Development of new methods to stimulate the production of antimicrobial peptides in the larvae of the black soldier fly *Hermetia illucens*

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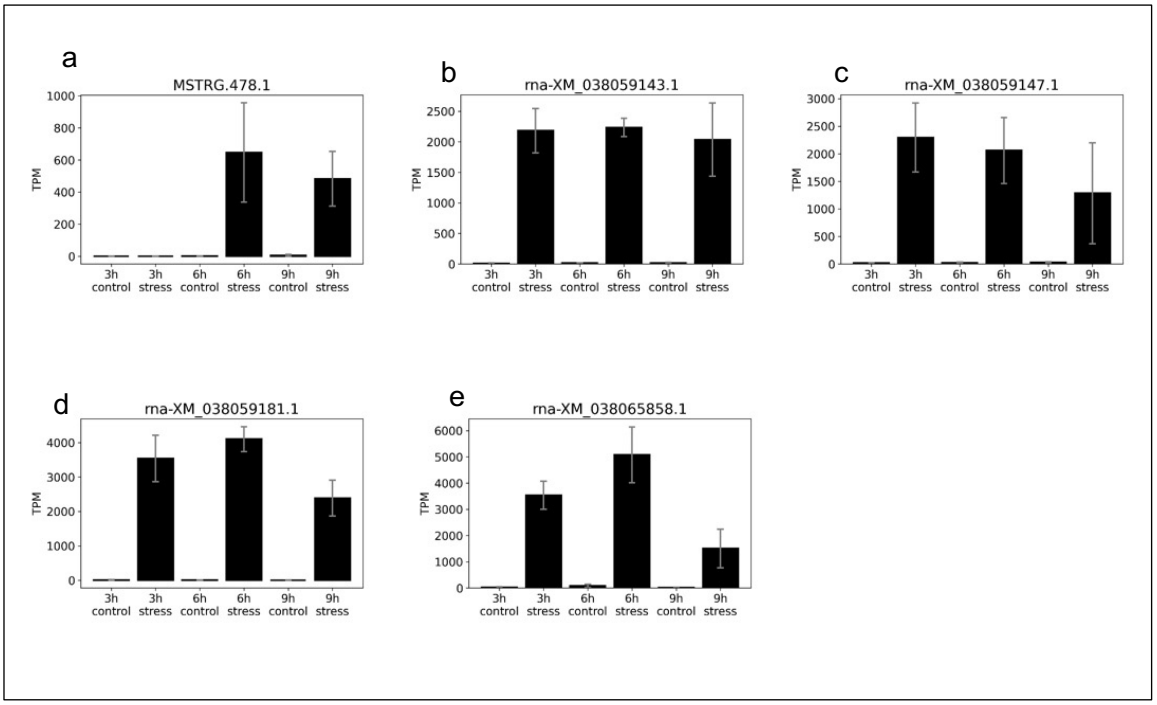
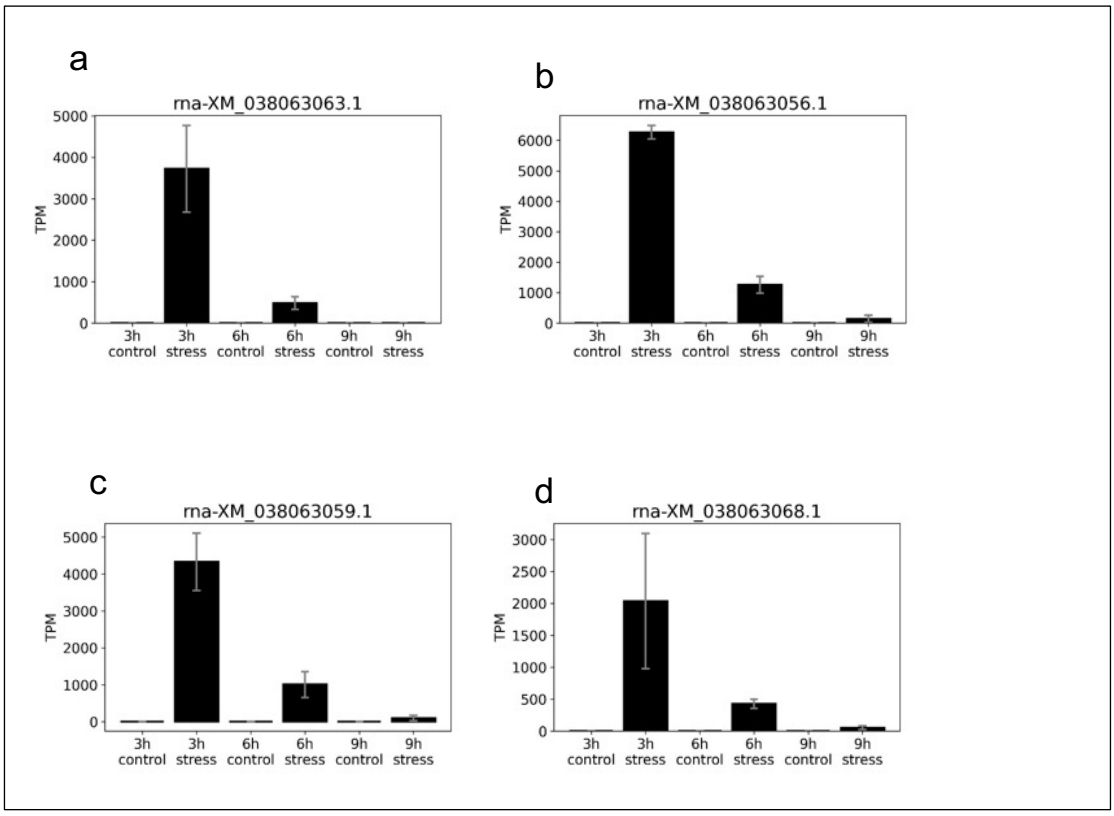
Supplementary information



