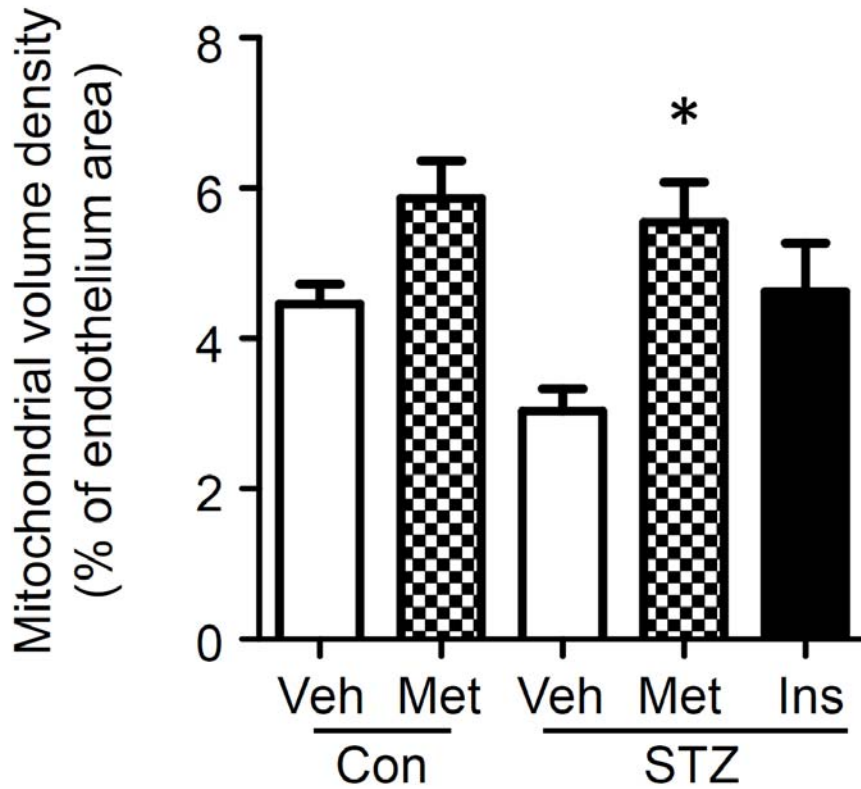


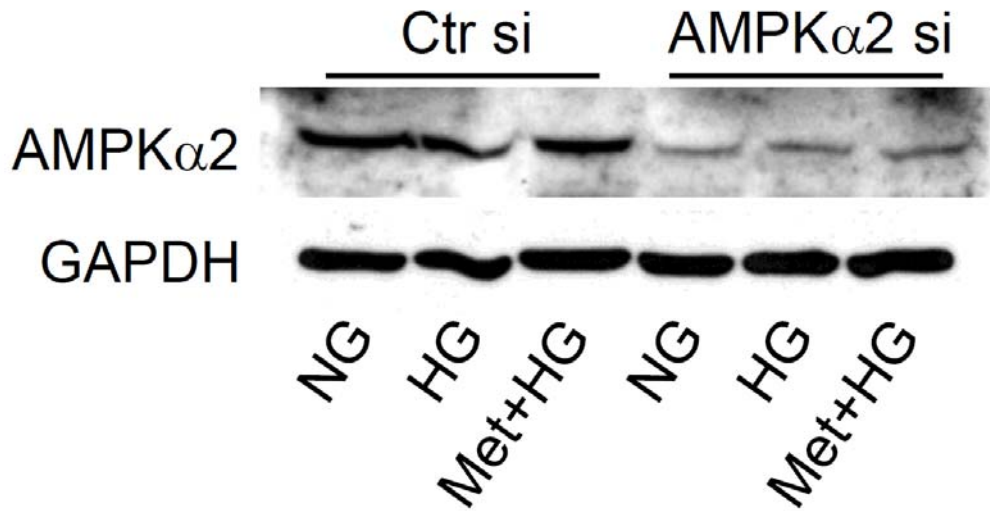
SUPPLEMENTARY DATA

Supplementary Figure 1. Mitochondrial volume density for the aortic endothelium. STZ-induced diabetic WT mice were administrated with metformin (300 mg/kg/d) for 4 weeks or intraperitoneally injected with insulin (STZ+Ins, 0.5 U/kg, twice per day) for 14 days. Quantification of mitochondrial volume density in aortic endothelium. n = 6 mice, at least 50 mitochondria per mice were analyzed. * $P < 0.05$ vs. Veh.



