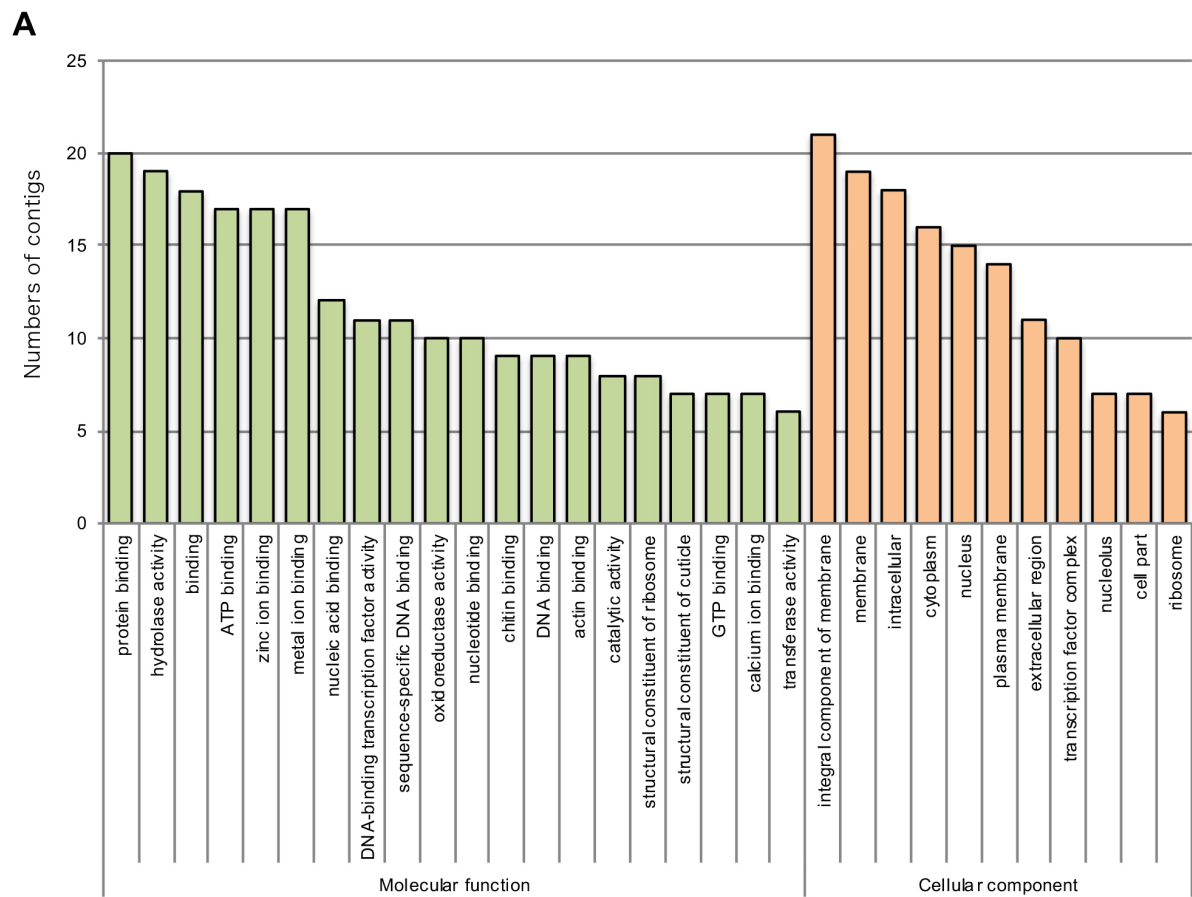


**Fig. S1. Signalling pathways of insect immunity, after Hillyer (2016), Lindsay and Wasserman (2014) and Anthoney et al. (2018).**

Schematic diagram of Toll, Imd, and JAK/STAT signalling pathways for insect immunity. Infectious pathogens, including gram-positive bacteria, fungi, and yeasts, are recognised by PGRP-SA and PGRP-SD, which activate clip-domain serine proteinases (clip-SPs) to catalyse pro-Spz to Spz. Matured Spz binds to Tolls and activates NF- $\kappa$ B transcription factors DI and Dif via MyD88, Tube, Pelle, and TRAF6. DAMPs released from injured cells activate clip-SPs. Gram-negative bacteria are recognised by PGRP-LC via Imd and activate NF- $\kappa$ B transcription factor Rel. Insect cytokine Upd binds to interleukin receptor Dome and activates transcription factor STAT via Janus kinases Hop (Anthoney et al., 2018; Hillyer, 2016; Lindsay and Wasserman, 2014).

Abbreviations: JAK/STAT, Janus kinase/signal transducer and activator of transcription protein; NF- $\kappa$ B, nuclear factor kappa-B; DI, dorsal; Dif, dorsal-related immunity factor; Dome, Domeless; MyD88, myeloid differentiation primary response 88; TRAF6, TNF receptor-associated factor 6; DAMPs, damage-associated molecular patterns; Psh, Persephone; PGRP, Peptidoglycan recognition protein; Spz, Spatzle; Hop, Hopscotch; Imd, Immune deficiency; Rel, Relish; Upd, Unpaired.

