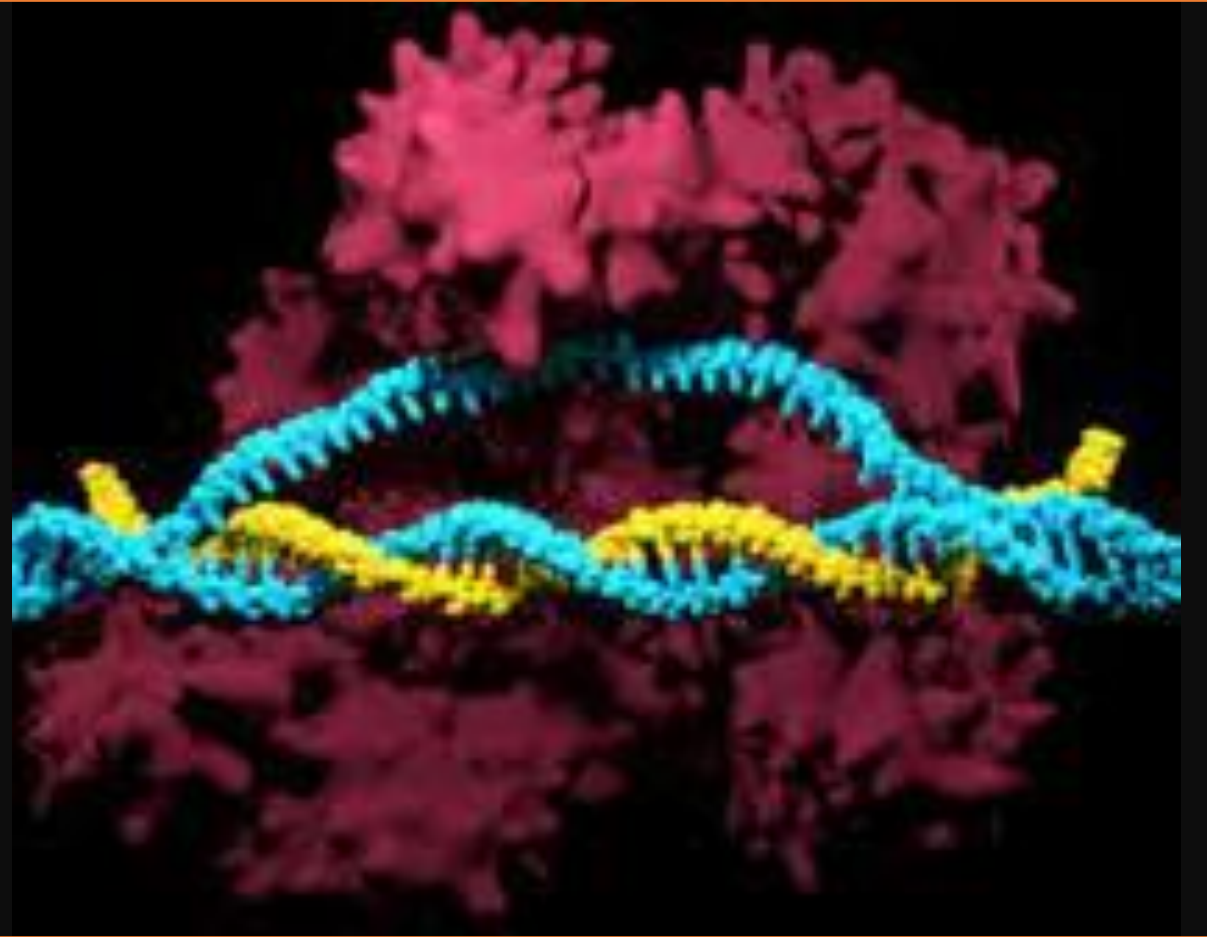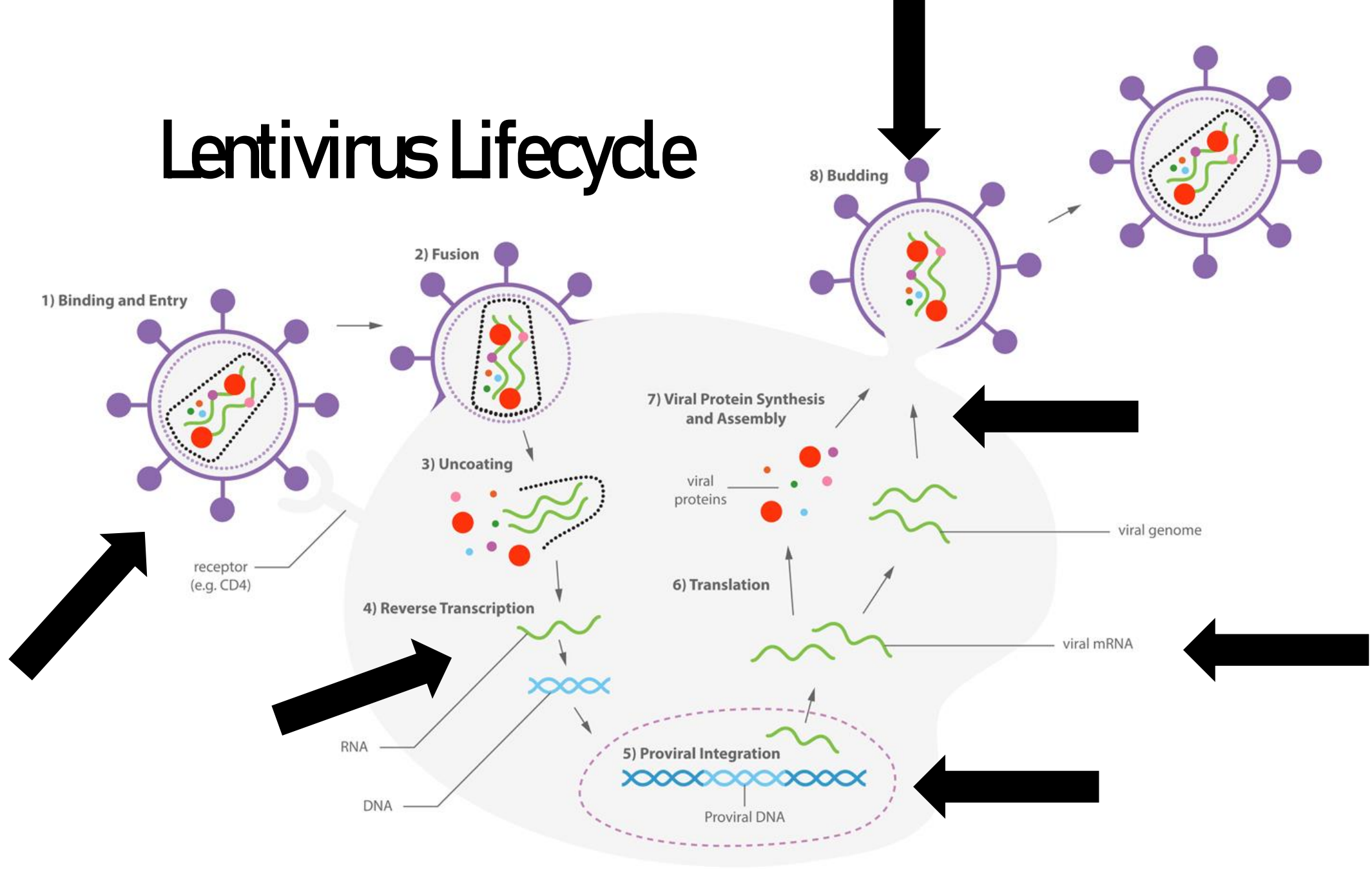A genome-wide CRISPR screen identifies host factors that regulate SARS-CoV-2 entry
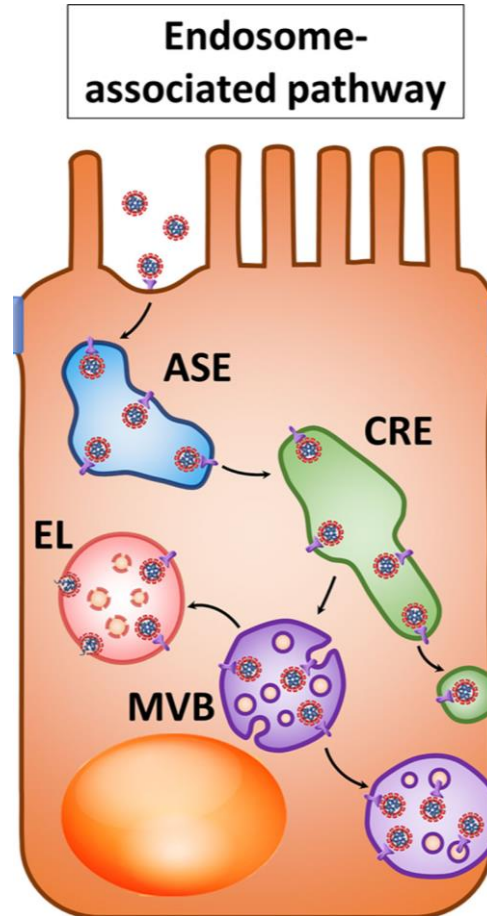
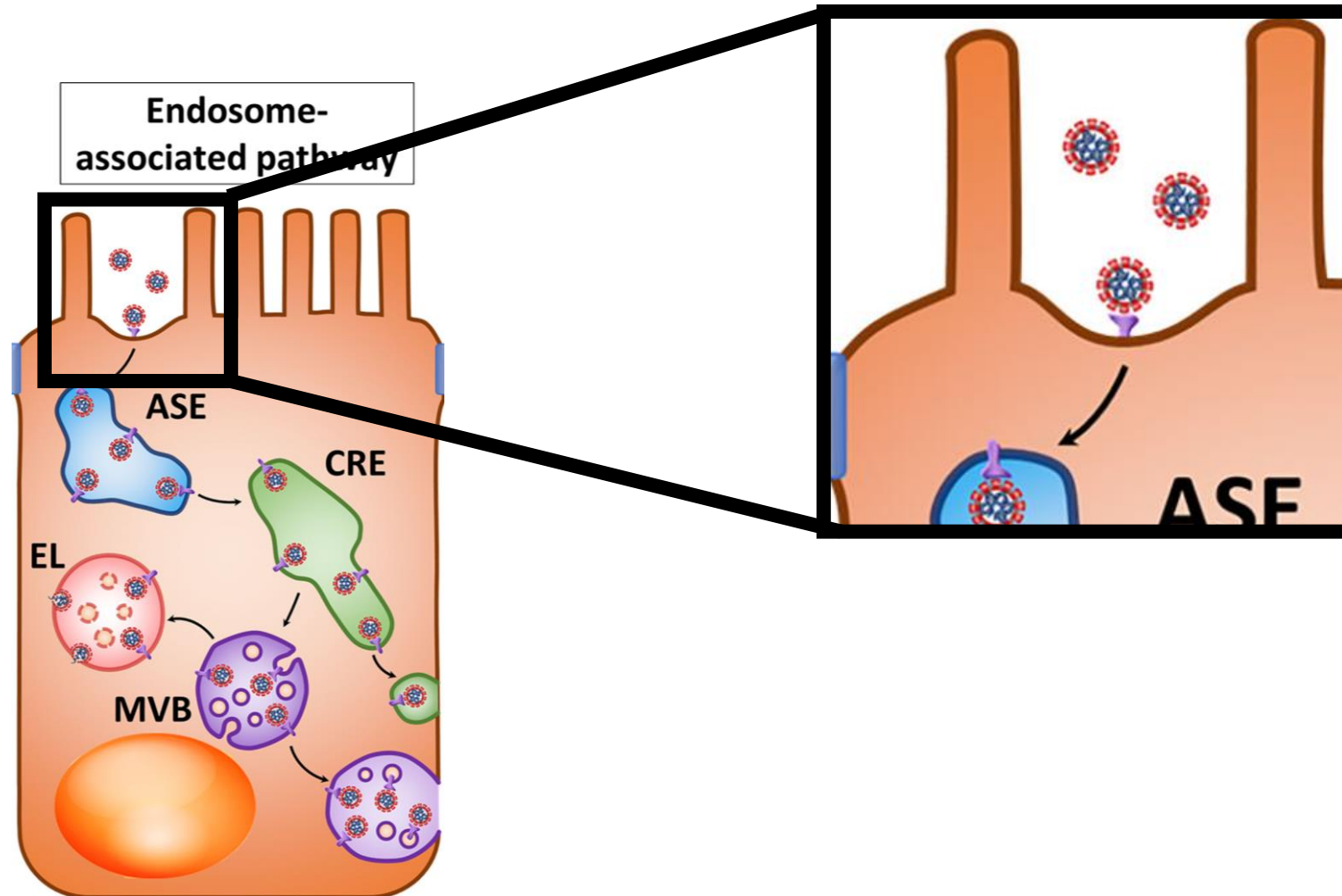THE LENTIVIRUS SYSTEM

Lentivirus Lifecycle

What is the SARS-CoV-2 Endosomal Pathway?

Knyazev et al.

What is the SARS-CoV-2 Endosomal Pathway?

Knyazev et al.

What is the SARS-CoV-2 Endosomal Pathway?
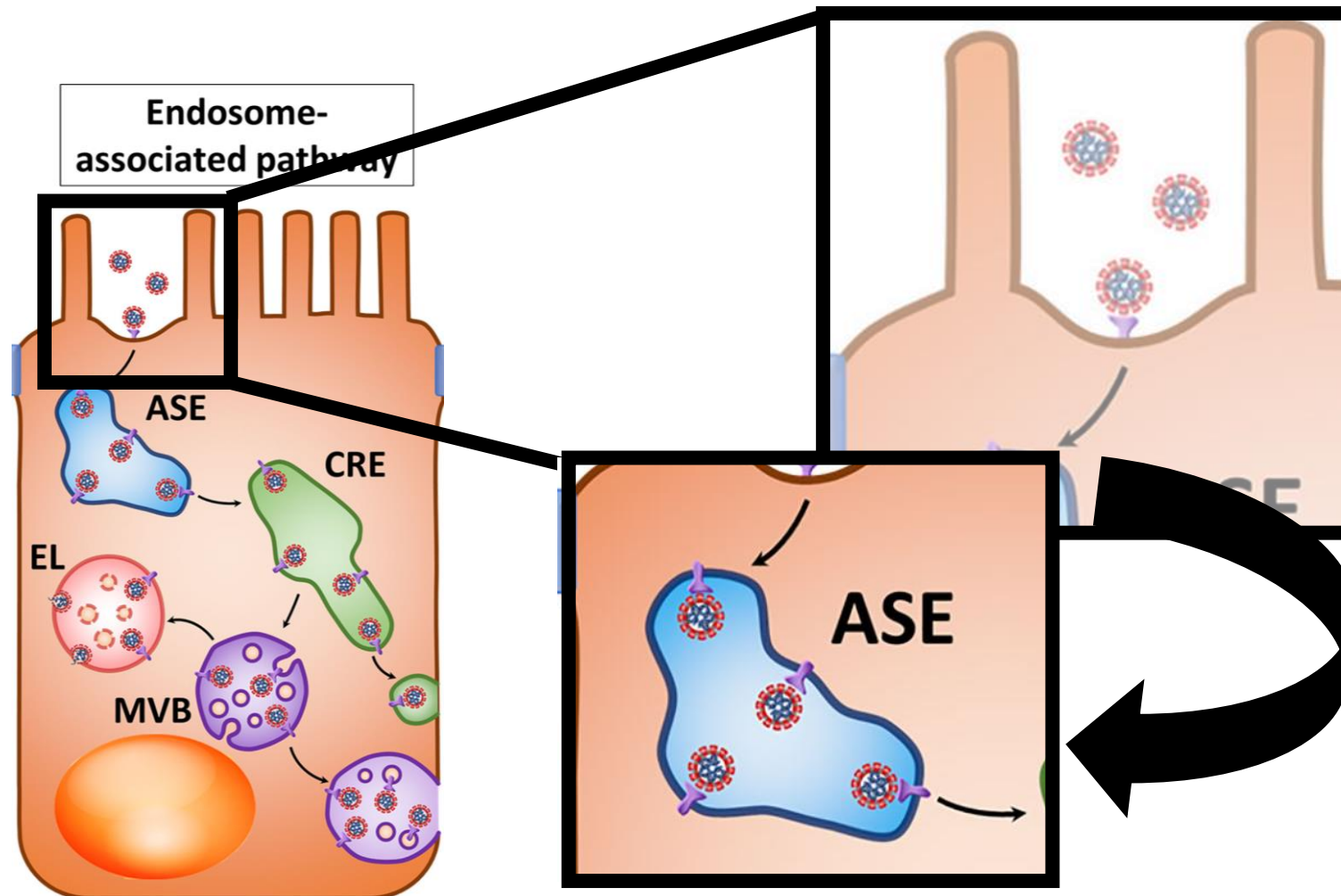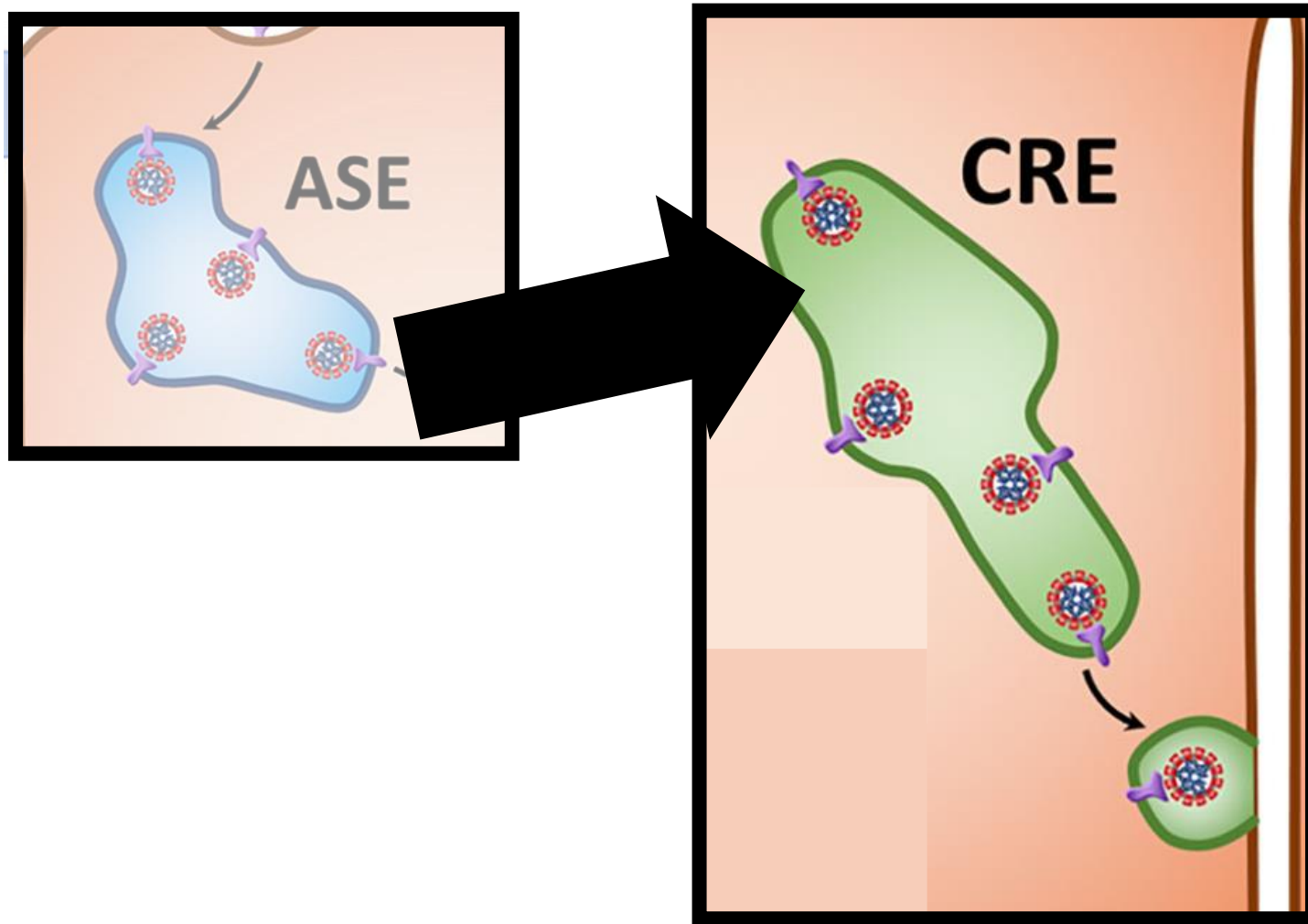

Knyazev et al.

What is the SARS-CoV-2 Endosomal Pathway?

Knyazev et al.

What is the SARS-CoV-2 Endosomal Pathway?

Knyazev et al.

