

Molecular Cell

Supplemental Information

**Polyamine Control of Translation Elongation
Regulates Start Site Selection on the
Antizyme Inhibitor mRNA via Ribosome Queuing**

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Figures S1 to S6

Tables S1 to S6

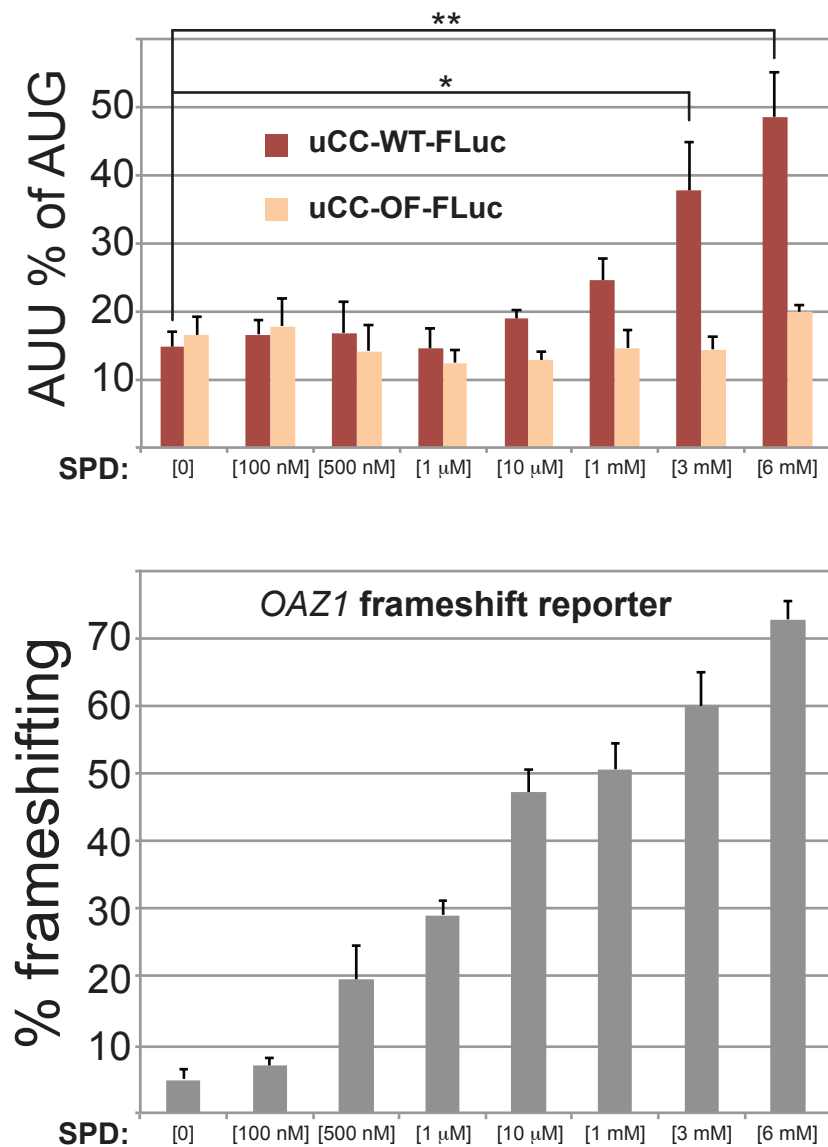


Figure S1. Dose-dependent stimulation of AZIN1 uCC translation and antizyme frameshifting by spermidine – Related to Figure 1A

The indicated uCC-Luc reporters from Figure 1A (upper panel) or a human antizyme OAZ1 dual-luciferase frameshift reporter (lower panel) were transfected in DFMO-treated HEK293T cells and then incubated in the presence of the indicated concentrations of spermidine.

(Upper panel) The percent AUU initiation and normalization to a co-transfected Renilla luciferase reporter initiated by AUG in perfect context were calculated as described in Figure 1A.

(Lower panel) +1 frameshifting was calculated relative to an in-frame reporter. Each data point represents the mean of four biological replicates, each done in duplicate. Error bars denote standard deviation. * $p < 0.05$, ** $p < 0.01$ (Student's two-tailed t-test, $n=4$).

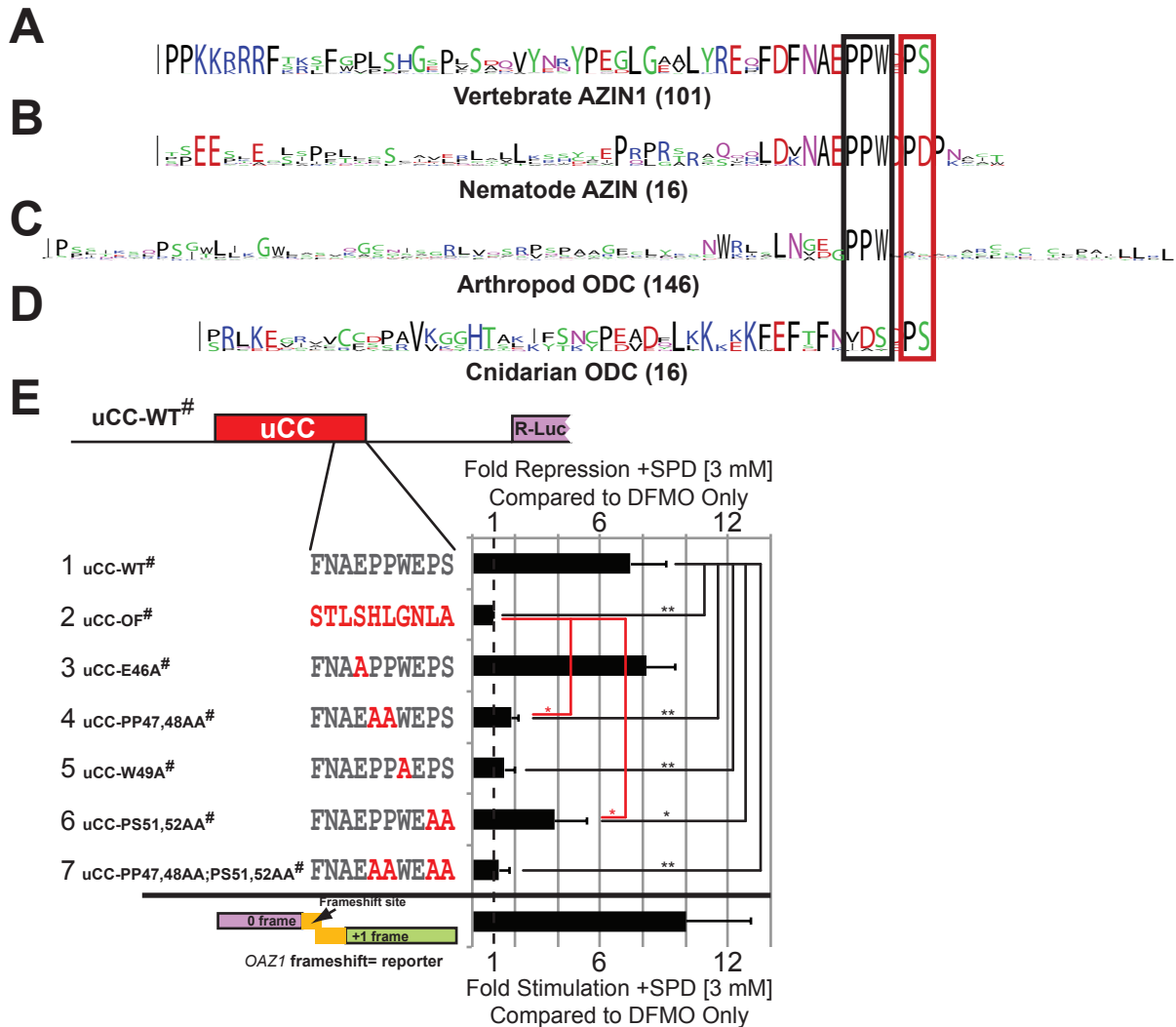


Figure S2. Phylogenetic comparisons reveal that PPW and PS-stop are highly conserved, but discrete, motifs in metazoan homologs of ODC/AZIN and these motifs mediate polyamine regulatory effects of the AZIN1 uCC – Related to Figures 1B, 1C

