Genome-wide screening of genes involved in programming diapause in the next generation in silkworm, Bombyx mori (Lepidoptera: Bombycidae)

Egi, Yuichi

Sakamoto, Katsuhiko

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Table S1. Primers used in real-time quantitative PCR.

Gene name	Forward primer	Reverse primer
2-iminobutanoate/2-iminopropanoate deaminase	5'-ACAAAGTTTCGCCCAAAATG-3'	5'-TGTCCGCTAAAATTGCTTGA-3'
Cuticular protein hypothetical 26	5'-TGTGAGACAACACAAAGCACA-3'	5'-AACTGGGGAGTAGCCGAAGT-3'
Cuticular protein RR-2 motif 58	5'-AAGATCAGCAATGGCAGCTAA-3'	5'-CGAGAAGCTGCTGTGTTCAG-3'
Cuticular protein RR-2 motif 75	5'-TCCAACAACATGGCAGCTAA-3'	5'-GATGTCTCCGCTTTGTTGGT-3'
Eukaryotic translation initiation factor 5B	5'-CCGTAACAAACAAGTCTCAAAAGA-3'	5'-GGGAGACGTCATTCTCTATTTCT-3'
F-box/SPRY domain-containing protein 1	5'-GGGGCTGGAATCTTGTTGAT-3'	5'-CAAATGAGAGGGTGTGACGA-3'
Facilitated trehalose transporter Tret1	5'-TCCTAATGAGAGCGGACACC-3'	5'-GAGCCCATCGATACAGCAAT-3'
Fungal protease inhibitor F-like	5'-GGCCGCCAAACAATACTTTA-3'	5'-AGTCCAATGGGCATTTTCAG-3'
Glucose-1-phosphatase	5'-CCGCATTCCCTGACTGTAAT-3'	5'-GCATTTCCTCGATTTCTTCG-3'
Juvenile hormone acid methyltransferase	5'-GAGCACGTCGATCGATTTAT-3'	5'-CAACGTTTTGCATTGCACTT-3'
Juvenile hormone epoxide hydrolase	5'-TACCAGCATCAAACCCTTCG-3'	5'-ACCCGTATTTGAAGCCAACA-3'
Krüppel homolog 1	5'-GAAACAATTTCGTTCTTCAGGTGACG-3'	5'-TCGTGCGTGTGCTGTAAGCG-3'
Low molecular lipoprotein 30K pBmHPC-6	5'-CCACAACAAAATTGCATTCG-3'	5'-TCGGTGGACATGATCTTGAA-3'
Low molecular lipoprotein 30K pBmHPC-12	5'-CACCGGTGACTACGACAGTG-3'	5'-CTCCATGGTGTTCCGTCTCT-3'
Low molecular lipoprotein 30K pBmHPC-19	5'-GATTACGACAGTGCGGTTGA-3'	5'-CCCTGGAGCCAAAGTTGATA-3'
Low molecular lipoprotein 30K pBmHPC-23	5'-GGCTCCATCATCCAGAATGT-3'	5'-AACAATTTCCTGTCCGTTGC-3'
Low molecular mass 30 kDa lipoprotein 19G1-like	5'-TCTCGCTTTGACGCTGAGTA-3'	5'-TGTTCTCCCACAGAGCAATG-3'
Mevalonate kinase	5'-GAGCAGCCAAGATTTTACGG-3'	5'-TAGCGCCTTTCTTGAACGAT-3'
MIF-like protein mif-2	5'-AAGGCTGTGCAAAAATCACC-3'	5'-CAGGTTACCAATCGATTCCAG-3'
Ommochrome-binding protein (*LOC101737984*)	5'-CCGCAACGGCAAGATATAAT-3'	5'-CGATATAGACCGTCGCTTCC-3'
Ommochrome-binding protein (*LOC101745519*)	5'-TCGACTACCAAACCAGCACA-3'	5'-ATACCCGAATTGATGCCAGA-3'
Phosphoribosyl pyrophosphate synthetase	5'-CACAGATCCAGGGCTTCTTC-3'	5'-GATTCCGGTCATCCTCTTCA-3'
Proton-coupled folate transporter	5'-GCTGATGGTTGGACCTCTGT-3'	5'-CCAAAAGACCGACGACGATA-3'
rp49	5'-TCAATCGGATCGCTATGACA-3'	5'-ATGACGGGTCTTCTTGTTGG-3'
Sex-specific storage-protein SP1	5'-AGCGATTAGTGGTGGCTACG-3'	5'-GTCGGCTGGAGGATATGGT-3'
THO complex subunit 1	5'-CGGTCGGCGATATTGTTAAG-3'	5'-CTCCAACGAAGGCATGAAAT-3'
Uncharacterized LOC101743046	5'-GACCGCTGACAAGGTCACTA-3'	5'-AGGGCATGAAACCTCATACG-3'
Uncharacterized LOC101744235	5'-GATGTCGCAGCAGTCAGTGT-3'	5'-TTCGTCACGTTTGTGGATGT-3'
Uncharacterized LOC105843021	5'-GGCGTAAGGTGTGGAGTGAT-3'	5'-TCTGCATGACCCTTTGTTCA-3'
Uncharacterized LOC110385080	5'-TCCCGGCTACAAGTTTGAGT-3'	5'-GACCCGGCAACAATAGAAAA-3'

Table S2. Primers used in the PCR to make DNA templates for double-stranded RNA synthesis.

Gene name	Forward primer	Reverse primer
GFP (*Enhanced green fluorescent protein*)	5'-**TAATACGACTCACTATAGGGAGA**CCTGAAGTTCATCTGCACCAC-3'	5'-**TAATACGACTCACTATAGGGAGA**ACGAACTCCAGCAGGACCAT-3'
Juvenile hormone acid methyltransferase	5'-**TAATACGACTCACTATAGGGAGA**CCTCGAGGAACATGCGAATA-3'	5'-**TAATACGACTCACTATAGGGAGA**GTATGCGAGAGTGTGCGGTA-3'
Krüppel homolog 1	5'-**TAATACGACTCACTATAGGGAGA**GCCATAAGACTTTTGCTGTGC-3'	5'-**TAATACGACTCACTATAGGGAGA**TCTCCCGTGTGAGTACGAGA-3'
Proton-coupled folate transporter	5'-**TAATACGACTCACTATAGGGAGA**ATACACTGCTCACCGGAACC-3'	5'-**TAATACGACTCACTATAGGGAGA**GCCGATGAAAGGGAATACAA-3'
Uncharacterized LOC110385080	5'-**TAATACGACTCACTATAGGGAGA**TCCTCATGTCGGCGGTACT-3'	5'-**TAATACGACTCACTATAGGGAGA**CGAATCTATCGCCATAATAATCTTT-3'

Underlined sequences are the T7-promotor.

Table S3. Genes expressed more abundantly in producers of diapause than non-diapause eggs under three experimental conditions.

