Cardiac hypertrophy is initiated as an adaptive response to sustained overload but progresses pathologically as heart failure ensues. Here we report that genetic loss of APJ, a G-protein-coupled receptor, confers resistance to chronic pressure overload by markedly reducing myocardial hypertrophy and heart failure. In contrast, mice lacking apelin (the endogenous APJ ligand) remain sensitive, suggesting an apelin-independent function of APJ. Freshly isolated APJ-null cardiomyocytes exhibit an attenuated response to stretch, indicating that APJ is a mechanosensor. Activation of APJ by stretch increases cardiomyocyte cell size and induces molecular markers of hypertrophy. Whereas apelin stimulates APJ to activate Gαi and elicits a protective response, stretch signals in an APJ-dependent, G-protein-independent fashion to induce hypertrophy. Stretch-mediated hypertrophy is prevented by knockdown of β-arrestins or by pharmacological doses of apelin acting through Gαi. Taken together, our data indicate that APJ is a bifunctional receptor for both mechanical stretch and the endogenous peptide apelin. By sensing the balance between these stimuli, APJ occupies a pivotal point linking sustained overload to cardiomyocyte hypertrophy.

G-protein-coupled receptors (GPCRs) have been widely implicated in the control of cardiac function. These receptors couple to heterotrimeric GTP-binding proteins of the Gαi, Gαq, Gαq/11, and Gβγ families, and transduce the GPCR signal to intracellular targets. Numerous studies have linked Gαq to increased contractility, Gαq/11 to pathological hypertrophy, and Gβγ to cardioprotection. APJ is a GPCR identified as the receptor for the adipokine apelin. Apelin-activated APJ signals through Gαi, exerting a positive effect on cardiac contractility and a vasodilator activity that counteracts angiotensin II-induced atheroma. Apelin administration blunts progression to hypertrophy (Supplementary Fig. 1 and Supplementary Tables 2 and 3) and apelin-KO mice show susceptibility to heart failure (also see Supplementary Fig. 1 and Supplementary Table 1). Thus, apelin and its receptor APJ are emerging as potential therapeutic targets.

We examined the response of APJ knockout (APJ-KO) mice to sustained pressure overload by transaortic constriction (TAC). Although deletion of APJ resulted in some prenatal lethality, all viable APJ-KO mice showed normal adult appearance and cardiovascular parameters at baseline (Supplementary Table 4). However, APJ-null animals were resistant to the pathological hypertrophic response to TAC (Fig. 1a–d) observed both in wild type (WT) and in apelin-KO mice (Supplementary Fig. 1g–i). APJ-KO mice responded to TAC by initially increasing cardiac mass, but the maladaptive progression to dilated ventricular hypertrophy was blunted shortly after injury (Supplementary Table 4). The protective effect persisted long-term (Fig. 1a, b, g, h) in all parameters measured, including diminished cardiomyocyte size (Fig. 1c, d), reduced fibrosis (Fig. 1e, f), sustained cardiac contractility (Fig. 1g) relative to WT and apelin-KO mice (Supplementary Tables 1 and 4) and reduced heart weight/body weight ratio (Fig. 1h). Baseline cardiac contractility, measured as percentage fractional shortening, was approximately 38% across genotypes. After 90 days of TAC, percentage fractional shortening decreased to 22 ± 2% in WT, 23 ± 1% in apelin KO mice, but remained at 34 ± 2% in APJ-KO mice (P = 0.01 between APJ-KO and WT mice) (Fig. 1a–d). Percentage fractional shortening decreased to 22 ± 2% in WT, 23 ± 1% in apelin KO mice, but remained at 34 ± 2% in APJ-KO mice (P = 0.01 between APJ-KO and WT mice) (Fig. 1a–d). Apelin-KO mice (Supplementary Fig. 1g–i). APJ-KO mice responded to TAC (Fig. 1a–d) observed both in wild type (WT) and in APJ-null animals were resistant to the pathological hypertrophic response to TAC as shown by echocardiographic analysis.

