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2 Supporting Information for

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4 Downregulation of a transcription factor associated with 5 resistance to Bt toxin Vip3Aa in the invasive fall armyworm

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7 Minghui Jin^{1,2†}, Yinxue Shan^{1†}, Yan Peng^{1,3†}, Wenhui Wang^{2†}, Huihui Zhang⁴, Kaiyu Liu⁴, David
8 G. Heckel⁵, Kongming Wu^{2*}, Bruce E. Tabashnik⁶, Yutao Xiao^{1*}

9 ¹Shenzhen Branch, Guangdong Laboratory of Lingnan Modern Agriculture, Genome Analysis
10 Laboratory of the Ministry of Agriculture and Rural Affairs, Agricultural Genomics Institute at
11 Shenzhen, Chinese Academy of Agricultural Sciences, Shenzhen, China; ²The State Key
12 Laboratory for Biology of Plant Disease and Insect Pests, Institute of Plant Protection, Chinese
13 Academy of Agricultural Sciences, West Yuanmingyuan Road, Beijing, China; ³College of Plant
14 Science and Technology, Huazhong Agricultural University, Wuhan, China; ⁴Institute of
15 Entomology, School of Life Sciences, Central China Normal University, Wuhan, China;
16 ⁵Department of Entomology, Max Planck Institute for Chemical Ecology, Jena, Germany;
17 ⁶Department of Entomology, University of Arizona, Tucson, AZ 85721

18 *Corresponding authors: Kongming Wu. Email: wukongming@caas.cn; Yutao Xiao. Email:
19 xiaoyutao@caas.cn

20 †These authors contributed equally to this work.

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26 **Tables S1 to S9**

27 **Table S1. Responses of strain DH-R (selected with Vip3Aa) and its parent susceptible**
 28 **strain DH-S of *S. frugiperda* to Vip3Aa, Cry1Ab, Cry1Fa, and Cry2Ab.**

Toxin	Strain	n ^a	LC ₅₀ ^b (95% FL ^c)	Slope (SE) ^d	RR ^e
Vip3Aa	DH-S	336	0.021 (0.011 - 0.038)	2.6 (0.7)	1.0
	DH-R	336	4.32 (1.5 - 10.9)	3.9 (0.7)	206 ^f
Cry1Ab	DH-S	192	0.22 (0.067 - 0.75)	2.0 (0.6)	1.0
	DH-R	192	0.27 (0.15 - 0.48)	1.4 (0.2)	1.2
Cry1Fa	DH-S	192	0.001 (0.00040 - 0.0094)	2.0 (0.5)	1.0
	DH-R	192	0.002 (0.00013 - 0.018)	2.1 (0.4)	2.2
Cry2Ab	DH-S	192	0.12 (0.065 - 0.21)	3.6 (0.6)	1.0
	DH-R	192	0.19 (0.11 - 0.33)	3.5 (0.5)	1.6

29 ^a Number of larvae tested.

30 ^b Concentration (µg toxin per cm² diet) killing 50% of larvae.

31 ^c 95% fiducial limits.

32 ^d Slope of the concentration-mortality line and its standard error.

33 ^e Resistance ratio = LC₅₀ for DH-R divided by LC₅₀ for DH-S for the same toxin.

34 ^f LC₅₀ of Vip3Aa significantly greater for DH-R than DH-S based on non-overlap of 95% FL.

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36 **Table S2. Survival from first instar to adult eclosion of *S. frugiperda* strains DH-R and DH-**
 37 **S on Bt and non-Bt corn.**

Corn type	Strain	Survival (%) ^a	SE (%)	Relative survival ^b
Non-Bt	DH-S	60.7	1.0	1.00
Non-Bt	DH-R	61.6	5.3	1.00
Vip3Aa	DH-S	0.0	0.0	0.00
Vip3Aa	DH-R	16.2	0.8	0.26
Cry1Ab	DH-S	1.4	0.6	0.02
Cry1Ab	DH-R	2.8	0.0	0.05
Cry1Ab + Vip3Aa	DH-S	0.0	0.0	0.00
Cry1Ab + Vip3Aa	DH-R	0.0	0.0	0.00

38 ^a Mean based on three replicates with 72 larvae per replicate (216 larvae per value for survival)

39 ^b Survival on Bt corn divided by survival on non-Bt corn for the same strain.

