Supplemental Information

Controlling tissue patterning by translational regulation of signaling transcripts through the core translation factor eIF3c.

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Sup_Fig.1

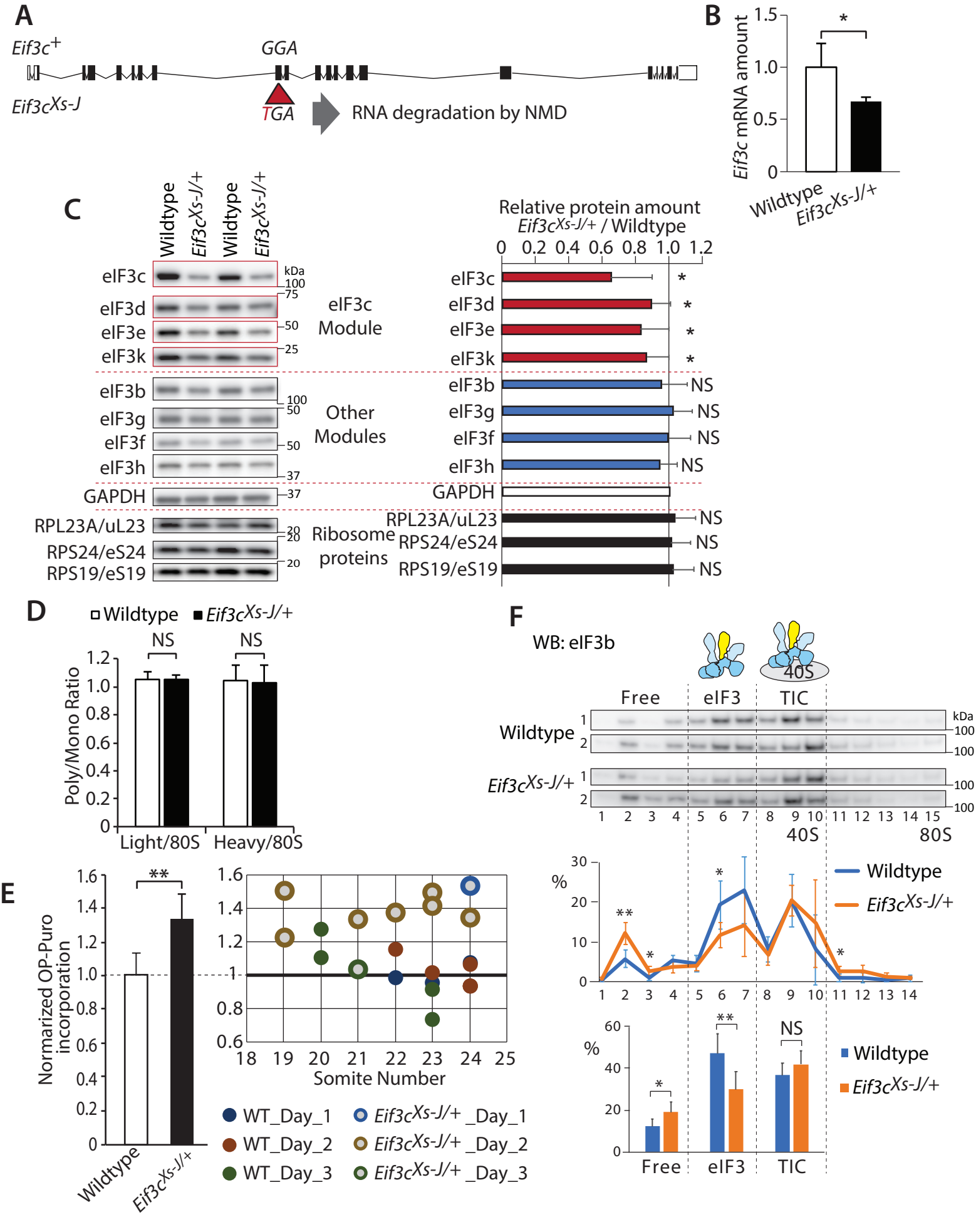
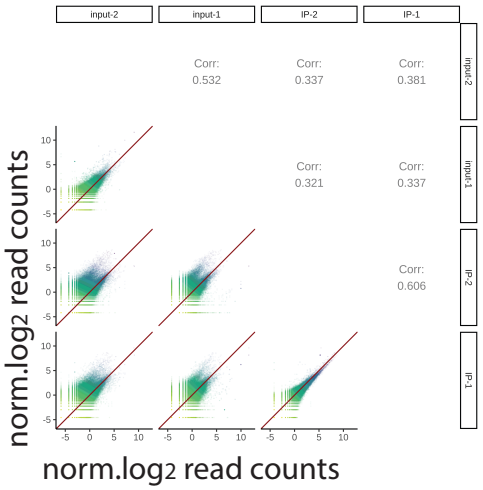

