Supplementary Figures

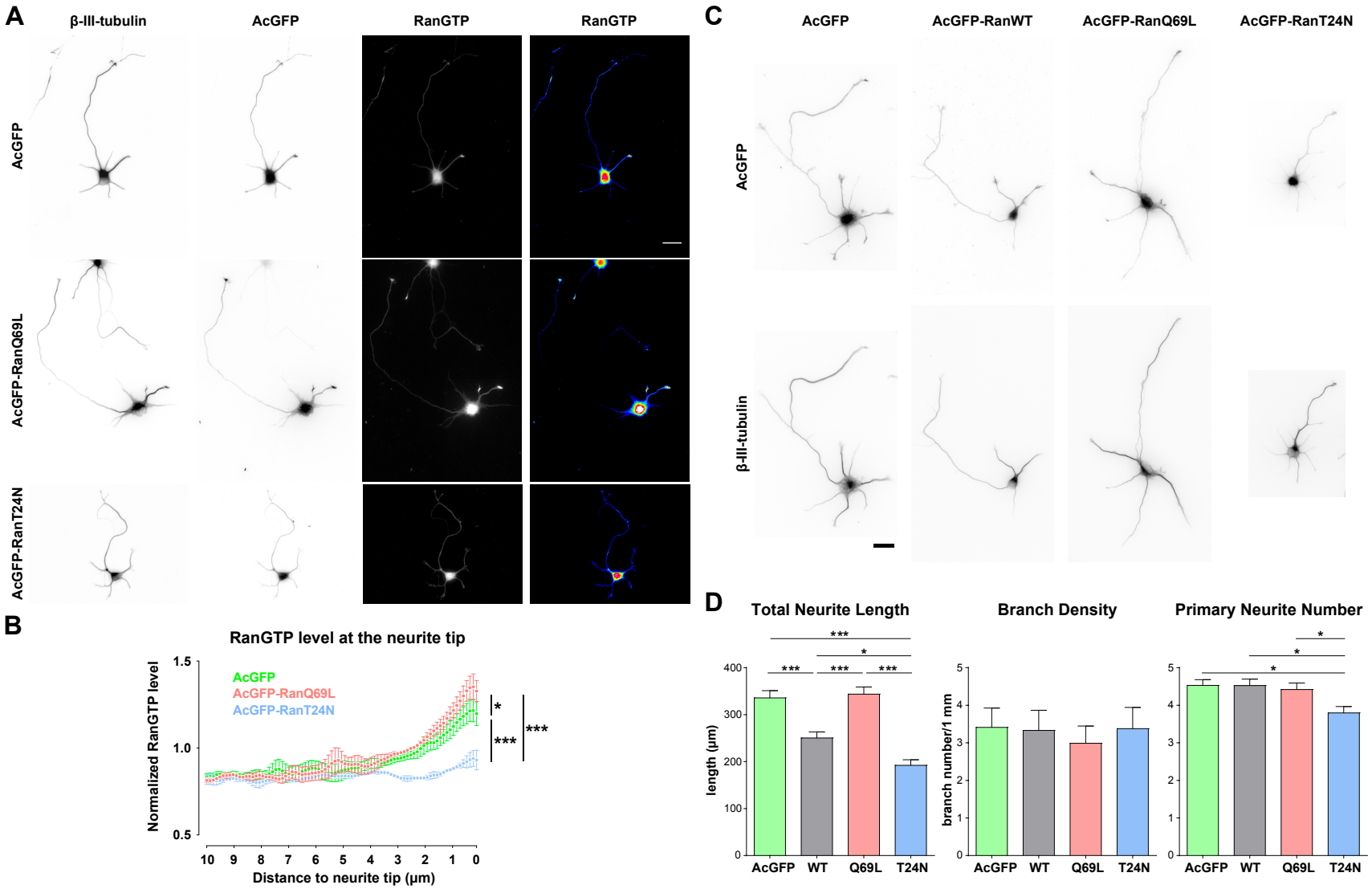


Figure S1. Ran mutants influence the level of RanGTP at the neurite tip and neuronal morphogenesis.
(A) Representative images of 2DIV low-density dissociated hippocampal neurons expressing AcGFP, AcGFP-RanQ69L, or AcGFP-RanT24N. β-III-tubulin, AcGFP, RanGTP, and pseudo-colored RanGTP channels are shown. All images have the same scale and the scale bar represents 20 μm. (B) Normalized RanGTP level linescans at the neurite tip (AcGFP is shown in green, AcGFP-RanQ69L in red, and AcGFP-RanT24N in blue). RanGTP intensity 0~10 μm from the neurite tip was normalized to the average RanGTP intensity along the entire neurite. The dots and error bars indicate mean and SEM from 3 independent experiments. At least 119 neurites from 3 independent experiments from each condition were analyzed. *** $p<0.001$, * $p<0.05$, two-way ANOVA followed by Tukey post-hoc test (RanGTP level 0~1 μm from the tip of the neurite). (C) Representative images of 2DIV low-density dissociated hippocampal neurons expressing AcGFP, AcGFP-RanWT, AcGFP-RanQ69L, or AcGFP-RanT24N. AcGFP (top) and β-III tubulin (bottom) channels are shown. All images have the same scale and the scale bar represents 20 μm. (D) Quantification of total neurite length (left), branch density (middle), and primary neurite number (right) in Ran expressing neurons. * $p<0.05$, *** $p<0.001$, one-way ANOVA followed by Tukey's post-hoc test. Error bars represent SEM from 3 independent experiments.

