Supporting Information

Hajdusek *et al.* 10.1073/pnas.0807961106

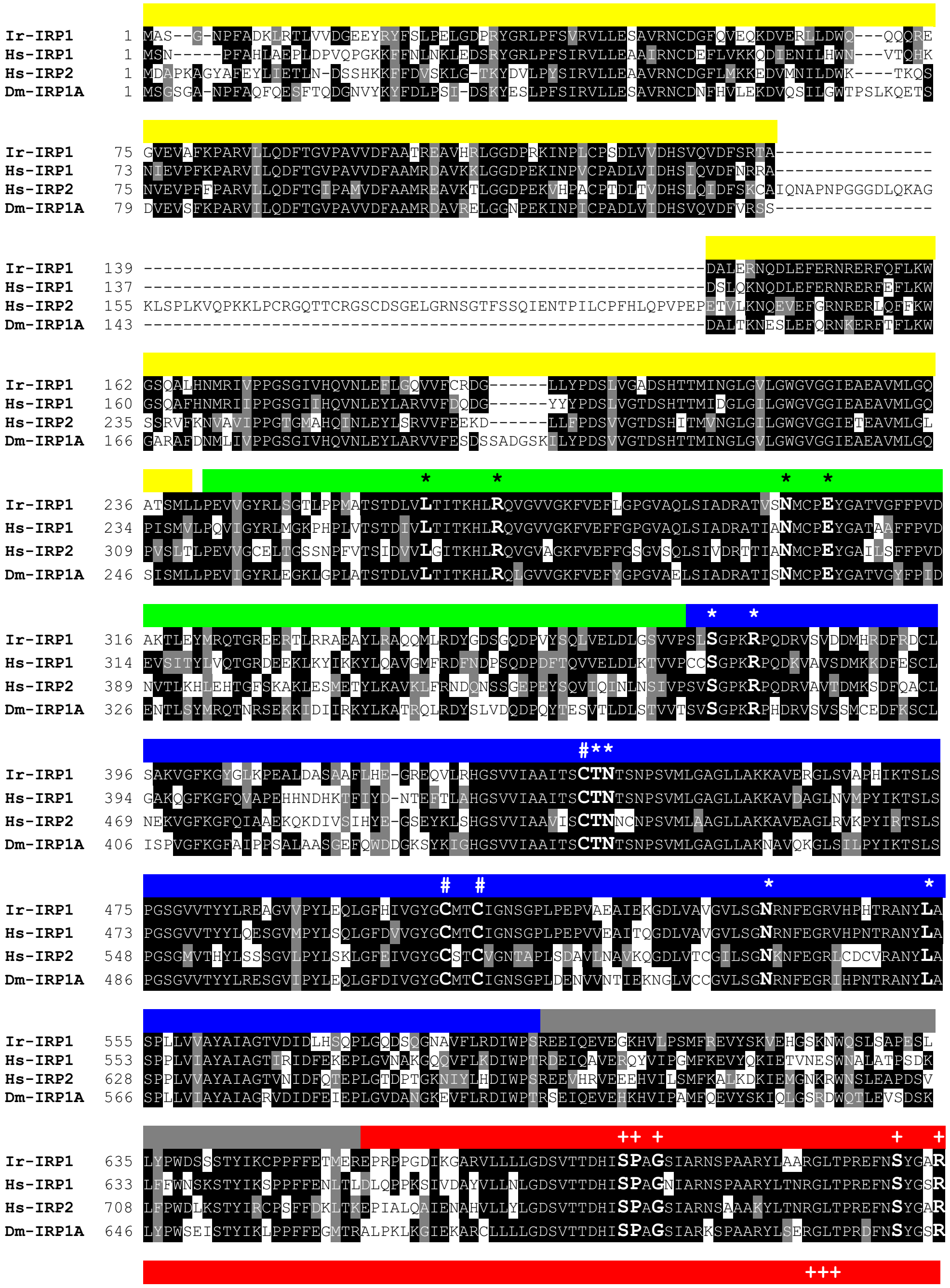

Fig. S1. Multiple alignment of *Ixodes ricinus* IRP1 with human IRP1, IRP2 and *Drosophila* IRP1. The domains are colored according to the human c-aconitase/IRP1 crystal structure (Dupuy *et al.* (2006) *Structure* 14:129–139). #, residues binding Fe-S cluster in active c-aconitase. *, IRP1 residues interacting with A15, G16, and U17 of the iron responsive element head. +, residues interacting with conserved C8 of iron responsive stem (according to Walden *et al.* (2006) *Science* 314:1903–1908). Ir-IRP1 (*Ixodes ricinus* EU885952), Hs-IRP1 (*Homo sapiens* NP_002188), Hs-IRP2 (*H. sapiens* AAI17484), Dm-IRP1A (*Drosophila melanogaster* CAB93519).

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  1 atttttcagacgcacgaaagacagatcagcacccgctcgatccacatccatcatgaagcaatttgtggtcatccttgcgctcatcggcgc  90
  1                                                      M  K  Q  F  V  V  I  L  A  L  I  G  A  13

 91 agcgacgtcagggaacaatttgttcgaaaacctggacaagtacccgcttcaagatgaatgtcaagcagcgctgcaggaacacatcaatgt  180
 14   A  T  S  G  N  N  L  F  E  N  L  D  K  Y  P  L  Q  D  E  C  Q  A  A  L  Q  E  H  I  N  V  43

181 cgaaatgcacgcaagcctcgtatatatgcagatggcggcacacttcgacaacaacaaagtggctcggaagggtttcagtactttctttgc  270
