SUPPLEMENTARY INFORMATION

MATERIALS & METHODS

Materials: Monoclonal antibodies to cleaved Y^{397}-FAK, Y^{402}-Pyk_2, Y^{118}-paxillin, phospho-$ERK_{1/2}$, phospho-p38 MAPK, phospho-$JNK_{1/2}$ and non-phospho-PTEN (antibody that detects endogenous levels of PTEN only when dephosphorylated at S^{380}, T^{382} and T^{383}) used for Western blot analysis were obtained from Cell Signaling. Pyk_2, paxillin and PTEN monoclonal antibodies were from BD Biosciences. β-actin monoclonal antibody was from Sigma Aldrich and FAK, $ERK_{1/2}$, p38 MAP kinase, and $JNK_{1/2}$ antibodies were from Santa Cruz Biotechnology. All other chemicals were from standard suppliers.

Collagen Analysis. After hemodynamic measurements, the heart was arrested in diastole and the LV inclusive of the septum and right ventricle was dissected and weighed. LV was then sectioned serially into five rings perpendicular to the longitudinal axis of the heart, fixed in formalin, then embedded in paraffin. Light microscopic examination (400×) was performed on 5 μm sections stained with picrosirius red (PSR) to measure the percent of interstitial collagen present in the myocardium as previously described [1,2]. The stained sections were digitized and analyzed (Bioquant) to determine the percent area of collagen staining from 30 randomly chosen fields within the myocardial section. Collagen volume fraction was calculated as the sum of all areas stained positive for Sirius red divided by the sum of all myocardial areas in each rat. Areas of the myocardium including vessels, scar tissue, or slide preparation artifacts were excluded from collagen volume fraction analysis.

Neonatal rat cardiomyocyte (NRCM) isolation: Myocytes were isolated from the ventricles of neonatal Sprague-Dawley rats by collagenase digestion as previously described [3]. The isolation

efficiency was approximately 3-5 x10^6 cells/heart. Viability as determined by trypan blue exclusion test was approximately 80-90%. After 30 minutes of preplating (to eliminate non-myocyte cell contamination), myocytes were plated on collagen precoated dishes at a density of 160,000/cm^2 in 10% fetal bovine serum DMEM supplemented with 1 mmol/liter L-glutamine, antibiotic/antimycotic solution, and 100 μmol/L 5-bromo-2-deoxyuridine (BrdU). Plating efficiency which corresponds to the number of cells that was attached to the culture plate was about 60% and the myocytes display spontaneous contractile activity within 24 hrs of plating.

Expression of adenoviral vectors: Production of recombinant adenoviruses carrying wild-type FAK and Y^{397}-FAK were described elsewhere [3]. Wild-type β_1-AR was provided by Dr. Koch WJ (Thomas Jefferson University), wild-type PTEN was provided by Dr. Sussman MR (San Diego State University) and dominant negative PTEN, a catalytically inactive PTEN in which cysteine 124 has been mutated to serine (C/S), was provided by Dr. Kontos CD (Duke University). Adenoviral vectors were purified using a kit from Virapur and titrated using BD Adeno-X rapid titer kit (BD Bioscience). After 24 hrs of plating, NRCMs were infected at the indicated plaque-forming units (pfu)/cell in DMEM for 2 hrs, then 5% fetal bovine serum DMEM was added, and cells were incubated for an additional 24 hrs. Serum-free DMEM/F-12 medium was changed 1 hr before the start of the experiments.

Immunoprecipitation and immunoblot analysis: Extraction of proteins from heart tissue samples or cultured cells was performed as described previously [3]. Briefly, lysates were cleared by centrifugation at 12,000 rpm and the supernatants (800 μg of protein/ml) were subjected to immunoprecipitation with corresponding antibodies. After overnight incubation at 4 °C, protein A- or G-agarose beads were added and left for an additional 3 hrs. Immunocomplexes were then subjected to SDS-PAGE followed by Western blot analysis according to methods published

previously or to the manufacturer's instructions [3]. Each panel in each figure represents results from a single gel exposed for a uniform duration, with bands detected by enhanced chemiluminescence and quantified by laser scanning densitometry.

Terminal deoxynucleotidyl transferase (TdT) and tropomyosin immunolabeling. Three sections from each LV cut perpendicularly to the major axis of the heart were sampled. TdT assay was performed using kit from Promega. Positive myocytes were counted throughout the LV and were expressed as percentage of the total number of cardiomyocyte nuclei as determined by DAPI (Molecular probes) and tropomyosin (Sigma) staining.