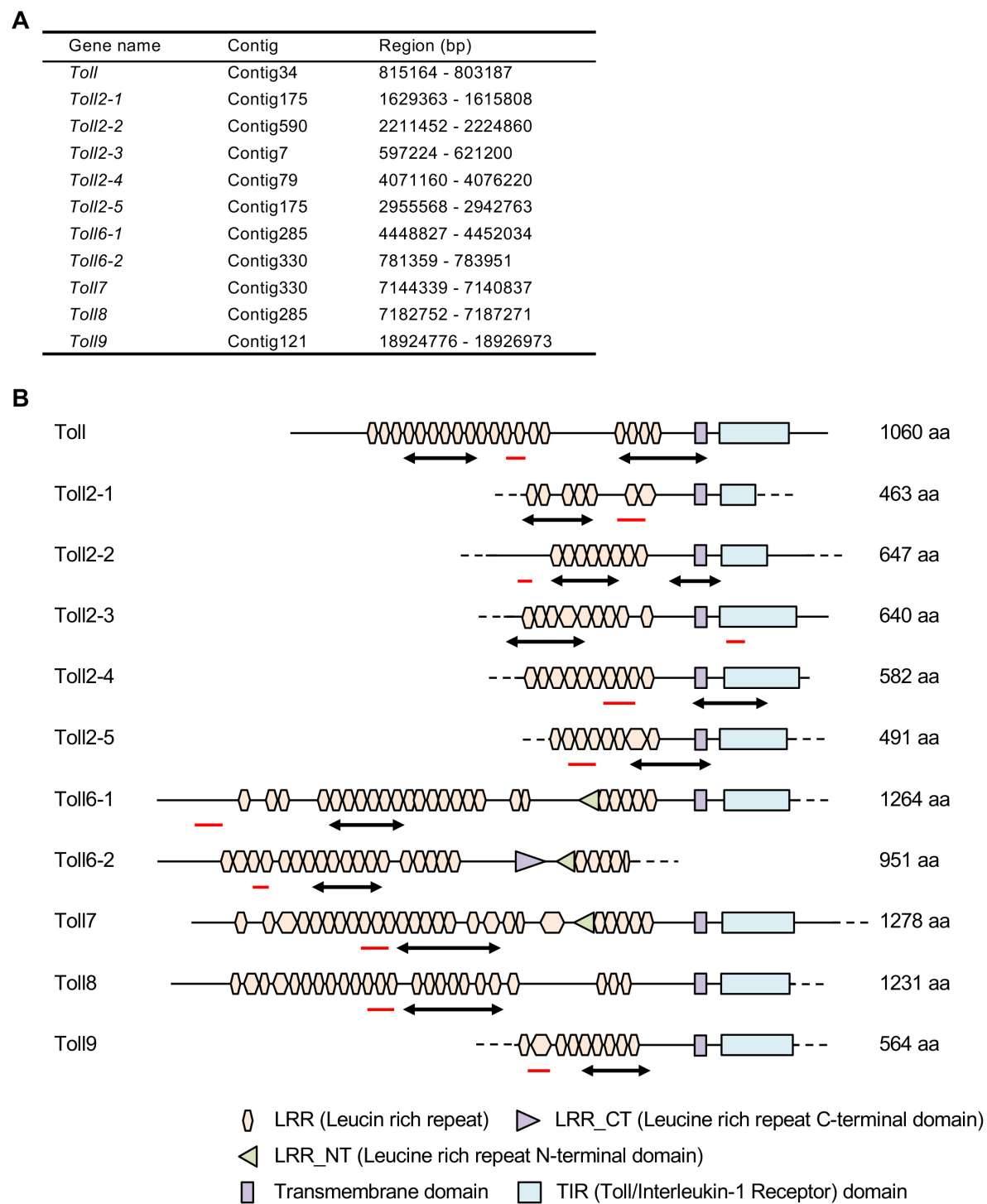


B

contig_ID	Sequence description	Length	0 hpa		3 hpa		RPKM Ratio 3h/0h
			595,425 reads in total	# Reads	RPKM	519,961 reads in total	
<b>Transcription factors</b>							
isotig04802	kayak	2,559	23	15.09	101	75.91	5.03
isotig11345	dna-binding protein d-ets-4	2,174	16	12.36	38	33.62	2.72
isotig06051	ets homologous factor-like	2,648	24	15.22	54	39.22	2.58
isotig16044	ap-1	1,030	38	61.96	72	134.44	2.17
<b>VEGF signalling</b>							
isotig15859	Platelet-derived and vascular endothelial growth factors	1,060	2	3.17	25	45.36	14.31
isotig13937	pvf3 cg34378-pd	1,380	3	3.65	17	23.69	6.49
isotig18768	vascular endothelial growth factor a-a-like	705	5	11.91	15	40.92	3.44
isotig03363	vascular endothelial growth factor receptor 1-like	6,760	29	7.2	71	20.2	2.81
isotig14929	vascular endothelial growth factor receptor 1 isoform x2	1,200	15	20.99	34	54.49	2.6
isotig14209	pdgf vegf receptor	1,325	7	8.87	13	18.87	2.13
<b>IGF signalling</b>							
isotig09133	tribbles homolog 2	4,891	136	46.7	254	99.88	2.14
<b>FGF signalling</b>							
isotig11083	dual specificity protein phosphatase mpk3-like	2,311	10	7.27	25	20.81	2.86
isotig11152	fgfr1 oncogene partner 2 homolog	2,269	10	7.4	21	17.8	2.41
<b>TGF-β signalling</b>							
isotig14196	transforming growth factor beta regulator 1	1,328	6	7.59	18	26.07	3.43
isotig03246	smad nuclear-interacting protein 1	2,945	17	9.69	30	19.59	2.02
<b>Wnt signalling</b>							
isotig12204	disheveled-associated activator of morphogenesis 2	1,823	10	9.21	34	35.87	3.89
<b>Toll signalling</b>							
isotig06664	Toll2-5	2,309	17	12.37	35	29.15	2.36
isotig12616	unc93-like	1,697	11	10.89	22	24.93	2.29
isotig05588	Relish	4,770	27	9.51	52	20.97	2.21
isotig09244	Toll8 (slit homolog 2)	4,522	43	15.97	82	34.87	2.18
isotig17867	Toll2-2	814	9	18.57	17	40.17	2.16
isotig11861	serine protease easter	1,956	45	38.64	79	77.68	2.01

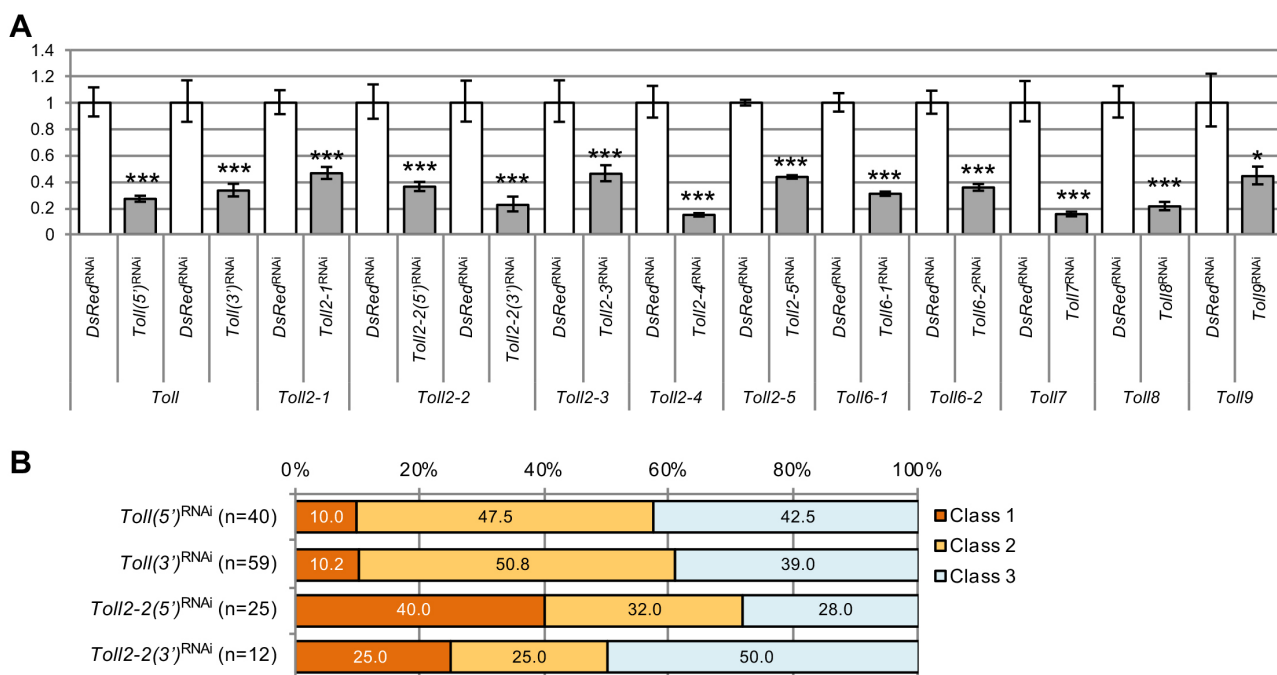
**Fig. S2. GO annotations of RNA-seq results.**

(A) Graphs show 20 and 11 most frequently counted GO terms of molecular function and cellular components by annotations of the transcripts with upregulated expression in RLs. (B) Selected signalling pathway genes upregulated in RLs (3 hpa) compared with NLs (0 hpa). Note that contigs are assembled sequences of reads obtained from RLs and NLs, and isotigs are assembled sequences of contigs.



