Varma et al., http://www.jcb.org/cgi/content/full/jcb.201304197/DC1

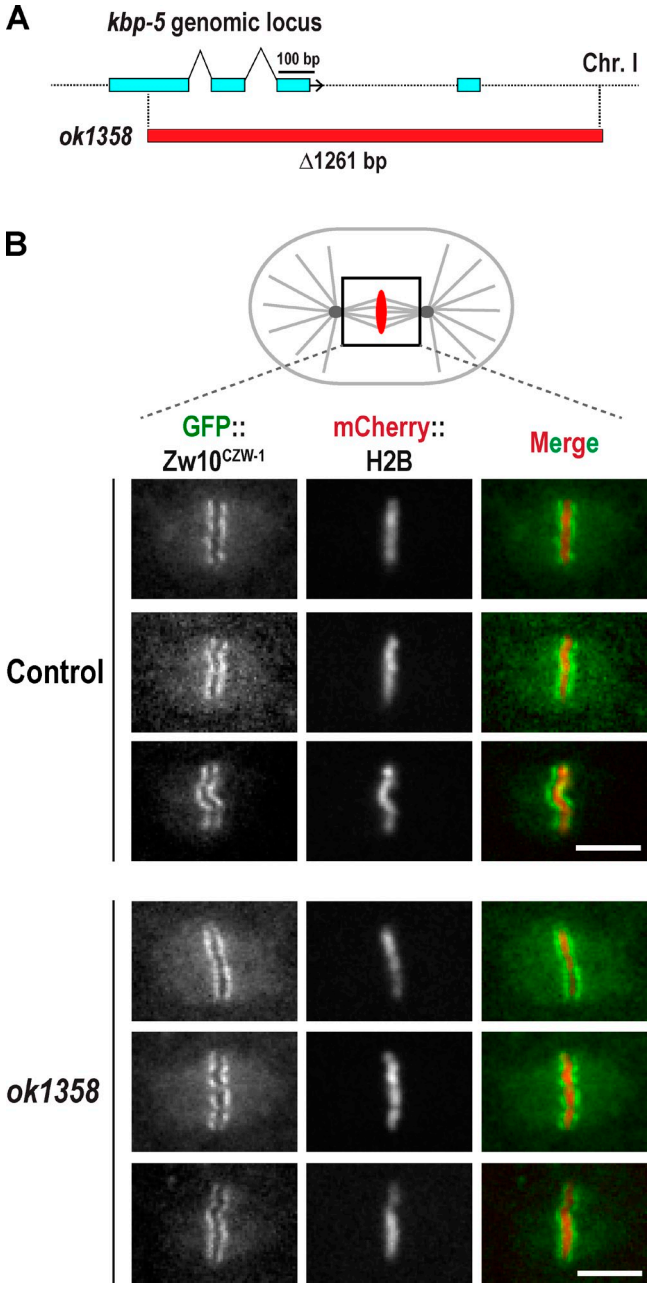


Figure S1. **RZZ localization to kinetochores is normal in *C. elegans* mutants lacking the Zwint1 homologue KBP-5.** (A) Schematic of the *kbp-5* genomic locus and the *ok1358* deletion mutant allele. Chr., chromosome. (B) Images showing kinetochore localization of GFP-Zw10 in metaphase one-cell-stage embryos expressing mCherry-H2B. Three examples are shown for both control (top) and the *ok1358* mutant (bottom) allele. Bars, 5 µm.

