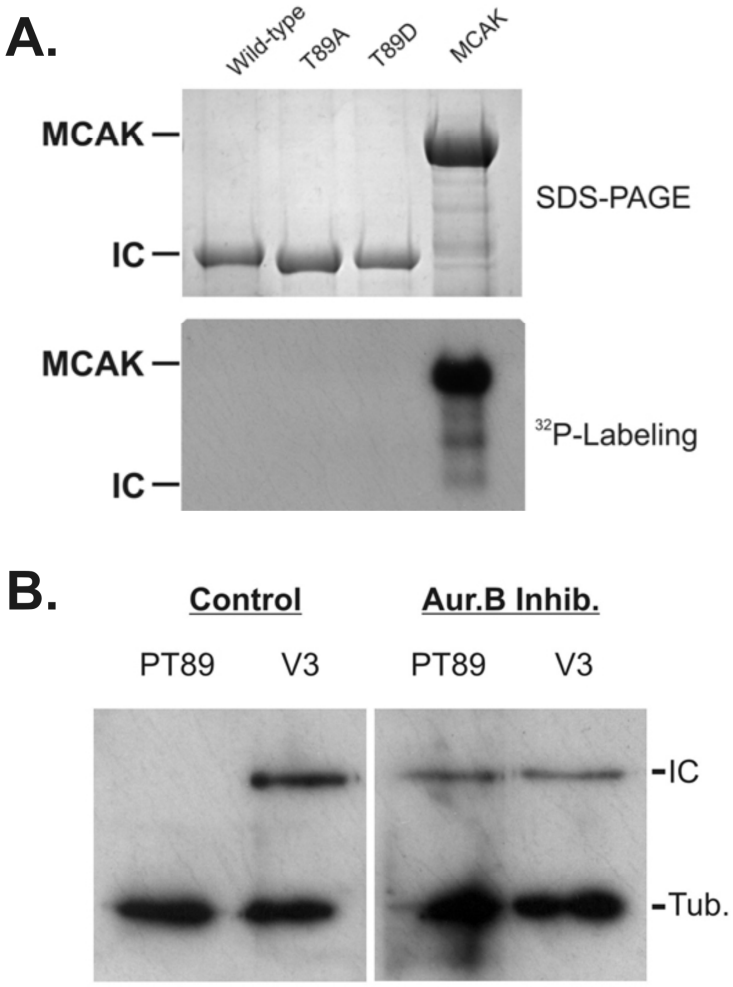
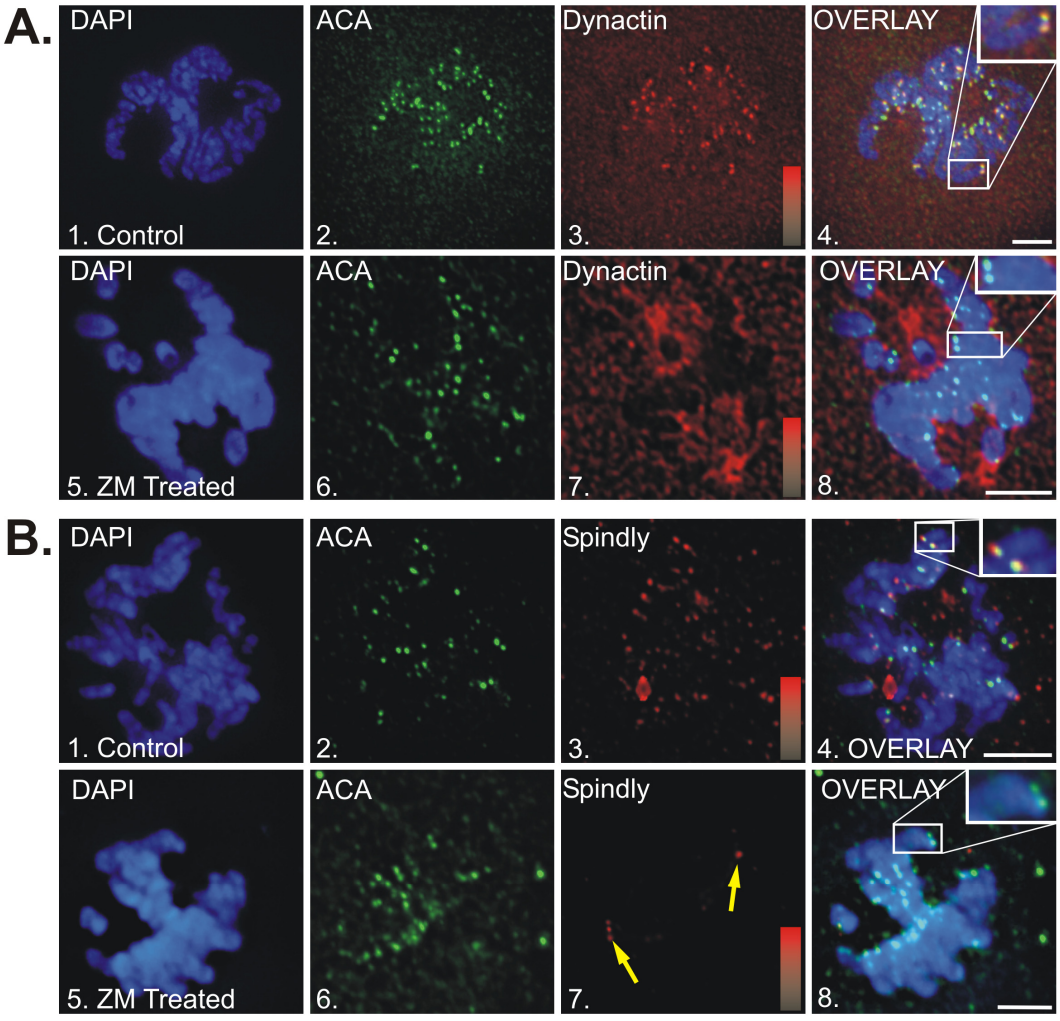
Supplemental Figure 1A&B.

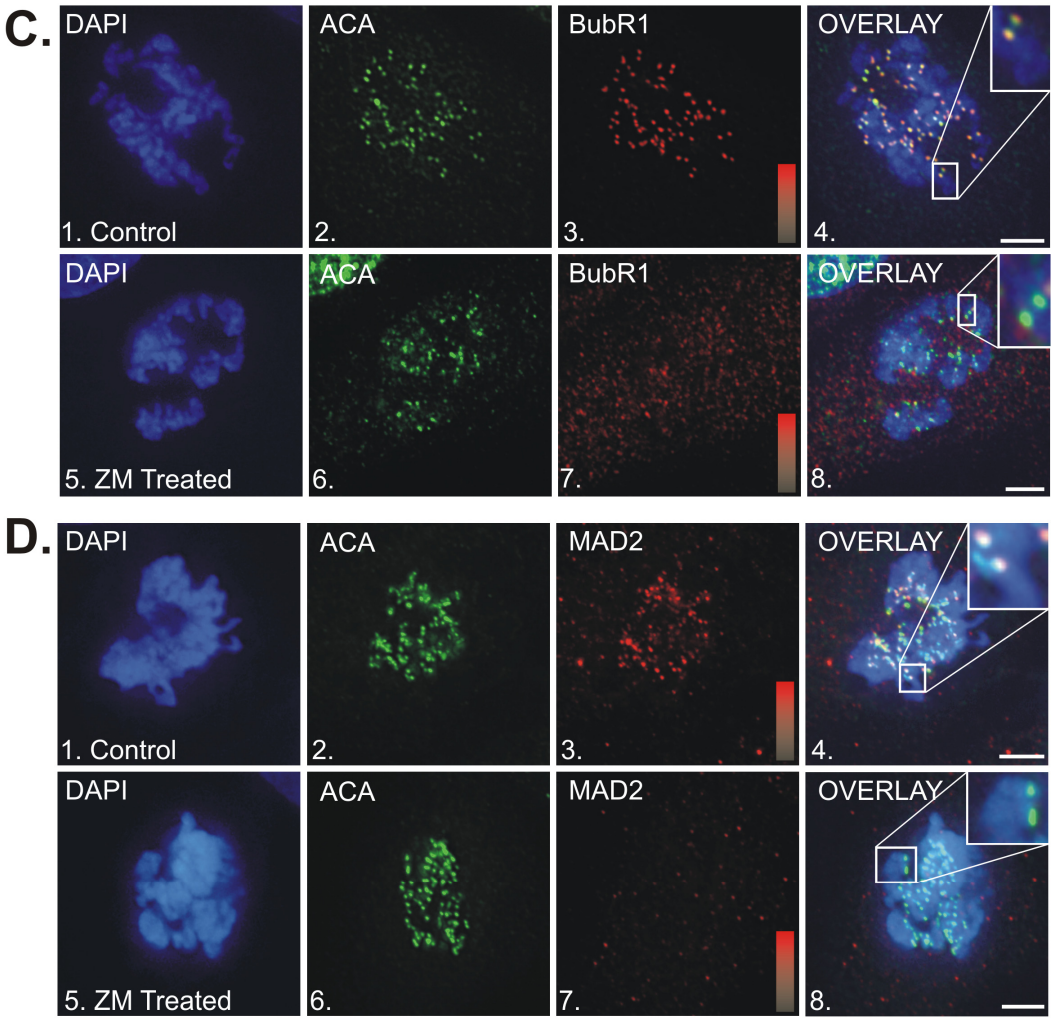


Supplemental Figure 1: A) SDS-PAGE analysis of *in vitro* kinase reactions of recombinant dynein ICs (wild type or T89A/D) or MCAK. CBB staining (top panel) confirms equal loading, whereas ^{32}P-labeling (bottom panel) indicates phosphorylation of MCAK but not ICs. **B)** Western blot analysis of pT89-dynein and total dynein (V3) in RIPA extracts from control and ZM-treated NRK2 cells. α-tubulin was used as a loading control.

