

Supplementary Methods

DNA Constructs, RNA Synthesis, and Zebrafish Lines. An N-terminal Avi-EGFP fusion construct was generated by PCR primer extension and cloned into pcGlobin (Ro et al., 2004) using BamHI and XhoI restriction sites. *rpl10a* (Fang et al., 2013) was cloned into the pcGlobin-N-Avi-EGFP vector. mRNA was generated using the mMessage mMachin kit (Invitrogen) and injected into one-cell stage zebrafish embryos. *Avi-EGFP-rpl10a* was sub-cloned into pT2KXIG in (Urasaki et al., 2006) to generate pT2KXIG in-MCS-Avi-EGFP-rpl10a (addgene.org/58380, Fig. S5). A 4 kb fragment of the *actc1b* promoter (Higashijima et al., 1997) was cloned by PCR into the pT2KXIG in-MCS-Avi-EGFP-rpl10a construct and used to generate the line *Tg(actc1b:Avi-EGFP-rpl10a)^{s973}*. *birA* (addgene.org/20857) was fused by PCR to EGFP and sub-cloned into pT2KXIG in to generate pT2KXIG in-MCS-BirA-EGFP (addgene.org/58377, Fig. S5). tagRFP was subcloned in place of EGFP to generate pT2KXIG in-MCS-BirA-tagRFP (addgene.org/58378, Fig. S5) The *ubb* promoter (addgene.org/27320) was sub-cloned into pT2KXIG in-MCS-BirA-EGFP to generate the line *Tg(ubb:birA-EGFP)^{s972}*.

Mass Spectrometry and Protein Identification. Peptides were analyzed by using nano-LC-ESI-MS/MS maXis Impact UHR-TOF (Bruker, Bremen, Germany) coupled with a 2D-LC Dionex UltiMate 3000 (Thermo). Spots were excised manually and proteins were digested with trypsin by in-gel digestion. The gel pieces were washed twice with distilled water and then shrunk with 100 % acetonitrile. The proteolytic digestion was performed by the addition of 8 μ l of modified trypsin (Promega) suspended in 50 mM NH_4HCO_3 cold buffer. Proteolysis was performed overnight at 37°C. The supernatant was collected and the eluates were kept at – 20°C prior to analysis.

The digests were separated by reverse-phase liquid chromatography using a 75 μ m X 150 mm reverse phase Thermo column (Acclaim PepMap 100 C18) in an Ultimate 3000 liquid chromatography system. Mobile phase A was 95 % of 0.1 % formic acid in water and 5 % acetonitrile. Mobile phase B was 0.1 % formic acid in acetonitrile. The digest (15 μ l) was injected, and the organic content of the mobile phase was increased linearly from 5 % B to 40 % B in 25 min and from 40 % B to 100 % B in 5min. The column effluent was connected to a Captive Spray (Bruker). In survey scan, MS spectra were acquired for 0.5 s in the m/z range between 50 and 2200. The 10 most intense peptides ions 2+ or 3+ were sequenced. The collision-induced dissociation (CID) energy was automatically set according to mass to charge (m/z) ratio and charge state of the precursor ion. MaXis and Thermo systems were piloted by Compass HyStar 3.2 (Bruker).

Peak lists were created using DataAnalysis 4.0 (Bruker) and saved as an XML file for use with ProteinScape 3.1 (Bruker) with Mascot 2.4 as the search engine (Matrix Science). Enzyme specificity was set to trypsin, and the maximum number of missed cleavages per peptide was set at one. Carbamidomethylation was allowed as fixed modification, oxidation of methionine and Gln – pyro-Glu were allowed as variable modification. Mass tolerance for monoisotopic peptide window was 5 ppm and MS/MS tolerance window was set to 0.05 Da. The peak lists were searched against the NCBI nr database.

Antibodies, Staining, Blotting, Gel Shift Assays, and Protein Purification. Anti-GFP antibodies (Aves Lab), anti-Tubb (Abcam), anti-Rpl7a (Genetex), and streptavidin-HRP (Pierce) were used in blotting experiments. Streptavidin gel shift assays were performed

by lysing 10 embryos in Laemmli buffer, boiling for 5 minutes, then incubating with 30 µg streptavidin (Sigma) prior to SDS-PAGE and anti-GFP blotting. Protein Staining was done with Sypro Ruby and visualized on a blue light trans-illuminator (Invitrogen) or using Coomassie Blue G-250 (Bio-Rad) as indicated. Avi-EGFP was purified by harvesting dechorionated and deyolked embryos, boiling in 1% SDS, and diluting samples with PBS. Streptavidin agarose was then incubated with samples for 30 minutes at room temperature after which the resin was washed twice and boiled with Laemmli buffer containing 1mM biotin for 10 minutes prior to SDS-PAGE. Whole-mount streptavidin staining was performed on PFA-fixed (4%, overnight at 4°C) embryos at 24 hpf. Embryos were permeabilized with proteinase K (10 µg/ml for 5 minutes), and fixed again with 4% PFA for 15 min in PBS-Tween 20 (0.1%). After PBS washes, embryos were blocked in PBS with 0.3% Triton X-100 and 4% BSA for 2 hours at room temperature. Staining was performed in blocking buffer with streptavidin-Alexa Fluor 647 (2 µg/ml, Life Technologies) overnight at 4°C followed by washes with PBS 0.3% Triton X-100.

