Supplementary information

A molecular network of conserved factors keeps ribosomes dormant in the egg

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# equal contribution

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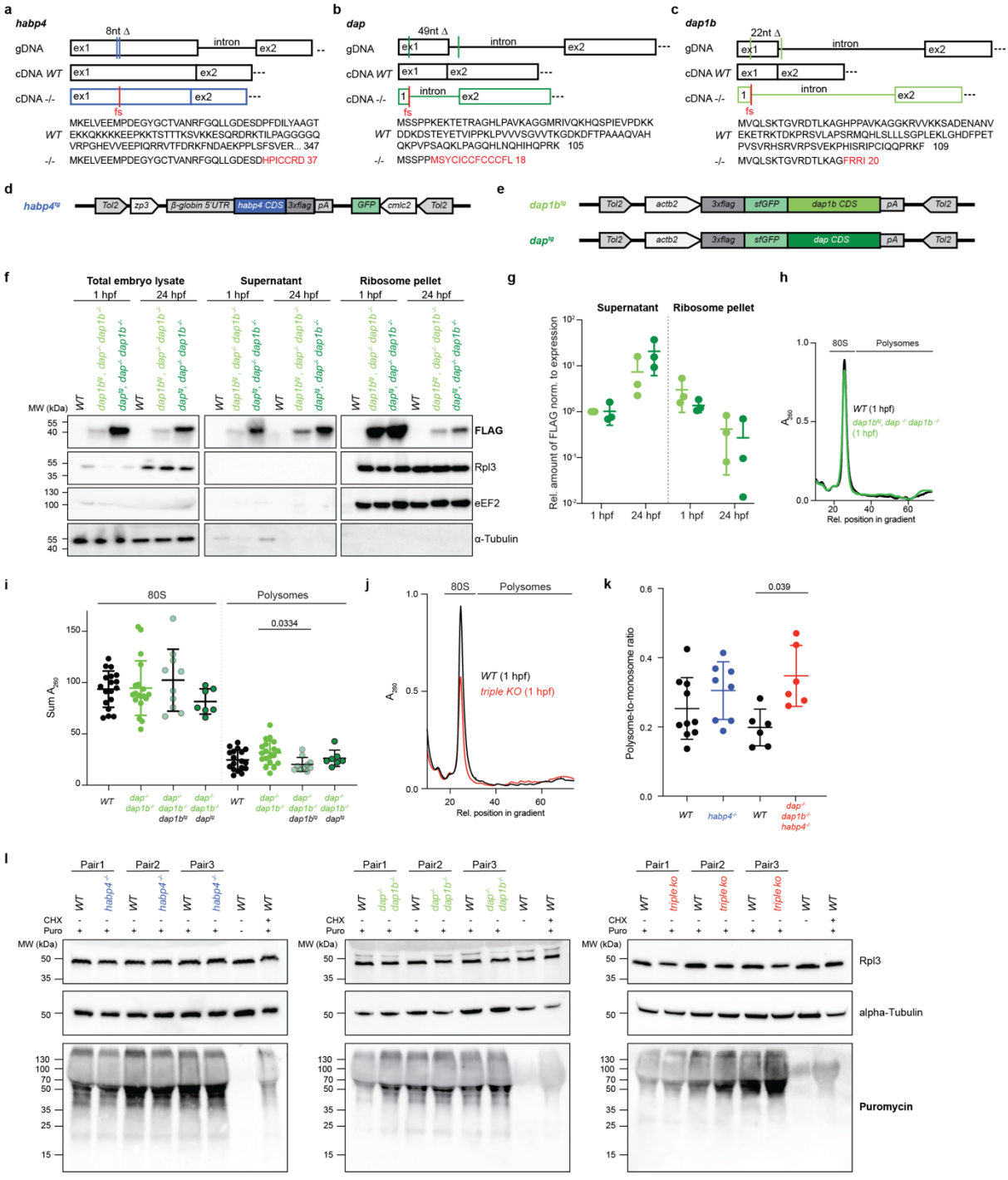
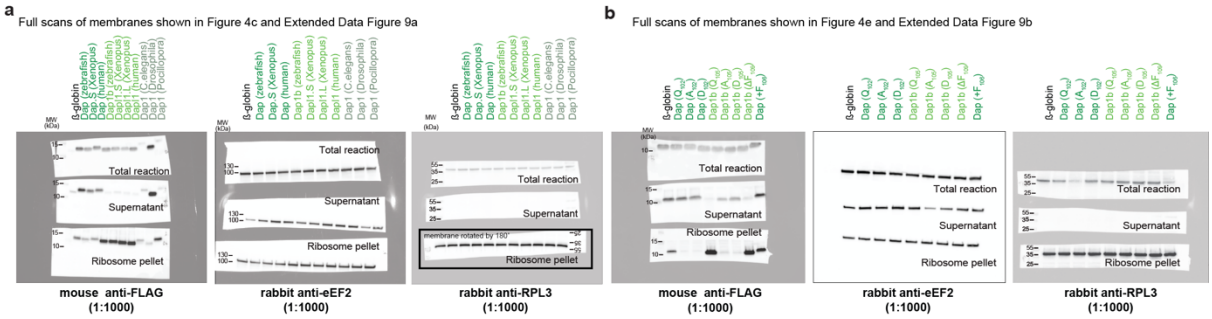
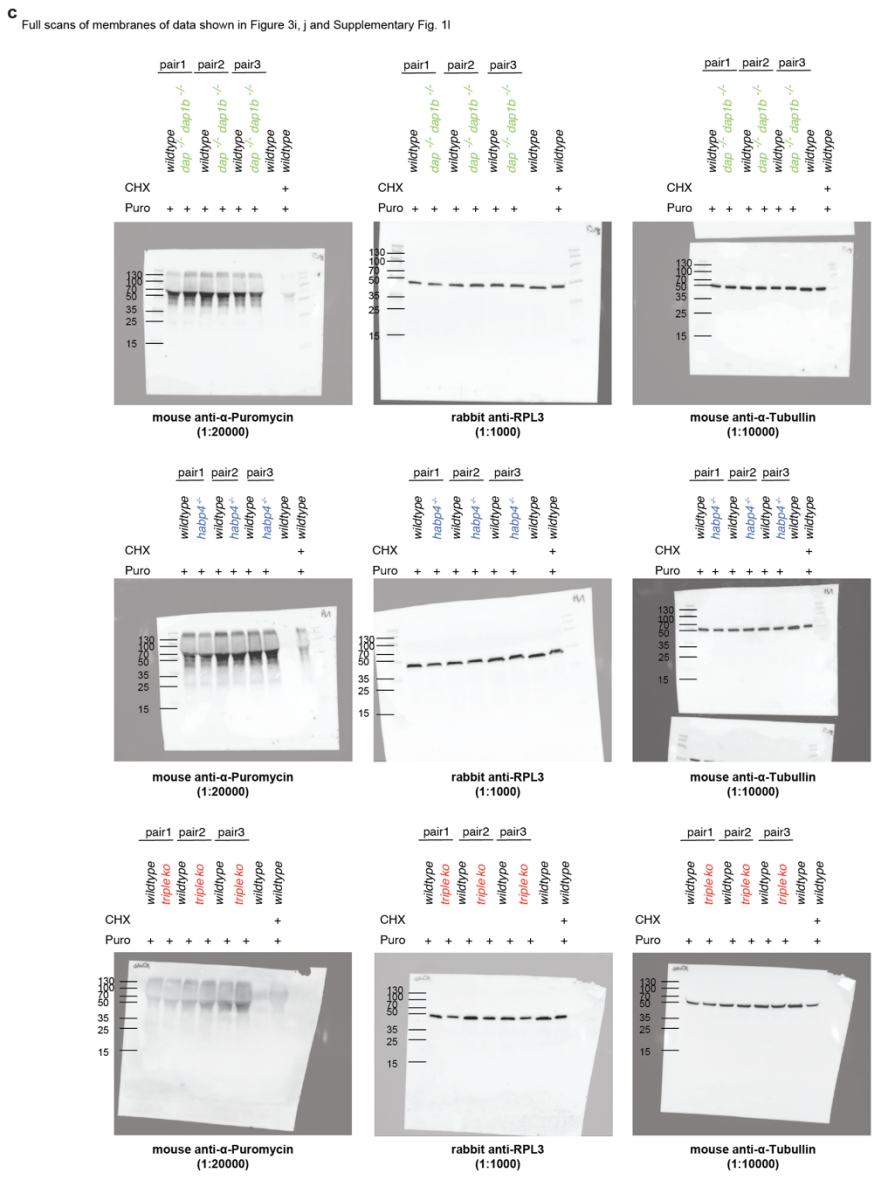


