

**An African tick flavivirus forming an independent clade exhibits unique exoribonuclease-resistant RNA structures in the genomic 3'-untranslated region**

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Supplementary Table S1. Summary of ticks captured in Zambia

| Location | Date           | Tick species                               | Sex    | Number of ticks |
|----------|----------------|--------------------------------------------|--------|-----------------|
| Isoka    | November, 2017 | <i>Amblyomma variegatum</i>                | Male   | 35              |
|          |                |                                            | Female | 25              |
|          |                | <i>Argas walkarea</i>                      | NI     | 20              |
|          |                | <i>Rhipicephalus (Boophilus) microplus</i> | Male   | 5               |
|          |                |                                            | Female | 41              |
|          |                | <i>Rhipicephalus (Boophilus) sp.</i>       | Female | 1               |
|          |                | <i>Haemaphysalis leachi</i>                | Female | 1               |
|          |                | <i>Rhipicephalus lunulatus</i>             | Male   | 39              |
|          |                |                                            | Female | 94              |
|          |                | <i>Rhipicephalus sanguennius</i>           | Male   | 6               |
|          |                |                                            | Female | 6               |
|          |                | <i>Rhipicephalus zambeziensis</i>          | Male   | 26              |
|          |                |                                            | Female | 21              |
|          |                | <i>Rhipicephalus sp.</i>                   | Male   | 1               |
|          |                |                                            | Female | 1               |
| Mpulungu | November, 2017 | <i>Amblyomma variegatum</i>                | Male   | 23              |
|          |                |                                            | Female | 19              |
|          |                | <i>Amblyomma pomposum</i>                  | Male   | 3               |
|          |                |                                            | Female | 2               |
|          |                | <i>Rhipicephalus (Boophilus) microplus</i> | Female | 5               |
|          |                | <i>Rhipicephalus (Boophilus) sp.</i>       | Female | 7               |
|          |                | <i>Haemaphysalis leachi</i>                | Male   | 6               |
|          |                |                                            | Female | 3               |
|          |                | <i>Rhipicephalus appendiculatus</i>        | Male   | 9               |
|          |                |                                            | Female | 3               |
|          |                | <i>Rhipicephalus camicasi</i>              | Male   | 1               |
|          |                | <i>Rhipicephalus compositus</i>            | Female | 1               |
|          |                | <i>Rhipicephalus evertsi evertsi</i>       | Female | 1               |
|          |                | <i>Rhipicephalus guilhoni</i>              | Male   | 2               |
|          |                | <i>Rhipicephalus lunulatus</i>             | Male   | 2               |
|          |                |                                            | Female | 8               |
|          |                | <i>Rhipicephalus muhsamae</i>              | Male   | 2               |
|          |                | <i>Rhipicephalus sanguennius</i>           | Male   | 26              |
|          |                |                                            | Female | 10              |
|          |                | <i>Rhipicephalus simpsoni</i>              | Male   | 1               |
|          |                | <i>Rhipicephalus sulcatus</i>              | Male   | 4               |
|          |                |                                            | Female | 2               |
|          |                | <i>Rhipicephalus zambeziensis</i>          | Male   | 5               |
|          |                |                                            | Female | 2               |
|          |                | <i>Rhipicephalus sp.</i>                   | Male   | 14              |
|          |                |                                            | Female | 19              |
| Samfya   | January, 2018  | <i>Amblyomma variegatum</i>                | Male   | 13              |
|          |                |                                            | Female | 23              |

|                                            |        |     |
|--------------------------------------------|--------|-----|
| <i>Rhipicephalus (Boophilus) microplus</i> | Male   | 4   |
|                                            | Female | 30  |
| <i>Rhipicephalus appendiculatus</i>        | Female | 1   |
| Total                                      |        | 573 |

NI; Not identified

Supplementary Table S2. Primers used in this study

| Primer               | Sequence (5'-3')                         | Reference  |
|----------------------|------------------------------------------|------------|
| Flavi all S          | TACAACATgATggggAARAgAgARAA               | 1          |
| DEN4                 | TACAACATgATgggRAAACgTgAGAA               |            |
| Flavi all AS2        | gTgTCCCAgCCNgCKgTgTCATCWgC               |            |
| MPFV-5RACE-R         | gattacgccaagcttGAACACATGTGCTCCGAGGGCTAG  |            |
| MPFV-5RACE-nested-R2 | gattacgccaagcttCTGGCCTGTATTGCTCTCCGTCTC  | This study |
| MPFV-3RACE-F         | gattacgccaagcttCAAAGCGCAATCCACTAGGGGAGTG |            |
| 5'FAM-xrRNA1-R-      | [FAM]-AGACGGTCAGCTCTTCCTGC               |            |
| 5'FAM-xrRNA2-R       | [FAM]-AGTTCTTTCTGCTATACAGG               |            |

