SI Material and Methods

Insect Rearing. *Spodoptera littoralis* larvae were reared on artificial diet (47.25 g/L wheat germ, 67.3 g/L brewer's yeast, 189 g/L corn meal, 6.75 g/L ascorbic acid, 0.75 g/L cholesterol, 0.5 g/L propyl 4-hydroxybenzoate, 3 g/L methyl 4-hydroxybenzoate, 1.25 g/L wheat germ oil, 33.75 g/L agar and 3 g/L vitamin mix (1.2 g/Kg vitamin B1, 2.6 g/Kg vitamin B2, 2.5 g/Kg vitamin B6, 40 g/Kg choline, 10 g/Kg pantothenic acid, 32 g/Kg inositol, 0.25 g/Kg biotin, 2.5 g/Kg folic acid, 5 g/Kg 4-aminobenzoic acid, 0.5 mg/Kg vitamin B12, 10 g/Kg glutathione, 2.1 g/Kg vitamin A, 0.25 g/Kg vitamin D3, 24 g/Kg vitamin E, 0.25 g/Kg vitamin K, 25 g/Kg vitamin C in dextrose)), at 25 ± 1°C, 70 ± 5% R.H., and under a 16:8 h light/dark period.

Cry1Ca Toxin Preparation and Toxicity Bioassays. The Cry1Ca protein was produced in a recombinant *Bacillus thuringiensis* (*Bt*) strain EG1081, kindly supplied by Ecogen Inc. Production and purification of Cry1Ca was done as previously described (1). The purity of the Cry1Ca preparation was checked by SDS-PAGE. Prior to use, Cry1Ca was dialyzed overnight, at 4°C in 50 mM sodium carbonate buffer, pH 9.0. After dialysis, toxin concentration was determined by Bradford assay (2), using bovine serum albumin as standard.

The experimental larvae were singly isolated in multi-well plastic trays (Bio-Ba-32, Color-Dec, Italy), containing artificial diet, covered with perforated plastic lids (Bio-Cv-4, Color-Dec), and maintained under the rearing conditions reported above. For the first three days, the upper surface (1 cm^2) of the artificial diet (0.3 cm^3) was uniformly overlaid with 50 µL of water suspension of Xentari™ (Valent BioSciences), a bioinsecticide based on *Bt* subsp. *aizawai* and containing several Cry toxins (Cry1Aa, Cry1Ab, Cry1Ca, Cry1Da, and Cry2Ab), or of purified Cry1Ca toxin (the component of Xentari™ most active against *S. littoralis* (3)) dissolved in 50 mM sodium carbonate buffer at pH 9.0, which was prepared as described above. Control larvae were reared on artificial diet overlaid with 50 µL sodium carbonate buffer or water, as appropriate. Experimental larvae

were maintained on artificial diet, replaced every 24 h, and daily inspected for survival, until pupation. The experimental procedure is schematically summarized in Fig. S7.

To determine the 50% lethal concentration (LC_{50}) of Xentari™ and Cry1Ca toxin, the bioassay described above was carried out at 5 different concentrations plus a control using 16 larvae for each experimental condition. Mortality was assessed after 8 days and Probit analysis (4) was performed with the POLO-PC program (LeOra Software, Berkeley, CA, USA), to determine LC_{50} values, 95% fiducial limits and toxicity increase ratio (TI) for each experimental treatment.

qRT-PCR. Total RNA was isolated from experimental samples by using TRIzol Reagent (Invitrogen), according to the manufacturer's instructions. The concentration and purity of total RNA were determined using a Varioskan™ Flash Multimode Reader (Thermo Scientific, Waltham, MA, USA). Differential relative expression of considered genes was assessed by means of SYBR-Green qRT-PCR, using the primer pairs reported in Table S6. Relative gene expression data were analyzed using the $2^{\Delta\Delta CT}$ method. For validation of the ΔΔCt method the difference between the Ct value of AMPs, *lysozyme 1a* or *16S rRNA* and the Ct value of *β-actin* transcripts [ΔCt = Ct(sample)-Ct (β-Actin)] was plotted versus the log of two-fold serial dilutions (200, 100, 50, 25 and 12.5 ng) of the purified RNA samples. The plot of log total RNA input versus ΔCt displayed a slope lower than 0.1 (*attacin 1*: Y=1.3987 + 0.0144X, R^2 = 0.0565; *gloverin*: Y=1.3567 + 0.013X, R^2 =0.0715; *lysozyme 1a*: Y=1.690 + 0.0124X, R^2 =0.0313; *16S rRNA*: Y=1.447 + 0.0128X, R^2 =0.0576), indicating that the efficiencies of the two amplicons were approximately equal.

Bioinformatics analysis of the *16S rRNA* gene sequencing data. Raw reads were filtered according to the 454 processing pipeline. Sequences were then analyzed and further filtered by using QIIME 1.7.0 software (5) as previously described (6). Operational Taxonomic Units (OTU), defined by a similarity of 97%, were picked using the uclust method (7), and the representative sequences were submitted to the RDPII classifier (8) to obtain the taxonomy assignment and the

relative abundance of each OTU using the Greengenes *16S rRNA* gene database (9). Alpha diversity and beta diversity were evaluated through QIIME as previously reported (10).

Light Microscopy and Transmission Electron Microscopy. The experimental larvae, obtained as described in the main text, 5 days after the onset of the experiment were chilled on ice and dissected to isolate the midgut. Synchronous starved controls were also processed to assess the impact on midgut tissues by feeding cessation following intoxication. The samples were fixed in 4 % glutaraldehyde (in 0.1 M Na-cacodylate buffer, pH 7.4) overnight, at 4 °C, and embedded in an Epon/Araldite 812 mixture as described previously (11). Semi-thin sections were stained with crystal violet and basic fuchsin and observed by using a Nikon Eclipse Ni-U microscope equipped with a DS-5 M-L1 digital camera system (Nikon, Tokyo, Japan). Thin sections, stained with uranyl acetate and lead citrate, were observed by using a Jeol JEM-1010 electron microscope (Jeol, Tokyo, Japan) and images were acquired with an Olympus Morada digital camera (Olympus, Münster, Germany).