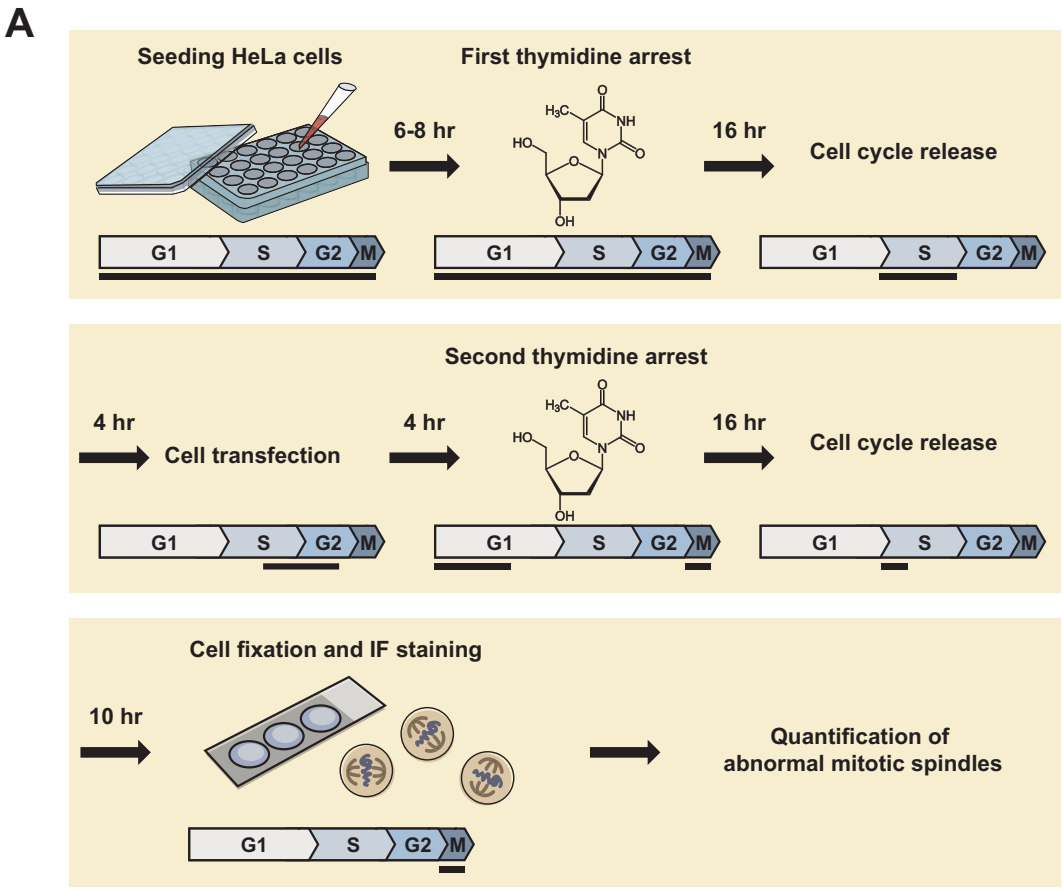


Figure S2. Functional validation of RanTRAP system in HeLa cells.
(A) Schematic procedure of double thymidine arrest to synchronize HeLa cells. The black horizontal bars indicate where in the cell cycle most cells are. (B) Representative images of synchronized mitotic HeLa cells with normal or abnormal spindles. Cells were stained with α-tubulin antibody (DM1A) and DAPI. The spindle morphology was categorized using the following criteria. A mitotic spindle containing two spindle poles and aligned chromosomes was categorized as "metaphase" spindle. A mitotic spindle containing two spindle poles and segregated chromosomes was categorized as "anaphase/telophase" spindle. A mitotic spindle containing two spindle poles and misaligned chromosomes was categorized as "bipolar spindle with misaligned chromosomes". A mitotic spindle containing more than two spindle poles was categorized as "multipolar spindle". A mitotic spindle containing only one spindle pole and radially arranged chromosomes was categorized as "monopolar spindle". The scale bar represents 10 μm. (C) Quantification of abnormal spindle occurrence. More than 50 mitotic HeLa cells were analyzed per condition per repeat. * $p<0.05$, ** $p<0.01$, *** $p<0.001$, one-way ANOVA followed by Tukey's multiple comparison test. Error bars represent SEM from 3 independent experiments.

