pathways in gene groups shared by all three conditions and unique for IFNβ and IFNγ. See also Tables S1–S3.
RESULTS

To investigate renal cytokine-induced genetic programs, we conducted unbiased RNA-seq analyses on HPPT cells treated for 12 hr with IFNα, IFNβ, IFNγ, or IL-1β. A total of 746 genes were significantly induced by IFNα, 1169 by IFNβ, 1280 by IFNγ, and 2190 by IL-1β (Tables S1 and S2). Next, we investigated the degree of interferon response overlap. IFNα induced expression of 58 unique genes, while IFNβ and IFNγ induced 482 and 710 genes, respectively (Figure 1A). The overlap between all three interferons (320 genes) was enriched for interferon response genes (Figure 1B and Table S3), while gene sets unique for IFNβ and IFNγ were more diverse (Figures 1C and 1D). Next, we focused on IFNβ. It statistically significantly altered the most diverse signaling pathways as identified by Gene Set Enrichment Analysis (GSEA) (Table S3); it is also a known antiviral used against COVID-19. Additionally, several public transcriptomic data sets from cells treated with IFNβ are available to help elucidate interferon-regulated genetic programs specific for renal epithelium.

First, we compared whether expression patterns in patients with AKI (Park et al., 2020) and patients with severe COVID-19 (Desai et al., 2020) bear resemblance to those stimulated in HPPT cells by IFNβ. To our knowledge, only one RNA-seq data set with human ischemia-reperfusion AKI data is publicly available (Park et al., 2020). Similarly, only one renal data set from patients with COVID-19 could be found for our comparison, differentiating between severe and nonsevere disease (Desai et al., 2020). IFNβ stimulation of HPPT cells resulted in upregulation of 981 unique genes compared with other conditions (Figures 2A and 2B). AKI resulted in increased expression of 2566 genes including 156 shared with IFNβ-stimulated HPPT cells (Figures 2A and 2C; Table S3). Expression of 35 genes was induced in both severe COVID-19 and IFNβ-treated HPPT cells (Figures 2A and 2D; Table S3). Although more genes were shared between IFNβ-HTHP and AKI than between IFNβ-HPPT and COVID-19, genes involved in the interferon signaling pathways were preferentially activated in IFNβ-HPPT cells and COVID-19 compared to IFNβ-HPPT cells and AKI (Figures 2C and 2D). We also visualized the genes identified as involved in interferon signaling pathway and overlapping between conditions to see whether similar fold increases in expression can be observed (Figures 2E and 2F). The degree of gene induction varied between data sets, possibly reflecting differences in sample type and technical preparation.

Next, to elucidate cell-specific and common genes induced by IFNβ in primary cells and cell lines, we compared our HPPT data (12-hr in vitro IFNβ treatment) with similarly treated human primary lung epithelium (Lee et al., 2021) and the PH5CH8 hepatocyte cell line (Forero et al., 2019) (Figure 3). A total of 685 genes were uniquely activated in HPPT cells, 203 in the lung cells, and 198 in the liver, showing tissue-specific gene induction by cytokine stimulation (Figures 3A and 3B). IFNβ-stimulated renal cells shared 444 genes with the lung and 195 with the liver, and 154 genes were common for all three tissues. They are enriched in various immune hallmarks in GSEA analysis (Figures 3C and 3D), and average fold read increase of genes most upregulated in HPPT cells did not correlate with other tissues (Figures 3E and 3F).

To gauge the extent of IFNβ response through the JAK/STAT pathway, we compared gene expression of cells stimulated by IFNβ to cells cultured additionally with the JAK inhibitor ruxolitinib (Figure 4, Table S3). Immune response genes were significantly enriched in the gene set upregulated by IFNβ, and their expression was dampened by ruxolitinib in RNA-seq analysis. We identified interleukins (IL4R, IL15), toll-like receptors (TLR2, TLR4), interferon regulatory factors (IRF1, IRF7, IRF9), interferon-induced proteins (IFIT1, IFIT2, IFIT3, IFI44), and chemokine receptors (CXCL10, CXCL11) as significantly upregulated by type I interferons (Figure 4, Table S2). Extended GSEA analyses for all data presented here can be found in Table S3.

