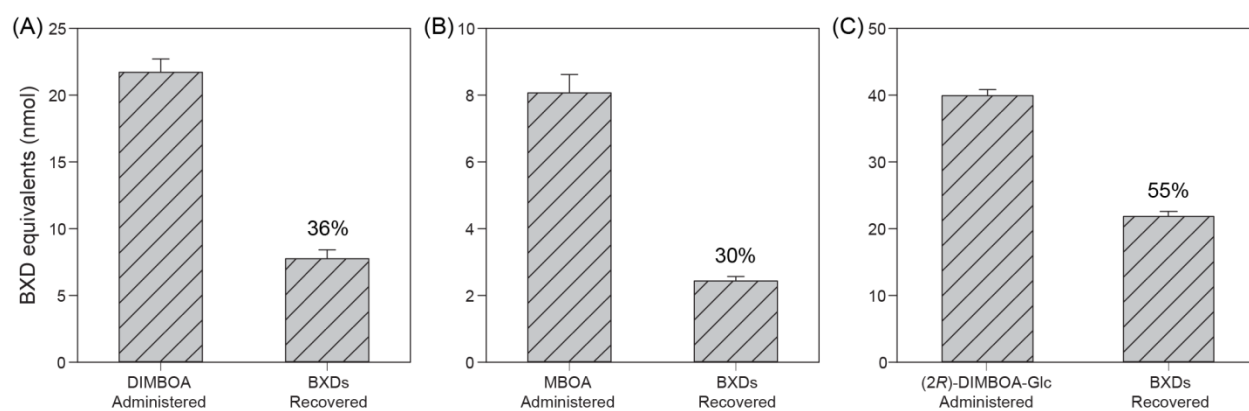


## SUPPLEMENTARY INFORMATION

### **The fall armyworm *Spodoptera frugiperda* utilizes specific UDP-glycosyltransferases to inactivate maize defensive benzoxazinoids**

Bhawana Israni, Felipe C. Wouters, Katrin Luck, Elena Seibel, Seung-Joon Ahn, Christian Paetz, Maximilian Reinert, Heiko Vogel, Matthias Erb, David G. Heckel, Jonathan Gershenzon, Daniel Giddings Vassão



**Supplementary figure 1. Recovery of BXD metabolites in *S. frugiperda* frass.** Overall rates of recovery calculated after oral administration of DIMBOA, MBOA and (2R)-DIMBOA-Glc (n=5-6, mean  $\pm$  SEM).

signal peptide cleavage site

SIUGT33F28 1 MKI L C I T L L L S F ANYD E AARI LAVFPNPSI SHQVFRPTQELARRGHEVTITDPVFPEG-KAPANLIEIDVH--DI SY--  
SIUGT33B25 1 MSTAL S L L L L S VSI MYS D AARI LAMFPVPSI SHQVFRPYTQELARRGHVTVITPDPAFPKG-GTPANLIEIDVH--DFS Y--  
SIUGT42C10 1 MKFNNI L I L F L S A L F S Q V L S L N I L G V F P Y Q G K S H F F E A P Y K E L A R R G H N I T V I S Y E P L K E P I D N Y H D I S L A G K T K --  
SIUGT40R13 1 NALAI F L F L G L L L S S C S A Y A L V F G M P S T S H F I L G N G V R N L R G C H E V T I T P L E Y K N P P N L R Q I N V N S N F D --  
SIUGT46A15 1 MRTL A L L L V L A F A A N V Q S A R I L G F P H T C K S H Q M T D P L L K T L A E R G H V T V V S F L P L K N P P A N Y I I V S E L G L A A --  
SIUGT40L8 1 MKHKV L T S I C H L L S L L I S S E A L R I L V C P M T S K S H S L G Y G V N R L E A C H E V H I T S P N G K V Q N L I E I D V S S I A D --  
SIUGT47A12 1 MRPV L L W L L L L A W A A S A A A R L A V L P T N T K S H A Y A G R L L D A L A R D H L T I V S H P M K N P R P N V H Q I S L A G T I P --  
SIUGT40F19 1 MEKL I C F L F C A L V S L C S C A Y K I L A V F P V P S P S H G I G D N M K H L L N A C H E V T I T P N A S C K S N P K L H I V D V T D H H S --  
SIUGT33V6 1 N S L V K L T V A S L A S I P T S D G A K I L G F P F P S I S H Q V F R P L M Q E L A R R G H E V T I T P D P A F P K G G T P A N L I E I D V H D A S Y --  
HaUGT47A2 1 MVI T S L L L W A V A A R A A R L A V L P T N T K S H A Y A G R L L E A L A R R D H L T V T V H P M K N P P P N V Q Q I S L A G T I P --  
HsUGT2B7 1 M S V K W S V I L L L Q S F C S S G N C G K V L V W A A E Y S H W N I K T L D E L I C R G H E V T I A S S A S I L F D P N S S A L K E I Y P T S L T K

cleavage site

SIUGT33F28 81 DW R L K I F L A S T K G N K D D V V Q A M T A V T A L T A V D V Q L K O E K V L N L I R D K S K F D L L L V E A C V  
SIUGT33B25 80 E M W Q S F Y K V T S T G E K D L N Q Q M T T A F T I I V D I V E M Q L K D A V Q T L K E E K F D L L L V E A C V  
SIUGT42C10 77 I L E D V F P I Y R S Y W T I I Q I S F L T N S G Q E N C I T L E D E N V Q N L W K T K Q F D L V L V E L F N  
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SIUGT33V6 83 R V H E Q F V G T P K G H K G N F V D F N I F N N K I V D V E L K O V E V Q L L N D K N Q T F D L I F A B A M M  
HaUGT47A2 72 E I T N N T V Q H D S I K P D F I N E Q M K E C D A C S T A A K V P A V K A L F N S T E T F D L V I V E V F G  
HsUGT2B7 84 T E L E N F I M Q Q I K R W S D L P D T F W L Y F S Q Q E M S I F G D I T R K F C K D V V S N K F M K V Q E S F D V I F A I A I F

