Supplementary data:

Table S1. Oligonucleotides used for amplifying *M. sexta* Spätzle-1 DNA.

Table S2. Oligonucleotides used in real-time PCR.

Table S3. Mass spectrometry identification of plasma proteins induced after Spätzle-C108 injection.

Fig. S1. Sequence of the region of the *M. sexta* proSpätzle gene encompassing the open reading frame. Deduced amino acid sequence encoded by exons is shown below the DNA sequence. The one-letter code for each amino acid residue is aligned with the second nucleotide of the corresponding codon. The 5' and 3' boundary of each intron is indicated in **bold**. The alternative 3' splicing sites resulting in variants proSpätzle-1A and proSpätzle-1B are shown in *italic.* Numbers left of the alignment indicate amino acid position with respect to the mature proSpätzle after the signal peptide in Fig.1.

Fig. S2. Alignment of amino acid sequences of insect Spätzle cystine knot domains. Sequences were aligned using the program Clustal W. The amino and carboxyl ends, which were highly variable, were left not included in the analysis. Asterisks (*) below the alignment indicate identical residues at that position while colons (:) indicate conservative substitutions. Accession numbers for the sequences are as follows: Aa_Spz1A, XM_001663577; Aa_Spz2, XM_001659134; Aa_Spz3, XM_001653272; Aa_Spz4, EF173379; Aa_Spz5, XM_001654288; Aa_Spz6, XM_001655868; Ag_Spz2, XM_557166; Ag_Spz3, XM_317093; Ag_Spz4, XM_317626; Ag_Spz5, XM_308593; Ag_Spz6, XM_314007; Bm_Spz1, NM_001114594; Dm_Spz1A, NM_079802; Dm_Spz2, NM_168090; Dm_Spz3, NM_135316; Dm_Spz4, NM_135660; Dm_Spz5, NM_139496; Dm_Spz6, NM_138117; Ms_Spz1A, GQ249944; NV_XP001606369, XP_001606369; NV_XP001607462, XP_001607462; NV_XP001605307, XP_001605307; NV_XP001599503, XP_001599503; Tc_GLEAN010504, GLEAN010504 from the Human Genome Sequencing Center at Baylor College of Medicine; Tc_GLEAN05940, NM_001160153; Tc_GLEAN06726, XM_0018106514; Tc_GLEAN13304, XM_965700; Tc_GLEAN16368_XM_961942.

Fig. S3 Analysis of the relative abundance of two *M. sexta* spätzle isoforms. (A) RT-PCR was used to amplify two isoforms using hemocyte cDNA from day 2 fifth instar larvae collected 24 hours after injection with curdlan. The positions of the two isoforms are indicated on the right. The lane numbers indicate samples obtained from three different insects. (B) The net intensities (the sum of the pixel values minus background) of each band corresponding to two isoforms in (A) were determined using Kodak Digital Science^{TM} 1D Image Analysis Software 2.0. The bars represent mean ± S.D. (n=3).

Table S1 Oligonucleotides used for amplifying *M. sexta* Spätzle DNA

Forward primers	**Reverse primers**
Spätzle cDNA cloning:	
5’ RACE: 5′-CTCCTGCGGACCCAACCTCTGGGCGAT-3′	
3’ RACE: 5′-CGCCCAGAGGTTGGGTCCGCAGGAGGACA-3′	
Amplification of the complete Spätzle open reading frame:	
5’-AGTTATGGCCTGGATCCAGC-3’	5’-CAGTTCATATGATTGTCAAT-3’
Amplification of Spätzle genomic DNA encompassing the open reading frame:	
5’-CGCCCAGAGGTTGGGTCCGCAGGAGGACA-3’	5’-GGAAGCTTTCATATGATTGTCA-3’
5’-ACGTACCCGAAGTGCCAGAGGA-3’	5’-CTCCTGCGGACCCAACCTCTGGGCGAT-3’
5’-ACAACCTGACAGACGGATAGCGGGACGG-3’	5’-GTTTCAGGGTAGTCAATTTCGC-3’
Amplification of the region differentiating two Spätzle isoforms :	
5’-GAAGAACCGAGACAACGTGC-3’	5’-GATTTTCTTGCATTCCTCTGGC-3’
Amplification of the region encoding Spätzle-C108 for production of recombinant protein:	
5’-GTCCATGGTGGGTCCGCAGGA-3’	5’-CGCTCGAGTCAATGATGATGATGATGATGTATGATTGTCAAT-3’
Added *Nco*I site is underlined.	Added *Xho*I site is underlined. The reverse complement sequence for 6 histidine residues inserted in front of the stop codon is double-underlined.
Amplification of full-length proSpatzle, including signal peptide with restriction sites for cloning in pFastBac:	
5’-ATACTAGTATGGCCTGGATCCAGCA-3’	5’-CGCTCGAGTCAATGATGATGATGATGATGTATGATTGTCAAT-3’
Added *Spe*I site is underlined.	Added *Xho*I site underlined. The reverse complement sequence for 6 histidine residues inserted in front of the stop codon is double-underlined.

