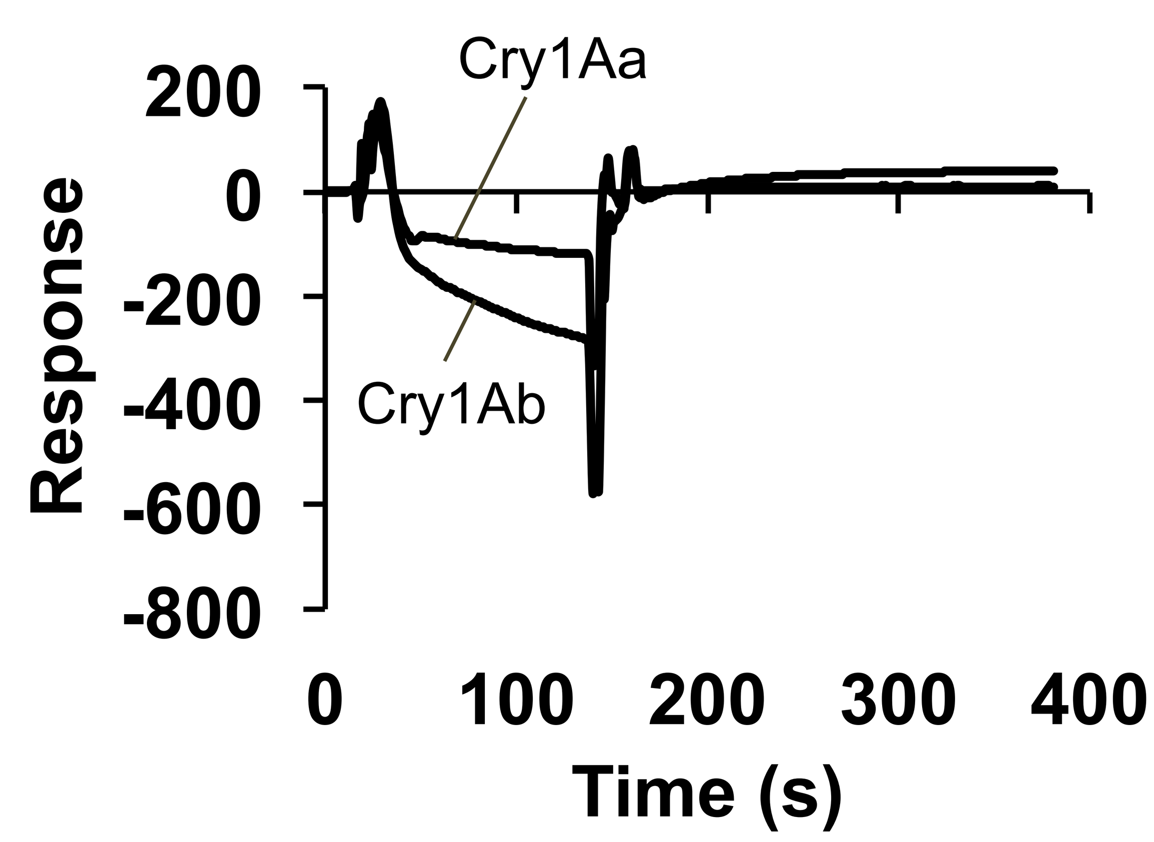

Figure S1. Binding analysis of Cry1A toxins to contaminating proteins from EGFP-expressing Sf9 cells. Membrane proteins from EGFP-expressing Sf9 cells (2L culture) were incubated with an anti-FLAG-tag-antibody-conjugated gel and proteins which non-specifically bound the gel were collected and immobilized on a CM5 sensor chip. 400 nM Cry1Aa and Cry1Ab toxins were injected over the sensor chip of Biacore J as analytes.

