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Supplemental information

Succinate metabolism in the retinal pigment epithelium uncouples respiration from ATP synthesis

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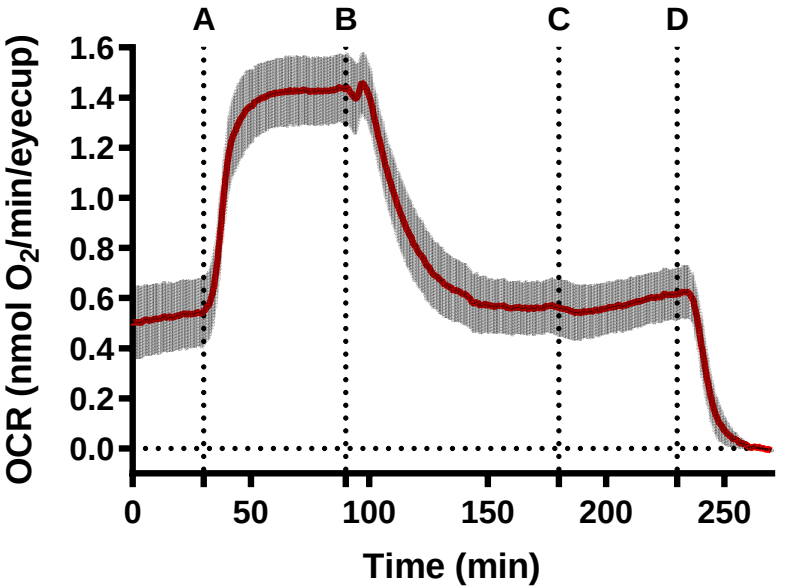

A: +5 mM succinate
B: -5 mM succinate
C: 500 μM *cis*-epoxysuccinate
D: KCN

Figure S1. SUCNR1 Agonism is insufficient to increase oxygen consumption.

Related to figure 1. We determined ex vivo O_2 consumption rate (OCR, mean±SEM) in ex vivo eyecup tissue exposed to 5 mM glucose (baseline), (A) 5 mM glucose + 5 mM succinate, (B) 5 mM glucose alone (to show the succinate effect is reversible), (C) with 5 mM glucose and 500 μM *cis*-epoxysuccinate (a SUCNR1 agonist with 10x greater potency than succinate) (Geubelle et al., 2017), and (D) KCN (to confirm that OCR in this experiment was mitochondrial). SUCNR1 agonism did not clearly affect OCR while succinate did. This suggests that OCR is not a consequence of SUCNR1-dependant protein signaling (n=4).

