Replication of single viruses across the kingdoms, Fungi, Plantae and Animalia

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Summary (201 words)

It is extremely rare that a single virus crosses host barriers across multiple kingdoms. Based on phylogenetic and paleo-virological analyses, it has previously been hypothesized that single members of the family *Partitiviridae* could cross multiple kingdoms. *Partitiviridae* accommodates members characterized by their simple bi-segmented double-stranded RNA genome; asymptomatic infections of host organisms; the absence of an extracellular route for entry in nature; and collectively broad host range. Herein, we show the replicability of single fungal partitiviruses in three kingdoms of host organisms: Fungi, Plantae and Animalia. Betapartitiviruses, originally isolated from a persistently infected phytopathogenic fungus (*Rosellinia necatrix*), could replicate in protoplasts of the carrot (*Daucus carota*), *Nicotiana benthamiana* and *Nicotiana tabacum*, in some cases reaching a level detectable by agarose gel electrophoresis. Moreover, betapartitiviruses showed more robust replication than the tested alphapartitiviruses. One of the fungal betapartitiviruses, RnPV18, could persistently and stably infect carrot plants regenerated from virion-transfected protoplasts. Both alpha- and betapartitiviruses, although with different host preference, could replicate in two insect cell lines derived from the fall armyworm *Spodoptera frugiperda* and the fruit fly *Drosophila melanogaster*. Our results indicate the replicability of single partitiviruses in members of three kingdoms, and provide insights into virus adaptation, host jumping and evolution.

Keywords: cross-kingdom infection, partitivirus, mycovirus, Fungi, Animalia, Plantae

Significance (<120 words)

Viruses generally have narrow host ranges due to constraints imposed at various steps of infection, and they rarely cross kingdom barriers. We have found that single fungal partitiviruses with a bipartite double-stranded RNA (dsRNA) genome can replicate in tobacco cells transfected with purified particles and systemically infect whole carrot plants regenerated from transfected protoplasts. There are five partitivirus genera based on phylogeny and hosts. Members of *Betapartitivirus* showed greater replication in plant cells than members of *Alphapartitivirus*. Both alpha- and beta-partitiviruses were capable of entering spontaneously without any transfection reagent and replicating in two insect cell lines from *Spodoptera frugiperda* and *Drosophila melanogaster*. Our results indicate the replicability of single dsRNA viruses in members of three terrestrial kingdoms: Fungi, Plantae and Animalia.

There are several cases in which viruses that naturally infect wild vertebrates undergo multi-step host-jump (spill-over) eventually into humans and cause epidemics and/or pandemics, as exemplified by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) and Ebola virus(1). Frequent host-jumps are envisaged from recent virus-hunting studies using metagenomic and metatranscriptomic approaches and subsequent phylogenetic analyses(1). Based largely on molecular phylogeny, diverse eukaryotic viruses are predicted to have been transferred horizontally among taxonomically distant organisms(2). In general, these viruses are assumed to switch from one organism to another within single kingdoms, but rarely do so across kingdoms. It is a tenet of modern virology that every virus can infect only a limited number of organisms, whether its range is broad or narrow.

Plants, fungi and animals have their own evolutionary histories and different cellular environments and antiviral immune systems(3-7). These distinct systems are assumed to serve as barriers that prevent cross-kingdom infection and the spread of single viruses at various steps of viral life cycles. However, several viruses have long been known to naturally infect multiple kingdoms of hosts. For example, plant rhabdoviruses, tospoviruses and plant reoviruses, which cause serious diseases in important crops, can replicate in and be transmitted by arthropod vectors(8-11). Recently, there have been several reports of cross-kingdom infections by diverse single viruses between fungi and insects and between plants and fungi(12-18). These studies suggest that single viruses can shuttle naturally or experimentally between two kingdoms of organisms. There are more examples of cellular-level trans-kingdom replication by single viruses(15, 19). An insect RNA virus, Flock House virus (FHV), can replicate at the cellular level in the yeast *Saccharomyces cerevisiae* and plant protoplasts(20, 21). FHV requires plant viral cell-to-cell movement proteins (MPs) to achieve systemic infection in whole plants(22). Nevertheless, there are very few reports showing a tri-kingdom systemic infection by a single virus, which goes against the dogma of virology.

To address whether a single virus can infect or replicate in organisms across Animalia, Plantae and Fungi, we utilized fungal partitiviruses(23). Members of the family *Partitiviridae*, are a group of viruses omnipresent in plants and fungi; two of the five genera (*Alphapartitivirus* and *Betapartitivirus*) accommodate both plant and fungal viruses. Furthermore, partitiviruses or partiti-like viruses have recently been reported from insects(24-26), oomycetes(27), and bacteria(28, 29). Partitiviruses have a bi-segmented, bi-particulate double-stranded RNA (dsRNA) genome of ~ 4 kilobase pairs (kbp)(30, 31). Each segment of ~ 2 kbp encodes an RNA-dependent RNA polymerase (RdRP) or a capsid protein (CP)(32, 33). In general, partitiviruses induce symptomless infections and show efficient vertical transmission through seeds or spores without an extracellular route for entry. Although there is no way to mechanically inoculate plant partitiviruses, virion transfection methods are available for some fungal partitiviruses(34-36). Partitiviruses are predicted to have switched hosts at an evolutionary time scale

between plants and associated fungi based on the fact that fungal and plant partitiviruses phylogenetically cluster together in a single clade(37). In support of this notion, a fungal partitivirus could replicate in protoplasts derived from a few plant species(19). It is of great interest that fungal partitivirus–related sequences have been detected in plant nuclear genomes. These endogenization events of partitiviruses, dating several tens million years ago, represent their infection records and imply horizontal transfer of partitiviruses between the two kingdoms(38, 39).

All of the above-mentioned considerations, observations and reports prompted us to hypothesize that some extant partitiviruses may be able to replicate in organisms of the three kingdoms: Animalia, Plantae and Fungi. The objective of this study is to show cross-kingdom infection of single fungal partitiviruses and antiviral RNA interference (RNAi) or RNA silencing against them with different virus-derived small RNA (vsiRNA) profiles in the kingdoms Animalia, Plantae and Fungi.

Results

Greater replication levels of fungal betapartitiviruses in plant cells than alphapartitiviruses. First, we tested the replicability of fungal partitiviruses in single plant cells (plant protoplasts). To this end, we attempted to transfect *Nicotiana benthamiana* protoplasts by two fungal partitiviruses (RnPV18 and RnPV19) with and without using polyethylene glycol (PEG), as some isometrical plant viruses were earlier reported to be able to enter and replicate in protoplasts without any transfection-promoting reagent(40). Consequently, PEG was found to provide much higher transfection efficiency approximately by 8-fold, while transfection without PEG also resulted in replication of RnPV18 to a certain level (*SI Appendix*, Fig. S1). Thus, PEG-mediated virion transfection was conducted in the subsequent experiments. We largely used six Rosellinia necatrix partitiviruses (RnPVs), three fungal alphapartitiviruses (RnPV2, RnPV10, and RnPV19) and three fungal betapartitiviruses (RnPV6, RnPV11 and RnPV18) isolated from the phytopathogenic filamentous ascomycete, *R. necatrix* (Fig. 1*A* and *SI Appendix*, Table S1). These viruses were isolated from independent Japanese fungal strains, except for RnPV18 and RnPV19, which were isolated from a single co-infected fungal strain (W442). They accumulated comparably in this strain and were molecularly and biologically characterized(41). Three plant species tested as fungal partitivirus hosts were carrot (*Daucus carota* subsp. *sativus*), and wild and cultivated tobacco species (*N. benthamiana* and *N. tabacum*, cultivar Bright Yellow 2 [BY-2]) protoplasts (*SI Appendix*, Table S1). Carrot has been recognized as a model plant in tissue culture and transformation, and regeneration methods through somatic embryogenesis are well established(42, 43). *N. benthamiana* is an important model plant to study virus–host interactions due to its susceptibility to virus infection(19, 44, 45). The entire experimental procedure is illustrated in Fig. 1*A*.

We purified the virions from singly or multiply infected *R. necatrix* fungal strains to transfect the

protoplasts of host organisms (*SI Appendix*, Table S1). We investigated virus replication with time-course analysis using agarose gel electrophoresis, northern blotting and reverse transcription–quantitative polymerase chain reaction (RT-qPCR) (*SI Appendix*, Table S1). We selected four time points (0, 24, 48 and 72 h post-transfection [hpt]) for the study with plant protoplasts. We compared virus accumulation at different timepoints to that at 0 hpt, which we arbitrarily set as 1 in RT-qPCR. We transfected *N. benthamiana* protoplasts with three betapartitiviruses (RnPV6, RnPV11 and RnPV18) and three alphapartitiviruses (RnPV2, RnPV10 and RnPV19). Comparison of the virus accumulation using RT-qPCR revealed that RnPV11 accumulation was approximately 100-fold and ~450-fold higher at 48 and 72 hpt than at 0 hpt (Fig. 1*B*). The tested alphapartitiviruses (RnPV2 and RnPV10) showed little or no replication (Fig. 1*B* and *SI Appendix*, Fig. S2*A*). The greater replication of betapartitiviruses was validated with another set of partitiviruses, namely RnPV18 and RnPV19 that coinfected a single fungal strain, *R. necatrix* W442(41). This experimental setting allowed us to eliminate the possibility that different particle inoculum preparations with different competency might have caused dissimilar replication profiles. RnPV18 dsRNA1 accumulation increased steadily to ~2,500-fold at 72 hpt, while RnPV19 replication was lower (114-fold) (Fig. 1*C*). Particle preparations from W442 contained equal amounts of RnPV18 and RnPV19 particles based on agarose gel electrophoresis of their genomic dsRNA (see lane dsRNA VP in Fig. 1*E*). The RT-qPCR results were congruent with the agarose gel electrophoretic analyses of the total RNA fractions. There were distinct genomic dsRNA bands of RnPV6 (faint), RnPV11 (Fig. 1*D* and *SI Appendix*, Fig. S2*B* and *C*) and RnPV18 in all the three replicates at 48 hpt, with increased intensity at 72 hpt. However, no band was likely visible for RnPV2, RnPV10 and RnPV19 (Fig. 1*E*). The above results showed that replication of betapartitiviruses (RnPV6, RnPV11 and RnPV18) was much greater than that of alphapartitiviruses (RnPV2, RnPV10 and RnPV19) or that of authentic plant partitiviruses.

There was a similar trend for alpha- and betapartitiviruses in *N. tabacum* BY-2 as well as in carrot protoplasts. Based on the RT-qPCR results, it was obvious that betapartitiviruses replicated considerably, unlike the alphapartitiviruses. RnPV11 (a betapartitivirus) in BY-2 showed an over 100-fold increase in accumulation at 72 hpt compared with 0 hpt (*SI Appendix*, Fig. S3*A* and *B*). Likewise, replication of the betapartitivirus RnPV18 was higher than RnPV19 in carrot, registering a ~20-fold increase at 72 hpt compared with the 2-fold increase of RnPV19 (*SI Appendix*, Fig. S3*C*). RnPV2 (an alphapartitivirus) replication was not detectable in carrot protoplasts (*SI Appendix*, Fig. S3*D*). It was interesting that we observed the dsRNA band of RnPV11 at 72 hpt in tobacco protoplasts (*SI Appendix*, Fig. S3*B*), a sign of high-levels of replication.

These combined results indicate that fungal betapartitiviruses can replicate much better than fungal alphapartitiviruses in plant protoplasts.

Stable persistent infection of carrot plants regenerated from RnPV18-transfected protoplasts. Carrot protoplasts could host fungal betapartitiviruses (RnPV18 and RnPV11) (*SI Appendix*, Fig. S3*C* and *D*). We tested whether plantlets or plants could maintain the fungal betapartitivirus stably in every tissue. To this end, we regenerated plants from carrot protoplast cells transfected with RnPV18 (a betapartitivirus) plus RnPV19 (an alphapartitivirus) via callus formation and somatic embryogenesis (Fig. 2*A*). We screened almost 50 calluses for virus infection, and obtained three RnPV18-positive calluses (Fig. 2*B*) but not RnPV19-positive calluses. At several subsequent stages of regeneration—for example, callus formation, somatic embryogenesis, then shoot and root regeneration stages—we selected virus-positive samples by RT-PCR. We obtained seven plants from four independent calluses and grew them in sterilized soil in pots. We systematically detected RnPV18 in leaves and roots by RT-PCR; it was stably maintained for several months in the laboratory conditions (Fig. 2*C*). We also observed virus particles of ~30 nm in diameter in a semi-purified preparation from carrot plants, similar to those from carrot calluses (Fig. 2*D* and *E*). Surprisingly, we noted variable levels of virus accumulation across different regenerated RnPV18-infected carrot plantlets via northern blotting (*SI Appendix*, Fig. S4).

We were interested to determine whether RnPV18 caused symptoms and was vertically transmitted in persistently infected plants. Eight RnPV18-infected carrot plants independently regenerated were monitored and appeared to be heathy before the flowering stage after vernalization at 4°C for three months. However, most of the eight vernalized virus-infected plants suddenly wilted and died shortly before flowering (*SI Appendix*, Fig. S5*A*), despite that all of three virus-free carrot plants regenerated through somatic embryogenesis, which were grown under the same growth conditions in parallel with virus-infected plants, produced self-pollinated seeds with germinability (*SI Appendix*, Fig. S5*B*). Only from one RnPV18-infected plant, we managed to obtain immature seeds which turned out to be unable to germinate. Importantly each of these immature seeds tested RnPV18-positive when examined by RT-PCR (*SI Appendix*, Fig. S5*C*). To determine whether the sudden death was induced by RnPV18 or whether RnPV18 could be vertically transmitted through seeds required further experiments.

Sequence analyses showed that the RnPV18 genomic segments isolated from infected calluses and immature seeds were nearly identical. Only one non-synonymous substitution at dsRNA2 nucleotide map position 1714 was detected. When compared between the immature seed-derived RnPV18 genomic sequences and that previously reported from the original fungal host strain (LC517384 for dsRNA1 and LC517386 for dsRNA2), a total of six substitutions were observed on the coding regions: three nonsynonymous (map positions 434, 1622, and 1637) and one synonymous substitutions (map position 2215), and one nonsynonymous (map position 1714) and one synonymous mutation (map position1613)

in dsRNA1 and dsRNA2, respectively. Whether these sequence differences imply viral adaptions to the new plant host remains unknown.

Replication of fungal partitiviruses in animals (insect cells). First, we tested two reagents, X-treme GENE HP DNA and the ProteoJuice Protein transfection reagent, for their ability to introduce RnPV18 and RnPV19 into Sf9 cells from the fall armyworm (*Spodoptera frugiperda*). We determined the fold change at different time points with reference to 0 hpt, set as 1, similarly to the plant protoplast transfection experiments. We assessed replication of the viruses with time course analysis using RT-qPCR, agarose gel electrophoresis and northern blotting. The protein transfection reagent failed to greatly promote infection of Sf9 cells by RnPV18 or RnPV19, while there was a slight increase in RnPV18 (4.7-fold) and RnPV19 (14-fold) accumulation at 72 hpt in Sf9 cells when we used the DNA transfection reagent (*SI Appendix*, Fig. S6). Surprisingly, treatment of Sf9 cells with purified particles in the absence of a reagent allowed the highest replication of both RnPV18 and RnPV19 (10- and 70-fold, respectively) (*SI Appendix*, Fig. S6). We tested the possibility that fungal partitiviruses were able to replicate in Sf9 cells and S2 cells from the fruit fly (*Drosophila melanogaster*), using RnPV11 (a betapartitivirus), RnPV18 (a betapartitivirus) and RnPV19 (an alphapartitivirus) with three time points (0, 24, 96 hpt). RnPV19 showed a 63-fold increase at 96 hpt, while RnPV18 only rose 9-fold in Sf9 cells (Fig. 3*A*). These finding suggest that the viruses could efficiently enter spontaneously and multiply in Sf9 cells. We performed experiments with S2 cells without a transfection reagent. Differently from the results in Sf9 cells, RnPV18 replicated highly compared with RnPV19. There was a 225-fold increase in virus accumulation for RnPV18 at 96 hpt compared with an 18-fold change for RnPV19 (Fig. 3*B*). Likewise, RnPV11 showed a 35-fold change at 96 hpt compared with 0 hpt in S2 cells; there was lower replication in Sf9 cells (2.75-fold) (*SI Appendix*, Fig. S7).