catalytic residues

SIUGT33F28 143 RP A L V F S H I Y K V P I Q S S F M A T F D N Y A N I G A P I H F F L Y A I T R O R I Q N I T M E K I T E F N D F M L N R I Y D S M M E K E N  
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SIUGT42C10 135 S D C A L A F Q L G A P V I G L T S H L M P W H Y E D Y G I Q Y N P S V E M F L E G G T K P T L Y C R I E R S I L H I Y F I Y H A L T C O R R N N  
SIUGT40R13 139 S E L A S Y A I Y D O P E I W S P V E V N A Q S V G L Y D V F P N P A I T T D A A S Y T P P P T E L E R E E I V T R S D N Y N D Y M Y E P T E E K Y Q R L I V  
SIUGT46A15 146 S D C M L M I Y Y L K A P Y S S S S A I M W G S D R G V T D N P S V E M F L S E F T S Q I T F E L O R E N T L M N Y Y K I W F Y A I Q M K E K  
SIUGT40L8 144 N E M N A G P A L E N C P L I W C S T E H W Q S M R V D G I T N E A Y T L D I F T H N K P L N F V C R A E G I K V A K K A A Q V L I L N Q E F R A Y S I Y P  
SIUGT47A12 136 S D C F L P L G K Y V G A P V I G L S S V P L P W L N E Q G N P E A T A V E S Y V G Y G Q R I T M E R F A N T M A V I A K M I Y Y K S Q I P S  
SIUGT40F19 141 L D L Y S T F P A V E Q Q Y W F S T I V E H M M M N L H S P M F A N G D Y Y A N I P P F N F M R V Q E I V L Q T G L Y H H N D Y Y O R E A V Y L K H V  
SIUGT33V6 145 RP A L V L S H I Y N A P V I L S S Y G T F S D N Y A V M A P I H F F L Y F S S R R L H S T S L W D K G H L Y D Y R I E I Q N S Y V E E N  
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N-terminal domain Interdomain linker C-terminal domain

SIUGT33F28 220 E M I R S H F K D I P P S E L N N V D L F L N V N P M F E S I R P V P P S V I Y G G L H Q V P H K P L P K D L Q T Y M D S S K N G V I Y V S F G I N D P T K I P A D  
SIUGT33B25 216 E A K L F G P D P T I S E L K N N V H L F L N A H P I W E G N R P V P P S V I Y G G L H Q K P Q E L P A D L K Q L D S S K N G V I Y S F G I N Q P S L I P D  
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HaUGT47A2 211 A S D R L F G P K P I E S L A E Y S L V L S N S H F S I N E V R P L P A L V E G G L H L D S P V L P R Q Q L D A S T E G V I Y S F G S I S R I E T I P S  
HsUGT2B7 234 Q F Y S E V L G R P T I T S E T M G K A D M L T R N S W N F Q P Y P L P N D F V G G L H C K P A K P L P K E M E T Q S S G E N G V I S L G S M S N T E

UGT signature motif

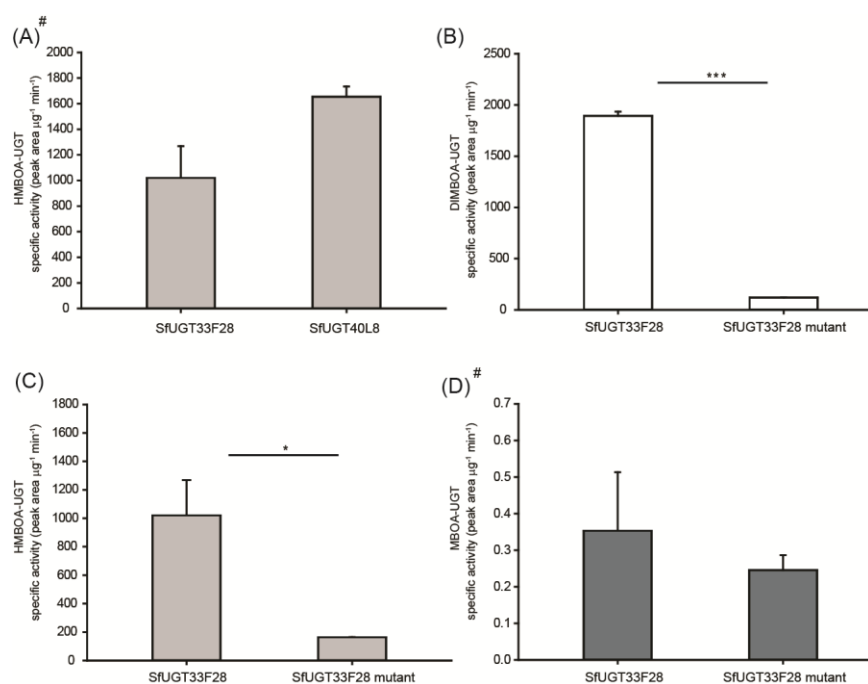
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SIUGT42C10 301 Y Q A I D A S F S Q L P V K V E E K N L P G N K N I Y S N V P O N E I L A H P K V L A F Y S H C G I L E T E A I H Y G V P M G M I F G D Q P N A A A  
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SIUGT46A15 316 K E E I I N A L S K I K O R V I W K Y E D S D E E G T I T G N I L K V K V P O Y E L L O H E K I A F I A H G G L I G M T E A I S A G K P M I V P F I G D Q W N E A A A  
SIUGT40L8 317 M K S I L N I F S K Y K O T V I W K F E S D M K D N V P A N V H L K V A P O S I L A H P S L K L F I T H G G L S T S E A I H Y G I P L V G I P V M A D Q V N M I S  
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SIUGT33V6 301 K I A M F R A F S R L P Y D L V K V I N K D E L P G R T D N I K I S K V L P O S O L L I H P K I K A F I T G G L Q S T D E A I T A R V P L I G I P M F G D Q W N V V K Y  
HaUGT47A2 298 T A Q I F N V L S E L P C I V F I K W D R M A R N L T V P D N A Y T D W I P O H A T L C H P N K L F I S H G G L I G T O E A A C G V P M L M V P L Y A D C A N A R A  
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negatively charged aa (D or E)

