(A)

```
  1 ATGTTTTGGTCGATGTTATGCTTCAACAGTGATTGAACGAGCATCGCCAAACTGCTTTCC
 61 TCTCTCCCCCAAATCTTTCTCACTGCATATTTCTTGAAGAAAGTACAGTTGGACGTTCCG
121 TTCCGGAAATAACCGACGAAAATGGCCGCTACTCAACCCCGCCAAAACTACCACATCGAT        13
                      M  A  A  T  Q  P  R  Q  N  Y  H  I  D
181 TGCGAAGCCCGCATCAACAAGCAGATCAACATGGAACTGTACGCGAGCTACGTCTACACC
      C  E  A  R  I  N  K  Q  I  N  M  E  L  Y  A  S  Y  V  Y  T        33
241 TCAATGGCCTATTACTTTGACCGTGACGACGTGGCCCTGCCGGGCTTCCACAAGTTCTTC
      S  M  A  Y  Y  F  D  R  D  D  V  A  L  P  G  F  H  K  F  F        53
301 AAGAAGAGCAGCGAGGAGGAGCGTGAGCACGCTGCCAAGCTGATGAAGTACCAGAACATG
      K  K  S  S  E  E  R  E  H  A  A  K  L  M  K  Y  Q  N  M           73
361 CGCGGGGGCCGCGTGGTGCTGCAGCCCATCCAGAAGCCCGCCCAGGACGAGTGGGGCTCG
      R  G  G  R  V  V  L  Q  P  I  Q  K  P  A  Q  D  E  W  G  S        93
421 GGCCTGGACGCCATGCAGGCCTCCCTGGAGCTCGAGAAGTCCGTCAACCAGTCGCTGCTG
      G  L  D  A  M  Q  A  S  L  E  L  E  K  S  V  N  Q  S  L  L       113
481 GACTTGCACAAGCTGGCCACCGACCACAACGATGCCCAGCTGTGCGACTTCCTGGAGAGC
      D  L  H  K  L  A  T  D  H  N  D  A  Q  L  C  D  F  L  E  S       133
541 GAGTACTTGGAGGAGCAGGTGAAGGCCATCAAGGAGCTGTCCGACTACGTGACCAACCTG
      E  Y  L  E  E  Q  V  K  A  I  K  E  L  S  D  Y  V  T  N  L       153
601 AAGCGTGTGGGGCCCGGCCTGGGCGAGTACATGTTCGACAAGGAGACCCTCGGAGGAGAC
      K  R  V  G  P  G  L  G  E  Y  M  F  D  K  E  T  L  G  G  D       173
661 GACTGAGGAGGAGGAGGAGGTGGAGGCGCCTGTTTTGTTGGATGCAAGGCCCGCACTCGT
      D  *                                                            174
721 TGTGTGCTATCCTGTAATTGGCCCCCCCATCCGACTCCACCACCCGAGAACAATAAAAAT
781 ATAGCTGAAGAGGGAAAAAAAAAAAAAAAAAAAAA
```

(B)

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   1 ACTCGAGCCCAGTGGTCCGCCGGCGCTCTGACCAGCAGCACCATGCTCCCGATCCTGATCTTCGCGTTTGCGCTGCTCTGCGTCGCCTCC
                                         M  L  P  I  L  I  F  A  F  A  L  L  C  V  A  S        16
  91 GCAGGGAACAACCTGAACGAGCAAGTGAACCAGAACAAGTATTTCCTGCACGACGATCGCGGCTTGGGCTGCAAGACGAGATCAACGCG
      A  G  N  N  L  N  E  Q  V  N  Q  N  K  Y  F  L  H  D  R  C  R  L  G  L  Q  E  Q  I  N  A    46
 181 GAGCTTCACGCAAGCTTGGTCTACATGCAGATGGCCGCCTACCTGGGCAACAACAAGGTGGCCCGTGCGGGCTTCGCTCGCTTCTTCAGC
      E  L  H  A  S  L  V  Y  M  Q  M  A  A  Y  L  G  N  N  K  V  A  R  A  G  F  A  R  F  F  S    76
 271 GACCAGTCAAGTGAGGAGCGCGAGGCACGCCCAGAAGCTGGTGGACTACGTGAACCTTCGCGGCGGCACCGTGAGCAACGTGAATGTGGAC
