Supplementary data

Table S1. Silk-associate genes

Gene Name	Description	Gene ID	Group
Fib-H	fibroin heavy chain precursor	693030	silk proteins
Fib-L	Fibroin light chain	693047	silk proteins
P25	fibroin P25	100146105	silk proteins
sericin 1	sericin 1-like isoform X1	101740082	silk proteins
sericin 2	sericin 2-isoform 2 precursor	100379325	silk proteins
sericin 3	sericin 3 precursor	100136948	silk proteins
SGF-1/Fkh	silk gland factor 1	692754	transcription factors
Awh	arrowhead PA	100862786	transcription factors
Ldb	LIM-domain binding protein	101742826	transcription factors
Lcaf	single-stranded DNA-binding protein 3-like	101738257	transcription factors
SGF-3/POUM1	silk gland factor 3	693025	transcription factors
FMBP-1	fibroin-modulator-binding protein-1	692516	transcription factors
Sage	helix-loop-helix protein 2-like	101741094	transcription factors
Dimm	class A basic helix-loop-helix protein 15-like	101736973	transcription factors
Antp	antennapedia-like proteinue protein	692747	transcription factors
inv	homeobox protein invected	693024	transcription factors
en	segmentation polarity homeobox protein engrailed-like	101739896	transcription factors
Pax3/7	protein gooseberry-like	101741981	transcription factors
seroin 1	seroin 1 precursor	692598	protease inhibitors
BmSPI16	serine protease inhibitor 16 precursor	100272180	protease inhibitors
LOC101736771	putative proteasome inhibitor-like isoform X1	101736771	protease inhibitors
BmSPI28	serine protease inhibitor 28	101737176	protease inhibitors
BmSPI45	SCO-spondin-like	101739956	protease inhibitors
BmSPI44	SCO-spondin-like	101742053	protease inhibitors
LOC692611	BCP inhibitor precursor	692611	protease inhibitors
BmSPI2	serine protease inhibitor 2	692573	protease inhibitors
BmSPI37	fungal protease inhibitor F-like precursor	101738296	protease inhibitors
BmSPI40	fungal protease inhibitor F-like	101738036	protease inhibitors
BmSPI39	SCO-spondin-like	101738036	protease inhibitors
BmSPI38	fungal protease inhibitor F-like	101738162	protease inhibitors
BmSPI36	fungal protease inhibitor F-like isoform X1	101738434	protease inhibitors
BmSPI3	serine protease inhibitor 3 precursor	693100	protease inhibitors
BmSPI68	serine protease inhibitor dipetalogastin-like	101747078	protease inhibitors
BmSPI5	serine protease inhibitor 5 precursor	692688	protease inhibitors
BmSPI4	serine protease inhibitor 4 precursor	692644	protease inhibitors

Table S2. Genes in JH signaling pathway with m^{6}A methylation

chr	strand	log2FC	pvalue	padj	Annotation	Gene ID	Gene Name
NC_051380.1	-	2.54	0	0	CDS	732865	*4-nitrophenylphosphatase*
NC_051380.1	-	7.44	0	0	UTR5	101743457	*Phosphoglycolate phosphatase 2*
NC_051359.1	+	2.29	0	0	UTR3	100101204	*Hmg-r*
NC_051360.1	-	2.05	0	0	UTR3	100101206	*Mppd*
NC_051359.1	+	3.27	0	0	UTR3	100101204	*Hmg-r*
NC_051373.1	-	2.6	0	0	UTR3	100101205	*Mk*
NC_051380.1	-	2.16	0	0	CDS	101743457	*Phosphoglycolate phosphatase 2*
NC_051376.1	-	2.37	0	0	UTR5	100101203	*Hmg-s*
NC_051376.1	-	2.83	0	0	UTR5	692988	*Aldehyde dehydrogenase*
NC_051378.1	+	2.46	0	0	UTR3	692835	*Mpk*
NC_051376.1	-	3.84	0	0	UTR3	100101203	*Hmg-s*
NC_051373.1	-	3.56	0	0	UTR5	100101205	*Mk*