Figure 1 | APJ-KO mice are protected from hypertrophy after TAC. a, Anatomical view (a) and histological sections (b) of WT and APJ-KO mice 90 days after surgery. c, Cell membrane staining (wheat germ agglutinin). d, Quantification from c. e, Trichrome staining (fibrosis in blue, stars). f, Quantification of f. g, Fractional shortening (FS%) decreased in WT mice after TAC, but did not change significantly in the APJ-KO mice. h, Heart weight/body weight ratio (HW/BW) at baseline and in TAC-operated mice, 90 days after surgery (see Supplementary Table 4 for details). Error bars, s.e.m.*P < 0.05 between indicated groups, analysis of variance.
and WT) (Fig. 1g and Supplementary Tables 1 and 4). In summary, both WT and apelin-KO mice presented clear signs of heart failure after 90 days of TAC, whereas APJ-KO mice were almost unaffected. The maintenance of cardiac function in the APJ-KO demonstrates that the expression of APJ is necessary to elicit heart failure in response to pressure overload.

The different responses of apelin-KO and APJ-KO mice to TAC imply that either apelin can act independently of APJ, or that APJ transduces a signal independently of apelin. We tested the first hypothesis by infusing APJ-KO mice with apelin (285 μg.kg⁻¹ per 24 h) and examining two readouts: contractility under TAC and vascular tone. Notably, apelin infusion did not increase cardiac contractility (percentage fractional shortening) in TAC-APJ-KO mice, in contrast to the characteristic improvement seen in TAC-WT animals (Supplementary Fig. 2a). In the absence of apelin infusion, endogenous levels of apelin in blood increased after TAC from 1 to 2 ng ml⁻¹ (Supplementary Fig. 2b). In the absence of apelin infusion, endogenous levels of apelin in blood increased after TAC from 1 to 2 ng ml⁻¹ and that rise was not different in WT and APJ-KO mice, making it unlikely that the protection achieved in the APJ-KO is due to hyper-activation of Gα₁₉ (Supplementary Fig. 2c–f), further indicating that apelin activity requires APJ.

Because the mechanical properties of the heart change markedly during pressure overload, and the structurally related angiotensin receptor (AT-1) can act as a mechanosensor, we asked whether APJ responds to mechanical stretch. Initially these experiments were challenging as cultured cardiomyocytes consistently downregulated the expression of endogenous APJ (Supplementary Fig. 3a), and studies had to be restricted to freshly isolated adult cardiomyocytes. We mimicked the effect of pressure overload by using a carbon fibre technique to stretch cardiomyocytes and evaluated their Frank-Starling gain (FSG). FSG is a dimensionless metric of the force that can be recruited by stretch. Freshly isolated adult cardiomyocytes from WT mice showed a significantly higher FSG than cardiomyocytes from APJ-KO mice (Fig. 2a–c). Treatment with apelin decreased the FSG in WT cardiomyocytes but showed no effect in APJ-KO cells (Fig. 2d). Therefore, apelin modulated the response to stretch only in cardiomyocytes with APJ receptors.

Engineered cells stably expressing human APJ (API-HEK) responded to apelin by increasing the content of phosphorylated extracellular signal-regulated kinase (pERK) (Fig. 3a, b) whereas parental (HEK) cells showed no significant change. Stretch also increased pERK content in cells expressing APJ (Fig. 3a, b), pERK levels, therefore, reflect the cellular response to APJ activation by stretch or apelin. pERK was therefore used as a simple readout of APJ activation. The Gi₃, inhibitor PTX blocked the ability of apelin, but not stretch, to induce APJ-dependent phosphorylation of ERK (Fig. 3a, b), first suggesting that separate mechanisms link stretch and apelin to APJ intracellular signalling.

