Cell, Volume *128*

Supplemental Data

AU-Rich-Element-Mediated Upregulation of Translation by FXR1 and Argonaute 2

Shobha Vasudevan and Joan A. Steitz

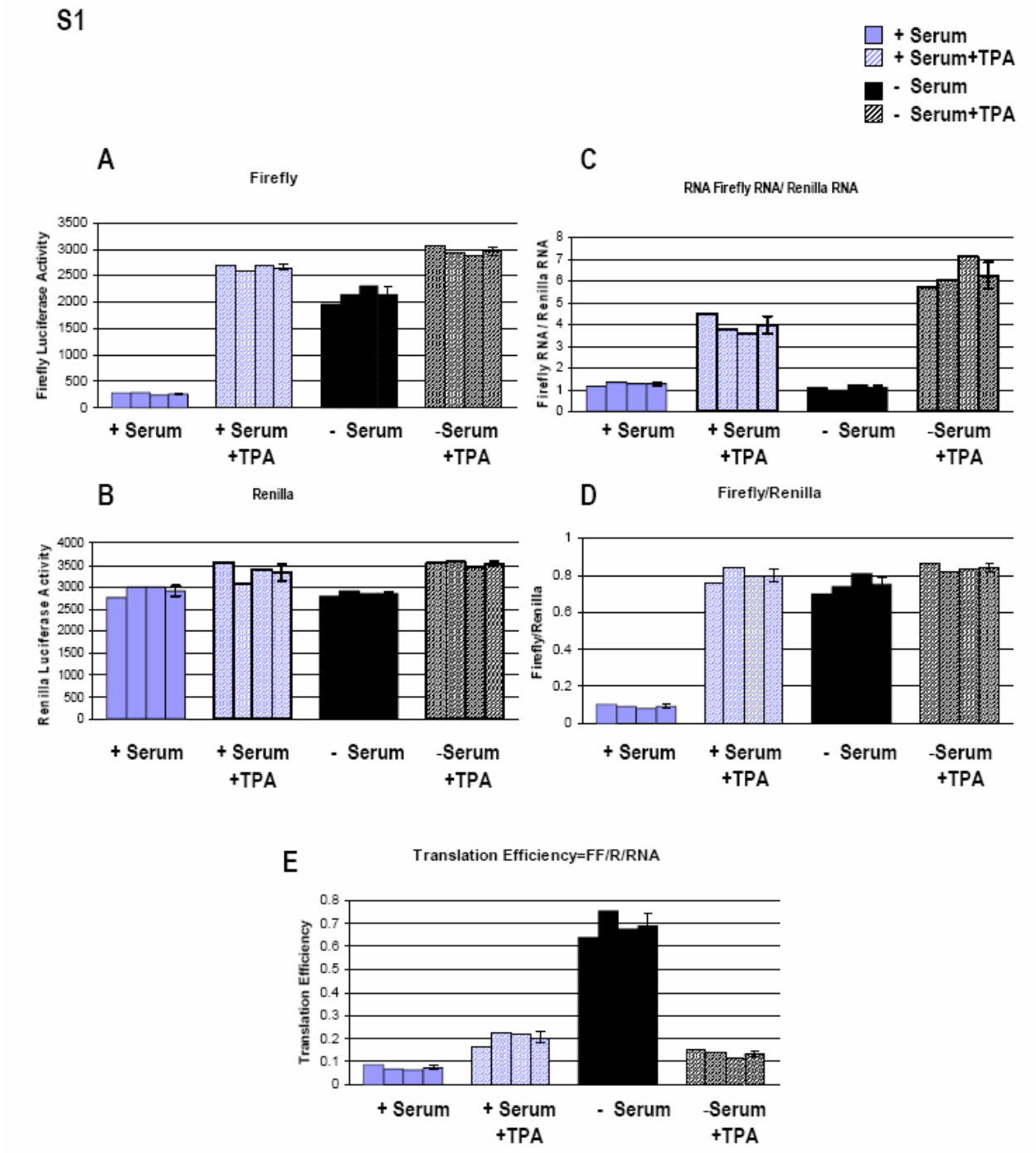


Figure S1.

The first three bars of each set represent three repeats (three independent experiments) of (A) Firefly values, (B) Renilla values, (C) RNA values, (D) Firefly/Renilla values, and (E) Translation Efficiency as reported in Figure 1D. The fourth bar of each set (with error bars) gives an average of the three repeats for each condition (colored the same as in Figure 1D). There is no normalization in A–C. Also see Table S1 and S2 for the numbers. Note that there is an eight-fold

difference between the Firefly numbers of cells that were serum-grown (+Serum) and serum-starved (–Serum), as observed in (A). These numbers are reflected in the final translation efficiency arrived at in Figure 1D and also shown in (E). The final translation efficiency arrived in Figure 1D and also depicted in (E) for the serum-grown and -starved conditions results from first dividing the numbers from the +Serum and –Serum sets in (A), which show at least an eight-fold difference, by the Renilla numbers of the same sets in (B), which are not significantly different. This yields the ratio of Firefly to Renilla as shown in (D), where the values maintain the fold difference observed with the Firefly numbers. These ratios are then divided by the RNA numbers in (C), also shown in the northern blot in Figure 1C. Since the RNA values between the two serum conditions are not significantly different, the final translation efficiency remains representative of the Firefly values observed in (A). Therefore, the change between these two conditions is at the translation level for the ARE reporter and is not an artifact of normalization by either the cotransfected Renilla control values or by the RNA levels. The values in panels (A)–(E) are averages from at least three transfections ± SD.

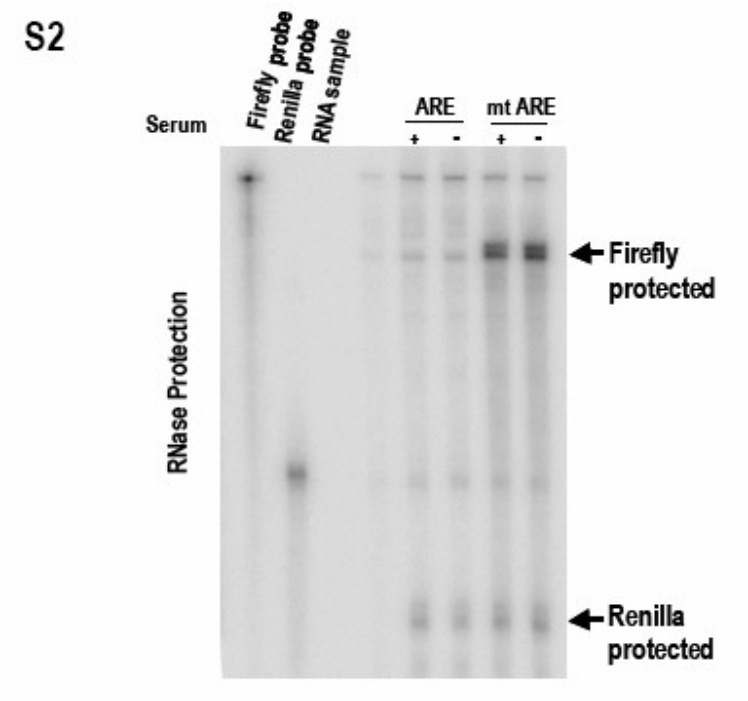


Figure S2.

RNase Protection Analysis (RPA) of the ARE and mt ARE luciferase reporters (described in Figure 1A) expressed in serum-grown or -starved conditions using Firefly and Renilla luciferase probes as described in Figure 3B and Experimental Procedures.

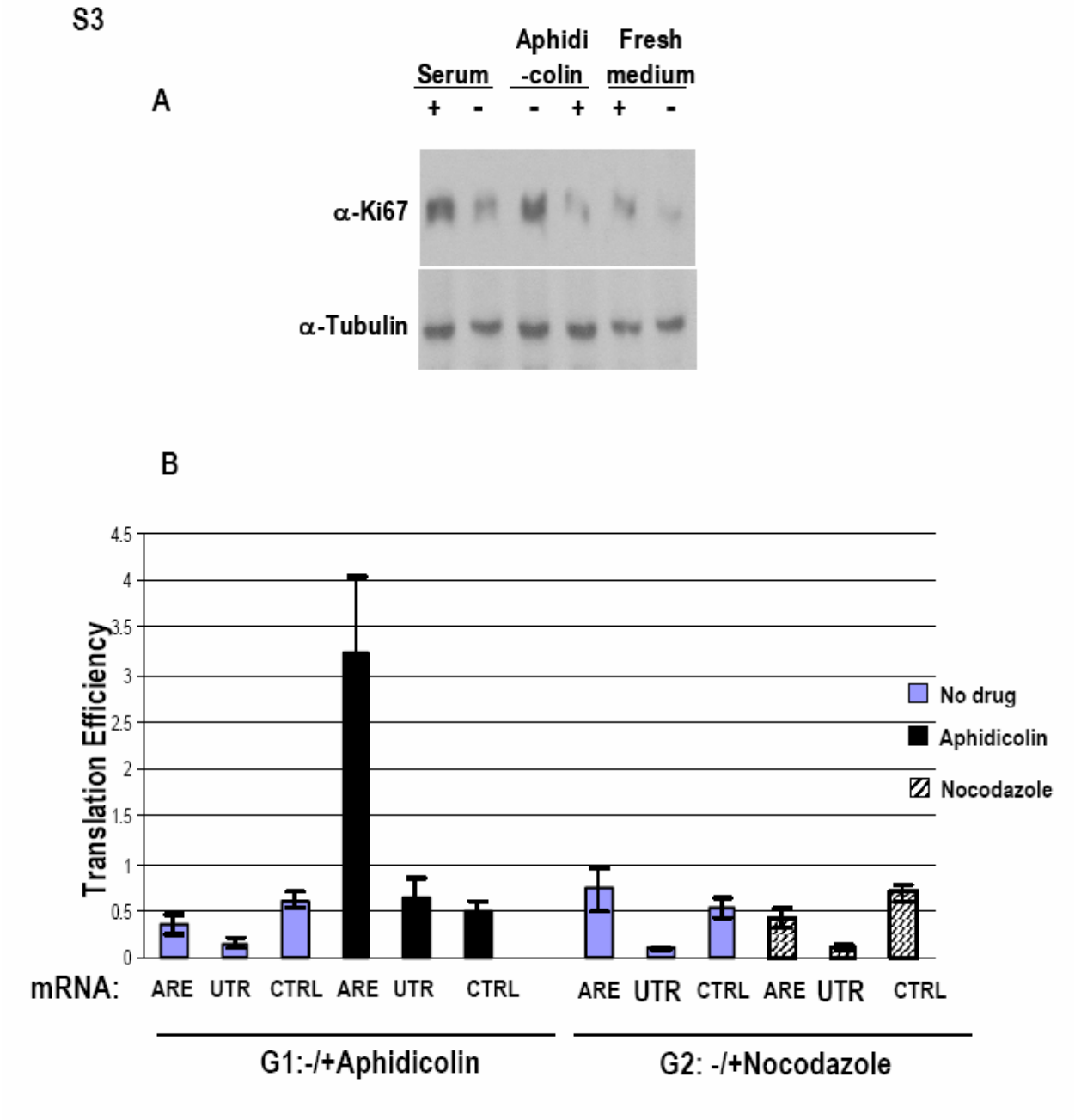


Figure S3.

(A) Western blot analysis of Ki67, a marker for proliferating cells in serum-grown versus -starved cells (Schafer, 1998), in DMSO- versus aphidicolin-treated cells, and fresh medium versus saturated cells. Anti-tubulin antibody was used as a loading control.

(B) G1 arrest by aphidicolin treatment, but not G2 arrest upon nocodazole treatment, leads to translation upregulation. The dark bars represent either aphidicolin treatment (the first set of bars marked G1) or nocodazole treatment (the second set of bars marked G2), while the light

bars are growing, untreated cells. The values in this panel are averages from at least three transfections ± SD.

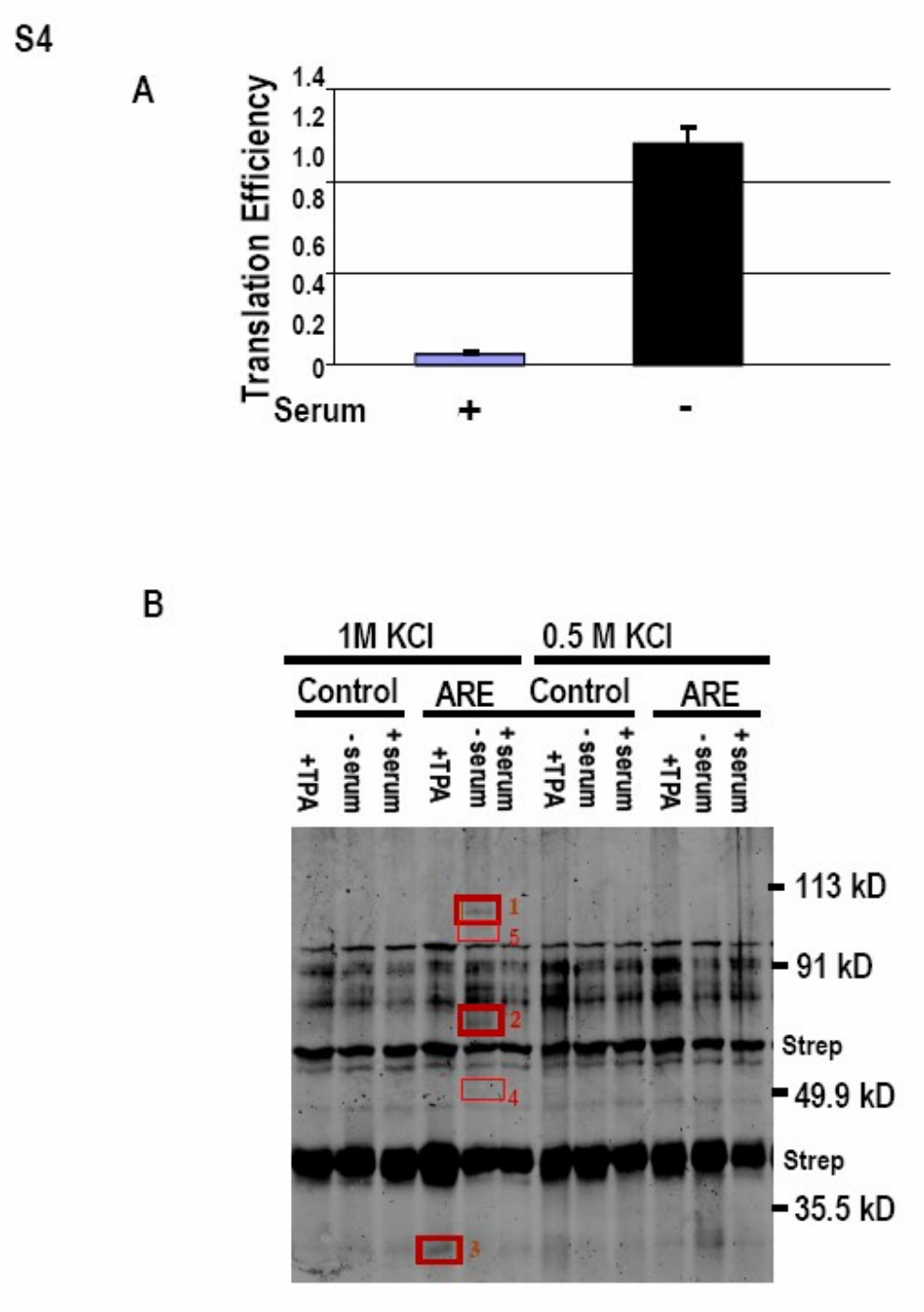


Figure S4.

