## Supporting Information

# CaM Kinase II regulates cardiac hemoglobin expression through histone phosphorylation upon sympathetic activation

Ali Reza Saadatmand,[a,b,c,1] Viviana Sramek,[a,b,1] Silvio Weber,[c] Daniel Finke,[a,b,d] Matthias Dewenter,[a,b] Carsten Sticht,[e] Norbert Gretz,[e] Till Wüstemann,[a,b] Marco Hagenmueller,[a,b] Stephan R. Kuenzel,[c] Stefanie Meyer-Roxlau,[c] Martin Kramer,[c] Samuel Sossalla,[b,f,g] Lorenz Lehmann,[a,b,d] Susanne Kämmerer,[c] Johannes Backs,[a,b,1,2] and Ali El-Armouche[c,1,2]

[a]Institute of Experimental Cardiology, University of Heidelberg, 69120 Heidelberg, Germany

[b]German Centre for Cardiovascular Research (DZHK) - partner site Heidelberg/Mannheim/Göttingen, 10785 Berlin, Germany

[c]Department of Pharmacology and Toxicology, Medical Faculty, Technische Universität Dresden, 01307 Dresden, Germany

[d]Department of Cardiology, University of Heidelberg, 69120 Heidelberg, Germany

[e]Center for Medical Research, Medical Faculty Mannheim, Heidelberg University, 68167 Mannheim, Germany

[f]Department of Internal Medicine II, University Hospital Regensburg, 93042 Regensburg, Germany

[g]Heart Research Center Göttingen, Department of Cardiology & Pneumology, University Medical Center Göttingen, Göttingen, Germany.

[1]A.R.S. and V.S.; J.B. and A.E.-A. contributed equally to this work.

[2]To whom correspondence may be addressed. Email: johannes.backs@med.uniheidelberg.de or ali.el-armouche@tu-dresden.de.

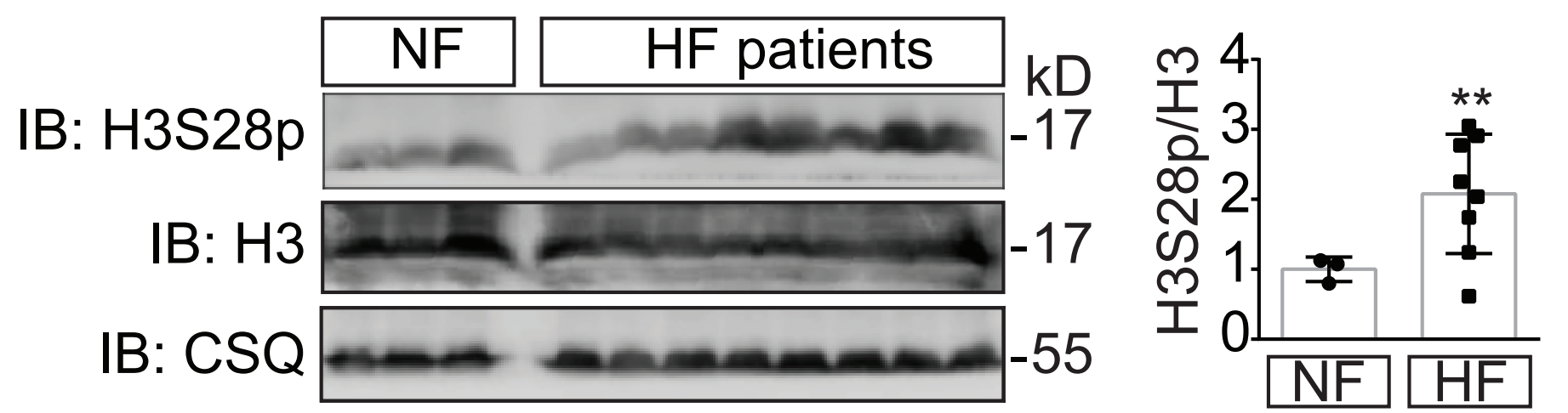
NF
HF patients
kD
IB: H3S28p
-17
IB: H3
-17
IB: CSQ
-55
H3S28p/H3
4
3
2
1
0
**
NF
HF

**Figure S1. Higher H3S28p in human failing hearts.** Immunoblot analysis from left ventricular tissue of non-failing donor hearts (NF; n = 3) and from patients with end-stage HF (n = 8). *p < 0.05 HF vs. NF.

