Supplementary information

Cardiac specific PRMT1 ablation causes heart failure through CaMKII dysregulation

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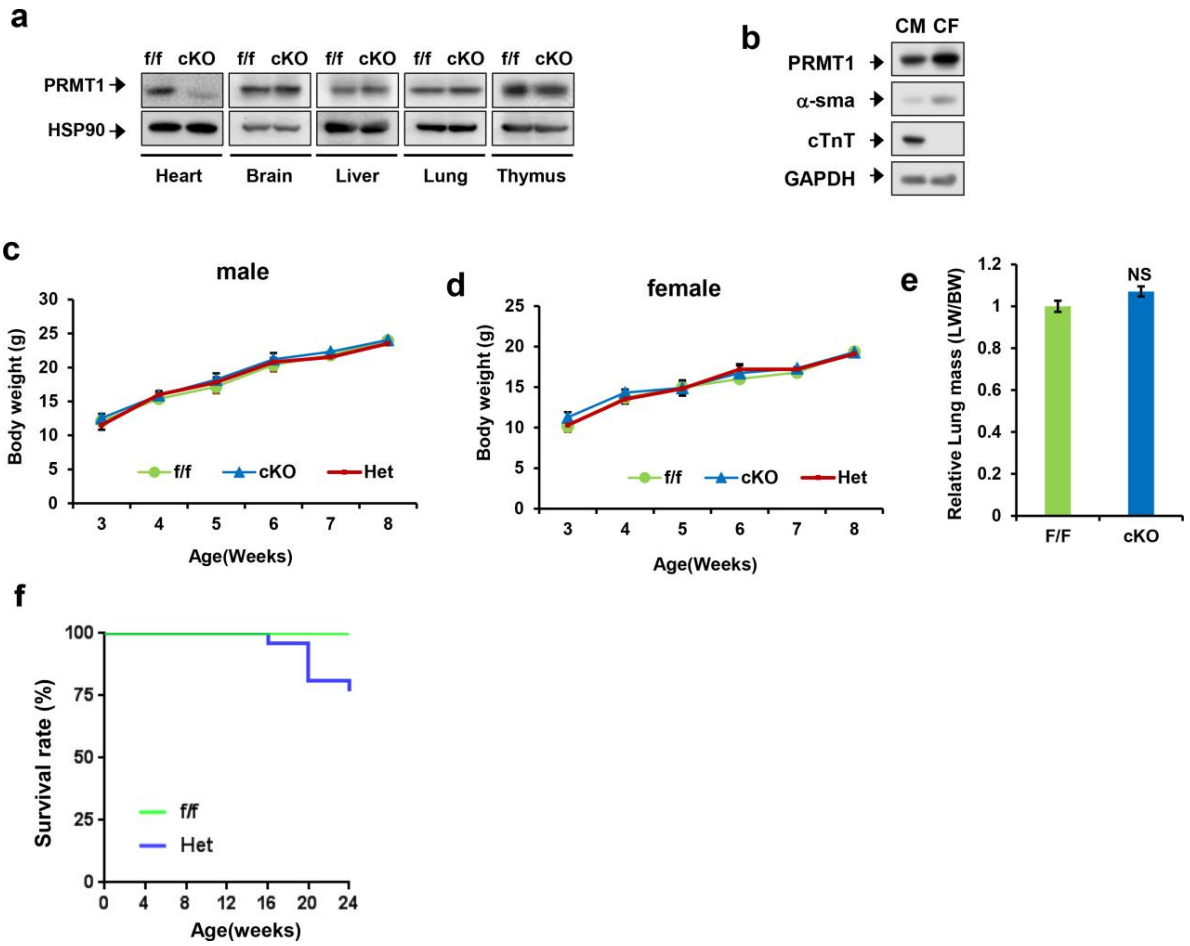
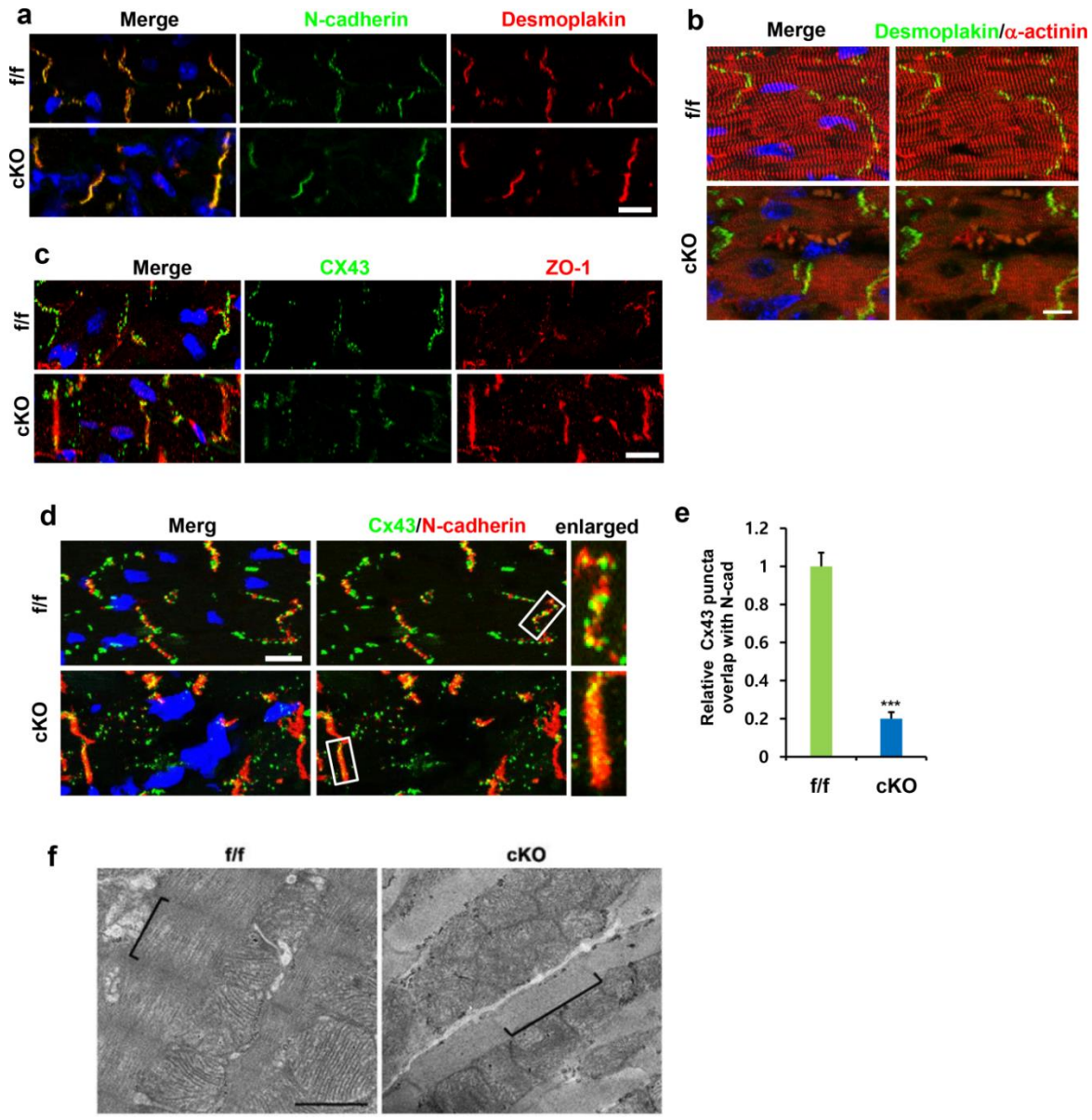