Table S2 Oligonucleotides primers used in real-time PCR

Gene	ID	Forward primer	Reverse primer
attacin-1	DQ072728	5’-GCAGGCGACGACAAGAAC-3’	5’-ATGCGTGTTGGTAAGAGTAGC-3’
attacin-2	AY232304	5’-CGTGTCGAACTTCTTAAAGCC-3’	5’-CCTCTTCCACAACAACC-3’
cecropin-6	AY232302	5’-CCGTGTTTTATTCTTCGTCTTC-3’	5’-AATCCTTTGACCTGCACCC-3’
βGRP-2	AY135522	5’-CTGGCGATGGCTTCTACC-3’	5’-CCGTGAAGTCGTGGATACC-3’
hemolin	M64346	5’-CAACCAAGCAACAACACAGG-3’	5’-CAGCACAGGCATCTTCTCC-3’
IML-2	AF242202	5’-ACAACACCTTCCAGCGTCTATG-3’	5’-TTGGCGTAACCTGCTTCTTCC-3’
lysozyme	S71028	5’-GTGTGCCTCGTGGAGAATG-3’	5’-ATGCCTTGGTGATGTCGTC-3’
moricin	AY232301	5’-TGCTTTCTTTAACCTTTGTCCTC-3’	5’-TATTCTAACACAGCCTATAATGCG-3’
spätzle	GQ249944	5’-AGTGACCAGTAAGCCAACAAC-3’	5’-CGAAGAGCCAAACGAGTAAATG-3’
rpS3	U12708	5’-TGCGTTTCATCATGGAGTC-3’	5’-TCCTTGCCTGAGAAGTACG-3’

Table S3 Mass spectrometry identification of plasma proteins induced after *M. sexta* Spätzle-C108 injection

Band number	Protein name	Accession number	Predicted MW (Da)	Mascot Score	MSMS Peptides matched
1	attacin-1	gi|67906420	24289	208	3
2	lysozyme	gi|233964	13975	346	11
3	cecropin B	Contig 2488*	3254	53	1
3	cecropin A	Contig 5774*	2875	42	1

* *M. sexta* cecropin A and B sequences are partial sequences obtained from *M. sexta* ESTs available at http://entoplp.okstate.edu/profiles/7231contigs.pdf.

