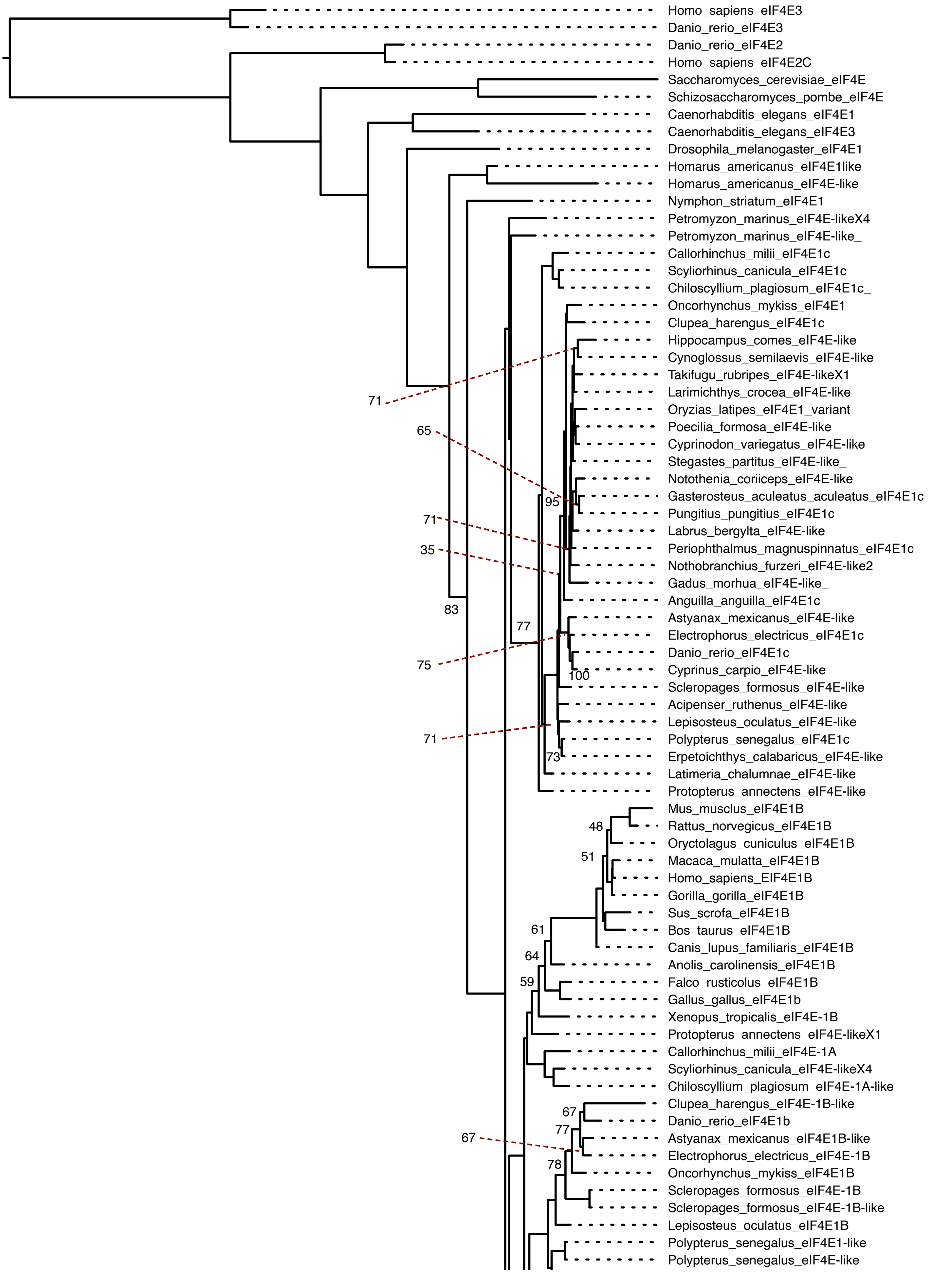
Figure S1A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Schizosaccharomyces_pombe_eIF4E
Caenorhabditis_elegans_eIF4E1
Caenorhabditis_elegans_eIF4E3
Drosophila_melanogaster_eIF4E1
Homarus_americanus_eIF4E1like
Homarus_americanus_eIF4E-like
Nymphon_striatum_eIF4E1
Petromyzon_marinus_eIF4E-likeX4
Petromyzon_marinus_eIF4E-like_
Callorhinchus_milii_eIF4E1c
Scyliorhinus_canicula_eIF4E1c
Chiloscyllium_plagiosum_eIF4E1c_
Oncorhynchus_mykiss_eIF4E1
Clupea_harengus_eIF4E1c
Hippocampus_comes_eIF4E-like
Cynoglossus_semilaevis_eIF4E-like
Takifugu_rubripes_eIF4E-likeX1
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Notothenia_coriiceps_eIF4E-like
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Pungitius_pungitius_eIF4E1c
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Scyliorhinus_canicula_eIF4E-likeX4
Chiloscyllium_plagiosum_eIF4E-1A-like
Clupea_harengus_eIF4E-1B-like
Danio_rerio_eIF4E1b
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Electrophorus_electricus_eIF4E-1B
Oncorhynchus_mykiss_eIF4E1B
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Figure S1A continued

