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Supplemental Material to:

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Spatial regulation of Aurora A activity during mitotic spindle assembly requires RHAMM to correctly localize TPX2

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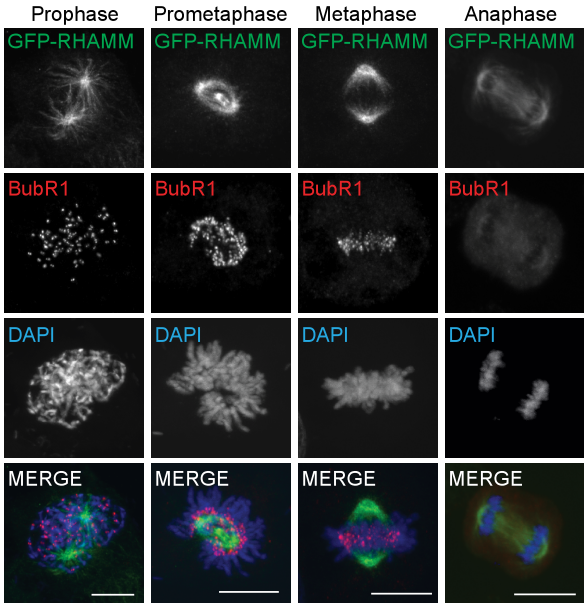
Supplemental Figure 1

A



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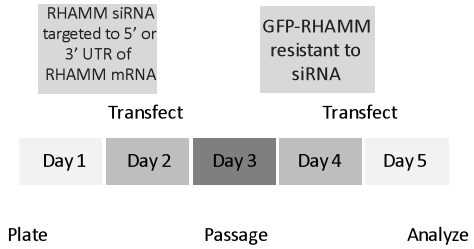

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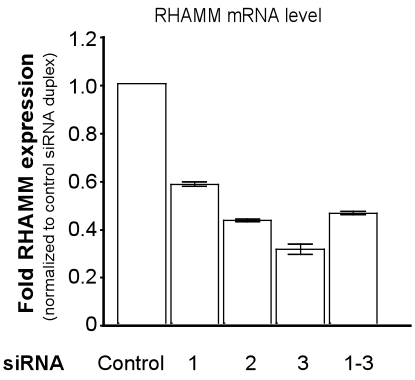

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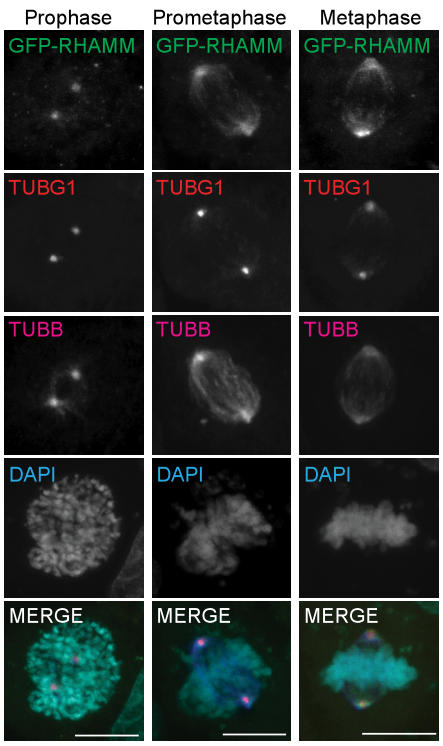

