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Supplemental Information

Succinate Can Shuttle Reducing Power from the Hypoxic Retina to the O_2-Rich Pigment Epithelium

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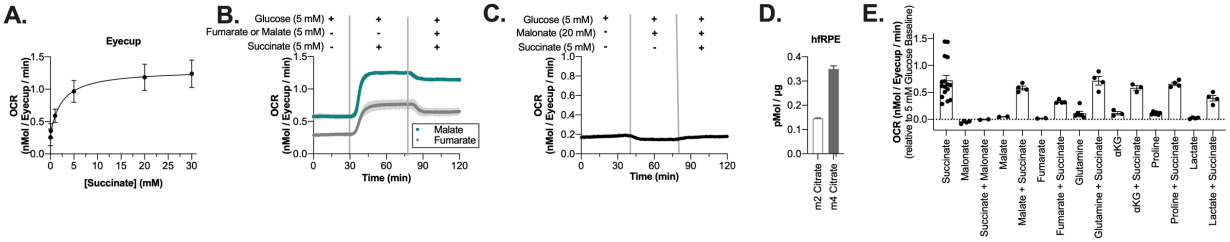
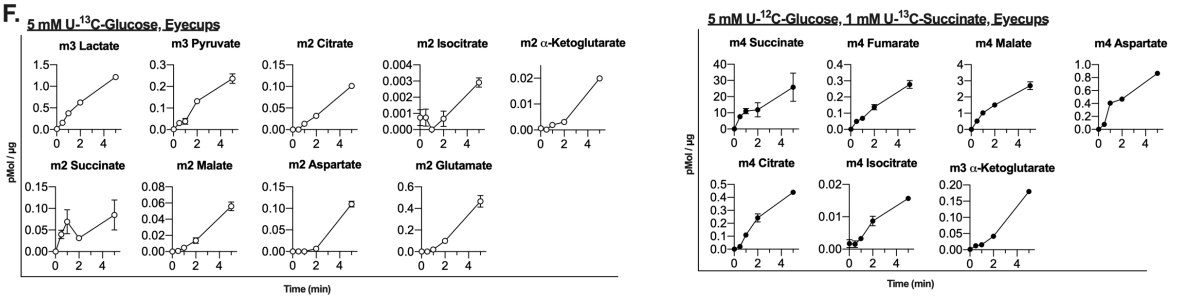
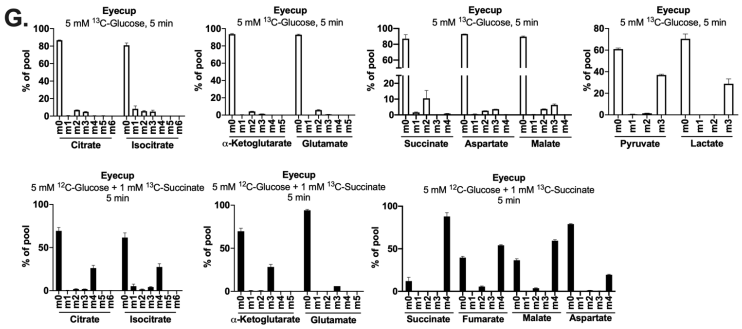
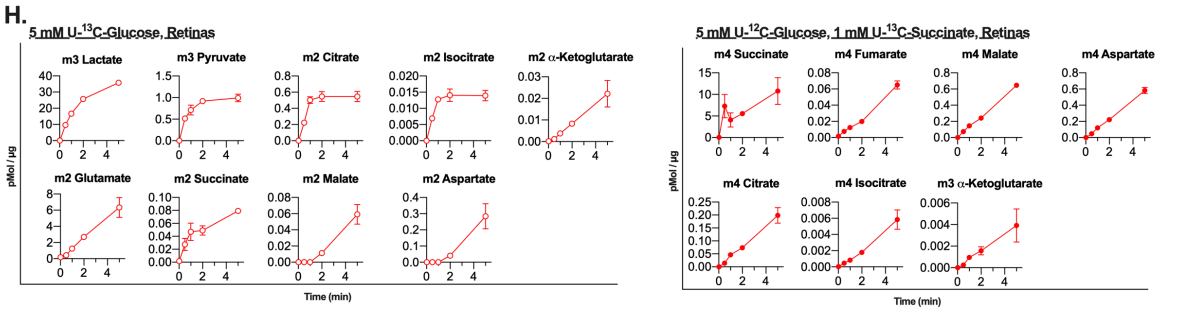
Figure S1


