Supplemental Data

Global Transcriptional Repression

in *C. elegans* Germline Precursors

by Regulated Sequestration of TAF-4

Tugba Guven-Ozkan, Yuichi Nishi, Scott M. Robertson, and Rueyling Lin

Supplemental Experimental Procedures

OMA-1/TAF-4 pull-down

MBP-tagged OMA-1 FL and MBP-tagged OMA-1 N-terminal fragment (amino acids 1 – 117) were purified from *E. coli* using amylose resin (New England Biolabs) according to the manufacturer's protocol (Nishi, et al, 2005).

Phosphorylation of purified proteins was carried out in vitro with human DYRK2 (Upstate/Millipore). Our previous work had shown that human DYRK2 phosphorylated OMA-1 with the same specificity as *C. elegans* MBK-2 and with significantly higher efficiency. Approximately 1 μg of MBP-tagged protein was added to 0.5μg active HsDYRK2 in 25μL kinase buffer (25mM Hepes, pH 7.6; 5mM $MgCl_2$; 5mM $MnCl_2$; 0.5mM DTT; 10mM β-glycerol phosphate) including final 500μM cold ATP, and incubated at 37°C for 30 minutes.

^{35}S-labelled full-length TAF-4 was generated in rabbit reticulocyte lysates according to suppliers instructions (Promega TnT T7 Quick Coupled System; 50 μL reaction volumes). In vitro phosphorylated OMA-1 protein was added to the TnT reaction without further purification, mixed, and incubated at room temperature for 30 minutes followed by 4°C for 1-2 hours. Amylose resin slurry was prepared in MBP binding buffer (200 mM NaCl, 20 mM Tris-HCl pH 7.4, 1 mM EDTA, 1 mM DTT) and preincubated for 1 hour at 4°C with 1% BSA in binding buffer. 20μl of resin slurry was then added, mixed, and the incubation at 4°C continued for an additional 2-3 hours. Resin was spun down in a tabletop microcentrifuge (low speed for 30-45 seconds) and washed 1 time with cold binding buffer, then 4-5 x with cold binding buffer supplemented to final 750 mM NaCl, then 1x with cold binding buffer, and finally eluted with 2x 10 μL cold binding buffer containing 100mM maltose. The eluted material was loaded onto a 10% polyacrylamide gel. The gel was stained (SimplyBlue Safestain, Invitrogen), dried, and exposed to single-side emulsion film (Kodak BioMax MR) at -80°C.

Rescue assay

Two OMA-1Δ46-80::GFP-expressing lines were generated by microparticle bombardment: TX1162 [*oma-1(te33); teIs108(P_{oma-1} oma-1Δ46-80::gfp)*], in which the transgene is integrated, and TX1155 [*oma-1(te33); teEx559(P_{oma-1} oma-1Δ46-80::gfp)*], a non-integrated line. The same results were obtained in both cases.

Approximately 200 L1 larvae were placed on each *oma-2(RNAi)* plate. These animals were scored for the Oma phenotype three days later when they had become adults. No animal with Oma phenotype was observed with TX864 [*oma-1(te33); teIs76(*$P_{oma\text{-}1}$ *oma-1::gfp)*] , TX1155, or TX1162 treated with *oma-2(RNAi)*, whereas 100% of *oma-1(te33);oma-2(RNAi)* animals were Oma. To score for rescue of embryonic lethality, only two L1 larvae were placed on each *oma-2* feeding RNAi plate. The number of larvae and dead embryos on a total of three plates were scored 5 days later.

