

Neuronal migration requires nuclear deformability controlled by Lamin B1 during cortical development

Minkyung Shin¹, Shunichi Ishida², Jinseon Yu^{3,4}, Misato Iwashita¹, Gyeong-un Jang¹, Giulia Ramazzotti⁵, Stefano Ratti⁵, Jong-Cheol Rah^{3,4}, Yohsuke Imai², Yoichi Kosodo^{1,4}

¹Neural Regeneration Lab, Korea Brain Research Institute (KBRI), Daegu, Republic of Korea ²Graduate School of Engineering, Kobe University, Kobe, Japan ³Neurophysiology Lab, Korea Brain Research Institute (KBRI), Daegu, Republic of Korea ⁴Brain sciences, Daegu Gyeongbuk Institute of Science & Technology (DGIST), Daegu, Republic of Korea ⁵Cellular Signalling Laboratory, Anatomy Centre, Department of Biomedical and Neuromotor Sciences (DIBINEM), University of Bologna, Bologna, Italy

SUMMARY

Radial neuronal migration in the developing cortex emerges from a precise interplay between cell-intrinsic programs and the surrounding tissue. Unlike cancer or immune cells, migrating cortical neurons operate under four defining constraints: their origin and destination are predetermined; movement must conclude within a narrow developmental window; a multipolar-to-bipolar transition enables engagement with radial glial fibers; and locomotion proceeds through exceptionally tight interstitial spaces formed by ECM, axons, and neighboring cells.

As in other cell types, nuclear translocation is pivotal because the nucleus is the largest and stiffest organelle. Newborn neurons express minimal Lamin A, the canonical stiffness regulator, but do express Lamin B isoforms, especially LB1, prompting the hypothesis that **LB1 tunes nuclear mechanics and thereby limits nucleokinesis *in vivo***.

We focused on the cortical plate (CP), where earlier-born neurons create physical barriers for later migrants. Three-dimensional mapping at E18.5 revealed that the mean distance to each nucleus's three nearest neighbors (~7.27 μm) is smaller than the mean nuclear diameter (~8.32 μm), indicating that neurons must deform their nuclei to traverse subnuclear-sized gaps.

To test causality, we mosaically increased LB1 *in vivo*. LB1 overexpression delayed migration and led to neuronal arrest beneath or below layer 2/3, consistent with progressively tighter spacing toward the upper CP. At P0, LB1-enriched nuclei were more spherical, and AFM confirmed increased nuclear stiffness. A computational model varying nuclear-membrane stiffness showed that higher stiffness nearly abolishes nuclear deformation and results in migration failure. The model further predicted that nuclear elongation peaks immediately before a maximal rise in cell velocity and then relaxes. *In vivo* time-lapse imaging reproduced this sequence, although relaxation persisted, likely because dense packing in the CP impedes full recoil, highlighting a departure from "nuclear pistoning" described for cancer cells in microchannels.

Collectively, substantial nuclear deformation is required for efficient radial migration through the CP's constrictions. Excess LB1 stiffens the nucleus, diminishes deformation, and hinders movement, identifying LB1-dependent nuclear mechanics as a principal rate-limiting factor in the spatially confined *in vivo* cortex.

Fig. 3. A simulation model of neuronal migration to analyze the effects of varied nuclear stiffness.