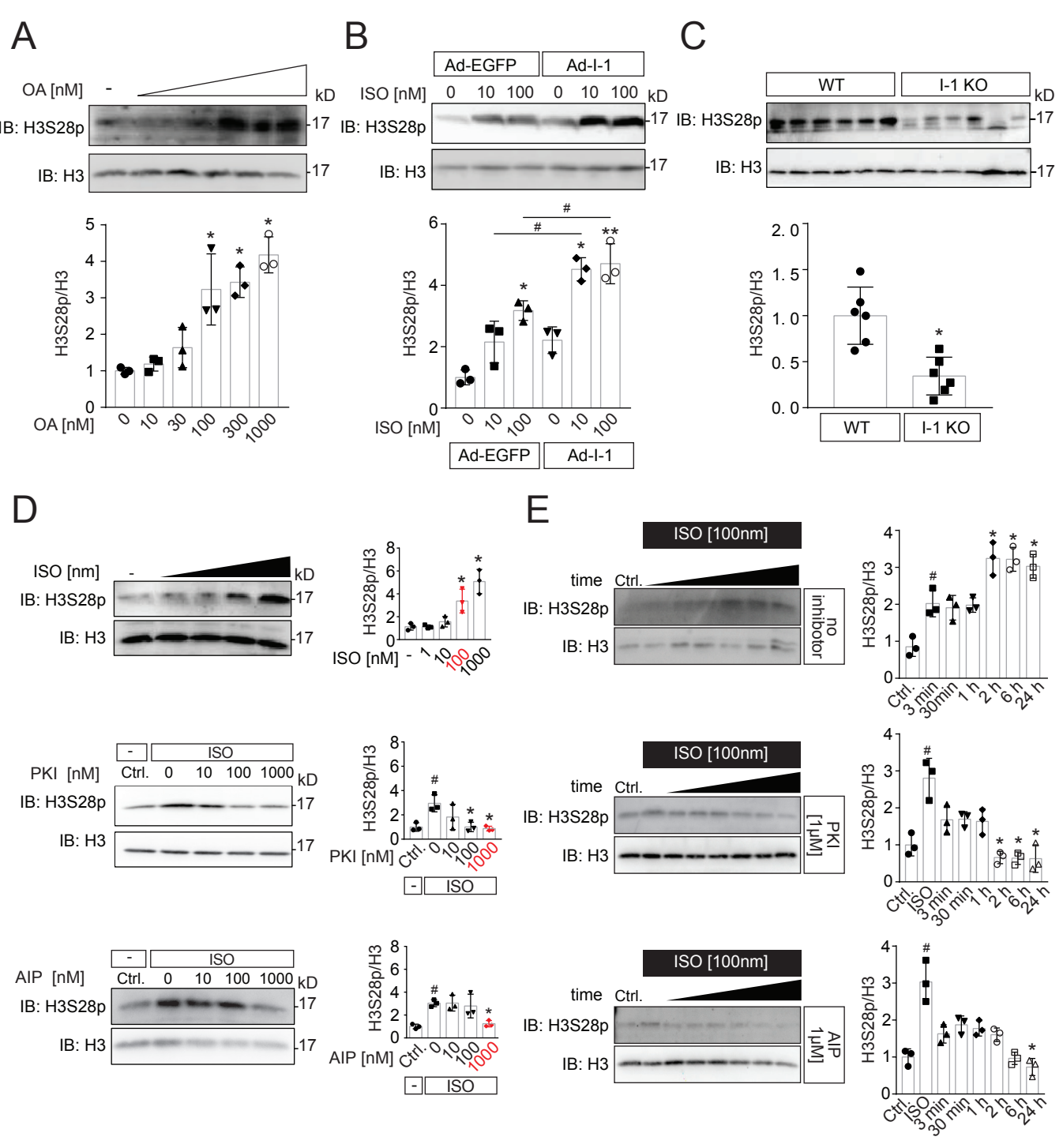
A
OA [nM]
-
kD
IB: H3S28p
17
IB: H3
17
H3S28p/H3
OA [nM]
0 10 30 100 300 1000
B
Ad-EGFP
Ad-I-1
ISO [nM]
0 10 100 0 10 100
kD
IB: H3S28p
17
IB: H3
17
H3S28p/H3
ISO [nM]
0 10 100 0 10 100
Ad-EGFP
Ad-I-1
C
WT
I-1 KO
kD
IB: H3S28p
17
IB: H3
17
H3S28p/H3
WT
I-1 KO
D
ISO [nm]
-
kD
IB: H3S28p
17
IB: H3
17
H3S28p/H3
ISO [nM]
- 1 10 100 1000
PKI [nM]
Ctrl. 0 10 100 1000
kD
IB: H3S28p
17
IB: H3
17
H3S28p/H3
PKI [nM]
Ctrl. 0 10 100 1000
-
ISO
AIP [nM]
Ctrl. 0 10 100 1000
kD
IB: H3S28p
17
IB: H3
17
H3S28p/H3
AIP [nM]
Ctrl. 0 10 100 1000
-
ISO
E
ISO [100nm]
time
Ctrl.
IB: H3S28p
IB: H3
no inhibitor
H3S28p/H3
Ctrl. 3 min 30min 1 h 2 h 6 h 24 h
ISO [100nm]
time
Ctrl.
IB: H3S28p
IB: H3
PKI [1μM]
H3S28p/H3
Ctrl. ISO 3 min 30 min 1 h 2 h 6 h 24 h
ISO [100nm]
time
Ctrl.
IB: H3S28p
IB: H3
AIP 1μM]
H3S28p/H3
Ctrl. ISO 3 min 30 min 1 h 2 h 6 h 24 h

**Figure S2. H3S28p is catecholamine-sensitive.** Representative immunoblots and H3S28p quantification from protein lysates of NRVMs or left ventricular tissue under different conditions normalized to total H3. **(A)** NRVMs upon increasing concentrations of okadaic acid (OA, n= 3; *p < 0.05). **(B)** NRVMs with increasing concentrations of isoprenaline (Iso) upon adenoviral expression of the constitutively active phosphatase inhibitor-1 (Ad-I-1) for inhibition of PP1 or recombinant eGFP control adenovirus (n= 3; *p < 0.05 vs 0; $^{\#}$p < 0.05). **(C)** LV tissues of I-1 wild type (WT) or I-1 knockout (KO) mice (n = 6; *p < 0.05). **(D)** NRVMs upon stimulation with increasing Iso concentrations (3 min, upper panel) or pretreatment (30 min) with increasing concentrations of PKI (PKA inhibitor, middle panel) or AIP (CaMKII inhibitor, lower panel) following Iso (100 nM, 3 min). (n= 3; *p < 0.05 vs 0, $^{\#}$p < 0.05 vs control). **(E)** NRVMs upon Iso (100 nM) at different time points (upper panel) or pre-treated with PKI (middle panel) and AIP (lower panel) for 30 min following Iso at different time points. (n= 3; *p < 0.05 vs 3 min, $^{\#}$p < 0.05 vs control).

