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Supporting Information for

Maintenance of Persistent Transmission of a Plant Arbovirus in its Insect Vector Mediated by the Toll-Dorsal Immune Pathway

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Supplemental Materials and Methods

Insects

The populations of both RSV-free and RSV-infected *L. striatellus* are reared on the susceptible rice seedlings (cv Wuyujing No. 3) in a temperature-controlled room maintained at 25 ± 1°C with a relative humidity of 70-80%, and a photoperiod of 14h light:10h dark. The RSV infection ratio of the planthopper population is approximately 80% that is monitored every 3-4 generations using reverse transcription polymerase chain reaction (RT-PCR) as previously described[1, 2].

Extraction and Injection of RSV-infected or RSV-free Crude Extracts of *L. striatellus*

To prepare the crude extracts, ten adult *L. striatellus* of RSV-infected or RSV-free insects were ground into a fine powder using a sterilized grinding rod, and subsequently suspended in 200 μl of 1×PBS placed in a 1.5mL tube. Following centrifugation at 4℃ and 12,000 rpm for 10 minutes, the supernatant was carefully removed. This process was repeated three times to obtain the final crude extracts. To investigate the effects of RSV on gene expression in *L. striatellus*, 20 nl of RSV-infected or RSV-free crude extracts were separately injected into the ventral thorax of non-viruliferous 2nd instar *L. striatellus*, with each biological replicate comprising 10 individuals. Samples were collected at 2, 4, 6, and 8 dpi (to assure that RSV has almost passed the latent period in *L. striatellus*), and quantitative analysis of gene expression was performed subsequently.

Double-Stranded RNA Synthesis and Injection

The *LsDorsal*, *LsZN708*, and other gene fragments of *L. striatellus* were amplified using gene-specific primers ligated with a T7-promoter sequence, while a green fluorescent proteins (GFP) fragment was used as control. The primers used for the amplification were listed in Supplementary Table 1. Double-stranded RNA (dsRNA) was synthesized using the T7 RiboMAX Express RNAi System (Promega, USA) following the manufacturer's instructions. The quality of synthesized dsRNA was evaluated using agarose gel electrophoresis. Each planthopper was injected with 40 nl of dsRNA into the insect ventral thorax with a glass needle[2,3]. Additionally, a mixture of ds*LsDorsal*/ds*LsZN708*/ds*GFP* with RSV crude extracts in a 1:1 ratio was injected into non-viruliferous *L. striatellus*. Subsequently, 10-15 individuals (with each individual serving as one biological replicate) were collected at 3 and 6 dpi for subsequent experiments. The relative expression of *LsDorsal*, *LsZN708* and *RSV-NP* were determined using RT-qPCR.

Total RNA Isolation and Quantitative Real-Time PCR

The RNA extraction procedure followed standard TRIzol protocol (Takara, Japan) for both

insects and rice plants. The quality of total RNA samples was quantified and assessed using a NanoDrop spectrophotometer (Thermo Scientific, USA). To synthesize the first strand of complementary DNA (cDNA), 1,000 ng of total RNA was used with HiScript ®II Q RT SuperMix for qPCR (+gDNA wiper) (Vazyme, China) following the manufacturer's protocol. For quantitative real-time PCR (qPCR), 10 µl reaction agents were prepared, comprising 0.5 µl of template cDNA, 5 µl of Hieff ® qPCR SYBR Green PCR Master Mix (YESEN, China), 0.2 µl of 1 µM forward and reverse primers, and 4.1 µl of ddH_2O. The qPCR experiments were conducted using a LightCycler® 480 II (Roche, Switzerland). Thermal cycling conditions encompassed an initial denaturation step at 95°C for 5 minutes, followed by 40 cycles of denaturation at 95°C for 30 seconds, annealing at 60°C for 30 seconds, and extension at 70°C for 30 seconds. Data analysis utilized the $2^{-\Delta\Delta CT}$ method, and statistical significance was determined at $P < 0.05$ (*) and $P < 0.01$ (**) levels using TTEST.

Cross-linking of Antibodies and Fluorescein

The process of cross-linking RSV-NP antibody with fluorescein (Invitrogen, USA) involved the following steps: A dialysis bag was boiled in ultrapurewater for three minutes and subsequently rinsed twice with ultrapure water Following this, 100 µl of purified antibodies were added into the dialysis bag which was then dialyzed in 1 L of 0.1M $NaHCO_3$ buffer on a magnetic grate at 4℃. The buffer was refreshed every 4 hours for a total of three times. Afterwards, TRITC or FITC fluorescent powder was gradually added into the dialyzed antibodies with continuous vortexing and incubated for 2 hours in the dark on a shaker. Finally, the conjugated antibodies were dialyzed three times in 1×PBS buffer and were then stored in a -40℃ freezer.

Immunohistochemistry

The *L. striatellus* insects were injected with a mixture of ds*LsDorsal*, ds*LsZN708*, or ds*GFP* and RSV crude extracts. The salivary glands, guts, and ovaries were dissected in cold 1×PBS buffer, followed by fixation in 4% paraformaldehyde at room temperature for 4 hours. Each of the samples were washed three times for 5 minutes with 1×PBST buffer. Subsequently, 2% TritonX-100 was added to increase the permeability of tissue cells for 30 minutes. The samples were then washed three times for 5 minutes again with 1×PBST buffer. A polyclonal fluorescent conjugated antibody against RSV-NP (diluted 1:100 in 1% BSA) was added to the samples, which were then incubated overnight at 4℃. The tissue samples were then stained with DAPI (Abcam, England), and fluorescence images were captured using a SP8 X confocal microscope (Leica, German).

ChIP-qPCR Analysis

Chromatin immunoprecipitation (ChIP) was performed using the SimpleChIP® Plus Enzymatic Chromatin IP-Magnetic Beads (Cell Signaling Technology, MA, USA) following the manufacturer's instructions. One hundred and fifth instars of non-viruliferous *L. striatellus* were homogenized in 1 mL of phosphate-buffered saline (PBS) supplemented with protease inhibitor (PIC) and treated with 1.5% formaldehyde solution for 15 minutes. The cross-linking reaction was stopped by incubation in 100 μl of 10× glycine for 5 minutes at room temperature. The tissues were washed twice with 1 mL of PBS containing PIC and were separated into a single-cell suspension using a Dounce homogenizer. The cells were resuspended in a mixture of 1× buffer A supplemented with 0.5 mmol/L DTT and 1× PIC, followed by a 10 minute incubation on ice. Subsequent isolation of nuclei were achieved via centrifugation, and the obtained nuclei were resuspended in 1× buffer B supplemented with 0.5 mmol/L DTT. Micrococcal nuclease (0.5μl) was added to the resuspended solution to digest DNA to a size range of 150-900bp. Next, 10 μL of 0.5 mol/L EDTA was added to the solution and incubated for 10 minutes on ice to end DNA digestion. Cross-linked chromatin was collected from the supernatant post ultrasonic lysis. The cross-linked chromatin was diluted with 400 μL of 1× ChIP containing 1× PIC, and 10 μL was taken as the 2% input DNA. The LsDorsal polyclonal antibodies (10 μL) were added to the above dilution and incubated overnight at 4℃. Then, 30 μL of ChIP-Grade Protein G Magnetic beads were inoculated for 2 hours. The protein G magnetic beads were precipitated using a magnetic rack, and subsequent washes were performed using 1× ChIP low and high salt buffers containing NaCl. The solution was then precipitated using the magnetic rack and the supernatant was removed. Elution buffer (1× ChIP) was added to 2% input DNA and IP samples, and then washed at 65℃ for 30 minutes. The collected chromatin supernatant was added to 6 μL 5M NaCl and 2 μL Proteinase K, and then incubated for 2 hours at 65℃. DNA binding buffer (750 μL) was added to each DNA sample and purified using a DNA purification kit according to the manufacturer's instructions. Specific primers (around 80-160 bp) were designed for the promoter of the target gene of *LsDorsal*, and qPCR was used to conduct quantitative analysis using chromatin DNA as a template. The IP efficiency was manually calculated using the percentage of input samples and the following formula. The signal obtained from each immunoprecipitation was expressed as a percentage of the total input sample chromatin. Input =$2\%\times 2^{(C[T]\ 2\%input\ sample\ -C[T]\ IP\ sample)}$, C[T]=CT=PCR Reaction Ct.

Yeast One-Hybrid Assay

The yeast one-hybrid assay was used to determine the binding of LsDorsal to the promoters of target genes. The promoter fragments of target genes and LsDorsal were cloned to the pAbAi and pGAD-T7 vector, respectively. The bacterias were cultivated at 37 °C incubator on selective medium

with appropriate antibiotic. Positive clones were selected and sequenced. The constructed pAbAi plasmids were linearized with BstBI enzyme and transformed into Y1H gold competent cells. Then the yeast strain was cultivated on selective medium SD/-Ura. The concentration of AbA was determined based on the absence of yeast colonies growing on SD/-Ura/AbA medium to prevent activation of the target promoter by endogenous yeast proteins. The pGAD-T7-*LsDorsal* and pGAD-T7 (negative control) were then transformed into yeast competent cell containing target gene promoter. Then the yeast strain with different constructs combinations was grown on selective media SD/-Leu. Additionally, positive control plasmids, pGAD-T7-*Lec* and pAbAi-*P53*, were also included. Positive clones were selected on SD/-Leu/AbA medium. Images were taken after 3 days of incubation at 30 °C.

Yeast Two-Hybrid Assay

Autophagy related genes *Atg8*, *Atg3*, *Atg5*, *Atg9*, *Atg12*, *ULK1* and *Sqstm*1 in *L. striatellus* were cloned into the DNA-binding domain of the vector pGBD-T7 to create bait plasmids. *LsZN708* was cloned into the activation domain of the yeast vector pGAD-T7. Autophagy gene libraries and pGAD-T7-*LsZN708* were co-transformed into yeast competent cells AH109. Then the different combinations of constructs were grown on selective media SD/-Leu/-Trp, and interactions were detected with SD/-Ade/-His/-Leu/-Trp medium. Pictures were taken after 3 days of incubation at 30 °C. Positive clones were selected on SD/- Ade /-His/- Leu/-Trp medium. The Y2H interaction assay of pGAD-T7-*LsDorsal*, pGAD-T7-*LsRHD-n*, pGAD-T7-*LsRHD_dimer* with pGBD-T7-*LsMSK2* or proteins encoded by rice viruses were performed as described above.