Recent research (Blume et al., 2020; Lee et al., 2021; Ng et al., 2020; Onabajo et al., 2020) revealed the presence of an alternative promoter expressing deltaACE2 (dACE2), a short isoform of ACE2, within intron 9 of the ACE2 gene. Although some studies found the presence of dACE2 RNA in healthy kidney tissue and tumors (Ng et al., 2020; Onabajo et al., 2020), its structure, function, and the presence of regulatory

Figure 2. Comparison of signaling pathways induced by IFNβ stimulation of HPPT cells, and human kidneys during AKI and COVID-19 infection
(A) Venn diagram of unique and common genes induced by IFNβ, AKI, and COVID-19. (B–D) GSEA results of p values and FDR in top 10 significantly represented pathways in gene groups unique for HPPT and shared between HPPT and COVID-19 or AKI. (E and F) Comparison of fold increase in expression of shared genes involved in IFN signaling in HPPT vs AKI and COVID-19 (in relation to untreated, healthy, and mild COVID-19 samples, respectively). See also Tables S1–S3.
Kidney – unique genes

CXCL10
ZBP1
IDO1
OASL
IFIT1
IFIT2
CMKP2
ISG15
IFIT3
XAF1
SEC7M1
OAS3
BATF2
ISG20
IFIT3

Kidney/Lung – shared genes

UV RESPONSE UP
IL6 JAK STAT3 SIGNALING
TNFA SIGNALING VIA NFKB
APOPTOSIS
INFLAMMATORY RESPONSE
ALLOGRAFT REJECTION
COMPLEMENT
INTERFERON GAMMA RESPONSE
INTERFERON ALPHA RESPONSE

Kidney/Liver – shared genes

APOPTOSIS
UV RESPONSE UP
TNFA SIGNALING VIA NFKB
Kras SIGNALING UP
IL6 JAK STAT3 SIGNALING
COMPLEMENT
INFLAMMATORY RESPONSE
ALLOGRAFT REJECTION
INTERFERON ALPHA RESPONSE
INTERFERON GAMMA RESPONSE

Fold changes for specific genes compared to control conditions.
elements, as well as cytokine inducibility in kidney cells, have not been investigated. First, we assayed the levels of ACE2 and dACE2 mRNA after cytokine treatment using quantitative real-time polymerase chain reaction (qRT-PCR) (Figure 5 and S1). We analyzed the expression of total ACE2, serine protease TMPRSS2 (which primes viral S protein), and the transcription factor STAT1 to investigate JAK/STAT pathway activation (Figures 5A–5C and S1). While ACE2 mRNA was increased 6- and 13-fold by IFNα and IFNβ, respectively, expression of the serine protease TMPRSS2 was not affected by them, but rather was elevated by IL-1β, indicating its regulation by an independent pathway. Expression of STAT1 was strongly upregulated after interferon treatment. To examine the expression changes of full-length ACE2 (flACE2) and dACE2 by IFNs, we performed qRT-PCR with isoform-specific primers (Figures 5D and S5). While flACE2 was elevated 3-fold, a 300-, 590-, and 27-fold upregulation of dACE2 was detected upon IFNα, IFNβ, and IFNγ treatment, respectively. Similar increase in gene expression was observed in the studies cited earlier (Table S4).

To understand the regulation of the ACE2 locus by IFNβ and to identify putative genetic control elements of dACE2 in HPPT cells, we conducted ChIP-seq (Figures 6A–6E) for H3K27ac (active chromatin), H3K4me1 (enhancers), H3K4me3 (promoter marks), and RNA polymerase II loading (Pol II), as well as used available DNase hypersensitive sites (DHS) data set (Thurman et al., 2012).

Candidate regulatory elements were identified at upstream and intronic regions of the ACE2 locus (Figures 6A–6C). H3K27ac marks and Pol II loading were enriched in the alternative exon 1c in intron 9, the first coding exon of dACE2. An increase in RNA-seq reads was detected after treatment, supporting the potential for the presence of a regulatory element (Figures 6C, 6F, and S2). In contrast, full-length ACE2 promoter marks, which seem to be more pronounced in the kidney than in the lung (Lee et al., 2021), were reduced by IFNβ stimulation. The STAT1 locus served as a control for the ChIP-seq and after interferon treatment increased H3K4me3 promoter marks and polymerase II binding can be seen, reflecting gene activation (Figure 6D). To confirm the presence of dACE2, we amplified and sequenced the novel dACE2 transcript and confirmed that exon 1c is spliced to exon 10 of ACE2 (Ng et al., 2020; Onabajo et al., 2020) (Figure S2A). Two TATA-box-like sequences were identified to exon 10 of ACE2 (Ng et al., 2020; Onabajo et al., 2020) (Figure S2A). Two TATA-box-like sequences were identified to exon 10 of ACE2 (Ng et al., 2020; Onabajo et al., 2020) (Figure S2A). Two TATA-box-like sequences were identified to exon 10 of ACE2 (Ng et al., 2020; Onabajo et al., 2020) (Figure S2A).