Fig. S1

```
    ACAACCTGACAGACGGATAGCGGGACGGTCAGCACAATACGAACATTTAAGAACAAACGAGAGGTCTCTCCCGGTCTACAGCGAGACCCA
 23   Q  P  D  R  R  I  A  G  R  S  A  Q  Y  E  H  L  R  T  N  E  R  S  L  P  V  Y  S  E  T  Q

    GAGGATACAAGCAGAAGAGAGAAGAAGACACAGTTCGAGACTAGAAGAACCGAGACAACGTGCTGAGAATGGTTCATATAAGATATTGAA
 53   R  I  Q  A  E  E  R  R  R  H  S  S  R  L  E  E  P  R  Q  R  A  E  N  G  S  Y  K  I  L  N

    TAACCCTCCGAAACCCTGTATTACTAATAGGAGAAGTAAGTGACAAATTCACACTGAAATTCATCATGATGCTGGCAAAATATTGGCGTA
 83   N  P  P  K  P  C  I  T  N  R  R

    TTGGTCAAAACATTTGCGGCTATTGGGCAAAGCAATATCGACTTTGTATTTTTCTAAACAGAGCTGACGTTACATCATTCAAATAAAGCA
    AATATTCGCTCAAATACGCCAAGTATAGTGCCAGCATAAGAATCTATGCAGACGGTGTACTTATACGCTTTGTTAAATCGCTTCGGAAGC
    ATTTAAAGGGCACAGTAAATATGATTCAACCAAAAACTCATTTTACAAAGAAAATTTGTGCGCCTGAAAAACGCGCAGAGATAATATTGA
    ATCGCGGTTTGCATGGGTCTTGAAACATTTATAATAACTAATAATTTATGATGCTTGGTCGCAATCTTAACGGTTCCTTCTTTCTTCTTT
    TGCTTCATTCTATTTCCTAATATATAAAATCTAAAAACATTTAGAGCGCACTCAAAACCATTCTAGGAACGCACGCGTTGCATCTAATGT
    ATATAGGCCTAAAATCTTTTTTTTTCTTTCAATAGCACGCGAAATTGACTACCCTGAAACTATTGGTCAAATTGATTCGTCGAATGATAGG
 94                                    T  R  E  I  D  Y  P  E  T  I  G  Q  I  D  S  S  N  D  R-Spz1B
                                                                     S  Q  I  D  S  S  N  D  R-Spz1B

    GTAGTGTTCCCCGGTCCGACTTCAGAAAGGTCGTACGTACCCGAAGTGCCAGAGGAATGCAAGAAAATCGGCATATGCGACAGTATACCG
113  V  V  F  P  G  P  T  S  E  R  S  Y  V  P  E  V  P  E  E  C  K  K  I  G  I  C  D  S  I  P

    AATTACCCAGAAGAACACGTAGCTAATATTATATCTCGAGTAAGTTTCAATTTATTAAAAACTACTTGTCTTTAGAAAGTGATATAATTG
143  N  Y  P  E  E  H  V  A  N  I  I  S  R

    ATGTTCAAATGATGTCTTATTAAGGTAGATATGATTAAAATTCAAGTAAGCTTTCATTTGAATCGAACAATATTTTAATTATTTTAAACT
    TCTTTGTATCTTATCGAATTCTTATCTTTAGCGATCATTGTTAAGTTCGAATCAGAACATCAATAGTTTAATTAATAATCCTAAACGTTT
    CAAAATACGATAATTTGAATAAATCCAAAACATTTCTGTTTTAATTTCAGCTTGGAGACAAAGGAAAAGTATTACAAATAGACGAACTGG
156                                                     L  G  D  K  G  K  V  L  Q  I  D  E  L

    ACGTATCAGACACTCCAGATATCGCCCAGAGGTTGGGTCCGCAGGAGGACAACATGGAACTATGTAGCTTTAGAGAAAAGGTAAATACAC
