Supplementary Figure Legends

Figure S1. Purification of recombinant VSS proteins.

SDS-PAGE and CBB staining for recombinant VSS proteins purified by nickel-chelating affinity chromatography. CrPV-1A, 17.5 kDa; DCV-1A, 11.3 kDa; FHV-B2, 11.6 kDa. A control experiment confirmed that the residual contaminating proteins do not have any effects on the target cleavage activity by Ago2-RISC (data not shown).

Figure S2. CrPV-1A inhibits target cleavage independently of the GC content.

(A) Additional GC pairs between the guide strand and the target RNA in the seed region or the 3′ supplementary region.

(B) Target cleavage assay in S2 cell lysate with 0.05–0.5 μM CrPV-1A for the series of guide-target configurations in A.

(C) Quantification of the cleavage assay in B. Error bars indicate the SD from three independent experiments.

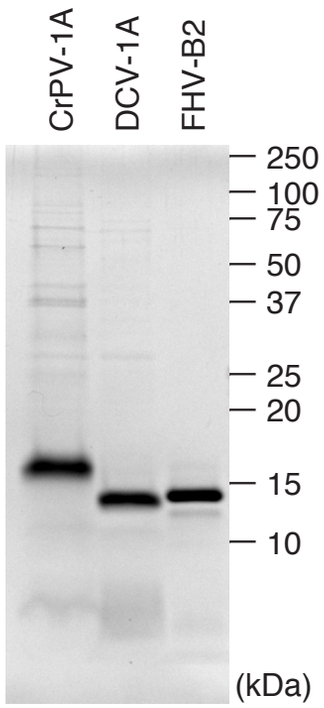
Figure S3. DCV-1A does not inhibit target binding by Ago2-RISC.

Target binding assay by immunopurified Ago2-RISC with 1 μM CrPV-1A, DCV-1A, or BSA.

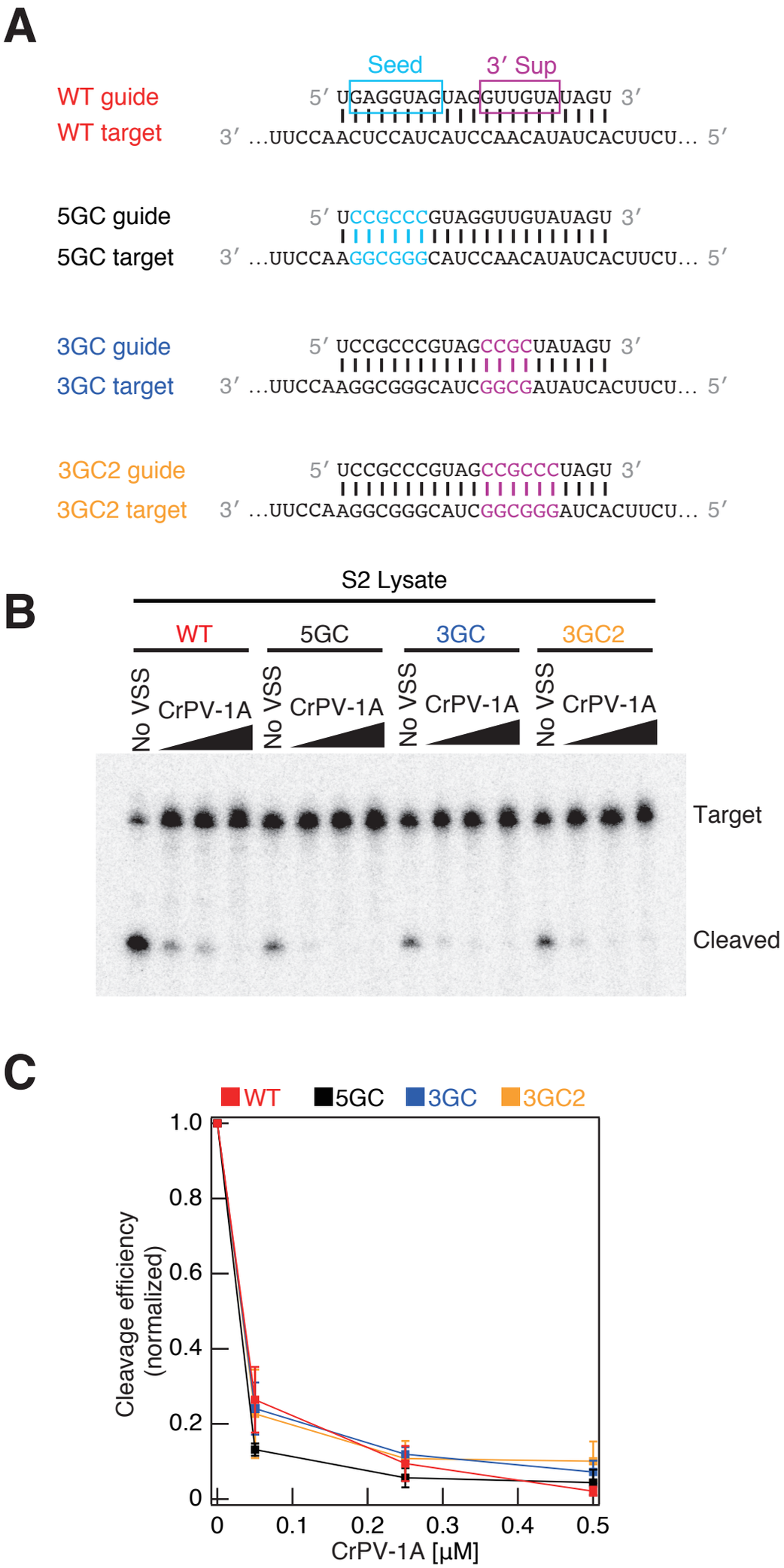
Figure S4. CrPV-1A preferentially inhibits target binding by Ago2-RISC.