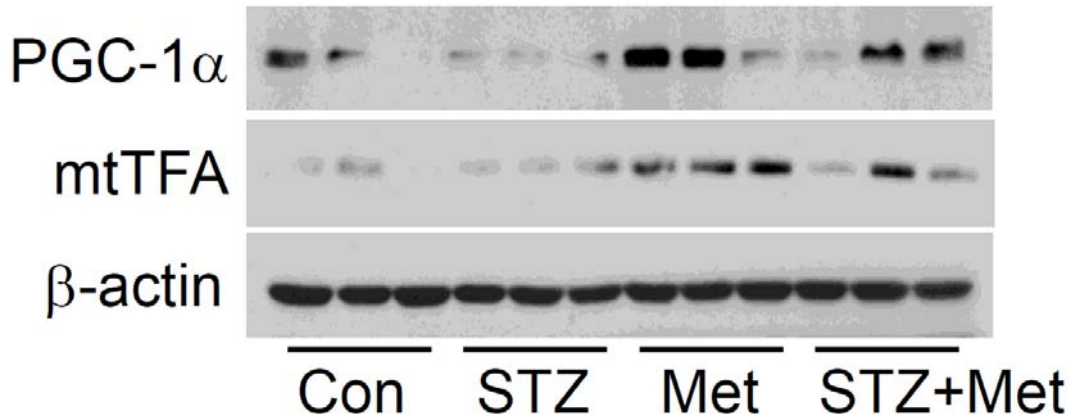
SUPPLEMENTARY DATA

Supplementary Figure 2. AMPK α 2 siRNA transfection silences AMPK α 2 expression. HUVECs were transfected with control siRNA or AMPK α 2 siRNA for 48 h, then pretreated with 2 mM metformin for 2 h and cultured with high glucose medium for 24 h.



SUPPLEMENTARY DATA

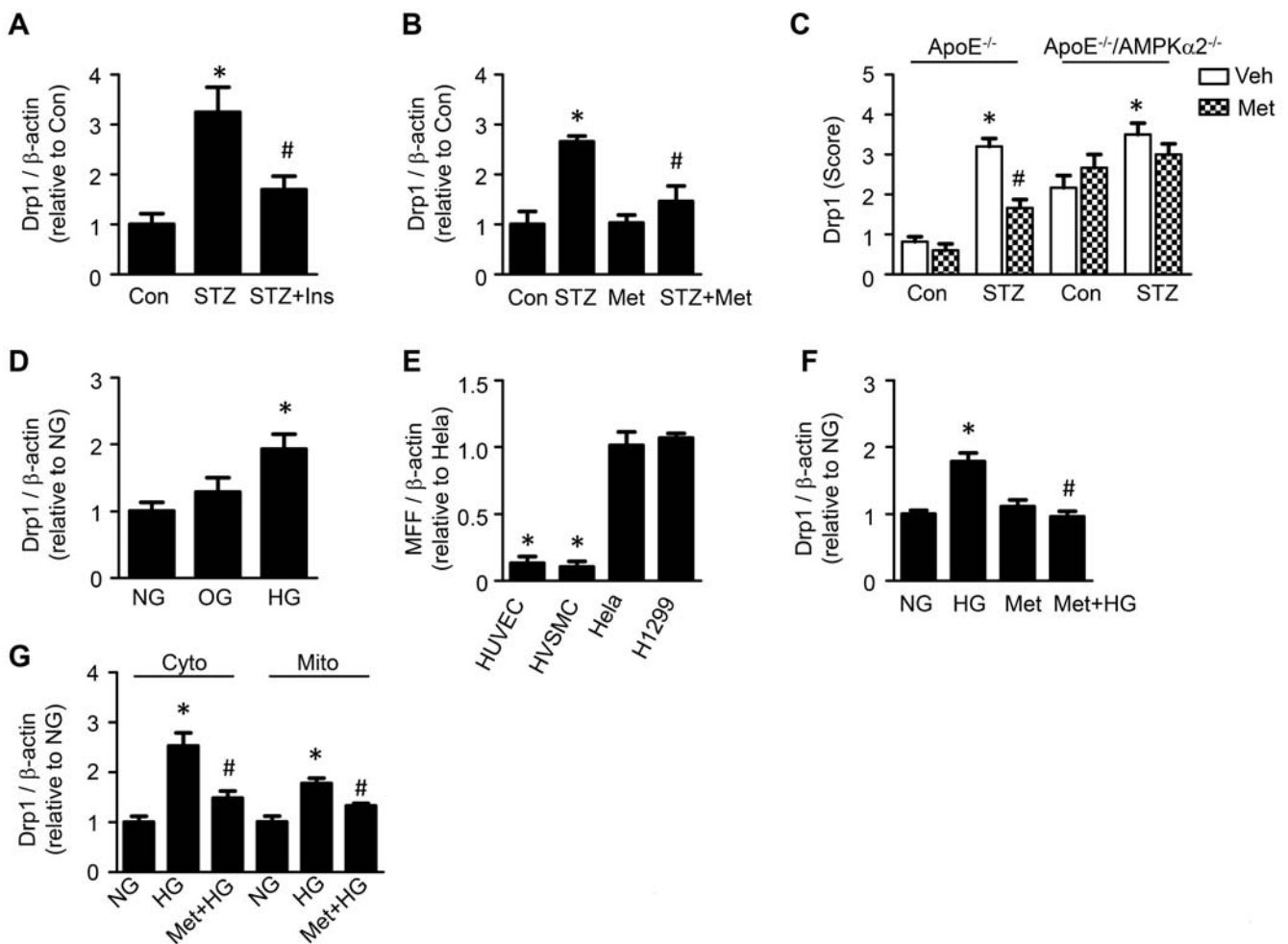
Supplementary Figure 3. Metformin increased the expression of PGC-1 α and mtTFA. STZ-induced diabetic WT mice were administered metformin (300 mg/kg/d) in drinking water for 4 weeks. Mitochondrial biogenesis-related proteins, including peroxisome proliferator-activated receptor gamma coactivator 1-alpha (PGC-1 α) and mitochondrial transcription factor A (mtTFA) in the aorta were analyzed by western blotting.



