

Supplementary Information

RNA-seq analyses of the midgut from blood- and serum-fed *Ixodes ricinus* ticks

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Table S1. The summary of Miseq the reads after removal of Illumina primers and trimming low base quality (smaller than 20).

Library name	Meal	Time in days	Total number of sequences	Total number of residues	Average length	Median size	L50
B1	Blood	3	2,155,570	623,472,674	289.2	300	300
B2	Blood	3	3,413,789	983,125,506	287.9	300	300
B3	Blood	3	1,913,967	547,583,431	286.0	300	300
B4	Blood	3	2,747,297	786,753,771	286.3	300	300
B5	Blood	8	3,203,379	903,713,423	282.1	300	300
B6	Blood	8	2,371,577	677,111,683	285.5	300	300
B7	Blood	8	3,108,306	892,945,075	287.2	300	300
B8	Blood	8	4,172,957	1,188,549,527	284.8	300	300
S1	Serum	3	3,213,133	912,458,003	283.9	300	300
S2	Serum	3	3,118,604	878,902,497	281.8	300	300
S3	Serum	3	3,007,399	840,317,863	279.4	300	300
S4	Serum	3	3,228,533	903,949,950	279.9	300	300
S5	Serum	8	3,064,434	866,807,467	282.8	300	300
S6	Serum	8	2,395,662	670,246,143	279.7	300	300
S7	Serum	8	2,601,754	726,967,206	279.4	300	300
S8	Serum	8	2,507,908	678,757,731	270.6	300	300
Total			46,224,269	13,081,661,950			
Average			2,889,017	817,603,872	283		
Minimum			1,913,967	547,583,431			
Maximum			4,172,957	1,188,549,527			

Table S2. The summary of Hiseq the reads after removal of Illumina primers and trimming low base quality (smaller than 20).

Library name	Meal	Feeding time in days	Total number of sequences	Total number of residues	Average length	Median size	L50
B1	Blood	3	9,629,494	1,190,648,679	123.7	125	125
B2	Blood	3	15,544,323	1,860,018,389	119.6	125	125
B3	Blood	3	11,884,809	1,320,412,881	111.1	125	125
B4	Blood	3	12,704,580	1,479,349,109	116.4	125	125
B5	Blood	8	15,348,566	1,766,877,794	115.1	125	125
B6	Blood	8	11,105,251	1,303,445,535	117.3	125	125
B7	Blood	8	14,939,645	1,754,368,386	117.4	125	125
B8	Blood	8	15,951,632	1,878,440,011	117.7	125	125
S1	Serum	3	15,033,927	1,821,330,422	121.1	125	125
S2	Serum	3	13,825,806	1,651,700,131	119.5	125	125
S3	Serum	3	15,959,483	1,902,584,122	119.2	125	125
S4	Serum	3	14,125,114	1,692,400,404	119.8	125	125
S5	Serum	8	11,945,337	1,470,248,650	123.0	125	125
S6	Serum	8	10,181,106	1,255,324,249	123.3	125	125
S7	Serum	8	10,753,913	1,325,093,390	123.2	125	125
S8	Serum	8	7,485,714	917,067,908	122.5	125	125
Total			206,418,700	24,589,310,060			
Average			12,901,169	1,536,831,879	119		
Minimum			7,485,714	917,067,908			
Maximum			15,959,483	1,902,584,122			

Table S3: List of oligonucleotides used in this work for RT-qPCR analyses of direct and independent validations, tissue profile expression of Day-3 up-regulated contigs, and tissue profile expression of Day-8 up-regulated contigs

