

## Supplementary Section

### **Alternative splicing contributes to the coordinated regulation of ferritin subunit levels in**

#### ***Bactrocera dorsalis* (Hendel)**

Xuan-Zhao Jiang, Lin Cong, Jin-Zhi Niu, Wei Dou & Jin-Jun Wang\*

Key Laboratory of Entomology and Pest Control Engineering, College of Plant Protection,  
Southwest University, Chongqing 400715, P. R. China

**Correspondence:** Dr. Jin-Jun Wang, College of Plant Protection, Southwest University,  
Chongqing 400715, P. R. China. Tel: +86-23-68250255; Fax: +86-23-68251269. E-mail:  
[wangjinjun@swu.edu.cn](mailto:wangjinjun@swu.edu.cn) or [jjwang7008@yahoo.com](mailto:jjwang7008@yahoo.com).

Supplementary figures and figure legends

Poly (A) tail ▼

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\* L Q K D F L F E G L A

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I L Q I L D T V S

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NF-kappaB

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NF-kappaB

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T M Q

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A N V A V T L L L V A A L L K M M

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C/EBP Pit-1a HNF-1 C/EBP GR H-TSS1 Oct-1 Sp1 TBP  
**AACTAACTTAAATCTTAAACTGTTAAAGAAGTTTGCTGACGAGCTAGACTAGACTAAGCGTTGGCGGAGA****CACGGAAA**

**Exon 3**  
**TATGTTGACCATATCTTCCGTTT**GC AACCGGAACTACTCAATTACGCAATCATATAGCGGACTATTATCTCAAGAT

**Iron responsive element**  
CGCAATCATATAGCGGACTATTATCTCAAGATCTGTGTTACCAGCAT**GT** TGAATCATTAGTGTTTAACTAACTTAATA

TGTTTAACTAACTTAACTTATAAACTGAACTTTCCTTTTAGAAAAAC**CGCCGCGACAGCATTTT**GAGAGAAAACAACA

H-TSS2  
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Oct-1 AP-1 Sp1 TBP C/EBP GATA-1  
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CGCGATCGCTCATGAACGTGTGCAACCCAGAAAGCGGTGAG**TG**AACATACATGCATACACAAAAGCAATAGCAAATA

**BR-C Z4**  
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**BR-C Z4**  
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**BR-C Z4** **BR-C Z3**  
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**BR-C Z1** **BR-C Z4**  
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C/EBP ICSBP Oct-1 L-TSS1 TBP GATA-1 HNF-3

BR-C Z3

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BR-C Z3

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BR-C Z4 BR-C Z1

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M K P F

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T A L T L F A G I I A L V S A D N Y A C H S N V T V

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G C S N S A S Exon C

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NF-kappaB

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T P V V C H S R Y G G I E N V E S T I Q S Y I N L N

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L H K S Y Q Y L L L S S F Y N S Y Q K N R P G F N K

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L Y R D L S D R S F D D A I D L I K H V T R R G G Q

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S G F V N D L S K I M N Q P D P S L G I Y L F D Q Y

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L E K Q \*

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▼ Poly (A) tail: AAAAAAAAAAAAAAAAAAAAAAAAAAAAAA

