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Supplementary Materials for

β-Arrestin–Biased Agonism of the Angiotensin Receptor Induced by Mechanical Stress

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The PDF file includes:

		Treatment			
Tagged Protein		Unstimulated	AngiotensinII	Isoproterenol	Stretch
AT1R-HA (N=4)		2.5 ± 0.2%	88.5 ± 2.7%*	-	77.0 ± 4.6%*
β1AR-flag (N=4)		1.7 ± 0.5%	-	83.7 ± 5.5%*	1.9 ± 0.4%
βarr1-YFP (N=5)		1.8 ± 0.3%	82.0 ± 2.9%*	-	66.0 ± 2.2%*
βarr2-YFP (N=5)		1.5 ± 0.4%	84.0 ± 2.4%*	-	66.5 ± 4.3%*
EGFR -GFP (N=4)	control siRNA	2.3 ± 0.2%	77.6 ± 3.5%*,†	-	77.7 ±5.5%*,†
	βarr1/2 siRNA	2.5 ± 0.6%	12.0 ± 2.7%		15.0 ± 0.5%

Table S1**.** Quantification of receptor internalization and β-arrestin translocation. Internalization was detected by the presence of intracellular aggregates. Data shown is for 4-5 independent experiments. In each experiment, 80-100 cells were counted for a given treatment. *, P<0.001 vs. unstimulated of the same fluorescent tagged protein. †, P<0.001 control siRNA vs. βarr1/2 siRNA with AngII or stretch conditions. Angiotensin II (1 μM) and Isoproterenol (1 μM).

