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Supplemental Data
Regulation of MBK-2/DYRK by CDK-1 and the Pseudophosphatases EGG-4 and EGG-5 during the Oocyte-to-Embryo Transition

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Figure S1: DYRK alignments
Alignment of DYRK2 from human (AAH06375), *Drosophila* (NP_523564 Smell impaired isoform A) and *C. elegans* (F49E11.1a). Kinase domain is in bold. Subdomains are colored. All TP and SP motifs are highlighted. TP motif at 470 in *C. elegans* MBK-2 is not required for phosphorylation by hCdk1 *in vitro* assay (data not shown).

Figure S2: The amino-terminal domain of MBK-2 is phosphorylated *in vivo*.
(A) GFP:MBK-2(1-109) was immunoprecipitated from worms and western blotted with an anti-GFP antibody. CIP denotes treatment of the IP with Calf alkaline phosphatase, *cdk-1(RNAi)* denotes treatment of the worms with feeding RNAi.
(B) Same as in A, except that the worms were harvested as gravid hermaphrodites with 4 or more embryos in their uteri (adults) or as younger hermaphrodites with 0-1 embryos in their uteri (late L4-young adults).

Figure S3: Localization and expression of GFP:MBK-2 transgenes
(A) Gonads of live hermaphrodites expressing GFP:MBK-2 wild-type, K196R, S68A, S68E and activation loop (Y325F, Y327F) mutants. Western showing that the transgenic proteins are expressed at similar levels.
(B) Immunoprecipitation showing that GFP:MBK-2(WT), S68A and S68E interact with EGG-4/5 with similar affinities. Transgenic proteins were immunoprecipitated from whole worm extracts using anti-GFP antibody. Immunoprecipitates were run on a NuPAGE 4-12% gel, and Western blotted with anti-EGG-4/5 antibody. Input is $1/100^{th}$ of the IP.

Figure S4: S68 is not required for MBK-2 activity when expressed in *E. coli*
MBP:MBK-2 fusions were partially purified from *E. coli* on amylose resin. Relative kinase activity was calculated by measuring ^{32}P incorporation in MEI-1, expressed as a ratio over the signal obtained for wild-type MBP:MBK-2 at 60 min (set to 1). In the case of Cdk1 addition, MBP:MBK-2 was pre-incubated with hCdk1 and cold ATP in kinase buffer for 30 min (same conditions as in Fig. 2A), before washing and performing the kinase assay on MEI-1 as above. Error bars represent standard deviation from 3 independent experiments. We do not know how the activity of *E. coli*-made MBP:MBK-2 compares to that of MBK-2 immunoprecipitated from worm or mammalian cells.

Figure S5: EGG proteins contain a PTP domain.
Alignment of EGG-3, EGG-4, EGG-5 and the human phosphatase PTN7. The PTP domain is highlighted in bold. Phosphatase active site is pink and putative destruction

