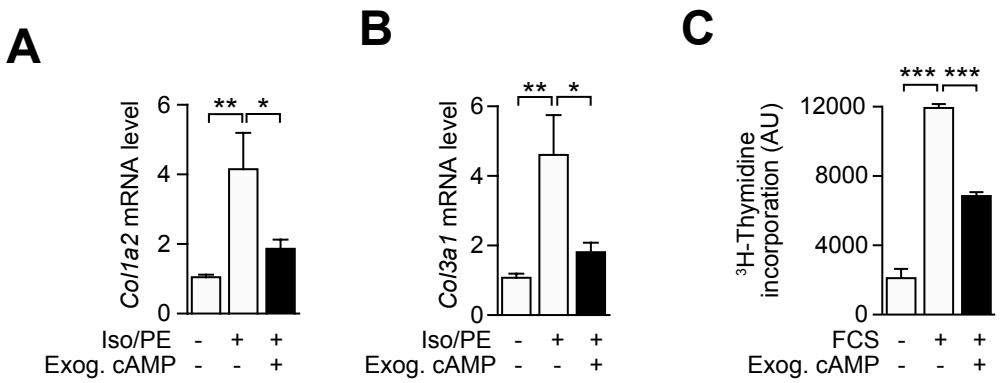
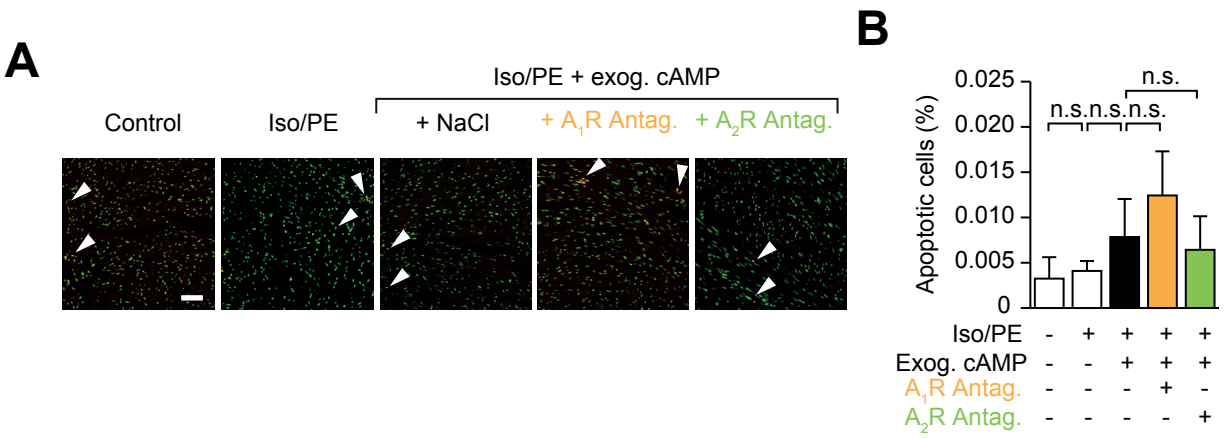
Supplemental Figure 1



