## Supplementary Information

# Foot-and-mouth disease virus 3A hijacks Sar1 and Sec12 for ER remodeling in a COPII-independent manner

**Heng-Wei Lee[1], Yi-Fan Jiang[1,2], Hui-Wen Chang[1,2], Ivan-Chen Cheng[1]* ***

*[1] School of Veterinary Medicine, National Taiwan University, Taipei, 106, Taiwan*

*[2] Graduate Institute of Molecular and Comparative Pathobiology, School of Veterinary Medicine, National Taiwan University, Taipei, 106, Taiwan*

Correspondence: ivancheng@ntu.edu.tw (I.-C.C)

Supplementary Figures S1-S6

Supplementary Table S1

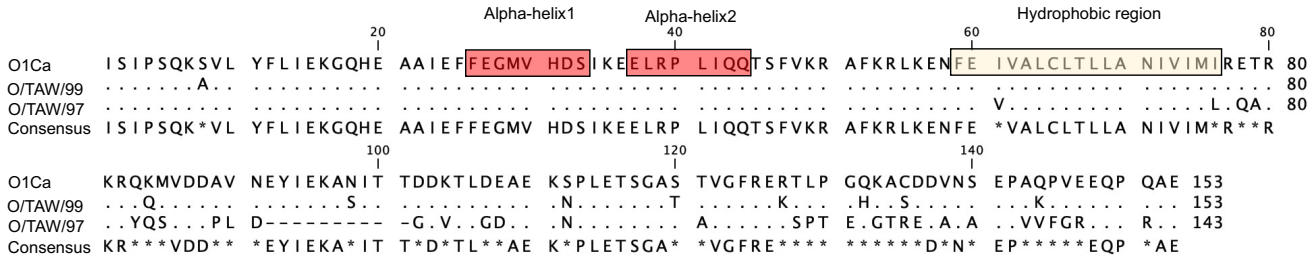
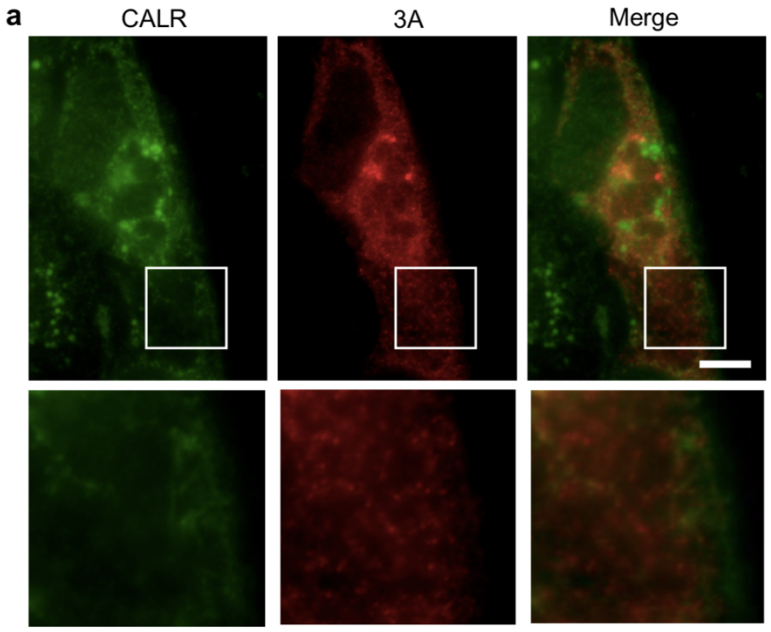
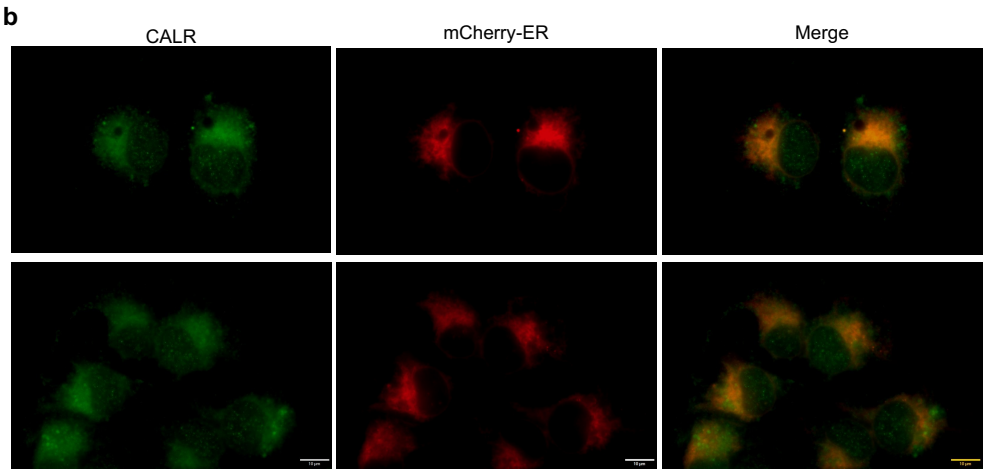
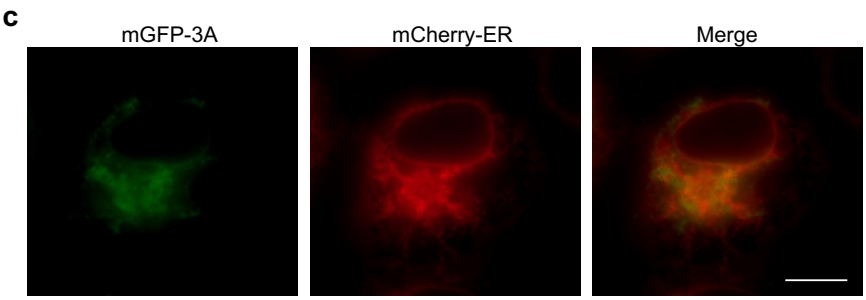
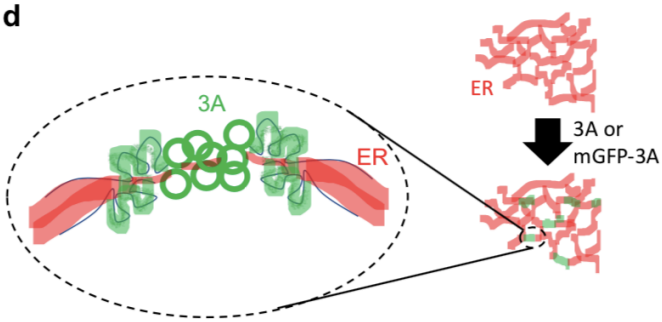