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98 **Table S1. Statistical analysis of the data reported in figures 1D, 3, S2.**

Figure 1D and S2			
	interaction	dsRNA treatment	immune challenge
Figure 1D			
attacin 1	$F_{3,66}$=0.36; *P*=0.784	$F_{1,66}$=0.13; *P*=0.7182	$F_{3,66}$=198.13; *P*<0.0001
gloverin	$F_{3,66}$=0.07; *P*=0.9734	$F_{1,66}$=1.53; *P*=0.2295	$F_{3,66}$=39.58; *P*<0.0001
lysozyme 1a	$F_{3,66}$=0.16; *P*=0.9241	$F_{1,66}$=0.07; *P*=0.7989	$F_{3,66}$=95.60; *P*<0.0001
Figure S2A (midgut)			
attacin 1	$F_{3,66}$=0.09; *P*=0.9647	$F_{1,66}$=0.36; *P*=0.5523	$F_{3,66}$=83.23; *P*<0.0001
gloverin	$F_{3,66}$=0.21; *P*=0.8874	$F_{1,66}$=0.33; *P*=0.5701	$F_{3,66}$=61.58; *P*<0.0001
lysozyme 1a	$F_{3,66}$=0.34; *P*=0.7935	$F_{1,66}$=0.48; *P*=0.4941	$F_{3,66}$=62.38; *P*<0.0001
Figure S2B (fat body)			
attacin 1	$F_{3,66}$=0.45; *P*=0.7177	$F_{1,66}$=0.04; *P*=0.8351	$F_{3,66}$=27.86; *P*<0.0001
gloverin	$F_{3,66}$=0.39; *P*=0.7618	$F_{1,66}$=0.61; *P*=0.9605	$F_{3,66}$=26.16; *P*<0.0001
lysozyme 1a	$F_{3,66}$=1.56; *P*=0.2062	$F_{1,66}$=0.25; *P*=0.6189	$F_{3,66}$=32.16; *P*<0.0001

Figure 3				
	interaction	dsRNA treatment	Cry1Ca treatment	Time
Figure 3*A* and *B*				
	$F_{3,176}$=0.08; *P*=0.924	$F_{1,176}$=22.65; *P*<0.0001	$F_{3,176}$=45.65; *P*<0.0001	$F_{3,176}$=310.36; *P*<0.0001
Figure 3*C* and *D*				
	$F_{2,81}$=24.63; *P*<0.0001	$F_{1,81}$=30.63; *P*<0.0001	$F_{2,81}$=16.94; *P*<0.0001	$F_{2,81}$=0.09; *P*=0.965

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Table S2. Microbial viable counts.

	Log CFU/mL ± S.D.*
***102 Sl* dsRNA**	
Control (M)	6.68 ± 0.20 [a]
Control (H)	4.28 ± 0.29 [b]
0.2 µg protein/cm^2 (H)	5.94 ± 0.32 [b]
1.2 µg protein/cm^2 (H)†	8.73 ± 0.23 [c]
***GFP* dsRNA**	
Control (M)	6.48 ± 0.44 [a]
Control (H)	4.22 ± 0.20 [b]
1.2 µg protein/cm^2 (H)	5.42 ± 0.16 [c]
6.6 µg protein/cm^2 (H)†	8.68 ± 0.01 [d]

Number of bacteria was assessed in the midgut content (M) and in the haemolymph (H) of *Spodoptera littoralis* larvae treated with *GFP* dsRNA or *102 Sl* dsRNA and exposed to different doses of Cry1Ca.

*Data are expressed as mean of Log CFU (Colony Forming Units)/ml ± S.D. of two independent experiments. Within the same type of treatment, different letters denote statistically significant differences (One-Way ANOVA; P<0.0001, F=56.61, df=3, n= 4 for *102 Sl* dsRNA and P<0.0001, F=238.62, df=3, n= 4 for *GFP* dsRNA treated larvae).

†LC_{50} of Cry1Ca.

Table S3. Alpha diversity measures.

	Alpha diversity*		
	Chao 1	Shannon	Good's coverage (%)
***GFP* dsRNA**			
Control (M)	852.28	2.62	99
Control (H)	1800.40	3.80	98
1.2 µg protein/cm^2 (M)	769.92	2.87	99
1.2 µg protein/cm^2 (H)	1672.79	4.04	98
6.6 µg protein/cm^2 (M) #	1065.13	3.48	98
6.6 µg protein/cm^2 (H) #	1040.08	2.80	99
***102 Sl* dsRNA**			
Control (M)	724.45	2.24	99
Control (H)	2200.32	4.67	98
0.2 µg protein/cm^2 (M)	717.44	2.49	99
0.2 µg protein/cm^2 (H)	2480.00	4.93	98
1.2 µg protein/cm^2 (M) #	687.24	2.42	99
1.2 µg protein/cm^2 (H) #	1183.64	2.50	99

Average observed diversity and estimated sample coverage was calculated, in 3 independent experiments, for *16S rRNA* amplicons of midgut content (M) and haemolymph (H) samples of *Spodoptera littoralis* larvae, treated with *102 Sl* dsRNA or *GFP* dsRNA and exposed to different doses of Cry1Ca.

*Chao 1 richness, Shannon diversity, and Good's coverage were calculated with Qiime at the 3% distance level. # LC_{50} of Cry1Ca.