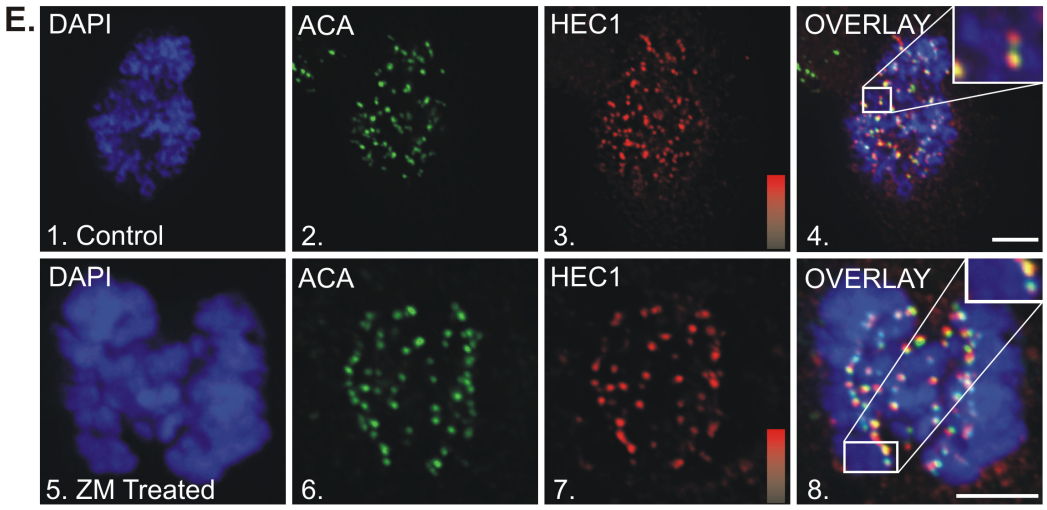
Supplemental Figure 2 A&B.

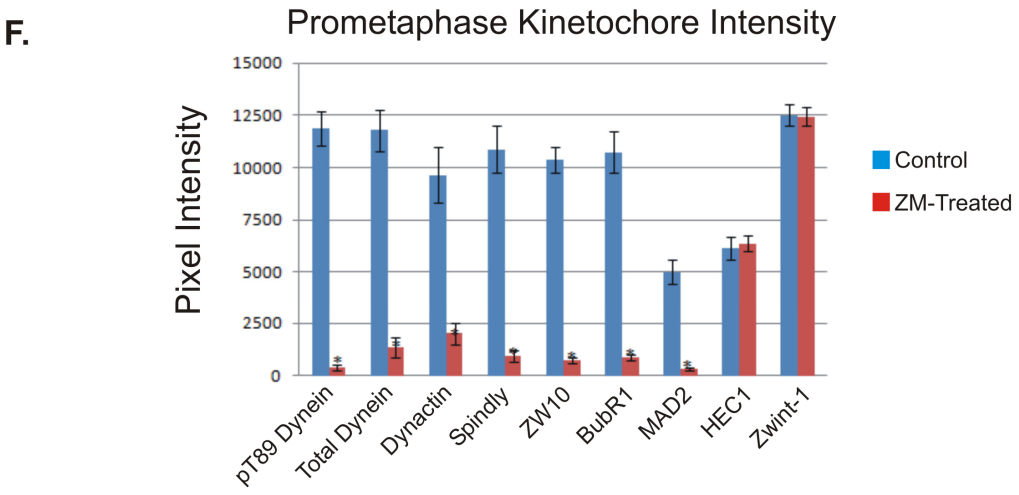


Supplemental Figure 2 C&D.



