Supplemental Information: Govindaraghavan, Lad and Osmani

Table S1: List of strains used in this study

Strain Name	Genotype (all strains also carry *veA1*)	Source
MG190	*nimA7; pyrG89; argB2; ndc80-CR::pyroA^{AF} (pyroA4?); tubA-GFP; chaA1; GCP3-GFP::riboB^{AF} (riboA1?)*	MG183 X CDS719
MG300	*tubA-GFP; An-H1-chRFP::pyroA^{AF}; pyrG89; argB2; nirA14?; nicA2? nicB8?*	MG210 X HA365
MG298	*nimA7; H1-chRFP::pyroA^{AF} (pyroA4); argB2; GFP-tub; pyrG89; nirA14?; nicA2? nicB8?; wA2* or *wA3*	MG210 X HA365
MG224	*argB2; pyrG89; sE15; GFP-tub; CR-AN162; pyroA4; nicA2? nicB8?; nirA14?*	MG199 X MC39
MG227	*nimA7; argB2; sE15; GFP-tub; CR-AN162; pyroA4; pyrG89; nicA2? nicB8?; nirA14?; wA2 or wA3*	MG199 X MC39
MG228	*nimA7; argB2; sE15; GFP-tub; CR-AN162; pyroA4; pyrG89; nicA2? nicB8?; nirA14?; wA2 or wA3*	MG199 X MC39
MG294	*nimA7; argB2; AN162-GFP; fib-CR::pyrG^{AF} (pyrG89); sE15?; nirA14?, wA3*	MG198 X SO1064
MG296	*argB2; AN162-GFP; fib-CR::pyrG^{AF} (pyrG89); sE15?; nirA14?; wA3*	MG198 X SO1064
MG321	*nimA7; GFP-tub; nup49-chRFP::pyroA^{AF} (pyroA4?); argB2; pyrG89; nirA14?; sE15?; nicA2? nicB8?; wA3*	MG199 X CDS838
MG378	*nimA7; GFP-tub; HP1-chRFP::pyroA^{AF} (pyroA4); argB2; pyrG89; nicA2? nicB8?; sE15?; nirA14?; wA3*	MG199 X KF28
MG273	*argB2; pyrG89; ΔyA::NLS-DsRed; GFP-tub; pyroA4; nicA2? nicB8?*	MG199 X AY02
MG229	*nimA7; argB2; pyrG89; ΔyA::NLS-DsRed; GFP-tub; pyroA4; nicA2? nicB8?*	MG199 X AY02
R153	*pyroA4; wA3*	(1)
MG313	*nimA7; ΔAn-mad2::pyrG^{AF}(pyrG89); pyroA4; GFP-tub; ΔyA::NLS-DsRed; nicA2? nicB8?; yA2*	MG229 X CDS611
MG310	*nimA1; argB2; pyroA4; pyrG89; GFP-tub; ΔyA::NLS-DsRed; nicA2? nicB8?; fwA1/wA2*	MG273 X SO338
MG308	*nimA5; GFP-tub; ΔyA::NLS-DsRed; pyroA4; pyrG89; wA2 or wA3*	MG273 X CDS616
MG381	*nimA7; ΔAn-mad2::pyrG^{AF}(pyrG89); pyroA4; nirA14?; nicA2? nicB8?; chaA1*	MG277 X CDS629
CDS790	*nimA7; argB2; wA3*	SO114 x CDS735
CDS629	*Δmd2A::pyrG (pyrG89); pyroA4; chaA1*	CDS565 x CDS459 (2)

Note: Question marks (?) refer to nutritional markers mutations that have not been tested for, but may be present in the strain.

1. **Osmani AH, McGuire SL, O'Donnell KL, Pu RT, Osmani SA.** 1991. Role of the cell-cycle-regulated NIMA protein kinase during G2 and mitosis: evidence for two pathways of mitotic regulation. Cold Spring Harbor symposia on quantitative biology **56:**549-555.
2. **De Souza CP, Hashmi SB, Nayak T, Oakley B, Osmani SA.** 2009. Mlp1 acts as a mitotic scaffold to spatially regulate spindle assembly checkpoint proteins in Aspergillus nidulans. Molecular biology of the cell **20:**2146-2159.

