Supplementary Figures

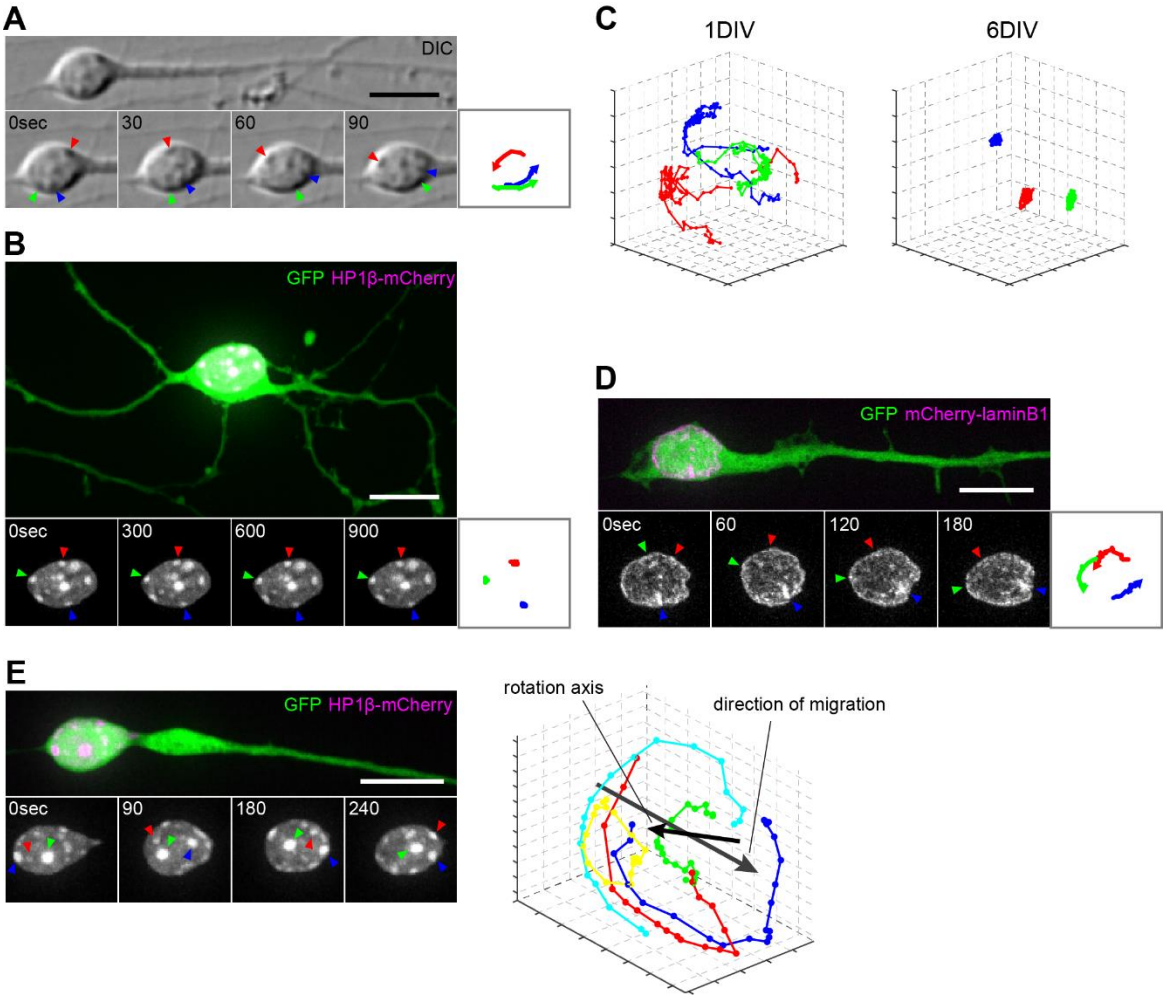


Fig. S1. The nucleus rotates in migrating cerebellar granule cell (A) Time-lapse sequence of DIC images of a migrating CGC in vitro. Colored arrowheads indicate the positions of nucleoli. Trajectories of individual nucleoli are shown in the right-most panel. (B) Time-lapse sequences of a post-migration CGC expressing GFP (green) and HP1β-mCherry (magenta) at 6 DIV (Movie 1). Colored arrowheads indicate the positions of HP1β spots on the nuclear surface. Trajectories of individual spots are shown in the right-most panels. (C) 3D plots of 30-min trajectories of HP1β-mCherry spots in the nucleus of a migrating CGC at 1 DIV and post-migratory CGC at 6 DIV. Positions of the HP1β-mCherry spots were detected from reconstructed 3D images and tracked at 15-sec intervals. Colored dots and lines indicate the trajectories of individual spots. Spots show

dynamic and coordinated motion in migrating CGCs, while they remain stationary at the same relative positions in post-migratory CGCs. (D) Time-lapse sequence of a CGC expressing GFP (green) and mCherry-laminB1 (magenta) in vitro. Colored arrowheads indicate the positions of laminB1 spots. (Movie 2). (E) Time-lapse sequence of a nucleus rotating around an axis parallel to the substrate plane (*left*) and 3D plots of the trajectories of HP1β-mCherry spots (*right*). Scale bars: 10 μm

