

Supplementary Materials

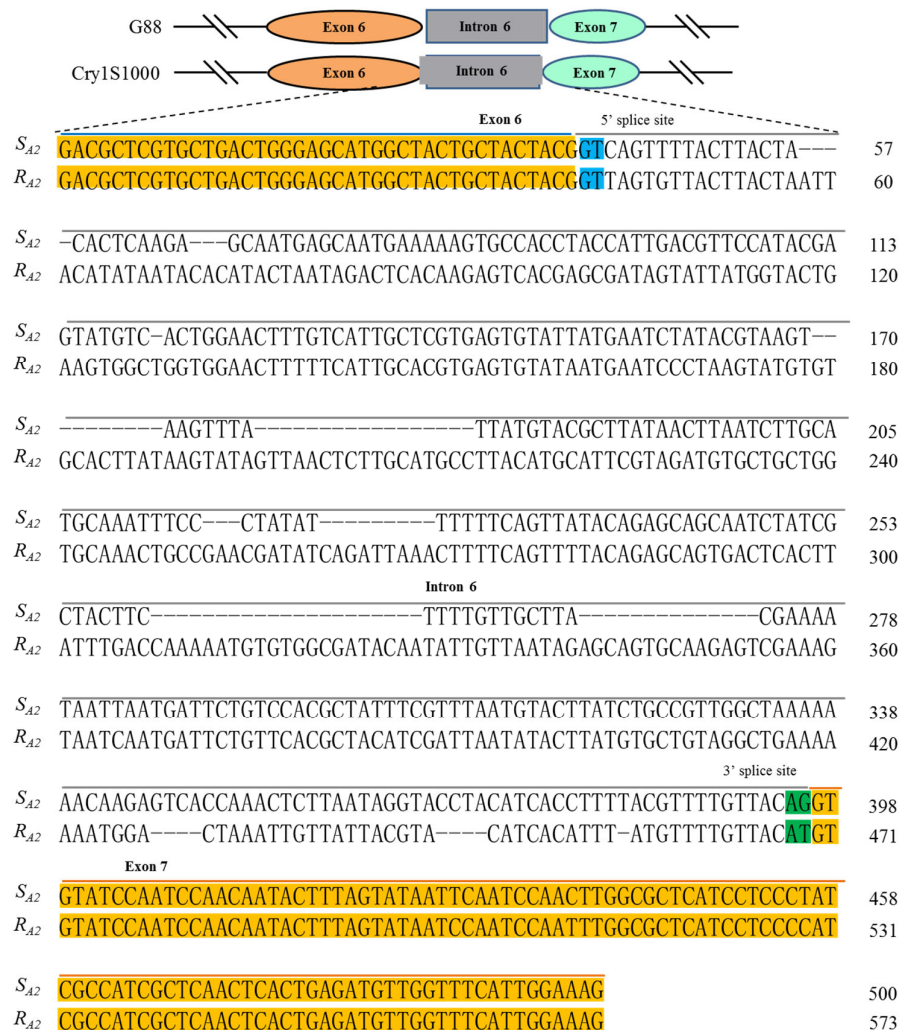


Figure S1. *R_{A2}* mutation of *PxABCC2* from the Cry1S1000 strain. Alignment of gDNA sequences of the *S_{A2}* allele and the *R_{A2}* allele. Sequences highlighted in orange are the exons 6 and 7.