To investigate whether flACE2 and dACE2 are regulated through the JAK/STAT pathway by interferon signaling, we used the JAK inhibitor ruxolitinib (Figures 5F, 5G, and S1). dACE2 and STAT1 levels elevated by IFNβ were ablated by ruxolitinib treatment, while no significant changes to full-length ACE2 expression were observed.

DISCUSSION

Our study presents a broad overview of genetic programs stimulated by interferons in renal proximal tubules and compares them with other transcriptomic data. We show robust cytokine response with 1169 genes significantly induced by IFNβ and efficient quenching of gene expression by JAK inhibitor ruxolitinib. Some of those genes are known regulators of renal injury, belonging to divergent pathways, either driving inflammation similar to IRF1 or TLR4 (Wang et al., 2009; Wu et al., 2007) or attenuating it similar to IL4 and IL15 signaling.
There is evidence that type I interferons may contribute to renal damage after ischemic AKI, suggesting that common pathways between IFNβ-treated HPPT cells and AKI could be found (Freitas et al., 2011). Conversely, we expected only a small overlap with SARS-CoV-2-induced genes, as IFNβ has antiviral activity. While we indeed found that interferon stimulation and AKI shared more upregulated genes, interferon-treated cells and COVID-19 samples shared more genes identified as interferon signaling related.

Figure 4. IFNβ-induced immune response genes and their downregulation by ruxolitinib

(A–H) Relative mRNA expression levels of multiple immune genes: (A) STAT gene family, (B) Toll-like receptors, (C) interleukins, (D and E) interferon-induced genes, (F) interferon-regulated factors, (G) CXCL chemokine family, and (H) Janus kinase 2 measured by RNA-seq. Individual data points as well as mean ± SEM of independent biological replicates (n = 3–6) are shown. Significance was analyzed with one-way ANOVA followed by Tukey’s multiple comparisons test. *p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001. See also Tables S1–S3.

(Eini et al., 2010; Zhang et al., 2017). There is evidence that type I interferons may contribute to renal damage after ischemic AKI, suggesting that common pathways between IFNβ-treated HPPT cells and AKI could be found (Freitas et al., 2011). Conversely, we expected only a small overlap with SARS-CoV-2-induced genes, as IFNβ has antiviral activity. While we indeed found that interferon stimulation and AKI shared more upregulated genes, interferon-treated cells and COVID-19 samples shared more genes identified as interferon signaling related.
by GSEA analysis. This may not reflect overall trend owing to diversity of AKI and only suggest the degree of similarity with ischemia-reperfusion. We also show uniqueness of renal response when compared with the lung and liver, with over half of the genes upregulated in the kidney being tissue specific. We did not observe significant regulation of \(\text{TMPRSS2} \), protease involved in SARS-CoV-2 infection, by interferons. Instead, we saw upregulation of \(\text{TMPRSS2} \) expression after IL-1\(\beta\) treatment. Both IL-1\(\beta\) and \(\text{TMPRSS2} \) were reported to be

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Figure 5. Differences in induction of total ACE2, full-length ACE2, and dACE2 after cytokine treatment
(A) ACE2, TMPRSS2, and STAT1 mRNA levels from control and experimental cells were measured by qRT-PCR and normalized to GAPDH levels. Relative mRNA levels of (D) full-length ACE2 (flACE2), (E) dACE2, after cytokine treatment. (F) flACE2, (G) dACE2 in cells treated with JAK-inhibitor ruxolitinib or vehicle, alone or together with IFN\(\beta\). Individual data points as well as mean $\pm$ SEM of independent biological replicates ($n=3$) are shown. One- or two-way ANOVA followed by Tukey’s multiple comparisons test were used to evaluate the statistical significance of differences relative to untreated cells. ****p < 0.0001. See also Figures S1 and S2.
downregulated in nasal basal epithelium after azithromycin treatment (Renteria et al., 2020), reinforcing potential for the link between them. Our study of ACE2 locus revealed coregulation of ACE2 and TMEM27, which may have additional significance for the kidney, as TMEM27 gene encodes collectrin, ACE2 homolog, primarily expressed in renal proximal tubule, and collecting duct (Mount, 2007). Collectrin, similarly to ACE2, regulates blood pressure (Chu and Le, 2014) and amino acid transport (Malakauskas et al., 2007). Overall, those results may serve as an important stepping-stone toward further elucidation of kidney-specific genetic programs.

