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Supplemental information

Parsing the role of NSP1 in SARS-CoV-2 infection

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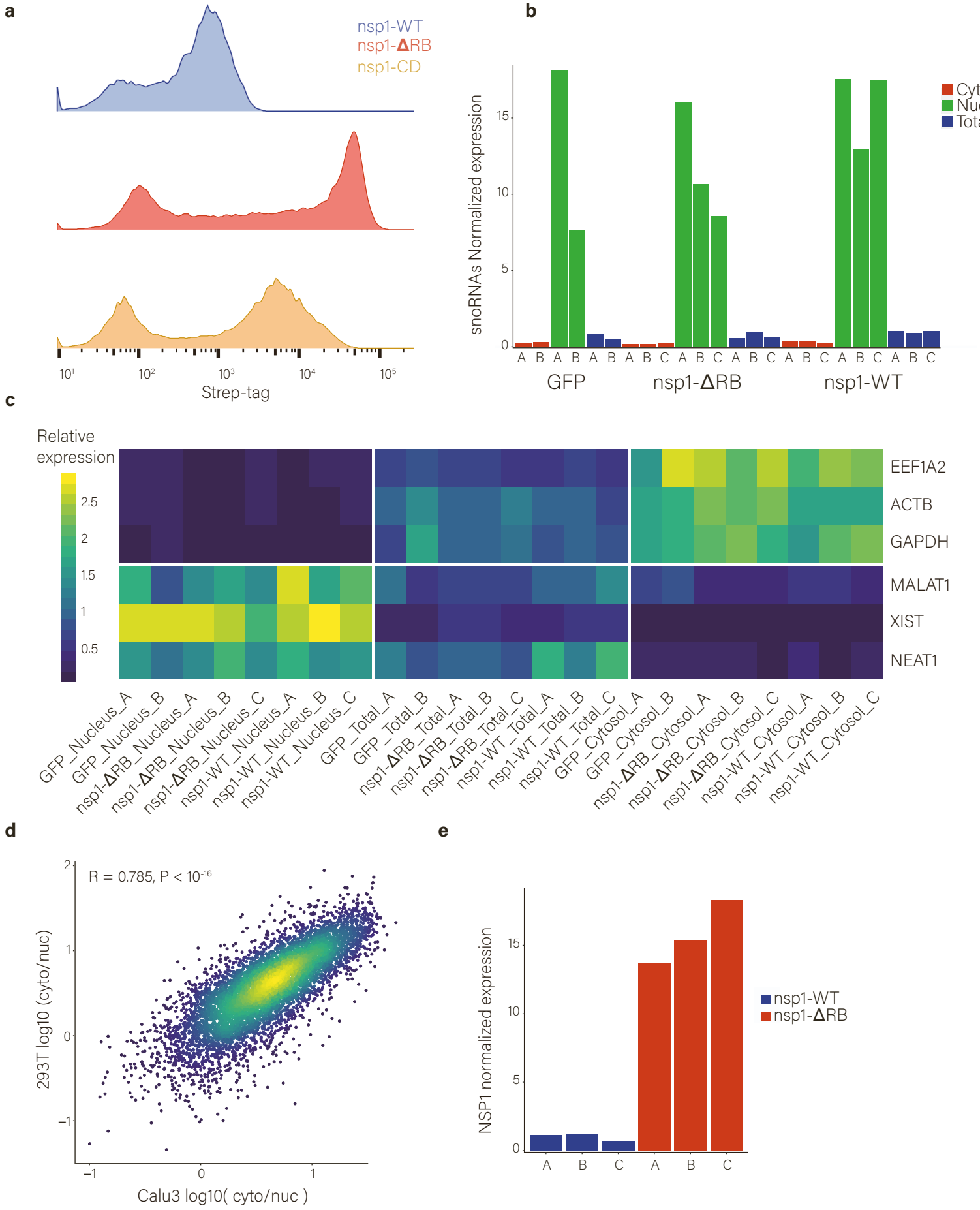
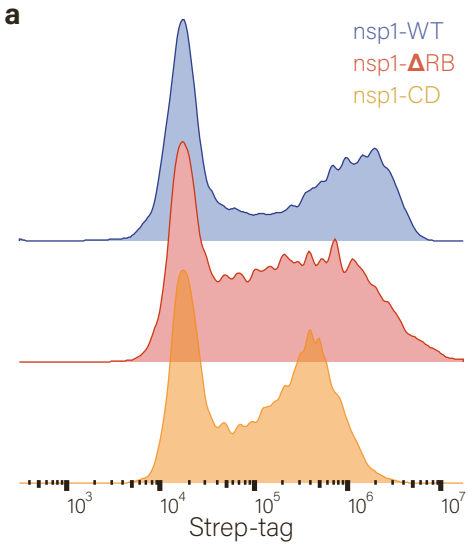
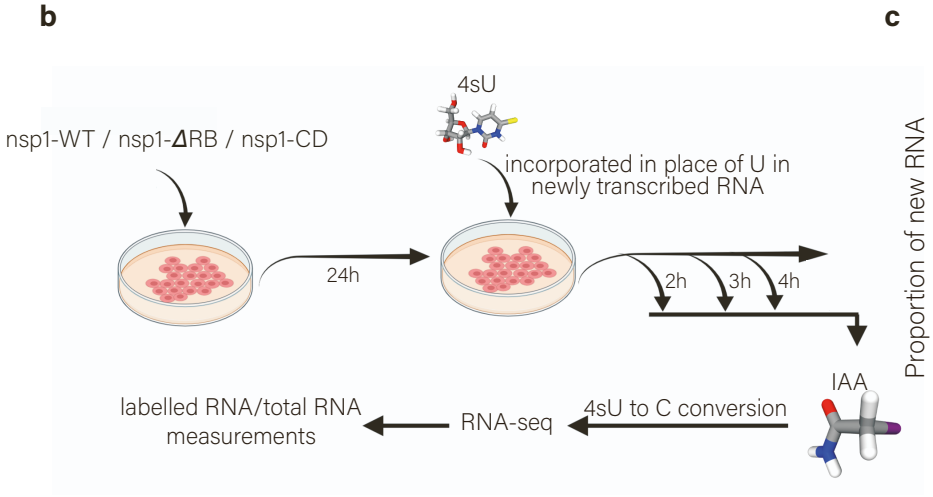
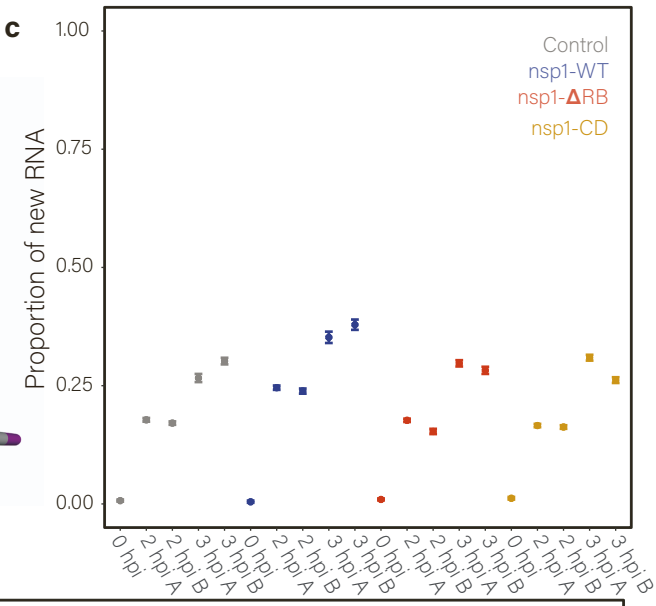

