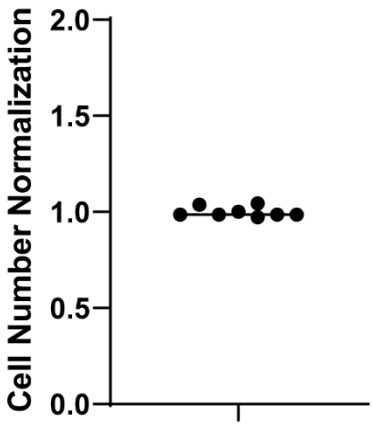
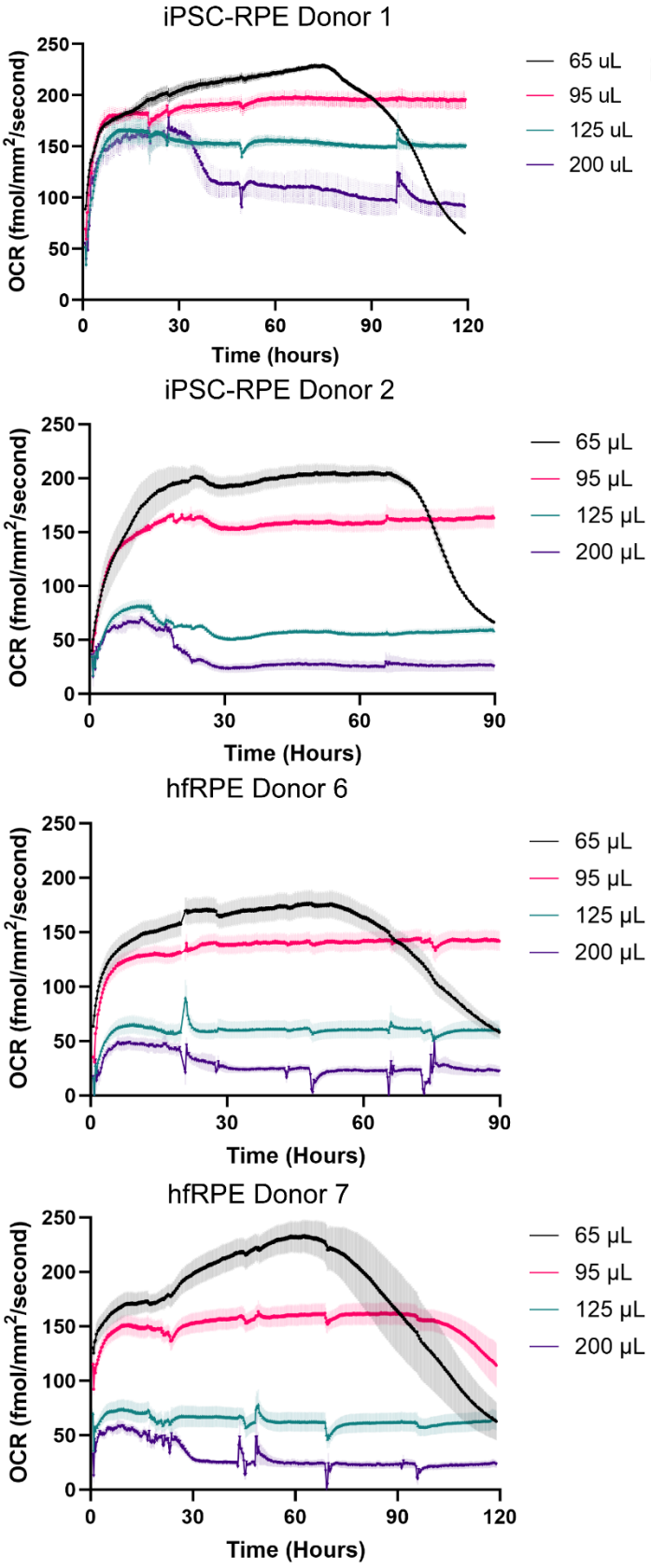
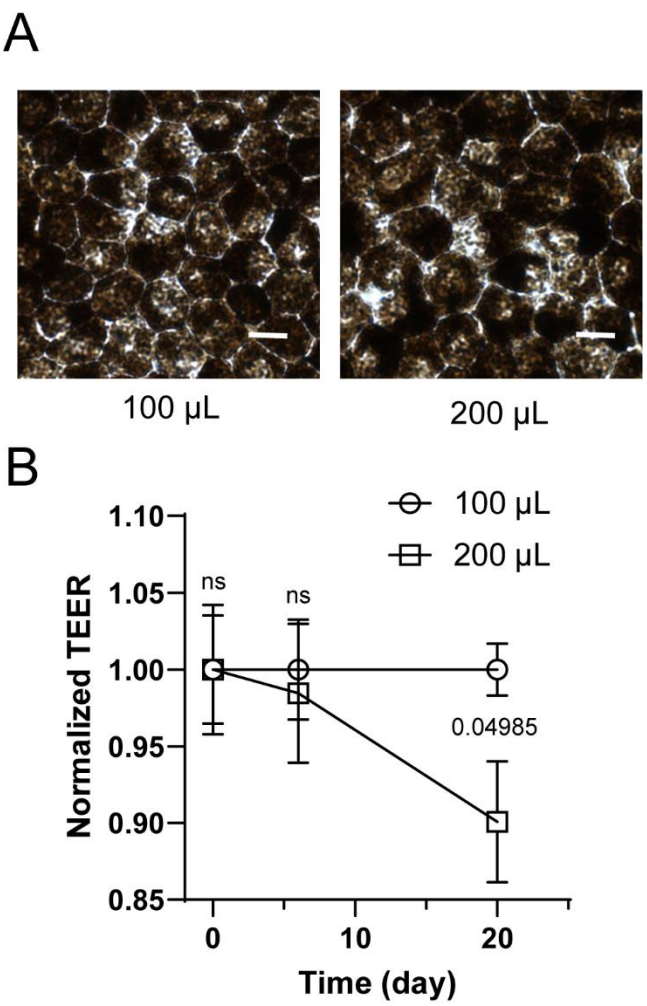


