SUPPORTING INFORMATION

Patterning neuroepithelial cell sheet via a sustained chemical gradient generated by localized passive diffusion devices

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Supporting Tables

Table 1. List of antibodies used in immunocytochemistry assays

Antibody	Source	Vendor	Catalog #	Concentration
PAX6	Mouse	Abcam	ab78545	5 µg/ml
NKX2.1	Rabbit	Abcam	ab76013	0.3 µg/ml
Alexa Fluor® 488	Goat anti-rabbit	Invitrogen	A11034	5 µg/ml
Alexa Fluor® 555	Goat anti-mouse	Invitrogen	A21424	5 µg/ml

Supporting Figures

Fig. S1

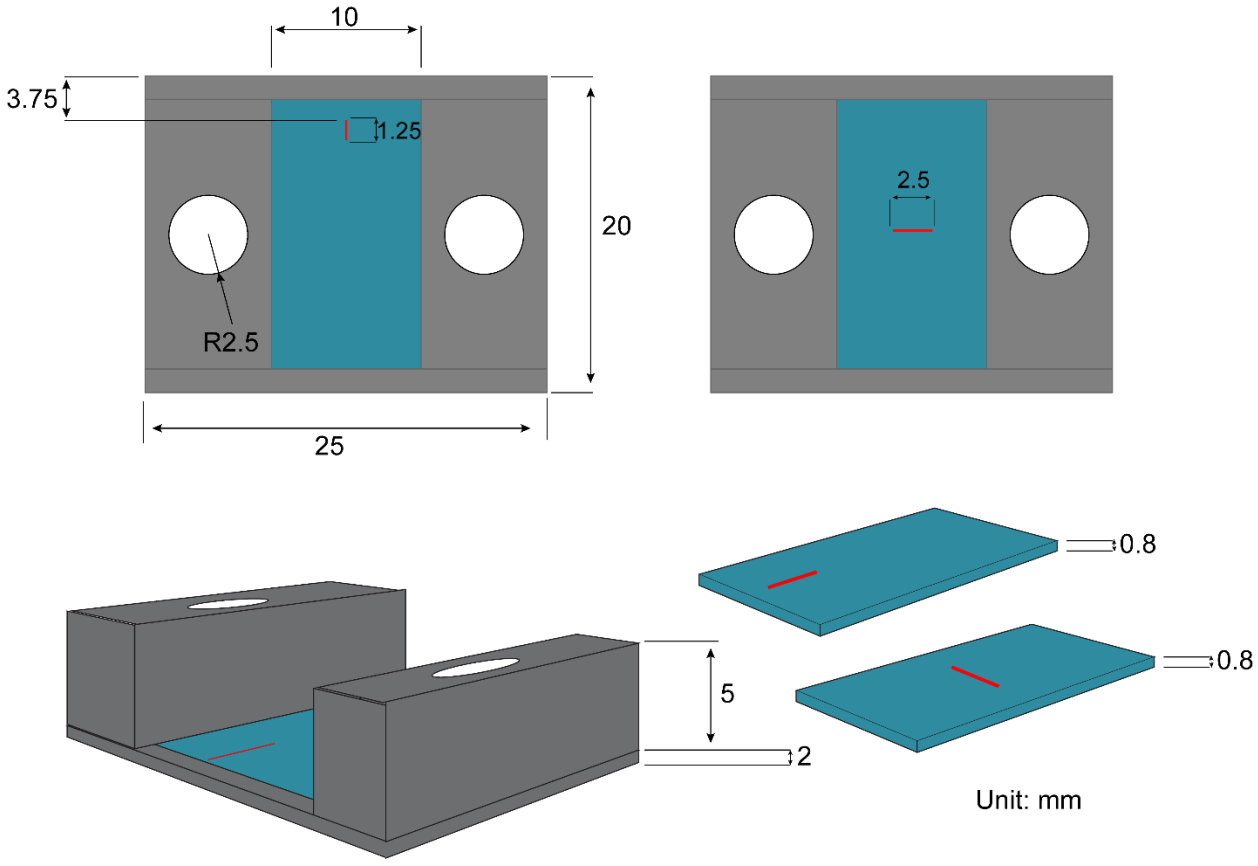

Fig. S1. The dimensions of the LPaD device. Top view schematic of cell culture device (up, left) and gradient measurement device (up, right), 3D schematic of device (bottom, left) and PDMS film with channel silt (bottom, right) and dimensions.