A

	Percentage lost by RNAi					
	Knl1 KD		Zwint1 KD		Hec1 KD	
	RZZ	Mad1	RZZ	Mad1	RZZ	Mad1
Prometaphase	99%	99%	57%	35%	51%	91%
Nocodazole	98%	85%	61%	37%	11%	11%*

* from Deluca et al., 2003

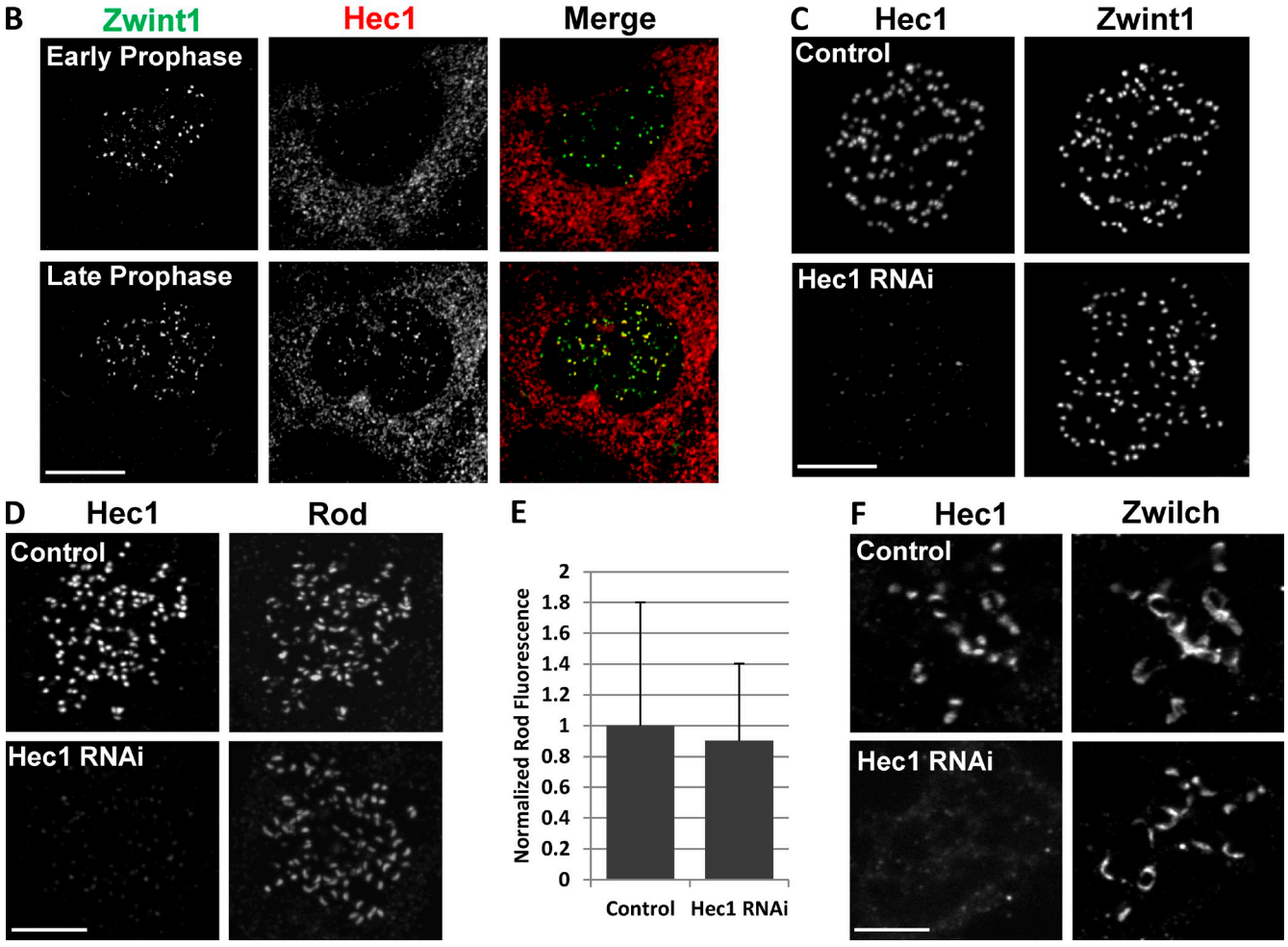
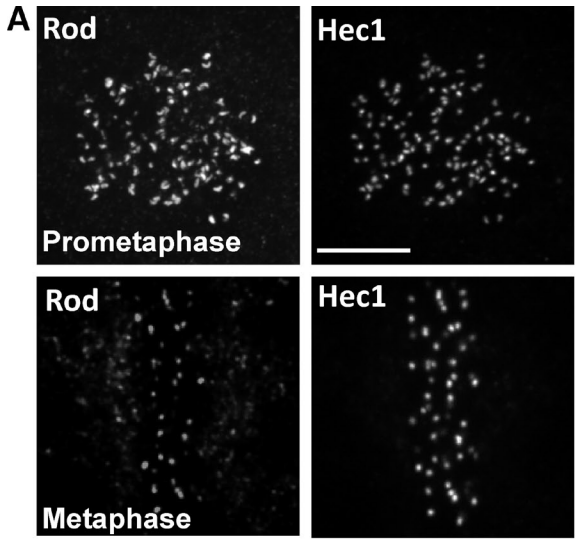
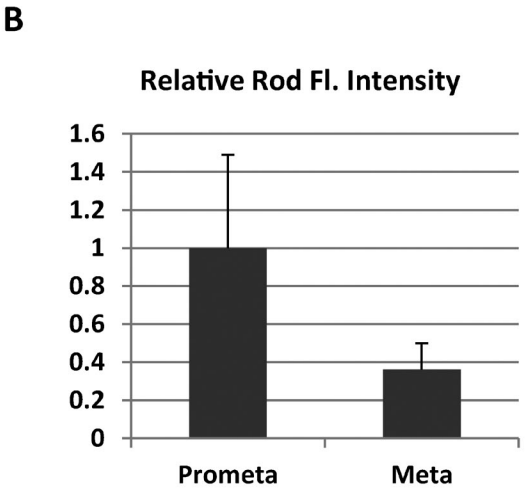
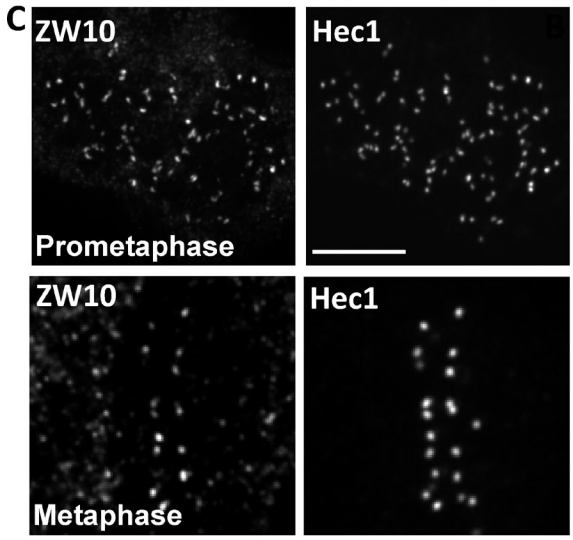
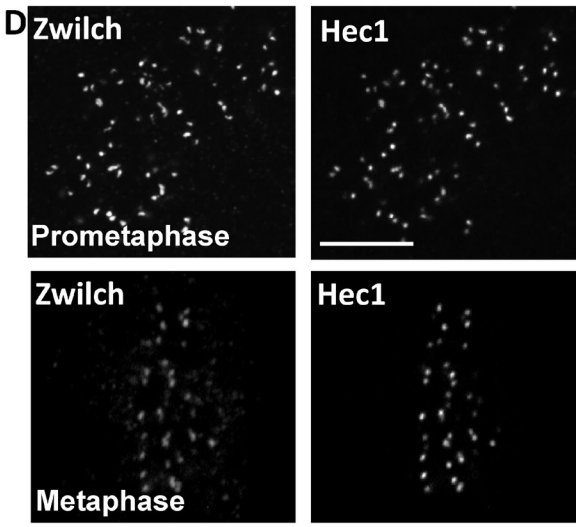


