Supporting Information

Perez-Moreno *et al.* 10.1073/pnas.0807301105

SI Text

Tissue Histology and Immunomicroscopy. Engrafted skins were frozen and embedded in OCT compound or fixed overnight with buffered 4% paraformaldehyde (PFA) at 4°C and embedded in paraffin. Skin sections (10 μM) were stained with hematoxylin and eosin or processed for immunoreactions. Slides were blocked with PBS solution, 0.3% Triton X-100, 1% BSA, 5% normal goat serum, 5% normal donkey serum, or MOM Basic kit (Vector Labs). Human skin samples embedded in paraffin were commercially acquired (SK801; US Biomax). Primary Abs used were: p120 (mouse mAb C-term; Zymed), E-cadherin (rat mAb, E-cad; ECCD-2; M. Takeichi, RIKEN Center for Developmental Biology, Japan), β-catenin (mouse mAb; Sigma), α-catenin (rabbit pAb; Sigma), β4-integrin (rat mAb; BD-Pharmigen), keratin 5 (rabbit pAb Ker5, 1:100; E.F.), keratin 6 (rabbit pAb Ker6 1:100; P. Coulombe, John Hopkins School of Medicine), phospho-NF-kB p65 (rabbit pAb; Cell Signaling), β-actin (mouse mAb; Sigma), BrdU (rat mAb; Abcam), pericentrin (mouse mAb; BD Biosciences), and β-tubulin (mouse mAb; Santa Cruz Biotechnology). Secondary Abs used were conjugated to FITC or Texas red (Jackson Labs) or biotin (Vector Labs). Unless indicated, dilutions were according to the manufacturer's instructions.

Cell Culture and Cell Adhesion Assays. Primary $p120^{fl/fl}$ mKers were isolated from P0 back skins. Cells (75×10^3) were transduced twice with 1×10^{11} cfu Adenovirus-GFP (controls) or Adenovirus-Cre-GFP (KO; Cell Biolabs). p120 expression was completely lost 72 h after infection and the transient GFP expression was lost 96 h after infection. For cell adhesion assays, coverslips were coated with fibronectin (FN; 10 μg/ml), matrigel (10 μg/ml) or collagen I (10 μg/ml; BD Biosciences) overnight at 4°C. Afterward, they were blocked with 10 mg/ml heat-inactivated BSA for 1 h at 32°C. Cells were plated in triplicate, fixed after 30 min of plating, stained with TRITC-phalloidin and DAPI, and counted by using Metamorph software (Universal Imaging).

Proliferation, Colony Formation Assays, and Cell Cycle Analysis. For proliferation assays, 75×10^3 cells were plated in triplicate on six-well plates coated with FN, grown in E low calcium medium, and trypsinized and counted at indicated times. To analyze colony formation properties, 500 cells were plated in triplicate on six-well plates coated with FN, and after 10 d, cells were fixed with 4% PFA and stained with 1% rhodamine B (Sigma).

For cell cycle analysis, mKers were synchronized as follows: for G1/S block, 2 mM thymidine was added to the media for 14 h. After washing with PBS solution, cells were allowed to recover for 8 h in medium supplemented with 10 μM deoxycytidine, and then treated again with 2 mM thymidine for 14 h. For G2/M block after G1/S block, cells were washed with PBS solution and then treated with 40 μg/ml nocodazole for 6 h. Nocodazole was washed out, and samples were collected at 1 h, 2 h, 4 h, and 8 h. Cells were fixed with EtOH, stained with propidium iodide, and analyzed on a FACScalibur system (BD Biosciences). Cell cycle analysis was performed by using FlowJo software.

Electron Microscopy. Skin samples were fixed for at least 1 h in 2% glutaraldehyde, 4% formaldehyde, and 2 mM $CaCl_2$ in 0.05 M sodium cacodylate buffer, and then processed for Epon embedding. Samples were visualized with a Tecnai 12-G2 transmission electron microscope (FEI).

NF-κB Luciferase Reporter Assays. Cells (1×10^4 per T24 well) were transfected with 500 ng of NF-κB-luc reporter construct (Path-Detect; Calbiochem), and 100 ng of Renilla luciferase plasmid (Promega) by using Fugene6 (Roche Applied Science). Twenty-four hours after transfection, cells were incubated with 20 ng/ml TNF-α for 6 h and then lysed, and firefly and Renilla luciferase values were measured by using the Dual-Luciferase reporter assay system (Promega). Normalized firefly luciferase values are reported.