Supplementary Figure 1. Features and further analyses of mutants and transgenic lines used in this study. a-c, Scheme showing deletions and predicted coding sequences in *habp4* (**a**), *dap* (**b**) and *dap1b* (**c**) mutants. **d,** Scheme of the construct used to express *Habp4* under the control of the germline specific *zp3 promoter* (*habp4*^{tg}). **e,** Scheme of the constructs used to express *Dap1b* and *Dap* under the control of the *actb2 promoter* (*dap*^{tg}/*dap1b*^{tg}). **f,** Representative western blots of transgenic flag-tagged Dap and Dap1b in total embryo lysates, supernatants and ribosome pellets. For uncropped images of membranes see **Supplementary Fig. 2e**. **g,** Quantification of Dap/Dap1b levels in ribosome and supernatant at 1 and 24 hours post-fertilization (hpf). Flag signal was normalized to total lysate and to

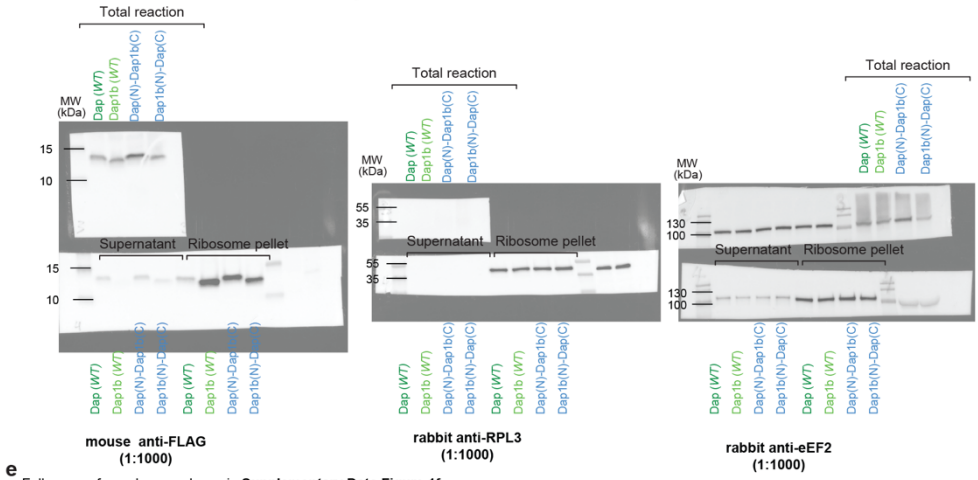
the loading controls eEF2 and Rpl3 (n = 3 independent experiments). **h,** Representative polysome profiles from 1 hpf embryos derived from *WT* and *dap1b*^{tg}, *dap*$^{-/-}$, *dap1b*$^{-/-}$ parents. **i,** Quantification of monosomes and polysomes from 1 hpf embryos (*WT*: n = 18; *dap*$^{-/-}$, *dap1b*$^{-/-}$: n = 19; *dap1b*^{tg}, *dap*$^{-/-}$, *dap1b*$^{-/-}$: n = 10; *dap*^{tg}, *dap*$^{-/-}$, *dap1b*$^{-/-}$: n = 7). **j,** Representative polysome profiles from 1 hpf *WT* and *triple KO* embryos. **k**, Quantification of polysome-to-monosome ratios of polysome profiles from 1 hpf embryos (*WT*: n = 10; *habp4*$^{-/}$: n = 8; *WT* and *dap*$^{-/-}$, *dap1b*$^{-/-}$, *habp4*$^{-/-}$ (*triple KO*): n = 6 in both). **l**, Representative western blots of single, double and *triple KO* embryos from 3 independent crosses injected with puromycin (experiments were performed twice with similar results, see quantifications in **Fig. 3i-j**). Rpl3 and Tubulin are used for normalization. CHX: cycloheximide; Puro: puromycin. For uncropped images of membranes see **Supplementary Fig. 2c**. For **i** and **j**, n are biologically independent samples. Statistical analysis was performed with two-sided Mann-Whitney (**g** and **j**) and Kruskal-Wallis followed by Dunn's two-sided test (**i, k**; for **i**, statistical significance was calculated separately for 80S and polysomes). In **g**, **i** and **k**, data are represented as scattered dot plots with mean and SD.