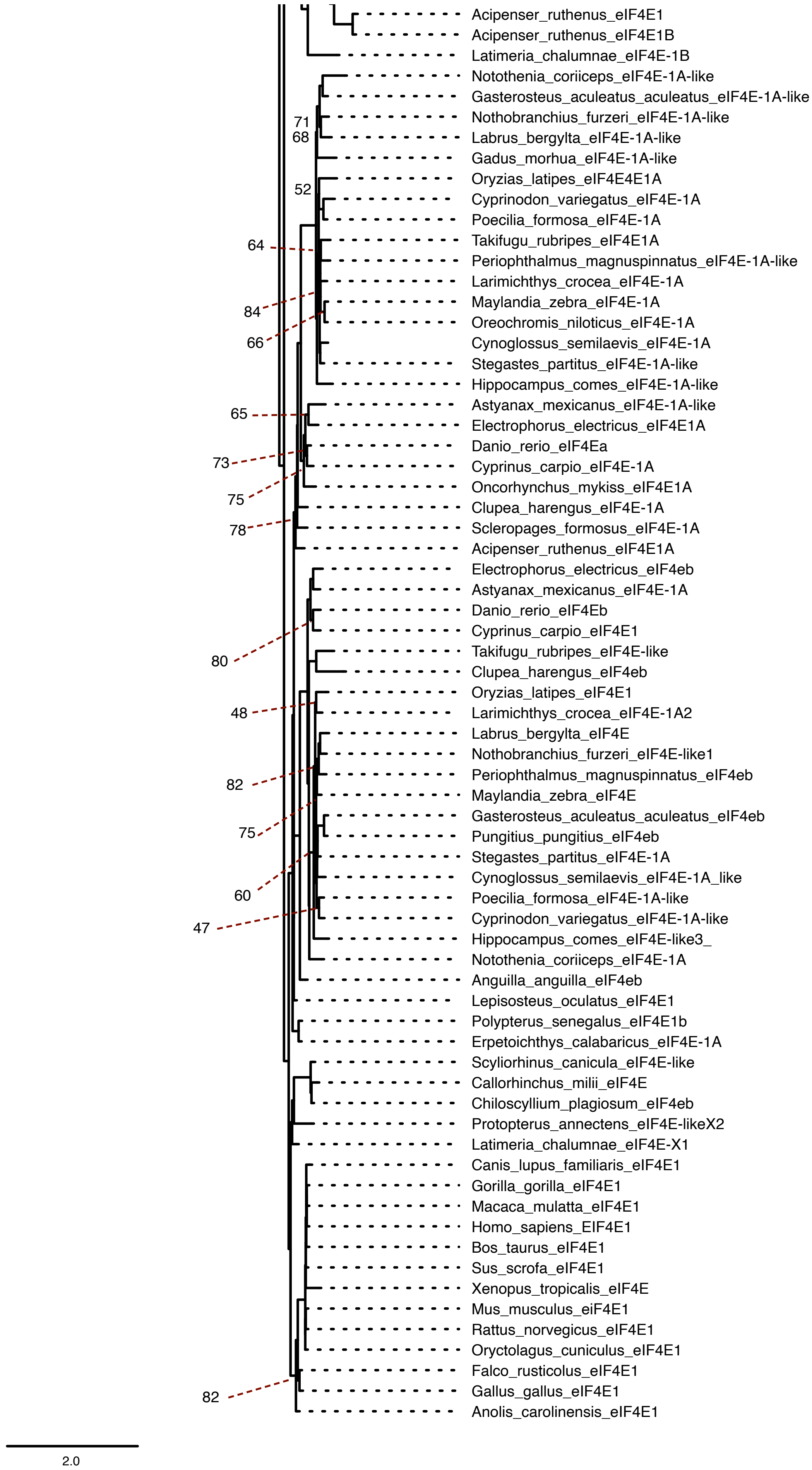

Figure S1B

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Oncorhynchus      NKWALWYFKNDKSKSWTENLRLIAKFDTVEDFWALYNHIQQPSKLGFGCDYCLFKDGVKP 98
Oryzias           NRWALWYFKNDKTKSWTENLRLISKFDTVEDFWALYNHIQQPSKLVLGCDYCLFKDGIKP 94
Gadus             NKWALWYFKNDKSKSWTENLRLISKFDTVEDFWALYNHIQQPSKLGFGCDYCLFKDGIKP 93
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Pungitius         NRWALWYFKNDKSKSWTENLRLISKFDTVEDFWALYNHIQQPSKLGYGCDYSLFKDGIKP 94
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Takifugu          NRWALWYFKNDKSKSWTENLRLISKFDTVEDFWALYNHIQQPSKLGFGCDYCLFKDGIKP 93
Labrus            NRWALWYFKNDKSKSWTENLRLISKFDTVEDFWALYNHIQQPSKLGFGCDYCLFKDGIKP 94
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Poecilia          NRWALWYFKNDKSKSWTENLRLISKFDTVEDFWALYNHIQQPSKLGFGCDYSLFKDGIKP 93
Cyprinodon        NRWALWYFKNDKSKSWTENLRLISKFDTVEDFWALYNHIQQPSKLGYGCDYSLFKDGIKP 93
Stegastes         NRWALWYFKNDKSKSWTENLRLISKFDTVEDFWALYNHIQQPSKLGFGCDYCLFKDGIKP 93
Maylandia         NRWALWYFKNDKSKSWTENLRLISKFDTVEDFWALYNHIQQPSKLGFGCDYCLFKDGIKP 93
Oreochromis       NRWALWYFKNDKSKSWTENLRLISKFDTVEDFWALYNHIQQPSKLGFGCDYCLFKDGIKP 93
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Latimeria         NKWALWYFKNDKSKSWTENLRLIAKFDTVEDFWALYNHIQQPSKLQFGCDYCLFKDGIKP 116
Clupea            NRWALWYFKNDKSKSWTENLRLIAKFDTVEDFWALYNHIQQPSKLGFGCDYCLFKDGIKP 117
Danio             NRWALWYFKNDKSKSWTENLRLISKFDTVEDFWALYNHIQQPSKLGFGCDYCLFKDGIKP 96
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Astyanax          NRWALWYFKNDKSKSWTENLRLISKFDTVEDFWALYNHIQQPSKLGFGCDYCLFKDGIKP 114
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Scleropages       NRWALWYFKNDKSKSWSENLRLIAKFDTVEDFWALYNHIQQPSKLGFGCDYCLFKDGIKP 95
Acipenser         NKWALWYFKNDKSKSWTENLRLIAKFDTVEDFWALYNHIQQPSKLGFGCDYCLFKDGIKP 95
Lepisosteus       NRWALWYFKNDKSKSWTENLRLIAKFDTVEDFWALYNHIQQPSKLGFGCDYCLFKDGIKP 95
Polypterus        NRWALWYFKNDKSKSWTENLRLIAKFDTVEDFWALYNHIQQPSKLGFGCDYCLFKDGIKP 95
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Petromyzon        NRWALWFYKNDKSKSWQANLRLITKVDTVEDFWALYNHIQVASRLMPGCDYSLFKDGIEP 102
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Sharks