BrdU Incorporation and TUNEL Assays. Mice carrying skin grafts were injected i.p. with 50 μg/gr BrdU (Sigma-Aldrich) and killed after 2 h. BrdU staining was performed as described for immunofluorescence with a pervious 30-min incubation in 1N HCl at 37°C. For *in vitro* studies, mKers were treated with BrdU for 1 h, and its incorporation was detected by using the BrdU Flow Kit (PharMingen). Cell analysis was performed by using a FACScalibur system (BD Biosciences). For TUNEL assays, cells and skin sections were processed and stained according to the ApopTag fluorescein direct *in situ* apoptosis detection kit protocol (Chemicon).

Active Ras/Rho Pull-Down Assays. The activity of Ras and RhoA was determined in newborn epidermis as described (15). Briefly, Ras-binding domain or Rhotekin Rho-binding domain GST recombinant proteins were used to pull down active GTP-bound Ras or RhoA from epidermal lysates, respectively, by using glutathione-coupled Sepharose beads. Active and total amounts of proteins were detected by immunoblot by using the specific antibodies H-Ras (rabbit pAb, sc-520; Santa Cruz Biotechnology) and RhoA (mouse mAb, sc-418; Santa Cruz Biotechnology).

Live Imaging Microscopy. For live cell imaging, cells were cultivated in a glass-bottom culture dish. They were transfected twice with an histone 2B–GFP expression construct under control of the hK14 promoter. Imaging was performed in a Zeiss/Perkin-Elmer wide-field/spinning disk confocal microscope (40×) at 1 frame/3 min. For bright-field live cell imaging, control and KO mKers were infected with 1×10^{11} cfu adenovirus-null, adenovirus-L63 RhoA (i.e., active Rho), or adenovirus-N17 RhoA (i.e., negative Rho; Cell Biolabs) or electroporated with DA ROCK or treated with 15 μM ROCK inhibitor Y-27632. Cells were imaged with an Olympus microscope (20×) for 12 h at 1 frame/min and processed in Metamorph (Universal Imaging).

