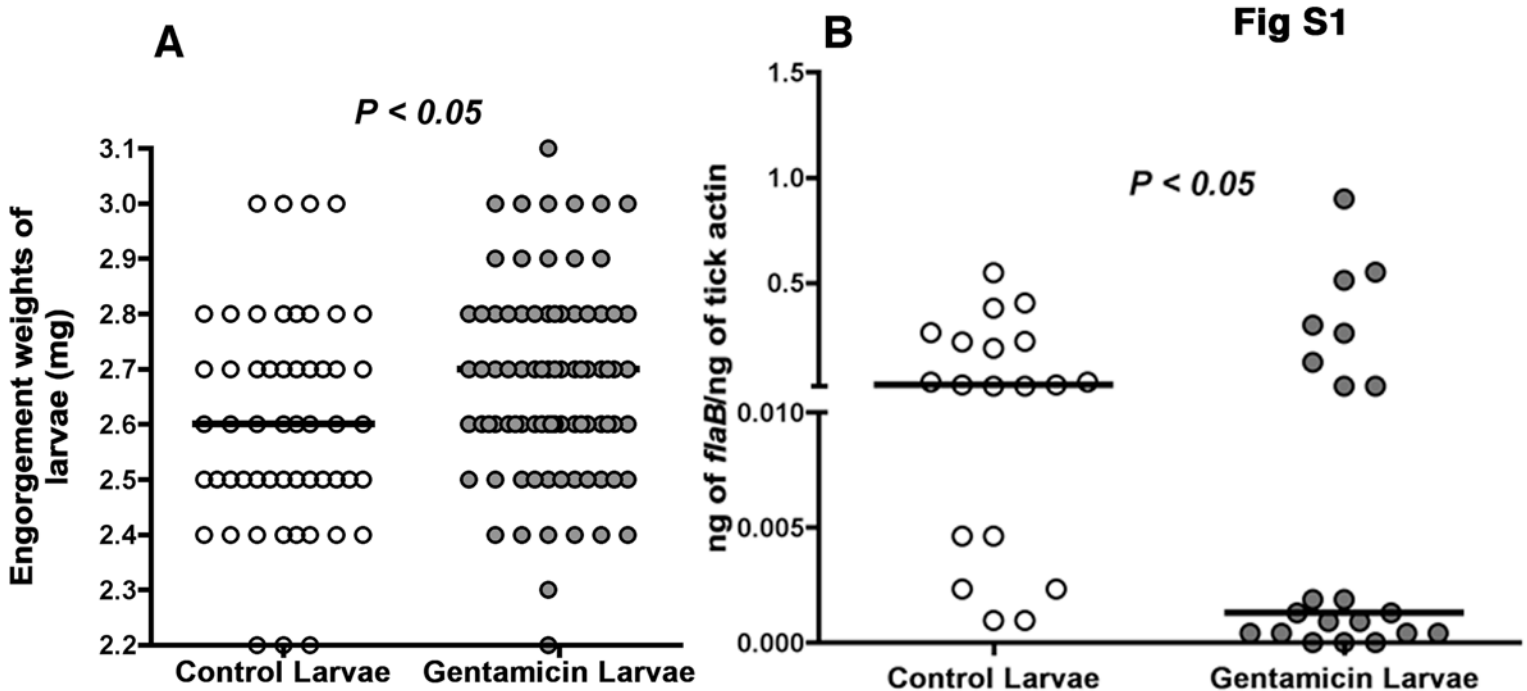
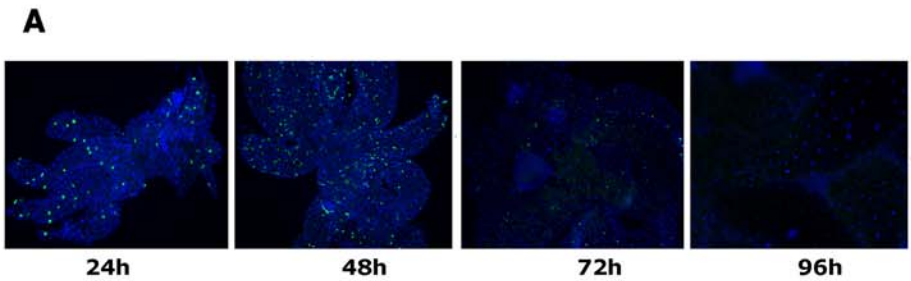
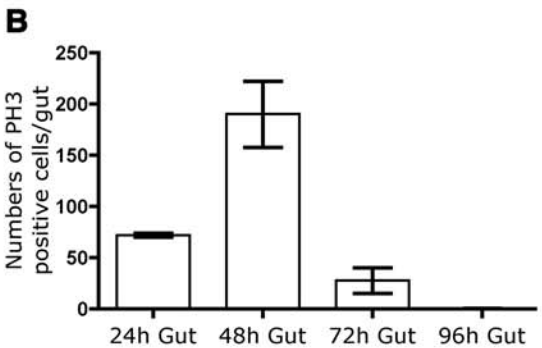
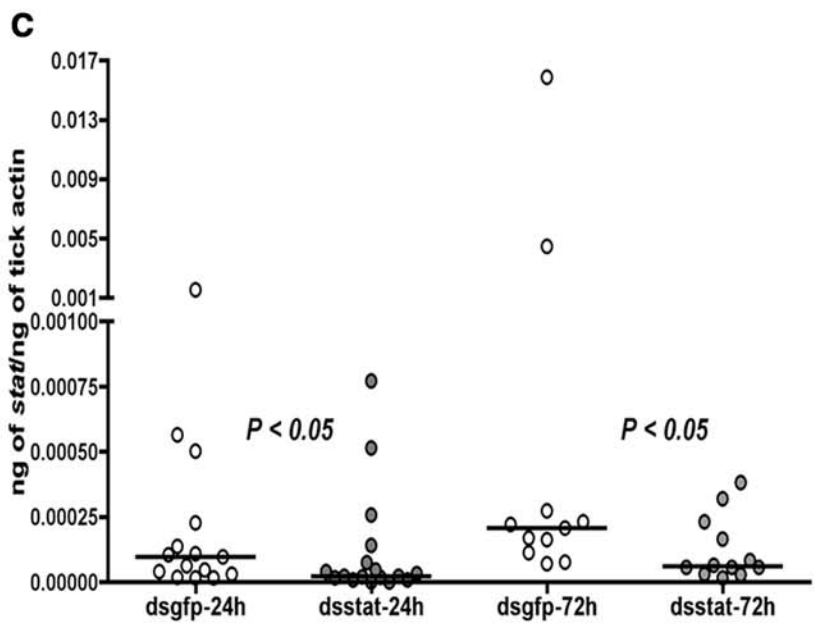