SUPPLEMENTARY DATA

Supplementary Figure 4. Metformin treatment reduces Drp1 expression and translocation into mitochondria.

A-B: STZ-induced diabetic WT mice were intraperitoneally injected with 0.5 U/kg insulin for 14 days (A) or administered metformin (300 mg/kg/d) in drinking water for 4 weeks (B). Quantification of Drp1 expression in the aorta. *n* = 5 mice. **C:** Quantification of positive staining for Drp1 in thoracic aortic sections from diabetic ApoE^{-/-} and ApoE^{-/-}/AMPKα2^{-/-} mice treated with metformin. *n* = 5 mice. **D:** HUVECs were treated with HG for 24 hours, mannitol as osmotic control. Quantification of Drp1 expression in the HUVECs. *n* = 4 independent experiments. **E:** Quantification of MFF expression in the HUVECs, HVSMCs HeLa cells, and H1299 cells. *n* = 3 independent experiments. **F-G.** HUVEC were pretreated with 2 mM metformin for 2 h, followed by stimulation with high glucose for 24 h. Quantification of Drp1 expression in the cell lysis, cytoplasmic and mitochondrial fractions from HUVECs. *n* = 3 independent experiments. **P* < 0.05 vs. Con or NG, #*P* < 0.05 vs. without metformin treatment.



SUPPLEMENTARY DATA

Supplementary Table 1. Antibody suppliers, catalog number and molecular weight.

Antibody	Application	Supplier	Catalog number	Molecular weight (kDa)
3-NT	IHC	EMD Millipore	06-284	
8-OHdG	IHC	Abcam	62623	
Drp1	IHC	ThermoFisher Scientific	PA1-16986	
ICAM-1	IHC, IF	Scant Cruz	7891	
VCAM-1	IHC, IF	Scant Cruz	8304	
p-ACC	WB	Cell Signaling Technology	3661	280
p-AMPK	WB	Cell Signaling Technology	2535	63
AMPK α 1	WB	Scant Cruz	130394	63
AMPK α 2	WB	Scant Cruz	19129	63
β -actin	WB	Scant Cruz	47778	43
COX IV	WB	Abcam	14744	17
Drp1	WB	Scant Cruz	271583	78-82
GAPDH	WB	Scant Cruz	137179	37
Fis1	WB	Enzo Life Science	Alx-210-1037	17
LDH	WB	Scant Cruz	33781	35
MFN2	WB	Abcam	56889	86
MFN	WB	Scant Cruz	515648	25-39
mtTFA	WB	Scant Cruz	23588	25
OPA1	WB	Abcam	42364	80-100
PGC-1 α	WB	Scant Cruz	13067	91

SUPPLEMENTARY DATA

Supplementary Table 2. Effects of metformin (Met) on blood glucose, lipid profiles and body weight in diabetic ApoE^{-/-} and ApoE^{-/-}/AMPKα2^{-/-} mice.

	ApoE ^{-/-}				ApoE ^{-/-} /AMPKα2 ^{-/-}			
	Con	Met	STZ	STZ + Met	Con	Met	STZ	STZ + Met
Body weight (g)	26.3±0.7	26.0±0.5	23.5±0.3*	23.3±0.2*	25.9±0.5	25.1±0.6	23.7±0.6*	23.0±0.6*
Glucose (mg/dL)	146±6	135±10	428±56*	444±52*	140±10	130±6	443±29*	395±30*
Cholesterol (mmol/L)	7.4±0.6	7.0±0.3	10.1±0.7*	9.0±0.8*	7.4±0.3	7.0±0.5	9.4±0.8*	10.8±2*
Triglycerides (mmol/L)	1.0±0.2	1.1±0.3	0.9±0.1	1.1±0.1	1.1±0.1	1.1±0.2	1.0±0.1	1.2±0.1

N = 8–10; *P < 0.05 vs. Con.

SUPPLEMENTARY DATA

Supplementary Table 3. Effects of mdivi-1 on blood glucose, lipid profiles, and body weight in diabetic ApoE^{-/-} mice.

	Con	Con + Mdivi-1	STZ	STZ + Mdivi-1
Body Weight (g)	26.3±0.7	25.0±0.6	23.6±0.9	23.8±0.9
Glucose (mg/dL)	156±6	149±9	469±55*	457±43*
Cholesterol (mg/dL)	7.8±0.2	10.1±0.5	12.4±1.0*	15.2±0.8*
Triglycerides (mg/dL)	0.9±0.1	0.9±0.1	1.0±0.1	1.1±0.0

N = 10; **P* < 0.05 vs. Con.