Supplementary Information

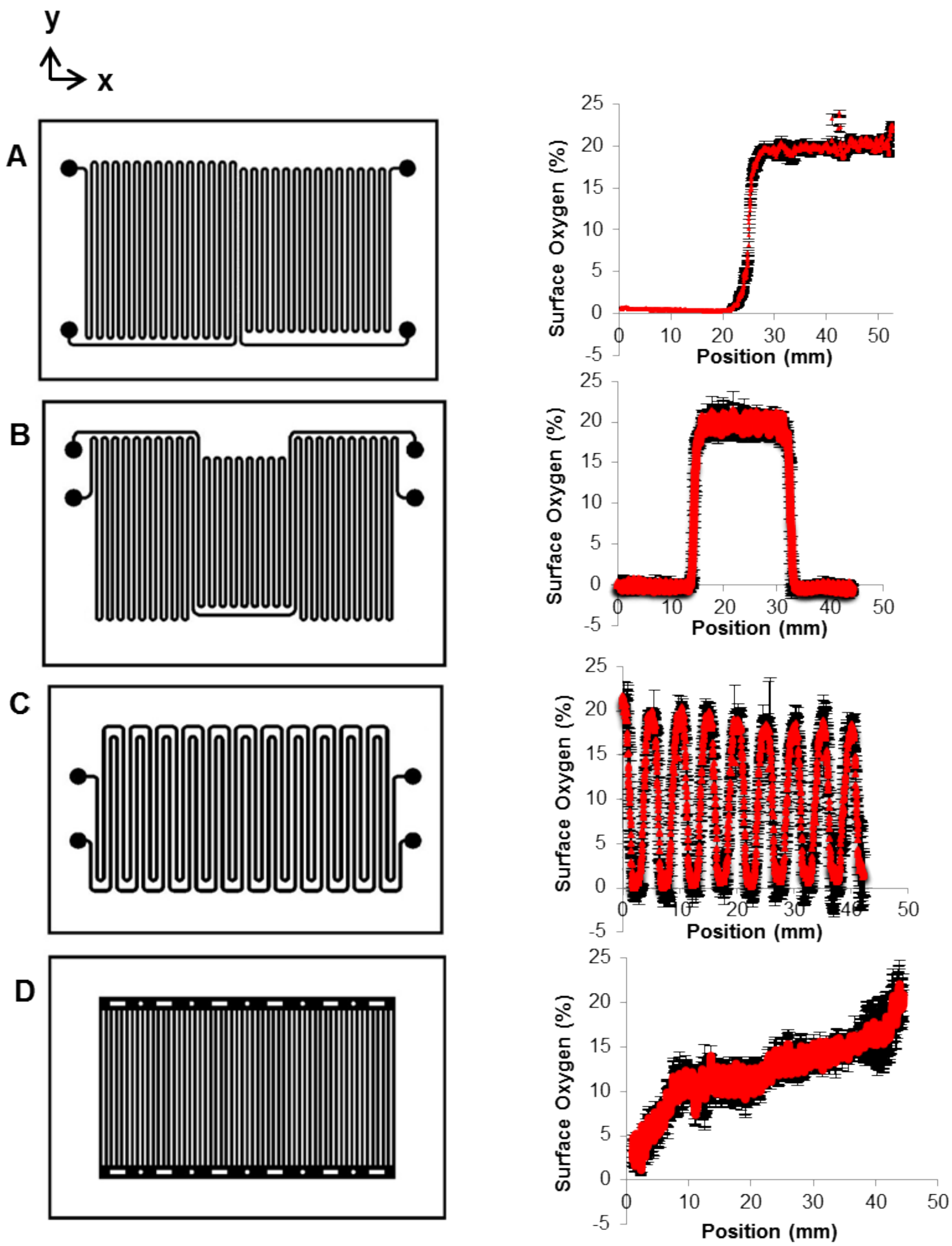

FIGURE S1. The microfluidic network design can generate a variety of oxygen landscapes. The gas network channel design and the corresponding surface oxygen profile as a function of position in the x-direction is shown for the (A) binary dual condition, (B) square wave, (C) oscillating, and (D) linear oxygen landscape.

The design elements in black (left column) represent the crosslinked SU-8 photoresist pattern on a master. The devices functioned via constant perfusion of compressed gas through the gas network channels and diffusion across the 100 µm PDMS membrane on which cells were cultured. By using different gas compositions, distinct oxygen profiles were achieved. The dual condition binary landscape (A) was created using two microfluidic perfusion networks (500 µm wide and 300 µm deep) separated by a 500 µm wide gap. A gas composition of 5% CO_2, balanced nitrogen flowed through the left network and 5% CO_2, balanced air flowed through the right network. The square wave landscape (B) consisted of two separate networks (500 µm wide and 300 µm deep) with 500 µm spacing between channels. The outer network was supplied with 5% CO_2, balanced nitrogen and the central, inner network was supplied with 5% CO_2, balanced air. The oxygen profile for an oscillating oxygen landscape (C) was created with a channel pattern of two interdigitated serpentine networks (one perfused with 5% CO_2, balanced air and the other perfused with 5% CO_2, balanced nitrogen), resulting in oscillations in the x-direction. Channels were 625 µm wide and 300 µm deep with 625 µm spacing between channels. A near linear gradient (D)was generated using 5 oxygen compositions. Five inlet and five outlet ports (3.2 mm diameter) were punched at the sites of the five small, white circles (1 mm diameter) which served as guides to center the punch placement. Oxygen compositions of 0%, 5%, 10%, 15%, and 21% O_2 were introduced into the inlet ports from left to right, respectively. The design was a single chamber (200 µm deep) with a series of PDMS walls (500 µm wide) spaced 500 µm apart. The different gas compositions mix and perfuse the spaces separated by the PDMS walls to distribute a relatively linear gradient in the x-direction.

Of these different landscapes demonstrated, only the linear gradient (D) requires more than two different inlet gas compositions. We were not able to achieve one steady linear gradient across the entire device with only two gas compositions due to the scale of the device (see Fig. 2A of the manuscript for dimensions). Due to the large scale of the overall device (centimeters as opposed to millimeters or micrometers) to accommodate enough cellular material for standard biological techniques, the ‘conventional’ source and sink methods for creating a linear gradient will not work. Although we have constant perfusion of different oxygen compositions across the PDMS membrane, there is also exchange with the environment above the media in the open-well format (Figure 3D), creating another source and sink at this large scale. For this reason, equilibration of gas contents in the microfluidic network with those of the surrounding, ambient environment cannot be opposed in a device with this large of an area without providing multiple inlet gas compositions to mix spatially in the device.