

Online Supplemental Data

Preservation of mitochondrial function in explanted fresh hearts

To test the preservation of mitochondrial function in BIOPS over time, freshly explanted nonfailing heart tissue was kept in BIOPS at 4°C, with respirometry experiments performed on the day of explant and 3 days later. Online Figure 1 A-C shows respirometry in nonfailing tissue with additions of pyruvate, malate plus ADP, glutamate (CI), and succinate (CI+CII). No statistical difference in oxygen flux was observed with all substrates and inhibitors, (including those not shown) during this 3-day period. After 7 days in BIOPS, there were still no significant differences in oxygen flux compared to 0-day values (data not shown). When HF samples were tested on the day of explant and 3 days after explant, however, oxygen flux was significantly higher at the latter time point (Online Fig. 1D-F). Based on this finding, all high resolution respirometry experiments were initiated within 6 hours of explant.

Elamipretide restores mitochondrial oxygen flux in failing human heart

Representative traces from saponin permeabilized HF fibers of one patient are shown in Figure 2 where the top tracing (Online Fig. 2A) is from untreated fibers and the bottom tracing (Online Fig. 2B) from fibers incubated in elamipretide. Elamipretide treatment seemed to increase the rate of mass-corrected oxygen flux (shown by the thick red line) compared to untreated fibers, especially with the addition of ADP and glutamate.

Derivation of Supercomplex Coupling Control Ratio and Respiratory Control Ratio:

Two mathematical constructs used in respirometry analysis are the coupling control factor ratio for the mitochondrial supercomplex (SC CCF) and the respiratory control ratio (RCR).

$$SC\ CCF = \frac{glut}{succ - glut} = \frac{CI}{C(I + II) - CI}$$

$$RCR = \frac{ADP}{PM},$$

where *glut*, *succ* and *ADP* are the oxygen fluxes after the addition of glutamate, succinate, and ADP respectively, *PM* is the flux after the addition of pyruvate and malate. The SC CCF is the inverse expression of the contribution of CII, which is not in the supercomplex, compared to the contribution of CI+CII supported oxidative phosphorylation. The RCR is directly proportional to the level of mitochondrial coupling between respiration and phosphorylation.

Online Figure Legends

Online Figure 1 Mitochondria Functional Preservation with BIOPS. Cardiac tissue was preserved in BIOPS bio preservation solution for up to 3 days and high resolution respirometry performed to determine the effect of incubation time on mitochondrial function. (A-C) Nonfailing hearts treated with (A) pyruvate, malate plus ADP; (B) subsequent addition of glutamate acting through CI; (C) subsequent addition of succinate additionally activating CII either measured on Day 0 or Day 3. (D-F) Failing heart under the same conditions as the first three panels. N=4-5/group. ADP, adenosine diphosphate; C, complex; O₂, oxygen.

Online Figure 2 **High Resolution Respirometry Traces.** Respirometry of an (A) untreated, and (B) elamipretide treated permeabilized fiber samples from a failing heart. Thin blue line is oxygen concentration and thick red line is oxygen flux. Stepwise additions to the chamber are shown in boxes between traces. For concentrations of additions, see Methods. ADP, adenosine diphosphate; FCCP, Carbonyl cyanide-*p*-trifluoromethoxyphenylhydrazine; HF, heart failure; O₂, oxygen.

Online Figure 3 **High Resolution Respirometry of Treated and Untreated Heart Fibers through Complex II .** Oxygen flux normalized to mass in permeabilized heart fibers from untreated failing (HF) vs nonfailing (NF) human hearts with addition of rotenone following additions of malate, pyruvate, ADP, glutamate, succinate and FCCP (see methods for concentrations). (A) There was no significant difference between flux through CII in untreated nonfailing vs. failing hearts. Elamipretide treatment had no effect on oxygen flux through CII of nonfailing (B) or failing (C) samples. Untreated hearts in B and C are shown with closed circles and treated hearts with open circles; n=5 nonfailing, n=16 failing.

