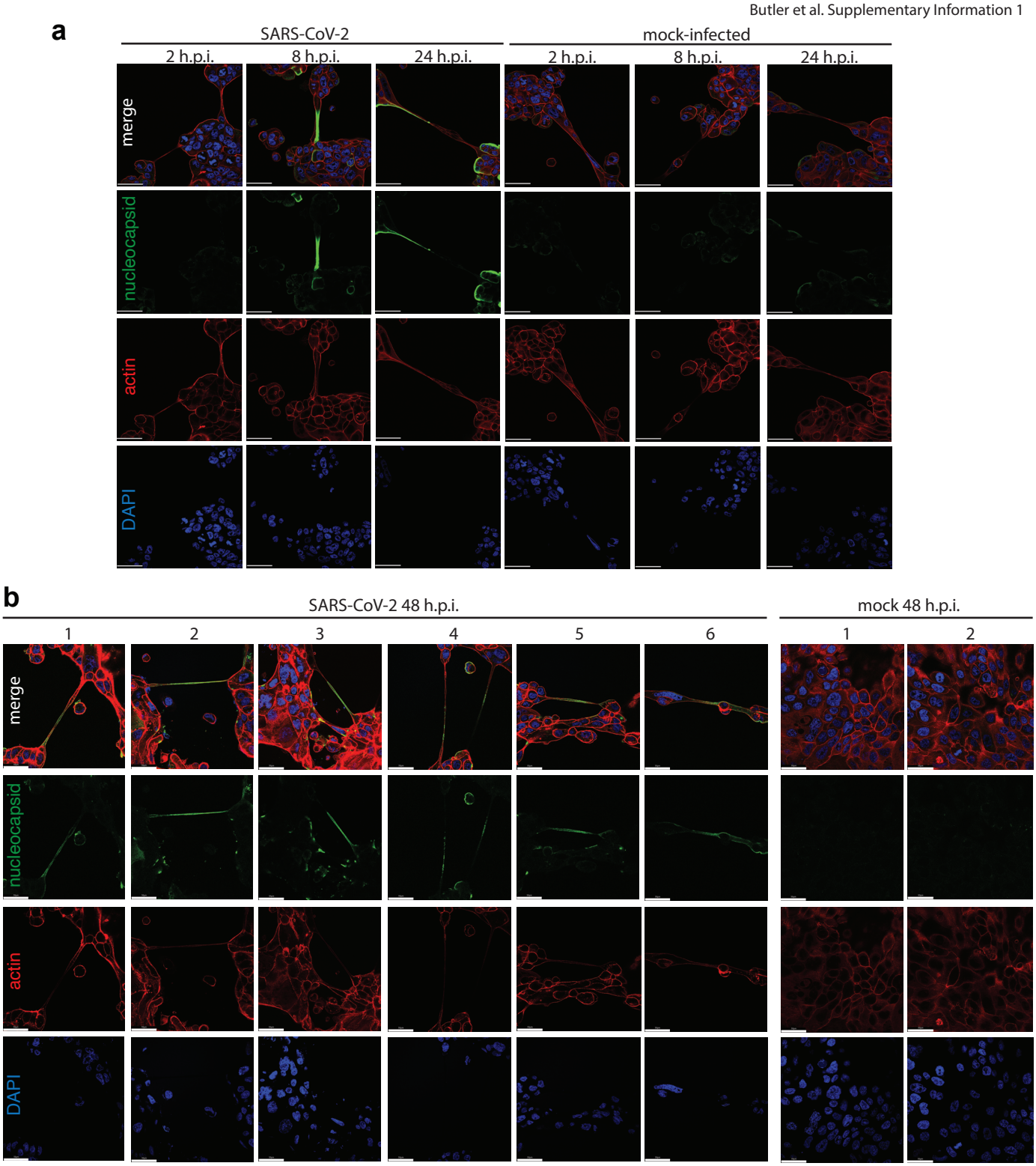
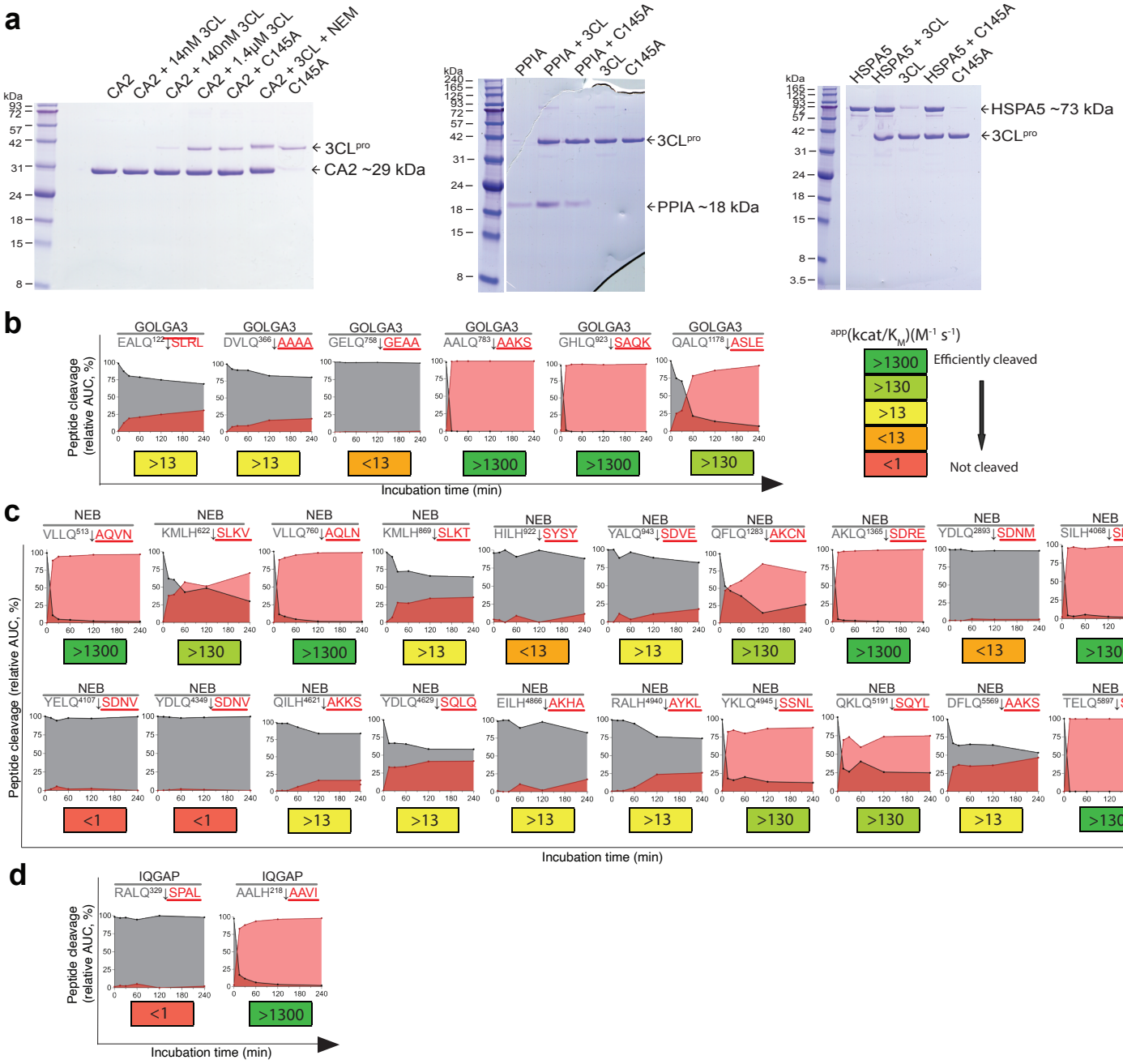
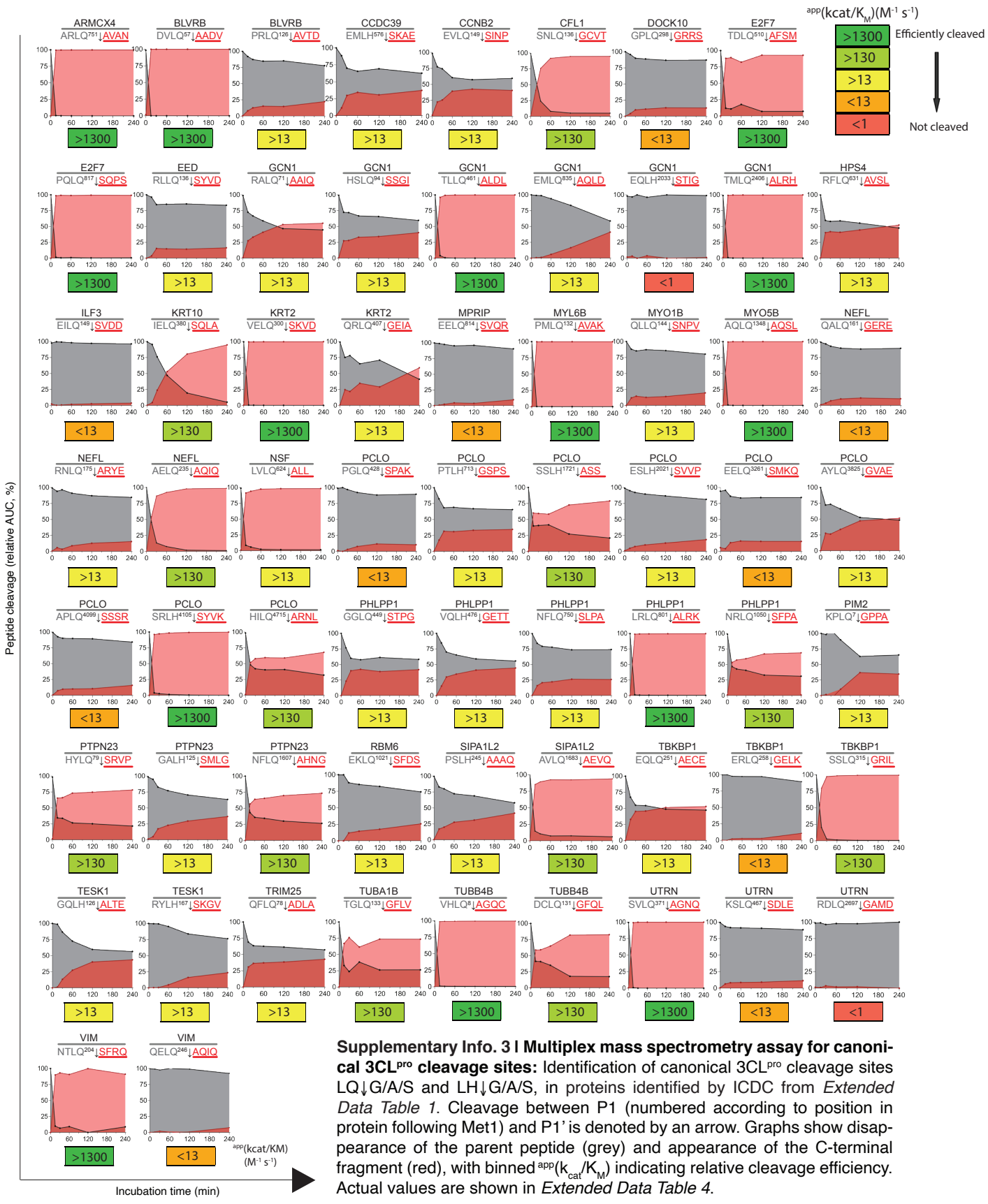