Figure 2 | APJ mediates a stretch response that can be modulated by apelin. a, b, Representative force measurements (arbitrary units) for end-diastolic and end-systolic length–tension relationships in adult cardiomyocytes from WT (a) and APJ-KO (b) mice plotted against diastolic length (normalized to unstrained length). Cells were paced at 1 Hz. c, FSG attained by dividing the active force by the passive force from experiments in a and b plotted as a function of diastolic length (n = 8 WT and 7 APJ-KO cardiomyocytes). d, Average FSG (at 1.02 sarcomeric length) from c is shown for APJ-KO and WT both with (+) and without (−) 10 nM apelin administration (n = 6 for WT + apelin and n = 7 for APJ-KO + apelin). Error bars, s.e.m.

Figure 3 | Stretch activation of APJ enhances β-arrestin signalling while reducing G-protein signalling. a, b, Immunoblot (a) and quantification (b) of ERK from APJ stably transfected (API-HEK) and parental (HEK) cells treated for 5 min with 100 nM apelin or stretch in absence or presence of PTX (Gα₁₉ inhibitor), n = 3. c, d, Effect of 1 μM apelin (c) or stretch (d) on cAMP levels. Isoproterenol (iso, 1 μM) was used to elevate cAMP artificially to study Gα₁₉ activation, n = 4. e, f, G-protein (Gα₁₉, and Gα₁₂/13) activation by apelin and effect of stretch (red) in CHO cells expressing APJ and Gα₁₉. Receptor stimulation activated the promiscuous Gα₁₂, phospholipase-Cβ, and caused the accumulation of IP1 (representative of three experiments, n = 4 samples). f, Full range of β-arrestin binding to APJ under either physiological or pharmacological doses of apelin. g, Data points for 0–10⁻¹⁰ M apelin, RLU, relative light units. Error bars, s.e.m. *p < 0.05 between indicated groups, analysis of variance.
We next examined whether activation of APJ by stretch or apelin differentially modulates G-protein-generated second messengers. Activation of Gz_1 is associated with inhibition of adenylyl-cyclase and reduced cAMP, whereas activation of Gz_3 increases cAMP. cAMP did not increase after application of stretch or apelin, arguing against activation of adenylyl-cyclase (and Gz_1) (Fig. 3c, d, no isoproterenol conditions). In contrast, when isoproterenol was used to elevate the intracellular concentration of cAMP, apelin addition decreased cAMP levels in the APJ-HEK cells (Fig. 3c). This effect of apelin was partly inhibited by PTX, consistent with the involvement of Gz_3.

Apelin did not modify isoproterenol-stimulated cAMP formation in untransfected HEK controls, showing that the decrease is mediated by APJ. These data agree with previous reports and demonstrate that Gz_3 transduces the signal initiated by apelin binding to APJ. In contrast, stretch reduced cAMP in parental HEK as well as in APJ-HEK cells (Fig. 3d), indicating that, although stretch can activate Gz_3, signalling, this response is not mediated through APJ.

The Gz_3 subunit couples any activated GPCR to phospholipase C, which results in accumulation of inositol phosphates (IP1), and thus provides a general readout of G-protein activation. Stretch did not

