## Supplementary Appendix

## Trimetazidine and Exercise Provide Comparable Improvements to High Fat Diet-induced Muscle Dysfunction through Enhancement of Mitochondrial Quality Control

Wenliang Zhang<sup>1</sup>, Baiyang You<sup>1</sup>, Yaoshan Dun<sup>1,2</sup>, Dake Qi<sup>3</sup>, Ling Qiu<sup>1</sup>, Jeffrey W. Ripley-Gonzalez<sup>1</sup>, Fan Zheng<sup>1</sup>, Siqian Fu<sup>1</sup>, Cui Li<sup>1</sup> and Suixin Liu<sup>1,4</sup>\*

- 1. Division of Cardiac Rehabilitation, Department of Physical Medicine & Rehabilitation, Xiangya Hospital of Central South University, Changsha, Hunan, China.
- 2. Division of Preventive Cardiology, Department of Cardiovascular Medicine, Mayo Clinic, Rochester, MN, USA.
- 3. College of Pharmacy, University of Manitoba, Winnipeg, Canada
- 4. National Clinical Research Center for Geriatric Disorders, Xiangya Hospital of Central South University, Changsha, Hunan, China.

<sup>\*</sup> Corresponding Author. Suixin Liu, MD, Ph.D., Division of Cardiac Rehabilitation, Department of Physical Medicine & Rehabilitation, Xiangya Hospital of Central South University, 87 Xiangya Road, Changsha, Hunan, 410008, P.R. China. E-mail, liusuixin@csu.edu.cn (SXL); +86 (731)84327174

This supplementary appendix was created by the authors to provide all original gels/blots images for review.

Supplementary Fig.1 is the original gel/blots shown in figure 1 of the main manuscript, Supplementary Fig.2 is the original gel/blots shown in figure 2 of the main manuscript and so on.

In **Fig. 3.** TMZ promotes PA-attenuated mitochondrial quality control (MQC) and functions in muscle cells. The original data presents P-AMPK, PGC-1α, LC3II/LC3I, BNIP3, DRP1, MFN1 and CS protein expressions were determined in four groups, we just cropped the right most group in the data analysis. In **Fig. 5.** TMZ regulates MQC and improves mitochondrial functions in HFD mice. The original data presents PGC-1α, LC3II/LC3I, PINK1, DRP1 and MFN1 and CS protein expressions were determined in five groups, we just cropped the right most group in the data analysis.

Supplementary Fig. 1. TMZ prevents palmitate (PA)-induced muscle cell damage

Atrogin1	GAPDH
14 24 54 - 6 LBX0 22 - 12	-16APDH-1
	-12 GAPPH-2
FBX033-3 55-	-3E CAPAH-3

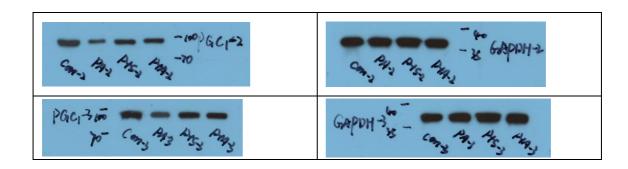
MuRF1	GAPDH
GOT RIM63 = -35	-16 APDH-1
- 15 - 15 - 15 - 15 - 15 - 15 - 15 - 15	-32 GAPPH-2
TRUM63-3 wa	-3E GAPAH-3

MyHC-II	GAPDH
14 24 54 - 150 MAHPA	-16 APDH-1
MyH2-2	-12 GAPPH-2
(4) FT EV QI @	-31 CAPPH-3

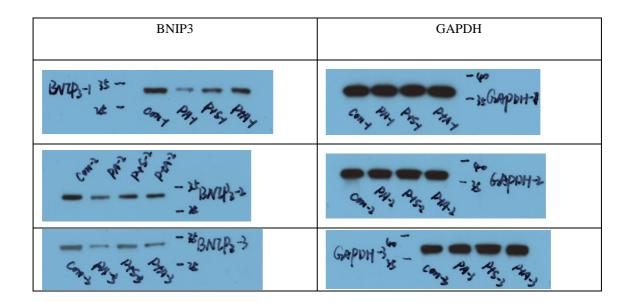
Supplementary Fig. 2. TMZ promotes PA-attenuated mitochondrial quality control (MQC) and functions in muscle cells

P-AMPK	GAPDH
AMPK-1	-40 -8 (PAPDH-1 -1 =1 2 10-1 (P-7m2)
(19-70) ys_	(5APDY-26- (O-7m2) #-
AMPK-37 (6)-796135 1 =- 1 =- 1 =- 1	-3-3 3-3 -3 -3 -3 -3 -3 -3 -3 -3 -3 -3 -

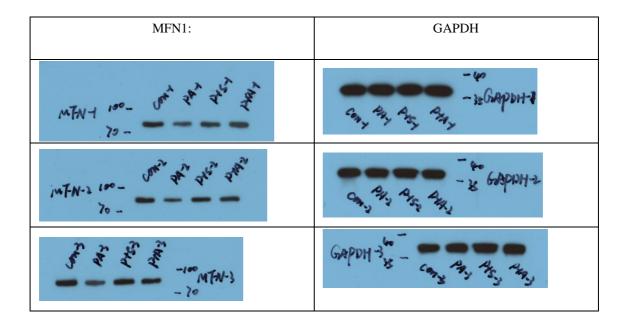
PGC-1α	GAPHD
Can, Pay 45, Pay -10	- up - 32 GAPDH-1



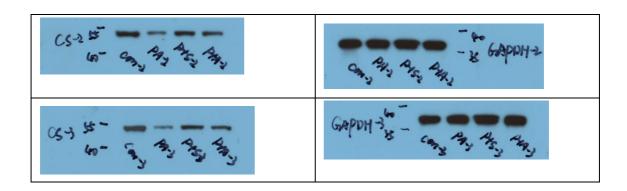
LC3II/LC3I	GAPDH
FEEE E E - 15 LCVB1	-40 -32 GAPDH-1
1000 U- \$ \$ \$ \$ \$ \$	Constitution of the GAPINH-2
FEEE -	GAPDH 335 Cang May Mys. May.