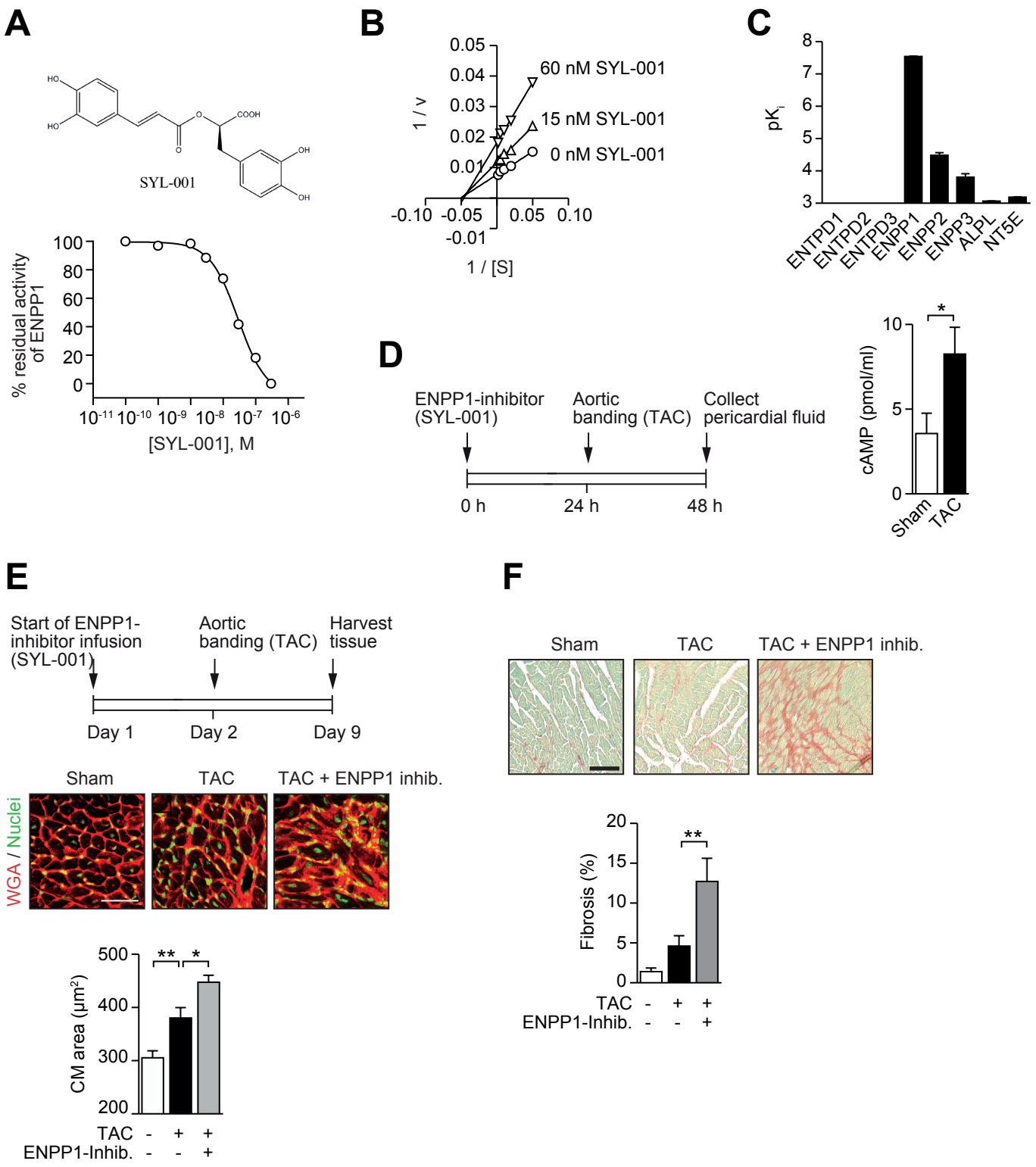
Supplemental Figure 1.
Extracellular cAMP reduces the activation and proliferation of cardiac fibroblasts. (**A and B**) Quantitative PCR analysis of mRNAs encoding Col1a2 (A) and Col3a1 (B) in myocardial tissue from mice after 7 days of Iso/PE infusion (30 mg/kg/day each) in presence of absence of exogenous cAMP (30 mg/kg/day). n = 9-10 mice per group. (**C**) ^{3}H-Thymidine incorporation by adult rat cardiac fibroblasts treated with 10% FCS and exogenous cAMP. Time of incubation: 48 h. n = 6 independent experiments. * $p<0.05$; ** $p< 0.01$;*** $p <0.001$ determined by one-way ANOVA with Bonferroni's post hoc analysis.