Lungfish

Seahorse

Lamprey

Spotted Gar – recently diverged from teleost

Gray bichir – dragon eel – similar to lungfish

Mudskippers – technically teleost but use fins as legs

Reedfish – bichir – snake fish

Eel

Teleost

Figure S1B continued

```
Callorhinchus     MWEDDKNKKGGRWLMTLTKQQRHNDLDRYWLETLLCLIGEAFDEHSDEVCGAVVNVRPKG 163
Scyliorhinus      MWEDDKNKKGGRWLMTLNKQQRHNDLDRYWLETLLCLIGEAFDEHSDEVCGAVVNVRPKG 165
Chiloscyllium     MWEDDKNKRGGRWLMTLNKQQRHNDLDRYWLETLLCLIGEAFDEFSDEVCGAVVNVRPKG 165
Protopterus       MWEDELNKQGGRWLITLNKQQRHNDLDRYWLETLLCLIGEAFNDYSDDVCGAVVNVRPKG 159
Oncorhynchus      MWEDDKNKLGGRWLMTLSKQQRQIDLDRYWMETLLCLIGESFDEASEDVCGAVVNVRPKG 158
Oryzias           MWEDDKNKLGGRWLMTLNKQ-KHNDLDRYWMETLLCLVGESFDDASEEVCGAVVNVRHKG 153
Gadus             MWEDDRNKLGGRWLMTLNKQQRHNDLDRYWMETLLCLVGESFDESSEDVCGAVVNVRPKG 153
Notothenia        MWEDDRNKLGGRWLMTLNKQQRHNDLDRYWMETLLCLVGESFDAASEDVNGAVVNVRPKG 166
Anguilla          MWEDDRNKLGGRWLMTLNKQQRHNDLDRYWMETLLCLIGESFDEASDDVCGAVVNVRPKG 155
Gasterosteus      MWEDDRNKLGGRWLMTLNKQQRHNDLDRYWMETLLCLVGESFDEASEDVCGAVVNVRPKG 152
Pungitius         MWEDDRNKLGGRWLMTLNKQQRHNDLDRYWMETLLCLVGESFDEASEDVCGAVVNVRPKG 154
Nothobranchius    MWEDERNKLGGRWLITLNRQQRHNDLDRYWMETLLCLVGESFDEASDDVCGAVVNVRPKG 153
Takifugu          MWEDDRNKLGGRWLMTLNKQQRHNDLDRFWMETLLCLVGESFDEASDDVCGAVVNVRPKG 153
Labrus            MWEDDRNKLGGRWLMTLNKQQRHNDLDRYWMETLLCLVGESFDEASEDVCGAVVNVRPKG 154
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Cyprinodon        MWEDDRNKLGGRWLMTLNKQQRHNDLDRYWMETLLCLVGDSFDEASEDVCGAVVNVRPKG 153
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Oreochromis       MWEDDRNKLGGRWLMTLNKQQRHNDLDRYWMETLLCLVGESFDEASEDVCGAVVNVRPKG 153
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Erpetoichthys     MWEDDRNKLGGRWLITLSKQQRHNDLDRYWMETLLCLIGESFDEASEDVCGAVVNVRPKG 156
Hippocampus       MWEDDRNKLGGRWLMTLNKVQRHNDLDRYWMETLLCLVGESFDEASDDVCGAVVNVRHKA 160
Petromyzon        MWEDERNKRGGRWLITLTKTQRHSDLDRYWLETLLCLIGEAFDDHSDDVCGAVVNVRPKA 162
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Sharks
Lungfish
Seahorse
Lamprey
Spotted Gar – diverged from teleost
Gray bichir – dragon eel – appears lungfish
Mudskippers – technically teleost but use fins as legs
Reedfish – bichir – snake fish
Eel
Teleost

Figure S1B continued

```
Callorhinchus     DKISIWTGNCQSREAVTSIGQSYKERLGLPMKALIGYQSHDDTSSKSGSTTKNLYTV   220
Scyliorhinus      DKIAIWTGNCQNREAIVSIGQLYKERLGLSLKALIGYQSHDDTSSKSGSTTKNLFSV   222
Chiloscyllium     DKIAIWTGNCQNRDAVVSIGQLYKERLGLSLKALIGYQSHDDTSSKSGSTTKNLFSV   222
Protopterus       DKLSIWTTNCQNREAVVSIGQSYKERLGLPLKPVIGYQSHDDTSTKSGSTTKNLYSA   216
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Gadus             DKIAIWTSNCQNRDAIVTIGAGYKERLCLPSKPLISYQSHDDTSSKSGSTTKNMYSV   210
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Takifugu          DKIAIWTSNCQNREAIMTIGQLYKERLNIPIKAMLGYQSHDDTSSKSGSTTKNMYSI   210
Labrus            DKISIWTSNCQNRDAIMTIGQLYKERLTVPIKALIGYQSHDDTSSKSGSTTKNMYSV   211
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Poecilia          DKISIWTSNCQNREAIMTIGQLYKERLNIPIKAMIGYQSHDDTSSKSGSTTKNMYSV   210
Cyprinodon        DKISIWTSNCQNREAIMTIGQLYKERLNLPIKAMIGYQSHDDTSSKSGSTTKNMYSV   210
Stegastes         DKIAIWTSNCQNREAIMTIGQLYKERLNLPIKAMIGYQSHDDTSSKSGSTTKNMYSV   210
Maylandia         DKISIWTSNCQNRDAIMTIGQLYKERLNLPMKAMIGYQSHDDTSSKSGSTTKNMYSV   210
Oreochromis       DKISIWTSNCQNRDAIMTIGQLYKERLNLPMKAMIGYQSHDDTSSKSGSTTKNMYSV   210
Larimichthys      DKIAIWTSNCQNREAIMTIGQLYKERLNIPIKAMIGYQSHDDTSSKSGSTTKNMYSV   210
Latimeria         DKIAIWTTSCQNREAIMSIGQSYKERLGLPLKALIGYQSHDDTSSKSGSTTKNMYTV   233
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Danio             DKIAIWTGNCQNRDAIMTIGQQYKERLSLPSKTLIGYQSHDDTSSKSGSTTKNMYSV   213
Cyprinus          DKISIWTGNCQNRDAIMTIGQQYKERLSLPIKTLIGYQSHDDTSSKSGSTTKNMYSV   213
Astyanax          DKIAIWTGNCQNRDAIMTIGLQYKERLNLPIKTLIGYQSHDDTSSKSGSTTKNMYSV   231
Electrophorus     DKIAIWTCNCQNRDAIMTIGQQYKERLNLPIKTLIGYQSHDDTSSKSGSTTKNMYSV   212
Scleropages       DKIAIWTVNCQNREAIMTIGQQYKERLNVPVKSLIGYQSHDDTSSKSGSTTKNMYTV   212
Acipenser         DKLAIWTGNCENKEAIMTIGQQYKERLSVPSKALIGYQSHDDTSSKSGSTTKNIFTV   212
Lepisosteus       DKISIWTGNCQNKEAIMTIGQQYKERLNVPNKALLGYQSHDDTSSKSGSTTKNMYTV   212
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Hippocampus       DKIAIWTSNCQNREAIMKIGETYKDLLSIPSKAMLGYQSHDDTSSKSGSTTKNMYSI   217
Petromyzon        DKIAVWTADCDNRESVVGIGRVYKDRLALPPRIIIGYQSHTDTATKSGSSTKNMFTV   219
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Sharks