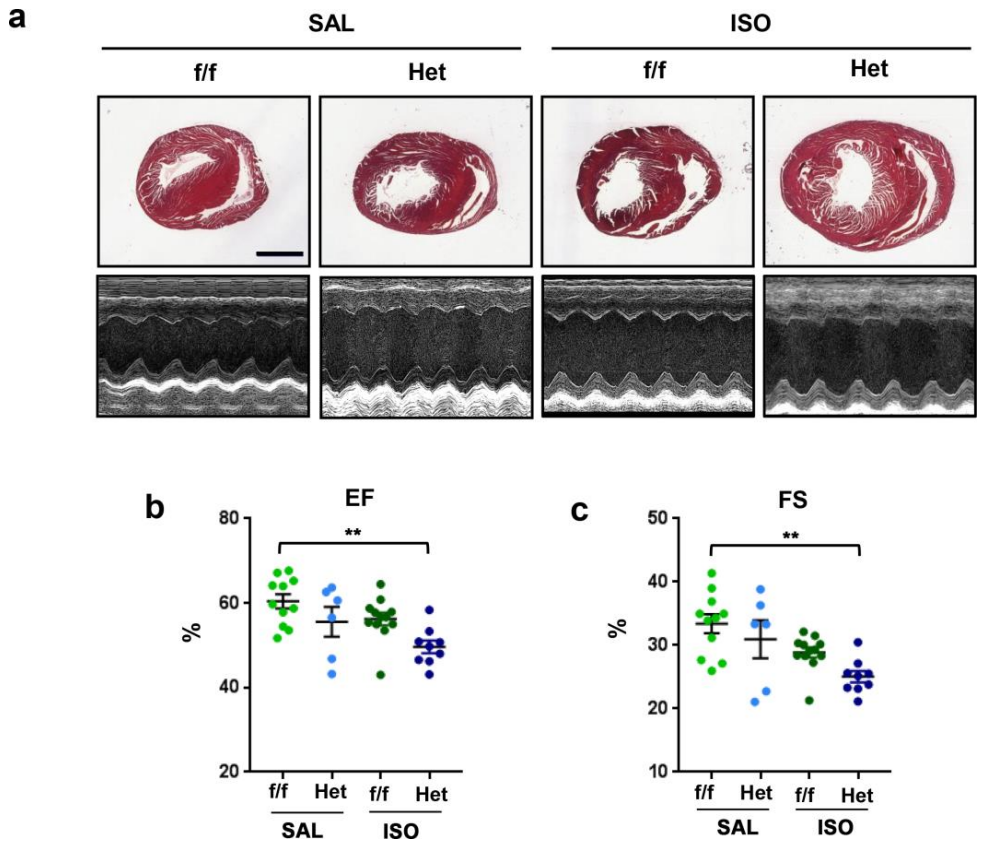
Supplementary figure 1.

(**a**) Protein analysis for PRMT1 expression in heart, brain, liver, lung and thymus isolated from f/f and cKO mice. (**b**) Immunoblotting for PRMT1 level in newborn rat cardiomyocytes (CM) and cardiac fibroblasts (CF). (**c, d**) Body weights of f/f , Het and cKO male and female mice from 3 weeks to 8 weeks. Data represent means ±SD. n=24. (**e**) Relative lung weight (LW)/body weight (BW) of 6-7 weeks-old f/f and cKO mice. n=5 for each group. Data represent means ±SD. (**f**) Survival rate of $PRMT1^{f/f}$ (f/f, n=30), $PRMT1^{f/cKO}$ (Het, n=30) mice.