A.
Eyecup
OCR (nMol / Eyecup / min)
[Succinate] (mM)
B.
Glucose (5 mM)
Fumarate or Malate (5 mM)
Succinate (5 mM)
Malate
Fumarate
Time (min)
C.
Glucose (5 mM)
Malonate (20 mM)
Succinate (5 mM)
Time (min)
D.
hfRPE
pMol / µg
m2 Citrate
m4 Citrate
E.
OCR (nMol / Eyecup / min) (relative to 5 mM Glucose Baseline)
Succinate
Malonate
Succinate + Malonate
Malate
Malate + Succinate
Fumarate
Fumarate + Succinate
Glutamine
Glutamine + Succinate
αKG
αKG + Succinate
Proline
Proline + Succinate
Lactate
Lactate + Succinate


F.
5 mM U-13C-Glucose, Eyecups
m3 Lactate
m3 Pyruvate
m2 Citrate
m2 Isocitrate
m2 α-Ketoglutarate
m2 Succinate
m2 Malate
m2 Aspartate
m2 Glutamate
pMol / µg
Time (min)
5 mM U-12C-Glucose, 1 mM U-13C-Succinate, Eyecups
m4 Succinate
m4 Fumarate
m4 Malate
m4 Aspartate
m4 Citrate
m4 Isocitrate
m3 α-Ketoglutarate
pMol / µg
Time (min)


G.
Eyecup
5 mM 13C-Glucose, 5 min
% of pool
Citrate
Isocitrate
α-Ketoglutarate
Glutamate
Succinate
Aspartate
Malate
Pyruvate
Lactate
Eyecup
5 mM 12C-Glucose + 1 mM 13C-Succinate
5 min
Citrate
Isocitrate
α-Ketoglutarate
Glutamate
Succinate
Fumarate
Malate
Aspartate


H.
5 mM U-13C-Glucose, Retinas
m3 Lactate
m3 Pyruvate
m2 Citrate
m2 Isocitrate
m2 α-Ketoglutarate
m2 Glutamate
m2 Succinate
m2 Malate
m2 Aspartate
pMol / µg
Time (min)
5 mM U-12C-Glucose, 1 mM U-13C-Succinate, Retinas
m4 Succinate
m4 Fumarate
m4 Malate
m4 Aspartate
m4 Citrate
m4 Isocitrate
m3 α-Ketoglutarate
pMol / µg
Time (min)

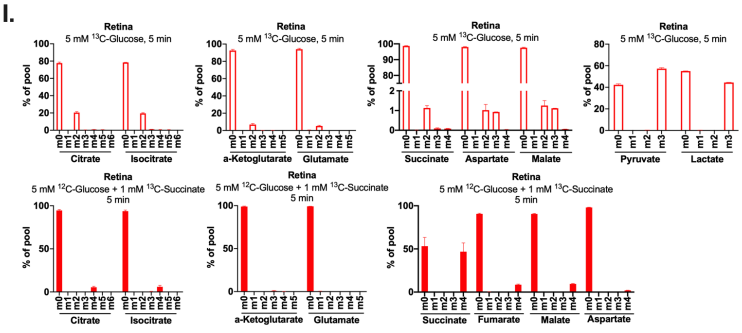

I.
Retina
5 mM 13C-Glucose, 5 min
% of pool
Citrate
Isocitrate
a-Ketoglutarate
Glutamate
Succinate
Aspartate
Malate
Pyruvate
Lactate
Retina
5 mM 12C-Glucose + 1 mM 13C-Succinate
5 min
Citrate
Isocitrate
a-Ketoglutarate
Glutamate
Succinate
Fumarate
Malate
Aspartate

