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Supplementary Information for

Vertical transmission in *Caenorhabditis* nematodes of RNA molecules encoding a viral RNA-dependent RNA polymerase

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Supplementary Information Text

Extended Material and Methods

Nematode culture handling and bleaching
Nematode strains were isolated from natural samples as in (1), keeping the associated microbiome (no bleaching of the population). Strains of male-female *Caenorhabditis* species were originally derived from a mated female or a male-female pair. The animals were cultured on standard *C. elegans* Normal Growth Medium agar plates at 20-25°C seeded with *E. coli* OP50, and frozen at -80°C and in liquid nitrogen, as in (2). The cultures can then be thawed and regrown. A strain list is available as Table S1. *C. zanzibari* is a new species described in (3).

Screening for viral sequences by RNA sequencing
RNA were prepared from mixed-stage populations of wild strains of *Caenorhabditis* and of a few other bacterivorous genera. Strains JU1396 and QG551 were individually indexed and sequenced (2 x 150 nt) on Illumina HiSeq. Strain JU2557 was pooled with four other strains and given a common index. Likewise JU3236 was pooled with four other strains with a common index. These pools were sequenced on an Illumina MiSeq (2 x 250 nt). Sequences were assembled and contigs with similarity to viruses were identified using VirusSeeker (4). From the pooled samples, individual *Caenorhabditis* strains from the positive pools were then screened by RT-PCR using the viral RNA sequences. Sequence reads are available at SRA under accession numbers SRR8869242 to SRR 8869245 (Bioproject PRJNA531652).

For JU2557, two small contigs found by sequencing were used to design two primers JU2557-bunya-F1 and JU2557-bunya-R2 (Table S2) that were used to amplify a 2340 nt fragment by RT-PCR. The *C. zanzibari* bunya-like RNA was found in a pool of five strains; infection of JU3236 was identified by RT-PCR. Using primers JU3236-bunya-F1 and JU3236-bunya R1, an amplicon of 1538 nt was generated by RT-PCR.

Blastp using RdRP proteins was performed on NCBI database (https://blast.ncbi.nlm.nih.gov/Blast.cgi) e.g. on 26 Dec 2018.

3' RACE sequencing
To extend the JU1396 contig, 3' RACE was performed as described (5). PolyA polymerase was used to add poly(A) tails to the RNA. Qiagen one-step RT-PCR was then used with a specific primer and an oligo dT primer and the resulting amplicons were cloned and Sanger sequenced.

Strand analysis
Following quality filtering of the raw sequence data from JU1396 and QG551 RNAseq, identical PCR copies (required the exact same 5' terminus and 3 or fewer mutations in the first 70 nt) were de-duplicated using CD-hit-dup (6). The remaining unique reads were then mapped to either JU1396 or QG551 bunya-like contigs and counted.

Alignments and phylogenetic analyses
Sequence alignments were performed using MUSCLE (7) and default parameters in MEGA7 (8). The sequence relationships were inferred in MEGA7 (8) using the Maximum Likelihood method based on the Jones-Taylor-Thornton (JTT) matrix-based model (9) and tested using bootstraps. All positions containing gaps and missing data were eliminated. Initial tree(s) for the heuristic search were obtained automatically by applying Neighbor-Join and BioNJ algorithms to a matrix of pairwise distances estimated using a JTT model, and then selecting the topology with superior log likelihood value. Domain structure was queried in NCBI Domain Architecture Retrieval Tool.

Bleach treatment
Bleaching of the nematode culture was performed as in (2). This bleach treatment eliminates the horizontally transmitted microbial organisms that are sensitive to bleaching (noda-like viruses (10), bacteria, yeasts, microsporidia, etc.). The *Caenorhabditis* embryos resist bleaching because of their egg shell. Only symbionts that are present in the egg are transmitted following a bleaching

protocol. For example, we previously reported that *C. brenneri* JU1396 harbors a microsporidian parasite, which is horizontally transmitted and removed by the treatment (11).

RT-PCR

RNAs of the different bleached strains were extracted using Trizol/Chloroform. 1 µg of total RNA was used to amplify the different viruses by a one-step RT-PCR with the Superscript III (Life Technologies), the GoTaq polymerase (Promega) and the oligonucleotides listed in Table S2, using the following conditions: 65°C 2 min, 50°C 50 min for the reverse transcriptase reaction and 98°C 30 sec, 58°C 30 sec, 72°C 30 sec for 40 cycles for the PCR reaction. The PCR reactions were performed in parallel on genomic DNA in order to test for the presence of an integrated DNA copy of the viruses.

Whole-genome sequencing (WGS) and mapping to *Caenorhabditis* reference genomes and viral contigs

WGS data from isolated DNA was generated on a NextSeq 500 and consisted of 1x75 bp single-ended reads for the four wild *Caenorhabditis* isolates: JU1396 (*C. brenneri*), JU2557 (*C. remanei*), JU3236 (*C. zanzibari*), QG551 (*C. remanei*). These sequences are available at the NCBI SRA database under accessions SRR9206839 to SRR9206841 (Bioproject PRJNA531652). Reference genome FASTA and GFF3 files for *C. brenneri* and *C. remanei* were downloaded from UCSC (https://genome.ucsc.edu/). For *C. zanzibari*, coding sequences FASTA and GFF3 files were available and downloaded from the Caenorhabditis Genomes Project (http://download.caenorhabditis.org/). WGS data for each wild isolate was aligned to the six detected viral-like contigs with bowtie version 1.2.2 using the -v alignment mode to allow 3 mismatches per alignment (bowtie -S -p 20 -v 3) (12).