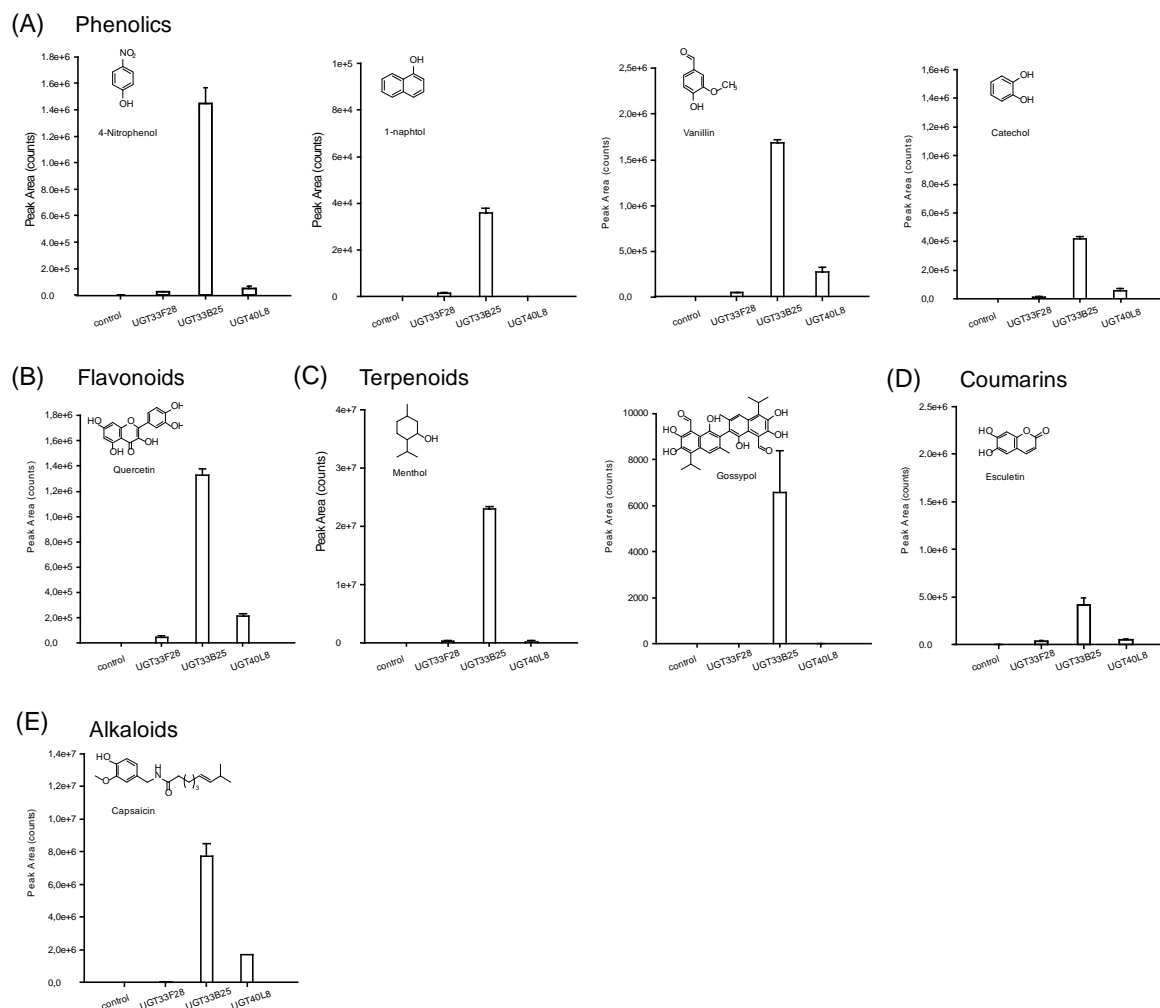
transmembrane domain cytoplasmic tail

SIUGT33F28 483 T L L G L A T I S V L F V L R S I Y K M F G S T V T G T V T S K P K K A R S  
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SIUGT42C10 475 V I L A I A I F F Y S L K T V I S L V R R R E D R Y Y E R K T K  
SIUGT40R13 489 L L L V F L A K L L L R N Y R R P K I V N S K K D K K N  
SIUGT46A15 492 V L I S F F L L L I R I L N Q I L R L F G A S T N K K E K L H  
SIUGT40L8 493 F I A V L I V L K A V K Y L M K R N A T N K K E K S H  
SIUGT47A12 481 L T A V A L T I Y I L K L F R Y L C T C R W P K E K L V F E K R L L R K N I S F F L C I L W K Y K A P N  
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SIUGT33V6 486 T L T A I I T T A T I L L A Y I Y D Q L R Y I A I I K I K R A  
HaUGT47A2 477 L T A I A L T I F I L K L F R Y L C T C K W P K E K L V F E K R F K R N I S F F L C I L W K Y K R T N  
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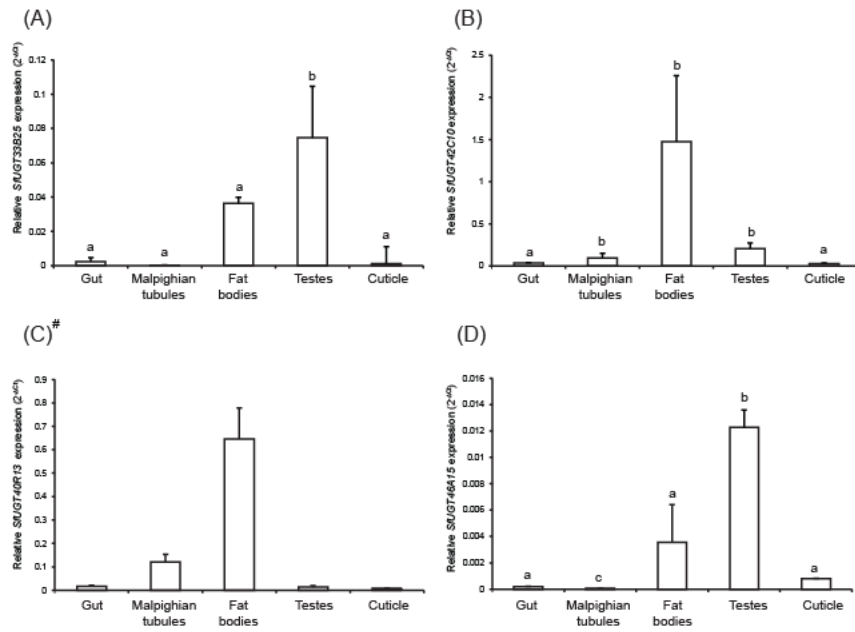
**Supplementary figure 2. Multiple sequence alignment of functionally characterized *S. frugiperda* UGTs active towards benzoxazinoids.** The primary protein structures of SfUGTs with potential function in benzoxazinoid detoxification are compared to the human UGT2B7 and the insect UGT47A2 from *H. armigera*. The predicted signal peptide, UGT signature motif, transmembrane domain and cytoplasmic tail are shown as bars on top of the alignment. The catalytic residues, putative sheets as well as regions (donor binding regions/ DBR1 and 2, yellow box) and residues (\*) responsible for sugar-donor binding in the C-terminal domain were predicted based on the crystal structure and mutational analysis reported for UGT2B7.