a
nsp1-WT
nsp1-ΔRB
nsp1-CD
Strep-tag
b
snoRNAs Normalized expression
Cyto
Nuc
Total
GFP
nsp1-ΔRB
nsp1-WT
c
Relative expression
EEF1A2
ACTB
GAPDH
MALAT1
XIST
NEAT1
GFP_Nucleus_A
GFP_Nucleus_B
nsp1-ΔRB_Nucleus_A
nsp1-ΔRB_Nucleus_B
nsp1-ΔRB_Nucleus_C
nsp1-WT_Nucleus_A
nsp1-WT_Nucleus_B
nsp1-WT_Nucleus_C
GFP_Total_A
GFP_Total_B
nsp1-ΔRB_Total_A
nsp1-ΔRB_Total_B
nsp1-ΔRB_Total_C
nsp1-WT_Total_A
nsp1-WT_Total_B
nsp1-WT_Total_C
GFP_Cytosol_A
GFP_Cytosol_B
nsp1-ΔRB_Cytosol_A
nsp1-ΔRB_Cytosol_B
nsp1-ΔRB_Cytosol_C
nsp1-WT_Cytosol_A
nsp1-WT_Cytosol_B
nsp1-WT_Cytosol_C
d
R = 0.785, P < 10-16
293T log10 (cyto/nuc)
Calu3 log10(cyto/nuc)
e
NSP1 normalized expression
nsp1-WT
nsp1-ΔRB

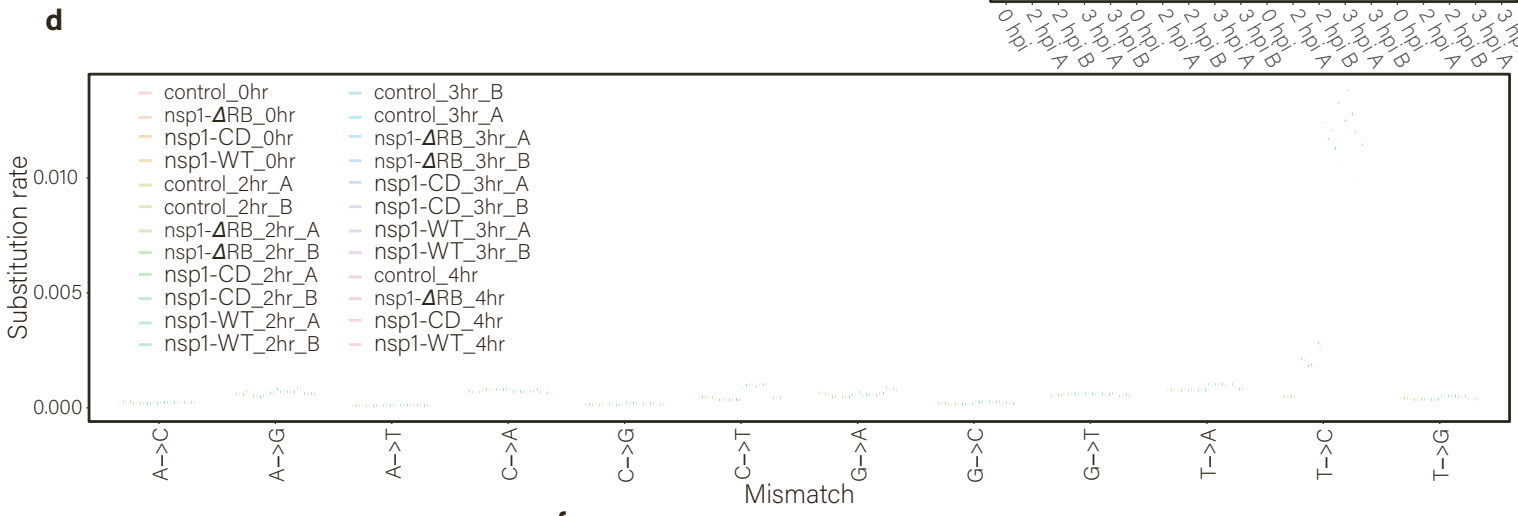
Figure S1. Nsp1 inhibits its own expression, related to Figures 1 and 2.

