Chan et al., http://www.jcb.org/cgi/content/full/jcb.201306083/DC1

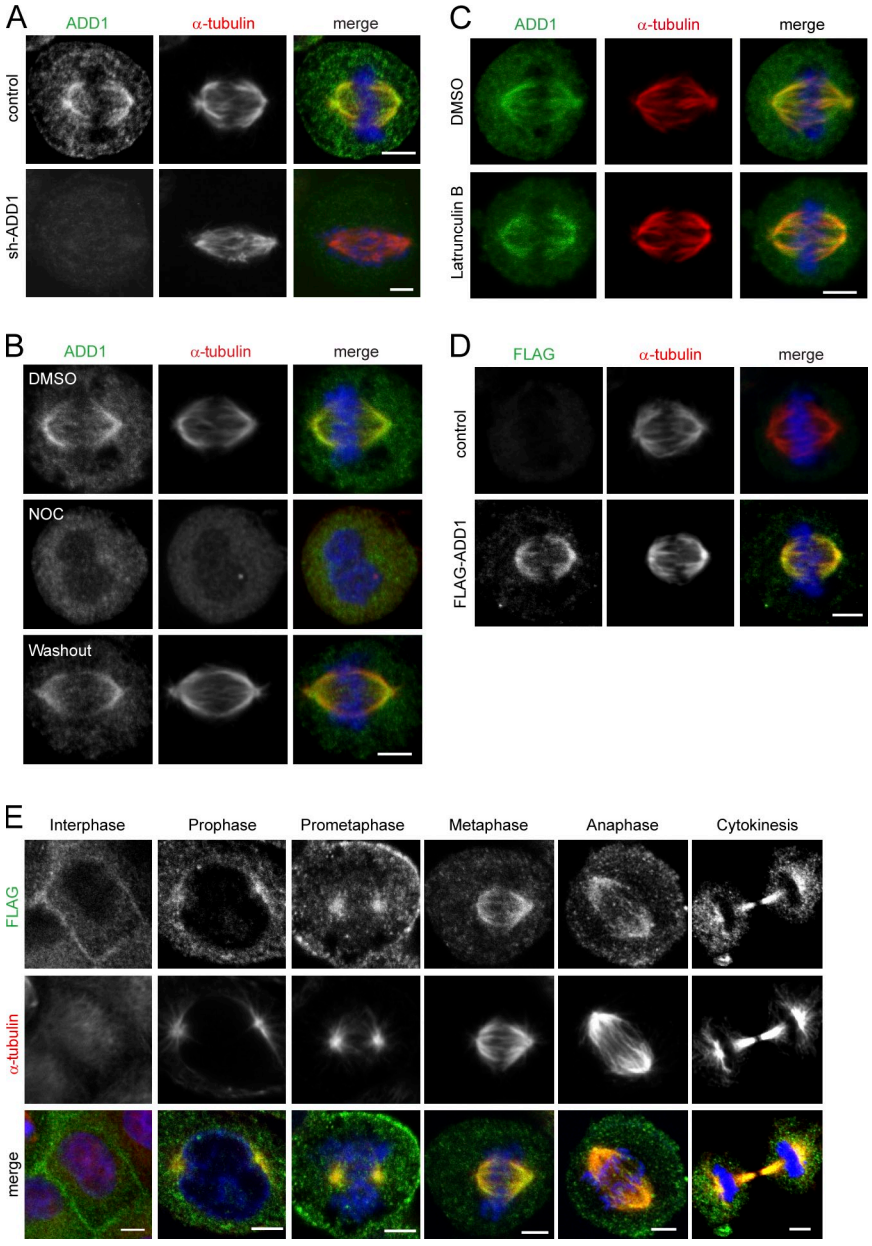


Figure S1. **Association of ADD1 with mitotic spindles.** (A) The signal of anti-ADD1 antibody in immunofluorescent staining is specific to ADD1. HeLa cells with or without ADD1 depletion (sh-ADD1) were probed with the anti-ADD1 antibody, anti–α-tubulin antibody, and DAPI for DNA (blue). (B) Mitotic HeLa cells were treated with DMSO (as a control) or nocodazole (NOC) to disrupt microtubules for 20 min followed by extensive washing to remove nocodazole to allow spindle assembly (washout) before fixation. Fixed cells were stained for ADD1, α-tubulin, and DNA (blue). (C) Mitotic HeLa cells were treated with DMSO (as a control) or 5 µM latrunculin B to disrupt F-actin for 30 min before fixation. Fixed cells were stained for ADD1, α-tubulin, and DNA (blue). (D) The signal of anti-FLAG antibody in immunofluorescent staining is specific to FLAG-ADD1. HeLa cells transfected with or without (control) FLAG-ADD1 were probed with the anti-FLAG antibody, anti–α-tubulin antibody, and DAPI for DNA (blue). (E) Representative confocal images show the association of FLAG-ADD1 with mitotic spindles throughout mitosis and with midzone microtubules during cytokinesis. Bars, 5 µm.