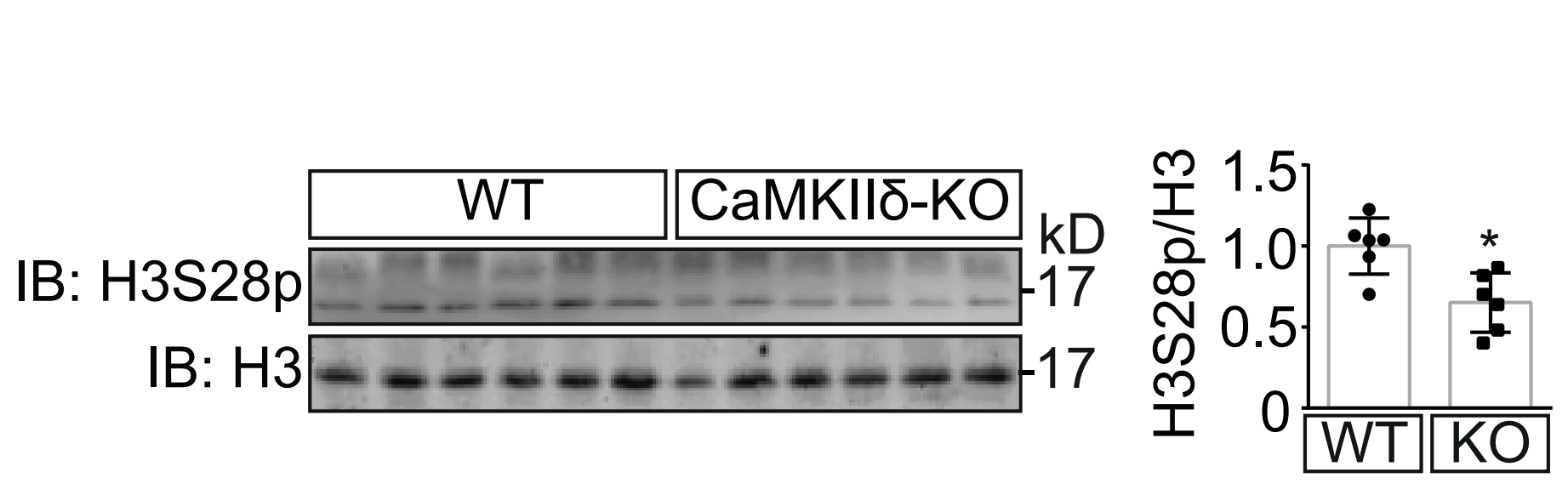
WT
CaMKIIδ-KO
kD
IB: H3S28p
17
IB: H3
17
H3S28p/H3
1.5
1.0
0.5
0
*
WT
KO

**Figure S3. CaMKII is required for H3S28p.** Immunoblot and quantification showing lower cardiac H3S28p in CaMKIIδ-KO compared to WT (n = 6, *p < 0.05).

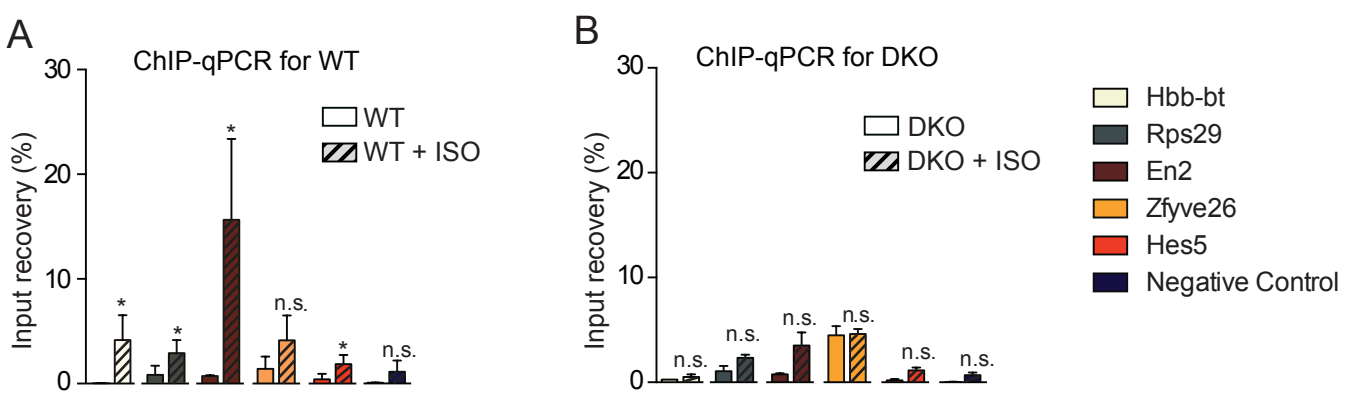
A
ChIP-qPCR for WT
Input recovery (%)
30
20
10
0
WT
WT + ISO
*
*
*
n.s.
*
n.s.
B
ChIP-qPCR for DKO
Input recovery (%)
30
20
10
0
DKO
DKO + ISO
n.s.
n.s.
n.s.
n.s.
n.s.
n.s.
Hbb-bt
Rps29
En2
Zfyve26
Hes5
Negative Control

**Figure S4.** ChIP-qPCR assay was performed with anti-H3S28p antibody followed by qPCR, showing increased H3S28p enrichment at the promoters of *Hbb-bt* and *Rps29* and intergenic region of *En2*, *Zfyve26* and *Hes5* (5 selected genes from the ChIPseq gene list). The results were calculated as recovery Input, the relative amount of pulldown enrichment was compared to input and represented as average ± SEM (*$p<0.05$, $n=3$). n.s. indicates not significant.