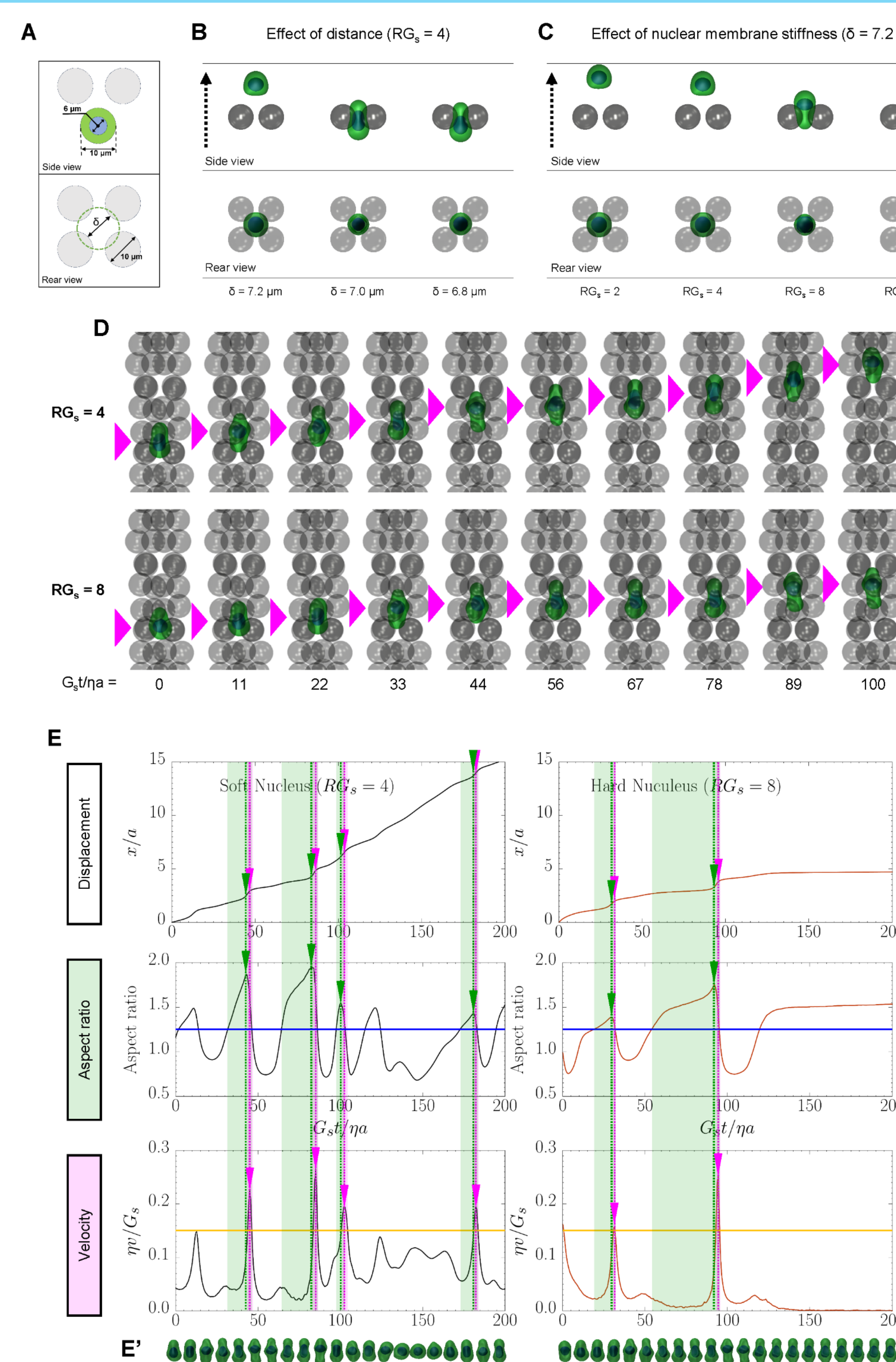


Fig. 5. Increased stiffness due to LB1-OE impairs cell migration through narrow physical constraints.