We further confirmed accumulation of RnPV18 and RnPV19 in Sf9 and S2 cells at three time points (0, 24 and 96 hpt) by northern blotting. The results were in accordance with the RT-qPCR data. The RnPV19 dsRNA1 (mRNA) band was detectable in total RNA fractions from Sf9 cells transfected with RnPV18 plus RnPV19, whereas no RnPV18 dsRNA1 (mRNA) band was observed (Fig. 3*C*). We observed the inverse northern blot pattern for transfection of S2 cells. RnPV18 replicated highly as opposed to RnPV19 in S2 cells (Fig. 3*D*). The RnPV11 band was detectable in the total RNA fraction from S2 cells transfected with RnPV11 but was undetected in Sf9 cells (*SI Appendix*, Fig. S7).

We also tested two different mammalian cell lines: Vero cells originated from African green monkey kidney and BHK-21 cells derived from baby Syrian hamster kidney. However, no or potentially very low replication of RnPV18 or RnPV19 was observed in these cells, regardless of the use of a transfection reagent or of the temperature for cell culture (28°C or 37°C) (*SI Appendix*, Fig. S8).

In summary, RnPV19 (an alphapartitivirus) could infect Sf9 insect cells and replicate more efficiently than RnPV18 (a betapartitivirus). However, in S2 cells, the opposite was true: betapartitivirus replicated much highly than alphapartitivirus.

Fungal partitiviruses are targeted by the RNA silencing in the original and new experimental host organisms. RNA silencing is the primary antiviral defense pathway in plants, fungi and insects. We compared the profiles of RnPV18- and RnPV19-derived small interfering RNAs (siRNAs) among three kingdoms of host organisms. These viruses have been shown to induce RNA silencing at different levels in an experimental filamentous ascomycete host *Cryphonectria parasitica*(46). RnPV18 induced antiviral RNA silencing in *C. parasitica* to a much greater extent than RnPV19. We analyzed the siRNA produced in *N. benthamiana* and *D. melanogaster* S2 cells 48 hpt with RnPV18 and RnPV19. Both partitiviruses were targeted by the silencing machinery in the tested organisms as judged by the production of viral siRNAs (vsiRNAs). *Nicotiana benthamiana* and *D. melanogaster* produced vsiRNAs in response to the cellular-level infection. *Nicotiana benthamiana* protoplasts produced a peak of 21-nucleotide (nt) vsiRNAs derived from RnPV18 and RnPV19. There was a reduction of one or two orders of magnitude in vsiRNAs derived from RnPV19 relative to those from RnPV18 (~740 and ~10 reads vs. ~27,000 and ~1800 reads for dsRNA1 and dsRNA2, respectively), likely reflecting the lower level of replication in *N. benthamiana* (Figs. 4*A* and *B, SI Appendix*, Fig. S9*A* and *B*). In *D. melanogaster* S2 cells, we detected vsiRNAs with a peak of 21 nt, while the number of reads was considerably low (Fig. 4*A* and *B, SI Appendix*, Fig. S9*A* and *B*). We also analyzed vsiRNAs in the natural host fungus *R. necatrix* strains persistently infected with the viruses (Figs. 4*A* and 4*B, SI Appendix*, Fig. S9*A* and S9*B*). In *R. necatrix*, infection by RnPV19 led to comparable production of vsiRNAs (~10,000 and ~70 reads for dsRNA1/dsRNA2) with a peak of 20-nt to those from RnPV18 (~6,000 and ~2,600 reads for dsRNA1/dsRNA2) (Fig. 4*A* and *B, SI Appendix*, Fig. S9*A* and S9*B*). The vsiRNA peak in this fungus was at 1 nt shorter than those in *D. melanogaster* and *N. benthamiana*. The vsiRNAs derived from negative-sense strands were slightly greater in amount than those from the positive-sense strands in all virus/host combinations except RnPV18 vsiRNAs in *N. benthamiana*, which provided an almost equal number of reads regardless of the virus and host organisms. Another interesting finding was a consistently higher amount of RdRP-encoding dsRNA1-derived siRNAs than CP-encoding dsRNA2-derived vsiRNAs in all tested host organisms. Mapping of vsiRNAs to their genomic RNAs indicated different but similar profiles between the host organisms (Figs. 4*C* and *D, SI Appendix*, Fig. S9*C* and *D*). For example, we detected some common hot spots for *N. benthamiana* and *D. melanogaster*, but also detected many others at different map positions for the two organisms *N. benthamiana* and *D. melanogaster*. Another interesting difference was found in A or U preference for the 5' termini of 20–22

nt vsiRNA; vsiRNAs with 5'-U or -A was much greater in *R. necatrix* (over 90%) than in fruit fly (~40–60%) or *N. benthamiana* (~40-60%) (Figs. 4*E* and *F*, *SI Appendix*, Fig. S9*E* and *F*).

These results strongly suggest that fungal partitiviruses are targeted by anti-viral RNA silencing in newly established host cells likely in a different manner.

Discussion

The family *Partitiviridae* accommodates plant-, fungus-, and protozoa-infecting members(30, 31). Partiti-like viruses as infectious entity have also been isolated from insects(25). Furthermore, partiti-like viruses have recently been reported from prokaryotic bacteria(28, 29). This study compellingly showed the replicability of a few partitiviruses in cells of three kingdoms, Fungi (original host, *R. necatrix*), Plantae (*N. tabacum*, *N. benthamiana* and carrot) and Animalia (*S. frugiperda* and *D. melanogaster*, at least their cultured cell lines) (*SI Appendix*, Fig. S10). We have previously shown that all the partitiviruses used in this study cross sub-class barriers to replicate efficiently and systemically in a filamentous fungal model host, *C. parasitica*(41). Interestingly, the members of the single host kingdoms Plantae and Animalia showed different degrees of susceptibility to members of the two partitivirus genera (Figs. 1 to 3). Extant partitiviruses have likely retained their ability to replicate in fungi, plants and insects during the course of evolution, which may reflect the corresponding ability that ancestor partitiviruses might have possessed. In this perspective, it is of interest to speculate that partitiviral ancestors might have constituted the virome of the last universal common ancestor (LUCA) and been lost from the archaea given the lack of any extant RNA viruses in this domain of life, as inferred for another group of RNA viruses, leviviruses, known as members of the RNA phage family *Leviviridae*(47). This study should inspire a lot of novel research and poses interesting open questions, as discussed below.

Plant partitiviruses generally induce asymptomatic infections like many other persistent plant viruses including positive-sense (+) single-stranded RNA ([+]ssRNA) viruses (mitoviruses and endornaviruses) and dsRNA viruses (chrysoviruses and totiviruses)(23, 48). Some plant persistent RNA viruses have been suggested to exert beneficial effects such as drought tolerance and innate immune response activation in host plants(49, 50). However, no inoculation method was developed for these persistent viruses. The method developed in this study for partitivirus inoculation is useful for preparing other crops systemically infected by other persistent viruses and examining them for possible tolerance to abiotic and abiotic stresses.

There should be robust barriers against trans-kingdom replication by single viruses. Our study showed that the intracellular conditions of the three kingdoms are suitable for the essential replication processes of partitiviruses such as RNA replication and probably virion assembly. Namely, we found that host factors required for these viral processes are commonly supplied by the three kingdoms and that the

antiviral defense is not sufficient to fully restrict partitivirus cellular-level replication. While it remains unknown how a single partitivirus can adjust to and subsequently replicate in cells from different kingdoms, the replication strategy of the dsRNA partitivirus as a dsRNA virus may be related to this phenomenon. Partitiviruses are packaged together with their dsRNA and RdRP, and are believed to replicate inside the particles in infected cells like other known dsRNA viruses, as has been observed *in vitro*(51). This replication strategy appears not to require many essential host factors for partitivirus replication, unlike the plethora of essential host factors required for efficient replication of positive-sense (+)RNA viruses(52-54). Of course, we cannot rule out the possibility that essential host factors have been conserved between host organisms and thus support partitivirus replication.

Of the newly detected hosts, the plant hosts allowed for persistent infection at the individual level by the betapartitivirus RnPV18 but not by the alphapartitivirus RnPV19. This finding is expected given the greater susceptibility of plant protoplasts to fungal betapartitiviruses. Researchers have suggested that CPs from alphapartitiviruses are localized in the nucleus of plant and fungal cells, while those of betapartitiviruses are localized in the cytoplasm(55). It should be clarified whether this difference in the subcellular distribution of partitivirus CP is related to the observed extent of replication between the two genera. Of note, the insect virus FHV, which has a (+)RNA genome, has been shown in separate studies(20, 21) to replicate in *S. cerevisiae* and plant protoplasts. However, FHV requires plant viral MPs to achieve systemic infection of whole plants(22). In general, plant viruses require MPs and other viral proteins, typically CP, for systemic infections. However, plant partitiviruses violate this rule and do not encode MP required for cell-to-cell movement. It is believed that plant partitiviruses achieve systemic infection via divisions of embryogenic cells carrying vertically transmitted viruses(36). In this study, carrot protoplasts that had been transfected with virus could retain the virus during their development into plants, similarly to authentic plant partitiviruses (Fig. 2).

RNA silencing is the primary antiviral defense that commonly operates in plants, fungi and insects. While the key players of RNA silencing are conserved across the three kingdoms, there are some differences. For example, only one Dicer and Argonaute (or called as Dicer-like or Argonaute-like proteins) participate in antiviral RNA silencing in the model insect and fungal hosts *D. melanogaster* and *C. parasitica*(3, 6, 56), while in plants multiple Dicer and Argonaute proteins function redundantly(57, 58). Partitiviruses are targeted by RNA silencing in fungi(41, 46, 59), and a fungal partitivirus CP has recently been identified as an RNA silencing suppressor(60). However, little is known about anti-partitivirus RNA silencing and counter-defense by partitiviruses in other kingdoms of hosts. We found that *N. benthamiana* protoplasts support partitivirus replication better than carrot or *N. tabacum* BY-2 protoplasts (Figs. 1 and *SI Appendix*, Figs. S2 to S3). When comparing between BY-2 and *N. benthamiana* cells, the accumulation of RnPV11 was considerably higher in *N. benthamiana* cells than in

BY-2 cells (compare ~450-fold vs. ~110-fold for the dsRNA1 band at 72 hpt in Fig. 1 vs. *SI Appendix*, Fig. S3). *Nicotiana tabacum* is expected to have more robust antiviral RNA silencing than *N. benthamiana* that supports replication of diverse plant viruses, partly because of the deficiency of one of the RNA-silencing genes(45). Furthermore, the vsiRNA production with peaks at 21 nt substantiate that the tested partitiviruses were targeted in the insect cells as they were in the plant and fungal cells. Interestingly, we observed different vsiRNA distribution profiles with different hot spots in different hosts—that is, the natural fungal host *R. necatrix*, the experimental plant host *N. benthamiana* and insect S2 cells (Fig. 4, and *SI Appendix*, Fig. S9). This finding may reflect the possible difference in recognition and dicing pattern for partitivirus dsRNA or its structured mRNA by Dicer or Dicer-like protein in the two host kingdoms.

Another finding of great impact is that fungal partitiviruses (RnPV18 and RnPV19) could infect insect cells without a transfection reagent (Fig. 3 and *SI Appendix*, Fig. S6). This phenomenon raises an intriguing question: How do these partitivirus particles enter insect cells? Partitiviruses have no membranous envelope. Generally, non-enveloped animal viruses enter host cells via one of three mechanisms after initial contact with their receptor molecules, whether specific or non-specific: macropinocytosis, clathrin-mediated endocytosis and caveolin mediated endocytosis(61). We initially anticipated that the examined fungal partitiviruses lacked a receptor-binding attachment to enter the insect cells. Thus, the observation that addition of purified fungal partitivirus particles to insect cells without a transfection reagent allowed for virus infection was very surprising. Further investigation is required to determine how fungal partitivirus virions can enter cultured cells from two different classes of insects and how they accomplish membrane penetration to initiate replication in the cytosol post entry. It should be noted that some plant viruses can infect plant protoplasts in the presence(62) or absence(40) of the polycation poly-L-ornithine which is assumed to enhance pinocytosis. This study also showed that RnPV18 can initiate the infection of *N. benthamiana* protoplasts without the polycation or PEG (*SI Appendix*, Fig. S1), as in the case for insect cells. All of these observations likely support the intrinsic ability of partitiviruses to penetrate cells of different host kingdoms by atypical entry mechanisms.

Materials and Methods

Fungal strains, viral strains and insect cell lines. All fungal and viral strains used in this study are listed in *SI Appendix*, Table S1. All partitiviruses were originally isolated from Japanese strains of the phytopathogenic fungus *R. necatrix*. Three alphapartitiviruses (RnPV2, RnPV10, RnPV19) and three betapartitiviruses (RnPV6, RnPV11 and RnPV18) were utilized. Two insect cell lines, *Spodoptera frugiperda* Sf9 and the fruit fly *Drosophila melanogaster* S2 were generously given by Prof. Yoshiharu

Matsuura (Research Institute for Microbial Disease, Osaka University) and purchased from Thermo Fisher Scientific Inc., respectively.

Virus particle purification. Fungal partitivirus particles were purified from approximately 10–40 g (wet weight) of mycelia of the natural host strains of *R. necatrix* cultured in potato dextrose broth (Difco) following the method described by Chiba et al.(35). The mycelia were ground to a powder using a mortar and pestle in liquid nitrogen, and homogenized in 3 or 4 volumes of 0.1 M sodium phosphate, pH 7.0 (PB), β-mercaptoethanol (b-ME) (1/1000 volume) and carbon tetrachloride (CCl_4) (1/4 volume). Homogenates were centrifuged at 2,000 *g* for 20 min (Kubota Model 2800), and the supernatant was re-clarified twice using CCl_4. Virus particles were pelleted by centrifugation at 15,000 x g for 20 minutes (Beckman Coulter Avanti J-26S xp) after stirring in sodium chloride and PEG 6000 at a concentration of 1% and 8%, respectively for 2–3 h on ice. The resultant pellets were suspended in 10 mL of 0.05 M PB (pH 7.0) and centrifuged at 7,600 *g* for 10 min. The supernatant was centrifuged through a 20% sucrose cushion in Beckman 70Ti rotor at 100,000 *g* for 2 h at 4°C. The pellet was eventually resuspended in 100–500 μL of 0.05 M PB (pH 7.0) and subjected to sucrose density (10%–40%) gradient centrifugation at 150,000 *g* for 2 h at 4°C using a Beckman SW41Ti rotor, or used as inocula after resuspended in distilled water filtering through a 0.22 μm mesh filter (Millipore). Virus particle fractions at the bottom third of the gradient were collected and further spun down for pelleting purposes. The pelleted particles were stored at –80°C until use, after filtering through a 0.22 μm mesh filter (Millipore) and quantifying protein absorbance at 280 nm in distilled water.