Expression, Purification and Antibody Preparation of Recombinant Proteins

RSV-NS4, *LsMSK2-C*, *LsDorsal*, *LsAtg8* and *LsZN708* were cloned into pET-28a vector to generate His-tagged recombinant protein. Likewise, *LsRHD-n* was cloned into pGEX-6P1 vector to generate GST-tagged recombinant proteins, and *LsDorsal*, *LsRHD-n*, *RSV-NS4* were cloned into pMAL-c5X vector to generate MBP-tagged recombinant proteins. Specific primers used for cloning were listed in Supplementary Table 1. All recombinant proteins were then transformed into *Escherichia coli* BL21. The bacteria was cultivated at 37 °C with shaking at 220 rpm. When the OD600 reached 0.6-0.8, the bacteria solution was added with 1M isopropyl β-D-thiogalactoside (IPTG) in a 1:1000 ratio induced for 8 hours at 16°C before centrifugation for 10 minutes at 4°C with 8000 rpm. The cells were resuspended with cold 1×PBS buffer and sonicated for 30 minutes on ice. The supernatant was retained for pull-down or Co-IP assays. The recombinant tagged proteins were

purified from supernatant using Ni-NTA Agarose (Qiagen, German) or GST-fusion protein purified magnetic beads (Beaver, China) or MBPSep Dextrin Agarose Resin (New England Biolabs, USA) according to manufacturer's instructions. Additionally, polyclonal rabbit antibodies of purified protein LsDorsal, LsAtg8 and LsZN708 were prepared by Huabio in Hangzhou, whereas polyclonal rabbit antibodies of RSV-NP and RSV-NS4 were well stored in our laboratory. The monoclonal tag antibodies for His, GST and MBP were purchased from Invitrogen (California, USA), and the actin antibody was purchased from Huabio (Hangzhou, China).

Pull Down Assays

The MBP beads were incubated with the purified MBP-tagged LsZN708 protein at 4℃ for 4 hours with vortical mixing, while the MBP-tagged protein served as a negative control. After centrifugation at 4℃ and 3100 rpm for 5 minutes, the beads were washed with cold 1×PBS buffer for three times. Next, the beads enriched MBP-tagged ZN708 protein or empty MBP-tagged protein were co-incubated vortically with His-tagged Atg8 protein at 4℃ overnight with the addition of 100 μM protease inhibitor cocktail (Roche, Basel, Switzerland). Subsequently, the beads were washed with cold 1×PBS buffer three times and then eluted by MBP wash buffer. Then the results were detected using western blotting. The interactions between LsRHD-n and RSV-NS4 or LsMSK2-C protein were verified using the same method (the purified beads and elution buffer used vary depending on the labels).

Western Blotting

Proteins were extracted from *L. striatellus* using 1× lysis buffer and separated using 8%-15% SDS-PAGE. The separated proteins were then transferred onto a PVDF membrane using eBlot L1 (Genscript, China) after soaking in methanol for 10 seconds. The transferred PVDF membrane was then blocked with 5% skimmed milk powder (diluted in PBST) for 2 hours at room temperature. Primary antibodies (at a 1:5000 ratio) were added to the membrane and incubated for 2 hours at room temperature or overnight at 4 ℃. Subsequently, secondary antibodies (either Goat anti-Rabbit IgG HRP or Goat anti-mouse IgG HRP) were added to a fresh block reagent with a 1:10000 ratio and incubated for 1 hour at room temperature with shaking. Thereafter, the PVDF membrane was washed thrice with 1× PBST buffer. Detection of the PVDF membrane was performed using the SuperSignal™ West Pico PLUS Chemiluminescent Substrate (Thermo Scientific, USA) according to the manufacturer's instructions and imaged using the Amersham Imager 680 (Cellular Technology Ltd, USA).

Co-Immunoprecipitation (Co-IP) Assay

Protein extraction from *L. striatellus* was performed using GTEN reagents containing protease inhibitor and DTT, and supplemented with TritonX-100 to achieve a final concentration of 0.5%. After homogenization, the extracted protein was subjected to immunoprecipitation with protein A agarose beads conjugated with RSV NS4 antibody. Incubation was carried out at 4℃ for 4 hours, followed by six washes using IP buffer. Co-immunoprecipitation was then analyzed by SDS-PAGE and Western Blotting using a specific LsDorsal antibody for detection.

Bimolecular Fluorescence Complementation Assays

To further confirm protein interactions, the complete *LsDorsal* gene sequence was inserted into both pCV-nYFP and pCV-cYFP expression vectors. These vectors, designated as pCV-nYFP-*LsDorsal* and pCV-cYFP-*LsDorsal*, were transformed into *Agrobacterium tumefaciens* GV3101 through a heat transfer method and co-transformed with pCV-cYFP-*NS4* and pCV-nYFP-*NS4* into *Nicotiana benthamiana*, respectively. The YFP fluorescence signal was then visualized and recorded using a Nikon confocal microscope (Nikon, Japan), providing visual confirmation of the interactions between these proteins.

Competitive Binding Assay

The competitive binding assay was performed to investigate the competitive relationship between LsMSK2, RSV-NS4, and LsRHD-n. Recombinant protein GST-tagged RHD-n was incubated with GST beads for 4 hours at 4℃ and the MBP-tagged NS4 protein and His-tagged MSK2-C protein with different amount were added with several different order for incubation at 4℃ for 4 hours. After centrifugation and washing steps, the proteins bound to the beads were detected by Western Blotting using antibodies specific to the His-tag, GST-tag and MBP-tag.

Nuclear and Cytoplasmic Proteins Extraction

Nuclear and cytoplasmic proteins were meticulously extracted from non-viruliferous or viruliferous *L. striatellus* adults using a Nuclear and Cytoplasmic Extraction kit (Beyotime, China). Specifically, fifty insects were homogenized in a cold blend of cytoplasmic extraction reagents A and B, mixed in a ratio of 20:1, along with a protease inhibitor. After incubation in an ice bath for 15 minutes, the supernatant was retained as the cytoplasmic protein. Furthermore, the remaining precipitate was treated with nuclear extraction reagent, and the supernatant was retained as the nuclear protein following centrifugation at 12,000 ×g for 10 minutes at 4°C. The extracted cytoplasmic and nuclear protein fractions were then subjected to Western blotting analysis. The blots were probed with

a p-Dorsal antibody (Abcam, England) as well as reference antibodies for nuclear and cytoplasmic proteins, histone H3 and GAPDH, respectively (Huabio, China).

Phosphorylation Assay in Vitro

To elucidate the relationship between LsMSK2 kinase and LsDorsal, an in vitro phosphorylation assay was conducted using the pIMAGO®-biotin Phosphoprotein Detection Kit (TYMORA). The reaction mixture consisted of 25mM Tris-Hcl (pH=7.5), 10 mM DTT, 100 μM ATP, 100 μM $CaCl_2$, and 2-5 μg of protein, with His-tagged MSK2-C protein maintained at a constant amount and varying concentrations of His-tagged Dorsal protein. After incubation for 30 minutes, the mixture was treated with 5×loading buffer and boiled for 10 minutes. Following a 5 minutes cooling interval, 1×IAA was added, and the sample was further incubated in darkness for 15 minutes. The phosphorylation status of the protein was detected by western blotting.

Membrane Feeding

The viruliferous *L. striatellus* adults were carefully confined in glass tubes and subjected to a unique feeding container with a two-layer parafilm system. The open side of the tubes was covered with a thin layer of parafilm infused with a NF-kB inhibitor, QNZ, and mixed with 20% sucrose solution, and a second layer of parafilm was carefully placed on top. The effectiveness of the inhibitor was evaluated by comparing the results to the negative control, which was treated with Dimethyl Sulfoxide (DMSO). After 48 hours, the insects were allowed to feed on the mixture, with the QNZ reagent being renewed every 12 hours. After the feeding period, the samples were carefully collected and subjected to western blotting analysis using a polyclonal rabbit anti-Atg8 antibody.

RSV Acquisition Rates for *L. striatellus* and Viral Disease Incidence Rates in Rice

To assess RSV acquisition rates in *L. striatellus*, second instar nymphs injected with a mixture of RSV crude extracts and ds*LsDorsal*, ds*LsZN708*, or ds*GFP* were fed on healthy rice seedlings for three or six days, and the relative transcript of *RSV-NP* in *L. striatellus* was measured using RT-qPCR. For viral disease incidence rates, instar nymphs were similarly injected and fed on healthy rice for four days. Each instar nymph of *L. striatellus* was then transferred to individual glass tube containing single rice seedling (about 30 replicates in total) for RSV transmission experiment. After four days of feeding, the insects were removed for total RNA extraction. Fifteen days later, disease symptom of single rice seedling was recorded. Meanwhile, the total RNA of them were extracted and disease incidence rates of the rices were calculated using RT-PCR.

References:

1. J. Li *et al*., Characterization of rice black-streaked dwarf virus- and rice stripe virus-derived siRNAs in singly and doubly infected insect vector *Laodelphax striatellus*. *PLoS One* **8**, e66007 (2013).
2. Y. J. He *et al*., Activation of toll immune pathway in an insect vector induced by a plant virus. *Front. Immunol*. **11**, 613957 (2020).
3. H. J. Xu *et al*., Two insulin receptors determine alternative wing morphs in planthoppers. *Nature* **519**, 464–467 (2015).

