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SUPPLEMENTARY DISCUSSION

Bt toxin receptor rationale. A conserved region proximal to the plasma membrane has been experimentally identified to be a required region for Cry1Ac binding and toxicity[1,2]. Sequence alignment of this region from cadherins of various lepidopteran species suggests a short ~50-amino acid motif, the toxin-binding region (TBR), in cadherin-like proteins that is necessary for Cry1Ac binding and insecticidal activity (Extended Data Fig. 4). In *T. ni*, binding of Cry1Ac to cadherin can occur when the protein is solubilized from the cell membrane and denatured to expose the transmembrane domain and other regions that are not natively exposed[3,4]. However, Cry1Ac does not bind the cadherin-like protein in its native form in the brush border membranes of the midgut of *T. ni,* as a Cry1Ac-resistant *T. ni* strain with no alteration of the cadherin-like protein at genomic, transcriptional, and protein levels exhibits an absence of Cry1Ac binding sites in the midgut brush border membranes[3-5]. Indeed, resistance to Cry1Ac in *T. ni* is not mediated through lack of binding to the cadherin-like receptor, but instead is associated with the *ABCC2* gene and down-regulation of the aminopeptidase *APN1*[3,4,6]. Consistent with this view, lepidopteran ABCC2 receptors have been shown to be sufficient for mediating Cry toxin activity in insect cells,[7] and Cry1Ac resistance in *T. ni* is not dependent on the *T. ni* cadherin-like receptor TnCAD[4], as we confirm experimentally below. We hypothesized that evolved Cry1Ac mutants that bind TnCAD with high affinity would establish binding of Cry1Ac to the cadherin in the midgut *T. ni*, and may be able to overcome *T. ni* resistance mechanisms involving mutations of the ABCC2 and APN1 receptors or their regulatory sequences.

SUPPLEMENTARY REFERENCES

1 Dorsch, J. A. *et al.* Cry1A toxins of Bacillus thuringiensis bind specifically to a region adjacent to the membrane-proximal extracellular domain of BT-R(1) in Manduca sexta: involvement of a cadherin in the entomopathogenicity of Bacillus thuringiensis. *Insect biochemistry and molecular biology* **32**, 1025-1036, (2002).

2 Xie, R. *et al.* Single amino acid mutations in the cadherin receptor from Heliothis virescens affect its toxin binding ability to Cry1A toxins. *The Journal of biological chemistry* **280**, 8416-8425, (2005).

3 Tiewsiri, K. & Wang, P. Differential alteration of two aminopeptidases N associated with resistance to Bacillus thuringiensis toxin Cry1Ac in cabbage looper. *Proceedings of the National Academy of Sciences of the United States of America* **108**, 14037-14042, (2011).

4 Zhang, X., Tiewsiri, K., Kain, W., Huang, L. & Wang, P. Resistance of Trichoplusia ni to Bacillus thuringiensis toxin Cry1Ac is independent of alteration of the cadherin-like receptor for Cry toxins. *PloS one* **7**, e35991, (2012).

5 Wang, P. *et al.* Mechanism of resistance to Bacillus thuringiensis toxin Cry1Ac in a greenhouse population of the cabbage looper, Trichoplusia ni. *Applied and environmental microbiology* **73**, 1199-1207, (2007).

6 Baxter, S. W. *et al.* Parallel evolution of Bacillus thuringiensis toxin resistance in lepidoptera. *Genetics* **189**, 675-679, (2011).

7 Tanaka, S. *et al.* The ATP-binding cassette transporter subfamily C member 2 in Bombyx mori larvae is a functional receptor for Cry toxins from Bacillus thuringiensis. *The FEBS journal* **280**, 1782-1794, (2013).