Supplemental Table 1 Patient Data for Experiments involving Fresh Tissue

Failure Status*	Age	Sex	Diagnosis	EF [FS]	Inotrope	Milrinone	Digoxin	ACE-I	BB	Diuretic	Other Medications	Experiment
NF	0.04	M	HLHS	NL	N	N	N	Y	N	Y		O,E
NF	0.05	F	PA/IVS	[32]	N	N	N	N	N	Y	ASA,PGE	O,C,E
NF	0.17	F	PA/IVS	[32]	Y	N	N	N	N	Y	VP, PGE	O,C,E
NF	0.25	M	HCM	67	N	N	N	N	Y	N	AA	O,E
NF	4.7	F	RCM	63	N	N	N	N	N	Y	ASA	E
NF	7	M	HLHS, PLE	NL	N	N	N	N	Y	N	PDE5i, ASA	O,E
NF	10	F	TGA, VSDs, PLE	NL	N	Y	N	N	N	Y	PDE5i	O,E
NF	25	M	Donor	56	NA	NA	NA	NA	N A	NA		O,C,E
NF	26	M	Donor	NA	NA	NA	NA	NA	N A	NA		O
NF	52	F	Donor	55	NA	NA	NA	NA	N A	NA		O,E
NF	53	M	Donor	NA	NA	NA	NA	NA	N A	NA		O,C
NF	58	F	Donor	80	NA	NA	NA	NA	N A	NA		O,E
NF	62	F	Donor	65	NA	NA	NA	NA	N A	NA		E
NF	63	F	Donor	60	Y	N	N	N	N	N	VP	O,C
HF	0.11	F	DCM	11	N	Y	N	N	N	Y	PGE, MCRA	C
HF	0.2	F	MS s/p MVR x 2	31	N	Y	N	N	N	Y	ASA	O,E
HF	0.2	M	HLHS	Sv	N	Y	Y	N	N	Y	ASA	O
HF	0.5	F	HLHS/TA PVR s/p	M/S	N	Y	N	N	N	Y	PDE5i,MCR A	O,E

			Nw									
HF	0.6	M	MS/AS	mild	N	Y	N	N	N	Y		O,C
HF	1.5	M	TGA	30	N	Y	N	N	N	Y	MCRA, ASA	O,C,E
HF	4	F	TGA/VSD	23	N	Y	N	N	N	Y	MCRA, ASA	O,C
HF	14	F	DCM	22	Y	Y	N	N	N	Y		E
HF	14	F	DCM	VA D/22	Y	Y	N	N	N	Y	PDE5i,CCB, VP	E
HF	16	F	DCM	VA D/N A	N	N	N	Y	N	Y	CCB, ASA	O,E
HF	18	M	DCM	37	N	Y	Y	Y	Y	Y	VASODIL	O
HF	18	M	DCM	[11]	N	N	N	N	N	Y	PDE5i,MCR A,ASA	O,E
HF	18	M	TV	[25]	N	Y	N	N	N	Y		O,C
HF	20	F	DCM	17	Y	Y	N	N	N	Y	MCRA,VAS ODIL	O
HF	32	M	DCM	29	N	Y	N	N	N	Y	MCRA	O
HF	33	M	DCM	[14]	N	N	N	Y	N	Y	MCRA,CCB, ASA	O
HF	43	F	ARVC	**	NA	NA	NA	NA	N A	NA		O,C
HF	44	M	ARVC	**	NA	NA	NA	NA	N A	NA	AA	O,C
HF	47	M	DCM Becker's MD	39	N	N	Y	N	N	N	MCRA	E
HF	47	M	DCM	30	N	Y	N	N	N	Y	VASODIL	O
HF	53	M	ARVC	27	N	N	N	N	Y	N	MCRA,AA	C
HF	55	M	Sarcoidosis	22	N	N	N	N	Y	N	MCRA,AA	E
HF	58	M	ICM	20	Y	N	Y	N	N	Y	MCRA, VASODIL	E
HF	60	M	Sarcoidosis	Sv	N	N	N	Y	N	N	STAT,AA,CC	E