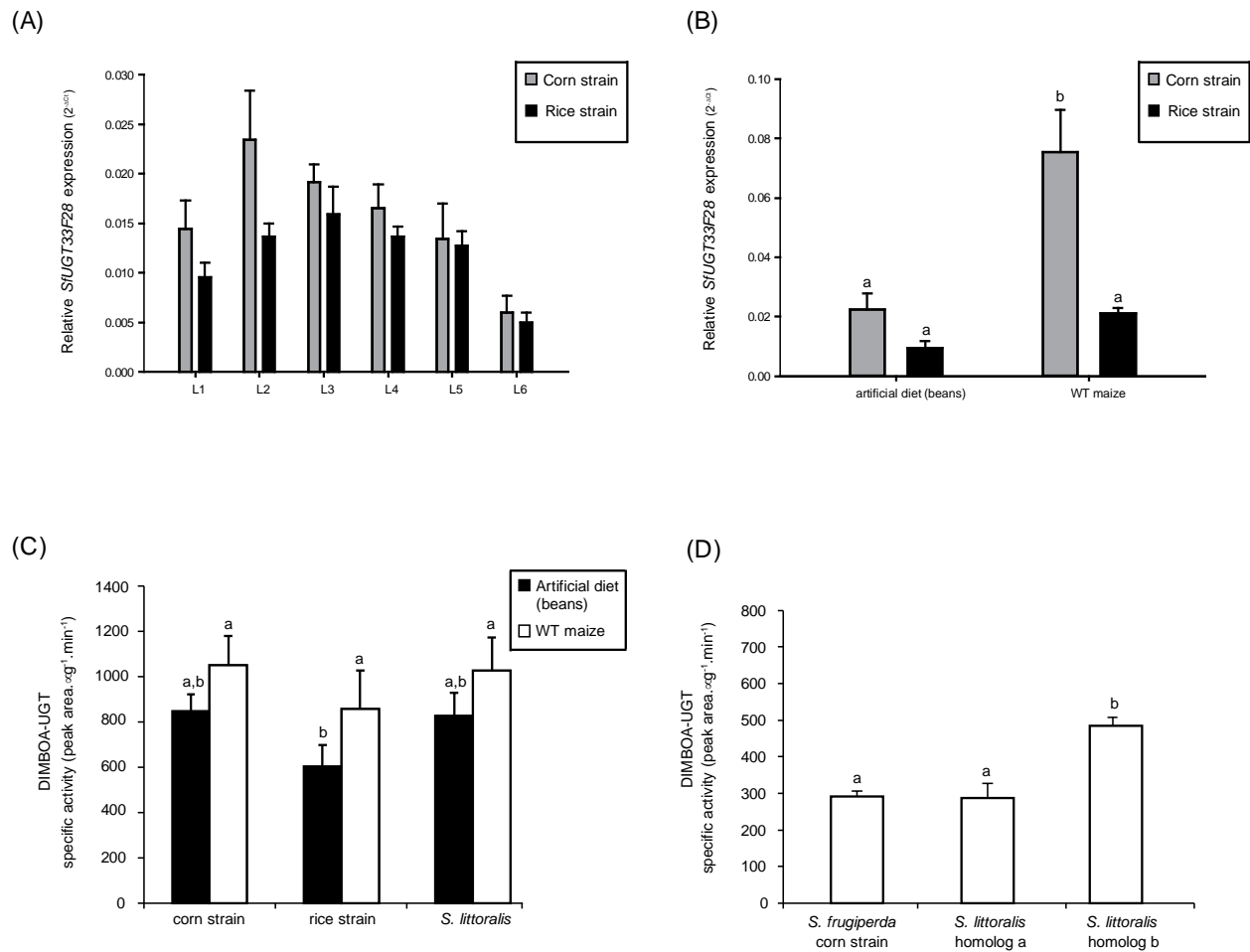
**Supplementary figure 3. Activity profiles of SfUGT33F28 and SfUGT40L8.** (A) SfUGT33F28 and SfUGT40L8 enzyme activities towards HMBOA, (B)-(D) SfUGT33F28 and SfUGT33F28-H34L site-directed mutant enzyme activities towards DIMBOA, HMBOA and MBOA respectively. Data are presented as mean  $\pm$  SEM (n=3). T-tests were performed to differentiate between the means (\* $P < 0.05$ , \*\*\* $P < 0.001$  according to the t-test used in the present study). #- no significant difference between the means.



**Supplementary figure 4. Comparison of glucosylation activities of SfUGT33F28, SfUGT33B25 and SfUGT40L8 towards different classes of UGT substrates.** The three DIMBOA-active UGT enzymes were additionally tested for activities towards a small panel of structurally diverse aglucone substrates classified generally as (A) phenolics, (B) flavonoid, (C) terpenoids, (D) coumarin and (E) alkaloid. Activities are expressed as raw peak areas from extracted LC-MS chromatograms.



**Supplementary figure 5. Relative expression profile of genes encoding MBOA glycosylating enzymes across various *S. frugiperda* tissues.** Relative transcript abundances of *SfUGT33B25* (A), *SfUGT42C10* (B), *SfUGT40R13* (C) and *SfUGT46A15* (D) were measured across different tissues of *S. frugiperda* by quantitative PCR (n=3, mean ± SEM). One way repeated measures ANOVA was performed and Holm-Sidak method was applied to carry out all pairwise comparisons. The differences between individual means are denoted as small letters (a-c). #- One way ANOVA shows no significant difference in expression levels among the tissue samples.