DRP1	GAPDH
Comp Pay Pray Play	Comp day day - 15- Crapph-1
DRP, 2 70 - Con, Any Area Army	CM, SH, SIS, SH,
Cany May May Pays	GAPUH 33 Cong My My My



CS	GAPDH
Con May May -40	-40 -35 GAPDH-1



Supplementary Fig. 3. TMZ prevents metabolic dysfunction and muscle atrophy in mice following high-fat diet feeding as exercise

Atrogin1	GAPDH
78×10>>> 40- SA, TRO, LITER ME	Con the state of t
FB X033 -2 40- Can, they stay after	GAPDH22- CM, 15, 45 TO
FBX037-342-	Constitution of the state of th

MuRF1	GAPDH
70.2M63-1 100_ CAN ARD ARD ARTHUR ?	Con, they citize they
TRIM63-242 - Ser Tray Route to Ser	GAPDH-24- CM, 180, 450 TO

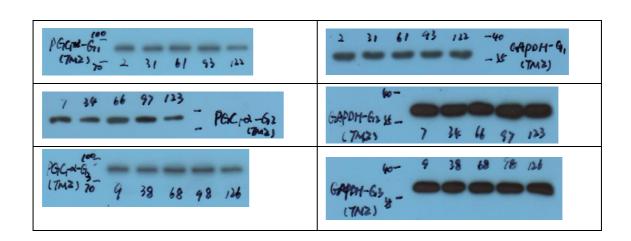


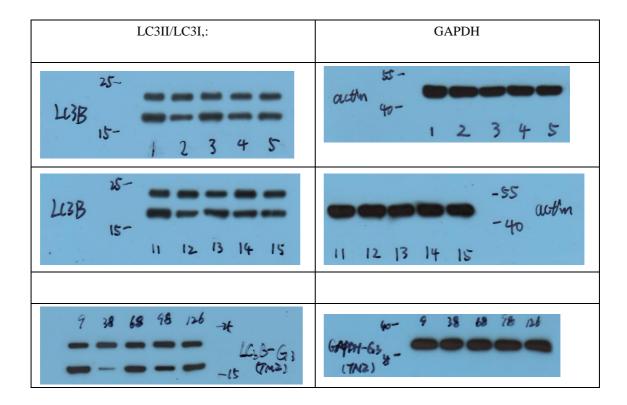
MyHC-II	GAPDH
149th + 10 - Con, they style thing	Con, they strike they
Mythe -1 Con they strong history	GAPDH-24- CM, 1751, AFE TOTAL
Myth -> - Con, they of they to the	- 60 - 100, 200 100 100 100 100 100 100 100 100 100

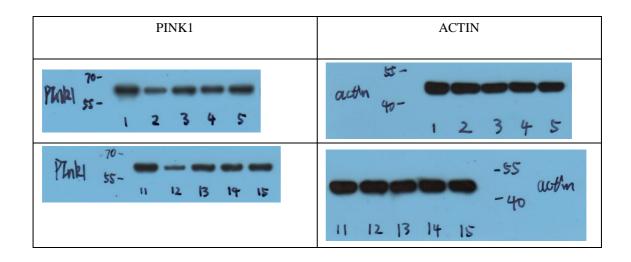
Supplementary Fig. 4. As exercise, TMZ regulates MQC and improves mitochondrial functions in HFD mice

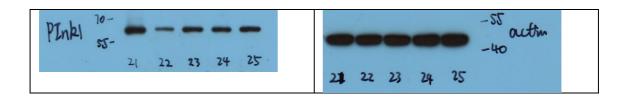
P-AMPK,:	GAPDH
PAMPK+ 30- (TM2) 5- 5 35 63 92	GAPOH4 40- 17m2) 35- 5 35 63 92
6 36 64 95 (IMD)	-40 -36 64 95 (TMZ)
8 39 67 97 (7m2)	6APDH-331-8 39 67 97

PGC-1α,:	GAPDH

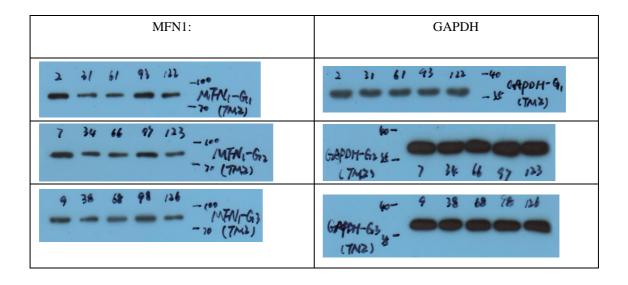








DRP1	GAPDH
DRP1-G1	2 31 61 43 122 -40 CAPOH-9, -35 (7M2)
100- 7 34 66 97 133 PRP-G2 =	GAPDH-G236 7 34 66 97 123
9 38 68 98 126 -100 -DRP1-G3 -70 (TM2)	GAPH-G3 (TNZ)



CS	GAPDH
2 31 61 93 132 -55-61 -10 (7043)	2 31 61 93 122 -40 CAPOH-91 -35 (TMZ)

