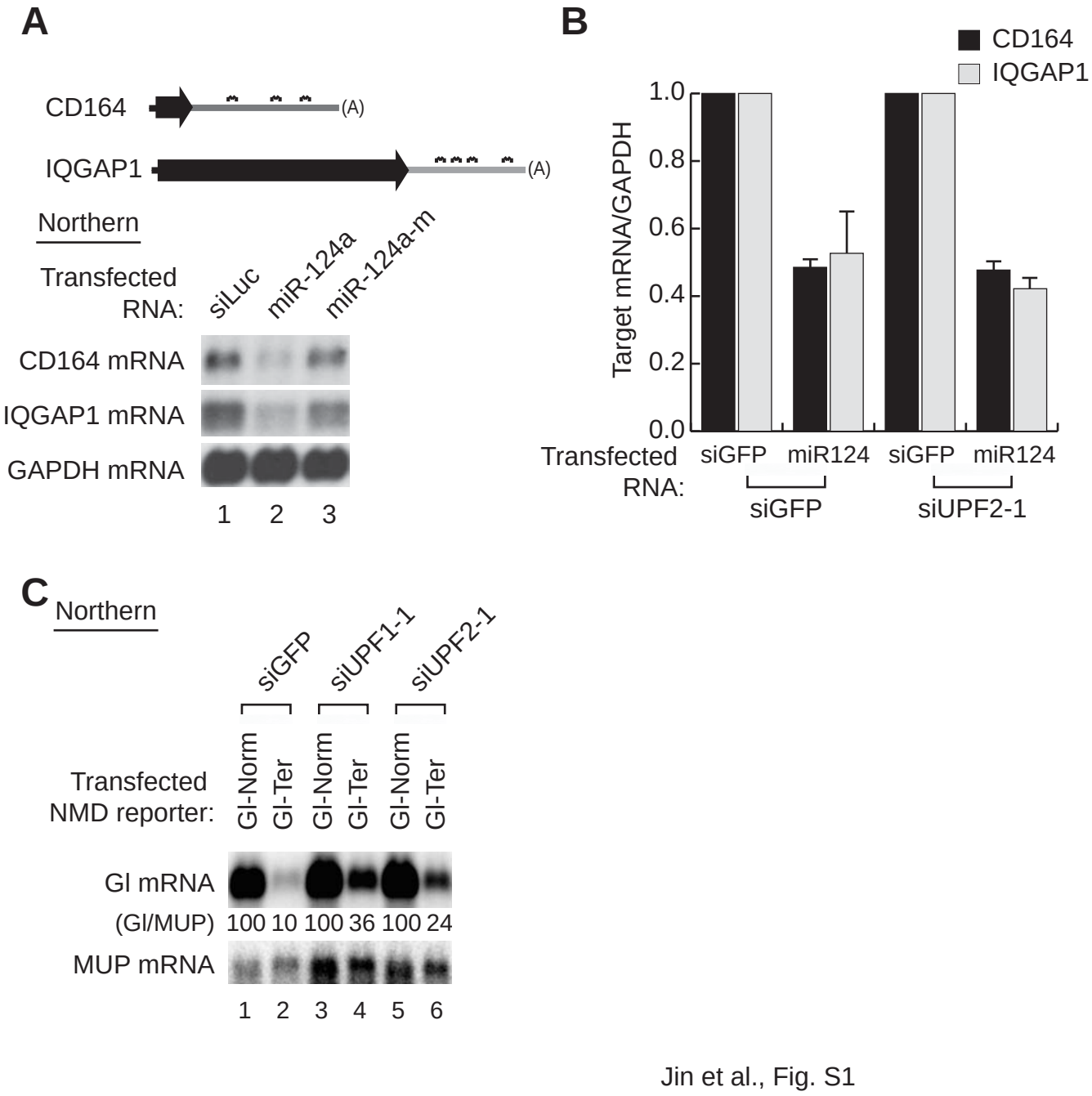


Jin et al., Fig. S1

Fig. S1. hUPF1 suppresses miRNA targets through a NMD-independent pathway. (A) Schematic representation of miR-124a targets and northern blot analysis. The short black lines indicate putative target sites for miR-124a. HeLa cells were transfected with control siRNA duplex (siLuc), miR-124a duplex, or mutant RNA duplex (miR-124a-m). (B) hUPF2 is not required for miRNA-induced mRNA decay. Graph: Quantification of the relative levels of the target mRNAs from northern blotting. Relative target mRNA levels were normalized against GAPDH mRNA levels (n=2, mean±SD). (C) Knckdown of hUPF1 or hUPF2 increases mRNA levels of the NMD substrate. siRNAs were cotransfected into HeLa cells with either nonsense-free normal reporter (GI Norm) or nonsense-containing NMD reporter (GI-Ter), along with an internal control (MUP). The mRNA levels were then measured by northern blotting.

