Supporting Information

Yanagida et al. 10.1073/pnas.1209166109

SI Materials and Methods

In Utero Electroporation. To label GABAergic interneurons, GE-directed in utero electroporation was performed at E12.5, as described with some modifications (1). Five 60-V pulses with a duration of 50 ms were applied at 600-ms intervals by using a forceps-shaped electrode (CUY650P2; NEPAGENE). Examination of electroporated site 1–4 d after electroporation revealed that radially oriented neurons in the cortico-striatal boundary were also labeled in some occasion, in addition to neurons in the GEs. Therefore, for imaging we focused on labeled cells located near the surface of the cortex and oriented tangentially in the dorsal part of the cortex to avoid imaging of excitatory neurons originating from this region. Expression vectors with fluorescent proteins (FPs) or fused FPs and a CAG promoter (pCAGGS; gift from J. Miyazaki, Osaka University, Osaka, Japan) (2) or a modified multicloning site [pCAGGS–MCS(–); subcloned by K. Nishida] were used at the following concentrations: 3.0 μg/μL tandem dimer Tomato (*tdTomato*; gift from R. Y. Tsien, University of California at San Diego, La Jolla; ref. 3) (pCAGGS–*tdTomato*), 3.0 μg/μL enhanced GFP (*EGFP*; Clontech) (pCAGGS–*EGFP*), 1.0 μg/μL *EGFP* with a nuclear localization sequence (nls) (pCAGGS–nls–*EGFP*; subcloned by Y. Tanabe), 3.0 μg/μL *EGFP* with a human galactosyltransferase sequence (GalT; Addgene; ref. 4) (pCAGGS–*GalT-EGFP*; subcloned by Y. Furukawa), 3.0 μg/μL *EGFP* or *tdTomato* with a membrane anchoring signal of the growth-associated protein 43 (GAP-43; subcloned by T. Kobayashi; refs. 5 and 6) (pCAGGS–*GAP-EGFP* or pCAGGS–*GAP-tdTomato*), 0.3 μg/μL monomer Kusabira-Orange (mKO1) (MBL) with pericentrin/AKAP 450 centrosomal targeting domain (PACT) (pCAGGS–PACT–*mKO1*; gift from F. Matsuzaki, RIKEN CDB, Kobe, Japan), 0.8 μg/μL monomer cherry (mCherry; gift from R. Y. Tsien, University of California at San Diego, La Jolla, CA ref. 3) (pCAGGS–*mCherry*), and 0.6 μg/μL mCherry with histone H2B (*H2B-mCherry*).

In Vivo Time-Lapse Imaging. The dam was anesthetized with urethane (1.5–2.0 g/kg) and placed on a metal plate. An incision was made in the abdomen of a pregnant mouse at E16.5, one uterine horn was partially exposed and embryos were removed from the uterus such that the umbilical cord remained connected to the dam.

Imaging at low magnification. An embryo was placed in a small container filled with agarose (01163-05; Nacalai Tesque) solution in PBS. After gelling of the agarose, the head of the embryo was exposed, and the scalp was carefully removed. A lump of agarose gel in which the embryo was embedded was dissected and glued by using an instant adhesive to a metal bar that was fixed to a magnetic stand on the metal plate. The metal plate was set on the stage of a confocal microscope (MRC-1024; Bio-Rad) housed in a temperature-controlled chamber. Images were obtained by using a 10×/0.30-N.A. dry objective lens (Olympus) at 30-min intervals at an excitation wavelength of 568 nm and an emission long-pass filter of 585 nm. The temperature at the surface of the head of the embryo was monitored every 30 min by using a noncontact temperature sensor. The color of the surface of the head was also monitored occasionally, to assure its health. Blood-flow through the vein of the brain of the embryo was observed under a dissecting microscope, both before and after the imaging to ensure the health of the brain.

