

Supplemental material

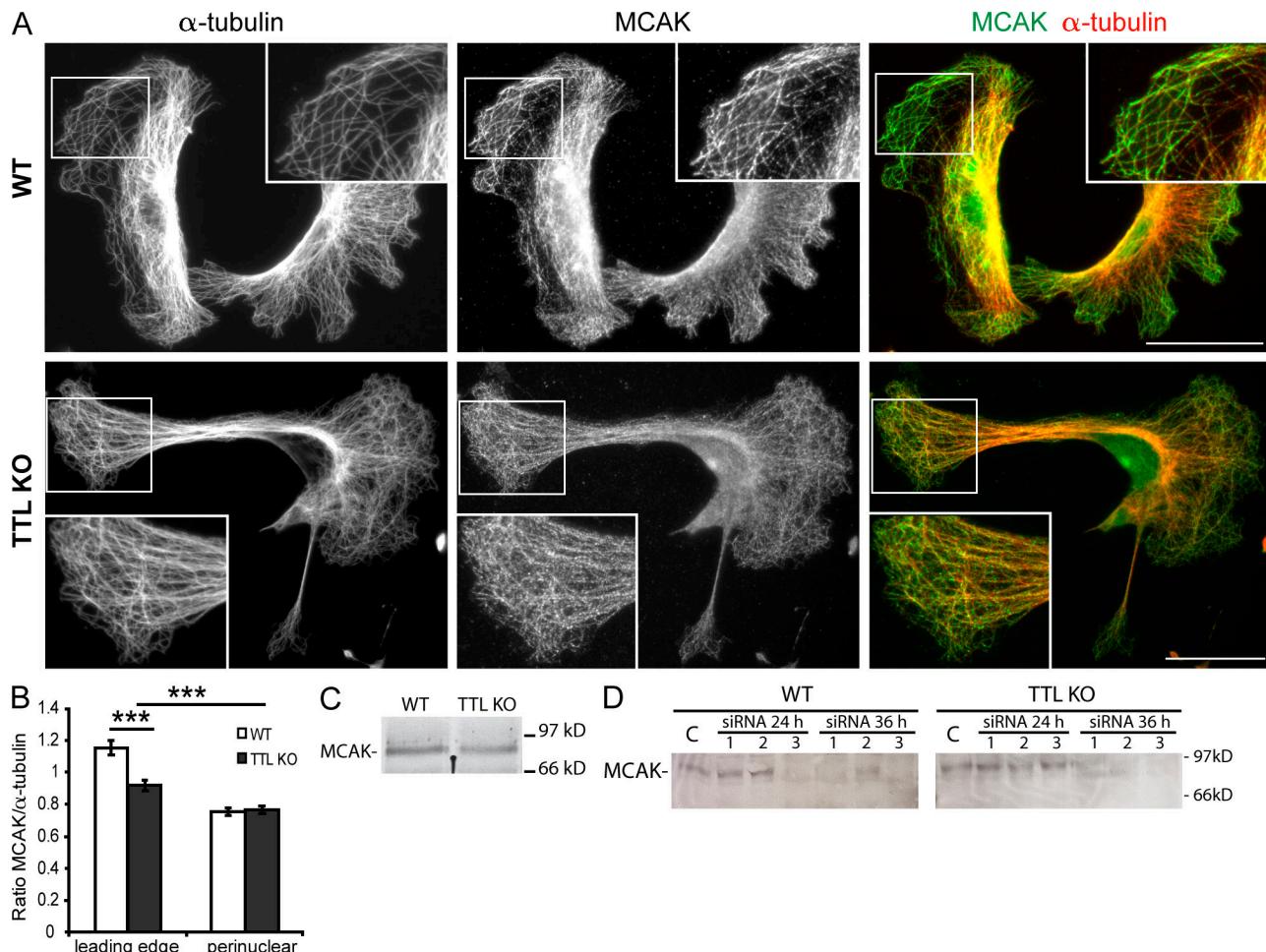
Peris et al., <http://www.jcb.org/cgi/content/full/jcb.200902142/DC1>

