SUPPLEMENTARY FIGURE LEGENDS

Supplementary Figure S1. (**A**) Alignment of amino acids sequences of Sas10 from human (hu, accession number: NM_020368.2), mouse (mu, accession number: NM_023054.2) and zebrafish (zf, accession number: NM_001004595.1) using the software Genedoc . The Sas10/C1D domain is highlighted by a blue underline. (**B**,**C**) The specificity test of a rabbit polyclonal antibody (Rb poly) (**B**) and a mouse monoclonal antibody (Mus mono) (**C**) against zebrafish Sas10 using total proteins extracted from the 5-dpf old WT and *sas10*⁴² (s^{42}) embryos. The anti-Sas10 monoclonal antibody also detected a non-specific band (lower band).

Supplementary Figure S2. (A) Alignment of amino acids sequences of Mpp10 from human (hu, accession number: NM_005791.2), mouse (mu, accession number: NM_026483.2) and zebrafish (zf, accession number: NM_001305543.1) using the software Genedoc. Red box, predicted Capn3 recognition sites; light blue underline, domain responsible for interaction with Utp3/Sas10 in yeast; purple underline, domain responsible for interaction with Imp3 in yeast; green underline, domain responsible for interaction with Imp3 in yeast; green underline, domain responsible for interaction with Imp3 in yeast; green underline, domain responsible for interaction with Imp3 in human. (B) The specificity test of a rabbit polyclonal antibody against zebrafish Mpp10 using total protein extracted from the 5-dpf old WT and $mpp10^{410}$ (m^{410}) embryos.

Supplementary Figure S3. *sas10* is essential for the organogenesis of digestive organs.(A) Whole-mount in situ hybridization (WISH) using the *sas10* probe on WT unfertilized

eggs and embryos at 6hpf, 12hpf, and 1dpf to 5dpf. (**B**) Upper panel: diagram showing the *sas10* genomic DNA. Red bar, exons; blue line, introns. Lower panels: showing the DNA sequence changes in *sas10⁴⁸* and *sas10⁴²* two mutant alleles which are mutated in the exon4 of the *sas10* gene (red letters, deletion; blue letters, insertion; small letters: intron). (**C**) Representative images showing the overall morphology of a 5dpf-old WT and *sas10⁴²* (*s*⁴²). Black arrow, cardiac region; red arrow, swimming bladder; blue arrow, eye. (**D-G**) WISH using the *prox1* (**D**) and *hhex* (**E**) probes on 30hpf- and 50hpf-old embryos, and the *gata6* (**F**) and *foxa3* (**G**) probes on 30hpf-, 50hpf- and 3dpf-old embryos as shown. The number of total embryos examined (as denominator) and the number of embryos exhibiting the displayed phenotype (as numerator) are shown at the bottom.

Supplementary Figure S4. *mpp10* is essential for the organogenesis of digestive organs. (A) Whole-mount in situ hybridization (WISH) using the *mpp10* probe on WT unfertilized eggs and embryos at 6hpf, 12hpf, and 1dpf to 5dpf. (B) Upper panel: diagram showing the *mpp10* genomic DNA. Red bar, exons; blue line, introns. Lower panels: showing the DNA sequence changes in $mpp10^{410}$ and $mpp10^{+2}$ two mutant alleles which are mutated in the exon2 of the mpp10 gene (red letters, deletion; blue letters, insertion). (C) Representative images showing the overall morphology of a 5dpf-old WT and $mpp10^{410}$ (m^{410}). Black arrow, cardiac region; red arrow, swimming bladder; blue arrow, eye. (D-G) WISH using the *prox1* (D) and *hhex* (E) probes on 30hpf- and 50hpf-old embryos, and the *gata6* (F) and *foxa3* (G) probes on 30hpf-, 50hpf- and 3dpf-old embryos. The number of total embryos genotyped (as denominator) and the number of embryos exhibiting the displayed phenotype (as numerator) are shown at the bottom. **Supplementary Figure S5.** Small liver phenotype in $sas10^{42}$ and $mpp10^{410}$ is due to cell cycle arrest. (**A**) Co-immunostainings of phosphorylated-Histone 3 (P-H3) and Bhmt were performed on cryosections of WT, $sas10^{42}$ and $mpp10^{410}$ embryos at 2.5dpf. (**B**) Histogram showing the statistic analysis of P-H3-positive cells (red) against total cells counted in the liver area (Bhmt-positive, green) and in the neural tube. For each genotype, at least 17 cryosections from three embryos were used in counting P-H3-positive cells. Bar: 20µm.

