DOI: 10.1002/a .21400

WILEY ARCHIVES OF INSECT BIOCHEMISTRY AND PHYSIOLOGY

ARTICLE

Knockdown of *TOR* causing ovarian diapause in a genetically stable brachypterous strain of *Nila a a a l gen*

Fangzhou Liu[1] | Kaiyin Li[1] | Wanlun Cai[1] | Jing Zhao[1] | Yulan Zou[2] | Hongxia Hua[1]

[1]H b il R U i i a i a S a i ab P Ma a K Lab a , C P a S i a T , H a A a U i i , W a , C i a

[2]C Li S i a T , H a A a U i i , W a , C i a

Correspondence
H a H a, C P a S i a T , H a A a U i i , W a 430070, C i a.
E ai: a @ ai. a .

G a :Na i a Na a S i F a- i C i a; G a b :31171846.

Abstract

B a (BPH), *Nilaparvata lugens* (S) (H i a: D - a i a), i a a i . BPH i a i a i i a a a i i a a i a i a . T a a i i a ai a , a i i

i i ■ i i , i i■a i ■ i BPH. A i i ■ i i i■ a i MF, i a i i ■ i i i■ a i BF (J , 1963; K i i , 1956). I ab i a i a ab , BPH i BF i ■ i a ■ i a i■ a i (S b , M i i a, Ya a ■ i, Ma a i, & Ma a, 2002); ab i a ■ i i , BPH i MF i ab i i a■i i a i a i (K i i , 1956).

I a i i ■ i , MF a a a i i i i a BF a (K i i , 1965). T i i ■ i■a b ■ i a a i a a , a a i a i i a i (C , C , Ya , & Y i , 1979). BPH i i i b *Insulin Receptor 1* a *Insulin Receptor 2*

TABLE 1 Li i

GenBank accession number	Name of primer	Primer sequences	Length of PCR product
JQ793898.1	*NlTOR-F*	GATCGGCATGAGGGAGGGAGACA	6,768 b
	NlTOR-R	CGACGACGGTACACTGCGTTTGG	
	1*NlTOR*-F	TAATACGACTCACTATAGGACCAG TGAAATGCTCGTAAACA	550 b
	1*NlTOR*-R	TAATACGACTCACTATAGGCCAGGTGCAGG TAATCGTCCAG	
	2*NlTOR*-F	TAATACGACTCACTATAGGTTGACGG TCACTCACTACTGCA	492 b
	2*NlTOR*-R	TAATACGACTCACTATAGGGCTCTTTGTT TCGTCCCATACC	
U76561	*GFP*-F	TAATACGACTCACTATAGGGTAAA CGGCCACAAGTTCAG	400 b
	GFP-R	TAATACGACTCACTATAGGTCGGC CATGATATAGACGTT	
EU179846.1	PCR-*NlActin1*-F	CCAACCGTGAGAAGATGACC	256 b
	PCR-*NlActin1*-R	GATGTCACGCACGATTTCAC	
JQ793898.1	PCR-*NlTOR*-F	AACGCCATGGAGGTGACAGG	143 b
	PCR-*NlTOR*-R	ATGAGGCGCCAGTTGAGCAG	
KY827832	PCR-*NlE74B*-F	AACAACATAATAGGCACAGTC	175 b
	PCR-*NlE74B*-R	GGAATGGCGAAGAAGTATC	
FJ263049.1	PCR-*NlEcR*-F	GCCAGAAAGTACGACGTGAA	234 b
	PCR-*NlEcR*-R	TTGGATCTTCTCCACCTTCC	
19JF345255	PCR-*NlFoxA*-F	GCGGAGGTTATGTTGTGTTGTA	193 b
	PCR-*NlFoxA*-R	CTGAGCCTTGTAGCATGTTGAA	
AB353856.1	PCR-*NlVg*-F	TCTTCATCATCCTCCTCCTCTTC	173 b
	PCR-*NlVg*-R	TCCTGGTTGTTGTCATTGTCATT	

2.3 | Synthesis of dsRNA

T b - a RNA (RNA) i *Nl1TOR*. T RNA (1*NlTOR*, 550 b) a b 2,764 a 3,313 i , a RNA (2*NlTOR*, 492 b) a b 1,736 a 2,227 i . T7 a a a a 5′- - i i . T i a i DNA *NlTOR*. T i PCR a i i T7 a a b 5′- a a RNA i . QIA TM PCR i a i i (C N .: 28104, Q a- , D , G a) i PCR . RNA i i a T7 R b MAX E RNA i S (C N .: P1700, P a, Ma i) i a a ' i i . *GFP* (G Ba a - i . U76561) RNA (*GFP*) a a a i . T i i RNA a 1*NlTOR*-F/R a 2*NlTOR*-F/R (Tab 1).

2.4 | RNAi using microinjection

T i - i a (6 12) BPH RNA i i i i i a b b L i a . (2015) i a Na i 2000 i (WPI, Sa a a). A i a 300 RNA (1*NlTOR*, 2*NlTOR*, GFP) a i i a a i a a a . A a 250 a i a a i a a a (i ., i a 750 i a RNA). T a a b b L i a . (2015). T i a BPH a a i a i i . A a i , i a a RT-PCR a a i a 3, 6, 7, 9, 11 a a i i .

2.5 | Developmental duration and ovary observation

Ea BPH a i RNA a b a 6 i i a a i i i . N a i i - a . T BPH a i i i i - i i (WPI, Sa a a) a 1, 3, 5, a 8 a a a , i 1 × a -b a i (PBS) b a- i i 4% a i 1 × PBS 20 i a a . D i a i a i 10 i i 0.2% T i -X 100 (C N .: T9824, Sa a a a, S i a) i 1 × PBS. A a i , a i - a i a O (SZX16, O , T , Ja a) a P90 P D i i a Ca a (P90, N i , T , Ja a). R i i BPH i a b .

2.6 | qRT-PCR

TRI a (C N .: 15596018, I i , Ca ba) a a a RNA. *Actin1* (G Ba a i b EU179846.1) a a a i a . SYBR P i E Ta (C N .:RR420A, Ta a a, Da a , C i a) a RT-PCR a a i a ABI P i 7300 i (A i B i) a i a a ' i i . Ea a i i a i a i a i a 100 300 b PCR (Tab 1), a PCR a i a i i a i a i a a . O i i 85 110% i a a 98% a i i RT-PCR a a i . Ea RNA a a a i b i a i , a i a i i a a a a a a . T b i a a a a . T a i i a a a a i L i a a S i (2001). S i RT-PCR a i Tab 1.

2.7 | Sequence comparison and phylogenetic relationship

P ba a i a i , i , i b i b - i i i M a E i a G A a i (MEGA) a . T b a a i i 1,000 a .

2.8 | Data analysis

A a a a a i SPSS i 18. O - a ANOVA a a a i b a , a a - a ANOVA a a a i a RNA. P a a a i b a i a a a i .

3 | RESULTS

3.1 | *NlTOR* expression patterns during different developmental stages in BS and MS BPH

T a *NlTOR* a i b a a a i BPH; i a i a a i i a a . D i a a , *NlTOR* i a i i a i BS a MS a i a , b i i a i i *NlTOR* i a a a . T *NlTOR* i i BS a i i a i a i MS a a 1, 2, a 4 a a

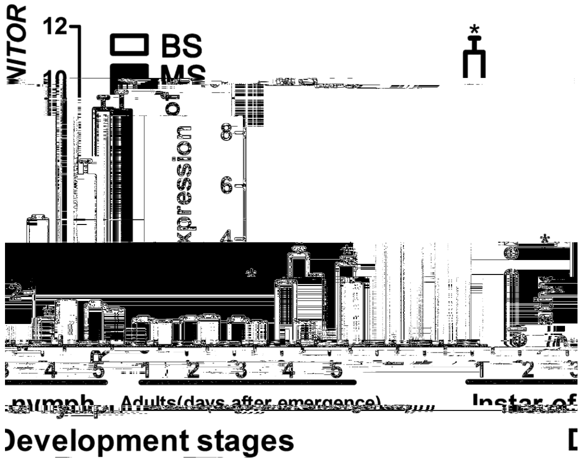

FIGURE 1 T a i i a *NITOR* a i a a MS a BS; BPH *actin1* a a a . T i a a i a i a a BS. T a a i a ba b i a a . E ba i a a a . Ba ab i a a i i i i a b BPH MS a BS, a i i - a ANOVA ($P < 0.05$)

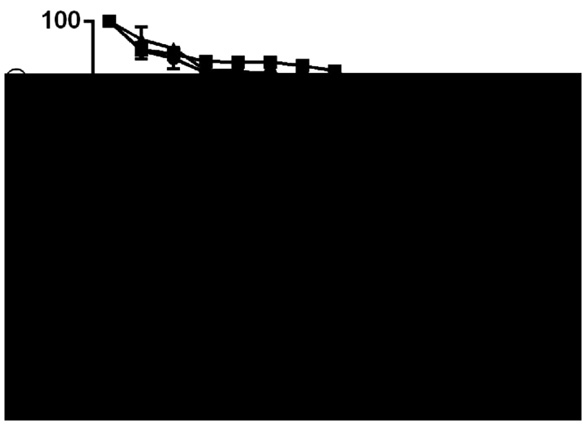

FIGURE 2 S i a a BS BPH i 1*NITOR* a 2*NITOR* i i . *GFP* i i a a . Ea i 45 . S i a *GFP*, 1*NITOR*, a 2*NITOR* a a i a - a ANOVA

, a *NITOR* i a i BS a MS 3 a a (F i 1). T *NITOR* i i i i i a a a 5 a b BS a MS (F i 1).

3.2 | Effects of knockdown of *NITOR* on the survival of BS BPH

B a i *NITOR* i BS a i i a i a i MS a a 1, 2, a 4 a a , *NITOR* a i i BS i i a *NITOR* i i a- a i BPH.

A i i RNA (1*NITOR*, 2*NITOR*, a *GFP*) i i - i a , i a a a i *NITOR* a *GFP* i i 1 7 a a i i ($F_{2,6} = 0.538$; $P = 0.609$; F i 2). I a , i a a i a i *NITOR* a i i a a a i a i *GFP* 8 15 a a i i (BS: $F_{2,6} = 12.568$; $P = 0.007$; F i 2). F 1 7 a a i i , a

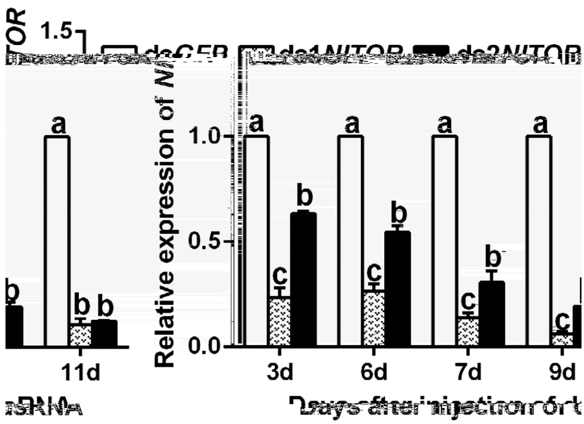

FIGURE 3 T a i i *NITOR* a i ◙ i RNA i i - i a i BS. E ba i ◙a a a . T i a a i a i a i ◙ i *GFP*. Ba ab i i i ◙a a i i ◙a i ◙ a a a a a i i - a ANOVA (*P* < 0.05). T a a i a ba b i - ◙a ◙a

BPH i a a , i 8 15 a a i ◙ i , a BPH i a a . T i i ◙a a *NITOR* a a ◙ BPH a b BPH .

3.3 | Effects of dsRNA injection on *NITOR* gene expression in BS

I BS, i ◙ i 1*NITOR* a 2*NITOR* i i - i a i i ◙a i *NITOR* RNA a a a i i . F 3 11 a a i ◙ i *NITOR*, a ◙ i *NITOR* ◙ a i i ◙a b 36.9 94.1% (F i 3) (*P* <

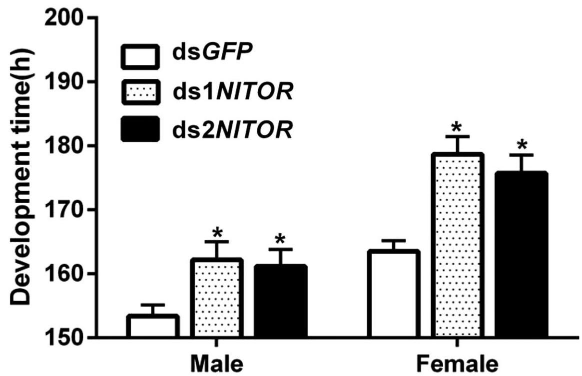

FIGURE 4 T 1*NITOR* a 2*NITOR* i i a i BS BPH i i a a . A ba i a a a a i i a . Ba ab i i i a a i i a i a a , a i i - a ANOVA (*P* < 0.05)

3.6 | Knockdown of *NITOR* inhibited ovarian development

W a a BS i a i 1*NITOR* a i a a i . H , a BS i i *GFP* a i a a a b a (ab 100 / a). T , i a i a a 1, 3, 5, a 8 a a a i a . T a a a a i b i a i a a a b 1*NITOR* i i , a i a i a a b a 3, 5, a 8 a . A i a i i a BPH a a b L (2011), a i *NITOR*- a BS a a i i a b a i i . B a , *GFP*- a a a i - a , a i a a b a 3, 5, a 8 a (F i 6). T a a i BS a i a i *NITOR* a i i a a a- ba C a . (1979).

3.7 | Effects of in vivo knockdown of *NITOR* on the expression of *NlFo A* and *NlVg* related to ovarian development

P i i a a *FoxA* a *Vg* (*Vitellogenin*) a i i a a (D a ., 2011). T i *NlFoxA* a *NlVg* i i a b RT-PCR a 1, 3, a 5 a a . A 1, 3, a 5 a a , *NlFoxA* i i a a i 1*NITOR* a b 95.9, 89.7, a 25.5%, i (F i 7A) i a i . A 1, 3, a 5 a a , *NlVg* i i a a i 1*NITOR* a i i a b 71.6, 60.8, a 51.2%, i (F i 7B), a (*P* < 0.05, - a ANOVA).

4 | DISCUSSION

TOR a i i a i a i a a , i , a a i i i a i (O a a ., 2000; Z a a ., 2000). I i , *TOR* a i i (L a ., 2016; Ma a ., 2009; Pa a ., 2006). *NITOR* a b a i i a a i BPH (L a ., 2016; Z a i a ., 2015; Z a ., 2017). D i i a i , a BPH i a a i i - a , a i , i a a (C a ., 1979). W a i a i b *NITOR* a BPH i a a i . I , a *NITOR* i

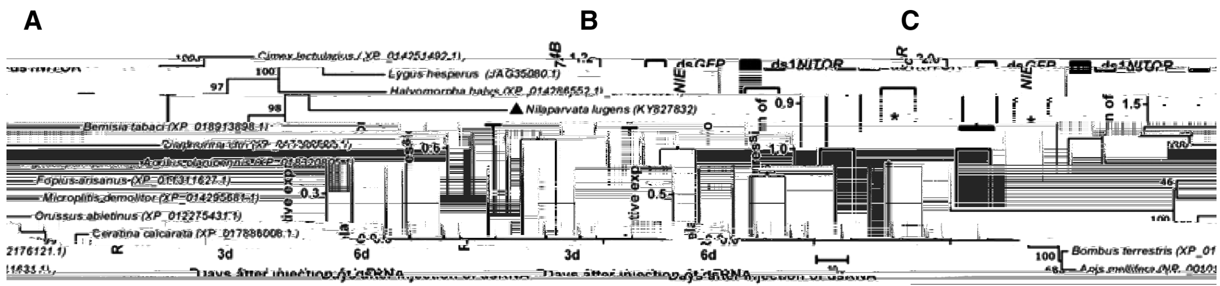

FIGURE 5 P ecdysone-induced protein 74EF isoform B (E74B) a i i i a i *NlE74B* a *NlEcR* a *1NITOR* i i i i -i a i BS. (A) T a i i b - i i (NJ) i MEGA a , b a a i i 1,000 a , a a i a i . T ba i a 10 b i i i . T G Ba a i . a E74B a i i i i a . (B a C) T i *NlE74B* a *NlEcR* a *1NITOR* i i i -i a i BS. A ba i a a a a i i a . T i a a i a i a i i i *GFP*. Ba ab i a a i i i i a b a a a , a i i - a ANOVA ($P < 0.05$)

WILEY ARCHIVES OF INSECT BIOCHEMISTRY AND PHYSIOLOGY

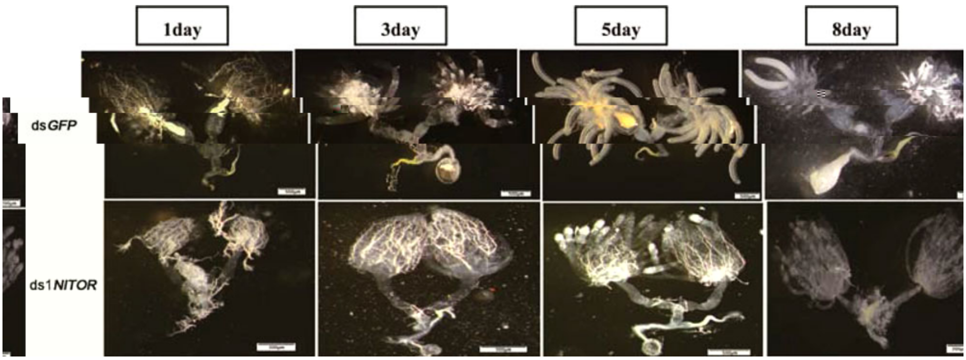

FIGURE 6 E 1*NITOR* a . 1*NITOR*- a a (300 /i) a a- a i b i i a 1, 3, 5, a 8 a a . *GFP* (300 / i) a i a a a a 1, 3, 5, a 8 a a

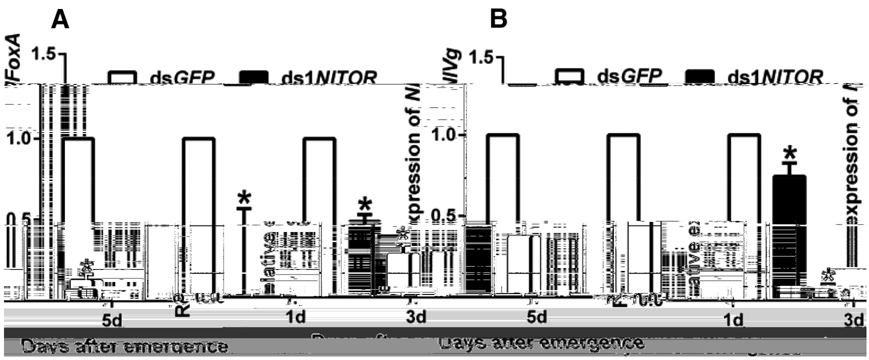

FIGURE 7 T a i i *NlFoxA* a *NlVg* a i i RNA. (A) T a i i *NlFoxA* a i i 1*NITOR* i a i i a . (B) T a i i *NlVg* a i i 1*NITOR* i a i i a . E ba i a a a . T i a a i a i a i i *GFP*. Ba ab i a a i i i i a b a a a a i i - a ANOVA ($P < 0.05$). T a a i a ba b i a a

a a a , i i i i *NITOR* b MS a BS a a a a a 1, 2, a 4 a i . I i *NITOR* i BS a a i BS BPH a i a a i a i a . T i i i a i b *NITOR* a BPH i a .

BPH i i i b *Insulin Receptor 1* a *Insulin Receptor 2* a a a (X a ., 2015), i a i a i a i a a i a i b a . T i a a a i i i a , a a a BS b i i a i . A 5 a i i , a BPH a i i i a MF (MS, ~85% MF) a a BPH a i i BF (BS, 100% BF) i ab. T a i i a- i *NITOR* i a i i a b a . T a a i i , i *NITOR* i a a a a b 73.7%. T , BS a a RNA- a b i i i a i *NITOR*. T i i i a i i a i *NITOR* i i a a a a i i a i .

T *NITOR* i i a 300 , i b a a i a a i X , X , L , Z a , Z , a Z a (2015). L i a (2015) a 300 *NlapA*; , *NlapA* a a BPH. T , b i a a i i a b i b b *NITOR* i . I a , *NITOR* a a BPH a , BPH . W i a a a *NlTOR* a a i a i a a i i i i *NlTOR* i a a i b i .

P i a a *NlTOR* a a a a i JH a i - a a (*NlJHAMT*), a a a i JH III RNA i(*NlTOR*) a a a a RNA i, i a i a TOR a a i JH b i i a AA - a V i i *N. lugens* (L a ., 2016). O a *NlTOR* a i i a a . *NlTOR* - a i i a a i *NlE74B* a *NlEcR*. T a i i a TOR a a , JH b i i , a i a a a b i .

I , *NlTOR* a i *NlFoxA* a *NlVg*. T i a - a i Z a i a . (2015). W a i a TOR i a i i i BPH i , a i i a i i a a i i a a b a i i *NlFoxA* a *NlVg*.

ACKNOWLEDGMENTS

T i a b a a Na i a Na a S i F a i C i a (N . 31171846).

LITERATURE CITED

C , R., C , X., Ya , L., & Y i , X. (1979). B a a a a i a i i i . *Acta Entomologica Sinica*, *22*, 280 288. (I C i).

D , X., Z a , Y., Z a , J., S , Z., C , J., C , J., & Z a , W. (2011). F a a i i a i i a a a i b a , *Nilaparvata lugens* (S). *BMC Molecular Biology*, *12*, 53.

J , S. (1963). A a i i a a i i a i - i b a , N i a a a a . *Japanese Journal of Applied Entomology and Zoology*, *7.1*, 45 48.

K i i , R. (1956). E i i a a i i a i i - a a a - . *Nature*, *178*(4534), 641 642.

K i i , R. (1965). S i i a i a i i a i b a , *Nilaparvata lugens* (S a). *Bulletin of the Shikoku National Agricultural Experiment Station*, *13*, 101 106.

L i , F., L i, K., L i, J., H , D., Z a , J., H , Y., … H a, H. (2015). *Apterous A* a i i , b i a i a a i i *Nilaparvata lugens*. *Scientific Reports*, *5*, 10526.

L i a , J. K., & S i , T. D. (2001). A a i a i i a a i a - i a i a i PCR a $2^{-\Delta\Delta C}$, *Methods*, *25*, 402 408.

L , F. (2011). T a a a i i a a a i b a . *Chinese Journal of Applied Entomology*, *48*, 1394 1400.

L , K., C , X., L i , W., & Z , Q. (2016). TOR a a - a i i a i - a i i *Nilaparvata lugens* (S). *International Journal of Molecular Sciences*, *17*(4), 438.

Ma a , S. A. P., Ra a a, D., H a a a, P. V., & S a a a , S. G. J. N. (2012). E a a i b a *Nilaparvata lugens* (S a) i a i *Oryza nivara* i a i i S i La a. *Proceedings of International Forestry & Environment Symposium*, *15*, 172 175.

Ma , J. L., C b , J., & B , X. (2009). *Target of rapamycin* (TOR) a a i i i a i a i - i i . *Journal of Biological Chemistry*, *284*, 5506 5513.

M a, S., & T , S. (1992). Ma i a a i a i b i i i i a b i i i i i b a - , *Nilaparvata lugens* (H a: D a i a). *Applied Entomology and Zoology*, *27*, 445 445.

O a , S., M a , J., Ra i , T., T a , G., & Ha , E. (2000). G a b i a a a i a i TOR, *Drosophila* *target of rapamycin*. *Genes & Development*, *14*, 2689 2694.

Pa , J. H., A a , G. M., Ha , I. A., & Ra i , A. S. (2006). GATA a a a i i a a i a i

S b , S., M i i a, H., Ya a , J., Ma a , M., & Ma a, M. (2002). F a i a a a i i - a i b a , *Nilaparvata lugens* S a i . *Japanese Journal of Applied Entomology and Zoology*, *46*(3), 135 143.

T , C. S. (2002). E - a 2000. *Insect Biochemistry and Molecular Biology*, *32*(2), 113 120.

X , H., X , J., L , B., Z a , X., Z , J., H , S., … Z a , C. (2015). T i i i a a i i i a . *Nature*, *519*, 464 467.

Y , R., X , X., L a , Y., T a , H., Pa , Z., J i , S., … Z a , W. (2014). T i i a a a RNA i-ba . *International Journal of Biological Sciences*, *10*(10), 1171 1180.

Z a , Y., S , Z., Z a , J., Ka , K., C , J., & Z a , W. (2015). A i a i TOR i a i a a b a i a i i . *Scientific Reports*, *5*, 10694.

Z a , H., S a , J. P., N , J. C., R i a , C., & N , T. P. (2000). R a i a b *Drosophila* a a a i TOR. *Genes & Development*, *14*, 2712 2724.

Z , J., X , J., L , B., H a , H., X , H., & Z a , C. (2017). E RNA i a *NITOR* i i a N ia a a a . *Journal of Insect Physiology*, *98*, 149 159.

How to cite this article: L i F, L i K, Ca i W, Z a J, Z Y, H a H . K *TOR* a i a a a a i a a ab b a a i *Nilaparvata lugens*. *Arch Insect Biochem Physiol*. 2017;95: 21400. :// i /10.1002/a .21400