Supporting Information

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SI Materials and Methods

Fish Husbandry and Collection of WT and Morphant Embryos. Zebrafish embryos were maintained at 28.5 °C and staged as described (1). The WT strain was a hybrid of the AB and TU lines. Fertilized embryos, obtained at the one-cell stage, were either collected as WT or used for morpholino-microinjection experiments as described (2). All animal experiments were carried out using protocols approved by the Weill Cornell Medical College Institutional Animal Care and Use Committee review board.

Isolation of Polysomes from Zebrafish Embryos and Polysome Profiling. We typically generated lysates from 300 embryos per gradient, generating an adequate amount of cell-free extracts (5–10 A_{260} units) to yield a well-defined polysome profile and 30–50 μg of total RNA from the polysome fractions. Staged embryos were treated with pronase in system water at 2 mg/mL for 4–5 min at 28.5 °C. Embryos were dechorionated by passing through a Pasteur pipette, were washed three or four times with system water, and then were treated with 0.1 mg/mL cycloheximide at room temperature for 5 min. After transfer to an Eppendorf tube on ice, they were washed three times with E2 embryo medium (15 mM NaCl, 0.7 mM $NaHCO_3$, 0.5 mM KCl, 0.15 mM KH_2PO_4, 2.7 mM $CaCl_2$, 0.05 mM Na_2HPO_4, and 1 mM $MgSO_4$) containing 0.1 mg/mL cycloheximide. The embryos were deyolked by pipetting through a P200 yellow tip using 1 mL of E2/cycloheximide buffer. After centrifugation at 1,500 rpm in an Eppendorf 5424 centrifuge for 30 s, the deyolked embryos were collected as a pellet, and the supernatant containing the dissolved yolk was discarded. One milliliter of E2/cycloheximide medium was added to this pellet, and the embryos were dissociated by pipetting up and down repeatedly through a P200 yellow tip. After centrifugation at 1,500 rpm for 2 min, the dissociated embryos were collected again as a pellet and typically were saved in liquid N_2 until enough embryos were collected (~300 per gradient). Subsequently, each embryo pellet was resuspended with 500 μL freshly prepared ice-cold lysis buffer [20 mM Tris·HCl (pH 7.5), 30 mM $MgCl_2$, 100 mM NaCl, 0.25% (vol/vol) Nonidet P-40, 100 μg/mL cycloheximide, 0.5 mM DTT, and 1 mg/mL heparin] on ice for 10 min. The lysate was centrifuged at 15,000 × *g* for 20 min, and the cellular debris was discarded. The supernatant (~500 μL) was loaded onto an 11-mL 10–50% (wt/vol) linear sucrose gradient prepared in TMS buffer [20 mM Tris·HCl (pH 7.53), 5 mM $MgCl_2$, 140 mM NaCl] and centrifuged at 40,000 rpm for 2.5 h in a Beckman SW41 rotor. Each sample was passed through an ISCO gradient fractionator (Teledyne) attached to an ISCO spectrometer (model 640) (Teledyne). Approximately 20 fractions per gradient were collected (500 μL per fraction), and the A_{254} profile was determined. Subsequently, 100 μL of 20% SDS was added to each fraction, and the samples were stored at −20 °C until RNA was extracted using TRIzol reagent (Invitrogen).

Isolation of Total RNA from Polysomal and Nonpolysomal Fractions. Individual polysomal fractions (typically fractions 11–20, previously frozen at −20 °C in the presence of SDS) were incubated at 65 °C for 10 min to disrupt ribosomes. RNA was extracted from each sample with 1 mL of TRIzol reagent as directed by the manufacturer. The aqueous layer from each fraction was pooled, and 1.5 volumes of 75% EtOH were added (total volume ~25–30 mL). The solution was passed through an RNeasy-Mini (Invitrogen) column (700 μL at a time) using centrifugation at 10,000 rpm for 30 s at room temperature. The column was washed twice with 500 μL of RPE buffer (Invitrogen) at 10,000 rpm for 30 s at room temperature, and the RNA was eluted with nuclease-free water.

To isolate total RNA from individual nonpolysomal fractions (typically fractions 1–10), individual fractions were incubated at 65 °C for 10 min and then were pooled and subsequently were divided equally into two 14-mL polypropylene tubes (Sarstedt). The aqueous layer was obtained from each tube using 5 mL TRIzol reagent for each tube following the manufacturer's directions and was transferred to two different 14-mL tubes. The total RNA from each aqueous layer was precipitated using 3 mL isopropyl alcohol for each tube, and the supernatant was discarded. The precipitated RNA from each tube was redissolved in a minimum volume of RNase-free water. After adding a 1/10th volume of 3 M sodium acetate (pH 5.5) and two volumes of 100% ethanol, RNA was reprecipitated, and this purified RNA precipitate was dissolved in nuclease-free water.

RNA Sequencing and Analysis of the Sequencing Data. The cDNA libraries were generated using standard Illumina kits. The input RNA and library quality were assessed by Qubit PicoGreen and Agilent Bioanalyzer assays. Samples were bar-coded and sequenced on either an Illumina GIIx or HiSeq sequencer for 54 cycles. Representative quality control analyses of our RNA samples are given in Fig. S2, and a tabular characterization of the RNA samples used for this study is given in Table S1. The data were passed through an established Illumina pipeline to convert raw image data to base calls, provide quality scores at each base, and align sequences to the zebrafish genome (Zv9) using a University of California Santa Cruz genome browser mirror. Phred Quality Scores of the read sequences for the samples were >30 for all base positions. As an example, representative Phred scores and alignment results of one of our samples are given in Fig. S2. The transcript abundance of each gene, measured in reads per kilobase per million reads (RPKM) values, was quantified for the samples, and using these values, the change of translational state for each transcript was calculated as $[(X_{mo}/X_{wt})_{poly}/(X_{mo}/X_{wt})_{total}]$, where X_{mo} and X_{wt} represent the abundance, in RPKM units, of the same transcript in the total RNA pool in the morphants and WT embryos, respectively.

