

Supplementary Materials

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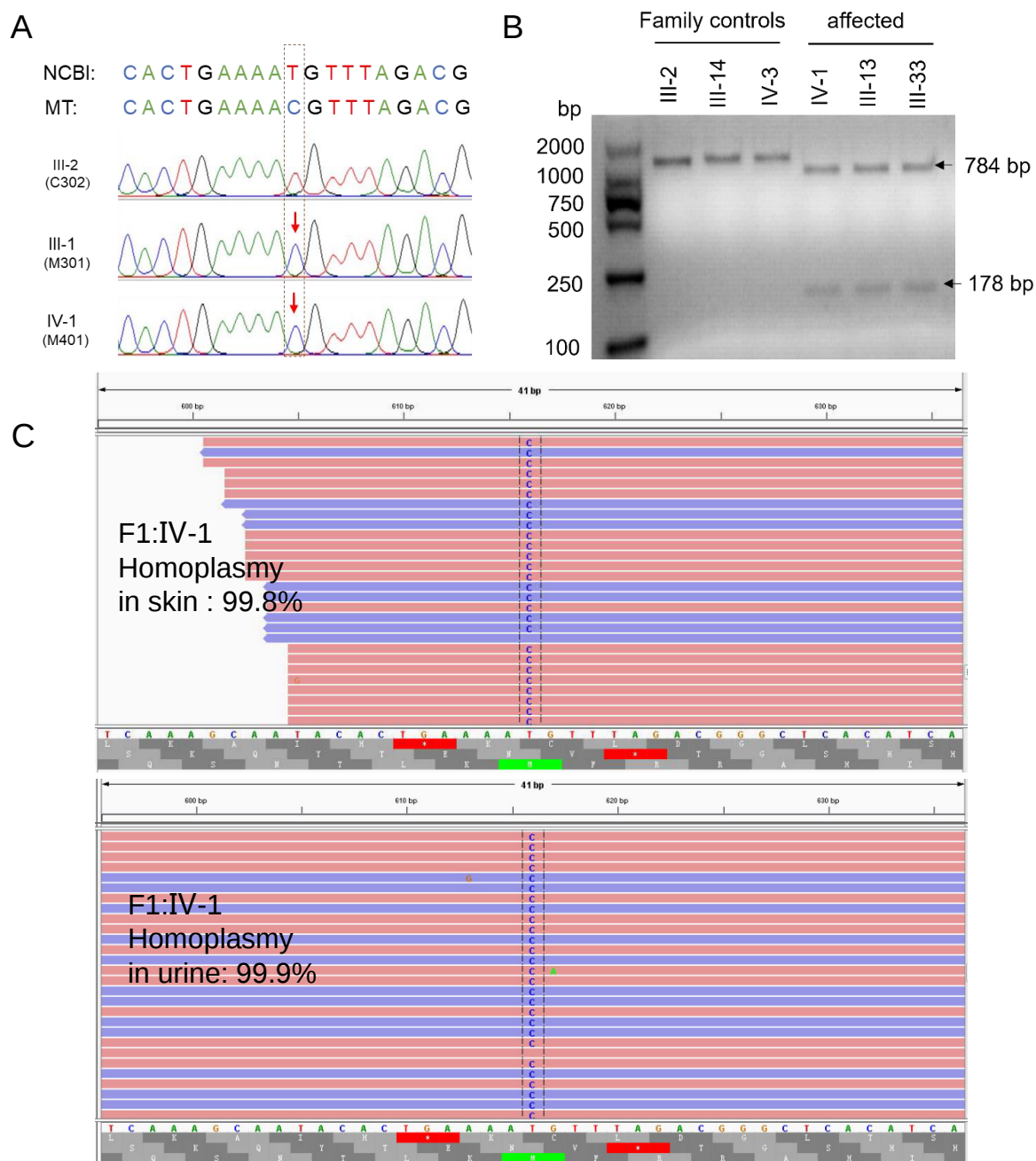


Fig S1 (related to Fig 1) Identification of the homoplasmic m.616 T>C in family 1.(A) Variant m.616T>C was confirmed by Sanger sequencing. Samples from unaffected control individual (father, III-2) and two affected individuals (mother, III-1 and proband, IV-1). (B) RFLP (restriction fragment length polymorphism) analysis of PCR fragments of samples from patients and control subjects, digested with enzyme Acl I and then analyzed by electrophoresis through 3% agarose gel. The 962 bp segments with m.616 T>C was segmented into two fragments, 784bp and 178bp. (C) Homoplasmic m.616 T>C in skin and urine samples of proband by high-throughput sequencing.

