iScience, Volume *26*

Supplemental information

Mutating novel interaction sites in NRP1 reduces SARS-CoV-2 spike protein internalization

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Supplementary Materials for

Title: Mutating novel interaction sites in NRP1 reduces SARS-CoV-2 spike protein internalization

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Figure(s). S1 to S6

Fig. S1: Immunodetection shows no difference in the cellular localization of mutant NRP1 compared to wild-type NRP1, Related to Fig. 1. Vero-E6 cells were transiently transfected with indicated N-terminal HA tagged NRP1 constructs for 30 hr. Detection of ZO-1 (red) and HA-antibody (green) were done as described in "Methods" without permeabilizing the cells. Images were acquired using Zeiss LSM 710 confocal microscope outfitted with a 63x objective. Scale bars represent 20 μm.

Fig. S2: Mutation in NRP1 impacts on interaction between NRP1 and SARS-CoV-2 spike protein, Related to Fig. 2. 293T and Vero-E6 cells were transfected with the indicated HA-NRP1 (wild type and mutants) and Flag-SARS-CoV-2-$S1^{493-685}$ constructs. Cells were lysed 30 hr post transfection, and the interaction between HA-NRP1 and Flag-SARS-CoV-2-$S1^{493-685}$ was analyzed. The relative amount of spike protein pulled down with NRP1 was normalized directly to immunoprecipitated NRP1 (a and c) or to