

# Strongyloides ratti



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## SUPPLIMENTARY MATERIAL

**Table S1.** Mean Ct (threshold cycle) of *Sra-hsp-17.1* and *Sra-hsp-17.2* compared to the housekeeping control gene, *Sra-gapdh* in iL3, parasitic and free-living *S. ratti* female stages.

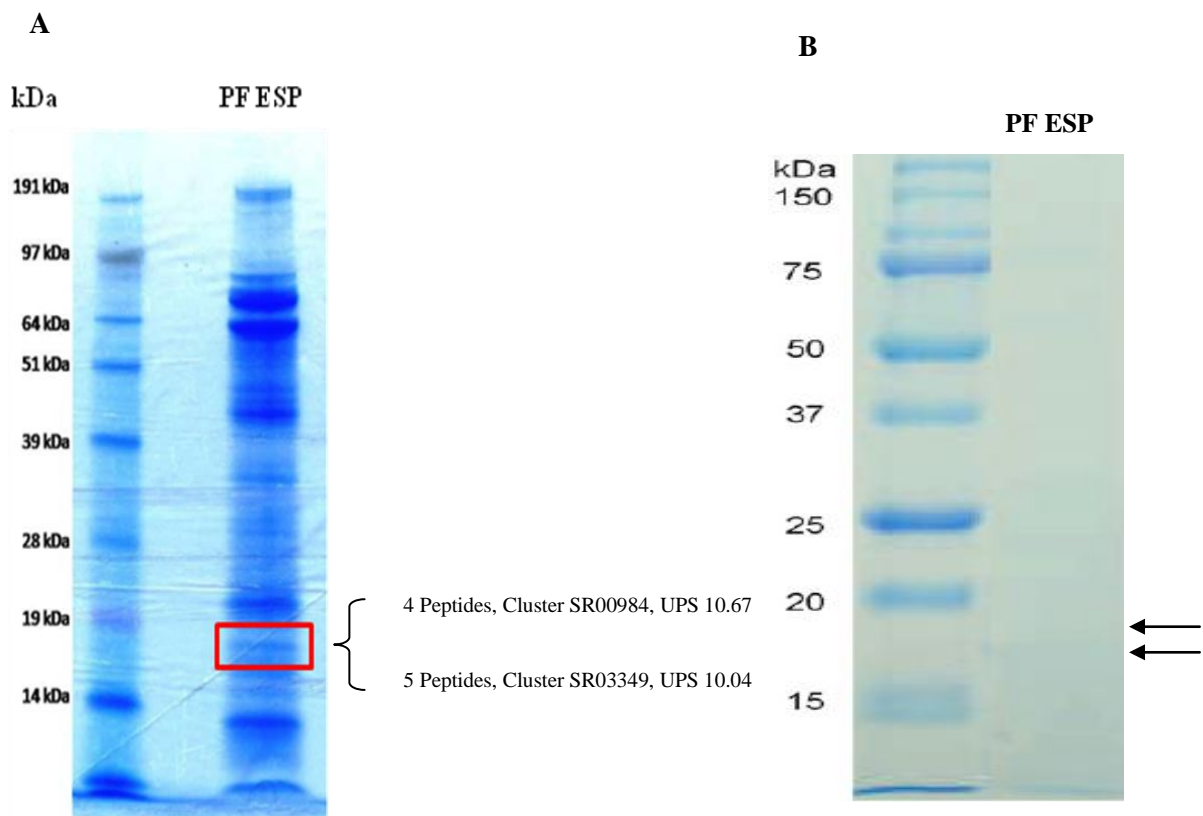
The data were expressed for two independent experiments. Ct values are means  $\pm$  S.D.