      D  Q  S  S  E  E  R  E  H  A  Q  K  L  V  D  Y  V  N  L  R  G  G  T  V  S  N  V  N  V  D   106
 361 ATGCCGGCGACGGCGACCTGGATGAGCGTGCTGGACACGCTGCAGGCTGCCCTGGCCCTCGAGCACCAGGTCACCAACCGGCTGCACGGA
      M  P  A  T  A  T  W  M  S  V  L  D  T  L  Q  A  A  L  A  L  E  H  Q  V  T  N  R  L  H  G   136
 451 CTGCACGGCGCTGGCCGCCGACCACTGCCGAGATCCCCAGATGACCGACTTCCTGGAGCAGGAGTTCCTGGCTGAGCAGATGCACTCGATC
      L  H  A  L  A  A  D  H  C  R  D  P  Q  M  T  D  F  L  E  Q  E  F  L  A  E  Q  M  H  S  I   166
 541 GACAAGCTGCAGCGGCTCATCACGCAGCTGCAGAACATGGACACGCGGGCTCGGCGAGTTCCTCTTGGATCGGGAGCTGCGTCGCGAAGAC
      D  K  L  Q  R  L  I  T  Q  L  Q  N  M  D  T  G  L  G  E  F  L  L  D  R  E  L  R  R  E  D   196
 631 AGCGACAAATAAACCGCAGGACTCTCGCTGCGAGCGGCTCTGCGCGGCGCGGCGTCCTTCTGCACTTTCTGGGGGGGTGTGAGGCAAAAATG
      S  D  K  *                                                                              199
 721 CCACCCTCACCGCTTCGACACACAACCAAGCAATCAATGAACAGTCTCCATCCCAAATGCTGCATGTTTGCGGGCCGCACAATGTTCCACG
 811 CAACTTGTTCCAAAGAATACCATTTCAATTATTATATGCCCAACCTCCTCCTGTCACACGAGCACTTTCAATCCGGATTTGAGAGATAGA
 901 GATAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGA
 991 GAGAGAGAGAGAGAGAGAGAGAGAGAAACTATAT  TTAGAACCAAAGGAGGTTCAGGCAACCCAGTTAAAGGTCGTCTTCAGTGAATGCTGAACT
1081 CAGCTCTAGTGCGGGTTTTGATGTATTGCACTAGATCTAGCTGCTCCTCGAAAGTGGAGGTGAAAGTCAGCCAGGTTGGGTCGACGGGATA
1171 TGGCAGCTGCGTCGGTGTGTTGAAGTGAGAAATGCTGGGAATAGTGTAMGGACATTCCCACAGGACATGTTGCTGATTAGGATATCCACC
1261 ACAGTGTTTGCAGGCTGGTGGTTCTTGTGCTCTTCCTTTAGAGAAAAGATATCGTCTAGCTGGGGTGCATGTCAGGTTAGTCTGTGCTCG
1351 TCTCAGTAATACTGATTGCTTGATTTCTGCCTGTCTAGGTTAGGGTGAGGTGGAGGTAATTCTCTTCGGTTCAGTTTAAGGTCTCTTAGT
1441 GTATTGTTCATGATGCCACGACAATTGGACCTCTCCAGGTGTTCGGTGTCCGGATTCTTAACTCACGGGCGAGCGTGCTTGCCCGAACGT
1531 TGCCAAACATCTCCGTGTGGCCATTTGTCCAAATAATATCGTAGTGATGATGACAGTGACGATTAAATAAACTCGTGCTGTATCTTTAAAAA
1621 AAAAAAAAAAAAA
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Fig. S1. Complete nucleotide and amino acid sequences of *Haemaphysalis longicornis* ferritin1 (A) and ferritin2 (B). The start and stop codons are underlined by dotted lines. The amino acid residues within the ferroxidase center loop are enclosed in boxes. The 3′ UTR ends with a 20-bp polyadenylation tail after the putative polyadenylation signal, AATAAA (double-underlined). For A, the putative iron-responsive element (IRE) in the 5′ UTR is underlined, and the predicted glycosylation site is enclosed in a gray-shaded box. For B, the signal peptide is enclosed in a dashed box.