A
Eif3c+
GGA
Eif3cXs-J
TGA
RNA degradation by NMD
B
Eif3c mRNA amount
1.5
1.0
0.5
0
*
Wildtype
Eif3cXs-J/+
C
Wildtype
Eif3cXs-J/+
Wildtype
Eif3cXs-J/+
kDa
eIF3c
eIF3d
eIF3e
eIF3k
eIF3b
eIF3g
eIF3f
eIF3h
GAPDH
RPL23A/uL23
RPS24/eS24
RPS19/eS19
100
75
50
25
100
50
50
37
37
20
20
20
eIF3c Module
Other Modules
Ribosome proteins
Relative protein amount
Eif3cXs-J/+ / Wildtype
0 0.2 0.4 0.6 0.8 1.0 1.2
*
NS
D
Wildtype
Eif3cXs-J/+
NS
NS
Poly/Mono Ratio
1.2
1.0
0.8
0.6
0.4
0.2
0
Light/80S
Heavy/80S
E
**
Normarized OP-Puro incorporation
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0
Wildtype
Eif3cXs-J/+
Somite Number
18 19 20 21 22 23 24 25
WT_Day_1
WT_Day_2
WT_Day_3
Eif3cXs-J/+ _Day_1
Eif3cXs-J/+ _Day_2
Eif3cXs-J/+ _Day_3
F
WB: eIF3b
40S
Free
eIF3
TIC
Wildtype
Eif3cXs-J/+
kDa
100
100
100
100
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15
40S
80S
%
30
20
10
0
Wildtype
Eif3cXs-J/+
1 2 3 4 5 6 7 8 9 10 11 12 13 14
60
40
20
0
NS
Free
eIF3
TIC

Figure S1. Characterization of molecular phenotype of *Eif3c*$^{Xs\text{-}J/+}$ mice. Related to Figure 1.

(A) Showing spontaneous point mutation from G to T in the *Eif3c*$^{Xs\text{-}J}$ allele, which create a nonsense stop codon, which induces nonsense mediated decay (NMD). **(B)** RT-qPCR in wildtype and *Eif3c*$^{Xs\text{-}J/+}$ E11.5 embryos for *Eif3c* mRNA normalized by *Actb* mRNA (t-test, n≥4). (Error bars represent s.d.; *P<0.05) **(C)** Western blotting for eIF3 subunits in wildtype and *Eif3c*$^{Xs\text{-}J/+}$ E9.5 whole embryos. Right are quantification by ImageJ normalized by GAPDH protein (t-test, n≥6). (Error bars represent s.d.; *P<0.05) **(D)** Shown are the quantification of polysome as actively translating ribosomes from Figure 1A. Light or heavy polysomes were normalized by 80S monosome amount. Error bars represent s.d. NS: not significant (t-test, n≥4). **(E)** Global translation activity in E9.5 whole embryo of Wildtype (n=13) or *Eif3c*$^{Xt\text{-}J/+}$ (n=8). Global protein synthesis was monitored by O-propargyl-puromycin (OP-Puro), in E9.5 whole embryos quantified by flow cytometry as the fluorescence activity of incorporated OP-Puro in nascent protein. Global protein synthesis in *Eif3c*$^{Xs\text{-}J/+}$ mutant embryos did not decrease and, if anything, a subtle increase is observed (t-test, **p<0.01) Right: Shown are individual data points. Each dot indicates individual embryos. Filled circles are wildtype and open circles are *Eif3c*$^{Xs\text{-}J/+}$ embryos. Each color indicates the day of the assay. **(F)** Fractionation of the eIF3-40S translation initiation complex (TIC). The eIF3 complex in each fraction was monitored by the eIF3b subunit whose amount did not change in *Eif3c*$^{Xt\text{-}J/+}$ embryos (Figure S1C). eIF3b signal from the fraction containing the eIF3 complex was reduced by around 20%, and correspondingly a slight increase in eIF3b in the free fraction not part of the translation initiation complex was also observed. In good agreement with the normal global protein synthesis in *Eif3c*$^{Xt\text{-}J/+}$ embryos (Figure 1A and S1D-E), the eIF3-40S TIC amount was not changed between Wildtype and *Eif3c*$^{Xt\text{-}J/+}$ embryos. (t-test; **p<0.01; *p<0.05; NS, not significant)