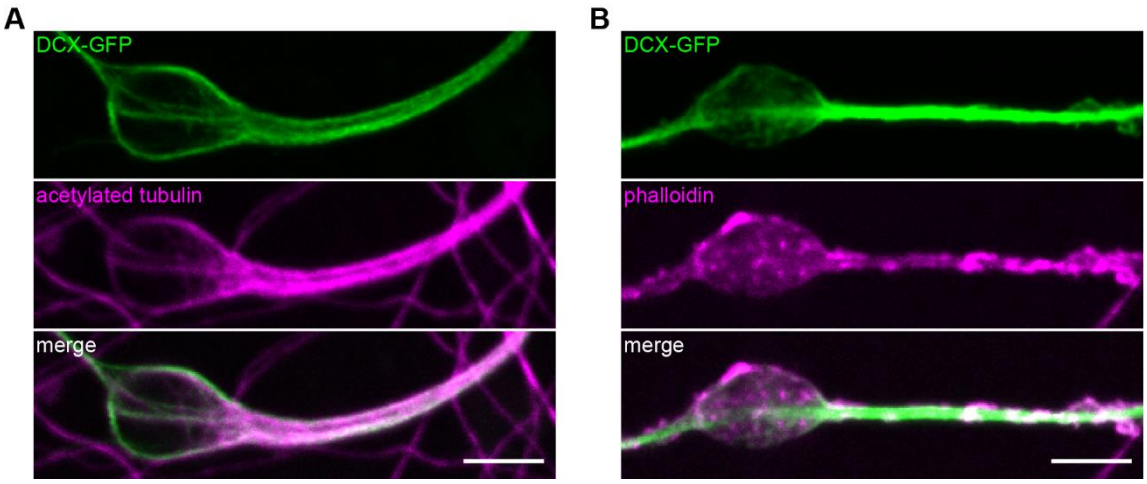


Fig. S2. DCX-GFP co-localizes with microtubules but not with actin. (A) Representative images of a CGC expressing DCX-GFP (green) fixed with methanol, and stained with acetylated tubulin (magenta). (B) Representative images of a CGC expressing DCX-GFP (green) fixed with PFA, and stained with Rhodamine-phalloidin (magenta). Scale bars: 5 μm

