# Znf598-mediated Rps10/eS10 ubiquitination contributes to the ribosome ubiquitination dynamics during zebrafish development

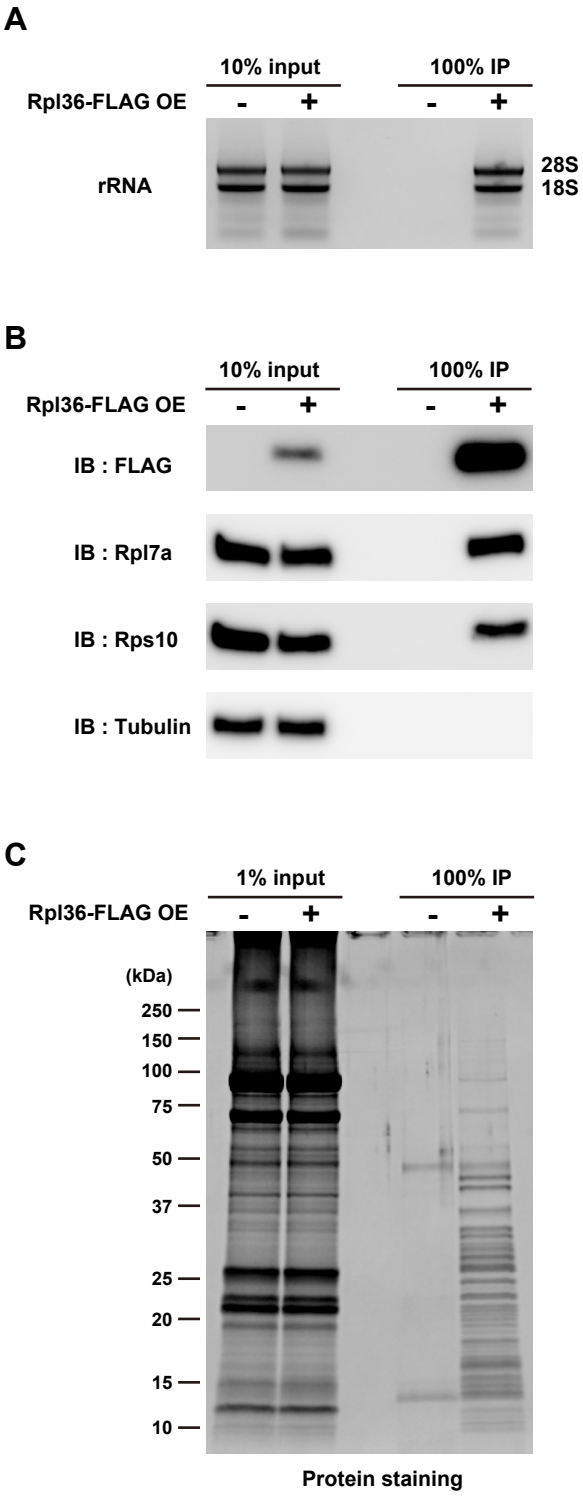
**Ugajin et al.**

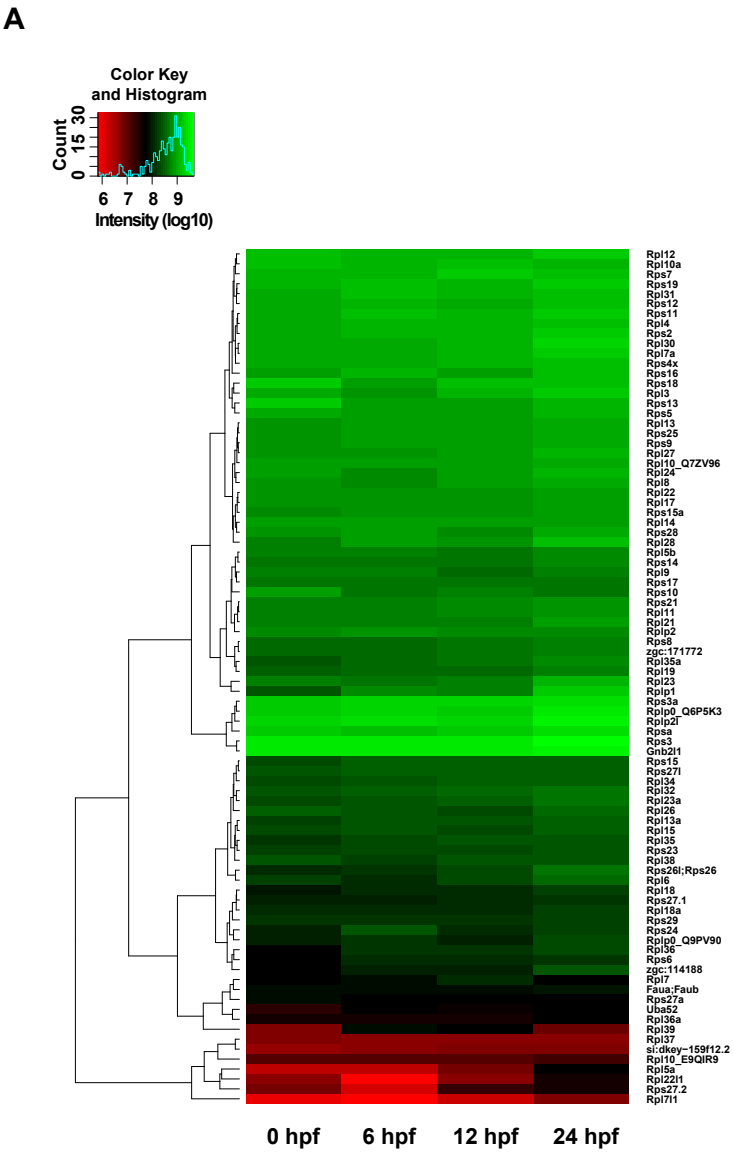
**Supplemental Materials**

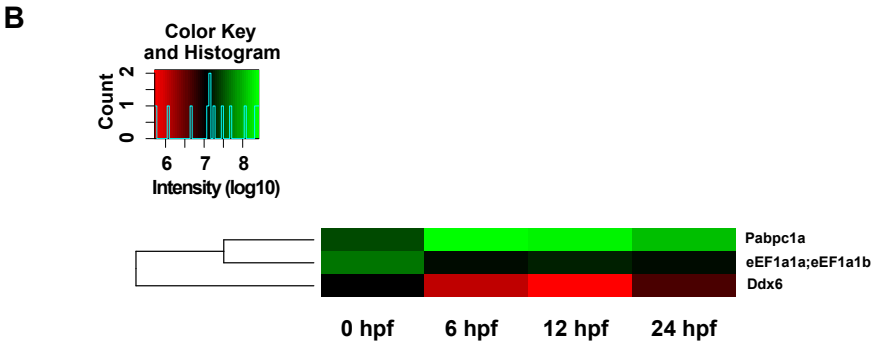
Supplemental Figures S1-S6

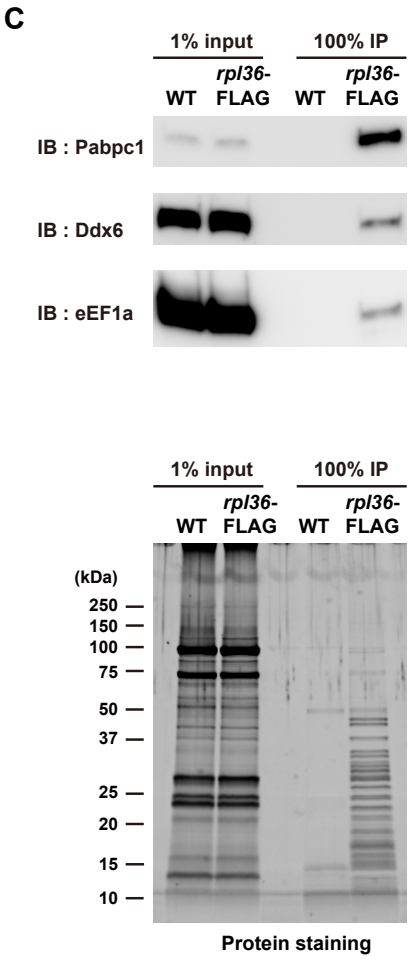
Supplemental Figure legends S1-S6

Supplemental Tables S1-S4

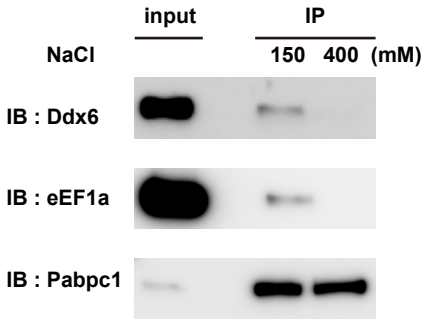

A
10% input
100% IP
Rpl36-FLAG OE
-
+
-
+
rRNA