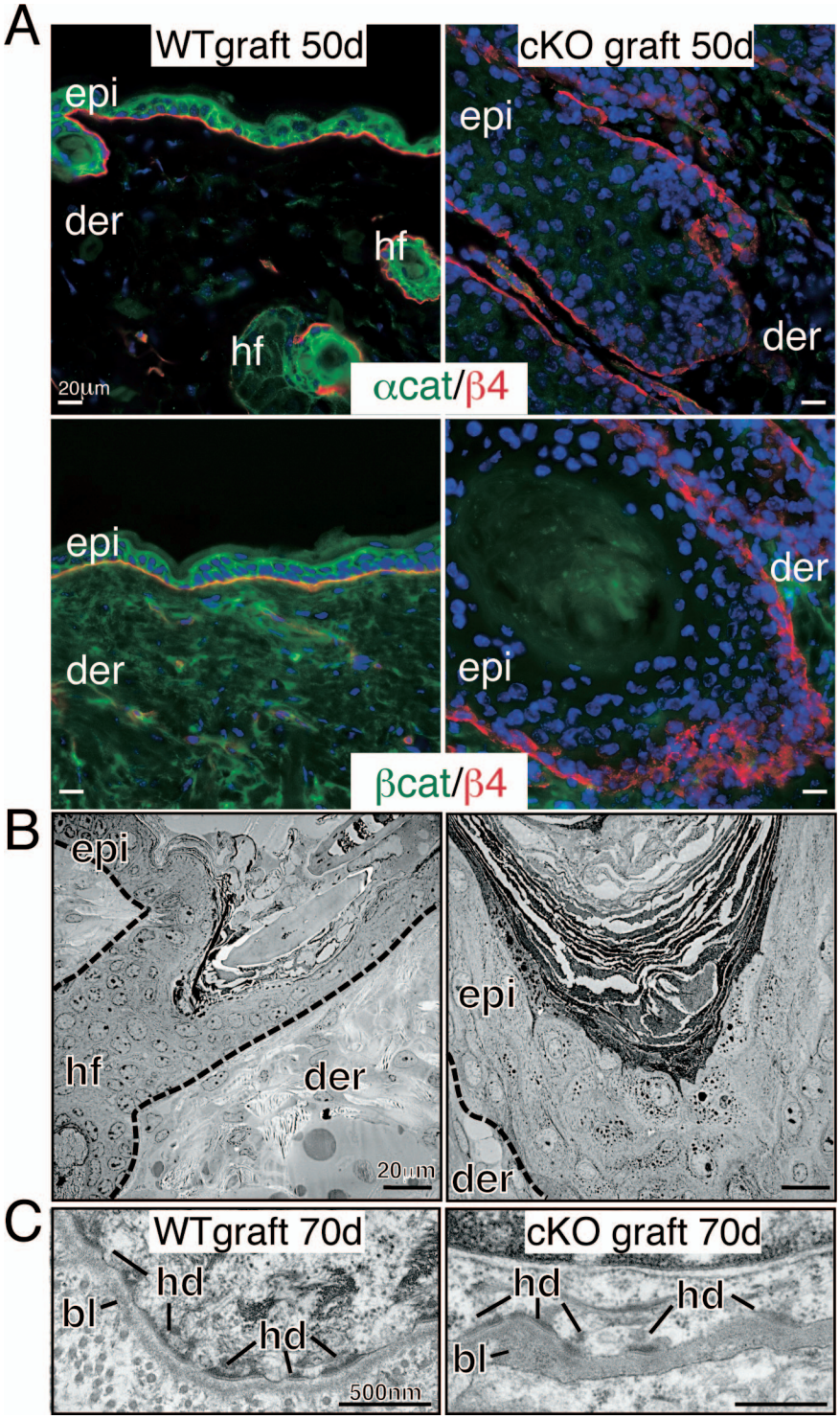


Fig. S1. Cell boundaries are maintained in the absence of p120, with reductions of α- and β-catenin. (*A*) Immunohistochemical analysis of α- and β-catenin show reductions in their expression in cKO skin grafts. (*B*) Semithin sections of WT and cKO skin grafts. Note the absence of gaps between epidermal cells. (*C*) EM of ultrathin sections show the epidermal–dermal boundaries. Note that there are no alterations in hemidesmosomes, and that basal lamina is not disrupted in cKO grafts.