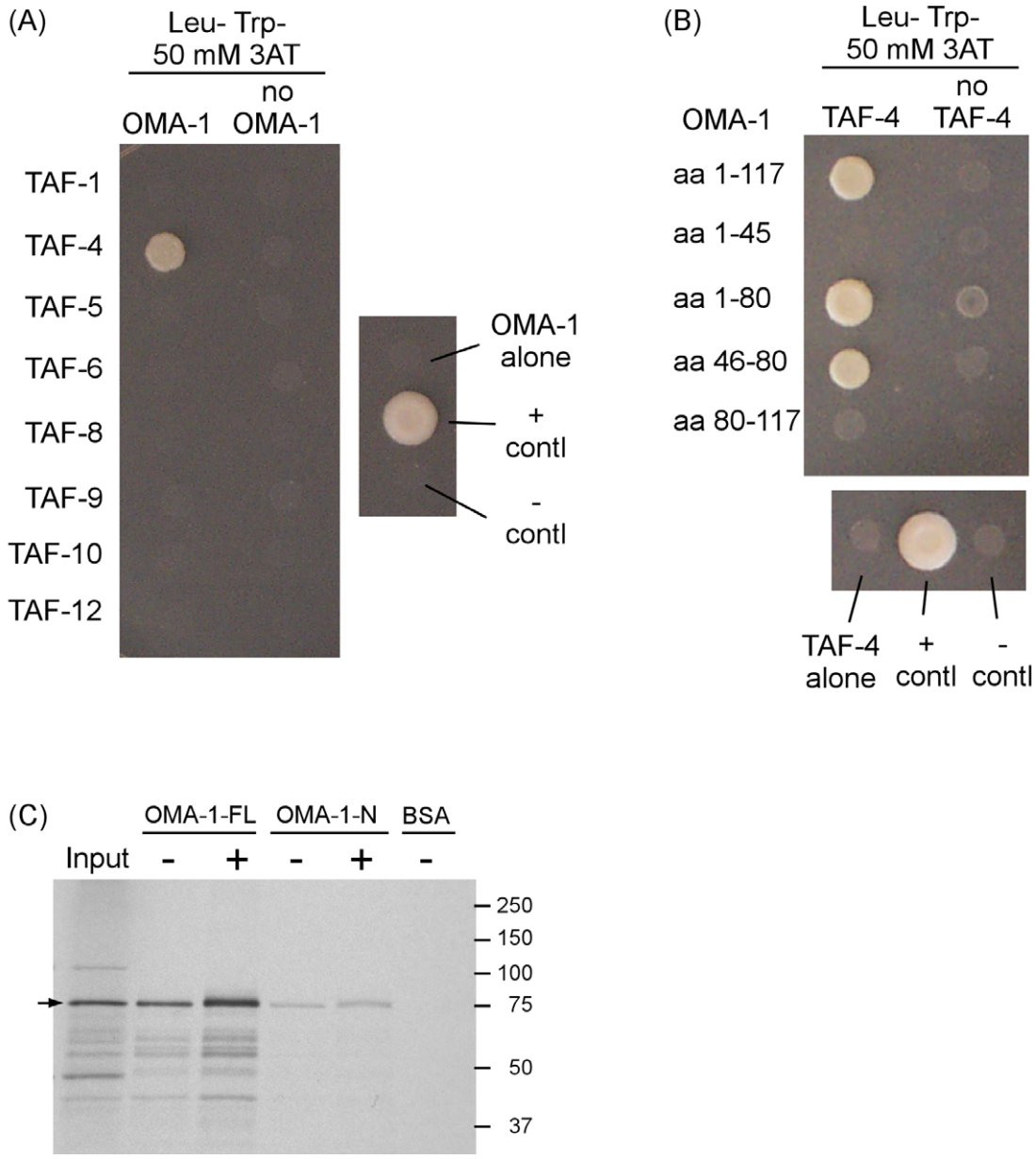
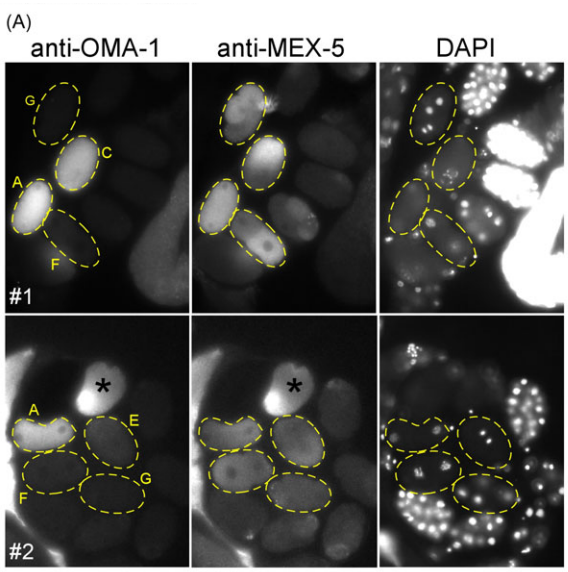
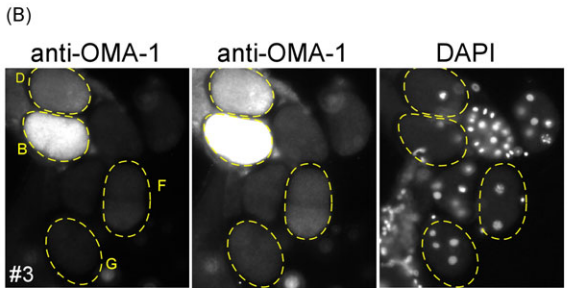