A
P < 0.05
Engorgement weights of larvae (mg)
3.1
3.0
2.9
2.8
2.7
2.6
2.5
2.4
2.3
2.2
Control Larvae
Gentamicin Larvae
B
Fig S1
P < 0.05
ng of flaB/ng of tick actin
1.5
1.0
0.5
0.010
0.005
0.000
Control Larvae
Gentamicin Larvae

Fig S2


A
24h
48h
72h
96h


B
Numbers of PH3 positive cells/gut
250
200
150
100
50
0
24h Gut
48h Gut
72h Gut
96h Gut


C
ng of stat/ng of tick actin
0.017
0.013
0.009
0.005
0.001
0.00100
0.00075
0.00050
0.00025
0.00000
P < 0.05
P < 0.05
dsgfp-24h
dsstat-24h
dsgfp-72h
dsstat-72h

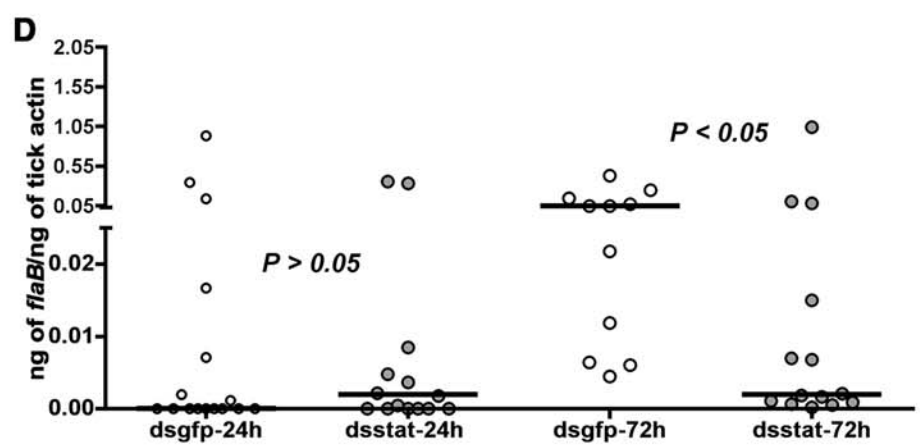

D
ng of flaB/ng of tick actin
2.05
1.55
1.05
0.55
0.05
0.02
0.01
0.00
P > 0.05
P < 0.05
dsgfp-24h
dsstat-24h
dsgfp-72h
dsstat-72h

