Van der Horst and Khanna, Supplementary Data

Movie S1. Time-lapse movie of asynchronously growing $Pin1^{+/+}$ MEFs. Phase contrast images of cells were captured every 10 min. The display rate is 30 frames per second. Still images of this video are shown in Figure 1*A*.

Movie S2. Time-lapse movie of asynchronously growing $Pin1^{-/-}$ MEFs. Phase contrast images of cells were captured every 10 min. The display rate is 30 frames per second. Still images of this video are shown in Figure 1*A*.

Movie S3. Time-lapse movie of control siRNA-transfected HeLa cells expressing EB1-GFP. Phase contrast images of cells were captured every 2 min. The display rate is 30 frames per second. Still images of this video are shown in Figure 2*A*.

Movie S4. Time-lapse movie of Pin1 siRNA-transfected HeLa cells expressing EB1-GFP. Phase contrast images of cells were captured every 2 min. The display rate is 30 frames per second. Still images of this video are shown in Figure 2*A*.

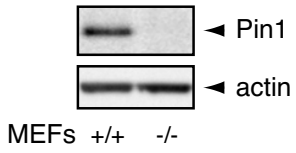

Figure S1. Expression of Pin1 in MEFs. $Pin1^{+/+}$ and $Pin1^{-/-}$ MEFs were lysed and total lysates were analyzed for levels of Pin1 by Western blotting. Actin was used as a loading control.

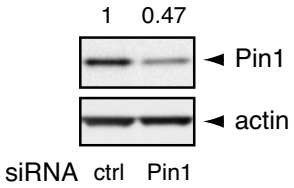

Figure S2. Depletion of Pin1 in HeLa cells by Pin1 siRNA. HeLa cells were transfected with control or Pin1 siRNA and total lysates were analyzed for levels of Pin1 by Western blotting. Actin was used as a loading control.

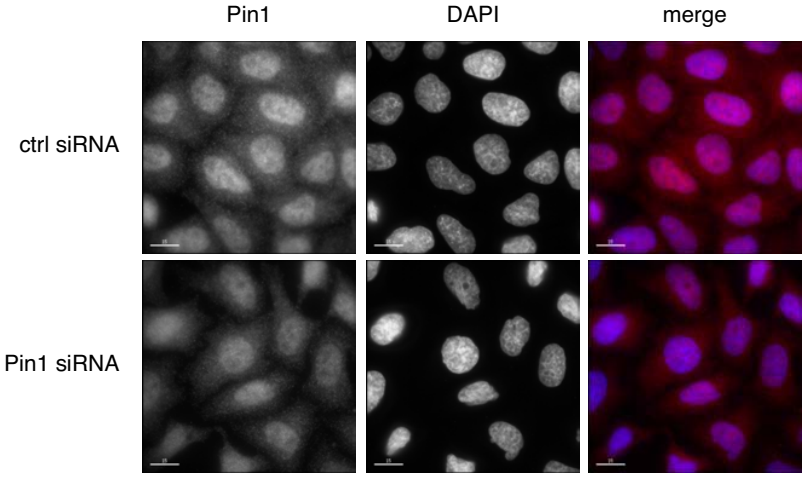

Figure S3. Pin1 depletion by siRNA in HeLa cells. Cells transfected with control or Pin1 siRNA were stained for Pin1 (monoclonal antibody) and DNA (DAPI). Representative examples are shown. Pin1 expression was estimated to be downregulated by ~60% in about 80% of cells. Bar, 15 μm.

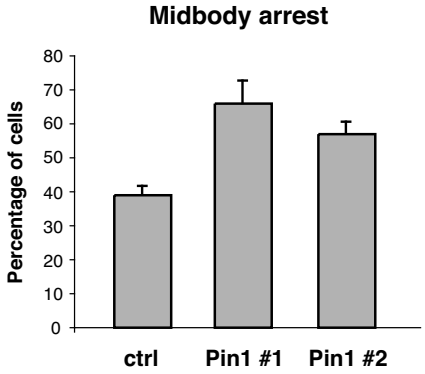

Figure S4. Depletion of Pin1 in HeLa cells causes a midbody arrest. Experiments were performed as in Figure 2*D*. Pin1 #1 is the Pin1 siRNA used throughout the paper; here, an additional siRNA (Pin1 #2) was used, which showed a similar effect on the number of cells displaying a midbody arrest as Pin1 #1. Mean±SD of four experiments (fifty cells with a midbody per experiment). The differences are statistically significant, p=0.0076 (Pin1 #1) and p=0.0052 (Pin1 #2).