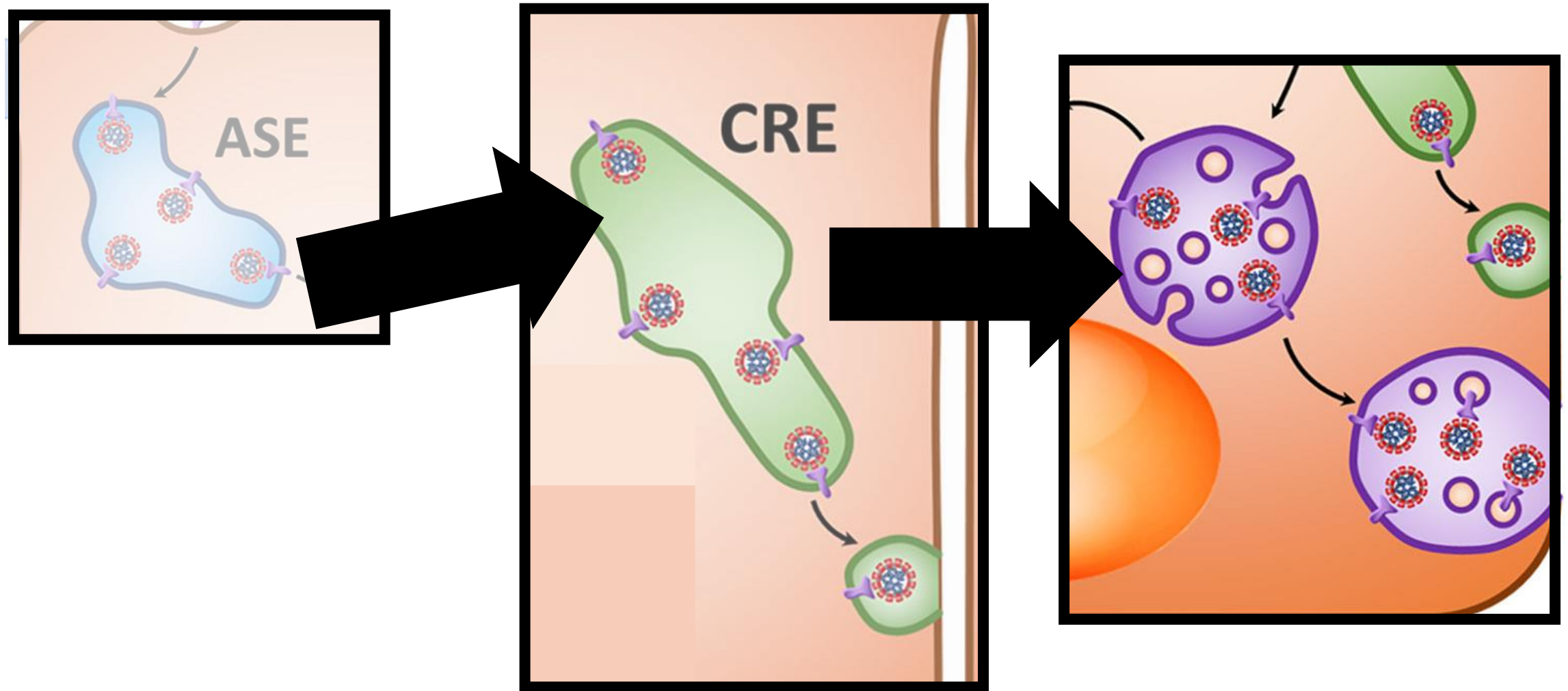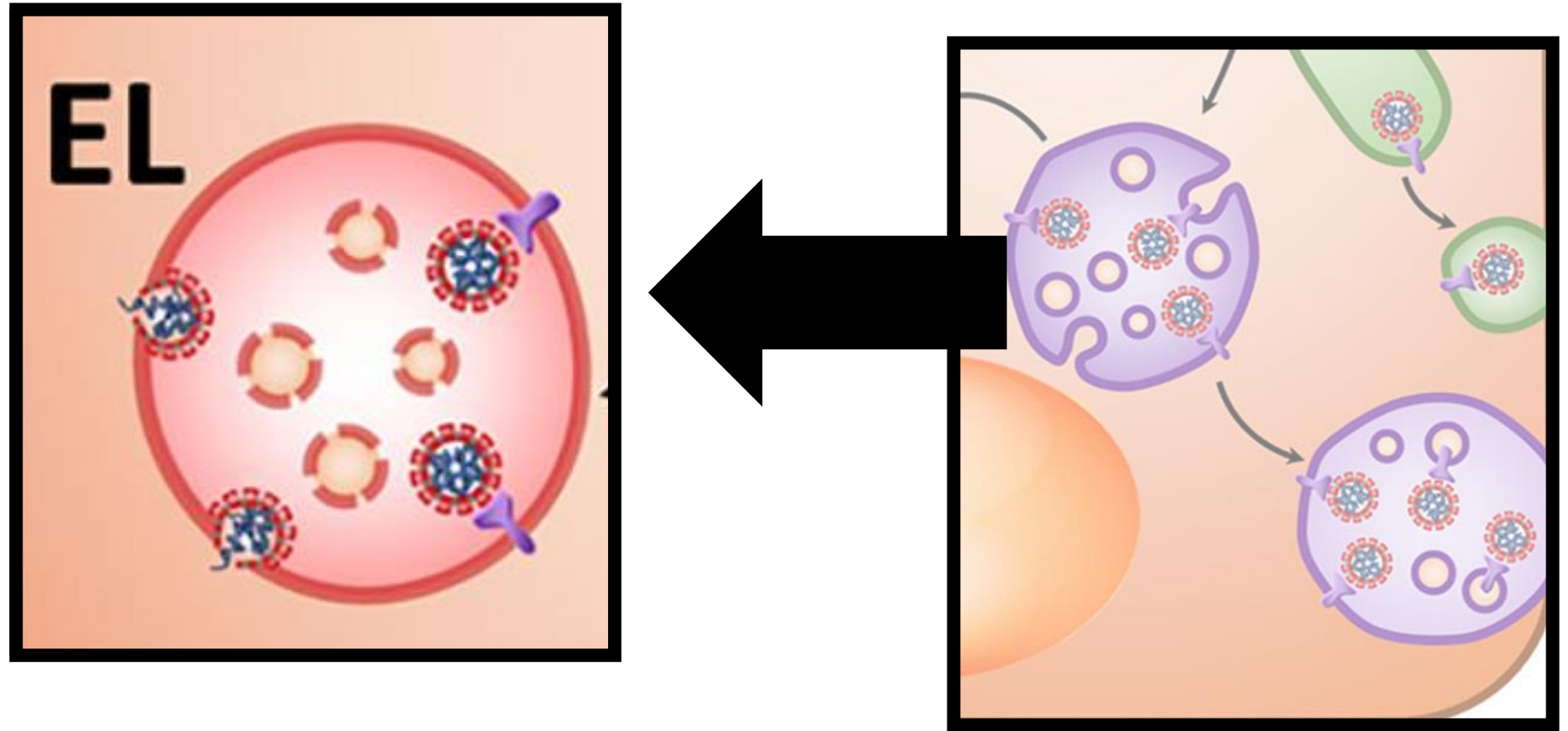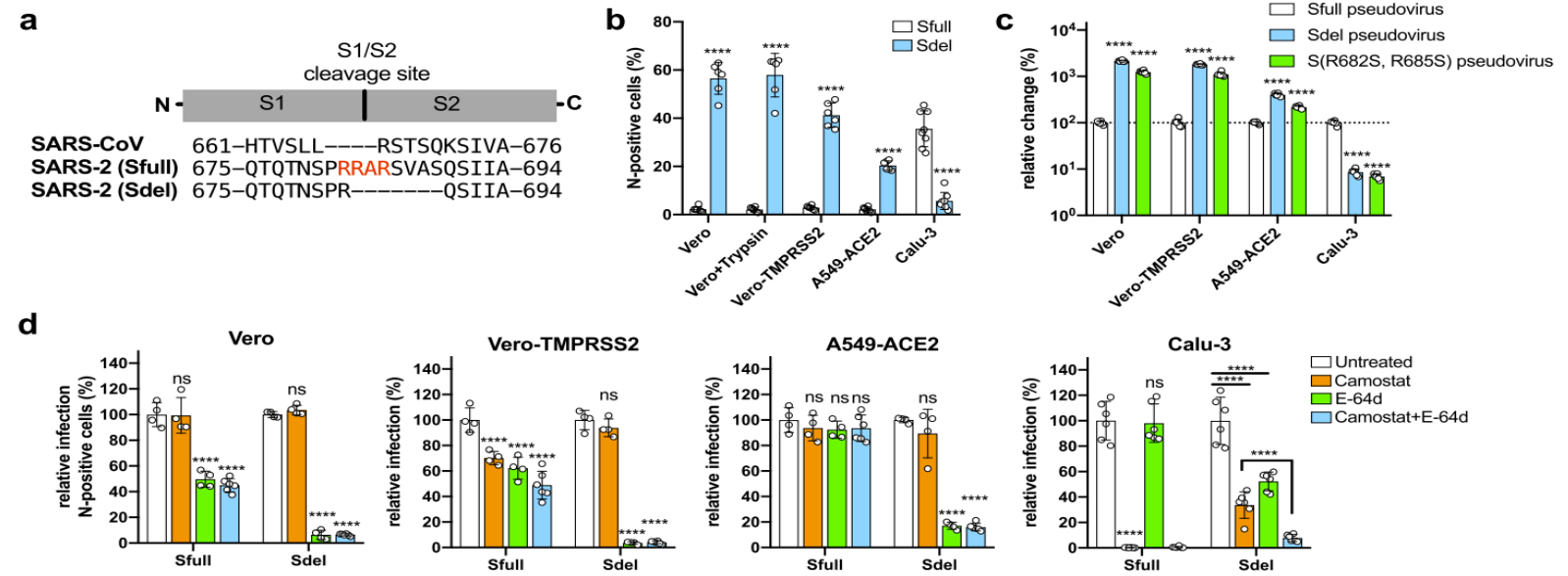# What is the SARS-CoV-2 Endosomal Pathway?

Knyazev et al.

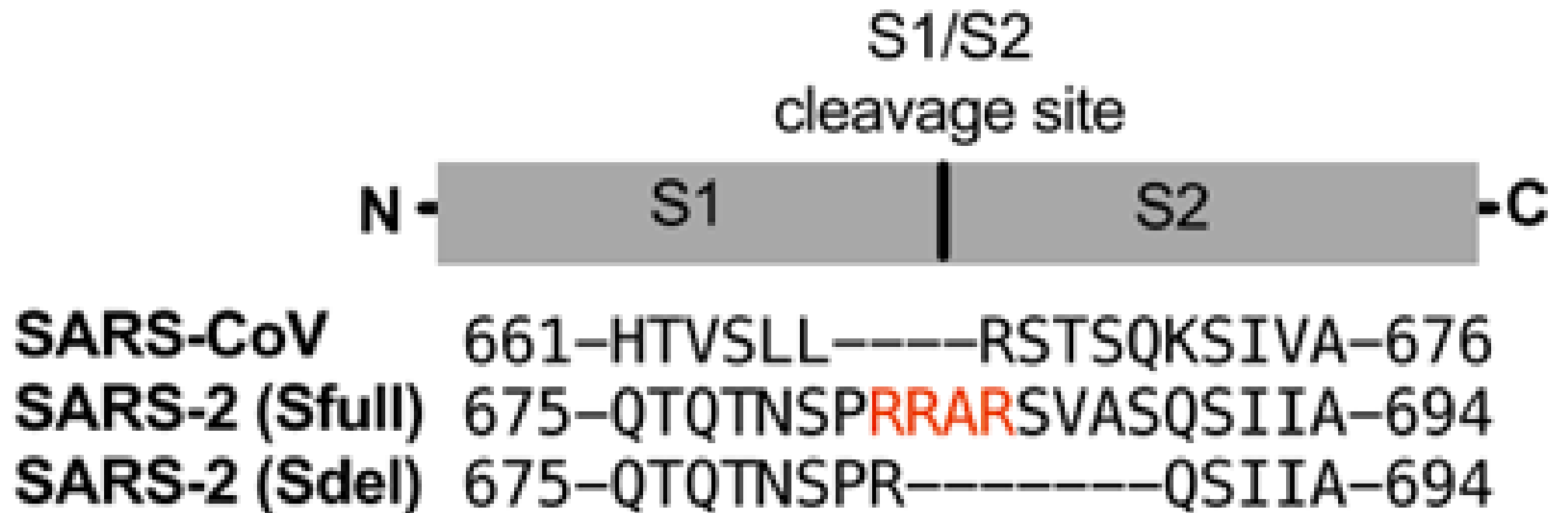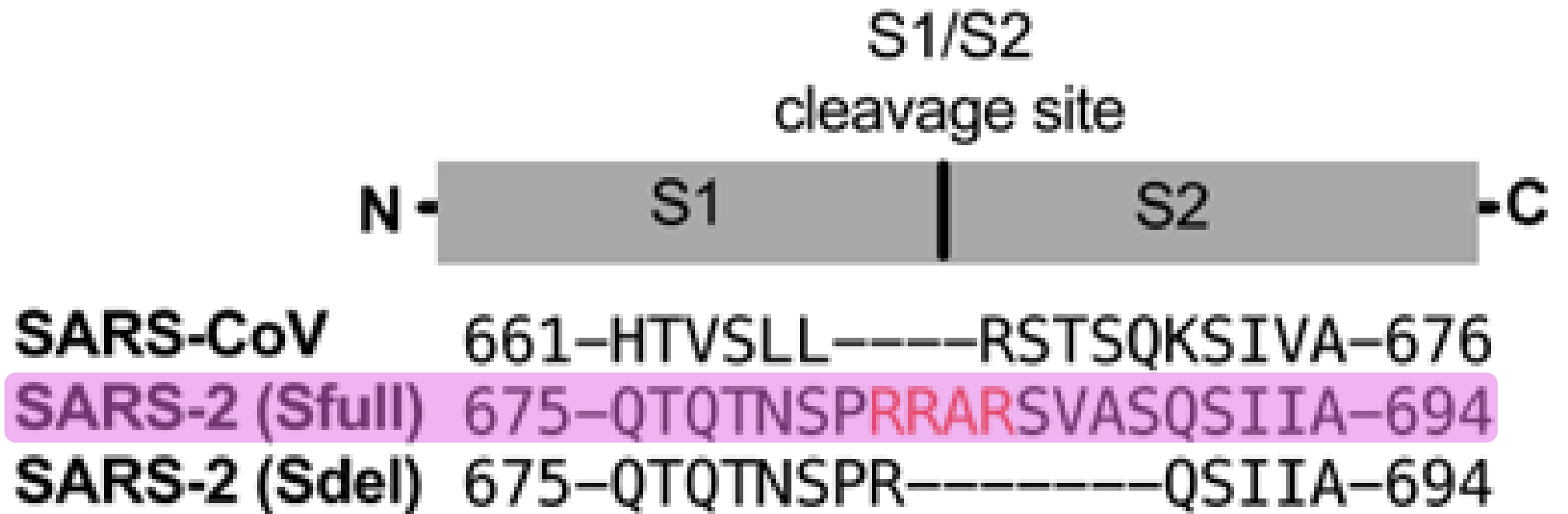# How does the deletion at S1/S2 boundary of spike protein propel virus to enter cells through endosomal pathway?
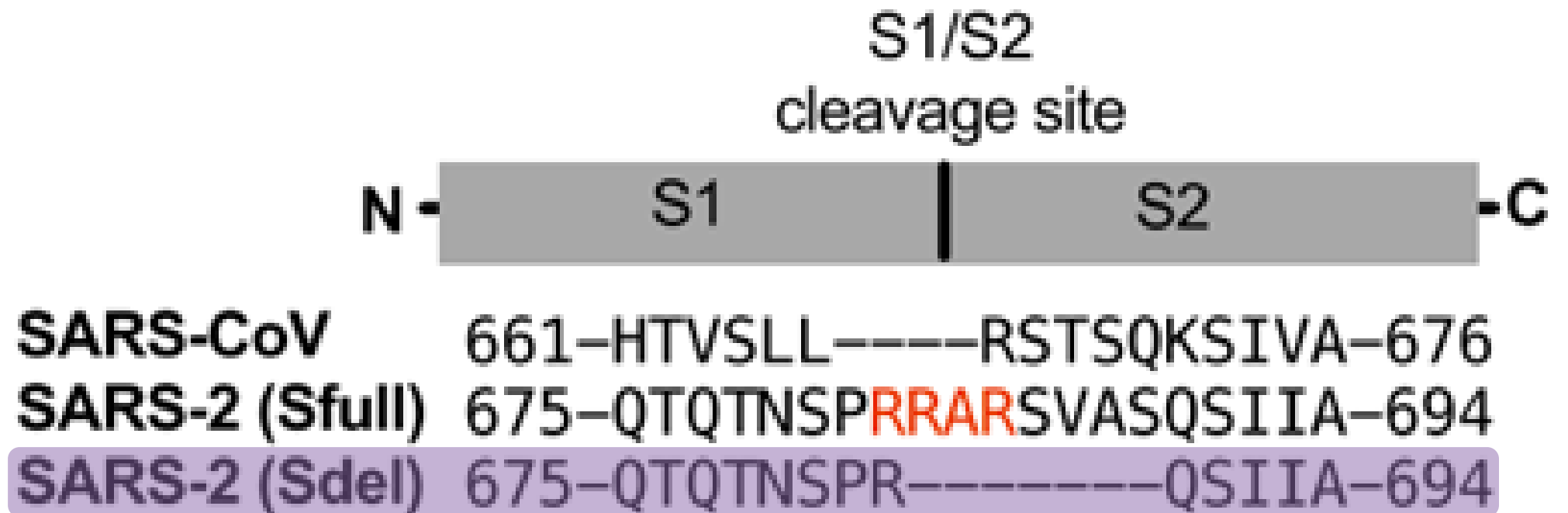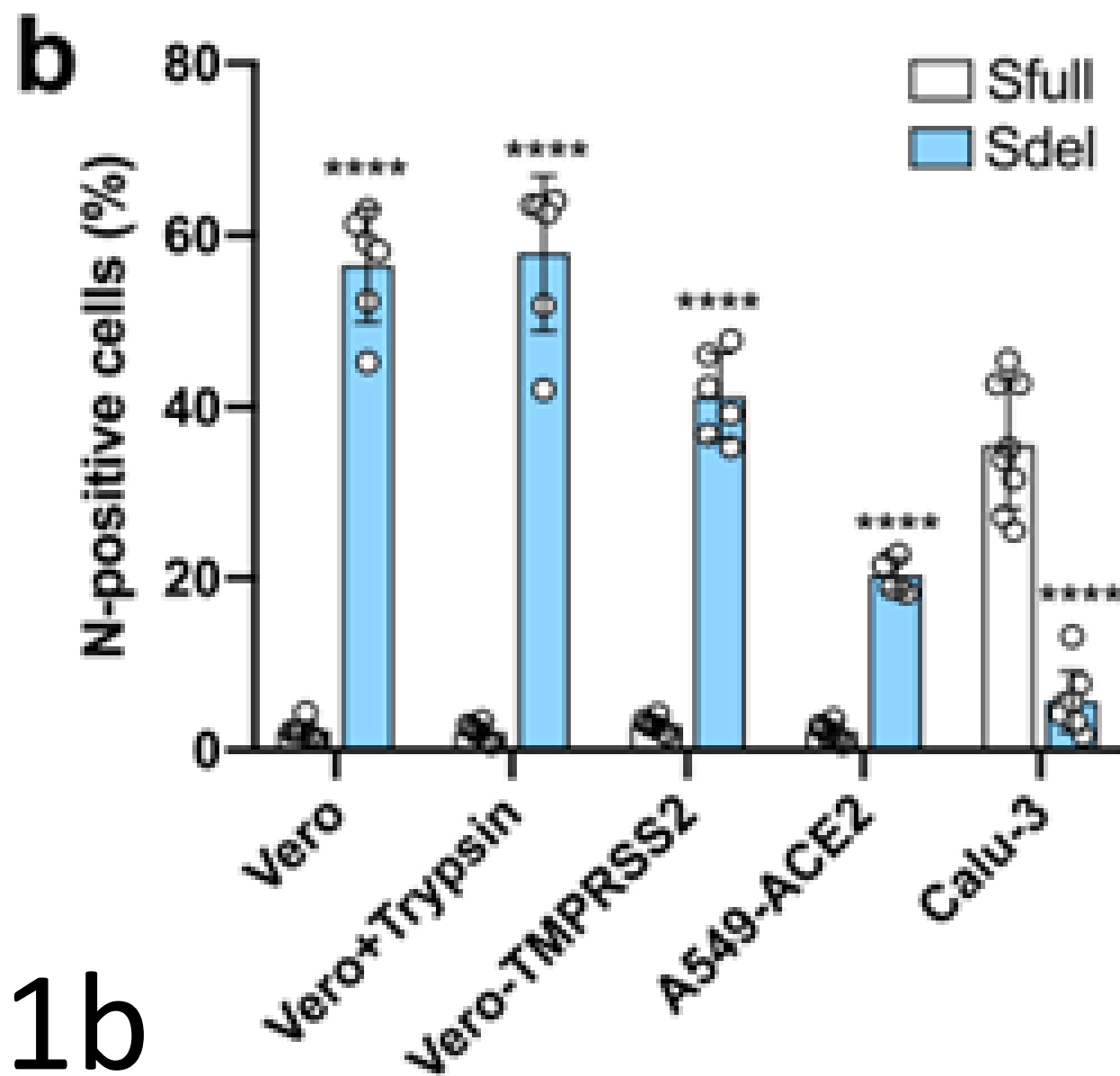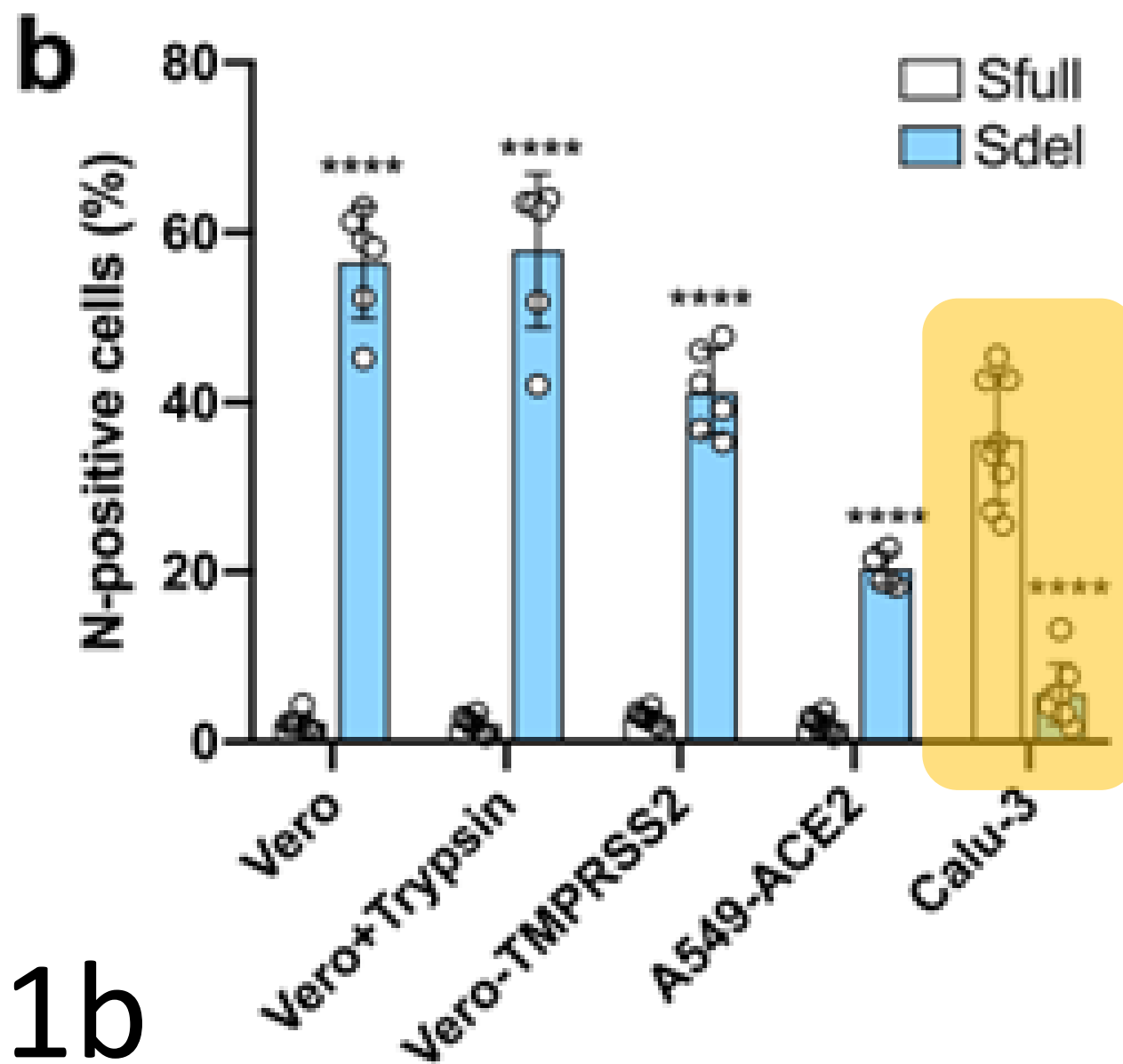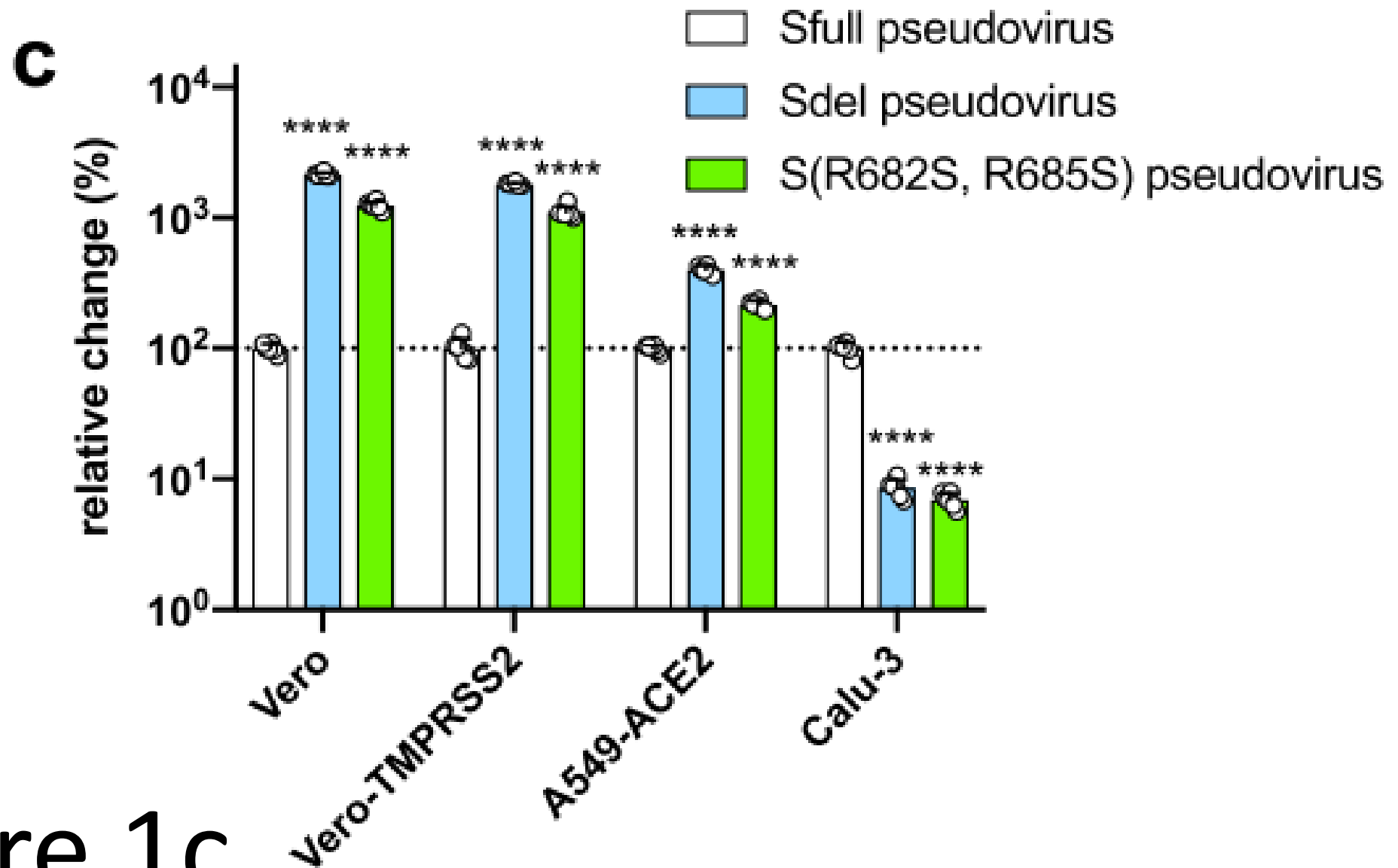
Figure 1a

