SUPPLEMENTARY FIGURE LEGENDS

Supplementary Figure S1. Impact of each individual *OIP5-AS1*-directed siRNA on *OIP5-AS1* abundance and cell number. HeLa cells were transfected with individual *OIP5-AS1*-directed siRNAs (Table 1, each at a concentration of 50 nM), as well as with miR-424. The number of cells from each group was counted 5 days after transfection **(A)**, and the levels of *OIP5-AS1* were assessed after RNA extraction and RT-qPCR analysis 3 days following transfection **(B)**.
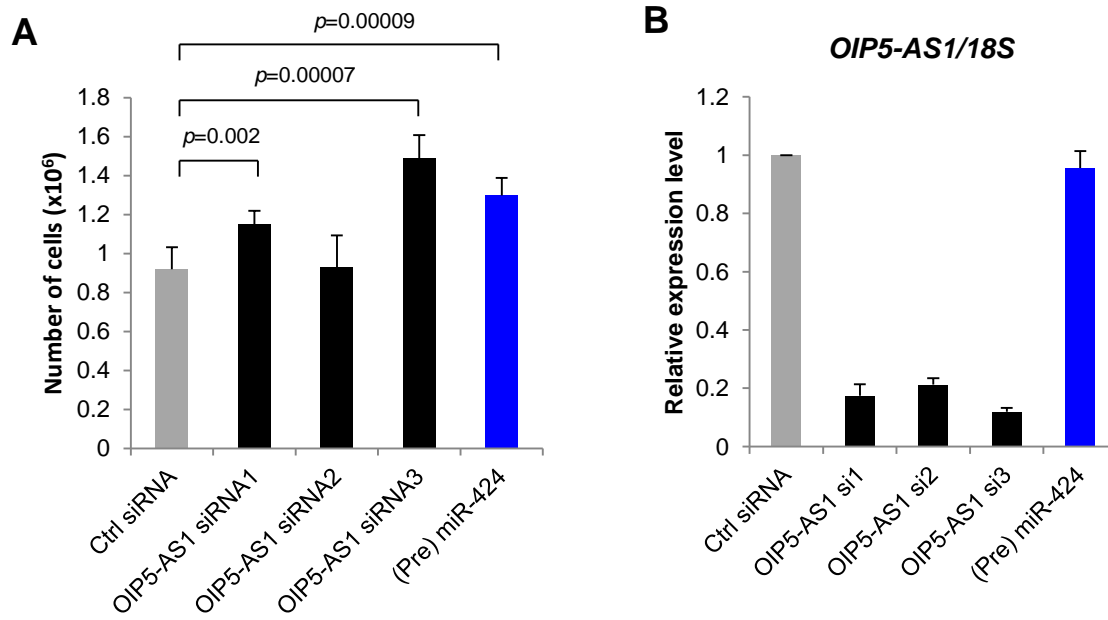
Supplementary Figure S2. microRNAs associated with *OIP5-AS1*. **(A)** HeLa whole-cell lysates were subjected to AGO2 RIP analysis. The relative enrichment of *OIP5-AS1* in AGO2 IP was calculated relative to the levels of *OIP5-AS1* in IgG IP. **(B)** Following MS2 pulldown from cells expressing different HuR levels (as explained in Figure 3), miRNAs associated with *OIP5-AS1-MS2* were identified by RT-qPCR analysis using specific primers (Table 3). Arrowheads indicate nine miRNAs showing significant changes in interaction with *OIP5-AS1-MS2* RNA (enrichment relative to the levels in MS2 IP samples) in cells with silenced HuR (transfected with HuR siRNA in final 50 µM) relative to control cells (transfected with Ctrl siRNA in final 50 µM). The levels of bound RNA were normalized to *U6* RNA levels in each sample. **(C)** To assess the relative abundance of *OIP5-AS1* in the nucleus and the cytoplasm of HeLa cells, the cytoplasmic and nuclear components were fractionated using the NE-PER nuclear and cytoplasmic extraction reagent (Thermo Fisher Scientific); after RT-qPCR analysis, the abundance of molecules in the nucleus and the cytoplasm was calculated on a '*per* µg' basis in each compartment.

