

Supplemental Figure Legends:

Supplemental Figure 1. RhoA and phosphorylated myosin light chain accumulate at the equatorial region in late anaphase cells. (A, B) LLCpK1 cells were fixed in TCA and stained with antibodies to RhoA. In control cells (A), RhoA localizes all along the cortex in metaphase and becomes restricted to the equatorial region in anaphase cells. In nocodazole treated cells, RhoA is located at the equator and at non-equatorial sites (arrows). (C, D) Cells were fixed and stained for F-actin using fluorescent phalloiden (left) and for phosphorylated myosin LC (right) in control (C) and nocodazole (D) treated cells. Asterisks mark the location of the chromosomes in anaphase cells. Bars = 10 μ m.

Supplemental Figure 2. Wave-like behavior of cortical actin in mitotic cells. (A). Selected images from a time-lapse sequence of the ventral cortex of a mitotic LLC-Pk1 cell expressing GFP-actin that has been treated with nocodazole. Arrowheads show the motion of GFP-actin. (B). Selected images from a time-lapse sequence of an LLC-Pk1 cell in mitosis following photobleaching. Dotted lines show the bleached region; notice the wave-like activity that contributes to recovery (arrowheads). Time in min:sec. Bars = 10 μ m.

Supplemental Figure 3. Organization of TDRFP-Myosin RLC in blebbistatin treated cells. (A) The cell was treated with blebbistatin, and nocodazole, in the continued presence of blebbistatin, was added. Cortical TDRFP-Myosin RLC accumulates in a broad band near the equator; myosin elsewhere on the cortex did not show contractile activity. (B) For comparison, a cell expressing TDRFP-Myosin RLC that was treated with nocodazole, showing the dynamic mislocalization of myosin to the polar cortex (arrowheads). In this cell, nocodazole was added relatively late in anaphase, so the accumulation of myosin RLC in the non-equatorial cortex was less extensive than in Figure 5C.

Supplemental Figure 4. Mislocalization of RhoA in nocodazole treated cells. LLCpK1 cells were fixed in TCA and stained with antibodies to RhoA. In control cells (A), RhoA localizes along the membrane in metaphase and becomes restricted to the equatorial region in anaphase cells. In nocodazole treated cells (B), RhoA is located at the equator and at non-equatorial sites (arrowheads). Bar = 10 μ m. Astrisks mark the location of the chromosomes in the anaphase cells.

On-line supplemental Videos

Movie 1. Control LLC-Pk1 cell expressing GFP-actin. Width of field 42um; elapsed time = 42 min.

Movie 2. Control LLC-Pk1 cell expressing Ce-GFPRhoA. Width of field, 42 um; elapsed time = 40 min.

Movie 3. LLC-Pk1 cell expressing GFP-actin that has been treated with nocodazole > 2 minutes following anaphase onset. Width of field, 70um; elapsed time = 23 min.

Movie 4. LLC-Pk1 cell expressing GFP-actin that has been treated with nocodazole immediately following anaphase onset. Width of field, 55um; elapsed time = 21 min.

Movie 5. Fluorescence recovery after photobleaching of a nocodazole treated anaphase LLC-Pk1 cell expressing GFP-actin. Width of field 55um; elapsed time = 2.5 min.

Movie 6. Fluorescence recovery after photobleaching of a nocodazole treated mitotic LLC-Pk1 cell expressing GFP-actin. Width of field 50um ; elapsed time = 4 min.

Movie 7. Control LLC-Pk1 cell expressing TDRFP-MRLC. Width of field, 25um; elapsed time = 46 min.

Movie 8. LLC-Pk1 cell expressing TDRFP-MRLC and treated with nocodazole > 2 min following anaphase onset. In this movie the first few frames are focused on the accumulation of MRLC at the equator. As soon as contractile activity is observed away from the ring, the focus shifts to the non-equatorial cortex. Width of field 67; elapsed time = 16 min.

Movie 9. LLC-Pk1 cell expressing Ce-GFPRhoA and treated with nocodazole at > 2 minutes after anaphase onset, the polar region is shown. Width of field, 86um; elapsed time = 4 min.