Lungfish

Seahorse

Lamprey

Spotted Gar – diverged from teleost

Gray bichir – dragon eel – appears lungfish

Mudskippers – technically teleost but use fins as legs

Reedfish – bichir – snake fish

Eel

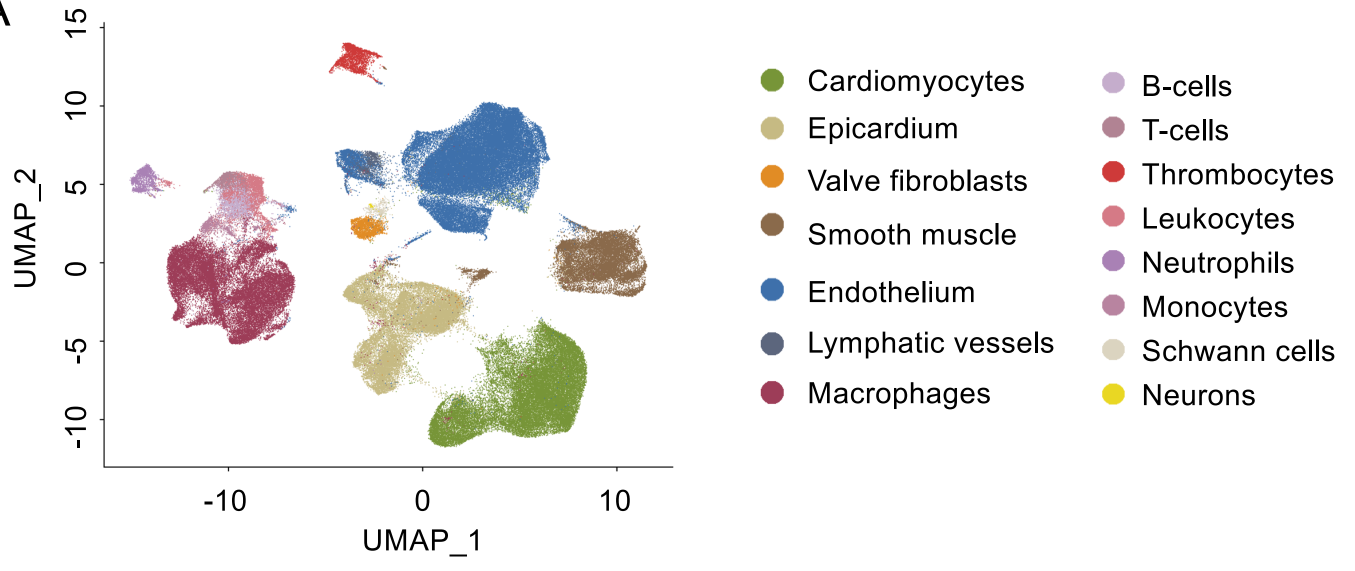
Teleost

Fig. S1. Phylogeny and conservation of eif4E orthologs.

(A) Shown is the phylogeny of Eif4e1c orthologues (all 53 species with 142 homologs) estimated using maximum likelihood using IQ-TREE. Nodes with bootstrap support < 0.85 are marked with their respective values, all other nodes had support values of 0.85 or higher. (B) ClustalW sequence alignment of Eif4e1c from aquatic vertebrate. Species names are color-coded by type with the legend on the bottom. Conserved Eif4e1c specific residues are highlighted in red bold with similar residues highlighted in pink.

Figure S2

A



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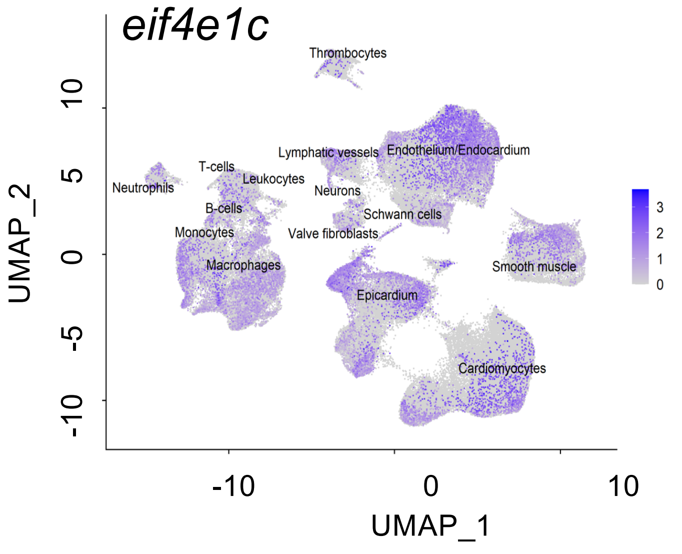


Cell-types	% *eif4e1c* (+) cells	% *eif4ea* (+) cells	% *eif4eb* (+) cells
Cardiomyocytes	4.58	1.45	4.47
Epicardium	17.51	7.98	14.46
Valve fibroblasts	7.49	3.39	10.97
Smooth muscle	9.56	5.50	9.86
Endothelium	10.64	4.44	7.27
Lymphatic vessels	12.86	4.36	8.18
Macrophages	13.67	4.53	5.36
B-cells	3.78	1.19	0.89
T-cells	1.94	0.91	0.67
Thrombocytes	2.69	0.96	1.69
Leukocytes	8.30	2.26	2.56
Neutrophils	5.89	2.26	2.39
Monocytes	11.49	6.42	3.54
Schwann cells	8.35	4.85	6.80
Neurons	42.86	14.29	2.04