Supplemental Figure 2



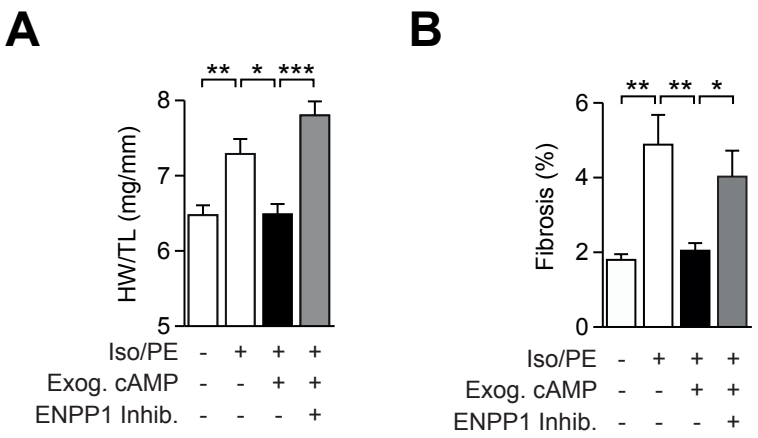
Supplemental Figure 2.
Exogenous cAMP does not alter cardiomyocyte apoptosis in vivo. (**A**) Representative pictures of TUNEL staining in mouse hearts of animals after 7 days of infusion with Iso/PE (30 mg/kg/day each), exogenous cAMP (30 mg/kg/day), an A_1 adenosine receptor antagonist (PSB-16P, 5 mg/kg/day) or an A_{2A} adenosine receptor antagonist (MSX-3, 5 mg/kg/day). (**B**) Quantification of data from (A). n = 6-8 mice/group. NS: not significant, determined by one-way ANOVA with Holm-Sidak's post hoc analysis.

Supplemental Figure 3



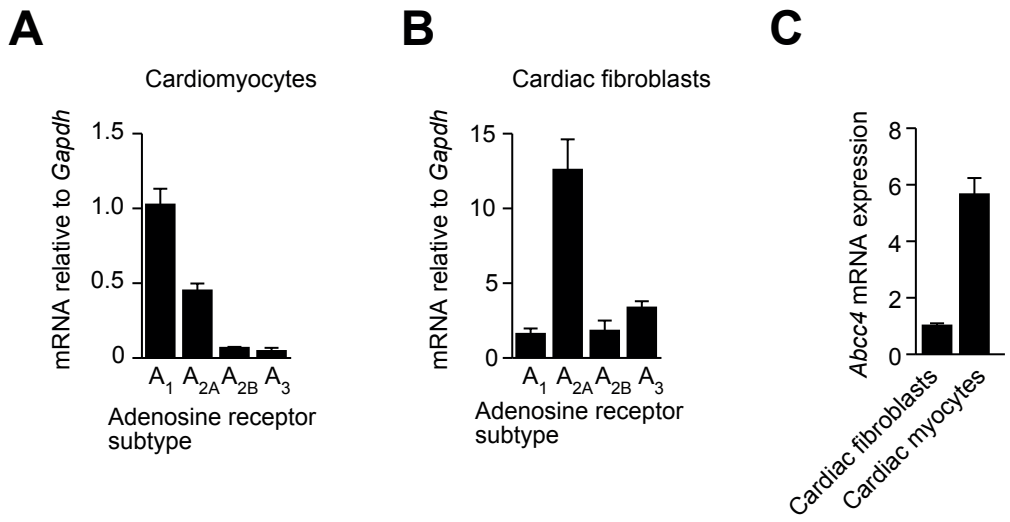
Supplemental Figure 3.
Inhibition of the cAMP-metabolizing enzyme ectonucleotide pyrophosphatase/phosphodiesterase 1 (ENPP1) exacerbates TAC-induced cardiac remodeling. (**A**) Concentration-inhibition curve for SYL-001 at human ENPP1 (Ki = 27 ± 0.8 nM, n = 3). (**B**) Lineweaver-Burk plot of ENPP1 inhibition by SYL-001. [S], substrate concentration of ATP (µM); v, velocity of enzyme (nmol/min/mg protein). (**C**) Potency of SYL-001 at various human enzymes: Ectonucleoside Triphosphate Diphosphohydrolases (ENTPD1-3), ENPP1-3, tissue-nonspecific alkaline phosphatase (ALPL) and ecto-5'-nucleotidase (NT5E). Data are given as means ± SEM of pKi values. The compound was inactive at ENTPD1-3 at concentrations up to 1 mM. (**D**) Quantification of endogenous cAMP in the pericardial fluid of mice after chronic cardiac pressure overload. 8 weeks old mice were subjected to transverse aorta constriction (TAC) and to sham surgeries. To prevent the degradation of cAMP, all mice received infusions with an inhibitor that blocks ENPP1 activity (SYL-001, 7.5 mg/kg/day) one day before TAC and sham surgeries. 24 h after surgery, mice were sacrificed and the pericardium was incised and washed with 350 µl PBS to collect pericardial fluid. 50 µl thereof were used for cAMP quantification. * p <0.05. (**E-F**) ENPP1 inhibition exacerbates TAC-induced cardiomyocyte hypertrophy and cardiac fibrosis. (**E**) (Top row) Wheat germ agglutinin staining of left ventricle tissue (scale bar: 50 µm) after the indicated treatments and (Bottom row) quantitative analysis. (**F**) Myocardial tissue sections after staining with Sirius Red and Fast Green counterstaining and quantification of myocardial fibrosis. Scale bar: 100 µm. n = 6-8 mice/group.