In each species, we identified two single copy genes as size-comparable controls (for the viral-like contigs), which we likewise aligned with WGS data. Reference GFF3 files were parsed to find genes in Wormbase that were similar in length (+/- 10%) to each viral contig. To identify if the gene was single copy, a nucleotide BLAST (version 2.7.1) alignment (blastn -num_threads 16 -evalue 1e-4) of the putative single copy gene to the reference was performed. WGS data then was aligned to each single copy gene using bowtie (bowtie -S -p 20 -v 3). The relaxed alignment parameters were to account for divergence between wild worm and reference genomes.

RNA secondary structure predictions

To predict the secondary structure of the ends of the RNA sequences, we used RNAfold (13), available at http://rna.tbi.univie.ac.at//cgi-bin/RNAWebSuite/RNAfold.cgi.

Single-molecule fluorescent in situ hybridization (smFISH)

Single-molecule FISH was performed essentially as described (14, 15) with probes labelled with Quasar 670 or CAL Fluor Red 610 (Table S2; Biosearch Technologies), except that the hybridization solution contained 20% formamide. One plate of mixed-stage animals was harvested and washed in nuclease-free water. The animals were spun down at 3000 rpm and resuspended in 1 ml of fixative solution (4% formaldehyde Sigma #533998, in PBS), followed by rotation at room temperature for 40 min. The pellet was then washed twice with 1 ml PBS and finally resuspended in 1 ml of 70% ethanol. These samples were stored at least one night at 4°C.

For hybridization, the fixed animals were pelleted and washed with 1 ml of Wash Solution (20% formamide Ambion #AM9342, in SSC 2X Ambion AM#9770). The animals were resuspended in 100 µl of hybridization solution (10% dextran sulfate Sigma #D6001, 2 mM vanadyl-ribonucleoside complex Sigma #94742, 0.02% RNAse-free BSA Sigma #A3059, 50 µg *E. coli* tRNA Roche #10109541001, 20% formamide, SSC 2X) with 1 µl of probes from a 1 µM stock. The samples were incubated in the dark at 30°C overnight. The next day, 1 ml of wash solution was added and the samples were centrifuged, resuspended in 1 ml of wash solution and incubated for 30 min at 30°C. The samples were then centrifuged again, resuspended in 1 ml of wash solution with 1.5 µl of DAPI (5 ng/µl; Sigma #1.24653) and incubated for 30 min at 30°C. Before imaging, the samples were resuspended in GLOX buffer (10% glucose, Tris-HCl 2M, SSC 2x) supplemented with 1 µl of glucose oxidase (Sigma #G0543) and 1 µl of catalase (from *Aspergillus niger*, Sigma #C315).

For embryo staining, cultures were bleached to select for embryos, after which the embryos were frozen by immersion into liquid nitrogen for 1 minute to crack the eggshells and then incubated 20 minutes on ice. The samples were then treated as above, starting with the wash in fixative solution.

Images were acquired using a Zeiss AxioImager M1, equipped with a Pixis 1024B camera (Princeton Instruments) and a Lumen 200 metal arc lamp (Prior Scientific). A Z-stack of 31 sections was recorded, which helped assess the sex and anatomy of the nematode; a single stack is shown in the figures. As is usual with the single-molecule FISH, the single-molecule signal is visible just above the background staining as a dot. As the signal of the dot is only slightly above the background and each animal presents a different level of background, it was not possible to treat each animal on the same scale in the same manner. Instead, we adjusted the minimum threshold using the Brightness and Contrast function in Image J so as to make the background in the animal appear clearly in the final figure - especially in the negative controls of Figures S1-S3.

Transmission through crosses

Crosses were performed using a single female and a single male per replicate. All crosses and sample processing were performed in parallel. Their descendants were scored in the adult stage by FISH after 7 days at 23°C for the presence of the bunyaviral RNA from JU1396.

RNA interference assays

The *Cbn-unc-22* and *Cre-pos-1* fragments were amplified with primers containing the attB1 and attB2 Gateway (Invitrogen) cloning sites and inserted into pDONR221 according to the BP reaction's protocol (Life Technologies). A LR Reaction was then performed to insert the fragments into the pDEST L4440 Gateway vector. For *Cbn-cyk-4,* the genomic fragment was amplified with primers containing the NcoI and NotI restriction sites and cloned into the L4440 vector previously digested by NcoI and NotI. The *Cre-unc-22* RNAi plasmid was previously described in (16). The GFP plasmid is that used in (10), a gift from Eric Miska's laboratory.

PCR products were generated using T7 primers on the plasmids described above and used as a template to perform in-vitro transcription reactions using the MEGAscript kit (Ambion).

Double-stranded RNAs were injected at 50 ng/µl into both gonadal arms of young adult females. After injection, a male was added onto the plate with the injected female and both were transferred onto a new plate the day after. For *unc-22,* the progeny was scored for twitching uncoordinated larvae; for *pos-1* and *cyk-4,* the progeny was scored for dead embryos.

Statistical analysis was performed in R version 3.4.1 (17).