Gene name	Reference sequence	Egg thermal stimulation			Egg illumination stimulation			Larval photoperiod stimulation		
		25 ℃ (Diapause)	18 ℃ (Non-diapause)	Fold change 25 ℃ / 18 ℃	Light (Diapause)	Darkness (Non-diapause)	Fold change Light / Darkness	LD12:12 (Diapause)	LD20:4 (Non-Diapause)	Fold change LD 12:12 / LD 20:4
Eukaryotic translation initiation factor 5B	XM_004927891.4	17.25	6.60	2.61	14.47	6.60	2.19	33.03	12.00	2.75
Facilitated trehalose transporter Tret1	XM_021350554.1	22.75	0.29	78.45	28.71	0.29	99.00	28.42	0.24	118.42
Juvenile hormone acid methyltransferase	NM_001043436.1	2.73	0.72	3.79	4.34	0.72	6.03	1.53	0.24	6.38
Krüppel homolog 1	NM_001177861.1	15.25	3.32	4.59	15.63	3.32	4.71	15.77	8.07	1.95
Mevalonate kinase	NM_001099829.1	34.58	16.18	2.14	51.98	16.18	3.21	31.95	7.79	4.10
Proton-coupled folate transporter	XM_004933326.3	2.73	1.40	1.95	3.88	1.40	2.77	3.45	0.80	4.31
THO complex subunit 1	XM_021352392.2	5.85	1.73	3.38	4.57	1.73	2.64	23.28	14.29	1.63
Uncharacterized LOC101743046	XM_012694982.3	2.90	1.54	1.88	3.07	1.54	1.99	2.73	1.28	2.13
Uncharacterized LOC110385080	XM_021347590.2	2.90	0.87	3.33	4.11	0.87	4.72	1.81	0.56	3.23

Gene expression in larval brains was compared between diapause- and non-diapause-eggs producers using cap analysis of gene expression (CAGE). Expression of listed genes was ≥ 1.5-fold higher in producers of diapause eggs than non-diapause eggs under three conditions. In egg thermal stimulation experiment, diapause- and non-diapause-egg producers were induced by incubating eggs at 25°C and 18°C, respectively. In egg illumination stimulation experiment, diapause- and non-diapause-egg producers were induced by incubating eggs under continuous light and continuous darkness, respectively. In larval photoperiod stimulation experiment, diapause- and non-diapause-egg producers were induced by rearing larvae under LD12:12 and LD20:4, respectively. Values quantified by CAGE are numbers of reads (counts) per million counted at transcription start sites for each gene. LD, light/dark.

Table S4. Genes expressed more abundantly in producers of non-diapause than diapause eggs under three experimental conditions.

Gene name	Reference sequence	Egg thermal stimulation			Egg illumination stimulation			Larval photoperiod stimulation		
		25 ℃ (Diapause)	18 ℃ (Non-diapause)	Fold change 18 ℃ / 25 ℃	Light (Diapause)	Darkness (Non-diapause)	Fold change Darkness / Light	LD12:12 (Diapause)	LD20:4 (Non-Diapause)	Fold change LD 20:4 / LD 12:12
2-iminobutanoate/2-iminopropanoate deaminase	XM_004931425.2	0.91	4.48	4.92	2.89	4.48	1.55	0.68	6.02	8.85
Cuticular protein hypothetical 26	NM_001173280.1	0.13	31.83	244.85	0.12	31.83	265.25	0.64	15.17	23.70
Cuticular protein RR-2 motif 58	NM_001173228.1	3.03	11.46	3.78	5.90	11.46	1.94	4.05	9.11	2.25
Cuticular protein RR-2 motif 75	NM_001173214.1	1.91	2.89	1.51	1.45	2.89	1.99	0.88	2.13	2.42
F-box/SPRY domain-containing protein 1	XM_004933671.3	0.43	2.36	5.49	0.00	2.36	NA	3.69	24.16	6.55
Fungal protease inhibitor F-like	XM_004924342.4	8.97	92.25	10.28	36.18	92.25	2.55	5.90	18.38	3.12
Glucose-1-phosphatase	XM_004930711.4	0.52	1.64	3.15	0.64	1.64	2.56	0.40	1.61	4.03
Juvenile hormone epoxide hydrolase	NM_001043736.2	0.91	1.93	2.12	1.27	1.93	1.52	1.12	1.81	1.62
Low molecular lipoprotein 30K pBmHPC-6	NM_001044021.2	28.43	222.78	7.84	65.06	222.78	3.42	20.59	119.86	5.82
Low molecular lipoprotein 30K pBmHPC-12	NM_001101726.1	53.73	245.02	4.56	126.30	245.02	1.94	30.91	221.17	7.16
Low molecular lipoprotein 30K pBmHPC-19	NM_001101727.1	18.89	118.83	6.29	33.57	118.83	3.54	8.63	47.44	5.50
Low molecular lipoprotein 30K pBmHPC-23	NM_001101728.2	4.03	8.71	2.16	4.57	8.71	1.91	2.65	13.29	5.02
Low molecular mass 30 kDa lipoprotein 19G1-like	NM_001279380.1	33.28	156.63	4.71	68.94	156.63	2.27	15.49	108.58	7.01
MIF-like protein mif-2	XM_004932734.3	0.82	1.30	1.59	0.75	1.30	1.73	0.48	2.93	6.10
Ommochrome-binding protein (*LOC101737984*)	XM_004923674.4	1.60	2.84	1.78	1.27	2.84	2.24	0.60	4.46	7.43
Ommochrome-binding protein (*LOC101745519*)	XM_021347804.1	1.82	5.59	3.07	3.70	5.59	1.51	1.28	9.55	7.46
Phosphoribosyl pyrophosphate synthetase	NM_001047016.1	0.48	1.01	2.10	0.58	1.01	1.74	0.20	1.32	6.60
Sex-specific storage-protein SP1	NM_001113276.3	10.53	49.40	4.69	27.32	49.40	1.81	5.42	41.22	7.61
Uncharacterized LOC101744235	XM_004929359.4	0.48	1.06	2.21	0.41	1.06	2.59	0.12	1.28	10.67
Uncharacterized LOC105843021	XM_012697974.2	1.99	6.98	3.51	0.00	6.98	NA	0.00	6.42	NA

Gene expression in larval brains was compared between diapause- and non-diapause-eggs producers using cap analysis of gene expression (CAGE). Expression of listed genes was ≥ 1.5-fold higher in producers of non-diapause eggs than diapause eggs under three conditions. In egg thermal stimulation experiment, diapause- and non-diapause-egg producers were induced by incubating eggs at 25°C

and 18°C, respectively. In egg illumination stimulation experiment, diapause- and non-diapause-egg producers were induced by incubating eggs under continuous light and continuous darkness, respectively. In larval photoperiod stimulation experiment, diapause- and non-diapause-egg producers were induced by rearing larvae under LD12:12 and LD20:4, respectively. Values quantified by CAGE are numbers of reads (counts) per million counted at transcription start sites for each gene. NA, fold change could not be calculated due to denominator of 0. LD, light/dark.