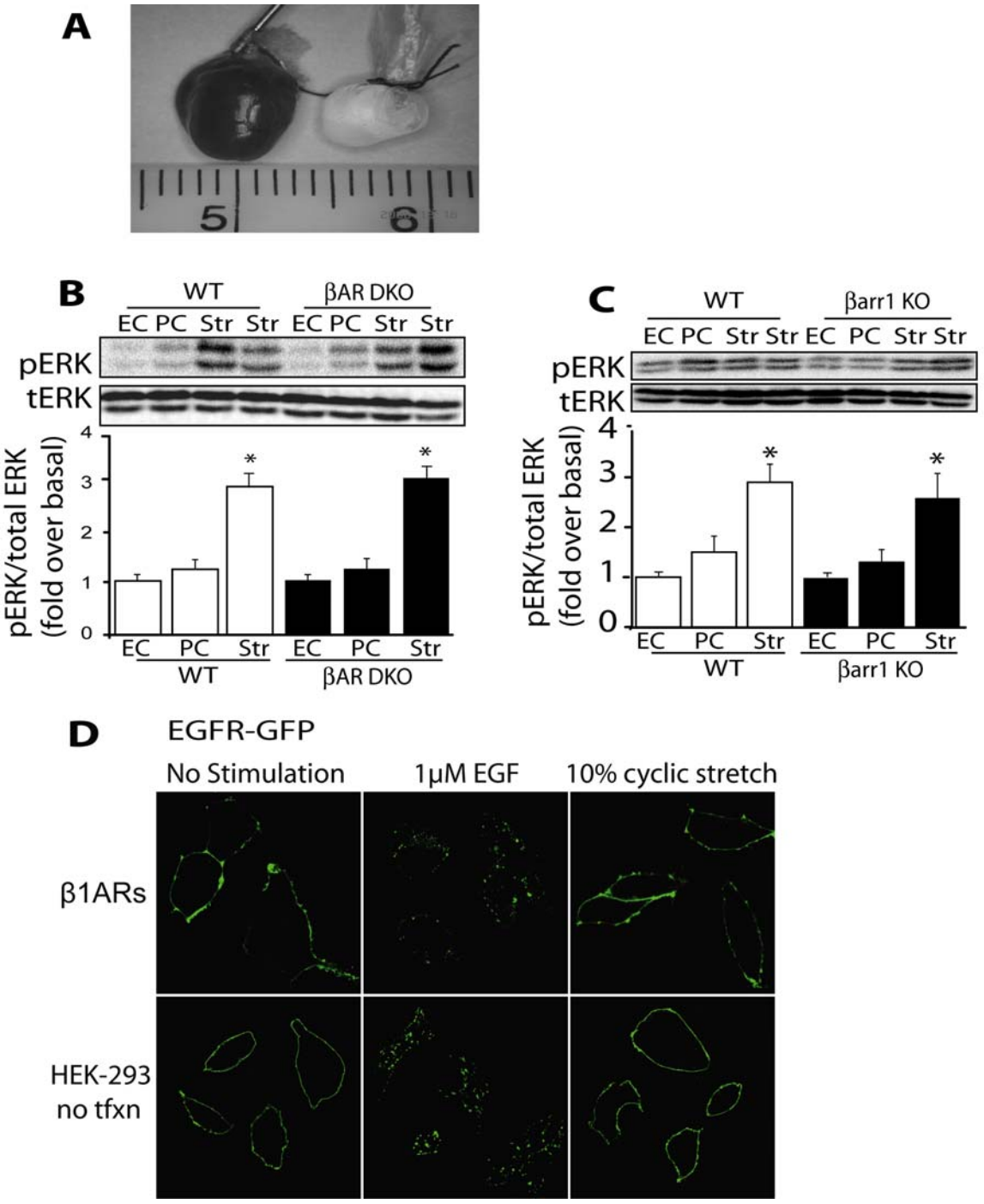

Fig. S1: βARs are not involved in stretch-mediated signaling. **(A)** A hand-made balloon was placed into the left ventricle through the mitral valve and inflated to achieve diastolic stretch on the heart. **(B** and **C)** Mechanical stretch-mediated ERK phosphorylation does not require βARs or β-arrestin1. **(D)** β1ARs are not involved in mechanical stretch-mediated signaling as seen by the absence of EGFR-GFP internalization into intracellular aggregates, indicating lack of β1AR- mediated EGFR transactivation. No tfxn: No transfection.