Although our study demonstrates that dACE2 expression is activated by interferon treatment, further work is needed to identify whether viral infection enhances dACE2 expression in the kidney. Although several studies have identified dACE2 after SARS-CoV-2 infection in vitro, its biological role remains...
unknown. Based on lung epithelium cell data, it is proposed that its extracellular enzymatic and viral spike protein-binding domains are truncated, resulting in partial loss of its carboxypeptidase function. The dACE2 promoter may be a remnant of a retroviral ISG (Ng et al., 2020). Blume (Blume et al., 2020) reports lack of increase of ACE2 or dACE2 after SARS-CoV-2 stimulated BCi-NS1.1 lung cells. Onabajo (Onabajo et al., 2020) similarly shows lack of their upregulation in lung Calu3 cell line, but colon cancer Caco-2 and T84 lines exhibited slightly increased dACE2 expression after SARS-CoV-2 exposure. This may in part be due to tissue-specific cytokine regulation of ACE2 and dACE2. A standardized and validated detection method of both ACE2 isoforms, as well as understanding of regulatory elements present in ACE2 locus, is necessary to forward this topic. This is especially true for studies at the protein level, as detection methods such as Western blot are contradictory between reports (Blume et al., 2020; Ng et al., 2020). In our attempts to investigate protein levels of dACE2 using Western blot, we were able to observe a 50-kDa band; however, its presence and intensity were not consistent between various anti-ACE2 antibodies (data not shown). We summarized current knowledge on factors causing dACE2 upregulation in Table S4.

In our study, we present an in-depth analysis and comparison of interferon-stimulated human proximal tubule cells and other experimental data sets, providing insight into genetic pathways driving response to stimuli affecting renal health. In addition, by comparing our data sets with other similarly treated cells, we show unique renal regulation of interferon response. We also identified several putative regulatory elements controlling ACE2, as well as confirmed the presence of dACE2 in renal epithelium. We describe reliance of dACE2 expression on the JAK/STAT pathway, which may be of clinical importance, as JAK inhibitors are currently used to treat COVID-19 (Cao et al., 2020). Our study strengthens current knowledge about cytokine signaling in renal epithelium, and we believe that it can become a basis for further transcriptomic studies reaching beyond the current efforts to thwart and understand the COVID-19 pandemic.

**Limitations of the study**

There are several limitations to our study. First, the availability of only a single public RNA-seq data set from patients with AKI is a clear limitation, as AKI is an extremely diverse condition. Additionally, there are no large-scale renal COVID-19 transcriptomics data. Second, reliance on a single batch of primary cells may introduce bias. Third, in an in vivo setting, JAK inhibitors have a broader effect than inhibition of genes induced by IFNβ and may affect other cytokines and genetic programs. Finally, the belief in direct SARS-CoV-2 infection of the kidney is not universal despite published evidence.

**STAR METHODS**

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**SUPPLEMENTAL INFORMATION**

Supplemental information can be found online at https://doi.org/10.1016/j.isci.2021.102928.
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AUTHOR CONTRIBUTIONS

Conceptualization and methodology: J.J., H.K.L., J.W., and L.H.; Formal analysis and validation, data curation, and visualization: J.J. and H.K.L.; Investigation: J.J.; Resources: L.H.; Writing – original draft: J.J. and H.K.L.; Writing – review and editing: J.J., H.K.L., J.W., and L.H.; Supervision, administration, and funding acquisition: J.W. and L.H. All authors approved the final version of the manuscript.

DECLARATION OF INTERESTS

The authors declare no competing interests.

