

Supplementary Table 1. Thickness of old and newly forming cuticles from LPMO15-1-deficient *T. castaneum* and *L. migratoria*.

Species	Developmental stage analyzed	Cuticles	Thickness of cuticle (μm)		<i>p</i> value
			<i>dsTcVer</i>	<i>dsTcLPMO15-1</i>	
<i>T. castaneum</i>	Penultimate instar larvae	old larval cuticle	1.86 $\pm$ 0.09	4.50 $\pm$ 0.28	1.89E-07
		new larval cuticle	3.30 $\pm$ 0.12	4.00 $\pm$ 0.48	0.18
	Pharate pupae	old larval cuticle	1.51 $\pm$ 0.12	6.05 $\pm$ 0.33	1.66E-13
		new pupal cuticle	3.09 $\pm$ 0.21	3.23 $\pm$ 0.11	0.54
	Pharate adult	old pupal cuticle	3.36 $\pm$ 0.32	7.27 $\pm$ 0.14	4.52E-09
		new adult cuticle	7.10 $\pm$ 0.25	7.03 $\pm$ 0.17	0.81
Species	Developmental stage analyzed	Cuticles	Thickness of cuticle (μm)		<i>p</i> value
			<i>dsGFP</i>	<i>dsLmLPMO15-1</i>	
<i>L. migratoria</i>	Pharate adult	old nymphal cuticle	14.66 $\pm$ 0.22	29.86 $\pm$ 0.77	3.67E-15
		new adult cuticle	8.79 $\pm$ 0.16	8.46 $\pm$ 0.34	0.39

Data are shown as the mean value $\pm$ SE (n = 9-15).

Supplementary Table 2. Primers used for cloning and dsRNA synthesis.

Gene	Genbank Accession #	Direction*	Sequence (5'-3')**	Product size (bp)	Note
<i>TcLPMO15-1</i>	MZ636451	F	ATGCTAGTGCCATTATCACG	1,014	cloning
		R	TTAATTCGTTGAAAATTCTTCG		
		F	(T7)-CAACGGCAAATGCGGCCT	295	dsRNA
		R	(T7)-GGCTTTCTTCTTCCGTCT		
<i>TcVer</i>	AY052390	F	(T7)-CTGAGCGAAGAATGTGGGAT	603	dsRNA
		R	(T7)-GGTTAAAGCTTCTCGACCG		
<i>LmLPMO15-1</i>	MZ440879	F	GGAGCAGCGGCGACACCACG	1,704	cloning
		R	CGCAAATTAAGCTGTTTCAGC		
		F	(T7)-AAGTTGAGGTGGACCTGACG	413	dsRNA
		R	(T7)-TGCGGAAGTCTCGGTAGTAGA		
<i>OfLPMO15-1</i>	MZ440880	F	TCGCGCCATTCAAACTTCG	2,120	cloning
		R	poly(T)		
<i>GFP</i>	CVU76561	F	(T7)-GCACCATCTTCTTCAAGGA	231	dsRNA
		R	(T7)-GATGTTGTGGCGGATCTT		

*F, forward; R, reverse.

**T7, T7 RNA polymerase recognition sequence (TAATACGACTCACTATAGGG).

Supplementary Table 3. Accession numbers of insect LMPO15-1s used for the amino acid sequence alignment and phylogenetic analysis.