Supplemental Figure 4



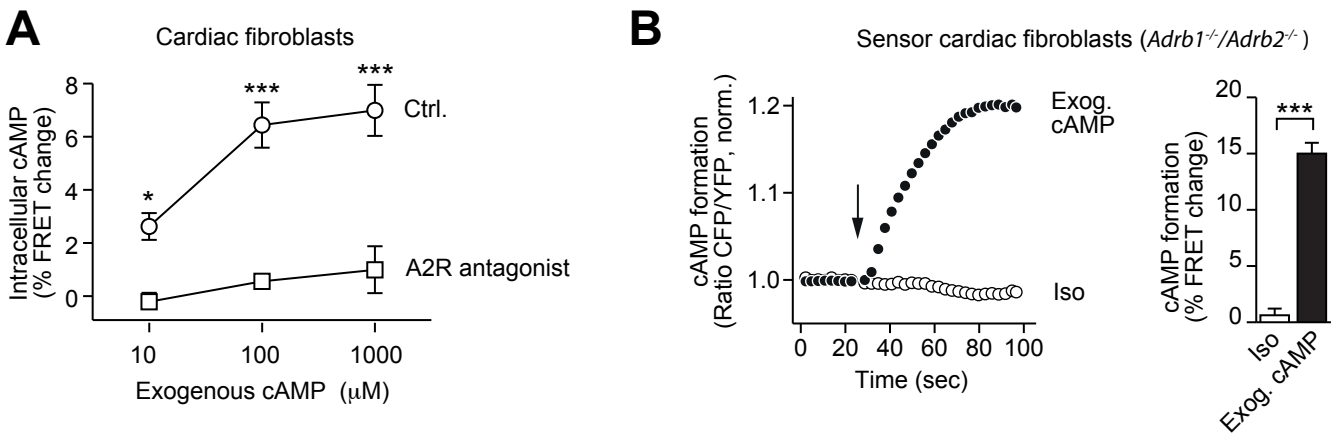
Supplemental Figure 4.
Extracellular cAMP requires processing by ENPP1 to exert antihypertrophic effects. (**A**) Ratio of heart weight to tibia length (HW/TL) and (**B**) quantification of myocardial fibrosis of mice chronically infused with Iso/PE (30 mg/kg/day each), exogenous cAMP (30 mg/kg/day) and the ENPP1 antagonist (SYL-001, 7.5 mg/kg/day). After 7 days, mice were sacrificed for the assessment of cardiac hypertrophy. n = 5-8 mice per group. * $p<0.05$; ** $p< 0.01$; *** $p <0.001$; determined by one-way ANOVA with Bonferroni's (A) and Holm-Sidak's (B) post hoc analysis.