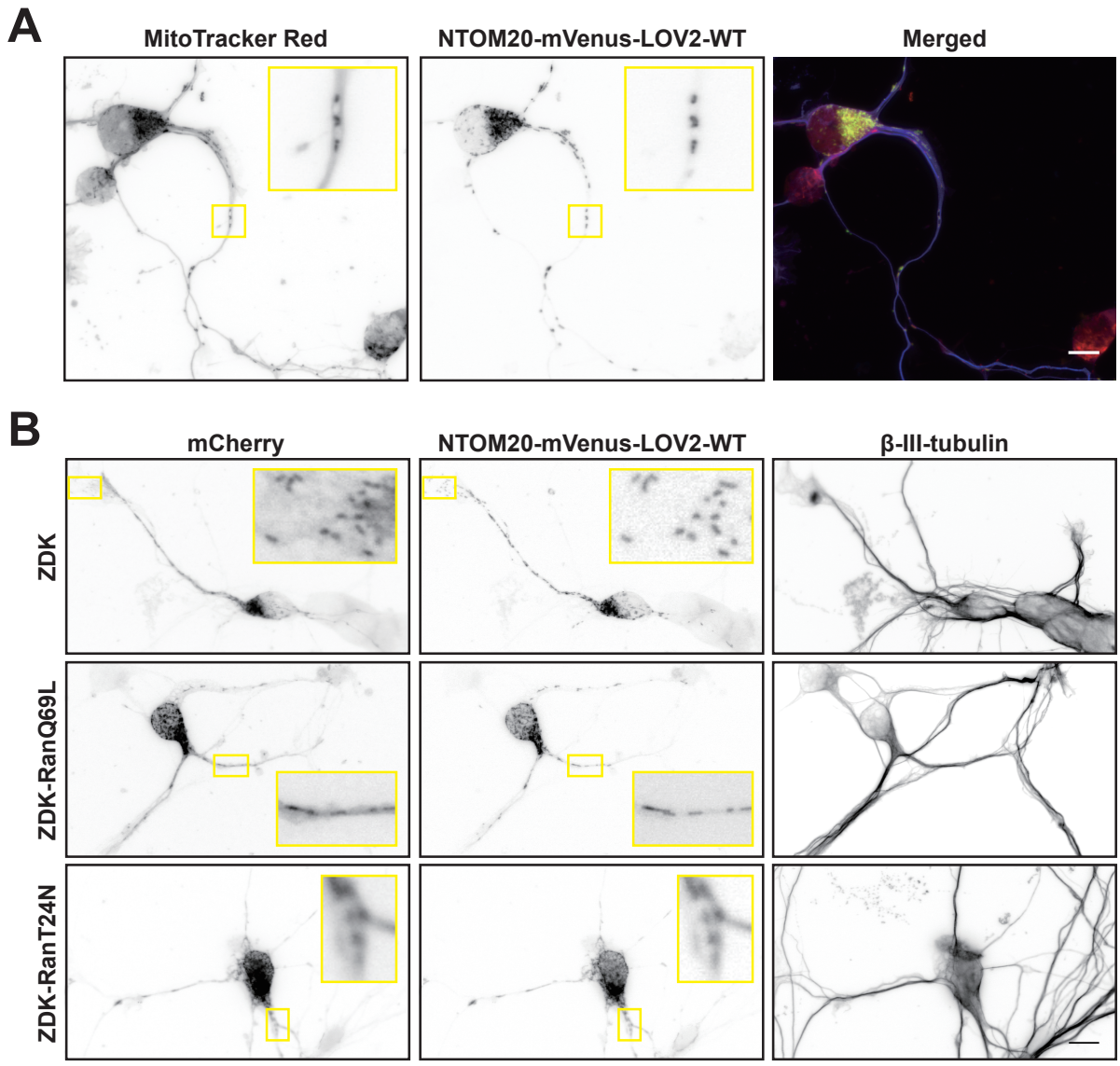


Figure S3. The RanTRAP system targets Ran to mitochondria along the neurite.
(A) Representative images of a mouse cortical neurons transfected with plasmid expressing NTOM20-mVenus-LOV2-WT (green in merged) at 2DIV, fixed and stained with MitoTracker Red (red in merged) and β-III-tubulin antibody (blue in merged) at 4DIV. (B) Representative images of mouse cortical neurons transfected with plasmids expressing NTOM20-mVenus-LOV2-WT and mCherry-ZDK (or mCherry-ZDK-RanQ69L or mCherry-RanT24N) at 2DIV, fixed and stained with the β-III-tubulin antibody at 4DIV. Insets show the magnified view of yellow boxed areas. All scale bars represent 10 μm.

