Influenza virus mRNA trafficking through host nuclear speckles

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Supplementary Information

Supplementary Figures

Supplementary Figure 1

a) A/Texas/36/91

b) A/Puerto Rico/8/34

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Supplementary Figure 1. Influenza M mRNA is enriched at nuclear speckles while NS mRNA does not accumulate at these nuclear bodies. (a) A549 cells were infected with A/Texas/36/91 or (b) A/Puerto Rico/8/34 at MOI 10. After 4h, cells were fixed and M mRNA molecules were labeled by RNA-FISH. Speckles were marked by immunofluorescence with SON antibody. Insets are enlargement of areas showing nuclear speckles. Images are representative of three independent experiments. Scale Bar = 10µm. (c) NS and M1 mRNA distribution was monitored at 2h, 3h, 4h and 6h during infection at MOI 10. A set of 40 probes labeled with Quasar 570 covering the entire NS mRNA were used together with the M1 Quasar 670 probes to label the M1 mRNA. Nuclear speckles were labeled by immunofluorescence with SON antibody. DNA was labeled with Hoechst. Images are representative of three independent experiments. Scale Bar = 10µm.
Supplementary Figure 2. Specificity of M1, M2 and mRNA3 Probes. (a) A549 Cells were either transfected with a plasmid encoding full length M1 mRNA with mutations in the splicing acceptor (SA) site (Plasmid M1 SA Mut) or with a plasmid encoding the M2 mRNA (Plasmid M2) as depicted. After 14 h, RNA was purified and subjected to reverse transcription. cDNA was
further amplified with primers spanning the M2 mRNA splice sites and products were run on agarose gel and sequenced. M1 and M2 specific bands are marked. (b) A549 cells were transfected with the Plasmid M1 SA mutant or Plasmid M2. After 14 h, cells were fixed and RNA-FISH was performed using the M1 probes. M1 or M2 proteins were labeled by immunofluorescence. Note untransfected cells in the Merge panel, DNA stained with Hoechst, that do not express M1. (c) A549 cells were transfected with the M1 and M2 plasmids. After 14 h, cells were fixed and RNA-FISH was performed using the M2 probe. M1 or M2 proteins were labeled by immunofluorescence. Note untransfected cells in the Merge panel, DNA labeled with Hoechst, that do not express M2. (d) A549 cells were untreated (Control) or treated with Meayamycin for 2h and then infected with WSN at MOI 3 for 6h. Infection was carried out in the presence of Meayamycin in cells pre-treated with Meayamycin. RNA was purified and analyzed as in a. (e) Cells were untreated (Control) or treated with Meayamycin and infected as in d. After 6h post-infection, cells were fixed and RNA-FISH was performed using the M1 probes and M2 probe. Speckles were marked with SC35 and DNA was stained with Hoechst. (f) A549 Cells were transfected with a plasmid encoding mRNA3 (Plasmid + mRNA3). After 14 h, RNA was purified and subjected to reverse transcription. cDNA was amplified with mRNA3 specific primers or primers spanning the M2 mRNA splice sites that can amplify both M1 and M2 mRNAs. Products were run on agarose gel and the mRNA3 specific band is marked. (g) A549 cells were transfected with plasmids encoding mRNA3, M1, and M2. After 14 h, cells were fixed and RNA-FISH was performed using the mRNA3 probe. Immunofluorescence was also carried out with antibody to label both M1 and M2 proteins. (h) A549 cells were infected for 4h and then fixed for RNA-FISH using the specific mRNA3 and M1 mRNA probes. Speckles were marked by immunofluorescence with SON antibody. DNA was labeled with Hoechst. Merge shows mRNA3, Speckle, and DNA channels. Data presented in a, d and f, and images presented in b,c,e,g and h are representative of three independent experiments. Scale Bars = 10µm.
Supplementary Figure 3. NS1 promotes M1 mRNA splicing at nuclear speckles and nuclear export in A549 cells. (a) FISH fluorescent signal quantification in nuclear speckles,
