

Supplemental Table 1. Echocardiogram of cardiac function at baseline and after adrenergic stimulation in β_2 AR-flox and CKO mice.

	Flox (baseline)	CKO (baseline)	Flox (ISO)	CKO (ISO)
EF %	59.1 ± 0.81	58.9 ± 0.98	95.26 ± 0.61***	93.93 ± 0.90***
FS %	30.45 ± 0.50	30.54 ± 0.67	69.39 ± 1.43***	66.80 ± 1.94***
IVS d (mm)	0.65 ± 0.026	0.66 ± 0.019	0.89 ± 0.018	0.94 ± 0.024
IVS s (mm)	1.01 ± 0.019	1.03 ± 0.024	1.56 ± 0.041	1.71 ± 0.042
LVID d (mm)	3.32 ± 0.11	3.58 ± 0.085	2.91 ± 0.046	3.08 ± 0.114
LVID s (mm)	2.31 ± 0.088	2.49 ± 0.059	0.89 ± 0.045***	1.02 ± 0.073***
LVPW d (mm)	0.747 ± 0.021	0.78 ± 0.028	0.86 ± 0.023	0.92 ± 0.058
LVPW s (mm)	0.94 ± 0.031	1.02 ± 0.027	1.694 ± 0.028***	1.73 ± 0.092***
LV mass (mg)	72.3 ± 3.56	80.1 ± 5.03	80.68 ± 2.09*	96.37 ± 6.21*
LV mass (corrected) (mg)	57.9 ± 2.87	64.1 ± 4.03	64.55 ± 1.67*	77.10 ± 4.97*
LV Vol; d (μL)	48.02 ± 3.44	54.17 ± 3.04	32.44 ± 1.22***	37.59 ± 3.26***
LV Vol; s (μL)	19.85 ± 1.65	22.21 ± 1.27	1.54 ± 0.21***	2.30 ± 0.43***
HR (beats/min)	474 ± 4	477 ± 6	541 ± 9***	542 ± 11***

EF, ejection fraction, FS, fractional shortening, IVS d, interventricular septal end diastole; IVS s, interventricular septal end systole; LVID d, left ventricular internal diameter end diastole; LVID s, left ventricular internal diameter end systole; LVPW d, left ventricular posterior wall end diastole; LVPW s, left ventricular posterior wall end systole; LV mass, left ventricular mass; LV Vol d, left ventricular volume end diastole, LV Vol s, left ventricular volume end systole. Data represent mean ± SEM. N=8. * $P < 0.05$ and *** $P < 0.001$, vs baseline within the same colony group, respectively. P value determined by one-way ANOVA with Turkey post hoc test.

Supplemental Table 2. Hemodynamic parameters of β_2 AR-flox and cKO working hearts.

Groups	Flox		cKO	
	Vehicle	ISO	Vehicle	ISO
Parameters				
HR (min ⁻¹)	225.80 ± 69.63	511.60 ± 74.05*	334.80 ± 6.02	432.20 ± 6.53
CF (ml min ⁻¹)	2.08 ± 1.17	0.46 ± 0.51*	2.48 ± 0.7	1.20 ± 1.06*#
AF (ml min ⁻¹)	1.92 ± 1.17	3.56 ± 0.52*	1.52 ± 0.7	2.80 ± 1.06*#
CO (ml min ⁻¹)	4.00 ± 0.01	4.02 ± 0.04	4.00 ± 0.01	4.00 ± 0.02
LVDP (mm Hg)	28.68 ± 7.85	7.62 ± 2.31*	28.54 ± 4.56	12.94 ± 2.04*#

HR, heart rate; CF, coronary flow; AF, aortic flow; CO, cardiac output. LVDP, left ventricle developed pressure. Data represent mean ± SEM. N = 4-6, * $P < 0.05$ vs vehicle or isoproterenol group within the same colony group. # $P < 0.05$ vs the same condition in β_2 AR-flox colony, respectively. P value determined by one-way ANOVA with Turkey post hoc test.