Figure 1a

Figure 1a

b

Figure 1b

Figure 1b

Figure 1c

Figure 1c

Figure 1d

d

Vero

Vero-TMPRSS2
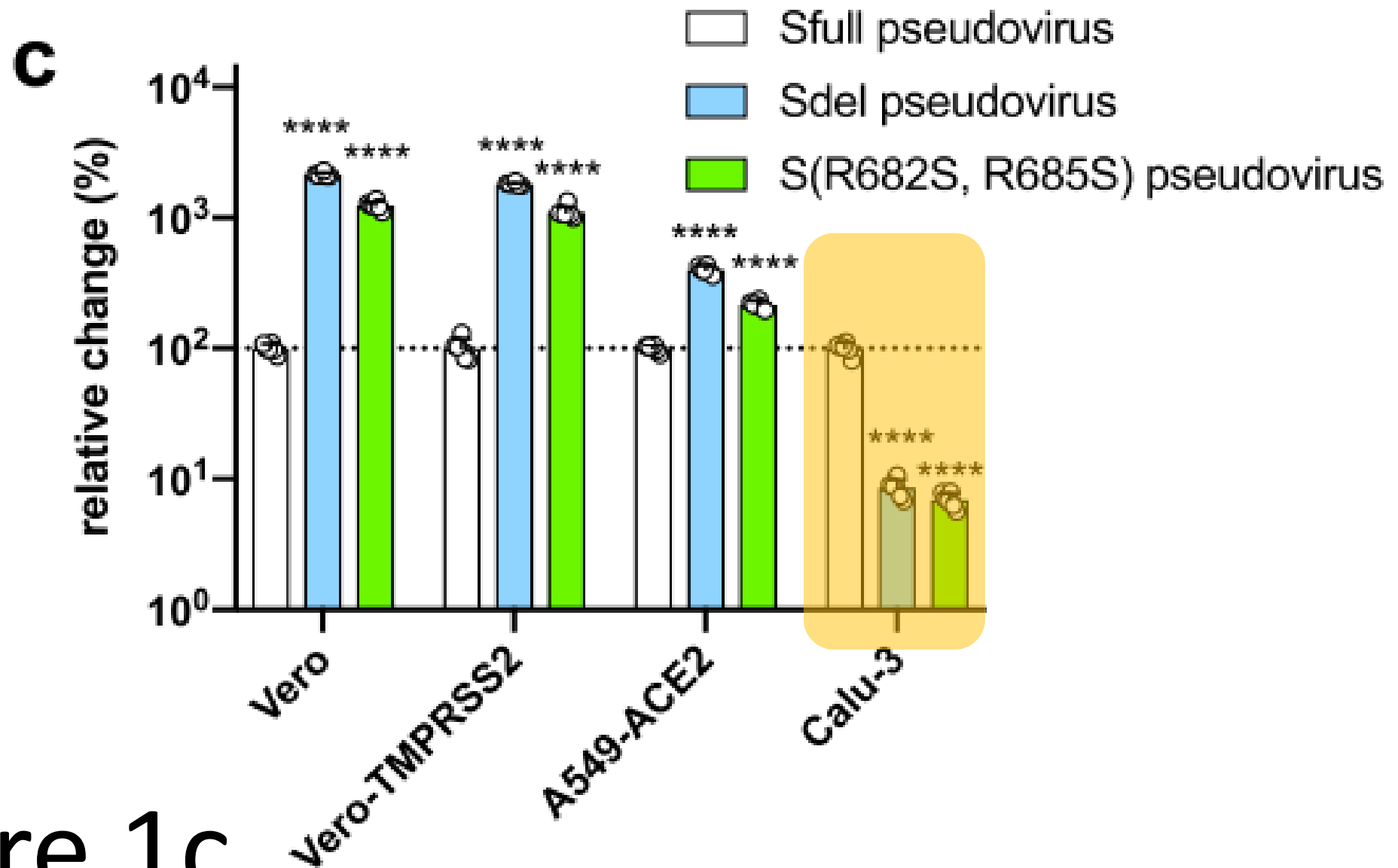
Untreated
Camostat
E-64d
Camostat+E-64d
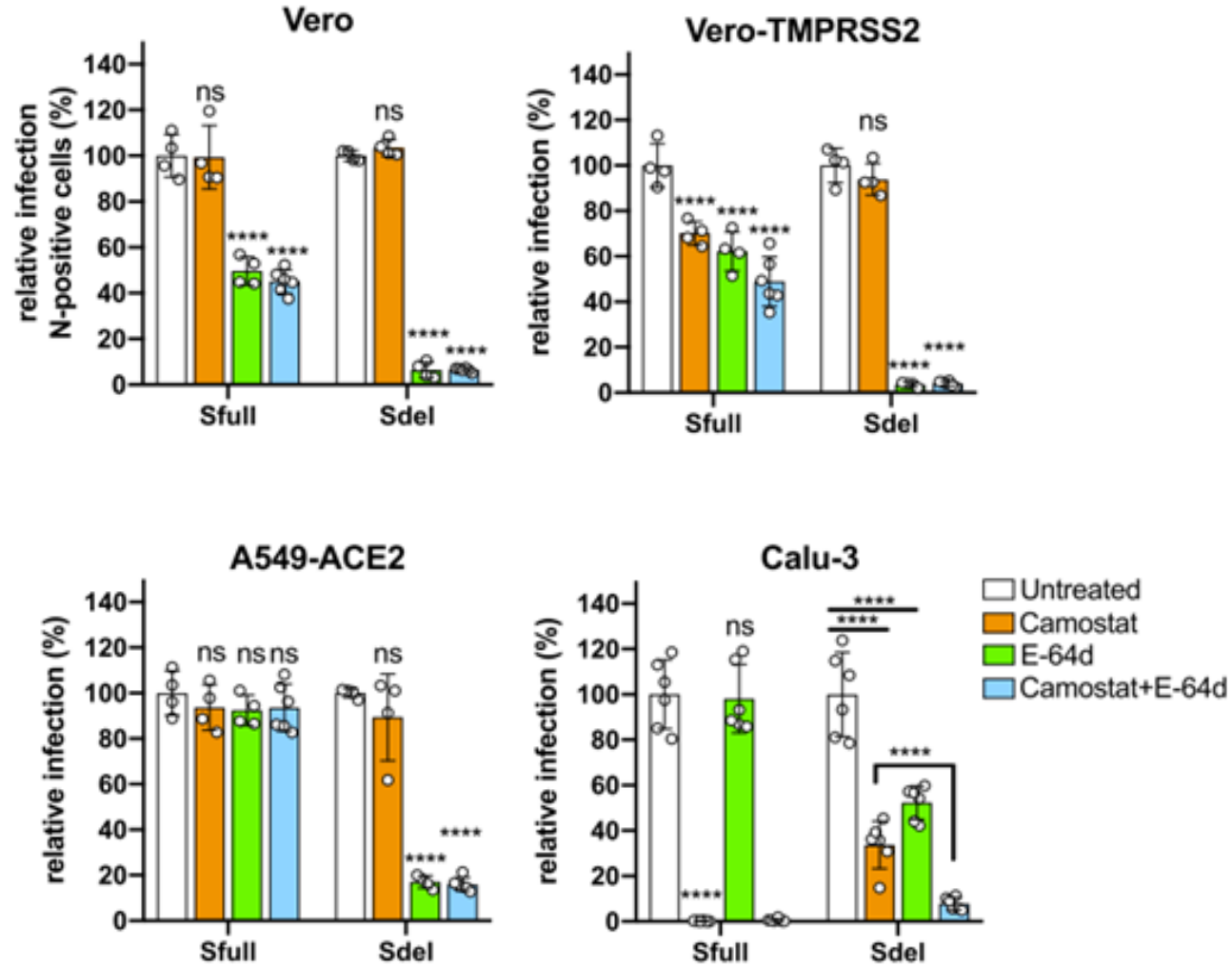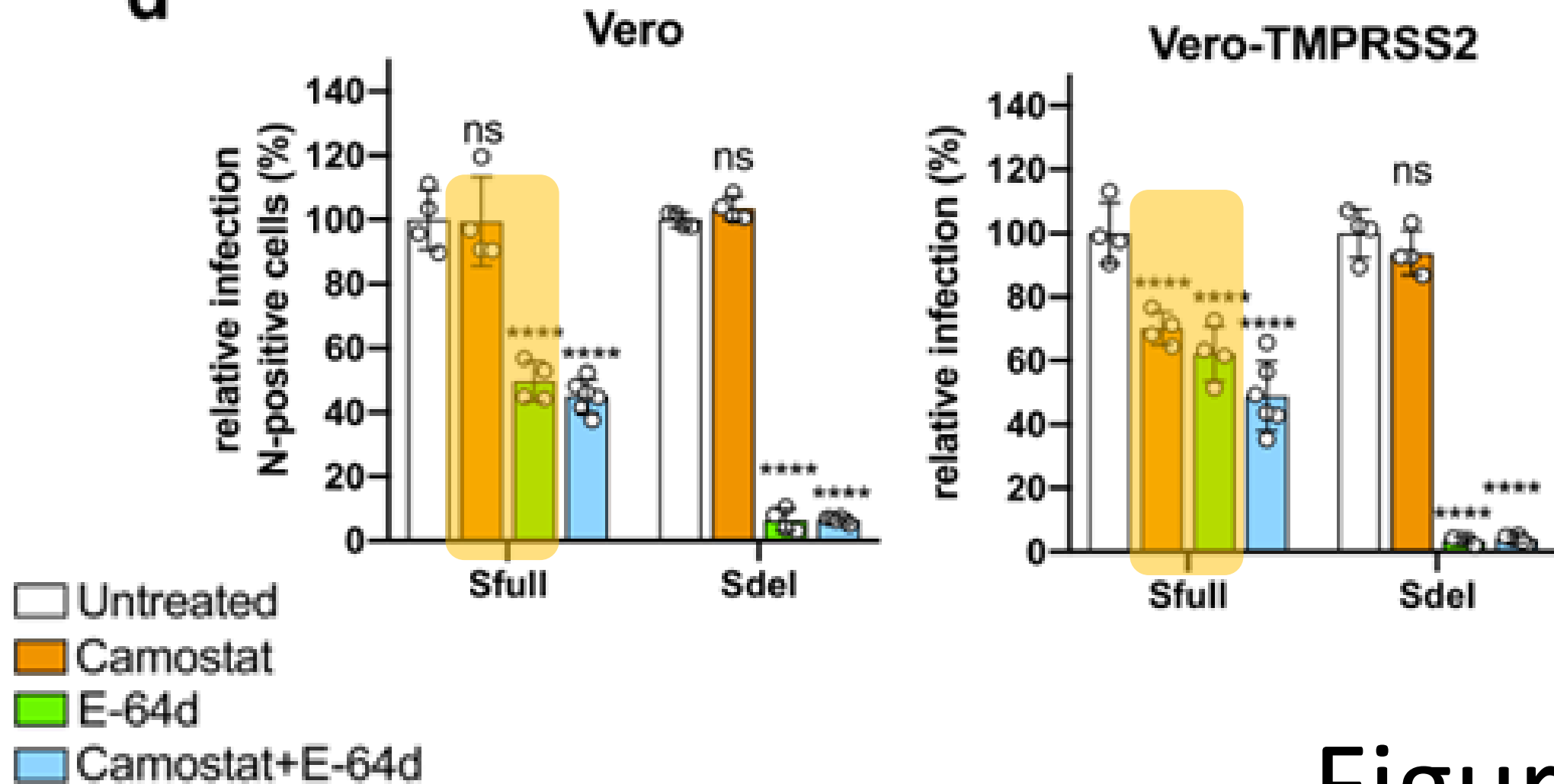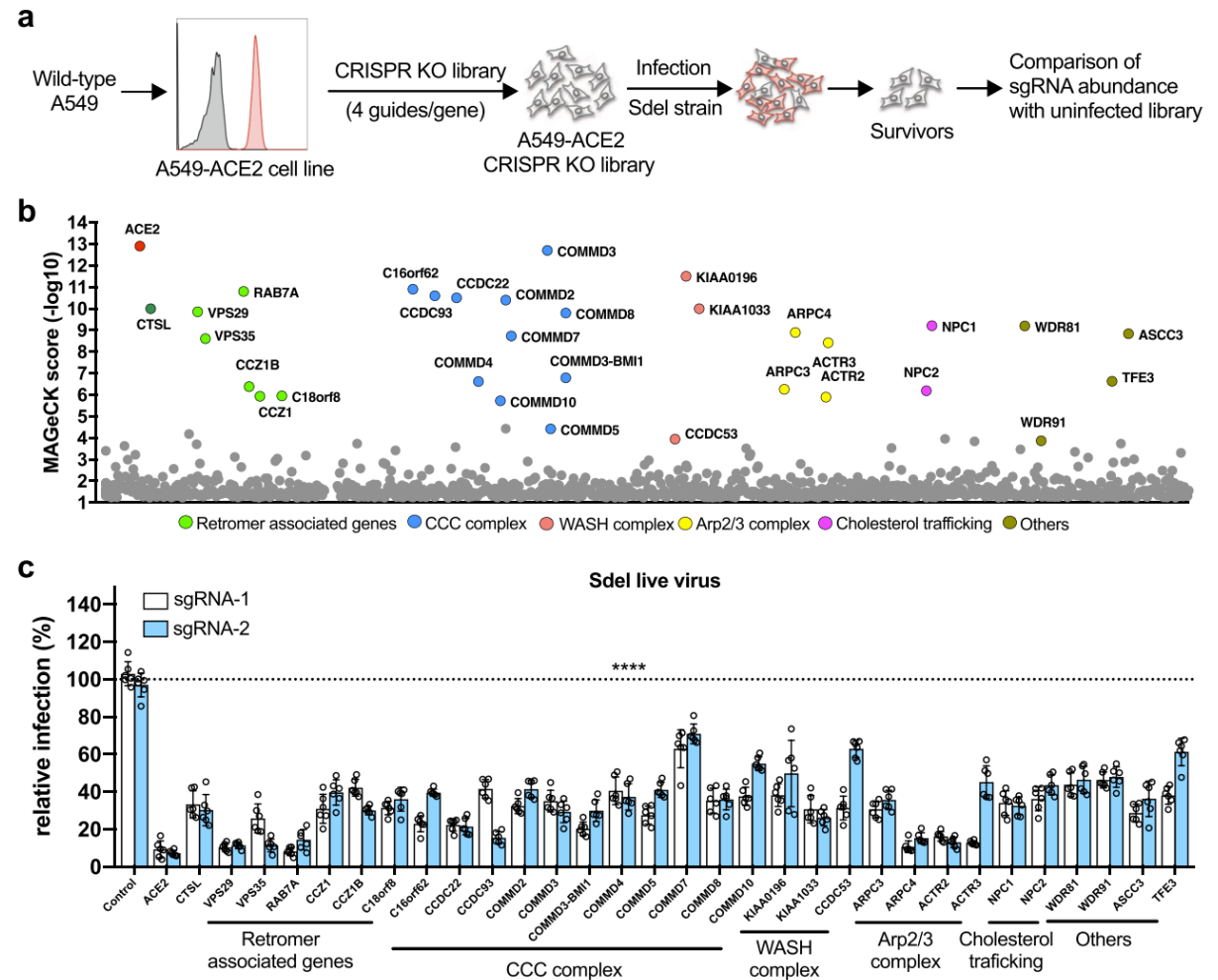
Figure 1d

How did the genome-wide CRISPR/Cas9 screen identify host factors with Sdel virus used as a model?

a

Wild-type A549 → A549-ACE2 cell line → CRISPR KO library (4 guides/gene) → A549-ACE2 CRISPR KO library → Infection Sdel strain → Survivors → Comparison of sgRNA abundance with uninfected library
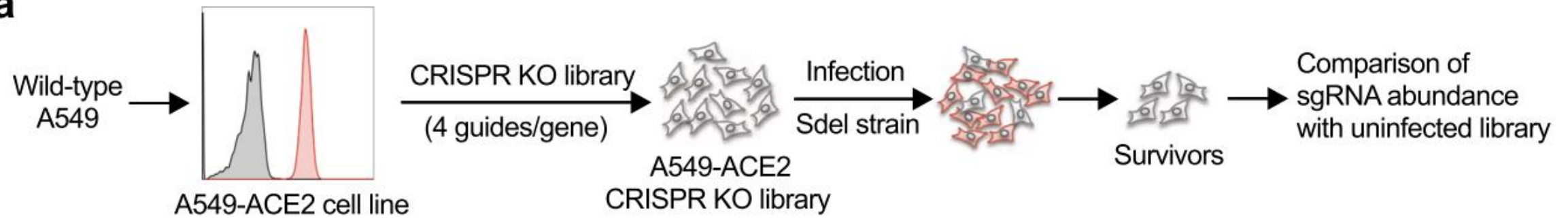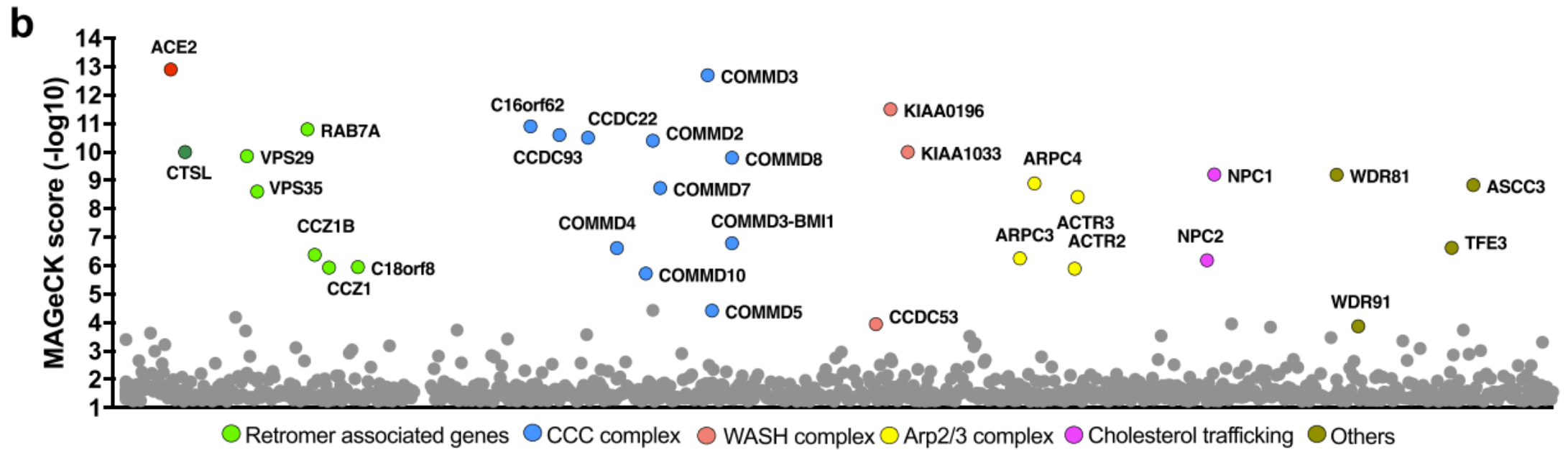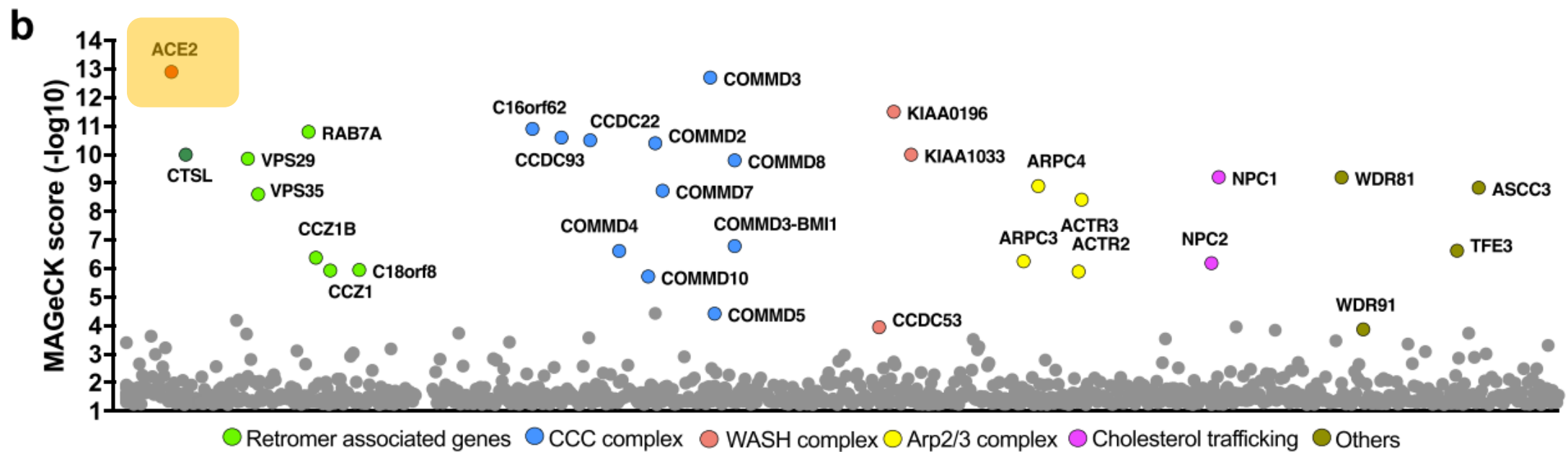
Figure 2a