Fig S3A

ISCW006076-*peritrophin-1*

GGGGGGAGGGAGGGAGGAGGAGGGATTGAATAAAAGGGACACGAAGTAGAACGTTCATTATGCTCCTATTC
TGCTCGATAAAAAAAAAAAAAGAATCTTTTTTGGTGGTTGTTGTTTTGAAGACAGTTGTTTTTCTTCCCCA
TTTTTACACACGGGAAGCAATGTATCCAATTAAAGCATTCCTTCCGTGCACACGTCCATGTGAAGAAAACA
AAAAATATTGGCGGATGTAGTTACAAAATCTCAAAACTGCTCACTATGGGAGGCAAATATTTTATAGTATG
TTTATTTTTATGGAATTACTTAAGCAACGAGTGAGAATTATTTCGGTGTTCTTCGAAACTATGCATTGTTC
ACACGAAAATGGCACACGATTCGTTATTGATACAATGTAATTGAACGGCAGAAAAGAAAAAAAGAAAATCT
AGAGCGGGGTTGTCCCGGTCCAAAATGGACGTCTCAATGGGGGAATGAATGAAGGGGAAGGGAAGCAGAAA
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AGATAAGTACCTTCCTCCTTTTTTTTTATTTTTAAACACCGGGGAAAGAATGCTACAAATTGAAGCATTCC
TTCCGTGCACACGTCTACTTGGGAAAAAATGTATTGGCAG*ATGTACCGACAGAAGAAGCTCAAAATTTGTC*
TGTG

ISCW013030-*peritrophin-2*

ATGGCGCCATTGTGGTCTCTCGTTGTAAAAGATTTTCAGAGATATCAAGGCGTACGTCATAAGATGTCGCA
GGTGTGCCAACGTACAATTTTGCACATGGGAAAAAAAAAATAGAGCAGACAAGAGCAGTCGTGGAATAACAT
AACAAATTAAGAACACGTGAATGAGACGCGTCTTGTGTTTTTGTGACTTACTATTGGACTCAGCTTTTATT
GCATTTCGAAGAAAAAATGTAAACACAGCGTCTTCTTACCCAAGGCAGCAATCACATACGAAATGAAAATT
GCTAAGAATTCCTCATCTAAACAATGTGCTGCATATTACGCGGAACGTTTTATAATAAAGCAAACAAACAC
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AACGACCTTTCAGAAAACTGCATTAGTGAAAAGCCGGAAACAGAAGGCGTCAAGAATTTTGTCTGATTCGA
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TCATGCTTTGCATTTTTGTTCGCATACTTAGTAAGCTCTGCGTATGGCGCTAAGTCAAGCTTTGTACTTAC
CGCTGTGCCAGGCGGTTAAATGCCAGACCTATACTCCTTCAAAGCTATAGGCGATGGTGCAATAATTTATA
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ACAAAGACCTCTTCAGCAACCCGAGCAGTCCCCAAAGAACGGGAGGCAGCGGCGGGTACCGGACGAGCGAC
GGACGAGAACCGGGCGAAGAGGCGCCCCCCGAAAAGTGAAGCAAAAACCGCGTAACCTCGTTCCCGGCGCT
TGCATTCCAGCCATCCGGTTTTCGCGGGGCGAGGGAAAAAAACTGTAAATTTGTTCCTCAGCATCTCTAGA
CAAGAACCCCCGCCAGGCACAATTGTGGAAGCGTTCCCGCTCTTCCAAGTCATCTCCAGGTTTGCATCTGT
GCCGGCGTCGTCGTCAAGA*ATGGCTCCCACGTCAACGCTTCTGGCTTTCCTCGGCG*

ISCW007687-*peritrophin-3*

GGCACACGGAGTTGCAAAATAGTTACGTTGCATAGTGGTTCGGCAGCCCTATTGCACTTCGCTCGCCAGGA
AAACCCGAAGACAGTGATCTTGAAGATGGGCTTGTATAATAACATGTTTGAAACCGAACAACTATAGATAT
CAGAAAAACACGTGTCTTATCTCTATAATTCGGCACTGCTAGAACCTGAGATAATAAAATTAGGAAAGAAG
ACGTTAAACTTTTTTGTTTGTTCAAGAGTCGCCAGCTCCCTTGCGCGATCACTTTTCTTTTAGTTATCTAA
ATCTGGCCGGGTAGGCGAGCGAGGGCGCAGATTTAGTTGTAGCTACTTCACTTGAAGAAATAAAGCAACAT
GCCAGTGAGATAATTCAAGTAAGACTTTCGTTATTCGGCGTTCGGAAGTGACCCATAATTGAACATCAGTG
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ACACCGGCATTAAAATGGCGGAGCCCAGGGGTCGTAATTACAACAAACGTGTATACGTCCAAGATATATTG
GCATTTTTCACAAATCATTTTGTATCCATTTTTTTTAGAAGGAGGATCACCTGGAGGTCCAGGACAACCTG
GGGAGCCCGGTGAACCAGGACAGCCCGGGGGACCGGGCCAGCCTGGAGGTCAAGGGCCACCCGGAGGTCAA
GGACAGCCCGGAGGACAGGGTCGACCAGGAGAGCCCGGTCAACCTGGAGTCGCTCCTCCCGCCACTGATGT
CACCTCGCCAAAGCAG

