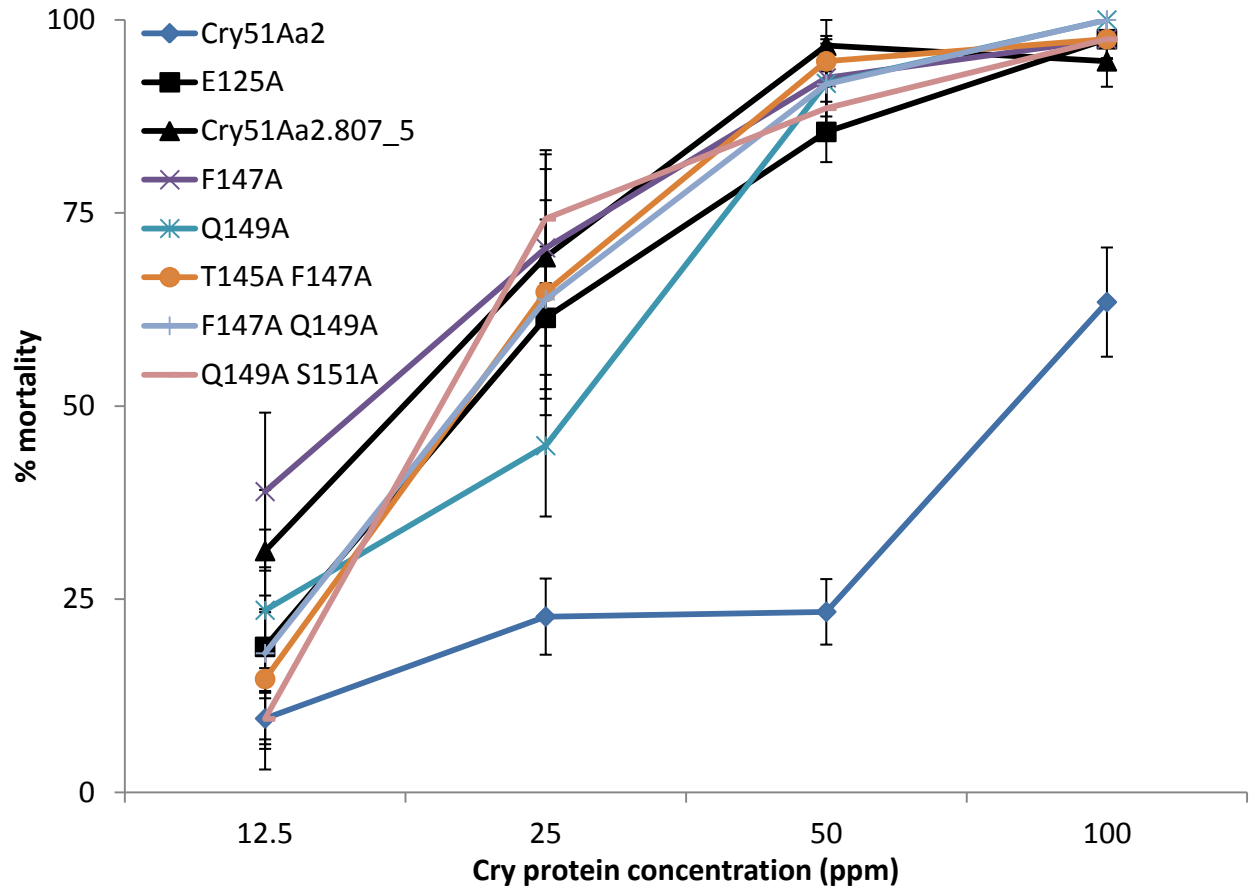


Supplementary Fig. 1. Stereo image of β -sheet structure and density map in Cry51Aa2: Residues displayed from left to right are 65-72, 169-177, and 259-266. The map is a 2Fo-Fc map contoured at 1σ level