Table S3. Genes in JH signaling pathway with significantly different expression levels after knocking down *METTL3*

Gene Name	Gene_ID	log2_(siM3_/_NC)	q value_(siM3_/_NC)
Hmg-r	100101204	0.77127354	0.02232639
Mk	100101205	0.47927833	0.04957009
Juvenile hormone epoxide hydrolase 3	100307010	-0.8515536	0.01615363
Cce-6	100500760	-2.0811432	0.00957523
Phosphoglycolate phosphatase 2	101743457	2.27616462	6.65E-12
Oxidoreductase	101746691	0.59403586	0.04533903
Oxidoreductase like	105842801	0.84949213	0.00871797
Fps	692433	-1.4330574	1.25E-07
Jhe1	692579	3.25449722	6.85E-05
Jheh2	692686	-1.4282115	1.28E-07
Aldehyde dehydrogenase	692988	-0.6329876	0.01897601
4-nitrophenylphosphatase	732865	2.61377615	1.57E-17

Table S4. The tendency of *BmSPI4*, *BmSPI5*, *sericin1*, and *sericin2* expression level and m^6A modification regulated by knocking down *METTL3* and JHA treatment.

Treatment	Gene name	m^6A modification level	Expression level
Knocking down *METTL3*	*BmSPI4*	Down	Down
	BmSPI5	Down	Up
	sericin1	Down	Up
	sericin2	Down	UP
JHA	*BmSPI4*	Up	Up
	BmSPI5	Down	Up
	sericin1	Up	Down
	sericin2	Down	Up

Table S5. Primers used in the study

Primers	Sequence (5'-3')	Purpose
BmMETTL3-F	CCCTATGGCACTATGTCGG	qRT-PCR of *METTL3*
BmMETTL3-R	CGGTTCTGATGATGCGTTG	qRT-PCR of *METTL3*
seroin1-F	TTCCTCATCCACGGTCAACG	qRT-PCR of *seroin1*
seroin1-R	TTCTTCGACGGCTTTTCCGT	qRT-PCR of *seroin1*
BmYTHDF3-F	AACAGCGGCATTTGGACAAC	qRT-PCR of *YTHDF3*
BmYTHDF3-R	GGCATACCAGGCCCTTTCTT	qRT-PCR of *YTHDF3*
LOC101736771-F	ATTGCCAAGAGCTGCGGT	qRT-PCR of *LOC101736771*
LOC101736771-R	GCCTTCTTCCCGGGATGG	qRT-PCR of *LOC101736771*
BmSPI4-F	GCGCGTCTGGGAATACGA	qRT-PCR of *BmSPI4*
BmSPI4-R	CGTGTTGACTCGGAGCCA	qRT-PCR of *BmSPI4*
BmRPL49-F	CAGGCGGTTCAAGGGTCAATAC	qRT-PCR of *BmRpl*
BmRPL49-R	TGCTGGGCTCTTTCCACGA	qRT-PCR of *BmRpl*
BmSPI5-F	TCGATCAGACCGCGAACG	qRT-PCR of *BmSPI5*
BmSPI5-F	TCTGGAGCGTCGTCCTGA	qRT-PCR of *BmSPI5*
Ldb-F	GCGGCGTCTCAGAGTTGT	qRT-PCR of *Ldb*
Ldb-R	CAGTCTGCCTTCCGTGCA	qRT-PCR of *Ldb*
sercin2-F	AAGAGCGGCGTCACAGTC	qRT-PCR of *sercin2*
sercin2-R	ACTCTTGCGTTCCGAGCC	qRT-PCR of *sercin2*
BmSPI4-clone-F	ctcggtaccgagctcggatccATGTGTCTTTTAAAATTTTTGGTTTTG	vector construction
BmSPI4-clone-R	gtgatgatgaccggtacgcgtGTAAAGTGATGGCTCCGTATAAAAGC	vector construction
BmSPI5-clone-F	ctcggtaccgagctcggatccATGTATTCAATCGCGTTCGTTCT	vector construction
BmSPI5-clone-R	gtgatgatgaccggtacgcgtATAGACAGTCGGTTTTGAATAAATACCA	vector construction
Pcold-YTHDF3-His-F	catatcgaaggtaggcatatgATGTCAGCAGGCGTGTCAGAT	Protein expression
Pcold-YTHDF3-His-R	agactgcaggtcgacaagcttTTACCTATAGTAATCATCATATTGTTTTGCA	Protein expression
pIZ/V5-BmSPI4-F	GAACAACAGCTGCCGGAGTAAC	qRT-PCR of *BmSPI4-Mut*
pIZ/V5-BmSPI4-R	TGGTGATGGTGATGATGACCGG	qRT-PCR of *BmSPI4-Mut*
pIZ/V5-BmSPI5-F	TGATATAGAGGTCACGGAATCGGG	qRT-PCR of *BmSPI5-Mut*
pIZ/V5-BmSPI5-R	TGGTGATGGTGATGATGACCGG	qRT-PCR of *BmSPI5-Mut*

