Electronic Supplementary Information

Light-inducible Activation of Target mRNA Translation in Mammalian Cells

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Materials and Methods

DNA Constructs:

All primers for PCR were synthesized by Integrated DNA Technologies Inc. (IL, USA). Sequences of primers, DNA fragments (GenScript USA Inc., NJ, USA) and TaqMan probes (Biosearch Technologies Inc., CA, USA) used in this study are listed in Tables S1, S2 and S3. Standard molecular biology protocols were used to make the different plasmids.

All final constructs were made in mammalian expression vector pVITRO2-hygro-mcs (Invivogen Inc., CA, USA), which has two multiple cloning sites (MCS1 and MCS2) under the control of two separate promoters. Briefly, for the construction of pλ-CIBN, annealed oligonucleotides encoding the 22 amino residues from the RNA binding domain of λ phage protein N (λN)[1] were ligated into MCS2 of pVITRO2-hygro-mcs vector in between *Bgl* II and *BstB* I sites. CIBN was amplified by PCR from pCIBN(deltaNLS)-pmEGFP which was a gift of Chandra Tucker (Addgene plasmid # 26867)[2], and cloned downstream of λN peptide in between the *BstB* I and *Xho* I sites. Plasmid pCIBN was constructed similarly. Plasmids pλ-eIF4E and pλ-LacZ were constructed by cloning eIF4E and LacZ downstream of λN. LacZ and eIF4E were

PCR amplified from pHA-eIF4E which was a gift of Dong-Er Zhang (Addgene plasmid # 17343)[3] and pET101/d/LacZ (Life Technologies Corporation, CA, USA) respectively and cloned in between the *BstB* I and *Nhe* I sites. Plasmid peIF4E was constructed similarly. For construction of pCIBN-MS2, first the MS2 sequence was amplified from pMS2-GFP which a gift of Robert Singer (Addgene plasmid # 27121)[4], and cloned in between the *Age* I and *Kpn* I sites of pCIBN-pmEGFP. This intermediate plasmid was then used as a template to amplify the complete coding sequence for CIBN-MS2, and the amplification product was inserted in between the *Bgl* II and *Xho* I sites of MCS2. The CRY2PHR and eIF4E WT required for the generation of pCRY2PHR-eIF4E were amplified from pCRY2PHR-mCherryN1 which was a gift of Chandra Tucker (Addgene plasmid # 26866)[2] and pHA-eIF4E. CRY2PHR was cloned into *Fsp* I and *BstB* I sites of MCS2 followed by insertion of eIF4E in between the *BstB* I and *Nhe* I sites of MCS2 of pVITRO2. A similar procedure was used to construct pCRY2PHR.

Fluorescent marker proteins were inserted in the MCS1 of CIBN and CRY2PHR based plasmid constructs -- DsRed (in between the *Age* I and *Sal* I sites) and CFP (in between the *Age* I and *Mlu* I sites). DsRed and CFP were obtained by PCR-amplification from plasmids pSIREN-RetroQ-DsRed-Express (Clontech Laboratories Inc.,CA, USA) and pCAG-CFP which was a gift of Connie Cepko (Addgene plasmid # 11179)[5] respectively.

Bicistronic reporter vectors encoding GFP as an upstream cistron and firefly luciferase (LUC) as a downstream cistron were generated. For construction of pGFP-(n)boxB-LUC, the GFP was PCR-amplified from pUb-R-GFP which was a gift of Nico Dantuma (Addgene plasmid # 11939).[6] The luciferase was PCR-amplified from pGL3-Promoter (Promega Corporation,WI, USA) vectors. GFP was cloned in between the *Fsp* I and *BstB* I sites of MCS2, followed by insertion of luciferase in between the *Bgl* II and *Nhe* I sites. DNA inserts encoding n = 1, 2, and 6

boxB sequences were cloned in between the GFP and luciferase into *BstB* I sites. For construction of pGFP-6MS2-LUC, GFP was first inserted into *Fsp* I and *BstB* I sites. Then a MS2-6X fragment, obtained from *BstB* I and *Bgl* II digestion of pSL-MS2-6X which was a gift of Robert Singer (Addgene plasmid # 27118)[7], was ligated in between the *BstB* I and *Bgl* II sites. Finally, luciferase was inserted in between the *Bgl* II and *Nhe* I sites of MCS2.