Figure S1. Endogenous MCAK distribution and expression levels in WT and TTL KO fibroblasts. (A) Immunofluorescence images of fibroblasts labeled with α -tubulin (red) and MCAK (green) antibodies. MCAK is enriched in lamellipodial extensions in both WT and TTL KO cells. However, MCAK seems to colocalize with the MT network (inset) in WT cells extensions, whereas it shows a more diffuse pattern in TTL KO cell extensions (inset), which is compatible with a diminished lateral association of MCAK with MTs. Note that MCAK comets, which are unaffected by tubulin detyrosination (Peris et al. 2006. *J. Cell Biol.* doi:10.1083/jcb.200512058), are not detected with MCAK antibody. (B) Quantitative analysis of MCAK versus MT signals at the leading edge and in the perinuclear region of cells. Ratio of MCAK versus α -tubulin fluorescent signals in a fixed size region located near the leading edge or close to the nucleus. Data are expressed as mean values \pm SEM for 74 WT fibroblasts and 70 TTL KO fibroblasts from three independent experiments (***, P < 0.001 using a parametric t test). The ratio MCAK/ α -tubulin in lamellipodial extensions was lower in TTL KO cells compared with WT cells. (C) Western blot analysis of MCAK content in WT or TTL KO fibroblast extracts. Equal amounts of proteins were loaded in each lane. (D) Western blot analysis of MCAK levels in WT or TTL KO fibroblast transfected with Stealth siRNA Negative Control or with 3 different commercial MCAK siRNAs (1, MSS232130; 2, MSS232131; and 3, MSS232132). Equal amounts of proteins were loaded in each lane. Because MCAK depletion is almost complete 36 h after transfection of siRNA 3, such conditions have been used in all MCAK depletion experiments. Bars, 50 μ m.