Quantitative Real-Time PCR. Using RNA purified from the total RNA or polysome fractions, first-strand cDNA synthesis was performed using equivalent amounts of starting RNA from all samples (Superscript III kit; Invitrogen). The cDNA was analyzed with the Light Cycler 480 II SYBR Green Master Mix (Roche) and using the Light Cycler 480 II system (Roche). All samples were prepared in triplicate. The PCR cycle conditions used were 95 °C for 5 min, followed by 40 cycles of 95 °C for 10 s, 54 °C for 10 s, and 72 °C for 15 s The crossing point (Cp) values were determined as described (3). To validate the RNA-sequencing (RNA-seq) data using qualitative real-time PCR (qPCR), the translational state change $[(X_{mo}/X_{wt})_{poly}/(X_{mo}/X_{wt})_{total}]$, was calculated after each term in the equation was normalized with respect to β-actin or TATA-binding protein (*tbp*). For all other cases, Cp values were normalized to the transcript levels of β-actin, and the morphant transcript levels were quantified considering WT levels as 1. The primers used are listed below.

Generation of Chimeric Crystallin Gamma 2d Isoform Constructs. Details of the generation of chimeric crystallin gamma 2d isoform 7 (crygm2d7) constructs are provided in Fig. S6. Two separate FLAG-tagged constructs containing either the *crygm2d7*-5′ UTR (with control β-actin 3′ UTR) or the *crygm2d7*-3′ UTR (with

control β-actin 5′ UTR) were prepared also. To do so, the previously described (Fig. S6) C-terminally FLAG-tagged construct in pBluescript plasmid (Agilent) was used as a template, and two separate PCR reactions were performed using two distinct sets of PCR primers (P7/P8 and P9/P10; see primer list below) so that the two PCR products contained KpnI/XhoI and KpnI/HindIII as flanking restriction enzyme sites. The cloning reactions were performed using these restriction enzyme sites to generate the desired constructs into the destination pBluescript vector. The resulting two plasmids (plasmid V1 containing only the 5′ UTR of *crygm2d7* and plasmid V2 containing only the 3′ UTR of *crygm2d7*) were used as destination vectors for subsequent insertion of control 3′ or 5′ β-actin UTRs, respectively. For this purpose, two PCR reactions were performed from 1-d-post-fertilization (dpf) zebrafish cDNA using two sets of primers (P11/P12 and P13/P14 in the primer list below), resulting in two PCR products containing the *β-actin* 3′ UTR (flanked by XhoI/SacI sites) or the *β-actin* 5′ UTR (flanked by KpnI sites), respectively. After two subsequent cloning reactions using the above restriction enzyme combinations, the 3′ or the 5′ UTR of *β-actin* was inserted into vector V1 or vector V2, respectively.

Generation of Chimeric pvalb1-FLAG Constructs. A C-terminally–tagged parvalbumin 1 (pvalb1)-*FLAG* plasmid construct was generated using a strategy similar to that described in Fig. S6. The final construct was incorporated into a pBluescript vector using the restriction enzymes KpnI, XhoI, and XbaI for the two-step cloning reactions. The gene-specific primers used for this strategy (pv1, pv2, pv3, and pv4) are given in the primer list below.

Analysis of Chimeric crygm2d7 Constructs. The pBluescript plasmids containing either the C-terminally–tagged or N-terminally–tagged *crygm2d7* cDNAs as well as pBluscript plasmids containing either the 5′ UTR or 3′ UTR of *crygm2d7* were linearized by HindIII digestion. Each FLAG-tagged mRNA was synthesized in vitro using T7 RNA polymerase (mMessage mMachine T7 μLtra kit; Invitrogen), and the resulting RNA (100 pg per injection) was injected into fertilized eggs in combination with control or eukaryotic initiation factor 3, sub-unit ha (eif3ha) morpholinos. Stage-matched embryos (~30) were collected at 20–22 h post fertilization (hpf) and were dechorionated using forceps, transferred to Eppendorf tubes, washed with E2 medium, and deyolked in a solution of 55 mM NaCl, 1.8 mM KCl, and 1.25 mM $NaHCO_3$, containing protease inhibitor mixture (Complete Mini; Roche).

For Western blotting, 39 μL of each protein extract was mixed with 6 μL of 10× reducing agent (Invitrogen) and 15 μL of 4× SDS sample buffer (Invitrogen) and was incubated at 70 °C for 10 min. Each sample (50 μL) was subjected to SDS-10% PAGE. The samples were transferred to a PVDF membrane using the iBlot Dry Blotting System (Invitrogen). After washing in PBS with Tween 20 (PBST), membranes were blocked using 5% milk/PBST for 1 h at room temperature and were incubated with primary antibodies [1:1,000 for anti-FLAG HRP-conjugated antibody (Sigma); 1:5,000 dilution for anti–β-actin antibody (Millipore)] at 4 °C overnight. The anti-FLAG signal was detected using ECL reagent (Millipore). For β-actin, the membrane first was incubated with anti-mouse HRP-coupled antibody [1:2,000 dilution (GE Healthcare)] for 1 h at room temperature before detection with the ECL reagent. Signals were quantified using the BioSpectrum UVP Multispectral Imaging System.

Generation of Luciferase Constructs and Dual Luciferase Assay. To generate the plasmid carrying the *Renilla* luciferase (RLUC) ORF, which was used as a test construct in the dual luciferase assay, the N-terminal and C-terminal FLAG-tagged *crygm2d7* plasmid constructs were digested separately with XhoI and HindIII. The longer fragment of N-terminal plasmid was used as the destination vector, and the shorter fragment of C-terminal plasmid was used as the insert to perform a cloning reaction to generate an intermediate plasmid that contained both the 5′ and the 3′ UTRs of *crygm2d7* mRNA (without any ORF sequence) joined by a single XhoI restriction enzyme site. In a separate PCR, the RLUC-ORF was amplified from the pRL-CMV vector flanked by two XhoI sites on each side (primer set P15/P16, given in the primer list, below). By using XhoI enzyme in the following cloning reaction RLUC-ORF was inserted between the 5′- and 3′-*crygm2d7* UTRs of the intermediate plasmid to obtain the final construct. The control *Firefly* luciferase (FLUC) ORF, obtained by digesting pGL3-Basic vector with XbaI/XhoI, was cloned into the $pCS2^+$ vector. The test RLUC mRNA and the control FLUC mRNA were prepared in vitro after linearizing the constructs with HindIII and NotI enzymes, respectively. After these mRNAs along with the control or *eif3ha* morpholinos were injected into one-cell-stage embryos, dual luciferase assays were performed according to manufacturer's protocol (Promega).