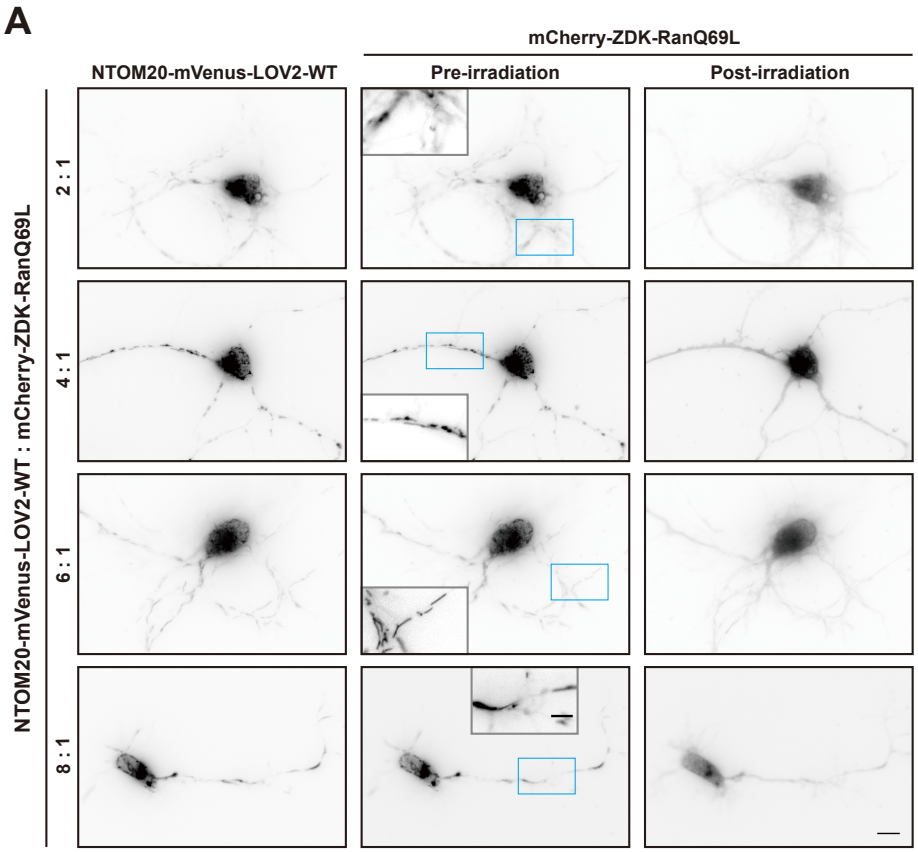


Figure S4. Optimizing the molar ratio of RanTRAP plasmids in neurons.
(A) Representative live cell images of 4DIV mouse cortical neurons co-expressing NTOM20-mVenus-LOV2-WT and mCherry-ZDK-RanQ69L. Neurons were transfected with various molar ratios of plasmid expressing NTOM20-mVenus-LOV2-WT and mCherry-ZDK -RanQ69L (LOV2:ZDK = 2:1, 4:1, 6:1, 8:1) at 2DIV, incubated for 2 days before subjected to live cell imaging. All images have the same scale and the scale bar represents 10 μm. The image insets show the magnified and contrast enhanced view of the area enclosed by the blue box, and the scale bar in the inset represents 5 μm. (B) Representative images showing the ROI (blue circle) where cytosolic mCherry-ZDK-RanQ69L level was quantified. Both live cell images show 4 DIV mouse cortical neurons expressing NTOM20-mVenus-LOV2-WT and mCherry-ZDK-RanQ69L for 2 days. Blue circular ROIs (1.1 μm in diameter) indicate where cytosolic mCherry-ZDK-RanQ69L signal was quantified from. The scale bar represents 4 μm. (C) Quantification of cytosolic mCherry-ZDK-RanQ69L level before and after photoactivation in selected ROI along the neurite in various molar ratio conditions. In the molar ratio 8:1 condition, most of the transfected neurons are unhealthy. As a result, this condition was excluded from the quantification. 13, 30, and 19 neurites were quantified in 2:1, 4:1, and 6:1 conditions, respectively. Box plot shows median, first and third quartile, and whiskers extend to the entire range of data. *** $p<0.001$, one-way ANOVA followed by Tukey's multiple comparison test. Since no statistically significant difference can be detected between the 4:1 and 6:1 condition, the molar ratio (4:1) that produced the higher signal of mCherry-ZDK-RanQ96L was chosen for subsequent experiments.