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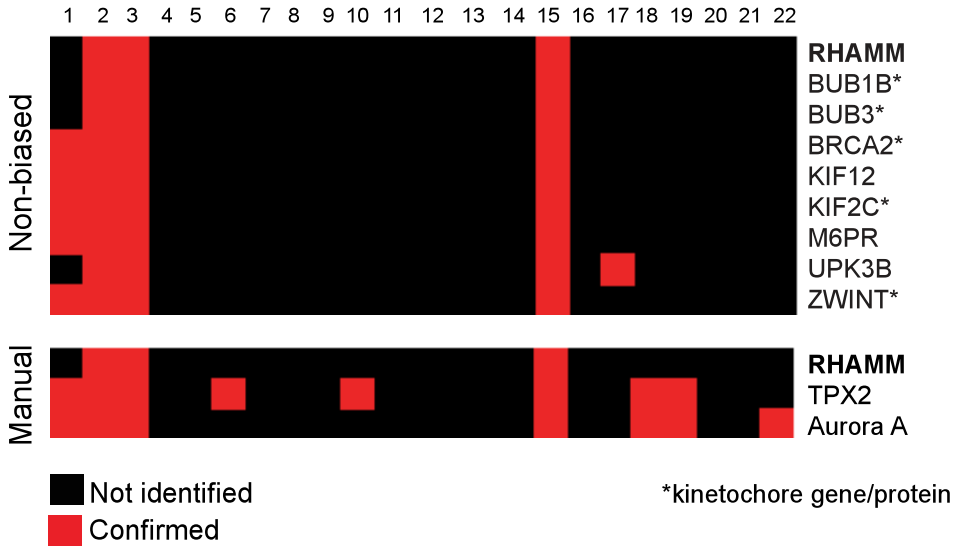
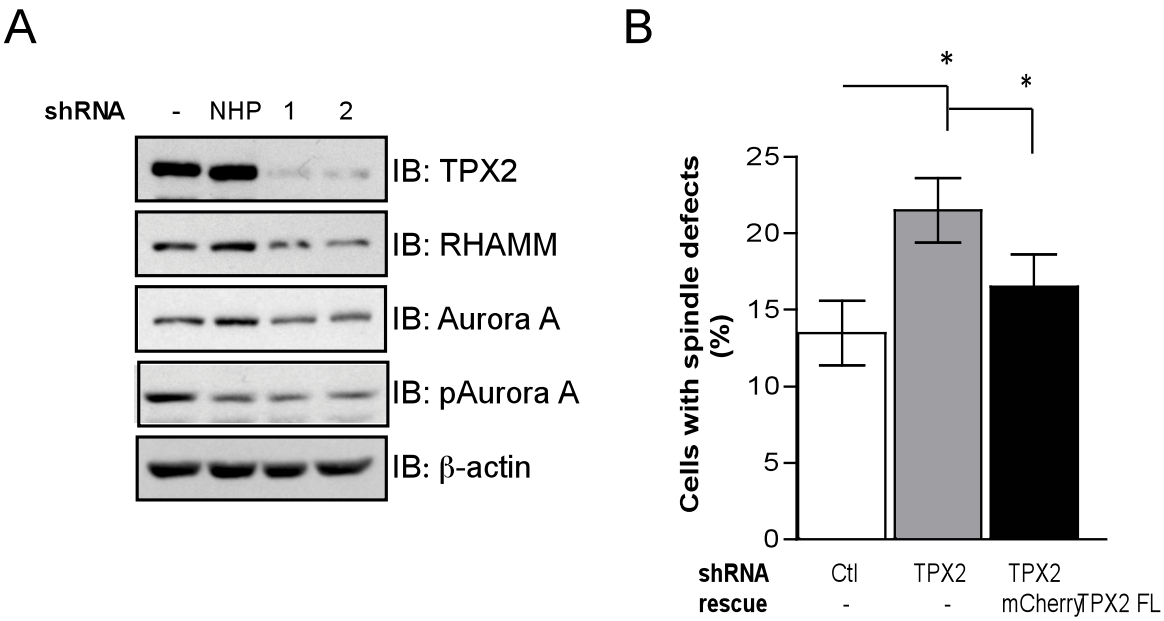