Multiple sequence alignment (three blocks):

Block 1 (residues 1–~80):

```
H. longicornis FER1   1                  MAA?------------QPRQNYH?DCEA?INKQIN?E?YASYVY?SMA?YYFDRDDVALPGF?HKF?K?S?   58
H. longicornis FER2   1  M?LP-----ILIFAF?A??LCV?SAGNNLN?-----QVN?NKYFL?DRCRLGLQ?QIN??LHAS?VYM??AA?LGNNK?VARAG?RARF?SDQSS   80
I. ricinus FER1       1                  MAA?------------QPRQNYH?DCEA?INKQIN?E?YASYVY?SMA?CYFDRDDVALPGF?HKF?KCS?   58
I. ricinus FER2       1  M?-----QFVVIL?A??IGA?TSGNNLF?-----NLD--KYPLQDEC?AALQEF?NVE?HAS?VYM??AAH?TNNK?VARK?GF?ST?FBAENSK   77
R. sanguineus         1                  MAA?------------QPRQNYH?DCEA?INKQIN?E?YASYVY?SMA?YYFDRDDVALPGF?HKF?K?S?   58
O. moubata            1                  MAN?------------QPRQNFL?DCEA?INKQIN?E?YASYVY?SMA?YYFDRDDVALPGF?HKF?K?S?   58
A. aegypti            1  M?KSVFFC?VVAITV?A?ILSIYQETAQAQ?QTVGATDNYQWDSVDDC?LAALHP?QINKF?DAS?I?YLK??AAYB?QEK?NLPGF?EKF?FHAAA   90
D. melanogaster       1  M?K?----LIASLLL?AVV?QAYGDF?CSLAVPE?TKDW?VDM?DACIKGMRN?QIQEF?NASY?QYLA??GAY?SRD?T?NR?P?GBAEH?F?KAAK   85
H. sapiens            1                  MTT?S??S------------QVR?NYH?D?SEA?INKQIN?E?YASYVY?SMS?YYFDRDDVA?KN?ARY?LHQS?   61
```

Block 2 (residues ~59–~174):

```
H. longicornis FER1   59  EEREHA?KLM?YQNMRG----GRV??QPF?Q--KF?AQD--EWGSGLD?QAS?LELEK?VNQSLLD?LHKLA?DHN--?AQLCDFLE?SEYLE?   138
H. longicornis FER2   81  EEREHA?CKLVDYVN?RG----G?VSNVNVD--M?ATA--TN??SVLDTIQAA?A?LEH?VTNR?HGLF?LA?DHCR-?P?Q?TDFLE?QEF??A?   161
I. ricinus FER1       59  EE?EHA?KLM?AYQNKRG----GRV??QPF?A--KF?AQD--EWGSGLE?QQAA?LELEK?VNQSLLE?LHKLAT?EKD--?GQLCDFLE?GNY??N?   138
I. ricinus FER2       78  EEREHA??RIIDYINK?G----STV??SV??--MELIT--TW??SVLC?A?LRDA?ISLEN?RVTNR?HAV??KIA?DEECK-?P?Q?MD?F??E?FLE?   158
R. sanguineus         59  EEREHA?KLM?KYQNMRG----GRV??QPF?Q--KF?SQD--EWGAGLD?QAA?LELEK?VNQSLLD?LHKLA?DHN--?AQLCDFLE?SEYLE?   138
O. moubata            59  EEREHA?KLM?KYQNMRG----GRV??QPF?Q--KF?SQD--EWGSGLD?QAA?LELEK?VNQSLLD?LFRIA?DHN--?AQLCDFLE?SEY?F?   138
A. aegypti            91  EEREH?GIK?LIEYAL?MRGKAPVDKHFFKLNYD?HEV??TVT-----?T?E?SA?L?ETA?QKEVE?VTK?SIRGV??A?CE?DG-SNDFH?D?Y?L?TGF?YH?DE   174