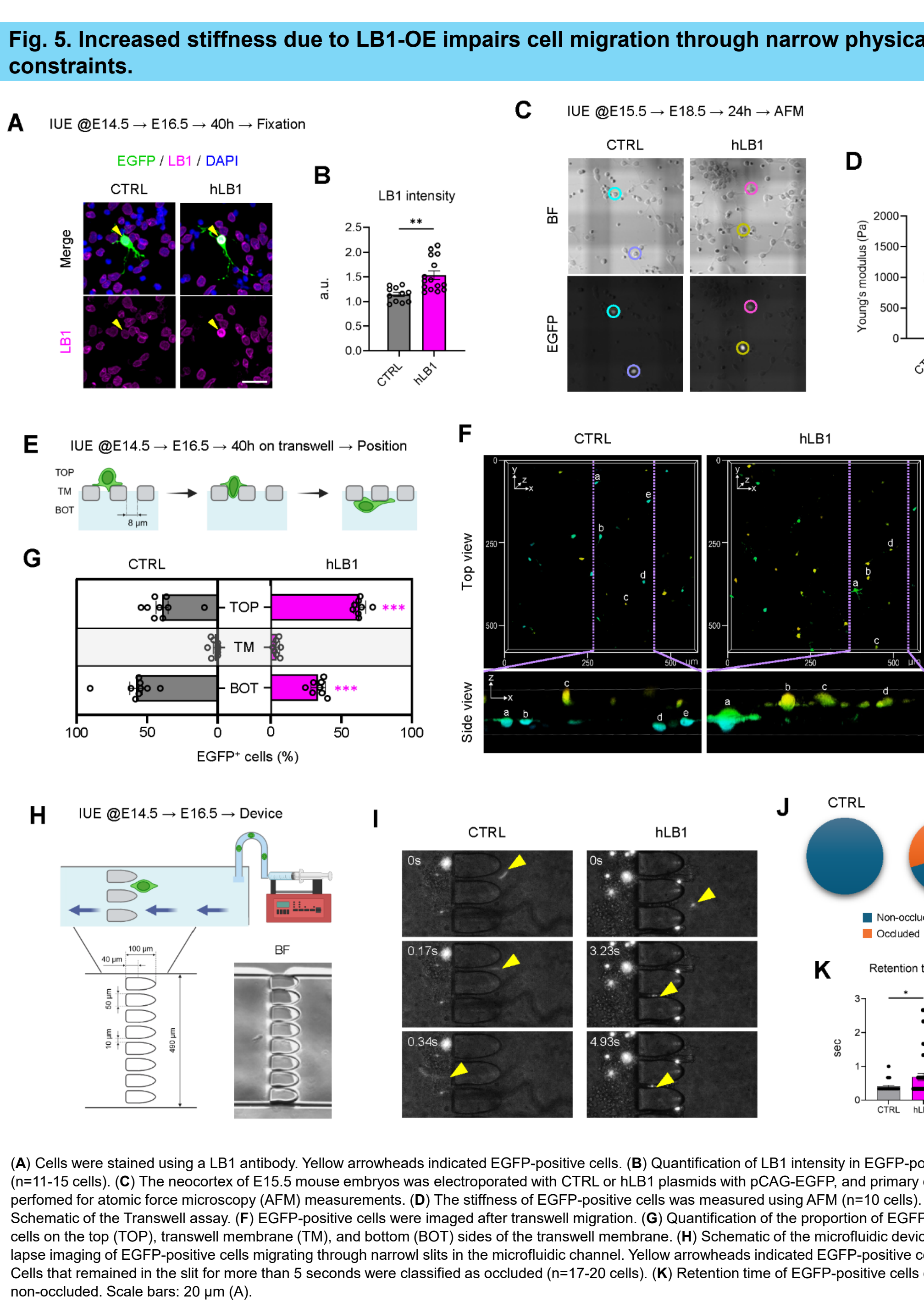