List of primers

A) Primers used for qPCR experiments:

β-ac, beta actin	Fp – 5′-GACAACGGCTCCGGTATG Rp – 5′-CATGCCAACCATCACTCC
tbp, tata binding protein	Fp – 5′-CGGTGGATCCTGCGAATTA Rp – 5′-TGACAGGTTATGAAGCAAAACAACA
crygm2d3, crystallin gamma, 2d3	Fp - 5′-CATCAGGTCTTGCCGTATGA Rp – 5′-TCTGACCTCCGAAGTTCTCC
crygm2d4, crystallin gamma, 2d4	Fp – 5′-ATCAGGTCCTGCCGTATGAT Rp – 5′-CATCTGACCTCCAAAGTTCTCC
crygm2d12, crystallin gamma, 2d12	Fp – 5′-ATGCACAGGGGATCCTACAG Rp – 5′-TAACGGTCCATGATGCTGTC
eno2, enolase 2	Fp – 5′-CCCCCTGTACCGTCATATTG Rp – 5′-GAGCCTCCGTTGATCACATT
ckmt2, creatine kinase, mitochondrial 2	Fp – 5′-CCGTGAAGTAGAGCGTGTGA Rp – 5′-TGCTGCTGCTCTTGTTCAGT
crygm2d7-FLAG, FLAG tagged (C-terminal) crystallin gamma, 2d7	Fp – 5′-GGCCTGGACAATACAGGAAC Rp - 5′-AGCTTGTCATCGTCGTCCTT

FLAG-crygm2d7, FLAG tagged (N-terminal) crystallin gamma, 2d7	Fp - 5′-AAGGACGACGATGACAAGGT Rp - 5′-ACGGCTCATGTAGGAGGAGA
crygm2d7-FLAG (only *crygm2d7*-5′-UTR and beta-acting 3′-UTR):	Fp - 5′-GGCCTGGACAATACAGGAAC Rp – 5′-AGCTTGTCATCGTCGTCCTT
crygm2d7-FLAG (only *crygm2d7*-3′-UTR and beta-actin 5′-UTR):	Fp – 5′-GGCCTGGACAATACAGGAAC Rp - 5′-AGCTTGTCATCGTCGTCCTT
28S rRNA, taken from ref. (4):	Fp – 5′-CCTCACGATCCTTCTGGCTT FP – 5′-AATTCTGCTTCACAATGATA
18S *rRNA*, taken from ref. (5):	Fp – 5′-TCGCTAGTTGGCATCGTTTATG Rp – 5′-CGGAGGTTCGAAGACGATCA
cu855779.1, unannotated gene	Fp – 5′-ACTCCACTGCCAGATGTTCC Rp – 5′-TGCTAGCTTCTCCTCCTTGC
zgc:101859, unannotated gene	Fp – 5′-GGGATATGAGCACCACGACT Rp – 5′-CTCCACATGGTAGCCCAGAT
bscl2, Berardinelli-Seip congenital lypodistrophy 2	Fp – 5′-GGTGTGGATGAGATGGAAGG Rp – 5′-CCGTGGGCATAAAGGAATAA
slc25a4, solute carrier family, 25a4	Fp – 5′-GAGAGAGTTCACCGGTCTCG Rp – 5′-TAATGCCCTGGACTGACACA
atp2a1l, ATPase *atp2a* like	Fp – 5′-TGAACCTGAGATGGGAAAGG Rp – 5′-CACCTCCACAATGTCACCAG
pvalb1, parvalbumin 1	Fp – 5′-CTGCCGACTCCTTCGACTAC Rp – 5′-TGTTGTCCTGGTCGATGATG
hspb2, heat shock protein b2	Fp – 5′-GGTACCGAGTGTTGCTGGAT Rp – 5′-CATGCTGGTCCATTCTCTGA
crygm2d5, crystalline gamma, 2d5	Fp – 5′-TACATGAGCCGCTGTCACTC Rp – 5′-CAGCATACTCTCCCCTCCTG
crygm2d8, crystalline gamma, 2d8	Fp – 5′-CAGGAGGGGAGAGTATGCTG Rp – 5′-CTGTAGGATCCCCTGTGCAT
crygm2d11, crystalline gamma, 2d11	Fp – 5′-CTTCCAGGGTCGCTCTTATG Rp – 5′-AGCCACTCTCCACTCTGCAT
crygm2d9, crystalline gamma, 2d9	Fp – 5′-CCAGTCCTGTCATGTGATGG Rp – 5′-TGCCACCATAATTGCTGAAG
si:ch211-212n6.8, unannotated gene	Fp – 5′-TACATGAGCCGCTGTCACT Rp – 5′-CAGCATACTCTCCCCTCCTG
and2, actinodine 2	Fp – 5′-TTCATGGAGACCAACAACCA Rp – 5′-TTGAAATTGGCACCCTCTTC
si:ch211-212n6.16, unannotated gene	Fp – 5′-GCAGTCAGTCAACAGGCAGA Rp – 5′-ACCCTGGAAGTTCCTGTCCT
zgc:86723, unannotated gene	Fp – 5′-TACATGAGCCGCTGTCACTC Rp – 5′-CAGCATACTCTCCCCTCCTG
si:ch211-212n6.18, unannotated gene	Fp – 5′-GAGCAGGCCAATTACAGAGG Rp – 5′-GAACCTCATGCCACCAAAGT
si:ch211-212n6.17, unannotated gene	Fp – 5′-TACATGAGCCGCTGTCACTC Rp – 5′-CAGCATACTCTCCCCTCCTG
flg1, fibrinogen like	Fp – 5′-GATGGCAGTCTGTCGTTTGA Rp – 5′-TCATTCCCCAACCAGAACTC
zgc:165347, unannotated gene	Fp – 5′-ATGCACAGGGGATCCTACAG Rp – 5′-AACGGTCCATGATGTTGTCA
slc18a3, unannotated gene	Fp – 5′-CCATCATCCCGGACTACCT Rp – 5′-GAACAGAACCCCGATCTGAA
si:dkey-17e16.15, unannotated gene	Fp – 5′-TGAGAGAGGAGAGCCAGAGC Rp – 5′-GCGGCTTTAGTGTCCAGAAG
crygmxl2, crystalline gamma, xl2	Fp – 5′-CTACAACAGGCCCGAGATGT Rp – 5′-GGTAGCCAAAGCGATCGTAG