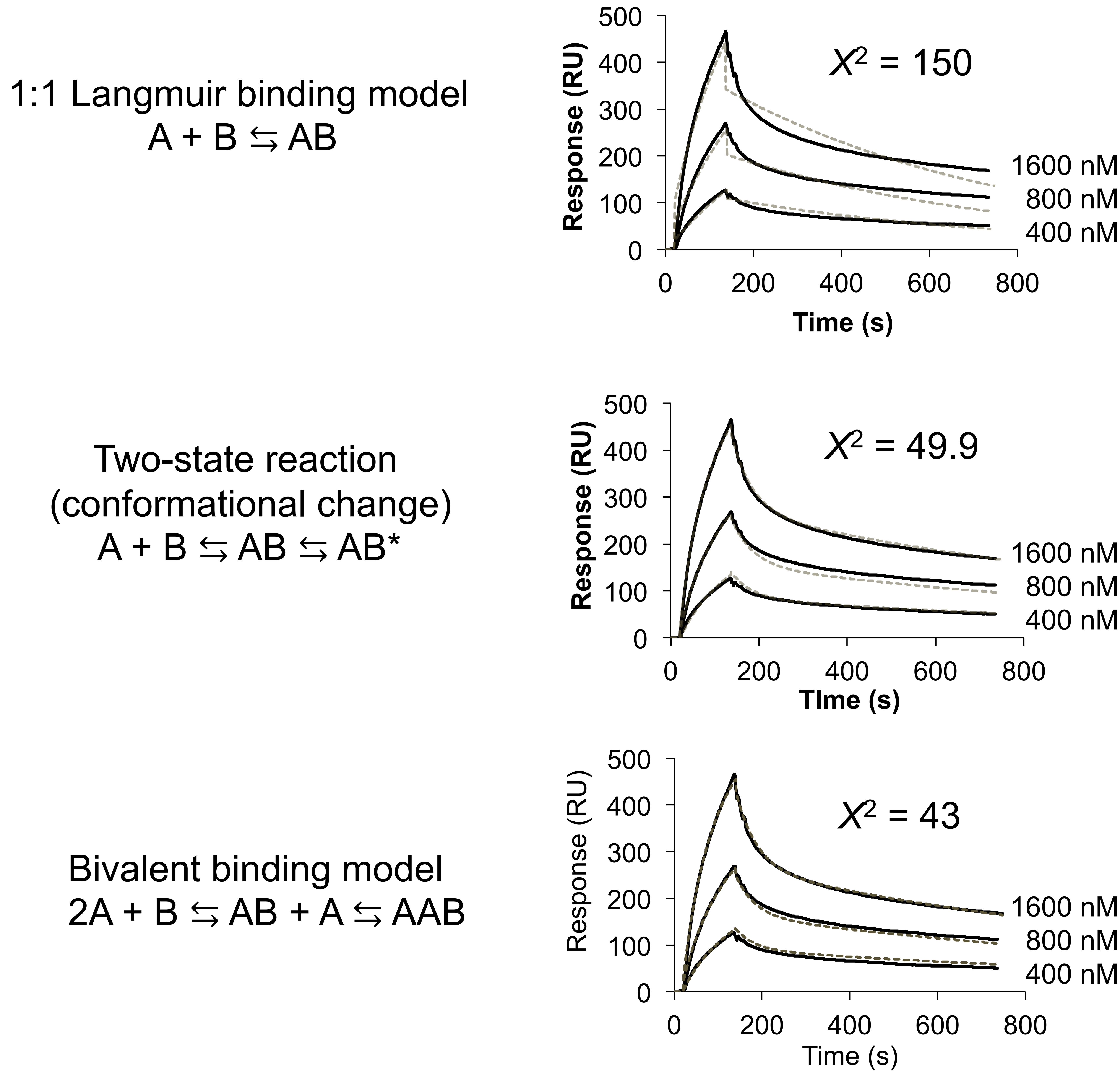

Figure S2. Curve fitting of the sensorgram displaying the binding response between the Cry1Aa toxin and BmABCC3-FLAG. Purified BmABCC3 was immobilized on a CM5 sensorchip of Biacore; 400, 800, and 1600 nM Cry1Aa toxins were applied, respectively. The thick black lines indicate the actual response curves, and the thin gray dotted lines show fitting curves based on binding models. X^2 indicates the chi-square value.