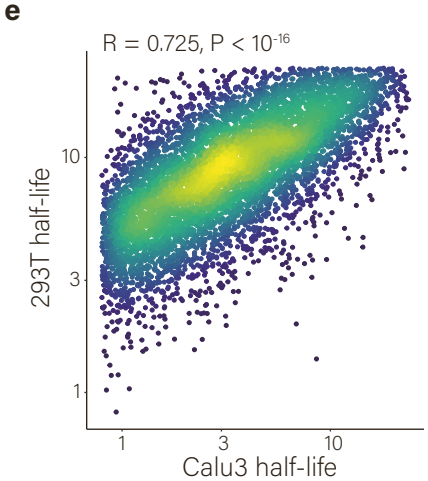
a. Flow cytometry analysis of the Strep tag expression in cells transfected with nsp1-WT, nsp1-ΔRB, or nsp1-CD containing strep-tag at the C-terminus. **b**. Normalized sum of the expression of snoRNAs in the nuclear (Nuc), cytosolic (Cyto), and total fractions of 293T cells transfected with nsp1-WT, nsp1-ΔRB, or GFP with A, B and C representing biological replicates **c**. Heatmap showing the relative expression of cellular mRNAs (EEF1A2, ACTB, GAPDH) and nuclear lncRNAs (MALAT1, XIST, NEAT1) in the nuclear (left panels), cytosolic (right panels), and total (middle panels) fractions of 293T cells transfected with nsp1-WT, nsp1-ΔRB, or GFP. **d**. Scatter plot of transcript cyto/nuc ratios calculated from fractionation measurements in 293T cells transfected with plasmid expressing GFP relative to cyto/nuc ratios measured in Calu3 cells (Finkel et al., 2021a). R (pearson) and two-sided P value are presented. **e**. Expression of transcripts encoding nsp1 calculated from RNA-seq data. Expression of nsp1-WT transcripts or nsp1-ΔRB transcripts was normalized to NEAT1 RNA (a nuclear long non coding RNA), and nsp1-ΔRB expression relative to the mean of nsp1-WT samples are presented.


a
nsp1-WT
nsp1-ΔRB
nsp1-CD
10^3
10^4
10^5
10^6
10^7
Strep-tag


b
4sU
nsp1-WT / nsp1-ΔRB / nsp1-CD
incorporated in place of U in
newly transcribed RNA
24h
2h
3h
4h
IAA
labelled RNA/total RNA
measurements
RNA-seq
4sU to C conversion


c
Control
nsp1-WT
nsp1-ΔRB
nsp1-CD
Proportion of new RNA
1.00
0.75
0.50
0.25
0.00
0 hpi
2 hpi A
2 hpi B
3 hpi A
3 hpi B


d
control_0hr
nsp1-ΔRB_0hr
nsp1-CD_0hr
nsp1-WT_0hr
control_2hr_A
control_2hr_B
nsp1-ΔRB_2hr_A
nsp1-ΔRB_2hr_B
nsp1-CD_2hr_A
nsp1-CD_2hr_B
nsp1-WT_2hr_A
nsp1-WT_2hr_B
control_3hr_B
control_3hr_A
nsp1-ΔRB_3hr_A
nsp1-ΔRB_3hr_B
nsp1-CD_3hr_A
nsp1-CD_3hr_B
nsp1-WT_3hr_A
nsp1-WT_3hr_B
control_4hr
nsp1-ΔRB_4hr
nsp1-CD_4hr
nsp1-WT_4hr
Substitution rate
0.010
0.005
0.000
A->C
A->G
A->T
C->A
C->G
C->T
G->A
G->C
G->T
T->A
T->C
T->G
Mismatch


e
R = 0.725, P < 10^-16
293T half-life
10
3
1
1
3
10
Calu3 half-life

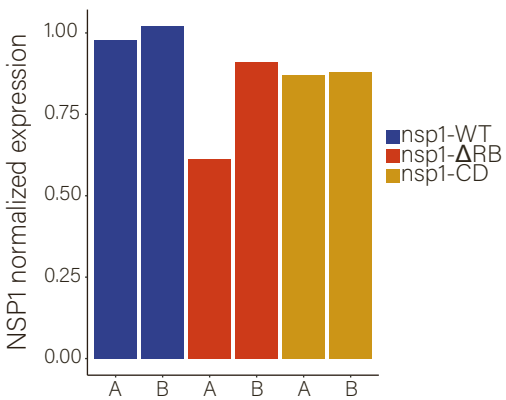

f
NSP1 normalized expression
1.00
0.75
0.50
0.25
0.00
A
B
A
B
A
B
nsp1-WT
nsp1-ΔRB
nsp1-CD

Figure S2. Subcellular fractionation and SLAM-seq measurements in nsp1 expressing cells, related to Figure 2.

a. Flow cytometry analysis of the Strep tag-positive cells, in cells transfected with plasmid expressing nsp1-WT, nsp1-ΔRB, or nsp1-CD, all of which were cloned downstream to the CoV2 viral 5' leader sequence. **b**. Experimental design of the SLAM-seq measurements and the half-life calculation. **c.** Rates of nucleotide substitutions demonstrate efficient conversion rates in 4sU-treated samples compared to non-treated cells (no 4sU). **d**. Proportion of new-to-total RNA (NTR) in 293T cells transfected with plasmid expressing GFP, nsp1-WT, nsp1-ΔRB, or nsp1-CD as calculated by GRAND-SLAM(Jürges et al., 2018). **e**. Scatter plot of transcript half-lives calculated from SLAM-seq measurements in 293T cells transfected with plasmid expressing GFP relative to transcript half-lives in Calu3 cells(Finkel et al., 2021a). Pearson's R and two-sided P-value are presented. **f**. Expression levels of nsp1 transcripts calculated from RNA-seq data. Nsp1 expression in each of the conditions (nsp1-WT, nsp1-ΔRB, or nsp1-CD) was normalized to NEAT1 expression, and nsp1 expression relative to the mean of nsp1-WT samples are presented.