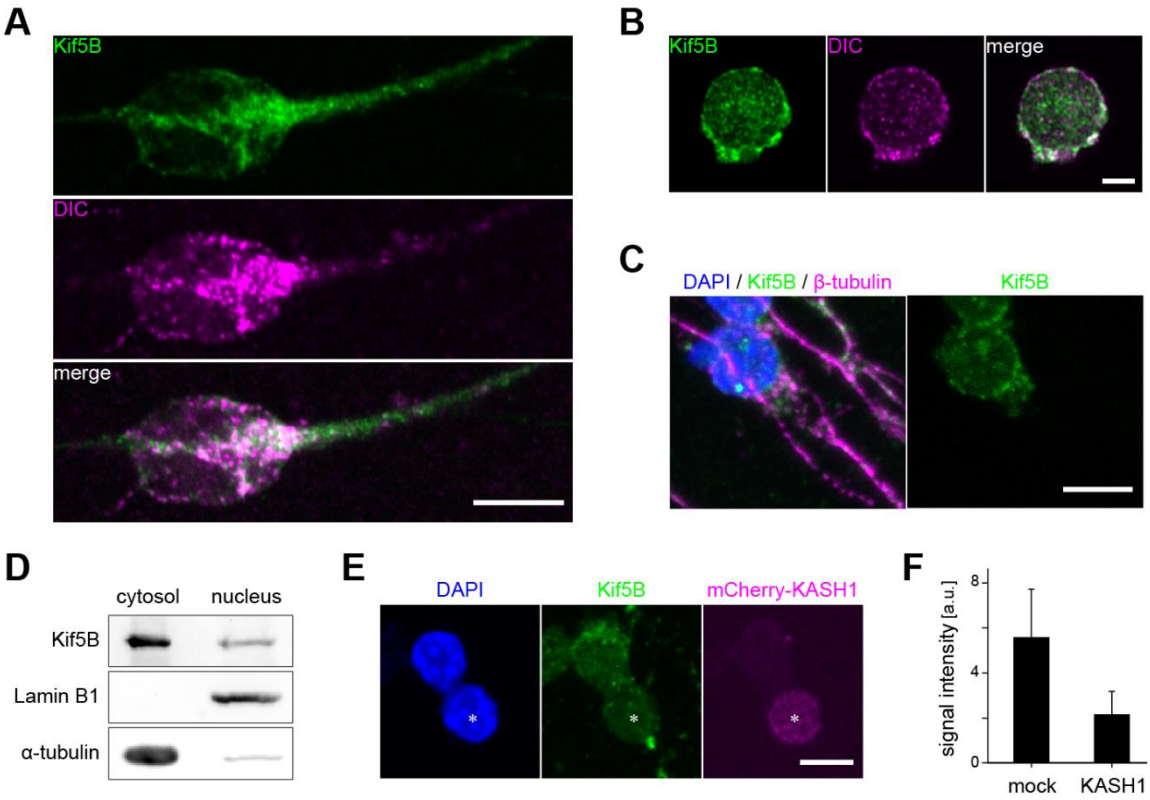


Fig. S3. Distribution of Kif5B and dynein in CGCs. (A) Super-resolution images of a migrating CGC stained with Kif5B (green) and dynein intermediate chain (magenta). Kinesin and dynein signals partially overlap. Scale bar: 5 μm. (B) Super-resolution images of a migrating CGC treated with hypotonic buffer. Kinesin (green) and dynein (magenta) signals partially overlap on the nuclear envelope. Scale bar: 2 μm. (C) Immunostaining of migrating CGCs with Kif5B (green) and β–tubulin (magenta). Nucleus was labeled with DAPI (blue). Multicolor image (left) shows a z-projection, while Kif5B image (right) shows a single section. Scale bar: 5 μm. (D) Subcellular localization of Kif5B in CGCs. Cerebella from P4 mice were fractionated into cytosolic and nuclear fractions that were then subjected to western blotting with indicated antibodies. Kif5B signal was detected in both the cytosol and nucleus. (E) Kif5B staining (green) in a CGC expressing mCherry-KASH1 (magenta). Asterisk indicates the transfected cell. Nucleus was labeled with DAPI (blue). Scale bar: 5 μm. (F) Quantification of Kif5B signal on the nuclear envelope in the CGCs transfected with either mock or mCherry-KASH1. n = 10 cells for each group; mean±s.e.m.

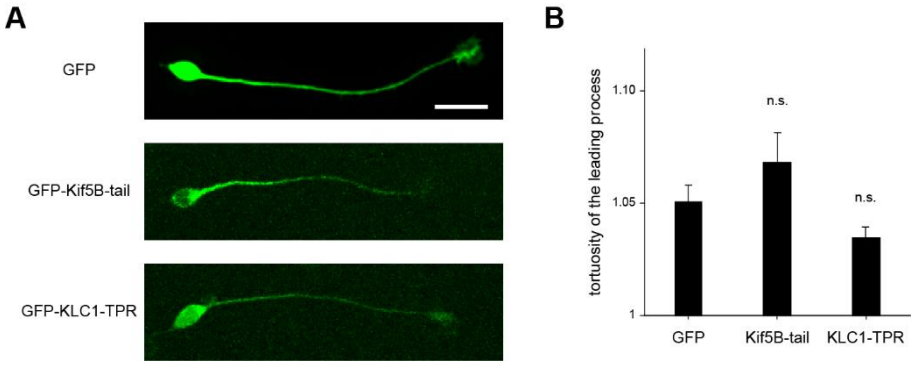


Fig. S4. Inhibition of kinesin-1 by overexpressing dominant-negative mutants does not affect cell morphology. (A) Representative images of CGCs expressing GFP, GFP-Kif5B-tail, or GFP-KLC1-TPR. Scale bar: 20 μm. (B) Quantification of the morphology of the leading process. The tortuosity was defined as L/d, where L is the length of the leading process, and d is the distance between the ends of the leading process. n = 30 cells for each group; mean±s.e.m. ; $p>0.05$, t-test.

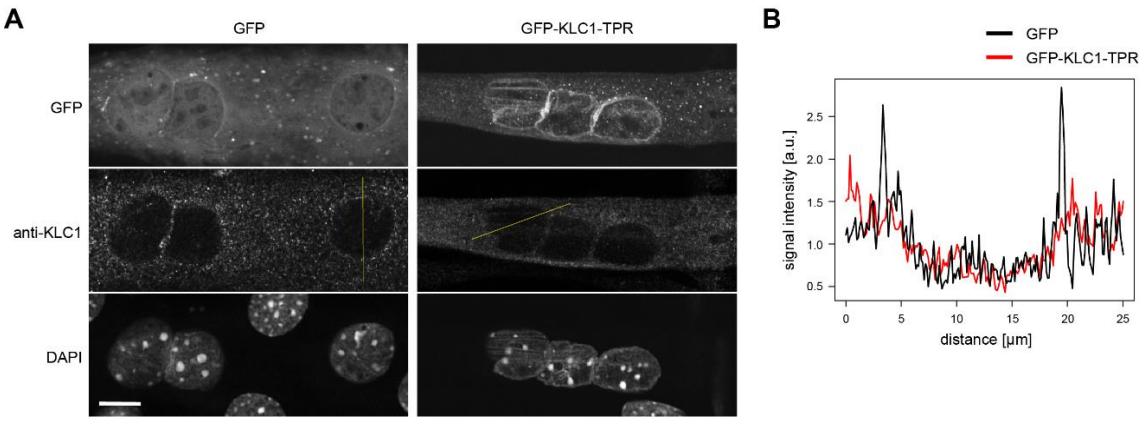


Fig. S5. KLC1-TPR overexpression delocalizes endogenous KLC1 from the nuclear envelope (A) Representative images of differentiated C2C12 cells expressing control GFP (left) or GFP-KLC1-TPR (right). Endogenous KLC1 was stained with an anti-KLC1 antibody that does not recognize GFP-KLC1-TPR. Scale bar: 10 µm. (B) The signal intensity of KLC1 immunofluorescence along the diameter of the nucleus (yellow lines in A). Signal peaks were observed on the nuclear envelope in GFP-expressing cells, while it disappeared in the cells expressing GFP-KLC1-TPR.

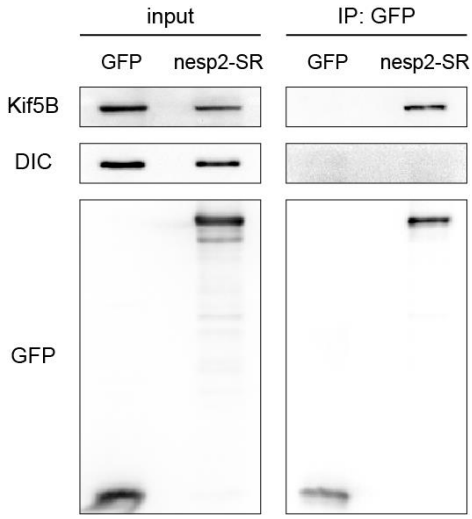


Fig. S6. GFP-nesp2-SR interacts with kinesin but not with dynein. Co-immunoprecipitation of HEK293T cells expressing GFP or GFP-nesp2-SR with anti-GFP. GFP-nesp2-SR precipitated Kif5B but not DIC.