D. melanogaster       86  EEREH?GSK?LVEYLSMRGQLTE?GVSD??IN---?VF?TVA?K?QEWTI?GA?AA?LSDA?IDLP?IK??TKS?IRK?I?IQTCENKPY??HYH??VD?YL?TGVYL?E?   171
H. sapiens            62  EEREHA?KLM?KLDQN?RG----GRI?F?QDI?--KF?KDCD?--DWES?GLN?AME?CA?IH?LEKN?VNQSLLD?LHKLA??DKN--?P?HLCDF?I?E?THY?N?   141
```

Block 3 (residues ~139–209):

```
H. longicornis FER1  139  QVKA?IKELSDYV?NLKRVG-P--GLGEYMFD?KETL?GG--DD   174
H. longicornis FER2  162  Q?MHS?DF?QRLI?Q?QNMD---T?LGE?FLLD?R?LRREDSDK   199
I. ricinus FER1      139  QVEA?IKELS?YV?NLKRVG-P--GLGEYMFD?KETL?S---G   172
I. ricinus FER2      159  QVNS?DF?QRMI?V?SNMD---S?T?GEY?LLD?R?LGDKKEF   196
R. sanguineus        139  QVKA?IKELSDYV?NLKRVG-P--GLGEYMFD?KETL?S---D   172
O. moubata           139  QVEA?IKELSDYV?QLKRVG-P--GLGEYMFD?KETL?S---D   172
A. aegypti           175  QHKGQR?E?AEK?AT?L?KMKKSAPF?GEF?I?FDKNH?M?   209
D. melanogaster      172  QLHGQR?E?AGKL?TL?KMMDTNGE?GEF?I?FDKTL   205
H. sapiens           142  QVKA?IKELGDHV?N?RKMGAPES?GLAE?Y?FDKH?TLGDS-DNES   183
```

|	H/FER1		H/FER2	
	Id.	Sm.	Id.	Sm.
H. longicornis FER1			40	14
H. longicornis FER2	40	14		
I. ricinus FER1	84	5	36	16
I. ricinus FER2	36	15	56	20
R. sanguineus	94	2	38	16
O. moubata	87	5	37	31
A. aegypti	28	19	29	22
D. melanogaster	30	16	31	38
H. sapiens	66	12	34	21

Fig. S2. Multiple alignment of the amino acid sequences of *H. longicornis* ferritins with other tick ferritins, human ferritin, and selected invertebrate ferritins. Identical residues are shaded with black, while similar residues are shaded with gray. The percent identity (Id.) and similarity (Sm.) were calculated and are summarized in a table. The GenBank accession numbers for the ferritin sequences are as follows: *H. longicornis* FER1, AY277905; *H. longicornis* FER2, AB734098; *Ixodes ricinus* FER1, AF068224.1; *I. ricinus* FER2, EU885951.1; *Rhipicephalus sanguineus*, AY277907.1; *Ornithodoros moubata*, AF068225.2; *Aedes aegypti*, XM_001654469.1; *Drosophila melanogaster*, AF145125.1; and *Homo sapiens*, NM_002032.2.
