## **Supplementary materials**

Wei Sun et al. Molecular cloning and characterization of *ecdysone oxidase* and *3-dehydroecdysone-3α-reductase* involved in the ecdysone inactivation pathway of silkworm, *Bombyx mori* 



Figure S1. Enzymic interconversions of ecdysone, 3-dehydroecdysone and 3-epiecdysone. EO represents the Ecdysone Oxidase;  $3DE-3\beta$ -reductase represents the 3-dehydroecdysone- $3\beta$ -reductase;  $3DE-3\alpha$ -reductase represents the 3-dehydroecdysone- $3\alpha$ -reductase

A DmEO: BmGMC2: BmEO: SIEO:	MGDQRVHKPRTAKTRTLRVNRILLPMLLLFSGETTSUVTQUFSVDSSCLGISULQSVAIALMUSSLAUANNTAUBLOHEPPEDRLEIESVDYTVVCAGSAGSIVASRUSULCQVRVLLDBEQLDEDSSETFGLTGA <mark>D</mark> HHDERYMBLEEAMPUPKCCUAVASN : :	163 98 119 119
DmEO: BmGMC2: BmEO: SIEO:	HGCVUUHGRUNGGGGAINGNOIPESRENRRUNSTOUDITIONE TYSELOORINPSYNOPIKUNLKLANTINSE-SAEHOOPENKOPLIAGATEGTHEOPENTOUDERGASSARLVUANDOVNERGNUKUTGAOVOR 2: GAYKLSTEKALGGGSTINEITHE GKOUDDTASYL DOSISSA VERTERRUD DOOTASHADYETE PROVERIEDOATHNIJKA SEITVETTIONADDWGCAEASYLIGD CYEUSIAY YM KKTEDUUYVITELLAEK 2: GIEOOPERKILGGSTANHIWA: GFPSONEDASIAC-ETUUTIVUKINERKIEHTOSILTENKEITOVERG ANEWSTN VESKKELOAFELGEKTINDIV ASHAD	301 248 272 272
DmEO: BmGMC2: BmEO: SIEO:	VLDNAAGSRATEVIYTL-NEVEHTAKTLGEVILSAGTLUSAKLLLLSGIG REELORINHTTHODLEVGRNLODHG <mark>E</mark> VPLFILFGSNCAVNSTRDPTENPYAPISITOVILDNOK PLASGFYNNGINNSSSSSRGEPDHYVAHTLLPKGSTGSFGYLGF 2: IIIGNDTAKEVILKLASG-KINYTASREVILSAGTNTFKLLNISGIG ADHUKSNGIDVIKDEVGGGUDHNLGIVWKLEESTATSET	463 389 424 425
DmEO: BmGMC2: BmEO: SIEO:	RPOLIQAQQDILQXGDUQ <mark>INDSULRELSHGNYSLSENISADQAKTENHYGEAVOQQTILCYNRYIQKUSKIRPERCGLOUKEPDHEDDTDAADSDDY'L-GYTRYFYD<mark>EDESTGTCRUAPRKGVDSQENGGWDERLRYHGYKGLRYDASINFELPA</mark> :: TDEL SNFSESVAGRUOFISLUTSQERERYDDLSSDTDOKLYTESEFTSDFTDFTDITLYTURTHTUTYTSATFODIK/ELADFGAFEGEEDVSSDTYK-GYTRSTTHHFFYSTGAU</mark>	625 540 573 574
DmEO: BmGMC2: BmEO: SIEO:	GITTICFANNICGRC ON LUDREANNEVIQEC : 657 : ANTLAGTINABRISDIRIEYSI	
B		
SI3DE-3a- Bm3DE-3a Dm3DE-3a Tc3DE-3a Am3DE-3a	tereductase : -MFANKWVIVTGCSSCIGATVEAVIEGASVIEVGENQAFI KEVESK QQHGANIFAR KAUSKHEEAKIIV GTVDKFGKLDVLVNNAGIIREASVIEPTIGTEE : : Gareductase : SSTNKVVIVTGSSSGIGASA VHAKEGADVAIVGENVKVDNVAREQQHGANIFAR KAUSKHEEAKIIV GTVDKFGKLDVLVNNAGIIREASVIEPTIGTEE : : Gareductase : SISKVVIVTGSSSGIGAST HAKEGADVAIVGENVAN EATKKSIK - GTQAEIVVADVKDADA - IV GTIAFGGIDVLVNNAGIIGKGUIDLI I-reductase : MSAGKVVITGASSGIGATT KQAGIGASIAIAGENIDNIKEVASC NSPSNKPLIITGEINEKTEDILETISHYGRLDVLVNAGIGKGUIDU - SIETTNI-QYD : : Gareductase : MSAGKVVITGASSGIGATT KQAGIGASIAIAGENIDNIKEVASC NSPSNKPLIITGEINEKTEDILETISHYGRLDVLVNNAGIGKG SIETTNI-QYD : : Gareductase : MSAGKVVITGASSGIGATT KQAGIGASIAIAGENIDNIKEVASC NSPSNKPLIITGEINEKTEDILETISHYGRLDVLVNNAGIGKG SIETTNI-QYD : :	107 108 103 107 105
SI3DE-3a- Bm3DE-3i Dm3DE-3i Tc3DE-3a Am3DE-3i SI3DE-3a- Bm3DE-3i Dm3DE-3a Am3DE-3a	20 *	107 108 103 107 105 211 211 210 214 212