RT-PCR and Microarray Profiling. For RT-PCR, cDNA was generated from equal amounts of total RNA using Maxima reagents (Thermo Scientific) and qPCR analysis was performed on an Illumina Eco system using SYBR Green (Thermo Scientific). Primer pairs are listed in Table S3. For qPCR, 3 technical replicates were performed for each TRAP experiment and averaged. To determine relative enrichment, fold differences between bound mRNA and input mRNA were calculated by subtracting threshold values and adjusting for amounts added to the reverse transcription reaction. Fold enrichment was then normalized to *act1b*. Two-color labeling and microarray hybridization was performed by Mogene using Zebrafish V3 44K gene expression arrays (Agilent Technologies) with 50 ng of RNA purified by TRAP. Amplification was performed with the NuGEN Pico Amp kit. Data were acquired using Agilent Feature Extraction with LOWESS dye normalization. Normalization across arrays was done using GeneSpring 11.5 default values. The data have been deposited at NCBI (GEO GSE59355).

References

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- Higashijima, S., Okamoto, H., Ueno, N., Hotta, Y. and Eguchi, G.** (1997). High-Frequency Generation of Transgenic Zebrafish Which Reliably Express GFP in Whole Muscles or the Whole Body by Using Promoters of Zebrafish Origin. *Dev. Biol.* **192**, 289–299.
- Ro, H., Soun, K., Kim, E.-J. and Rhee, M.** (2004). Novel vector systems optimized for injecting in vitro-synthesized mRNA into zebrafish embryos. *Mol. Cells* **17**, 373–376.
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Fig. S1

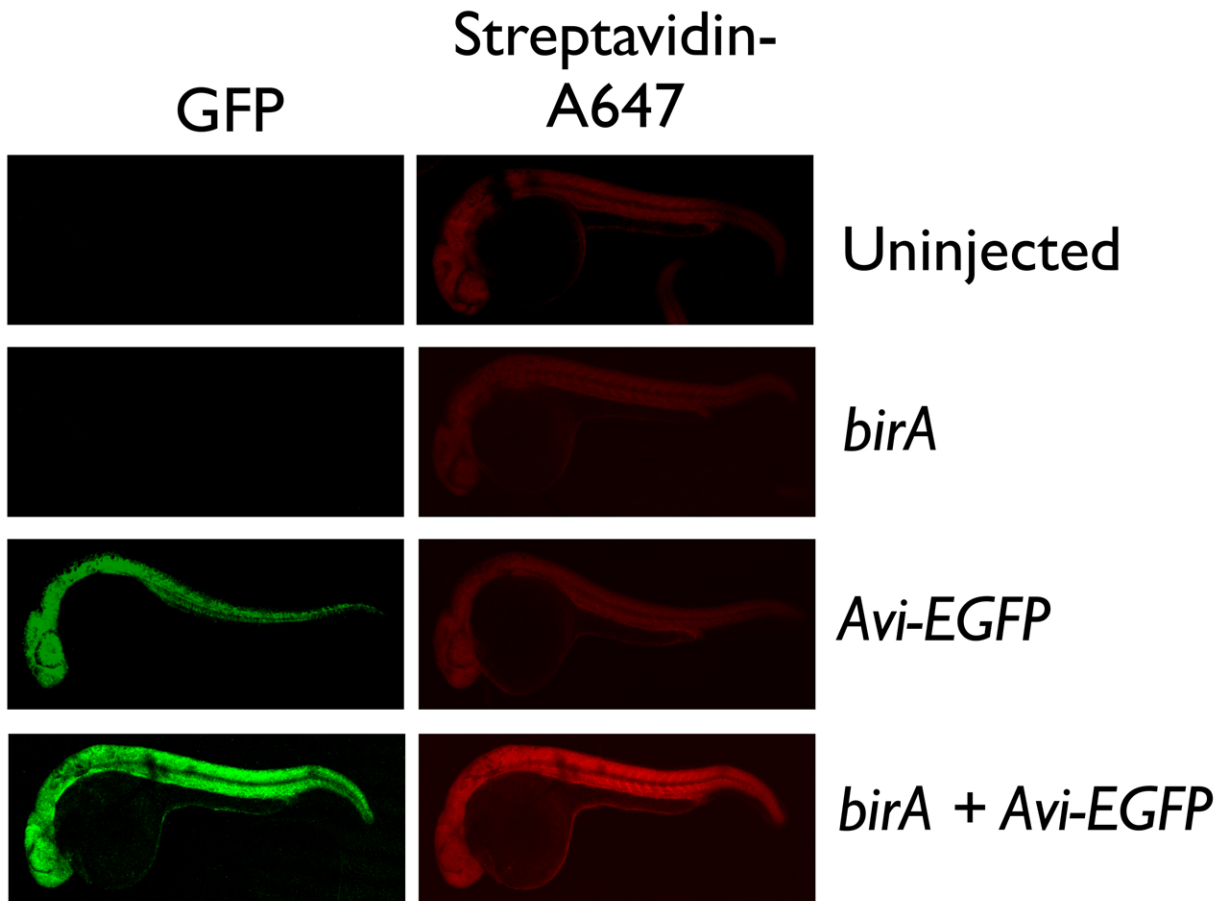


Fig. S1. Streptavidin-conjugated Alexa Fluor 647 staining of embryos expressing Avi-tagged EGFP. 24 hpf embryos were injected with either *BirA*, *Avi-EGFP*, or both *birA* and *Avi-EGFP* mRNA and stained with streptavidin-Alexa Fluor 647.