Stage	<i>Sra-gapdh</i>	<i>Sra-hsp-17.1</i>	<i>Sra-hsp-17.2</i>
iL3	15,365 $\pm$ 0,1341	20,5433 $\pm$ 0,0273	26,8967 $\pm$ 0,0103
PF	15,4 $\pm$ 0,0782	15,6233 $\pm$ 0,0121	19,1833 $\pm$ 0,0288
FF	15,3767 $\pm$ 0,304	18,04 $\pm$ 0,041	22,06 $\pm$ 0,0434

**Table S2.** List of nucleotide primers

Primer name	5' - 3' sequence
Oligo dT-T7I	GAGAGAGGATCCAAGTACTAATACGACTCACTATAGGGGAGATT24
Oligo dT-T7II	GAGAGAGGATCCAAGTACTAATACGACTCACTATAGG
SL-1	GGTTTAATTACCCAAGTTTGAG
af1	ATGAACGACCGTTGGATGA
ar1	TTATTTTCTATATTCAATTGGGAC
bf1	ATGTTTGACAACCACATGATGACACC
br1	TTACTTAAACTTGATAGGAATATTTTTTCCC
af2	AACGACAAGGAATTCAGAGTCAA
bf2	CCATTCACTCGTATGCCACTT
ar2	ACGGACGAAACTTCTTTGGA
br2	TGGGTGGGTGTCAGCAAAT
af3	AACGACAAGGAATTCAGAGTCAA
ar3	ACGGACGAAACTTCTTTGGA
bf3	CCATTCACTCGTATGCCACTT
br3	TGGGTGGGTGTCAGCAAAT
af4	AAGCTTgatgatgatgataaaATGAACGACCG
ar4	GGATCCTTATTTTCTATATTCAATTGGGAC
bf4	CATATGgatgatgatgataaaATGTTTGACAAC
br4	GGATCCTTACTTAAACTTGATAGG
gapdhf	GTACCACTAACTGTTTAGCTCC
gapdhr	GCACCTCTCCATCTCTCC
PJC45f	ATACGACTCACTATAGGGGAATTG

**Fig. S1.** (A) SDS-PAGE of excretory/secretory products from *S. rattii* PF after 250-fold concentration and mass spectrometric data analyses by ProteinPilot search engine v2.0.1, identifying *Sra*-HSP-17s peptides, in the respective 18-19 kDa-excised protein band. UPS= unused protein score. (B) SDS-PAGE of excretory/secretory products from *S. rattii* PF after inhibition of protein release by 70  $\mu$ M cycloheximide.



**Fig. S2.** Sequence identification. (A) Alignment of the two *hsp-17* clusters identified by ProteinPilot search engine v2.0.1 from the ESP of the PF (SR03349 and SR00984) and a third alternative cluster SR01014. Symbols: identical residues are shaded in black blocks, conserved residues in two clusters are shaded in grey. (B) Alignment of *Cel-hsp-17a* (acc. No Q20660) and *Cel-hsp-17b* (acc. No Q7JP52) isoforms (differ nucleotides are in bounder). (C) Alignment of the identified full length cDNAs of *Sra-hsp-17.1* and *Sra-hsp-17.2*. Identical residues underlined with asterisks.