Supplementary Figure S3. Further characterization of the influence of miR-424 on *OIP5-AS1* levels. **(A)** Seventy-two hours after either silencing HuR or overexpressing miR-424, HeLa whole-cell lysates were used for AGO2 RIP and the relative enrichment in *OIP5-AS1* bound to AGO2 (relative to control IgG IP) was measured by using RT-qPCR analysis. **(B)** To determine the steady-state level of mature and precursor (pre)-*OIP5-AS1* after overexpressing miR-424, total RNA from HeLa cells transfected with miR-424 was tested by RT-qPCR analysis using primers that spanned intron-exon regions of pre-*OIP5-AS1* (GTATTTCTAGGTGTCTCTATG and CAGAACAAAAAGAAAAGTGGC) as well as primers TGCGAAGATGGCGGAGTAAG and TAGTTCCTCTCCTCTGGCCG amplifying an internal exonic region. **(C)** Cells overexpressing miR-424 were treated with actinomycin D to block *de novo* transcription and the levels of *OIP5-AS1, GAPDH* mRNA*,* and *7SL* (a stable lncRNA) were assessed by RT-qPCR analysis, normalized to *18S* rRNA levels, which had also been quantified by RT-qPCR analysis, and plotted using a semi-log scale.
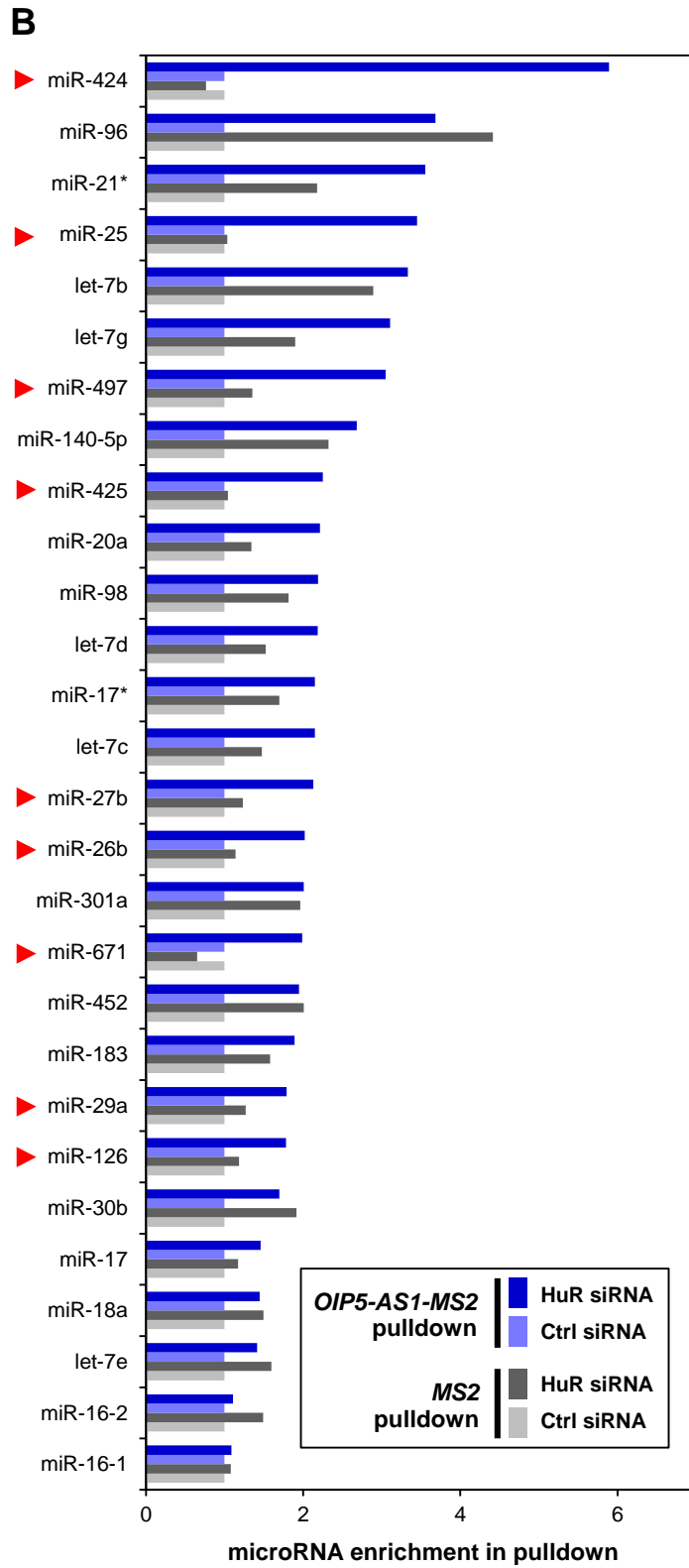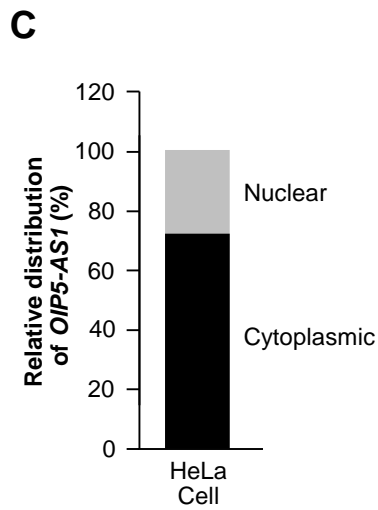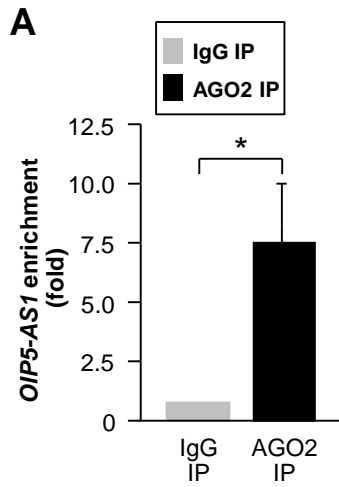
