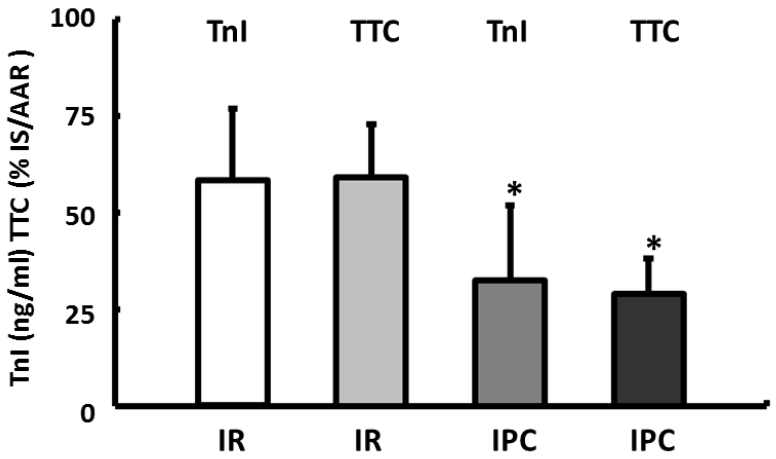
SUPPLEMENTAL MATERIAL

Table S1. Functional and morphological data assessed by echocardiography.

	Sham	IR	IPC	IPoC
FS (%)	59.8±11.9	42.9±10.3*	49.8±13.9	32.2±12.0*
IVSd (mm)	1.65±0.50	1.74±0.32	1.83±0.40	1,52±0.23
LVPWd (mm)	1.62±0.37	1.84±0.22	1.90±0.34	1.86±0.32
LVPWs (mm)	2.53±0.34	2.71±0.44	2.86±0.47	2.61±0.60
LVIDd (mm)	5.25±0.89	5.75±1.28	5.52±0.94	6.03±0.58
LVIDs (mm)	2.69±1.32	3.23±0.90	2.88±1.10	3.43±0.63