Supplementary Table S3. Putative polyprotein cleavage sites of Mpulungu flavivirus and other representative flaviviruses

| Virus                         | Cleavage site (protease) |                          |                 |                    |                      |               | NS1/NS2A<br>(Unknown) |
|-------------------------------|--------------------------|--------------------------|-----------------|--------------------|----------------------|---------------|-----------------------|
|                               | C/CTHD<br>(VSP)          | AnchC/prM<br>(Signalase) | pr/M<br>(Furin) | M/E<br>(Signalase) | E/NS1<br>(Signalase) |               |                       |
| Mpulungu flavivirus           | AGRRR / ANTGQ            | SLALG / AHVFM            | RIAER / SLSVT   | APAYG / TQCLG      | LGVGA / DVACG        | GFVYA / WALEV |                       |
| Ngoye virus                   | -                        | -                        | -               | -                  | -                    | -             |                       |
| Louping ill virus             | RGKRR / SVTNW            | GMTLA / ATVRK            | SRTRR / SVLIP   | APVYA / SRCTH      | LGVGA / DVGCA        | SMVVA / DNGEL |                       |
| Tick borne encephalitis virus | RGKRR / SATDW            | GMTLA / ATVRK            | SRTRR / SVLIP   | APVYA / SRCTH      | LGVGA / DVGCA        | SMVVA / DNGEL |                       |
| Omsk hemorrhagic fever virus  | RGKRR / STTDW            | SIALA / ATVRK            | TRSRR / SVLIP   | APAYA / SRCTH      | LGVGA / DVGCA        | SMVVA / DNGEL |                       |
| Langat virus                  | RGSRR / TTIDW            | GMCLT / ATVRR            | SRSRR / SVLIP   | APAYA / SRCTH      | LGVGA / DVGCA        | SMVVA / DNGAL |                       |
| Alkhurma virus                | RGKRR / STTGL            | TLVIS / ATIRR            | GRSRR / SVSIP   | APTYA / TRCTH      | LGVGA / DMGCA        | SMVLA / DNGAM |                       |
| Kyasanur forest disease virus | RGKRR / STTGL            | TLVFS / ATVRR            | GRNRR / SVSIP   | APTYA / TRCTH      | LGVGA / DMGCA        | SMVLA / DNGAM |                       |
| Powassan virus                | RGRRR / SGVDW            | TMAMA / TTIHR            | SRGKR / SVVIP   | GPVYA / TRCTH      | MGVGA / DYGCA        | SMVVA / DNGAL |                       |
| Deer tick virus               | RGRRR / SGVDW            | AMAMA / TSIHR            | FRGRR / SVVIP   | GPVYA / TRCTH      | MGVGA / DYGCA        | SMVMA / DNGAM |                       |
| Kadam virus                   | NGKRK / SAGGG            | SVAGA / AVVVQ            | RRQTR / SVHIT   | APAYA / SRCVH      | LGVGA / DIGCA        | SQVLA / FNDGL |                       |
| Meaban virus                  | GRNRR / GGGSF            | AIAG / AVLME             | KRHRR / ELSIS   | VPAYA / SRCVH      | LGVGA / DYGCA        | STVLA / MTDGF |                       |
| Saumarez Reef virus           | GRNRR / GPGGM            | GGCMG / ALVMT            | SRARR / SISIT   | VPAYA / SKCVH      | LGVGA / DYGCA        | STVLA / MTDGL |                       |
| Apoi virus                    | KGGRR / GGKSV            | PIALS / AVVMN            | TRTRR / DVTIQ   | APAYA / STCVS      | TGVVG / EIGCM        | GLVMA / FDEEP |                       |
| Rio Bravo virus               | KKQRR / GGTES            | TGLMA / MQVSQ            | HRLKR / SLSIT   | APSYS / TQCVN      | TGVMG / DHGCA        | GLVYA / GSMTA |                       |
| Zika virus                    | ERKRR / GADTS            | TTAMA / AEITR            | RRSRR / AVTLP   | APAYS / IRCIG      | TAVSA / DVGCS        | SMVTA / GSTDH |                       |
| West Nile virus               | KQKKR / GGKTG            | ASVGA / VTLSN            | RRSRR / SLTVQ   | APAYS / FNCLG      | VNVHA / DTGCA        | SQVNA / YNADM |                       |
| Yellow fever virus            | SRKRR / SHDVL            | LMTGG / VTLVR            | RRSRR / AIDL    | GPAYS / AHCIG      | LGVGA / DQGCA        | SWVTA / GEIHA |                       |
| Yokose virus                  | KRKRR / SSVSC            | VTVGA / LQIGR            | RRNRR / SVALT   | APAYS / THCTN      | TGVGA / EQACA        | SWVSA / GEGRM |                       |
| Cell fusing agent virus       | LESRR / TTGDP            | VLCGC / VVIDM            | KREKR / SREPP   | TTVKG / EFVEP      | YYVRA / DLGCG        | GKANA / QSDFR |                       |
| Kamiti River virus            | LEKQR / SGPNL            | GLCYG / EMLRY            | VRRRR / APQPQ   | NVVKA / SSIEP      | RSVSA / DVGCG        | GKAHA / CSDFR |                       |
| Tamana bat virus              | QKRQK / SSGGY            | MVIFC / GYQSG            | HRTRR / SVTET   | ILVIA / QFYLAD     | EVVAA / DKYVL        | NVVKA / SKMKN |                       |