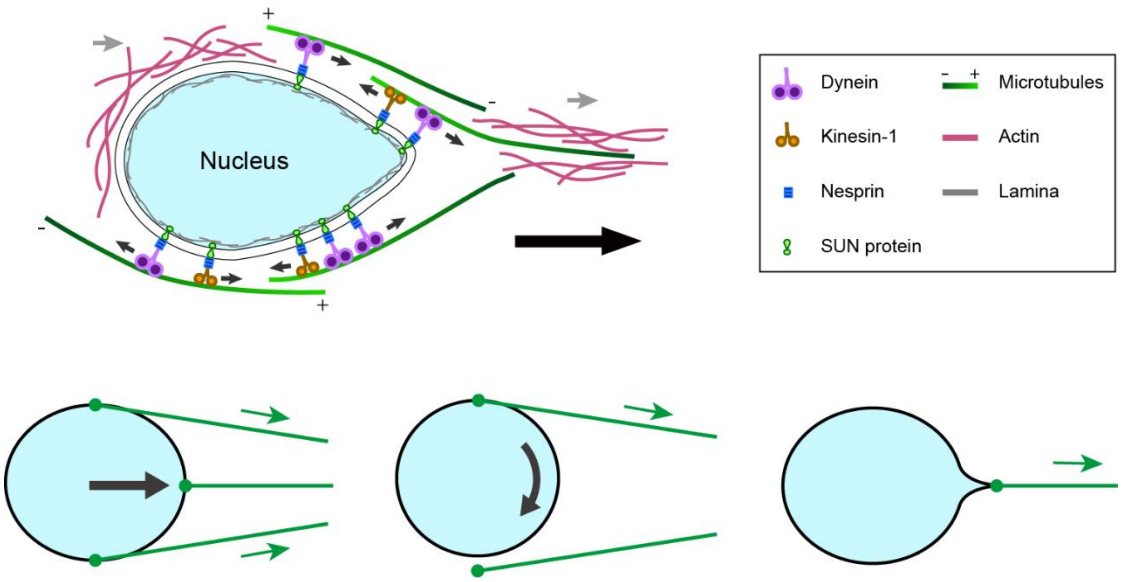
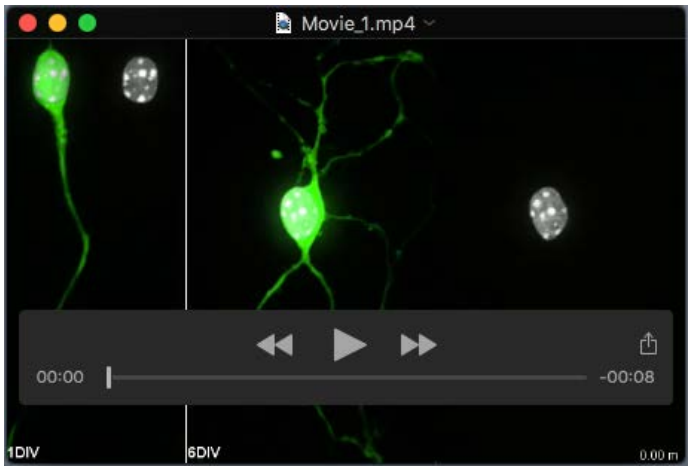
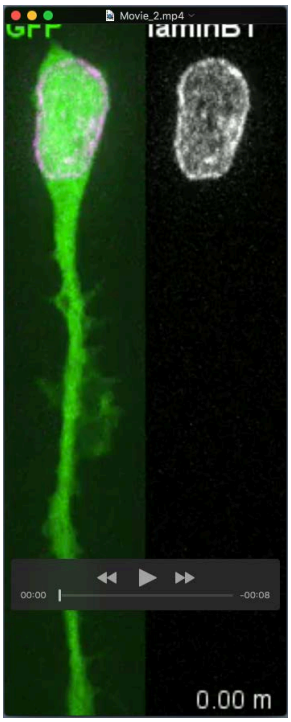


Fig. S7. Model of cytoskeletal control of nuclear dynamics in CGCs. The nucleus migrates toward the leading process (*right*). Dynamic arrays of parallel (minus-end to leading process) and fewer antiparallel (minus-end to trailing process) microtubules cover the nuclear envelope. Dynein and kinesin interact with nesprins at the nuclear envelope and generate a point force toward both anterior and posterior directions, which may drive frequent switches of rotation direction. Dynein along the parallel microtubules may serve as the predominant motor and cause net displacement toward the leading process. Actomyosin-based force may function independently of nesprins during nuclear translocation. Nuclear translocation, rotation, and deformation are induced depending on the positions of microtubule-dependent force.