 44   E  M  H  A  S  L  V  Y  M  Q  M  A  A  H  F  D  N  N  K  V  A  R  K  G  F  S  T  F  F  A  73
      *                    *

271 cgagaactccaaagaggaacgtgagcacgcccaaaagatcatcgactacatcaacaagaggggcagcaccgtctcgctcgtcaatatcga  360
 74   E  N  S  K  E  E  R  E  H  A  Q  K  I  I  D  Y  I  N  K  R  G  S  T  V  S  L  V  N  I  D  103
                  *  *        *

361 catgcccctgatcaccacttggaaatccgttttgcaagcgctgcgagatgccatcagcttggagaacaaagtgaccaacaagctccacgc  450
104   M  P  L  I  T  T  W  K  S  V  L  Q  A  L  R  D  A  I  S  L  E  N  K  V  T  N  K  L  H  A  133
                                                                  *

451 cgtgcacaagattgccgatgaggagtgcaaagaccctcagctcatggacttcatcgagagcgagttcttggaggagcaagtgaattccat  540
134   V  H  K  I  A  D  E  E  C  K  D  P  Q  L  M  D  F  I  E  S  E  F  L  E  E  Q  V  N  S  I  163
                                                                                 *

541 tgacaagctgcagcgaatgattacagtactcagtaacatggactccggcacgggggagtacctgttggacagagagctgctcggagacaa  630
164   D  K  L  Q  R  M  I  T  V  L  S  N  M  D  S  G  T  G  E  Y  L  L  D  R  E  L  L  G  D  K  193

631 gaaggaattttaagcacttgcttttcagtagaatcccaattcccaaggcgtgtcgctgggtcggcgacaaatcaaggtgtattgaaggac  720
194   K  E  F stop                                                                                197

