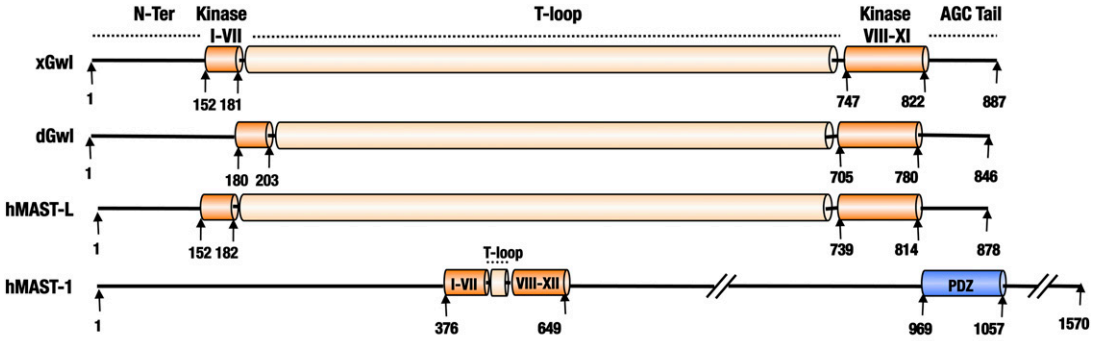
Supporting Information

Burgess et al. 10.1073/pnas.0914191107



% Sequence Homology			
	d-Gwl	x-Gwl	H-Mast1
hMast-l	50,2	65,7	10,4

Fig. S1. Sequence homology among *Xenopus* Greatwall (xGwl), *Drosophila* Gwl (dGwl), human microtubule-associated serine/threonine kinase-like (hMAST-L), and human MAST1 (hMAST1). Shown are the kinase domains of all four proteins. xGwl, dGwl, and hMAST-L have a kinase domain separated by a very long T-loop (> 500 amino acids), whereas the MAST1 protein contains a conventional T-Loop of about 30 amino acids. Neither xGwl, dGwl, nor hMAST-L contains a PDZ domain, a characteristic motif of the MAST family of proteins. Finally, hMAST-L has a much higher sequence homology with dGwl and xGwl compared with the hMAST1 protein.

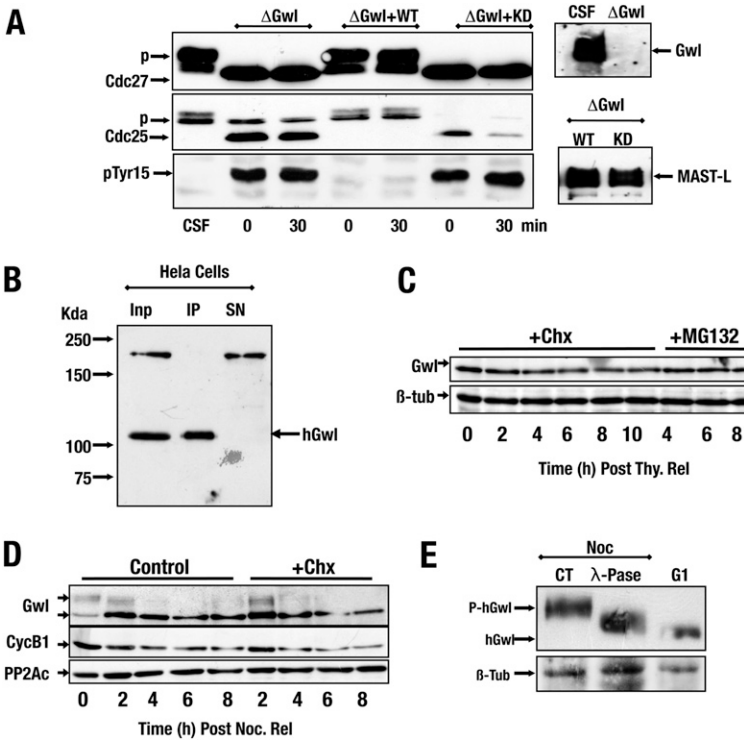
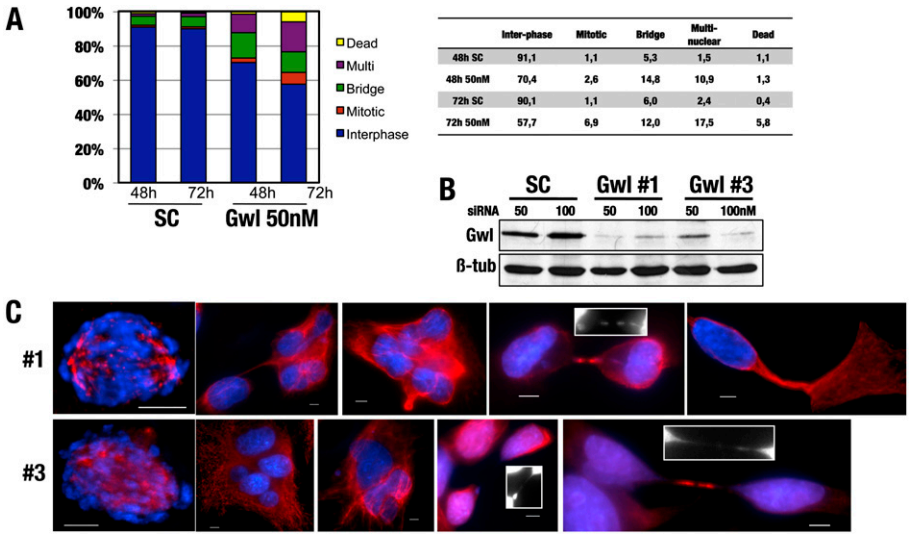