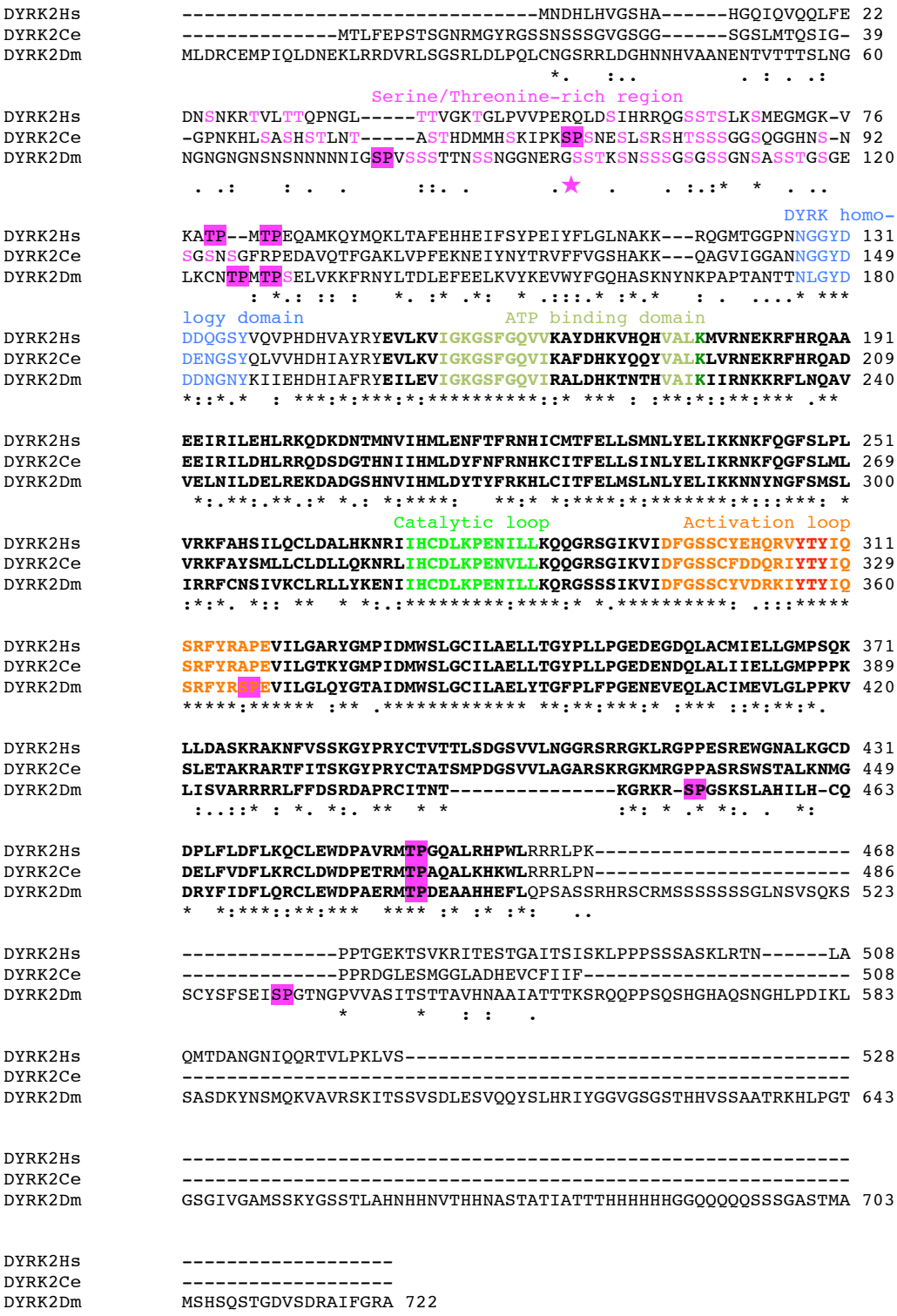
boxes (RXXL) are green. The first two RXXL motifs in EGG-3 are required for degradation *in vivo* (Stitzel et al., 2007).

Figure S6: EGG-4/5 are required to inhibit MBK-2 in metaphase of meiosis I and co-localize with EGG-3 on subcortical puncta.
A. *egg-4/5(RNAi)* zygote in metaphase of meiosis I co-stained for P-MEI-1 and DAPI.
B. Western blot of whole worm extracts blotted with anti-MBK-2 and anti-EGG-4/5 sera. Predicted molecular weights of EGG-4 and MBK-2 and are 83 kDa and 55 kD, respectively.
C. Zygote in Meiosis II co-stained with DAPI (DNA), anti-EGG-3 and anti-EGG-4. Two sections are shown: one near the cortex and one near the center of the embryo (cytoplasm). At this stage, EGG-3 appears on puncta throughout the embryo. In contrast, EGG-4 appears on puncta near the cortex and around the sperm pronucleus (right most signal in cytoplasm section), but less so in the cytoplasm.