Supplementary Figure S6. (A) $sas10^{42}$ and $mpp10^{410}$ mutant embryos did not show abnormal apoptotic activities. TUNEL assay was performed on cryosections of WT, $sas10^{42}$ and $mpp10^{410}$ embryos at 5dpf. The liver area was defined by immunostaining of Bhmt (green). Compared to the WT embryos, no abnormal apoptotic activities were observed in $sas10^{42}$ and $mpp10^{410}$ mutant embryos. DNaseI treated embryos were used as the positive control for apoptotic cells (red). At least 16 cryosections from three embryos were examined. Bar: 50µm. (B) Northern blot analysis of rRNA precursors using the 18S-F (left panel) and 18S-B (right panel) probes in 5dpf-old WT, $sas10^{42}$ (s^{42}) and $mpp10^{410}$ (m^{410}) embryos. 28S: loading control.

Supplementary Figure S7. (A) Sas10 directly interacts with Mpp10. *sas10* and *mpp10* coding sequences were cloned to the vector harboring the Gal4-activation domain (AD-V, vector; AD-S, AD-Sas10; AD-M, AD-Mpp10) or the vector harboring DNA-binding domain (BD-V, vector; BD-S, BD-Sas10; BD-M, BD-Mpp10), respectively, for yeast two-hybrid experiments as shown. Mpp10 showed an auto-activation activity (AD-V + BD-M

panel). (B) His-tagged Capn3 and Capn3^{C129S} (C129S) expressed in the SF9 cells were purified using the Ni-NTA agarose beads. Proteins in different purification process were stained by Coomassie brilliant blue. CE, total protein extract from the cultured cells; FT, flow-through protein; wash1-3, supernatant collected after the beads being washed with washing buffer; elute1, eluted protein from the beads. Red arrow, Capn3; Black arrow, Capn3^{C129S}. Smaller fragments in the elute1 sample likely represent the cleaved or early termination of translation products harboring the His-tag. (C) Western blot of Capn3 and Capn3^{C129S} in different purification process using the anti-Capn3 antibody. Since this anti-Capn3 antibody was raised using the C-terminal 610-640 aa as the antigen, it cannot detect C-terminus-truncated Capn3 observed in the elute1 shown in (B). WT Capn3 exhibited quick autocleavage after purification and majority of WT Capn3 displayed a smaller sizes compared with Capn3^{C129S} (C129S). *, cleaved Capn3 fragments. (D,E) His-tagged Mpp10 (D) and Mpp10-X (E) expressed in the *E.coli* were purified using the Ni-NTA agarose beads. Proteins in different purification process were stained by Coomassie brilliant blue. none-induced, total protein from *E.coli* without IPTG induction (as a negative control); induced, total protein from *E.coli* with IPTG induction; supernatant, soluble proteins after centrifugation of the cell lysates; pellet, insoluble proteins after centrifugation of the cell lysates; FT, flow-through proteins; wash1-3, supernatant after the beads being washed with washing buffer; elute1-5, eluted proteins from the beads. Red arrow, Mpp10; Black arrow, Mpp10-X.

Supplementary Figure S8. Mpp10 is a Capn3 target. (A) Cell lysates containing Capn3 or its inactive mutant Capn3^{C129S} (C129S) were mixed with Ni-NTA beads purified Mpp10.

Reaction mixture containing 3 mM Ca²⁺ was incubated at 37°C for different time intervals (minutes) as indicated. (**B**) Cell lysates containing Capn3 and Mpp10 were mixed and placed on ice for 0, 10 and 30 min. (**C**) Western blot of Mpp10, Capn3b and β -Actin in the WT and *capn3b*^{-/-} mutant. Total proteins were extracted from 5dpf-old embryos. Value below the figures indicated the relative intensity of Mpp10/Actin where WT was taken as 1.0.

Supplementary Figure S9. Nucleolus-localisation of Mpp10 depends on Sas10. (**A**) *HA-Sas10* plasmid transfected 293T cells were co-stained with antibodies against Sas10 and HA-tag. *Myc-Mpp10* plasmid transfected 293T cells were co-stained with antibodies against Mpp10 and Myc-tag. Untransfected cells were used as the negative control. Bar, 10µm. (**B**) HA-Sas10, Myc-Mpp10 were over-expressed alone or together in 293T cells. The cells were subjected to immunostaining using antibodies against HA-tag, Myc-tag or Fib as indicated. Bar, 10µm. (**C**) Immunostaining of HA-Sas10 or HA-Sas10-s5 (lacking the Mpp10-interacting domain) over-expressed in 293T cells using antibodies against HA-tag or Fib as indicated. Bar, 10µm.

