Electronic Supplement Information

Supplementary Table 1. Model parameters for the oxygen transport and consumption at 37 ℃.

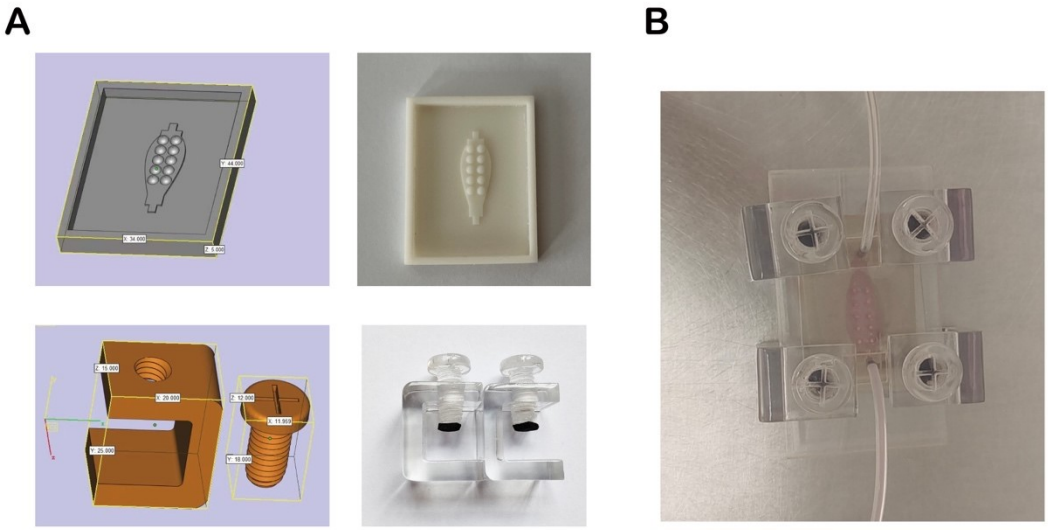
Parameters	Values
Henry's constant for oxygen	$K_{H,O_2} = 1.32 \times 10^{-3}\, mol \cdot m^{-3} mmHg^{-1}$
Oxygen partial pressure in the atmosphere	$p_{O_2} = 159\ mmHg$
Oxygen concentration for a fresh medium	$c = 0.21\ mol/m^3$
Critical oxygen concentration	$c_{cr} = 1.0 \times 10^{-4}\ mol/m^3$
Michaelis-Menten constant	$K_m = 0.66 \times 10^{-3}\ mol/m^3$
Maximum oxygen consumption rate	$R_{max} = 2.0 \times 10^{-3}\, mol \cdot m^{-3} \cdot s^{-1}$
The diffusion constant of oxygen	$D = 2.68 \times 10^{-9}\ m^2/s$
Flow rate	$Q = 10, 15, 35, 50{,}70\ \mu l/h$
Fluid density	$\rho = 993\ kg/m^3$
Dynamic viscosity	$\eta = 0.7 \times 10^{-3}\ Pa \cdot s$
Top fluidic layer height	$h_{top} = 100\ \mu m$
Membrane height	$h_{memb} = 10\ \mu m$
Bottom fluidic layer height	$h_{bot} = 100, 200, 400, 600, 800\ \mu m$
Diameter of the cell spheroid	$D_{cell} = 0.8\ mm$
The short shaft of ellipse microwell	$a = 1\ mm$
The long shaft of ellipse microwell	$b = 1.25\ mm$

Supplementary Table 2. The information of the primary antibodies.

