

SUPPLEMENTARY MATERIAL

Table S1.

Gene-Specific primers

Degenerate primers

Sirt2-F1 CCMGACTTCCGTAGTCC	Sirt2-F2 AACCACAGGCKATATT	Sirt2-R1 TCCCCCATCCSAGTCT
Sirt2-R2 CCSGCCTTCTCACGGTT	Sirt2-F3 GAATTATTTCAGGAAG	
PGAM-F1 TGTKATGATYCGTCATGG	PGAM-F2 GAATGGAAYCAGAARAA	
PGAM-F3 GCWGAAGGCTATCAGTT	PGAM-R2 AACATTGTTCCAGTAWGG	
PGAM-R1 GGCAGRITCAAYTCCAT		
PK-F1 ATCTGTACYATYGGACC	PK-F2 GTGCTVGAGAAGATGAT	
PK-R1 GGGTASTCGCCCTTGGC	PK-R2 ATACCCAGATCATCCACG	
PK-R3 TTGATGTTCTTBCCTTC		
PEPCK-F1 GAAGGATGGCTSGCCGA	PEPCK-F2 GACGACATMGCCTGGATG	
PEPCK-R1 CGGAACCAGTTBACGTG	PEPCK-R2 GCCGAAGTTGTAGCCGAA	
PEPCK-R3GTGGCCTCGSWCCTCAT		

Overexpression primers

Sirt2 F CCGGAATTCTATGTCTGCAAATTCACCGCCAGG
Sirt2 R CCGCTCGAGCGTCATAGTTCGGGCTCCTGTGG
PK F CGGGATCCATGGTGTATACTAATACTATTACGAT
PK R CCGGAATTCATTCCGCTTGGATGACACGCATGGT
PGAM F CGGGATCCACATGGGACGTCAAAGGAAAATT
PGAM R CCCTCGAGCGCTTGGCCTTGCCTTGGGAAGCAAC
PEPCK F CCGGAATTCATGTTGCACCTGCAGGCTGACC
PEPCK R CCGCTCGAGGGTGACTGTTGGACATTTTCTAAG

Prokaryotic expression primers

Sirt2 F CCGGAATTCGTGGAGGCTCACGGCACCTTC
Sirt2 R CCGCTCGAGGCTCCTGTGGCGTGACGTG

RNA interference primers

Sirt2-Ri-F1 GGATCCTAATACGACTCACTATAGGGTGTCTTCGGCGAGAGTCTC
Sirt2-Ri-F2 GTG TTCTTCGGCGAGAGTCTC
Sirt2-Ri-R1 GGATCCTAATACGACTCACTATAGGTAGTTC GGG CTCCTGTGGCGT
Sirt2-Ri-R2 TAGTTCGGGCTCCTGTGGCGT
GFP-Ri-F1: GGATCCTAATACGACTCACTATAGGAAGGGCGAGGAGCTGTTACCG
GFP-Ri-F2: AAGGGCGAGGAGCTGTTACCG
GFP-Ri-R1: GGATCCTAATACGACTCACTATAGGCAGCAGGACCATGTGATCGCGC
GFP-Ri-F2: CAGCAGGACCATGTGATCGCGC

Primers used for PCR in this study. M=A/C; R=A/G; W=A/T; S=G/C; Y=C/T; K=G/T; V=A/G/C; H=A/T/C; B=G/T/C; D=G/A/T.