(A) Weblogo representation of amino acid conservation of uCCs in 101 orthologs of vertebrate AZIN1. (B) Conservation of uCCs in 16 homologs of AZIN in nematodes. Though the nematode uCC is an ortholog of the AZIN1 uCC, the nematode AZIN is not a true ortholog of vertebrate AZIN1 and instead arose as a result of a gene duplication that occurred independently in the nematode lineage (Ivanov et al., 2010b; Kurosinski et al., 2013).

(C) Conservation of uCCs in 146 arthropod ODC mRNAs.

(D) Conservation of uCCs in 16 cnidarian ODC mRNAs. In all WebLogos the starting residue is shown as isoleucine (corresponding to the AUU start codon); however, presumably translation is initiated by Met-tRNA^{iMet}. The PPW and PS-stop motifs are boxed in black and red, respectively.

(E) (Top) Schematic of the AZIN1 reporter; the # symbol in the names of the reporters indicates that AUG start codons of the three conventional uORFs were mutated to AAA. The full 5' leader of mouse *azin1* mRNA with the AUG start codons of the three conventional uORFs mutated to AAA was fused upstream of Renilla luciferase. The “wild type” and “out-of-frame” reporters were described in Figure 6B, and for each reporter the amino acid changes relative to the wild type uCC are in red. HEK293T cells were pretreated, transfected and incubated as in Figure 1, and reporter normalization was performed as in Figure 6B. The fold repression in the presence of 3 mM SPD was calculated relative to the reporter in which the last 10 codons are out-of-frame (set at “1”). (Bottom) The human antizyme OAZ1 dual-luciferase frameshift reporter was examined in parallel and +1 frameshifting was calculated relative to an in-frame reporter. The fold stimulation indicates the increase in percent frameshifting in 3 mM vs. no SPD. Each data point represents the mean of four biological replicates, each done in duplicate. Error bars denote standard deviation. * $p < 0.05$, ** $p < 0.01$ (Student’s two-tailed t-test, $n = 4$).

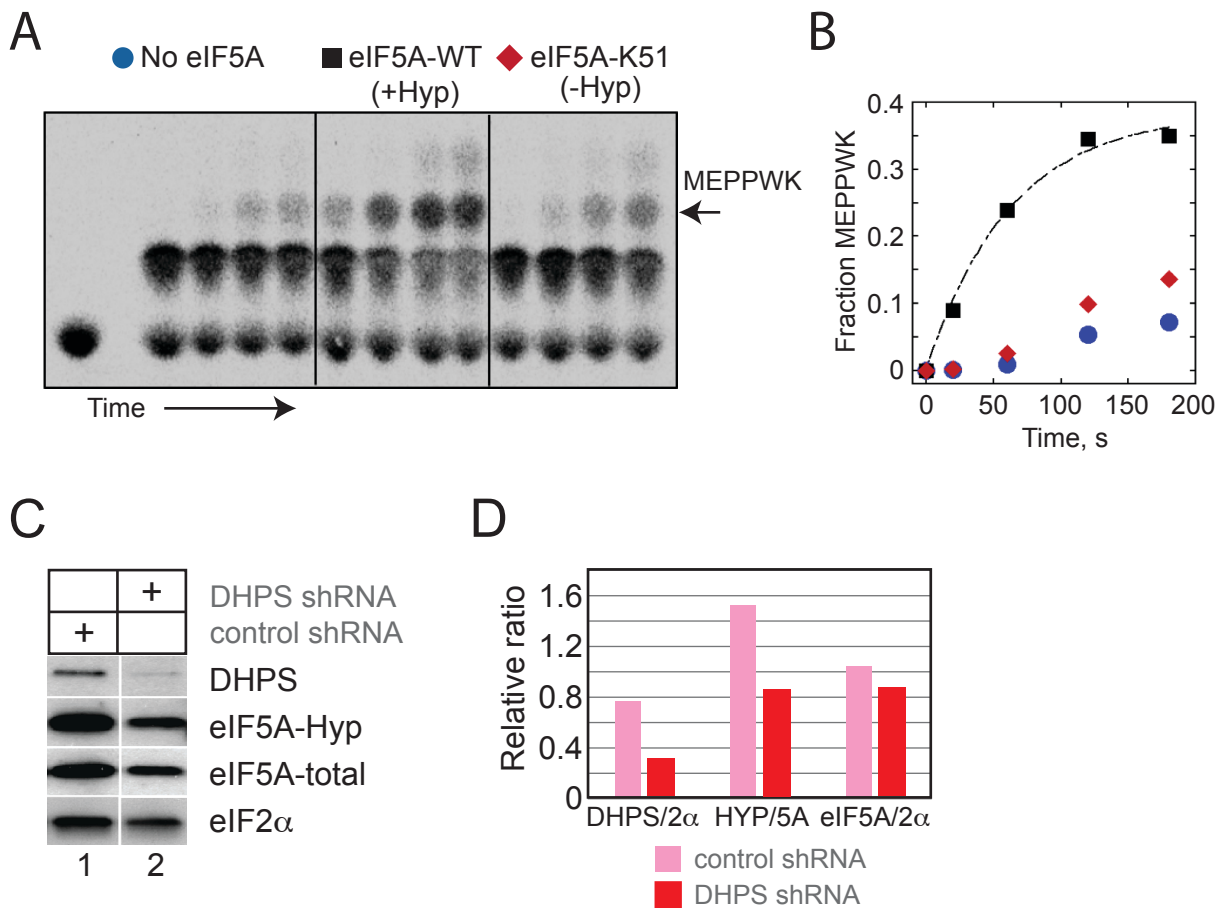


Figure S3. Hypusination of eIF5A is required for in vitro synthesis of MEPPWK peptide and *DHPS* shRNA transfections reduce hypusination of eIF5A in cells – Related to Figures 4A,4G

(A) Electrophoretic thin-layer chromatography (TLC) analysis of MEPPWK peptide synthesis in yeast in vitro reconstituted elongation assays performed in the presence of no eIF5A, hypsinated eIF5A (eIF5A-WT) or unmodified form of eIF5A (eIF5A-51K), as indicated.

(B) Fraction of MEPPWK synthesis in each reaction was plotted and fit to a single exponential equation (black squares, +WT hypsinated eIF5A; red diamonds, +unmodified eIF5A-K51; blue circles, -eIF5A).