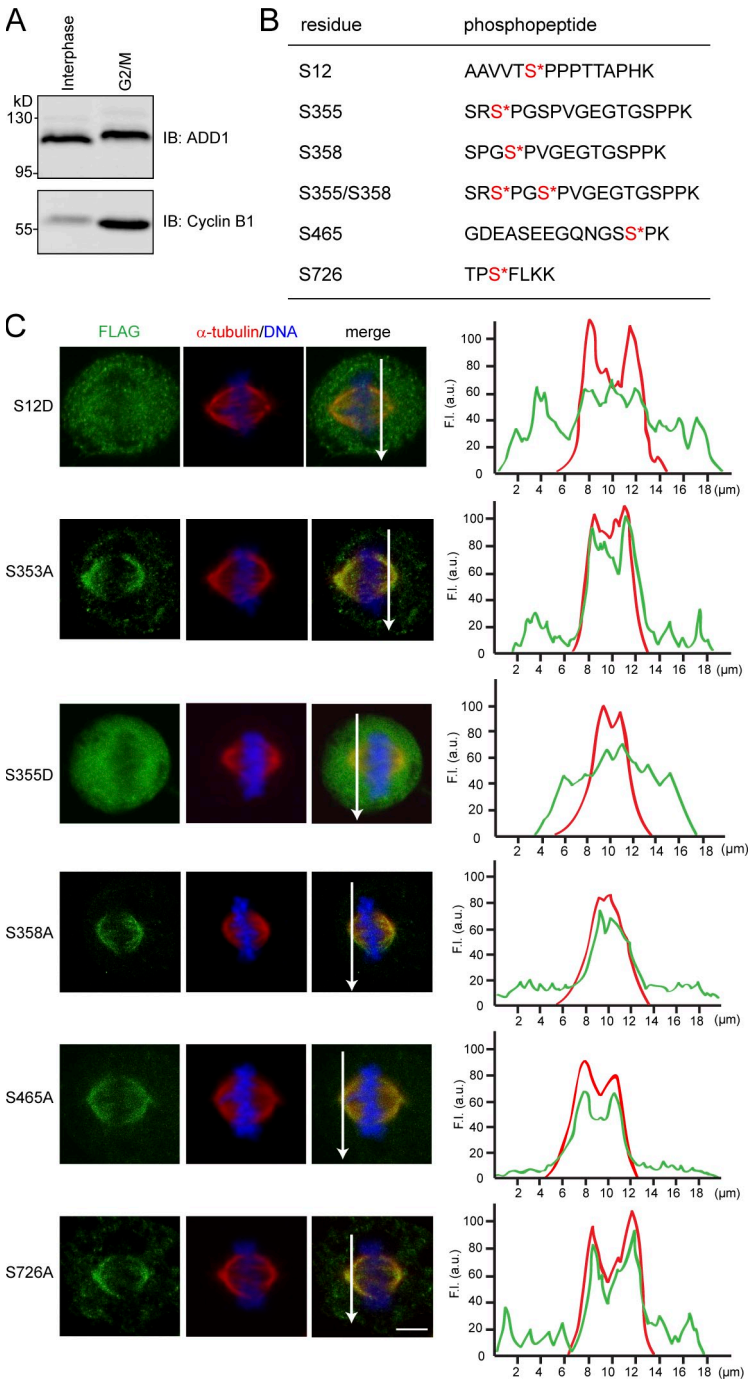


Figure S2. **Identification of ADD1 phosphorylation sites in mitosis and their importance in association with mitotic spindles.** (A) The molecular mass of ADD1 increases in the G2/M phase. IB, immunoblot. (B) Phosphorylation sites of ADD1 from the cells arrested in the G2/M phase were identified by MS. Asterisks in red indicate phosphorylation sites. (C) HeLa cells transiently expressing FLAG-ADD1 and mutants were stained for FLAG-ADD1, α-tubulin, and DNA. (left) Representative confocal images from the cells in metaphase are shown, which were from a single experiment out of three repeats (n > 160). (right) Graphs show the relative fluorescence