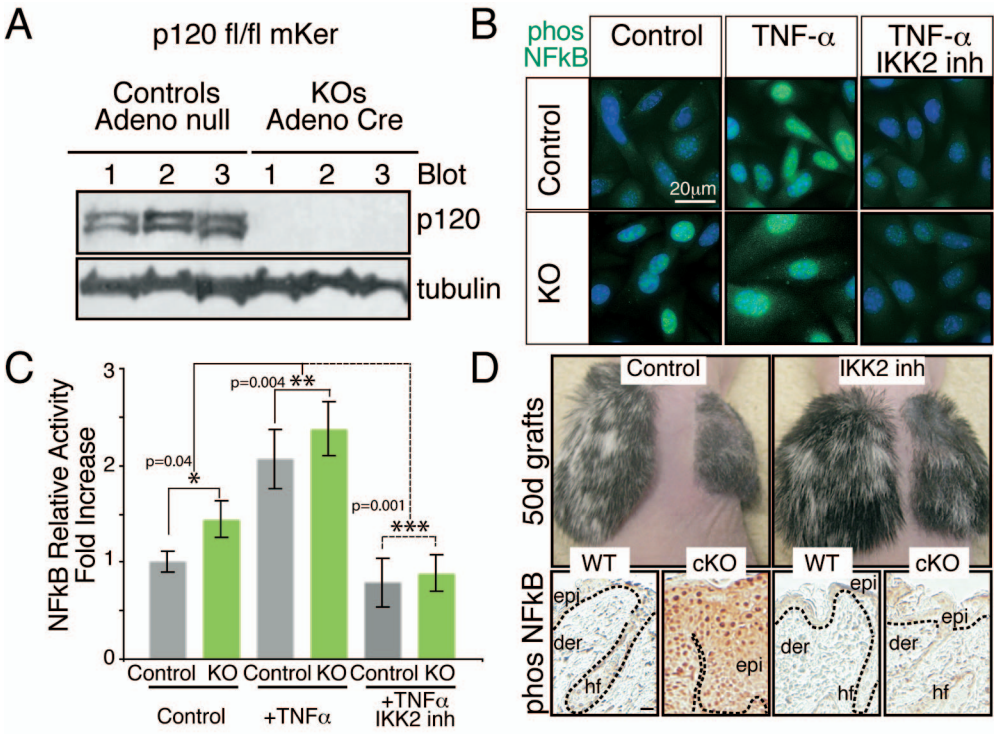
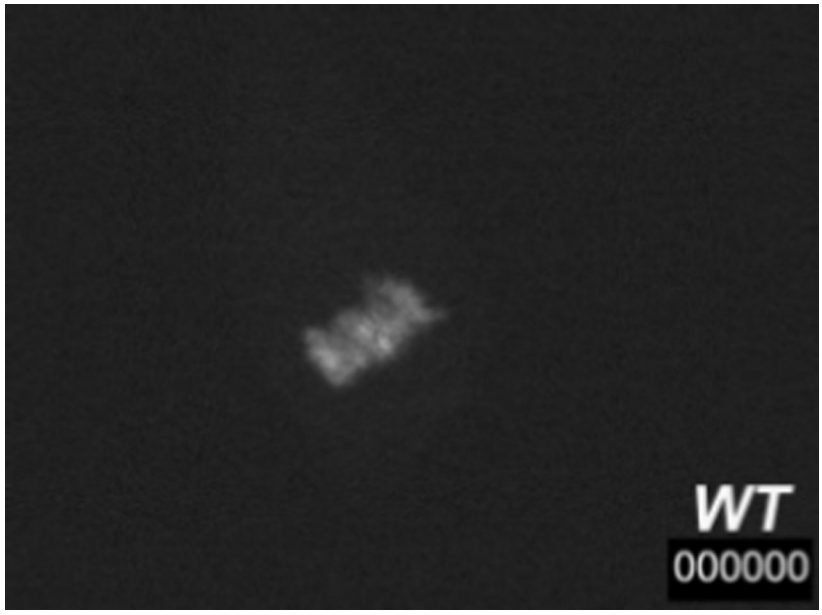
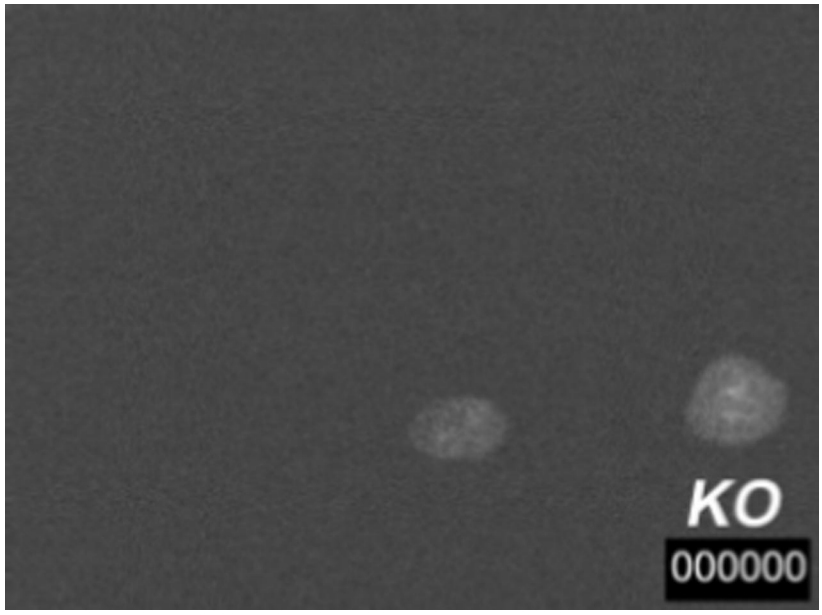


Fig. S2. NF-κB activation mediated by loss of p120 is dampened by the specific inhibition of IKK2. (*A*) *In vitro* recombination of p120$^{fl/fl}$ mKers with adenovirus Cre. Expression of the levels of p120 after 72 h of infection is shown. Note that, at that time, the expression of p120 catenin is completely absent. (*B*) p120-null keratinocytes display an increase in nuclear phos-NF-κB. This distribution is enhanced by TNF-α but completely blocked after the addition of IKK2 inhibitor IV. (*C*) NF-κB luciferase reporter assays on cultured keratinocytes with and without TNF-α and IKK2 inhibitor IV. (*, Student *t* test statistical *P* values.) (*D*) Skin grafts treated with IKK2 inhibitor IV display a reduced level of phospho-nuclear NF-κB.