Fig. S2

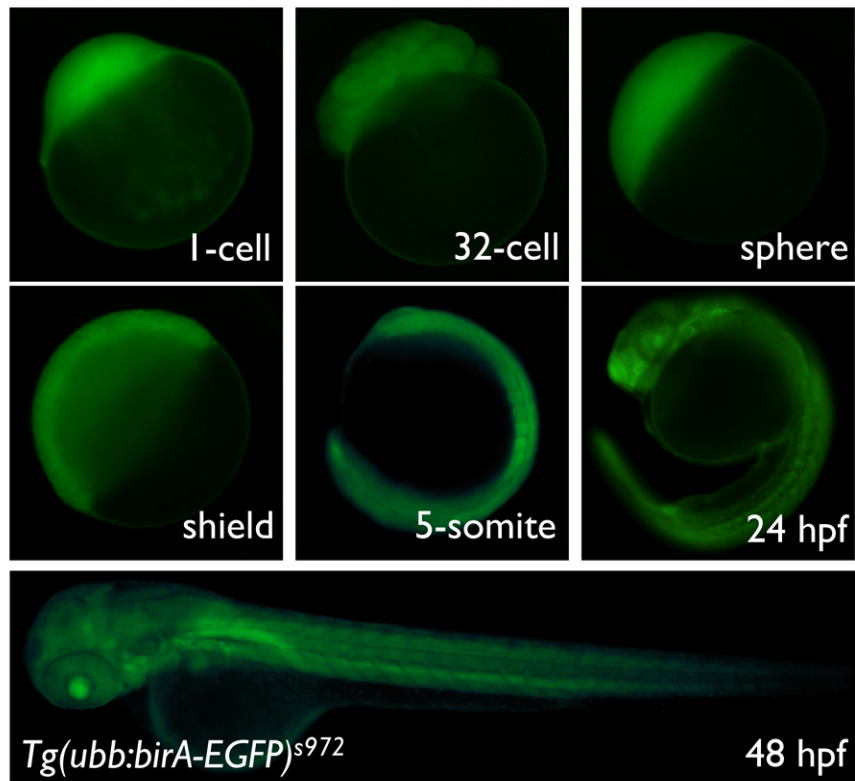


Fig. S2. Fluorescence images of *Tg(ubb:birA-EGFP)* expression at the indicated developmental stages.

Fig. S3

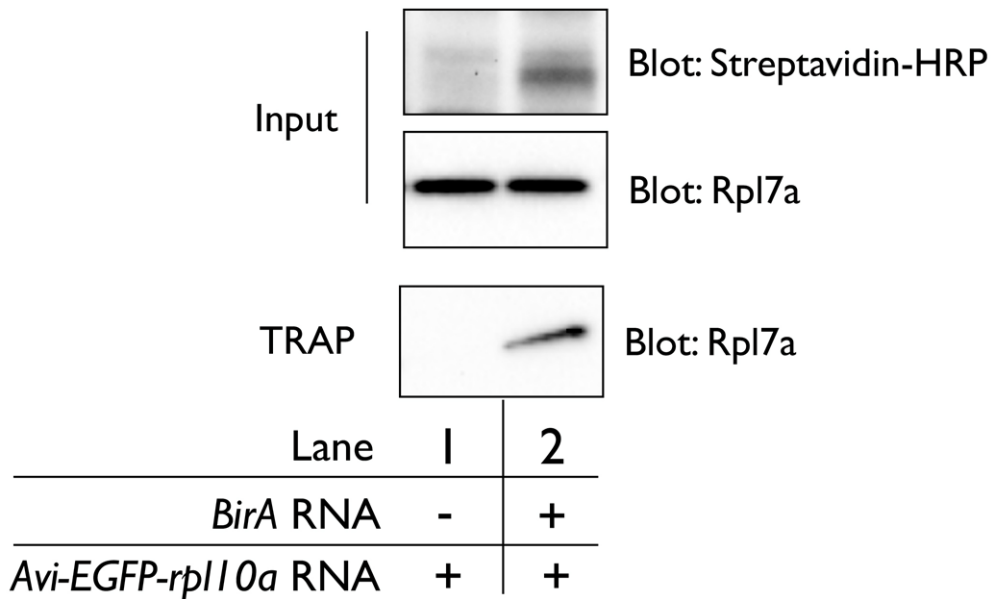


Fig. S3. Rpl7a immunoblotting of TRAP samples. Embryos injected with RNA for either *Avi-EGFP-rpl10a* or both *BirA* and *Avi-EGFP-rpl10a* were harvested at 24 hpf. Following TRAP, samples were subjected to SDS-PAGE, and analyzed by immunoblotting with anti-Rpl7a antibodies. Input samples for the TRAP were also blotted with anti-Rpl7a antibodies and streptavidin-HRP to confirm biotinylation.

