

## Supplementary Data

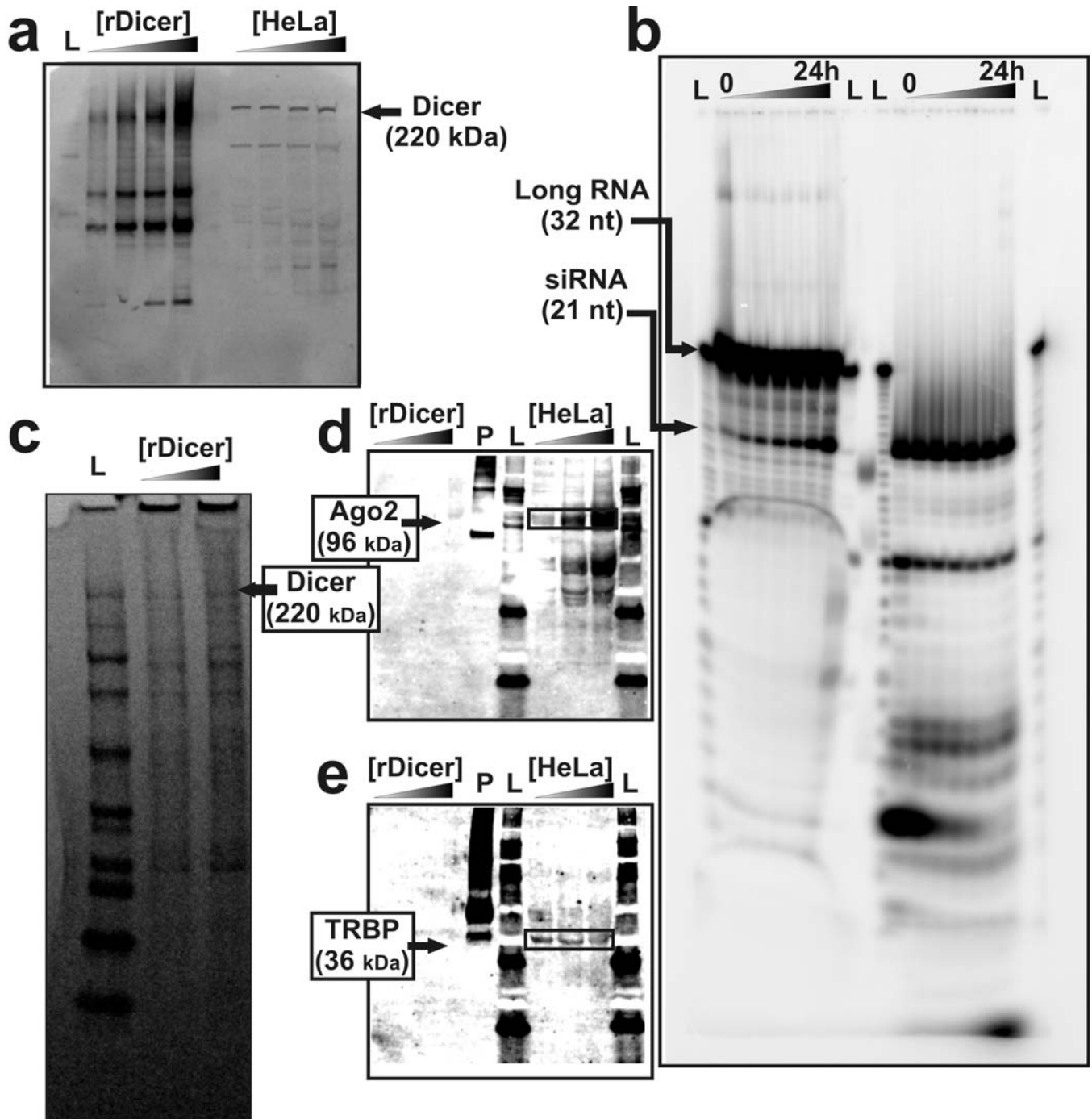
# **Viral RNAi suppressor reversibly binds siRNA to outcompete Dicer and RISC via multiple-turnover**