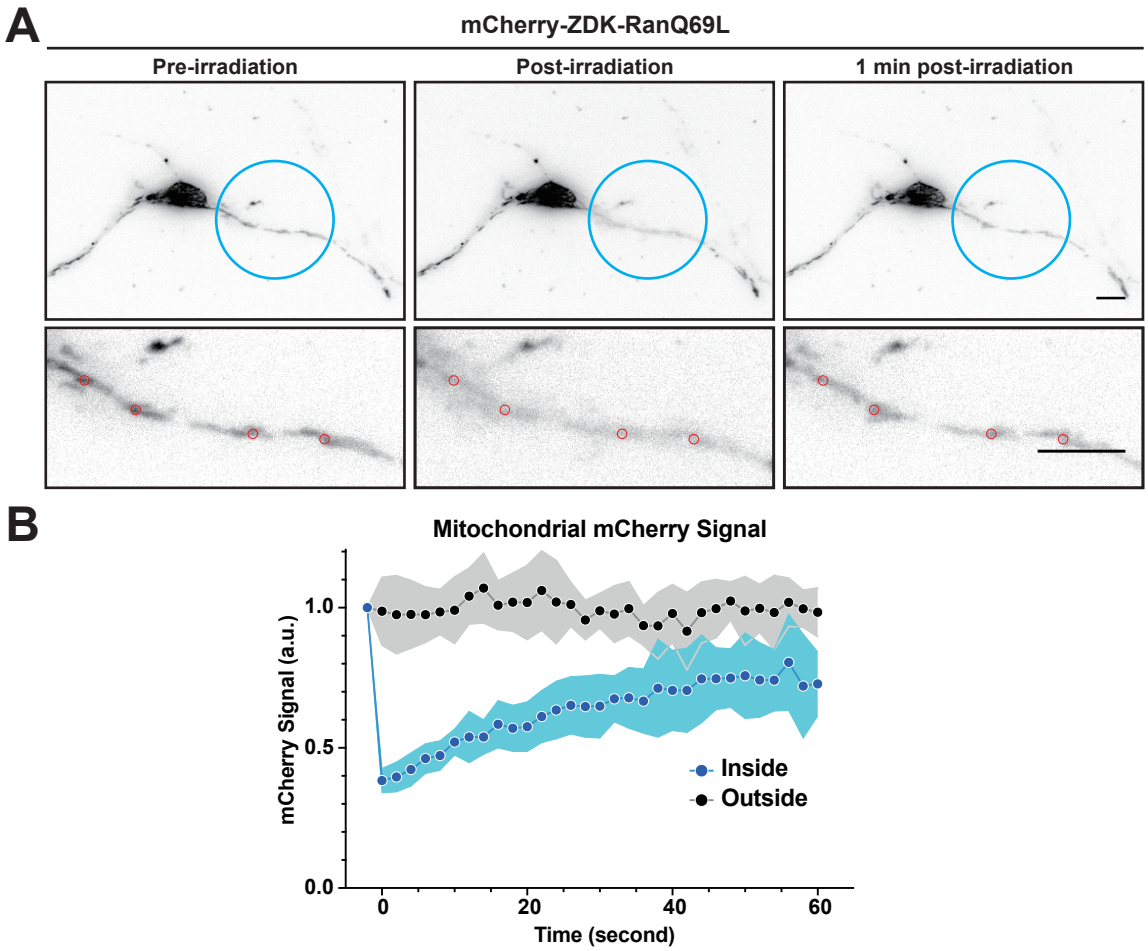


Figure S5. Local release of RanGTP-mimic mutant can be achieved in neurons.
(A) Mouse hippocampal neurons were co-transfected with plasmids expressing NTOM20-mVenus-LOV2-WT and mCherry-ZDK-RanQ69L at 3DIV, incubated for 1 days before subjected to live cell imaging. The blue circle indicated the region of photoactivation. Images on the bottom row show magnified images from the photoactivated region. The mCherry-ZDK-RanQ69L signal before (left), immediately after photoactivation (center), and 1 minute after photoactivation (right) are shown. All scale bars represent 10 μm. (B) Quantification of the mitochondria-localized mCherry-ZDK-RanQ69L signal over time from panel A. 4 selected ROIs (red circles in panel A) on the mitochondria inside (blue line) and outside (black line) the region of photoactivation are analyzed. Dots and shaded areas indicate mean and SD. Note that mCherry-ZDK-RanQ69L only dissociated from the mitochondria inside the photoactivated region.