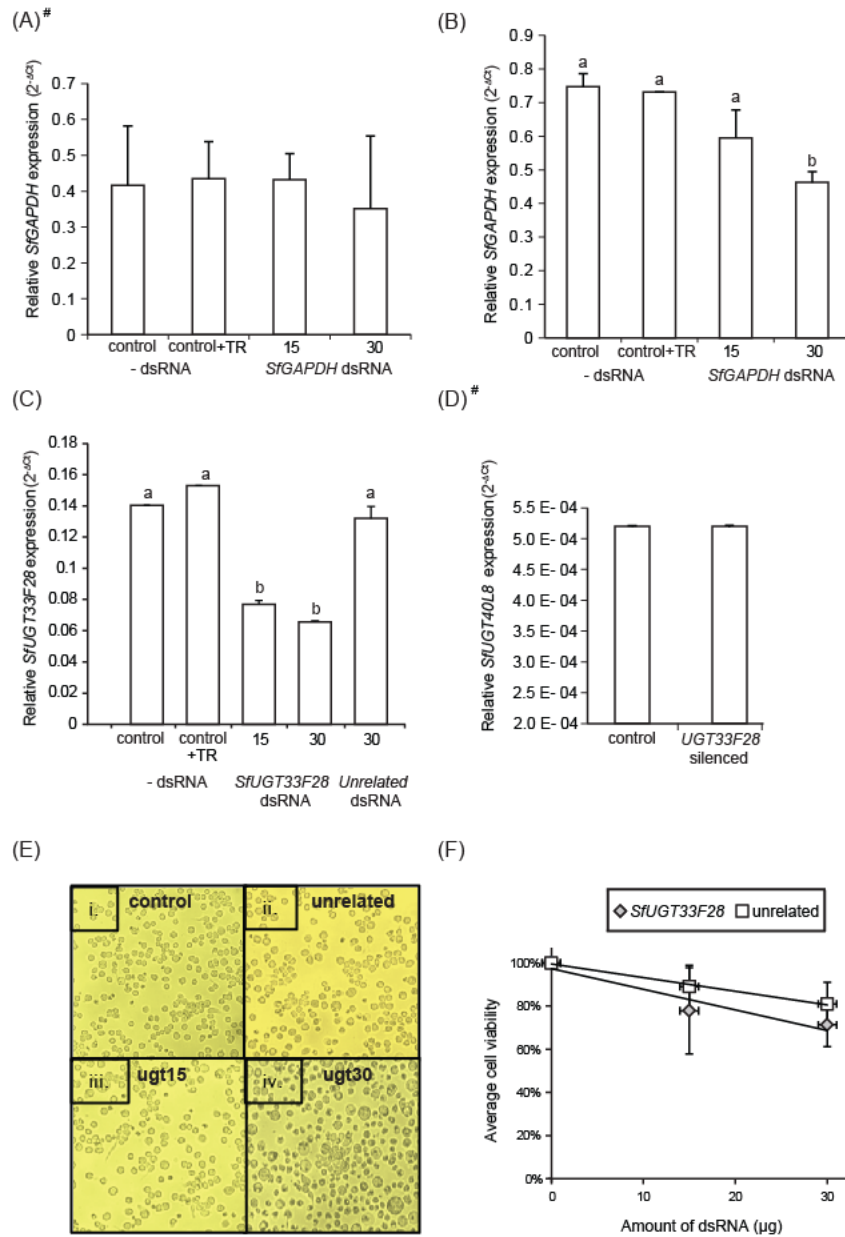


**Supplementary figure 6. Expression profile of *SfUGT33F28* and comparative account of UGT33F28 mediated glycosylation activity in two *Spodoptera* species.** (A), Relative expression levels of *SfUGT33F28* across larval stages of corn and rice strain FAW caterpillars maintained on bean-based artificial diet, (B) Relative expression levels of *SfUGT33F28* during the L2 larval stage in *S. frugiperda* corn and rice strain caterpillars fed on artificial diet and maize, (C) *in vitro* enzyme activity assays towards DIMBOA from midgut extracts prepared from *S. frugiperda* corn strain, rice strain and *S. littoralis* caterpillars fed on artificial diet and corn leaves, (D) specific activity measured towards DIMBOA from crude microsomal extracts prepared from *T. ni* insect cell cultures heterologously producing *S. frugiperda* corn strain UGT33F28 and *S. littoralis* UGT33F28 homologs (SIUGT33F28 a and b). Data are presented as mean  $\pm$  SEM (n=3). One way ANOVA was performed and Holm-Sidak method was applied to carry out all pairwise comparisons. The differences between individual means are denoted as small letters (a-b).

