SUPPLEMENTARY MATERIAL

Detailed Methods

Animal models

The generation of the *AKAP150*-/- mouse[25] and *AKAP150* loxP-targeted (fl) mouse[26] was described previously. AKAP150fl/fl mice were crossed with αMHC-Cre mice[27] to generate cardiac-specific AKAP150-deficient mice. The NFAT-luciferase reporter transgenic mouse, also described previously,[28] was crossed with the AKAP150-/- mouse. All experimental procedures with animals were performed in accordance with the guidelines of the Institutional Animal Care and Use Committee of University of Washington.

Echocardiography, TAC, MI, and isoproterenol infusion

Mice were anesthetized with 2% isoflurane by inhalation. Echocardiographic imaging was performed with a VisualSonics Vevo 2100 imaging system as described previously.[29] M-mode ventricular dimensions were averaged from 3-5 beats. Fractional shortening (FS) was calculated using ventricular dimensions in end of systole and diastole (LVES and LVED, respectively): FS = [(LVED - LVES)/LVED] x 100 (%).[29] Transverse aortic constriction (TAC) was performed to produce cardiac pressure overload in mice using a 27 gauge needle as previously described.[30] Sham-operated mice underwent the same procedure without aortic constriction. Pressure gradients (PG; mm Hg) across the aortic constriction were calculated from the peak blood velocity (*Vmax*) (m/s) measured by Doppler: PG = 4 x $Vmax^2$. The surgical procedure for myocardial infarction (MI) injury in the mouse has been described previously and infarct area was assessed using 2% triphenyltetrazolium chloride in saline and 2% Evan's blue dye infusion.[31] For isoproterenol infusion, Alzet 2002 osmotic pumps (Durect) loaded with isoproterenol (60 mg/kg/day in phosphate-buffered saline) or phosphate-buffered saline were implanted subcutaneously for 14 days.[31] Mice were sacrificed by CO2 asphyxiation.

Histological analysis, cell size measurement, and TUNEL

For histological analysis, mouse hearts were fixed in 10% formalin/phosphate-buffered saline and dehydrated for paraffin embedding. Fibrosis was assessed with Masson's Trichrome staining on paraffin sections. Blue collagen staining was quantified using MetaMorph 6.1 software as described previously.[29] For cell surface area measurements, membranes were stained with TRITC- or FITC- labeled lectin from Triticum vulgaris (Sigma), and nuclei were labeled with TO-PRO 3 iodine (Molecular Probes, Carlsbad, CA). Cellular areas were quantified with ImageJ 1.33 software (Scion Corp., Frederick, MD). Terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) in paraffin sections was performed with a TMR Red In Situ Death Detection Kit (Roche Diagnostics) or an ApopTag Peroxidase In Situ Apoptosis Detection Kit (Millipore) according to the manufacturer's instructions.[29]

Luciferase reporter assays in mouse hearts

Luciferase reporter assays from mouse hearts were performed as described.[7] Briefly, hearts were removed from NFAT-luciferase transgenic mice in the AKAP150+/+ or AKAP150-/- background. Hearts were homogenized in 1 ml luciferase assay buffer (100 mM KH_2PO4, pH 7.8, 0.5% Nonidet P-40, and 1 mM DTT). Homogenates were centrifuged at 3,000 *g* for 10 min at 4°C and the supernatants assayed for luciferase activity as described previously.[28,30]

Cell cultures

Ventricular myocytes were isolated using a Langendorff perfusion apparatus from adult mice euthanized with pentobarbital (100 mg/kg given IP) as previously described.[32] The isolated ventricular myocytes were kept in Tyrode's solution containing (mM): 140 NaCl, 5 KCl, 10 HEPES, 10 glucose, 2 $CaCl_2$, and 1 $MgCl_2$; pH = 7.4 with NaOH, and used 0.5–8 h after isolation. Primary neonatal rat cardiomyocytes were prepared from hearts of 1- to 2-day-old Sprague-Dawley rat pups anesthetized by 2-4% isloflurane inhalation followed by decapitation.[30] Cells were grown in M199 medium containing 100 U/ml of

penicillin-streptomycin and 2 mM L-glutamine without serum for 24 h before infection. Adenoviral infections were performed as previously described at a multiplicity of infection of 10 to 50 plaque forming units per ml. Adβgal, AdΔCnA, AdNFAT-luciferase reporter, and AdNFATc1-GFP have been previously described.[30]