		<u>Identity to SR03349</u>
SR03349	-----ACCACATAAGTTATTAAATATTTCT	25 (100%)
SR01014	ACATCTATATAAACACTATTTTTCCATTTTATCACCACATAAGTTATTATAATTTCT	60 (98%)
SR00984	-----GTTATTGTCT-----	12 (64%)
SR03349	ACTGATPATTTTTTGCTACGAAAGTTTTTGCTATTTATTTATTCGCTATCTTTGACAACCAC	85
SR01014	ACTGATPATTTTTTGCTACGAAAGTTTTTGCTATTTATTTATTCGCTATCTTTGACAACCAC	120
SR00984	AAATACTPATTTTTT-CTATCTTGTACTCTACTGCTTTTAAATC---ATC---AAGACCCT	65
SR03349	ATGATGACACCATTCACTCGTATGCCACTTACCTCCTGTCCTACAACAGTA-AGTACGT	144
SR01014	ATGATGACACCATTTACTCGTATGCCACTTACCTCCTGTCCTACAACAGTA-AGTACGT	179
SR00984	TGATGACTCCATTTGTCGGTATCCTCTCTCTGTTTGTCC--ATTAGGATACGGTGGAC	123
SR03349	CAATGAAT-----GGAT---CGTCTATGAAACGCTACATGGCTAATACTCTTT-	191
SR01014	TAATGAAT-----GGAT---CGTCTATGAAACGCTACATGGCTAATACTCTTT-	226
SR00984	CAAGCAACCTCTTCAACGAGATGAACATGT--TGGAGCGCAAAATGATGAACTCCCTCAA	181
SR03349	CATGGGCAACA-AATCTCTTATGGAAAGCCATAAATTTGCTGACACCACCCAGATATT	250
SR01014	CATGGGCAACA-AATCTCTTATGGAAAGCCATAAATTTGCTGACACCACCCAGATATT	285
SR00984	CATGGTTGATCTAATCTC--ACCAACACATGGAACTTATGAAACCATGTCCAGAGGTA	239
SR03349	ATCGAATGACAAGGAATTCAAAGTCAAATGGACGTATCACACTTTAGTCCAGATGAA	310
SR01014	ATCGAATGACAAGGAATTCAAAGTCAAATGGACGTATCACACTTTAGTCCAGATGAA	345
SR00984	GTAAATGACAAGGAATTCAGAGTCAAATGGATGTATCTCACTATGACCAATGAA	299
SR03349	TTGAAAGTCACTTCAGAGATGGATACCTCCAAGTTGAAGGTAATCATGAAGAGAAGACT	370
SR01014	TTGAAAGTCACTTCAGAGATGGATACCTCCAAGTTGAAGGTAATCATGAAGAGAAGACT	405
SR00984	TTGAAAGTCACTTCAGAGATTAACCTCCAAGTTGAAGGTAATCATGAAGAGAAAAGT	359
SR03349	GACAAATATGGAACCATCCAAAGAAGCTTTGTAAAGAACTATTTCTTTACCACCAAACTTC	430
SR01014	GACAAATATGGAACCATCCAAAGAAGCTTTGTAAAGAACTATTTCTTTACCACCAAACTTC	465
SR00984	GACAAATATGGAACCATCCAAAGAAGCTTTGTAAAGAACTATTTCTTTACCACCAAAAGGACTC	419
SR03349	AATGAAGACGACTCCGTCTGTGAGATTTCAAAGGATGCTGTTTTAACTGTTGGAGTTGCC	490
SR01014	AATGAAGACGACTCCGTCTGTGAGATTTCAAAGGATGCTGTTTTAACTGTTGGAGTTGCC	525
SR00984	ACAGAGGAGATGTCAAAAAGTGAACCTTACTAAGGACGGTGTCTTACAGTCCGGAGGTAA	479
SR03349	AAACTTGCCATCGGAGAGAAAAGGGGAAAAATATTCCATCAAGTTTAAGTAAAAGATT	550
SR01014	AAACTTGCCATCGGAGAGAAAAGGGGAAAAATATTCCATCAAGTTTAAGTAAAAGATT	585
SR00984	AAACTTGCCATCGGAGAGAAAAGGGGAAAAATATTCCATCAAGTTTAAGTAAAAGATT	539
SR03349	AAAT-ATTATATAATGAGTTTTTATTTCTTTTGTACC---ATGNNN-----	591
SR01014	AAAT-ATTATATAATGAGTTTTTATTTCTTTTGTACC---ATGTTATACGGTAAAGTTCTA	641
SR00984	AGATTATTTTATAATGTTGATTAAATTTATTTATATCTATATATTTTT-----TTATT	591
SR03349	-----	
SR01014	TTATGATGCT--TTTTTGTAGTTTCTTTCTACAAAATAAATGTGAAATTTTTAAA	695
SR00984	TTATGGGACTAATTTTCGTAGCCTTT-----AAATAAAAATCTTTTACGTAATA	640

## B

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Cel-hsp-17a ATGGATCGTCGTTTTCCACCATTCTCCCCATTCTTTAACCATGGCCSCAGATTCTTCGAT 60
Cel-hsp-17b ATGGATCGTCGTTTTCCACCATTCTCCCCATTCTTTAACCATGGCC---GATTCTTCGAT 57
*****