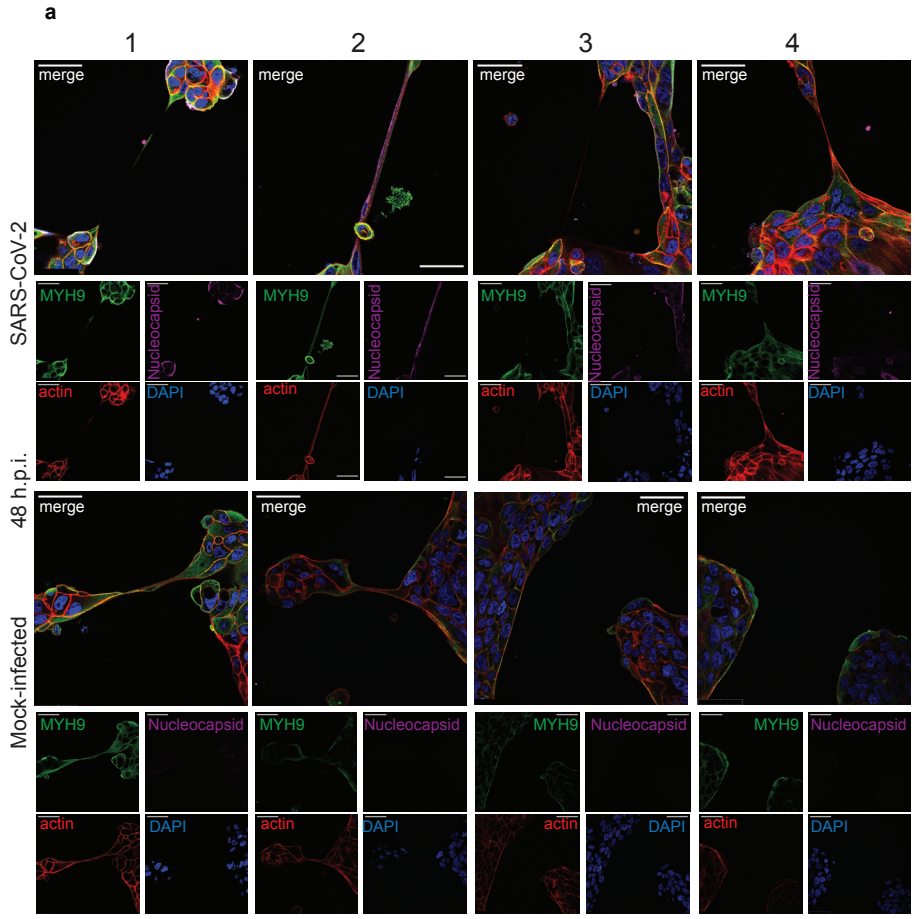
Supplementary Info. 1 I SARS-CoV-2 nucleocapsid protein was detected in TNTs and colocalised with actin in infected human lung epithelial cells: Supporting data for Fig. 1. Calu-3 cells were stained for viral nucleocapsid (SARS Coronavirus NP Monoclonal Antibody, Thermo Scientific #MA5-29981) at 1:250 dilution, together with phalloidin and DAPI to visualise the actin cytoskeleton and nuclei respectively. **a** Nucleocapsid protein was visible in TNTs from 8 h.p.i. Merged infected panels (2, 8, 24 h.p.i) are shown in Fig. 1e.; **b** 48 h.p.i. All LUT 0-255. Merged infected panels 1-3 are shown in Fig. 1f. Representative images ($n > 10$), confocal images at 63X magnification, scale bar 50 μm. Images were captured using identical settings. Individual channels were merged using Image J (https://imagej.net/ij/). The intensity of the phalloidin channel in panel (a) was uniformly adjusted in Image J from LUT 0-255 to 0-150.



