

Supplementary Information

Cardioprotective Effects of Short-Term Empagliflozin Treatment in *db/db* Mice

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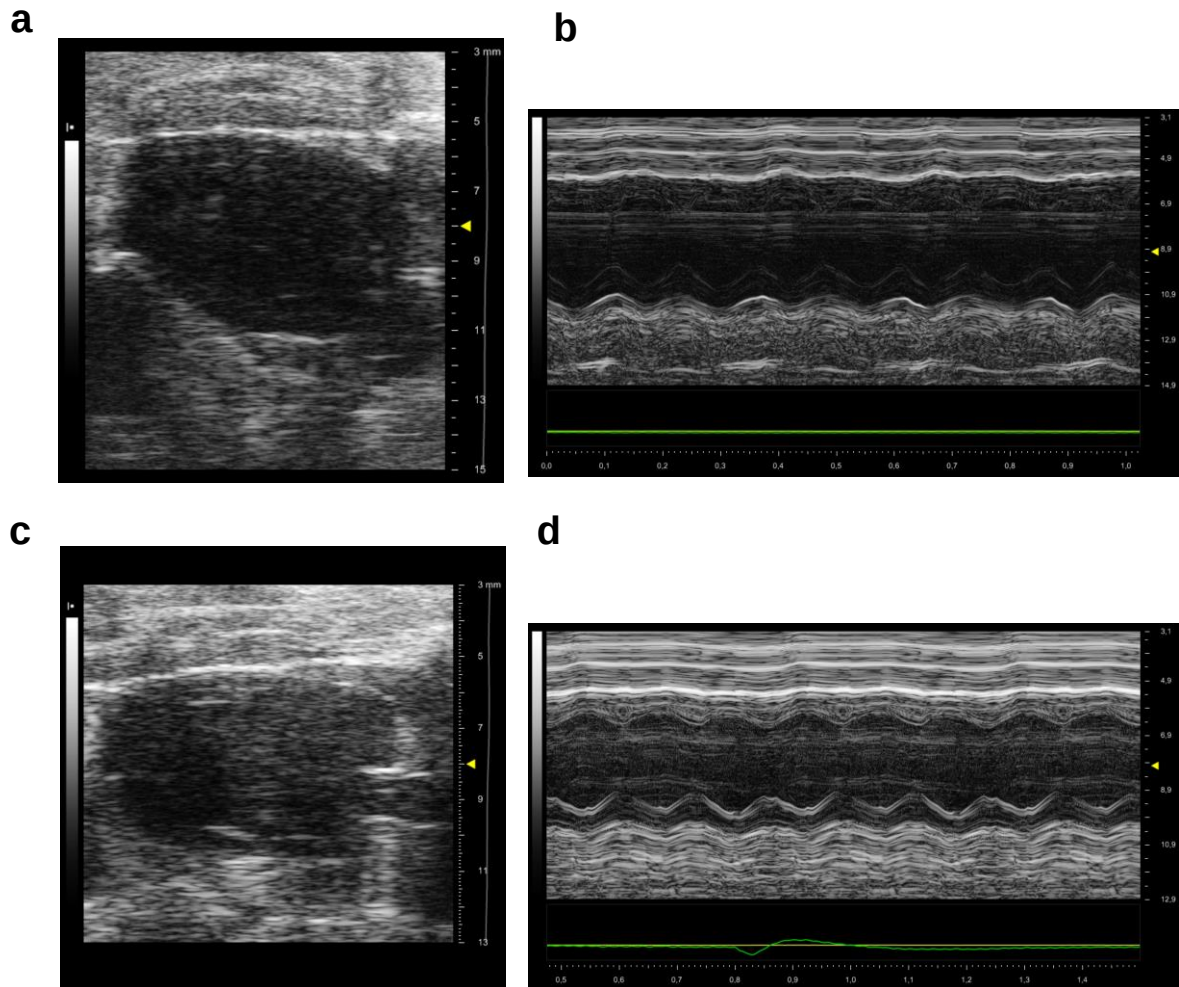
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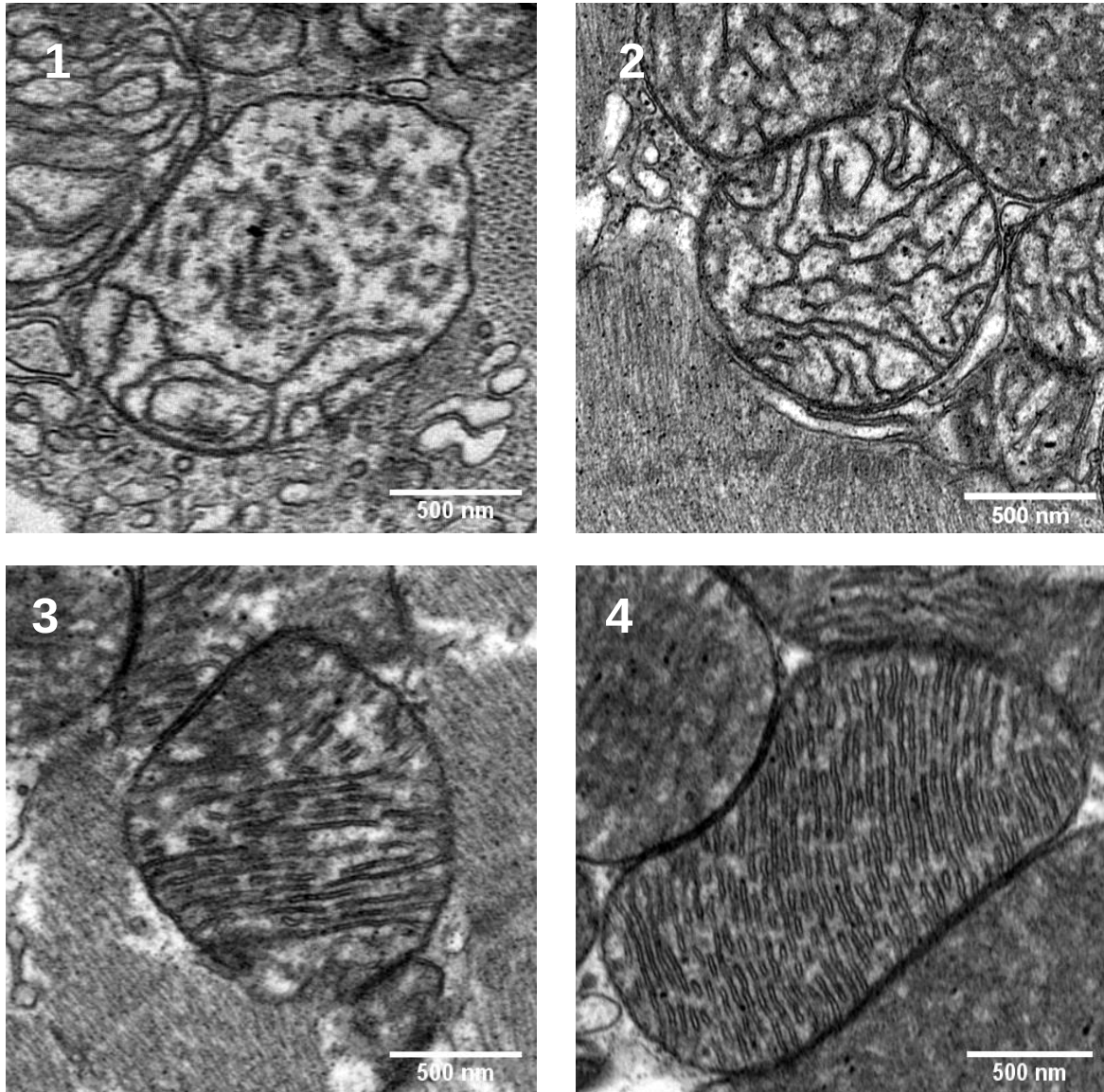
SI Figure 1,

