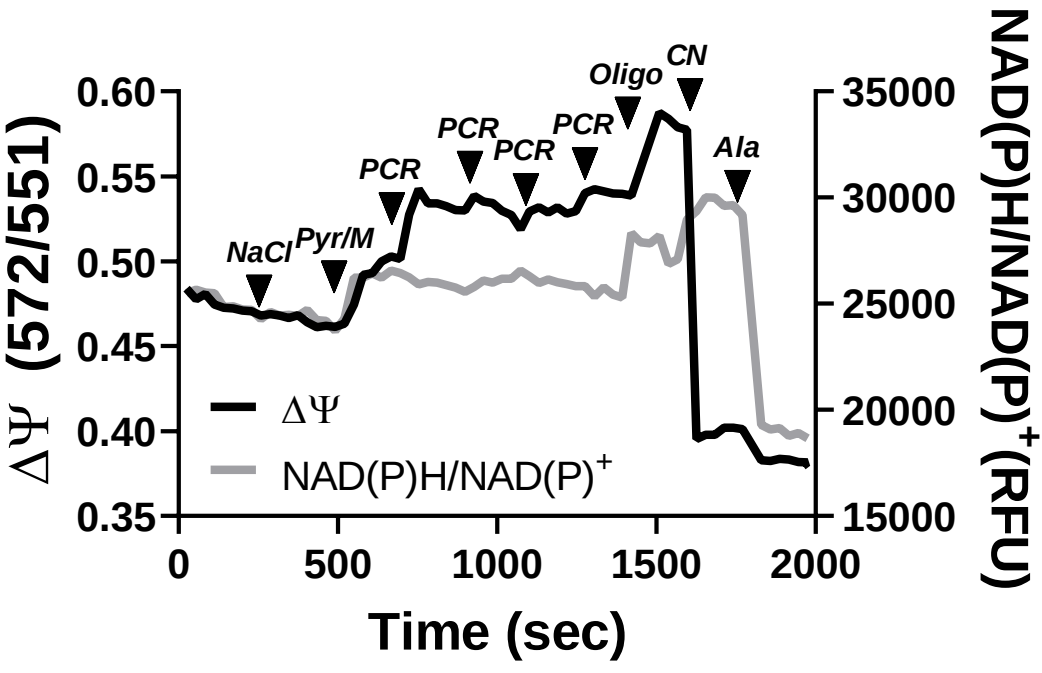
**Supplementary Figure 1, related to the Experimental Methods.** Representative trace of ΔΨ (depicted as the 572/551 ratio) and NAD(P)H/NAD(P)$^+$ redox (expressed as RFU, Ex:Em, 350:450). Final values for each measurement were normalized based on the values recorded following a final addition of alamethicin ('Ala').

