Supplementary Figure 1. (A) HSP90 inhibitors destabilise Argonaute 2 in human cells. Hela (left panel) and U2OS (right panel) cells were treated with geldanamycin (GD) and its derivative (17AAG) for 16 hours with the indicated concentrations. Endogenous Ago2 level was assayed with Western blotting. βActin was used as a loading control. The relative normalized levels of hAgo2 are presented on the top of the panels. **(B) Ago3 is also sensitive to geldanamycin.** Hela cells were transfected with empty FLAG plasmid or FLAG-tagged human Ago3. Cells were treated with DMSO (M) or geldanamycin (GD, 10μM for 16 hours). Overexpressed Ago3 level was monitored with FLAG antibody. βActin was used as a loading control. **(C) HSP90 prevents proteosomal degradation of human Argonautes.** After HeLa cells were treated with geldanamycin (10μM for 16 hours) a proteasome inhibitor (MG132) was added to cells (20μM) for the indicated times. Endogenous hAgo1 and hAgo2 levels were assayed with western blotting. Tubulin was used as a loading control.

Supplementary Figure 2. (A) Graph shows the quantification of the Western blot presented in Figure 2A. The relative hAgo2 and GW182 protein levels between the mock and geldamycin treated cells were plotted at the indicated times after treatment. **(B)** Relative miR-21 level in geldanamycin treated cell as it was described in Figure 2B. **(C)** Representative Northern blots of *let-7*, miR-21, and U6 that were the basis of the quantifications presented in Figure 2A and Supplementary Figure 2A.

Supplementary Figure 3. (A) HeLa cells were treated with geldanamycin (10μM for 16 hours) and transfected with luciferase reporters that measure RNAi mediated by endogenous *let-7*. The dual luciferase data obtained from the geldanamycin treated

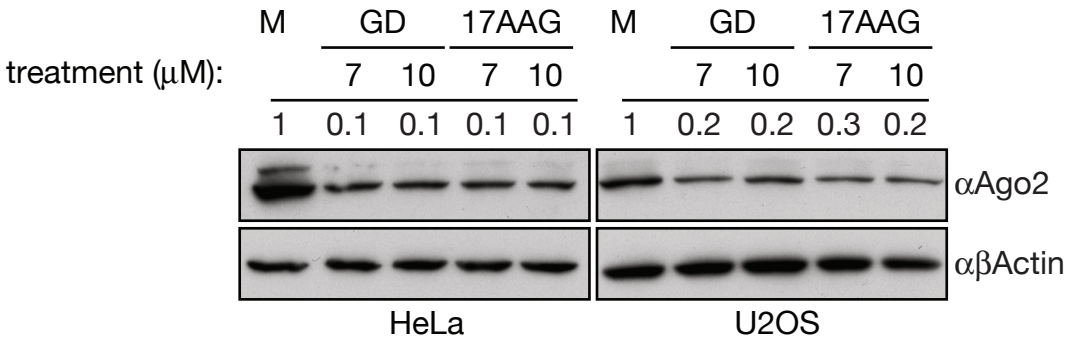
cells (GD) were normalized to the luciferase reading of the mock treated cells (M). The error bars represent the standard error of 3 experiments. *let-7*X1: Firefly luciferase plasmid contain one perfect complementary site for *let-7a*. *let-7*X1m: similar reporter, only the cleavage site and the 2nd and 3rd base of the seed sequence was mutated. (**B, C**) Control Western blots show that gedanamycin treatments successfully affected hAgo2 leves in the Dual Luciferase assays presented in (A) and Figure 2C. The numbers are represent the relative hAgo2 levels normalized to Ago2 level of the mock treated cells. (**D**) Affinity purification were carried out with biotinylated control 2′-*O*-methyl oligo (c) and biotinylated 2′-*O*-methyl oligo complementary to human *let-7a* (*let-7*) in mock and geldanamycin treated (10μM for 16 hours) HeLa cells. The bound fractions were assayed for the presence of hAgo2 and HSP90 with Western blotting. 10% of total lysate was loaded and half of the bound fraction.

Supplementary Figure 4. (A) A repeated experiment of Figure 3A. **(B), (C)** A repeat of the experiment described in Figure 3B,C, with the only difference that data were normalized to the lowest non-effective siRNA concentrations. (**D)** The decrease of hAgo2 level shows that in the experiments presented in Figure 3B and C the geldanamycin treatment was successful. hAgo level was monitored with Western blotting and the relative levels of hAgo2 compared to actin is presented on the top of the panel.

