Supplemental information

Systematic analysis of SARS-CoV-2 infection
of an ACE2-negative human airway cell

Figure S1
Figure S1. Expression of ACE2 across cell line models, related to Figure 1. A, Unsupervised hierarchical clustering of upper quartile-normalized RNA-seq reads. Normalized RNA-seq reads were aligned to the GRCh38 and Vervet-African green monkey genomes and quantified with Salmon (v1.3.0). As indicated, RNA-seq data were generated at UNC or Washington University or obtained from the Sequence Read Archive (SRA). B, Read counts for ACE2, GAPDH and ACTB across the indicated cell models.
Figure S2
Figure S2. Visualization of SARS-CoV-2 replication and spread in H522 cells, related to Figure 1. A, Representative images of H522 cells infected with SARS-CoV-2 at the indicated time points and MOIs. H522 cells were fixed and stained for SARS-CoV-2 RNA (green) by RNAscope reagents and Nucleocapsid (N) protein (red) and imaged by confocal microscopy (n=2). B, Quantification of vRNA puncta in H522 cells infected with variable MOIs of SARS-CoV-2. Greater than 150 cells per sample from 5 different fields were counted in a blinded manner from a representative experiment.
**Figure S3**

**A**

ACE2 CRISPR KO Polyclonal Populations

<table>
<thead>
<tr>
<th></th>
<th>H522</th>
<th>H522</th>
<th>H522</th>
<th>Calu-3</th>
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<tbody>
<tr>
<td>ACE2</td>
<td>WT-1</td>
<td>ACE2</td>
<td>ACE2</td>
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<tr>
<td></td>
<td>+</td>
<td>+</td>
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<td>+</td>
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</tbody>
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500 bp - 300 bp - 200 bp -

Parental band - Cleaved bands

Cleavage Efficiency: 44% 51%

Detection Enzyme

**B**

ACE2 CRISPR KO Monoclonal Populations

**Ref:** 5'-AACTTTTCCAGTACTGTAGATGGTGCTCATTGT

ACE2<sup>+/-</sup> MC: 5'-AACTTTTCCAGTACTGTAGATGGTGCTCATTGT

ACE2<sup>+</sup> MC-1: 5'-AACTTTTCCAGTACTGTAGATGGTGCTCATTGT

ACE2<sup>+</sup> MC-2: 5'-AACTTTTCCAGTACTGTAGATGGTGCTCATTGT

ACE2<sup>+</sup> MC: 5'-AACTTTTCCAGTACTGTAGATGGTGCTCATTGT

**Cut site**

**D**

NRP1 CRISPR KO Monoclonal Populations

**Ref:** 5'-TTTCTAGCCGGTCGTAGCGACAGAACATCCCCC

NRP1<sup>+/+</sup> MC: 5'-TTTCTAGCCGGTCGTAGCGACAGAACATCCCCC

NRP1<sup>+</sup> MC-1: 5'-TTTCTAGCCGGTCGACAGAACATCCCCC

NRP1<sup>+</sup> MC-2: 5'-TTTCTAGCCGGTCGACAGAACATCCCCC

**Cut site**

**E**

siRNA knockdown efficiency

**Relative mRNA (siRNA vs Mock)**

Mock 1 2 3 4

NT siAXL siNR1

2.0 1.5 1.0 0.5 0.0
Figure S3. ACE2 and NRP1 knockout via CRISPR in H522 and Calu-3 cell lines, related to Figure 3. **A**, Genomic Cleavage Detection Assay (Invitrogen) was performed following the manufacturer’s protocol on ACE2 WT or ACE2 KO CRISPR modified polyclonal cells. **B**, Sanger sequencing of genomic ACE2 at exon 3. Unique monoclonal populations of H522 ACE2 KO’s were aligned to the human genome (‘Ref’; hg38). The red dashed lines indicate small deletions within exon 3 of ACE2. **C**, Monoclonal populations of NRP1 CRISPR cells were infected with SARS-CoV-2 at an MOI: 1 i.u./cell and cell associated vRNA levels quantitated at 4 and 72 hpi (n= 3-6). **D**, Sanger sequencing of genomic NRP1 at exon 4. Unique monoclonal populations of H522 NRP1 KO’s were aligned to the human genome (‘Ref’; hg38). The red dashed lines indicate small deletions within exon 4 of NRP1. **E**, NRP1 and AXL siRNA knockdown efficiencies in H522 cells relative to Mock was assessed by Q-RT-PCR 48 h post transfection (n=3-5).
Figure S4

RNA copies relative to DMSO (%)
Figure S4. Comparative analysis of infection pathways in H522 and other permissive cells, related to Figure 4. H522, H522-ACE2 and Vero E6 cells were pre-treated with bafilomycin A (vATPase inhibitor), SGC-AAK1-1 (clathrin-mediated endocytosis inhibitor), E64D (endosomal cathepsins inhibitor), apilimod (PIKfyve inhibitor), or camostat mesylate (TMPRSS2 inhibitor) for 1 h and then infected with SARS-CoV-2 in the presence of the inhibitors. Cell-associated SARS-CoV-2 RNA was detected by qRT-PCR 24 hpi and normalized to DMSO treated cells (n≥3). * indicates p<0.05, ** indicates p<0.01, and *** indicates p<0.001 compared to DMSO treated controls where significance was determined using two-way ANOVA and the Dunnett correction for multiple comparisons.
Figure S5. Protein interaction networks of differentially expressed proteins in H522 cells infected with SARS-CoV-2, related to Figure 6. Protein complexes of differentially expressed H522 and SARS-CoV-2 proteins. Complexes and functions were extracted from the CORUM database. The colors correspond to the whole cell proteomic clusters identified in ‘Fig. 6D’.
Figure S6
Figure S6. siRNA knockdown efficiency for viral sensing pathways in H522 cells, related to Figure 7. qRT-PCR for each gene targeted by siRNA in H522 cells. Knockdown efficiency was calculated compared to a non-targeting (NT) control. H522 cells were infected with SARS-CoV-2 24 hpi and RNA was collected 24, 96, and 120 hpi. TLR8 mRNA was not detected across the three time points.