G Protein-Coupled Receptor Kinase 2 Ablation in Cardiac Myocytes Before or After Myocardial Infarction Prevents Heart Failure

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Abstract

Myocardial G protein-coupled receptor kinase 2 (GRK2) is a critical regulator of cardiac β-adrenergic receptor (βAR) signaling and cardiac function. Its upregulation in heart failure (HF) may further depress cardiac function and contribute to mortality in this syndrome. Preventing GRK2 translocation to activated βAR with a GRK2-derived peptide that binds $G_{\beta\gamma}$ (βARKct) has benefited some models of HF, but the precise mechanism is uncertain, as GRK2 is still present and βARKct has other potential effects.

We generated mice in which cardiac myocyte GRK2 expression was normal during embryonic development, but was ablated after birth (αMHC-Cre × GRK2 fl/fl), or only after administration of tamoxifen (αMHC-MerCreMer×GRK2 fl/fl) and examined the consequences of GRK2 ablation before and after surgical coronary artery ligation on cardiac adaptation after myocardial infarction. Absence of GRK2 prior to coronary artery ligation prevented maladaptive post-infarction remodeling and preserved βAR responsiveness. Strikingly, GRK2 ablation initiated 10 days after infarction increased survival, enhanced cardiac contractile performance, and halted ventricular remodeling.

These results demonstrate a specific causal role for GRK2 in post-infarction cardiac remodeling and HF and support therapeutic approaches of targeting GRK2 or restoring βAR signaling by other means to improve outcomes in HF.

Keywords

heart failure; myocardial infarction; conditional gene targeting; GRK2

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INTRODUCTION

The role of myocardial β-adrenergic receptor (βAR) signaling in heart failure (HF) is controversial, with abundant data supporting dominant effects that are either pathological, or beneficial. Pathological effects are strongly suggested by the cardiomyopathies that develop under conditions of chronic βAR activation after long-term catecholamine administration [1] or as a consequence of high-levels of overexpressed cardiac β_1 or β_2-ARs [2, 3]. Likewise, enhanced signaling from overexpression of the βAR signal transducer $G_{\alpha s}$ causes HF [4]. Most importantly, however, are the human patient data indicating that βAR signaling is deleterious in HF: Genetic polymorphisms that enhance myocardial sympathetic tone are independent risk factors for HF [5], and pharmacological βAR antagonism has been known for over two decades to strikingly prolong life when used as primary therapy in HF [6].

Despite the strength of data in experimental systems and the human condition that excessive βAR signaling can contribute to HF, there continue to be those who advocate modulating βARs in HF as a form of therapy [7]. This viewpoint is based upon observations that two transgenic mouse lines overexpressing low levels of β_2-AR in the heart, which were developed in two independent laboratories, demonstrated enhanced contractile function without myocardial disease [8, 9], and β_2-ARs can improve function of cardiomyopathy models [10, 11]. Another group has suggested that transgenic or adenoviral-mediated overexpression of the downstream βAR signaling effector, adenylyl cyclase, has similar effects [12]. Also consistent with this idea are studies pioneered in our laboratory where prevention of typical HF-associated G protein-coupled receptor kinase 2 (GRK2)-mediated desensitization and downregulation of myocardial βARs has rescued numerous genetic [13, 14] and physiological [15, 16] models of HF.

The reasons for the apparently contradictory data sets relating to βAR signaling in failing myocardium are unknown, but understanding them will be essential to fully optimizing HF treatment. Regarding GRK2 inhibition specifically, concerns have been expressed about the specificity and true mechanism of action of the βARKct GRK2 inhibitor used in many of these studies [17]. It is also not entirely clear that GRK2 is the best target in HF, as GRK3, GRK5, and GRK6 are also expressed in the heart, and forced expression of GRK5 had similar desensitization effects on myocardial βARs as does forced expression of GRK2 [18]. Indeed, cardiac-specific ablation of GRK2 in the early embryo actually sensitized adult mice to catecholamine cardiomyopathy, rather than conferring protection, which was interpreted as evidence for pathological effects of βAR signaling [19]. These results raise additional questions about whether GRK2 inhibition may have different effects in different experimental HF models, or at different time points relative to the induction of HF.

To address these issues we created mice in which the GRK2 gene could be selectively ablated in cardiac myocytes in a temporally-defined manner, and compared the consequences of heart-specific GRK2 ablation before and after induction of HF by myocardial infarction.