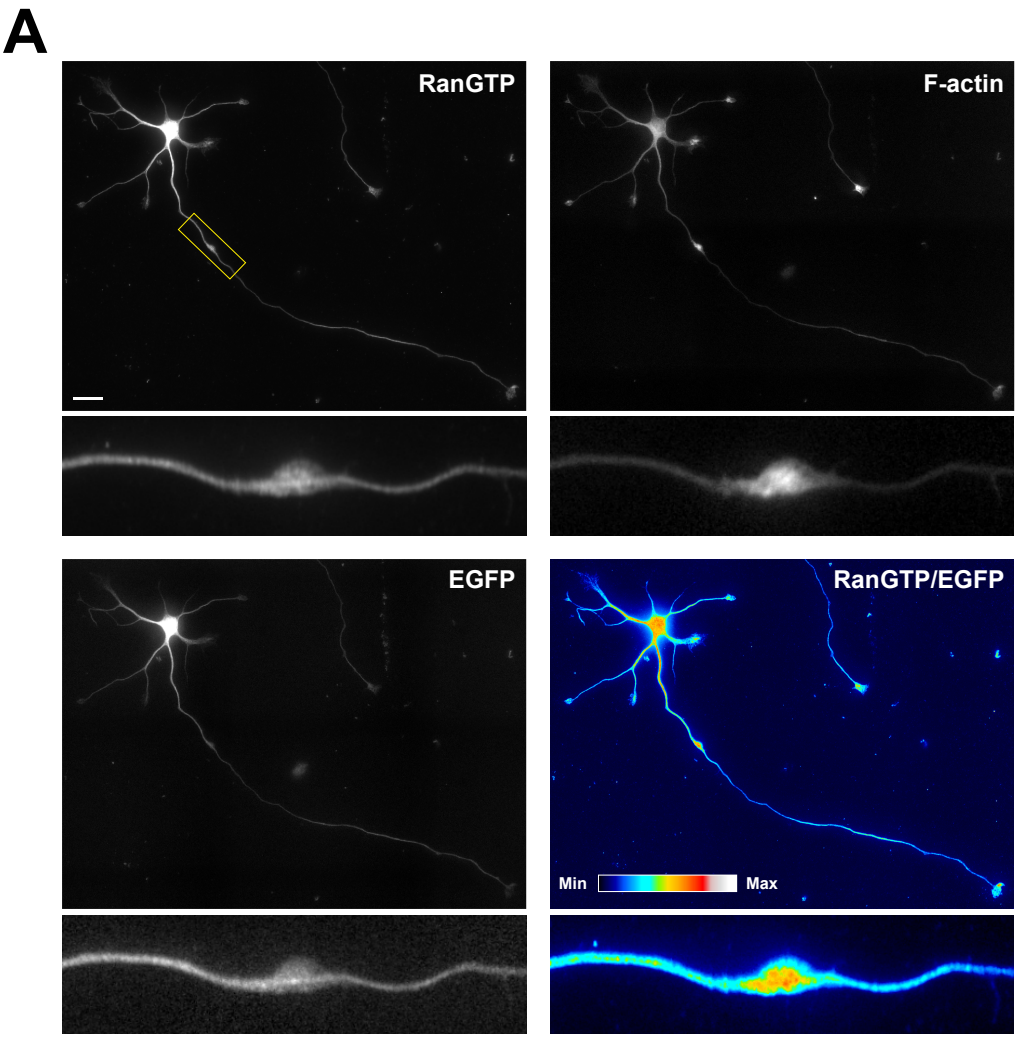
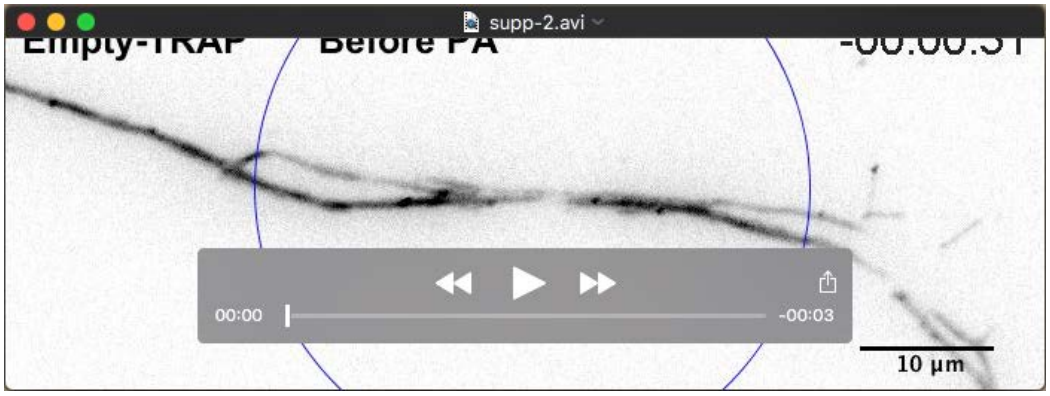


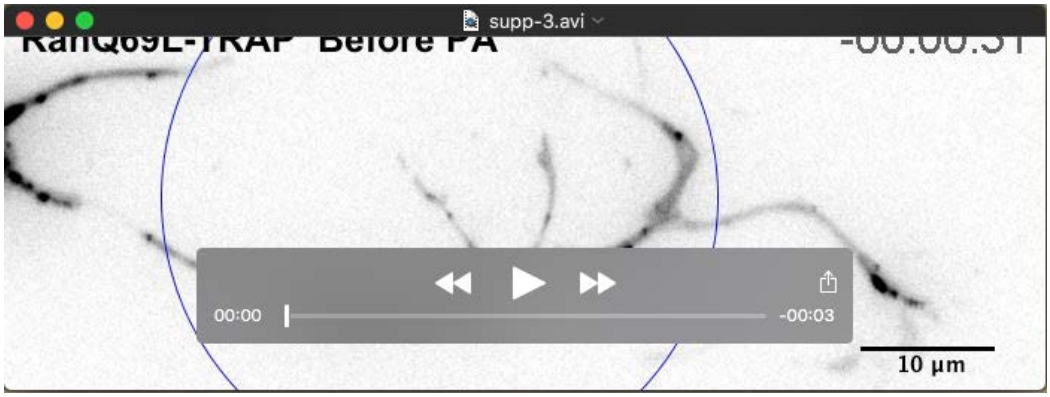
Figure S6. RanGTP level is elevated in the actin wave in neurons.

(A) Representative image of a 2DIV mouse hippocampal neurons expressing EGFP (bottom left) and immunofluorescence stained with antibody against RanGTP (top left) and phalloidin (top right). The ratio image of RanGTP/EGFP was pseudo-colored (bottom right). The boxed area was used to generated the magnified images. All images have the same scale and the scale bar represents 20 µm. (B) Cytoplasmic volume-normalized RanGTP intensity linescan along a 40 µm stretch centered at the actin wave (red) or a random location without the actin wave (blue) in 2DIV hippocampal neurons. The origin of the X-axis is selected using the neurite location with the highest phalloidin staining, negative or positive value of the X-axis indicate the neurite region towards or away from the soma. Dots and shaded areas indicate mean and SEM collected from 47 neurites, *** $p<0.001$, two-way ANOVA followed by Sidak post-hoc analysis between the red and the blue curves.