(C) Western blot analysis of whole cell lysates from HEK293T cells pre-treated in 1 mM DFMO for 4 days, then transfected with control shRNAs or shRNAs against human *DHPS*. Transfectants were incubated an additional 48 hr in DFMO and then incubated for another 24 hr in DFMO supplemented with 1 M SPD. Immunoblot analysis of whole cell lysates was performed using antibodies against *DHPS*, hypsinated eIF5A, total eIF5A, or total eIF2α, as indicated.

(D) Relative levels of *DHPS*, hypsinated eIF5A, total eIF5A, and total eIF2α from panel (C) were determined by quantitative densitometry and NIH ImageJ software.

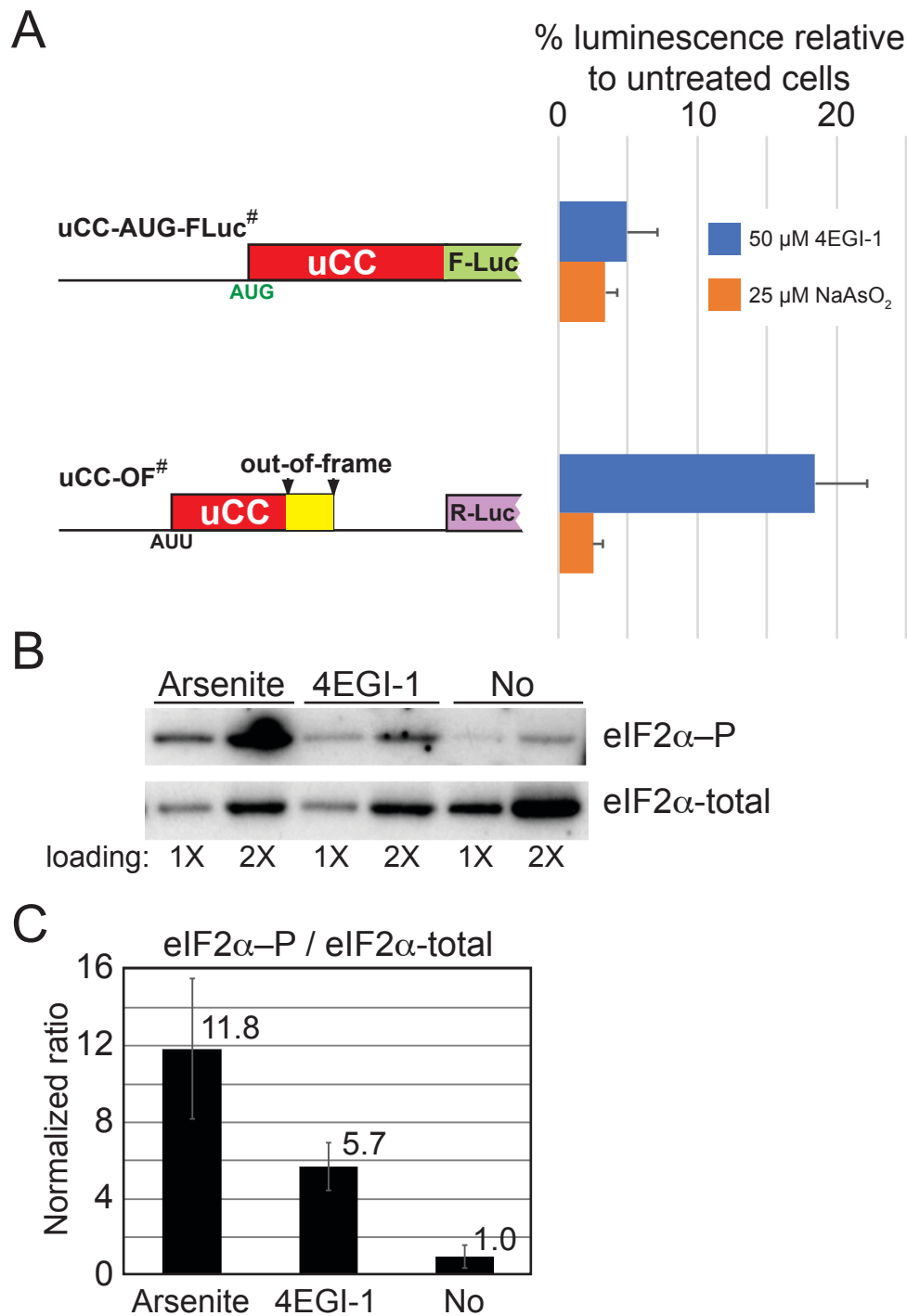


Figure S4. Arsenite and 4EGI-1 treatments substantially impair luciferase activities from control reporters and impact eIF2 α phosphorylation – Related to Figures 6 and S8

(A) The indicated control reporters, AUG-initiated uCC firefly luciferase fusion and “out-of-frame” uCC upstream of Renilla luciferase, which were used in Figures 6A-C and Figure S5A, respectively, were transfected in HEK293T cells. Luminescence of lysates from cells treated with 25 μ M arsenite or 50 μ M 4EGI-1 were compared to untreated cells.

(B) Western blot analysis of whole cell lysates from HEK293T cells treated with either 50 μ M 4EGI-1, as in Figures 6A-C, or 25 μ M arsenite, as in Figure S5B. Two different amounts of each cell extract differing by a factor of 2 were loaded in successive lanes and subjected to immunoblot analysis using rabbit monoclonal anti-eIF2 α (phospho-Ser51) or rabbit monoclonal anti-eIF2 α antibodies.

(C) Relative levels of phosphorylated and total eIF2 α from panel (A) were determined by quantitative densitometry and NIH ImageJ (1.46r) software and are shown as the average of the quantifications obtained from the two dilutions. Error bars are standard deviations; n=2 for arsenite and 4EGI-1, and n=4 for no treatment.

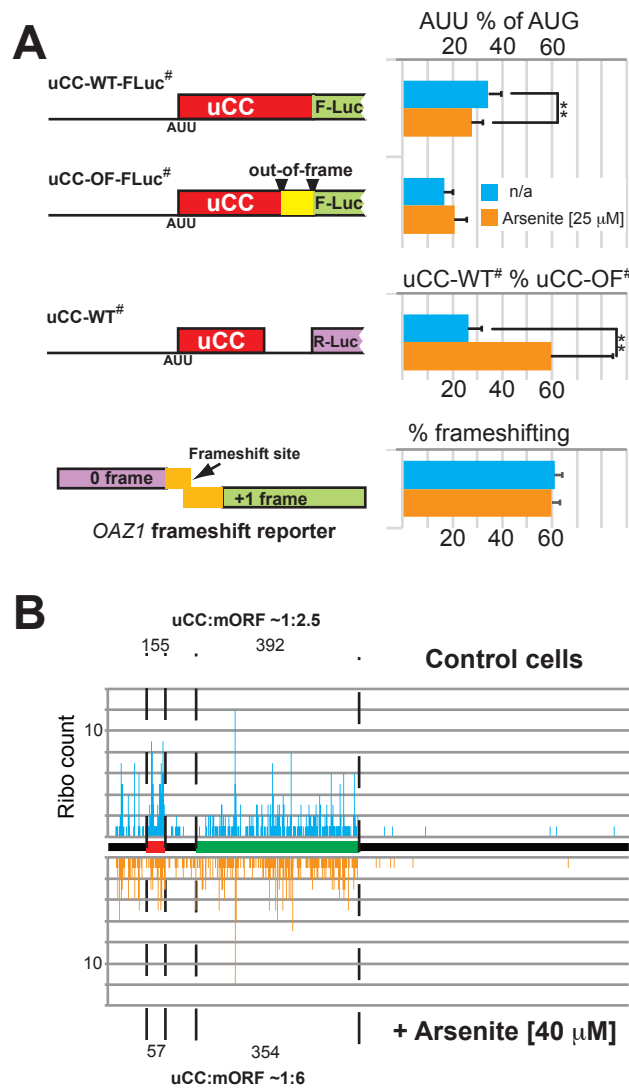


Figure S5. Arsenite induces derepression of AZIN1 mORF translation – Related to Figure 6A-C