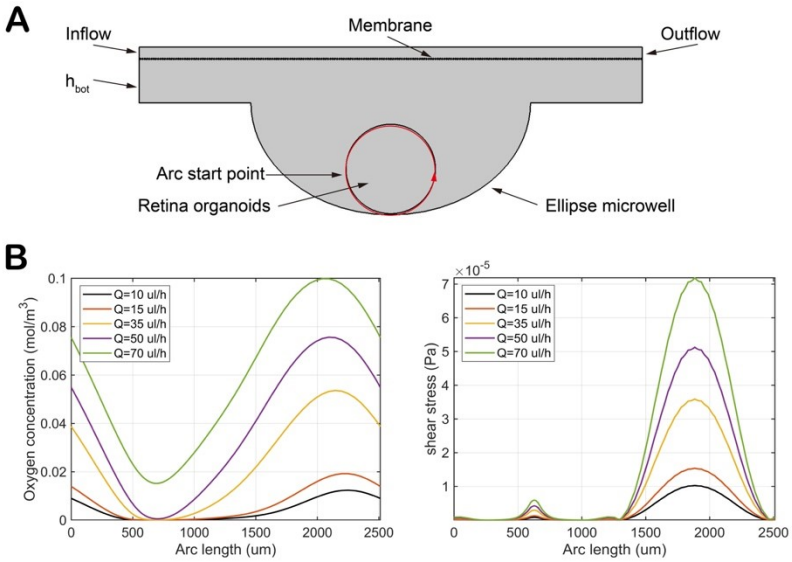
Antigen	Host	Source	Catalogue Number	IHC dilution
Brn3a	Rabbit	Abcam	ab245230	1:200
Atoh7	Rabbit	NOVUS	NBP1-88639	1:400
ZO-1	Rabbit	Invitrogen	402200	1:500
Cleaved-caspase 3	Rabbit	CST	#9661	1:400
CRX	Mouse	NOVUS	H00001406-M02	1:400
RX	Mouse	Santa Cruz	sc-271889	1:50
CHX10	Mouse	Santa Cruz	sc-365519	1:50
Tuj1	Rabbit	Abcam	ab18207	1:500
Tuj1	Mouse	Abcam	ab78078	1:500
Pax6	Mouse	Biolegend	862002	1:200
Pax6	Rabbit	Abcam	ab195045	1:500
HuC/D	Mouse	Invitrogen	A21272	1:200
HuC/D	Rabbit	Abcam	ab184267	1:400
Recoverin	Rabbit	Millipore	AB5585	1:400
Ki67	Rabbit	Abcam	ab66155	1:500
CCND1	Rabbit	ABclonal	A19038	1:500
Phospho-Vimentin	Mouse	MBL	D076-3	1:500
AP2α	Rabbit	CST	#3215	1:200
Islet1	Rabbit	Abcam	ab20670	1:500
RBPMS	Rabbit	Abcam	ab152101	1:500

Supplementary Table 3. The information of Primers.

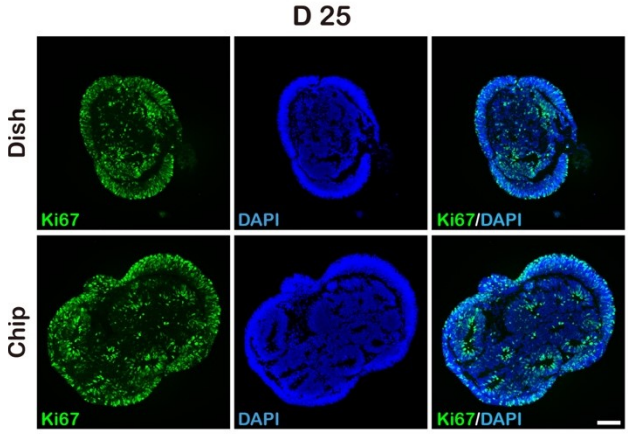
Name	Forward primer	Reverse primer	ID
KCNN1	TCGGGGAAACCCTCAAATGTG	CCATGACGACGATGCCAAAC	NM_001386974.1
KCNN2	GGCGTCGCTGTATTCCTTAG	TATTCTCCAGTCATCTGCTCCA	XM_047417166.1
KCNH1	AGTGGCCCCTCAAAACACG	CTATCTGAGCATTCCCCAACAC	NM_002238.4
KCNH6	TCCGCTGCCTGGTAGATGT	GGTCCTCGAAGTTGAGAATGAAC	NM_011525313.2
KCNK1	TTCTGGAAACCTTCTGTGAACTC	AGTTGGTCATGCTCTATGATGTG	NM_002245.4
CRX	GCCCCACTATTCTGTCAACG	GTCTGGGTACTGGGTCTTGG	NM_000554.6
Recoverin	GACGGTAACGGGACCATCAG	GATCTTCTCGGCTCGCTTTT	NM_002903.3
Tuj1	CAGCAAGGTGCGTGAGGAGTA	TGCGGAAGCAGATGTCGTAGA	NM_00119181.2
GAPDH	CCATGTTCGTCATGGGTGTGA	CATGAGTCCTTCCACGATACCA	NM_002046
TRPV3	TGGGCAGGTTCATCAACGC	CCTTCGTGTTGGTACTTGGGG	NM_001258205.2
LAMC3	GCTCCGAGGAATGCACGTT	TGTCATCGCACTGGAGGTGTA	NM_006059.4
COL6A1	ACACCGACTGCGCTATCAAG	CGGTCACCACAATCAGGTACTT	NM_001848.3
COL6A2	TACGGAGAGTGCTACAAGGTG	GGTCCTGGGAATCCAATGGG	NM_058175.3



