Supporting Information

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SI Materials and Methods

Virus and Virus-Like Particle Preparation. Cultured DL-1 cells were grown in Schneider's media (Sigma) containing 15% fetal bovine serum (Gibco) using standard laboratory procedures. A total of 8×10^7 cells in a volume of 2 mL were infected with flock house virus (FHV) at a multiplicity of infection of 1 and rocked for 1 h at room temperature before plating into 10 mL total of Schneider's insect media. Cells were harvested two days postinfection.

Sf21 cells were cultured in TC-100 media (Invitrogen) supplemented with 10% fetal bovine serum (Gibco) following standard laboratory procedures. Full-length alpha protein from FHV RNA 2 or *Nudaurelia capensis* omega virus (NωV) RNA 2 was expressed from the pBacPAK9 baculovirus vector as described in ref. 1. Expressed alpha protein spontaneously assembles into viral capsids encapsidating cellular RNA. Cells were harvested three days postinfection for FHV virus-like particles (VLPs) and six days postinfection for NωV VLPs.

Virions and VLPs were purified using a series of sucrose gradients as is well-established (2) in buffers containing 50 mM Hepes pH 7.0 for FHV VLPs and virions, and Tris pH 7.6 and 250 mM NaCl for NωV VLPs. Particles were first spun at 40,000 rpm for 2.5 h onto a 30% sucrose cushion. The pellet was resuspended and then applied to a 10–40% sucrose gradient and spun at 40,000 rpm for 1.5 h. Fractions from the sucrose gradient were removed and analyzed by SDS-PAGE. Fractions containing only viral capsid proteins were pooled and extensively washed on a 100 kDa molecular weight cutoff (MWCO) centrifugal concentrator. This sample was then applied to the top of a second 10–40% sucrose gradient and spun at 40,000 rpm for 1.5 h. Again, fractions from the sucrose gradient were removed and analyzed by SDS-PAGE. Fractions containing only viral capsid proteins were pooled and extensively washed on a 100 kDa MWCO centrifugal concentrator. After this extensive purification, no cellular proteins could be detected by Coomassie blue stain on an SDS-PAGE gel (Fig. S1).

Negative-Stain Electron Microscopy. Approximately 0.2 μg of virion or VLP was loaded onto grow-discharged 200-mesh carbon-coated Cu/Pd grid (Agar Scientific). After one minute at room temperature, grids were then washed with 40 μL 2% uranyl-acetate and left to air-dry. Images were taken using a CM-100 transmission electron microscope (Phillips) at 80 eKv.

RNA Preparation. Purified FHV or VLP particles were disrupted at room temperature by incubation in 0.1% SDS and 0.1 M NaCl for 15 min. RNA was extracted from the disrupted particles using an equal volume of acid phenol followed by three washes with 100% chloroform. RNA was then ethanol-precipitated in the presence of 100 mM sodium acetate pH 5.3. RNA pellets were washed in 70% ethanol, dried, and resuspended in pure water.

Directional RNAseq. One microgram of RNA was prepared for next-generation sequencing using a modified version of the Illumina protocol (www.illumina.com) where 12 cycles of PCR were performed and standard TruSeq adapters and TruSeq barcoded primers were used. A final size selection was performed by native agarose gel electrophoresis to yield a library of inserts 50–150 bases in length suitable for 40 base single-read sequencing. The library was extracted from the agarose gel using standard oligo purification columns. The prepared library was then loaded onto an Illumina HiSeq v2 single-read flow cell, standard cluster generation was performed on a Cbot and sequenced for 40 bases of the insert and 7 bases of the index read using standard HiSeq sequencing reagents. Reads were processed using CASAVA 1.8 and demultiplexed based on index sequences.