Figure 2b

Figure 2b

c

Sdel live virus

relative infection (%)

sgRNA-1 (white bars)
sgRNA-2 (blue bars)

****

Genes: Control, ACE2, CTSL, VPS29, VPS35, RAB7A, CCZ1, CCZ1B, C18orf8, C16orf62, CCDC22, CCDC93, COMMD2, COMMD3, COMMD3-BMI1, COMMD4, COMMD5, COMMD7, COMMD8, COMMD10, KIAA0196, KIAA1033, CCDC53, ARPC3, ARPC4, ACTR2, ACTR3, NPC1, NPC2, WDR81, WDR91, ASCC3, TFE3

Retromer associated genes | CCC complex | WASH complex | Arp2/3 complex | Cholesterol traficking | Others
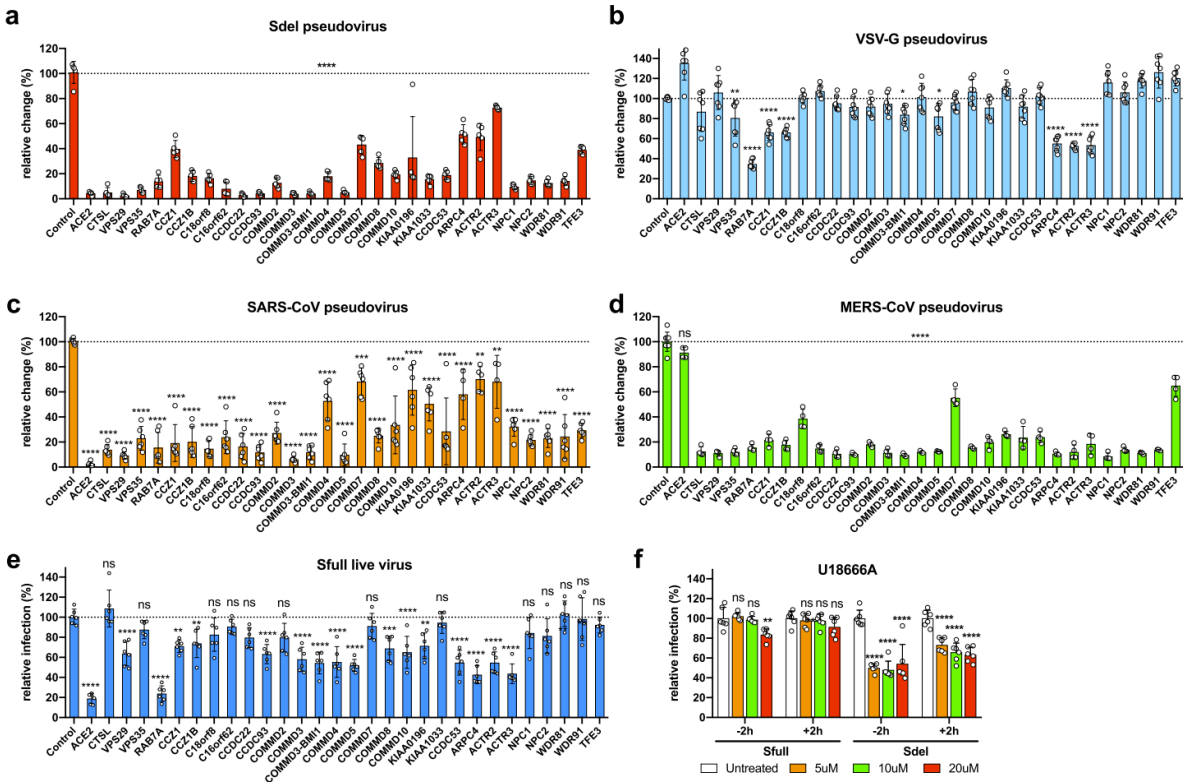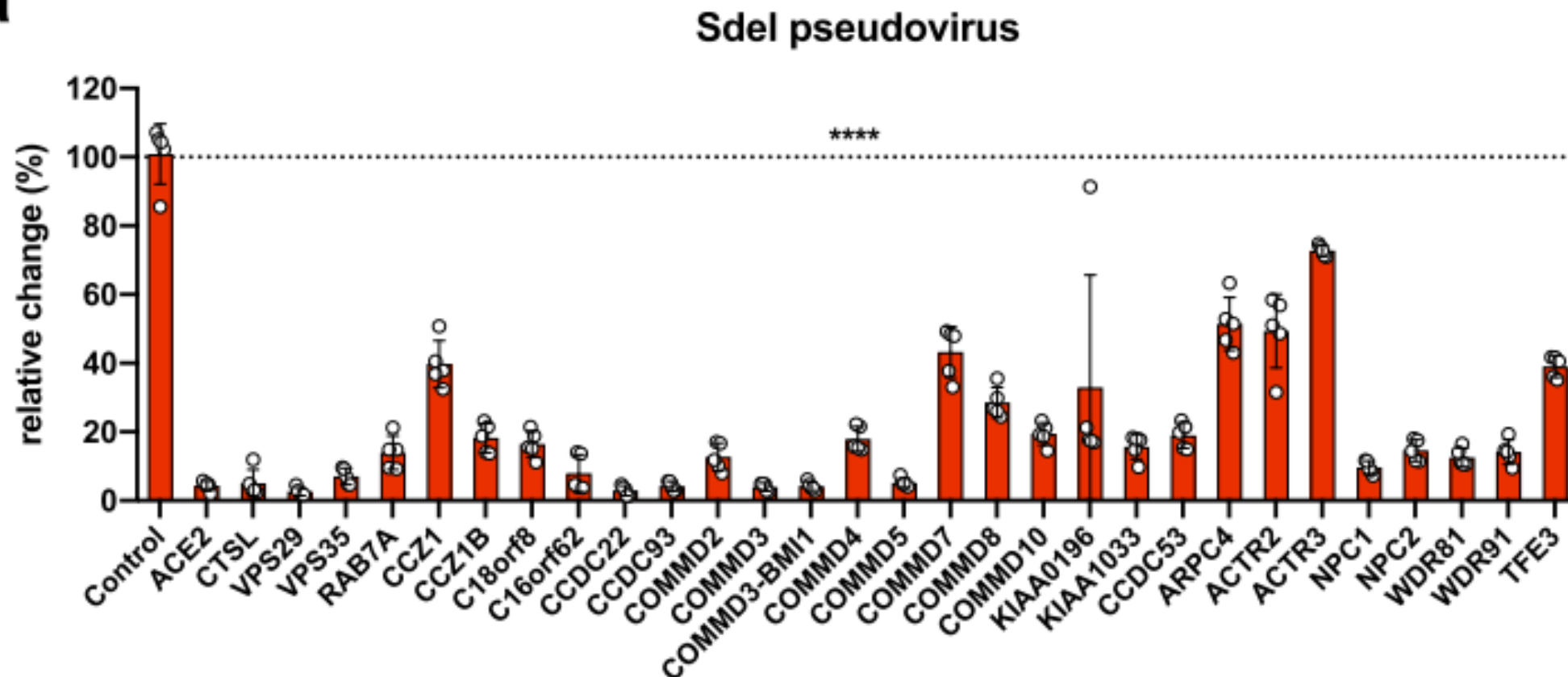
Figure 2c

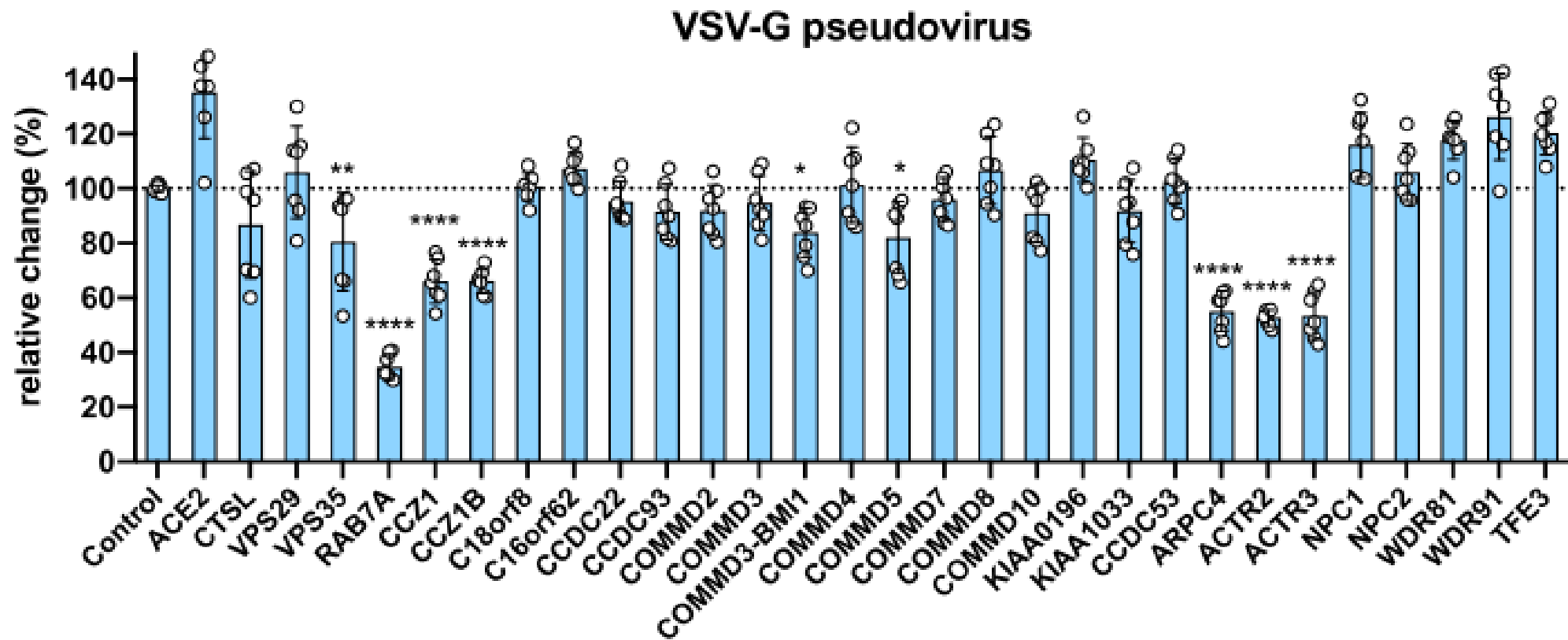What genes are identified that are required for the endosomal cell entry of SARS-CoV-2, SARS-CoV, and MERS-CoV?

Figure 3a

Figure 3b

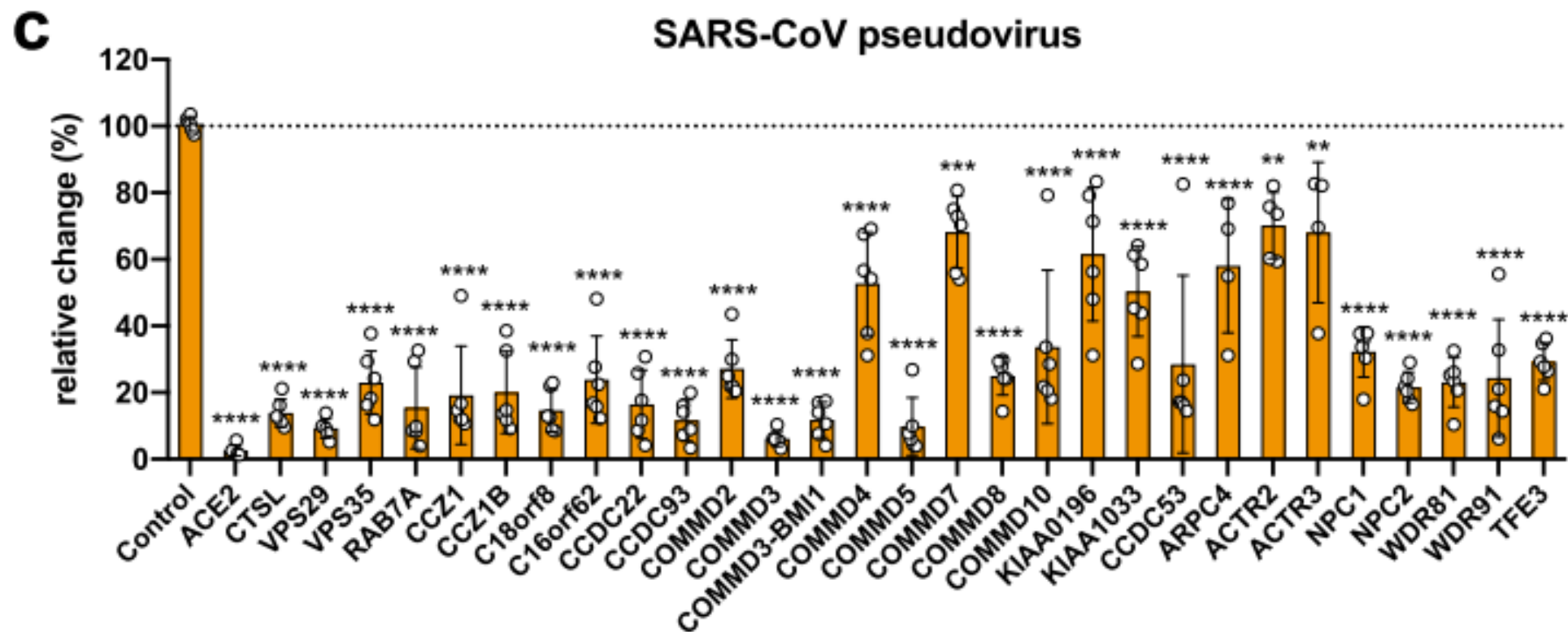Figure 3c

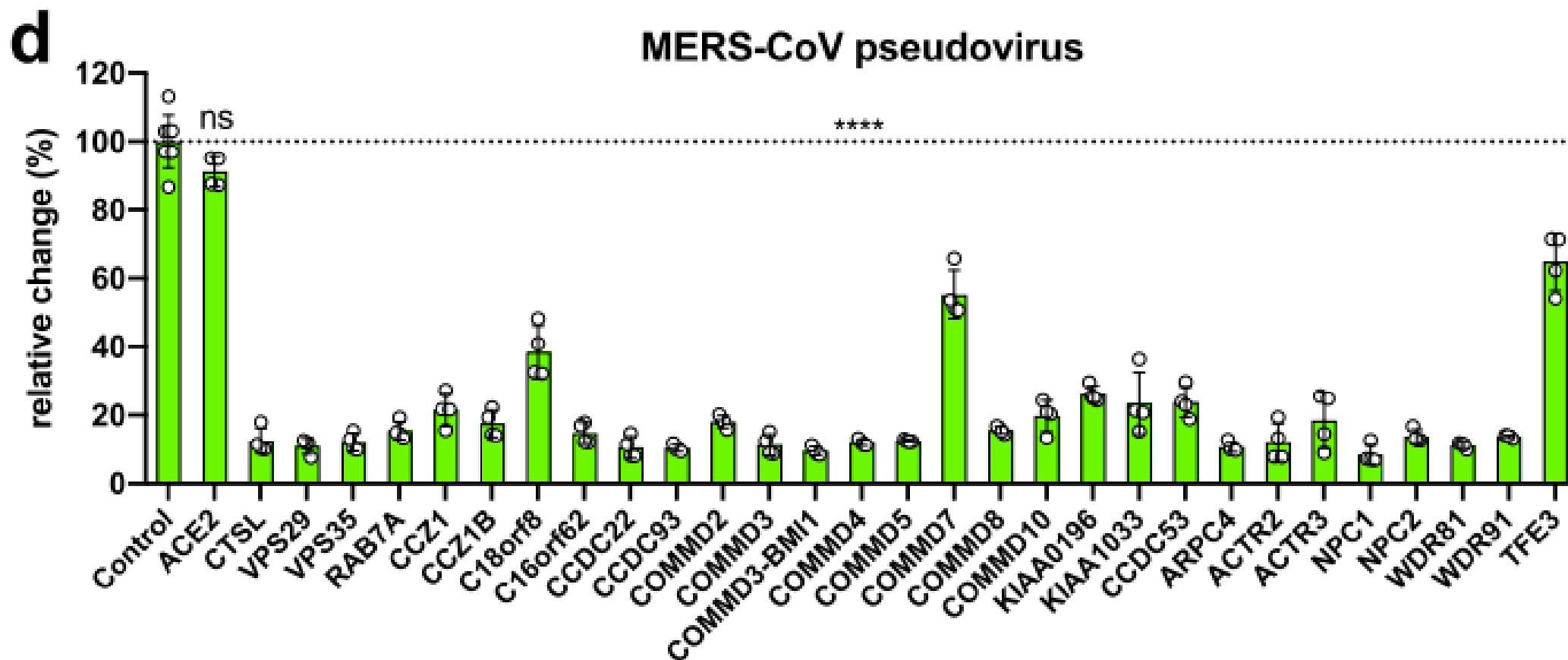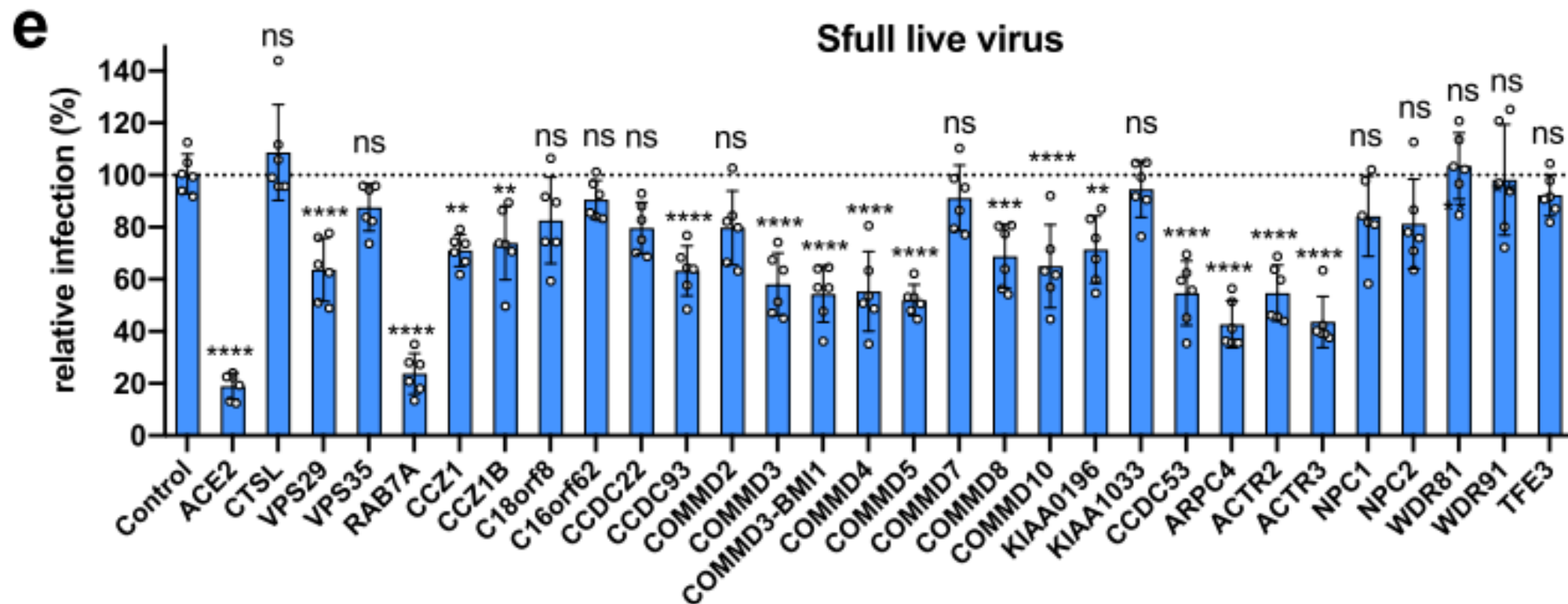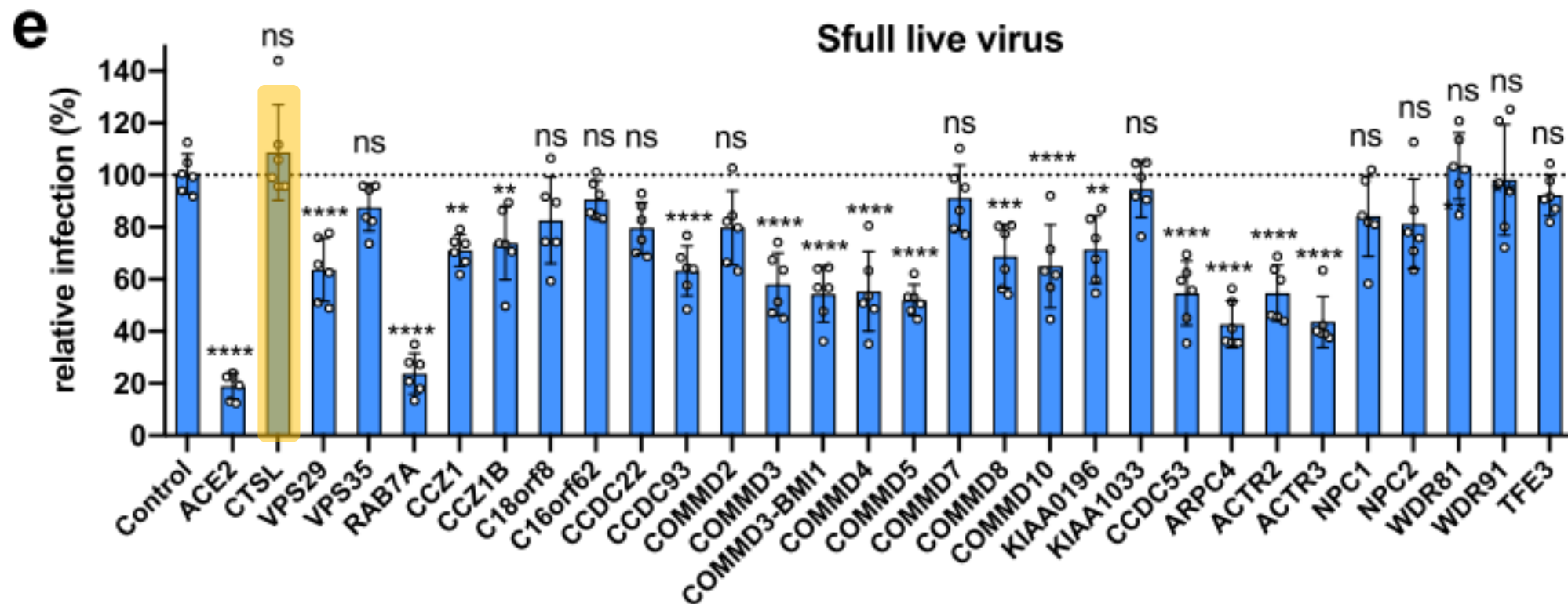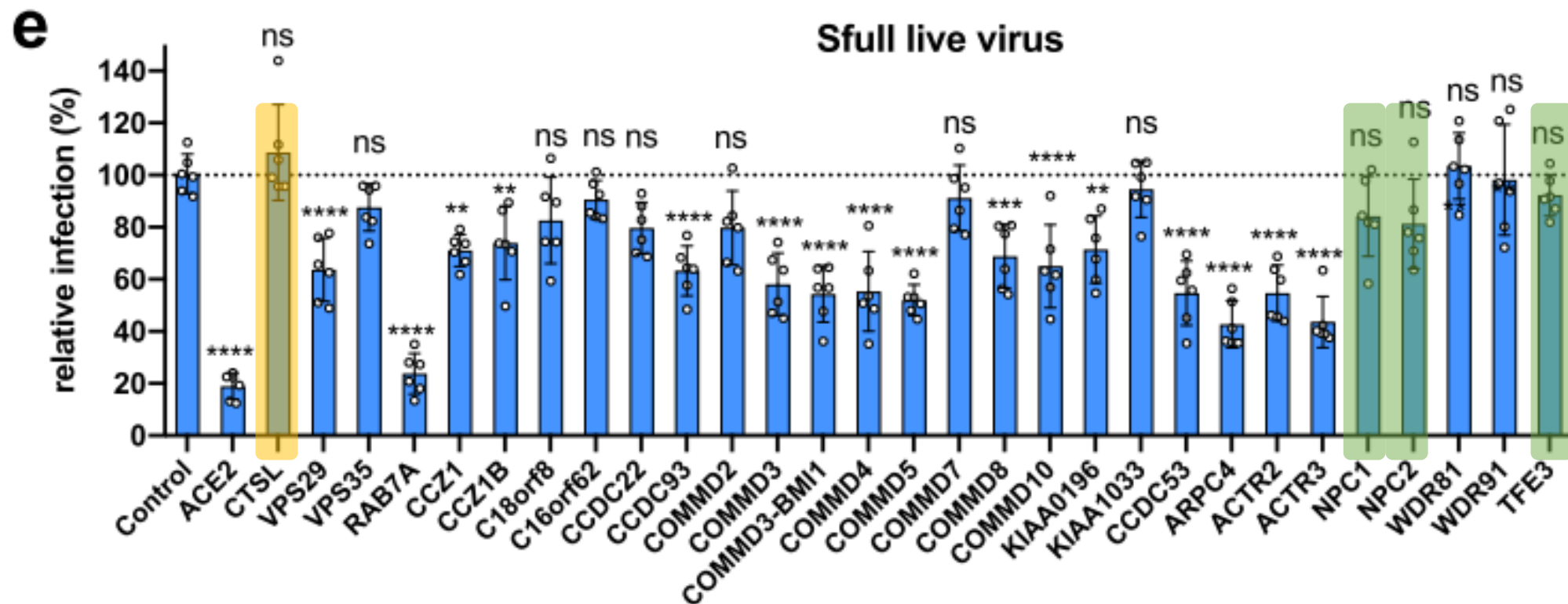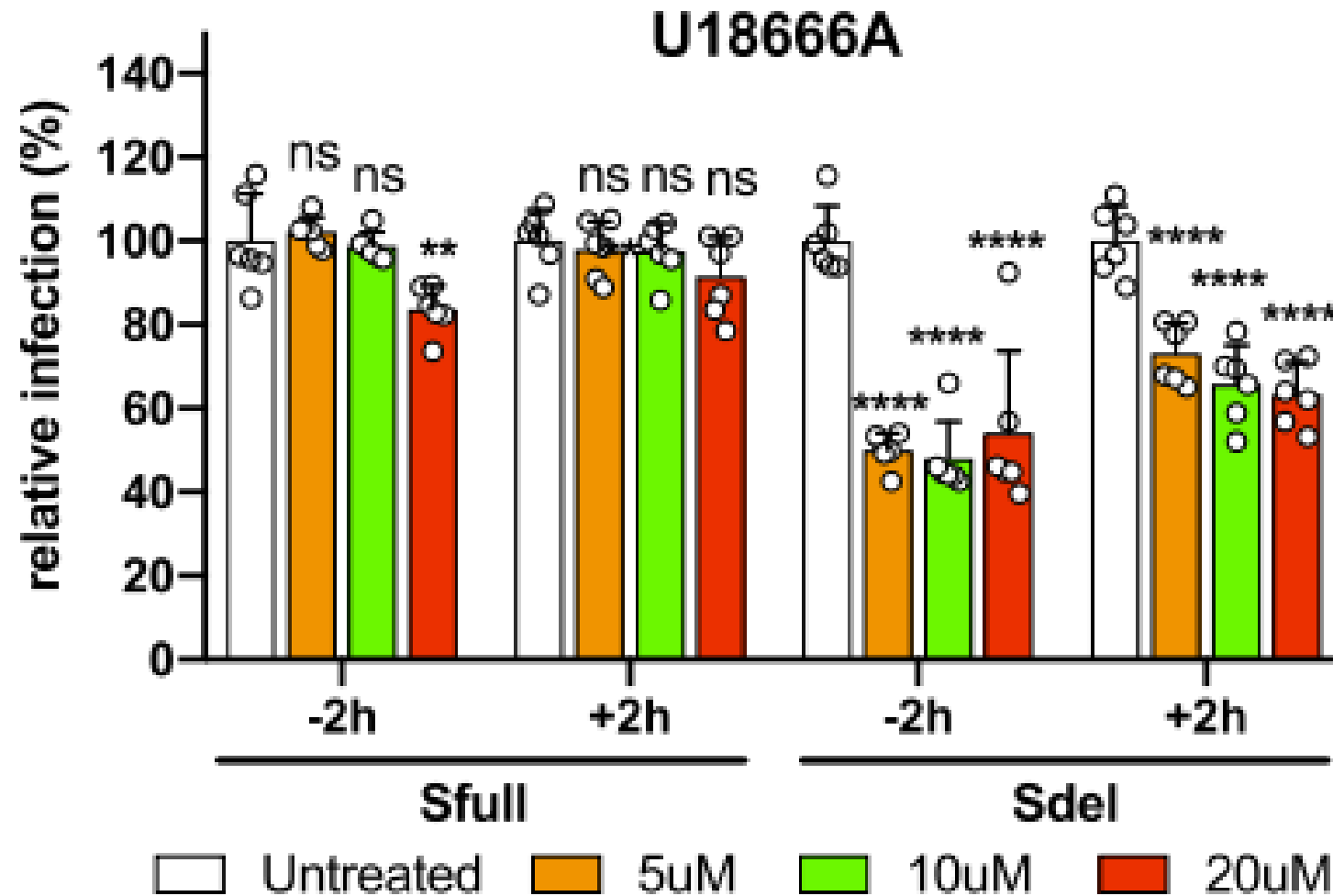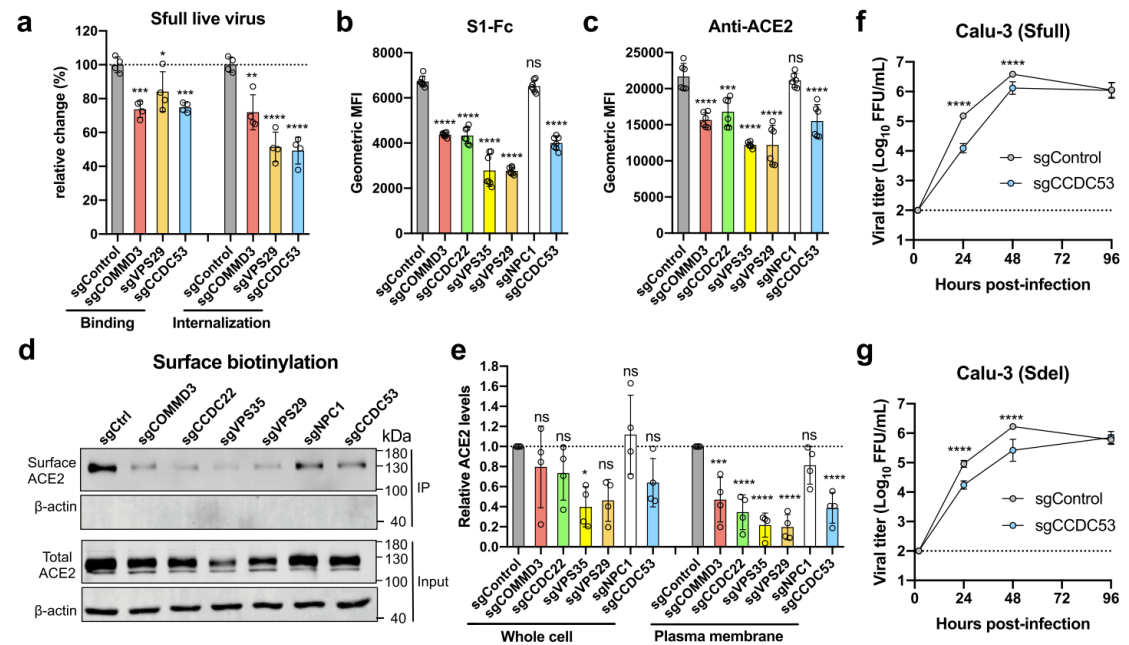Figure 3d