ROUGH GALLEY PROOF

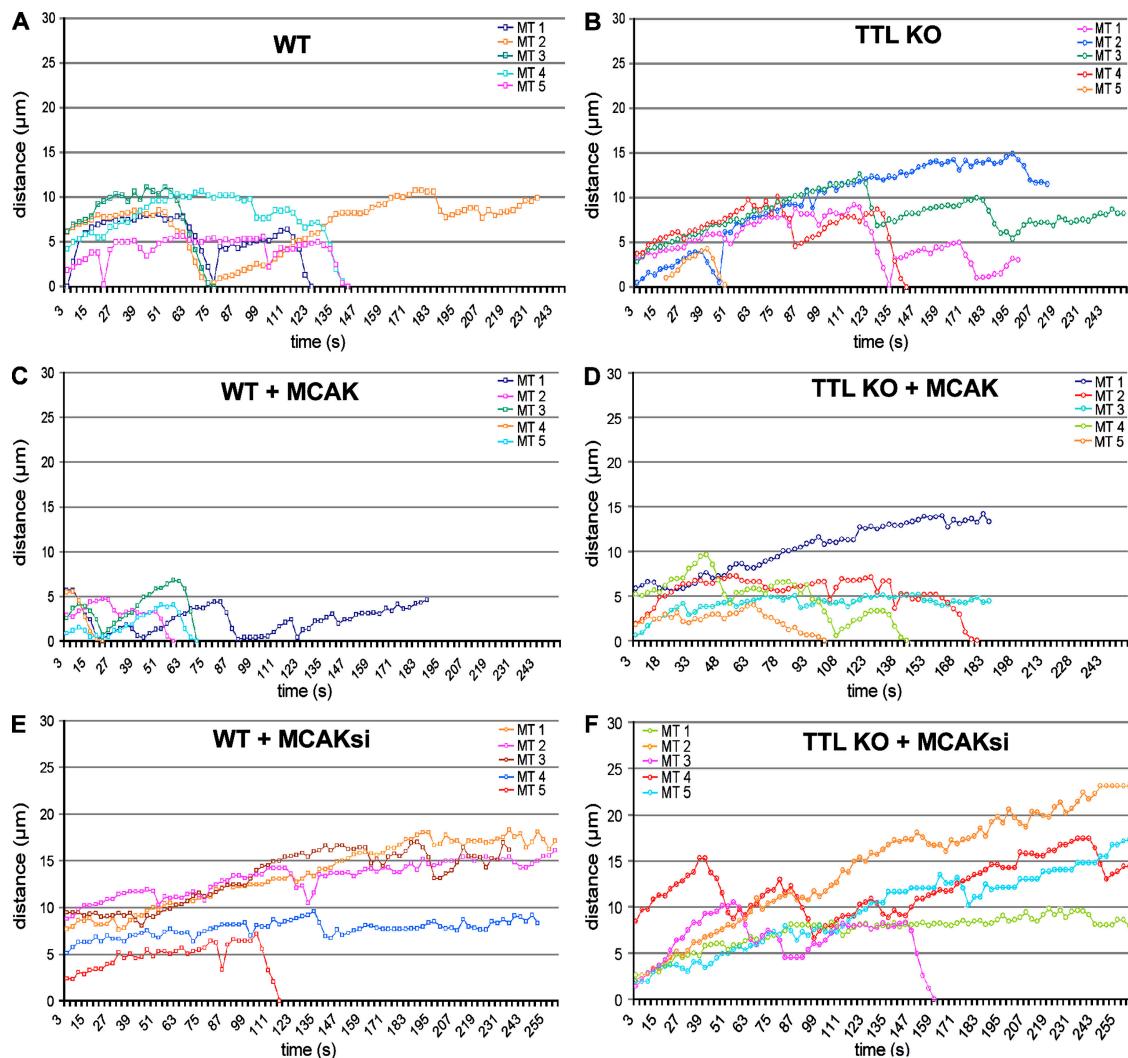
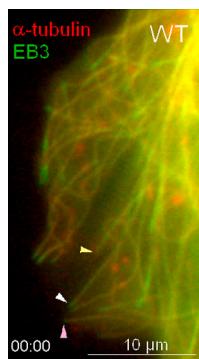
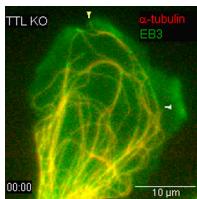


Figure S2. Analysis of MT dynamic instability. Representative life history plots of individual MTs in various conditions are shown as indicated. The corresponding statistical analysis is shown in Fig. 1 D. (A and B) MT rescues are more frequent in TTL KO cells compared with WT. As a result, MT catastrophes leading to extensive depolymerization are a more frequent occurrence in WT cells than in TTL KO cells. (C and D) MCAK overexpression leads to more precocious large MT catastrophes in WT cells. In TTL KO cells, MCAK overexpression reduces the number of rescues and increases the time spent pausing by polymers at the expense of the time spent growing. (E and F) MCAK depletion with siRNAs induces an attenuation of MT dynamic instability behavior in both genotypes, with a decrease in the time spent shrinking and an increase in the time spent pausing. As a result of MCAK depletion, MT dynamic instability behavior becomes similar in both genotypes.

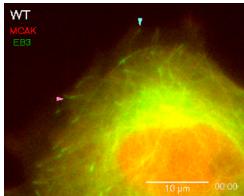


Video 1. Time-lapse video microscopy of a lamellipodial extension of a WT fibroblast expressing m-cherry α -tubulin and GFP-EB3. Representative MTs are marked with arrowheads. When MTs reach the cell edge, MT tips stop growing and often undergo a complete depolymerization until disappearance from the peripheral area (effective catastrophe; white and yellow arrowheads). Only a small proportion of MTs follow the leading edge for a while before depolymerizing (pink arrowhead). Images from the red and green channels were taken every 3 s for 3 min. Time is expressed in minutes:seconds. The video is shown at 6 frames/s. Bar, 10 μ m.