Transcript	Contig no.	Forward primer (5'-3')	Reverse primer (5'-3')
<i>gst</i>	Ir-114935	ggttcgtcctgtgggaaag	actgtccggggcgtactt
<i>gst</i>	Ir-113744	ttatgcatttggatgcttcg	gccctgtcagggttggtcata
<i>sult</i>	Ir-110976	ggtcgacagagttccctacg	tcccacgggttacgatagac
<i>ache</i>	Ir-108903	cgtttctcatgagtggcaca	tggactgcactctcgaac
<i>Na⁺-bile acid c-t</i>	IrSigP-109984	atggcatcgtggcaactc	gggatggaccctagctc
<i>ph-gpx</i>	IrSigP-109202	ctggaagtacctcaaggaaag	ctgtccattcttgtccacca
<i>pm-chbp</i>	Ir-113572	cgacaacagctatcggatga	aggtgtggttagcgacgaatc
<i>cyp450</i>	IrSigP-117992	gcgaaggagcaggttctct	agatttggttcgggtccag
<i>m20</i>	Ir-107651	caacatctccatgcttcgac	cattaagcatcacccatga
<i>cyp450</i>	IrSigP-112182	ctcaggagaccgctgagttt	gcgttatattgocggaaaag
<i>unknown</i>	IrSigP-112102	gtgctacaaagccaacgaca	ggacgggtggaggaaggtt
<i>unknown</i>	Ir-111905	atgagccattatcctgacg	gacacggtgagagtggctcg
<i>unknown</i>	IrSigP-107775	acgggaatgaacttgacagc	cgacaatgtttcggattctg
<i>unknown</i>	IrSigP-111681	ctgaaaggagtgaccgttgc	ggccgacaccttgttgaa
<i>plA2</i>	Ir-111829	gacaactccgacgacaacatc	agaactggcgggtcatctttg
<i>smpd</i>	Ir-103540	gccctcatcaagctgctc	ctcgtccttgttagaggctatgg
<i>a-smase</i>	Ir-110985	agtcgggtcccatcaacag	gccactggacgggtgtagttt
<i>lip</i>	Ir-108861	catcttttcgactccgtct	cgggagtagagcatgaacttg
<i>pep synthase</i>	IrSigP-110295	tcgagtacaaatggcagcac	cacgtcagactgcaccatct
<i>grp</i>	IrSigP-109251	caggaagaaacgggcaagta	ccggagggtccttctggtc
<i>grp</i>	Ir-109833	tggttctaagggcgagaaaag	aaacctggtagtccatttctgc
<i>sult</i>	Ir-114115	cttacgactgttgcgtgtcc	cgaactggtaagcaggggaag
<i>plc</i>	IrSigP-114509	cctagtggcgatggatttct	ttagcatcgatggcaacatt
<i>ak</i>	Ir-98671	ggcaacactagacaagctgga	agcgacttgcagtccttagc
<i>hsd17b8</i>	Ir-100072	tcacaagtggatgtccggta	caccacgatgcttaaaggaag
<i>vmat</i>	IrSigP-107534	ggattctacatccgggacac	gcagaaccacgtcactgt
<i>acyl-CoA synthetase</i>	Ir-121498	gttgactgcgcaactcgtc	aacgaagtccttgagtttctcg
<i>jh-acid mt</i>	Ir-113115	gaaagaaatggtcgcagacg	tggactagcggggtaaacag
<i>gpx</i>	Ir-97790	ccagaagatcgaggatgaacg	aacgacccttgaggaaagtgt
<i>kunitz 80</i>	Ir-113156	atgttccggcgctacaaat	cgcagcctccgtaaatgt
<i>vitellogenin 2</i>	IrSigP-108669	cctcacttccctccagacttc	ccaggagcaagtgcaggac
<i>org. anion trans.</i>	Ir-109200	gaatgttgctgtcggctcttg	tgccgtggattccgtaag
<i>hsd17b8</i>	Ir-118795	gcaagtgtgggtatgccttt	gacttcgtgaacgcaatgac
<i>15-oh-pg-dh</i>	Ir-113345	tcgttgatcaataatgcagggtg	aagcaggggtccacagaatg
<i>sec14</i>	Ir-113452	caattgtccggcatctga	tttgttccgtgaaggtggat
<i>ache</i>	IrSigP-113423	gaacctacgacgcaacgact	gtggtttcttcacgttctcaagt
<i>ir-cp6</i>	Ir-108832	catcgaggatcacgagagg	tgatcatcatcttggagtacgg
<i>ef</i>	IrSigP-108204	acgaggctctgacggaag	cacgacgcaactccttcac
<i>actin</i>	Ir-107867	cgacatcaaggagaagctctg	gtcgggaagctcgtaggac