Imaging at high magnification. The embryo was placed in a small stage made of agarose, which was then placed in a small plastic cylinder-shaped container (Fig. S1). The embryo was immobilized with gallamine triethiodide (0.1 mg/g, i.p.). To adjust the position of the embryo head, a small amount of superabsorbent polymer was mounted on embryos after addition of PBS. After filling the container with agarose solution, the head was exposed and the scalp was carefully removed. The anesthetized dam and embryo, which were placed together on a metal plate, were set on the stage of a two-photon microscope (TCS-SP5 MP; Leica Microsystems) housed in a temperature-controlled chamber. A Ti-sapphire pulsed laser (MaiTai; Spectra-Physics) was tuned to 940 nm to excite EGFP and tdTomato simultaneously. Images were acquired by using a 20×/1.0-N.A. water-immersion objective lens (HCX APO 20× 1.0 W; Leica Microsystems) at 10- or 8-min intervals by using 525/50 nm (for EGFP) and 585/40 nm (for tdTomato) emission filters. Scattered EGFP fluorescence or autofluorescence caused by illumination with a mercury lamp allowed us to monitor the flow of red blood cells as shadow spots. Blood-flow was monitored every hour, as was the temperature of the objective lens tip by a noncontact temperature sensor. The parietal cortex was chosen for both low- and high-magnification imaging.

Dissociated Culture. GE explants electroporated with *GAP-EGFP* and *GAP-tdTomato* at E12.5 were prepared from E14.5 embryos. Cells were dissociated by pipetting in Neurobasal medium with 2% (vol/vol) B27 supplement (all from Invitrogen) and were cultured on polylysine-coated coverslips in 24-well plates for 1 d.

Immunohistochemistry. Cultured dissociated cells were fixed in 4% paraformaldehyde (PFA) in phosphate buffer (PB) (0.1 M, pH 7.4) for 15 min. Cells were treated with 0.1% Triton X-100 in PBS (0.1 M, pH 7.4; PBS-T) containing 5% (vol/vol) normal goat serum (NGS) for 1 h. Subsequently, cells were incubated with a mouse monoclonal anti-GM130 (1:400; BD Bioscience) antibody diluted in PBS-T with 5% (vol/vol) NGS for 1 d at 4 °C, followed by incubation with goat Alexa Fluor 633 anti-rabbit IgG (1:200; Invitrogen) overnight at 4 °C. Images were captured by using a confocal microscope (FV1000; Olympus).

Localization of the Centrosome and Golgi Apparatus. To examine the localization of the centrosome and Golgi apparatus, GE-directed in utero electroporation of PACT–*mKO1*, *GalT-EGFP*, and *mCherry* or *H2B-mCherry* was performed at E12.5. Four days later (E16.5), embryos were perfused with 4% (wt/vol) PFA in PB and postfixed for 2 h at room temperature (RT). Subsequently, their brains were immersed in 30% (wt/vol) sucrose in PB overnight and embedded in optimal cutting temperature compound (Tissue Tek; Sakura Finetek). Sections were cut at 20–30 μm by using a cryostat (MICROM HM500M; Carl Zeiss). Images were captured by using a confocal microscope (TCS SP2 AOBS or TCS SP5; Leica Microsystems).

To confirm the localization of the Golgi apparatus, the surface of the neocortex was exposed, and images were captured by using a two-photon microscope (TCS SP5 MP; Leica Microsystems) with a 20×/1.0-N.A. water-immersion objective lens (HCX APO 20 × 1.0 W; Leica Microsystems).

Image Processing. Images were processed with minor contrast and brightness adjustments by using Adobe Photoshop (Version 7.0; Adobe) or Adobe Photoshop CS4 (Version 11.0; Adobe), and movies were assembled by using ImageReady (Version 7.0; Adobe) or MetaMorph (Version 6.1; Molecular Devices). Projection images were created by stacking 10–20 confocal images taken at 10-μm intervals along the *z* axis every 30 min by using a dry objective lens. For high-magnification imaging, Z stacks were created from 10–20 images taken at 2 or 3 μm with intervals

of 8 or 10 min. Images that were substantially distorted because of embryo movement were excluded from the analysis. However, those with only minor distortions, also possibly caused by embryo movement, were corrected by focusing on a few cells, reference cells, in the imaging field. We corrected the position and orientation of movie frames by carefully comparing the position and orientation of these cells between neighboring frames. The detection of cell movement in directions that deflected from the direction of their LPs led us to conclude that the apparent movement of these cells was not genuine but occurred as a consequence of a drift (namely due to embryo movement). Therefore, we adjusted such movements by shifting the image frames so that the cells should move in the direction of their LPs.