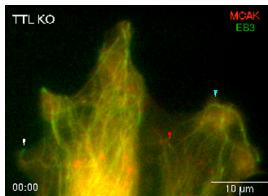
ROUGH GALLEY PROOF



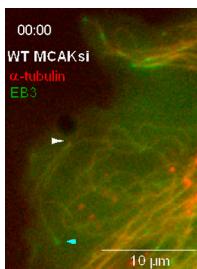
Video 2. Time-lapse video microscopy of a lamellipodial extension of a TTL KO fibroblast expressing m-cherry α -tubulin and GFP-EB3. Representative MTs are marked with arrowheads. MT tips in TTL KO cells do not pause upon reaching the membrane but, instead, continue to grow. As a result, as they approach the membrane, they turn away from the cell edge or sometimes they run along the plasma membrane (white arrowhead). Occasionally, when some MTs contact the leading edge, they seem to push the membrane forward for a while (yellow arrowhead). This phenotype has also been observed in KIF2A KO cells. Images from the red and green channels were taken every 3 s for 3 min. Time is expressed in minutes:seconds. The video is shown at 6 frames/s. Bar, 10 μ m.



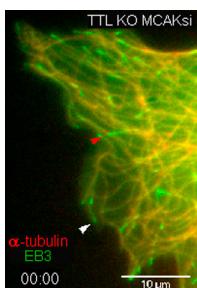
Video 3. Time-lapse video microscopy of a lamellipodial extension of a WT fibroblast expressing m-cherry MCAK and GFP-EB3. Representative MTs are marked with arrowheads. Each arrowhead indicates a different MT plus end. MT behavior is perturbed with MCAK overexpression leading to a complete depolymerization until disappearance from the lamellipodial extension after membrane contact. Images from the red and green channels were taken every 3 s for 3 min. Time is expressed in minutes:seconds. The video is shown at 6 frames/s. Bar, 10 μ m.



Video 4. Time-lapse video microscopy of a lamellipodial extension of a TTL KO fibroblast expressing m-cherry MCAK and GFP-EB3. Representative MTs are marked with arrowheads. MT behavior is perturbed with MCAK overexpression in TTL KO cells. Some MTs undergo catastrophes after contact with the cell membrane (light blue arrowhead), some spend time pausing (red arrowhead), and only a small proportion follows the leading edge and never shrinks (white arrowhead). Images from the red and green channels were taken every 3 s for 3 min. Time is expressed in minutes:seconds. The video is shown at 6 frames/s. Bar, 10 μ m.



Video 5. Time-lapse video microscopy of a lamellipodial extension of a WT fibroblast treated with MCAK siRNA for 36 h and expressing m-cherry α -tubulin and GFP-EB3. Representative MTs are marked with arrowheads. MCAK depletion induced an attenuation of MT dynamics in WT cells. When MTs reach the cell edge, they turn away and grow continuously, alternating phases of growing and pausing (white and light blue arrowheads). Images from the red and green channels were taken every 3 s for 3 min. Time is expressed in minutes:seconds. The video is shown at 6 frames/s. Bar, 10 μ m.



Video 6. Time-lapse video microscopy of a lamellipodial extension of a TTL KO fibroblast treated with MCAK siRNA for 36 h and expressing m-cherry α -tubulin and GFP-EB3. Representative MTs are marked with arrowheads. MCAK depletion induced an attenuation of MT dynamics in TTL KO cells. When MTs reach the cell edge, they turn away and continue polymerizing, alternating phases of growing and pausing (white and red arrowheads), as observed in WT siRNA-treated cells. Images from the red and green channels were taken every 3 s for 3 min. Time is expressed in minutes:seconds. The video is shown at 6 frames/s. Bar, 10 μ m.