Cel-hsp-17a GACGTCGACTTTGATCGCCACATGATCCGACCATACTGGGCCGATCAAACAATGCTCACT 120
Cel-hsp-17b GACGTCGACTTTGATCGCCACATGATCCGACCATACTGGGCCGATCAAACAATGCTCACT 117
*****

Cel-hsp-17a GGACACCGAGTAGGAGATGCTATTGATGTTGTGAACAACGATCAAGAATACAATGTATCT 180
Cel-hsp-17b GGACACCGAGTAGGAGATGCTATTGATGTTGTGAACAACGATCAAGAATACAATGTATCT 177
*****

Cel-hsp-17a GTAGATGTTTCACAGTTTGAACCAGAAGAGTTGAAAGTGAACATTGTTGATAATCAGCTA 240
Cel-hsp-17b GTAGATGTTTCACAGTTTGAACCAGAAGAGTTGAAAGTGAACATTGTTGATAATCAGCTA 237
*****

Cel-hsp-17a ATCATCGAAGGAAAGCATAACGAGAAGACTGATAAATATGGACAGGTAGAGCGTCACTTT 300
Cel-hsp-17b ATCATCGAAGGAAAGCATAACGAGAAGACTGATAAATATGGACAGGTAGAGCGTCACTTT 297
*****

Cel-hsp-17a GTCCGGAAGTATAACCTTCCAACAGGGGTCCGTCCAGAACAATCAAGTCCGAATTGAGC 360
Cel-hsp-17b GTCCGGAAGTATAACCTTCCAACAGGGGTCCGTCCAGAACAATCAAGTCCGAATTGAGC 357
*****

Cel-hsp-17a AACAAATGGAGTGCTCACTGTCAAATATGAGAAGAATCAGGAACAGCAGCCAAAATCCATT 420
Cel-hsp-17b AACAAATGGAGTGCTCACTGTCAAATATGAGAAGAATCAGGAACAGCAGCCAAAATCCATT 417
*****

Cel-hsp-17a CCAATCACAATTGTGCCAAAGAGAACTGA 450
Cel-hsp-17b CCAATCACAATTGTGCCAAAGAGAACTGA 447
*****
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## C

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Sra-hsp-17.1 ATG---AACGACCGTTGGATGACTCCATTTGTCCGTGATCCTCTCTCTGTTTGTCC--AT 55
Sra-hsp-17.2 ATGTTTGACAACCACATGATGACACCATTCACTCGTATGCCACTTACTCCTGTCCCTAC 60
*** ** * ***** ** * * * * *

Sra-hsp-17.1 TAGGATACGGTGGACCAGCAAACCTCTTCAACGAGATGAACATGTT--GGAGCGTAAAT 113
Sra-hsp-17.2 AAGAGTA-AGTACGTCAATGAAT-----GGGAT---CGTGCTATGAAACGCTACAT 107
** ** * ** * * * * * * * * * *

Sra-hsp-17.1 GATGAACTCCCTCAACATGGTTGATCGTAATCTC--ACCAACAACATGGAAGTTATGGAA 171
Sra-hsp-17.2 GGCTAATACTCTTT-CATGGGCAAACA-AATCTTTATGGAAGCCATAAATTTGCTGAC 165
* ** * * * ***** * * ***** * * * * * **

Sra-hsp-17.1 CCATGTCCAGAGGTAGTAAATAACGACAAGGAATTCAGAGTCAAATGGATGTATCTCAC 231
Sra-hsp-17.2 ACCCACCCAGATATTATCGACAATGACAAGGAATTCAAAGTCAAGATGGACGTATCACAC 225
* ***** * * * * * ***** ***** ***** **

Sra-hsp-17.1 TATGGACCAAATGAATTGAAGGTTACCGTTAGAGATAACTACCTCCAAGTTGAAGGAAA 291
Sra-hsp-17.2 TTTAGTCCAGATGAATTGAAAGTACCTTCAGAGATGGATACCTCCAAGTTGAAGGTAAT 285
* * * * * ***** ** * * * * * ***** ***** ***** **

