

## SUPPLEMENTARY DATA

### microRNA/Argonaute2 regulates nonsense-mediated mRNA decay

Junho Choe, Hana Cho, and Yoon Ki Kim \*

\*Correspondence: [yk-kim@korea.ac.kr](mailto:yk-kim@korea.ac.kr) (Y.K.K)

Tel: 82-2-3290-3410

## SUPPLEMENTARY MATERIALS AND METHODS

### Plasmid construction

To construct eIF4E-BP1, an EcoRI/XhoI fragment of pCMV-Myc (Clontech) was ligated to a PCR-amplified eIF4E-BP1 cDNA fragment. The full-length human eIF4E-BP1 cDNA was amplified using pOTB7-eIF4E-BP1 (21C Human Gene Bank, Genome Research Center, KRIBB, Korea) and two oligonucleotides: 5'-  
GGAATTCGGGATCCGATGTCCGGGGGCAGCAGCTGCAGCCAG-3' (sense) and 5'-  
CCCTCGAGAAAGCTTTTAAATGTCCATCTCAAAGTGTGACTC-3' (antisense), where the underlined nucleotides specify the EcoRI and XhoI sites, respectively. The resulting PCR product was digested with EcoRI and XhoI. PCR amplification was carried out using the Advantage-HF2 PCR Kit (Clontech).

pCI-λN-HA was generated using pCI-λN-HA-Ago2 (Kiriakidou *et al.*, 2007; Pillai *et al.*, 2004) that was digested with EcoRI and NotI and self-ligated.

Tethering NMD reporter plasmids phRL-GI-5BoxB Norm and Ter were constructed by inserting the β-globin (GI) gene (either Norm or 39Ter), which was PCR-amplified from

pmCMV-Gl Norm or 39Ter (Cho *et al.*, 2009; Kim *et al.*, 2005), into the site immediately upstream of the stop codon of the *Renilla* luciferase (RLuc) cDNA in plasmid RL-5BoxB (Kiriakidou *et al.*, 2007).

To generate miRNA binding site-containing NMD reporter plasmids pRL-Gl-CXCR4 Norm and Ter, pRL-Gl Norm or Ter was first constructed, which contains a chimeric RLuc-Gl (either Norm or 39Ter) gene. The Gl Norm or 39Ter DNA fragment was amplified by PCR using pmCMV-Gl Norm or 39Ter (Cho *et al.*, 2009; Kim *et al.*, 2005). Then, a CXCR4-6xBulge fragment from pcDNA3.1-FLuc-CXCR4-6xBulge (a gift from Sung Key Jang) was inserted into the 3'UTR.

pRL-Gl-CAT-1A Norm and Ter and pRL-Gl-CAT-1C Norm and Ter were constructed by inserting a CAT-1A or CAT-1C fragment obtained from pRL-CAT-1A (Bhattacharyya *et al.*, 2006) or pRL-CAT-1C (Bhattacharyya *et al.*, 2006) into the XbaI and NotI sites of the 3'UTR of pRL-Gl Norm or Ter.

To construct plasmid pCI-F, which encodes firefly luciferase (FLuc) cDNA, the XbaI/Klenow-filled NheI fragment from pCI-Neo (Promega) was ligated to an XbaI/Klenow-filled NcoI fragment containing the FLuc cDNA.

To construct pcDNA3-FLAG-CBP20, a BamHI/HindIII fragment of pcDNA3-FLAG (Kim *et al.*, 2009) was ligated to a PCR-amplified CBP20 cDNA fragment. The full-length human CBP20 cDNA was amplified using pCNS-D2-CBP20 (21C Human Gene Bank, Genome Research Center, KRIBB, Korea) and two oligonucleotides: 5'-CGGGATCCGATGTCGGGTGGCCTCCTGAAGGCGCTG-3' (sense) and 5'-CCCAAGCTTTCACTGGTTCTGTGCCAGTTTTCCATAG-3' (antisense), where the underlined nucleotides specify the BamHI and HindIII sites, respectively. The resulting PCR product was digested with BamHI and HindIII.