$[Ca^{2+}]_i$ measurements and cell shortening

Ventricular myocytes were loaded with the membrane-permeable acetoxymethyl-ester form of Fluo-4 (Fluo-4 AM) for measurement of $[Ca^{2+}]_i$ as previously described.[33] All experiments were performed using an Olympus Fluo View 1000 confocal microscope equipped with a 60x oil-immersion lens. All images were analyzed using ImageJ. Field stimulation was performed via two platinum wires (0.5 cm separation) placed at the bottom of the perfusion chamber. An IonOptix Myopacer (IonOptix Corp, Milton, MA, USA) was used to deliver square voltage pulses (4 ms duration) with an amplitude of 5 V at a frequency of 1 Hz and Ca^{2+} transients were measured. After reaching steady state, SR Ca^{2+} load was assayed by measuring the caffeine-induced Ca^{2+} transient evoked by rapid application of 20 mM caffeine to the cells via a picospritzer.[32] Contraction was measured by measuring the cell's longitudinal axis before and during field stimulation and caffeine application. These experiments were also performed in Tyrode's solution containing 100 nM isoproterenol.

Western blotting

Protein extraction from mouse heart or cultured cardiomyocytes and subsequent Western blotting followed by enhanced chemiluminescence detection were performed as previously described.[29,30] The following antibodies were used: Anti-AKAP150 (sc-6445), anti-RYR2 (sc-13942), anti-pSer16-phospholamban (Ser16; sc-12963R), anti-PKA IIα reg (sc-908), and anti-glyceraldehyde-3-phosphate dehydrogenase (GAPDH; sc-25778) antibodies were from Santa Cruz Biotechnology (Santa Cruz, CA); anti-SERCA2 (9580), anti-phospholamban (8495), anti-TnI (13083), anti-phospho-TnI (Ser23/24; 4004),