(A) (Top two constructs) To monitor uCC translation, HEK293T cells were transfected with the indicated uCC-Luc reporters and incubated for 18 hr in DMEM supplemented with 1 mM aminoguanidine and 1 mM SPD in the absence or presence of the 25 μM NaAsO₂. Percent AUU initiation was calculated as described in Figure 1A, except that no normalizing co-transfected Renilla reporter was used. The # symbol in the names of the reporters indicates that the AUG start codon of uORF1 was mutated to AAA. (Third construct) To monitor AZIN1 synthesis, the full 5' leader of mouse azin1 mRNA with the AUG start codons of the three conventional uORFs mutated to AAA (as denoted by the # symbol in the name of the reporter) was fused upstream of Renilla luciferase. HEK293T cell transfectants were grown +/- 25 μM NaAsO₂ as described above. Wild type reporter activity was calculated as a percent of a reporter containing the out-of-frame uCC mutation (i.e. constitutively derepressed). (Bottom construct) To monitor levels of free polyamines, a dual-luciferase human antizyme OAZ1 frameshifting reporter was assayed in cells treated as described above. Percent +1 frameshifting was calculated relative to a dual-luciferase in-frame reporter. Each data point represents the mean of eight biological replicates, each performed in triplicate. Error bars denote standard deviation. **p < 0.01 (Student's two-tailed t-test, n=8).

(B) Ribosome footprint raw data was obtained from (Andreev et al., 2015). Ribosome protected fragments from HEK293T cells grown under control conditions or in the presence of 40 μM sodium arsenite for 30 min were mapped to the mRNA of AZIN1. Total (not normalized) fragment counts were aligned to the AZIN1 mRNA assuming a 15-nt offset from the 5' end of protected fragments to the ribosomal A-site. Quantified fragment counts mapping to the uCC and mORF, red and green rectangles, respectively, and their relative ratio under each condition are indicated above (control conditions) and below (arsenite conditions) the ribosome profiles.