**Figure S1.** Alignment of the amino acid sequence of 3A among O1Campos (O1Ca, NCBI reference sequence CAC86575), O/TAW/99, and O/TAW/97 virus strains. The red boxes represent alpha-helix1 and alpha-helix2, while the yellow box indicates the hydrophobic region.


a
CALR
3A
Merge


b
CALR
mCherry-ER
Merge


c
mGFP-3A
mCherry-ER
Merge


d
3A
ER
ER
3A or
mGFP-3A

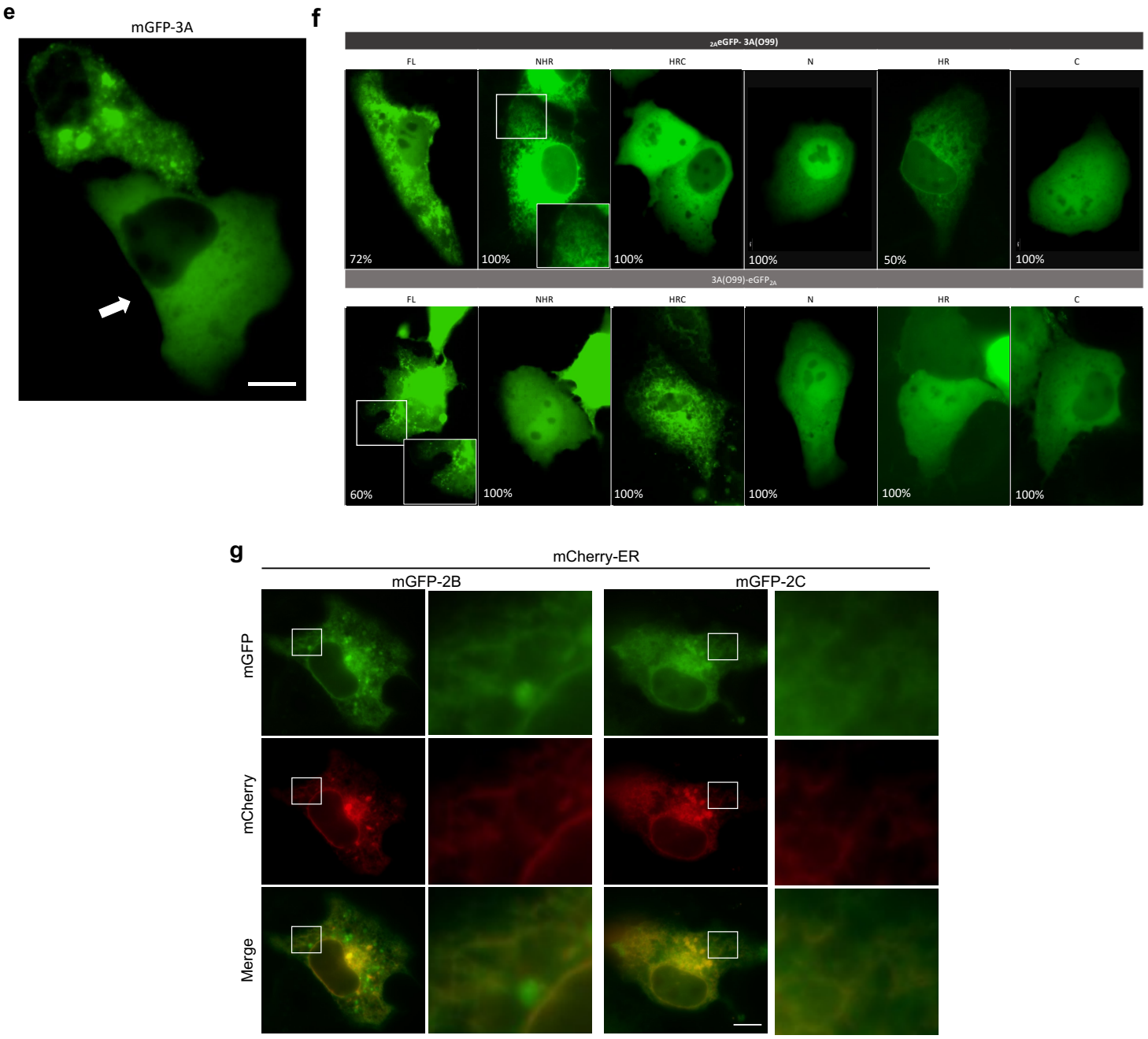



**Figure S2.** (**a**) Double immunofluorescence to 3A (red) and calreticulin (green) revealed with QA2 MAb and anti-calreticulin (CALR) rabbit antibody, followed by appropriate secondary antibodies (Alexa 594 conjugated anti-mouse antibody and FITC conjugated anti-rabbit antibody). (**b**) PK-15 cells expressing mCherry-ER were fixed and stained with anti-CALR rabbit antibody, followed by anti-rabbit antibody coupled with FITC. (**c**) After 3 hr post-transfection, PK-15 cells co-expressing mGFP-3A and mCherry-ER were examined by fluorescence microscopy. (**d**) The diagram depicts the modification of ER by 3A and the limitation of fluorescence microscopy. (**e**) PK-15 cells expressing mGFP-3A showed two different groups, diffuse-type (white arrow) and cells with multiple punctae. (**f**) The images from variant truncated 3A fused to eGFP. The N- or C-terminal eGFP was also fused to the 2A peptide of FMDV, which exhibited