| Virus                         | Cleavage sites (protease) |                   |                   |                  |                        |                   |
|-------------------------------|---------------------------|-------------------|-------------------|------------------|------------------------|-------------------|
|                               | NS2A/NS2B<br>(VSP)        | NS2B/NS3<br>(VSP) | NS3/NS4A<br>(VSP) | NS4A/2K<br>(VSP) | 2K/NS4B<br>(Signalase) | NS4B/NS5<br>(VSP) |
| Mpungu flavivirus             | RAQRR / AALGD             | PGNRR / ADLVG     | QALRR / SAGDI     | PGGQR / GVLDN    | GLVYC / NEMGL          | RDGRR / GAGHM     |
| Ngoye virus                   | -                         | -                 | HALRR / SAGDI     | PGGQR / GVLDN    | GMVYC / NEMGL          | RDGRR / GAGHM     |
| Louping ill virus             | HRGRR / SFSEP             | RSSRR / SDLVY     | ASGRR / SFGDV     | TGKQR / SSDDN    | GLVAA / NEMGF          | SGGRR / GGSDG     |
| Tick borne encephalitis virus | HRGRR / SFSEP             | RSSRR / SDLVF     | ASGRR / SFGDV     | AGKQR / SSDDN    | GLVAA / NEMGF          | SGGRR / GGSEG     |
| Omsk hemorrhagic fever virus  | RRDRR / SFSEP             | RSARR / SDLVF     | ASGRR / SLGDM     | AGKQR / SSDDN    | GLVAA / NEMGF          | SGTRR / GGSEG     |
| Langat virus                  | SRGRR / SFNEP             | GSPRR / TDLVF     | ASGRR / SVGDV     | TGKQR / SSDDN    | GMVAA / NEMGL          | TGTRR / GGSEG     |
| Alkhurma virus                | RRNRR / SFSEP             | SSGRR / SELVF     | ASGRR / SVGDV     | PGKQR / SSDDN    | GLVTA / NEMGM          | TGTRR / GGADG     |
| Kyasanur forest disease virus | RRNRR / SFSEP             | GSGRR / SELVF     | ASGRR / SVGDV     | PGKQR / SSDDN    | GLVTA / NEMGM          | TGTRR / GGAEG     |
| Powassan virus                | GRGRR / SLSEP             | SSTRR / TDLVF     | ASGRR / SAVDI     | PGKQR / SGEDN    | GLVAA / NELGY          | QGARR / GGAEG     |
| Deer tick virus               | GRGRR / SLSEP             | SSARR / TDLVF     | ASGRR / SAMDI     | PGKQR / SGEDN    | GLLAA / NELGY          | QGARR / GGAEG     |
| Kadam virus                   | FGKQR / SLEEP             | SRNRR / SDLIF     | AESRK / SASLV     | PGTQR / SGEDT    | GAVAA / NELGW          | GPTRR / GLTGG     |
| Meaban virus                  | HGNKR / SVSDA             | GSERR / SDLVW     | AEGRR / GVGRG     | PGTQR / SFEDN    | GLVAA / NEMGW          | ARDRR / GPGST     |
| Saumarez Reef virus           | RSGRR / SVADA             | NDTRR / GDLVW     | AEGRR / SIGGG     | PGTQR / SFEDN    | GIVAA / NEMGW          | QRDRR / GPGSA     |
| Apoi virus                    | RSGQR / SVDPI             | RSIQK / SNTSF     | AKGKR / SGMTI     | EGMQR / TQVDS    | AAVVA / NEMGF          | SENRR / GVSSS     |
| Rio Bravo virus               | HRGQR / ATDYT             | DATQR / SIIVF     | AQMRR / SGVLL     | EGMQR / TQIDS    | VTVVA / NEMRL          | RSDRR / GIVTS     |
| Zika virus                    | RSGKR / SWPPS             | KTGKR / SGALW     | AAGKR / GAALG     | PEKQR / SPQDN    | GLITA / NELGW          | LVKRR / GGGTG     |
| West Nile virus               | PNRKR / GWPAT             | QYTKR / GGV LW    | ASGKR / SQIGL     | PEKQR / SQT DN   | SAVAA / NEMGW          | PGLKR / GGA KG    |
| Yellow fever virus            | IFGRR / SIPVN             | RGARR / SGDVL     | AEGRR / GAAEV     | PGQQR / SIQDN    | SAVAA / NELGM          | KTGRR / GSANG     |
| Yokose virus                  | NGKVR / SIDWT             | YTKQR / SNILW     | ATTTR / SITAV     | TGMQR / SIQDN    | ALIVA / NENGY          | QANRR / GGTGS     |
| Cell fusing agent virus       | NI                        | TANNR / SDDL      | WETRK / VSIDF     | SIGNR / SYMDS    | CGVLA / WEMRM          | NQFRA / LEKST     |
| Kamiti River virus            | NI                        | SEQNR / SDDL      | WDTRK / LSIEF     | AIGNR / SYMDS    | CGVLA / WEMRL          | NQFRA / LEKST     |
| Tamana bat virus              | NI                        | NLRDK / SKGLI     | PLVQR / VFSGI     | GITQR / EKSTG    | YYILA / DGEIL          | KTTQR / FRSSI     |