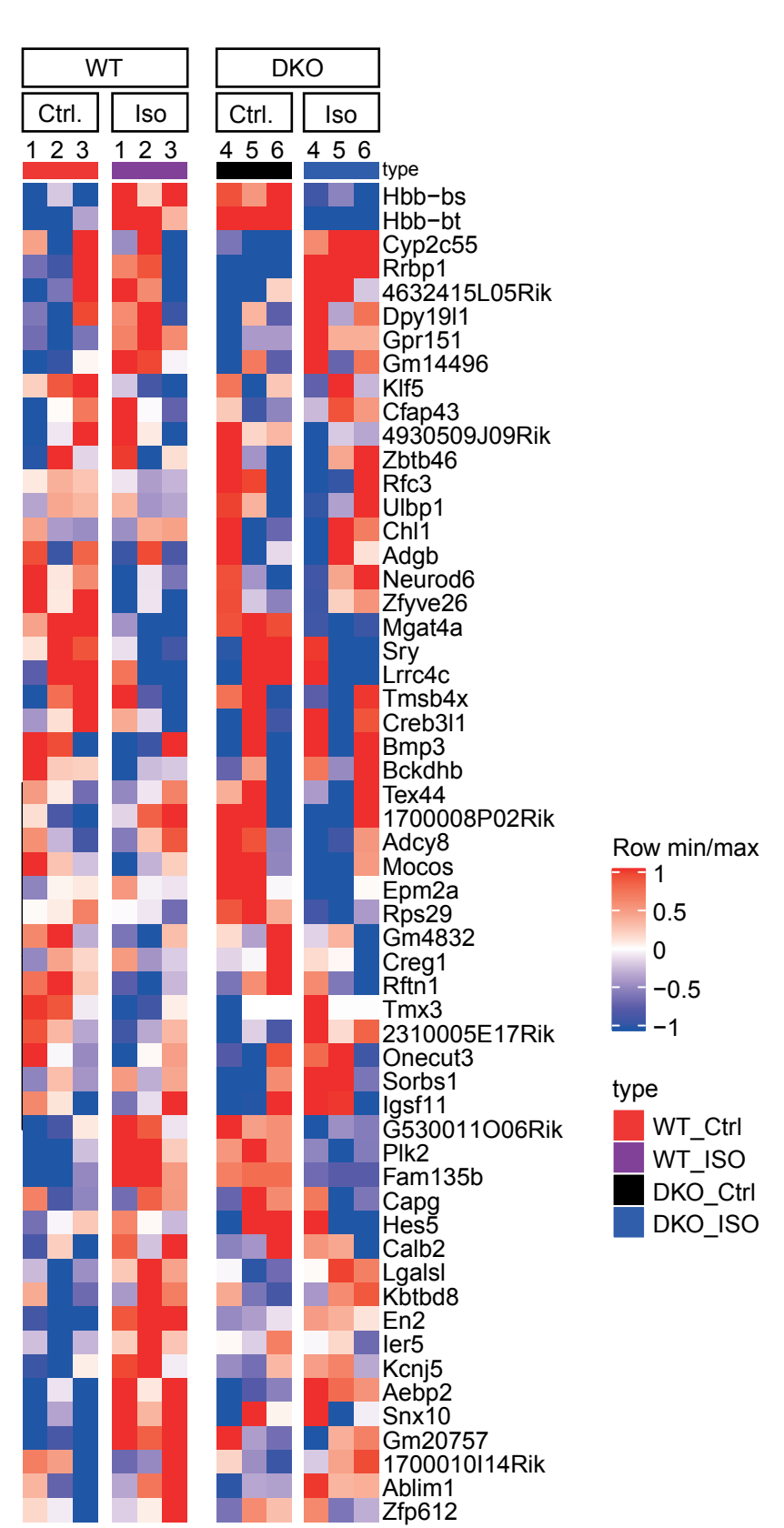
WT
DKO
Ctrl.
Iso
Ctrl.
Iso
1 2 3
1 2 3
4 5 6
4 5 6
type
Hbb−bs
Hbb−bt
Cyp2c55
Rrbp1
4632415L05Rik
Dpy19l1
Gpr151
Gm14496
Klf5
Cfap43
4930509J09Rik
Zbtb46
Rfc3
Ulbp1
Chl1
Adgb
Neurod6
Zfyve26
Mgat4a
Sry
Lrrc4c
Tmsb4x
Creb3l1
Bmp3
Bckdhb
Tex44
1700008P02Rik
Adcy8
Mocos
Epm2a
Rps29
Gm4832
Creg1
Rftn1
Tmx3
2310005E17Rik
Onecut3
Sorbs1
Igsf11
G530011O06Rik
Plk2
Fam135b
Capg
Hes5
Calb2
Lgalsl
Kbtbd8
En2
Ier5
Kcnj5
Aebp2
Snx10
Gm20757
1700010I14Rik
Ablim1
Zfp612
Row min/max
1
0.5
0
−0.5
−1
type
WT_Ctrl
WT_ISO
DKO_Ctrl
DKO_ISO

**Figure S5.** Affymetrix gene microarray (gene chip) analysis of cDNA in AMVMs obtained from WT and DKO mice w/o 24 h Iso stimulation. Heatmap depicting changes in gene expression of corresponding ChIPseq gene list presented in Table S1. Genes are ordered based on hierarchical clustering.

## Tables

**Tab. S1. Identification of CaMKII-dependent H3S28p target genes.** List of 68 peaks with the corresponding chromosome (Chr), the distance from TSS, the nearest gene and Iso-induced fold-regulation in WT; significantly, enriched/diminshed regions in WT but not DKO are named CaMKII-dependent H3S28p target regions (black) and significantly, enriched/diminished regions in WT and DKO are named CaMKII-independent H3S28p target regions (red).

| peak | Chr | Distance to TSS | Nearest Gene | WT-Iso/WT (fold) | DKO-Iso/DKO (fold) |
|---|---|---|---|---|---|
| **1** | 9 | -125064 | 2310003N18Rik | 15 | 2 |
| **2** | 15 | -117045 | Adcy8 | 15 | -1 |
| **3** | X | -110519 | G530011O06Rik | -6 | -1 |
| **4** | 11 | -105549 | Lgalsl | 26 | 1 |
| **5** | 5 | -97055 | Bmp3 | 25 | 3 |
| **6** | 6 | -83667 | Kbtbd8 | 2 | -1 |
| **7** | 5 | -83395 | Rfc3 | 1 | -1 |
| **8** | 2 | -69511 | Creb3l1 | -1 | 4 |
| **9** | 13 | -60744 | 2310005E17Rik | 31 | 2 |
| **10** | 18 | -47201 | Mir6356 | -6 | 2 |
| **11** | 5 | -46663 | En2 | 21 | 1 |
| **12** | 9 | -38950 | Dpy19l1 | -3 | 4 |
| **13** | 6 | -38945 | Capg | 14 | 0 |
| **14** | 10 | -30491 | Onecut3 | 22 | 2 |
| **15** | 3 | -28011 | 4632415L05Rik | 37 | 2 |
| **16** | 1 | -26510 | Mgat4a | 13 | 0 |
| **17** | Y | -17624 | Sry | 15 | 1 |
| **18** | 19 | -16302 | Cyp2c55 | 16 | 2 |
| **19** | 8 | -15298 | 2610005L07Rik | 8 | 2 |
| **20** | 4 | -13858 | Hes5 | 31 | 2 |
| **21** | 10 | -12868 | Gm20757 | 21 | 1 |
| **22** | 8 | -12856 | Gm21119 | 5 | 1 |
| **23** | 9 | -11957 | Kcnj5 | 27 | 3 |
| **24** | 9 | -11193 | Kcnj5 | 22 | 0 |
| **25** | 9 | -10460 | Kcnj5 | 15 | 5 |
| **26** | 15 | -10075 | Fam135b | 16 | 0 |
| **27** | 9 | -9203 | Bckdhb | 13 | -1 |
| **28** | 3 | -6074 | Mannr | 4 | 0 |
| **29** | 1 | -4108 | Creg1 | 13 | -3 |
| **30** | 2 | -3943 | Zbtb46 | 22 | 2 |
| **31** | 1 | -2947 | Ier5 | 7 | 1 |
| **32** | 12 | -262 | Rps29 | 6 | 0 |
| **33** | 7 | 79 | Hbb-bs | 7 | 2 |
| **34** | 7 | 79 | Hbb-bt | 8 | 0 |
| **35** | X | 1788 | Tmsb4x | 10 | 0 |
| **36** | 17 | 1917 | Rn45s | 24 | 11 |
| **37** | 19 | 5073 | Sorbs1 | 20 | 0 |
| **38** | 17 | 5476 | Rn45s | 24 | 7 |
| **39** | 19 | 5515 | Ablim1 | 13 | 1 |
| **40** | 10 | 7244 | Epm2a | 17 | 1 |