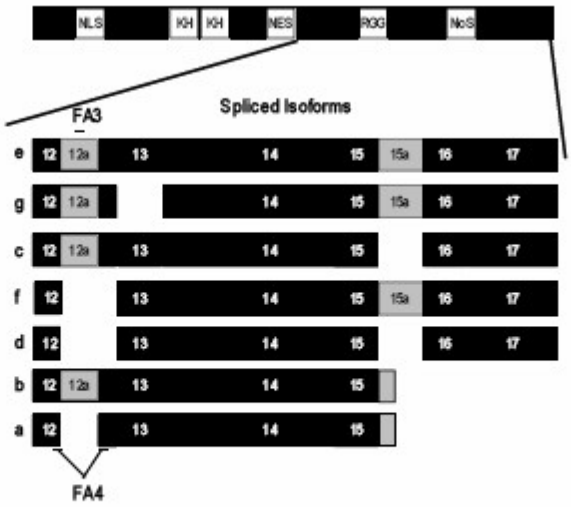
(A) The S1-tagged ARE reporter tested for translation efficiency in serum-grown and -starved cells. The S1-tagged ARE reporter exhibits serum-regulated translation similar to the untagged ARE reporter (Figure 1D), arguing that the aptamer does not interfere with

formation of the translation regulatory complex on the ARE. The values in this panel are averages from at least three transfections ± SD.

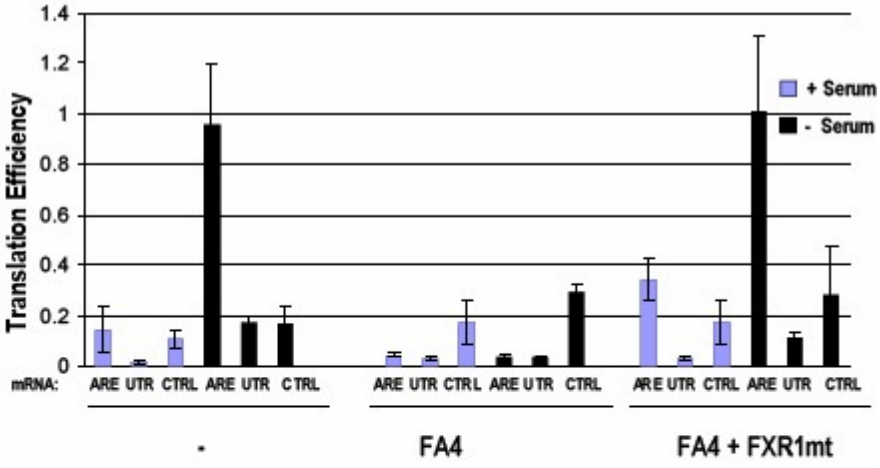
(B) Sypro (Invitrogen) stained gel of the 1000 mM (1M KCl) fraction from the purification of S1-tagged ARE and Control reporters, as depicted in Figure 3, from serum-grown, serum-starved and TPA-treated cells. Bands specific to the ARE RNP are boxed. Also see Table S4. Note that some strong bands are common to all RNPs, while the two abundant bands marked at 42 kD and 55 kD by "Strep" represent streptavidin, which was used in the purification. Boxed band 2 was identified as FXR1.

S5

A


NLS
KH
KH
NES
RGG
NoS
Spliced Isoforms
FA3
FA4
e
g
c
f
d
b
a

B


Translation Efficiency
1.4
1.2
1
0.8
0.6
0.4
0.2
0
+ Serum
- Serum
mRNA:
ARE UTR CTRL ARE UTR CTRL
ARE UTR CTRL ARE UTR CTRL
ARE UTR CTRL ARE UTR CTRL
-
FA4
FA4 + FXR1mt

C

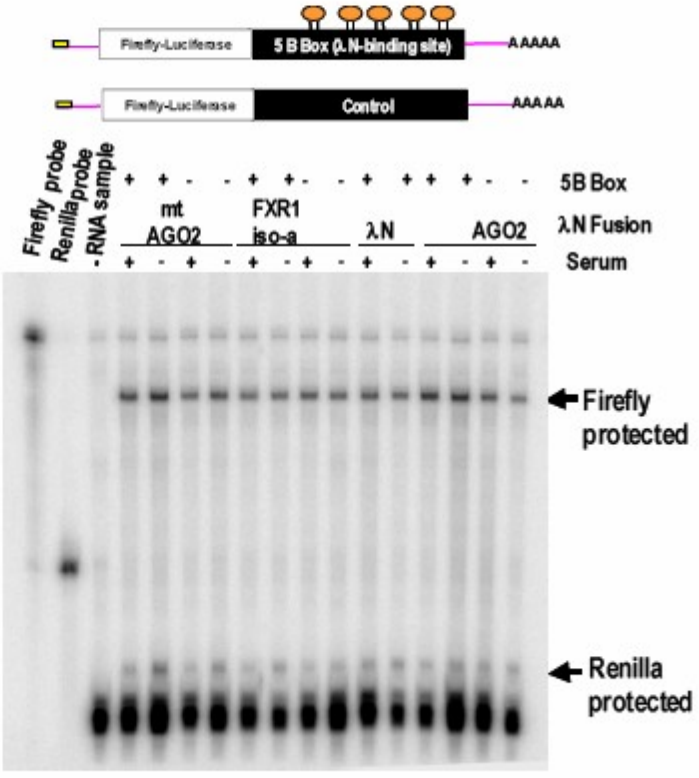

Firefly-Luciferase
5 B Box (λ N-binding site)
AAAAA
Firefly-Luciferase
Control
AAAAA
Firefly probe
Renilla probe
- RNA sample
5B Box
λN Fusion
Serum
mt AGO2
FXR1 iso-a
λN
AGO2
Firefly protected
Renilla protected

Figure S5.

(A) The seven spliced isoforms of FXR1 are shown (Kirkpatrick et al., 1999) along with the target sites for the shRNAs against FXR1: FA3 targets isoforms containing exon 12a (iso-b, -c, -g and -e), while FA4 (which straddles the splice junction) should target iso-a, -d and -f best (but could diminish all isoforms). Iso-a and -b are predicted to be ~65 kD and are expressed in our cells as determined by RT-PCR (not shown).

(B) The lack of serum starvation-induced upregulation in an FXR1 knockdown background (FA4) can be rescued by exogenous expression of an FXR1 clone harboring silent mutations at the knockdown site (FXR1mt). Exogenous expression of the same clone in a serum-grown FXR1-knockdown background does not lead to the overexpression phenotype at these low concentrations, suggesting that this is a true complementation of the FXR1 knockdown. The values in this panel are averages from at least three transfections ± SD.

(C) RPA assessing the levels of the 5B Box luciferase reporter (Figures 4E and 6A) expressed in serum-grown or -starved cells with mutant AGO2 (mt AGO2), FXR1 iso-a, or AGO2, all co-expressed as λN fusion proteins. The CTRL reporter was used in the 5B Box –lanes.

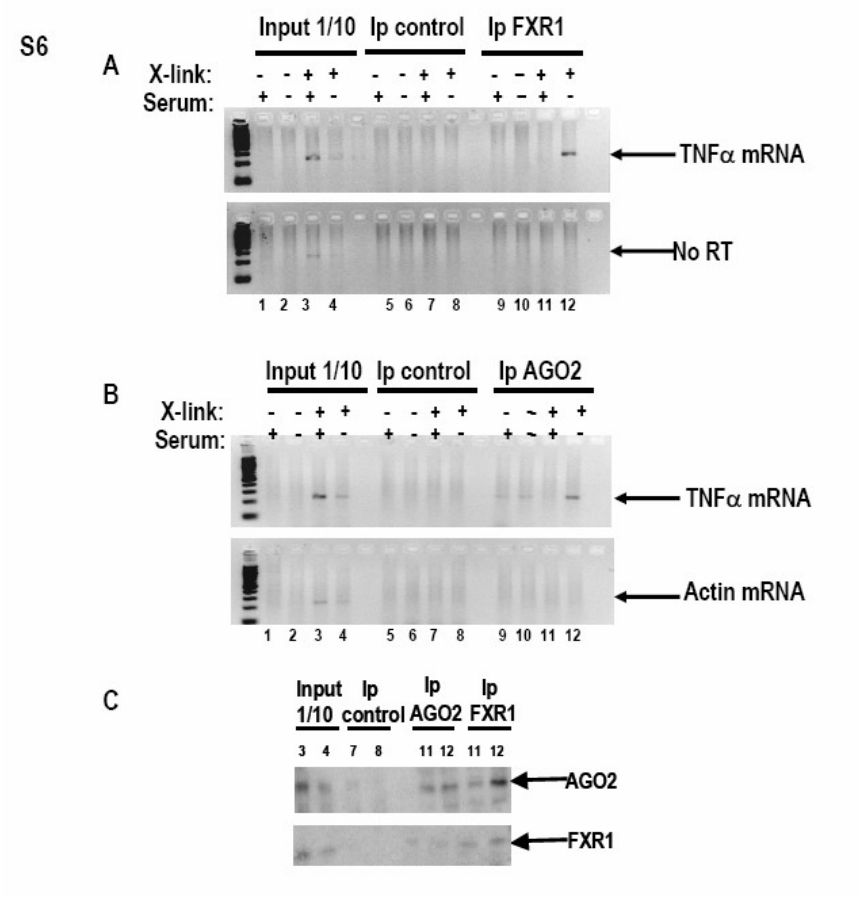


Figure S6.

The endogenous TNFα transcript forms a serum starvation-induced complex with AGO2 and FXR1 in THP-1 monocytes. Immunoprecipitation using anti-AGO2, anti-FXR1 or a control rabbit polyclonal antibody and cell extracts of non-crosslinked (lanes 1, 2, 5, 6, 9 and 10) or formaldehyde-crosslinked (lanes 3, 4, 7, 8, 11 and 12) THP-1 monocytes grown in serum or serum-starvation conditions was followed by RNA and protein analyses.

(A) Immunoprecipitation with antibody against FXR1 or

(B) antibody against AGO2 was followed by RT-PCR analysis for detection of either TNFa (top panel) or actin (lower panel) mRNA, as marked by the arrows. RT-PCR for TNFα mRNA utilized three primer sets [amplifying between the stop codon and 240 nt into the 3′-UTR (between 865–1104 nts); between 932–1139 nts in the 3′-UTR (shown); and in the coding region between 550–770 nts] and 12 cycles of touchdown PCR followed by 15–18 cycles at 54°C. The no RT-treated samples shown in the lower panel in (A) served as a control. Actin

mRNA was present in the input but not in the immunoprecipitates shown in the lower panel in (B) and served as the negative control. 100bp ladder was run as a marker.

(C) Western blot analysis of the above crosslinked samples (lanes 3, 4, 7, 8, 11 and 12) from monocytes grown in serum or serum-starvation conditions to demonstrate the immunoprecipitation efficiency.