Representative images from echocardiography. (a) left parasternal long-axis B-mode and (b) M-mode image of an untreated mouse. (c) left parasternal long-axis B-mode and (d) M-mode image of an EMPA treated mouse.



SI Figure 2,

Mitochondrial Cristae Score



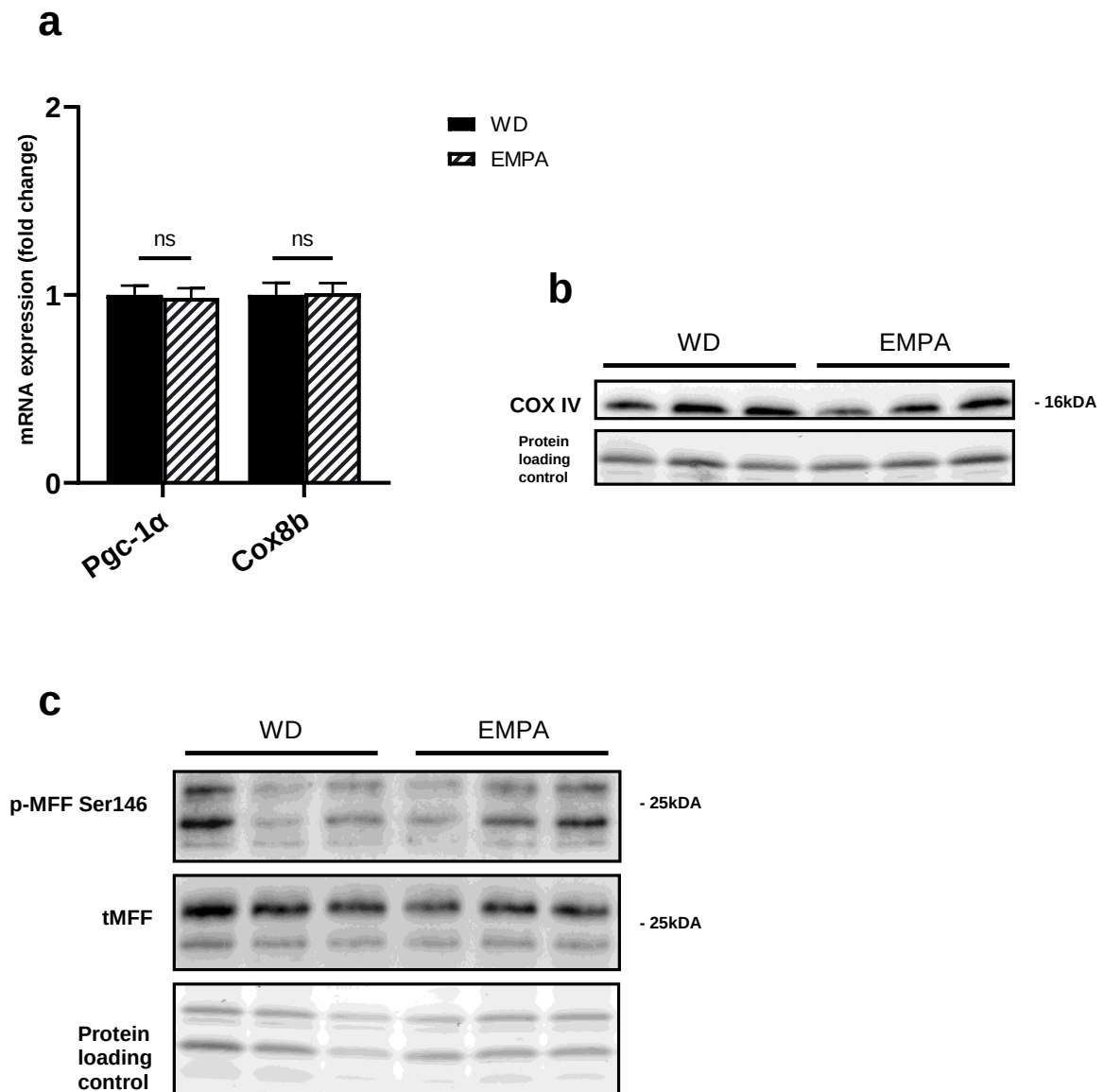
Mitochondrial Cristae Score (1-4)

- 1 - distorted, swollen looking mitochondria, no clear cristae structure
- 2 - rounded irregular cristae, widened mitochondrial matrix
- 3 - clearly visible cristae structure, white spots and some areas of widened matrix
- 4 - well defined, regular cristae, mitochondria look densely packed with cristae