MATERIALS AND METHODS

Experimental animals

Conditional mice bearing floxed GRK2 alleles (GRK2 fl/fl) have previously been described [19]. Transgenic mice overexpressing Cre-recombinase protein fused to two mutant estrogen receptor ligand-binding domains under the control of the αMHC promoter (αMHC-MerCreMer) [20] were received from The Jackson Laboratory (JAX Mice and Services, Bar Harbor, Maine, USA). Homozygous mice with the floxed GRK2 alleles were crossed with αMHC-MerCreMer mice and resulting αMHC-MerCreMer × GRK2 fl/fl mice and GRK2 fl/fl, as well as αMHC-MerCreMer, were studied. To induce Cre recombination and subsequent

deletion of GRK2, adult αMHC-MerCreMer × GRK2 fl/fl transgenic mice were treated with tamoxifen (Tmx) (Sigma-Aldrich, St. Louis, Missouri, USA) as described previously [20].

In addition αMHC-Cre mice [21] were bred on to the GRK2 fl/fl background to generate cardiac GRK2-deleted mice, initiated by the activation of the αMHC-promoter. αMHC-Cre × GRK2 fl/fl mice and GRK2 fl/fl were included in the study. All animal procedures and experiments were performed in accordance with the guidelines of the IACUC of Thomas Jefferson University. All animals were bred and maintained on a C57Bl/6 background.

Model of LV myocardial infarction

MI was induced by high ligation of the left anterior descending coronary artery (LAD) as described previously [22] and more detailed in the online expanded methods supplement.

Echocardiographic and hemodynamic analysis of cardiac function

LV ventricular function was assessed by transthoracic echocardiography and LV hemodynamics in anesthetized mice as described previously [23] and more detailed in the online expanded methods supplement. For measurements of LV hemodynamics all recordings were performed with a closed chest approach at baseline and after increasing doses of isoproterenol (Sigma-Aldrich, St. Louis, Missouri, USA) as described previously [23].

Isolation of cardiac myocytes

Adult mouse cardiac myocytes were isolated from sham and infarcted GRK2 fl/fl and αMHC-Cre × GRK2 fl/fl mice as previously described [24] and more detailed in the online expanded methods supplement.

Single myocyte contractility studies

Isolated cardiac myocytes were stimulated in an electrical field, and continuously flushed with tyrodes containing 1mM $CaCl_2$ without (baseline) and with $10^{-8}M$ isoproterenol (isoproterenol-stimulation). Single-cell contractions were measured by video edge detection (Fluorescence and Contractility System, IonOptix, Milton, Massachusetts, USA) .

RNA isolation, reverse transcription and quantitative real-time RT-PCR

Isolated LV cardiac myocytes (rescue-study) or myocardial tissue from the remote zone (prevention-study) was snap-frozen. Analysis was carried out 35 days post MI/ 21 days after the last dose of Tmx (rescue-study) or 28 days post MI (prevention study). Quantitative real-time polymerase chain reaction was performed as described previously [22] and more detailed in the online expanded methods supplement.

Western blot analysis

Western blotting was performed as described previously [22] and more detailed in the online expanded methods supplement. Cardiac protein levels of GRK2 (sc-562, C-15, Santa Cruz Biotechnology, 1:5,000), α-actin (A7811, Sigma-Aldrich, 1:5,000) and calsequestrin (CSQ) (208915; Calbiochem; 1:10,000), were assessed in cardiac myocyte cellular preparations.

Statistical analysis

Data are generally expressed as mean ± SEM. An unpaired two tailed t-test, a one-way ANOVA and a two-way repeated measurement ANOVA was performed for between-group comparisons. Survival analysis was performed by the Kaplan-Meier method and between-group differences in survival were tested by the log-rank test. For all tests, a *P* value < 0.05 was considered significant.