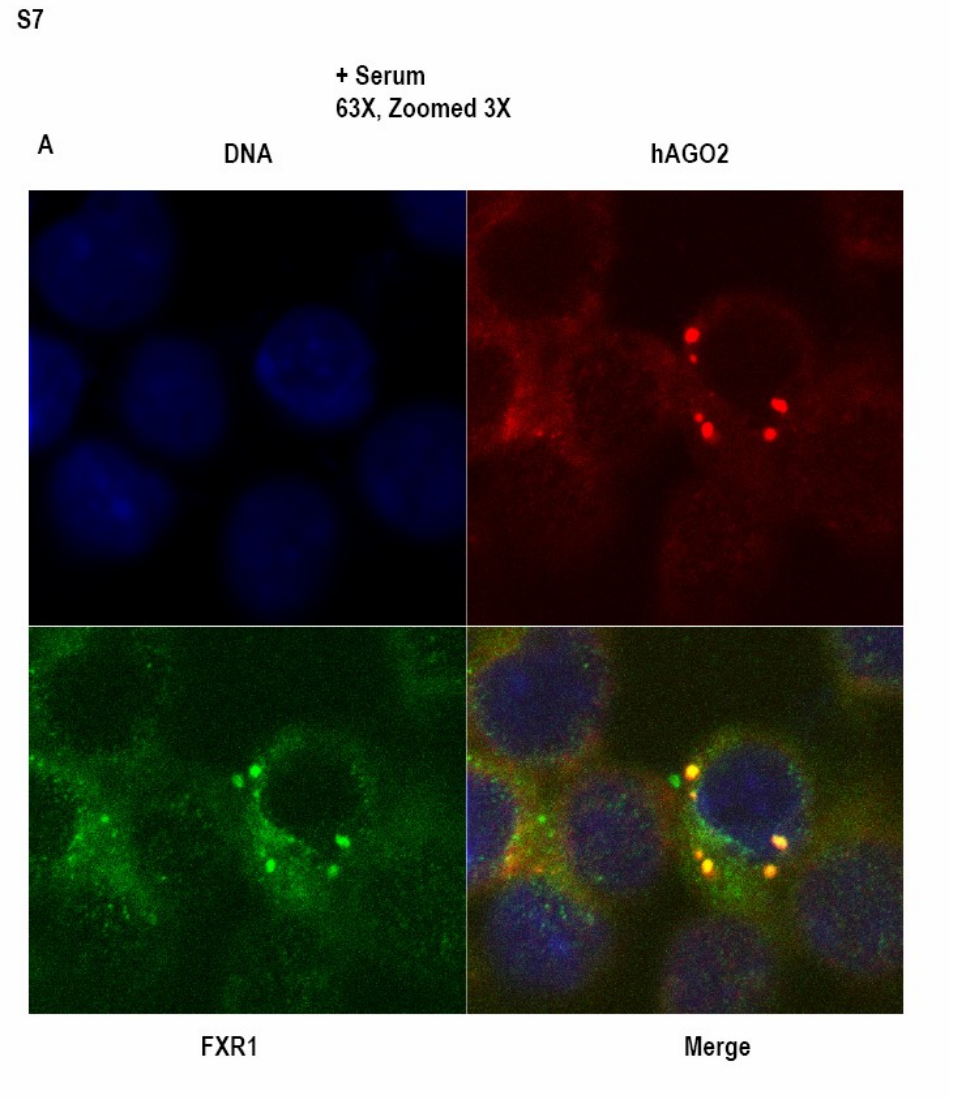


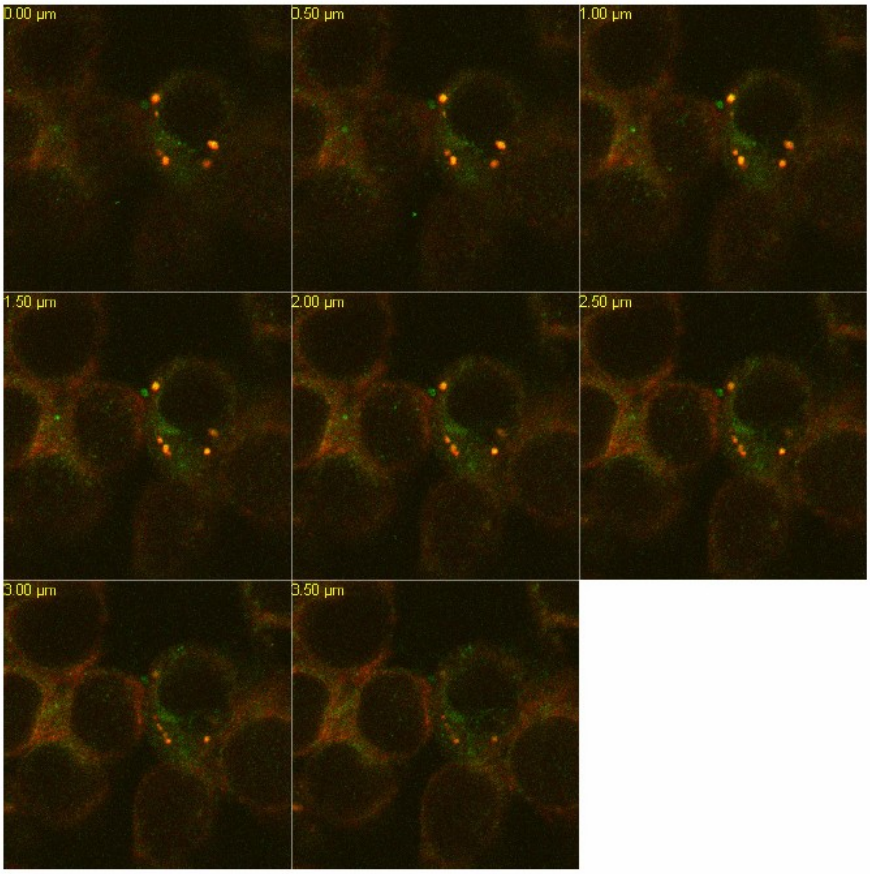
Figure S7.

Colocalization of FXR1 with AGO2 and hDCP1A, a P body marker. Confocal images shown in Figure 5B were magnified to show detail.

(A) +Serum cells at 63X, zoomed three times to show colocalization of some of the AGO2- and FXR1-containing bodies.

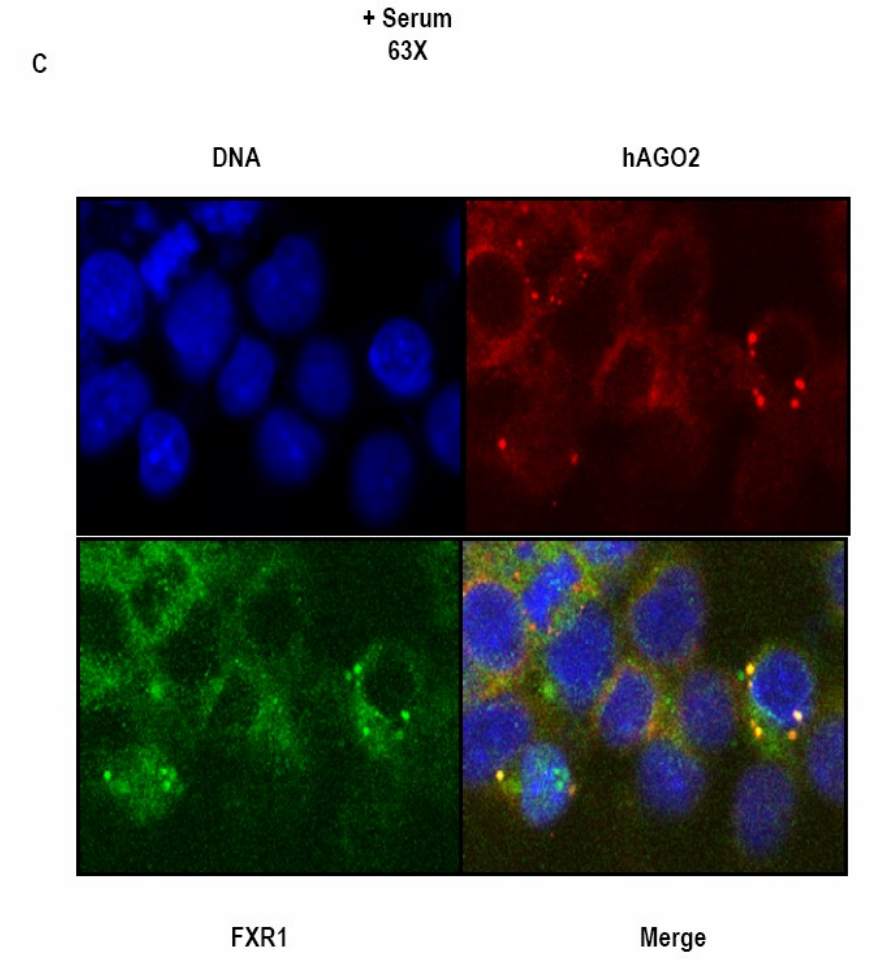
S7
B

Z stack of merged + Serum image of AGO2 (red) and FXR1 (green) bodies showing o.5 μm sections. 63X zoomed three times.



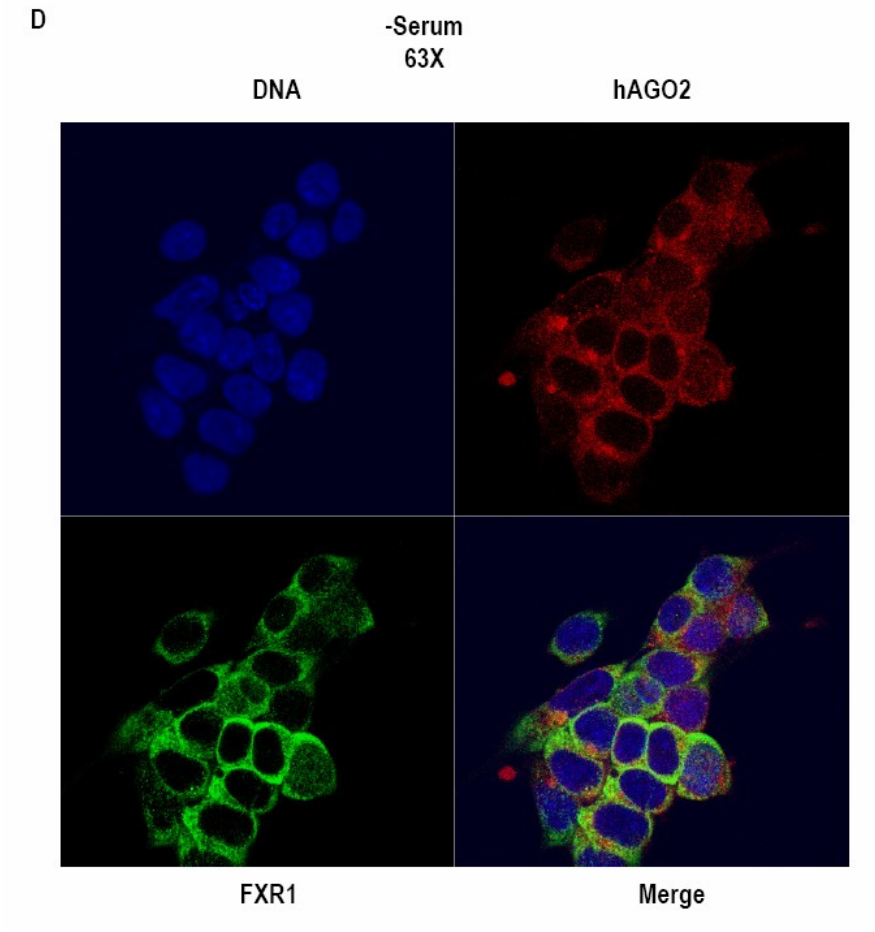
(B) Z stack analysis of the field in (A) to show that AGO2 and FXR1 remain colocalized through a stack of eight 0.5μm sections.

S7



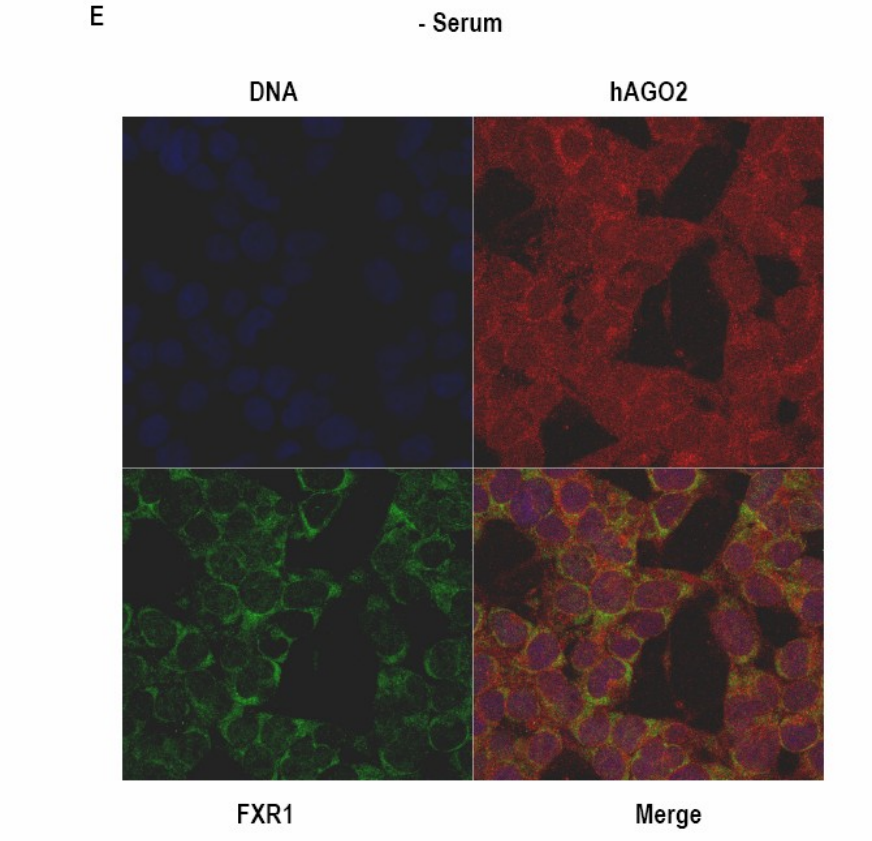
(C) Magnified version of +Serum cells demonstrating AGO2 and FXR1 colocalization in Figure 5B (top panel).

S7



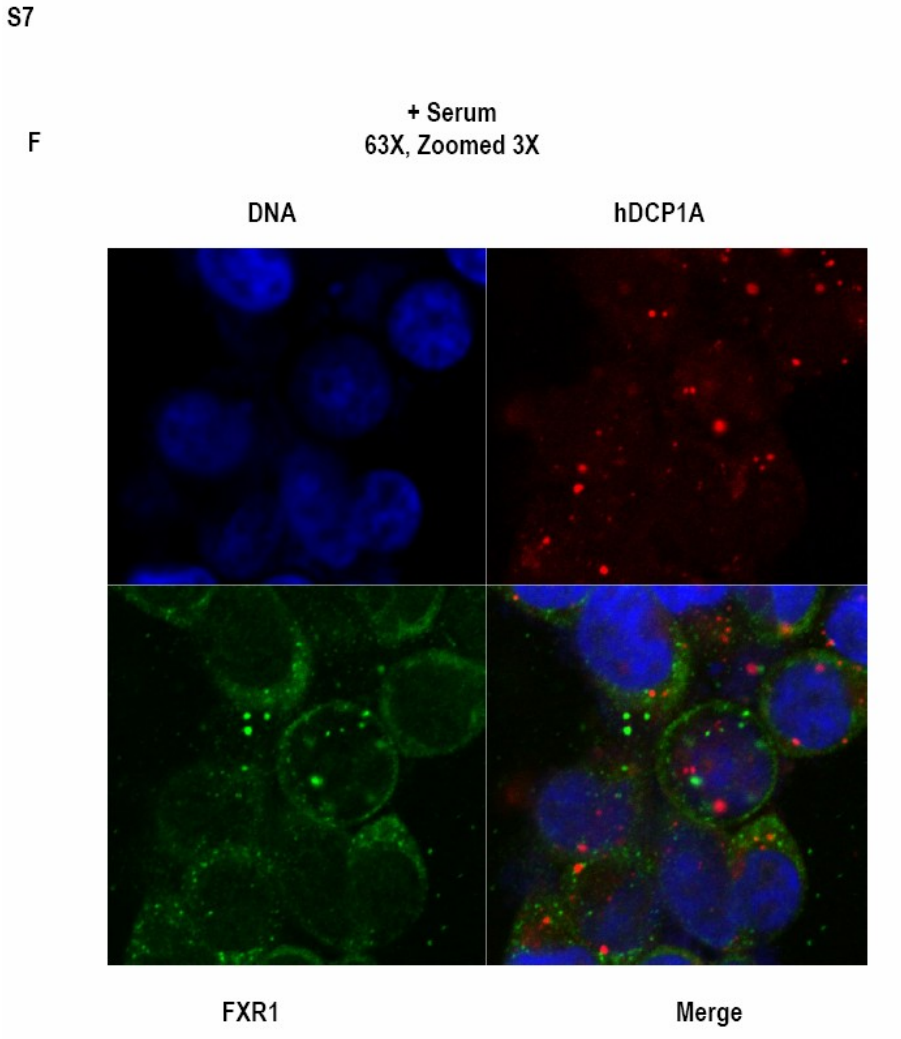
(D) –Serum cells at 63X showing no bodies formed by AGO2 and FXR1 in serum-starved growth conditions.