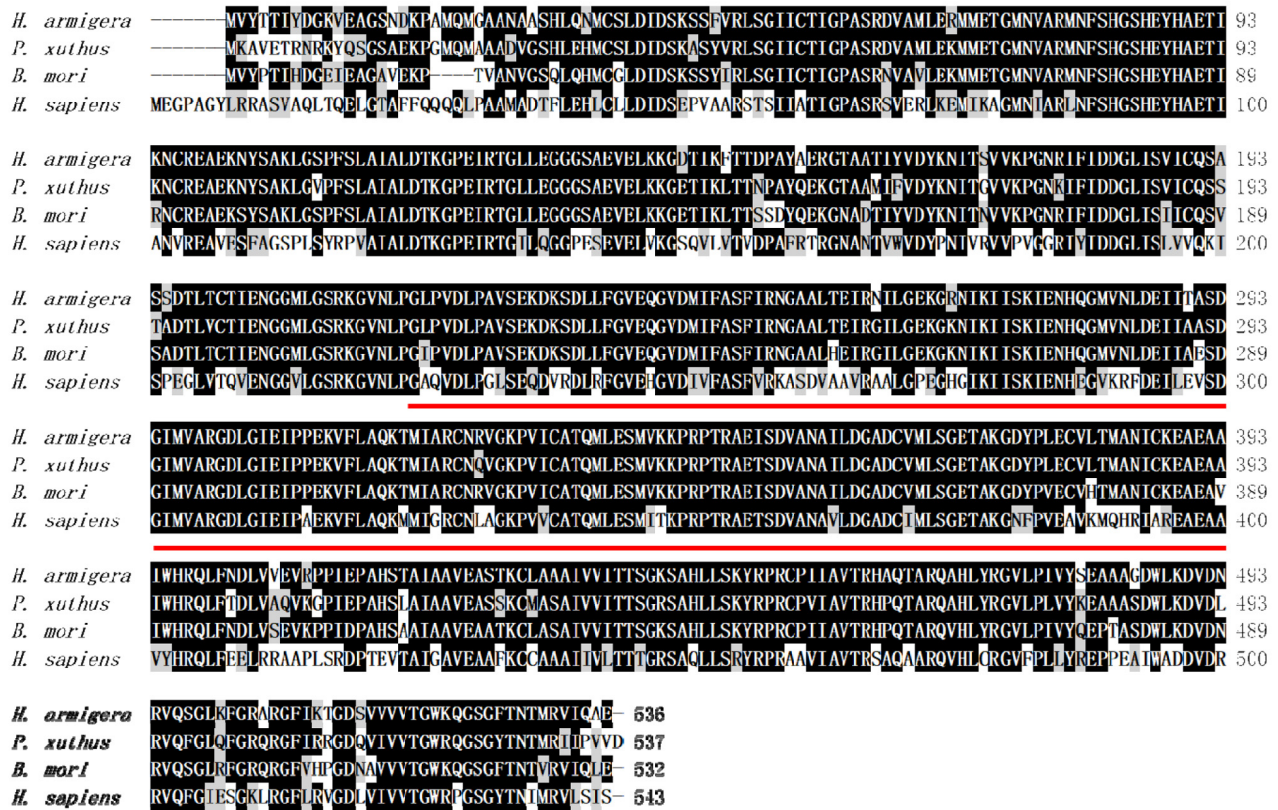


Figure S1. Homology comparison to other known PK proteins. The *H. armigera* PK amino acid sequence has high identity with PKs of other species: *P. xuthus* (89%), *B. mori* (87%), and *H. sapiens* (57%). Black shading represents ≥50% sequence identity. *H. armigera*, GenBank™ number KY412773; *P. xuthus*, XP_013164662.1; *B. mori*, XP_004931176.2; *H. sapiens*, AY352517.1. The red line below the amino acid sequence shows peptide synthesized as an immunogen.

