Supplementary data:

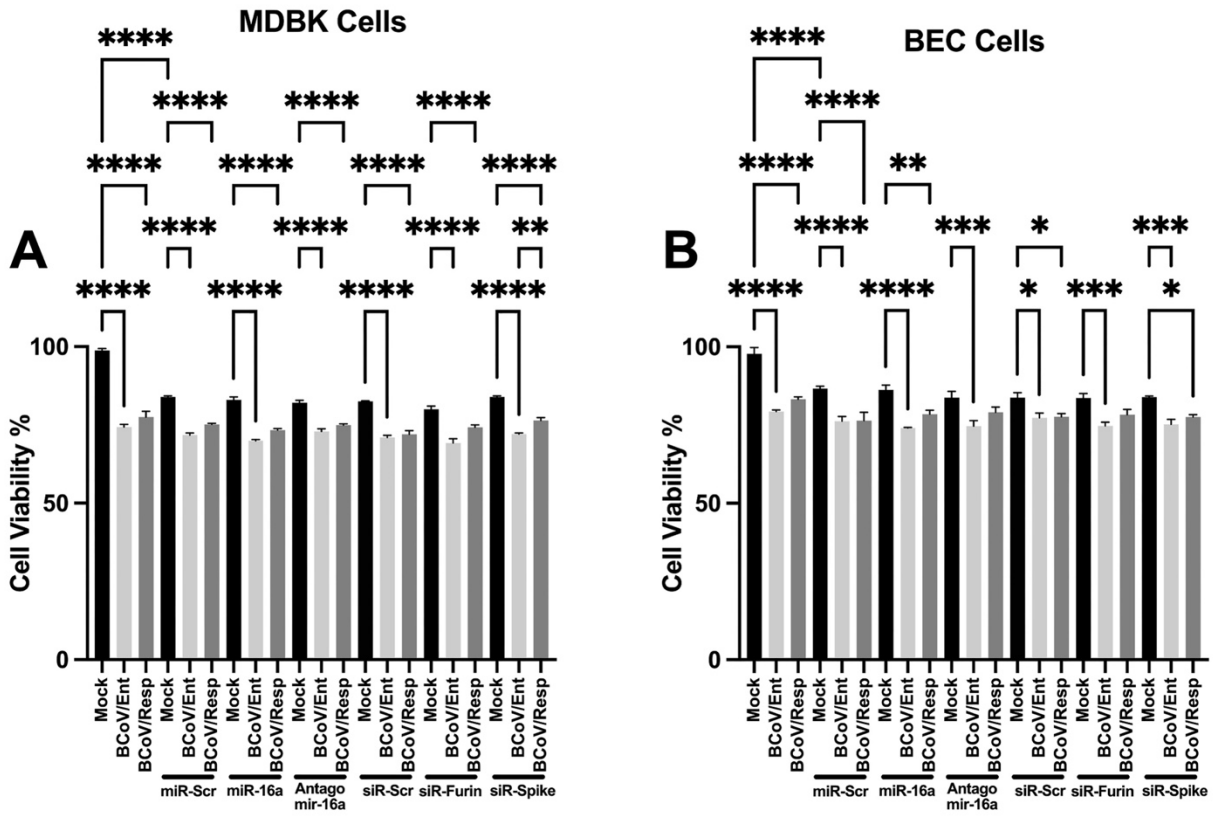


Fig S1: The cell viability assay analysis of cells transfected (with various types of miRNA mimics, antagomirs, or siRNA molecules) or infected with BCoV isolates.

(A) The MTT assay was conducted to assess the cell condition in the MDBK; **(B)** or the BEC cells prior to the BCoV infection or transfection with various oligonucleotides based on the type of the experiment. Each type of cells was grown independently on the 96-well plates and infected with either the BCoV/Ent or the BCoV/Resp, or transfected with the (miR-Scr, miR-16a, Antagomir-16a, siRNA-Scr, siRNA-Furin and siRNA of the BCoV/S). The cell viability after infection was calculated by comparing virus-infected cells with the mock, and the cell viability after transfection was calculated by comparison with the transfected-miR-Scr-Mock cells with the mock (no infection/transfection). The statistical significance of each infected or transfected group was compared to its mock-treated group of cells. All the experiments were performed in triplicate. The significance of the data was determined by one-way ANOVA with Dunnett's multiple comparison test and indicated as * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, and **** $p < 0.0001$.

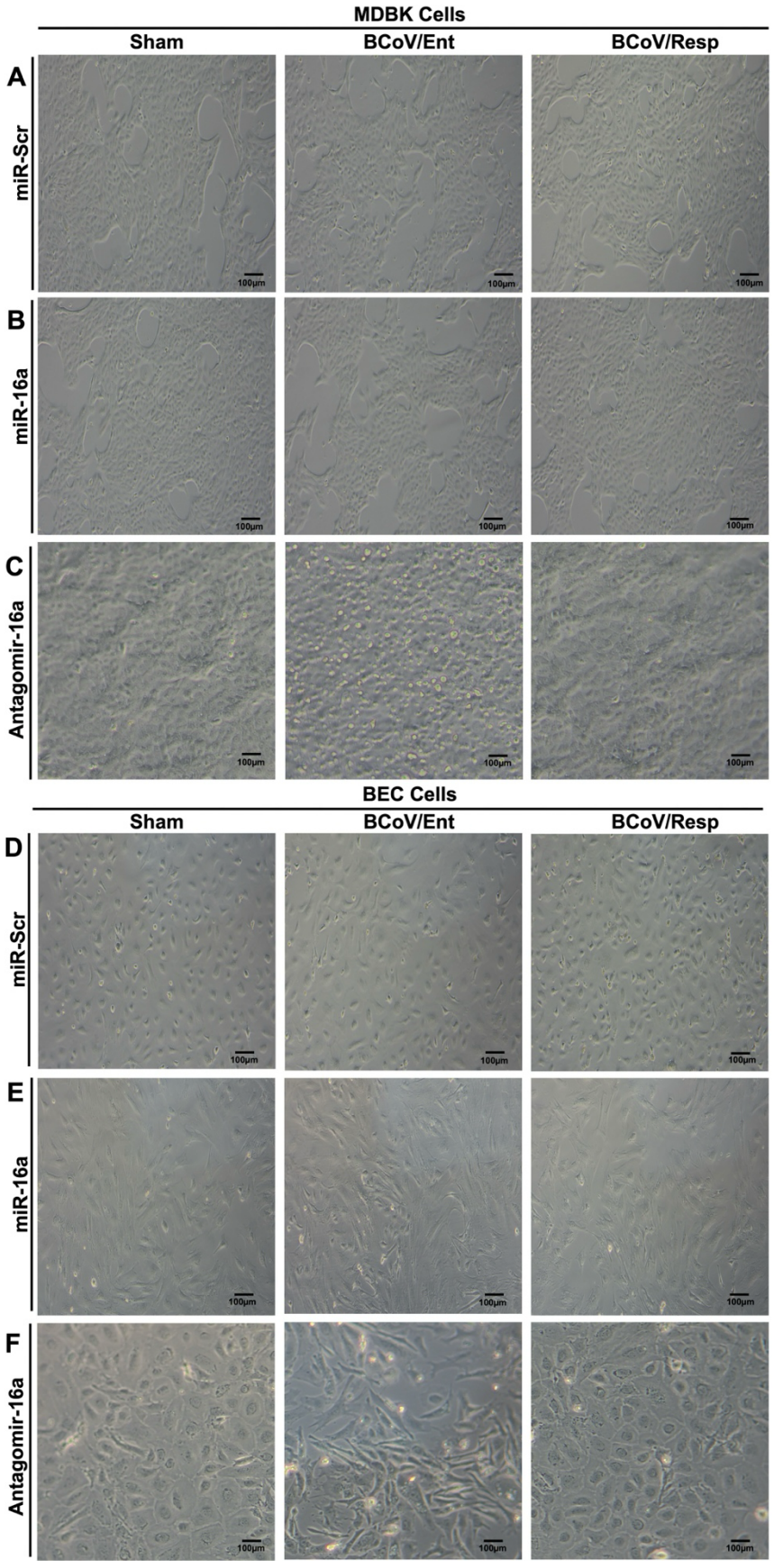

MDBK Cells
Sham
BCoV/Ent
BCoV/Resp
A
miR-Scr
B
miR-16a
C
Antagomir-16a
BEC Cells
Sham
BCoV/Ent
BCoV/Resp
D
miR-Scr
E
miR-16a
F
Antagomir-16a
100μm