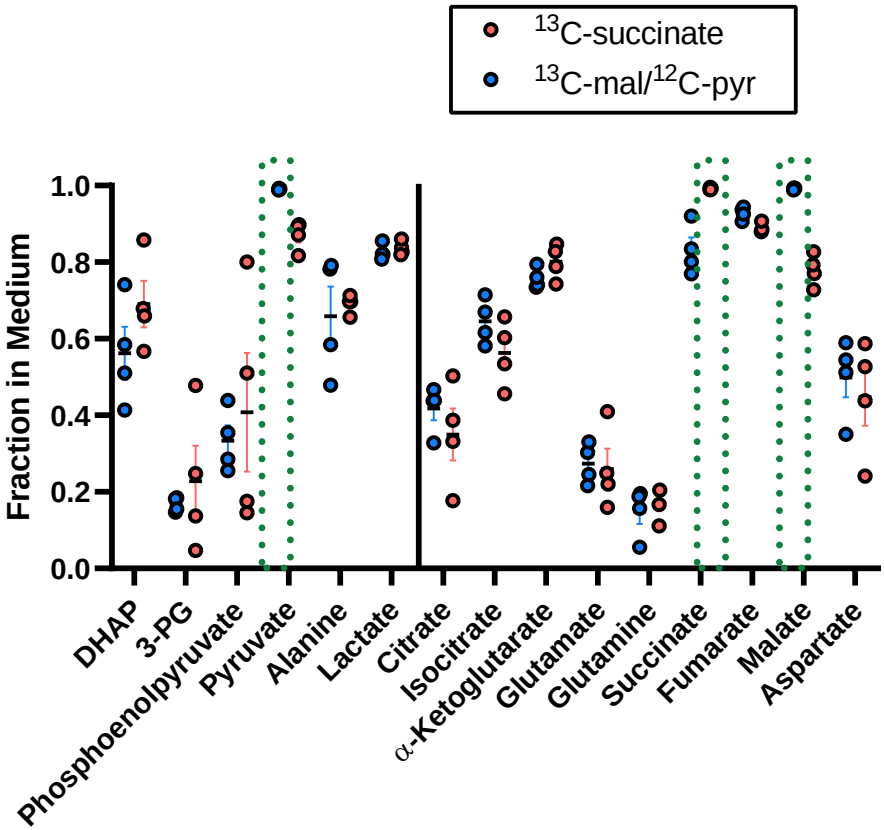

Figure S2. Metabolite compartmentalization between eyecup tissue and incubation medium. Related to Figure 2. We incubated eyecup tissue in 200 μL of KRB with either 5 mM ^{12}C-glucose, 1 mM ^{13}C malate (blue-filled circles), and 1 mM ^{12}C-pyruvate or 5 mM ^{12}C-glucose and 1 mM ^{13}C-succinate (red-filled circles) for 10 minutes. We snap-froze tissue and media samples in liquid N_2 before measuring metabolite levels. These levels are presented as a fraction of tissue + media for the same sample. A black line seperates glycolytic intermediates from TCA cycle intermediates and anapleurotic metabolites. Green dotted boxes surround metabolites that in the experiment were supplied in excess. If cells were fully permeabile, metabolites would be equally partitioned between media samples and tissue. Instead, some metabolites (3-phosphoglycerate, phosphoenolpyruvate, glutamate, glutamine) are preferentially present in tissues, while others (lactate α-ketoglutarate, pyruvate, malate, fumarate) are partially equillibrated with incubation medium. These data suggest that if cells are damaged and permeabilized, the propotion of those cells is at most 16% of cells in the tissue, as only 16% of the most compartmentalized metabolite (glutamine) is present in media samples.