NI: Not identified, VSP: viral serine protease

Supplementary Table S4. Flavivirus reference sequences used for compositional analyses in this study

| Group | Accession | Flavivirus                        | Seq. length | Reference |
|-------|-----------|-----------------------------------|-------------|-----------|
| ISFV  | AB488408  | Aedes flavivirus                  | 11064       | 2         |
| ISFV  | KX148546  | Anopheles flavivirus variant 1    | 10588       | 3         |
| ISFV  | KX148547  | Anopheles flavivirus variant 2    | 10529       | 3         |
| ISFV  | KX669689  | Calbertado virus                  | 10733       | 4         |
| ISFV  | AB377213  | Culex flavivirus                  | 10837       | 2         |
| ISFV  | HE574574  | Culex theileri flavivirus RP 2011 | 10538       | 2         |
| ISFV  | KT599442  | Culiseta flavivirus               | 10864       | 5         |
| ISFV  | M91671    | Flavivirus sp.                    | 10695       | 2         |
| ISFV  | JQ268258  | Hanko virus                       | 10158       | 2         |
| ISFV  | AY149905  | Kamiti River virus                | 11375       | 2         |
| ISFV  | KY320649  | La Tina virus                     | 10050       | 4         |
| ISFV  | KX907452  | Menghai flavivirus                | 10897       | 6         |
| ISFV  | KP688058  | Mercadeo virus                    | 10938       | 2         |
| ISFV  | KC464457  | Mosquito flavivirus               | 10865       | 2         |
| ISFV  | AB981186  | Mosquito flavivirus               | 10863       | 2         |
| ISFV  | JQ957875  | Nienokoue virus                   | 10878       | 7         |
| ISFV  | KY345399  | Ochlerotatus caspius flavivirus   | 10370       | 8         |
| ISFV  | KC505248  | Palm Creek virus                  | 10095       | 2         |
| ISFV  | KT192549  | Parramatta River virus            | 10893       | 2         |
| ISFV  | FJ644291  | Quang Binh virus                  | 10865       | 2         |
| ISFV  | MH899446  | Sabethes flavivirus               | 10585       | 9         |
| ISFV  | KU201526  | Xishuangbanna aedes flavivirus    | 10884       | 10        |
| ISFV  | GQ165809  | Nakiwogo virus                    | 10122       | 2         |
| ISFV  | KX245154  | Cuacua virus                      | 9747        | 11        |
| MBFV  | AY898809  | Alfuy virus                       | 10960       | 2         |
| MBFV  | KF917535  | Aroa virus                        | 10248       | 2         |
| MBFV  | HQ644143  | Bagaza virus                      | 10794       | 2         |
| MBFV  | KM225264  | Bainyik virus                     | 10648       | 2         |
| MBFV  | JF312912  | Baiyangdian virus                 | 10278       | 2         |
| MBFV  | KU308380  | Bamaga virus                      | 10203       | 12        |
| MBFV  | DQ859056  | Banzi virus                       | 10182       | 2         |
| MBFV  | DQ859057  | Bouboui virus                     | 10173       | 2         |
| MBFV  | AY632536  | Bussuquara virus                  | 10815       | 2         |
| MBFV  | KF917536  | Cacipacore virus                  | 10284       | 2         |
| MBFV  | JQ308185  | Chaoyang virus                    | 10733       | 2         |
| MBFV  | KC692512  | Dengue virus 1                    | 10735       | 2         |
| MBFV  | KC294208  | Dengue virus 2                    | 10723       | 2         |
| MBFV  | KF973479  | Dengue virus 3                    | 10569       | 2         |