SIUGT33F28a	1	NEIFIIYLICLSIFASHDEAARI LAVFPNPSI SHQVFRPLTQELAKRGHEVTI TTDPV
SIUGT33F28b	1	NEIFIIYLICLSIFASHDEAARI LAVFPNPSI SHQVFRPLTQELAKRGHEVTI TTDPV
SIUGT33F28 corn strain	1	MKILICITLLLSIFANYDEAARI LAVFPNPSI SHQVFRPLTQELARRGHEVTI TTDPV
SIUGT33F28 rice strain	1	MKILICITLLLSIFANHDEAARI LAVFPNPSI SHQVFRPLTQELARRGHEVTI TTDPV
*****		
SIUGT33F28a	61	FPEGKAPANLTEI DVHDI SYDI WKI FLASTKGNKDDVFAMSAI VNALTAVVDVQLKDE
SIUGT33F28b	61	FPEGKAPANLTEI DVHDI SYDI WKI FLASTKGNKDDVFAMSAI VNALTAVVDVQLKDE
SIUGT33F28 corn strain	61	FPEGKAPANLTEI DVHDI SYDI WKI FLASTKGNKDDVVCAMTAI VTALTAVVDVQLKDE
SIUGT33F28 rice strain	61	FPEGKAPANLTEI DVHDI SYDI WKI FLASTKGNKDDVVLAMTAI VTALTAVVDVQLKDE
*****		
SIUGT33F28a	121	RVQNLI RDKSKQFDLLLEACVRPALVFSHI YKIPVI QISSFMATFDNYANI GAPI HPFL
SIUGT33F28b	121	RVQNLI RDKSKQFDLLLEACVRPALVFSHI YKIPVI QISSFMATFDNYANI GAPI HPFL
SIUGT33F28 corn strain	121	KVLNLI RDKSKQFDLLLEACVRPALVFSHI YKIPVI QISSFMATFDNYANI GAPI HPFL
SIUGT33F28 rice strain	121	KVLNLI RDKSKQFDLLLEACVRPALVFSHI YKIPVI QISSFMATFDNYANI GAPI HPFL
*****		
SIUGT33F28a	181	YPALTRQRQLNLTMI EKI KEFYNDFMLNRLYANNMEKENEMLRSHFKDI PPI SELNNNV
SIUGT33F28b	181	YPALTRQRQLNLTMI EKI KEFYNDFMLNRLYANNMEKENEMLRSHFKDI PPI SELNNNV
SIUGT33F28 corn strain	181	YPALTRQRQLNLTMI EKI TEFYNDFMLNRLYDSNMEKENEMLRSHFKDI PPI SELNNNV
SIUGT33F28 rice strain	181	YPALTRQRQLNLTMI EKI TEFYNDFMLNRLYDSNMEKENEMLRSHFKDI PPI SELNNNV
*****		
SIUGT33F28a	241	MLFLNVNPMFEGI RPVPPSVVYLGGLHQVPHKPLPKDLQTYMSSKNGVI YVSFGTNVDP
SIUGT33F28b	241	MLFLNVNPMFEGI RPVPPSVVYLGGLHQVPHKPLPKDLQTYMSSKNGVI YVSFGTNVDP
SIUGT33F28 corn strain	241	MLFLNVNPMFEGI RPVPPSVVYLGGLHQVPHKPLPKDLQTYMSSKNGVI YVSFGTNVDP
SIUGT33F28 rice strain	241	MLFLNVNPMFEGI RPVPPSVVYLGGLHQVPHKPLPKDLQTYMSSKNGVI YVSFGTNVDP
*****		
SIUGT33F28a	301	TKLPADRI EVLVKTLSQLPYDI LKWNGDVLPGRTANI RAKWLPOPDLLI HPKCLKFI T
SIUGT33F28b	301	TKLPADRI EVLVKTLSQLPYDI LKWNGDVLPGRTDNI RAKWLPOPDLLI HPKCLKFI T
SIUGT33F28 corn strain	301	TKLPADRI EVLVKTLSQLPYDI LKWNGDVLPGTPTNI KAKWLPOPDLLI HPKCLKFI T
SIUGT33F28 rice strain	301	TKLPADRI EVLVKTLSQLPYDI LKWNGDVLPGTPTNI KAKWLPOPDLLI HPKCLKFI T
*****		
SIUGT33F28a	361	QAGLQSTDEAI TAGVPLVAI PMFGDCSFNAERYENFKI GKRLSMDSLTVGEFTNAI NTVI
SIUGT33F28b	361	QAGLQSTDEAI TAGVPLVAI PMFGDCSFNAERYENFKI GKRLSMDSLTVGEFTNAI NTVI
SIUGT33F28 corn strain	361	QAGLQSTDEAI TAGVPLVAI PMFGDCSFNAERYEYFKI GKRLSMEKLTVEFTNAI NTVI
SIUGT33F28 rice strain	361	QAGLQSTDEAI TAGVPLVAI PMFGDCSFNAERYEYFKI GKRLSMEKLTVEFTNAI NTVI
*****		
SIUGT33F28a	421	NDESYRENI VKLRTL I QDEPMSPLERAVVWTEHVL RHGGARHL RGPAAANMSWAEYLELEL
SIUGT33F28b	421	NDESYRENI VKLRTL I QDEPMSPLERAVVWTEHVL RHGGARHL RGPAAANMSWAEYLELEL
SIUGT33F28 corn strain	421	NDKSYRENI VKLRTL I QDEPMSPLERAVVWTEHVL RHGGARHL RGPAAANMSWAEYLELEL
SIUGT33F28 rice strain	421	NDKSYRENI VKLRTL I QDEPMSPLERAVVWTEHVL RHGGARHL RGPAAANMSWAEYLELEL
*****		
SIUGT33F28a	481	VFTLLLGGLAVI SVLFV I LRSLYKI I FGSTV----TSKPKKAKRS
SIUGT33F28b	481	VFTLLLGGLAVI SVLFV I LRSLYKI I FGSTV----TSKPKKAKRS
SIUGT33F28 corn strain	481	VFTLLLGGLAVI SVLFV I LRSLYKIMFGSTVTSVTSTKPKKAKRS
SIUGT33F28 rice strain	481	VFTLLLGGLAVI SVLFV I LRSLYKIMFGSTVTSVTSTKPKKAKRS
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**Supplementary figure 7. Protein sequence alignment of SfUGT33F28 from *S. frugiperda* host differentiated strains with UGT33F28 homolog from *S. littoralis* using Clustal omega.**





**Supplementary figure 8. Optimization of RNAi-mediated knock-down of *SfUGT33F28* in cultured Sf9 cells showing endogenous glycosylation activity.** (A-B) time course quantitative analysis of silencing on Sf9 cells treated with 15  $\mu$ g and 30  $\mu$ g of dsRNA against *SfGAPDH*, 24 and 48 hours post transfection (TR: controls treated with transfection reagent), (C) quantitative analysis of *SfUGT33F28* silencing on Sf9 cells transfected with 15  $\mu$ g and 30  $\mu$ g dsRNA specific to *UGT33F28* and an unrelated dsRNA control (TR: controls treated with transfection reagent), 48 hours post transfection, (D) quantitative *SfUGT40L8* expression analysis in control and ds*UGT33F28* transfected Sf9 cells to rule out conditional functional compensation under lack of *SfUGT33F28* gene product, (E) cell morphology of Sf9 control and ds*UGT33F28* treated cells, (F) MTT cytotoxicity assay for determination of cell viability in control and dsRNA transfected Sf9 cells. Data are presented as mean  $\pm$  SEM (n= 3-5 for RNAi treatments in Sf9 cells). One way

ANOVA was performed and Tukey's test was applied for all pairwise comparisons (A, B, C). The differences between the individual means are denoted as small letters (a-b). T-tests were performed to differentiate between the means (D). #- no significant difference in expression levels among the samples.