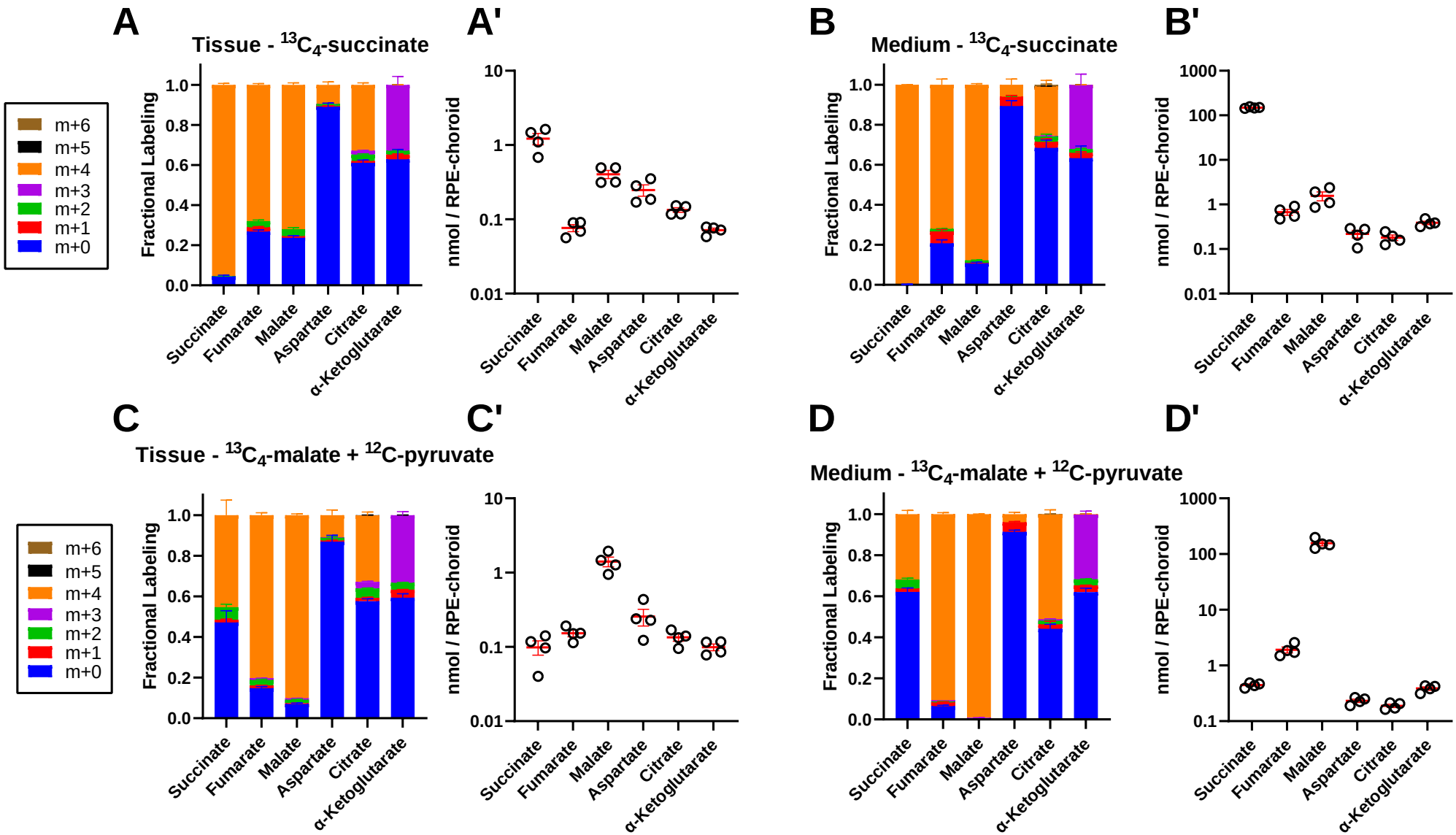

Figure S3. TCA cycle metabolite amounts and isotopic distribution following a 10 minute incubation in $^{13}C_4$-succinate or $^{13}C_4$-malate/^{12}C-pyruvate.
Related to fiigure 3. Each metabolic substrate was provided at 1 mM, in addition to 5 mM ^{12}C-glucose. A-D indicate the fraction of an indicated metabolite pool carrying one ^{13}C (m+1), two ^{13}C (m+2), etc. A'-D' indicate the total amount of each metabolite quantified in the tissue or culture medium. A, A', B, and B' were tissue or media incubated in $^{13}C_4$-succinate. C, C', D, and D' were tissue or media incubated in $^{13}C_4$-malate and ^{12}C-pyruvate. A, A', C, and C' are tissue samples, and B, B', D, and D' are matched media samples. All data show mean and SEM, with n=4 RPE-choroid tissue or media samples.