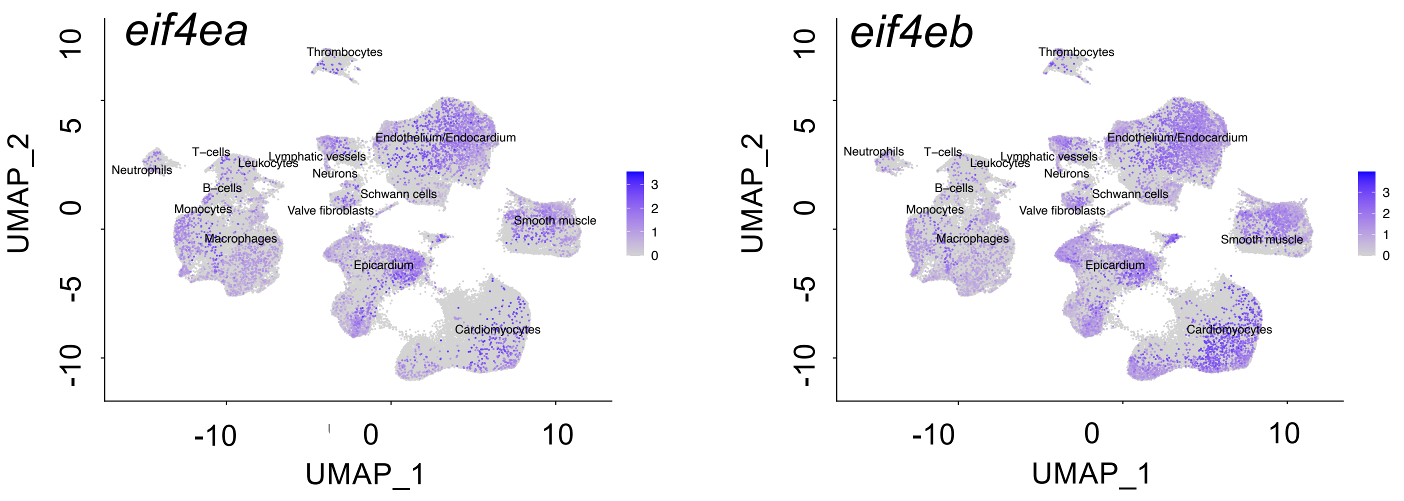


Fig. S2. EIF4E homologs are expressed in all cell-types within zebrafish hearts. (A) UMAP representation of single-cell RNA-seq data and clustering results of zebrafish hearts. (B) UMAP plots depicting *eif4e1c*, *eif4ea* and *eif4eb* expression. Right - Table showing the distribution of *eif4e1c* expressing cells in each cell clusters.

Figure S3

A

Cross	Stage	N	WT	HETS	HOMS	chi²	p value	Significant?
Δeif4e1c HETS X HETS	3dpf	190	51	85	54	1.94	0.33	no
	4wpf	410	109	203	98	0.629	0.73	no
	8wpf	630	207	312	111	29.3	4.31E-07	yes
	>3mpf	1868	599	951	318	84.9	3.22E-19	yes
Δeif4e1c HOMS ♀ X HETS ♂	3dpf	107	N/A	58	49	0.757	0.384	no