nucleoplasm, and cytoplasm. RNA-FISH signal in nuclear speckles was captured in the detected volume of speckles (white regions in Detection) marked by SON antibody. RNA-FISH signal in the nucleus was captured in the detected volume of nuclei (Blue region in Detection) marked by Hoechst staining. The remaining cellular RNA-FISH signal is cytoplasmic (Orange region in Detection). Images depicting the detection approach are representative of three independent experiments. Scale Bar = 5µm. (b) A549 cells were infected with WSN or WSN ΔNS1 at MOI 10. After 4h, cells were fixed and M mRNA molecules were labeled by RNA-FISH. Speckles were marked by immunofluorescence with SON antibody. Insets are enlargement of areas showing nuclear speckles. Scale Bar = 10µm. (c) M mRNA intensity at speckles after infection of A549 cells with WSN or WSN ΔNS1 was quantified as shown in a. M mRNA intensity sum at speckles was normalized to the intensity sum in the nuclear compartment. Normalized intensities at speckles of WSN virus infected cells were set to 1 and the relative fold change in WSN ΔNS1 infected cells is shown. Values are mean ± s.d. measured in 21 WSN and 23 WSN ΔNS1 infected cells. p.i.- post infection. (d) RT-qPCR was performed to quantify M2/M1 mRNA ratio in WSN or WSN ΔNS1 A549 infected cells at MOI 1 for 6h. The average M2/M1 mRNA ratio from cells infected with WSN was set to 1 and the relative average M2/M1 mRNA ratio in WSN ΔNS1 infected cells is shown. Values are mean ± s.d. ratios from three independent experiments. (e and f) M mRNA intracellular distribution in A549 cells infected with WT WSN or WSN ΔNS1 at MOI 10. After 6h post-infection, cells were fixed and M mRNA was labeled by RNA-FISH. The nucleus was marked by Hoechst DNA staining. Images were taken with a 20x lens. Scale Bar = 10µm. (f) Intensities were measured in the nucleus (N) and cytoplasm (C), as shown in a, and the C/N ratio was calculated. Values are mean ± s.d. of 446 WSN and 241 WSN ΔNS1 infected cells. **T test p value < 0.01. (g and h) A549 cells were infected with WSN or WSN ΔNS1 at MOI 3. After 9h post-infection, cells were fixed, M mRNA was labeled by RNA-FISH, and immunofluorescence microscopy was performed with antibodies specific to M1 protein (g) and M2 protein (h). Scale Bar = 10µm. (i) Cells were infected as in g.
and cell extracts were subjected to western blot with antibodies specific to the depicted proteins. Asterisk marks a non-specific band. Western blot image presented in i and images presented in b,e,g and h are representative of three independent experiments.
Supplementary Figure 4. Viral M mRNA localization but not cellular GAPDH mRNA localization is influenced by the host factors NS1-BP, hnRNP K and SON. (a) Immunofluorescence microscopy performed with antibodies specific to hnRNP K and NS1-BP show a pool of these proteins at nuclear speckles. Scale bar = 10µm. (b) A549 cells were transfected with control siRNA or NS1-BP siRNA for 72h. RT-qPCR with specific NS1-BP primers shows the NS1-BP knockdown efficiency. (c) A549 cells were transfected with control siRNA or hnRNP K siRNA for 72 h. RT-qPCR with specific hnRNP K primers demonstrate hnRNP K knockdown efficiency. The average NS1-BP/GAPDH mRNA ratio (b) or hnRNP K/GAPDH mRNA ratio (c) from cells treated with Control siRNA was set to 1 and the relative average NS1-BP/GAPDH or hnRNP K/GAPDH mRNA ratio is shown, respectively. Values are mean ± s.d. ratios from three independent experiments. (d) A549 cells were transfected with control siRNA, hnRNP K siRNA or NS1-BP siRNA. After 72h, cells were infected with WSN at MOI 10 for 4h. Cells were then fixed for M mRNA labeling by RNA-FISH. Nuclear speckles were marked with SON antibody. Insets show an enlarged nuclear speckle region. Scale bar = 10µm. (e and f) The distribution of M mRNA at speckles in e and in the nuclear and cytoplasm (f) was quantified as shown in Supplementary Figure 3a. (e) The intensity sum in speckles of cells transfected with control siRNA were set to 1 and the relative fold changes in NS1-BP and hnRNP K siRNA transfected cells is shown. (f) The C/N ratio values of control versus NS1-BP and hnRNP K siRNA transfected cells were obtained. Values are mean ± s.d. of at least 26 cells analyzed for each treatment. ** T test p value < 0.01. Scale Bar = 10µm. (g) A549 cells were transfected with control siRNA, NS1-BP siRNA, or hnRNP K siRNA. After 72h, cells were then fixed for GAPDH mRNA labeling by RNA-FISH. GAPDH mRNA molecules were labeled with forty-eight FISH probes labeled with Quasar 570. DNA was labeled with Hoechst staining and nuclear speckles were marked with SON antibody. Scale Bar = 10µm. (h) A549 cells were transfected with control siRNA or SON siRNA. After 48h, cells were fixed for GAPDH mRNA labeling by RNA-FISH. SON protein was labeled by immunofluorescence with SON antibody.
DNA was labeled with Hoechst staining. Images presented in a, d, g, and h are representative of three independent experiments. Scale Bar = 10µm.

