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Supplemental Information

Functional interrogation of a SARS-CoV-2 host protein interactome identifies unique and shared coronavirus host factors

H.-Heinrich Hoffmann, Francisco J. Sánchez-Rivera, William M. Schneider, Joseph M. Luna, Yadira M. Soto-Feliciano, Alison W. Ashbrook, Jérémie Le Pen, Andrew A. Leal, Inna Ricardo-Lax, Eleftherios Michailidis, Yuan Hao, Ansgar F. Stenzel, Avery Peace, Johannes Zuber, C. David Allis, Scott W. Lowe, Margaret R. MacDonald, John T. Poirier, and Charles M. Rice

SUPPLEMENTAL FIGURE TITLES AND LEGENDS

Supplementary Figure S1. Cell type selection and screen QC, Related to Figures 1 and 2.

(A) Expression profile distributions of all 332 interactome genes in Huh-7.5 or Calu-3 cells are shown in red. The expression distributions for all genes are also shown with coronavirus entry factors highlighted.

(B) Crystal violet staining of infected cells at 7 days post infection. Huh-7.5 or Calu-3 cells were infected with SARS-CoV-2 (MOI = 0.5 PFU/cell) or endemic coronaviruses HCoV-OC43 (MOI = 1 PFU/cell), HCoV-229E (MOI = 0.5 PFU/cell), and HCoV-NL63 (MOI = 0.01 PFU/cell) at either 33 ºC or 37 ºC.

(C) Species accumulation curve for each sgRNA observed more than 10 times for actual (blue) and projected sequenced reads (orange). Horizontal dashed line indicates library size. Vertical dashed line indicates number of reads sequenced.

(D) Heatmap of Pearson correlation coefficients of normalized sgRNA counts.

(E) Receiver operating characteristic (ROC) curves

(F) Bar charts indicating the area under the curve (AUC) for each ROC curve in E.

Supplementary Figure S2. Validation of candidate host factors with HCoV-229E, HCoV-NL63, and HCoV-OC43, Related to Figure 3.

(A) Candidate validation in Huh-7.5 cells in the context of infection by HCoV-229E. Cells were grown at 37 °C and infected using 1,250 HCoV-229E PFU/well. Plates were quantified at 24 hours post-infection. Data represent the average ± S.E.M. of n=4 replicates per target gene. Significance for viral infection percentage values was evaluated using t-test (2-tailed, unpaired) as compared to non-targeting (NT) controls (set at 100%). * denotes $p < 0.05$.

(B) Candidate validation in Huh-7.5 cells in the context of infection by HCoV-NL63. Cells were grown at 33 °C and infected using 100 HCoV-NL63 PFU/well. Plates were quantified at 72 hours post-infection. Data represent the average ± S.E.M. of n=4 replicates per target gene. Significance for viral infection percentage values was evaluated using t-test (2-tailed, unpaired) as compared to non-targeting (NT) controls (set at 100%). * denotes $p < 0.05$.

(C) Candidate validation in Huh-7.5 cells in the context of infection by HCoV-OC43. Cells were grown at 33 °C and infected using 15,000 HCoV-OC43 PFU/well. Plates were quantified at 72 hours post-infection. Data represent the average ± S.E.M. of n=4 replicates per target gene. Significance for viral infection percentage values was evaluated using t-test (2-tailed, unpaired) as compared to non-targeting (NT) controls (set at 100%). * denotes $p < 0.05$.

(D) Heatmap representation of data from panels A-C. Entry receptors not used by the corresponding virus were not tested and are highlighted with an “X”.