Quantitative analysis. ***Trajectories, direction, and rate of migration.*** Analyses were performed by using four embryos from which stable recordings were obtained up to 8.5, 9.5, 10, or 13 h. To analyze the trajectories of migrating neurons, the center of the cell body, which was defined as the midpoint of the minor axis of neurons, was tracked by using a confocal microscope with a 10×/0.30-N.A. dry objective lens. Some cells occasionally shifted parallel to the direction of their LPs. Such cells were excluded from the analysis. After the end of imaging, we monitored the blood flow, and when it was significantly reduced, data corresponding to the last 2 h of recording were discarded. In addition, samples in which an ensemble of neurons observed in an imaging field exhibited a 2-h rate of migration that was less than the minimum migration rate during the initial 6 h of recording were deemed as deteriorated, and their corresponding data during this out-of-range period and those recorded thereafter were discarded. The average rate of migration of the neuronal ensemble was quantified by using migrating neurons tracked for initial 3 h of the onset their imaging.

To analyze the direction of migration, interneurons migrating at an average rate of >6 μm/h were used. A line was drawn that connected the position of a cell at the outset of the imaging with its position 2 h later. The direction was categorized into 12 sectors on a horizontal plane. To analyze its nuclear movement, the centroid of an interneuron's nls–EGFP signals was tracked.

Localization of the Golgi apparatus in fixed preparations. To quantify the distribution of the Golgi apparatus in fixed neurons, the soma was divided into 2-μm-wide bins set perpendicular to the line drawn from the base of the LP to the posterior edge of the cell. The position of the Golgi apparatus was determined as the point with the highest GalT–EGFP fluorescence. The resulting points were categorized into three equal-sized subregions of the soma, "front," "middle," and "rear" (Fig. S3), and the LP. To determine the position of the Golgi apparatus in the LP, the distance between the position of the highest GalT–EGFP fluorescence intensity in the LP and the front edge of the soma was measured.

Changes in the localization of the Golgi apparatus during nucleokinesis. To quantify the relationship between translocation of the soma and that of the Golgi apparatus, the displacement of the soma and Golgi apparatus was analyzed in two successive frames of movies. A circle was inscribed in the contour of the soma, and the center of the circle was defined as the center of the soma. A line was drawn that passed through two centers of somata of two successive movie frames. The position of the Golgi apparatus was defined as the point with the highest integrated EGFP fluorescence intensity along the axis of the line. The distance between the center of the soma and the position of the Golgi apparatus was calculated in each pair of successive frames. Because the shape of the soma changes during migration, the localization of the Golgi apparatus within the soma was determined by referring to the average size of the soma, which was determined by analyzing the major diameter of nls–GFP-labeled nuclei of neurons undergoing a displacement of >3 μm. Neurons showing a deflection from the direction of their LP were eliminated from the analysis. All quantifications were performed with the aid of MetaMorph (Version 6.1 or 7.7).

1. Tanaka DH, et al. (2009) Random walk behavior of migrating cortical interneurons in the marginal zone: Time-lapse analysis in flat-mount cortex. *J Neurosci* 29:1300–1311.
2. Niwa H, Yamamura K, Miyazaki J (1991) Efficient selection for high-expression transfectants with a novel eukaryotic vector. *Gene* 108:193–199.
3. Shaner NC, et al. (2004) Improved monomeric red, orange and yellow fluorescent proteins derived from Discosoma sp. red fluorescent protein. *Nat Biotechnol* 22: 1567–1572.
4. Cole NB, et al. (1996) Diffusional mobility of Golgi proteins in membranes of living cells. *Science* 273:797–801.
5. Liu Y, Fisher DA, Storm DR (1994) Intracellular sorting of neuromodulin (GAP-43) mutants modified in the membrane targeting domain. *J Neurosci* 14:5807–5817.
6. Moriyoshi K, Richards LJ, Akazawa C, O'Leary DDM, Nakanishi S (1996) Labeling neural cells using adenoviral gene transfer of membrane-targeted GFP. *Neuron* 16:255–260.