119 **Table S4. Relative abundances of microbial taxa.**

	Incidence* (%)											
	GFP dsRNA						*102 Sl* dsRNA					
Taxon	**Control H**	**1.2 μg/cm² H**	**6.6 μg/cm² H**	**Control M**	**1.2 μg/cm² M**	**6.6 μg/cm² M**	**Control H**	**0.2 μg/cm² H**	**1.2 μg/cm² H**	**Control M**	**0.2 μg/cm² M**	**1.2 μg/cm² M**
Acetobacter sp.	52.559	47.156	4.980	1.182	0.712	0.831	42.556	36.689	5.000	0.875	2.659	0.449
Acetobacter orleanensis	6.634	6.923	1.376	0.368	0.359	0.439	6.081	7.566	1.498	0.480	0.941	0.272
Acetobacteraceae	0.198	0.014	0	0	0	0.005	0.056	0.010	0	0	0	0
Acidovorax sp.	0.008	0.018	0	0	0.006	0.125	0.015	0.029	0	0	0	0.010
Acinetobacter sp.	2.565	0.294	0	0	0.006	0.005	0.030	0.039	0.010	0	0	0.005
Actinobacteria	0.012	0.047	0.003	0.008	0	0	0.139	0.128	0.003	0.004	0.005	0
Actinomyces oris	0.113	0	0	0	0	0.005	0.256	0.007	0	0	0	0
Bacilli	0.093	0.165	0.029	0.030	0.046	0.078	0.090	0.189	0.026	0.011	0.022	0.005
Betaproteobacteria	0.020	0.125	0.003	0	0	0.031	0.105	0.277	0.003	0	0	0.005
Carnobacteriaceae	0.028	0.004	0	0	0	0.026	0.045	0.228	0.003	0	0	0.005
Caulobacteraceae	0.532	0.047	0	0	0	0	0.023	0.039	0.003	0	0	0
Clostridium sp.	0.464	1.142	26.672	54.104	60.839	51.203	1.437	0.567	20.890	50.157	68.031	32.838
Comamonadaceae	0.012	0.057	0.003	0	0	0.058	0.045	0.140	0	0	0	0
Corynebacterium sp.	0.008	0.025	0.003	0	0.006	0.005	0.075	4.800	0.010	0.004	0	0
Deltaproteobacteria	0.561	0.263	0.075	0.015	0.011	0.335	1.775	5.325	0.071	0.014	0.011	0.015
Enterobacteriaceae	0.496	0.075	3.304	2.649	1.721	1.788	0.387	0.061	3.566	3.238	1.408	4.618

Continued table S4

	***GFP* dsRNA**						***102 Sl* dsRNA**					
Taxon	**Control H**	**1.2 μg/cm^2 H**	**6.6 μg/cm^2 H**	**Control M**	**1.2 μg/cm^2 M**	**6.6 μg/cm^2 M**	**Control H**	**0.2 μg/cm^2 H**	**1.2 μg/cm^2 H**	**Control M**	**0.2 μg/cm^2 M**	**1.2 μg/cm^2 M**
Enterococcus sp.	3.404	16.019	6.017	7.512	11.189	18.328	8.070	10.524	2.337	1.441	5.323	3.324
Enterococcus lactis	0.540	2.126	0.382	0.565	0.456	2.777	1.200	1.690	0.117	0.160	1.267	0.425
Escherichia sp.	0.113	0.032	0.016	0.008	0	0.010	0.335	0.201	0.013	0.004	0	0
Gammaproteobacteria	0.552	3.275	0.085	0.049	0.034	0.878	2.159	5.742	0.052	0.011	0.049	0.025
Hyphomicrobium sp.	0.141	0.476	0.026	0.019	0.011	0.251	0.478	1.386	0.023	0	0.016	0.005
Hyphomicrobium zavarzinii	0.077	0.254	0.026	0	0	0.068	0.165	0.557	0.006	0	0.005	0
Klebsiella oxytoca	0.149	0.014	0	0.004	0	0.005	0.034	0.005	0	0	0	0
Lactobacillus brevis	3.496	2.187	0.603	0.042	0.040	0.110	5.111	2.644	0.544	0.014	0.049	0.099
Lactobacillus crispatus	0.032	0.018	0	0	0	0.005	0.034	0.152	0.016	0	0	0
Lactobacillus pentosus	18.055	8.144	0.956	0.110	0.108	0.178	9.518	6.164	1.184	0.132	0.043	0.084
Lactobacillus sp.	0.177	0.089	0.016	0	0.006	0	0.154	0.091	0.032	0	0	0
Lactococcus lactis	0.379	0.072	0.013	0.008	2.404	0.852	0.406	0.255	0.146	0.004	0.027	0.815
Leuconostoc sp.	0.452	0.766	0.228	0.004	0	0.021	0.824	1.803	0.252	0.014	0.011	0.015
Limnobacter sp.	0.758	3.293	0.098	0.042	0.046	0.596	2.396	6.706	0.081	0.018	0.054	0.005
Mycobacterium sp.	0.016	0.079	0.003	0	0	0.016	0.038	0.155	0.003	0	0	0
Nocardioidaceae	0.073	0.183	0	0	0	0.037	0.173	0.419	0.006	0	0	0

Continued table S4

	***GFP* dsRNA**						***102 Sl* dsRNA**					
Taxon	**Control H**	**1.2 µg/cm² H**	**6.6 µg/cm² H**	**Control M**	**1.2 µg/cm² M**	**6.6 µg/cm² M**	**Control H**	**0.2 µg/cm² H**	**1.2 µg/cm² H**	**Control M**	**0.2 µg/cm² M**	**1.2 µg/cm² M**
Propionibacterium sp.	0.218	0.100	0.013	0.004	0.040	0.037	0.703	0.390	0.026	0	0	0.005
Proteobacteria	0.121	0.677	0.042	0.023	0.091	0.042	0.444	0.800	0.032	0.021	0.043	0.044
Pseudonocardia sp.	0	0	0	0	0	0	0.109	0.007	0	0	0	0
Serratia sp.	0.847	0.229	53.080	30.137	18.527	16.356	1.685	0.125	62.123	40.717	16.930	54.963
Staphylococcus hominis	0.081	0.011	0	0	0	0.042	0.105	0.064	0.003	0	0	0
Staphylococcus sp.	0.181	0.011	0.007	0	0.011	0.031	0.068	0.049	0.010	0	0	0
Streptococcus sp.	0.891	0.021	0.042	0.061	0.091	0.199	2.854	0.150	0.123	0.057	0.082	0.069
Other	4.827	3.258	1.895	3.058	3.242	4.225	9.819	3.819	1.786	2.626	3.023	1.901