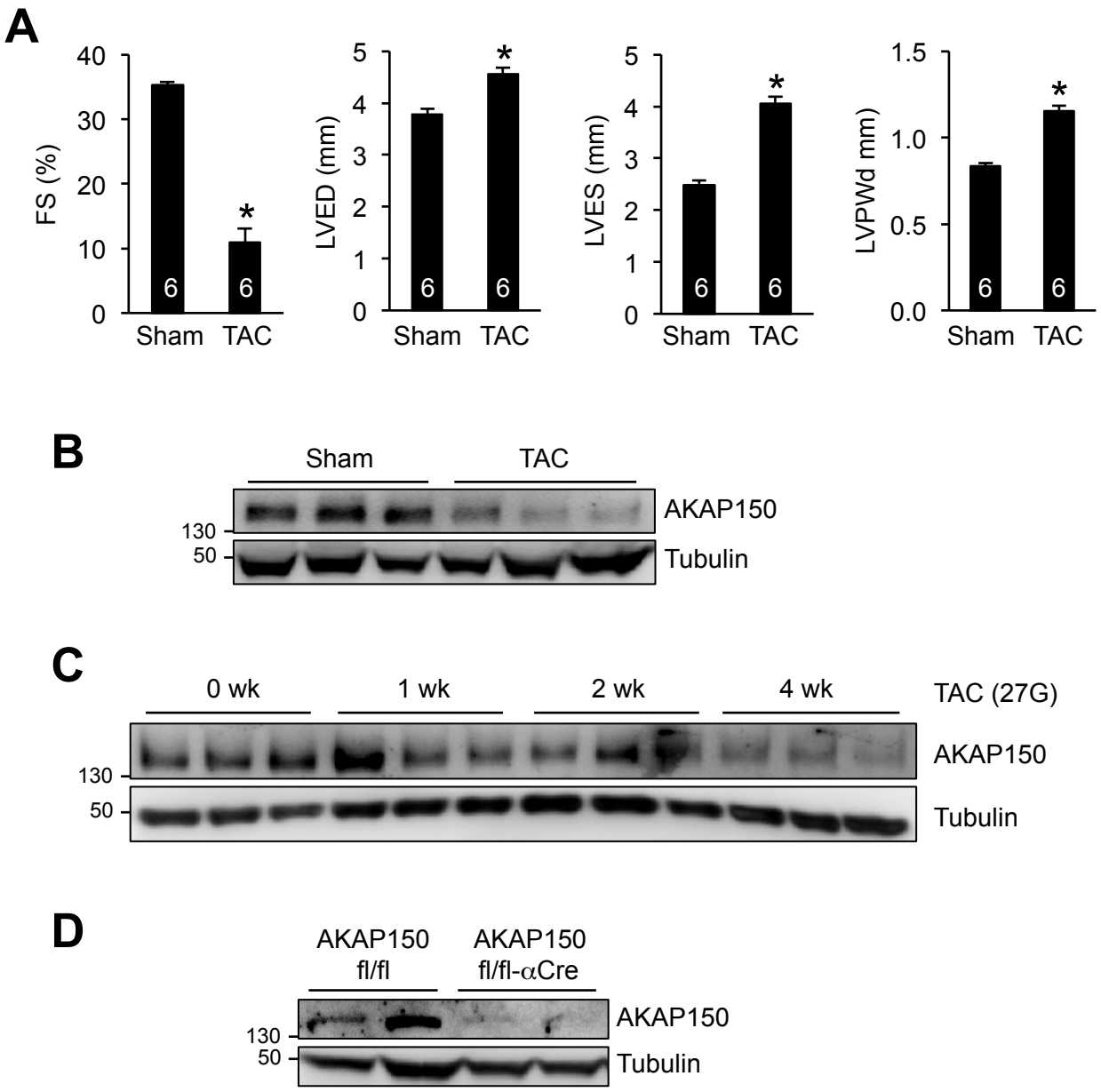
and anti-calcineurin A (2614) were from Cell Signaling Biotechnology (Beverly, MA); anti-pRYR2 Ser2808 (A010-30) was from Barilla (Leeds, UK).