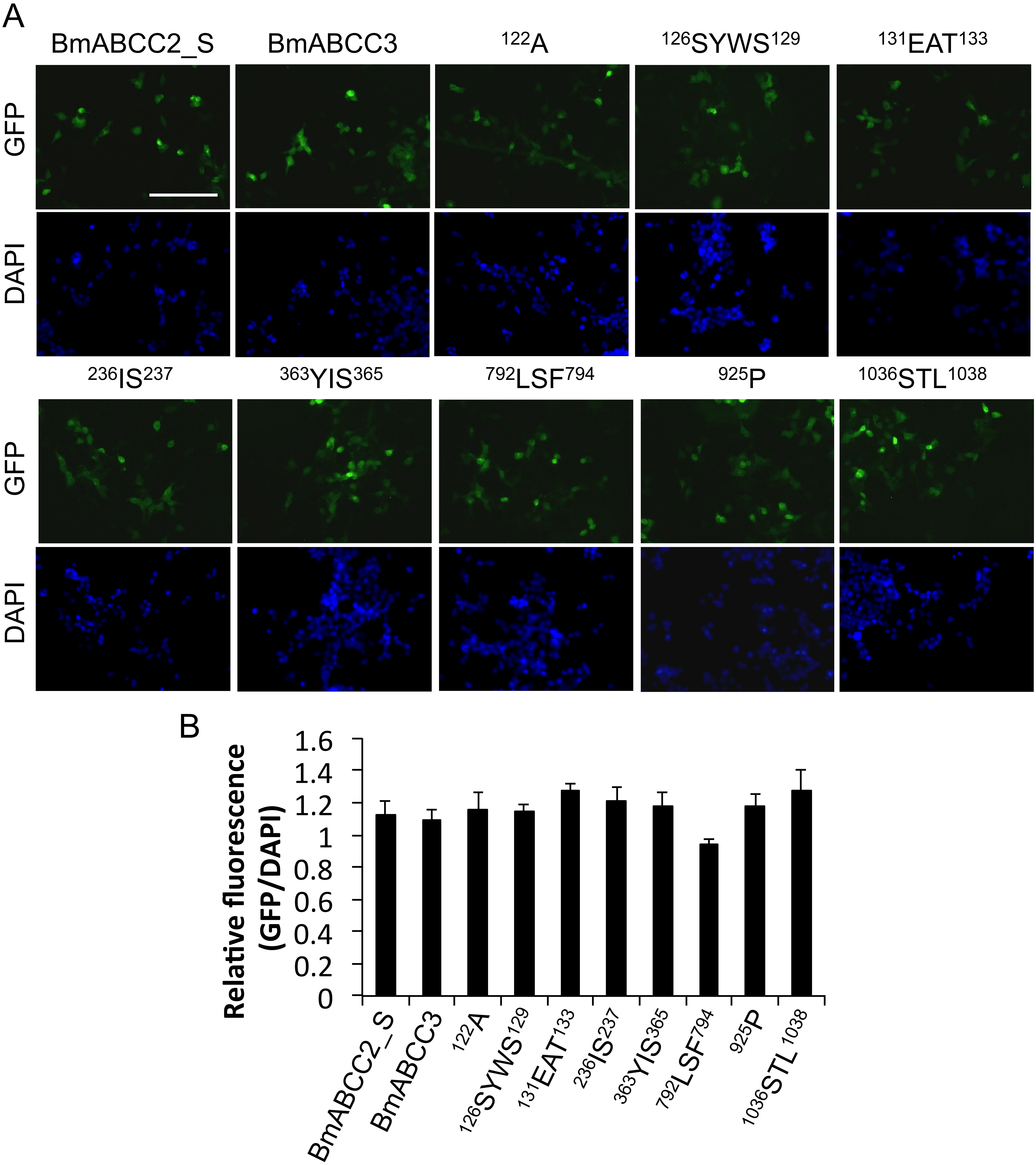

Figure S3. Expression levels of BmABCC3 mutants in HEK293T cells. (A) HEK293T cells were transfected with BmABCC3 mutant expression vectors. EGFP, which was fused to the C-termini of the BmABCC3 mutants, and DAPI, which stains nuclear DNA, were visualized under a fluorescence microscope after 48 h as described in the Materials and methods. Scale bar indicates 20 µm. (B) The GFP/DAPI ratio indicating the per-cell expression levels of ABCC3 mutants was calculated using the fluorescence intensities of three fields of view, including images in (A). Error bars indicate standard error.

Table S1. Primers used in this study

Purpose	Name	Sequence
Human cell expression	3.1_BmABC_GFP_F	ccacccGGATCCGATATGAATAGTGATGGGAGAGC
	3.1_BmABC_GFP_R	GCTCACCATTTTTTCTGTATTTCTACCAA
	BmABC_GFP_FLAG_F	CAGAAAAAATGGTGAGCAAGGGCGAGGA
	BmABC_GFP_FLAG_R	ttgtagtcCTTGTACAGCTCGTCCATGC
	GFP_FLAG_3.1_F	TGTACAAGgactacaaagaccatgacgg
	GFP_FLAG_3.1_R	GAATTCGGTACCGATcttgtcatcgtcatccttgt
	BmABCC3_GFentryF	CCACCCGGATCCGATATGGGTGTTGGAAGTGAAAA
	BmABCC3_GFentryR	GCCCTTGCTCACGATTCTCATGTTTTCTTTCAGAT
Insect cell expression	BmABCC2_F1	CGCtctagaATGGACTACAAAGACCATG
	BmABCC2_R1	TCATGGTCTTTGTAGTCCATTTTTTCTGTATTTCTACCAA
	BmABCC2_F2	TTGGTAGAAATACAGAAAAAATGGACTACAAAGACCATGA