**Supplementary Figure 5**
Supplementary Figure 5. Meamycin treatment of cells depleted of Aly/REF reveals a relationship between M mRNA localization and splicing at nuclear speckles. (a) A549 cells were uninfected or infected with WSN at MOI 10 for 5h and subjected to immunofluorescence microscopy using Aly/REF, HA and SON antibodies. Merge shows Aly/REF, Speckle and DNA channels. (b) A549 cells were transfected with control siRNA or UAP56 siRNA for 48h. RT-qPCR was carried out with specific UAP56 primers and demonstrates the UAP56 knockdown efficiency. (c) A549 cells were transfected with control siRNA or Aly/REF siRNA for 48h. RT-qPCR was performed with specific Aly/REF primers and shows Aly/REF knockdown efficiency. The average UAP56/GAPDH mRNA ratio (b) or Aly/REF/GAPDH mRNA ratio (c) from cells treated with Control siRNA was set to 1 and the relative average UAP56/GAPDH or Aly/REF/GAPDH mRNA ratio is shown, respectively. Values are mean ± s.d. ratios from three independent experiments. (d) A549 cells were transfected with control siRNA or UAP56 siRNA for 48h and then infected with WSN at MOI 1 for 9h and 10h. Cell extracts were subjected to western blot with antibodies specific to M1 and M2 proteins. Data is representative of three independent experiments. (e) A549 cells were transfected with Aly/REF siRNA for 48h and then infected with WSN at MOI 10. After 1h, virus was removed and 0.1% DMSO (control) or 50 nM meamycin was added to the cells for 3h. Then, media was removed and the following 3 treatments were performed for an additional 4h: 1) Media containing 0.1% DMSO was added to cells pre-treated with 0.1% DMSO (No Treatment Control); 2) Media containing 0.1% DMSO was added in cells pre-treated with meamycin (Meamycin & Release); 3) 50 nM meamycin was added to cells pre-treated with meamycin (Meamycin). Purified RNA was subjected to RT-PCR with specific primers to detect M1 and M2 mRNAs. RNA products were loaded on agarose gel. Data show three independent experiments for each depicted condition. (f) A549 cells were processed as in e except that at the end of the infection process cells were fixed for M1 and M2 mRNA labeling by RNA-FISH. Nuclear speckles were marked with SON antibody. Merge shows the M2 mRNA, Speckle, and DNA channels. Insets are enlargement of
areas showing nuclear speckles. Intensity of the M2 mRNA channel shown at the left inset at Meayamycin&Release was intensified. Inset at Meayamycin&Release Merge contains the intensified M2 mRNA channel with the Speckle and DNA channels. Images presented in a and f are representative of three independent experiments. Scale bars = 10µm.