|      |          |                                              |       |    |
|------|----------|----------------------------------------------|-------|----|
| MBFV | JN983813 | Dengue virus 4                               | 10649 | 2  |
| MBFV | JQ086551 | Donggang virus                               | 10791 | 2  |
| MBFV | JQ920422 | Duck egg drop syndrome virus                 | 10990 | 2  |
| MBFV | KF573582 | Duck flavivirus KPS54A61/THA                 | 10990 | 2  |
| MBFV | KC990542 | Duck Tembusu virus                           | 10990 | 2  |
| MBFV | DQ859060 | Edge Hill virus                              | 10206 | 2  |
| MBFV | AY632537 | Entebbe bat virus                            | 10236 | 2  |
| MBFV | KM361634 | Fitzroy River virus                          | 10807 | 13 |
| MBFV | AY632538 | Iguape virus                                 | 10922 | 2  |
| MBFV | AY632539 | Ilheus virus                                 | 10755 | 2  |
| MBFV | KC692067 | Ilomantsi virus                              | 10365 | 2  |
| MBFV | KC734550 | Israel turkey meningoencephalomyelitis virus | 10792 | 2  |
| MBFV | AF254452 | Japanese encephalitis virus                  | 10970 | 2  |
| MBFV | DQ859066 | Jugra virus                                  | 10173 | 2  |
| MBFV | AY632540 | Kedougou virus                               | 10723 | 2  |
| MBFV | AY632541 | Kokobera virus                               | 10874 | 2  |
| MBFV | EU082200 | Koutango virus                               | 10302 | 2  |
| MBFV | KT934798 | Kunjin virus                                 | 10302 | 2  |
| MBFV | FJ606789 | Lammi virus                                  | 10727 | 2  |
| MBFV | AF161266 | Murray Valley encephalitis virus             | 11014 | 2  |
| MBFV | KF917538 | Naranjal virus                               | 10290 | 2  |
| MBFV | KC788512 | New Mapoon virus                             | 10864 | 2  |
| MBFV | KJ210048 | Nhumirim virus                               | 10891 | 2  |
| MBFV | EU159426 | Nounane virus                                | 10755 | 2  |
| MBFV | JX236040 | Ntaya virus                                  | 10943 | 2  |
| MBFV | KJ152564 | Paraiso Escondido virus                      | 10761 | 2  |
| MBFV | DQ859067 | Potiskum virus                               | 10173 | 2  |
| MBFV | AY632542 | Rocio virus                                  | 10794 | 2  |
| MBFV | DQ859062 | Saboya virus                                 | 10173 | 2  |
| MBFV | DQ837642 | Sepik virus                                  | 10793 | 2  |
| MBFV | JX477686 | Sitiawan virus                               | 10278 | 2  |
| MBFV | KF917541 | Sokoluk virus                                | 10242 | 2  |
| MBFV | DQ859064 | Spondweni virus                              | 10290 | 2  |
| MBFV | DQ525916 | St. Louis encephalitis virus                 | 10940 | 2  |
| MBFV | KF917540 | Stratford virus                              | 10245 | 2  |
| MBFV | EU879061 | T'Ho virus                                   | 10937 | 14 |
| MBFV | KM225265 | Torres virus                                 | 10735 | 2  |
| MBFV | DQ859065 | Uganda S virus                               | 10182 | 2  |
| MBFV | KJ438721 | Usutu virus                                  | 11065 | 2  |
| MBFV | EU707555 | Wesselsbron virus                            | 10814 | 2  |
| MBFV | DQ080055 | West Nile virus                              | 11018 | 2  |

|        |          |                                        |       |    |
|--------|----------|----------------------------------------|-------|----|
| MBFV   | EU082199 | Yaounde virus                          | 10293 | 2  |
| MBFV   | JN811142 | Yellow fever virus                     | 10862 | 2  |
| MBFV   | AB114858 | Yokose virus                           | 10857 | 2  |
| MBFV   | KF268948 | Zika virus                             | 10788 | 2  |
| MBFV   | EU078325 | Barkedji virus                         | 10582 | 2  |
| MBFV   | KY320648 | Kampung Karu virus                     | 10311 | 4  |
| MBFV   | KY290249 | Long Pine Key virus                    | 10380 | 4  |
| MBFV   | MF139576 | Marisma mosquito virus                 | 10848 | 4  |
| MBFV   | MF139575 | Nanay virus                            | 10804 | 4  |
| MBFV   | KJ152564 | Paraiso Escondido virus                | 10761 | 2  |
| NKV    | AF160193 | Apoi virus                             | 10116 | 2  |
| NKV    | KJ469370 | Batu Cave virus                        | 10131 | 2  |
| NKV    | KJ469371 | Jutiapa virus                          | 10125 | 2  |
| NKV    | AJ242984 | Modoc virus                            | 10600 | 2  |
| NKV    | AJ299445 | Montana myotis leukoencephalitis virus | 10690 | 2  |
| NKV    | KJ469372 | Phnom Penh bat virus                   | 10131 | 2  |
| NKV    | AF144692 | Rio Bravo virus                        | 10140 | 2  |
| NKV    | AF285080 | Tamana bat virus                       | 10053 | 15 |
| TBFV   | JF416955 | Alkhumra hemorrhagic fever virus       | 10749 | 2  |
| TBFV   | AF311056 | Deer tick virus                        | 10800 | 2  |
| TBFV   | DQ235145 | Gadgets Gully virus                    | 10251 | 2  |
| TBFV   | DQ235153 | Greek goat encephalitis virus          | 10245 | 2  |
| TBFV   | DQ235146 | Kadam virus                            | 10215 | 2  |
| TBFV   | KF815940 | Kama virus                             | 10688 | 2  |
| TBFV   | AY863002 | Karshi virus                           | 10653 | 2  |
| TBFV   | JF416958 | Kyasanur forest disease virus          | 10774 | 2  |
| TBFV   | EU790644 | Langat virus                           | 10943 | 2  |
| TBFV   | KP144331 | Louping ill virus                      | 10880 | 2  |
| TBFV   | DQ235144 | Meaban virus                           | 10266 | 2  |
| TBFV   | KT224355 | Negishi virus                          | 10605 | 2  |
| TBFV   | AY193805 | Omsk hemorrhagic fever virus           | 10787 | 2  |
| TBFV   | KT224351 | Powassan virus                         | 10742 | 2  |
| TBFV   | DQ235149 | Royal Farm virus                       | 10254 | 2  |
| TBFV   | DQ235150 | Saumarez Reef virus                    | 10269 | 2  |
| TBFV   | KP144332 | Spanish goat encephalitis virus        | 10870 | 2  |
| TBFV   | DQ235152 | Spanish sheep encephalitis virus       | 10245 | 2  |
| TBFV   | U27491   | Tick borne encephalitis virus          | 11141 | 2  |
| TBFV   | DQ235151 | Turkish sheep encephalitis virus       | 10245 | 2  |
| TBFV   | KF815939 | Tyuleny virus                          | 10673 | 2  |
| Marine | MK473878 | Crangon crangon flavivirus             | 11434 | 16 |
| Marine | MF776369 | Cyclopterus lumpus virus               | 11454 | 17 |