Figure S1. OMA-1/TAF-4 interaction assays.
(A). OMA-1-N interaction with a series of TAF proteins under high stringency conditions: -Leu, -Trp and plus 50mM 3-amino-1,2,4-triazole (3-AT). Note exclusive interaction between OMA-1-N and TAF-4. (B). TAF-4 versus different portions of OMA-1-N. Amino acid residues 46-80 of OMA-1 are both necessary and sufficient for interaction with TAF-4. OMA-1 and TAF proteins are expressed from the bait and prey vectors, respectively. Positive and negative controls are the E and A strains from Invitrogen. (C) Autoradiography of the ^{35}S-labelled full-length TAF-4 pulled down by MBP::OMA-1 FL or MBP::OMA-1-N that have (+) or have not (-) been pre-phosphorylated by the human DYRK2. Input = 5% of the amount used for pull down. Arrow: full-length TAF-4. Note higher amount of ^{35}S-TAF-4 pulled down following DYRK2 phosphorylation of OMA-1. BSA used as a negative control.





(C)

Symbol	Embryonic Stages	#1	#2	#3
A	1-cell, decondensation	100	100	-
B	1-cell, pronuclear migration	-	-	100
C	1-cell, pronuclei meet	95	-	-
D	1-cell, mitotic metaphase	-	-	34
E	1-cell, mitotic anaphase	-	21	-
F	2-cell	9	9	7
G	4-cell	0	0	0

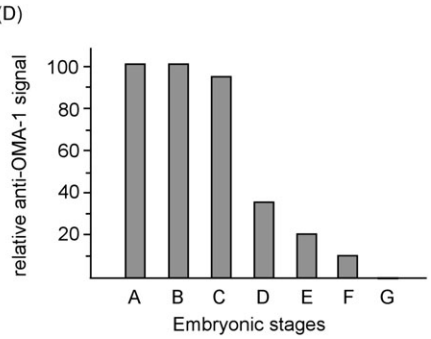
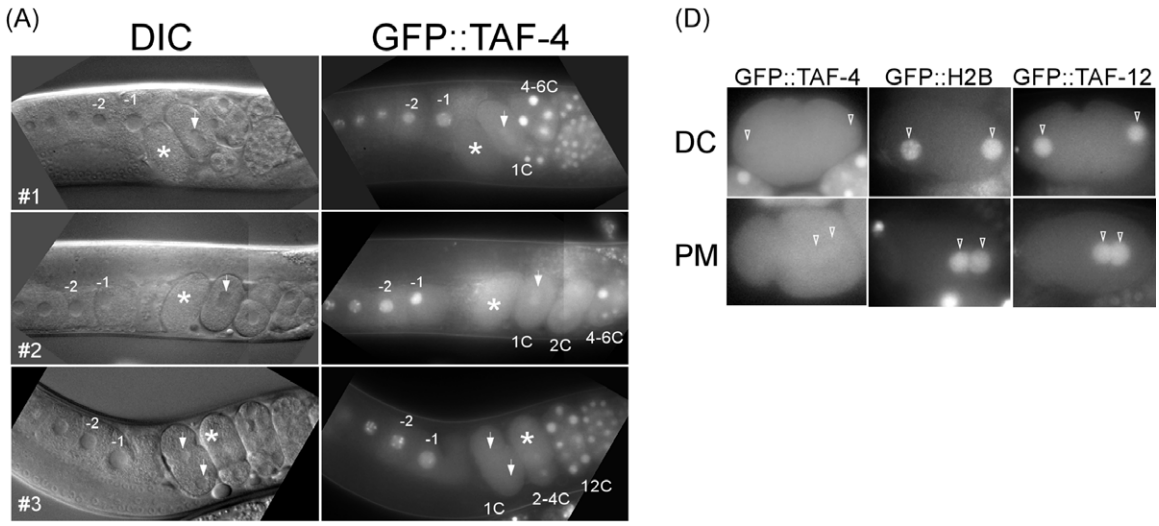