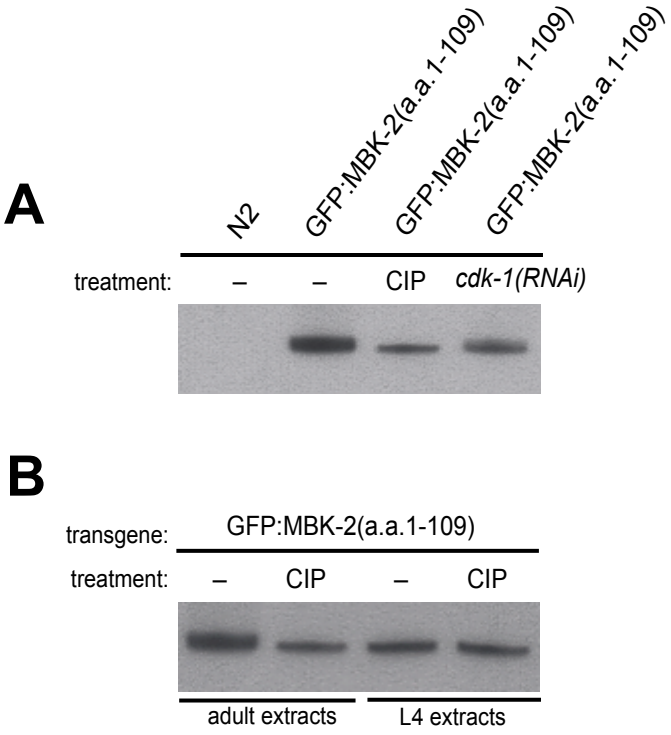
Figure S7: EGG-3 uses its phosphatase domain to interact with the amino-terminus of MBK-2.
Extracts from *E. coli* expressing the indicated FLAG-tagged MBK-2 or EGG-3 fusions were pulled down with glutathione-sepharose beads coupled to the indicated GST-tagged MBK-2 or EGG3 fusions and immunoblotted with an anti-FLAG antibody. Input is $1/50^{th}$ of the pull-down. EGG-3(237-513) is phosphatase domain, MBK-2(1-166) is amino-terminus, MBK-2(166-508) is the kinase domain (see Sup. Figs. 1 and 5).

Figure S8: EGG-4 inhibits MBK-2 kinase activity in a dose-dependent manner
Indicated molar excess of FLAG-tagged EGG-3, EGG-4 and EGG-4(H599A,G603A, R606A) were added to MBP:MBK-2 kinase reactions and the amount of P-MEI-1 was quantified after 30 minutes (as in Fig. 3). Levels are expressed as percentage of P-MEI-1 observed with MBP:MBK-2 alone (first lane, set to 100%).

