

## *Supplementary Material*

### **Alphavirus nsP3 co-localization with BIN1 in cytoplasmic granules**

While all alphavirus nsP3s (except SAV) have at least one proline-rich motif (**Figure 2**), it was experimentally confirmed that CHIKV and SINV can bind BIN1 whereas EEEV and VEEV cannot (Frolov et al., 2017). To investigate co-localization of nsP3 of additional alphaviruses with BIN1, the nsP3 proteins of twenty alphaviruses fused to eGFP were transiently expressed in mammalian cells. The cells were fixed and stained for BIN1 by IFA (**Figure S1**). NsP3-BIN1 granular co-localization was quantified and represented as percentage cells with observed co-localization. All twenty alphavirus nsP3s were grouped based on the number of proline-rich motifs and high-affinity proline-rich motifs are indicated with an asterisk (**Figure S2**).

Many alphavirus nsP3s localized with BIN1 in cytoplasmic granules to a certain degree. A high percentage of nsP3-BIN1 co-localization (>50%) was observed for BFV, BEBV, MAYV, GETV and RRV<sup>QML</sup> (**Figure S2**). Moderate nsP3-BIN1 co-localization (20-50%) was observed for SESV-, CHIKV-, ONNV-, SINV-, and RRV<sup>T48</sup>. The nsP3s of SAV, VEEV, EILV, EEEV, SFV and WHAV showed very low to no co-localization with BIN1. TAFV nsP3 showed nuclear localization and MIDV and UNAV nsP3 showed diffuse cytoplasmic localization, therefore nsP3-BIN1 co-localization in cytoplasmic granules could not be determined for these viruses (**Figure S1**). A clear division was observed regarding the ability of nsP3s to co-localize with BIN1. All alphavirus nsP3s that also contained an FGDF-like motif co-localized with BIN1 to a certain extent. In contrast, alphavirus nsP3s with an Agenet-like domain binding motif did not co-localize with BIN1 at all, despite the presence of a proline-rich motif.

The HVD of nsP3 contains several highly conserved motifs of which PxPxPR is often present multiple times. A previous study reported that all alphavirus nsP3s contain this motif accept those of African origin (Aaskov et al., 2011). However, all alphaviruses in our study including those of African origin (except SAV) contain at least one copy of the PxPxPR motif (**Figure 2**). Co-immunoprecipitation of BIN1 with SINV and CHIKV nsP3 indicated direct binding between these molecules (Frolov et al., 2017). Here we showed that most alphavirus nsP3s indeed did co-localize with BIN1 when expressed in Vero cells. The nsP3s of BEBV, BFV, CHIKV, EILV, GETV, MAYV, ONNV, RRV<sup>QML</sup>, RRV<sup>T48</sup>, SESV, SINV, SFV, and WHAV all showed co-localization with BIN1.

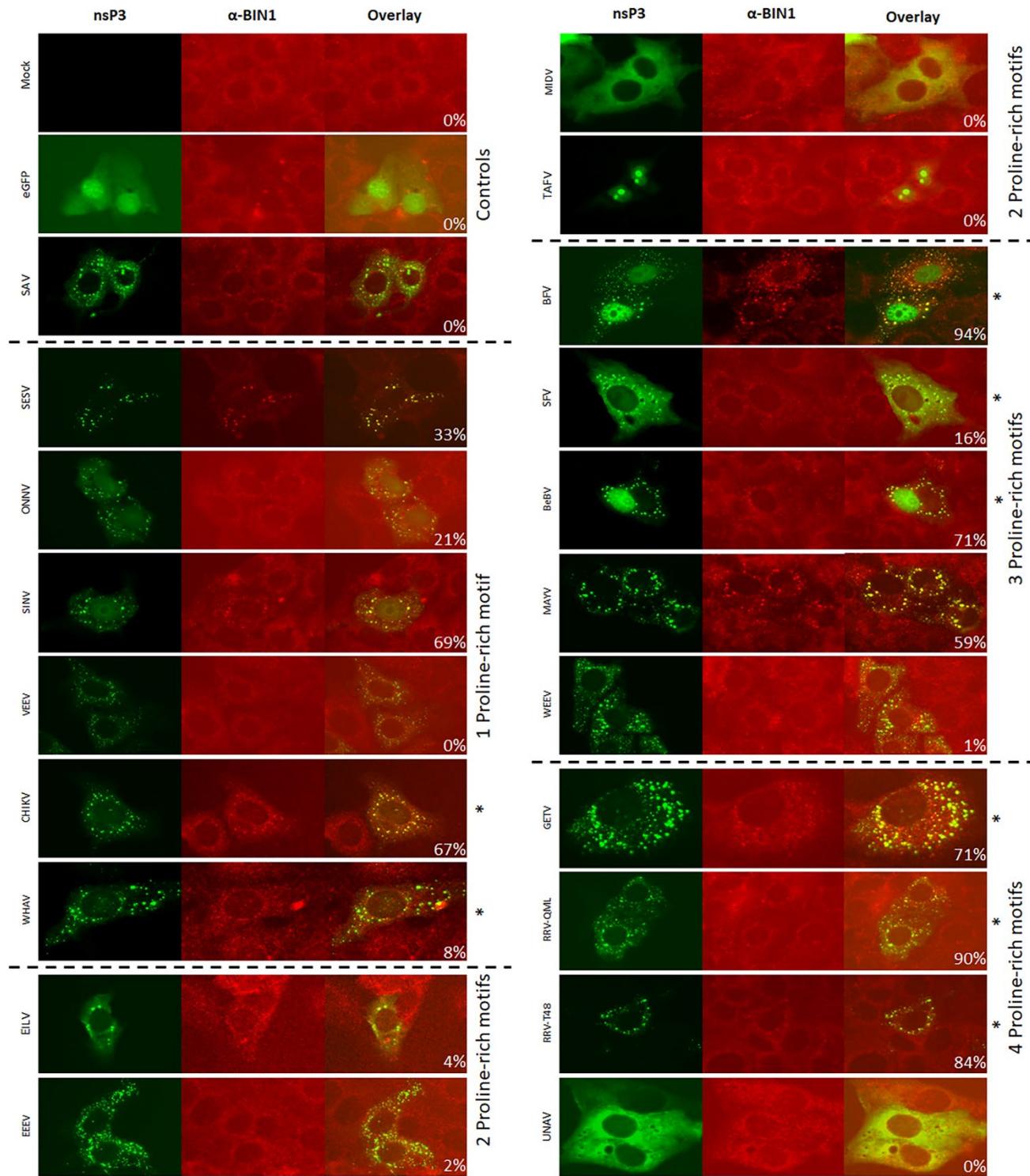
Although the proline-rich motif in alphaviruses nsP3s is often referred to as the PxPxPR motif, multiple alternative BIN1 binding motifs exist, some of which are present in the HVD of nsP3. The motif P[I/V][P/A]PPR[R/K/P][R/K][R/K] was identified having the highest affinity for BIN1 (Gotte et al., 2018). This is comparable with the results from this study; BEBV-, BFV-, CHIKV-, GETV-, and RRV-nsP3 all carry the high affinity proline-rich motif and showed a relatively high co-localization with BIN1 compared to moderate co-localization between BIN1 and nsP3 of viruses carrying other PxPxPR motifs (**Figure S2**, co-localization in ~40-70 % vs ~10-40 % of cells). Two exceptions were observed as the nsP3s of WHAV and SFV both carry the high-affinity motif, but co-localization with BIN1 was relatively poor (**Figure S2**, in ~10-20 % of cells). However, in immunoprecipitation experiments SFV nsP3 does interact directly with BIN1 and this interaction is also required for effective SFV RNA

replication (Neuvonen et al., 2011). Many of the cellular functions attributed to BIN1 stem from its ability to remodel cellular membranes (Habermann, 2004; Wu et al., 2014). Alphaviruses replicate in cytoplasmic viral factories or spherules, which are constructed from membrane invaginations (Froshauer et al., 1988). It has been hypothesized that BIN1 and related proteins help form or stabilize the membrane structures required for alphavirus RNA replication (Neuvonen et al., 2011).