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43 **Table S3. Larval survival of *S. frugiperda* strains DH-R, DH-S, and their F₁ progeny on diet**
 44 **treated with 0.4 µg Vip3Aa per cm² diet.**

Strain or cross ^a	Survival (%) ^b	Dominance (<i>h</i>) ^c
DH-S	0.0	
DH-R	96.6	
DH-S♀ X DH-R♂	13.7 ^d	0.142
DH-S♂ X DH-R♀	10.8 ^d	0.111

45 ^a F₁ progeny were obtained from reciprocal mass crosses between DH-S and DH-R.

46 ^b Sample sizes were 48 larvae per strain and 96 larvae from each cross.

47 ^c $h = (\text{Survival of F}_1 \text{ progeny} - \text{survival of DH-S}) / (\text{Survival of DH-R} - \text{survival of DH-S})$;

48 $h = 0$ indicates completely recessive resistance, $h = 1$ indicates completely dominant resistance

49 ^d Survival did not differ significantly between the progeny from the two reciprocal crosses (Fisher's
 50 exact test, $P = 0.66$)

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52 **Table S4. Fine-scale mapping: five markers on chromosome 27 tightly linked with**
 53 **resistance to Vip3Aa in strain DH-R of *S. frugiperda*.**

Marker	Site (Mb)	<i>rr</i> ^a	<i>rs</i> ^a	<i>ss</i> ^a	χ^2	<i>P</i> ^b
1	10.01	65	22	9	89.9	6.9E ⁻²⁰
2	10.29	77	13	6	150.0	5.8E ⁻³⁴
3	10.33	89	7	0	225.8	3.4E ⁻⁴⁹
4	10.48	75	17	4	139.4	7.9E ⁻³²
5	10.63	60	29	7	70.8	2.5E ⁻¹⁵

54 ^a *rr*, homozygous for the marker from DH-R; *ss*, homozygous for the marker from DH-S; *rs*,
 55 heterozygous. Based on analysis of 96 larvae that were F₂ progeny (obtained from a single-pair
 56 mating between F₁ from DH-R and DH-S) and survived exposure to diet with 0.5 µg Vip3Aa per
 57 cm² diet.

58 ^b Probability based on the null hypothesis of random assortment *rr* : *rs* : *ss* = 1:2:1.

Table S5. Relative abundance of mRNA in larval midguts of *S. frugiperda* strains DH-R and DH-S for nine genes from 10.29 to 10.63 Mb on chromosome 27.

Complete gene name	Gene ID	Mb	Count ^a		FPKM ^b		FPKM log ₂ (DH-R/DH-S)	<i>P</i> ^c
			DH-S	DH-R	DH-S	DH-R		
<i>membrane associated ring-CH-type finger 2</i> ^d	118279256	10.41 -10.42	0	0	0	0	NA	NA
<i>nuclear pore glycoprotein p62-like (nup62)</i>	118279300	10.39 -10.40	47	39	1.47	1.13	-0.39	0.50
<i>checkpoint protein HUS1-like</i> ^e	118279287	10.38 -10.39	4	3	0.30	0.23	-0.37	0.80
<i>myeloblastosis (myb)</i>	118279242	10.33 - 10.38	100	42	2.05	0.74	-1.46	0.01
<i>pif1 5'-to-3' DNA helicase</i> ^d	118279132	10.30 - 10.31	0	0	0	0	NA	NA
<i>pif1 5'-to-3' DNA helicase</i> ^d	118279131	10.30 - 10.30	0	0	0	0	NA	NA
<i>Mariner</i> ^d	118279130	10.29 - 10.29	0	0	0	0	NA	NA
<i>attacin-like</i> ^e	118279240	10.29 - 10.29	3	4	0.25	0.37	0.57	0.24
<i>attacin-like</i> ^d	118279268	10.29 - 10.29	0	0	0	0	NA	NA

^a Mean of normalized counts from three replicates of 24 fourth instar larvae for each strain

^b Fragment per kilobase of transcript per million mapped reads

^c based on Wald test

^d No counts, NA: not applicable, no mRNA was detected

^e Mean count less than 5, categorized as not expressed

Table S6. Two-way ANOVA for effects of strain (DH-R vs. DH-S), trial, and their interaction on *Sfmyb* promoter activity^a

Source	df	Sum of squares	Mean Square	F	P
Strain	1	884.73	884.73	54.88	8.18E-06
Trial	2	42.10	21.05	1.31	0.31
Interaction	2	72.66	36.33	2.25	0.15
Error	12	193.45	16.12		
Total	17	1192.94	70.17		

^aThree replicates were done for each of the three trials for each strain.