Supplementary Figure S10. (**A**,**B**) *HA-sas10* or *HA-sas10-s5* mRNA was injected into one-cell stage WT and $sas10^{42}$ (s^{42}) embryos, respectively. Total proteins were extracted at 28 hour post injection (hpi) for Western blot of Mpp10, Sas10 and Tub (**A**). The signal of maternal Sas10 in s^{42} were low but still distinguishable. The nonspecific band detected by Mpp10 antibody is likely due to protein extraction without deyolk. Embryos were also fixed and sectioned for co-immunostaining of Mpp10 and Fib (**B**). The big box shows the high magnification view of the nucleoli in the small box. The nucleolus-localisation of Mpp10 was only detected in WT and s^{d2} injected with *HA-sas10* mRNA but not in s^{d2} and s^{d2} injected with *HA-sas10-s5* mRNA. Bar, 5µm. (C) *Myc-Mpp10* was co-expressed with HA-Imp3 or HA-Imp4 in 293T cells for Co-IP analysis using an anti-HA antibody. Mpp10 was detected with its specific antibody and Imp3 and Imp4 were detected with anti-HA antibody.

REAGENT or RESOURCE	SOURCE	IDENTIFIER	
Antibodies			
Mouse monoclonal Bhmt antibody	Huabio	Hangzhou, China, Clone# D5G5	
Mouse monoclonal Fibrillarin antibody	Abcam	Cat# ab4566; Clone# 38F3	
Mouse monoclonal α-Tubulin antibody	Beyotime	Cat# AT819-1; Clone# B-5-1-2	
Mouse monoclonal HA antibody	Beyotime	Cat# AH158; Clone# HA-7	
Mouse monoclonal Sas10 antibody	Huabio	Hangzhou, China, Clone# E4D4	
Rabbit polyclonal β-Actin antibody	Huabio	Hangzhou, China, Clone# 1207-1	
Rabbit polyclonal Sas10 antibody	Huabio	Hangzhou, China, Clone# 3382	
Rabbit polyclonal Mpp10 antibody	Huabio	Hangzhou, China, Clone# 3570	
Rabbit polyclonal Def antibody	Huabio	Hangzhou, China, Clone# 3203L	
Rabbit polyclonal Capn3 antibody	Abcam	Cat# ab103250; Clone# N/A; RRID: N/A	
Rabbit polyclonal HA antibody	Sigma-Aldrich	Cat# H6908; Clone# N/A; RRID: I2149	
Rabbit polyclonal C-Myc antibody	Huabio	Cat# 0912-2; Clone# N/A; RRID: N/A	
Chemicals			
AciI (SsiI) restriction enzyme	Thermo	Cat# ER1791	
	Scientific		
Albumin Bovine Fraction V	HXbio	Cat# D332	
Anti-Digoxigenin-AP Fab fragments	Roche	Cat# 11093274910	
Adhesion Microscope Slides	Citoglas	Cat# 188105	
Anti-HA-Tag mAb (Agarose conjugated)	Abmart	Cat# M20013	
Anti-Myc-Tag mAb (Agarose conjugated)	Abmart	Cat# M20012	
Benzyl alcohol	Sigma-Aldrich	Cat# 402834	
Benzyl benzoate	Sigma-Aldrich	Cat# B6630	
CDP-Star	Roche	Cat# 12041677001	
Cell lysis buffer for Western and IP	Beyotime	Cat# P0013	
cOmplete, EDTA-free	Roche	Cat# 16829800	
Difco TM Yeast Nitrogen Base w/o Amino Acids	Becton,	Cat# 291940	
	Dickinson and		
	Company		
DMEM High Glucose	Biological	Cat# 06-1055-57-1ACS	
	Industries		
DNase I	Thermo	Cat# EN0521	
	Scientific		
Fetal Bovine Serum	Gibco	Cat# 10270	