Gasterosteus aculeatus AZIN1-2 IPPKKK---KRS--VPSFC--FSASVYNNYPEDLGASLYREPPDFNAEPPWDPS
 Trematomus bernacchii AZIN1-2 IPPKKK---KRS--VPSFC--FSANVYNNYPEDLGSLLHREPPDFNAEPPWDPS
 Oreochromis niloticus AZIN1-2 IPPKKK---KRS--VPSFC--FSADVYNNYPEDLGAALYREPPDFNAEPPWDPS
 Morone chrysops AZIN1-2 IPPKKK---KRS--VPSFC--FSADVYNNYPEDLGAALYREPPDFNAEPPWDPS
 Micropterus salmoides AZIN1-2 IPPKKK---KRS--VPSFC--FSADVYNNYPEDLGAALYREPPDFNAEPPWDPS
 Neolamprologus brichardi AZIN1-2 IPPKKK---KRS--VPSFC--FSADVYNNYPEDLGAALYREPPDFNAEPPWDPS
 Haplochromis burtoni AZIN1-2 IPPKKK---KRS--VPSFC--FSADVYNNYPEDLGAALYREPPDFNAEPPWDPS
 Maylandia zebra AZIN1-2 IPPKKK---KRS--VPSFC--FSADVYNNYPEDLGAALYREPPDFNAEPPWDPS
 Lates calcarifer AZIN1-2 IPPKKK---KRS--VPSFC--FSADVYNNYPEDLGAALYREPPDFNAEPPWDPS
 Morone saxatilis AZIN1-2 IPPKKK---KRS--VPSFC--FSADVYNNYPEDLGAALYREPPDFNAEPPWDPS
 Miichthys miiuy AZIN1-2 IPPKKK---KRS--VPSGC--FSADVYNNYPEDLGAALYREPPDFNAEPPWDPS
 Tripterygion delaisi AZIN1-2 IPPKKK---KRS--VPSVC--FSPDVYNNYPEDLGEALYREPPDFNAEPPWDPS
 Oryzias latipes AZIN1-2 IPSKKK---KRS--VPCPR--ASSPNVYNNYPEDLGAALYREPPDFNAEPPWDPS
 Notobranchius furzeri AZIN1-2 IPSKEK---KCS--VCRFR--PSVEVCNNYPEDLGAALYREPPDFNAEPPWDPS
 Takifugu rubripes AZIN1-2 IPPKKK---KHS--LPTFC--VSADVYNNYPKDLGAALYKESDFNAEPPWDPS
 Gadus morhua AZIN1-2 IPPKKK---KGS--IPSFC--LSAHAYN--YPEGLKALYREPPDFNAEPPWDPS
 Salmo salar AZIN1-2 IPPKRR---KRS--TPNIG--FYSHVYNYYPEGLAALRREPPDFNAEPPWDPS
 Oncorhynchus tshawytscha AZIN1-2 IPPKRR---KRS--TPNIG--FYSHVYNYYPEGLAALRREPPDFNAEPPWDPS
 Oncorhynchus mykiss AZIN1-2 IPPKRR---KRS--TPNIG--FYSHVYNYYPEGLAALRREPPDFNAEPPWDPS
 Osmerus mordax AZIN1-2 IPPKRR---KRS--TPNFG--FLSHVYNYYPEGLAALYREPPDFNAEPPWDPS
 Petromyzon marinus AZIN1 IPPKKRRFRKRSFGTSLGV--YTEKVNIYPE--GRRLYQEQDFNAEPPWEPES
 Danio rerio AZIN1 IPPKKRRFRKRSFRPLSHGSPSLSRVYKRYPEGLGEALYREDFNAEPPWDPS
 Pimephales promelas AZIN1 IPPKKRRFRKRSFRPLSHGSPSLSRVYKRYPEGLGEALYREDFNAEPPWDPS
 Sinocyclocheilus angustiporus Zacco platypus AZIN1 IPPKKRRFRKRSFRPLSHGSPSLSRVYKRYPEGLGEALYREDFNAEPPWDPS
 Ictalurus furcatus AZIN1 IPPKKRRFRKRSFRPLSHGSPSLSRVYKRYPEGLGEALYREDFNAEPPWDPS
 Tetraodon nigroviridis AZIN1 IPPKKRRFRKTFWPLSHGSPVSAQVYERYVPGGLAALYREDFNAEPPWDPS
 Takifugu rubripes AZIN1 IPPKKRRFRKTFWPLSHGSPVSAQVYERYVPGGLAALYREDFNAEPPWDPS
 Morone chrysops AZIN1 IPPKKRRFRKTFWPLSHGSPVSAQVYERYVPGGLAALYREDFNAEPPWDPS
 Morone saxatilis AZIN1 IPPKKRRFRKTFWPLSHGSPVSAQVYERYVPGGLAALYREDFNAEPPWDPS
 Dicentrarchus labrax AZIN1 IPPKKRRFRKTFWPLSHGSPVSAQVYERYVPGGLAALYREDFNAEPPWDPS
 Maylandia zebra AZIN1 IPPKKRRFRKTFWPLSHGSPVSAQVYERYVPGGLAALYREDFNAEPPWDPS
 Tripterygion delaisi AZIN1 IPPKKRRFRKTFWPLSHGSPVSAQVYERYVPGGLAALYREDFNAEPPWDPS
 Miichthys miiuy AZIN1 IPPKKRRFRKTFWPLSHGSPVSAQVYERYVPGGLAALYREDFNAEPPWDPS
 Oreochromis niloticus AZIN1 IPPKKRRFRKTFWPLSHGSPVSAQVYERYVPGGLAALYREDFNAEPPWDPS
 Neolamprologus brichardi AZIN1 IPPKKRRFRKTFWPLSHGSPVSAQVYERYVPGGLAALYREDFNAEPPWDPS
 Haplochromis burtoni AZIN1 IPPKKRRFRKTFWPLSHGSPVSAQVYERYVPGGLAALYREDFNAEPPWDPS
 Ophthalmotilapia ventralis AZIN1 IPPKKRRFRKTFWPLSHGSPVSAQVYERYVPGGLAALYREDFNAEPPWDPS
 Lates calcarifer AZIN1 IPPKKRRFRKTFWPLSHGSPVSAQVYERYVPGGLAALYREDFNAEPPWDPS
 Micropterus salmoides AZIN1 IPPKKRRFRKTFWPLSHGSPVSAQVYERYVPGGLAALYREDFNAEPPWDPS
 Monopterus albus AZIN1 IPPKKRRFRKTFWPLSHGSPVSAQVYERYVPGGLAALYREDFNAEPPWDPS
 Paralichthys olivaceus AZIN1 IPPKKRRFRKTFWPLSHGSPVSAQVYERYVPGGLAALYREDFNAEPPWDPS
 Notobranchius furzeri AZIN1 IPPKKRRFRKTFWPLSHGSPVSAQVYERYVPGGLAALYREDFNAEPPWDPS
 Osmerus mordax AZIN1 IPPKKRRFRKTFWPLSHGSPVSAQVYERYVPGGLAALYREDFNAEPPWDPS
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 Gasterosteus aculeatus AZIN1 IPPKKRRFRSETFLPSHGFPVSAQVYERYVPGGLAALYREDFNAEPPWDPS
 Gadus morhua AZIN1 IPPKKRRFRSKSLWPLSHGSPVSAQVYERYVPGGLAALYREDFNAEPPWDPS
 Sebastes goodei AZIN1 IPPKKRRFRSKSLWPLSHGSPVSAQVYERYVPGGLAALYREDFNAEPPWDPS
 Trematomus bernacchii AZIN1 IPPKKRRFRSESLWPLSHGSPVSAQVYERYVPGGLAALYREDFNAEPPWDPS
 Fundulus heteroclitus AZIN1 IPPKKRRFRSKSFRPLSHGSPVSAQVYERYVPGGLAALYREDFNAEPPWDPS
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 Oncorhynchus mykiss AZIN1 IPPKKRRFRSETFPLSHGSLFSLQVYNYCYPEGLGASLYREDFNAEPPWDPS
 Salmo salar AZIN1 IPPKKRRFRSETFPLSHGSLFSLQVYNYCYPEGLGASLYREDFNAEPPWDPS
 Esox lucius AZIN1 IPPKKRRFRSETFWPLSHGSLFVQVYNYCYPEGLGASLYREDFNAEPPWDPS
 Hynobius chinensis AZIN1 IPPKKRRFRSRSFWPFSHGG--FSLKVFN--YPEGLGEALYREDFNAEPPWEPES
 Ovis aries AZIN1 IPPKKRRFRTRLFGPLSHGE--LSDQVYN--YPEGLGEVLYREDFNAEPPWEPES
 Bos taurus AZIN1 IPPKKRRFRTRLFGPLSHGE--LSDQVYN--YPEGLGEVLYREDFNAEPPWEPES
 Sus scrofa AZIN1 IPPKKRRFRTRLFGPLSHGE--LSDQVYN--YPEGLGEVLYREDFNAEPPWEPES
 Spermophilus lateralis AZIN1 IPPKKRRFRTRLFGPLSHGE--LSDQVYN--YPEGLGEVLYREDFNAEPPWEPES
 Homo sapiens AZIN1 IPPKKRRFRTRLFGPLSHGE--LSDQVYN--YPEGLGEVLYREDFNAEPPWEPES
 Pan troglodytes verus AZIN1 IPPKKRRFRTRLFGPLSHGE--LSDQVYN--YPEGLGEVLYREDFNAEPPWEPES
 Canis familiaris AZIN1 IPPKKRRFRTRLFGPLSHGE--LSDQVYN--YPEGLGEVLYREDFNAEPPWEPES
 Pongo pygmaeus AZIN1 IPPKKRRFRTRLFGPLSHGE--LSDQVYN--YPEGLGEVLYREDFNAEPPWEPES
 Camelus bactrianus AZIN1 IPPKKRRFRTRLFGPLSHGE--LSDQVYN--YPEGLGEVLYREDFNAEPPWEPES
 Capra hircus AZIN1 IPPKKRRFRTRLFGPLSHGE--LSDQVYN--YPEGLGEVLYREDFNAEPPWEPES
 Monodelphis domestica AZIN1 IPPKKRRFRTRLFGPLSHGE--LSDQVYN--YPEGLGEVLYREDFNAEPPWEPES
 Trichosurus vulpecula AZIN1 IPPKKRRFRTRLFGPLSHGE--LSDQVYN--YPEGLGEVLYREDFNAEPPWEPES
 Macropus eugenii AZIN1 IPPKKRRFRTRLFGPLSHGE--LSDQVYN--YPEGLGEVLYREDFNAEPPWEPES
 Otlemur garnettii AZIN1 IPPKKRRFRTRLFGPLSHGE--LSDQVYN--YPEGLGEVLYREDFNAEPPWEPES
 Loxodonta africana AZIN1 IPPKKRRFRTRLFGPLSHGE--LSDQVYN--YPEGLGEVLYREDFNAEPPWEPES
 Ochotona princeps AZIN1 IPPKKRRFRTRLFGPLSHGE--LSDQVYN--YPEGLGEVLYREDFNAEPPWEPES
 Macaca fascicularis AZIN1 IPPKKRRFRTRLFGPLSHGE--LSDQVYN--YPEGLGEVLYREDFNAEPPWEPES
 Dasypus novemcinctus AZIN1 IPPKKRRFRTRLFGPLSHGE--LSDQVYN--YPEGLGEVLYREDFNAEPPWEPES
 Macaca mulatta AZIN1 IPPKNIRFRTRLFGPLSHGE--LSDQVYN--YPEGLGEVLYREDFNAEPPWEPES
 Rattus norvegicus AZIN1 IPPKKRRFRTRLFGPLSHGE--LSDQVYN--YPEGLGEVLYREDFNAEPPWEPES
 Mus musculus AZIN1 IPPKKRRFRTRLFGPLSHGE--LSDQVYN--YPEGLGEVLYREDFNAEPPWEPES
 Cavia porcellus AZIN1 IPPKKRRFRTRLFGPLSHGE--LSDQVYN--YPEGLGEVLYREDFNAEPPWEPES
 Ctenomys sociabilis AZIN1 IPPKKRRFRTRLFGPLSHGE--LSDQVYN--YPEGLGEVLYREDFNAEPPWEPES
 Atractaspis aterrima AZIN1 IPPKKRRFRTKSFGPLSHGK--LSDQVYN--YPEGLGEVLYREDFNAEPPWEPES
 Crocodylus horridus AZIN1 IPPKKRRFRTKSFGPLSHGK--LSDQVYN--YPEGLGEVLYREDFNAEPPWEPES
 Boiga irregularis AZIN1 IPPKKRRFRTKSFGPLSHGK--LSDQVYN--YPEGLGEVLYREDFNAEPPWEPES
 Misonax fulvius AZIN1 IPPKKRRFRTKSFGPLSHGK--LSDQVYN--YPEGLGEVLYREDFNAEPPWEPES
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 Crocodylus adamantinus AZIN1 IPPKKRRFRTKSFGPLSHGK--LSDQVYN--YPEGLGEVLYREDFNAEPPWEPES
 Trachemys scripta elegans IPPKKRRFRTKSFGPLSHGK--LSDQVYN--YPEGLGEVLYREDFNAEPPWEPES
 Anolis carolinensis AZIN1 IPPKKRRFRTKSFGPLSHGK--LSDQVYN--YPEGLGEVLYREDFNAEPPWEPES
 Gallus gallus AZIN1 IPPKKRRFRTKSFGPLSHGK--LSDQVYN--YPEGLGEVLYREDFNAEPPWEPES
 Meleagris gallopavo AZIN1 IPPKKRRFRTKSFGPLSHGK--LSDQVYN--YPEGLGEVLYREDFNAEPPWEPES
 Carduelis chloris AZIN1 IPPKKRRFRTKSFGPLSHGK--LSDQVYN--YPEGLGEVLYREDFNAEPPWEPES
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 Callorhynchus milii AZIN1 IPPKKRRFRTKSFGPLSHGK--LSDQVYN--YPEGLGEVLYREDFNAEPPWEPES
 Leucoraja erinacea AZIN1 IPPKKRRFRTKSFGPLSHGK--LSDQVYN--YPEGLGEVLYREDFNAEPPWEPES
 Xenopus laevis AZIN1 IPPKKRRFRPNKSGPLPHGG--FVSKAYN--YPEGLGKALYREDFNAEPPWQPS
 Xenopus tropicalis AZIN1 IPPKKRRFRPKSFGPLPHGG--FVSKAYN--YPEGLGKALYREDFNAEPPWQPS
 Xenopus laevis AZIN1-2 IPPKKRRFRPKSFGPLPHGG--FVSKAYN--YPEGLGKALYREDFNAEPPWQPS
 Rana clamitans AZIN1 IPPKKRRFRPKSFGPLPHGG--FVSKAYN--YPEGLGKALYREDFNAEPPWQPS
 Pseudacris regilla AZIN1 IPPKKRRFRPKSFGPLPHGG--FVSKAYN--YPEGLGKALYREDFNAEPPWQPS
 Anguilla japonica AZIN1 IPPKKRTAFTKTFWPLSYGA--ALPVGINGCAG--GAELHREQDFNAEPPWKPS
 Danio rerio AZIN1-2 IPPKSRFRPSDARDFSR-----VYVNL--YENSLGTSVYREDFNAEPPWQPS
 Pimephales promelas AZIN1-2 IPPKRRFRFNSALEFSH-----VFNVL--YENSLGTSVYREDFNAEPPWQPS
 Ictalurus furcatus AZIN1-2 IPPKKRRFRNPTGNFSR-----LLVT--RQNCQTGAVVCREQDFNAEPPWQPS