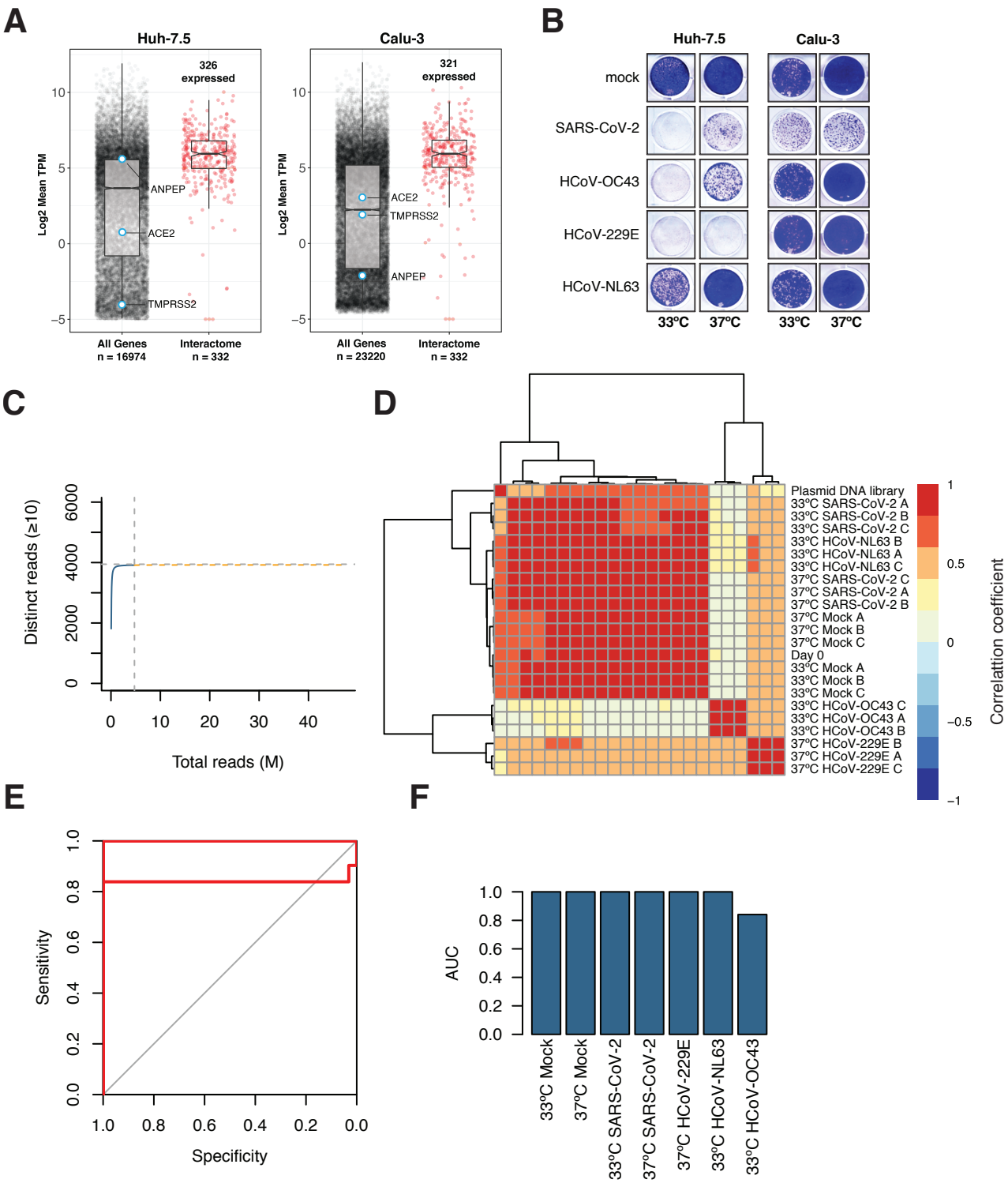
Supplementary Figure S3. Detection and quantification of coronavirus infection via immunofluorescence, Related to Figure 3.

