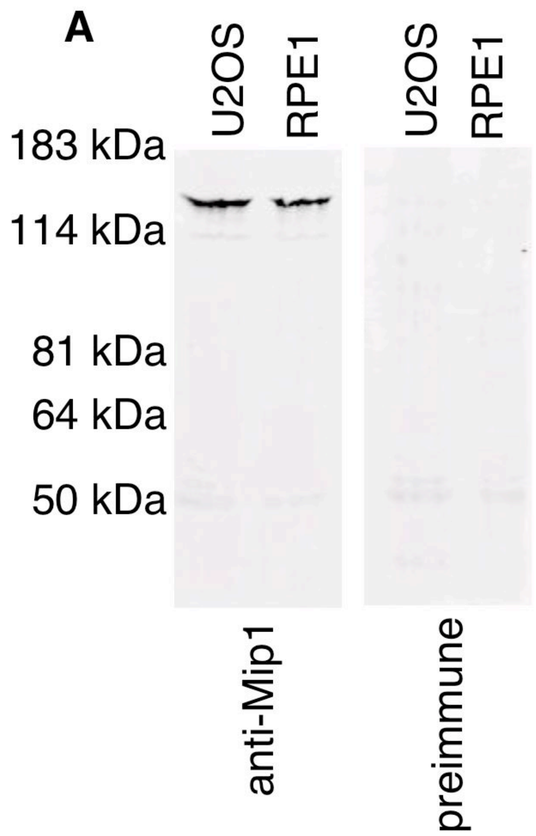
Supplemental Figure S1. Mip1 is required to maintain proper ploidy. A. Affinity purified rabbit anti-Mip1 antibody recognizes a single band in immunoblots from whole cell lysates of U2OS and RPE1 cells. B. U2OS cells were treated with either Mip1 or lamin specific siRNAs for 36 hours and cell lysates were analyzed by SDS-PAGE and immunoblotting with anti-Mip1 and anti-lamin antibodies. C. Cells were subjected to siRNAs as in B, fixed, analyzed with anti-tubulin immunofluorescence and stained with DAPI to visualize nuclei. At least 100 cells were counted per experiment and the average number of nuclei per cell for four experiments was plotted using the standard deviation for error bars. D. Examples of cells treated in B.

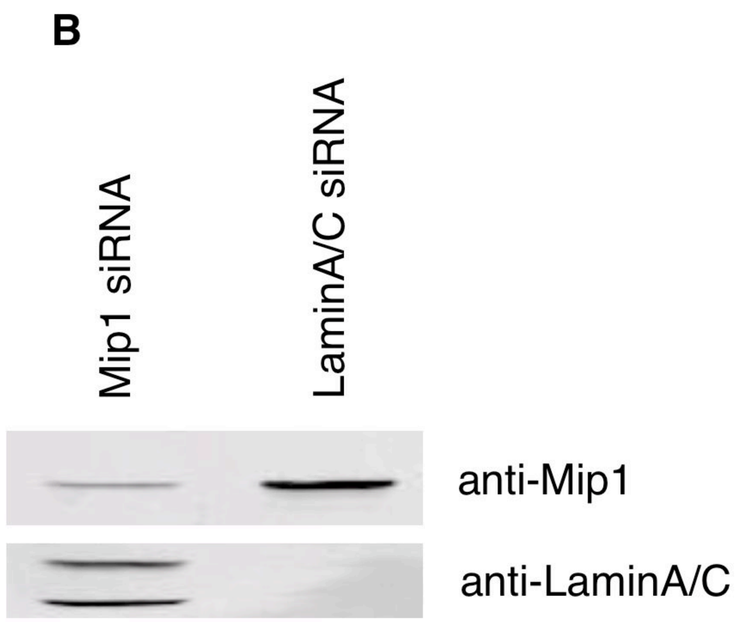
Supplemental Figure S2. Loss of Mip1 does not perturb the centrosome duplication or checkpoint functions of hMps1. A. U2OS cells were treated with Mip1 specific siRNAs for 36 hours and then nocodazole was added to the cultures. After 12 hours in nocodazole, cells were fixed and recruitment of Mad2 to kinetochores was assayed by immunofluoresence. At least 100 cells were counted in three independent experiments and the average was plotted using the standard deviation for error bars. B. Examples of cells treated in A. C. U2OS cells were treated with Mip1 specific siRNAs for 36 hours and then arrested in S-phase by the addition of hyroxyurea. Reduplication of centrosomes was quantified using gamma-tubulin immunofluorescence and plotted as in A. D. Examples of cells treated in C.