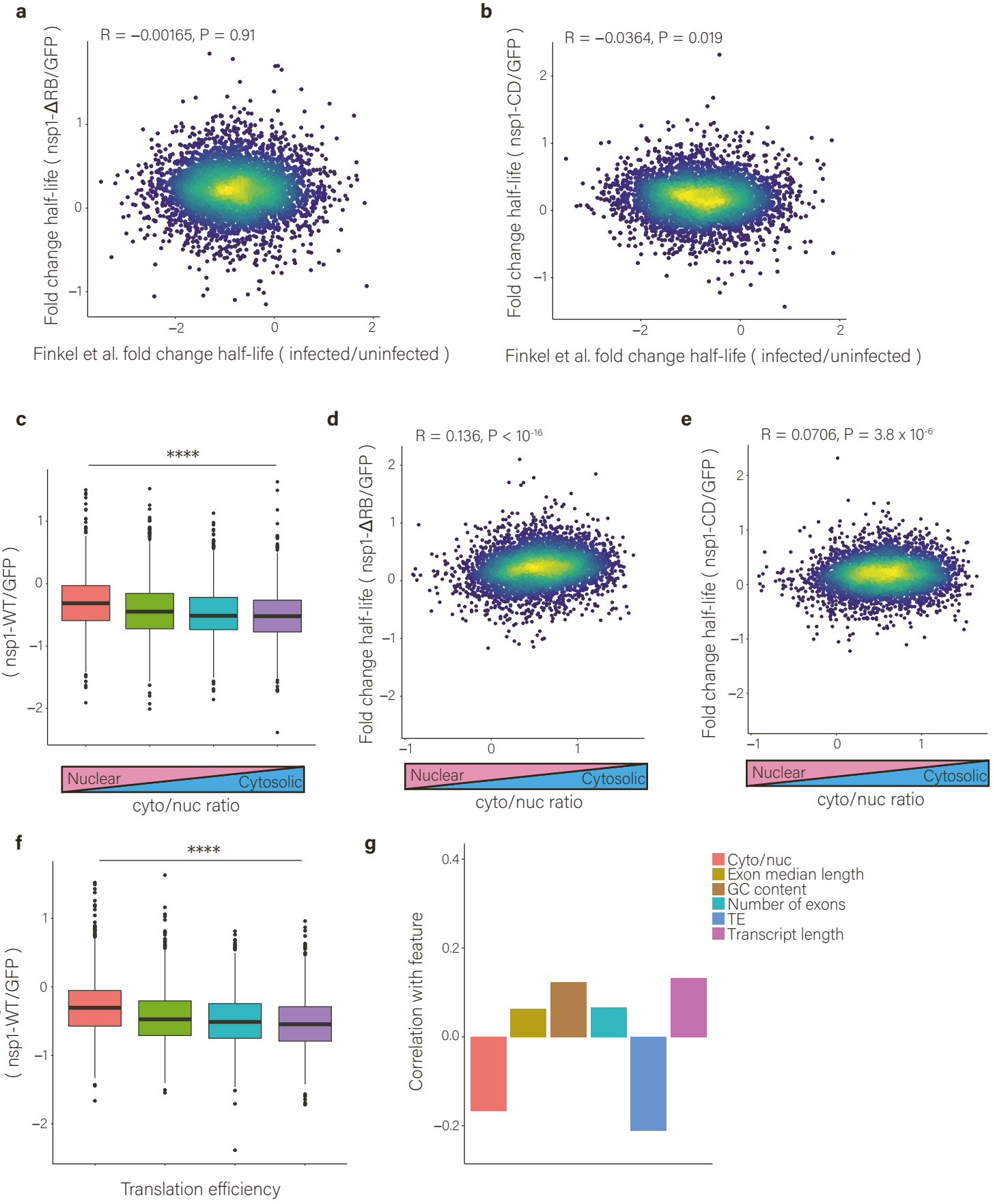

a
R = −0.00165, P = 0.91
Fold change half-life (nsp1-ΔRB/GFP)
Finkel et al. fold change half-life (infected/uninfected)
b
R = −0.0364, P = 0.019
Fold change half-life (nsp1-CD/GFP)
Finkel et al. fold change half-life (infected/uninfected)
c
****
Fold change half-life (nsp1-WT/GFP)
Nuclear
Cytosolic
cyto/nuc ratio
d
R = 0.136, P < 10-16
Fold change half-life (nsp1-ΔRB/GFP)
Nuclear
Cytosolic
cyto/nuc ratio
e
R = 0.0706, P = 3.8 x 10-6
Fold change half-life (nsp1-CD/GFP)
Nuclear
Cytosolic
cyto/nuc ratio
f
****
Fold change half-life (nsp1-WT/GFP)
Translation efficiency
g
Correlation with feature
Cyto/nuc
Exon median length
GC content
Number of exons
TE
Transcript length

Figure S3. Analysis of gene expression in cells expressing nsp1-ΔRB or nsp1-CD, related to Figure 2.

a and b. Scatter plot of fold change in transcript half-lives in 293T cells transfected with plasmid expressing nsp1-ΔRB (**a**) or nsp1-CD (**b**) relative to those transfected with plasmid expressing GFP and the fold change transcript half-lives in uninfected and infected cells(Finkel et al., 2021a). Pearson's R and two-sided P values are presented. **c**. The nsp1-WT/GFP half-life fold-change compared to the cytosolic/nuclear ratio, divided into 4 bins. P values for two tailed t-tests between extreme bins are presented, **** = $p<0.0001$. **d and e**. Scatter plot of fold change in transcript half-lives in 293T cells transfected with a plasmid expressing nsp1-ΔRB (**d**) or nsp1-CD (**e**) relative to those transfected with a plasmid expressing GFP and cyto/nuc ratios of uninfected cells (Finkel et al., 2021a). Pearson's R and two-sided P values are presented. **f**. The nsp1-WT/GFP half-life fold-change compared to translation efficiency, divided into 4 bins. P values for two tailed t-tests between extreme bins are presented. **g**. Correlation between gene features and the fold change of transcript half-lives between 293T transfected with a plasmid expressing nsp1-WT or control plasmid.