the total amount of spike protein expressed in the cells and then to immunoprecipitated NRP1 (b and c). n = 2 biological replicates; error bars, SEM; ns, no significance; *, $p<0.05$; **,$p < 0.005$; ***, $p < 0.0005$ were calculated by one way ANOVA.

Fig. S3: SARS-CoV-2 $S1^{493-685}$ colocalization with NRP1 is cysteine dependent in HeLa cells, Related to Fig. 3. (a) Representative images of colocalization studies between Flag-SARS-CoV-2-$S1^{493-685}$ and different HA-NRP1 constructs by confocal immunofluorescence microscopy in

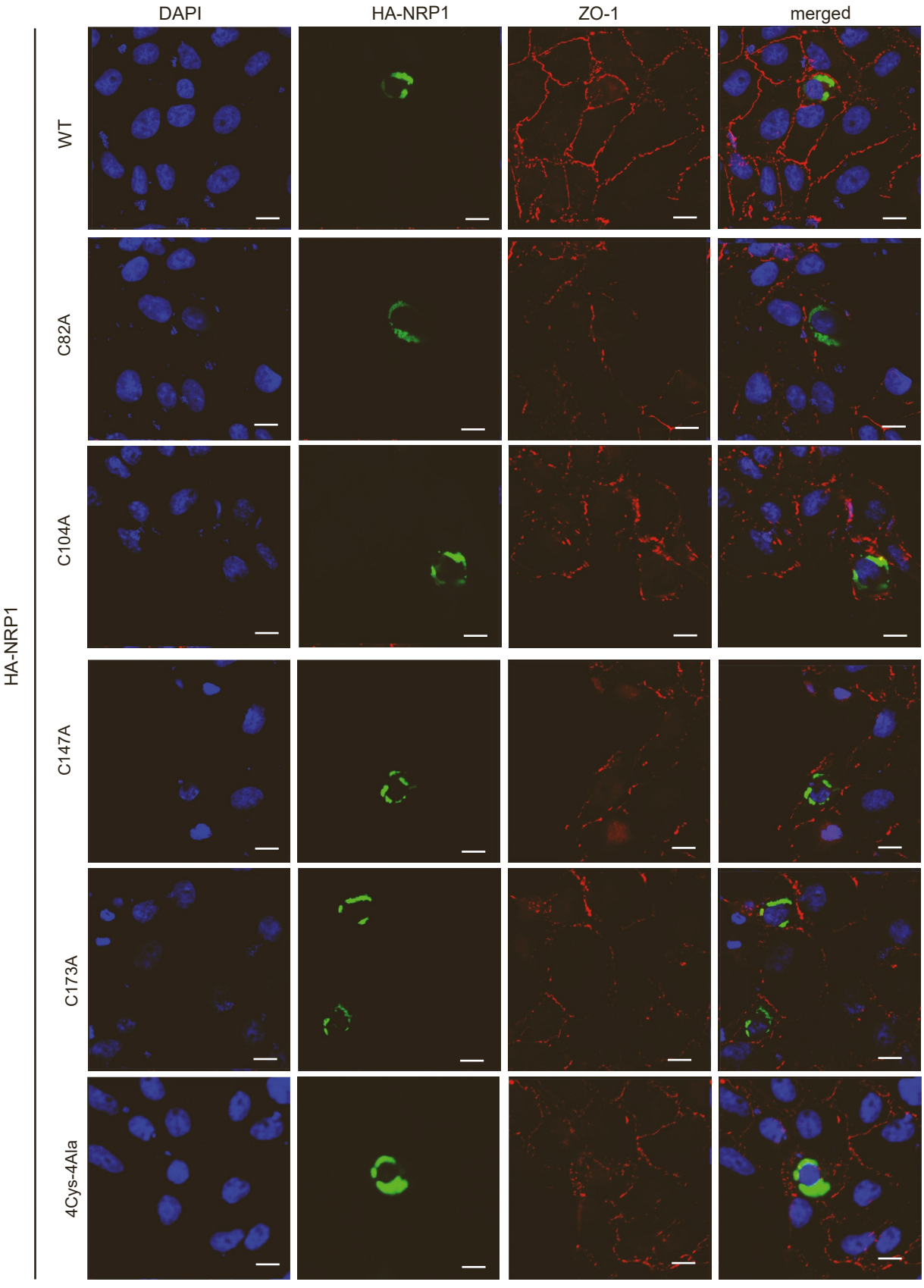
HeLa cells. The cells were transiently transfected with Flag-SARS-CoV-2-S1$^{493-685}$ and different mutants of HA-NRP1 as indicated. 30 hours post-transfection cells were fixed, mounted and protein expression patterns were visualized using a Zeiss LSM 710 confocal microscope. Scale bars represent 50 μm. The images shown are representative from three independent biological experiments (average 100 cells were observed per experimental condition per replicate). (b) Quantification of the HeLa cells expressing Flag-SARS-CoV-2-S1$^{493-685}$ in the presence of indicated HA-NRP1 constructs. Data are represented as mean ± SD, n = 3 (average 100 cells were observed for each condition per experiment); ns, no significance; *, p<0.05; **,p < 0.005; ***, p < 0.0005 were calculated by one-way ANOVA.