| | | | | | |
|---|---|---|---|---|---|
| **41** | 19 | 10664 | Cfap43 | 14 | 2 |
| **42** | 2 | 16904 | Rrbp1 | -3 | 0 |
| **43** | 18 | 21290 | Gpr151 | 31 | 0 |
| **44** | 2 | 21799 | Gm14496 | 1 | -2 |
| **45** | 18 | 23604 | Mocos | 27 | 1 |
| **46** | 8 | 24345 | Gm21119 | 9 | 3 |
| **47** | 8 | 26889 | Zfp612 | 13 | -2 |
| **48** | 7 | 30796 | Vmn2r31 | 4 | 1 |
| **49** | 8 | 31592 | Calb2 | 14 | 0 |
| **50** | 1 | 34777 | 1700019O17Rik | 4 | 0 |
| **51** | 12 | 36835 | Zfyve26 | 24 | 3 |
| **52** | 17 | 42690 | 1700010I14Rik | 29 | 3 |
| **53** | 3 | 48026 | 4930509J09Rik | -3 | 0 |
| **54** | 6 | 50438 | Neurod6 | 11 | 0 |
| **55** | 6 | 65826 | Aebp2 | 14 | 0 |
| **56** | 18 | 92247 | Tmx3 | -10 | -6 |
| **57** | 17 | 95079 | Gm4832 | 4 | 1 |
| **58** | 12 | 109065 | Mir3072 | 27 | 1 |
| **59** | 10 | 136857 | Adgb | 15 | 2 |
| **60** | 6 | 138322 | Chl1 | -6 | 1 |
| **61** | 3 | 141792 | 1700008P02Rik | 2 | 1 |
| **62** | 16 | 150400 | Igsf11 | -3 | 9 |
| **63** | 14 | 166257 | Klf5 | 28 | 2 |
| **64** | 6 | 169125 | Snx10 | 18 | -1 |
| **65** | 13 | 214506 | Plk2 | 23 | 1 |
| **66** | 17 | 249246 | Rftn1 | 26 | 3 |
| **67** | | 573774 | Ulbp1 | 6 | 9 |
| **68** | 2 | 1197463 | Lrrc4c | -9 | -5 |

**Tab. S2.** List of 23 peaks with the corresponding chromosome (Chr), the distance from TSS, the nearest gene and Iso-induced fold-regulation in DKO; significantly, enriched/diminshed regions in WT but not DKO are named CaMKII-dependent H3S28p target regions (black) and significantly, enriched/diminished regions in WT and DKO are named CaMKII-independent H3S28p target regions (red).

| peak | Chr | Distance to TSS | Nearest Gene | WT-Iso/WT (fold) | DKO-Iso/DKO (fold) |
|---|---|---|---|---|---|
| 1 | 12 | -125799 | Rab10os | -12 | -5 |
| 2 | 18 | -122466 | Tmx3 | 0 | 1 |
| 3 | 1 | -55926 | 1700007P06Rik | -2 | -6 |
| 4 | X | -35533 | Pak3 | -6 | 4 |
| 5 | 2 | -32192 | Gad2 | 1 | 17 |
| 6 | 9 | 724 | Mir101c | 1 | 19 |
| 7 | 9 | 2818 | Mir101c | 0 | 14 |
| 8 | 9 | 8176 | Mir101c | -1 | 15 |
| 9 | 9 | 12606 | Mir101c | -3 | 19 |
| 10 | 9 | 16303 | Mir101c | -1 | 10 |
| 11 | 2 | 21806 | Gm14496 | 1 | -2 |
| 12 | 9 | 22673 | Mir101c | 0 | 11 |
| 13 | 9 | 24306 | 4933422A05Rik | -14 | -8 |
| 14 | 9 | 27053 | Mir101c | 7 | 13 |
| 15 | 9 | 28531 | Mir101c | 0 | 13 |
| 16 | 9 | 31240 | Mir101c | -1 | 11 |
| 17 | 9 | 33829 | Mir101c | -3 | 11 |
| 18 | 9 | 36577 | Mir101c | -5 | 8 |
| 19 | 18 | 92245 | Tmx3 | -10 | -6 |
| 20 |  | 573775 | Ulbp1 | 6 | 9 |
| 21 |  | 661756 | Mucl1 | 6 | 21 |
| 22 | 2 | 1194943 | Lrrc4c | -13 | 0 |
| 23 | 2 | 1199132 | Lrrc4c | -16 | -6 |

**Tab. S3.** Patient characteristics of samples in Figure S1 and supplemental Figure 5: DCM, ICM, dilatative or ischaemic cardiomyopathy; EF, ejection fraction (%); PCW, Pulmonary Capillary Wedge Pressure (mmHg); CI, cardiac index (min-1·m-2); MRB, mineralocorticoid receptor blocker, Dig, digitalis glycosides; Cat, catecholamines; ACE/AT-RB, ACE inhibitors, Diu, diuretics, BB, betablocker; LVE DD, left ventricular enddiastolic diameter (mm). No further data of patients are available.