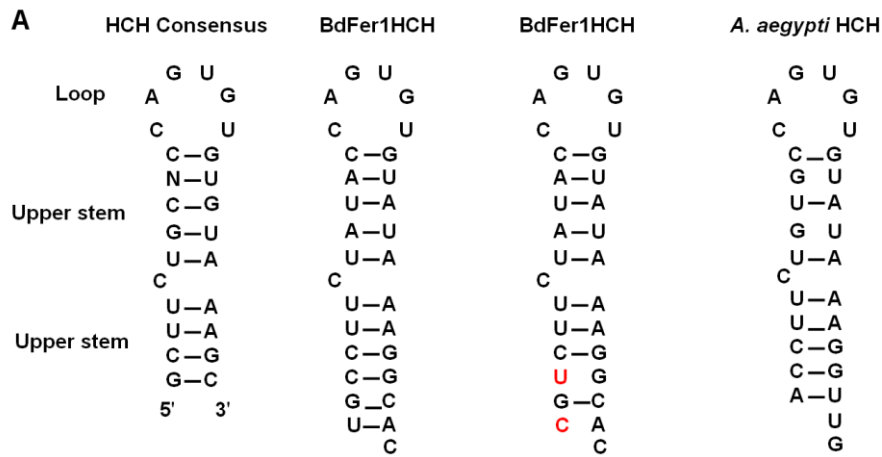
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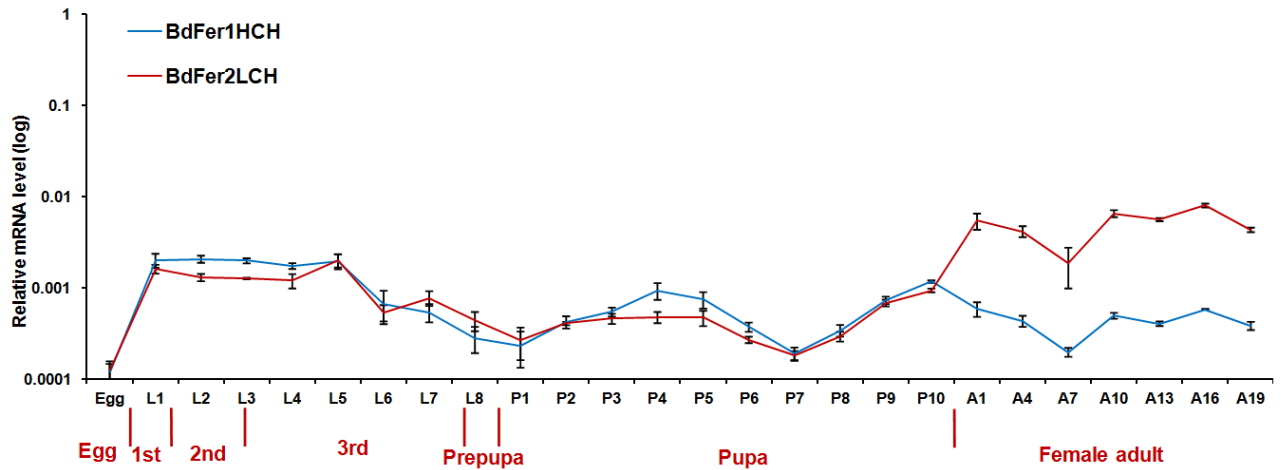
**Supplementary Figure 1: Genomic DNA sequences and TFBSs analysis of *BdFer1HCH* and *BdFer2LCH*.** The exons, untranslated regions and sequences between the transcription start sites (TSS) of *BdFer1HCH* and *BdFer2LCH* are shown as uppercase letters. Introns are shown as lowercase letters. For the coding regions, the encoded amino acids are denoted below the corresponding nucleotides. The terminal codon is represented using an asterisk. The amino acids implicated in ferroxidase center are shaded in red. The putative N-Glycosylation sites are underlined with red lines. Alternatively spliced exons are shaded in gray. The iron responsive element (IRE) is highlighted in black and the consensus hexanucleotide loop (5'-CAGUGU-3') is bolded. The splicing consensus sequences gt-ag are in bold. Sequences between the TSSs are shown as double strands. Broken arrows indicate the transcription direction. H-TSS1 and H-TSS2 in *BdFer1HCH* results from alternative promoters. The canonical polyadenylation signals and those identified in this study are underlined; those uncanonical are double underlined. The amino acids in the putative cleaved signal peptide are boxed. The poly (A) sites are indicated with "▼" and the added poly (A) tails are also shown. The putative transcription factor binding sites (TFBSs) are shown on the sense strands, underlined and denoted with the corresponding names.



**B**

Species	Gene names	Subunit type	Ferroxidase center	Signal peptide	N-Glycosylation site	IRE	Number of introns
	<i>BdFer1HCH</i>	HCH	+	+	+	+	3
<i>B. dorsalis</i>	<i>BdFer2LCH</i>	LCH	-	+	+	-	1

**Supplementary Figure 2: The structure of iron responsive elements and features of ferritin of *Bactrocera dorsalis*.** (A) Comparison of the iron responsive elements (IREs) of *B. dorsalis* and *A. aegypti* with the consensus IRE motif. The potential RNA editing sites are shown in red. (B) Features of *B. dorsalis* ferritins.



**Supplementary Figure 3: Developmental stage expression profiling of *BdFer1HCH* and *BdFer2LCH*.** RT-qPCR analysis was performed on the relative mRNA levels at four developmental stages including one sample of eggs, eight samples of 1–8-day-old larvae, ten samples of 1–10-day-old pupae, as well as seven samples of 1–19-day-old female adults at 3-day intervals. Results are represented as mean  $2^{-\Delta Ct}$  values of three independent biological replicates  $\pm$  SEM. 1st, first instar larvae; 2nd, second instar larvae; 3rd, third instar larvae.