Figure S2. Quantitative analysis of OMA-1 expression in early *C. elegans* embryos. (A). Double-staining of various-stage embryos with anti-OMA-1 (left) and anti-MEX-5 (center) antibodies. Right: corresponding DAPI-staining. Above: embryo field #1; below: embryo field #2. Embryos subjected to quantification of the anti-OMA-1 signal are outlined by a dashed yellow line. A – H designation: see (C) below. Asterisk: embryo not quantified due to an abnormal background signal. (B). Anti-OMA-1 staining of embryo field #3. Center panel is an enhanced version of the left panel to emphasize the weak anti-OMA-1 signal observed in 2-cell embryos versus background signal in 4-cell and older embryos. (C). Quantification of total pixel intensity in embryos outlined in (A) – (C), as percentile of that in the earliest 1-cell embryo in each field. A – E: early to late 1-cell embryos (exact stage as indicated); F: 2-cell embryos; G: 4-cell embryos. (D). Graphical presentation of the quantification in (C). Relative signal intensity versus developmental stage. A – G as in (C).



(B)

	N/C					N					total		
	wildtype			*mbk-2 (RNAi)*		wildtype			*mbk-2 (RNAi)*		wildtype		
	#1	#2	#3	#1	#2	#1	#2	#3	#1	#2	#1	#2	#3
-2 oocyte	1.08	0.89	1.23	--	--	0.92	0.7	0.96	--	--	1.03	0.72	0.62
-1 oocyte	1	1	1	1	1	1	1	1	1	1	1	1	1
1C embryo	**0.53**	**0.39**	**0.48**	**1.18**	**0.94**	**0.98**	**0.67**	**0.72**	**1.15**	**1.48**	**1.43**	**1.11**	**1.21**
2C embryo		0.52		1.45	1.33		0.64		1.71	2.18	--	1.10	1.11
>4C embryo	1.01	0.67	1.67	--	--	1.79	1.27	1.19	--	--	--	--	--

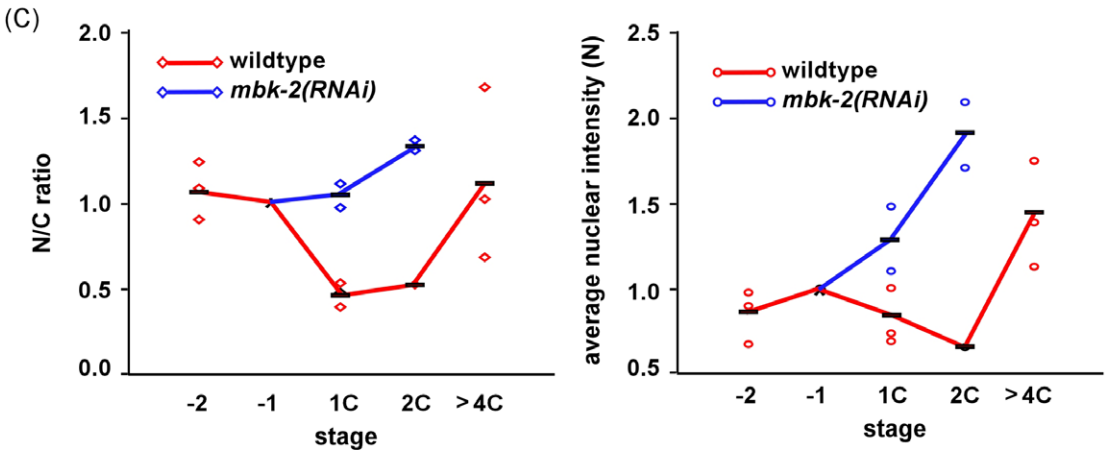


Figure S3. Quantification of GFP::TAF-4 fluorescence in oocytes and early embryos.