The construction details for pcDNA3-FLAG-eIF4E were previously reported (Kim *et*

*al.*, 2009).

### ***In vivo* UV cross-linking followed by immunoprecipitation**

Cos-7 cells ( $2 \times 10^7$ ) were exposed to ultraviolet (UV;  $250 \text{ mJ/cm}^2$ ) for 5 min in 4 ml of ice-cold DMEM supplemented with 10% FBS using a Bio-Link Crosslinker (Vilber Lourmat). Total-cell extracts were obtained from cells either exposed or not exposed to UV, and incubated at  $65^\circ\text{C}$  for 3 min with addition of 0.1% SDS to dissociate non-covalent interactions. The IPs were performed as previously described (Cho *et al.*, 2009; Kim *et al.*, 2005), except that NET2 buffer containing 800 mM of NaCl was used.

### **SUPPLEMENTARY FIGURE LEGENDS**

**Supplementary Fig S1.** Endogenous Ago2 and eIF4AIII associate with FLAG-CBP20 *in vivo*. Cos-7 cells were transiently transfected with plasmid expressing either FLAG-CBP20 or FLAG-eIF4E. Two days after transfection, cells were either exposed to UV for 5 min to induce cross-linking between RNA and proteins *in vivo* (+ UV exposure) or not exposed to UV (- UV exposure). Each extract was incubated at  $65^\circ\text{C}$  for 3 min to dissociate noncovalent interactions. IPs were then performed using anti-FLAG antibody. Samples before or after IP were analyzed by Western blotting using indicated antibodies.

**Supplementary Fig S2.** Downregulation of endogenous eIF4AIII, Upf1, or Y14 abrogates NMD of GI-5BoxB mRNAs and GI-CXCR4 mRNAs. HeLa cells were transiently transfected with 100 nM eIF4AIII siRNA [5'-r(CGAGCAAUCAAGCAGAUCA)d(TT)-3'], Upf1 siRNA [5'-r(GAUGCAGUUCCGCUCCAUU)d(TT)-3'], Y14 siRNA [5'-r(UCCAGCCUUCAACAGAGCG)d(TT)-3'], or nonspecific Control siRNA [5'-r(ACAAUCCUGAUCAGAAACC)d(TT)-3']. Two days later, cells were retransfected with

0.1  $\mu$ g of either tethering NMD construct or miRNA-targeted NMD construct, and 0.1  $\mu$ g of reference plasmid pCI-F. After an additional day, protein and RNA were purified. **(A)** Western blotting of endogenous eIF4AIII. The result showed that the level of endogenous eIF4AIII was downregulated to 10% of normal, where normal is defined as the level in the presence of nonspecific Control siRNA. The level of endogenous GAPDH served to control for variations in protein loading. **(B)** Semi-quantitative RT-PCR of Gl-5BoxB Norm and Ter mRNAs. The levels of Gl-5BoxB mRNAs were normalized to the levels of FLuc mRNAs. Normalized levels of Gl-5BoxB Norm mRNA in the presence of each siRNA were set to 100%. **(C)** Western blotting of endogenous Upf1 and Y14. The levels of endogenous Upf1 and Y14 were downregulated to 0.1% and 2% of normal. The level of endogenous  $\beta$ -actin served to control for variations in protein loading. **(D)** Semi-quantitative RT-PCR of Gl-5BoxB Norm and Ter mRNAs. The levels of Gl-5BoxB mRNAs were normalized to the levels of FLuc mRNAs. Normalized levels of Gl-5BoxB Norm mRNA in the presence of each siRNA were set to 100%. **(E, F)** As in Supplementary Fig S2B and S2D, respectively, except that miRNA-targeted NMD construct, pRL-Gl-CXCR4 Norm or Ter was transfected.