and3, actinodine 3	Fp – 5′-GGCACACAGAAGGGCTCTAT Rp – 5′-CTGGTATTTCGATGCGTCAG
mipb, major intrinsic protein, lens fibre b	Fp – 5′-TGGCACTTAACACGCTTCAG Rp – 5′-GAAAACGCAGACCACCAACT
crygm2d13, crystalline gamma, 2d3	Fp – 5′-GGTCCTGCCGTATGATCTCT Rp – 5′-ACGGTCCATGATGTTGTCAC
slc35a5, solute carrier family, 35a5	Fp – 5′-AGTTCTCGGGCAATGAAGAA Rp – 5′-CACCCGTACTGACATCACCA
dap, death associated protein	Fp – 5′-AGCTGCCTGTTGTGGTCTCT Rp – 5′-AAGGCACAGGTTTCTGATGG
hoxb2a, homeo box 2a	Fp – 5′-CCAACACGCAGCTACTTGAA Rp – 5′-CTTGTCGCTCGGTTAGATCC
kifc1, kinesin family member C1	Fp – 5′-ACATGGAGGCCAAAGTTCAG Rp – 5′-AATATCAGCGGTGCGTCTTT
cwf19l1, CWF-19 like protein	Fp – 5′-GCTGCTGTGTGTTGGTGACT Rp – 5′-ACTGGCTGCACCGAGAATAC
B) Primers used to generate cDNAs for *in situ* hybridization: *crygm2d3*, crystalline gamma, 2d3	Fp – 5′-AGTGTATGGGCGACTGTGGT Rp – 5′-ACATCCTGCCTCTGTAGTGGG
crygm2d12, crystalline gamma, 2d12	Fp - 5′-ATTGTATGGGCGACTGTGGT Rp – 5′-ACATTCTGCCTCTATAGTGGGG

C) Primers used to generate *crygm2d7* and luciferase constructs:
Gene specific primer, GSP (5′-RACE) – 5′-ACATCCTGCCTCTGTAGTGGG

Gene specific primer, GSP (3′-RACE) – 5′-AGTGTATGGGCGACTGTGGT
P1 – 5′-GGGGTACCAAACAGCCAGAATCAGCT
P2 – 5′-CCCAAGCTTTAATGTATAGAAATTGTTTTATTGAAACT
P3 - 5′-CCGCTCGAGGACTACAAGGACGACGATGACAAGGTCACCTTCTTTGAGGACAGGAACTTC
P4 – 5′-CCGCTCGAGCTTCATGTTTGTGCTGTCAGTGGT
P5 – 5′-CCGCTCGAGTAGAGTTTCAATAAAACAATTTCTATA
P6 - 5′-CCGCTCGAGCTTGTCATCGTCGTCCTTGTAGTCGTACCAAGAGTCCATGATACGCCTCAT

To generate constructs containing only 5′-UTR of *crygm2d7* (with 3′-UTR sequences of beta-actin serving as control):
P7 – 5′-GGGGTACCAAACAGCCAGAATCAGCTTCTCCC
P8 – 5′-CCGCTCGAGCTACTTGTCATCGTCGTCCTTGTA
P11 – 5′-CCGCTCGAGACAGAACTGTTGCCACCT
P12 – 5-CGAGCTCTTGTGTGGTTTTACATGTGCACGT

To generate constructs containing only 3′-UTR of *crygm2d7* (with 5′-UTR sequences of beta-actin serving as control):
P9 – 5′-GGGGTACCATGAAGGTCACCTTCTTT
P10 – 5′-CCCAAGCTTTAATGTATAGAAATTGTTTTATTGAAACTCTACTCG
P13 – 5′- GGGGTACCATTGTGAGTTTTCAGTGCACGCTG
P14 – 5′-GGGGTACCGGCTGTGTATTAGTAGGTTAT

For Renilla luciferase (RLUC) and Firefly luciferase (FLUC) constructs:
P15 – 5′-CCGCTCGAGACTTCGAAAGTTTATGATCCAGAA
P16 – 5′-CCGCTCGAGTTGTTCATTTTTGAGAACTCGC

Primers used to generate *pvalb1* constructs:
pv1 – 5′-GGGGTACCACACCTCGACTAGCTCCTTTGCTT
pv2 – 5′ CCGCTCGAGCTTGTCATCGTCGTCCTTGTAGTCAGCC
TTTACAAGAGCAGCAAACTCTTC
pv3 – 5′-CCGCTCGAGTAAATTTTCATCCGACCAAGACCC
pv4 – 5′-GCTCTAGATTACACCAAAACTATATTTATTCC

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2. Choudhuri A, Evans T, Maitra U (2010) Non-core subunit eIF3h of translation initiation factor eIF3 regulates zebrafish embryonic development. Dev Dyn 239(6):1632–1644.
3. Livak KJ, Schmittgen TD (2001) Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) Method. *Methods* 25(4):402–408.
4. Delaunay F, Thisse C, Marchand O, Laudet V, Thisse B (2000) An inherited functional circadian clock in zebrafish embryos. *Science* 289(5477):297–300.
5. McCurley AT, Callard GV (2008) Characterization of housekeeping genes in zebrafish: Male-female differences and effects of tissue type, developmental stage and chemical treatment. *BMC Mol Biol* 9:102.