Small RNA sequencing and analysis

Mixed-stage populations from two 55 mm diameter plates were harvested in M9 (2) and centrifuged. 800 µl of TRIzol (Invitrogen) were added to each worm pellet and the mixes were snap-frozen in liquid nitrogen before being stored at -80 °C. The next day, total RNAs were extracted by adding 200 µl of chloroform to the mix. After a 15-minute centrifugation at 13,000 rpm, the upper phase was collected. This step was repeated twice. RNAs were then precipitated overnight at -20°C with 500 µl isopropanol and 1 µl glycogen. The next day RNAs were pelleted at 13,000 rpm for 30 minutes and the pellets washed twice in 75% ethanol. The pellets were air dried and dissolved in nuclease-free water. RNA concentrations were estimated using the Nanodrop (Thermofisher)

In order to generate 5'-end independent small RNA libraries, 800 ng of total RNAs were treated with 5'-polyphosphatase (Epicenter/Illumina) for 30 minutes. Libraries were generated using the NEBNext® Small RNA Library Prep Set for Illumina® following the manufacturer's instructions from step 1 to 15. Migration on denaturing polyacrylamide (Novex™ TBE-Urea Gels 6% from ThermoFisher) was used for the library size selection. Bands between the DNA ladder fragments at 147 and 160 bp were extracted from the gel. Library quality control was performed using a Bioanalyser (Agilent) with the Agilent High Sensitivity DNA Kit, before being sequenced using an Illumina NextSeq System machine to generate 75-nucleotide single-end reads. Sequence reads are available at SRA under BioProject PRJNA551618, accessions SRX6374058-SRX6374060.

For sequence analysis, adaptors were removed from the Fastq files using the program Cutadapt v1. Fastq sequences were trimmed to leave reads of length between 15 and 34 nucleotides, which were aligned to the vRNA genome using bowtie-0.12.8 (12), allowing for one mismatch. We extracted the counts of reads grouped according to their length and the identity of their first nucleotide. Plots of the proportions of small RNAs grouped according to their length and their first nucleotide were generated to illustrate the small RNA population differences between generations. For calculation of % of reads mapping to each RNA genome, data were normalized to available RNA genome sequence length by dividing the number of reads by genome length for each RNA segment and then by the sum of these for all three segments. The graphs of read positions among the genomes were made in R version 3.4.1 (17).