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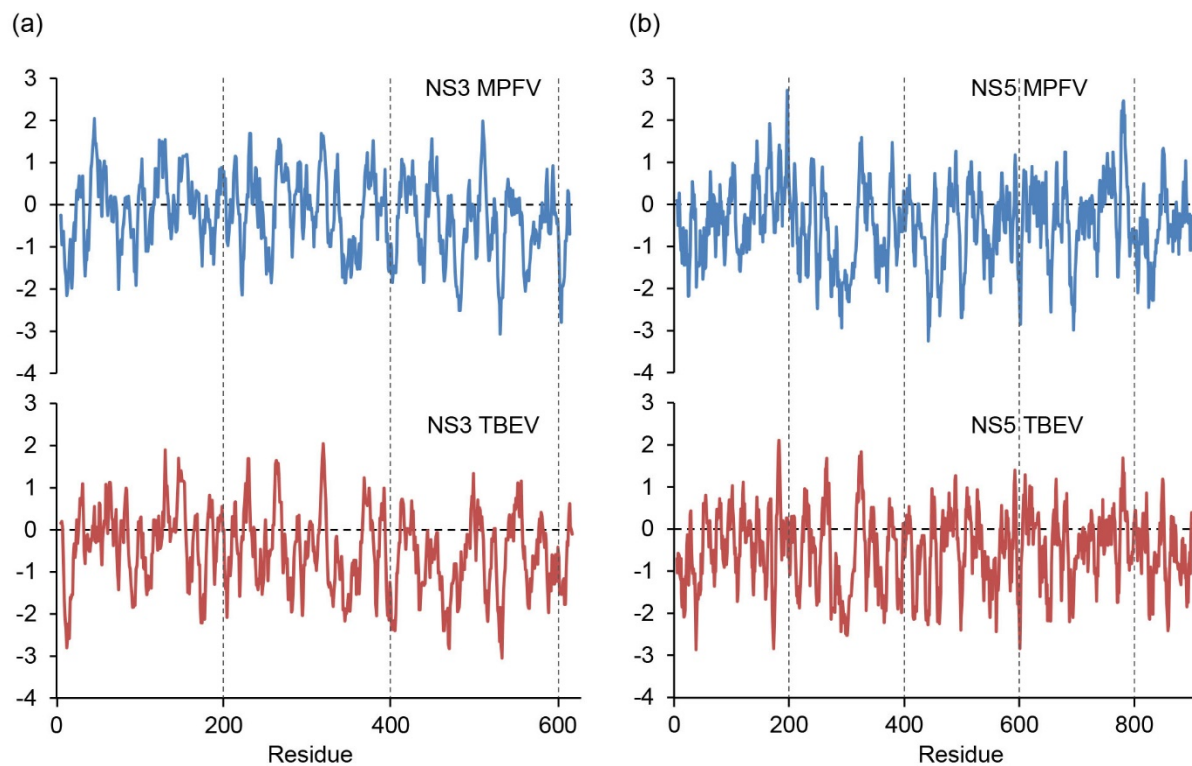


Fig. S1

Comparison analyses of hydropathy profile. Amino acid hydropathy values of NS3 (a) and NS5 (b) were determined by Kyte and Doolittle scale. Those of Mpulungu flavivirus (MPFV) were compared with tick-borne encephalitis virus (TBEV). Negative scores mean a hydrophilic domain. A sliding window size of nine amino acids.

