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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 ~10 ng/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.

<table>
<thead>
<tr>
<th>Mutation</th>
<th>Oligonucleotide 1</th>
<th>Oligonucleotide 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>K4700R</td>
<td>GTTCTACACCTGGcGATCGGGGCGTGG</td>
<td>CCAGGCGACGTACCAAGGTTAGAAC</td>
</tr>
<tr>
<td>Y4701F</td>
<td>GTTCTACACCTGAAGTCCGCGGCGTGG</td>
<td>CCAGGCGCCAGACTCAAGGTTAGAAC</td>
</tr>
<tr>
<td>I4790C</td>
<td>GTATCCTGCGTgtCTGATCGGGTACTAGGATTTGAAGG</td>
<td>CCTTCAAATGGTATCCCGATCGAaGcACCGCGATAC</td>
</tr>
<tr>
<td>S4919L</td>
<td>CTCTTCTCTGTACGtCTGTTGCTACTCTCTGCTGATGCGGCG</td>
<td>GCCCCATCACAGAAGAAGAAGTACCAAGaAGCTACAGGAAAGAG</td>
</tr>
<tr>
<td>V4945M</td>
<td>CGCTCATCTGTGGAGCGGGCCTaTcGTTCAAGACCGTGAGG</td>
<td>CCTCAACGCTCTGAAACCGAGGCGTCAACAGATGAGCG</td>
</tr>
<tr>
<td>RFCLM (MULTI)</td>
<td>CTCGGCATGAAAGTCTACACCTGGAGCTAGCGGGCGCTGGTGCTGGCC</td>
<td>No reverse primer required in Lightning Multi Reaction</td>
</tr>
<tr>
<td></td>
<td>GCACCTATAGTATCGGGGTCTACTAGCGGGTACTCCATTTGAAGG</td>
<td>TCCGGC</td>
</tr>
<tr>
<td></td>
<td>CAGTACACAGAACACTCTTCTGTACCTCCTCTGGTACTTCTCGTTCTCTGT</td>
<td></td>
</tr>
</tbody>
</table>
Supplementary Figure S1: Px RyR 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