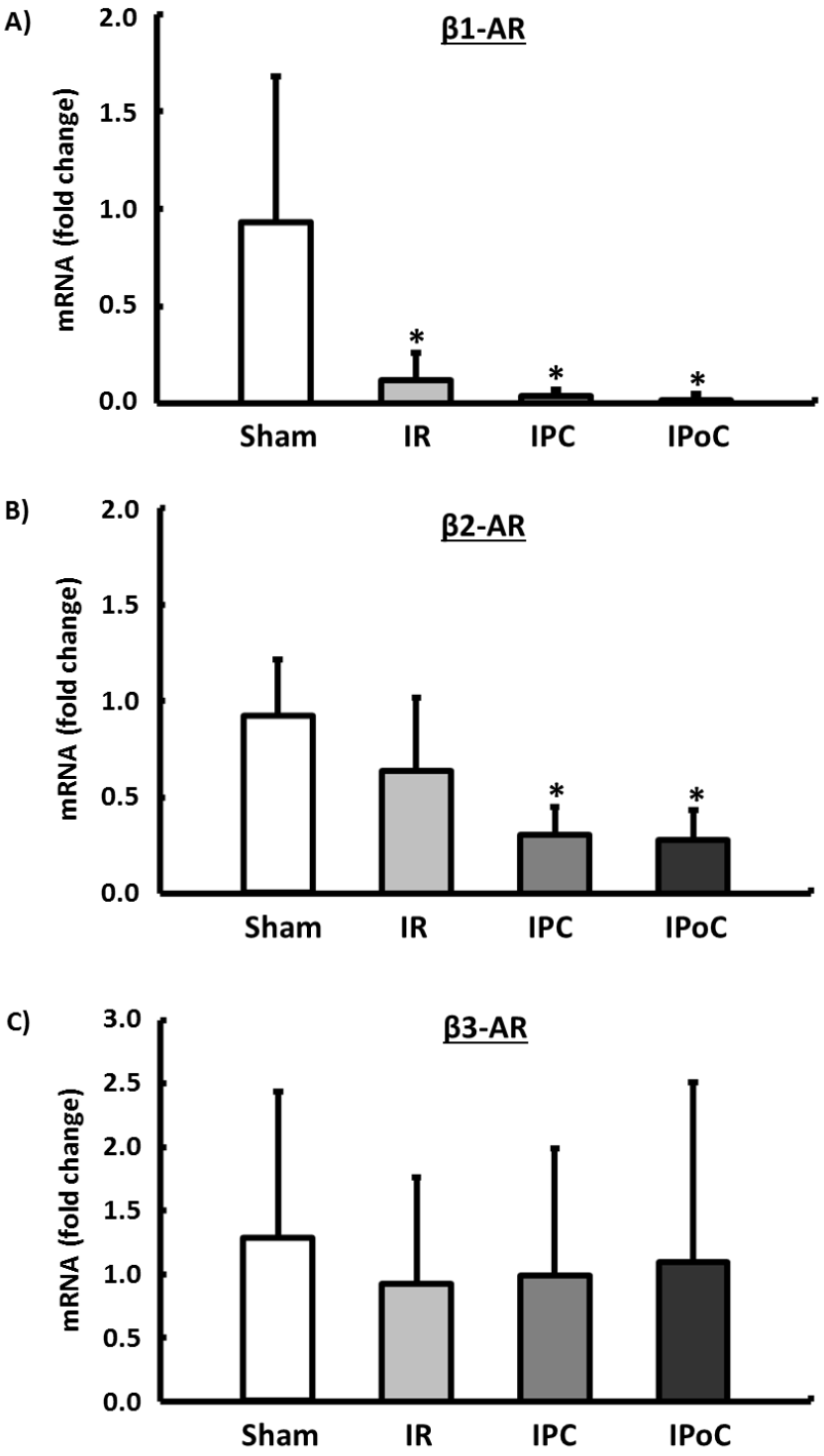
FS (%): fractional shortening, IVSd (mm): end-diastolic interventricular septum thickness, LVPWd (mm): left ventricular posterior wall thickness in diastole, LVPWs (mm): left ventricular posterior wall thickness in systole, LVIDd (mm): left ventricular internal diameter in diastole, LVIDs (mm): left ventricular internal diameter in systole. Data are means ± S.D. of n=6-10 animals. *, p≤0.05 vs. Sham.

Figure S1. The validity of TnI as a marker to determine the infarct size.