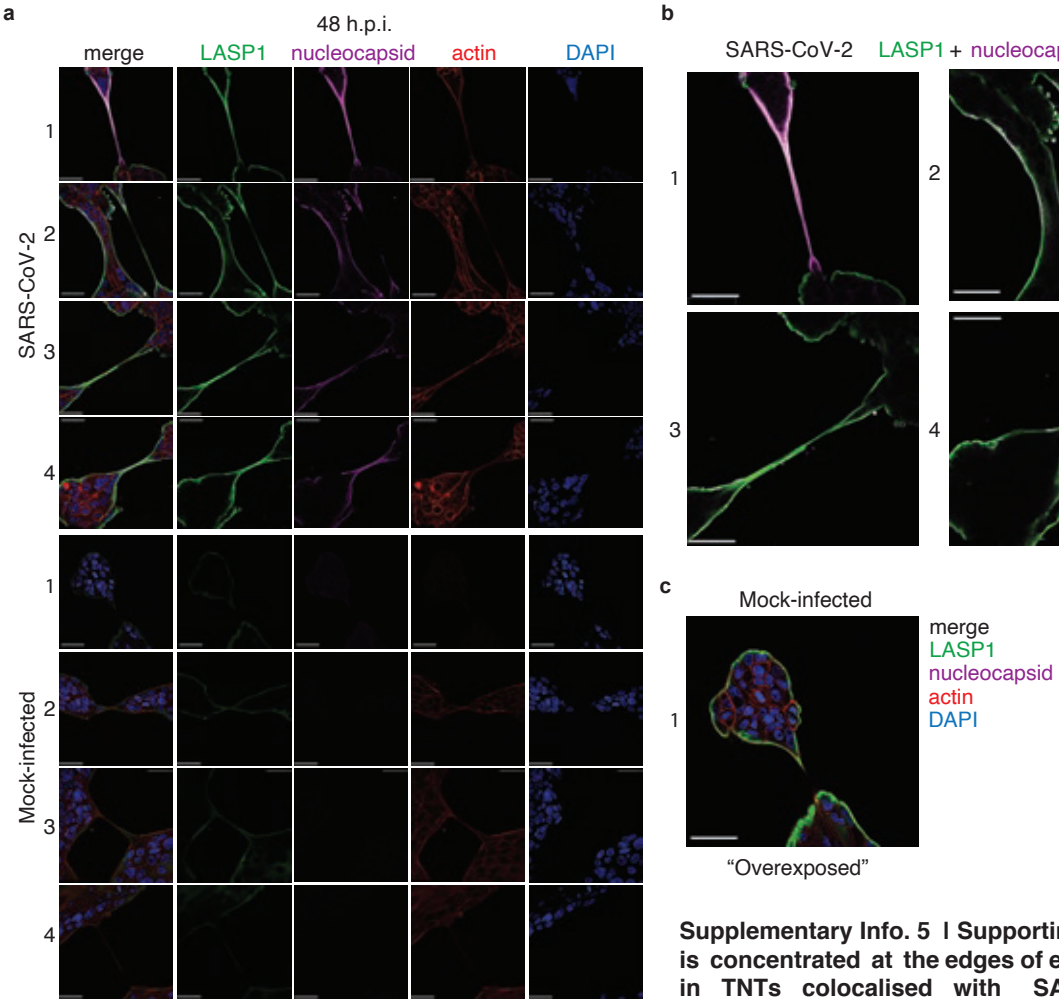
Supplementary Info. 2 I a Proteins from the 3CL^{pro} interactome identified by ICDC that lack canonical cleavage sites were resistant to cleavage: Recombinant human proteins were incubated at a 5:1 molar ratio with active 3CL^{pro} or inactive 3CL^{pro}-C145A overnight at 37 °C. The cysteine protease inhibitor N-ethylmaleimide (NEM) (at a high, 5 mM, final concentration) was included as an additional control. NEM caused an upward shift in molecular weight of all proteins in the lane. Samples were electrophoresed on 12% or 4-12% SDS-PAGE gels and stained with Coomassie R250 dye. Estimated molecular weights of the recombinant proteins are shown. Proteins analysed were: Peptidyl-prolyl cis-trans isomerase A (PPI1A) (R&D Systems #3589-CA), Endoplasmic reticulum chaperone BiP (Heat shock protein A5, HSPA5) (Prospec #hsp-044-b), and Carbonic anhydrase 2 (CA2) (Prospec #enz-420, lot # 618PCARA2), none of which have predicted 3CL^{pro} cleavage sites. **Supporting data for Extended Data Table 4:** Graphs for the multiplex mass spectrometry assay for **b** the known 3CL^{pro} substrate GOLGA3, **c** Nebulin (NEB), and **d** GTPase-activating-like protein 1 (IQGAP1) from which $^{app}(k_{cat}/K_M)$ was calculated. Cleavage of canonical 3CL^{pro} cleavage sites LQ↓G/A/S and LH↓G/A/S, where cleavage between P1 (numbered according to position in protein following Met1) and P1' is denoted by an arrow. Graphs show disappearance of the parent peptide (grey) and appearance of the C-terminal fragment (red), with binned apparent $^{app}(k_{cat}/K_M)$ indicating relative cleavage efficiency. Actual $^{app}(k_{cat}/K_M)$ values are shown in *Extended Data Table 4*.

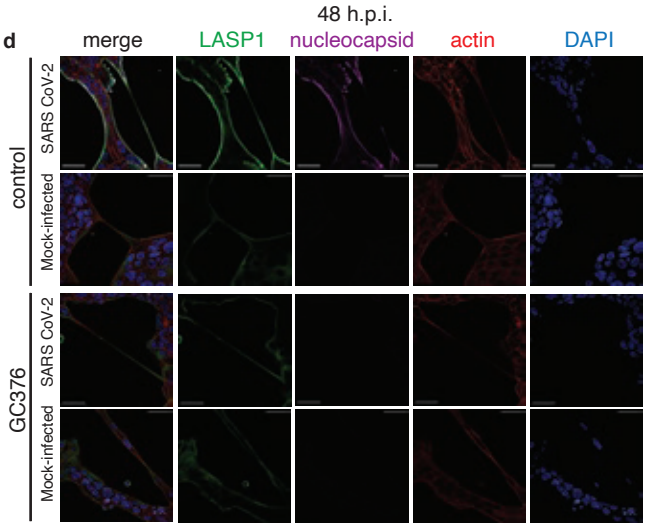


Supplementary Info. 3 | Multiplex mass spectrometry assay for canonical 3CL^{pro} cleavage sites: Identification of canonical 3CL^{pro} cleavage sites LQ↓G/A/S and LH↓G/A/S, in proteins identified by ICDC from *Extended Data Table 1*. Cleavage between P1 (numbered according to position in protein following Met1) and P1' is denoted by an arrow. Graphs show disappearance of the parent peptide (grey) and appearance of the C-terminal fragment (red), with binned $^{app}(k_{cat}/K_M)$ indicating relative cleavage efficiency. Actual values are shown in *Extended Data Table 4*.