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# STAR METHODS

## KEY RESOURCES TABLE

| REAGENT or RESOURCE | SOURCE | IDENTIFIER |
|---------------------|--------|------------|
| **Antibodies**      |        |            |
| Anti-Trimethyl-Histone H3 (Lys4) | Millipore | Cat# 07-473; RRID:AB_1977252 |
| Anti-RNA polymerase II CTD repeat | Abcam | Cat# ab5408; RRID:AB_304868 |
| Anti-Histone H3K27ac | Active Motif | Cat# 39133; RRID:AB_2561016 |
| Anti-Histone H3K4me1  | Active Motif | Cat# 39297; RRID:AB_2615075 |
| **Chemicals, peptides, and recombinant proteins** | | |
| Human IFNα | Stem Cell Tech. | Cat# 78077 |
| Human IFNβ | Peprotech | Cat# 300-02BC |
| Human IFNγ | Peprotech | Cat# 300-02 |
| Human IL-1β | Peprotech | Cat# 200-01B |
| Human TNFα | Peprotech | Cat# 300-01A |
| Human IL-6 | Peprotech | Cat# 200-06 |
| Ruxolitinib | Peprotech | Cat# 9414958 |
| **Critical commercial assays** | | |
| SsoAdvanced Universal Probes Supermix | Bioline | Cat# 172-5281 |
| PureLink RNA Mini Kit | Invitrogen | Cat# 12183018A |
| SuperScript III First-Strand Synthesis SuperMix | Invitrogen | Cat# 18080-400 |
| NEBNext Ultra II DNA Library Prep Kit | New England Bio | Cat# E7645L |
| TruSeq total RNA Library Prep Kit | Illumina | Cat# 20020597 |
| **Deposited data** | | |
| Original ChIP-seq data – cytokine-stimulated human proximal tubule cells | NCBI GEO data set | GSE161915 |
| Original RNA-seq data – cytokine-stimulated human proximal tubule cells | NCBI GEO data set | GSE161916 |
| RNA-seq – human AKI kidney | NCBI GEO data set | GSE142077 |
| RNA-seq – human COVID-19 kidney | NCBI GEO data set | GSE150316 |
| RNA-seq – lung epithelium + IFNβ | NCBI GEO data set | GSE161665 |
| RNA-seq – liver epithelium + IFNβ | NCBI GEO data set | GSE115198 |
| CTCF ChIP-seq - HEK293 | NCBI GEO data set | GSE68976 |
| DHS - Human Renal Cortical Epithelial cells | NCBI GEO data set | GSE29692 |
| Hi-C - human adrenal gland | Northwestern University Yue Lab database | [http://3dgenome.fsm.northwestern.edu/view.php](http://3dgenome.fsm.northwestern.edu/view.php) |
| Human reference genome NCBI build 37, GRCh37 | Genome Reference Consortium | [http://www.ncbi.nlm.nih.gov/projects/gencode/index.html](http://www.ncbi.nlm.nih.gov/projects/gencode/index.html) |
| **Experimental models: Cell lines** | | |
| Human Primary Proximal Tubule (HPPT) Cells | ATCC | Cat# PCS-400-010 |
| **Oligonucleotides** | | |
| Human GAPDH Taqman probe mix | Thermo Fisher | Hs002786624_g1 |
| Human ACE2 Taqman probe mix | Thermo Fisher | Hs01085333_m1 |
| Human TMPRSS2 Taqman probe mix | Thermo Fisher | Hs01122322_m1 |
| Human STAT1 Taqman probe mix | Thermo Fisher | Hs01013996_m1 |
| Custom dACE2 qRT-PCR probe | Eurofins Genomics | N/A |

(Continued on next page)
**RESOURCE AVAILABILITY**

**Lead contact**
Further information and requests for resources and reagents should be directed to and will be fulfilled by the Lead Contact, Lothar Hennighausen (lotharh@nih.gov).

**Materials availability**
This study did not generate new unique reagents.

**Data and code availability**
The original RNA-seq and ChIP-seq data from HPPT cells were submitted to the Gene Expression Omnibus under GEO: GSE161917 (ChIP-seq - GEO: GSE161915, RNA-seq - GEO: GSE161916), and are publicly available. Accession numbers are also listed in the key resources table. Additional supplemental items are available from Mendeley Data: https://doi.org/10.17632/zhvggb5b44.1. DHS from human renal cortical epithelial cells and CTCF ChIP-seq from HEK293 cells were obtained under GEO: GSE29692 and GEO: GSE68976, respectively. RNA-seq data for IFNβ-stimulated lung and liver cell were obtained from GEO: GSE161665 and GEO: GSE115198, respectively. Human AKI and COVID-19 data were found under GEO: GSE142077 and GEO: GSE150316, respectively. Hi-C data from human adrenal gland tissues were obtained from Hi-C data browser (http://3dgenome.fsm.northwestern.edu/view.php).