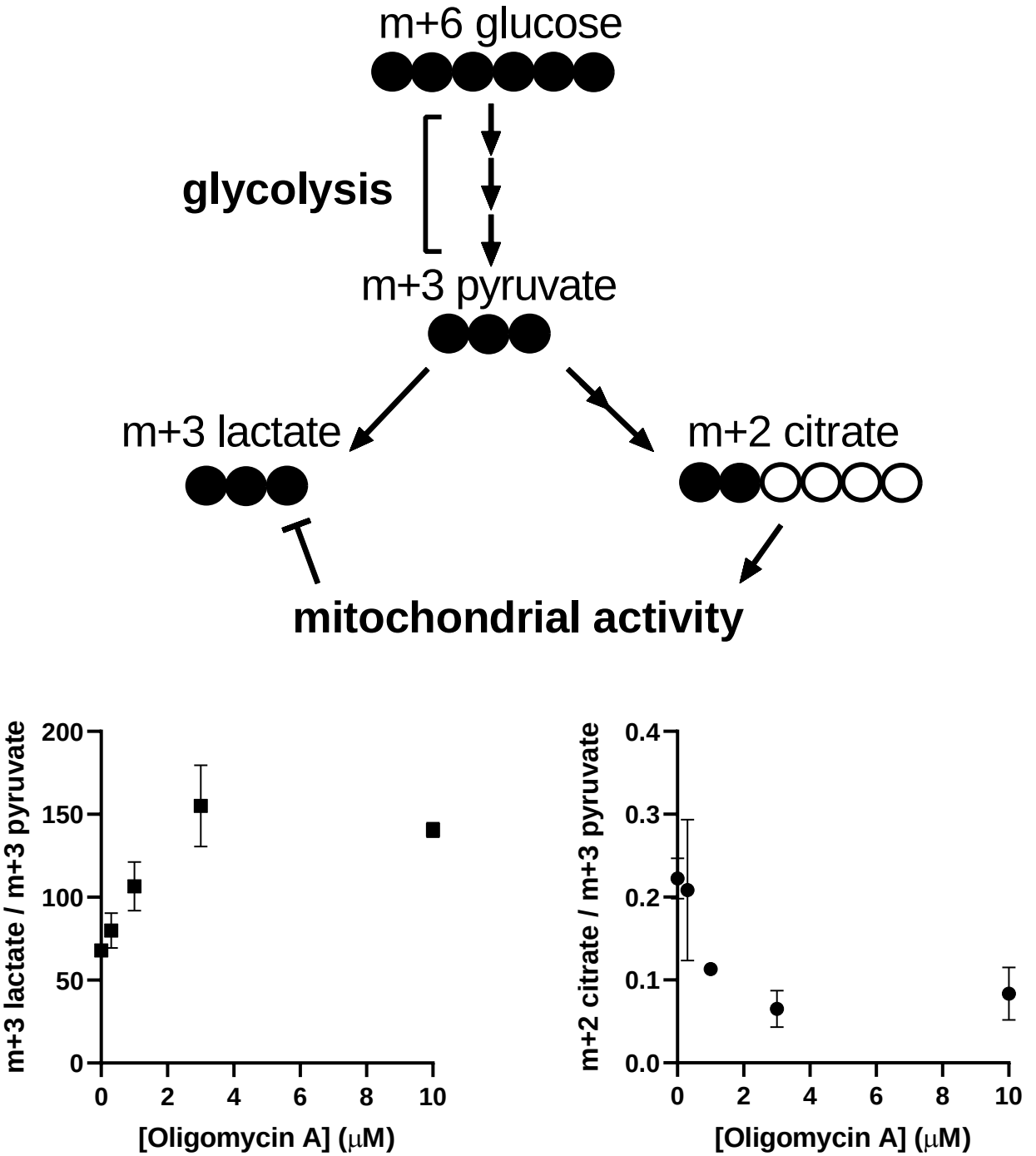

Figure S4. Determination of oligomycin concentrations for eyecup tissue. Related to figure 4. To determine a concentration of oligomycin that yields a maximal effect on mitochondrial activity, we incubated eyecup tissue in 5 mM U-^{13}C-glucose (m+6 labeled) for 10 minutes along with 0, 0.3, 1, 3, or 10 μM oligomycin. Glycolysis yields m+3 pyruvate from m+6 glucose, which can either become m+3 lactate or acetyl-CoA then citrate (both m+2). When mitochondria are active (without oligomycin inhibiting ATP-synthase), pyruvate is made into citrate. When mitochondria are inactive, pyruvate is not oxidized to acetyl-CoA and is instead reduced to lactate. We looked at formation of m+3 labeled lactate / m+3 pyruvate or m+2 labeled citrate / m+3 pyruvate as a function of [oligomycin], and at 3 or 10 μM oligomycin these ratios reach a steady state that is distinct from untreated controls.