Figure S2. **Hec1 is not required for Zwint1–RZZ recruitment in nocodazole-treated cells.** (A) Summary of the analysis of kinetochore recruitment of the RZZ complex and Mad1 in normal and nocodazole-treated prometaphase HeLa cells. KD, knockdown. (B) HeLa cells in early prophase (top) or late prophase (bottom) were immunostained using anti-Zwint1 and anti-Hec1 antibodies. (C) Control and Hec1 siRNA–treated HeLa cells were immunostained using anti-Zwint1 and anti-Hec1 antibodies. (D) HeLa cells as in C were immunostained using anti-Rod and anti-Hec1 antibodies. (E) Rod fluorescence was normalized relative to that of Hec1 in control and Hec1-depleted cells. n = 130 kinetochores; $P > 0.4$ (not significant). Error bars are SD from the means. (F) Control and Hec1 siRNA-treated Ptk2 cells were immunostained using anti-Zwilch and anti-Hec1 antibodies. Bars, 5 µm.









E

% Protein levels (retained)		
RZZ Component	Prometa-phase (PM)	Metaphase (relative to PM)
Rod	100%	35%
Zw10	100%	45%
Zwilch	100%*	50%*

*also from Gassmann et al., 2010

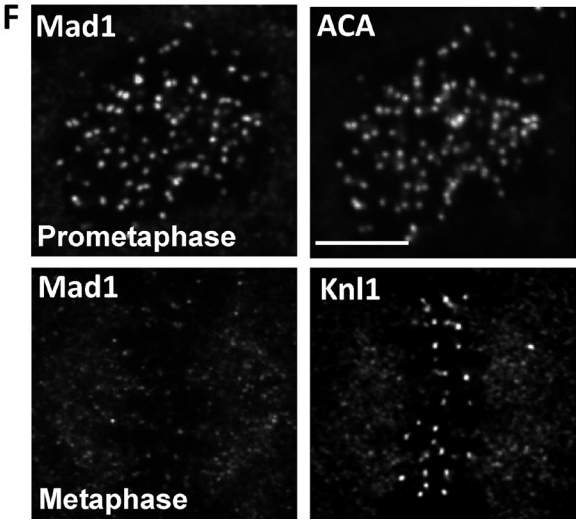
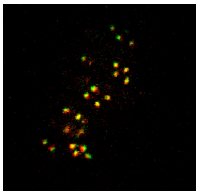
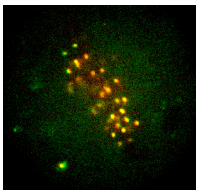


Figure S3. **Components of the RZZ complex are retained substantially at metaphase kinetochores unlike Mad1.** (A) HeLa cells in prometaphase (top) or metaphase (bottom) were immunostained using anti-Rod and anti-Hec1 antibodies. (B) Rod fluorescence (Fl.) was normalized relative to that of Hec1 in prometaphase (Prometa; $n = 65$) versus metaphase cells (Meta; $n = 100$). $P < 0.001$. Error bars are SD from the means. (C) HeLa cells as in A were immunostained using anti-Zw10 and anti-Hec1 antibodies. (D) HeLa cells as in A were immunostained using anti-Zwilch and anti-Hec1 antibodies. (E) Summary of the analysis of the levels of RZZ complex retained at prometaphase and metaphase kinetochores of HeLa cells. (F) HeLa cells as in A were immunostained using anti-Mad1 and either the anti-ACA (top) or Knl1 antibody (bottom). Bars, 5 µm.



Video 1. **Example of metaphase HeLa cells expressing Hec1-GFP/mCherry–CENP-C used to make live Delta measurements.** Double thymidine-synchronized HeLa cells were transfected with cDNA plasmids encoding Hec1-GFP and mCherry–CENP-C and subjected to continuous tandem two-color live imaging during metaphase using an inverted microscope (TE300). Videos were generated from merged z stacks of images obtained from both the channels in which each frame was played for 1/6 s.



Video 2. **Example of metaphase HeLa cells expressing Zwint1-GFP/Hec1-tdTomato used to make live Delta measurements.** Double thymidine-synchronized HeLa cells were transfected with cDNA plasmids encoding Zwint1-GFP and Hec1-tdTomato and subjected to continuous tandem two-color live imaging during metaphase using an inverted microscope (TE300). Videos were generated from merged z stacks of images obtained from both the channels where each frame was played for 1/6 s.