International Journal of
Molecular Sciences

MDPI

Supplementary

>TmPGRP-SA
TACGGCCGGGGATTTCCAAGTTGCATCCGATCAGAGGACATCATAACATAAAGTCTGCAT
AAAGTAAAACAGTGAATAGGTCACATTTGTCGTTCAACTAACTGCTAGATATTGTTGTTT
TGTTACA
>>>>>>>>>>>*Tm*PGRP-SA_FW_Cloning
ATGCACGGAGTGTTGTGTTTTTTATTAAGTTTTGTGCGTGTTTATCAAATTTCGGCTCTC
TCAGGTTCTACGATACCAAGAATATGTCCTGAAATTATTAGTCGGACAAGATGGGGGGCG
AGAACTCCATTAGAAGTGGATTATTCATTAATTCCCATTGAAAATGTCGTTGTTCATCAT
>>>>>>>>>>>>>*Tm*PGRP-SA_Fw_dsRNA
ACTGTAACTCATACATGCGACTCGGAAAGCGAATGTGCAACTCTTTTGAGAAATGTTCAG
AATTTTCACATGGAAAACTTAGAATTTCATGACATAGGATACAACTTTTTGGTTGCAGGT
GACGGACAAATATACGAAGGAGCGGGTTGGCATAAAGTTGGAGCGCATACCAGAGGCTAC
AATACAAGATCCTTGGGATTAGCCTTTATTGGCAACTTCACAAGCCAACTACCAGTCCAA
AAACAGCTTAAAGTTGCTAAAGATTTTCTTCAATGCGGAGTTGAACTGGGAGAATTAAGT
*Tm*PGRP-SA_REV_dsRNA<<<<<<<<<<<<<
*Tm*PGRP-SA_FW_qPCR>>>>>>>>>>>>>>>
AAAAATTATAAATTATTTGGAGCACGCCAAGTGAGTTCGACAAGCAGCCCTGGACTGAAA
TmPGRP-SA_REV_qPCR<<<<<<<<<<<<<<<<<
CTCTACCGTGAACTGCAAGATTGGCCCCATTTCACCAGATCTCCTCCTAAATAA
<<<<<*Tm*PGRP-SA_REV_Cloning<<<<<<<<<<<<<<<<
ATTCATCAATTGTTCAACAATTGTTGTATTTTATATTATTGTTTTATGTCATTAAATAAT
TGAATTATCG

MHGVLCFLLSFVRVYQISALSGSTIPRICPEIISRTRWGARTPLEVDYSLIPIENVVVHH
TVTHTCDSESECATLLRNVQNFHMENLEFHDIGYNFLVAGDGQIYEGAGWHKVGAHTRGY
NTRSLGLAFIGNFTSQLPVQKQLKVAKDFLQCGVELGELSKNYKLFGARQVSSTSSPGLK
LYRELQDWPHFTRSPPK*

Figure S1. The full length cDNA sequence of *TmPGRP-SA*. The position of all primers used for real-time analysis and dsRNA synthesis are indicated. The cloning primer sequences are presented in green, qPCR primer sequences in blue, and double-stranded RNA primer sequences in red.

