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1 Area cooling enables thermal positioning and manipulation of single cells

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1 Section 1 Temperature measurement

2 **Temperature calibration**

Thermal cycles and temperature calibration were performed using the temperature control system (heat sink and Peltier element) with a precision of 1 °C. To minimize photobleaching, a controlled shutter was used to excite the Rhodamine B only during measurements. To get thermal stabilization, a 10 min wait was made after attaining each new temperature before making measurements. At each temperature (23, 25, 30, 35, 40, 45, 50, 55, 60 °C), five images were captured, each with an exposure time of 160 ms. Based on these data, the relationship between temperature and fluorescence was established.

Temperature control system	Heat sink	
	Height PDMS: 50 µm	

- 11 Figure S1. Setup for temperature calibration
- 12 Notes: Based on the room temperature and humidity in the lab, setting the cooling
- 13 temperature at 15°C was suitable for long-time observation. Under lower temperatures,

- 1 extra strategies are needed to reduce dew formation (operation time reduction or humidity
- 2 reduction).
- 3
- 4 Section 2 1D particle migration



- 5
- 6 Figure S2 Images of 5 μm particle migration after 1 min (heat power, 0.18W; DC voltage
- 7 amplitude 3 V; cooling temperature, 15 °C; sample chamber height, 500 μm). Scale bar,
- 8 250 µm



- 1
- 2 Figure S3 Parameters of geometric structure: height of the chamber (H_c) and thickness of
- 3 the glass (h_t) .



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Figure S4. Mean velocity of 10 μm particles at different chamber heights and glass
thicknesses. Heat power, 0.18 w; applied voltage, 3 V; STC, 15 °C (mean±SD, N= 3).

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8 Section 3 Cell viability test



2 Figure S5. Process for cell viability test.



5% CO₂

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T: 37°C

- Figure S6. Statistical treatment for cell number. The average growth ratio in four sections 4
- 5 was defined as the growth ratio for one chamber.



2 Figure S7. Initial shape and size of cells after different treatments on the thermal
3 manipulation platform. Black scale bar, 400 μm. White scale bar, 200 μm.



6 Figure S8. The number of trapped cells after different treatments (these are connected

- 1 cells in the center area of the microheater)
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3 Supplementary Video list

- 4 Video S1 2D particle (5 μm) manipulation
- 5 Video S2 2D particle (10 µm) manipulation
- 6 Video S3 2D cell manipulation

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