Figure S1: Additional oxygen consumption experiments and raw traces (related to Figure 1)

A. Oxygen consumption of eyecup tissue perifused with 0, 0.1, 1, 5, 20, and 30 mM succinate in the presence of 5 mM glucose (n=3 chambers, error bars indicate SEM).
B. Oxygen consumption of eyecups perifused with media containing 5 mM glucose, then 5 mM glucose + 5 mM fumarate (gray) or malate (teal), then 5 mM glucose + 5 mM fumarate or malate + 5 mM succinate (n=2 chambers, error bars indicate SEM).
C. Raw trace of eyecups supplied with 5 mM glucose, then 5 mM glucose + 20 mM malonate, then 5 mM glucose + 20 mM malonate + 5 mM succinate. (n=2 chambers, error bars indicate SEM)
D. Citrate production in human fetal RPE (hfRPE) supplied with either 5 mM U-^{13}C-glucose alone (m2 Citrate) or 5 mM ^{12}C-glucose + 1 mM U-^{13}C-succinate (m4 Citrate) for 2 minutes (n=2 plates of cells, error bars indicate SEM).
E. Compiled change in OCR for eyecups supplied with various metabolites (relative to 5 mM glucose condition alone). All metabolites were supplied at 5 mM, with the exception of malonate which was supplied at 20 mM. All metabolites were supplied in the presence of 5 mM glucose. Succinate, malonate, and succinate + malonate conditions are duplicated from figure 1D for ease of comparison (each dot represents measurements taken from a single chamber, error bars indicate SEM).
F. Accumulation of labeled TCA cycle metabolites in eyecups supplied with 5 mM ^{12}C-glucose alone (left panel) or 5 mM ^{12}C-glucose + 1 mM U-^{13}C-succinate (right panel) (n=2 eyecups per time point, error bars indicate SEM).
G. Full isotopic distribution of metabolites in eyecups at the 5 minute time point from the experiments described in (F) (n=2 eyecups per time point, error bars indicate SEM).
H. Accumulation of labeled TCA cycle metabolites in retinas supplied with 5 mM ^{12}C-glucose alone (left panel) or 5 mM ^{12}C-glucose + 1 mM U-^{13}C-succinate (right panel). Of note, equilibration of α-ketoglutarate with the large, unlabeled glutamate pool in retinas makes causes the specific activity of the ^{13}C tracer to drop for metabolites after and including α-ketoglutarate in retinas supplied with U-^{13}C-glucose (Du et al., 2013) (n=2 retinas per time point, error bars indicate SEM).
I. Full isotopic distribution of metabolites in retinas at the 5 minute time point from the experiments described in (H) (n=2 retinas per time point, error bars indicate SEM).