Figure S6. uCCs from AZIN1 orthologs in vertebrates contain naturally occurring deletions/insertions between the start codon and conserved C-terminus – Related to Figures 1B and 5
 Amino acid sequence alignment, generated by ClustalX, of 101 vertebrate AZIN1 uCCs. Species names are on the left. The uCCs in the two paralog of AZIN1 in boney fish differ in length by 7 residues. The starting residue is shown as isoleucine (corresponding to the AUU start codon); however, presumably translation is initiated by Met-tRNA_i^{Met}. Deletions are represented by dashes highlighted in magenta.

Table S1. Relative reporter mRNA levels – Related to Figures 1A, 1C

plasmid #	plasmid name	treatment	FFLuc RNA levels (860 DFMO set at 1)		FFLuc mRNA levels (all DFMO values set at 1)	
				stdev		stdev
854	uCC-WT-Fluc AUU	DFMO	1.19	0.23	1.00	0.19
854	uCC-WT-Fluc AUU	3 mM SPD	5.37	4.00	4.51	3.36
860	uCC-WT-Fluc AUG	DFMO	1.00	0.06	1.00	0.06
860	uCC-WT-Fluc AUG	3 mM SPD	4.08	1.23	4.07	1.23
862	uCC-OF-Fluc AUU	DFMO	1.17	0.17	1.00	0.14
862	uCC-OF-Fluc AUU	3 mM SPD	3.43	0.15	2.92	0.13
868	uCC-OF-Fluc AUG	DFMO	1.10	0.03	1.00	0.03
868	uCC-OF-Fluc AUG	3 mM SPD	2.99	1.28	2.71	1.16
2171	uCC-PP47,48AA-FLuc AUU	DFMO	0.65	0.08	1.00	0.13
2171	uCC-PP47,48AA-FLuc AUU	3 mM SPD	2.84	1.18	4.39	1.82
2138	uCC-PP47,48AA-FLuc AUG	DFMO	0.87	0.07	1.00	0.08
2138	uCC-PP47,48AA-FLuc AUG	3 mM SPD	3.08	0.14	3.52	0.16

Relative reporter mRNA levels for experiments in Figure 1A,C determined by qPCR. Normalization controls set at 1 are highlighted in yellow. Standard deviation based on two biological replicates.

Table S2. Relative reporter mRNA levels – Related to Figure 3B

plasmid #	plasmid name	treatment	FFLuc RNA levels (2260 no Arg set at 1)		FFLuc mRNA levels (all no Arg values set at 1)	
				stdev		stdev
2255	uCC-AAP-Fluc AUU	no Arg	0.80	0.08	1.00	0.10
2255	uCC-AAP-Fluc AUU	25 mM Arg	1.52	0.41	1.90	0.51
2260	uCC-AAP-Fluc AUUG	no Arg	1.01	0.17	1.00	0.17
2260	uCC-AAP-Fluc AUG	25 mM Arg	2.06	0.60	2.05	0.60
2256	uCC-AAP-D12N-Fluc AUU	no Arg	0.51	0.08	1.00	0.16
2256	uCC-AAP-D12N-Fluc AUU	25 mM Arg	0.70	0.16	1.39	0.31
2261	uCC-AAP-D12N-Fluc AUG	no Arg	0.86	0.09	1.00	0.11
2261	uCC-AAP-D12N-Fluc AUG	25 mM Arg	1.89	0.23	2.19	0.26

Relative reporter mRNA levels for experiments in Figure 3B determined by qPCR. Normalization controls set at 1 are highlighted in yellow. Standard deviation based on two biological replicates.