28S
18S
B
10% input
100% IP
Rpl36-FLAG OE
-
+
-
+
IB : FLAG
IB : Rpl7a
IB : Rps10
IB : Tubulin
C
1% input
100% IP
Rpl36-FLAG OE
-
+
-
+
(kDa)
250
150
100
75
50
37
25
20
15
10
Protein staining


A
Color Key and Histogram
Count
0 15 30
6 7 8 9
Intensity (log10)
Rpl12
Rpl10a
Rps7
Rps19
Rpl31
Rps12
Rps11
Rpl4
Rps2
Rpl30
Rpl7a
Rps4x
Rps16
Rps18
Rpl3
Rps13
Rps5
Rpl13
Rps25
Rps9
Rpl27
Rpl10_Q7ZV96
Rpl24
Rpl8
Rpl22
Rpl17
Rps15a
Rpl14
Rps28
Rpl28
Rpl5b
Rps14
Rpl9
Rps17
Rps10
Rps21
Rpl11
Rpl21
Rplp2
Rps8
zgc:171772
Rpl35a
Rpl19
Rpl23
Rplp1
Rps3a
Rplp0_Q6P5K3
Rplp2l
Rpsa
Rps3
Gnb2l1
Rps15
Rps27l
Rpl34
Rpl32
Rpl23a
Rpl26
Rpl13a
Rpl15
Rpl35
Rps23
Rpl38
Rps26l;Rps26
Rpl6
Rpl18
Rps27.1
Rpl18a
Rps29
Rps24
Rplp0_Q9PV90
Rpl36
Rps6
zgc:114188
Rpl7
Faua;Faub
Rps27a
Uba52
Rpl36a
Rpl39
Rpl37
si:dkey-159f12.2
Rpl10_E9QIR9
Rpl5a
Rpl22l1
Rps27.2
Rpl7l1
0 hpf
6 hpf
12 hpf
24 hpf

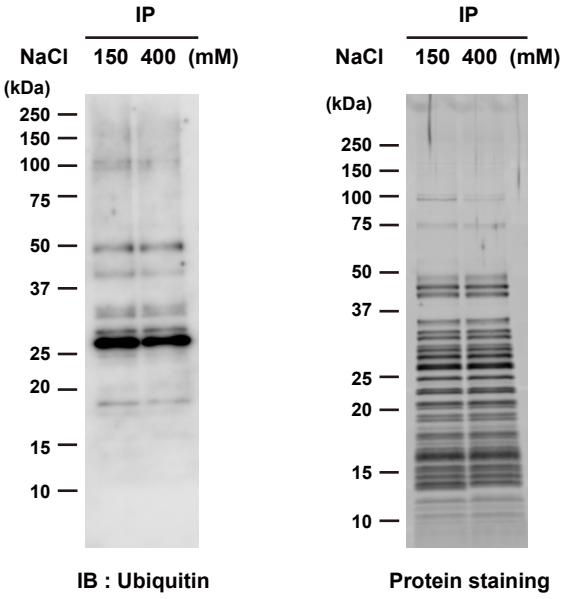

B
Color Key and Histogram
Count
0 1 2
6 7 8
Intensity (log10)
Pabpc1a
eEF1a1a;eEF1a1b
Ddx6
0 hpf
6 hpf
12 hpf
24 hpf