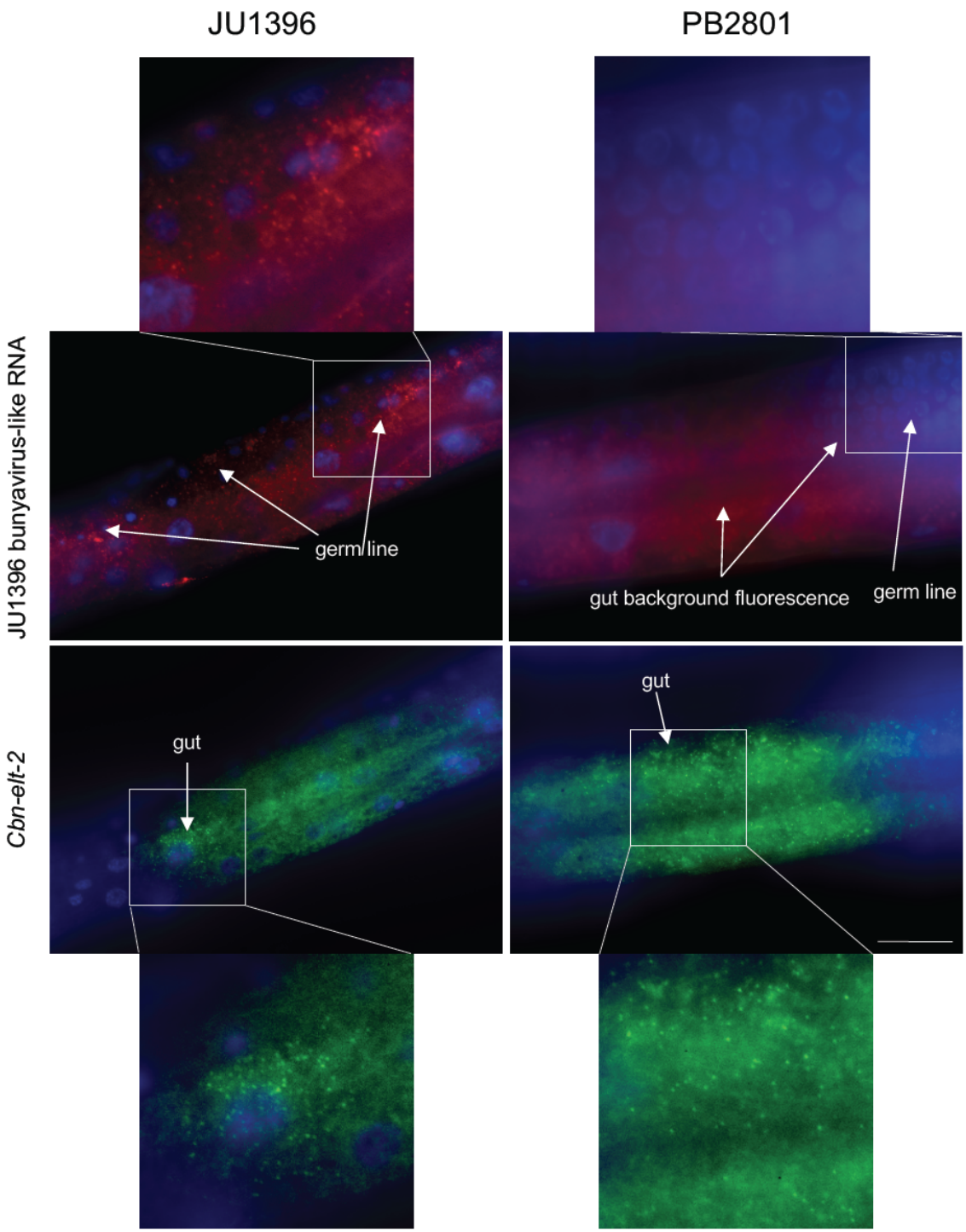

Figure S1. The bunyaviral-like RNA is detected by smFISH in *C. brenneri* JU1396 but not in *C. brenneri* PB2801. Fluorescent in situ hybridization against the plus strand of the JU1396 bunya-like viral sequence (labeled with CAL Fluor Red 610, shown in red), DAPI staining of nuclei (blue) and positive control with *Cbn-elt-2* probes (labeled with Quasar 670, shown in green). Bar: 50 micrometers, same magnification for all four main panels. A zoom of a region is shown next to each panel, in the germ line for the bunyaviral-like RNA, in the gut for *Cbn-elt-2*. The RNAs are detected as dots only slightly above the background fluorescence. Each picture was treated differently as the background level differed in each animal and probe combination, making sure the background was apparent, especially in the negative controls.

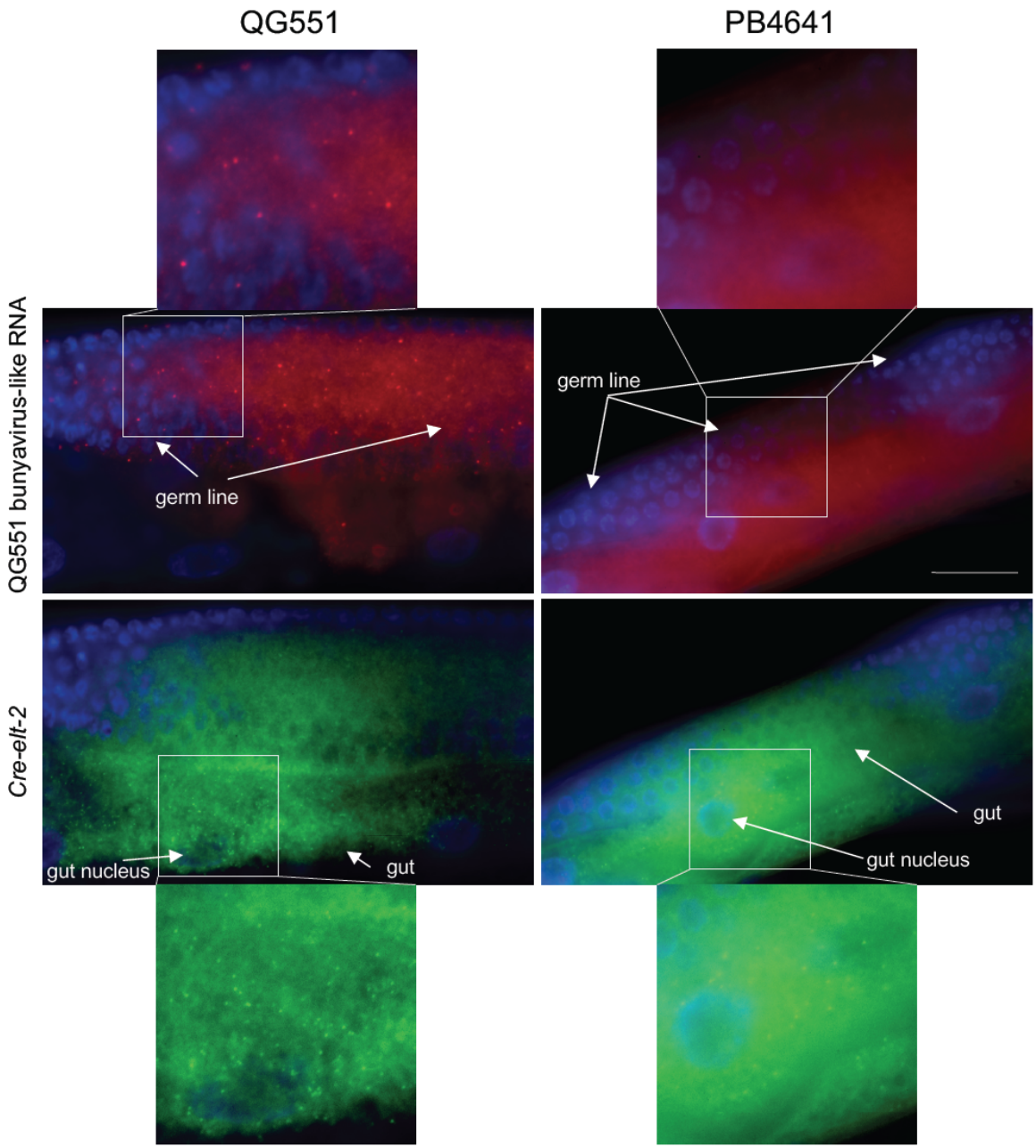

Fig. S2. The bunyaviral-like RNA is detected by smFISH in *C. remanei* QG551 but not in *C. remanei* PB4641. Fluorescent in situ hybridization against the plus strand of the QG551 bunya-like viral sequence (labeled with Quasar 670, shown in red), DAPI staining of nuclei (blue) and positive control with *Cre-elt-2* probes (labeled with CAL Fluor Red 610, shown in green). Bar: 50 micrometers, same magnification for all four main panels. A zoom of a region is shown next to each panel, in the germ line for the bunyaviral-like RNA, in the gut for *Cre-elt-2*. The RNAs are detected as dots above the background fluorescence.