Fig. S4

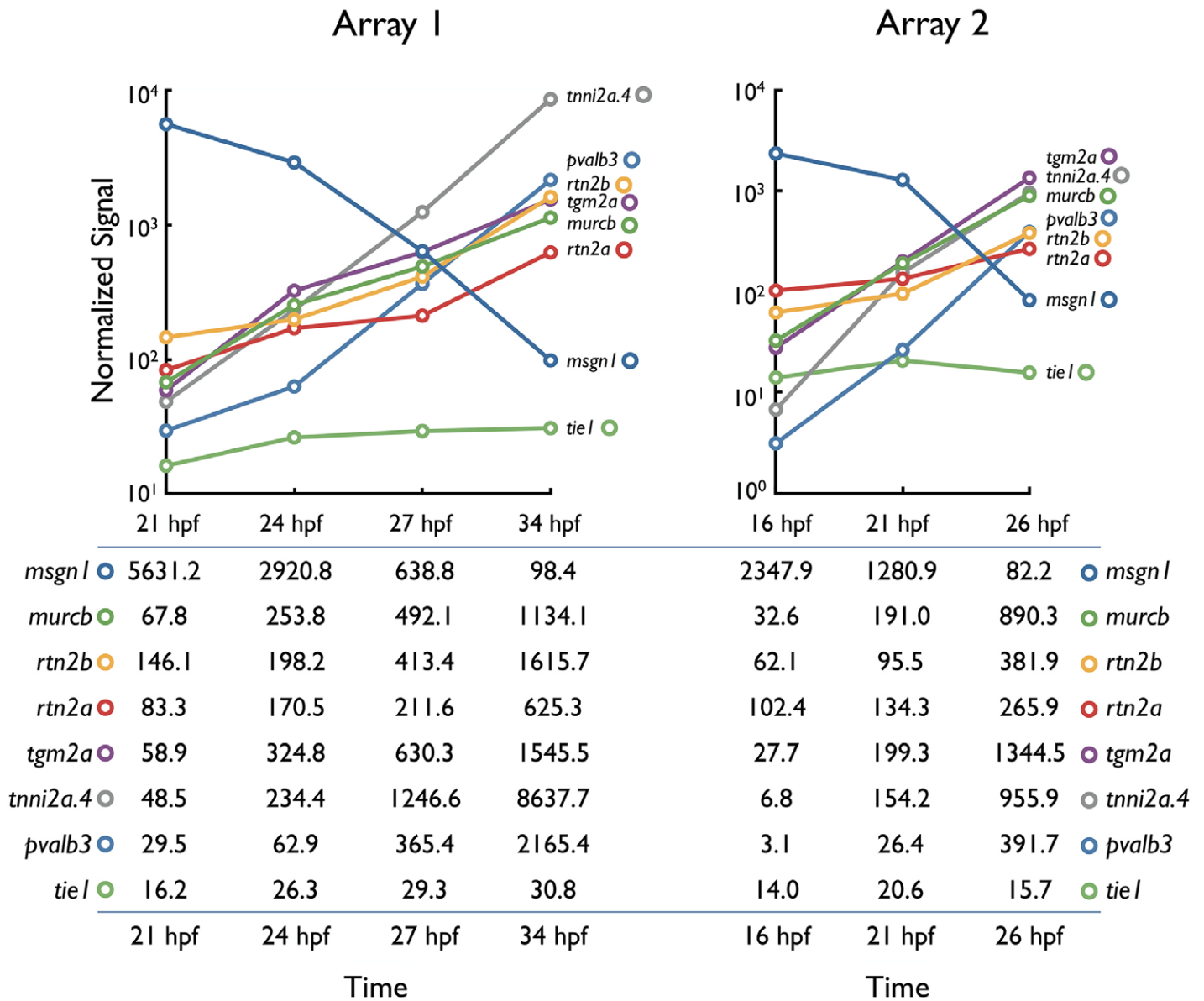


Fig. S4. Microarray expression data from two skeletal muscle TRAP time courses. Relative mRNA expression data from selected genes (log scale, y-axis) from skeletal muscle-specific TRAP (*Tg(ubb:birA-EGFP)^{s972}* crossed to *Tg(actc1b:Avi-EGFP-rpl10a)^{s973}*) assayed by microarray hybridization. Array 1 is reproduced from Fig. 4B. Array 2 represents expression from a second time course, TRAP, and microarray experiment. *tie1* is an endothelial-specific gene and is minimally expressed. *mesogenin (msgn1)* is an example of a gene whose expression is down-regulated during skeletal muscle development.

Fig. S5. Combinatorial Biotin Ligase - Avi-tag TRAP Protocol

Clone promoters of interest into TRAP vectors and generate transgenic lines

Image transgenic lines to confirm expression of BirA and Avi-EGFP-Rpl10a

Cross Tg(promoter a:birA-EGFP) to Tg(promoter b:Avi-EGFP-rpl10a) fish

Collect embryos and add 50 μ M biotin to eggwater

Incubate embryos to desired developmental stage, then wash out biotin

Collect and lyse embryos in polysome buffer

Bind labeled polysomes to streptavidin agarose

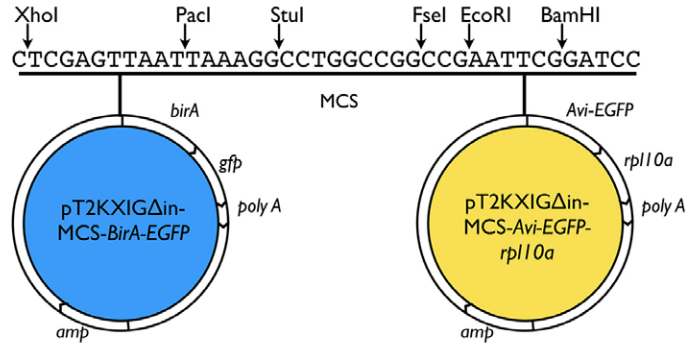
Transfer beads to column and wash 3 times

Elute RNA and ribosomal proteins

Extract RNA with Trizol

Purify and concentrate RNA on a micro spin column

Elute RNA and measure concentration



Dissolve biotin at 50 mM in DMSO then add to eggwater at a final concentration of 50 μ M. Change biotin-containing eggwater every 24 hours. Always make a fresh biotin solution and we recommend storing powdered biotin under inert gas.