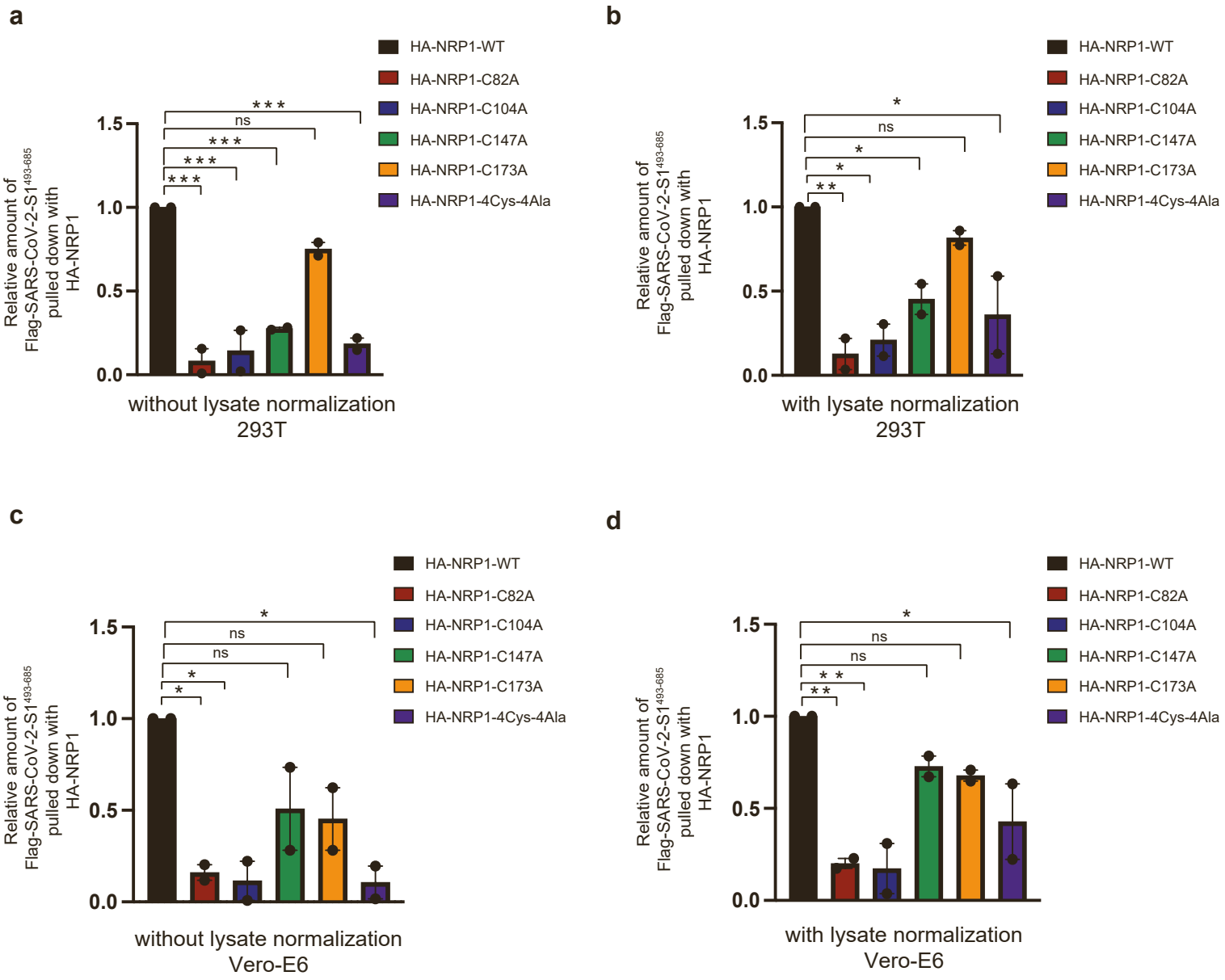
Fig. S4: Detection of interaction of NRP1 and SARS-CoV-2 spike protein in cells, Related to Fig. 3. Quantification of the Vero-E6 cells expressing Flag-SARS-CoV-2-S1$^{493-685}$ in the presence of indicated HA-NRP1 corresponds to Fig 3. Data are represented as mean ± SD, n = 3 (average 100 cells were observed for each condition per experiment), and *,p<0.05; **,p < 0.005; ***, p < 0.0005; and ****, p < 0.0001 were calculated by one-way ANOVA.