Supplemental Figure 2E&F.





Supplemental Figure 2: Impact of Aurora B Inhibition of Recruitment of Dynein-associated Proteins to Kinetochores. A) NRK2 cells stained for chromatin (DAPI-blue), ACA (green), and dynactin (red-p150^{Glued}) in controls (panels 1-4) and ZM-treated cells (panels 5-8). Overlay panels reveal colocalization of dynactin with ACA in controls but not ZM-treated cells (insets). Intensity gradients represent a range of 0–12,000 for dynactin staining. Average pixel intensity was 9641 +/- 1333 for controls and 2000 +/- 486 for ZM-treated cells ($p<0.0001$). Mag. bar = 5μm. **B)** NRK2 cells stained for chromatin (DAPI-blue), ACA (green), and spindly (red) in controls (panels 1-4) and ZM-treated cells (panels 5-8). Overlay panels reveal colocalization of spindly with ACA in controls but not ZM-treated cells (insets). A population of spindly remains at the spindle poles (arrows) after ZM-treatment (panel 7). Intensity gradients represent a range of 0–

13,000 for spindly staining. Average pixel intensity was 10880 +/- 1107 for controls and 910 +/- 278 for ZM-treated cells ($p<0.0001$). Mag. bar = 5μm. **C)** NRK2 cells stained for chromatin (DAPI-blue), ACA (green), and BubR1 (red) in controls (panels 1-4) and ZM-treated cells (panels 5-8). Overlay panels reveal colocalization of BubR1 with ACA in controls but not ZM-treated cells (insets). Intensity gradients represent a range of 0–12,500 for BubR1 staining. Average pixel intensity was 10750 +/- 973 for controls and 900 +/- 141 for ZM-treated cells ($p<0.0001$). Mag. bar = 5μm. **D)** HeLa cells stained for chromatin (DAPI-blue), ACA (green), and MAD2 (red) in controls (panels 1-4) and ZM-treated cells (panels 5-8). Overlay panels reveal colocalization of Mad2 with ACA in controls but not ZM-treated cells (insets). Intensity gradients represent a range of 0–5,500 for Mad2 staining. Average pixel intensity was 5001 +/- 600 for controls and 327 +/- 70 for ZM-treated cells ($p<0.0001$). Mag. bar = 5μm. **E)** HeLa cells stained for chromatin (DAPI-blue), ACA (green), and HEC1 (red) in controls (panels 1-4) and ZM-treated cells (panels 5-8). Overlay panels reveal colocalization of HEC1 with ACA in both controls and ZM-treated cells (insets). Intensity gradients represent a range of 0–6,600 for HEC1 staining. Average pixel intensity was 6138 +/- 536 for controls and 6367 +/- 361 for ZM-treated cells ($p=0.2534$). Mag. bar = 5μm. **F)** Statistical Analysis of Kinetochore Protein Accumulation After AurB Inhibition. Proteins analyzed during prometaphase as part of this study are compared in control and ZM-treated cells. Asterisks indicate statistically-significant differences.

Supplemental Figure 3.

A.

M	Y	SEQ	B	M
129	129	Q	2369	128.1
167	296	S	2241	167
181	477	T	2060	181
57	534	G	1896	57
115	649	D	1839	115
101	750	T	1724	101
131	881	M	1623	131
57	938	G	1492	57
156.1	1094	R	1435	156.1
115	1209	D	1279	114
98	1307	P	1165	98
57	1364	G	1067	58
99	1462	V	1009	99
167	1630	S	910	167
147	1777	F	743	147
128.1	1905	K	596	128.1
71	1976	A	468	71
99	2075	V	397	98
57	2132	G	299	58
113	2245	L	241	113.1
128	2373	Q	128	128.1

B.