S7



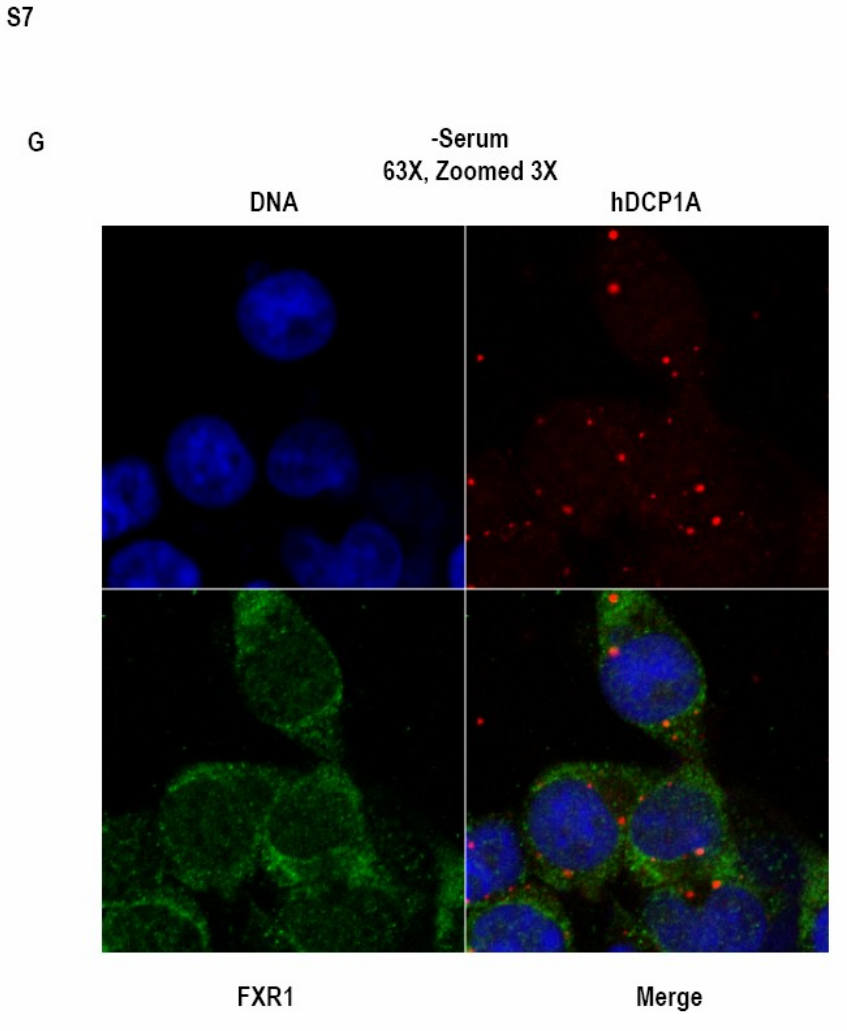
(E) Larger field of –Serum cells showing no bodies containing AGO2 and FXR1 in serum-starved growth conditions.

S7



(F) +Serum cells zoomed three times to show that P bodies (hDCP1A) and FXR1 bodies do not significantly colocalize.

S7



(G) –Serum cells zoomed three times to show that P bodies persist in serum-starved growth conditions, whereas FXR1 (shown here) and AGO2 (seen in D-E) form smaller bodies and appear more diffuse.

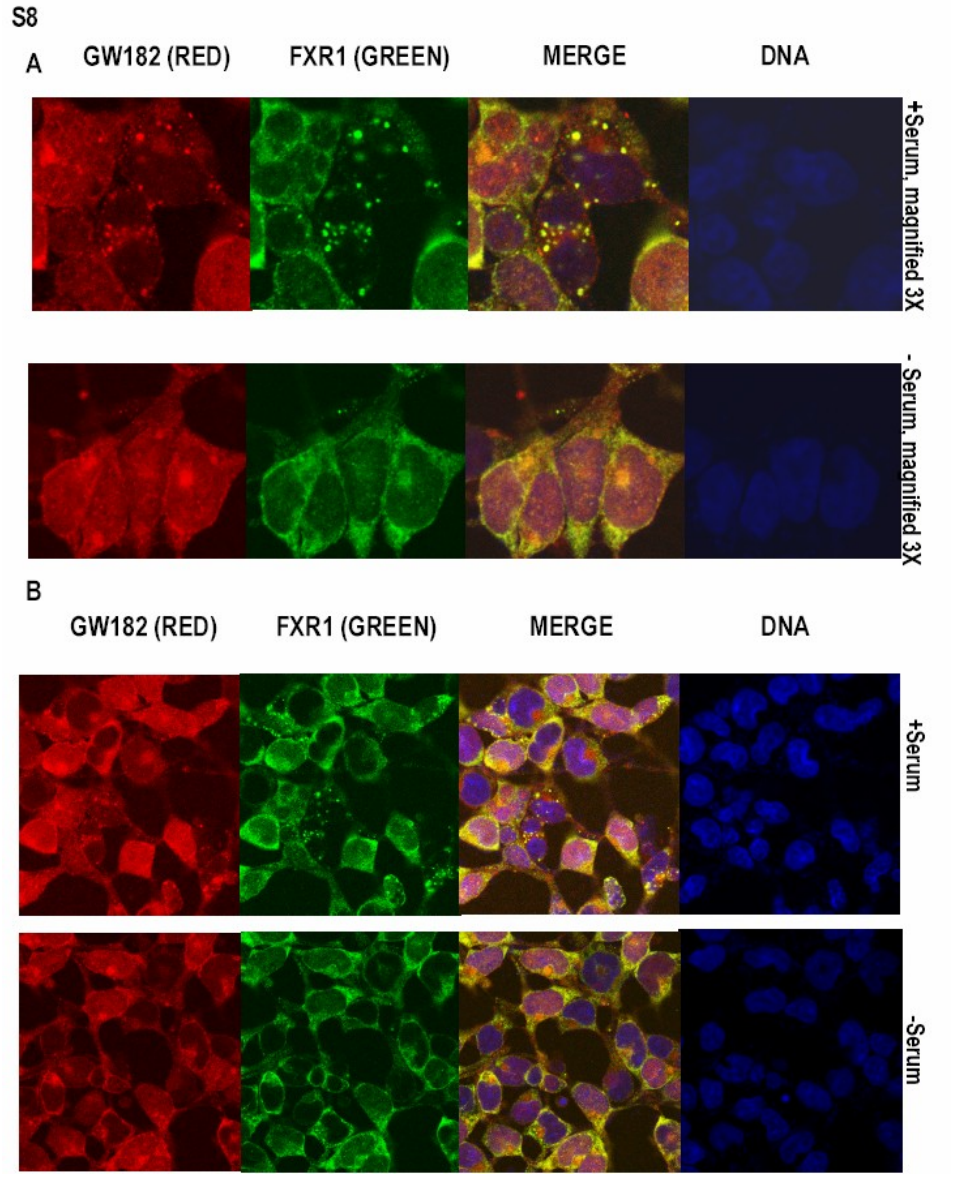
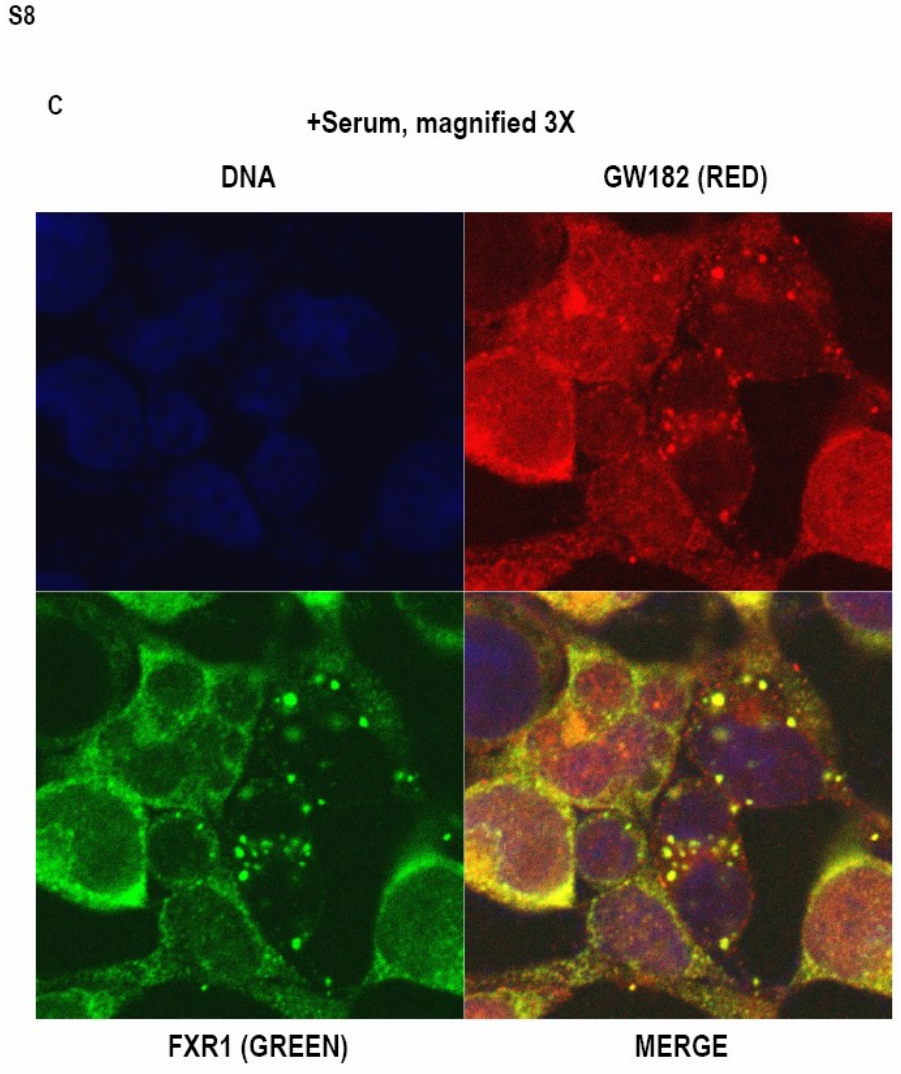


Figure S8.

Confocal images taken with a 63X objective as in Figure 5B, demonstrating colocalization of GW182 (red) with FXR1 (green). The DNA is stained blue by TO-PRO3. GW182 was detected with 2D6 and 4B6 antibodies (Jakymiw et al., 2005; Yang et al., 2004), while FXR1 was detected using the antibody from S. Warren (Jin et al., 2004). Note that the serum-grown cells are asynchronous.

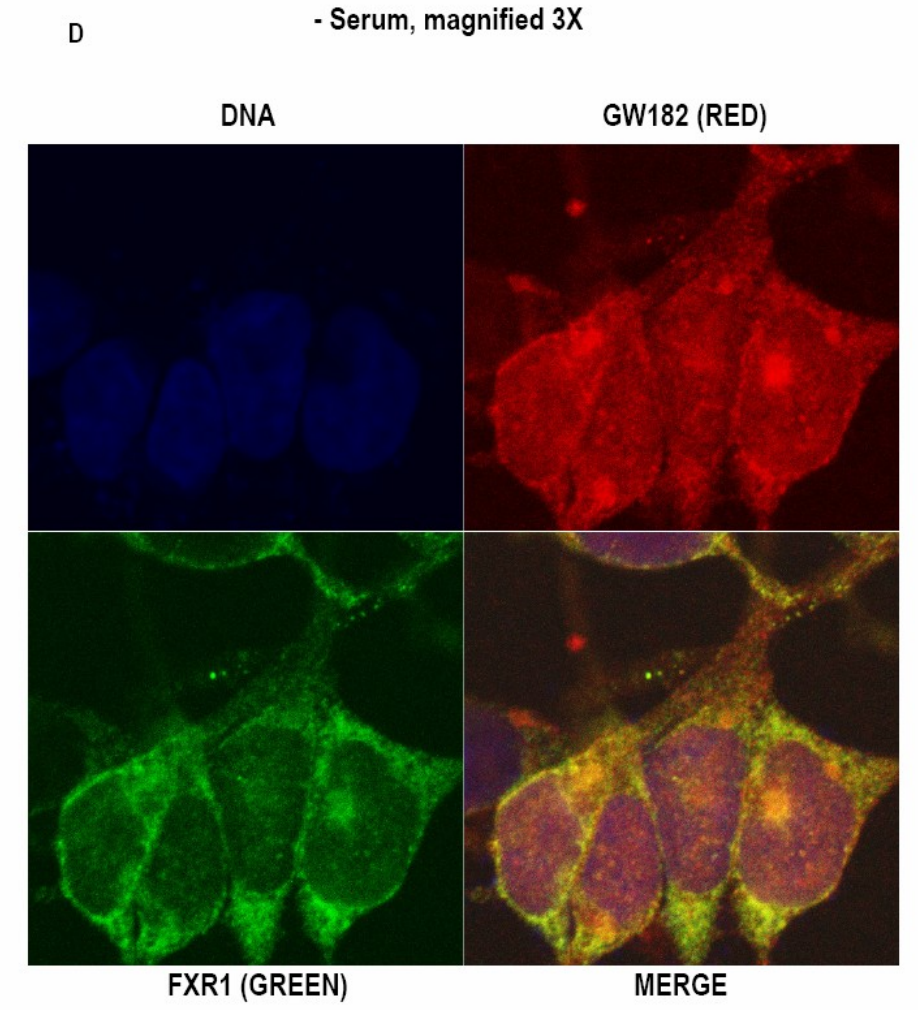
(A) Images at 3X magnification. The top panel shows images of cells grown in serum, while the lower panel shows images of cells grown under serum-starvation conditions.