Figure S2

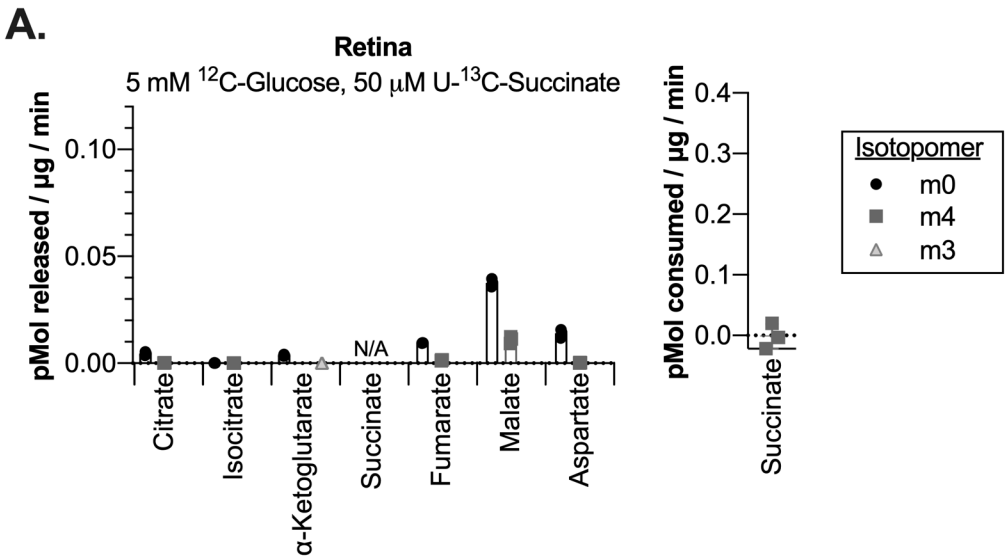


Figure S2: Metabolites exported by retinas supplemented with succinate (related to Figure 2)

A. Rate of TCA cycle metabolite release by retinas incubated in 5 mM ^{12}C-glucose + 50 μM U-^{13}C-succinate for 30 minutes. The rate of succinate consumption by retinas during incubation is shown on the right panel (n=3 retinas, error bars indicate SEM).