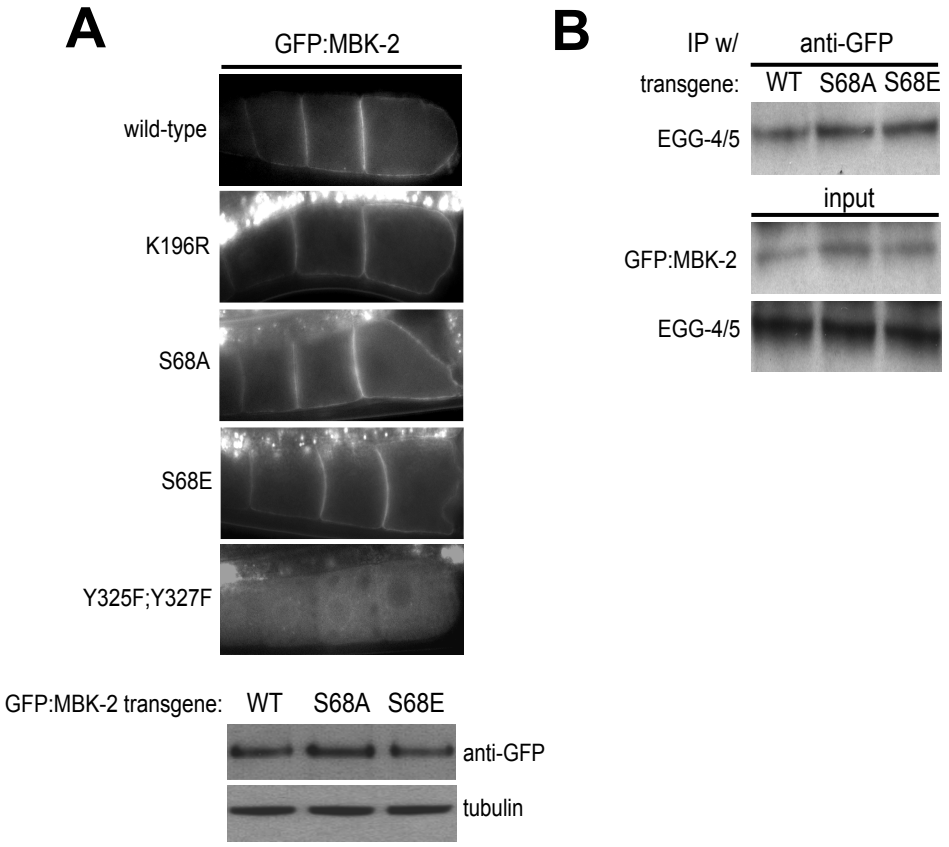
Supplementary Figure 1



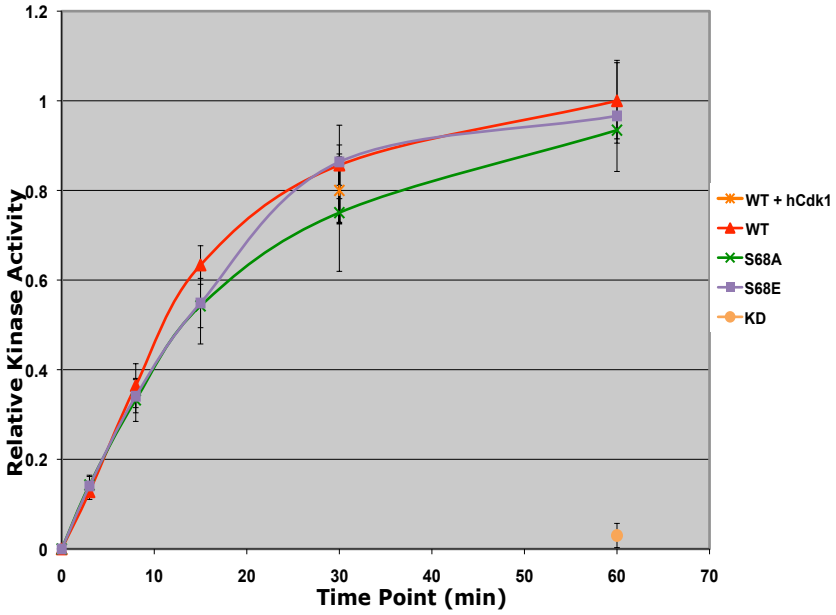
Supplementary Figure 2



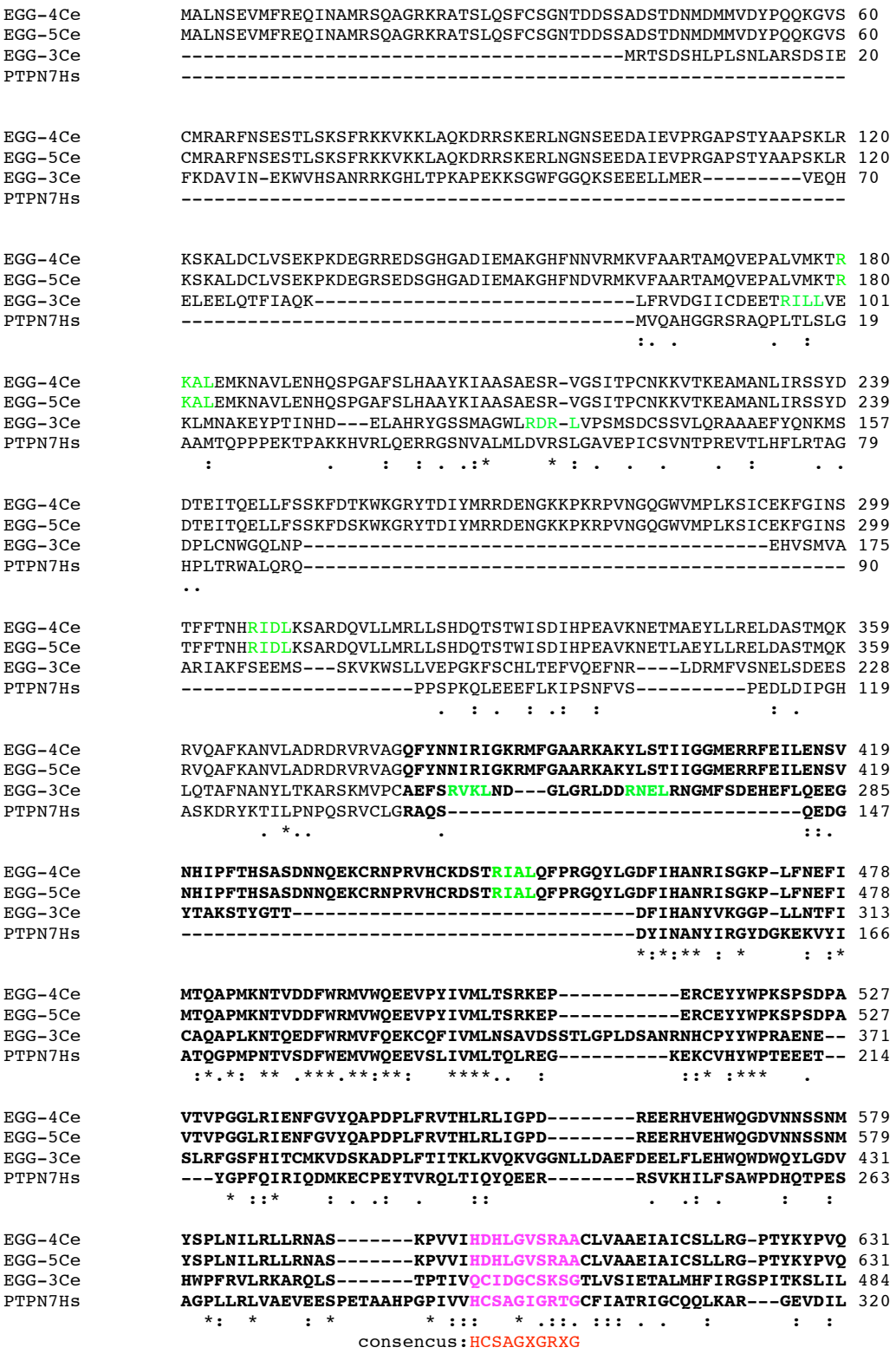
Supplementary Figure 3



Supplementary Figure 4



Supplementary Figure 5



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EGG-4Ce         RAVQFLRQRRPFSIETPMQYIFVHRLVAFFFRDVIGSAKELDVDYERWLQERSERMFLDD 691
EGG-5Ce         RAVQFLRQRRPFSIETPMQYIFVHRLVAFFFRDVIGSAKELDVDYERWLQERSERMFLDD 691
EGG-3Ce         QSCVFVRLQRRLSVSSVLLYLFIYRVILRWIEPYVNKWYHRAALGLRFKSIG----FIQK 540
PTPN7Hs         GIVCQLRLDRGGMIQTAEQYQFLHHTLALYAGQLPEEPSP---------------------- 360
                     :*  *   :.:   * *::: :  :      .

EGG-4Ce         LAAPIPGYRLLSPRADPDIVRMVGRPERPNYRREAPDCVGEMPNKVATVDGILSPAKSVF 751
EGG-5Ce         LAAPIPGYRLLSPRADPDIVRMVGRPERPNYRREAPDCVGEMPNKVAAVDGILSPAKSVF 751
EGG-3Ce         YNAMIQEFSRITPAY---------------------------------------------- 555
PTPN7Hs         ------------------------------------------------------------