**Figure 4** | APJ activation through mechanical stretch elicits cardiac hypertrophy. a–l. ANF immunostaining (white) and nuclear DAPI staining (blue) of rat neonatal ventricular cardiomyocytes transduced with rat APJ (Ad-APJ–GFP) or control GFP (Ad–GFP, green). m. Quantification of ANF (% of total nuclei) in a–l (n = 250–350 cells). n. Quantitative polymerase chain reaction analysis of the ratio between β- and α-MHCs, as an independent index of hypertrophy (n = 3–5 samples). o. Mean cell sizes in a–l (n = 24 cells). p. Responsiveness of transfected cells to apelin treatment (n = 4–5). q. ANF expression in cardiomyocytes in the presence of conditioned media from stretched cardiomyocytes (n = 3). r. Apelin enzyme-linked immunosorbent assay of conditioned media (12 h) from cardiomyocytes (CM) non-stretched and stretched. s. Apelin standard curve of CHO APJ–β-arrestin2 (siRNA) and CHO APJ–β-arrestin2 (siRNA) + apelin (pmol mg–1 protein). t. Mean cell sizes in a–l (n = 250–350 cells) conditioned with α-MHC or β-MHC mRNA (%). u. Mean cell sizes in a–l (n = 250–350 cells) conditioned with α-MHC or β-MHC mRNA (%). v. Mean cell sizes in a–l (n = 250–350 cells) conditioned with α-MHC or β-MHC mRNA (%). w. Mean cell Sizes in a–l (n = 250–350 cells) conditioned with α-MHC or β-MHC mRNA (%). x. Mean cell Sizes in a–l (n = 250–350 cells) conditioned with α-MHC or β-MHC mRNA (%). y. Mean cell Sizes in a–l (n = 250–350 cells) conditioned with α-MHC or β-MHC mRNA (%). z. Mean cell Sizes in a–l (n = 250–350 cells) conditioned with α-MHC or β-MHC mRNA (%).
increase IP$_1$ production whereas apelin did so in a dose-responsive manner (Fig. 3e). Notably, for any given concentration of apelin, stretch consistently reduced G-protein activation (Fig. 3e). There was a decrease in maximal levels and a shift in half-maximum effective concentration (EC$_{50}$) from 5.1 $\times$ 10$^{-9}$ to 5.5 $\times$ 10$^{-8}$ when both stimuli were applied simultaneously (Fig. 3e). These experiments demonstrate that APJ activation by stretch is largely G-protein independent and that stretch interferes with apelin-mediated G-protein activation by APJ.

Using a β-arestin/APJ complementation assay, apelin was found to induce a dose-dependent increase in β-arestin signalling, as expected (Fig. 3f). Stretch in the absence of apelin boosted β-arestin signalling twofold more than baseline (Fig. 3g). Notably, stretch increased β-arestin recruitment in response to apelin at all doses tested (Fig. 3g). Taken together, stretch causes APJ to signal less effectively/bi twofold more than baseline (Fig. 3g). Notably, stretch increased stretch interferes with apelin-mediated G-protein activation by APJ.

To investigate whether these distinct mechanisms of APJ action differentially affect cardiac hypertrophy, we examined cardiomyocyte cell growth and the expression of molecular markers of pathological hypertrophy in neonatal rat ventricular cardiomyocytes$^{23,24}$. These cardiomyocytes in culture respond to mechanical stretch$^{25}$, mainly through the angiotensin II receptor AT-1 and the endothelin1 receptor ET-A$^{25}$, which are also GPCRs. To test the specific effect of APJ in cardiac stretch/hypertrophy, we used pharmacological inhibitors of AT-1 (100 nM candesartan) and ET-A (300 nM BQ123), hereafter labelled as ‘inhibitors’. To overcome APJ downregulation during cardiomyocyte culture, we re-established APJ expression by adenoviral transduction (about 90% efficiency) with either control GFP (Ad–GFP) or an APJ–GFP fusion protein (Ad–APJ–GFP). Hypertrophy was assessed by the characteristic increase in perinuclear immunolocalization of atrial natriuretic factor (ANF) (Fig. 4a–m). Remarkably, apelin treatment did not increase ANF immunostaining, neither in Ad–APJ–GFP- nor in Ad–GFP-infected cells (Fig. 4d–f, m). In contrast, stretch applied in the presence of inhibitors significantly increased the number of perinuclear ANF$^+$ cells in APJ-restored cardiomyocytes (27 ± 1%), but not in control cardiomyocytes infected with Ad–GFP (5.8 ± 2.1%) (Fig. 4g–i, m). Co-stimulation with apelin and stretch reduced the number of ANF$^+$ cells (Fig. 4j–l, m). Stretch also induced specific changes in the expression of other molecular markers of hypertrophy, including an increased ratio of β- to α-myosin heavy chain (β-MHC/α-MHC) (Fig. 4n) and increased cell size (Fig. 4o). Similar to its effects on ANF, the addition of apelin also attenuated effects of stretch on other parameters of hypertrophy (Fig. 4m–o). The induction of ANF by stretch was PTX insensitive, but the ability of apelin to antagonize stretch-induced hypertrophy was prevented by treatment with PTX (Fig. 4w).

