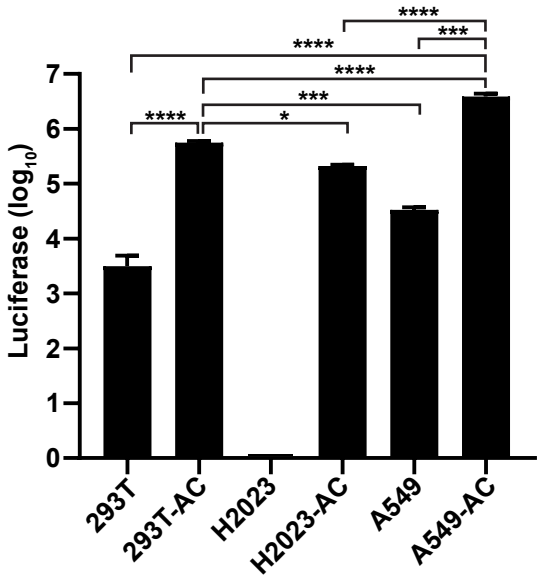
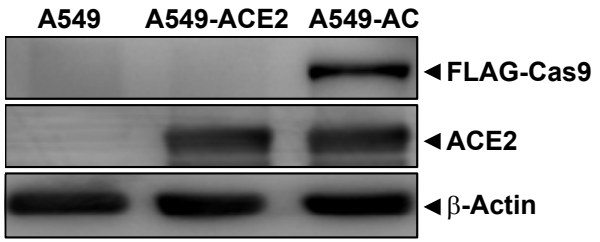
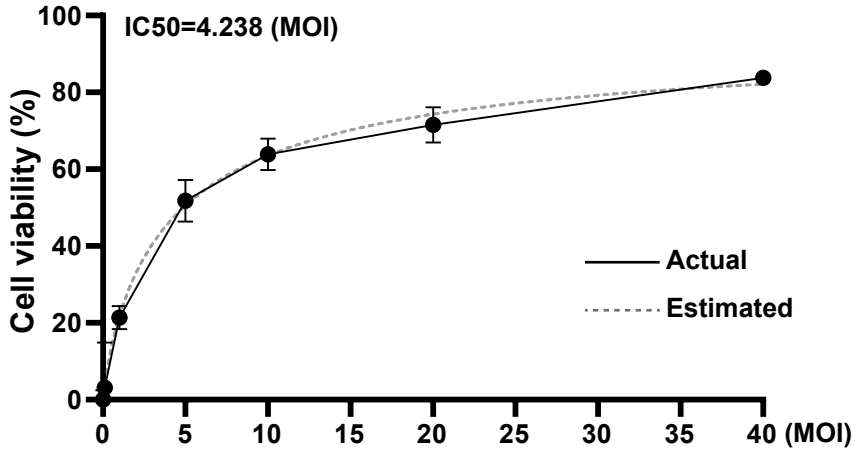
a


Luciferase (log_{10})
0
1
2
3
4
5
6
7
****
****
****
***
****
***
*
293T
293T-AC
H2023
H2023-AC
A549
A549-AC