C
1% input
100% IP
WT
rpl36-FLAG
WT
rpl36-FLAG
IB : Pabpc1
IB : Ddx6
IB : eEF1a
1% input
100% IP
WT
rpl36-FLAG
WT
rpl36-FLAG
(kDa)
250
150
100
75
50
37
25
20
15
10
Protein staining

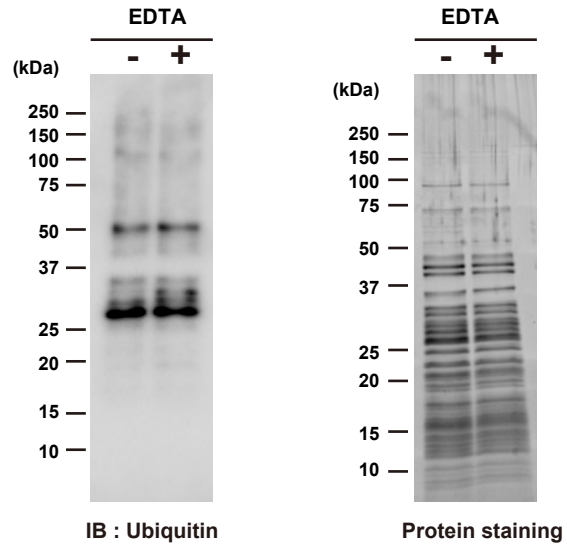
A

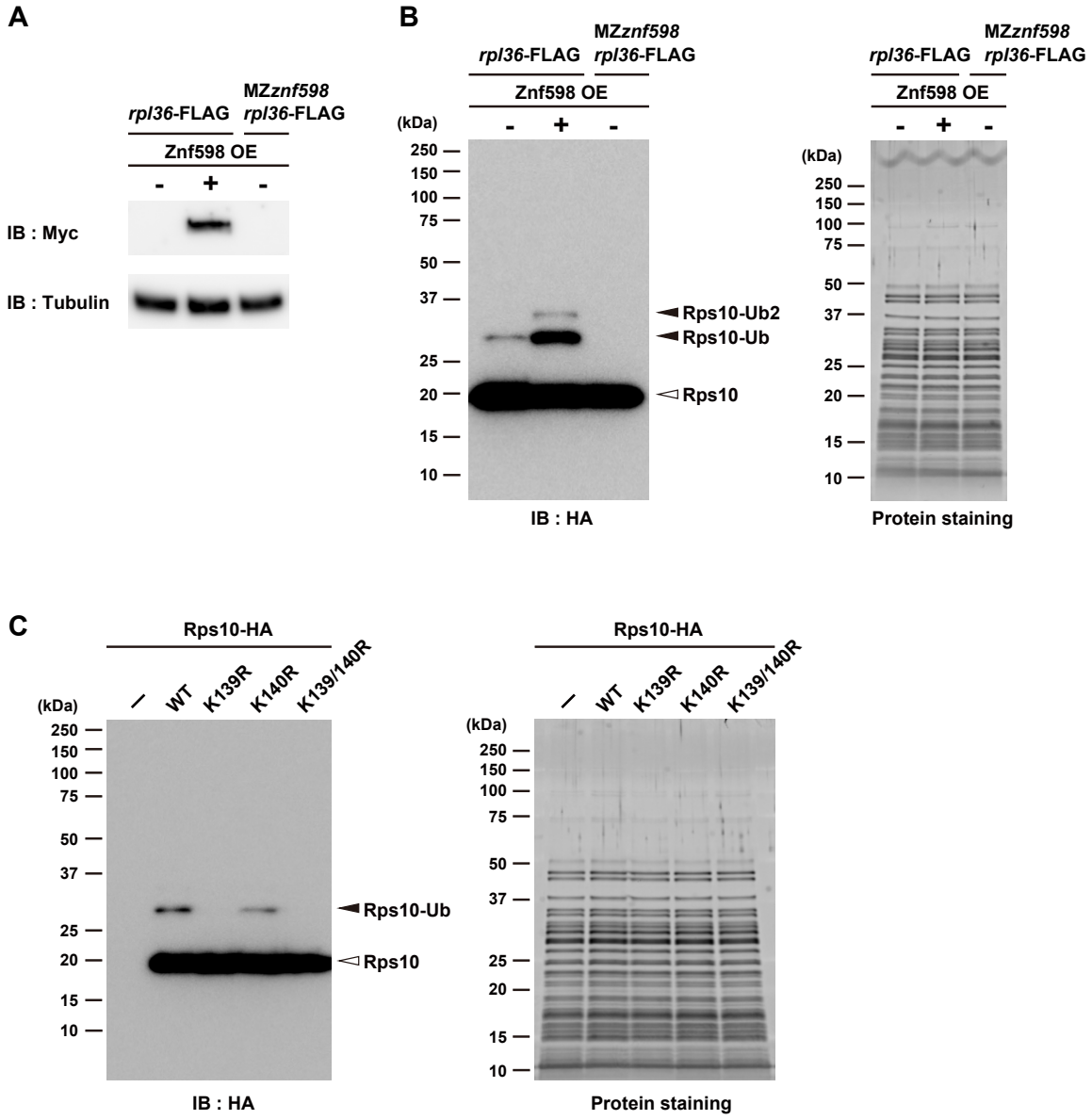

input
IP
NaCl
150 400 (mM)
IB : Ddx6
IB : eEF1a