Relative abundance (%) of OTUs was assigned to the genus/species level based on *16S rRNA* pyrosequencing of RNA samples directly extracted from midgut content (M) and haemolymph (H) of *Spodoptera littoralis* larvae, treated with *102 Sl* dsRNA or *GFP* dsRNA and exposed to different doses of Cry1Ca. *Only OTUs with a relative abundance of 0.1% in at least 1 sample are reported.

Table S5. Microbial viable counts in the haemolymph as affected by gavage.

	Log CFU/mL ± S.D.
***GFP* dsRNA**	4.22 ± 0.20[1]
***102 Sl* dsRNA**	4.28 ± 0.29[1]
No dsRNA	4.00 ± 1.67[2]

Number of bacteria were assessed in the haemolymph of *Spodoptera littoralis* experimental control larvae (i.e. not exposed to Cry1Ca toxin) treated with *GFP* dsRNA or *102 Sl* dsRNA or unmanipulated (No dsRNA), in order to assess any unintended effect of the gavage procedure on gut permeability to bacterial cells. [1]Data are expressed as mean of Log CFU (Colony Forming Units)/ml ± S.D. of two independent experiments, each based on a pool of 8 larvae. [2]Data are expressed as mean of Log CFU (Colony Forming Units)/ml ± S.D. obtained from haemolymph of 10 larvae, separately analyzed.

Table S6. Primers for qRT-PCR analyses (F: Forward, R: Reverse).

Primer name	Sequence (5'-3')	Accession numbers
Attacin 1 - F	CGTTCTTAGACCGCAAGGAC	*attacin 1* FQ971100.1
Attacin 1 - R	CACGGAAGTGGTCGGGCT	
Gloverin - F	GGCAGCACGGACGATTCTT	*gloverin* FQ965511.1
Gloverin - R	CCGAGGTTGGTGCTGTCTCCGTT	
Lysozyme - F	ATGAGGGATTGGGTGTGCC	*lysozyme 1a* FQ961692.1
Lysozyme - R	TGGAACAGGCCGTAGTCCCG	
16S Ribosomal RNA - F	ACTCCTACGGGAGGCAGC	
16S Ribosomal RNA - R	ATTACCGCGGCTGCTGGC	