**Figure S1**

| | **Age** | **Gender** | **EF** % | **PCW** mm HG | **CI** $min^{-1} \cdot m^{-2}$ | **MRB** | **Dig** | **Cat** | **ACEi** | **Diu** | **BB** | **LVED D mm** |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| DCM | 61 | m | 25 | 18 | 2.89 | + | + | - | + | + | + | 76 |
| DCM | 56 | m | 29 | 25 | 1.24 | + | + | - | + | + | + | 67 |
| DCM | 63 | m | 15 | 17 | 2.69 | + | - | - | + | + | + | 65 |
| DCM | 61 | m | 20 | 29 | 1.72 | + | + | - | + | + | + | 67 |
| ICM | 56 | m | 20 | 10 | 2.1 | - | - | - | + | + | + | 84 |
| ICM | 56 | m | 20 | 29 | 2.49 | - | + | - | + | + | + | 75 |
| ICM | 57 | m | 23 | 28 | 2.77 | - | + | - | + | + | + | n.a, |
| ICM | 56 | m | 20 | 22 | n.a. | + | + | + | + | + | - | 69 |

**Figure 5**

| | **Age** | **Gender** | **EF** % | **PCW** mm HG | **CI** $min^{-1} \cdot m^{-2}$ | **MRB** | **Dig** | **Cat** | **ACEi** | **Diu** | **BB** | **LVE DD mm** |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| ICM | 62 | m | 20 | 12 | 2.33 | + | + | | -/- | + | + | n.a. |
| ICM | 56 | f | 27 | 25 | 1.91 | + | - | - | +/- | + | + | n.a. |
| ICM | 52 | m | 35 | 25 | 1.56 | + | - | - | +/- | + | + | 77 |
| ICM | 66 | f | 25 | 21 | 1.75 | + | - | - | + | + | + | 63 |
| DCM | 61 | M | 25 | 18 | 2,89 | + | + | - | +/- | + | + | 70 |
| DCM | 56 | M | 29 | 25 | 1,24 | + | + | - | +/- | + | + | |
| DCM | 59 | M | 20 | 12 | 2,3 | + | - | - | -/- | + | + | |
| DCM | 42 | F | 25 | 30 | 1,1 | - | - | - | -/- | + | - | |

Tissue from healthy donor hearts derived from patients that could not be transplanted for technical reasons. Control subjects had no history of heart disease and had normal LV function. Patient characteristics are not available for healthy organ donors.

## Material and Methods

**Animal experiments**. CaMKIIδ transgenic (CaMKIIδ-TG), global CaMKIIδ single (CaMKIIδ-KO) and cardiac-specific CaMKIIγ/CaMKIIδ double knockout (DKO) have been described before [1, 2].The animal experiments of the study were performed according to the European Community guiding principles in the care and use of animals (2010/63/UE, 22 September 2010) and were authorized by the "Niedersächsisches Landesamt für Verbraucherschutz und Lebensmittelsicherheit" (Germany). As previously described [3], the β-blocker metoprolol (270 µg/g/d, Sigma-Aldrich, #M5391) was orally given via drinking water for 8 to 10 weeks starting at the age of 4 weeks.

**Patients**. The study conforms to the Declaration of Helsinki and was approved by the ethic committee of the University Medical Center Goettingen (Az. 31/9/00). Frozen myocardial samples were obtained from healthy donor hearts that could not be transplanted for technical reasons. Control subjects had no history of cardiac disease and had normal LV function. Diseased tissue samples from left ventricles were collected from explanted hearts of patients with end stage dilative or ischaemic HF (n = 8) who had given written informed consent. A detailed list of patient characteristics is shown in Supporting Information (**SI Appendix, Table S2**).

**Material.** Reagents for cell culture were purchased from Gibco Life technologies. Cells were grown and maintained in a humidified incubator with 5% $CO_2$-95% room air at 37°C. Isoprenaline (Iso) and AIP (Autocamptide-2 Related Inhibitor Peptide) were from Sigma, Okadaic acid (OA) from Calbiochem and PKI (5-24) from Santa Cruz.

**Cardiac tissue/cell preparation, SDS-Gel electrophoresis and immunoblotting.** Human cardiac tissue or Isolated rat cardiomyocytes were homogenized in lysis buffer containing: 30 mMTris/HCl (pH 8.8), 5 mM EDTA, 3 mM NaF, 3% SDS and protease and phosphatase inhibitor cocktail (Roche Diagnostics). Total protein extracts were separated by SDS-PAGE and subsequently transferred onto nitrocellulose membranes. After blocking in TBST with 5% milk (AppliChem) membranes were probed overnight at 4°C with the respective primary antibody: anti-H3 (ab70550, Abcam), anti-H3S28p (#9713, Cell Signaling), anti-calsequestrin (PA1-913,Thermo Scientific), total CaMKII (BD Biosciences, 611292), anti-α-globin (abcam, ab191183),

anti β-globin (LifeSpan BioSciences, LS-C294453) or anti-GAPDH (sc-47724, Santa CruzBiotechnology). After washing and incubation with appropriate secondary antibodies for 1 h, chemiluminescence was detected using a Fusion FX imaging system (VilberLourmat). Band intensities were quantified using Fusion-CaptAdvancesoftware (VilberLourmat).

**Isolation, cell culture, adenoviral infection and pharmacological treatment of neonatal rat ventricular myocytes (NRVM).** NRVM were Isolated from newborn rats (1-3 days old) as previously described [4]. Briefly, the animals were sacrificed by decapitation and the hearts were quickly excised. Cardiac cells from ventricular tissues were dissociated by digestion with 0.5% trypsin. To separate cardiomyocytes from non-myocytes, Isolated cells were pre-plated for 60 min and a discontinuous Percoll gradient (GE-Healthcare) was performed. The purified myocytes were plated and maintained in 6- or 12-well plates at a density of 700,000 or 300,000 cells/well in Dulbecco's modified Eagles' medium (DMEM) (GIBCO Laboratories) supplemented with 10% FCS and antibiotics (100 U/ml penicillin G and 100 mg/ml streptomycin). Before pharmacological treatment, NRVM were kept in serum-free DMEM overnight and then incubated with isoprenaline (Iso, Sigma) at different concentrations (1 nM to 1000 nM) or for different time periods (3 min to 24 h) to induce β-adrenergic stimulation. To inhibit CaMKII activity, cells were treated with KN93 (422711, Calbiochem) at different concentrations (10 nM to 1000 nM) or for different time periods (3 min to 24 h). Okadaic acid (OA, Calbiochem) at different concentrations (0, 10, 30, 100, 300 and 1000 nM) was added for 20 min to induce phosphatase inhibition. Control cells were treated with vehicle (DMSO 1%).