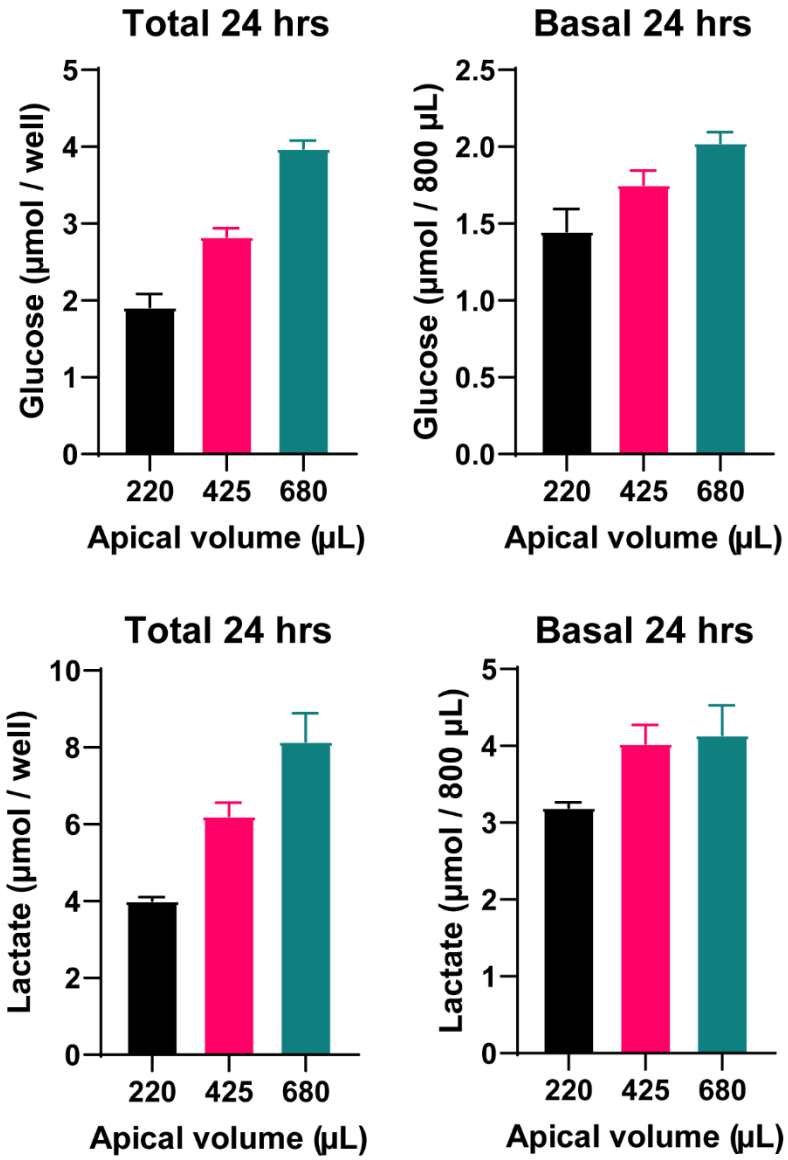
Supplemental Figure 1. Number of highly differentiated confluent RPE cells is consistent between wells. Manually counted cell numbers from same size images randomly taken in the center of 8 hfRPE culture wells were normalized to the average (donor 1).