Several control experiments confirmed that APJ is directly involved in the response to stretch: neonatal rat ventricular cardiomyocytes treated with forskolin responded to apelin only upon reconstitution of APJ expression, as monitored by their ability to decrease intracellular cAMP levels (Fig. 4p). The possibility that stretch induces the secretion of factors that might indirectly activate hypertrophy through APJ was considered but seems unlikely because conditioned medium from APJ-transfected cardiomyocytes did not induce ANF expression (Fig. 4q). Moreover, the concentration of apelin in the media remained unchanged (approximately 5 ng ml$^{-1}$) with or without stretch (Fig. 4r), and conditioned media from stretched cells could not activate β-arestin recruitment (Fig. 4s). Single-cell analysis of low multiplicity APJ–GFP transduced cells showed that cardiomyocytes require APJ to induce ANF upon stretch (Fig. 4t–v, note perinuclear ANF immunostaining only in GFP$^+$ cells in panel v), further demonstrating that APJ activation through stretch is sufficient to elicit cardiac hypertrophy. Previous studies showed that APJ interacts with AT-1 and apelin antagonizes AT-1 function$^{11}$. As shown in Fig. 4x, in the presence of AT-1 inhibitors, only those cells expressing APJ responded to stretch by significantly increasing perinuclear ANF expression. In the absence of AT-1 inhibitors, APJ-transduced and non-transduced cells reached the same maximal level of ANF expression (Fig. 4y). Thus, blocking AT-1 does not impair the ability of APJ to respond to stretch, indicating that APJ alone is sufficient to transduce a stretch-induced hypertrophy signal.

Notably, siRNAs specific for β-aretin1 or β-aretin2 (Fig. 4z) blocked the stretch induction of hypertrophic markers (β-MHC/α-MHC (Fig. 4z) and ANF (not shown)) with an additive effect when both siRNAs were used together. These data substantiate the model that APJ signalling through β-aretin mediates stretch-induced myocardial hypertrophy.

In summary, the mechanono-response of APJ is necessary (blunted hypertrophic response to TAC of APJ-KO mice, Fig. 1) and sufficient (stretch induction of ANF expression occurs in cells expressing APJ, Fig. 4) to trigger myocardial hypertrophy in a β-aretin-dependent manner (Fig. 4z). Apelin does not induce hypertrophy, but instead blunts stretch-mediated hypertrophy (Fig. 4j–o), suggesting the ability of apelin to override pathological signalling from stretch. At a mechanistic level, APJ transduces apelin and stretch signalling differently. The response to apelin seems to be G-protein-PTX sensitive, whereas that induced by stretch is PTX insensitive and G-protein independent in the absence of exogenous apelin (Fig. 3). Stretch profoundly affects apelin signalling, diminishing G-protein activation while augmenting β-aretin recruitment (Fig. 3e–g). These data indicate that APJ integrates apelin and stretch stimuli, biasing the levels of G-protein signalling versus β-aretin recruitment accordingly.

These results have implications for the consideration of APJ as a drug target, because APJ/stretch can be pathological. Therefore, a beneficial effect will be obtained not by general apelin receptor agonism, but rather by selectively inhibiting the ability of APJ to respond to mechanical stretch or by blocking its interaction with molecules that initiate pathological signalling cascades.

**METHODS SUMMARY**

All experiments were performed in accordance with relevant guidelines and regulations. The Sanford-Burnham Medical Research Institute’s Animal Care & Use Program is accredited by Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC International), and a Multiple Project Assurance A3053-1 is on file in the Office of Laboratory Animal Welfare, Department of Health and Human Services. APJ and apelin KO mice. APJ-KO mice were obtained from Deltagen. Apelin-KO mice are described elsewhere$^{13}$. Both APJ and apelin lines (male and female) were in C57Bl/6 genetic background in a 99%–100% purity, as demonstrated by microsatellite analysis (Radil). For detailed methods, see Supplementary Information.