Sup_Fig.2


A
input-2
input-1
IP-2
IP-1
Corr: 0.532
Corr: 0.337
Corr: 0.381
Corr: 0.321
Corr: 0.337
Corr: 0.606
norm.log2 read counts
norm.log2 read counts

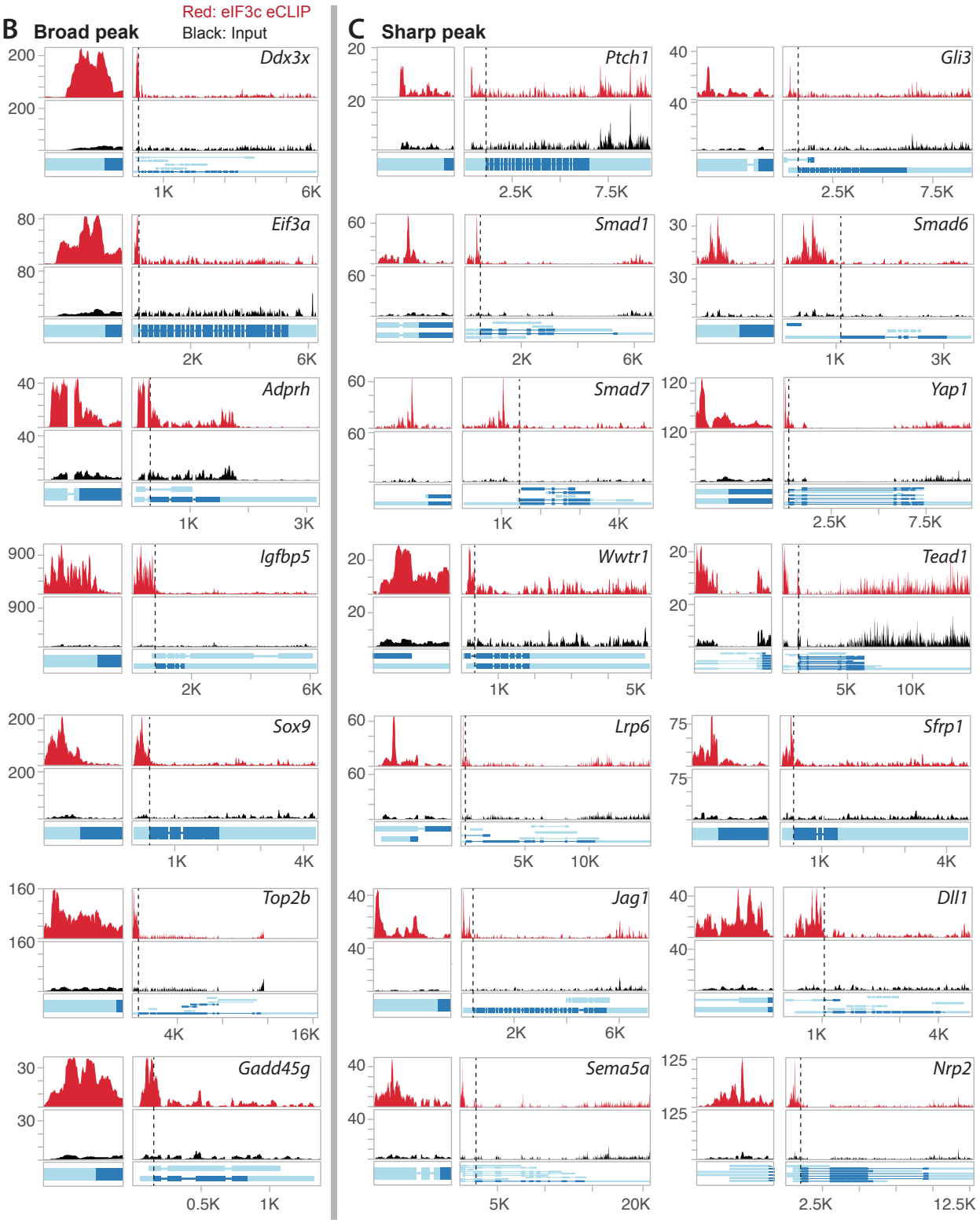

B Broad peak
Red: eIF3c eCLIP
Black: Input
Ddx3x
Eif3a
Adprh
Igfbp5
Sox9
Top2b
Gadd45g
C Sharp peak
Ptch1
Gli3
Smad1
Smad6
Smad7
Yap1
Wwtr1
Tead1
Lrp6
Sfrp1
Jag1
Dll1
Sema5a
Nrp2

Figure S2 Basic analysis of the eIF3c eCLIP-seq dataset. Related to Figure 2.

(A) Plot of pairwise correlations of all CLIP and Input sequence datasets confirms the consistency of biological replicates. **(B-C)** eIF3c eCLIP (Red) and input (Black) plots of several transcripts having broad enrichment in 5'-UTRs (B) and transcripts having distinct sharp peak in 5'-UTRs (C).