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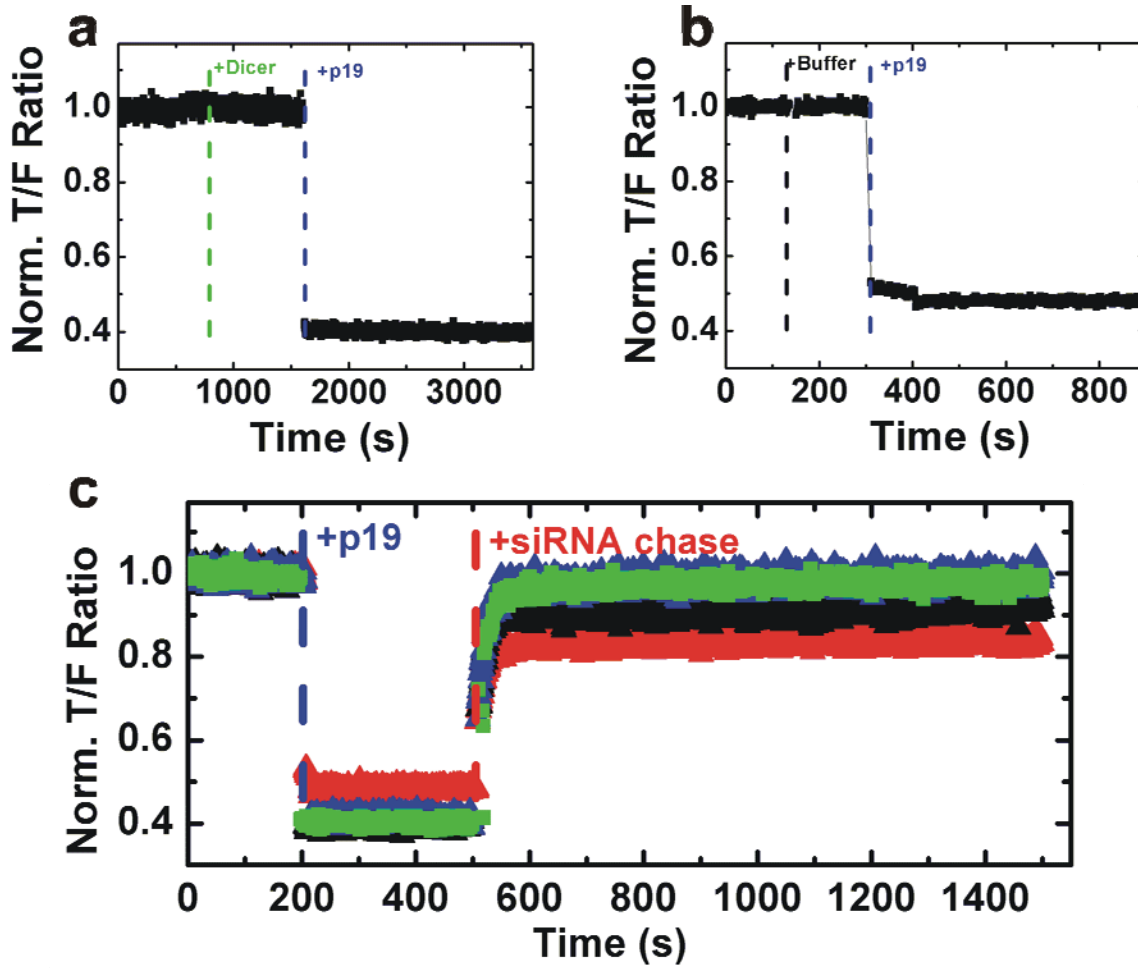
Rawlings et al, Figure S1



**Fig. S1.** Characterization of recombinant (r)Dicer and HeLa cell extract. (a) Denaturing Western blot against Dicer showing the presence of full length Dicer protein (218 kDa) in both extracts. Lane 1 (from left, marked “L”): Full-Range Rainbow Molecular Weight Marker (RPN800E). Lanes 2-5: Recombinant Dicer (Genlantis) – 3, 6, 9, and 12  $\mu$ L, respectively. Lanes 6-9: Cytosolic HeLa cell extract – 3, 6, 9, and 12  $\mu$ L, respectively. The multiple bands