Alignment of RNAseq Reads. Poor quality reads containing any base with a PHRED score < 20 and reads containing fragments of the RNA adaptor sequence were removed and trimmed, respectively, using the FastX Toolkit (http://hannonlab.cshl.edu/fastx_toolkit/). The quality of the short reads in the datasets was assessed using the FastQC package (http://www.bioinformatics.bbsrc.ac.uk/projects/fastqc/). Alignment of the short reads to reference genomes was performed using algorithms packaged in the CLC Genomics Workbench 5.1. The *Drosophila melanogaster* reference genome r5.22 was downloaded from the FlyBase repository (http://flybase.org/). Functional annotations (refFlat) for mRNA sequences in the *D. melanogaster* genome were obtained from the University of California, Santa Cruz genome browser web site (http://genome.ucsc.edu/) and alignments of reads to exonic, intronic, and intergenic regions were determined using SAMMate (http://sammate.sourceforge.net/) (3). Expression sequence tags (ESTs) and sequence fragments from *Spodoptera frugiperda* cell lines were downloaded from the *S. frugiperda* database: Spodobase (http://bioweb.ensam.inra.fr/spodobase/) (4). Sequences for the FHV RNA 2 (NC_004144), FHV RNA 1 (NC_004146), NωV RNA 2 (S43937), the *Attacus ricini* 45S rDNA (AF463459), and the Baculovirus genome (NC_001623) were obtained from National Center for Biotechnology Information (NCBI). The *S. frugiperda* microsatellite library (187 sequences) (5) was obtained from NCBI.

De Novo Assembly of Contigs. Reads that did not align to the reference genomes were assembled de novo into contigs with the Velvet package (http://www.ebi.ac.uk/~zerbino/velvet/) (6) using a hash length of 21. Contig alignment and finishing was carried out and inspected using SeqMan Pro (DNAStar Lasergene) (http://www.dnastar.com/). For finishing, contigs with overlapping sequences of greater than 21 bp with at least 95% sequence identity were conjoined. Contigs <200 bp were discarded. From the VLP datasets, a library of contigs was generated containing 336 sequences ranging from 200 to 5,730 bp (Dataset S1).

RT-PCR. Approximately 100 μg FHV virions in 100 μL were treated overnight at room temperature with 4 U DNase I (NEB) and 500 ng RNase A (Roche) in NEB DNase I buffer (10 mM Tris pH 7.6, 2.5 mM $MgCl_2$, 0.5 mM $CaCl_2$) to remove any traces of nonencapsidated DNA or RNA. The particles were then extensively washed with 50 mM Hepes pH 7.0 using a 100 kDa MWCO centrifugal concentrator to remove the enzymes. Encapsidated RNA was then extracted as described. Purified RNA was reversed transcribed into cDNA using RT Superscript III (Invitrogen) and amplified using standard PCR techniques using the primers indicated in Table S1. PCR products were analyzed by standard electrophoresis on a 0.8% agarose gel stained with ethidium bromide. Bands corresponding to PCR products of the expected length for each analysis were gel-extracted using a Qiagen Gel Extraction kit and the purified PCR products were submitted for sequencing (Eton Biosciences) using the PCR primers indicated in Table S1.

1. Schneemann A, Dasgupta R, Johnson JE, Rueckert RR (1993) Use of recombinant baculoviruses in synthesis of morphologically distinct virus-like particles of flock house virus, a nodavirus. *J Virol* 67:2756–2763.
2. Schneemann A, Marshall D (1998) Specific encapsidation of nodavirus RNAs is mediated through the C terminus of capsid precursor protein alpha. *J Virol* 72:8738–8746.
3. Xu G, et al. (2011) SAMMate: A GUI tool for processing short read alignments in SAM/BAM format. *Source Code Biol Med* 6:2.
4. Negre V, et al. (2006) SPODOBASE: An EST database for the lepidopteran crop pest *Spodoptera*. *BMC Bioinf* 7:322.
5. Arias RS, Blanco CA, Portilla M, Snodgrass GL, Scheffler BE (2011) First microsatellites from *Spodoptera frugiperda* (Lepidoptera: Noctuidae) and their potential use for population genetics. *Ann Entomol Soc Am* 104:576–587.
6. Zerbino DR, Birney E (2008) Velvet: Algorithms for de novo short read assembly using de Bruijn graphs. *Genome Res* 18:821–829.