Any additional information required to reanalyze the data reported in this paper is available from the lead contact upon request.

**EXPERIMENTAL MODEL AND SUBJECT DETAILS**

**Cell culture**
HPPT cells (ATCC® PCS-400-010™) from a male donor were cultured in low-serum medium consisting of Renal Epithelial Cell Basal Medium (ATCC® PCS-400-030™) with Renal Epithelial Cell Growth Kit (ATCC® PCS-400-040™), Penicillin-Streptomycin-Amphotericin B Solution (ATCC® PCS-999-002™), and Phenol red (ATCC® PCS-999-001™) added. Cells were obtained at passage 2, cultured according to the manufacturer’s instructions, and used between passages 4 and 6. In addition to characteristic cobblestone growth pattern when confluent, cells were confirmed to express several proximal tubule markers including γ-glutamyltransferase-1 and SLC3a1 as assessed with RNA-seq (Lee et al., 2015).
METHOD DETAILS

Cytokine stimulation

Cells were stimulated with IFNα (Stem Cell Technologies), IFNβ, IFNγ, TNFα, IL-6, and IL-1β (all obtained from Peprotech) for 12 hours in concentration of 10 ng/ml. Cells were treated with ruxolitinib (Peprotech) at 10 μM for 12 hours together with IFNβ. At least three biological replicates were prepared for all experiments.

RNA isolation and qRT-PCR

After cytokine stimulation, cells were washed twice with phosphate buffered saline (PBS) before RNA isolation to remove medium and debris. mRNA was isolated using PureLink™ RNA Mini Kit (Invitrogen), and 500 ng was transcribed into cDNA using SuperScript™ III First-Strand Synthesis SuperMix (Invitrogen). qRT-PCR reaction was prepared with SsoAdvanced Universal Probes Supermix (Bio-Rad) and the following Taqman probes (ThermoFisher): GAPDH (Hs02786624_g1), ACE2 (Hs01085333_m1), TMPRSS2 (Hs01122322_m1), and STAT1 (Hs01013996_m1) or the following primers: dACE2 forward: 5’ GGAAGCAGGCTGGGACAAA 3’, dACE2 reverse: 5’ AGCTGTCAGGAAGTCGTCCATT 3’, ACE2 forward: 5’ GGCCGAC TCCAGGTACTTAT 3’, ACE2 reverse: 5’ GGATATGCCCTATCTCATGATG 3’. Custom qRT-PCR probe sequences were as follows: ACE2: 5’ [6-FAM] ATGGACGACTTCCTGACAG [MGBE/Quencher] 3’, dACE2: 5’ [6-FAM] AGGGAGGATCCTTATGTG [MGBE/Quencher] 3’. Reaction conditions were as follows: initial denaturation for 3 minutes at 95°C and 40 cycles of 10 seconds at 95°C and 30 seconds at 60°C.

PCR amplification

ACE2 PCR was performed with cDNA obtained as described earlier. Fifty nanograms of cDNA was used in the following reaction: initial denaturation – 3 minutes, 98°C and 35 cycles of denaturation – 30 seconds at 98°C, annealing – 30 seconds at 58°C, extension – 72°C for 2 minutes, ending with final extension of 72°C for 10 minutes. Amplified fragments were run on a 1.5% agarose gel with 100-kb DNA ladder to assess product size. Bands were cut out, and PCR products cleaned with MinElute Gel Extraction Kit (Quiagen) and Sanger sequenced by Quintara Biosciences. Primers used were as follows: dACE2 forward: 5’-TGTGAGAGCCTTAGGTTGGATTCC-3’, dACE2 reverse: 5’-TCTCTCCTTGGCCATGTTGT-3’ (Onabajo et al., 2020).

RNA-seq library preparation and data analysis

mRNA was prepared as described earlier and quality assessed using Bioanalyzer 2100 (Agilent). Samples with adequate RIN values were transcribed into libraries using TruSeq total RNA Library Prep Kit according to the manufacturer’s instructions. Libraries were pooled in equimolar amounts and sequenced using HiSeq 2000 (Illumina).