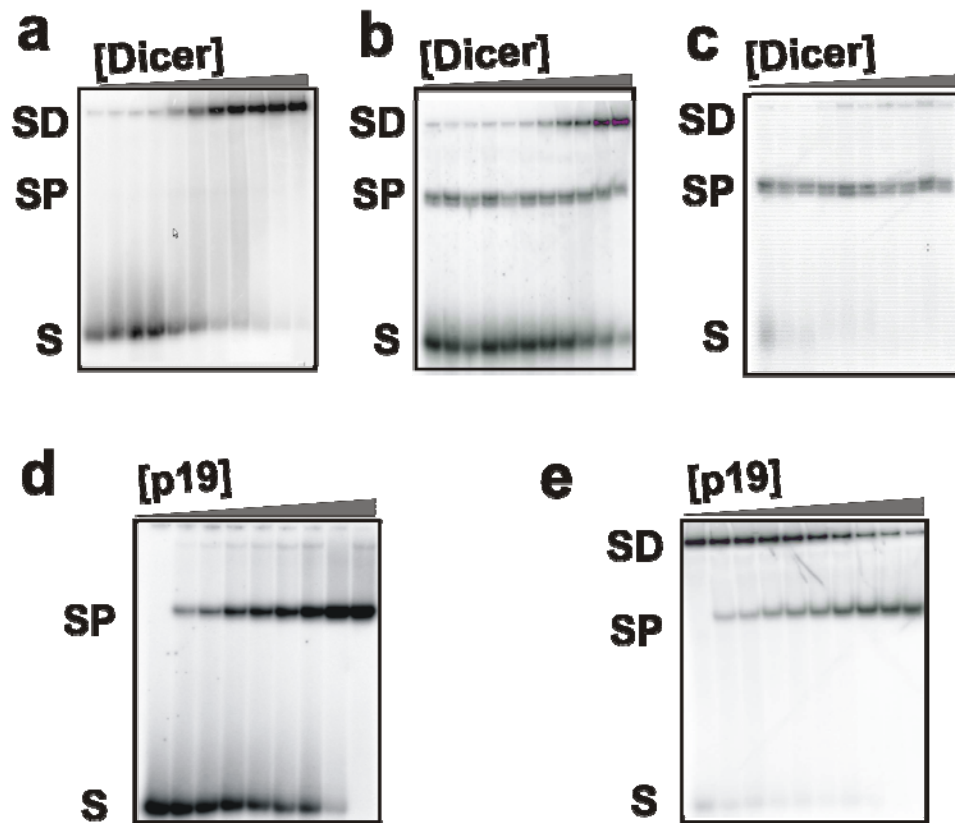
seen on the Western blot may result from degradation of Dicer or cross-reactivity of the antibody. (b) Cleavage assay measuring the activity of recombinant Dicer. Recombinant Dicer was incubated with radio-labeled double-stranded 32 nucleotide RNA for 1, 3, 5, 7, 9, 12, and 24 h in the Genlantis supplied buffer (lanes 2-8 from left to right) and in the near-physiologic standard buffer (lanes 12-18) used for the Dicer assays (50 mM Tris-Acetate, pH 7.4, 80 mM KCl, 20 mM NaCl, 1 mM MgCl<sub>2</sub>, 1 mM DTT, 0.02% (v/v) Tween 20). In the company-supplied buffer, cleavage was observed over the recommended incubation time of 12 h. In the near-physiologic buffer, all RNA was 100% cleaved into mature siRNA as early as 1 h after incubation. Lane L: Sequencing ladder from partial digestion of single stranded 32 nucleotide RNA with alkali-ladder. (c) Coomassie stained 4-12% SDS-PAGE gel. Lane 1 (from left, marked "L"): Full-Range Rainbow Molecular Weight Marker (RPN800E). Lane 2: Recombinant Dicer (Genlantis) – 5 μL. Lane 3: Recombinant Dicer (Genlantis) – 10 μL. Fraction of full length Dicer was calculated as 5 ± 2% (d) Western blot of recombinant Dicer and HeLa cell extract against Ago2. Lane P: Recombinant EIF2C2-GST tag protein; 67.58 kDa (Abnova- positive control). (e) Western blot of recombinant Dicer and HeLa cell extract against TRBP. Lane P: Recombinant TRBP-His Tag protein; 52 kDa (Abcam- positive control).