Immunoprecipitation

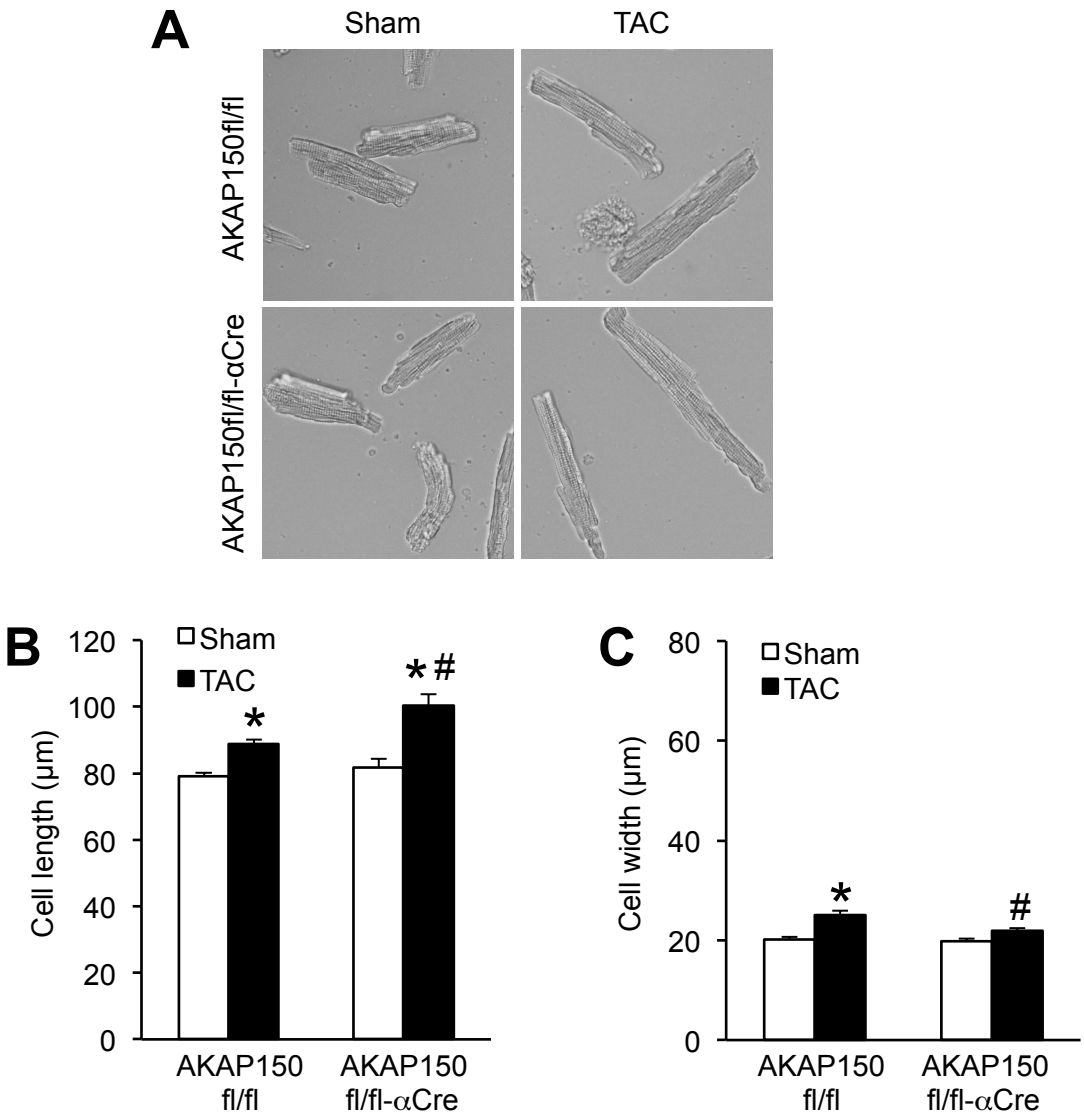
Cells were lysed at 4°C in lysis buffer (50 mM Tris-HCl [pH 7.5], 150 mM NaCl, 1mM EDTA, 10 mM NaF, 1 mM sodium vanadate, 0.5% NP-40) containing protease inhibitor cocktail (Roche). Whole cell lysates were cleared by centrifugation at 18,000 x *g* for 10 min and then incubated with the indicated antibodies and protein A/G-PLUS agarose beads (Santa Cruz Biotechnologies) overnight at 4°C. The beads were washed extensively with binding buffer, and the proteins were resolved on an 8-12% SDS-PAGE for subsequent Western blotting.[29,30]

Statistics

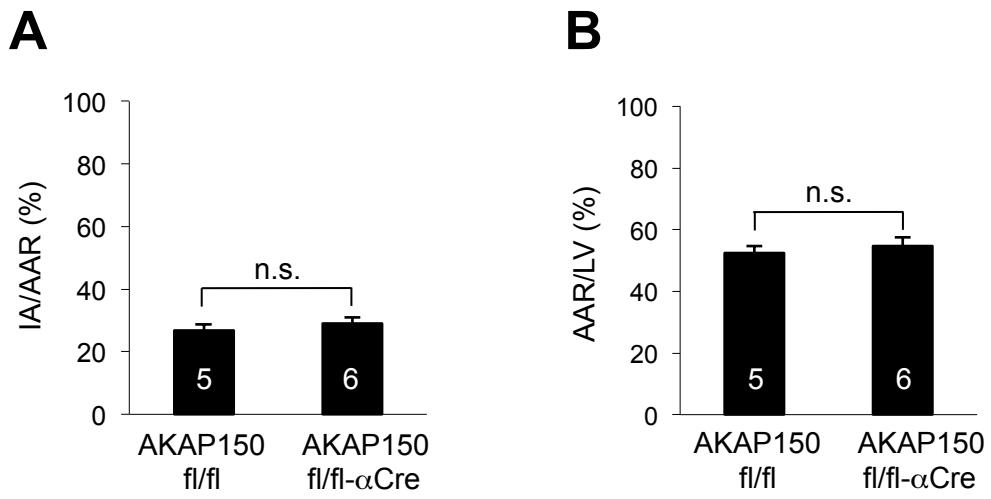
Sample size was estimated by conducting pilot experiments and power analysis. Results are presented as means ± SEM. Mann–Whitney U-test or Kruskal-Wallis test with post hoc Mann–Whitney U-test and Bonferroni's correction was used for studies with small sample sizes. Two-way nested ANOVA with Tukey's post hoc test was used for calcium and contractility data. $P<0.05$ was considered statistically significant.