**Supplementary Fig S3.** Gl-CAT-1C mRNA, but not Gl-CAT-1A mRNA, is efficiently targeted for NMD. Huh-7 cells were transiently cotransfected with 0.05  $\mu$ g of pRL-Gl-CAT-1A or pRL-Gl-CAT-1C harboring Norm or Ter, and 0.05  $\mu$ g of pCI-F. **(A)** Semi-quantitative RT-PCR of Gl-CAT-1A mRNAs and Gl-CAT-1C mRNAs. **(B)** Translational efficiencies of Gl-CAT-1A Norm mRNA and Gl-CAT-1C Norm mRNA.

**Supplementary Table S1.** A subset of cellular NMD substrates escapes NMD in a miRNA/Ago2-dependent manner. **(A)** Transcripts commonly downregulated upon downregulation of Ago2 in two independently performed microarray analyses. The fold-

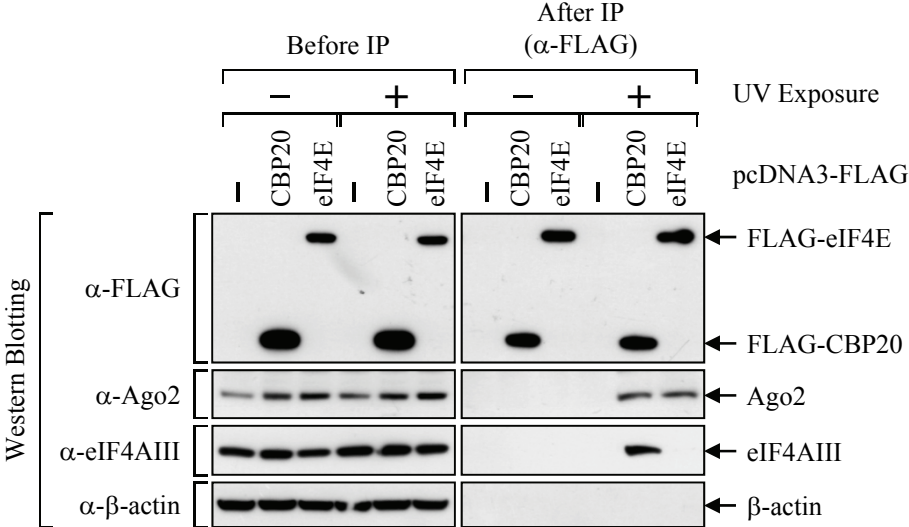
changes of transcripts downregulated upon downregulation of either Ago2 or Upf1 are depicted in the Ago2 or Upf1 column, respectively. The ratios of the levels of transcript upon downregulation of both Ago2 and Upf1 to the levels of transcript upon downregulation of Ago2 are depicted in the Ago2&Upf1/Ago2 column. Cellular transcripts downregulated more than 1.8-fold upon downregulation of Ago2 and restored more than 1.8-fold upon downregulation of both Ago2 and Upf1 are highlighted in blue. The ranges of fold-changes are depicted as standard deviation ( $\pm$ ) in each column. The presence of uORF at the 5'UTR is depicted in the uORF column. **(B)** Transcripts commonly upregulated upon downregulation of Ago2 in two independently performed microarray analyses.