Movies



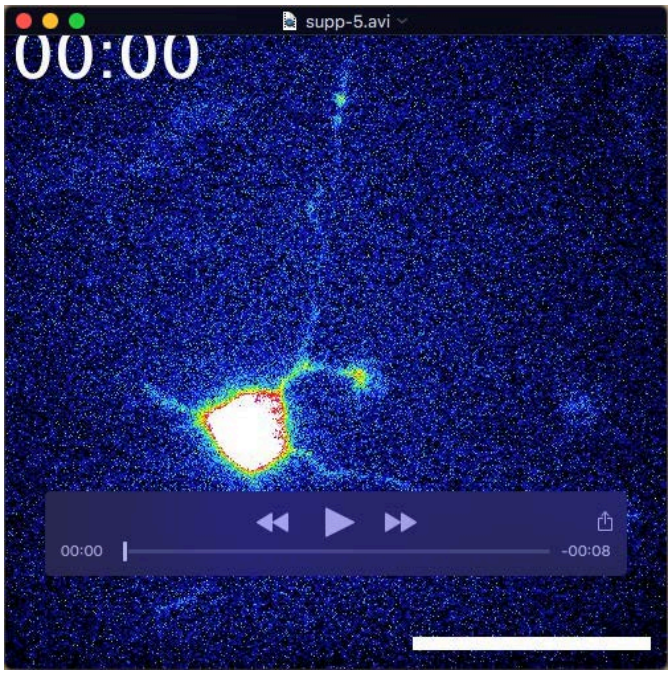
Movie 1. Non-centrosomal microtubule nucleation within the photoactivation region before and after photoactivation in a neuron expressing Empty-TRAP reagent.
Time-lapse video of a 4DIV hippocampal neuron expressing EB3-mCherry and Empty-TRAP before and after photoactivation. Only the EB3-mCherry channel is shown and the photoactivation region is indicated by the blue circle. The time stamp is expressed as hour:minute:second.
https://figshare.com/s/666c60c72185096510de



Movie 2. Non-centrosomal microtubule nucleation within the photoactivation region before and after photoactivation in a neuron expressing RanQ69L-TRAP reagent.
Time-lapse video of a 4DIV hippocampal neuron expressing EB3-mCherry and RanQ69L-TRAP before and after photoactivation. Only the EB3-mCherry channel is shown and the photoactivation region is indicated by the blue circle. The time stamp is expressed as hour:minute:second.
https://figshare.com/s/c5f185244b46f7f5084f

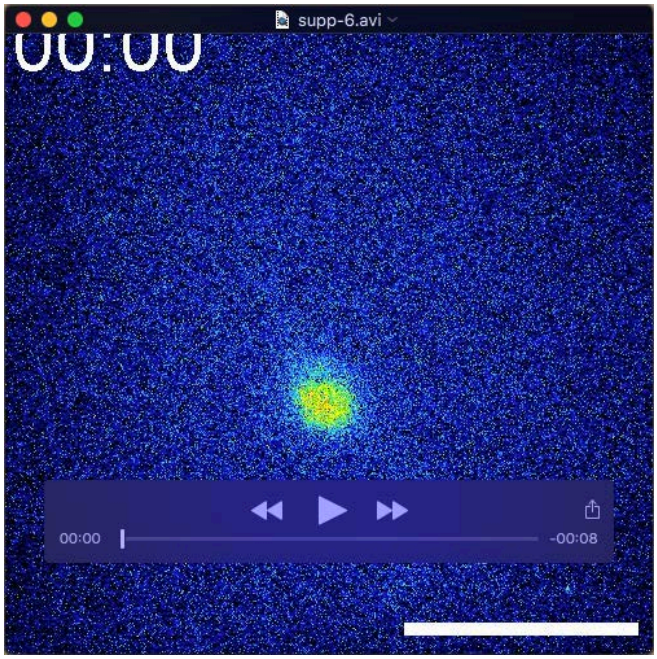
Journal of Cell Science • Supplementary information

Movie 3. Non-centrosomal microtubule nucleation within the photoactivation region before and after photoactivation in a neuron expressing RanT24N-TRAP reagent.
Time-lapse video of a 4DIV hippocampal neuron expressing EB3-mCherry and RanT24N-TRAP before and after photoactivation. Only the EB3-mCherry channel is shown and the photoactivation region is indicated by the blue circle. The time stamp is expressed as hour:minute:second.
https://figshare.com/s/14ac455f4bbf624e47a9