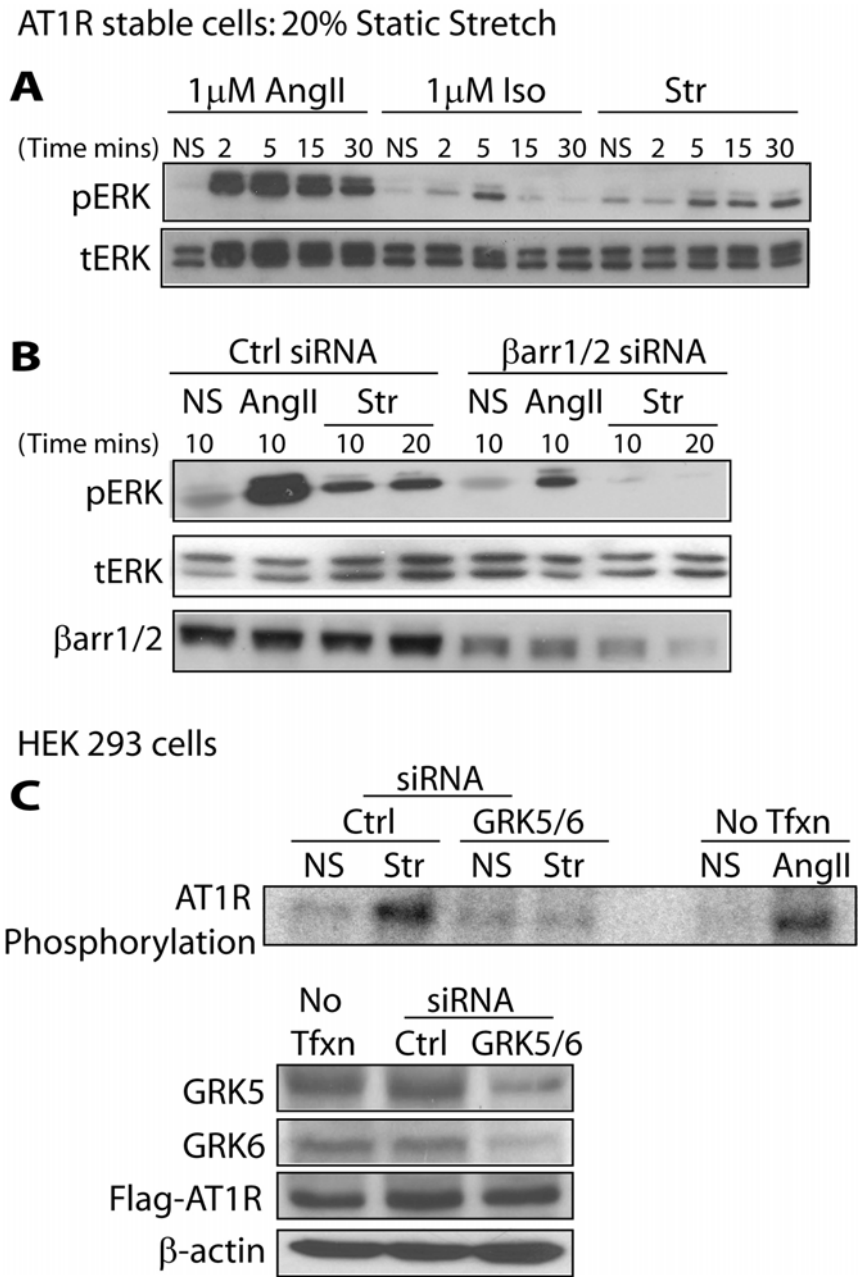

Fig. S2. Time course of stretch-mediated ERK phosphorylation. **(A)** Cells stably expressing the AT1R were stimulated with 1 μM AngII, 1 μM isoproterenol, or 20% static stretch for various time points. Lysates were immunoblotted for phosphorylated ERK. N=4 independent experiments with 2-3 million cells per experiment. **(B)** AT1R stable cells were transfected with control or βarr1/2 siRNA and stimulated with 1 μM AngII or 20% static stretch for 10 or 20 minutes. Cell lysates were blotted for phosphorylated ERK. N=3 separate experiments containing 2-3 million cells per experiment. **(C)** Cells were transfected with the indicated siRNAs and a plasmid containing the PKC phosphorylation defective AT1R mutant. After stimulation with 1 μM AngII or mechanical stretch for 10 min, receptor phosphorylation was visualized. No Tfxn: no transfection.

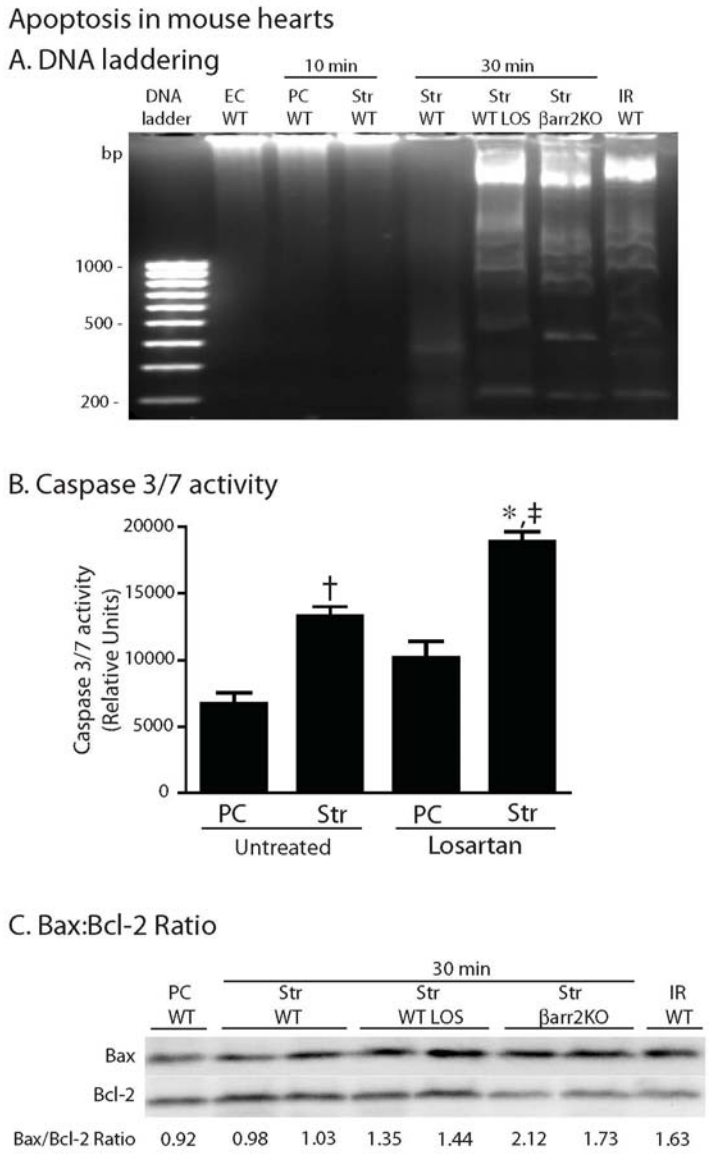

Fig. S3. Increased markers of apoptosis in mouse hearts treated with losartan. **(A)** After the indicated treatments, DNA from mouse hearts was isolated and separated by agarose gel electrophoresis. DNA fragmentation is evident as multimers of small sized fragments due to cleavage of the DNA. Ischemia and reperfusion injury (IR), which was 30 min ischemia followed by 45 min reperfusion, was the positive control for apoptosis. **(B)** Caspase 3/7 activity was determined in extracts hearts treated or not with losartan and subjected to mechanical stretch. Capase3/7 activity is increased in the presence of losartan. N=4 hearts in each group. Data are presented as mean±SE. †, P=0.001, untreated Str compared to untreated PC. *P=0.005 Losartan Str compared to untreated Str. ‡, P<0.0001, Losartan Str compared to Losartan PC. **(C)** Mechanical stretch in the presence of losartan increases the ratio of Bax/Bcl-2 protein abundance. LOS: losartan.