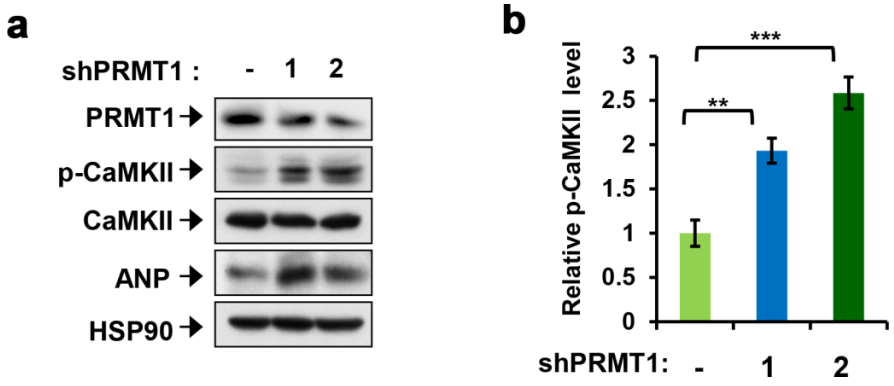
Supplementary figure 2

(**a**) Immunostaining of cardiac tissue of 8 weeks-old f/f and cKO mice for N-cadherin and Desmoplakin. Scale bar: 50µm. Note enhanced and broadened staining pattern of N-cadherin and Desmoplakin in cKO cardiomyocyte. (**b**) Immunohistochemistry for α-Actinin and Desmoplakin localized at intercalated disc in 8 weeks-old cardiac tissue Scale bar: 50 µm. Note the disarrayed α-actinin positive cytoskeletal structures. (**c**) Representative image for Cx43 and ZO-1 immunostaining. Scale bar: 50µm. Note thickened ZO-1 immunopositive signal at intercalated disc of cKO cardiomyocyte. (**d**) Immunostaining for Cx43 and N-cadherin as a marker of intercalated disc. Boxed areas are enlarged. Scale bar: 50µm. Note reduced Cx43 signal at intercalated disc and scattered Cx43 in the cytoplasmic area of cKO cardiomyocyte. (**e**) Quantification of Cx43 signal overlapping with N-cadherin signal. Mean values of at least 9 fields per each. n=3. Data represent means ±SD. $^{***}P<0.001$. (**f**) Transmission electron microscopy picture showing alterations in cytoskeletal organization and mitochondria structures in 8 weeks-old cKO heart section, compared to the f/f. Scale bar: 1µm. The bracket depicts a sarcomere. Note the stretched sarcomere in cKO heart, relative to the f/f heart.



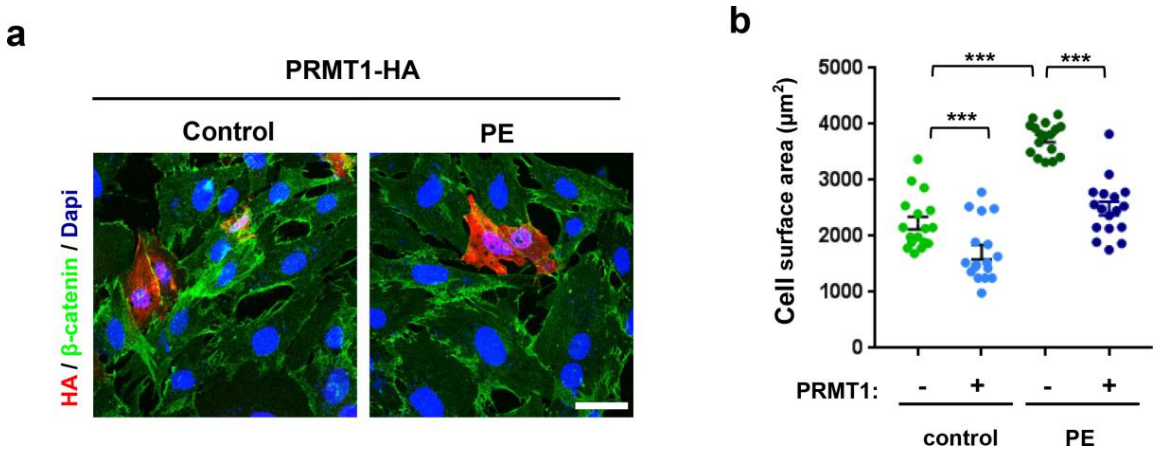
Supplementary figure 3

(**a**) The histological analysis and the short axis transthoracic M mode echocardiographic tracings from control f/f and PRMT1 heterozygous mice treated with isoproterenol for 2 weeks starting from 2 months of the age. Scale bar: 2mm. (**b, c**) The analysis of echocardiographic parameters; the ejection fraction and the fractional of shortening. n=11 (f/f, SAL), 6 (Het, SAL), 12 (f/f, ISO), 9 (Het, ISO). Data represent means ±SEM, one-way ANOVA. $^{**}P<0.005$.



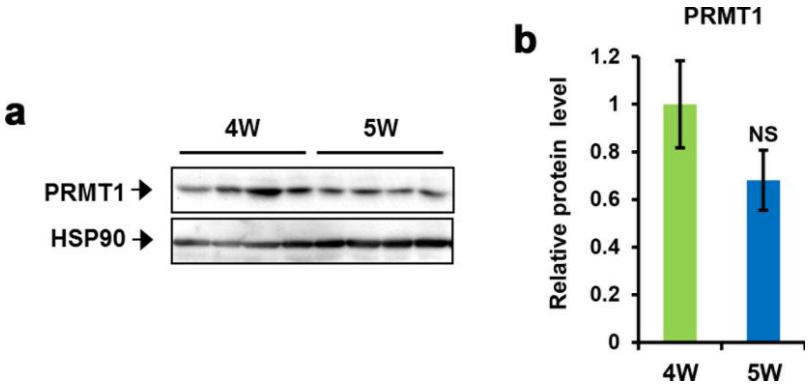
Supplementary figure 4

(**a, b**) Immunoblot analysis for PRMT1 and CaMKII in NRVM treated with PRMT1-targeted shRNAs. Relative levels of p-CaMKII. n=3. Data represent means ±SD. $^{**}P<0.01$, $^{***}P<0.001$, Student's *t*-test.



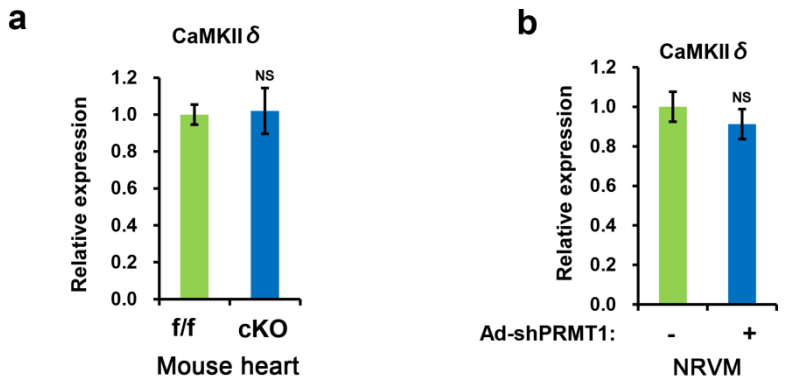
Supplementary figure 5

(**a**) Representative image of PRMT1-HA overexpressing H9C2 cardiomyocytes in response to vehicle or PE treatment. Scale bar: 50μm. (**b**) Quantification of surface area of cells similar shown in panel a. n=30. Data represent means ±SD. $^{***}P<0.001$, Student's *t*-test.