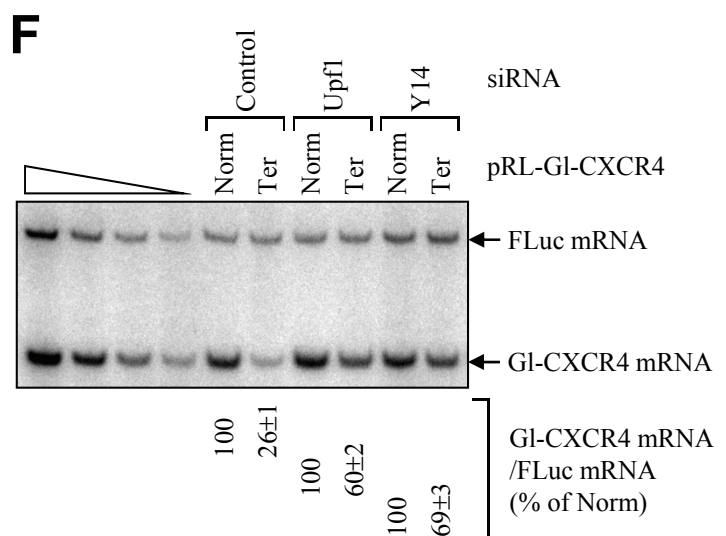
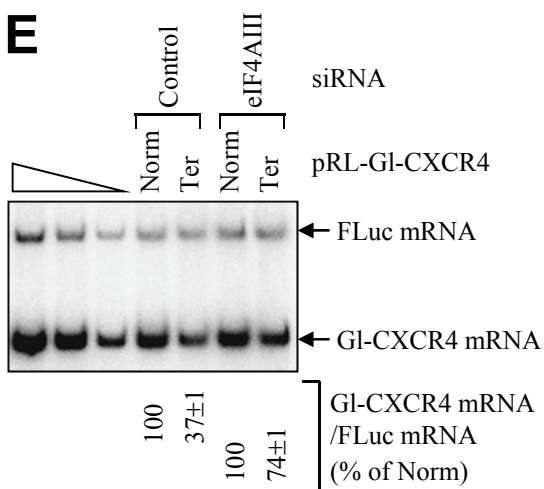
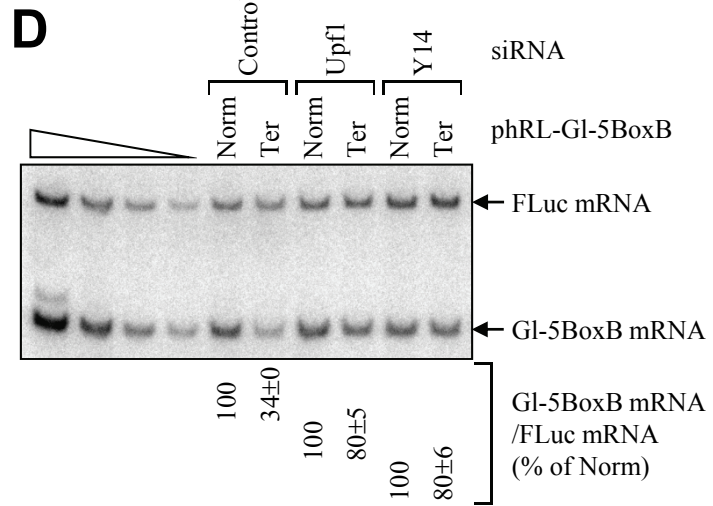
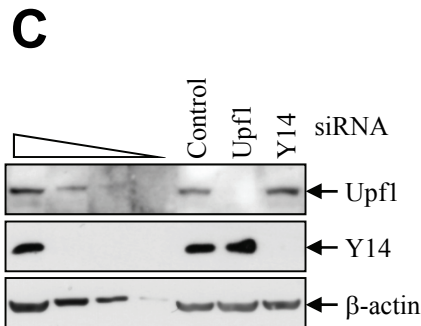
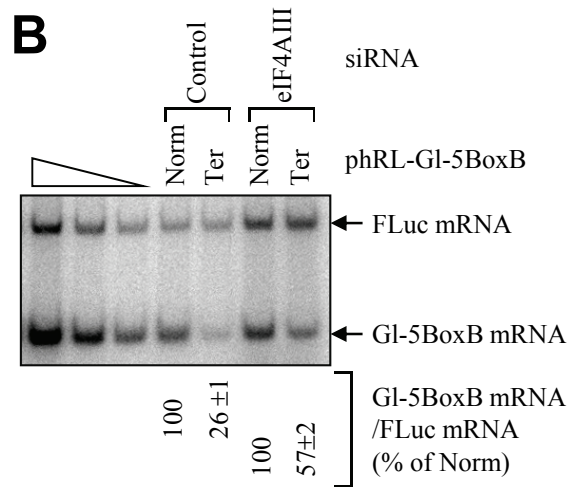
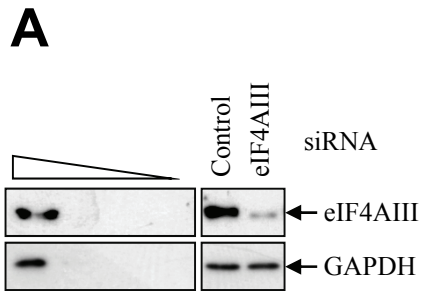
#### **SUPPLEMENTARY REFERENCES**

