

Supplementary Figure 1. Effects of chronic treatment with empagliflozin on systolic blood pressure, blood ketone, blood glucose and body weight in cytoATP-Tg mice. a-b Graph of the blood glucose before treatment with empagliflozin (a) (+/+ [control]: n = 10, db/db [control]: n = 8, db/db [EMPA]: n = 10; EMPA: empagliflozin) and after 8-week treatment with empagliflozin (b) (+/+ [control]: n = 10, db/db [control]: n = 8, db/db [EMPA]: n = 10). \*p = 0.000. c-d Graph of the blood ketone before treatment with empagliflozin (c) (+/+ [control]: n = 10, db/db [control]: n = 8, db/db [EMPA]: n = 10) and after 8-week treatment with empagliflozin (d) (+/+ [control]: n = 10, db/db [control]: n = 8, db/db [EMPA]: n = 10). \*p = 0.005, 0.004 each. e Graph of the body weight change ratio after 8 weeks (+/+ [control]: n = 10, db/db [control]: n = 8, db/db [EMPA]: n = 10. f-g Graph of the systolic blood pressure before treatment with empagliflozin (f) (+/+ [control]: n = 10, db/db [control]: n = 8, db/db [EMPA]: n = 10) and after 8-week treatment with empagliflozin (g) (+/+ [control]: n = 8, db/db [EMPA]: n = 10) and after 8-week treatment with empagliflozin (g) (+/+ [control]: n = 10, db/db [control]: n = 8, db/db [EMPA]: n = 10).



## Supplementary Figure 2. Autofluorescence in the hearts did not have a significant effect on measurement of FRET and GFP. a-d Fluorescence intensity of FRET (a, c) and GFP (b, d) in a live heart of a cytoATP-Tg mouse (FRET, Fluorescence Resonance Energy Transfer). Higher brightness indicates stronger intensity. Scale bar: 2 mm. e-h Fluorescence intensity of FRET (e, g) and GFP (f, h) in a live heart of a mitoATP-Tg mouse. Higher brightness indicates stronger intensity. Scale bar: 2 mm. i-l Autofluorescence intensity of FRET (i, k) and GFP (j, l) in a live heart of a wild-type mouse. Higher brightness indicates stronger intensity. Scale bar: 2 mm. m Graph of the intensity of FRET and GFP in each mouse (cytoATP: n = 3, mitoATP: n = 3, wild type: n = 3).



Supplementary Figure 3. Effects of single treatment with empagliflozin on blood glucose and blood ketone in db/db; mitoATP-Tg mice. a-b Graph of the blood glucose (a) and ketone (b) levels in db/db; mitoATP-Tg mice after 4 h of fasting followed by 3 h of control or empagliflozin (30 mg/kg b.w., EMPA) treatment (db/db [control]: n = 4, db/db [EMPA]: n = 4; EMPA: empagliflozin). \*p = 0.000, 0.009 each.



Supplementary Figure 4. Single treatment with empagliflozin did not increase cytosolic ATP levels in the heart of T2D model mice. a Schematic illustration of the treatment regimen (EMPA: empagliflozin). b-c Representative fluorescence images of a live heart from each condition. Warmer colors indicate higher ATP concentrations. Scale bar: 2 mm. d FRET/GFP ratio indicating the amount of ATP in the heart under each condition (db/db [control]: n = 6, db/db [EMPA]: n = 6). There was no significant difference between the control and EMPA groups. e The FRET/GFP ratio was examined 1 h after the addition of control ( $1.3 \pm 0.006$ , n = 32) or 10 nM ( $1.3 \pm 0.009$ , n = 34), 100 nM ( $1.3 \pm 0.007$ , n = 33) or 1000 nM ( $1.2 \pm 0.011$ , n = 34) empagliflozin (EMPA) to mature cardiomyocytes isolated from 8-week-old db/db; cytoATP-Tg mice. There was no significant difference between the control and EMPA groups.