**Figure S2.** Multiple sequence alignment of EO and 3DE-3DE-3 $\alpha$ -reductase proteins. (A) Multiple sequence alignment of EO. The red box means conserved ecdysone binding residues. (B) Multiple sequence alignment of 3DE-3 $\alpha$ -reductase. Identical and similar residues have been highlighted in black and grey, respectively. SIEO represents the Ecdysone Oxidase from the *S. littoralis*; DmEO represents the Ecdysone Oxidase from the *D. melanogaster*. SI3DE-3 $\alpha$ -reductase represents the 3DE-3 $\alpha$ -reductase from the *S. littoralis*.



Figure S3. Phylogenetic tree of EO and 3DE-3DE-3α-reductase in insects. (A) The phylogenetic tree of EO. (B) The phylogenetic tree of 3DE-3α-reductase. Abbreviations: Dm: D. melanogaster; Ag: *Anopheles gambiae*; Cele: *Caenorhabditis elegans*; Ecol: *Escherichia coli*; Tc: *Tribolium castaneum*; Am: *Apis mellifera*; SI: *Spodoptera littoralis*; Ct: *Comamonas testosteroni* S44; Ps: *Pseudomonas sp.* B-0831; Sh: *Shewanella halifaxensis* HAW-EB4; Rn: *Rattus norvegicus*; Mum: *Mus musculus*; Bt: *Bos taurus*; Ho: *Homo sapiens*; Mam: *Macaca mulatta*; Pt: *Pan troglodytes*. GMC: Glucose-Methanol-choline oxidoreductases family; CHD: choline dehydrogenase; SDR: short-chain dehydrogenases/reductases (SDR) superfamily; Sro: Shroud; 3α-HSDs: 3α- hydroxysteroid dehydrogenases.





Figure S4. HPLC and Mass spectrum of the ecdysone and 3-epiecdysone (FAP). (A) the HPLC of the ecdysone and 3-epiecdysone (FAP). (B) the mass spectrum of the ecdysone. (C) the mass spectrum of the 3-epiecdysone (FAP). FAP means the first additional peak in the Figure 3D.



Figure S5. Negative control of Immunohistochemical analysis. (A) Immunohistochemical analysis in the midgut using control antiserum. (B) Immunohistochemical analysis in the Malpighian tubule using control antiserum. Bar, 200 μm. The control antiserum was collected from the mice immunized with PBS. Blue fluorescence represents the nuclei.