IB : Pabpc1

B

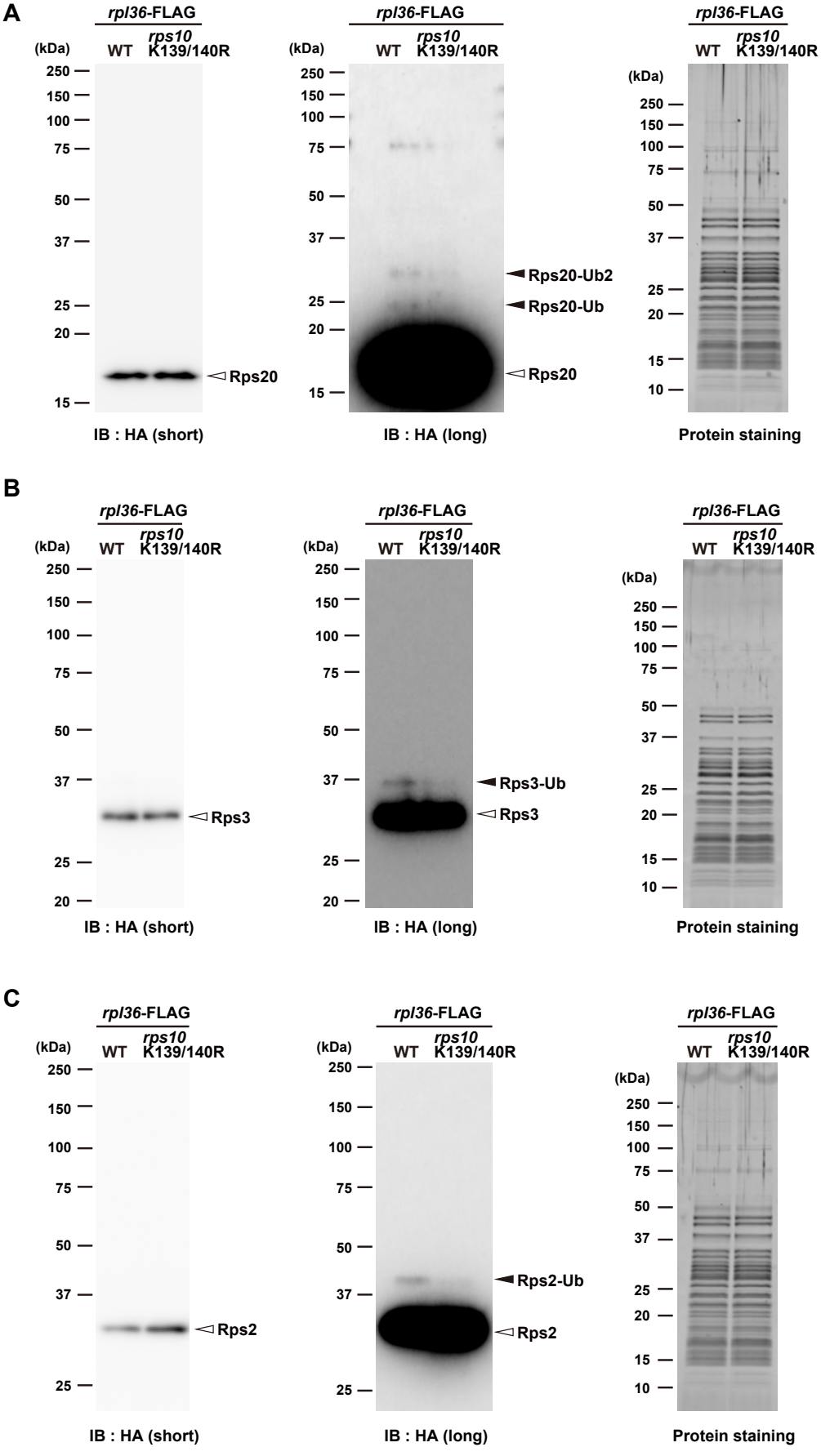

IP
NaCl 150 400 (mM)
(kDa)
250
150
100
75
50
37
25
20
15
10
IB : Ubiquitin
IP
NaCl 150 400 (mM)
(kDa)
250
150
100
75
50
37
25
20
15
10
Protein staining

C


EDTA
- +
(kDa)
250
150
100
75
50
37
25
20
15
10
IB : Ubiquitin
EDTA
- +
(kDa)
250
150
100
75
50
37
25
20
15
10
Protein staining


A
rpl36-FLAG
MZznf598 rpl36-FLAG
Znf598 OE
- + -
IB : Myc
IB : Tubulin
B
rpl36-FLAG
MZznf598 rpl36-FLAG
Znf598 OE
- + -
(kDa)
250
150
100
75
50
37
25
20
15
10
Rps10-Ub2
Rps10-Ub
Rps10
IB : HA
Protein staining
C
Rps10-HA
– WT K139R K140R K139/140R
Rps10-Ub
Rps10
IB : HA
Protein staining