Received 9 March; accepted 25 May 2012.
Published online 18 July; corrected online 15 August 2012 (see full-text HTML for details).

1. Levy, D., Garrison, R. J., Savage, D. D., Kannel, W. B. & Castelli, W. P. Prognostic implications of echocardiographically determined left ventricular mass in the Framingham Heart Study. *N. Engl. J. Med.* 322, 1561–1566 (1990).
2. Knowlton, K. L. et al. The alpha 1A-adrenergic receptor subtype mediates biochemical, molecular, and morphologic features of cultured myocardial cell hypertrophy. *J. Biol. Chem.* 268, 15374–15380 (1993).
3. Rapacciuolo, A., Esposito, G., Prasad, S. V. & Rockman, H. A. G protein-coupled receptor signaling in vivo in cardiac overload. *Acta Physiol. Scand.* 173, 51–57 (2001).
4. Rockman, H. A., Koch, W. J. & Lefkowitz, R. J. Seven-transmembrane-spanning receptors and heart function. *Nature* 415, 206–212 (2002).
5. O’Dowd, B. F. et al. A human gene that shows identity with the gene encoding the angiotensin receptor is located on chromosome 11. *Gene* 136, 355–360 (1993).
6. Tatemoto, K. et al. Isolation and characterization of a novel endogenous peptide ligand for the human APJ receptor. *Biochem. Biophys. Res. Commun.* 251, 471–476 (1998).
7. Szokodi, I. et al. Apelin, the novel endogenous ligand of the orphan receptor APJ, regulates cardiac contractility. *Circ. Res.* 91, 434–440 (2002).
8. Ashley, E. A. et al. The endogenous peptide apelin potently improves cardiac contractility and reduces cardiac loading in vivo. *Cardiovasc. Res.* 65, 73–82 (2005).
9. Jia, Y. X. et al. Apelin protects myocardial injury induced by isoproterenol in rats. *Regul. Pept.* 133, 147–154 (2006).
10. Siddiqui, K. et al. Apelin protects against angiotensin II-induced cardiovascular fibrosis and decreases plasminogen activator inhibitor type-1 production. *J. Hypertens.* 29, 724–731 (2011).
11. Chun, H. J. et al. Apelin signaling antagonizes Ang II effects in mouse models of atherosclerosis. *J. Clin. Invest.* **118**, 3343–3354 (2008).

12. Kuba, K. et al. Impaired heart contractility in apelin gene-deficient mice associated with aging and pressure overload. *Circ. Res.* **101**, e32–e42 (2007).

13. Charo, D. N. et al. Endogenous regulation of cardiovascular function by apelin-APJ. *Am. J. Physiol. Heart Circ. Physiol.* **297**, H1904–H1913 (2009).

14. Scott, I. C. et al. The G protein-coupled receptor Agtr1b regulates early development of myocardial progenitors. *Dev. Cell* **12**, 403–413 (2007).

15. Frank, D. et al. Gene expression pattern in biomechanically stretched cardiomyocytes: evidence for a stretch-specific gene program. *Hypertension* **51**, 309–318 (2008).

16. Zou, Y. et al. Mechanical stress activates angiotensin II type 1 receptor without the involvement of angiotensin II. *Nature Cell Biol.* **6**, 499–506 (2004).

17. Irbe, G. et al. Axial stretch of rat single ventricular cardiomyocytes causes an acute and transient increase in Ca^{2+} spark rate. *Circ. Res.* **104**, 787–795 (2009).

18. Boilsdorff, C., Lookin, O. & Kohl, P. Assessment of contractility in intact ventricular cardiomyocytes using the dimensionless ‘Frank-Starling Gain’ index. *Pflügers Arch.* **462**, 39–48 (2011).