Supplemental Figure 5



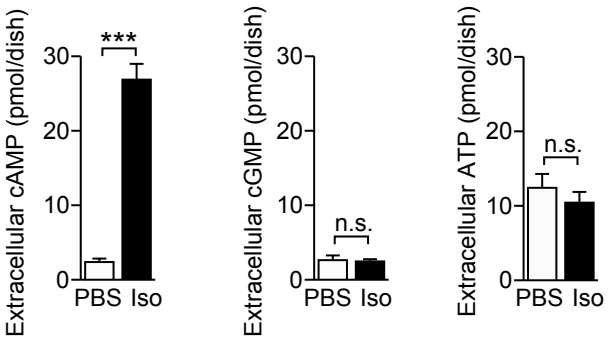
Supplemental Figure 5.
Quantification of endogenous adenosine receptor subtype expression in isolated adult mouse cardiomyocytes (**A**) and cardiac fibroblasts (**B**). (**C**) Quantitative PCR analysis of *Abcc4* mRNA by qPCR in RNA isolated from neonatal rat cardiac fibroblasts and cardiomyocytes. n=3-6 experiments in duplicate.

Supplemental Figure 6



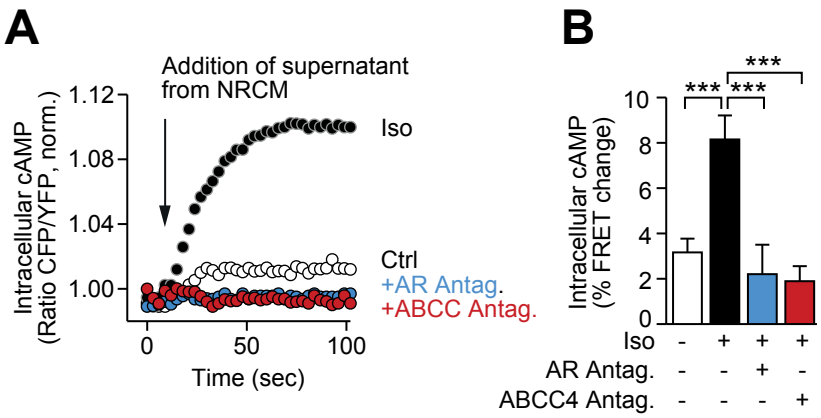
Supplemental Figure 6.
Extracellular cAMP induces the formation of intracellular cAMP in cardiac fibroblasts. (**A**) Intracellular cAMP was detected in neonatal rat cardiac fibroblasts infected by an adenovirus to express the EPAC-FRET sensor. Intracellular cAMP formation occured as a concentration-dependent response to exogenously provided cAMP. An antagonist against adenosine receptor type 2A (ZM-241385, 100 nM) suppressed this response (n ≥ 17 cells per group). ** p< 0.01; *** p <0.001 determined by one-way ANOVA with holm-Sidak's post hoc analysis. (**B**) Insensitivity of *Adrb1/Adrb2*-deficient sensor cardiac fibroblasts to isoproterenol, but not to exogenous cAMP. (Left) Representative FRET measurement in sensor cardiac fibroblasts, isolated from adult Adrb1$^{-/-}$/Adrb2$^{-/-}$ mice and stimulated with cAMP (100 μM) or with Iso (10 μM). Arrow indicates the addition of Iso or exogenous cAMP. (Right) Quantification of the changes in cAMP-FRET ratio. n=13-19 cells. *** p< 0.001 determined by Mann-Whitney test.