RESULTS

Defined loss of GRK2 in cardiac myocytes after MI rescues the HF phenotype

Survival of HF is improved after loss of GRK2—To gain insight into whether GRK2 ablation could be therapeutic in hearts already in HF, we used Tmx-inducible GRK2 KO (αMHC-MerCreMer × GRK2 fl/fl) mice. These mice along with αMHC-MerCreMer mice as controls were subjected to coronary artery ligation and loss of GRK2 was induced by 5 consecutive days of Tmx injections (on days 10–14 post-MI) (Fig. 1A). GRK2 levels in these two groups of mice were similar until after Tmx induction (Fig. 1B). We found similar mortality in both mice until ~12–13 days post-MI (Fig. 1C). Interestingly, following the Tmx-injections survival in Tmx-treated αMHC-MerCreMer × GRK2 fl/fl mice was significantly preserved compared to the continued death seen in Tmx-treated control mice (Fig. 1C). Thus, the loss of cardiac GRK2 after MI prevents the significant mortality seen chronically in HF mice with normal levels of GRK2 in their hearts.

Loss of cardiac GRK2 in already established HF rescues cardiac function—We next examined whether loss of cardiac GRK2 after MI could reverse the cardiac dysfunction, remodeling and reduced contractility associated with HF. In these studies αMHC-MerCreMer × GRK2 fl/fl, αMHC-MerCreMer, and GRK2 fl/fl mice were subjected to coronary artery ligation and cardiac function and dimensions were measured by serial echocardiography for up to 4 months after MI (Fig. 2). Importantly, all groups of mice 10 days post-MI had similar levels of chamber dilatation and cardiac dysfunction compared to pre-MI measurements (Fig. 2A and B). The 3 sham groups showed no alterations in cardiac function or dimensions at any time-point. Following loss of GRK2 in αMHC-MerCreMer × GRK2 fl/fl mice, echocardiography at 42 and 120 days post-MI showed stabilization of LV remodeling (Fig. 2A). Further, significant enhancement of cardiac function (as measured by FS [%]) was observed after the loss of cardiac GRK2 compared to both groups of Tmx-treated control mice (αMHC-MerCreMer and GRK2 fl/fl) (Fig. 2B). These salutary effects, which were clearly associated with the loss of GRK2, could be observed throughout the time span studied, showing beneficial long-term therapeutic effects.

Measurements of LV hemodynamics 120 days post MI showed preserved responsiveness to β-AR stimulation in infarcted Tmx-treated αMHC-MerCreMer × GRK2 fl/fl mice, whereas infarcted Tmx-treated αMHC-MerCreMer mice revealed an impaired β-AR reserve compared to sham mice (Fig. 2C and D). LVEDP was significantly lower in infarcted αMHC-MerCreMer × GRK2 fl/fl mice as compared to infarcted αMHC-MerCreMer mice (Fig. 2E).

Loss of GRK2 in post MI failing cardiac myocytes reduces activation of the fetal gene program—RT-PCR analysis was performed 5 weeks post MI (3 weeks after the last dose of Tmx) on cardiac myocytes isolated from the LV. Interestingly, less induction of selected fetal genes (atrial natriuretic peptide/ANP, brain natriuretic peptide/BNP, and β myosin heavy chain/β-MHC) was found in infarcted Tmx-treated αMHC-MerCreMer × GRK2 fl/fl mice as compared to post-MI Tmx-treated αMHC-MerCreMer cardiac myocytes, which showed significant up-regulation of these genes compared to sham-controls (Fig. 3).

We also examined expression of GRK mRNA post-MI in cardiac myocytes isolated from the LV. As expected in control Tmx-treated αMHC-MerCreMer mice, MI produced a significant increase in GRK2 expression over sham levels, whereas infarcted Tmx-treated αMHC-MerCreMer × GRK2 fl/fl mice showed significantly lower GRK2 levels (Fig. 3D). Interestingly, GRK5 mRNA was selectively upregulated in infarcted Tmx-treated αMHC-MerCreMer mice (supplemental Fig. 5).

Loss of GRK2 before myocardial infarction prevents the development of HF

Loss of cardiac myocyte GRK2 before MI reduces infarct-related mortality—To study the consequence of GRK2 loss on the development of HF, adult (8 week old) αMHC-Cre × GRK2 fl/fl mice and their corresponding littermate controls (GRK2 fl/fl) were subjected to coronary artery ligation and subsequent MI (Fig. 4A). αMHC-Cre × GRK2 fl/fl mice have an approximate 80% loss of cardiac myocyte GRK2 (Fig. 4B). Of note, infarct size was not different 24 hours or 28 days after MI between mice with normal levels of GRK2 and the GRK2 KO mice (supplemental Fig. 2). Interestingly, survival was significantly improved in male αMHC-Cre × GRK2 fl/fl mice subjected to MI compared to control GRK2 fl/fl mice (see Fig. 4C). There was a trend towards improved survival in infarcted αMHC-Cre × GRK2 fl/fl mice of both sexes (p=0.06) (supplemental Fig. 3). The differences in survival were present, despite only the αMHC-Cre × GRK2 fl/fl mice and not the control mice in this study (GRK2 fl/fl) possessed Cre recombinase, which has previously been proven to contribute to cardiac pathology [25].

