Supplementary Information

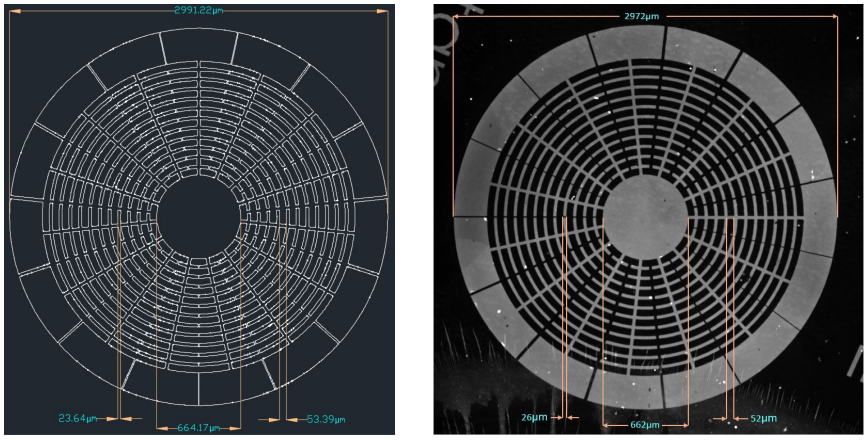
S1. Additional experimental setup information

a. Cell Culture

We obtain RFP-labeled MDA-MB-231 human breast carcinoma cells (GenTarget) and maintain them according to the manufacturer's instructions. Briefly, growth media is prepared using Dulbecco's Modified Eagle Medium with GlutaMAX (Gibco) supplemented with 10% Fetal Bovine Serum (Gibco), 1% 100x Penicillin-Streptomycin (Gibco), and 0.1 mM Non-Essential Amino Acid (ThermoFisher). Cells are kept in culture flasks in a tissue culture incubator at 37°C and 5% CO_2.

b. On-Stage Incubation

This growth media is prepared using Leibovitz' L-15 media with GlutaMAX (Gibco) supplemented with 10% Fetal Bovine Serum (Gibco), 1% 100x Penicillin-Streptomycin (Gibco), and 0.1 mM Non-Essential Amino Acid (ThermoFisher). L-15 media is chosen to better maintain the viability of cells over extended imaging sessions. Samples are kept in petri dishes within an Ibidi on-stage incubator while the microscope is running.



(a) Measurements of the pattern in CAD Design software.

(b) Measurement in a fluorescently-labeled fibrinogen micropattern.

Figure S1: Geometric characterization of a micropatterned circular maze pattern. (a): Dimensions in the CAD design used for creating the laser etched quartz mask. (b): Dimensions in a micropatterned labyrinth composed of a PEG background and fluorescent-labeled fibrinogen.

c. Preparing Seeded Micropatterns

We prepare micropatterns on 22mm diameter circular glass coverslips. Coverslips are first coated with 200 μL of 0.1 mg/mL PLL-g-PEG in HEBES buffer solution. After 30 minutes, the coverslips are washed off with ddH20 in order to maintain a monolayer of PLL-g-PEG on the coverslip surface, and then placed atop a laser etched quartz mask. The mask and coverslips are irradiated with UV-C light (254 nm) in a UV Ozone Cleaner for 10 minutes to remove PLL-g-PEG in the pattern of circular mazes. The coverslips are then coated with 50 μg/mL fibrinogen 647 in 0.1M Sodium Bicarbonate. After 30 minutes, the coverslips are washed off with ddH20 to reveal fibrinogen filling the PLL-g-PEG cavities.

Several factors, such as gap between quartz mask and coverslips, uneven UV illumination, and small particles on the surfaces, may cause missing segments in the micropatterned labyrinth. We find the cell motility characteristics, such as average speed and persistence, are not affected by these defects in the micropatterns.