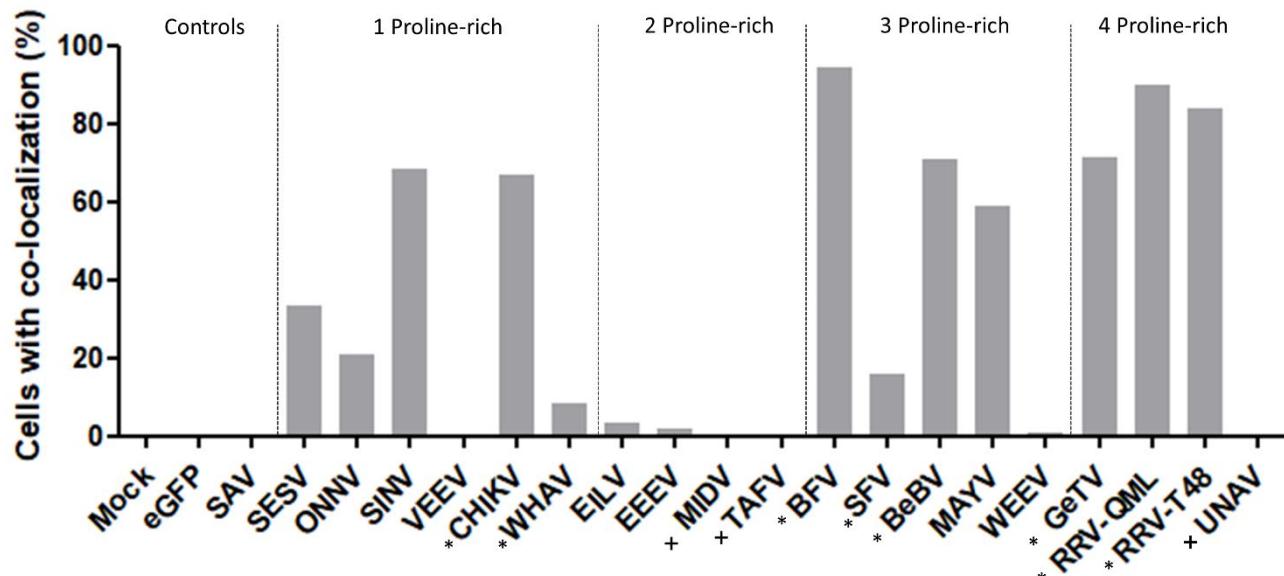
Interestingly, the HVD of nsP3s of EEEV, VEEV and WEEV did not co-localize with BIN1 despite that all these viruses contained proline-rich BIN1 binding motifs (**Figure 2, S1 and S2**). Similarly, immunoprecipitation of EEEV-nsP3 and VEEV-nsP3 did not co-precipitate BIN1 whereas the nsP3s of CHIKV and SINV did (Frolov et al., 2017). Why the interaction between BIN1 and V/W/EEV-nsP3 is absent despite the presence of BIN1 binding sites remains elusive. Perhaps the proposed interaction between BIN1 and the nsP3s of V/W/EEEV is short lived and only exists during a specific stage of virus replication.

## References

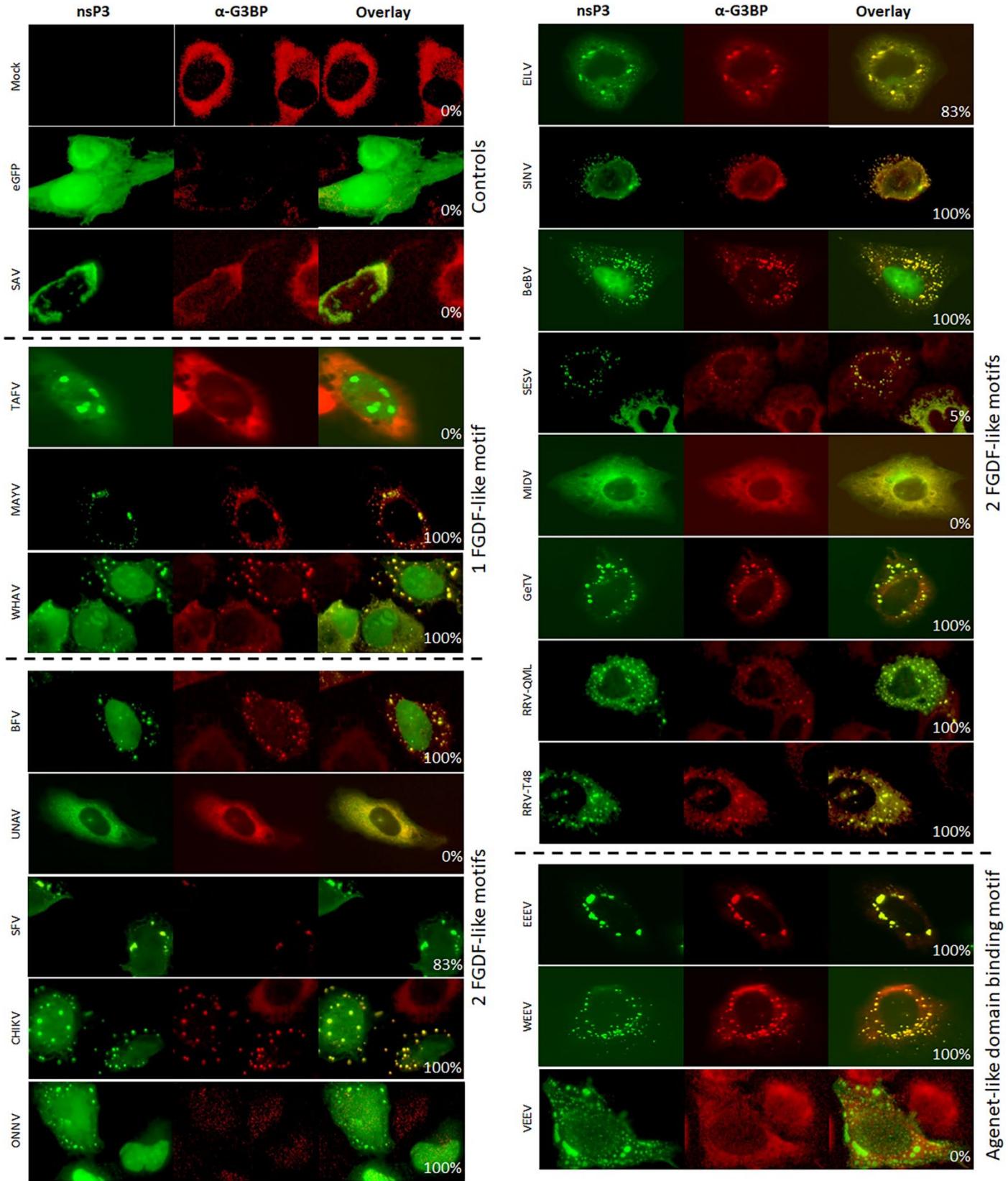
- Aaskov, J., Jones, A., Choi, W., Lowry, K., and Stewart, E. (2011). Lineage replacement accompanying duplication and rapid fixation of an RNA element in the nsP3 gene in a species of alphavirus. *Virology* 410(2), 353-359. doi: 10.1016/j.virol.2010.11.025.
- Frolov, I., Kim, D.Y., Akhrymuk, M., Mobley, J.A., and Frolova, E.I. (2017). Hypervariable Domain of Eastern Equine Encephalitis Virus nsP3 Redundantly Utilizes Multiple Cellular Proteins for Replication Complex Assembly. *Journal of Virology* 91(14), 1-22. doi: 10.1128/jvi.00371-17.
- Froshauer, S., Kartenbeck, J., and Helenius, A. (1988). Alphavirus RNA replicase is located on the cytoplasmic surface of endosomes and lysosomes. *J Cell Biol* 107(6 Pt 1), 2075-2086. doi: 10.1083/jcb.107.6.2075.
- Gotte, B., Liu, L., and McInerney, G.M. (2018). The Enigmatic Alphavirus Non-Structural Protein 3 (nsP3) Revealing Its Secrets at Last. *Viruses* 10(3). doi: 10.3390/v10030105.
- Habermann, B. (2004). The BAR-domain family of proteins: a case of bending and binding? *EMBO Rep* 5(3), 250-255. doi: 10.1038/sj.embo.7400105.
- Neuvonen, M., Kazlauskas, A., Martikainen, M., Hinkkanen, A., Ahola, T., and Saksela, K. (2011). SH3 domain-mediated recruitment of host cell amphiphysins by alphavirus nsP3 promotes viral RNA replication. *PLoS Pathog* 7(11), e1002383. doi: 10.1371/journal.ppat.1002383.
- Wu, T., Shi, Z., and Baumgart, T. (2014). Mutations in BIN1 associated with centronuclear myopathy disrupt membrane remodeling by affecting protein density and oligomerization. *PLoS One* 9(4), e93060. doi: 10.1371/journal.pone.0093060.