Incubate embryos until desired developmental stage is reached. Wash away unincorporated biotin which would block streptavidin binding sites on the column by first anesthetizing fish, collecting them in a strainer, rinsing with egg water, and transferring them to a new dish containing fresh egg water. Incubate fish for 30 minutes. Prepare polysome buffer and streptavidin agarose columns or dry ice to snap freezing samples. Prepare cycloheximide fresh each time by dissolving 10 mg/ml in DMSO. Store under inert gas.

Label a clean, RNase-free, 1.5ml tube for each sample and add 100 μ l streptavidin agarose slurry. Add 1 ml polysome buffer to equilibrate the column. Incubate at room temperature for 10 minutes, pellet beads, remove supernatant, and place tubes on ice.

Collect embryos into a 1.5 ml tube, place on ice, wash once in cold Ringer's solution, and pellet embryos. Tail bud and younger embryos should be de-yolked by gentle pipetting with a p200, followed by pelleting of cells, and one wash in Ringer's. Snap freeze on dry ice or continue with the TRAP.

Lyse fish in 0.5 ml ice-cold polysome buffer with the aid of a clean pestle. Incubate on ice for 10 minutes then centrifuge at 4 $^{\circ}$ C for 5 min at 8000 g.

Remove 20 μ l of the supernatant and add to 1 ml Trizol for the input sample, if desired. Add remaining supernatant to polysome buffer equilibrated beads. Gently rotate at 4 $^{\circ}$ C for one hour. Prepare Wash and Elution Buffers.

Apply beads to an empty column and drain by gravity flow. Wash 3 times with 1 ml ice cold wash buffer.

Place spin column in a clean 1.5 ml tube. Elute RNA with 100 μ l elution buffer. Elute a second time with 100 μ l. Immediately add 1 ml Trizol to eluate.

Extract RNA by adding 200 μ l chloroform. Shake tubes and centrifuge at 12,000 g for 10 min at 4 $^{\circ}$ C. Prepare binding buffer for Zymo Clean & Concentrate RNA columns. Remove aqueous phase to a fresh 2 ml tube*.

Add two volumes of Binding Buffer per volume of aqueous phase. Mix. Add to the RNA column in multiple, successive loads. Wash column 3 times with 0.7 ml 80% ethanol 10 mM Tris pH 7.5*. With each wash, cap and invert column several times. Centrifuge 30 seconds.

Dry column by centrifugation. Transfer the column to a fresh tube and dry again.

Transfer the column to a fresh tube. Elute twice with 6 μ l water.

Notes:

Use clean, RNase-free tubes and barrier pipette tips and solutions.
*Deviates from manufacturer's recommendations
Catalog Numbers:
Pestles: Axygen PES-15-B-SI
Empty columns: BioRad 732-6204
RNA columns: Zymo R1016
Cycloheximide: Cell Signaling 2112s
Biotin: Thermo 29129
Streptavidin Agarose: Pierce 20359
RNasin: Promega N2511

Calcium free Ringer's:
116 mM NaCl
2.9 mM KCl
5.0 mM Hepes 7.2
Plasmids available from addgene.org
58377
58378
58380

Polysome Buffer:
10 mM Hepes pH 7.4
150 mM KCl
5 mM MgCl₂
1% Triton X-100
add fresh each time:
0.5 mM DTT
0.5 mM PMSF
10 units/ml RNasin
100 μ g/ml cycloheximide

Wash Buffer:
10 mM Hepes
300 mM KCl
5 mM MgCl₂
1% Triton X-100
add fresh each time:
0.5 mM DTT
0.5 mM PMSF
10 units/ml RNasin
100 μ g/ml cycloheximide

Elution Buffer:
1 M NaCl
10 mM Tris pH 7.5

Zymo RNA Column Binding Buffer:
1 volume binding buffer concentrate
1 volume 95% ethanol
(0.5 ml each per column is generally sufficient)

Table S1.**Mass spectrometric identification of ribosomal proteins from the skeletal-muscle specific TRAP.**