Order	Name	Species	Accession number
Coleoptera	AtLPMO15-1	<i>Aethina tumida</i>	XP_019876627
	AgILPMO15-1	<i>Anoplophora glabripennis</i>	XP_018574059
	DpLPMO15-1	<i>Dendroctonus ponderosae</i>	XP_019763018
	LdLPMO15-1	<i>Leptinotarsa decemlineata</i>	XP_023029101
	NveLPMO15-1	<i>Nicrophorus vespilloides</i>	XP_017786569
	OtLPMO15-1	<i>Onthophagus taurus</i>	XP_022909937
	TcLPMO15-1	<i>Tribolium castaneum</i>	MZ636451
Lepidoptera	BmLPMO15-1	<i>Bombyx mori</i>	XP_004926014
	HaLPMO15-1	<i>Helicoverpa armigera</i>	XP_021180988
	MsLPMO15-1	<i>Manduca sexta</i>	XP_030035714
	OfLPMO15-1	<i>Ostrinia furnacalis</i>	MZ440880
	PxLPMO15-1	<i>Papilio xuthus</i>	XP_013180709
	PrLPMO15-1	<i>Pieris rapae</i>	XP_022121259
	TnLPMO15-1	<i>Trichoplusia ni</i>	XP_026747704
Hymenoptera	AmLPMO15-1	<i>Apis mellifera</i>	XP_026298239
	NvLPMO15-1	<i>Nasonia vitripennis</i>	XP_001602613
Diptera	AaLPMO15-1	<i>Aedes aegypti</i>	XP_021696109
	Aa/LPMO15-1	<i>Aedes albopictus</i>	XP_019525791
	AgLPMO15-1	<i>Anopheles gambiae str. PEST</i>	XP_310852
	CcLPMO15-1	<i>Ceratitis capitata</i>	XP_012157461
	DmLPMO15-1	<i>Drosophila melanogaster</i>	NP_001188545
	HiLPMO15-1	<i>Hermetia illucens</i>	CAD7090791
	MdLPMO15-1	<i>Musca domestica</i>	XP_019893458
Orthoptera	AspLPMO15-1	<i>Atractomorpha sp</i>	GDYX01023215
	LmLPMO15-1	<i>Locusta migratoria</i>	MZ440879
	SgLPMO15-1	<i>Schistocerca gregaria</i>	GHHP01008411
	SpLPMO15-1	<i>Schistocerca piceifrons</i>	GIOR01128883
Hemiptera	ApLPMO15-1	<i>Acyrtosiphon pisum</i>	XP_016660633
	AgoLPMO15-1	<i>Aphis gossypii</i>	XP_027852856
	AlLPMO15-1	<i>Apolygus lucorum</i>	KAF6203625
	ClLPMO15-1	<i>Cimex lectularius</i>	XP_014240666
	DnLPMO15-1	<i>Diuraphis noxia</i>	XP_015368218
	MpLPMO15-1	<i>Myzus persicae</i>	XP_022170584
	NilLPMO15-1	<i>Nilaparvata lugens</i>	XP_039292167
	RmLPMO15-1	<i>Rhopalosiphum maidis</i>	XP_026816188
Isoptera	CsLPMO15-1	<i>Cryptotermes secundus</i>	XP_023723964
	ZnLPMO15-1	<i>Zootermopsis nevadensis</i>	XP_021922611
Zygentoma	TdLPMO15-1	<i>Thermobia domestica</i>	GASN01008505

Supplementary Table 4. Primers used for real-time qPCR analysis.