(B) Images at 1X magnification of serum-grown and serum-starved cells as in (A).



(C) Three-fold magnified images of cells shown in the top panel of (A) (grown in serum).

S8



(D) Three-fold magnified pictures of images of cells shown in the lower panel of A (grown in serum-starved conditions). The foci vary in size in all images of serum-grown cells because the cells are asynchronous and GW bodies are known to grow in size and number through the cell cycle (Ikeda et al., 2006).

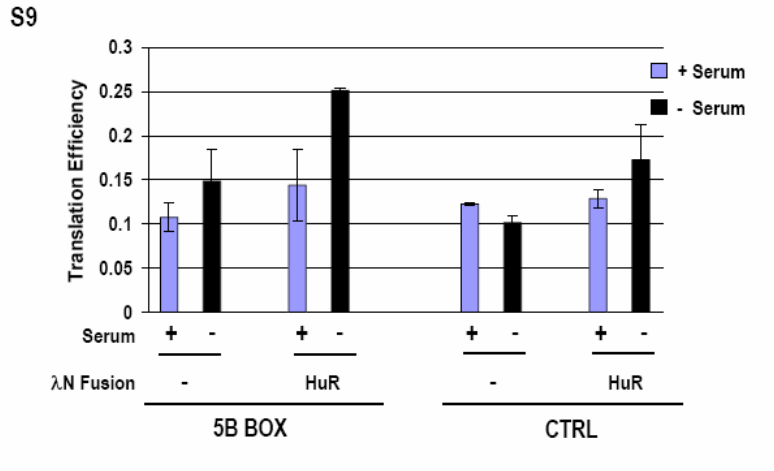


Figure S9.

Tethering HuR increases the translation efficiency of the 5B Box luciferase reporter less than two-fold compared to the λN empty vector control upon serum starvation. The CTRL reporter without the 5 B boxes does not respond significantly to the presence of λN-HuR. The values in this figure are averages from at least three transfections ± SD.

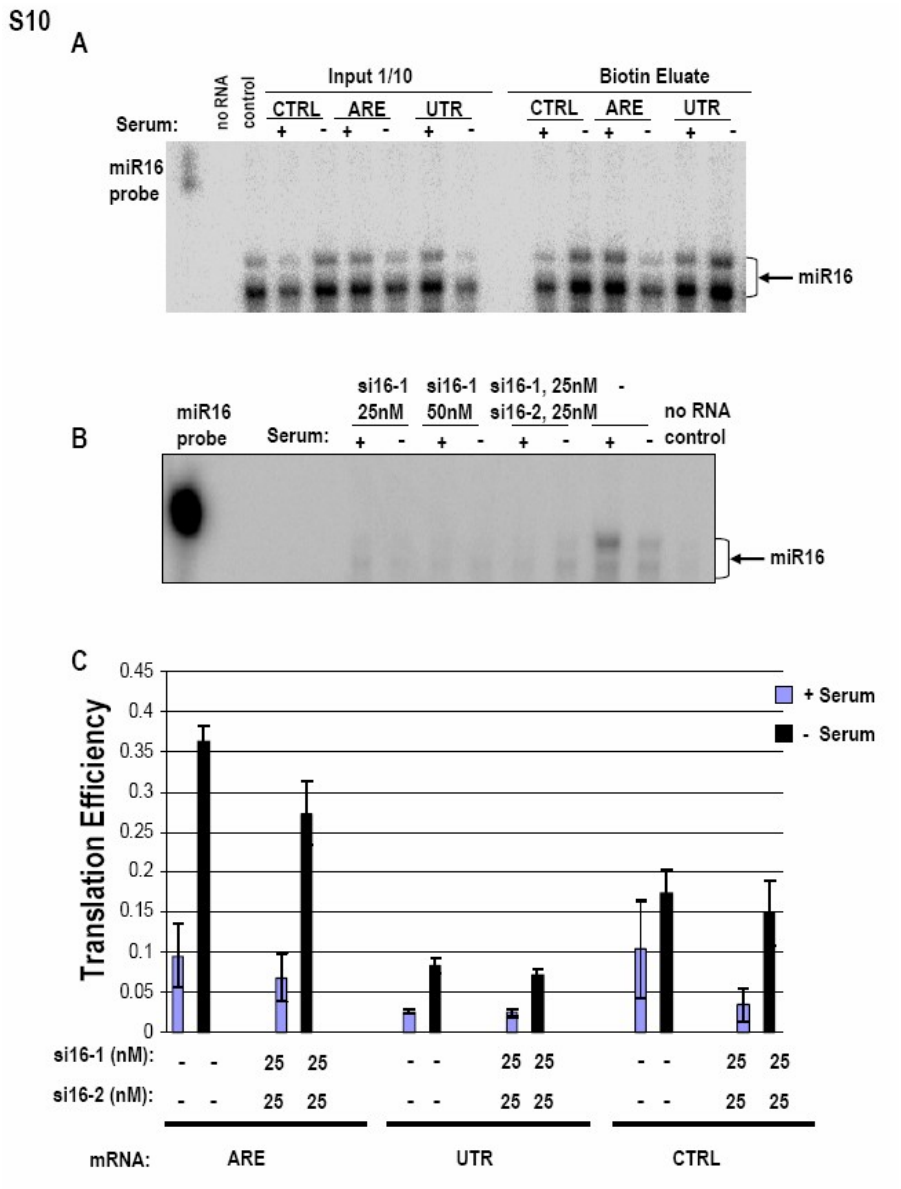


Figure S10.

MicroRNA miR16 is not involved in this translation regulation of the ARE and UTR reporters. (A) RPA of the aptamer affinity-purified RNPs, as described in Figure 3, for the CTRL, ARE, and UTR reporters. Note that Jing et al. (2005) did not show association with the 34-nt ARE; rather, a second AU-rich sequence in the UTR was predicted by them to be associated with miRNA 16. The bands corresponding to mature miR16 are marked: two bands appear in both the control (total RNA from the Ambion RPA kit) and after affinity purification, likely due to breathing during the RPA hybridization. In multiple attempts, we found miR16 equally

present in the CTRL versus ARE and UTR RNPs when affinity purification was performed as in Figure 3, suggesting non-specific association with our reporters.

(B) RPA of control-treated cells and cells knocked down for miR16-1 and miR16-2 using siRNAs at concentrations described in Jing et al. (2005). Phosphorimager quantitation suggests more than two-fold knockdown was achieved.

(C) Translation Efficiency of the ARE, UTR and CTRL reporters upon knockdown of miR16-1 and miR16-2 is not significantly altered. Higher concentrations of either siRNA revealed non-specific effects on the CTRL reporter (not shown). The values in this panel are averages from at least three transfections ± SD.

Table S4

JNK2	ARE and UTR RNPs
TOB1	ARE and UTR RNPs
GSTPI	ARE RNP
FXR1	ARE and UTR RNPs
AGO2	ARE and UTR RNPs
HUR	ARE and UTR RNPs
TTP	ARE and UTR RNPs

Table S4.

List of proteins identified so far by mass spectrometry or by western analyses that are associated specifically with the ARE and/or UTR reporters upon formaldehyde-crosslinked affinity purification either in serum-starved or in both conditions. HuR and TTP were identified by western analyses as ARE-binding proteins that should be present on the ARE/UTR complexes, while AGO2 was initially identified by western analysis as an FXR1-interacting protein.

Supplemental Experimental Procedures

Cell Lines, Growth Conditions and Transfections

HEK293 and THP-1 monocytes were obtained from ATCC. Cells were maintained in DMEM with 10% FBS. Transfections were performed with the Trans-It 293 kit (MirusBio) for HEK293 cells or the Nucleofector (Amaxa) for THP-1 cells according to the manufacturers' instructions. All plasmids were cleared of endotoxin using the EndoGO kit (MirusBio) prior to transfection. 12–18 hours post-transfection, cells were washed and 1) the medium replaced with either complete medium with 10% serum or with complete medium without serum for 18 hours [where indicated, 50ng/ml TPA (Sigma) and 2.5 uM Io (Calbiochem) were added]; 2) the medium replaced with complete medium for 48 hours followed by another medium change to only one set for 6 hours; or 3) the medium replaced with complete medium containing 4 μg/ml of aphidicolin (Sigma) or an equal volume of the solvent for another 36 hours, followed by a medium change for 6 hours for the solvent samples prior to harvesting the cells.

Plasmids

The following plasmids were used:

pcDNA3 vector was cut with BamHI to insert the Firefly ORF as a PCR-amplified product using oligos 1 and 2, generating CTRL (Figure 1A). The Renilla reporter and the template for PCR amplification of Firefly were obtained from Ruiz-Echevarria et al. (1998). Firefly and Renilla reporter expression was under control of the CMV promoter. Oligos 3 and 4 (ARE), as well as oligos 5 and 6 (mt ARE), were phosphorylated by T4 polynucleotide kinase, annealed and inserted into the EcoRI site of pBluescript (SK+). These plasmids were then used to generate XhoI-NotI fragments containing the inserts, which were cloned downstream of the Firefly ORF in CTRL to obtain the ARE and the mt ARE reporters, respectively. The TNF 3′-UTR was

amplified by RT-PCR using oligos 7 and 8 and inserted as a XhoI-NotI fragment into CTRL to obtain the UTR reporter. The CTRL, ARE and UTR vectors were cut with ApaI, and oligos 9 and 10 annealed and inserted to obtain the S1 aptamer-tagged vectors. The λN vector and 5B Box plasmids were obtained from M. Hentze (Baron-Benhamou et al., 2004). The 5B Box sequence was PCR-amplified with oligos 11 and 12 and inserted as a NotI-SalI/XhoI fragment into the CTRL vector to obtain the Firefly 5B Box reporter. The FXR1 iso-a clone was obtained from Open Biosystems and amplified using oligos 13 and 14 and cut with XhoI-NotI to clone into the λN vector as λN-FXR1 iso-a Flag. The λN-tagged AGO2 and PRPΔ (mt AGO2) plasmids were obtained from R. Pillai and W. Filipowicz (Pillai et al., 2004). Oligos 15 and 16 were used to amplify oligos 17, 18, 28 and 29, restriction-digested with EcoRI and XhoI, and cloned into the shRNA vector pshag-MAGIC2 (Open Biosystems) to produce the FA3, FA4, shAGO2-1-8 and shAGO2-2-6 knockout plasmids against FXR1 and AGO2, as described (Paddison et al., 2004).

Oligonucleotides used:
1. 5′-CGCGGATCCATGGAAGACGCCAAAAAC-3′
2. 5′-CGCGGATCCTTACAATTTGGACTTTCC-3′
3. 5′AATTCTCTATTTATATTTGCACTTATTATTTATTATTTATTTATTATTTATTTATTTGCTTATGAATGTATTTATTTG-3′
4. 5′AATTCAAATAAATACATTCATAAGCAAATAAATAAATAATAAATAAATAATAAATAATAAGTGCAAATATAAATAGAG-3′
5. 5′AATTCTCTATGTATATGTGCACTTATTATGTATTATGTATGTATTATGTATGTATTTGCTTATGAATGTATGTATTTGG-3′
6. 5′AATTCCAAATACATACATTCATAAGCAAATACATACATAATACATACATAATACATAATAAGTGCACATATACATAGAG-3′
7. 5′-AAGGAAAAAAGCGGCCGCGGAGGACGAACATCCAACC-3′
8. 5′-CCGCTCGAGCTCGCCACTGAATAGTAGGGC-3′
9.5′-CCTCGAGACCGACCAGAATCATGCAAGTGCGTAAGATAGTCGCGGGCCGGGGGGCC-3′
10. 5′-CCCCGGCCCGCGACTATCTTACGCACTTGCATGATTCTGGTCGGTCTCGAGGGGCC-3′
11. 5′-AAGGAAAAAAGCGGCCGCTAAGCTCGCTTTCTTGCTGTCCAATTTC-3′
12. 5′-ACGCGTCGACAGGCAGAATCCAGATGCTCAAGGCCC-3′
13. 5′-CCGCTCGAGATGGCGGAGCTGACGGTG-3′
14. 5′AAGGAAAAAAGCGGCCGCTTACTTGTCATCGTCGTCCTTGTAGTCATCACAT

CTTTTGCCTAGCCC-3′
15. 5′-CAGAAGGCTCGAGAAGGTATATTGCTGTTGACAGTGAGCG-3′
16. 5′-CTAAAGTAGCCCCTTGAATTCCGAGGCAGTAGGCA-3′
17. 5′TGCTGTTGACAGTGAGCGCGTACCGTCTCTTCTGTACAAGTAGTGAAGCCAC
AGATGTACTTGTACAGAAGAGACGGTACTTGCCTACTGCCTCGGA-3′
18. 5′TGCTGTTGACAGTGAGCGAACAGATTGGTTCTAGGTCTTATAGTGAAGCCAC
AGATGTATAAGACCTAGAACCAATCTGTCTGCCTACTGCCTCGGA-3′
28. 5′TGCTGTTGACAGTGAGCGCCCCGGCTCTTCTGCACTGACATAGTGAAGCCAC
AGATGTATGTCAGTGCAGAAGAGCCGGGTTGCCTACTGCCTCGGA-3′
29. 5′TGCTGTTGACAGTGAGCGATGGCAGGACAAAGATGTATTATAGTGAAGCCA
CAGATGTATAATACATCTTTGTCCTGCCACTGCCTACTGCCTCGGA-3′

Extract Preparation and Purification of S1-Tagged mRNP

The S1-tagged aptamer-bearing ARE and CTRL reporter constructs were transfected along with the REN control into $5x10^8$ HEK293 cells or THP-1 cells, resulting in 80% transfection efficiency for HEK293 and ~40% for THP-1 cells by GFP analysis. After growth in the various serum conditions described above, the medium was replaced with 0.2% formaldehyde in PBS (37% grade from J.T.Baker) for 10 minutes at 37°C (Niranjanakumari et al., 2002). The cells were harvested, washed and resuspended in lysis buffer (150mM KCl, 10mM Hepes 7.4, 3mM MgCl2, 2mM DTT, 10% glycerol, 0.5% NP-40) for 10 minutes on ice, then sonicated and clarified by centrifugation at 2000g for 5 minutes. The extracts were precleared with avidin beads that do not bind the aptamer (Srisawat and Engelke, 2002), followed by DEAE sepharose fractionation with 150–1000mM salt (usually KCl, but NaCl gave the same results). Fractions were assayed by RPA to detect the RNA. The 1M fraction, which was enriched for the Firefly RNA, was then bound to streptavidin pre-blocked with tRNA and glycogen for 4 hours, subjected to ten washes with salt concentrations increasing to 300 mM KCl in the binding buffer with tRNA, glycogen and 2% NP-40, followed by elution for 1 hour with 5mM biotin as described (Srisawat and Engelke, 2002). After heat inactivation at 65°C for 45 minutes (Niranjanakumari et al., 2002), the RNA and proteins were assayed by RPA and Sypro (Molecular Probes, Invitrogen) staining, respectively, and specific bands sent for mass spectrophotometric analysis (Columbia University Protein Core Facility).