**Fig. S3. Genomic loci of *Gryllus* Toll genes and their domain structures.**

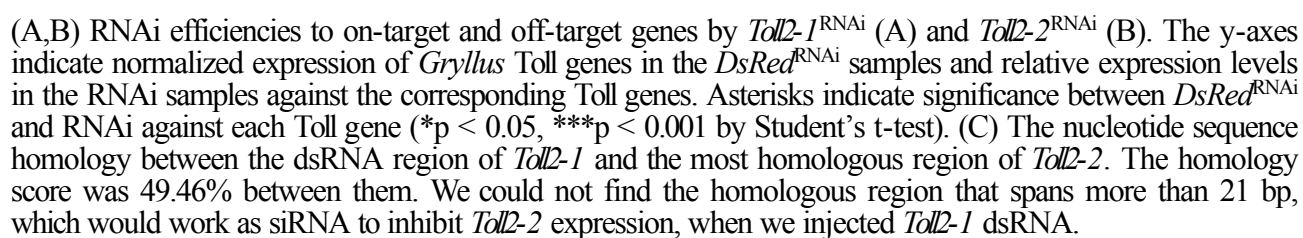
(A) Physical positions of *Gryllus* Toll genes spanning the genome contigs. Note that three pairs of Toll genes (*Toll2-1* and *Toll2-5*, *Toll6-1* and *Toll8*, and *Toll6-2* and *Toll7*) are located at the different regions of same contigs. (B) Schematic diagram of *Gryllus* Toll proteins is shown. Domains were predicted by Protein BLAST at NCBI web BLAST (<https://blast.ncbi.nlm.nih.gov/Blast.cgi>) and SMART website (<http://smart.embl-heidelberg.de/>). Dotted lines indicate regions that we have not cloned. Double-headed arrows and red lines indicate regions for RNAi and qPCR, respectively. To verify the specificity of RNAi against *Toll* or *Toll2-2*, we compared the phenotype using dsRNAs corresponding to two independent regions of each gene. The phenotypic effects by RNAi against *Toll*(5'), which correspond to an extracellular LRR region, and *Toll*(3'), which corresponds to an extracellular region and transmembrane domain were not significantly different ( $p > 0.05$ , Fisher's exact test) (see Fig. S4B). The similar results were obtained for *Toll2-2*<sup>RNAi</sup>. We therefore used the *Toll*(5') and *Toll2-2*(5') fragments for all subsequent analyses.

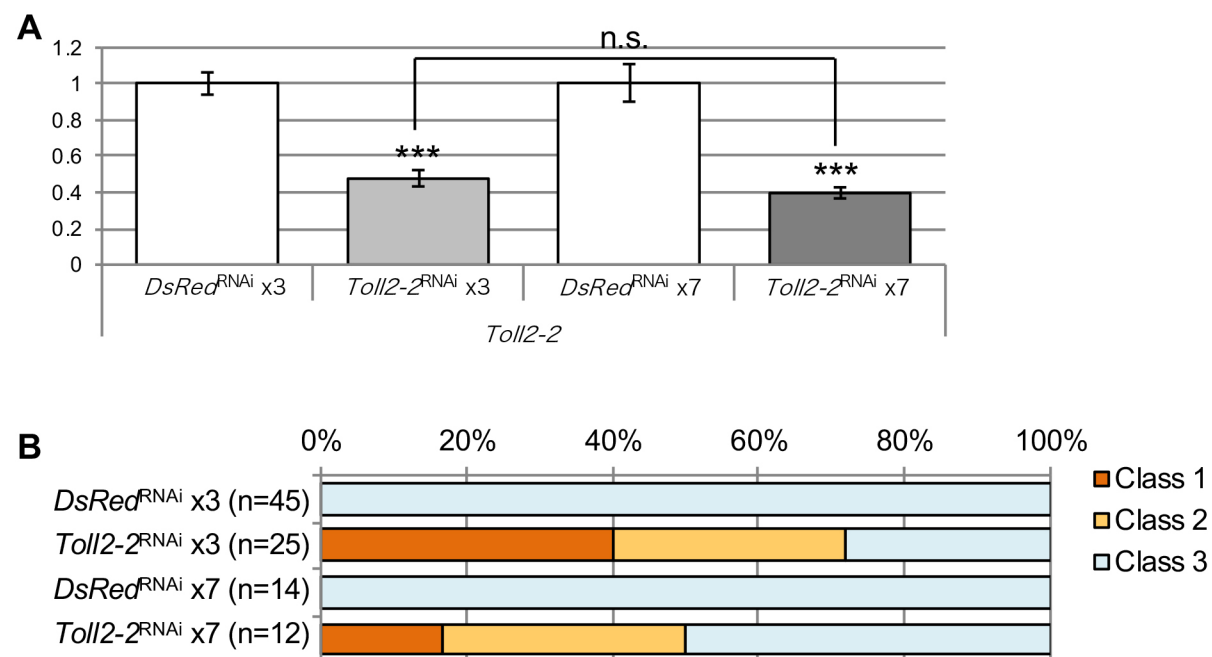


**Fig. S4. Efficiencies of RNAi for *Gryllus* Toll genes.**

(A) RNAi efficiencies to endogenous on-target genes, revealed by qPCR. Two independent regions of *Toll* (*Toll(5')* and *Toll(3')*) and *Toll2-2* (*Toll2-2(5')* and *Toll2-2(3')*) were used for RNAi experiments to observe phenotype reproducibility. The y-axis indicates normalized expression of *Gryllus* Toll genes in the *DsRed*<sup>RNAi</sup> samples and relative expression levels in the RNAi samples against the corresponding Toll genes. Asterisks indicate significance between *DsRed*<sup>RNAi</sup> and RNAi against Toll genes (\* $p < 0.05$ , \*\*\* $p < 0.001$  by Student's t-test). (B) Graph shows the percentage of class 1, class 2, and class 3 phenotypes obtained by RNAi against two independent regions of *Toll* and *Toll2-2* genes. Numbers of RNAi-treated individuals are shown by  $n$ . The phenotype ratios of *Toll(5')*<sup>RNAi</sup> and *Toll(3')*<sup>RNAi</sup> were similar ( $p = 0.954747$ , Fisher's exact test) and those of *Toll2-2(5')*<sup>RNAi</sup> and *Toll2-2(3')*<sup>RNAi</sup> were also similar ( $p = 0.462691$ , Fisher's exact test).