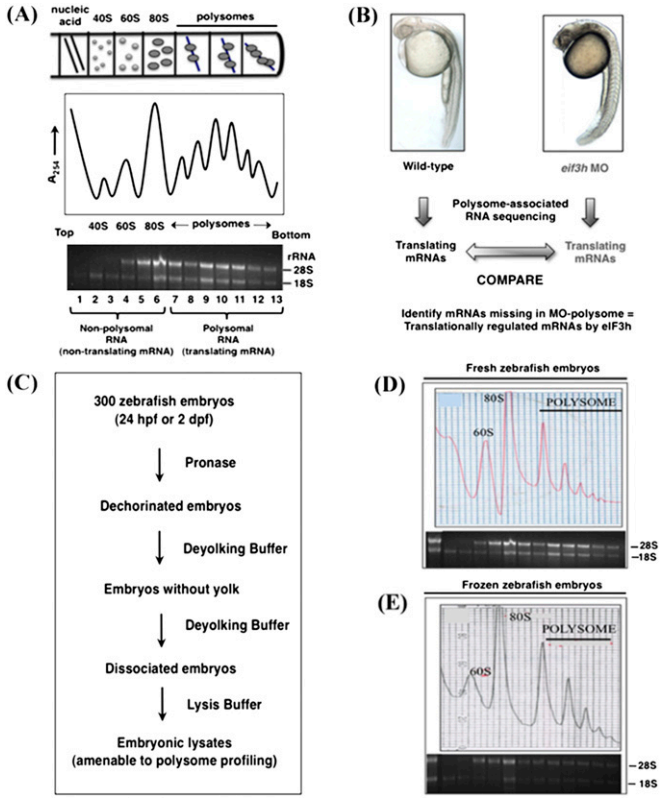

Fig. S1. Polysome profile analysis and the strategy of subsequent RNA-seq of polysome-associated mRNAs in zebrafish embryos. (*A*) A cell-free extract prepared from actively translating cells is loaded onto a linear 10–50% sucrose gradient and subjected to velocity gradient centrifugation. Free 40S, 60S, and 80S ribosomes, in addition to polysomes, are size fractionated from the translationally inactive mRNAs and messenger ribonuclear protein (MRNP) particles. The A_{254} profile is analyzed with an attached UV-absorbance monitor. The position and integrity of the ribosomal components in the separated fractions are demonstrated by isolating total RNA from individual fractions and analyzing aliquots by gel electrophoresis. The presence of 18S, 28S, or equimolar 18S and 28S ribosomal RNAs (rRNAs) identifies the positions of 40S, 60S, and 80S ribosomes, respectively. Polysomal fractions also yield both 18S and 28S rRNAs and consist of translating mRNAs. (*B*) To identify genes that are translationally regulated, stage-matched WT and morphant embryos were collected, and whole-cell lysates were processed as in *A*. Both total RNA and polysome-associated RNA were used to generate cDNA libraries that were analyzed by deep sequencing to identify transcripts underrepresented in the morphant samples (in the polysome, but not the total RNA samples). Here we compared *eif3ha* and control samples at 24 hpf. (*C*) Flowchart indicating the protocol optimized to obtain reproducible polysome profiles from cell-free extracts of zebrafish embryos. (*D*) A typical polysome profile prepared from fresh (nonfrozen) WT embryos. (*E*) A representative polysome profile prepared from frozen WT embryos. The positions of 40S, 60S, and 80S ribosomes are indicated.