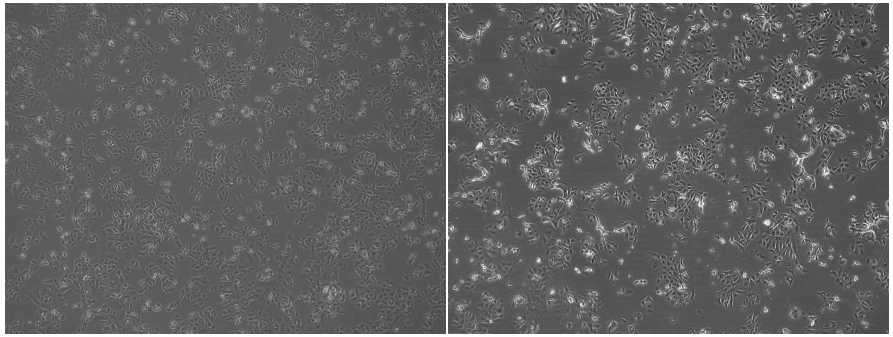
b


A549
A549-ACE2
A549-AC
FLAG-Cas9
ACE2
β-Actin

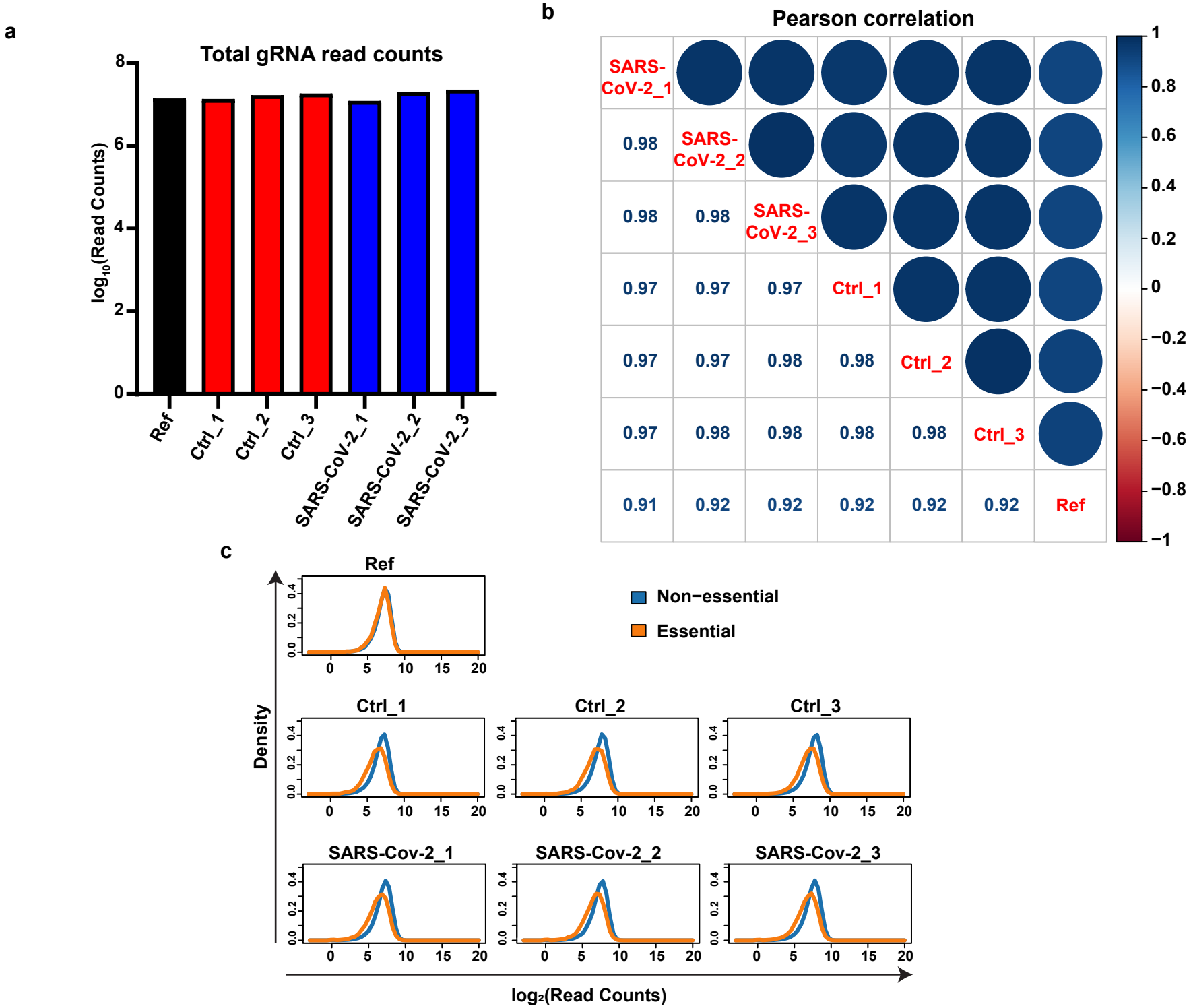
c


IC50=4.238 (MOI)
Cell viability (%)
0
20
40
60
80
100
0
5
10
15
20
25
30
35
40 (MOI)
Actual
Estimated

