## **Supplementary File**

## Exercise promotes cardiac hydrogen sulfide biosynthesis and mitigates pyroptosis to prevent high-fat diet induced diabetic cardiomyopathy

Sumit Kar<sup>1</sup>\*, Hamid R. Shahshahan<sup>1</sup>\*, Bryan T. Hackfort<sup>1</sup>\*, Santosh K. Yadav<sup>1</sup>, Roopali Yadav<sup>1</sup>, Tyler N. Kambis<sup>1</sup>, David J. Lefer<sup>2</sup>, Paras K. Mishra<sup>1</sup>

## Affiliations:

<sup>1</sup>Department of Cellular and Integrative Physiology, University of Nebraska Medical Center, Omaha, NE, USA

<sup>2</sup>Cardiovascular Center of Excellence, Louisiana State University Health Sciences Center, New Orleans, LA 70112

## **Corresponding author:**

Paras Kumar Mishra, Ph.D., F.A.H.A. F.C.V.S. Department of Cellular and Integrative Physiology University of Nebraska Medical Center Omaha, NE-68198, USA Phone: 402-559-8524 Fax: 402-559-4438 Email: paraskumar.mishra@unmc.edu

\*Authors contributed equally

Exercise	<u>Warm-up</u>		Run		<u>Cool-down</u>	
regimen	Time	Speed	Time	Speed	Time	Speed
	(min)	(M/min)	(min)	(M/min)	(min)	(M/min)
Pre-training	5	8	5, 20,	10, 10	5	8
1-4 days			30, 40	12, 12	5	8
Week 1	5	8	50	12	5	8
Week 2-20	5	8	50	15	5	8

**Table S1.** Exercise training protocol: Regimen for the exercise pre-training and training.



ITT 18 week



ND HFD HFDEX EX

**Figure S1. 18 weeks treatment of high-fat diet induces a diabetic phenotype in mice. A, B.** Intraperitoneal glucose tolerance test (GTT) at 18-week treatment in the four groups of mice: normal diet (ND), high-fat diet (HFD), HFD mice on exercise training (HFDEX) and ND mice on exercise training (NDEX). Glucose clearance was decreased in HFD but improved with EX. **C, D.** Intraperitoneal insulin tolerance test (ITT) at 18-week treatment. Insulin resistance was higher in HFD as compared to ND group which was also improved with EX. **E.** Glucose transporter type 4 (GLUT-4) expression in the heart was slight reduced with HFD treatment but not statistically significant. \*, *P*<0.05 between ND and HFD. #, *P*<0.05 between HFDEX and HFD. All values expressed as mean±SEM with dots representing each animal. Repeated measures one-way ANOVA and Tukey's post-hoc test was used for statistical analysis.





Α



**Figure S3. Expression of endoplasmic reticulum stress markers BiP and CHOP in obese mice with exercise training.** All values expressed as mean±SEM with dots representing each animal. One-way ANOVA and Tukey's post-hoc test was used for statistical analysis.