YEASEN	Cat# 20502ES10	
Thermo	Cat# 88529	
Scientific		
Biological	Cat# 02-024-1ACS	
Industries		
Sigma-Aldrich	Cat# P4338	
SignaGen	Cat# 100688	
Aidlab	Cat# RN01	
Bio-light	Cat# BL-025TE-00	
Biotech		
New England	Cat# R0151S	
Biolabs		
Gibco	Cat# 10902088	
Beyotime	Cat# C3206	
Beyotime	Cat# P0013	
Roche	Cat# 11277065910	
Roche	Cat# 11277073910	
Trans	Cat# DW101-02	
Roche	Cat# 12156792910	
Sunky	Cat# 61804	
Axygen	Cat# 156	
Santa Cruz	Cat# SC-8656-R	
Axygen	Cat# 155	
Clontech	Cat# PT4084-1	
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ATCC	Cat# CRL-3216; RRID:	
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·	•	
Singapore	N/A	
This paper	N/A	
This paper	N/A	
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This paper	N/A	
This paper	N/A	
This paper	N/A	
	Thermo ScientificBiological IndustriesIndustriesSigma-AldrichSigmaGenAidlabBio-light BiotechNew England BiolabsGibcoBeyotimeBeyotimeRocheTransRocheSunkyAxygenSanta CruzAxygenClontechSingaporeThis paperThis paperThis paperThis paper	

5'-GACAGTGATGATCCTGAAGCGT-3'			
Primers for sas10 mutant ID, Reverse:	This paper	N/A	
5'-TCTAGATCGCTCTCCATGTCTG-3'			
Sequencing primers 1 for sas10, Forward:	This paper	N/A	
5'-AGAGGAAGACGAGGCTACAA-3'			
Sequencing primers 2 for sas10, Forward:	This paper	N/A	
5'-GCGCTTCTATAACGCCATGG-3'			
Primer: Sas10(1-470aa), Forward:	This paper	N/A	
5'-CGCGGATCCATGGTCCGAGCAAGA-3'			
Primer: Sas10(1-470aa), Reverse:	This paper	N/A	
5'-TCCCCCGGGGAGTTTGACACTTCT-3'			
Primer: 5'ETS probe, Forward:	This paper	N/A	
5'- CCGGTCTACCTCGAAAGTCC-3'			
Primer: 5'ETS probe, Reverse:	This paper	N/A	
5'-CGAGCAGAGTGGTAGAGGAA-3'			
Primer: ITS1 probe, Forward:	This paper	N/A	
5'-CTCGGAAAACGGTGAACCTG-3'			
Primer: ITS1 probe, Reverse:	This paper	N/A	
5'-GTGTTCGTTTCAGGGTCCGA-3'			
Primer: 18S F probe, Forward:	This paper	N/A	
5'-ATCGCTCCATCCGTTACTTG-3'			
Primer: 18S F probe, Reverse:	This paper	N/A	
5'-GCGCCTAAAGTACCATCGAA-3'			
Primer: 18S F probe, Forward:	This paper	N/A	
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Primer: 18S F probe, Reverse:	This paper	N/A	
5'-CCTCGTTGATGGGAAACAGT-3'			
Primer: Sas10(1-150aa), Reverse:	This paper	N/A	
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Primer: Sas10(100-320aa), Forward:	This paper	N/A	
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Primer: Sas10(200-470aa), Forward:	This paper	N/A	
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Primers for <i>mpp10</i> mutant ID, Forward:	This paper	N/A	
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Primers for <i>mpp10</i> mutant ID, Reverse:	This paper	N/A	
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Sequencing primers 1 for <i>mpp10</i> , Forward:	This paper	N/A	

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Sequencing primers 2 for <i>mpp10</i> , Forward:	This paper	N/A
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Sequencing primers 3 for <i>mpp10</i> , Forward:	This paper	N/A
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Primer: Mpp10(1-695aa), Forward:	This paper	N/A
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Primer: Mpp10 (1-440aa), Reverse:	This paper	N/A
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Primer: Mpp10 (231-600aa), Reverse:	This paper	N/A
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Primer: Mpp10 (211-220aa deletion), Forward:	This paper	N/A
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Primer: Imp3, Forward:	This paper	N/A
5'- CGGGATCCGTACGTAAATTAAAATTTCACGAGC-3'		
Primer: Imp3, Reverse:	This paper	N/A
5'- CGGAATTCTTAGGCCATGAGGTCAAAGT-3'		
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Primer: Imp3, Reverse:	This paper	N/A