Caspase-3 Assay. Caspase 3 activity was measured with CaspACE assay system (Promega, Madison, WI). In brief, LV lysates were prepared by dounce homogenization in lysis buffer provided with the kit. The lysates were centrifuged at 15,000g for 20 minutes at 4 ^{o}C, and the supernatants containing 100 μg protein were used for caspase-3 activity assay using specific fluorogenic conjugated substrate MCA-Val-Asp-Gln-Met-Asp-Gly-Trp-Lys-(DNP)-NH_2.

Apoptotic cell death ELISA. Cell death detection ELISA kit (Roche Applied Science, Indianapolis, IN) was used to quantitatively determine the apoptotic DNA fragmentation by measuring the cytosolic histone-associated mono- and oligo-nucleosomes fragments associated with apoptotic cell death.

REFERNCES

[1] Kolpakov MA, Seqqat R, Rafiq K, Xi H, Margulies KB, Libonati JR, et al. Pleiotropic effects of neutrophils on myocyte apoptosis and left ventricular remodeling during early volume overload. J Mol Cell Cardiol. 2009;47(5):634-45.

[2] Chancey AL, Brower GL, Peterson JT, Janicki JS. Effects of matrix metalloproteinase inhibition on ventricular remodeling due to volume overload. Circulation. 2002;105(16):1983-8.

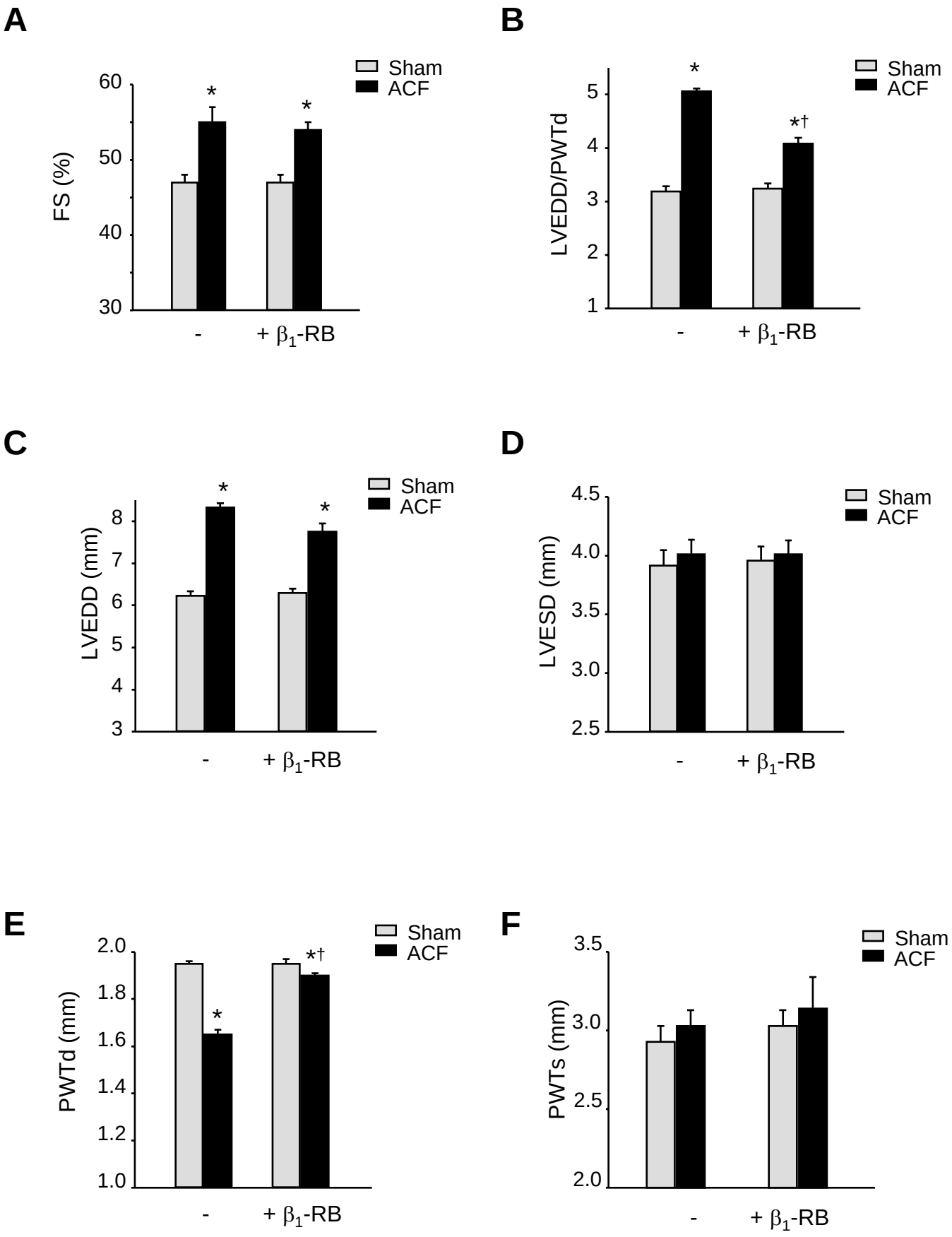
[3] Rafiq K, Kolpakov MA, Abdelfettah M, Streblow DN, Hassid A, Dell'Italia LJ, et al. Role of protein-tyrosine phosphatase SHP2 in focal adhesion kinase down-regulation during neutrophil cathepsin G-induced cardiomyocytes anoikis. J Biol Chem. 2006;281(28):19781-92.

