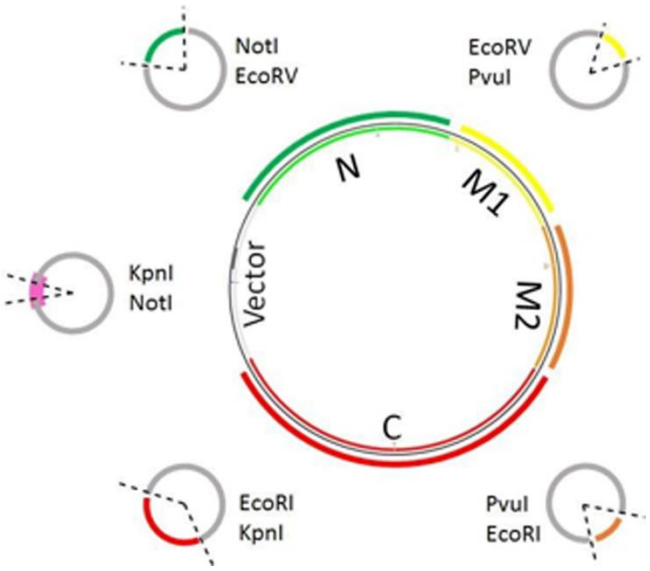
Supplementary Data

Supplementary Methods M1

To facilitate fragment reassembly, a hybrid plasmid vector was used, composed of the multiple-cloning-site (MCS) from pcDNA3.1(-) (Invitrogen, Carlsbad, CA, USA), plus the addition of two extra restriction enzyme cut sites (HindIII and MluI cut sites) at the 5′ and 3′ ends of the MCS respectively, spliced into a pIZ/V5-His plasmid vector (Thermo-Fisher Scientific, Waltham, MA, USA) in place of its original MCS. The four fragments (N, M1, M2 and a modified C) to be re-incorporated into the linearised hybrid pIZ/V5-His plasmid vector where then re-assembled into a whole intact full-length cDNA corresponding to the PxRyR ORF by ligation in the presence of the cut hybrid pIZ/V5-His plasmid vector. The total DNA concentration in the ligation reaction was maintained at ~10ng/ul. The addition of HindIII and MluI cut sites at the 5′ and 3′ ends of the pcDNA3.1(-) MCS, allowed the amplified sequence reassembled in the hybrid vector to be easily transferred across into pIZ/V5-His.

Supplementary Table S1: Primers used to generate amino acid substitutions in the *P. xylostella* RyR.

Mutation	Oligonucleotide 1	Oligonucleotide 2
K4700R	GTTCTACACCTTGcgGTACGTGGCGCTGG	CCAGCGCCACGTACcgCAAGGTGTAGAAC
Y4701F	GTTCTACACCTTGAAGTtCGTGGCGCTGG	CCAGCGCCACGaACTTCAAGGTGTAGAAC
I4790C	GTATCGCTGGCTtgtCTGATCGGGTACTAGGATTTGAAGG	CCTTCAAATGGTAGTACCCGATCAGacaAGCCAGCGATAC
S4919L	CTCTTTCCTGTACTtaCTGTGGTACTTCTCGTTCTCTGTGATGGGC	GCCCATCACAGAGAACGAGAAGTACCACAGtaAGTACAGGAAAGAG
V4945M	CGCTCATCTGTTGGACGTGGCTaTgGGGTTCAAGACGTTGAGG	CCTCAACGTCTTGAACCCcAtAGCCACGTCCAACAGATGAGCG
RFCLM (MULTI)	CCTCGCCAGGAAGTTCTACACCTTGAAGTACGTGGCGCTGGTGCTGGCC GCACTCTATAGTATCGCTGGCTATACTGATCGGGTACTACCATTTGAAGG TCCCGC CGATCACAGACAACTCTTTCCTGTACTCTCTGTGGTACTTCTCGTTCTCTGT	No reverse primer required in Lightning Multi Reaction



Supplementary Figure S1: *PxRyR* construct fragment assembly and restriction sites used for cutting out and re-ligating. The WT-PxRyR sequence was digested into four fragments, as labelled in the diagram. N ('N-terminus', green) - 4061bp M1 (yellow) - 2350bp M2 (orange) - 2684bp C ('C-terminus', red) - 6326bp Vector (pIZ/V5-His) - 2900bp