Plant protoplast preparation and virion transfection. Plant protoplasts were prepared from two solanaceous species: *N. tabacum* and *N. benthamiana*. *N. tabacum* BY-2 cell suspensions were a generous gift of Dr. Takayuki Sasaki at IPSR, Okayama University. Plant protoplasts were prepared from two solanaceous species: *N. tabacum* and *N. benthamiana*. Protoplasts were generated from tobacco BY-2 cell cultures as described by Watanabe et al. (63) with slight modifications. The cell cultures were washed twice with mannitol solution (0.4 M mannitol, 20 mM $MgCl_2$), and then digested with 1% (w/v) Cellulase Onozuka RS (Duchefa, the Netherlands) and 0.1% (w/v) Pectolyase Y-23 (Duchefa) (pH 5.5) dissolved in the mannitol solution for 3 h at 25°C in the dark with gentle mixing at 45 rpm (Taitec Bio-Shaker BR-40LF). The protoplasts were then washed two times as before and resuspended in MMG solution (pH 5.7) containing 0.4 M mannitol, 15 mM $MgCl_2$, and 5 mM 2-(*N*-morpholino)ethanesulfonic acid, (MES) at a concentration of 2×10^6 cells/mL.

Protoplasts were isolated from 5–6-week-old *N. benthamiana* plants using the protocol described by Ambrós et al.(64) and Hyodo et al.(65) with modifications. Two to four fully expanded leaves were

picked and kept in a 9-cm Petri dish. The leaves were cut into 1 mm strips and incubated in an enzyme solution containing 1.0% (w/v) Cellulase RS and 0.5% (w/v) Macerozyme R-10 prepared in MMC buffer [0.5 M mannitol, 10 mM $CaCl_2$ and 5 mM MES (pH 5.7)]. The samples were incubated overnight at 22 ± 2°C in the dark. The digested leaves were filtered through four layers of gauze using a Buchner funnel into a 50 mL Falcon tube. The protoplasts were pelleted at 46 *g* for 3 min (Kubota Model KN-70), centrifuged once more after suspension in 10 mL of MMC solution and then resuspended at a concentration of 3×10^6 cells/mL and incubated on ice for 3 h before transfection.

Plant protoplasts were transfected as described by Hyodo et al.(66) with slight modifications. Protoplasts (400 µL) were mixed with 40 µL of virus particles. Approximately 100 µL of the mixture was aliquoted into four tubes, each representing a different time point. Transfection was performed with or without PEG solution with 0.4 M mannitol only used in the latter. In the case of PEG-mediated transfection, 200 µL of PEG solution (40% [w/v] PEG 4000, 0.4 M mannitol, 200 mM $Ca(NO_3)_2$) was added, and the mixture was diluted with 2 mL of dilution buffer (0.4 M mannitol, 125 mM $CaCl_2$, 5 mM KCl, 5 mM glucose, and 1.5 mM MES, pH 5.7) and incubated on ice for at least 15 min. The transfected cells were washed with 2 mL of dilution buffer. Finally, the protoplasts were resuspended in incubation buffer W5 (154 mM NaCl, 125 mM $CaCl_2$, 5 mM KCl, and 2 mM MES, pH 5.7) at 22 ± 2°C in the dark. For the 0 hpt time point, virus-free protoplasts and one virus-transfected sample were picked and immediately processed for total RNA extraction. The process was stopped at the ethanol precipitation stage and the samples were stored at –80°C until sample collection at the last timepoint (72 hpt). This experiment was repeated three times for all the viruses involved.

Virion transfection of insect and mammalian cells. The Sf9 cell line of *S. frugiperda* (fall armyworm) was cultured as described previously by Suzuki et al.(67). The S2 cell line of *D. melanogaster* was purchased from Thermo Fisher Scientific Inc. (Carlsbad, CA, USA) and cultured according to the manufacturer's instructions. These cultured cells were seeded into 35-mm dishes ($0.5\sim1.0 \times 10^6$/dish). The medium was replaced with 1.5 mL of serum-free Sf900-II (Thermo Fisher Scientific Inc.) (for Sf9 cells) or Schneider's *Drosophila* medium (Thermo Fisher Scientific Inc.) (for S2 cells). Purified virus particles were diluted 100-fold by either by Sf900-II or SDM and incubated at room temperature for 15 min. This particle solution (100 µL) was dropped into a dish of seeded cells. For transfection with transfection reagents, virus particles were preincubated with 5 µL of X-tremeGene DNA Transfection Reagent (Roche) for 10 min at room temperature and mixed with 50 µL of either of the serum-free media, and later with virus particle solution diluted 50-fold with the serum-free media. For ProteoJuice Protein Transfection Reagent (Novagen), 6 µL each of the virus particle solution and reagent was mixed and left

442 at room temperature for 20 min. After adding 1.4 mL of serum-free media, the virus particle/reagent
443 mixture was dropped onto the seeded cells.
444 We have tested two mammalian cell lines: Vero E6 cells originated from African green monkey
445 kidney and BHK-21 cells derived from baby Syrian hamster kidney. These cells were cultured in medium
446 DMEM (Sigma-Aldrich, St. Louis, MO, USA, D6429) supplemented with 10% fetal bovine serum (FBS)
447 at 37℃ and 5% CO_2 on a regular basis. For virus inoculation, 10^5 cells per 35-mm dish were seeded and
448 maintained for a half a day in DMEM supplemented with 2% FBS, which was then replaced with
449 serum-free DMEM. All types of media were supplemented with 2% non-essential amino acid (Thermo
450 Fisher Scientific Inc.) and 1% penicillin-streptomycin (Thermo Fisher Scientific Inc.). Cells were
451 inoculated with the virus inoculum solution as for insect cells in the presence or absence of transfection
452 reagent *Trans*IT (Mirus Bio, Madison WI, USA) and maintained at 28°C or 37°C. Total RNA was
453 extracted with the aid of Isogen II (Nippon Gene, Tokyo, JAPAN).
454
455 **Plant regeneration from carrot protoplasts.** Protoplasts of carrot (a western-type cultivar
456 Koyasu-sanzun) were isolated from calluses according to Grzebelus et al.(42) with slight modifications.
457 Carrot calluses were generated from hypocotyl segments and maintained on a solidified Gamborg's B5
458 basal medium and vitamins(68), supplemented with 1.0 mg/L 2,4-D and 30 g/L sucrose (pH 5.7;
459 abbreviated as B5DS). The calluses were suspended in 25 mL of enzyme mixture and digested overnight
460 (14–16 h) in the dark at 26 °C with gentle shaking (30 rpm). The enzyme mixture consisted of 1% (w/v)
461 Cellulase Onozuka R-10 (Duchefa), 0.1% (w/v) Pectolyase Y-23 (Duchefa), 20 mM MES, 5 mM $CaCl_2$
462 and 0.6 M mannitol (Thermo Fisher Scientific Inc.). The pH was adjusted to 5.6 with NaOH and
463 filter-sterilized (0.22 μm, Millipore).
464 The released protoplasts were separated from undigested tissue by filtration through a test sieve
465 (125 μm aperture, Tokyo Screen) into a 50-mL Falcon tube and centrifuged at 100 *g* for 5 min. The pellet
466 was resuspended in 8 mL of a solution containing 0.5 M sucrose and 1 mM MES, and overlaid with 2 mL
467 of W5 solution(42). After centrifugation at 145 *g* for 10 min, the purified protoplasts at the interphase
468 were washed twice with W5 solution. The protoplasts were then transfected with or without virus
469 particles using PEG solution(66). The transfected protoplasts were washed with B5D culture medium
470 composed of B5 basal medium and vitamins, 74 g/L glucose, 1 mg/L 2,4-D and 0.2 mg/l trans-zeatin and
471 74 g/L glucose (B5DZG), and centrifuged at 100 *g* for 5 min. The protoplast density was adjusted to 1 ×
472 10^6/mL. Aliquots (200 μL) of the protoplast solution were mixed carefully with an equal volume of a
473 solution containing 2.8% sodium alginate (Sigma-Aldrich) in 0.4 M mannitol. The mixture was plated on
474 $CaCl_2$-agar containing 20 mM $CaCI_2$, 0.4 M mannitol and 1% agar. After incubation for 1 h at room
475 temperature, the thin layers were carefully removed with fine scalpels and transferred into small (6 cm)

476 Petri dishes containing 4 mL of B5DZG culture medium. The culture was incubated in the dark at 20–
477 25°C. The liquid medium was refreshed every 10 days.
478 After 2 months, depolymerization of calcium alginate layers was conducted in 20 mM sodium
479 citrate solution containing 0.2 M mannitol (pH 5.6). Within 2 h, the proembryonic masses (PEM) were
480 released, washed with liquid B5D and transferred to B5D medium solidified with 0.8% agar for callus
481 multiplication. Shoot and root induction through somatic embryogenesis was achieved by sub-culturing
482 the calluses in hormone-free B5S media containing 30 g/L sucrose (instead of 74 g/L glucose) with a 16-h
483 photoperiod. Small plantlets with one to two true leaves and roots were transferred to glass jar containing
484 rooting media (B5S with or without 1 mg/L IAA) and grown for 4–6 weeks. The plants were further
485 grown in potting soil covered with a plastic bag for 1 week and then maintained in the soil with a 16-h
486 photoperiod at 20–22°C.
487 Virus particles were semi-purified from carrot calluses and regenerated plants by a conventional
488 method, and subjected to electron microscopy.
489
490 **RNA preparations and analyses.** Total RNA was extracted from carrot plant tissue using the
491 PureLink® Plant RNA Reagent as described by the manufacturer (Thermo Fisher Scientific., Inc.,
492 Waltham, MA, USA). The phenol-chloroform method was used in the total RNA preparation from *N.*
493 *benthamiana*, BY-2 cells, carrot protoplasts and insect cells (Sf9 and S2) (69). Cells were sedimented at
494 46 *g* for 3 min and the supernatant was discarded. Fungal total RNA was prepared similarly(41). The
495 extraction buffer SDS-STE (2% SDS in 200 mM NaCl, 100 mM Tris-HCl, and 4 mM EDTA, pH 8.0) or
496 glycine buffer was added and the mixture was vortexed. Then, phenol/chloroform/isoamyl alcohol (PCI)
497 (25:24:1) was added immediately and was vortexed. The homogenates were centrifuged at 22,140 *g*
498 (Kubota Model 3700) for 10 min at 4°C. The supernatant was mixed with chloroform/isoamyl alcohol
499 and centrifuged in the same conditions. RNA was ethanol precipitated in 0.3 M sodium acetate, pH 5.2
500 (1/10 volume), pelleted by centrifugation and washed with 70% ethanol. To eliminate DNA
501 contamination, the sample was treated with RQ1 DNase I in 10×RQ DNase buffer at 37°C for 1 h. The
502 samples were phenol-chloroform treated and precipitated in 0.3 M sodium acetate (pH 5.2) and 70%
503 ethanol.
504 Northern blotting was performed according to Eusebio-Cope and Suzuki(70). The
505 single-stranded (ss) RNA fraction was isolated from the plant or insect host cells using the
506 phenol-chloroform and chloroform protocol and later enriched using 2 M lithium chloride followed by
507 chloroform extraction and precipitation. The heat-denatured ssRNA (8–20 μg) was electrophoresed in a
508 1% agarose gel under denaturing conditions and capillary-transferred onto a Hybond-N+ nylon
509 membrane (GE Healthcare Life Sciences, Pittsburgh, PA, USA) and fixed by a UV cross-linker (CL-1000,

510 UVP, Upland, CA, USA). Hybridization was accomplished using digoxigenin (DIG)-labeled DNA
511 probes amplified from complementary DNA (cDNA) by PCR using specific primers targeting both
512 partitivirus dsRNA1 and dsRNA2 listed in *SI Appendix*, Table S2 (PCR DIG labeling mix; Roche,
513 Risch-Rotkreuz, Switzerland). The prehybridization and hybridization steps were performed based on the
514 instructions provided by the supplier (Roche). Two low- and high-stringency washes were carried out in
515 2× SSC (1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate) and 0.1× SSC, respectively.
516 Chemiluminescent signals of each probe-RNA hybrid were detected using ready-to-use CDP-Star
517 (Roche) in the Image Quant LAS 4000 system (GE Healthcare Life Sciences).
518 For the time-course analysis, complementary DNA (cDNA) was prepared in one of three ways.
519 cDNA was synthesized from 0.5 µg of total RNA with a ReverTra Ace qPCR RT kit (Toyobo, Osaka,
520 Japan). Standard methods using M-MLV or Superscript III previously reported by Chiba et al.(71) and
521 Hyodo et al.(65) were also utilized. RT-qPCR was performed using the CFX Connect™ Real-Time PCR
522 Detection System (Bio-Rad), LightCycler® 480 and qTOWER3/G (Roche) with THUNDERBIRD
523 SYBR qPCR Mix (Toyobo). The gene-specific primer sequences are listed in *SI Appendix*, Table S2. The
524 relative normalized expression values of virus RNAs were determined using the comparative Ct method
525 ($2^{-\Delta\Delta Ct}$ method) with respective reference genes for host organisms. Two sets of primers were used for
526 each virus designed from the RdRP and CP coding genes. The internal control genes were the protein
527 phosphatase 2A (PP2A) gene for *N. benthamiana*, the elongation factor 1-alpha gene for *N. tabacum*
528 BY-2 cells, the actin gene for carrot, the ribosomal protein L13 (RPL13) and/or elongation factor 1-alpha
529 genes for Sf9 and ribosomal protein 32 (RPL32) and/or the alpha-tubulin gene for S2 insect cells (*SI*
530 *Appendix*, Table S2). The experiment was repeated at least three times using different batches of
531 protoplasts with at least two technical replicates.
532
533 **NGS of host sRNAs.** For deep sequencing of vsiRNA, total RNA was extracted from plant protoplasts
534 (*N. benthamiana*) or insect cultured cells (S2 and Sf9) transfected with partitiviral virions from the *R.*
535 *necatrix* strain W442 (a strain coinfecting with RnPV18 and RnPV19). Each sample was harvested 72 hpt
536 for plant protoplasts or 96 hpt for insect cells. Total RNA from the virus-infected *R. necatrix* W442 strain
537 was used as the natural host–virus combination. Small RNA cDNA library construction and subsequent
538 deep RNA sequencing were conducted by Macrogen Inc. (Tokyo, Japan) with the Illumina platform
539 (HiSeq 2000; 50-bp single-ends reads). Raw reads (total read numbers ranging from 21.2–33.2 Mbp)
540 were trimmed to remove the adapter and filtered for low-quality sequences. After size filtering (15–32 nt
541 in length), the remaining reads in each library were mapped to the partitivial dsRNA genomes
542 (RnPV18:LC517384, LC517385; RnPV19: LC517386, LC517387)(41) using CLC Genomic Workbench

(version 11; CLC Bio-Qiagen). The mapped virus-derived small RNA reads were then subjected to further in-depth analysis using the MISIS-2 program(72).

Data Availability. All study data are included in the article and/or *SI Appendix*.