Fig S2: Morphology of some bovine cells transfected with either the Scr-miRNA or the miRNA-16a mimics and then infected with either the BCoV/Ent or the BCoV/Resp isolates. **(A)** Results of the morphological examination of the MDBK cells transfected with miR-Scr as the relevant control followed by BCoV enteric (Ent), and BCoV respiratory (Resp)-infection. **(B)** Morphological examination of the MDBK cells transfected with the miRNA16a, followed by BCoV/Ent-, and BCoV/Resp-infection. **(C)** Morphological examination of the MDBK cells transfected with Antagomir-16a, followed by BCoV/Ent-, and BCoV/Resp-infection. **(D)** Results of the morphological examination of BEC cells transfected with miR-Scr followed by infection with BCoV/Ent- and BCoV/Resp-infection. **(E)** Results of the morphological examination of BEC cells transfected with the miRNA16a mimics followed by the BCoV/Ent, or the BCoV/Resp-infection. **(F)** Morphological examination of the BEC cells transfected with Antagomir-16a, followed by BCoV/Ent-, and BCoV/Resp-infection. The miR-Scr, miRNA16a, and Antagomir-16a was transfected for 24 hours, followed by 48-72 hours post-infection with BCoV. All the images were captured using 10x magnification power of light microscopy. The scale represents 100μm.

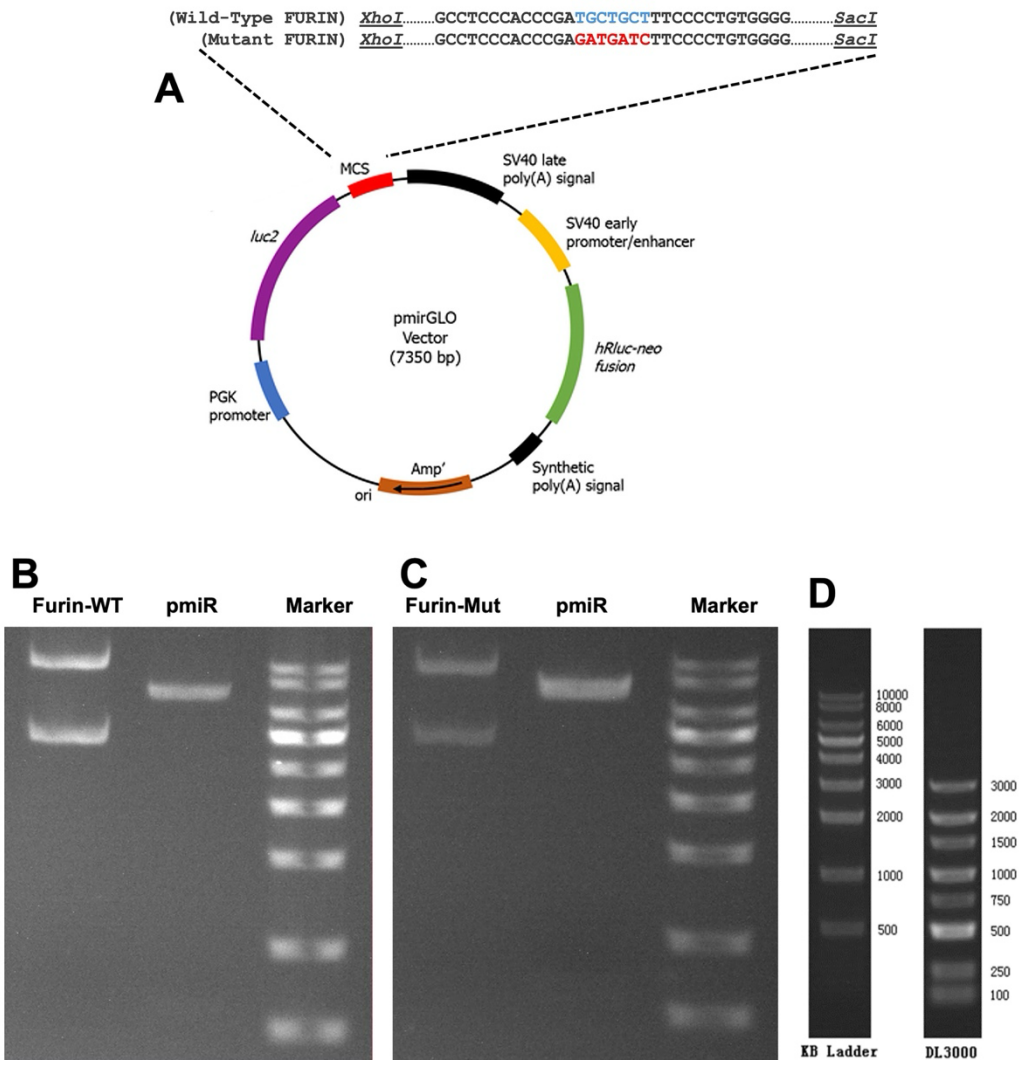


Fig S3: The genetic map showing the design, construction, and confirmation of the pmiR-GLO dual-luciferase reporter plasmids containing either the wild-type or mutated host cell Furin-3'UTR constructs.

(A) Diagram showing the genetic map of the luciferase reporter plasmids. A fragment of the 3'UTR of Furin wild-type (WT) carrying the miRNA16abinding site (indicated in blue) was inserted into the *XhoI* and *SacI* restriction enzyme sites of the pmirGLO luciferase vector. The seed region of Furin was mutated by site-directed mutagenesis to produce the mutant (MUT) Furin (indicated in red). **(B)** Confirmation of the plasmid construct via restriction enzyme digestion by agarose gel electrophoresis. (Furin-WT: double digested with *XhoI* and *SacI*; empty pmiR-GLO vector; marker). **(C)** Confirmation of the mutant construct via agarose gel electrophoresis. (Furin-Mut: double digested with *XhoI* and *SacI*; empty pmiR-GLO vector; marker). Approximately 200-1000 ng of plasmid was digested at 37°C for 30-60 minutes and analyzed through a 1% agarose gel. **(D)** The maps of the reference ladders were used to identify the size of the target fragments (DL3000 and KB Ladder).

