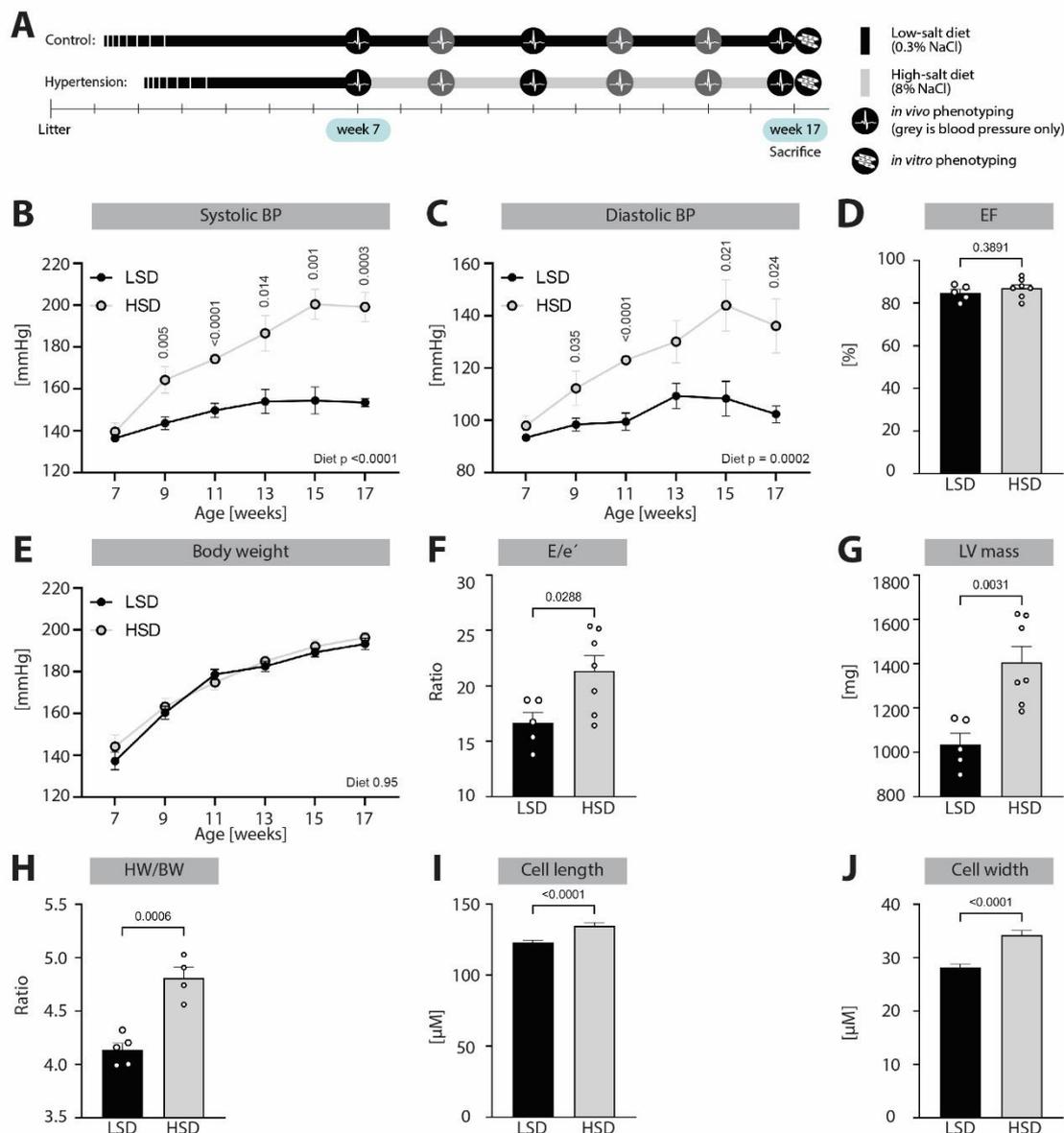


# $\beta$ -adrenergic receptor stimulation maintains NCX-CaMKII axis and prevents overactivation of IL6R-signaling in cardiomyocytes upon increased workload

## Supplementary materials

Information on the diet plan and in vivo and hypertrophy phenotyping of hypertensive rats is shown in Figure S1.



**Figure S1.** Dahl salt-sensitive rats develop hypertension, diastolic dysfunction and cardiac hypertrophy upon high-salt diet (HSD, 8% NaCl) feeding compared to low-salt diet (LSD, 0.3% NaCl). (A) Schematic overview of feeding and experimental plan of Dahl salt-sensitive rats, which were purchased at 4–5 weeks of age and fed either a LSD or HSD diet starting at the age of 7 weeks for 10 weeks thereafter. Blood pressure measurements were carried out biweekly, and echocardiographic phenotyping was performed at baseline (7 weeks) and presacrifice (17 weeks). (B) Systolic and (C) diastolic blood, (D) ejection fraction (EF), (E) body weight curve, (F) ratio of peak early Doppler transmitral flow velocity [E] to myocardial tissue Doppler velocity [e'], (G) left ventricular (LV) mass, (H) ratio of heart (HW) to body weight (BW), (I) cell length and (J) cell width. Data are

presented as mean  $\pm$  SEM ( $N = 5\text{--}11/4\text{--}7$  rats for LSD/HSD, and for I and J,  $n = 203/124$  cells for LSD/HSD). Indicated  $p$ -values were calculated by unpaired  $t$ -test (**D,F-I**), Mann–Whitney test (**J**), multiple unpaired  $t$ -tests (vertical  $p$ -values in **B** and **C**) or mixed-effect analysis (**B,C** and **E** bottom left diet effect).

Relevant information on the employed TaqMan™ Gene Expression Assays is provided in Table S1. Additional information on transcript IDs and RNA levels (including fold change) of microarrays performed on RNA isolated from *Dahl salt-sensitive rats* is provided in Table S2.

**Table S1.** List of TaqMan™ Assays (Thermo Fisher Scientific, Waltham, MA, USA) used for qPCR in *NRVCMs*.

Target Gene Name	TaqMan™ Gene Expression Assay ID	LOT
Regulator of Calcineurin 1 ( <i>RCAN1</i> )	Rn00596606_m1	1733914
Interleukin-6 Receptor ( <i>IL6R</i> )	Rn01495381_m1	1975678
Transforming Growth Factor beta 1 ( <i>TGFB1</i> )	Rn00572010_m1	1984434
<i>GAPDH</i>	Rn01775763_g1	1992407

**Table S2.** Additional microarray information for RNA analysis of *Dahl salt-sensitive rats* on either high-salt (HSD) or low-salt diet (LSD) for 10 weeks.

ID	HSD Avg (log2)	LSD Avg (log2)	Fold Change	$p$ -Value	FDR $p$ -Value	Gene Symbol
TC0600001773.rn.1	13.06	13	1.04	0.5803	0.8688	Slc8a1
TC1200000643.rn.1	19.03	19.03	−1	0.5656	0.8627	Atp2a2
TC0500001897.rn.1	15.3	14.21	2.13	0.0144	0.1954	Nppb
TC0100003865.rn.1	8.83	9.07	−1.18	0.0597	0.3863	Adrb1
TC0400000061.rn.1	8.62	3.99	24.8	$7.44 \times 10^{-5}$	0.0081	Il6
TC0200004086.rn.1	6.95	6.51	1.36	0.0001	0.0115	Il6r