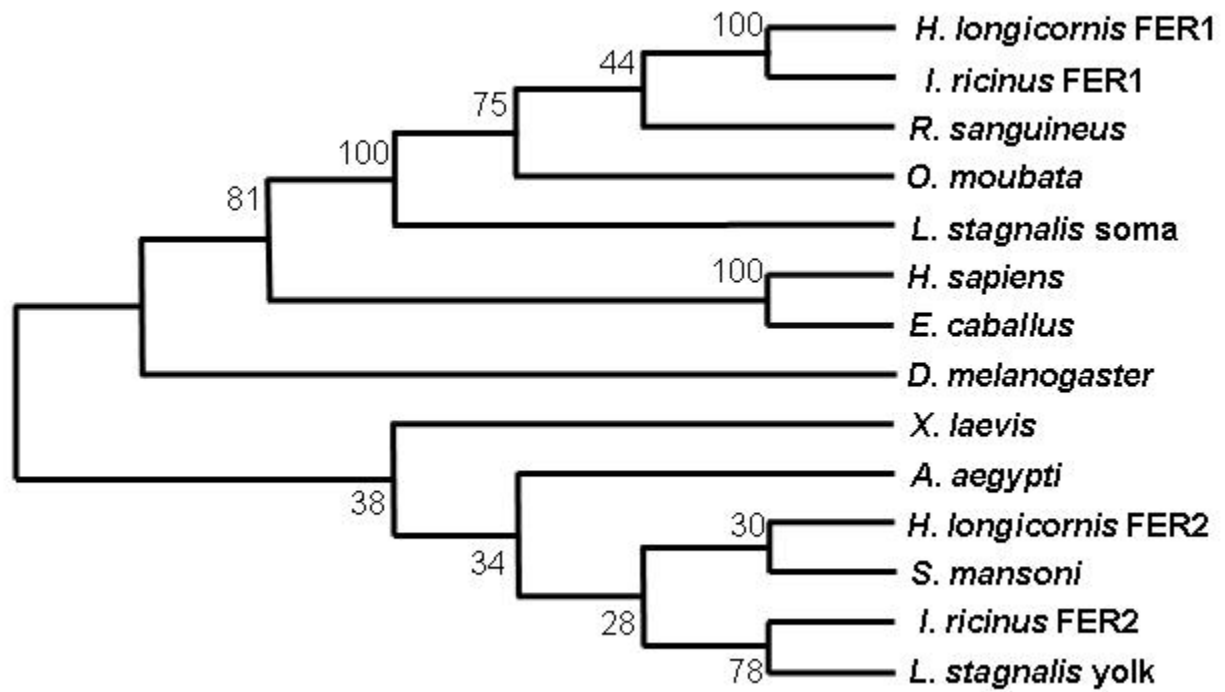
Fig. S3. Phylogenetic tree of ferritins from different species of ticks, selected vertebrates and invertebrates. A dendogram was constructed by the neighbor-joining method based on the amino acid sequences of ferritins. Bootstrap values are shown on the lineage of the tree. GenBank accession numbers are as follows: *H. longicornis* FER1, AY277905; *H. longicornis* FER2, AB734098; *I. ricinus* FER1, AF068224.1; *I. ricinus* FER2, EU885951.1; *R. sanguineus*, AY277907.1; *O. moubata*, AF068225.2; *A. aegypti*, XM_001654469.1; *D. melanogaster*, AF145125.1; *H. sapiens*, NM_002032.2; *Equus caballus*, AY112742.1; *Lymnaea stagnalis* yolk ferritin, AY112742.1; *L. stagnalis* soma ferritin, X56778.1; *Schistosoma mansoni*, XM_002576034.1; and *Xenopus laevis*, NM_001086111.1.