Rawlings et al, Figure S2



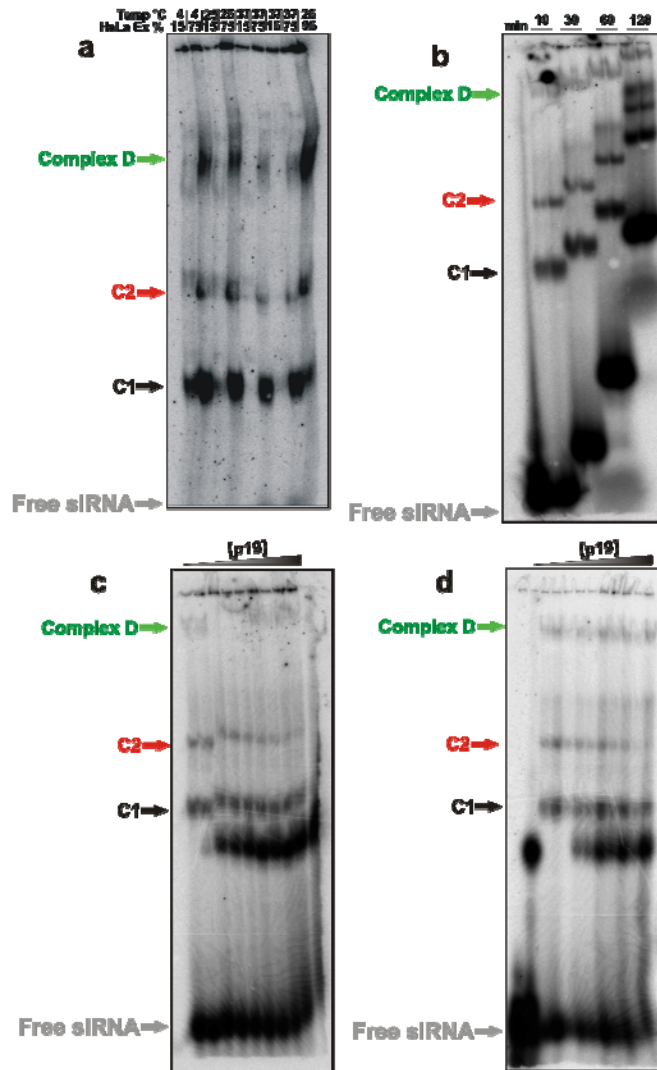
**Fig. S2.** Concentration dependence of siRNA:p19 dissociation kinetics. (a, b) Doubly-labeled siRNA (50 nM) after addition of Dicer (5 nM green dotted line) or Buffer (black dotted line) respectively, followed by addition of p19 (500 nM, blue dotted line). (c) Doubly-labeled siRNA (50 nM) was bound by p19 (500 nM, blue dotted line) after which excess unlabeled siRNA was added at concentrations of 500 nM (red), 750 nM (black), 1.5  $\mu$ M (green), 3  $\mu$ M (blue), respectively.

Rawlings et al, Figure S3



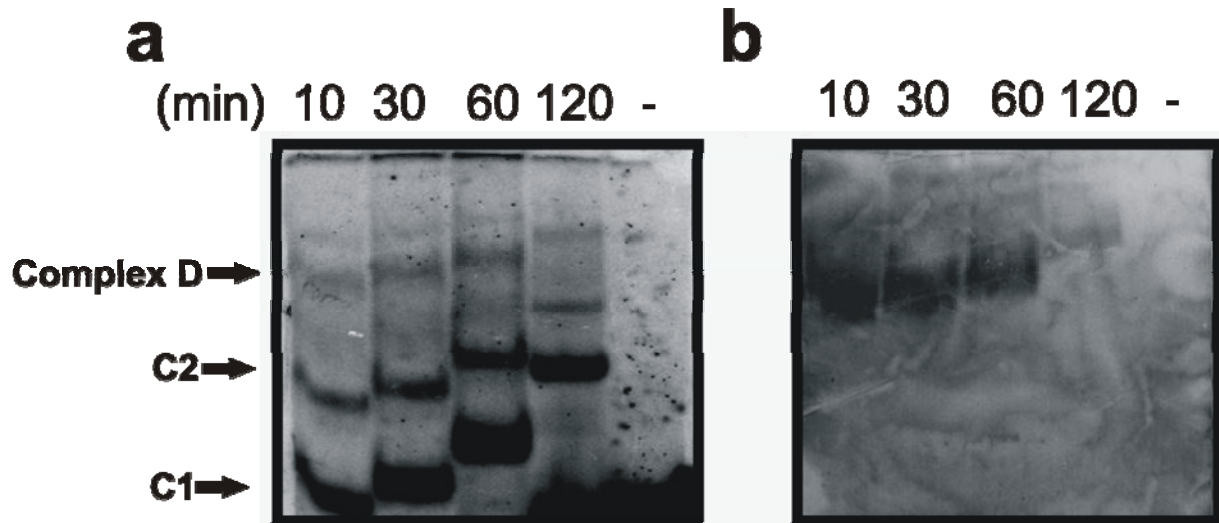
**Fig. S3.** Competition between p19 and human Dicer for siRNA binding as shown by EMSAs. siRNA (S) labeled with  $[\gamma\text{-}^{32}\text{P}]$  ATP are bound to (a) Recombinant human Dicer (D) (Genlantis Inc.) where Dicer concentrations are 0, 0.16, 0.25, 0.5, 2.5, 5, 10, 16, 50, 100, 200 nM, respectively. Samples were incubated for 30 min before loading onto a 12% nondenaturing polyacrylamide gel. (b) siRNA pre-incubated with p19 at a concentration of 0.16 nM for 30 min followed by Dicer addition as described above. (c) siRNA pre-incubated with p19 at a concentration of 2.56 nM for 30 min followed by Dicer addition as described above. (d) p19 only (P) where p19 concentrations are 0, 0.02, 0.04, 0.08, 0.16, 0.32, 0.64, 1.28, 2.56, 5.12 nM, respectively. Samples were incubated for 30 min before loading onto a 12% nondenaturing polyacrylamide gel. (e) siRNA pre-incubated with Dicer at a concentration of 140 nM for 30 min followed by p19 addition as described in panel d.