Supplementary Figure 6

VERO Cells

Supplementary Figure 6. Knockdown Efficiency of UAP56 and Aly/REF in Vero Cells. (a) Vero cells were transfected with control siRNA or UAP56 siRNA for 48h. RT-qPCR was carried out with specific UAP56 primers and demonstrates the UAP56 knockdown efficiency. (b) Vero cells were transfected with control siRNA or Aly/REF siRNA for 48h. RT-qPCR was performed with specific Aly/REF primers and shows Aly/REF knockdown efficiency. The average UAP56/GAPDH mRNA ratio (a) or Aly/REF/GAPDH mRNA ratio (b) from cells treated with Control siRNA was set to 1 and the relative average UAP56/GAPDH or Aly/REF/GAPDH mRNA ratio is shown, respectively. Values are mean ± s.d. ratios from three independent experiments.
Supplementary Figure 7. Raw data from western blots and agarose gels. Data is labeled according to the corresponding figures in the main text and supplementary information. Selected areas show where the gels and membranes were spliced.
Supplementary Video 1. M mRNA is localized at nuclear speckles-3D. A549 cells were infected with WSN at M.O.I 10 and were fixed at 4h post-infection. Twenty-eight *0.3µm 3D sections of M mRNA FISH (Red) and SC35 IF (Green) images were reconstructed and the 3 dimensions are presented.

Supplementary Methods

Infection Media:
EMEM, 12% BSA (Gibco), 0.5 µg/ml TPCK treated trypsin (Worthington), 1% HEPES (Gibco).

Influenza Growth Media:
DMEM, 0.3% BSA, 100 units/ml penicillin, 100 µg/ml streptomycin and 0.5 µg/ml TPCK treated trypsin.

Plasmids

M1 Splice Acceptor (SA) mutant plasmid: The PDZ-M1 (SA Mutated) plasmid was generated by gene synthesis of the PR8 M full segment (Invitrogen) containing the splice acceptor site mutated (gaaatttgcaggc) to (gaGaaCttAcaAgc) and subsequently cloned into the PDZ expression vector between the EcoRI and NheI restriction sites.

M2 Plasmid: M2 open reading frame from WSN was cloned into pcDNA3.1 using the BamHI and NotI restriction sites.
**mRNA<sub>3</sub> Plasmid:** mRNA<sub>3</sub> from WSN was cloned into pcDNA3.1 using the BamHI and NotI restriction sites.

**siRNA oligos:**
siRNA siGENOME SMARTpool against NS1-BP, hnRNP K and siGENOME non-targeting siRNA #3 (Dharmacon, Thermo Fisher Scientific); ON-TARGETplus siRNA against SON and ON-TARGETplus Non-targeting Control #2 (Dharmacon, Thermo Fisher Scientific); MISSION siRNAs were used to target Aly/REF, UAP56 and MISSION siRNA Universal Negative Control #2 (Sigma-Aldrich).

**Inhib Buffer:**
0.2% triton, 1mM DTT + 200 units/ml RNase inhibitor (Roche).

**Primary antibodies:**
SC35 (ab11826, abcam), hnRNP K (ab20343, abcam), SON (GTX129778, GeneTex), Prp8 antibody- ((H-300): sc-30207, Santa Cruz), Aly/REF (A9979, Sigma-Aldrich), M1 (ab22396, abcam), M2 (MA1-082, ThermoFisher Scientific), UAP56 (SAB1307254, Sigma), hnRNP U (ab10297, abcam), Anti-influenza A virion polyclonal antibodies (Meridian Life Science, Inc.), NS1-BP was previously described<sup>1</sup>, anti M1&M2 was previously described<sup>2</sup>, HA CR9114 was previously described<sup>3</sup>, NS1 antibody was previously described<sup>4</sup>.

**Secondary antibodies:**
Alexa488, Alexa546, or Alexa647 labeled goat anti-mouse, anti-rabbit, and anti-human antibodies (Life technologies).

