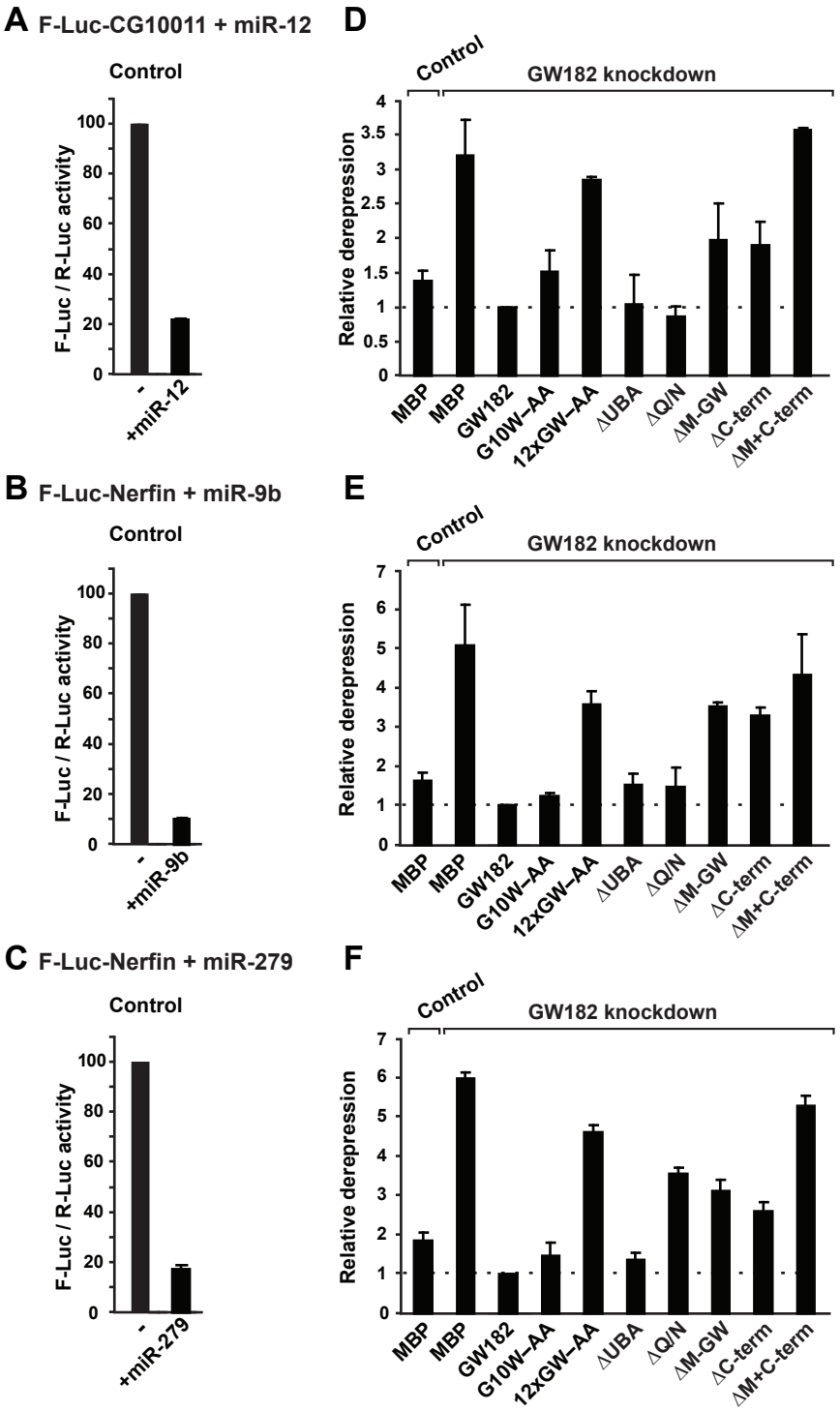