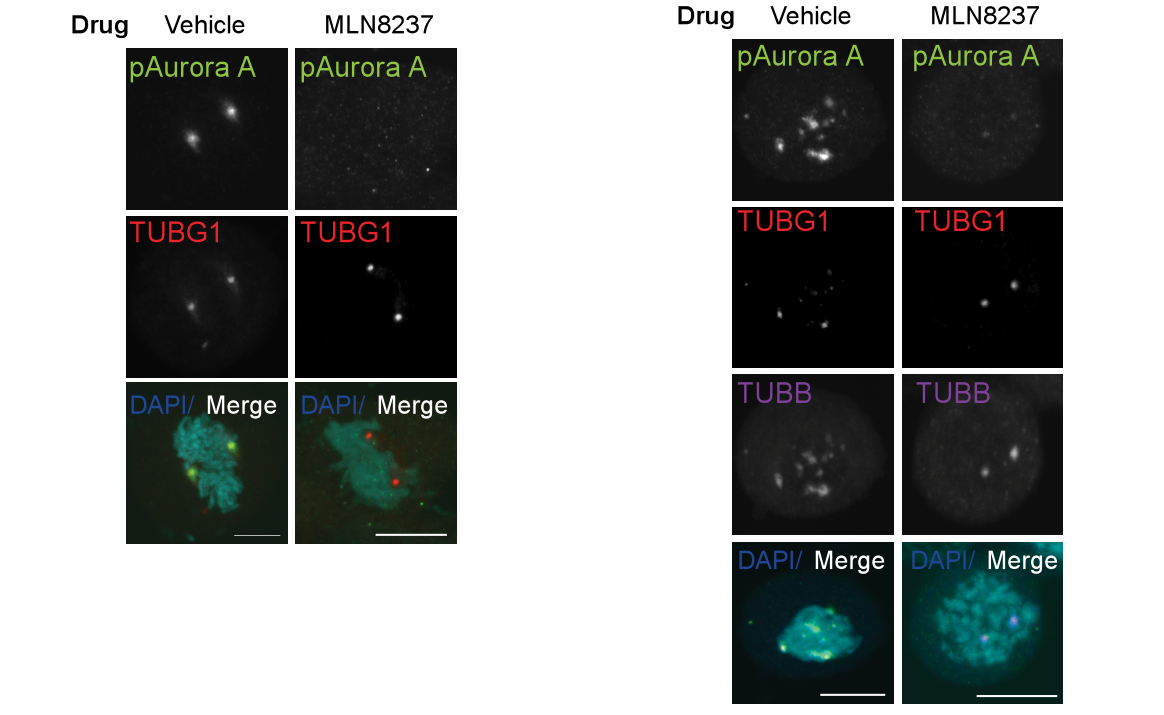
Depletion Phenotypes

1. Nuclei stay close together
2. Strange nuclear shape
3. Segregation problems
4. Cell migration
5. Metaphase delay
6. Cell death
7. Metaphase alignment problems
8. Pulsating nuclei
9. Small nucleus
10. Prometaphase delay
11. Condens. followed by decondens
12. Large nucleus
13. Failure in decondens
14. Binuclear
15. Polylobed
16. Large
17. Dynamic change
18. Mitotic delay
19. Grape
20. Migration (speed)
21. Migration (distance)
22. Increased proliferation

Supplemental Figure 1. RHAMM is a spindle assembly factor that can be depleted by siRNA treatment and rescued with exogenous GFP-RHAMM. A) GFP-RHAMM localization at kinetochores (BubR1) occurred transiently during prophase, and was not observed after nuclear envelope breakdown. Scale bars= 10μm. **B)** Schematic protocol for siRNA-mediated silencing of endogenous RHAMM and rescue with exogenous GFP-RHAMM. **C)** Quantitative PCR confirmed RHAMM mRNA depletion by siRNA, with 42% loss by 3' UTR-A (#1), 57% loss by 3'UTR-B (#2), 69% loss by 5' UTR (#3) and 54% loss by pooled siRNA (1-3). Expression fold differences were normalized to control siRNA. (mean ± s.d., n= 3) **D)** Exogenous GFP-RHAMM in RHAMM-silenced cells localized along the spindle fibers and at the spindle poles, similar to endogenous RHAMM. Scale bars= 10μm. **E)** In the MitoCheck consortium, non-biased alignment of HMMR/RHAMM depletion phenotypes highlighted significant overlap with a number of kinetochore-associated gene products (asterisks), such as strange nuclear shape, chromosome segregation problems and polylobed nuclei. Manual comparison between HMMR/RHAMM, Aurora A and TPX2 revealed similar morphological abnormalities, such as strange nuclear shape and chromosome segregation problems.

Supplemental Figure 2





Supplemental Figure 2. pAurora A specific inhibitor, MLN8237, is sufficient to inhibit kinase activity. A) TPX2 was efficiently silenced in cells treated with two separate shRNA targeting TPX2, compared to control shRNA (NHP) or untreated cells. RHAMM, Aurora A and pAurora A levels were reduced in TPX2-silenced lysates. β-actin levels confirmed equal loading. **B)** Aberrant spindle figures (multipolar spindle, disorganized spindle, lagging and unattached chromosomes) were significantly more frequent in TPX2-silenced cells. Rescue with mCherry-TPX2 full-length transgene was sufficient to reduce these aberrant phenotypes. (mean ± s.d., n= 2, **P*< 0.05) **C)** MLN8237 treatment significantly reduced pAurora A specific immuno-staining at the spindle poles (TUBG1). Scale bars= 10μm. **D)** Following nocodazole treatment, pAurora A localization to non-centrosomal microtubule assembly sites was abolished with MLN8237 treatment. Scale bars= 10μm.