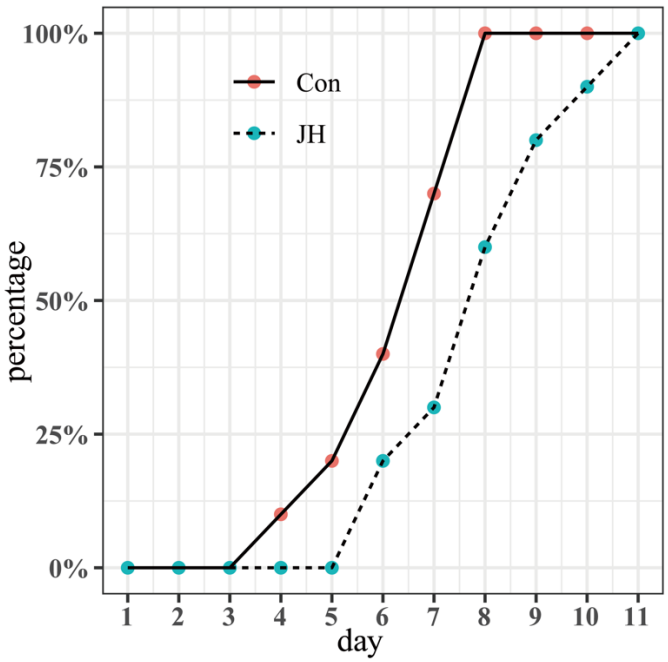

Figure S1. The length of 5th instar period of control and JHA treated silkworm larvae

