**Empagliflozin ameliorates obesity-related cardiac dysfunction by regulating Sestrin2-mediated AMPK-mTOR signaling and redox homeostasis in high-fat induced obese mice**

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**Supplemental Material**

**Body composition analysis**

Body composition of the mice were measured by EchoMRI body composition analyser (EchoMRI LLC, Houston, USA).

**Oral glucose tolerance test (OGTT) and insulin tolerance test (ITT)**

After a 6h fasting, the mice were given glucose (2 mg/g) via an oral gavage or humulin (0.75 U/kg) intraperitoneally, respectively. Blood glucose levels were measured by tail vein puncture with a blood glucometer (AimStrip Plus, USA) at the different time point (0, 30, 60, 90 and 120 min).

**Biochemical Analysis**

The mice were anesthetized with 3% isolurane and blood was collected from heart. Blood was centrifuged and plasma was stored at −80 °C until determination. Plasma lipids were measured by. Fasting insulin levels were measured with a mouse insulin ELISA kit. Plasma FFA levels were measured with a using colorimetric assays (BioVision, USA, Cat. #K612-100). Plasma adiponectin and leptin levels were measured using ELISA kits (R&D, Cat. #MRP300 and #MOB00).

**Western blotting**

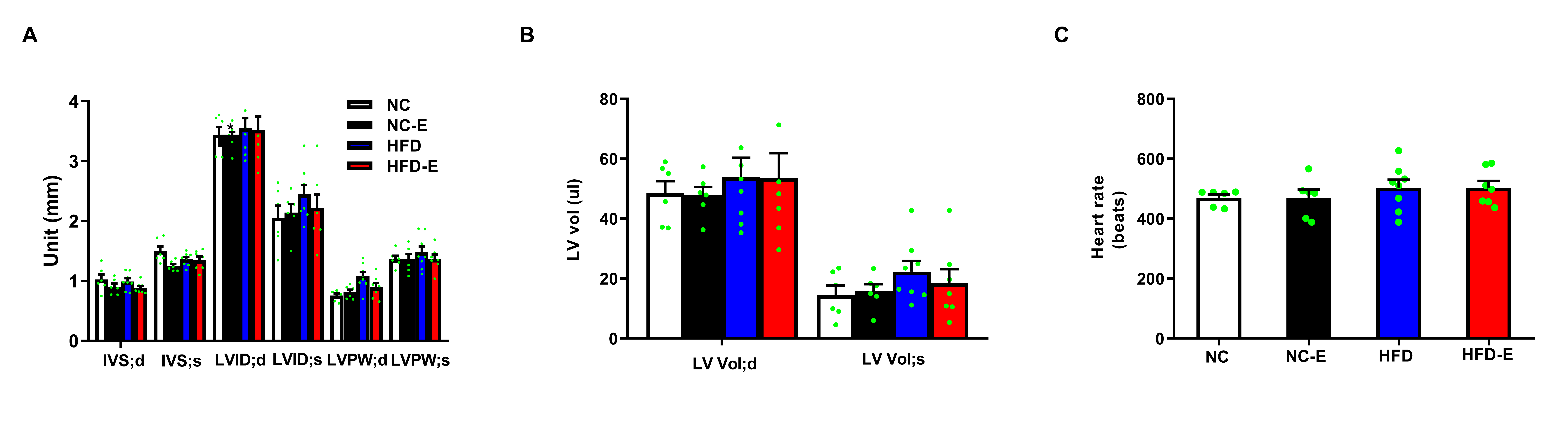
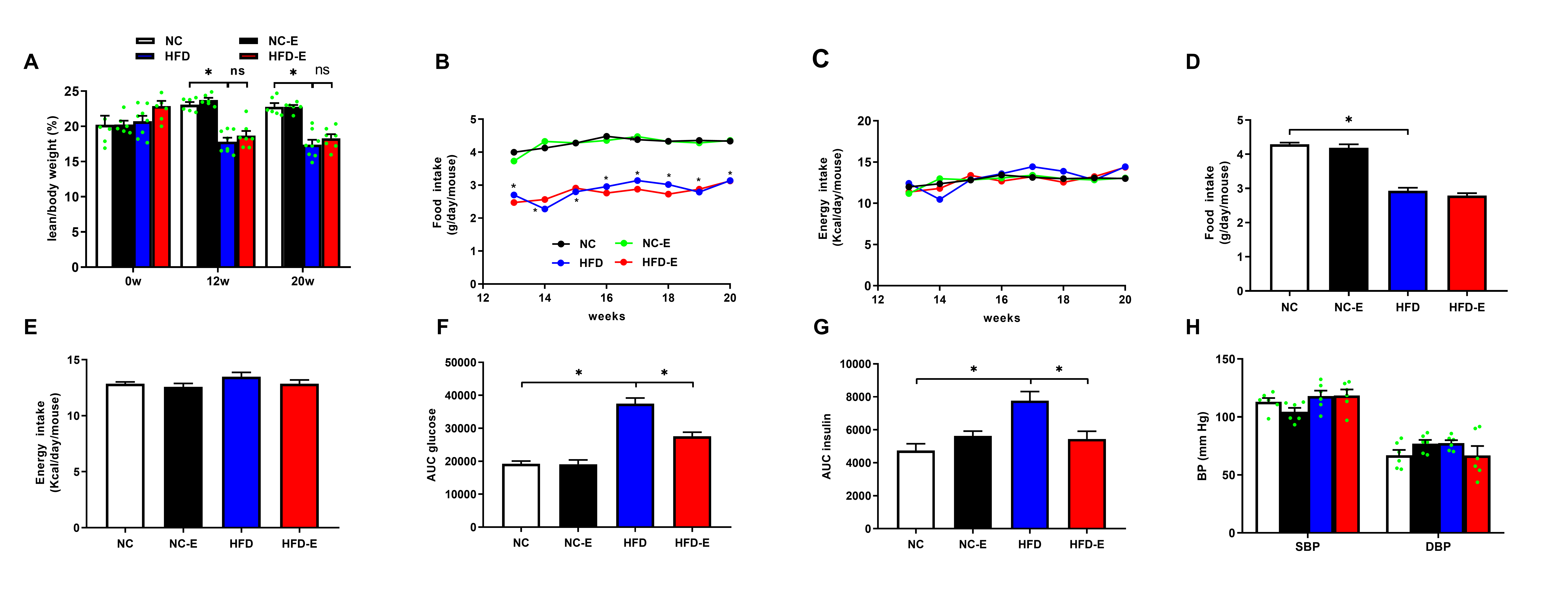
The primary antibodies are as followings: p-AMPK (Thr172) (#2535), AMPKα (#5831), p-mTOR (#2971), mTOR (#2972s), Nrf2 (#12721), HO-1(#70081), MCP-1 (#2027), p-NF-κB-p65 (#3033), p-p70 S6 kinase (#9205S), PI3K p110α (#4249), p-Akt (Ser473) (#4060) and Akt (#4691) were purchased from Cell Signalling Tech (USA); p-eNOS (Ser1177) (#ab215717), eNOS (#ab199956) were obtained from Abcam (USA)；Sestrin2 (#10795) was purchased from Proteintech (USA).