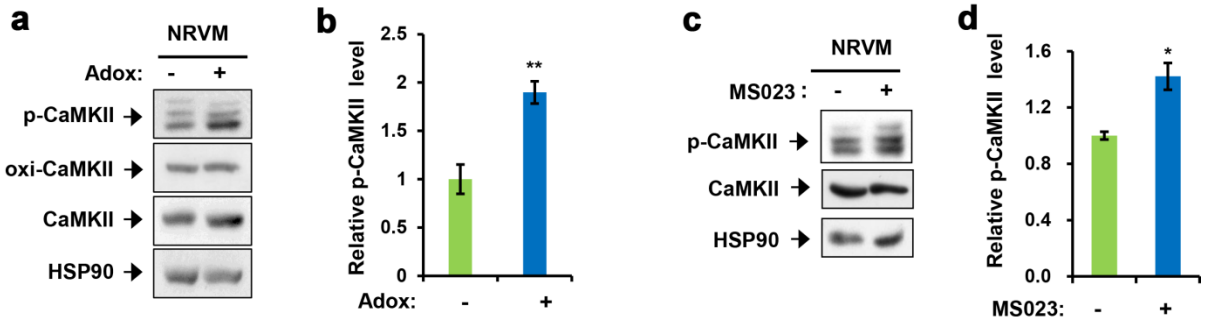
Supplementary figure 6

(a) Immunoblot analysis for PRMT1 proteins in heart lysates from 4 weeks and 5 weeks-old mice. (**b**) Quantification of the relative signal intensity shown in panel a. Data represent means ±SD. NS; not significant.



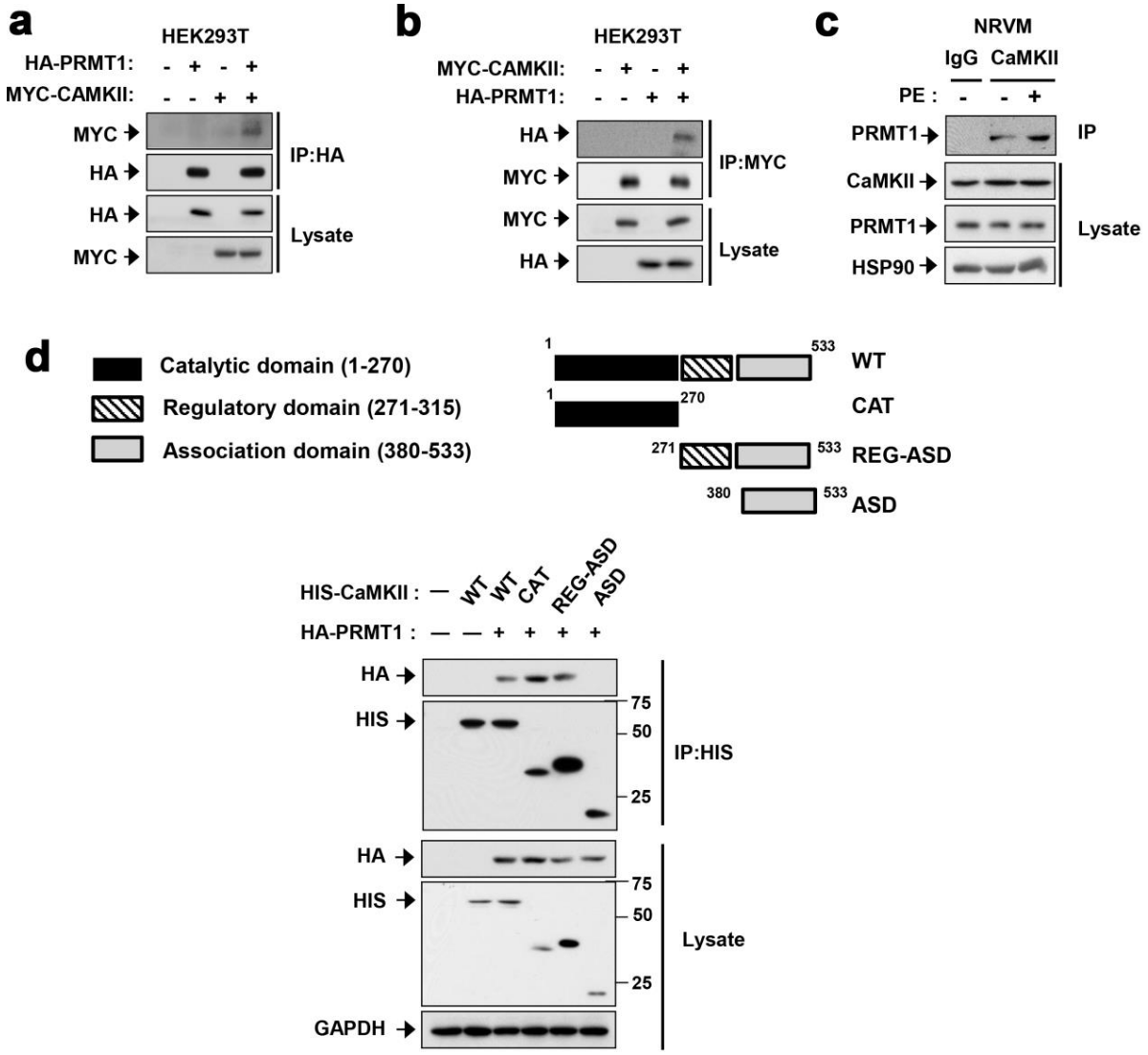
Supplementary figure 7

(**a, b**) The transcript level of CaMKIIδ was examined in f/f and cKO hearts (**a**) or control or shPRMT1-expressing NRVM cells (**b**) by quantitative RT-PCR. n=3. NS: not significant.