Table S3. Relative reporter mRNA levels – Related to Figures 6A, 6B, 6D

plasmid #	plasmid name	treatment	FFLuc RNA levels (2324 no 4EGI-1 set at 1)		FFLuc mRNA levels (all no 4EGI-1 values set at 1)	
				stdev		stdev
2109	uCC-WT-Fluc# AUU	no 4EGI-1	1.09	0.44	1.00	0.40
2109	uCC-WT-Fluc# AUU	50 μ M 4EGI-1	1.80	1.25	1.65	1.15
2324	uCC-WT-Fluc# AUG	no 4EGI-1	1.00	0.04	1.00	0.04
2324	uCC-WT-Fluc# AUG	50 μ M 4EGI-1	1.07	0.55	1.07	0.55
5475	uCC-OF-Fluc# AUU	no 4EGI-1	1.04	0.34	1.00	0.33
5475	uCC-OF-Fluc# AUU	50 μ M 4EGI-1	1.36	0.01	1.31	0.01
5476	uCC-OF-Fluc# AUG	no 4EGI-1	1.14	0.21	1.00	0.18
5476	uCC-OF-Fluc# AUG	50 μ M 4EGI-1	1.73	0.36	1.52	0.32

plasmid #	plasmid name	treatment	RLuc RNA levels (5320 no 4EGI-1 set at 1)		RLuc mRNA levels (all no 4EGI-1 values set at 1)	
				stdev		stdev
5320	uCC-WT#	no 4EGI-1	1.00	0.07	1.00	0.07
5320	uCC-WT#	50 μ M 4EGI-1	2.15	0.05	2.14	0.05
5321	uCC-OF#	no 4EGI-1	0.76	0.19	1.00	0.26
5321	uCC-OF#	50 μ M 4EGI-1	1.82	0.19	2.41	0.25

plasmid #	plasmid name	treatment	RLuc RNA levels (5817 DFMO set at 1)		RLuc mRNA levels (all DFMO values set at 1)	
				stdev		stdev
5802	AAA-AUU WT	DFMO	0.53	0.17	1.00	0.33
5802	AAA-AUU WT	6 mM SPD	2.45	0.17	4.67	0.32
5803	AUU-AAA WT	DFMO	0.39	0.02	1.00	0.06
5803	AUU-AAA WT	6 mM SPD	2.14	0.04	5.50	0.11
5817	AAA-AAA OF	DFMO	1.00	0.89	1.00	0.89
5817	AAA-AAA OF	6 mM SPD	1.06	0.02	1.06	0.02

Relative reporter mRNA levels for experiments in Figure 6A (plasmids 2109-5476), Figure 6B (plasmids 5320-5321), and Figure 6D (plasmids 5802-5817) determined by qPCR. Normalization controls set at 1 for each group are highlighted in yellow. Standard deviation based on two biological replicates.

Table S4. Oligonucleotides used in this study – Related to STAR Methods

Name	Sequence
AZIUTR/S2	CCAAAGCTTCTCTCGCCGCGGTGTTTCCG
UTRD2/A m5 BamHI	CCAGGATCCAGCTAGGTTCCCAAGGTGGCTC
E50del/A	CCAGGATCCCCAAGGTGGCTCAGCGTTG
PPAA/A	CCAGGATCCGCTAGGTTCCCAAGCTGCCTCAGCGTTGAAG
PSAA/A	CCAGGATCCGGCAGCTTCCCAAGGTGGCTCAGCG
WA/A	CCAGGATCCGCTAGGTTCCGCAGGTGGCTCAGCGTTGAAG
EA/A	CCAGGATCCGCTAGGTTCCCAAGGTGGCGCAGCGTTGAAGTCG
AAPLNG/A	CCAGGATCCGCTGTTGAGAGCTCTCCAGAGATGATCGCTCAGGTAAT
AAP/A	CCAGAGATGATCGCTCAGGTAATCCTGTGATGTCAGCACTTCTCCTAGGCCTTC
AAPM/A	CCAGAGATGATCGCTCAGGTAATCCTGTGATGTCAGCACTTCTCCTAGGCCTTC
OCC1197	ACGTCGTCCCCGCAGCCTAAAGCCCAGGCCGCCTTGGGTATT
OCC1198	AATACCCAAGGCGGCCTGGGCTTTAGGCTGCGGGGACGACGT
AZIUTR/S2	CCAAAGCTTCTCTCGCCGCGGTGTTTCCG
AZIM2/A	CCACTCGGGTTTCATCTCAGCCGTATCCACAAAGCCGAAAGTTTTAAACCAGGA
AUU/S	CATCCCTCTTTAAAAATTCCGCCGAAAAGA
AUU/A	TCTTTTTCGGCGGAATTTTTAAAGAGGGATG
M5M6/S	GAACCTAGCTTGAAAATAGGGGGTTCCA
M5M6/A	TGGAACCCCTATTTTCAAGCTAGGTTT
M1M2M6PPAA/S	CTTCAACGCTGAGGCAGCTTGGGAACCTAGCTGAAAATAGGGGGTTTC
M1M2M6PPAA/A	GAACCCCTATTTTCAGCTAGGTTCCCAAGCTGCCTCAGCGTTGAAG
M1M2M6PSAA/S	CCACCTTGGGAAGCTGCCTGAAAATAGGGGGTTCCATCTCC
M1M2M6PSAA/A	GGAGATGGAACCCCTATTTTCAGGCAGCTTCCCAAGGTGG
M1M2M6W49A/S	CTGAGCCACCTGCGGAACCTAGCTGAAAATAGGGGGTTCC
M1M2M6W49A/A	GGAACCCCTATTTTCAGCTAGGTTCCGCAGGTGGCTCAG
M1M2M6E46A/S	CTTCAACGCTGCGCCACCTTGGGAACCTAGCTGAAAATAGGGGGTTTC
M1M2M6E46A/A	GGAACCCCTATTTTCAGCTAGGTTCCCAAGGTGGCGCAGCGTTGAAG
M1M2M6PPPSAAAA/S	CAACGCTGAGGCAGCTTGGGAAGCTGCCTGAAAATAGGGGGTTCC
M1M2M6PPPSAAAA/A	GGAACCCCTATTTTCAGGCAGCTTCCCAAGCTGCCTCAGCGTTG
M1M2M6W49ASP/S	CGCTGAGCCACCTGCGGAACCTCCATGAAAATAGGGGGTTCC
M1M2M6W49ASP/A	GGAACCCCTATTTTCATGGAGGTCCGCAGGTGGCTCAGCG
hAZ1WT/S	GTCCCTCGAGGGTCTCCCTCCACTGCTGTAGTAAC
hAZ1WT/A	GTCCAGATCTTGAAAGATTGTGATCCCTCTGACTATT