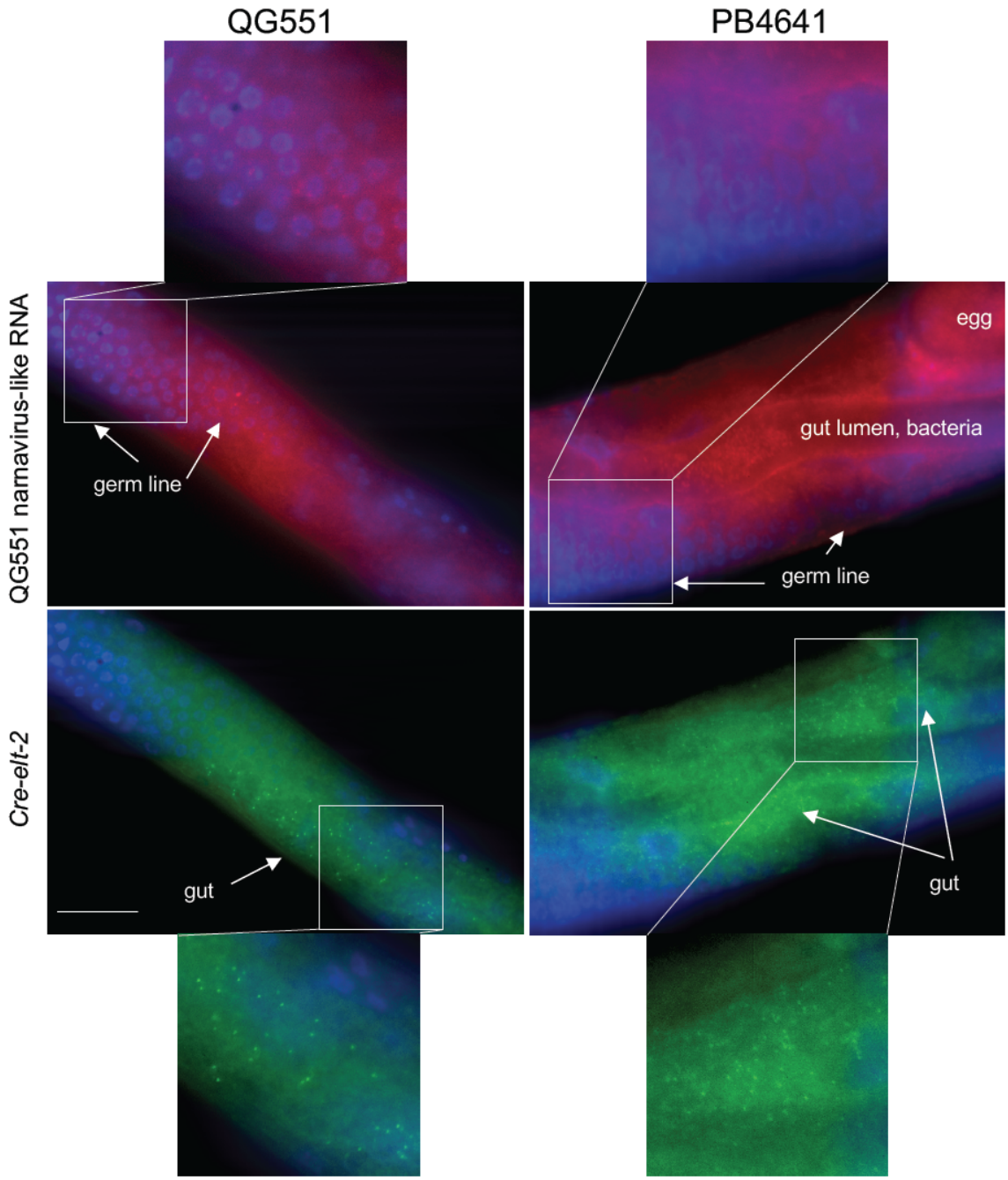

Fig. S3. The narnaviral-like RNA is detected by smFISH in *C. remanei* QG551 but not in *C. remanei* PB4641. Fluorescent in situ hybridization against the minus strand of the QG551 narna-like viral sequence (labeled with Quasar 670, shown in red), DAPI staining of nuclei (blue) and positive control with *Cre-elt-2* probes (labeled with CAL Fluor Red 610, shown in green). Bar: 50 micrometers, same magnification for all four main panels. A zoom of a region is shown next to each panel, in the germ line for the narnaviral-like RNA, in the gut for *Cre-elt-2*. The RNAs are detected as dots above the background fluorescence.