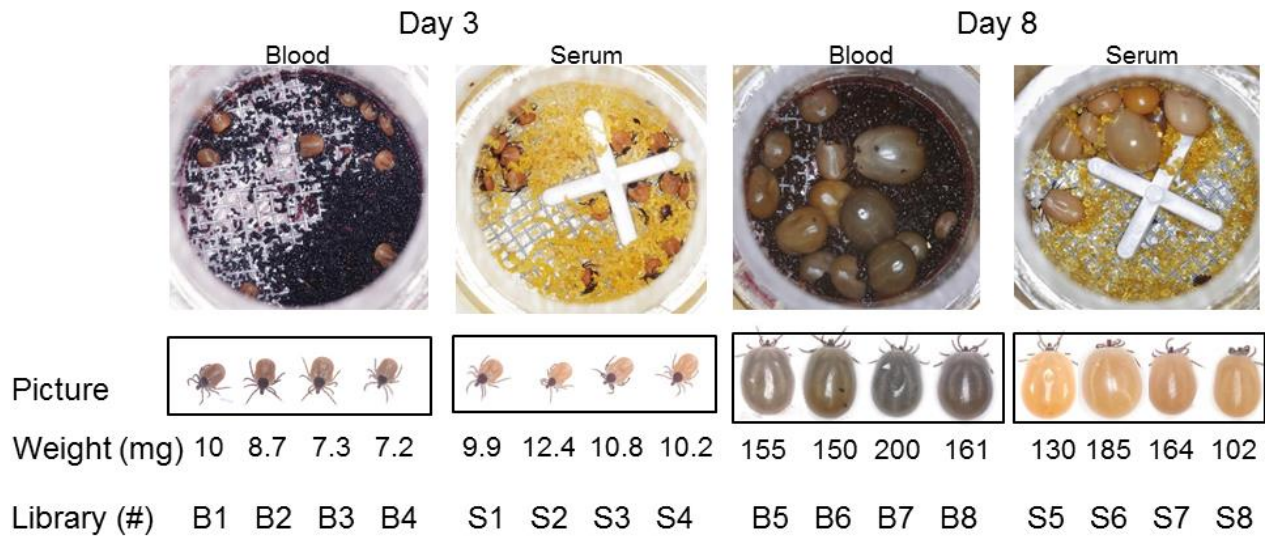
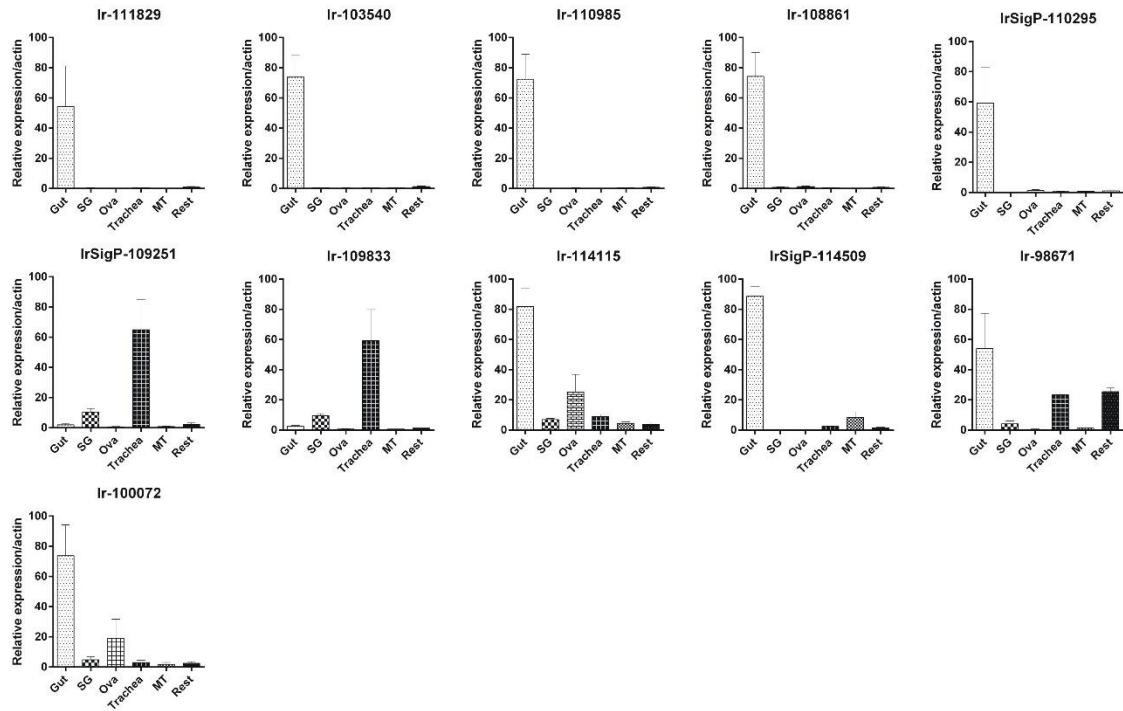