Sup_Fig.3

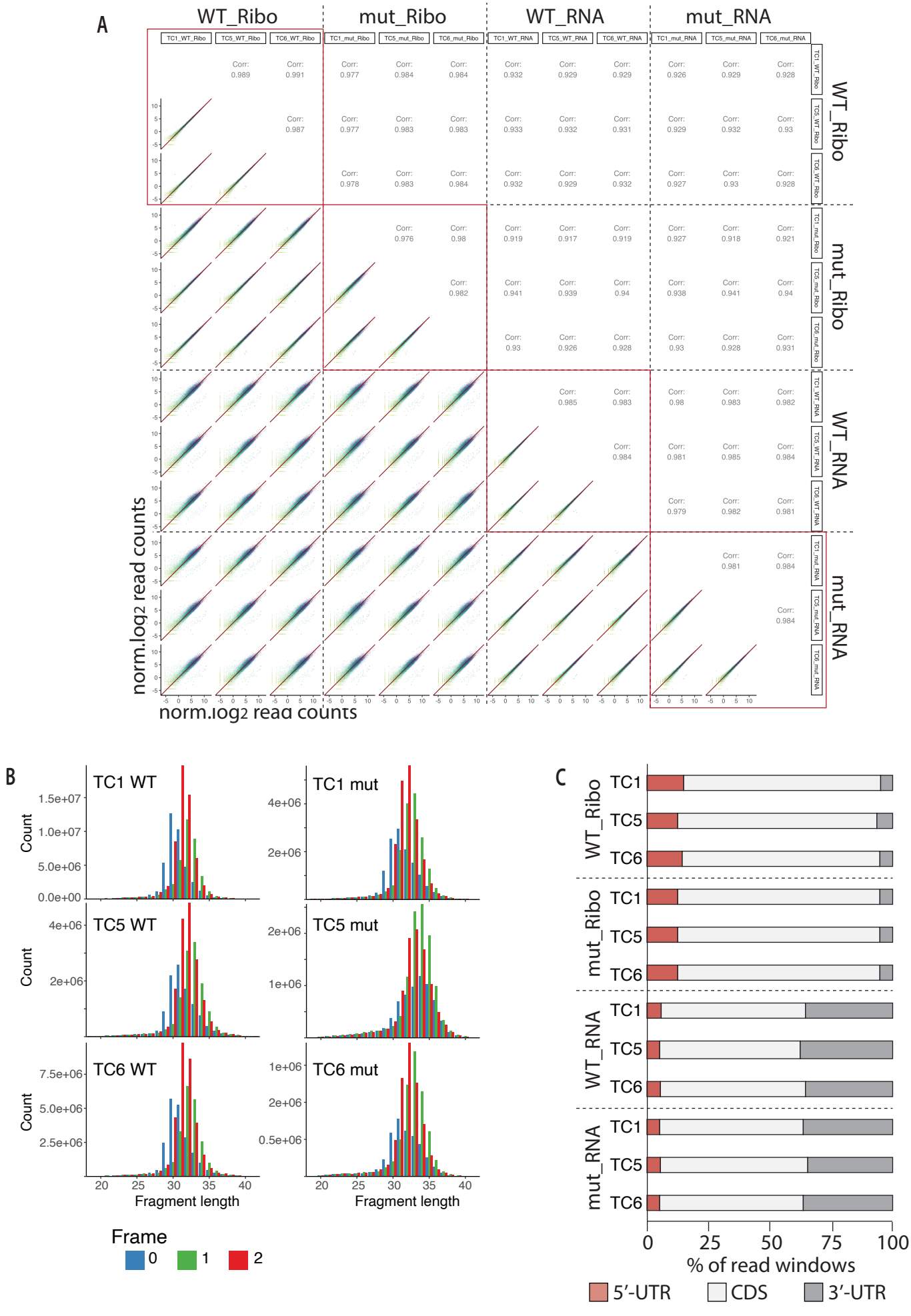

A
WT_Ribo
mut_Ribo
WT_RNA
mut_RNA
norm.log2 read counts
B
TC1 WT
TC1 mut
TC5 WT
TC5 mut
TC6 WT
TC6 mut
Count
Fragment length
Frame
0
1
2
C
WT_Ribo
mut_Ribo
WT_RNA
mut_RNA
TC1
TC5
TC6
% of read windows
5′-UTR
CDS
3′-UTR

Figure S3 Basic analysis of the ribosome profiling dataset from wildtype and *Eif3c*$^{Xs\text{-}J/+}$ *embryos*. Related to Figure 2.

(A) Plot of pairwise correlations of all Ribo-seq and RNA-seq datasets confirms the consistency of biological replicates. **(B)** Read length and CDS reading frame distribution for ribosome protected fragments (RPF) aligning to annotated ORFs in a representative library. **(C)** Proportion of reads aligning to CDS, 5'-UTR, and 3'-UTR regions from Ribo-seq and RNA-Seq libraries. CDS enrichment for ribosome protected fragments are expected.