Figure 3e

Figure 3e

Figure 3e

Figure 3f

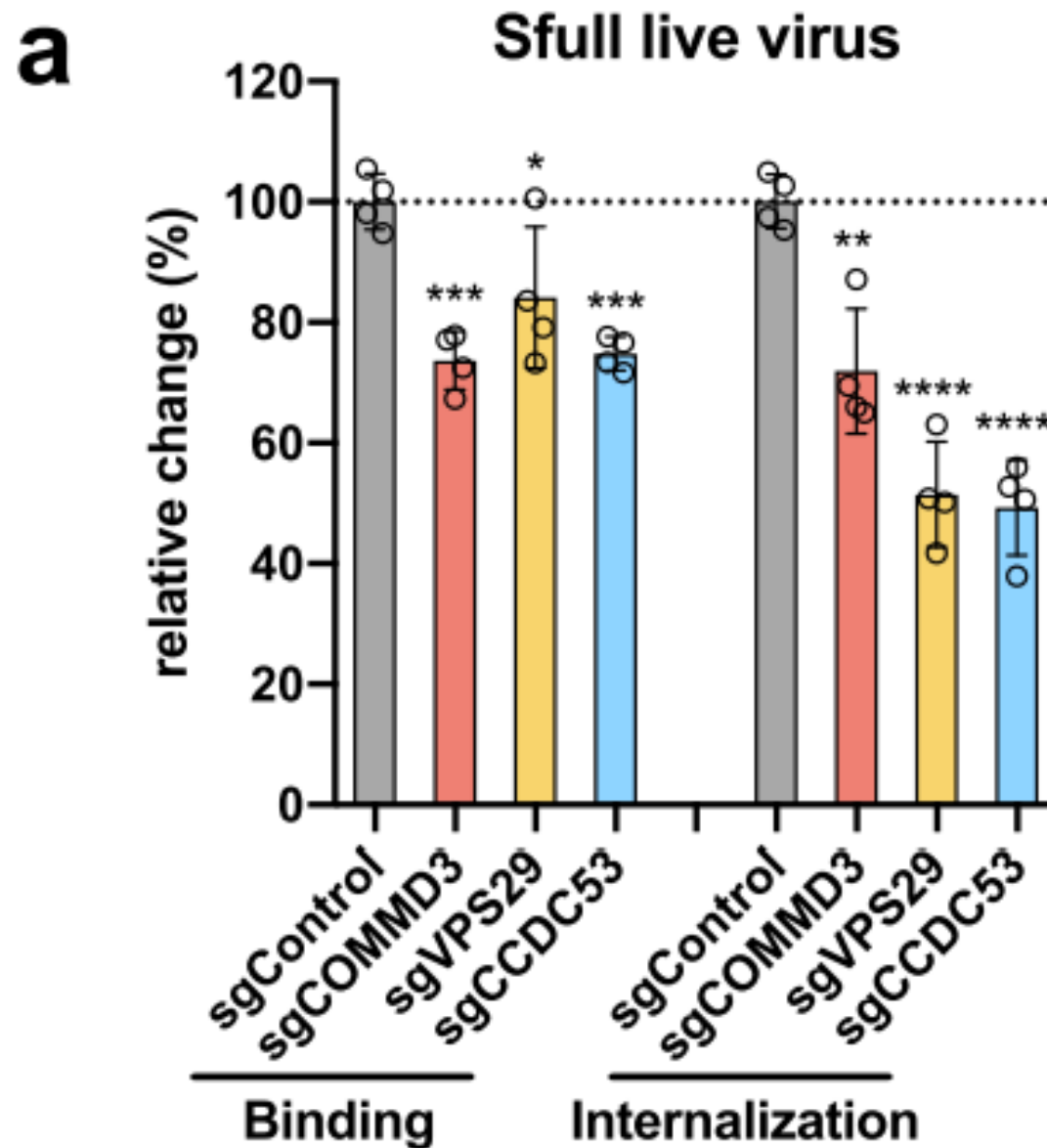# Which host genes that regulate the surface expression of receptor ACE2 were identified?
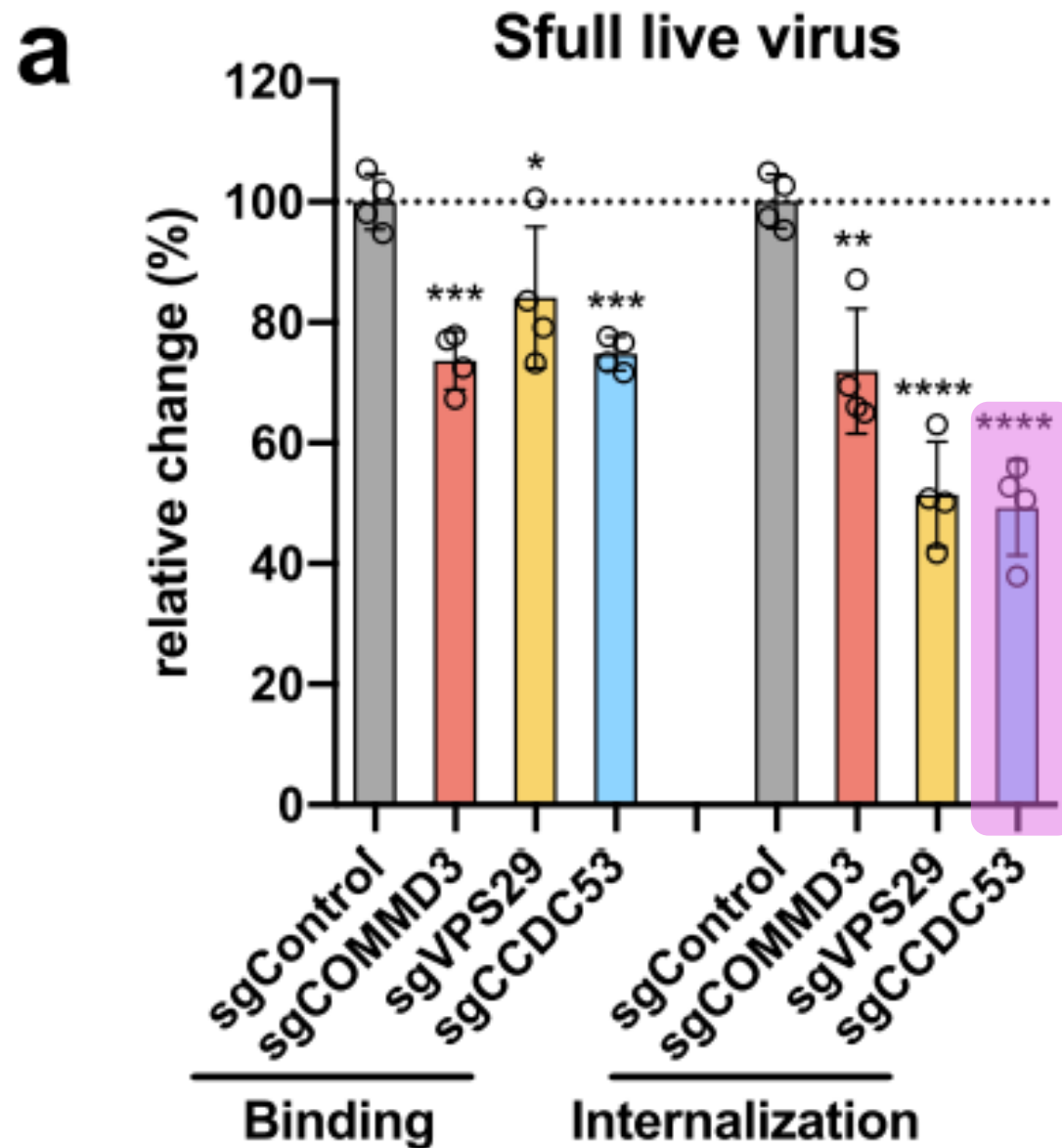
a Sfull live virus