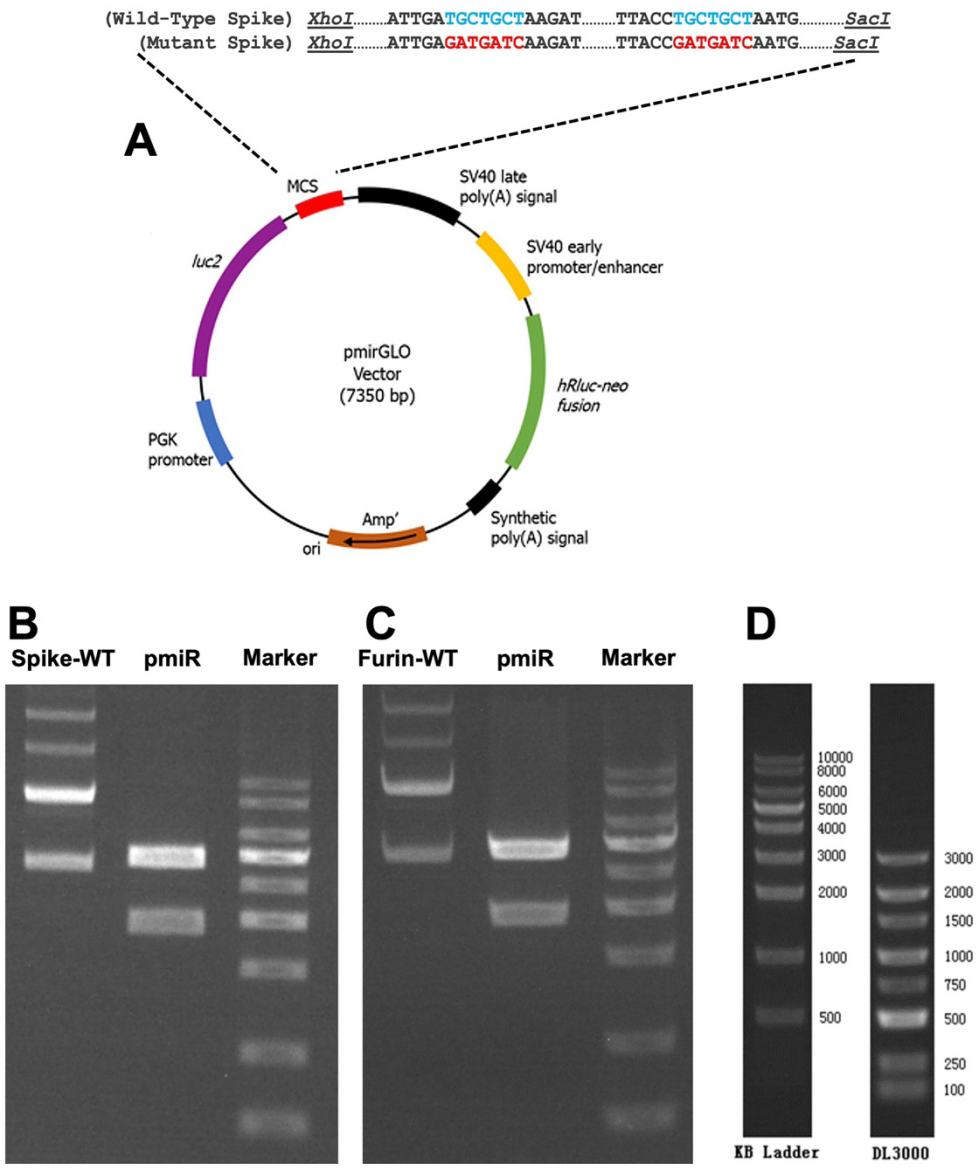


Fig S4: The genetic map showing the design, construction, and confirmation of the pmiR-GLO dual-luciferase reporter plasmids containing either the wild-type or mutated BCoV-Spike gene.

(A) Two fragments of the BCoV-Spike wild-type sequences carrying the miRNA16a binding site (indicated in blue) were inserted into the *XhoI* and *SacI* restriction enzyme sites of the pmirGLO luciferase vector. The site-directed mutagenesis mutated the seed regions of BCoV-Spike is to produce the mutant BCoV/S (indicated in red). **(B)** Confirmation of the plasmid construct via restriction enzyme digestion by agarose gel electrophoresis. (Spike-WT: double digested with *XhoI* and *SacI*; empty pmiR-GLO vector; marker). **(C)** Confirmation of the mutant construct via agarose gel electrophoresis. (Spike-Mut: double digested with *XhoI* and *SacI*; empty pmiR-GLO vector; marker). Approximately 200-1000 ng of plasmid was digested at 37°C for 30-60 minutes and analyzed through a 1% agarose gel. **(D)** The maps of the reference ladders were used to identify the size of the target fragments (DL3000 and KB Ladder).

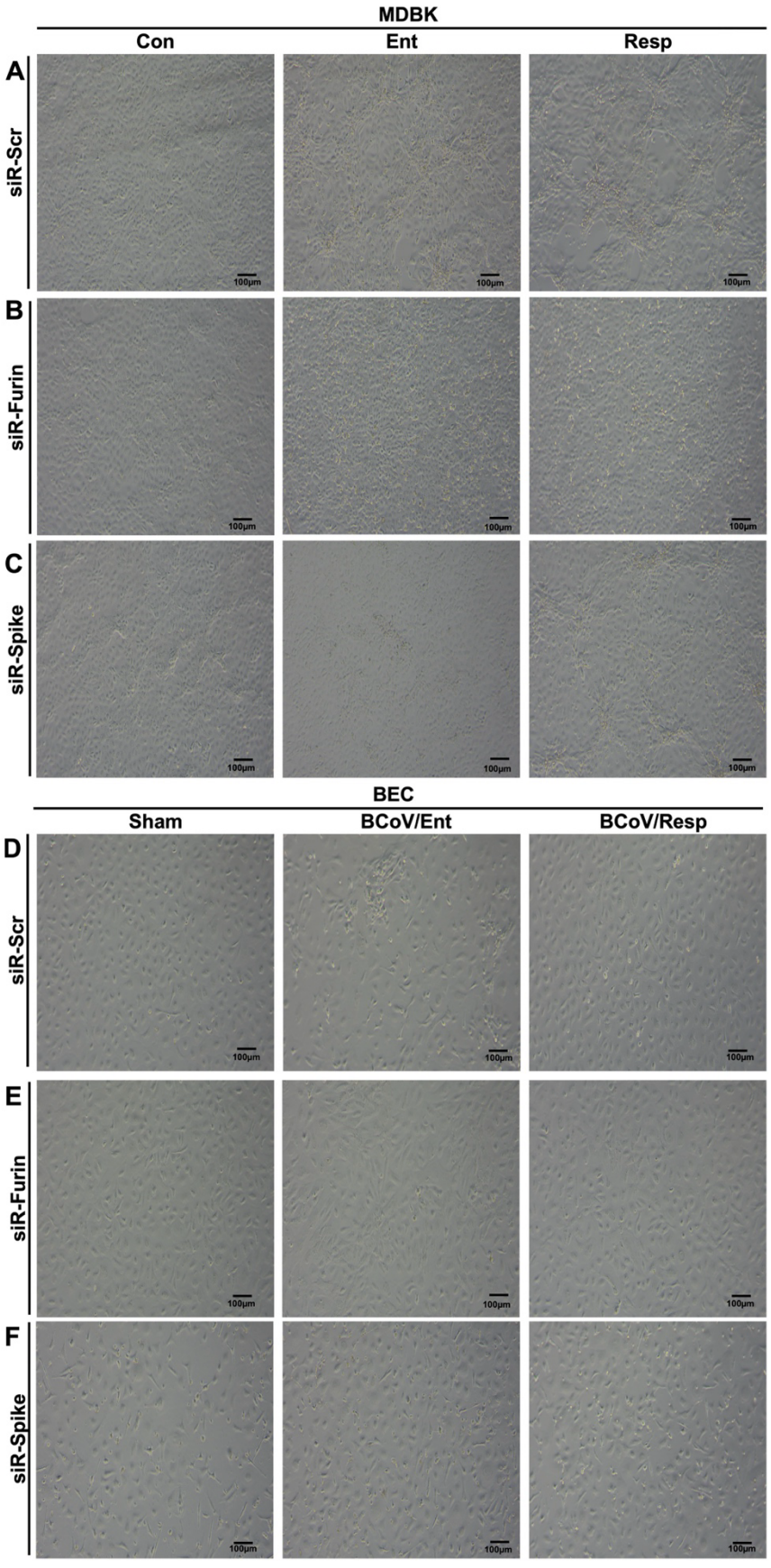
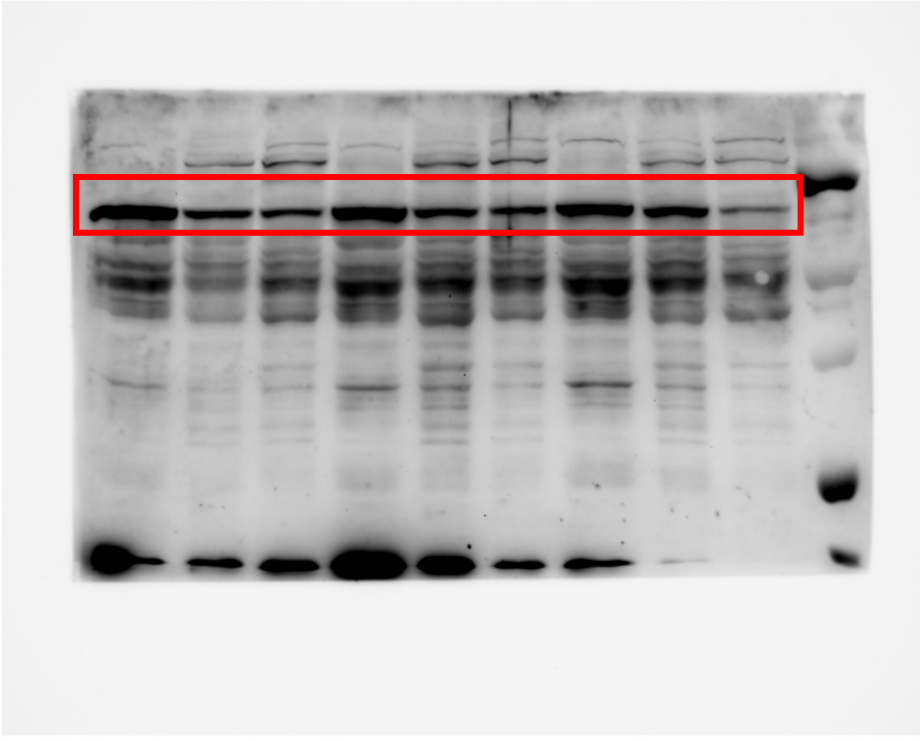