Figure S1 Verification of CAGE data by RT-qPCR.

Relative mRNA amount of genes screened by CAGE was measured in brains of fifth instar larvae of producers of diapause eggs (white column) and those of non-diapause eggs (shaded column). The CAGE findings showed that 9 genes (A-C; Tables S3) were expressed more abundantly in producers of diapause eggs and 20 genes (D-H; Table S4) more abundantly in those of non-diapause eggs, compared with each other under three experimental conditions. However, the expression of only 4 genes coincided with that assessed by RT-qPCR (Fig. 1). In egg thermal stimulation experiment (Egg-Thermal), we generated diapause- and non-diapause-egg producers by incubating eggs at 25°C and 18°C, respectively. In egg illumination stimulation experiment (Egg-Illumi), we generated diapause- and non-diapause-egg producers by incubating eggs under continuous light and continuous darkness, respectively. In larval photoperiod stimulation experiment (Larval-Photoperi), we generated diapause- and non-diapause-egg producers by rearing larvae under LD12:12 and LD20:4, respectively. Data are shown as means ± S.E.M. (n = 3–4: independent samples). The higher value of each transcript between diapause- and non-diapause-egg producers is expressed as 1.0 under each condition (Welch's t-test, $^{*}P < 0.05$, $^{**}P < 0.01$). CAGE, cap analysis of gene expression; LD, light/dark; RT-qPCR, real-time quantitative polymerase chain reaction.

Figure S1

A

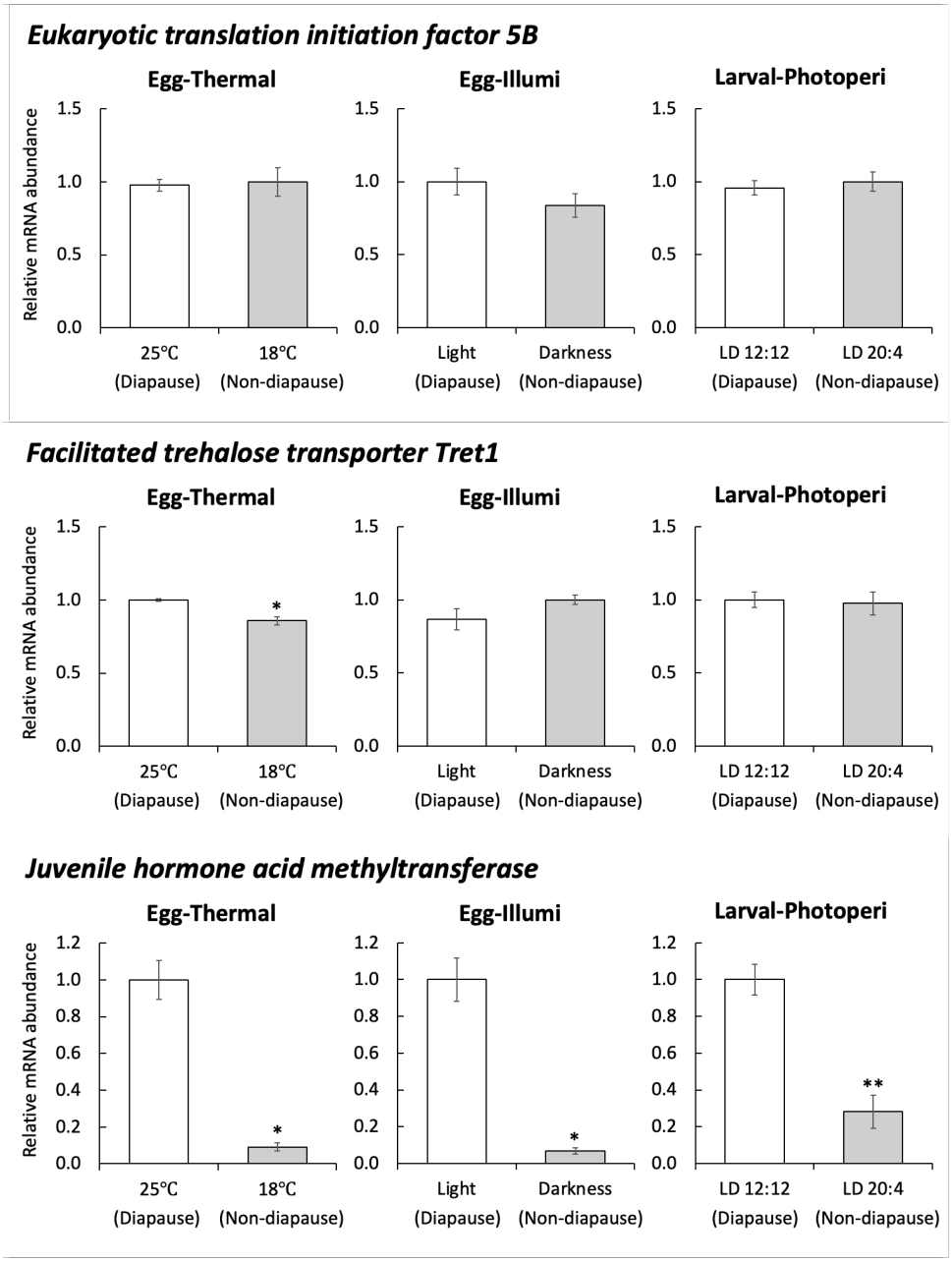

Eukaryotic translation initiation factor 5B
Egg-Thermal
Egg-Illumi
Larval-Photoperi
Relative mRNA abundance
1.5
1.0
0.5
0.0
25℃ (Diapause)
18℃ (Non-diapause)
Light (Diapause)
Darkness (Non-diapause)
LD 12:12 (Diapause)
LD 20:4 (Non-diapause)
Facilitated trehalose transporter Tret1
Egg-Thermal
Egg-Illumi
Larval-Photoperi
Relative mRNA abundance
1.5
1.0
0.5
0.0
*
25℃ (Diapause)
18℃ (Non-diapause)
Light (Diapause)
Darkness (Non-diapause)
LD 12:12 (Diapause)
LD 20:4 (Non-diapause)
Juvenile hormone acid methyltransferase
Egg-Thermal
Egg-Illumi
Larval-Photoperi
Relative mRNA abundance
1.2
1.0
0.8
0.6
0.4
0.2
0.0
*
*
**
25℃ (Diapause)
18℃ (Non-diapause)
Light (Diapause)
Darkness (Non-diapause)
LD 12:12 (Diapause)
LD 20:4 (Non-diapause)