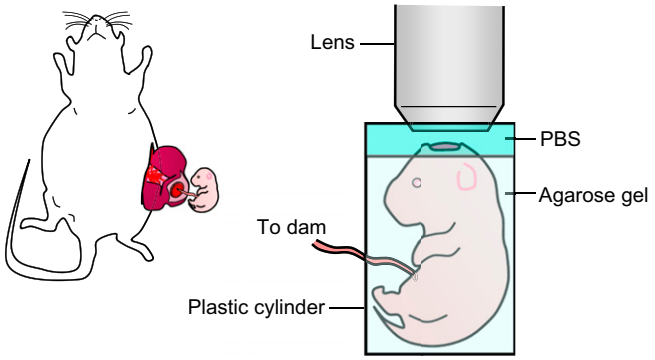


Fig. S1. In vivo imaging of mouse embryos. Schematic illustration of mouse embryo in vivo imaging is shown. The dam was anesthetized, and an incision was made in its abdomen to partially expose one uterine horn and to remove embryos from the uterus while keeping the umbilical cord attached to the dam. For immobilization, an embryo was placed in a small plastic container filled with agarose solution. Migrating neurons in the cortex was observed through the skull.

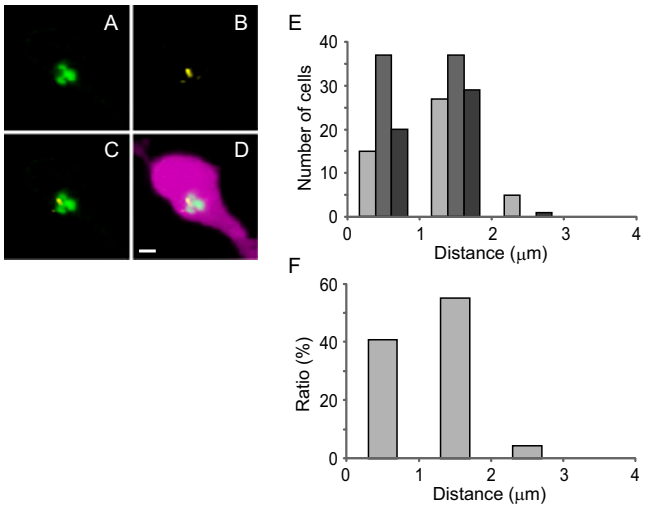


Fig. S2. Close association of the centrosome with the Golgi apparatus. (*A*) GalT–EGFP fluorescence showing localization of the Golgi apparatus in an interneuron. (*B*) PACT–mKO1 fluorescence showing localization of the centrosome in an interneuron. (*C*) Merged view of *A* and *B*. (*D*) mCherry fluorescence of the neuron shown in *A*–*C*, showing the morphology of the neuron. (*E* and *F*) The shortest distance between the Golgi apparatus and the centrosome. Most Golgi apparatuses were localized within a distance of 2 μm from the centrosome. Constructs carrying the centrosome-targeted mKO1 fusion protein (PACT–*mKO1*), *GalT-EGFP*, and *mCherry* (or *H2B-mCherry*) were coelectroporated into the MGE of E12.5 embryos, and cortical sections were prepared 4 d later. The three different color bars in *E* show the number of cells from each brain (*n* = 171 cells, 3 brains). (Scale bar, 2 μm.)