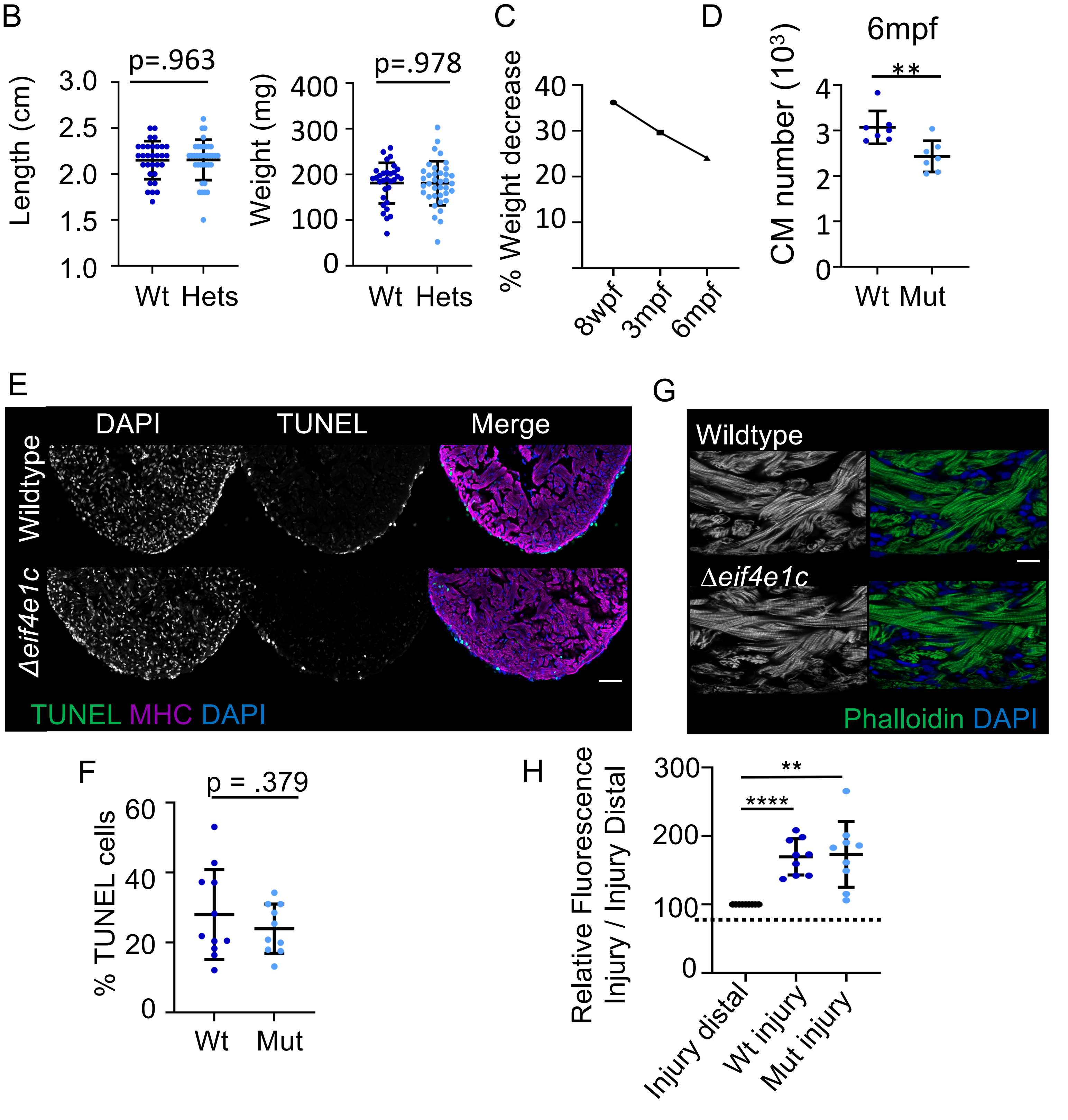
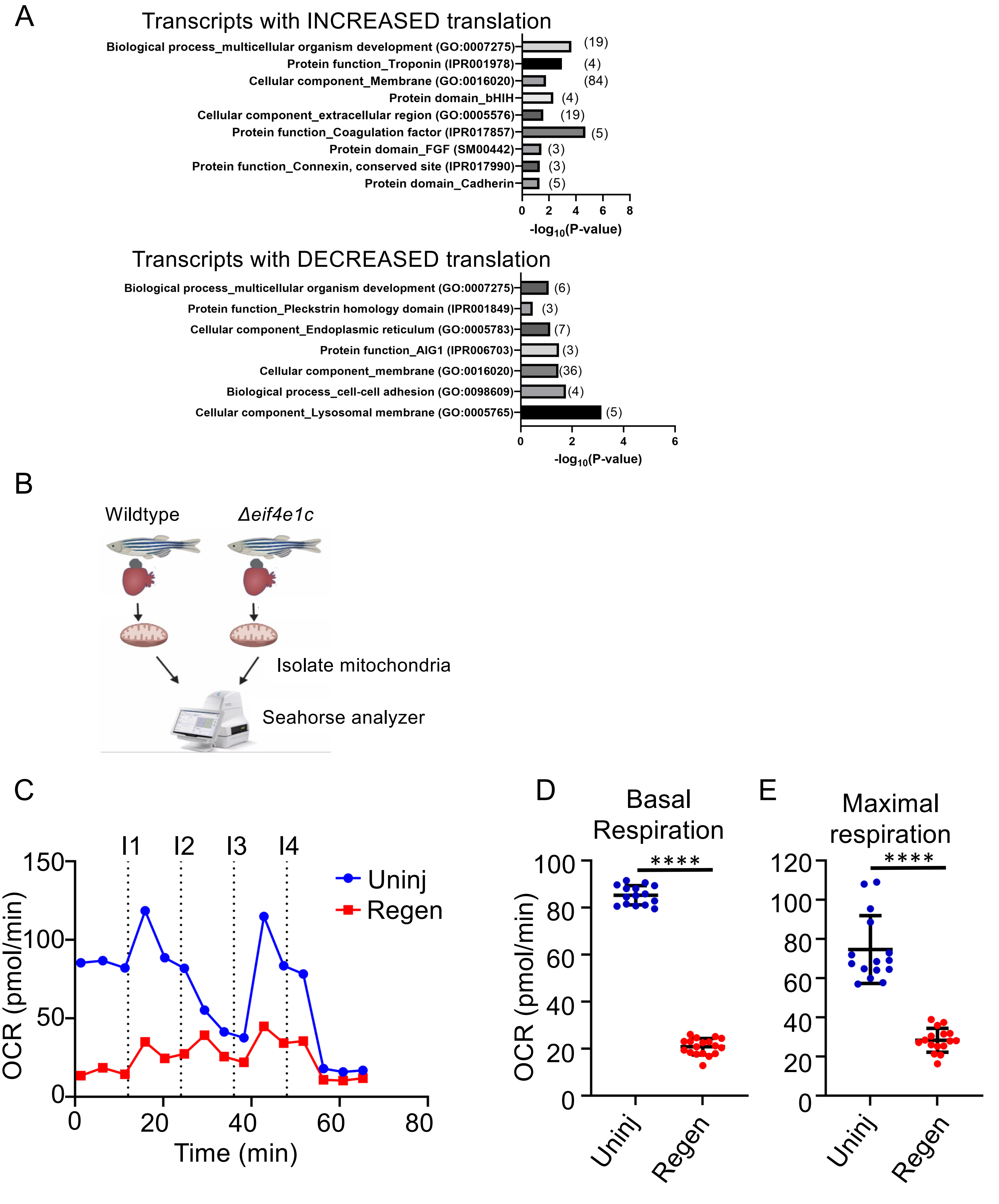


Fig. S3. Heterozygotes for D*eif4e1c* were compared for length and weight with their wildtype siblings. (A) Table showing survival of progeny from crosses of Deif4e1c heterozygote carriers. Clutches of fish were genotyped at the listed development stage (dpf, wpf and mpf represent days-, weeks- or months-post-fertilization). Significant deviations from Mendelian ratios were calculated using chi^2 and the resulting p-value is shown. (B) Fish were grown together, genotyped at the listed stage (top label) and immediately measured from jaw to the caudal fin bifurcation (Average length WT= 2.15cm and mutant= 2.15cm with N = 31 vs 37; Welch's t-test, p-value =0.963,). After measuring length, fish were dried and weighed (Average weight: wildtype = 181.0mg and mutant = 180.7mg with N = 31 vs 37; Welch's t-test, p-value = 0.978,). (C) The percent weight decrease between mutant and wildtype fish was calculated for each of the time points (D) CM numbers for wildtype and D*eif4e1c* mutants were counted at 6mpf from cryosections stained with an antibody for Mef2c (Scale bar = 100mm). The numbers of Mef2c positive cells were counted with MIPAR (average wildtype = 3070 and mutant = 2432 with N = 7 vs 7; Welch's t-test, p-value = 0.0055). (D) TUNEL staining of zebrafish hearts (Scale bar = 50mm). (F) Quantification of TUNEL stain showed no increase in the number of cells undergoing apoptosis in adult hearts (mean: wildtype = 28.03, mutant = 23.98; Welch's t-test, p-value = .379, N = 11 vs 10) (G) Phalloidin staining of wildtype (top) and mutant (bottom) zebrafish hearts. Left – Phalloidin in gray scale. Right – Merge of phalloidin with DAPI (Scale bar = 10mm). (H) Quantification of Eif4ea/b immunostaining from Figure 3G (wildtype mean increase (blue) = 1.70, Mann-Whitney p-value < 0.0001, N = 9 vs 9; mutant mean increase (light blue) = 1.73, Mann-Whitney p-value = 0.0018, N = 9 vs 9). Horizontal black bars display the mean (middle) or standard error (top and bottom).

