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	WT baseline	WT TAC	β3-/- baseline	β3-/- TAC
ESV, μL	11 ± 1	12 ± 1	11 ± 1	17 ± 2*
CO, mL/min	11 ± 2	11 ± 2	11 ± 2	9 ± 1*
WTh, mm	0.75 ± 0.11	1.02 ± 0.13*	0.78 ± 0.14	0.89 ± 0.12

WT=wild type, TAC=transverse aortic constriction, ESV=LV end systolic volume, CO=LV cardiac output=LV stroke volume x heart rate), WTh=LV wall thickness, LV=left ventricle.

Table S1. Echocardiographic measurements of WT and β_3 (-/-) mice after 4 wk TAC. Age-matched WT mice and β_3 (-/-) mice were subjected to 4 wk TAC (n=4/group). Echocardiographic measurements were made prior to banding and then again 4 wk later prior to sacrifice as described in 'Materials and Methods'. Data are reported as mean ± SEM. *=$p<0.05$ vs. corresponding baseline.

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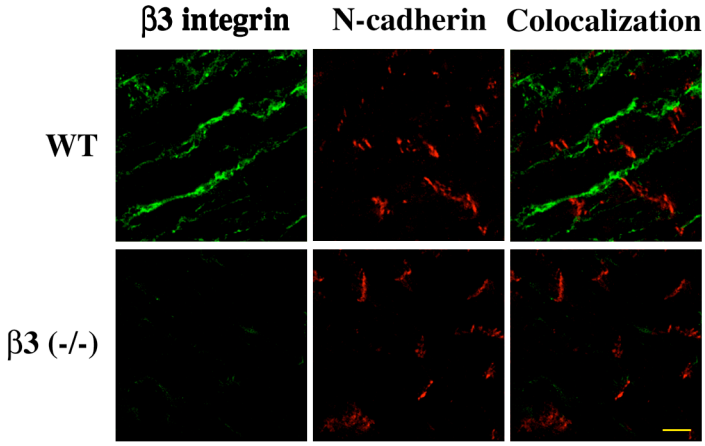

Figure S1. β_3 integrin distribution in WT and β_3 (-/-) mice hearts. LV tissue sections from age-matched WT mice and β_3 (-/-) mice were stained with anti-β_3 integrin (green) and N-cadherin antibodies. Scale bar=10 μM.

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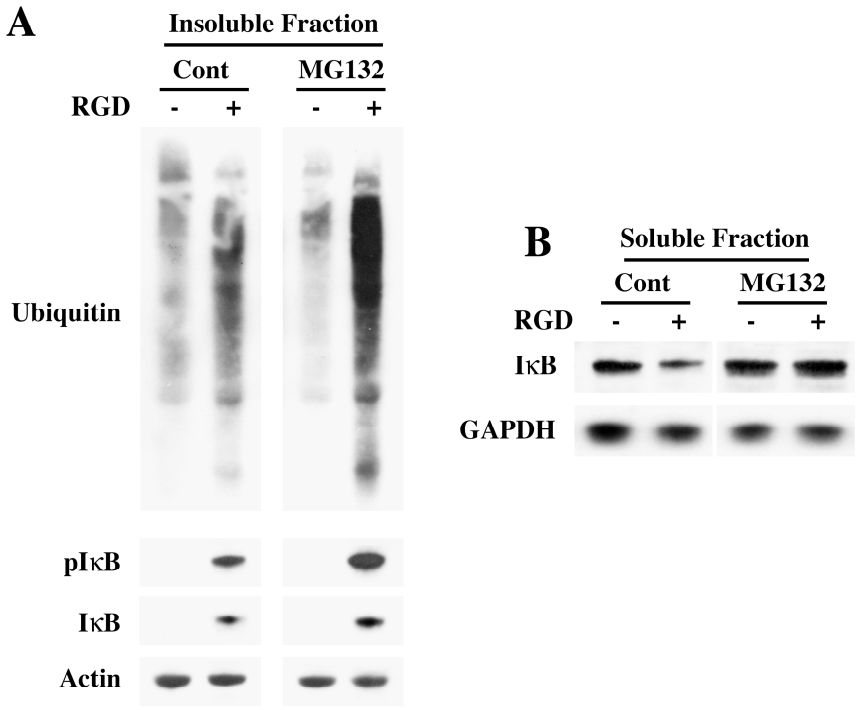