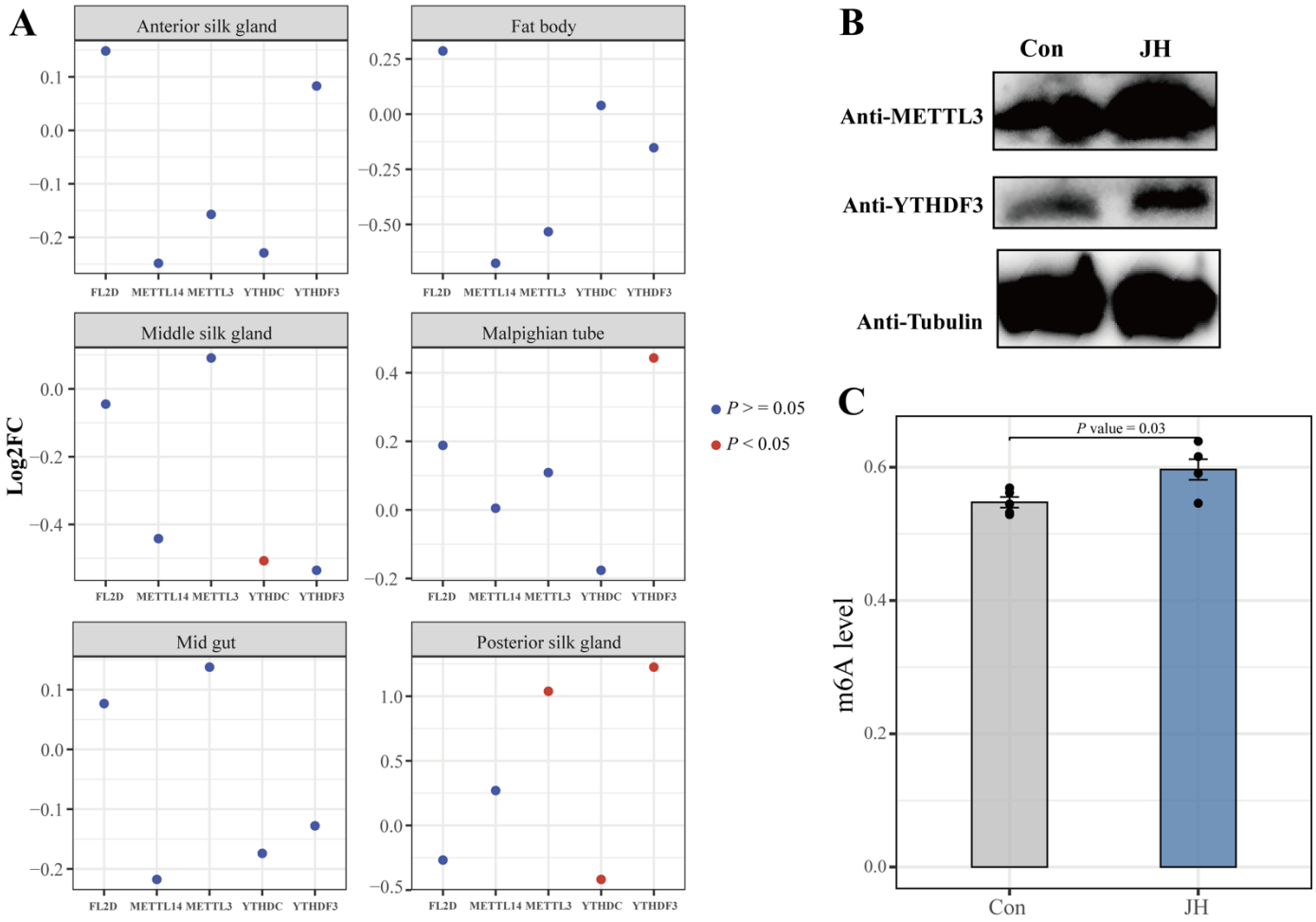

Figure S2. (A) Expression of m^{6}A-related genes, *FL2D*, *METTL14*, *METTL3*, *YTHDC*, and *YTHDF3* in silkworm posterior silk gland, anterior silk gland, fat body, middle silk gland, midgut, and malpighian tube after JHA treatment for 24 h. (B) Protein expression of METTL3 and YTHDF3 in silkworm PSG after JHA treatment for 24 h were measured by western blot analysis. (C) m^{6}A abundance of silkworm PSG after JHA treatment for 24 h.