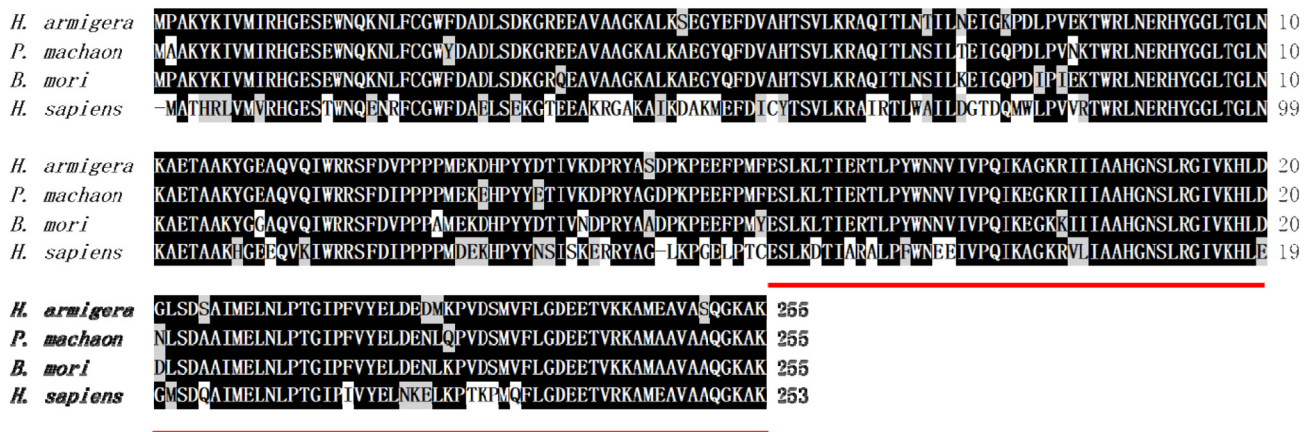


Figure S2. Homology comparison to other known PGAM proteins. The *H. armigera* PGAM amino acid sequence has high identity with PGAMs of other species: *P. machaon* (92%), *B. mori* (92%), and *H. sapiens* (69%). Black shading represents ≥50% sequence identity. *H. armigera*, GenBank™ number KY387804; *P. machaon*, XP_014361246.1; *B. mori*, NP_001037540.1; *H. sapiens*, NP_000281.2. The red line below the amino acid sequence shows peptide synthesized as an immunogen.