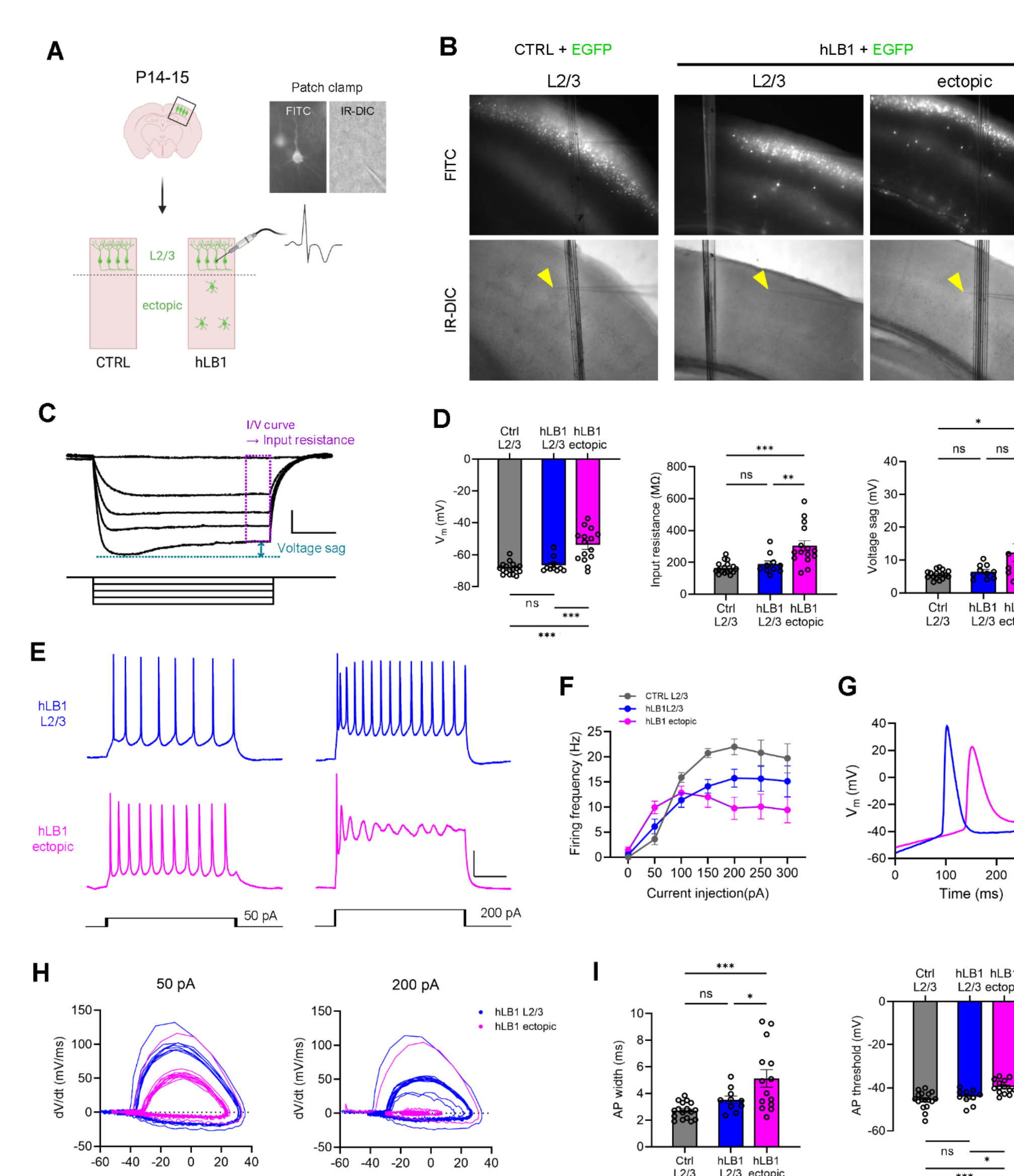