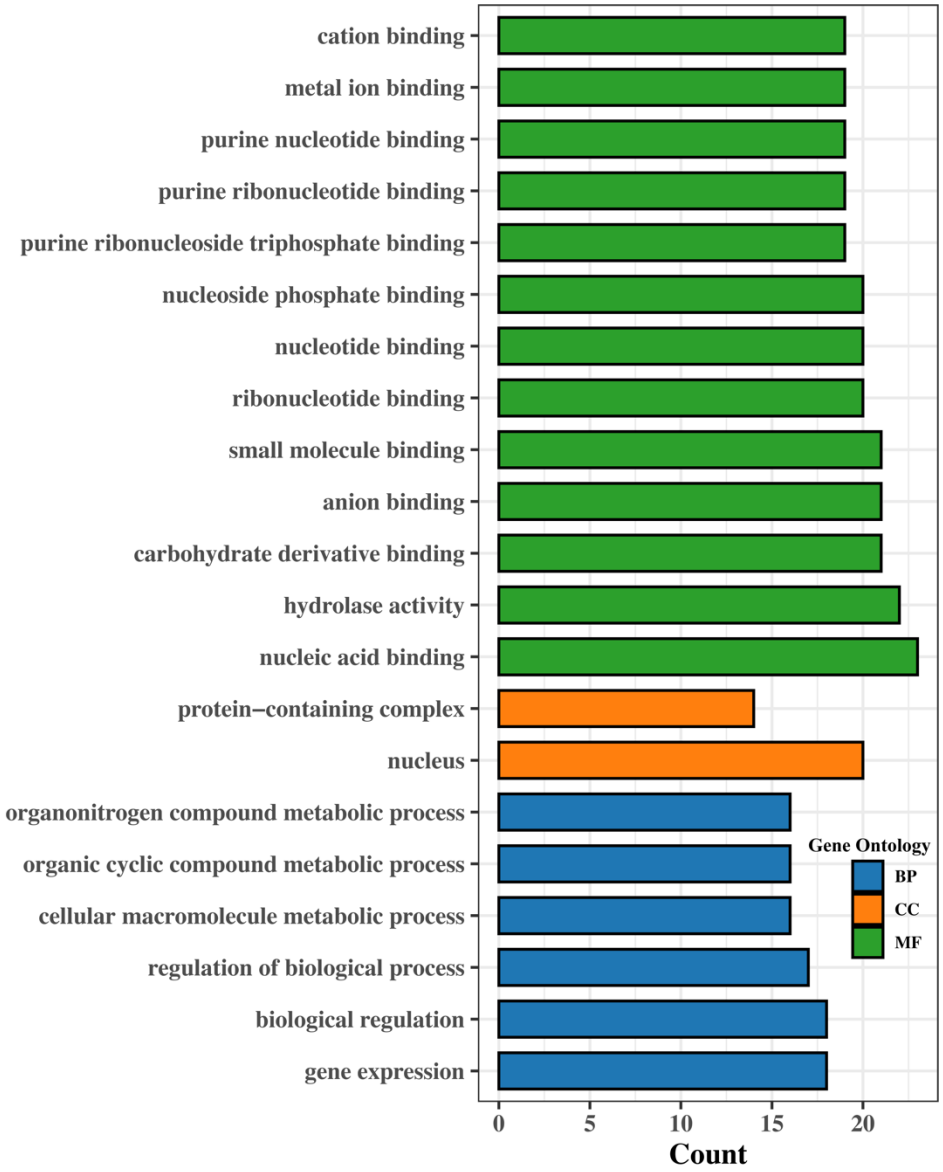

Figure S3. GO analysis of different expressed and different m^6A modification genes identified in transcriptome sequencing and m6A sequencing data. GO terms were grouped into three classes, Biological Process (BP), Cellular Compounds (CC), and Molecular Function (MF).