721 tcaaactgcaagaaacactttctggttgcctagaaaacattcatatgtattggttaaacgcttcaaaactttccctatcccaactatcct  810
811 atttgttttcaaacttcccactgattttagttttgacgaattctggggagcaggggcatctgttgcaataaaaggtcaaacgacagcaa  900
901 aaaaaaaaaaaaaaaaaaaaaaaaaaaa                                                                928
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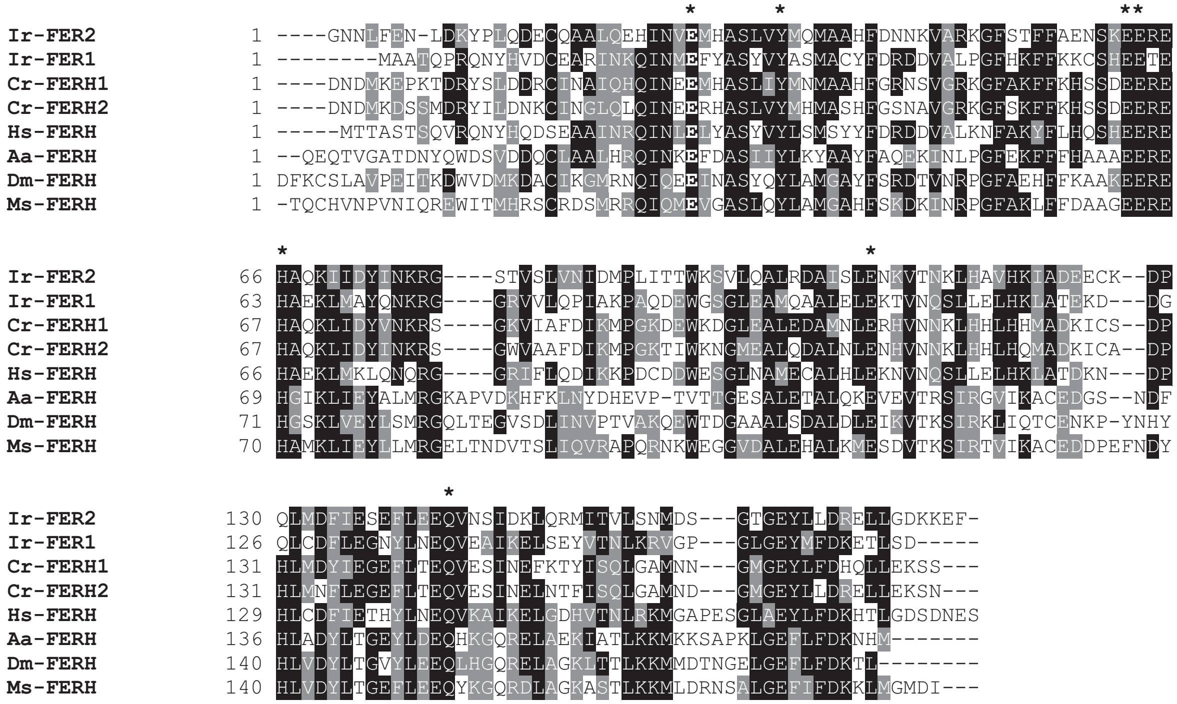
Fig. S2. Secreted ferritin (fer2) cDNA and deduced amino acid sequence. The signal peptide is in italics, highlighted by green. Cysteine residues are marked in yellow. Asterisks and red color depict the conserved residues of ferroxidase centers of heavy chain ferritins [Hempstead *et al.* (1997) *J Mol Biol* 268:424–448].



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                                                 *      *                          **
Ir-FER2        1 ----GNNLFEN-LDKYPLQDECQAALQEHINVEMHASLVYMQMAAHFDNNKVARKGFSTFFAENSKEERE
Ir-FER1        1 --------MAATQPRQNYHVDCEARINKQINMEFYASYVYASMACYFDRDDVALPGFHKFFKKCSHEETE
Cr-FERH1       1 ----DNDMKEPKTDRYSLDDRCINAIQHQINEEMHASLIYMNMAAHFGRNSVGRKGFAKFFKHSSDEERE
Cr-FERH2       1 ----DNDMKDSSMDRYILDNKCINGLQLQINEERHASLVYMHMASHFGSNAVGRKGFSKFFKHSSDEERE
Hs-FERH        1 -----MTTASTSQVRQNYHQDSEAAINRQINLELYASYVYLSMSYYFDRDDVALKNFAKYFLHQSHEERE
Aa-FERH        1 --QEQTVGATDNYQWDSVDDQCLAALHRQINKEFDASIIYLKYAAYFAQEKINLPGFEKFFFHAAAEERE
Dm-FERH        1 DFKCSLAVPEITKDWVDMKDACIKGMRNQIQEEINASYQYLAMGAYFSRDTVNRPGFAEHFFKAAKEERE
Ms-FERH        1 -TQCHVNPVNIQREWITMHRSCRDSMRRQIQMEVGASLQYLAMGAHFSKDKINRPGFAKLFFDAAGEERE