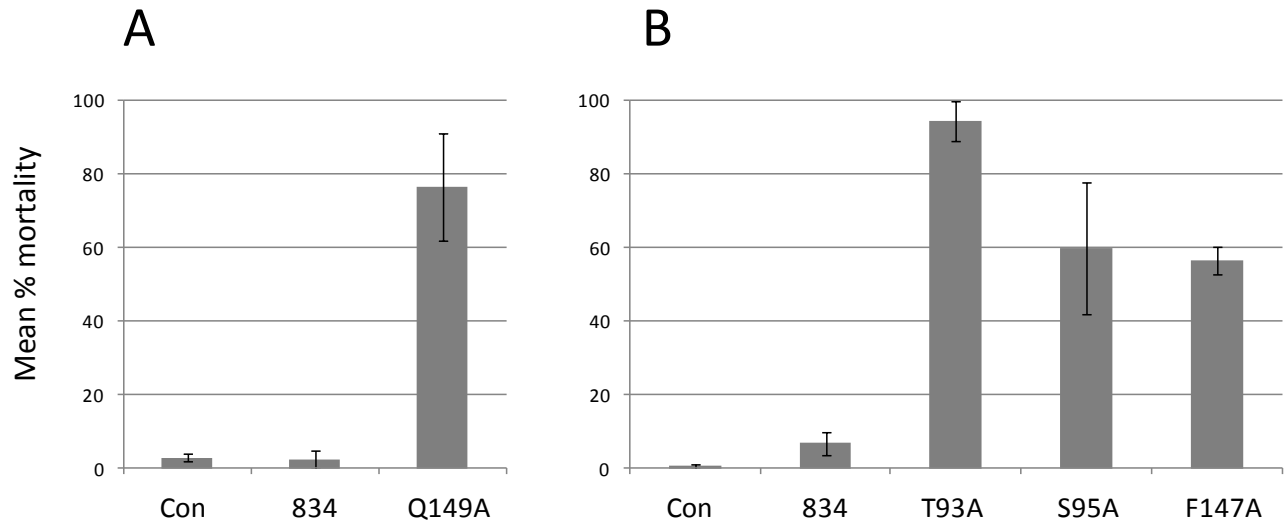
Supplementary Fig. 2. Cry51Aa1 (ref. 13) and Cry51Aa2 display a high level of structural similarity. Cry51Aa1 (1.65 Å resolution; cyan); Cry51Aa2 (2.28 Å resolution; red); r.m.s.d.=0.57 Å for 291 alpha carbon pairs.



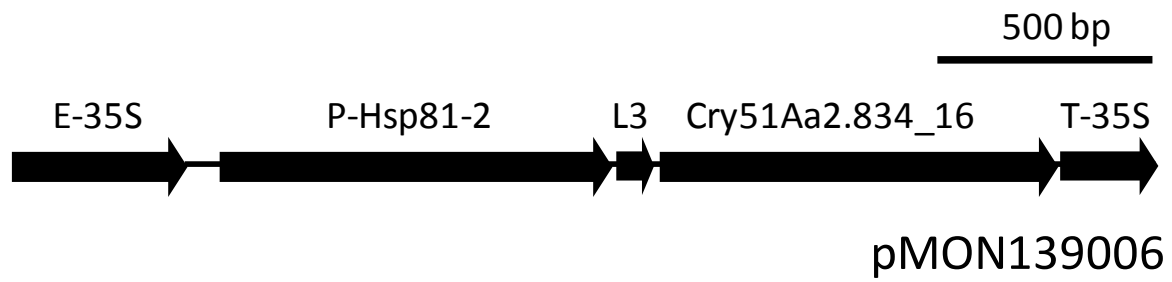
Supplementary Fig. 3. Concentration-response assay demonstrating improved activity of select Cry51Aa2 variants towards *L. hesperus*. Data shown as mean \pm s.e.m. ($n=24$)

TIC853	MAILDLKSLV	LDAINYWGPK	NNNGIQGYNF	NYPISERQID	TSIITSTHSR	LMPHDLTIPQ	60
Cry51Aa1	LAILDLKSLV	LNAINYWGPK	NNNGIQGGDF	GYPISEKQID	TSIITSTHPR	LIPHDLTIPQ	63
Cry51Aa2	MAILDLKSLV	LNAINYWGPK	NNNGIQGGDF	GYPISEKQID	TSIITFTHPR	LIPYDLTIPQ	60
	*	*	**	*	*	* *	60
TIC853	NLETIFTTQ	VLTNNTDVQQ	SQTVSFSKKT	TTTTSTSTTD	GWTEGGRISD	TLEENVSVSI	120
Cry51Aa1	NLETIFTTQ	VLTNNTDLQQ	SQTVSFAKKT	TTTTSTSTTN	GWTEGGKISD	TLEEKVSVSI	123
Cry51Aa2	NLETIFTTQ	VLTNNTDLQQ	SQTVSFAKKT	TTTTSTSTTN	GWTEGGKISD	TLEEKVSVSI	120
		*	*	*	*	*	120
TIC853	PFIGAGGAKN	STTIEANVAH	NSSTTTSQQA	STEIEWNISQ	PVLVPPRKQV	VATLVIMGGD	180
Cry51Aa1	PFIGEGGGKKN	STTIEANFAH	NSSTTTFQQA	STDIEWNISQ	PVLVPPRKQV	VATLVIMGGN	183
Cry51Aa2	PFIGEGGGKKN	STTIEANFAH	NSSTTTFQQA	STDIEWNISQ	PVLVPPSKQV	VATLVIMGGN	180
	* *	*	*	*	*	*	180
TIC853	FTVPMDLITT	IDSTQ---HF	TGYPILTWIE	NPEHNVGRF	LSWFFANWPN	LPSEFGSLNS	237
Cry51Aa1	FTIPMDLMTT	IDSTE---HY	SGYPILTWIS	SPDNSYNGPF	MSWYFANWPN	LPSGFGPLNS	240
Cry51Aa2	FTIPMDLMTT	IDSTE HYSHY	SGYPILTWIS	SPDNSYSGPF	MSWYFANWPN	LPSGFGPLNS	240
	* *	**** *	*	*	* * * * *	* *	
TIC853	DNTITYKGSV	VSRISAGVYA	TVRFDQYAIN	NLRTIEKTWY	ARHGLHNGK	KISINNVTEM	297
Cry51Aa1	DNTVITYTGSV	VSQVSAGVYA	TVRFDQYDIH	NLRTIEKTWY	ARHATLHNGK	KISINNVTEM	300
Cry51Aa2	DNTVITYTGSV	VSQVSAGVYA	TVRFDQYDIH	NLRTIEKTWY	ARHATLHNGK	KISINNVTEM	300
	* *	**	* *	*	*	*	
TIC853	APTSPPIERN	306					
Cry51Aa1	APTSPIKTN	309					
Cry51Aa2	APTSPIKTN	309					
	**	309					