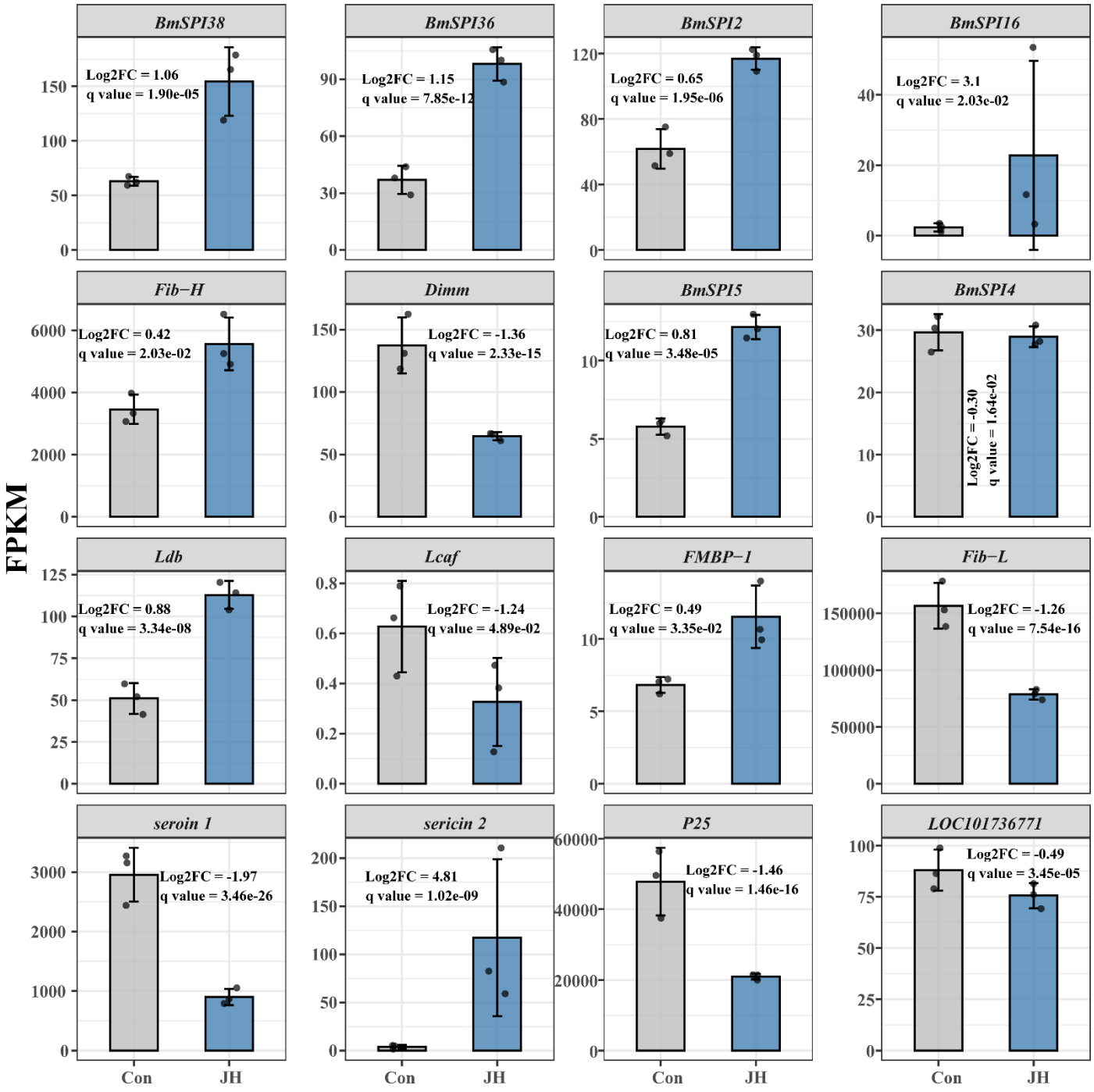

Figure S4. Many silk-associated genes have significantly different expression level in silkworm PSG after JHA treatment for 24 h.

