SUPPLEMENTARY DATA

Insulin inhibits cardiac contractility by inducing a G_i-biased β_2 adrenergic signaling in hearts

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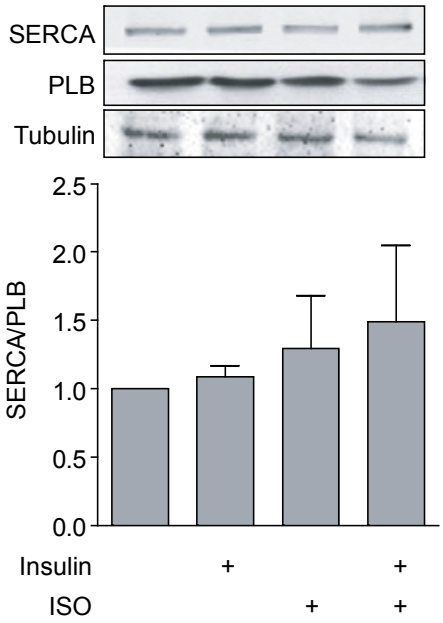
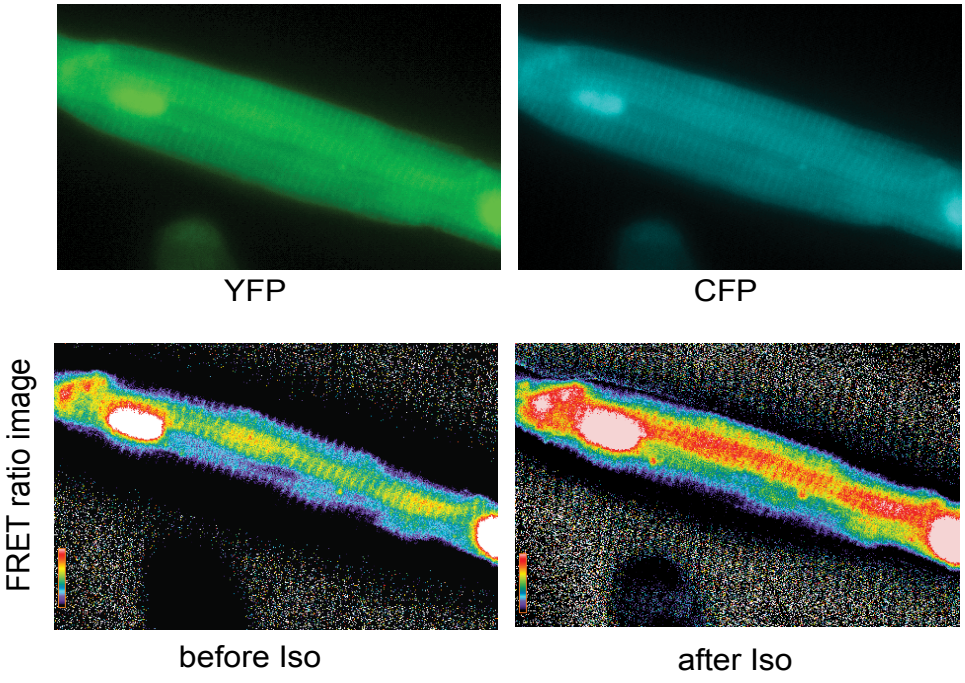
Supplementary Figure 1. Expression of SERCA and PLB in hearts after perfusion with insulin and isoproterenol were detected by Western blot. The SERCA/PLB ratio was calculated and normalized relative to control and plotted in bar graph below. Bar graphs represent mean +/- SD from 5-8 hearts.



Supplementary Figure 2. Representative images of the expression of the PKA biosensor AKAR3 in adult rat myocytes. The ratio of YFP/CFP was acquired before and after stimulation with isoproterenol. The images show striated patterns of PKA activity in adult rat myocytes.



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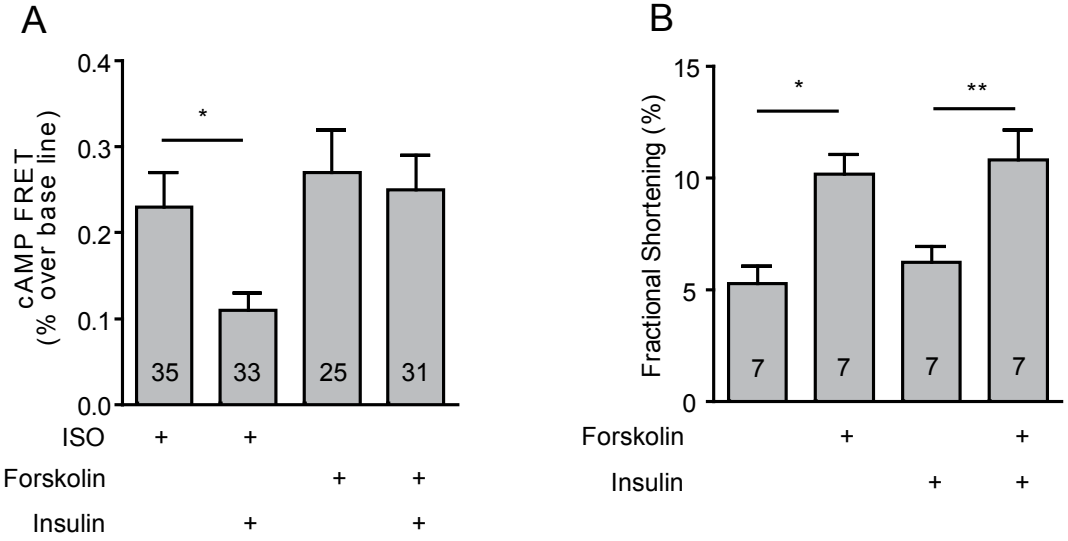
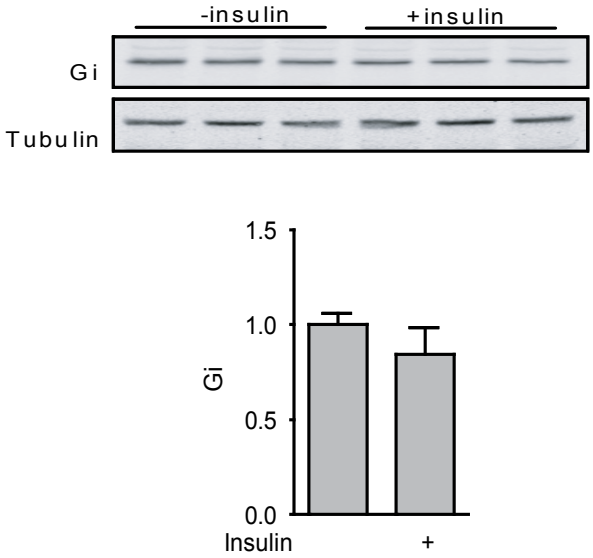
Supplementary Figure 3. A. Neonatal cardiac myocytes expressing cAMP biosensor ICUE3 were treated with insulin (100 nM) and/or forskolin (1 mM) as indicated. The changes in ICUE3 FRET ratio were recorded, and the maximal responses were plotted. B. Adult cardiac myocytes were stimulated with insulin (100 nM) and/or forskolin (1 mM) as indicated. The fractional shortening was measured and the maximal shortening plotted. Bar graphs represent mean +/- SD, and N represents the number of cells detected. * < 0.05 and ** < 0.01 by one-way ANOVA followed by Tukey's post hoc test.