MDBK
Con
Ent
Resp
A
siR-Scr
B
siR-Furin
C
siR-Spike
BEC
Sham
BCoV/Ent
BCoV/Resp
D
siR-Scr
E
siR-Furin
F
siR-Spike
100μm

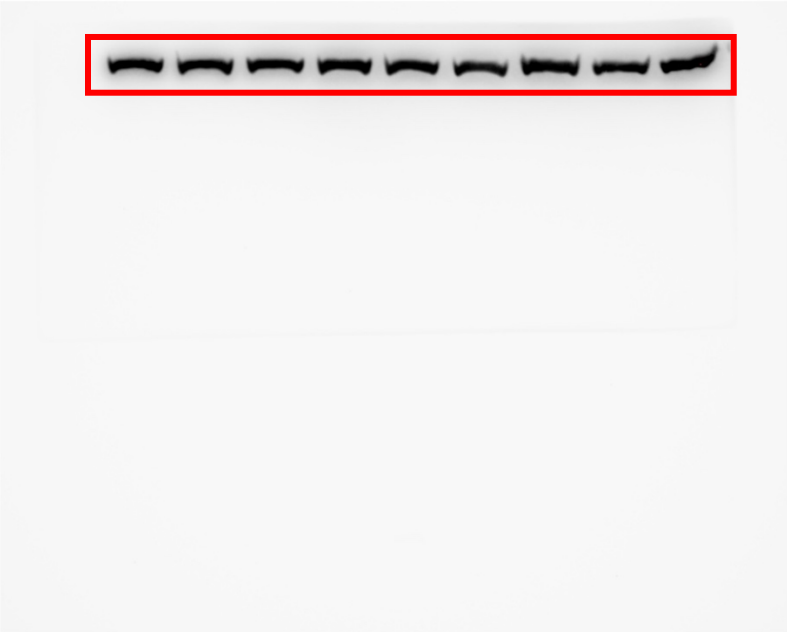
Fig S5: Morphology of the MDBK and BEC cells transfected with Scr-siRNA, siRNA-Furin, or siRNA-BCoV-S glycoprotein.

(A) Morphological observation of the MDBK cells transfected with Scr-siRNA in the control (Mock) group of cells and infected with either BCoV/Ent or the BCoV/Resp isolates. **(B)** Morphological observation of the MDBK cells transfected with siRNA-Furin and then infected with either BCoV/Ent or BCoV/Resp. **(C)** Morphological observation of the MDBK cells transfected with siRNA-BCoV-S and then infected with either BCoV/Ent or BCoV/Resp isolates **(D)** Morphological observation of BEC cells transfected with the Scr-siRNA, BCoV/Ent or BCoV/Resp isolates. **(E)** Morphological observation of BEC cells transfected with the siRNA-Furin and then infected with either the BCoV/Ent or the BCoV/Resp isolates. **(F)** Morphological observation of BEC cells transfected with the siRNA-BCoV-S and then infected with either the BCoV/Ent or the BCoV/Resp isolates. Cells were transfected with siRNA-Furin or siRNA-BCoV-Spike for 24 hours, followed by 48-72 hours post-infection with BCoV. All the images were captured using 10x magnification power of light microscopy. The scale represents 100µm.

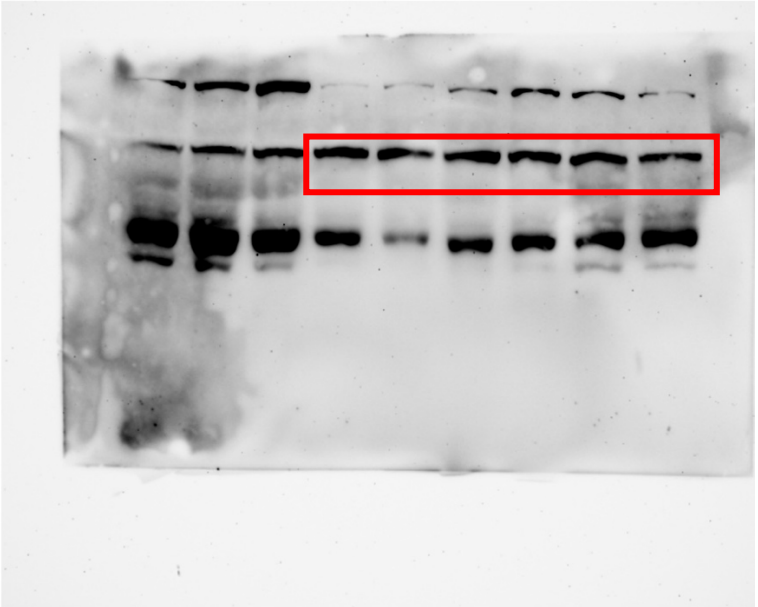
Original Western Blot and Gel Electrophoresis Images:



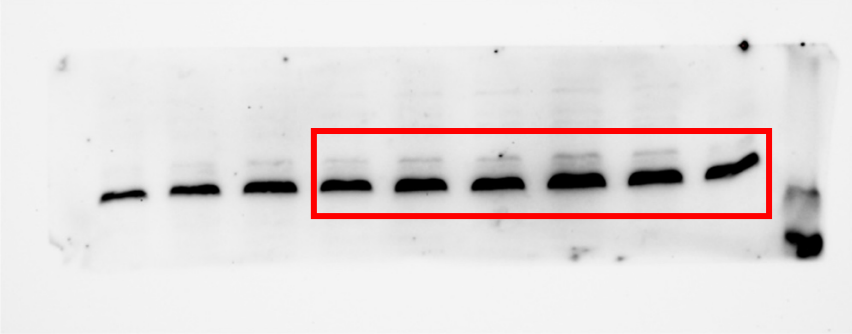
1: Original Western Blot (WB) image for Furin (in red box), corresponding to the data shown in Figure 3D of the manuscript.