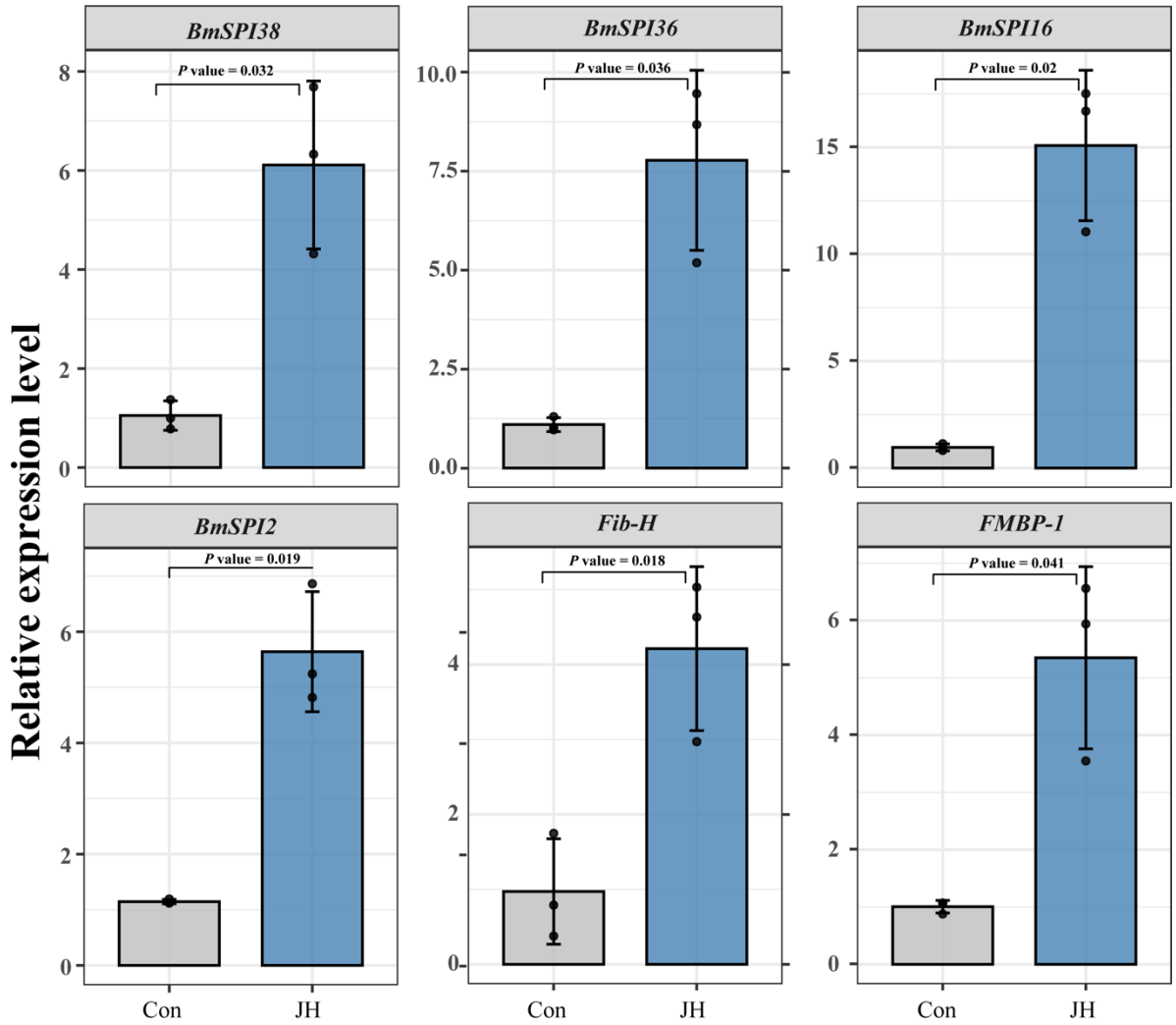

Figure S5. Six silk-associated genes were randomly selected for performing qPCR to identify the result of Transcriptome and MeRIP-sequencing data.