Supplementary Figures 1-10

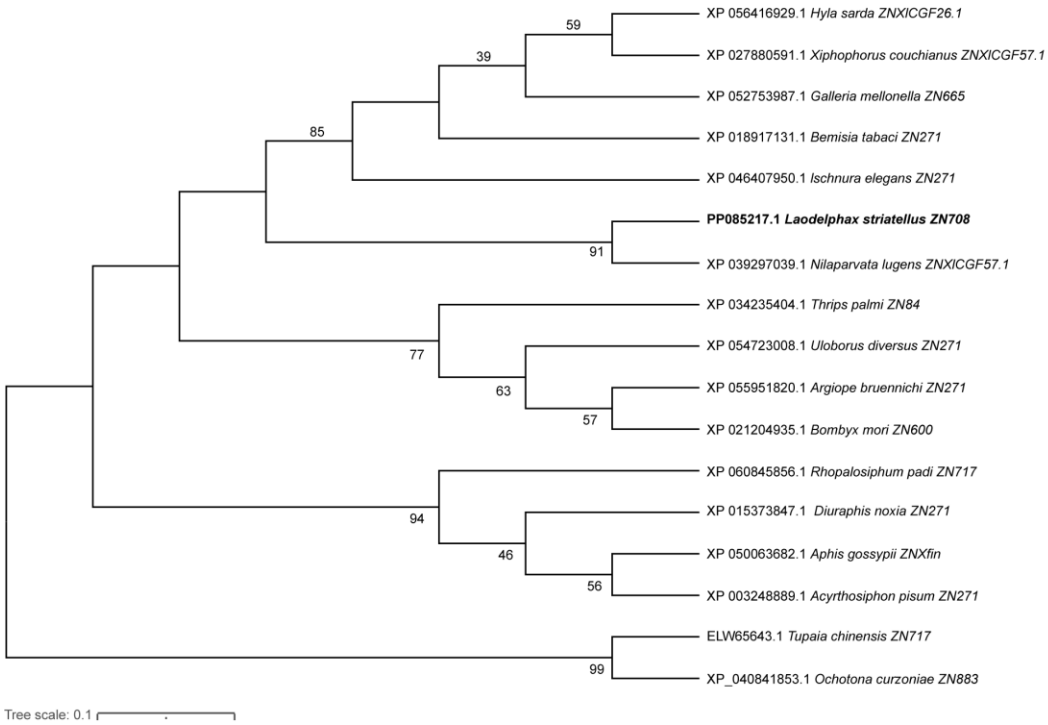

Fig. S1. Phylogenetic Analysis of ZN708 in Various Arthropods and Mammals. The phylogenetic tree was constructed using Mega 7.0 and optimized with iTOL software. ZN708 of *Laodelphax striatellus* is indicated with black bold font.

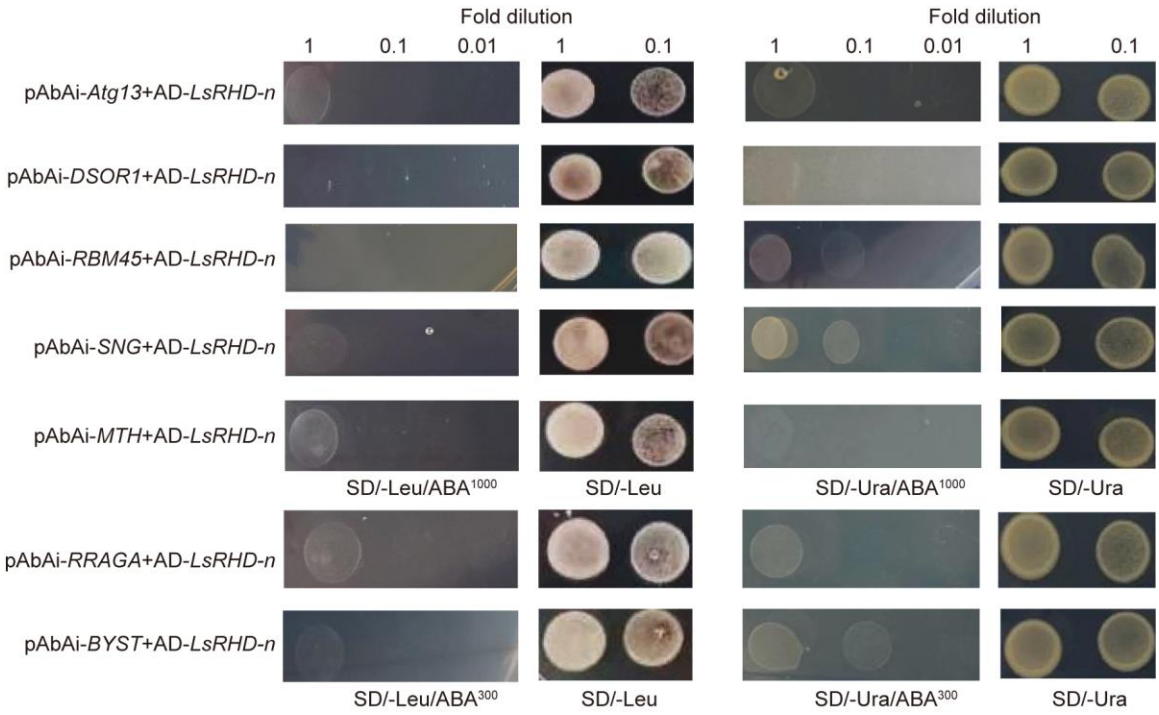

Fig. S2. Interactions between LsDorsal and the Promoter Regions of Candidate Target Genes (*LsATG13*, *LsDSOR1*, *LsRBM45*, *LsSNG*, *LsMTH*, *LsPRAGA*, and *LsBYST*) via the yeast one-hybrid. The promoters of candidate target genes were constructed to pAbAi vector, and *LsRHD-n* fragment was constructed to pGAD-T7 vector (AD-*LsRHD-n*). ABA screening concentration was set at 300 ng/μL or 1000 ng/μL (inhibiting self-activation). The self-activation assay was performed on selective medium SD/-Ura. The different combinations of constructs transformed into yeast cells were grown on selective medium SD/-Leu, and interactions were detected on SD/-Leu/-ABA$^{300/1000}$. The images were taken after 3 days of incubation at 30 ℃.

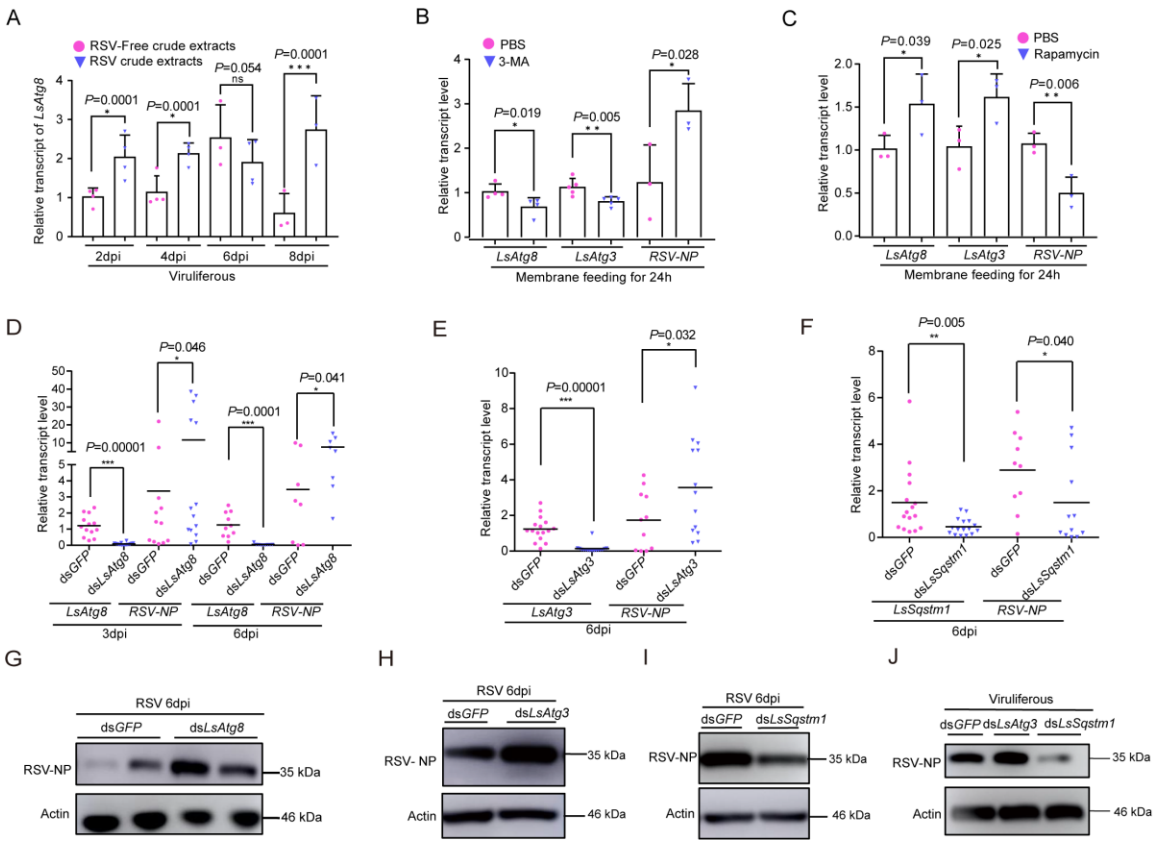

Fig. S3. Autophagy Pathway was Involved in the Antiviral Response of *L. striatellus* against RSV Infection. (A) Detection of *LsAtg8* expression injected with crude extracts of RSV-free and RSV-infected *L. striatellus* at various time points. (B and C) Effects of autophagy inhibitor (3-MA) or activator (Rapamycin) treatment on accumulation levels of RSV in viruliferous *L. striatellus* through membrane feeding. PBS was used as negative control. (D) Effects of *LsAtg8* knockdown on the expression levels of RSV-NP transcripts in *L. striatellus* at 3 and 6 dpi. Injection of ds*GFP* and RSV crude extracts was used as negative control. (E and F) Effects of *LsAtg3* (E) and *LsSqstm1* (F) knockdown on the expression of *RSV-NP* transcripts in *L. striatellus* at 6 dpi. (G) The protein level of RSV-NP in *L. striatellus* injected with ds*LsAtg8* and RSV crude extracts at 6 dpi. (H and I) The protein level of RSV-NP in *L. striatellus* injected with ds*LsAtg3* (H) or ds*LsSqstm1* (I) and RSV crude extracts at 6 dpi. (J) Protein expression of RSV-NP in viruliferous *L. striatellus* injected with ds*LsAtg3* or ds*LsSqstm1*. Three biological replicates were performed for each of the experiment (A collection of 10-15 *L. striatellus* was used as one replicate). The *t* test method was used for significance analysis. * represents significant difference ($P < 0.05$), ** and *** represent extremely significant difference ($P < 0.01$ and $P < 0.001$). The error bars represent the standard error (SE) of the mean.