(A) Target binding assay by immunopurified Ago1-RISC or Ago2-RISC. The radiolabeled target RNA was perfectly complementary to the guide strand as in Figure 2B. CrPV-1A was added at the concentrations of 0.01–1.0 μM in the target binding assay. Mock indicates mock immunopurification from naive S2 lysate.

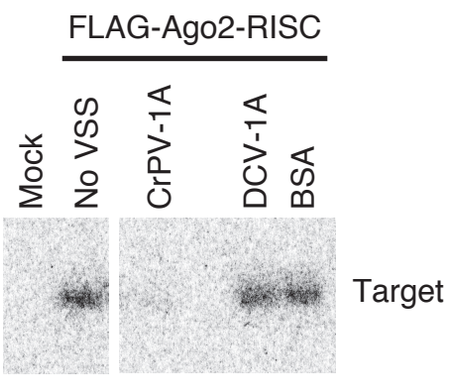
(B) Quantification of the binding assay in A. Error bars indicate the SD from three independent experiments.



Supplementary Fig. 1

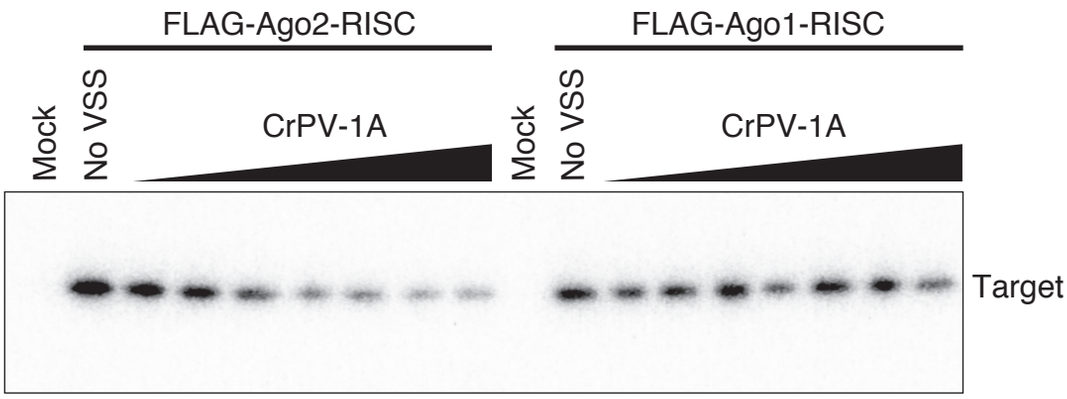


Supplementary Fig. 2

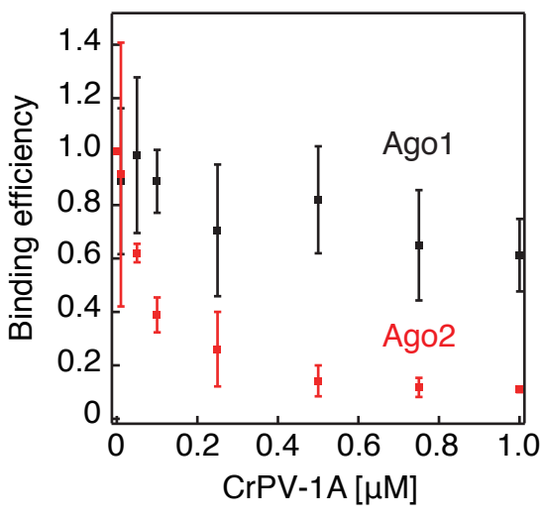


Supplementary Fig. 3

A



B



Supplementary Fig. 4