ISCW013029-*peritrophin-4*

GACTTTGAACACAGATTCTGGACTCAAGTTAACGAGGTTGCCCAAAATATGTACCTATTGCCTCTCCGACG
TAAGATACTTAATGCACCTATGGAAAGTTCTCTAGAAAAGAACCCCAAAGGAAATATCTTGCCTTTTCACA
AAAAAAAAATTGTTTGTTGTACTTGTGTTTTTTTAGACAATTTCTCTCCGGTTACTACGATAGTTTCTTTA
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TTGCCCATTCCTATATTATTTCAGGTTCCGTCGGGTGTTTCCACCACTGATTCTGCCGATGTCGACGAATG
ACAGCCTTCCACAAACTTCGACACCATGGTTCAAGTCCAAGTCATGTCCATCAATGCGCTGGTGAAGAAAG
TGTCACTGCCTCATGGAGACACCGTTTGTTGCGTAGCCTGTAAATAAAGATGCGATTAAATTTTACAATGC
CCAATAAAACACGAGAATATATAAGCAGCATTACTCTGTTATCACCAGAACTTTAGCGCCCCTTCACCTCC
GTGCCCTCCCACAGGGAGACGTTATCTCGCTTGATAGGTAGTGGCAACAACGGTGTGGCAAATATGGTTAC
CAACAACTTAGGAAACGAAGAGTGCCGCAAGTCCTGAGGACCTCACTATTTCTCCCCAGCCAATGAGGTTG
CGGCATTAGCCCAGAATACCACCAAAGAACGAATCAGAAAGCAAGAAGCGTGTTCATAATAGGCCCCACCC
TTCGTTTTTTAAGGTCTCAGTGACTGTGGAGGCGATGACCAGGCGCTTATTCAGCAGCTAGTTCAAGGCTT
CTCTGGAGTAACTGTAAAGTTTGTAAACAAACAAGGAGCACTGTCACATATAGCTACTTACACACTGAAAG
GACAAATGTAAAATAATAAATATAACAAAAAAAAGAACTATCCAAAATTGAATTCTGGTTTGCAAAATTTG
CGGCAGTACTTTGTTCGTATCCGACGAATTCGTCATATTTATAGCGCGTTCTTGAGTCGGTGAATCTCGAT
GCCATTTTTTGCGTACGAAGAATCGTATCGCAAATTCTGACGGAAACGTTGTTGGTTATTGTTATTGTCAT
TCAATCATTTATATGAGTTAAGAGCAGTGAGCAGCCACCCTGGAGTGAGCTCAACAGCGTCTCAATCTTGC
GGCTCACGTTTTCACCAAATCATCCTCGCTAGAAAGCTATTATACAAAAATATTGCTGGTAGTACTTTCCT
CTTGCCTAAAAAAAGAAAAAGCAACCATAAGCAGAAGACATTTTTGGGTCGATCCCGGTTCAATTATTTGA
ACTCATGCCACGGCTTCACTCCGACTGTAGTATAGAGTTTTTCTTTTCTCTTTCGCATCCTTTTTTAGCGT
CTTACATTTTTATCAAAGTATATATACTCTCACCAGAAACAAAGCTACGTTAGAGTCTAGCTTGCATATTT
TTTGACTTGTATCTGGAGTACCACCTGAAGGGTGAGGCACAAGAAATGAATTTCGAAACCGATGTGATTGT
ACAACTTGTTAAGTAACGTCAGTGGTAATCTAGCAATCGGCGATGGCTTAAGAACTACTGAGAATGGGAAA
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CTATATTGATAATGAAGATGTTCGATGATCGCCACCCCCCGTCGTCTTGAAGCTGTCAGGGGCTATAACTC
AACATAATCGCCGCAAGCGAAAACCCGTTTGAACAGGTTGTAGGAGCAGCAGCCAGACGGTGACAAATCTG
TTAGAACTGTCCCACTCGAAATCTACCAAAGCCAAACACAAACCGGGTCTATCGTACGGCTGCGCATAATG
AACACGTAGACGCCTCTACCAGGTGACATGACGATGTCTACCAGAA