Figure S4



Development • Supplementary information

Fig. S4. (A) Gene ontology categories were discovered using the DAVID on-line platform. Each category is listed on the left with the identifier in parentheses. Shown are GO categories with p-value < 0.05, x-axis is $-\log_{10}$(p-value). Parentheses to the right of the bar indicate numbers of genes identified in each group. (B) Cartoon depicting experimental set-up for Seahorse analysis. Mitochondria were isolated from hearts dissected from wildtype and mutant fish and then processed on the Seahorse analyzer from Agilent. (C) Shown is a time-course of oxygen consumption rates (OCR) from mitochondria measured by the Seahorse analyzer. Hashed lines indicate time points of drug injection. The first injection (I1) is of ADP to stimulate respiration, the second injection (I2) is of oligomycin to inhibit ATP synthase (complex V) decreasing electron flow through transport chain, the third injection (I3) is of FCCP to uncouple the proton gradient, and the fourth injection (I4) is of antimycin A to inhibit complex III to shut down mitochondrial respiration. (D) Basal respiration is calculated as the OCR average after addition of ADP (I1 to I2) subtracting the non-mitochondrial respiration after injection of antimycin A (after I4) (mean: wildtype uninjured= 85.30 and wildtype regenerating= 20.90; Welch's t-test p value < 0.0001; N= 15,18). (E) Maximal respiration is calculated as the OCR average after FCCP addition (I3 to I4) subtracting the non-mitochondrial respiration after injection of antimycin A (after I4)(mean: wildtype uninjured= 74.64 and wildtype regenerating= 28.34; Mann-Whitney p-value < 0.0001; N= 15,18). For panels C-E, individual data points are represented by dots, uninjured is in blue, regenerating is in red, and horizontal black bars display the mean (middle) or standard error (top and bottom). Wildtype – Blue, Mutant – light blue.

Figure S5

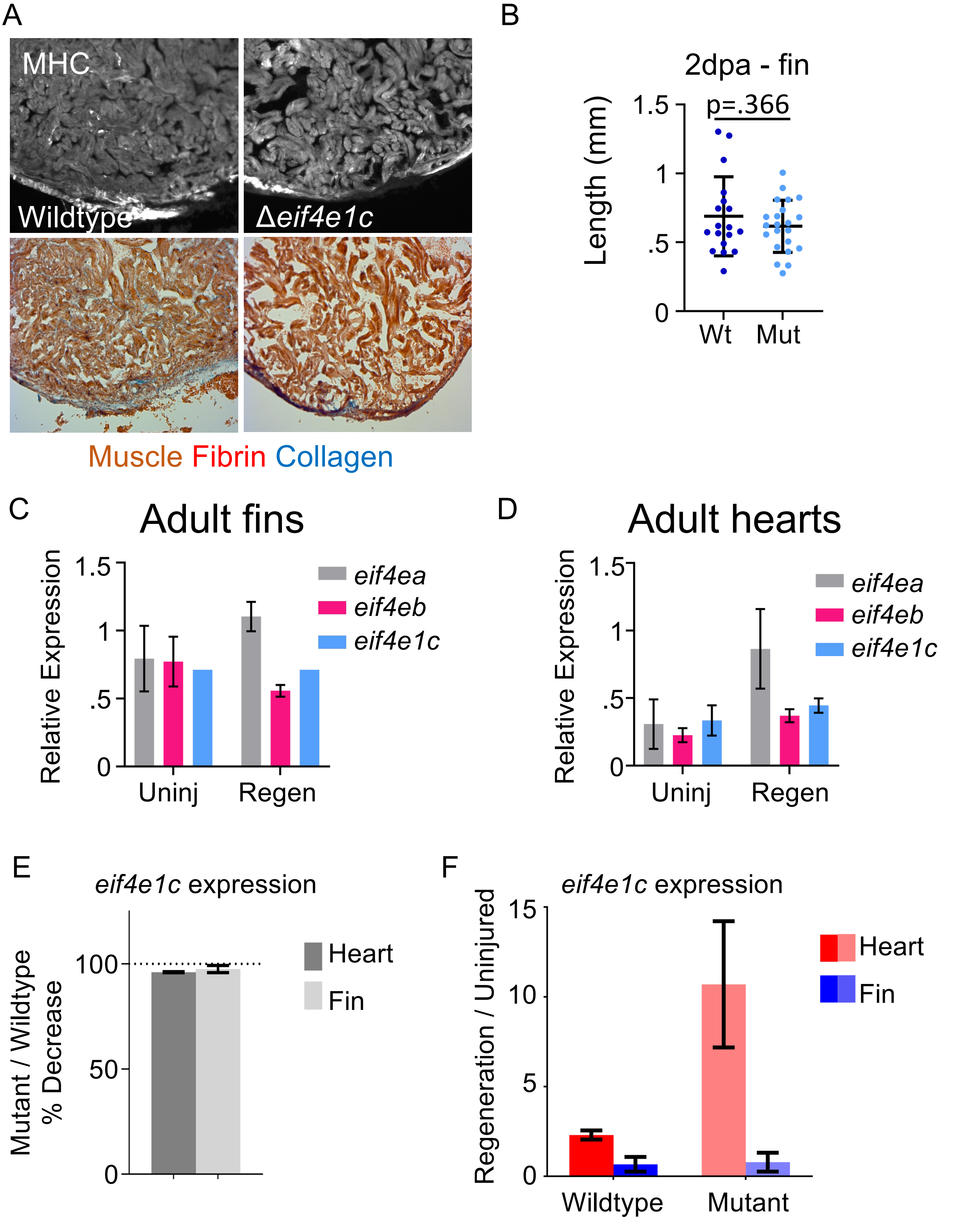

A
MHC
Wildtype
Δeif4e1c
Muscle Fibrin Collagen
B
2dpa - fin
p=.366
Length (mm)
1.5
1
.5
0
Wt
Mut
C
Adult fins
Relative Expression
eif4ea
eif4eb
eif4e1c
Uninj
Regen
D
Adult hearts
Relative Expression
eif4ea
eif4eb
eif4e1c
Uninj
Regen
E
eif4e1c expression
Mutant / Wildtype
% Decrease
100
50
0
Heart
Fin
F
eif4e1c expression
Regeneration / Uninjured
15
10
5
0
Heart
Fin
Wildtype
Mutant