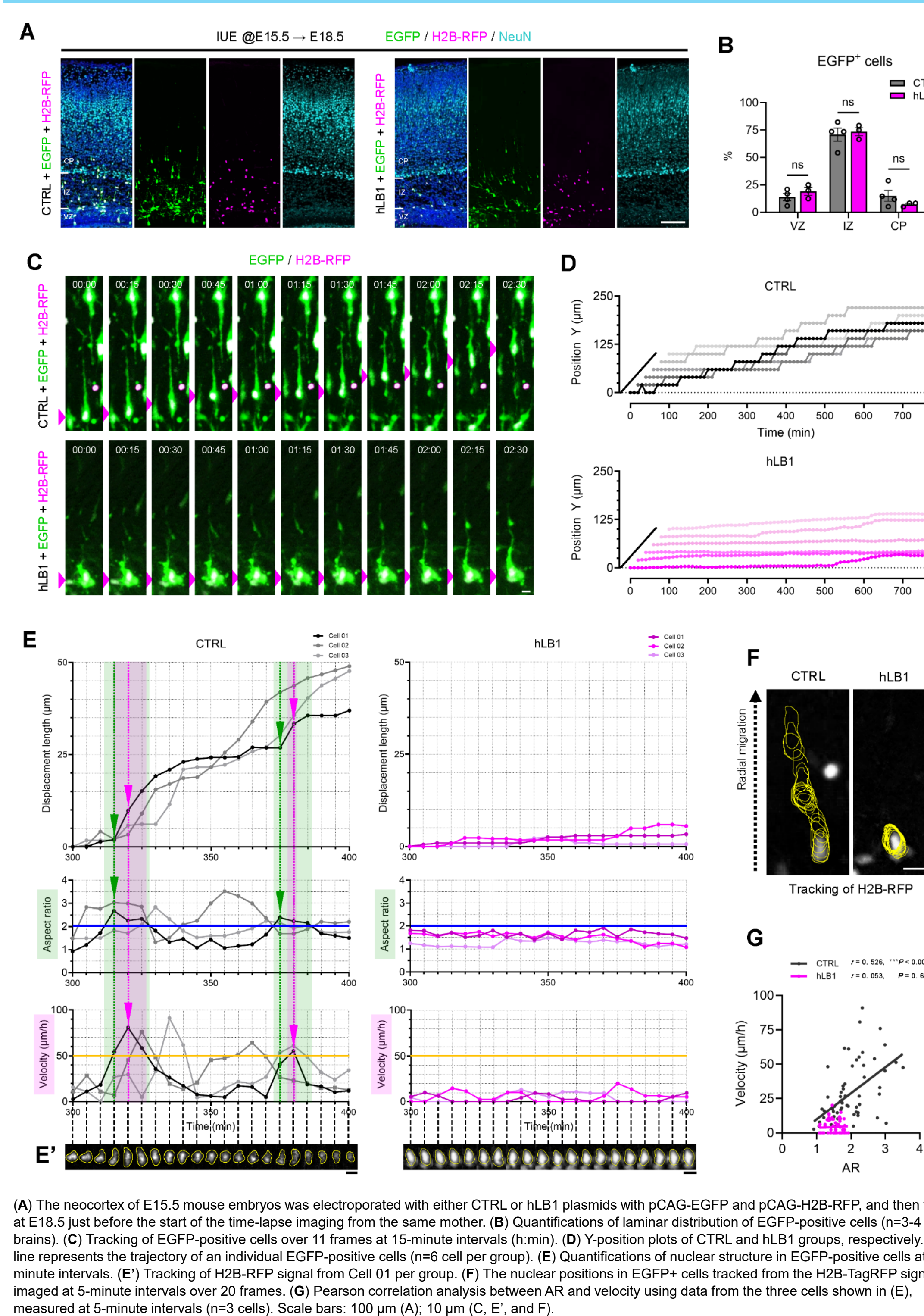