Fig. S2. hGwl protein levels remain constant throughout the cell cycle. (*A*) WT or kinase dead (KD) MAST-L mRNAs were translated or not (ΔGwl) in CSF (M-phase frog egg) extracts that were then depleted of endogenous Gwl (ΔGwl) using *Xenopus* anti-Gwl antibodies. The levels of endogenous Gwl before (CSF) and after depletion (ΔGwl), as well as the WT and KD MAST-L levels in translated CSF extracts after depletion of *Xenopus* Gwl are shown. The ability of hGwl to rescue the mitotic exit was assessed by analyzing the phosphorylation of Cdc27, Cdc25, and pTry15 on Cdc2. (*B*) Asynchronously growing HeLa cells were lysed and used for immunoprecipitation with anti-hGwl antibodies. (*C*) HeLa cells, synchronized in G1/S by thymidine, were treated with (Chx) or without 100 μg/mL of cycloheximide or MG132 (12.5 μM) for the indicated times. (*D*) Mitotic HeLa cells, synchronized by nocodazole shake-off, were treated with 100 μg/mL cycloheximide (Chx) for the indicated time. (*E*) Nocodazole-arrested HeLa cell lysates were treated with (λ-PPase) or not (CT) with lambda phosphatase and the electrophoretic mobility of hGwl in these two samples was compared.



	Inter-phase	Mitotic	Bridge	Multi-nuclear	Dead
48h SC	91,1	1,1	5,3	1,5	1,1
48h 50nM	70,4	2,6	14,8	10,9	1,3
72h SC	90,1	1,1	6,0	2,4	0,4
72h 50nM	57,7	6,9	12,0	17,5	5,8

Fig. S3. Similar phenotypes were observed when different sequences of siRNA were used to knockdown hGwl in HeLa cells. (*A*) Quantification of cells with apparently correct interphase (Interphase), mitotic cells (Mitotic), interphase cells presenting DNA bridges (Bridge), multinucleated cells (Multi), and dead cells (Dead) from immunofluorescence data in Fig. 1*G*. A minimum of 300 cells were counted for each condition. (*B*) Asynchronous HeLa cells were transfected with 50 or 100 nM of Scramble or hGwl siRNA sequences #1 and #3 (*Materials and Methods*) and the levels of hGwl were analyzed 24 h later by Western blot. (*C*) Asynchronous HeLa cells treated as in *A* were analyzed by immunofluorescence for β-tubulin (red) and DAPI (blue). (Scale bars, 5 μm.)

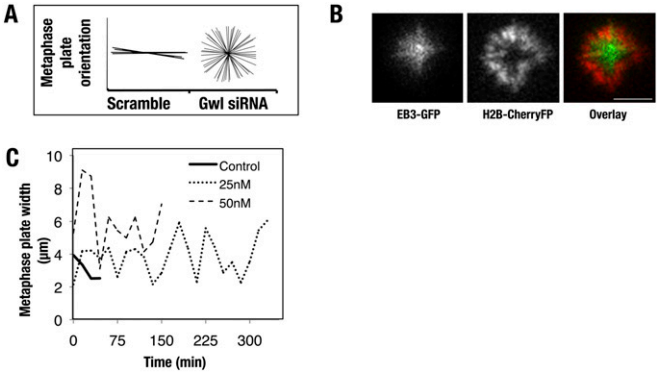


Fig. S4. hGwl knockdown results in spindle rotation. (*A*) The orientation of the metaphase plate during each frame of the film of cells with the rotation phenotype in Fig. 2 were represented and then compiled into a radial projection. (*B*) In some cases, hGwl siRNA-treated cells showed a clear Rosetta phenotype, indicating that the cell has rotated 90° through the *z* axis. (*C*) The thickness of the metaphase plate was measured as hGwl-depleted cells (25 and 50 nM) or siRNA Scramble-treated cells (control) progressed through mitosis. The oscillating changes in width are further evidence of rotations through the *z* axis in these cells.