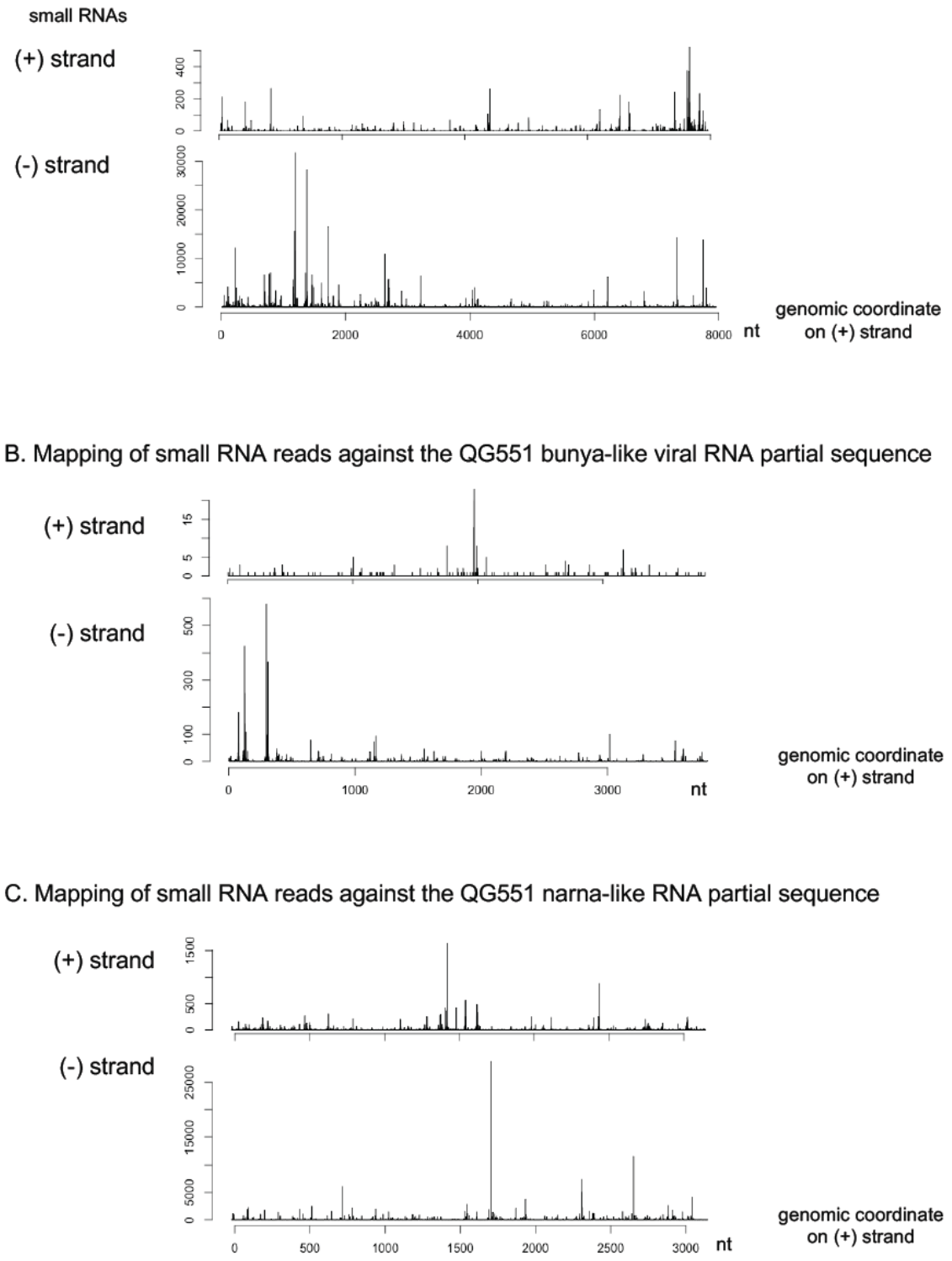

Fig. S4. Mapping of small RNAs along the viral molecules. (A) JU1396 bunya-like viral sequences. (B) QG551 bunya-like viral sequences. (C) QG551 narna-like viral sequences. The graphs show the number of reads mapping at a given position on the coding (+) or non-coding (-) strand. Note that the graphs are not at the same scale.

Table S1. Strain list.

