Supplemental Materials

Molecular Biology of the Cell

Takada et al.

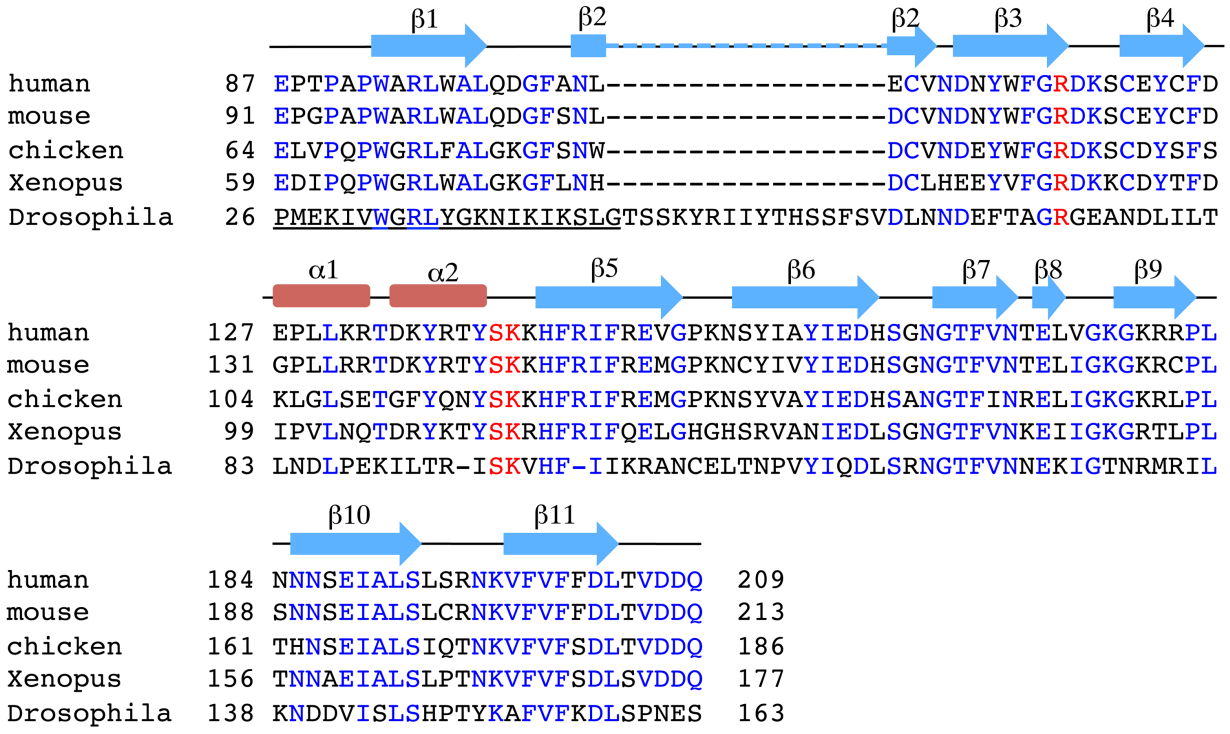


Figure S1. Sequence alignment and secondary structure elements of Chk2 FHA domain. FHA domain sequence alignment of human Chk2 (CHEK2) isoform a (GenBank: NP_009125), mouse Chk2 (AAH56617.1), chicken Chk2 (ABL14109.1), and *Xenopus laevis* Cds1 (AAI66130.1). The alignment and the secondary structure elements indicated above the sequences are based on a study by Cai *et al.*(Cai *et al.*, 2009). *Drosophila* Mnk long-form (BAA28755.1) used in this study was added to the alignment. It has 17 amino acids insertion in the position of β2. This insertion is missing in Mnk short-form, an alternative splicing product (Loki isoform b: NP_477218.1) (Oishi *et al.*, 1998). Human Chk2 isoform c (NP_001005735.1) has 44 amino acids insertion at the same position (not shown). Blue letters are amino acid residues conserved across four or more orthologs. Red letters indicate conserved arginine, serine, and lysine (R73, S95, and K96 in *Drosophila* Mnk). These residues in human Chk2 (R117, S140, and K141) form a total of five hydrogen bonds with pThr phosphate in binding phosphopeptide (Li *et al.*, 2002). R73 was substituted to alanine in EGFP-R73A, and R73, S95, and K96 were substituted to alanine in EGFP-RSK to disrupt phosphopeptide binding of Mnk-FHA domain. Underline indicates amino acid 26-46 that is deleted in EGFP-Δ[26-46] variant.