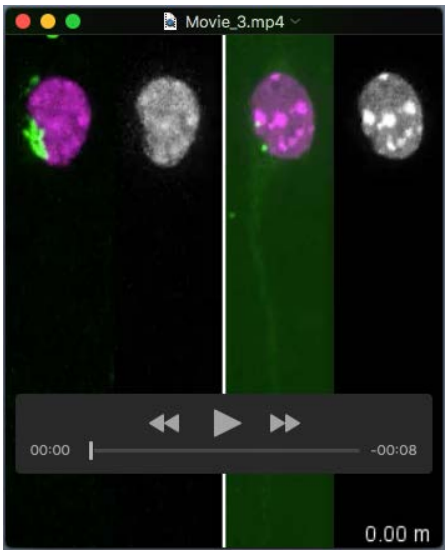
Supplementary Movies



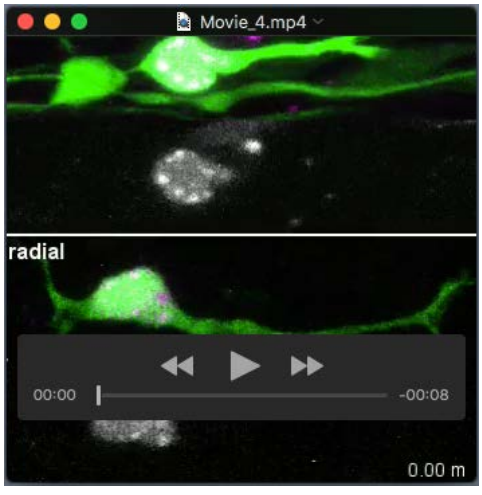
Movie 1. Nuclear dynamics of a migrating and a post-migratory CGC in vitro. Time-lapse movies of a CGC expressing GFP (green) and HP1β-mCherry (magenta) at 1 DIV (*left*) or 6 DIV (*right*). Images were acquired every 15 sec for 1 hr.



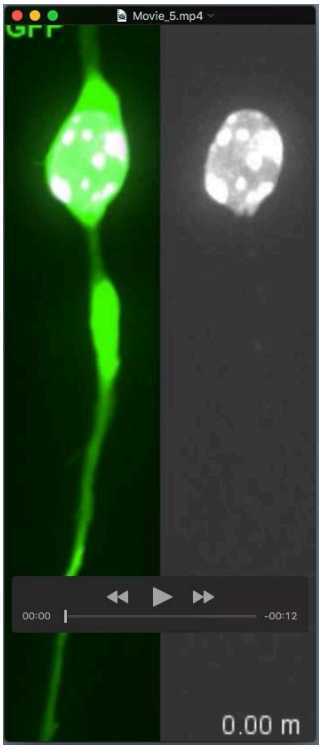
Movie 2. Dynamics of the nuclear envelope labeled with laminB1 in a migrating CGC in vitro. Time-lapse movie of a CGC expressing GFP (green) and mCherry-laminB1 (magenta) in vitro. Images were acquired every 15 sec for 1 hr.



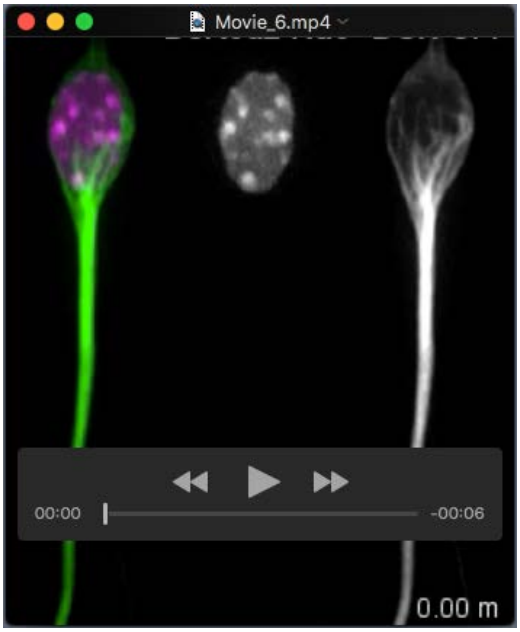
Movie 3. Dyanimcs of the Golgi apparatus and centrosome in migrating CGCs in vitro. Time-lapse movies of CGCs expressing HP1β-mCherry (magenta) together with AcGFP-Golgi (green; *left*) or Cetn2-GFP (green; *right*) in vitro. Images were acquired every 15 sec for 1 hr.



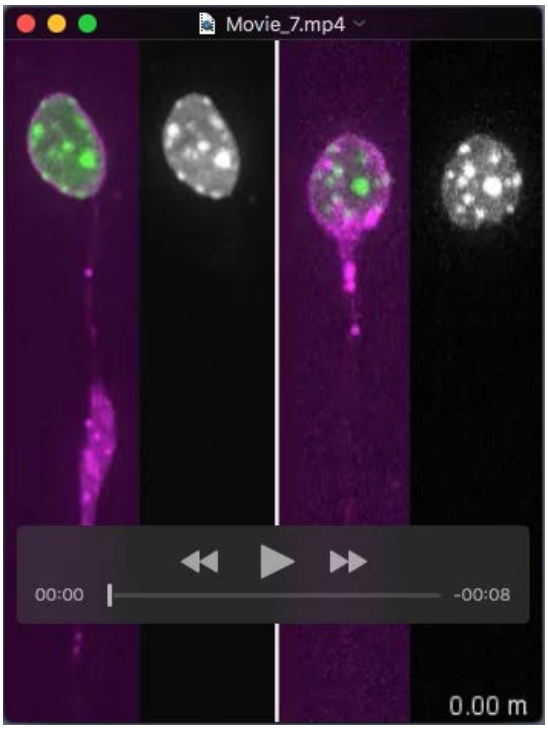
Movie 4. Nuclear dynamics of migrating CGCs in an organotypic slice. Time-lapse movies of tangential (*upper*) or radial (*lower*) migration of a CGC expressing GFP (green) and HP1β-mCherry (magenta) in an organotypic slice. Images were acquired every 30 sec (tangential) or 15 sec (radial) for 1 hr.