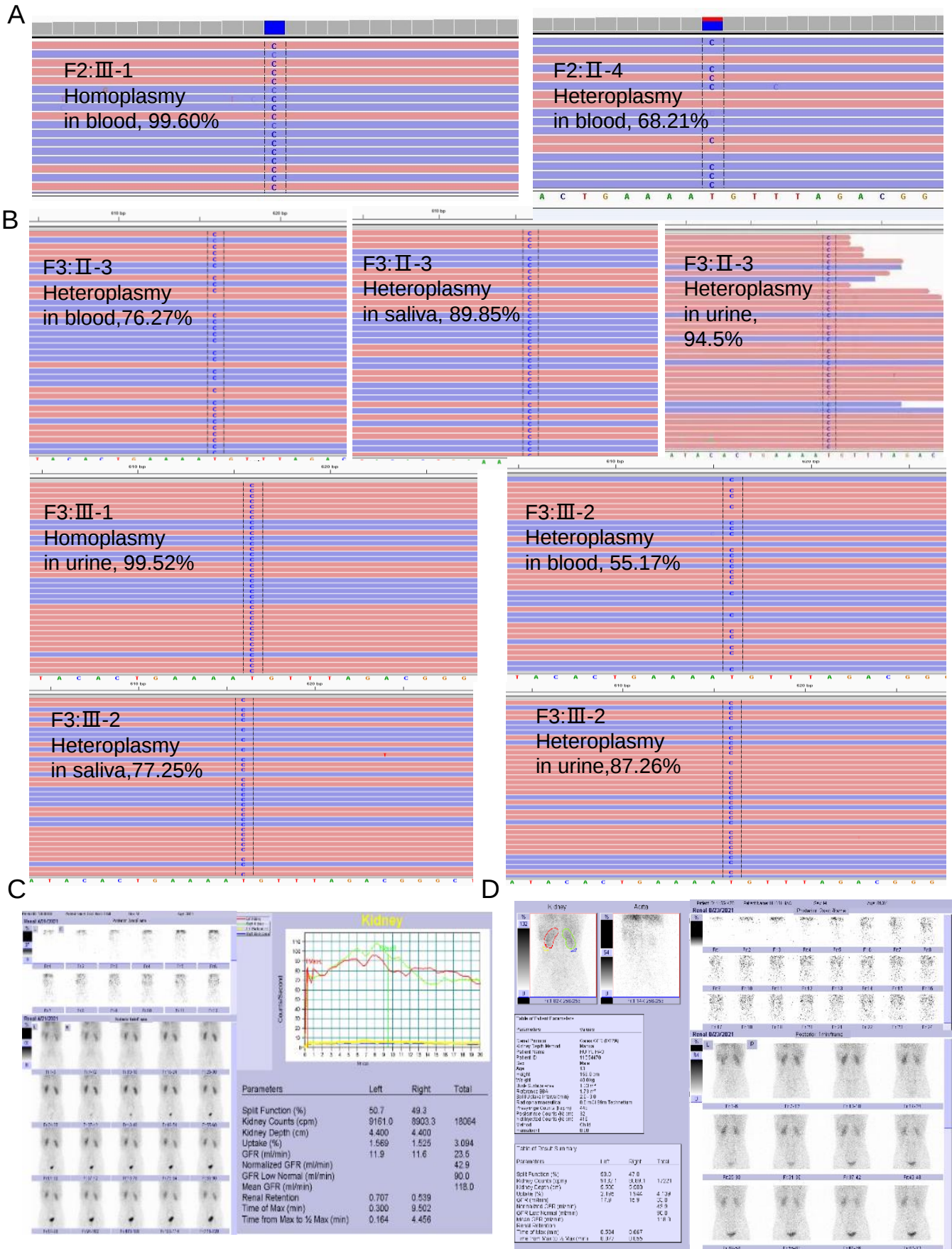


Fig S2 (related to Fig 1) Heteroplasmy m.616 T>C and renal function by DTPA test . Homoplasmic and heteroplasmic m.616T>C, confirmed by high-throughput sequencing in different tissues. Heteroplasmic m. 616T>C was present in individuals F2:II-4(A) ; F3:II-3 and patient F3:III-2 (B). DTPA test of proband 2 (C) and of proband 3 (D).