Fig. 6. Ectopic positioning of hLB1-OE neurons alters intrinsic electrophysiological properties.



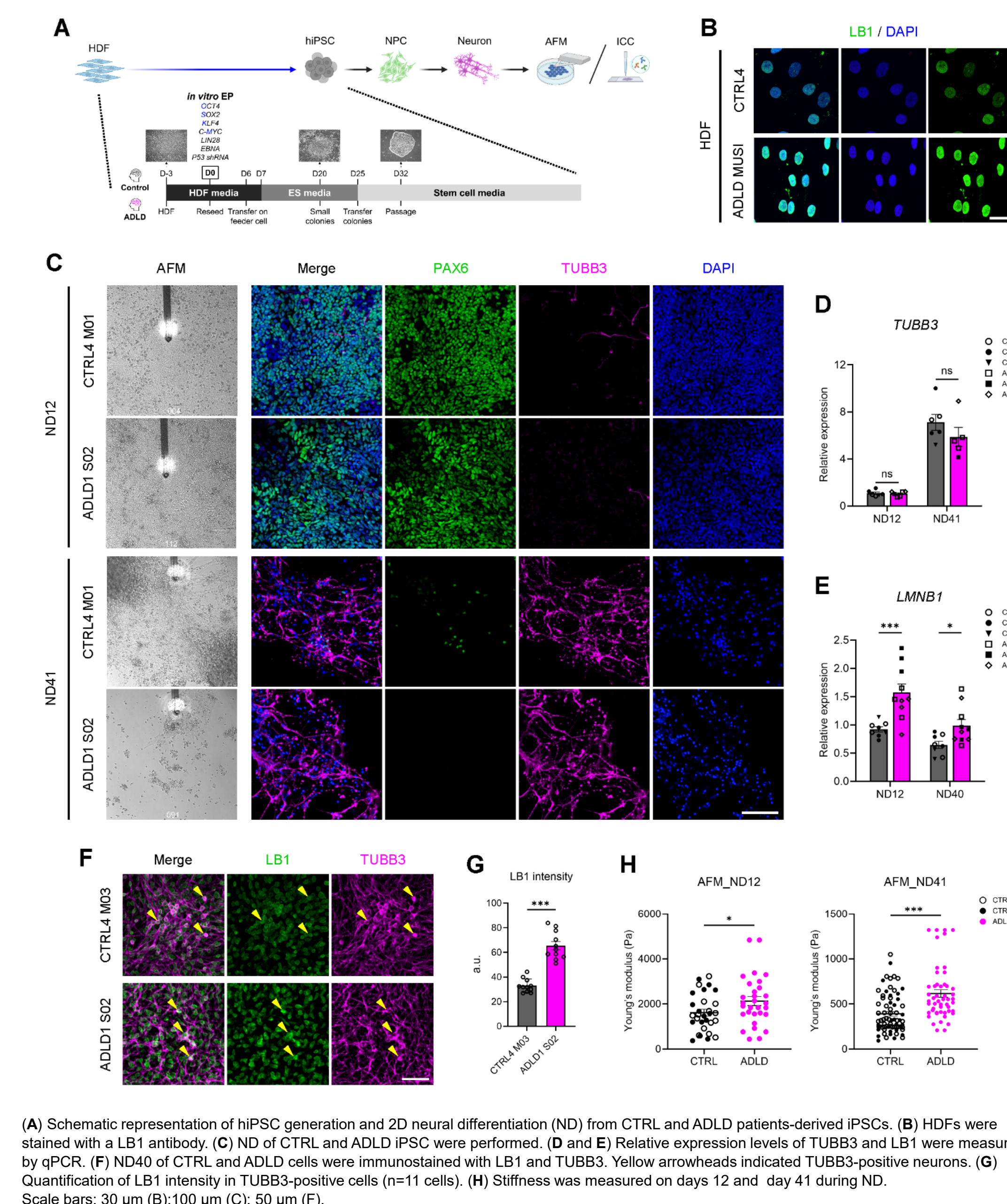
(A) Schematic of whole-cell patch-clamp recordings. (B) Representative confocal and IR-DIC images. (C) Representative voltage responses to hyperpolarizing and depolarizing current steps. (D) Quantification of resting membrane potential (left), input resistance (middle), and voltage sag (right) of EGFP-positive cells. (E) Representative traces showing action potential (AP) frequencies in response to current injection (0-300 pA in 100 pA steps for 600 ms). (F) Quantification of firing frequencies in response to 600-ms current injections (0-300 pA in 50 pA steps). (G) Overlay of representative first AP traces evoked by 100 pA current injection. (H) Representative phase-plane plots (dV/dt vs. membrane potential). (I) Quantification of AP properties across groups. Scale bars: 400 μm (B).