Cell transfection and luciferase assay

HEK293T cells (ATCC, VA, USA) were grown in Dulbecco's modified Eagle's medium (Life Technologies Corporation, CA, USA) supplemented with 10% heat-inactivated fetal bovine serum (FBS) and 1% penicillin/streptomycin (Life Technologies Corporation, CA, USA) at 37°C and 5% CO_2. When 80-90% confluent, cells were trypsinized with 0.05% trypsin (Life Technologies Corporation, CA, USA) for transfection. For RNA tether function assay, HEK 293T cells were plated into 96-well plate at a density of 50,000 cells per well the day before transfection. For each well, 200 ng reporter, 100 ng effector and 100 ng pRSF plasmids were mixed with 1 μL lipofectamine 2000 reagent (Life Technologies Corporation, CA, USA) and used for transfection. Forty-eight hours after transfection, luciferase activity was measured. For light illumination experiments, HEK 293 T cells were seeded in 6-well plates at a density of 1,000,000 cells per well the day before transfection. For each well, 1000 ng reporter, 500 ng pCRY2PHR-eIF4E and 500 ng pλ-CIBN plasmids were mixed with 5 μL lipofectamine 2000 reagent (Life Technologies Corporation, CA, USA) and used for transfection. Twenty-four hours after transfection, cells were harvested, mixed well and plated to 96-well plates and kept under dark and light condition for another 48 hours before performing luciferase assay.

Luciferase activity was determined using the Luciferase Assay Kit (Promega Corporation,WI, USA) and VICTOR™ X3 Multilabel Plate Reader (PerkinElmer, USA) following the manufacturer's instructions. The luminescence of each sample was measured by integrating for 1 s and there was no delay time.

RNA extraction and complementary DNA preparation

RNA was extracted from different cell samples by using RNeasy Mini Kit (Qiagen Inc., CA, USA), according to the manufacturer's protocol. Extracted RNA was treated with DNaseI and tested on 2% agarose gel. RNA concentration was measured spectrometrically, and 500 ng of RNA from each sample was used for cDNA synthesis by using ImProm-II Reverse Transcription system (Promega Corporation,WI, USA) according to the manufacturer's protocol. After the completion of reaction, cDNA was diluted to a concentration of 10 ng/µL and kept at -20 °C.

Quantitative real-time polymerase chain reaction (QRT-PCR)

Forward and reverse primers were obtained from Integrated DNA Technologies Inc. (IL, USA). TaqMan probes for luciferase and 18S (house keeping gene) were purchased from BioSearch Technologies (CA, USA). Nucleotide sequences of the primers and probes are shown in supplementary table 1. QRT-PCR was run on LightCycler 489 (Roche, Basel, Switzerland) in a 96-well plate. Brilliant QRT-PCR Master Mix was purchased from Agilent Technologies Inc., (CA, USA) and used according to the manufacturer's guidelines. Briefly, 12.5 µL of master mix, and 0.2 mM of the primers and TaqMan probe, and 1 µL of cDNA as template were used in a final reaction volume of 25 µL. Samples, which included no-template controls, were run in triplicate on a single plate. Two-step cycling protocol was run and cycling conditions were as

follows: initial incubation (95 °C for 10 min) followed by 70 amplification cycles (95 °C for 15 s, 60 °C for 1 min). The QRT-PCR analysis was based on data from three independent experiments.

Light illumination

Blue-light pulses were administered by a custom LED array light source. The blue LED (472 nm, www.superbrightLEDs.com) array was placed on top of a 96-well plate and the LED array was powered using an Arduino Uno microcontroller programmed to pulse illumination for 30 seconds with a 30 second gap between consecutive pulses. The yellow LEDs (595 nm), red LEDs (630 nm) and white LEDs were purchased from SuperbrightLEDs and assembled into LED arrays used for light illumination.