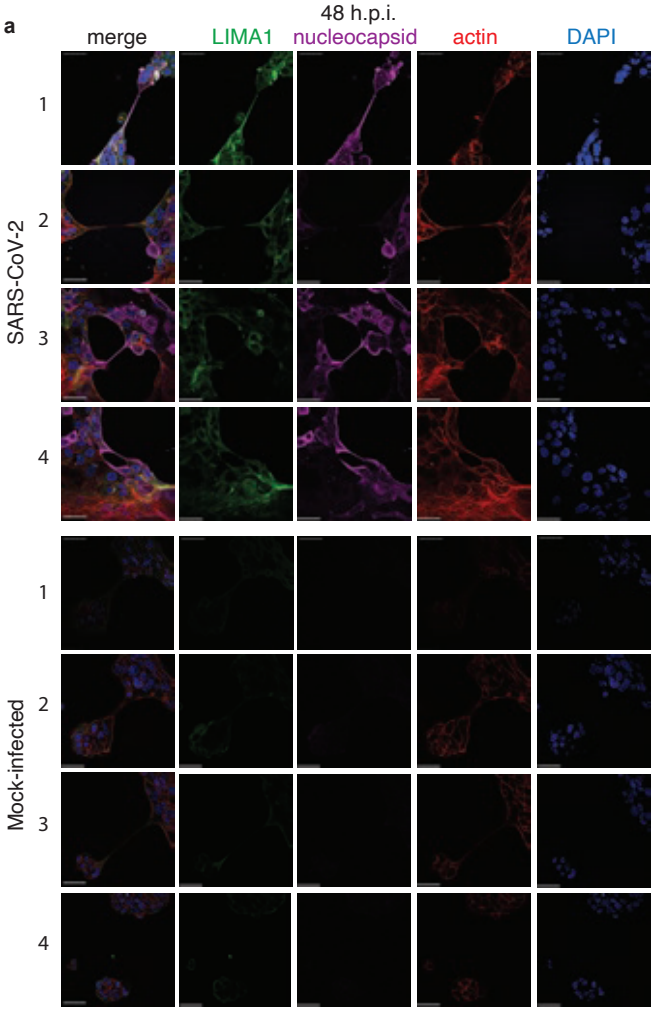


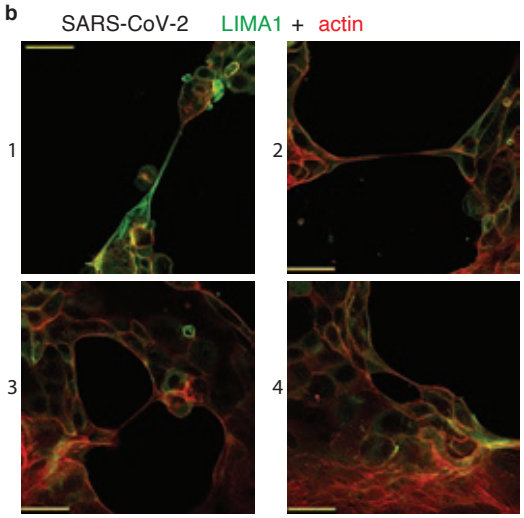
Supplementary Info. 4 | Supporting Data for Fig 2i: a Myosin-9 (MYH9) colocalises with actin and with SARS-CoV-2 nucleocapsid protein at the epithelial cell colony periphery. Calu3 cells were infected with SARS-CoV-2 at a MOI of 1.0 or mock-infected. Cells were fixed 48 h.p.i. and stained with anti-human MYH9 antibody (Thermo-Scientific PA5-27506, 1:500 dilution, 488-conjugated secondary antibody, green), anti-SARS nucleocapsid antibody (Thermo-Scientific MA5-29981, 1:250 dilution, 633-conjugated secondary antibody, magenta), nuclei were stained with DAPI (blue), and actin was stained with 594-conjugated phalloidin (red). The 4 channels were merged for the largest panels using Image J. Slides were imaged at 63X magnification by confocal microscopy, scale bar = 50 μm: Intensities (Look-up Tables (LUT) were adjusted uniformly for infected and mock panels where necessary in Image J as follows: MYH9 (LUT 0-60, except panel 1 LUT 0-100), nucleocapsid (LUT 0-100, except panel 1 LUT 0-150), phalloidin (LUT 0-150, except panel 1 LUT 0-60) and DAPI (not adjusted). White denotes colocalization of MYH9 and viral nucleocapsid protein at the colony periphery and in the TNTs. Yellow indicates colocalisation of MYH9 and actin. Panel 1 (infected) is shown in Fig. 2i.

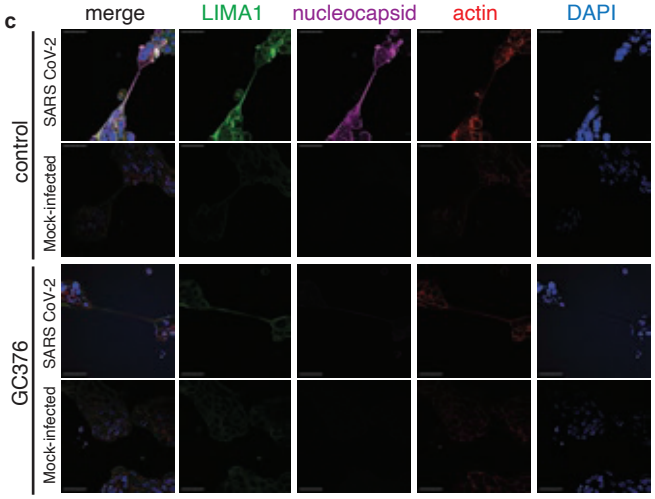




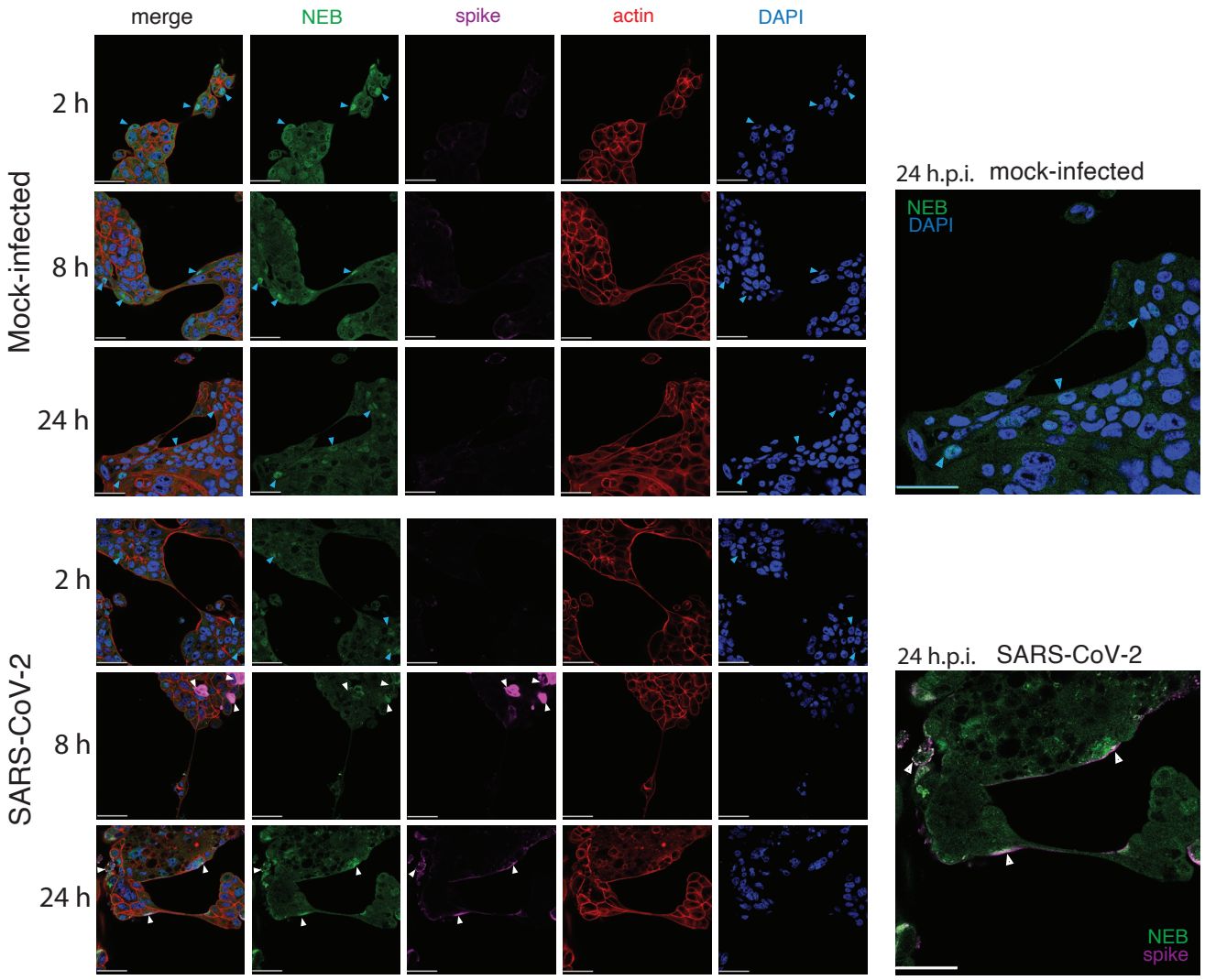
Supplementary Info. 5 | Supporting Data for Fig.3a: LASP1 is concentrated at the edges of epithelial cell colonies and in TNTs colocalised with SARS-CoV-2 nucleocapsid protein and appears to be upregulated in infection. Calu-3 cells were infected with SARS-Co-2 at a MOI of 1.0 or mock-infected and then fixed 48 h.p.i. Cells were stained with: anti-LASP1 antibody (ABclonal A3941, 1:100 dilution, 488-conjugated secondary antibody , green), anti-SARS nucleocapsid antibody (Thermo Scientific MA5-29981, 1:250 dilution, 633-conjugated secondary antibody, magenta), actin was stained with 594-conjugated phalloidin (red), and nuclei were stained with DAPI (blue). Confocal images were acquired using identical settings. Images were merged using Image J (https://imagej.net/ij/). Scale bars, 50 μm. **a** LASP1 colocalised with nucleocapsid at the colony edge, and appeared to be more prevalent than in mock-infected cells. No intensity adjustments were made. Panel 2 (infected) is shown in Fig. 3a(vii); **b** SARS-CoV-2 replicates 1-4 with the LASP1 and nucleocapsid channels merged showing colocalisation of LASP1 with viral nucleocapsid protein at the colony periphery; **c** The color intensity (Look-up-tables, LUT) of mock-infected panel 1 (merge) was adjusted ("overexposed" for LASP1 LUT 0-40 and phalloidin LUT 0-30 (DAPI, and nucleocapsid protein unaltered, LUT 0-255) in ImageJ to show that LASP1 was present around the colony periphery as in infected cells, but at lower levels; **d** The $3CL^{pro}$ inhibitor GC376 decreased the intensity of LASP1 staining, suggesting that $3CL^{pro}$ activity is required for the increased intensity of LASP1 seen in infection. After incubation with SARS-CoV-2 for 2h, the supernatant was replaced with fresh medium containing GC376 (10 μM final concentration), cells were fixed 48 h.p.i. Infected panel 2 and mock infected panel 3 from a are shown for comparison. No intensity adjustments were made.



