**Supporting Information**

**Supporting Methods**

*Real-Time GRK Activity Assay*

FRET was calculated as %F, the percentage of CFP-excited fluorescence detected as YFP emission, corrected for both background signal and for spectral bleed-through:

$$FRET\ (\%F) = 100 \bullet \frac{FRETc}{CFP + FRETc}$$

$$FRETc = FRET - 0.43 \bullet CFP - 0.24 \bullet YFP$$

Assuming pseudo-first-order receptor-β-arrestin association kinetics, the FRET signal %F was modeled as an exponential function of time (GraphPad), with rate constant $k_{obs}$ and equilibrium amplitude $\%F_{max}$, where $k_{obs}$ is a relative measure of GRK activity and $\%F_{max}$ depends on expression of $AT_{1A}$R-mCFP and β-arrestin2-mYFP, the affinity of the interaction between the two molecules, and the average distance and orientation between CFP and YFP in the interacting state:

$$\%F = \%F_{\max} \bullet (1 - e^{-k_{obs} \bullet t})$$

*Cardiomyocyte Isolation and Functional Assays*

Purified cardiomyocytes were treated with relevant drugs, and were then subjected to electrical pacing at 1 Hz under visualization with an inverted microscope (Nikon Eclipse TE300). Cyclic shortening and re-lengthening of single cells was assessed in real-time using video edge detection (i.e., cardiomyocyte length as a function of time). % Fractional shortening, an index of systolic function, was calculated directly from these measurements [(length at end-diastole - length at end-systole)/length at end-diastole]. Cardiomyocyte end velocities were derived from

the measured length-time relationships, and their minima (maximum contraction; $-dL/dt_{max}$) and maxima (maximum relengthening; $+dL/dt_{max}$) are reported as indices of systolic and diastolic function, respectively. In an individual experiment (i.e., a single animal), 10-15 cardiomyocytes were analyzed for each experimental condition. A mean result was calculated for these cells, and represents a single data point. This was performed for each experiment (i.e., individual animal), and the mean and SEM for a particular experimental condition were determined using n as the number of animals (i.e., independent experiments).

**Supporting Figure Legends**

**Fig. 6.** $AT_{1A}R$-dependent changes in cardiomyocyte systolic and diastolic function mediated by the natural agonist ANG and the biased agonist SII. (A) Absence of effects of ANG and SII on $-dL/dt_{max}$ of cardiomyocytes from $AT_{1A}R$ KO mice. $-dL/dt_{max}$ of cardiomyocytes from contemporaneous WT (n=4 animals; black bars) and $AT_{1A}R$-deficient (KO) (n=7 animals; white bars) mice, under conditions of pacing alone (Basal), or additional exposure to 10 μM ANG or SII as indicated. (B) Absence of effects of ANG and SII on $+dL/dt_{max}$ of cardiomyocytes from $AT_{1A}R$ KO mice. $+dL/dt_{max}$ of cardiomyocytes from contemporaneous WT (n=4 animals; black bars) and $AT_{1A}R$-deficient (KO) (n=7 animals; white bars) mice, under conditions of pacing alone (Basal), or additional exposure to 10 μM ANG or SII as indicated. In 4 experiments (i.e., 4 individual animals), KO cardiomyocytes were additionally stimulated with 1 μM isoproterenol (Iso). *$p < 0.05$ by one-way ANOVA with *post hoc* Bonferroni test relative to pertinent Basal; **$p < 0.05$ by one-way ANOVA with *post hoc* Bonferroni test relative to pertinent $AT_{1A}R$ KO (identical stimulation condition). Data displayed are mean ± SEM. See *Materials and Methods* for experimental details.

**Fig. 7.** Differential effects of PKC antagonism on changes in cardiomyocyte systolic and diastolic function in response to ANG and SII. $-dL/dt_{max}$ (A) and $+dL/dt_{max}$ (B) of cardiomyocytes from WT mice (n=4), without (black bars) or with (white bars) pretreatment with the PKC inhibitor Ro-31-8425 (1 μM), under conditions of pacing alone (Basal), or additional exposure to 10 μM ANG or SII as indicated. (A and B) (*Left*) Absolute values for each variable under indicated stimulation conditions. (*Right*) Percentage change in each variable in response to ANG or SII, relative to pertinent Basal. *$p < 0.05$ by one-way ANOVA with *post hoc* Bonferroni test relative to pertinent Basal (*Left*) or ANG (*Right*); **$p < 0.05$ by one-way ANOVA with *post hoc* Bonferroni test relative to identical stimulation condition (*Left* only). Data displayed are mean ± SEM. See *Materials and Methods f*or experimental details.