Ugajin et al., Figure S5



Ugajin et al., Figure S6

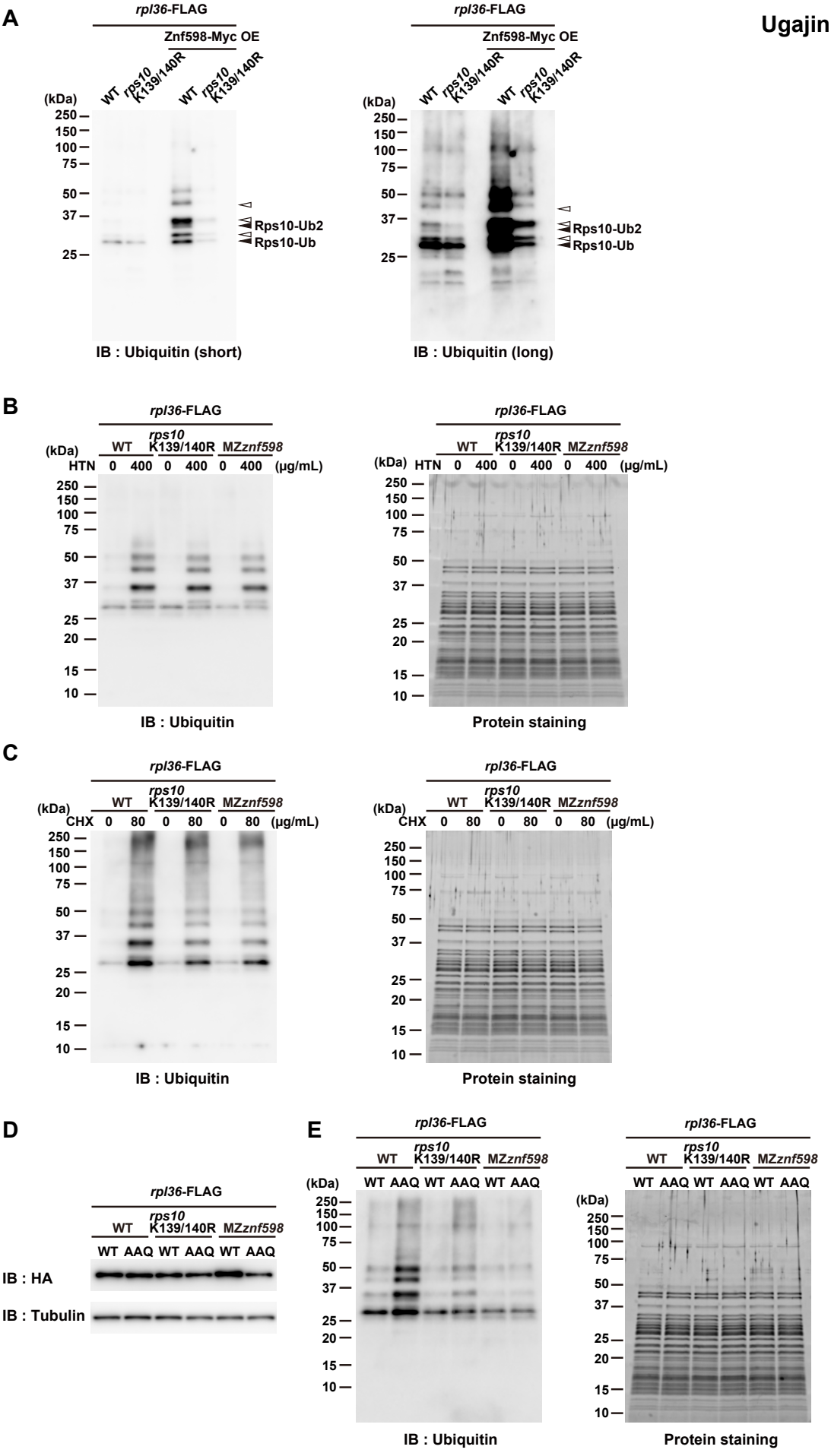


**Figure S1. Exogenously overexpressed Rpl36-FLAG enables us to purify the assembled ribosomes. Related to Figure 1.**

(A-C) Validation of ribosome purification using exogenously overexpressed Rpl36-FLAG. Total lysates (input) and FLAG-immunoprecipitants (IP) in the presence (+) or absence (-) of Rpl36-FLAG overexpression were subjected to RNA electrophoresis (A), immunoblotting analysis using antibodies against the indicated proteins (B), and protein staining (C).

**Figure S2. The *rpl36*-FLAG strain enables us to purify the ribosome complex engaging in translation. Related to Figure 1.**

(A-B) Heat map showing ribosomal proteins (A) or a part of the copurified proteins (B) identified by the mass spectrometry analysis. The log10 scale is used to display the mean signal intensity of two biological replicates. The developmental time points are indicated below as hours post-fertilization (hpf). (C) Analysis of the copurified proteins detected by mass spectrometry analysis in (B). Total lysates (input) and FLAG-immunoprecipitants (IP) obtained from wild-type (WT) and *rpl36*-FLAG embryos at 24 hpf were subjected to immunoblotting analysis using antibodies against the indicated proteins (upper) and protein staining (lower).