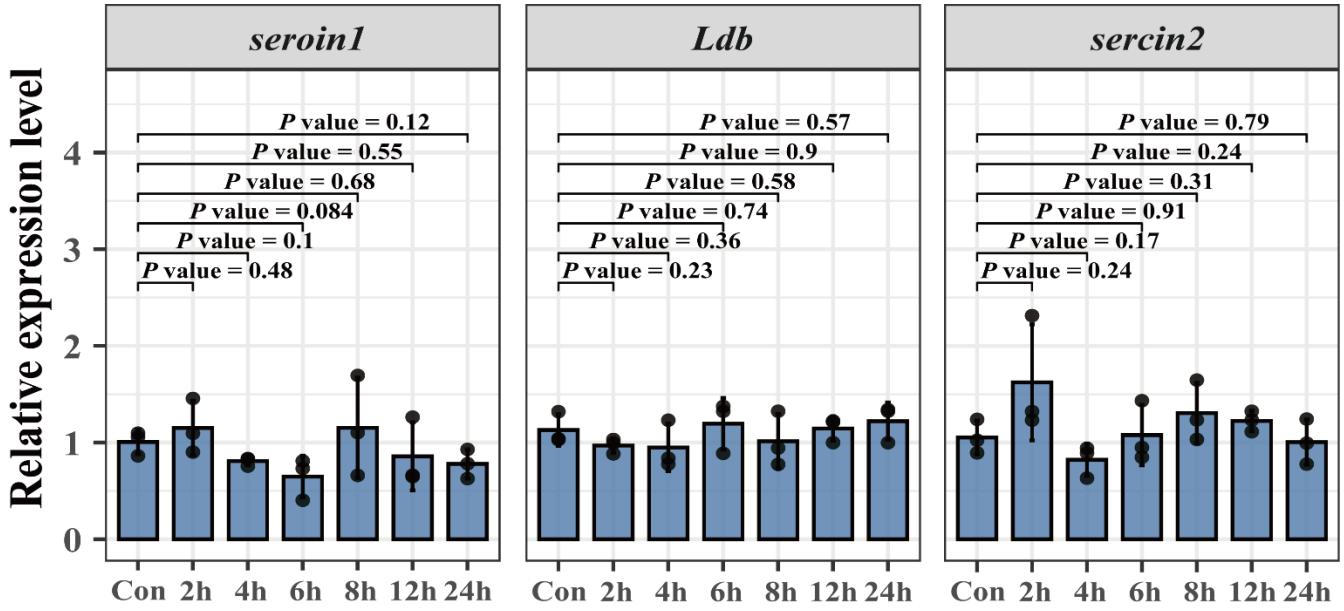

Figure S6. qPCR results of *seroin 1*, *Ldb*, and *sericin 2* in BmN cells after JHA treatment for 2 h, 4 h, 6 h, 8 h, 12 h, and 24 h.

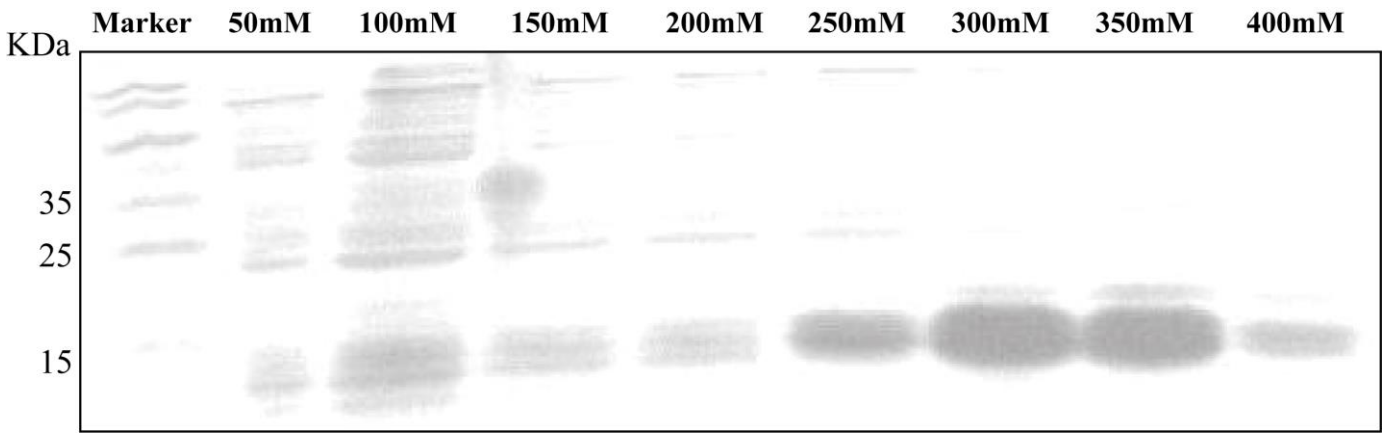

Figure S7. Recombinant His-tagged BmYTHDF3 protein were purified by nickel chromatography. The purified BmYTHDF3 were subjected to SDS-PAGE through a 10% SDS-polyacrylamide gel, and then stained with CBB. Molecular mass markers are shown to the left of the gel.