- Bhattacharyya SN, Habermacher R, Martine U, Closs EI, Filipowicz W (2006) Relief of microRNA-mediated translational repression in human cells subjected to stress. *Cell* **125**: 1111-1124
- Cho H, Kim KM, Kim YK (2009) Human proline-rich nuclear receptor coregulatory protein 2 mediates an interaction between mRNA surveillance machinery and decapping complex. *Mol Cell* **33**: 75-86
- Kim KM, Cho H, Choi K, Kim J, Kim BW, Ko YG, Jang SK, Kim YK (2009) A new MIF4G domain-containing protein, CTIF, directs nuclear cap-binding protein CBP80/20-dependent translation. *Genes Dev* **23**: 2033-2045
- Kim YK, Furic L, Desgroseillers L, Maquat LE (2005) Mammalian Staufen1 recruits Upf1 to specific mRNA 3'UTRs so as to elicit mRNA decay. *Cell* **120**: 195-208
- Kiriakidou M, Tan GS, Lamprinaki S, De Planell-Saguer M, Nelson PT, Mourelatos Z (2007) An mRNA m7G cap binding-like motif within human Ago2 represses translation. *Cell* **129**: 1141-1151

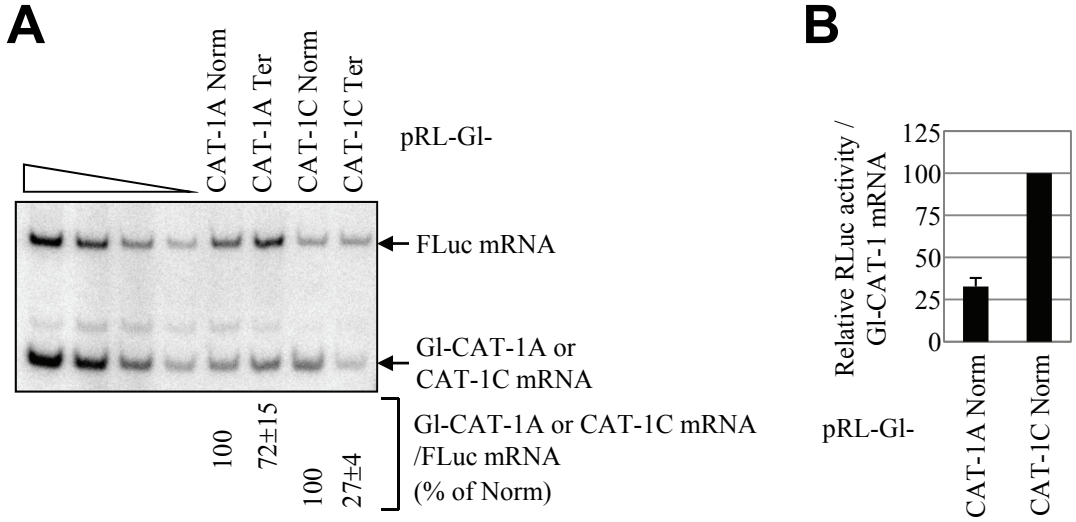
Pillai RS, Artus CG, Filipowicz W (2004) Tethering of human Ago proteins to mRNA mimics the miRNA-mediated repression of protein synthesis. *RNA* **10**: 1518-1525