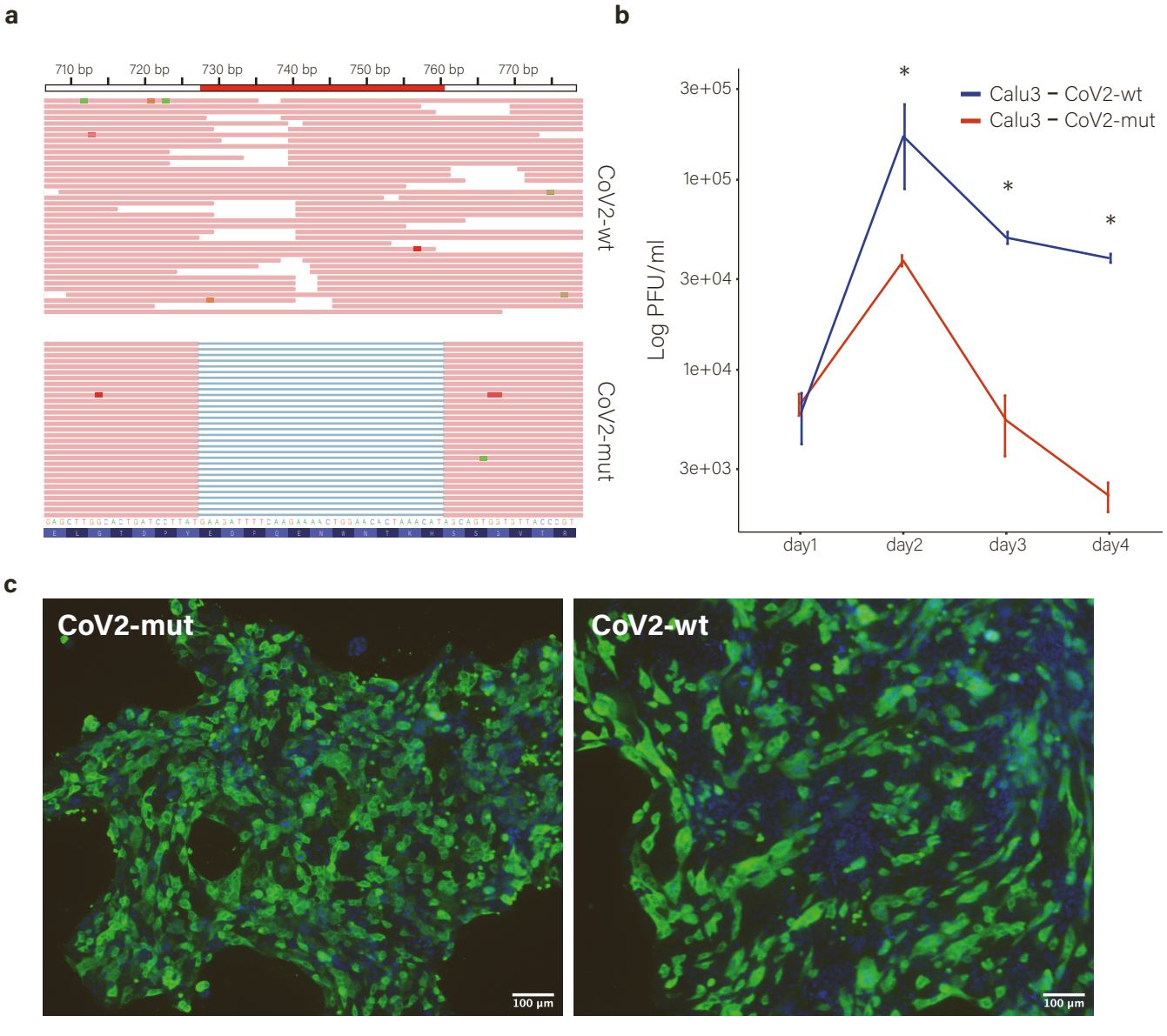

a
710 bp
720 bp
730 bp
740 bp
750 bp
760 bp
770 bp
CoV2-wt
CoV2-mut
b
Log PFU/ml
3e+05
1e+05
3e+04
1e+04
3e+03
Calu3 – CoV2-wt
Calu3 – CoV2-mut
*
*
*
day1
day2
day3
day4
c
CoV2-mut
CoV2-wt
100 µm
100 µm

Figure S4. CoV2-mut characterization, related to Figure 3.

a, RNA reads spanning the deletion region in nsp1 (nt 728-760) in the SARS-CoV-2-WT (CoV2-wt) or in SARS-CoV-2 carrying nsp1-ΔRB mutant (CoV2-mut). **b**. Viral titers in supernatant collected from Calu3 cells infected with CoV2-wt or with CoV2-mut (MOI=0.01) at 1, 2, 3, and 4 dpi. The titers of the CoV2-wt infection were higher than the titers of the CoV2-mut infection at all timepoints (two tailed t-test, where * = $p<0.01$). Three replicates are presented. **c**. Microscopy images of Calu3 cells infected with CoV2-wt or CoV2-mut at an MOI of 3 at 8hpi stained with antisera against SARS-CoV-2 (green) and DAPI (blue).