	BmABCC2_R2	CTAtctagaCTTGTCATCGTCATCCTTGTAATC
	BmABCC3_HindF	actaagcttATGGGTGTTGGAAGTGAAAA
	BmABCC3_XhoR	actctcgagTCTCATGTTTTCTTTCAGAT
BmABCC3 mutants	BmABCC3_EAT_inverseF	ACAGTGGAAGCGACTATTACGCAAATGGAAGCGGT
	BmABCC3__EAT_inverseR	CGTAATAGTCGCTTCCACTGTCCAATACGTGAGCA
	BmABCC3_LSF_inverseF	TACTGGCTCAGTTTCTGGACGAATGCAATGGCA
	BmABCC3_LSF_inverseR	CGTCCAGAAACTGAGCCAGTAGTCAGCGCCAGC
	BmABCC3_AELL_inverseF	TGTTATTTgcaGAGCTGCTCACGTATTGGACAGT
	BmABCC3_AELL_inverseR	AGCTCtgcAAATAACAGAGGTGTTATAATACGTAA
	BmABCC3_SYWS_inverseF	CTCtCGTATTGGtCAGTGGATCCGCCTATTACGC
	BmABCC3_SYWS_inverseR	CTGaCCAATACGaGAGCAGCTCTCCAAATAACAG
	BmABCC3_ECL2IS_inverseF	GGCTACaTTtcAGCTGGTGTTGCAGCTCT
	BmABCC3_ECL2IS_inverseR	ACCAGCTgaAAtGTAGCCCAGATAGCAGACAG
	BmABCC3_ECL3YIS_inverseF	AACAATaCATcAgCGCTGCACAACTCAATATCACA
	BmABCC3_ECL3YIS_inverseR	AGCGCTGATGTATTGTTGTAAGGGATAAATCACAG
	BmABCC3_ECL5Y_inverseF	TCGCACTGccTTGGACATTGATTCCTTCTGT
	BmABCC3_ECL5Y_inverseR	AATGTCCAAggCAGTGCGATTGCGTTCAA
	BmABCC3_ECL6STL_inverseF	GACTTTaGTAcCcTCATTGCCGTGGGAAGTG
	BmABCC3_ECL6STL_inverseR	GAgGgTACtAAAGTCTATAAAGATGAACACTAAAATAAC
qPCR	BmABCC2_RTF	TCTTGCCATTGCAAGTTTGTCT
	BmABCC2_RTR	AGCGATGCCTTGTTGTTGA
	BmABCC3_RTF	ATTGATGCCTGCTGGTTCGAA
	BmABCC3_RTR	ACGTTTTACCAGATTGTTGCTA
	ActinA3_RTF	CGTACCACCGGTATCGTGCT
	ActinA3_RTR	GAGGATCTTCATGAGGTAGTCGGTC