Supplementary Figure S4. Relative levels of HuR and relative binding of HuR to *OIP5-AS1* in the cytoplasm and the nucleus. **(A)** HeLa whole-cell lysates were fractionated into cytoplasmic and nuclear components using NE-PER nuclear and cytoplasmic extraction reagent (Thermo Fisher Scientific). The relative abundance of HuR in the cytoplasm and the nucleus was assessed by Western blot analysis and the quality and concentration of proteins in the different fractions were determined by monitoring the levels of specific markers, including GAPDH (a cytoplasmic protein), hnRNAC1/2 (a nuclear protein), and ACTB (a protein present in both compartments). **(B)** Relative levels of HuR in each cell compartment; approximately 8% is cytoplasmic, 92% nuclear. **(C)** To assess the number of HuR molecules per individual HeLa cell, recombinant protein MBP-HuR was used to prepare a reference standard curve, and HuR in HeLa cell lysates from known number of cells was assayed by Western blot analysis in order to calculate the number of molecules per cell. **(D)** Number of HuR molecules per cell in the cytoplasm and nucleus. **(E)** Cytoplasmic and nuclear lysate (150 µg each) was used for HuR RIP analysis. One hour after incubation with anti-HuR antibody, RNA was extracted from the complexes and RT-qPCR analysis was used to determine the levels of *OIP5-AS1*. Enrichments were calculated relative to the amount of *OIP5-AS1* in each IgG IP sample.