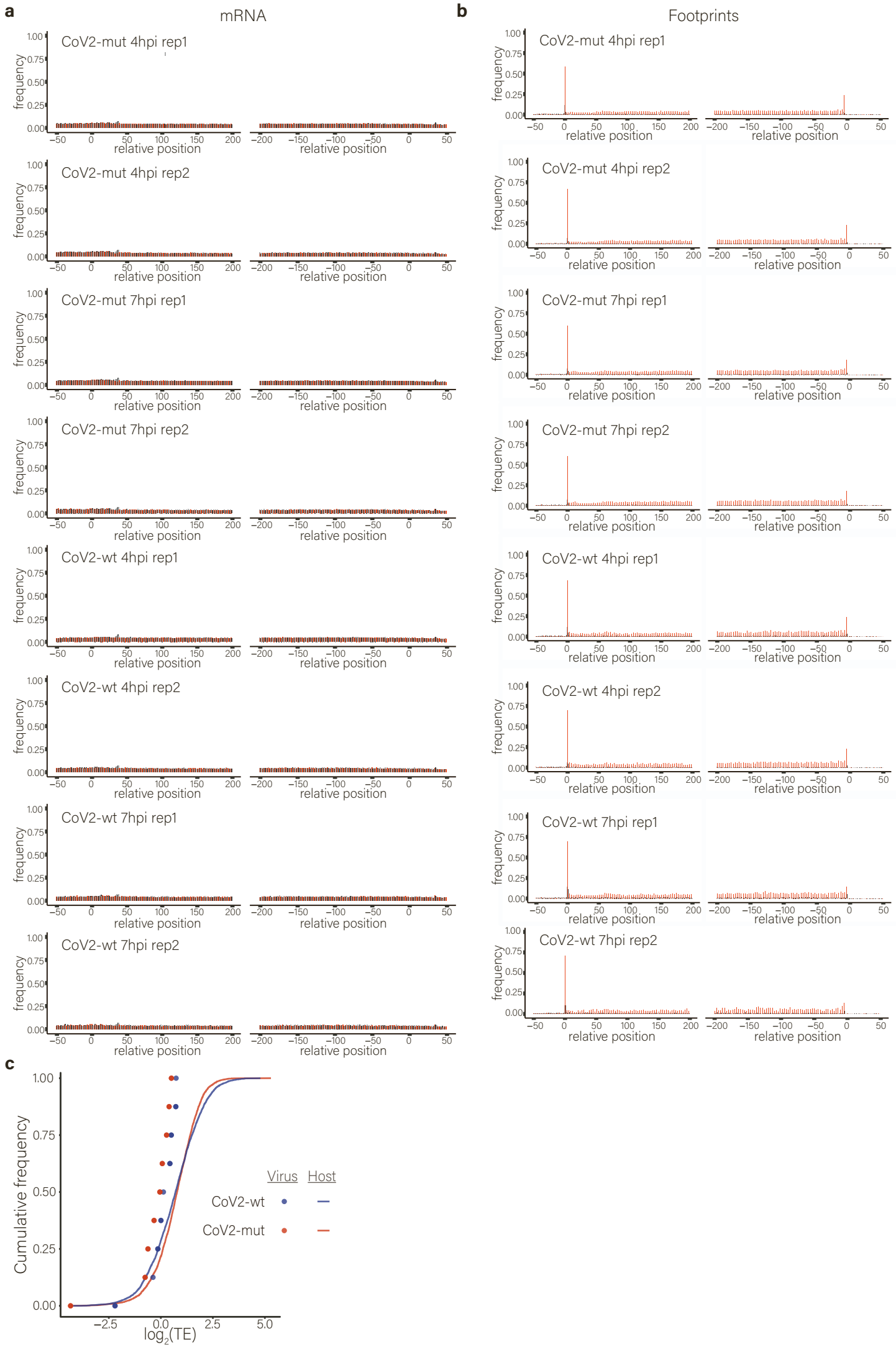
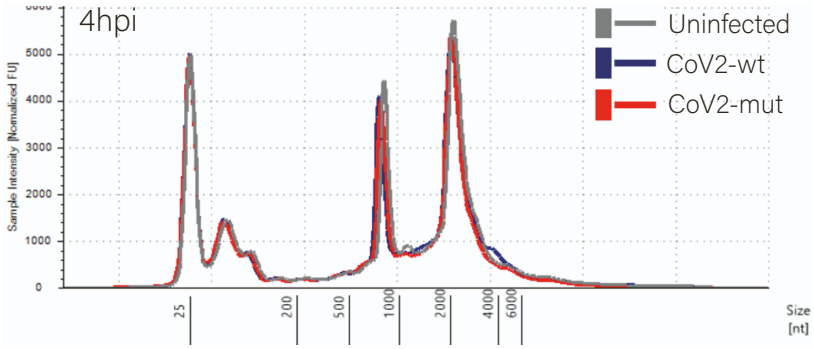

a
mRNA
b
Footprints
CoV2-mut 4hpi rep1
CoV2-mut 4hpi rep2
CoV2-mut 7hpi rep1
CoV2-mut 7hpi rep2
CoV2-wt 4hpi rep1
CoV2-wt 4hpi rep2
CoV2-wt 7hpi rep1
CoV2-wt 7hpi rep2
frequency
relative position
c
Cumulative frequency
log2(TE)
Virus
Host
CoV2-wt
CoV2-mut

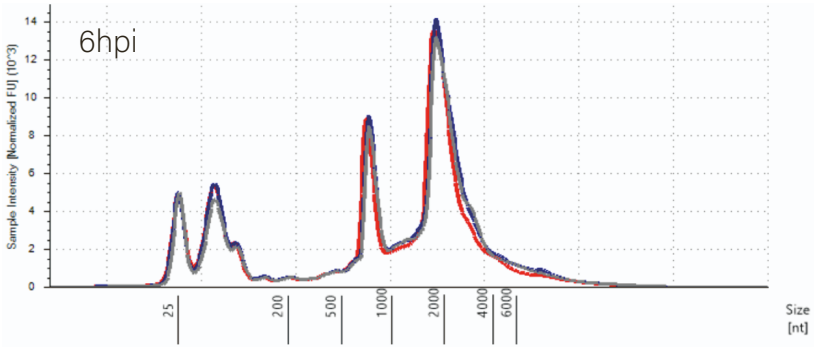
Figure S5. Ribosome profiling measurements in CoV2-wt-infected cells and Cov2-mut-infected cells, related to Figure 4.

a and b. Metagene analysis for the mRNA (**a**) and footprint (**b**) samples. **c**. Cumulative frequency of human (line) and viral (dots) genes according to their relative TE in cells infected with CoV2-wt (blue) or with CoV2-mut (red) at 7 hpi. TE was calculated from ribosome profiling in parallel to mRNA sequencing, and is defined as the ratio of ribosome footprints to mRNA for a given gene. Each dot represents one of nine major viral mRNA species.