## Rawlings et al, Figure S4



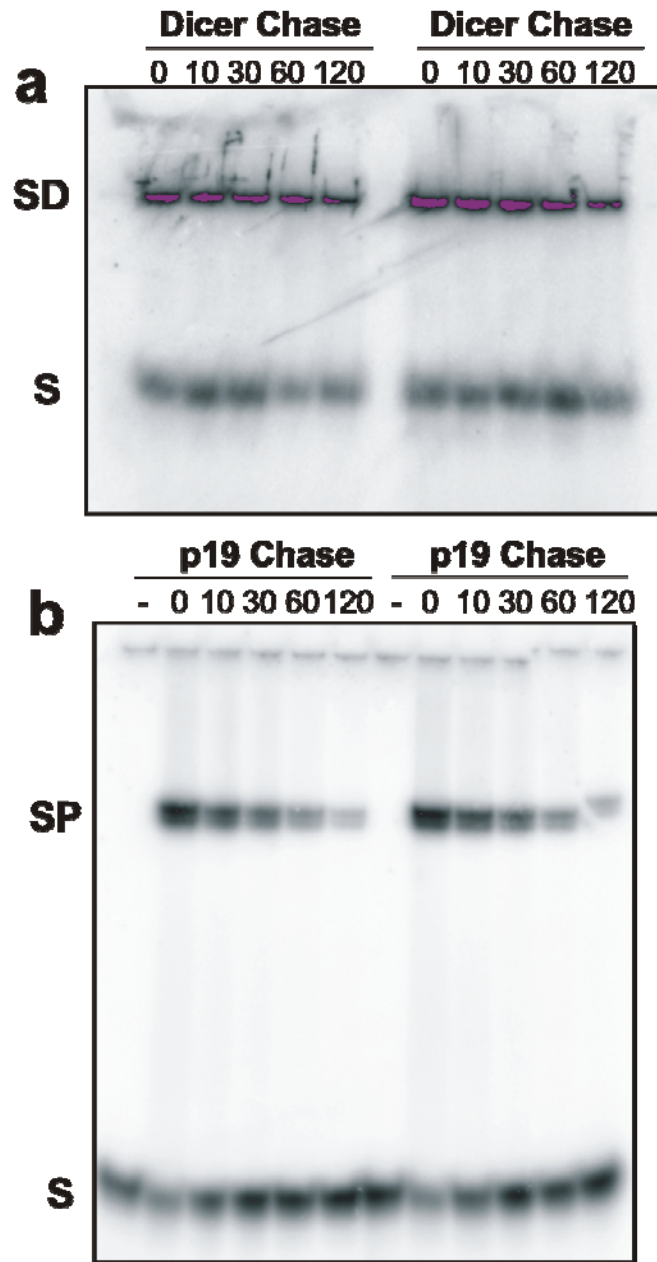
**Fig. S4.** Comparison of siRNA complex formation for different HeLa cell extract concentrations and temperatures. (a) (lanes 1-2) 15% or 75% (v/v) HeLa extract conditions incubated with siRNA for 1 h at 4 °C, (lanes 3-4) incubated at 25 °C, (lanes 5-8) incubated at 37 °C, and (lane 9) 93% (v/v) HeLa cell extract in the absence of an ATP generating system and in supplied buffer (20 mM HEPES-Na pH 7.9, 42 mM ammonium sulfate, 0.2 mM EDTA, 0.5 mM DTT and 20% glycerol). The effect of incubation temperature and HeLa extract concentration on complex formation was limited. The same migration patterns are seen under all conditions. (b) Formation of complexes D, C2, and C1 after 10, 30, 60 and 120 min incubation of siRNA in 50% cytosolic HeLa cell extract. Samples were loaded onto a running 4% native polyacrylamide gel, leading to the indicated differences in migration. (c) Formation of complexes after 2.5 h incubation of siRNA in 50% (v/v) cytosolic HeLa cell extract, and increasing concentrations of p19. (d) Formation of complexes after 2 h incubation of siRNA in 50% (v/v) cytosolic HeLa cell extract, followed by the addition of increasing concentrations of p19 and further incubation for 30 min.

