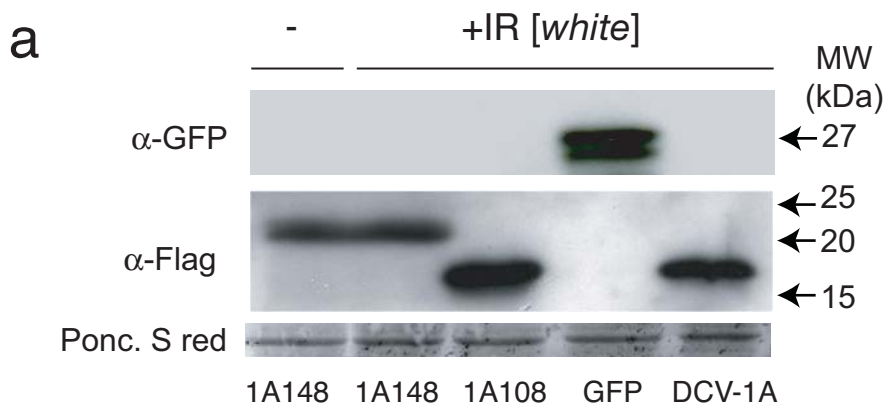
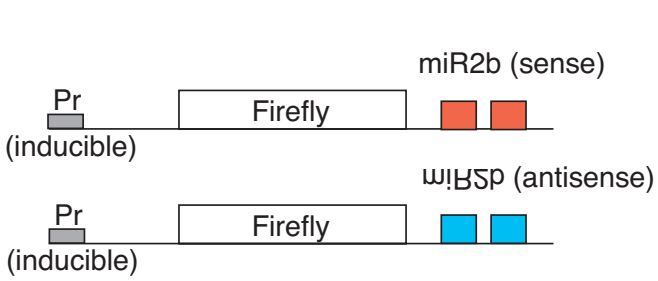
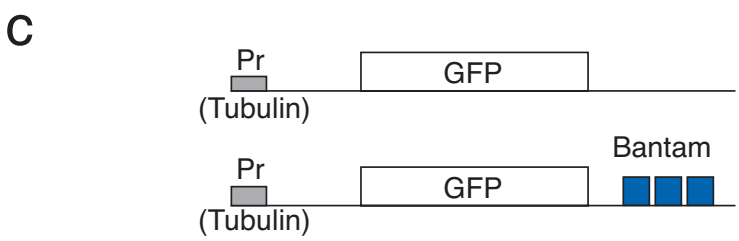
Supplementary Fig. 1
Nayak et al.







Supplementary Fig. 1 (a) Expression of the suppressor protein in transgenic flies expressing CrPV-1A148 or CrPV-1A108, or DCV-1A99 or GFP and an inverted repeat [IR] directed against the *white* gene was analyzed by western blot analysis. Suppressor proteins were tagged with Flag tag and were detected using monoclonal anti-Flag antibodies (α-Flag). Green fluorescent protein (GFP) expression was determined using rabbit polyclonal antibodies (α-GFP). Loading control for each lane was verified by Ponceau S red (Ponc. S red) staining. (b) Firefly luciferase DNA reporter carries 2 copies of microRNA 2b (miR2b) target sequence in the 3′ untranslated region (UTR) either in sense or antisense orientation. The expression of the reporter gene is under the control of a metallothionein inducible promoter (Pr) (c) GFP DNA reporter contains a tubulin promoter (Pr) and with or without three complementary bantam micro RNA target sequence in the 3′ UTR.