Figure S1









Supplementary Table S1A. Transcripts downregulated in Ago2-depleted HeLa cells

Gene Symbol	Definition	Accession No.	Fold changes			uORF
			Ago2	Ago2&Upfl	Ago2&Upfl / Ago2	
PRNP	prion protein (p27-30) (Creutzfeldt-Jakob disease, Gerstmann-Strausler-Scheinker syndrome, fatal familial insomnia) (PRNP), transcript variant 2, mRNA.	NM_183079.2	0.29±0.00	0.54±0.12	1.88±0.42	+
LAMC1	laminin, gamma 1 (formerly LAMB2) (LAMC1), mRNA.	NM_002293.2	0.33±0.00	0.47±0.03	1.42±0.08	
PCK2	phosphoenolpyruvate carboxykinase 2 (mitochondrial) (PCK2), nuclear gene encoding mitochondrial protein, transcript variant 1, mRNA.	NM_004563.2	0.37±0.02	0.56±0.06	1.51±0.06	
ASNS	asparagine synthetase (ASNS), transcript variant 1, mRNA.	NM_133436.1	0.38±0.00	0.80±0.30	2.1±0.77	+
SLC38A1	solute carrier family 38, member 1 (SLC38A1), mRNA.	NM_030674.2	0.38±0.03	0.53±0.06	1.54±0.49	+
STC2	stanniocalcin 2 (STC2), mRNA.	NM_003714.2	0.39±0.02	1.06±0.05	2.76±0.31	+
MAP1LC3B	microtubule-associated protein 1 light chain 3 beta (MAP1LC3B), mRNA.	NM_022818.3	0.41±0.02	0.72±0.02	1.79±0.16	+
RNGTT	RNA guanylyltransferase and 5'-phosphatase (RNGTT), mRNA.	NM_003800.1	0.41±0.01	0.46±0.02	1.12±0.07	+
INHBE	inhibin, beta E (INHBE), mRNA.	NM_031479.3	0.41±0.04	0.68±0.03	1.68±0.23	
ECM2	extracellular matrix protein 2, female organ and adipocyte specific (ECM2), mRNA.	NM_001393.2	0.42±0.02	0.58±0.07	1.39±0.26	+
MLXIPL	MLX interacting protein-like (MLXIPL), transcript variant 3, mRNA.	NM_032953.2	0.42±0.01	0.55±0.09	1.30±0.16	
TRIB3	tribbles homolog 3 (Drosophila) (TRIB3), mRNA.	NM_021158.3	0.43±0.02	0.80±0.14	1.86±0.25	+
PSPH	phosphoserine phosphatase (PSPH), mRNA.	NM_004577.3	0.43±0.01	0.66±0.04	1.53±0.05	+
MAPK6	mitogen-activated protein kinase 6 (MAPK6), mRNA.	NM_002748.2	0.43±0.05	0.68±0.15	1.57±0.17	+
HSCARG	NimrA-like family domain containing 1 (NMRAL1), mRNA.	NM_020677.2	0.43±0.01	0.49±0.02	1.14±0.07	+
IL8	interleukin 8 (IL8), mRNA.	NM_000584.2	0.44±0.02	0.47±0.06	1.08±0.08	
CCL20	chemokine (C-C motif) ligand 20 (CCL20), mRNA.	NM_004591.1	0.44±0.02	0.46±0.04	1.05±0.04	
NUPR1	nuclear protein 1 (NUPR1), transcript variant 1, mRNA.	NM_001042483.1	0.44±0.06	1.14±0.29	2.66±1.04	+
PCK2	phosphoenolpyruvate carboxykinase 2 (mitochondrial) (PCK2), nuclear gene encoding mitochondrial protein, transcript variant 2, mRNA.	NM_001018073.1	0.44±0.08	0.65±0.06	1.51±0.17	
HIST1H2BD	histone cluster 1, H2bd (HIST1H2BD), transcript variant 2, mRNA.	NM_138720.1	0.44±0.02	0.86±0.24	1.98±0.43	
MGC33212	hypothetical protein MGC33212 (MGC33212), mRNA.	NM_152773.2	0.45±0.03	0.61±0.20	1.33±0.36	
LOC203547	hypothetical protein LOC203547 (LOC203547), mRNA.	NM_001017980.1	0.46±0.02	0.66±0.00	1.45±0.08	
CYP4V2	cytochrome P450, family 4, subfamily V, polypeptide 2 (CYP4V2), mRNA.	NM_207352.2	0.46±0.03	0.57±0.00	1.26±0.06	
RAB21	RAB21, member RAS oncogene family (RAB21), mRNA.	NM_014999.1	0.46±0.00	0.55±0.06	1.20±0.15	
GPT2	glutamic pyruvate transaminase (alanine aminotransferase) 2 (GPT2), mRNA.	NM_133443.1	0.46±0.01	1.28±0.06	2.75±0.18	+
MT1F	metallothionein 1F (MT1F), mRNA.	NM_005949.1	0.47±0.04	0.35±0.16	0.74±0.28	
C5orf15	chromosome 5 open reading frame 15 (C5orf15), mRNA.	NM_020199.1	0.47±0.01	0.72±0.09	1.55±0.23	+
GIPCI	GIPC PDZ domain containing family, member 1 (GIPC1), transcript variant 3, mRNA.	NM_202468.1	0.47±0.03	0.73±0.10	1.54±0.12	
P8	p8 protein (candidate of metastasis 1) (P8), mRNA.	NM_012385.1	0.47±0.04	1.11±0.33	2.38±0.90	+
PSAT1	phosphoserine aminotransferase 1 (PSAT1), transcript variant 2, mRNA.	NM_021154.3	0.48±0.04	0.72±0.19	1.50±0.29	
TNFRSF11B	tumor necrosis factor receptor superfamily, member 11b (osteoprotegerin) (TNFRSF11B), mRNA.	NM_002546.2	0.48±0.01	0.76±0.20	1.59±0.37	+
DBT	dihydroipoamide branched chain transacylase E2 (DBT), mRNA.	NM_001918.1	0.48±0.01	0.73±0.09	1.52±0.21	
STK39	serine threonine kinase 39 (STE20/SPS1 homolog, yeast) (STK39), mRNA.	NM_013233.1	0.49±0.03	0.58±0.06	1.18±0.19	
IRAK2	interleukin-1 receptor-associated kinase 2 (IRAK2), mRNA.	NM_001570.3	0.49±0.01	0.82±0.04	1.67±0.04	
RWDD4A	RWD domain containing 4A (RWDD4A), mRNA.	NM_152682.1	0.49±0.01	0.65±0.07	1.32±0.17	
GOLPH2	golgi phosphoprotein 2 (GOLPH2), transcript variant 1, mRNA.	NM_016548.2	0.50±0.03	0.63±0.00	1.26±0.06	