Rawlings et al, Figure S5



**Fig. S5.** Non-denaturing western blot detection of Dicer. (a) A scaled-up version of the non-denaturing time course gel in (Figure 4B), imaged for radio-labeled siRNA, yields sufficient complexes for Western blot detection of Dicer. (b) Western blot of a similar non-denaturing gel to (a) probed with a rabbit primary antibody against Dicer, followed by a goat anti-rabbit secondary antibody, conjugated with horseradish peroxidase. Samples were loaded onto a running gel, leading to the observed differences in migration.

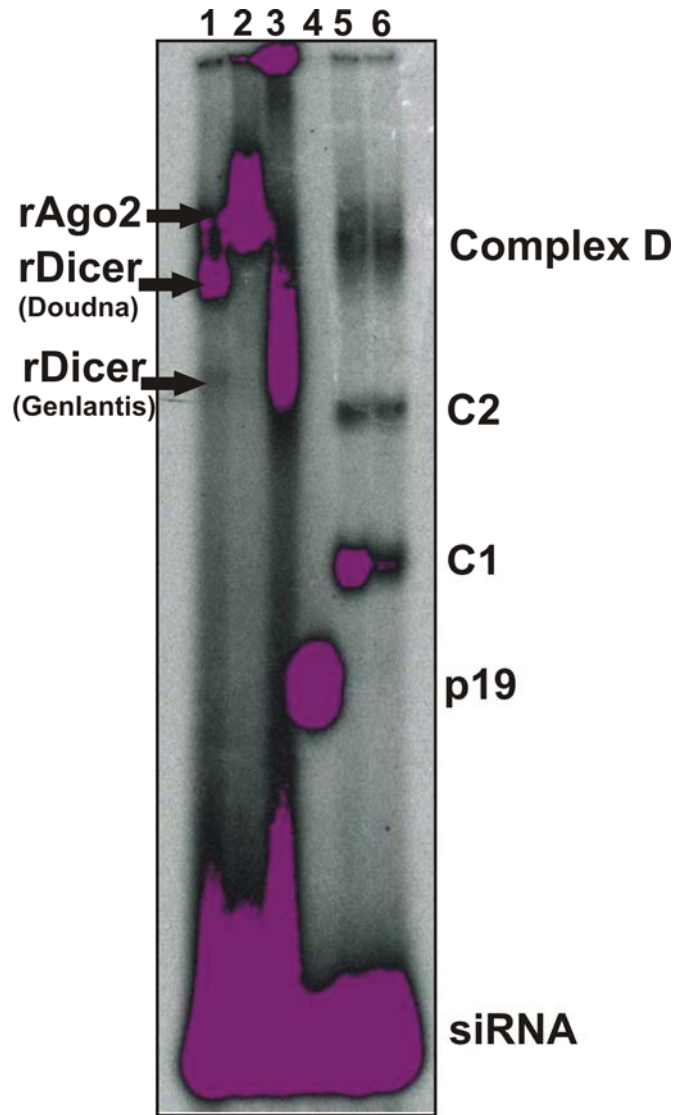
Rawlings et al, Figure S6



**Fig. S6.** Gel-based dissociation chase assays. Duplicate gel chase experiments were done in standard buffer with (a) 70 nM recombinant Dicer or (b) 10 nM of p19 added to 5'-<sup>32</sup>P labeled siRNA before addition of 750 nM unlabeled siRNA chase at 0, 10, 30, 60 and 120 min.