self-cleavage activity. It was originally designed for a cistronic co-expression system; however, we found that, if other protein genes that served 2A as linkage were added, the cleavage was not complete (data not shown).

Therefore, these plasmids were not used for further construction. However, mGFP-3A and $_{2A}$eGFP-3A truncated versions showed identical results; we thought 2A peptide would not interfere with the localization of 3A. Similar to mGFP-3A, 72% and 60% of $_{2A}$eGFP-3A and 3A- eGFP$_{2A}$ showed punctate patterns, respectively, while the others were diffuse-type. In addition, $_{2A}$eGFP-NHR and HRC- eGFP$_{2A}$ both showed clear reticular patterns. Only 50% of $_{2A}$eGFP-HR expressing cells appeared as reticular pattern; the others were diffuse. The other truncations distributed diffusely in the cytoplasm and nucleus. (**g**) PK-15 cells co-expressing mCherry-ER with mGFP-2B or mGFP-2C were examined for colocalization test. Scale bar, 10 μm.

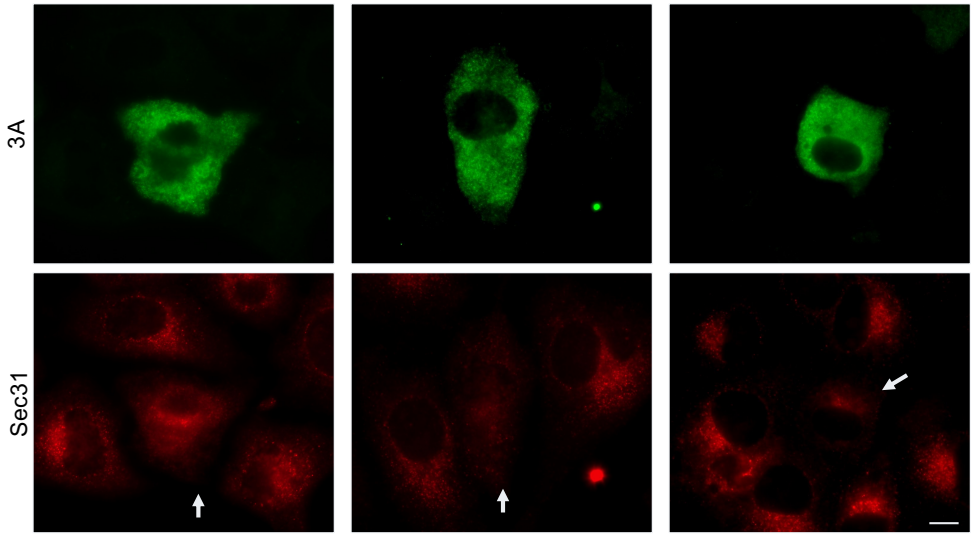



**Figure S3.** The transfected cells were stained with anti-Sec31A rabbit antibodies, followed by anti-rabbit secondary antibodies (red). Finally, 3A was identified with QA2-Dylight488 (green). The Sec31A signal in 3A-expressing cells (white arrows) was dispersed within the cytoplasm compared to non-transfected cells. Scale bar, 10 μm.