Table S1: PCR primers and siRNA sequences

	Sequence
Truncations	
RHAMM^{FL}	Forward (F1): 5' GGGGACAAGTTTGTACAAAAAAGCAGGCTCCACCATGGTGAGCAAGGGCGAG 3' Reverse: 5' GGGGACCACTTTGTACAAGAAAGCTGGGTCTTACTTCCATGATTCTTGAC 3'
RHAMM$^{1-623}$	Forward: F1 Reverse: 5' GGGGACCACTTTGTACAAGAAAGCTGGGTCCTATCTTATTTTATTTAGCTGTTCCTGAGCTGCACC 3'
RHAMM$^{1-679}$	Forward: F1 Reverse: 5' GGGGACCACTTTGTACAAGAAAGCTGGGTCCTATGGTGTTTGGTACCTAGAACTTAATTCAAT 3'
RHAMM$^{623-724}$	Forward:5'GGGGACAAGTTTGTACAAAAAAGCAGGCTTGAAGGAGATAGAACCATGAGAGATTCATATGCTAAATTATTGG 3' Reverse: 5' GGGGACCACTTTGTACAAGAAAGCTGGGTCTTACTTCCATGATTCTTGAC 3'
TPX2^{FL}	Forward (F2): 5' GGGGACAAGTTTGTACAAAAAAGCAGGCTCCACCATGGTGAGCAAGGGCGAG 3' Reverse: 5' GGGGACCACTTTGTACAAGAAAGCTGGGTCGTTTAAACTCTTTCTTCACCGGCA 3'
TPX2$^{1-319}$	Forward: F2 Reverse: 5' GGGGACCACTTTGTACAAGAAAGCTGGGTCTAATTCATCAAATGTTCTTTTCTTTCCT 3'
TPX2$^{40-783}$	Forward:5'GGGGACAAGTTTGTACAAAAAAGCAGGCTCGAAGGAGATAGAACCATGAATTTGGAGAATAAGTTACTGGGG 3' Reverse (R1): 5' GGGGACCACTTTGTACAAGAAAGCTGGGTCGTTTAAACTCTTTCTTCACCGGCA 3'
TPX2$^{319-783}$	Forward:5'GGGGACAAGTTTGTACAAAAAAGCAGGCTCGAAGGAGATAGAACCATGGAAACAGTTTCTACATATGTGCCC 3' Reverse: R1
Mutagenesis	
RHAMM^{L629R}	Forward: 5' GATTCATATGCTAAAcgATTGGGTCATCAGAATTTG 3' Reverse: 5' TCTTATTTTATTTAGCTGTTCCTGAGCTGCACC 3'
RHAMM^{L645R}	Forward: 5' AAGCATGTTGTGAAGcgGAAAGATGAAAATAGCCAACTC 3' Reverse: 5' GATTTTTTGTTTCAAATTCTGATGACCCAATcgTTTAGC 3'
RHAMM^{L663R}	Forward: 5' AAACTCCGCTGTCAGCgTGCTAAAAAAAAACAAAGTGAG 3' Reverse: 5' TGATACTTCCGATTTGAGTTGGCTATTTTCATCTTTCcg 3'
qRT-PCR	
HMMR cDNA	Forward: 5' TGTGCTTCAGATCAAGTGG 3' Reverse: 5' CGTTGTGTTCTCTATTCCTG 3'
TBP cDNA	Forward: 5`TGCACAGGAGCCAAGAGTGAA 3' Reverse: 5`CACATCACAGCTCCCCACCA 3'
RT-PCR conditions	35 cycles at 95^{o}C for 60s, 60^{o}C for 30s and 72^{o}C for 30s
qPCR conditions	5 cycles at 95^{o}C for 5 minutes, 60^{o}C for 60s and 72^{o}C for 60s, and 35 cycles at 95^{o}C for 60s, 60^{o}C for 30s and 72^{o}C for 30s
siRNA	
siHMMR 1	Sense: 5' GAAAUAAGGACAAGCCUAAUU 3' Antisense: 5' PUUAGGCUUGUCCUUAUUUCUU 3'
siHMMR 2	Sense: 5' GCAAAUACCUCCUCCCUAAUU 3' Antisense: 5' UUAGGGAGGAGGUAUUUGCUU 3'
siHMMR 3	Sense: 5' UGGCUUUCCAAUUGGCUAAUU 3' Antisense: 5' PUUAGCCAAUUGGAAAGCCAUU 3'
Control	AllStars Negative Control siRNA (proprietary), Qiagen
shRNA	
TPX2	5' CCGAGCCTATTGGCTTTGATT 3'
Control	pLKO.1 - TRC control (Plasmid 10879), Addgene