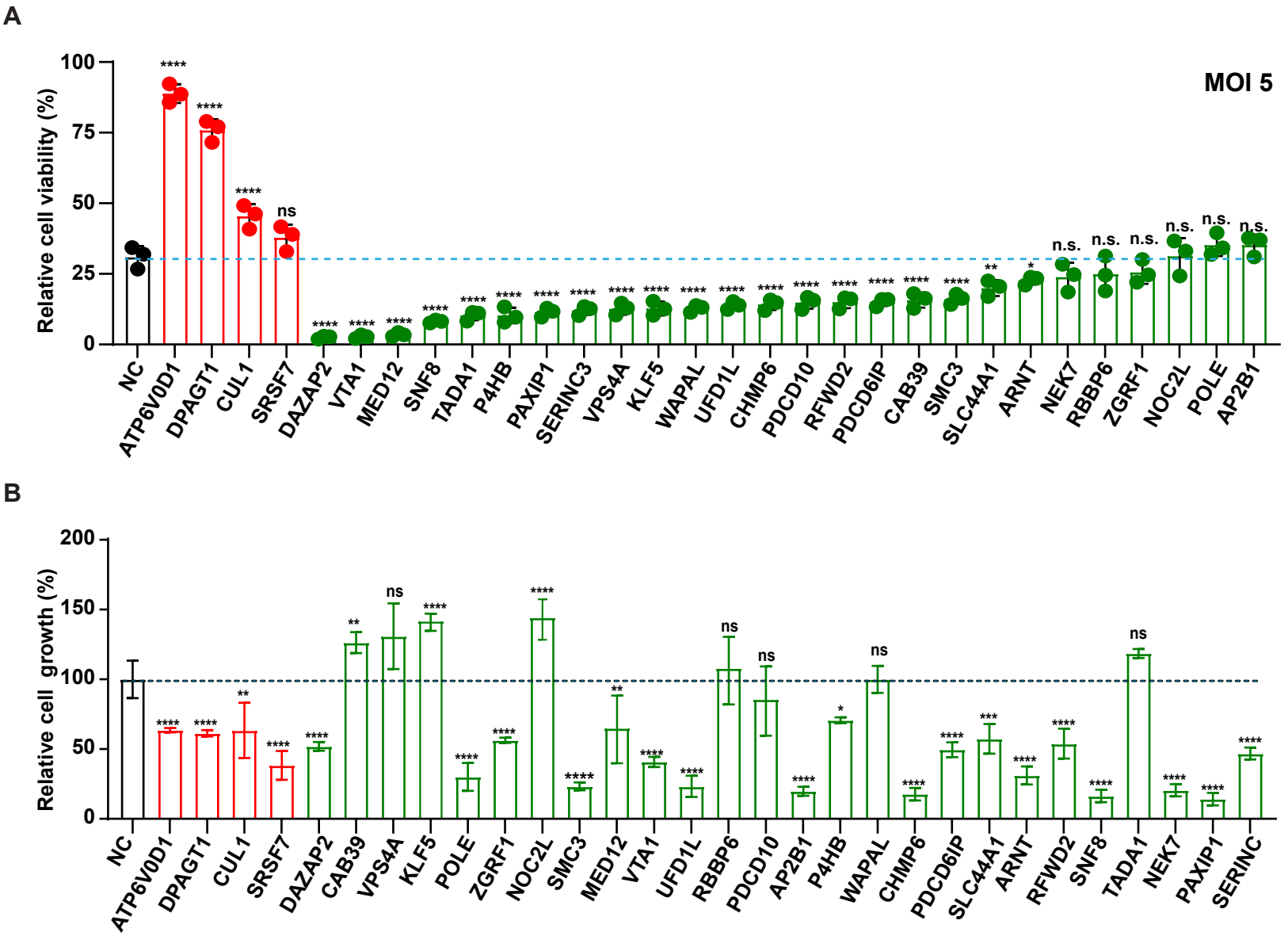
d


A549-AC
with pooled gRNA library
Before Infection
Post-Infection 48hrs

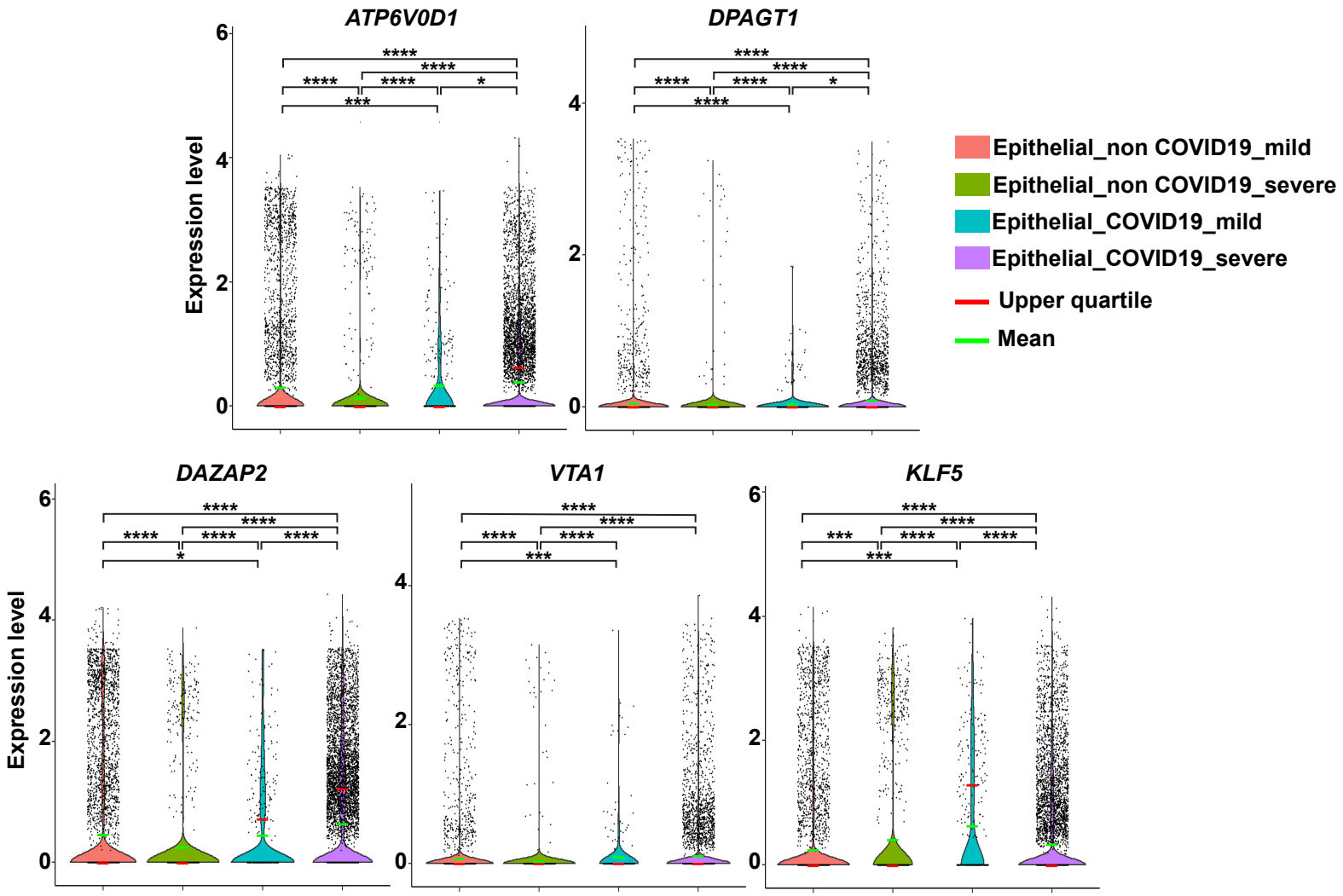
Extended Data Figure 1. Establishment of genome-wide CRISPR/Cas9 dropout screens for SARS-CoV-2 infection (a) Evaluation of permissiveness of human epithelial cell lines for SARS-CoV-2 infection. Human epithelial cells were infected with recombinant SARS-CoV-2-Nluc at MOI=0.2 and luciferase signals were 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
log10(Read Counts)
0
2
4
6
8
Ref
Ctrl_1
Ctrl_2
Ctrl_3
SARS-CoV-2_1
SARS-CoV-2_2
SARS-CoV-2_3
b
Pearson correlation
SARS-CoV-2_1
SARS-CoV-2_2
SARS-CoV-2_3
Ctrl_1
Ctrl_2
Ctrl_3
Ref
0.98
0.98 0.98
0.97 0.97 0.97
0.97 0.97 0.98 0.98
0.97 0.98 0.98 0.98 0.98
0.91 0.92 0.92 0.92 0.92 0.92
1
0.8
0.6
0.4
0.2
0
−0.2
−0.4
−0.6
−0.8
−1
c
Ref
Ctrl_1
Ctrl_2
Ctrl_3
SARS-Cov-2_1
SARS-Cov-2_2
SARS-Cov-2_3
Non−essential
Essential
Density
log2(Read Counts)
0.0
0.2
0.4
0
5
10
15
20

