JHou et al. Extended Data Figure 1

(a) Bar graph showing luciferase activity (log10) for different cell lines: 293T, 293T-ACE2, H2023, H2023-ACE2, A549, and A549-ACE2. The y-axis represents luciferase activity, and the x-axis represents different cell lines. The data is presented as mean ± standard error of the mean (SEM).

(b) Western blot analysis showing the expression of different markers: FLAG-Cas9, ACE2, and β-Actin. The blot includes samples labeled A549, A549-ACE2, and A549-AC.

(c) Graph depicting cell viability (%) against MOI (multiplicity of infection). The IC50 is 4.238 (MOI). The graph includes actual and estimated data points.

(d) Image showing a comparison of A549 cell line before and after infection with pooled gRNA library, indicating the post-infection 48 hours condition.
Extended Data Figure 1. Establishment of genome-wide CRISPR/Cas9 dropout screens for SARS-CoV-2 infection
(a) Evaluation of permissiveness of human epithelial cell lines for SARS-CoV-2 infection. Human epithelial cells were infected with recombinant SARS-CoV-2-Nluc at MOI=0.2 and luciferase signals were measured at 24-h post-infection. (b) Expression of ACE2 and Cas9 expression in A549-AC cells. Anti-human ACE2 and anti-flag antibodies were used to determine the level of ACE and FLAG-tagged Cas9, respectively. (c) Dose effect of SARS-CoV-2 on CPE. A549-AC cells were infected with different MOIs (ranging from 0.1 to 40) of recombinant SARS-CoV-2. 48 hours after infection, the viabilities of infected cells were measured. A four-parameter nonlinear regression method was used to generate the estimated dose-response curve and to calculate the MOI for 50% of cell lysis. (d) Representative images of cells before and after viral infection. Typical bright field images of pooled A549-AC cells with the gRNA library were illustrated before (left panel) and after (right panel) SARS-CoV-2 infection. Samples were triplicated in experiments. The comparisons with statistical significance were indicated. *p<0.05; ***p<0.001; ****p<0.0001.
a) Total gRNA read counts

b) Pearson correlation

- SARS-CoV-2_1
- SARS-CoV-2_2
- SARS-CoV-2_3
- Ctrl_1
- Ctrl_2
- Ctrl_3
- Ref

Non-essential
Essential

JHou et al. Extended Data Figure 2
Extended Data Figure 2. Quality evaluation of results from the genome-wide CRISPR dropout screen. (a) Raw read counts of total gRNAs in samples collected from the genome-wide CRISPR dropout screen. (b) Correlations of gRNA frequencies across experimental samples. Pairwise Pearson correlation analyses were performed between two distinct samples. (c) Distribution of gRNAs targeting essential and non-essential genes in the reference sample (upper panel), the control samples (middle panel) and the SARS-CoV-2 infected samples (bottom panel).
Extended Data Fig. 3. Phenotypes of knocking out putative host factors in lung epithelial cells. (a) Effects of knocking out putative host factors on CPE at the high MOI condition. A series of A549-AC cell lines with related gRNA expression were infected with the recombinant SARS-CoV-2 at MOI=5 and cell viability was measured at 48 hours post-infection. (b) Effects of knocking out putative host factors on in vitro growth. Equal numbers of genetically modified A549-AC cells were seeded and cultured for 48 hours in vitro. The relative changes in cell numbers of A549-AC cells with gene-specific KD were calculated by normalizing with the number of A549-AC cells expressing non-targeting gRNA. Statistical significance between the gRNAs and NC was determined by one-way ANOVA with repeated measurements. *p<0.05; **p<0.01; ***p<0.001; ****p<0.0001. n.s., not significant.
JHou et al. Extended Data Figure 4
Extended Data Fig. 4. Comparisons of expression levels of identified host factors in lung epithelial cells from pneumonia patients. The mRNA expression levels of two pro-viral factors (ATP6V0D1 and DPAGT1) and three anti-viral factors (DAZAP2, VTA1, and KLF5) in epithelial cells in bronchoalveolar lavage fluids were extracted from published single cell RNA-seq datasets of pneumonia patients. Patients were stratified by their diagnosis (COVID-19 and non-COVID-19) and severity (mild and severe). Log transformed read counts to each host factor in lung epithelial cells from different patient groups were illustrated. The comparisons with statistical significance were indicated. *p<0.05; ***p<0.001; ****p<0.0001.
Extended Data Fig. 5. Successful gene-specific perturbations in A549-AC cells. (a) Inhibition of mRNA expression of target genes in A549-AC cells by gene-specific gRNAs. (b) Inhibition of expression of target proteins in A549-AC cells by gene-specific gRNAs. (c) Increased mRNA expression of target genes in gene-specific overexpression (OE) A549-AC cells. (d) Increased expression of target protein in gene-specific OE A549-AC cells. The comparisons with statistical significance were indicated. ***p<0.001; ****p<0.0001.
Extended Data Table 1. List of pro-viral host factors identified from SARS-CoV-2 dropout screens.

Extended Data Table 2. List of anti-viral host factors identified from SARS-CoV-2 dropout screens.

Extended Data Table 3. Summary of published genome-wide gRNA SARS-CoV-2 screens for integrative studies. Publicly available datasets from genome-wide CRISPR screens based on the CPE of SARS-CoV-2 on human epithelial cells were extracted and used to evaluate the performance of identified hits. The evaluation criteria for each dataset were listed.

Extended Data Table 4. Evaluation of 30 identified host factors in two independent validation experiments.

Extended Data Table 5. List of DNA sequences to generate gRNAs targeting 30 identified host factors for validation.

Extended Data Table 6. List of primer information to determine mRNA expression by real-time PCR.