intensity (F.I.) of the lines that were scanned by confocal microscopy. a.u., arbitrary units. Bar, 5 μm.

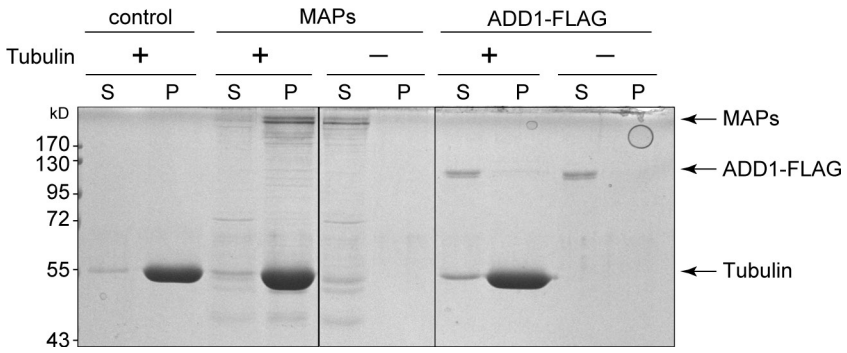
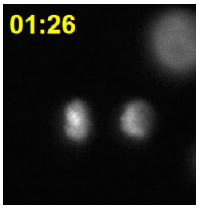


Figure S3. **ADD1 does not directly interact with microtubules in vitro.** Purified FLAG-ADD1 or microtubule-associated proteins (MAPs) were incubated with (+) or without (−) polymerized tubulins. After centrifugation, the proteins in the supernatants (S) or pellets (P) were fractionated by SDS-PAGE and were stained by Coomassie blue. Black lines indicate that intervening lanes have been spliced out.

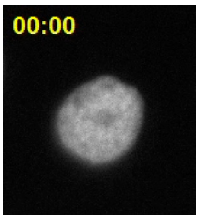
Table S1. **Mutagenic primers used in this study**