169 D  V  S  D  T  P  D  I  A  Q  R  L  G  P  Q  E  D  N  M  E  L  C  S  F  R  E  K

    AAAACTGTTTTTTGTTTCATTTTTCCACATACATCTAGCAAGATTATTAACCAGATAACTAACTCTTCTCTTTCATACTTTATATTTATA
    ACTTCCTGTTTACAATACCTATATGGAAGAGTTAATACTTAATAATGTCAACTTCGAATCGAATTTAGATCGATGAACACTTAGAATTCG
    ACATTCGGAACTTCTTTTGAATCCTTAAGGTAAAATTTTTCTGTCCAGTGTGGTTGAGGACATATTAATATTCCCTCTAGTATTGAACGG
    TATGGTCAGTAAATGGTATTACCATCAGGTGACCCAAATGCTTATTTTCTCTCTTTTATATCAATAACTTTATTTGACGTTCAATTATTT
    ATCAACGTTTGAACTTATCCTCTTTGTTGCAGATTTTTTACCCCAAGGCAGCGCCAGACAAAGATGGAAATTGGTTCTTCGTTGTGAATT
196                                  I  F  Y  P  K  A  A  P  D  K  D  G  N  W  F  F  V  V  N

    CAAAAGAAAACCCAGTACAGGGTTATAAAGTTGAAATTTGCGAGTAAGTTTATTGTGTTAATTCTATTGATAATAGAGCCCTTAGGTAGA
215 S  K  E  N  P  V  Q  G  Y  K  V  E  I  C  D

    GACGTACAAATCGCTCGCGTGACGGCAATATCACTACGTAGTATAATTATCTATTCAATGATATCACCAAGAATATTAACATGCAAAAGT
    TTCAAAGTACTCAGTAACATTAACACGTGCTCGTTCTCCGGGATATCAAAAACTCTTAAACACTTGCATTAATTTCGGTTAGATTTTACG
    ACCAGCTAGTTGCTTTATATTAATCTCCTCTAGCCTTGTGTATTCCAATCTTTGAGACAATAAACCATATCGAACGTCATATCATATGTT
    TAAGTAATTAAAGAATTATAAAGCATTAATGACCTTCAAACTGAAACATAACTATTGTTGTACACTGCTGCGTGGCGAGAGACGTACTCC
    TAAATATAGTTCGAGCCCCCAAAATATCATTATTATTTCTTACCATCATGTCTTTACAACGTTAATGACGAATGTCGGAAATCCCTCCCG
    TCATTTGACGTCATTAATTTGTATCTAATTGGAGATCATTGCCTGTCGTACACGAGTTTTTATTCCATTGTGAGTGGGTAATATTACTCG
    TCCTTCAACGGAGAGGGCTCTGTACCGTAGGACGTTAGCCCACGGCGCCAGAATGAAGATAAAATGTGACAAAACTGATTTATTTATAAA
    CTTCACAGAGATTTAGTACATGATCTTTAGGTATTTCTGCCATCAAGCAGGAGCTTGCATGCGTGGTTGTGTTCCGATTTGAAGAAATTA
    TATTATGCACTTTAATTACTGAGCATTAAGAGACTTATTATCTTACACCAGGGCGGCTTAAGCAGTACAGTATCCAGCAATGATTTGTAA
    TTTTAAATGCTGTTGTTAGTATGTGTCGACGGTAGTCGCTCACCATCAGGGGACCACCATTAAACTAGTAAAACTAGATTTTTTTTTCCA
    GCCGTCAGCAATTACCATGCGCGGAGTTCGCGAGCTTCCAACAGGGATATGAAGCGAGGTGCATCCAGAAATACGTTCGCCGGACCATGT
230    R  Q  Q  L  P  C  A  E  F  A  S  F  Q  Q  G  Y  E  A  R  C  I  Q  K  Y  V  R  R  T  M

    TGGCGTTGGATCCCAAGGGTCAGATGACCGACATGCCCCTTAAAGTGCCCAGCTGTTGCTCATGCGGGCCAAATTGACAATCATATGA
259 L  A  L  D  P  K  G  Q  M  T  D  M  P  L  K  V  P  S  C  C  S  C  V  A  K  L  T  I  I  *
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Fig. S2