Figure S2

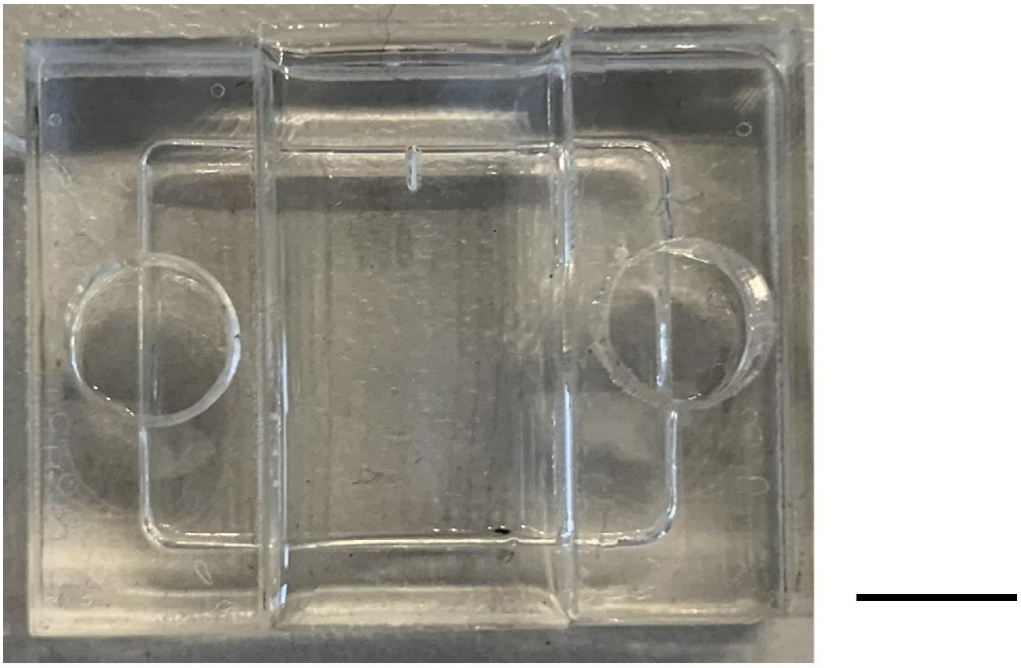

Fig. S2. Photograph showing acrylic LPaD device. Scale bar, 5 mm.

Figure S3.