## Supplementary tables

### Supplementary Table 1 Primers used in cDNA full-length cloning and genomic DNA sequence cloning

Primer names	Primer sequence (5'→3')
<b>Primers used in RACE</b>	
BdFer1HCH-3'RACE	TACTCCGCACAGAAATACAGTAAG
BdFer1HCH-5'RACE(1)	TCAGCGAAACCGGGACGATTGAC
BdFer1HCH-5'RACE(2)	CTCTTTCTATGGCGTGTCTC
BdFer2LCH-3'RACE	TGACCCACAGACCGCTCACTACAT
BdFer2LCH-5'RACE	AACCGGGACGATTCTTCTGGTATG
NUP	AAGCAGTGGT AACAAACGCAGAGT
<b>Primers used to amplify ORF</b>	
BdFer1HCH-F	ACAGAGGCGGTTGCGAATCAG
BdFer1HCH-R	TGAATCGCAGTAGCCACATG
BdFer2LCH-F	GGGTCATTGAAACACATCTG
BdFer2LCH-R	TTCTCGCACCAAGTATCACA
<b>Primers used to amplify genomic DNA</b>	
a-F	CAAGGCTTACTGTATTTCTGTGC
a-R	GATCAATGCCTCCAACATCTAC
b-F	AAGAAGTGCTCAGCGAAACC
b-R	CGAAGGGTAGTAGAGGCAGG
c- F	CCGCTCTTGTCATTGAGG
c- R	GCATTGGAGTCGTGTGTATG
d-f	CAATATGTCGGCCTCGTATGATTC
d-r	AACCGGGACGATTCTTCTGGTATG
e-F	AATTAAAGCCAAAGTTATGAA
e-R	CAAACAAATTTAGTTACATCGT
f-F	CCGCTCTTGTCATTGAGG
f-R	TTCTCGCACCAAGTATCACA
g-F	TGTCATTTGAGCTCCAATTG
g-R	GGGTCATTGAAACACATCTG

F: forward; R: reverse



**Supplementary Table 2 Primers used in identifying multiple transcripts and RT-PCR analysis**

Primer names	Primer sequence (5'→3')	Products
<b>Primers used in RT-PCR for <i>BdFer1HCH</i></b>		
HCH-F	GATCAATGCCTCCAACATCTAC	BdFer1HCH
HCH-R	AGTGTGCTCAATTTGCCAGC	
H-TSS2-F	CAACAAAGAGAGTTTTACGACAGC	H2
H-TSS2-R	AACTGCGGCTAATAATTTTCATC	
H-TSS3-F	GCATTGGAGTCGTGTGTATGG	H3
H-TSS3-R	CTCTTTCTATGGCGTGTCTCTAC	
<b>Primers used in RT-PCR for <i>BdFer2LCH</i></b>		
LCH-F	AATTAAGCCAAAGTTATGAA	BdFer2LCH
LCH-R	CAAACAAATTTAGTTACATCGT	
LCH'-F	AATTAAGCCAAAGTTATGAA	BdFer2LCH'
LCH'-R	AGTTGGGGTGCTGACTGATG	
L-TSS2-F	GACCAGACACAGGCATTATAAGC	L2
L-TSS2-R	AGGACTTGTGCAGATTCAAATTG	
L-TSS2'-F	GACCAGACACAGGCATTATAAGC	L2'
L-TSS2'-R	AGTTGGGGTGCTGACTGATG	

F: forward; R: reverse.