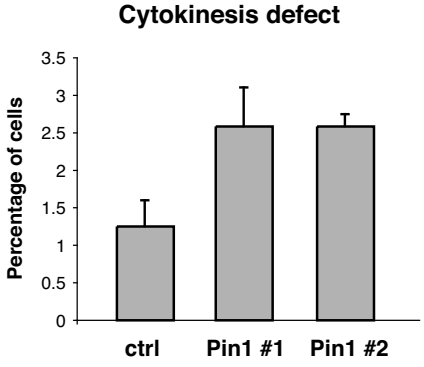

Figure S5. Depletion of Pin1 in HeLa cells causes a cytokinesis defect. Experiments were performed as in Figure S4. Here, the percentage of cells in the total population displaying a midbody arrest was analyzed (300 cells per experiment). Mean±SEM of four experiments. The differences are statistically significant. p=0.0163 (Pin1 #1) and p=0.0109 (Pin1 #2).

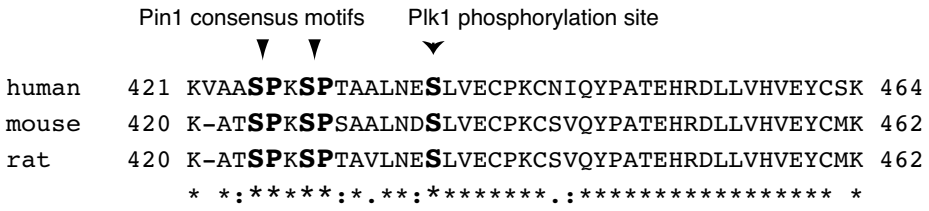

Figure S6. Alignment of the C-terminal region of human, mouse and rat Cep55 protein showing the conserved Pin1 consensus binding motifs (serines 425 and 428 in humans, both followed by a proline) and the conserved Plk1 phosphorylation site (S436 in humans).

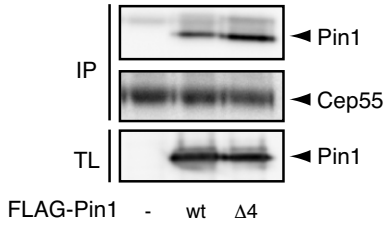

Figure S7. Wild-type Pin1 and a Pin1 isomerase mutant interact with Cep55. 293T cells expressing wild-type FLAG-Pin1 or a FLAG-Pin1 isomerase mutant (Δ4) were synchronized in mitosis using nocodazole. Cep55 was immunoprecipitated using α-Cep55 antibody and Western blots containing immunoprecipitates (IP) and total lysates (TL) were analyzed using FLAG-M2 antibody.

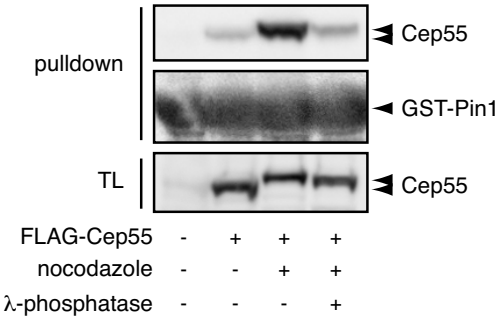

Figure S8. Pin1 interacts with Cep55 in a phosphorylation-dependent manner. GST-Pin1 was produced in BL21 cells, purified and incubated with lysates of 293T cells that were transfected with a FLAG-Cep55 expression plasmid. Samples were treated with nocodazole for 16 hours if indicated. The last sample was pretreated with λ-phosphatase for 30 min to remove phosphorylation. Pulldown samples and total lysates were analyzed for the presence of FLAG-Cep55 and GST-Pin1 by Western blotting using FLAG-M2 antibody.

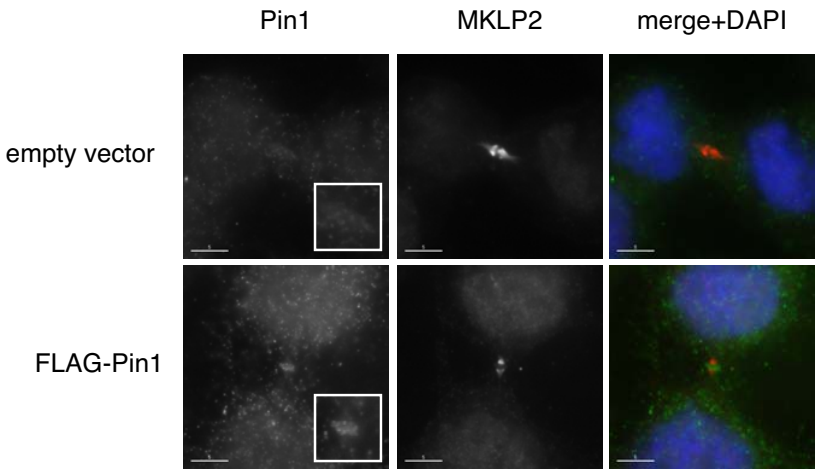

Figure S9. Localization of FLAG-Pin1 to the midbody ring. Asynchronous HeLa cells were transfected using empty vector or FLAG-Pin1 and stained for FLAG, MKLP2 and DNA (DAPI). Representative cells in cytokinesis are shown. Bar, 5 μm.