Supplementary Figure 2. Uncropped blots.

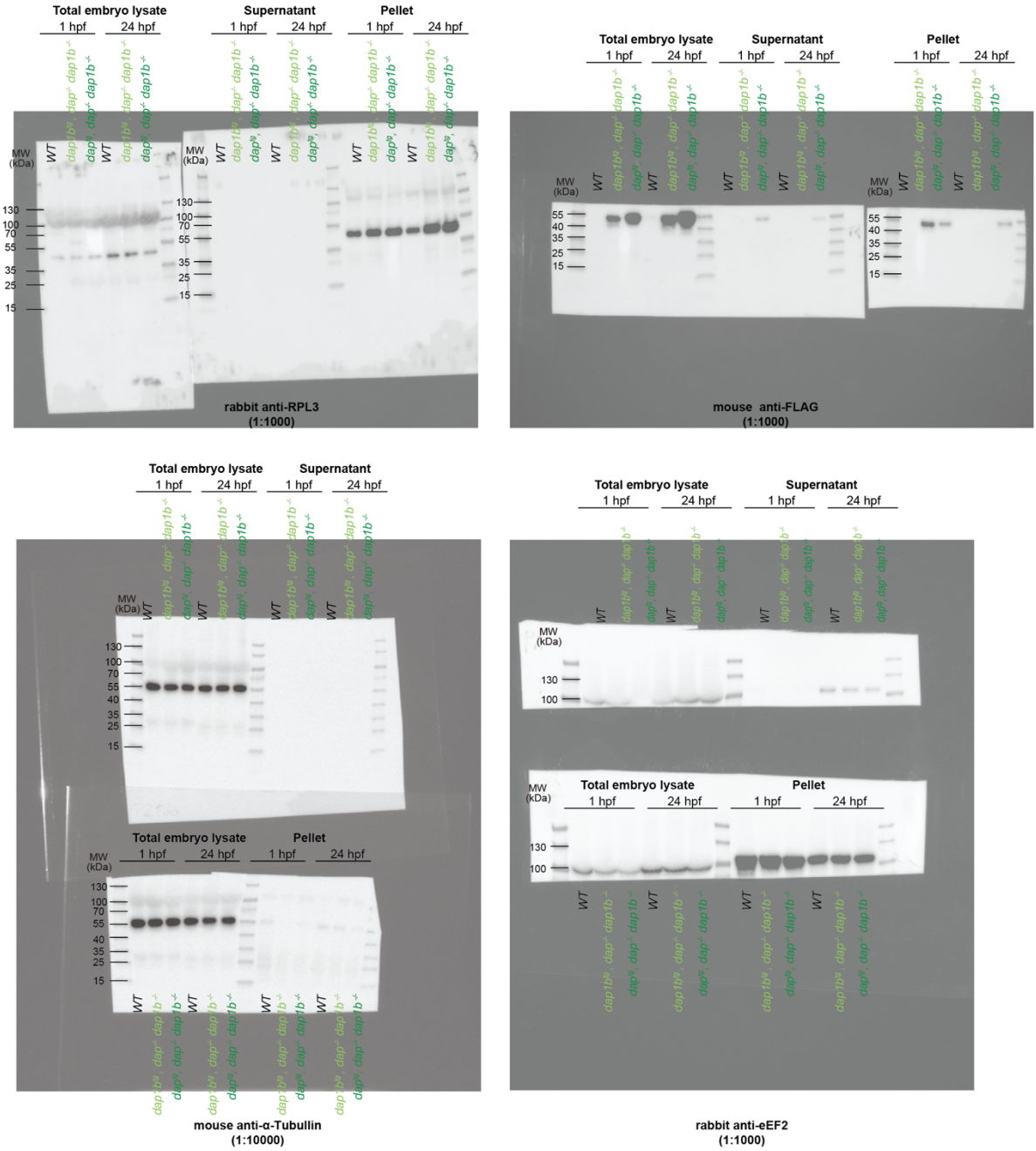




d
Full scans of membranes shown in **Extended Data Figure 9d**



e
Full scans of membranes shown in **Supplementary Data Figure 1f**



Supplementary Figure 2. Uncropped blots. Shown are composite images of Western blot membranes (except for the second image in panel **b**, where no colorimetric image was taken) prior to cropping for presentation in the figures (corresponding figure panels are indicated above the blot images). Antibodies and dilutions are indicated below the images in bold; MW (molecular weight), kDa (kilodalton). All *in vitro* transcription reactions analyzed by Western blot were controlled for loading by respective loading controls (Rpl3, eEF2 and alpha-Tubulin), which are included in the respective figures (**Supplementary Fig. 2a, b, d).** Equal loading of embryo lysates and fractions was controlled for by using equal amounts of embryos for each condition and loading of equal volumes. Loading controls are included in the respective panels in each figure (**Supplementary Fig. 2c, e)**. **a-b,** Individual membranes where cut at 70 and 25 kDa. Membranes with samples from the total reaction, supernatant and pellet fraction were imaged together. **c,** Full membranes for each experiment were stripped and analyzed with anti-Puromycin, anti-Rpl3 and anti-alpha-Tubulin antibody in the respective order. **d,** Individual membranes where cut at 70 and 25 kDa. Membranes with samples from the total reaction, supernatant and pellet fraction were imaged together. Unlabeled bands belong to an experiment which was conducted independently of the shown experiment. **e,** Membranes were partially cut (membranes analyzed with anti-eEF2 and anti-FLAG antibody).

Supplementary Table 1. Proteins differentially associated with ribosomes. Proteins significantly enriched or depleted in ribosomes isolated from 1 hpf zebrafish embryos versus zebrafish eggs, 3 hpf and 6 hpf embryos (permutation-based false discovery rates < 0.05).

See excel file

Supplementary Table 2. Cryo-EM data collection and refinement statistics. Data collection, refinement, B factors, RMSD deviations and validation scores of ribosome structures from 1 hpf zebrafish (PDB-7OYA), 6 hpf zebrafish (PDB-7OYB), *Xenopus* egg (PDB-7OYC) and rabbit reticulocyte lysate supplemented with recombinant zebrafish Dap1b (PDB-7OYD).