Sra-hsp-17.1 CATGAAGAGAAAAGTACAAAATACGGAACCATCCAAGAAGTTTCGTCCGTAATATGCT 351
Sra-hsp-17.2 CATGAAGAGAAAGAGTACAAAATATGGAACCATCCAAGAAGCTTTGTAAGAAAGTATCT 345
***** * ***** ***** ***** ** * * * * *

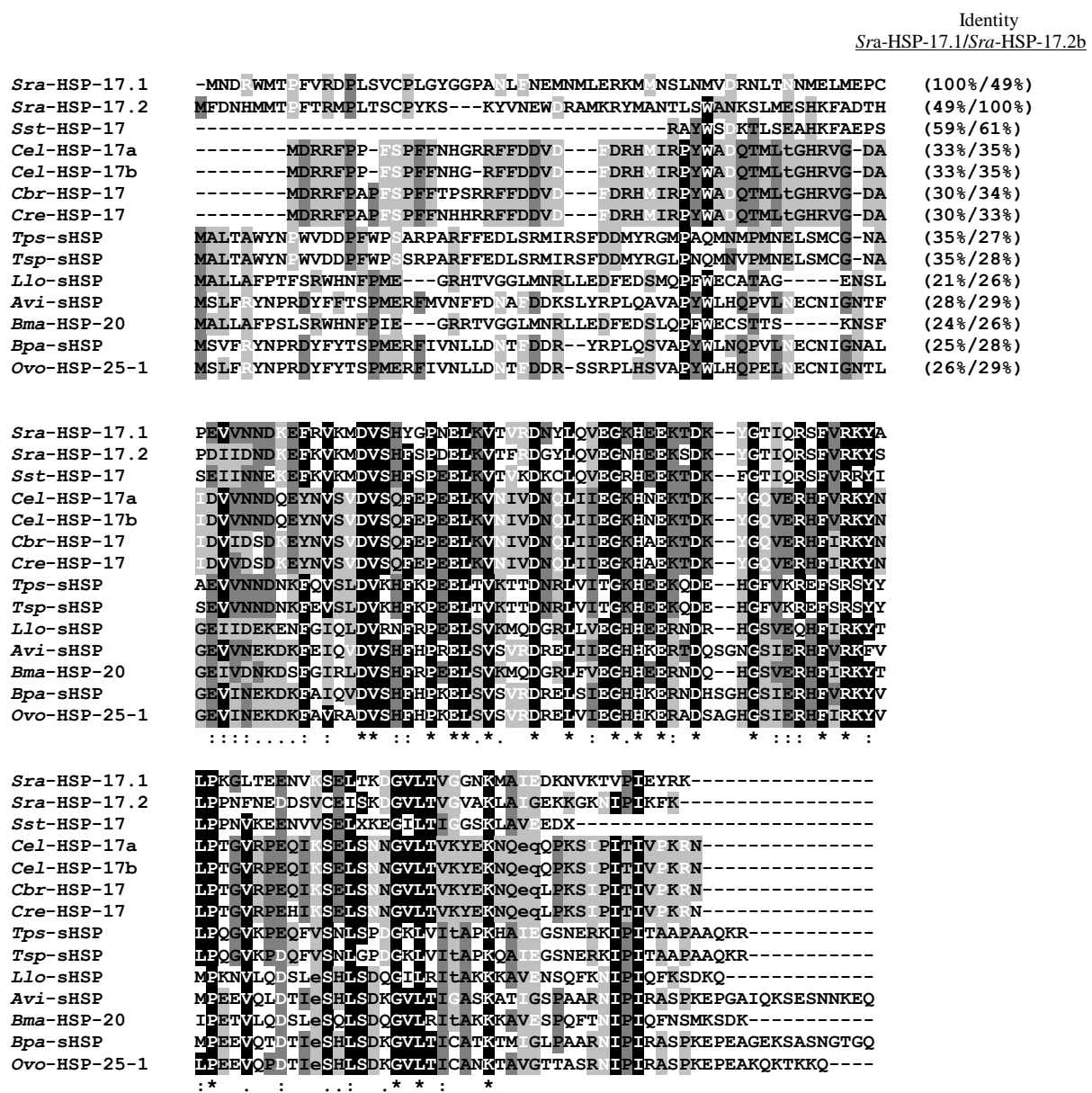
Sra-hsp-17.1 CTCCCAAAGGACTCACAGAGGAGAATGTCAAAGTGAAGTTACTAAGGACGGTGTCTC 411
Sra-hsp-17.2 TTACCACCAAAGTCAATGAAGACGACTCCGTCTGTGAGATTTCAAAGGATGGTGTTTTA 405
* * * * * * * * * * * * * * * * * * * * *