Figure S1. Overview of the experimental design and libraries designation. Libraries were prepared from individual first-generation siblings *Ixodes ricinus* females fed *in vitro* for 3 days or 8 days. Females were fed either reconstituted bovine blood or bovine serum. Similarly appearing tick females were forcibly removed at indicated time points. Weights of individual females and corresponding library names, used in Source data 1, are shown.

A)



B)

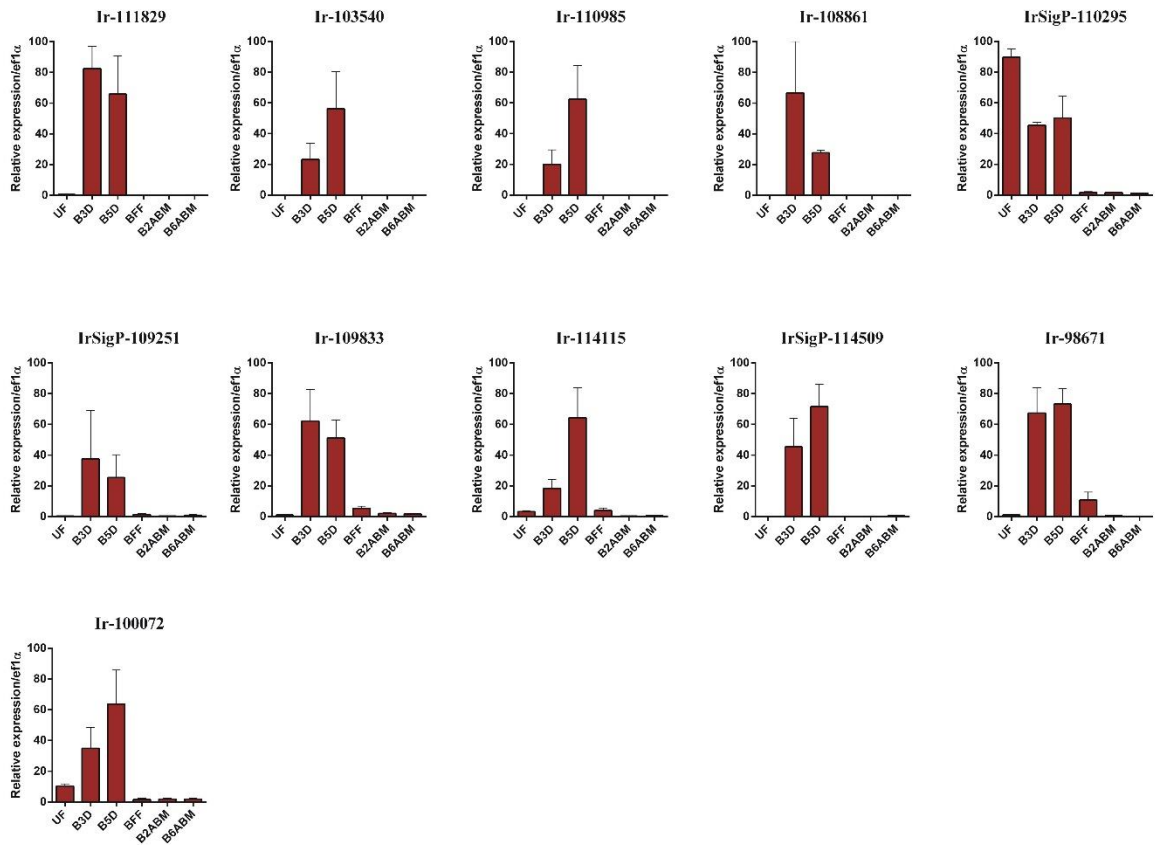
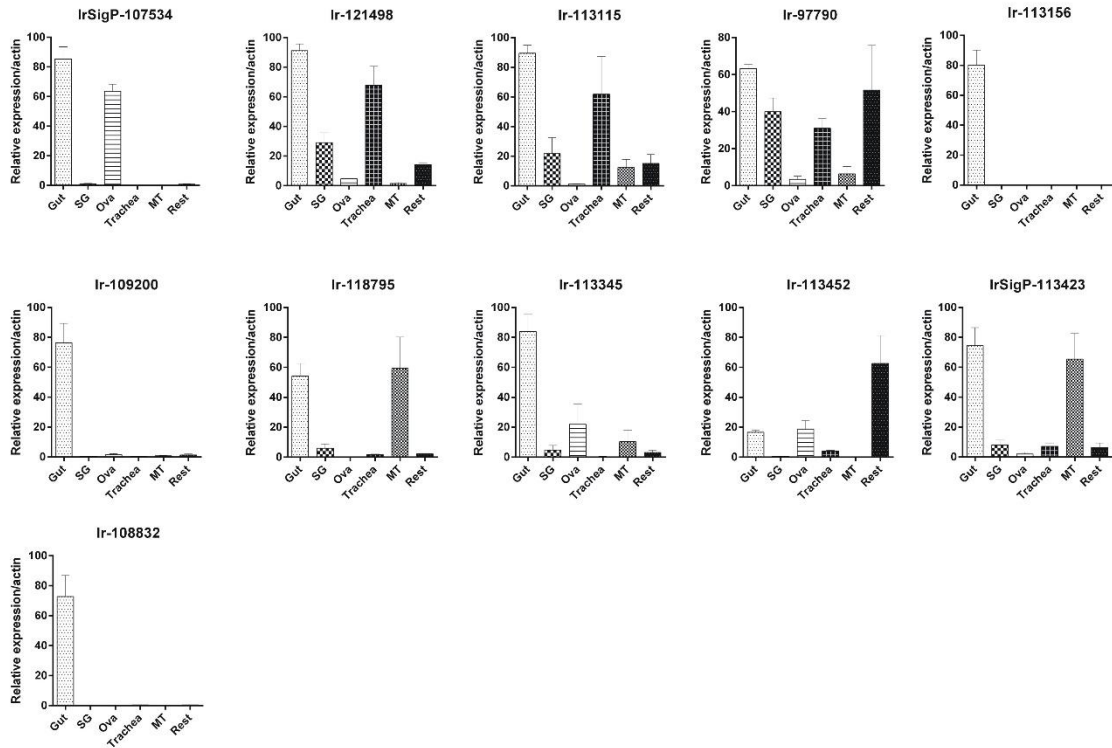


Figure S2. RT-qPCR profiles of genes over-represented in the gut of 3D ticks. **A)** cDNA samples were synthesised from RNA isolated from dissected tissues of *I. ricinus* females collected in wild, fed naturally for 3 days. SG - salivary glands, Ova - ovaries; Trachea - trachea-fat body complex; MT - Malpighian tubules; Rest - remaining tissues. Mean values and SEM are shown, n = 3 (biological replicates) **B)** cDNA samples were synthesised from RNA isolated from dissected gut of *I. ricinus* females before, during, and after feeding. UF - unfed, B3D - blood-fed for 3 days; B5D - blood-fed for 5 days; BFF - blood-fed for 9 days (fully fed); B2ABM - 2 days after blood-meal; B6ABM - 6 days after blood-meal. Mean values and SEM are shown, n = 3 (biological replicates).