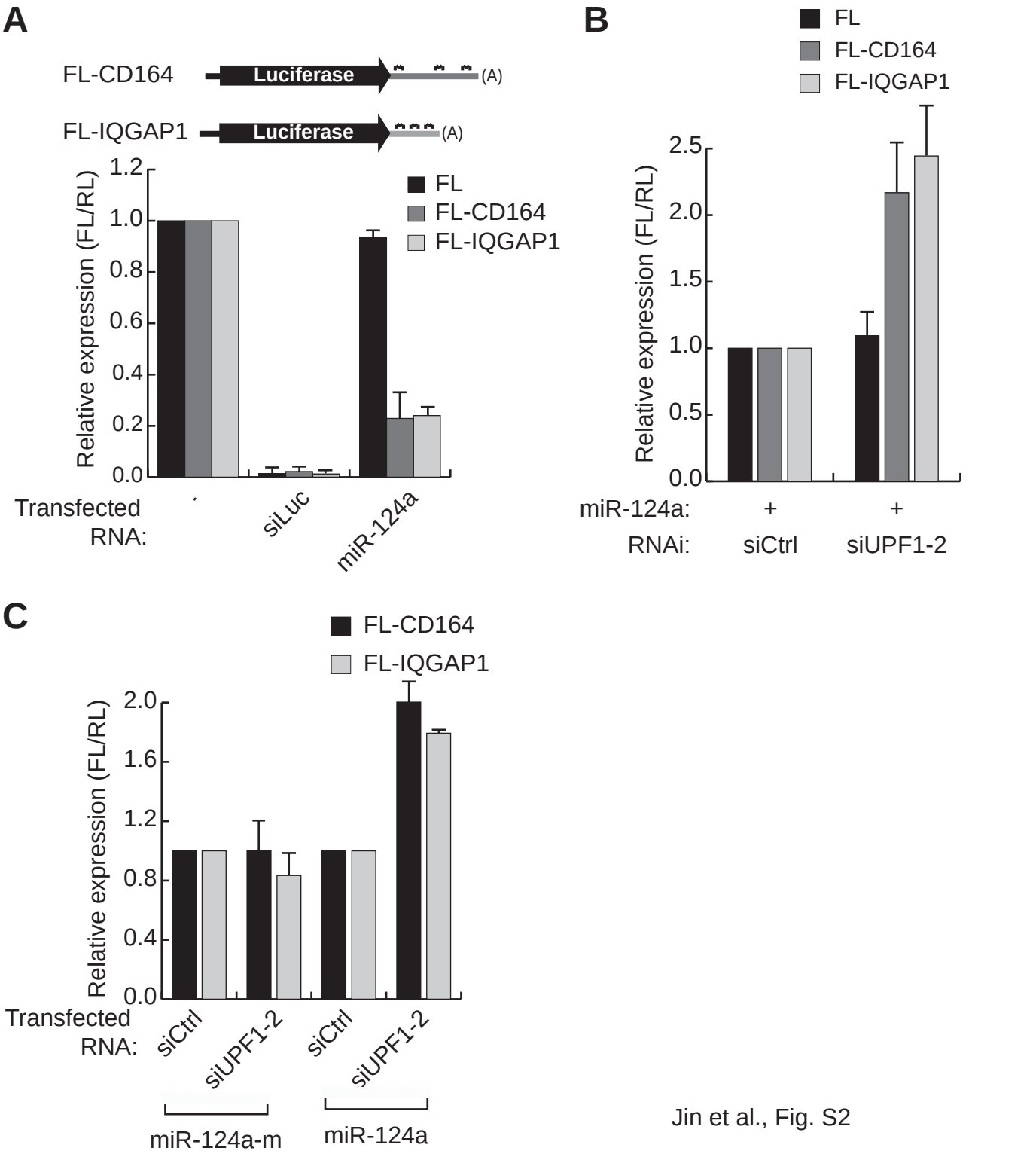
