Appendix

Immunofluorescence

Cells were grown on 10mm glass coverslips and fixed in 3.7% formaldehyde/0.5% Triton X-100 in PBS for 20 min. All primary antibodies were incubated at 4°C overnight and secondary antibodies were incubated for 2 hrs at room temperature. DAPI was added to all samples before mounting using Vectashield mounting fluid (Vector Laboratories). Images were acquired on a DeltaVision microscope (Deltavision Elite; Applied Precision), taking 200-nm z-stacks using a PlanApo N 60×/NA 1.42 objective (Olympus) and a high resolution CCD camera (Coolsnap HQ2; Photometrics). Images were deconvolved using Softworx (Applied Precision). Figures were generated by maximum intensity projection of entire cells using Softworx and ImageJ (National Institutes of Health). Brightness and contrast were adjusted with Photoshop 6.0 (Adobe).

Live cell microscopy

For cortical dynein localization, HeLa cells stably expressing GFP-DHC were plated in glass bottom dishes (Labtek) in Leibovitz L15 CO^2-independent medium (Gibco). Images were acquired on a Zeiss LSM510 META confocal microscope (Carl Zeiss) with a Plan Apochromat 63x 1.4NA objective with 1 µm z-stacks. Images were processed using ImageJ software. For GFP-LGN, mCherry-ARP1 and H2B-RFP localization experiments, cells were filmed on a DeltaVision microscope (Deltavision Elite; Applied Precision), in a permanently heated chamber. Cells were filmed in Leibovitz L15 CO^2-independent medium (Gibco). Images were acquired every 5 or 8 minutes using a 60x/NA1.42 oil objective (Olympus) and a high resolution CCD camera (Coolsnap HQ2; Photometrics). Z-stacks were acquired with 2.5 µm intervals. Images were processed using ImageJ software. For GFP-LGN localization experiment in the presence of Hoechst33342, images were taken within two hours of drug addition. Z-stacks were acquired with 1.0 µm intervals. Images were deconvolved using SoftWoRx (Applied Precision). Figures were generated by maximum intensity projection of 10 µm stacks. Graphs were generated with GraphPad Prism.

Micropatterning on glass

Adhesive fibronectin micropatterns on 42mm glass coverslips were produced using deep-ultraviolet illumination through a photomask according to a previously described protocol [1]. The photomask was custom-made (Delta Mask, the Netherlands), and was printed with rectangular shapes with a dimension of 20x80µm. Live cell imaging of micropatterned cells was described in full previously [2].

1. Azioune A, Storch M, Bornens M, Théry M, Piel M (2009) Simple and rapid process for single cell micro-patterning. *Lab Chip* **9**: 1640.
2. Tame MA, Raaijmakers JA, van den Broek B, Lindqvist A, Jalink K, Medema RH (2014) Astral microtubules control redistribution of dynein at the cell cortex to facilitate spindle positioning. *cc* **13**: 1162–1170.