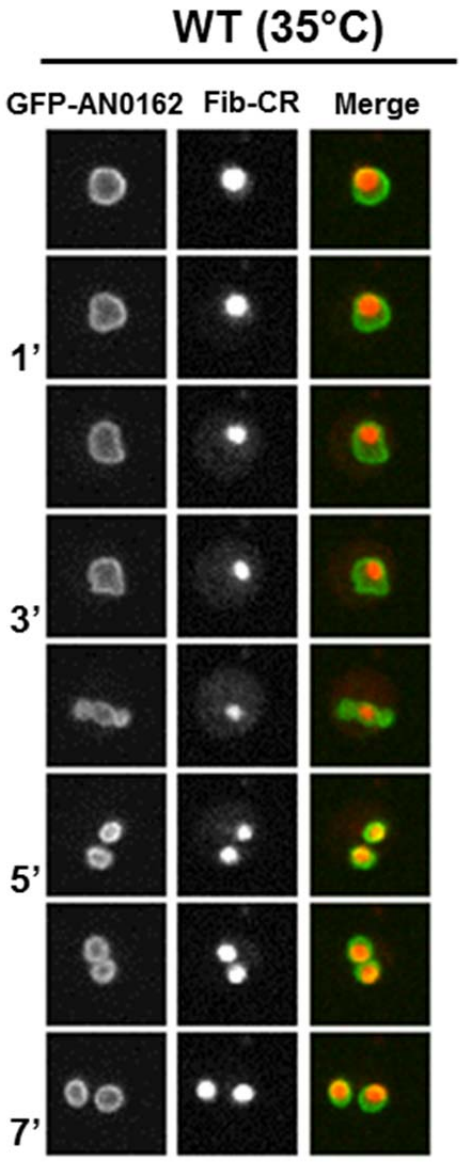

Fig S1: Normal nucleolar segregation and nuclear envelope dynamics during Wt (strain MG296) mitosis at 35°C. The nuclear envelope marked by GFP-AN162 exhibits a double restriction generating three membrane bound compartments at 4'. The nucleolar protein, fibrillarin-CR, is seen to locate within the middle compartment, and undergoes disassembly and reassembly to generate two daughter nucleoli.