## Table S1. List of primers

Gene	Sequence (5' to 3')	Purpose		
BmEO	S: <sup>a</sup> GGATCCATGGTTTGCGGGGTTG	Probe	synthesis	for
	AS: <sup>a</sup> GCGGCCGCTCATGCGACATTGAC	RT-PCR	and prokar	yotic
		expression	on	
	S: <sup>a</sup> GAATTCATGGTTTGCGGGTTG	Probe	synthesis	for
	AS: <sup>a</sup> GCGGCCGCTCATGCGACATTGAC	eukaryot	ic expression	
	S: CGTTTCGCAGCGGGTGTC	Probe	synthesis	for
	AS: CCGATTTGGGTTTCAGCAGG	quantitat	ive RT-PCR	
	S: TGGATCTCCGCCCGCCTCCAGTAGGAG	Probe	synthesis	for
	AS: CTCGGAGAGTCTGGCGGCGAGCGCAG	5'RACE		
	S: TGGCATTCCGTGGGTACTGCCGCT	Probe	synthesis	for
	AS: GCACCGGTTGTGATGATCGCGGAA	3'RACE		
$Bm3DE$ - $3\alpha$ -reductase	S: <sup>a</sup> <u>GGATCC</u> ATGAGTTTCACAAAT	Probe	synthesis	for
	AS: <sup>a</sup> GCGGCCGCTCATCTGAGCAGCAG	prokaryo	otic expression	n
	S: <sup>a</sup> <u>GAATTC</u> ATGAGTTTCACAAAT	Probe	synthesis	for
	AS: <sup>a</sup> GCGGCCGCTCATCTGAGCAGCAG	eukaryot	ic expression	
	S: AATGCCAGGGTTTCTGGCATACT	Probe	synthesis	for
	AS: CTTCAACGCCGTGGCAGCCCTA	RT-PCR	and quantit	ative
		RT-PCR		
	S: TGAGCACATCTAGCTTCCCGAAGGC	Probe	synthesis	for
	AS: CCGACTTGCGCACATTCCCGGGCCAC	5'RACE		
	S: TGTCAGAGCAAACATCATCAGTCCAGGTCT	Probe	synthesis	for
	AS: GACATTTTTAGGGCTGCCACGGCGTTGAAG	3'RACE		

<sup>a</sup>Restriction sites are underlined; S: sense primer; AS: antisense primer.

Table S2.	The	Genbank	Accession	Numbers	of the	proteins u	ised in	the ph	ylogenetic	analysis

	Protein name	Accession No.	Species
a*	DmEO	CG9504	Drosophila melanogaster
	DmGMC <sup>β2</sup>	CG9509	Drosophila melanogaster
	DmGMC <sup>β3</sup>	CG9512	Drosophila melanogaster
	AgGMC β4-1	AAAB01008844	Anopheles gambiae
	AgGMC β4-2	AAAB01008844	Anopheles gambiae
	AgGMC β4-3	AAAB01008844	Anopheles gambiae
	AgGMC β4-4	AAAB01008844	Anopheles gambiae
	SIEO	AY035784	Spodoptera littoralis
	BmGMC2	BGIBMGA000158 <sup>#</sup>	Bombyx mori
	Cele CHD	NP_495846	Caenorhabditis elegans
	Ecol CHD	NP_414845	Escherichia coli
b*	DmSDR	NP_610235	Drosophila melanogaster
	DmSDR 1	NP_647839	Drosophila melanogaster
	DmSDR 2	NP_729808	Drosophila melanogaster
	DmSDR 4	NP_001163844	Drosophila melanogaster
	Dm-3DE-3α-reductase-like	NP_569875	Drosophila melanogaster
	DmSro-like	CG12068	Drosophila melanogaster
	TcSDR 1	XP_974493	Tribolium castaneum
	TcSDR 2	XP_969456	Tribolium castaneum
	TcSDR 3	XP_970029	Tribolium castaneum
	TcSDR 4	XP_969383	Tribolium castaneum
	Tc-3DE-3α-reductase-like	XP_974115	Tribolium castaneum
	TcSro-like	GA11371	Tribolium castaneum
	AmSDR 1	XP_624401	Apis mellifera
	AmSDR 2	XP_624353	Apis mellifera
	Am-3DE-3α-reductase-like	XP_394891	Apis mellifera
	AmSro-like	XP_001120169	Apis mellifera
	S1-3DE-3α-reductase	AF255341	Spodoptera littoralis

\*a represents the protein sequences used in the phylogenetic analysis of the ecdysone oxidase; \*b represents the protein sequences used in the phylogenetic analysis of the 3DE-3α-reductase. # represents the Accession No. of ecdysone oxidase identified by Yang et al (2011) in SilkDB.