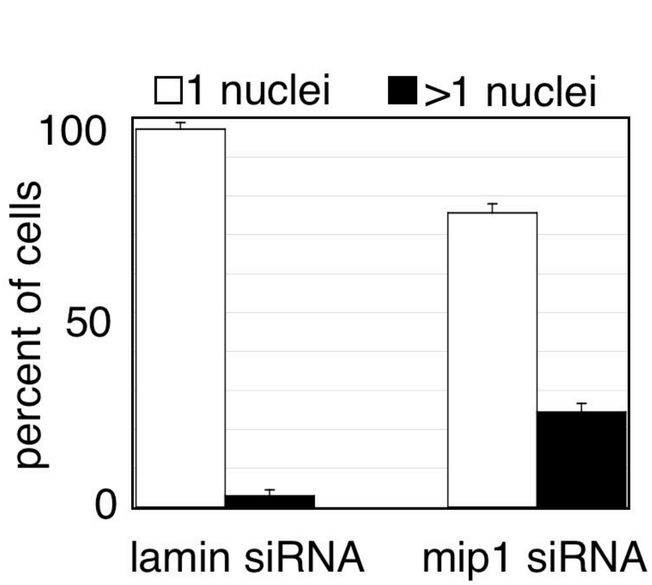
Supplemental Movie 1. A HeLa cell expressing GFP-tubulin (green) and mRFP-Cenp-B (red) was analyzed by time-lapse microscopy 48 hours after treatment with control siRNAs. Images were collected every 20 seconds and are played at 120X real time.

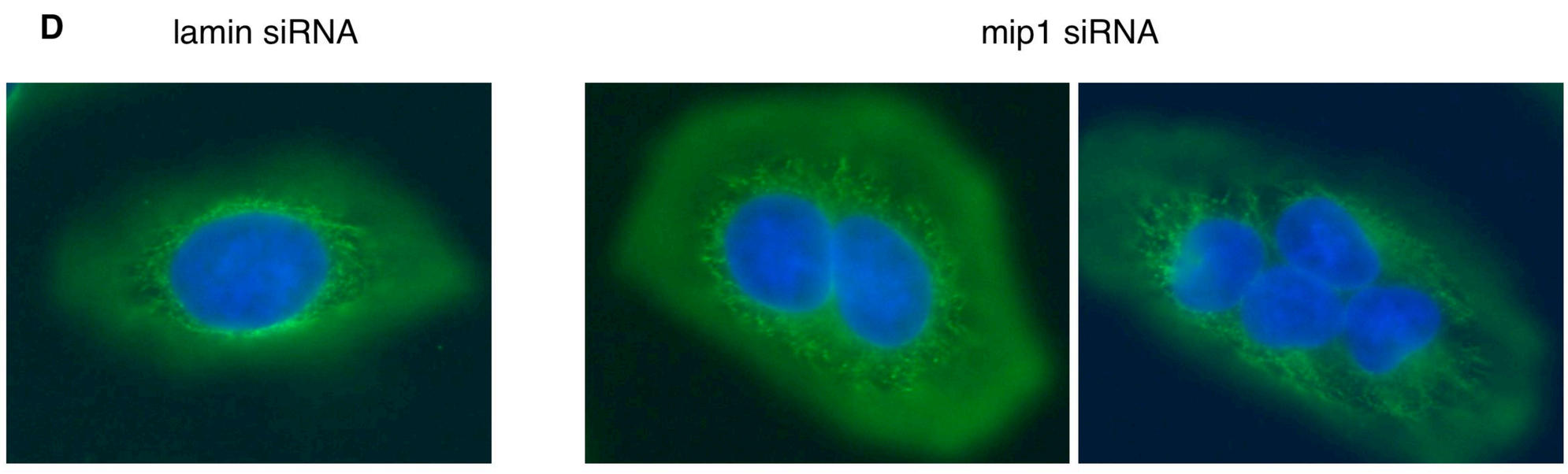
Supplemental Movie 2. A HeLa cell expressing GFP-tubulin (green) and mRFP-Cenp-B (red) was analyzed by time-lapse microscopy 48 hours after treatment with Mip1 siRNAs. Images were collected every 30 seconds and are played at 120X real time.

Supplemental Figure S1. MIP1 siRNA causes multinucleate cells









Supplemental Figure S2. Mip1 knockdown does not prevent spindle assembly checkpoint or centrosome re-duplication functions