Figure S1

B

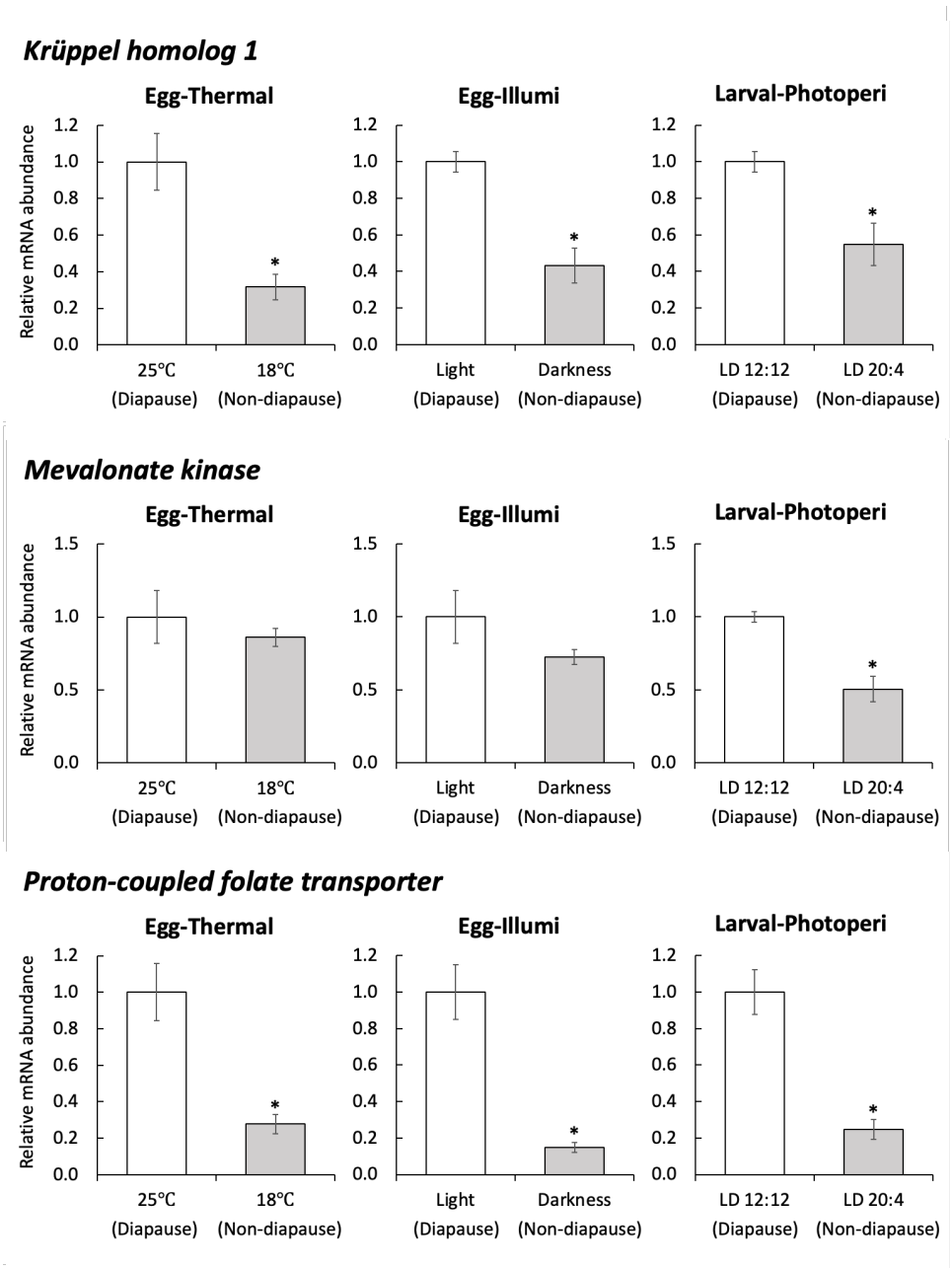
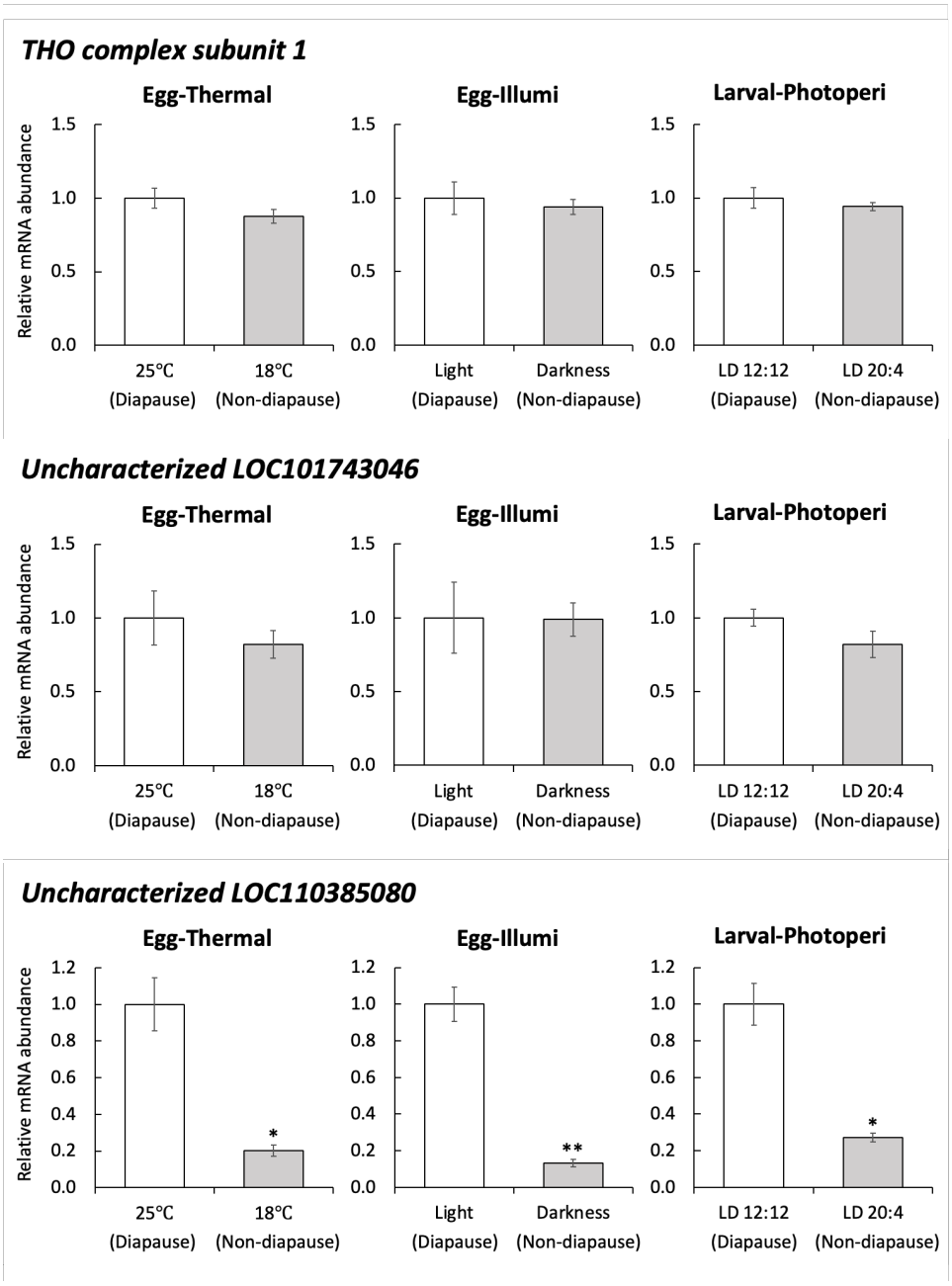