											B	
HF	60	F	DCM	6	Y	N	N	N	N	N	MCRA, VASODIL	O
HF	61	M	ICM	21	N	N	Y	N	N	Y	MCRA,VAS ODIL,ASA	O
HF	62	M	ICM	15	Y	Y	N	N	N	Y	AA, ASA	O,C
HF	65	M	ICM	27	N	N	N	N	N	Y	MCRA,STAT ,VASODIL,A SA	O
HF	66	M	ARVC	28	Y	N	N	Y	N	Y	MCRA	O
HF	67	M	DCM	15	Y	N	Y	N	N	Y	MCRA,VAS ODIL	C
HF	68	M	DCM	21	N	N	Y	N	Y	Y	MCRA	E
HF	71	M	ICM	22	Y	N	Y	N	N	N	MCRA,STAT	O

**Abbreviations for Failure Status column:* NF, nonfailing; HF, heart failure.

Abbreviations for Sex column: M, male; F, female

Abbreviations for Diagnosis column: ARVC, arrhythmogenic right ventricular cardiomyopathy; AS, aortic stenosis; DCM, dilated cardiomyopathy; HCM, hypertrophic cardiomyopathy; HLHS, hyperplastic left heart syndrome; ICM, ischemic cardiomyopathy; MD, muscular dystrophy; MS, mitral stenosis; MVR, mitral valve replacement; Nw, Norwood; PA/IVS, pulmonary atresia with intact ventricular septum; PLE, transplanted secondary to refractory protein losing enteropathy; RCM, restrictive cardiomyopathy; RV, right ventricle; s/p, status post; Sv RVF, severe right ventricular failure; TAPVR, total anomalous pulmonary venous return; TGA, transposition of the great arteries; TV, transplant vasculopathy; VSD, ventricular septal defect.

Abbreviations for EF [FS] column: EF, ejection fraction (%); FS, fractional shortening (%) shown in brackets; M/S, moderate to severe; NA, not available; NL, normal; Sv, severe; VAD, ventricular assist device; **Class IV symptoms with low cardiac output/ cardiac index by right heart catheterization.

Abbreviations for Medications columns: AA, anti-arrhythmic; ACE-I, angiotensin converting enzyme inhibitor; ASA, aspirin; BB, beta blocker; CCB, calcium channel blocker; MCRA, mineralocorticoid receptor antagonist; N, not taking medication; NA, not

available; PDE5i, phosphodiesterase 5 inhibitor; PGE, prostaglandin E; STAT, statin; VASODIL, vasodilator; VP, vasopressin; Y, taking medication.

Abbreviations for Experiment column: C, cardiolipin study; E, enzyme study; O, Oroboros O2K study.

Supplemental Table 2 Patient Data for Experiments involving Frozen Tissue for BN-PAGE

Failure Status*	Age	Sex	Diagnosis	EF [FS]	Inotrope	Milrinone	Digoxin	ACE-I	BB	Diuretic	Other Medications
NF	1.3	F	Donor	49	Y	N	N	N	N	N	VP
NF	3	M	Donor	NA	Y	N	N	N	N	N	VP
NF	3	F	Donor	NA	Y	N	N	N	N	N	VP, DDAVP
NF	7	M	Donor	NA	Y	N	N	N	N	N	DDAVP, T4
NF	8	F	Donor	NA	N	N	N	N	N	N	
NF	9	F	Donor	NA	Y	N	N	N	N	Y	VASOPR, DDAVP, T4, VASODIL
NF	9	M	Donor	NA	N	N	N	N	N	N	DDAVP, T4
NF	10	M	Donor	NA	Y	N	N	N	Y	N	VP
NF	14	M	Donor	NA	Y	N	N	N	Y	N	T4, DDAVP, VASODIL
NF	17	F	Donor	60	N	N	N	N	N	N	
NF	23	F	Donor	NA	Y	N	N	N	N	N	VP
NF	30	M	Donor	mild	Y	N	N	N	N	Y	T4
NF	35	F	Donor	67	Y	N	N	N	Y	Y	T4
NF	48	F	Donor	>55	N	N	N	N	N	N	VASOPR, T4
NF	51	F	Donor	60	Y	N	N	N	Y	Y	VASODIL, CCB, DDAVP
NF	53	M	Donor	70	Y	N	N	N	N	N	VP, PGE
NF	56	F	Donor	NA	N	N	N	N	N	Y	T4