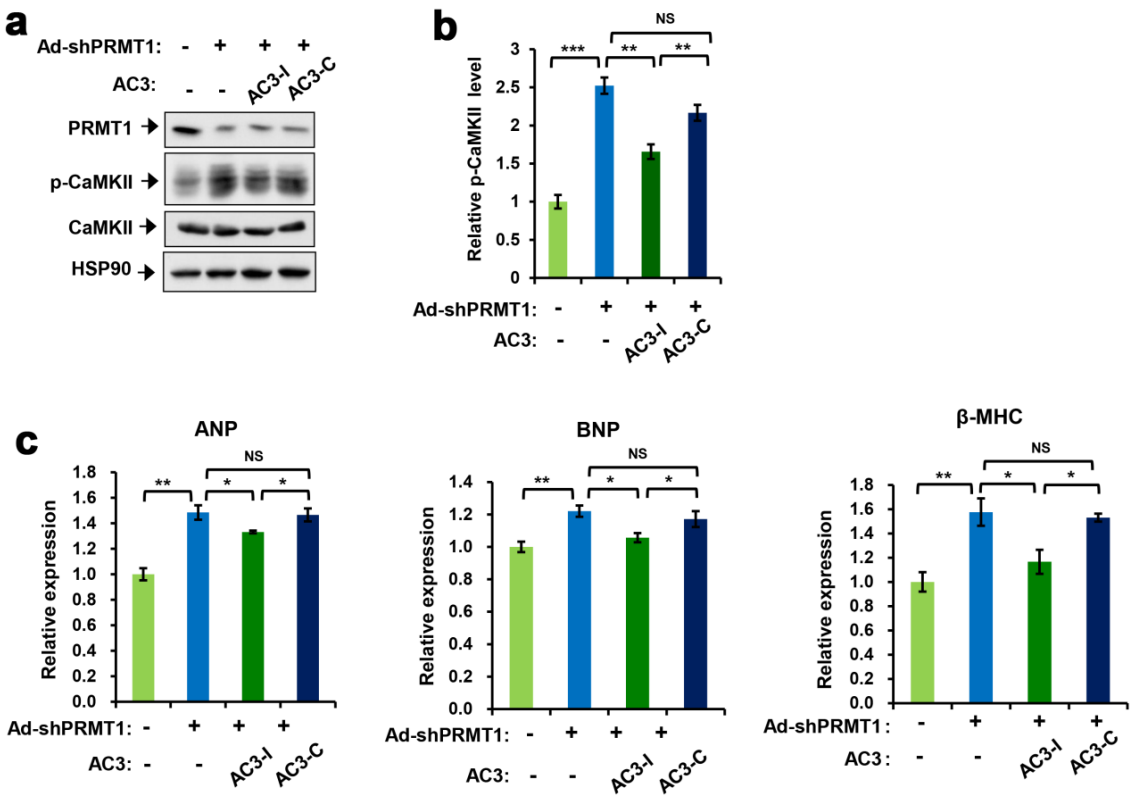
Supplementary figure 8

(**a, b**) Immunoblot analysis for CaMKII proteins in NRVM treated with a pan-PRMT inhibitor Adox for 4 hours and quantification of the relative signal intensity. The value from the vehicle treatment was set to 1. n=3. Data represent means ±SD. $^{**}P<0.01$, Student's *t*-test. (**c, d**) Immunoblot assay for CaMKII proteins in NRVM treated with MS023, an inhibitor for type1 PRMT family and quantification of the signal intensity of p-CaMKII in panel c. n=3. Data represent means ±SD. $^{*}P<0.05$, Student's *t*-test.



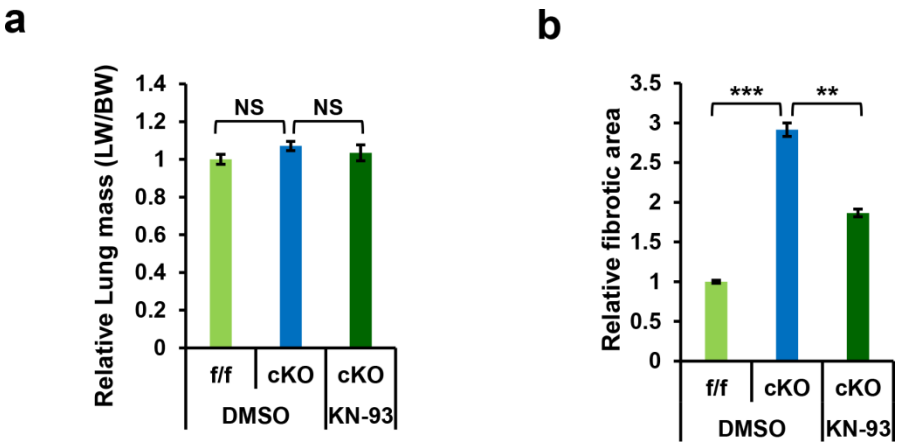
Supplementary figure 9

(**a, b**) Coimmunoprecipitation of PRMT1 and CaMKII in 293T cells transfected with HA-PRMT1 and Myc-CaMKII. (**c**) Coimmunoprecipitation of PRMT1 with CaMKII in NRVM in response to PE. (**d**) Coimmunoprecipitation of HIS-tagged CaMKII fragments (aa1-270, catalytic domain; 271-315, association domain, aa380-533; regulatory-association domain, aa-271-533) with HA-PRMT1.



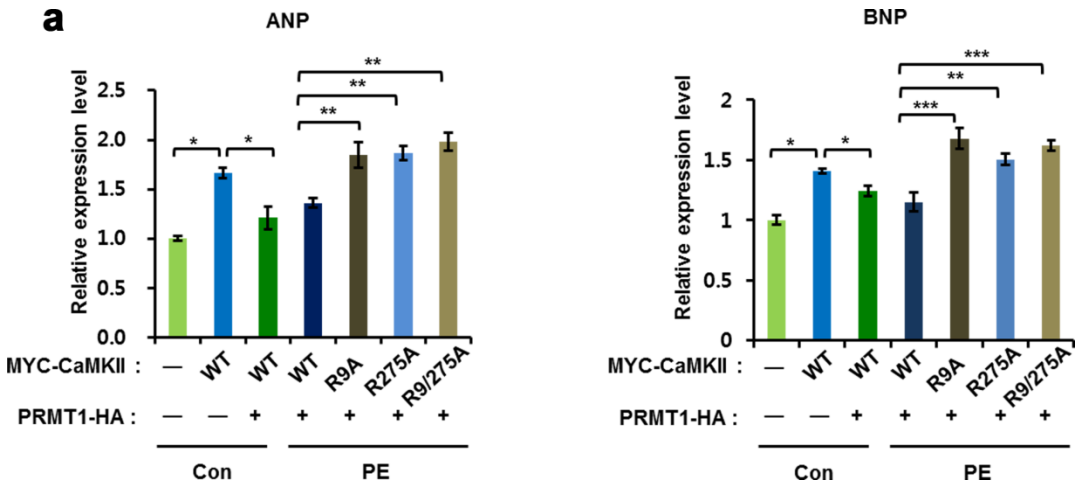
Supplementary figure 10

(**a**) Immunoblot analysis of Ad-control or Ad-shPRMT1-infected NRVM cells and treated with AC3-I and AC3-C peptides for 12 hours. (**b**) quantification of relative p-CaMKII level. n=3. Data represent means ±SD. $^{**}P<0.01$, $^{***}P<0.001$, Student's *t*-test. (**c**) qRT-PCR analysis of ANP, BNP and β-MHC levels in PRMT1-depleted NRVM treated with AC3 peptides. n=3. Data represent means ±SD. $^{*}P<0.05$, $^{**}P<0.01$.