The raw data were subjected to QC analyses using the FastQC tool (version 0.11.9) (https://www.bioinformatics.babraham.ac.uk/projects/fastqc/). Trimmomatic (version 0.36) (Bolger et al., 2014) was used to assess total RNA-seq read quality, and STAR RNA-seq (version 2.5.4a) (Dobin et al., 2013) using 50-bp paired-end mode was used to align the reads (hg19). HTSeq (Anders et al., 2015) was used to retrieve the raw counts, and R package DESeq2 (Huber et al., 2015; Love et al., 2014) was used to normalize data. Additionally, the RUVSeq (Risso et al., 2014) package was applied to remove confounding factors. A minimum of five reads was an additional basis for filtering artifacts. The visualization was done using dplyr (https://CRAN.R-project.org/package=dplyr) and ggplot2 (Risso et al., 2014). Significantly differential expressed genes with an adjusted p-value (pAdj, FDR) below 0.05 and a fold change > 2 for upregulated genes were categorized using GSEA (https://www.gsea-msigdb.org/gsea/msigdb). Sequence read numbers were calculated using Samtools (Li et al., 2009) software with sorted bam files.

ChIP-seq library preparation and data analysis

Cells were washed twice with PBS and fixed with 0.75% formaldehyde in Dulbecco’s Modified Eagle Medium (DMEM) for 10 minutes in room temperature. Next, glycine was added to quench fixation in a final concentration of 125 mM, and plates were incubated in room temperature for another 10 minutes. Cells were then scraped and centrifuged at 4°C, for 1 minute, at 3000 rpm, and then washed twice with cold PBS. Pellets were resuspended in 2 ml of Farnham Lysis Buffer with protease inhibitors and incubated on ice for 10 minutes. Then, the cells were pelleted again at 4°C, for 5 minutes, at 3500 rpm and resuspended in TE buffer with protease inhibitors. Chromatin was sonicated for 3 minutes using a probe sonicator.
Finally, after centrifugation at 4°C, 13000 g for 10 minutes, the supernatant was used for immunoprecipitation.

Briefly, 600-1000 μg of chromatin was incubated with antibody-coated Dynabeads™ Protein A (Invitrogen) at 4°C overnight. The beads were then washed with radioimmunoprecipitation assay buffer (RIPA), high-NaCl RIPA, LiCl buffer, and PBS. Next, DNA was eluted from the beads and reverse cross-linked by incubating with proteinase K at 65°C overnight. DNA was then purified with MinElute PCR Purification Kit (Quiagen), and library preparation was performed according to the manufacturer’s instructions for NEBNext® Ultra™ II DNA Library Prep Kit for Illumina® (New England Biotechnology). Proper library size distribution with a peak in 300- to 500-bp range was confirmed using Bioanalyzer 2100 (Agilent), libraries pooled and sequenced with HiSeq 2000 (Illumina). Antibodies used were as follows: anti-Trimethyl-Histone H3 (Lys4) (Millipore, 07-473), Anti-RNA polymerase II CTD repeat (Abcam, ab5408), Anti-Histone H3K27ac (Active Motif, 39133), Anti-Histone H3K4me1 (Active Motif, 39297).

Quality filtering and alignment of the raw reads was performed using Trimmomatic (Bolger et al., 2014) (version 0.36) and Bowtie (Langmead et al., 2009) (version 1.1.2), with the parameter ‘-m 1’ to keep only uniquely mapped reads, using the reference genome hg19. Picard tools (Broad Institute, Picard, http://broadinstitute.github.io/picard/) 2016 was used to remove duplicates and subsequently, and Homer (Heinz et al., 2010) (version 4.8.2) software was applied to generate bedGraph files. Integrative Genomics Viewer (Thorvaldsdottir et al., 2013) was used for visualization.

**QUANTIFICATION AND STATISTICAL ANALYSIS**

Statistical analysis of data was performed using Prism 8. First, normal distribution of data was assessed. Next, statistical significance was evaluated with 1-way or 2-way ANOVA followed by Tukey’s multiple comparisons, or a T-test, depending on the experimental setup. n and points on a graph always represent biological replicates – seeded wells of a 6-well culture plate. Values of *p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001 were considered statistically significant.