Figure 4a

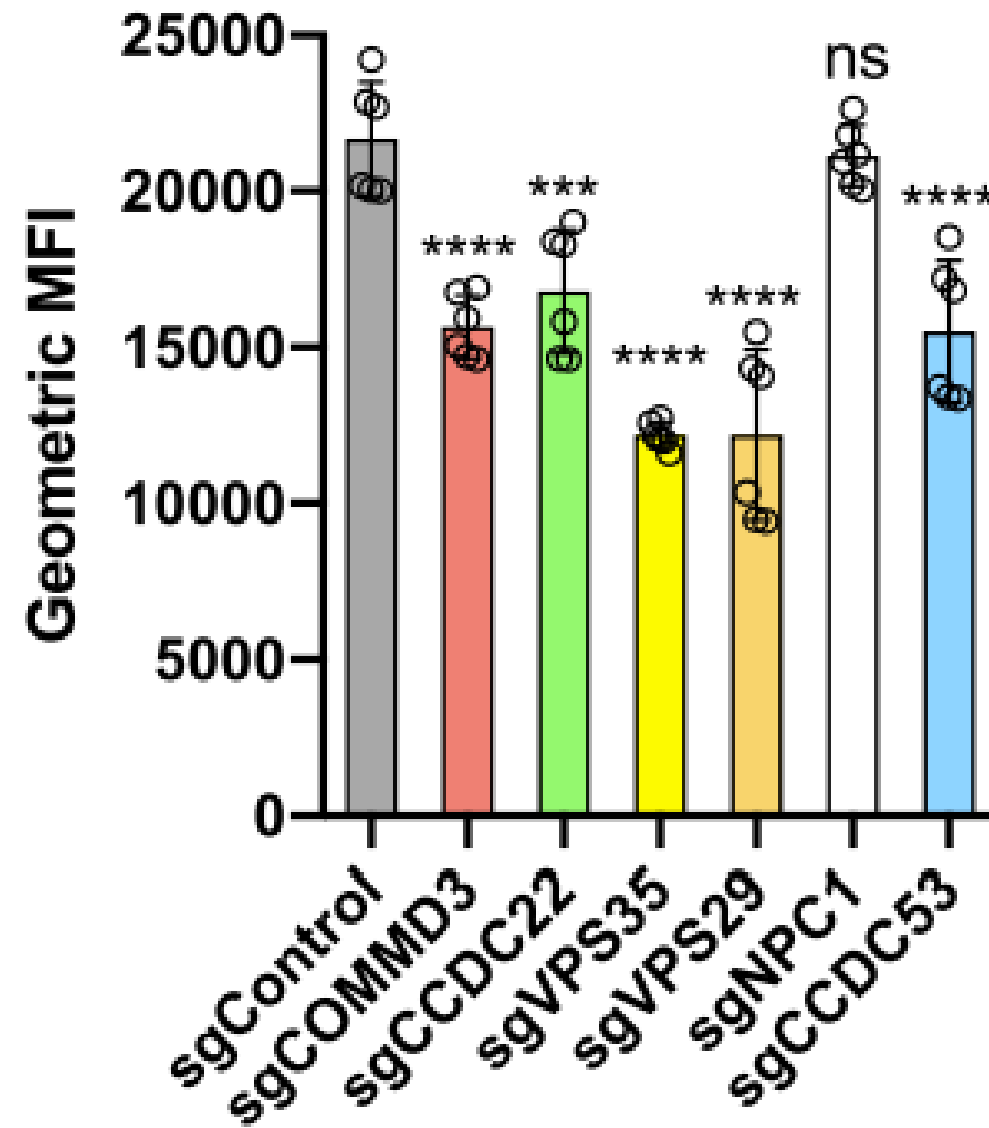**Sfull live virus**

Figure 4a

b
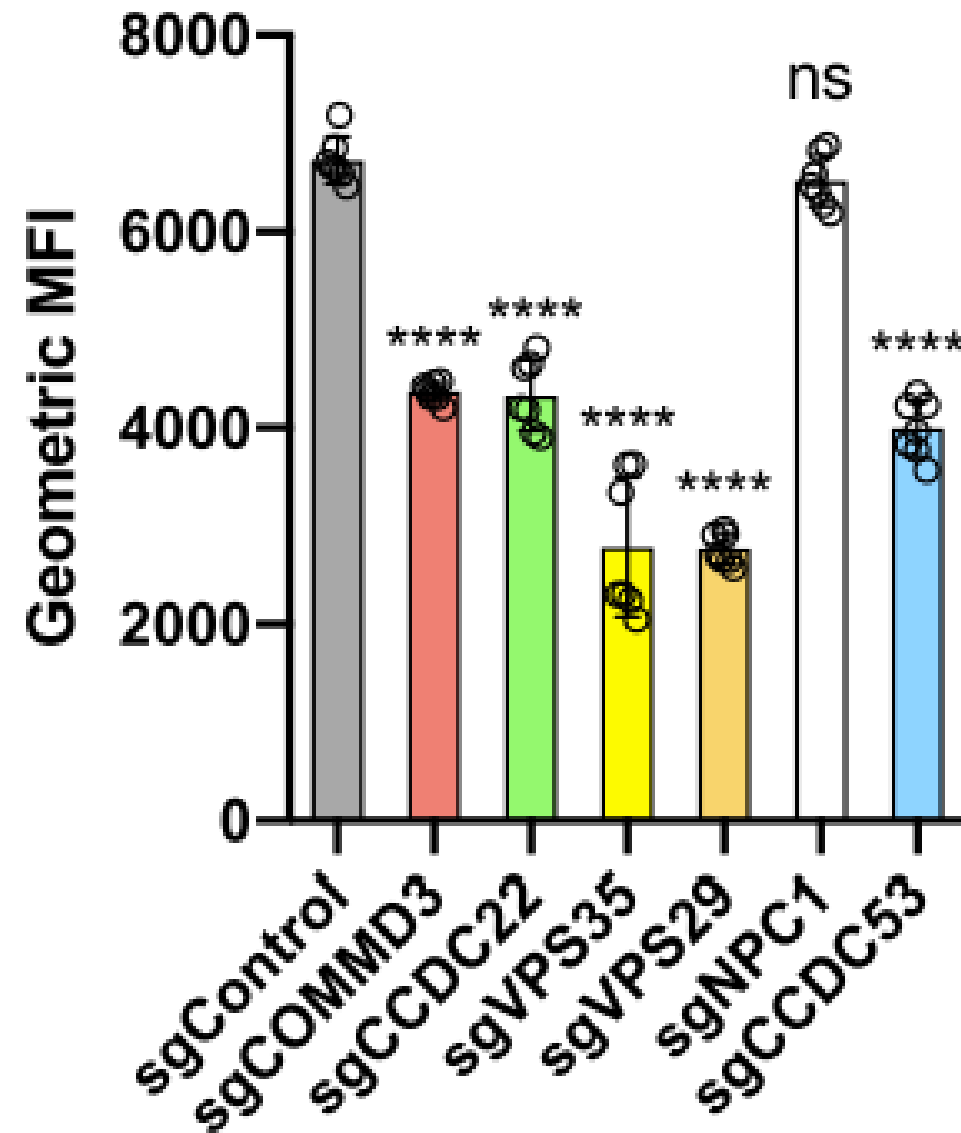
S1-Fc

Figure 4b

b

S1-Fc
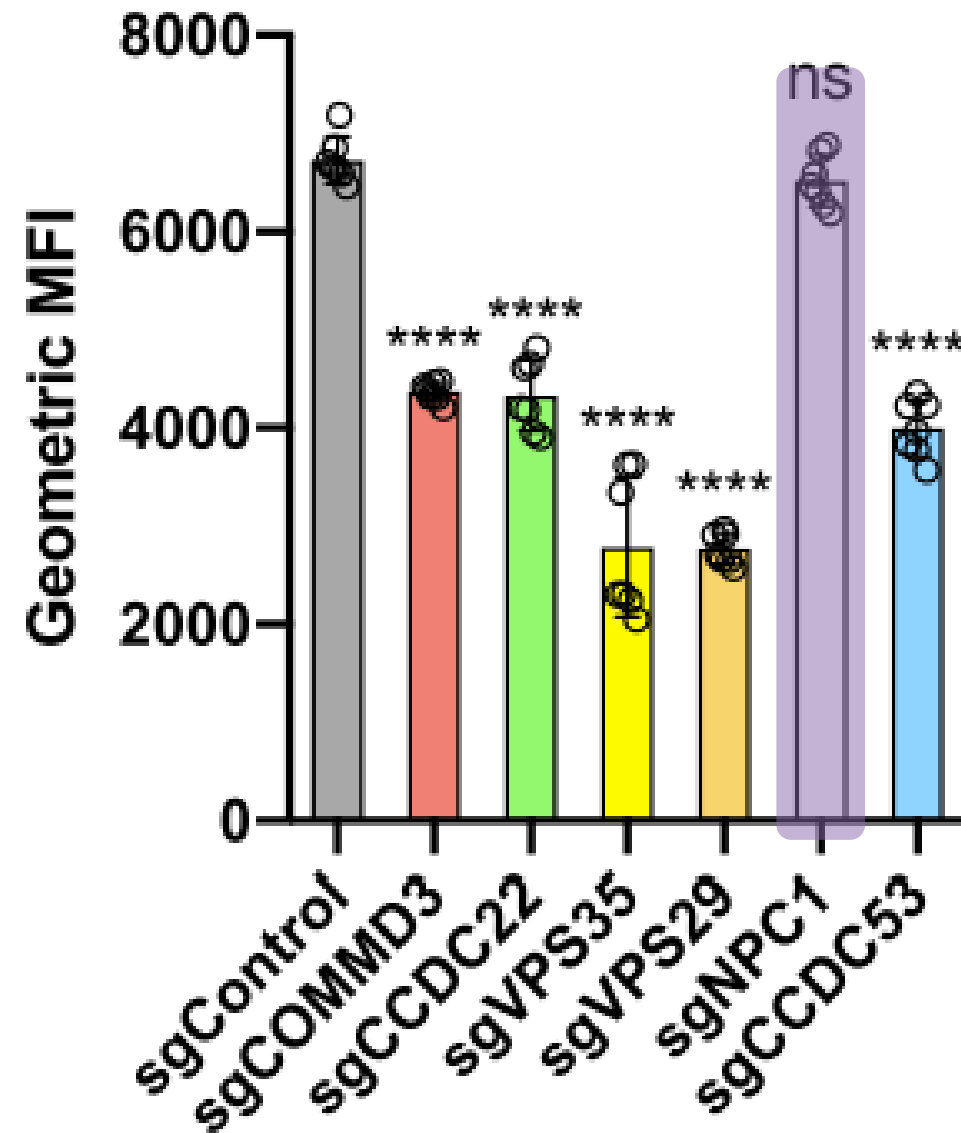
Figure 4b

C Anti-ACE2

Figure 4c

C Anti-ACE2

Figure 4c

b S1-Fc

c Anti-ACE2

d

Surface biotinylation

Figure 4d

Figure 4e

f

Calu-3 (Sfull)

Viral titer (Log$_{10}$ FFU/mL)

**** ****

sgControl
sgCCDC53

Hours post-infection

g

Calu-3 (Sdel)

Viral titer (Log$_{10}$ FFU/mL)

