Electronic Supplementary Material (ESI) for Analytical Methods.

Electronic Supplementary Materials for

Generating linear oxygen gradients across 3D cell cultures with Block-Layered Oxygen Controlled Chips (BLOCCs)

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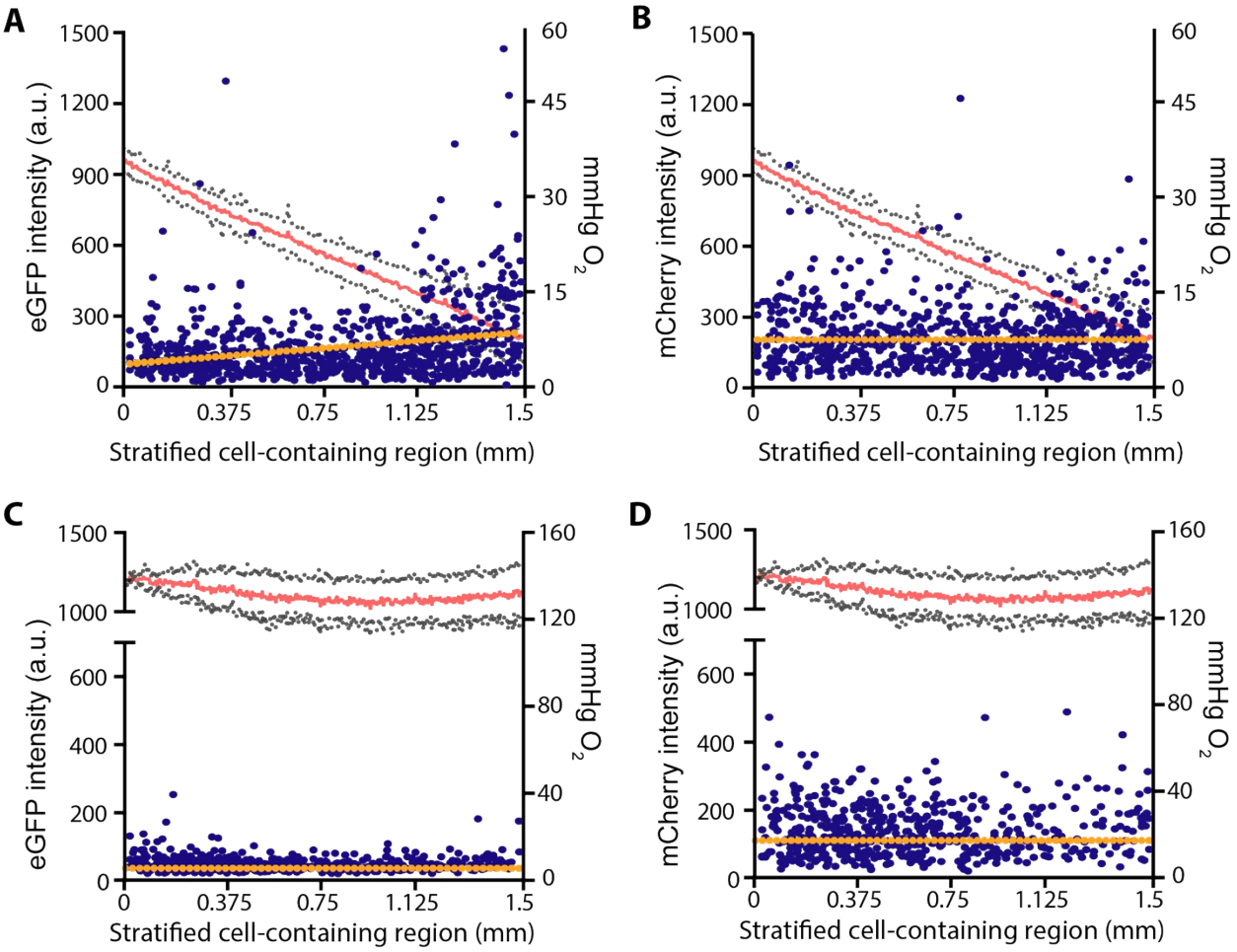

Figure S1. eGPF and mCherry fluorescence intensities of single M231-HRE cells at their respective location in the cell-containing regions of the BLOCCs. Each plot contains the corresponding oxygen profile plot across the cell-containing region, represented as the average (solid line) and standard deviation (dots) of the oxygen tension. A) eGFP and B) mCherry fluorescence intensities in an oxygen gradient formed from a deoxygenated gas mixture and a gas mixture containing 5% O_2. The eGFP expression exhibited a significant correlation relative to x-axis placement under the oxygen gradient (Pearson's $r = 0.263$, $P < 0.0001$). The mCherry expression did not exhibit a significant correlation relative to cellular position. C) eGFP and D) mCherry fluorescence intensities under ambient conditions, in the absence of an oxygen gradient. There was no significant correlation for either fluorescent protein relative to cellular position.

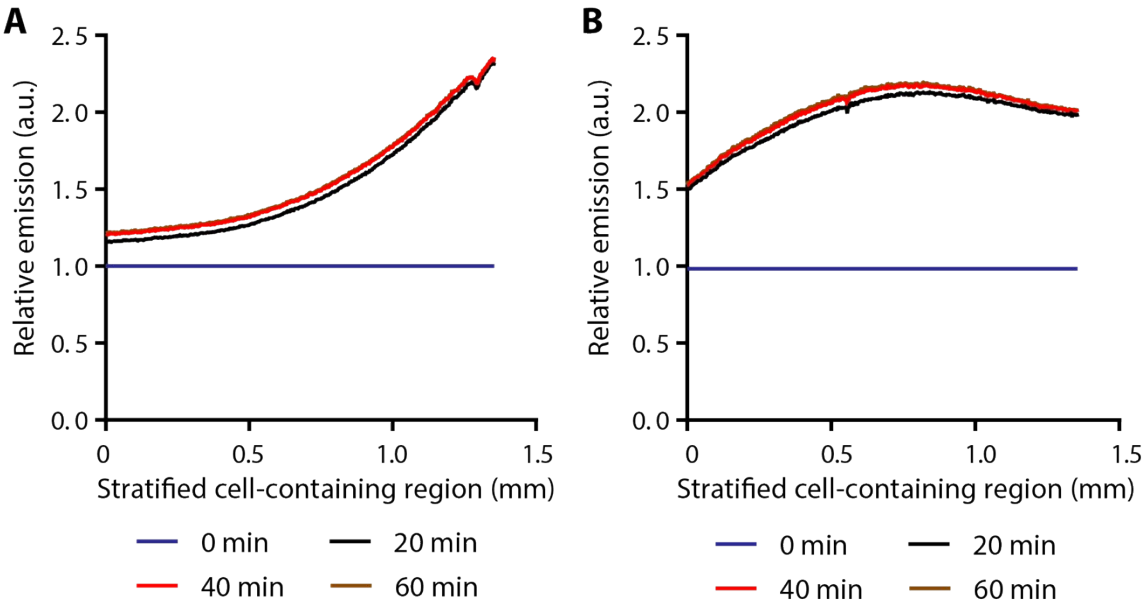

Figure S2. Equilibration times for generating stable oxygen gradients in the BLOCCs generated by flowing either A) 20% or B) 5% O_2 gas mixtures through the left fluidic channel and a 0% O_2 gas mixture through the right channel straddling the cell-containing region (component F, Figure 1 of main text). Emission intensities represent plot profiles parallel to the oxygen gradients as imaged directly from the oxygen sensing films. Fluorescence intensity values were normalized to the initial reading at ambient conditions. Both gas mixtures reached a steady state gradient within 60 min.