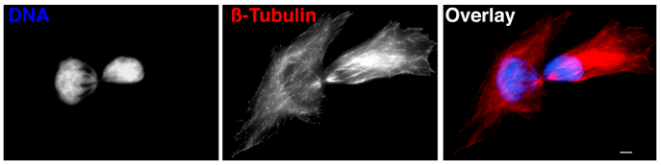


Fig. S5. Greatwall knockdown results in cells attempting to pinch through multiple DNA bridges. HeLa cells were transfected with 50 nM of hGwl siRNA and analyzed by immunofluorescence as per Fig. 3*D*. Multiple DNA bridges can be clearly seen, indicating that cell has attempted to pinch through the DNA and has failed to properly separate sister chromatids during anaphase. (Scale bar, 5 μm.)

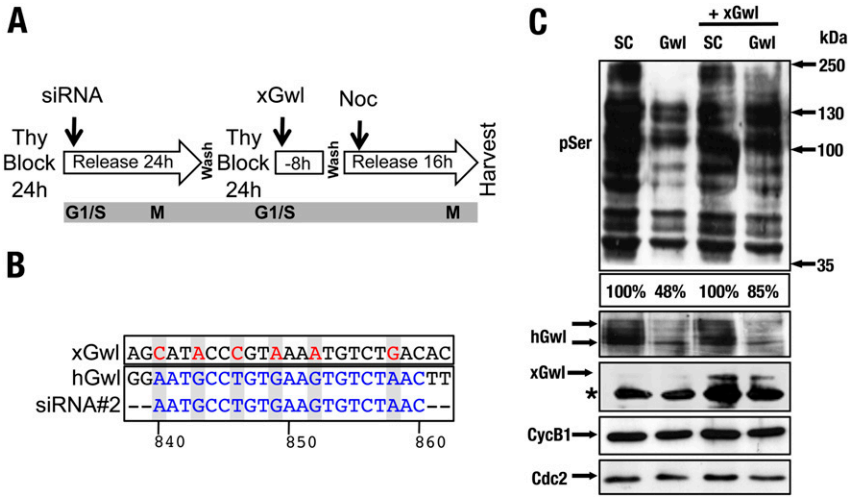


Fig. S6. Decrease of cyclin B-Cdc2 substrate phosphorylation in Gwl knockdown cells is rescued by the cotransfection of a siRNA-resistant plasmid encoding *Xenopus* Gwl. (*A*) HeLa cells were synchronized in G1/S by thymidine and transfected with a dose of 50 nM siRNA (sequence #2) for 24 h. The siRNA was removed and cells were then blocked again for 24 h with thymidine. Eight hours before release, cells were transfected with a resistant plasmid coding for *Xenopus* Gwl. (*B*) Picture showing the siRNA #2 used in this experiment targeting sequence from 840 to 860 of hGwl. The corresponding sequence of *Xenopus* Gwl is detailed. Cells were washed three times with media and released into fresh media supplemented with nocodazole (100 ng/mL) for 16 h to capture mitotic cells. (*C*) Phosphorylation of cyclin B-Cdc2 substrates as well as human Gwl, *Xenopus* Gwl, cyclin B1, and Cdc2 levels were analyzed by Western blot. The pSer staining was equalized against Cdc2 and the percentage of staining in each condition was indicated. * Denotes a nonspecific band.

