

Supplemental Table1: siRNA's used for RNAi experiments

MobKL1A	5'-GGUAAAUGUUGAUACGAAUU-3' 5'-GCACCAAAGUAUAUUGAUUUU-3' 5'-GCAGAUUGGAACGAACAUAUU-3' 5'-AUGAAUGGGUUGCAGUUAAUU-3'
MobKL1B	5'-GAGACAAGCUGUUAUGUUGUU-3' 5'-UUAACCAGAUAACAUGUUUU-3' 5'-CAAAGACUAUUCUAAAGCGUU-3' 5'-GAAGCAAGCUGUCCAGUCAUU-3'
INCENP	5'-GUCAUGAAGUCCUUUAUUA-3' 5'-GGAUGGAUCUGAAUAGCGA-3' 5'-CAACGGAAAUAACUCGUGG-3' 5'-CGGAAGAAGAGACGGAUUU-3'
PLK	5'-GCACAUACCGCCUGAGUCU-3' 5'-CCACCAAGGUUUUCGAUUG-3' 5'-GCUCUCAAUGACUCAACA-3' 5'-UCUCAAGGCCUCCUAAUAG-3'
BubR1	5'-CAAUACAGCUUCACUGAUUU-3' 5'-GCAAUGAGCCUUUGGAUUAUU-3' 5'-GAAACGGGCAUUUGAAUAUU-3' 5'-GAUGGUGAAUUGUGGAAUAUU-3'
Control	5'-UAGCGACUAAACACAUCAA-3'

Supplemental Table 2: Primers used for Quantitative PCR experiments.

MobKL1A NM_173468	5'-ggtgatgctgaactgtgagg-3' 5'-cctcaatecctaccctcag-3'
MobKL1B NM_018221	5'-tctgaggaccgaagatgagc-3' 5'-ccgacaatacaacggactccc-3'
MobKL2B NM_024761	5'-ctaaccaagcagcctgaacc-3' 5'-gggatgtagagaaaaccggt-3'
MobKL2C NM_145279	5'-tggagtctgtctcccatcc-3' 5'-cgataggtgtccctctgacc-3'
MobKL2A NM_130807	5'-cagggtttcaccgtgttagc-3' 5'-ccttgctacaggtgaaacga-3'
GAPDH NM_002046	5'-caatgacccttcattgacc-3' 5'-gctctagggaggttttagtt-3'
ACTIN NM_001101	5'-agaaaatctggcaccacacc-3' 5'-cgatagggacatgaggagac-3'

Supplemental Table 3: List of organisms, gene names and ascension numbers used to construct the dendrogram in Figure 1A.

Organism	Gene Name	Alternative Gene Names	Ascension #
<i>G. lamblia</i>	MOB1	ATC 50803	XM_001709385
<i>S. cerevisiae</i>	MOB1	Mob1p, YIL106W	NP_012160
<i>S. pombe</i>	MOB1	SPBC428	NM_001021098
<i>H. sapiens</i>	MOBKL1A	MATS2, MOB4A, Mob1B	NM_173468
<i>M. musculus</i>	MOBKL1A	MGC129510, MGC129511	NM_026735
<i>X. tropicalis</i>	MOBKL1A	Mats2, Mob1b, Mob4A	NM_001079104
<i>D. rerio</i>	MOBKL1A	zgc:56156, zgc:77872	NM_200200
<i>D. rerio</i>	MOBKL1B	zgc:111840, C2orf6, MOB1, MOBK1B, Mob4B, cb512	NM_214783
<i>M. musculus</i>	MOBKL1B	MOB1, MOB4B, Mobk1b	NM_145571
<i>H. sapiens</i>	MOBKL1B	MATS1, MOB1, MOBK1B, Mob4B, C2orf6, FLJ10788, FLJ11595	NM_018221
<i>X. tropicalis</i>	MOBKL1B	MOB1, Mats1, Mob4b, Mobkl1b	NM_001017026
<i>D. melanogaster</i>	MATS	DMob1, Dme1, SCG13852	NM_142784
<i>S. purpuratus</i>	MOB1	Mob1	XM_787632
<i>A. thaliana</i>	MOB1	AT5G45550, AT5G20430	NM_118023
<i>O. sativa</i>	MOB1B		NM_001071066
<i>O. sativa</i>	MOB1A		NM_001057076
<i>D. rerio</i>	MOBKL2A	zgc:56681, zgc:77552	NM_199716
<i>X. tropicalis</i>	MOBKL2A	Mobkl2c, moblak	NM_001011080
<i>H. sapiens</i>	MOBKL2A	MOB LAK, MOB-LAK	NM_130807
<i>M. musculus</i>	MOBKL2A	MOB1	NM_172457
<i>M. musculus</i>	MOBKL2B	MGC28359, Mob3b	NM_178061
<i>H. sapiens</i>	MOBKL2B	Mob3B	NM_024761
<i>X. tropicalis</i>	MOBKL2B	Mob3B, Mobkl2a	NM_001016616
<i>M. musculus</i>	MOBKL2C	MOB3C	NM_175308
<i>D. rerio</i>	MOBKL2C	zgc:92408	NM_145729
<i>D. melanogaster</i>	MOB1	Mob3, DMob3, Mob3-PA	NM_0135520
<i>T. brucei</i>	MOB1B		XM_841088
<i>T. brucei</i>	MOB1A		XM_841087
<i>L. major</i>	MOB1		XM_001680815

Supplemental figure legends

Supplemental Figure 1. GFP-Mob1C and Mob1D localization in HeLa cells.