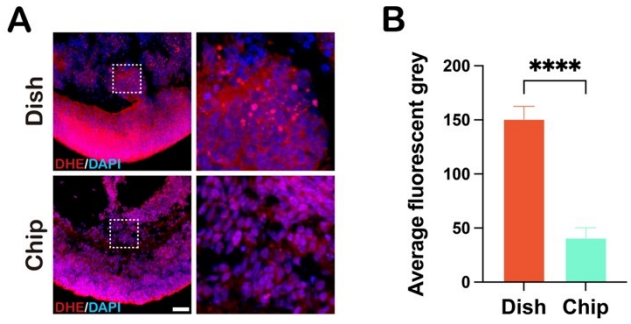
Supplementary Fig. 1 3D printing molds and images of the assembled CPMC device. (A) 3D printed molds of microwells and U clamps. (B) Experiment setup for culturing ROs in a microfluidic chip.



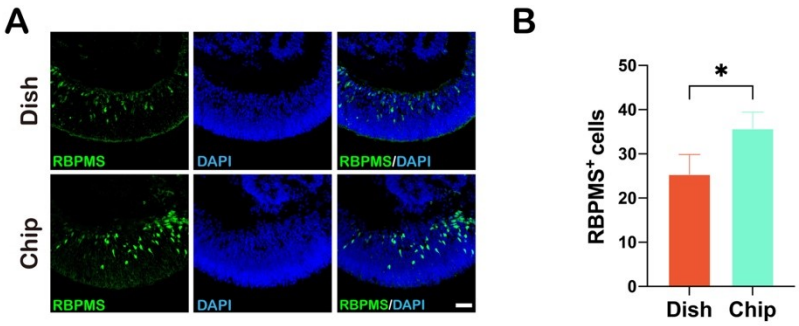
Supplementary Fig. 2 Simulation of oxygen in the simplified version of the microfluidic chip with different flow rates. (A) The geometrical conditions of the simulated microfluidic chip. The path along the cell spheroid surface is marked in red. (B) Distribution profiles of the oxygen concentration and FSS along the path marked in red under different flow rates ($Q = 10, 15, 35, 50, 70\ \mu l/h$) after steady-state conditions have been reached. For this simulation, the bottom channel height is fixed at $h_{bot} = 400\ \mu m$.