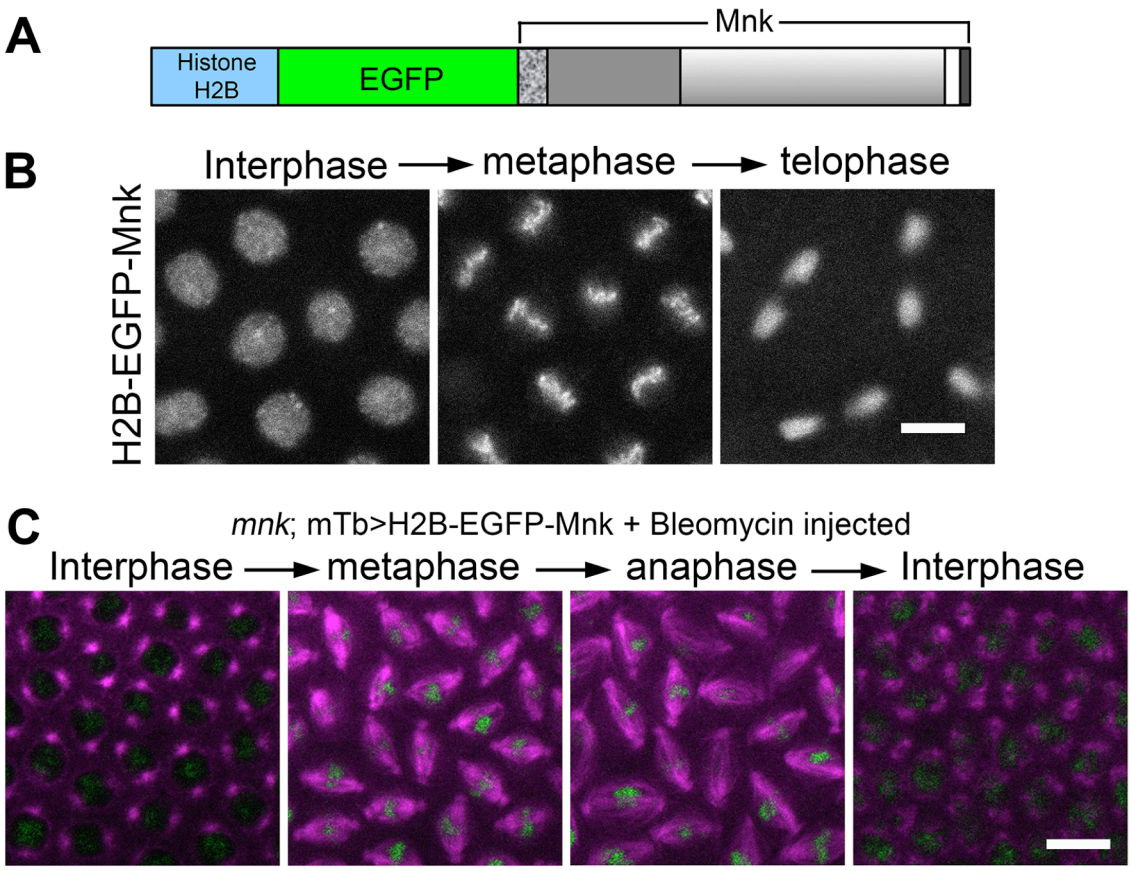