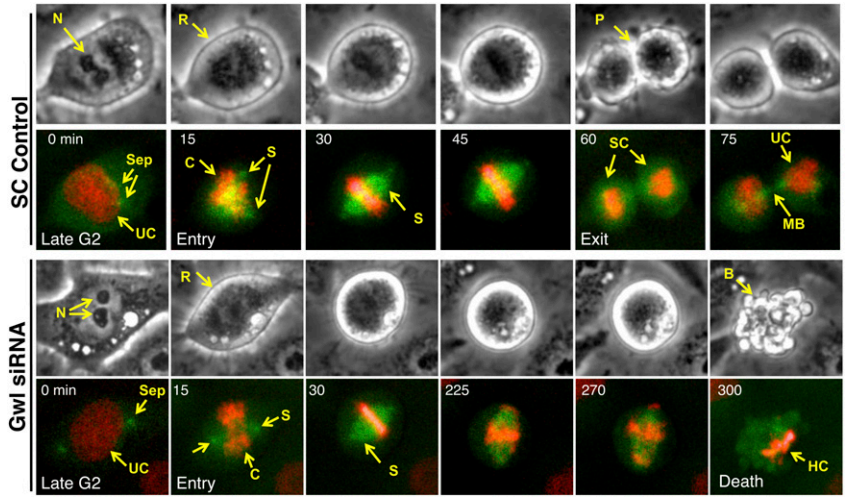
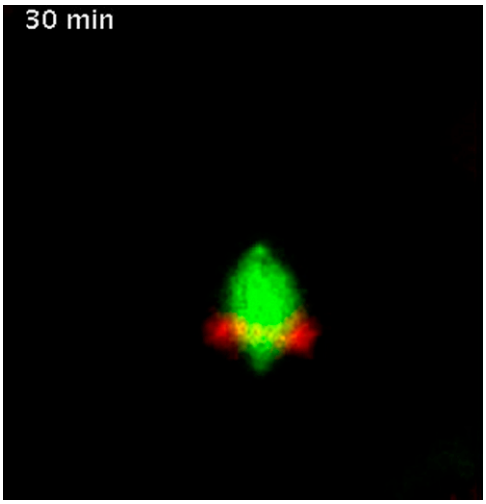
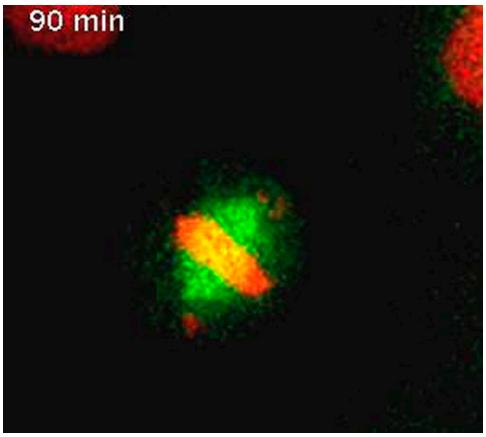


Fig. S7. Late G2 phase cells were identified by the presence of uncondensed chromosomes (UC), partial centrosome separation (Sep), and the presence of nucleoli. In all cases, mitotic entry was determined by the following visual cues, loss of nucleoli (N) and rounding up (R) of the cell from the culture dish in phase/contrast, cross referenced with the presence of condensed chromosomes (C) and a mitotic spindle in the fluorescence channel (S). Exit was scored based on when cells attempted to perform anaphase: the separation (SC) or loss of condensation of chromosomes (UC) and presence of a midbody (MB) in the fluorescence channel or pinching of the membrane (P) under phase/contrast. Cell death was scored by the first appearance of severe membrane blebbing (B), cross-referenced with hyper condensed chromatin (HC).



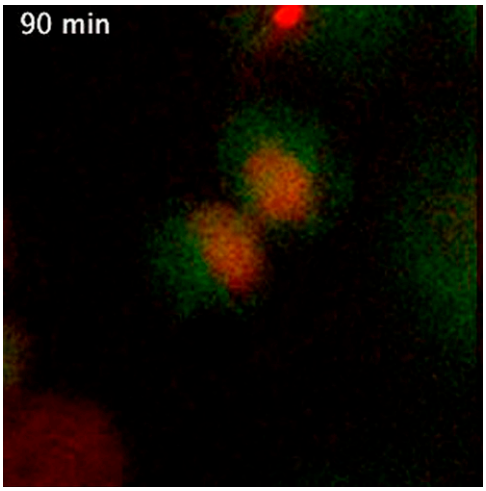
Movie S1. Scramble siRNA-Control. HeLa cells stably expressing H2B-Cherry FP (red) and EB3-GFP (green) were transfected with 50 nM of siRNA (Scramble), then synchronized using thymidine. Movies were started 5 h after release and frames were taken every 15 min.