KCNGB1	potassium voltage-gated channel, subfamily G, member 1 (KCNGB1), transcript variant 1, mRNA.	NM_002237.2	0.51±0.05	0.54±0.02	1.05±0.07	
ARL1	ADP-ribosylation factor-like 1 (ARL1), mRNA.	NM_001177.3	0.51±0.02	0.66±0.08	1.31±0.03	
EIF2C2	eukaryotic translation initiation factor 2C, 2 (EIF2C2), mRNA.	NM_012154.2	0.51±0.09	0.50±0.04	0.98±0.04	
JUB	jub, ajuba homolog (Xenopus laevis) (JUB), transcript variant 2, mRNA.	NM_198086.1	0.51±0.02	0.90±0.24	1.77±0.54	+
UBE2Q1	ubiquitin-conjugating enzyme E2Q (putative) 1 (UBE2Q1), mRNA.	NM_017582.5	0.51±0.08	0.62±0.03	1.23±0.12	
C16orf63	chromosome 16 open reading frame 63 (C16orf63), mRNA.	NM_144600.1	0.51±0.02	0.66±0.09	1.29±0.02	
MIRPS10	mitochondrial ribosomal protein S10 (MIRPS10), nuclear gene encoding mitochondrial protein, mRNA.	NM_018141.2	0.52±0.01	0.57±0.06	1.09±0.10	
CCDC23	coiled-coil domain containing 23 (CCDC23), mRNA.	NM_199342.1	0.53±0.00	0.60±0.12	1.14±0.15	
C20orf45	chromosome 20 open reading frame 45 (C20orf45), mRNA.	NM_016045.1	0.53±0.00	0.61±0.09	1.15±0.15	
RPS23	ribosomal protein S23 (RPS23), mRNA.	NM_001025.4	0.53±0.06	0.63±0.05	1.20±0.23	
ERRF1	ERBB receptor feedback inhibitor 1 (ERRF1), mRNA.	NM_018948.2	0.53±0.05	0.82±0.08	1.55±0.00	+
EMR2	egf-like module containing, mucin-like, hormone receptor-like 2 (EMR2), transcript variant 2, mRNA.	NM_152916.1	0.53±0.00	0.60±0.06	1.14±0.11	
PAK2	p21 (CDKN1A)-activated kinase 2 (PAK2), mRNA.	NM_002577.3	0.53±0.01	0.68±0.08	1.28±0.17	
CXCL2	chemokine (C-X-C motif) ligand 2 (CXCL2), mRNA.	NM_002089.1	0.53±0.04	0.84±0.09	1.57±0.06	+
LARP6	La ribonucleoprotein domain family, member 6 (LARP6), transcript variant 1, mRNA.	NM_018357.2	0.54±0.02	0.61±0.16	1.12±0.26	
WARS	tryptophanyl-tRNA synthetase (WARS), transcript variant 4, mRNA.	NM_213646.1	0.54±0.01	0.65±0.00	1.20±0.01	+
DDIT3	DNA-damage-inducible transcript 3 (DDIT3), mRNA.	NM_004083.4	0.54±0.07	1.03±0.17	1.90±0.09	+
RBM28	RNA binding motif protein 28 (RBM28), mRNA.	NM_018077.1	0.55±0.04	0.66±0.01	1.20±0.06	