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713

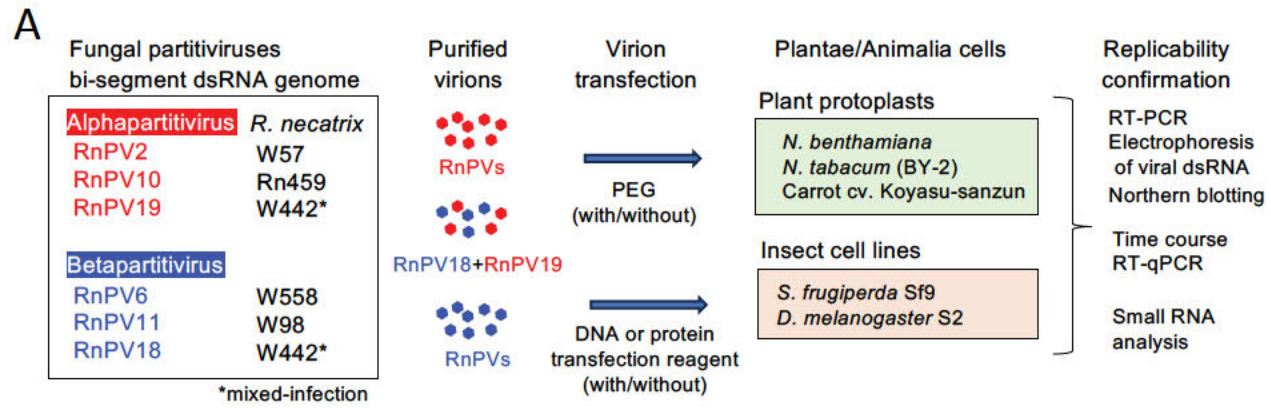
714 **Figure legends**
715

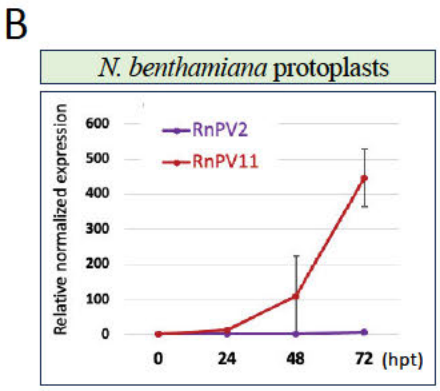
716 **Fig. 1. Replication of fungal partitiviruses in *N. benthamiana* protoplasts. (A) Experimental**
717 **procedure.** See the Materials and Methods section for the details of viruses, original fungal host strains,
718 experimental host organisms and methodologies utilized. **(B, C)** Relative quantification of RnPV2 and
719 RnPV11 **(B)**, and RnPV18 and RnPV19 **(C)** viral RNA by RT-qPCR. Virus contents at different time
720 points post-transfection were quantitatively compared; the value at 0 hpt was arbitrarily assigned as 1.
721 The data are presented as the fold change of viral RNA accumulation. **(D, E)** Agarose gel electrophoretic
722 analysis of total RNA extracted from transfected *N. benthamiana* protoplasts with RnPV11 **(D)** and
723 RnPV18 and RnPV19 **(E)**. Total RNA fractions obtained at different time points were electrophoresed in
724 1.3% agarose gel using 1 × TAE buffer. Only RnPV18 and RnPV11 dsRNA is visible in the gel. Note
725 that the two dsRNA genomic segments (dsRNA1 and dsRNA2) of RnPV11, RnPV18 and RnPV19
726 comigrate in agarose gel. A size standard (1-kb DNA ladder) was electrophoresed in parallel (lane M).
727 VF refers to the virus-free sample. Mean values and standard errors were calculated from three biological
728 replicates, each with three technical replicates, in this and subsequent figures. hpt refers to hours
729 post-transfection.
730

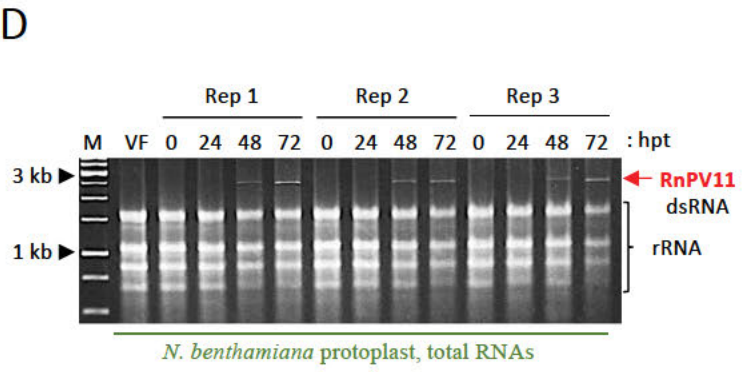
731 **Fig. 2. Replication of carrot calluses and plants by RnPV18. (A)** Procedure for the regeneration of
732 carrot plants from protoplasts transfected by RnPV18 and RnPV19. **(B)** Agarose gel electrophoresis of
733 RT-PCR products derived from RnPV18 in different carrot calluses. The numbers circled with red are
734 positive for the RnPV18 infection. **(C)** RT-PCR analysis of systemic infection of different carrot plant
735 tissues by RnPV18. Two carrot plants (Virus free and Carrot 12-3C), derived from a virus-free callus and
736 RnPV18-infected callus (No. 12), respectively, were subjected to the analysis. L1–L4 denote leaf
737 samples, and R1 denotes a root sample. Total RNA fractions obtained from fungal strain W442 (Rn
738 RNA-W442) and plant or callus line derived from untransfected carrot protoplasts (NTC) served as a
739 positive and a negative control. **(D, E)** Electron micrographs of RnPV18 particles purified from carrot

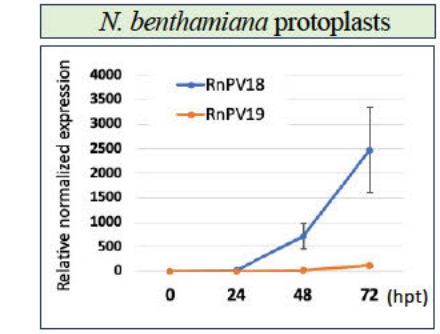
740 callus **(E)** and plant leaves **(E)**. The samples were examined using a Hitachi electron microscope model
741 H-7650. The white arrows point to typical virus-like particles.
742
743 **Fig. 3. Replication of RnPV18 and RnPV19 RNA in Sf9 and S2 insect cells. (A, B)** Relative
744 quantification of RnPV18 and RnPV19 RNA in Sf9 **(A)** and S2 **(B)** cells. RT-qPCR was performed as
745 described in the Fig. 1 legend at different time points post transfection (0, 24 and 96 hpt). **(C, D)** Northern
746 blotting of RnPV18 **(upper rows in C, D)** and RnPV19 mRNA **(lower rows in C, D)** in army worm Sf9
747 **(C)** and fruit fly S2 **(D)** cells. Equal amounts of single-stranded RNA fractions (20 µg) from insect cells
748 were subjected to northern blotting. DIG-labeled probes specific to RnPV18 and RnPV19 RdRP
749 encoding dsRNA1 were used. W97 is a virus-free *R. necatrix* strain (VF), while W442 is the natural host
750 of RnPV18 and RnPV19, for which a much smaller amount (1 µg) of RNA was probed.
751
752 **Fig. 4. Properties of RnPV18- and RnPV19-derived small RNAs in three different host organisms.**
753 **(A, B)** Size distribution of RnPV18 dsRNA1-**(A)** and RnPV19 dsRNA1-derived siRNAs **(B)** produced in
754 plant, fungal and insect cells. Comparison of siRNAs (sense and antisense, 15–32 nucleotides) derived
755 from the RdRP-coding dsRNA1 genomic segments in *N. benthamiana* cells (top row), insect S2 cells
756 (middle row) and the original host *R. necatrix* W442 mycelia (bottom row) in each panel. *N. benthamiana*
757 and fruit fly S2 cells were harvested at three days post transfection for high through-put small RNA
758 sequencing, while *R. necatrix* W442 mycelia were collected after culturing in potato dextrose broth for
759 one week. Read numbers were normalized against one million small RNA reads. **(C, D)** Hot spot of
760 RnPV18 dsRNA1- (**C**) and RnPV19 dsRNA1-derived small RNAs (**D**) produced in *N. benthamiana*
761 (Nb), fruit fly S2 (S2) and *R. necatrix* (Rn) cells, are mapped to their genomes on the left. The vsiRNA
762 reads were not normalized. **(E, F)** Ratios in percentage of the 5'-terminal nucleotides of RnPV18
763 dsRNA1-**(E)** and RnPV19 dsRNA1-derived siRNAs **(F)** are shown in graph form on the right. See *SI*
764 *Appendix*, Fig. S*9* for the properties of the CP-coding genomic segment (dsRNA2).
765

Fig. 1


A
Fungal partitiviruses
bi-segment dsRNA genome
Alphapartitivirus
R. necatrix
RnPV2
W57
RnPV10
Rn459
RnPV19
W442*
Betapartitivirus
RnPV6
W558
RnPV11
W98
RnPV18
W442*
*mixed-infection
Purified virions
RnPVs
RnPV18+RnPV19
RnPVs
Virion transfection
PEG (with/without)
DNA or protein transfection reagent (with/without)
Plantae/Animalia cells
Plant protoplasts
N. benthamiana
N. tabacum (BY-2)
Carrot cv. Koyasu-sanzun
Insect cell lines
S. frugiperda Sf9
D. melanogaster S2
Replicability confirmation
RT-PCR
Electrophoresis of viral dsRNA
Northern blotting
Time course RT-qPCR
Small RNA analysis


B
N. benthamiana protoplasts
Relative normalized expression
RnPV2
RnPV11
0
100
200
300
400
500
600
0
24
48
72 (hpt)


D
Rep 1
Rep 2
Rep 3
M VF 0 24 48 72 0 24 48 72 0 24 48 72 : hpt
3 kb
1 kb
RnPV11
dsRNA
rRNA
N. benthamiana protoplast, total RNAs


C
N. benthamiana protoplasts
Relative normalized expression
RnPV18
RnPV19
0
500
1000
1500
2000
2500
3000
3500
4000
0
24
48
72 (hpt)

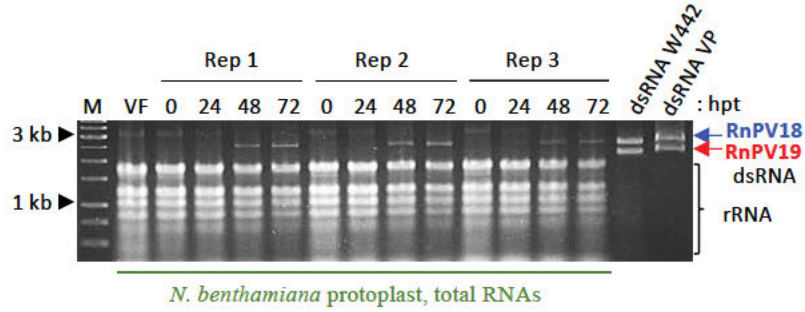

E
Rep 1
Rep 2
Rep 3
dsRNA W442
dsRNA Vp
M VF 0 24 48 72 0 24 48 72 0 24 48 72 : hpt
3 kb
1 kb
RnPV18
RnPV19
dsRNA
rRNA
N. benthamiana protoplast, total RNAs

Fig. 2

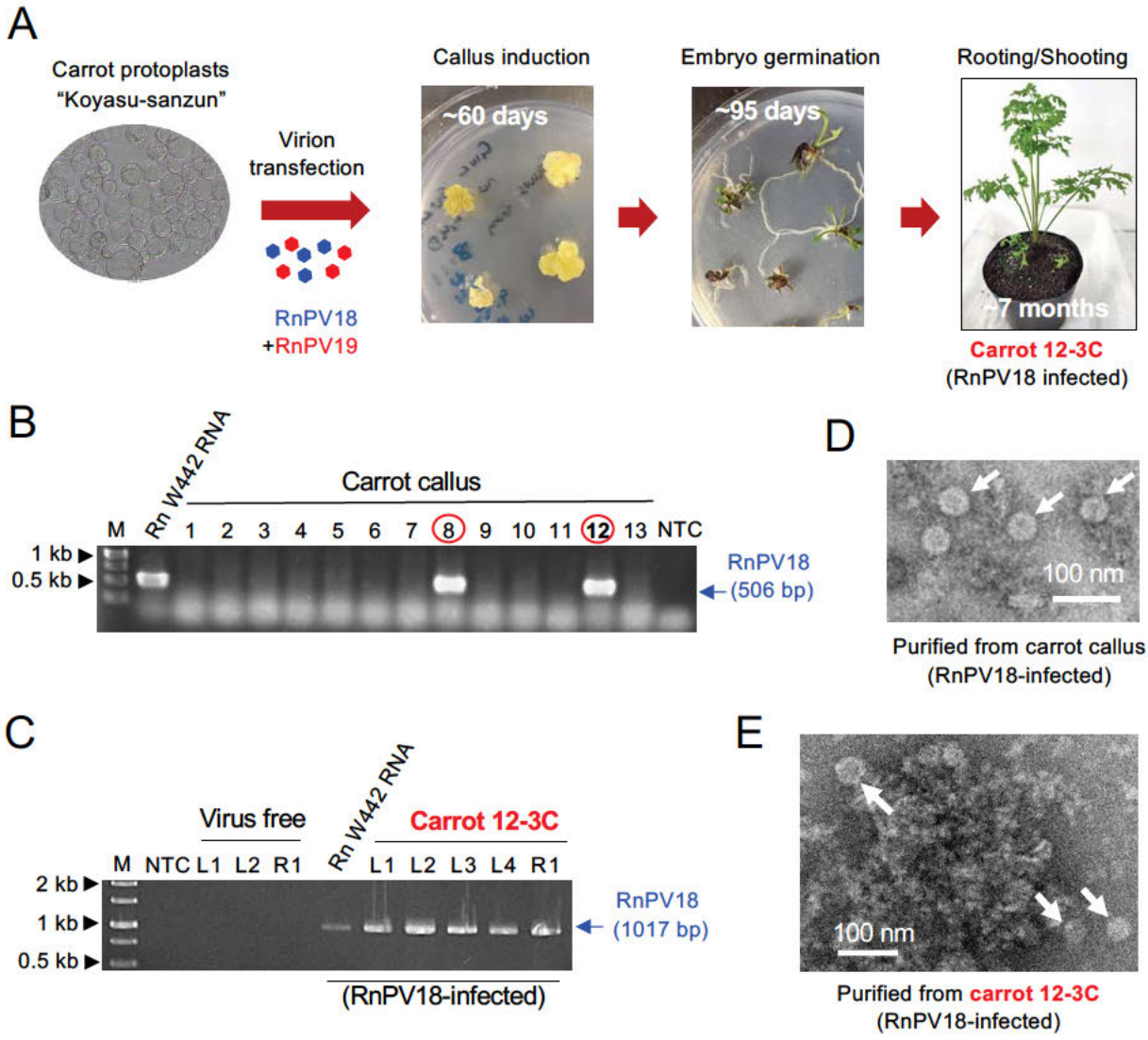

A
Carrot protoplasts
"Koyasu-sanzun"
Virion
transfection
RnPV18
+RnPV19
Callus induction
~60 days
Embryo germination
~95 days
Rooting/Shooting
~7 months
Carrot 12-3C
(RnPV18 infected)
B
Rn W442 RNA
Carrot callus
M 1 2 3 4 5 6 7 8 9 10 11 12 13 NTC
1 kb
0.5 kb
RnPV18
(506 bp)
C
Virus free
Rn W442 RNA
Carrot 12-3C
M NTC L1 L2 R1 L1 L2 L3 L4 R1
2 kb
1 kb
0.5 kb
RnPV18
(1017 bp)
(RnPV18-infected)
D
100 nm
Purified from carrot callus
(RnPV18-infected)
E
100 nm
Purified from carrot 12-3C
(RnPV18-infected)