Fig. S5: NRP1 interaction with co-receptor Plexin-A1 is cysteine dependent, Related to STAR Methods. (a) 293T cells were transfected with HA-NRP1-WT or HA-NRP1-4Cys-4Ala together with Flag-Plexin-A1. Cell lysates were then immunoprecipitated with anti-Flag antibody and blotted with anti-HA or anti-Flag antibody. (b) Quantification of the band intensities ($n = 2$). Immunoprecipitated HA-NRP1 band intensities were normalized to the respective Flag-Plexin-A1 IP bands and then further normalized to HA-NRP1-WT control. Data are represented as mean ±

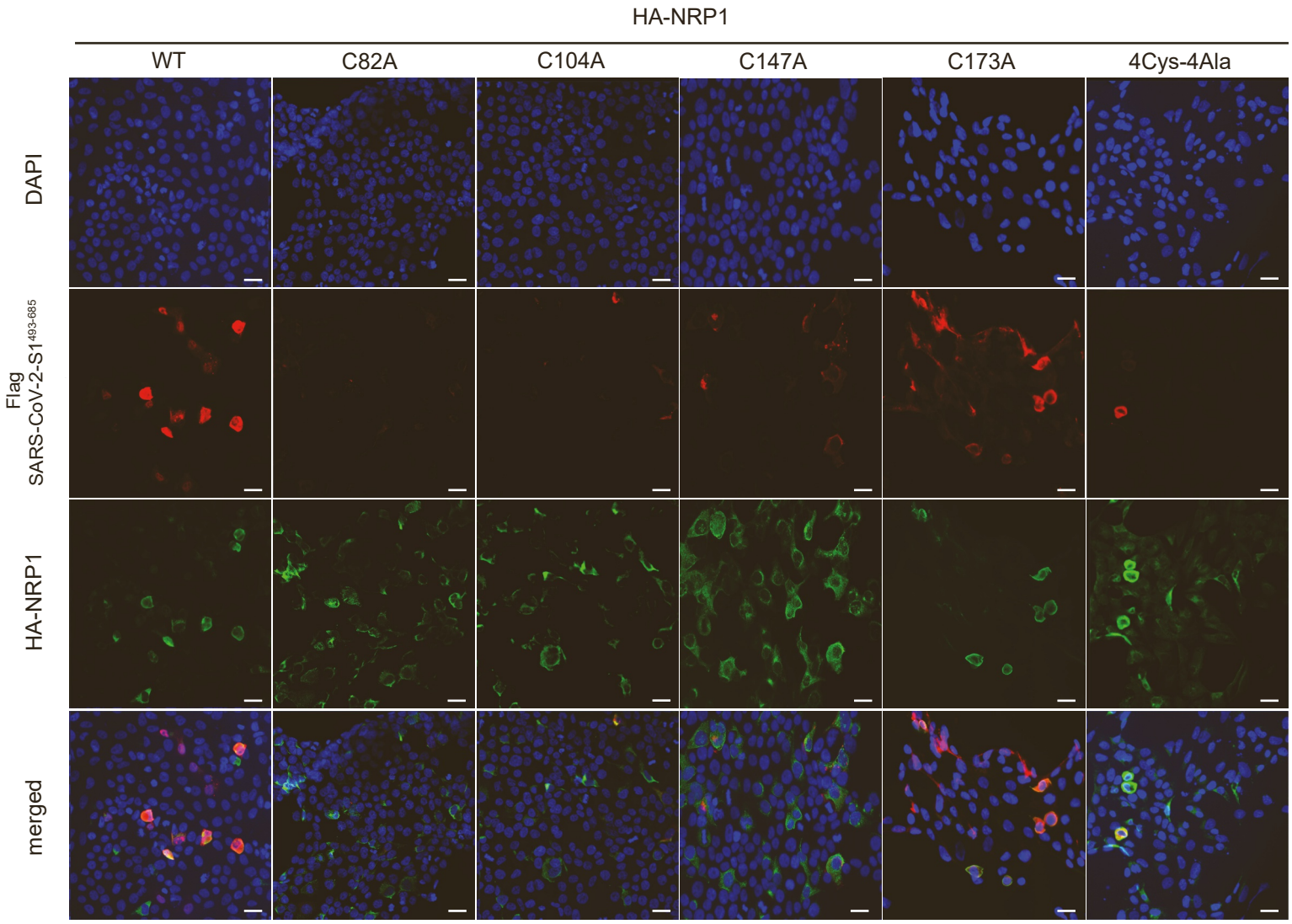
SD, and ***, $p < 0.0005$ (Student's t test). (c) Representative images of colocalization studies between Flag-tagged Plexin-A1 protein and indicated HA-NRP1 constructs by confocal immunofluorescence microscopy in HeLa cells. The cells were transiently transfected with Flag-Plexin-A1 and different constructs of HA-NRP1 as indicated. 30 hours post-transfection cells were fixed, mounted and protein expression patterns were visualized using a Zeiss LSM 710 confocal microscope. Scale bars represent 50 μm. The images shown are representative from three independent biological experiments (average 100 cells were observed per experimental condition per replicate).

Fig. S6: Attenuated colocalization of NRP1 mutant with endolysosomal marker LAMP1, Related to STAR Methods. Representative image of Vero-E6 cells transfected with different HA-NRP1 constructs (both WT and 4Cys-4Ala) and then coimmunostained with DAPI (blue), antibody specific to HA-tagged NRP1 protein (HA, red) and anti-LAMP1 (green). Cells were visualized using a Zeiss LSM 710 confocal microscope outfitted with a 63x objective. Scale bars represent 10 μm.