Movie S1



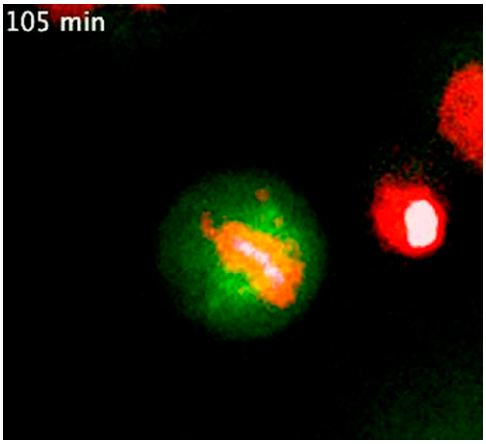
Movie S2. Greatwall siRNA-Segregation Defect. HeLa cells stably expressing H2B-Cherry FP (red) and EB3-GFP (green) were transfected with 50 nM of hGwl siRNA, then synchronized using thymidine. Movies were started 5 h after release and frames were taken every 15 min. Movie shows a typical cell that displayed chromosome segregation defects and subsequent formation of a multinuclear cell.

Movie S2



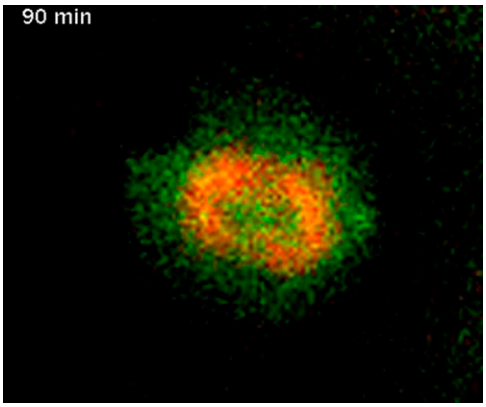
Movie S3. Greatwall siRNA-Mitotic Arrest. HeLa cells stably expressing H2B-Cherry FP (red) and EB3-GFP (green) were transfected with 50 nM of hGwl siRNA, then synchronized using thymidine. Movies were started 5 h after release and frames were taken every 15 min. Movie shows a typical cell that arrested during mitosis, underwent spindle rotation, and failed to properly congress all chromosomes to the metaphase plate, finally undergoing cell death after ~8 h.

Movie S3



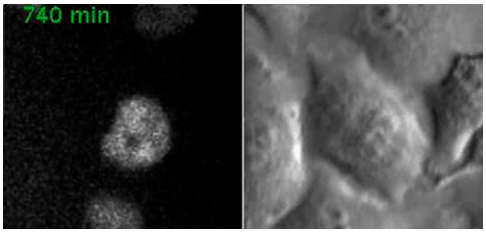
Movie S4. Greatwall siRNA-Mitotic Delay. HeLa cells stably expressing H2B-Cherry FP (red) and EB3-GFP (green) were transfected with 50 nM of hGwl siRNA, then synchronized using thymidine. Movies were started 5 h after release and frames were taken every 15 min. Movie shows a typical cell that delayed during mitosis with significant problems on chromosome congression. Cell finally exited mitosis and presented impaired chromosome segregation, resulting in visible chromosome bridges, fractionation of the nucleus, and the formation of a multinuclear cell.

Movie S4



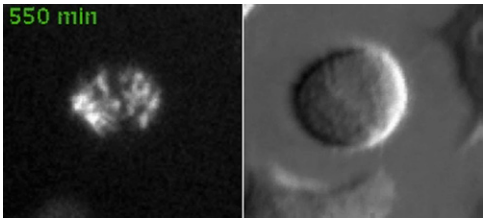
Movie S5. Greatwall siRNA-No Metaphase Plate. HeLa cells stably expressing H2B-Cherry FP (red) and EB3-GFP (green) were transfected with 50 nM of hGwl siRNA, then synchronized using thymidine. Movies were started 5 h after release and frames were taken every 15 min. Movie shows a typical cell that fails to form a metaphase plate. Cells pass directly from a prometaphase to an anaphase without chromosome congression. Premature anaphase occurs and the cell pinches through the mass of DNA forming multiple DNA bridges.

Movie S5



Movie S6. HeLa cells stably expressing H2B-Cherry FP and EB3-GFP were transfected with 100 nM of hGwl siRNA, synchronized by thymidine block, and 6 h after release followed by time-lapse microscopy. Frames were taken every 10 min. Indicated time corresponds to minutes after release. Movie shows a typical cell that had a long delay in G2 and 13 h 30 min after release condensed chromosomes but failed to form a metaphase plate.

Movie S6



Movie S7. HeLa cells stably expressing H2B-cherry FP and EB3-GFP were transfected with 100 nM of hGwl siRNA and synchronized by thymidine block, and 6-h after release, cells were treated with 500 nM of okadaic acid and followed by time-lapse microcopy. Frames were taken every 10 min. Indicated time corresponds to minutes after release. Movie shows a typical Gwl depleted cell that enters mitosis with normal kinetics.

Movie S7