

SUPPLEMENTAL FIGURE LEGEND

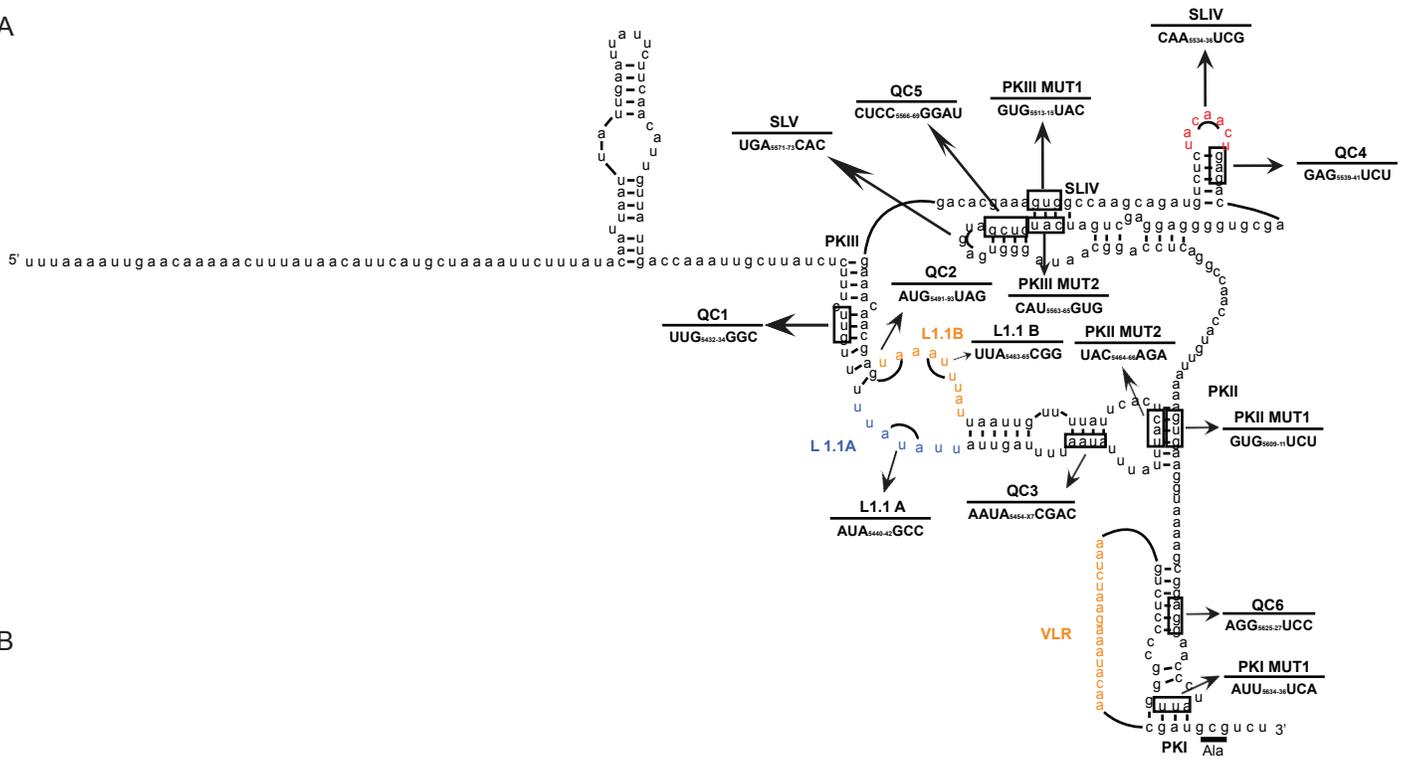
Figure S1: Predicted secondary model of BaDV-2 IGR IRES. A) Specific nucleotides were mutated to validate the predicted secondary structure of BaDV-2 IGR IRES. B) The luciferase activities were measured through luciferase assays for each mutation via bicistronic reporter RNA assays in Sf-21 extracts. A one-way ANOVA statistical test was used to determine the p value and thus the significance levels. Groups were normalized to bicistronic RNAs containing WT BaDV-2. * $p < 0.05$, ** $p < 0.01$. “n.s” denotes the difference is not significant between the experimental groups and WT ($p > 0.05$). Shown are the averages from at least three independent experiments \pm standard deviation.

Figure S2: BaDV-2 IGR IRES activity in mock-infected and CrPV-infected cells. Bicistronic reporters were transfected into S2 cells followed by mock infection or CrPV infection (MOI = 20). Cells were collected 6 hours post transfection. Bicistronic RNAs tested contain the wild-type CrPV, mutant CrPV (CC6214-5 to GG to disrupt PKI basepairing), wild-type BaDV-2 full-length IGR IRES (WT BaDV-2), BaDV-2 3' DEL 5 and BaDV “minimal” IRES (double deletion at both 3' and 5' end. A) Renilla luciferase activities and B) ratio of Renilla/Firefly luciferase activities were measured and normalized to wild-type CrPV with mock infection. A paired t-test was used to determine the p value and thus the significance levels. * represents $p < 0.05$, ** represents $p < 0.01$. “n.s” denotes the difference is not significant between the control groups and the experimental groups ($p > 0.05$). Shown are the averages from at least three independent experiments \pm standard deviation.

Figure S3: SHAPE reactivity of BaDV-2 IRES. In vitro transcribed RNA was analyzed for SHAPE reactivity. Denatured vs folded RNA in the presence or absence of NMIA were analyzed by amplicon next-gen sequencing (UBC Sequencing and Bioinformatics Consortium). Relative

SHAPE reactivities for each nucleotide are depicted with colors indicated to the bottom right and are from three independent experiments.

A



B