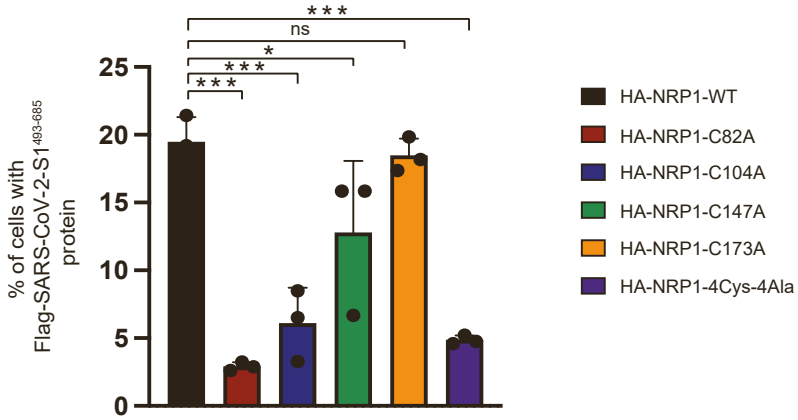

DAPI
HA-NRP1
ZO-1
merged
WT
C82A
C104A
C147A
C173A
4Cys-4Ala
HA-NRP1
Vero-E6

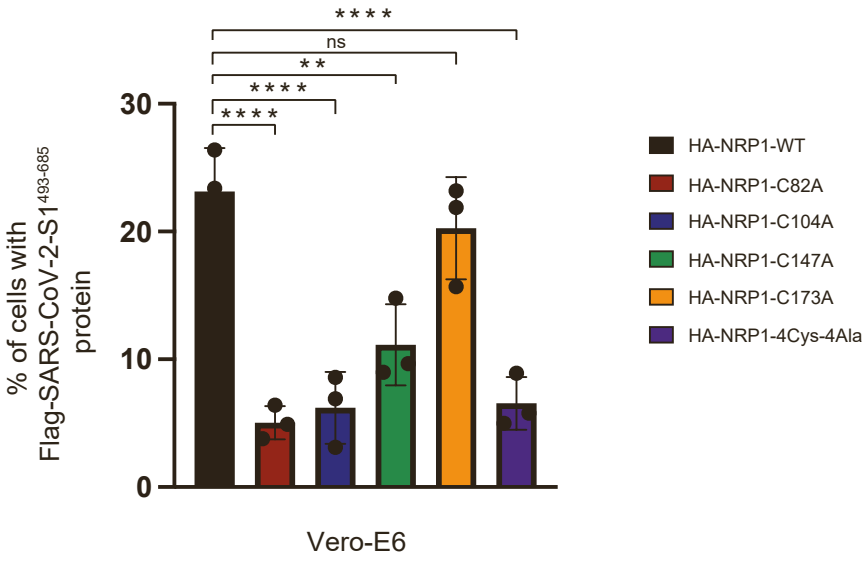

a
b
c
d
HA-NRP1-WT
HA-NRP1-C82A
HA-NRP1-C104A
HA-NRP1-C147A
HA-NRP1-C173A
HA-NRP1-4Cys-4Ala
Relative amount of Flag-SARS-CoV-2-S1493-685 pulled down with HA-NRP1
without lysate normalization
293T
with lysate normalization
293T
without lysate normalization
Vero-E6
with lysate normalization
Vero-E6
ns
0.0
0.5
1.0
1.5

a

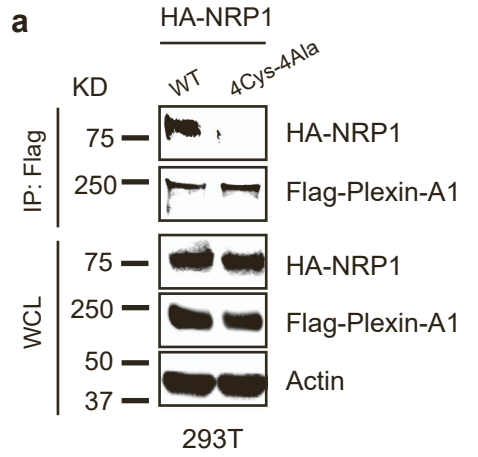

HA-NRP1
WT
C82A
C104A
C147A
C173A
4Cys-4Ala
DAPI
Flag SARS-CoV-2-S1493-685
HA-NRP1
merged
HeLa