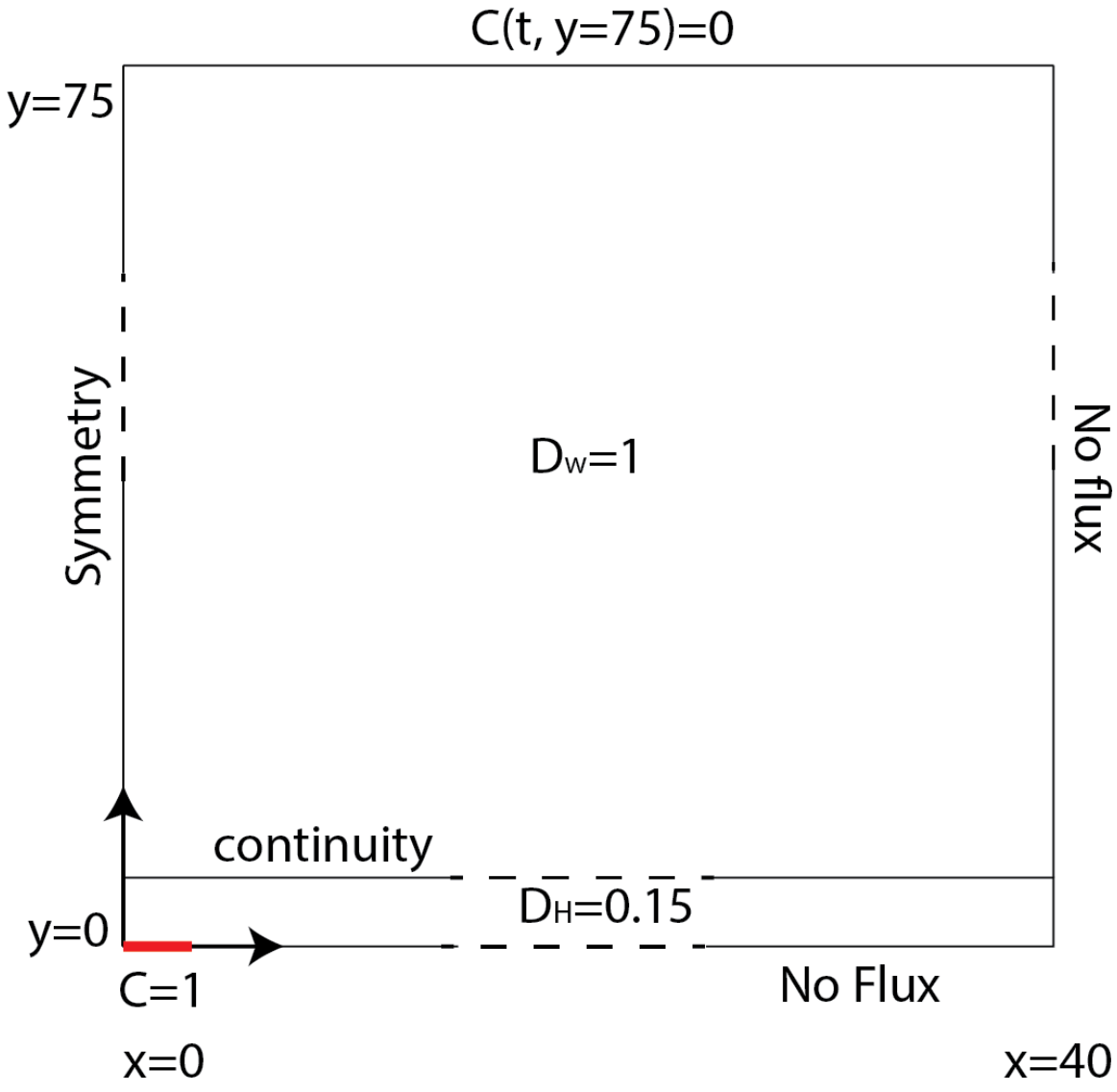

Fig. S3. Schematic of boundary conditions for simulation

Figure S4

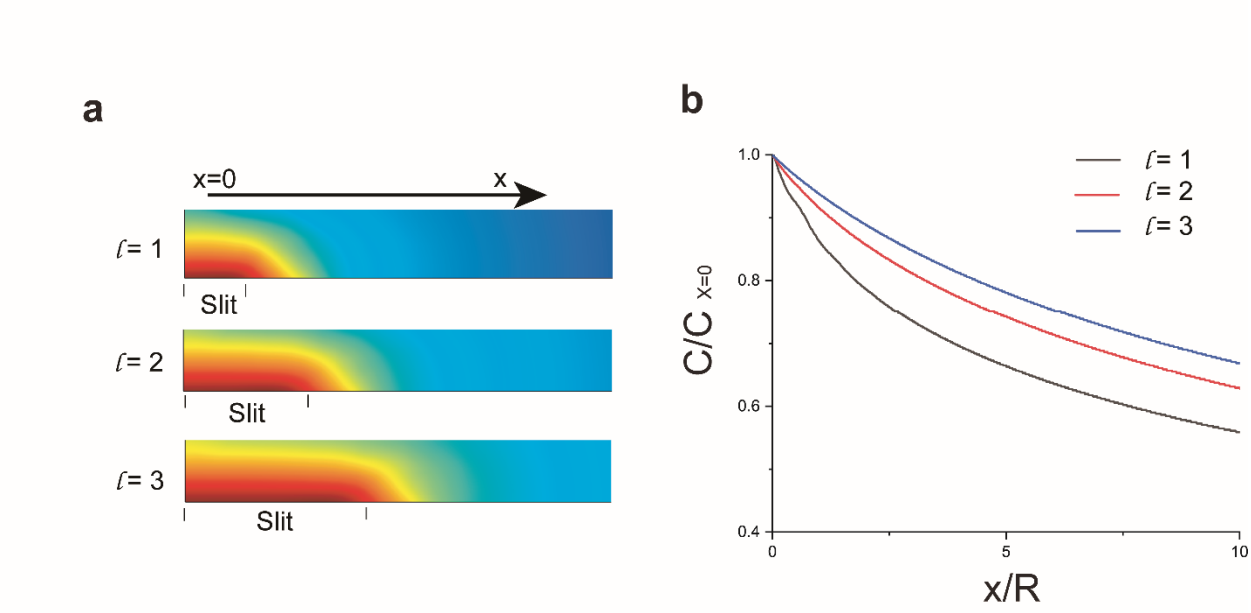

Fig. S4. The effect of channel size on the gradient formation. (a) Simulated gradient profile at simulation time equivalent to 24 hr with different channel size. The width of slit, *l*, is 1, 2, and 3, respectively. (b) Simulated gradient profiles plotted against normalized distance from the center of the slit; x = 0 at the slit center, *R* was the width of slit at *l*=1.