EGG-4Ce         EF 753
EGG-5Ce         EF 753
EGG-3Ce         --
PTPN7Hs         --
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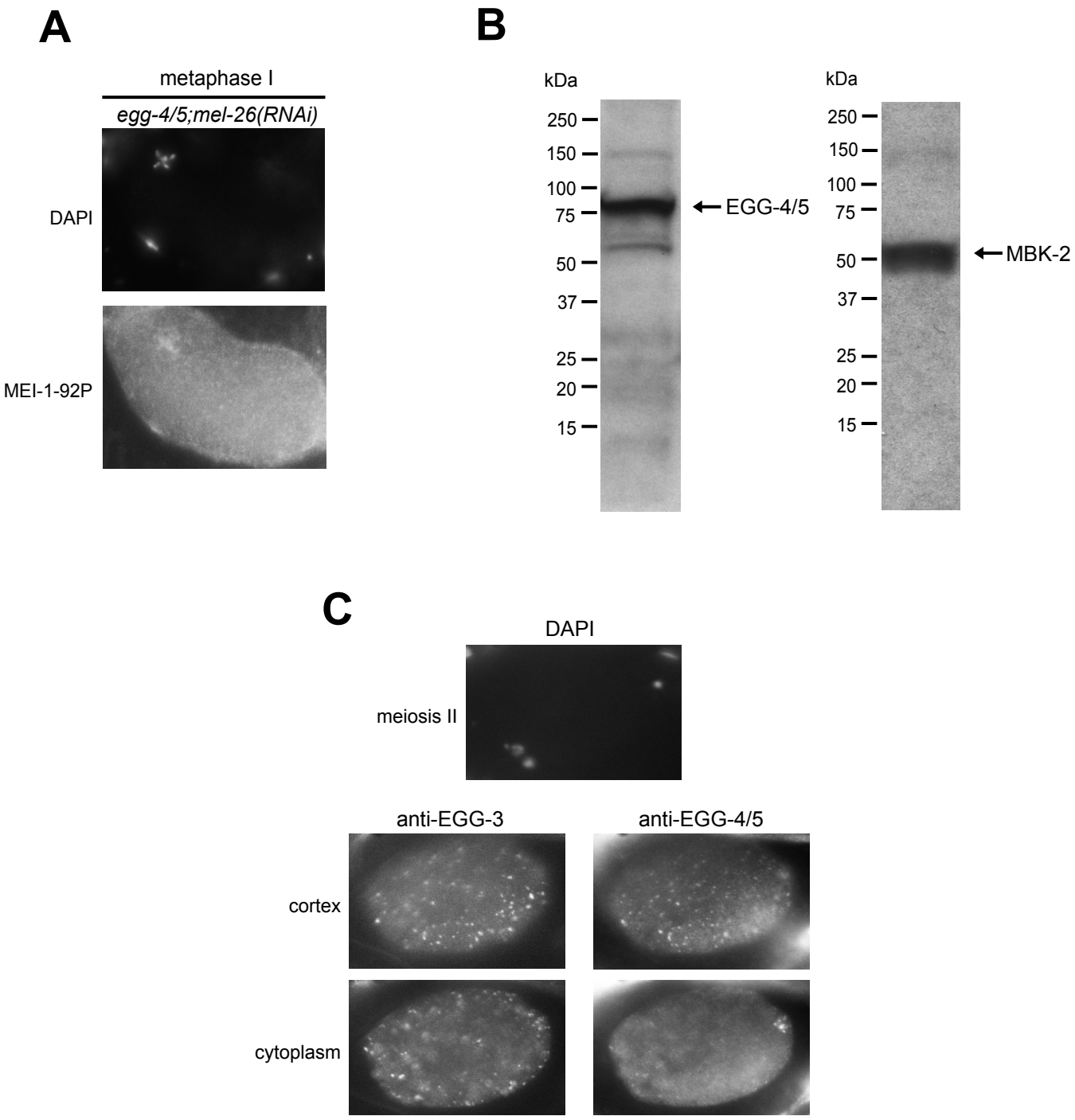
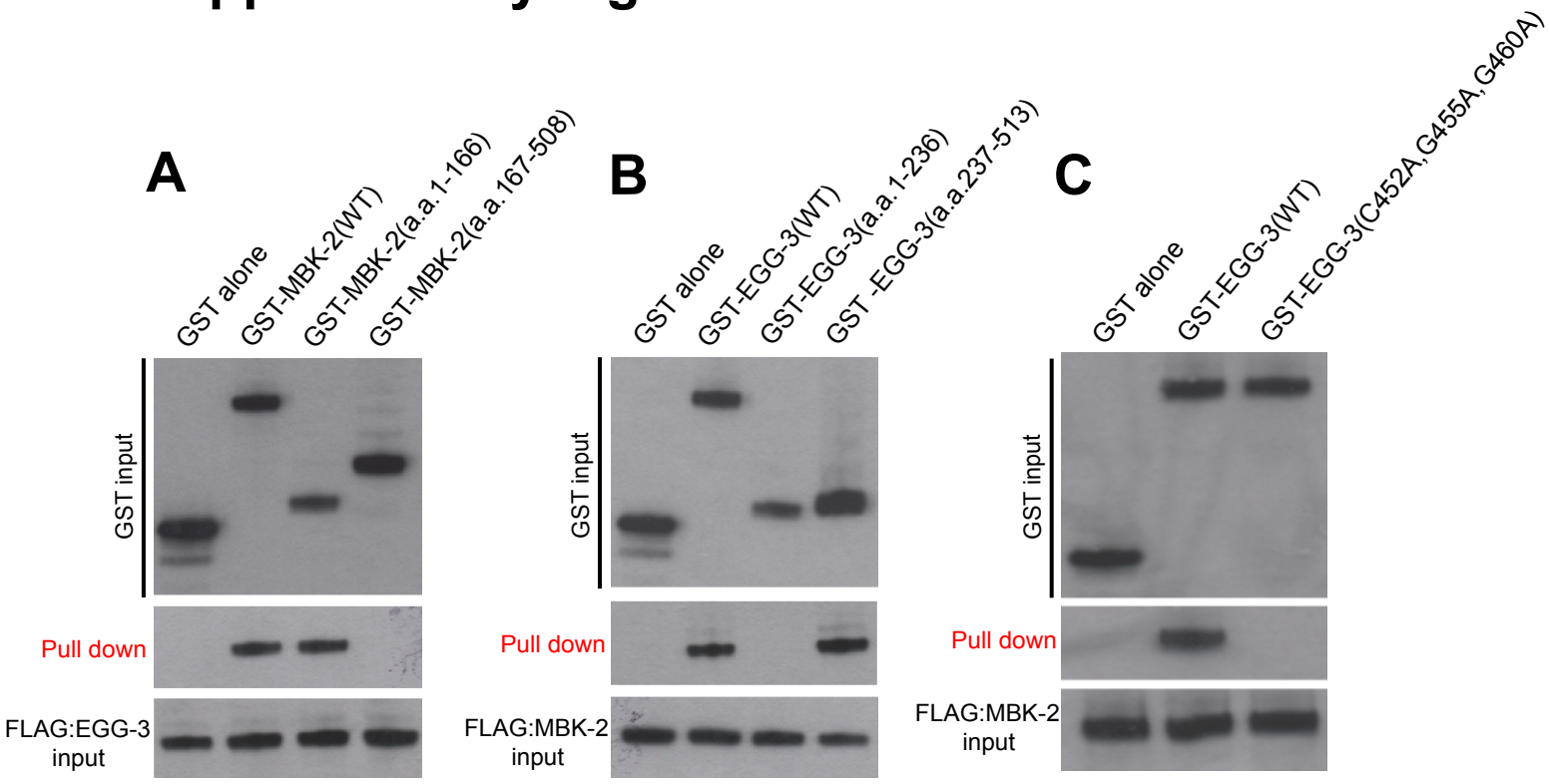
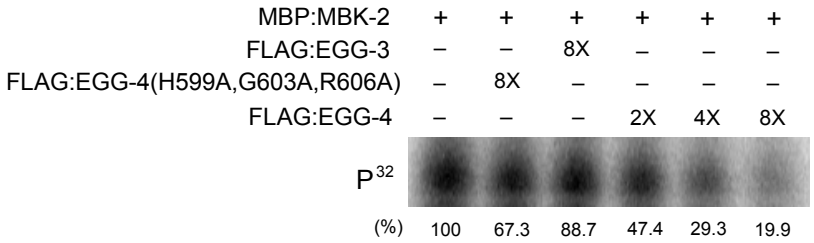
Supplementary Figure 6