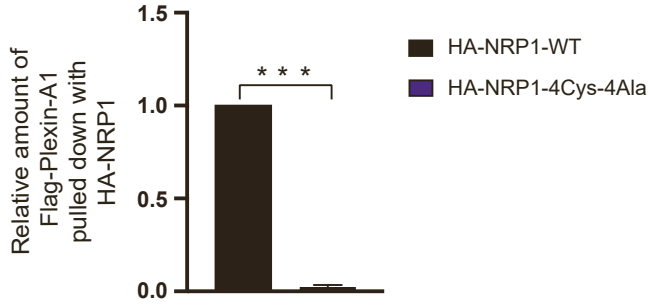
b

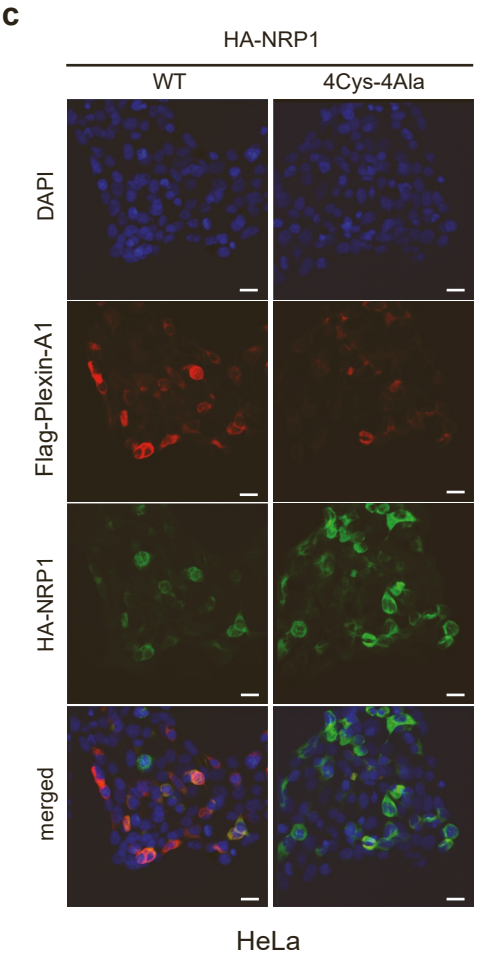

***
ns
*
***
***
25
20
15
10
5
0
% of cells with Flag-SARS-CoV-2-S1493-685 protein
HA-NRP1-WT
HA-NRP1-C82A
HA-NRP1-C104A
HA-NRP1-C147A
HA-NRP1-C173A
HA-NRP1-4Cys-4Ala

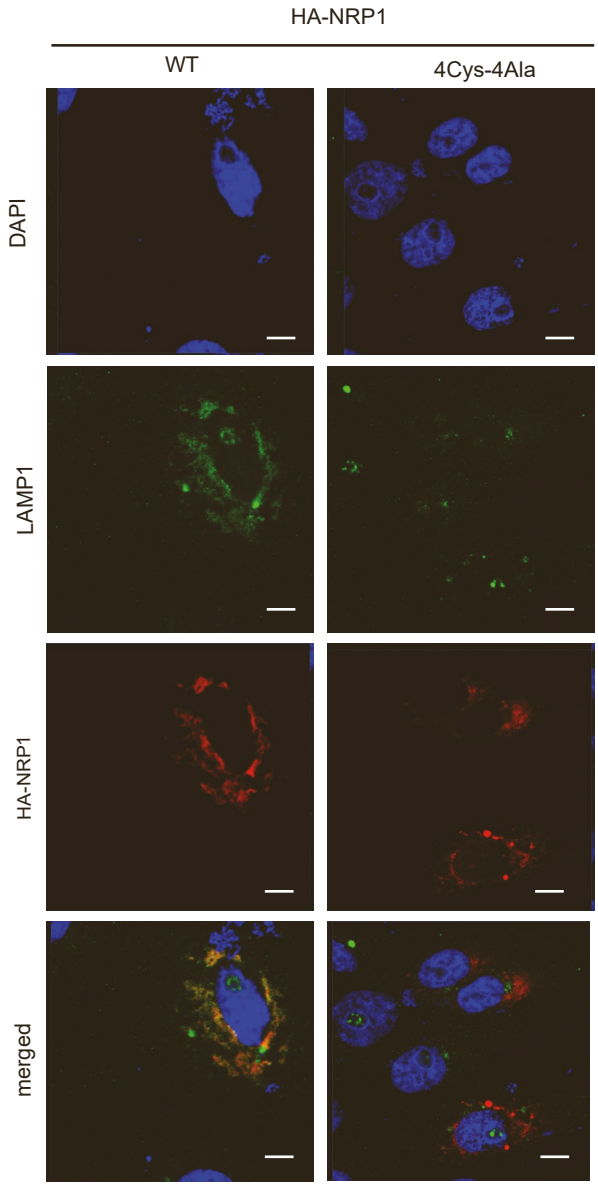

****
ns
**
****
****
30
20
10
0
% of cells with Flag-SARS-CoV-2-S1493-685 protein
Vero-E6
HA-NRP1-WT
HA-NRP1-C82A
HA-NRP1-C104A
HA-NRP1-C147A
HA-NRP1-C173A
HA-NRP1-4Cys-4Ala

Pal et al., Supplemental Figure 5








HA-NRP1
WT
4Cys-4Ala
DAPI
LAMP1
HA-NRP1
merged
Vero-E6