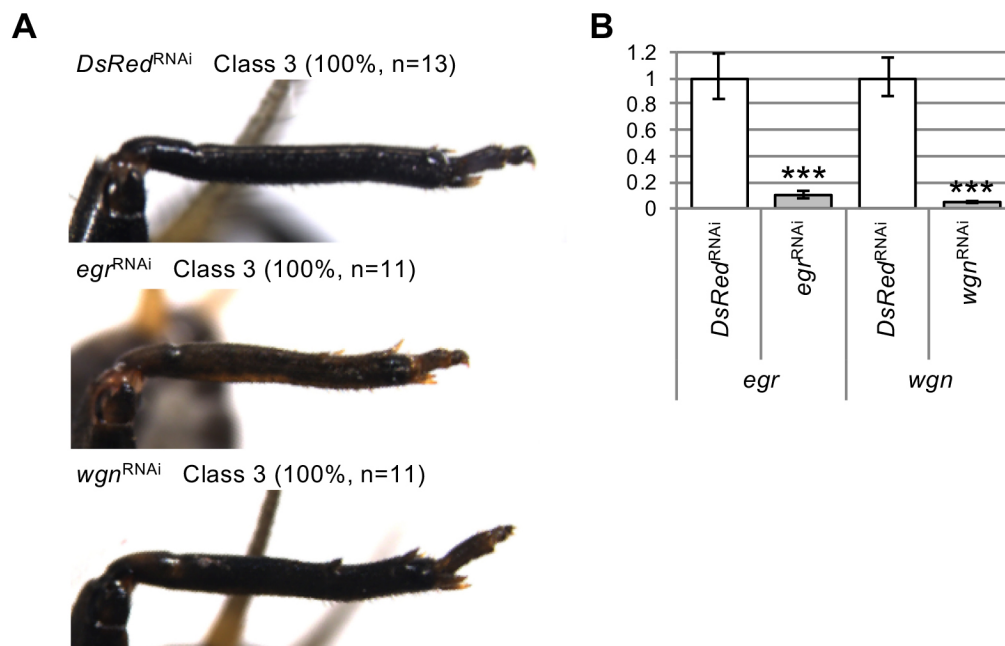






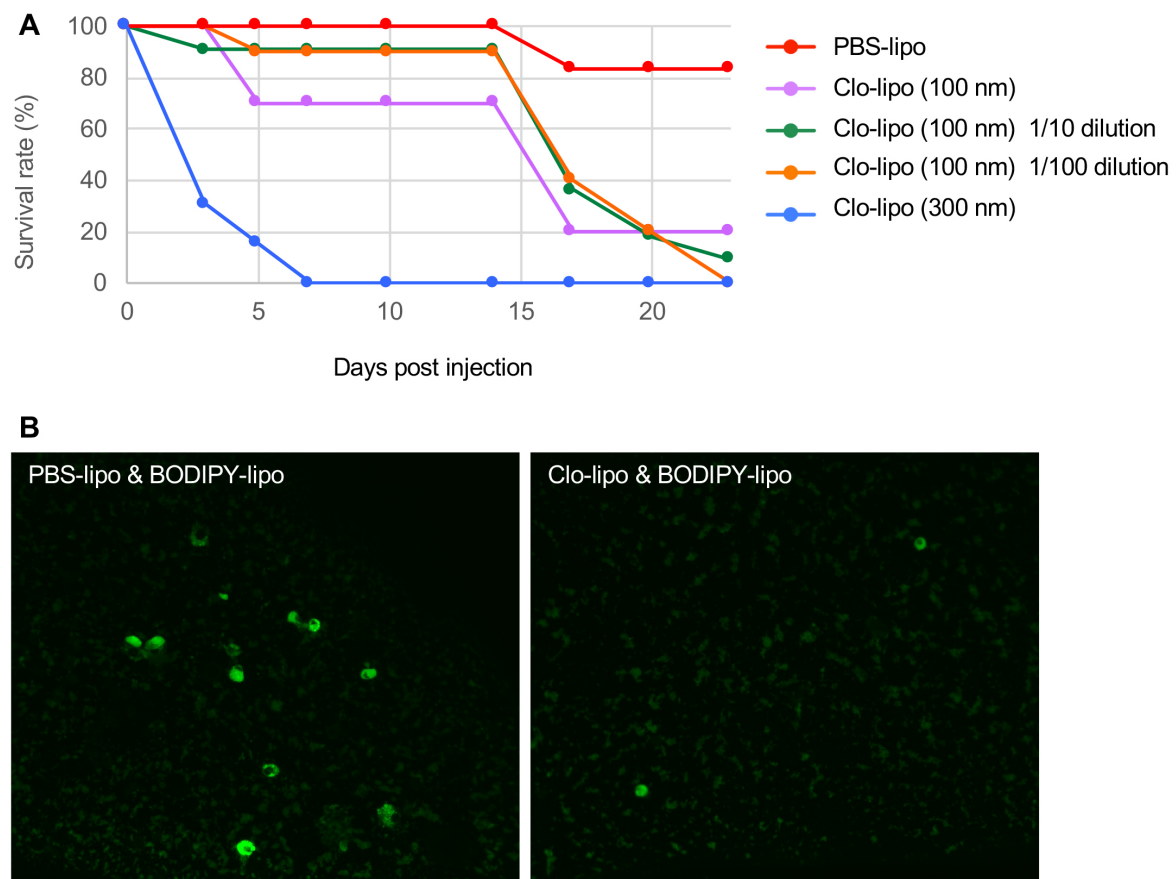
**Fig. S6. RNAi efficiency and phenotype ratio of *Toll2-2*<sup>RNAi</sup> in different dsRNA dose.**

(A) Relative expression levels of *Toll2-2* after *Toll2-2*<sup>RNAi</sup> x3 (207 nL dsRNA injection) or *Toll2-2*<sup>RNAi</sup> x7 (483 nL dsRNA injection) to see RNAi efficiencies. The y-axis indicates normalized expression of *Toll2-2* in the *DsRed*<sup>RNAi</sup> samples and relative expression levels in the *Toll2-2*<sup>RNAi</sup> samples. 207 nL or 483 nL of *DsRed* dsRNA were injected into control nymphs. Relative expression levels of *Toll2-2* in *Toll2-2*<sup>RNAi</sup> x3 and *Toll2-2*<sup>RNAi</sup> x7 were significantly reduced compared with respective control experiments (\*\*\*p < 0.001 by Student's t-test), but reduction of *Toll2-2* expression by *Toll2-2*<sup>RNAi</sup> x7 was not significant to that by *Toll2-2*<sup>RNAi</sup> x3 (n.s.; not significant). (B) Phenotypic ratios of RNAi by *Toll2-2*<sup>RNAi</sup> x3 or *Toll2-2*<sup>RNAi</sup> x7. Ratios of class 1 and class 2 were not significantly changed by *Toll2-2*<sup>RNAi</sup> x7 compared with *Toll2-2*<sup>RNAi</sup> x3 (p > 0.05, Fisher's exact test).