**** ****

sgControl
sgCCDC53

Hours post-infection
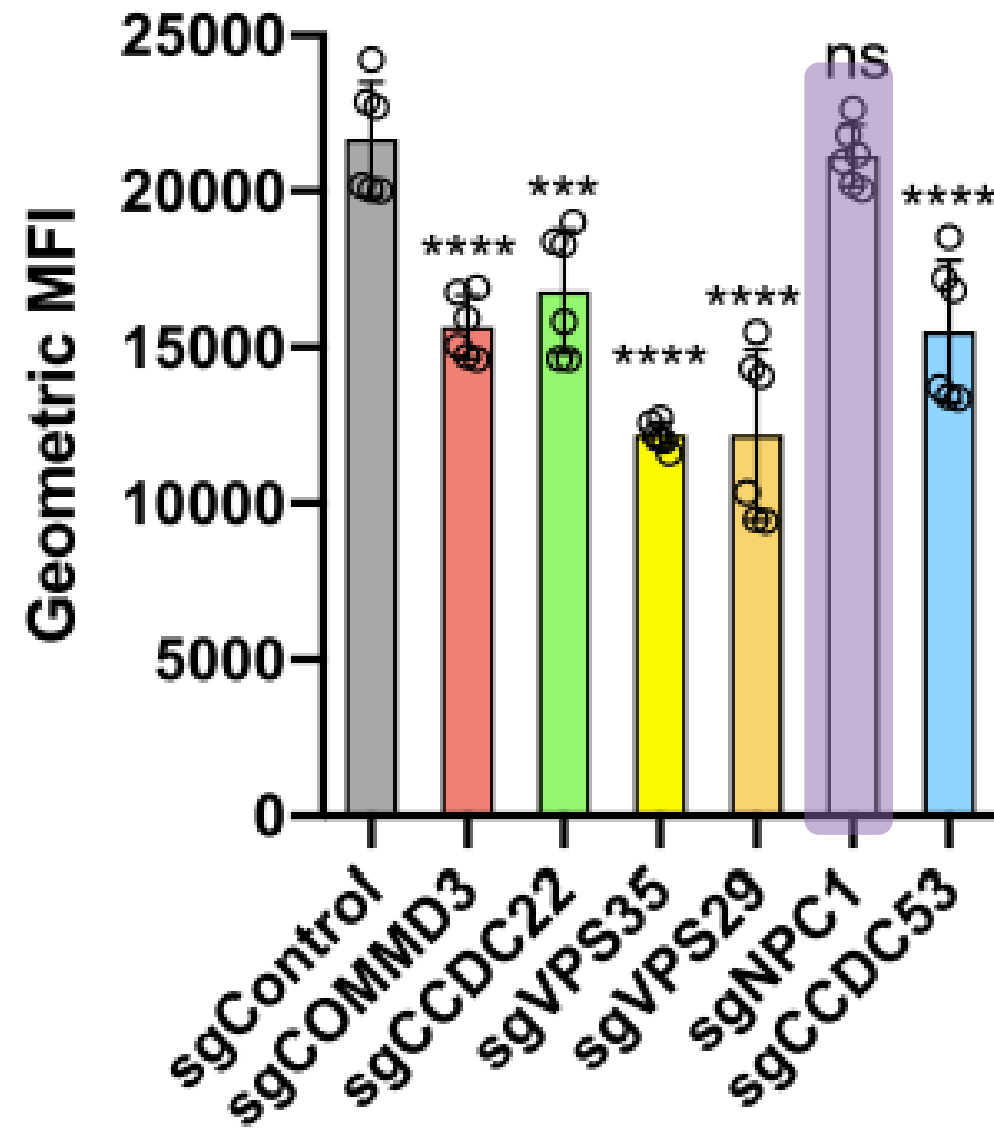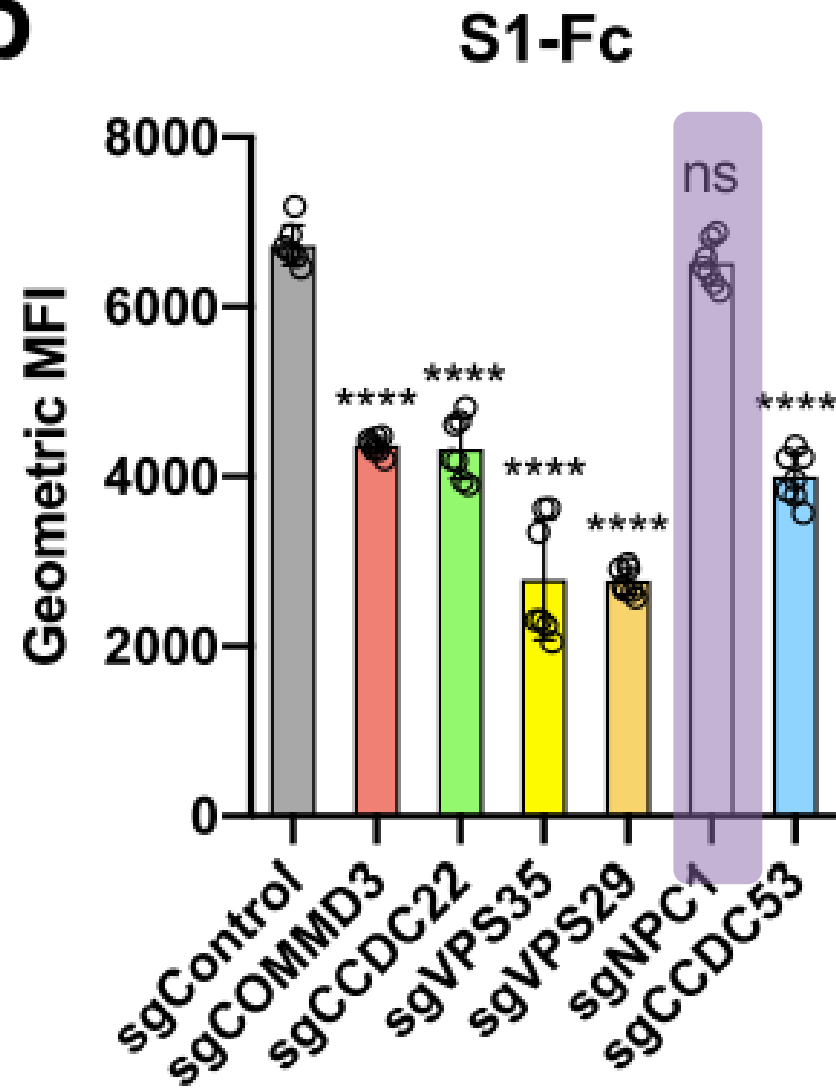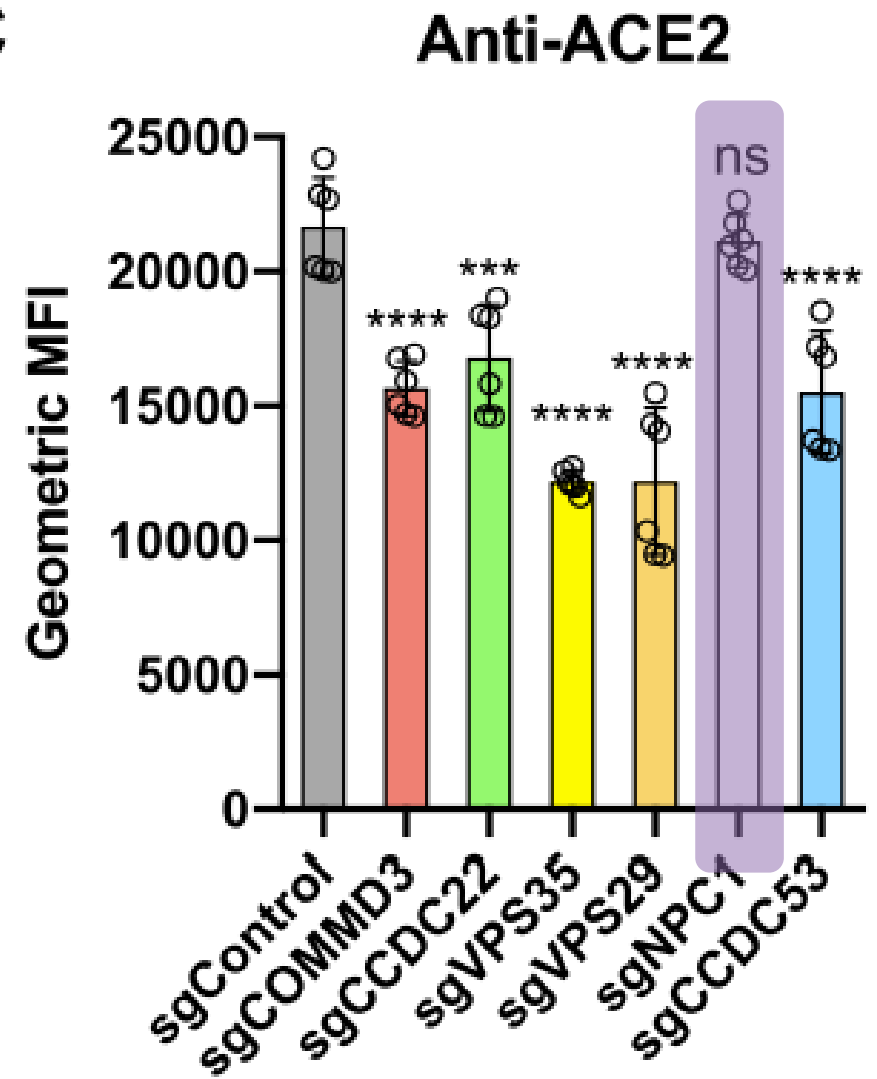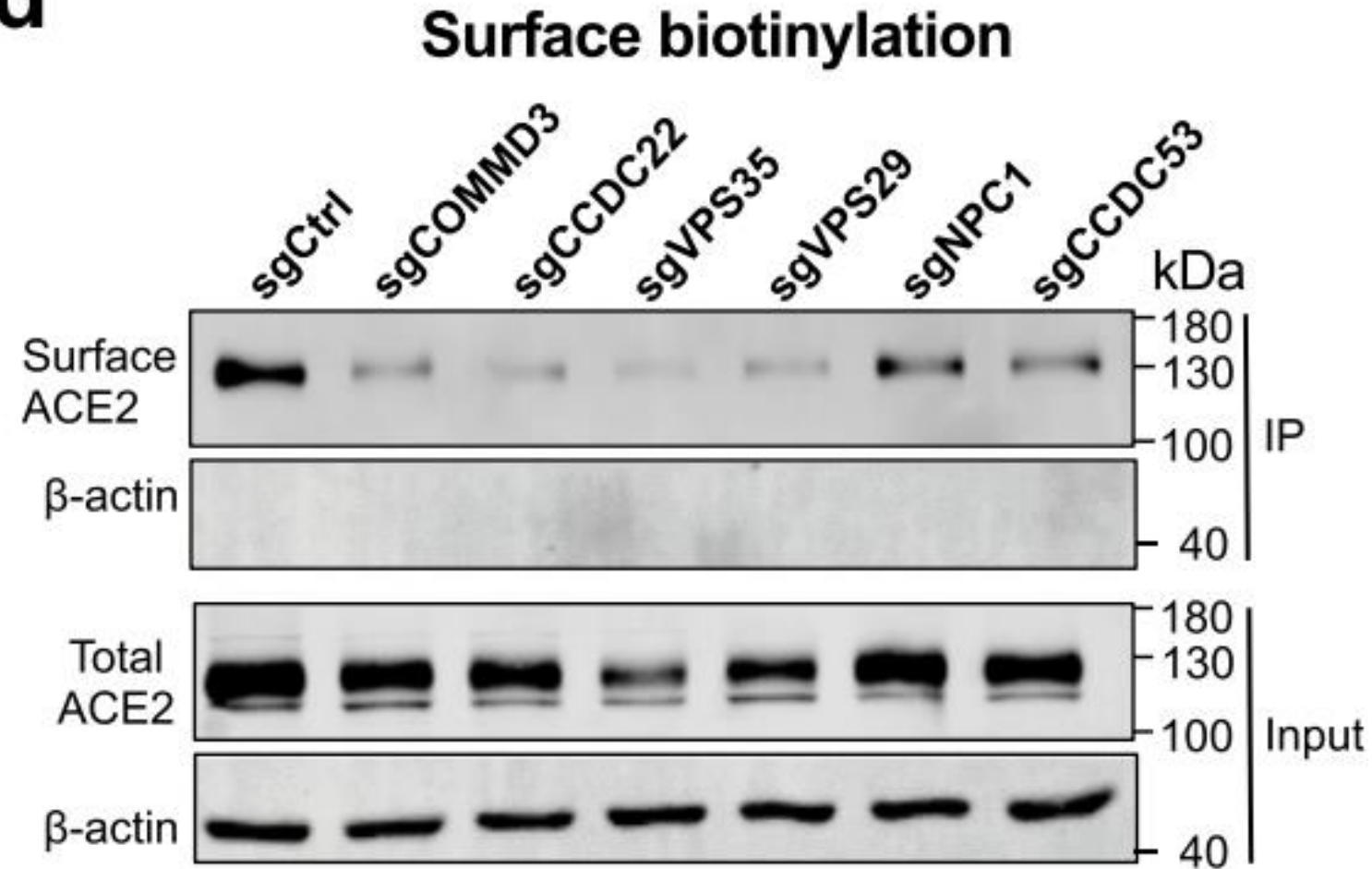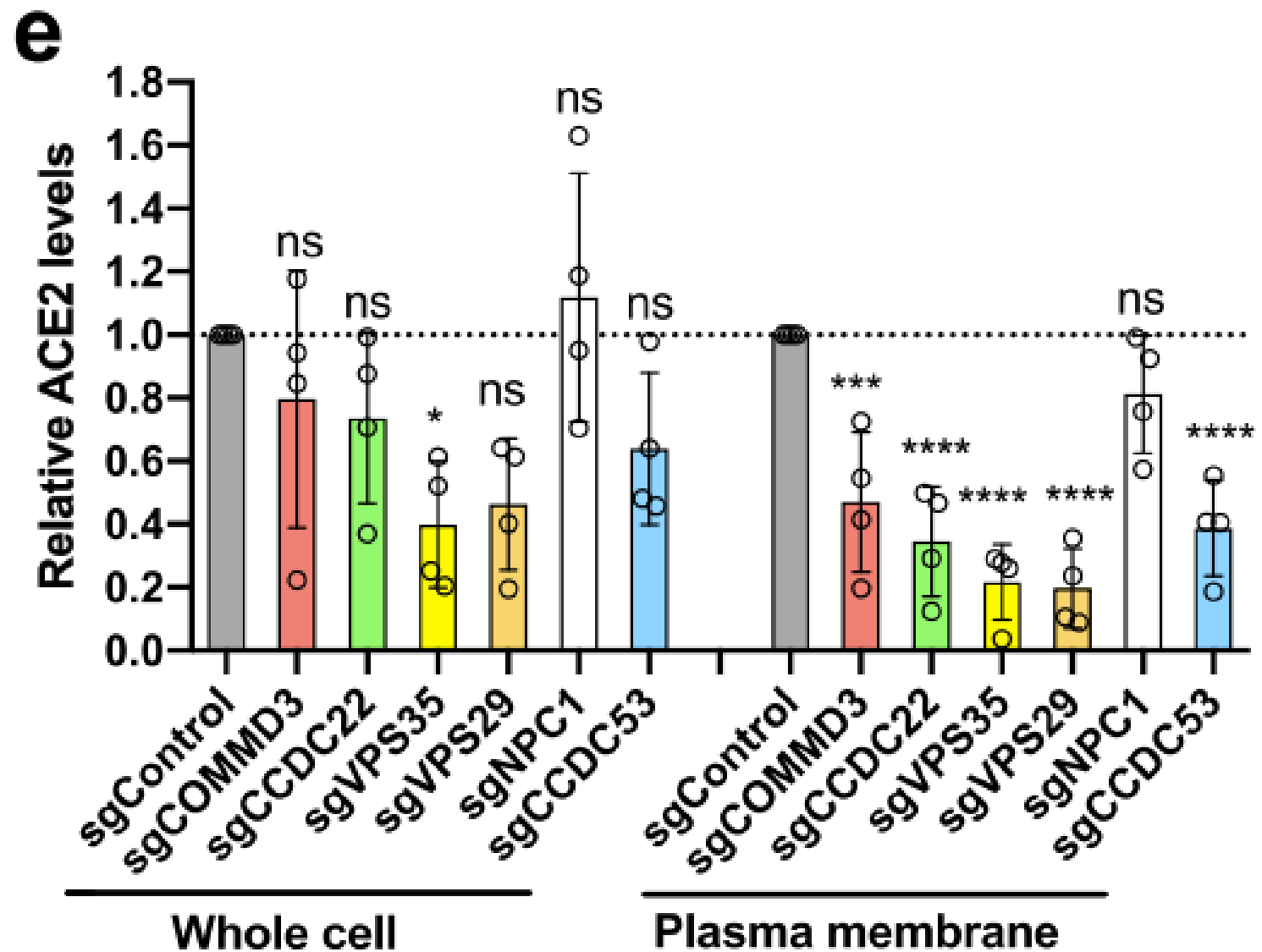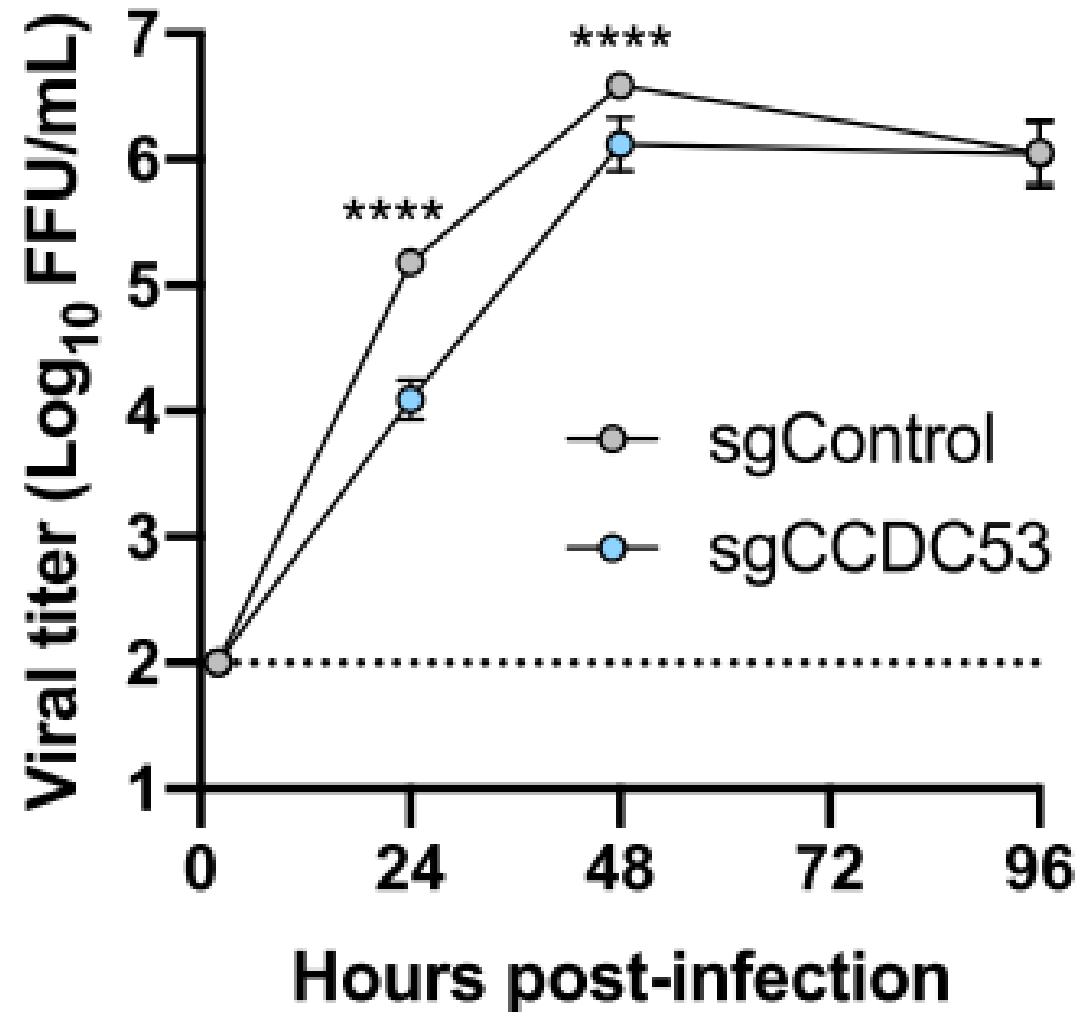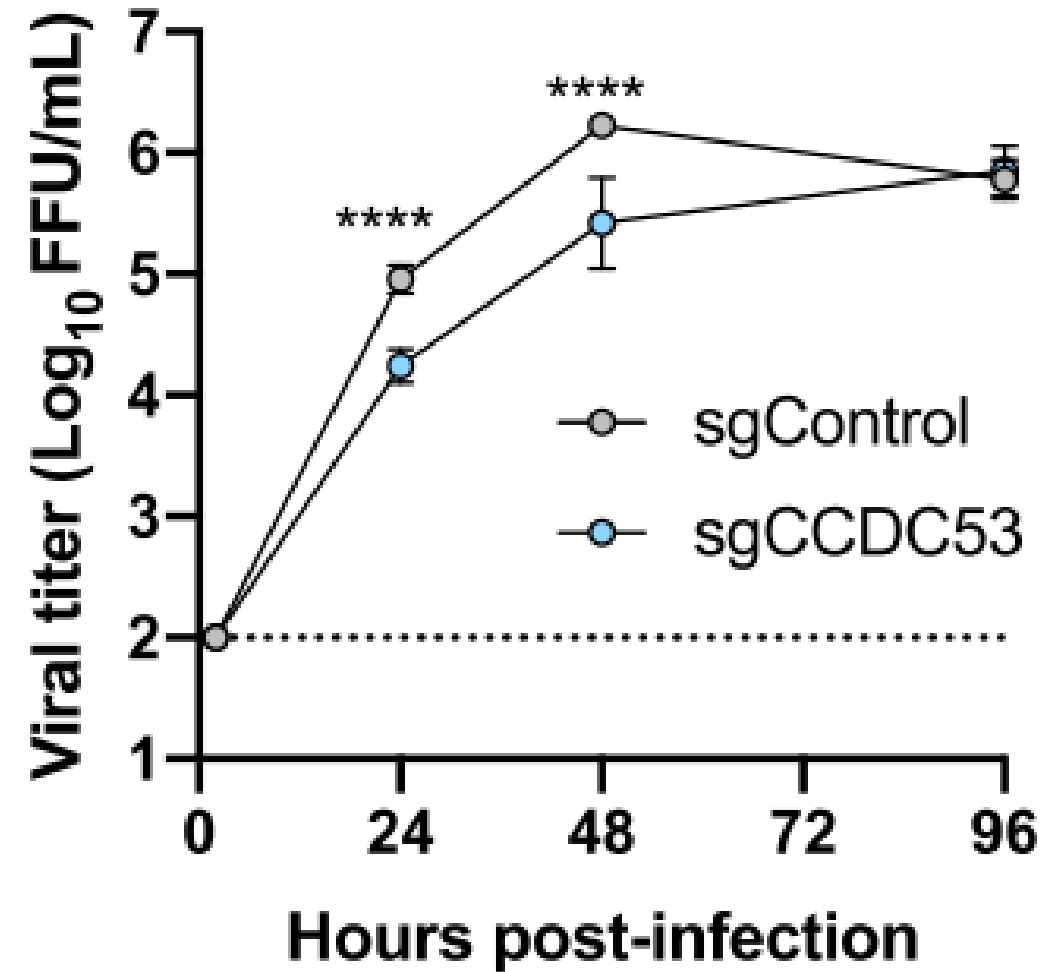
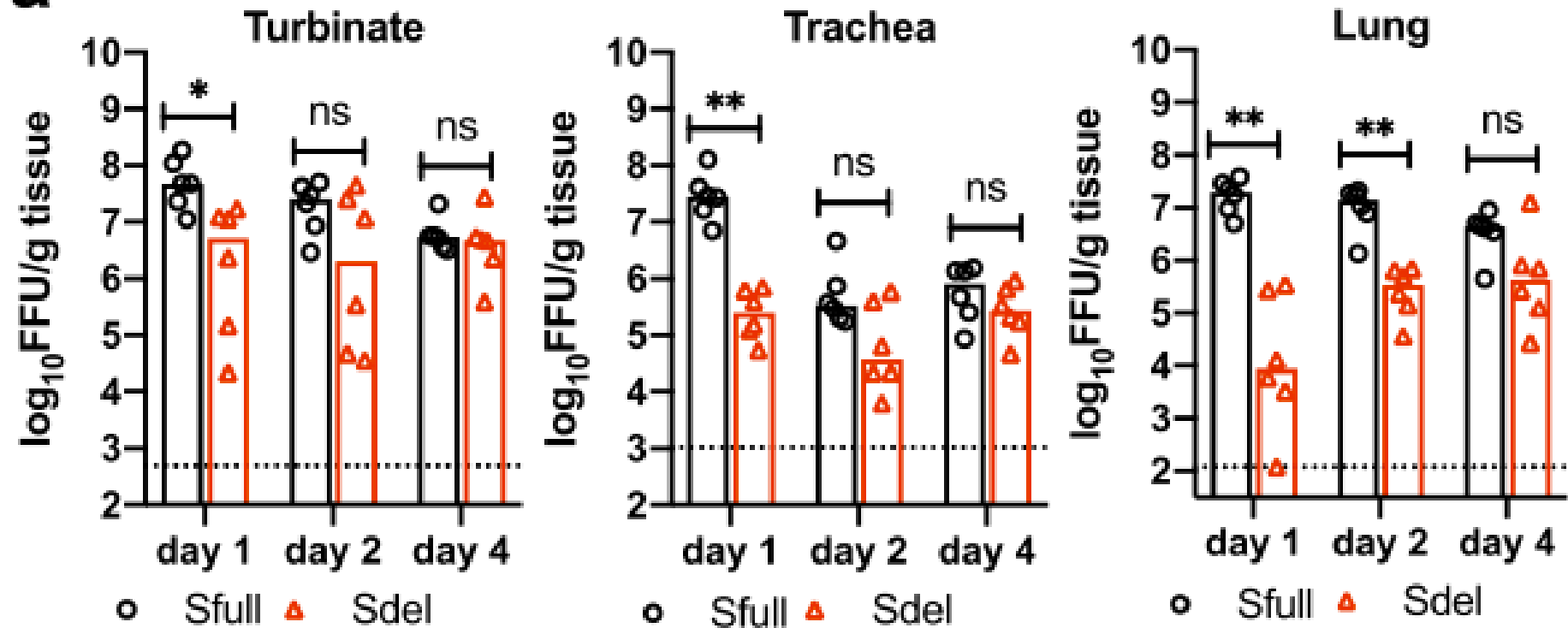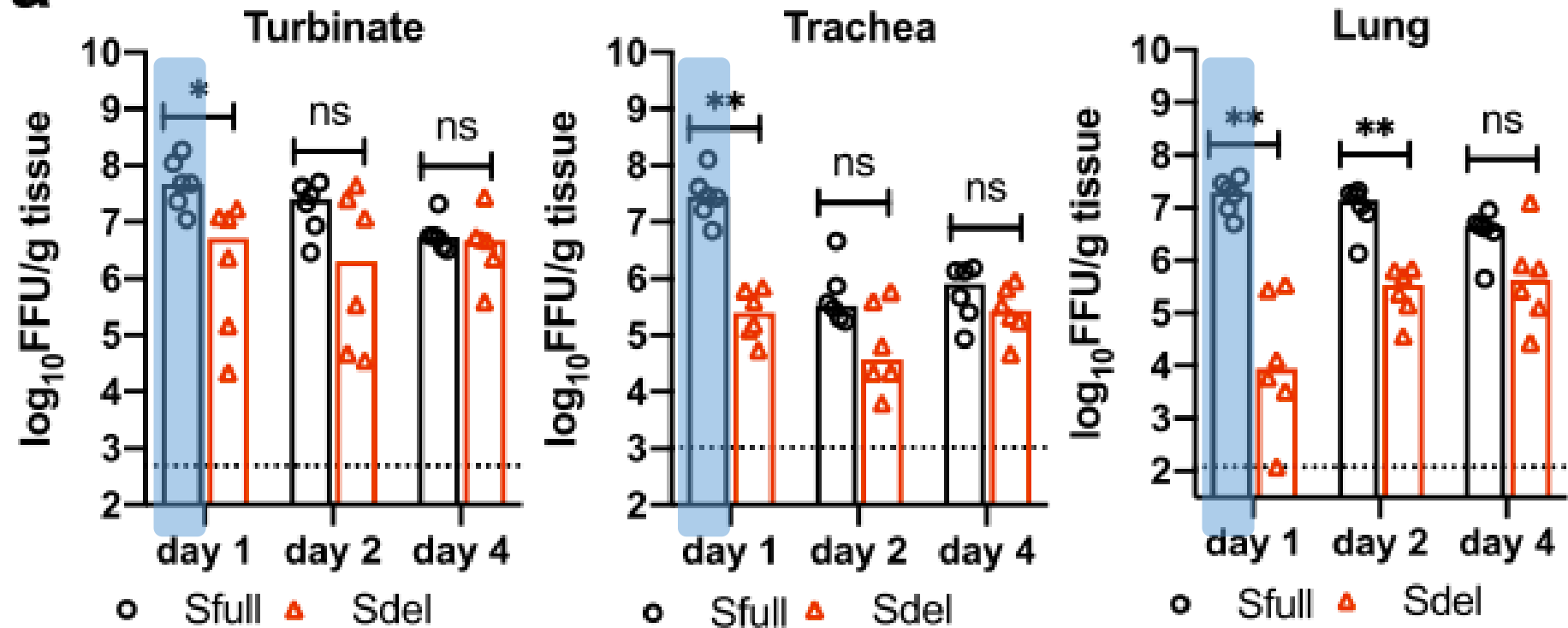How does switching the virion entry pathway modulate the infection and transmission in hamsters?
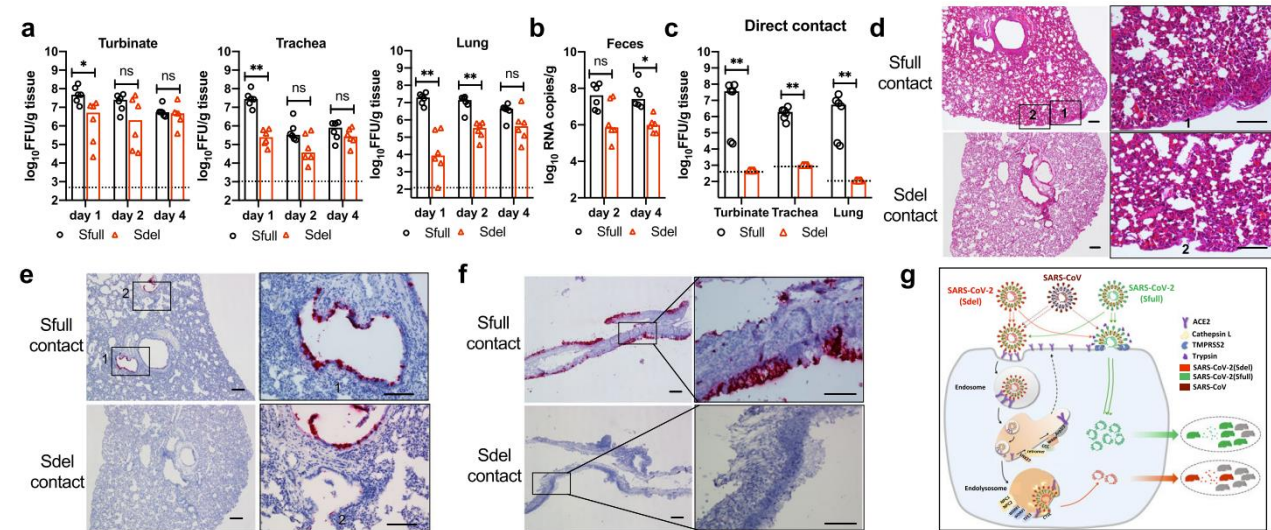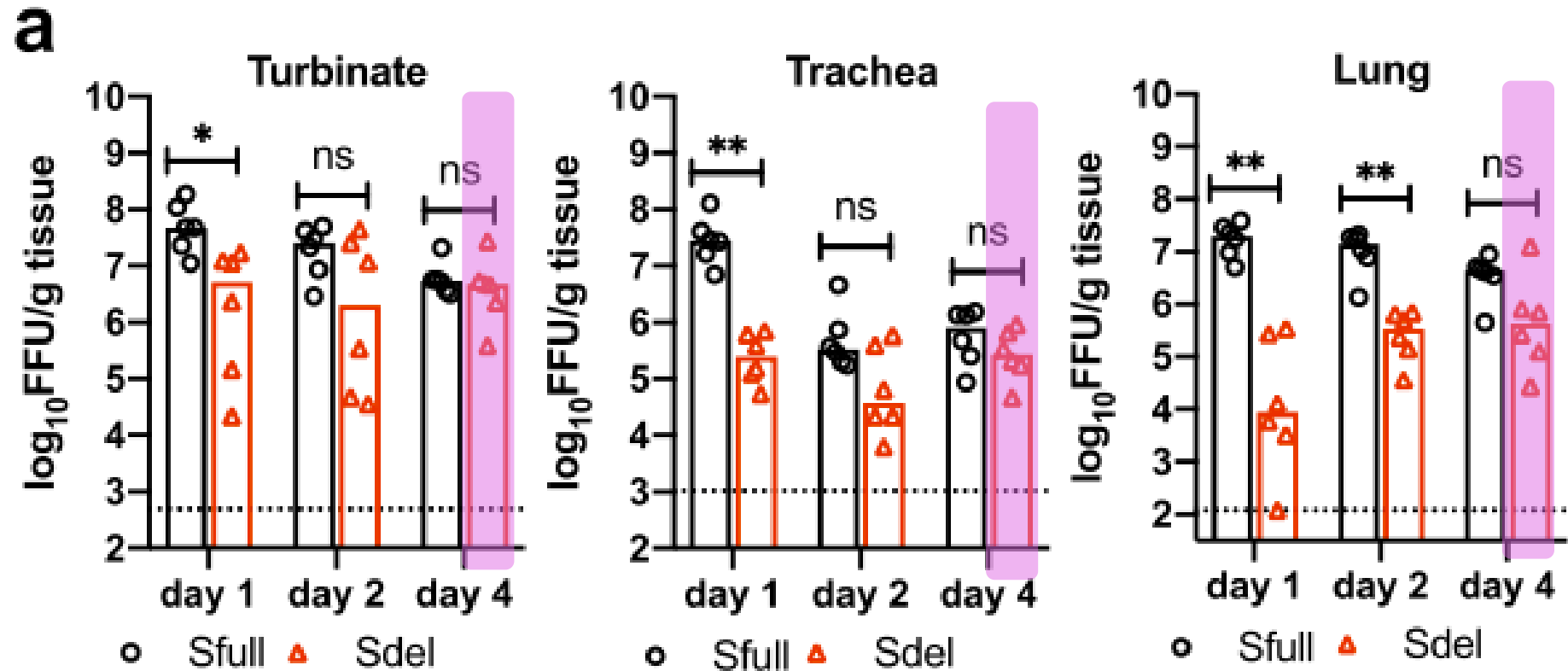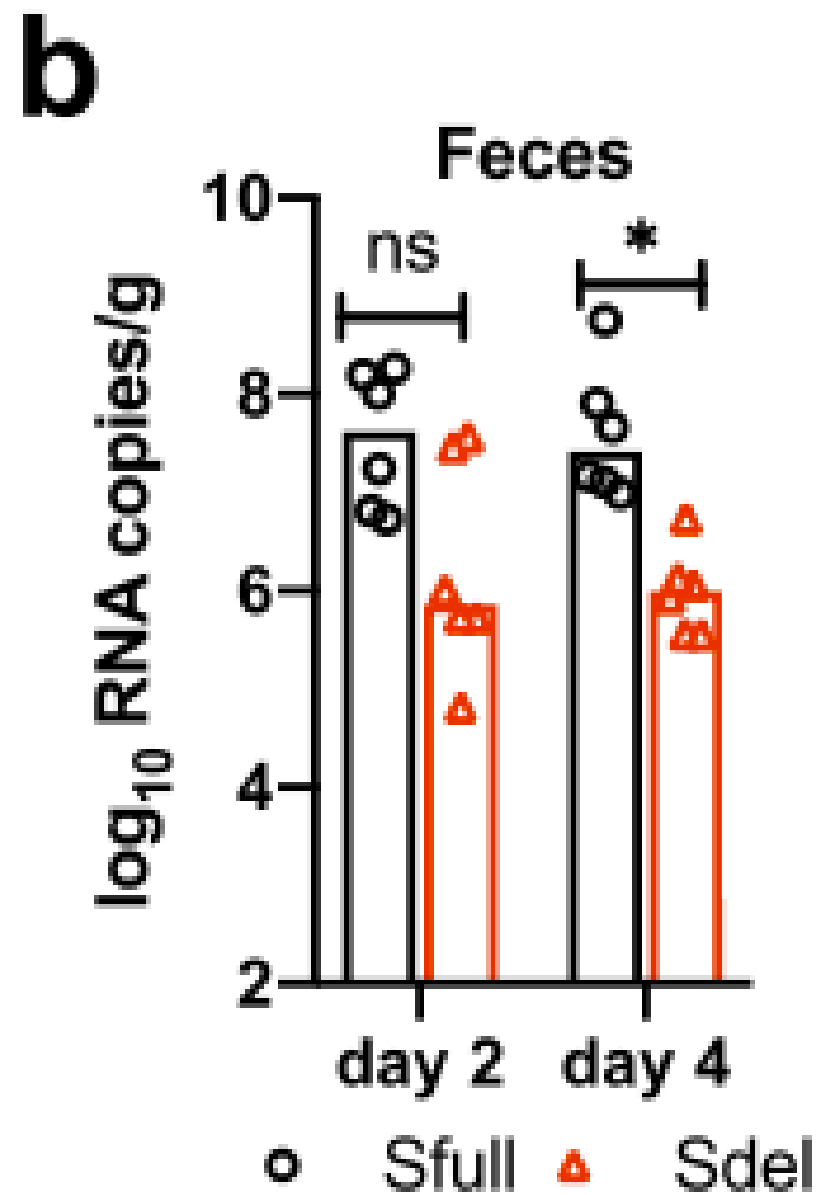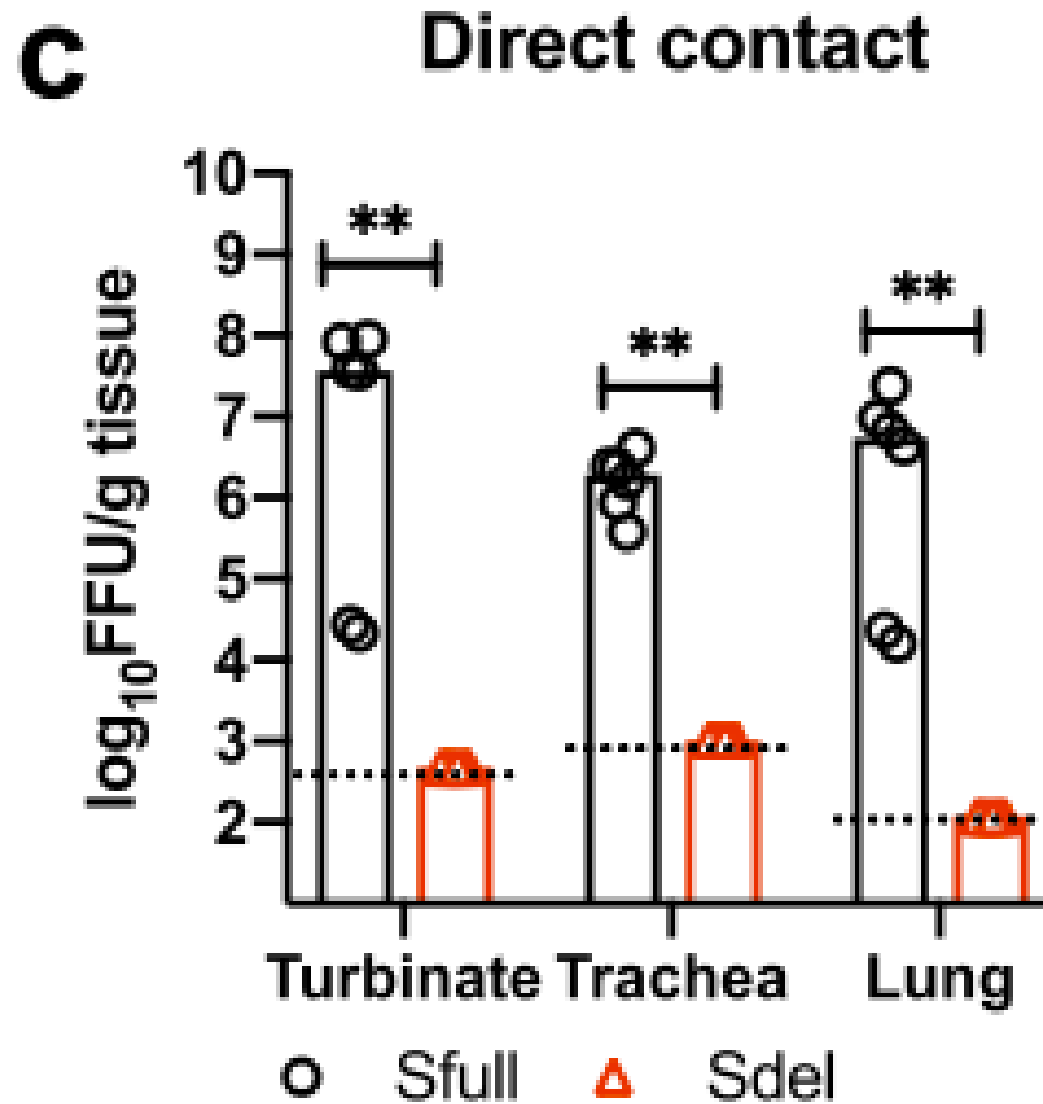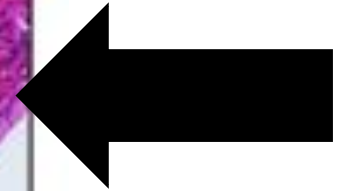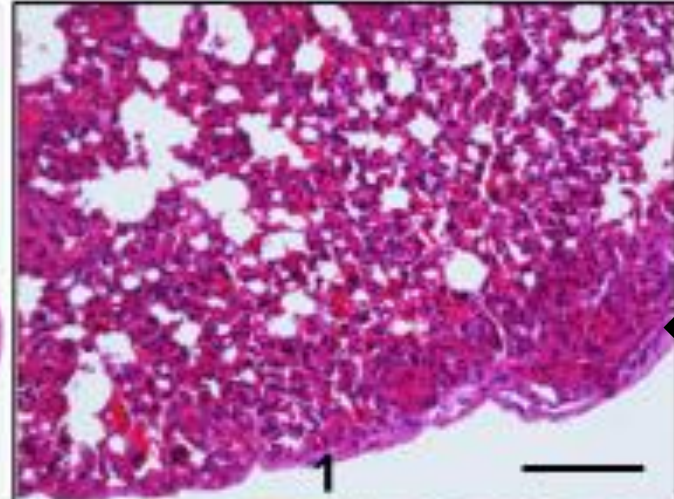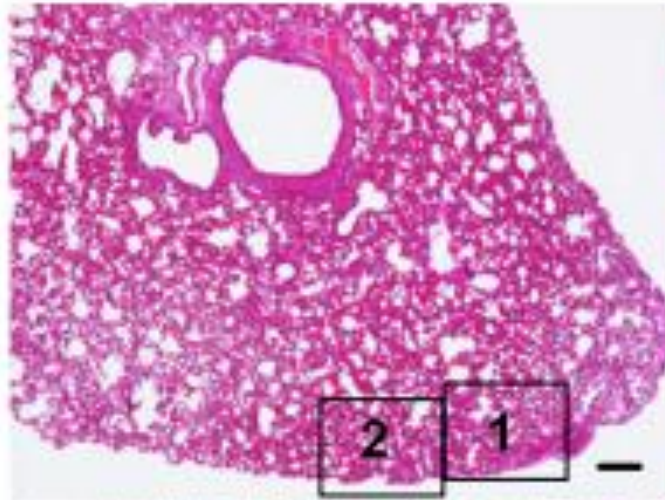
Figure 5a