Figure S5

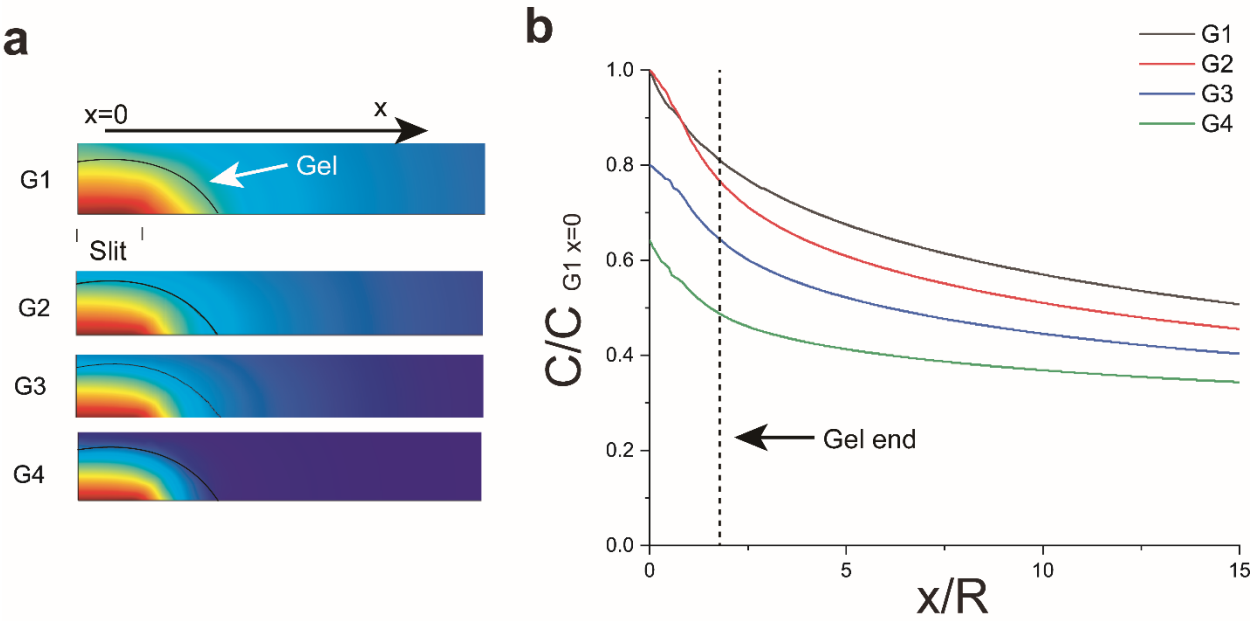

Fig. S5. The effect of gel diffusivity on gradient formation. (**a**) Simulated gradient profile at simulation time equivalent to 24 hr with different gel diffusivities. The gel diffusivities are$1.2 \times 10^{-6}\ cm^2/s$, $9 \times 10^{-7}\ cm^2/s$, $6 \times 10^{-7}\ cm^2/s$,and $3 \times 10^{-7}\ cm^2/s$ for device G1 to G4, respectively. (**b**) Simulated gradient profiles plotted against normalized distance from the center of each slit; x = 0 at the slit center.