Cells are extracted from flasks by replacing growth medium with Gibco TrypLE^{TM} Express and placing in the tissue culture incubator for 10 minutes before centrifuging at 123g for 5 minutes. Cells are diluted in growth medium to a chosen density between 5 cells/uL and 500 cells/uL. 200uL of the cell-growth medium mixture is then taken and pipetted onto the coverslip surface and gently shaken for the liquid to cover as much of the coverslip surface as possible. Samples are kept in 35mm Ibidi μ-dishes in a tissue culture incubator at 37°C and 5% CO_2.

d. Staining and Imaging

After the sample has been in the incubator for 6-8 hours, 1μL of 1mM SYTO 16 Green Nucleic Stain is diluted in 1.33mL standard growth medium and vortexed for 10 seconds. This creates a solution of 750nM dye, and all of the growth medium in the sample is replaced with the 1.33mL of solution. The sample is returned to the incubator for 15 minutes. During this time, a 1.5mL aliquout of on-stage incubation growth medium is thawed from frozen. After the 15 minutes is over, the dye solution is removed and replaced with a wash of 1mL of growth medium. This growth medium is removed and the 1.5mL of on-stage incubation growth medium is added onto the sample. Samples are imaged on a fluorescence microscope using TXR, I3, and Y5 filter cubes to capture the RFP cell cytoplasm, nuclear stain, and Fibrinogen 647 maze respectively. Images are taken every 30 minutes for 24-36 hours before the sample is removed and discarded.

2. Cell Tracking

In order to quantify the cell position and path data, we used the plugin 'Trackmate' in the image processing package Fiji, also known as ImageJ.

Trackmate identifies the cells' locations in each frame by fitting circles to their coordinates and algorithmically matching circles frame-by-frame to determine unique cell paths. Drift in substrate is accounted for by auto-correlating images of the fibrinogen maze and subtracting these displacements from cell positions.

S2. Additional Experimental Results

While the typical doubling time for MDA-MB-231 cells is 22 hours, we find cell number does not increase in typical experimental recordings. As shown in Fig. S4, there is no evident increase of cell density over the course of 26 hours.

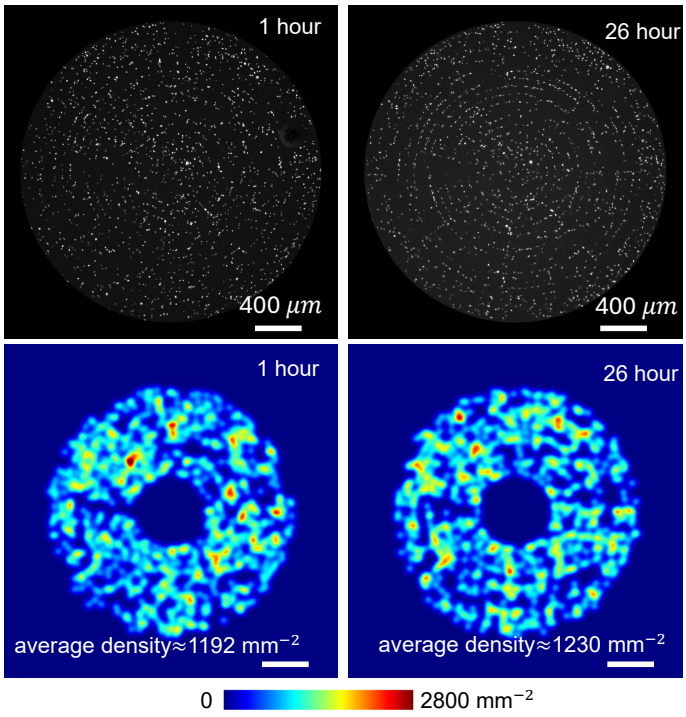


Figure S2: Spatial-temporal variations of cell density. Top: fluorescent images showing nucleus-stained MDA-MB-231 cells at 1 hour and 26 hours after being seeded on a micropatterned labyrinth. Bottom, cell density estimated using a Gaussian Kernel.

The micropatterned labyrinth contains circular tracks of various radius. To examine if radius of curvature affects the nematic order, we compute spatially resolved nematic order parameter (in same manor as Fig. 2C of main text) over different radial positions. As shown in Fig. S3, there is no statistical difference between the mean nematic order in different radial positions.

In computing the spatial map of nematic order, we use a circular sampling window of radius 75 μm. The size of the sampling window is chosen to include a sufficient number of cells. To examine the effect of window size, we reanalyzed the data with windows of radius 50 μm. Because the track-to-track distance is 55 um, the window size ensures cells on two different radial ring tracks are not mixed in sampling. As shown in the figure below, the normalized area