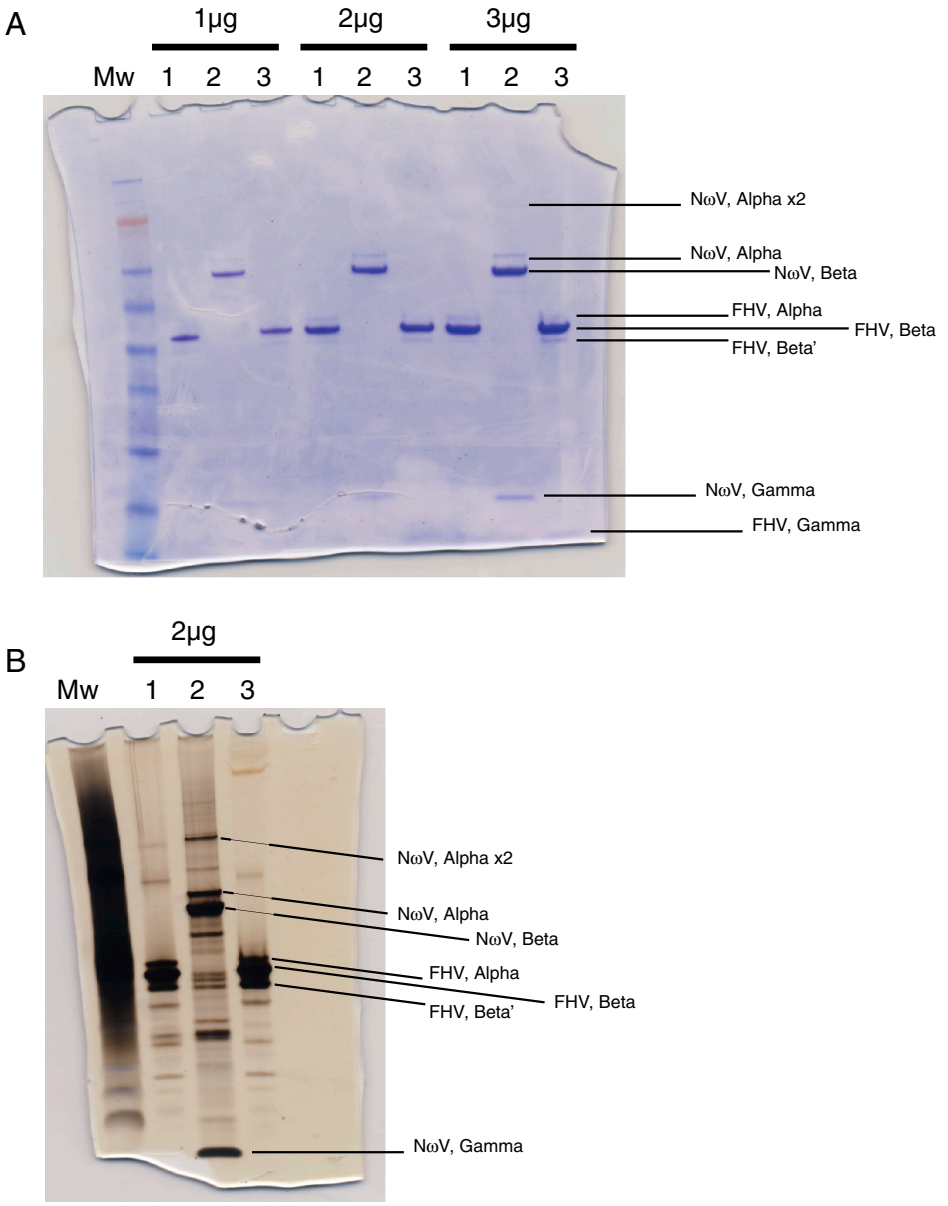

Fig. S1. Virus and virus-like particles are exceptionally pure, as judged by SDS-PAGE gel electrophoresis. For all gel lanes: 1, FHV VLPs; 2, NωV VLPs; and 3, FHV virions. (*A*) One, two and three micrograms of each sample were analyzed by SDS-PAGE gel electrophoresis and stained with Coomassie blue (30 min stain and overnight destain). (*B*) Trace protein contaminants are only visible after silver staining (Invitrogen Silver Quest—can detect >0.3 ng BSA). Two micrograms of each sample were analyzed, stained, and fully developed (eight minutes developing time). Identities of known bands are indicated appropriately. NωV alpha x2 is an apparent capsid dimer—as judged from molecular weight and by positive Western blotting. Beta is an apparent oxidation product of the beta capsid protein previously observed whose abundance seems to be correlated to pH.