Statistical Analysis

The statistical significance (p value) used to compare the samples with/without light exposure was determined using a one sample t-test; the statistical significance (p value) used to compare the samples with matched/mismatched ligand-aptamer combination in response to light exposure was determined using a Student's t-test. Error bars represent the standard deviation of at least 3 independent experiments.

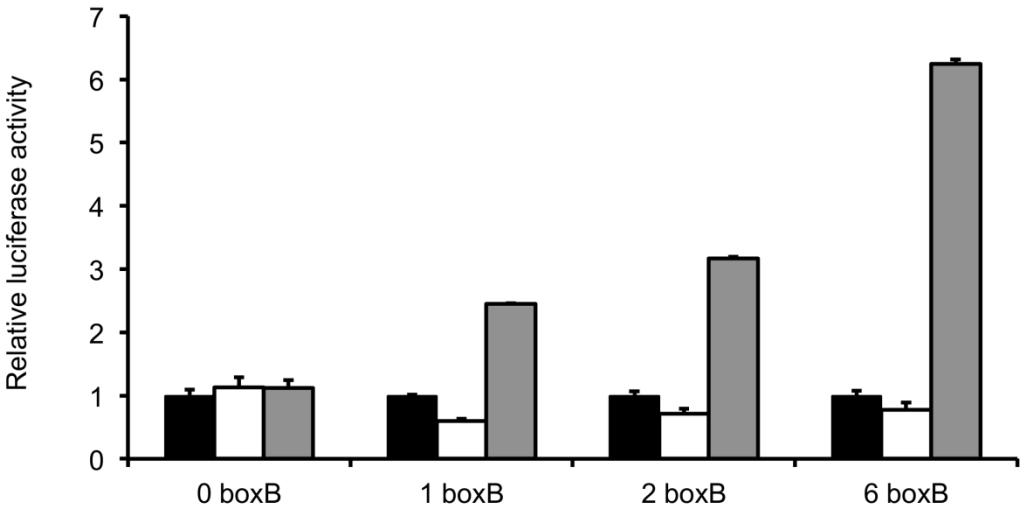

Figure S1. Influence of the number of repeats of the boxB aptamer in the bicistronic reporter on the efficiency of protein translation. Luciferase activity was characterized for cells expressing one of the reporters and either eIF4E (Black bars), the λ-LacZ fusion protein (white bars) (a fusion of λN peptide and the control protein LacZ), or the λ-eIF4E fusion protein (grey bars) (a fusion of λN peptide and eIF4E). The luciferase activity of the cells expressing the reporter and eIF4E protein was set as 1.