Loss of GRK2 in cardiac myocytes before MI reduces the extent and progression of cardiac dysfunction—*In vivo* cardiac function was assessed by echocardiography prior to and 28 days after MI (or sham). 28 days after MI, control GRK2 fl/fl mice displayed significantly depressed systolic function and enlarged cardiac chambers as compared to pre-MI values (Fig. 5A and 5B). In striking contrast, αMHC-Cre × GRK2 fl/fl mice subjected to MI did not display the same degree of cardiac deterioration (Fig. 5A and B).

Loss of myocyte GRK2 before MI improves LV hemodynamics and preserves βAR responsiveness—Marked desensitization of cardiac βARs is considered a hallmark of the failing heart [26–28]. To determine whether the loss of GRK2 in cardiac myocytes can reverse dysfunctional βAR signaling, we measured *in vivo* LV hemodynamics 28 days after MI at baseline and after isoproterenol. LV contractility (as measured by dP/dt_{max}) and LV relaxation (as measured by LV dP/dt_{min}) after isoproterenol were significantly impaired in control GRK2 fl/fl mice compared to sham control mice demonstrating a loss of inotropic reserve consistent with HF (Fig. 5C and 5D). Similarly, hemodynamic studies on αMHC-Cre × GRK2 fl/fl mice revealed signs of cardiac dysfunction at baseline; however, responses to isoproterenol were significantly improved compared to post-MI control mice showing improved contractile reserve and preserved βAR signaling in hearts without GRK2 (Fig. 5C and D). Finally, elevated LV end diastolic pressure (LVEDP) in control post-MI GRK2 fl/fl mice was significantly lowered in mice with GRK2 expression lost (Fig. 5E).

Loss of GRK2 is associated with a lower extent of cardiac hypertrophy and normalized gene expression after MI—Heart-to-body weight ratio (HW/BW-ratio) was significantly increased 28 days post-MI in control GRK2 fl/fl mice compared to corresponding sham-operated animals (Fig. 5F). Although αMHC-Cre × GRK2 fl/fl mice subjected to MI displayed LV hypertrophy compared to sham-operated controls, the extent of hypertrophy was significantly less than that observed in GRK2 fl/fl mice subjected to MI (Fig. 5F).

Consistent with decreased hypertrophy post-MI in GRK2 KO mice, RT-PCR analysis of the remote (non-ischemic) portion of the LV showed significantly less induction of selected fetal genes (atrial natriuretic peptide/ANP, brain natriuretic peptide/BNP, and β myosin heavy chain/β-MHC) as seen in post-MI control hearts, which showed significant up-regulation of these genes compared to sham-controls (Fig. 6A–C). Up-regulation of the mRNAs encoding matrix metalloproteinase 9 (MMP-9) and Collagen-1 (Coll-1) in the non-ischemic LV zone were also seen in post-MI GRK2 fl/fl mice indicating adverse remodeling, however in GRK2 KO mice, these changes in MMP-9 and Coll-1 were not seen (Fig. 6D and 6E). These

observations in combination with the attenuation of LV chamber dilation (see above) argue in favor of significant repression of adverse LV remodeling by the loss of GRK2 in myocytes.

We also examined myocardial expression of GRK mRNA post-MI in remote (non-ischemic) sections of the LV. In agreement with the data obtained from the rescue-study, MI led to a significant increase in GRK2 expression over sham levels, which was absent in GRK2 KO mice (Fig. 6F). Interestingly, GRK5, another major GRK found in the heart [29], which was up-regulated in post-MI control (GRK2 fl/fl) hearts compared to sham was not significantly changed in the hearts of post-MI αMHC-Cre × GRK2 fl/fl mice (Fig. 6G). The results in wild-type mice were somewhat expected as GRK5 has been shown to be up-regulated in HF as well as GRK2, however, the fact that the GRK2 KO mice had no increase in GRK5 argues for a lack of compensation by this GRK. Of note, GRK3 a close homologue of GRK2 was not altered in any of our groups post MI or sham (data not shown).