Supplemental Figure 7



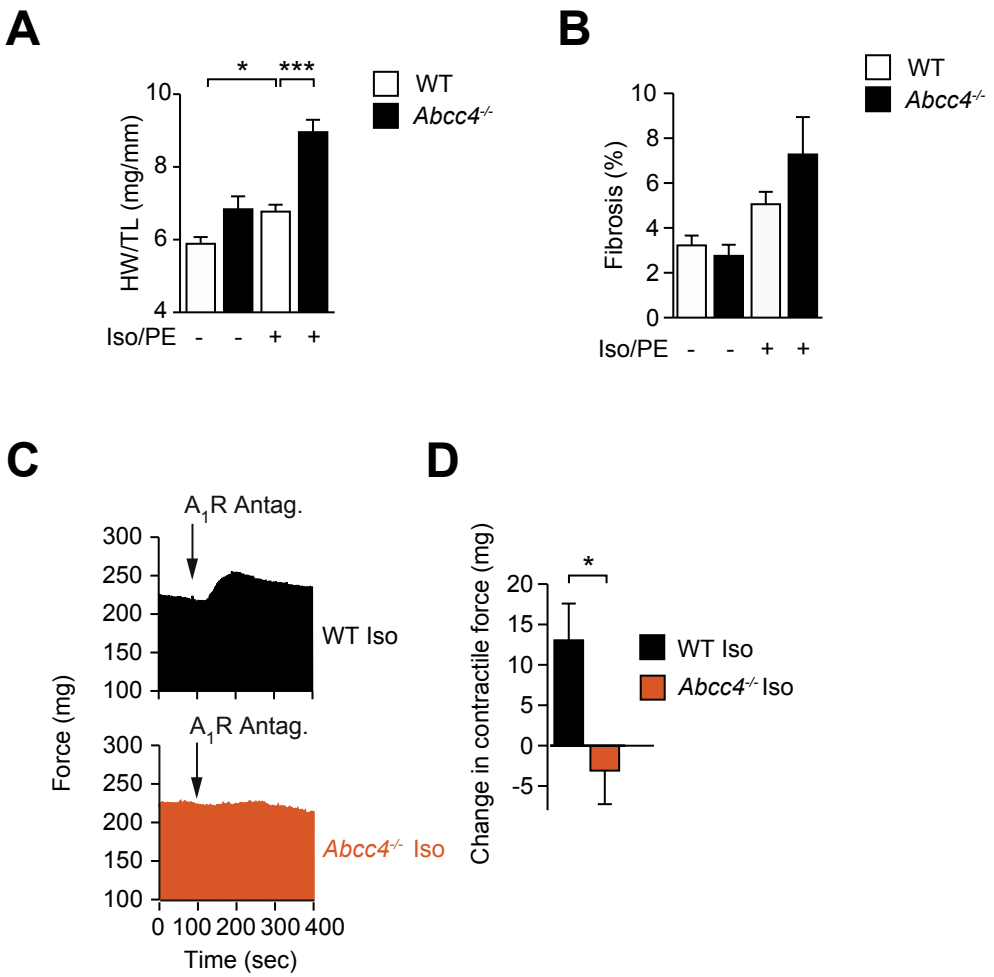
Supplemental Figure 7.
Quantification of nucleotide efflux from neonatal rat cardiac myocytes (NRCM) after β-adrenoceptor stimulation in vitro. NRCM were cultured for 24 h and then stimulated for 5 min with Iso (10 μM) or mock-treated with PBS. To block degradation of cAMP or cGMP, IBMX was added (300 μM) together with Iso. As for the quantification of ATP, the E-NTPDase inhibitor POM-1 was added (30 μM) together with Iso. n = 4-5 experiments in duplicate. ** $p < 0.01$; NS, not significant vs. PBS determined by unpaired t test with Welch's correction.