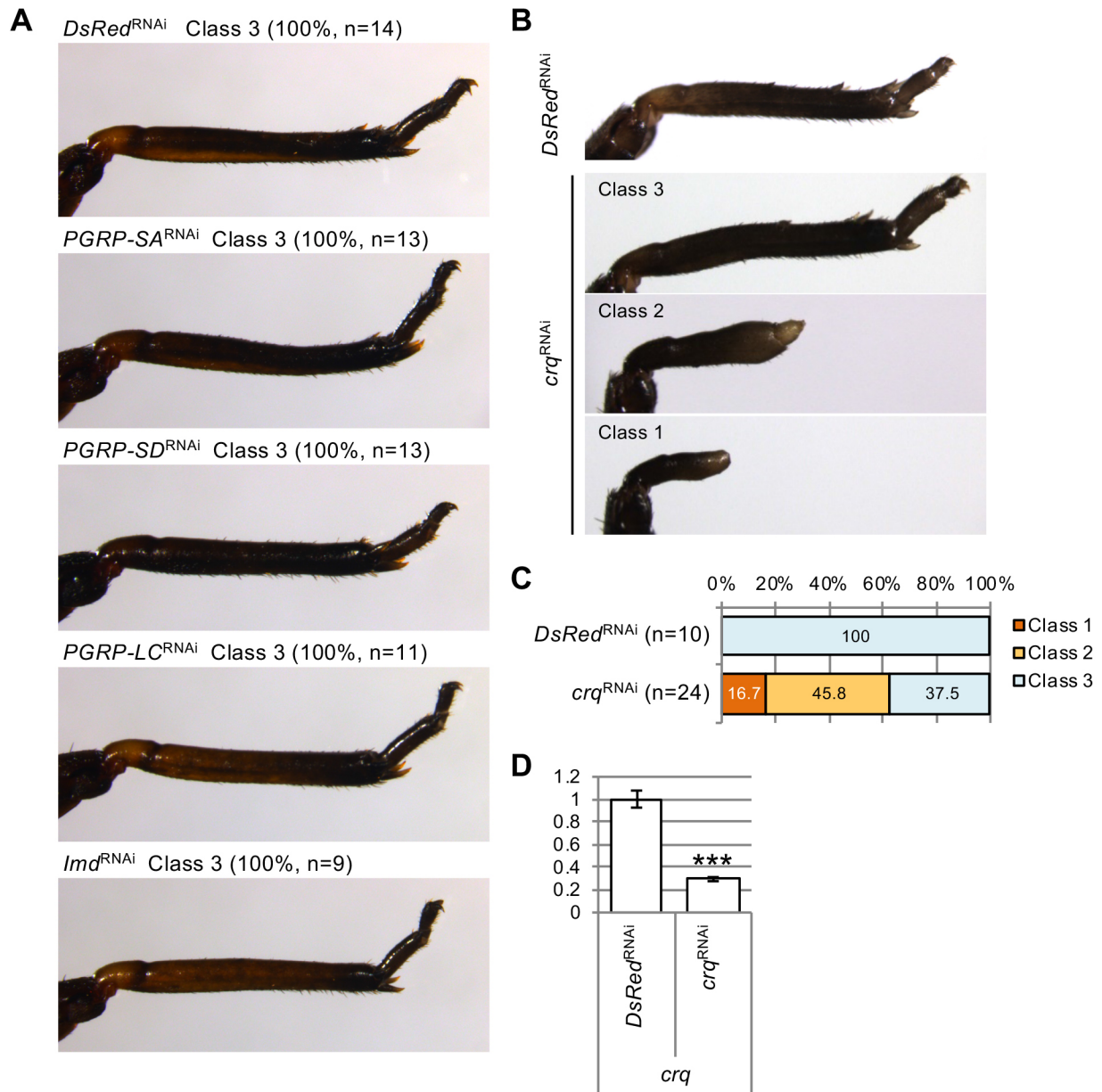
**Fig. S7. Phenotypes of RNAi for *egr* and *wgn*.**

Typical morphology of regenerating legs of *DsRed*<sup>RNAi</sup>, *egr*<sup>RNAi</sup>, or *wgn*<sup>RNAi</sup> crickets at the fifth instar. Note that these RNAi crickets regenerated the lost part normally, and no RNAi crickets showed class 1 or 2 phenotypes. (B) Efficiency of RNAi against *egr* and *wgn*. The y-axis indicates normalized expression of *egr* and *wgn* in the *DsRed*<sup>RNAi</sup> samples and relative expression levels in the *egr*<sup>RNAi</sup> and *wgn*<sup>RNAi</sup> samples. Asterisks indicate significance between *DsRed*<sup>RNAi</sup> and RNAi against *egr* or *wgn* (\*\*\**p* < 0.001 by Student's *t*-test).