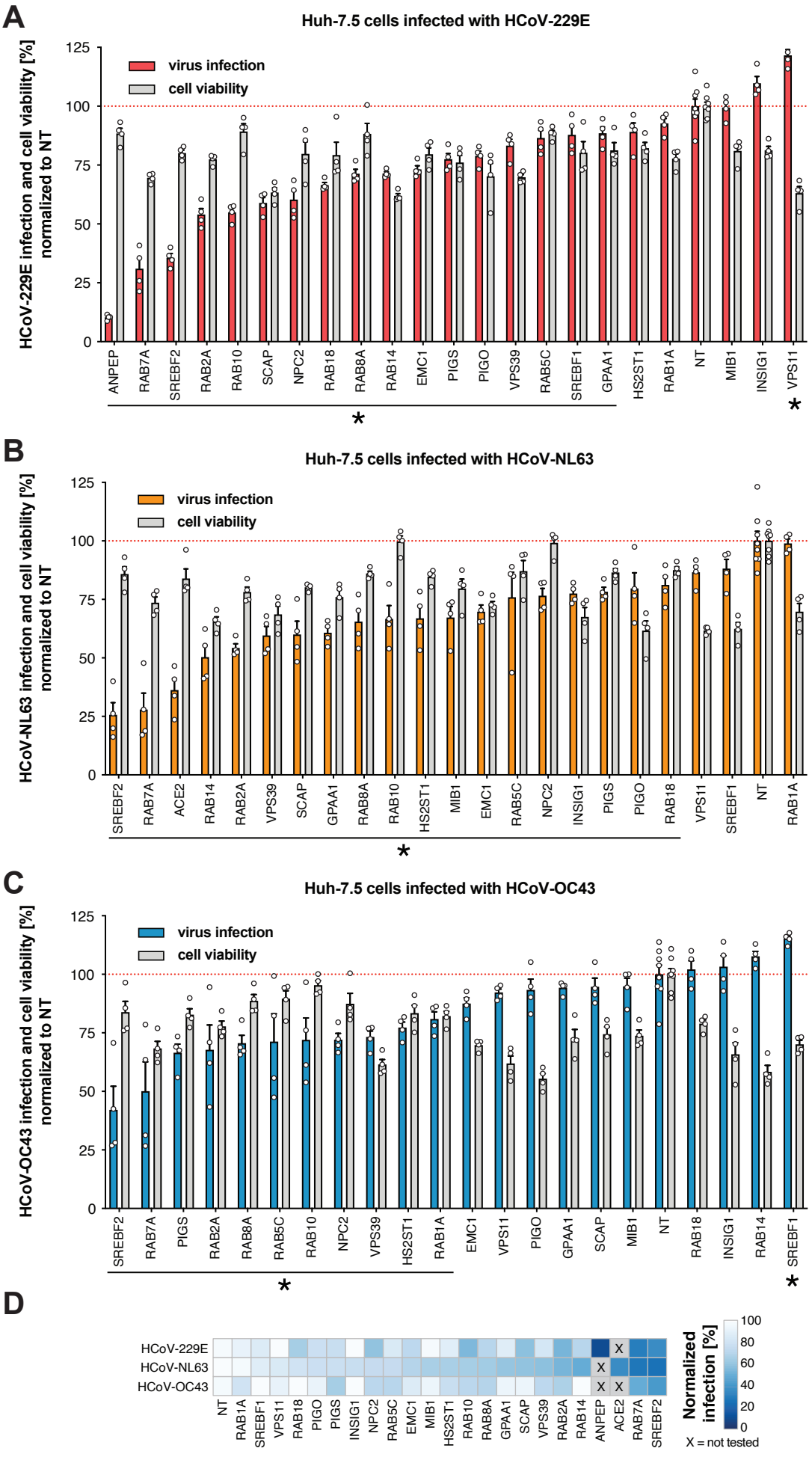
(A) Detection and quantification of SARS-CoV-2 infection by immunofluorescent staining for SARS-CoV-2 N protein. Scale bars are 70um.

(B) Detection and quantification of HCoV-229E infection by immunofluorescent staining for dsRNA. Scale bars are 70um.

Figure S4. High confidence CRISPR hits are expressed in the airway, Related to Figure 5.

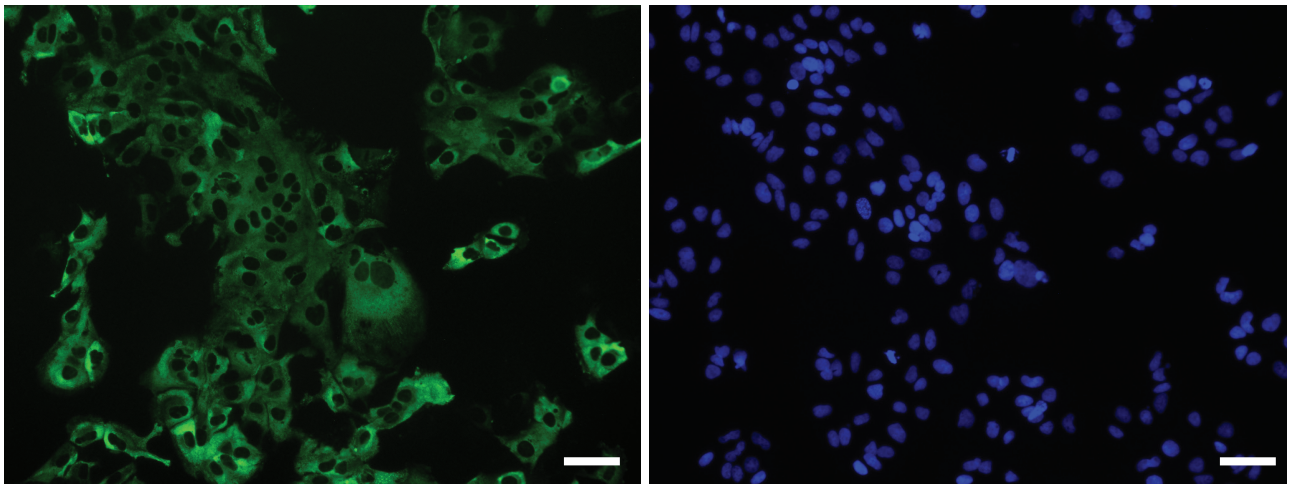
(A-B) DotPlots depicting mean expression z-scores for enriched (A) or depleted (B) CRISPR hits per indicated cell type cluster from upper respiratory tract scRNAseq in humans from (Chua et al., 2020). ACE2 and SARS-CoV-2 susceptible cell types are highlighted in red.


A
Huh-7.5
Calu-3
326 expressed
321 expressed
Log2 Mean TPM
ANPEP
ACE2
TMPRSS2
All Genes n = 16974
Interactome n = 332
All Genes n = 23220
Interactome n = 332
B
Huh-7.5
Calu-3
mock
SARS-CoV-2
HCoV-OC43
HCoV-229E
HCoV-NL63
33°C
37°C
C
Distinct reads (≥10)
Total reads (M)
D
Plasmid DNA library
33°C SARS-CoV-2 A
33°C SARS-CoV-2 B
33°C SARS-CoV-2 C
33°C HCoV-NL63 B
33°C HCoV-NL63 A
33°C HCoV-NL63 C
37°C SARS-CoV-2 C
37°C SARS-CoV-2 A
37°C SARS-CoV-2 B
37°C Mock A
37°C Mock B
37°C Mock C
Day 0
33°C Mock A
33°C Mock B
33°C Mock C
33°C HCoV-OC43 C
33°C HCoV-OC43 A
33°C HCoV-OC43 B
37°C HCoV-229E B
37°C HCoV-229E A
37°C HCoV-229E C
Correlattion coefficient
E
Sensitivity
Specificity
F
AUC
33°C Mock
37°C Mock
33°C SARS-CoV-2
37°C SARS-CoV-2
37°C HCoV-229E
33°C HCoV-NL63
33°C HCoV-OC43