Figure S3

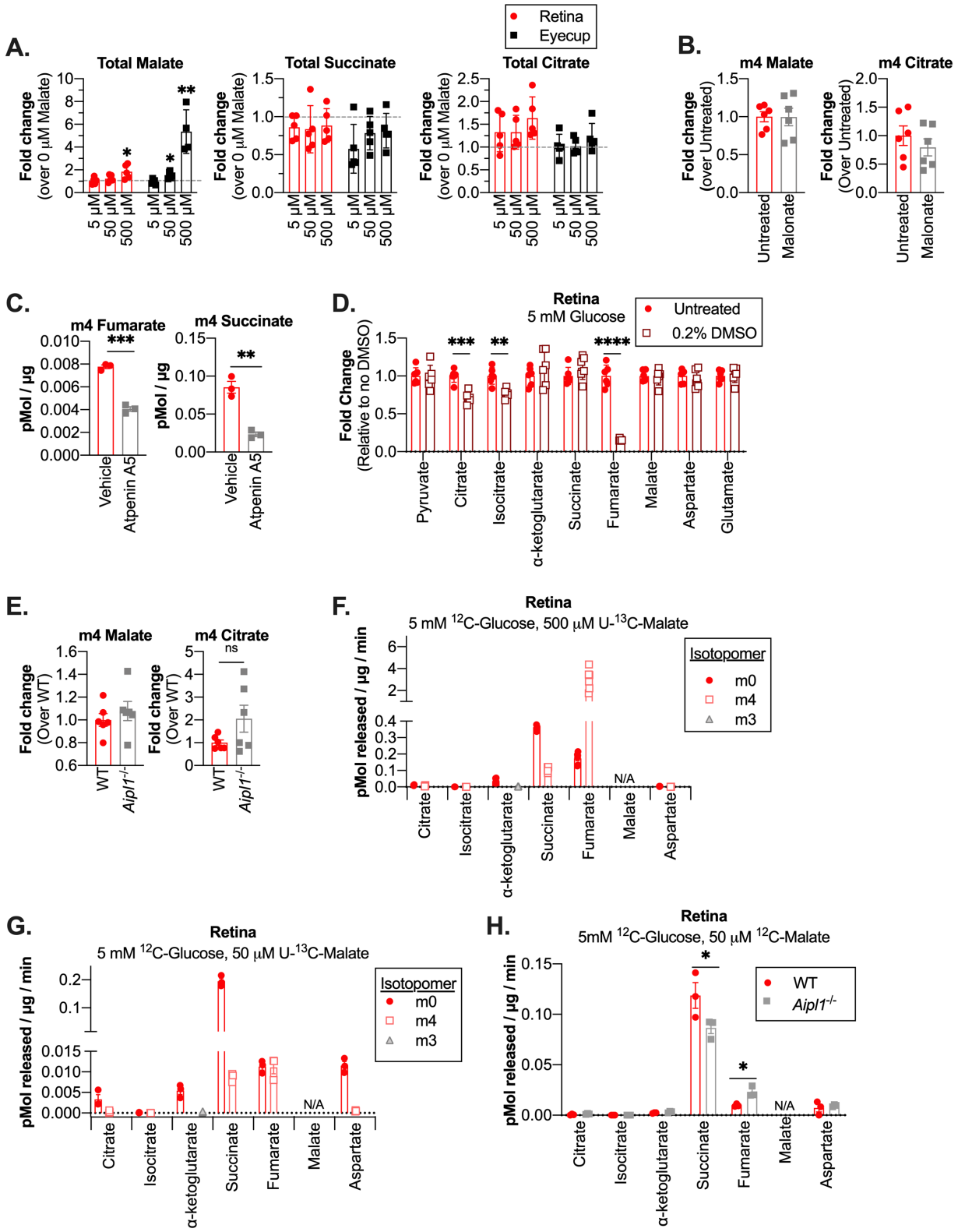
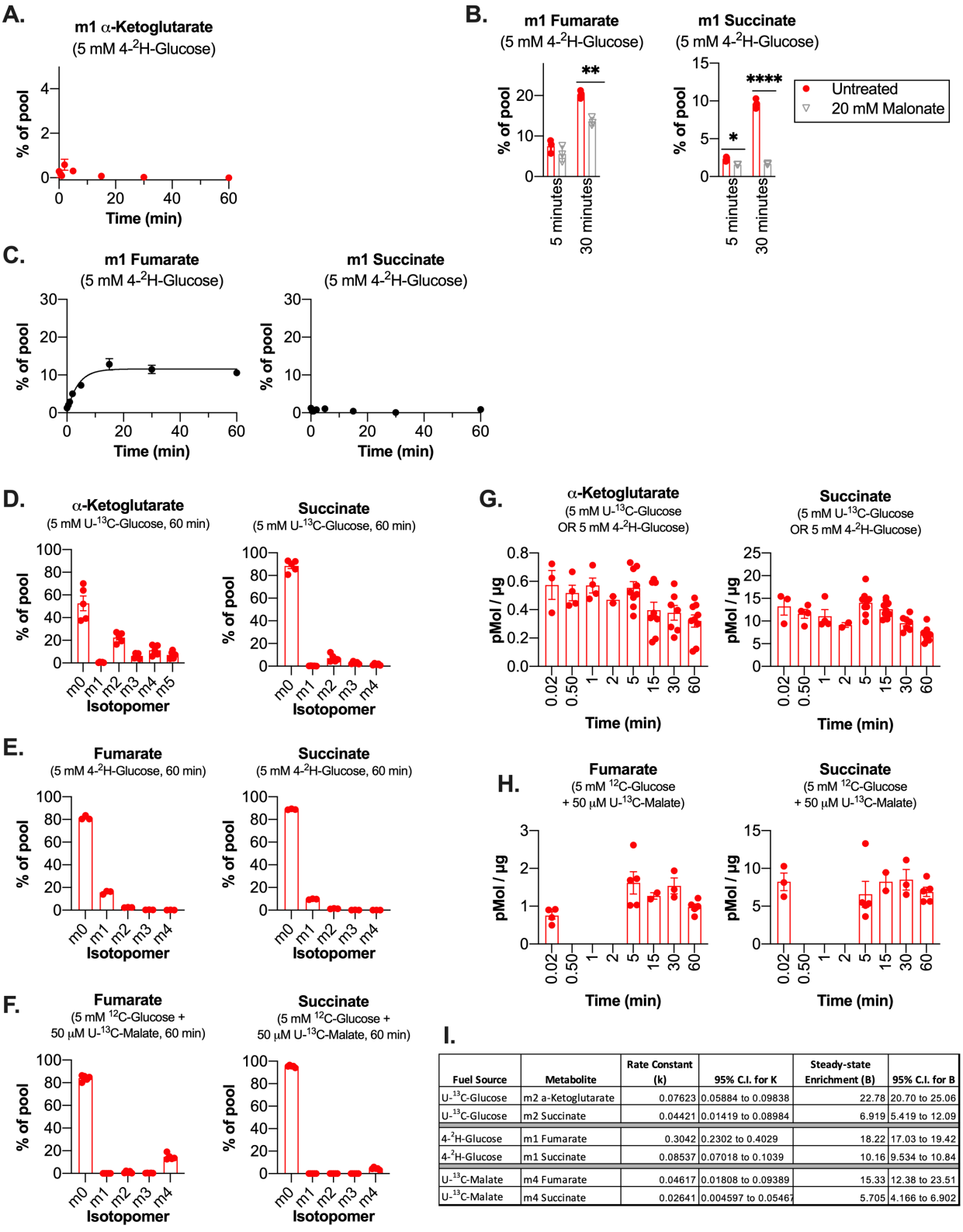