Sup_Fig.4

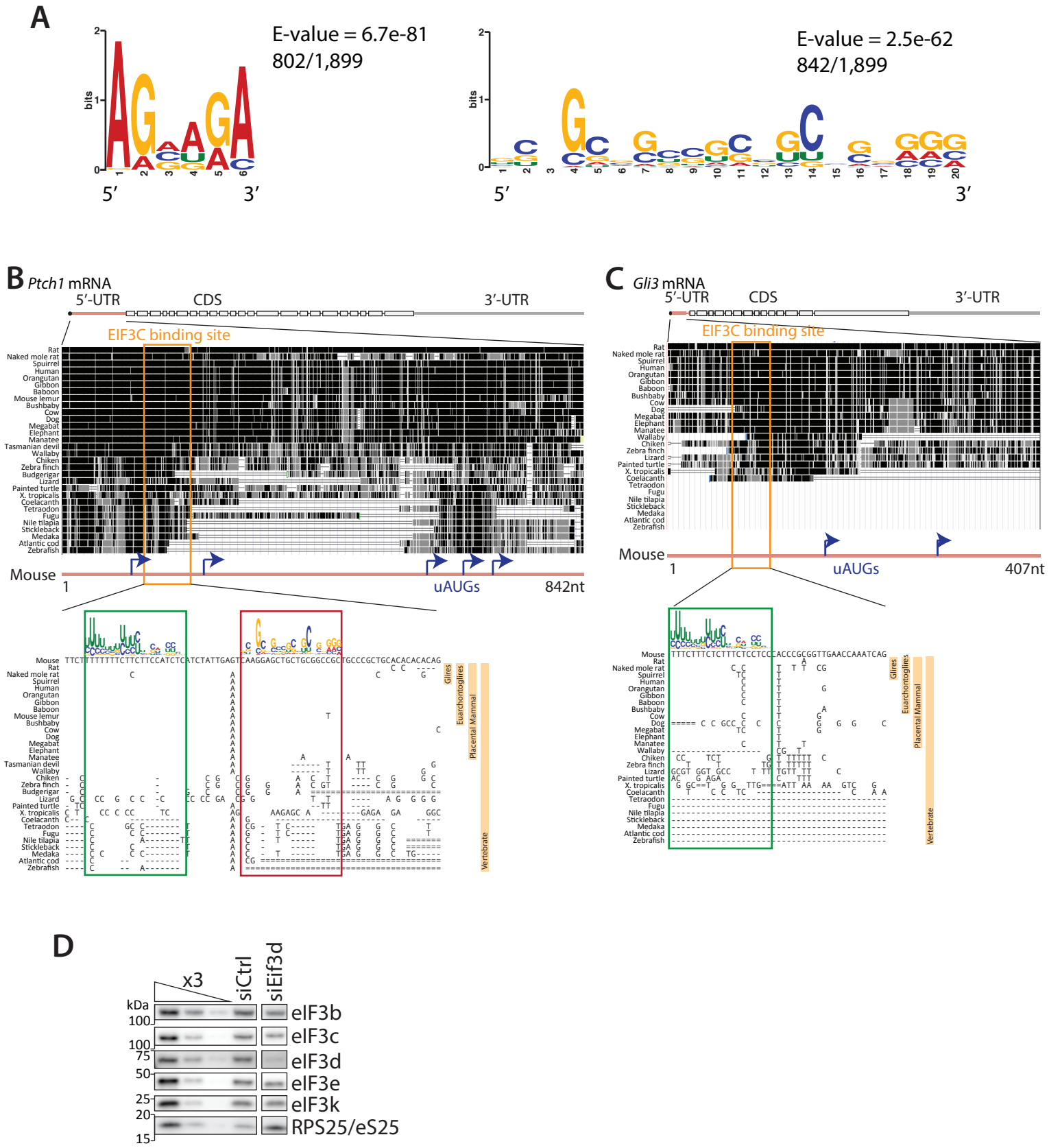

A
E-value = 6.7e-81
802/1,899
E-value = 2.5e-62
842/1,899
bits
5′
3′
B Ptch1 mRNA
5′-UTR
CDS
3′-UTR
EIF3C binding site
Mouse
1
uAUGs
842nt
C Gli3 mRNA
5′-UTR
CDS
3′-UTR
EIF3C binding site
Mouse
1
uAUGs
407nt
Glires
Euarchontoglires
Placental Mammal
Vertebrate
D
x3
siCtrl
siEif3d
kDa
100
100
75
50
25
20
15
eIF3b
eIF3c
eIF3d
eIF3e
eIF3k
RPS25/eS25

Figure S4. The eIF3 binding motifs in 5'-UTRs are highly conserved across vertebrates. Related to Figure 3.

(A) MEME motif analysis identified AG and GC rich motifs in the distinct eIF3 binding peak. (B-C) Alignment of the mouse *Ptch1* (B) or *Gli3* (C) 5'-UTRs across other vertebrate species from UCSC genome browser. The eIF3 binding sites are zoomed in and showing conservation in nucleotide resolution. Pyrimidine (UC) rich and GC rich motifs are shown in Green and Red rectangles, respectively. **(D)** Western blotting against several eIF3 subunits shows specific siRNA knock down of the eIF3d subunit by siRNA.