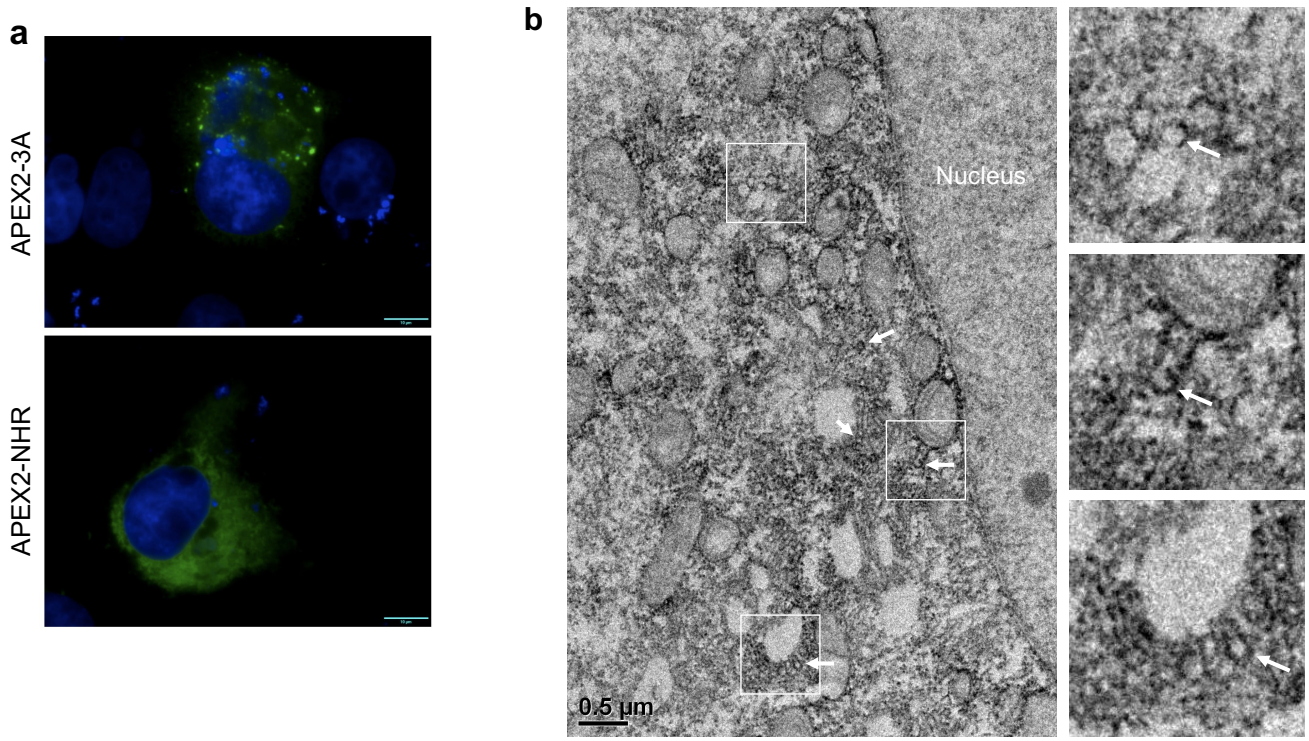



**Figure S4.** (**a**) The patterns of APEX2-3A and APEX2-NHR were examined under IFA by QA2 MAb. (**b**) The PK-15 cells' ultrastructure for expression of APEX2-N2HRC in TEM. The white arrows indicate modified structures around DAB reaction products.