Krüppel homolog 1
Egg-Thermal
Egg-Illumi
Larval-Photoperi
Relative mRNA abundance
25°C (Diapause)
18°C (Non-diapause)
Light (Diapause)
Darkness (Non-diapause)
LD 12:12 (Diapause)
LD 20:4 (Non-diapause)
Mevalonate kinase
Egg-Thermal
Egg-Illumi
Larval-Photoperi
Relative mRNA abundance
25°C (Diapause)
18°C (Non-diapause)
Light (Diapause)
Darkness (Non-diapause)
LD 12:12 (Diapause)
LD 20:4 (Non-diapause)
Proton-coupled folate transporter
Egg-Thermal
Egg-Illumi
Larval-Photoperi
Relative mRNA abundance
25°C (Diapause)
18°C (Non-diapause)
Light (Diapause)
Darkness (Non-diapause)
LD 12:12 (Diapause)
LD 20:4 (Non-diapause)

Figure S1

C


THO complex subunit 1
Egg-Thermal
Egg-Illumi
Larval-Photoperi
Relative mRNA abundance
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1.0
0.5
0.0
25°C (Diapause)
18°C (Non-diapause)
Light (Diapause)
Darkness (Non-diapause)
LD 12:12 (Diapause)
LD 20:4 (Non-diapause)
Uncharacterized LOC101743046
Egg-Thermal
Egg-Illumi
Larval-Photoperi
Relative mRNA abundance
1.5
1.0
0.5
0.0
25°C (Diapause)
18°C (Non-diapause)
Light (Diapause)
Darkness (Non-diapause)
LD 12:12 (Diapause)
LD 20:4 (Non-diapause)
Uncharacterized LOC110385080
Egg-Thermal
Egg-Illumi
Larval-Photoperi
Relative mRNA abundance
1.2
1.0
0.8
0.6
0.4
0.2
0.0
*
**
*
25°C (Diapause)
18°C (Non-diapause)
Light (Diapause)
Darkness (Non-diapause)
LD 12:12 (Diapause)
LD 20:4 (Non-diapause)

Figure S1

D

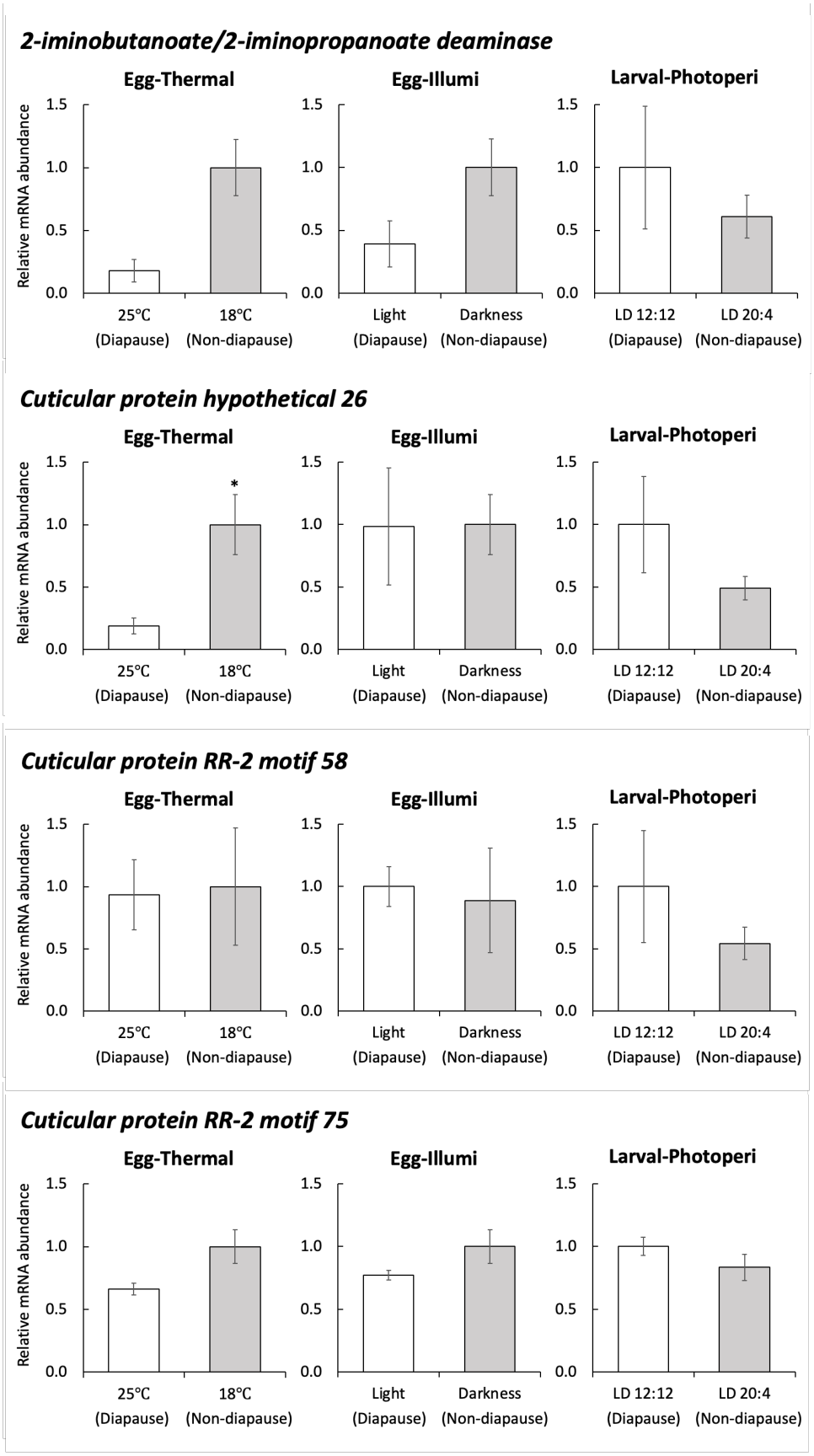

2-iminobutanoate/2-iminopropanoate deaminase
Egg-Thermal
Egg-Illumi
Larval-Photoperi
Relative mRNA abundance
1.5
1.0
0.5
0.0
25°C (Diapause)
18°C (Non-diapause)
Light (Diapause)
Darkness (Non-diapause)
LD 12:12 (Diapause)
LD 20:4 (Non-diapause)
Cuticular protein hypothetical 26
*
Cuticular protein RR-2 motif 58
Cuticular protein RR-2 motif 75