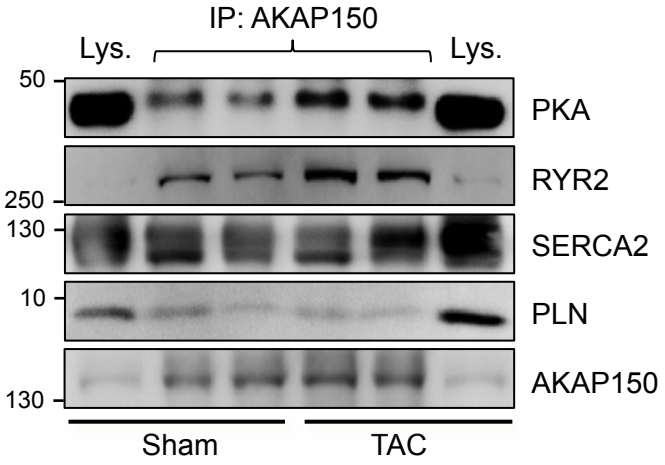
Supplemental Figure 1. Decreased AKAP150 expression in the heart following pressure overload. *(A)* Echocardiographic assessment of cardiac function from wild-type mice subjected to TAC (27-gauge) or sham surgery for 4 weeks. **P*<0.05 versus corresponding Sham. Mann–Whitney U-test was used. *(B)* Western blot for AKAP150 from cardiomyocytes isolated from 3 mice each after TAC or sham surgery for 4 weeks. *(C)* Western blot for AKAP150 in cardiac extracts following 0-4 weeks of TAC stimulation. n=3 mice for each. *(D)* Western blots for AKAP150 from cardiomyocytes isolated from two AKAP150fl/fl and two AKAP150fl/fl-αCre mice.