Figure S2. Integrin activation by RGD causes ubiquitin-mediated degradation of IκB. Plated feline cardiomyocytes were pretreated with ± MG132 (10 μM) for 30 min prior to overlaying collagen ± RGD (9 mM) for 1 h. Cardiomyocytes were extracted from collagen via collagenase digestion, and Triton-insoluble (A) and -soluble (B) fractions were prepared. Western blots were performed on insoluble and soluble fractions with anti-ubiquitin, -IκB, and -pIκB (S32/S36) antibodies; loading was normalized to actin and GAPDH, respectively.

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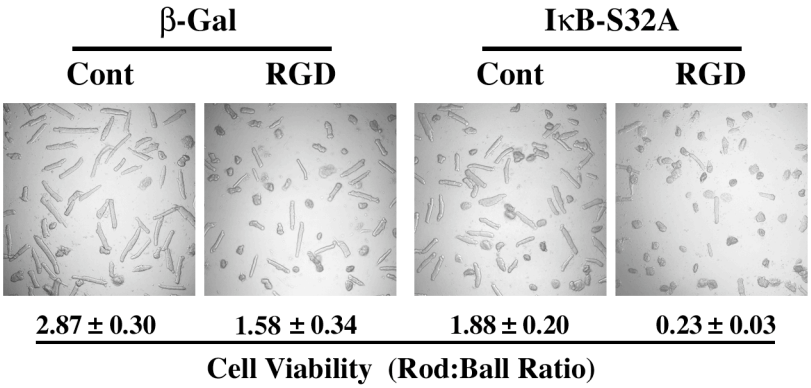

Figure S3. Inhibiting NFκB during integrin stimulation by RGD causes cell death of cardiomyocytes. Murine cardiomyocytes were infected with either Ad-β-Gal (MOI 150) or Ad-IκB-S32A (MOI 150) for 36 h before stimulating with 6 mM RGD in three-dimensional collagen environment. To emphasize the importance of NFκB survival transcription, stimulation time with RGD was extended for 2 h. Representative light microscope images of cardiomyocytes in collagen gel are shown with corresponding cell viability calculations, represented by the ratio of healthy rod-shaped cardiomyocytes (rods) vs. dead cells (balls) shown below for each group from duplicate experiments. At least 500 nuclei were counted from 10 fields per each experimental condition. Data represent mean ± SEM.

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Supplementary Materials and Methods

Reagents

Additional antibodies used in supplementary data at 1:1000 dilution unless indicated. Anti-β_3 integrin antibody (F11 clone) for confocal immunostaining was obtained from (BD Biosciences, Sand Diego , CA).

Preparation of Cell and Tissue Lysates

Triton X-100 soluble and insoluble fractions were prepared from fresh tissue and cell samples as established previously (1, 2). Briefly, 50 mg tissue or 100,000 cells were homogenized in ice cold Triton X-100 extraction buffer (1 ml or 120 μl respectively); (final concentration 100 mM Tris-HCl, pH 7.4, 10 mM EGTA, 2% Triton X-100, 0.5 mM phenylmethylsulfonyl fluoride, 10 μg/ml leupeptin, 10 μg/ml aprotinin, 2 μg/ml pepstatin, 2 μM E-64, and 200 μg/ml p-aminobenzamidine, 1 μM okadaic acid, 10 mM β-glycerophosphate, and 1 mM sodium orthovanadate). The homogenate was centrifuged at 14,000 x g to obtain a supernatant and pellet. The pellet was reextracted with extraction buffer to remove any remaining detergent-soluble proteins, pelleted, resuspended, and boiled in 0.5 ml (120 μl for cells) of 1X SDS sample buffer to obtain the Triton X-100 insoluble fraction, which contains cytoskeletal-associated proteins. The supernatant of tissue samples was spun at 100,000 x g for 2.5 h at 4ºC, forming a pellet of membrane skeleton proteins. The membrane skeletal protein fraction showed no changes in Ub and therefore was not used for this study. Furthermore, this step was not done with cell samples, which do not produce a pellet at this step. The supernatants from the high-