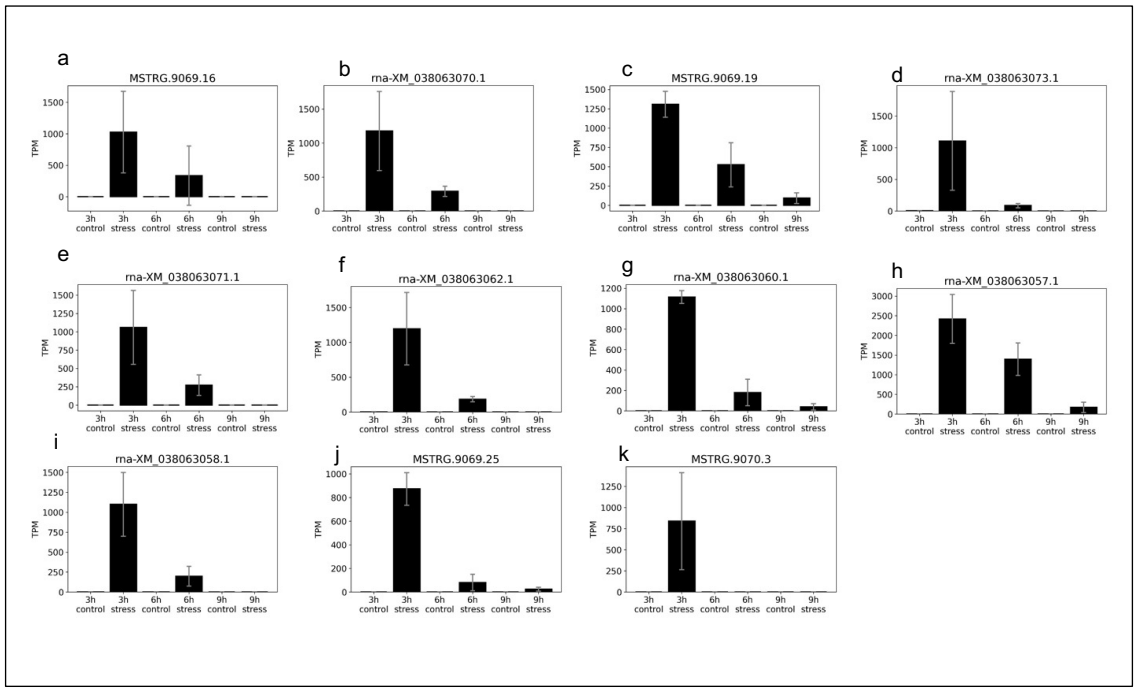
Supplementary figure S1 The expression of the transcripts annotated to Defencin (NM_078948.3). The y-axis indicates the ratio of the average Transcripts Per Kilobase Million (TPM) values for each transcript between the control and thermal injury groups.

