

## Expanded View Figures

**Figure EV1. Binding data for analysis of seed-supplementary bridging region.**

- A Variant-1 of miRNA-122 (with an AU-rich supplementary region) shown paired to target RNAs containing bridging regions of various lengths.
- B Fraction target bound at equilibrium versus Ago-miRNA concentration. Calculated  $K_D$  values indicated.
- C Variant-2 of miRNA-122 (with GC-rich supplementary region) shown paired to target RNAs containing bridging regions of various lengths.
- D Fraction target bound at equilibrium versus Ago-miRNA concentration. Calculated  $K_D$  values indicated.

Data information: All plotted data are the means of at least three independent replicates. Error bars indicate SEM.

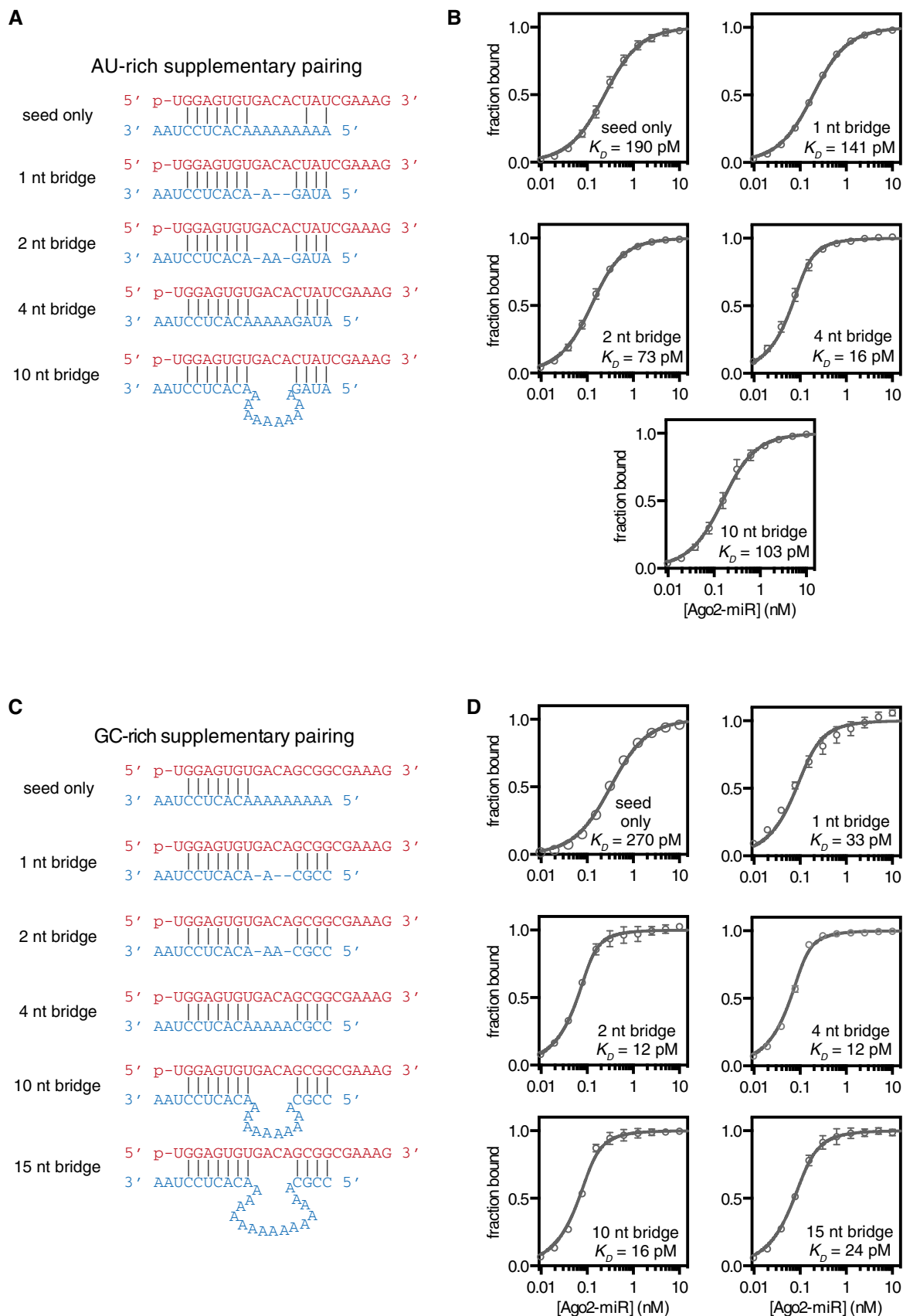
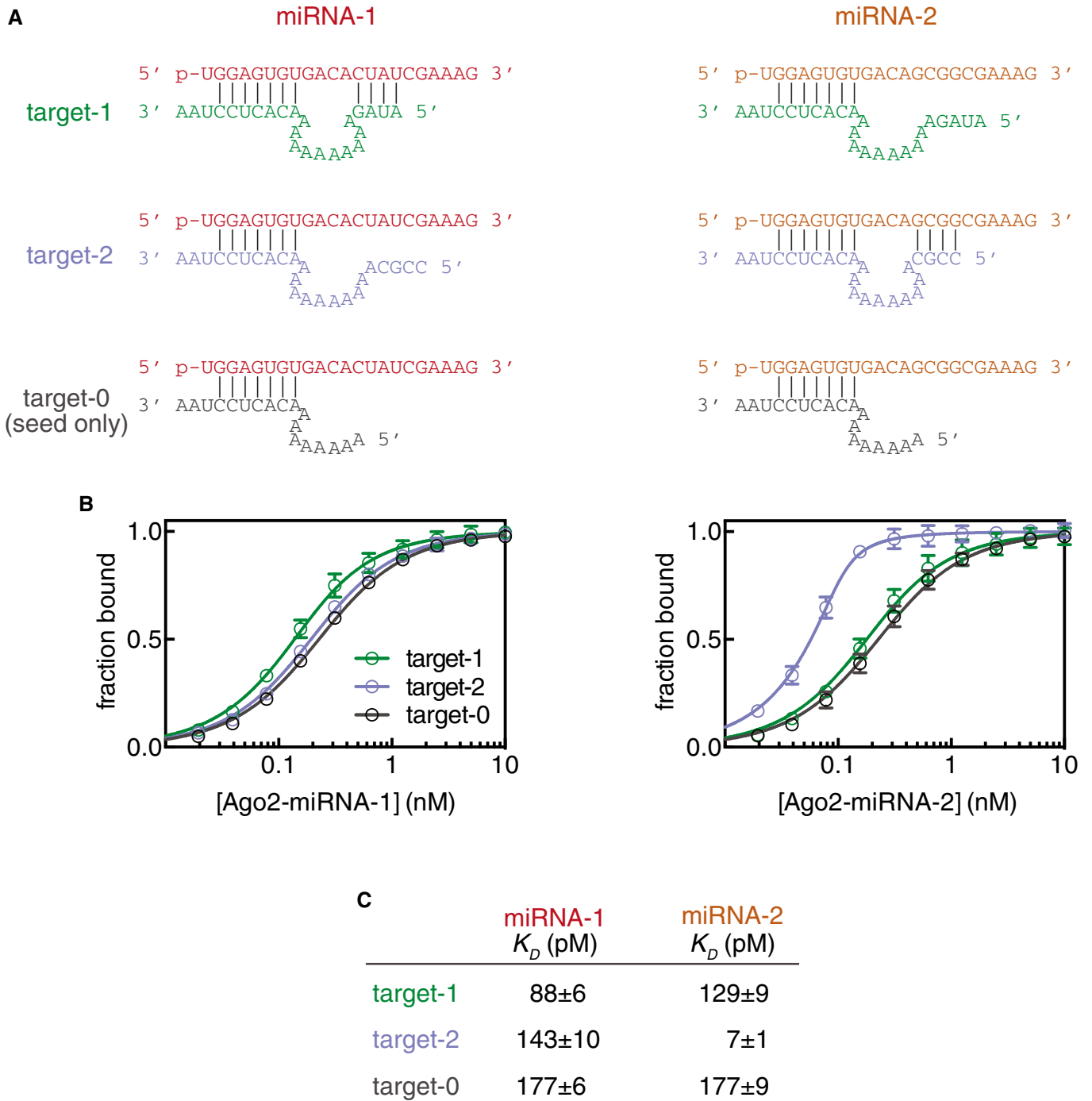


Figure EV1.