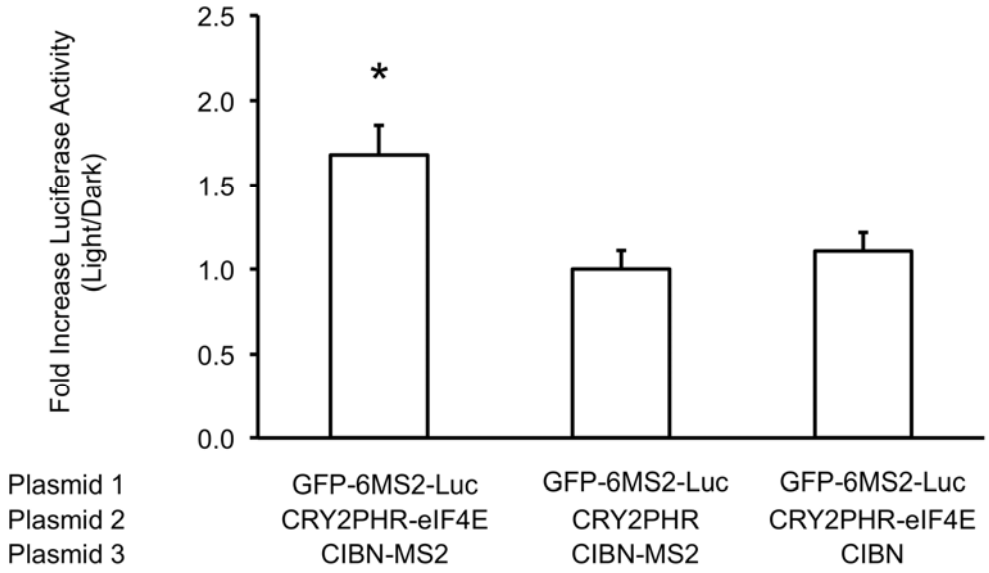

Figure S2. Influence of blue light on the luciferase activity of HEK 293T cells transfected with 3 plasmids: i) reporter (GFP-6MS2-luc) containing 6 MS2-binding aptamers; ii) CRY2PHR-eIF4E or CRY2PHR; and iii) CIBN-MS2 or CIBN. (*$p < 0.005$ vs. dark ; $n = 6$).

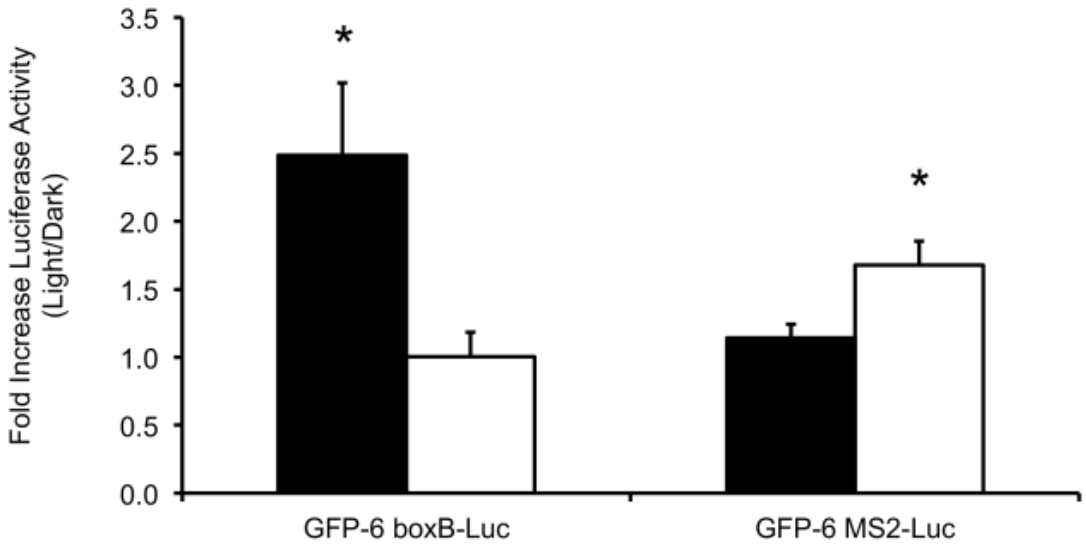

Figure S3. Influence of tether and binding site sequence on light-inducible activation of translation. Luciferase activity of HEK 293T cells transfected with 3 plasmids: i) a luciferase reporter containing either 6 boxB aptamers or 6 MS2 aptamers; ii) CRY2PHR-eIF4E; and iii) CIBN-tether fusion protein (λ-CIBN (black bars) or CIBN-MS2 (white bars)). Significant luciferase activation was found only in cells transfected with CRY2PHR-eIF4E, CIBN-tether and the reporter containing the tether's cognate aptamer. (*$p < 0.005$ vs. mismatched tether; $n \geq 6$.)

Table S1. Primers used in this study.

