480 **Fig S1. NLS prediction in the S protein of pathogenic coronaviruses.** All categories of NLS
481 motifs were searched in the S protein sequence using the web-based program PSORT II
482 (https://psort.hgc.jp/form2.html) [27] for the S protein ORF amino acid sequence of SARS-CoV-
483 2 (USA/WA-CDC-WA1/2020 isolate, GenBank accession no. MN985325) (Query 1), SARS-CoV
484 (Urbani strain, GenBank accession no. AY278741) (Query 2), or MERS-CoV (GenBank accession
485 no. NC_019843.3) (Query 3).

486

Fig S2. Detection of the nuclear translocation of S mRNA and **S protein.** Confocal images of SARS-CoV-2-infected airway epithelium (described in Fig 2) were analyzed for spot-to-spot colocalization using Imaris image analysis software (Oxford Instruments). The left panel shows the confocal images, the middle panel shows spot-to-spot colocalization, and the right panel shows merged confocal images and spot-to-spot colocalization. Spot-to-spot colocalization between the nucleus and S protein or S mRNA is indicated by a different color. The images represent multiple independent cross sections of the SARS-CoV-2-infected airway epithelium (from 3 independent donors).

Fig S3. The translocation of S mRNA and S protein includes both the inside and outer surface of the nucleus. From the images shown in Fig 3, the signals of S mRNA and S protein were plotted in the graph by Imaris image analysis software. The distance and intensity of all S mRNA or S protein from the nuclear surface (considered 0) were plotted. A negative value indicates that S mRNA or S protein resides inside the nucleus. The higher the negative value is, the farther the distance from the nuclear surface. In contrast, a positive value indicates that S mRNA or S protein resides on the nucleus surface and beyond in the cytoplasm. The higher the positive value is, the farther the distance from the nuclear surface.

Fig S4. The SARS-CoV S protein does not translocate into the nucleus. A four-week pseudostratified airway epithelium was infected with SARS-CoV at an MOI of 0.1 for four days, fixed, paraffin-embedded and sectioned at a thickness of 5 μm for immunohistochemistry slide preparation. S protein (shown in cyan) was detected by immunofluorescence-based protein

509 detection using SARS-CoV/SARS-CoV-2 S specific rabbit polyclonal primary antibody and anti-
510 rabbit secondary antibody. The confocal image was analyzed for spot-to-spot colocalization using
511 Imaris image analysis software. The left panel shows a confocal image, the middle panel shows
512 spot-to-spot colocalization, and the right panel shows merged confocal images and spot-to-spot
513 colocalization. The images represent multiple independent cross sections of SARS-CoV-infected
514 airway epithelium (at least two donors).

515

516 **Fig S5. S mRNA and S protein colocalization was spatially evident in all possible ways inside**
517 **the infected cell.** The confocal images shown in Figs. 3 & 4 were further visualized at a higher
518 magnification to detect S mRNA and S protein colocalization spatially. S protein and S mRNA
519 distribution and colocalization in the cytoplasm (top panel), on the nuclear surface (middle panel)
520 and inside the nucleus (bottom panel). The specific region of colocalization is indicated by a white
521 spot. The colors were made translucent to show colocalization. Scale bar 0.2 μm.

522

523 **Fig S6. NLS motif prediction in the N protein of pathogenic coronaviruses.** All categories of
524 NLS motifs were searched in the N protein sequence using the web-based program PSORT II
525 (https://psort.hgc.jp/form2.html) [27] for the N protein ORF of SARS-CoV-2 (USA/WA-CDC-
526 WA1/2020 isolate, GenBank accession no. MN985325) (Query 1), SARS-CoV (Urbani strain,
527 GenBank accession no. AY278741) (Query 2), or MERS-CoV (GenBank accession no.
528 NC_019843.3) (Query 3).

529

Fig S7. Nuclear translocation of the N protein of pathogenic coronaviruses. Four-week pseudostratified airway epithelium was infected with SARS-CoV-2, SARS-CoV, or MERS-CoV at an MOI of 0.1 for four days, fixed, paraffin-embedded and sectioned at a thickness of 5 μm for immunohistochemistry slide preparation. SARS-CoV-2 or SARS-CoV N protein (green) was detected by a SARS-CoV/SARS-CoV-2 N protein-specific antibody. Similarly, the MERS N protein (green) was detected by the MERS N protein-specific antibody. The nucleus (shown in blue) was detected by DAPI staining. The confocal images were analyzed for spot-to-spot colocalization. The left panel shows a confocal image, the middle panel shows spot-to-spot colocalization, and the right panel shows merged confocal images and spot-to-spot colocalization. Spot colocalization between the nucleus and N protein is indicated by a different color. The images represent multiple independent technical replicates from at least one independent experiment for one donor (donor #1).

Fig S8. NLS motif distribution in the N protein in different pathogenic coronaviruses. The sequences of the N protein of the SARS-CoV-2 N protein (nCoV-WA1-2020, GenBank accession no. MN985325), SARS-CoV N protein (Urbani Strain, GenBank accession no. AY278741), and MERS-CoV N protein (HCoV-EMC/2012, GenBank accession no. NC_019843) by NCBI's constraint-based multiple alignment tool COBALT [25]. All categories of NLS motifs are shown in the colored rectangle box: pat4: green; pat7: blue; bipartite 1: black; bipartite 2: orange.

S1 dataset 1. Prediction of SARS-CoV-2 S protein and genome interaction

S2 dataset 2. Prediction of SARS-CoV-2 N protein and genome interaction