HeLa cells were transfected with GFP-Mob1C and Mob1D, and were fixed 24 hr post-transfection. Panels A-H show optical sections of cells in prometaphase, metaphase, anaphase, and late cytokinesis, respectively. Bar, 10 μm .

Supplemental Figure 2. Localization of GFP-tagged and endogenous Mob1A.

A. HeLa cells transiently expressing GFP-Mob1A were counterstained for endogenous Mob1A with an antibody directed against the N-terminus of MobKL1B (A-L).

Additionally, HeLa cells processed for endogenous Mob1 and tubulin (M-O) to visualize Mob1 localization in the absence of the GFP-tagged construct. Note that in the optical sections shown in Panels A, D and M, one or both spindle poles are not visible in the focal plane. Bar, 10 μm . **B.** Confirmation of the specificity of the Mob1A antibody was confirmed by western blot. **C.** Transient expression of untagged EGFP in HeLa cells. Bar, 10 μm .

Supplemental Figure 3: Regulation of Mob1C and Mob1D recruitment to spindle poles.

GFP-Mob1C and GFP-Mob1D-expressing HeLa cells were transfected with non-targeting control (left panels)- and Plk1 siRNA (right panels). Twenty-four hours post-siRNA transfection, cells were processed for localization of γ tubulin and Plk1. Similar to Mob1A, in the absence of Plk1, cells formed a monopolar spindle, and while both

Mob1C and Mob1D localization was lost from the centrally located spindle pole while kinetochore localization was unaffected (D, J, O and U). Bar, 10 μ m.

Supplemental Figure 4: Confirmation of Plk1 and INCENP depletion in HeLa cells.

GFP-Mob1A-expressing HeLa cells were transfected with non-targeting control, Plk1, or INCENP siRNA. Twenty-four hours post-siRNA transfection, cells were lysed for Western blotting and probed for the target proteins, and then reprobbed for tubulin as a loading control.

Supplemental Figure 5. Regulation of Mob1C and Mob1D recruitment to kinetochores.

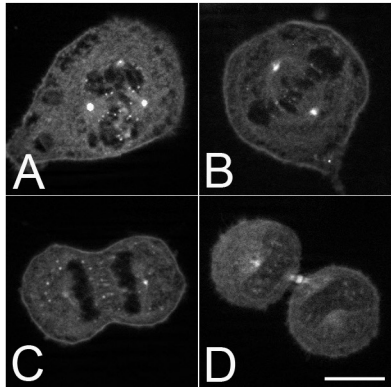
GFP-Mob1C and GFP-Mob1D-expressing HeLa cells were transfected with non-targeting control and INCENP siRNA. Twenty-four hours post- transfection, cells were processed for localization of Hec1 and INCENP. As was found with GFP-Mob1A, when INCENP was depleted, Mob1C and Mob1D no longer localized on the kinetochore, but spindle pole localization was unaffected (D, J, O and U). Bar, 10 μ m.

Supplemental Figure 6: Mob1C and Mob1D localization to the kinetochore is lost when Aurora B is inhibited.

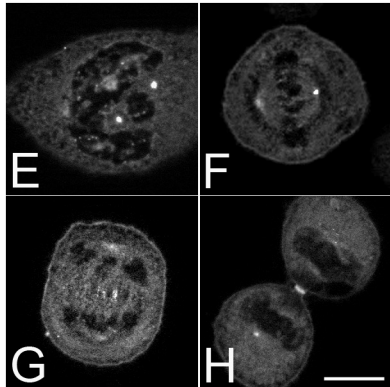
GFP-Mob1C and GFP-Mob1D-expressing HeLa cells were transfected were treated with 200nM nocodazole for 3hr, and then released into 0.1% DMSO (A-D and I-L) or 5 μ M ZM477439 (E-H and M-P) for an additional 30min. Cells were fixed and counter-stained with phospho- (Ser10) histone H3 (B, F, J and N). Note that while spindle pole