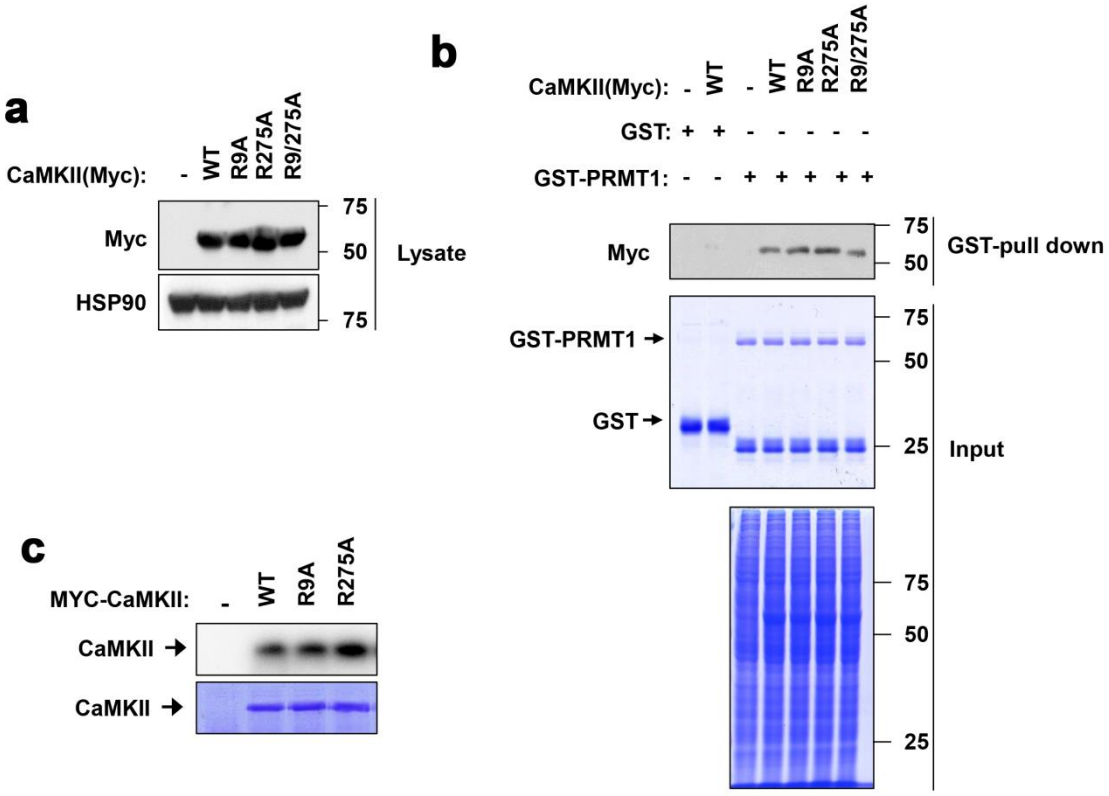
Supplementary figure 11

(**a**) Relative lung mass of f/f and cKO mice treated with vehicle or KN-93. Data represent means ±SD. NS: no significance. (**b**) Quantification of fibrotic area in panel i of figure 6. n=3. Data represent means ±SD. $^{**}P<0.01$, $^{***}P<0.001$, Student's *t*-test.