NF	61	M	Donor	61	Y	N	N	N	N	Y	VP, STAT, VASOPR
NF	62	F	Donor	NA	Y	N	N	N	N	N	VP, VASOPR
HF	0.5	F	DCM	20	N	Y	N	N	Y	Y	AA, ASA, MCRA
HF	0.6	M	DCM	VAD/ NA	Y	Y	N	N	N	Y	PDE5i, ASA, AA
HF	0.7	F	DCM	20	N	Y	Y	N	N	Y	MCRA, AA
HF	3	F	DCM	41	N	Y	N	Y	N	Y	ASA, MCRA, T4
HF	3	M	DCM	14	N	Y	Y	Y	N	Y	AA, ASA
HF	4	F	DCM	28	N	Y	N	Y	N	Y	ASA
HF	4	F	DCM	27	N	Y	Y	Y	Y	Y	ASA
HF	5	M	DCM	[16]	N	N	N	N	Y	Y	PDE5i, AA. ASA
HF	5	M	DCM	[4]	N	Y	N	Y	Y	Y	MCRA
HF	9	M	DCM	VAD/ 50	N	N	N	N	N	N	AA, ASA
HF	12	F	DCM	25	N	Y	N	Y	N	Y	ASA
HF	24	M	DCM	15	Y	N	N	N	N	Y	VASODIL
HF	31	M	DCM	26	N	N	N	Y	Y	N	ASA
HF	44	F	DCM	VAD/ NA	Y	N	N	N	N	N	CCB
HF	45	M	DCM	26	N	N	N	N	Y	Y	MCRA, AA
HF	46	F	DCM	16	N	N	Y	N	Y	Y	MCRA, ASA
HF	51	F	DCM	10	Y	N	N	N	N	N	
HF	53	M	DCM	21	N	N	N	Y	Y	N	ASA
HF	54	F	DCM	15	N	N	N	N	N	Y	PDE5i, MCRA, VASODIL