Fig. 4. Time-lapse imaging to assess the correlation between neuronal migration and nuclear deformability in brain tissue.



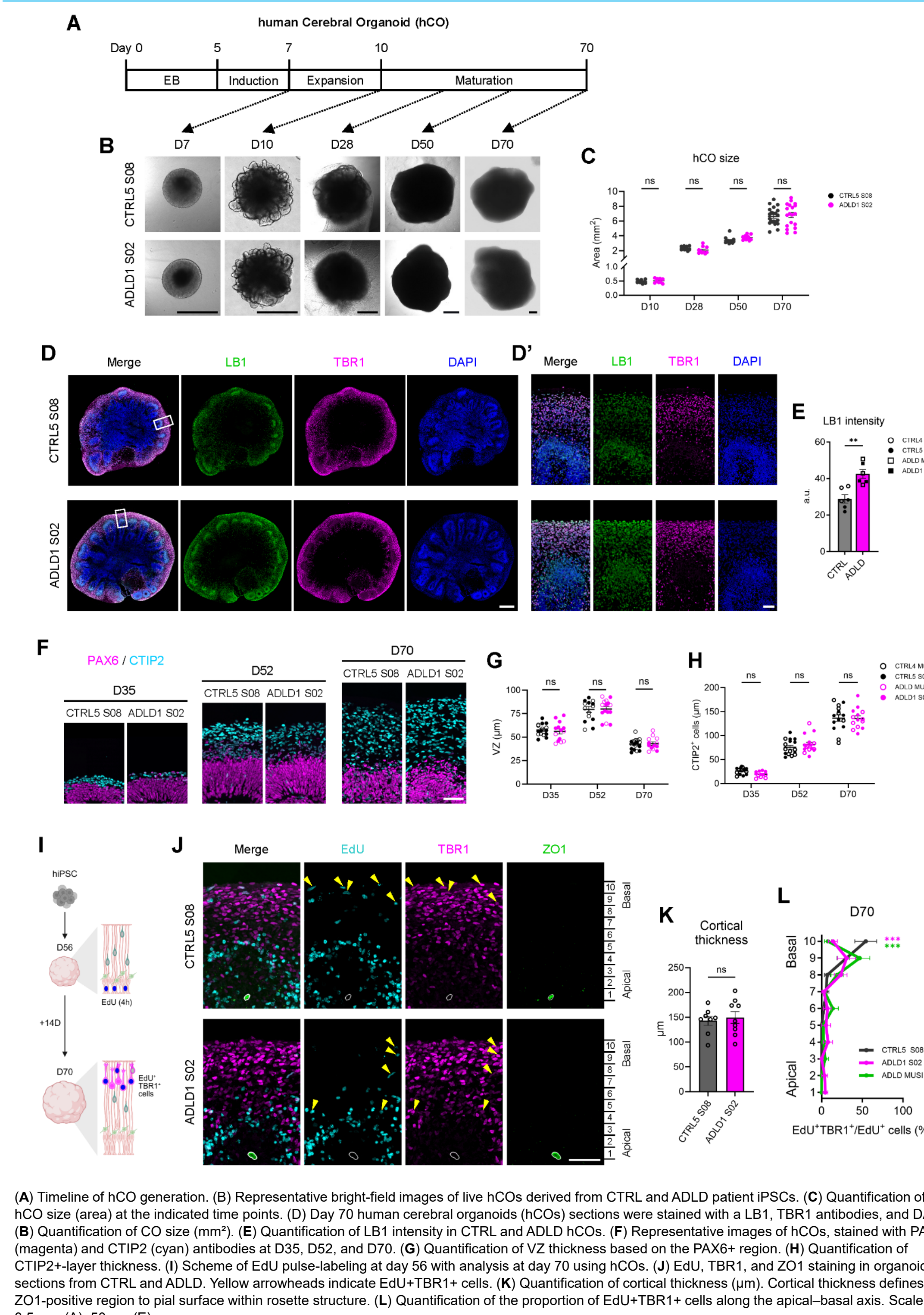
(A) The neocortex of E15.5 mouse embryos was electroporated with either CTRL or hLB1 plasmids with pCAG-EGFP and pCAG-H2B-RFP, and then fixed at E18.5 just before the start of the time-lapse imaging from the same mother. (B) Quantifications of laminar distribution of EGFP+ cells (n=3-4 brains). (C) Tracking of EGFP+ cells over 11 frames at 15-minute intervals (h:min). (D) Y-position plots of CTRL and hLB1 groups, respectively. Each line represents the trajectory of an individual EGFP+ cell (n=6 cell per group). (E) Quantifications of nuclear structure in EGFP+ cells at 5-minute intervals. (F) Tracking of H2B-RFP signal from Cell 01 per group. (G) The nuclear positions in EGFP+ cells tracked from the H2B-RFP signal are imaged at 5-minute intervals over 20 frames. (G) Pearson correlation analysis between AR and velocity using data from the three cells shown in (E), measured at 5-minute intervals (n=3 cells). Scale bars: 100 μm (A); 10 μm (C, E, and F).