Figure 5a

Figure 5a

Figure 5b

Figure 5c

d Sfull contact

Sdel contact

Figure 5d

d
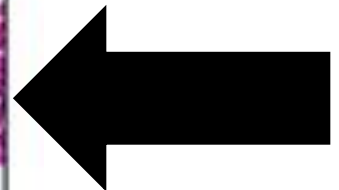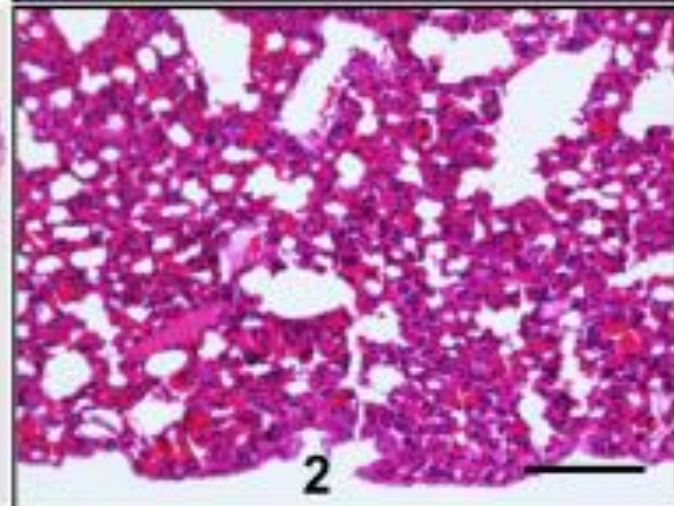
Sfull contact
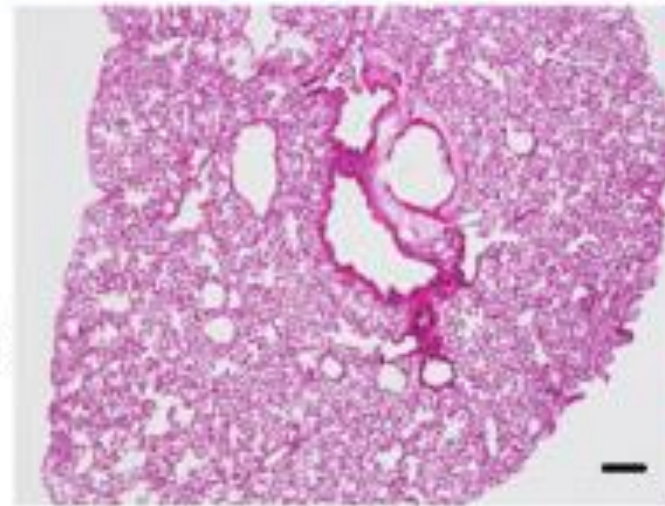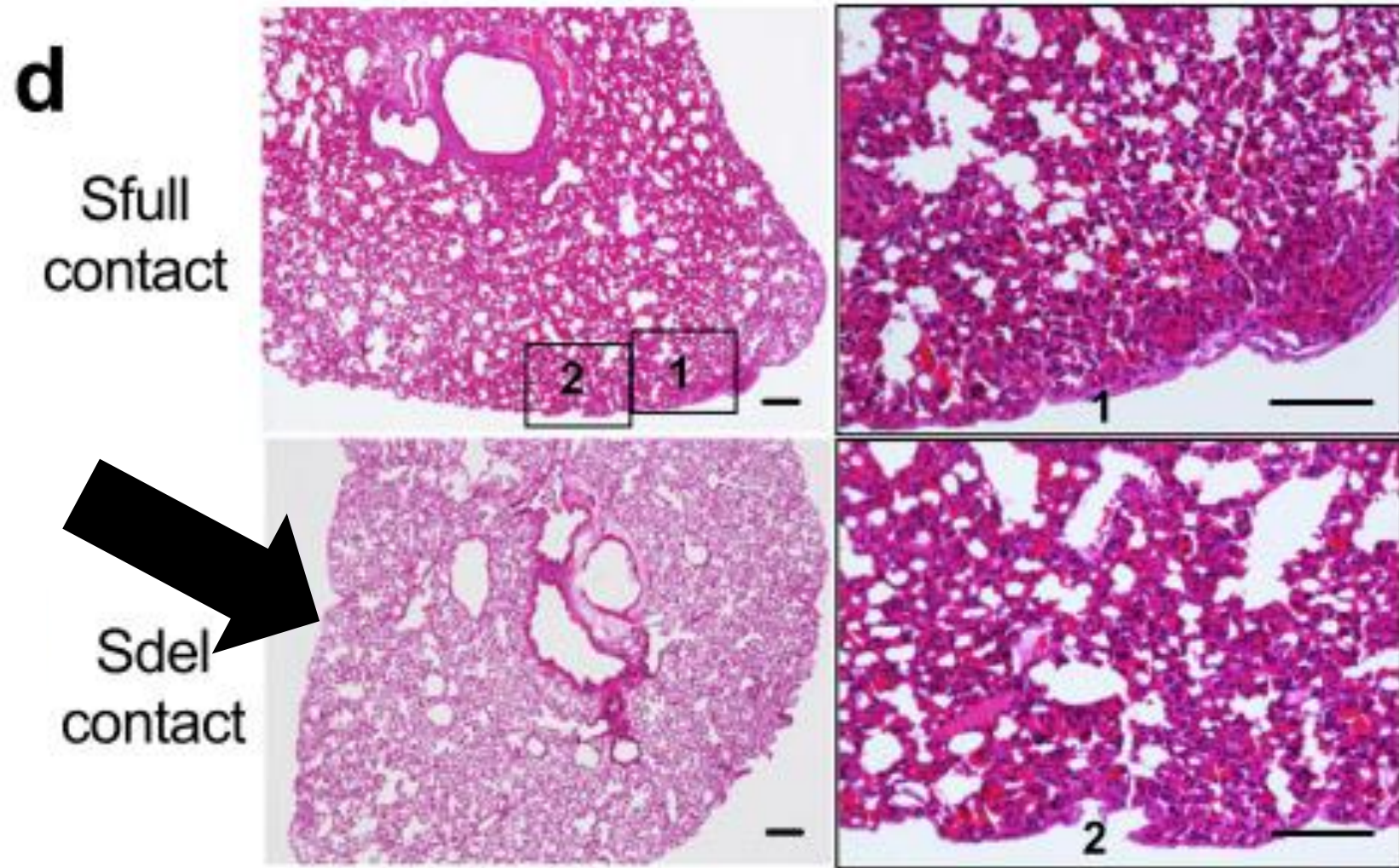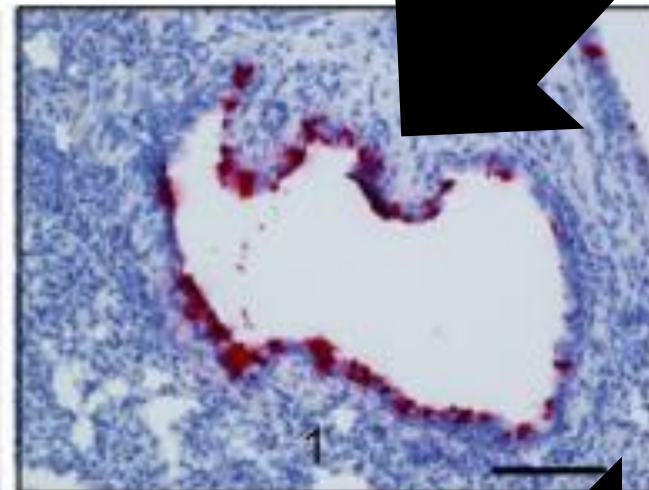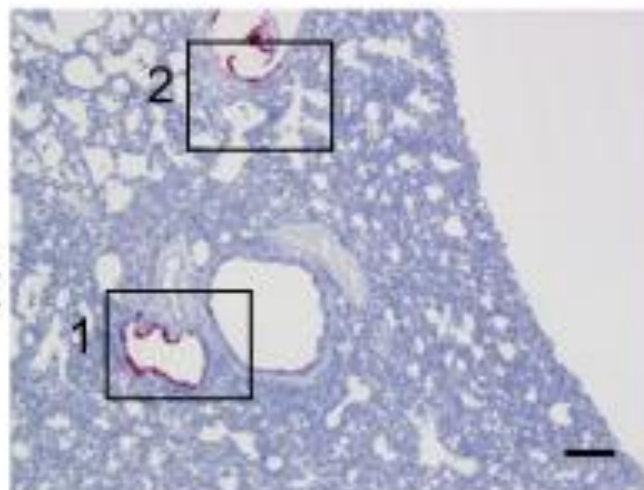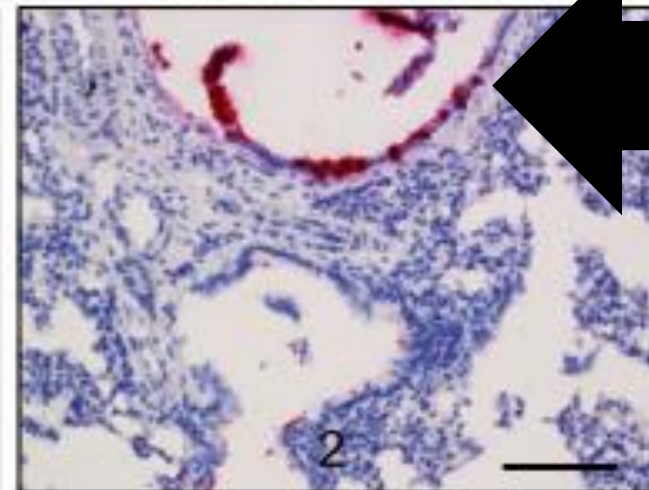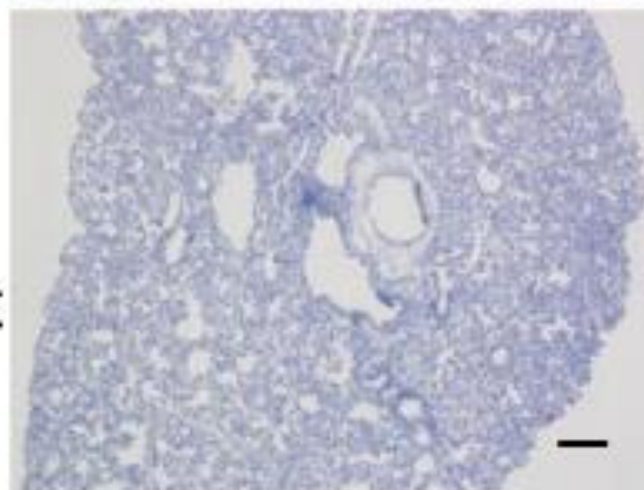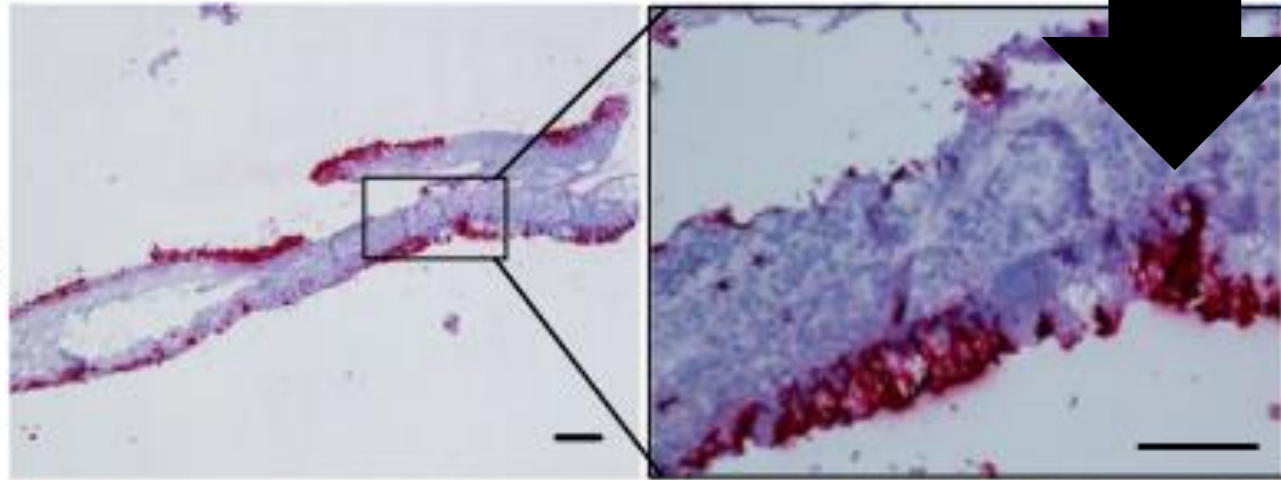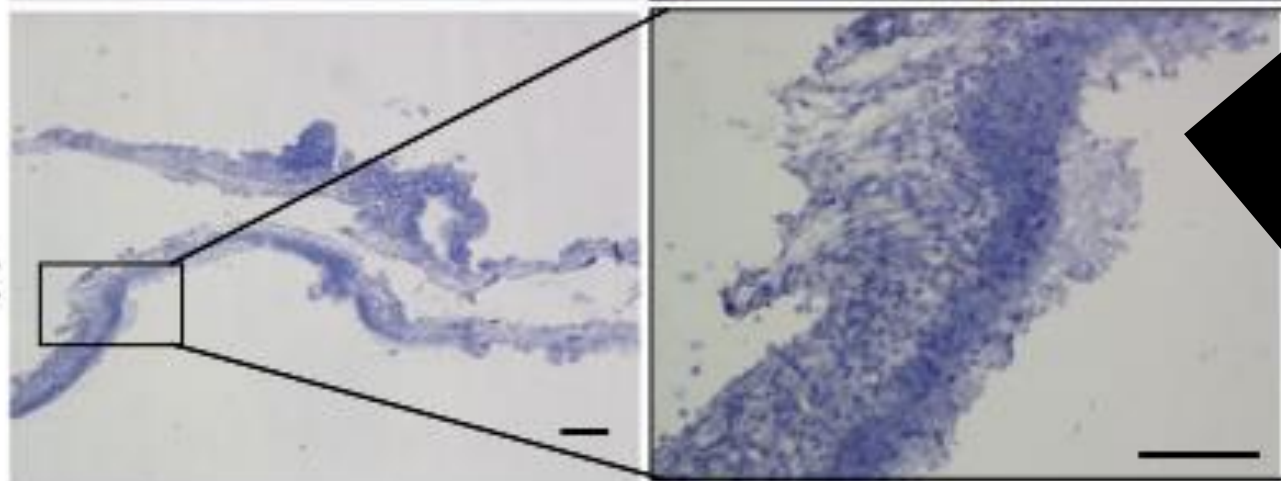
Sdel contact

Figure 5d

Figure 5e

f

Sfull contact

Sdel contact

Figure 5f

Figure 5g

a

S1/S2 cleavage site

N – | S1 | S2 | – C

SARS-CoV   661–HTVSLL————RSTSQKSIVA–676
SARS-2 (Sfull) 675–QTQTNSPRRARSVASQSIIA–694
SARS-2 (Sdel) 675–QTQTNSPR———————QSIIA–694

d Surface biotinylation

sgCtrl sgCOMMD3 sgCCDC22 sgVPS35 sgVPS29 sgNPC1 sgCCDC53 kDa

Surface ACE2
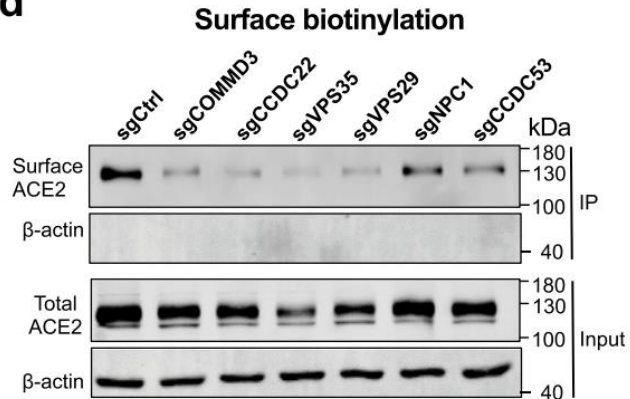β-actin   IP
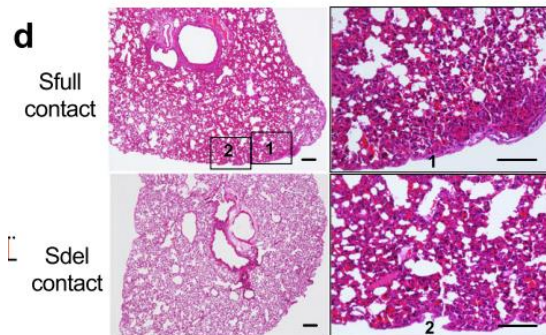
Total ACE2
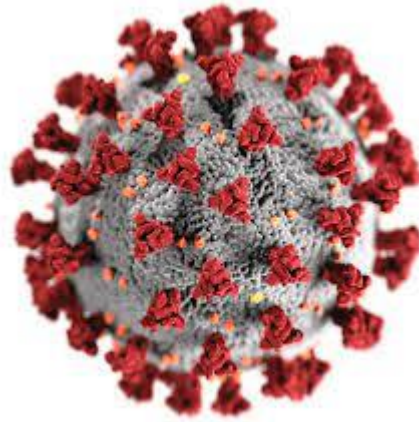β-actin   Input

d

Sfull contact

Sdel contact
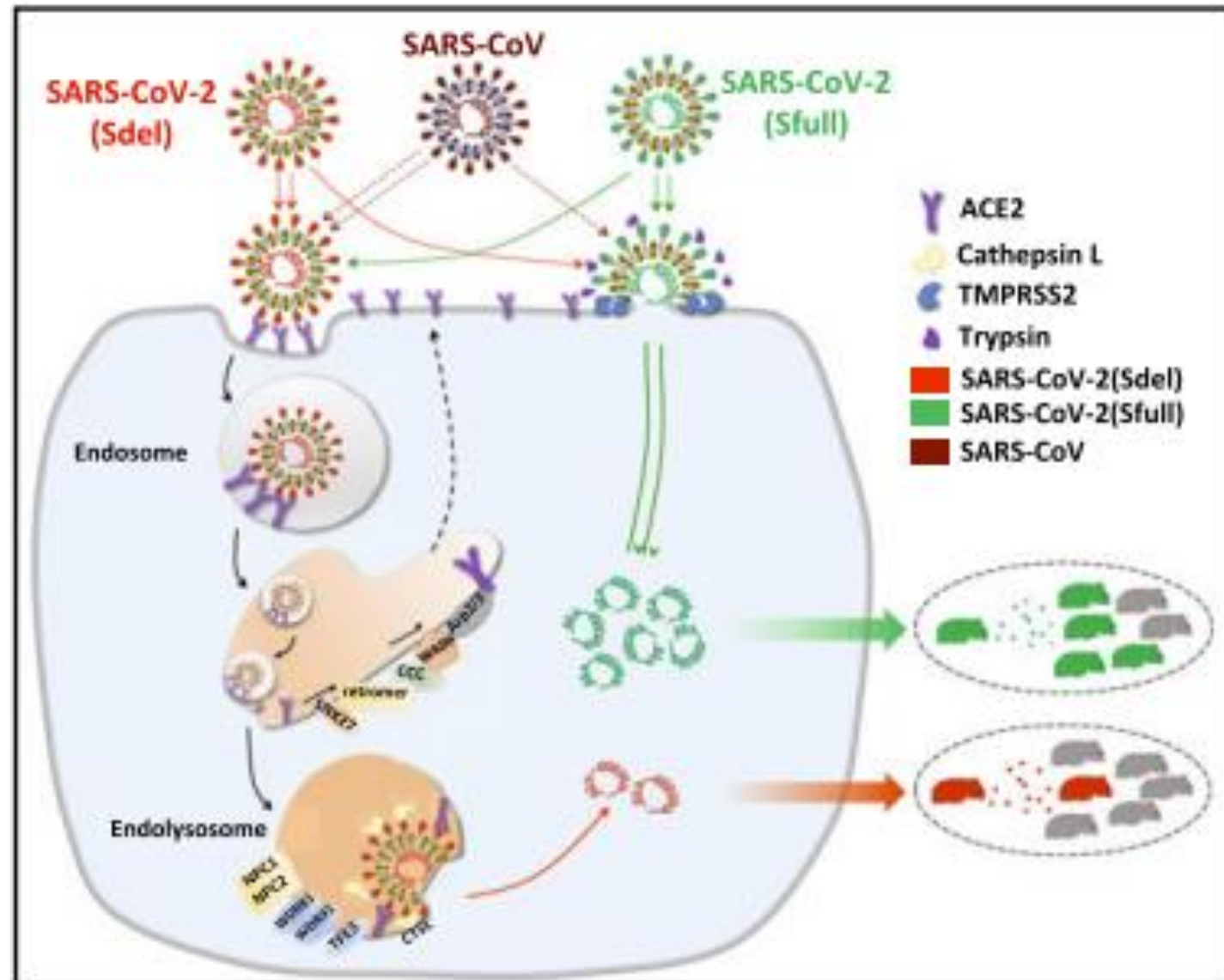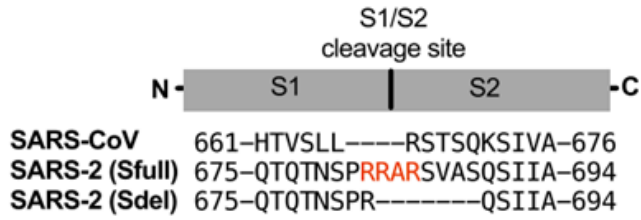
Summary

- Sequences at S1/S2 boundary of SARS-CoV-2 spike protein modulate:
  - Entry pathways
  - Infectivity
  - Transmissibility

- Host genes that regulate viral entry have been identified

- The spike deletion mutant virus can be used as a tool

Future Directions

Questions?