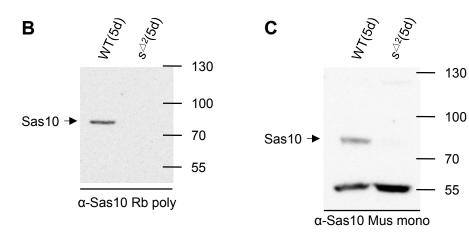
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Recombinant DNA			
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pCS2+-HA-Sas10(1-320aa)	This paper	N/A	
pCS2+-HA-Sas10(100-320aa)	This paper	N/A	
pCS2+-HA-Sas10(200-470aa)	This paper	N/A	
pCS2+-HA-Sas10(226-307aa deletion)	This paper	N/A	
pCS2+-Mpp10	This paper	N/A	
pCS2+-HA-Mpp10	This paper	N/A	
pCS2+-Myc-Mpp10	This paper	N/A	
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pCS2+-Myc-Mpp10(1-440aa)	This paper	N/A	
pCS2+-Myc-Mpp10(230-560aa)	This paper	N/A	
pCS2+-Myc-Mpp10(440-695aa)	This paper	N/A	
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pCS2+-Myc-Mpp10(230-600aa)	This paper	N/A	
pCS2+-Myc-Mpp10(230-650aa)	This paper	N/A	
pCS2+-Myc-Mpp10(1-440aa, 211-220aa deletion)	This paper	N/A	
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pcDNA ⁺ -HA-Capn3	(29)	N/A	
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pcDNA+- Capn3C129S	(29)	N/A	
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pFastBac-Capn3C129S	(29)	N/A	
pGAD-Sas10	This paper	N/A	
pGAD-Mpp10	This paper	N/A	
pGBK-Sas10	This paper	N/A	
pGBK-Mpp10	This paper	N/A	
Software and Algorithms			
Motif Scan	MyHits database	https://myhits.isb-sib.ch/cgi- bin/motif_scan	

Α

hu MVGRSRRRGAAKWAAVRAKAGPTLTDENGDDLGLPPSPGDTSYYQDQVDDFHEARSRAAL mu MVKKSRRRGAAQWAAVRAQAGLTATDENEDDLGLPPSPGDSSYYQDQVDEFHEARSRAVL zf MVRARRAVKKPRTKKQQEYDSDDPEAYTNEAIPDKKSSSYVEDKVDEFHNEKISKLL	A 61
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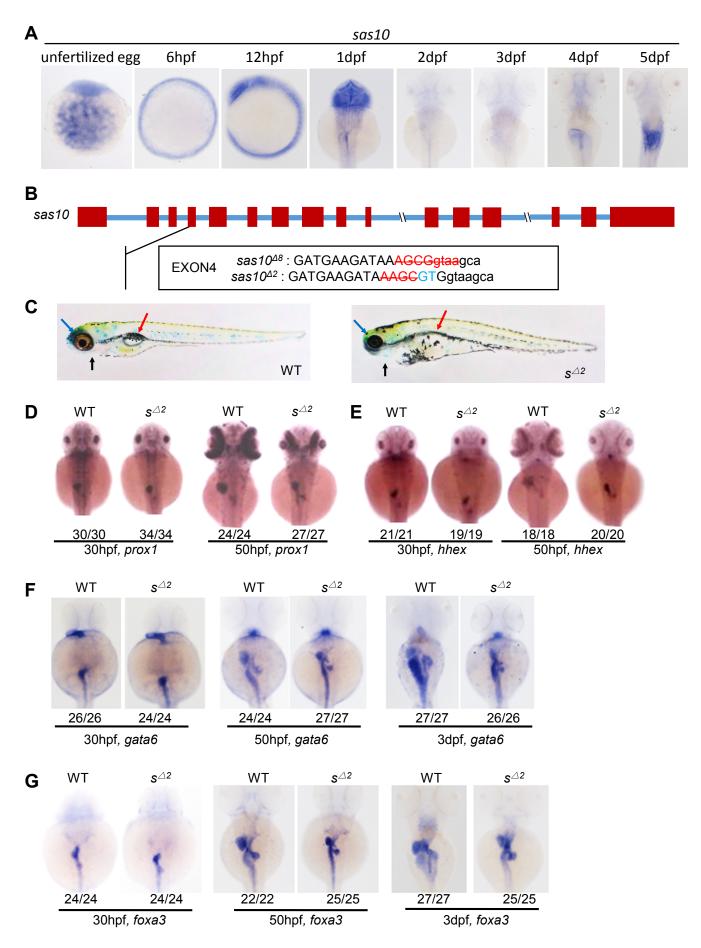
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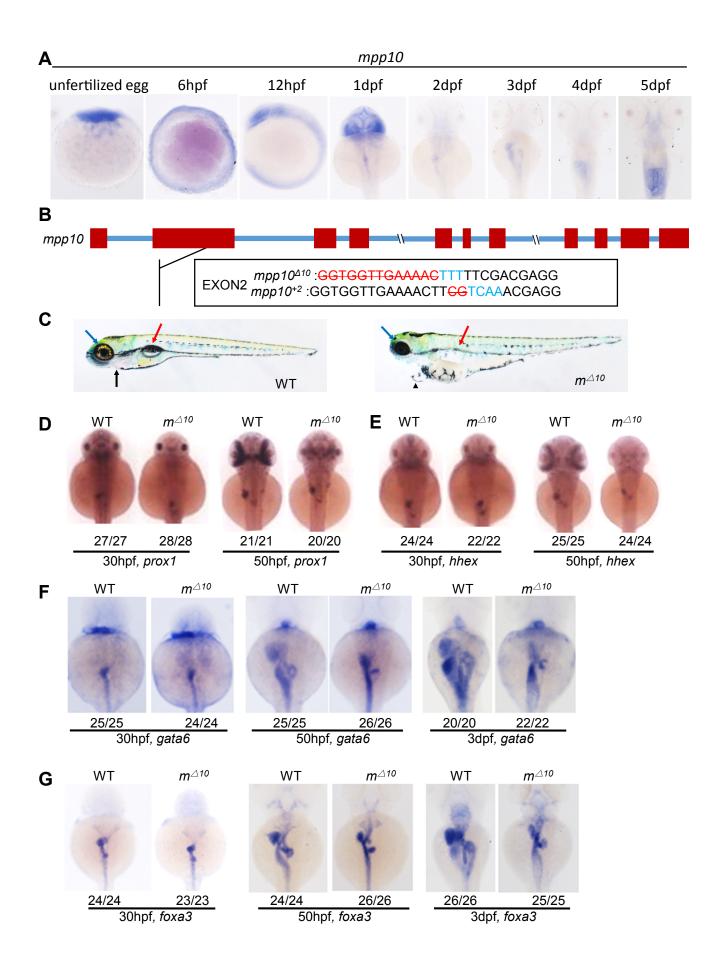
Sas10/Utp3/C1D family