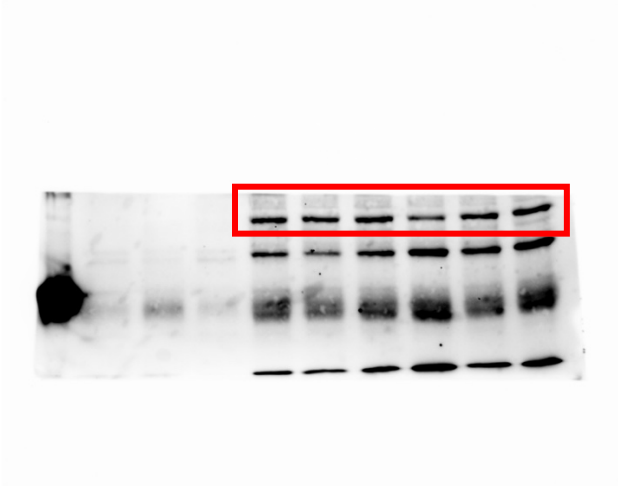
2: Original WB image for B-actin (in red box), corresponding to the data shown in Figure 3D of the manuscript.



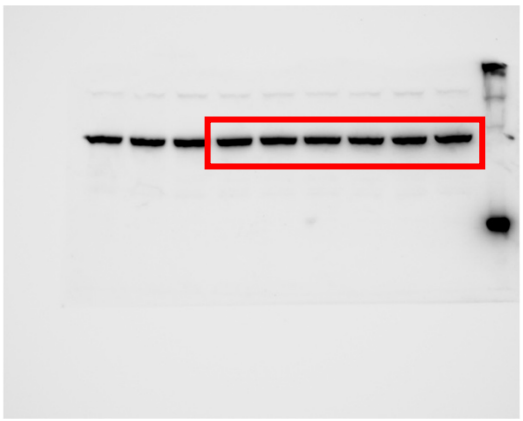
3: Original WB image for Furin (in red box), corresponding to the data shown in Figure 3G of the manuscript.



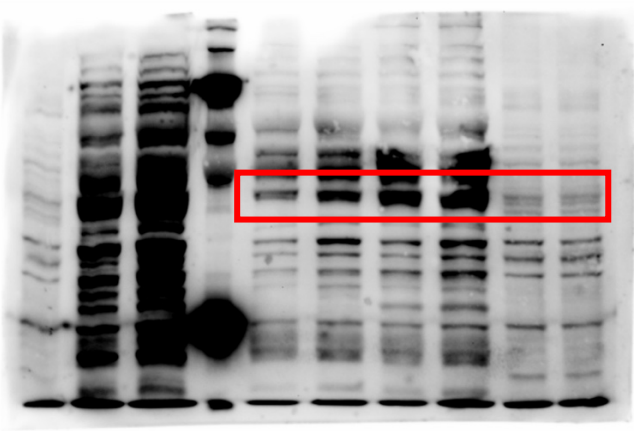
4: Original WB image for B-actin (in red box), corresponding to the data shown in Figure 3G of the manuscript.



5: Original WB image for Furin (in red box), corresponding to the data shown in Figure 4B of the manuscript.

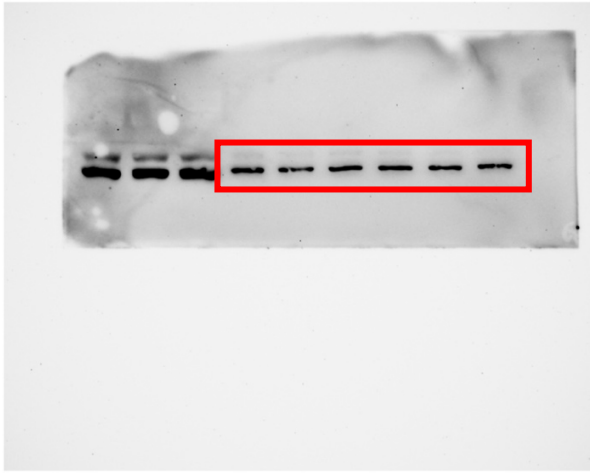


6: Original WB image for B-actin (in red box), corresponding to the data shown in Figure 4B of the manuscript.



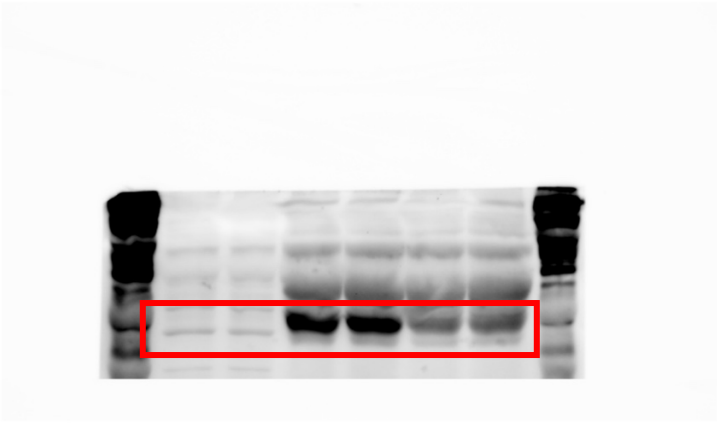
7: Original WB image for BCoV-Nucleocapsid (in red box), corresponding to the data shown in Figure 5F of the manuscript.

8: Original WB image for BCoV-Spike (in red box), corresponding to the data shown in Figure 5F of the manuscript.



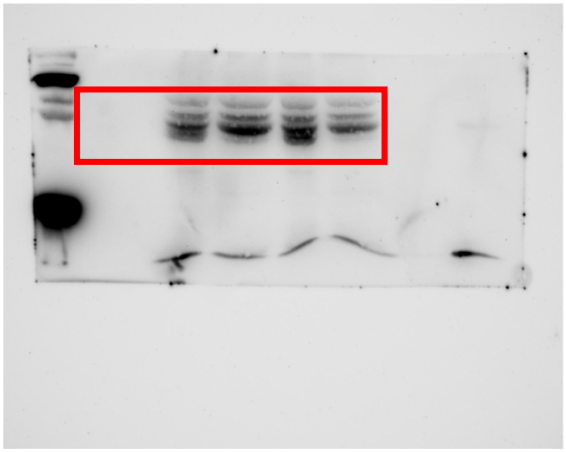
9: Original WB image for B-actin (in red box), corresponding to the data shown in Figure 5F of the manuscript.

10: Original WB image for BCoV-Nucleocapsid (in red box), corresponding to the data shown in Figure 5I of the manuscript.