Gene	Genbank Accession #	Direction*	Sequence (5'-3')	Product size (bp)
<i>TcLPMO15-1</i>	MZ636451	F	GACCCACATCACGTCAAAGA	91
		R	CTTGCCGACGCTGTAAT	
<i>TcLPMO15-2</i>	XM_015980870	F	GGCGTTCCTCTTCGTCTTT	105
		R	TGAGGCGTATCGAAACCATATC	
<i>TcLPMO15-3s</i>	XM_015981790	F	ACGGAAGAAGAGGACATGATAC	95
		R	GCAGTGTAGGTCCATCTCAAA	
	XM_015981792	F	CAACGTCTGGAAGACAGACTAC	99
		R	CCAGTTCCGTAATCCACCTAA	
	XM_015981791	F	GAAGCTTGCAAACGGTGATAAA	119
		R	GATAATGCCAACGCAAGACAC	
	XM_963550	F	GCTTCCAGATGGGTTGACTTG	98
		R	CTTCTTGGTAACTCCCGTCTTC	
	XM_963476	F	AAATGGCGAGCCGAGATAC	100
		R	GACACATCTGTCGCACTTCA	
	XM_008201743	F	GTGCTCATTGTGTGTGAGATG	94
		R	TCTTGAGACCCACATCCTTTG	
	XM_970659	F	TTGCTGATGGTTCCTCGAAATA	106
		R	CACCTCAAGACACACCTATCA	
<i>TcCHT5</i>	AY675073	F	AAATGGGACGAACACGGCAAAGTC	120
		R	CCCACCGTAGCCCTTTGATTTGAT	
<i>TcCHT10</i>	DQ659250	F	ATTGGTCAAGGCGTTGAACTGGG	104
		R	AAGTCTTCAAGAGCGGGTGTTTGC	
<i>TcNAG1</i>	EF592536	F	CCGATAGTGCAGTGTTGAT	98
		R	CTCGGCTTCTCTCCATGTATTC	
<i>TcCHS-A</i>	AY291475	F	GGTGGTCGATCCGGATTATTACGA	97
		R	GTTGACTTGCGTTTCGTGCGTCGTT	
<i>TcCHS-B</i>	AY291477	F	TGCAGTGGTTTCGAAAGGGACAAC	154
		R	AATGAGGAGCCAGATCCATTGCAC	
<i>TcRpS6</i>	NM_001172390	F	ACGCAAGTCAGTTAGAGGGTGCAT	81
		R	TCCTGTTGCGCTTTACGCACGATA	
<i>LmLPMO15-1</i>	MZ440879	F	CTACTTCATCCCGCCAGGTA	80
		R	GGGTGCACGTCACGAAAGG	
<i>LmLPMO15-2</i>	GEZB01012450	F	ACACTTGTTGCGGTCTGTGC	116
		R	CGTGTCTCGGAGGTGGAAC	
<i>LmLPMO15-3</i>	OK181108	F	TTCCGCACCTGCTCAGACAT	90
		R	CACGTTGTAGTCCGCGTTGG	
<i>LmChtl</i>	KM397371	F	CATCAAAGCGAAGGGCTACGGC	92
		R	AGATTAGTGCGTCCTTCGGGCCA	
<i>LmChtlI</i>	KU757433	F	CTTTCAAACCACGAGGCTTACTC	158
		R	ATGACCAGTCTGCTTATCCCAAT	
<i>LmNAG1</i>	JX888720	F	CCCTCGCAGAACGTTTGTG	133
		R	TGTAGACACCACTCAGGCTCGAT	
<i>LmCHS-A</i>	GU067730	F	GCACTGTTGTTCTCATCGTATTC	121
		R	ACCAAACCAATTGGCTCAAG	
<i>LmCHS-B</i>	JQ901491	F	CAGCCTTCCGCATAGACAACT	150
		R	CGGCCATCATAACCAATGAAT	
<i>LmRp49</i>	GETS01057474	F	CGTAAACCGAAGGGAATTGA	115
		R	GAAGAACTGCATGGGCAAT	

*F, forward; R, reverse.