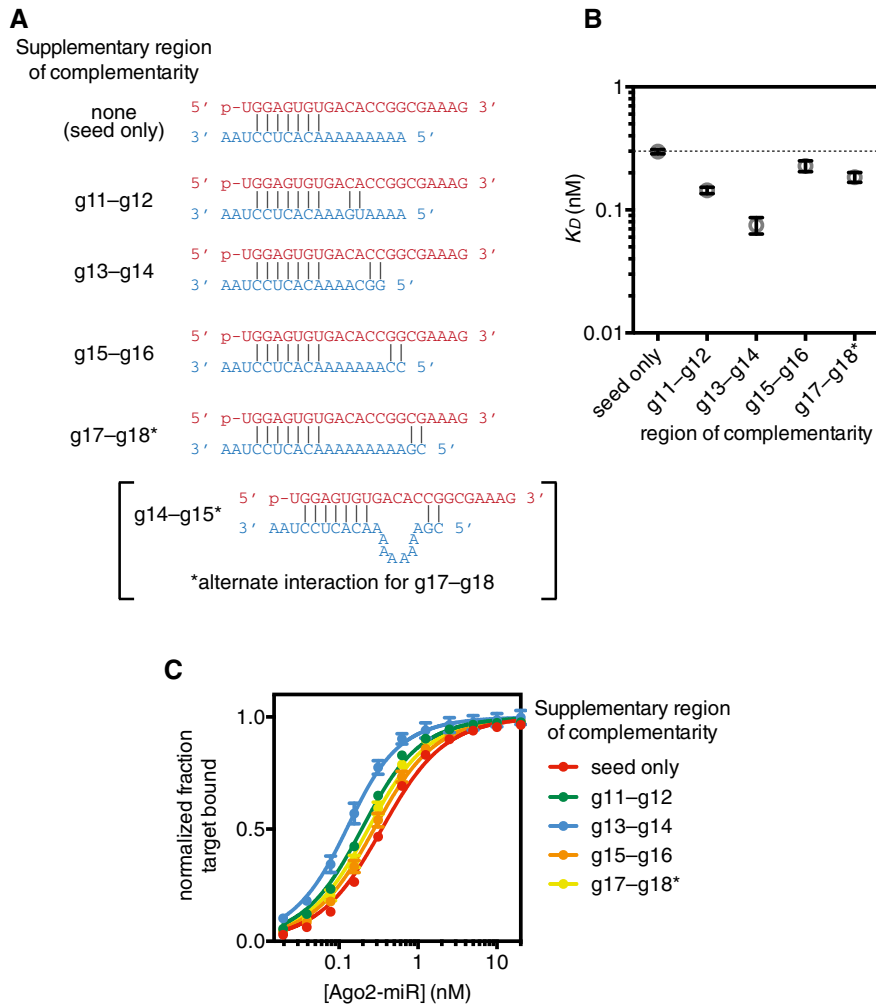
**Figure EV2. Cross-comparison of miRNA-122 and supplementary target variants.**

A Predicted base pairing interactions between two variants of miR-122 (miRNA-1 and miRNA-2) and three target RNAs (targets 1, 2 and 0).

B Fraction target bound plotted as a function of Ago2-miRNA concentration.

C Calculated dissociation constants ( $K_D$ ) from data shown in (B).

Data information: Values represent means ± SEM from three independent experiments.



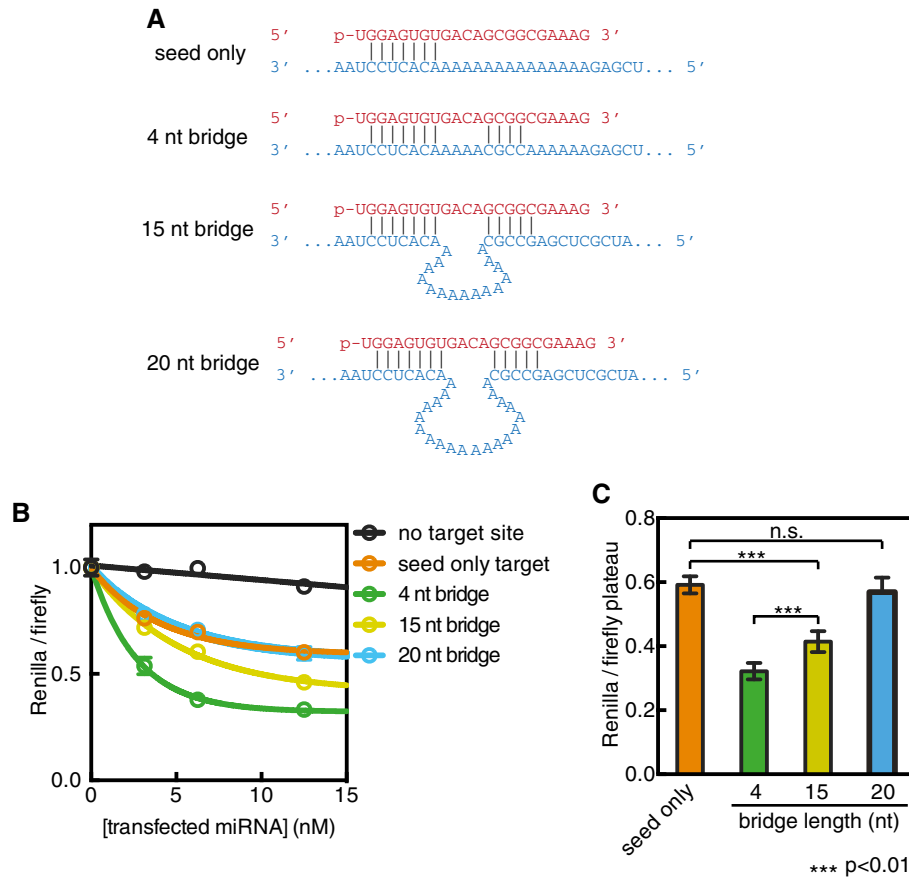
**Figure EV3. Analysis of di-nucleotide supplementary pairing.**

