Abstract
Background: The brown planthopper (Nilaparvata lugens Stål) is a notorious rice pest in many areas of Asia. Study on the molecular mechanisms underlying its development and reproduction will provide scientific basis for effective control. SPARC (Secreted Protein, Acidic and Rich in Cysteine) is one of structural component of the extracellular matrix, which influences a diverse array of biological functions. In this study, the gene for SPARC was identified and functionally analysed from N. lugens.

Results: The result showed that the NlSPARC mRNA was highly expressed in fat body, hemolymph and early embryo. The mortality increased significantly when NlSPARC was downregulated after RNA interference (RNAi) in 3 ~ 4th instar nymphs. Downregulation of NlSPARC in adults significantly reduced the number of eggs and offspring, as well as the transcription level of NlSPARC in newly hatched nymphs and survival rate in progeny. The observation with microanatomy on individuals after NlSPARC RNAi showed smaller and less abundant fat body than that in control. No obvious morphological abnormalities in the nymphal development and no differences in development of internal reproductive organ were observed when compared with control.

Conclusion: NlSPARC is required for oviposition and nymphal development mainly through regulating the tissue of fat body in N. lugens. NlSPARC could be a new candidate target for controlling the rapid propagation of N. lugens population. Our results also demonstrated that the effect of NlSPARC RNAi can transfer to the next generation in N. lugens.

Keywords: Nilaparvata lugens, SPARC, RNAi, Parental RNAi, Fat body

Introduction
Rice brown planthopper (Nilaparvata lugens Stål) (Hemiptera: Delphacidae), a major sap-sucking pest, causes the wilting and complete drying of rice (Oryza sativa), a condition known as 'hopperburn' under heavy infestations [1]. Strategies for controlling N. lugens include breeding resistant cultivars and using synthetic pesticides. However, continued cultivation of resistant varieties has allowed the emergence of new virulent biotypes of N. lugens that can overcome existing resistance genes [2, 3]. The use of synthetic pesticides can kill natural predators and ultimately develops insecticide resistant biotypes [4, 5]. Therefore, more economical, targeted, and sustainable control methods are highly needed to pest management. The discovery of important functional genes required for the growth and development of pests through research is an effective strategy to explore potential targets for pest control.

SPARC (Secreted Protein, Acidic and Rich in Cysteine) is one of the structural components of the extracellular matrix, which has been shown to bind calcium and collagens, modulate cell–matrix interactions and influence a
diverse array of biological functions. SPARC has been the topic of a number of reviews [6–8]. In vertebrate species it participates in bones mineralization or regulating cell proliferation in some cancer types. SPARC mis-regulation perturbs the function of many extracellular matrices and associated tissues, and correlates with cancer progression [9, 10]. SPARC has been studied only in a limited number of invertebrate species. In the nematode Caenorhabditis elegans, downregulation of SPARC expression using RNA interference (RNAi) demonstrated it affects the function of a variety of tissues and is required for completion of normal development [11]. In the cnidian Nematostella vectensis, four SPARC orthologs have been identified and their expression is restricted to the endoderm in early stages of development [12]. Among insects, SPARC has been thoroughly studied in the fly Drosophila melanogaster with results revealing multiple functions in oogenesis, vitellogenesis, embryo development, heart formation and cardiomyopathies [13–18]. In the cockroach Blattella germanica, depletion of SPARC does not allow the follicular cells to complete mitosis, resulting in a great alteration of the ovarian follicle cytoskeleton and disability of females for oviposition [19]. However, no studies on SPARC in N. lugens have been reported to date.

In this study we cloned the SPARC gene from N. lugens. The contribution of SPARC to the development and reproduction in N. lugens was investigated by using microanatomy and RNAi technology to knock down its expression in different developmental stages, including early and late instar nymphs, newly emerged adults and gravid females.

Results

NISPARC identification

Based on N. lugens transcriptome data constructed in our laboratory and genome data from National Center for Biotechnology Information (assembly ASM1435652v1), one specific PCR primer pairs for SPARC were designed and used to clone this gene from N. lugens. The cDNA of N. lugens SPARC (NISPARC) consists of 1288 nucleotides, including the 5′ and 3′ untranslated regions, and an open reading frame of 891 nucleotides (from 138 bp to 1028 bp). It encodes a protein of 296 amino acids, with a theoretical molecular weight of 34.7 kDa and an isoelectric point of 5.3. The cDNA sequences of NISPARC were deposited in GenBank under accession number MZ983402. Blast against NCBI showed that NISPARC shared high identity with the SPARC predicted from Thrips palmi (up to 70.9% identity with an E value 4e−74 and 45% coverage). Blast analysis with N. lugens genome data showed that NISPARC was located on chromosome 2 and was composed of at least 7 exons.