TcLPMO15-1

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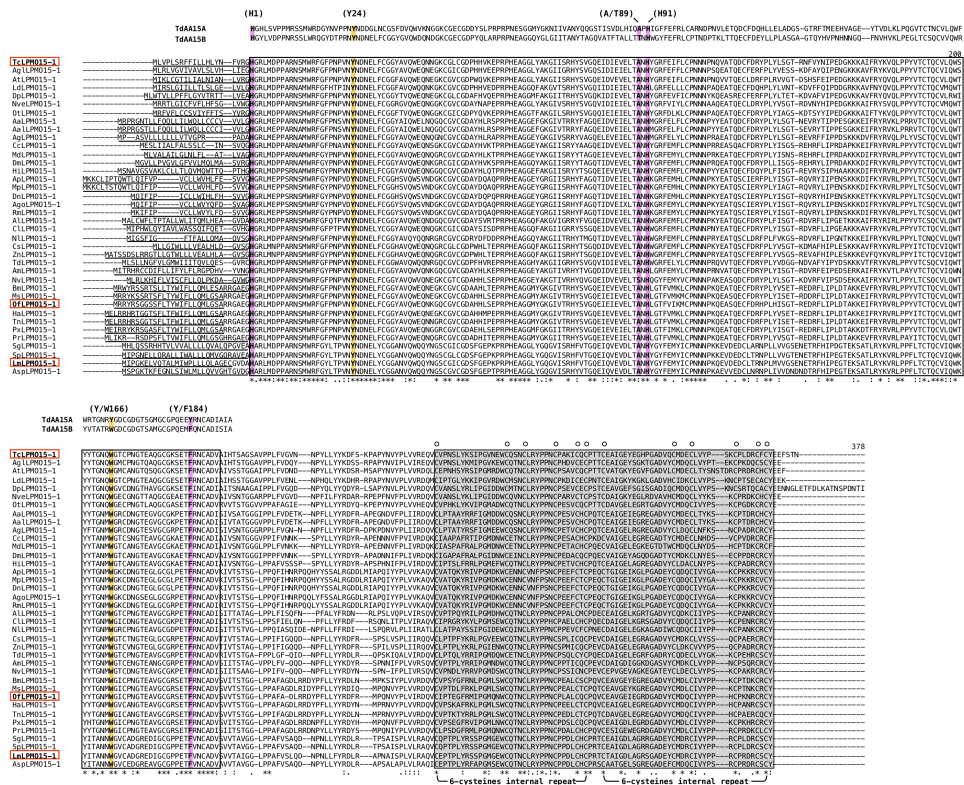
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241 N P N L L F Y R D L R A P N A I S P L V 260
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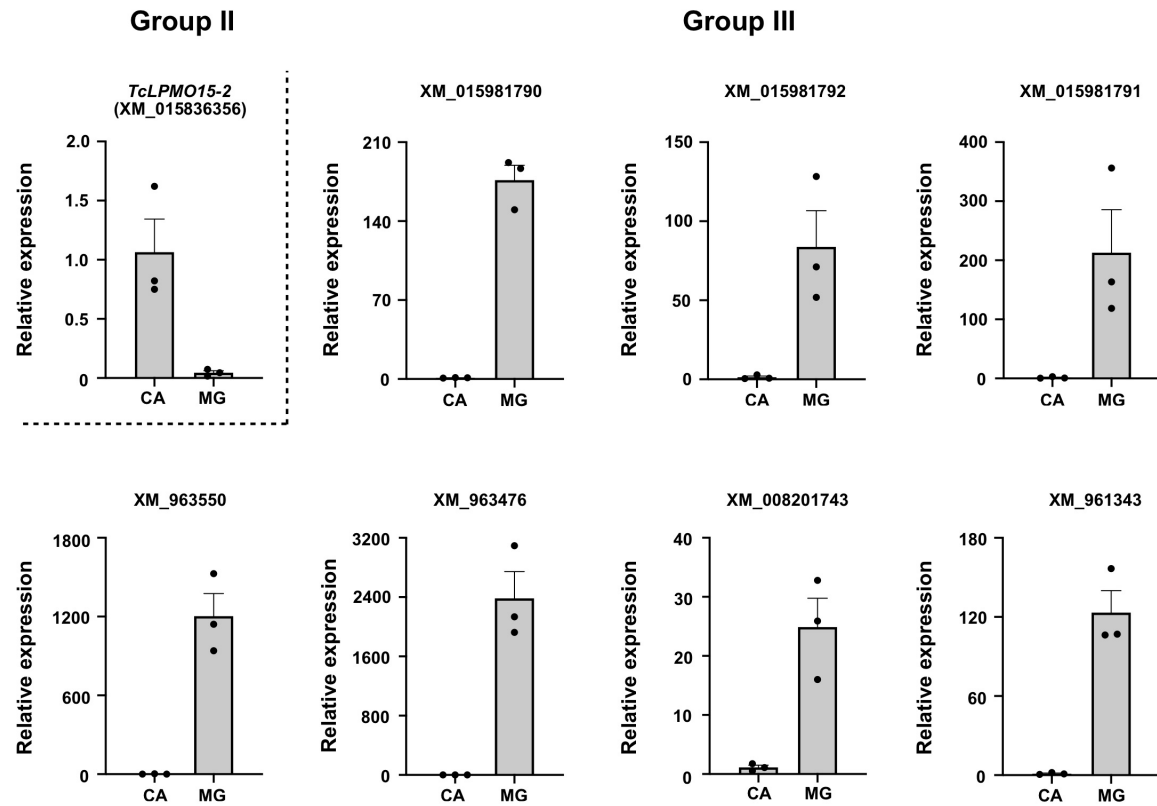
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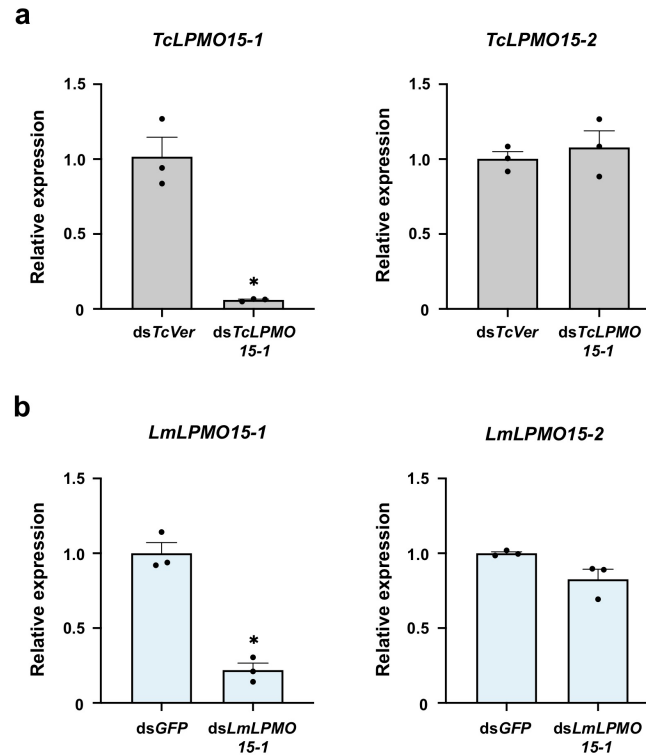
Supplementary Fig. 1. Nucleotide and deduced amino acid sequences of *TcLPMO15-1*, *LmLPMO15-1* and *OaLPMO15-1*. Predicted secretion signal peptide sequences and the putative LPMO catalytic domains are bolded and boxed, respectively. The conserved cysteine-rich motifs identified at the C-terminus of insect LPMO15-1 sequences is indicated by gray highlight.