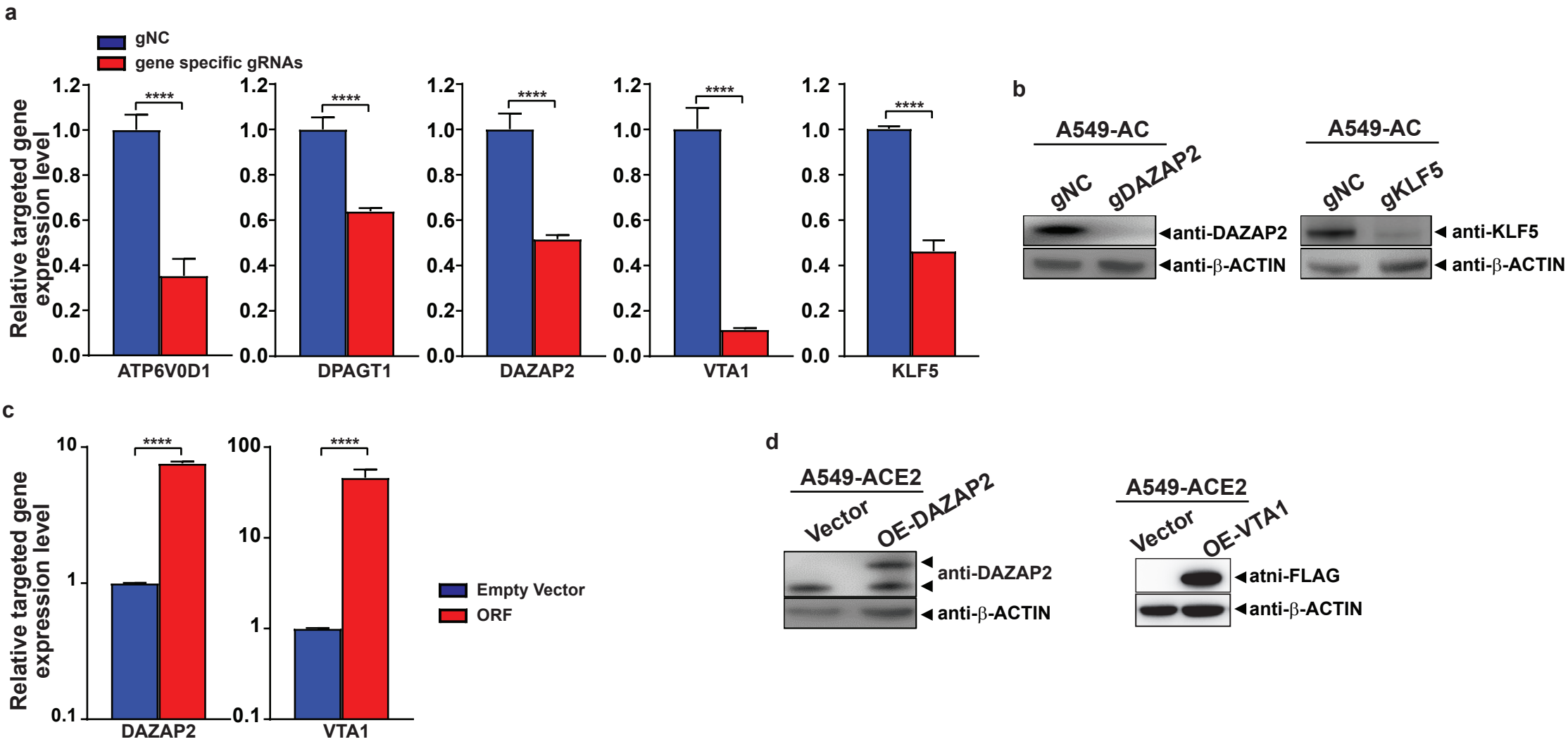
Extended Data Figure 2. Quality evaluation of results from the genome-wide CRISPR dropout screen. (a) Raw read counts of total gRNAs in samples collected from the genome-wide CRISPR dropout screen. (b) Correlations of gRNA frequencies across experimental samples. Pairwise Pearson correlation analyses 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).