(A). Fluorescence in one proximal gonad arm of three different GFP::TAF-4 transgenic worms. Developmental progression from left to right. Arrows: 1-cell embryo pronuclei. Oocytes are numbered according to their position relative to the spermatheca; -1 = oocyte immediately adjacent to spermatheca undergoing maturation prior to ovulation and fertilization. Embryos with white asterisk are not scored due to a lack of visible nuclei or an artificial background signal. (B). Quantification of GFP::TAF-4 fluorescence in the oocytes and early embryos presented in panel (A). Pixel intensities for nuclei were

determined by ImageJ from 16-bit images and averaged over the entire nucleus. Pixel intensities for cytoplasm were determined in multiple locations (n = 6-8) of the same oocyte/embryo and averaged. The average pixel intensity in the distal gonad was used for background subtraction. Values for nuclear to cytoplasmic ratio (N/C), total nuclear signal (N), and total cell/embryo signal (total) are presented relative to the -1 oocyte in each worm, which is assigned a value of 1. N/C and N values are also given for two *mbk-2(RNAi)* worms. Image for worm #2 is presented in Figure 6A; image for worm #1 is not presented. (C). Graphical presentation of nuclear/cytoplasmic ratios versus developmental stage. Note that both the total nuclear signal as well as the nuclear signal to cytoplasmic signal ratio drops in the wildtype 1-cell and 2-cell embryos relative to the proximal oocytes and later embryos, and that this drop does not occur in the corresponding *mbk-2(RNAi)* embryos. (D). Higher magnification images of 1-cell embryos at pronuclei decondensation (DC) or pronuclei meeting (PM) expressing GFP::TAF-4, GFP::H2B, or GFP::TAF-12. Arrowhead points to pronuclei. Note that GFP::TAF-4 fluorescence is ubiquitous throughout the embryo.