**Supplementary table 1. *UGT* expression levels in Sf9 cells.** Illumina reads from the Sf9 transcriptome (Shu et al., 2017) were downloaded from GenBank SRA Archives SRR5892097 on November 6, 2019. CLC Genomics Workbench 12.0.4 was used for assembly of the 87 Mio paired-end (2 x 100 bp) reads, mapping, and TPM/RPKM expression calculations. For the assembly, the graph parameters were set for automatic word size, a bubble size of 150, mismatch cost of 2, and insertion and deletion cost of 3. Mappings were performed with default parameter settings except for the length fraction (0.8), the similarity fraction (0.92) and the maximum number of hits for a read (5). RPKM indicates Reads per Kilobase of transcript per Million mapped reads, a normalized index of transcript expression. n.d., not enough reads to assemble a contig for RPKM calculations. When this was the case, the number of reads was estimated by inspection of the output of NCBI blastn against the Sequence Read Archive SRR5892097. Three housekeeping genes with different expression levels are shown for comparison: *LDH* (lactate dehydrogenase), *eIF4A* (eukaryotic initiation factor 4A) and *RpL5* (ribosomal protein L5).

Name	Contig RPKM	Contig Reads
<i>SfUGT33B24</i>	4.1	1,181
<i>SfUGT33B25</i>	4.1	1,181
<i>SfUGT33F28</i>	125.5	115,108
<i>SfUGT33F29</i>	4.8	824
<i>SfUGT33F31</i>	0.8	226
<i>SfUGT33F34</i>	2.5	774
<i>SfUGT33J12</i>	3.3	1,075
<i>SfUGT33S2</i>	1.7	404
<i>SfUGT33T9</i>	1.0	146
<i>SfUGT33V6</i>	0.2	22
<i>SfUGT33V7</i>	25.5	23,693
<i>SfUGT39B21</i>	n.d	0
<i>SfUGT40D16</i>	2.6	854
<i>SfUGT40D17</i>	3.8	1,119
<i>SfUGT40F17</i>	n.d	11
<i>SfUGT40F19</i>	1.8	810
<i>SfUGT40L8</i>	n.d	13
<i>SfUGT40M10</i>	n.d	4
<i>SfUGT40M11</i>	n.d	0
<i>SfUGT40M8</i>	1.6	656
<i>SfUGT40Q8</i>	6.9	2,488
<i>SfUGT40R11</i>	8.2	2,946
<i>SfUGT40R12</i>	19.4	7,997

<i>SfUGT40R13</i>	30.2	11,726
<i>SfUGT41B7</i>	6.7	578
<i>SfUGT41D8</i>	219.4	80,133
<i>SfUGT42B13</i>	n.d	6
<i>SfUGT42C10</i>	n.d	1
<i>SfUGT44A18</i>	0.3	17
<i>SfUGT46A15</i>	0.4	34
<i>SfUGT46B5</i>	0.5	47
<i>SfUGT47A12</i>	3.6	375
<i>SfUGT48D4</i>	n.d	27
<i>SfUGT50A15</i>	n.d	16
<i>SfeIF4A</i>	28.2	15,061
<i>SfLDH</i>	228.2	110,961
<i>SfRpL5</i>	1605.4	342,697

**Supplementary table 2. Summary of SfUGT33F28 glycosylation activities observed across a spectrum of substrates.** ANOVA was employed based on the net amounts of glucoside produced by UGT33F28 after subtracting what was produced by the corresponding amount of non-transfected control (NTC). When the glucoside formation did not differ significantly from NTC, the enzyme was considered not active (-) towards the substrate. The activity was scored on a scale of 1 to 3, 1 corresponding to the lowest and 3 corresponding to the highest activity.

Substrate	SfUGT33F28 enzyme activity
<b>Benzoxazinoid-related</b>	
Indole	-
IAA	3
BOA	-
6-OH-BOA	-
MBOA	-
DIMBOA	3
HMBOA	3
<b>Phenolic compounds</b>	
4-nitrophenol	1
1-naphthol	-
Salicyl aldehyde	-
Vanillin	1
Catechol	-
L-Tyrosine	-
L-DOPA	-
Dopamine	-
<b>Flavonoids</b>	
Quercetin	-
<b>Terpenoids</b>	
Menthol	-
Gossypol	-
<b>Coumarins</b>	
Esculetin	1
<b>Alkaloids</b>	
Capsaicin	-