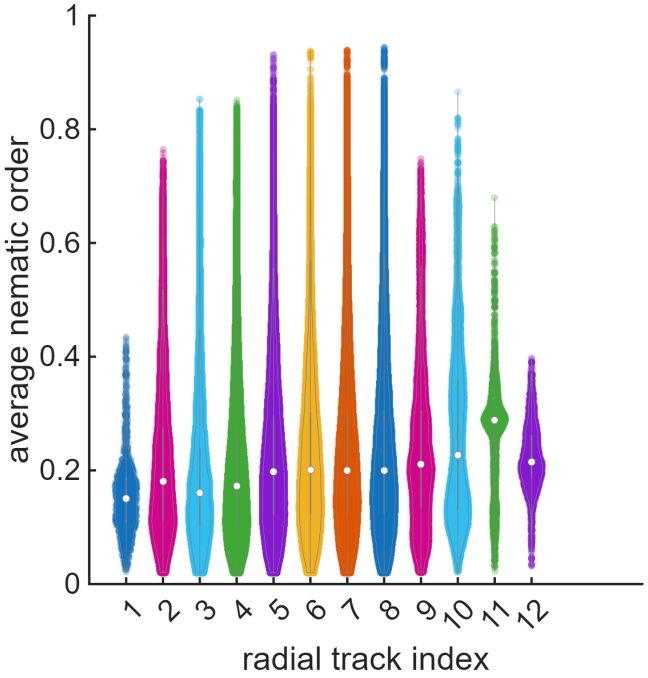


Figure S3: The distribution of nematic parameter sampled at different radial locations in the micropatterned labyrinth. Here radial track index 1 correspond to the circular track closest to the center island. The data includes more than 14,000 trajectories from 25 experiments. ANOVA test shows the mean nematic order can not be distinguished statistically.

significantly increases over time, consistent with the main text (Fig. 2D). The higher level of fluctuations is expected as the number of cells in a window is reduced.

For most trajectories, the net displacement can be approximated as a power-law function with time, $\Delta r \sim t^{\alpha}$. To obtain the exponent α, we perform linear fitting of $log\Delta r$ with $logt$. Fittings with R-square less than 0.8 is excluded (about 8% of all trajectories excluded). The distribution of the fitted exponent is shown in Fig. S5. We empirically classify trajectories with exponent between 0.4-0.6 as patroller cells, and trajectories with exponent 0.9 and 1.1 as explorer cells.

To examine how intracellular mechanotransduction affects the active nematic dynamics, we treat the cells with 10 μM Blebbistatin which inhibits myosin II contractility. Cell morphology shows elongated protrusions (Fig. S6A), while motility is significantly reduced compared with untreated cells (Fig. S6B). Concurrently, the high nematic region (HNR) occupies a much smaller fraction of area, although still demonstrates moderate increase over time (Fig. S6C). Because the HNR is small, we modify our data analysis procedure to identify

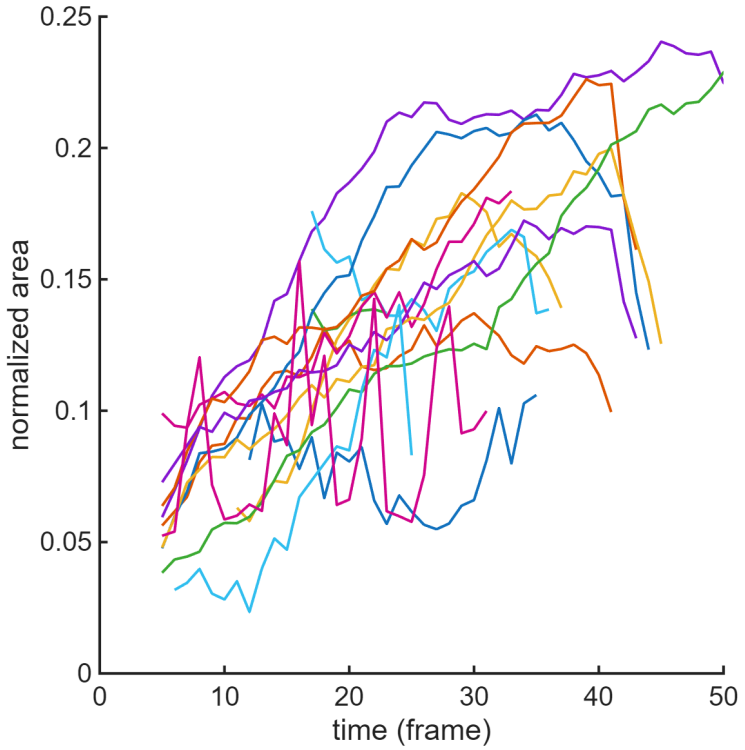


Figure S4: The temporal evolution of the area of high nematic regions A_{HNR} normalized by the area of cell-accessible surface A_{maze}. Each curve represents one experimental recording. Compared to the main text Fig. 2D, these results uses a smaller sampling window. The radius of sampling window is 50 μm, as compared with 75 μm in the main text.

the explorer and patroller cells in the entire field of view, and not just within HNR. The average position of each cell is shown in (Fig. S6D), where 16% of cells are explorers and 12% of cells are patrollers. There is an increased percentage of explorer cells, consistent with elongated cell morphology.