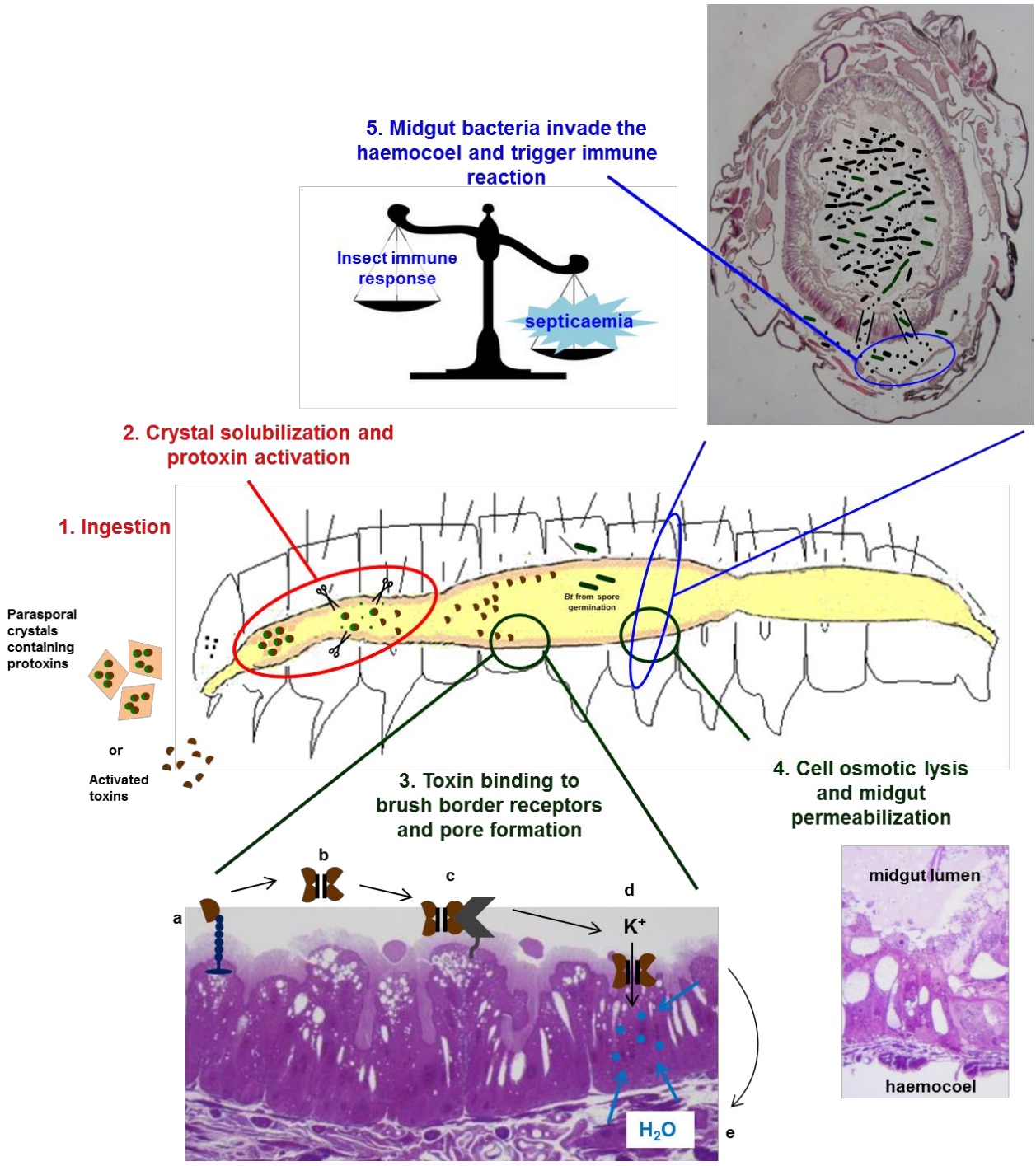


Figure S1. *Bacillus thuringiensis* (*Bt*) mode of action. *Bt* has been extensively used for insect control as a spraying formulate (containing spores and crystals of Cry protoxins) and, more recently, to develop *Bt*-crops, expressing active Cry toxins. After ingestion by susceptible insects (1), crystals are solubilized in the midgut lumen and protoxins are cleaved to produce activated toxins (2). The toxin crosses the peritrophic matrix and binds to specific primary receptors

146 (cadherins) located on the brush border membrane of the midgut cells (3a). Toxin binding to
147 cadherins is associated with a small proteolytic cleavage, followed by the formation of toxin
148 oligomers (3b) that bind to secondary receptors (aminopeptidase N and alkaline phosphatase
149 isoforms) (3c). Binding to these proteins results in toxin insertion into the membrane, pore
150 formation and cation influx into midgut cells (3d). Ion movement promotes a water influx (3e) and
151 osmotic cell shock (4). Tissue damage (4) causes gut function paralysis and the passage of midgut
152 bacteria to the body cavity (i.e. haemocoel) (5). The entrance of bacteria into the haemocoel triggers
153 an immune reaction that, depending on the host immunocompetence and the degree of gut damage,
154 may be able to limit the infection process, or may fail to block the onset of a lethal septicaemia.
155

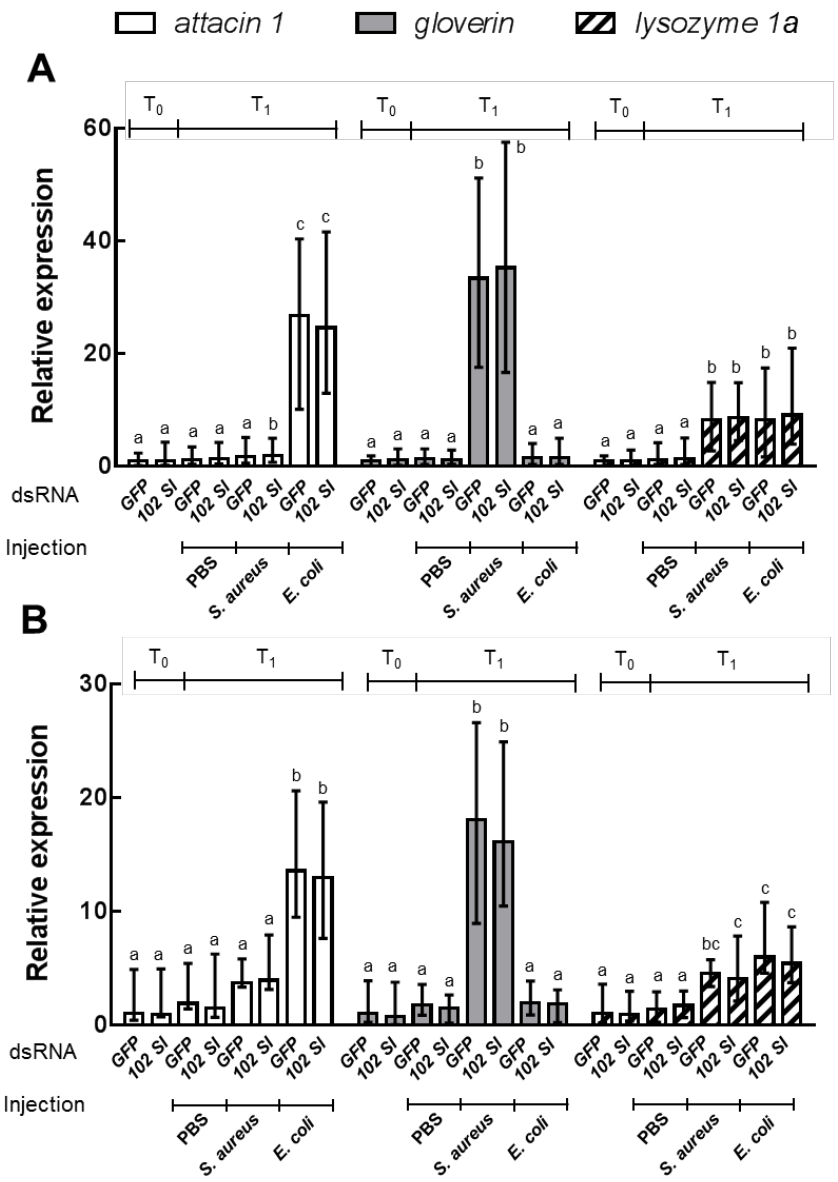


Figure S2. Humoral immune response by *Spodoptera littoralis* larvae as affected by RNAi mediated silencing of the immune gene *102 Sl*. The transcript level of the genes encoding the humoral effectors considered was significantly enhanced by the immune challenge, both in the midgut (*A*) (*attacin 1* $F_{3,66}$=83.23, *P*<0.0001; *gloverin* $F_{3,66}$=61.58, *P*<0.0001; *lysozyme 1a* $F_{3,66}$=62.38, *P*<0.0001) and in the fat body (*B*) (*attacin 1* $F_{3,66}$=27.86 P<0.0001; *gloverin* $F_{3,66}$=26.16, *P*<0.0001; *lysozyme 1a* $F_{3,66}$=32.16, *P*<0.0001), but was not influenced by gene silencing (Table S1). The values reported are the mean ± S.E.; T_0 is the time at the injection, T_1 is

18 hours after injection; different letters denote significant differences between treatments compared within each gene considered.