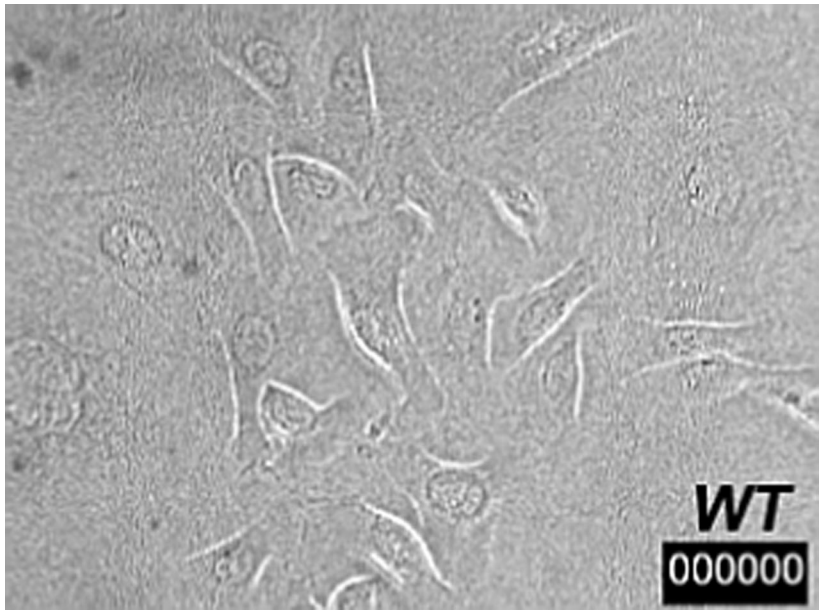
Movie S1. Time-lapse fluorescence microscopy of a representative control mKer. Histone 2B–GFP expressing control mKer completes cytokinesis by 30 to 45 min. Time in min is indicated in the lower right corner.

Movie S1 (AVI)



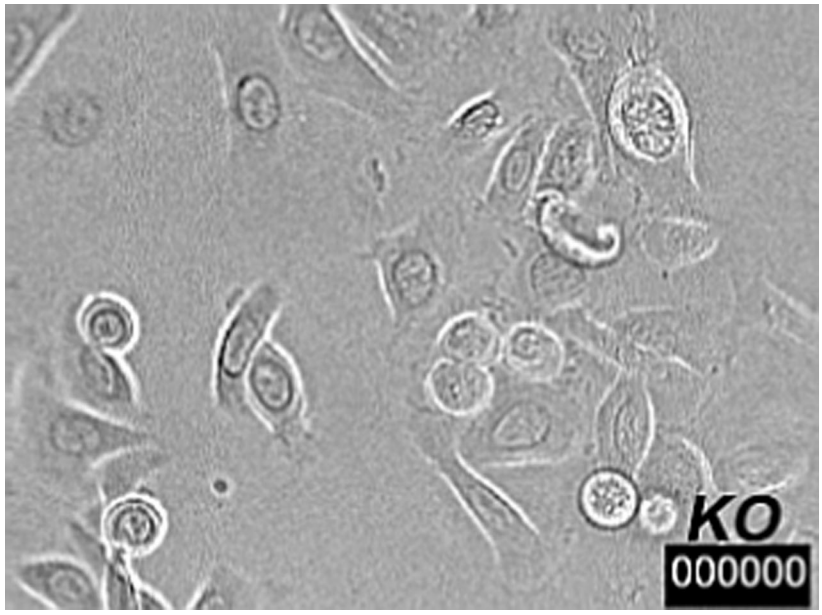
Movie S2. Time-lapse fluorescence microscopy of a representative p120-null mKer. Histone 2B–GFP expressing p120-null mKer proceeds to mitosis but fails to complete cytokinesis. Time in min is indicated in the lower right corner.