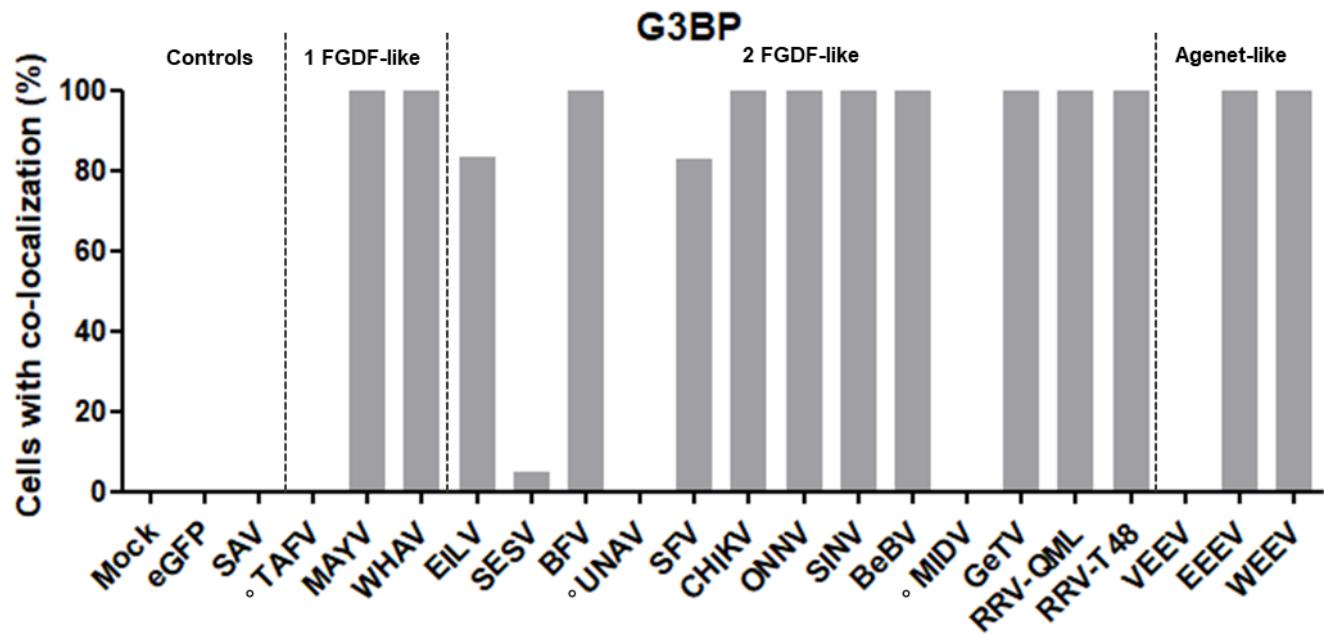
**Figure S1. Alphavirus nsP3 co-localization with BIN1.** Vero cells were transfected with alphavirus nsP3-eGFP, stained with antibodies for BIN1 and visualized by immunofluorescence. Green indicates localization of nsP3, red represents BIN1. NsP3-BIN1 co-localization is visualized in yellow in the overlay. The percentages in the overlay images represent co-localization percentage of all counted cells. An asterisk indicates the presence of the high affinity proline-rich motif; P[I/V][P/A]PPR[R/K/P][R/K][R/K].



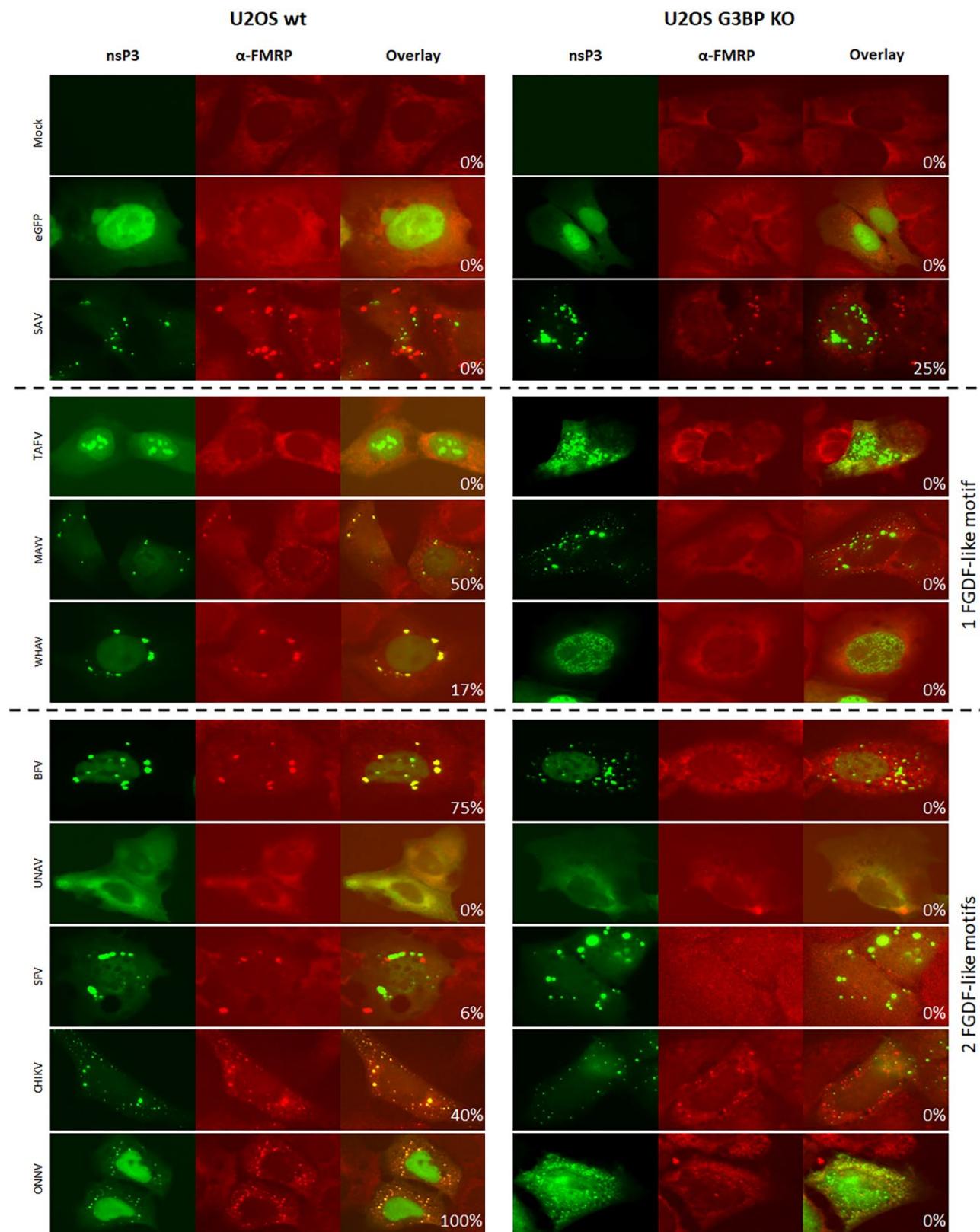
**Figure S2. NsP3-BIN1 co-localization percentages.** Vero cells with co-localization of nsP3 and BIN1 are represented as co-localization percentages. Bars represent the mean co-localization pattern of 30-50 transfected Vero cells. A distinction is made for alphaviruses with one, two, three or four proline rich motifs (PxPxPR). An asterisk indicates the presence of the high affinity proline-rich motif P[I/V][P/A]PPR[R/K/P][R/K][R/K]. In absence of cytoplasmic granules, no co-localization could be determined, these nsP3-eGFPs are indicated with an open circle.