**Fig. S8. Effects of plasmatocyte depletion.**

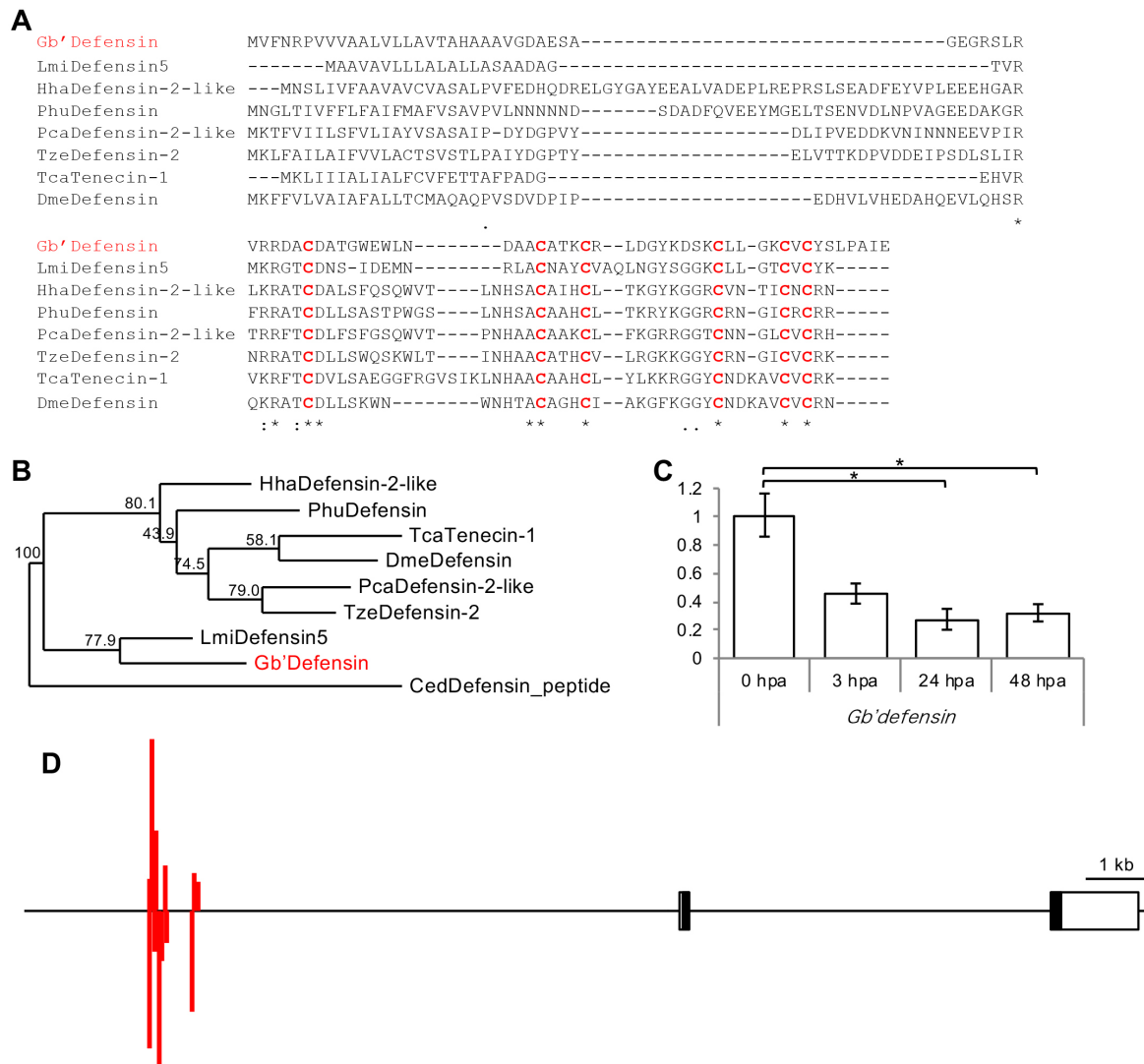
(A) Survival curve of PBS-lipo, Clo-lipo (100 nm) or Clo-lipo (300 nm) injected cricket nymphs. The mean lifespan of Clo-lipo (100 nm) injected crickets was 15 days, which was longer than that of Clo-lipo (300 nm) injected crickets (2.5 days). The short lifespan of Clo-lipo (300 nm) injected crickets was an obstacle to observing regeneration processes. Thus, we used Clo-lipo (100 nm) to deplete the plasmatocytes in this study. PBS was used as the diluent. (B) Plasmatocytes in the haemolymph were visualised by BODIPY-lipo incorporation in the PBS-lipo injected or Clo-lipo injected crickets.



**Fig. S9. Phenotypes after RNAi to PAMPs and cellular debris recognising molecule genes.**

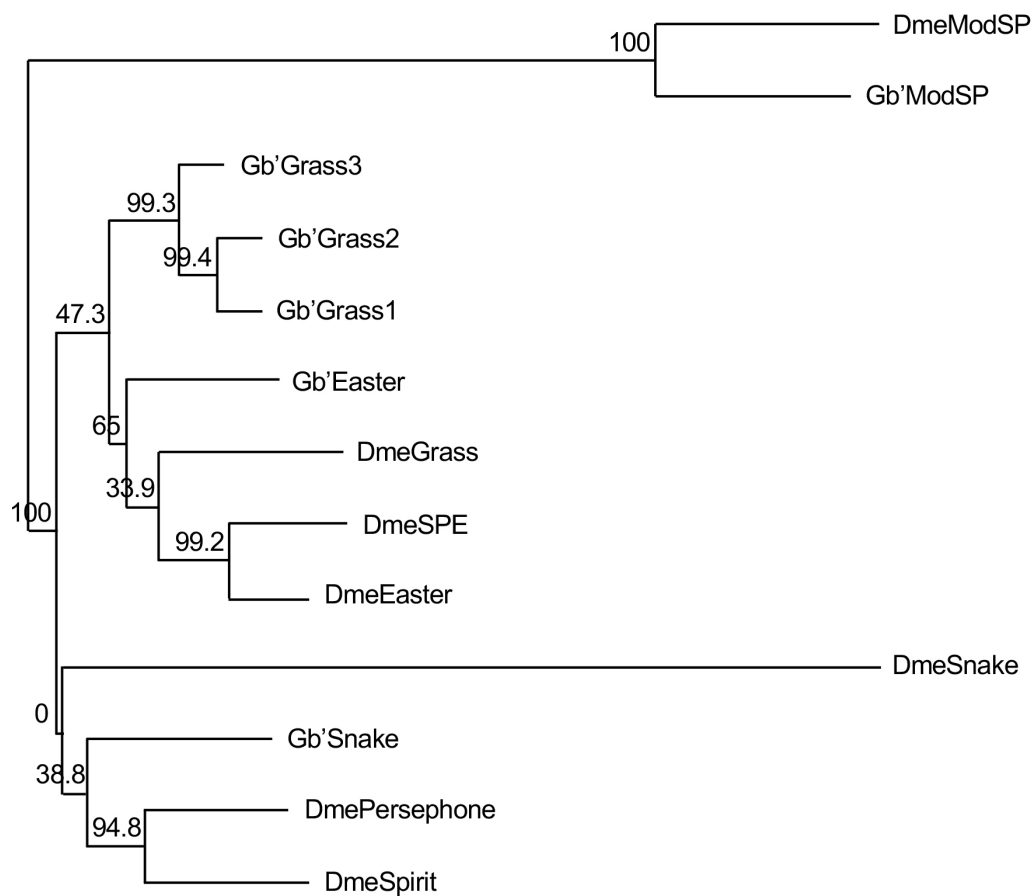
(A) Typical morphology of regenerating legs of *DsRed*<sup>RNAi</sup>, *PGRP-SA*<sup>RNAi</sup>, *PGRP-SD*<sup>RNAi</sup>, *PGRP-LC*<sup>RNAi</sup>, or *imd*<sup>RNAi</sup> crickets at fifth instar. Note that all of *PGRP-SA*<sup>RNAi</sup>, *PGRP-SD*<sup>RNAi</sup>, *PGRP-LC*<sup>RNAi</sup>, or *imd*<sup>RNAi</sup> crickets show normal leg regeneration. (B-D) Phenotypes and efficiency of RNAi for *crq*. (B) Morphology of regenerating legs of *DsRed*<sup>RNAi</sup> and *crq*<sup>RNAi</sup> crickets at fifth instar. (C-D) Phenotype ratio (C) and efficiencies (D) of *DsRed*<sup>RNAi</sup> and *crq*<sup>RNAi</sup>. *crq*<sup>RNAi</sup> significantly decreased the amount of *crq* transcripts to 29.4% in regenerating legs at 48 hpa. The y-axis indicates normalized expression of *crq* in the *DsRed*<sup>RNAi</sup> and a relative expression level in the *crq*<sup>RNAi</sup>. Asterisks indicate significance between *DsRed*<sup>RNAi</sup> and *crq*<sup>RNAi</sup> samples (\*\*\*)  $p < 0.001$  by Student's t-test).