Supplementary Fig. 2
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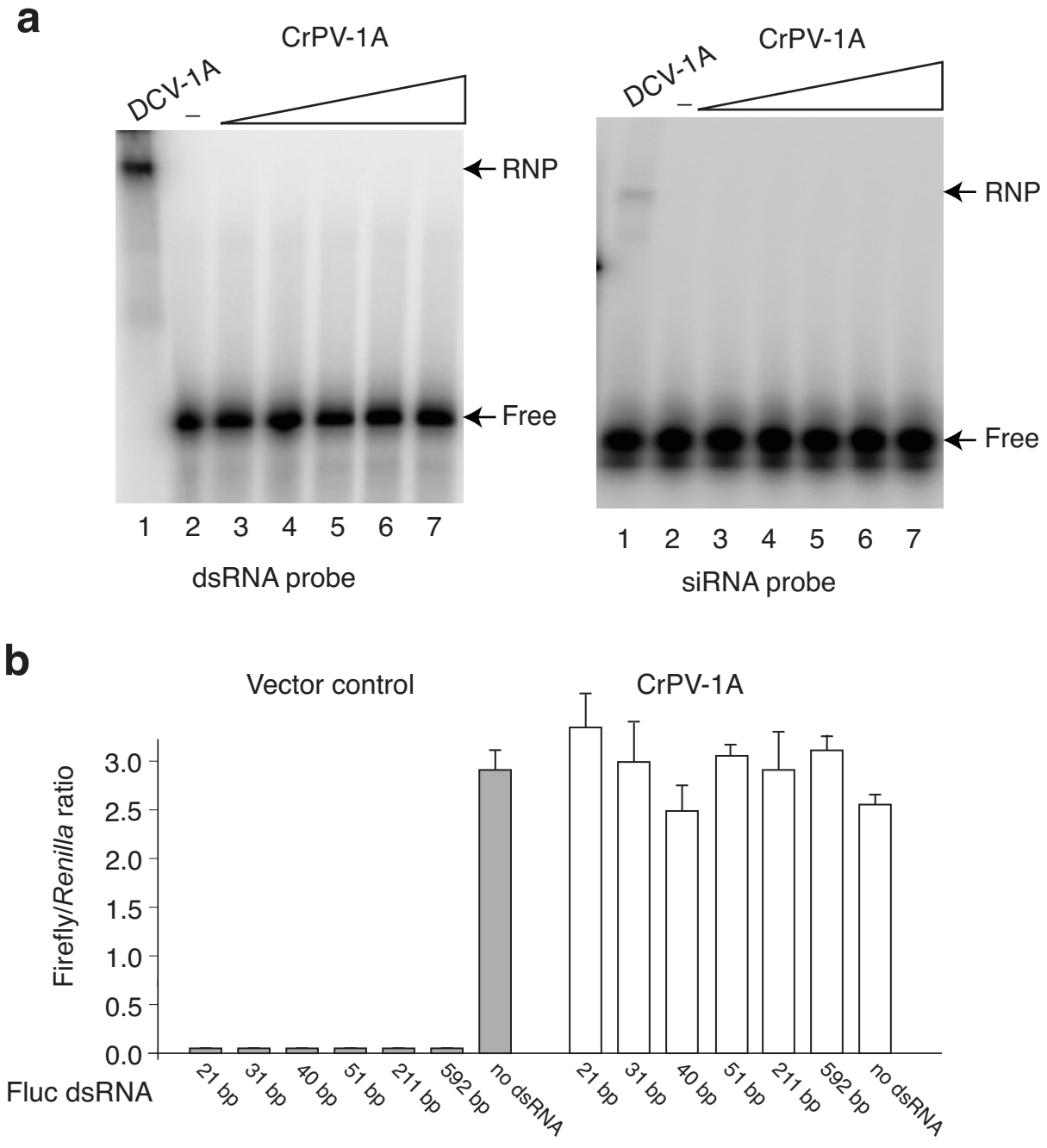

Supplementary Fig. 2 CrPV-1A does not bind RNA and RNAi inhibition by CrPV-1A is independent of dsRNA size. (a) Electrophoretic mobility shift assay was performed using purified recombinant protein with either 200 bp dsRNA probe (left panel) or 21 bp siRNA probe (right panel). Each probe was incubated with buffer (lane 2) or fixed concentration of DCV-1A (0.35 μM, lane 1) and increasing concentration of CrPV-1A [lanes 3 (0.11 μM), lane 4 (0.3 μM), lane 5 (0.6 μM), lane 6 (0.9 μM), lane 7 (1.2 μM)]. Ribonucleoprotein (RNP) complexes were analyzed using native polyacrylamide gel electrophoresis (b) RNAi suppression assay was carried out as described (Fig. 1d) using different sizes of dsRNA that include 21bp, 31bp, 40bp, 51bp, 211bp, 592bp and no dsRNA control. Silencing was expressed by taking the ratio of FIrefly to *Renila* luciferase expression in presence of either F-luc dsRNA or no dsRNA control.