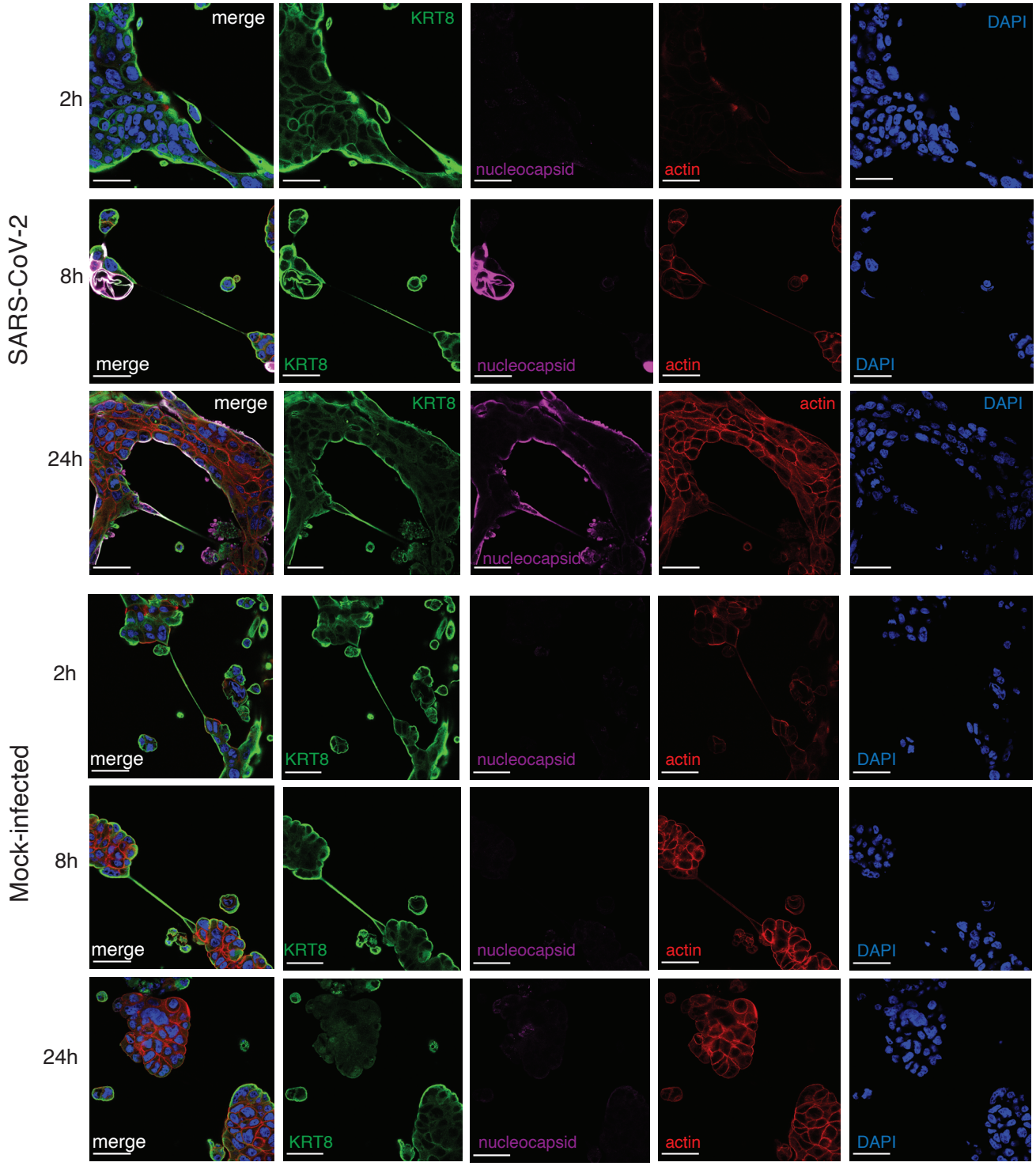




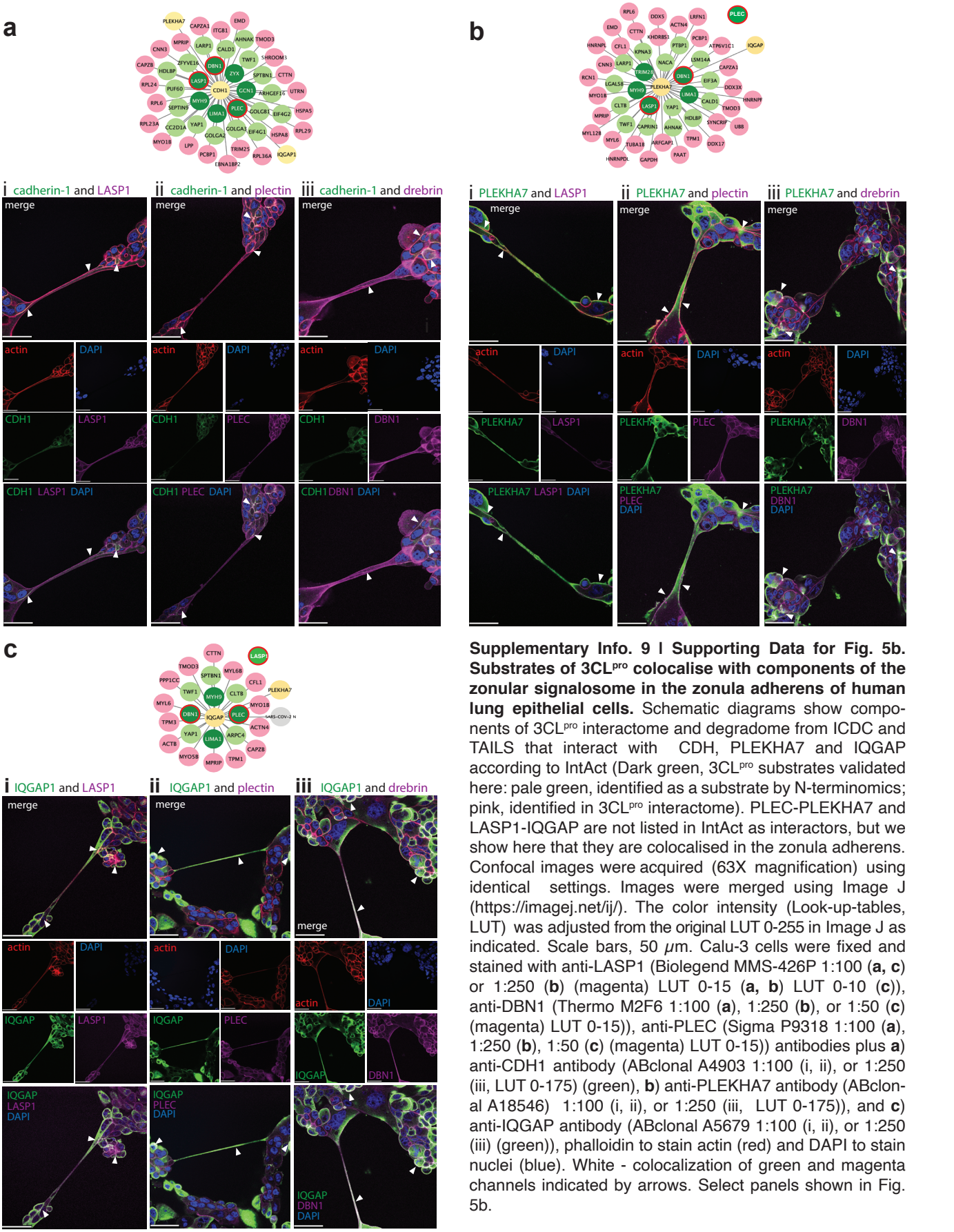
Supplementary Info. 6 | Supporting Data for Fig 3b. LIMA1 is cytoplasmic and is colocalised with actin at the edges of epithelial cells. Calu-3 cells were infected with SARS-CoV-2 at a MOI of 1.0 or mock-infected and were fixed 48 h.p.i. Cells were stained with anti-human LIMA1 antibody (ABclonal A11682, 1:100 dilution, 488-conjugated secondary antibody, green), anti-SARS nucleocapsid antibody (Thermo-Scientific MA5-29981, 1:250 dilution, 633-conjugated secondary antibody, magenta), actin was stained with 594-conjugated phalloidin (red), and nuclei were stained with DAPI (blue). Confocal microscopy images were acquired using identical settings (63X magnification). scale bars, 50 μm. Representative images (n = 4, 1 - 4) are shown. Channels were merged using Image J (https://imagej.net/ij/). The color intensity (Look-up-tables, LUT) of infected and mock-infected panels 2 - 4 (confocal images captured at the same time) was adjusted in Image J: LIMA1 LUT 0-50, nucleocapsid LUT 0-40. Panel 1 images and all DAPI and actin staining were not adjusted (LUT 0-255). Infected panel 1 is shown in Fig. 3b(vi). White denotes some colocalisation of LIMA1 and viral nucleocapsid protein at the colony periphery and in the TNTs, but **b** LIMA1 colocalised more extensively with actin (yellow, shown in panels on the right); **c** The $3CL^{pro}$ inhibitor GC376 decreased the intensity of LIMA1 staining, suggesting that the increase is due to $3CL^{pro}$ in infection. After incubation with SARS-CoV-2 for 2h, supernatant was replaced with fresh medium containing GC376 (10 μM final concentration). Cells were fixed 48 h.p.i. Infected panel 1 and mock infected panel 1 from a are shown for comparison. No intensity adjustments were made.