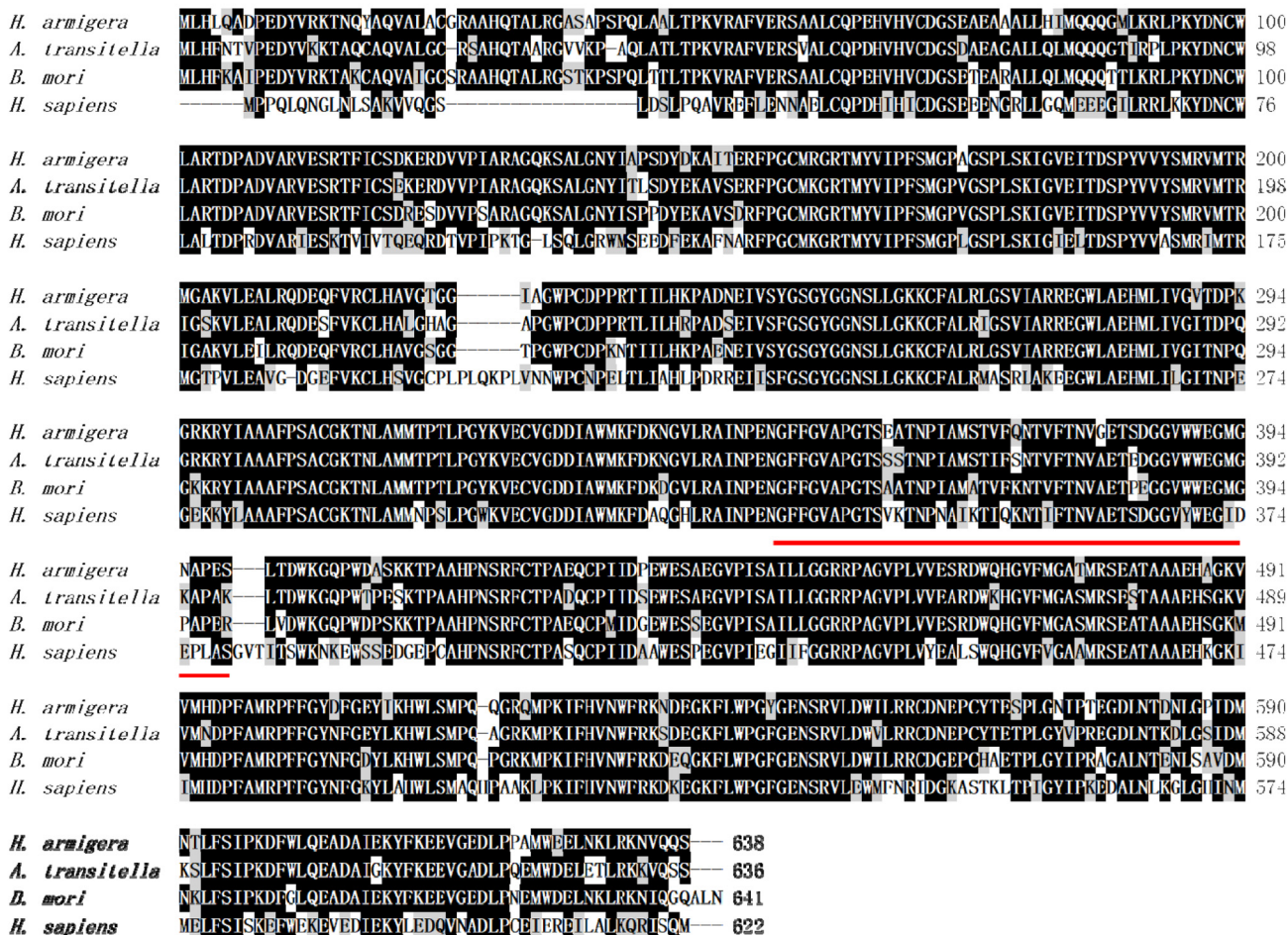


Figure S3. Homology comparison to other known PEPCK proteins. The *H. armigera* PEPCK amino acid sequence has high identity with PEPCKs of other species: *A. transitella* (85%), *B. mori* (86%), and *H. sapiens* (59%). Black shading represents $\geq 50\%$ sequence identity. *H. armigera*, GenBankTM number AFK28502.1; *A. transitella*, XP_013191765.1; *B. mori*, NP_001040542.1; *H. sapiens*, NP_002582.3. The red line below the amino acid sequence shows peptide synthesized as an immunogen.

A	Position of site	Flanking residues
PK	146	EVELKKGDTI-K-FTTDPAYAER
	174	VDYKNITSVV-K-FGNRIFDIDG
	271	NILGEKGRNI-K-IISKIENHQG
	275	EKGRNIKIIS-K-IENHQGMVNL
	310	GD LGIEIPPE-K-VFLAQKT MIA
	500	DVDNRVQSG L-K-FGRARGFIKT
PGAM	39	ELSEKGTEEA-K-RGAKAIKDAK
	43	KGTEEAKRGA-K-AIKDAKMEFD
	46	EEAKRGAKAI-K-DAKMEFDICY
	49	KRGAKAIKDA-K-MEFDICYTSV
	100	RHYGGLTGLN-K-AETA AKHGEE
	106	TGLNKAETAA-K-HGEEQVKIWR
	113	TAAKHGEEQV-K-IWRRSFDIPP
179	EEIVPQIKAG-K-RVLI AAHGNS	
PEPCK	91	LHIMQQQGM L-K-RLPKYDNCWL
	95	QQQGM LKRLP-K-YDNCWLARTD
	204	SMRV MTRMGA-K-VLEALRQDEQ
	264	GYGGNSLLGK-K-CFALRLG SVI
	294	HMLIVGV TDP-K-CRKR YIAAAF
	297	IVGV TDPKGR-K-RYIAAAFPSA
	540	VNWF RKND EG-K-ELWPGYGENS
613	QEADAIEKYF-K-EEVGEDLPPA	

B		
PK	<i>H. armigera</i> :	DLGIEIPPEK VFLAQK
	<i>H. sapiens</i> :	DLGIEIPAEK VFLAQK
PGAM	<i>H. armigera</i> :	HYGGLTGLNKAETA AK
	<i>H. sapiens</i> :	HYGGLTGLNKAETA AK
PEPCK	<i>H. armigera</i> :	LKRLPKYDNCWLA
	<i>H. sapiens</i> :	LRRLK KYDNCWLA

Figure S4. Predicted acetylation site of the metabolic enzymes. (A) Potential acetylation sites of PK, PGAM, and PEPCK using a PSKAcePred software. (B) Predicted acetylation sites of PK, PGAM, and PEPCK compared with known acetylation sites. The red amino acids show the predicted acetylation sites and the green amino acids show the flanking residues. *H. sapiens*, *Homo sapiens*.

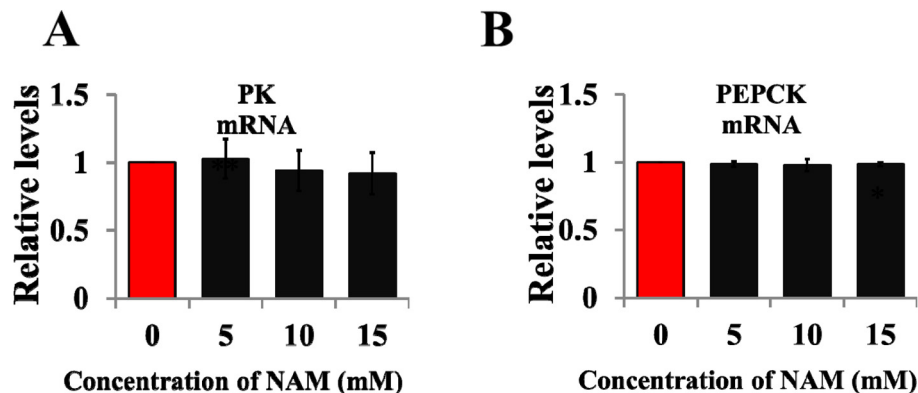


Figure S5. Effect of NAM treatment on PK and PEPCK mRNA levels. HzAm1 cells were treated with 0, 5, 10, 15 mM NAM for 48 h. Total RNA was extracted and PK and PEPCK mRNA levels were detected by qPCR using *actin* as an internal standard. Each point represents the means \pm S.D. of three independent replicates. *, $p < 0.05$; **, $p < 0.01$ (determined by independent t-test).