Loss of GRK2 normalizes βAR responsiveness of single cardiac myocytes post-MI—Since the loss of GRK2 in cardiac myocytes resulted in preserved in vivo LV contractility globally post-MI without affecting infarct size, we explored whether the beneficial effect was due to improved β-AR signaling and contractile function in single cardiac myocytes. Functional recordings of cardiac myocyte twitches were obtained at 2 Hz (field stimulation), 20–22°C, and 1 mM extracellular Ca^{2+} ($[Ca^{2+}]e$). Fig. 7A shows representative steady state twitches from cardiac myocytes (from sham and infarcted GRK2 fl/fl and αMHC-Cre × GRK2 fl/fl mice) under baseline conditions (left panel) and following isoproterenol stimulation (10^{-8}M) (right panel). Isolated cardiac myocytes from post-MI αMHC-Cre × GRK2 fl/fl mice demonstrated significantly enhanced FS, improved rate of cell shortening (+dL/dt), and improved rate of re-lengthening (−dL/dt) under isoproterenol stimulation compared to control, post-MI GRK2(fl/fl) myocytes (Fig. 7B and 7C). These results indicate that β-AR responsiveness post-MI is preserved by the loss of GRK2.

DISCUSSION

The incidence of HF is increasing and its long-term prognosis has remained at 50% survival after five years despite the broad application of life-saving anti-neurohormonal pharmacological therapies with ACE inhibition and β-blockade over two decades ago [6, 30]. Thus, more efficient HF therapeutics are needed, which may be achieved by identifying non-traditional molecular targets downstream of the neurohormonal ligand-receptor interaction. For a number of years we have proposed that GRK2, a kinase that regulates the signaling activity and number of βAR in the heart, and that itself is regulated in HF, might represent such a non-traditional target [26]. Indeed, transgenic and adenoviral expression of a peptide inhibitor of GRK2 translocation, βARKct, has benefited a number of experimental HF models [13, 14, 31]. Mechanistically however, questions have arisen about the specific effects of βARKct, and about the specific pathophysiological role of GRK2 in heart disease. In the current studies we attempted to unambiguously define the role of GRK2 in post-ischemic HF and to assess the efficacy of its genetic ablation in a highly relevant model of HF, myocardial infarction, and at clinically relevant times in the course of the disease, i.e. both before and after the inciting ischemic event. Finally, we did this in a manner such that, for the first time, any major effects of GRK2 gene ablation on embryonic cardiac development [19, 32] would not confound our results, as cardiac myocyte-specific GRK2 gene ablation was induced around the second postnatal day with the activation of the α-MHC promoter or the application of Tmx in adult animals. In short, our data contains definitive evidence that GRK2 contributes to ischemic HF, and demonstrate a critical role for this kinase in the disease. Moreover our data show the striking efficacy of restoring cardiac βAR signaling homeostasis by GRK2 ablation either before or after myocardial infarction.

GRK2 is the critical GRK in the failing heart

Using constitutive (starting post-natally) αMHC-Cre or inducible αMHC-MerCreMer mice we were able to test the role of GRK2 expression in adult cardiac myocytes in both the development of, and rescue of ischemic cardiomyopathy. The use of these mice is necessary since global GRK2 KO mice die *in utero* [19, 32]. Interestingly, mortality post-MI was similar between groups until the Tmx treatment when the induced loss of GRK2 prevented further death up to 120 days post-MI. Thus, the loss of GRK2 expression in myocytes after MI, when HF was already established, clearly led to significantly improved survival chronically after MI. Furthermore, loss of GRK2 before MI significantly reduced mortality in male GRK2KO mice. In addition, GRK2 abolishment prevented LV dilatation and deterioration of cardiac function that is seen in control mice post-MI. This is the first study showing that controlled loss of GRK2 in failing cardiac myocytes rescues a model of established HF and directly demonstrates that minimizing GRK2 activity in the failing heart is beneficial. Although the loss of GRK2 was not complete, this loss of HF-associated upregulation of cardiac myocyte GRK2 essentially restored *in vivo* cardiac systolic and diastolic function. Our data strongly suggest GRK2 as the primary GRK critically involved in the functional regulation of the failing heart.