**Fig. 8.** Effects of deficiency of β-arrestin2 on changes in cardiomyocyte systolic and diastolic function in response to ANG and SII. (A) WT cardiomyocytes display positive inotropic and lusitropic responses to ANG and SII, as assessed by changes in $-dL/dt_{max}$ and $+dL/dt_{max}$. $-dL/dt_{max}$ (*Left*) and $+dL/dt_{max}$ (*Right*) of cardiomyocytes from a series of WT mice (n=12 animals) analyzed contemporaneously with the experiments in Figs. 4 and 5 (see also *B* and Fig. 9), under conditions of pacing alone (Basal), or additional exposure to 10 μM ANG or SII as indicated. (*Upper*) Absolute values for each variable under indicated stimulation conditions. (*Lower*) Percentage change in each variable in response to ANG or SII, relative to Basal. (B) β-arrestin2 KO cardiomyocytes display severely defective positive inotropic and lusitropic responses to SII, but unaffected responses to ANG, as assessed by changes in $-dL/dt_{max}$ and $+dL/dt_{max}$. $-dL/dt_{max}$ (*Left*) and $+dL/dt_{max}$ (*Right*) of cardiomyocytes from β-arrestin2 KO mice (n=5 animals), under conditions of pacing alone (Basal), or additional exposure to 10 μM ANG

or SII as indicated. (*Upper*) Absolute values for each variable under indicated stimulation conditions. (*Lower*) Percentage change in each variable in response to ANG or SII, relative to Basal. (C) β-arrestin1 KO cardiomyocytes display equivalent positive inotropic and lusitropic responses to ANG and SII, as assessed by changes in $-dL/dt_{max}$ and $+dL/dt_{max}$. $-dL/dt_{max}$ (*Left*) and $+dL/dt_{max}$ (*Right*) of cardiomyocytes from β-arrestin1 KO mice (n=5 animals), under conditions of pacing alone (Basal), or additional exposure to 10 μM ANG or SII as indicated. (*Upper*) Absolute values for each variable under indicated stimulation conditions. (*Lower*) Percentage change in each variable in response to ANG or SII, relative to Basal. *$p < 0.05$ by one-way ANOVA with *post hoc* Bonferroni test relative to Basal, and between ANG and SII when relevant (*Above* in all panels; in *B*, the * for ANG thus represents significance relative to both Basal and SII, whereas in *A* and *C*, the * for either ANG or SII represents significance relative to Basal), and by Student's paired t-test between ANG and SII (*Below* in all panels). Data displayed are mean ± SEM. See *Materials and Methods* for experimental details.

**Fig. 9.** Effects of deficiency of specific GRK isoforms on changes in cardiomyocyte systolic and diastolic function in response to ANG and SII. (*A* and *B*) GRK5 KO cardiomyocytes display positive inotropic and lusitropic responses to ANG and SII, as assessed by changes in $-dL/dt_{max}$ and $+dL/dt_{max}$. $-dL/dt_{max}$ (*Left*) and $+dL/dt_{max}$ (*Right*) of cardiomyocytes from GRK5 KO mice (n=5 animals), under conditions of pacing alone (Basal), or additional exposure to 10 μM ANG or SII as indicated. (*A*) (*Upper*) Absolute values for each variable under indicated stimulation conditions. (*Lower*) Percentage change in each variable in response to ANG or SII, relative to Basal. (*B*) GRK6 KO cardiomyocytes display severely defective positive inotropic and lusitropic responses to SII, but unaffected responses to ANG, as assessed by changes in $-dL/dt_{max}$ and

+dL/$dt_{max}$. -dL/$dt_{max}$ (*Left*) and +dL/$dt_{max}$ (*Right*) of cardiomyocytes from GRK6 KO mice (n=5 animals), under conditions of pacing alone (Basal), or additional exposure to 10 μM ANG or SII as indicated. (*Upper*) absolute values for each variable under indicated stimulation conditions. (*Lower*) Percentage change in each variable in response to ANG or SII, relative to Basal. (*C*) GRK2 +/- cardiomyocytes display augmented positive inotropic responses to SII. -dL/$dt_{max}$ (*Left*) and +dL/$dt_{max}$ (*Right*) of cardiomyocytes from GRK2 +/- mice (n=5 animals) mice under conditions of pacing alone (Basal), or additional exposure to 10 μM ANG or SII as indicated. (*Upper*) Absolute values for each variable under indicated stimulation conditions. (*Lower*) Percentage change in each variable in response to ANG or SII, relative to Basal. *$p < 0.05$ by one-way ANOVA with *post hoc* Bonferroni test relative to Basal, and between ANG and SII when relevant (*Upper* in *A*-*C*; in *B*, the asterisk for ANG thus represents significance relative to both Basal and SII, whereas in *A* and *C*, the asterisk for either ANG or SII represents significance relative to Basal), and by Student's paired t-test between ANG and SII (*Below*); **$p < 0.05$ by ANOVA with *post hoc* Bonferroni test for SII relative to both Basal and ANG (*C Upper*). Data displayed are mean ± SEM. See *Materials and Methods* for experimental details.