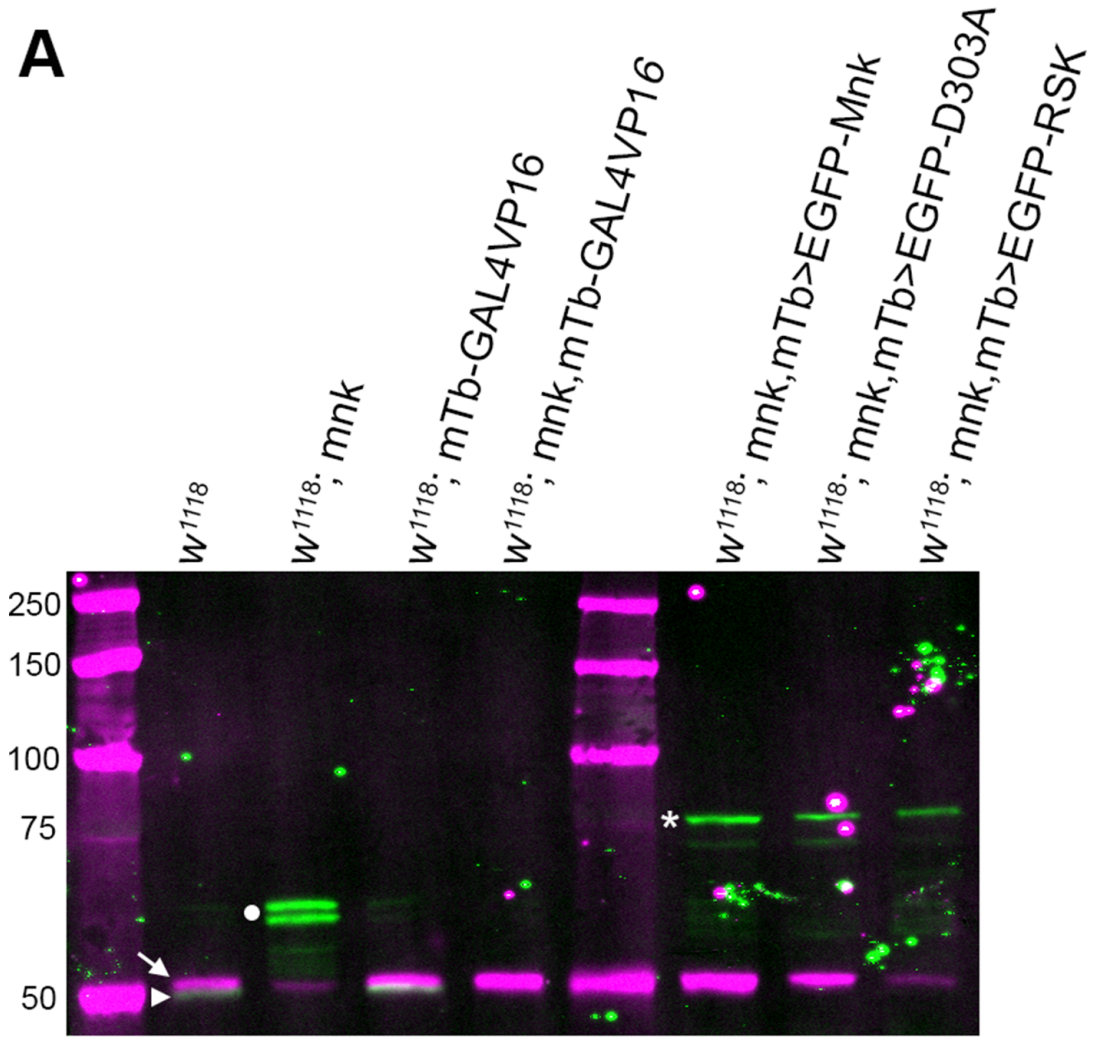