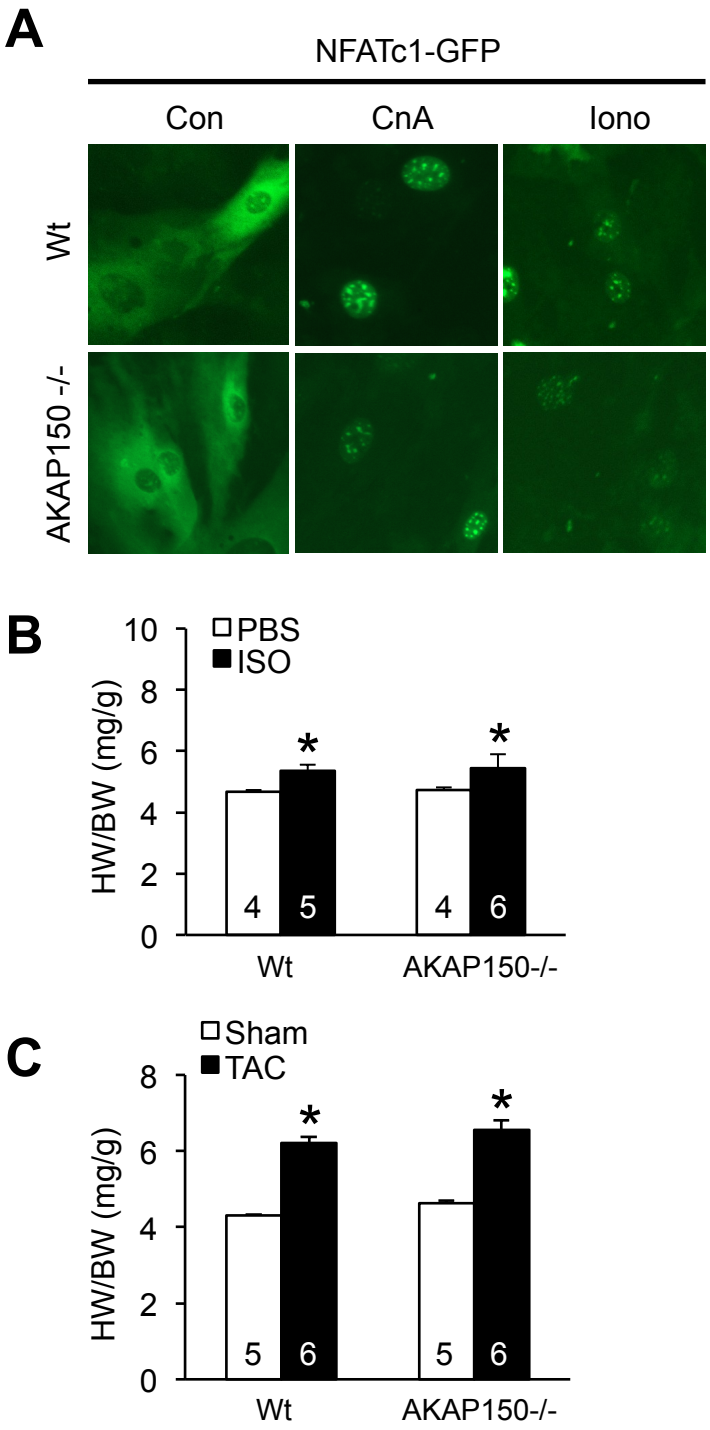
Supplemental Figure 2. Cardiomyocytes from AKAP150-deficient mouse display elongation after TAC. *(A)* Representative images of isolated cardiomyocytes from hearts of the indicated mice subjected to 4-week TAC or sham surgery. *(B)* and *(C)*, Cardiomyocyte length and width from the indicated mice subjected to 4-week TAC or sham surgery. Two-way nested ANOVA with Tukey's post hoc test was used. **P*< 0.05 versus corresponding Sham. #*P*<0.05 versus AKAP150fl/fl TAC. n=200 cells from 3 mice in each group.



Supplemental Figure 3. Left ventricular ischemic injury in AKAP150-deficient and control mice after acute ischemia/reperfusion. *(A)* Infarct area normalized to area at risk (IA/AAR) in the hearts of the indicated mice after 60 minutes of ischemia followed by 24 hours of reperfusion. *(B)* Area at risk (AAR) normalized to the perfused area of the left ventricle (LV) in the indicated mice. Mann–Whitney U-test was used. n.s. indicates non-significance.



Supplemental Figure 4. Pressure overload increases the interaction of AKAP150 with PKA and RYR2. Western blotting for the indicated proteins was performed after immunoprecipitation with an anti-AKAP150 antibody from cardiac extracts of mice subjected to TAC or sham surgery for 1 week.



Supplemental Figure 5. Effects of AKAP150 deletion on NFATc1 translocation and cardiac hypertrophic response. *(A)* Fluorescent images from mouse embryonic fibroblasts derived from Wt or AKAP150-/-mice, which are infected with an adenoviral vector encoding NFATc1-GFP (green) followed by stimulation with 1 μmol/l ionomycin (Iono) or vehicle control, or infection with an adenovirus encoding active calcineurin (CnA). *(B)* HW/BW from AKAP150+/+ or AKAP150-/- mice subjected to isoproterenol (ISO) or phosphate-buffered saline (PBS) infusion for 2 weeks. **P*<0.05 versus corresponding Sham. *(C)* HW/BW from Wt or AKAP150-/- mice subjected to 27-gauge TAC or a sham procedure for 1 week. Kruskal-Wallis test followed by post hoc Mann–Whitney U-test was used. **P*<0.05 versus corresponding Sham.