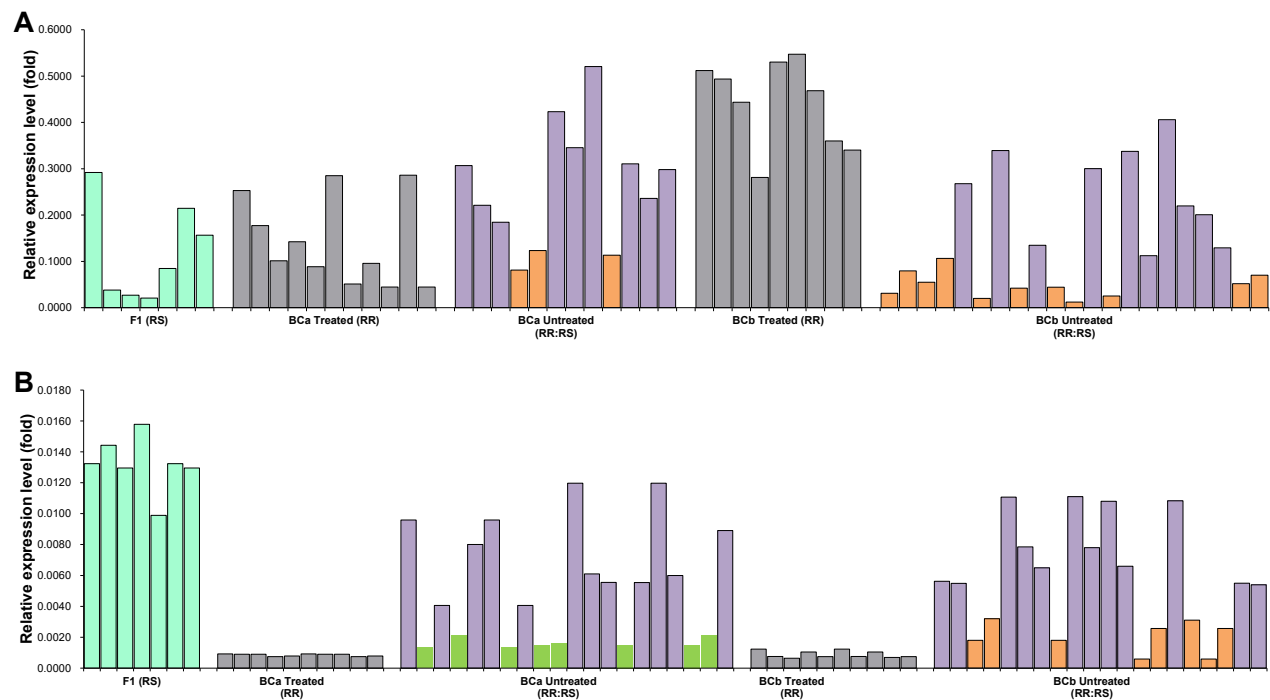


Figure S2. Analysis of the linkage between resistance to Cry1Ac and reduced *Pxpolycalin* (A) and *PxABCC2* (B) expression levels in the Cry1S1000 strain of *P. xylostella*. Expression levels of *PxABCC2* and *Pxpolycalin* in individual midgut from larvae of the F₁, without Cry1Ac-selected (untreated) and with Cry1Ac-selected (treated) backcross families (backcross family a and b) are shown relative to levels in the susceptible (G88) strain.

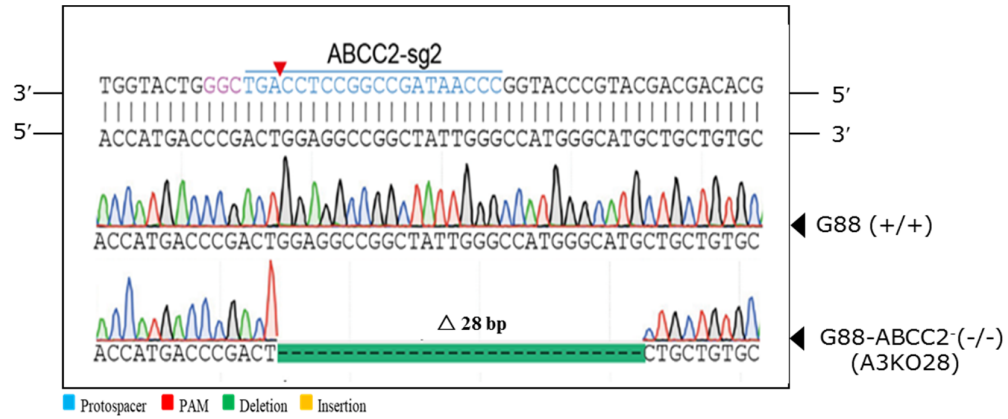


Figure S3. Mutagenesis of *PxABCC2* induced by CRISPR/Cas9. Partial sequences from the G88 and homozygous *PxABCC2* mutant showing the indels at the target sequence (ABCC2-sg2) in exon 3 of *PxABCC2*.

Table S1. Genetic linkage of the R_{A2} allele with Cry1Ac resistance.

Generation	N ^a	Genotype ^b	
		$R_{A2}S_{A2}$	$R_{A2}R_{A2}$
F ₁ (G88♀ x Cry1S1000♂)	10	10	0
Backcross family a with toxin treated	12	0	12
Backcross family a without toxin treated	24	14	10
Backcross family b with toxin treated	12	0	12
Backcross family b without toxin treated	24	15	9

^a Total number of adults used for genotyping.

^b For the genetic linkage analysis, we used Fisher's exact test. The observed genotype frequencies of *PxABCC2* on diet treated with Cry1Ac toxin differed significantly from the expected genotype frequencies on the untreated diet ($\chi^2 = 11.46$, $df = 1$, $P < 0.001$ for Backcross family a; $\chi^2 = 12.86$, $df = 1$, $P < 0.000$ for Backcross family b).