

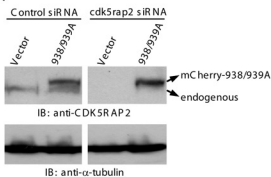
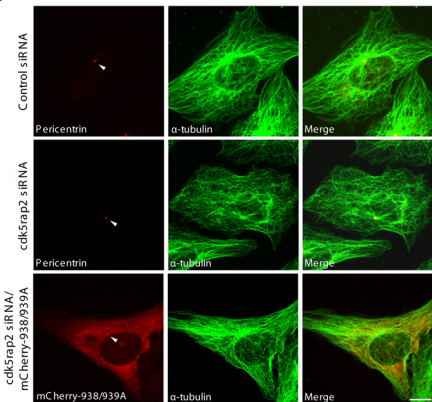
Supplemental Movie Legends

Supplemental movie 1. Cellular CDK5RAP2 displays dynamic behaviors. Time-lapse microscopy was conducted using U2OS stably expressing GFP-CDK5RAP2. mCherry- α -tubulin was transiently expressed at low levels. Images were acquired on live cells at every 4 sec. Shown are the movies of the boxed areas.

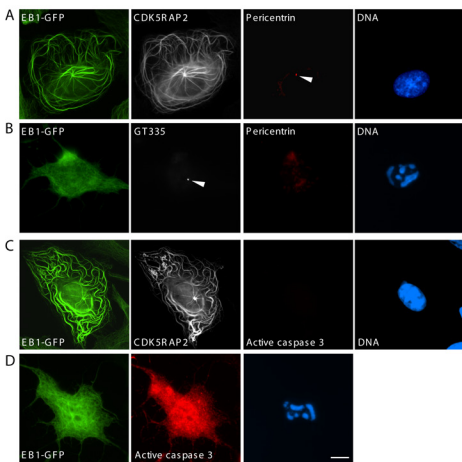
Supplemental movie 2. The EB1-binding deficient mutant does not have plus-end tracking property. Images were acquired with U2OS stably expressing GFP-CDK5RAP2(L938A/P939A). mCherry- α -tubulin was expressed at low levels. Images were acquired on live cells at every 4 sec. Shown are the movies of the boxed areas.

Supplemental movie 3. Tip tracking of GFP-CDK5RAP2 depends on microtubule dynamics. The stable cells of GFP-CDK5RAP2 were imaged before and 1 min after treatment with 0.1 μ M nocodazole. Images were acquired at every 4 sec. mCherry- α -tubulin was transiently expressed.

Supplemental movie 4. Depletion of EB1 inhibits the dynamic behavior of CDK5RAP2. The stable line of GFP-CDK5RAP2 was transfected with control or *eb1*-targeting siRNA. Images were acquired at every 4 sec. mCherry- α -tubulin was transiently expressed. Shown are the movies of the boxed areas.

A**B**

Supplemental Figure 1. U2OS cells were transfected with control or *cdk5rap2*-targeting siRNA. The CDK5RAP2(L938A/P939A) construct (938/939A) contains silent mutations within the siRNA-targeted sequence. (A) The cell extracts were probed by immunoblotting (IB). (B) The cells were stained for MTs (anti- α -tubulin) and pericentrin. Arrows mark the centrosomes.



Supplemental Figure 2. Expression of CDK5RAP2 in selected EB1-GFP stable lines does not induce apoptosis. U2OS cells stably expressing EB1-GFP (Clone E2) were transiently transfected with Flag-CDK5RAP2 (A and C) or treated overnight with 1 μ M staurosporine (B and D). The cells were then subjected to immunostaining. Ectopically expressed CDK5RAP2 was detected with an anti-Flag antibody; centrosomes were labeled with the centriole antibody GT335; nuclear DNA was labeled with Hoechst 33258. Arrows point to centrosomes. Bar, 10 μ M.