Figure S1

E

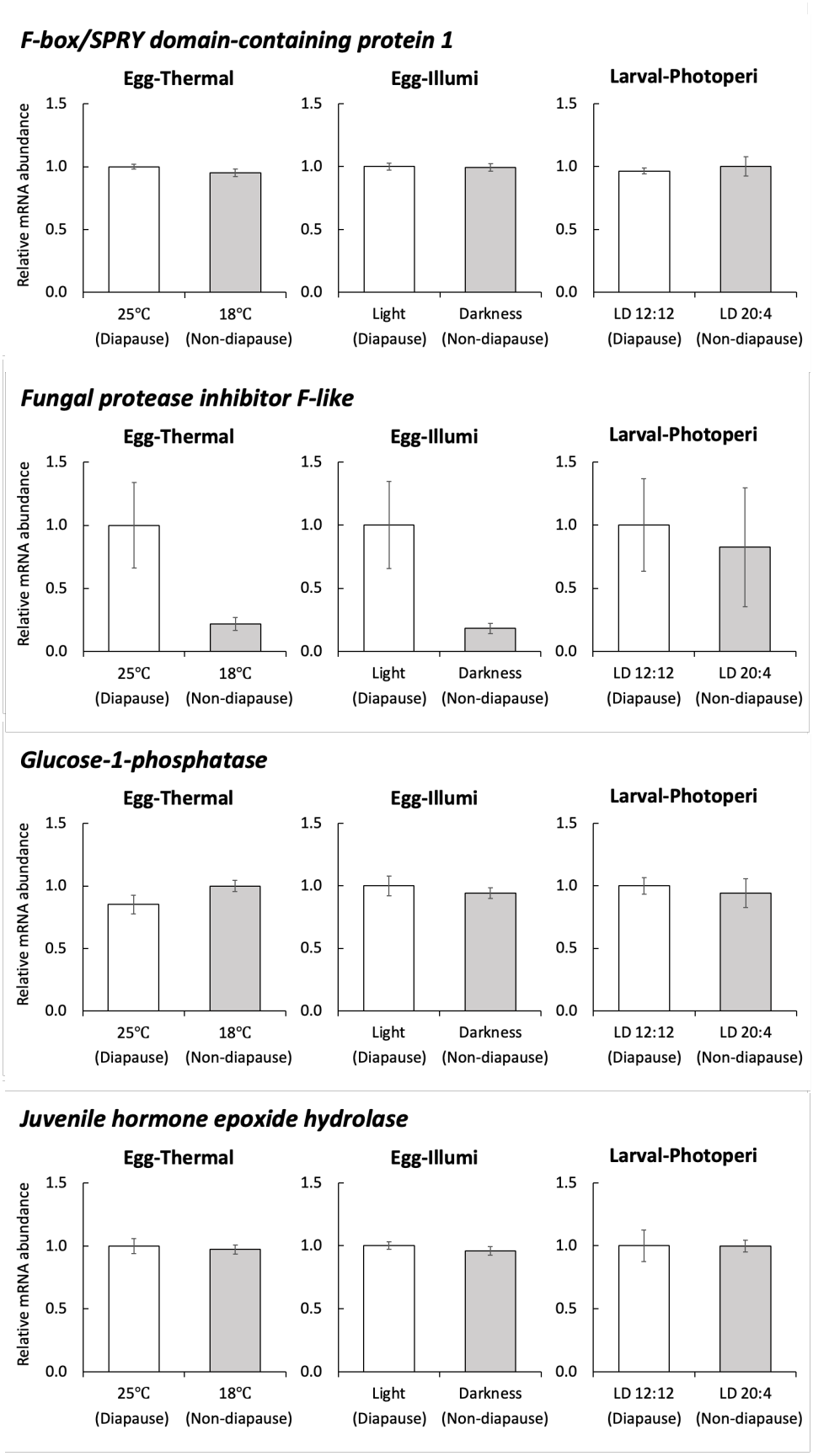

F-box/SPRY domain-containing protein 1
Egg-Thermal
Egg-Illumi
Larval-Photoperi
Relative mRNA abundance
1.5
1.0
0.5
0.0
25°C (Diapause)
18°C (Non-diapause)
Light (Diapause)
Darkness (Non-diapause)
LD 12:12 (Diapause)
LD 20:4 (Non-diapause)
Fungal protease inhibitor F-like
Egg-Thermal
Egg-Illumi
Larval-Photoperi
Relative mRNA abundance
25°C (Diapause)
18°C (Non-diapause)
Light (Diapause)
Darkness (Non-diapause)
LD 12:12 (Diapause)
LD 20:4 (Non-diapause)
Glucose-1-phosphatase
Egg-Thermal
Egg-Illumi
Larval-Photoperi
Relative mRNA abundance
25°C (Diapause)
18°C (Non-diapause)
Light (Diapause)
Darkness (Non-diapause)
LD 12:12 (Diapause)
LD 20:4 (Non-diapause)
Juvenile hormone epoxide hydrolase
Egg-Thermal
Egg-Illumi
Larval-Photoperi
Relative mRNA abundance
25°C (Diapause)
18°C (Non-diapause)
Light (Diapause)
Darkness (Non-diapause)
LD 12:12 (Diapause)
LD 20:4 (Non-diapause)