Movie S2 (AVI)



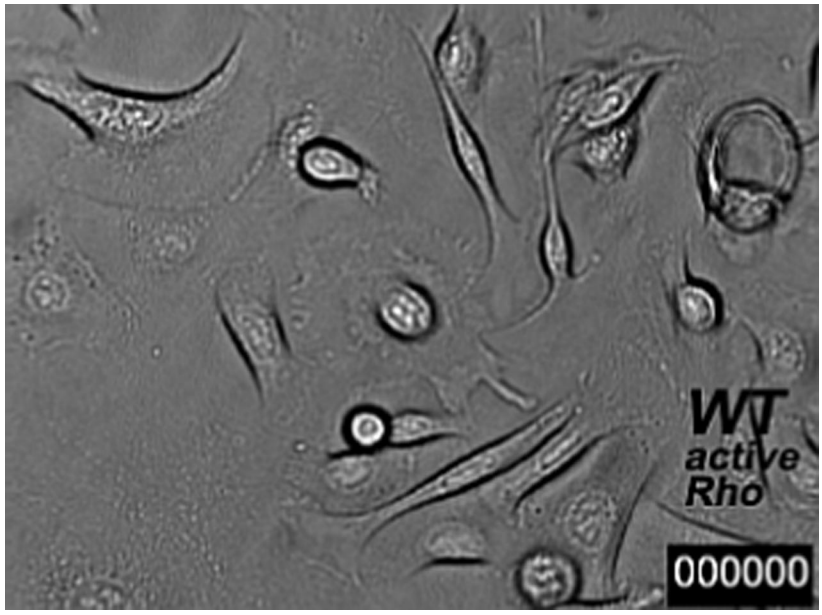
Movie S3. Time-lapse bright-field microscopy of control mKers. Complete cytokinesis of mKers is seen by 30 to 45 min. Time in min is indicated in the lower right corner.

Movie S3 (AVI)



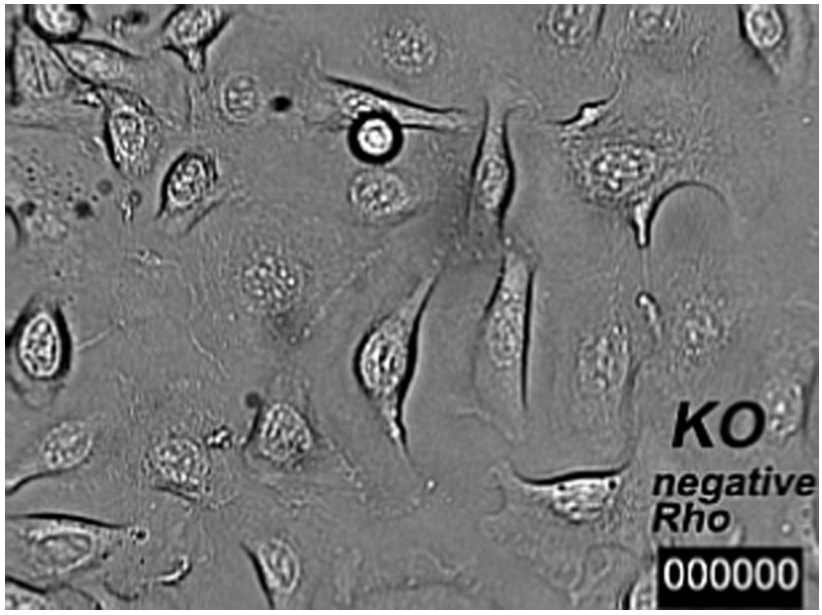
Movie S4. Time-lapse bright-field microscopy of p120-null mKers. mKers stay longer in mitosis and exit mitosis as binucleated cells. Time in min is indicated in the lower right corner.

Movie S4 (AVI)



Movie S5. Time-lapse bright-field microscopy of control mKers expressing a constitutively active form of RhoA. mKers exit mitosis as multinucleated cells. Time in min is indicated in the lower right corner.

Movie S5 (AVI)



Movie S6. Time-lapse bright-field microscopy of p120-null mKers expressing a constitutively dominant negative form of RhoA. p120-null mKers progress through this mitotic event when they are rescued by dampening the activity of RhoA. Time in min is indicated in the lower right corner.

Movie S6 (AVI)