**RNA extraction and qRT-PCR**

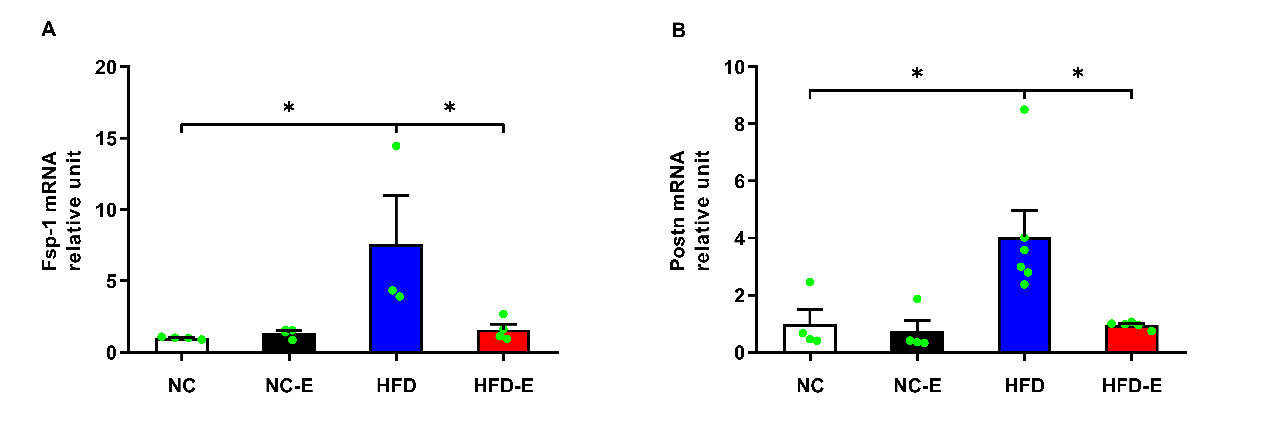
Primer sequence used for qPCR as follows:

|  |  |  |
| --- | --- | --- |
| **Primer** | **Forward** | **Reverse** |
| *GAPDH* | GGTTGTCTCCTGCGACTTCA | TGGTCCAGGGTTTCTTACTCC |
| *mtDNA* | CCGCAAGGGAAAGATGAAAGA | TCGTTTGGTTTCGGGTTTC |
| *Sesn2* | GCCTTCCGAAGTCTTGGTCAGTG | AGCCTCTGGATCAGCGAGTAGC |
| *Nrf2* | CTGTGCTGCCAGAGGTCCTTAATG | GGAACAGTGAGGTGCCAGTAACG |
| *Postn* | GCCCTCCACTCCCTTCCTGAC | GGATGCTGATGCCTTCTTGAGACC |
| *FSP-1* | GTGGGTCCTGCCTGTGTATTGC | AGGTGGTTCATTGTGGTTCCGTAC |
| *HO-1* | CCTGCTGATTCTCCTCTCCTCCTC | AAGCCTTCTCTGGACACCTGACC |
| *Catalase* | TTGGTGCCTTGGTCACTGTGTTAG | GACTGGAATGCTTCGTGGCTCTC |
| *GCLM* | CTGGCTGTCCTGGAACTCACTTTG | GAGGCGGAGGCAGAGGTAGAG |

**Supplemental Figure 1. Effects of EMPA treatment on body weight, body composition and biochemical parameters.** (A) Body lean/body weight (n=6-8/group)**;** (B) Weekly food intake (n=5/group)**;** (C) Weekly energy intake (n=5/group)**;** (D) Average food intake each day (n=5/group)**;** (E) Average energy intake each day (n=5/group)**;** (F) glucose AUC from OGTT (n=5/group)**;** (G) glucose AUC from ITT (n=5/group)**;** (H) blood pressure (n=6/group)；\*p<0.05.



**Supplemental Figure 2. Effects of EMPA treatment on cardiac function.** (A) Interventricular septal (IVS) dimensions, LV internal dimensions (LVID), LV posterior wall thickness (LVPW); (B) LV volume (LV vol); (C) Heart rates. n= 6-8/group.

**Supplemental Figure 3. Effects of EMPA treatment on Fsp-1 (A) and Postn (B).** n=3-6/group. \*p<0.05.



**Supplemental Figure 4. Effects of EMPA treatment on phosphorylation of acetyl coenzyme A carboxylase (ACC).** n=3/group. \*p<0.05.



**Supplemental Figure 5. Effects of EMPA treatment on mTOR, Nrf2/HO-1 and NF-κB p65 activity in isolated cardiomyocytes from HFD mice.** n=3/group. \*p<0.05.