                 *                                             *
Ir-FER2       66 HAQKIIDYINKRG----STVSLVNIDMPLITTWKSVLQALRDAISLENKVTNKLHAVHKIADEECK--DP
Ir-FER1       63 HAEKLMAYQNKRG----GRVVLQPIAKPAQDEWGSGLEAMQAALELEKTVNQSLLELHKLATEKD---DG
Cr-FERH1      67 HAQKLIDYVNKRS----GKVIAFDIKMPGKDEWKDGLEALEDAMNLERHVNNKLHHLHHMADKICS--DP
Cr-FERH2      67 HAQKLIDYINKRS----GWVAAFDIKMPGKTIWKNGMEALQDALNLENHVNNKLHHLHQMADKICA--DP
Hs-FERH       66 HAEKLMKLQNQRG----GRIFLQDIKKPDCDDWESGLNAMECALHLEKNVNQSLLELHKLATDKN---DP
Aa-FERH       69 HGIKLIEYALMRGKAPVDKHFKLNYDHEVP-TVTTGESALETALQKEVEVTRSIRGVIKACEDGS--NDF
Dm-FERH       71 HGSKLVEYLSMRGQLTEGVSDLINVPTVAKQEWTDGAAALSDALDLEIKVTKSIRKLIQTCENKP-YNHY
Ms-FERH       70 HAMKLIEYLLMRGELTNDVTSLIQVRAPQRNKWEGGVDALEHALKMESDVTKSIRTVIKACEDDPEFNDY

                              *
Ir-FER2      130 QLMDFIESEFLEEQVNSIDKLQRMITVLSNMDS---GTGEYLLDRELLGDKKEF-
Ir-FER1      126 QLCDFLEGNYLNEQVEAIKELSEYVTNLKRVGP---GLGEYMFDKETLSD-----
Cr-FERH1     131 HLMDYIEGEFLTEQVESINEFKTYISQLGAMNN---GMGEYLFDHQLLEKSS---
Cr-FERH2     131 HLMNFLEGEFLTEQVESINELNTFISQLGAMND---GMGEYLLDRELLEKSN---
Hs-FERH      129 HLCDFIETHYLNEQVKAIKELGDHVTNLRKMGAPESGLAEYLFDKHTLGDSDNES
Aa-FERH      136 HLADYLTGEYLDEQHKGQRELAEKIATLKKMKKSAPKLGEFLFDKNHM-------
Dm-FERH      140 HLVDYLTGVYLEEQLHGQRELAGKLTTLKKMMDTNGELGEFLFDKTL--------
Ms-FERH      140 HLVDYLTGEFLEEQYKGQRDLAGKASTLKKMLDRNSALGEFIFDKKLMGMDI---
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Fig. S3. Multiple alignment of *I. ricinus* secreted ferritin2 (FER2) with intracellular ferritin1 (FER1), human ferritin and selected invertebrate ferritins. The mature proteins without signal peptides are aligned. Asterisks depict conserved residues of ferroxidase centers of heavy chain ferritins (Hempstead *et al.* (1997) *J Mol Biol* 268:424–448). Ir-FER2 (*Ixodes ricinus* EU885951, heavy chain), Ir-FER1 (*Ixodes ricinus* AAC19131, heavy chain), Cr-FERH1 (*Carcinoscorpius rotundicauda* AY691510, heavy chain), Cr-FERH2 (*Carcinoscorpius rotundicauda* AY691511, heavy chain), Hs-FERH (*Homo sapiens* AF088851, heavy chain), Aa-FERH (*Aedes aegypti* AF126431, heavy chain), Dm-FERH (*Drosophila melanogaster* NP_733358, heavy chain), Ms-FERH (*Mandusca sexta* AAK39636, heavy chain).