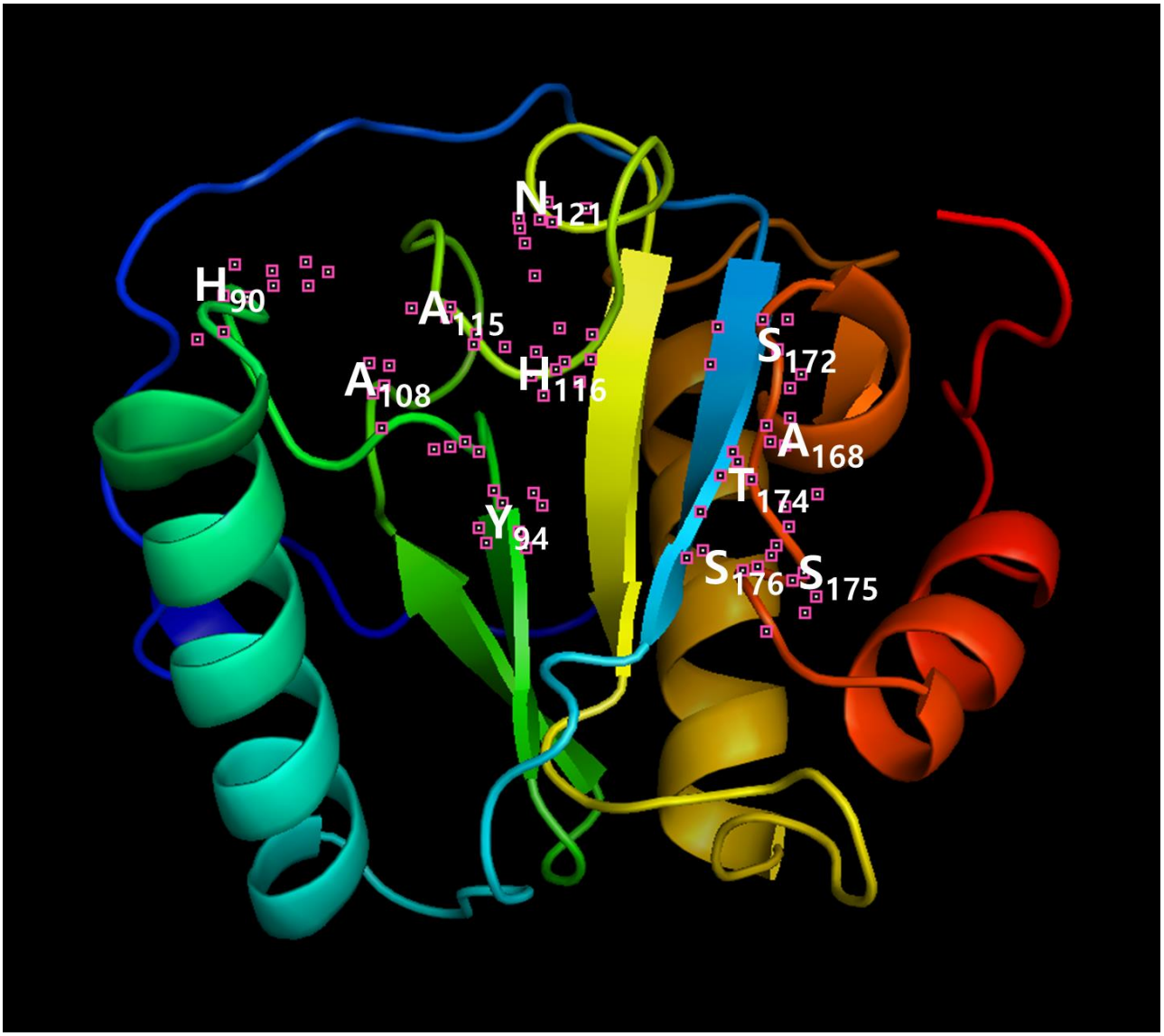


Figure S2. 3D structure of *Tm*PGRP-SA. SWISS-MODEL and PyMOL visualized the amidase catalytic site (active site) and chemical (substrate) binding site amino acid residues (N-terminal: blue, C-terminal: red). The conserved amino acids are shown in small pink squares and name of the amino acids is represented in white color.

	*Tc*PGRP-SA	*Lm*PGRP-SA	*Gb*PGRP-SA	*Bc*PGRP-SA	*Bk*PGRP-SA	*Bi*PGRP-SA	*Ds*PGRP-SA	*Dm*PGRP-SA	*Db*PGRP-SA	*Bl*PGRP-SA	*Zc*PGRP-SA	*Hs*PGLYRP3	*Ej*PGRP-SA	
*Tm*PGRP-SA	76	50	43	37	37	38	43	44	43	43	45	38	27	
*Tc*PGRP-SA		52	42	40	40	41	45	46	42	42	44	38	26	
*Lm*PGRP-SA			56	42	42	42	49	49	49	47	48	43	26	
*Gb*PGRP-SA				44	44	47	43	42	41	44	44	36	25	
*Bc*PGRP-SA					100	91	40	41	38	42	43	36	23	
*Bk*PGRP-SA						91	40	41	38	42	43	36	23	
*Bi*PGRP-SA							41	41	39	42	43	36	21	
*Ds*PGRP-SA								98	69	66	66	38	31	
*Dm*PGRP-SA									68	66	66	38	31	
*Db*PGRP-SA										69	69	35	27	
*Bl*PGRP-SA											91	38	26	
*Zc*PGRP-SA												38	26	
*Hs*PGLYRP3													26	
*Ej*PGRP-SA														

% Identity

Figure S3. Percent identity with full-length ORF of *TmPGRP-SA* and other insects.

