Figure S1: Specificity of the Rab6A’-dependent metaphase block

A) (a) 72 h after transfection with siRNAs that target Rab6A, Rab6A’ or control siRNA, cell lysates were subjected to western-blottting analysis and probed with an anti-Rab6 antibody (top) or anti β-tubulin antibody (bottom). The remaining band after depletion of Rab6A or Rab6A’ corresponds to the isoform which has not been depleted, since the anti-Rab6 antibody recognizes both isoforms (Del Nery et al., in preparation). (b) Quantification of the effects of the depletion of Rab6A or Rab6A’ with siRNA. Depletion of Rab6A’ leads to a strong metaphase block, whereas depletion of Rab6A only slightly impairs progression through mitosis.

Rab6A and Rab6A' differ in only three amino acids: one conservative substitution (I instead of V at position 62) and two nonconservative changes (the "TV" motif: A for T and A for V at positions 87 and 88, respectively). We have previously shown that the functional differences between the two isoforms, especially their interaction with RK6, are dependent on residue A or T at position 87 (Echard et al., 2000). To test the specificity of Rab6A’ siRNA, a Rab6A mutant was generated in which the T87V88 motif of Rab6A was replaced by the corresponding A87A88 motif of Rab6A’ (see Del Nery et al., in preparation). This Rab6A’”AA” mutant, termed Rab6A’ siResistant (siRes), thus behaves likely as Rab6A’, but should be resistant to Rab6A’ siRNA and be still recognized by Rab6A siRNA.

A) We first tested the sensitivity of the GFP-Rab6A’ siRes mutant expressing cells to Rab6A and Rab6A’ siRNAs by western-blot and immunofluorescence. 72 h after transfection with control, Rab6A, Rab6A’ or Rab6A/A’ siRNAs, HeLa cells stably expressing GFP-Rab6A’ siRes were subjected to western-blot analysis and probed with an anti-Rab6 or with anti-GAPCenA antibodies. As expected, expression of GFP-Rab6A’ siRes was not decreased after treatment with Rab6A’ siRNA, whereas its expression was completely lost after treatment with Rab6A siRNA. Same results were obtained by immunofluorescence: GFP-Rab6A’ siRes staining was not decreased after treatment with Rab6A’ siRNA, whereas the corresponding staining was completely lost after treatment with Rab6A siRNA.

B) GFP-Rab6A’wt or GFP-Rab6A’ siRes cells were treated with control or Rab6A’ siRNAs and imaged by videomicroscopy for 72 h. The mitotic index was calculated at times 48, 72 and 82 h. An increase in the percentage of the mitotic index was observed from 72 h after transfection in GFP-Rab6A’ wt cells treated with Rab6A’ siRNA. This increase reflects the
metaphase block quantified from videomicroscopy experiments (see Fig. 1). In the case of GFP-Rab6A’ siRes expressing cells treated with Rab6A’ siRNA, the mitotic index was similar to that of control cells. These results indicate that the Rab6A’-dependent metaphase block is specific for Rab6A’ siRNA.

Finally, another Rab6A’ siRNA (Young et al., 2005) targeted against a different region of Rab6A’ mRNA also led to a metaphase block (data not shown).

**Figure S2: Localization of BubR1 in cells blocked in metaphase**

Confocal images of metaphasic cells co-transfected for 72 h with mtGFP (used here as a reporter gene) and either control or Rab6A’ shRNA. Cells were co-stained with a CREST serum (left), anti-BubR1 antibody (middle) and DAPI (right). Arrowheads indicate kinetochores labelled with BubR1 antibodies. Bar, 10 µm. In the majority of cell transfected with Rab6A’ shRNA (68% as compared to 27% in control cells), BubR1 was present on at least two kinetochores (arrowhead). Bar, 10 µm.

**Figure S3: Quantification of the number of the co-localized structures present at kinetochores using the Spot Detection Software**

A) Representative 2D plane of deconvolved images of CREST (a, c), Mad2 (b) or p150Glued (d) labelling in a Rab6A’ shRNA transfected cell. Images were then processed (named images a’-d’) with the Spot Detection Software, developed in our Laboratory as a Metamorph module. Based on wavelet transforms, this software (described in (Langevin et al., 2005)) allows the detection of small “spot like” structures in 3D, even in presence of bigger and brighter structures and of a non homogeneous and noisy background. B) Logical AND (yellow) in the region 1 and 2 between either (a) CREST (green, a1’) and Mad2 (red, b1’) or (b) CREST (green, c2’) and p150Glued (red, d2’) labelling was then calculated in order to keep the colocalization regions between the two labels. The number of remaining objects was automatically counted in 3D with the Spot Detection Software. Structures with an area smaller than 5 voxels were not taken in account.
Movie 1: Rab6A’ shRNA treated cells display a metaphase block.

HeLa cells depleted of Rab6A’ were analysed by time-lapse phase contrast videomicroscopy up to 72 h following transfection with shRNAs. Some cells (arrow) entered mitosis normally, rounded up, but were subsequently blocked in mitosis and died after several hours (arrow). The numbers correspond to the time in minutes after the beginning of the recording.

Movie 2: Details of a metaphase block in Rab6A’ shRNA treated cells.

Note the alignment of chromosomes on the metaphase plate during the metaphase block (arrowhead). The numbers correspond to the time in minutes after the beginning of the recording.
Figure S1
Figure S2