**Fig. S10. Amino acid homology, phylogenetic tree and expression of *Gb'defensin*.**

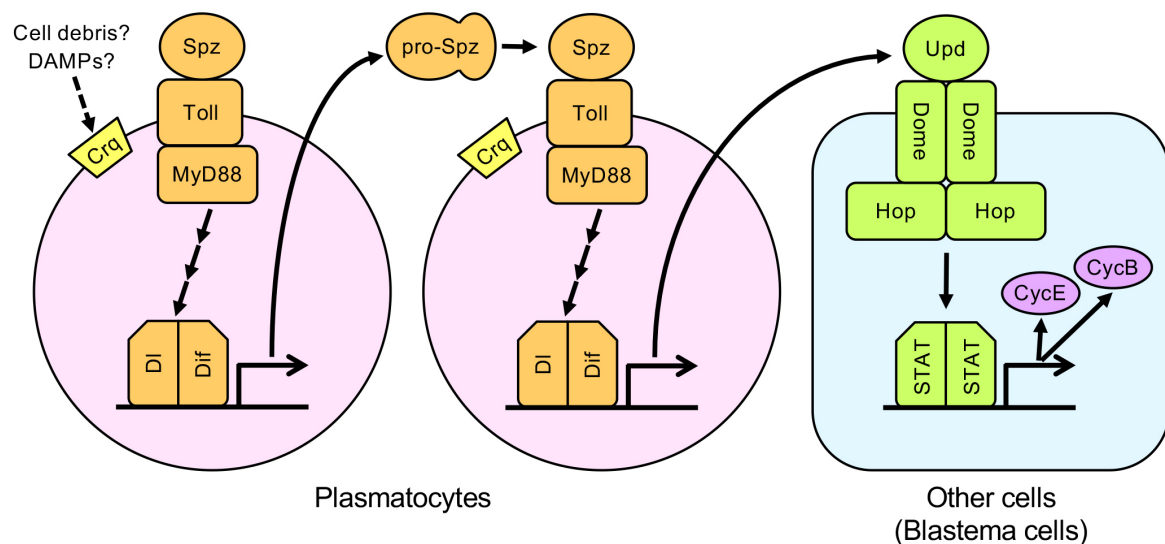
The *Gryllus bimaculatus* genome contains a single *defensin* gene. (A) *Gb'defensin* encodes 84 amino acids, with six evolutionarily conserved cysteine residues, shown in red. The N-terminal region of *Gb'Defensin* has diverged from the Defensins of other insects, but the C-terminal domain contains six evolutionarily conserved cysteine residues. (B) The phylogenetic tree indicates that *Gb'Defensin* is evolutionarily close to the grasshopper *Locusta migratoria* Defensin. *Centruroides edwardsii* was selected as the outgroup. (C) Temporal expression changes of *Gb'defensin* during leg regeneration, as revealed by qPCR. The y-axes indicate normalized expression at the 0 hpa and relative expression levels at 3, 24 and 48 hpa. Asterisks indicate significance of expression changes (\* $p < 0.05$ ) by Tukey's test. (D) Spatial distribution of NF- $\kappa$ B binding sites (dl(var.2)) in the upstream region of *Gb'defensin*, by using Cister website (<http://www.ijdb.ehu.es/web/>), are indicated by red lines. Black boxes and white boxes represent coding and non-coding regions, respectively. Red bar lengths indicate probabilities. Gb, *Gryllus bimaculatus*, Lmi, *Locusta migratoria*, Tze, *Trachymyrmex zeteki*, Pca, *Polistes canadensis*, Hha, *Halyomorpha halys*, Phu, *Pediculus humanus*, Tca, *Tribolium castaneum*, Dme, *Drosophila melanogaster*. CedDefensin\_peptide (*Centruroides edwardsii*) was selected as an outgroup.



**Fig. S11. Phylogenetic tree of Toll signalling-related proteinases**

Phylogenetic tree of Toll signalling-related serine proteinases in *Gryllus* and *Drosophila*. *Gryllus* genome contains ModSP, Easter, and Snake homologues, and three Grass paralogues (Grass1, Grass2 and Grass3). We could not find *Drosophila* SPE and Persephone homologues in the *Gryllus* genome.





**Fig. S12. Roles of Toll signalling during leg regeneration.**

Schematic representation of Toll signalling during regeneration. In the plasmatocytes, Crq, and probably also Toll2-2, recognises apoptotic cells and cell debris caused by amputation and activates Toll signalling. Activated Toll signalling induces the *spz/spz2* expression that activates Toll signalling in surrounding plasmatocytes to lead accumulation of plasmatocytes in regenerating leg, and *upd* expression that activates JAK/STAT signalling in other cells including blastema cells mediated by the expression of Cyclin E and Cyclin B, which promote cell proliferation during regeneration.

**Table S1. Comparison of expressed contigs between regenerating legs (3 hpa) and non-regenerating legs (0 hpa).** Comparison of RPKM values of contigs between regenerating legs and non-regenerating legs. Contigs are ordered by RPKM values of regenerating legs.

[Click here to download Table S1](#)

**Table S2. Blast results of contigs only expressed in or upregulated in the regenerating legs.** Blast annotations are listed. Contigs are ordered by ratios of RPKM values of between regenerating legs and non-regenerating legs.

[Click here to download Table S2](#)