Primer	Template	Sequence
For-Lambda-CIBN	pCIBN(deltaNLS)-pmEGFP	5'TTCGAAATGAATGGAGCTATAGGAGGTGACCTTTTGC3'
Rev-Lambda-CIBN	pCIBN(deltaNLS)-pmEGFP	5'CTCGAGTTATACATGAATATAATCCGTTTTCTCCAATTCCTTCGT3'
For-MS2	pMS2-GFP	5'ACCGGTGGGTGGCGGTGGCTCTGGTGGCGGTGGCTCTATGGCTTCTAACTTTACTCAGTTCGTTCTCGTC3'
Rev-MS2	pMS2-GFP	5'GGTACCGTTTAAACTTAGTAGATGCCGGAGTTTGCTGCG3'
For-CIBN-MS2	CIBN-MS2 in pCIBN-pmEGFP	5'AGATCTATGAATGGAGCTATAGGAGGTGACCTTTTGC3'
Rev-CIBN-MS2	CIBN-MS2 in pCIBN-pmEGFP	5'CTCGAGTTAGTAGATGCCGGAGTTTGCTGCG3'
For-CRY2PHR	pCRY2PHR-mCherryN1	5'TGCGCAATGAAGATGGACAAAAAGACTATAGTTTGGTTTAGAAGAG3'
Rev-CRY2PHR	pCRY2PHR-mCherryN1	5'TTCGAATGCTGCTCCGATCATGATCTGTG3'
For-DsRed	pSIREN-RetroQ-DsRed-Express	5'ACCGGTATGGCATCCTCCGAGGACGTCATCAAGGAG3'
Rev-DsRed	pSIREN-RetroQ-DsRed-Express	5'GTCGACCTACAGGAACAGGTGGTGGCGG3'
For-CFP	pCAG-CFP	5'ACCGGTCGCCACCATGGTGAGCAAGGGC3'
Rev-CFP	pCAG-CFP	5'ACGCGTTTACTTGTACAGCTCGTCCATGCC3'
For-Ub-R-GFP	pUb-R-GFP	5'CAACAACAATGCGCAATGCAGATCTTCGTGAAGACTCTGACTGGT3'
Rev-Ub-R-GFP	pUb-R-GFP	5'CAACAACAATTCGAATTACTTGTACAGCTCGTCCATGCCGA3'
For-Luc	pGL3-Promoter	5'AGATCTGCCACCATGGAAGACGCCAAAAACATAAAGAAAGG3'
Rev-Luc	pGL3-Promoter	5'GCTAGCTTACACGGCGATCTTTCCGC3'
For-eIF4E	pHA-eIF4E	5'CAACAACAATTCGAAATGGCGACTGTCGAACCGG3'
Rev-eIF4E	pHA-eIF4E	5'CAACAACAAGCTAGCTTAAACAACAAACCTATTTTTAGTGGTGGAGCCG3'

For-LacZ	pET101/d/LacZ	5’CAACAACAACAATTCGAAATGATAGATCCCGTCGTTTTACAAC3’
Rev-LacZ	pET101/d/LacZ	5’CAACAACAACAAGCTAGCTCATTTTTGACACCAGACCAACTG3’
λN-A	None[a]	5'GATCTGCCACCATGGACGCCCAGACCAGGAGAAGAGAGAGGAGAGCCGAGAAGCAGGCCCAGTGGAAGGCTGCCAACGGAGGCAGCTT3'
λN-B	None[b]	5'CGAAGCTGCCTCCGTTGGCAGCCTTCCACTGGGCCTGCTTCTCGGCTCTCCTCTCTCTTCTCCTGGTCTGGGCGTCCATGGTGGCA3'

a,b λN-A, λN-B were annealed together and used as the insert.

Table S2. DNA fragments used in this study.