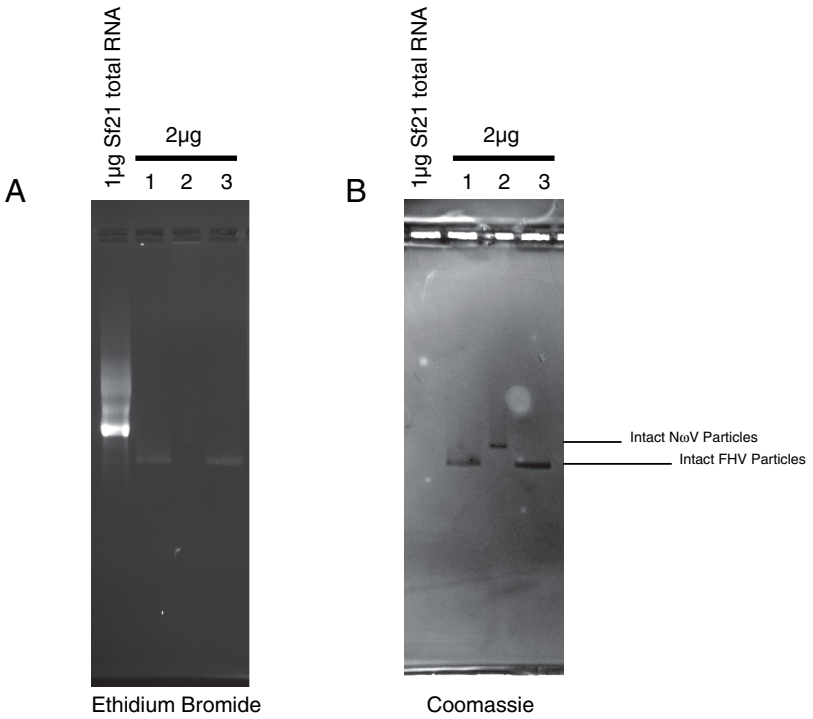

Fig. S2. Virus and virus-like particles show no evidence of contamination with copurified RNAs, as judged by native agarose gel electrophoresis. One microgram of total RNA purified from Sf21cells and 2 µg of each intact virus particle were analyzed by native agarose gel electrophoresis (0.8% agarose) and visualized by (*A*) ethidium bromide (prestained) and (*B*) Coomassie blue (1 h poststain with overnight destain). For all gel lanes: 1, FHV VLPs; 2, NωV VLPs; and 3, FHV virions. Virus particles migrate as one single band. Ethidium bromide staining of the virus particles is very poor as the ethidium bromide cannot easily penetrate the viral capsid to intercalate with the encapsidated RNA. Extra bands corresponding to copurified contaminant RNAs cannot be visualized.