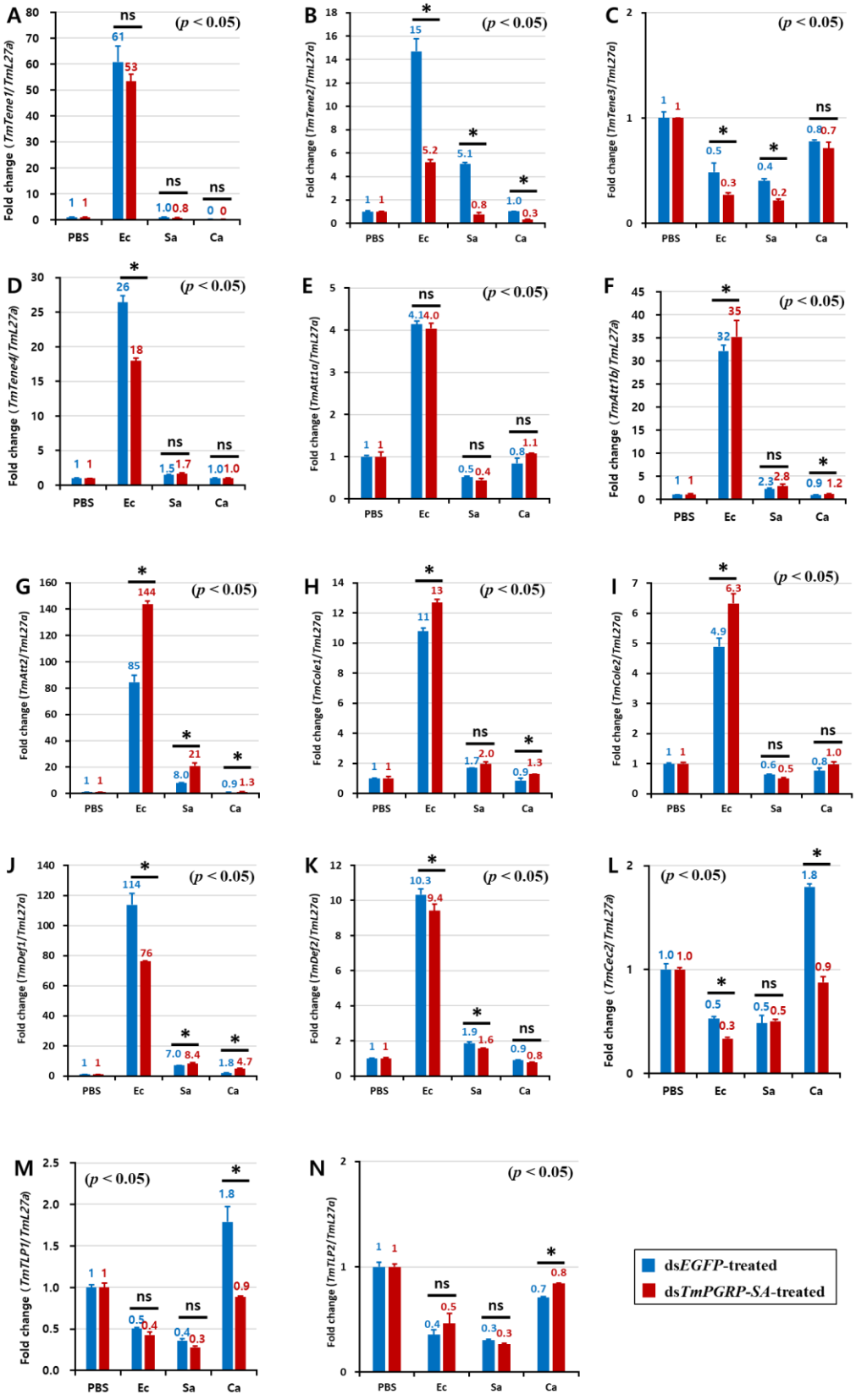


Figure S4. AMP expression levels in the *TmPGRP-SA*-knockdown *T. molitor* larval hemocytes upon *E. coli* (Ec), *S. aureus* (Sa), and *C. albicans* (Ca) infections. Healthy larvae (10th–12th instar) were injected with ds*TmPGRP-SA* and infected with a suspension of *E. coli*, *S. aureus*, or *C. albicans* on the second day post-dsRNA injection. PBS-injected larvae were used as controls. The expression level of the AMP genes *TmTenecin-1* (**A**), *TmTenecin-2* (**B**), *TmTenecin-3* (**C**), *TmTenecin-4* (**D**), *TmAttacin1a* (**E**),

TmAttacin-1b (**F**), *TmAttacin-2* (**G**), *TmColeoptericin-1* (**H**), *TmColeoptericin-2* (**I**), *TmDefensin1* (**J**), *TmDefensin2* (**K**), *TmCecropin-2* (**L**), *TmThaumatin-like protein-1* (**M**), and *TmThaumatin-like protein-2* (**N**) were measured using qRT-PCR and compared with the ds*EGFP* treated groups. ds*EGFP* was used as negative control and *TmL27a* as an internal control. The numbers above the bars indicate AMP expression levels. All experiments were repeated three times with similar results. Statistical analysis was performed using Student's t-tests (* $p < 0.05$) and ns: no significant.