Supplementary Fig. 3
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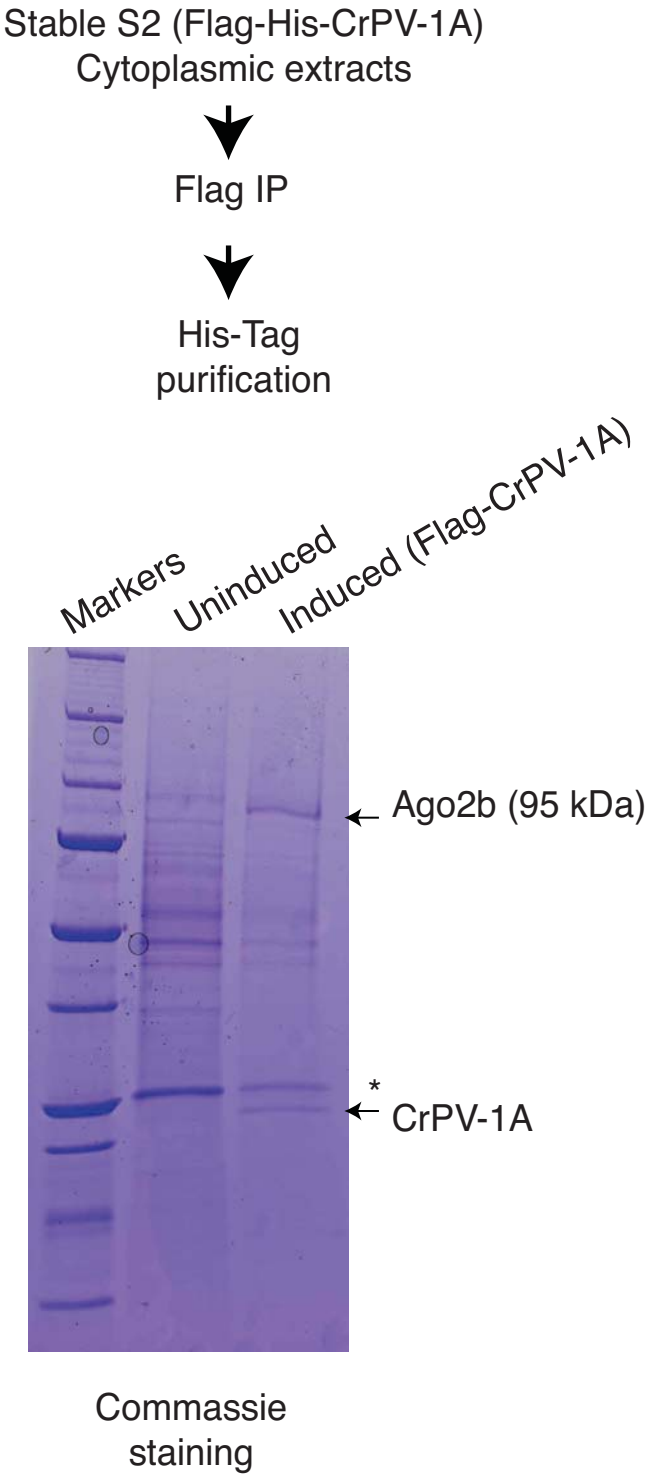

Supplementary Fig. 3 CrPV-1A-Argonaute 2 complex purification from S2 cells. Stable S2 cells either expressing Flag-His-tagged CrPV-1A (induced with CuSO4) or uninduced control was lysed. After two steps affinity purification (Flag IP and His tag purification) the eluted sample was coomassie stained. Bands with asterisks in the coomassie gel represent the host protein co-purified both in the un-induced (mock, lane 2) and induced sample (lane 3). Bands with ⊠ (bottom and top) in lane 3 were excised for mass spectrometry analysis. Data analysis revealed the bands to be CrPV-1A and 95 kDa isoform of Argonaute 2 (Ago2b) respectively.