A)



B)

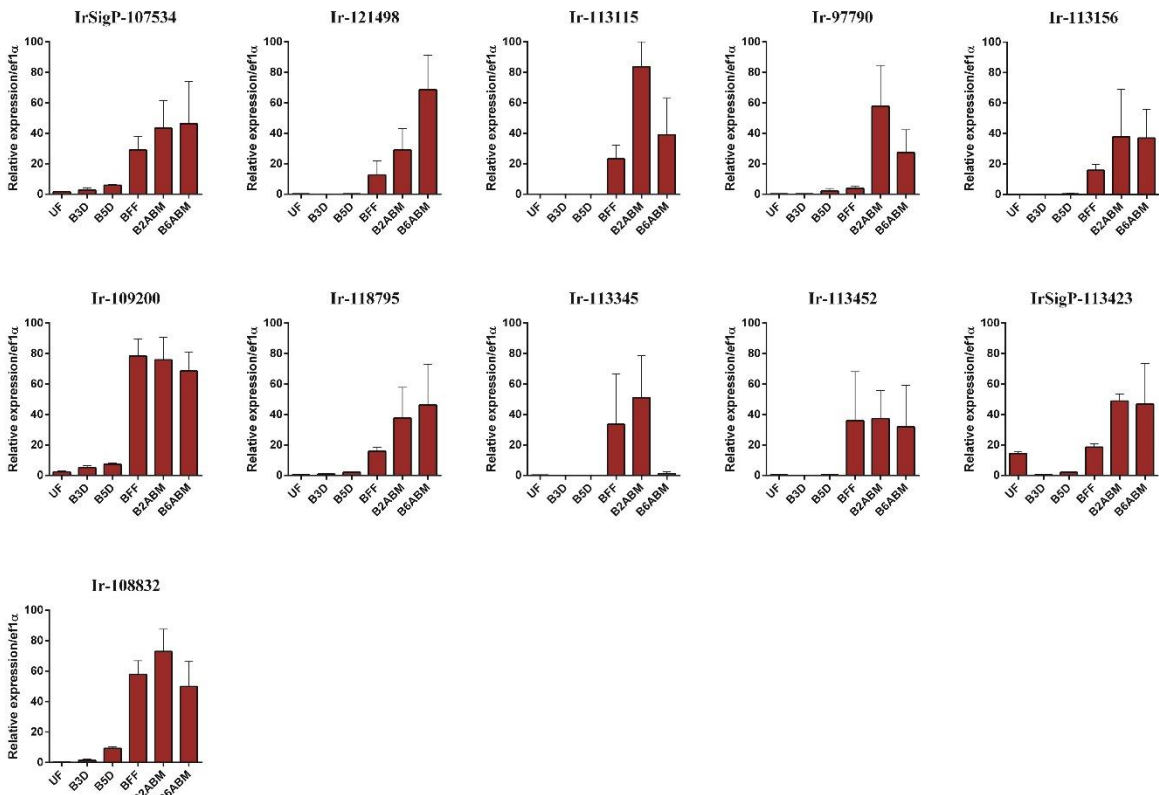


Figure S3. RT-qPCR profiles of genes over-represented in the gut of 8D ticks. **A)** cDNA samples were synthesised from RNA isolated from dissected tissues of *I. ricinus* females collected in wild, fed naturally for 3 days. SG - salivary glands, Ova - ovaries; Trachea - trachea-fat body complex; MT - Malpighian tubules; Rest - remaining tissues. Mean values and SEM are shown, n = 3 (biological replicates) **B)** cDNA samples were synthesised from RNA isolates of dissected gut of *I. ricinus* females before, during, and after feeding. UF - unfed, B3D - blood-fed for 3 days; B5D - blood-fed for 5 days; BFF - blood-fed for 9 days (fully fed); B2ABM - 2 days after blood-meal; B6ABM - 6 days after blood-meal. Mean values and SEM are shown, n = 3 (biological replicates).