ISCW024120-*peritrophin-5*

TGCTGAGTAGACAGCTGACATATGCAGGCTCCCTCCATATGCGGCTAGCCACTGAGATGTACATTAAGAAA
TACACGTATTGCAGATTTTGAATCGGCGTTCTCTCATCCAGAAATAATCGAATGAGAGAACATAGAAGACTC
AACCTTCAAGGCGTAGGTCTAATTAGCGTTTTGACTTGAGGAAGTACTCGAGGTTCACGCGAAGAGTATGG
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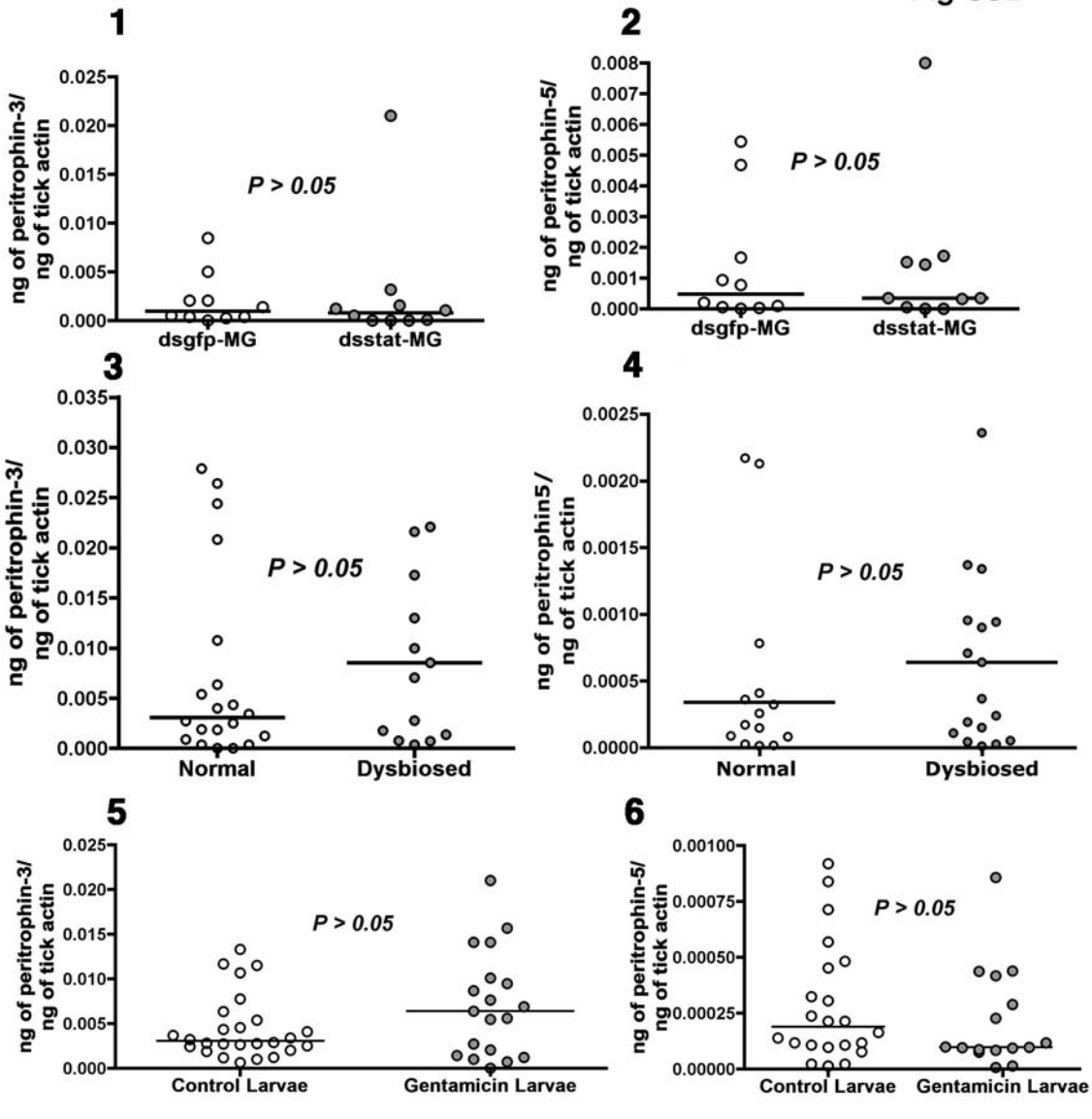
TGCACGCGCGTTCCTGCATGTATAGTGCGAA

Fig S3B


1
ng of peritrophin-3/ ng of tick actin
0.025
0.020
0.015
0.010
0.005
0.000
P > 0.05
dsgfp-MG
dsstat-MG
2
ng of peritrophin-5/ ng of tick actin
0.008
0.007
0.006
0.005
0.004
0.003
0.002
0.001
0.000
P > 0.05
dsgfp-MG
dsstat-MG
3
ng of peritrophin-3/ ng of tick actin
0.035
0.030
0.025
0.020
0.015
0.010
0.005
0.000
P > 0.05
Normal
Dysbiosed
4
ng of peritrophin5 / ng of tick actin
0.0025
0.0020
0.0015
0.0010
0.0005
0.0000
P > 0.05
Normal
Dysbiosed
5
ng of peritrophin-3/ ng of tick actin
0.025
0.020
0.015
0.010
0.005
0.000
P > 0.05
Control Larvae
Gentamicin Larvae
6
ng of peritrophin-5/ ng of tick actin
0.00100
0.00075
0.00050
0.00025
0.00000
P > 0.05
Control Larvae
Gentamicin Larvae

SUPPLEMENTAL FIGURE LEGENDS

Suppl Figure 1 related to Figure 1. Dysbiosis alters larval feeding and *B. burgdorferi* colonization. A. Engorgement weights of buffer-treated (Control) and gentamicin-treated (Gentamicin) larvae fed on *B. burgdorferi*-(Bb914) infected C3H mice. **B**. qRT-PCR analysis of *B. burgdorferi* burden in engorged buffer-treated (Control) and gentamicin-treated (Gentamicin) larvae fed on GFP-*B. burgdorferi* (Bb914)-infected C3H mice. Each data point in A through B represents pools of 5 larvae. Horizontal bars represent the median, and mean values significantly different in a two-tailed non-parametric Mann-Whitney test ($P < 0.05$) indicated.