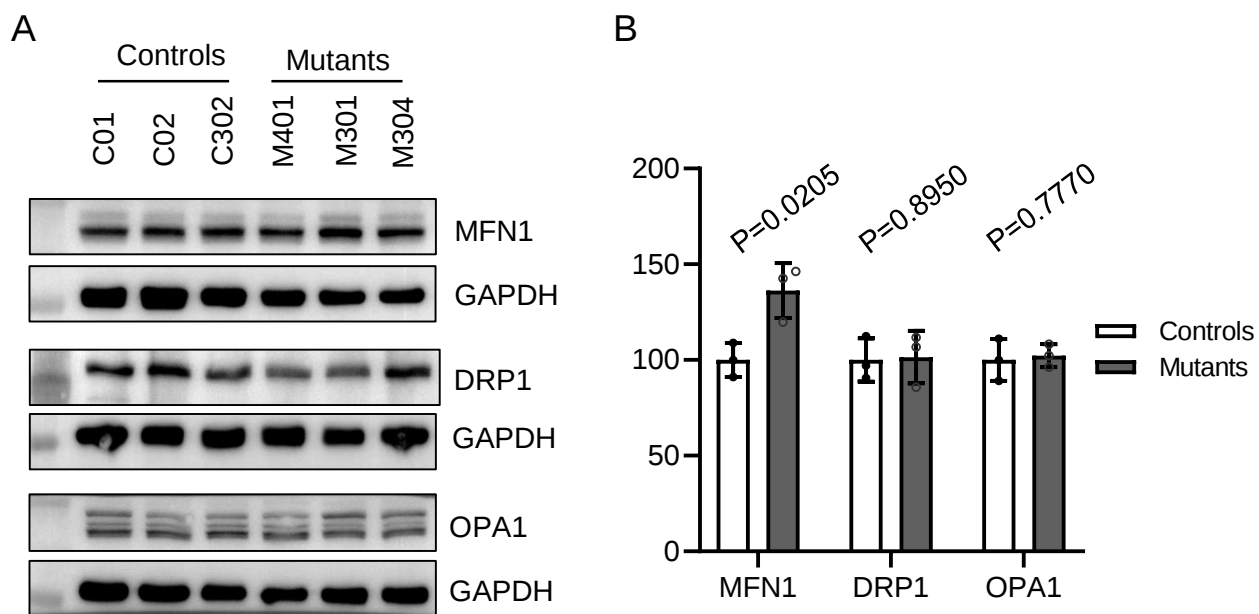


Fig S3 (related to Fig 2) The expression levels of mitochondria shaping proteins. (A) Western blotting assay for shaping proteins, MFN1, OPA1 and DRP1. (B) Quantification analysis. *, $P < 0.05$.

Table S1 Mitochondrial DNA variants and haplogroups in probands and three control subjects

Gene	Position	rCRS ^a	F1:IV-1 (D4)	F1:III-2 (G4)	F2:III-1 (F2)	F3:III-1 (B5)	C01 (D4)	C02 (D4)	Previously reported ^b
D-loop	73	A	G	G	G	G	G	G	Yes
	103	G				A			
	146	T				C			
	152	T	C			C	C	C	Yes
	189	A				G			
	204	T				C			
	249	A			-				
	260	G		A					Yes
	263	A	G	G	G	G	G	G	Yes
	310	T	CTC	CCTC	CTC	CTC	TC	TC	Yes
	385	A					G		Yes
	489	T	C	C			C	C	Yes
	16093	T		C		C		G	Yes
	16129	G	A				A	A	Yes
	16140	T				C			
	16183	A				C			
	16189	T				C			
	16203	A			G				
	16209	T			C				
	16223	C	T	T			T	T	Yes
	16263	T					C		Yes
	16278	C		T					Yes
	16286	C	T						Yes
	16291	C			T				
	16304	T			C				
	16311	T			C				
	16325	T		C					Yes
	16335	A			G				
	16362	T	C	C			C	C	Yes
	16519	T			C	C	C	C	Yes
tRNA ^{Phe}	616	T	C		C	C			Yes
12S rRNA	709	G		A		A			Yes
	750	A	G	G	G	G	G	G	Yes
	1005	T			C				
	1438	A	G	G	G	G	G	G	Yes
	1598	G				A			
16S rRNA	1824	T			C				
	2526	C						A	
	2706	A	G	G	G	G	G	G	Yes
	3010	G	A				A	A	Yes