Fig. 3

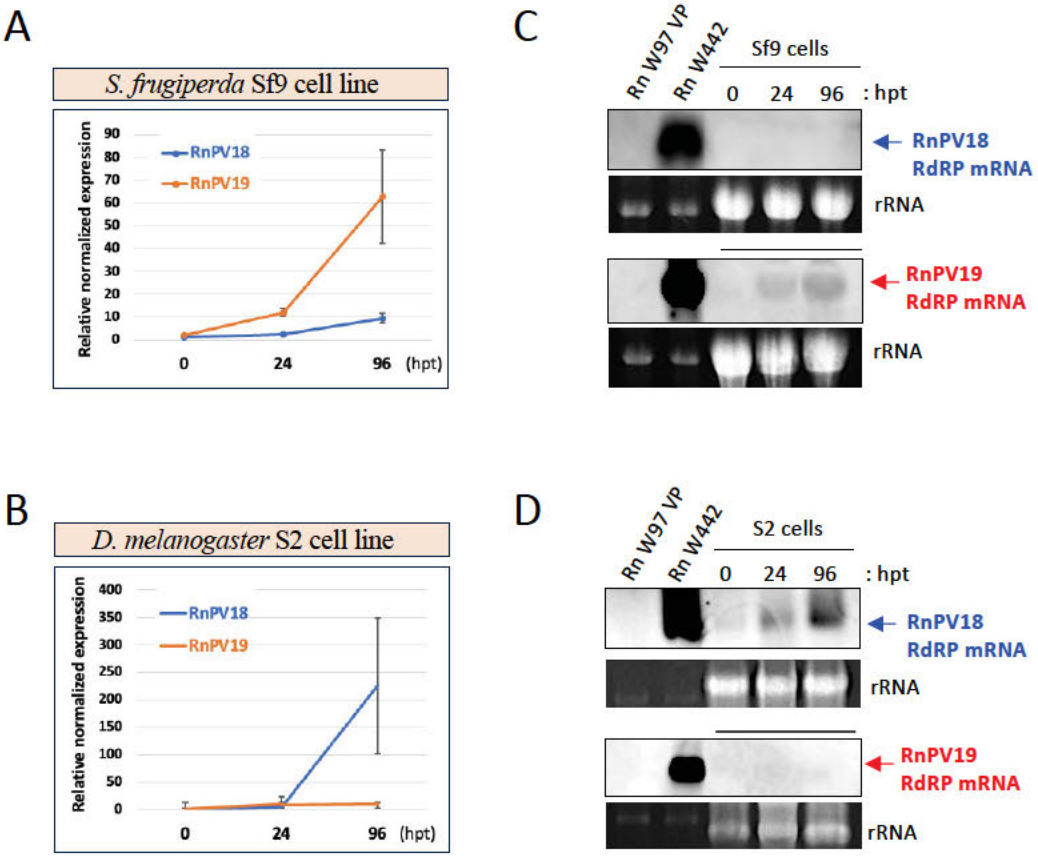
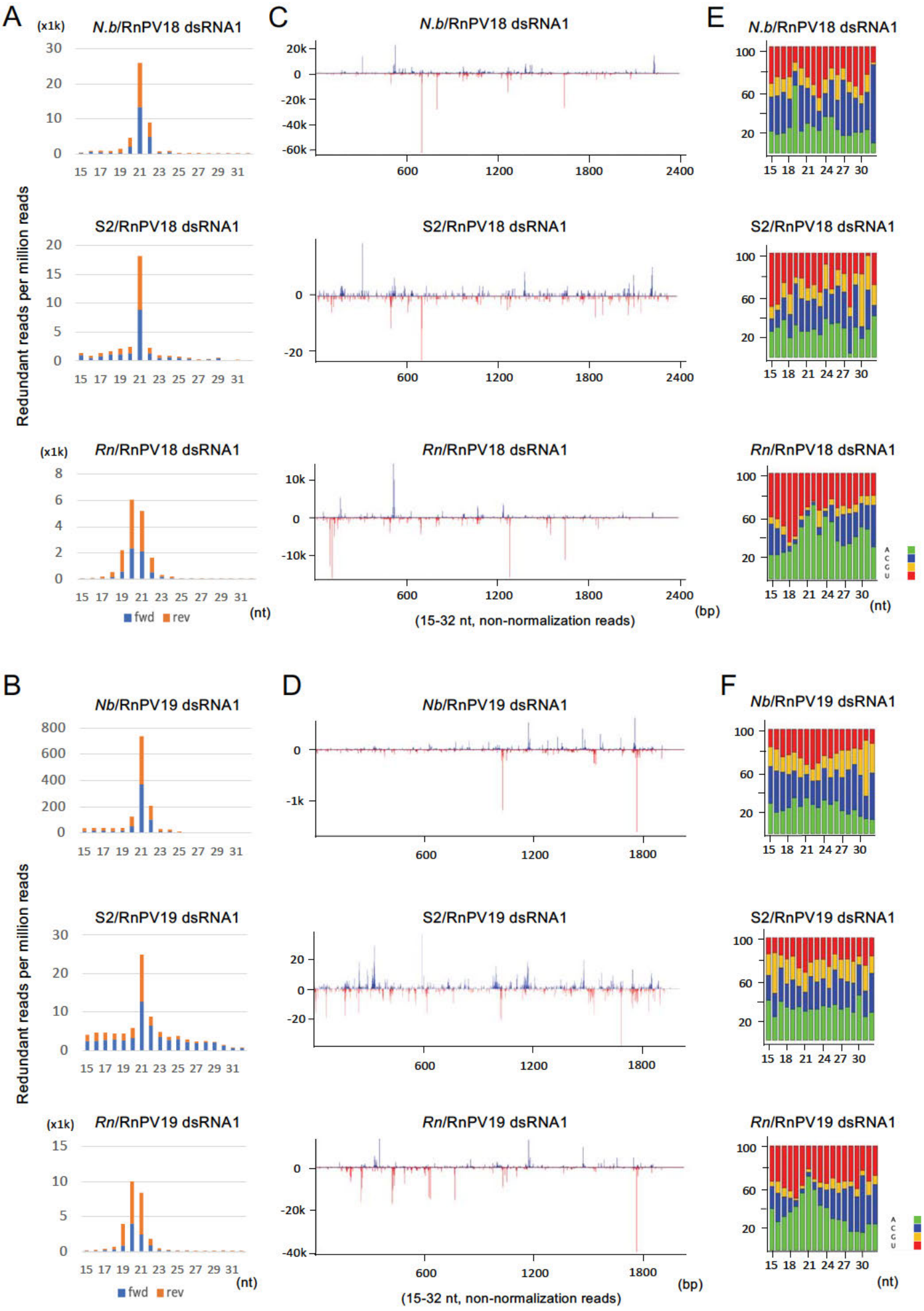

A
S. frugiperda Sf9 cell line
Relative normalized expression
RnPV18
RnPV19
0
24
96 (hpt)
B
D. melanogaster S2 cell line
Relative normalized expression
RnPV18
RnPV19
0
24
96 (hpt)
C
Rn W97 VP
Rn W442
Sf9 cells
0 24 96 : hpt
RnPV18 RdRP mRNA
rRNA
RnPV19 RdRP mRNA
rRNA
D
Rn W97 VP
Rn W442
S2 cells
0 24 96 : hpt
RnPV18 RdRP mRNA
rRNA
RnPV19 RdRP mRNA
rRNA

Fig. 4


A
C
E
B
D
F
(x1k)
N.b/RnPV18 dsRNA1
S2/RnPV18 dsRNA1
Rn/RnPV18 dsRNA1
Nb/RnPV19 dsRNA1
S2/RnPV19 dsRNA1
Rn/RnPV19 dsRNA1
Redundant reads per million reads
fwd
rev
(nt)
(bp)
(15-32 nt, non-normalization reads)
A
C
G
U

Supplementary Information for

Replication of single viruses across the kingdoms, Fungi, Plantae and Animalia

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Data information:

Supplemental figures, 10.

Supplemental tables, 2.

Figure legends

Fig. S1. Effects of polyethylene glycol (PEG) on transfection of *N. benthamiana* protoplasts. (A, B) Relative RNA accumulation of RnPV18 **(A)** and RnPV19 **(B)** in *N. benthamiana* protoplasts were compared between transfection with or without PEG at different time points 24, 48, 72 h post-transfection (hpt) with respect to time point 0 hrs. Reverse transcription–quantitative polymerase chain reaction (RT-qPCR) with the dsRNA1-specific primers (*SI Appendix*, Table S2) was conducted as described in the Materials and Methods. The data is presented as the fold change of viral RNA accumulation.

Fig. S2. Replication of fungal partitiviruses in *N. benthamiana* protoplasts. (A) Relative quantification of RnPV6 and RnPV10 viral RNAs in *N. benthamiana* protoplasts at different time points were quantified as described in the Fig. 1 legend. Reverse transcription–quantitative polymerase chain reaction (RT-qPCR) data were used. The data are presented as the fold change of viral RNA accumulation. **(B)** Agarose gel electrophoresis of total RNA extracted from transfected *N*. *benthamiana* protoplasts with RnPV6 virions at different time points. **(C)** Inverted image of agarose gel in **B**. RnPV6 (2462 bp) dsRNA is visible in the 1.3% agarose gel and marked with arrows. Size standard was electrophoresed in parallel (lane M). Lane dsRNA VP was loaded with dsRNA from RnPV6 virus particles (VP) purified from strain W558. VF refers to virus free sample and hpt hours post-transfection. White and black arrows indicate virus dsRNA.

Fig. S3. **Replication of RnPV11, RnPV18 and RnPV19 RNA in tobacco BY-2 and carrot protoplasts. (A, C, D)** Partitiviral RNA in transfected tobacco BY-2 **(A)** or carrot **(C, D)** protoplasts was quantified as described in the Fig. 1 legend. RT-qPCR data were used. The data are presented as the fold change of viral RNA accumulation. **(B)** Agarose gel electrophoresis of total RNA extracted from transfected tobacco BY-2 protoplasts at the different time points. The white arrow indicates virus dsRNA. A size standard (1-kb DNA ladder) was electrophoresed in parallel (lane M) using 1 × TAE buffer. VF refers to the virus free sample. See the Fig. 1 legend for details.

Fig. S4. RnPV18 detection in carrot plants. Equal amounts of total RNA was obtained from carrot plants regenerated from transfected and untransfected (virus-free [VF]) protoplasts, and subjected to northern blotting. RNA1 and RNA2 of RnPV18 were detected using specific DIG-labeled DNA probes. Differently numbered plant lines were derived from different independent calluses. Plants 12-1 to 12-3 were from three calluses originated from a single callus 12, while plant lines 12-3A, -3B and -3C were

regenerated from an embryogenic callus no. 12-3. rRNA refers to 28S ribosomal RNA stained by ethidium bromide, serving as a loading control.

Fig. S5. Detection of RnPV18 in single immature seeds produced in an infected carrot plant. (A) A dying RnPV18-infected carrot plant (145 days post vernalization). The inset shows immature seeds from the dying carrot plant. **(B)** RnPV18-free carrot plant from a callus after virion transfection. The inset shows mature seeds harvested from the virus-free (VF) carrot plant. **(C)** RT-PCR detection of RnPV18 (PV18) from single seeds. from the VF and RnPV18-infected plants. Total RNA was isolated from individual seeds harvested from these VF and RnPV18-infected carrot plants, and subjected to RT-PCR with RnPV18 dsRNA1-specific primers. Amplified products were analyzed in a 1% agarose gel using the 1 × TAE buffer system. M is a 1 kb marker (Thermofisher). +ve indicates positive control, i.e., RNA obtained from RnPV18-infected carrot plant leaves. The expected amplicon size is 1017 bp.

Fig. S6. Effects of the transfection reagents on infection of Sf9 insect cells by RnPV18 and RnPV19. Virus accumulation levels were quantitatively compared at 0 and 72 h post-transfection by RT-qPCR. Virus accumulation in cells transfected with or without (W/O) the DNA transfection reagent X-tremeGENE™ (X-tr) or protein transfection reagent Proteojuice™ (Prt) was examined in parallel at 72 h post-transfection. The data are represented as the fold change of viral RNA accumulation; the value at 0 h post-transfection was set as 1.

Fig. S7. Replication of RnPV11 in S2 and Sf9 insect cells. (A, C) Quantification of RnPV11 RNA accumulating in S2 **(A)** and Sf9 **(C)** cells by RT-qPCR using total RNA isolated at 0, 24, and 96 h post-transfection. **(B, D)** Northern blotting of RnPV11 replicated in insect cells. Equal amounts of ssRNA (20 μg) were obtained from natural host *R. necatrix* and transfected S2 **(B)** and Sf9 insect cells **(D)** and subjected to northern blotting. DIG-labeled probes specific to RnPV11 RdRP encoding RNA were used. Fungal ssRNA fractions (1 μg) isolated from W97 and W98 were also probed in parallel. W97 is a virus-free *R. necatrix* strain while W98 is the natural host of RnPV11.

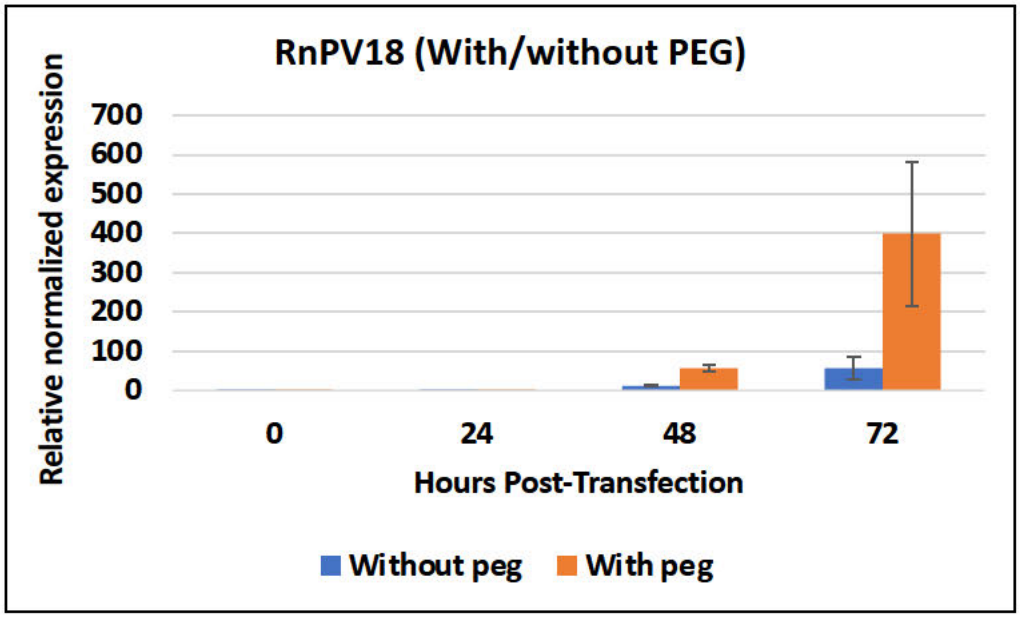
Fig. S8. Replication of RnPV18 or RnPV19 in mammalian cell lines (Vero and BHK-21). (A and B) Relative quantification of RnPV18 and RnPV19 accumulated in Vero (A) and BHK-21 cells (B) by RT-qPCR using total RNA isolated at 0, 24, and 96 h post-transfection with two different culture conditions at 28°C (top) and 37°C (bottom). Virion transfection was performed with or without a transfection reagent (Trans-IT). Relative quantification of viral RNA at different time points with respect to time point 0 hrs. The data is presented as fold change of viral RNA accumulation taken from three technical replicates.