adapted from Eisner et al., PNAS 2017

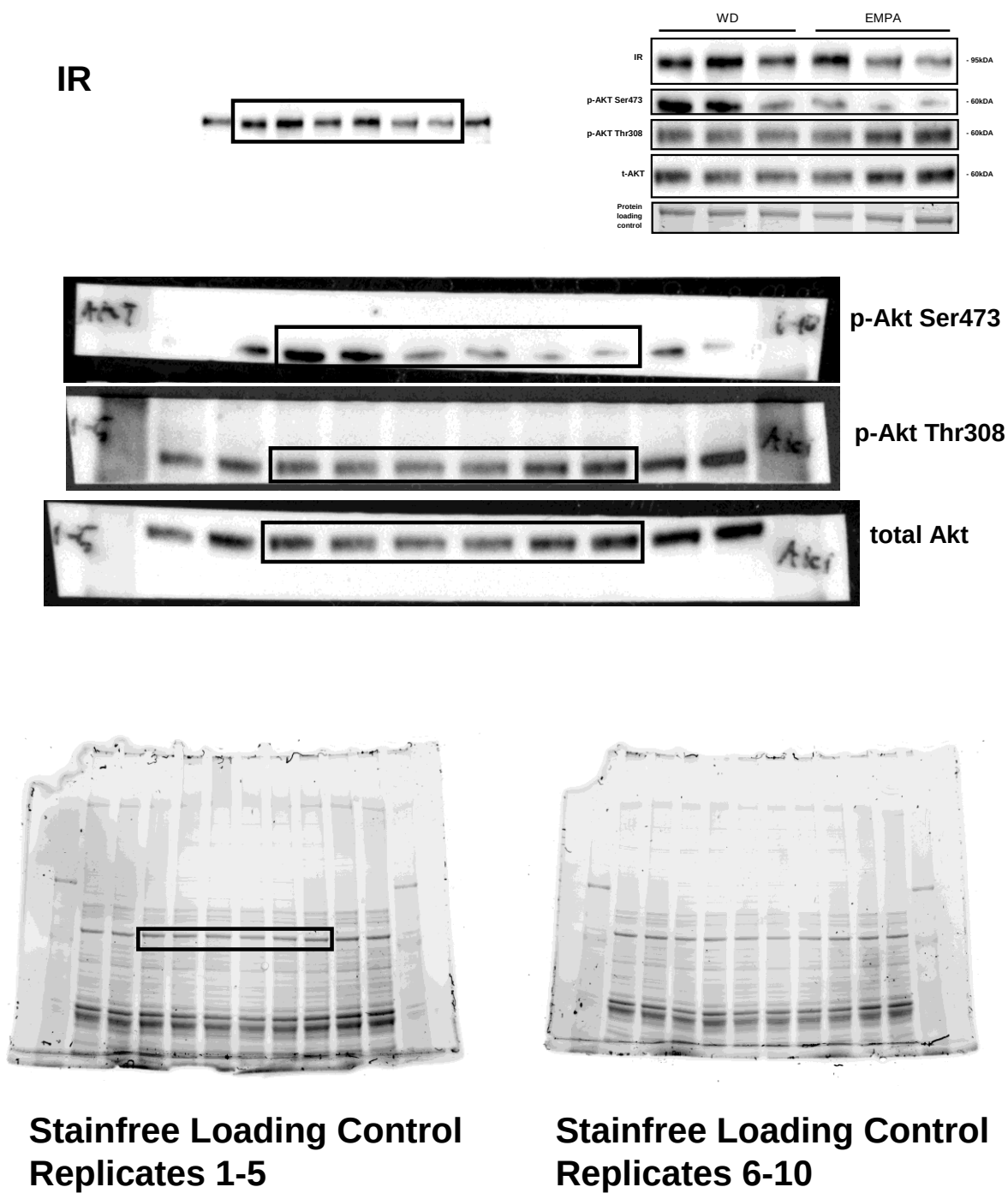
SI Figure 3,

Effects of short-term empagliflozin on the expression of Pgc-1 α , Cox 8b and Cox IV. (a) Pgc-1 α and Cox8b mRNA expression (n=8-9). (b) COX IV protein expression (n=8). (c) p-MFF Ser146 and MFF Protein expression (n=10). Data are expressed as means $\pm$ SEM, n.s. = not significant. Full-length images of cropped gels/blots are available in SI Figure 7. WD, Western type diet only; EMPA, Western type diet enriched with empagliflozin.