a, transcript for MSTRG.478.1; b, transcript for rna-XM_038059143.1;c, transcript for rna-XM_038059147.1;d, transcript for rna-XM_038059181.1;e, transcript for rna-XM_038065858.1.



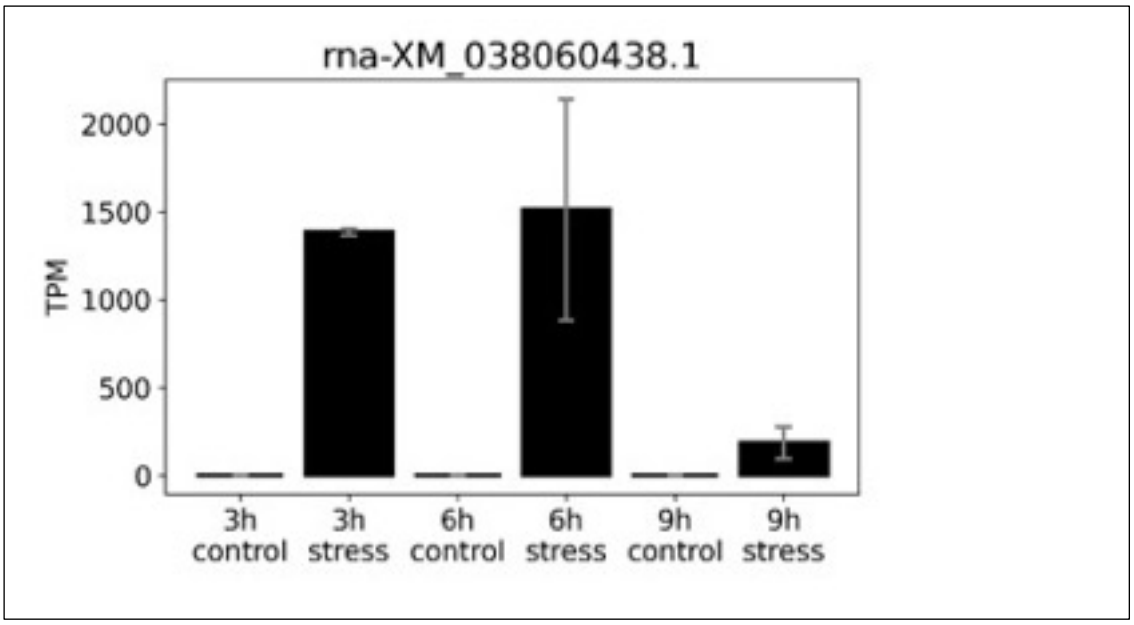
Supplementary figure S2 The expression of the transcripts annotated to Cecropin A2 (NM_079850.4). The y-axis indicates the ratio of the average Transcripts Per Kilobase Million (TPM) values for each transcript between the control and thermal injury groups.

a, transcript for rna-XM_038063063.1; b, transcript for rna-XM_038063056.1;c, transcript for rna-XM_038063059.1;d, transcript for rna-XM_038063068.1.