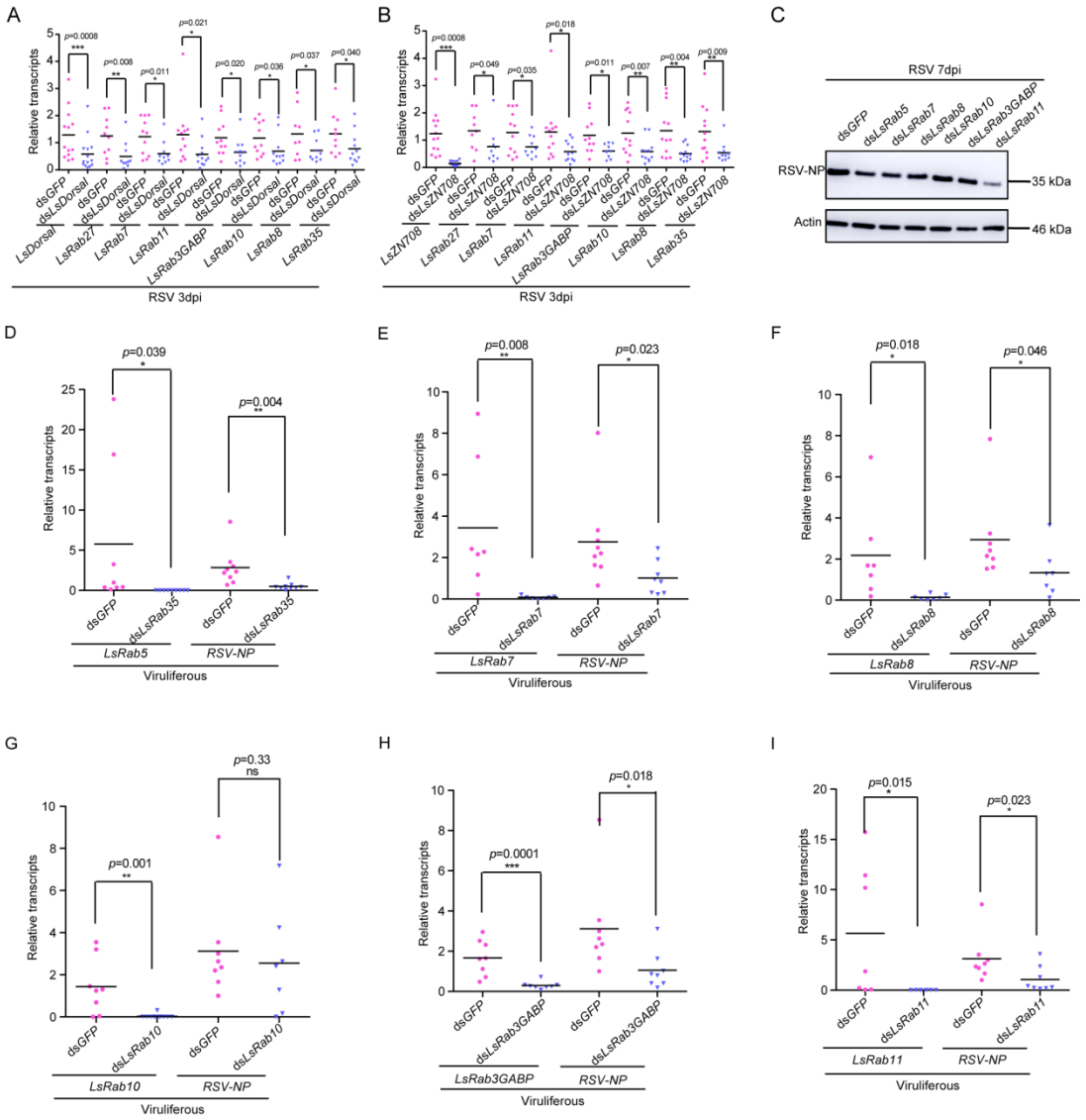

Fig. S4. Expression Level of Exosome Related Genes Potentially Regulated by Toll-Dorsal-ZN708 Pathway. (A and B) Relative transcript levels of exosome pathway related genes in non-viruliferous *L. striatellus* treated with ds*LsDorsal* (A) or ds*LsZN708* (B) and RSV crude extracts at 3 dpi. (C) Effects of exosome pathway related genes (*LsRab5*, *LsRab7*, *LsRab8*, *LsRab10*, *LsRab11*, and *LsRab3GABP*) knockdown on protein levels of RSV-NP in non-viruliferous *L. striatellus* treated with dsRNA and RSV crude extracts at 7dpi. (D-I) The transcriptional levels of the exosome pathway related genes corresponding to (C). Three biological replicates were performed for each experiment. Significance analysis was performed using the *t* test method. * represents a significant difference ($P < 0.05$), ** and *** represent extremely significant differences ($P < 0.01$ and $P < 0.001$). The error bars represent the standard error (SE) of the mean.

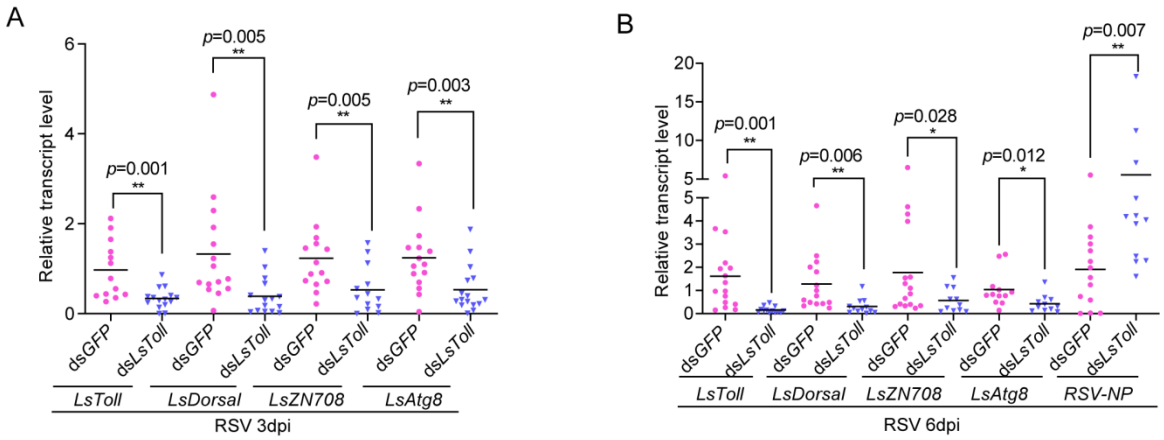

Fig. S5. Toll Receptor Participated in Anti-RSV by Regulating Dorsal-ZN708-Atg8 Pathway. (A and B) Effects of *LsToll* receptor knockdown on the transcriptional of *LsDorsal*, *LsZN708* and *LsAtg8* in non-viruliferous *L. striatellus* treated with dsRNA and RSV crude extracts at 3 (A) and 6 dpi (B). Three biological replicates were performed for each experiment. Significance analysis was performed using the *t* test method. * represents a significant difference ($P < 0.05$), ** represent extremely significant differences ($P < 0.01$). The error bars represent the standard error (SE) of the mean.

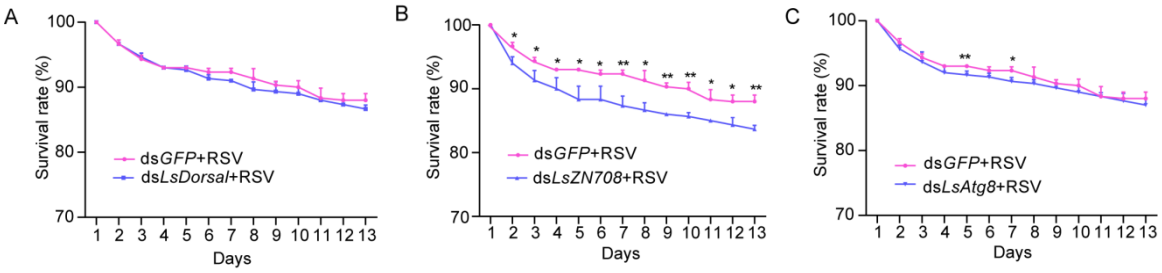

Fig. S6. Effects on the Survival Rate of *L. striatellus* after Knockdown of *LsDorsal*, *LsZN708* and *LsAtg8* with RSV Infection. (A-C) Survival rate of *L. striatellus* after treatment with ds*LsDorsal* (A), ds*LsZN708* (B) or ds*LsAtg8* (C) and RSV crude extracts for 13 days. Three biological replicates (approximately 100 *L. striatellus* for each replicate) were performed. Significance analysis was calculated using the *t* test method. * represents a significant difference ($P < 0.05$), ** represent extremely significant differences ($P < 0.01$). The error bars represent the standard error (SE) of the mean.