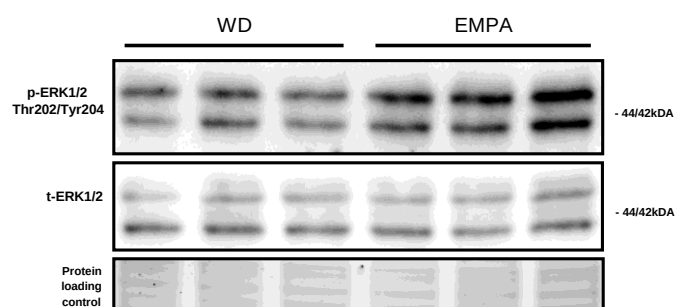
SI Figure 4a,

Full-length Western Blots of Figure 3

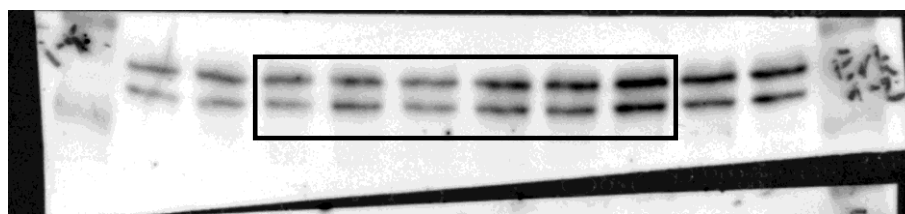


SI Figure 4b,

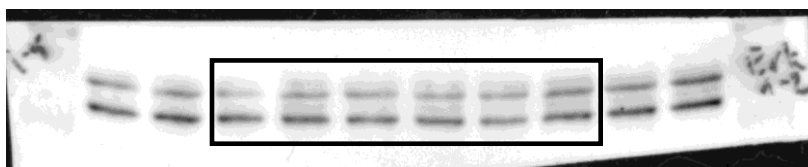
Full-length Western Blots of Figure 3



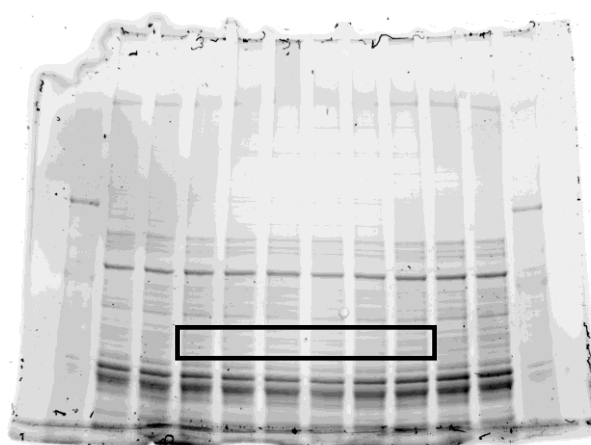
p-Erk 1/2