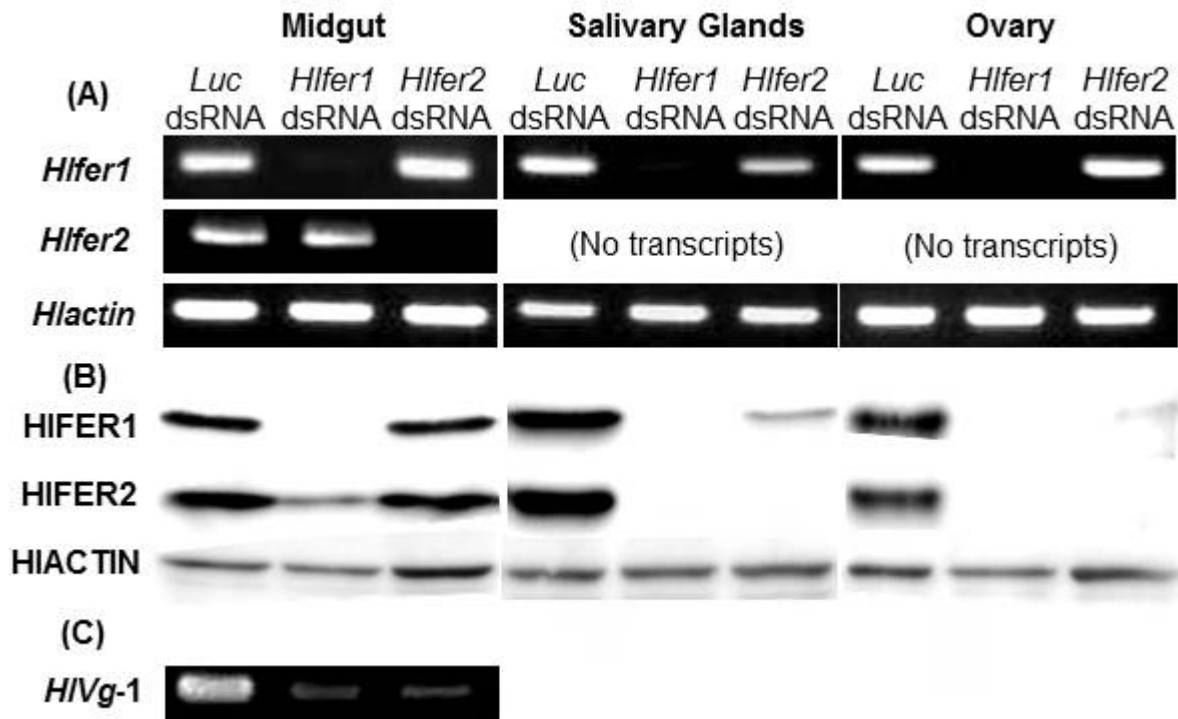
Fig. S4. Confirmation of *Hlfer* silencing by RT-PCR (A) and western blot analysis (B) in different organs of adult *H. longicornis*. After injection of *Hlfer1*, *Hlfer2* or *Luciferase* dsRNA, ticks were allowed to feed and were collected at 4 days post-attachment. The midgut, salivary glands and ovary were collected for RNA and protein extraction. Specific primers and anti-sera were used and are indicated on the left. No transcript was detected in the salivary glands and ovary using the *Hlfer2* primer (please refer to Fig. 1). RT-PCR was also performed to examine *HlVg-1* expression in the midgut after *Hlfer* silencing (C).
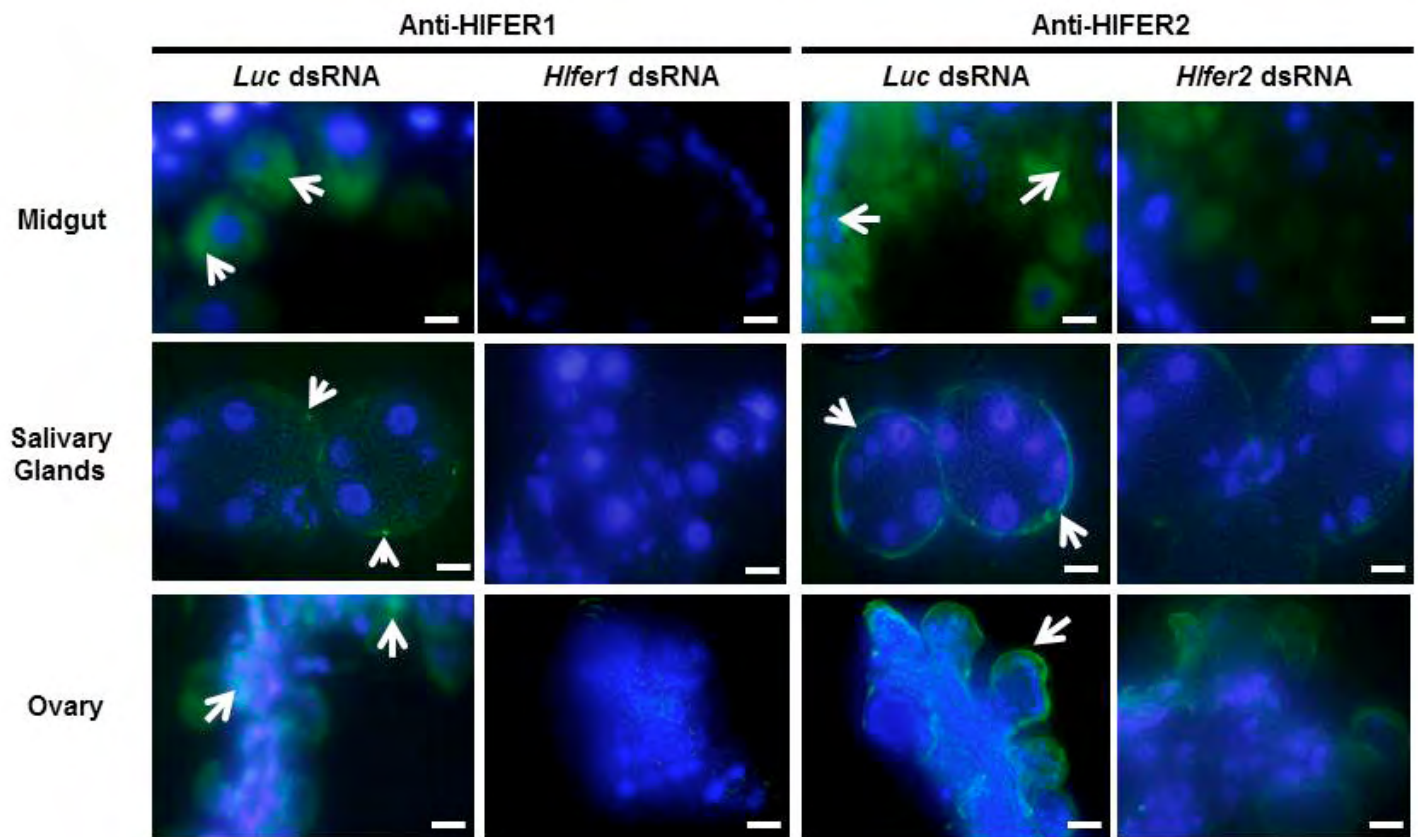
Fig. S5. IFAT after *Hlfer* silencing. Midgut, salivary glands and ovary were collected from *Hlfer1*, *Hlfer2* or *Luciferase* dsRNA-injected ticks after 4 days of feeding. Sections were prepared as for IFAT of normal organs (Fig. 7). Arrows point to positive fluorescence. Bars=20 µm.