HF	60	F	DCM	23	N	N	N	N	N	Y	ARB, MCRA
HF	63	M	DCM	28	N	N	N	Y	Y	Y	MCRA

**Abbreviations for Failure Status column:* NF, nonfailing; HF, heart failure.

Abbreviations for Sex column: M, male; F, female

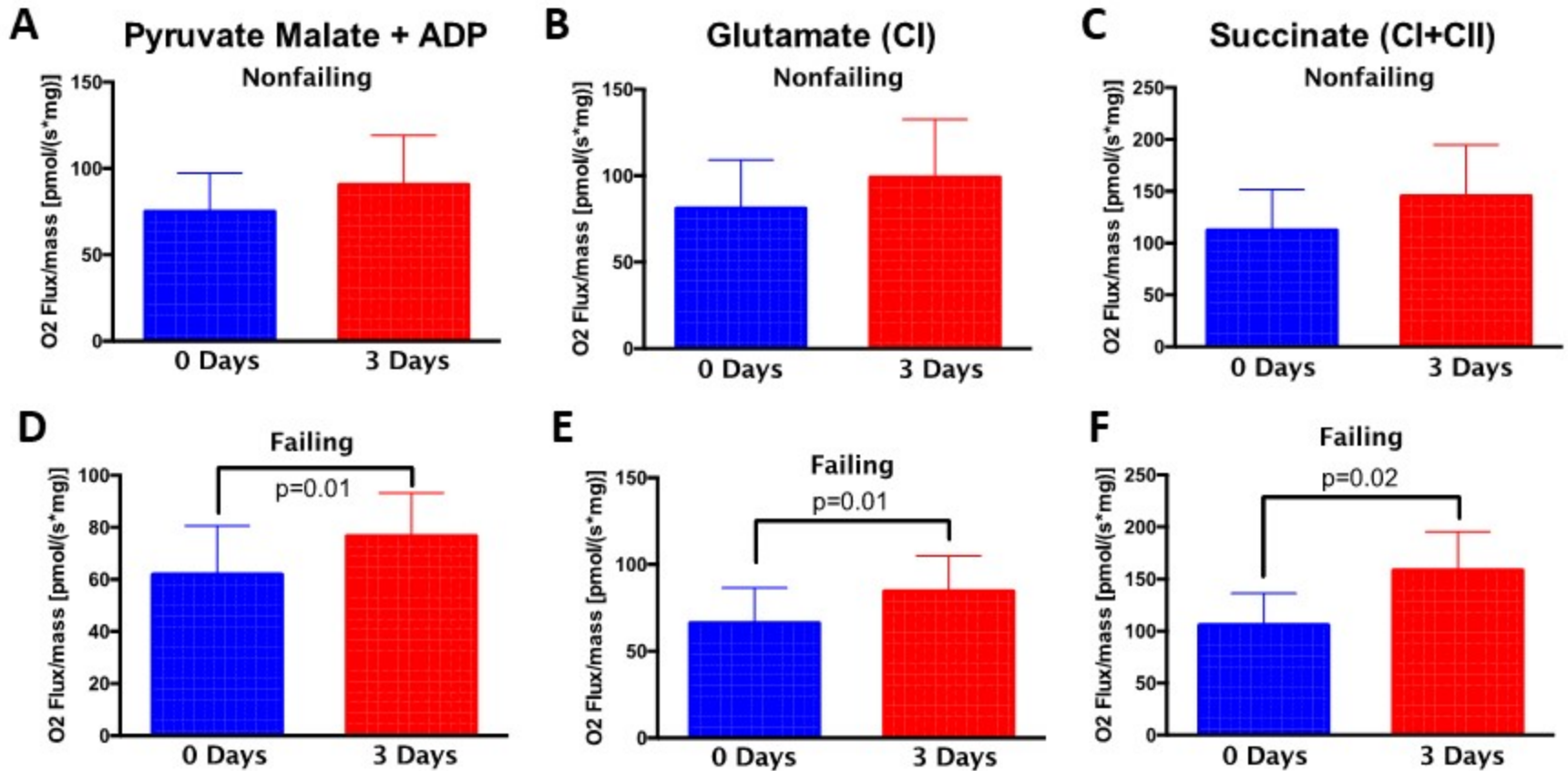
Abbreviation for Diagnosis column: DCM, dilated cardiomyopathy.

Abbreviations for EF [FS] column: EF, ejection fraction (%); FS, fractional shortening (%) shown in brackets; NA, not available; VAD, ventricular assist device.

Abbreviations for Medications columns: AA, anti-arrhythmic; ACE-I, angiotensin converting enzyme inhibitor; ARB, angiotensin receptor blocker; ASA, aspirin; BB, beta blocker; CCB, calcium channel blocker; DDAVP, desmopressin; MCRA, mineralocorticoid receptor antagonist; N, not taking medication; NA, not available; PDE5i, phosphodiesterase 5 inhibitor; PGE, prostaglandin E; T4, thyroid replacement; STAT, statin; VASOPR, vasopressor; VASODIL, vasodilator; VP, vasopressin; Y, taking medication.