Anti-FXR1 polyclonal antibodies were from S. Warren (Jin et al., 2004), E.W. Khandjian (Khandjian et al., 1998), anti-FXR1 6GB10 monoclonal antibody from G. Dreyfuss; anti-HuR monoclonal 3A2; anti-TTP H-120 polyclonal antibody and anti-HA monoclonal antibody from Santa Cruz Biotech, Inc.; anti-hUPF3 polyclonal antibody; anti-Flag monoclonal antibody from Sigma; anti-Ki67 antibody from Abcam Inc.; α-hDCP1A (Lykke-Andersen and Wagner, 2005); anti-GW182 (2D6 and 4B6; Jakymiw et al., 2005; Yang et al., 2004) from Abcam Inc.; anti-AGO2 polyclonal antibody and anti-tubulin monoclonal antibody from Upstate. For western blotting, soluble extracts were prepared by lysis on ice in hypotonic buffer (10mM NaCl, 10mM Hepes 7.4, 3mM MgCl2, 2mM DTT, 10% glycerol and 0.1% NP-40) for 10 minutes, followed by centrifugation at 2000g for 10 minutes; sonicated extracts were made as described above. For immunoprecipitations, sonicated extracts were prepared as described in the S1 tag purification, incubated with specific antibodies and exposed to either 25 μl of a 50% slurry of protein G beads (Amersham, AGO2, Flag and HA IP) or 50 μl of IgY beads (Aves Labs Inc., FXR1 IP), followed by eight washes with RIPA buffer (1% NP-40, 0.5% sodium deoxycholate, 0.1% SDS, 50 mM Tris 7.5 and 150 mM NaCl).

Immunofluorescence

Laser-scanning confocal immunofluorescence imaging was performed using an inverted Axiovert 200 LSM 510 Meta confocal microscope (Zeiss) at the Yale Cell and Confocal Microscopy and Imaging Facility. α-AGO2, α-FXR1, α-GW182 and α-hDCP1A were used to stain cells grown with serum or in serum-starved conditions. TO-PRO-3 (Blue, Invitrogen) staining identified the nuclei. Briefly, cells were fixed with 2% formaldehyde, permeabilized with methanol or 0.1% Tween 20, blocked, incubated and washed with 1% normal goat serum in PBS. Alexa Fluor 488 (green) or Alexa Fluor 594 (red; Invitrogen) goat anti-rabbit were used to detect AGO2 and hDCP1A, while FITC (green) goat anti-chicken (Abcam Inc.) or Alexa Fluor

555 and Alexa Fluor 594 (red) goat anti-mouse were used to detect FXR1 (FXR1 antibody from S.Warren or 6GB10 from G. Dreyfuss) and GW182.

RNA Analyses

Northern blots were performed on 10μg total RNA run on a 1% formaldehyde gel and probed with ^{32}P-labeled RNAs complementary to the 3′-end of the coding region of Firefly (-330 nts from the stop codon) or of Renilla (-90 nts from the stop codon; Figure 1). For all other figures, RPA was performed using the same probes and the protected samples run on 6% PAGE, dried and quantitated by a Storm 840 phosphorimager (Molecular Dynamics). All RNA analyses were performed in triplicate and standard deviations calculated for subsequent normalization of the translation assays. RT-PCR was performed using Superscript II (Invitrogen) and oligo dT priming following the protocol from Invitrogen. The primer pairs used were:

1. TNF4-1: 5′CCCTGAAAACAACCCTCAGA-3′

   TNF4-2: 5′AAGAGGCTGAGGAACAAGCA-3′

2. TNF3-1: 5′CTATCTGGGAGGGGTCTTCC-3′

   TNF3-2: 5′GGTTGAGGGTGTCTGAAGGA-3′

3. TNF2-1: 5′CCTGTGAGGAGGACGAACAT-3′

   TNF2-2: 5′AGGCCCCAGTTTGAATTCTT-3′

siRNAs against microRNA 16-1 and microRNA 16-2 were synthesized as described in Jing et al. (2005) and were transfected into HEK 293 cells to a final concentration of 25 nM.

Translation Assays

Luciferase activities were measured using a TD 20/20n (Turner BioSystems) and the Dual Luciferase Assay System (Promega) per the manufacturers' instructions. The Firefly to Renilla luciferase ratio was further normalized for both Firefly and Renilla mRNA levels to obtain the translation efficiency (Figure 1A).

Polysome analysis was performed as described in Ceman et al. (2003). Briefly, cytoplasmic lysates from cells grown in either +/- serum, treated with or without puromycin and 200mM salt as a control, were made as described in Ceman et al. (2003). Soluble cytoplasmic extracts were ultracentrifuged through a 10–50% sucrose gradient at 39000 rpm for 1 hr 20 minutes at 4°C. Fractions were collected with a continuous UV/VIS ISCO6 monitor.

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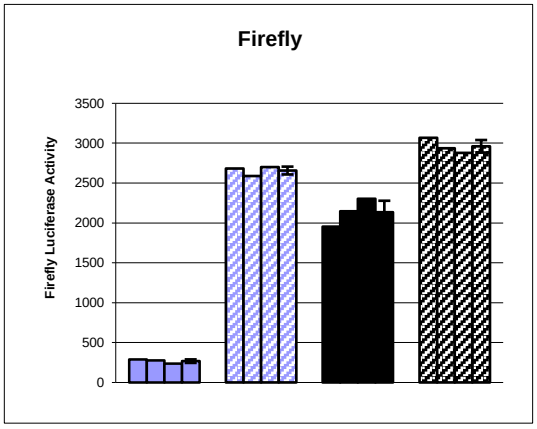
Firefly

289	2682	1956	3067	
275	2588	2145	2936	
237	2700	2305	2878	
267	2656.667	2135.333	2960.333	average
21.96968	49.10759	142.6425	79.05413	stdev
serum	tpa+serum	no serum	tpa-serum	

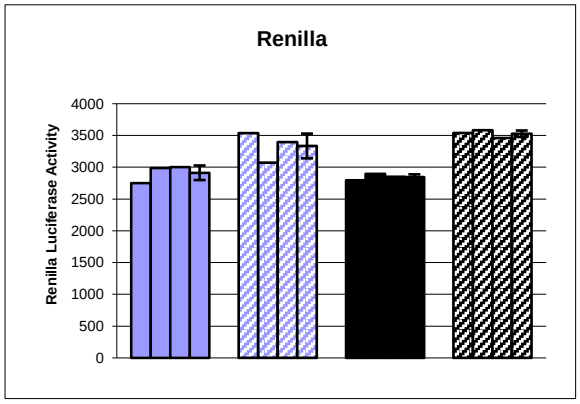


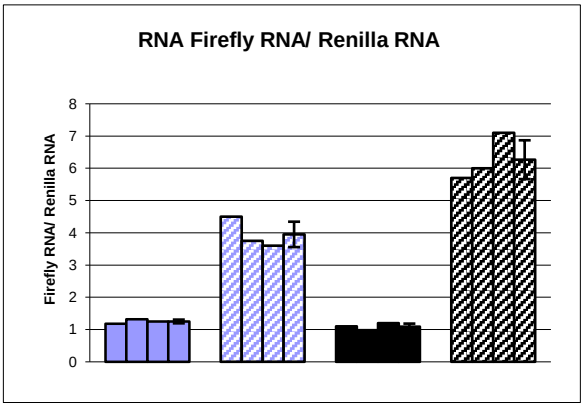
renilla

2751	3537	2794	3539	
2987	3072	2896	3583	
3001	3396	2850	3460	
2913	3335	2846.667	3527.333	average
114.6938	194.6741	41.70798	50.88768	stdev
serum	tpa+serum	no serum	tpa-serum	

RNA ratio

1.18	4.5	1.1	5.7
1.32	3.75	0.98	6
1.25	3.6	1.2	7.1
1.25	3.95	1.093333	6.266667
0.057155	0.3937	0.089938	0.601849
serum	tpa+serum	no serum	tpa-serum



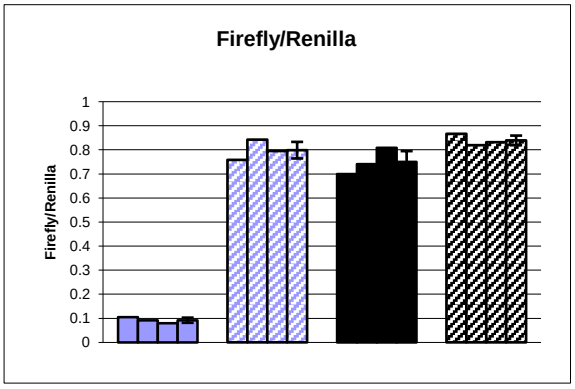


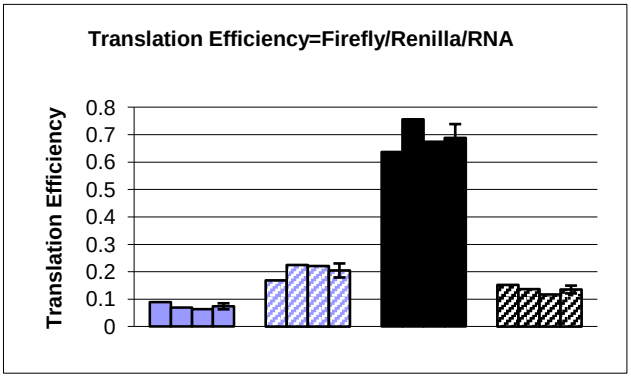
Firefly/Renilla

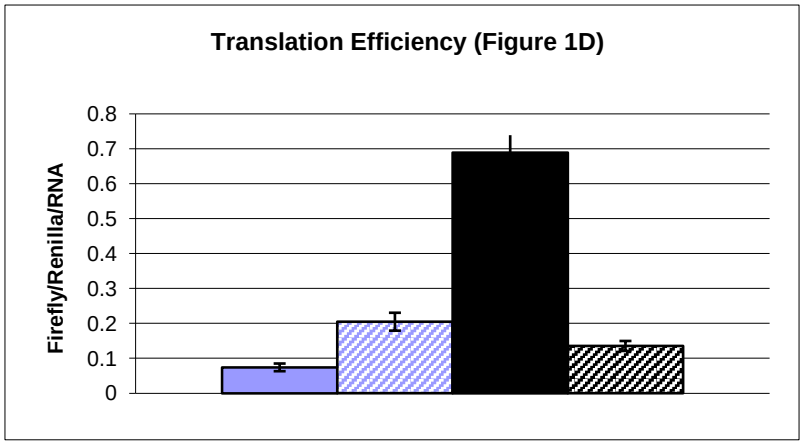
0.105053	0.75827	0.700072	0.866629	
0.092066	0.842448	0.740677	0.819425	
0.078974	0.795053	0.808772	0.831792	
0.092031	0.79859	0.74984	0.839282	average
0.010647	0.034457	0.044847	0.019985	stdev
serum	tpa+serum	no serum	tpa-serum	

Translation Efficiency= Firefly/Renilla/ RNA

0.089028	0.168504	0.636429	0.15204
0.069747	0.224653	0.755793	0.136571
0.063179	0.220848	0.673977	0.117154
0.073984	0.204668	0.688733	0.135255
0.01097	0.025619	0.049835	0.014273
serum	tpa+serum	no serum	tpa-serum







Firefly RNA/Renilla RNA Ratio

1.18	1.7	4.5	1.1	1.9	5.7	trial 1
1.32	1.5	3.75	0.98	1.88	6	trial 2
1.25	1.8	3.6	1.2	1.766	7.1	trial 3
1.25	1.666667	3.95	1.093333	1.848667	6.266667	average
0.057155	0.124722	0.3937	0.089938	0.059022	0.601849	stdev
serum + DMSO	serum no DMSO	tpa+serum TPA	no serum+ DMSO	no serum no DMSO	tpa no serum TPA	