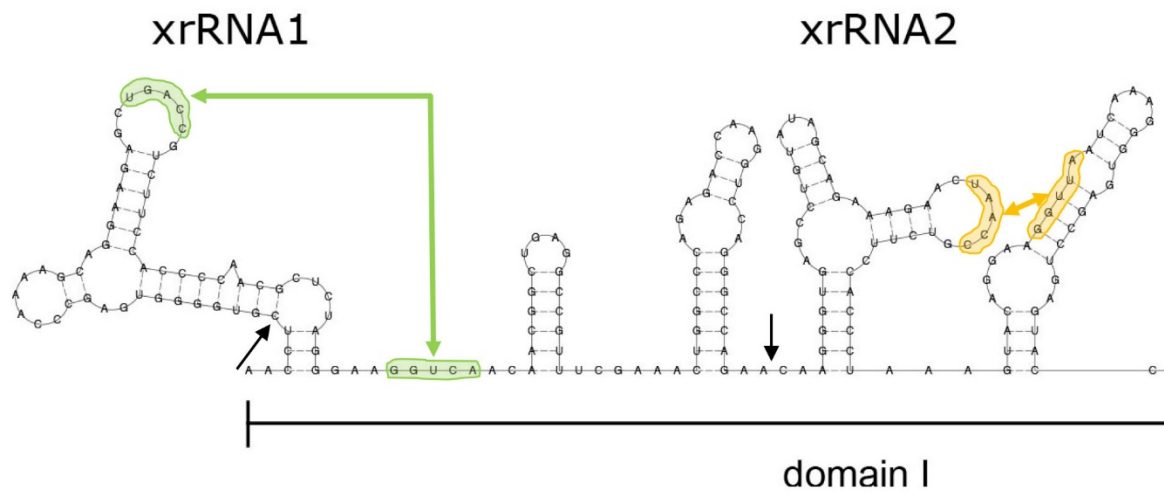


Fig. S2

Characterization of xrRNA1 and xrRNA2 derived from Mpulungu flavivirus (MPFV). Secondary structures of relevant parts of the 3'-UTR of MPFV. The mapped Xrn1 halt sites is indicated with black arrows. Green and orange arrows indicate potential pseudoknot interactions between complementary regions in xrRNA1 and xrRNA2, respectively.