Supplementary Figure 4. The expression of G_i protein was examined in wildtype hearts perfused with either saline or insulin (1 nM) for 30 min. Bar graphs represent mean +/- SD from 5-8 hearts.



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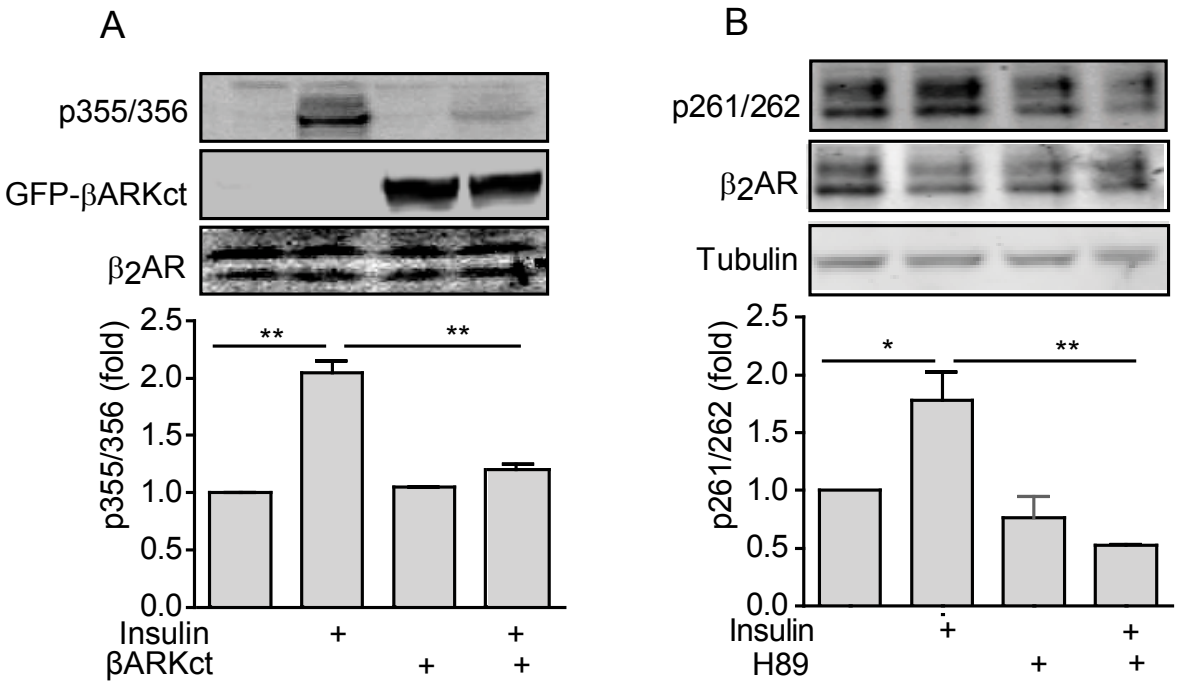
Supplementary Figure 5. A. β_2AR and the GRK2 inhibitor GFP-βARKct were co-expressed in H9c2 cardiomyoblasts before stimulation with insulin (100 nM, 5 min) as indicated. The phosphorylation of β_2AR at serine 355/356 was detected in cell lysates. B. β_2AR was expressed in H9c2 cardiomyoblasts before treatment with insulin (100 nM, 5 min) in the presence or absence of PKA inhibitor H89 (10 mM). The phosphorylation of β_2AR at serine 261/262 was detected in cell lysates. Bar graphs represent mean +/- SD, and N represents the number of cells detected. * < 0.05 and ** < 0.01 by one-way ANOVA followed by Tukey's post hoc test.



Supplementary Figure 6. Adult rat myocytes were treated with insulin (100 nM) and the PDE3 inhibitor cilostamide (cilo) (100 nM) before stimulation with isoproterenol (100 nM). The maximal contractile shortening was measured and plotted. Bar graphs represent mean +/- SD, and N represents the number of cells detected. * < 0.05 and ** < 0.01 by one-way ANOVA followed by Tukey's post hoc test.