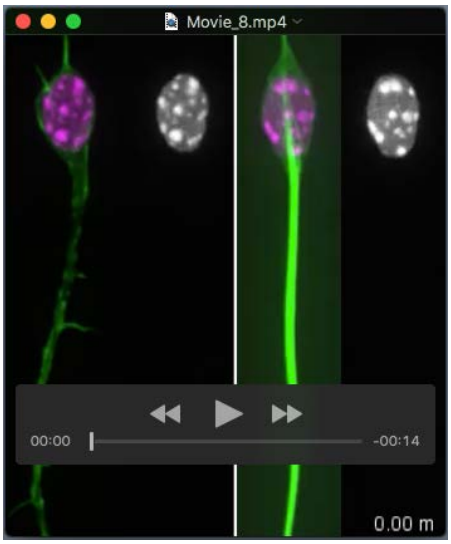
Movie 5. Sharp peaks temporarily formed in the nuclear envelope of a migrating CGC. Time-lapse movie of a CGC expressing GFP (green) and HP1β-mCherry (magenta) in vitro. Images were acquired every 15 sec for 1 hr.



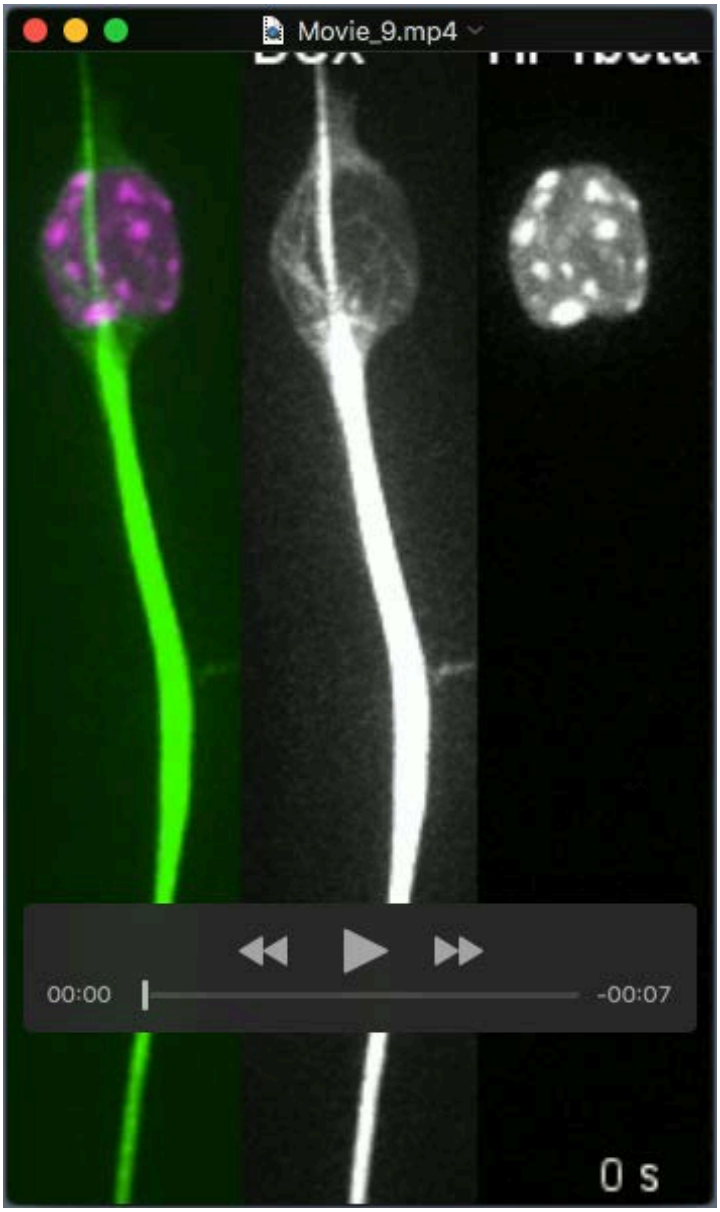
Movie 6. Nuclear shape change during translocation and rotation in a migrating CGC. Time-lapse movie of a CGC expressing DCX-GFP (green) and DsRed2-Nuc (magenta) in vitro. Images were acquired every 15 sec for 1 hr.



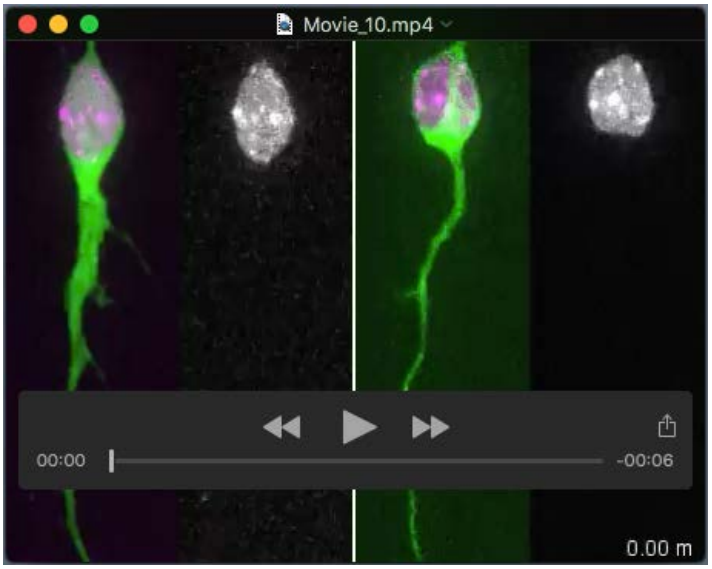
Movie 7. Dominant negative inhibition of KASH domain proteins affects the nuclear dynamics of CGCs. Time-lapse movies of CGCs expressing HP1β-GFP (green) together with mCherry-KASH1 (magenta; *left*) or mCherry-KASH1ext (magenta; *right*) in vitro. Images were acquired every 15 sec for 1 hr.