Strain	Species	Isolation
CB5161	*Caenorhabditis brenneri*	Isolated in Trinidad by David Hunt.
JU1323	*Caenorhabditis brenneri*	Isolated by MAF from rotting coconut and wood sampled in Estuary Island Resort, Poovar, Kerala, India on 19 Dec 2007.
JU1326	*Caenorhabditis brenneri*	Isolated by MAF from rotting banana leaves and stems sampled on the shore of backwaters a few km from Allepey, Kerala, India on 24 Dec 2007.
JU1327	*Caenorhabditis brenneri*	Isolated by MAF from rotting pineapples (large heap) sampled in a plantation in Kanjirapally, Kerala, India on 26 Dec 2007 (ca. 70 m from JU1329).
JU1329	*Caenorhabditis brenneri*	Isolated by MAF from fallen banana tree (fruit, leaf, stem, flower) sampled in a plantation in Kanjirapally, Kerala, India on 26 Dec 2007.
JU1379	*Caenorhabditis brenneri*	Isolated by MAF from rotting *Alpinia purpurata* (red ginger) flowers sampled in the island of La Réunion by Mr and Mrs Robert on 22 Jan 2008.
JU1396	*Caenorhabditis brenneri*	Isolated from rotting noni fruits, 50 km SW from Medellin, Colombia, sampled March 2008. About 50 meters from JU1397-9.
JU1397	*Caenorhabditis brenneri*	Isolated from rotting orange fruits, 50 km SW from Medellin, Colombia, sampled March 2008. About 50 meters from JU1396, 3-4 meters from JU1398.
JU1398	*Caenorhabditis brenneri*	Isolated from rotting orange fruits, 50 km SW from Medellin, Colombia, sampled March 2008. About 50 meters from JU1396.
JU1813	*Caenorhabditis brenneri*	Isolated from small fruits sampled in Montabo near Cayenne, French Guiana, on 17 Nov 2009 by MAF and Christian Braendle.
JU1815	*Caenorhabditis brenneri*	Isolated from rotten seeds/fruits sampled near the Comté river at the cross with RN2 road, French Guiana, on 18 Nov 2009 by MAF and Christian Braendle..
JU1816	*Caenorhabditis brenneri*	Isolated from rotten lemons sampled in Cacao, French Guiana, on 19 Nov 2009 by MAF and Christian Braendle.
JU1817	*Caenorhabditis brenneri*	Isolated from rotten kumquat sampled in Cacao, French Guiana, on 19 Nov 2009 by MAF and Christian Braendle.
JU1886	*Caenorhabditis brenneri*	Isolated from unidentified rotting fruit. Sampled by Chloe Yujin Kim in the region of Krabi nearby the Sai Tai beach in Thailand on 02 Dec 2009.
JU2557	*Caenorhabditis remanei*	Isolated from rotting apples sampled on 26 Sep 2013 in Illkirch (Bas-Rhin), France. Same sample (several apples) as JU2556-60. Isofemale line started from a L4 female and a male on 27 Sep 2013.
JU2558	*Caenorhabditis remanei*	Isolated from rotting apples sampled on 26 Sep 2013 in Illkirch (Bas-Rhin), France. Independent female from the natural population.
JU2559	*Caenorhabditis remanei*	Isolated from rotting apples sampled on 26 Sep 2013 in Illkirch (Bas-Rhin), France.
JU2560	*Caenorhabditis remanei*	Isolated from rotting apples sampled on 26 Sep 2013 in Illkirch (Bas-Rhin), France.
JU3235	*Caenorhabditis zanzibari*	Isolated from rotting *Morinda citrifolia* fruits sampled by Amir Yassin in M'Tsangamouji, Mayotte. -12.76, 45.09 in agricultural landscape
JU3236	*Caenorhabditis zanzibari*	Isolated from rotting *Morinda citrifolia* fruits sampled by Amir Yassin in M'Tsangamouji, Mayotte. -12.76, 45.09 in agricultural landscape
LKC28	*Caenorhabditis brenneri*	Isolate from which the inbred line for sequencing (PB2801) was derived. Isolated in Costa Rica, at a plant nursery, from roots of *Liriope*.
NIC42	*Caenorhabditis brenneri*	Isolated from rotten *Ficus* fruit sampled at the CNRS Biological Station in Nouragues Forest, French Guiana, on 24 Nov 2009 by MAF and Christian Braendle.
PB4641	*Caenorhabditis remanei*	Inbred line used for genome sequencing.
QG548	*Caenorhabditis remanei*	Isolated from a rotting *Alpinia* flower collected by Taniya Kaur on 20 May 2011 in Okinawa, Japan. Seaside House garden path, Okinawa, Japan.
QG549	*Caenorhabditis remanei*	Isolated from a rotting small round fruit collected by Taniya Kaur on 20 May 2011 in Okinawa, Japan. Seaside House adjacent to the main wooden deck.
QG551	*Caenorhabditis remanei*	Isolated from a rotting fruit collected by Taniya Kaur on 20 May 2011 on hilly hiking trail, Okinawa, Japan.
SB280	*Caenorhabditis brenneri*	Isolated from Guadeloupe.
ZF1137	*Caenorhabditis brenneri*	Isolated by Takao Inoue from a rotten fruit sampled ca. November 2010 in Singapore.

Table S2. List of oligonucleotides.

A. RT-PCR probes

name	sequence	strand
JU1396-bunya-F1	GTCTTTATGTGCGTGATGACTTGC	minus
JU1396-bunya-R1	TCATCTGACTTCCAAACGTTCCAA	minus
QG551-bunya-F1	CCTCCAGAGTGTATGCGGAT	plus
QG551-bunya-R1	CGGTGTCTCTGCAATGATGG	plus
JU2557-bunya-F1	GACATTCTCTCGCTTGCAAC	plus
JU2557-bunya-R1	ACCAGCATGTTGAGACTTCT	plus
JU3235-bunya-F1	CTCCACTACAGAATGATTGGCTGC	plus
JU3235-bunyaR1	GGT TGC ACA ATT GAG CCA AAC	plus
JU3236-bunya-F1	CTAATTAACTCCTGGTACATTGGTTATGCAAAAAATAAG	plus
JU3236-bunyaR1	ACACAAAAGTGAGCTCCATTTTCTGTC	plus
QG551-narnavirus-F1	CCGTACACTGGACCTGTTCT	minus
QG551-narnavirus-R1	CTGATGAAGTTGATCCTCTC	minus
QG551-sobemovirus-F1	CGACGTACCGATACCCAGAA	plus
QG551-sobemovirus-R1	CCCTCGAAGACTAGCAGGAG	plus
Cbn-actin-F	GTCCTCTCCCTCTACGCTTC	
Cbn-actin-R	TCGTAGGACTTCTCGAGGGA	
Cre-actin-F	TCCTTACCCTCGAGTACCCA	
Cre-actin-R	GGCGGGTGGGAAAACATATC	