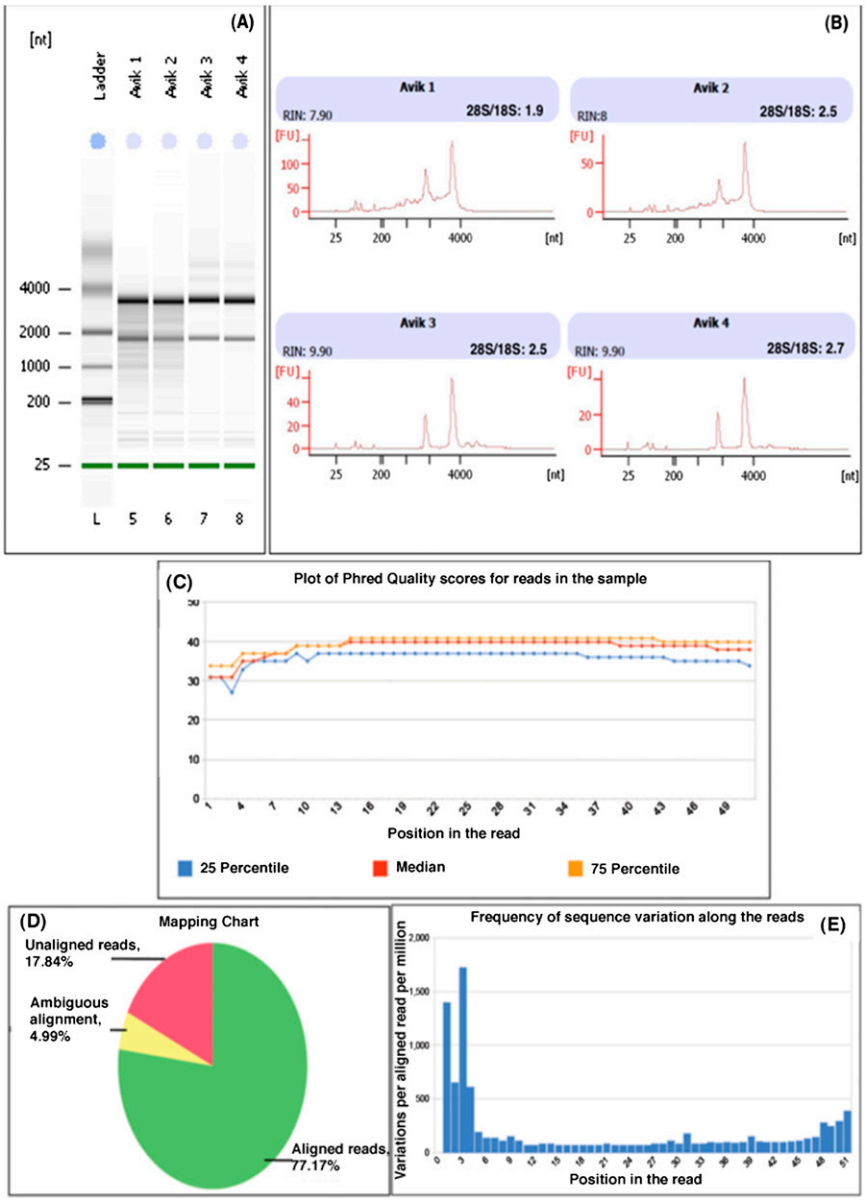

Fig. S2. Quality control of the total RNA and RNA-seq data using RNA isolated from the polysomal fractions as well as from the whole embryos. A representative analysis of four RNA samples is shown. Avik1, WT polysomal RNA at 2 dpf; Avik2, *eif3ha* polysomal RNA at 2 dpf; Avik3, WT total RNA at 2 dpf; Avik4, *eif3hba* total RNA at 2 dpf. (*A*) The appearance of rRNA bands during bioanalysis. (*B*) The quantitative calculation of the 28S/18S ratio and the corresponding RNA Integrity Number (RIN) values obtained. (*C*) The plot of median base quality (Phred Quality Score) at each position of all of the reads present in the RNA sample. The base quality is calculated under three different probability scenarios and plotted accordingly [blue, 25th percentile; red, 50th percentile (median); yellow, 75th percentile]. Phred Scores >30 for all of the base positions indicate that good-quality reads are present in the sample. (*D*) The mapping chart obtained after aligning the read-sequences to the reference zebrafish genome. The percentage of reads unambiguously aligned (green, 77.17%), ambiguously aligned (yellow, 4.99%), and unable to be aligned (red, 17.84%) are indicated. An alignment >70% is considered a good alignment score. (*E*) The alignment chart showing the frequency of sequence variation along the reads obtained after the read-sequences were aligned to the reference genome. Each position in the read should be associated with least possible variance. The alignment chart shown is representative of a well-aligned sample.

Tissue/Organ	# Genes
Lens	~35
Hind Brain	31
Retina	27
Cranial Ganglion	25
Diencephalon	23
Telencephalon	18
Neural Tube	18
Basal Plate Mid-brain	12
Myotome	~36

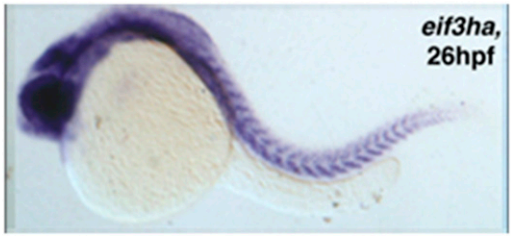

Fig. S3. Deregulated transcripts are expressed in the organ systems that are consistent with the spatial expression patterns and morphant phenotypes of eif3ha morphants. Shown is a Database for Annotation, Visualization and Integrated Discovery (DAVID) gene ontogeny analysis of gene sets deregulated from polysomes in the eif3ha morphant embryos. Shown below are the in situ hybridization patterns showing the spatial expression of eif3ha at 24 hpf indicating a correlation between the tissue association of the transcripts and the expression domains (see also ref. 2).