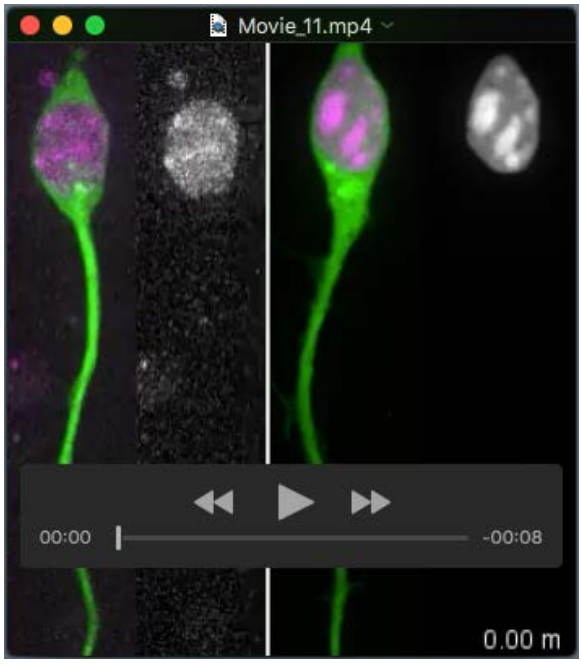
Movie 8. Nuclear dynamics of CGCs treated with drugs. Time-lapse movies of a CGC expressing HP1β-mCherry (magenta) together with LifeAct-2xGFP (green; *left*) or DCX-GFP (green; *right*). The cell was treated with 20 µM cytochalasin B (*left*) or 1 µM nocodazole (*right*). Images were acquired every 15 sec for 40 min (before drug treatment) and 1 hr (after drug treatment).



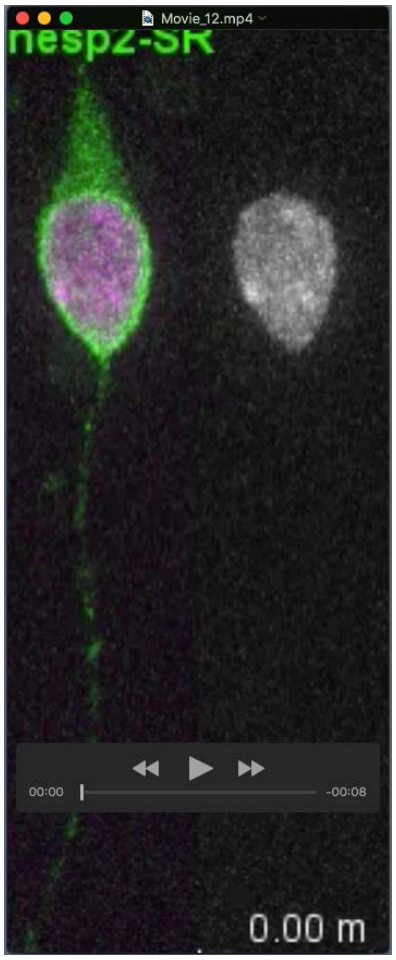
Movie 9. Microtubule dynamics during nuclear rotation. Time-lapse movie of a CGC expressing DCX-GFP (green) and HP1β-mCherry (magenta) in vitro. Images were acquired every 15 sec for 525 sec.



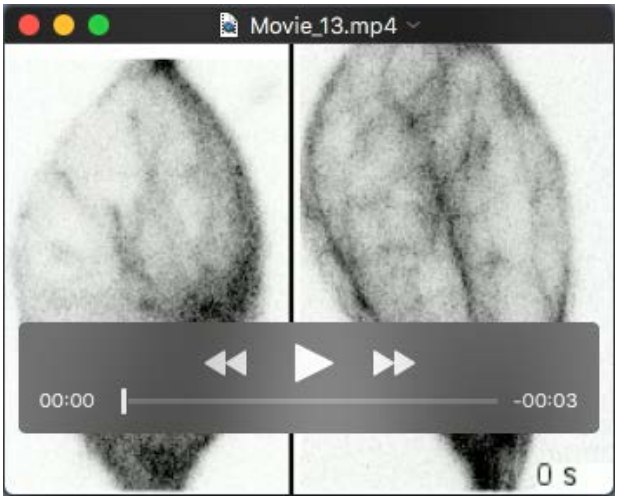
Movie 10. Inhibition of dynein by dominant negative Lis1 or p150^{Glued} affects the nuclear dynamics of CGCs. Time-lapse movies of CGCs expressing HP1β-mCherry (magenta) toghether with GFP-Lis1N (green; *left*) or GFP-p150-CC1 (green; *right*). Images were acquired every 15 sec for 1 hr.



Movie 11. Inhibition of kinesin-1 by dominant negative Kif5 or KLC affects the nuclear dynamics of CGCs. Time-lapse movies of CGCs expressing HP1β-mCherry (magenta) together with GFP-Kif5B-tail (green; *left*) or GFP-KLC1-TPR (green; *right*). Images were acquired every 15 sec for 1 hr.