Supplementary Figure S5. Pulldown assay using biotinylated *OIP5-AS1* (full-length and internal segment) RNAs. **(A)** Full-length *OIP5-AS1* and a partial internal segment (Δ5'3') of *OIP5-AS1* were biotinylated by *in vitro* transcription, and 3 µg RNA from each group was incubated with 20 ng of purified MBP-HuR recombinant protein at room temperature for 30 min. After an additional incubation for 30 min with 50 µl streptavidin beads (Invitrogen) and washes with 1× TENT (20 mM Tris-HCl, pH 8.0, 20 mM EDTA, pH 8.0, 500 mM NaCl, and 1% Triton X-100) buffer, the levels of bound HuR were assessed by Western blot analysis and quantified using ImageJ. Negative control lanes include 'Beads' only and mouse *Gapdh* 3'UTR. Primers used for amplification of mouse *Gapdh* from genomic DNA were ttctaatacgactcactatagggGCTCTCAATGACAACTTTGT and GGGTGCAGCGAACTTTATTG (where lowercase letters represent the T7 RNA polymerase promoter sequence). **(B)** The quality and concentration of the *in vitro*-transcribed biotinylated RNAs were assessed by electrophoresis through 1% agarose gels. **(C)** *Green*, HuR binding sites on *OIP5-AS1* identified using the tools starBASE v2.0 and GSM738185.bed.gz; *yellow*, two additional binding sites identified by Kishore and colleagues (45).
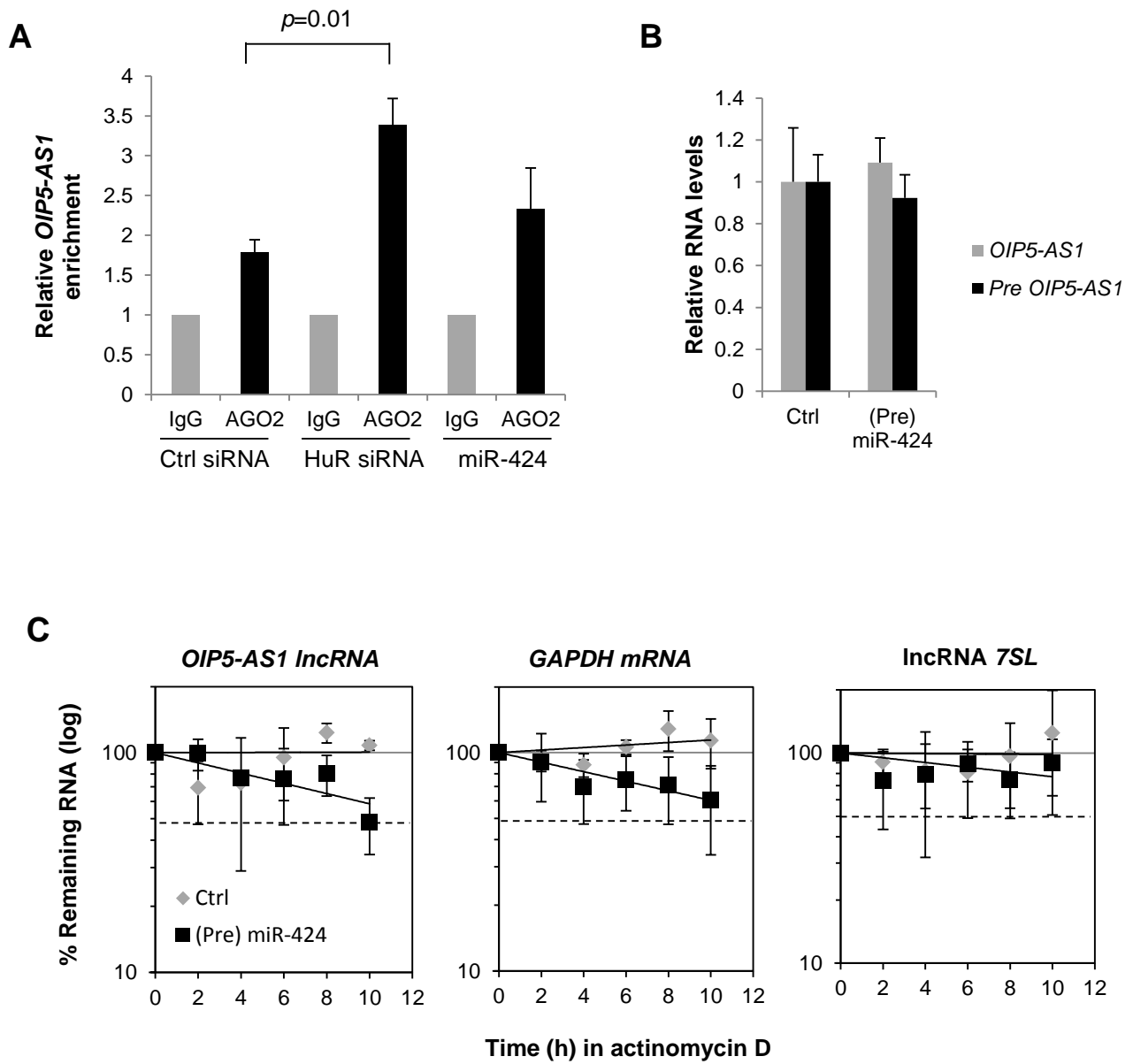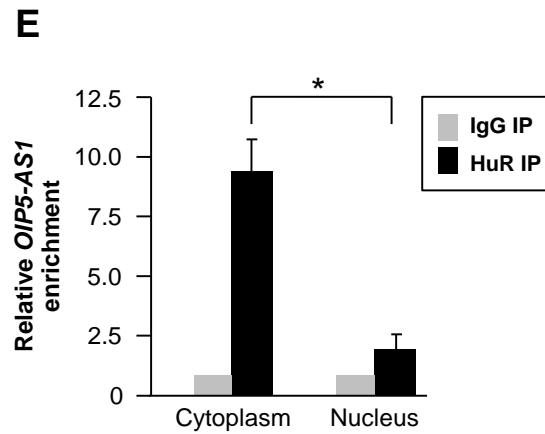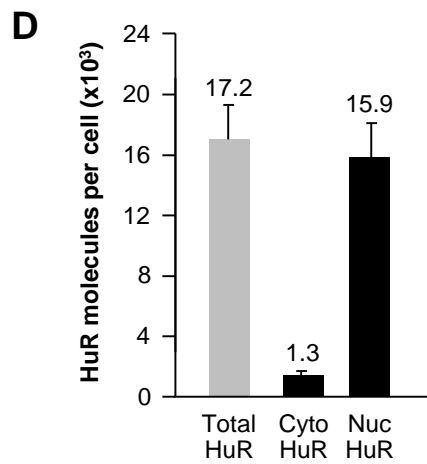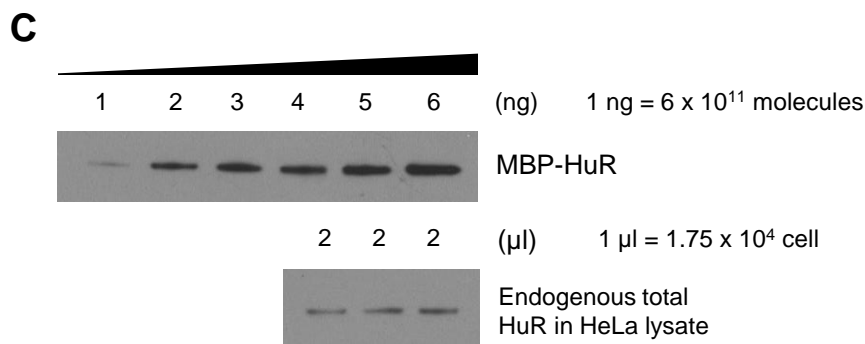
Kim et al. Supplementary Figure S1

Kim et al. Supplementary Figure S2

Kim et al. Supplementary Figure S3

A

B

C

Time (h) in actinomycin D

Kim et al. Supplementary Figure S4

A

Endogenous total
HuR in HeLa lysate

GAPDH (cytoplasmic)

hnRNPC1/2 (nuclear)

ACTB (total)

B

C

1 ng = 6 x 10^11 molecules

MBP-HuR

1 µl = 1.75 x 10^4 cell

Endogenous total
HuR in HeLa lysate

D

E

Kim et al. Supplementary Figure S5

A

B

C

OIP5-AS1