To study PP1 influence on histone H3S28 phosphorylation, PP1-inhibitor-1 (I-1) was used as previously described [5]. Therefore, Isolated NRVM were cultured for 48 h and then incubated in serum-free DMEM containing the control adenovirus (Ad-EGFP) or the I-1 adenovirus (Ad-I-1) for 6 h. After further cultivation for 12 h, NRVM were stimulated with increasing concentrations of Iso (0, 10 and 100 nM) for 3 min and then used for western blot experiments.

**Isolation, cell culture and pharmacological treatment of primary adult mouse ventricular myocytes.** Adult mouse ventricular myocytes (AMVMs) from double knockout mice deficient for CaMKIIδ and CaMKIIγ (CaMKII DKO) or corresponding wild type (WT) mice were enzymatically Isolated using the Langendorff perfusion as

previously described [2]. Cells were placed on the laminin-coated cell culture plate and maintained in fresh complete medium. Afterwards cardiomyocytes were stimulated with 0.1 µM isoprenaline (Iso, Sigma) for 24 h.

**RNA procedures**. Total RNA was Isolated from cultured cardiomyocytes or ventricular tissue using trizol reagent procedure (Life technologies). cDNA synthesis from 1 µg of RNA was carried out using cDNA synthesis kit (Thermo scientific). Quantitative real-time PCR (qPCR) was performed with Universal Probe Library (Roche) by using SensiFAST Probe Lo-ROX (Bioline) kit and detection on a 7500 Fast Cycler (Applied Biosystems). Primers used for quantitative RT-PCR analysis were: *Hbb-bt* sense 5´-gtgacaagctgcatgtggat-3´ and antisense 5´-gtgaaatccttgcccaggt-3´; *Hba-a1* sense 5´-tgacagactcaggaagaaacca-3´ and antisense 5´-gggaagctagcaaacatcctt-3´; *Gapdh* mix Mm 99999915-91 lot: 1703331 E9.

**Affymetrix Gene expression.** Gene expression profiling (*n* = 3 per group) was performed using Affymetrix-Gene-Chip Mouse Gene 2.0 ST Array (902118, Thermo Fisher Scientific). Biotinylated antisense cRNA was then prepared according to the Affymetrix standard labeling protocol. Afterwards, hybridization on the chip was performed using a Gene-Chip Hybridization Oven 640, then dyed using the Gene-Chip Fluidics Station 450 and scanned with a Gene-Chip Scanner 3000 (accession number of Affymetrix: GSE131796). All protocols and equipment were from Affymetrix (Santa Clara, CA, USA).

**Chromatin Immunoprecipitation Assay.** After β-adrenergic stimulation with Iso for 24 h, adult cardiomyocytes were washed twice with PBS and fixed with 1% (v/v) formaldehyde for 10 min at room temperature with slow rocking. Crosslinking was terminated by adding glycine to a final concentration of 250 mM. For chromatin extraction the cells were submitted to different chemical lysis buffers (Lysis Buffer 1: 50 mM Hepes-KOH pH 7.5; 140 mM NaCl; 1 mM EDTA 0.2 ml; 10% glycerol; 0.5% NP-40; 0.25% Triton X-100; Lysis Buffer 2: 200 mM NaCl; 1 mM EDTA (pH 8); 0.5 mM EGTA (pH 8); 10 mM Tris pH 8) and the samples were rotated in each buffer for 10 min at 4 °C. Consequently the lysates were transferred to the next lysis buffer (50 mM Tris pH 8; 0.1 % SDS; 0.95 % NP40; 0.1 % Na-deoxycholate; 10 mM EDTA; 150 mM NaCl) and sonicated in polymethylpentene tubes (TPX, Diagenode) for a total of 40 sonication cycles using an automated sonication system (Bioruptor, Diagenode). Sonication cycles were performed at the high energy levels in 30 sec sonication and

30 sec pause mode. The resulting chromatin fragment length was adjusted to 150-200 bp. The ChIP analysis was conducted using the semi-automated IP-Star system (Diagenode) following the manufactures´ guidelines. IP buffers and magnetic beads for histone mark precipitation were purchased at Diagenode. For pulldown, we used 1 µg of chromatin for each sample and the immunoprecipitation antibody anti-H3S28p (#9713, Cell Signaling). The chromatin samples were subjected to RNAse (Quiagen) and Proteinase K treatment (Roche). Reverse crosslinking was performed at 65 °C for 6 h. Chromatin concentrations were evaluated using Qubit® dsDNA HS assay kit (Thermo Fisher Scientific). The DNA was ethanol precipitated and the DNA was suspended in TE-buffer. Chromatin quality was assessed by agarose gel electrophoresis. In order to prepare the samples for high-throughput sequencing on an Illumina HiSeq2000 instrument (Illumina), the NEBNext DNA Library Prep Master Mix Set for Illumina (E6040) was used to prepare libraries of the individual samples and the respective controls according to manufacturers' protocol (ChIPseq data: E-MTAB-8011). ChIP-qPCR was also conducted to examine and validate the enrichment of phosphorylation of H3S28 in some regions identified by ChIP-Seq. Five exemplary regions were selected. Among them promoter regions of *Hbb-bt* and Rps29 were amplified from the immunoprecipitated and non-immunoprecipitated chromatin using the following pair primers:

| **target** | **Sense (5′ -3′)** | **Antisense (5′ -3′)** |
|---|---|---|
| *Hbb-bt* | GCCTCACCACCAACTTCATC | TGTTGTGTTGACTTGCAACCT |
| *Rps29* | GGAGTTCTGGGCTGTAGTGC | ATCAGCACGGGAGTTTTGAC |

The intergenic regions of other 3 target genes were amplified using the following pair primers:

| **target** | **Sense (5′ -3′)** | **Antisense (5′ -3′)** |
|---|---|---|
| *En2* | GGGGAGAGTCATACGAACACA | TTCAGGACACTACGAAGACCTG |
| *Zfyve26* | ATCCTGCCCAAACACTCTTG | ACACACACACACACACACACAC |
| *Hes5* | CACACACACCTGGCTGATCT | TGACCAAACTTCAGTCACTCC |
| *Fosl* | CCCCCGTGGTGCAAGTGGTT | TGGCGGCTGCGGTTCTGACT |

**Immunocytofluorescence Analysis**. Cells were fixed with Histofix 4 % (Carl Roth, Gmbh, Karlsruhe) and permeabilized with 0.05 % Triton X-100 in PBS for 5 min. After

washing with PBS and blocking with Roti-block (Carl Roth, GmbH, Karlsruhe) for 1 h at room temperature (RT), the cells were incubated with primary antibodies (anti-H3S28, #9713 Cell Signaling; anti-α actinin, A5044 Sigma) overnight at 4 ºC followed by sequential incubation with secondary antibodies (anti-mouse Alexa Fluor 488, anti-rabbit Alexa Fluor 633, Invitrogen) for 1 h at RT. For counterstaining of nuclei, DAPI (Sigma) was used.