Figure S6. Homology comparison to other known Sirt2 proteins. The *H. armigera* Sirt2 amino acid sequence has high identity with Sirt2s of other species: *A. transitella* (83%), *P. xuthus* (84%), and *B. mori* (81%). Black shading represents $\geq 50\%$ sequence identity. *H. armigera*, GenBank™ number KY363351; *A. transitella*, XP_013184462.1; *P. xuthus*, XP_013171078.1; *B. mori*, NP_001036937.1.

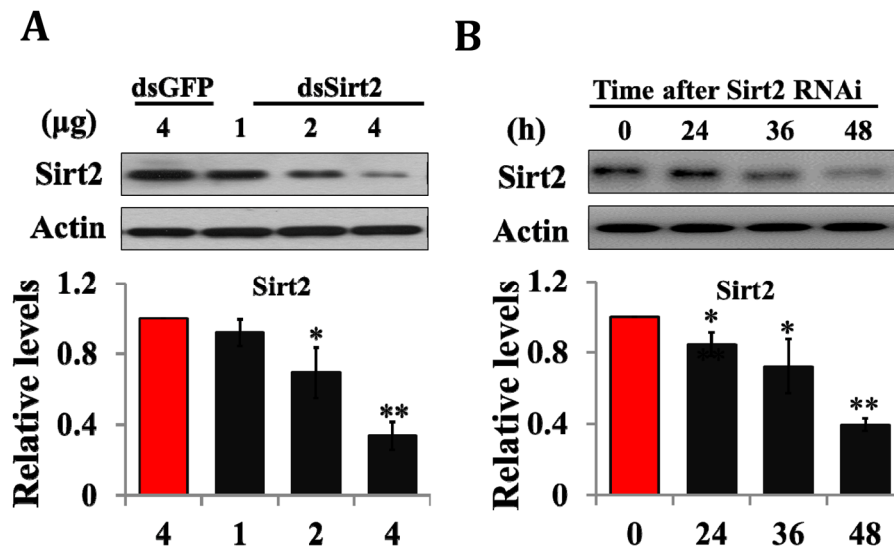


Figure S7. Efficiency of Sirt2 knockdown. (A) HzAm1 cells were transfected with 1, 2, and 4 μg Sirt2 dsRNA or 4 μg GFP dsRNA for 48 h. (B) HzAm1 cells were transfected with 4 μg Sirt2 dsRNA for 0, 24, 36, and 48 h. Protein (20 μg for Sirt2) was extracted from the cells for immunoblotting with the anti-Sirt2 antibody. Protein bands were quantified and normalized to the levels of *H. armigera* actin (5 μg). Each point represents the means \pm S.D. of three independent replicates. *, $p < 0.05$; **, $p < 0.01$ (determined by and independent t-test).

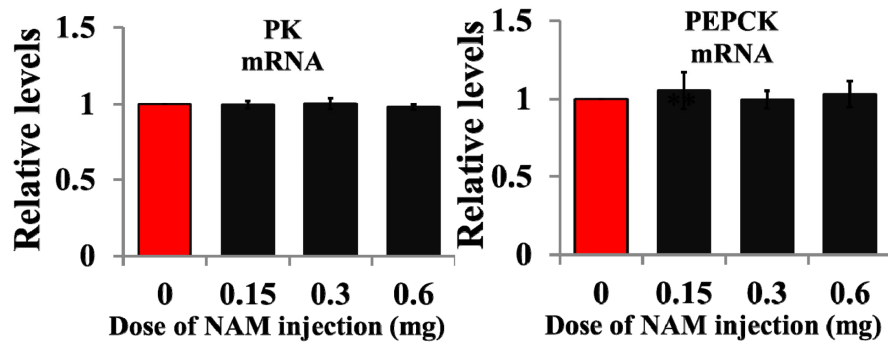


Figure S8. Effect of NAM injection on *PK* and *PEPCK* mRNA levels. Day-1 nondiapause-destined pupae were injected with NAM and pupal brains were dissected 48 hours after injection. *PK* and *PEPCK* mRNA levels were detected by qPCR using *actin* as an internal standard. Each point represents the mean \pm S.D. of three independent replicates. *, $p < 0.05$; **, $p < 0.01$ (determined by and independent t-test).