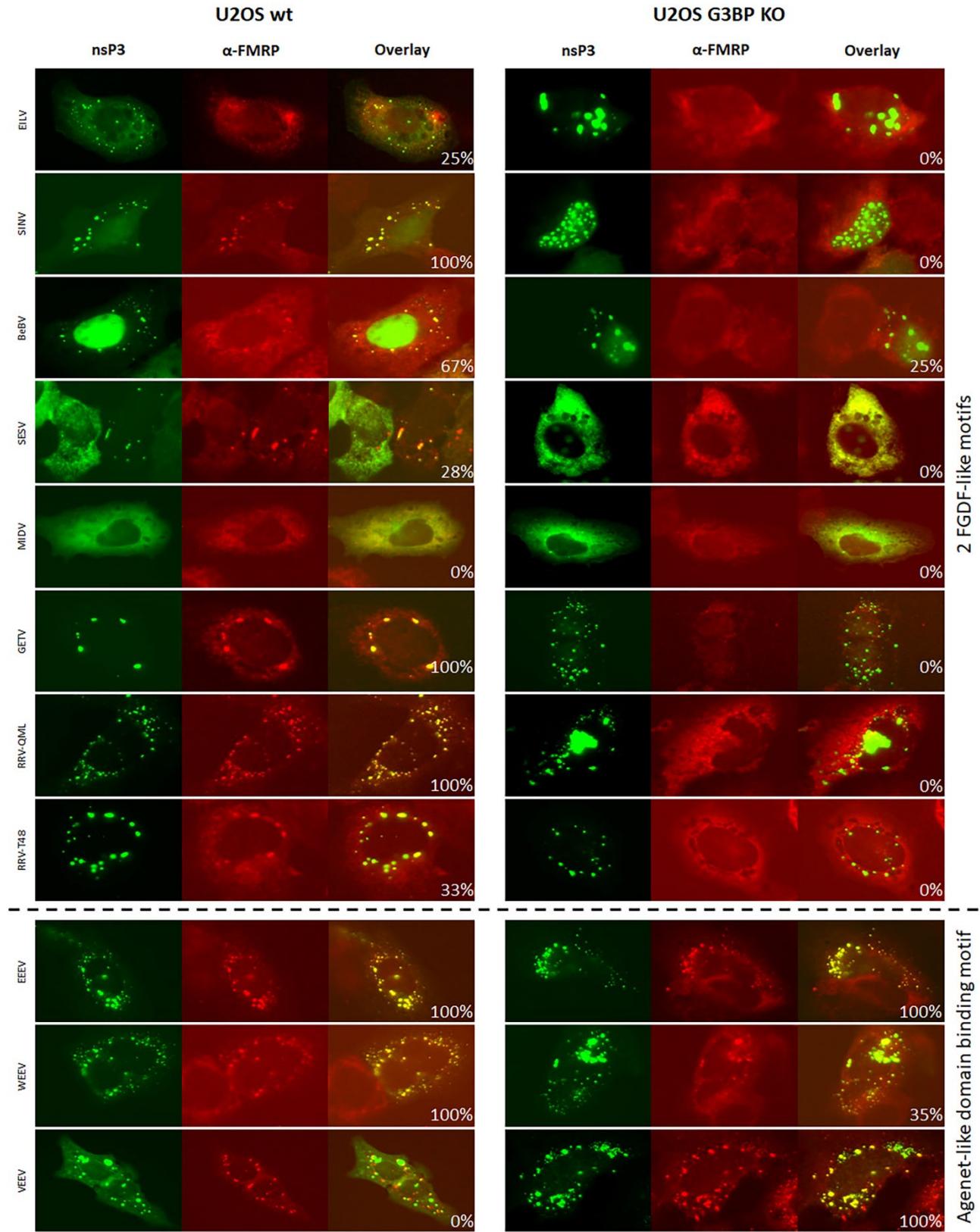


**Supp Figure S3. Alphavirus nsP3 co-localization with G3BP in U2OS cells.** U2OS cells were transfected with alphavirus nsP3-eGFP, stained with antibodies for G3BP and visualized by immunofluorescence. Green indicates localization of nsP3, red represents G3BP. NsP3-G3BP co-localization is visualized in yellow in the overlay. The percentages in the overlay images represent co-localization percentage of all counted cells. A distinction is made for alphaviruses with one or two FGDF-like motifs or the presence of one or two Agenet-like domain binding motifs.

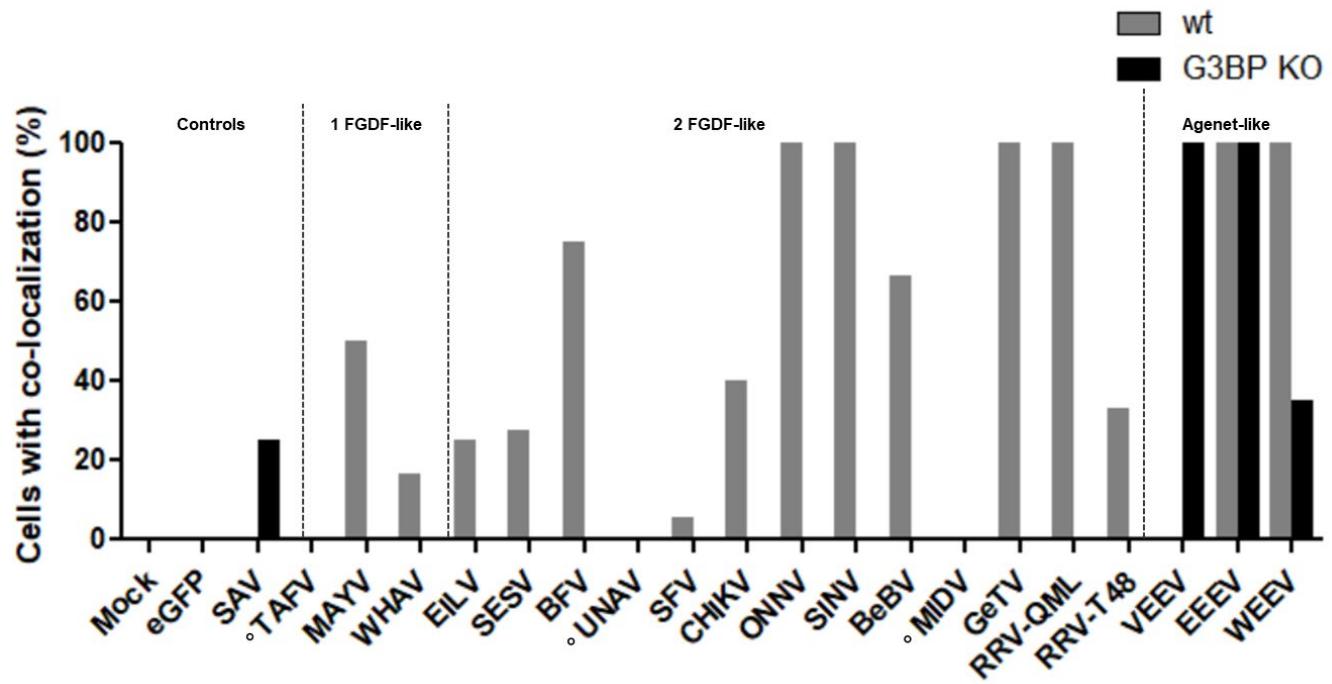


**Supp. Figure S4. NsP3-G3BP co-localization percentages in U2OS cells.** Cells with co-localization of nsP3 and G3BP are represented as co-localization percentages. Bars represent the mean co-localization pattern of 30-50 transfected U2OS cells. A distinction is made for alphaviruses with one or two FGDF-like motifs or the presence of one or two Agenet-like domain binding motifs. In absence of cytoplasmic granules, no co-localization could be determined, these nsP3-eGFPs are indicated with an °.





**Supp. Figure S5. Alphavirus nsP3 co-localization with FMRP1 in U2OS wt and U2OS G3BP KO cells.** U2OS wt and U2OS G3BP KO cells were transfected with alphavirus nsP3-eGFP, stained with antibodies for FMRP1 and visualized by immunofluorescence. Green indicates localization of nsP3, red represents FMRP1. NsP3-FMRP1 co-localization is visualized in yellow in the overlay. The percentages in the overlay images represent co-localization percentage of all counted cells. A distinction is made for alphaviruses with one or two FGDF-like motifs or the presence of one or two Agenet-like domain binding motifs.



**Supp. Figure S6. NsP3-FMRP1 co-localization percentages in U2OS wt and U2OS G3BP KO cells.** Cells with co-localization of nsP3 and FMRP1 are represented as co-localization percentages. Bars represent the mean co-localization pattern of 20-80 transfected U2OS wt and HeLa G3BP KO cells. A distinction is made for alphaviruses with one or two FGDF-like motifs or the presence of one or two Agenet-like domain binding motifs. In absence of cytoplasmic granules, no co-localization could be determined, these nsP3-eGFPs are indicated with an °.

**NsP3 gene sequences of the 20 alphaviruses used in this study.**

&gt; SAV

```

atggcaccggggttacagagtccctcaacaggaacatcatcactgcgcgaagaggaagtcttggtaacgccgtaacagtaac
ggcagaccggcgatggtgtgtggcgctcacggcggttcgggacgcattcccaacggtgcatcggcgccggaa
aacgcggcttggccaggactcgaggccaccatcatacgcagccggagctgacttcagagaagtgcacgaagaaacc
ggcgccgcacagctaagagcagcataccgtgcggccgtacccatgtactgcacccgtatcaccagtgcgcacatcccc
ttgctgagttacacacatcttccaacggcgaaacacagactggAACAGTGCCTCGGCATGGTGGAGGCGTCACACG
acagaatgcgacgtcaccatctattgtggccaacaacatggcgtaaggattcagcaactgtacgtacgcacgtacgcccgc
gaagagttcgacgaggagggtggtagaaagaggaagcatgaagctgtacgtacgtatacgagacgcgtgtcc
agttcgggtgacgaaacgggtgtgggtccaaacatagcactctagctggaaaggccaggatatagtgccttacggcgac
cgccagatccctttgtcgacgaaacgggtgtgggtccaaacatccatgtcgatgtcgtaattgaagcggcttggctaagacc
aaagaagccaaacgccaactcatcgactacgaggcaacacacctcgacgtcctaaagagacgtgtccggtaacgac
ataccgttaggcacgcctctagcctgcgcgtgtatttatgcctgcgcgttatgcgcattcaattgcgcactaaca
catccatgtcgacgtacccgtgcgtatctgcacccgtgcgtatccgcacgcgtgtccgggttcg
caaaaggccgcaggaagggtttgtgttatgcgcattcaattgcgcactaaca
catccatgtcgacgtacccgtgcgtatctgcacccgtgcgtatccgcacgcgtgtccgggttcg
tgcacgggtgagagcacctgcagaagaacctagaccgtgcgtatctgcacccgtgcgtatccgcacgcgtgtccgggttcg
caaccttaggcggcaactgtggctggcagactgcgcgtgcgcgtatccgcacgcgtgtccgggttcg
acacccgcgcgggtcggtgcgggtcgctgcgcgtatccgcacgcgtgtccgggttcg
cagtcacgcgtcacctcatcagcgggatccatactgcgcgtgcgcgtatccgcacgcgtgtccgggttcg
ggcagcgtccacggcatagtgta
cgcagcgcgcgtccatgcgcgtgcgcgtatccgcacgcgtgtccgggttcg
cagtcacgcgttcagccggcatagaactgcgtgcgcgcgtatccgcacgcgtgtccgggttcg
cagttcggcgcgcgtgagagactaggccgcaggacttagagactggagggatcagagcatggcagtca
gactagcttcgc
tccggctcgctgatggtagggagacaccgcgtatggctatagccaatgttctgcacatcaggacacgggctccgaaccatca
agccgcggcgccctctgtgaggacgcggcgacagaggcaacgagacggccctggagggcattga