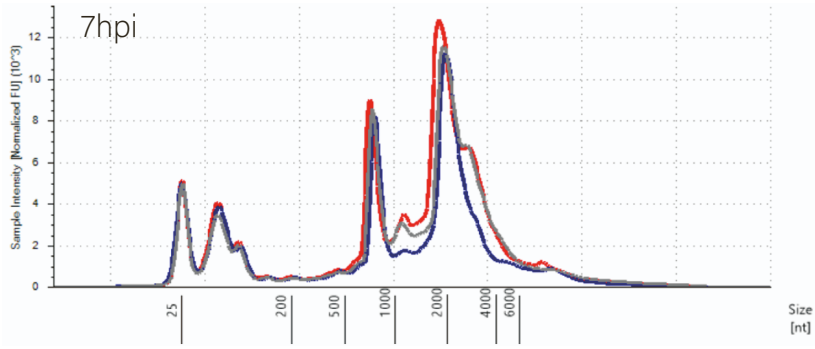
a


4hpi
Uninfected
CoV2-wt
CoV2-mut
Sample Intensity [Normalized FU]
Size [nt]

b


6hpi
Sample Intensity [Normalized FU] (10^-3)
Size [nt]

c


7hpi
Sample Intensity [Normalized FU] (10^-3)
Size [nt]

d

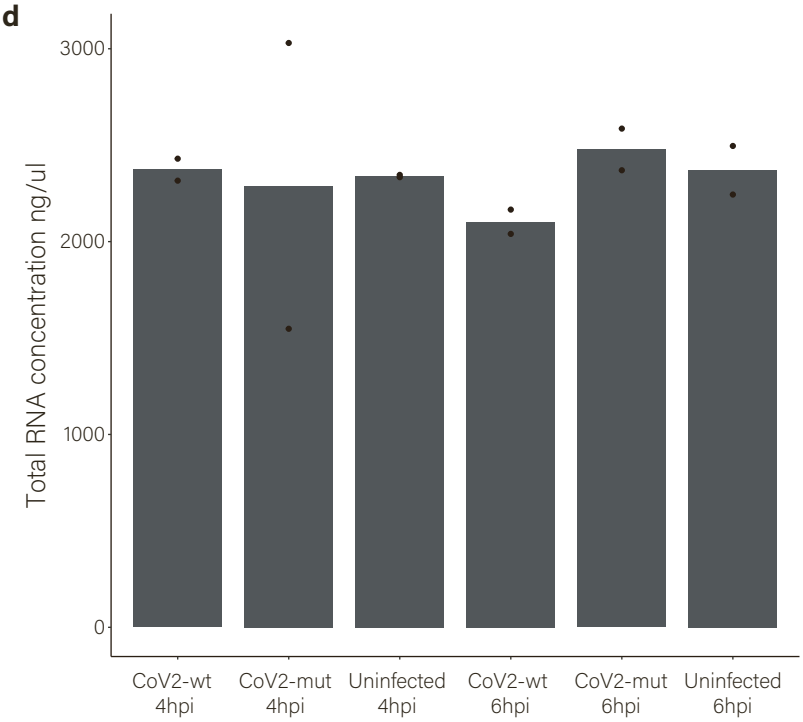

Total RNA concentration ng/ul
CoV2-wt 4hpi
CoV2-mut 4hpi
Uninfected 4hpi
CoV2-wt 6hpi
CoV2-mut 6hpi
Uninfected 6hpi

Figure S6. Amounts of RNA in CoV2-wt and CoV2-mut infected cells, related to Figure 5.

a-c. The concentration of the 18S and 28S ribosomal RNAs were measured in uninfected Calu3 cells and Calu3 cells infected with CoV2-wt or CoV2-mut at 4 hpi (a), 6 hpi (b), or 7 hpi (c) by using a TapeStation system. **d.** Total RNA was measured in uninfected Calu3 cells and in CoV2-wt or CoV2-mut infected Calu3 cells at 4 and 6 hpi by Qubit Fluorometer. Two replicates are presented.

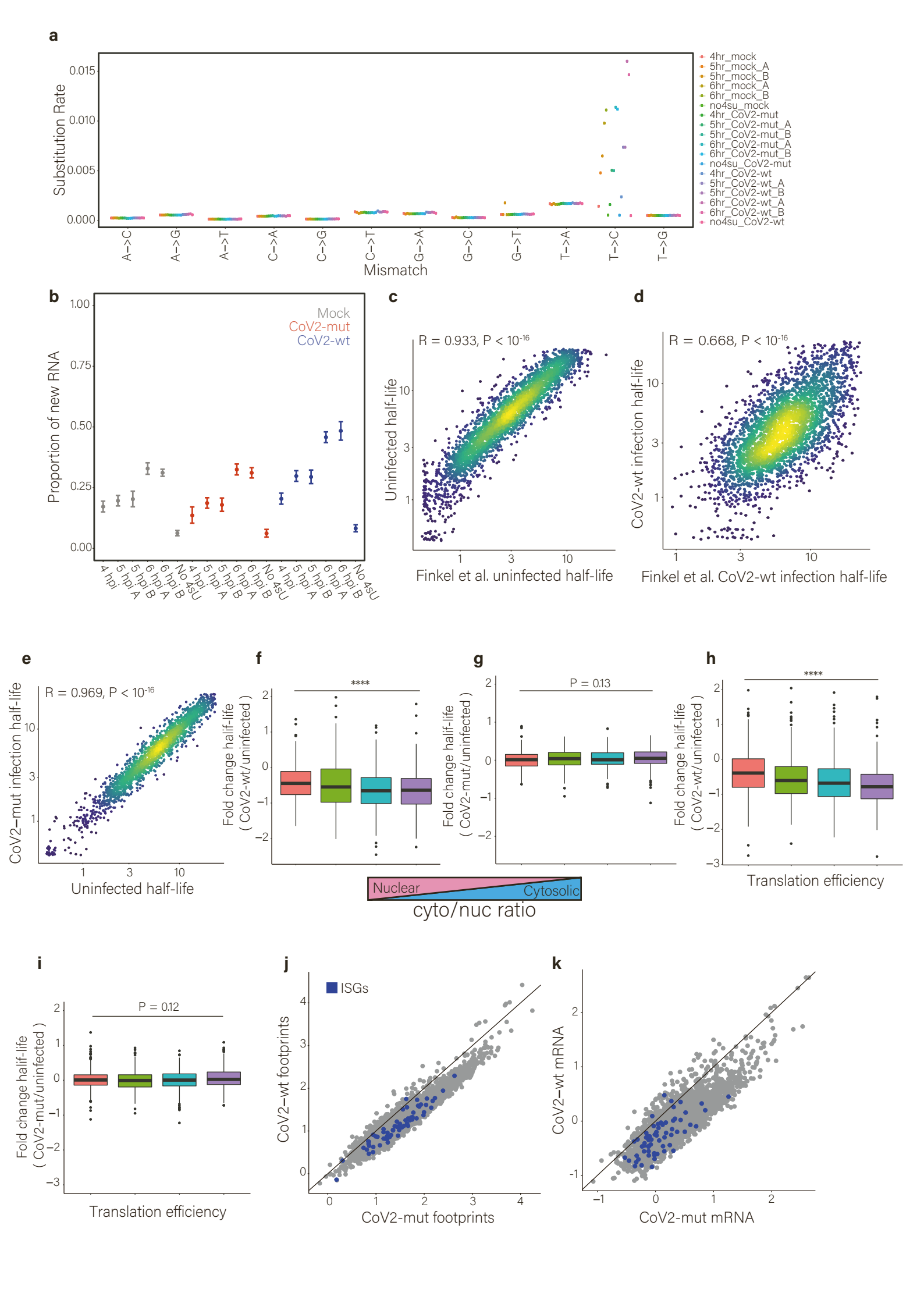

a
Substitution Rate
0.015
0.010
0.005
0.000
A->C
A->G
A->T
C->A
C->G
C->T
G->A
G->C
G->T
T->A
T->C
T->G
Mismatch
4hr_mock
5hr_mock_A
5hr_mock_B
6hr_mock_A
6hr_mock_B
no4su_mock
4hr_CoV2-mut
5hr_CoV2-mut_A
5hr_CoV2-mut_B
6hr_CoV2-mut_A
6hr_CoV2-mut_B
no4su_CoV2-mut
4hr_CoV2-wt
5hr_CoV2-wt_A
5hr_CoV2-wt_B
6hr_CoV2-wt_A
6hr_CoV2-wt_B
no4su_CoV2-wt
b
Proportion of new RNA
1.00
0.75
0.50
0.25
0.00
Mock
CoV2-mut
CoV2-wt
4 hpi
5 hpi A
5 hpi B
6 hpi A
6 hpi B
No 4sU
c
R = 0.933, P < 10-16
Uninfected half-life
Finkel et al. uninfected half-life
d
R = 0.668, P < 10-16
CoV2-wt infection half-life
Finkel et al. CoV2-wt infection half-life
e
R = 0.969, P < 10-16
CoV2−mut infection half-life
Uninfected half-life
f
****
Fold change half-life (CoV2-wt/uninfected)
g
P = 0.13
Fold change half-life (CoV2-mut/uninfected)
Nuclear
Cytosolic
cyto/nuc ratio
h
****
Fold change half-life (CoV2-wt/uninfected)
Translation efficiency
i
P = 0.12
Fold change half-life (CoV2-mut/uninfected)
Translation efficiency
j
ISGs
CoV2−wt footprints
CoV2-mut footprints
k
CoV2−wt mRNA
CoV2-mut mRNA

Figure S7. SLAM-seq measurements in CoV2-wt-infected cells and CoV2-mut infected cells, related to Figure 5.

a. Rates of nucleotide substitutions demonstrate efficient conversion rates in 4sU-treated samples compared to non-treated cells (no 4sU). **b**. Proportion of new-to-total RNA (NTR) in Calu3 cells infected with CoV2-wt or CoV2-mut as calculated by GRAND-SLAM(Jürges et al., 2018). **c** and **d**. Scatter plot of transcript half-lives in (**c**) uninfected Calu3 and (**d**) CoV2-wt-infected cells relative to our previous half-lives measurements from uninfected and infected cells (Finkel et al., 2021a). Half-lives lower than 0.8 h or higher than 24h were omitted. Pearson's R and two-sided P value are presented. **e**. Scatter plot of transcript half-lives in CoV2-mut-infected Calu3 cells relative to half-lives in mock infected cells. Pearson's R and two-sided P values are presented. **f-i.** The CoV2-wt/uninfected (**f,h**) or CoV2-mut/uninfected (**g,i**) half-life fold-change compared to the cytosolic/nuclear ratio (**f,g**) or translation efficiency (**h,i**) which are divided into 4 bins. P values for two tailed t-tests between extreme bins are presented, **** = $p<0.0001$. **j-k**. Scatter plot of footprints (**j**) or mRNA (**k**) measurements in CoV2-wt-infected cells compared to CoV2-mut-infected cells. ISGs are marked in blue.