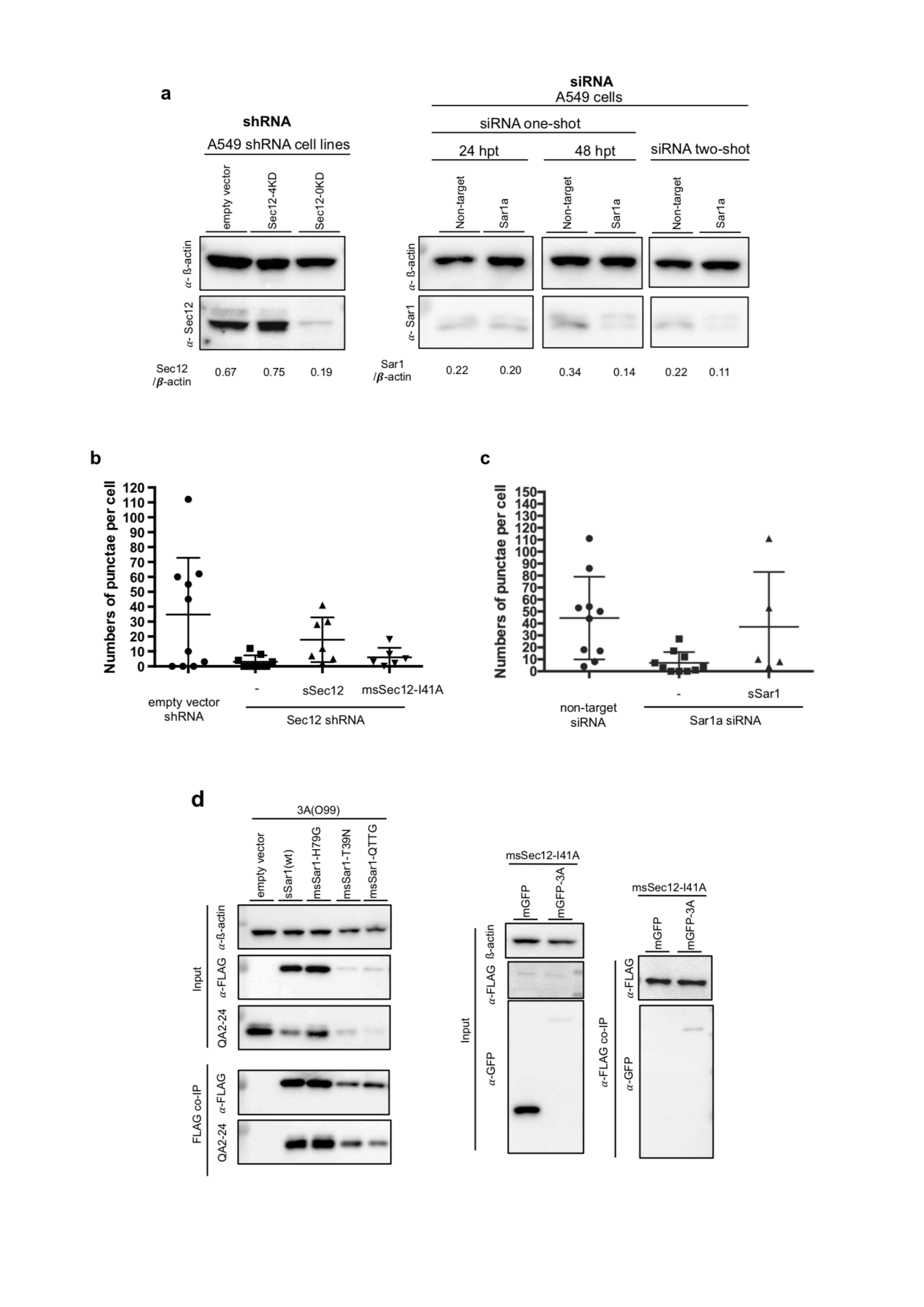

a
shRNA
A549 shRNA cell lines
empty vector
Sec12-4KD
Sec12-0KD
α- ß-actin
α- Sec12
Sec12 /β-actin
0.67
0.75
0.19
siRNA
A549 cells
siRNA one-shot
24 hpt
48 hpt
siRNA two-shot
Non-target
Sar1a
α- ß-actin
α- Sar1
Sar1 /β-actin
0.22
0.20
0.34
0.14
0.22
0.11
b
Numbers of punctae per cell
empty vector shRNA
-
sSec12
msSec12-I41A
Sec12 shRNA
c
Numbers of punctae per cell
non-target siRNA
-
sSar1
Sar1a siRNA
d
3A(O99)
empty vector
sSar1(wt)
msSar1-H79G
msSar1-T39N
msSar1-QTTG
Input
α-ß-actin
α-FLAG
QA2-24
FLAG co-IP
α-FLAG
QA2-24
msSec12-I41A
mGFP
mGFP-3A
Input
α-FLAG
ß-actin
α-GFP
α-FLAG co-IP
α-FLAG
α-GFP