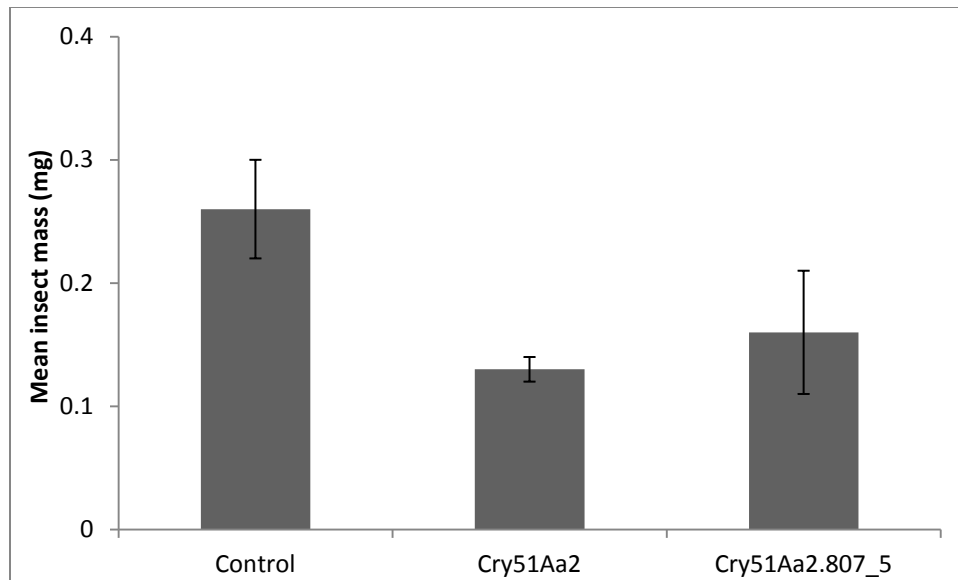
Supplementary Fig. 4. Alignment of the Cry51Aa1, Cry51Aa2, and TIC853 (ref. 14) amino acid sequences. Amino acid differences are denoted by the asterisk.