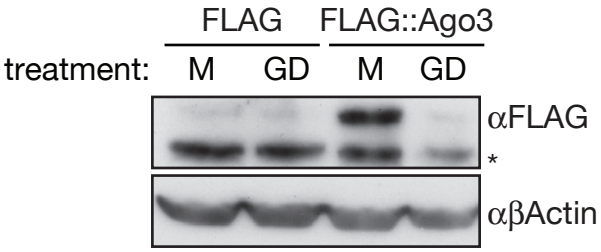
Supplementary Figure 5. Stabilizing hAgo2 with additional siRNAs cannot recover P-bodies after geldanamycin treatment. (A) The experiment was carried

out as it has been described in Figure 4A. P-bodies were visualized with immunofluorescence using a GW182 antibody. DAPI was used for counter-staining DNA. **(B)** Quantification of (A) The number of cells and P bodies were manually counted in five fields of view for each of the treatments to obtain the average number of P-bodies per cell in a field of view. Error bars represent standard error. The standard error was calculated with representing the number of fields of view that was used to determine the average number of P bodies per cell.

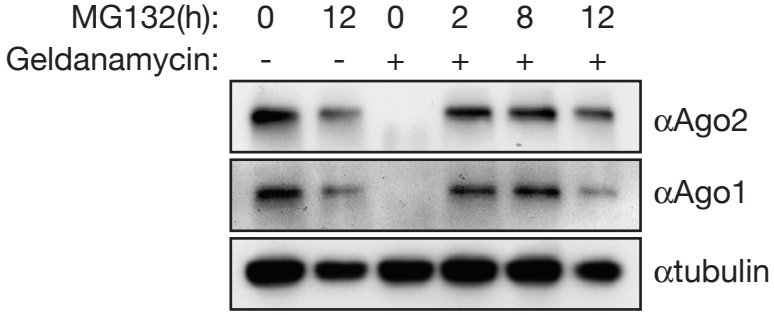
A

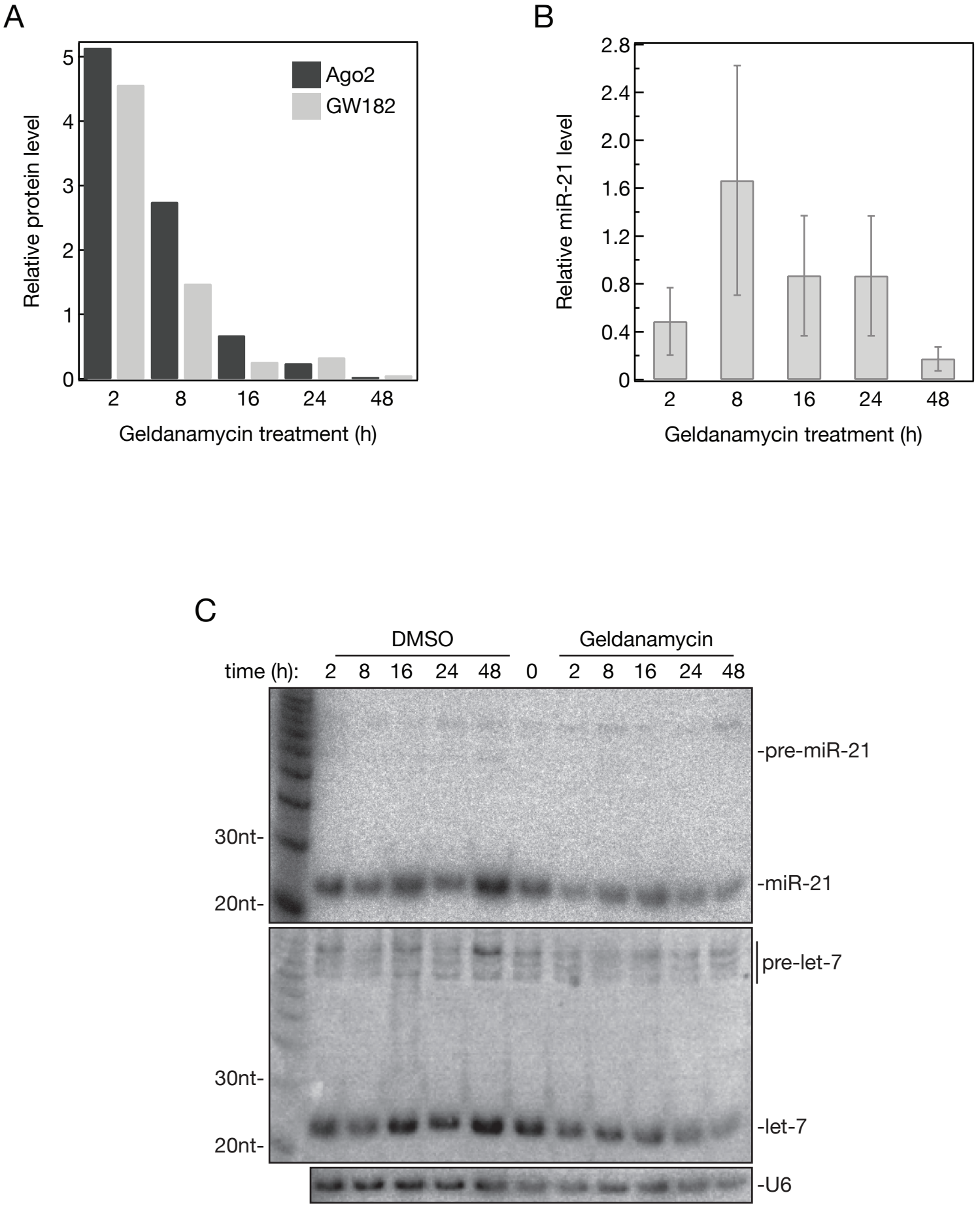

M
GD
17AAG
M
GD
17AAG
treatment (μM):
7
10
7
10
7
10
7
10
1
0.1
0.1
0.1
0.1
1
0.2
0.2
0.3
0.2
αAgo2
αβActin
HeLa
U2OS