**Figure S5.** (**a**) For Sec12 knockdown, A549 cells were transfected with pLAS2w.Ppuro (empty vector), Sec12-4 (target sequence: GTGTGCTTCAACCACGATAAT) or Sec12-0 (target sequence: GCTGGCCTAAAGATGCAATAA), followed by puromycin selection for more than 2 weeks. The cell lysates were examined by western blotting. The Sec12 knockdown cell line was successfully established for Sec12-0 and named A0 cells. For Sar1 knockdown, due to failure to establish the shRNA cell line, siRNA (target sequence: CCAGTTCCTAGGACTCTACAA) was chosen as an alternative. After siRNA transfection for 24 hr or 48 hr, cell lysates were examined by western blotting. To elevate knockdown efficiency, we performed double transfection at a 24 hr interval. Cells were harvested at the next 24 hours (after the last transfection of siRNA), which was adopted for the following experiments. The band intensities for indicated proteins were quantified in ImageJ software and standardized by ß-actin. (**b, c**) The numbers of punctae were quantified in different condition for knockdown and re-expression assay. (**d**) HTK cell lysates, co-expressing 3A and wild-type (or mutants) of sSar1, were applied to co-immunoprecipitation assay. All mutants of sSar1 preserved the ability to interact with 3A. Empty vector: pcDNA-3.1(+). Similarly, in PK-15 cell lysates, the I41A mutation of Sec12 would not abrogate the interaction with 3A, as proved by mGFP-3A.

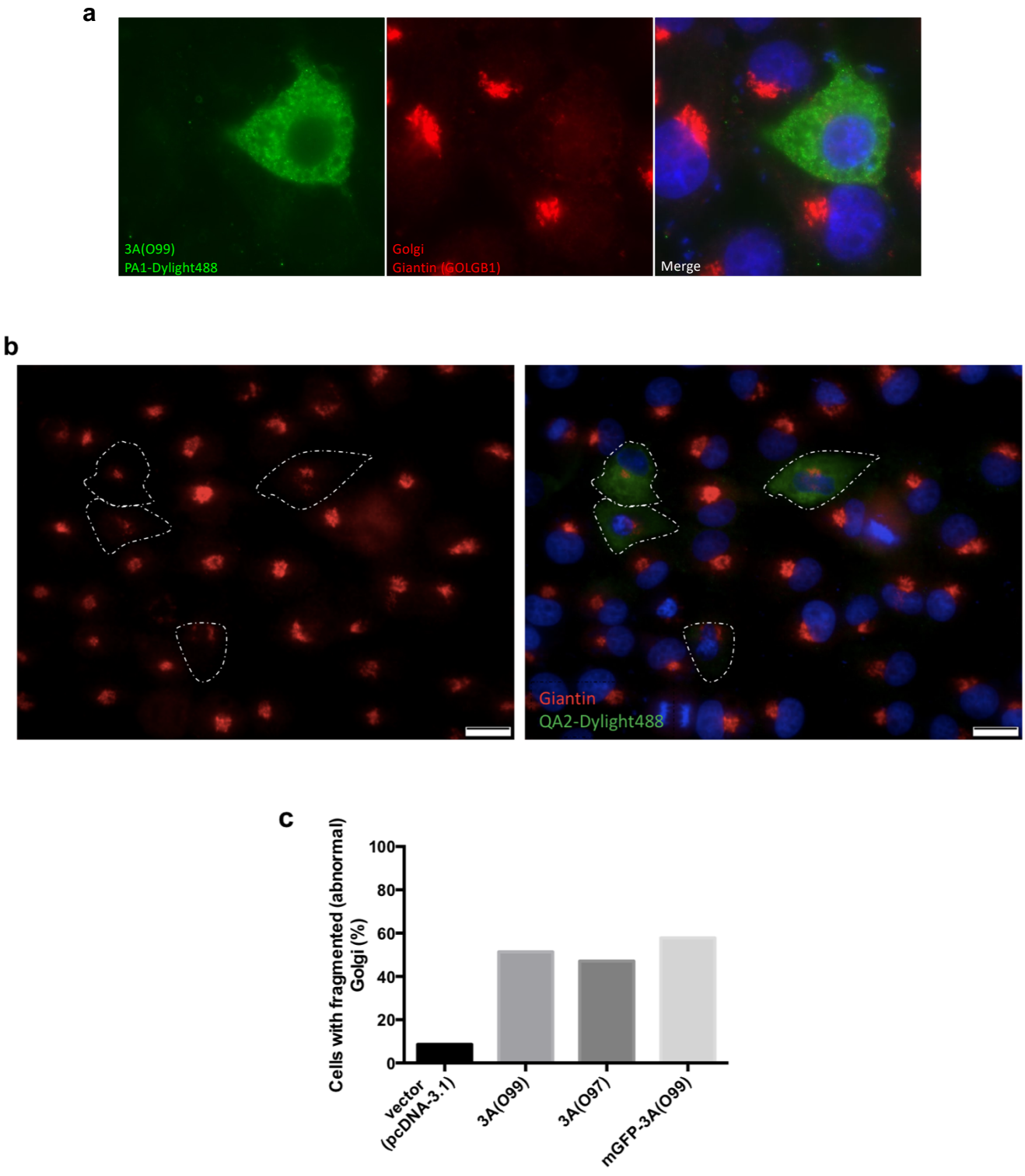



**Figure S6.** FMDV 3A expression resulted in the dispersal of the Golgi, shown by IFA. (**a**, **b**) The fixed PK-15 expressing 3A(O99) were subsequently incubated for anti-giantin rabbit antibodies, anti-rabbit antibodies-Alexa Fluor 594, and PA1-Dylight488 or QA2-Dylight488. Chromosomes were stained with Hoechst 33258. (**c**) PK-15 cells were transfected for 3A(O99), 3A(O97), and mGFP-3A(O99). Golgi integrity was examined in about 100 cells for each group, with mitotic cells excluded.