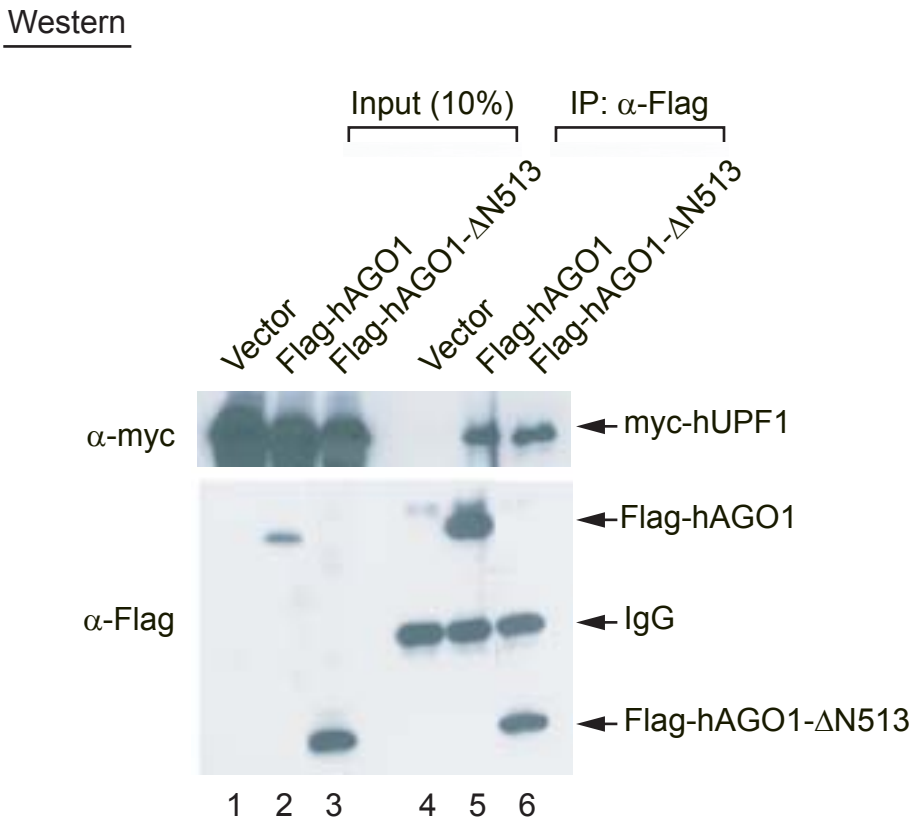