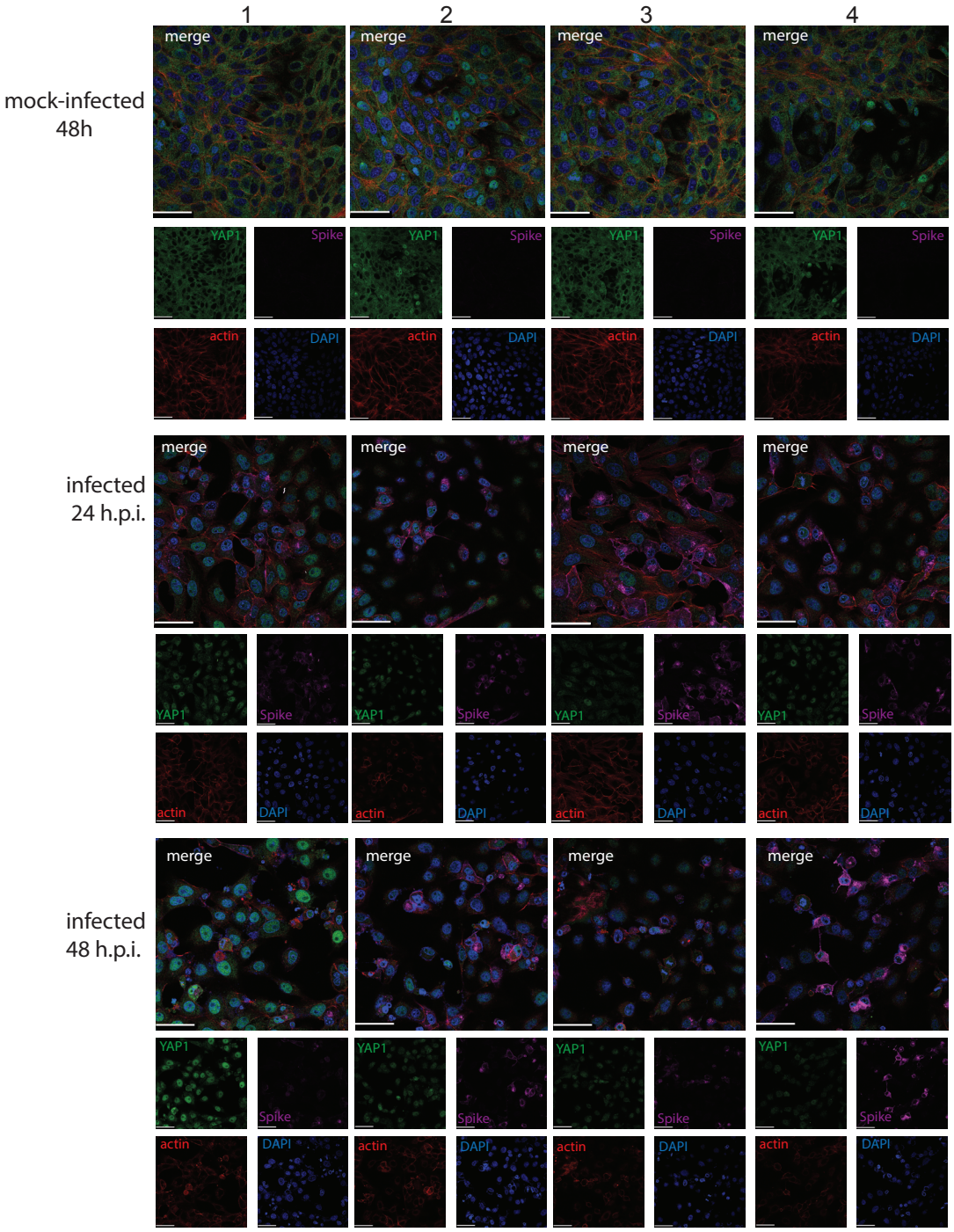
Supplementary Info. 7 | Supporting Data for Fig. 4f: Nebulin (NEB) is colocalized at discrete plasma membrane regions with SARS-CoV-2 spike protein in infected lung epithelial cell colonies Calu-3 cells were infected with SARS-CoV-2 at a MOI of 1.0 or mock-infected, fixed 2, 8 or 48 h.p.i. and stained with anti-human NEB antibody (Chicken IgY, MyoMedix, #NEB-1gamma, 1:100 dilution, 488-conjugated secondary antibody, green), SARS-CoV-2 (2019-nCoV) Spike Antibody (Rabbit MAb, Sino Biological, #40150-R007-100), 1:100 dilution, 633-conjugated secondary antibody, magenta), nuclei were stained with DAPI (blue), and actin was stained with 594-conjugated phalloidin (red). Confocal images (63X magnification) were acquired using identical settings. Images were merged using Image J (https://imagej.net/ij/). Scale bar 50 μm. The color intensity (Look-up-tables, LUT) of the 488-channel (nebulin) was uniformly adjusted to LUT 0-150. Other channels were not adjusted (LUT 0-255). In mock-infected cells and infected cells at 2 h.p.i., nebulin is present in several nuclei (blue arrows). After longer infection times, nebulin is colocalised with spike protein at sub-plasma membrane locations (white arrows).