Supplemental Table 3. Echocardiogram of cardiac function at baseline and after adrenergic stimulation in WT and GRK-KI mice.

	WT (baseline)	GRK KI (baseline)	WT (ISO)	GRK-KI (ISO)
EF %	57.10 ± 2.11	54.14 ± 2.40	95.08 ± 2.82***	93.46 ± 3.137***
FS %	29.42 ± 1.38	27.40 ± 1.47	70.31 ± 8.45***	66.54 ± 6.298***
IVS d (mm)	0.77 ± 0.029	0.73 ± 0.041	0.666 ± 0.234	0.831 ± 0.087
IVS s (mm)	1.18 ± 0.035	1.17 ± 0.019	1.159 ± 0.491	1.602 ± 0.289
LVID d (mm)	3.61 ± 0.069	3.47 ± 0.059	2.358 ± 1.056	3.418 ± 0.182
LVID s (mm)	2.52 ± 0.087	2.62 ± 0.059	1.001 ± 0.54*	1.146 ± 0.222*
LVPW d (mm)	0.73 ± 0.035	0.73 ± 0.045	0.806 ± 0.375	0.647 ± 0.100
LVPW s (mm)	1.03 ± 0.036	1.02 ± 0.054	1.477 ± 0.617	1.647 ± 0.188
LV mass (mg)	95.71 ± 3.85	86.2 ± 7.98	91.08 ± 22.43	82.00 ± 13.39
LV mass (corrected) (mg)	76.57 ± 3.08	68.96 ± 6.38	62.86 ± 9.74	65.60 ± 10.72
LV Vol; d (μL)	55.07 ± 2.56	49.88 ± 2.01	33.95 ± 8.956*	48.24 ± 6.032
LV Vol; s (μL)	25.27 ± 1.90	24.09 ± 1.10	3.373 ± 3.059***	3.164 ± 1.398***
HR (beats/min)	478 ± 4	482 ± 7	566 ± 29***	583 ± 30***

EF, ejection fraction, FS, fractional shortening, IVS d, interventricular septal end diastole; IVS s, interventricular septal end systole; LVID d, left ventricular internal diameter end diastole; LVID s, left ventricular internal diameter end systole; LVPW d, left ventricular posterior wall end diastole; LVPW s, left ventricular posterior wall end systole; LV mass, left ventricular mass; LV Vol d, left ventricular volume end diastole, LV Vol s, left ventricular volume end systole, Data represent mean ± SEM. N = 6-7. * $P < 0.05$ and *** $P < 0.001$, vs baseline within the same colony group, respectively. P value determined by one-way ANOVA with Turkey post hoc test.

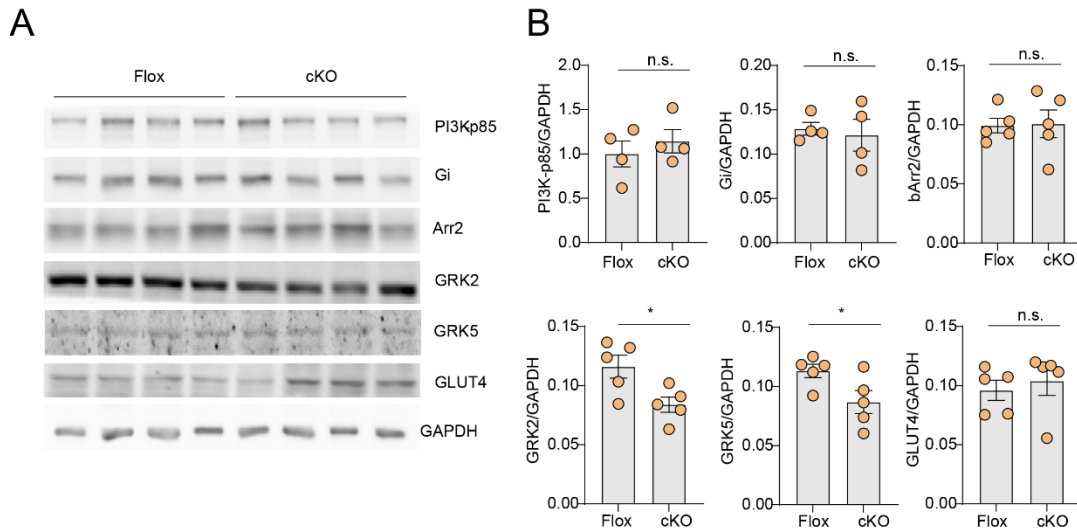
Supplemental Table 4. Hemodynamic parameters of WT and GRK-mut working hearts.