```
                208                                                        277
M. musculus     LQNKLVISEGKAEDKDVKGRALTAKSKSP------------------------------
R. rattus       LQNKLVISESKADDKDVKGPALPPKSP-------------------------------
H. sapein       LQGKLLFPEAEAEAENLPDDKPQQPTRPQEQSTGDTMGRDPGVSFKAVGLQPAGDVNLP
                **.**::.*.:*: ::: .      :
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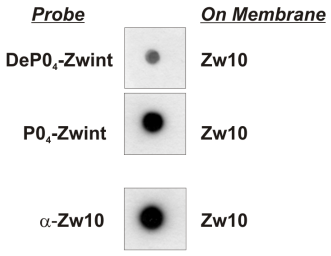
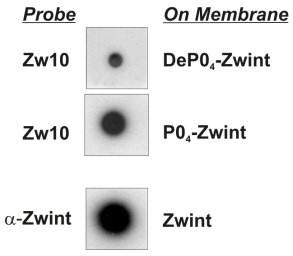
C. Zwint OL

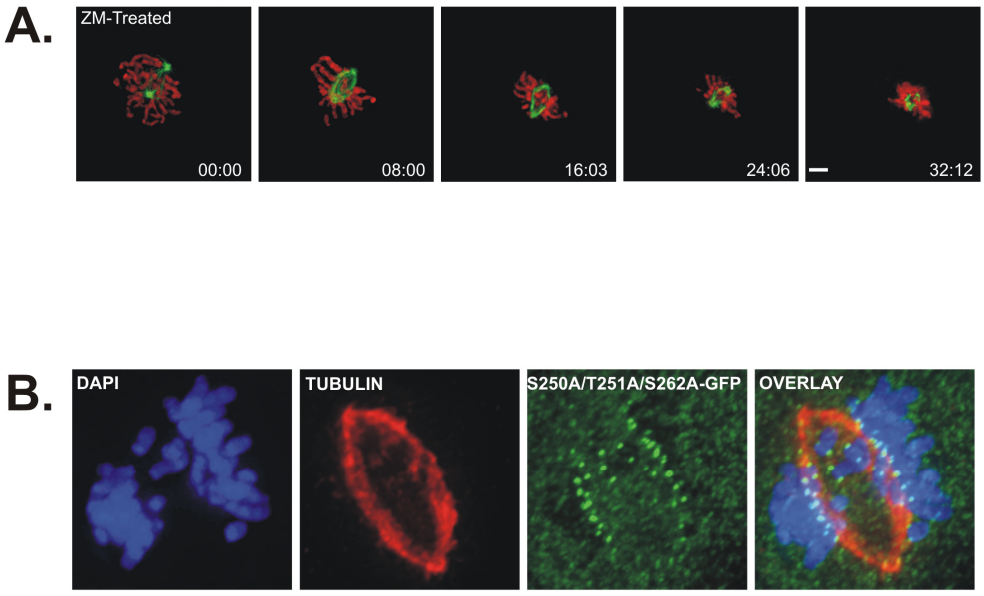


D. ZW10 OL



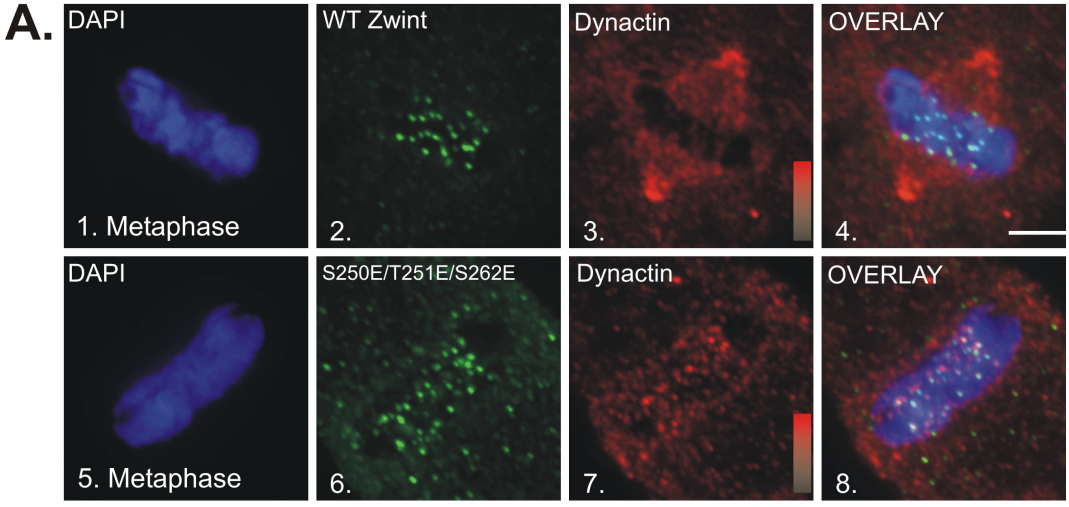
Supplemental Figure 3: Phosphorylation Analysis of Zwint-1. A) Fragmentation data of C-terminal EndoGlu-C fragment of phosphorylated Zwint-1. The peptide sequence is presented with Y and B fragment data. Species containing S250, T251 and S262 are each shifted by 80 Da for each phosphate. **B)** Sequence alignment of C-terminal sequences from mouse, rat and human zwint-1. Phosphorylation sites in human zwint-1 are indicated in red, whereas potential sites in rodent sequences are indicated in yellow. **C)** Recombinant ZW10 spotted onto PVDF membranes was probed with recombinant zwint-1 or phosphorylated zwint-1. **D)** Recombinant zwint-1 or phosphorylated zwint-1 was spotted onto PVDF membranes and probed with recombinant ZW10.