speed spin (tissue) and low spin (cell) were then mixed with equal volume 2X SDS sample buffer and used for the analyses of Triton X-100 soluble proteins.

Mouse cell isolation

We used a modified procedure (3) for adult mouse cell isolation. Briefly, mice (25–35 g) were anesthetized using isoflurane and heparinized (200 IU, i.p.). The heart was quickly removed from the chest and the aorta retrogradely perfused at constant pressure (70 mm Hg) at 37ºC for 5 min with a calcium-free Krebs-HEPES buffer containing (in mM) 113 NaCl, 4.7 KCl, 0.6 KH_2PO_4, 0.6 $NaHPO_4$, 1.2 Mg_2SO_4, 0.032 phenol red, 12 $NaHCO_3$, 10 $KHCO_3$, 10 HEPES, 30 taurine, 10 2,3-butanedione monoxime (BDM) and 5.5 glucose at pH 7.4 and then in enzyme buffer containing Liberase blendzyme (0.25 mg/mL) (Roche), trypsin (0.14 mg/mL) and calcium (12.5 μM). The heart was removed from the cannula when palpably flaccid at 8-10 min. The LV including the septum was separated, minced and gently agitated to disperse cardiomyocytes in the HEPES buffer containing 5% fetal bovine serum for 5 min and then suspended in HEPES buffer with gradually increasing calcium concentrations in five steps from 12.5 to 62 to 112 to 212 to 500 to 1000 μM at room temperature. After gravity sedimentation, the cardiomyocytes were resuspended in Modified Eagle's Medium (MEM) with Hank's balanced salt solution containing 1.3 mM calcium supplemented with 0.1 mg/mL BSA, 1% ITS, 10 mM BDM and penicillin (100 U/mL) at 37ºC in a 5% CO_2 incubator.

Primers for RT-PCR

Primers used were: luciferase: Forward-CATCTTCGACGCAGGTGTC and Reverse-GACTGGCGACGTAATCCAC (4); 18S: Forward-TATGGTTCCTTTGGTCGCTC and Reverse-GGTTGGTTTTGATCTGATAAATG; cIAP1: Forward-AGGTCATTGCTGGCGTTC

and Reverse-AAGGGACAAGTCTTTCTCTGG (5); and GAPDH: Forward-AGGTCATCCCAGAGCTGAAC and Reverse-CCTGCTTCACCACCTTCTTG (6).

Preparation of nuclear fraction from tissue

Nuclear isolation was performed as previously described (7) with Nuclear and Cytoplasmic Extraction Kit (NE-PER, Pierce). Briefly, 50 mg ventricular tissue was homogenized using Tekmar Tissuemizer for 45 s in 1 ml PBS with protease and phosphatase inhibitor cocktails (Sigma). The homogenate was centrifuged at 1000 x *g* for 8 min. To the pellet, 500 µl of cytoplasmic extraction buffer I (CERI) with inhibitors was added. After suspending the pellet, the solution was incubated on ice for 15 min, followed by the addition of 27 µl CERII. The contents were incubated on ice for 2 min, centrifuged at 16,000 x *g* for 15 min, and the supernatant (cytoplasmic proteins) was mixed with equal volume of 2X SDS-sample buffer. The pellet was washed with PBS, suspended in 250 µl of nuclear extraction buffer with inhibitors and incubated on ice for 40 min with occasional vortexing. After centrifugation at 16,000 x *g* for 15 min, the supernatant, consisting of nuclear proteins, was mixed with equal volume of 2X SDS sample buffer.

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