19. Civelli, O. GPCR deorphanzations: the novel, the known and the unexpected transmitters. *Trends Pharmacol. Sci.* **26**, 15–19 (2005).

20. Means, C. K., Miyamoto, S., Chun, J. & Brown, J. H. S1P1 receptor localization and transient increase in Ca^{2+} in cardiomyocytes: evidence for a stretch-specific gene program. *Hypertension* **51**, 309–318 (2008).

21. Masri, B., Morin, N., Pedeberrade, L., Knibiehler, B. & Audigier, Y. The apelin receptor is coupled to Gi1 or Gi2 protein and is differentially desensitized by apelin fragments. *J. Biol. Chem.* **283**, 11954–11963 (2008).

22. Kostenis, E. Is Gα12 the optimal tool for fishing ligands of orphan G-protein-coupled receptors? *Trends Pharmacol. Sci.* **22**, 560–564 (2001).

23. Knowlton, K. U. Divergent pathways mediate the induction of ANF transgenes in neonatal and hypertrophic ventricular myocardium. *J. Clin. Invest.* **96**, 1311–1318 (1995).

24. Rockman, H. A. et al. Molecular and physiological alterations in murine ventricular dysfunction. *Proc. Natl Acad. Sci. USA* **91**, 2694–2698 (1994).

25. Sedoskina, J. & Izumo, S. Mechanical stretch rapidly activates multiple signal transduction pathways in cardiac myocytes: potential involvement of an autocrine/paracrine mechanism. *EMBO J.* **12**, 1681–1692 (1993).

26. Sadoshima, J., Xu, Y., Slayter, H. S. & Izumo, S. Autocrine release of angiotensin II mediates stretch-induced hypertrophy of cardiac myocytes in vitro. *Cell* **75**, 977–984 (1993).

27. Mappi, G., Vuotienaho, O., Marttila, M. & Ruskaaho, H. Endothelin-1 is involved in stretch-induced early activation of B-type natriuretic peptide gene expression in atrial but not in ventricular myocytes: acute effects of mixed ET(A)/ET(B) and AT1 receptor antagonists in vivo and in vitro. *Circulation* **96**, 3053–3062 (1997).

Supplementary Information is linked to the online version of the paper at www.nature.com/nature.

Acknowledgements. We are thankful to N. Ling, S. Zhao and F. Abdel-Wahhab for technical assistance; E. Sergienko for help optimizing arrestin assay; E. Adamson for reading the manuscript; and M. Querol for graphics design. This work was supported by Wyeth Sponsored Research Agreement and National Institutes of Health (NIH) grant RO1HL086879 to P.R.L.; NIH grants R37HL059902 and R01HL083463 and the Sanford Children’s Center to M.M.; NIH grant RO1HL054732, grants from the Ellison Medical Foundation and the Muscular Dystrophy association to R.B., NIH grant (NS05422) and Florida Department of Health grant 06-01-NR-09 to L.H.S., and NIH grants RO1HL28143, P01 HL085577 to J.H.B. M.C.S. has received support from the California Institute for Regenerative Medicine (clincial fellow), the Italian Ministry of Research and Education and the Italian Society of Cardiology (SIC and Sanofi-Aventis Foundation). C.H. holds an American Heart Association Postdoctoral Award, S.R. is a Sanford Children’s Health Research Center fellow. P.K. is supported by SAF2010-15050 Ministerio de Ciencia e Innovación (MICINN) Spain.

Author Contributions. M.S.C. and C.H. designed, performed experiments, analysed data and prepared the manuscript. C.E.W., S.R., S.A.M., K.W., S.M., J.W., N.H.P., T.A. and M.S.C. and C.H. designed, performed experiments, prepared the manuscript. P.K. designed and supervised experiments, and analysed data and prepared the manuscript.

Author Information. Reprints and permissions information is available at www.nature.com/reprints. The authors declare no competing financial interests. Readers are welcome to comment on the online version of this article at www.nature.com/nature. Correspondence and requests for materials should be addressed to P.R.L. (prlozano@stanford.edu).