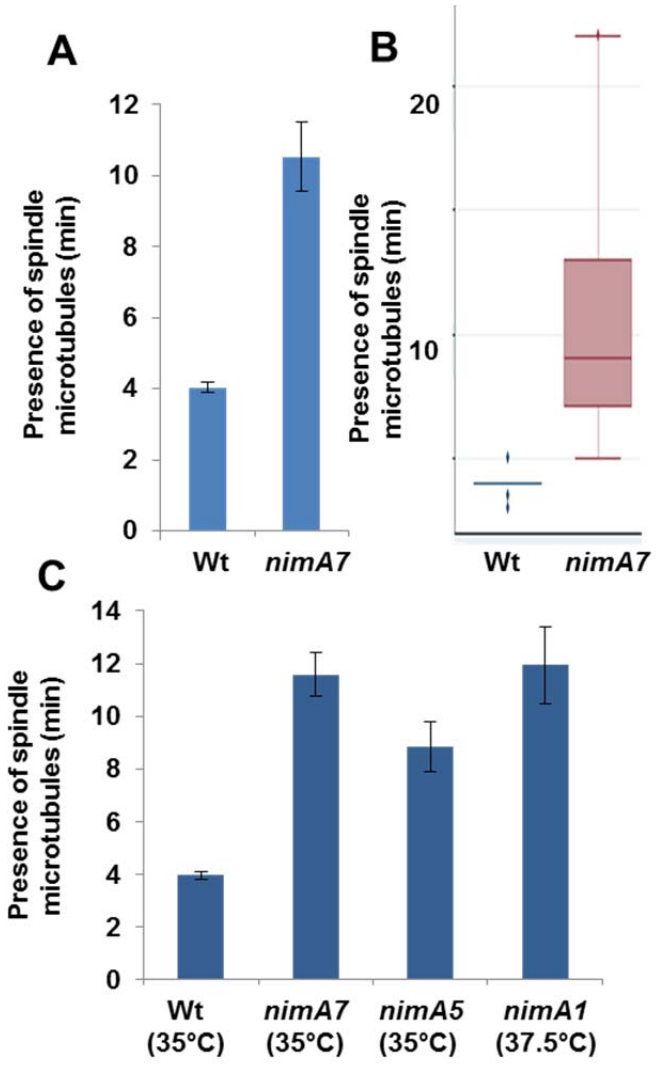

Fig S2: Cells with reduced NIMA function show a prolonged mitosis. (A) Quantitation of the average time spent in mitosis measured by spindle formation in Wt (n=13) and *nimA7* (n=21) cells at 35°C. Strains: Wt – MG224, *nimA7* – MG190, MG227, and MG229. (B) Box plot showing the distribution of time in mitosis for Wt and *nimA7* cells. The bottom and top of the box represent the 25th and 75th percentile and the band near the middle of the box is the 50th percentile (or the median). (C) The mitotic delay in cells with partial NIMA function is independent of the *nimA* allele used. The quantitation of time in mitosis for Wt (n=15) and three different temperature sensitive alleles of *nimA* – *nimA7* (n=19), *nimA5* (n=12) and *nimA1* (n=31), at their respective semi-permissive temperatures, as indicated. Strains: Wt – MG224, *nimA1* – MG310, *nimA5* – MG308, *nimA7* – MG229.

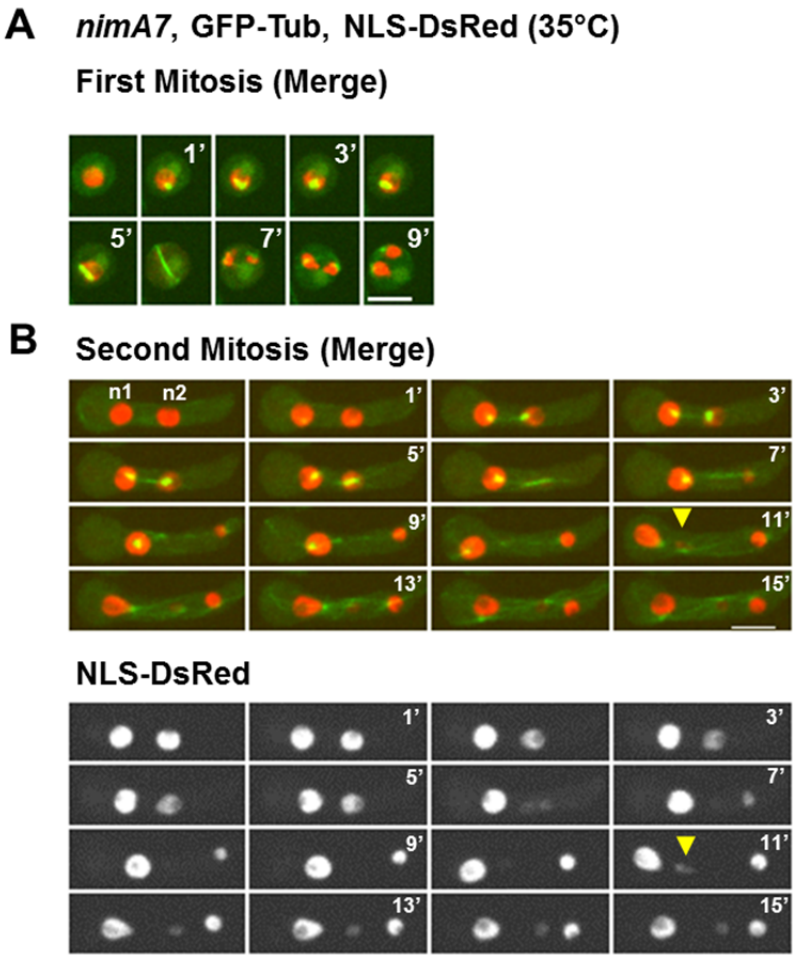

Fig S3: Apparently successful first mitosis in *nimA7* cells (A) may be followed by a defective second mitosis (B) (71%, n=7). The same cell going through two rounds of mitosis is shown. Nuclear transport is marked by NLS-DsRed and spindle formation by GFP-Tub (strain MG229). In (A), mitosis is apparently successful with two daughter nuclei formed. In (B), both nuclei attempt the second mitoses and start to form mitotic spindles. However, the spindle in the nucleus to the left (n1) fails to elongate and this nucleus does not release its NLS-DsRed. The nucleus to the right (n2) completes mitosis after spindle formation and NLS-DsRed release but generates two nuclei of unequal size with the arrowhead indicating the micronucleus formed. Bars, 5μm.