| Small Subunit Protein | Gene Name | Accession Number | Description | Mascot Score | Peptides |
|------------------------------|------------------|-------------------------|--|---------------------|-----------------|
| SA | <i>rpsa</i> | gi 41054972 | ribosomal protein SA [Danio rerio] | 376.65 | 51 |
| S2 | <i>rps2</i> | gi 47086117 | ribosomal protein S2 [Danio rerio] | 113.52 | 5 |
| S3 | <i>rps3</i> | gi 37595356 | ribosomal protein S3 [Danio rerio] | 846.98 | 138 |
| S3A | <i>rps3a</i> | gi 41152457 | ribosomal protein S3A [Danio rerio] | 832.99 | 61 |
| S4 | <i>rps4x</i> | gi 53933236 | ribosomal protein S4, X-linked [Danio rerio] | 597.65 | 57 |
| S5 | <i>rps5</i> | | | | |
| S6 | <i>rps6</i> | gi 28630207 | ribosomal protein S6 [Scyliorhinus canicula] | 84.22 | 10 |
| S7 | <i>rps7</i> | gi 41152175 | ribosomal protein S7 [Danio rerio] | 208.17 | 10 |
| S8 | <i>rps8</i> | gi 47550881 | ribosomal protein S8 [Danio rerio] | 380.39 | 41 |
| S9 | <i>rps9</i> | gi 198285475 | ribosomal protein S9 [Salmo salar] | 474.78 | 37 |
| S10 | <i>rps10</i> | gi 148235024 | 40S ribosomal protein S10 [Xenopus laevis] | 204.41 | 13 |
| S11 | <i>rps11</i> | gi 47087057 | 40S ribosomal protein S11 [Danio rerio] | 186.2 | 9 |
| S12 | <i>rps12</i> | gi 119568395 | ribosomal protein S12, isoform CRA_c [Homo sapiens] | 37.94 | 1 |
| S13 | <i>rps13</i> | gi 50344812 | 40S ribosomal protein S13 [Danio rerio] | 314.67 | 15 |
| S14 | <i>rps14</i> | gi 41152464 | ribosomal protein S14 [Danio rerio] | 365.26 | 31 |
| S15 | <i>rps15</i> | gi 12733945 | 40S ribosomal protein S15a [Platichthys flesus] | 24.85 | 1 |
| S15A | <i>rps15a</i> | gi 12733945 | 40S ribosomal protein S15a [Platichthys flesus] | 24.85 | 1 |
| S16 | <i>rps16</i> | gi 115529347 | ribosomal protein S16 [Danio rerio] | 429.71 | 21 |
| S17 | <i>rps17</i> | gi 41053565 | 40S ribosomal protein S17 [Danio rerio] | 196.17 | 13 |
| S18 | <i>rps18</i> | | | | |
| S19 | <i>rps19</i> | gi 41152179 | ribosomal protein S19 [Danio rerio] | 159.94 | 4 |
| S20 | <i>rps20</i> | gi 47086001 | 40S ribosomal protein S20 [Danio rerio] | 123.79 | 7 |
| S21 | <i>rps21</i> | gi 41055255 | 40S ribosomal protein S21 [Danio rerio] | 115.05 | 3 |
| S23 | <i>rps23</i> | | | | |
| S24 | <i>rps24</i> | gi 220678148 | ribosomal protein S24 [Danio rerio] | 243.04 | 7 |
| S25 | <i>rps25</i> | | | | |
| S26 | <i>rps26</i> | gi 12641796 | 40S ribosomal protein S26 [Platichthys flesus] | 126.68 | 17 |
| S27 | <i>rps27</i> | gi 226958509 | 40S ribosomal protein S27-like [Danio rerio] | 139.58 | 5 |
| S27A | <i>rps27a</i> | gi 226358625 | ribosomal protein S27a [Hypophthalmichthys nobilis] | 18.76 | 1 |
| S28 | <i>rps28</i> | gi 183212473 | ribosomal protein S28 [Xenopus borealis] | 56.36 | 3 |
| S29 | <i>rps29</i> | | | | |
| S30 | <i>rps30</i> | gi 149518006 | 40S ribosomal protein S30-like, partial [Ornithorhynchus anatinus] | 48.17 | 1 |
| | | | | | |
| Large Subunit Protein | Gene Name | Accession Number | Description | Mascot Score | Peptides |
| L3 | <i>rpl3</i> | gi 60688481 | Ribosomal protein L3 [Danio rerio] | 428.42 | 17 |