Parameters	WT		GRK-mut KI	
	Vehicle	ISO	Vehicle	ISO
HR (min ⁻¹)	261.32 ± 72.68	479.6 ± 77.8*	283.3 ± 88.64	373.64 ± 81.17
CF (ml min ⁻¹)	3.56 ± 0.22	2.98 ± 0.22*	2.92 ± 0.4	2.38 ± 0.36*
AF (ml min ⁻¹)	0.46 ± 0.25	1.03 ± 0.26*	1.12 ± 0.43	1.65 ± 0.32*
CO (ml min ⁻¹)	4.02 ± 0.06	4.01 ± 0.05	4.04 ± 0.09	4.03 ± 0.05
LVDP (mm Hg)	26.8 ± 8.57	6.72 ± 1.32*	29.45 ± 3.51	14.31 ± 4.67*

HR, heart rate; CF, coronary flow; AF, aortic flow; CO, cardiac output. LVDP, left ventricle developed pressure. Data represent mean ± SEM. N = 4-6, * $P < 0.05$ vs vehicle or isoproterenol group within the same colony group. P value determined by one-way ANOVA with Turkey post hoc test.

Supplemental Figure 1. Biochemical characterization of flox and β_2 AR-CKO hearts. (A) Western blots showing the expression of Gi, PI3K-p85, GLUT4, GRK2, GRK5, and β arrestin2 expression in flox and β_2 AR-CKO hearts. (B) The Western blots were quantified using ImageJ, and AUs were defined as the ratio of intensity of the protein of interest over the intensity of GAPDH. Data representing mean \pm SEM, $n = 4$. * $P < 0.05$ and ** $P < 0.01$ were analyzed using Student's t -test.

Figure S1



Supplemental Figure 2. Biochemical characterization of flox and β_2 AR-CKO hearts. (A) Western blots showing the baseline phosphorylation of mTOR, PDK, AMPK, and AS160 in Flox and β_2 AR-CKO hearts. (B) The Western blots were quantified using ImageJ, and AUs were defined as the ratio of phosphorylated PDK over the intensity of total PDK or as the ratio of phosphorylated mTOR, AMPK, and AS160 over the intensity of GAPDH. Data representing mean \pm SEM, n = 4. *P* values were analyzed using Student's *t*-test.

Supplemental Figure 3. Detection of ISO-induced Akt phosphorylation in flox and β_2 AR-CKO AVMs. (A) Western blots showing phosphorylation of Akt at Ser473 and Ser474 mouse AVMs after stimulation with ISO (100 nM, 10 minutes). (B) The Western blots were quantified using ImageJ, and AUs were defined as the ratio of intensity of phosphorylated Akt over the intensity of total Akt. Data representing mean \pm SEM, n = 3. **P* < 0.05 and ***P* < 0.01 were analyzed using two-way ANOVA with Tukey's multiple comparison test.