Point mutation	Direction of primers	Sequences of primers
S12A	Forward	5′-GCTGCGGTGGTGACCGCACCACCCCCGACCAC-3′
	Reverse	5′-GTGGTCGGGGGTGGTGCGGTCACCACCGCAGC-3′
S12E	Forward	5′-CGTGCTGCGGTGGTAACCGAGCCACCCCCGACCACAGC-3′
	Reverse	5′-GCTGTGGTCGGGGGTGGCTCGGTCACCACCGCAGCACG-3′
S12D	Forward	5′-GCTGCGGTGGTGACCGATCCACCCCCGACCACA-3′
	Reverse	5′-TGTGGTCGGGGGTGGATCGGTCACCACCGCAGC-3′
S355A	Forward	5′-GTACAAAGCCAAGTCCCGTGCCCCAGGGTCTCCGGTAGGG-3′
	Reverse	5′-CCCTACCGGAGACCCTGGGGCACGGGACTTGGCTTTGTAC-3′
S355E	Forward	5′-GTACAAAGCCAAGTCCCGTGAGCCAGGGTCTCCGGTAG-3′
	Reverse	5′-CTACCGGAGACCCTGGCTCACGGGACTTGGCTTTGTAC-3′
S355D	Forward	5′-TACAAAGCCAAGTCCCGTGACCCAGGGTCTCCG-3′
	Reverse	5′-CGGAGACCCTGGGTCACGGGACTTGGCTTTGTA-3′
S353A	Forward	5′-GAAGTACAAAGCCAAGGCCCGTTCCCCAGGG-3′
	Reverse	5′-CCCTGGGGAACGGGCCTTGGCTTTGTACTTC-3′
S358A	Forward	5′-TCCCGTTCCCCAGGGGCTCCGGTAGGGGAAG-3′
	Reverse	5′-CTTCCCCTACCGGAGCCCCTGGGGAACGGGA-3′
S358D	Forward	5′-CCCGTTCCCCAGGGGATCCGGTAGGGGAAG-3′
	Reverse	5′-CTTCCCCTACCGGATCCCCTGGGGAACGGG-3′
S465A	Forward	5′-GAGGAAGGGCAGAATGGAAGCGCTCCCAAGTCGAAGACTAAGTCG-3′
	Reverse	5′-CGACTTAGTCTTCGACTTGGGAGCGCTTCCATTCTGCCCTTCCTC-3′
S465D	Forward	5′-GGAAGGGCAGAATGGAAGCGATCCCAAGTCGAAGACTAAG-3′
	Reverse	5′-CTTAGTCTTCGACTTGGGATCGCTTCCATTCTGCCCTTCC-3′
S726A	Forward	5′-GAAGTTCCGTACCCCGGCCTTTCTGAAGAAGAGCAAGAAGAAG-3′
	Reverse	5′-CTTCTTCTTGCTCTTCTTCAGAAAGGCCGGGGTACGGAACTTC-3′

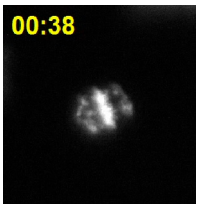
The mutagenic bases are denoted by underlines.



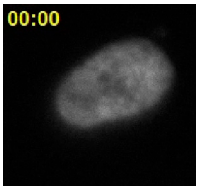
Video 1. **Control HeLa cells.** HeLa cells stably expressing mCherry-H2B were used to trace chromosome movement during mitosis. Images were recorded by time-lapse fluorescent microscopy using a microscope (Axio Observer.D1). Frames that were taken every 3 min for 2 h are displayed at 5 frames per second (fps).



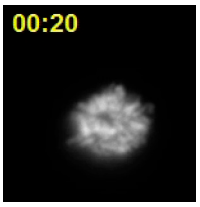
Video 2. **HeLa cells expressing shRNA to luciferase.** HeLa cells expressing mCherry-H2B were infected by lentiviruses expressing shRNA to luciferase**.** Images were recorded by time-lapse fluorescent microscopy using a microscope (Axio Observer.D1). Frames that were taken every 3 min for 1.6 h are displayed at 5 fps.



Video 3. **Type I mitotic defect caused by ADD1 depletion.** HeLa cells expressing mCherry-H2B were infected by lentiviruses expressing shRNAs to ADD1. Images were recorded by time-lapse fluorescent microscopy using a microscope (Axio Observer. D1). Frames that were taken every 3 min for 6.1 h are displayed at 5 fps.



Video 4. **Type II mitotic defect caused by ADD1 depletion.** HeLa cells expressing mCherry-H2B were infected by lentiviruses to deplete ADD1. Images were recorded by time-lapse fluorescent microscopy using a microscope (Axio Observer.D1). Frames that were taken every 3 min for 8.3 h are displayed at 5 fps.



Video 5. **Type III mitotic defect caused by ADD1 depletion.** HeLa cells expressing mCherry-H2B were infected by lentiviruses to deplete ADD1. Images were recorded by time-lapse fluorescent microscopy using a microscope (Axio Observer.D1). Frames that were taken every 3 min for 7.2 h are displayed at 5 fps.