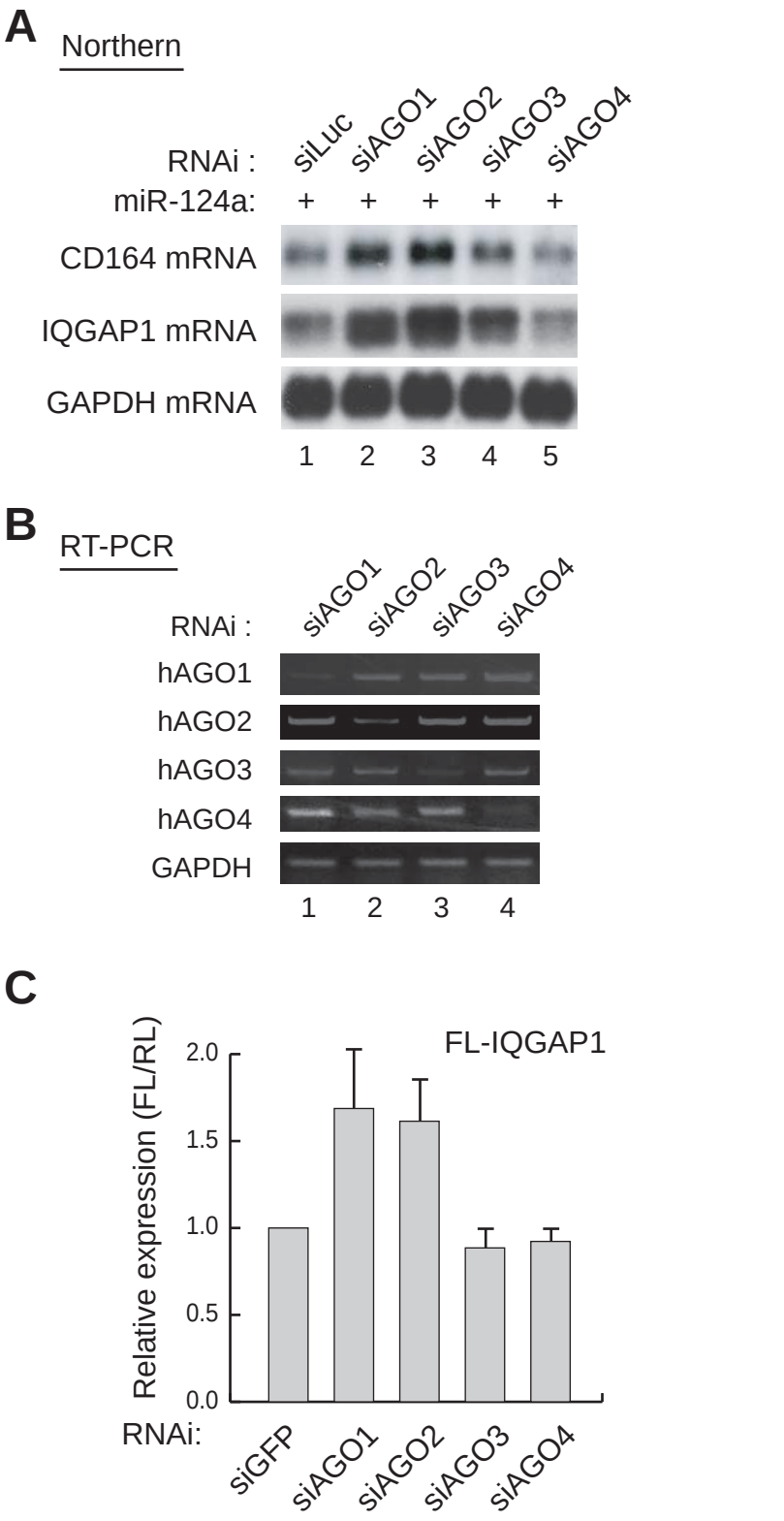