Fig. S5. (A) Images of sectioned ventricles at 28dpa are stained for Myosin heavy chain (MHC) to indicate cardiac muscle. Shown below are the same sections stained with AFOG which shows muscle (orange), Fibrin (red) and Collagen (blue). Hearts from 5 wildtype and 5 Δ*eif4e1c* mutants were ranked by injury size. Shown are representative hearts near the averages of the groups. (B) Caudal fins were amputated ~50% and the blastema were imaged 48 hours later for Δ*eif4e1c* mutants and their wildtype siblings. The length of the blastema was measured from the tip of the 3rd ray using the Zeiss microscope software. There was no significant difference between the groups (2dpa average wildtype = 0.689mm and mutant = 0.617mm; Welch's t-test, p-value = 0.366, N = 18 vs 22). Horizontal black bars display the mean (middle) or standard error (top and bottom). (C) Expression of each transcript was normalized to *mob4* in uninjured fins (left) and fins undergoing regeneration (right). (D) same as (C) but for the heart. (E) RT-qPCR analysis of *eif4e1c* from uninjured hearts (r dark gray) and fins (light gray) in Δ*eif4e1c* mutants. Ct values are normalized first to *mob4* and then to the wildtype values (hashed line). (F). RT-qPCR analysis of *eif4e1c* during regeneration of the heart (red) and the fin (blue). Wildtype is shown in dark colors (red and blue) and Δ*eif4e1c* mutants are shown in light colors (pink and light blue).

Figure S6

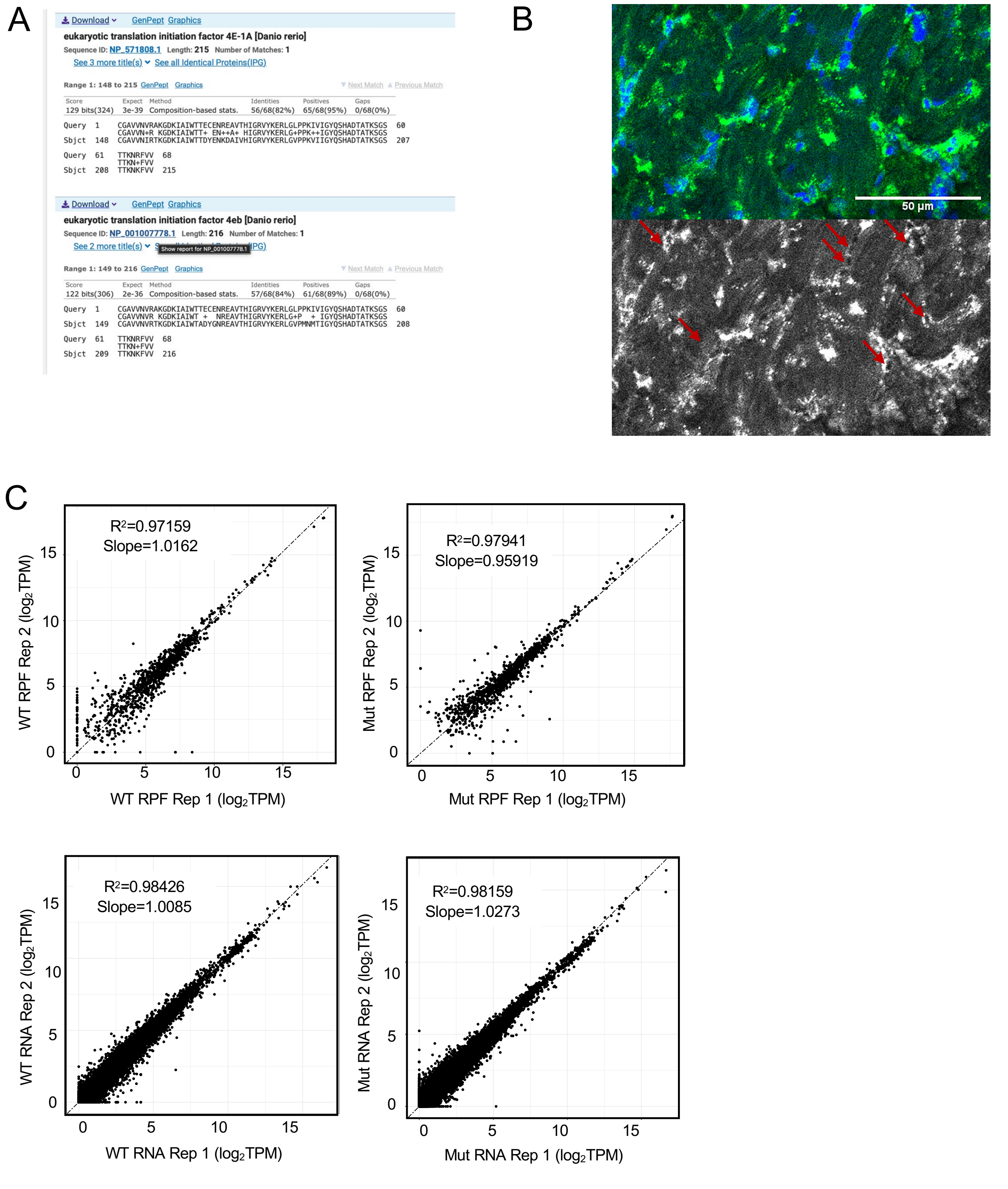


Fig. S6. (A) Region of human EIF4E1 (query) used to raise ab33768 is nearly identical in both paralogs of zebrafish canonical Eif4e1 (subject). (B) Immunofluorescence staining with ab33768 (green) on zebrafish hearts produces the expected cytoplasmic staining pattern (nuclei – blue). (C) Shown are the correlations between replicates for ribosome profiling (top) and RNA sequencing of the inputs (bottom) for both wildtype (left) and Δ*eif4e1c* mutant (right) samples.