Table S5. Plasmids used in this study – Related to STAR Methods

Construct #	Construct name	start	template	S primer	AS primer/s
862	uCC-OF-FLuc (AUU)	AUU	phRL-M5	AZI/UTR-S2	UTRD2/A m5 BamHI
868	uCC-OF-FLuc (AUG)	AUG	phRL-M4M5	AZI/UTR-S2	UTRD2/A m5 BamHI
2121	uCC-PS51,52DD-FLuc (AUU)	AUU	phRL-WT	AZI/UTR-S2	E50del/A
2171	uCC-PP47,48AA-FLuc (AUU)	AUU	phRL-WT	AZI/UTR-S2	PPAA/A
2125	uCC-PS51,52AA-FLuc (AUU)	AUU	phRL-WT	AZI/UTR-S2	PSAA/A
2127	uCC-W49A-FLuc (AUU)	AUU	phRL-WT	AZI/UTR-S2	WA/A
2326	uCC-E50A-FLuc (AUU)	AUU	phRL-WT	AZI/UTR-S2	EA/A
2135	uCC-PS51,52DD-FLuc (AUG)	AUG	phRL-M4	AZI/UTR-S2	E50del/A
2138	uCC-PP47,48AA-FLuc (AUG)	AUG	phRL-M4	AZI/UTR-S2	PPAA/A
2140	uCC-PS51,52AA-FLuc (AUG)	AUG	phRL-M4	AZI/UTR-S2	PSAA/A
2141	uCC-W49A-FLuc (AUG)	AUG	phRL-M4	AZI/UTR-S2	WA/A
2361	uCC-E50A-FLuc (AUG)	AUG	phRL-M4	AZI/UTR-S2	EA/A
2255	uCC-AAP-FLuc (AUU)	AUU	phRL-WT	AZI/UTR-S2	AAPLNG/A + AAP/A
2256	uCC-AAP-D12N-FLuc (AUU)	AUU	phRL-WT	AZI/UTR-S2	AAPLNG/A + AAPM/A
2260	uCC-AAP-FLuc (AUG)	AUG	phRL-M4	AZI/UTR-S2	AAPLNG/A + AAP/A
2261	uCC-AAP-D12N-FLuc (AUG)	AUG	phRL-M4	AZI/UTR-S2	AAPLNG/A + AAPM/A
2109	uCC-WT-FLuc#	AUU	phRL-M1	AZI/UTR-S2	UTRD2/A BamHI
2324	uCC-WT-FLuc#	AUG	phRL-M1M4	AZI/UTR-S2	UTRD2/A BamHI
5375	uCC-OF-FLuc#	AUU	uCC-OF-FLuc (AUU)	OCC1197	OCC1198
5376	uCC-OF-FLuc#	AUG	uCC-OF-FLuc (AUG)	OCC1197	OCC1198
5320	uCC-WT#	N/A	phRL-M1M2M3M6	AZIUTR/S2; AUU/S	AUU/A; AZIM2/A
5321	uCC-OF#	N/A	phRL-M1M2M5	AZIUTR/S2; M5M6/S	M5M6/A; AZIM2/A
5325	uCC-E46A#	N/A	phRL-M1M2	AZIUTR/S2; M1M2M6E46A/S	M1M2M6E46A/A; AZIM2/A
5322	uCC-PP47,48AA#	N/A	phRL-M1M2	AZIUTR/S2; M1M2M6PPAA/S	M1M2M6PPAA/A; AZIM2/A
5324	uCC-W49A#	N/A	phRL-M1M2	AZIUTR/S2; M1M2M6W49A/S	M1M2M6W49A/A; AZIM2/A
5323	uCC-PS51,52AA#	N/A	phRL-M1M2	AZIUTR/S2; M1M2M6PSAA/S	M1M2M6PSAA/A; AZIM2/A
5326	uCC-PP47,48AA;PS51,52AA#	N/A	phRL-M1M2	AZIUTR/S2; M1M2M6PPPSAA AA/S	M1M2M6PPPSAAAA/A; AZIM2/A
3007	hAZ1-WT	N/A	AZ-1wt	hAZ1WT/S	hAZ1WT/A
3008	hAZ1-IF	N/A	AZ-1if	hAZ1WT/S	hAZ1WT/A
5802	uCC-AAA-AUU-WT#	N/A	see Table S6	see Table S6	see Table S6
5803	uCC-AUU-AAA-WT#	N/A	see Table S6	see Table S6	see Table S6
5817	uCC-AAA-AAA-OF#	N/A	see Table S6	see Table S6	see Table S6

Start, initiating codon for uCC; S (sense) and AS (antisense) primers for PCR.

Table S6. Synthetic inserts used to generate 5' extended *azin1* uCC reporters – Related to STAR Methods

Plasmid name (#)	Synthetic sequence cloned between HindIII and Accl sites of plasmids “5320” or “5321”
uCC-AAA-AUU-WT# (5802)	<p><u>AAGCTTAGTTAAAA</u>AAACCGAGTAAACTGGTGGCTCTTCAAATTTGATTGGGCTGCTCGCTTCAGCC CCAAAGTTAGCCTACCTAAAACAAAACCTATCTCTCCAGGGTCTTCTAAGTCAGAGACATCAAACCTG GACCTTCTGGATTACAGGCCAAATTAGCAAGTTTAAAGAAAGTCCACCAAGAAACGCAGAAAAATTCCGC CGAAAAAGAGAAGACGCTTTACCCGACTCTTTGGGCCGTTATCTCACGTAAGTATCAAGGTTACAAGAC AGGTTTAAAGGAGACCAATAGAAACTGGGCTTGTCGAGACAGAGAAGACTCTTGCGTTTCTGATAGGCAC CTATTGGTCTTACTGACATCCACTTTGCCTTTCTCTCCACAGGGCGAACTTTCTGACCGAGTATAC</p>
uCC-AUU-AAA-WT# (5803)	<p><u>AAGCTTAGTTAAAA</u>ATTCCGAGTAAACTGGTGGCTCTTCAAATTTGATTGGGCTGCTCGCTTCAGCC CCAAAGTTAGCCTACCTAAAACAAAACCTATCTCTCCAGGGTCTTCTAAGTCAGAGACATCAAACCTG GACCTTCTGGATTACAGGCCAAATTAGCAAGTTTAAAGAAAGTCCACCAAGAAACGCAGAAAAAAACCGC CGAAAAAGAGAAGACGCTTTACCCGACTCTTTGGGCCGTTATCTCACGTAAGTATCAAGGTTACAAGAC AGGTTTAAAGGAGACCAATAGAAACTGGGCTTGTCGAGACAGAGAAGACTCTTGCGTTTCTGATAGGCAC CTATTGGTCTTACTGACATCCACTTTGCCTTTCTCTCCACAGGGCGAACTTTCTGACCGAGTATAC</p>
uCC-AAA-AAA-OF# (5817)	<p><u>AAGCTTAGTTAAAA</u>AAACCGAGTAAACTGGTGGCTCTTCAAATTTGATTGGGCTGCTCGCTTCAGCC CCAAAGTTAGCCTACCTAAAACAAAACCTATCTCTCCAGGGTCTTCTAAGTCAGAGACATCAAACCTG GACCTTCTGGATTACAGGCCAAATTAGCAAGTTTAAAGAAAGTCCACCAAGAAACGCAGAAAAAAACCGC CGAAAAAGAGAAGACGCTTTACCCGACTCTTTGGGCCGTTATCTCACGTAAGTATCAAGGTTACAAGAC AGGTTTAAAGGAGACCAATAGAAACTGGGCTTGTCGAGACAGAGAAGACTCTTGCGTTTCTGATAGGCAC CTATTGGTCTTACTGACATCCACTTTGCCTTTCTCTCCACAGGGCGAACTTTCTGACCGAGTATAC</p>

Extension sequence derived from *Trip12* is shown in blue letters. Sequence of synthetic intron within the uCC is shown in red letters. Nucleotides of the uCC are highlighted in yellow. AUU near-cognate start codons are highlighted in green. Non-cognate AAA codons are highlighted in red. *HindIII* and *Accl* cloning sites are underlined.