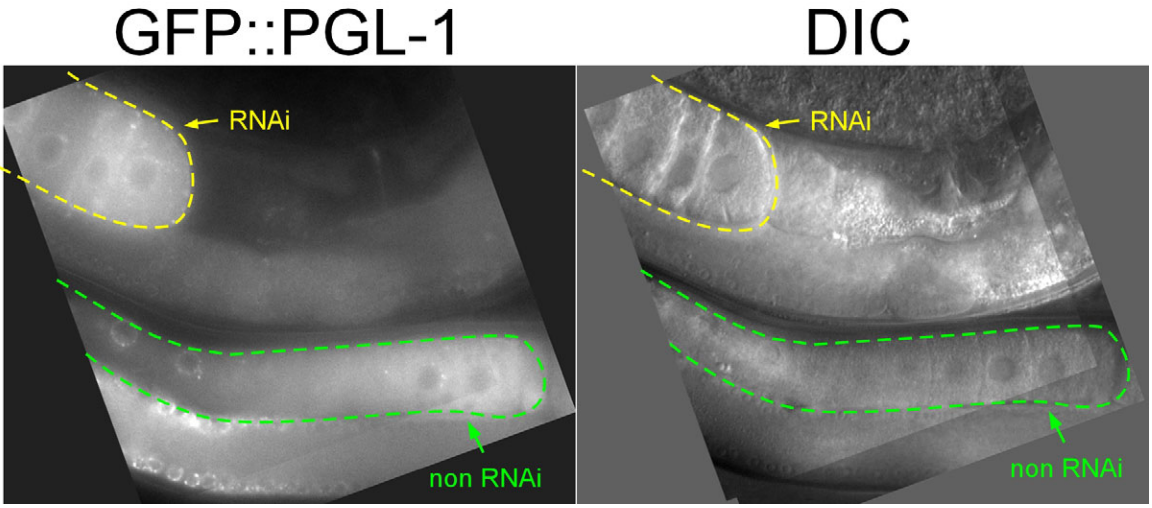


Figure S4. GFP::PGL-1 levels are unaffected by OMA protein depletion
Left panel: GFP::PGL-1 fluorescence in two proximal gonad arms, one subjected to *oma-1(RNAi);oma-2(RNAi)* (yellow dashed), and the other without RNAi (green dashed). GFP::PGL-1 is expressed from the same vector that we express GFP::TAF-4. Note that both fluorescence intensity and subcellular localization of GFP::PGL-1 are unchanged following RNAi. Right panel: corresponding DIC image.