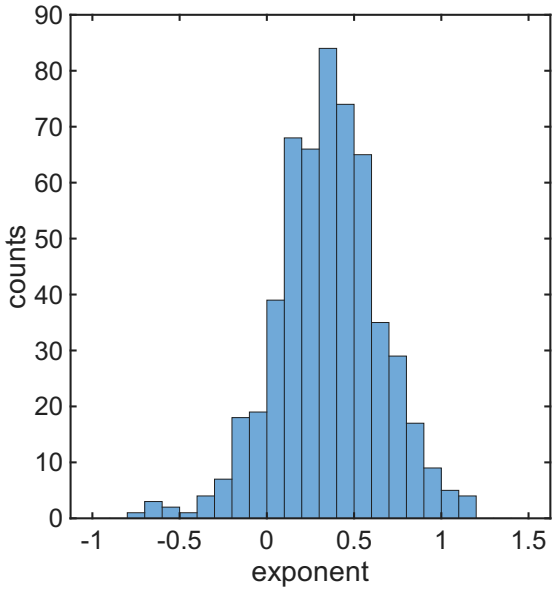


Figure S5: The histogram illustrates the distribution of exponents that quantify the scaling relationship between net displacement and time.

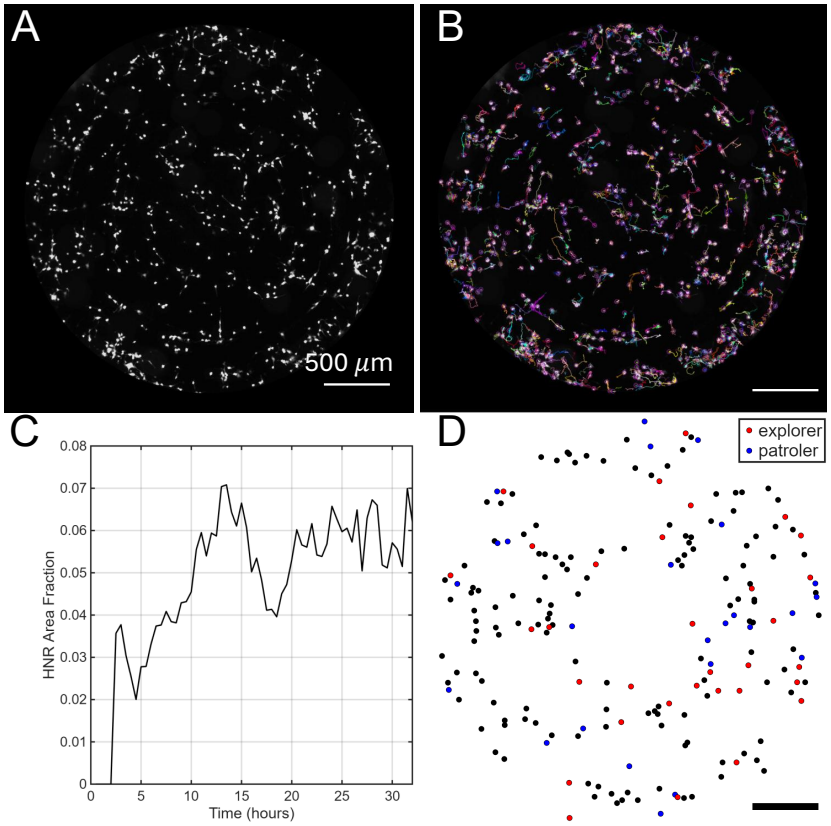


Figure S6: (A) MDA-MB-231 cells are labeled with SYTO 16 Green Nucleic Stain and treated with 10 μM Blebbistatin after seeding on micropatterned labrynth. (B) Trajectory of the cells over 32 hours of recording. (C) Fraction of high nematic region (HNR) grows over time, although the overall fraction is still significantly smaller compared with untreated cells (Fig. 2D main text). (D) Average position of explorer (red), patroller (blue), and other cells (black).