Table S7. Number of promoters in *S. frugiperda* with each of 12 DNA motifs that bound SfMyb and occur in promoters of *D. melanogaster*^a

Motif no.	Sequence ^b	Potential target genes
1	GACGCGT	670
2	CCGGCCC	621
3	ACCACCC	1279
4	TTCTTGT	388
5	CCCGTGC	867
6	GTCCTTC	513
7	TGAATCC	847
8	CGTGGTC	448
9	CGTGGCC	538
10	CACGTGT	1046
11	CCGGAAA	571
12	TGACCTA	956

^a For *D. melanogaster* from the CIS-BP Database: <http://cisbp.cabr.utoronto.ca/TFTools.php>

^b 18 motifs bound SfMyb in the yeast one-hybrid assay but are not identified in *D. melanogaster* promoters by the CIS-BP Database: GATGGGC, TACGTTT, AAGGAGA, GTACTCG, TCGGCGA, CGGCGA, CCCCCT, GCGCTCG, CGGAGGA, GATGCAG, TCTCCGT, AACGTCC, CTAGTG, ATGATCA, ATCCGCG, GCGCATG, GCCGGAC, GGCGGAG

Table S8. 27 target genes of the Sfmyb transcription factor that are candidates for contributing to resistance to Vip3Aa.

Gene ID	Binding motifs	log2(DH-R/DH-S) ^a	<i>P</i> ^b	log2(dsMyb/dsGFP) ^c	<i>P</i> ^b	Protein in DH-S ^d	Protein location ^e	Annotation ^f
118279751	1,5	NA ^g	6.2E-14	-1.5	2.3E-03	yes	membrane	<i>serine protease stubble-like</i>
118274591	8	-5.9	1.3E-32	-1.1	2.0E-03	yes	membrane	uncharacterized
118265051	2	-5.0	1.2E-21	-1.3	2.1E-03	yes	membrane	<i>peste</i>
118272752	3,9,10	-1.9	3.9E-15	-1.0	4.1E-03	yes	membrane	<i>NPC1</i>
118277657	7	-1.1	4.1E-02	-1.1	2.4E-03	yes	membrane	<i>Tret1</i>
118268968	5,7,9,10	1.0	3.2E-02	1.5	4.0E-06	yes	membrane	<i>SLC4A2</i>
118272562	5	2.5	6.4E-19	1.4	3.4E-02	yes	membrane	<i>LPCAT</i>
118280306	3,5	-3.0	1.0E-02	-1.7	2.1E-02	yes	cytoplasm	<i>GMFB</i>
118273900	9	-2.0	1.4E-03	-1.5	7.7E-04	yes	cytoplasm	<i>N-like</i>
118270409	1	-1.8	5.4E-04	-1.4	1.5E-03	yes	cytoplasm	<i>atlastin-like</i>
118271576	5,7	-1.1	1.1E-02	-1.7	4.3E-03	yes	cytoplasm	<i>WASF3</i>
118274833	3	1.3	1.4E-05	1.1	1.8E-02	yes	cytoplasm	uncharacterized
118271777	3	2.2	2.1E-08	1.1	1.3E-02	yes	cytoplasm	<i>GST2</i>
118272063	5,7	2.3	6.2E-11	1.5	4.3E-03	yes	cytoplasm	<i>SET domain-containing</i>
118277508	12	-7.3	7.9E-23	-1.4	3.9E-03	yes	extracellular	<i>AGRN</i>
118273225	12	-5.5	2.3E-30	-1.4	4.7E-03	yes	extracellular	<i>pnliprp2</i>
118263806	1	-4.2	7.5E-11	-1.4	2.2E-02	yes	extracellular	<i>arylsulfatase B-like</i>
118261877	2,6,9,10	-1.2	6.9E-03	-1.0	2.1E-03	yes	nucleus	<i>Afdh</i>
118280234	2,3,10	-1.1	3.2E-02	-1.6	1.2E-02	yes	nucleus	<i>Usp2</i>
118270463	1,8,11	3.0	1.6E-14	2.4	7.7E-05	yes	nucleus	uncharacterized
118270199	12	-3.3	4.8E-07	1.9 ^h	1.6E-03	yes	cytoplasm	uncharacterized
118276924	2,5	-3.2	5.1E-04	1.4 ^h	1.9E-02	yes	cytoplasm	<i>ommochrome-binding</i>
118263030	9	-3.1	1.7E-10	1.1 ^h	1.0E-02	yes	cytoplasm	uncharacterized
118263167	10	-5.0	5.9E-26	1.2 ^h	6.9E-03	yes	extracellular	uncharacterized
118266038	1,3,5,7,10,11	-2.3	4.0E-06	-1.3	2.7E-03	no	mitochondria	<i>farnesyl pyrophosphate synthase</i>
118263545	6,11	-4.1	1.0E-12	-1.8	4.1E-03	no	membrane	<i>robo1</i>
118278607	2,3,9,10	-1.4	2.3E-02	1.6 ^h	4.1E-02	no	nucleus	<i>lava lamp</i>