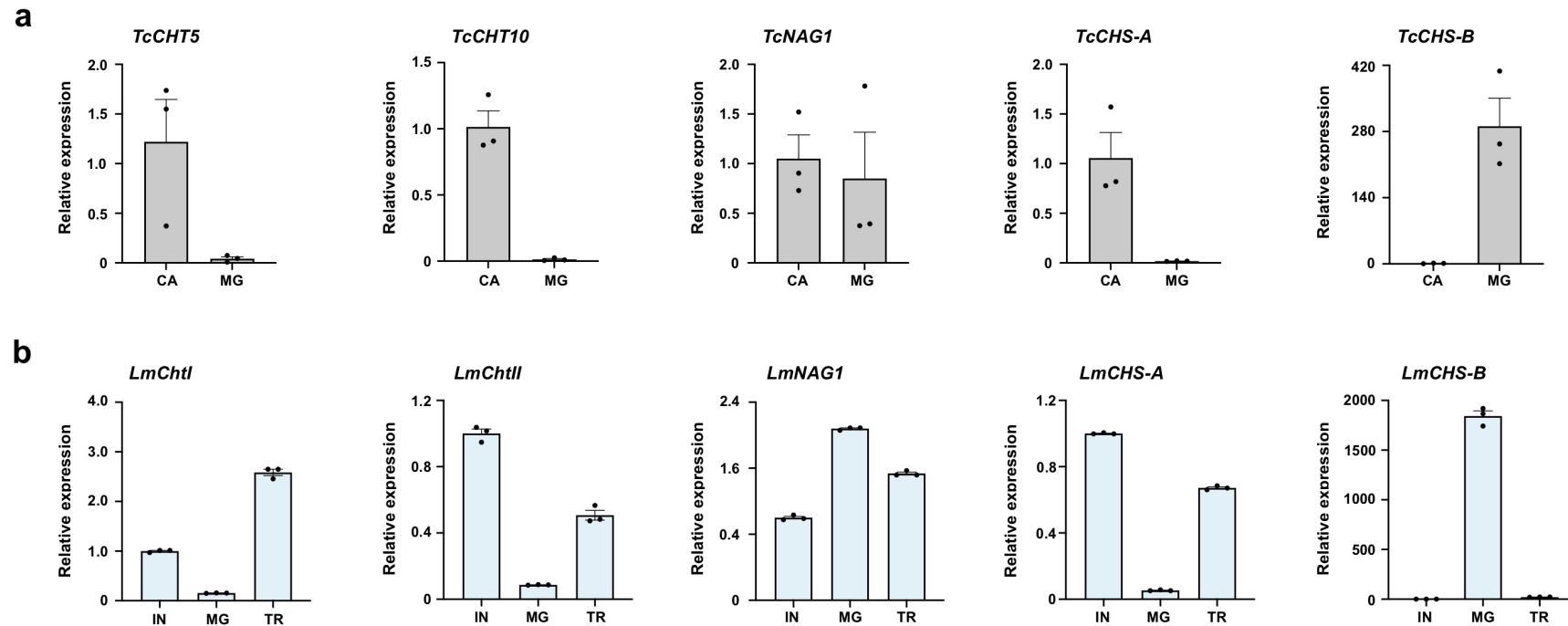
Supplementary Fig. 2. Amino acid sequence alignments of insect LPMO15-1s. Multiple protein sequence alignment of LPMO15-1s from several lepidopteran, coleopteran, hymenopteran, dipteran, hemipteran and orthopteran species was made using ClustalW software. Symbols located under the alignment indicate identical (*), highly conserved (:), and conserved residues (.). Amino acid sequences of the catalytic domains of the *TdAA15A* and *TdAA15B* are also shown above the alignment. Predicted signal peptides and the putative LPMO15 catalytic domains are underlined and boxed, respectively. The four conserved amino acids (corresponding to H1, H91, A/T89 and Y/F184 in *TdAA15A* and *TdAA15B*) involved in the copper binding and the two corresponding to Y24 and Y/W166 in *TdAA15A* and *TdAA15B*) involved in the substrate binding are highlighted in magenta and yellow, respectively. Gray highlight indicates C-terminal stretches consisting of two “6-cysteines-containing internal repeats (C-X₁₅-C-X₃-C-X₆₋₉-C-X₄-C-X₁-C)” where X is any amino acid residue. The 6-cysteines in each repeat are indicated by open circles.



