Supplemental Figure 1

a. Short circuit current (Isc) tracings following treatment with the A$_{2B}$ adenosine receptor inhibitor PSB603 (10µM) and addition of 2‘3’ cGAMP, 3’3’ cGAMP, cAAG (20µM) and Forskolin (Fsk) (20µM).

b. Short circuit current (Isc) tracings following treatment with the CD73 inhibitor APCP (1mM) and addition of 2‘3’ cGAMP, Ap4A (20µM) and Forskolin (Fsk) (20µM).

c. Short circuit current (Isc) tracings following treatment with the ENPP1 inhibitor STF1084 (10µM) and addition of 2‘3’ cGAMP, 3’3’ cGAMP (20µM) and 5’-AMP (20µM).
Supplementary Methods: Custom Transwell Short-circuit Current Apparatus (Thiagarajah Lab)
(http://thiagarajahlab.com/tools/)

Summary Description

A device to securely hold electrodes and wires in place to carry out transepithelial electrophysiology using 0.33cm² transwell permeable support inserts while also giving the user a port access to the bottom reservoir and clear access to the top of the transwell. In addition, it has a plate that secures each sampling cell and is compatible with 24-well plate format heaters.

Overview images of device

Figure 1: A) 3D rendering of transwell holding cells inserted into securing plate. B) Top down view of transwell holding cells inserted into securing plate. C) Actual setup with electrodes in proper sockets and in 24 well plate heater. D) Single transwell holding cell close up with electrodes and wires in place. E) Close up of inside chamber to show electrode placement when in socket.
Figure 2: Key measurements given for the following views A) slanted view of 3D rendered image of securing plate. B) Top down view of 3D rendered image securing plate. C) Slanted view of 3D rendered image of holding cell. D) 3D rendered image of holding cell with a sagittal cut away plane added (left) and coronal cut away plane added (right).
Creation

- Transwell holding cells were 3D printed in PLA with a Prusa i3 Mk3s printer at stock 0.20mm quality settings with the following basic settings:
  - 0.4mm nozzle
  - 0.20 mm layer height
  - 25% infill
  - 3 layers in for vertical and horizontal shells (already thick enough to be watertight, but this is added insurance most likely it will operate perfectly fine with default shell layers)
  - 220C extrude temp (touch hotter to get PLA to flow slightly more to fill in gaps a little better)
  - 60C bed temp
  - Print cell from back to front (figure 3)
- Plate frame was 3D printed in PLA with a Prusa i3 Mk3s printer with same settings as above, but with the following changes:
  - 215C first layer, 210C after that
  - 0.30 mm layer height
  - Print plate frame from bottom to top (figure 3)

Figure 3: A) Plate frame on print bed with correct printing orientation. B) Transwell holding cell on print bed with correct printing orientation.
Electrodes and Voltage Clamp Unit

This device was used with a Physiological Instruments voltage clamp system (figure 4). The system is compatible with any voltage amplifier but the electrode sockets were designed specifically for the Physiological Instruments P2020 Ag/AgCl electrodes.

Figure 4: A) Physiological Instruments DM MC8 single channel electrode input. B) Physiological Instruments MC8 voltage clamp unit.
Tips and Notes

• When printing the transwell holding cell, print back to front even if your printer can successfully print it differently. An accidental discovery when designing and printing this apparatus was that the upper electrode hole “sagging” actually caused the electrodes to become very stable in their sockets and fixed an stability issues in holding the upper electrodes (figure 5).

![Figure 5: Black arrow points to “sagging” upper electrode socket due to gravity while printing.](image)

• The transwell holding cells sit very tightly in the holding frame. It is possible that after printing, the holding frame sockets will be too tight. An easy solution to this is to file down the inside walls of the sockets.
• The holding frame was designed to fit extremely tight into a 24-well plate heater block. This makes everything more stable while conducting the experiment, however makes it harder to remove out of the heating block.

Files

Design files are available by request (jay.thiagarajah@childrens.harvard.edu)

1. .Stl files if you want to use your own slicer program to generate the .gcode for your printer.
2. Prusa .gcode files if you want to use the exact files used while printing with our Prusa i3 Mk3s printer
3. Fusion 360 file if you desire to see other dimensions that are not listed or modify it for your specific use.