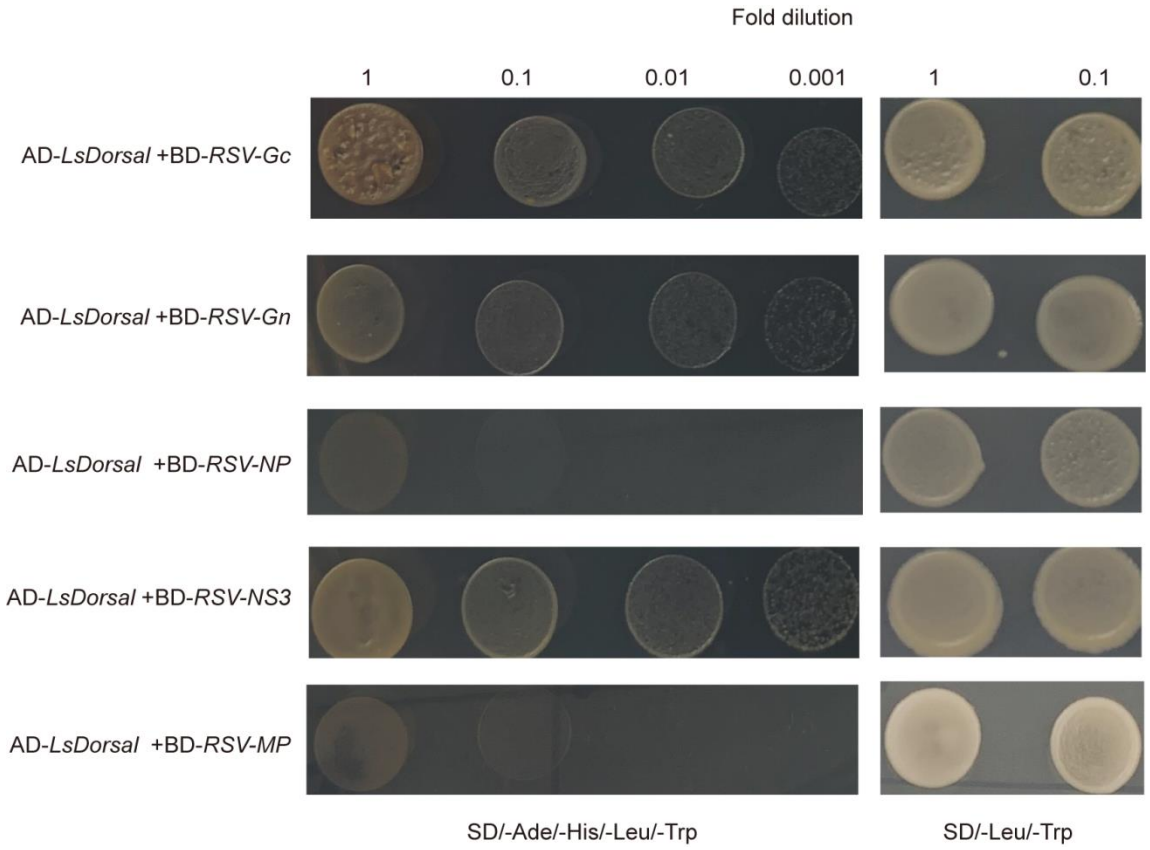

Fig. S7. Interactions between LsDorsal and RSV-encoded Proteins (RSV-Gc, RSV-Gn, RSV-NP, RSV-NS3 and RSV-MP proteins) via the yeast one-hybrid. The different combinations of constructs transformed into yeast cells were grown on selective medium SD/-Leu/-Trp, and interactions were detected on SD/-Ade/-His/-Leu/-Trp. The images were taken after 3 days incubation at 30 ℃.

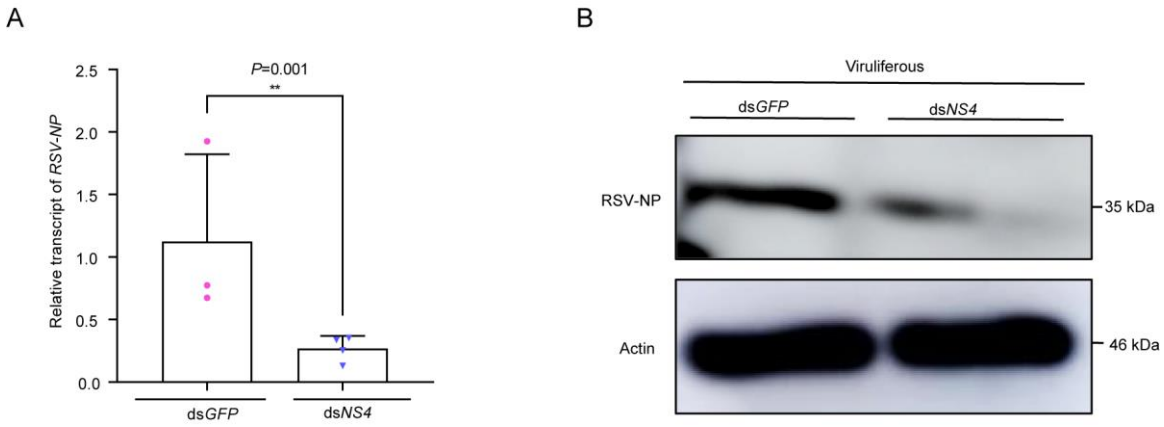

Fig. S8. *NS4* Knockdown Decreased the Expression of RSV-NP in *L. striatellus.* (A and B) Effects of *NS4* knockdown on RSV-NP expression at the levels of transcription (A) and protein (B) in viruliferous *L. striatellus*. Three biological replicates were performed. Significance analysis was calculated using the *t* test method. ** represent extremely significant differences ($P < 0.01$). The error bars represent the standard error (SE) of the mean.

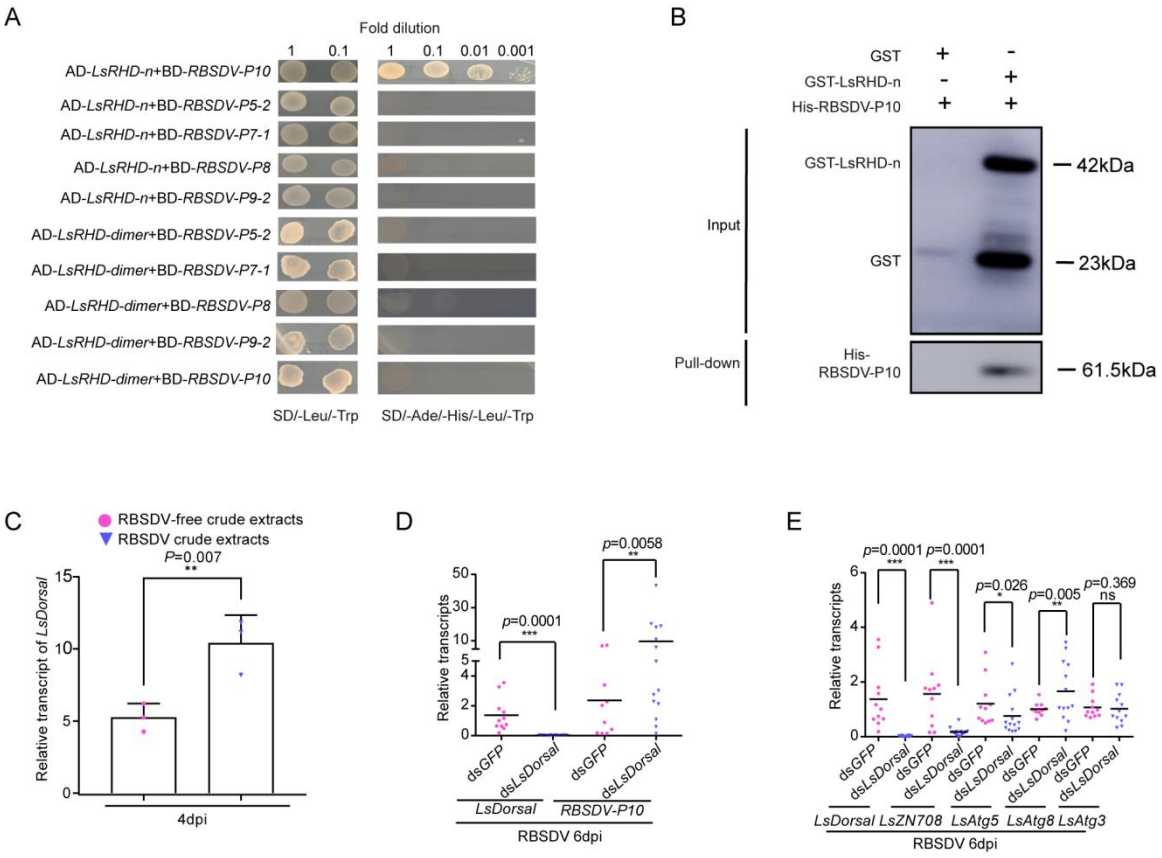

Fig. S9. The Broad-spectrum for the Antiviral Roles of Toll Pathway in Planthoppers against Rice Viruses. (A) Interaction verification between LsRHD-n and RBSDV P10 by yeast two-hybrid assay. (B) An in vitro pull-down assay confirmed the interaction between LsRHD-n and RBSDV P10. GST-RHD-n protein was used to pull-down with His-RBSDV-P10. His-RBSDV-P10 was further detected with anti-His antibody. (C) Relative transcript level of *Dorsal* in *L. striatellus* treated with RBSDV crude extracts at 4 dpi. (D and E) Effects of *LsDorsal* knockdown on the accumulation levels of *RBSDV-P10* (D) and the transcript levels of *LsZN708*, *LsAtg5*, *LsAtg8* and *LsAtg3* (E) in *L. striatellus* at 6 dpi.