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Dm_Spz3            CESKIEIVTPYWASNSAGKIRAIVNTQH----FEQAIHQEVCSN-TQTPRCEG----------ECGCEQ
Ag_Spz3            CESKIEIVTPYWASNSAGKIRAIVNTQH----FEQAIHQEVCSN-TQTPRCEG----------ECGCEQ
Aa_Spz3            CESKIEIVTPYWASNSAGKVRAIVNTQH----FEQAIHQEVCSK-TQTSRCSG----------DCGCEQ
Tc_GLEAN05940      CESKVEIVTPYWASNSAGKIRAIVNTQH----FEQAIHQEVCAK-VRTNRCSG----------DCGCEQ
Aa_Spz4            CPVKEEVVAPFWANNTRGEVLALLNLYP----FEQYVHWEKCAYELKQMYCRE----------GCRCEQ
Ag_Spz4            CPVKEEVVAPFWANNTRGEVLALLNLYP----FEQYVHWEKCTHELKQMYCRE----------GCRCEQ
Dm_Spz4            CPVKDEVVAPFWANNTRGEVLALLNLYP----FEQYVHWEKCTHEFKQMFCRD----------GCRCEQ
Nv_XP001605307     CPVKEEVVAPFWANNTRGEVLALLNLYP----FEQYVHWEKCTNENKQMYCRD----------GCRCEQ
Tc_GLEAN06726      CPVKEEVVAPFWANNTRGEVLALLNMYP----FEQYVHWEKCTHEHRQMYCRD----------GCRCEQ
Aa_Spz2            CPSIIRYARPQKARSATGEWKYIVNTG----EHTQTLRLEKCTTPQDSCTYLT-------DNFRSRCVQ
Ag_Spz2            CPSIIRYARPQKARSATGEWKYIVNTG----EHTQTLRLEKCTTPQDSCTYLT-------DNFRSRCVQ
Dm_Spz2            CQSVVRYARPQKAKSASGEWKYIVNTG----QHTQTLRLEKCSNPVESCSYLA-------QTYRSHCSQ
Nv_XP001607462     CPSLVQYARPQLARAASGVWKYIINTG----EHTQTLRLEKCSTPRTSCSFIS-------ENYKSSCMQ
Ms_Spz             CSFREKIFYPKAAPDKDGNWFFVVNSKE---NPVQGYKVEICDRQQLPCAEFAS----FQQGYEARCIQ
Bm_Spz             CDFRVQIMTPLAAQSDDLKWYHVLNFNE---NPLQGFRVEICNTTSTGCAKFVT----MENNYNPKCVQ
Tc_GLEAN01054      CTSISKTIFPRVGINKNNKWKVIINQEED--GYIQGIRTEICRKAN---------------GYITSCKQ
Nv_XP001606369     CEATEKVVYPKVAQSKDKEWLFVVNQE----GFSQGVRVETCGKENNACNLIEG----FAEGYKTVCKQ
Dm_Spz1A           CRSIRKLVYPKKGLRADDTWQLIVNND----EYKQAIQIEECEGADQPCDFAAN----FPQSYNPICKQ
Aa_Spz1A           CESRERLIHPRSGFNTDNRTIMIINTK----EYMQGVRIETCSSQGQPCVKLN------PLFGKTECRQ
Aa_Spz5            CQTREMYVTPQAALNTKGNWMYIVNHEE----SRQLVKAEIC--TSSECSNLCS----LPNGYNSRCEQ
Ag_Spz5            CSVRERYITPQTALNTKGNWMFVVNQEN----SRQLVKTEIC--ASTECSNLCS----FPIGYSSRCEQ
Dm_Spz5            CQTTSQFITPQAALNSRGNWMFVVNEQNT---ARQMVKAELC--ASNTCSNLCE----LPNGYNSRCEQ
Tc_GLEAN13304      CTSRSQYIMPRAALNNKGNWMYVVNMPELDNRFSQLVKSETC--ASQTCSGLCG----LPVGYTSRCEQ