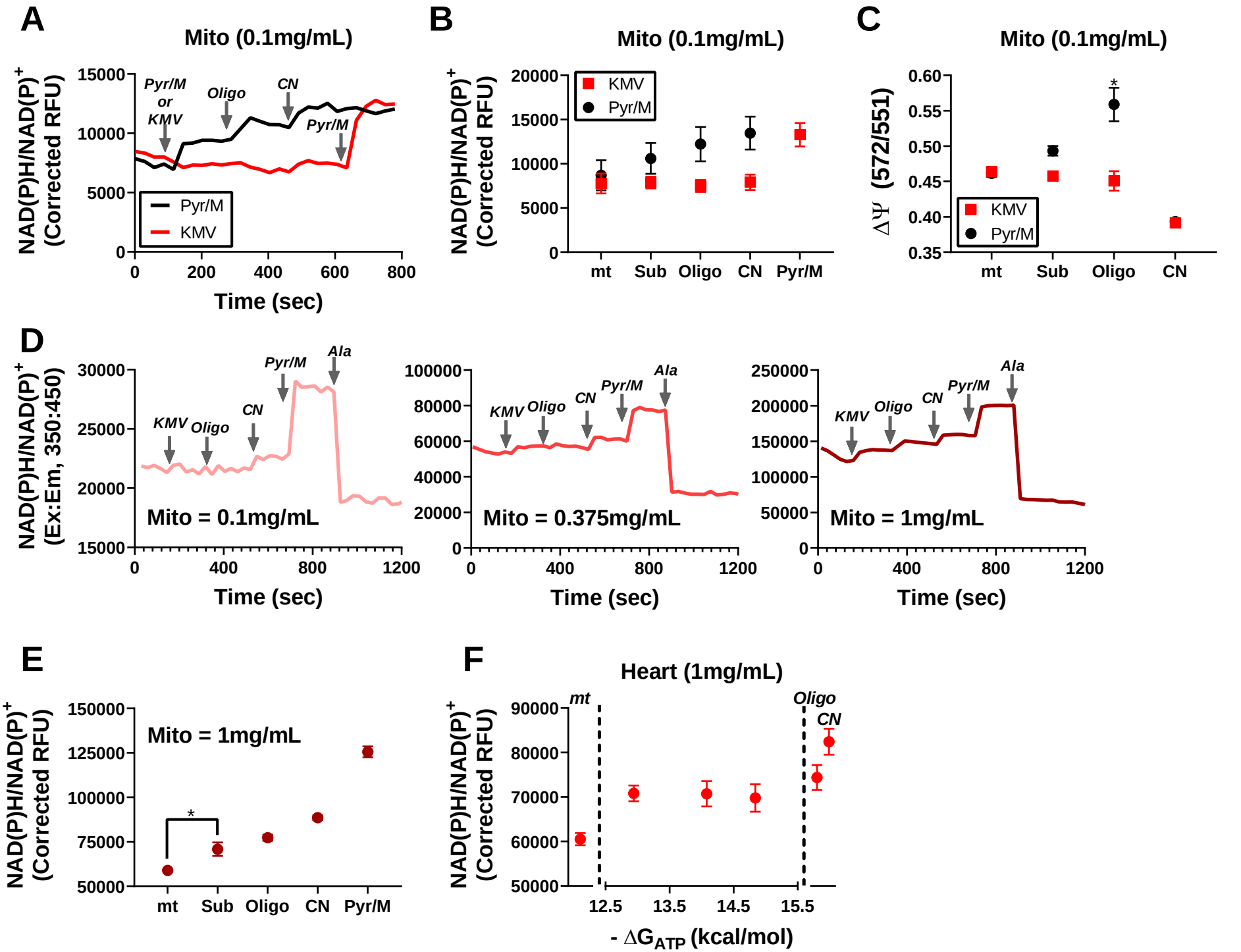
**Supplementary Figure 2, related to Figure 2.** All experiments were performed in isolated heart mitochondria at varying concentrations (0.1, 0.375, 1mg/mL) incubated with the CK clamp established with a starting PCR concentration of 1mM. Bicarbonate was present for all experiments. (**A**) Representative trace depicting NAD(P)H/NAD(P)$^+$ redox following the sequential addition of KMV or Pyr/M, Oligo and cyanide (CN). Pyr/M was added to KMV energized mitochondria at the end of the protocol. (**B**) Quantification of the data depicted in panel A. (**D**) Representative trace depicting NAD(P)H/NAD(P)$^+$ redox following the sequential addition of KMV, Oligo, CN, Pyr/M and Ala. The mitochondrial concentration for each experiment was 0.1mg/mL, 0.375mg/mL or 1mg/mL. (**E**) Quantification of the data depicted in panel C for 1mg/mL mitochondria. Note the increase in redox state from mitochondria alone ('mt') to KMV ('Sub'). (**F**) Relationship between NAD(P)H/NAD(P)$^+$ redox and ATP free energy in KMV energized mitochondria (1mg/mL). Data are mean ± SEM, n=4/group, *P<0.05.

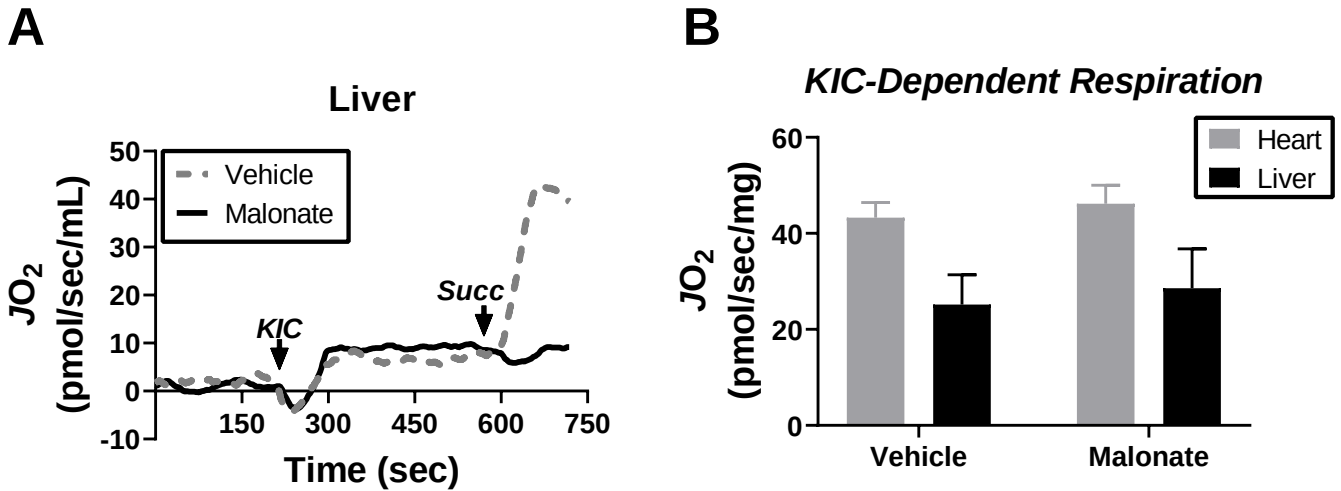
**Supplementary Figure 3, related to Figure 3.** All experiments were performed in isolated heart and liver mitochondria incubated with the CK clamp established with a starting PCR concentration of 21mM. Bicarbonate was present for all experiments. Experiments were performed either in the presence of 5mM malonate or vehicle control. (**A**) Representative trace depicting $JO_2$ in liver mitochondria in the absence and presence of 5mM malonate in response to KIC (2mM) and succinate (1mM). (**B**) Quantification of the KIC-dependent $JO_2$ in the absence and presence of 5mM malonate in heart and liver mitochondria. KIC-dependent respiration was quantified by subtracting the oxygen consumption prior to the addition of KIC from the rates recorded in response to KIC. Data are mean ± SEM, n=3-4/group.