Figure S2. Histone H2B-EGFP-Mnk expression in early *Drosophila* embryos. (A) Schematic diagram of human Histone H2B-conjugated EGFP-Mnk construct. (B) Localization of H2B-EGFP-Mnk during the syncytial blastoderm stage without DNA damage. The fusion protein was expressed in w^{1118} (wild-type) embryos using nanos-GAL4VP16 driver. It is tethered to chromatin throughout the cell cycle. Frames were selected from Movie S22. Bar: 10μm. (C) Expression of H2B-EGFP-Mnk did not restore centrosome inactivation, mitotic delay, and nuclear dropping responses to DNA damage in *mnk* mutant embryos. H2B-EGFP-Mnk was expressed in *mnk* mutant embryos using mTb-GAL4VP16 driver. The embryo was injected with bleomycin and Rhodamine-Tubulin. Frames were selected from a time-lapse laser scanning confocal microscope movie. EGFP is shown in green, rhodamine is shown in magenta. Bar: 10μm.

A



B

Blot	Endogenous Mnk	EGFP-Mnk (N=6)	EGFP-D303A (N=4)	EGFP-RSK (N=5)
1	1.00	1.10	2.07	4.21
2	1.00	1.85	1.05	
3	1.00	1.80	1.53	4.31
4	1.00	1.50		2.34
5	1.00	2.33		3.96
6	1.00	0.680	0.860	3.77
Average		1.54	1.38	3.72
SD		0.59	0.54	0.80

Figure S3. (A) Western blot of *Drosophila* embryonic lysate. This membrane is the blot 1 shown in B. EGFP-Mnk, EGFP-D303A, and EGFP-RSK (green: indicated by *) in transgenic embryos and endogenous Mnk (green: indicated by arrowhead) in w^{1118} embryos were detected with anti-Mnk antibody and quantified as described in Materials and Methods. α-Tubulin (magenta: indicated by arrow in w^{1118} lysate) in each sample was detected by anti-α-Tubulin antibody, quantified, and used as the loading control. Double green bands indicated by circle are non-specific protein bands detected with the anti-Mnk antibody, which are usually detected weakly in embryonic lysate samples. (B) Summary of quantitative Western blot analyses. Six independent blots were analyzed. On each Western blot, the expression level of each EGFP-fusion protein relative to the endogenous Mnk was calculated (see Materials and Methods). SD: standard deviation.

Table S1. Length of mitosis after bleomycin injection.

	mTb-GAL4VP16	mnk, mTb-GAL4VP16	EGFP-Mnk	EGFP-D303A	EGFP-Δ[1-25]	EGFP-Δ[26-46]	EGFP-Δ[47-163]	EGFP-Δ[470-476]		EGFP-R73A	EGFP-RSK
								CNI	No CNI		
1	**20.17***	5.33	**11.33***	5.17	6.5	5.17	5.5	**15.33**		5.5	5
2	**28.83***	5.83	**15.67***	5.17	5.5	5.67	5.33	**11**		5.5	5.3
3		6.67	**13.83***	8	5.67	6.67	6.17		6.67	6.5	8.17
4		5.83	**22.33***	6.33	6	6.5	6.17		6.33	5.5	6.83
5			**4.33**	6	6.33	4.33	5.33		8	5.33	5.67
6			**19.17***		6		4.5		7		5.33
7					5.83				4.5		
8					6				6.17		
9									6.33		
10									4.5		
AVE	24.5*	5.92	14.44*	6.13	5.98	5.67	5.5	13.17	6.43	5.67	6.06
SD		0.55		1.16	0.33	0.96	0.71	4.17	1.11	0.47	1.21

Transgenes were expressed in *mnk* mutant using mTb-GAL4VP16. Bleomycin (50μg/mL) was injected with Rhodamine-labeled Tubulin (5mg/mL) during cycle 11, 12, or 13. The injected embryo was imaged by confocal microscopy starting immediately after injection. Time-lapse recordings were analyzed using ImageJ. CNI: centrosome inactivation; AVE: average length of mitosis from NEB (nuclear envelope breakdown) to NEF (nuclear envelope formation); SD: standard deviation. Numbers with asterisk (*) indicate time between NEB and the end of recording (minutes). These embryos showed significant mitotic delay thus, the recordings were stopped during anaphase-like state. Numbers in bold indicate that these embryos showed CNI and nuclear dropping.