```

&gt; SESV

```

atggccccgaactatcgtgttcagagccgacatacgtaaaacgacaactgaagcagtggtaacgcccaacccctg
ggagtgcggggcgccagggtgtgtggctatcgctagacagtggctaaaggattccggcaggtaaaatgcaggttggt
gagtgtaaagcggttagtaacagatgatcaccatactgcatactgtggccctgacttcgtaagactggggaaaaggag
ggcgcacgcgtctggcgatggcctaccagaattgcgcgtcagaggcgtccctccttagggtccacacccatgtatccca
ctattatccaccggcatatattcagctggtagatagattagatcagtcgtacccatgtggctatccgcacgcgtgtcc
accaacatagaggttcatatattgttagagataaaacatggagatctcgcatttgagagtgatttagtacgcgggacact
gtggaggaattggtagagggaaaatgtggactgtcagatgaaatcgtaagagtcgcgtatccgcgtatccgcacgcgtgtcc
gaagggtatagctccaccactggatcaatctactcatttcgcgttaccaaggtttcatcagacagctgtggacgtggcg
gagatcaaaggccctgtggccaaaggaaccagaggccaaatgagcagatgtaaattacattctggggatagcatgaaccag
atacgagacaatgcgggtggagatataggcactacatccctccatgtacggtaccatgtttatgtccttatgcgcatt
actgcagaaagagtgtacagactgcgttgcgccttaagagacttcgttgcgttgcgcgttgcgcgttgcgcgttgcgcgtt
cgtattactggagtgcacgggtgcgcgttgcgcgttgcgcgttgcgcgttgcgcgttgcgcgttgcgcgttgcgcgtt
gaggaggttcgctgagacctgcgcgttagcttcgcgttgcgcgttgcgcgttgcgcgttgcgcgttgcgcgttgcgcgtt
ttgatagacagtgcgtggattccattgcactccgtcaagttgcgcgttgcgcgttgcgcgttgcgcgttgcgcgttgcgcgtt
cctccaccgcgcgtatgatcacccctgcgggtgcgcgttgcgcgttgcgcgttgcgcgttgcgcgttgcgcgttgcgcgtt
caagcgctgtcgcaatttctttggtagttgaaccaggagagcccgatagactgcgtccgaaagcaggcatctaga
gaggccccgtctattacattggagatttcaggaaaatgaggtggacagtgatgttagcgcgcgcgcgattactaagtca
caacgtaggcgttaggagacgcgaaggcgcaagatccgcgtctaggcagcgttgcgcgttgcgcgttgcgcgttgcgcgtt

```

&gt; SFV

atggcaccatcctacagagttaagagagcagacatagccacgtgcacagaagccgctgtggtaacgcagctaaccggcgt  
ggaactgtagggatggcgatgcaggccgtggcgaagaaatggccgtacgcctaaggggagcagcaacaccagtggc  
acaattaaaacagtcatgtcggtcgtacccgtatccacgcttaggcctaatttcgtccacgactgaagccgaa  
ggggaccgcgaattggcgctgttacccggcagtgccgcgaagtaaacagactgtcactgagcagcgttagccatccg  
ctgctgtccacaggagtgttacggcggcgaagagataggctgcagcaatccctaaccatctattcacagcaatggacgc  
acggacgctgacgtgaccatctactgcagagacaaaagttggagaagaaaatccaggaagccattgacatgaggacggc  
gtggagttgctcaatgatgacgtggagctgaccacagacttggtgagactgcacccggacagcgcctgggtggcgttaag  
ggctacagtaccactgacgggtcgctgtactctgtactttgaaggtacgaaattcaaccaggctgtattgatatggcagag  
atactgacgttgtggccagactgcaagaggaaacgaacagatatgcctatacgctggcgaaaacaatggacaaacatc  
agatccaaatgtccgtgaacgattccgattcatcaacacccctcaggacagtgccctgcgtgccgctacgcaatgaca  
gcagaacggatgccccgccttaggtcacaccaagttaaaagcatgggtttgctcatctttcccccggaaataccat  
gtagatggggtgcaaggttaagtgcgagaaggttctcctgttgcacccgacggtacccctactgttagtcccgaaag  
tatgccgcacactacgacggaccactcagatcggtcgttacgagggttgacttggactggaccaccgactcgtttccact  
gccagcgataccatgtcgctaccctcgacttgcgtgtgacatcgactcgatctacgagccaatggctccatagtagtg  
acggctgacgtacaccctgaaccccgaggcatcgccgacactggcggcagatgtgcacccctgaaccccgacaccatgtggac  
ctcgagaacccgattcctccaccgcgcccgaagagagactgcataccctgcctccgcggcggagcggaccggccggc  
ccgagaaagccgacgcctgccccaggactgcgtttaggaacaagctgccttgcgttgcacttgcgacgacacgag  
gtcgatgcgttggcctcggattacttcggagacttcgacgcgttgcactaggccgcgggtgcata

> SINV

gcctcataccgcacaaaagggagaatattgtgactgtcaagagggaaagcagttgtcaacgcagccaatccgctggtaga  
ccaggcgaaggagtctggcgtgccatctataaacgttggccgaccgtttaccgattcagccacggagacaggcaccgc  
agaatgactgtgtgccttagaaagaaagtgtatccacgcggcgtggccctgatttccgaaagcaccaggaaagcagaacgc  
aaattgtctacaaaacgcctaccatgcgtggcagacttagtaatgaacataacatcaagtctgtgcgcattccactgcta  
tctacaggcattacgcagccggaaaagaccgccttgaagtatcacttaactgcttgcacaaccgcgttagacagaactgac  
gcggacgtaaccatctattgcctggataagaagtggaaagaatcgacgcggcactccaacttaaggagtgtgtaaca  
gagctgaaggatgaagatatggagatcgacgtgatgttagtatggattcatccagacagttgtctgaagggaaagaaaggga  
ttcagtactacaaaaggaaaattgtattcgacttcgaaggcaccatccatcaagcagcaaaagacatggcggagata  
aaggcctgttccctaattgaccaggaaagtaatgaacaactgtgtgcctacatattgggtgagaccatggaagcaatccgc  
aaaaagtgcggcgtgaccataaccgtcgtagccgcggaaaacgttgcgtgccttgcattgtatgcctgacgc  
gaaagggtccacagacttagaagaacgtcaaagaagttagtgcctgtttaatccgcacactccgcattcggtccggcgt  
aagaatgttcagaaggttcagtgacgaaagttagtgcctgtttaatccgcacactccgcattcggtccggcgt  
atagaagtgcagaacacgcctaccgcctcgcacaggccggaggaggccccgaagttgttagcgcacaccgtcaccatct  
acagctgataaacacgccttgatgtcacaaggacatctcactggatattggatgcacagttgtctgc  
tttagcggatcgacacttattacttagttagtgcacggatgtgtcgtaggcatttcactagagatgtgacacc  
cagggtgtggcgtacggtcatgcgtccaaagagcctgcccattccaccgcacaggctaaagaagatggccgc  
gcagcggcaagaaaagagccactccaccggcaagcaatagctgtgatgtccctccaccccttgc  
tccctcgatcaatttcgacggagagacggccgcaggcagcgtacaaccctggcaacaggccccacggatgtgc  
atgtcttcggatcggttccacggagatgtgatgtgacgtgcggcagactgtgatgtccgaaccctgc  
tcatttgcacccggcgaagtgaactcaattatgcgtccgatcagccgtatcttcctacgc  
cgcaggagcaggaggactgaatat

> BeBV

atggcaccctcgtacagggttaagcgtgcagacatcgctactagcgaggaagaggcagttgtaaacgcggccaaacgc  
ggtagaccggagacggagtgtgcagagccatccaccgaaagtggcctgaggccttcgtggcgtgccacggcggaccgc  
acggcaaaaaccataaaggcgacacatcatccatgcgtccgcggaccgaactttcgtccacccaaagaacaggag  
ggtgataagttgtcgccggagcgtaccgagcggtagccaaagaaagtatccatgcgtccgcggaccgaactttc  
ctgctgtctacggcattatggcggtgtttaagaccgaatgttaccagtcgttgcatttttaccgcgttggatgc  
accgacgcagatgtgttatgcaggagacacgtggagacgaagatccaggaggcgtatgcgc  
gtggagctagtgtccgtatgaaatggactgcagacagacttagtccgcgtccatcccgatagc  
gactgttagtgggacggc  
ggttacagcactactgacggtaaatgttattcataccctagaggccaccaagttccatcaatgtgc  
gtatggcag  
attctgtattatggccgaacaccaggaggc  
gaatgac  
actata