**Supplementary Table 3 Primers used in RT-qPCR**

Primer names	Primer sequence (5'→3')
BdFer1HCHq-F	CTGCGAGAAGACACCCAAC
BdFer1HCHq-R	AGTGTGCTCAATTTGCCAGC
BdFer2LCHq-F	CGGACCGCTCTTTCGATGAT
BdFer2LCHq-R	AGTTGGGGTGCTGACTGATG
Diptericinq-F	CAGTTCCTTGCTTATCCCAGAGA
Diptericinq-F	GCCCAAATGTTGCGAATAC
EF1 $\alpha$ -F	CGTTGGTGTCAACAAGATGG
EF1 $\alpha$ -R	TGCCTTCAGCATTACCTTCC
18S-F	GCGAGAGGTGAAATTCTTGG
18S-R	CGGGTAAGCGACTGAGAGAG
SD-F	CAAGTGCAAAGGTGTGTTGG
SD-R	GTCGCAATGACGGTGTTATG
$\alpha$ Tub-F	CGCATTTCATGGTTGATAACG
$\alpha$ Tub-R	GGGCACCAAGTTAGTCTGGA
$\beta$ TUB-F	TTACATTTCTTTATGCCTGGTTTC
$\beta$ TUB-R	CATTTGTTTCGTCCACTTCCTTC
RPE-F	GTTACGGCGAGGACAGTTTG
RPE-R	CACGCAATGCTTCACGACC
ACT-F	GTGTGATGGTTGGTATGGGA
ACT-R	GGCTGGGGAGTTGAAGGTTT
RPL13-F	CAGTTGTACGTTGCGAGGAATT
RPL13-R	TCTTGATGGAGCACGGGAG

F: forward; R: reverse

**Supplementary Table 4 Stability estimation of candidate reference genes**

Ranking order	Gene names	Geomean of ranking values in whole larvae after iron overload	Gene names	Geomean of ranking values in midgut after iron overload	Gene names	Geomean of ranking values in <i>E. coli</i> challenged larvae
1	<i><math>\alpha</math>-tubulin</i>	1.57	<i><math>\alpha</math>-tubulin</i>	1.41	<i><math>\alpha</math>-tubulin</i>	1.50
2	<i>18S ribosomal RNA</i>	1.73	<i>actin 2</i>	1.86	<i>ribosomal protein L13</i>	2.21
3	<i>actin 2</i>	2.00	<i>ribosomal protein L13</i>	2.45	<i>actin 2</i>	3.46
4	<i>ribosomal protein L13</i>	4.23	<i>elongation factor-1a</i>	3.94	<i>18S ribosomal RNA</i>	3.98
5	<i>succinate dehydrogenase</i>	4.61	<i><math>\beta</math>-tubulin</i>	4.16	<i>succinate dehydrogenase</i>	4.14
6	<i>elongation factor-1a</i>	5.73	<i>18S ribosomal RNA</i>	5.73	<i>elongation factor-1a</i>	4.16
7	<i>RNA polymerase II</i>	7.00	<i>RNA polymerase II</i>	7.24	<i>RNA polymerase II</i>	5.38
8	<i><math>\beta</math>-tubulin</i>	8.00	<i>succinate dehydrogenase</i>	7.74	<i><math>\beta</math>-tubulin</i>	8.00