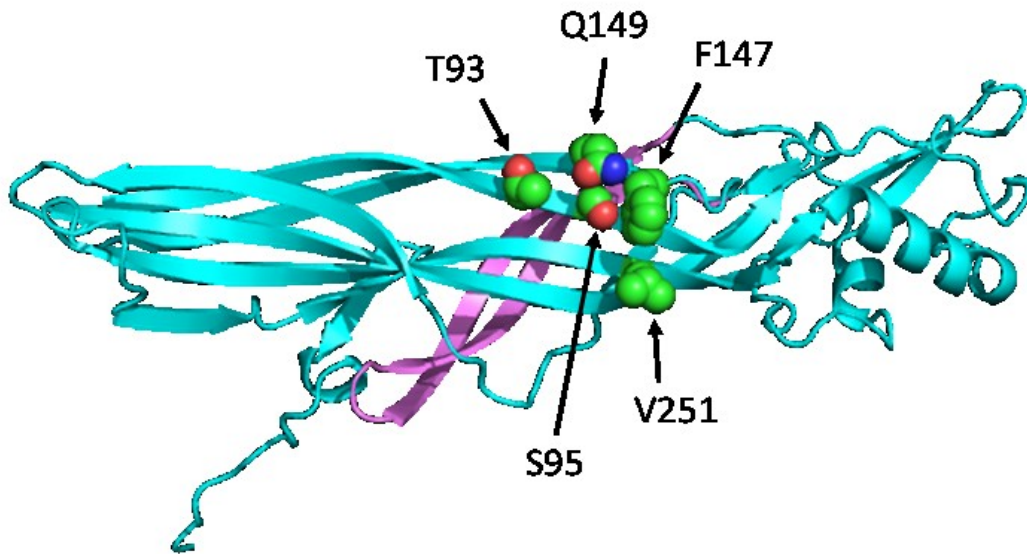
Supplementary Fig. 5. Bioassays demonstrating improved activity of select Cry51Aa2.834 alanine substitution variants towards *L. hesperus*. Proteins were presented in the diet at a concentration of 3.1 ppm. Con = buffer negative control; 834 = parental Cry51Aa2.834 protein. Data shown as mean \pm s.e.m. ($n=24$). Panels A and B summarize data from two independent tests.



Supplementary Fig. 6. Schematic diagram of the Cry51Aa2.834_16 expression cassette contained on the plant transformation vector pMON139006. E- enhancer, P-Promoter, L- leader and T-terminator.



Supplementary Fig. 7. Bioassay demonstrating that the HYS deletion in Cry51Aa2.807_5 protein does not impact activity towards *Lygus lineolaris*. Proteins were presented in the diet at a concentration of 100 ppm and the assay scored for mean insect mass. Control = buffer negative control; Data shown as mean \pm s.e.m. ($n=40$).



Supplementary Fig. 8. Alanine substitution sites in the wild-type Cry51Aa2 resulting in increased activity towards *Lygus hesperus* are located on three adjacent β strands in close proximity to each other. The putative beta-pore-forming loop is colored magenta.

Supplementary Table 1. Data collection, phasing and refinement statistics for SeMet-Cry51Aa1-L11M: MAD data collection for structure solution and high resolution data for refinement

	Native	Crystal 1			Crystal 2
Data collection					
Space group		P43212			P43212
Cell dimensions					
<i>a</i> , <i>b</i> , <i>c</i> (Å)		54.8, 54.8,			55.6, 55.6,
		208.9			209.0
α , β , γ (°)		90°, 90°,			90°, 90°,
		90°			90°
		<i>Peak</i>	<i>Inflection</i>	<i>Remote</i>	
Wavelength		0.97972	0.97996	0.97228	1.00
Resolution (Å)		50-2.75	20-2.80	20-2.8	53.7-2.28
R_{sym} or R_{merge}		0.109	0.110	0.105	0.08
$I / \sigma I$		34.0	32.3	37.3	17.4
Completeness (%)		99.9%	100%	99.9%	95.9%
Redundancy		22.6	22.6	22.6	4.7
Refinement					
Resolution (Å)		53.7-2.75			53.7-2.28
No. reflections		9165			15297
$R_{\text{work}} / R_{\text{free}}$		0.218/0.304			0.238/0.295
No. atoms		2456			2437
Protein		2390			2390
Ligand/ion		-			-
Water		66			47
<i>B</i> -factors					
Protein		42.1			41.4
Ligand/ion		-			-
Water		31.7			41.3
R.m.s deviations					
Bond lengths (Å)		0.014 Å			0.019 Å
Bond angles (°)		1.60°			1.80°

Supplementary Table 2. Cry51Aa2 alanine scan variants showing increased mortality compared to the wild-type Cry51Aa2 protein towards *Lygus hesperus* in artificial diet feeding assays

Amino acid substitution	Strategy	Test concentration (ppm)
S95A, F147A	Combinatorial	12.5
E125A	Alanine scan	25
E125A, F147A	Combinatorial	12.5
E125A, Q149A	Combinatorial	12.5
G128A	Alanine scan	25
I134A	Alanine scan	25
E135S	Alanine scan	25
T145A	Alanine scan	25
T145A, F147A	Double Ala scan	25
F147A	Alanine scan	25
F147A, Q149A	Double Ala scan	25
F147A, S159A	Combinatorial	12.5
F147A, L187A	Combinatorial	12.5
Q149A	Alanine scan	25
Q149A, S151A	Double Ala scan	25
Q149A, T182A	Combinatorial	12.5
D153A	Alanine scan	25
S159A	Alanine scan	25
V175A	Alanine scan	25
T182A	Alanine scan	25
L187A	Alanine scan	25
I123A, A139S	Combinatorial	25