Suppl Figure 2 related to Figure 3. Mitotic activity in nymhpal guts at different times of feeding. **A**. Immunofluorescence microscopy of nymphal gut fed for 24, 48, 72 and 96 h on C3H mice showing PH3 positive signal (green/Alexa 488). Nuclei stained with DAPI (Blue). **B.** Quantification of PH3 positive signal/gut at 24, 48, 72 and 96 h of feeding. Magnification x10. Each data point in B represents one gut. Horizontal bars represent the median, and mean values significantly different in a two-tailed non-parametric Mann-Whitney test ($P < 0.05$) indicated. Quantitative-RT-PCR analysis of: **C**. Expression levels of *stat;* and **D**. *B. burgdorferi* burden in dsgfp and dsstat RNA-injected nymphs at 24 and 72 h of feeding. Each data point in C-D represents a pool of 2-3 nymph guts. Horizontal bars represent the median and mean values significantly different in a two-tailed non-parametric Mann-Whitney test ($P < 0.05$) indicated.

Suppl Figure 3 related to Figure 4. Canonical Stat-binding sites in the 5'untranslated regions of *Ixodes. scapularis* Peritrophin encoding genes. A. *In silico* analysis of 2000 base pairs upstream of putative Peritrophin-encoding genes ISCW006076 (peritrophin-1) and ISCW013030 (peritrophin-2), ISCW007687 (peritrophin-3), ISCW013029 (peritrophin-4) and ISCW024120 (peritrophin-5) extracted from the I. *scapularis* genome scaffold reveals at canonical STAT binding sites (Highlighted in pink). The first 35 base pairs of the translational start site is shaded grey. **B.** Quantitative-RT-PCR analysis of expression levels of *peritrophin-3, and 5* in replete: guts of dsgfp and dsstat RNA-injected nymphs (**1-2**); dysbiosed and normal larvae (**3-4**); and gentamicin-exposed and normal larvae **(5-6).** Horizontal bars represent the median and mean values significantly different in a two-tailed non-parametric Mann-Whitney test ($P < 0.05$) indicated. In 1-2, each data point represents a pool of 2-3 nymphal guts. In 3-6, each data point represents a pool of 5 larvae.

Supplemental Table 1. Primers utilized in this study

Gene Name	Forward primer sequence	Reverse primer sequence
Double stranded RNA primers		
peritrophin-1	taatacgactcactatagggcgtgtcttattcctgtc	taatacgactcactatagggcgtaatggtcgtgatgtc
gfp	taatacgactcactatagggcgacgtaaacggccacaagtt	taatacgactcactatagggcgcgggtcttgtagttgccgtc
stat	taatacgactcactatagggccctggattgaagacaag	taatacgactcactatagggcaccactcctgtatctg
Quantitative PCR primers		
peritrophin-1	atgccgaataaggtcgactg	agtggaatctggacggaatg
peritrophin-2	agaagggattggccttcaac	tacaggctacgcaacaaacg
peritrophin-3	tgtgacaagacgacctccag	catcgagttcttggcctgt
peritrophin-4	acctgtcgacggatgtgact	cctcgcacgtgtagctgtag
peritrophin-5	acctgggattccagtgtcc	ccagccgtgcacgttgag
16s rRNA	agagtttgatcctggctcag	catgctgcctcccgtaggagt
socs-2	agcatctgtgtagactag	gatagtcattcacgtagtc
stat	aggtcaaggtgtccatcatc	gatactccattgttcctgtgttg
EMSA primers		
Biotperitrophinprobe-1F	Biotin-ggaattacttaagcaacgagtgagaattatttc Ggtgttcttcgaaactatgcattgttcacacgaaaatggca cacgattcgttattgatacaatgtaattgaac	Biotin-gttcaattacattgtatcaataacgaatcgtgtgccattttcg Tgaacaatgcatagtttcgaagaacaccgaaataattctca ctcgttgcttaagtaattcc
Non-specific probe-1F	agaacctctg tctgcatctg	agaagggaatttaaataag
RNA-FISH primers		
senseperitrophin-1	gcgtgtcttattcctgtc	taatacgactcactatagggcgtaatggtcgtgatgtc
sensestat	ccctggattgaagacaag	taatacgactcactatagggcaccactcctgtatctg
antisenseperitrophin-1	taatacgactcactatagggcgtgtcttattcctgtc	cgtaatggtcgtgatgtc
antiense stat	taatacgactcactatagggccctggattgaagacaag	gcaccactcctgtatctg

Supplemental Experimental Procedures

Mice and ticks

Female C3H/HeN mice (4 - 6 weeks old) were purchased from NIH/NCI and all animal experiments were performed according to the protocols approved by the Institutional Animal Care and Use Committee at the Yale University School of Medicine. *I. scapularis* nymphs and larvae were obtained from a tick colony maintained in the laboratory in an incubator at 23°C with 85% relative humidity and a 14/10 h light/dark photo period regimen.