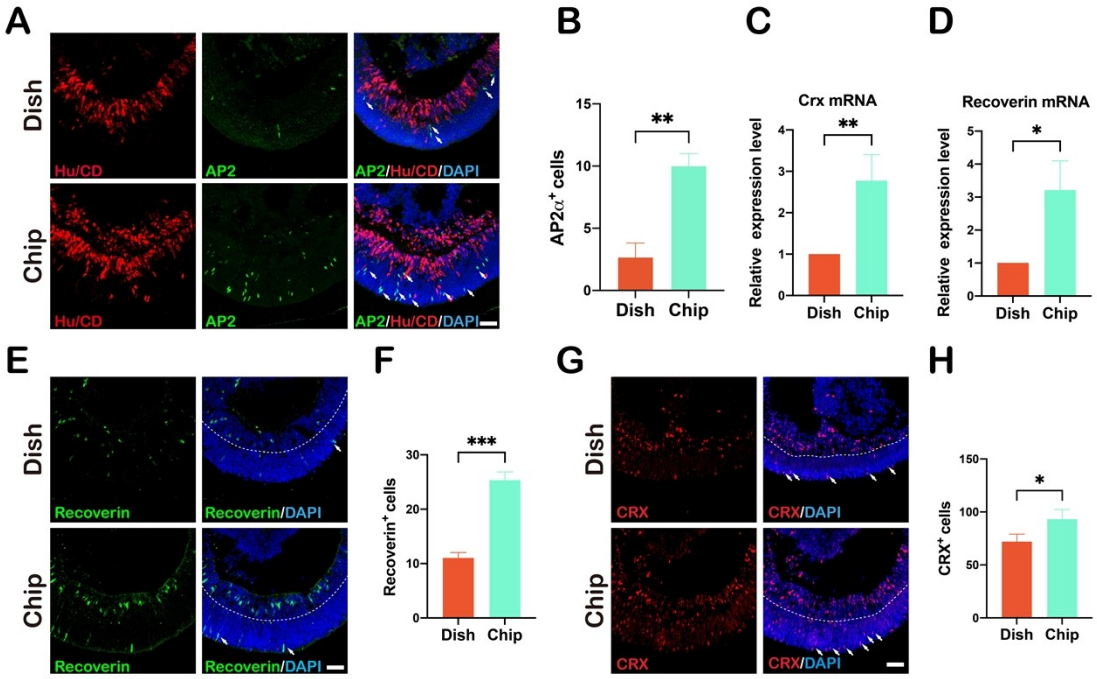
Supplementary Fig. 3 Representative images for Ki67$^+$ proliferative cells in the central rosette-like structure of the whole ROs. Scale bars, 100 μm.



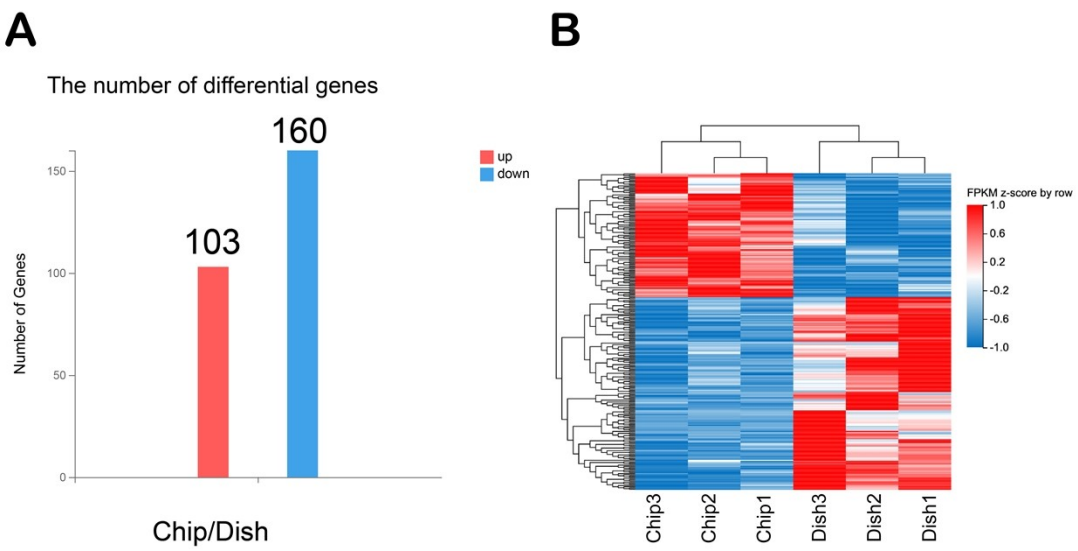
Supplementary Fig. 4 The expression of DHE in the interior of ROs. A. Representative images of DHE staining in the NRs of the ROs. The white dotted line shows the 5X magnification of the original images. Scale bar, 50 μm. B. Quantitation analysis of the average fluorescent grey of DHE in the interior of ROs. Data are mean ± SEM. *n* = 3, three independent experiments. ****$p < 0.0001$.