Fig. S9. Properties of small RNAs derived from the CP-coding genomic segment in plant, fungal and insect cells. (A, D) Size distribution of RnPV18- **(A)** and RnPV19-derived siRNAs **(B)**. Comparison of siRNAs (sense and antisense) derived from CP-coding dsRNA2 in *N. benthamiana cells* (*N.b*), insect S2 cells (S2), and the original host *R. necatrix* W442 mycelia (*R.n*). *Nicotiana benthamiana* and fruit fly S2 cells were harvested at three days post transfection for high through-put small RNA sequencing, while *R. necatrix* W442 mycelia were collected after culturing in potato dextrose broth for one week. Read numbers were normalized against one million small RNA reads **(A, B)**. **(C, D)** Hot spots of RnPV18- (**C**) and RnPV19-derived small RNAs (**D**) produced in *N. benthamiana* (*Nb*), fruit fly S2 cells (S2), and *R. necatrix* (*Rn*) are mapped to their genomes on the left. **(E, F)** Ratios in percentage of the 5'-terminal nucleotides of vsiRNAs are shown in graph form. The codes for host organisms are the same as above. See Fig. 4 for the properties of the RdRP-coding genomic segment (dsRNA1).

Fig. S10. Summary of the replicability confirmation study in organisms across three kingdoms.

Supplementary Fig. S1

A.



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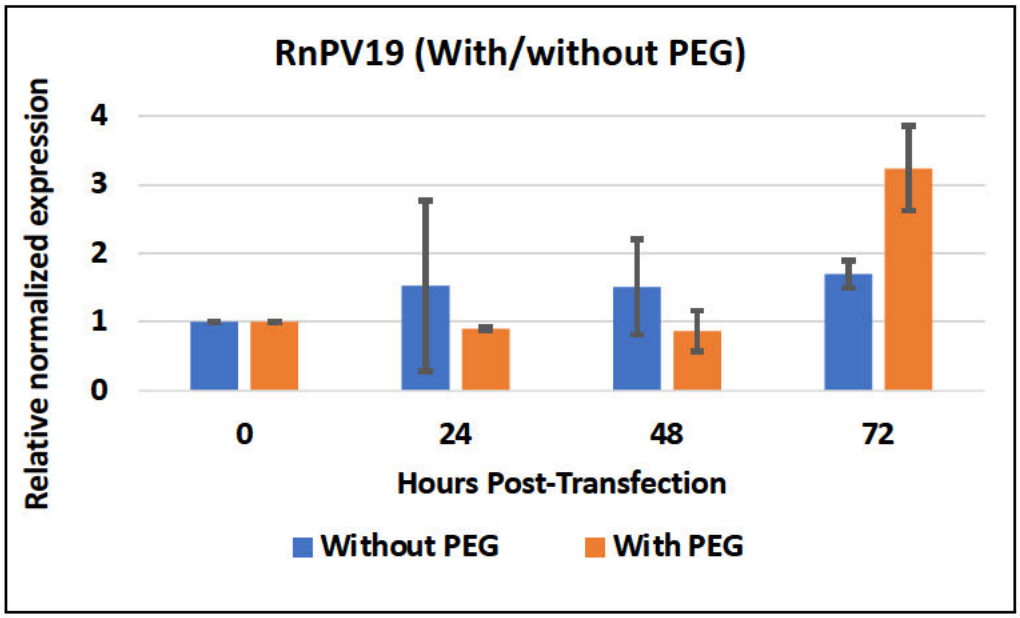
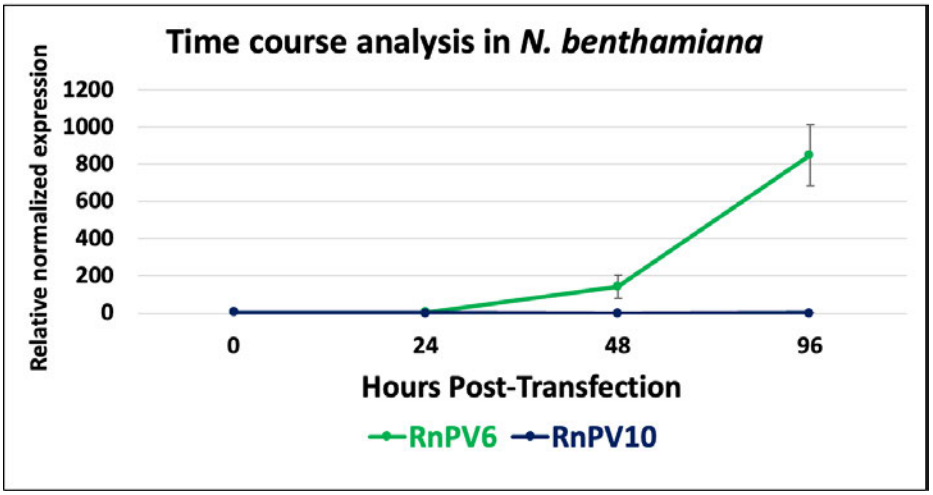


Supplementary Fig. S2

A



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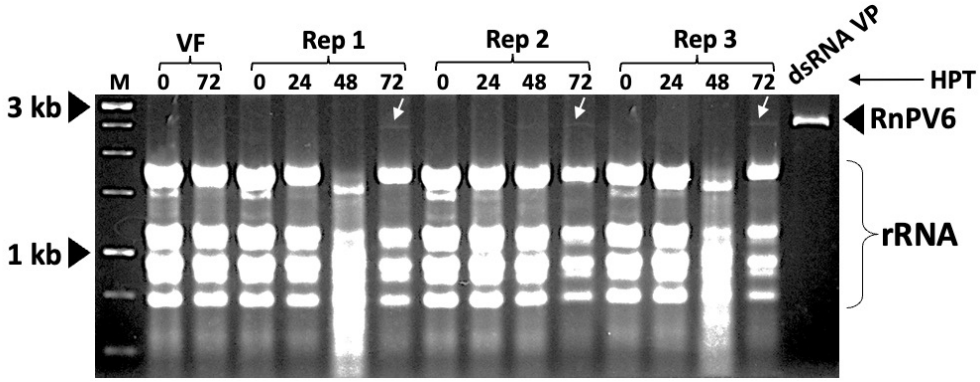
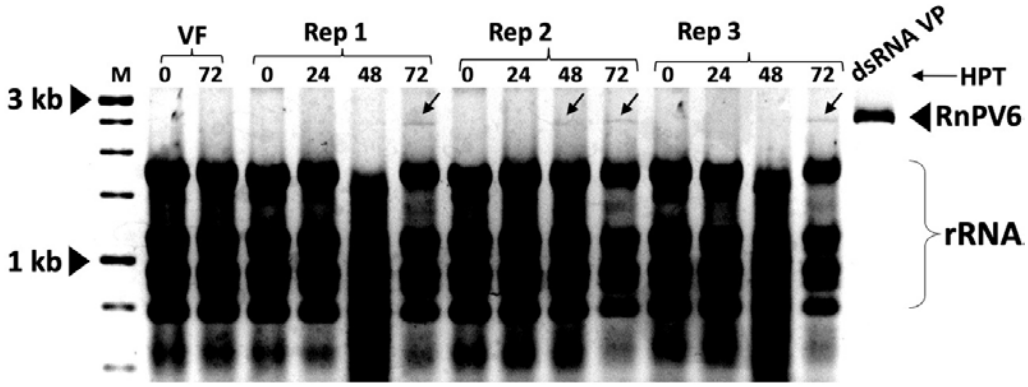
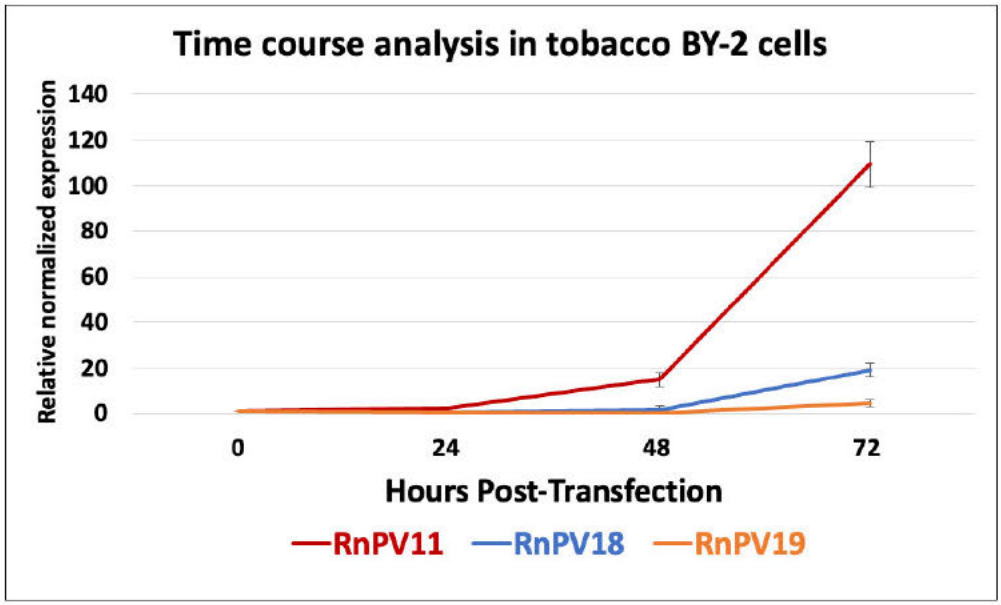