A.
Retina
Eyecup
Total Malate
Total Succinate
Total Citrate
Fold change (over 0 µM Malate)
5 µM
50 µM
500 µM
B.
m4 Malate
m4 Citrate
Fold change (over Untreated)
Untreated
Malonate
C.
m4 Fumarate
m4 Succinate
pMol / µg
Vehicle
Atpenin A5
D.
Retina
5 mM Glucose
Untreated
0.2% DMSO
Fold Change (Relative to no DMSO)
Pyruvate
Citrate
Isocitrate
α-ketoglutarate
Succinate
Fumarate
Malate
Aspartate
Glutamate
E.
m4 Malate
m4 Citrate
Fold change (Over WT)
WT
Aipl1-/-
ns
F.
Retina
5 mM 12C-Glucose, 500 µM U-13C-Malate
pMol released / µg / min
Isotopomer
m0
m4
m3
N/A
G.
Retina
5 mM 12C-Glucose, 50 µM U-13C-Malate
H.
Retina
5mM 12C-Glucose, 50 µM 12C-Malate
WT
Aipl1-/-

Figure S3: Additional validation of tracers used to observe reverse SDH activity in retinas (related to Figure 3)

A. Total malate, succinate, and citrate levels in retinas and eyecups incubated in 5 mM ^{12}C-glucose and 5, 50, or 500 μM U-^{13}C-malate for 5 minutes (n=5 retinas or eyecups per concentration, error bars indicate SEM).
B. m4 malate and m4 citrate levels in retinas incubated in 5 mM ^{12}C-glucose + 50 μM U-^{13}C-malate in the presence or absence of malonate for 5 minutes (relative to untreated) (n=6 retinas, error bars indicate SEM).
C. m4 fumarate and m4 succinate levels in retinas incubated in 5 mM ^{12}C-glucose + 50 μM U-^{13}C-malate and either 2 μM Atpenin A5 or vehicle (0.2% DMSO) for 5 minutes (n=3 retinas, error bars indicate SEM).
D. Total metabolite levels in retinas incubated in 5 mM ^{12}C-glucose in the presence or absence of 0.2% DMSO for 5 minutes (n=6 retinas, error bars indicate SEM).
E. m4 malate and m4 citrate produced by WT and *Aipl1*$^{-/-}$ retinas supplied with 5 mM ^{12}C-glucose + 50 μM U-^{13}C-malate for 5 minutes (n=6 retinas, error bars indicate SEM).
F. Rate of TCA cycle metabolite release by retinas incubated in 5 mM ^{12}C-glucose + 500 μM U-^{13}C-malate for 60 and 90 minutes (n=6 retinas, 3 per time point, error bars indicate SEM).
G. Rate of TCA cycle metabolite release by retinas incubated in 5 mM ^{12}C-glucose + 50 μM U-^{13}C-malate for 30 minutes (n=3 retinas, error bars indicate SEM).
H. Rate of TCA cycle metabolite release by WT and *Aipl1*$^{-/-}$ retinas incubated in 5 mM ^{12}C-glucose + 50 μM ^{12}C-malate for 30 minutes (n=3 retinas, error bars indicate SEM).

Figure S4



I.