| | | | | | |
|------|---------------|--------------|---|--------|----|
| L4 | <i>rpl4</i> | gi 54261775 | 60S ribosomal protein L4 [Danio rerio] | 500.09 | 18 |
| L5 | <i>rpl5</i> | gi 50344868 | ribosomal protein L5 [Danio rerio] | 301.57 | 29 |
| L6 | <i>rpl6</i> | gi 62202562 | Ribosomal protein L6 [Danio rerio] | 187.71 | 24 |
| L7 | <i>rpl7</i> | | | | |
| L7A | <i>rpl7a</i> | gi 41152461 | 60S ribosomal protein L7a [Danio rerio] | 425.05 | 24 |
| L8 | <i>rpl8</i> | gi 116488054 | 60S ribosomal protein L8 [Scophthalmus maximus] | 197.92 | 14 |
| L9 | <i>rpl9</i> | gi 3088347 | ribosomal protein L9 [Homo sapiens] | 30.72 | 1 |
| L10 | <i>rpl10</i> | | | | |
| L10A | <i>rpl10a</i> | | | | |
| L11 | <i>rpl11</i> | gi 209737976 | 60S ribosomal protein L11 [Salmo salar] | 79.61 | 7 |
| L12 | <i>rpl12</i> | gi 60551102 | Ribosomal protein L12 [Danio rerio] | 260.25 | 15 |
| L13 | <i>rpl13</i> | gi 47086477 | 60S ribosomal protein L13a [Danio rerio] | 149.33 | 8 |
| L13A | <i>rpl13a</i> | gi 47086477 | 60S ribosomal protein L13a [Danio rerio] | 149.33 | 8 |
| L14 | <i>rpl14</i> | | | | |
| L15 | <i>rpl15</i> | gi 31322590 | ribosomal protein L15 [Coturnix japonica] | 97.11 | 2 |
| L17 | <i>rpl17</i> | gi 47086529 | 60S ribosomal protein L17 [Danio rerio] | 102.23 | 4 |
| L18 | <i>rpl18</i> | gi 51010947 | 60S ribosomal protein L18 [Danio rerio] | 253.34 | 18 |
| L18A | <i>rpl18a</i> | gi 41055022 | 60S ribosomal protein L18a [Danio rerio] | 178.4 | 9 |
| L19 | <i>rpl19</i> | | | | |
| L21 | <i>rpl21</i> | gi 50344966 | ribosomal protein L21 [Danio rerio] | 119.57 | 6 |
| L22 | <i>rpl22</i> | gi 83415112 | 60S ribosomal protein L22 [Danio rerio] | 337.05 | 23 |
| L23 | <i>rpl23</i> | gi 41282078 | 60S ribosomal protein L23 [Danio rerio] | 58.7 | 3 |
| L23A | <i>rpl23a</i> | | | | |
| L24 | <i>rpl24</i> | gi 318101831 | 60S ribosomal protein L24 [Ictalurus punctatus] | 179.63 | 14 |
| L26 | <i>rpl26</i> | gi 47085861 | 60S ribosomal protein L26 [Danio rerio] | 159.06 | 9 |
| L27 | <i>rpl27</i> | gi 41054351 | 60S ribosomal protein L27 [Danio rerio] | | |
| L27A | <i>rpl27a</i> | gi 41053327 | 60S ribosomal protein L27a [Danio rerio] | 89.77 | 8 |
| L28 | <i>rpl28</i> | gi 41055030 | ribosomal protein L28-like [Danio rerio] | 79.55 | 3 |
| L29 | <i>rpl29</i> | | | | |
| L30 | <i>rpl30</i> | gi 119612175 | ribosomal protein L30, isoform CRA_a [Homo sapiens] | 163.07 | 12 |
| L31 | <i>rpl31</i> | | | | |
| L32 | <i>rpl32</i> | | | | |
| L34 | <i>rpl34</i> | | | | |
| L35 | <i>rpl35</i> | | | | |
| L35A | <i>rpl35a</i> | | | | |
| L36 | <i>rpl36</i> | gi 47086131 | 60S ribosomal protein L36 [Danio rerio] | 101.43 | 8 |
| L36A | <i>rpl36a</i> | | | | |
| L37 | <i>rpl37</i> | | | | |
| L37A | <i>rpl37a</i> | | | | |
| L38 | <i>rpl38</i> | | | | |
| L39 | <i>rpl39</i> | | | | |
| L40 | <i>rpl40</i> | | | | |
| L41 | <i>rpl41</i> | | | | |
| LP0 | <i>rplp0</i> | | | | |
| LP1 | <i>rplp1</i> | | | | |
| LP2 | <i>rplp2</i> | gi 154426308 | 60S acidic ribosomal protein P2 [Danio rerio] | 283.67 | 36 |

Table S2.
Detailed information on genes from skeletal muscle TRAP highlighted in Fig. 4.

| Gene Symbol | Gene Name | 21 hpf | 34 hpf | Fold Change | zfin link |
|-----------------|--|--------|---------|-------------|---|
| <i>mespaa</i> | <i>mesoderm posterior aa</i> | 203.1 | 1.2 | 166.9 | http://zfin.org/ZDB-GENE-000406-8 |
| <i>her7</i> | <i>hairy and enhancer of split related-7</i> | 110 | 1.5 | 74.7 | http://zfin.org/ZDB-GENE-000427-6 |
| <i>cdx1a</i> | <i>caudal type homeo box transcription factor 1 a</i> | 114.6 | 1.7 | 68.8 | http://zfin.org/ZDB-GENE-050510-1 |
| <i>msgn1</i> | <i>mesogenin 1</i> | 5631.2 | 98.4 | 57.2 | http://zfin.org/ZDB-GENE-030722-1 |
| <i>tbx16</i> | <i>T-box gene 16</i> | 692.4 | 15.3 | 45.4 | http://zfin.org/ZDB-GENE-990615-5 |
| <i>rippy2</i> | <i>rippy2</i> | 203 | 4.9 | 41.6 | http://zfin.org/ZDB-GENE-060113-2 |
| <i>lft1</i> | <i>lefty1</i> | 668.3 | 24.6 | 27.2 | http://zfin.org/ZDB-GENE-990630-10 |
| <i>her1</i> | <i>hairy-related 1</i> | 207.1 | 8.3 | 25.1 | http://zfin.org/ZDB-GENE-980526-125 |
| <i>her5</i> | <i>hairy-related 5</i> | 136.7 | 7.3 | 18.7 | http://zfin.org/ZDB-GENE-990415-90 |
| <i>cdx4</i> | <i>caudal type homeo box transcription factor 4</i> | 2127.1 | 124.3 | 17.1 | http://zfin.org/ZDB-GENE-980526-330 |
| <i>wnt8a</i> | <i>wingless-type MMTV integration site family, member 8a</i> | 146.4 | 10.2 | 14.3 | http://zfin.org/ZDB-GENE-980526-332 |
| <i>tbx6</i> | <i>T-box gene 6</i> | 965.4 | 70.6 | 13.7 | http://zfin.org/ZDB-GENE-020416-5 |
| <i>ved</i> | <i>ventrally expressed dharma/bozozok antagonist</i> | 3285.3 | 280.5 | 11.7 | http://zfin.org/ZDB-GENE-030813-1 |
| <i>rtn2a</i> | <i>reticulon 2a</i> | 83.3 | 625.3 | 7.5 | http://zfin.org/ZDB-GENE-060420-1 |
| <i>rtn2b</i> | <i>reticulon 2b</i> | 146.2 | 1615.7 | 11.1 | http://zfin.org/ZDB-GENE-060331-95 |
| <i>acta1b</i> | <i>actin, alpha 1b, skeletal muscle</i> | 2872.6 | 47773.2 | 16.6 | http://zfin.org/ZDB-GENE-030131-55 |
| <i>murc</i> | <i>muscle-related coiled-coil protein b</i> | 67.8 | 1134.1 | 16.7 | http://zfin.org/ZDB-GENE-041212-87 |
| <i>ckmb</i> | <i>creatine kinase, muscle b</i> | 1368.8 | 26470.4 | 19.3 | http://zfin.org/ZDB-GENE-040426-2128 |
| <i>myhz2</i> | <i>myosin, heavy polypeptide 2, fast muscle specific</i> | 609 | 12955.6 | 21.3 | http://zfin.org/ZDB-GENE-020604-1 |
| <i>myoz1b</i> | <i>myozenin 1b</i> | 176.6 | 3830.3 | 21.7 | http://zfin.org/ZDB-GENE-040718-146 |
| <i>atp2a1</i> | <i>ATPase, Ca⁺⁺ transporting, cardiac muscle, fast turnover</i> | 2180.5 | 48618.4 | 22.3 | http://zfin.org/ZDB-GENE-020905-1 |
| <i>tgm2a</i> | <i>transglutaminase 2, C polypeptide A</i> | 58.9 | 1545.5 | 26.2 | http://zfin.org/ZDB-GENE-040912-78 |
| <i>mylpfb</i> | <i>myosin light chain, phosphorylatable, fast skeletal muscle</i> | 1147.7 | 36526.4 | 31.8 | http://zfin.org/ZDB-GENE-040912-115 |
| <i>pvalb3</i> | <i>parvalbumin 3</i> | 29.5 | 2165.4 | 73.5 | http://zfin.org/ZDB-GENE-040426-945 |
| <i>tnnt3a</i> | <i>troponin T3a, skeletal, fast</i> | 42.2 | 3865.7 | 91.7 | http://zfin.org/ZDB-GENE-000322-3 |
| <i>tnni2a.4</i> | <i>troponin I, skeletal, fast 2a, tandem duplicate 4</i> | 48.5 | 8637.8 | 178 | http://zfin.org/ZDB-GENE-040625-119 |

