# Supporting Information

**Terasaki et al. 10.1073/pnas.1013155108**

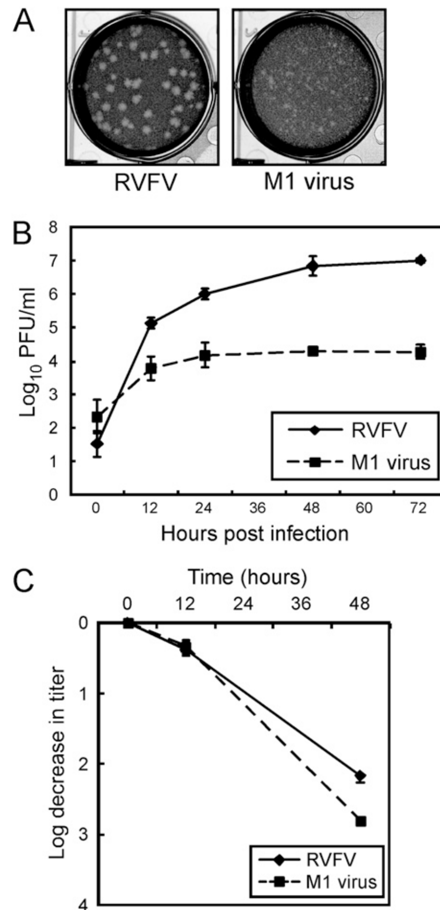


**Fig. S1.** Plaque phenotypes (*A*), growth kinetics (*B*), and the thermal inactivation of the infectivity of RVFV and M1 virus at 42 °C. (*A*) Vero cells were infected with the MP-12 strain of RVFV or M1 virus. After virus adsorption for 1 h, cells were overlayed by media containing 0.45% Noble agar, Dulbecco's modified eagle medium, 10% tryptose phosphate broth, and 10% FBS. Plaques were stained with neutral red at 3 d p.i. (*B*) Vero cells were infected with RVFV and M1 virus at a multiplicity of infection (MOI) of 0.01, and the culture supernatants were collected at various times p.i. Virus titers were determined by plaque assays. (*C*) RVFV and M1 virus were incubated at 42 °C in medium containing Dulbecco's modified eagle medium and 10% FBS. At indicated times of incubation, virus infectivities were determined by plaque assays and the data are expressed as mean ± SD of three experiments. The infectivities of both viruses after 12 h incubation had no statistically significant difference, whereas the infectivity of RVFV was statistically higher than that of M1 virus after 48 h incubation ($P < 0.01$; Student's *t* test).

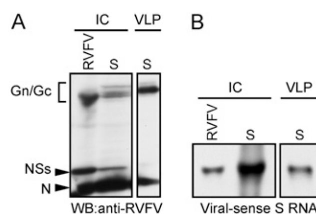


**Fig. S2.** Analysis of S RNA packaging into VLPs. BSR-T7/5 cells were cotransfected with protein expression plasmids, each expressing L protein, N protein and the viral envelope Gn and Gc proteins, and plasmid-expressing antiviral-sense S RNA (S). RVFV, RVFV-infected cells. VLPs, intracellular RNA, and intracellular proteins were collected at 3 d posttransfection. (*A*) Western blot analysis of virus-specific intracellular proteins (IC) and VLP proteins. (*B*) Northern blot analysis of virus-specific intracellular RNA (IC) and viral RNA packaged into VLPs by using an RNA probe that hybridizes with viral-sense S RNA.

**Fig. S3.** Analysis of VLPs carrying L, M, and S RNAs and those carrying L2, M, and S RNAs. (*A*) VLPs, intracellular RNAs, and intracellular proteins were collected from cells supporting the replication of L, M, and S RNAs (lane 1) or from those supporting the replication of L2, M, and S RNAs (lane 2) at 3 d posttransfection. (*Left*) Western blot analysis of virus-specific intracellular (IC) and VLP proteins. Mock, mock-infected cells; RVFV, RVFV-infected cells. (*Middle* and *Right*) Northern blot analysis of virus-specific intracellular RNAs (IC) and viral RNAs in VLPs. *Top*, *Middle*, and *Bottom* represent viral-sense L RNA or L2 RNA, viral-sense M RNA, and viral-sense S RNA, respectively.

**Fig. S4.** Characterization of M1 virus. (*A*) RVFV (lane 1) and M1 virus (lane 2) were recovered from plasmid-transfected cells and then inoculated into BSRT7/5 cells without dilution. Intracellular RNAs were extracted at 24 h p.i. and subjected to Northern blot analysis by using RNA probes, each of which hybridizes with viral-sense L RNA (L), viral-sense M RNA (M), or viral-sense S RNA (S). (*B*) Vero cells were inoculated with RVFV (lane 1) or M1 virus (lane 2) at an MOI of 0.1. The culture medium was harvested at 2 d p.i. and released viruses were purified as described in *Materials and Methods*. RNAs were extracted from purified viruses and subjected to Northern blot analysis by using RNA probes, each of which hybridizes with viral-sense L RNA (L), viral-sense M RNA (M), or viral-sense S RNA (S). (*C*) Vero cells were inoculated with RVFV and M1 virus at an MOI of 0.1. The culture medium was harvested at 2 d p.i. and clarified by centrifugation at 3,000 rpm for 15 min in a tabletop centrifuge. Then, virus particles were partially purified by two subsequent untracentrifugation on a discontinuous sucrose gradient consisting of 20, 30, 50, and 60% (wt/vol) sucrose by using a Beckman SW28 rotor; the sample was first centrifuged at 26,000 rpm for 3 h, and the virus particles at the interface of 30 and 50% sucrose were further centrifuged at 26,000 rpm for 18 h. The virus particles at the interface of 30 and 50% sucrose were collected, diluted and then further applied on a continuous sucrose gradient of 20–60% sucrose. The samples were centrifuged at 26,000 rpm for 18 h. Subsequently, 12 fractions were collected, and the sucrose density in each fraction was measured (*Top*). Virus particles in the each fraction were pelleted through a 20% sucrose cushion at 38,000 rpm for 2 h by using a Beckman SW41 rotor. The viruses in the pellet were dissolved in sample buffer and subjected to Western blot analysis by using an anti-RVFV polyclonal antibody.