Figure S6

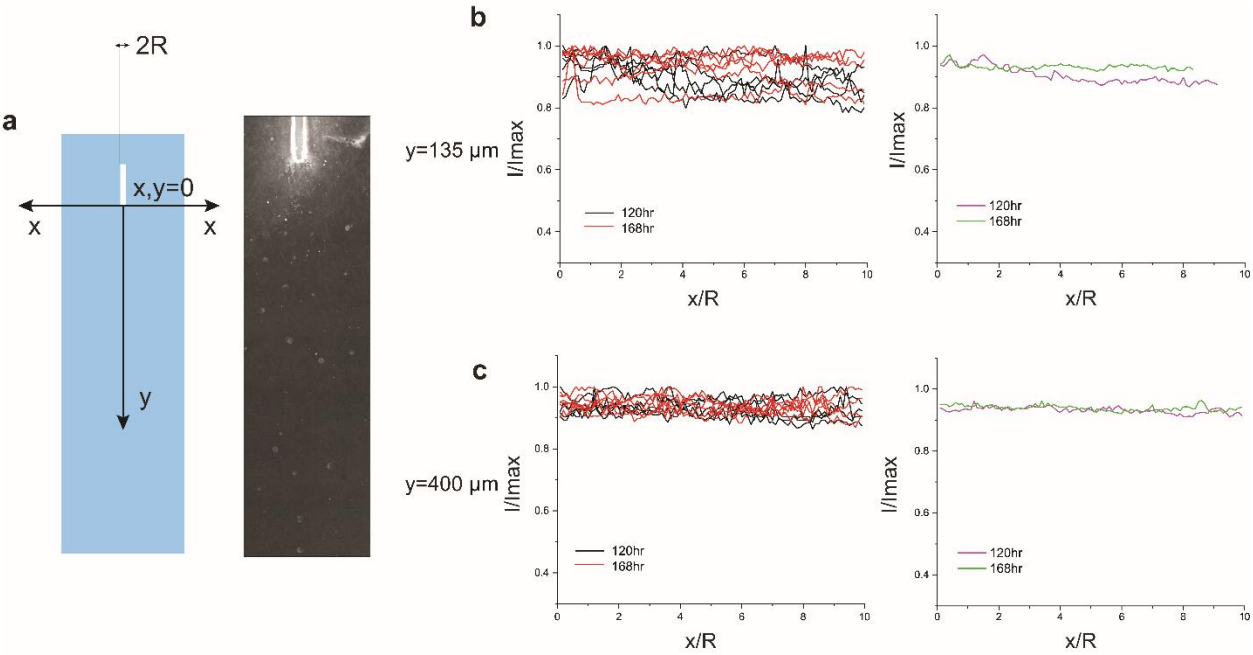

Fig. S6. The calibration of DAPI gradient along the width of the cell culture region. (**a**) Schematic and fluorescence images of the cell culture area of a device used for stem cell experiments. white line: diffusive channel. $x,y = 0$ at the end the channel. (**b**) Normalized individual (right) and average (left) intensity profiles from n devices and m images at 120hr ($n = 3$, $m = 5$) and 168 hr ($n = 3$, $m = 6$) time points plotted against normalized distance along x direction; $x = 0$ on the midline ($x = 0$, $y = 135\mu m$) as shown in panel. Two sample t-test for the linear fits of individual samples has been performed, P=0.168, no significant differences between 120 hr and 160 hr time points. (**c**). Normalized individual (right) and average (left) intensity profiles from n devices and m images at 120 hr ($n = 3$, $m = 5$) and 168 hr ($n = 3$, $m = 6$) time points plotted against normalized distance along x direction; $x = 0$ on the midline ($x = 0$, $y = 400\mu m$) as shown in panel. Two sample t-test for the linear fits of individual samples has been performed, P=0.863, no significant differences between 120 hr and 160 hr time points.