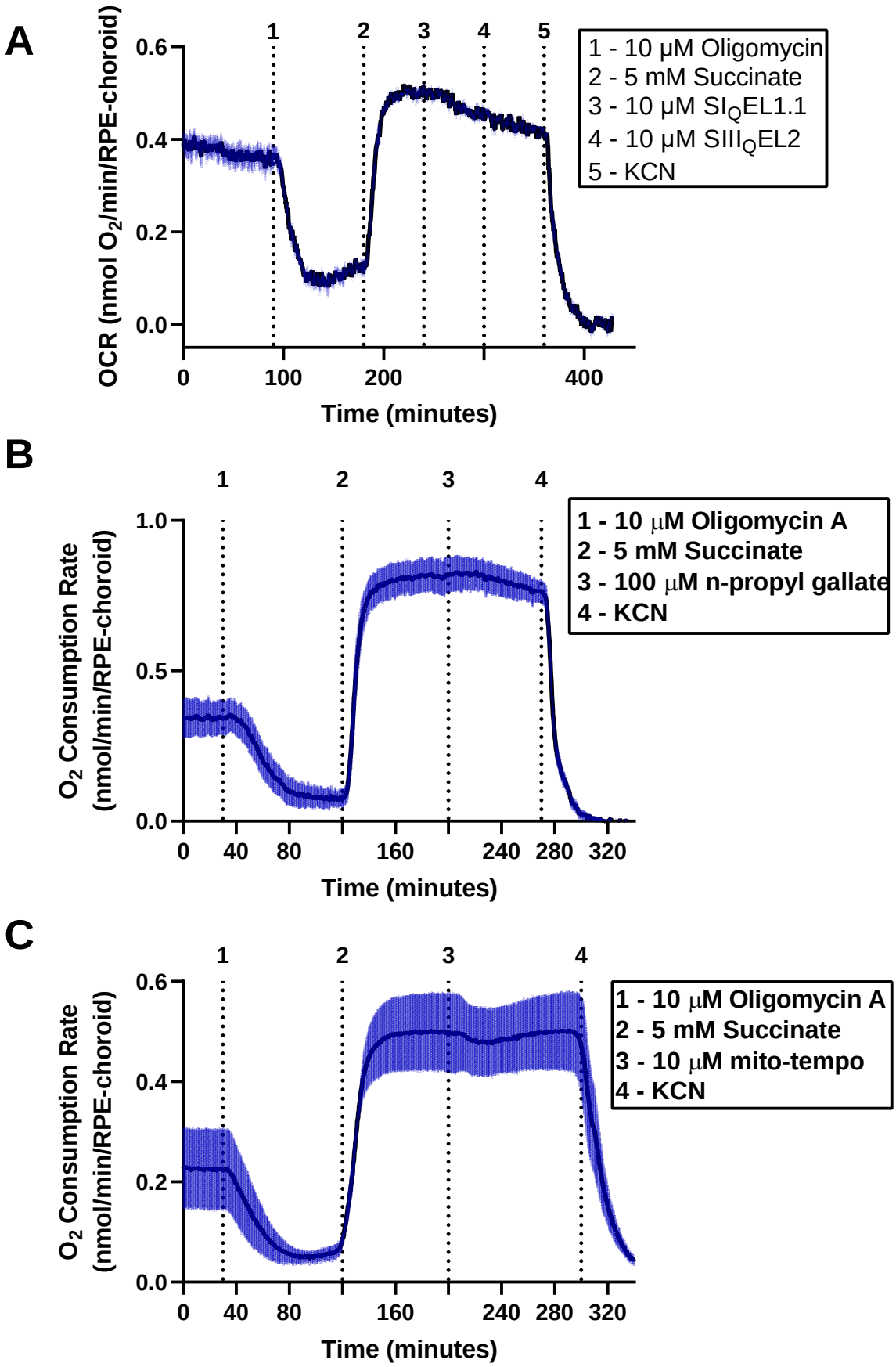

Figure S5. Supressors of electron leak and antioxidants do not clearly decrease succinate-driven uncoupled respiration. Related to figure 4. OCR measurements of eyecup tissue exposed to oligomycin then succinate was subjected to either (a) supressors of electron leak at sites I_Q or III_Q on the electron transport chain (n=3), (b) the antioxidant n-propyl gallate (n=4), or (c) the antioxidant mito-Tempo (n=4). For panel a, $SI_QEL1.1$ and $SIII_QEL2$ are provied as seperate treatments with each including oligomycin and succinate but not the other supressor of electron leak. For panels b and c, each treament is provided in addition to past treatments. All experiments are performed in freshly dissected C57BL6/J mouse RPE-choroid prepartations perifused with 5 mM glucose. Data are displayed as mean (black line) ± SEM (blue).