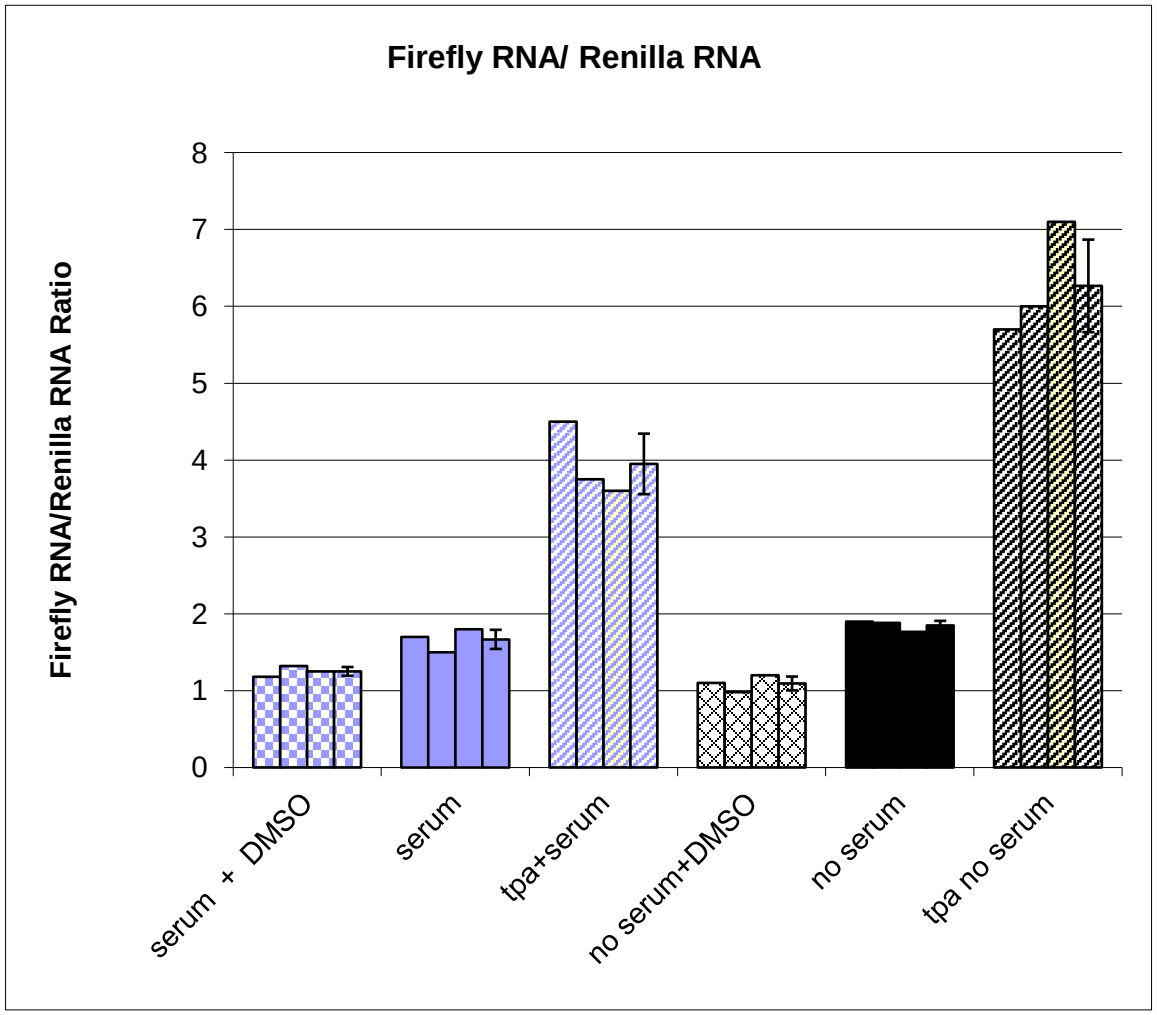
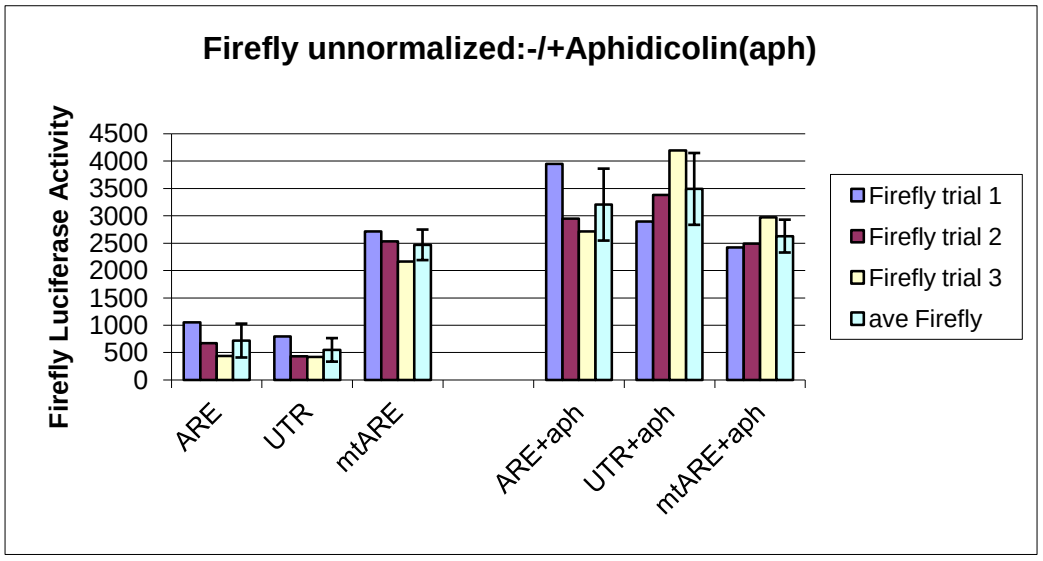


Table S3

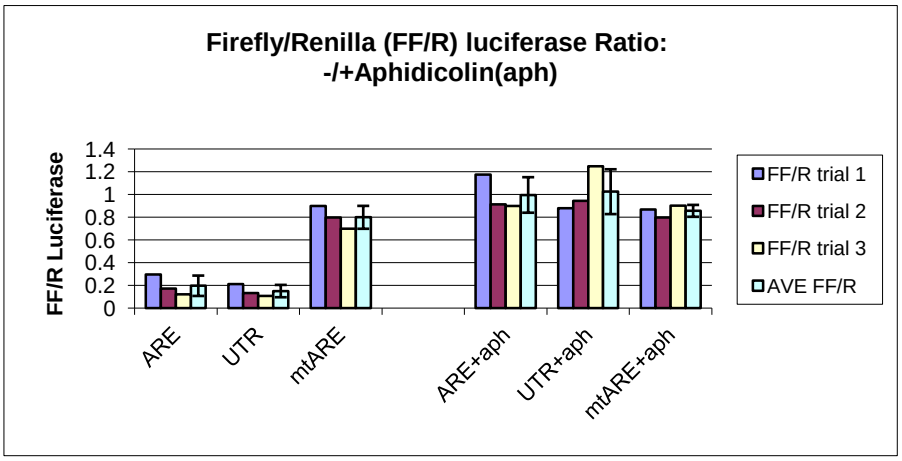
Figure 2A	**trial 1**			**trial 2**		
	Firefly (FF	Renilla-R	Ratio	**Firefly**	Renilla	Ratio
no aphidicolin						
ARE	1051	3542	0.296725	670.548	3876	0.173
UTR	798	3765	0.211952	432.782	3254	0.133
mtARE	2712.911	3011	0.901	2530.267	3166	0.7992
+aphidicolin (aph)						
ARE	3951	3357	1.176944	2949.801	3227	0.9141
UTR	2895.86	3290	0.880201	3383.023	3575	0.9463
mtARE	2424.099	2786	0.8701	2489.508	3115	0.7992

	trial 3		
	Firefly	Renilla	Ratio
no aphidicolin			
ARE	438.495	3565	0.123
UTR	419.04	3880	0.108
mtARE	2164.953	3095	0.6995
+aphidicolin			
ARE	2712.585	3017	0.8991
UTR	4195	3356	1.25
mtARE	2973.389	3299	0.9013

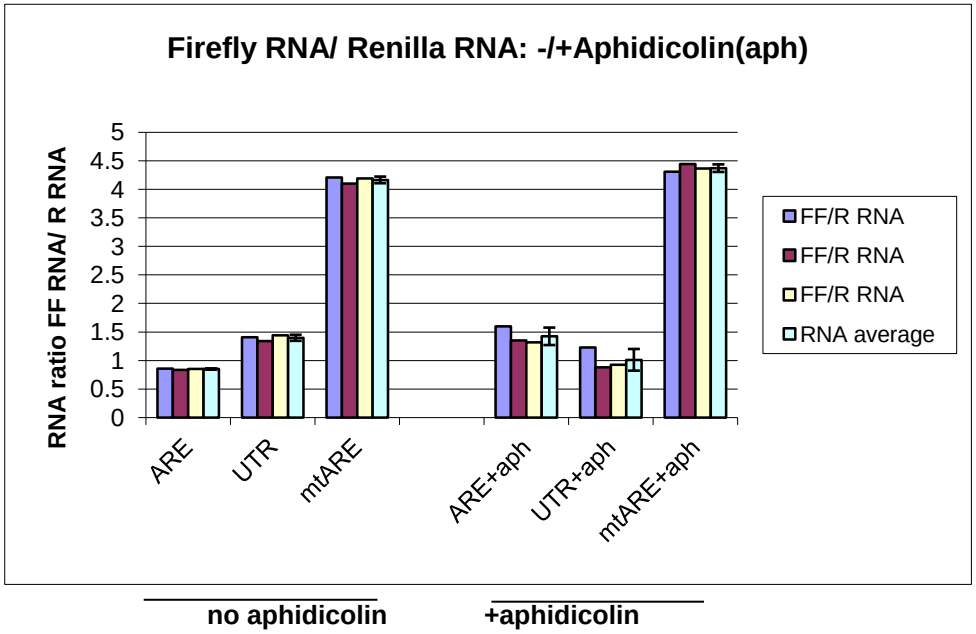
Firefly tria	**Firefly tria**	**Firefly tria ave**	**Firefly std dev**	
				no aphidicolin
1051	670.548	438.495	720.0143	309.2342
798	432.782	419.04	549.9407	214.9355
2712.911	2530.267	2164.953	2469.377	279.0078
				+aphidicolin(aph)
3951	2949.801	2712.585	3204.462	657.3107
2895.86	3383.023	4195	3491.294	656.3027
2424.099	2489.508	2973.389	2628.999	300.0385



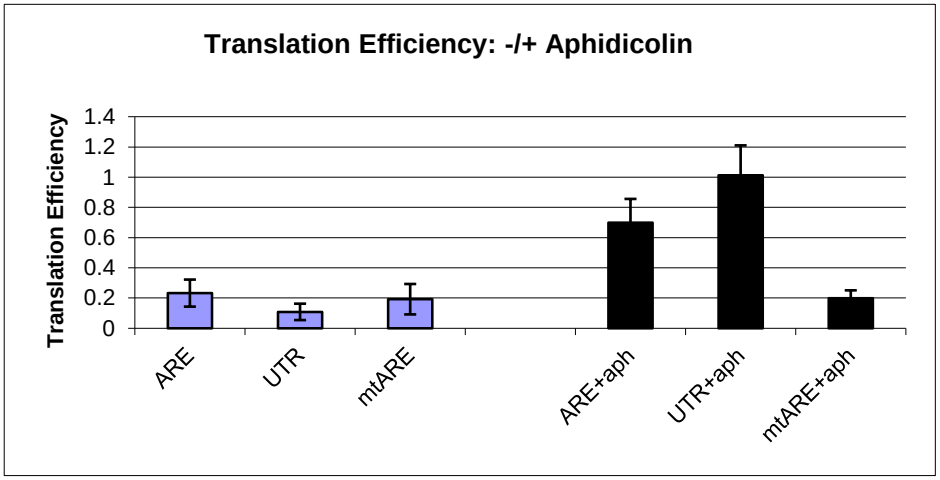
FF/R RATI	FF/R trial 1	FF/R trial 2	FF/R trial 3	AVE FF/R	STD DEV	no aphidicolin
ARE	0.297	0.173	0.123	0.197667	0.089584	ARE
UTR	0.212	0.133	0.108	0.151	0.054286	UTR
mtARE	0.901	0.7992	0.6995	0.7999	0.100752	mtARE
						+aphidicolin(aph)
ARE+aph	1.177	0.9141	0.8991	0.996733	0.156296	ARE
UTR+aph	0.8802	0.9463	1.25	1.0255	0.197212	UTR
mtARE+ap	0.8701	0.7992	0.9013	0.856867	0.052321	mtARE



FF/R RNA trial 1	FF/R RNA trial 2	FF/R RNA trial 3	RNA avera	RNA std dev	no aphidicolin
0.859	0.838	0.8548	0.8506	0.011112	ARE
1.41	1.34	1.444	1.398	0.053028	UTR
4.2087	4.1	4.1896	4.1661	0.058035	mtARE
					+aphidicolin(aph)
1.6	1.352	1.32	1.424	0.153258	ARE
1.23	0.881	0.9244	1.0118	0.190209	UTR
4.31	4.442	4.364	4.372	0.066363	mtARE



	trial 1	trial 2	trial 3	ave Ratio	RNA avera	Translation Efficienc	std dev/er
ARE	0.297	0.173	0.123	0.197667	0.8506	0.2324	0.089584
UTR	0.212	0.133	0.108	0.151	1.398	0.108	0.054286
mtARE	0.901	0.7992	0.6995	0.7999	4.1661	0.192	0.100752
ARE+aph	1.177	0.9141	0.8991	0.996733	1.424	0.6998	0.156296
UTR+aph	0.8802	0.9463	1.25	1.0255	1.0118	1.0135	0.197212
mtARE+ap	0.8701	0.7992	0.9013	0.856867	4.312	0.198396	0.052321



LEGEND: blue bars= no aphidicolin/dmso black bars=aphidicolin

	ave FF/R F	RNA avera	Translatio	std dev/ er	RNA std d	average st A
ARE	0.197667	0.8506	0.2324	0.089584	0.011112	0.050348
UTR	0.151	1.398	0.108	0.054286	0.053028	0.053657
mtARE	0.7999	4.1661	0.192	0.100752	0.058035	0.079394
ARE+aph	0.996733	1.424	0.6998	0.156296	0.153258	0.154777
UTR+aph	1.0255	1.0118	1.0135	0.197212	0.190209	0.193711
mtARE+ap	0.856867	4.312	0.198396	0.052321	0.066363	0.059342