Sra-hsp-17.1 ACAGTCGGAGGTAACAAGATGGCTATTGAGGACAAGAAATGTCAAGACTGTCCCAATTGAA 471
Sra-hsp-17.2 ACTGTTGGAGTTGCCAAACTTGCATCGGAGAGAAAAGGGAAAAAATATTCTTATCAAG 465
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Sra-hsp-17.1 TATAGAAAATAA 483
Sra-hsp-17.2 TTTA---AGTAA 474
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Identity (64%)
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**Fig. S3.** Alignment of the *Sra*-HSP-17.1 and *Sra*-HSP-17.2 aa sequences with other 12 related nematodes sHSPs based on CLUSTAL W, neighbor-joining. Sympols: identical (asterisks), highly conserved residues (>10) are shaded in black blocks, median conserved residues (7-9) in dark grey, low conserved residues (4-6) in light grey, no amino acid residue (dashes), conservation of residue size and hydrophathy (colons), conservation of size or hydrophathy (periods). Accession numbers are listed in Fig. 2.



**Fig. S4.** Alignment of the cloned and sequenced PCR products amplified from the PF cDNA and gDNA. Symbols: identical (asterisks) and introns (underlined). (A) *Sra-hsp-17.1*. (B) *Sra-hsp-17.2*. A single intron in each sHSP located in the second third of the ACDs ORFs. Both introns are phase 0, their splice sites sequences followed the GU-AG convention.

**A**

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gSra-hsp-17.1      ATGAACGACCGTTGGATGACTCCATTGTGCCGTGATCCTCTCTCTGTTGTCCATTAGGA 60
cSra-hsp-17.1      ATGAACGACCGTTGGATGACTCCATTGTGCCGTGATCCTCTCTCTGTTGTCCATTAGGA 60
*****

gSra-hsp-17.1      TACGGTGGACCAGCAAACCTCTTCAACGAGATGAACATGTTGGAGCGTAAATGATGAAC 120
cSra-hsp-17.1      TACGGTGGACCAGCAAACCTCTTCAACGAGATGAACATGTTGGAGCGTAAATGATGAAC 120
*****

gSra-hsp-17.1      TCCCTCAACATGGTTGATCGTAATCTCACCAACAACATGGAACCTTATGGAACCATGTCCA 180
cSra-hsp-17.1      TCCCTCAACATGGTTGATCGTAATCTCACCAACAACATGGAACCTTATGGAACCATGTCCA 180
*****

gSra-hsp-17.1      GAGGTAGTAAATAACGACAAGGAATTCAGAGTCAAATGGATGTATCTCACATGGACCA 240
cSra-hsp-17.1      GAGGTAGTAAATAACGACAAGGAATTCAGAGTCAAATGGATGTATCTCACATGGACCA 240
*****

gSra-hsp-17.1      AATGAATTGAAGGTTACCGTTAGAGATAACTACCTCCAAGTTGAAGGAAAACATGAAGAG 300
cSra-hsp-17.1      AATGAATTGAAGGTTACCGTTAGAGATAACTACCTCCAAGTTGAAGGAAAACATGAAGAG 300
*****

gSra-hsp-17.1      AAAACTGACAAATACGGAACCGTAAGTTTACTTTATACCTCTAAGTATTTAACTGTGGTA 360
cSra-hsp-17.1      AAAACTGACAAATACGGAACC-----ATCCAAAGAAGTTTCGTCCGTAATATGCTCTCCCA 321
*****

gSra-hsp-17.1      AAATCTATTCCTTTTAATTTTAGATCCAAAGAAGTTTCGTCCGTAATATGCTCTCCCA 420
cSra-hsp-17.1      -----ATCCAAAGAAGTTTCGTCCGTAATATGCTCTCCCA 357
*****