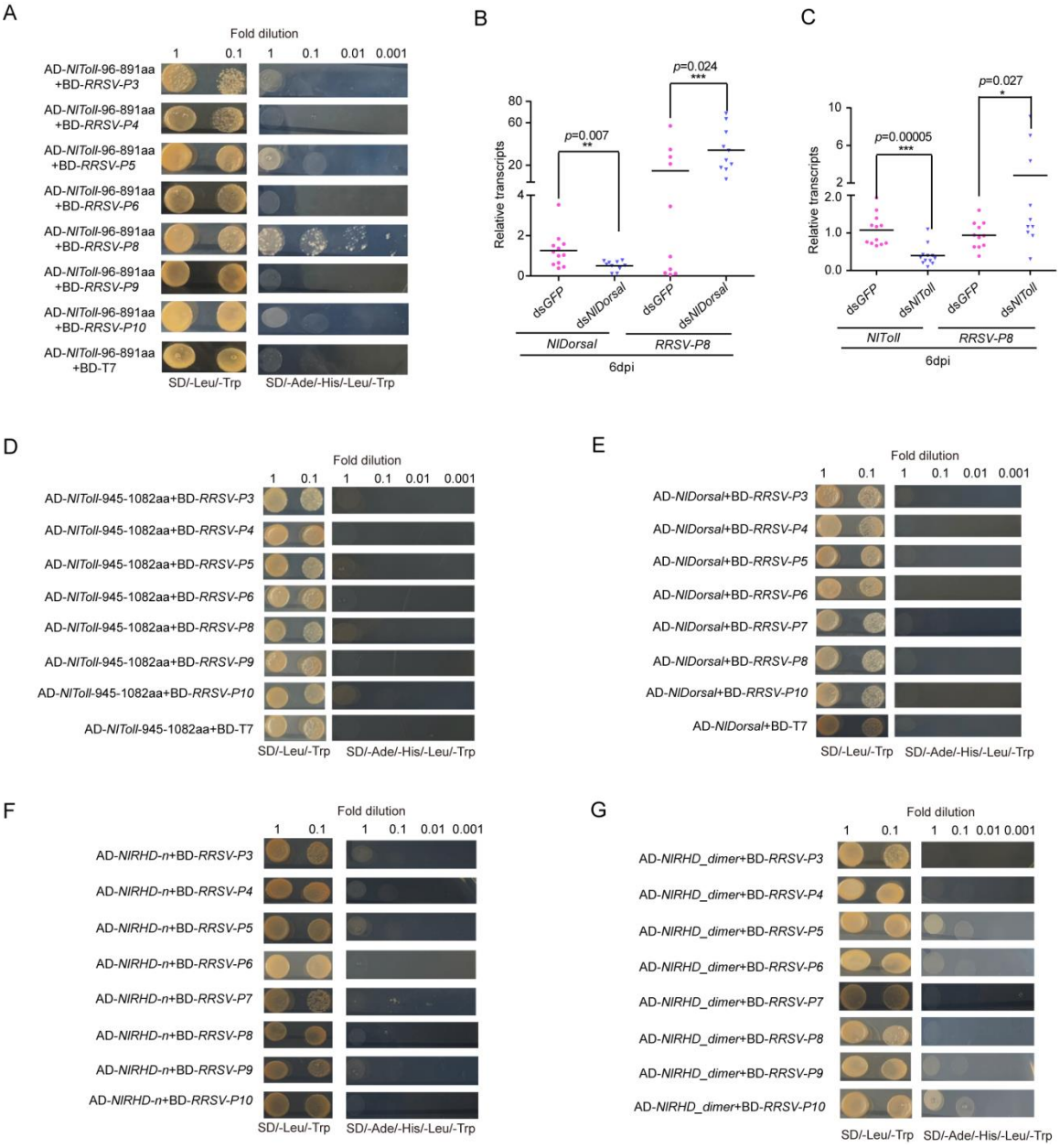

Fig. S10. Effect of Toll Pathway on RRSV in *N. lugens*. An interaction verification between specific region of NlToll, 96-891 aa and the P8 protein of RRSV *in N. lugens* (A), but not for NlDorsal (E-G) or the other region of NlToll (D). Effect of *NlDorsal* and *NlToll* knockdown on the accumulation level of RRSV in *N. lugens* treated with ds*NlDorsal* (B) or ds*NlToll* (C) and RRSV crude extracts at 6 dpi. Three biological replicates were performed, with 10-15 *N. lugens* for each replicate. Significance analysis was performed using the *t* test method, where * represents a significant difference ($P < 0.05$), ** and *** represent extremely significant differences ($P < 0.01$ and $P < 0.001$). The error bars represent the standard error (SE) of the mean.

Table S1 The primers used in this study.

Primer Name	Sequences（5’-3’）
For Yeast Two-Hybrid (Y2H) Assays	
pGAD-T7-*LsDorsal*-F	GACGTACCAGATTACGCTCATATGGACACGG ACATTGA
pGAD-T7-*LsDorsal*-R	GCAGCTCGAGCTCGATGGATCCCTATTTAACA GGAACATTG
pGAD-T7-*LsRHD-n*-F	GACGTACCAGATTACGCTCATATGATCACAGAGCAGCCGGCCT
pGAD-T7-*LsRHD-n*-R	GCAGCTCGAGCTCGATGGATCCCTATTTTTTTATCGTAGATGGGCTC
pGAD-T7-*LsRHD-dimer*-F	GACGTACCAGATTACGCTCATATGCTTGTAATTTGCAAACTAAGCG
pGAD-T7-*LsRHD-dimer*-R	GCAGCTCGAGCTCGATGGATCCCTA GGGCGTCAACTGGAACGG
pGAD-T7-*LsAidA*-F	GACGTACCAGATTACGCTCATATGGAGAGTGGTGGGGAAAATATA
pGAD-T7-*LsAidA*-R	GCAGCTCGAGCTCGATGGATCCCTACTCTACATCACTTCTAGTATTC
pGAD-T7-*LsRHD-n*-301-339aa-F	GACGTACCAGATTACGCTCATATGGGTAGCATCGACCTGAACG
pGAD-T7-*LsRHD-n*-301-339aa-R	GCAGCTCGAGCTCGATGGATCCCTATTTTTTATCGTAGATGGGCTC
pGAD-T7-*LsZN708*-F	GACGTACCAGATTACGCTCATATGGTTCTAAAAAAACTTTTCTCAA
pGAD-T7-*LsZN708*-R	GCAGCTCGAGCTCGATGGATCCTTATCCTGTGTGTATATTCCT
pGBD-T7-*LsZN708*-F	ATCTCAGAGGAGGACCTGCATATGGTTCTAAAAAAACTTTTCTCAA
pGBD-T7-*LsZN708*-R	GCCGCTGCAGGTCGACGGATCCTTATCCTGTGTGTATATTCCT
pGAD-T7-*LsAtg5*-F	GACGTACCAGATTACGCTCATATGGCTAATGACAGGGAAGTT
pGAD-T7-*LsAtg5*-R	GCAGCTCGAGCTCGATGGATCCTCACGTTGCACTGGTTTCAT
pGAD-T7-*LsAtg8*-F	GACGTACCAGATTACGCTCATATGAAATTCCAGTATAAAGAAGA
pGAD-T7-*LsAtg8*-R	GCAGCTCGAGCTCGATGGATCCTTAGAATTTATTTCCATAGAC
pGAD-T7-*LsAtg9*-F	GACGTACCAGATTACGCTCATATGGTGTGGTGTCCCGAG
pGAD-T7-*LsAtg9*-R	GCAGCTCGAGCTCGATGGATCCTTAGATCTCGTGTACAAACAATA
pGAD-T7-*LsAtg12*-F	GACGTACCAGATTACGCTCATATGGGAGACGAGCAACCAA
pGAD-T7-*LsAtg12*-R	GCAGCTCGAGCTCGATGGATCCCTATCCCCAAGCTTGACTCT
pGAD-T7-*LsSqstm1*-F	GACGTACCAGATTACGCTCATATGGAGTCGGTGTCTTTCAA
pGAD-T7-*LsSqstml* -R	GCAGCTCGAGCTCGATGGATCCTTAAGCGATAACACGACCAAAT
pGBD-T7-*LsAtg3*-F	ATCTCAGAGGAGGACCTGCATATGCAAAGTGTTATTAATACTGT
pGBD-T7-*LsAtg3*-R	GCCGCTGCAGGTCGACGGATCCTCACAGAGCTTTCGACCAC
pGBD-T7-*LsAtg5*-F	ATCTCAGAGGAGGACCTGCAT ATGGCTAATGACAGGGAAGTT
pGBD-T7-*LsAtg5*-R	GCCGCTGCAGGTCGACGGATCCTCACGTTGCACTGGTTTCAT
pGBD-T7-*LsAtg8*-F	ATCTCAGAGGAGGACCTGCATATGAAATTCCAGTATAAAGAAGA
pGBD-T7-*LsAtg8*-R	GCCGCTGCAGGTCGACGGATCCTTAGAATTTATTTCCATAGAC
pGBD-T7-*LsAtg9*-F	ATCTCAGAGGAGGACCTGCATATGGTGTGGTGTCCCGAG
pGBD-T7-*LsAtg9*-R	GCCGCTGCAGGTCGACGGATCCTTAGATCTCGTGTACAAACAATA
pGBD-T7-*LsAtg12*-F	ATCTCAGAGGAGGACCTGCAT ATGGGAGACGAGCAACCAA
pGBD-T7-*LsAtg12*-R	GCCGCTGCAGGTCGACGGATCC CTATCCCCAAGCTTGACTCT
pGBD-T7-*LsULK1*-F	ATCTCAGAGGAGGACCTGCATATGGAGATTGTAGGGGATTAC
pGBD-T7-*LsULK1*-R	GCCGCTGCAGGTCGACGGATCCTCAAGTCGATGTGTTGTTTAC
pGAD-T7-*NlToll*-96-891aa-F	GACGTACCAGATTACGCTCATATGGGCTGTCAGCTGGTGCCGGT
pGAD-T7-*NlToll*-96-891aa-R	GCAGCTCGAGCTCGATGGATCCTTAGCCTGTTGTCGGGATGGGACAC
pGAD-T7-*NlToll*-945-1082aa-F	GACGTACCAGATTACGCTCATATGTACGACGCTTTTGTCAGCT
pGAD-T7-*NlToll*-945-1082aa-R	GCAGCTCGAGCTCGATGGATCCTTACGGATGCGGCAGCGCGTA
pGAD-T7-*NlDorsal*-F	GACGTACCAGATTACGCTCATATGCACAAGCAAGCAGTGTGT
pGAD-T7-*NlDorsal*-R	GCAGCTCGAGCTCGATGGATCCCTATTTAACAGGCACATCA
pGAD-T7-*NlRHD-n*-F	GACGTACCAGATTACGCTCATATGATCACTGAGCAGCCTGCCTC
pGAD-T7-*NlRHD-n*-R	GCAGCTCGAGCTCGATGGATCCCTACTTTTTGTCATAGATGGGCTC
pGAD-T7-*NlRHD_dimer*-F	GACGTACCAGATTACGCTCATATGATCTGCAAACTGAGTGAAG
pGAD-T7-*NlRHD_dimer*-R	GCAGCTCGAGCTCGATGGATCCCTAGGGCGTCAACTGGAACGGCAGC
pGBD-T7-*LsMSK2-N*-F	ATCTCAGAGGAGGACCTGCAT ATG AAAGTCCTCGGTCAAGGCTCT
pGBD-T7-*LsMSK2-N*-R	GCCGCTGCAGGTCGACGGATCCTCAGATAAAGCTGAAGCCTCTGAA
pGBD-T7-*LsMSK2-C*-F	ATCTCAGAGGAGGACCTGCAT ATG GAATACGAACTAAGAGAAGA
pGBD-T7-*LsMSK2-C*-R	GCCGCTGCAGGTCGACGGATCCTCAGCTCACTGCTCGGTATGTTGCT
pGBD-T7-*LsMSK1*-F	ATCTCAGAGGAGGACCTGCATATGTCTAGCTTTGACCTGCTCAA
pGBD-T7-*LsMSK1*-R	GCCGCTGCAGGTCGACGGATCCTTAATCTATAACTATGGTTTCG
pGBD-T7-*LsPKAC*-F	GTACCAGATTACGCTCATATGGGCAATGCCGCAACGGCTA
pGBD-T7-*LsPKAC*-R	CAGCTCGAGCTCGATGGATCCTCAAAACTCGGCAAACTCCTTGGC
For Protein Purification	
pGEX-6P1-*LsRHD-n*-F	TCCAGGGGCCCCTGGGATCCATGATCACAGAGCAGCCGGCCT
pGEX-6P1-*LsRHD-n*-R	GTCAGTCACGATGCGGCCGCCTATTTTTTATCGTAGATGGGCTC
pMAL-c5X-MBP-*LsDorsal*-F	CACATATGTCCATGGGCGGCCGCATGGACACGGACATTGA
pMAL-c5X-MBP-*LsDorsal*-R	ACCTGCAGGGAATTCGGATCCCTATTTAACAGGAACATTG
pMAL-c5X-MBP-*LsRHD-n*-F	CACATATGTCCATGGGCGGCCGCATGATCACAGAGCAGCCGGCCT
pMAL-c5X-MBP-*LsRHD-n* -R	ACCTGCAGGGAATTCGGATCCCTATTTTTTATCGTAGATGGGCTC
pMAL-c5X-MBP-*NS4*-F	CACATATGTCCATGGGCGGCCGCATGCAAGACGTACAAAGGACA
pMAL-c5X-MBP-*NS4*-R	ACCTGCAGGGAATTCGGATCCCTATGTTTTATGAAGAAGAGGT
pMAL-c5X-MBP-*LsZN708*-F	CACATATGTCCATGGGCGGCCGCATGGTTCTAAAAAAACTTTTCTCAA
pMAL-c5X-MBP-*LsZN708*-R	ACCTGCAGGGAATTCGGATCCCTATCCTGTGTGTATATTCCT
pET-28a-*NS4*-F	CTGTATTTTCAGGGCCAT ATGCAAGACGTACAAAGGACA
pET-28a-*NS4*-R	ACGGAGCTCGAATTCGGATCC CTATGTTTTATGAAGAAGAGGT
pET-28a-*LsAtg8*-F	CTGGTGCCGCGCGGCAGCCATATGAAATTCCAGTATAAAGAAGA