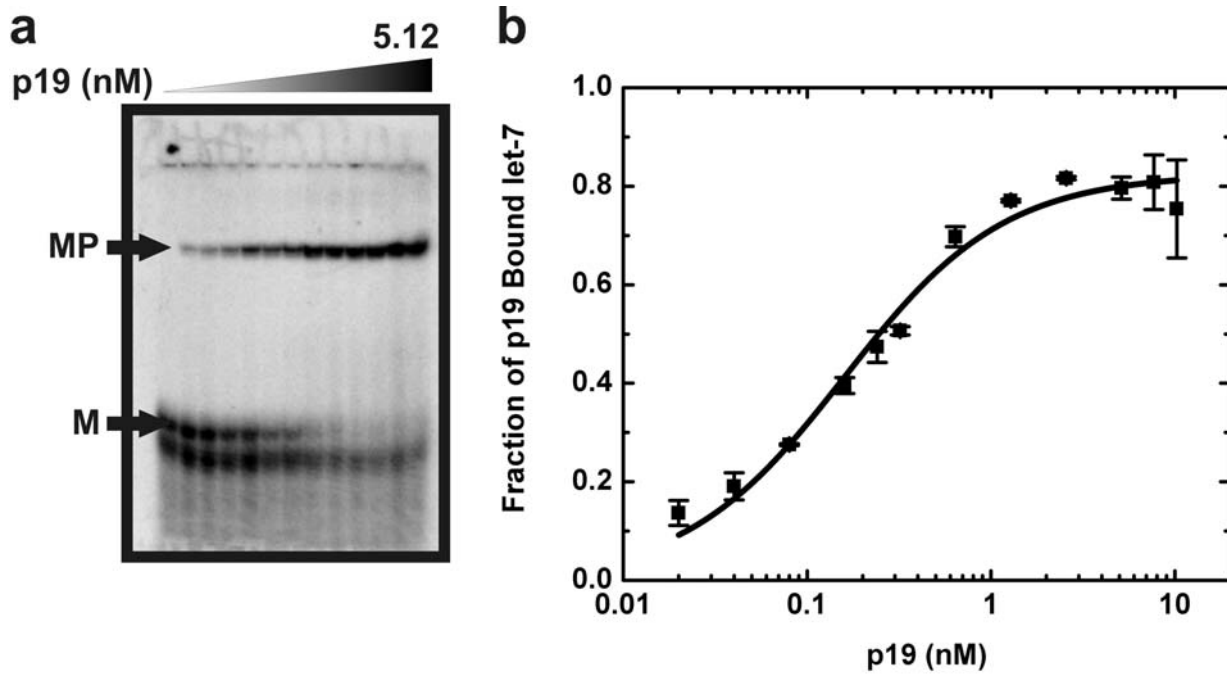


Rawlings et al, Figure S7



**Fig. S7.** Size comparison of protein-siRNA binding complexes. [ $\gamma$ - $^{32}$ P] ATP labeled siRNA incubated with: purified rDicer provided from the J. Doudna lab (lane 1); purified recombinant Ago2 protein provided from the J. Doudna lab (lane 2); rDicer (Genlantis Inc.; lane 3); purified p19 (lane 4); 15% (v/v) HeLa cell extract (lanes 5, 6). All samples were incubated for 30 min before loading. Nondenaturing 4% (w/v) polyacrylamide gel electrophoresis was used to resolve these complexes as described above. Although Dicer is a larger protein (MW 218 kDa) compared to Ago2 (MW 97 kDa), it has an isoelectric point of 5.47 compared to 9.39 for Ago2. This difference contributes to Ago2 running as an apparently slower migrating complex in buffer at pH 7.4.

Rawlings et al, Figure S8



**Fig. S8.** Complex formation between p19 and miRNA let7a as shown by EMSAs. miRNA (M) labeled with  $[\gamma\text{-}^{32}\text{P}]$  ATP is bound to p19 (P) to form the complex (MP). (a) Representative EMSA. (b) Analysis of binding by EMSA yields a p19-miRNA affinity of  $0.16 \pm 0.02$  nM.