gSra-hsp-17.1      AAAGGACTCACAGAGGAGAATGTCAAAGTGAACCTTACTAAGGACGGTGTCCACAGTC 480
cSra-hsp-17.1      AAAGGACTCACAGAGGAGAATGTCAAAGTGAACCTTACTAAGGACGGTGTCCACAGTC 417
*****

gSra-hsp-17.1      GGAGGTAACAAGATGGCTATTGAGGACAAGAATGTCAAGACTGTCCCAATTGAATATAGA 540
cSra-hsp-17.1      GGAGGTAACAAGATGGCTATTGAGGACAAGAATGTCAAGACTGTCCCAATTGAATATAGA 477
*****

gSra-hsp-17.1      AAATAA 546
cSra-hsp-17.1      AAATAA 483
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B

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gSra-hsp-17.2      ATGTTTGACAACCACATGATGACACCATTCACTCGTATGCCACTTACCTCCTGTCCCTAC 60
cSra-hsp-17.2      ATGTTTGACAACCACATGATGACACCATTCACTCGTATGCCACTTACCTCCTGTCCCTAC 60
*****

gSra-hsp-17.2      AAGAGTAAGTACGTCAATGAATGGGATCGTGTATGAAACGCTACATGGCTAATACTCTT 120
cSra-hsp-17.2      AAGAGTAAGTACGTCAATGAATGGGATCGTGTATGAAACGCTACATGGCTAATACTCTT 120
*****

gSra-hsp-17.2      TCATGGGCAAACAAATCTCTTATGGAAGCCATAAAATTTGCTGACACCCACCAGATATT 180
cSra-hsp-17.2      TCATGGGCAAACAAATCTCTTATGGAAGCCATAAAATTTGCTGACACCCACCAGATATT 180
*****

gSra-hsp-17.2      ATCGACAATGACAAGGAATTCAAAGTCAAGATGGACGTATCACACTTTAGTCCAGATGAA 240
cSra-hsp-17.2      ATCGACAATGACAAGGAATTCAAAGTCAAGATGGACGTATCACACTTTAGTCCAGATGAA 240
*****

gSra-hsp-17.2      TTGAAAGTCACCTTCAGAGATGGATACCTCCAAGTTGAAGGTAATCATGAAGAGAAGAGT 300
cSra-hsp-17.2      TTGAAAGTCACCTTCAGAGATGGATACCTCCAAGTTGAAGGTAATCATGAAGAGAAGAGT 300
*****

gSra-hsp-17.2      GACAAATATGGAACCGTAAGTTTATGCTACTTACTTAGTGTGTTATCTTATTTCGTTTT 360
cSra-hsp-17.2      GACAAATATGGAACC----- 315
*****

gSra-hsp-17.2      TAAGATCCAAAGAAGCTTTGTAAGAAAGTATTCTTTACCACCAAACCTCAATGAAGACGA 420
cSra-hsp-17.2      ---ATCCAAAGAAGCTTTGTAAGAAAGTATTCTTTACCACCAAACCTCAATGAAGACGA 371
*****

gSra-hsp-17.2      CTCCGTCTGTGAGATTTCAAAGGATGGTGTTTTAACTGTTGGAGTTGCCAAACTTGCCAT 480
cSra-hsp-17.2      CTCCGTCTGTGAGATTTCAAAGGATGGTGTTTTAACTGTTGGAGTTGCCAAACTTGCCAT 431
*****

gSra-hsp-17.2      CGGAGAGAAAAAGGGAAAAAATATTCCTATCAAGTTTAAGTAA 523
cSra-hsp-17.2      CGGAGAGAAAAAGGGAAAAAATATTCCTATCAAGTTTAAGTAA 474
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**Fig. S5.** Cross-reactivities in ELISAs titration of the (A) 1:3000 immune sera and (B) 1:300 purified antibodies against the respective *Sra*-HSP-17s, by using the recombinant *Sra*-HSP-17s as antigens. Sera from mock-immunized rats (1:50) served as controls.