## Supplementary Material

agatctagggtgcccaactgacaatgattcgttcaccaccggcgacggccatgtctgttagtgttatgaca  
gccgagagggtgaccagattacgcattgcaccataactaagtcggtcacagtcgttcttcatccctgcctaaatacaac  
gttggaaagggttcagagggtgaaatgcgagaagggttactgttgcgtccctcgatcacggcgttccatatcgtagacccatctgtc  
tacgtgttaacaccatacaggccgtatctcctcgatcacggcgttccatatcgtagacccatctgtc  
tcaatatatctgtcctccattctacaacagattcatgccgcgaaacacttccgtggacaacataactacggcattgcc  
gagattcaccctgtcccacccgaggtaacagacgctgcgtgcccggagcaagggtatgtccctgcggcacattaccgg  
gagcatcaagcaccctgtccccctcttagacccgaaagcgcgcagggcattggggctgccaggataaccaggatccggcg  
ccgagacattcaaaaggcacgtcccggtccggggcggaccatcccagaaccagttagggccgtgtccgagcagccgt  
gaactaccatggaaaatacagggttgcctggctgacttcggcattggcggaccctcgagcacaccagccgttgc  
ctggccatggggccggaggagtcgtcaggactatccatggcacttcggcgttgc

> BFV

atggcaccggcgtaacaggtaaaacgtggagacatttgcgaacgcccagaggatgcagtggtaatgcagcaaaccaacag  
ggagtgaagggtctggagggttgcgtcaattaccgttaagtggccggacgcatttcgggtatgcgtactccaaacccgg  
acaggcatttcgaaatccgtccaagataaaattgggtatccacgctgtcggcccaatttcgaaatgttcaagagg  
ggggacagagacactagcatctgtcttacagaggctgcagcagaaaatagtgtatggataaaaaattacaacagtgccgtcc  
ttactctccaccggcattatcgccggaggaaaaacagagatagaacactcgactcaaccatcttcacggcattcgacaat  
actgatgcagatgtgaccataattgcattggaaaaacatggggaaaaaggatgaggaggcaatcgatcaccggacttcg  
gttgagatgggtcaggatgacgtgcagggttgcgtggaggactgggtacgatcccttgcgttgcggatggatgg  
gggtacagtgccgacagccggcggactgtttccatctggaggatgacatccatcgactacggcgatcgccat  
atgcaagtgtgtggcccccctcaaaagactgtcaatgagcaaatagtggatcatccatcgactacccggat  
cggtggcaagtgtggcccccactgacgcccggacacaatgtggggccactgcgttgcgtatccatcgactac  
ccagagagatgttccatcgacttaaatgcacgaaacactacccatcgatcgccatccatcgactac  
attcaggggatgtcaggagataaaatgtgaaagatcatcgatcgactacccatcgactac  
atcgagacggatccatcgacttaaatgcacgaaacactacccatcgactac  
tcctcgattgggttctgcggcggagacacgagaccattccagccggggaggaccatccatcgactac  
agagcaccctgtcagaaccacaccgcctcaaaccaccgcgcacattcaccgtgcgtgcagaagtgcaccaagcacco  
cctacacctgtacccatcgactacccatcgactac  
ttcggagagcacggatgttgcggccttcggatccatcgactac  
ggagtggagttgaatga

> CHIKV

atggcaccgtgtaccgggtaaacgcattgcgcacatcgcaaaaggatgttagtcaacgcccaaccctcg  
ggctaccaggcgatggcgtctgttaaaggcgatataaaaaatggccggagtccctcaagaacacaggccgttgg  
accgcaaaagacactcatgtgcgttacatacccgtaatccatcgactcgatcgccatccatcgacttgc  
ggagacggggattggcggatcgactgttccatcgagaagtgcgtactcgactac  
ctctttccaccggcgtgtactctggaggggaaagacaggctgactcgactactaaaccacccatccatcg  
actgatgcagatgtgttatctactgcgcgacaaggagggtgggagaaggaaaatagctgaggccat  
gtgatccatcgacttcgatcgatcatccatcgactgcgtgcaccctgcacaggccatcgatccat  
gggataggcactacagaagggttacttgcgtacttccatcgatccatcgacttccatcg  
gtatacaccatgtggccaaaggcggaggctaatgcacgcggccatcgacttcgacttc  
aggcaaaaatgtcccaggatgcgcaggatgcacatcgatcgccgcggccatcgact  
cccgaaacgcgatcaccaggccatcgatgcgcggccatcgactgcacatcgact  
atagaaggaggatgcggaaaaggatcgatgcgttgcggccatcgact  
tataaaatcgccatcgatgcggccatcgactgcacgcggccatcgact  
ggtaggaaactggccgcctcgacttgcggccatcgactgcacaggccat  
acttgcctccatcgatgcggccatcgactgcggccatcgact  
agacgtggaaaacttgcgttgcggccatcgactgcacaggccat  
gcccgttgcgttgcggccatcgactgcacaggccat  
acagaaccgaatcgccatcgacttc



## Supplementary Material

ggggacagagagctagccgcgtatcgagctgtggctagcataatttagtaccaacaacataaaagagcgtcgactaccg  
ctgtgtccacaggcacccctccggcgtaaggacagactgacgcagtcttgaaccacttattcacggcactggacgca  
accgacgcagacgtggatatctactgcagagataaaaactggaaaagaagattcaggaagccatcgacaggcggacggca  
atcgagctgttatctgaagacgtgacccatggaaaccgatctggtagagtacacccggacagttgttagtcggcagaaat  
ggttacagtgcactgacggtaactgtactcttacccgtggggcacgaggttccaccagacggcggtcgacatggctgaa  
atatcaactttatggccaagactccaagatgctaaccgcagatctgcctataccgcctagggagacgtggatgcata  
cgcaactaaatgcccagttagaggacgcagattctgttacgccccggaaaacggtaccgtgttatgtcggtatgcgtatgacc  
gcggagcgggttgcgcagacttaggatgaataacacaaaaacatcatcgatgtgttatccctccattaccgaagttacagg  
atagaaggcgtgcagaaggtgaagtgtgaccgagtgctaattttgaccagaccgtcccgactagtaagtcccgagaaag  
tacatacagcagccgcggaaacagctggataatgtgagccctgacttctacgacgtcgacgggatccgcattgttatcc  
tcggaaacgcacccatgaaactgaaaggatctgttagccgaggtacataccgaacctccatccctccgcctgcgcacgtaga  
gcagccgtcgcccaactttagacaggatctggaaagtccaccggagatcgagccgtacgtggcacagcaagcagaggatcatg  
gtcttggagagggtcgacgacagacatacgatccgcctttagccgcacggcggccatcacaatgcccacttccaaacc  
cccagggttcgtaaggtcgctactgaaacctccattagaaccggaaagctcctatccggcaccaagaaagagaaccact  
agcaccccacccctccgcataaccccgaggatttcgttcccgaggatctgttagttaccgtggagccggaaagacccatagac  
atccaattcggtgacttggagccacgcgcggaaacaccaggaccgagatgtcagcacaggaatacagttcggtgacatc  
gactttaaccagtcc

> MAYV

atggctccagtgtatgcgcgtaaaaggccgcacattgcaaccgcattgaggacgcgggtggtaacgcggcaaccaccgc  
ggacaagtggcgacgggtctgcagagctgttagcgcacggaaagtggcctcaagccctccgcacgcacaccggcg  
accgcaaaaaccgtcaagtgcgcacgcggatccatcatccatcgccgtggccactttaacaatacatccgaggccag  
ggagatcgtgacttggcgcggcataccgcggccgtggcagcagagatcaaccgcactgtccataggtagtggcaatcc  
ctgcttctacaggatatttagtgcggaaagacagactgtcatcaatcactctcgatctgttgccagcaatggatacc  
aytgaggcacgggtcactatctactgcgcgataaaacgtggagccaaagataaaaccgtctgcggcaaccgcactgtcc  
actgaactgggtcagatgaattacagttgaagtcacactgacttagtccatccggacagtagcgttggggacgttcca  
gggtacagcactaccgcggactctgtactcctacatggaaaggatctacttccaccaggcggcttgcacatggctgag  
atcacgactttgtggccaaagactccaggatgcaaatgaacatattttgtatgcgttggccagaccatggacaatt  
cgtccagggtcccagggtgaaagacactgtgattcatcgactccacccaaagacgcgtccatgcctatgcgcata  
ccggagagactcacaagactacggatgcaccacacaaaagatttgcgttgcgtcttcagctgcggcaactatcgt  
ataccctggcgtgcaacgactgtgagttatgcgttgcagctccaccgcctccgcgtcactgcgt  
tacccgtggccaaactaccataagttgttgcgttgcgttgcgttgcgttgcgttgcgttgcgttgcgttgcgt  
cctgtatctggagggtcagccggaaatttgcgttgcgttgcgttgcgttgcgttgcgttgcgttgcgttgcgttgcgt  
cctacgcggcaacggcagaacttgcgttgcgttgcgttgcgttgcgttgcgttgcgttgcgttgcgttgcgttgcgt  
gtcaagtccagggtggaaagtacatcaaccacccatccaaccaccaacgcggccatcccgaccacgttaccgcgttgcgt  
cccgccaggctctagaagatacgtgcggccagggtggccgttgcgttgcgttgcgttgcgttgcgttgcgttgcgt  
ggggcaccgcactgaagaggagagcgatatttcgttgcgttgcgttgcgttgcgttgcgttgcgttgcgttgcgt  
tga