Figure S2

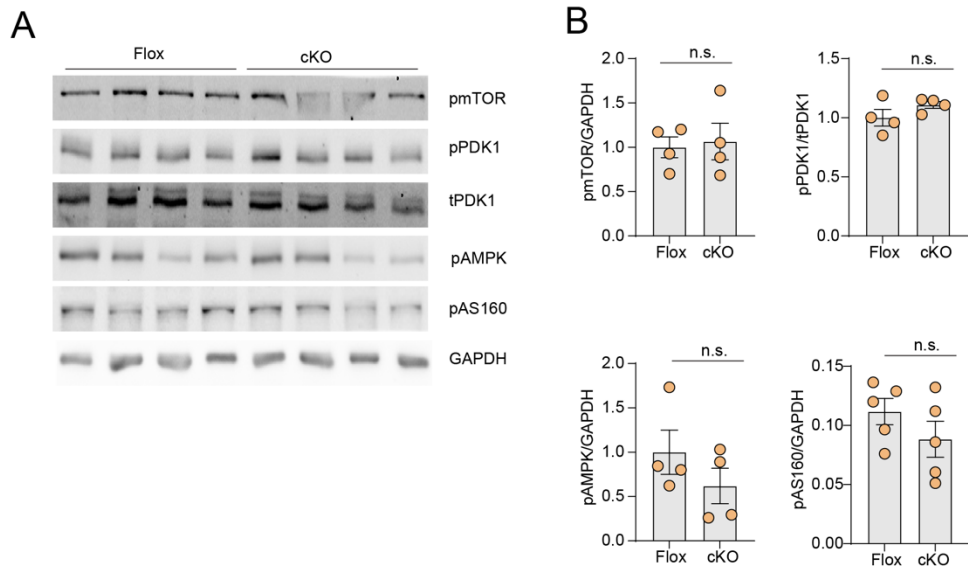
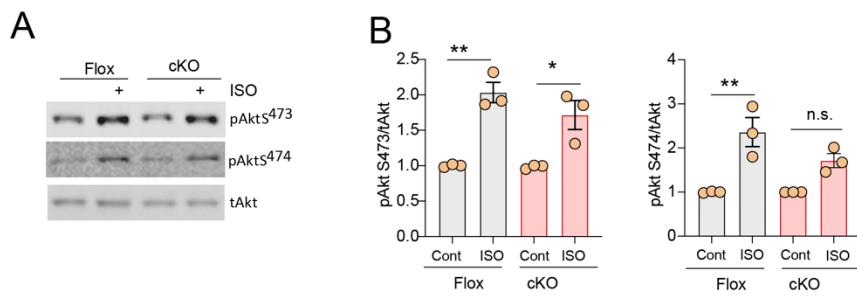


Figure S3



Supplemental Figure 4. Characterization of WT and GRK-mut β_2 AR expressing in β_2 AR-CKO AVMs. (A-B) β_2 AR-CKO myocytes expressing either WT or GRK-mut β_2 AR, the expression of the receptor was quantified. Data representing mean \pm SEM of individual cells from 3 isolations. * $P < 0.05$ were analyzed using Student's *t*-test.

Supplemental Figure 5. Biochemical characterization of WT and GRK-mut hearts. (A-B) Western blots showing the expression of Gi, PI3K-p85, GLUT4, GRK2, GRK5, and β arrestin2 expression in WT and GRK-mut hearts. (C-D) Western blots showing the baseline phosphorylation of Akt and AS160 in WT and GRK-mut hearts. The Western blots were quantified using ImageJ, and AUs were defined as the ratio of phosphorylated Akt over the intensity of total Akt or as the ratio of the protein intensity over the intensity of GAPDH. Data representing mean \pm SEM, n = 4. * $P < 0.05$ were analyzed using Student's *t*-test.

Figure S4

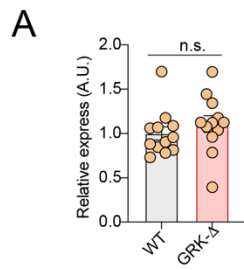


Figure S5