A
Huh-7.5 cells infected with HCoV-229E
HCoV-229E infection and cell viability [%] normalized to NT
virus infection
cell viability
125
100
75
50
25
0
ANPEP
RAB7A
SREBF2
RAB2A
RAB10
SCAP
NPC2
RAB18
RAB8A
RAB14
EMC1
PIGS
PIGO
VPS39
RAB5C
SREBF1
GPAA1
HS2ST1
RAB1A
NT
MIB1
INSIG1
VPS11
*
*
B
Huh-7.5 cells infected with HCoV-NL63
HCoV-NL63 infection and cell viability [%] normalized to NT
virus infection
cell viability
125
100
75
50
25
0
SREBF2
RAB7A
ACE2
RAB14
RAB2A
VPS39
SCAP
GPAA1
RAB8A
RAB10
HS2ST1
MIB1
EMC1
RAB5C
NPC2
INSIG1
PIGS
PIGO
RAB18
VPS11
SREBF1
NT
RAB1A
*
C
Huh-7.5 cells infected with HCoV-OC43
HCoV-OC43 infection and cell viability [%] normalized to NT
virus infection
cell viability
125
100
75
50
25
0
SREBF2
RAB7A
PIGS
RAB2A
RAB8A
RAB5C
RAB10
NPC2
VPS39
HS2ST1
RAB1A
EMC1
VPS11
PIGO
GPAA1
SCAP
MIB1
NT
RAB18
INSIG1
RAB14
SREBF1
*
*
D
HCoV-229E
HCoV-NL63
HCoV-OC43
NT
RAB1A
SREBF1
VPS11
RAB18
PIGO
PIGS
INSIG1
NPC2
RAB5C
EMC1
MIB1
HS2ST1
RAB10
RAB8A
GPAA1
SCAP
VPS39
RAB2A
RAB14
ANPEP
ACE2
RAB7A
SREBF2
X
X
X
X
X
Normalized infection [%]
100
80
60
40
20
0
X = not tested