Figure S7

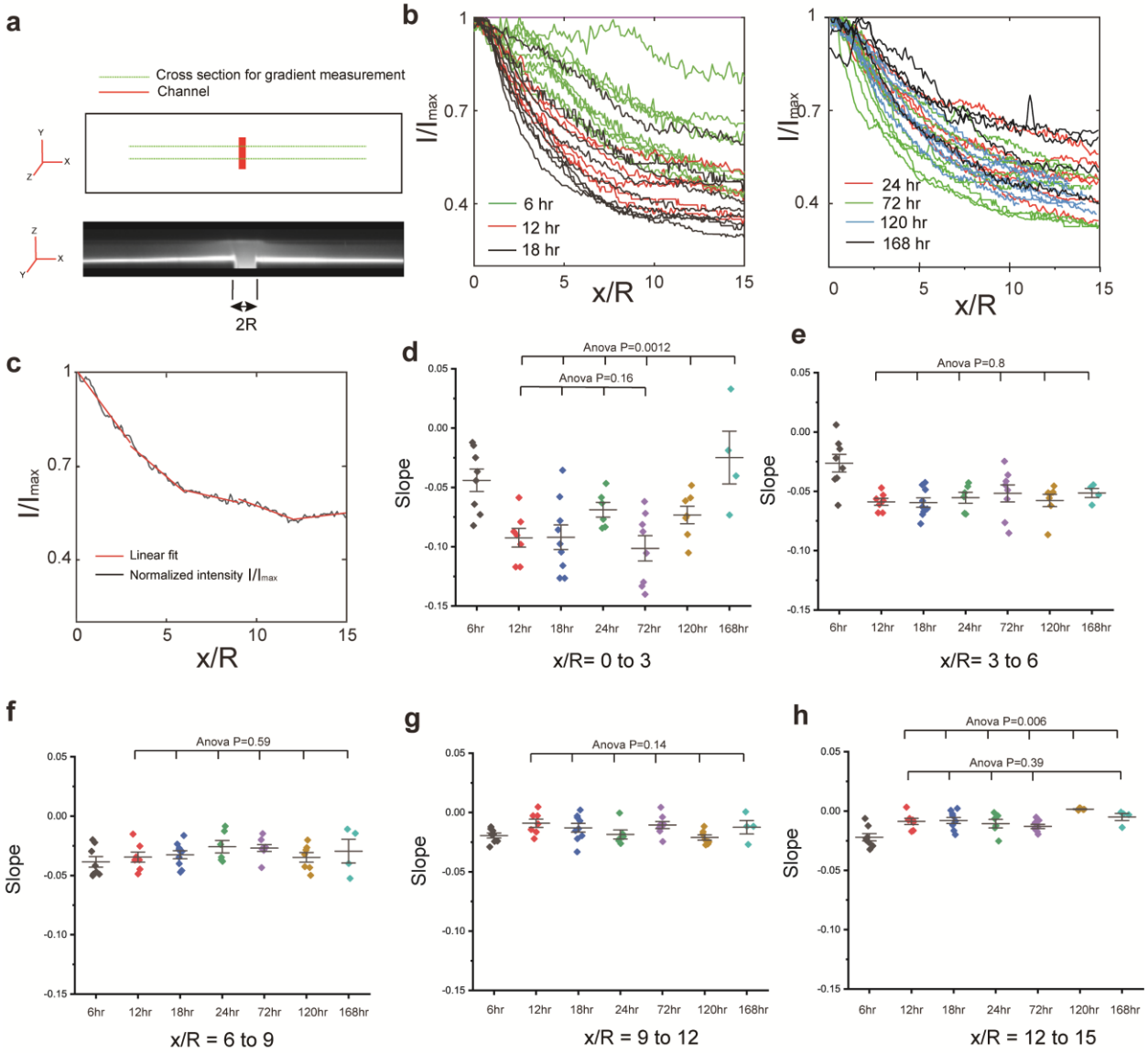

Fig. S7. Quantification of the stability of the gradient established in LPaD devices. (**a**) Schematic and fluorescence images showing the setup for calibration; (**b**) Normalized intensity profiles from different time points plotted against normalized distance from center of the slit. Raw intensity profiles measured from *n* devices and *m* images at 6 hr (*n* = 4, *m* = 8), 12 hr (*n* = 4, *m* = 6), 18 hr (*n* = 5, *m* = 8), 24 hr (*n* = 3, *m* = 6), 72 hr (*n* = 5, *m* = 8), 120 hr (*n* = 4, *m* = 7), and 168 hr (*n* = 3, *m* = 4). (**c**). Example linear fit of normalized intensity profiles. (**d**). Linear fit slope of normalized intensity data for 6 hr to 168 hr time point, x/R=0 to 3. No significant differences among 12 hr to 72 hr time points. (**e**). Linear fit slope of normalized intensity data for 6 hr to 168 hr time point, x/R=3 to 6. No significant differences among 12 hr to 168 hr time points. (**f**). Linear fit slope of normalized intensity data for 6 hr to 168 hr time point, x/R=6 to 9. No significant differences among 12 hr to 168 hr time points. (**g**). Linear fit slope of normalized intensity data for 6hr to 168 hr time point, x/R=9 to 12. No significant differences among 12 hr

to 168 hr time points. (**h**). Linear fit slope of normalized intensity data for 6 hr to 168 hr time point, x/R=12 to 15. No significant differences among 12 hr to 72 hr, and 168 hr time points.