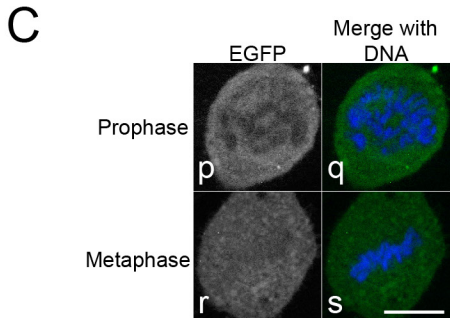
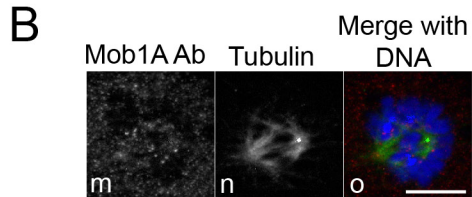
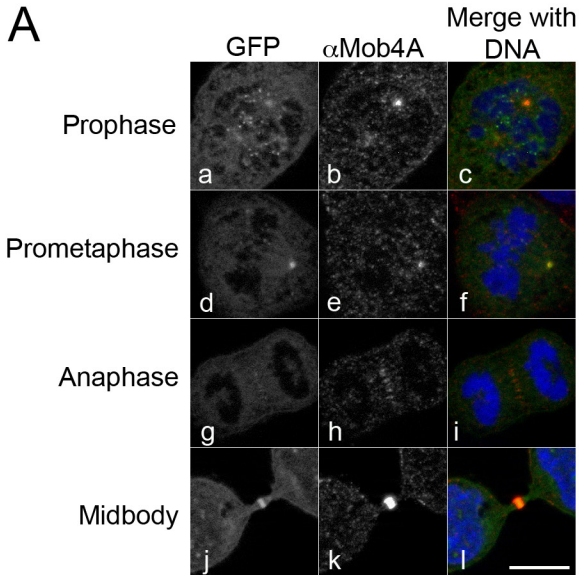
localization was maintained in cells with compromised Aurora B activity, kinetochore localization was lost (E and M). Bar, 5 μm .

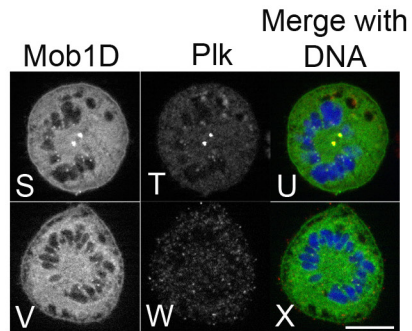
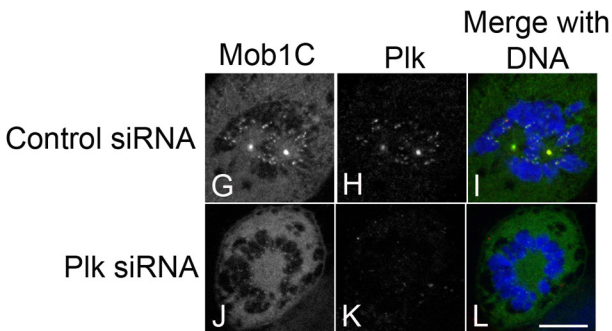
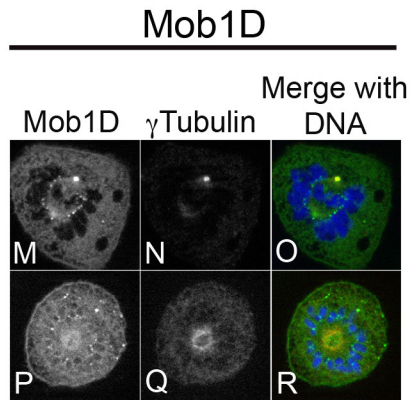
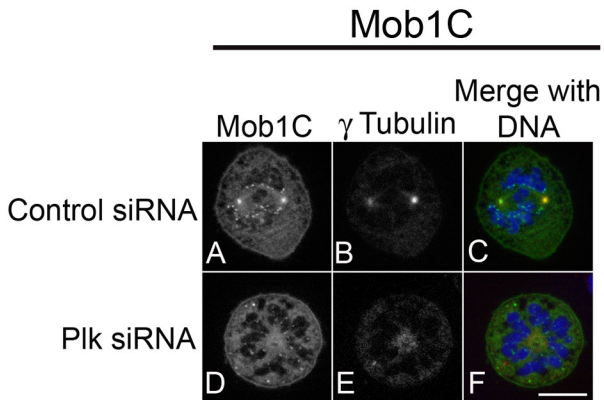
Mob1C



Mob1D







PIK RNAi

Control

PIK

PIK



Tubulin



INCENP RNAi

Control

INCENP

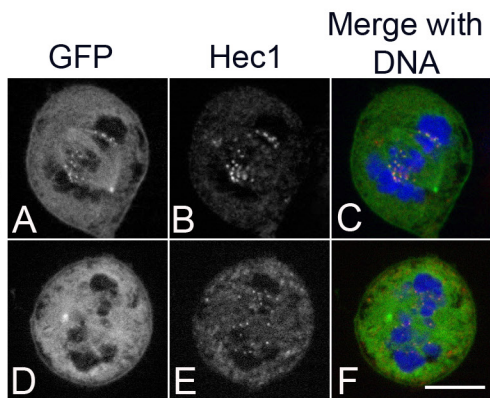
Incenp



Tubulin



Mob1C



Mob1D