454 pyrosequencing of DNA from larvae and nymphal guts

DNA was extracted from pools of ~20 unfed (total of ~200 unfed normal or dysbiosed larvae) or 5 fed larvae (a total of 50-75 fed normal or dysbiosed larvae) or from guts of individual laboratory reared or field-collected nymphs (10-15 nymphs) using the Genomic DNA Extraction Kit according to the manufacturer's instructions (Qiagen, Valencia, CA). The V2 variable region (V2) of the bacterial 16*S* rRNA was amplified from 1 μl of the purified genomic DNA from each sample using 12-base barcoded primers sets described by Hamady *et al* (Hamady et al., 2008). Briefly, 16S rRNA gene were amplified from individual nymphal guts or pools of five larval DNA sample, using the universal forward and reverse primer listed in Table S1, tagged additionally with a unique 12-base barcode to mark PCR products from individual samples. PCR reactions

were conducted using a Platinum PCR high-fidelity DNA polymerase (Invitrogen, CA). PCR amplifications were performed in triplicate for each sample essentially as described by Fierer et al (Fierer et al., 2008). Amplified bacterial 16*S* rRNA products were purified using the QIAquick Gel Extraction Kit (Qiagen, Valencia, CA), quantified using the Nanodrop 2000C (Thermoscientific, Wilmington, DE), pooled together in equimolar concentrations, ethanol precipitated and suspended in 80 μl of sterile RNAse and DNAse free water to a concentration of 8-10 ng /μl of each sample. The pooled DNA was sequenced on a 454 Life Sciences FLX Genome sequencer at Environmental Genomic Core Facility (University of South Carolina, SC) or on a 454 GS Junior system using the Roche/454 Titanium chemistry.

16S rDNA sequence analysis.

The sequences were analyzed using the "Quantitative Insights into Microbial Ecology (QIIME) pipeline (Caporaso et al., 2010). All sequences that were less than 200 bp or greater than 580 bp in length or with a quality score less than 25 were discarded. The remaining sequences were grouped into Operational Taxonomic Units (OTUs) at the species level by clustering them based on a sequence similarity score of 97% using the UCLUST program version 1.2.22 (Edgar, 2010). From sequences in each OTU the most abundant sequence was picked as the representative sequence for downstream analysis. OTUs were assigned biological taxonomy by referring to the Greengenes database [greengenes.lbl.gov] release February 4, 2011 using multiple sequence analysis in PyNAST with minimum sequence similarity of 75% and Ribosomal Database Project

(RDP) Bayes classifier version 2.2 with confidence level at 0.8. A phylogenetic tree was built using the representative sequences in all the OTUs using FastTree version 2.1.3 (Price et al., 2010). This tree was used to compute the weighted and unweighted UniFrac (Lozupone et al., 2006) distances between any two sequences in the OTUs. Groups of samples were compared based on their beta diversity statistics computed using the principal coordinate analysis (PCA) of UniFrac distance matrix (weighted and unweighted), after rarefying and jack-knifing to a depth of 100 sequences per subset, in the QIIME pipeline. To visualize the variation among groups of samples, PCA plots that best matched the pair-wise distances between sample groups were generated.

Collection of *I. scapularis* nymphs from Branford, CT

Questing *I. scapularis* nymphs collected by flagging or dragging between 2009 and 2010, June through August at Lake Gaillard, North Branford, CT, preserved in 70 % ethanol as described earlier (Hanincova et al., 2006), dissected and genomic DNA prepared from 15 ticks individually as described below.

Tick RNA isolation, quantitative RT-PCR and RNA interference

RNA and DNA isolation from nymphal guts and whole larvae and quantitative PCR analysis was essentially conducted as described earlier (Narasimhan et al., 2007). Fed and unfed nymphs and larvae were surface sterilized in 70 % ethanol and dissected on sterilized slides, using sterile razors, forceps, and water. Gut tissues from nymphal

ticks were collected in pools of 2-3 ticks into sterilized eppendorf tubes and whole larvae in pools of 5 processed for RNA isolation using Trizol (Invitrogen, CA). cDNA was synthesized using the iScript RT-PCR kit (BioRad, CA). DNA was isolated from unfed and fed larvae in pools of ~ 20 and 5 larvae respectively and bacterial loads in whole larvae quantified by Q-PCR analysis of DNA using SYBR Green Master Mix (Invitrogen, CA) and universal 16S rRNA gene primers targeting the V2 region of the 16S rRNA (Table S1) as described (Hamady et al., 2008); and for *B. burgdorferi* burden using *flaB* primers as described (Narasimhan et al., 2007). qRT-PCR for expression levels of *peritrophin1*, *2*, 3, 4, and 5, *stat*, *socs2*, were done using SYBR Green Master Mix (Invitrogen, CA) and primers listed in Table S1, and data normalized to tick actin (Narasimhan et al., 2007). Double stranded (ds) RNA targeting *stat* transcripts was generated as described earlier (Liu et al., 2012) and dsRNA targeting *peritrophin-1*, and control dsgfp RNA generated using primers listed in Table S1, and fed nymphal gut cDNA or pCAG-GFP plasmid (www.addgene.org) as templates respectively, and dsRNA injected into the analpore of nymphal ticks as described (Narasimhan et al., 2007).