Supplemental Table 1: LV hemodynamics in 2 days sham and ACF treated with vehicle or β1-RB.

	Sham (n=6)	**ACF (n=6)**	**Sham + β_1-RB (n=6)**	**ACF + β_1-RB (n=6)**
HR (b.p.m)	380 ±10	420 ±14*	365 ±7	378 ±10*†
MAP (mmHg)	86 ±4	72 ±4	82 ±3	71 ±3
LVESP (mmHg)	66 ±4	56 ±3	64 ±3	57 ±2
LVEDP (mmHg)	3 ±1	13 ±2*	3 ±1	8 ±2*
+dp/dt (mmHg/s)	8231 ±423	8259 ±325	7985 ±200	6971 ±438*†
-dp/dt (mmHg/s)	-7197 ±303	-6032 ±387*	-7212 ±416	-5661 ±312*†
LVW/BW (mg/g)	2.3 ±0.1	2.6 ±0.1*	2.5 ±0.1	2.8 ±0.1*

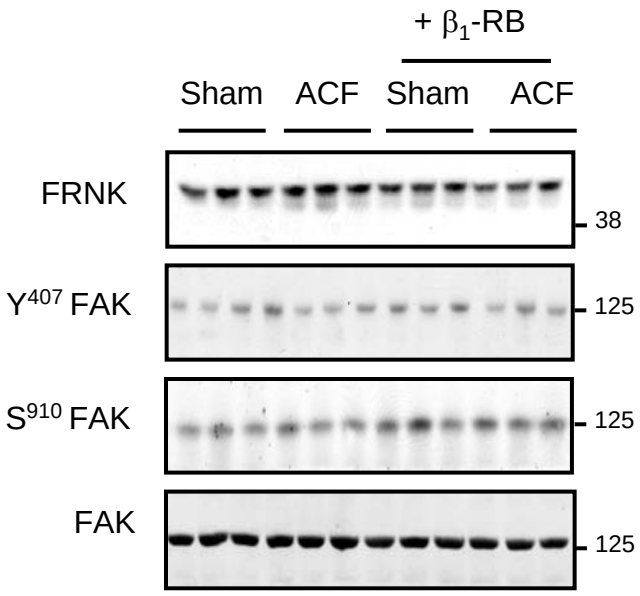
Values are mean ±SEM. HR, heart rate; MAP, mean arterial pressure; LVESP, LV end systolic pressure; LVEDP, LV end diastolic pressure, LVW, LV weight. *$P< 0.05$ vs. 2d sham; $^{\dagger}P< 0.05$ vs. 2d ACF.

Supplemental Figure S1: Effect of β_1-RB on cardiac remodeling induced by acute ACF. Indices of LV remodeling in sham or ACF rats treated with vehicle or β_1-RB for 2 days. (A) LV fractional shortening (FS). (B) LV end diastolic dimension (LVEDD)/posterior wall thickness (PWT)d. (C) LVEDD. (D) LV end systolic dimension (LVESD). (E) PWTd. (F) PWTs. Values are mean ± SEM. (n=6 for each experimental group). *P<0.05 vs. sham, and $^{\dagger}P$<0.01 vs. ACF.