B

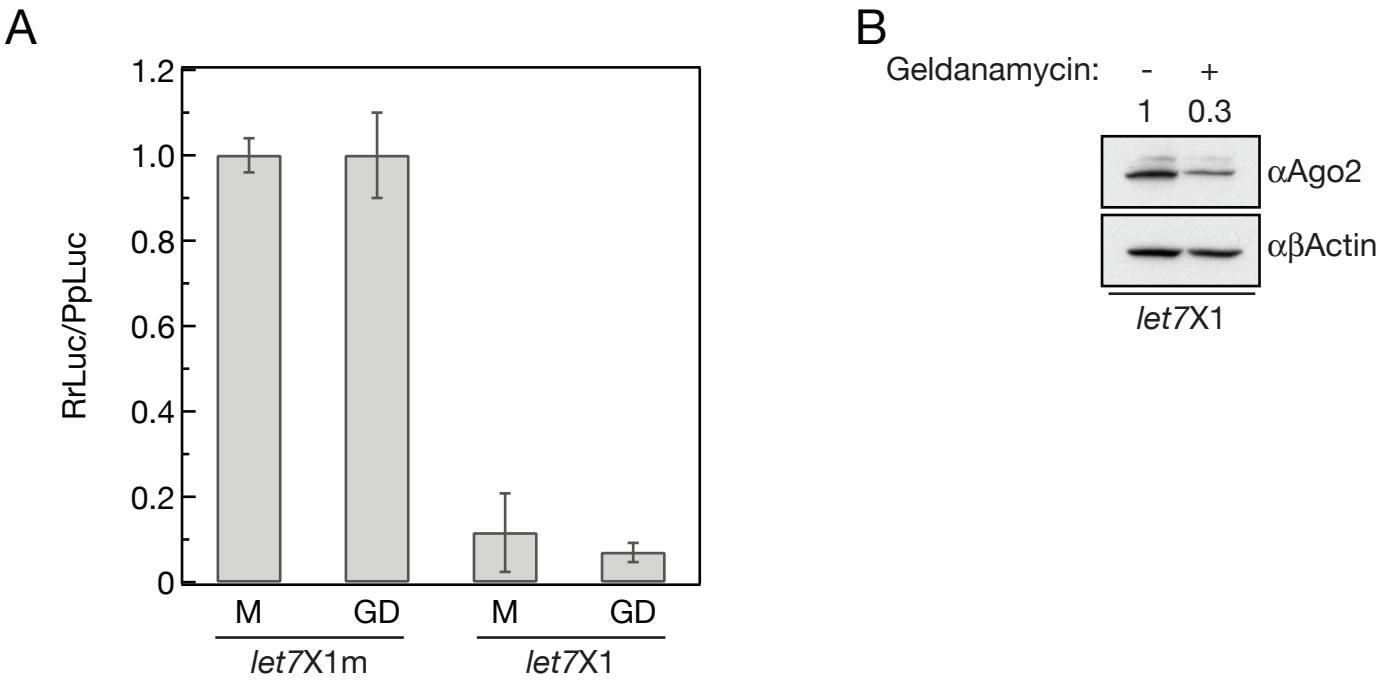

FLAG
FLAG::Ago3
treatment:
M
GD
M
GD
αFLAG
*
αβActin

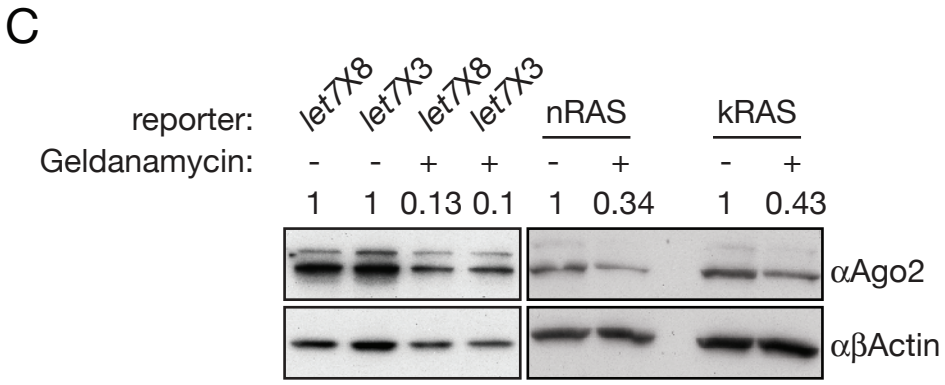
C

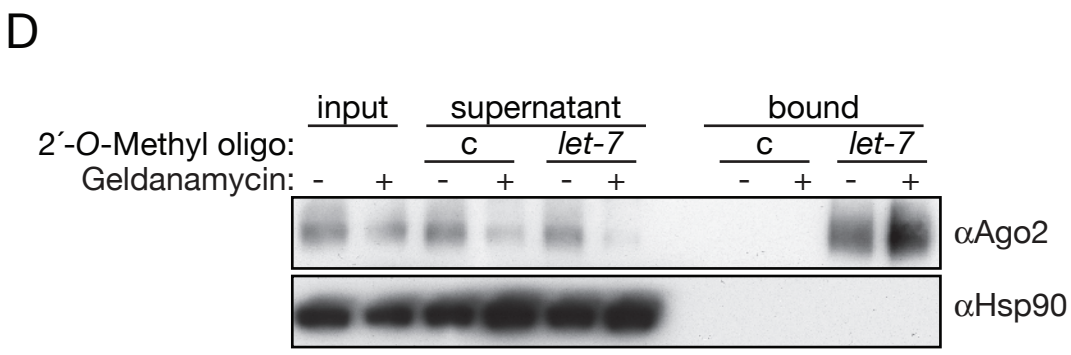

MG132(h):
0
12
0
2
8
12
Geldanamycin:
-
-
+
+
+
+
αAgo2
αAgo1
αtubulin

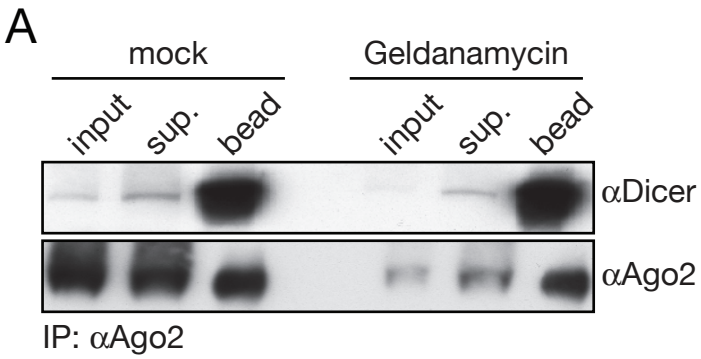

A
Relative protein level
Ago2
GW182
Geldanamycin treatment (h)
B
Relative miR-21 level
Geldanamycin treatment (h)
C
DMSO
Geldanamycin
time (h):
-pre-miR-21
-miR-21
pre-let-7
-let-7
-U6
30nt-
20nt-