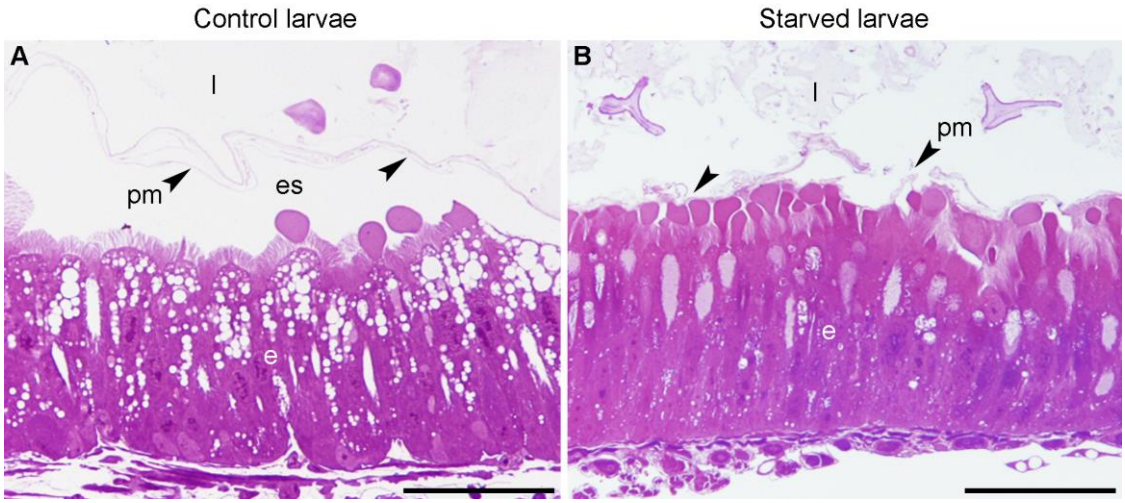


Figure S3. Midgut morphology of *Spodoptera littoralis* larvae as affected by starvation. Semi-thin cross-sections of the midgut epithelium isolated from day 1 of 5th instar larvae (*A*), and from larvae starved for 120 h, starting at the beginning of the 5th instar (*B*). The epithelial monolayer [e] appears intact under both experimental conditions, while starvation induces the disruption of the peritrophic matrix [pm] (arrowheads) and the disappearance of a well-delimited ectoperitrophic space [es] in the midgut lumen [l]. The abbreviations used are reported in square brackets. Bars: 50 μm.