C

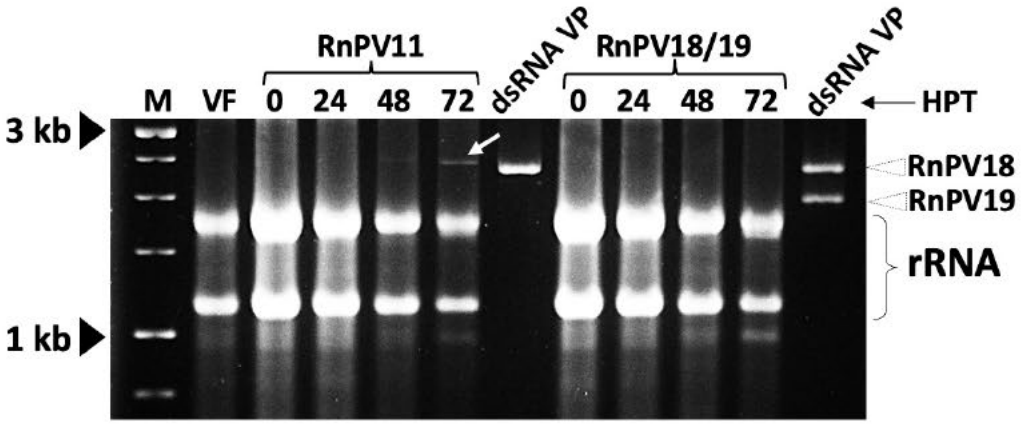


Supplementary Fig. S3

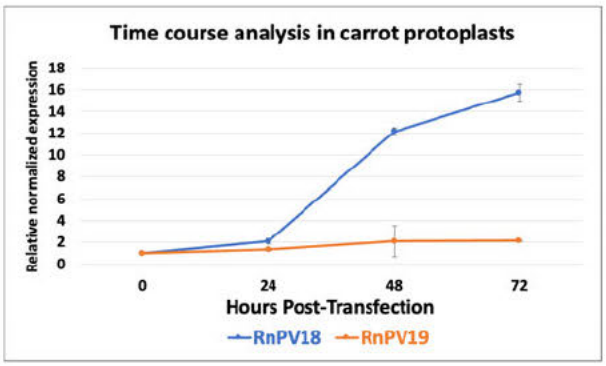
A



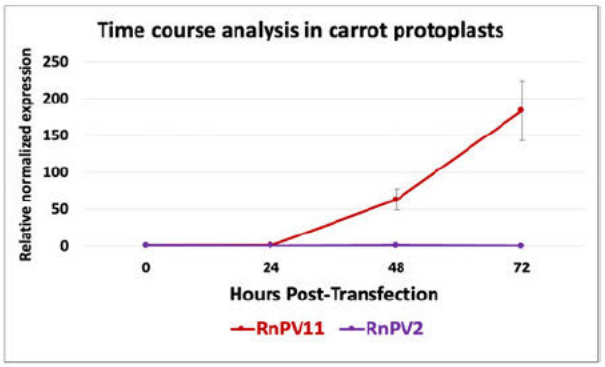
B



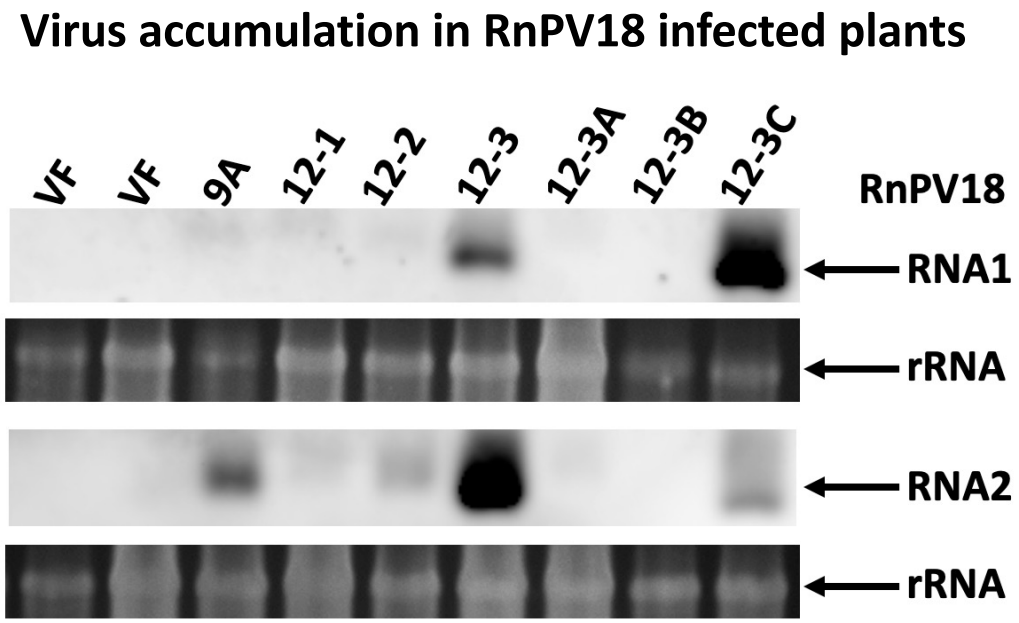
C



D

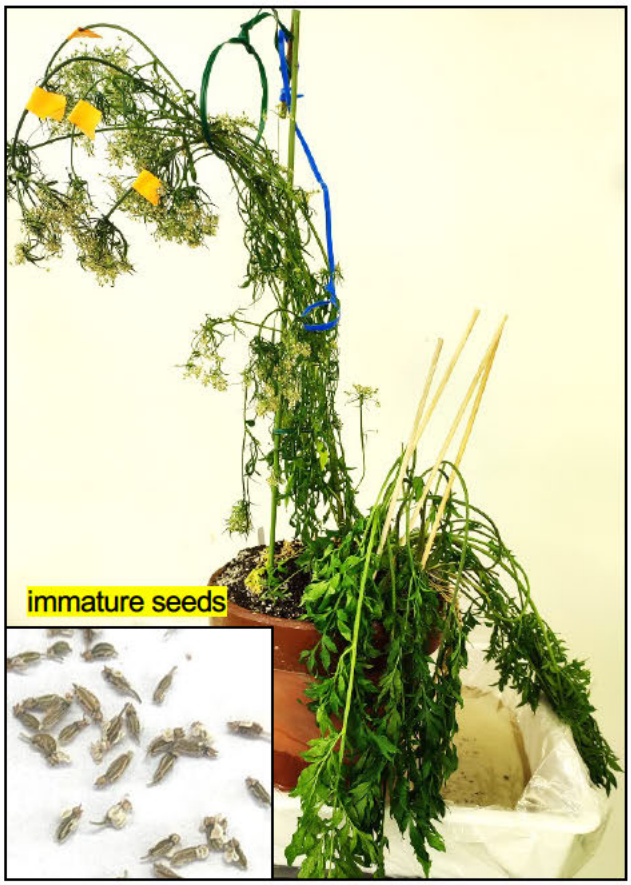


Supplementary Fig. S4



Supplementary Fig. S5

A

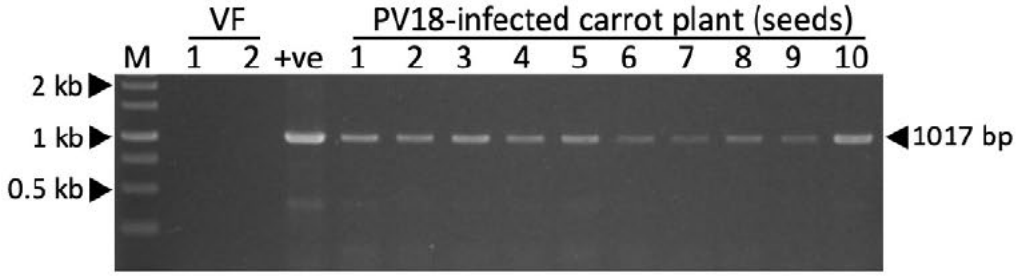


RnPV18-infected plant

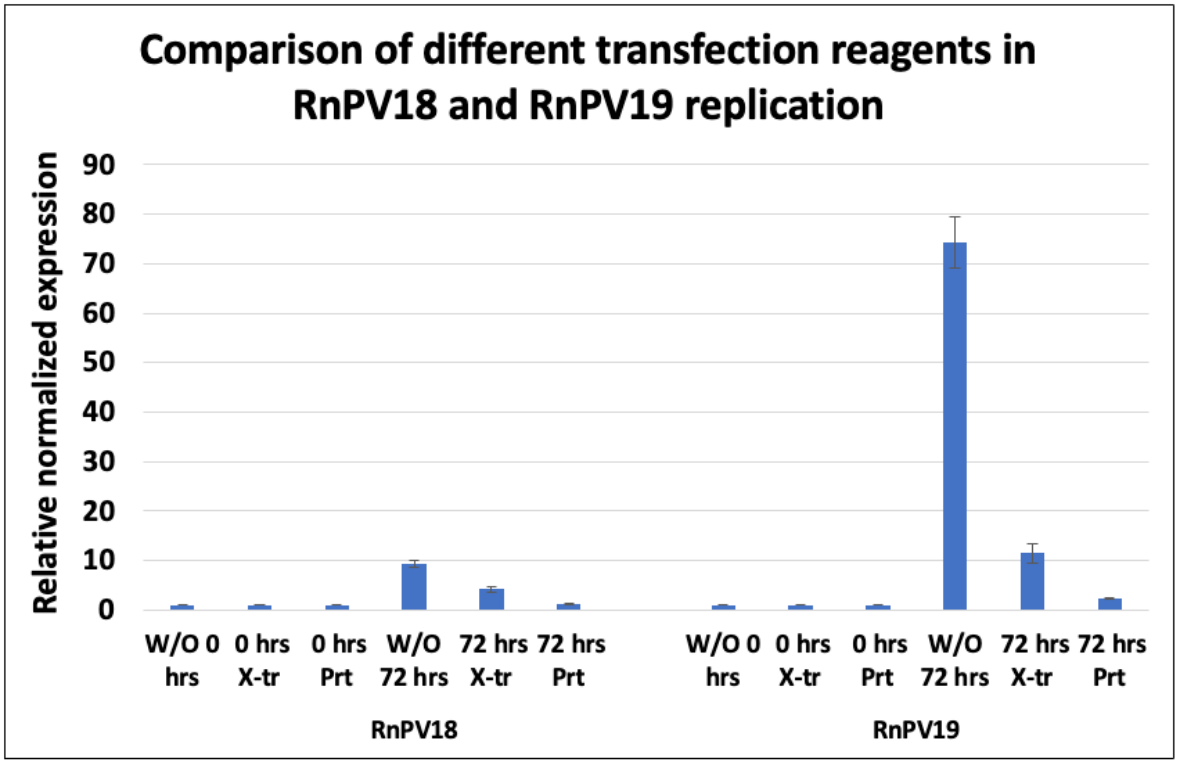
B

RnPV18-free (VF) plant

C

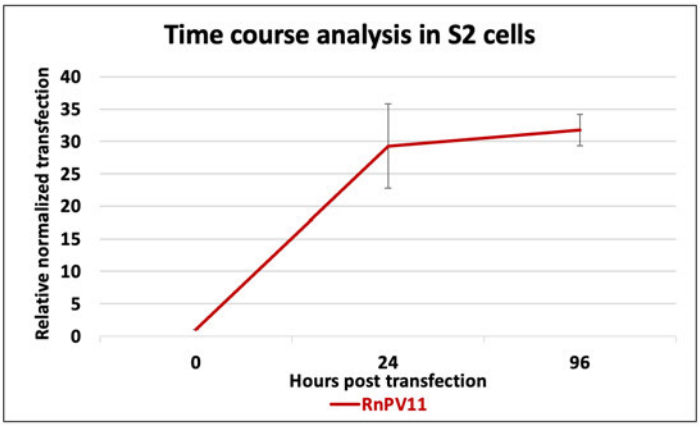


Supplementary Fig. S6



Supplementary Fig. S7

A



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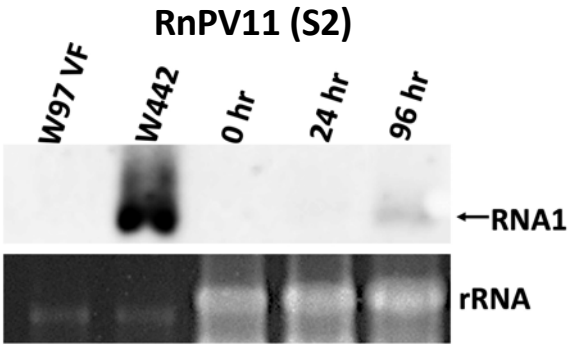

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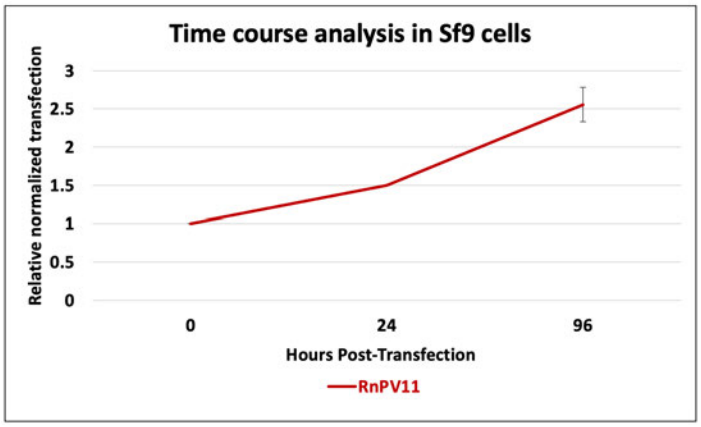

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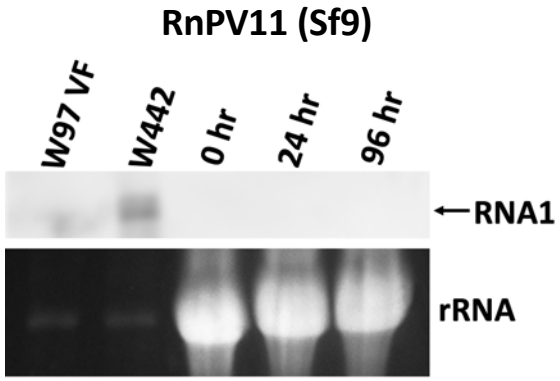
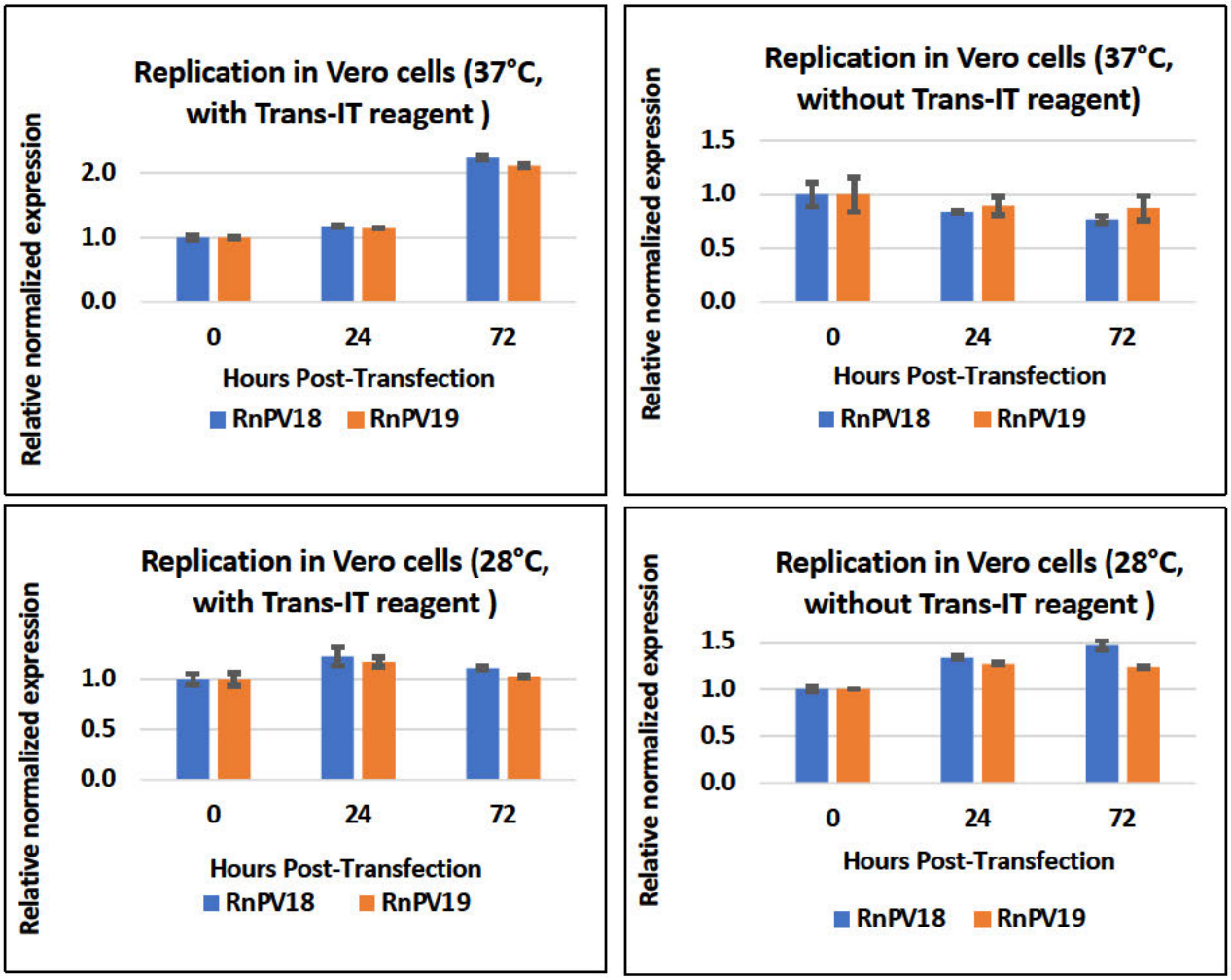


Supplementary Fig. S8

A



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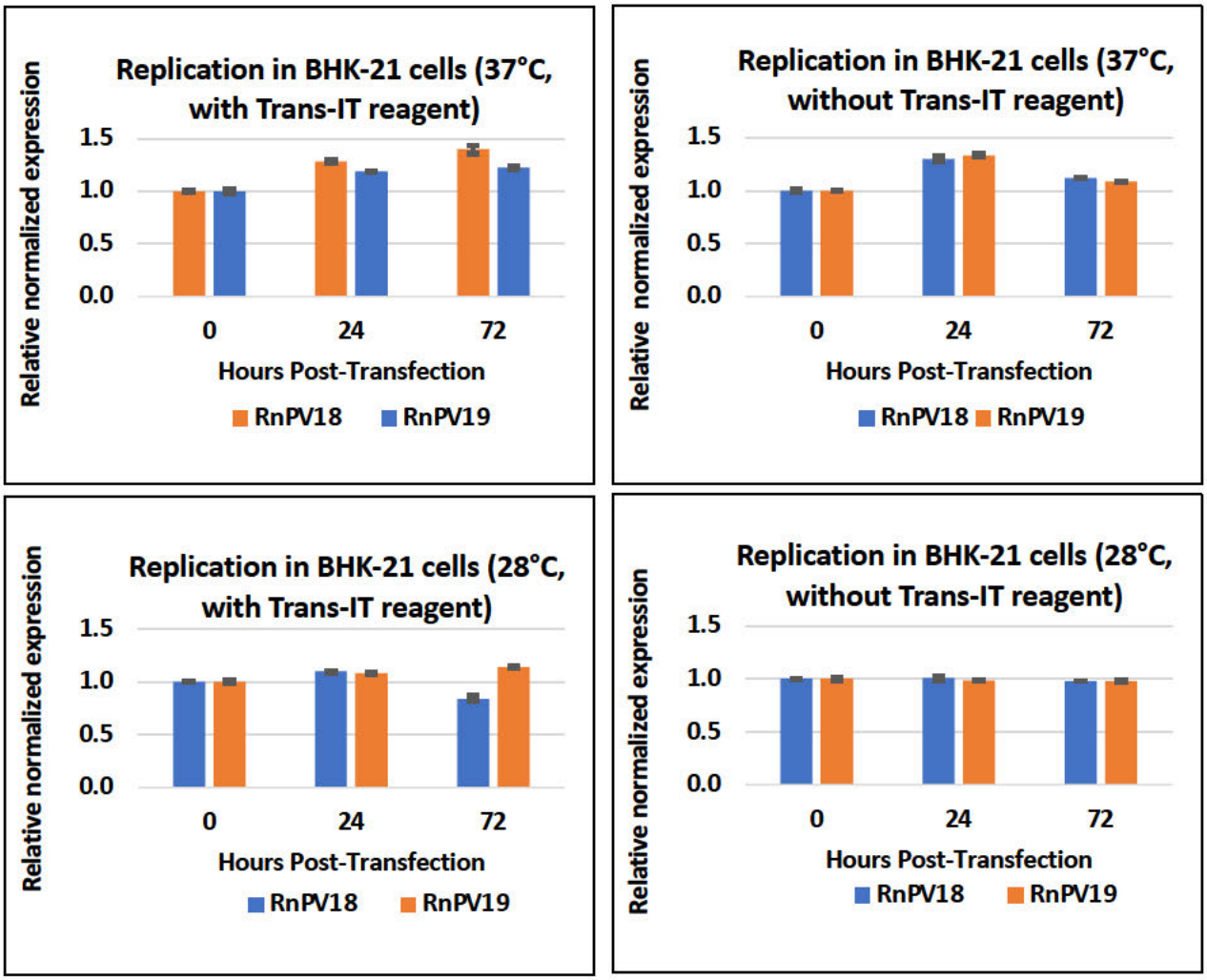
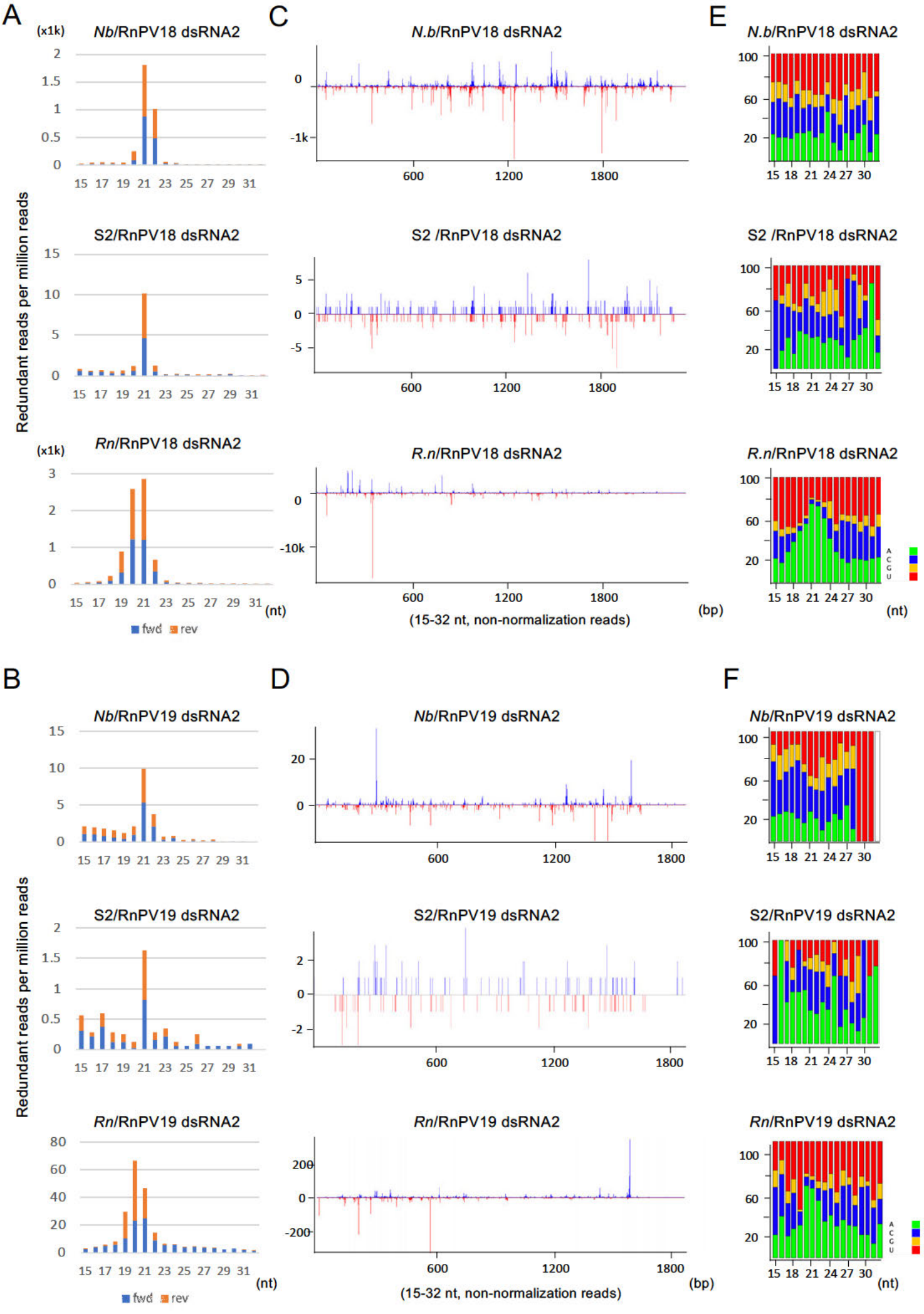