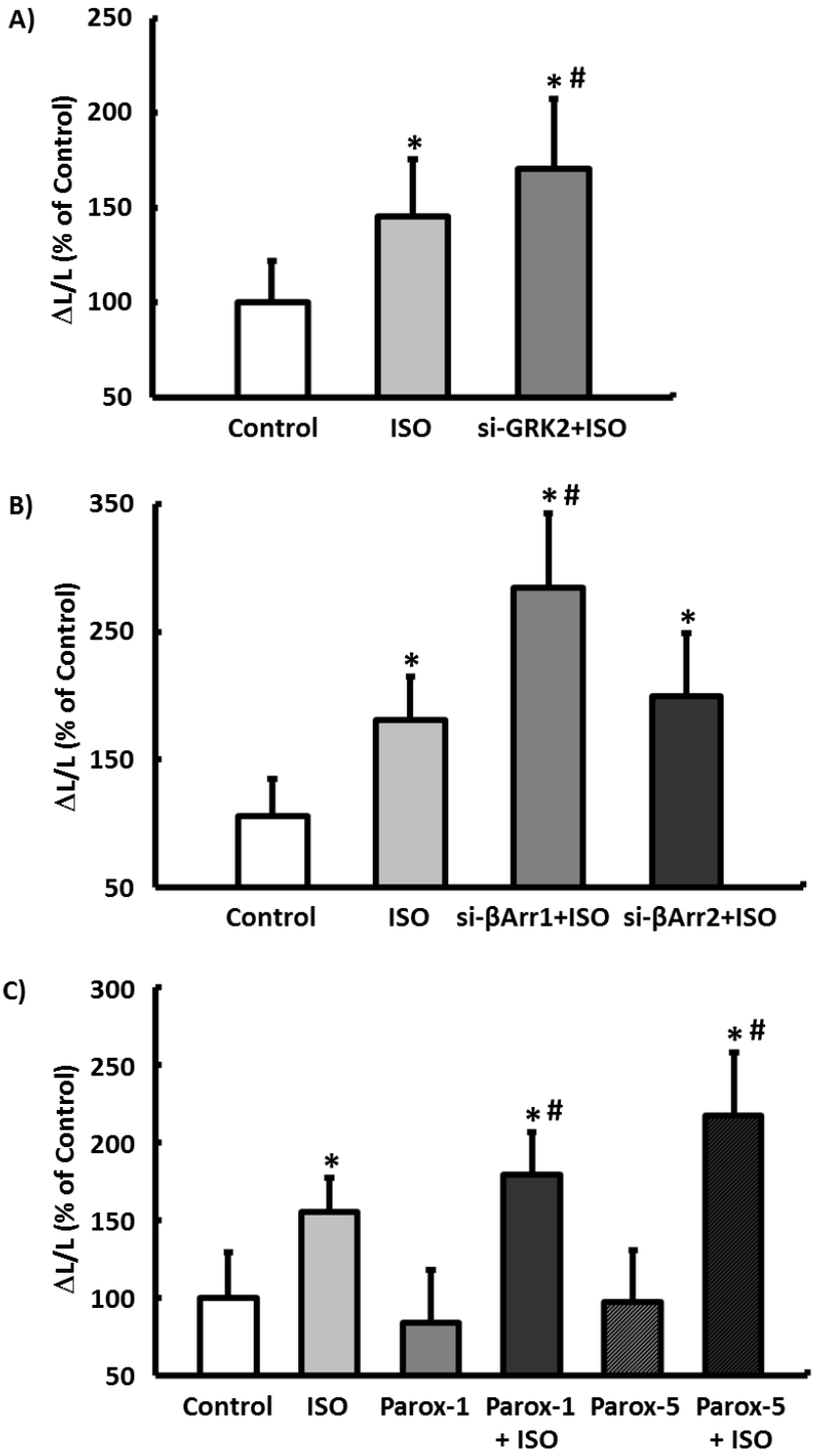
Plasma TnI values that were measured one hour after the start of the reperfusion confirm a reduction of the particular size of the infarct to the same magnitude as determined using TTC staining two hours after the start of the reperfusion. Data are means ± S.D. of n=6 hearts. *, $p \leq 0.05$ vs. IR.

Figure S2. Expression pattern of β-ARs in left ventricular tissue.



A-C) The mRNA expression of the β_1- and β_2-AR subtypes was reduced in the hearts in the IR, IPC and IPoC groups compared to the sham group seven days after the infarction. However, the expression of the β_3-AR remained stable and was unaffected by either IPC or IPoC. Data are means ± S.D. of n=6 hearts. *, p≤0.05 vs. Sham.

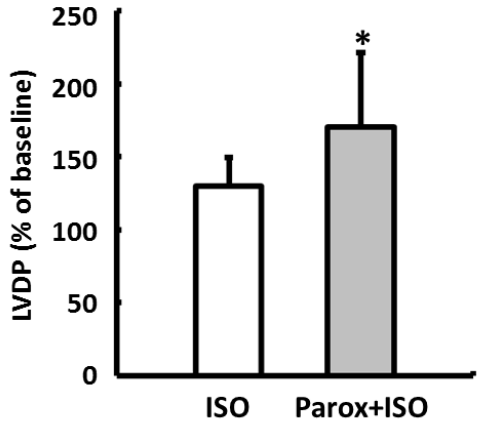
Figure S3. The effect of GRK2 and β-arrestin on the contraction of isolated ventricular cardiomyocytes.