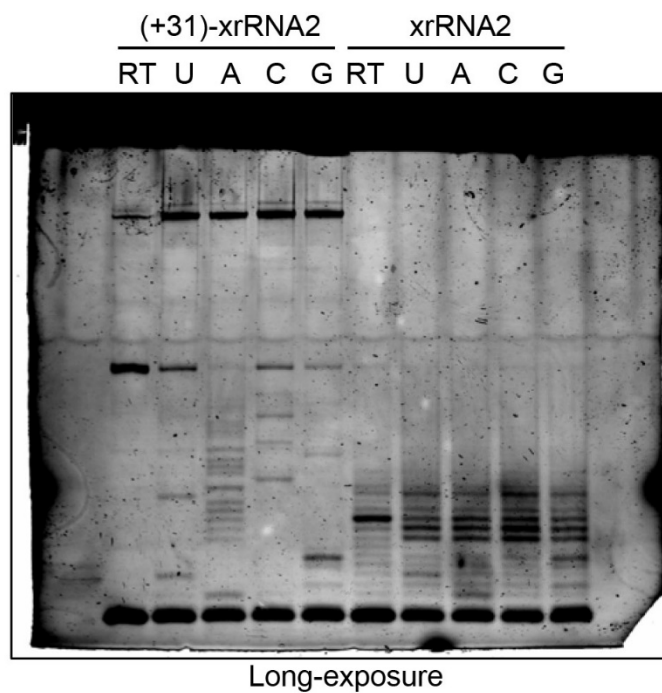
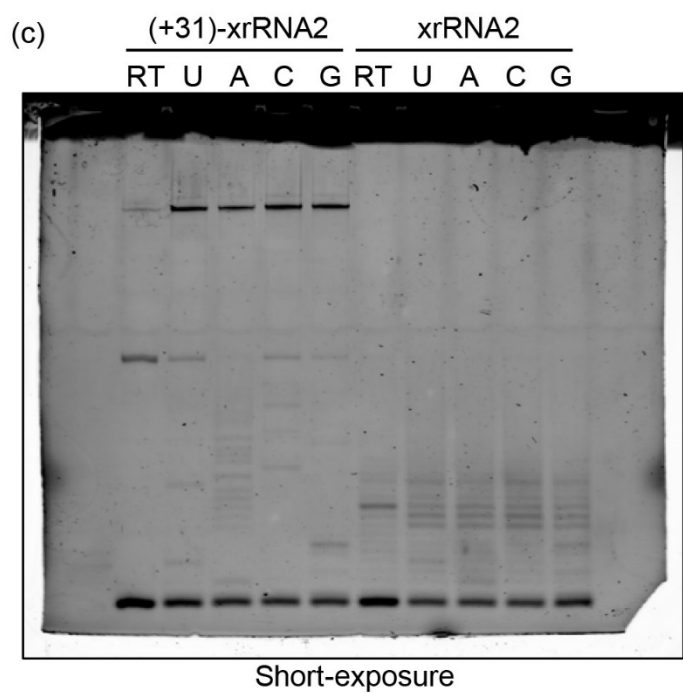
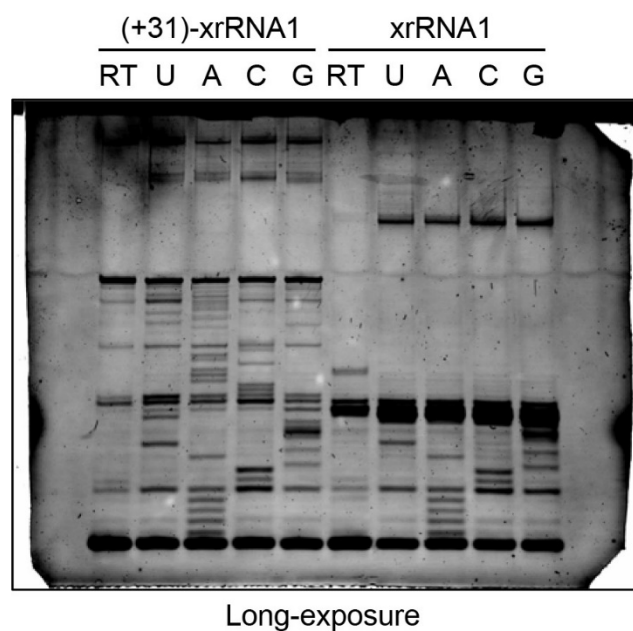
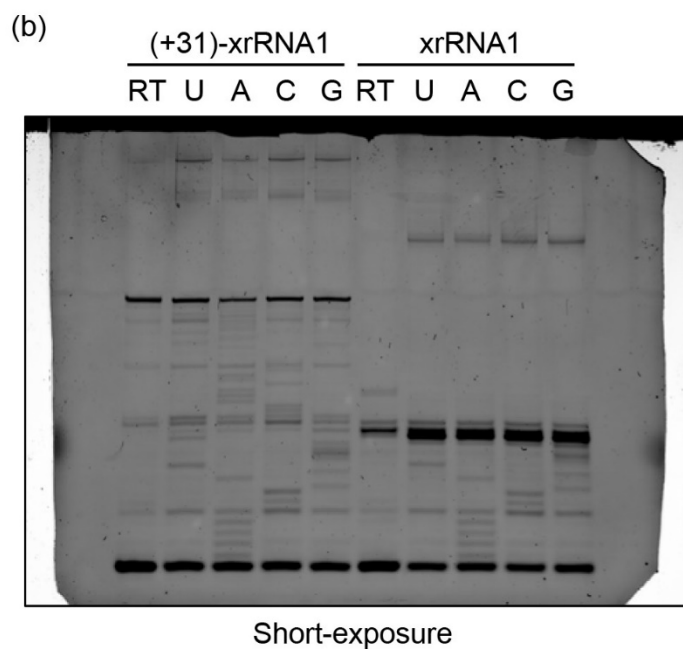
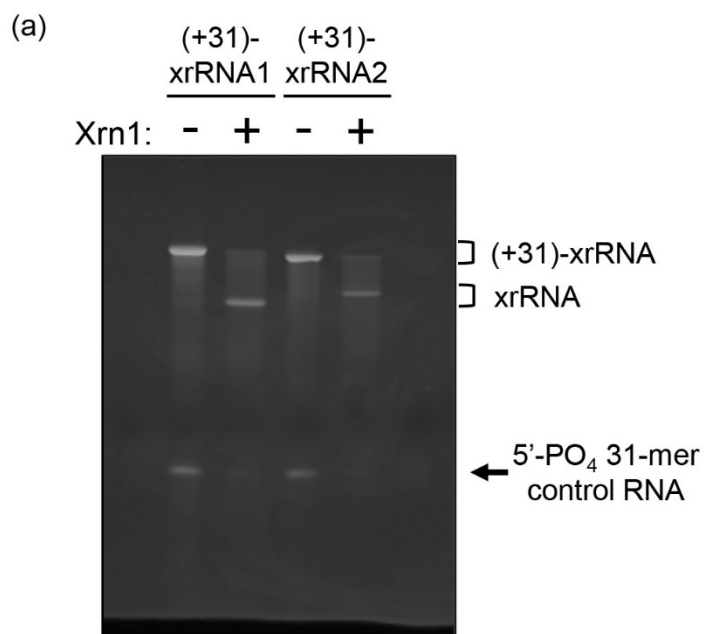


Fig. S3

Images of original full-length gels partly represented in Fig. 6 (c), 6 (d) and 6 (e). Images of full-length gels are represented in both short and long exposure conditions (b, c).