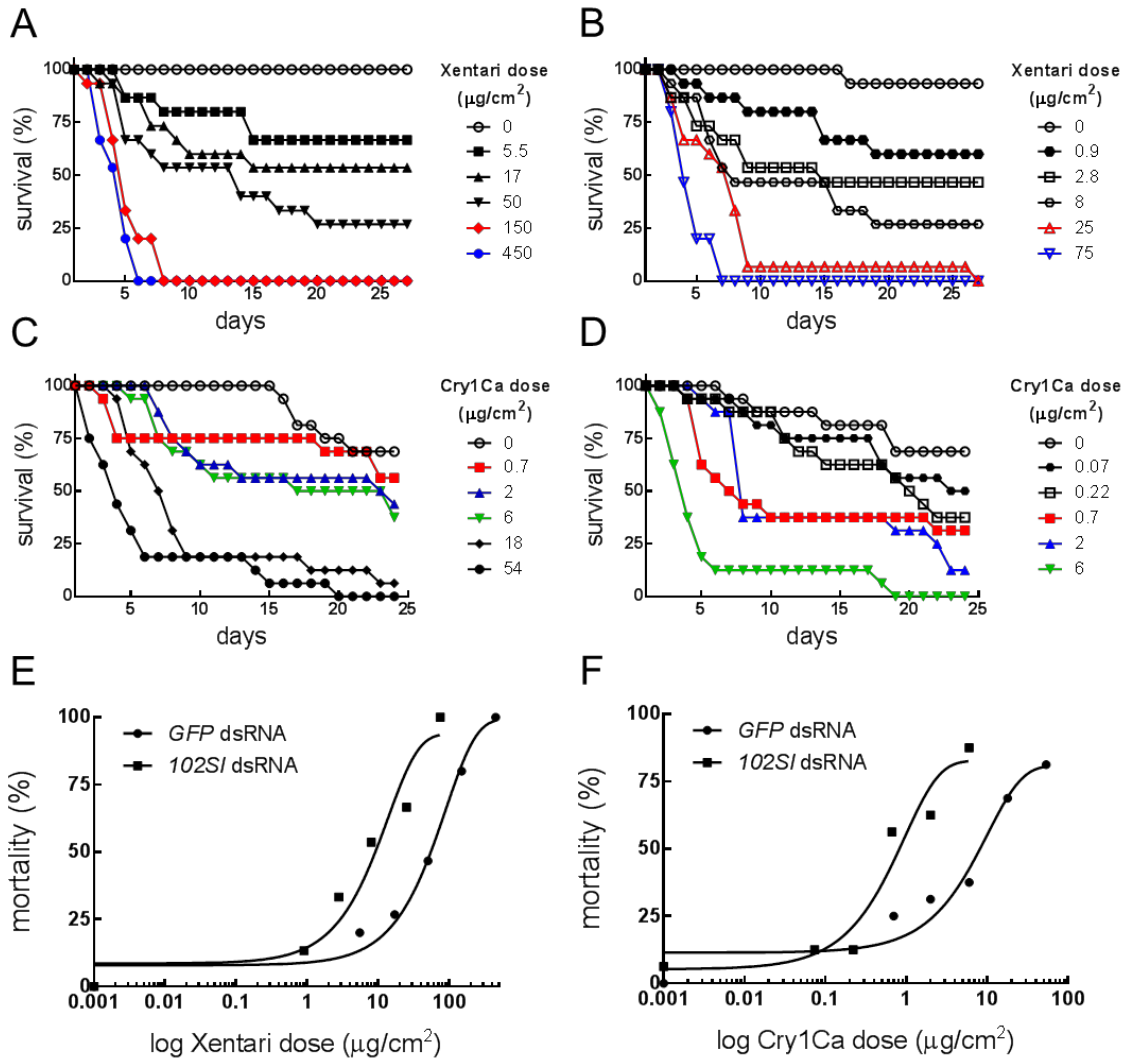
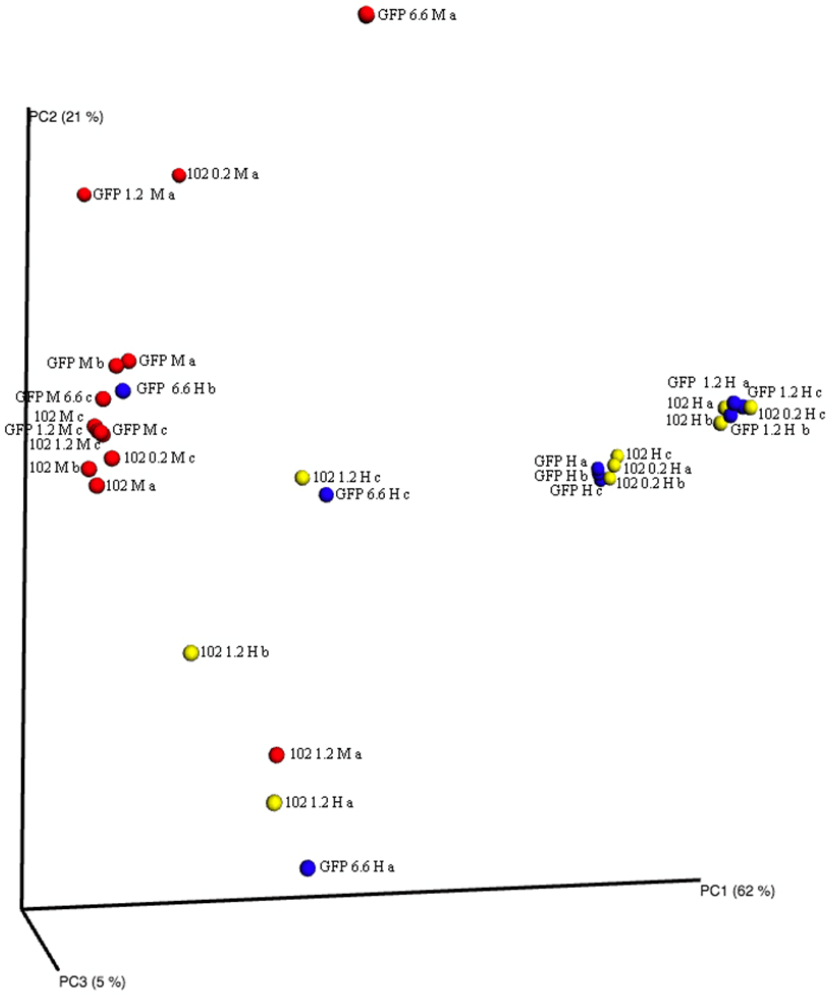


Figure S4. Survival of experimental larvae as affected by *Bt*, Cry1Ca toxin and gene silencing. Survival (*A*-*D*) and mortality (*E*, *F*) curves, at day 8 from the onset of the bioassay (when LC_{50} was calculated), recorded for *Spodoptera littoralis* larvae treated with *GFP* dsRNA (*A*, *C*) or *102 Sl* dsRNA (*B*, *D*), and exposed to different doses of Xentari™ or Cry1Ca. In *A* and *B*, the curves corresponding to doses that induced the two highest mortality levels in control and silenced larvae are represented with the same colors; in *C* and *D* colors are used for an immediate comparison of the different effects induced by identical doses.



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190 **Figure S5.** Phylogenetic beta diversity analysis. Principal Coordinates Analysis (PCoA) based on
191 weighted UniFrac distances for *16S rRNA* sequence data showing separation between midgut
192 content (M, red dots) and haemolymph (H) samples of *Spodoptera littoralis* larvae treated with *102*
193 *Sl* dsRNA (102, yellow dots) or *GFP* dsRNA (GFP, blue dots) and exposed to different doses of
194 Cry1Ca. Labels in samples IDs indicate replicate experiments (a, b, c).