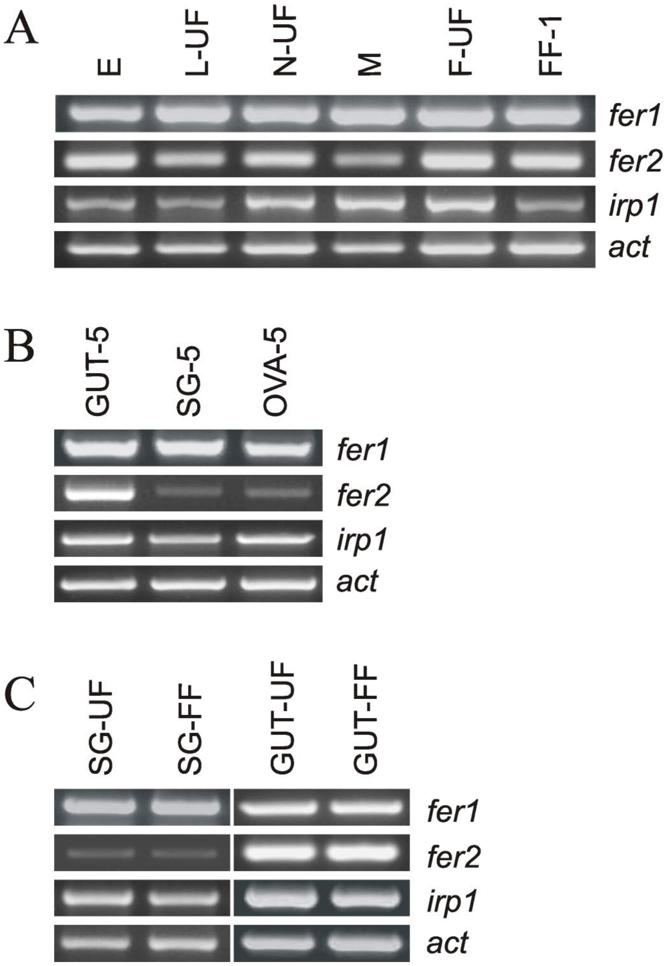

Fig. S4. Transcription profiles and dynamics of *fer1*, *fer2* and *irp1*. RT-PCR was performed on different cDNAs. Transcription levels of tick developmental stages (*A*), tissues of half engorged (fed for 5 days) ticks (*B*), and tissues of unfed and fully fed ticks (*C*). E, eggs; L, larvae; N, nymphs; M, males; F, females; GUT, gut tissue; SG, salivary glands; OVA, ovaries; UF unfed; FF, fully fed; FF-1, female fed for 1 day. Numbers indicate the days of feeding.

Table S1. Putative iron metabolism genes found in NCBI *I. scapularis* EST database using BLASTN search

Gene	Function	BLASTN search against EST *I. scapularis* (GB#)	BLASTX search homology	*E*
Ferritin	Iron storage	EW833235	Ferritin *Ixodes scapularis*	3×10^{-96}
		EW871480	Ferritin heavy chain-1b *Carcinoscorpius rotundicauda*	2×10^{-48}
Iron regulatory protein	Translation control factor/cytoplasmic aconitase	EW889997	Aconitase 1 *Mus musculus*	1×10^{-119}
Transferrin	Iron transport, immune function	EW961538	Transferrin 2 *Drosophila melanogaster*	6×10^{-63}
Divalent metal transporter	Metal (Mn, Zn, Cd, Fe, …) transport	EW887153	Divalent metal transporter *Homo sapiens*	7×10^{-85}

Table S2. Primers used

Gene	GenBank Accession no.	Primer name	Sequence, 5′ to 3′
Ferritin1	AF068224	FER1-F	CCAAACGGTTCGCTTTCCTC
		FER1-R	GAGCCCCACTCGTCCTGGG
Ferritin2	EU885951	FER2-F	TATATGCAGATGGCGGCAC
		FER2-R	GAGCTTGTTGGTCACTTTG
		FER2-Exp-F	CACCGGGAACAATTTGTTCGAAAAC
		FER2-Exp-R	GCAAGTGCTTAAAACTCCTTCTTG
Iron regulatory protein 1	EU885952	IRP1-F1	CAGCAAGAACTGGCAGAG
		IRP1-R1	CAGGTGCAGGGTGCGTGG
Actin	AJ889837	ACT-F	CCGTGCCGTGTTCCCCTCCAT
		ACT-R	CCGGCCGGCCAAGTCCAGA

Other Supporting Information Files

Alignment and Sequence Descriptors for Fig. 3 (ZIP)