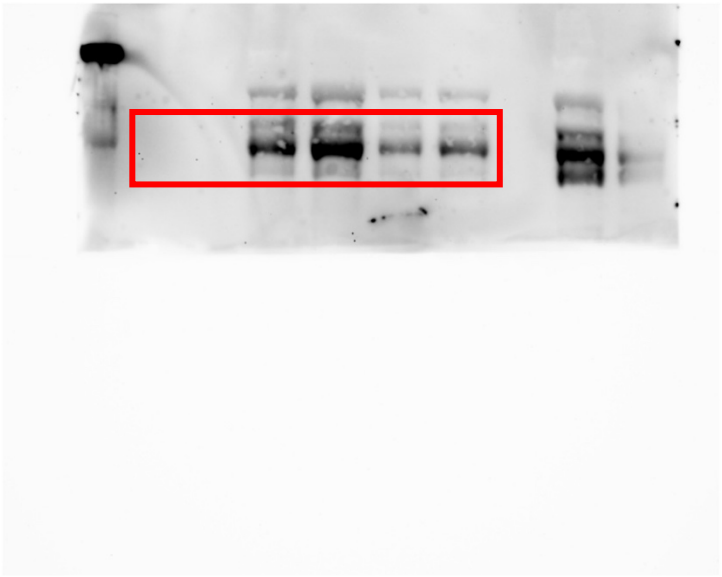


11: Original WB image for BCoV-Spike (in red box), corresponding to the data shown in Figure 5I of the manuscript.

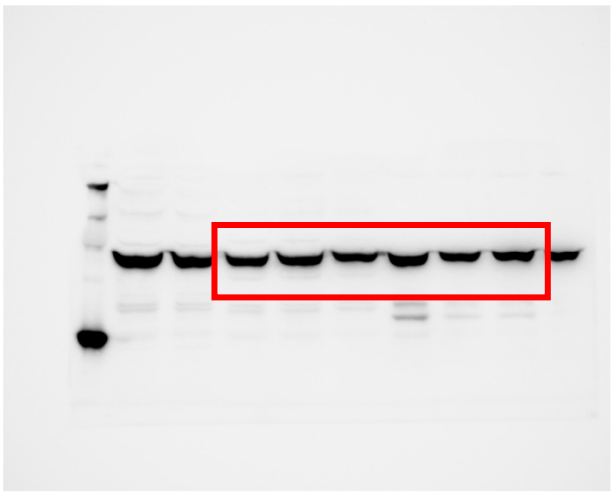
12: Original WB image for B-actin (in red box), corresponding to the data shown in Figure 5I of the manuscript.



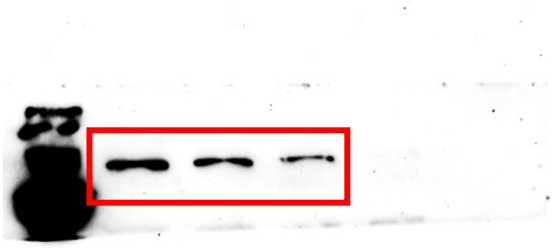
13: Original WB image for BCoV-Nucleocapsid (in red box), corresponding to the data shown in Figure 6C of the manuscript.



14: Original WB image for BCoV-Spike (in red box), corresponding to the data shown in Figure 6C of the manuscript.

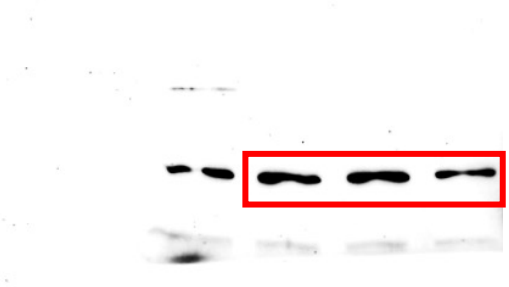


15: Original WB image for B-actin (in red box), corresponding to the data shown in Figure 6C of the manuscript.

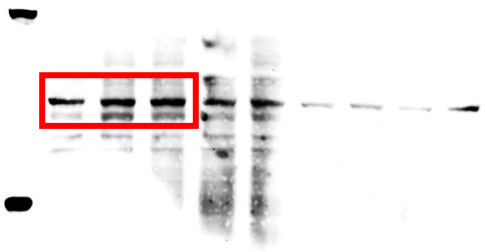


16: Original WB image for Furin (in red box), corresponding to the data shown in Figure 7B of the manuscript.

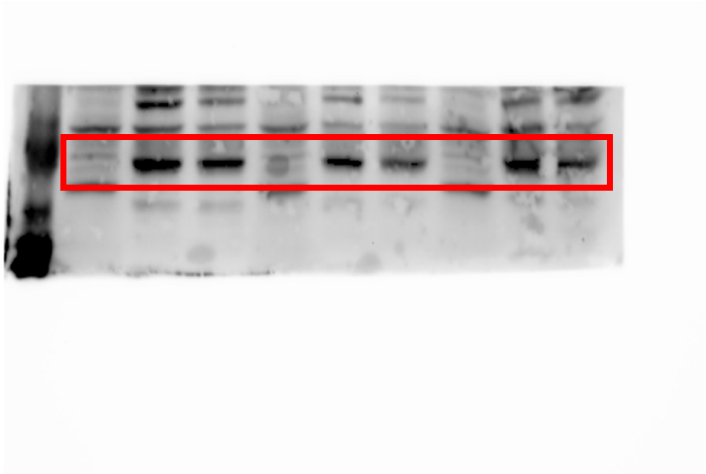
17: Original WB image for B-actin (in red box), corresponding to the data shown in Figure 7B of the manuscript.



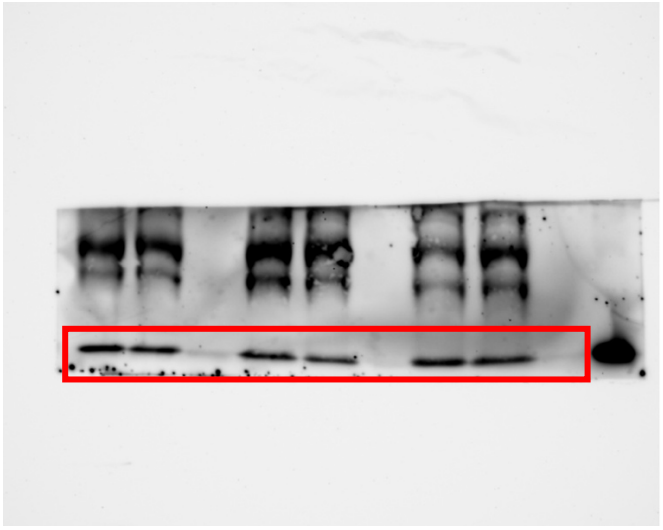
18: Original WB image for BCoV-Spike (in red box), corresponding to the data shown in Figure 7D of the manuscript.



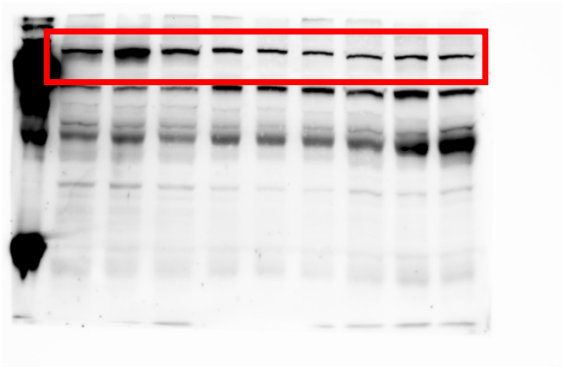
19: Original WB image for B-actin (in red box), corresponding to the data shown in Figure 7D of the manuscript.