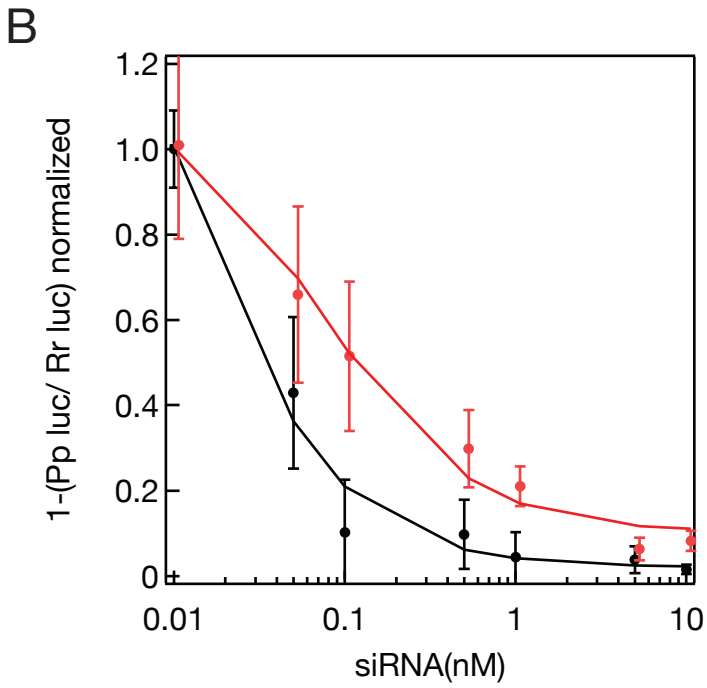
Supplementary Figure 3

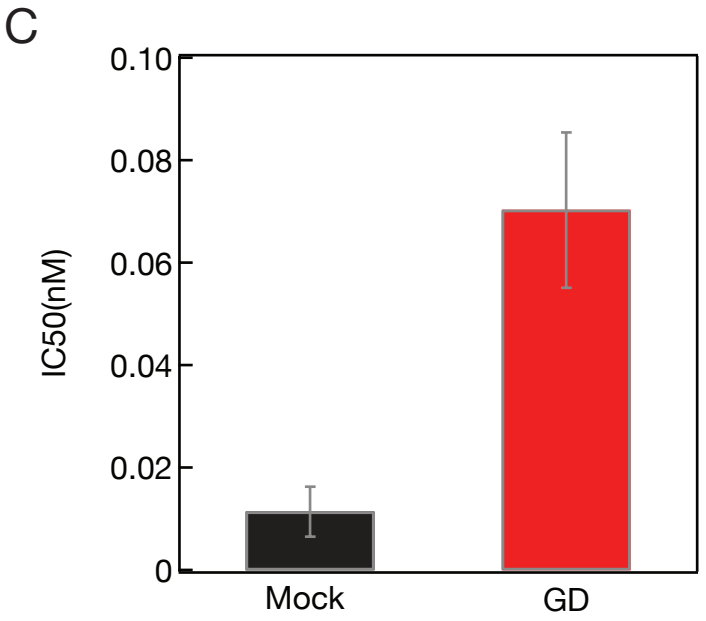


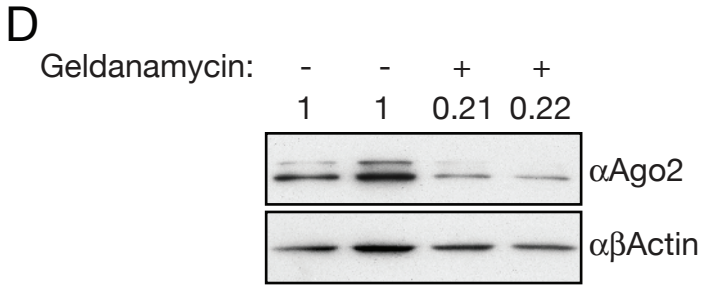





A
mock
Geldanamycin
input
sup.
bead
input
sup.
bead
αDicer
αAgo2
IP: αAgo2

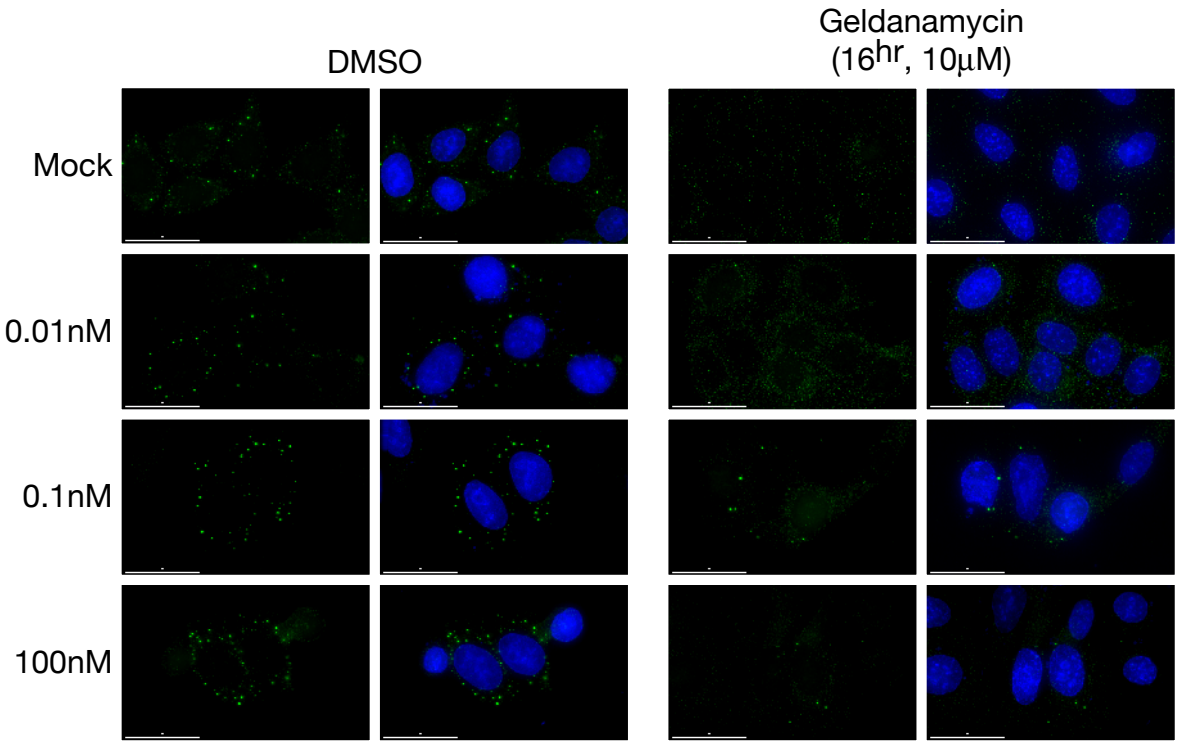