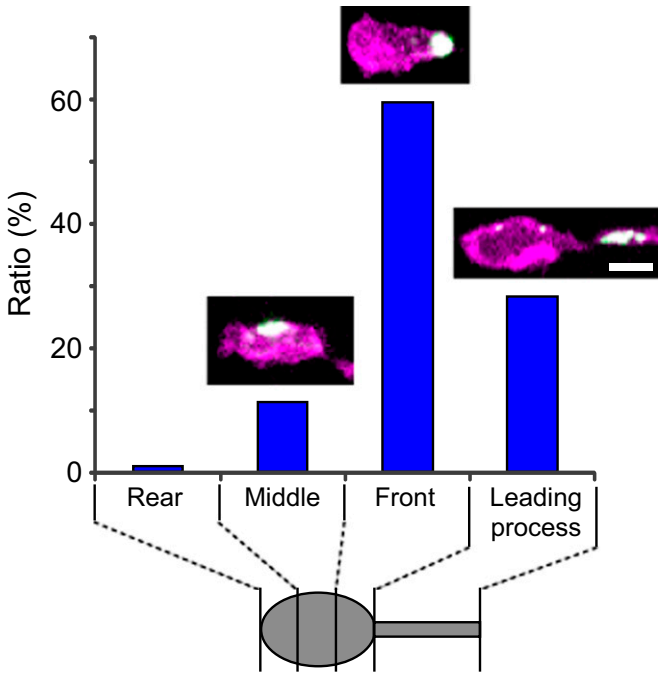
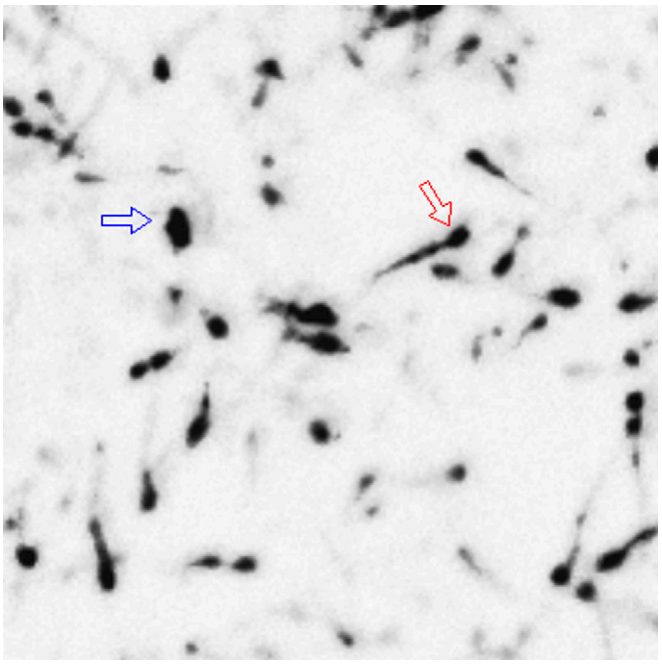
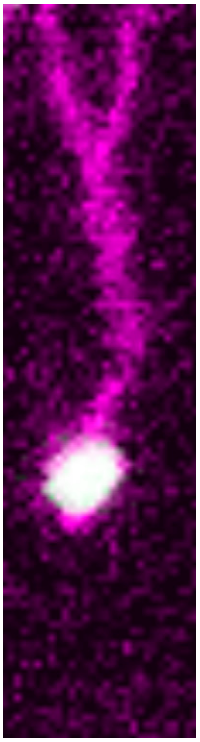


Fig. S3. Localization of the Golgi apparatus in interneurons. Localization of the Golgi apparatus was analyzed in neurons of E16.5 embryo fixed brains. The cellular profile of these interneurons was divided into four domains, as illustrated at the bottom of the figure (see *SI Materials and Methods* for details), and the proportion of the Golgi apparatus that localized in each domain was plotted (*n* = 4 brains, 206 cells). Photos show representative examples of the localization of the Golgi apparatus (white). Neurons were labeled by electroporation of *GalT-EGFP* (green) and *GAP-tdTomato* (magenta). (Scale bar, 10 μm.)



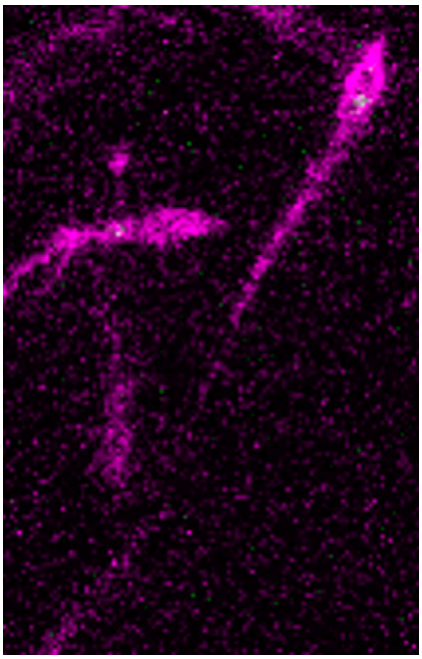
Movie S1. Migration of cortical interneurons in the MZ of an E16.5 living mouse embryo. The interneurons were labeled by electroporation of *tdTomato* and observed by using a confocal microscope. The interneurons migrated in all directions. The open red arrow points to the cell shown in Fig. 1*A*. The open blue arrow points to the cell exhibiting stationary behavior. The movie was acquired at 30-min intervals for 13 h.