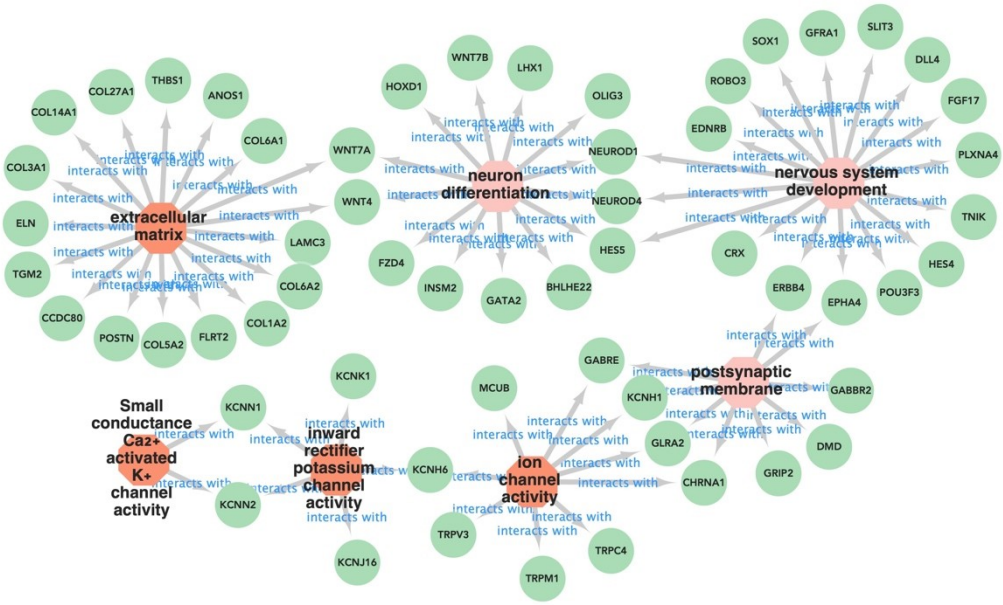
Supplementary Fig. 5 The expression of RBPMS$^+$ cells in the NRs at day 39. (A) Representative images of RBPMS$^+$ cells in the NRs. Scale bars, 50 μm. (B) Quantitation analysis for the number of RBPMS$^+$ cells in the NRs. Data are mean ± SEM. *n* = 3, three independent experiments. **p* < 0.05.



Supplementary Fig. 6 Development of amacrine cells and photoreceptors under perfused conditions. (A) Representative images of HuC/D^{-}AP2α^{+} cells in the NRs at day 39. Scale bar, 50 μm. (B) Quantitation analysis for the number of AP2α^{+} cells in the NRs. Data are mean ± SEM. *n* = 3, three independent experiments. $^{**}p < 0.05$. (C-D) Quantitation analysis for the mRNA levels of CRX and Recoverin in the ROs. Data are mean ± SEM. *n* = 3, three independent experiments. $^{*}p < 0.05$, $^{**}p < 0.01$. (E) Representative images of Recoverin^{+} cells in the NRs at day 39. White arrows indicate the migration of Recoverin^{+} cells to the apical side of the NRs. Scale bars, 50 μm. (F) Quantitation analysis for the number of Recoverin^{+} cells in the NRs. Data are mean ± SEM. *n* = 3, three independent experiments. $^{***}p < 0.001$. (G) Representative images of CRX^{+} cells in the NRs at day 39. White arrows indicate the migration of CRX^{+} cells to the apical side of the NRs. Scale bar, 50 μm. (H) Quantitation analysis for the number of CRX^{+} cells in the NRs. Data are mean ± SEM. *n* = 3, three independent experiments. $^{*}p < 0.05$.



Supplementary Fig. 7 The number of DEGs and cluster heat map of 39-day ROs in the dish and chip group. (A) The number of DEGs with 103 upregulation genes and 160 downregulation genes. (B) Cluster heat map of ROs in dish and chip group. *n* = 10, three independent experiments.



Supplementary Fig. 8 Cytoscape online software analyzed the gene-pathway interaction network between ion channels, ECM, and nervous system development.