Table S3.
Oligonucleotides used to generate in situ hybridization probes and as qPCR primers.

| Gene Symbol | Gene Name | Sequence |
|-------------------------------------|--|--|
| <i>in situ</i> probe primers | | |
| <i>tmg2a</i> | <i>transglutaminase 2, C polypeptide A</i> | 5'-AGTGACCCCAACACCAGCTGAAGACG 5'-TAGAAGCGCTCCATTATTAGGTCGCC |
| <i>rtn2a</i> | <i>reticulon 2a</i> | 5'-ATGTGGAGAAATGTGGAGCTCACTG 5'-GAAACAAGTCACTTTAAAACAAGTCAT |
| <i>rtn2b</i> | <i>reticulon 2b</i> | 5'-GTTTCAGAGATCAGGGACACATAG 5'-TTTTACAGATGAAATGTTAAAACAG |
| <i>murca</i> | <i>muscle-related coiled-coil protein a</i> | 5'-TCTCAAAGTGGCTCGTGACCACGG 5'-CATATTGAGTTTTTTTTAATACAT |
| <i>murcb</i> | <i>muscle-related coiled-coil protein b</i> | 5'-GCTTAAGCAGGCCTGACAGCCAC 5'-GCGGCGCGATTACGCAAATGAAG |
| qPCR primers | | |
| <i>actb1</i> | <i>actin, beta 1</i> | 5'-CGAGCAGGAGATGGGAACC 5'-CAACGGAAACGCTCATTGC |
| <i>tie1</i> | <i>endothelium-specific receptor tyrosine kinase 1</i> | 5'-AATTAATGCTCAAGGAGTTCGCC 5'-AGGCTGGATCTGTCTCTAGGACTCG |
| <i>cmlc2 (myl7)</i> | <i>myosin, light polypeptide 7, regulatory</i> | 5'-GCCCATAACTTCACTGTCTTCC 5'-CTGGTCAACCTCTTCTGCTG |
| <i>pax7a</i> | <i>paired box gene 7a</i> | 5'-GGCTACTTTACCAGGAACAG 5'-AGGTGTTGAGACTTCTAATGGG |
| <i>pax7b</i> | <i>paired box gene 7b</i> | 5'-AGTTCCTCAGTAAGTTCTATCAG 5'-CCTTTGTCTCCAGAATGCC |
| <i>efnb3b</i> | <i>ephrin B3b</i> | 5'-CTATAAACTGTACCTGGTTTCGTC 5'-GACCATATGGACTCTGTCCC |
| <i>myod1</i> | <i>myogenic differentiation 1</i> | 5'-AAACTACCAATGCTGACCGT 5'-AAATCCATCATGCCATCAGAG |
| <i>myog</i> | <i>myogenin</i> | 5'-TCAGTTCCTCAACCAGCAG 5'-ATCACTAGAGGACGACACCC |
| <i>myf5</i> | <i>myogenic factor 5</i> | 5'-GAACTACTACAGCCTGCCGA 5'-CCGCAGGATCTACAGAGGAC |
| <i>myf6</i> | <i>myogenic factor 6</i> | 5'-CTGACCCTTACACATACAACCTG 5'-ACACGGCTCCTTCTCTATGAC |
| <i>myl1</i> | <i>myosin, light chain 1, alkali; skeletal, fast</i> | 5'-TCCGGTGTGAAGCTCGATTCACCC 5'-CATAGGCAGGAAACCCTCAAAGTCC |