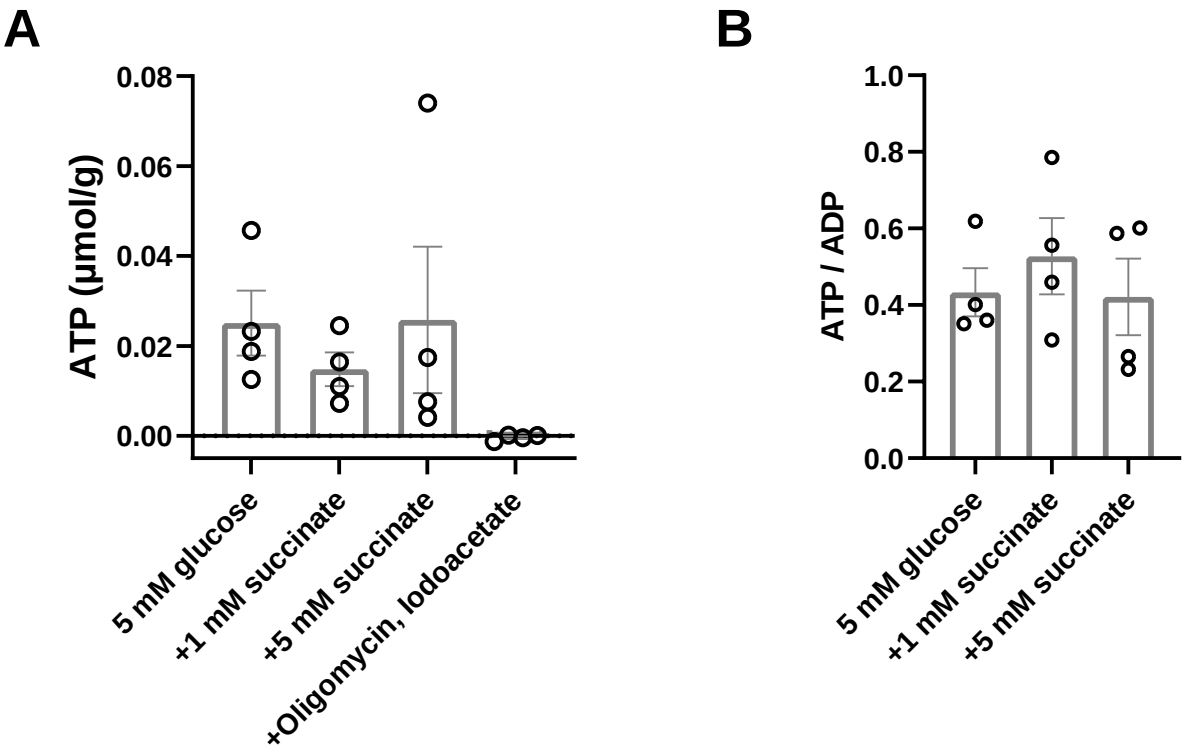

Figure S6. Succinate does not alter metrics of intracellular energetic status. Related to figure 4. Freshly dissected RPE-choroid preparations were incubated for 1 hour (at 37°C, 5% CO_2) in KRB supplemented with 5 mM glucose (control), 5 mM glucose and 1 mM succinate, 5 mM glucose and 5 mM succinate, or 5 mM glucose, 5 mM succinate, 20 µM oligomycin, and 20 µM iodoacetate (- control). We lysed tissue in boiling dH_2O to quench endogenous ATPase activity (Yang et al., 2002), then assessed ATP concentration and [ATP]/[ADP].