pET-28a-*LsAtg8*-R	ACGGAGCTCGAATTCGAATCCTTAGAATTTATTTCCATAGAC
pET-28a-*LsDorsal*-(1-500aa)-F	CTGGTGCCGCGCGGCAGCCATATGGACACGGACATTGAAGC
pET-28a-*LsDorsal*-(1-500aa)-R	ACGGAGCTCGAATTCGGATCC TCACACAGCATCCAGCGTTATCACT
pET-28a-*LsMSK2*-C-F	CTGGTGCCGCGCGGCAGCCAT ATG GAATACGAACTAAGAGAAGA
pET-28a-*LsMSK2*-C-R	ACGGAGCTCGAATTCGGATCCTCAGCTCACTGCTCGGTATGTTGCT
For DsRNA Synthesis	
ds-*LsDorsal*-1F	TAATACGACTCACTATAGGGAGAGCACAAGAATCAGCCAGGTAG
ds-*LsDorsal*-1R	TAATACGACTCACTATAGGGAGACCACAGCATCCAGCGTTATC
ds-*LsDorsal*-2F	TAATACGACTCACTATAGGGAGAGGATGCTGAGGAGGTGGTAG
ds-*LsDorsal*-2R	TAATACGACTCACTATAGGGAGAGGCTGATTCTTGTGCTCGTAG
ds-*LsZN708*-F	TAATACGACTCACTATAGGGAGATCAGACCACAACTACCAACATCC
ds-*LsZN708*-R	TAATACGACTCACTATAGGGAGACTTGCTGCCATGAACATGTGG
ds-*LsToll*-F	TAATACGACTCACTATA**G**GGAGA TTTGATGACGTCGATGGGCA
ds-*LsToll*-R	TAATACGACTCACTATA**G**GGAGA CCAATTTGCTGAGATCGGCG
dsRSV-*NS4*-F	TAATACGACTCACTATAGGGAGAATGCAAGACGTACAAAGGACA
dsRSV-*NS4*-R	TAATACGACTCACTATAGGGAGACTATGTTTTATGAAGAAGAGGT
ds*GFP*-F	TAATACGACTCACTATAGGGAGAACGTAAACGGCCACAAGTTC
ds*GFP*-R	TAATACGACTCACTATAGGGAGATGTTCTGCTGGTAGTGGTCG
ds-*LsRab5*-F	TAATACGACTCACTATAGGGAGAATGGCGAATCGTGGTGGGGCT
ds-*LsRab5*-R	TAATACGACTCACTATAGGGAGAACCCTCCTCCTCCTCCGC
ds-*LsRab7*-F	TAATACGACTCACTATAGGGAGAATGGCAACCAGAAAGAAAGTC
ds-*LsRab7*-R	TAATACGACTCACTATAGGGAGACTGTGTCGAGCAAGCGCGCACAG
ds-*LsRab11*-F	TAATACGACTCACTATAGGGAGAATGGGCACCCGTGATGACGAA
ds-*LsRab11*-R	TAATACGACTCACTATAGGGAGAAGACTGTTGCGCTCGGCGAAT
ds-*LsRab3GABP*-F	TAATACGACTCACTATAGGGAGACTCCAGGCTTCCCAGACTTG
ds-*LsRab3GABP*-R	TAATACGACTCACTATAGGGAGAGGTTGCCGGGAATTTGCATT
ds-*LsRab10*-F	TAATACGACTCACTATAGGGAGAATGGCGAAGAAAACATACGAT
ds-*LsRab10*-R	TAATACGACTCACTATAGGGAGAAATCGCTTCTCCTCGTTCTT
ds-*LsRab8*-F	TAATACGACTCACTATAGGGAGAATGGGAATTATGCTGGTTTAC
ds-*LsRab8*-R	TAATACGACTCACTATAGGGAGATGCTTCCTCTACATTGATTG
ds-*LsAtg8*-F	TAATACGACTCACTATA**G**GGAGAAGAGGAAAAGTGAAGGCGAGAA
ds-*LsAtg8*-R	TAATACGACTCACTATA**G**GGAGA GCATCTTCTGGTCTGAGGTGA
ds-*LsAtg3*-F	TAATACGACTCACTATA**G**GGAGAGACGTGGCGACTGCCAAGGAT
ds-*LsAtg3*-R	TAATACGACTCACTATA**G**GGAGATCACAGAGCTTTCGACCACAT
ds-*LsSqstm1*-F	TAATACGACTCACTATA**G**GGAGAAGCGAGATACGTCGCTTCTG
ds-*LsSqstm1*-R	TAATACGACTCACTATA**G**GGAGAGGCGCACAAATCGTAGTTGG
ds-*LsMSK2*-F	TAATACGACTCACTATAGGGAGAAAAGTCCTCGGTCAAGGCTCTTTCGG
ds-*LsMSK2*-R	TAATACGACTCACTATAGGGAGACGCCGGATTTCGTTTGAAGAGAACTCT
ds-*NlToll*-1F	TAATACGACTCACTATAGGGAGAATGTTGCGTCGCTGTAGGCATG
ds-*NlToll*-1R	TAATACGACTCACTATAGGGAGAGCTGTAGCTGATTTCGAGTT
ds-*NlToll*-2F	TAATACGACTCACTATAGGGAGAAGGGGCAACTTCATAACAGATC
ds-*NlToll*-2R	TAATACGACTCACTATAGGGAGACTTGAATAATTCATCTGGCA
ds-*NlDorsal*-1F	TAATACGACTCACTATAGGGAGAATCACTGAGCAGCCTGCCTC
ds-*NlDorsal*-1R	TAATACGACTCACTATAGGGAGACTTTTTGTCATAGATGGGCTC
ds-*NlDorsal*-2F	TAATACGACTCACTATAGGGAGAATCTGCAAACTGAGTGAAG
ds-*NlDorsal*-2R	TAATACGACTCACTATAGGGAGACCAACATAACTTCCATTAAATACCA
For qRT-PCR	
q-*LsDorsal*-F	ATCGTCGTCGAGAAAGCCC
q-*LsDorsal*-R	CATTGCTAGCACGTCCCTCCA
q-*LsZN708*-F	ACAGTTGTGAAGTTTGTGATAGA
q-*LsZN708*-R	AACTATAGGGTCTCTCTCCA
q-*LsToll*-F	AACCCTCTCAACTGCGACTG
q-*LsToll*-R	GTGACGGAATCGTGGTCACT
q-*LsAtg8*-F	CGTTATTCCCCCTACCAGCG
q-*LsAtg8*-R	TTTCCATAGACGTTCTCATCACT
q-*LsAtg3*-F	TCACGTGGATCCCACAACTG
q-*LsAtg3*-R	TCCTCGTCTAGAAGGCCACT
q-*LsSqstm1*-F	GGCCGACAACTACAAGGTGA
q-*LsSqstm1*-R	GGATGCGACGTCGGTATGAT
q-*LsGAPDH*-F	GTGTTGACTACATGGTCTACT
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q-*LsRab7*-F	CAGTGGTGCCAGAGCAAA
q-*LsRab7*-R	TTAACAGGAACAGTTTTC
q-*LsRab11*-F	CTTCATCGAAACGTCGGCGCTC
q-*LsRab11*-R	TCACTGGCAACACTGCTTACGA
q-*Ls3GABP*-F	TCTCATCCAAGGCCGAAACC
q-*Ls3GABP*-R	AGCAACTTCAGGCTCGACAA
q-*LsRab10*-F	CAGAGAACACGGAATACGGTT
q-*LsRab10*-R	CTAACAACATCGATTGACGCC
q-*LsRab8*-F	TTTTTCACACTGGCGAGGGAT