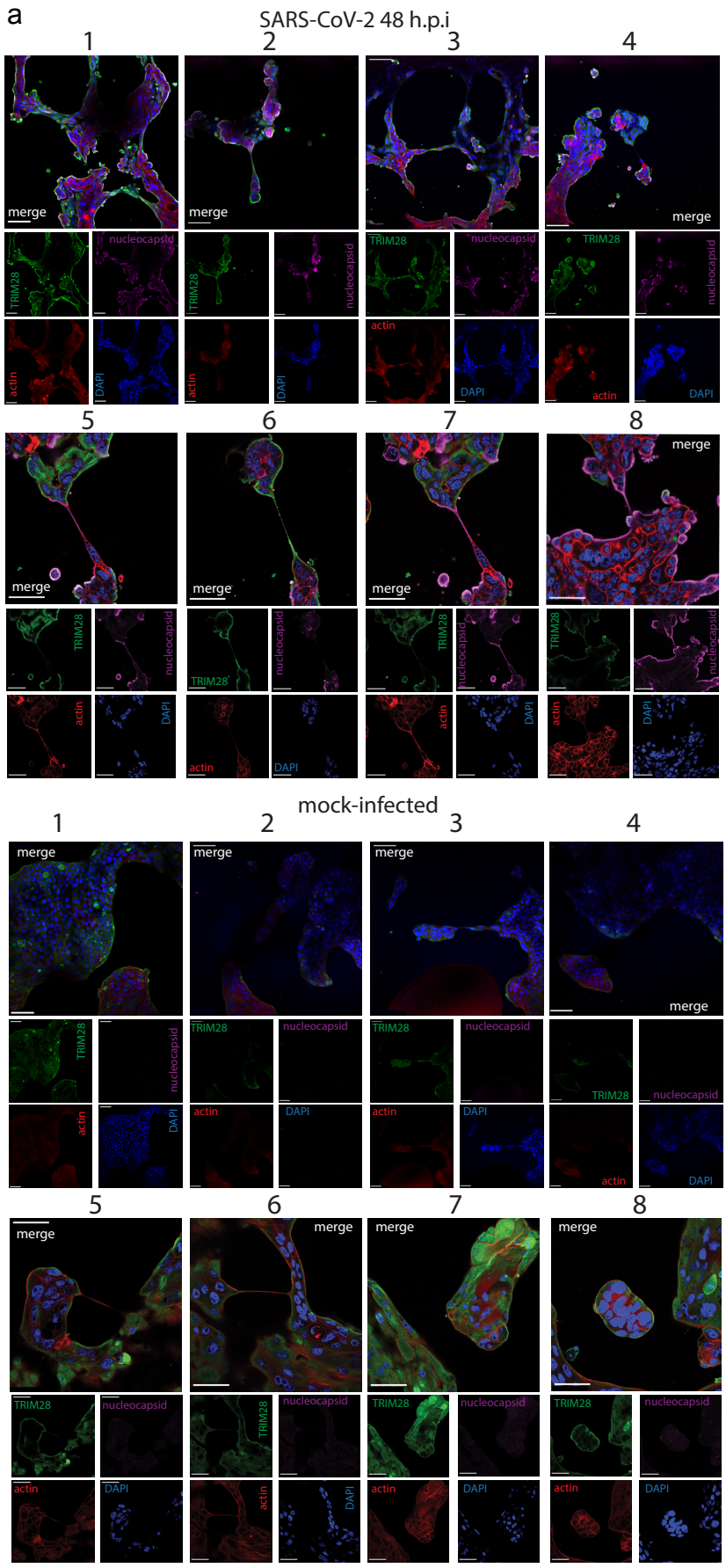
Supplementary Info. 8 | Supporting Data for Fig. 4j. The intermediate filament protein keratin-8 (KRT8) is localised to the boundary of epithelial cell colonies and TNTs and is colocalised with SARS-CoV-2 nucleocapsid protein. Calu-3 cells were infected with SARS-CoV-2 at a MOI of 1.0 or mock-infected and then fixed 2, 8 or 48 h.p.i. Cells were stained with anti-human KRT8 antibody (Thermo-Scientific PA5-32469, 1:250 dilution, 488-conjugated secondary antibody, green), anti-SARS nucleocapsid antibody (Thermo-Scientific MA5-29981, 1:250 dilution, 633-conjugated secondary antibody, magenta), actin was stained with 594-conjugated phalloidin (red) and nuclei were stained with DAPI (blue). Scale bar 50 μm. Confocal images were acquired using identical settings. Channels were merged using Image J (https://imagej.net/ij/), intensities were not adjusted. White areas represent colocalization of nucleocapsid and substrate in the merged images. Infected panels are shown in Fig 4j.

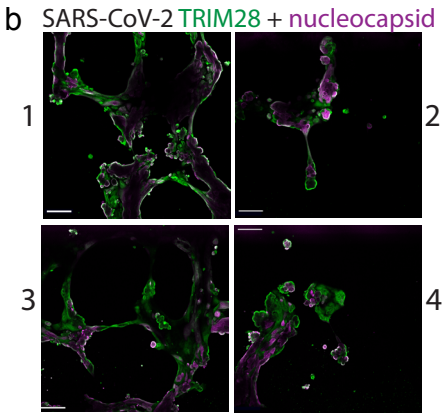


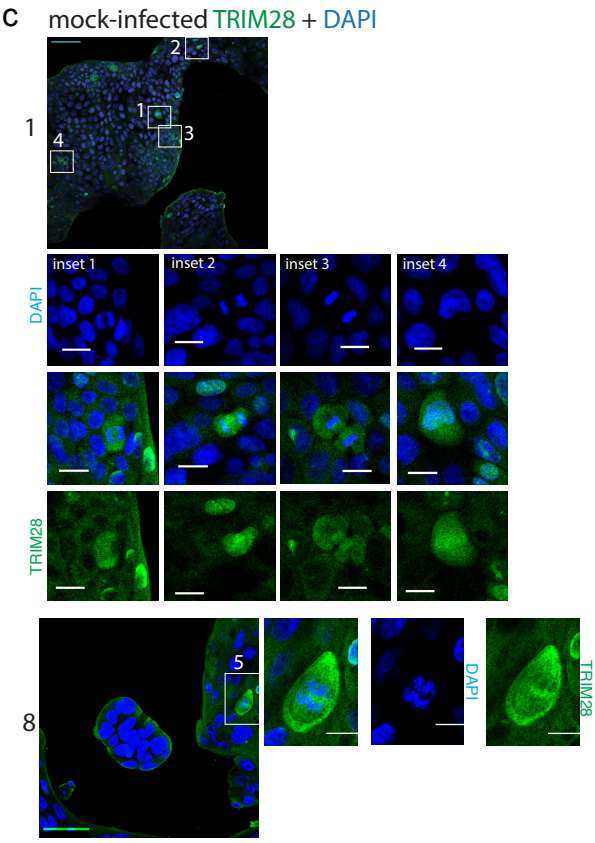
Supplementary Info. 9 l Supporting Data for Fig. 5b. Substrates of 3CL^{pro} colocalise with components of the zonular signalosome in the zonula adherens of human lung epithelial cells. Schematic diagrams show components of 3CL^{pro} interactome and degradome from ICDC and TAILS that interact with CDH, PLEKHA7 and IQGAP according to IntAct (Dark green, 3CL^{pro} substrates validated here: pale green, identified as a substrate by N-terminomics; pink, identified in 3CL^{pro} interactome). PLEC-PLEKHA7 and LASP1-IQGAP are not listed in IntAct as interactors, but we show here that they are colocalised in the zonula adherens. Confocal images were acquired (63X magnification) using identical settings. Images were merged using Image J (https://imagej.net/ij/). The color intensity (Look-up-tables, LUT) was adjusted from the original LUT 0-255 in Image J as indicated. Scale bars, 50 μm. Calu-3 cells were fixed and stained with anti-LASP1 (Biolegend MMS-426P 1:100 (**a, c**) or 1:250 (**b**) (magenta) LUT 0-15 (**a, b**) LUT 0-10 (**c**)), anti-DBN1 (Thermo M2F6 1:100 (**a**), 1:250 (**b**), or 1:50 (**c**) (magenta) LUT 0-15)), anti-PLEC (Sigma P9318 1:100 (**a**), 1:250 (**b**), 1:50 (**c**) (magenta) LUT 0-15)) antibodies plus **a**) anti-CDH1 antibody (ABclonal A4903 1:100 (i, ii), or 1:250 (iii, LUT 0-175) (green), **b**) anti-PLEKHA7 antibody (ABclonal A18546) 1:100 (i, ii), or 1:250 (iii, LUT 0-175)), and **c**) anti-IQGAP antibody (ABclonal A5679 1:100 (i, ii), or 1:250 (iii) (green)), phalloidin to stain actin (red) and DAPI to stain nuclei (blue). White - colocalization of green and magenta channels indicated by arrows. Select panels shown in Fig. 5b.