> MIDV

atggcaccatcatatagactgttaagaggcaatattcaccgcactccgcgcgtgtgtctagtaatcagctggcgtaac  
aacaaggctgcgcggatgttgcgttgcgttgcgttgcgttgcgttgcgttgcgttgcgttgcgttgcgttgcgttgcgt  
gacgcgttgcgttgcgttgcgttgcgttgcgttgcgttgcgttgcgttgcgttgcgttgcgttgcgttgcgttgcgt  
gatgccgcacccatcgactgtgttatagccgtggccgttgcgttgcgttgcgttgcgttgcgttgcgttgcgttgcgt  
tcaacggggacgttcgttgcgttgcgttgcgttgcgttgcgttgcgttgcgttgcgttgcgttgcgttgcgttgcgt  
gtcgatgtacgatatactgcgggataagtgtggaaaagaaaatccaagaggccattgtatgaggacggcaaccacgg  
ctgcttagatgacgcacaacggttatgaaagagactaaccagggtgcattctgtatgtcgttgcgttgcgttgcgt  
agcacgggtggacggacggctgcattcgatcttgcgttgcgttgcgttgcgttgcgttgcgttgcgttgcgttgcgt  
actctgtggccaaggagagagaagaagcgaacgcggacgacataacacactacgtccttgcgttgcgttgcgttgcgt  
aatgcccgggtggatgacaccgattcgccggaccaccatgcaccgtcccgatgcgttgcgttgcgttgcgttgcgt  
cgctacatagattcgccgcgcgcggatgttgcgttgcgttgcgttgcgttgcgttgcgttgcgttgcgttgcgttgcgt  
tga

ggcgtgcagagagtggcgtttcgctgtaatgtttaatcacacgttcacgcgttgtaagccctcgcaagtacagg  
gaaccgagcattagcagcgactcgtcatcctctggactgtctgttgacactggcataggctctgatttagtgcaggaa  
ccaatggaaaccctgtcaaccgaaccgtgtcatcgacttgcgactgtcgtagaggagacggccccgtcagactagaacgggtg  
gcccctgtggctgcacccgtcgagagccgcgcacacccttacttggcgcgggtttagcaccagttcccgcc  
cgtacatgcagtcgtcagaccccctgcggaaagaaaggccgcaccagaacacactgaaaggatttcgtcggcgatttagat  
ggcgtgcatggccatcatatatgacgacacttcggcggacttcggcgcggcgagttcgaacgtttaacgtcaga

> ONNV

atggctccgtcataccgtgtgaaacggatggacatcgcggaaaaacactgaggaatgcgtggtaaacgcccaatccacgc  
ggagtaccaggcgtggagttgttaaagccgtgtatgaaaatggccagactcattcagaaacagtgcacaccactgggg  
actgc当地atcatgtggcccaataccggcatccacgcgttaggtcataacttcaaacttctgaggctgaa  
gggatagggaaattggcgtatagagaagtgccgaaagaagttgtctaggcgttaggatgtgaggactgc当地  
ttgcttcaaccgggtgtactcaggaggcaagacagattgctcaatcactaaaccatcttcgcagcgtatggatttc  
acagatgcagatgtgtcatctattgcagggacaaggaaatgggagaagaagatcactgaagccatcactaagatccc  
gtagaactactagatgtcacatctcgttgatgcgttcacacttaggcgttccagacagcgcgtggcaggccc  
gggtacagcacagtagaggagcactctactttacctagagggacaagattccaccaactgtctgtagatattggctgag  
atatataccatgtggccaaacaaactgaagccaacgaacaggctcctataatgctctggggagagttatagatcc  
aggaaaaatgtcccgttagacgacggccgcattccctcggaaaacagttccgtgcctatgcggcatgc  
cctgaacgcaggatgcacgcattcgcgtcatgcataactactagcatcatgtgtcgtctttccgcggcaggaa  
atcgagggcgtgcaaaatgttcaaaagcactcttgcgttgcactcactgcgtgcaggacgc当地  
tataggcctgcggacgaatcatacagacacccatcactgaaagcgtgtcaggacgcacaactcgtcagtc  
aatatgtgaagcagtgccagttccctcggacttagaggctgtgacgc当地actatggactggccct  
acaagacaaagacacgactcatgcgttagtgcggaaaataactccctatggccagcggcactgtc  
actgtcgcactgagcgtatcaacacgacataactgcgtaccccatccaggcaccgctaggattgac  
ctcaacagatcacttcggagatttgccggaaatagacaacacctcctgacagggcattgacatttggggactt  
gagccaggtgaagtggaagagactgacggatagcgtggcaacatgc当地ggc  
gcagggttga

> RRV-QML

atggcaccctcataccgtgtgcgcaggaccgacattccgggcacgctgaagagggcgttgtcaatgc  
ggcacagtcggtatgggtttgcagacggcgtggcaaaaaatggccagactcattcaaggtgccc  
acggccaagtggcgtacaggccaaacggcatgaatgtcatccacgcgttaggcgc  
ggagacagagagtggccggcataccgtgcgtggcggtatcatcaatgc  
ctgttgacggagttctccggaggtaaagatagactgtcactaaatcat  
actgtttaccgcaatggacacc  
acggacgc当地tactgtcgaccaaaagc  
gtggaaattggatctgaagacatctcactcgactgtc  
gacccatccaggcaccgctaggattgac  
atataccatgtggccaaacttc  
aggacgc当地atgc  
agaacgaaatgc  
gctgaggagtgccgaggctcc  
attgaaaggcgtcc  
tacataccaggcc  
gatttacggtat  
caccactcg  
tcc  
cct  
c  
cg  
cg

## Supplementary Material

ttaccgtggagccggaggacattgacatccaattcgagatttgaaacacccgacaaaatccaattcggcgcacatcgat  
tttgcacaattctga

> RRV-T48

atggcacccataccgtgtcgtaggaccgacattccggcacgctgaagaggcggtttaatgccgccaacgcgaag  
ggcacagtgcgcgtgggttgcagagcggtgagaaaaatggccagactcctcaagggtccgcactccgtgggt  
acggctaagttgtacaggcaacggtatgaatgtcatccacgcgtggccgatatttcacggtagccatccgtgggt  
ggcacagagagttggccgcataccgtgccgtggccggattatcaatgttagtaacattaagagtgttagccatccct  
ctgttgcacggagttctccggaggtaaagatagagtcatgcactaaatcacctgtttaccgcaatggacacc  
acggacgctgacgttagtcatctattgccgcacaaaggctggagaagaaaaatccaggaggtatcgatcgcgcacc  
atggaaattggtatctgaagacatctcactcgagtctgacttgatacgggtacacccagatagttgcttgttaggcagaaaa  
ggttacagcataacagatggaaagctgcattcatacctggaaggtacccgcttcatcagactgcggtgacatggctgag  
atatctaccttgcggccaaacttcaggacgacaaacaaatatgctgtatgcattgggtgagagatatggacacgcac  
agaacgaaatgccctgttggaggacgcgttgcattgtccacgcctccgaaaacagttccgtgtctgttaggtacgc  
gctgagagagttggcaagacttcggatgaacaacactaaggccataattgtgtgtccctcccttaccgaagtt  
atggaaaggcgtccagaaggtcaagtgcgaccggagtctgattttggaccagacgggtccatctgttagtccaagg  
tacataccagccgcgcctctacgcacgcacataccgtgagcttgcattcactagtatccacaggatccgcgtgg  
ccatctgaggccacgtatgagaccatgaaagtagtagcagaggtgcaccactcggaccaccaggccgc  
cgtcgtgcgcagggtacgatgcaccaccaggacttggaaagtctgtgacatgcacaccccgattgcggcaagg  
atccccgtgtacgataccgcttgcattggagagatggcaattcctgcacaaggcgtatgcacccatacc  
ccacgggcagaaagggtcgtaaccgtgccggaccacgcattcagcgcgtcgcacgtacagactctct  
cctcgcgttctgagagcctcgatgcactgtgaccactagcgcgtggtagagttccctggcgcctgaagatctgg  
gtactcaccgagcctgtcactgcaaaatgcgcgagccgggttagttaccgtggagcctgaggacgttatcc  
ggagattttgaaacatccgacaaaatccaattcggcgcacatcgatttgcattctga

> TAFV

atggccccatcctacacagttgtcagggcaacattacggccacccgcgcacatgccgtgtcattccagtcacacc  
cacaagacggcgtgtacagatcctgttctaaaaatggggaccgcgttccacatggagtgggtcggaaggagg  
tacgcgtccgcacgcctgcccacactgcaggatgcgtaccgcgttccatcgcgcactcgaccgcactc  
taccgtcccatagcaaagggtggtcgttgcacgcacaaataccgtcccttcgcgtccgttattag  
catcaagaaaaatggcgcacatgtcgatgtcgacggagtactcaaccatctcgtaaccgcatt  
ggaccacactgcgcgtcgcacgtacaggactgtggacgcacgc  
ctggataaggacggcaccgaaagatgcgcgaaatgcgcgttgcacgcacgc  
gagatgcacgaagagactgtacttgggtacaccggacagttgccttaagaacc  
gtacgcgttgcacgcacacttgcactaataactaggggagactatt  
gaaggatccgcgcacgc  
aacccctccgttaccccccacagacacttgcacgc  
agcaattccgtgcacgcacacttgcacgc  
tgcttgcacgcacacttgcacgc  
cctatcgatctgcacgc  
tacaaaaccgttaagcgcacccacag  
ctgtcgctccttc  
cctgctaccc  
tattga