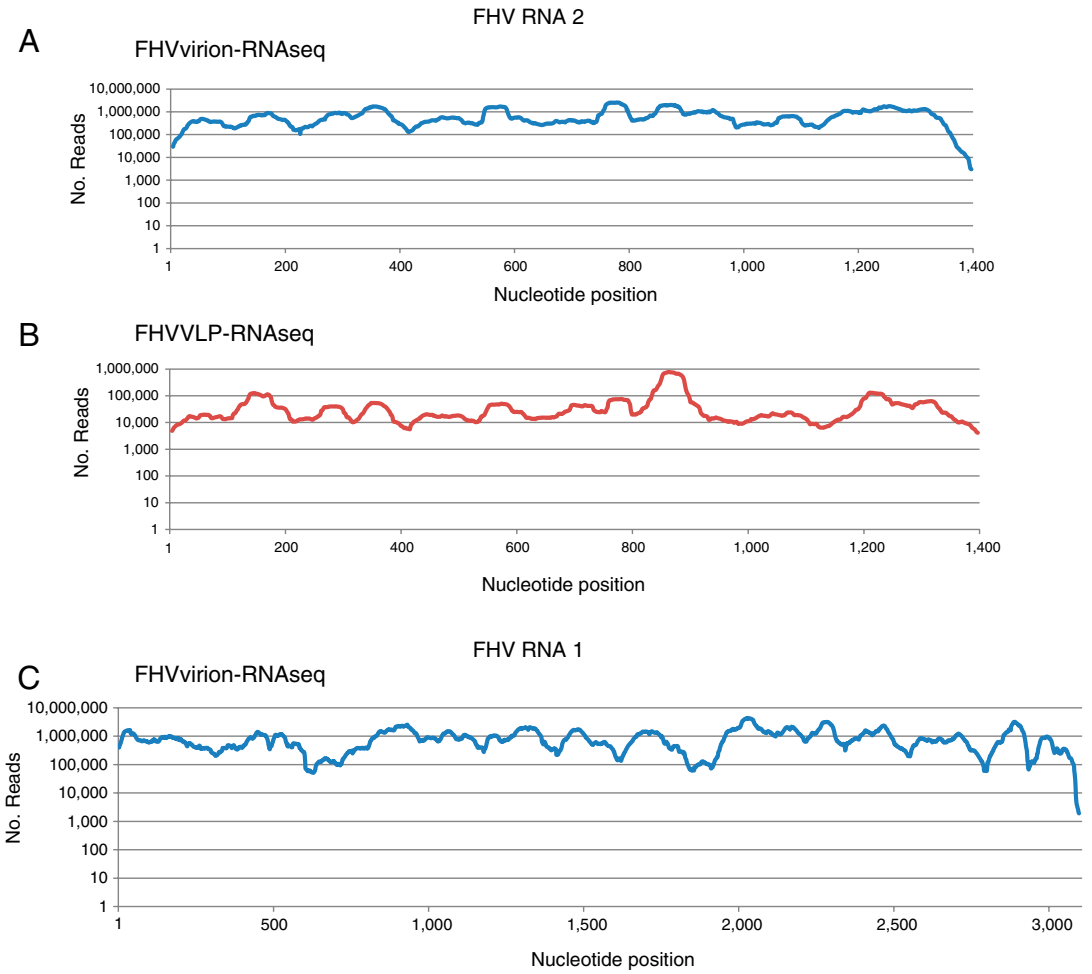

Fig. S3. Read Coverage over FHV genome indicated per base. Coverage over FHV RNA 2 for (*A*) FHVvirion-RNAseq and (*B*) FHVVLP-RNAseq datasets. (*C*) Coverage over FHV RNA 1 for FHVvirion-RNAseq.

Dataset S1. A file in FASTA format containing contigs assembled from the unaligned reads as described in the text. This library was used as a reference genome for read alignment for the FHVVLP-RNAseq and NωVVLP-RNAseq datasets detailed in Dataset S2 and Dataset S3, respectively.
Dataset S1 (TXT)

Dataset S2. Alignment details of FHVVLP-RNAseq reads to ribosomal DNA, Sf21 ESTs, FHV genome, Baculovirus genome, microsatellite marker library, and contig library. Color-coded: peach, viral RNA; green, mRNA; blue, rRNA; gold, transposon RNA; purple, noncoding RNA; and yellow, microsatellite markers from ref. 5.
Dataset S2 (XLSX)

Dataset S3. Alignment details of NωVVLP-RNAseq reads to ribosomal DNA, Sf21 ESTs, NωV RNA 2, FHV genome, Baculovirus genome, microsatellite marker library, and contig library. Color-coded: peach, viral RNA; green, mRNA; blue, rRNA; gold, transposon RNA; purple, noncoding RNA; and yellow, microsatellite markers from ref. 5.
Dataset S3 (XLSX)

Dataset S4. Alignment details of FHVvirion-RNAseq reads to *Drosophila melanogaster* genome (r5.22) and FHV genome. Color-coded: peach, viral RNA; green, mRNA; blue, rRNA; gold, transposon RNA; and purple, noncoding RNA.
Dataset S4 (XLSX)

Table S1. PCR primers

	Forward	Reverse
′1731′ retrotransposon ORF1	5′-gcc gga atg agt aac ctg ta	5′-tag ccc cgc tat cga gac ac
18S rRNA	5′-caa cgg gta acg ggg aat cag g	5′-ggt agt agc gac ggg cgg tgt g
7SL ncRNA	5′-ctg tga ggt ctg att gtg gga tgg	5′-tac gct atc cca cta ctg cct acc
Enolase mRNA	5′-act gcc tgc tcc tga agg tc	5′-ata atg att aga tca agg tt