The importance of GRK2 and β-arrestin for the β-AR–mediated positive inotropic response of the myocardium was investigated using a model of left ventricular cardiomyocytes isolated from three-month-old Wistar rats. GRK2, β-arrestin 1

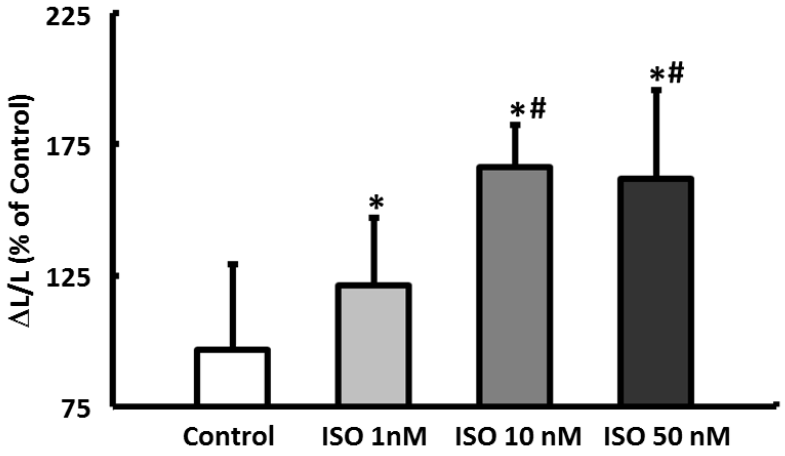
and β-arrestin 2 were selectively and effectively downregulated by transfection with siRNA over 24 hours (Figure S6A,B). The load free cell shortening before and after stimulation with ISO was then determined. **A)** In untreated cardiomyocytes ISO improved basal cell shortening by 45±9%. Cardiomyocytes in which the GRK2 was previously downregulated were sensitised to the β-adrenergic stimulus and showed an improvement in the cell shortening of about 70 ±15%. **B)** Specifically knocking down the β-arrestin 1 isoform improved the ISO-induced cell shortening by 57±12% while the β-adrenergic stimulation after successful knockdown of β-arrestin 2 did not differ significantly from ISO treated cells. Transfection of the cardiomyocytes with nonsense siRNA over 24 hours did not have any effect on cell shortening. **C)** The use of GRK2-specific siRNA was supplemented in the cell culture model described above by application of the selective GRK2 inhibitor paroxetine. After a 15-minute pre-incubation with paroxetine, the ISO-induced increase in cell shortening increased in a concentration-dependent manner from 55±8% to 79±12% (1 µM) and to 117 ±22% (5 µM). Data are means± S.D. of n=3 hearts. *, p≤0.05 vs. Control, #, p≤0.05 vs. ISO.

Figure S4. The functional relevance of GRK2 in ex-vivo perfused hearts.