195

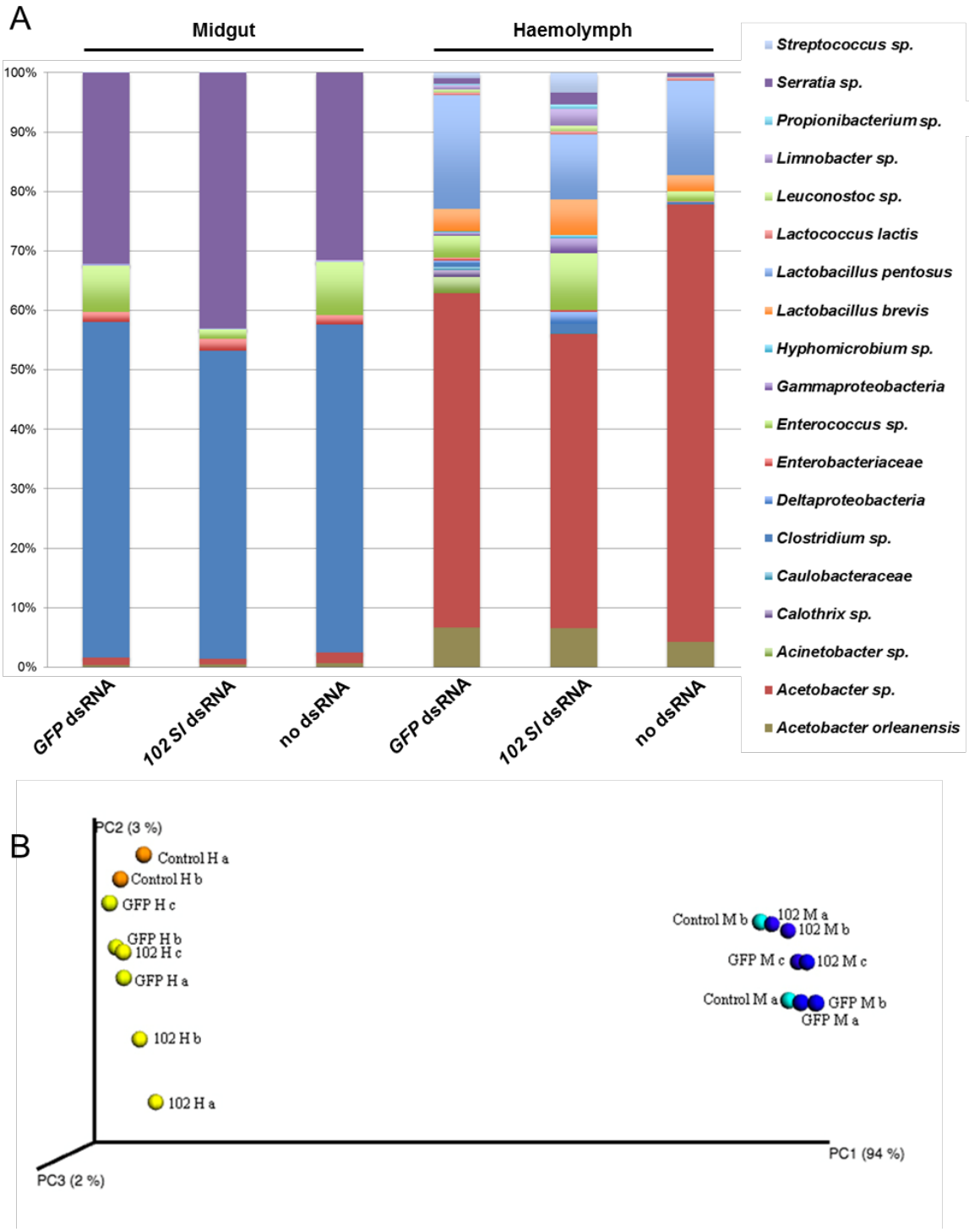

Figure S6. Impact of dsRNA gavage on midgut and haemolymph microbiota. (*A*) Incidence of the major bacterial taxonomic groups detected by pyrosequencing. Relative abundance of identified microbial taxa in the midgut content and haemolymph samples collected from *Spodoptera littoralis* experimental control larvae treated with *102 Sl* dsRNA, *GFP* dsRNA, or from unmanipulated controls, not exposed to dsRNA gavage. (*B*) Principal Coordinates Analysis (PCoA) based on

202 weighted UniFrac distances for *16S rRNA* gene sequence. The data show separation between
203 midgut (M, blue dots) and haemocoel (H, yellow dots) samples of *S*. *littoralis* larvae treated with
204 *102 Sl* dsRNA or *GFP* dsRNA, and separation of midgut (sky blue dots) and haemocoel (orange
205 dots) samples of untreated *S*. *littoralis* larvae. Labels in samples IDs indicate different replicate
206 experiments (a, b, c).
207

1. Bacterial counts by RT-PCR and nodulation assay during the bioassay

	Days						
	1st	2nd	3rd	4th	5th	6th	7th
morning	dsRNA[1]	dsRNA[1]	dsRNA[1]		Sampling[2] (T_1)	Sampling[2] (T_2)	Sampling[2] (T_3)
afternoon			Sampling[2] (T_0)				
			Cry1Ca administration[3]	Cry1Ca administration[3]	Cry1Ca administration[3]		

[1] Oral administration of 150 ng of *102 Sl* dsRNA or *GFP* dsRNA (control).

[2] Dissection of *S. littoralis* larvae and isolation of samples to process for bacterial counts (midgut, haemolymph and haemocytes) at different time points.

[3] Oral administration of Cry1Ca toxin through artificial diet.

2. Antimicrobial peptides (AMP) and lysozyme expression determined by RT-PCR

	Days			
	1st	2nd	3rd	4th
morning	dsRNA[1]	dsRNA[1]	dsRNA[1]	Sampling[2] (T_1)
afternoon			Sampling[2] (T_0)	
			Bacterial injection[3]	

[1] Oral administration of 150 ng of *102 Sl* dsRNA or *GFP* dsRNA (control).

[2] Dissection of *S. littoralis* larvae and isolation of samples to process for AMP and lysozyme expression profile (midgut, haemocytes and fat body) at different time points.

[3] Haemolymphatic injection of a PBS suspension of Gram positive (*Staphylococcus aureus*) or Gram negative (*Escherichia coli*) bacteria. PBS was injected into control larvae.

3. Metagenomic analysis and bacterial counts by culture-dependent method

	Days						
	1st	2nd	3rd	4th	5th	6th	7th
morning	dsRNA[1]	dsRNA[1]	dsRNA[1]				Sampling[3]
afternoon			Cry1Ca administration[2]	Cry1Ca administration[2]	Cry1Ca administration[2]		

[1] Oral administration of 150 ng of *102 Sl* dsRNA or *GFP* dsRNA (control).

[2] Oral administration of Cry1Ca toxin through artificial diet.

[3] Dissection of *S. littoralis* larvae and isolation of samples to process for metagenomics analysis (midgut content and haemolymph).

Figure S7. Schematic representation of the experimental conditions and timing adopted.