Supplementary Fig. 4
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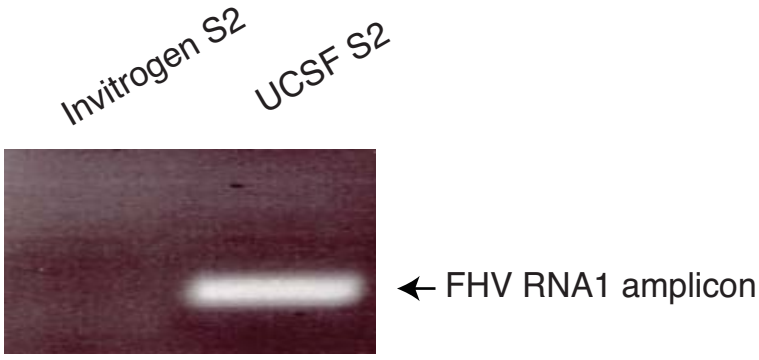

Supplementary Fig. 4 Detection of Flock House Virus (FHV) contamination in S2 cells. Total RNA from Invitrogen and UCSF S2 cells were isolated and cDNA were synthesized using Thermoscript RT enzyme (Invitrogen) and oligo-dT primer. cDNA templates were used for polymerase chain reaction (PCR) to amplify FHV specific RNA1 using primer set RNA1-F 5′-AGCAGCCATGGGAATGAGC and RNA1-R-5′ CTTCCGGTTGTTGGAAGGC. PCR analysis showed that UCSF S2 cells are persistently infected with FHV whereas Invitrogen cells are free of FHV. Based upon this observation we performed all the experiments using Invitrogen S2 cells.

Supplementary Fig. 5
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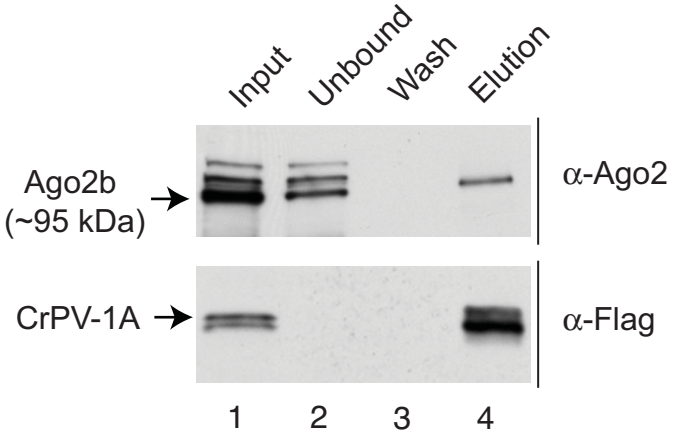

Supplementary Fig. 5 Purification profile of immunoprecipitation (IP) protocol for stable S2 (CrPV-1A-3FH) cells lines. S2 cells (10^8 cells) expressing CrPV-1A-3FH were resupended in IP buffer (see methods). Cytoplasmic extract (total 2 ml) was added to Flag-antibody conjugated magnetic beads, washed with IP buffer (2 mL) and eluted by boiling the beads in 100 µl Laemmli buffer. Equal volume (10 µl) of input (lane 1), unbound (lane 2), and wash (lane 3), 5 µl of elution (lane 4) was probed for Ago2 using rabbit polyclonal antibody (α-Ago2) (top panel). The same blot was stripped and probed for CrPV-1A using monoclonal anti-Flag antibody (α-Flag) (bottom panel). We observed that under our experimental conditions about 30% to 50% of Ago2 binds to the column, while majority of CrPV-1A (more than 95%) is retained. Accordingly, we observed a substantial amount of Ago2 in the unbound fraction (lane 2), while CrPV-1A is undetectable.