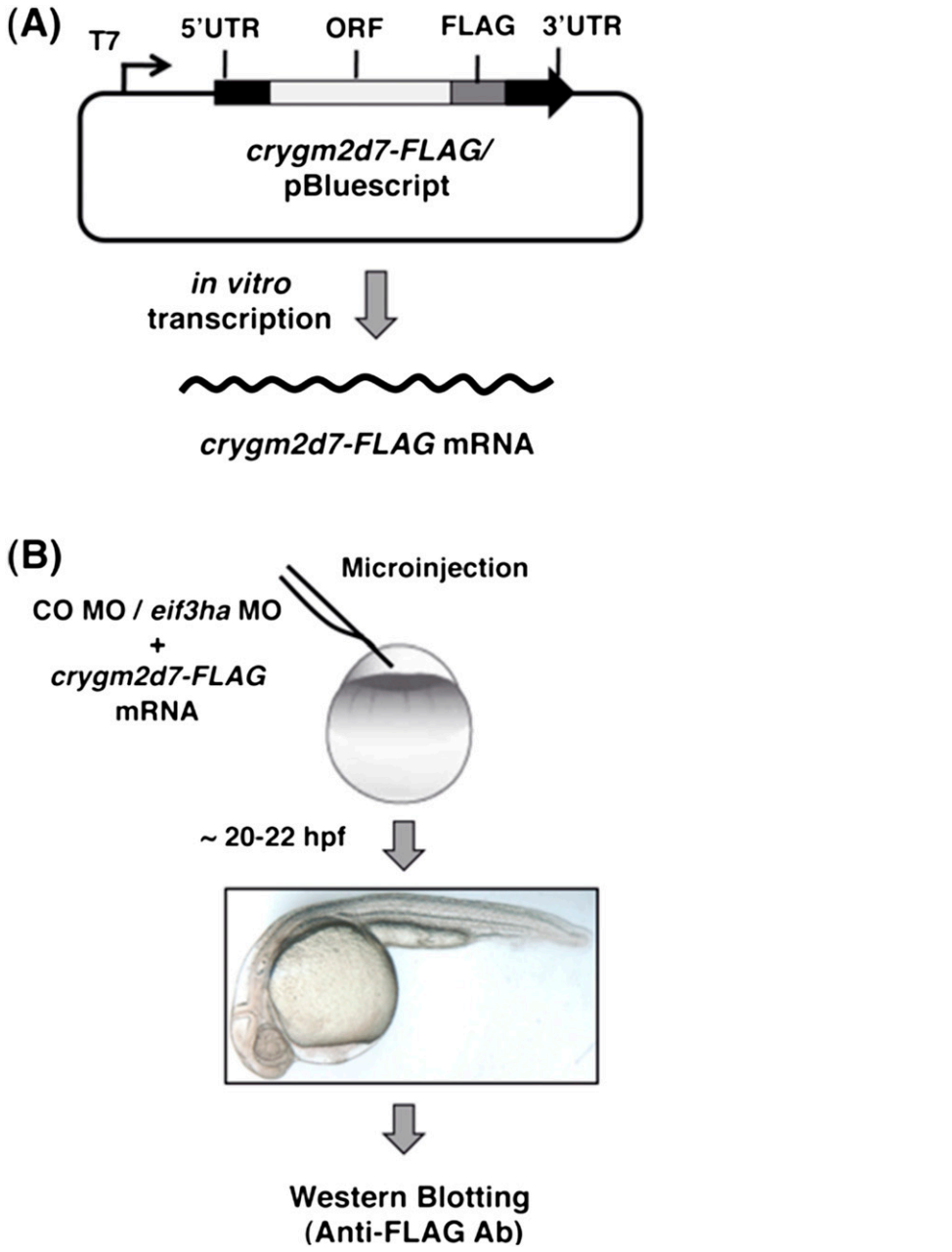

Fig. S4. The approach used to compare the synthesis of Crygm2d7 protein from the corresponding FLAG-tagged *crygm2d7* mRNA in stage-matched control and *eif3ha* morphant embryos. (*A*) Strategy used to generate mRNA by in vitro transcription encoding C-terminally Flag-tagged Crygm2d7. In an alternate construct, the FLAG tag was placed at the N terminus of the protein. (*B*) The purified RNA was injected along with either a control or *eif3ha*-specific MO, followed by Western blotting experiments of the embryo-derived lysates ~24 h later, using anti-FLAG antibody.

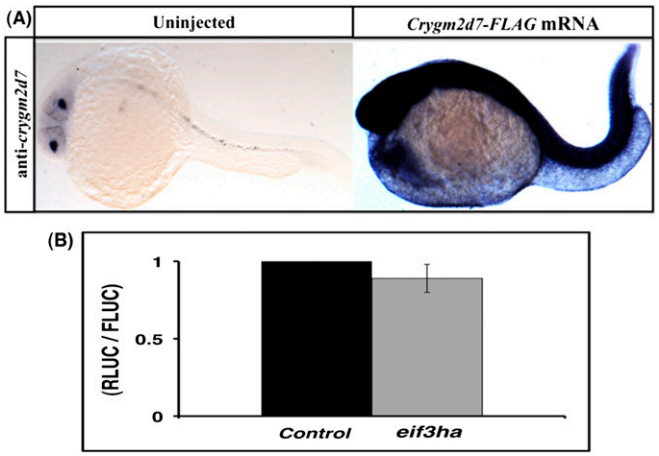

Fig. S5. In situ hybridization experiments demonstrating the spatial expression patterns of ectopically injected *crygm2d7-FLAG* mRNA. The *crygm2d7* UTR sequences are not sufficient to confer *eif3ha* dependency for translation. (*A*) Embryos were probed using an anti-*crygm2d7* RNA probe. Shown are representative 24 hpf embryos that had been injected with the *crygm2d7-FLAG* mRNA or stage-matched control uninjected embryos, as indicated (note that transcripts in the controls are restricted to the lens). (*B*) The renilla luciferase (RLUC) cDNA was flanked by the *crygm2d7* UTR sequences, and activity relative to firefly luciferase (FLUC) was measured in *eif3ha* and control morpholino-injected embryos. FLUC was used as control luciferase in this dual luciferase assay. Results shown are the mean from three independent experiments.