**Immunoprecipitation**. Total proteins from isolated NRVM were extracted in lysis buffer containing 20 mM Tris (pH 7.5), 150 mM NaCl, 1 mM EDTA, 1 % Triton X-100, 1 mM PMSF and protease inhibitor cocktail (Sigma). Protein extract (200 µg) was incubated either with anti-H3S28p or anti-H3 (1 : 25) and rotated for 6 h at 4 ºC followed by addition of protein G Sepharose beads 4 fast flow (GE-Healthcare) for overnight incubation at 4 ºC. Protein-antibody complexes were collected, washed with high salt wash buffer (500 mM NaCl with 1mM PMSF) and resuspended in SDS loading buffer. Eluted proteins were separated and blotted as described.

**In vitro Kinase Assay.** The active form of recombinant CaMKIIδ (Lot:1817913-D, NEBioLabs) was mixed in protein kinase buffer supplemented with 200 µM ATP, 2 mM CaCl2 and 1.2 µM calmodulin at 30 °C for 10 min. Then 2 µg of histone H3 synthetic peptides (H3S28 wild type) or H3A28 (mutant) was diluted in protein kinase buffer containing 200 µM ATP and incubated at 30 °C with the activated CaMKII to measure peptide kinase activity. The phosphorylated proteins were separated in SDS-PAGE and blotted as described before.

**Statistical Analysis**. Raw Fastq data was annotated to the mouse genome (mm9) using bowtie 2 alignments [6]. The resulting BAM files have been used for MACS (Model based analysis of ChIPseq) Peak Calling with the following settings: 16 fold enrichment and p value $10^{-3}$ [7]. ChIPseq data has been deposited in the ArrayExpress database (E-MTAB-8011).Only unique reads were subjected to further analysis. Precipitation peaks were normalized to the corresponding input sample. Differentially regulated peaks between both groups were calculated using the DiffBind Algorithm [8]. Consequently, the differential bound genomic sites were used for illustrations. The corresponding enrichment Scores were calculated within the DeeptoolsAnalysis as previously published and the differential bound Peaks could be ranked accordingly [9]. Annotations of genomic regions to their corresponding gene names and the determination of the distance to the TSS were performed with HOMER v4.8 [10].

**Analysis of Affymetrix gene chip.** A Custom CDF Version 22 with ENTREZ based gene definitions was used to annotate the arrays [11]. The Raw fluorescence intensity values were normalized applying quantile normalization and RMA background correction. Before performing the ANOVA, a batch normalization was used to remove the individual mouse variations. An ANOVA was performed to identify differential expressed genes using a commercial software package SAS JMP Genomics, version 7, from SAS (SAS Institute, Cary, NC, USA). A false positive rate of a=0.01 with FDR correction was taken as the level of significance. The raw and normalized data are deposited in the Gene Expression Omnibus database (http://www.ncbi.nlm.nih.gov/geo/; accession number of Affymetrix: GSE131796).

## References


[1] Zhang T, Maier LS, Dalton ND, Miyamoto S, Ross J, Jr., Bers DM, et al. The deltaC isoform of CaMKII is activated in cardiac hypertrophy and induces dilated cardiomyopathy and heart failure. Circ Res. 2003;92:912-9.

[2] Kreusser MM, Lehmann LH, Keranov S, Hoting MO, Oehl U, Kohlhaas M, et al. Cardiac CaM Kinase II genes delta and gamma contribute to adverse remodeling but redundantly inhibit calcineurin-induced myocardial hypertrophy. Circulation. 2014;130:1262-73.

[3] Dewenter M, Neef S, Vettel C, Lammle S, Beushausen C, Zelarayan LC, et al. Calcium/Calmodulin-Dependent Protein Kinase II Activity Persists During Chronic beta-Adrenoceptor Blockade in Experimental and Human Heart Failure. Circulation Heart failure. 2017;10:e003840.

[4] El-Armouche A, Bednorz A, Pamminger T, Ditz D, Didie M, Dobrev D, et al. Role of calcineurin and protein phosphatase-2A in the regulation of phosphatase inhibitor-1 in cardiac myocytes. Biochem Biophys Res Commun. 2006;346:700-6.

[5] El-Armouche A, Wittkopper K, Fuller W, Howie J, Shattock MJ, Pavlovic D. Phospholemman-dependent regulation of the cardiac Na/K-ATPase activity is modulated by inhibitor-1 sensitive type-1 phosphatase. FASEB J. 2011;25:4467-75.

[6] Langmead B, Salzberg SL. Fast gapped-read alignment with Bowtie 2. Nat Methods. 2012;9:357-9.

[7] Zhang Y, Liu T, Meyer CA, Eeckhoute J, Johnson DS, Bernstein BE, et al. Model-based analysis of ChIP-Seq (MACS). Genome biology. 2008;9:R137.

[8] Ross-Innes CS, Stark R, Teschendorff AE, Holmes KA, Ali HR, Dunning MJ, et al. Differential oestrogen receptor binding is associated with clinical outcome in breast cancer. Nature. 2012;481:389-93.

[9] Ramirez F, Dundar F, Diehl S, Gruning BA, Manke T. deepTools: a flexible platform for exploring deep-sequencing data. Nucleic acids research. 2014;42:W187-91.

[10] Heinz S, Benner C, Spann N, Bertolino E, Lin YC, Laslo P, et al. Simple combinations of lineage-determining transcription factors prime cis-regulatory elements required for macrophage and B cell identities. Mol Cell. 2010;38:576-89.

[11] Dai M, Wang P, Boyd AD, Kostov G, Athey B, Jones EG, et al. Evolving gene/transcript definitions significantly alter the interpretation of GeneChip data. Nucleic acids research. 2005;33:e175.