**Table S3. Primer sequences for gene cloning**

Targeted gene	Forward primer (5' -> 3')	Reverse primer (5' -> 3')	Amplicon size
<i>spz</i>	CATGAATGGAGAGAAATCATT	CATAACAAACACACACGATG	302
<i>spz2</i>	GTATCGCACTATAATCCTGACGAAT	GTGTGATTGACACACACAACAGT	436
<i>Toll(5)</i>	GGAAACAAAATCTCAAATCTAACAAAA	AAGCTCTTTTAGATACTCTGTGTCTCG	426
<i>Toll(3)</i>	TCAAGAATTACTTAATCCAACATTTC	ACCAAACTTTGACTTCATTTTGATAAC	524
<i>Toll2-1</i>	AATTATTGAATACCTGGATCTATCACG	TTTGTGTTGAGACATGTTCACTACTTC	520
<i>Toll2-2(5)</i>	ATAGGATTGAAATTGCTGATTATTACG	AGTTTGTGTTAGACCTTTAAATGCAATG	434
<i>Toll2-2(3)</i>	ACAACACTGGTTAGACGTCACAAAA	AAGCCTTTCTTTTCACTCTCCTTCTC	302
<i>Toll2-3</i>	CAACTTCCTACTGGGCTACCTG	GGATATGTTGCAAGTACTCACGTC	472
<i>Toll2-4</i>	CTTACATTATAGTAGGCGTGGTCCTC	AAAGAATAAACTGTAATGTTCCGCTAA	449
<i>Toll2-5</i>	GTTGAAATTACAGAACAATCTGTAGCA	ATGTTTTGAGAGATAAAGAACCAAGA	545
<i>Toll6-1</i>	GAAGTTTTCGATCTGTGCAATAATAAA	AACTAAGTACGTACAAACCGTTGAGAG	445
<i>Toll6-2</i>	AGATATCAAGAAGTGTACCTGCAGAA	AGGTCTTTGATTGATTGTTAGAGAG	411
<i>Toll7</i>	ACGATTAGCAATAATCTTCTCATTAGC	GTAGTCGAACCACTAAATGGTTATT	462
<i>Toll8</i>	AATCAGTTCCTAGATGTTCCCTGAAGTA	AAGTATACGACTGCACCTTGTGAT	598
<i>Toll9</i>	ACAATAATTTAAGAGAGCATTTAGGCA	AAAGAACTCAATCATTGGACAACTATC	407
<i>MyD88</i>	CAGTACCGAATTTATATGACATTCTCTC	CAACACATCATCTCTCGTTAATATTTT	404
<i>tube</i>	AAAAGATTAAGAATGATGCTGTCAGT	TAAGCTGATGTTCCAAATACTGTAGTG	431
<i>pelle</i>	CGGCATGATAATATACTTCTTTGTAT	ACTTTTGTAGACAACTGTTTGTCTCTT	389
<i>TRAF6</i>	AAATGAAGAGACTGACTTATTTCCAGA	TATATCACTCACAGTCCAAACAAGAAC	559
<i>dl</i>	AGCCAGTAGTACTCCAGATAACAAGAC	AGAAGCGAACTTGATATCTTCTTTAG	548
<i>Dif</i>	TCATCATCATCAATGAGTAATAAAAGC	TCCTTCTAAAAAGACTTGGAACATAA	486
<i>Rel</i>	ACAATGAATAGAGAAGAGCCATTTT	CAGTCAAAGCACTTTTCATATTGTTT	625
<i>upd</i>	GAGAACTTCAAAGAGAAATATGTCCAG	CCATTCATGTAGTCACGGTAGATTAG	455
<i>egr</i>	ACTTCGAAGGTAACGGAAAGC	CAGTGTGACCCAGTTTGACAAG	414
<i>wgn</i>	ACTAAGTTTGATGGTACCAGAACTCG	TTTATTGTTTTAACAACATATTTACTCCT	322
<i>lmd</i>	GATCCTCCCAGAGTTGAAATACAC	CAGTAACATCTGATACACCACCTCTTT	537
<i>PGRP-SA</i>	AGAATCGATTATATGGTGATTCCACT	GGATCTCCTGGAAGAGTGCTAGT	424
<i>PGRP-SD</i>	GTCTGGTGGAATATGAATCAGATAAT	CCTTCTTCTAAGAACTTCTGACACACT	418
<i>PGRP-LC</i>	TTTGGTAACAAAACCTTTTATAATGGC	ATGATAACATATGGCACTGGTGTAGTA	566
<i>crg</i>	TATGTTACAAAGACAGTGAAGGAGTTG	AGTGGTGTTAAGTATGATGAATTAGCC	566

**Table S4. Primer sequences for qPCR**

Targeted gene	Forward primer (5' -> 3')	Reverse primer (5' -> 3')	Amplicon size
<i>actin</i>	TTGACAATGGATCCGGAATGT	AAAAGTCCCTGGGTGCAT	64
<i>spz</i>	GCCAATTCAAACCACGCTTC	CATCCACGCCTCCTTCACA	81
<i>spz2</i>	GATACCCCGACCGTCGATAC	GCAGCAGGGTGGTGTAGAGA	81
<i>Toll</i>	ATCACTCATCTCCTCTACCC	AACCCAGCTAATCACC GTTT	134
<i>Toll2-1</i>	CGGAAGTGGGTGATGCGT	GATGACCTTCCTGCTGTGCT	147
<i>Toll2-2</i>	ATTCGATGATGGACTCTTTGTAGGA	CGTGGAGATGAAAAGCGGTAAAG	121
<i>Toll2-3</i>	GCCCTCCACCAAACCTC	TGACCATCTCAGATAAACATCACACA	145
<i>Toll2-4</i>	TGGAACCGTGGATTGAAGAGG	CAACATGACTGGGCTGAGGT	101
<i>Toll2-5</i>	TCCAAGACACTCATCACATCACT	TTGAATTGAAGGCAGTTGACACTC	105
<i>Toll6-1</i>	ACACCACCAACTTCAGCGT	GCGGGTCAGGTGGTAGAAG	119
<i>Toll6-2</i>	CCGCGATGACCATGGAGTT	GGTTGCGCGTGAGGTTG	150
<i>Toll7</i>	CAGAACTCCATCGGCTACATC	GTTAAACATCAACGGCCCTATTTCT	111
<i>Toll8</i>	AACACCTTTGCCTCTCTTTACAATC	AATGCTTCGGATGTTGTTGTATCT	138
<i>Toll9</i>	GCCACTCCAAACCTTCAAACA	ACTTTTGTTCCTTCTAATGTTCTCTCA	117
<i>cycE</i>	AGCAACAGGAGGAAGGAGCA	CCAGGGCAGCATAAGGAAAC	144
<i>cycB</i>	CCCAGGTGGAGGTCAAGAGA	GGAAATTCGTGGTCGGAAAA	132
<i>upd</i>	AGGTGCAAGTGCTGATGGTG	GCGACGTGCTGTTGTTTTGT	99
<i>dome</i>	CAGTGACGGAAGTTACCAATTCAT	TGTAAAAGCGAAGTACACCATTCAAT	81
<i>hop</i>	CCTCCTTCAGAATATGATCGTTCA	ACGATCCCAGGCCAGAAAA	91
<i>STAT</i>	TGGGCCAAGGGTTATCACTAGT	CCTGTGTGCGTCTGCGTAAA	81
<i>egr</i>	GATTCATACATATTCAAAGCTCCTACAATC	ACTGTTGTACCATGAAGAAAGCAA	100
<i>wgn</i>	TGAACTGCTAATTTGATGTTTACGAATG	GCCATTAAACCACGCACATGAA	119
<i>crq</i>	ATTGCTGGTCTCGGTGCTTT	GTTGCTGGTCTCTGGGTCTCT	88
<i>Gcm</i>	CGGTCTGTGTTGTGTCCTTTG	ATGCTGGAAGTGGGGATTGT	150

## References

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