iPSC-RPE Donor 1
OCR (fmol/mm2/second)
Time (hours)
65 uL
95 uL
125 uL
200 uL
iPSC-RPE Donor 2
OCR (fmol/mm2/second)
Time (Hours)
65 µL
95 µL
125 µL
200 µL
hfRPE Donor 6
OCR (fmol/mm2/second)
Time (Hours)
65 µL
95 µL
125 µL
200 µL
hfRPE Donor 7
OCR (fmol/mm2/second)
Time (Hours)
65 µL
95 µL
125 µL
200 µL

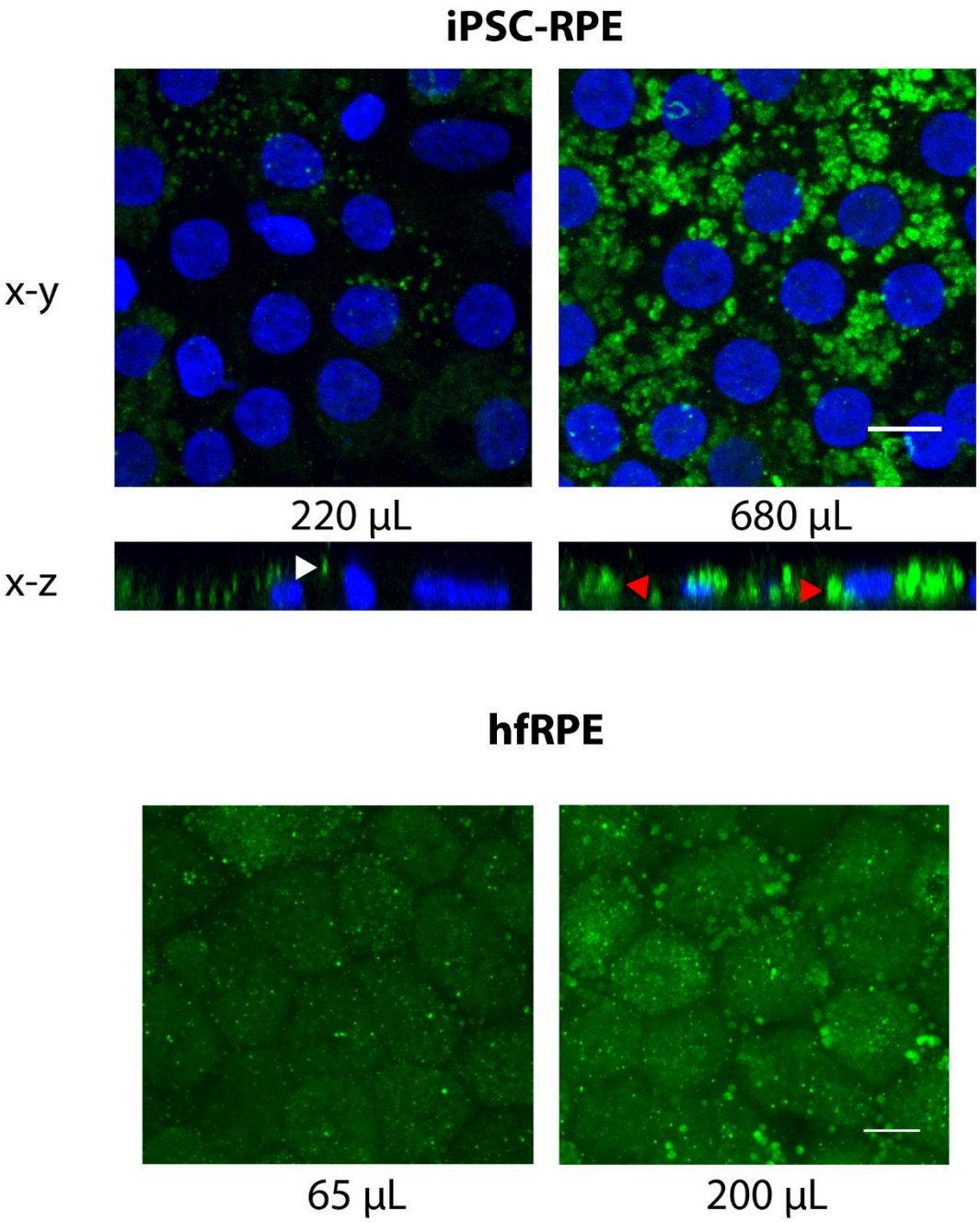
Supplemental Figure 2. Medium depth limits cellular O_2 availability and consumption rate in both iPSC-RPE and hfRPE cultures derived from multiple donors. OCR, as measured by Resipher device, over time for wells at different medium depths (65, 95, 125, 200 µL; n=6 wells/group). (Top) iPSC-RPE donors (iPSC-RPE donor 1, iPSC-RPE donor 2). (Bottom) Additional hfRPE donors (donor 6, donor 7). Spikes in the OCR represent opening and closing of the incubator door. Error bars represent +/- 1 standard deviation.