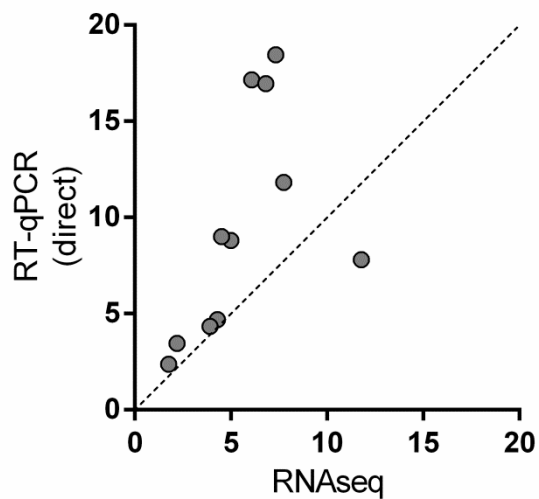


Figure S4. Correlation of direct RT-qPCR validation and RNA-seq data. Individual RT-qPCR fold change up-regulations (based on mean relative expression value from three biological triplicates) are plotted against RPKM up-regulations from RNA-seq data. Following contigs were used as representatives for validation: Ir-114935, Ir-110976, Ir-113572, Ir-108903, IrSigP-117992, IrSigP-109202, Ir-107651, IrSigP-112182, IrSigP-112102, Ir-111905, IrSigP-107775.

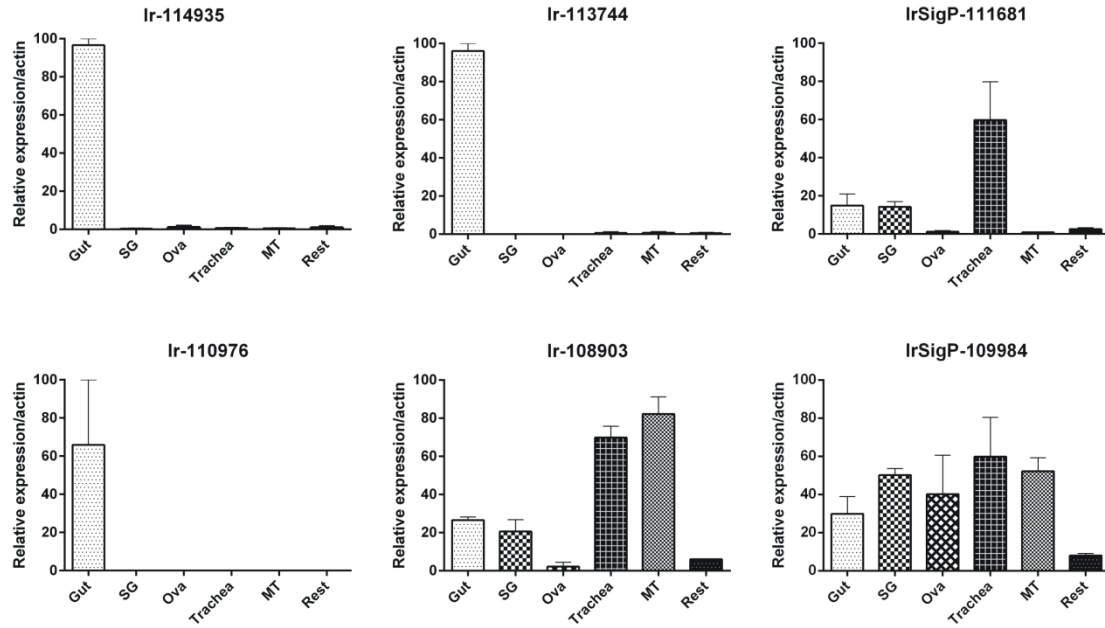


Figure S5. Tissue expression profile of differentially expressed genes in the gut of blood-fed and serum-fed ticks. Three sets of cDNA from selected tissues of naturally fed ticks for 3 days or 8 days were synthesised. Tissue expression profiling was analysed by RT-qPCR at the time-point of differential expression (Day 3 or Day 8). SG - salivary glands, Ova - ovaries; Trachea - trachea-fat body complex; MT - Malpighian tubules; Rest - remaining tissues. Mean values and SEM are shown, n = 3 (biological replicates).