Supplemental Figure 8



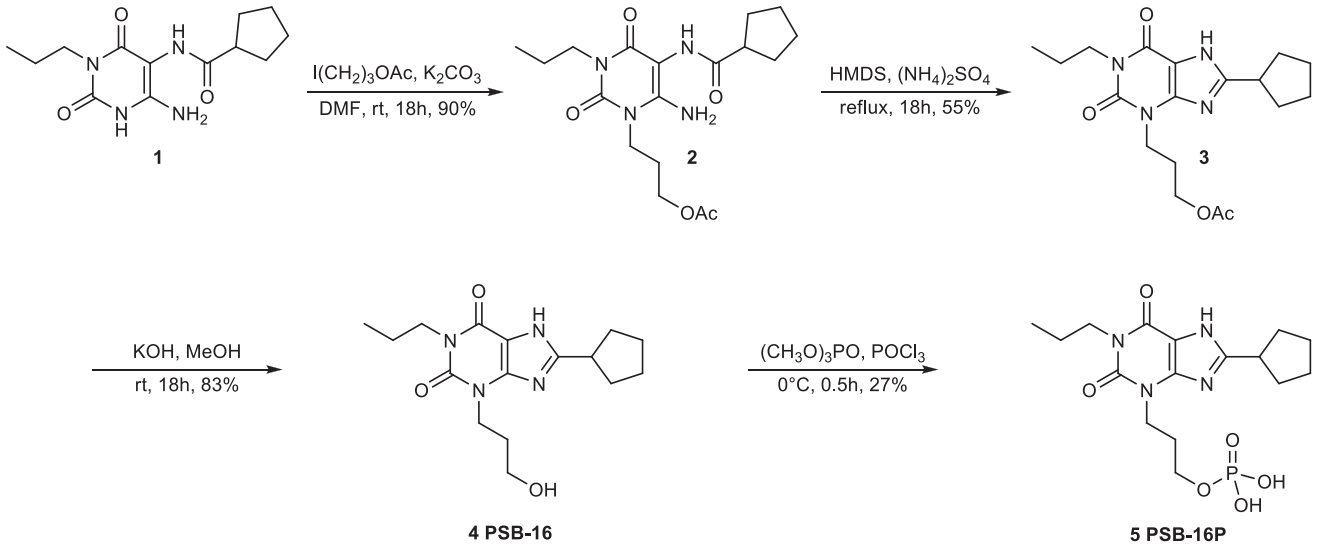
Supplemental Figure 8.
Conditioned medium from Iso-stimulated neonatal rat cardiomyocytes (NRCM) induces cAMP formation in sensor cardiac fibroblasts. The experiment was carried out analogous to Figure 3, except that no centrifugation of CM was applied, in order to prevent potential cell damage. Medium from cultured, Iso-treated NRCM was transferred onto *Adrb1*$^{-/-}$/*Adrb2*$^{-/-}$ CF expressing the cAMP sensor. FRET recordings from individual cells (**A**) and statistical assessment of data from multiple cells (**B**) indicate that the Iso-conditioned medium increased cAMP formation in CF more than in ctrl-conditioned medium. Pretreatment of the CM with the general ABCC antagonist (MK571, 50 µM), or addition of the adenosine receptor antagonist to CF (DPSPX, 10 nM) completely abolished the Iso effect of conditioned medium. Quantitative FRET data were obtained from at least 7 cells per group * $p<0.05$; ** $p< 0.01$; vs Iso determined by one-way ANOVA with Holm-Sidak's post hoc analysis.

Supplemental Figure 9



Supplemental Figure 9.
ABCC4 reduces cardiac remodeling and contractility. (**A-B**) ABCC4 is required to prevent catecholamine-induced cardiac remodeling. (**A**) Ratio of heart weight-to-tibia length (HW/TL) and (**B**) quantification of myocardial fibrosis of WT and *Abcc4*$^{-/-}$ mice (age of 8 months) after Iso/PE infusion (30 mg/kg/day each) for 7 days. (**C-D**) Contractility was analyzed on living myocardial slices from WT and *Abcc4*$^{-/-}$ mice in the presence of adenosine A_1 receptor antagonist DPCPX (100 nM). (**C**) Representative twitch force recordings from WT (black) and *Abcc4*$^{-/-}$ (orange) slices after incubation with Iso (100 nM) and DPCPX (100 nM). (**D**) Quantification of data. n= 5-6 mice per group. * p< 0.05 determined by unpaired t test with Welch's correction.

Supplemental Figure 10



Supplemental Figure 10.
Synthesis of the water–soluble phosphate prodrug PSB-16P of the A_1-selective antagonist PSB-16. See the Methods section for a detailed description of the synthesis.