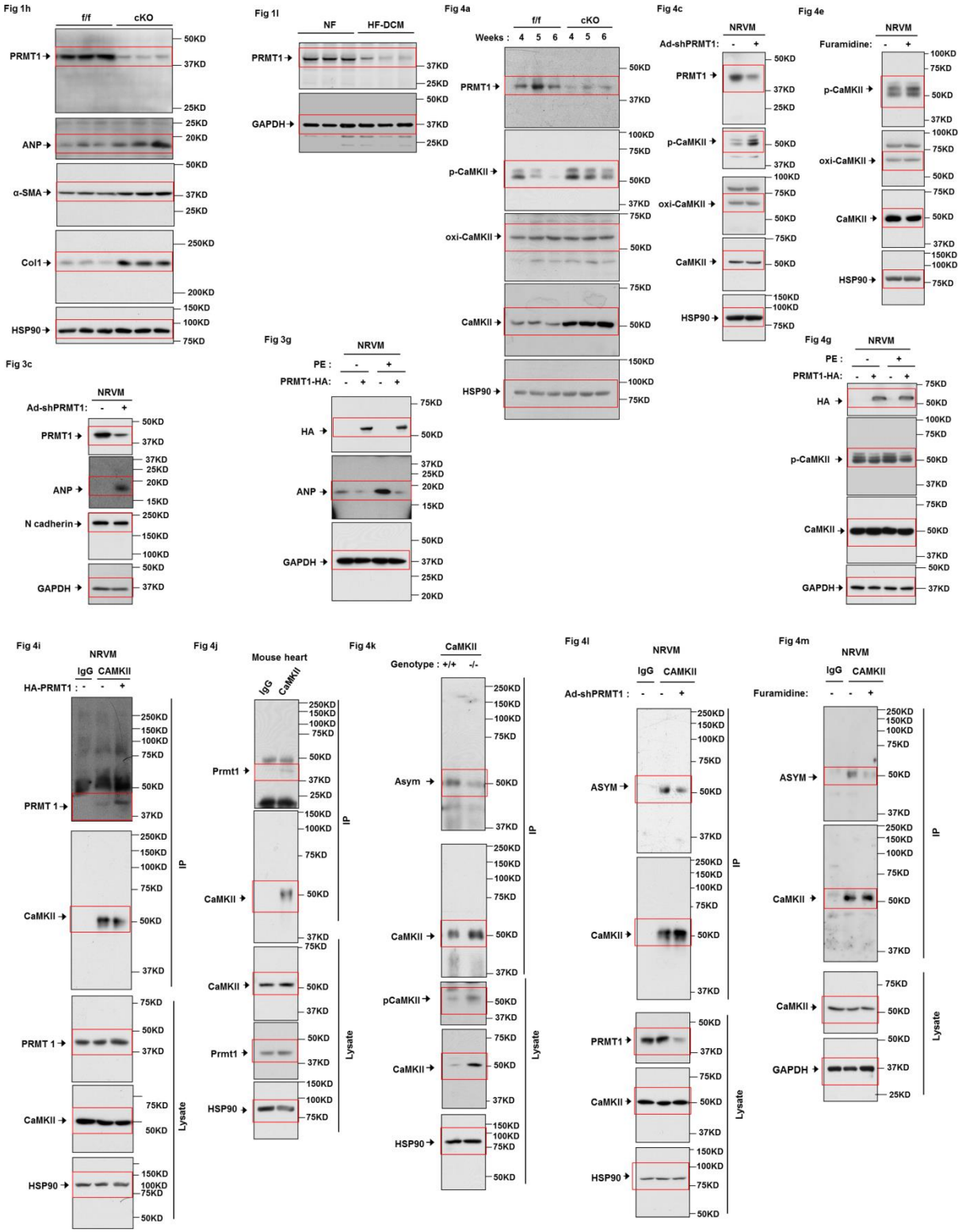
Supplementary figure 12

(**a**) Analysis of hypertrophic gene expression level by qRT PCR for WT and CaMKII mutant transfected NRVM in presence of PRMT1 overexpression and PE treatment for 12 hours. Data represent means ±SD. $^{*}P<0.05$, one-way ANOVA.



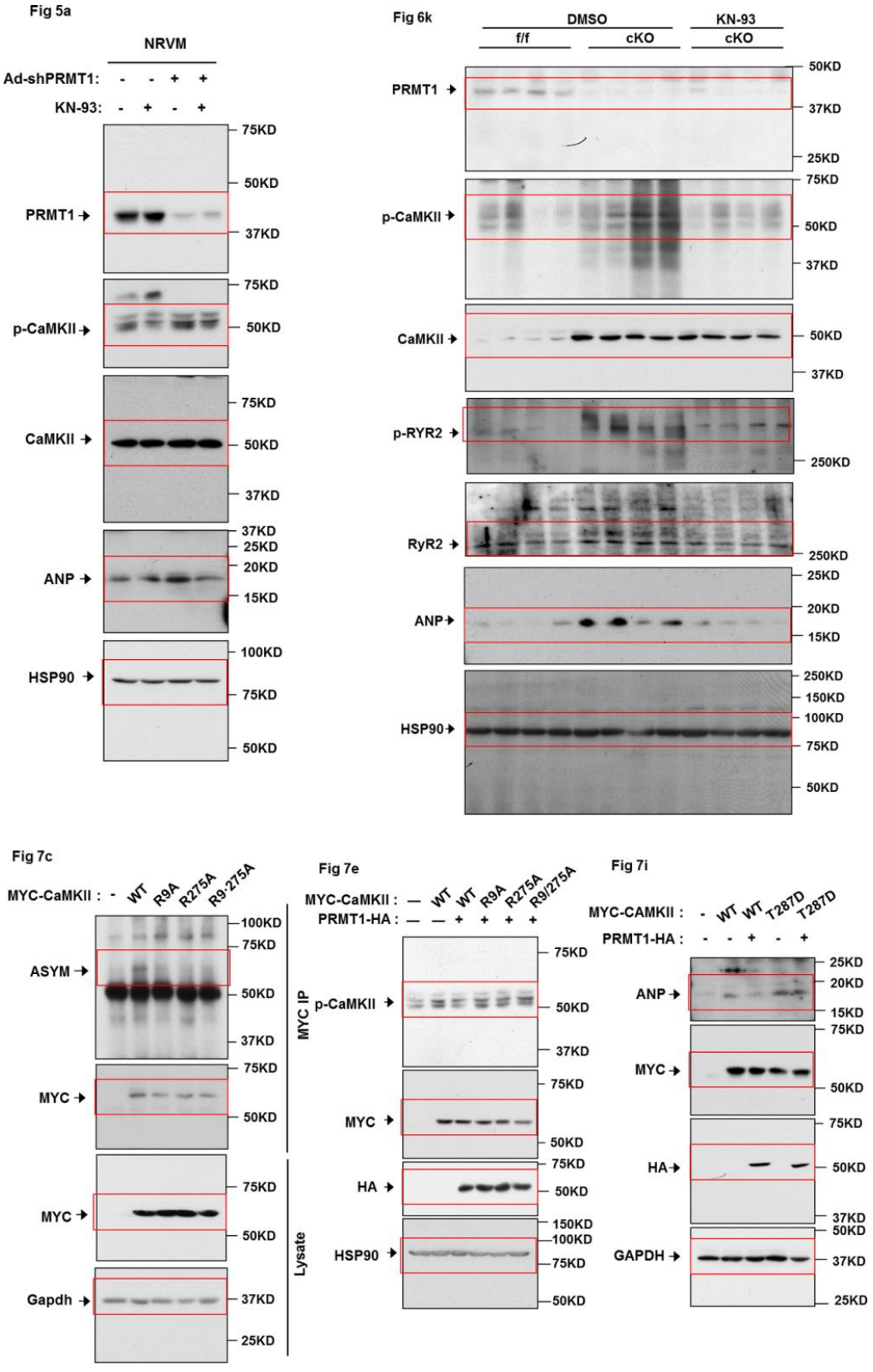
Supplementary figure 13Supplementary figure 13

(**a**) Immunoblot analysis for the expression of CaMKII proteins in 293T cells that were used for the pulldown assay. (**b**) Pull-down assay with purified GST and GST-tagged PRMT1 protein with 293T lysates shown in panel a. (**c**) In vitro kinase assay for autophosphorylation of CaMKII proteins.