A
MOI 5
Relative cell viability (%)
100
75
50
25
0
NC
ATP6V0D1
DPAGT1
CUL1
SRSF7
DAZAP2
VTA1
MED12
SNF8
TADA1
P4HB
PAXIP1
SERINC3
VPS4A
KLF5
WAPAL
UFD1L
CHMP6
PDCD10
RFWD2
PDCD6IP
CAB39
SMC3
SLC44A1
ARNT
NEK7
RBBP6
ZGRF1
NOC2L
POLE
AP2B1
B
Relative cell growth (%)
200
150
100
50
0
NC
ATP6V0D1
DPAGT1
CUL1
SRSF7
DAZAP2
CAB39
VPS4A
KLF5
POLE
ZGRF1
NOC2L
SMC3
MED12
VTA1
UFD1L
RBBP6
PDCD10
AP2B1
P4HB
WAPAL
CHMP6
PDCD6IP
SLC44A1
ARNT
RFWD2
SNF8
TADA1
NEK7
PAXIP1
SERINC

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.


ATP6V0D1
DPAGT1
DAZAP2
VTA1
KLF5
Expression level
Epithelial_non COVID19_mild
Epithelial_non COVID19_severe
Epithelial_COVID19_mild
Epithelial_COVID19_severe
Upper quartile
Mean

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 (ATP6V0D1and 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. *$p<0.05$; ***$p<0.001$; ****$p<0.0001$.


a
gNC
gene specific gRNAs
Relative targeted gene expression level
****
ATP6V0D1
DPAGT1
DAZAP2
VTA1
KLF5
b
A549-AC
gNC
gDAZAP2
anti-DAZAP2
anti-β-ACTIN
A549-AC
gNC
gKLF5
anti-KLF5
anti-β-ACTIN
c
Relative targeted gene expression level
DAZAP2
VTA1
Empty Vector
ORF
d
A549-ACE2
Vector
OE-DAZAP2
anti-DAZAP2
anti-β-ACTIN
A549-ACE2
Vector
OE-VTA1
atni-FLAG
anti-β-ACTIN

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.