Figure S8

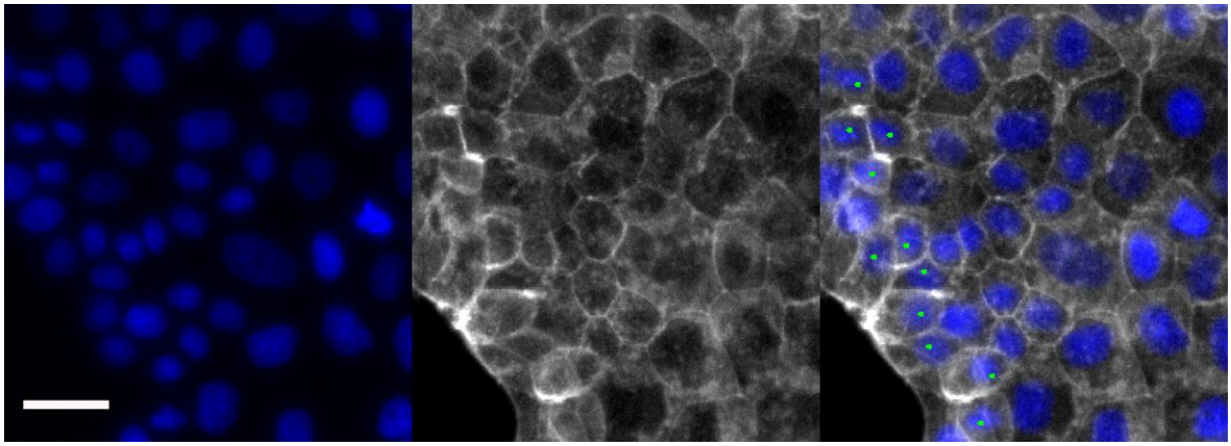

Fig. S8. MDCK cells were exposed to a Cyto D gradient for 24 hr, then fixed and stained with DAPI (blue) and Phalloidin (white). Cells with actin puncta were marked by cyan dots. Scale bar, 50 µm.