Fuel Source	Metabolite	Rate Constant (k)	95% C.I. for K	Steady-state Enrichment (B)	95% C.I. for B
U-^{13}C-Glucose	m2 a-Ketoglutarate	0.07623	0.05884 to 0.09838	22.78	20.70 to 25.06
U-^{13}C-Glucose	m2 Succinate	0.04421	0.01419 to 0.08984	6.919	5.419 to 12.09
4-^{2}H-Glucose	m1 Fumarate	0.3042	0.2302 to 0.4029	18.22	17.03 to 19.42
4-^{2}H-Glucose	m1 Succinate	0.08537	0.07018 to 0.1039	10.16	9.534 to 10.84
U-^{13}C-Malate	m4 Fumarate	0.04617	0.01808 to 0.09389	15.33	12.38 to 23.51
U-^{13}C-Malate	m4 Succinate	0.02641	0.004597 to 0.05467	5.705	4.166 to 6.902

Figure S4: Additional controls and isotopomers from extended ^{13}C-glucose, 4-^{2}H-glucose, and ^{13}C-malate time courses (related to Figure 4)

A. m1 α-ketoglutarate signal from retinas supplied with 5 mM 4-^{2}H-glucose for 0.02, 0.5, 1, 2, 5, 15, 30, and 60 minutes (n=3 retinas per time point, error bars indicate SEM).
B. % enrichment of m1 fumarate and m1 succinate in retinas incubated in 5 mM 4-^{2}H-glucose for 5 and 30 minutes, in the presence or absence of 20 mM malonate (n=3 retinas per time point, error bars indicate SEM).
C. % enrichment of m1 fumarate and m1 succinate in eyecups incubated in 5 mM 4-^{2}H-glucose for 0.02, 0.5, 1, 2, 5, 15, 30, and 60 minutes (n=3 eyecups per time point, error bars indicate SEM)
D. Fractional enrichment for all isotopomers of α-ketoglutarate and succinate in retinas supplied with 5 mM U-^{13}C-glucose for 60 minutes (n=5 retinas, error bars indicate SEM).
E. Fractional enrichment for all isotopomers of fumarate and succinate in retinas supplied with 5 mM 4-^{2}H-glucose for 60 minutes (n=3 retinas, error bars indicate SEM).
F. Fractional enrichment for all isotopomers of fumarate and succinate in retinas supplied with 5 mM ^{12}C-glucose + 50 μM U-^{13}C-malate for 60 minutes (n=5 retinas, error bars indicate SEM).
G. Total succinate and α-ketoglutarate levels in retinas supplied with 5 mM glucose for 0.02, 0.5, 1, 2, 5, 15, 30, and 60 minutes (n=3 to 9 retinas per time point, error bars indicate SEM).
H. Total fumarate and succinate levels in retinas supplied with 5 mM glucose + 50 uM malate for 0.02, 0.5, 1, 2, 5, 15, 30, and 60 minutes (n=3 to 5 retinas per timepoint, error bars indicate SEM).
I. Table of constants and confidence intervals calculated by Graphpad Prism using the equation $\%metabolite = B * (1 - e^{-kt})$ to fit each curve. B is the fractional enrichment at the steady state, k is the rate constant for the reaction, t is time, and %metabolite is the fractional enrichment at a given time.