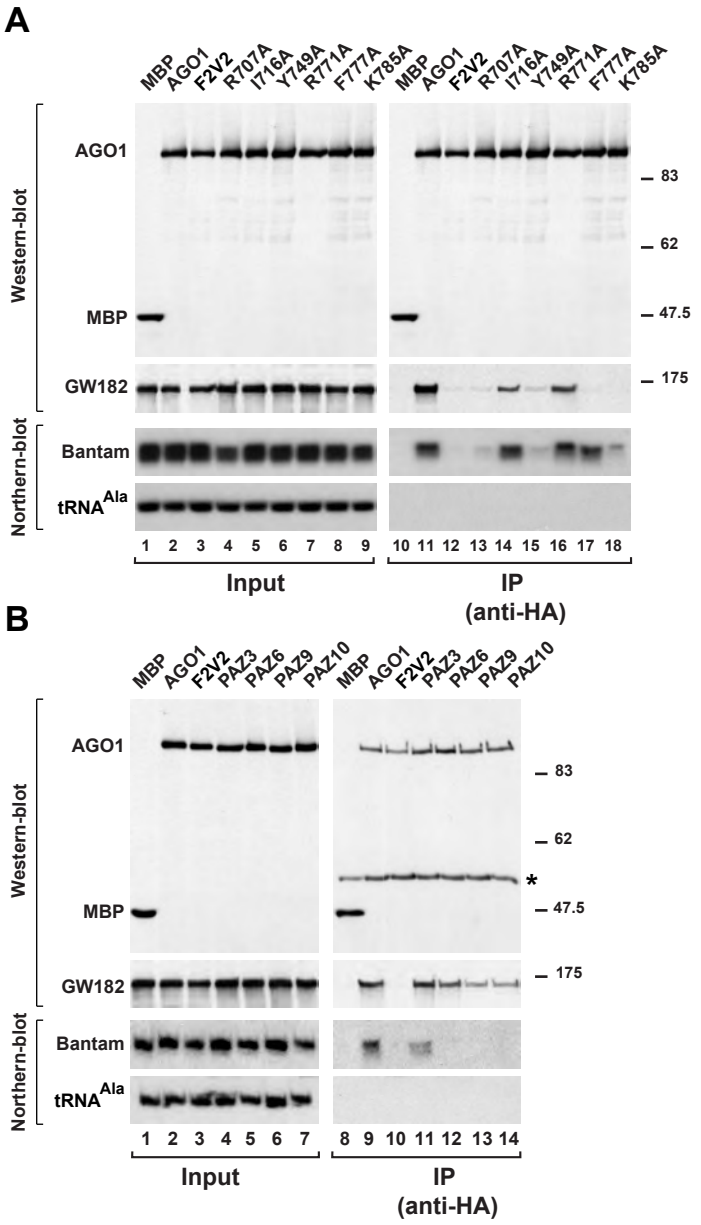
Eulalio et al. Supplemental Figure 1