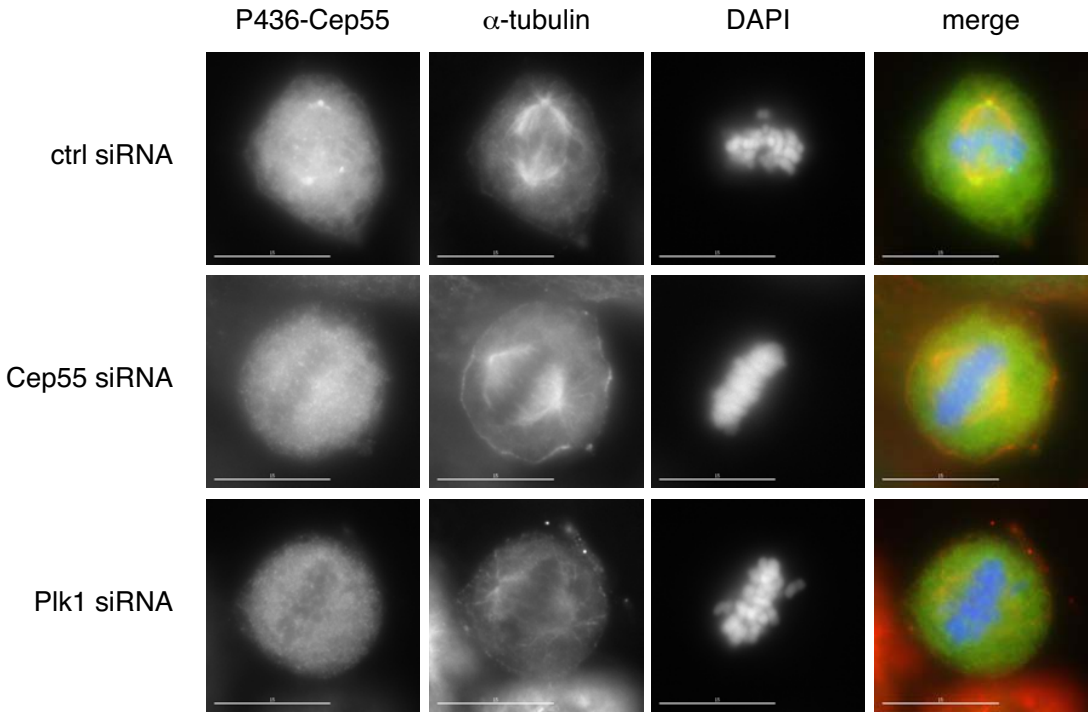

Figure S10. Specificity of the phospho-specific antibody against Cep55 phosphorylated on S436 by Plk1. Asynchronous HeLa cells were transfected using control, Cep55 or Plk1 siRNA and stained for P436-Cep55, α-tubulin and DNA (DAPI). Note that the centrosomal staining by the phospho-S436-Cep55 antibody in control cells is absent in both Cep55- and Plk1-depleted cells. Representative cells are shown. Bar, 15μm.

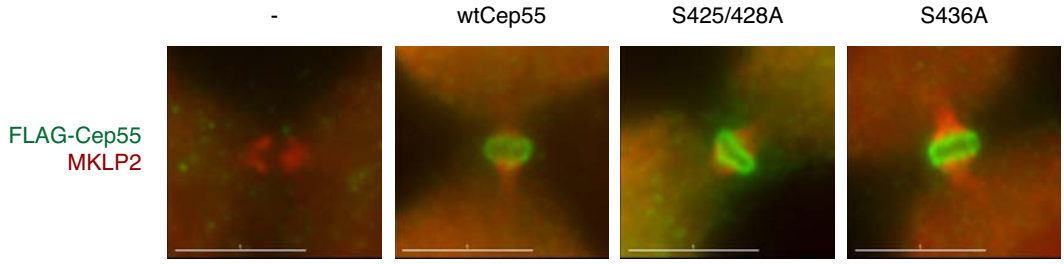

Figure S11. Cep55 phosphorylation mutants display midbody ring localization. Asynchronous HeLa cells transfected with the indicated FLAG-Cep55 constructs were stained using FLAG-M2 and MKLP2 antibodies. Representative examples of midbody rings in the different samples are shown. Bar, 5 μm.

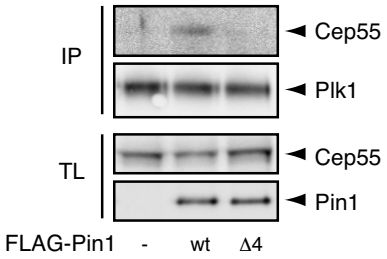

Figure S12. Wild-type Pin1 but not an isomerase mutant enhances the interaction between Plk1 and Cep55. Control HeLa cells and cells expressing wild-type FLAG-Pin1 or a FLAG-Pin1 isomerase mutant (Δ4) were synchronized in mitosis using nocodazole. Plk1 was immunoprecipitated and immunoprecipitates and total lysates were analyzed for Cep55, Pin1 and Plk1 levels.