Figure S1

F

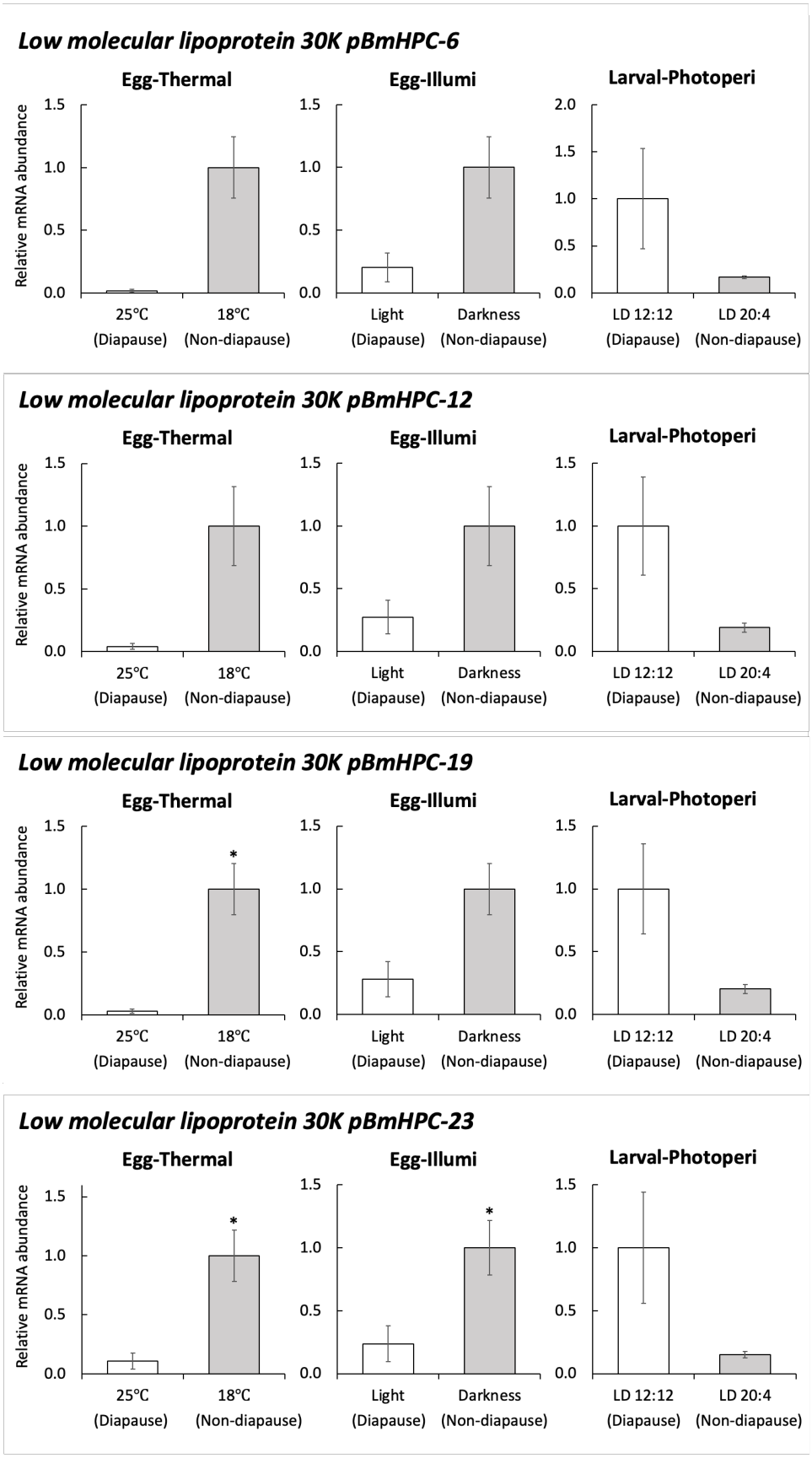

Low molecular lipoprotein 30K pBmHPC-6
Egg-Thermal
Egg-Illumi
Larval-Photoperi
Relative mRNA abundance
25°C (Diapause)
18°C (Non-diapause)
Light (Diapause)
Darkness (Non-diapause)
LD 12:12 (Diapause)
LD 20:4 (Non-diapause)
Low molecular lipoprotein 30K pBmHPC-12
Egg-Thermal
Egg-Illumi
Larval-Photoperi
Relative mRNA abundance
25°C (Diapause)
18°C (Non-diapause)
Light (Diapause)
Darkness (Non-diapause)
LD 12:12 (Diapause)
LD 20:4 (Non-diapause)
Low molecular lipoprotein 30K pBmHPC-19
Egg-Thermal
Egg-Illumi
Larval-Photoperi
Relative mRNA abundance
25°C (Diapause)
18°C (Non-diapause)
Light (Diapause)
Darkness (Non-diapause)
LD 12:12 (Diapause)
LD 20:4 (Non-diapause)
Low molecular lipoprotein 30K pBmHPC-23
Egg-Thermal
Egg-Illumi
Larval-Photoperi
Relative mRNA abundance
25°C (Diapause)
18°C (Non-diapause)
Light (Diapause)
Darkness (Non-diapause)
LD 12:12 (Diapause)
LD 20:4 (Non-diapause)