Movie S1



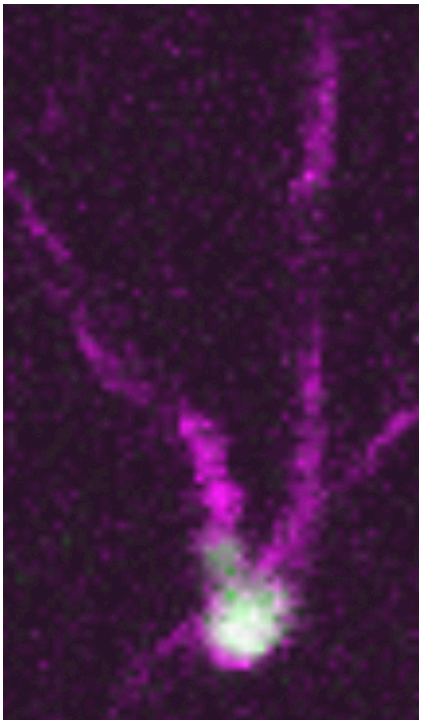
Movie S2. Nucleokinesis in a migrating interneuron. The nucleus was labeled by electroporation of nls–*EGFP* (green) and *GAP-tdTomato* (magenta). The movie was acquired at 10-min intervals for 2.8 h.

Movie S2



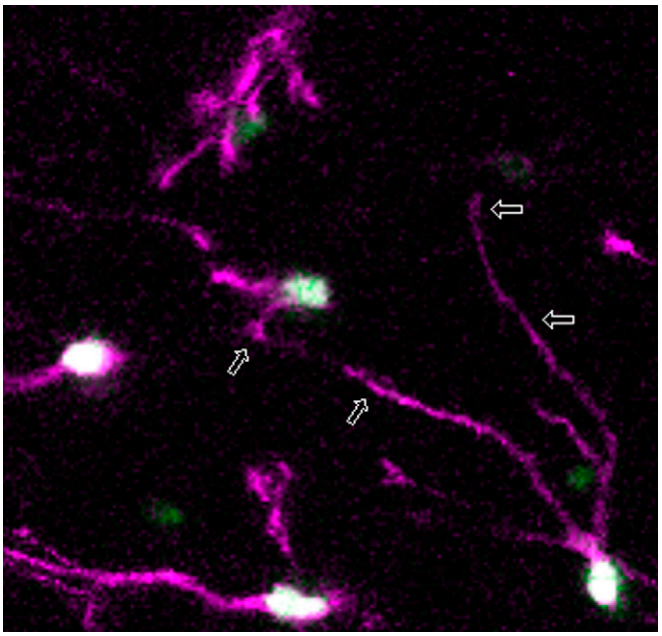
Movie S3. Dynamics of the Golgi apparatus in a migrating interneuron. The neuron was labeled by electroporation of *GalT-EGFP* (green) and *GAP-tdTomato* (magenta). The Golgi apparatus moved in parallel with the soma and was largely localized to the anterior part of the soma throughout the period of recording. The movie was acquired at 8-min intervals for 3.1 h.

Movie S3



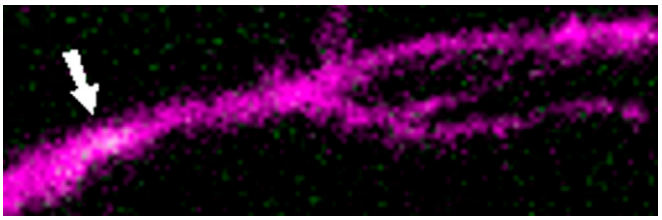
Movie S4. Deformation of nucleus showing a straddling behavior at the branching point. The neuron was labeled by electroporation of nls–*EGFP* (green) and *GAP-tdTomato* (magenta). The movie was acquired at 10-min intervals for 5 h.

Movie S4



Movie S5. Nucleokinesis at the branching point of the LP. When the nucleus reached the branching point of the LP, it moved into one of the branches, followed by retraction of the other. The neuron in focus is pointed to by arrows. The neuron was labeled by electroporation of nls–*EGFP* (green) and *GAP-tdTomato* (magenta). The movie was acquired at 10-min intervals for 3.8 h.

Movie S5



Movie S6. Dynamics of the Golgi apparatus when neurons reached the branching point of the LP. The neuron was labeled by electroporation of *GalT-EGFP* (green) and *GAP-tdTomato* (magenta). The arrow points to the Golgi apparatus. The movie was acquired at 8-min intervals for 4.5 h.

Movie S6