**Supplementary Table 1.** Table indicating the concentrations of each substrate combination and inhibitor used for all experiments.

| Substrate Conditions | Concentration |
|---|---|
| Pyruvate/Malate/Glutamate/Succinate/Octanoyl-carnitine (P/M/G/S/O) | Pyr: 5mM, G: 5mM, M: 2mM, S: 5mM, Oct: 0.2mM |
| Malate (Mal or M) | 2mM |
| Ketoisovalerate (KIV) | 2mM |
| Ketoisocaproate (KIC) | 2mM |
| Ketomethylvalerate (KMV) | 2mM |
| Malonate (Malo) | 5mM |
| Pyruvate (Pyr) | 5mM |
| Glutamate (G) | 10mM |
| Succinate (Succ) | 10mM |
| Octonoyl-Carnitine (Oct) | 0.2mM |
| Rotenone (Rot) | 0.005mM |
| Antimycin A (AntA) | 0.005mM |
| Carbonyl cyanide-p-trifluoromethoxyphenylhydrazone (FCCP) | 0.002mM |
| Cyanide (Cyn) | 10mM |
| Alamethicin (Ala) | 0.03mg/mL |
| Oligomycin (Oligo) | 0.005mM |
| Alpha-Ketoglutarate (AKG) | 10mM |
| NaCl | 3mM |
| BCKAs | KIC, KMV, KIV: 1mM |
| Isocitrate | 5mM |
| Carnitine (Carn) | 5mM |


ΔΨ (572/551)
NAD(P)H/NAD(P)+ (RFU)
Time (sec)
0.60
0.55
0.50
0.45
0.40
0.35
35000
30000
25000
20000
15000
0
500
1000
1500
2000
NaCl
Pyr/M
PCR
PCR
PCR
PCR
Oligo
CN
Ala
ΔΨ
NAD(P)H/NAD(P)+


A
Mito (0.1mg/mL)
NAD(P)H/NAD(P)+ (Corrected RFU)
Pyr/M or KMV
Oligo
CN
Pyr/M
Pyr/M
KMV
Time (sec)
B
Mito (0.1mg/mL)
NAD(P)H/NAD(P)+ (Corrected RFU)
KMV
Pyr/M
mt
Sub
Oligo
CN
Pyr/M
C
Mito (0.1mg/mL)
ΔΨ (572/551)
KMV
Pyr/M
mt
Sub
Oligo
CN
D
NAD(P)H/NAD(P)+ (Ex:Em, 350:450)
KMV
Oligo
CN
Pyr/M
Ala
Mito = 0.1mg/mL
Time (sec)
Mito = 0.375mg/mL
Time (sec)
Mito = 1mg/mL
Time (sec)
E
NAD(P)H/NAD(P)+ (Corrected RFU)
Mito = 1mg/mL
mt
Sub
Oligo
CN
Pyr/M
F
Heart (1mg/mL)
NAD(P)H/NAD(P)+ (Corrected RFU)
mt
Oligo
CN
- ΔGATP (kcal/mol)


A
Liver
Vehicle
Malonate
KIC
Succ
JO2
(pmol/sec/mL)
50
40
30
20
10
0
-10
150
300
450
600
750
Time (sec)
B
KIC-Dependent Respiration
Heart
Liver
JO2
(pmol/sec/mg)
60
40
20
0
Vehicle
Malonate