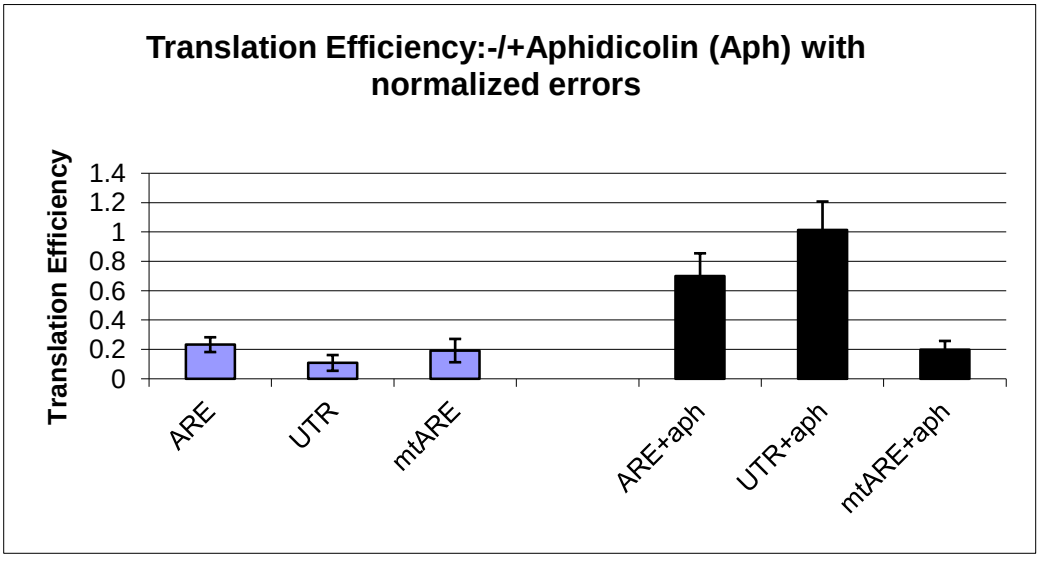
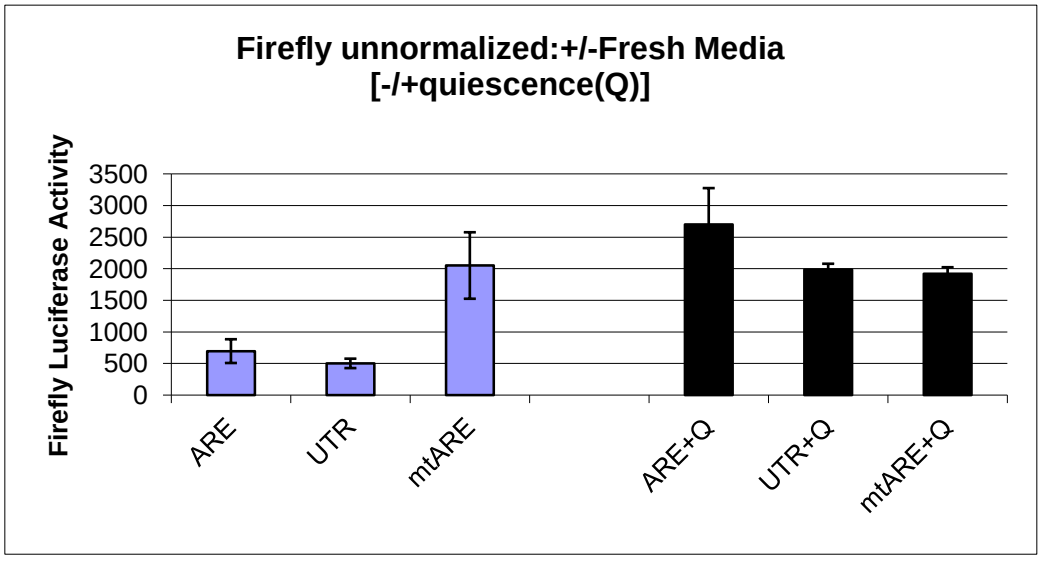


Figure 2B

	trial 1			trial 2		
	Firefly	Renilla	Ratio	**Firefly**	Renilla	Ratio
fresh medium						
ARE	799.44	3331	0.24	810.0128	3764	0.2152
UTR	486.59	3876	0.125539	583.299	3072	0.189876
mtARE	2175.6	3920	0.555	2503.66	3748	0.667999
no change to medium-quiescent(Q)						
ARE+Q	3144.698	3151	0.998	2905.05	3210	0.905
UTR+Q	1982.369	3299	0.6009	2080.75	2870	0.725
mtARE+Q	1885.888	2984	0.632	1837.86	2459	0.747401

	trial 3		
	Firefly	Renilla	Ratio
fresh medium			
ARE	479.36	3063	0.1565
UTR	436.77	3450	0.1266
mtARE	1473.857	2605	0.56578
no change to medium-quiescent(Q)			
ARE+Q	2047.872	2560	0.79995
UTR+Q	1896.048	3412	0.5557
mtARE+Q	2036.296	3391	0.6005

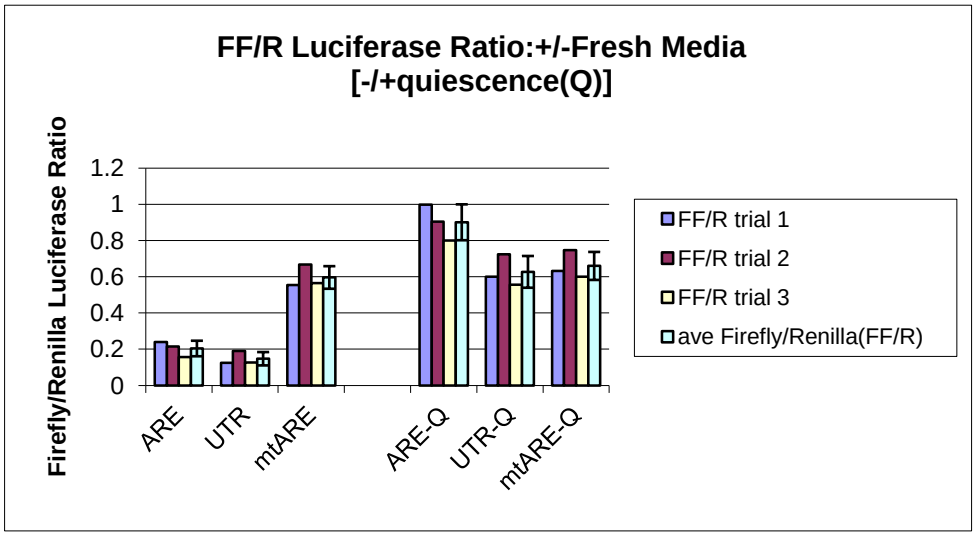
	Firefly-tria	**Firefly-tria**	**Firefly-tria**	**ave Firefly**	**std dev**
ARE	799.44	810.0128	479.36	696.2709	187.9247
UTR	486.59	583.299	436.77	502.2197	74.50437
mtARE	2175.6	2503.66	1473.857	2051.039	526.08
ARE+Q	3144.698	2905.05	2047.872	2699.207	576.6588
UTR+Q	1982.369	2080.75	1896.048	1986.389	92.4164
mtARE+Q	1885.888	1837.86	2036.296	1920.015	103.526



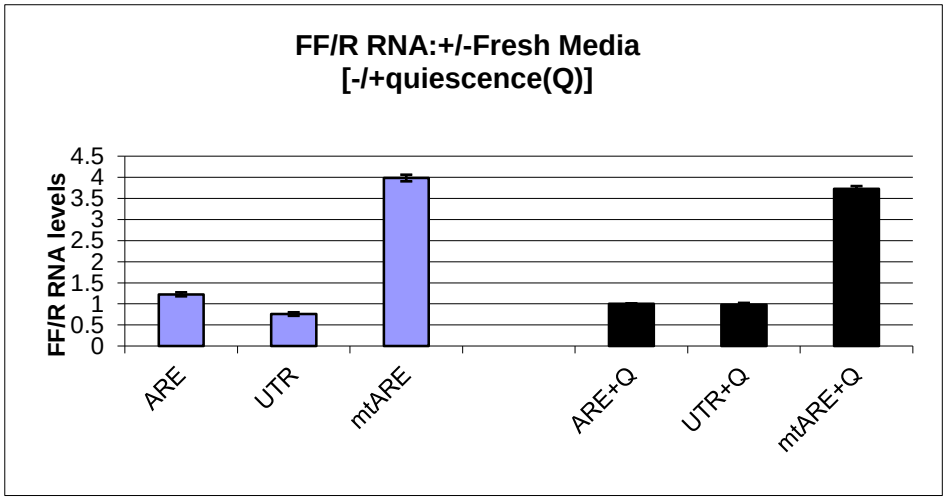
LEGEND blue bars=fresh medium black bars=quiescence/no media change/aged

FF/R of -/+quiescence

	FF/R trial 1	FF/R trial 2	FF/R trial 3	ave Firefly	std dev
ARE	0.24	0.2152	0.1565	0.2039	0.042882
UTR	0.12554	0.189876	0.12668	0.147365	0.03682
mtARE	0.555	0.668	0.56578	0.59626	0.062362
ARE-Q	0.998	0.905	0.79995	0.900983	0.099086
UTR-Q	0.6009	0.725	0.5557	0.6272	0.087661
mtARE-Q	0.632	0.7474	0.6005	0.659967	0.07734

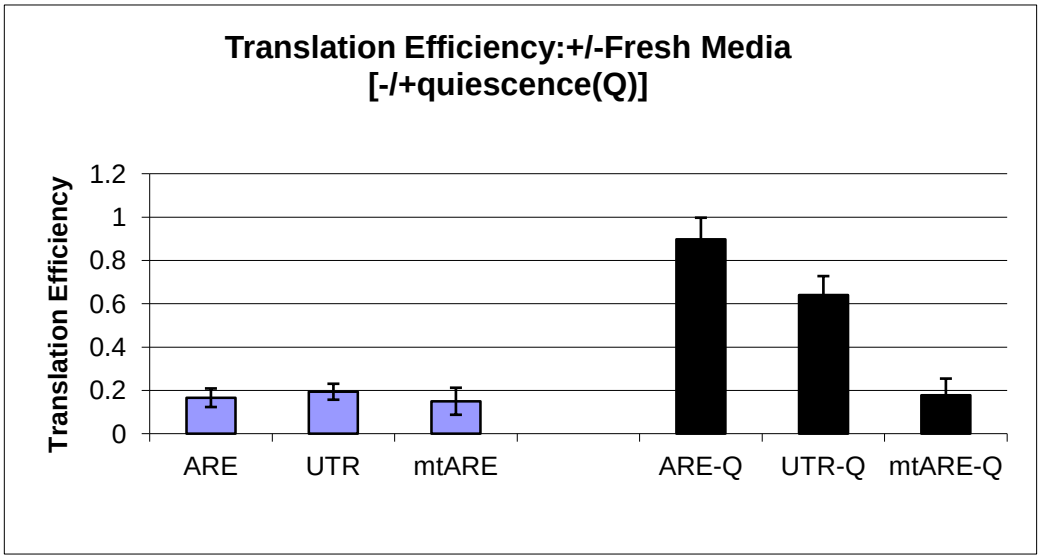


FF/R RNA trial 1	FF/R RNA trial 2	FF/R RNA trial 3	RNA avera	RNA std dev		fresh medium
1.176	1.2366	1.2681	1.2269	0.04681	ARE	ARE
0.7763	0.7132	0.7905	0.76	0.041147	UTR	UTR
4.0454	4.0003	3.8943	3.98	0.077568	mtARE	mtARE
						no change to medium-
1.007	1.0013	1.0016	1.0033	0.003208	ARE+Q	ARE+Q
1.004	0.93	1.006	0.98	0.043313	UTR+Q	UTR+Q
3.675	3.798	3.696	3.723	0.065795	mtARE+Q	mtARE+Q

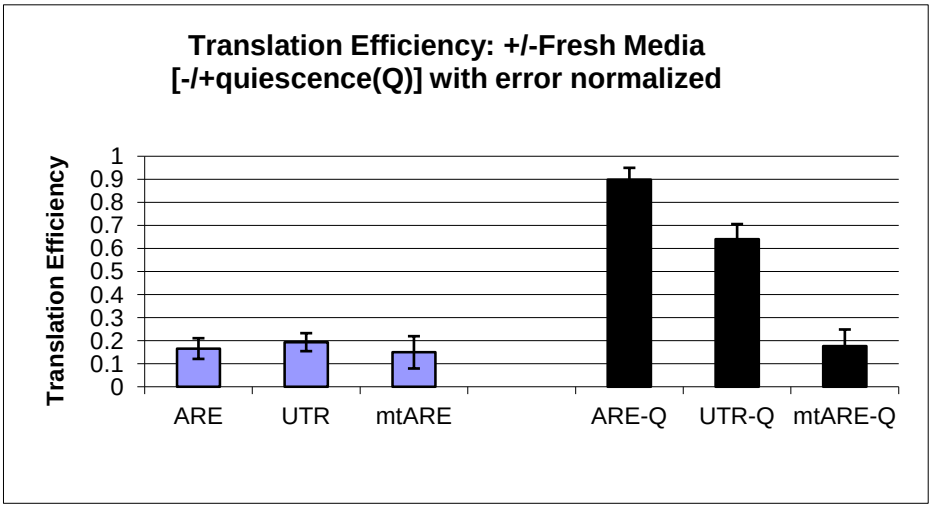


LEGEND blue bars=fresh medium black bars=quiescence/no media change/aged

	trial 1	trial 2	trial 3	ave Ratio	RNA avera	Translation Efficienc	std dev/ er
ARE	0.24	0.2152	0.1565	0.2039	1.2269	0.166191	0.042882
UTR	0.12554	0.189876	0.12668	0.147365	0.76	0.1939	0.03682
mtARE	0.555	0.668	0.56578	0.59626	3.98	0.15	0.062362
ARE-Q	0.998	0.905	0.79995	0.900983	1.0033	0.898	0.099086
UTR-Q	0.6009	0.725	0.5557	0.6272	0.98	0.64	0.087661
mtARE-Q	0.632	0.7474	0.6005	0.659967	3.723	0.17727	0.07734



ave FF/R F	RNA avera	Translatio	std dev/ er	RNA std d	average std dev
0.2039	1.2269	0.166191	0.042882	0.04681	0.044846
0.147365	0.76	0.193901	0.03682	0.041147	0.038984
0.59626	3.98	0.149814	0.062362	0.077568	0.069965
0.900983	1.0033	0.89802	0.099086	0.003208	0.051147
0.6272	0.98	0.64	0.087661	0.043313	0.065487
0.659967	3.723	0.177268	0.07734	0.065795	0.071568



LEGEND blue bars=fresh medium black bars=quiescence/no media change/aged

ror

quiescent(Q)

'ror