A

Huh-7.5 cells infected with SARS-CoV-2

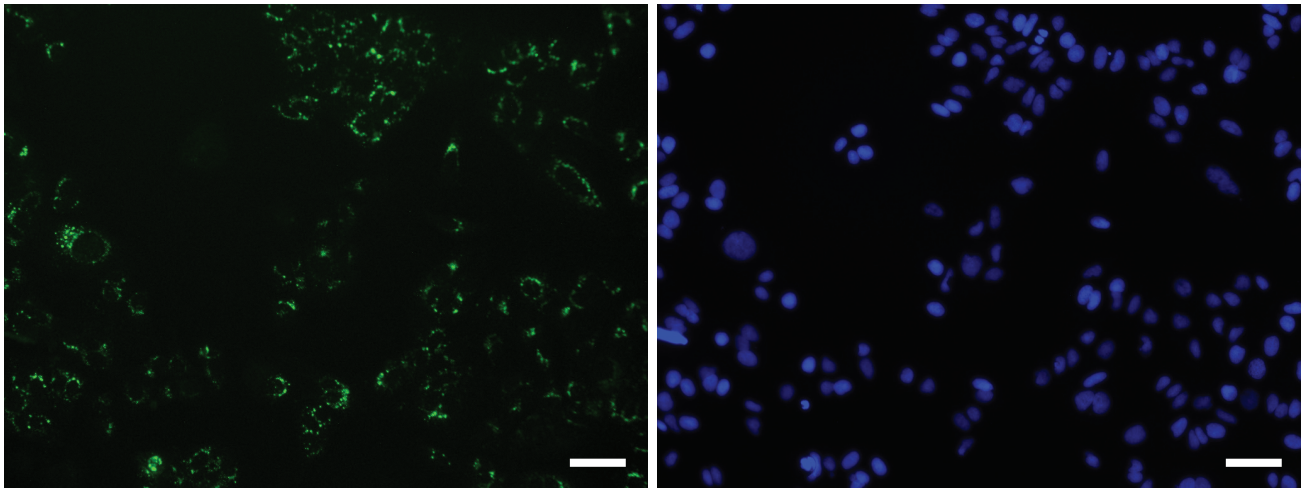


SARS-CoV-2 N Protein

DAPI

B

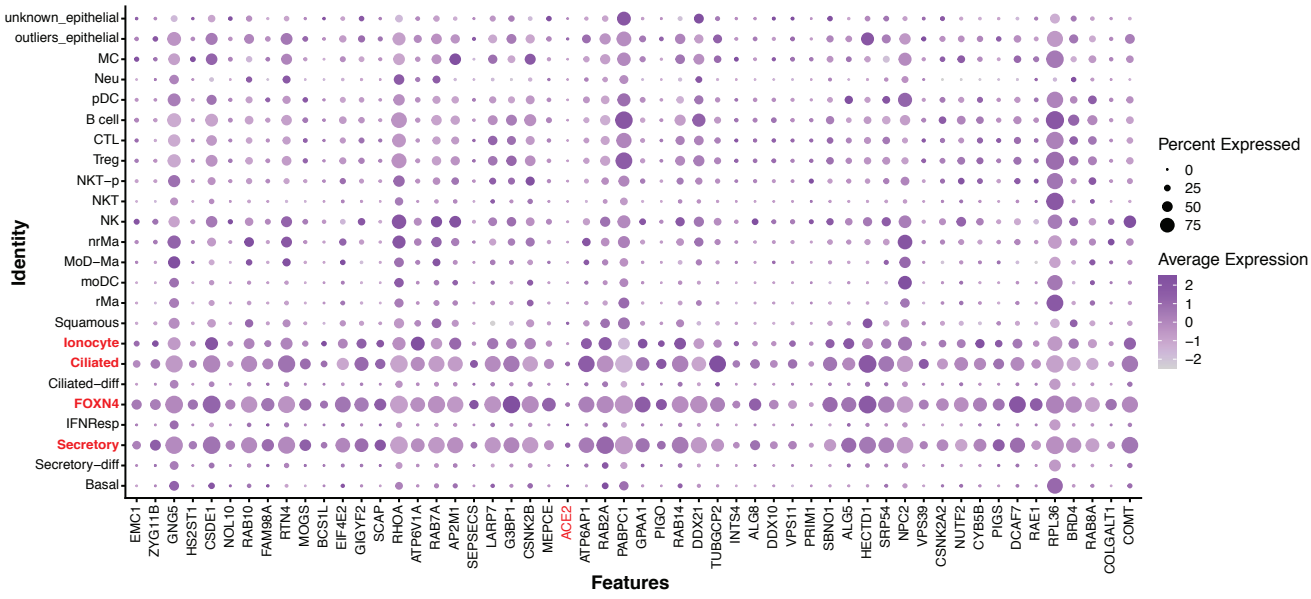
Huh-7.5 cells infected with HCoV-229E



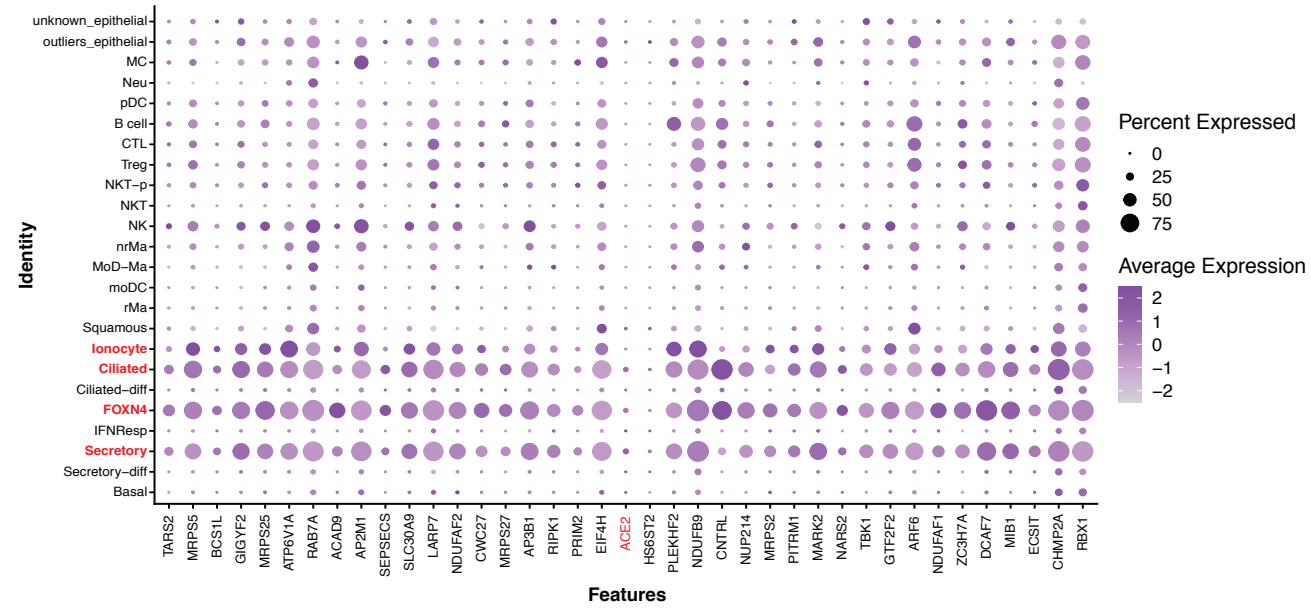
dsRNA

DAPI

A


Upper respiratory tract scRNAseq - Enriched high confidence CRISPR hits
Identity
unknown_epithelial
outliers_epithelial
MC
Neu
pDC
B cell
CTL
Treg
NKT–p
NKT
NK
nrMa
MoD–Ma
moDC
rMa
Squamous
Ionocyte
Ciliated
Ciliated–diff
FOXN4
IFNResp
Secretory
Secretory–diff
Basal
EMC1 ZYG11B GNG5 HS2ST1 CSDE1 NOL10 RAB10 FAM98A RTN4 MOGS BCS1L EIF4E2 GIGYF2 SCAP RHOA ATP6V1A RAB7A AP2M1 SEPSECS LARP7 G3BP1 CSNK2B MEPCE ACE2 ATP6AP1 RAB2A PABPC1 GPAA1 PIGO RAB14 DDX21 TUBGCP2 INTS4 ALG8 DDX10 VPS11 PRIM1 SBNO1 ALG5 HECTD1 SRP54 NPC2 VPS39 CSNK2A2 NUTF2 CYB5B PIGS DCAF7 RAE1 RPL36 BRD4 RAB8A COLGALT1 COMT
Features
Percent Expressed
0
25
50
75
Average Expression
2
1
0
−1
−2

B


Upper respiratory tract scRNAseq - Depleted high confidence CRISPR hits
Identity
unknown_epithelial
outliers_epithelial
MC
Neu
pDC
B cell
CTL
Treg
NKT–p
NKT
NK
nrMa
MoD–Ma
moDC
rMa
Squamous
Ionocyte
Ciliated
Ciliated–diff
FOXN4
IFNResp
Secretory
Secretory–diff
Basal
TARS2 MRPS5 BCS1L GIGYF2 MRPS25 ATP6V1A RAB7A ACAD9 AP2M1 SEPSECS SLC30A9 LARP7 NDUFAF2 CWC27 MRPS27 AP3B1 RIPK1 PRIM2 EIF4H ACE2 HS6ST2 PLEKHF2 NDUFB9 CNTRL NUP214 MRPS2 PITRM1 MARK2 NARS2 TBK1 GTF2F2 ARF6 NDUFAF1 ZC3H7A DCAF7 MIB1 ECSIT CHMP2A RBX1
Features
Percent Expressed
0
25
50
75
Average Expression
2
1
0
−1
−2