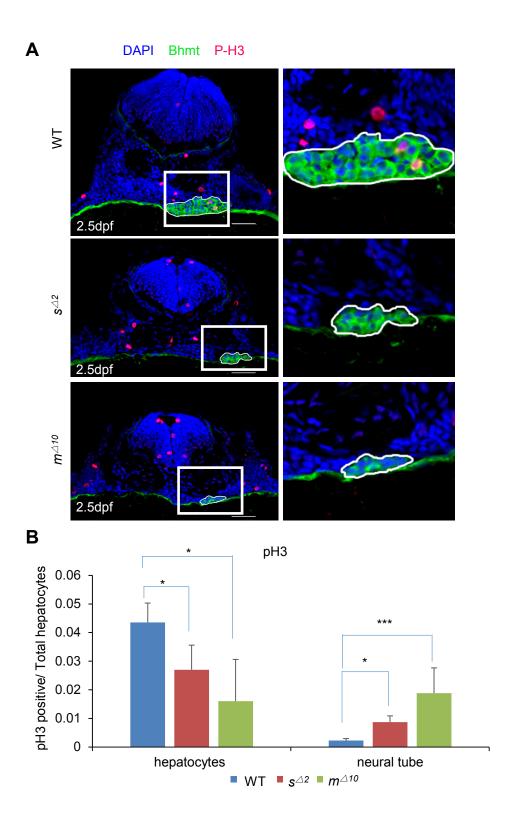


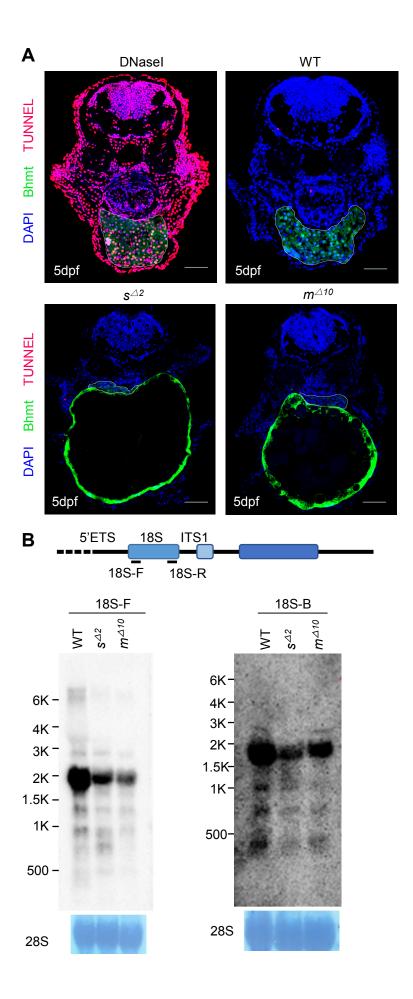
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hu PLQKLVIENFDDEQIWQQLELQNEPILQYFQNAVSETINDEDISLLPESEEQEREEDGSEI 122 mu plqkleINSFDDEQIWQQLELQNEPVLQYFQNAVSETVEDEDISLLPECEDEECEEDASEV 122 zf pleqLvvenfdeeqIwqelelqNSALLTHFEQAVEQAVTDDAITFLND-EDDDVEEDEEL 119	
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hu FEDIDSDEDEGGLFGSKKLKSGKSSRNLKYKDFFDPVESDED-ITNVHDDELDSN 290 mu FEDIDSDESEGGLFGRQKIKSNKSSRNLKYKDFFDPVESDED-ITGVDEELGPDE 292 zf FQDLPSDEDDELSFDKPVVKKKKLKNKSSRNMKYKDFFAPVDGEPDPIPDEEKELESNDDYE 295	
hu -KEDDEIAEEEAEELSISETDEDDDLQENEDNKQHKESLKRVTFALPDDAETEDTGVLNVK 350 mu -EKEEEEGFAEEADESISDTDEDNDLEEDENSDQHKGSLKRVTFALPDD-EAEDTSPLAVK 351 zf geeqdddeleeeyeedMneedFdMerddddigdKvRdgSRRVTFDLPddsegedvedilgg 356	
hu KNSDEVKSSFEKRQEKMNEKIASLEKELLEKKPWQLQGEVTAQKRPENSLLEETLHF 407 mu QESDEVKSSFEKRQEKMNEKIASLEKELLDKKPWQLQGEVTAQKRPENSLLEETLHF 408 zf KAKDVPKPESKSSFEKRQEKMVKQIEELENAALSEKPWQLSGEVTAQTRPENSMLEEDIAF 417	