Supplemental Figure 3. Higher media volumes cause only mild alterations in typical markers of RPE health. (A) Brightfield images show similar polygonal shape and pigmentation for hfRPE under 100 µL and 200 µL apical (basal 600 µL) media volume after 3 weeks. Scale bar = 10 µm. **(B)** TEER was measured at day 0, 7, and 21 days after culturing in 100 µL or 200 µL apical media (basal 600 µL), with subtle differences emerging only after 3 weeks (note Y-axis does not start at 0). TEER readings were normalized to starting TEER value for the day 0, 100 µL value (n=6, donor 2). Absolute TEER values across all wells, timepoints, and media volumes ranged from 819 to 1146 $\Omega*cm^2$. ns = not statistically significant.

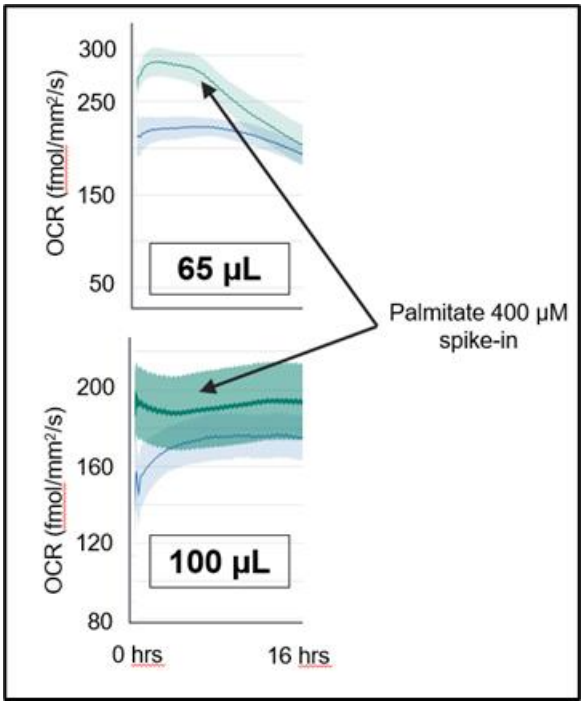


Supplemental Figure 4. Glycolysis is accelerated by increasing medium depth in human iPSC-RPE. Increasing medium volume limits cellular O_2 availability, increasing glucose consumption and lactate production. Left graphs, total glucose and lactate (apical and basal chambers combined). Right graphs, basal chamber only. Note that basal chamber analysis is not subject to confounding from mass action, as discussed in Figure 4. iPSC-RPE cultures are on 12-well Transwells, and volumes in the figure equivalently correspond to the volume/cell culture surface area ratios for hfRPE seen in Figure 2 (n=4, iPSC-RPE donor 1,3). *p≤0.05 for all graphs by one-way ANOVA.



Supplemental Figure 5. Media volume affects RPE lipid droplet size, number, and localization after lipid challenge in iPSC-RPE and an additional hfRPE donor. Immunostaining LD (ADRP/perilipin-2, green) for iPSC-RPE and hfRPE and nuclei (Hoechst 34580, blue) for iPSC-RPE under different media volumes containing palmitate. (Top) In iPSC-RPE, the larger LD that form under high volume are predominantly basolateral (red arrowhead). The smaller LD that form mainly under normoxia are more apically localized (white arrowhead). iPSC-RPE cultures are on 12-

well Transwells, and volumes in the figure equivalently correspond to the volume/cell culture surface area ratios for hfRPE seen in Figure 6 (iPSC-RPE donor 3). (Bottom) An additional hfRPE donor (donor 5) replicates the findings from Figure 6 and the iPSC-RPE cultures above. Cultures were in 24-well Transwells, which again equivalently correspond to the volume/cell culture surface area ratios for iPSC-RPE above and hfRPE in Figure 6. Scale bar= 10 µM.



Supplemental Figure 6. Unusually low media volumes may be required for metabolic processes that consume O_2 at a particularly high rate. Resipher plot of hfRPE in a 96-well with either 65 µL (top) or 100 µL (bottom) of media, after spike-in of 400 µM palmitate. Compared to wells that did not receive palmitate, there is only a modest rise in OCR for wells that received palmitate in 100 µL of media. In contrast, there is a dramatic spike in OCR for wells that received palmitate in 65 µL of media. β-oxidation of palmitate consumes significant oxygen, but the increase in OCR can only occur if media volume does not limit O_2 availability at the cells (hfRPE donor 5, n=6 for each condition).