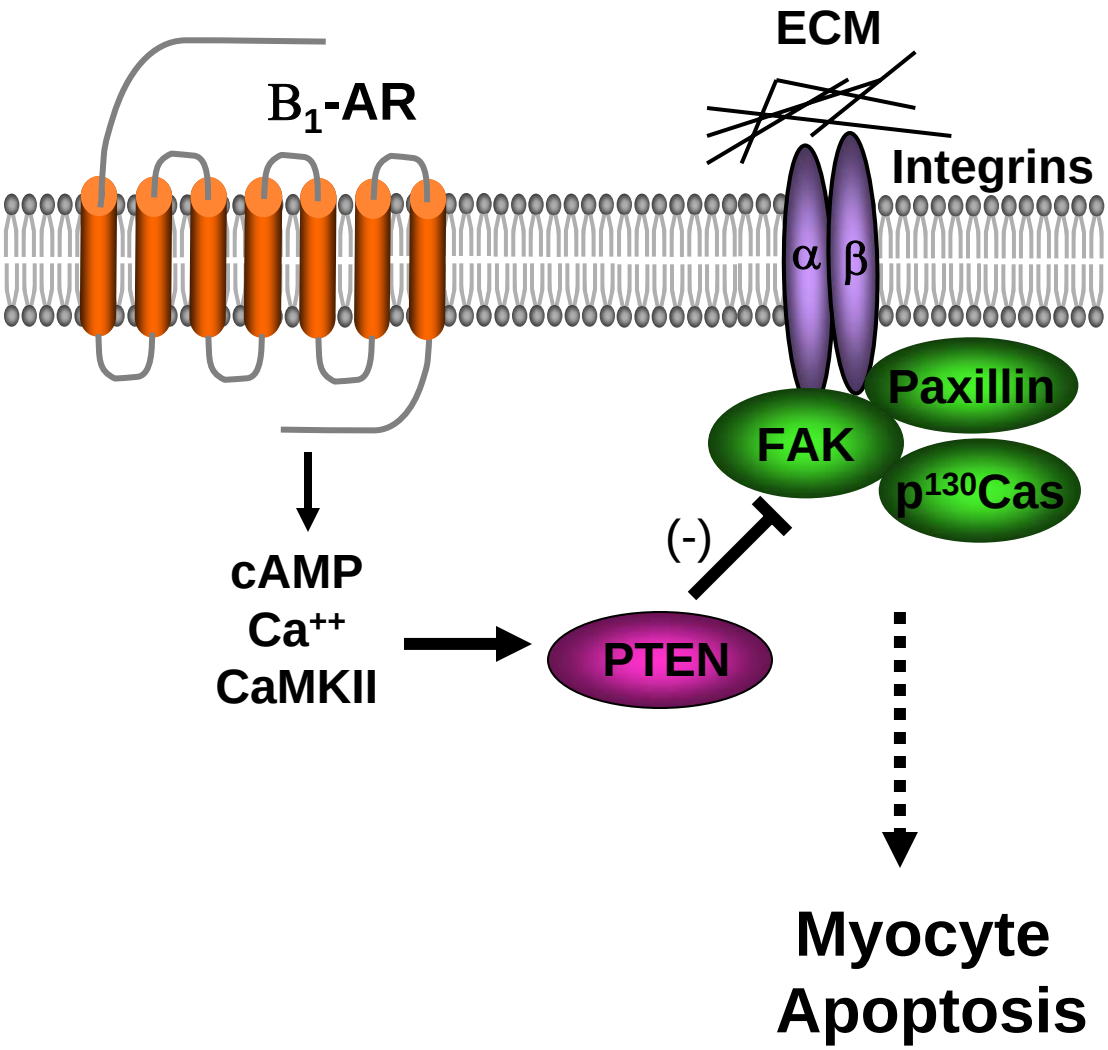
Supplemental Figure S1

Supplemental Figure S2: Acute ACF does not modulate FRNK expression, Y^{407} FAK or S^{910} FAK phosphorylation. LV lysates from sham or ACF animals treated with vehicle or β_1-RB for 2 days were immunoblotted with FRNK, Y^{407} FAK, S^{910} FAK FAK and FAK with each lane from a single gel exposed for the same duration.



Supplemental Figure S2

Supplemental Figure S3: Novel mechanisms whereby ß1-ARs regulates FA signaling downregulation and myocyte death



Supplemental Figure S3