	3206	C	T				T	T	Yes
ND1	3951	C				T			
	3970	C			T				
ND2	4483	C				G			
	4703	T				C			
	4769	A	G	G	G	G	G	G	Yes
	4883	C	T				T	T	Yes
	5108	T		C					Yes
	5178	C	A				A	A	Yes
tRNA ^{Ala}	5601	C		T					Yes
CO1	6392	T			C				
	6686	C					T		Yes
	6737	A		G					Yes
	7028	C	T	T	T	T	T	T	Yes
	7148	T	C						Yes
	7170	C				T			
	7185	C						A	
	7186	A						C	
	7389	T						C	
CO2	7600	G		A					Yes
	7828	A			G				
	8281	C				T			
	8282	C				-			
	8283	C				-			
	8284	C				-			
	8285	C				-			
	8286	T				-			
	8287	C				-			
	8288	T				-			
	8289	A				-			
ATP8	8414	C	T				T	T	Yes
	8473	T	C				C	C	Yes
ATP6	8545	G			A				
	8584	G				A			
	8701	A	G	G			G	G	Yes
	8748	C		T					Yes
	8829	C				T			
	8860	A	G	G	G	G	G	G	Yes
	8930	C				T			
	9128	T			C				
	9144	C		T					Yes
CO3	9377	A		G					Yes
	9540	T	C	C			C	C	Yes
	9575	G		A					Yes

	9845	T					C		Yes
	9950	T					C		
ND3	10310	G			A				
	10398	A	G	G		G	G	G	Yes
	10400	C	T	T			T	T	Yes
ND4L	10535	T			C				
	10586	G			A				
ND4	10873	T	C	C			C	C	Yes
	11650	A	G						Yes
	11719	G	A	A	A	A	A	A	Yes
ND5	12338	T			C				
	12361	A				G			
ND5	12705	C	T	T			T	T	Yes
	13563	A		G					Yes
	13708	G			A				
	13928	G			C				
ND6	14200	T		C					Yes
	14494	T	C						Yes
	14569	G		A					Yes
	14668	C	T				T	T	Yes
CYTB	14766	C	T	T	T	T	T	T	Yes
	14783	T	C	C			C	C	Yes
	14979	T	C				C	C	Yes
	15001	T				C			
	15043	G	A	A			A	A	Yes
	15223	C				T			
	15301	G	A	A			A	A	Yes
	15326	A	G	G	G	G	G	G	Yes
	15508	C				T			
	15662	A				G			
	15813	T			G				
	15824	A			C				
	15851	A				G			
	15852	T				G			
tRNA ^{Thr}	15889	T					C		Yes
	15927	G				A			

^a rCRS, revised Cambridge reference sequence (NC_012920).

^b See the online mitochondrial genome database MITOMAP (<https://www.mitomap.org/MITOMAP>).

MtDNA haplogroups were analyzed by MitoTool (<http://www.mitotool.org/>), belonged to D4 (F1:IV-1, C01 and C02), G2 (F1:III-2, father), F2 (F2:III-1) and B5 (F3:III-1) respectively. Probands: F1:IV-1, F2:III-1 and F3:III-1; Controls: F1:III-2, C01 and C02.