Supplementary Table S1B: Transcripts upregulated in Ago2-depleted HeLa cells

Gene Symbol	Definition	Accession No.	Fold changes			
			Ago2	Ago2&Upfl	Ago2&Upfl / Ago2 uORF	
TYRP1	tyrosinase-related protein 1 (TYRP1), mRNA.	NM_000550.1	2.28±0.27	2.30±0.34	1.01±0.03	
TMEM2	transmembrane protein 2 (TMEM2), mRNA.	NM_013390.1	2.25±0.11	2.29±0.01	1.02±0.06	+
C1orf41	chromosome 1 open reading frame 41 (C1orf41), mRNA.	NM_016126.1	1.99±0.01	1.95±0.83	0.98±0.41	
CCNE2	cyclin E2 (CCNE2), transcript variant 2, mRNA.	NM_057735.1	1.98±0.26	1.81±0.02	0.92±0.13	
BTG2	BTG family, member 2 (BTG2), mRNA.	NM_006763.2	1.96±0.17	1.56±0.20	0.79±0.03	
EMPI	epithelial membrane protein 1 (EMPI), mRNA.	NM_001423.1	1.89±0.10	1.74±0.07	0.92±0.09	
GABARAPL1	GABA(A) receptor-associated protein like 1 (GABARAPL1), mRNA.	NM_031412.2	1.89±0.11	3.80±0.09	2.02±0.17	
KLF2	Kruppel-like factor 2 (lung) (KLF2), mRNA.	NM_016270.2	1.85±0.09	2.93±0.27	1.58±0.07	
ACTC	actin, alpha, cardiac muscle (ACTC), mRNA.	NM_005159.3	1.80±0.19	2.04±0.09	1.13±0.07	+