Supplementary Figure 7



Supplementary Figure 8



transgene	genotype	(%) gonads			
		with **0 oocyte** positive for P-MEI-1	with **2 oocytes** positive for P-MEI-1	with **3 oocytes** positive for P-MEI-1	with **4 oocytes** positive for P-MEI-1
–	*mel-26(RNAi)* **(n=10)**	100%	0%	0%	0%
–	*egg-3(tm1191)* **(n=11)**	100%	0%	0%	0%
–	*mel-26(RNAi); egg-3(tm191)* **(n=16)**	100%	0%	0%	0%
–	*mel-26(RNAi); egg-4/5(RNAi)* **(n=65)**	86.2%	13.8%	0%	0%
–	*mel-26(RNAi); egg-3(tm1191); egg-4/5(RNAi)* **(n=32)**	87.5%	12.5%	0%	0%
GFP:MBK-2 (WT)	*mel-26(RNAi)* **(n=18)**	100%	0%	0%	0%
GFP:MBK-2 (WT)	*mel-26(RNAi); egg-3(RNAi)* **(n=14)**	100%	0%	0%	0%
GFP:MBK-2 (WT)	*mel-26(RNAi); egg-4/5(RNAi)* **(n=41)**	7.3%	68.3%	22%	2.4%
GFP:MBK-2 (S68E)	*mel-26(RNAi)* **(n=21)**	100%	0%	0%	0%
GFP:MBK-2 (S68E)	*mel-26(RNAi); egg-3(RNAi)* **(n=18)**	100%	0%	0%	0%
GFP:MBK-2 (S68E)	*mel-26(RNAi); egg-4/5(RNAi)* **(n=37)**	0%	24.3%	43.2%	32.4%
GFP:MBK-2 (S68A)	*mel-26(RNAi); mbk-2(pk1427); egg-4/5(RNAi)* **(n=21)**	100%	0%	0%	0%

Table S1: % gonads with 0,1,2,3 or more oocytes positive for P-MEI-1

Table S2: Strains Used in This Study				
Name	Description	Genotype	Reference	Note
JH1576	pie-1prom:GFP:MBK-2	*unc-119(ed3);axls1140[pJP1.02]*	Pellettieri et al, 2003	
JH1714	pie-1prom:GFP:MBK-2(K196R)	*unc-119(ed3);axls1227[pJP1.08]*	Pellettieri et al, 2003	
JH2298	pie-1prom:GFP:MBK-2(S68A)	*unc-119(ed3);axls*	this study	
JH2300	pie-1prom:GFP:MBK-2(S68E)	*unc-119(ed3);axls*	this study	
JH1872	pie-1prom:GFP:MBK-2(Y325FY327F)	*unc-119(ed3);axls[pJP1.57]*	J. Pellettieri thesis, 2004	unstable
JH1580	*mbk-2*(null)	*unc-24(e1772) mbk-2(pk1427)/nT1*	Pellettieri et al, 2003	
JH1866	*mbk-2*(null);pie-1prom:GFP:MBK-2	*unc-24(e1772) mbk-2(pk1427)/nT1; unc-119(ed3);axls1140[pJP1.02]*	Pellettieri et al, 2003	
JH2301	*mbk-2*(null);pie-1prom:GFP:MBK-2(S68A)	*unc-24(e1772) mbk-2(pk1427)/nT1; unc-119(ed3);axls*	this study	
JH2302	*mbk-2*(null);pie-1prom:GFP:MBK-2(S68E)	*unc-24(e1772) mbk-2(pk1427)/nT1; unc-119(ed3);axls*	this study	
JH2385	*egg-3*(deletion)(with Pharynx GFP *b*alancer); pie-1prom:GFP:MEI-1	*egg-3(tm1191)/mln1[dpy-10(e128) mls14];orls1*	Stitzel et al., 2007	
JH2399	pie-1prom:3XFLAG:MBK-2:6XHis	*unc-119(ed3);axls1813[pKC2.25]*	Stitzel et al., 2007	unstable
JH2585	pie-1prom:3XFLAG:MBK-2:6XHis(S68A)	*unc-119(ed3);axls[pKC2.35]*	this study	
JH1859	pie-1prom:GFP:MBK-2(1-109)	*unc-119(ed3);axls1859[pJP1.61]*	J. Pellettieri thesis, 2004	