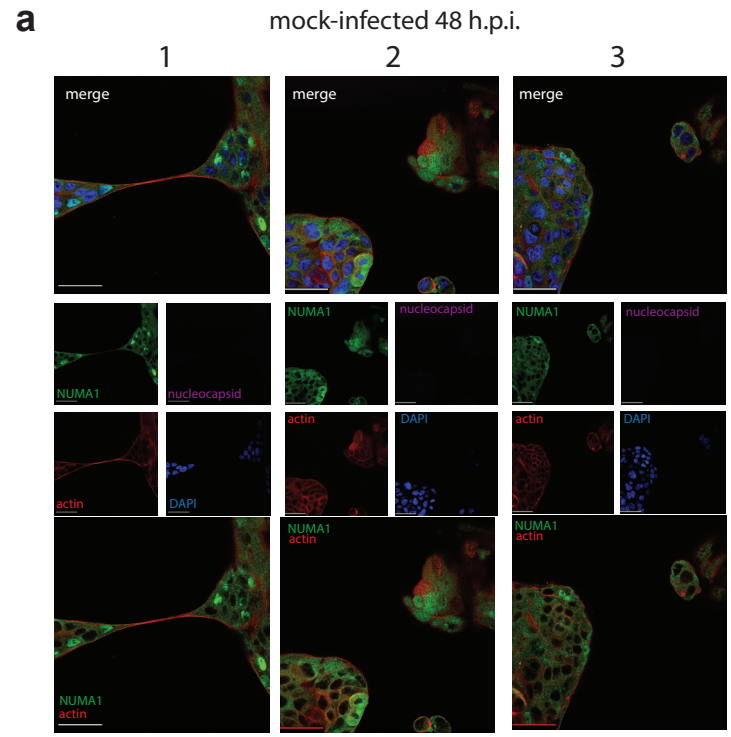
Supplementary Info. 10 | Supporting Data for Fig. 5f: SARS-CoV-2 infection of Vero E6 cells induces nuclear translocation of YAP1. Vero E6 cells were infected with SARS-CoV-2 for 24 or 48 h or mock-infected for 48 h and stained for YAP1 (green), viral spike protein (magenta), actin (phalloidin, red) and nuclei (DAPI, blue).Confocal images were acquired (63X magnification) using identical settings. Images were merged using Image J (https://imagej.net/ij/). The color intensity (Look-up-tables, LUT) was adjusted from the original LUT 0-255 in Image J for YAP1, LUT 0-80, and spike, LUT 0-80. In mock-infected cells, YAP1 is predominantly cytoplasmic, whereas in SARS-CoV-2 infected cells YAP1 is mainly nuclear suggesting that infection triggers nuclear translocation of YAP1. Panel 1 for mock and infected timepoints is shown in Fig 5f.

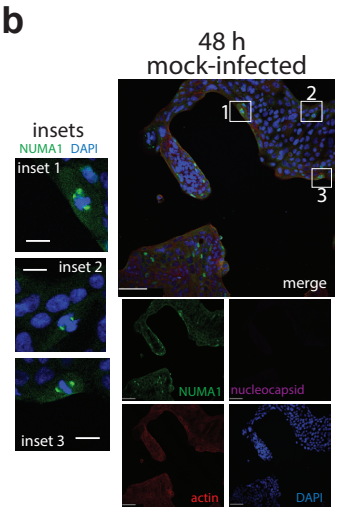


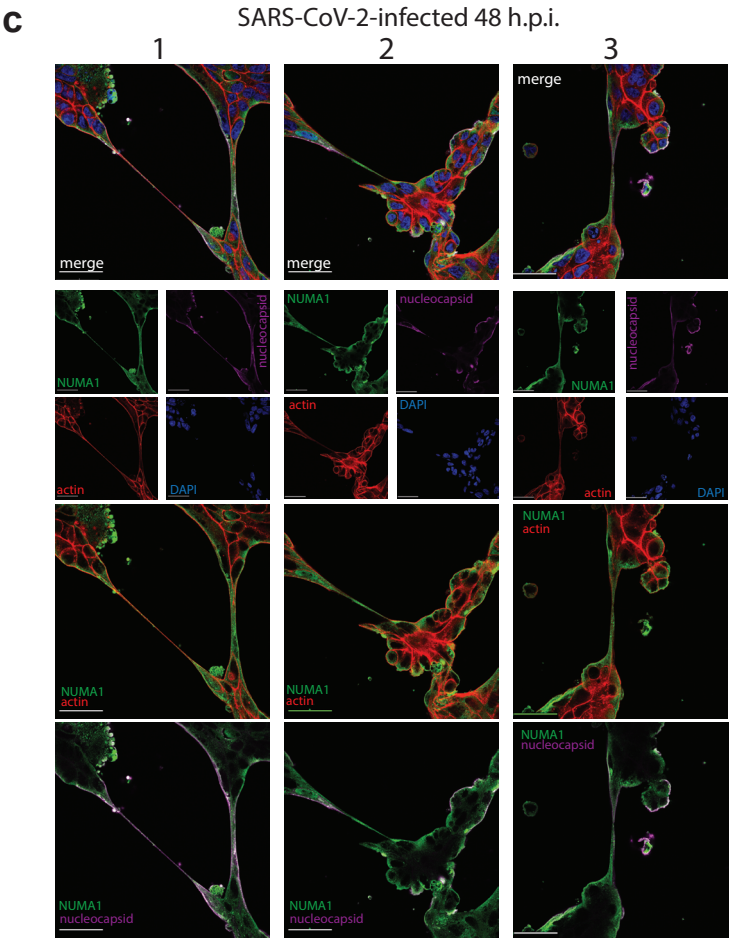




Supplementary Info. 11 | a TRIM28 lines the periphery of SARS-CoV-2-infected lung epithelial cells and b colocalises with nucleocapsid protein (white denotes colocalisation of TRIM28 and nucleocapsid, panels from a). **c TRIM28 was concentrated in the cytoplasm of cells with condensed DNA (insets), especially in anaphase (inset 5)**, panels from a. Calu3 cells were infected with SARS-CoV-2 at a MOI of 1.0 or mock-infected for 48 h. Images were captured by confocal microscopy: Representative fields are shown: 20X magnification $n = 4$, panels 1-4, scale bar 100 μm, 63X magnification $n = 4$, panels 5-8, scale bar 50 μm. Cells were stained with anti-human TRIM28 antibody (ABclonal A2245, 1:100 dilution, 488-conjugated secondary antibody, green), anti-SARS nucleocapsid antibody (Thermo-Scientific MA5-29981, 1:250 dilution, 633-conjugated secondary antibody, magenta), actin was stained with 594-conjugated phalloidin (red), and nuclei were stained with DAPI (blue). Image intensities were uniformly adjusted in Image J (see Source Data). Infected and mock panel 1 is shown in Fig. 6c. Image intensities were uniformly adjusted in Image J (see Source Data). Images from panels 1 to 8 were analysed to quantify TRIM28 staining at the colony periphery vs. the colony interior (see Source Data for quantitation and LUT).







Supplementary Info. 12 | NUMA1 translocates from the nucleus and cytoplasm to the colony periphery in SARS-CoV-2-infected lung epithelial cells. a Mock-infected cells show NUMA1 in the cytoplasm and **b** nuclei of anaphase cells, where it is associated with the bipolar spindle; **c** In infected cells, NUMA1 is enriched at the colony periphery where it is colocalized with viral nucleocapsid protein. Calu3 cells were infected with SARS-CoV-2 at a MOI of 1.0, cells were fixed 48 h.p.i. and stained with anti-human NUMA1 antibody (ABclonal A0527, 1:100 dilution), anti-SARS nucleocapsid antibody (Thermo-Scientific MA5-29981, 1:250 dilution), actin was stained with phalloidin, and nuclei were stained with DAPI. Confocal microscopy images were acquired using identical settings and channels were merged as indicated using ImageJ (https://imagej.net/ij/). **a** and **c,** 63X magnification, scale bar 50 μm $n = 3$, panels 1 - 3; **b**, 20X magnification, scale bar 100 μm, insets 20 μm. Panel 1 (a and c) and inset 3 (b) are shown in Fig. 6f and g respectively. The color intensity (Look- up-tables, LUT) was unifomly adjusted in ImageJ for infected and mock-infected imnages adjusted: NUMA1, green LUT 0-60, nucleocapsid, magenta, LUT 0-150. Actin and DAPI channels were not adjusted (LUT 0-255).