A Variant-2 of miRNA-122 (red) shown paired to target RNAs (blue) with supplementary interactions restricted to two adjacent base pairs. Two modes of interaction are possible for the g17-g18 target.

B Dissociation constants ( $K_D$ ) of the Ago2-miRNA complex binding to target RNAs in panel (A).

C Binding curves used to calculate  $K_D$  values in panel (B).

Data information: All plotted data are the means of at least three independent replicates. Error bars indicate SEM.



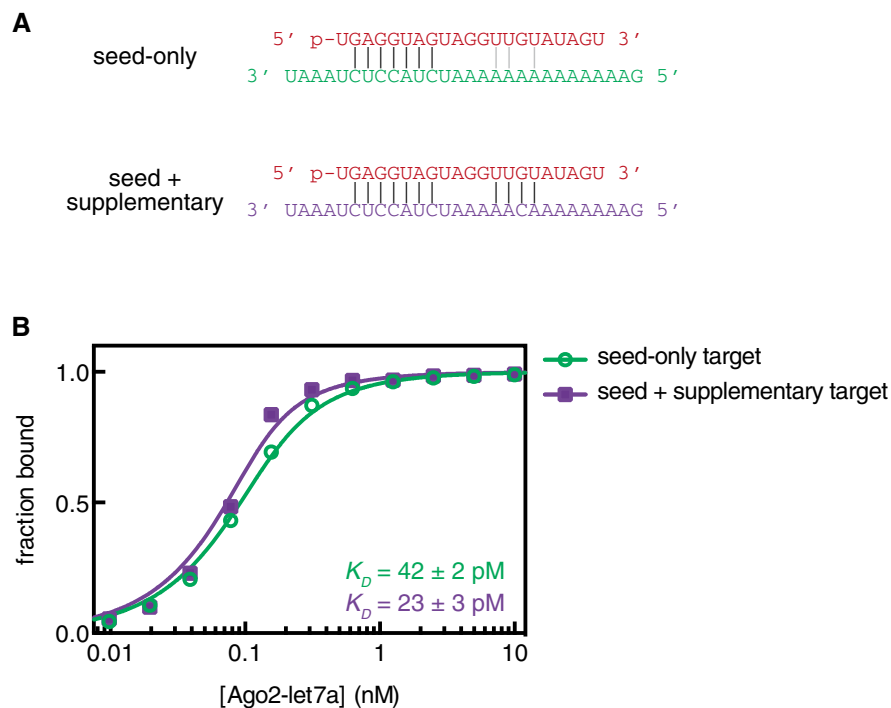
**Figure EV4. Impact of bridging region on target-reporter repression.**

A Variant-2 of miRNA-122 (red) shown paired to single target sites (blue) cloned into the 3' UTR of a Renilla luciferase reporter.

B Renilla luciferase levels (normalized to Firefly internal control) plotted as a function of miRNA concentration co-transfected into HEK 293 cells.

C Plateau values from panel (B) for targets with bridging regions of various lengths.

Data information: All plotted data are the means of at least four biological replicates. Error bars indicate SEM. *P* values of two-tailed, paired *t*-test are indicated.



**Figure EV5. Re-measurement of the affinity of a previously reported small RNA-target RNA pair.**

A let-7a (red) shown paired to seed-only (green) and seed plus supplementary (purple) target RNAs. RNA sequences and lengths are identical to those reported previously (Wee *et al*, 2012). Vertical black lines indicate predicted base pairing interactions. Gray lines indicate potential (previously unnoted) supplementary pairing interactions.

B Fraction target RNA bound versus Ago2-let7a concentration. Error bars indicate SEM. Dissociation constant ( $K_D$ ) values for the two targets are indicated with values representing means  $\pm$  SEM from three independent experiments.