> UNAV

atggcaccagcctatcggtgatcaggctgatattgcgaccagcaaggagcaggctgtggtaatgcggca  
ggcatcatcggtatggagtatgtcgatcggtcgacatggccacaggcg  
actgcacaaagacactgtggagtgtacgggttcatattatcc  
atccatgcgcgc  
acttgc  
cagtaac  
acatc  
cagac  
gaggaa

ggtagacagagacacctggccgcctacagagactatcgccgaaagtcaaccggcttcgtataaccacggtaatcccg  
ctccttcgacagggcgttcagcgccgaaagacagacttcgccaatcactgaaccacctttcacagcactagataca  
acagatgcagatgtaaacgatctactgcaggacagagactggaaaagaggataaggacgccccatcgacatgaggaccg  
caagagctggtcagtgcacaccacggaccttggaaagtggacttggtagactccaccccacagcagccatggccggaga  
ggctacagcaccactgtggtcactatactcctacttggaaaggcacaaaatttaccagggtgtcaatagatatggcagaa  
ataataaccatgtggctaaattggcagaagcaaacgagcagatatgtctatacgctatggcagacaatggacaatatt  
agagcttagatgccctgtggacattgcgaatctccacaccacaaaacagtgccctgttgcaggtacgccatgacc  
gcggagcgcgtgacacggctaaggatgcaccatagcaaggattcacagtctgtcatccacttccaactgcccgaatcc  
atagagggcatacagagactcaagtgtgacaaagtttgcgttgcaccacggtaccccttagtgcgttgcaggatcc  
tacgtggagcggcaacactactgtatctgcgggcagcgacgaacttgtccgcactcaccagtcgcgagtcaigt  
gcagagaccatgtgcacggatgggagttcgccgcggcgctcgcgcacccatgtggacttgggttcaacagccat  
ttgtatgtatctatgcacaatgttaccgcataatgtccctccattccaccacccaggccgaagagacgcacgcgc  
gctctgtggcgccgtccatcccgccggactcgaataacggtcacagcagacatacatgttaccgcggacttgc  
gtccccatccccagaacacacgcgtcccgacccatcgaggactcggcagttttaccgtggaccccaaggaaacgc  
ctaccggcaggggcgccgtgcctccccaaagaagtgttcgcagaccatcaggactgttagtactgttcaatgg  
tccttgcgtggatccagaggatattgatttggaaatttggaggcgccttggatgatcatcaccttcggagacttacc  
gaagaagaggtggcgtctcgacagtgaaggagtggaaatgccatggatgcacagcccagattaggcatatgc  
actttcgagatttctctgtacgactgtggaaaccgttaagctttcatcatctg

> VEEV

atggcacccatcatgtggcgaggggatattgcacggccaccgaaggagtgattataatgtctacagcaaa  
ggacaacctggcgagggggtgtggggagcgctgtataagaaattcccgaaagttcgattacagccatgc  
aaagcgcgactggtaaagggtcagctaaacatattcattcatgcgttaggacccaaacttcaacaaagttcg  
ggtagacaaacagttggcagaggcttatgagtcgcatacgacttcaacgataacaattacaatgt  
actgtgcagatgtactgcaggacaagaatggaaatgactctcaaggaagcactgtggtaggagagaagca  
gtggaggagatatgcataatccgacacttgcgtgacacaagcgtatggaaaacttctcatatttgg  
aaggatatacgagaaattaatgcatgtggccgttgcacacggcaatgacggatgtcatgtatatc  
actcatgcacccatgttcccgatccatcccgatccatcccgatccatcccgatccatcc  
atccatgcacccatgttcccgatccatcccgatccatcccgatccatcccgatccatcc  
ttggcttgcgtggatccagggatatttggaaatttggaggcgccttggatgatcatcac  
gaagaagaggtggcgtctcgacagtgaaggagtggaaatgccatggatgcac  
gggacacctgaacaaccaccacttataaccgaggatgagaccaggactagaac  
gaagaggatagcataagttgcgtcagatggccgcaccacccaggctgc  
tctgtatctagctcatctgtccattcctcatgcacccatcc  
ggagcttagcgtgaccaggcccccaacgtc  
gtgcctgcgcctcgacacttccatcccgatccatcccgatccatcc  
gcctgcgtcgagaaccaggcttagttccaccccccaggcgt  
ccgtcacgcactcttagcaggctcgatccatcccgatcc  
gaggagtttggggcgttcgtacacaacaatgacatcttctg

> WEEV

atggctccagcgtacagagactgtacagagggtgacattagcaagagcgctgaccaagctatcgtaatgt  
ggtaaccagggttccggagtgtgcggctgcactgtaccggaaaatggccggctgttt  
acggctagacttgtgaagcacgaaccgc  
gacccatcgatccatccatcccgatccatcccgatccatcc  
ctgtcaactgcacccatattctgtggcaagatcgactgt  
gatgccatgtcaccatattgtggataaacaatgg  
gaaatactggatgatgatgacagcc  
gttactccgtcaatgagg  
atccatgcacccatgtgg  
19

## Supplementary Material

cgctccaaatgcccagttagaggagttagaggcgtctgcacccactcacacttccatgcctgtgttaattacgtatgacg  
gctgagcgcgtatacagggtgcgtcgaaagaagaacagttcgccgtatgtcatcattccgttgccgaagtacagg  
atcacaggcgtgcagaagctacaatgcagcaaaccagtccgttcaggcgtcgtaaccaccggctgtacaccccaggaa  
tacgcggaaataattctagaaacgcaccatcgcaacaacgacaaccgtaatatgtgaaccaactgtgccagaacgtata  
cccagtcccggtgattctagagcacaaggatgcggaatcactgctatcgttggccgtctcgtagctctgccccaca  
cgctcgtaaccgcctggagcactatgacaggcggttgcgttacagctgacgtcatcaagcgaacacgtctacgtgg  
agcatccctagtgcctggcttgcgtccagctgcctctgacgatactgatcccactggagtattccaagtgcata  
ggcttgaagtgagaacacccatctgtacaggacctaactgcagagtgtgcgaggcctcgactggccgaaataatgca  
gacttcaataactgctccttccagttcttcggaccacagaccagtaccagcaccacggagacgccccatcccatcacct  
agatcgacggctccgcacctccagttccaaagccacgcagaactaagttaccaacaaccaccaggagtgcctaggcgatc  
tcagaagcgagttggacgactacgtcaacattccaactgacatatg

> WHAV

atggcgccatcgtaaaatcaaggagggaaacatcatcgaatgcaccgaagaagccgtcgtaacgcgtgccaacgcacta  
ggacgccccggagaaggggctgcaggcgattacaagaagtggccgaacagcttcaccgggttccgcaacagaagttaggg  
actgcaaaaatgaccacaaggcctaggcaagaaagtcatcatacgccgtcgaccggattttaaagaagcactctgaaagaagaa  
gcccttaaactgctcgagaatgcctaccacgcctcgagatattattaatgagaacaacatcaaattcgtggccattcca  
ttgctatcaactggtatatacgctcgaggaaaggacagactagagacttcttcgactgttgcggatccacagcgatggacagg  
acggacgcccacgtaacggtatactgccttgacaagaaatggcagcagcgaattgacgcagtccttagattgaaagaagag  
gtaacggagctaaagacgacgacatggaaattgtatgaggagctggatccaccctgacagctgttgcggatccac  
aaaggctttagcaccacccaaaggcaaactgtattcatacttcgaaggaaactaaatttaccaggcagcggaaagacatggca  
gaaatcaatgtattgttccagacaccattgaggctaacgagcaaattctgtatgttatattccggagaaagcatggaaagct  
atccgcggaaaatgccccgtcgactacaacccttcgtcaagtccggccaaaaccttaccctgctgtcatgtatgctatg  
acacctgagagggtgcataactcagaagcaacaatgtcaaagaaattacggatgctccacttccacttccaaaacat  
aaaatcaagaacgtacaacgaatccagggtttcaaaaatctgtttatccccagactccagctttgtacctgcacgt  
aagttcatagaaaccgaacccaaagaaacagaagacgatgcggctcagccggacccggcagtgccaggcagtt  
tcgaccggcggtcccacaacgtcagcaagaccgttagagttgataatattccgcagactcttaaccgaagtaacgcacacc  
tctgacgacattccgcacatacccttgcacacatctgtatgtcttagtacttcctcactgagctcggtttggactgcccc  
aatgttagtcgagggtcgaggcggaaattcagtcgtcccgacactccgggtggaccggccgagaaagaagaagtttagcact  
ttagcggcgctatcaagagcatctgacattcccatcgaatccaacccaccaatcactttggatcatttgaggatgg  
gaaatagacaacttgcagaagaagtgcacttcagaaccattatgtcggctcgttgcgaaccaggcgaagtcaacacgcctg  
atagaaaccaggcggagccaccacgttagggggcgcagacgtcgcaacaagaaccgcacaggagtattga