Fig. S2. hUPF1 suppresses the expression of reporters in a miRNA-dependent manner. Luciferase assay using the UTR reporter constructs. (A) Schematic representation of reporter mRNAs and luciferase assay. The 3' UTR fragment from either IQGAP1 or CD164 mRNA was inserted downstream of firefly luciferase (FL) coding sequences to generate reporter mRNAs, FL-IQGAP1 or FL-CD164, respectively. The firefly luciferase reporter construct and the control renilla luciferase (RL) construct were co-transfected into HeLa cells together with miR-124a or siLuc. The firefly luciferase activity (FL) was normalized against renilla luciferase activity (RL) (n=3, mean±SD). (B) HeLa cells were co-transfected with the FL reporter construct, the renilla luciferase (RL) plasmid, miR-124a, and either control siRNA or siRNA against hUPF1. Firefly luciferase activity (FL) was normalized against renilla luciferase activity (RL) (n=4, mean±SD). (C) The firefly luciferase reporter construct and the control renilla luciferase (RL) construct were co-transfected into HeLa cells, together with control siRNA or siRNA against hUPF1, and either miR-124a-m (mutant) or miR-124a (wild type). The firefly luciferase activity (FL) was normalized against renilla luciferase activity (RL) (n=3, mean±SD).



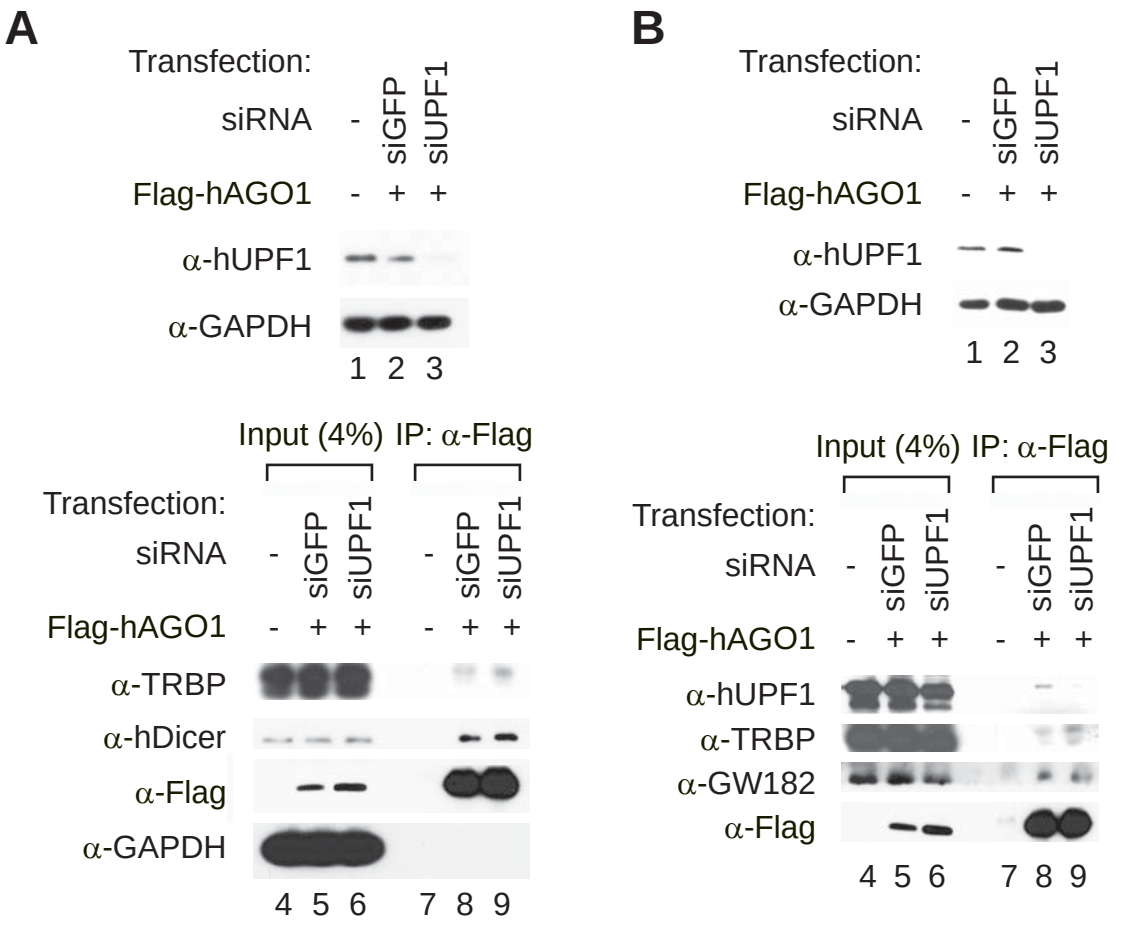
Jin et al., Fig. S3

Fig. S3. The N-terminal deletion mutant of hAGO1 interacts with hUPF1. Plasmid expressing myc-UPF1 was co-transfected into HEK293T cells with plasmid expressing either Flag-AGO1 or Flag-hAGO1-ΔN513 . Flag-tagged proteins were immunoprecipitated with anti-Flag antibody in Buffer D containing 150 mM KCl after RNase A treatment. Proteins present in the precipitates were analyzed by western blotting using anti-Flag or anti-myc antibody.