**Figure S3. Ubiquitination signals detected in Figure 2A mainly derive from core ribosome 40S components. Related to Figure 2 and Figure 3.**

(A) Comparison of interactions under normal (150 mM NaCl) and high salt (400 mM NaCl) wash conditions. Total lysate (input) and FLAG-immunoprecipitants (IP) were subjected to immunoblotting analysis using antibodies against the indicated proteins. Salt

concentrations are indicated above. (B) Comparison of ribosome ubiquitination levels under normal (150 mM NaCl) and high salt (400 mM NaCl) wash conditions. FLAG-immunoprecipitants in (A) were subjected to immunoblotting analysis with an anti-Ubiquitin antibody (left) and protein staining (right). Salt concentrations are indicated above. (C) Comparison of ribosome ubiquitination levels with or without EDTA incubation after purification. FLAG-immunoprecipitants obtained from EDTA-free conditions were incubated with or without EDTA and subjected to immunoblotting analysis with an anti-Ubiquitin antibody (left) and protein staining (right).

**Figure S4. Znf598 ubiquitinates K139 and K140 in Rps10/eS10. Related to Figure 4.**

(A-B) Detection of Rps10-HA ubiquitination. Total lysates were subjected to immunoblotting analysis of Znf598-Myc and Tubulin (A). FLAG-immunoprecipitants were subjected to immunoblotting analysis with an anti-HA antibody (B, left) and protein staining (B, right). White and black arrowheads indicate non-ubiquitinated or ubiquitinated Rps10-HA signals, respectively. (C) Validation of Rps10/eS10 ubiquitination sites. FLAG-immunoprecipitants were subjected to immunoblotting analysis with an anti-HA antibody (left) and protein staining (right). White and black arrowheads indicate non-ubiquitinated or ubiquitinated Rps10-HA signals, respectively.

**Figure S5. Rps20/uS10, Rps3/uS3, and Rps2/uS5 ubiquitination depend on Rps10/eS10 ubiquitination. Related to Figure 5.**

(A-C) Comparison of Rps20-HA (A), Rps3-HA (B), and Rps2-HA (C) ubiquitination levels in *rpl36*-FLAG and *rps10* K139/140R; *rpl36*-FLAG embryos. FLAG-

immunoprecipitants were subjected to immunoblotting analysis with an anti-HA antibody (left and middle) and protein staining (right). White and black arrowheads indicate non-ubiquitinated or ubiquitinated signals, respectively.

**Figure S6. Rps10/eS10 ubiquitination is a prerequisite for multiple ubiquitination events promoted by Znf598. Related to Figure 5.**

(A) Comparison of ribosome ubiquitination levels under Znf598 overexpression in *rpl36*-FLAG and *rps10* K139/140R; *rpl36*-FLAG embryos. FLAG-immunoprecipitants in Figure 5C were subjected to immunoblotting analysis with an anti-Ubiquitin antibody. Black arrowheads indicate putative ubiquitinated Rps10/eS10 signals. White arrowheads indicate reduced ubiquitination signals in *rps10* K139/140R; *rpl36*-FLAG embryos. (B-C) Comparison of ribosome ubiquitination levels under harringtonine (HTN) (B) and cycloheximide (CHX) (C) treatment in *rpl36*-FLAG, *rps10* K139/140R; *rpl36*-FLAG, and MZ*znf598*; *rpl36*-FLAG embryos. FLAG-immunoprecipitants were subjected to immunoblotting analysis with an anti-Ubiquitin antibody (left) and protein staining (right). Concentrations of drugs are indicated above. (D-E) Comparison of ribosome ubiquitination levels under eRF1-AAQ overexpressed conditions in *rpl36*-FLAG, *rps10* K139/140R; *rpl36*-FLAG, and MZ*znf598*; *rpl36*-FLAG embryos. Total lysates were subjected to immunoblotting analysis of eRF1-HA and Tubulin (D). FLAG-immunoprecipitants were subjected to immunoblotting analysis with an anti-Ubiquitin antibody (E, left) and protein staining (E, right).