Immunofluorescence microscopy

Guts from nymphal ticks were dissected, fixed in 4 % PFA for 20 minutes, washed 3 times in PBS /0.5 % Tween20 and blocked in PBS/0.5%Tween20, 5 % Fetal Calf Serum for 1 hour prior to incubation with polyclonal mouse anti-PH3 (AbCam, MA) antibody as described (Buchon et al., 2009b). Polyclonal mouse anti- PH3 binding was detected using Alexa Fluor 488-conjugated goat anti-mouse antibody (Invitrogen, CA).

Nuclei were stained with DAPI (Invitrogen, CA) and stained guts visualized under a Zeiss Axioscope Fluorescence Microscope (Carl Zeiss Inc, NY) at 10x magnification.

Artificial feeding of nymphs

Capillary feeding was performed as described earlier (Soares et al., 2005) with a solution of fluorescein-conjugated 500,000 MW dextran and Rhodamine-red-conjugated 10,000 MW dextran (Molecular probes, CA) mixed in equimolar concentrations (2 μM in PBS/0.2 % sucrose) for 1 h. The ticks were then dissected, and guts placed in a drop of ProLong™ Gold antifade reagent, cover-slipped and visualized immediately under a Zeiss LSM510 Confocal microscope at 40x magnification.

Electrophoretic Mobility Shift Assay (EMSA)

Oligonucleotides (100 bp long) complementary to the promoter region of *peritrophin-1* containing the conserved STAT-binding sites (Table S1) were synthesized at the Yale University oligonucleotide synthesis facility. Identical oligonucleotides synthesized without the biotinylation served as the specific competitor probe. The oligonucleotides were suspended in TE buffer, diluted to 1μg/50 μl and forward and reverse strands mixed, denatured at 95°C for 5 minutes in a heat block and annealed by allowing the heat block to slow cool to room temperature. The annealed probes were electrophoresed and probe purified using the QIAquick gel extraction kit (Qiagen, CA). A 100 bp DNA fragment of *B. burgdorferi flaB* (Genbank accession#AF416447)

spanning nucleotides 121 to 221 of the coding region (that does not contain any known STAT binding sites) was amplified using primers listed in Table S1 and *B. burgdorferi* N40 genomic DNA as template, and purified amplicon utilized as the non-specific competitor probe. The assay was conducted using the Light Shift Chemiluminescent EMSA kit (ThermoScientific, IL) as directed by the manufacturor. Recombinant STAT (0.5 ug) generated in the *Drosophila* expression system (Liu et al., 2012) was incubated with 20 ng of biotinylated probe in the presence or absence of 100 ng of unlabeled specific probe or unlabeled non-specific probe at room temperature for 30 min in binding buffer containing 5 % glycerol and 50 ng of poly (dI.dC) prior to electrophoresis on 4 % polyacrylamide gels (BioRad, CA) in 0.5X TBE (Tris-Boric acid-EDTA) buffer and transferred to positively charged nylon membranes (GE-Healthcare Life Sciences, NJ). Electrophoretic mobility of the probe was visualized using the Chemiluminescent Nucleic Acid Detection Module (Thermoscientific, IL).

RNA-Fluorescence in situ hybridization (RNA-FISH)

About 200 bp-long *stat* or *peritrophin-1* amplicons were generated using primers listed in Table S1 containing T7 promoter sequences at the 5' or 3' ends. Sense and antisense RNA complementary to *stat* and *peritrophin-1* were synthesized using stat or peritrophin-1 amplicons generated above as templates for *in vitro* transcription. Sense and antisense RNA synthesis, and Alexa Fluor reactive dye labeling of the RNA was performed as described in the FISH Tag^{TM} RNA multicolor kit (Invitrogen, CA) user manual. Nymphal ticks were allowed to feed on pathogen-free C3H mice for 48 h as

described above, guts dissected and fixed in 4 % PFA overnight. The guts were processed and hybridized to a cocktail of Alexa Fluor 488-labelled antisense *stat* and Alexa Fluor 594-labelled *peritrophin* antisense RNA as described in the FISH Tag^{TM} RNA multicolor kit user manual (Invitrogen, CA) and visualized at 25x or 63x magnification under a Zeiss LSM510 Confocal microscope. Control guts were labeled with a cocktail of Alexa Fluor 488-labelled sense *stat* and Alexa Fluor 594-labeled *peritrophin* sense RNA.

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