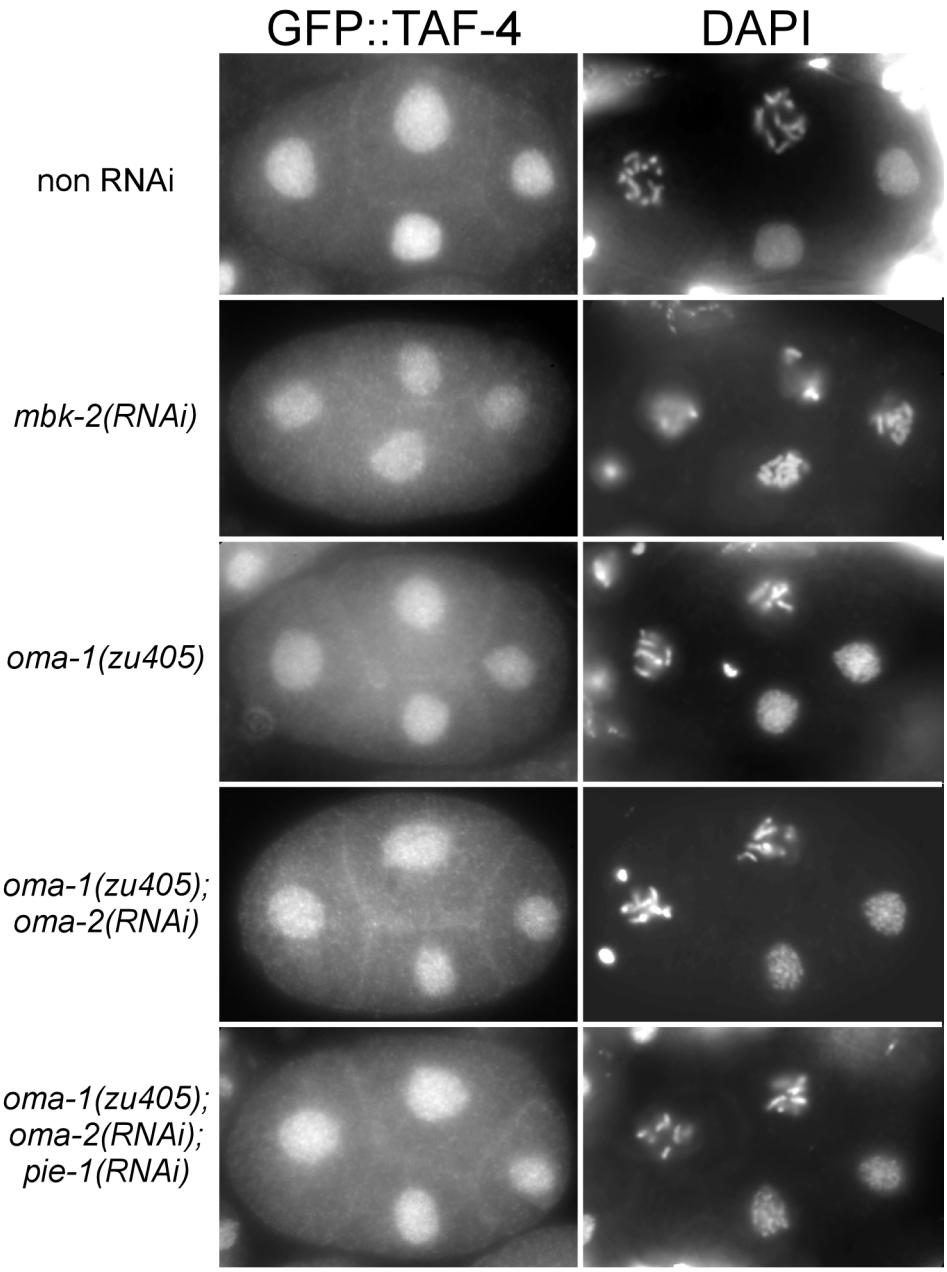


Figure S5. Nuclear localization of GFP::TAF-4 is unaffected by persisting OMA-1/2 in embryos with compromised phosphorylation by MBK-2
Anti-GFP (left-hand column) and DAPI (right-hand column) stainings in GFP::TAF-4 expressing 4-cell embryos in the following backgrounds: non RNAi, *mbk-2(RNAi)*, *oma-1(zu405)*, *oma-1(zu405);oma-2(RNAi)*, *oma-1(zu405);oma-2(RNAi);pie-1(RNAi)*.

Table S1. GFP::TAF-4 in *oma-1(RNAi);oma-2(RNAi)* embryos[a]

	Nuclear to Cytoplasmic Ratio[b]		Total Embryonic GFP
	non RNAi	*oma-1/2(RNAi)*	RNAi/non RNAi[c]
1-cell, decondensation	1.2	3.4	0.97
2-cell #1	ND[d]	4.5	1.16
2-cell #2	ND[d]	-	1.01
≥ 4C	3.2	4.9	1.09

[a.] Quantification of embryos shown in Figure 3C.
[b.] Average GFP intensity of nuclear signal versus cytoplasmic signal in fixed embryos.
[c.] Relative total GFP intensity in *oma-1(RNAi);oma-2(RNAi)* and non RNAi embryos of comparable stages
[d.] not determined, ratio not measured because cells are dividing.

Table S2. Sensitivity to the depletion of *oma-1* and *oma-2* in different genetic backgrounds.

Dilution of O1O2 RNAi bacteria[a]	Wildtype N2		TX903[b]		ET113[c]	
	Oma[d]	DEB[e]	Oma	DEB	Oma	DEB
1:3	100	100	100	100	100	100
1:9	100	95	100	90	100	95
1:33	30-40	50	100	67	30-50	50
1:100	0	0	70	50	<5	0
1:200	0	0	50	50	0	0
1:400	0	0	10-20	50	0	0
Non RNAi	0	0	0	0	0	0

[a.] *oma-1* and *oma-2* RNAi bacteria were prepared separately and diluted with non-RNAi bacteria HT115. The dilutions denote the factors to which RNAi bacteria were diluted in the final mix. Equal amounts of *oma-1* and *oma-2* RNAi bacteria were used.
[b.] TX903 (*teIs90 [$P_{pie\text{-}1}$gfp::taf-4]*).
[c.] ET113 (*$P_{pie\text{-}1}$gfp::CYB-1*).
[d.] percentage of adults worms with the Oma phenotype (n>300 in every dilution).
[e.] percentage of dead embryos (DEB) of total embryos released to plates (n≥ 100).

Table S3. Quantification of early embryonic stages by DAPI staining

	Meiosis I[a]	Meiosis II[b]	1-cell[c]	2-cell	4-cell	8-cell
TX864[d] (n=192) [e]	12.0[f]	9.9	27.6	20.8	17.7	12.0
TX1162[g];*oma-2(RNAi)* (n=215)	12.6	10.7	24.2	19.1	20.0	13.5

a. embryos between meiotic prophase I and anaphase I
b. embryos between meiotic prophase II and anaphase II
c. embryos between completion of meiosis II and first mitotic anaphase
d. *oma-1(te33); teIs76[$P_{oma\text{-}1}$ oma-1::gfp]*
e. total number of embryos scored.
f. percentile of the total number scored.
g. *oma-1(te33); teIs108[$P_{oma\text{-}1}$ oma-1Δ46-80::gfp]*