**Wash Buffer (WB):**
Nuclease Free Water, 2x SSC, 10% formamide.

**Hybridization Buffer (HB):**
WB + 100 mg/ml dextran sulfate sodium salt (Sigma).

**Florescent In Situ Hybridization (FISH) probes:**
Different probes were used in Florescent In Situ Hybridization (FISH) experiments:

- **M mRNA probes**- Forty-five 20nt DNA probes labeled with Quasar 570 (BIOSEARCH TECHNOLOGIES) were designed to hybridize with the Influenza WSN full length M segment mRNA. These probes can hybridize with both M1 mRNA and M1 cRNA, although M1 cRNA is present at very low levels as compared to M1 mRNA. M1 cRNA serves as template for the generation of M1 vRNA, which is not recognized by these probes. The signal detected by the 46 probes constitutes mostly M1 mRNA in addition to the alternative spliced forms M2 mRNA, mRNA3, and M4 mRNA. Hence, we label the signal detected with the 46 probes as M mRNA. While M1 mRNA can be detected at the single particle level, the spliced forms can only be detected in bulk due to their small sizes.

Here are the specific Full M probes sequences:

1. aatatctacctgcttttgct
2. tcggtagaagactcatctt
3. agagagaacgtacgtttcga
4. tgagggggcctgacgggacg
5. agtctctgtgcagatcggc
6. cttccctgcaaaagacatcttt
7. tgagaacctcaagatcggtg
8. ggtctttgctttagccttcc
9. cttatgcagaggtgacagga
10. tgaacacaatctcataaatc
11. cgctcactgggcaacaggtgag
12. aaagctctacgtgctgcagtc
13. tccatgtaagacagatttttg
14. tcatgttatgttggatctcc
15. cctatacagtttaactgcct
16. atgttatctcccttttaagc
17. gctatctcttttgcccatg
18. tgcaccagcagaataactga
19. tgagGCCcatacaactgc
20. acagcccccatctgcttga
21. ggcaaatgcacctcagttg
22. gtcacaggttcgcacatacc
23. cgatgtgagggtcagcaat
24. tgcaccatttggcctatgag
25. gtcgagtagtggttgg
26. gctagaaccatttcgttctc
27. catagccttagcgttagtc
28. tcactcgatccagccatttg
29. atccatggcctctgctgt
30. ttgcctgctgctgactagca
31. atgtttctcatgcctgtcagc
32. actggaagctaggtgacttcc
33. gaagatcatcttcttagacca
34. tgatagcctgcacaaatcc
35. catctgcacccttcaccggt
36. gaggagtagtatgaaatcg
37. tcaatgatatttgctgtcacat
38. ccacataatactgcaaga
39. tgaaaaaagacacacaaaga
40. ttaagcgagctaataatgca
41. aggccctttcttaaaaccgt
42. actctgacacctcctccgta
43. ttctgatatttcctctcat
M1 probes- Thirty one 20nt DNA probes labeled with Quasar 670 (BIOSEARCH TECHNOLOGIES) were designed to hybridize with the Influenza WSN M segment mRNA intron region. Here are the specific sequences:

1. cgggacgcatagagaggaacgt
2. cgatctcggctttgaggggg
3. aagacatcttcaagtctctg
4. aagatcgtggttcttccctg
5. tttagccattccatgagaac
6. aggtgacagggattgtcttg
7. atctaaatcccccttagtct
8. ggcacgggtgagcgtgaacac
9. tgcgctcgagttcccccgctca
10. agagcattttgacaaaacgc
11. atttgatctccgttcccat
12. gttaactgctttgttcatg
13. tccctcttaaagcttcctata
14. tttgcccccatggaatgtta
15. cagaataactgagttcattt
16. atacaactgcaagaatgcacc
17. catcctgtttatatgaggc
18. ccactcagtggtcagcc
19. gttgcgcataccaggccaa
20. ggagtcagcaatctgttcac
21. ttgcctatatgacagctgc
22. agtgatgttggttgctcac
23. cattctgtctctgtctgta
24. tagcgttagtgctgtgctaga
25. ccagccattgctccatagc
26. cttgcgttgtgtctcactgc
27. cttgacagcaatatccatg
28. atcgcctgcaccattttgc
29. aggalagtcccaatatgttctc
30. ctttagaccagcactggag
31. tgcaaaatattcaagaagatc
M2 probe- Single 20nt DNA probe labeled with *2 Quasar 570 fluorophores designed to hybridize with the Influenza WSN M segment mRNA M2 exon junction region. Here is the sequence:

1. tctgataggcgtttcgacct

mRNA3 probe- Single 20nt DNA probe labeled with *2 Quasar 570 fluorophores designed to hybridize with the Influenza WSN M segment mRNA3 exon junction region. Here is the sequence:

1. tctgataggcctgcttttgc

NS probes- Forty 20nt DNA probes labeled with Quasar 570 (BIOSEARCH TECHNOLOGIES) were designed to hybridize with the Influenza WSN full length NS segment mRNA. Here are the sequences:

1. tttgtcaccctgcttttgct
2. cagttttgtagtcattag
3. caatctacctgaagcttgta
4. tttgcgacagatgcacagaa
5. ctagttcttgcctgtcgaact
6. cgatcaaggagaatgggaatc
7. ggaccttctgatctggcgcga
8. agagtgcgtccttcttctct
9. ggctgtttcgatgtccagac
10. ctatttgccttcagcaggg
11. tcttccctcagaatccgctc
12. cattttaagtgcctcatcgg
13. atgcaggtacagaggccatg
14. gtcattcgtagttagtggaacg
15. gtcaccgtgacatttcctca
16. gctgaccgatgcatgagcagc
17. ctaagagggctgctcactt
18. gatcgcttgacatttcctgta
19. tcagttatggcattcttctc
20. aaaaactcatgaagtggctc
21. tattagttcctcagccggt
22. cttcggtagagccttctag
23. atttcgcacacatggtcctc
U5 snRNA probes- Five 20nt DNA probes labeled with Quasar 570 (BIOSEARCH TECHNOLOGIES) were designed to hybridize with the human U5 snRNA. Here are the sequences:

1) ctgaagagaaaccagagtat
2) aaaaagcgaagagattatgcg
3) ctctccacggaaatctttag
4) gggttaagactcagagttgt
5) gccaaagcaaggcctcaaaa

M vRNA probes- Forty four 20nt DNA probes labeled with Quasar 670 (BIOSEARCH TECHNOLOGIES) were designed to hybridize with the Influenza WSN M segment vRNA. Here are the sequences:

1. agagctggagtaaaaaacta
2. acgatggtcattttgtaac
3. cagcacaagtctggagtatgt
4. gagggagaataatcgaaagg
5. taccgaaggagtgccagagt
6. acgtttgaaagagggcct
7. tgcttatactgctgtttaa
8. tcttgaagcttttttttca
9. tcgcacttattatatgttg
10. atggcagcaaatatcattga
11. cgatctcaagtctctctcg
12. ggtcttaaagatgatcttct
13. gactcatctagctccagtg
14. tgcagggcgatgagaaccatt
15. gctagtcaggccaggggaaat
16. gcagcagcagggccatgggata
17. aatggcttgatcaggtgag
18. actacagcttaaggctatgga
19. gaacagaaatgtgctagcca
20. ccaacactaatacatgacat
21. catagggaaatgtgacacat
22. tgctgactccacgatcgtt
23. tatgcgcaccttgtgaaacag
24. actgatatgtcagttgtgct
25. caaacagaggtgggtgtgta
26. ccagttgtatgggctctata
27. aagttatctgtctgtgtgact
28. tggggccaaagaaatagcac
29. ttaagagggagataaacttc
30. gcagttaaactgtttaggaa
31. agatccaaataacatgggaca
32. aaaatgtctttaatgggaaac
33. ctgcagcagcagcgtctttt
34. caccgtgcccagtgagcggg
35. ttttaggattgtgtaacgt
36. ctgctacctctagcaaggg
37. atggctaaagacaagcacaa
38. ccagatcttcaggttctcatg
39. gatgcttttgagggaagaa
40. cgagatcgcagcagagcattt
41. tccgctcagccccccccaa
42. cgaaacgtaccttcctctctct
43. agatgagtcttttaacccgag
44. gcaaaagcaggtgatatgtg
**GAPDH probes** - Forty eight 20nt DNA probes labeled with Quasar 570 (BIOSEARCH TECHNOLOGIES) were designed to hybridize with the human GAPDH mRNA. Here are the sequences:

1. atttatagaaacgggggccc
2. agcagagagcagagcggag
3. cggctgactgtcagacagga
4. gctggcgacgcacaaagaaga
5. catgggtctgtgacgatgtg
6. ttgacctccagctctcctccct
7. gcgcacaataacgacacaaac
8. ttaaaagcagcctctgtgtgac
9. gcaacaataatccacctttacc
10. gtcaatgaggggtcattga
11. acatgtaaaccatgtagttg
12. ccatgggtgaatcatatattg
13. ctggcggtccatgggaatt
14. cattgatgacaagcttcctcccg
15. ctggaagatgtgtgtgatgggt
16. ctgatttttgagggtatctc
17. cagtggactccacagcgtac
18. ttctccatggtgtggtaagac
19. agagatgatgacccctttgg
20. acatggggccatcagcagag
21. tcatggctcacaccccatgac
22. ctggaggtttgtgtcatact
23. tgcgaggacatgtgctgtagtg
24. cagggtgtaagcagttgg
25. agttgtcatggatgaccccttg
26. atgagtctctccacagcatcc
27. agtgatggcatggactgtgg
28. catcccagctttctgggttg
29. tagagccagggatcatgcttc
30. tcagctcagggatcagcttg
31. tggcagtggtggacagcggag
32. gtcaggtccaccacgtgacac
33. ttgcaggggtttctaggtcag
34. cacctttctgttatgcacat
35. cacctggtgtcagtggtac
36. cgtggatgagtcgagcaggag
37. cgtcaaaaggctggaggtg
38. aaagtggtcgttgagggcaa
39. gtcataccagaaatgagct
40. tgtgtgtagccaaattcg
41. atgtggcccatgaggtccac
42. aggggtcttactcttgag
43. cagtgaggctctctcttc
44. actgagtgtgcaaggagacct
45. aactgtgaggagggagatt
46. ctctcaagggtcttacatg
47. atggtacatgacaaggtgcg
48. ttaactggttgagcacaaggg

Primers used in RT-qPCR:

M1:
Forward: ATCAGACATGAGAACAGAATGG,
Reverse: TGCCTGGCCTGACTAGCAATATC

M2:
Forward: CGAGGTCGAAACGCCTATCAGAAAC
Reverse: CCAATGATA TTTGCTGCAATGACGAG

GAPDH:
Forward: CGACCGGAGTCAACGGATTTGGTCG
Reverse: GGCAACAATATCCACTTTACCAGA

NS1-BP:
Forward: CGCTGGTAATCAACTGGGTGCAGCG
Reverse: ACCTCTTCCATCAGCTCTTCCA

hnRNP K:
Forward: AAGATATGGAAGAGGAAACAAGCA
Reverse: CATTGTAGTCTGTACGGAGAGC
SON:
Forward: TCGTGGTCAGTAAATTCCGG
Reverse: TTCATTTTGATAGACTCTGGC

Aly/REF:
Forward: GATCTTTTGCAGTGCTTC
Reverse: CTTCAGCGCTTCATTCCAGC

Aly/REF amplification in VERO cells:
Forward: GATCTTTTGCAGTGCTTC
Reverse: CTTCAGCGCTTCATTCCAGC

UAP56:
Forward: AGGCTGGAGTAGAGGGAAG
Reverse: AGGAACAGCAAAGGAAAACAAAG

UAP56 amplification in VERO cells:
Forward: AGGCTGGAGTAGAGGGAAGC
Reverse: AGGAACAGCAAAGGAAAATACAG

Primers used in RT-PCR and products loaded on agarose gels:

M1-M2 amplification
Forward: ATGAGTCTCTAACCAGGTCG
Reverse: TGGCAGCTCTCCGTAGAAGG

mRNA3 amplification
Forward: AGCAAAAGCAGGCTATCAAG
Reverse: TAGGTTTACTCCAGCTC
References: