



## Supplemented data

**Preparation of homogenate:** Frozen heart tissue was toughed in ice-cold A1 buffer (250 mM Sucrose, 0.5 mM Na<sub>2</sub>EDTA, 10 mM Tris, pH 7.4). The following steps were performed on ice. Approximately 20 mg tissue in 0.5 mL A1 buffer was cut into 1x1 mm pieces. After 10 min incubation at 4°C with 0.5 mg/mL trypsin (Sigma Aldrich), the buffer was removed, and the sample was washed twice with 2 mL A1. Thereafter, the tissue was transferred to a shredder tube (Oroboros Instrument, Innsbruck, Austria) containing 0.5 mL Mir05 (0.5 mM EGTA, 3 mM MgCl<sub>2</sub>·6H<sub>2</sub>O, 20 mM Taurine, 10 mM KH<sub>2</sub>PO<sub>4</sub>, 20 mM HEPES, 110 mM Sucrose, 50 mM K-lactobionate, 1 g/L BSA, pH 7.1). Tissue homogenate was made by placing the shredder tube in a shredder holder (Oroboros Instrument) and homogenize for 12 sec. at position 1 by using the PBI-Shredder SG3 (Oroboros Instrument). The homogenate was collected and centrifuged (Heraeus Biofuge, Thermo Scientific) at appr. 60 x g in 1 min. Finally, the supernatant was transferred to an Eppendorf tube and stored on ice until respiration analysis.

**Table S1.** mRNA expression of genes in cardiac tissue from C57BL/6J mice treated with 50ng/kg/min AngII (AngII<sub>50</sub>) or saline (sham) for two weeks.

	Sham	AngII <sub>50</sub>
<i>n</i>	7	10
<i>iNOS</i>	1 ± 0.15	0,7 ± 0.08
<i>ucp3</i>	1 ± 0.11	1.1 ± 0.17
<i>nppb</i>	1.0 ± 0.1	1.2 ± 0.2
<i>nppa</i>	1.0 ± 0.1	1.3 ± 0.2

Data are means ± SEM. *nppb*, natriuretic peptide b; *nppa*, natriuretic peptide A; iNOS, inducible nitric oxide synthase; *ucp3*, uncoupling protein 3. mRNA expression of the genes of interest was adjusted to the housekeeping gene hypoxanthine guanine phosphoribosyl transferase *hppt*.

**Table S2.** mRNA expression of genes in cardiac tissue from C57BL/6J mice treated with 400 ng/kg/min AngII (AngII<sub>400</sub>) or saline (sham) for two weeks.

	Sham	AngII <sub>400</sub>
<i>n</i>	9-10	9-10
<i>ppara</i>	1.00 ± 0.12	0.97 ± 0.05
<i>cd36</i>	1.00 ± 0.06	0.96 ± 0.03
<i>pdh4</i>	1.00 ± 0.13	0.73 ± 0.08
<i>ldh</i>	1.00 ± 0.04	0.87 ± 0.03 <sup>#</sup>
<i>hk</i>	1.00 ± 0.03	0.96 ± 0.05
<i>nox2</i>	1.00 ± 0.09	1.10 ± 0.11

Data are means ± SEM. *ppara*, peroxisome proliferator-activated receptor  $\alpha$ ; *cd36*, cluster of differentiation 36; *pdh4*, pyruvate dehydrogenase kinase 4; *ldh*, lactate dehydrogenase; *hk*, hexokinase; *nox2*, nicotinamide-adenine dinucleotide phosphate oxidase 2. <sup>#</sup> p < 0.05 compared to sham. mRNA expression of the genes of interest was adjusted to the housekeeping gene hypoxanthine guanine phosphoribosyl transferase *hppt*

**Table S3.** mRNA expression of genes in cardiac tissue from male (WT) and cs NOX2 transgenic (TG) mice treated with 400 ng/kg/min AngII (AngII<sub>400</sub>) for two weeks.

	WT AngII <sub>400</sub>	TG AngII <sub>400</sub>
<i>n</i>	4-6	5-6
<i>nppb</i>	1.00 ± 0.11	0.86 ± 0.10
<i>nppa</i>	1.00 ± 0.20	0.72 ± 0.22
<i>ppara</i>	1.00 ± 0.05	0.97 ± 0.03
<i>cd36</i>	1.00 ± 0.04	0.98 ± 0.02
<i>pd4</i>	1.00 ± 0.07	1.57 ± 0.13 <sup>#</sup>
<i>ldh</i>	1.00 ± 0.05	0.93 ± 0.04
<i>hk</i>	1.00 ± 0.02	1.00 ± 0.02
<i>nox2</i>	1.00 ± 0.06	33.51 ± 1.08 <sup>#</sup>

Data are means ± SEM. *nppb*, natriuretic peptide b; *nppa*, natriuretic peptide A; *ppara*, peroxisome proliferator-activated receptor  $\alpha$ ; *cd36*, cluster of differentiation 36; *pd4*, pyruvate dehydrogenase kinase 4; *ldh*, lactate dehydrogenase; *hk*, hexokinase; *nox2*, nicotinamide-adenine dinucleotide phosphate oxidase 2. mRNA expression of the genes of interest was adjusted to the housekeeping gene hypoxanthine guanine phosphoribosyl transferase *hppt*. #  $p < 0.05$  vs WT.

**Table S4.**

mRNA expression of the genes of interest was adjusted to the housekeeping gene hypoxanthine guanine phosphoribosyl transferase *hppt*.

	Gene Forward	Gene Accession Number	Reverse
<i>iNOS</i>	CAG-CTG-GGC-TGT-ACA	NM_010927.4	CAT-TGG-AAG-TGA-AGC-GTT
<i>ucp3</i>	TAC-CCA-ACC-TTG-GCT-AGA	NM_009464.3	GCC-TGG-CAA-TCT-TTT-GCT-T
<i>nppb</i>	CCA GTC TCC AGA GCA ATT CAA	NM_008726.5	GCC ATT TCC TCC GAC TTT T
<i>nppa</i>	CAC AGA TCT GAT GGA TTT CAA GA	NM_008725.3	CCT CAT CTT CTA CCG GCA TC
<i>ppara</i>	ACG ATG CTG TCC TCC TTG ATG	NM_0111446	GTG TGA TAA AGC CAT TGC CGT
<i>cd36</i>	TTG TAC CTA TAC TGT GGC TAA ATG AGA	NM_001159558.1	CTT GTG TTT TGA ACA TTT CTG CTT
<i>pd4</i>	TTC ACA CCT TCA CCA CAT GC	NM_013743.2	AAA GGG CGG TTT TCT TGA TG
<i>ldh</i>	CAT TGT CAA GTA CAG TCC ACA CT	NM_010699.2	TTC CAA TTA CTC GGT TTT TGG GA
<i>hk</i>	GAA GGG GCT AGG AGC TAC CA	NM_013820.3	CTC GGA GCA CAC GGA AGT T
<i>nox2</i>	TGAATGCCAGAGTCGGGATTT	NM_007807.5	CCCCCTCAGGGTTCTTGATT
<i>hppt</i>	TCC TCC TCA GAC CGC TTT T	NM_013556.2	CCT GGT TCA TCA TCG CTA ATC

Inducible nitric oxide synthase (*iNOS*); uncoupling protein 3 (*ucp3*); natriuretic peptide B (*nppb*); natriuretic peptide A (*nppa*); peroxisome proliferator-activated receptor  $\alpha$  (*ppara*); cluster of differentiation 36 (*cd36*); pyruvate dehydrogenase kinase 4 (*pd4*); lactate dehydrogenase (*ldh*); hexokinase (*hk*); nicotinamide-adenine dinucleotide phosphate oxidase 2 (*nox2*); hypoxanthine guanine phosphoribosyl transferase *hppt*.

**Table S5.** *Ex vivo* steady-state measurements of cardiac function from C57Bl/6J, WT and csNOX2 transgenic (TG) mice treated for two weeks with micro-osmotic pumps containing either Saline (sham), 50 or 400ng/kg/min Angiotensin II (AngII<sub>50</sub> and AngII<sub>400</sub>). The data are presented as mean  $\pm$  SEM.

	Sham	AngII <sub>50</sub>	Sham	AngII <sub>400</sub>	WT AngII <sub>400</sub>	TG AngII <sub>400</sub>
	<i>n</i> = 9	<i>n</i> = 11	<i>n</i> = 6	<i>n</i> = 9	<i>n</i> = 5	<i>n</i> = 6
BPM	389 $\pm$ 12	384 $\pm$ 10	349 $\pm$ 17	361 $\pm$ 13	361 $\pm$ 17	324 $\pm$ 16
CO (mL/min)	13.5 $\pm$ 0.7	12.2 $\pm$ 0.6	13 $\pm$ 1	13 $\pm$ 1	13.0 $\pm$ 0.9	13.4 $\pm$ 0.6
CF (mL/min)	2.8 $\pm$ 0.2	3.5 $\pm$ 0.2	3.4 $\pm$ 0.3	3.2 $\pm$ 0.2	4.0 $\pm$ 0.5	3.8 $\pm$ 0.3
DEVP (mmHg)	37 $\pm$ 2	37 $\pm$ 3	42 $\pm$ 3	41 $\pm$ 3	45 $\pm$ 3	52 $\pm$ 3
dp/dt <sub>max</sub> (mmHg/s)	1641 $\pm$ 85	1615 $\pm$ 106	1611 $\pm$ 110	1663 $\pm$ 84	1940 $\pm$ 48	1888 $\pm$ 62
dp/dt <sub>min</sub> (mmHg/s)	-844 $\pm$ 93	-950 $\pm$ 150	-821 $\pm$ 114	-718 $\pm$ 146	-1024 $\pm$ 96	-1008 $\pm$ 86

BPM beats per minute; CO, Cardiac Output; CF, Coronary Flow; DEVP, Aortic developed pressure; dp/dt<sub>max</sub> and dp/dt<sub>min</sub>, maximal and minimal first derivative of pressure over time, respectively.