Table S1. Gene-specific primers used for RT-PCR and dsRNA synthesis

Name	Sequence (5'→3')
HIfer1 forward	ATGGCCGCTACTCAACCCCG
HIfer1 reverse	TCAGTCGTCTCCTCCGAGGG
HIfer2 forward	ATGCTCCCGATCCTGATCTT
HIfer2 reverse	TTATTTGTCGCTGTCTTCGC
HIfer1 RNAi forward	CTGTACGCGAGCTACGTCTA
HIfer1 RNAi reverse	ACACGCTTCAGGTTGGTCA
HIfer1 T7 forward	GGATCCTAATACGACTCACTATAGGCTGTACGCGAGCTACGT
HIfer1 T7 reverse	GGATCCTAATACGACTCACTATAGGACACGCTTCAGGTTGGT
HIfer2 RNAi forward	CAAGTATTTCCTGCACGACC
HIfer2 RNAi reverse	GAGCCGCTGCAGCTTGTCGA
HIfer2 T7 forward	GGATCCTAATACGACTCACTATAGGCAAGTATTTCCTGCACG
HIfer2 T7 reverse	GGATCCTAATACGACTCACTATAGGGAGCCGCTGCAGCTTGT
Luc T7 forward	GTAATACGACTCACTATAGGGCTTCCATCTTCCAGGGATACG
Luc T7 reverse	GTAATACGACTCACTATAGGCGTCCACAAACACAACTCCTCC