Supplemental movie legends

Supplemental Movie 1. Spindle formation and mitosis in HeLa cells (GFP-tubulin and mCherry-histone H2B) transfected with control siRNA duplexes. 96 hours following transfection, mitosis was followed by time-lapse imaging for 14 hours with images captured at 15 minute intervals. At each time point, 7 images were captured through the Z-volume at 1.0 μm steps. A maximum intensity projection of the Z-volume is shown through time at 2 frames/second.

Supplemental Movie 2. Bipolar spindle formation is disrupted in HeLa cells (GFP-tubulin and mCherry-histone H2B) transfected with siRNA duplexes targeting RHAMM. 96 hours following transfection, mitosis was followed by time-lapse imaging for 16.5 hours with images captured at 15 minute intervals. At each time point, 7 images were captured through the Z-volume at 1.0 μm steps. A maximum intensity projection of the Z-volume is shown through time at 2 frames/second.

Supplemental Movie 3. Rotation of metaphase spindle occurs in HeLa cells (GFP-tubulin and mCherry-histone H2B) transfected with siRNA duplexes targeting RHAMM. 96 hours following transfection, mitosis was followed as described in Supplemental movie 2.

Supplemental Movie 4. The kinetics of mitosis were restored with exogenous GFP-RHAMM rescue in HeLa cells transfected with siRNA duplexes targeting RHAMM. Exogenous GFP-RHAMM was transfected into cells 48 hours after RHAMM siRNA transfection. 48 hours following rescue, the cells were labelled with Hoechst (blue) to visualize DNA. Mitosis was followed by time-lapse imaging for 5 hours with images captured at 15 minute intervals. At each time point, 7 images were captured through the Z-volume at 1.0 μm steps. A maximum intensity projection of the Z-volume is shown through time at 2 frames/second.

Supplemental Movie 5. Spindle microtubule regrowth occurred at centrosome and non-centrosomal spindle assembly sites in control cells. HeLa cells stably expressing GFP-tubulin were transfected with control siRNA duplexes, allowed to recover for 96 hours, and then exposed to nocodazole. The cells were given fresh media after nocodazole removal, and microtubule assembly was followed by time-lapse imaging for 40 minutes with images captured at 2 minute intervals. At each time point, 7 images were captured through the Z-volume at 1.0 μm steps. A maximum intensity projection of the Z-volume is shown through time at 2 frames/second.

Supplemental Movie 6. Spindle microtubule regrowth was disrupted in RHAMM-silenced cells. HeLa cells stably expressing GFP-tubulin were transfected with siRNA duplexes targeting RHAMM, allowed to recover for 96 hours, and then exposed to nocodazole. Following the removal of nocodazole, microtubule assembly was followed by time-lapse imaging as described in Supplemental movie 5.

Supplemental Movie 7. Spindle microtubule regrowth at non-centrosomal assembly sites was severely delayed in RHAMM-silenced cells. HeLa cells stably expressing GFP-tubulin were transfected with pooled siRNA duplexes targeting RHAMM, allowed to recover for 96 hours, and then exposed to nocodazole. The cells were given fresh media after nocodazole removal, and microtubule assembly was followed by time-lapse imaging for 52 minutes with images captured at 2 minute intervals. At each time point, 7 images were captured through the Z-volume at 1.0 μm steps. A maximum intensity projection of the Z-volume is shown through time at 2 frames/second.