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64 a Based on FPKM (fragments per kilobase of transcript per million mapped reads) from RNA-Seq; negative values
65 indicate downregulated in DH-R, positive values indicate upregulated in DH-R

66 b Probability from comparison between strains or treatments after adjustment for false discovery rate (1)

67 c Based on FPKM from RNA-Seq, negative values indicate downregulated in the dsMyb treatment, positive values
68 indicate upregulated in the dsMyb treatment

69 d Based on previously reported data from DH-S midguts (18) with the parameter Unique peptides ≥ 1 (2)

70 e Predicted from WoLF PSORT (<https://wolfsort.hgc.jp/>) and Phobius (<https://phobius.sbc.su.se/>)

71 f From *S. frugiperda* genome version ZJU_Sfru_1.1

72 (https://www.ncbi.nlm.nih.gov/datasets/genome/GCF_011064685.2/)

73 g No mRNA was detected in DH-R. DH-R/DH-S = 0 and the logarithm of 0 is undefined.

74 h DH-R upregulated relative to DH-S and dsSfmyb downregulated relative to dsGFP (control)

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Table S9. Chromosomal location and relative abundance of mRNA in larval midguts of *S. frugiperda* strains DH-R and DH-S for six genes previously reported to affect responses to Vip3Aa (the first three are putative Vip3Aa receptors, the last three putatively defend against Vip3Aa).

Complete gene name	Short gene name	Chromosome	Gene ID	Count ^a		FPKM ^b		FPKM log ₂ (DH-R/DH-S)	<i>P</i> ^c
				DH-S	DH-R	DH-S	DH-R		
Scavenger receptor-C	SRC	30	118280749	18.0	8.5	0.70	0.31	-1.20	0.27
Fibroblast growth factor receptor	FGFR	25	118277870	141.8	129.8	2.14	1.80	-0.26	0.17
Ribosomal S2	S2	5	118263612	355.0	148.0	29.45	11.65	-1.39	2.4E-07
Autophagy related gene 5	ATG5 ^d	4	118262572	119.0	70.3	8.60	4.71	-0.91	0.24
Phenoloxidase-activating enzyme-like	PAE	27 ^e	118279360	4.0	9.3	0.21	0.42	1.00	0.36
Phenoloxidase subunit 2-like	PO2 ^d	20	118275234	12.3	5.0	0.39	0.14	-1.48	0.06

77 ^a Mean of normalized counts from three replicates of 24 fourth instar larvae for each strain

78 ^b FPKM, fragment per kilobase of transcript per million mapped reads

79 ^c based on Wald test.

80 ^d Expression is numerically lower in DH-R than DH-S, which is opposite of the direction predicted if the putative defensive genes ATG5 and

81 PO2 contributed to resistance in DH-R.

82 ^e PAE is at 8.34 - 8.35 Mb on chromosome 27, which is outside the region linked with resistance in DH-R.

83 **SI References**

84

85 1. Y. Benjamini, Y. Hochberg, Controlling the false discovery rate - a practical and powerful approach to multiple testing. *J. Roy. Stat. Soc. B* **57**,
86 289-300 (1995).

87 2. B. C. Orsburn, Proteome discoverer – A community enhanced data processing suite for protein informatics. *Proteomes* **9**, 15 (2021).