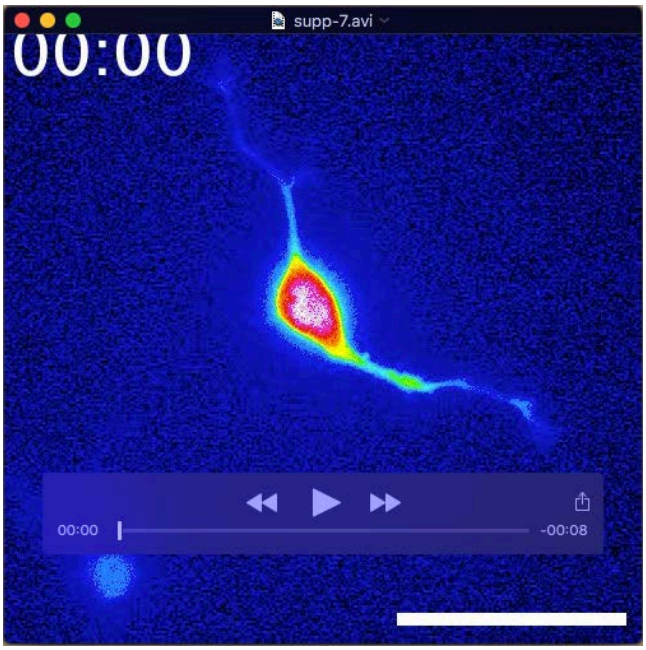


Movie 4. The motility of AcGFP-RanQ69L in a neuron actively generating actin waves.
Time-lapse video of a 2DIV cortical neuron expressing AcGFP-RanQ69L. The time stamp is expressed as hour:minute, and the scale bar represents 20 µm.
https://figshare.com/s/6f9f6194b710cfc04d7c

Journal of Cell Science • Supplementary information

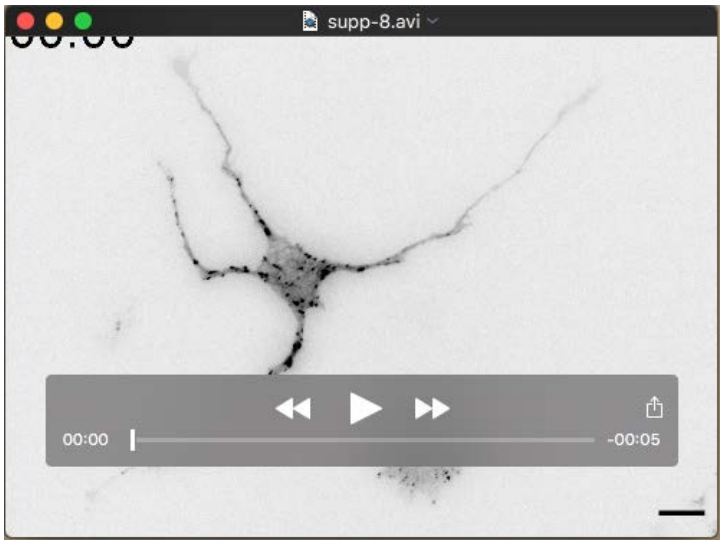


Movie 5. The motility of AcGFP-RanT24N in a neuron actively generating actin waves. Time-lapse video of a 2DIV cortical neuron expressing AcGFP-RanT24N. The time stamp is expressed as hour:minute, and the scale bar represents 20 µm.
https://figshare.com/s/82881e839edc8eba72f5

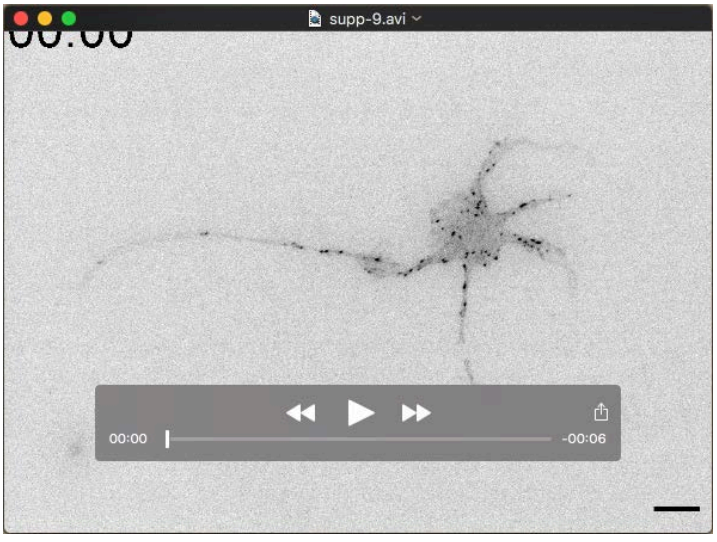


Movie 6. The motility of the cytosolic AcGFP in a neuron actively generating actin waves. Time-lapse video of a 2DIV cortical neuron expressing AcGFP. The time stamp is expressed as hour:minute, and the scale bar represents 20 µm.
https://figshare.com/s/eb87bee04f511a55cb7f

Journal of Cell Science • Supplementary information



Movie 7. Microtubule dynamics in a neuron treated with DMSO.
Time-lapse video of a 1DIV EB3-mCherry-expressing hippocampal neuron after DMSO treatment for 6 hours. The time stamp is expressed as minute:second, and the scale bar represents 10 µm.
https://figshare.com/s/f7cab1b7df933e66a1ea



Movie 8. Microtubule dynamics in a neuron treated with cytochalasin D.
Time-lapse video of a 1DIV EB3-mCherry-expressing hippocampal neuron after cytochalasin D treatment for 6 hours. The time stamp is expressed as minute:second, and the scale bar represents 10 µm.
https://figshare.com/s/97d28fb22d71e2fc868b

Journal of Cell Science • Supplementary information