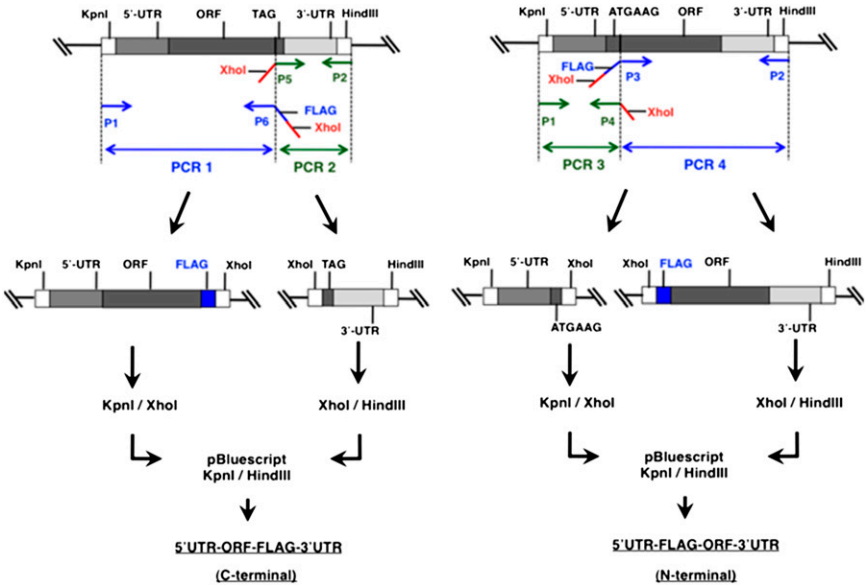

Fig. S6. Schematic details of the generation of C-terminal and N-terminal FLAG-tagged plasmid constructs. To determine the 5′ and 3′ UTR sequences of *crygm2d7* unambiguously, 5′ and 3′ RACE reactions were performed using the GeneRacer Kit with the SuperScript III RT and TOPO TA cloning kit for sequencing (Invitrogen). The gene-specific primers (GSP) used are given in the primer list. We first generated a full-length clone flanked by HindIII and KpnI restriction sites. Using this template *crygm2d7* cDNA, PCR was performed with forward and reverse primers P1 and P2, respectively. The cDNA (5′ UTR-ORF-3′ UTR) was cloned into the TOPO vector. The P1 and P2 primers contain internal restriction enzyme sites KpnI and HindIII, respectively; the sequences are given in the primer list. To introduce a C-terminal FLAG tag between the *crygm2d7* ORF and the 3′ UTR (5′ UTR-ORF-FLAG-3′ UTR), two separate PCR reactions were carried out using the *crygm2d* 5′ UTR-ORF-3′ UTR as the template and two different forward and reverse primer sets, PCR1 using primers P1 and P6, and PCR2 using primers P5 and P2 (primers given in primer list). P6 contains the FLAG tag and a XhoI restriction enzyme site, such that when the PCR product obtained with PI and P6 is cloned into the PCRII TOPO vector, a XhoI site is present downstream of the FLAG tag. The P1, P5, and P2 primers contain KpnI, XhoI, and HindIII restriction enzyme sites, respectively. The amplified product from PCR2 also was cloned into the PCRII TOPO vector. The pPCR1 and PCR2 clones were digested with KpnI/XhoI and XhoI/HindIII, respectively. The pBluescript final destination vector also was digested with KpnI and HindIII. All three products were ligated in a single reaction, placing the FLAG tag C terminal between the *crygm2d7* ORF and the 3′ UTR. To introduce an N-terminal FLAG tag, a strategy similar to that described for the C-terminal FLAG-tagged construct was used. Primers P1–P4 are the primers used for four independent PCR reactions. The inserted FLAG tags are shown in blue. Specific restriction enzyme sites that were used for individual cloning reactions are as indicated.

Table S1. Summary of RNA-seq analyses

Sample	Source	Type of RNA	RIN value	Ratio [28S/18S]	Total no. of reads	Average read length	% alignment to zebrafish genome
1	WT, 1dpf	Polysomal RNA	7.9	2.3	30,159,166	36	81.5
2	*eif3ha*, 1dpf	Polysomal RNA	7.7	2.0	30,234,137	36	82.47
3	WT, 1dpf	Total RNA	8.9	2.3	39,815,810	36	79.58
4	*eif3ha*, 1dpf	Total RNA	9.0	2.5	38,347,128	36	79.41

Table S2. TS change = $[(WT/MO)_{poly}/(WT/MO)_{total}]$

Gene name	Ensemble no.	TS change (RNA-seq)	TS change (qPCR-1)	TS change (qPCR-2)	TS change (qPCR-3)	Mean TS change (qPCR)
cu855779.1	ENSDARG00000012388	69.4	24.6	15.1	5.2	14.9
zgc:101859	ENSDARG00000014803	15.1	16.5	18.1	22.3	18.9
bscl2	ENSDARG00000025912	15.4	14.4	9.4	18.2	14.0
slc25a4	ENSDARG00000027355	33.1	25.2	40.2	23.3	37.9
atp2a1l	ENSDARG00000035458	19.4	9.6	12.9	11.8	11.4
pvalb1	ENSDARG00000037789	30.7	22.8	19.9	18.3	20.3
hspb2	ENSDARG00000052450	29.8	22.2	33.5	50.1	35.2
crygm2d5	ENSDARG00000069792	45.8	35.0	20.9	60.2	38.7
crygm2d8	ENSDARG00000069817	17.4	10.6	35.5	29.2	25.1
crygm2d11	ENSDARG00000069827	29.5	11.0	17.8	10.2	13
crygm2d9	ENSDARG00000073750	59.6	41.7	35.2	25.6	34.1
si:ch211-212n6.8	ENSDARG00000076693	58.2	34.3	93.1	85.5	70.9
and2	ENSDARG00000079302	24.4	12.9	8.9	10.3	10.7
si:ch211-212n6.16	ENSDARG00000086658	72.4	16.8	16.9	47.3	27
zgc:86723	ENSDARG00000086912	57.9	15.2	53.7	70.2	46.3
si:ch211-212n6.18	ENSDARG00000086917	47.2	21.8	28.8	22.7	24.4
si:ch211-212n6.17	ENSDARG00000087765	18.6	11.1	25.9	61.2	32.7
fgl1	ENSDARG00000087772	19.9	13.5	37.8	42.1	31.13
zgc:165347	ENSDARG00000088687	137.1	24.8	90.2	87.9	67.6
slc18A3 (1 of 2)	ENSDARG00000090189	72.0	40.6	85.8	28.8	51.7
si:dkey-17e16.15	ENSDARG00000094217	19.9	2.2	8.9	5.2	5.4
crygmxl2	ENSDARG00000074001	136.6	40.2	97.8	30.0	56.0
crygm2d13	ENSDARG00000057460	76.7	28.4	35.9	28.8	31.0
and3	ENSDARG00000056873	31.9	23.7	17.8	10.8	17.4
mipb	ENSDARG00000013963	18.9	7.6	3.9	20.6	10.7
slc35a5	ENSDARG00000000001	0.98	1.6	0.8	0.48	0.96
dap	ENSDARG00000000069	0.99	1.5	1.3	0.48	1.09
hoxb2a	ENSDARG00000000175	0.87	1.4	0.3	0.5	0.73
kifc1	ENSDARG00000001558	0.84	1.6	0.48	0.32	0.8
cwf19l1	ENSDARG00000002128	0.86	1.5	0.5	3.2	1.7

Validation data (qPCR) for the transcripts lost from polysomes (first 25, black) and control unchanged transcripts (last five, gray). TS change = Change in translation state; $(WT/MO)_{poly}$ = Change in polysomal abundance for individual mRNAs; $(WT/MO)_{total}$ = Change in abundance of individual mRNAs in total RNA. Gray font color represents mRNAs that do not show significant change of TS.

Other Supporting Information Files

Dataset S1 (XLS)