Table S2. Kinetic parameters of Cry toxins to silkworm ABC transporters

Cry toxin	Cry1Aa			Cry1Ab			Cry1Ca		Cry1Da		Cry3Bb	
ABCC transporter	BmABCC2_S	BmABCC3	BmABCC2_R	BmABCC2_S	BmABCC3	BmABCC2_R	BmABCC2_S	BmABCC3	BmABCC2_S	BmABCC3	BmABCC2_S	BmABCC3
ka1 (1/Ms)	3.24×10^{4}	6.46×10^{3}	4.54×10^{4}	4.36×10^{4}	4.44×10^{3}	1.77×10^{4}	2.34×10^{4}	7.71×10^{3}	2.52×10^{2}	1.01×10^{1}	2.83×10^{3}	5.33×10^{2}
kd1 (1/s)	5.94×10^{-3}	1.07×10^{-2}	1.08×10^{-2}	7.06×10^{-3}	1.41×10^{-5}	1.15×10^{-2}	2.10×10^{-2}	2.81×10^{-2}	1.80×10^{-2}	1.96×10^{2}	7.95×10^{-2}	1.61×10^{-2}
ka2 (1/s)	5.47×10^{-3}	6.36×10^{-3}	2.19×10^{-3}	1.91×10^{-3}	9.48×10^{-4}	1.27×10^{-2}	2.23×10^{-3}	7.89×10^{-3}	3.01×10^{-3}	3.66×10^{-3}	7.64×10^{-4}	2.12×10^{-3}
kd2 (1/s)	1.30×10^{-5}	1.16×10^{-3}	2.60×10^{-6}	3.06×10^{-6}	1.00×10^{-5}	4.87×10^{-4}	5.25×10^{-4}	9.52×10^{-4}	1.01×10^{-4}	1.00×10^{-3}	1.78×10^{-3}	2.80×10^{-6}

Table S3. Kinetic parameters of Cry toxins to ABCC3 mutants

Cry toxin Mutants	Cry1Aa ECL1 ^{129}EAT^{131}	Cry1Ab ECL1 ^{129}EAT^{131}	Cry1Aa ECL3 ^{363}YIS^{365}	Cry1Ab ECL3 ^{363}YIS^{365}
ka1 (1/Ms)	4.01×10^{4}	2.22×10^{3}	2.16×10^{4}	6.15×10^{3}
kd1 (1/s)	5.11×10^{-3}	3.04×10^{-2}	1.77×10^{-2}	2.68×10^{-2}
ka2 (1/s)	6.36×10^{-3}	2.18×10^{-3}	6.46×10^{-3}	7.57×10^{-4}
kd2 (1/s)	2.67×10^{-4}	4.73×10^{-6}	1.37×10^{-3}	1.88×10^{-4}