Table S2 Usage of Phenylalanine codon in human mitochondrial genes

Complex	Protein	Number of amino acids (AA)	Number of Phenylalanine codons	Density of Phenylalanine codons (%)	Decrease level of mitochondrial proteins (%)
Complex I	ND1	318	16	5.03	32.7
	ND2	347	15	4.32	NA
	ND3	115	8	6.96	NA
	ND4	459	20	4.36	33.6
	ND4L	98	3	3.06	NA
	ND5	603	38	6.30	NA
	ND6	174	8	4.60	NA
Complex III	Cyt b	380	24	6.32	33.8
Complex IV	CO1	513	41	7.99	23.2
	CO2	227	7	3.08	0
	CO3	261	24	9.20	32.7
Complex V	ATP6	226	13	5.75	NA
	ATP8	68	2	2.94	20.2

Table S3 Oligonucleotides for mt- tRNA probes and primers for mtDNA copy number assay

PRIMERS & PROBES	SEQUENCES (5'-3')
Primers	
16S-F	CACCCAAGAACAGGGTTGT
16S-R	TGGCCATGGGTATGTTGTAA
18S-F	TAGAGGGACAAGTGGCGTTC
18S-R	CGCTGAGCCAGTCAGTGT
Probes	
Phe	TGTTTATGGGGTGATGTGAGCCCGTCTAAA
Val	TCAGAGCGGTCAAGTTAAGTTGAAATCTCC
Leu-UUR	AGAAGAGGAATTGAACCTCTGACTGTAAAG
Met	TAGTACGGGAAGGGTATAACCAACATTTTC
Tyr	GGTAAAAAGAGGCCTAACCCCTGTCTTTAG
Ser-UCN	AAAGGAAGGAATCGAACCCCCCAAAGCTGG
His	AAATAAGGGGTCGTAAGCCTCTGTTGTCAG
Ser-AGY	GAGAAAGCCATGTTGTTAGACATGGGGGCA
Leu-CUN	ACTTTTATTTGGAGTTGCACCAAAATTTT
5S rRNA	GGGTGGTATGGCCGTAGAC

Table S4. Key resources in experiments

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Antibodies		
MTCO1	Abcam	Cat# ab14705
GAPDH	Abcam	Cat# ab8245
total OXPHOS human WB cocktail	Abcam	Cat# ab110411
MT-ND1	Proteintech	Cat# 19703-1-AP
ATP8	Proteintech	Cat# 26723-1-AP
LC3A	Cell Signaling Technology	Cat# 4599
TOM20	Abclonal	Cat# A19403
MT-ND4	Abclonal	Cat# A9941
MT-CYB	Abclonal	Cat# A17966
MTCO2	Abclonal	Cat# A3843
ATG12	Abgent	Cat# AP1816a
MTCO3	Novus Biologicals	Cat# NBP1-88063
KIM1	Abcam	Cat# ab228973
NGAL	Abcam	Cat# ab125075
AQP2	Santa Cruz	Cat# Sc-515770
Alexa Fluor 488 goat anti-mouse IgG	Abcam	Cat#ab150113
Alexa Fluor 594 goat anti-rabbit IgG	Abcam	Cat#ab150080
Goat anti mouse IgG(H+L) (HRP)	Beyotime	Cat# A0216
Goat anti rabbit IgG(H+L) (HRP)	Beyotime	Cat# A0208
Critical Commercial Assays		
CellTiter-Glo® Luminescent Cell Viability Assay kit	Promega	Cat# G7571
Lactate-Glo™ Assay	Promega	Cat# J5021
MitoSOX™ Red Mitochondrial Superoxide Indicator, for live-cell imaging	Invitrogen	Cat# M36008
JC-10 Mitochondrial Membrane Potential Assay Kit (Flow Cytometry)	Abcam	Cat# ab112133
TRIzol reagent	Invitrogen	Cat# 15596018
Genious 2X SYBR Green Fast qPCR Mix	Abclonal	Cat# RK21204
T7 RiboMAX™ Express Large Scale RNA Production System	Promega	Cat# P1320
Annexin V-FITC Apoptosis detection Kit with PI	BioLegend	Cat# 640914
Chemicals and plasmids		
Antimycin A	Sigma	Cat# A8674-50MG
Oligomycin	Sigma	Cat# 495455-10MG
FCCP	Sigma	Cat# C2920-10MG
Rotenone	Sigma	Cat# 45656-250MG
2-DG	Sigma	Cat# D8375-1G
ATP	MCE	Cat# HY-B2176