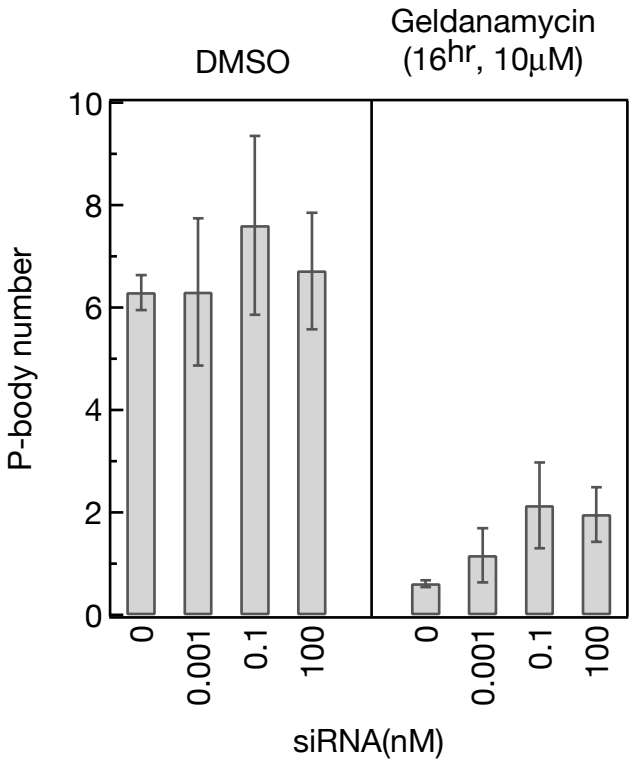

B
1-(Pp luc/ Rr luc) normalized
1.2
1.0
0.8
0.6
0.4
0.2
0
0.01
0.1
1
10
siRNA(nM)


C
IC50(nM)
0.10
0.08
0.06
0.04
0.02
0
Mock
GD


D
Geldanamycin:
-
-
+
+
1
1
0.21
0.22
αAgo2
αβActin

Supplementary Figure 5

A



B