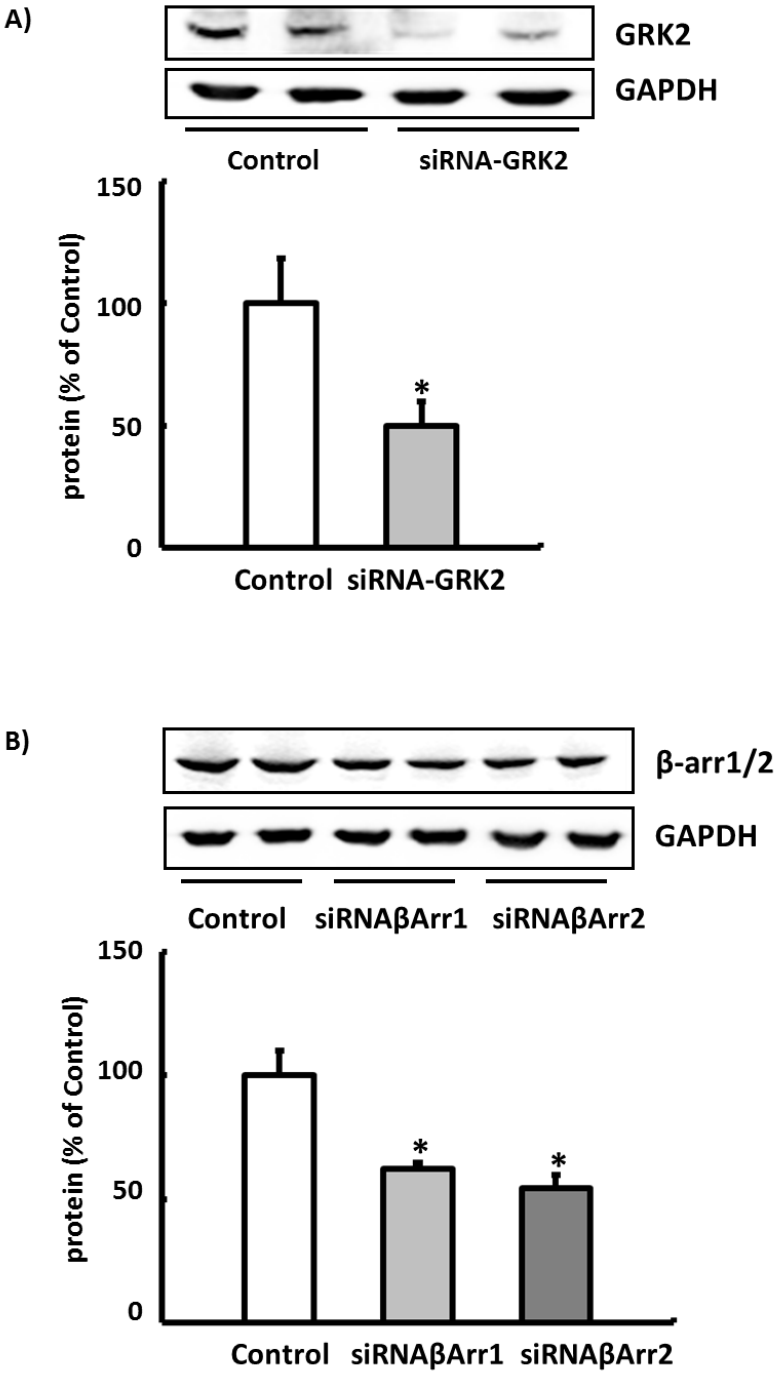
Using the Langendorff technique, rat hearts were initially perfused for 10 min with paroxetine and then stimulated for a further 5 min with ISO in the presence of paroxetine. Hearts that had not had their GRK2 inhibited responded to the β-adrenergic stimulation with an increase in the LVDP of 30±20% while the LVDP of hearts perfused with paroxetine increased by 71 ±51%. Data are means ± S.D. of n=4 hearts. *, p≤0.05 vs. ISO.

Figure S5. Concentration – response curves to isoprenaline (ISO) in isolated cardiomyocytes.



Left ventricular cardiomyocytes isolated from three-month-old Wistar rats were used to determine the ISO concentration used in all experiments to measure the β-AR–mediated positive inotropic response. The ISO-induced increase in cell shortening increased in a concentration-dependent, however, concentrations above 10 nM had no further effects. Data are means ± S.D. of n=3 hearts. *, $p \leq 0.05$ vs. Control, #, $p \leq 0.05$ vs. ISO 1 nM.

Figure S6. Western blot analysis to assess the efficiency of the siRNA-mediated knockdown.



A-B) Representative immunoblots and densitometric analysis: the expression of GRK2 (A), β-arrestin1 and β-arrestin2 (B) was analysed 24 hours after transfection of isolated cardiomyocytes. Data are means ± S.D. of n=3 hearts. *, p≤0.05 vs. Control.