Fig. 7. Establishment of ADLD patient-derived iPSCs and measurement of stiffness in NPCs and neurons.



(A) Schematic representation of hPSC generation and 2D neural differentiation (ND) from CTRL and ADLD patients-derived iPSCs. (B) HDFs were stained with a LB1 antibody. (C) ND of CTRL and ADLD IPSC were performed. (D and E) Relative expression levels of TUBB3 and LB1 were measured by qPCR. (F) ND40 of CTRL and ADLD cells were immunostained with LB1 and TUBB3. (G) Quantification of LB1 intensity in TUBB3-positive cells. (H) Stiffness was measured on days 12 and day 41 during ND. Scale bars: 30 μm (B); 100 μm (C); 50 μm (F).

Fig. 8. Impaired neuronal migration in brain organoids generated from ADLD patient-derived cells.



(A) Timeline of hCO generation. (B) Representative bright-field images of live hCOs derived from CTRL and ADLD patient iPSCs. (C) Quantification of live hCO size (area) at the indicated time points. (D) Day 70 human cerebral organoids (hCOs) sections were stained with a LB1, TBR1 antibodies, and DAPI. (E) Quantification of CO size (mm²). (F) Quantification of LB1 intensity in CTRL and ADLD hCOs. (G) Representative images of hCOs, stained with PAX6 (magenta) and CTIP2 (cyan) antibodies at D35, D52, and D70. (H) Quantification of VZ thickness based on the PAX6+ region. (I) Quantification of CTIP2+ layer thickness. (J) Scheme of EdU pulse-labeling at day 56 with analysis at day 70 using hCOs. (K) EdU, TBR1, and ZO1 staining in organoid sections from CTRL and ADLD. Yellow arrowheads indicate EdU+TBR1+ cells. (L) Quantification of cortical thickness (μm). Cortical thickness defines from ZO1-positive region to pial surface within rosette structure. (M) Quantification of the proportion of EdU+TBR1+ cells along the apical-basal axis. Scale bars, 0.5 mm (A); 50 μm (E).

FUNDING
This research was supported by KBRI basic research program through Korea Brain Research Institute funded by Korea government Ministry of Science and ICT (MSIT) (25-BR-01-03 and 25-BR-05-04 to Y.K., 25-BR-01-01 to J.R.), and by National Research Foundation of Korea (NRF) grant funded by Korea government Ministry of Science and ICT (MSIT) (2022R1A2C1002728 to Y.K. and 2022R1F1A1074446 to M.S.).