Supplementary Table S1. A list of cloning primers

| Primer name | Sequence (5' to 3') |
|---|---|
| (mGFP-, eGFP- or GST-) HindIII-3A-XhoI (or XbaI) | |
| HindIII-3A(O99)-F | AAAAAGCTTGCCACCATGGCCATCTCAATTCCTTCCCAAAAGG |
| XhoI-3A(O99)His-R | AACTCGAGTCAATGATGGTGGTGGTGGTGTTCAGCTTGTGGTTGTTCTTC |
| HindIII-3A(O97)-F | AAAAAGCTTGCCACCATGGCCATTTCAATCCCTTCCCAGAAG |
| XhoI-3A(O97)His-R | AACTCGAGTCAATGATGGTGGTGGTGGTGTTCAGCTCGCGGTTGTTC |
| XhoI-3A(O99)-R | AACTCGAGTCATTCAGCTTGTGGTTGTTCTTC |
| HindIII-3A(O97)C-F | AAAAAGCTTGCCACCATGGCCCGCCAAGCGCGCAAG |
| XbaI-3A(O99)N-R | AATCTAGATTAGTTTTCCTTCAGGCGC |
| HindIII-3A(O99)HR-F | AAAAAGCTTATGGCCTTTGAGATAGTTGCCCTGTG |
| XhoI-3A(O99)HR-R | AAACTCGAGTCAGATCATGATCACTATGTTTGCC |
| HindIII-3A(O99)C-F | AAAAAGCTTATGGCCCGCGAGACTCGCAAG |
| HindIII-3A(O99)a2-F | AAAAAGCTTGCCACCATGGCCATCCAGCAGACCTCATTTG |
| XbaI-3A(O99)a1-R | TTTCTAGATTAGAGAGGCCGGAGCTCC |
| (APEX2) XhoI-3A-XbaI | |
| XhoI-3A(O99)-F | AACTCGAGATGGCCATCTCAATTCCTTCCCAAAAGG |
| XbaI-3A(O99)-R | AATCTAGATTATTCAGCTTGTGGTTGTTCTTC |
| XbaI-3A(O99)HR-R | AATCTAGATTAGATCATGATCACTATGTTTGCC |
| XhoI-3A(O99)a2-F | AACTCGAGATGGCCATCCAGCAGACCTCATTTG |
| XbaI-3A(O99)tC1-R | AATCTAGATTACTCGTTCACTGCATCATCC |
| NheI-3A-HindIII (-GFP or -GST) | |
| NheI-3A(O97)-F | AAAGCTAGCATGATTTCAATCCCTTCCCAGAAG |
| HindIII-3A(O97)-R | AAAAAGCTTTTCAGCTCGCGGTTGTTC |
| NheI-3A(O99)-F | AAAGCTAGCATGGCCATCTCAATTCCTTCCCAAAAGG |
| HindIII-3A(O99)-R | AAAAAGCTTTTCAGCTTGTGGTTGTTCTTC |
| HindIII-3A(O99)N-R | AAAAAGCTTGTTTTCCTTCAGGCGC |
| NheI-3A(O99)HR-F | AAAGCTAGCATGGCCTTTGAGATAGTTGCCCTGTG |
| HindIII-3A(O99)HR-R | AAAAAGCTTGATCATGATCACTATGTTTGCC |
| NheI-3A(O99)C-F | AAAGCTAGCATGGCCCGCGAGACTCGCAAG |
| HindIII-3A(O99)tC1-R | AAAAAGCTTCTCGTTCACTGCATCATCC |
| NheI-3A(O99)tC2-F | AAAGCTAGCATGGCCTACATTGAGAAGGCAAGCATC |
| HindIII-3A(O99)a1-R | AAAAAGCTTGAGAGGCCGGAGCTCC |
| NheI-3A(O99)a2-F | AAAGCTAGCATGGCCATCCAGCAGACCTCATTTG |
| Vectors or others | |
| NheI-GFP-F | AAAGCTAGCATGGGCAGCAAGGGCGAGG |
| XhoI-HindIII-GFP-R | AACTCGAGTCAAAGCTTCTTGTACAGCTCGTCCATG |
| NheI-BamHI-d1D-F | AAAGCTAGCGCCACCATGGGATCCCACAAGCAGAGGATTGTGG |
| NheI-BamHI-d1D2A-F | AAAGCTAGCATGCTCGAGCACAAGCAGAGGATTGTGG |
| XhoI-d1D2A-R | TTTCTCGAGGGGCCCAGGGTTGG |