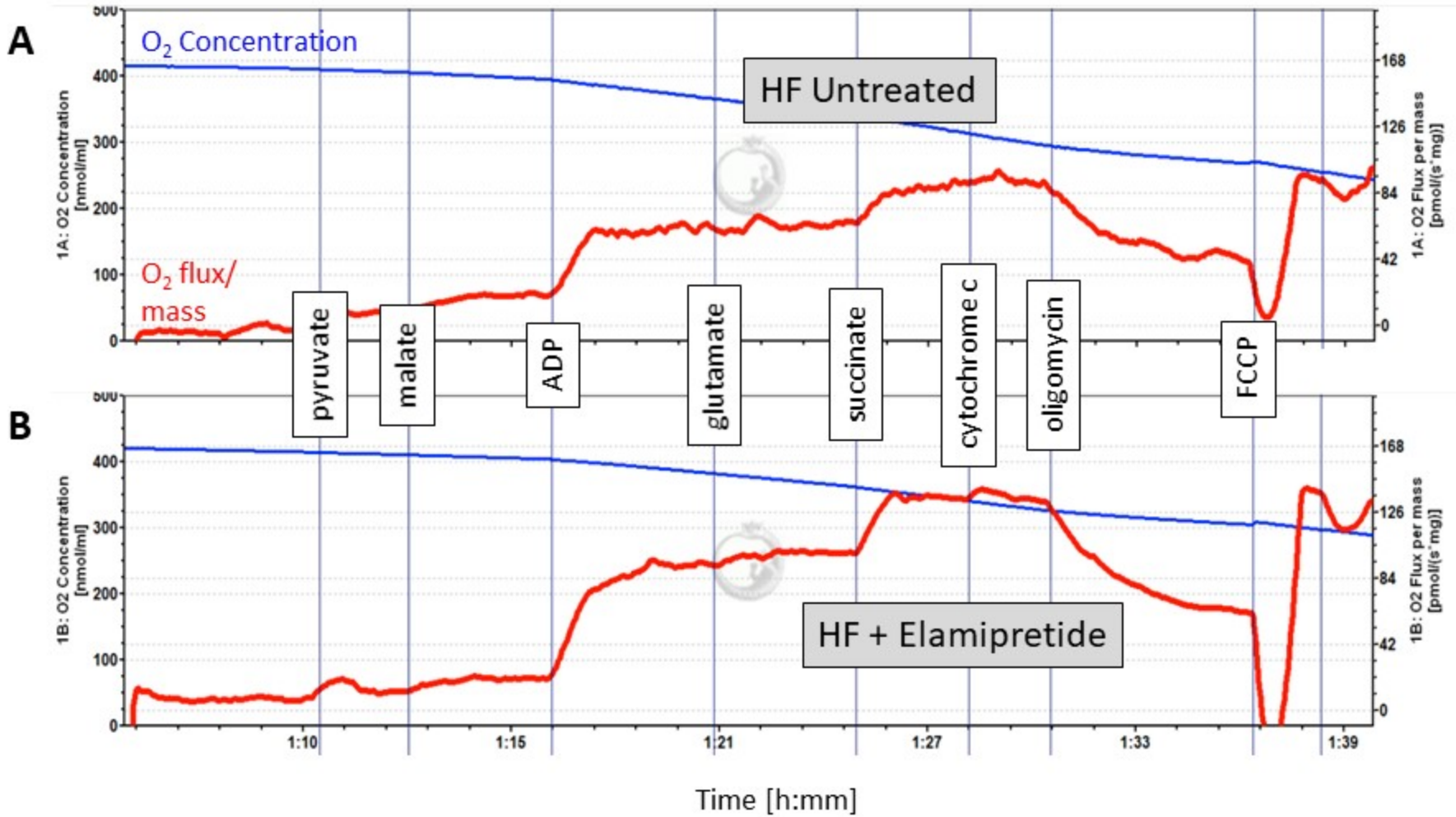
Online Figure 1

Mitochondria Functional Preservation with BIOPS



Online Figure 2

High Resolution Respirometry Traces



Online Figure 3

High Resolution Respirometry of Treated and Untreated Heart Fibers through Complex II