Figure S5

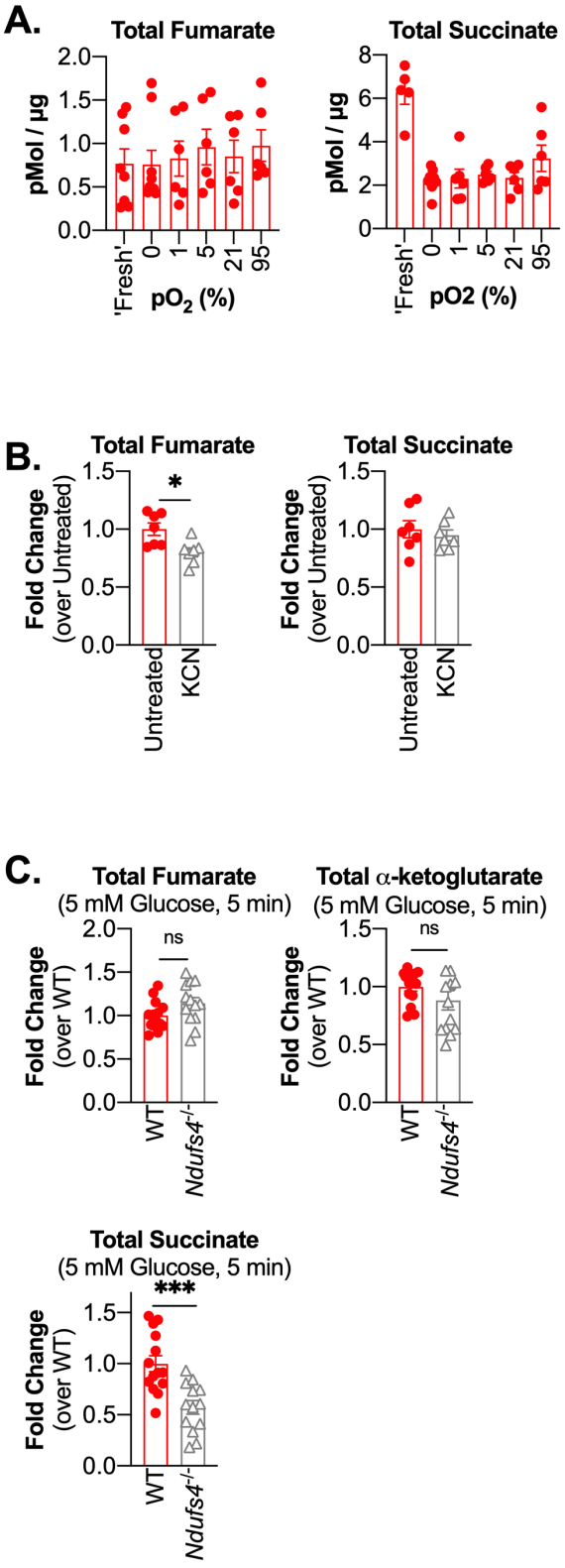

A.
Total Fumarate
pMol / µg
2.0
1.5
1.0
0.5
0.0
'Fresh'
0
1
5
21
95
pO2 (%)
Total Succinate
pMol / µg
8
6
4
2
0
'Fresh'
0
1
5
21
95
pO2 (%)
B.
Total Fumarate
Fold Change (over Untreated)
1.5
1.0
0.5
0.0
*
Untreated
KCN
Total Succinate
Fold Change (over Untreated)
1.5
1.0
0.5
0.0
Untreated
KCN
C.
Total Fumarate
(5 mM Glucose, 5 min)
Fold Change (over WT)
2.0
1.5
1.0
0.5
0.0
ns
WT
Ndufs4-/-
Total α-ketoglutarate
(5 mM Glucose, 5 min)
Fold Change (over WT)
1.5
1.0
0.5
0.0
ns
WT
Ndufs4-/-
Total Succinate
(5 mM Glucose, 5 min)
Fold Change (over WT)
1.5
1.0
0.5
0.0
***
WT
Ndufs4-/-

Figure S5: Total metabolite levels from incubations at varied pO_2 and *Ndufs4*$^{-/-}$ retinas (related to Figure 5)

A. Total fumarate and succinate levels in retinas incubated in 5 mM ^{12}C-glucose + 50 μM U-^{13}C-malate for 5 minutes. "Fresh" indicates that retinas were incubated immediately after dissection. "pO_2" indicates that retinas were incubated at the specified level of oxygen for 2 hours (in 5 mM ^{12}C-glucose) prior to incubation in labeled malate (n=6 to 9 retinas per pO2 condition, error bars indicate SEM).
B. Total fumarate and succinate levels in retinas supplied with 5 mM ^{12}C-glucose + 50 μM U-^{13}C-malate in the presence of 3 mM KCN (n=6 retinas, error bars indicate SEM).
C. Total fumarate, succinate, and α-ketoglutarate levels in WT and *Ndufs4*$^{-/-}$ retinas supplied with 5 mM glucose for 5 min (n=14 WT and 13 *Ndufs4*$^{-/-}$ retinas, error bars indicate SEM).