Supplementary Fig. 3. Tissue-specific expression of group II and group III LPMOs in *T. castaneum* by RT-qPCR. Transcript abundance of *TcLPMO15-2* (group II) and seven *TcLPMO15-3* (group III) genes in the carcass (CA) and midgut (MG) was determined by real-time qPCR. Total RNA was extracted from the tissues of *T. castaneum* larvae (n = 10). Expression levels of *TcLPMO15s* are presented relative to the levels of expression in carcass (CA). Data are shown as the mean values ± SE (n = 3)



Supplementary Fig. 4. Knockdown levels of transcripts of *TcLPMO15-1* and *LmLPMO15-1* genes by RT-qPCR. **a** cDNAs were prepared from total RNA isolated from three pooled day 0 pupae that had been injected with *dsTcLPMO15-1* at the last instar larval stage. Transcript levels of *TcLPMO15-1* and *TcLPMO15-2* in *dsTcLPMO15-1*-treated insects were presented relative to the levels in *dsTcVer*-treated controls. An asterisk indicates a significant difference in transcript levels of *TcLPMO15-1* ($p = 1.83\text{E-}03$, t -test) between samples. Data are shown as the mean value $\pm$ SE ($n = 3$). **b** cDNAs were prepared from total RNA isolated from three pooled integument of 5th instar day 5 nymphs 2 d after the second *dsLmLPMO15-1* injection. Expression levels of *LmLPMO15-1* and *LmLPMO15-2* in *dsLmLPMO15-1*-treated insects were presented relative to the levels in *dsGFP*-treated controls. An asterisk indicates a significant difference in transcript levels of *LmLPMO15-1* ($p = 7.93\text{E-}04$, t -test) between samples. Data are shown as the mean values $\pm$ SE ($n = 3$).



Supplementary Fig. 5 Tissue-specific expression of chitinases and chitin synthases in *T. castaneum* and *L. migratoria* by RT-qPCR. **a** Transcript abundance of *TcCHT5* (group I chitinase, AY675073), *TcCHT10* (group II chitinase, DQ659250), *TcNAG1* (β -N-acetylhexosaminidase, EF592536), *TcCHS-A* (chitin synthase-A, AY291475) and *TcCHS-B* (chitin synthase-B, AY291477) in the carcass (CA) and midgut (MG) was determined by real-time qPCR. Total RNA was extracted from the tissues of *T. castaneum* larvae (n = 10). Expression levels of each gene are presented relative to the levels of expression in carcass (CA). **b** To analyze spatial expression patterns of *LmCHTl* (group I chitinase, KM397371), *LmCHTII* (group II chitinase, KU757433), *LmNAG1* (JX888720), *LmCHS-A* (GU067730) and *LmCHS-B* (JQ901491), total RNA was extracted from the integument (IN), midgut (MG) and trachea (TR) from three *L. migratoria* 5th instar day 3 nymphs. Expression levels of each gene are presented relative to the levels of expression in integument (IN). All data are shown as the mean value $\pm$ SE (n = 3). See Supplementary Table 4 for the primer sequences used in this study.