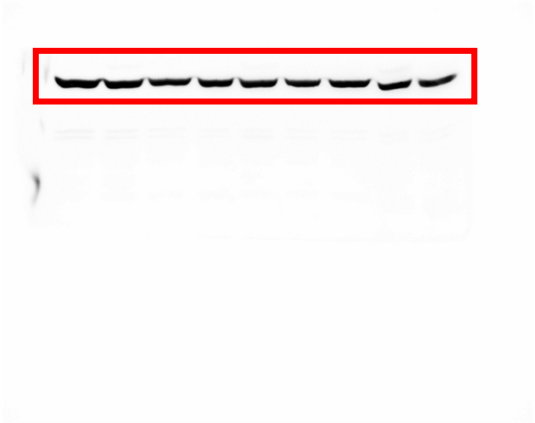
20: Original WB image for BCoV-Spike (in red box), corresponding to the data shown in Figure 8C of the manuscript.



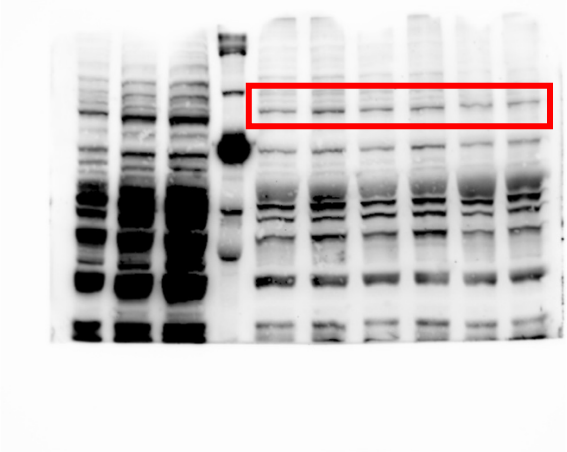
21: Original WB image for BCoV-Nucleocapsid (in red box), corresponding to the data shown in Figure 8C of the manuscript.



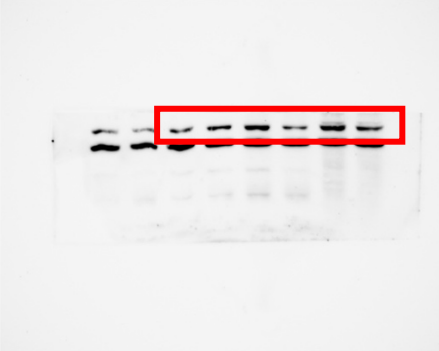
22: Original WB image for Furin (in red box), corresponding to the data shown in Figure 8C of the manuscript.



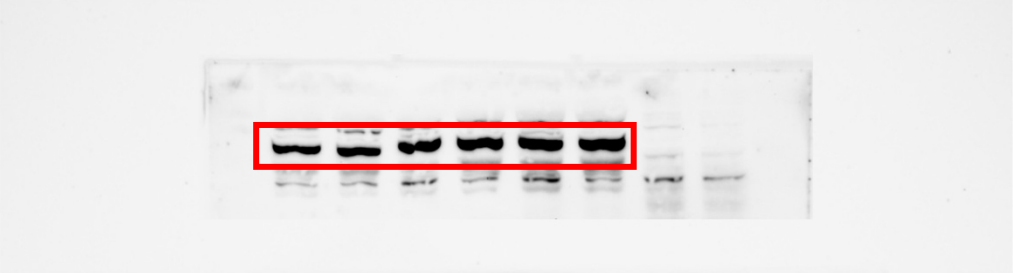
23: Original WB image for B-actin (in red box), corresponding to the data shown in Figure 8C of the manuscript.



24: Original WB image for ACE2 (in red box), corresponding to the data shown in Figure 9A of the manuscript.

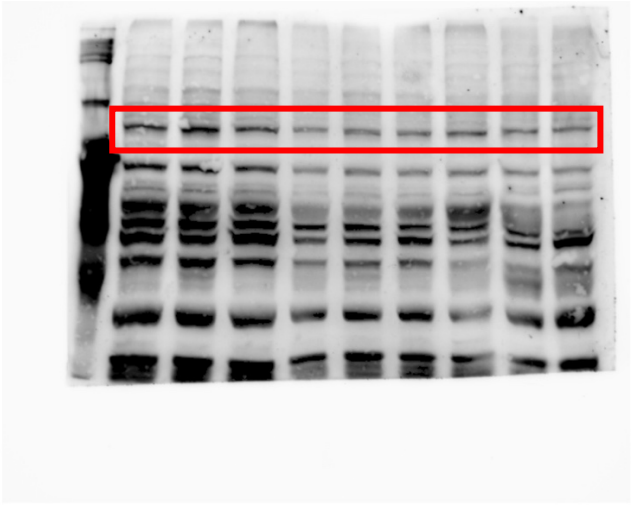


25: Original WB image for NRP1 (in red box), corresponding to the data shown in Figure 9A of the manuscript.

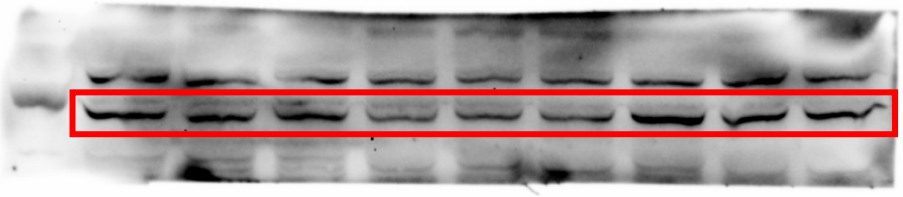


26: Original WB image for TMPRSS2 (in red box), corresponding to the data shown in Figure 9A of the manuscript.

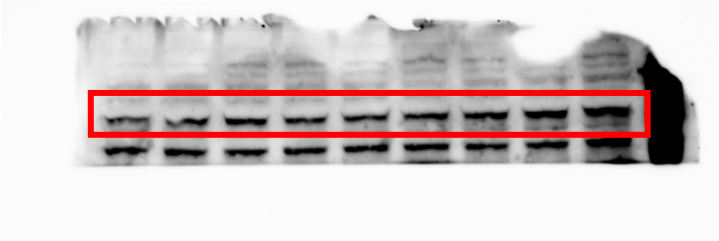
27: Original WB image for B-actin (in red box), corresponding to the data shown in Figure 9A of the manuscript.



28: Original WB image for ACE2 (in red box), corresponding to the data shown in Figure 10A of the manuscript.

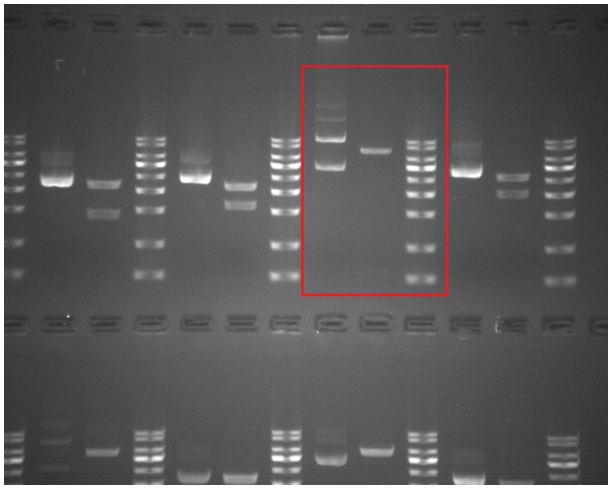


29: Original WB image for NRP1 (in red box), corresponding to the data shown in Figure 10A of the manuscript.