B. RNAi primers

name	sequence
unc22-Cbn-F	**AAAAAAGCAGGCT**GCTGGAAAGACTGCTGGAAC
unc22-Cbn-R	**CAAGAAAGCTGGGT**TACACTCGGTCCCTTTGACC
pos1-Cre-F	**AAAAAAGCAGGCT**CTACGAAACAGCCGATTCCG
pos1-Cre-R	**CAAGAAAGCTGGGT**GGTGGAACTGATTGCCGAAG
attB1F univ	GGGGACAAGTTTGTAC**AAAAAAGCAGGCT**
attB2R univ	GGGGACCACTTTGTA**CAAGAAAGCTGGGT**
cyk4-Cbn-Not-F	AG**GCGGCCGC**GTGGAACGGCTCTGAAACTG
cyk4-Cbn-Nco-R	TG**CCATGG**GGAACGAGCTAAAAGTGGGG

C. FISH probes

JU1396-bunya(-)-Quasar670	JU1396-bunya(+)-CALFluorRed610	QG551-bunya(+)-Quasar670	QG551-narna(-)-Quasar670	QG551-narna(+)-CALFluorRed610
taacaatgcagaggacggct	cagtagtgcgtgtatttctg	ctttcatcatctttgcttct	ttccgatgttattggaacgc	ggaaaccgagggatctgatg
gtgaaccgaagtcagaggta	gttcgatcatgttcacttgg	ggaaggaaatcgggaccttg	aacttcctcctaacgtgtaa	gtgttcaagtgtgtcacgat
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C. FISH probes (continued)

QG551-sobemo(+)-Quasar670	JU2557-bunya(+)-Quasar670	Cbn-elt-2-Quasar670	Cre-elt-2-CALFluorRed610
ggatgggatactcgttcaga	caagcgagagaatgtcctta	cattttccgcataagtggta	ttatccgagtaggtgttgtc
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tggtggacttctggtacatg		taggaatctccatcgacagc	tttcatcgtcttgaactggc
ccaaacacgtttttcatcgg			aaatctctagcagcagcttt
ttggtgtttggagagcagat			tatgagtcgttgtccacagc
gaagactagcaggagatcgc			

Table S3. FISH scoring of other *C. brenneri* and *C. remanei* strains.

Strain	species	sampling localization	comments	probe against	smFISH signal
JU1396	*C. brenneri*	50 kms from Medellin (Colombia)	reference	Colombia bunya-like virus minus strand	+
JU1397	*C. brenneri*	50 kms from Medellin (Colombia)	local set	idem	-
JU1398	*C. brenneri*	50 kms from Medellin (Colombia)	local set	idem	-
CB5161	*C. brenneri*	Trinidad	world collection	idem	+/-
LKC28	*C. brenneri*	Costa Rica	world collection	idem	-
NIC42	*C. brenneri*	French Guiana	world collection	idem	-
SB280	*C. brenneri*	Guadeloupe	world collection	idem	-
JU1323	*C. brenneri*	India	world collection	idem	-
JU1326	*C. brenneri*	India	world collection	idem	-
JU1327	*C. brenneri*	India	world collection	idem	-
JU1329	*C. brenneri*	India	world collection	idem	-
JU1379	*C. brenneri*	La Réunion	world collection	idem	-
JU1813	*C. brenneri*	French Guiana	world collection	idem	-
JU1815	*C. brenneri*	French Guiana	world collection	idem	-
JU1816	*C. brenneri*	French Guiana	world collection	idem	-
JU1817	*C. brenneri*	French Guiana	world collection	idem	-
JU1886	*C. brenneri*	Thailande	world collection	idem	-
ZF1137	*C. brenneri*	Singapore	world collection	idem	-
QG551	*C. remanei*	Okinawa (Japan)	reference	Okinawa bunya-like virus minus strand	+
QG548	*C. remanei*	Okinawa (Japan)	local set	idem	-
QG549	*C. remanei*	Okinawa (Japan)	local set	idem	-
JU2557	*C. remanei*	Illkirch (France)	reference	Illkirch bunya-like virus minus strand	+
JU2558	*C. remanei*	Illkirch (France)	local set	idem	-
JU2559	*C. remanei*	Illkirch (France)	local set	idem	-
JU2560	*C. remanei*	Illkirch (France)	local set	idem	-

The *C. brenneri* isolates were tested using the probe set against the minus strand of the JU1396 Colombia bunya-like RNA. The *C. remanei* isolates were tested using the probe sets against the minus strand of either the QG551 or the JU2557 bunya-like virus, as indicated. n=20 animals per strain.

Table S4. The JU1396 bunya-like viral RNA is transmitted by both males and females.

	Females			Males		
Cross	+	+/-	-	+	+/-	-
JU1396 female x JU1397 male, replicate 1	5	0	0	9	0	0
JU1396 female x JU1397 male, replicate 2	13	0	0	12	0	0
JU1396 female x JU1397 male, replicate 3	4	0	0	13	0	0
JU1397 female x JU1396 male, replicate 1	0	0	2	0	0	8
JU1397 female x JU1396 male, replicate 2	8	4	2	6	1	0
JU1397 female x JU1396 male, replicate 3	13	1	2	3	1	3
JU1396 female x JU1396 male	3	0	0	7	0	0
JU1397 female x JU1397 male	0	0	12	0	0	17

Each line represents the progeny of a cross of one female and one male. Only adults were scored, using exposure times of 2000 ms. f: female. m: male. -: no FISH signal. +: clear signal. +/-: weak signal, resembling that in CB5161.

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