q-*LsRab8*-R	TCAGAGCAGGGTGCAGCGCGA
q-*LsRab35*-F	TATTGACAGAGGATGCCCAAA
q-*LsRab35*-R	CTAACAACATTTGCTTTTT
q-*LsRab27*-F	GACTGATATACTTTGAGACAAG
q-*LsRab27*-R	TCAGCAACTGCATTTGGGTT
q-*NlToll*-F	TGTTCTGGATTTGACCAATAA
q-*NlToll*-R	AACCTTTTTGAAATTTTCCTG
q-*NlDorsal*-F	ATTGATAAGCAAGGCCACAA
q-*NlDorsal*-R	ATTTCCACTACCTGCTTCAAC
q-*NlActin*-F	GTGCGTGACATCAAGGAGAAGC
q-*NlActin*-R	GGAAGGAAGGCTGGAACAGAG
q-RSV-*NS4*-F	TGCAAGACGTACAAAGGACAA
q-RSV-*NS4*-R	TCTTCCGTGACTCTCTCTGG
q-RSV-*NP*-F	ACCACAATCTTCGATGCGCT
q-RSV-*NP*-R	CCAACCCGCTGACTTCAGAT
q-RRSV-*P8*-F	CCATCCATTCCGCCAGAGGAC
q-RRSV-*P8*-R	CTTATCTGTGTCAGCCTTGATAT
q-RBSDV-P10F	AACAACCGACCAACAATCAC
q-RBSDV-P10R	GAGCAGGAACTTCACGACAG
For Chip-qPCR	
Chip-q-*LsZN708*-F	GTGATAGAACATTCACTCAAA
Chip-q-*LsZN708*-F	TAAAGTCTCTCTCCAGTATG
Chip-q-*LsAtg13*-F	CGGTGCAATGACTAATTGGT
Chip-q-*LsAtg13*-F	GTCATTCATTCATGAGAAAC
Chip-q-*LsDSOR1*-F	GCCTAGCCTTATAATGCAGA
Chip-q-*LsDSOR1*-F	GGCCTTATCATGGCCAGGAAG
Chip-q-*LsRBM45*-F	AGCGCAGTTTTCGGGCTCAG
Chip-q-*LsRBM45*-F	CGGTATCCAAATAAGAGAAA
Chip-q-*LsSNG*-F	GTAGAGAAAAGTCTCATAT
Chip-q-*LsSNG*-F	TTATTTTGAGCAATTTTGAG
Chip-q-*LsMTH*-F	GCTTCAGCATGTACACAACA
Chip-q-*LsMTH*-F	ACGACGAGAAATTCGTCTAT
Chip-q-*LsRRAGA*-F	AGATCTTCACTCTGATGAAAT
Chip-q-*LsRRAGA*-F	AACCAGTATGAGAGCAATAAT
Chip-q-*LsBYST*-F	AGCTCAGGTCCCTTTCGGTA
Chip-q-*LsBYST*-F	AAATGAGATCCAAGCTCCCA
For Yeast One-Hybrid (Y1H) Assays	
pAbAi-*LsZN708*-F	AAGCTTGAATTCGAGCTCGTGATAGAACATTCACTCAGA
pAbAi-*LsZN708*-R	AAGCTTGAATTCGAGCTCGTGATAGAACATTCACTCAGA
pAbAi-*LsAtg13*-F	AAGCTTGAATTCGAGCTCTTGCTTAGGAAGCAGCACACA
pAbAi-*LsAtg13*-R	GCACATGCCTCGAGGTCGACGCGGCCTTCTTCTGCTAAAC
pAbAi-*LsDSOR1*-F	AAGCTTGAATTCGAGCTCTATGTGAATTACTGTGAC
pAbAi-*LsDSOR1*-R	GCACATGCCTCGAGGTCGACAGAAGATTGGGGAGCTTTCC
pAbAi-*LsRBM45*-F	AAGCTTGAATTCGAGCTCACCAGGGGGGGGCAATTTTCAT
pAbAi-*LsRBM45*-R	GCACATGCCTCGAGGTCGACCAAAATAATTGTTCCACCTA
pAbAi-*LsSNG*-F	AAGCTTGAATTCGAGCTCGCTAAATATCTCTGCTTGTA
pAbAi-*LsSNG*-R	GCACATGCCTCGAGGTCGACTTCAAGTACTATCTTATACG
pAbAi-*LsMTH*-F	AAGCTTGAATTCGAGCTCCATATCAGTGTAGACTACTG
pAbAi-*LsMTH*-R	GCACATGCCTCGAGGTCGACCACAGTGCTGCCCAACGGCT
pAbAi-*LsRRAGA*-F	AAGCTTGAATTCGAGCTCTAGTTTTGAACAATAGTTTGC
pAbAi-*LsRRAGA*-R	GCACATGCCTCGAGGTCGACGGGAAGAGTGGATCTGGTAA
pAbAi-*LsBYST*-F	AAGCTTGAATTCGAGCTCAAGTAGGGTTTCAGCATACTT
pAbAi-*LsBYST*-R	GCACATGCCTCGAGGTCGACCAGACCGGGAAGAGGAAGTTC
For BiFC Assays	
pCV-cYFP-*LsDorsal*-F	CTGTACAAGTCCGGAGTCGACATGGACACGG ACATTGA
pCV-cYFP-*LsDorsal* -R	GATCGGGGAAATTCGAGCTCCTATTTAACA GGAACATTG
pCV-nYFP-*LsDorsal*-F	ATCGAGGACTCCGGAGTCGACATGGACACGG ACATTGA
pCV-nYFP-*LsDorsal* -R	GATCGGGGAAATTCGAGCTCCTATTTAACA GGAACATTG
pCV-nYFP-*NS4*-F	ATCGAGGACTCCGGAGTCGACATGCAAGACGTACAAAGGACA
pCV-nYFP-*NS4*-R	GATCGGGGAAATTCGAGCTCCTATGTTTTATGAAGAAGAGGT
pCV-cYFP-*NS4*-F	CTGTACAAGTCCGGAGTCGACATGCAAGACGTACAAAGGACA
pCV-cYFP-*NS4*-R	GATCGGGGAAATTCGAGCTCCTATGTTTTATGAAGAAGAGGT
For Virus Detection	
RSV-*NP*-F	TGCCTCATCCTCGAAGAACTCC
RSV-*NP*-R	AGCCAGCCACTCTAGCTGATTT
RBSDV-*P10*-F	ACCCAGTCAAGACGCTCAGT
RBSDV-*P10*-R	CTGTTCCCGCCATAGACACT
RRSV-*P8*-F	GCAGACTAGGGATGTGCGTT
RRSV-*P8*-R	AATTGACGAGTCCTCTGGCG

>Nucleotide sequence of *Laodelphax striatellus* ZN708

ATGGTTCTAAAAAAACTTTTCTCAACACTAACTTACTTACAGGAAGAAGAGAATGGATGCAAG
AGTCAGTCCAGTCCTCTACAGACTCAACTTGTCAACTGGGATTGTCATAATCCAGACAACTA
CAGGCCGATAGCAGAATCACAAGATAAAAGACAATGTAGTGGGGGCATGGACACCTGTGAT
GGACTGGATCAAGATCAACACAACACAAATGATCATGATCAATGTTCAGACCACAACTACCA
ACATCCATTTGAAATGATAGTGCTCAAACAAGAGATAGTGGTTGAAGAAGAACAACATGTGG
GAATAGTTGAAGGAGGACTGCAAGTGGAAAAAGAAGAGATGGAAGATGAAAATTTCATGAAT
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GGAAGATTTAAATGAATTAGAATCAGATGGGGTATATAATTATGTTTCTACAATGATGCAAAT
CCAGGACATTTCACCTGTTATAGATACTAAAATTGTTGTTAATTCCAAAACTGTAAACAATTCT
ATTGTTGTGAATGAACAAGACTTTAGCGCAGAAGGTATTGGAGAACAAGGCAGTTCAACTCC
TGATGAGAGTGACAATAATAGTCCAGGCCAATTCAATCCACAAGTTCATGGCAGCAAGTCTT
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ACCAGACATTTACTGACACATACTGGAGAGAGACTTTACAGTTGTGAAGTTTGTGATAGAACA
TTTGCTGAGAAAGCATCTTTGACCAGACATTTACTTACACATACTGGAGAGAGACCTTACAGT
TGTGAATTTTGTGATAAAACATTTACTCGTAAATCTAGTCTTACCACACATTCACATACACATA
CTGGAGAAGGACTCTACAGTTGTGGAAATTGTGATAAATCATTCACTCATAAATCACATCTTA
CGGCCCATTTATATACACATACTGGAGAGAGACCCTATAGTTGTGAATTTTGTGATAAAACAT
ACATTTATAAGTCAAATCTCACCATGCATTTACGTACACATACTGGAGAAAGACCCTATACTT
GTGGAAATTGTGATAAGACATTCACTCAGAAATGCCATCTCATCTCACACATAAGGAATATAC
ACACAGGATAG

>Protein sequence of *Laodelphax striatellus* ZN708

MVLKKLFSTLTYLQEEENGCKSQSSPLQTQLVNWDCHNPDNYRPIAESQDKRQCSGGMDTCD
GLDQDQHNTNDHDQCSDHNYQHPFEMIVLKQEIVVEEEQHVGIVEGGLQVEKEEMEDENFMND
ETDTSNRFTIKQEHVEEETEEDLNELESDGVYNYVSTMMQIQDISPVIDTKIVVNSKTVNNSIVVN
EQDFSAEGIGEQGSSTPDESDNNSPGQFNPQVHGSKSYRHDRPYSCEYCDRTFTQNSNLTRHL
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HLLTHTGERPYSCEFCDKTFTRKSSLTTHSHTHTGEGLYSCGNCDKSFTHKSHLTAHLYTHTGE
RPYSCEFCDKTYIYKSNLTMHLRTHTGERPYTCGNCDKTFTQKCHLISHIRNIHTG*