hu DHAVRMAPVITEETTLQLEDIIKQRIRDQAWDDVVRKEKPKEDAYEYKKRLTLDHEKSKLS 468 mu DHAVRMAPVITEETTLHLEDIIKQRIRDQAWDDVERKEKPKEDAYEYKKRLTLDHEKSKLS 469 zf DQASRMAPAITEETTLQLEDIIKQRIKDQVWDDVVRKEKPKEEVFEYKKRLTLDHEKSKLS 478	
hu LAEIYEQEYIKLNQQKTAEEENPEHVEIQKMMDSLFLKLDALSNFHFIPKPPVPEIKVVSN 529 mu LAEIYEQEYLKLNQQKTEEEDNPEHVEIQKMMDSLFLKLDALSNFHFIPKPPVPEIKVVSN 530 zf LAEVYEQEYIKQTQEKKEEEENPAHVEIQKLMDSLFLKLDALSNFHFTPKPHVPEVKVVSN 539	
hu LPAITMEEVAPVSVSDAALLAPEEIKEKNKAGDIKTAAEKTATDKKRERRKKKYQKRMKIK 590 mu LPAITMEEVAPVSVSDAALLAPEEIKEKNKAGDLKTAAEKTATDKKRERRKKKYQKRLKIK 591 zf LPTITMEEVAPVNVSDATLLAPEEIKEKNKAGDILGDTEKTTTDKKRERRKKKKKLKRLKIH 600	
hu EKEKRRKLLEKSSVDQAGKYSKTVASEKLKQLTKTGKASFIKDEGKDKALKSSQAFFS 648 mu EKEKRKKLLEKNNPDQS-KSSRAAASEKLKQLTKTGKVSLLKDERKDKPLKSSQAFFS 648 ^{Zf} EREKRQKLKEAARGGADDGKKKKSKAEVEQTIKKLTKGGKAKVLTNDGMDKALRSSQAFFS 664	
hu KLQDQVKMQINDAK-KTEKKKKKRQDISVHKLKL 681 mu KLQDQVKMQINDAK-QPEKIKKKKQDISVHKLKL 681 zf _Q LQDQVRSQVKGSKDQTGKKKKKQKEISVNKLKL 695	
Capn3 recognize site Ct-Utp3 Yeast-Imp3 hu-Imp4 hu-Imp3	
B ⁽⁰ 5) ₁ ⁽⁰ 5) ₁ ⁽⁰ 5) ₁	
Mpp10 ► 130	
- 100	
70	
55 g-Map10 Rb poly	