DNA fragments	Backbone plasmid	Sequence
1 BoxB	pGFP-(n)boxB-LUC	5'TTCGAAAATGTAACTGTATTCAGC GATGACGAAATTCTTAGCTATTGTA ATCCTCCGAGGCCTCCGGTACCCGG GGATCCGGGCCCTGAAGAAGGGCC CTTTCCTTTTCTAGAGTCAGCTTCG ACGAGATTTTCAGGAGCTAAGGAA GCCACCATGGAAGACGCCAAAAAC ATAAAGAAAGGCCCGGCGCCATTC TATCCGCTGGAAGATGGAACCGCT GGAGAGCAACTGCATAAGGCTATG AAGAGATACGCCCTGGTTCCTGGA ACAATTGCTTTTACAGATGCACATA TCGAGGTGGACATCACTTACGCTGA GTACTTCGAA3'
2 BoxB	pGFP-(n)boxB-LUC	5'TTCGAAAATGTAACTGTATTCAGC GATGACGAAATTCTTAGCTATTGTA ATCCTCCGAGGCCTCCGGTACCCGG GGATCCGGGCCCTGAAGAAGGGCC CATATAGGGCCCTGAAGAAGGGCC CTATCGAGGATATTATCTCGAGTTT CCTTTTCTAGAGTCAGCTTCGACGA GATTTTCAGGAGCTAAGGAAGCCA CCATGGAAGACGCCAAAAACATAA AGAAAGGCCCGGCGCCATTCTATC CGCTGGAAGATGGAACCGCTGGAG AGCAACTGCATAAGGCTATGAAGA GATACGCCCTGGTTCCTGGAACAAT TGCTTTTACAGATGCACATATCGAG GTGGACATCACTTACGCTGAGTACT TCGAA3'
6 BoxB	pGFP-(n)boxB-LUC	5'TTCGAAAATGTAACTGTATTCAGC GATGACGAAATTCTTAGCTATTGTA ATCCTCCGAGGCCTCCGGTACCCGG GGATCCGGGCCCTGAAGAAGGGCC CATATAGGGCCCTGAAGAAGGGCC CTATCGAGGATATTATCTCGACTAA GTCCAACTACTAAACTGGGCCCTGA AGAAGGGCCCATATAGGGCCCTGA AGAAGGGCCCTATCGAGGATATTA TCTCGACTAAGTCCAACTACTAAAC TGGGCCCTGAAGAAGGGCCCATAT AGGGCCCTGAAGAAGGGCCCTATC GAGGATATTATCTCGAGTTTCCTTT

	TCTAGAGTCAGCTTCGACGAGATTT TCAGGAGCTAAGGAAGCCACCATG GAAGACGCCAAAAACATAAAGAAA GGCCCGGCGCCATTCTATCCGCTGG AAGATGGAACCGCTGGAGAGCAAC TGCATAAGGCTATGAAGAGATACG CCCTGGTTCCTGGAACAATTGCTTT TACAGATGCACATATCGAGGTGGA CATCACTTACGCTGAGTACTTCGAA 3'

Table S3. Primers and TaqMan probes for QRT-PCR used in this study.

Oligonucleotides	Gene	Sequence
Sense	Firefly luciferase	5'CGGAAAGACGATGACGGAAA 3'
Antisense	Firefly luciferase	5'CGGTACTTCGTCCACAAACA 3'
Probe	Firefly luciferase	5'-FAM490-CGTGGATTACGTCGCCAGTCAAGT -BHQ-3'
Sense	18 S	5'GTAACCCGTTGAACCCCATTC3'
Antisense	18 S	5'CCATCCAATCGGTAGTAGCGA3'
Probe	18 S	5'-CAL610-AAGTGCGGGTCATAAGCTTGCG-B HQ-3'

References

1 E. De Gregorio, T. Preiss, M. W. Hentze, *EMBO J.,* 1999, **18**, 4865-4874.

2 M. J. Kennedy, R. M. Hughes, L. A. Peteya, J. W. Schwartz, M. D. Ehlers, C. L. Tucker, *Nat. Methods,* 2010, **7**, 12-16.

3 F. Okumura, W. Zou, D. E. Zhang, *Genes Dev.,* 2007, **21**, 255-260.

4 D. Fusco, N. Accornero, B. Lavoie, S. M. Shenoy, J. M. Blanchard, R. H. Singer, E. Bertrand, *Curr. Biol.,* 2003, **13**, 161-167.

5 T. Matsuda, C. L. Cepko, *Proc. Natl. Acad. Sci. U. S. A.,* 2004, **101**, 16-22.

6 N. P. Dantuma, K. Lindsten, R. Glas, M. Jellne, M. G. Masucci, *Nat. Biotechnol.,* 2000, **18**, 538-543.

7 E. Bertrand, P. Chartrand, M. Schaefer, S. M. Shenoy, R. H. Singer, R. M. Long, *Mol. Cell,* 1998, **2**, 437-445.