The sequence of the NISPARC protein is similar with other insect SPARC orthologs, showing 87.5% and 70.6% identity to the SPARC from Laodelphax striatellus and Blattella germanica, respectively. When compared with SPARC from D. melanogaster, its identity decreases to 50.0%. As in other insect SPARC proteins, NISPARC is structured in the three characteristic domains, Domain I (the acidic domain in the N-terminal), Domain II (the follistatin-like domain, the conserved putative N-glycosylation site) and Domain III (EF-hand calcium binding domain). NISPARC sequences begin at the N-terminal methionine of the signal sequences (MERKAYLLFAL-LACFLILLIDVTSS) (Fig. 1). Domain I (residues 24–76) have 12 acidic residues, the overall acidity is reduced by the presence of 9 basic residues (pI 6.3). NISPARC contains 16 cysteine residues, 11 of which are conserved in domain II (residues 77–191). The C-terminal half in domain III is most highly conserved containing two EF-hand domains in all organisms. EF-hand II domain in NISPARC is stabilized by a disulfide bridge between cysteine residues 258 and 274 and an additional cysteine residue (286) located near the C-terminus which is found only in invertebrate SPARC proteins (Fig. 1). The name of the species, the corresponding accession numbers for SPARC, length and the pIs of Domain I are listed in Table S1.

To clarify the evolutionary relationship of NISPARC, we used a neighbor-joining tree construction program Mega 7 based on distances of 18 SPARC sequences from 18 arthropod including Coleoptera, Lepidoptera, Diptera, Hemiptera, Dictyoptera, Hymenoptera and Psocoda. The dendrogram obtained places the NISPARC with Hemiptera as a distinct cluster (Figure S1).

Developmental and tissue-specific expression of NISPARC in N. lugens

Egg and nymphal development normally lasts 6 ~ 7 days and 12 ~ 14 days, respectively. Nymphal development is comprised of five nymphal instars, from N1 to N5, spanning about 48 h for each. The RT-qPCR analysis revealed that NISPARC was expressed at all developmental stages of N. lugens. Expression of NISPARC was highest on average in 3- and 4-day-old eggs, then declined rapidly in 5- and 6-day-old eggs and reached to the lowest level in newly hatched nymphs (Fig. 2A). The results of the expression analysis of NISPARC in various tissues showed that the highest expression was in fat body and hemolymph and there was no significant difference between females and males (Fig. 2B).

Knock-down of NISPARC resulted in nymph mortality

Based on the sequence alignment of NISPARC, the regions (95–891 bp) of NISPARC with great divergence
from other insects were selected for the synthesis of dsRNA. Preliminary study conducted for dose-determination showed NISPARC expression was downregulated by more than 70% and the nymph mortality within 24 h was less than 20% after injection of dsNISPARC in nymphs with a concentration of 700 ng/μL (i.e., 70 ng dsRNA in each insect), which concentration was used for further study. In order to elucidate the function of NISPARC in nymphal development, 3rd instar and 4th instar nymphs were injected with dsNISPARC,
respectively. There was a significant increase in nymph mortality at day 8 after injection. The mortality was 36.0% and 46.4% at day 9 after injection in 3rd instar and 4th instar nymphs respectively, whereas it was 20.4% and 22.1% in control with dsGFP ($P = 0.0104$, $P = 0.0004$, respectively) (Fig. 3A).

*NlSPARC* mRNA levels were measured in 3rd instar nymphs after injection at day 4 and 9 and in tissues of adults developed from the injected 4th instar nymphs. Compared with dsGFP injection, in the 3rd instar nymphs, expression of *NlSPARC* significantly decreased by 18.4-fold at day 4 ($P = 0.0003$) and 4.4-fold at day 9 ($P = 0.0035$) after injection of dsNlSPARC (Fig. 3B). In the 4th instar nymphs, expression of *NlSPARC* significantly decreased by 24.2-, 20.6- and 7.3-fold in fat body, female and male internal reproductive organs respectively ($P = 0.0034$, $P = 0.0027$ and $P = 0.0088$) (Fig. 3B). After insect anatomy evaluation, we found that the fat body in adults injected with dsNlSPARC was smaller and less abundant than those from adults injected with dsGFP (Fig. 3C).

**Effects of NlSPARC knock‑down on reproduction and progeny**

In order to elucidate the function of *NlSPARC* in *N.lugens* reproduction, the 5th instar nymph, newly emerged adults and gravid females were injected with dsNlSPARC, respectively. As no difference between injection of dsGFP and no injection control in 5th instar nymphs was observed, the no injection control was omitted in other stages. The injection of dsNlSPARC did not significantly affect adult longevity and ovariole maturation. The number of offspring after injection of dsNlSPARC in the 5th instar nymph, newly emerged adults and gravid females was significantly reduced by 51.3%, 59.1% and 52.5%, respectively ($P = 0.0001$, $P = 0.0174$ and $P = 0.0300$). The number of eggs after injection of dsNlSPARC in the 5th instar nymph, newly emerged adults and gravid females was significantly reduced by 49.3%, 59.2% and 46.0%, respectively ($P = 0.0001$, $P = 0.0252$ and $P = 0.0467$), compared to dsGFP injection (Fig. 4A). No difference in hatch rate between the treatment and control was found (data not shown).
There was no significant difference in 10-day survival rate of F1 progeny between injection of dsNlSPARC and dsGFP in 5th nymphs. The 10-day survival rate of F1 progeny of newly emerged adults and 4-day old gravid females injected with dsNlSPARC respectively. Individuals treated with dsGFP were used as a control. The number of offspring and egg was calculated from fifteen biological replicates (Mean±SE). NlSPARC mRNA levels of 30 newly hatched nymphs and 20 10-day old mixed insects with 10 females and 10 males were analyzed by RT-qPCR with the 2^ΔΔCT method. Data is presented as mean±SE with five biological replicates. G1NHN, newly hatched nymphs from F1 progeny; G1 10d, 10d-old nymphs from F1 progeny. Different lowercase letters and ** above the bars indicate significant differences at P<0.05 and P<0.01 respectively among different treatments by the Tukey’s multiple range tests.

Fig. 4 Effects of dsNlSPARC on fecundity (A), survival rate of progeny (B) and expression level of NlSPARC in N. lugens (C). The 5th instar nymphs, newly emerged adults and 4-day old gravid females were injected with dsNlSPARC respectively. Individuals treated with dsGFP were used as a control. The number of offspring and egg calculated from fifteen biological replicates (Mean±SE). NlSPARC mRNAs levels of 30 newly hatched nymphs and 20 10-day old mixed insects with 10 females and 10 males were analyzed by RT-qPCR with the 2^ΔΔCT method. Data is presented as mean±SE with five biological replicates. G1NHN, newly hatched nymphs from F1 progeny; G1 10d, 10d-old nymphs from F1 progeny. Different lowercase letters and ** above the bars indicate significant differences at P<0.05 and P<0.01 respectively among different treatments by the Tukey’s multiple range tests.

NlSPARC mRNA levels were measured in F1 progeny with RNA extracted from 30 newly hatched nymphs and 20 mixed insects of 10 females and 10 males. Compared to dsGFP-injected control, the expression of NISPARC decreased significantly in newly hatched nymphs by 2.4, 9.0 and 6.4 times after injection of the 5th instar nymphs, newly emerged adults and gravid females, respectively (P<0.0001), whereas 10 days later no significant difference between treatment and control was found (Fig. 4C).

mRNA levels of NISPARC, NIVg (Vitellogenin), NIVgR (Vitellogenin receptors) and NIFOXO were also measured in newly emerged females developed from the injected 5th instar nymphs. Compared to dsGFP-injected control, the expression of NISPARC decreased
by 7.0 times and the expression of NIVg, NIVgR and NIFoxO showed no significant difference (Figure S2).

As the number of offspring decreased after injection of dsNlSPARC, we then dissected the adults and found that the ovarian tubules were easily dispersed and the amount and volume of fat body reduced compared to the control (dsGFP) (Fig. 5). There was no obvious difference in development of internal reproductive organ, ovarian tubules and egg granules between dsNlSPARC and dsGFP treatment (Fig. 5).

Discussion

SPARC is a secreted calcium-binding and collagen-binding glycoprotein that has functions in tissues remodelling during development [16]. Genome analysis has revealed a single copy of the Drosophila SPARC gene [13]. NlSPARC is encoded by a single gene and located in chromosome 2 in N.lugens. Comparative analysis of primary sequences of SPARC proteins from diverse species revealed functional conservation, including the high-affinity Ca\(^{2+}\) binding sites in domain III. As reported in Drosophila [13, 17], temporal and spatial expression data in N.lugens indicated that maximum levels of SPARC expression occur during embryonic development stages and in fat body and hemocytes.

Injection of dsNlSPARC in the 3rd or 4th instar nymphs caused a significant decrease in NlSPARC transcripts at 4 and 9 days after injection, especially in fat bodies and female internal reproductive organs. This result suggests that the genes expressed in fat body and internal reproductive organs can be down-regulated by RNAi in N.lugens. A notable result was that a significant reduction in NlSPARC expression was observed in newly hatched nymphs of the progeny after injection of dsNlSPARC in the 5th instar nymphs and adults, and 10 days later, the expression level returned to the same level as the control. This indicated that NlSPARC RNAi is still in effect in the next generation in N.lugens. This phenomenon was called parental RNAi (pRNAi), which is identified by determining whether dsRNA introduced into the body cavity resulted in gene inactivation in offspring embryos [20, 21]. The phenomenon of pRNAi, especially the genes involved in embryonic development has been confirmed in Tribolium castaneum [22], Acyrthosiphon pisum [23], Diabrotica virgifera virgifera and Euschistus heros [24]. Our result showed the pRNAi of SPARC also exists in N.lugens.

Down-regulation of the NlSPARC with 18.4-folds in 3rd instar nymphs resulted in the corrected mortality of 26.4%, with 6.4- and 9.0- folds in new hatched nymphs of F1 progeny resulted in the corrected mortality of 46.1% and 25.5%. With a 2.4-folds down-regulation of NlSPARC in new hatched nymphs of F1 progeny did not result significant mortality. The down-regulation fold of NlSPARC expression was lower in newly hatched nymphs than in 3rd instar nymphs, whereas the effect on mortality was more significant. In Drosophila, loss of SPARC alone leads to defective fat body basal laminae assembly, embryonic

![Fig. 5](image-url) Effects of dsNlSPARC on the internal reproductive organs. A 2-,4-, 6- day old females (F) and 4 day old males (M) with dsGFP treated N.lugens; B 2-,4-, 6- day old females (F) and 4 days old males (M) with dsNlSPARC treated N.lugens. Fat bodies are highlighted with red arrow. No obvious difference in development of internal reproductive organ, ovarian tubules and egg granules were found between the treatment injected with dsNlSPARC and the control injected with dsGFP. The ovarian tubules were easily dispersed and the amount and volume of fat body were reduced in dsNlSPARC treatment compared to the control (dsGFP).
lethality and larval lethality of the 2nd instar [14, 16, 25]. In C. elegans, a reduction in the amount of SPARC protein also resulted in embryonic or larval lethality in a substantial proportion of progeny, and no significant morphological abnormalities were observed [11].

In nematodes, developmental defects were observed during embryogenesis following SPARC deletion, while there appeared to be no changes in adult gonad or oocyte morphology, suggesting that the fertilization process was disturbed [11]. In D. melanogaster, there was an increase of its expression during previtellogenic stages, which indicated that SPARC play a role in oocyte development and vitellogenesis [13]. In Blattella germanica, SPARC is necessary to maintain the cytoskeleton of the follicular cells and depletion of SPARC disables females for oviposition [19]. Therefore, we selected three different developmental stages including the 5th instar nymphs, newly emerged adults and gravid females for RNAi to study the function of this gene in N. lugens reproduction. Knock-down of the NISPARC in these stages resulted in a reduction of offsprings and egg number, whereas the egg hatchability, early embryo development was not affected. The expression of genes NIVg, NIVgR and NIFoxO, which related closely to oocyte development and vitellogenesis, was not changed [26, 27]. This result implied that the fertilization process and vitellogenesis were not affected after knock-down the NISPARC. Microanatomy showed that down-regulation of NISPARC resulted in the decrease of the fat body, which is crucial for development and acts as the primary source of energy. Together with hemocytes, fat body is the major sources of basal laminae components during larval development [28]. This result showed that oviposition events and processes are affected after knock-down of NISPARC in N. lugens and this effect may be mainly achieved by regulating the fat body. More detailed experiments and further analyses of SPARC function are needed to reveal its precise role in the N. lugens.

Moderate control of population densities can help delay the rate of resistance evolution and is therefore considered a greener, gentler strategy for sustainable control. RNAi has been widely applied as a genetics tool for gene function analysis through the sequence-specific suppression of target gene. It is also regarded as a potential approach for insect pest management as it avoids the rapid formation of resistant populations and excessive use of chemical pesticides [29, 30]. Recently report showed that the sap-sucking insect pest can be effectively controlled by plastid-mediated RNAi including N. lugens [31]. RNAi of NISPARC showed mortality, especially in early nymphal stages and low fecundity. Although the decline in survival and the number of offspring was not drastic after RNAi of NISPARC in N. lugens, when it fed on transplastomic rice expressing dsNISPARC, the suppression of NISPARC expression persisted throughout the developmental period and across successive generations, the population of N. lugens will be controlled within a certain range. Compared with the gene with high mortality after down-regulation, NISPARC is more suitable as a candidate target gene for green control of N. lugens through RNAi, which meets the standards of green control by reducing initial population sizes, decreasing population growth rates of pest and controlling pests within the allowable economic loss density without affecting the biodiversity in rice fields [32]. Considering the high similarity of domains II and III of SPARC proteins in insects and thus possibly causing off-target effects in RNAi applications, designing dsRNA against the sequence of domain I can improve the specific selectivity for the control of the target pest N. lugens.

Materials and methods

Insects and tissue sampling

N. lugens were reared on rice variety Taichung Native 1 at 26 ± 2 °C under a 16 h light/8 h dark cycle. Six developmental stages: Eggs (200) within 1 ~ 6 days, and the individuals (50) from the first day of the 1st instar to the twelfth day of the 5th instar nymphs, newly emerged females (10) and male adults (10) as well as 4- day old females and males (10) were randomly selected. Adult females and males at 2 days after eclosion were immobilized by placing them in a freezer for 15 min, tissues including guts, salivary glands, fat bodies, hemolymph, legs, wings and teguments from 50 to 100 mixed sex adults were disected and internal reproductive organ from males (50) and females (50). Heads (20), abdomens (20) and thoraces (20) from males and females respectively were disected with tweezer. The number of insects in each sample was given in parentheses above. All samples were collected in triplicate. The samples were frozen in liquid nitrogen and stored at -80 °C prior to RNA extraction.

RNA extraction, cDNA preparation and RT-qPCR analysis

Total RNA extraction, cDNA synthesis, T-A clone and RT-qPCR analysis were conducted according to our previous study [33]. Total RNA was isolated from the whole bodies or tissues of N. lugens using RNeasy Mini Kit (Qiagen, Valencia, CA) following the manufacturer’s recommendations. Total RNA (0.5–1 µg) was used to synthesize first strand cDNA with ReverTra Ace qPCR RT Kit (Toyobo Co., Ltd., Japan). RT-qPCR was performed using SYBR green mix and a thermal cycler CFX-96 PCR detection system (Bio-Rad, Philadelphia, PA, USA). The primers used for clone are NISPARC-F: GACATTCCCTCAG TCCACGAGT and NISPARC-R: ATACGGTTACTG
GGTTATGAACA. NISPARC specific primers for RT-qPCR expression are qNISPARC-F: ACCTCTCCACCT CCCGAI TT and qNISPARC-R: TGCAAAACACACT TTGCCTTCT. Primers were synthesized by Shangya Biotechnology Co. RT-qPCR analysis was performed with at least three biological replicates, and three technical replications for each. Nlactin and Nl18S rDNA were used as the reference gene to normalize gene transcript levels [34]. Relative quantification of the transcripts was calculated by the $2^{-\Delta \Delta Ct}$ method [35].

**Phylogenetic studies**

The open reading frame (ORF) for NISPARC was predicted using DNASTAR software (DNASTAR Inc., Madison, USA) and the corresponding amino acid sequence of NISPARC was analyzed using SignalP 3.0 (http://www.cbs.dtu.dk/services/SignalP-3.0). The sequence domains were identified using the Conserved Domain Search Service from NCBI. Sequences used in the phylogenetic analysis were obtained by Blast from GenBank, using the SPARC protein sequences from *N.lugens* and *D.melanogaster* as query. A phylogenetic tree was constructed by MEGA version 7.0 (http://megasoftware.net/) using the neighbor-joining method. A Poisson-corrected distance was used and the data was bootstrapped for 1000 replicates.

**dsRNA synthesis and RNAi experiments**

To knock-down NISPARC for the assessment of the specificity of the phenotype, the dsRNA was synthesized in vitro using the MEGA script high-yield transcription kit (Applied Biosystems Inc., USA). And then quantified using a NANODROP™ 1000 spectrophotometer (Thermo Scientific, Franklin, MA) at 260 nm and analyzed by gel electrophoresis to determine purity. The primers used for dsNISPARC were dsNISPARC-F: TAATACGACTCACTATAGGG GAATAGAGCCGGAGCAGTG and dsNISPARC-R: TAATACGACTCATATAGGG TGCAAT GTTCCAGAGCCATGA. A dsGFP that targets the green fluorescent protein (GFP) gene (AB608314) was also produced and used as control.

The dsNISPARC or dsGFP was injected into the 3rd instar, 4th instar, 5th instar nymphs, newly emerged adult insects and gravid 4-day old females respectively according to the method described by Zhang et al.[36]. Gravid 4-day old females were collected as follow: virgin males and females (24 h old) were allowed to mate for 4 days. On day five, males were removed and the remaining females were used for injection. Each nymph was injected with 0.1 μL dsRNA solution into the thorax. Each adult insect was injected into the thorax with 0.2 μL dsRNA solution. The efficiency and specificity of RNAi were examined by RT-qPCR at day 4 or 9 after injection of the

3rd instar nymphs, different tissues of adults developed from injected the 4th instar nymphs or newly hatched nymphs from progeny of each paired parent.

For investigation of the survival, at least 30 individual nymphs were injected and reared on 30- to 35-day-old rice of Taichung Native1 in one cage at 28°C, 85% RH, and 16:8 (L: D) h darkness with three separated parallel replicates. The number of surviving nymphs was counted daily until 9 days after injection. The survival rate was calculated as the number of live insects divided by the starting number of insects. For investigation of the fecundity, each newly emerged female and male after injection were reared in one cage with 60-day-old rice of Taichung Native1 and allowed to oviposit until parents die. At least 15 pairs were successfully paired. Each gravid 4-day old female after injection was reared in one cage and allowed to oviposit for seven days. At the 7th day the females were dissected to collect under the stereomicroscope. Then the number of nymphs hatching from each female was counted on rice stems for 10–12 consecutive days until no further hatching was observed. Finally, rice stems were dissected and examined with a stereomicroscope, and the number of eggs was counted. At least fifteen biological replicates were used for statistical analysis.

**Data analysis**

The differences between control and RNAi treatments were analyzed using one-way analysis of variance followed by the Tukey’s multiple range tests for multiple comparisons using DPS software [37].

**Supplementary Information**

The online version contains supplementary material available at https://doi.org/10.1186/s12864-022-08903-z.

---

**Additional file 1: Table S1.** Information of protein sequences used in alignment.

**Additional file 2: Figure. S1.** Unrooted phylogenetic tree of NISPARC from *N.lugens* and representative insect species. An unrooted phylogenetic tree was constructed by the neighbour-joining tree construction program Mega 7. Evolutionary distances were computed using Poisson correction method. Branch support values (1000 bootstraps) for nodes are indicated only support values > 50% are shown. NISPARC is marked with filled triangle. All protein sequences (accession numbers, length and pl) obtained from GenBank was listed in Table S1.

**Additional file 3: Figure. S2.** Relative expression of NISPARC,NlVg,NlVgR, and NlFoxO in newly emerged adults developed from injected 5th instar nymphs. mRNA levels of NISPARC, NlVg, NlVgR, and NlFoxO from 5 newly emerged females were analyzed by RT-qPCR with the $2^{-\Delta \Delta Ct}$ method from three biological replicates (Mean ± SE). ** above the bars indicate significant differences at P < 0.01 among different treatments by the Tukey’s multiple range tests.

**Acknowledgements**

Not applicable
Authors’ contributions
WWX designed and wrote the manuscript. ZTH and FQ analyzed and revised the manuscript. WPJ and WQ performed the PCR and RNAi respectively. HJC and LFX performed the microanatomy. All authors read and approved the final manuscript.

Funding
This study was supported by Open Project Program (20210302) of State Key Laboratory of Rice Biology, the China Agriculture Research System (Grant No. CARS-01-38), Natural Science Foundation of China (32272538).

Availability of data and materials
The datasets generated and/or analysed during the current study are available in the [GenBank] repository, [GenBank:https://www.ncbi.nlm.nih.gov/gene under accession number MZ983402].

Declarations

Ethics approval and consent to participate
Not applicable.

Consent for publication
Not applicable.

Competing interests
The authors have no relevant financial or non-financial interests to disclose.

Received: 23 April 2022 Accepted: 22 September 2022 Published online: 03 October 2022

References
1. Bottrell DG, Schoenly KG. Resurrecting the ghost of green revolutions past: the brown planthopper as a recurring threat to high-yielding rice production in tropical Asia. J Asia-Pac Entomol. 2012;15(1):122–40.
2. Jing SL, Zhang L, Ma YH, Liu BF, Zhao Y, Yu HJ, et al. Genome-wide map-}

ation of calcineurin subunits A and B in development and fecundity of the brown planthopper. Sci China Life Sci. 2021;64:133–43.
3. Isabella AJ, Horne-Badovinac S. Dynamic regulation of basement mem-

brane protein levels promotes egg chamber reorganization in Drosophila. Dev Biol. 2015;406:212–21.
4. Isabella AJ, Horne-Badovinac S, Bodmer R, Ocor K. SPARC-dependent cardiomyopathy in Drosophila. Circ Cardiovasc Gene. 2016;9:119–29.
5. Irles P, Ramosa S, Pauachs MA. SPARC preserves follicular epithelium integrity in insect ovaries. Dev Bio. 2017;422:105–14.
6. Jorgensen JM, Talbot LK, Lefevre J. Changes in insecticide resistance and host range performance of planthoppers artificially selected to feed on resistant rice. Crop Prot. 2020;127:104963.
7. Kang K, Bu KK, Liu YK, Zhou ZS, Karthi S, Yang H, Li C. A review of physi-

ological resistance to insecticide stress in Nilaparvata lugens. 3 Biotech. 2022;12(3):84.
8. Kato T, Endo S, Kaza H. Toxicity of insecticides to predators of rice planthoppers: spiders, the mirid bug and the drynidy wasp. Appl Entomol Zool. 2000;35(1):177–87.
9. Koehler A, Desser S, Chang B, MacDonald J, Tepass U, Ringuette M. Molecular cloning and characterization of the matricellular protein Sparc/osteonectin in flatfish, Scophthalmus maximus, and its developmental stage dependent transcriptional regulation during metamorphosis. Genes. 2015;688:129–39.
10. Koehler A, Desser S, Chang B, MacDonald J, Tepass U, Ringuette M. Molecular evolution of SPARC: absence of the acidic module and expression in the endoderm of the starlet sea anemone, Nematostella vectensis. Dev Genes Evol. 2009;219:509–21.
11. Koehler A, Desser S, Chang B, MacDonald J, Tepass U, Ringuette M. Evolutionary conservation and association of SPARC with the basal lamina in Drosophila. Dev Genes Evol. 2002;212:124–33.
12. Koehler A, Desser S, Chang B, MacDonald J, Tepass U, Ringuette M. Loss of SPARC dysregulates basal lamina assembly to disrupt larval fat bod

homeostasis in Drosophila melanogaster. Dev Dyn. 2015;244:540–52.
13. Martinek N, Shabah J, Satthoff M, Ringuette M. Haemocyte-derived SPARC is required for collagen-IV-dependent stability of basal laminae in Drosophila embryos. J Cell Sci. 2008;121(124):1671–80.
14. Martinek N, Shabah J, Satthoff M, Ringuette M. Haemocyte-derived SPARC is required for collagen-IV-dependent stability of basal laminae in Drosophila embryos. J Cell Sci. 2008;121(124):1671–80.
15. Volk T, Wang S, Rottstein B, Paululait A. Matricellular proteins in develop-

ment: perspectives from the Drosophila heart. Matrix Biol. 2014;37:162–6.
16. Wang WX, Zhu TH, Wang L, Wei Q, Fu Q. Cloning and functional RNA interference analysis of vitellogenin receptor in Nilaparvata lugens (Stål). J Insect Physiol. 2015;73:20–9.
17. Wang WX, Zhu TH, Wang L, Wei Q, Fu Q. Cloning and functional RNA interference analysis of vitellogenin receptor in Nilaparvata lugens (Stål). J Insect Physiol. 2015;73:20–9.
18. Wang WX, Zhu TH, Wang L, Wei Q, Fu Q. Cloning and functional RNA interference analysis of vitellogenin receptor in Nilaparvata lugens (Stål). J Insect Physiol. 2015;73:20–9.
19. Wang WX, Zhu TH, Wang L, Wei Q, Fu Q. Cloning and functional RNA interference analysis of vitellogenin receptor in Nilaparvata lugens (Stål). J Insect Physiol. 2015;73:20–9.
20. Fire A, Xu S, Montgomery MK, Kostas SA, Driver SE, Mello CC. Potent and specific genetic interference by double-stranded RNA in Caenorhabditis elegans. Nature. 1998;398:806–11.
21. Timmons L, Fire A. Specific interference by ingested dsRNA. Nature. 1998;398:854.
22. Bucher G, Scholten J, Klinger M. Parental RNAi in Tribolium (Coleoptera). Curr Biol. 2002;12:R85R86.
23. Mao JJ, Liu YY, Zeng FR. Hunchback is required for abdominal identity supression and germ band growth in the pathenogenetic embryo-

genesis of the pea aphyrus, Aphis pisum. Arch Insect Biochem. 2013;84:209221.
24. Fishilevich E, Veléz AM, Khajuria C, Frey ML, Hamm RL, Wang H, et al. Use of chromatin remodelling ATPas as RNAi targets for parental control of western corn rootworm (Diabrotica virgifera virgifera) and Neotropical brown stink bug (Euschistus heros). Insect Biochem Molec. 2016;71:58–71.
25. Pastor-Pareja JC, Xu T. Shaping cells and organs in Drosophila by opposing roles of fat body-secreted Collagen IV and perlecan. Dev Cell. 2011;21:245–56.
26. Wang WX, Zhu TH, Wang L, Wei Q, Fu Q. Cloning and functional RNA interference analysis of vitellogenin receptor in Nilaparvata lugens (Stål). J Insect Physiol. 2015;73:20–9.
27. Dong Y, Chen WW, Kang K, Fang R, Dong YP, Liu K, Zhang WQ. FoxO directly regulates the expression of TORK6/kvttellogenin and modulate the fecundity of the brown planthopper. Sci China Life Sci. 2021;64:133–43.
28. Zhang YF, Xi YM. Fat body development and its function in energy stor-

age and nutrient sensing in Drosophila. J Tissue Sci Eng. 2015;06:1000141.
29. Chandra GS, Manamohan M, Sita T. Key to the successful RNA inter-

ference (RNAi) mediated management of agricultural pests. Int J Agric Sci. 2015;5(5):191–200.
30. Cai Q, He B, Kogel KH, Jin H. Cross-kingdom RNA trafficking and environ-

mental RNAi -- nature’s blueprint for modern crop protection strategies. Curr Opin Microbiol. 2018;46:58–64.
31. Dong Y, Wu M, Zhang Q, Fu J, Loiacono FV, Yang Y, Wang Z, Li S, Chang L, Rock R, Zhang J. Control of a sap-sucking insect pest by plastid-mediated RNA interference. Mol Plant. 2022. https://doi.org/10.1016/j.molp.2022.05.008.
32. Xu HY, Yang YJ, Lu YH, Zheng XS, Tian JC, Lai FX, Fu Q, Lu ZX. Sustainable management of rice insect pests by non-chemical-insecticide technolo-

gies in China. Rice Sci. 2017;24(2):61–72.
33. Wang WX, Zhu TH, Wang L, Wei Q, Fu Q. Cloning and functional analysis of calcineurin subunits A and B in development and fecundity of Nilaparvata lugens (Stål). Rice Sci. 2022;29(2):143–54.
34. Wang WX, Lai FX, Li KL, Fu Q. Selection of reference genes for gene expression analysis in Nilaparvata lugens with different levels of virulence on rice by quantitative real-time PCR. Rice Sci. 2016;21(6):305–11.
35. Livak KJ, Schmittgen TD. Analysis of relative gene expression data using real-time quantitative PCR and the 2^(-Delta Delta C(T)} method. Methods. 2001;25(4):248–49.
36. Zhang CX, Xue J, Ye YX, Jiang YQ, Zhuo JC, Huang HJ, Cheng RL, Xu H. Efficient RNAi of rice planthoppers using microinjection. Protocol Exchange. 2015;12(4):1–8.

37. Tang QY, Zhang CX. Data Processing System (DPS) software with experimental design, statistical analysis and data mining developed for use in entomological research. Insect Sci. 2013;20:254–60.

Publisher’s Note
Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.