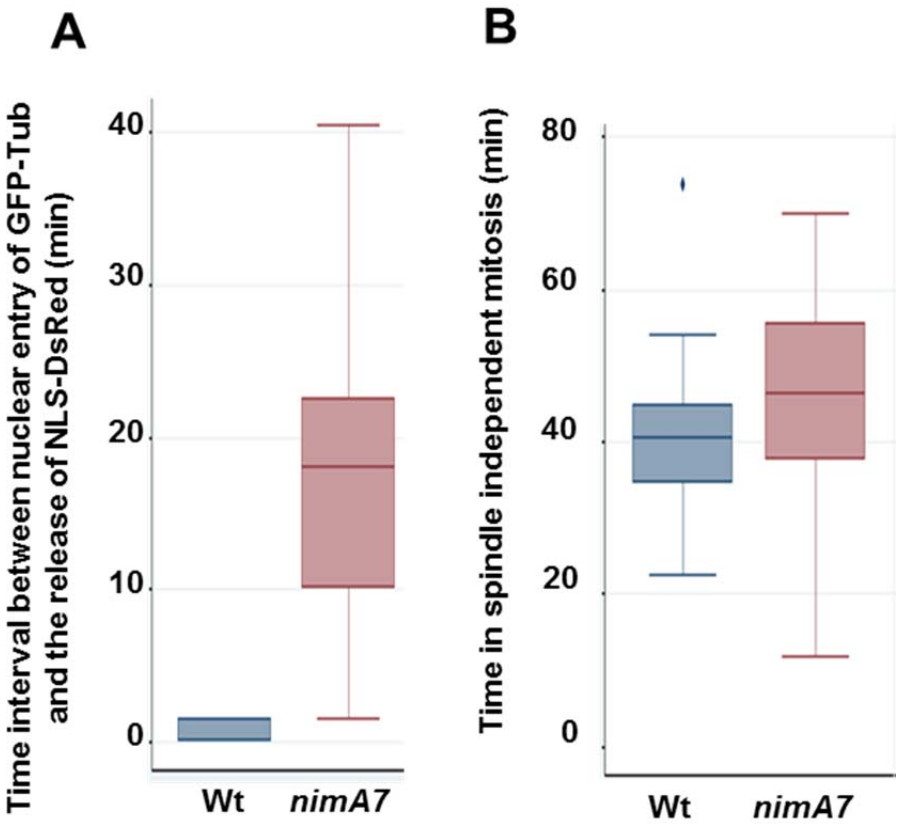

Fig S4: Mitotic arrest in *nimA7* cells in the absence of spindles (A) A box and whiskers plot showing the variability in the time interval between the entry of GFP-tub into the nucleus and the dispersal of NLS-DsRed in *nimA7* (n=41) cells at 35°C as compared to Wt cells (n=25). The difference in time interval between nuclear entry of GFP-Tub and the release of NLS-DsRed between Wt and nimA7 cells is highly significant (p value is 0.00 by unpaired t test). (B) A box and whiskers plot showing the length of SAC mediated mitotic arrest in the absence of spindles as represented by the time interval between the entry of GFP-tub into the nucleus and the reimport of NLS-DsRed at mitotic exit for Wt (n=25) cells and *nimA7* (n=41) cells at the semi-permissive temperature. The difference in mitotic arrest time between Wt and *nimA7* is not significant; p value > 0.01 using unpaired t-test. For (A) and (B), the bottom and top of the box represent the 25th and 75th percentile and the band near the middle of the box is the 50th percentile (or the median).

Supplementary Movie Information

Movie S1: The configuration of the nuclear envelope and nucleolus in cells with partial NIMA function. The movie shows two nuclei, one of which (on the right in frame 1) has a distinct protrusion of the nucleus visualized by the nuclear envelope marked in green with GFP-AN0162 and the nucleolus in red with fibrillarin-CR. This rotation was generated from a movie with 8 Z sections that are 0.8 μm apart and 10 times points that are 1 min apart. Each time point is rotated through 360° in 10 degree increments.