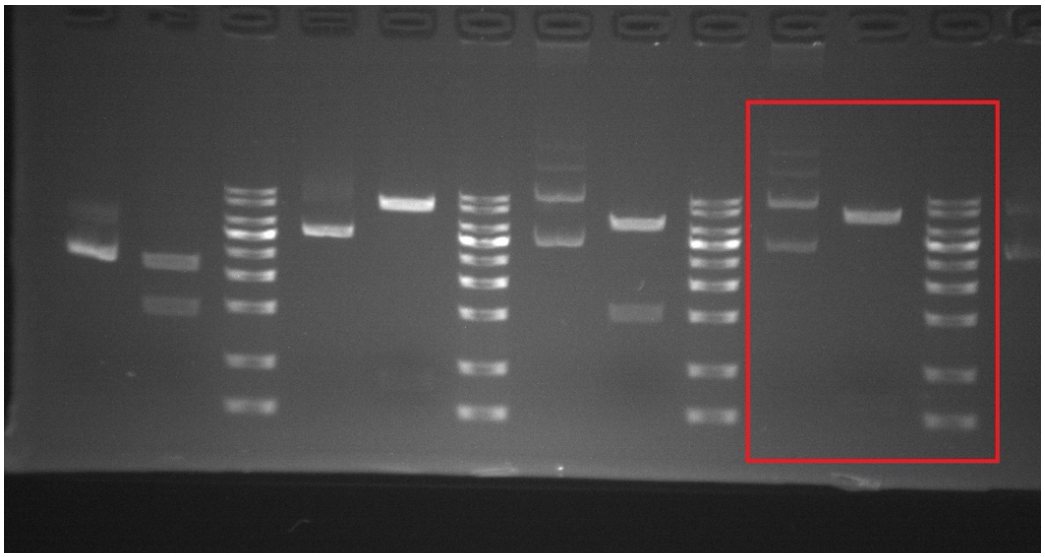


30: Original WB image for TMPRSS2 (in red box), corresponding to the data shown in Figure 10A of the manuscript.

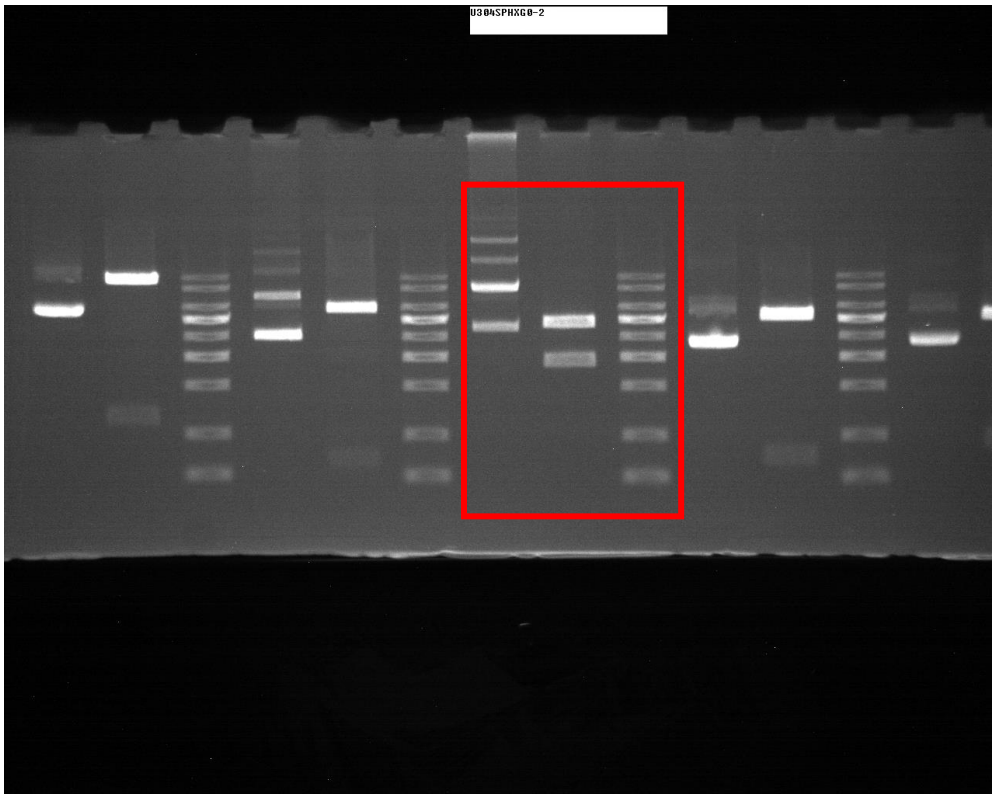
31: Original WB image for B-actin (in red box), corresponding to the data shown in Figure 10A of the manuscript.



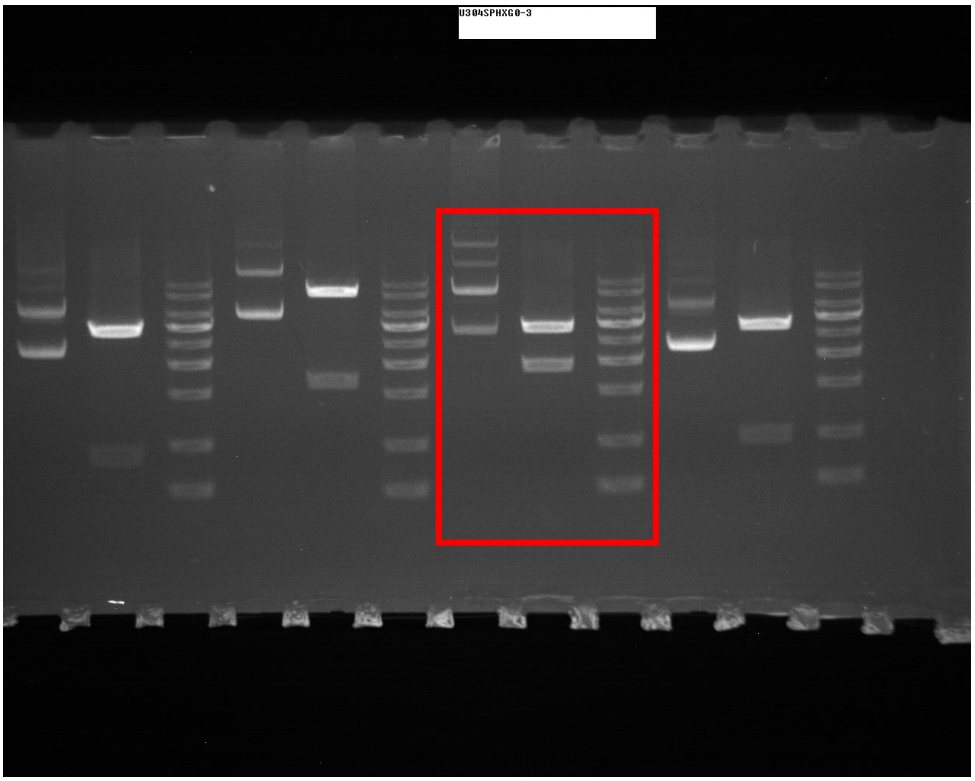
32: Original gel electrophoresis image for Furin wild-type construct (in red box), corresponding to the data shown in Figure S3B of the manuscript.



33: Original gel electrophoresis image for Furin mutant construct (in red box), corresponding to the data shown in Figure S3C of the manuscript.



34: Original gel electrophoresis image for Spike wild-type construct (in red box), corresponding to the data shown in Figure S4B of the manuscript.



35: Original gel electrophoresis image for Spike mutant construct (in red box), corresponding to the data shown in Figure S4C of the manuscript.