t-Erk 1/2

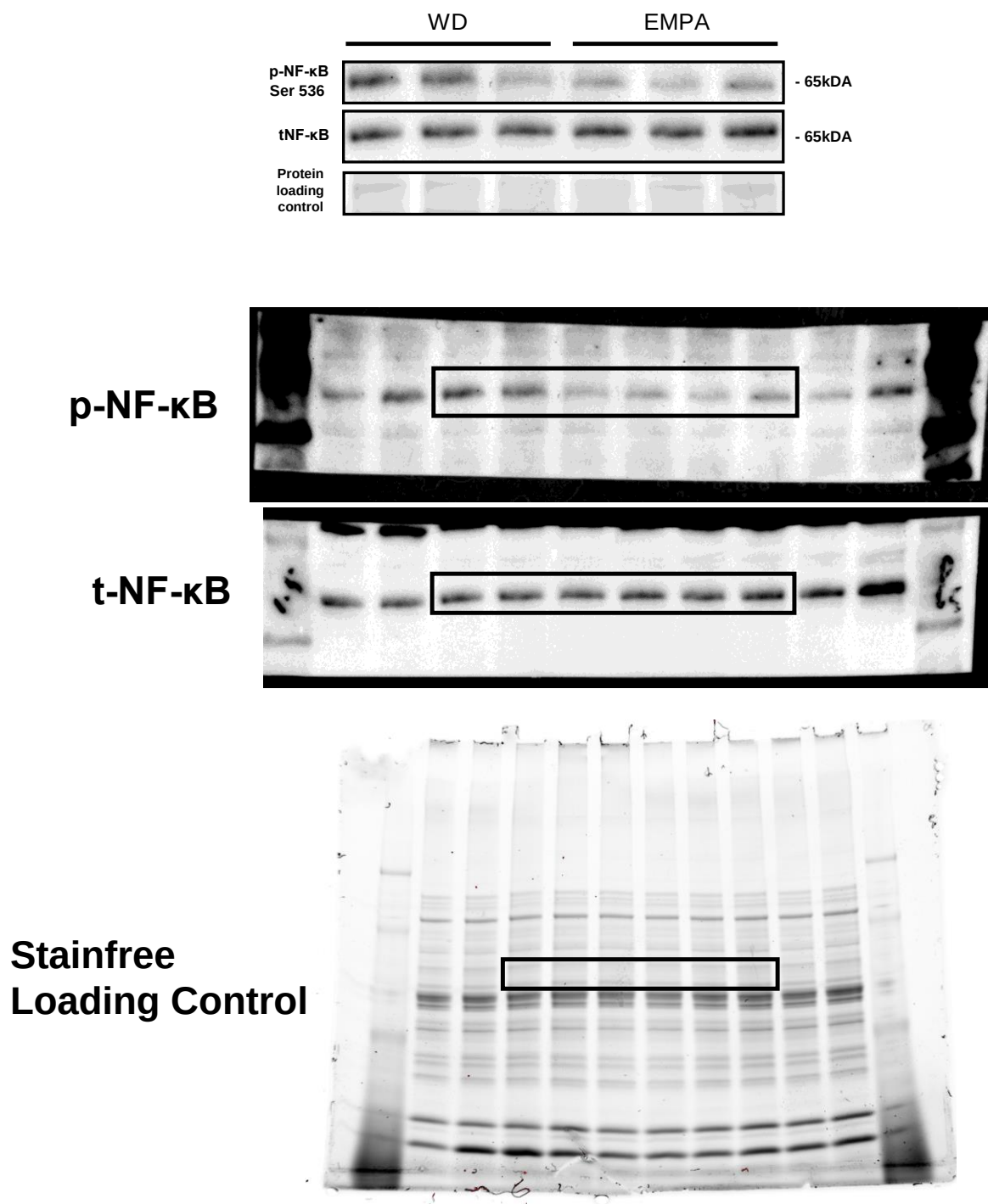


Stainfree
Loading Control



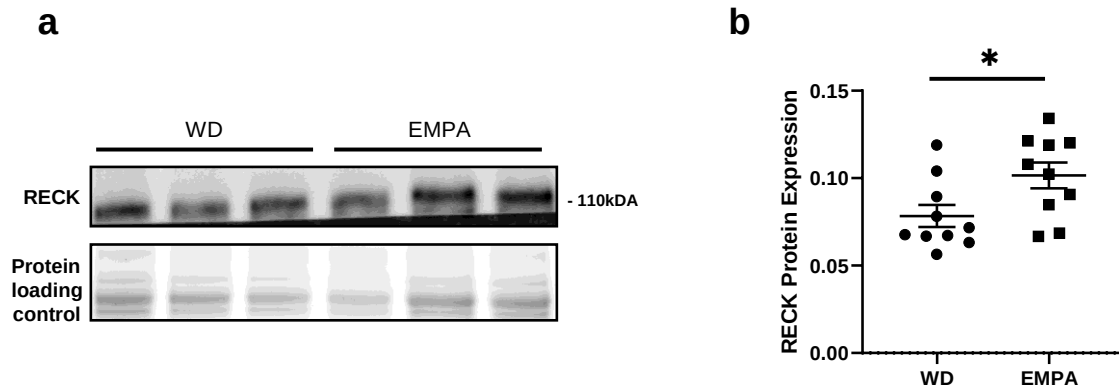
SI Figure 5,

Full-length Western Blots of Figure 4



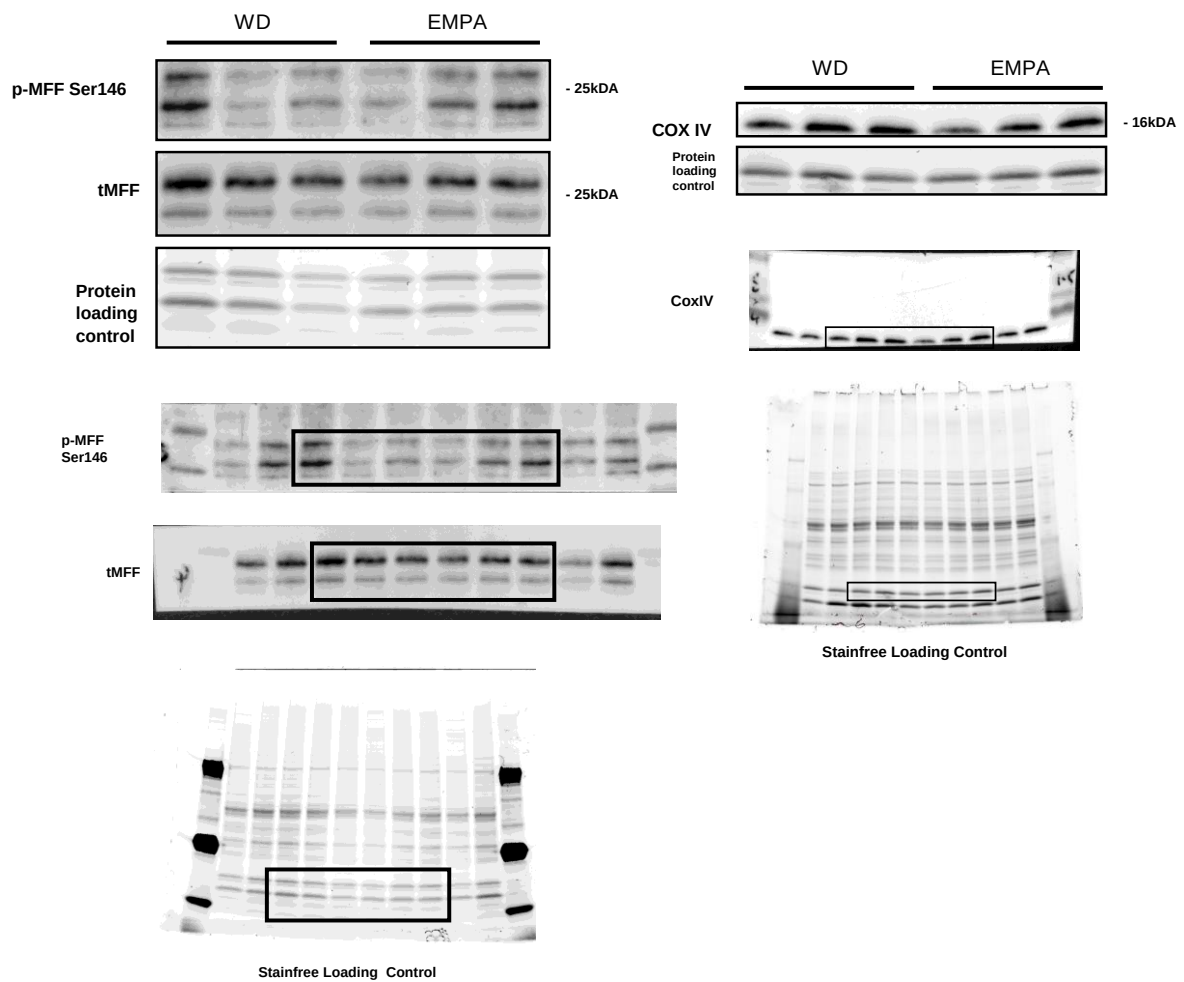
SI Figure 6,

Effects of short-term empagliflozin on RECK expression. **(a)** Representative Western Blot. **(b)** quantification of Western Blot. n=10. Data are expressed as means $\pm$ SEM, n.s. = not significant. Full-length images of cropped gels/blots are available in SI Figure 8. WD, Western type diet only; EMPA, Western type diet enriched with empagliflozin.