| | |
|---|---|
| NheI-HindIII-GFP-F | AAAGCTAGCATGAAGCTTATGAGCAAGGGCGAGG |
| BamHI-GFP-R | AAAGGATCCCTTGTACAGCTCGTCCATG |
| NheI-GST-F | AAAGCTAGCATGGCCTCCCCTATACTAGGTTATTG |
| BamHI-HindIII-GST-R | AAGGATCCTTAAAGCTTTTTTGGAGGATGGTCGC |
| HindIII-GST-F | AAAAAGCTTATGGCCTCCCCTATACTAGGTTATTG |
| XhoI-GST-R | AACTCGAGTTATTTTGGAGGATGGTCGC |
| NheI-CALR-mCherry-F | AAAGCTAGCATGGCCCTGCTATCCGTGCCGCTGCTGCTCGGCCTCCTCGGCCTGGCCGTCGCCGAGATGGTGAGCAAGGGC |
| HindIII-KDEL-mCherry-R | AAAAAGCTTTTACAGCTCGTCCTTCTTGTACAGCTCGTCC |
| BamHI-sSar1-F | TTGGATCCATGTCTTTCATCTTTGAGTGGATCTAC |
| 2B or 2C | |
| NheI-FLAG-2B-F | AAAGCTAGCATGGATTACAAGGATGACGACGATAAGGGTACCCCCTTCTTCTTCTCCGAC |
| EcoRI-stop-BamHI-2B-R | AAAGAATTCTTAGGATCCCTGCTTTTCTGCTCTCTCG |
| NheI-FLAG-KpnI-2C-F | AAAGCTAGCATGGATTACAAGGATGACGACGATAAGGGTACCCTCAAAGCACGTGACATCAAC |
| EcoRI-stop-BamHI-2C-R | AAAGAATTCTTAGGATCCCTGTTTAAATATCAGGTGGCTCG |
| Mutagenesis | |
| O973A-dNheI-F | GGAGACGAGTGGCGCCAGCGCTGTCGGTTTC |
| O973A-dNheI-R | GAAACCGACAGCGCTGGCGCCACTCGTCTCC |
| msSar1-H79G-F | TTTGATCTCGGTGGGGGTGAGCAAGCACGTCG |
| msSar1-H79G-R | CGACGTGCTTGCTCACCCCCACCGAGATCAAA |
| msSar1-T39N-F | ACAATGCAGGCAAAAACACTCTTTTACACAT |
| msSar1-T39N-R | ATGTGTAAAAGAGTGTTTTTGCCTGCATTGT |
| msSar1-QTTG-F | TTTGGGCTTTATGGAGCCGCCGCAGCAAAGGGGAATGTGACC |
| msSar1-QTTG-R | GGTCACATTCCCCTTTGCTGCGGCGGCTCCATAAAGCCCAAA |
| XhoI-msSar1-D198A-R | AACTCGAGTCAGGCAATATACTGGGAGAGCCAGC |
| sSec12-I41A-F | GCTGCCAAGACCGGTGCAAAGAACGGCGTGCAC |
| sSec12-I41A-R | GTGCACGCCGTTCTTTGCACCGGTCTTGGCAGC |
| m3A-49,50-F | CAGACCTCATTTGTGGCCGCCGCTTTTAAGCGCCT |
| m3A-49,50-R | AGGCGCTTAAAAGCGGCGGCCACAAATGAGGTCTG |
| m3A-53,54-F | GTGAAGCGCGCTTTTGCCGCCCTGAAGGAAAACTT |
| m3A-53,54-R | AAGTTTTCCTTCAGGGCGGCAAAAGCGCGCTTCAC |
| m3A-54,56-F | AAGCGCGCTTTTAAGGCCCTGGCGGAAAACTTTGAGAT |
| m3A-54,56-R | ATCTCAAAGTTTTCCGCCAGGGCCTTAAAAGCGCGCTT |
| m3A-80,81,82-F | CATGATCCGCGAGACTGCCGCCGCACAGCAGATGGTGGATG |
| m3A-80,81,82-R | CATCCACCATCTGCTGTGCGGCGGCAGTCTCGCGGATCATG |
| m3A-125,126,127-F | CACCACTGTTGGTTTCGCCGCCGCAACTCTCCCGGGACAC |
| m3A-125,126,127-R | GTGTCCCGGGAGAGTTGCGGCGGCGAAACCAACAGTGGTG |

Green: restriction site; Red: start codon or stop codon; Blue: tag or additional signal peptide; Yellow: mutation site