Movie 12. Nuclear dynamics of a CGC expressing a kinesin-binding nesprin mutant. Time-lapse movie of a CGC expressing GFP-nesp2-SR (green) and HP1β-mCherry (magenta) in vitro. Images were acquired every 15 sec for 1 hr.



Movie 13. Growing microtubules around the nucleus in migrating CGCs. Time-lapse movies of CGCs expressing GFP-EB3 in vitro. Images were acquired every 3 sec for 150 sec.

Supplementary Materials and Methods

Antibodies

Antibodies used for immunostaining and western blotting were as follows: rabbit anti-Kif5B (1:500-1000, ab167429), anti-Lamin B1 (1:500, ab16048), rat anti-α-tubulin (1:1000, ab6160) purchased from Abcam; mouse anti-dynein intermediate chain (1:500-1000, MAB1618), HRP-conjugated anti-rabbit IgG (1:5000, AP182P) or anti-rat IgG (1:10000, AP183P) purchased from Millipore; goat anti-KLC1 (1:200, sc-13361), mouse anti-GFP (1:2000, sc-9996) purchased from Santa Cruz; mouse anti-acetylated tubulin (1:4000, T6793), anti-β-tubulin (1:1000, T4026) from Sigma; rabbit anti-GFP (1:2000, A11122), Alexa488-conjugated anti-mouse IgG (1:400, A21202; 1:400, A11029) or anti-rabbit IgG (1:400; A11034), Alexa568-conjugated anti-mouse IgG (1:400; A11004), anti-rabbit IgG (1:400, A11011) or anti-goat IgG (1:400, A11057), Alexa633-conjugated anti-mouse IgG (1:400, A21052) purchased from Molecular Probes; HRP-conjugated anti-rabbit IgG (1:10000, 170-6515) or anti-mouse IgG (1:10000, 170-6516) purchased from Bio-Rad.

Subcellular fractionation

Cerebella from P4 ICR mice were dissociated. Harvested CGCs were resuspended in a hypotonic buffer (10 mM HEPES pH7.9, 10 mM potassium chloride, 1.5 mM magnesium chloride, 1 mM EDTA, 1 mM DTT, and 1x protease inhibitor cocktail (Thermo Fisher)) on ice for 5 min, homogenized with a Dounce homogenizer, and centrifuged at 400 G for 5 min. The pellet was washed and saved as the nuclear fraction. The supernatant was further centrifuged at 10000 G for 10 min, and the resulting supernatant was saved as the cytosolic fraction. Proteins from each fraction were subjected to SDS-PAGE and western blotting.

Immunostaining of CGCs

Migrating CGCs in reaggregated cultures were fixed with 4% paraformaldehyde (PFA) in PBS and permeabilized with 0.3% Triton X-100 in PBS. For kinesin and dynein staining on the nuclear envelope, CGCs were treated with a hypotonic buffer (10 mM

HEPES pH7.9, 10 mM potassium chloride, 1.5 mM magnesium chloride, 1 mM EDTA, 1 mM DTT) on ice for 5 min before fixation. For tubulin staining with DCX-GFP overexpression, CGCs were fixed with methanol at -20 °C. Cells were washed with PBS, blocked with 2% skim milk, 0.1% Tween 20 in PBS, and subjected for immunostaining. The nuclei were stained with 4'-6-diamidino-2-phenylindole (DAPI)(Life technologies). Images were acquired with a laser-scanning confocal microscope FV1000 (Olympus) through a 100x oil-immersion objective (N.A. 1.4), or with LSM 880 with Airyscan (ZEISS) through a 100x oil-immersion objective (N.A. 1.46).

C2C12 cell culture and immunostaining

C2C12 cells were plated on poly-D-lysine coated coverslips and maintained at 37°C/5% CO_2 in RPMI with 10% fetal bovine serum and penicillin-streptomycin. Plasmid transfection were performed with Lipofectamine 2000. To induce myogenic differentiation, the media was changed to Dulbecco's Modified Eagle Medium with 2% horse serum. Differentiated C2C12 cells were fixed with 4% PFA in PBS and blocked with 2% skim milk, 0.3% Triton X-100 in PBS. Immunostaining was then performed with KLC1 and GFP antibodies, and stained with DAPI. Images were acquired with a laser-scanning confocal microscope FV1000 (Olympus) through a 100x oil-immersion objective (N.A. 1.4).

HEK293T cell culture and co-immunoprecipitation

HEK293T (RCB2202, RIKEN BRC) cells were maintained at 37°C/5% CO_2 in D-MEM with 10% fetal bovine serum and penicillin-streptomycin. GFP constructs transfection were performed with Lipofectamine 2000. Twenty hours post-transfection, cells were harvested in a lysis buffer (50 mM Tris-HCl pH7.4, 75 mM sodium chloride, 2.5 mM magnesium chloride, 1 mM EDTA, 1 mM DTT, 0.1% Triton-X100, and 1x protease inhibitor cocktail (Thermo Fisher)). Lysates were pre-cleared with Protein A/G PLUS Agarose beads (Santa Cruz) for 1 hr at 4°C. Mouse GFP antibody were added to the supernatant and incubated for 2.5 hr at 4°C. The beads were added to the sample and incubated for 1 hr at 4°C. The beads were washed with the lysis buffer for four times and subjected to SDS-PAGE and western blotting.