Nv_XP001606369     CPTRSQFVTPKAALNNQGNWMYVVNLEDQ-NKHSQLVKSE----VSKHATGLCS----LPLGYTSKCQQ
Aa_Spz6            CPAKVEYATPVFAKNYQGSWRYVVQIPYEG-YFTQTVEVTRC--LQARCHYLDG-----------GCCD
Ag_Spz6            CPAKVEYATPVFAKNYQGSWRYVVQIPYEG-YFTQTVEVTRC--LQARCHYLDAPTAAASSAKANQHCD
Dm_Spz6            CPAKVEYATPVFAKNYQGAWRYVVQIPYEG-YFTQTVEVTRC--IQARCHYLDG-----------GCCD
Tc_GLEAN16368      CPAKVEYATPVFAKNYQGVWRYVVQIPYEG-YFTQTVEVTRC--LQSRCHYLDG-----------GCCD
                   *        *            :::         *                                 :

Dm_Spz3            KYKWHRLLAYDPDNDCKGIFMDWFLFPSCCVC
Ag_Spz3            KYKWHRLLAYDPDNDCKGIFMDWFLFPSCCVC
Aa_Spz3            KYKWHRLLAYDPDNDCKGIFMDWFLFPSCCVC
Tc_GLEAN05940      KYKWHRLLAYDPDNDCKGIFMDWFLFPSCCVC
Aa_Spz4            QYRLHRLLAYDPHNECRGIFSDWFRFPSCCIC
Ag_Spz4            QYRLHRLLAYDPHNECRGIFSDWFRFPSCCIC
Dm_Spz4            QYRLHRLLAYDPHNECRGIFSDWFRFPSSCIC
Nv_XP001605307     QYRLHRLLAYDPNNECRGIFSDWFKFPSCCIC
Tc_GLEAN06726      QYRLHRLLAYDPNNECRGIFSDWFRFPSCCVC
Aa_Spz2            IYNYHRLLSWDTARG---LHVDIFKVPTCCSC
Ag_Spz2            IYNYHRLLSWDTARG---LHVDIFKVPTCCSC
Dm_Spz2            VYNYHRLLSWDKVRG---LHVDIFKVPTCCSC
Nv_XP001607462     VYNYHRLLTWDSKLG---LHMDIFKVPTCCSC
Ms_Spz             KYVRRTMLALDPKGQ---MTDMPLKVPSCCSC
Bm_Spz             KFIFRKMKILSESGE---MIERSMKVPSCCSC
Tc_GLEAN01054      KYMLRRMMSLSENGD---PVPDTFQLPSACCC
Nv_XP001606369     KYIYRQLVALSTIGQ---LKPEKFRFPASCCC
Dm_Spz1A           HYTQQTLASIKSDGEL-DVVQNSFKIPSCCKC
Aa_Spz1A           LYHYRTLLAIDPQTN--QPYKEKFKLPSCCKC
Aa_Spz5            KFSQKRLLTLEADGQ--SLYVDTYWFPSCCVC
Ag_Spz5            RYVQKRLVTLDPTGR--TLYVDTYWFPSCCVC
Dm_Spz5            KFVQKRLIALQGNGQ--NLYTDTFWFPSCCVC
Tc_GLEAN13304      KYVQKRLVALEGAGN--ELYTDVFWFPSCCVC
Nv_XP001606369     QYVQKRLVALEGSGN--RLYTDVFWFPHGCMC
Aa_Spz6            GHDEIGCFQVR-------LYYDWFLIPGSCKC
Ag_Spz6            GHDEIGCFQVR-------LYYDWFLIPGSCKC
Dm_Spz6            GHDEIGCFQVR-------LYYDWFLIPGSCKC
Tc_GLEAN16368      GVDALGCFQVR-------LYYDWFLIPGSCKC
                                             *  * *
```
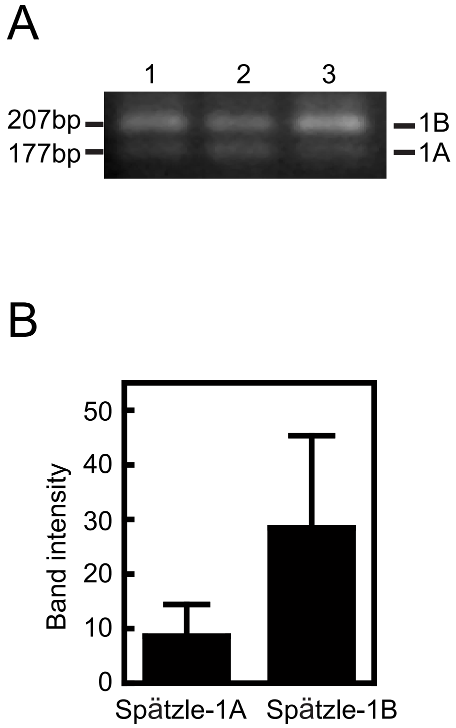

Fig. S3


A
1
2
3
207bp
177bp
1B
1A
B
Band intensity
50
40
30
20
10
0
Spätzle-1A
Spätzle-1B