Supplementary Fig. S9



Supplementary Fig. S10

Replicability confirmation in organisms across three kingdoms*.

Tested virus		Fungi	Plantae			Animalia (Insecta/mammalian)			
		*C.p.***	*N.b.*	BY-2	Carrot	*Dm* S2	Sf9	Vero	BHK-21
RnPV2	*Alpha-partitivirus*	++	(-)		(-)		?		
RnPV10			(-)						
RnPV19		+	+	(-)	(-)	(+)	+	(-)	(-)
RnPV6	*Beta-partitiviurs*	++	++	+					
RnPV11		+	++	++	+	+	(-)		
RnPV18		+	++	(+)	+	+	(+)	(-)	(-)

* Fungi: *C.p*, *C. parasitica*
Plantae: *N.b., N. benthamiana; N. tabacum* (BY-2); Carrot cv. Koyasu-sanzun ;
Animalia (Insecta): *D. melanogaster* S2; *S. frugiperda* Sf9 cells; (mammalian) :Vero; BHK-21 cells.
**Reference no, 2, 4, 5, 6.

++:dsRNA positive;+:Northern or vsiRNA positive;(+)RT-qPCR positive (~10-fold change) :(-): no or potentially very low accumulation

Supplementary Table S1. Partitiviruses replicable in organisms across three kingdoms.

Virus (Rosellinia necatrix partitiviruses)	**genus**	**Original host strain** (*R. necatrix* strain)	**Ref.**	**Accession No.**	**Confirmed Replicability in and by.**					
					Plantae			Animalia (Insecta/mammalian)		
					Carrot	*N. benthamiana*	*N. tabacum* (BY-2)	*D.melanogaster* (S2 cell)	*S. frugiperda* (Sf9 cell)	Vero or BHK-21
RnPV2	*Alpha-partitivirus*	W57	1	AB569997 AB569998	(RT-qPCR)	RT-qPCR				
RnPV10		Rn459	3	LC333736 LC333737		(RT-qPCR)				
RnPV19		W442*	4	LC517386 LC517387	(RT-qPCR)	(RT-qPCR) (dsRNA gel) siRNA	(RT-qPCR) (dsRNA gel)	(RT-qPCR) (Northern) siRNA	RT-qPCR Northern	(RT-qPCR)
RnPV6	*Beta-partitivirus*	W558	2	LC010952 LC010953		RT-qPCR dsRNA gel	RT-qPCR			
RnPV11		W98	4	LC517370 LC517371	RT-qPCR	RT-qPCR dsRNA gel	RT-qPCR dsRNA gel	RT-qPCR Northern	(RT-qPCR) (Northern)	
RnPV18		W442*	4	LC517384 LC517385	RT-qPCR Northern	RT-qPCR dsRNA gel siRNA	RT-qPCR (dsRNA gel)	RT-qPCR Northern siRNA	RT-qPCR (Northern)	(RT-qPCR)

*: mixed infection with RnPV18 and RnPV19.

(RT-qPCR) or (Northern) refers to no or potentially low virus accumulation.

Supplementary Table S2. List of primers used in this study

NO.	PRIMER NAME	PRIMER SEQUENCE	PURPOSE
1.	PV11-Rd-QRT-F	ACCGAAGACACAGACTACATCGTG	RnPV11 amplification in RT-qPCR
2.	PV11-Rd-QRT-R	ATCTGTGGTGCTTGGAGGGTTG	RnPV11 amplification in RT-qPCR
3.	PV2-Rd-QRT-F	ACGCACGCCAAGGAGAACTAGATT	RnPV2 amplification in RT-qPCR
4.	PV2-Rd-QRT-R	TCATTCTAGAGACGACCAGGCAGA	RnPV2 amplification in RT-qPCR
5.	PV11 QRT F2	AATCCCCTTGAACACAAGCG	RnPV11 amplification in RT-qPCR
6.	PV11 QRT R2	AGTGTCCTTTTGCCGTTTGC	RnPV11 amplification in RT-qPCR
7.	PV11 QRT F3	AAAATTCGCCCAGTCTACGC	RnPV11 amplification in RT-qPCR
8.	PV11 QRT R3	AGGTTTCAAGTCCGTGCATG	RnPV11 amplification in RT-qPCR
9.	Car Actin F	TCTCTATATGCTAGTGGCCGCAC	Internal control- Carrot
10.	Car Actin R	CATCTGTAAGATCACGGCCAGCAA	Internal control- Carrot
11.	PV2 QRT F2	AACTTCGCACTCACAGAACC	RnPV2 amplification in RT-qPCR
12.	PV2 QRT R2	AAGAGCGTCGCGAAATTGTG	RnPV2 amplification in RT-qPCR
13.	PV2 QRT F3	TTTCGTCGAGGCACACAAAG	RnPV2 amplification in RT-qPCR
14.	PV2 QRT R3	ATGCGGTGAATGGTTTCTCG	RnPV2 amplification in RT-qPCR
15.	PV18 QRT Beta F	TCTCCTGCGTTTTACGATGC	RnPV18 amplification in RT-qPCR
16.	PV18 QRT Beta R	ATTCATTGCGGACGCGAATG	RnPV18 amplification in RT-qPCR
17.	PV19 QRT Alpha F	TTCACTGAAGCGCACGAATC	RnPV19 amplification in RT-qPCR
18.	PV19 QRT Alpha R	TTGCGGGCGATGAAAAACAG	RnPV19 amplification in RT-qPCR
19.	NbPP2A F	GACCCTGATGTTGATGTTCGCT	Internal control- *N. benthamiana*
20.	NbPP2A R	GAGGGATTTGAAGAGAGATTTC	Internal control- *N. benthamiana*
21.	RnPV6 QRT F	AGCTCACGCTTCCTTTTCAC	RnPV6 amplification in RT-qPCR
22.	RnPV6 QRT R	ATGATTGTGCGGGCGTTTTC	RnPV6 amplification in RT-qPCR
23.	RnPV10 QRT F	AAGCACACTCCCATTGTTGC	RnPV10 amplification in RT-qPCR
24.	RnPV10 QRT R	GTTTGTTGAAAGCCGGAAGC	RnPV10 amplification in RT-qPCR
25.	Ef1 N.tabacum F	TGTTGAGATGCACCACGAAG	Internal control- Tobacco BY-2 cells
26.	Ef1 N.tabacum R	AAGCAACAAACCCACGCTTG	Internal control- Tobacco BY-2 cells
27.	Sf9 Ef1α F QRT	TGATTGCGCCGTACTCATTG	Internal control- Sf9 insect cells
28.	Sf9 Ef1α R QRT	TTGTTGACGCCCACAATCAG	Internal control- Sf9 insect cells
29.	PP2A N.tabacum F	ATTGCTTGCACCTGTGATGG	Internal control- Tobacco BY-2 cells
30.	PP2A N.tabacum R	AGGGATTGCAACACCTTTGC	Internal control- Tobacco BY-2 cells
31.	ECD Sf9 F	AAACGTCGTATTCCGAGCAC	Internal control- Sf9 insect cells
32.	ECD Sf9 R	TTGCACGTGTGTACTGCATC	Internal control- Sf9 insect cells
33.	EF2 Sf9 F	TCAAGAAATCCGACCCTGTC	Internal control- Sf9 insect cells
34.	EF2 Sf9 R	TCATGAAGAGACGGTTGTGC	Internal control- Sf9 insect cells
35.	RPL13 Sf9 F	ACCATTGATGCCTGTACAGC	Internal control- Sf9 insect cells
36.	RPL13 Sf9 R	AAACGCTTGGCACGAATACC	Internal control- Sf9 insect cells
37.	PV18 QRT cp F2	AAACTGCCCGCACCAAAAAC	RnPV18 amplification in RT-qPCR
38.	PV18 QRT cp R2	TGGCAATGCAGTCATGAAGG	RnPV18 amplification in RT-qPCR
39.	PV19 QRT cp F2	TGGTTTCACCTTCGCTGTTG	RnPV19 amplification in RT-qPCR
40.	PV19 QRT cp R2	AAATCGCGAGGCATTTTGGG	RnPV19 amplification in RT-qPCR
41.	PV11-QRT- CP-F	TGATCACCGCAAACGCAATC	RnPV11 amplification in RT-qPCR
42.	PV11-QRT- CP-R	AAATTCTCCAGCTGCGTTGG	RnPV11 amplification in RT-qPCR
43.	PV2-QRT- CP-F	AACTGACGACCGCACTTTTG	RnPV2 amplification in RT-qPCR
44.	PV2-QRT- CP-R	AGAAAGCGCGTTCTTCAGTC	RnPV2 amplification in RT-qPCR
45.	PV10-QRT- CP-F	TTCCGCCGCCAATATGTTTC	RnPV10 amplification in RT-qPCR
46.	PV10-QRT- CP-R	AAGCGGGAAAGACTTCATGC	RnPV10 amplification in RT-qPCR
47.	DM S2 Ef1A QRT F	TGACAAAAGCCGCTGAGAAG	Internal control- S2 insect cells
48.	DM S2 Ef1A QRT R	TGTTGTTGCTGCTGTTGCTC	Internal control- S2 insect cells
49.	DM S2 Atub QRT F	AGCGCACCATTCAATTCGTC	Internal control- S2 insect cells
50.	DM S2 Atub QRT R	TGTTGGACAACATGCACACG	Internal control- S2 insect cells
51.	DM S2 RPL32 QRT F	AAGTGTGCGGCTCGTATTTC	Internal control- S2 insect cells
52.	DM S2 RPL32 QRT R	TGCGCTTCTTCACGATCTTG	Internal control- S2 insect cells
53.	W442 Entol Rd F	CCATGTTGTAGAGGGAGTCAA	Northern blotting – RnPV19 (RdRP)
54.	W442 Entol Rd R	ACGAATCTTCCGAACCCCTC	Northern blotting – RnPV19 (RdRP)
55.	W442 RdRp2 F	ATGCTGCTGAATCCTACATCC	Northern blotting – RnPV18 (RdRP)
56.	W442 RdRp2 R	ATCGCGGACTTGTTCATCAGA	Northern blotting – RnPV18 (RdRP)

57.	W442 PV18 Cp F	ACGACATTCTCTTCGCTGCC	Northern blotting – RnPV18 (CP)
58.	W442 PV18 Cp R	TGTAAGGAATGGCGGAGTCG	Northern blotting – RnPV18 (CP)
59.	W442 PV19 Cp F	TCGTCGAAAGCACATTTGTGCC	Northern blotting – RnPV19 (CP)
60.	W442 PV19 Cp R	GCGGCAGCATAATCTCCGTTA	Northern blotting – RnPV19 (CP)
61.	4F	TAGACCATGTAGCAGAGCTGA	Northern blotting – RnPV11 (RdRP)
62.	4R	CAGTCTACGCTGTCGCTCT	Northern blotting – RnPV11 (RdRP)
63.	4R-2F	CTCTCGACTCTTACGGAAATC	Northern blotting – RnPV11 (RdRP)
64.	262F	TTGCGGATTGCGAACCACTGT	Northern blotting – RnPV11 (CP)
65.	262R	TGCCCTCTACACCGACAACA	Northern blotting – RnPV11 (CP)
66.	874F	GGTATGACCAAGACCTCTAAC	RnPV18 amplification in RT-PCR
67.	874R	AGTAGCGTAGACAGCGTTAAC	RnPV18 amplification in RT-PCR
68.	vBaction5	TTAGTTGCGTTACACCCTTTCTTG	Internal control- Vero cells
69.	vBaction3	TCACCTTCACCGTTCCAGTTT	Internal control- Vero cells
70.	vAlup53F	GGGTAAGGGCGGTTGTCAGT	Internal control- Vero cells
71.	vAlup53R	GGGAGGTCAAATAAGCAGAAG	Internal control- Vero cells
72.	bCdkn1βF	CAGCTTGCCGGAGTTCTACT	Internal control- BHK cells
73.	bCdkn1βR	ATGCCGGTCCTCAGAGTTTG	Internal control- BHK cells
74.	bG3pdhF	GACATCAAGAAGGTGGTGAAGCA	Internal control- BHK cells
75.	bG3pdhR	CATCAAAGGTGGAAGAGTGGGA	Internal control- BHK cells

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