**Supplementary table 3. List of primers used for the amplification of full length SfUGTs described in the study.**

<b>Gene</b>	<b>Forward primer (5'-3')</b>	<b>Reverse primer (5'-3')</b>
<i>SfUGT33V7</i>	AGGACACGTGCAATGTCGTTC	ACTCCTTTTAACCTTACCAGCG
<i>SfUGT40R1</i>	GTGCCGATAATGGCCTTAGTAT	ATTCCTTTTTTTACTATTTACAATTT
<i>SfUGT33F2</i>	TAGAAAATGAAGATATTAATTT	ACTACGTTTGGCTTTTTTAGGTT
<i>SfUGT40D1</i>	ATTGAGAATATGGAAAGAATG	GTTGTTCTTCTTTTGGCTTCC
<i>SfUGT50A1</i>	GCAAAAATGCATAGGTGGA	CAATTTCTTCTTTGTTGCCG
<i>SfUGT40F1</i>	CTGTCAAAAATGGAGATAATT	ATTCTTCTTAAGTTTTGGGCTCT
<i>SfUGT41D8</i>	ACAATGAAGCTAAGTATCCTGT	ATTCCTTTTCTTTTACTAGATTTCT
<i>SfUGT41B7</i>	CCGTTTCGAAATGAAGGCT	ATTCTTCTTCTTATGCGCTAGTCT
<i>SfUGT33B2</i>	TTGCGTGTAATCATGTCTACCG	ATTGTCTTTTAACCTACTATATTTCA
<i>SfUGT33F3</i>	CGCGTTACAATGTCGGTATTAC	ATTCGTTTCTTCTTCACACTAGTTT
<i>SfUGT42C1</i>	AATAAAAATGAAGTTTAATAAT	CTTAGTCTTGAACCTCATAATAACGA
<i>SfUGT44A1</i>	GCATTCAAAAATGACAAAACAG	TTCGAACCTTCACTCTTTTTTGAGGA
<i>SfUGT40Q8</i>	AGCAATAAAAATGAACAAATGG	ACTCCTTTTCTTCTTTTATCTTCAC
<i>SfUGT33F3</i>	ATAAAAATGTTATCTTTTGTGT	ATTATTTTTAAGTTTAGGAGTAGAA
<i>SfUGT40R1</i>	TTGTGCGTGCCAATAATGGCG	ATTCTTCTTATCCTTTTTCTTACTAT
<i>SfUGT40M1</i>	TGTGTCACAATGAGGTTGCCA	ATTAGTTTTCTTCTGATCATATTTTG
<i>SfUGT48D4</i>	GTAGCTGACATGAGGCGG	TTCGTTTTTCTTGTCTTCAAAATA
<i>SfUGT39B2</i>	AAAATGGACCCCCTAAAAATA	ATGTTTCTTCTGTTTTTTCGTTG
<i>SfUGT33J1</i>	CTCAAGATGGTTGGGTTT	ACTACTCTTCACTTTAGTCTTAATT
<i>SfUGT40L8</i>	TTTGTAATAATGAAGCATAAA	GTGACTCTTCTCTTTTTTATTAGTCG
<i>SfUGT33T9</i>	TCGATCACAATGTCTGTGC	ACTCCGTTTAATCTTAACATCACTA
<i>SfUGT42B1</i>	TCCATAATGAAGCCCGG	CGTTCGCTTCGCCTTTTT
<i>SfUGT46A1</i>	TTGCCACGATGCG	ATGCAACTTCTCCTTCTTATTCG
<i>SfUGT33F2</i>	AGCCAGACAATGAGGTATTTT	ACTACGTTTTGATTTTGATTTTTTAA
<i>SfUGT40R1</i>	ATTTGTAAAATGGCATTAGCAA	ATTCCTCTTCTTACTGTCTACAATTT
<i>SfUGT40M8</i>	AGAACAAAAATGGCGGATT	ATTCTTCTTCTCTTTTATATTCTTCT
<i>SfUGT40D1</i>	TCAGCCATCATGCAACG	ATTCTTCTTCTTTTTTCCCTCCTGTAT
<i>SfUGT40M1</i>	TGTGCCGTAATGAAGTGG	CATTATTTTCTTTTTGCATATTTTCA
<i>SfUGT33B2</i>	CGTTCGATAATGTTCTTCCC	ATTCTTCTTAAGTTTCTTCTGCTTTG
<i>SfUGT46B5</i>	TATCTCAAGATGCTACTCCCG	ACTCTGTTTAAGTTTCTCAGTCTTTT
<i>SfUGT47A1</i>	AGCACAGAGATGAGGCCG	ATTGGGTTTCACTTTGTACTTCC
<i>SfUGT40F1</i>	TTAATAATAATGGAAAAGTTA	ATTCTTCTTCTTTTTATATTCTTCT
<i>SfUGT33S2</i>	AGTGAGAACATGTTGCTGTGC	GTCTGCCTTAAGCTTCTTTAAAGT
<i>SfUGT33V6</i>	GAAACCAACATGTCTCTAGTA	TGCGCGCTTAATTTTAATAATAG

**Supplementary table 4. List of primers used for site-directed mutagenesis in the catalytic region of *SfUGT33F28*.**

Gene	Forward primer (5'-3')	Reverse primer (5'-3')
<i>SfUGT33F28</i> AT-TG	ACCCTTCAATCAGCCTGCA AGTGGTTTCCGA	TCGGAAAACCACTTGCAAGGCTGATT GAAGGGT

**Supplementary table 5. List of primers used for quantitative real time PCR (OGS2.2 gene annotation in brackets).**

Gene	Forward primer (5'-3')	Reverse primer (5'-3')
<i>SfUGT33F28</i> (GSSPFG0001823700 1/ UGT33-13)	GTCGTTCAACGCTGAGAGATA C	CGCAGTTTTACCATGTTCT CC
<i>SfUGT33B25</i> (GSSPFG0002458200 1/ UGT33-19)	CAACGAATGAAGAGGAATTCA AG	AGGCTGGTCGTACATCAC G
<i>SfUGT42C10</i> (GSSPFG0003536900 1/ UGT42-01-hap)	CCGCTGCTATCGAAGAAAAC	TCATGCCATACTTTGGATC G
<i>SfUGT40L8</i> (GSSPFG0000846600 1/ UGT40Lb)	AGCAAACATCGCTGGTTACC	TAAATGACGCCGTGTTTCG C
<i>SfUGT40R13</i> (GSSPFG0001460100 1/ GSSPFG00005183001 / UGT40-05)	TCGCTAGAGGCTTTGCTTTG	TCGGTCGTGGTAGATGTAG G
<i>SfUGT46A15</i> (GSSPFG0003214900 1/ GSSPFG00021909001 / UGT46-01)	TCGAAGGATTGCAGAGTGTG	TGCTGTGTCCAAAGGATCT G
<i>SfGAPDH</i> (GSSPFG0002813900 1/ GSSPFG00007681001 )	TGGATAACTTTGGCGAGAGG	TGTCAACCTTGACGCTTAC G
<i>SfRPL10</i> (GSSPFG0001220500 1)	ATTGGACAGCCCATCATGTC	AAGCCCCATTTCTTGGAGA C

**Supplementary table 6. List of primers used for the preparation of RNAi constructs.**

<b>Gene</b>	<b>Forward primer (5'-3')</b>	<b>Reverse primer (5'-3')</b>
<i>SfUGT33F</i> 28 RNAi	TAATACGACTCACTATAGGGC CAAGTACCTCATAAACCAT	CCCTATAGTGAGTCGTATTAGTCCA GGCAGAACATCA
<i>SfGAPDH</i> RNAi	TAATACGACTCACTATAGGGG AAGGGCATTCTTGACTA	CCCTATAGTGAGTCGTATTATTGGTC TGGATGTACTTG

Shu, B., Zhang, J., Sethuraman, V., Cui, G., Yi, X., and Zhong, G. (2017). Transcriptome analysis of *Spodoptera frugiperda* Sf9 cells reveals putative apoptosis-related genes and a preliminary apoptosis mechanism induced by azadirachtin. *Scientific reports* 7, 1-13.