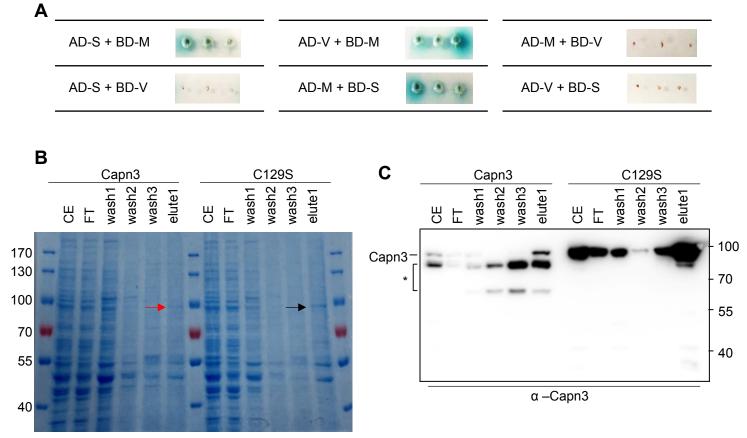
α-Mpp10 Rb poly



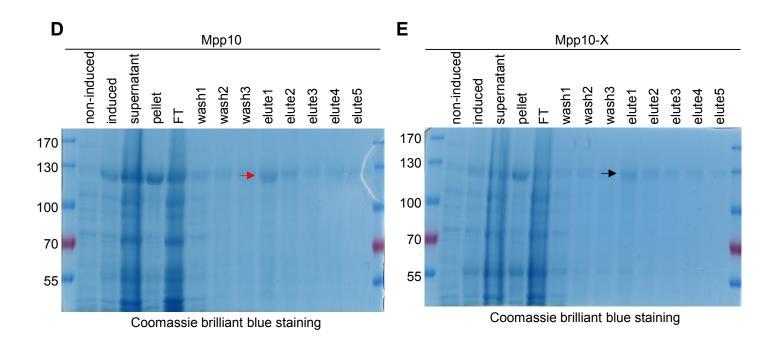


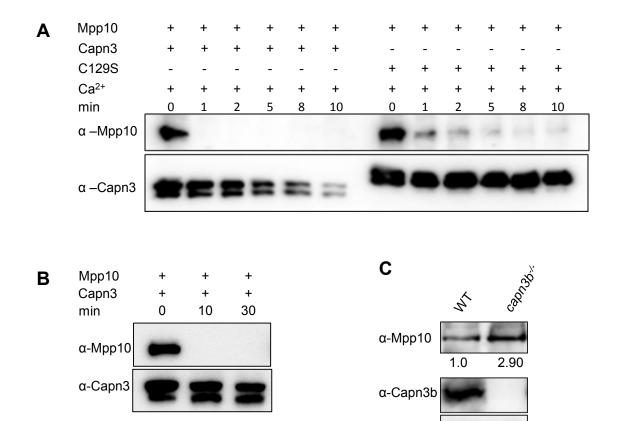






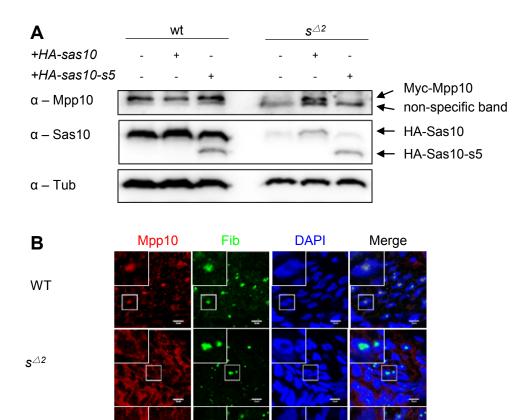
Coomassie brilliant blue staining





α-Actin

A +HA-Sas10	Sas10 🐧	HA 🌷	DAPI	Merge	Bright
untransfected control	Sas10	HA —	DAPI	Merge	Bright
+Myc-Mpp10	Mpp10	Myc	DAPI	Merge	Bright
untransfected control	Mpp10	Myc	DAPI	Merge	Bright
B +HA-Sas10	HA A	Fib	DAPI	Merge	Bright
+Мус-Мрр10	Myc Hyc Hon	Fib	DAPI	Merge	Bright
+HA-Sas10 Myc-Mpp10	HA or other	Fib	DAPI	Merge	Bright
+HA-Sas10 Myc-Mpp10	Myc	Êib _{19m}	DAPI	Merge	Bright
C +HA-Sas10	€R €]	Fib	DAPI	Merge	Bright
+HA-Sas10-s5		Fib	DAPI	Merge	Bright



~

S∕∆2

S⊿2

+HA-Sas10

+HA-Sas10-s5