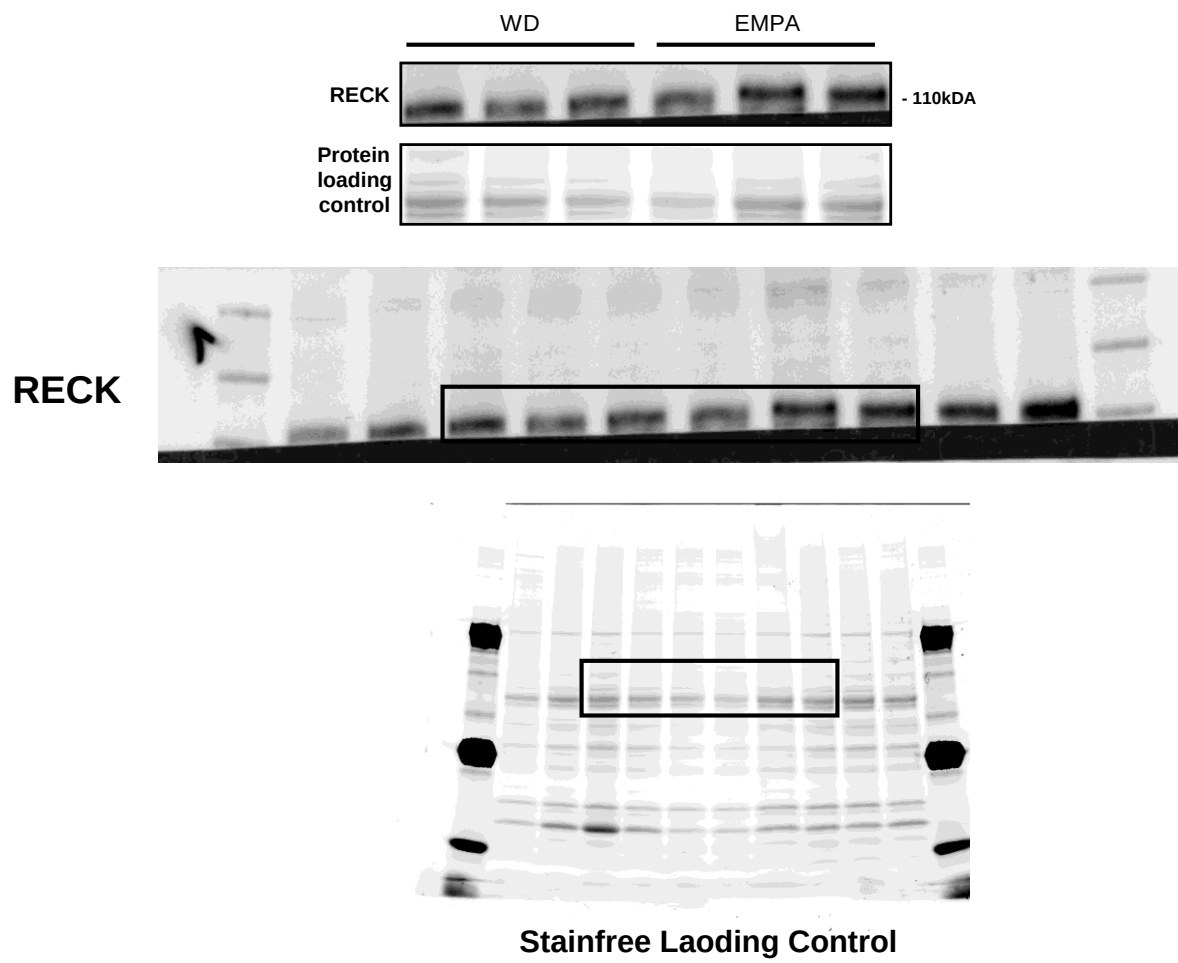
SI Figure 7,

Full-length Western Blots of SI Figure 3



SI Figure 8,

Full-length Western Blots of SI Figure 6



SI Table 1, Echocardiographic assessment of empagliflozin treated and control mice.

Ejection fraction (EF), Fractional shortening (FS), Left ventricular end diastolic diameter (LVEDd), Left ventricular end systolic diameter (LVESd), Heart rate and Left ventricular mass (LV mass). Data are expressed as mean $\pm$ SEM, n=4-5.

	WD	EMPA	p-value
EF (%)	45.43 $\pm$ 2.34	48.69 $\pm$ 2.45	0.427
FS (%)	36.69 $\pm$ 2.84	40.39 $\pm$ 3.04	0.405
LVEDd (mm)	3.95 $\pm$ 0.11	3.65 $\pm$ 0.14	0.125
LVESd (mm)	2.50 $\pm$ 0.12	2.17 $\pm$ 0.11	0.09
Heart rate (bpm)	428.4 $\pm$ 26.65	445.8 $\pm$ 27.22	0.865
LV mass (mg)	114.2 $\pm$ 9.90	101.3 $\pm$ 13.04	0.446

SI Table 2, Primer sequences used for qPCR

Gene	5' sequence	3' sequence
F 4/80	CTTTGGCTATGGGCTTCCAGT	GCAAGGAGGACAGAGTTTATCGTG
Ccl2	AGCTGTAGTTTTGTCACCAAGC	GTGCTGAAGACCTTAGGGCA
Ccr2	ATTCTCCACACCCTGTTTCG	GATTCCTGGAAGGTGGTCAA
IL10	GCTCTTACTGACTGGCATGAG	CGCAGCTCTAGGAGCATGTG
Mgl1	TGAGAAAGGCTTTAAGAACTGGG	GACCACCTGTAGTGATGTGGG
Mrc2	TACAGCTCCACGCTATGGATT	CACTCTCCCAGTTGAGGTACT

Gene	5' sequence	3' sequence	Probe
Glut4	CTCATGGGCCTAGCCAATG	GGGCGATTTCTCCACATAC	CATTGGCGCCTACTCAGGGCTAACATC
IL-6	TaqMan Gene Expression Assays 4331182 (Applied Biosystems)		

SI Table 3, Antibodies used for Western Blotting

Target		Used Dilution
Insulin Receptor beta	CellSignaling	1:1000
AKT	CellSignaling	1:1000
Phospho-AKT (Ser473)	CellSignaling	1:1000
Phospho-AKT (Thr308)	CellSignaling	1:500
ERK1/2 (p44/42)	CellSignaling	1:1000
Phospho ERK1/2 (Thr202/Tyr204)	CellSignaling	1:2000
NF-κB p65	CellSignaling	1:1000
Phospho-NF-κB p65 (Ser536)	CellSignaling	1:1000
Phospho-MFF (Ser146)	CellSignaling	1:1000
MFF	CellSignaling	1:1000
RECK	CellSignaling	1:1000
COX IV	Abcam	1:2000