Figure S1

G

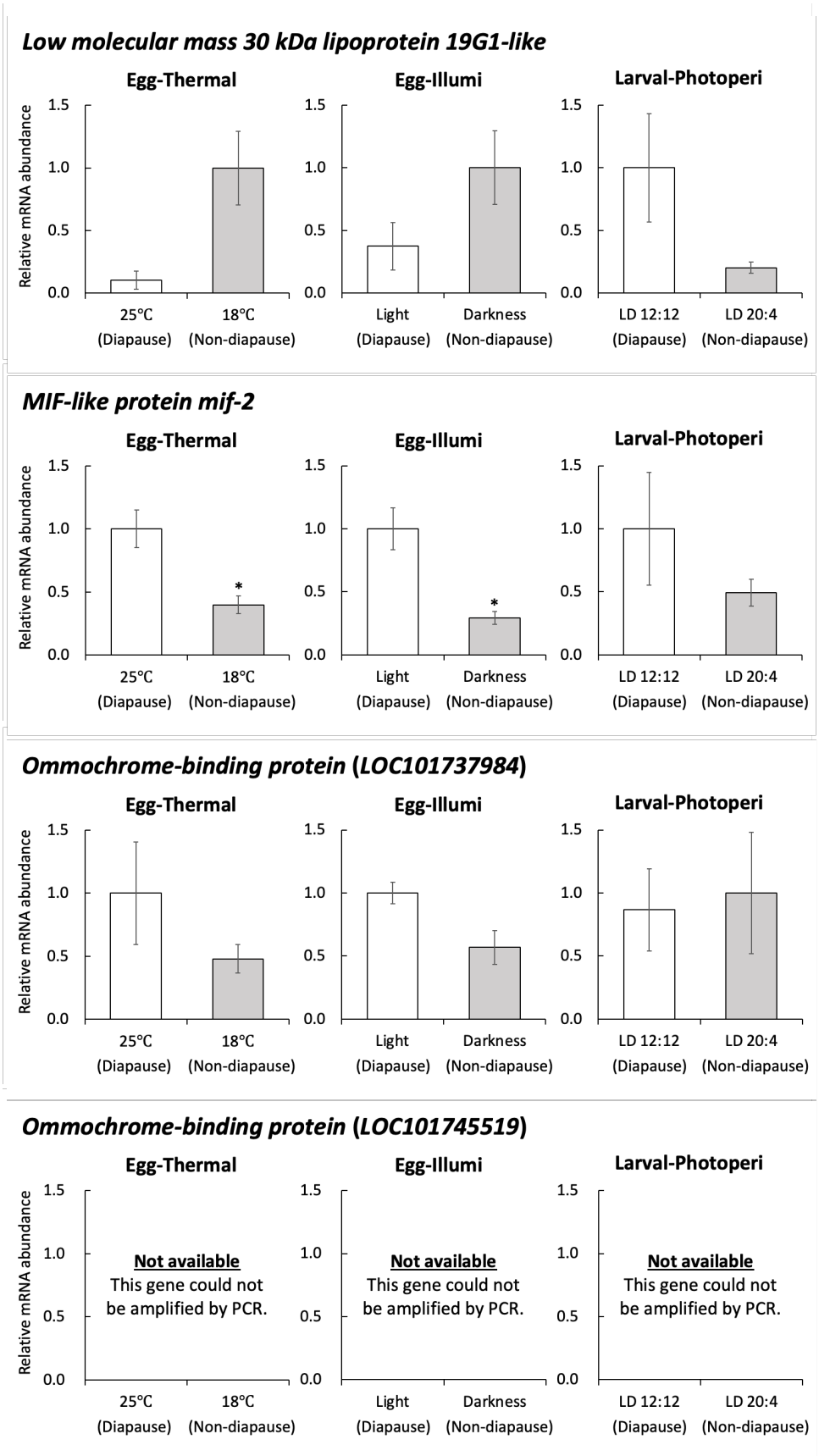
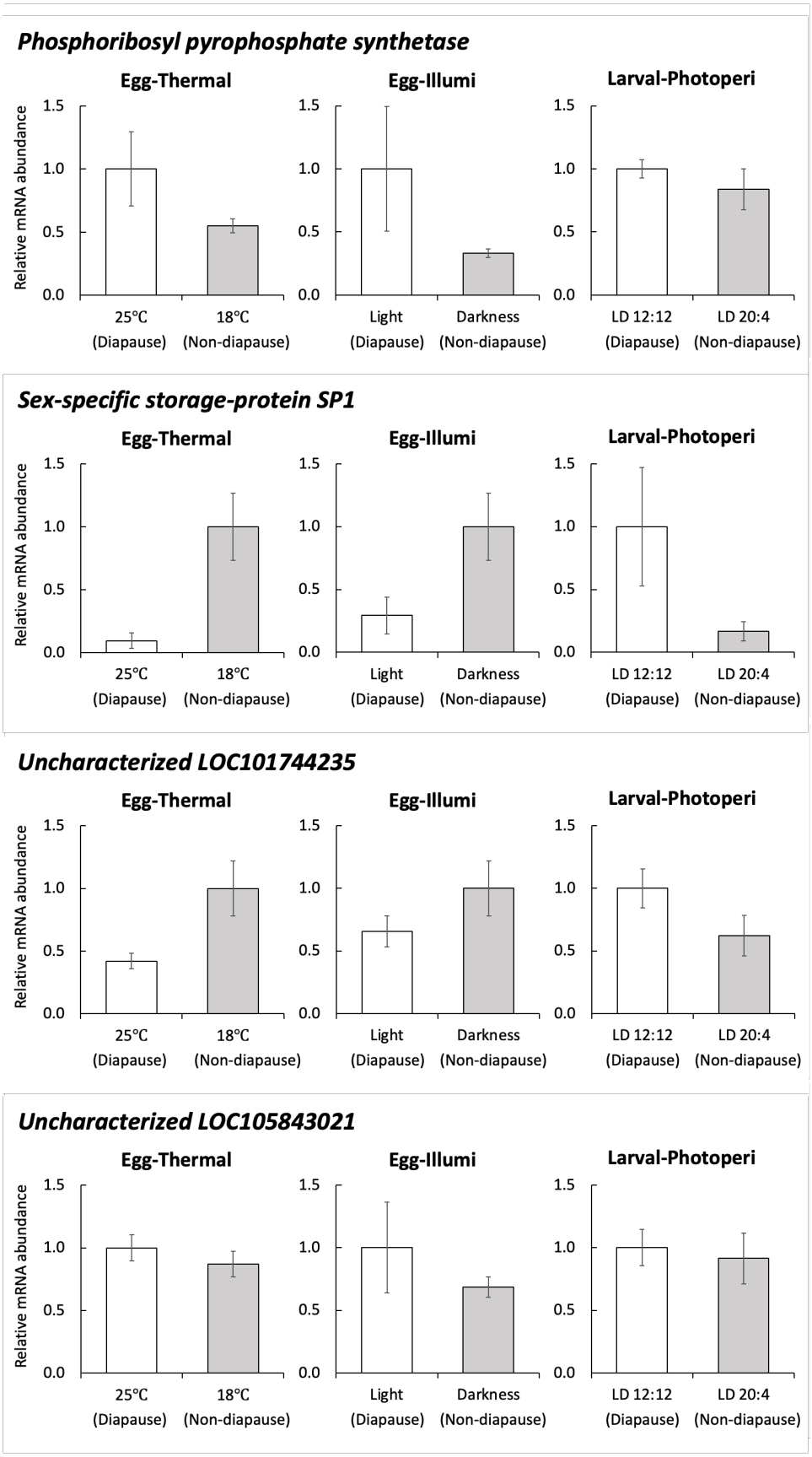

Low molecular mass 30 kDa lipoprotein 19G1-like
Egg-Thermal
Egg-Illumi
Larval-Photoperi
Relative mRNA abundance
1.5
1.0
0.5
0.0
25°C (Diapause)
18°C (Non-diapause)
Light (Diapause)
Darkness (Non-diapause)
LD 12:12 (Diapause)
LD 20:4 (Non-diapause)
MIF-like protein mif-2
*
*
Ommochrome-binding protein (LOC101737984)
Ommochrome-binding protein (LOC101745519)
Not available
This gene could not be amplified by PCR.

Figure S1

H


Phosphoribosyl pyrophosphate synthetase
Egg-Thermal
Egg-Illumi
Larval-Photoperi
Relative mRNA abundance
1.5
1.0
0.5
0.0
25°C (Diapause)
18°C (Non-diapause)
Light (Diapause)
Darkness (Non-diapause)
LD 12:12 (Diapause)
LD 20:4 (Non-diapause)
Sex-specific storage-protein SP1
Egg-Thermal
Egg-Illumi
Larval-Photoperi
Relative mRNA abundance
25°C (Diapause)
18°C (Non-diapause)
Light (Diapause)
Darkness (Non-diapause)
LD 12:12 (Diapause)
LD 20:4 (Non-diapause)
Uncharacterized LOC101744235
Egg-Thermal
Egg-Illumi
Larval-Photoperi
Relative mRNA abundance
25°C (Diapause)
18°C (Non-diapause)
Light (Diapause)
Darkness (Non-diapause)
LD 12:12 (Diapause)
LD 20:4 (Non-diapause)
Uncharacterized LOC105843021
Egg-Thermal
Egg-Illumi
Larval-Photoperi
Relative mRNA abundance
25°C (Diapause)
18°C (Non-diapause)
Light (Diapause)
Darkness (Non-diapause)
LD 12:12 (Diapause)
LD 20:4 (Non-diapause)