Jin et al., Fig. S4

Figure S4. hAGO1 and hAGO2 are required for miRNA-induced mRNA decay.
(A) Northern blotting of miR-124a targets (the CD164 and IQGAP1 mRNAs) following RNAi against Argonaute proteins (hAGO1, hAGO2, hAGO3, or hAGO4) in HeLa cells. siRNAs were transfected for 24 hours, and then transfected with miR-124a for additional 48 hours before harvesting. (B) RT-PCR to confirm the knockdown of Argonautes. (C) Luciferase assay to determine the effect of Argonaute protein depletion in HeLa cells. The cells treated with siRNAs against AGO were subsequently transfected with the firefly luciferase (FL) and control renilla luciferase (RL) reporter constructs, together with miR-124a. After an additional 24 hours, luciferase assay was performed. Experiments were performed in duplicates.



Jin et al., Fig. S5

Fig. S5. No effect of hUPF1 knockdown on the interaction between RISC components. (A, B) Immunoprecipitation (IP) followed by western blotting. HeLa cells were co-transfected with Flag-tagged hAGO1 and siRNAs against hUPF1 or GFP using Lipofectamine 2000. IP was carried out using anti-Flag antibody in the presence of RNase A. Endogenous proteins were detected using anti-hUPF1 antibody, anti-TRBP antibody, anti-hDicer antibody, anti-GW182 antibody, and anti-GAPDH antibody.

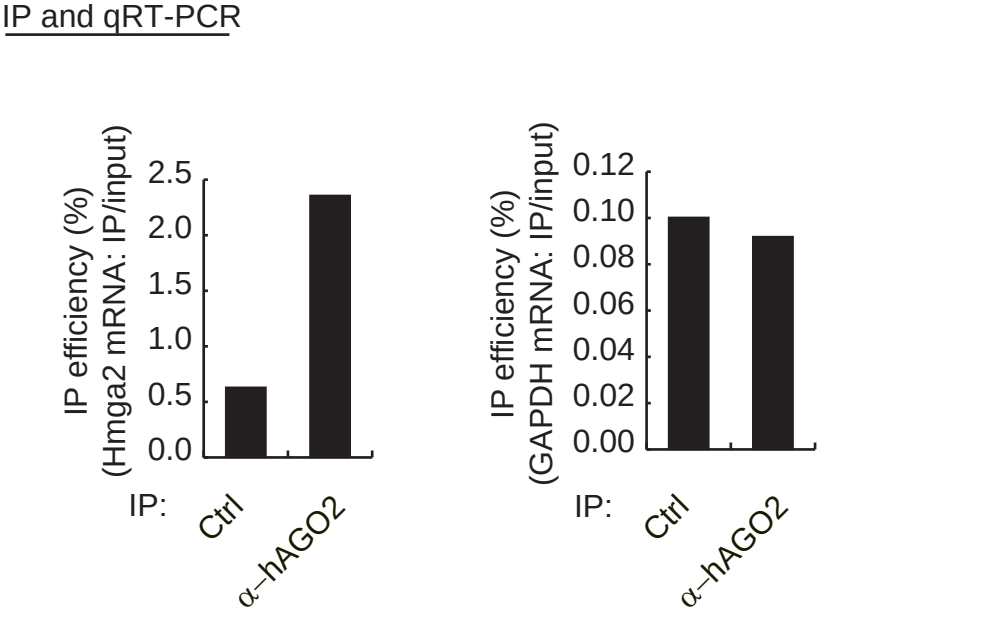


Fig. S6. Anti-hAGO2 antibody selectively precipitates Hmga2 mRNA (left), but not GAPDH mRNA (right). The hAGO2 complex was immunoprecipitated using anti-hAGO2 monoclonal antibody (4G8). Control antibody used in the immunoprecipitation was non-immune antibody (SP2/0). The mRNA levels were then measured by qRT-PCR.