Figure 3

Figure 3A

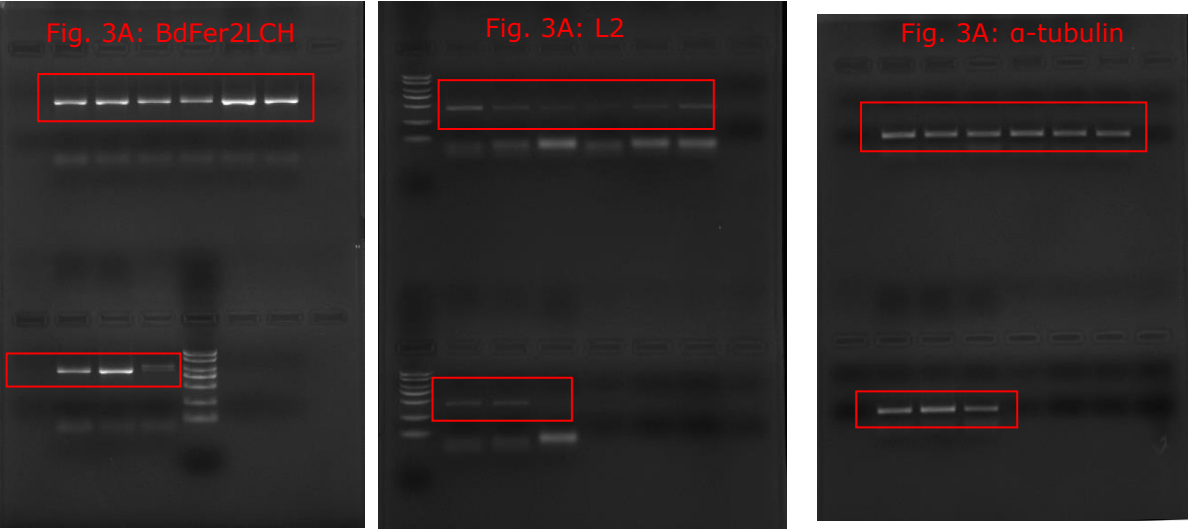


Figure 3B

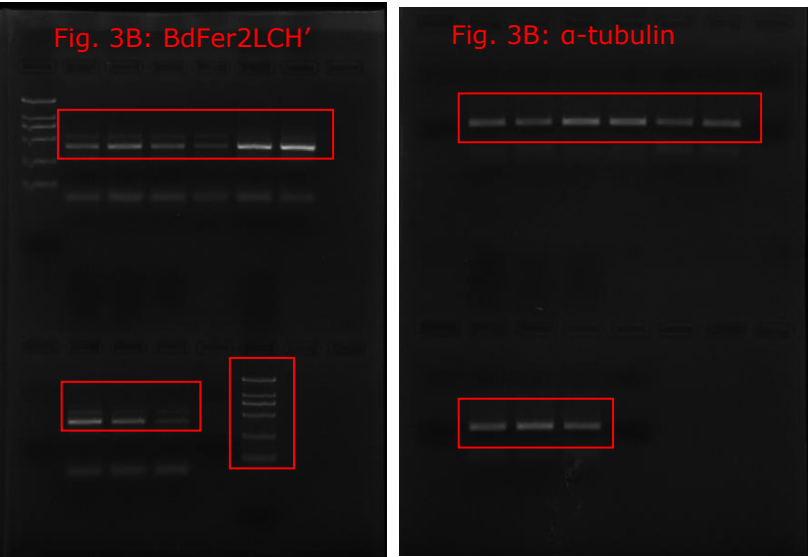


Figure 3C

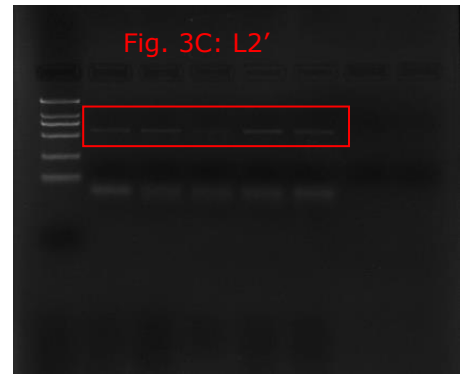


Figure 5

Figure 5C

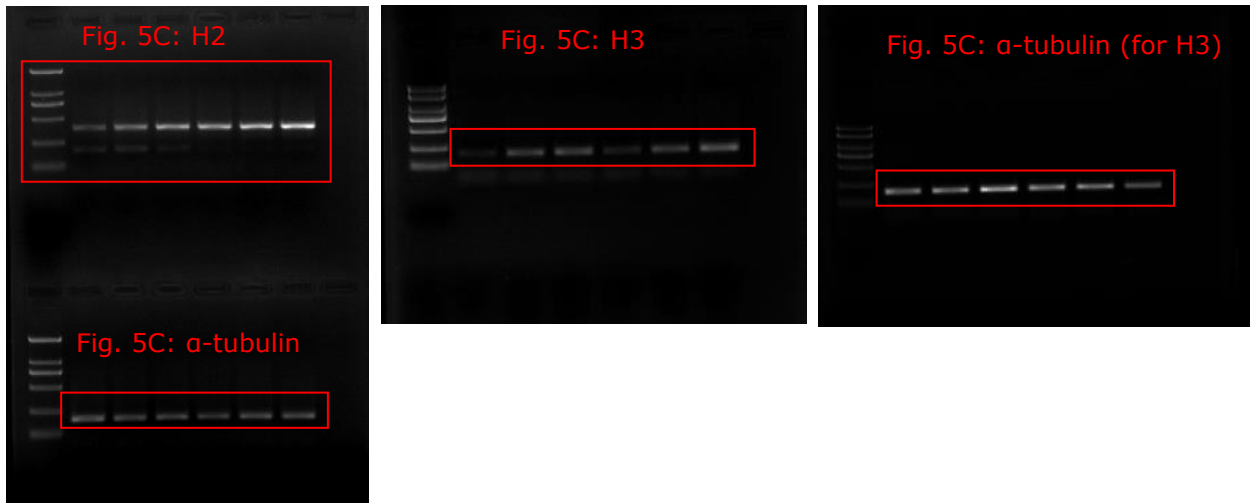


Figure 5D