Figure S9

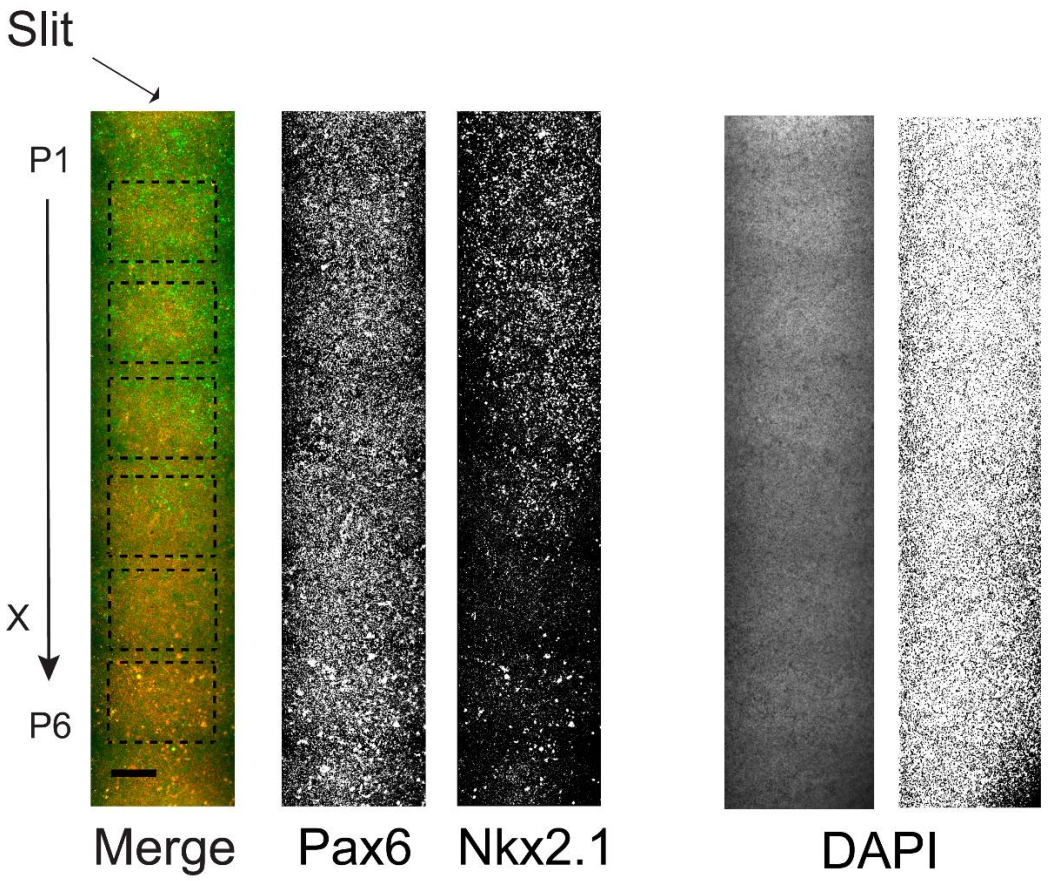

Fig. S9. Imaging processing for quantifying the percentage of PAX6 and NKX2.1 positive cells. From left to right: Original merge image of PAX6 and NKX 2.1 (red: PAX6, green: NKX 2.1), processed PAX6 image, processed NKX 2.1 image, original DAPI image, processed DAPI image, Scale bar: 200 μm. The sizes of the boxes (P1-P6) are 400 μm × 500 μm. Adjacent rectangles were set to be apart from 100 to 300 μm in the x-direction. Images were processed using ImageJ. Briefly, we first subtracted background by choosing the *Sliding paraboloid* with rolling ball radius set to be 50 pixels. We then adjusted the *Threshold* and used *Analyze Particle* function to automatically identify cell nuclei. Only particles over 50 pixels were counted.

Figure S10

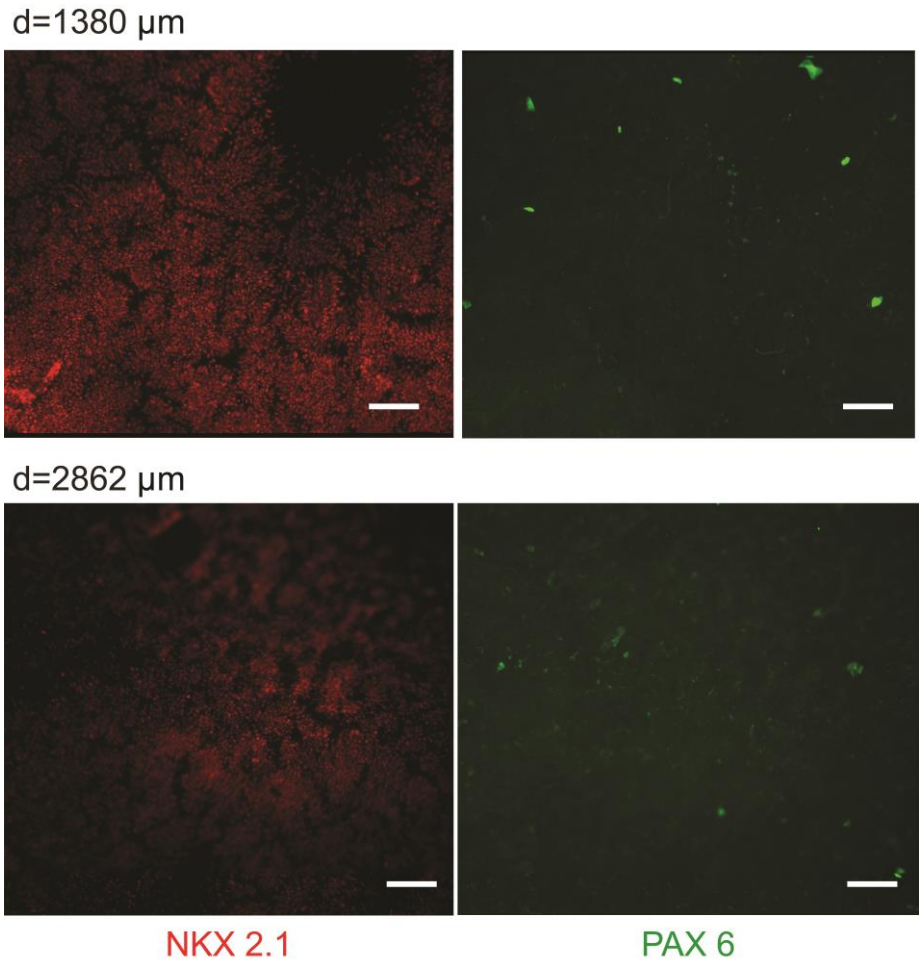

Fig. S10. Density effect on hPSCs response to Shh gradients. hPSCs were seeded at a lower density (25,000 cell/cm^2) and cultured on device in E6 medium with 100 nM LDN, 10 µM SB and 5 µM XAV and the same medium with the addition of 100 µM purmorphamine in the chemical chamber to create a gradient. Representative immunofluorescence images at different distances from the slit as indicated were shown. Scale bar, 200 µm.