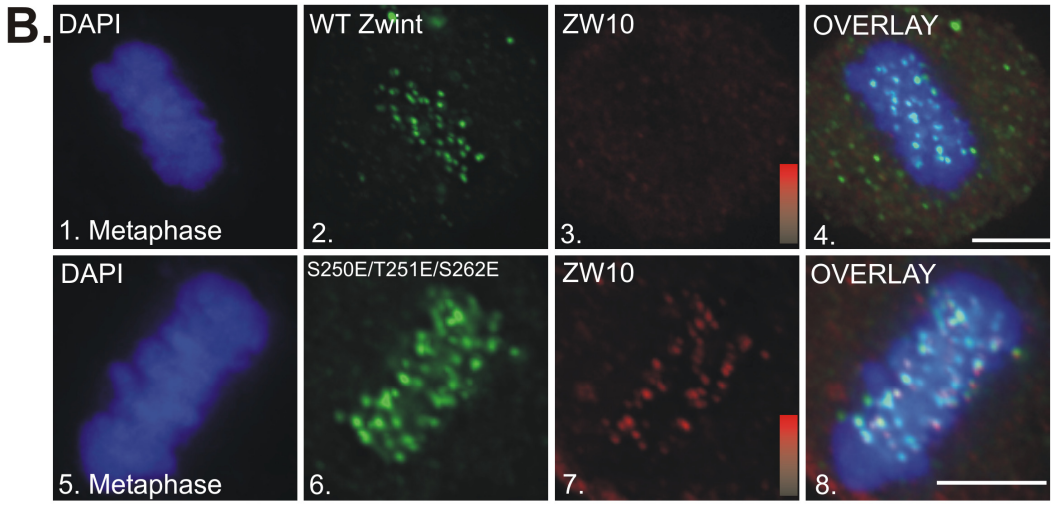
Supplemental Figure 4



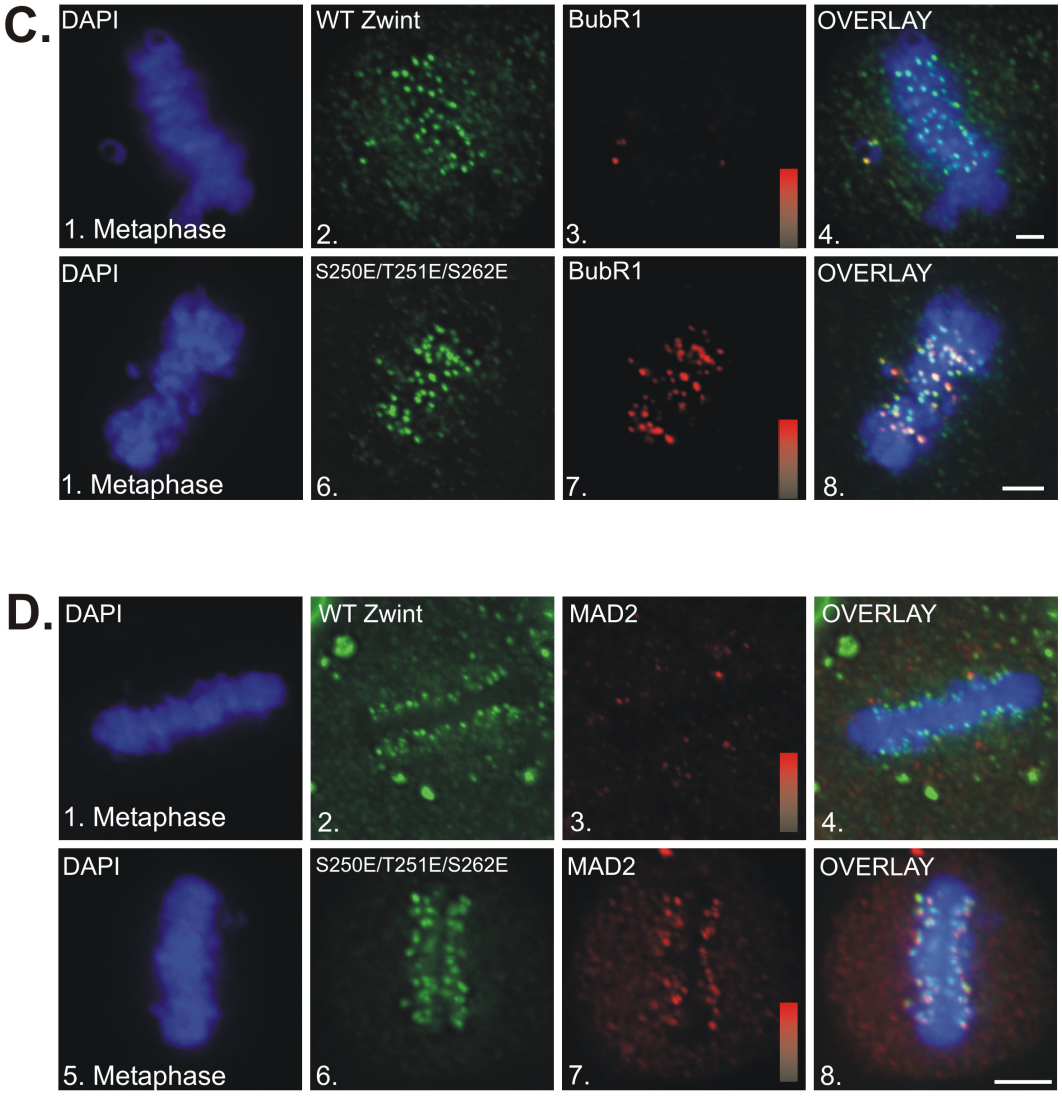
Supplemental Figure 4: Microtubule Attachment in Cells Expressing Triple-A Mutant Zwint-1. A) Timelapse imaging of NRK2 cells expressing mCherry-H2B and EGFP-tubulin after ZM-treatment. Cells treated with ZM display prometaphase arrest and eventual spindle collapse overt the time-lapse sequence. **B)** IFM analysis of cells expressing triple-A mutant zwint-1 demonstrate association of kinetochores with the mitotic spindle.

Supplemental Figure 5A&B.





Supplemental Figure 5C&D.



Supplemental Figure 5: Impact of Triple-E Mutant Expression on Dynein-driven Streaming at Metaphase. A) NRK2 cells (at metaphase) stained for chromatin (DAPI, blue) and dynactin (red) after expression of w.t. zwint-1-EGFP (panels 1-4) or triple-E mutant zwint-1-EGFP (panels 5-8). Intensity gradients correspond to intensity values of 0-10,000. Mag. bar = 5μm. **B)** NRK2 cells (at metaphase) stained for chromatin (DAPI, blue) and ZW10 (red) after expression of w.t. zwint-1-EGFP (panels 1-4) or triple-E mutant zwint-1-EGFP (panels 5-8). Intensity gradients correspond to intensity values of 0-10,000. Mag. bar = 5μm. **C)** NRK2 cells (at metaphase) stained for chromatin (DAPI, blue) and BubR1 (red) after expression of w.t. zwint-1-EGFP (panels 1-4) or triple-E mutant zwint-1-EGFP (panels 5-8). Intensity gradients correspond to intensity values of 0-11,000. Mag. bar = 5μm. **D)** NRK2 cells (at metaphase) stained for chromatin (DAPI, blue) and MAD2 (red) after expression of w.t. zwint-1-EGFP (panels 1-4) or triple-E mutant zwint-1-EGFP (panels 5-8). Intensity gradients correspond to intensity values of 0-13,000. Mag. bar = 5μm.