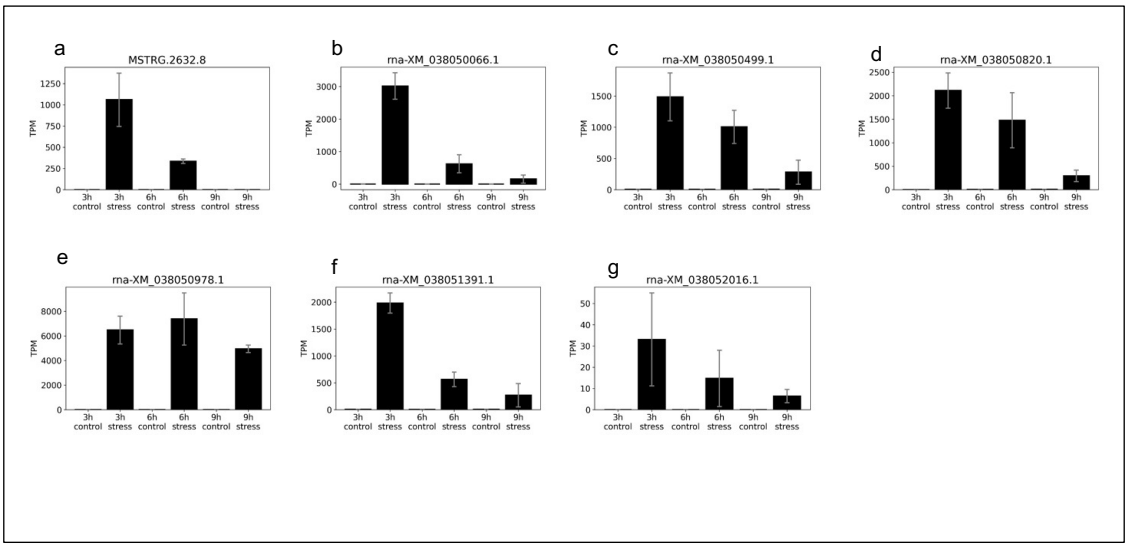


Supplementary figure S3 The expression of the transcripts annotated to Cecropin C (NM_079852.3). The y-axis indicates the ratio of the average Transcripts Per Kilobase Million (TPM) values for each transcript between the control and thermal injury groups.

a, transcript for MSTRG.9069.16; b, transcript for rna-XM_038063070.1;c, transcript for MSTRG.9069.19; d, transcript for rna-XM_038063073.1.; e, transcript for rna-XM_038063071.1.; f, transcript for rna-XM_038063062.1.; g, transcript for rna-XM_038063060.1.; h, transcript for rna-XM_038063057.1.; i, transcript for rna-XM_038063058.1.; j, transcript for MSTRG.9069.25; k, transcript for MSTRG.9070.3.

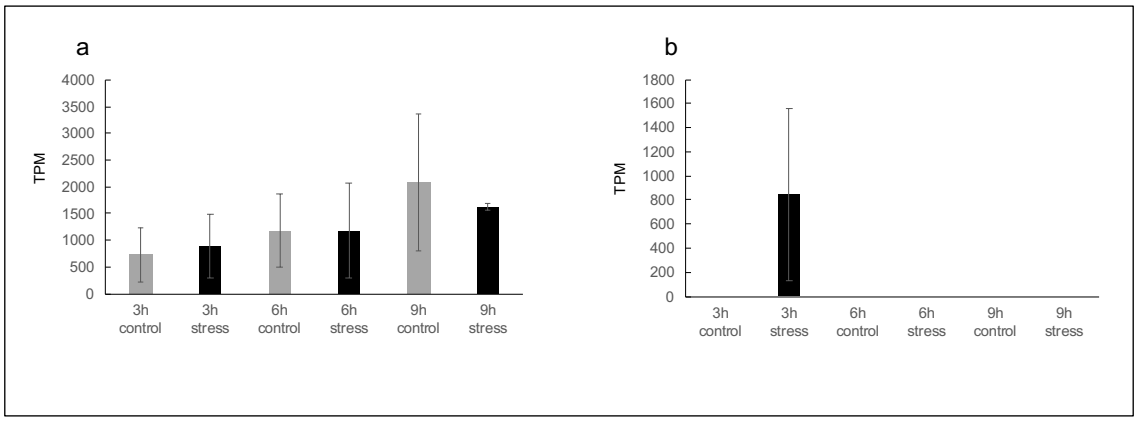


Supplementary figure S4 The expression of the transcripts annotated to Attacin-A (NM_079021.5). The y-axis indicates the ratio of the average Transcripts Per Kilobase Million (TPM) values for the transcript between the control and thermal injury groups.
The graph indicates the transcript for rna-XM_038060438.1.