	1 hpf zebrafish	**6 hpf zebrafish**	***Xenopus* egg**	**Rabbit + Dap1b**
PDB ID	7OYA	7OYB	7OYC	7OYD
Data collection				
Microscope	Thermo Fisher Titan Krios			
Acceleration Voltage (kV)	300			
Detector	Falcon 3 EC	Falcon 3 EC	Falcon 3 EC	K3
Magnification (nominal)	81.000	81.000	105.000	81.000
Defocus range (μm)	0.5-4	0.5 -3	0.2-2	0.4-3.2
Calibrates pixel size (Å/px)	1.1	1.04	0.82	1.07
Electron exposure ($e^-/Å^2$)	43	48	40	50
Exposure rate ($e^-/Å^2/s$)	43	48	40	50
Number of frames per movie	39	39	39	41
Collection software	Cryosparc 3.2.0	Cryosparc 3.2.0	Cryosparc 3.2.0	Cryosparc 3.2.0
Number of micrographs	17,040	11,860	9,832	9,127
Initial particle number	1,961,364	1,285,670	635,641	1,808,120
Final particle number	535,633	775,288	465,392	479,754
Map resolution (Å, FSC=0.143)	3.2	2.6	2.8	2.4
Refinement				
Software	Phenix 1.17.1	Phenix 1.17.1	Phenix 1.17.1	Phenix 1.17.1
Initial model(s)	4UG0 6MTE 5DAT	4UG0	4UG0 6MTE 5DAT	6MTE 5DAT 5GAK
Correlation coefficient (CC_{mask})	0.83	0.84	0.82	0.76
Map sharpening factor ($Å^2$)	-108.5	-89.2	-73.4	-50.2
Model composition (chains)	82	77	81	85
Non-hydrogen atoms	199,914	194,870	198,952	213,014
Protein residues	11,179	10,795	11,387	12,293
nucleotides	5,130	5,035	5,006	5,316
Ligands	MG: 202 ZN: 7	MG: 203 ZN: 7	MG: 134 ZN: 7	ZNA: 1 MG: 201 B8N: 1 ZNK: 1 ZN: 5 4AC: 2
B factors ($Å^2$, min/max/mean)				
Protein	4.4 / 281.9 / 53.3	12.8 / 350.6 / 51.7	4.4 / 300.2 / 53.4	4.4 / 244.8 / 82.7
RNA	13.9 / 234.1 / 53.1	13.9 / 205.7 / 56.6	13.9 / 205.7 / 54.6	20.0 / 333.2 / 81.7
Ligands	7.8 / 102.4 / 28.4	7.8 / 102.4 / 27.0	7.8 / 102.4 / 25.1	25.8 / 120.5 / 57.1
RMS deviations				
Bond length (Å) (#>4 sigma)	0.003	0.003	0.003	0.005
Bond angle (°) (#>4 sigma)	0.620	0.614	0.619	0.714
Validation				
MolProbity score	1.59	1.72	1.54	1.68
Clashscore	6.04	8.01	6.95	6.96
Poor Rotamers (%)	1.30	1.32	0.76	1.06
Cß deviations (%)	0.01	0.00	0.00	0.01
CABLAM outliers (%)	2.35	2.36	2.27	2.25
Favored (%)	97.03	96.86	97.10	96
Allowed (%)	2.97	3.14	2.90	4
Disallowed (%)	0	0	0	0

Supplementary Table 3. CryoDRGN analysis of ribosome particles. Classification was done based on the presence of eEF2, eIF5a and tRNA factors.

See excel file

Supplementary Table 4. Dap, Dap1b/Dapl1 and Habp4 crosslinks. Crosslinks identified in ribosome samples from 1 hpf zebrafish embryos and *Xenopus* eggs.

See excel file

Supplementary Table 5. List of primers. Oligonucleotide sequences used for the generation and genotyping of *dap*, *dap1b* and *habp4* mutants.

Oligo name	**Sequence (5' to 3')**
dap_gRNA1	GTCTCGGTTTTCTCTTTGGG
dap_gRNA2	CATGGGCATTACCAGCAGGAAGG
dap1b_gRNA1	GTCATCCACCTGCAGGTTTGGG
dap1b_gRNA2	GACGAAATCCCCAAACCTGCAGG
habp4_gRNA1	ATATAGGATGTCGAAAGGGTCGG
habp4_gRNA2	ACCTTGTCCGCCCCCACCTGCGG
	AAAAGCACCGACTCGGTGCCACTTTTTCAAGTTGATAA
common_tracer_oligo	CGGACTAGCCTTATTTTAACTTGCTATTTCTAGCTCTAAAAC
dap_gt_F	AGTCGACGGTAGTCTGCGTTAT
dap_gt_R	TCAAAAAGGTGCCATTTAGTGT
dap1b_gt_F	GTGTTTTTGTGAGAAGCGTTTG
dap1b_gt_R	CACCATTTGGTCAATAGACGTG
habp4_gt_F	GATGAGGGATATGGATGCACCGTGG
habp4_gt_R	TCAGTCCCTGCAGCATATAGG