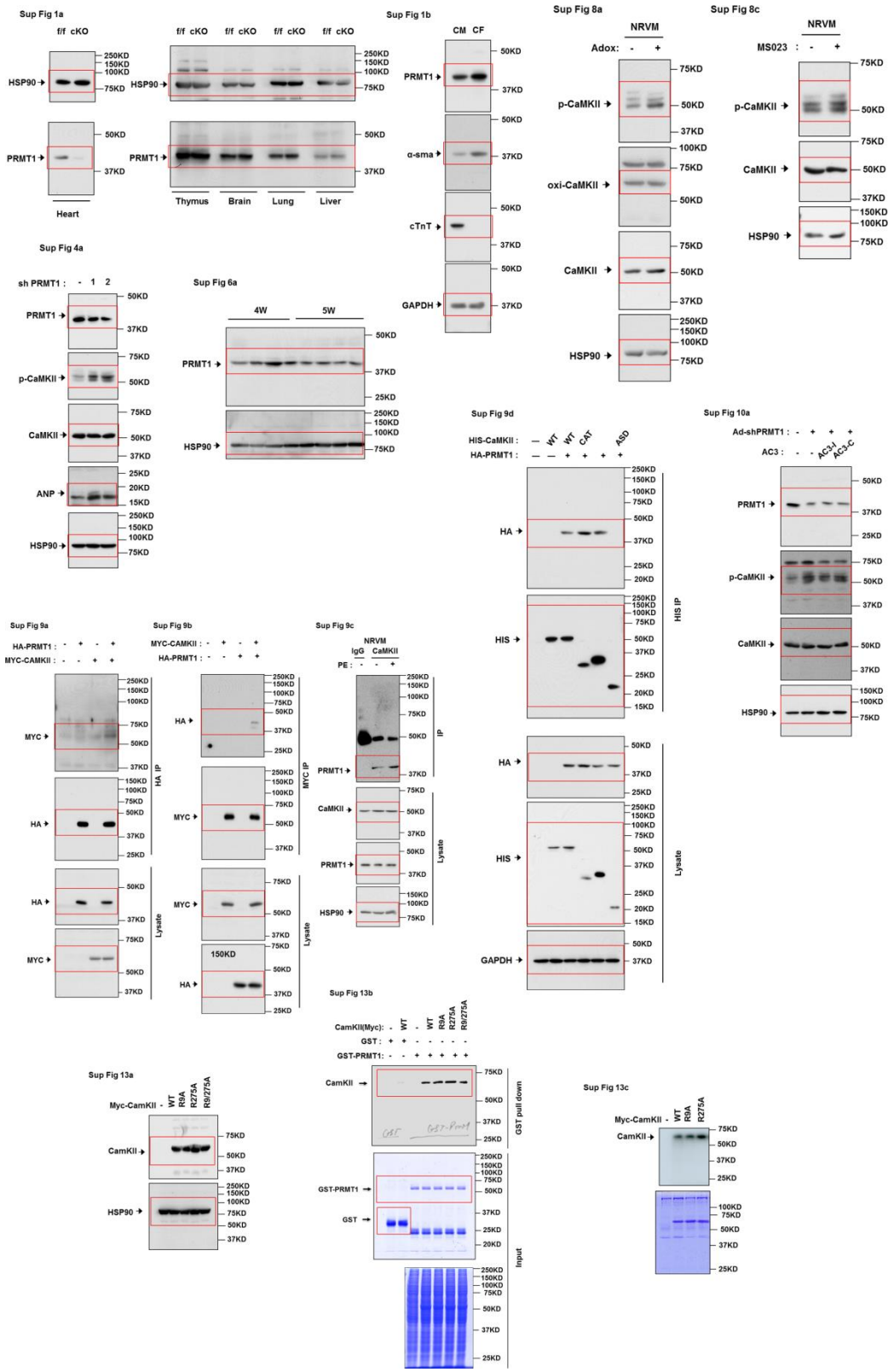
Supplementary figure 14

Uncropped scans of western blots from figure 1. h, l; figure 3. c, g; figure 4. a, c, e, g, i, j, k, l, m.



Supplementary figure 15

Uncropped scans of western blots from figure 5. a; figure 6. k; figure 7. c, e, i.



Supplementary figure 16

Uncropped scans of western blots from supplementary figure 1. a, b; supplementary figure 4. a; supplementary figure 6. a; supplementary figure 8. a, c; supplementary figure 9. a, b, c, d; supplementary figure 10. a; supplementary figure 13. a, b, c.

Supplementary Table 1. Primer sequences used in study.

Primer	Forward(5'→3')	Reverse(3'→5')
PRMT1 flox	GTG CTT GCC ATC AAG AGA TCC	ACA GCC GAG TAG CAA GGA GG
Myosin heavy chain 6 (WT)	CAA ATG TTG CTT GTC TGG TG	GTC AGT CGA GTG CAC AGT TT
Myosin heavy chain 6 (TG)	ATG ACA GAC AGA TCC CTC CTA TCT CC	CTC ATC ACT CGT TGC ATC ATC GAC
ANP(rat)	CCG TCT CAG AGA GAT GGA GG	ATC CTG TCA ATC CTA CCC CC
BNP(rat)	CAG CTC TCA AAG GAC CAA GG	GCA GCT TGA ACT ATG TGC CA
β-MHC(rat)	TGG CAC CGT GGA CTA CAA TA	TAC AGG TGC ATC AGC TCC AG
CaMKII delta(rat)	TGG CAT AGT TCA CAG GGA CC	TGC CAG CAA AAC CAA ACC AC
GAPDH(rat)	GAC ATG CCG CCT GGA GAA AC	AGC CCA GGAT GCC CTT TAG T
ANP(mouse)	CAG AAT CGA CTG CCT TTT CC	GGG GGT AGG ATT GAC AGG AT
BNP(mouse)	ACC CAG GCA GAG TCA GAA AC	ACA AGA TAG ACC GGA TCG GA
CaMKII delta(mouse)	AGG GAC CTG AAG CT GAG AA	GTG CCA GCA AAA CCA AAC CA
GAPDH(mouse)	CAG TGC CAG CCT CGT CCC GTA GA	CTG CAA ATG GCA GCC CTG GTG AC
CaMKII delta R9A	CTC GTC GGT GAA CGC GGT GCA GGT GGT G	CAC CAC CTG CAC CGC GTT CAC CGA CGA
CaMKII delta R275A	AGG CAA CAG TAG AAG CTT GAC AGA TCC ATG GGT GTT TCA GG	CCT GAA ACA CCC ATG GAT CTG TCA AGC TTC TAC TGT TGC CT