Supplementary figure S5 The expression of the transcripts annotated to Diptericin B (NM_079063.4). The y-axis indicates the ratio of the average Transcripts Per Kilobase Million (TPM) values for each transcript between the control and thermal injury groups.

a, transcript for MSTRG.2632.8; b, transcript for rna-XM_038050066.1;c, transcript for rna-XM_038050499.1; d, transcript for rna-XM_038050820.1.; e, transcript for rna-XM_038050978.1.; f, transcript for rna-XM_038051391.1.; g, transcript for rna-XM_038052016.1.



Supplementary figure S6 The expression of the transcripts annotated to phenoloxidase (XM_038046567.1). The y-axis indicates the ratio of the average Transcripts Per Kilobase Million (TPM) values for the transcript between the control and thermal injury groups. The graph indicates the transcript for XM_038046567.1

Supplementary Table S1. Identification of antimicrobial peptides by mass spectrometry.

Accession IDs	Description
AHH35100.1	Defensin-like peptide 4 precursor (*H. illucens*)
L7VIN3.1	Cecropin-like peptide 1 (*H. illucens*)
AHH35095.1	Cecropin-like peptide 2 (*H. illucens*)
AHH35096.1	Cecropin-like peptide 3 (*H. illucens*)

The hemolymph collected from the larvae after thermal injury was loaded on the NovexTM 10%–20% Tricine Gel (1.0 mm × 10 well) (Thermo Fisher Scientific). Tricine 10× SDS running buffer (Thermo Fisher Scientific) was used as a running buffer. Electrophoresis was carried out at 100 V for 130 min using a Zoom Dual Power system (Thermo Fisher Scientific) and stained with EzStain AQua (ATTO Co, Ltd.). The visualized bands were extracted and treated with lysyl endopeptidase, followed by sequencing of the amino acids using a High-Resolution Mass Spectrometer (LTQ Orbitrap XL ETD, Thermo Scientific).

Supplementary Table S2. SRA accession numbers for RNA-seq data

SRA accession numbers	Samples information
DRR426824	Control 1 for 3h
DRR426825	Control 2 for 3h
DRR426826	Control 3 for 3h
DRR426827	Thermal injury 1 for 3h
DRR426828	Thermal injury 2 for 3h
DRR426829	Thermal injury 3 for 3h
DRR426830	Control 1 for 6h
DRR426831	Control 2 for 6h
DRR426832	Control 3 for 6h
DRR426833	Thermal injury 1 for 6h
DRR426834	Thermal injury 2 for 6h
DRR426835	Thermal injury 3 for 6h
DRR426836	Control 1 for 9h
DRR426837	Control 2 for 9h
DRR426838	Control 3 for 9h
DRR426839	Thermal injury 1 for 9h
DRR426840	Thermal injury 2 for 9h
DRR426841	Thermal injury 3 for 9h

BioProject: PRJDB14676, DRA submission: DRA015412

Supplementary Table S3. Primers for RT-qPCR were used in this study.

Gene name	Forward (5'-3')	Reverse (5'-3')
Hirs18	CGATGGCAAGTACACACAGC	AATCCACCAACGCGACATTG
HiCLP1	CAGAACATTGGCTCCTTGCT	TTTCGTTGTTTTCGCTGTTG
HiDLP4	CATTGCCAACCTTTCCAACT	GTCACACCATCCTCCTCGTT
HiPPO	AGACTTGGAGCCGCTAATCA	GGGACCTATTTGGCTGCATA
HiPGRP-SA	CGTCCCCAATGTGATAATCC	TTATGCCAACCTACGCCTTC

Supplementary Table S4. Primers for cDNA cloning were used in this study.

Gene name	Forward (5'-3')	Reverse (5'-3')
HiCLP1	ATGAATTTCACTAAGCTTTTCGTT	TTATCCTTGTTGTGGTGGTCCA
HiDLP4	ATGGTCCATTGCCAACCTTTCCAA	CTATTTCCGGCAGTTGCAAACAGC