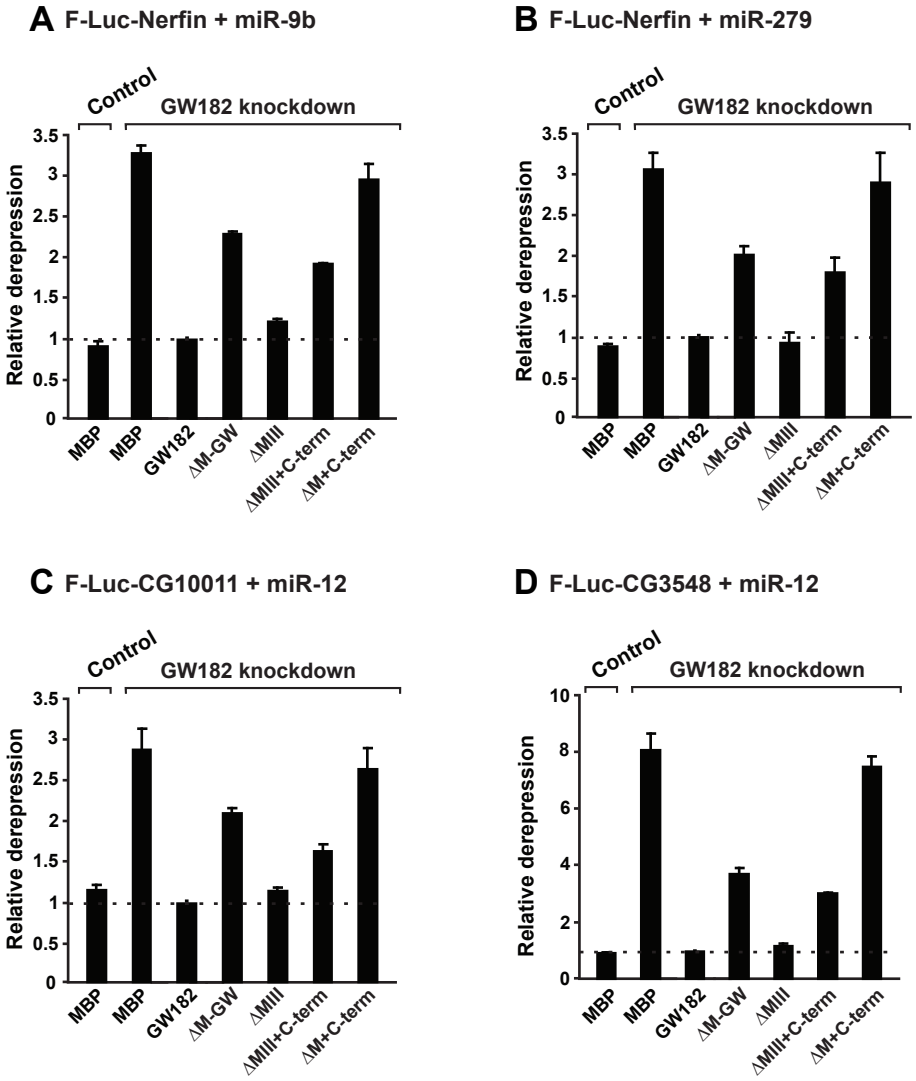


Supplemental FIGURE 1. Role of GW182 domains in silencing. (*A–F*) S2 cells were treated with dsRNA targeting the 5' and 3' UTRs of *GW182* mRNA. Control cells were treated with GFP dsRNA. These cells were subsequently transfected with a mixture of three plasmids: one expressing the indicated F-Luc reporters; another expressing miRNA primary transcripts (+miR-12, miR-9b or miR-279) or the corresponding empty vector (-); and a third expressing Renilla luciferase (R-Luc). Plasmids (1μg) encoding wild-type HA-GW182, HA-GW182 mutants or HA-MBP were included in the transfection mixtures, as indicated. Firefly luciferase activities were normalized to those of the Renilla luciferase transfection control and set to 100 in cells transfected with the empty vector (i.e. in the absence of the miRNAs). Panels (A–C) show normalized Firefly luciferase activities in the absence or presence of miRNAs in control cells. Panels (D–F) show relative fold derepression for each mutant tested relative to wild-type GW182. Mean values ± standard deviations from three independent experiments are shown.



Supplemental FIGURE 2. Mutations in AGO1 that uncouple miRNA and GW182 binding. (*A,B*) Lysates from S2 cells expressing HA-tagged versions of MBP, wild-type AGO1 or AGO1 mutants were immunoprecipitated using a monoclonal anti-HA antibody. Inputs (1.5%) and immunoprecipitates (30%) were analyzed by Western blotting using a polyclonal anti-HA antibody. Endogenous GW182 was detected by Western blotting using anti-GW182 antibodies. The association between HA-AGO1 and endogenous bantam was analyzed by Northern blotting. $tRNA^{Ala}$ served as a loading control. Asterisk indicates IgG cross-reactivity.

Eulalio et al. Supplemental Figure 3



Supplemental FIGURE 3. Role of GW182 motif III in silencing. (*A–D*) S2 cells were treated with dsRNA targeting the 5' and 3' UTRs of *GW182* mRNA. Control cells were treated with GFP dsRNA. These cells were subsequently transfected with a mixture of three plasmids: one expressing the indicated F-Luc reporters; another expressing miRNA primary transcripts (+miR-9b, miR-279 or miR-12) or the corresponding empty vector; and a third expressing Renilla luciferase (R-Luc). Plasmids (25ng) encoding wild-type HA-GW182, HA-GW182 mutants or HA-MBP were included in the transfection mixtures, as indicated. Firefly luciferase activities were normalized to those of the Renilla luciferase transfection control and set to 100 in cells transfected with the empty vector (i.e. in the absence of the miRNAs; not shown). Graphs show relative fold derepression for each protein relative to wild-type GW182. Mean values ± standard deviations from three independent experiments are shown.