Restoration of βAR-signaling in the failing heart by GRK2 ablation similar to effects of the GRK2 inhibitor peptide βARKct

Both early and late after the acute inciting event, our current data provide further support for the benefits of restoration or normalization of βAR-signaling in HF. The increased LV hemodynamic response and the improved contractility of isolated single LV cardiac myocytes to βAR stimulation in mice lacking cardiac myocyte GRK2 shows that signaling through this inotropic system is preserved despite the presence of a large MI. Moreover, this data is consistent with previous rescue studies using the βARKct and demonstrates and supports the primary mechanism of action of this $G_{\beta\gamma}$-sequestering peptide in HF therapeutics to be GRK2 inhibition. Further, our data strongly support that the beneficial effect of βARKct expression observed in several HF models [13, 14, 31] is indeed due to inhibition of membranous GRK2 activity (primarily targeting βAR desensitization) causing a restoration towards normalized signaling. In addition, previous studies with βARKct and our present study with conditional GRK2 ablation provide strong evidence that GRK2 is the critical GRK regulating inotropic responsiveness and adverse βAR desensitization after MI.

An apparent narrow therapeutic window for altering βAR-signaling in HF

GRK2 is a major regulator of myocardial βAR signaling and dysfunctional βAR signaling is a consequence of HF and might at least in part contribute to the progression of cardiac dysfunction as failing human myocardium is characterized by diminished βAR responsiveness, loss of overall cardiac and single myocyte contractility, and disruption of intracellular Ca^{2+}-cycling [26, 33]. Importantly, βAR derangements include the up-regulation of GRK2 [34, 35]. βAR stimulation in myocardium modulates intracellular Ca^{2+}-fluxes and Ca^{2+}-responsiveness of the sarcomere, thereby linking βAR signaling to cardiac myocyte contractility. Of note, chronic upstream activation of the βAR signaling cascade by cardiac overexpression of the β_1-AR [2] or the adenylyl cyclase stimulating G protein α-subunit ($G_{\alpha s}$) [4] led to the development of dilated cardiomyopathy in mice. Moreover, overexpression of PKA, a down-stream effector of βAR-G_s signaling also resulted in fatal dilated cardiomyopathy [36]. Despite the evidence showing detrimental effects of chronic βAR and down-stream activation in the heart, other studies manipulating βAR signaling have led to positive outcomes. This includes studies by us showing the prevention and rescue of HF using the βARKct [13, 14, 31] as well as overexpression of adenylyl cyclase VI that has been shown to increase contractile function in the heart as well as inhibiting the development of HF post-MI [37, 38]. Recently, β_1-AR transactivation of the epidermal growth factor (EGF) receptor mediated by β-arrestin was

shown to confer cardioprotection in stressed hearts [39]. Taken together, these genetic manipulations at different levels of βAR signaling demonstrate that the point of intervention is important to the function of the heart and suggest that it is more desirable to circumvent βAR desensitization than to simply facilitate βAR activation. Studies using the βARKct are consistent with this notion [13, 14, 31]. Overall, the data discussed and our data suggest a narrow therapeutic window for βAR-signaling in HF, and indicate that normalization of βAR-signaling, which can be best achieved by preventing their uncoupling and downregulation through GRK2 inhibition/ablation, might be the optimal approach, rather than forced overexpression of receptors or their downstream signaling effectors.

Conclusions

Protein kinases are rapidly emerging as a new pharmacological approach that may complement the existing drug classes targeting G protein-coupled receptors. Taken together, our current results show that targeted deletion and lowering of cardiac myocyte GRK2 activity leads to a novel protective and inotropic phenotype, which prevents post-ischemic HF and rescues a phenotype of established HF. Our results herein combined with our previous βARKct data add to the significance of the βAR system in the failing heart as it is clear that resensitization can positively affect cardiac function. The data would appear to indicate that βAR desensitization and down-regulation is indeed maladaptive. We have not ruled out that other G protein-coupled receptors signals, no doubt altered by the loss of GRK2, are also contributing to the above phenotypes, however the above described changes in βAR inotropic reserve are significant. Overall, our data demonstrate that GRK2 activity is pathogenic in HF and targeted inhibition or lowering its expression lead to beneficial effects for contractile function of the heart and this lone molecular change in the post-ischemic heart can prevent and rescue structural HF and offer novel benefits over existing HF therapies.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgements

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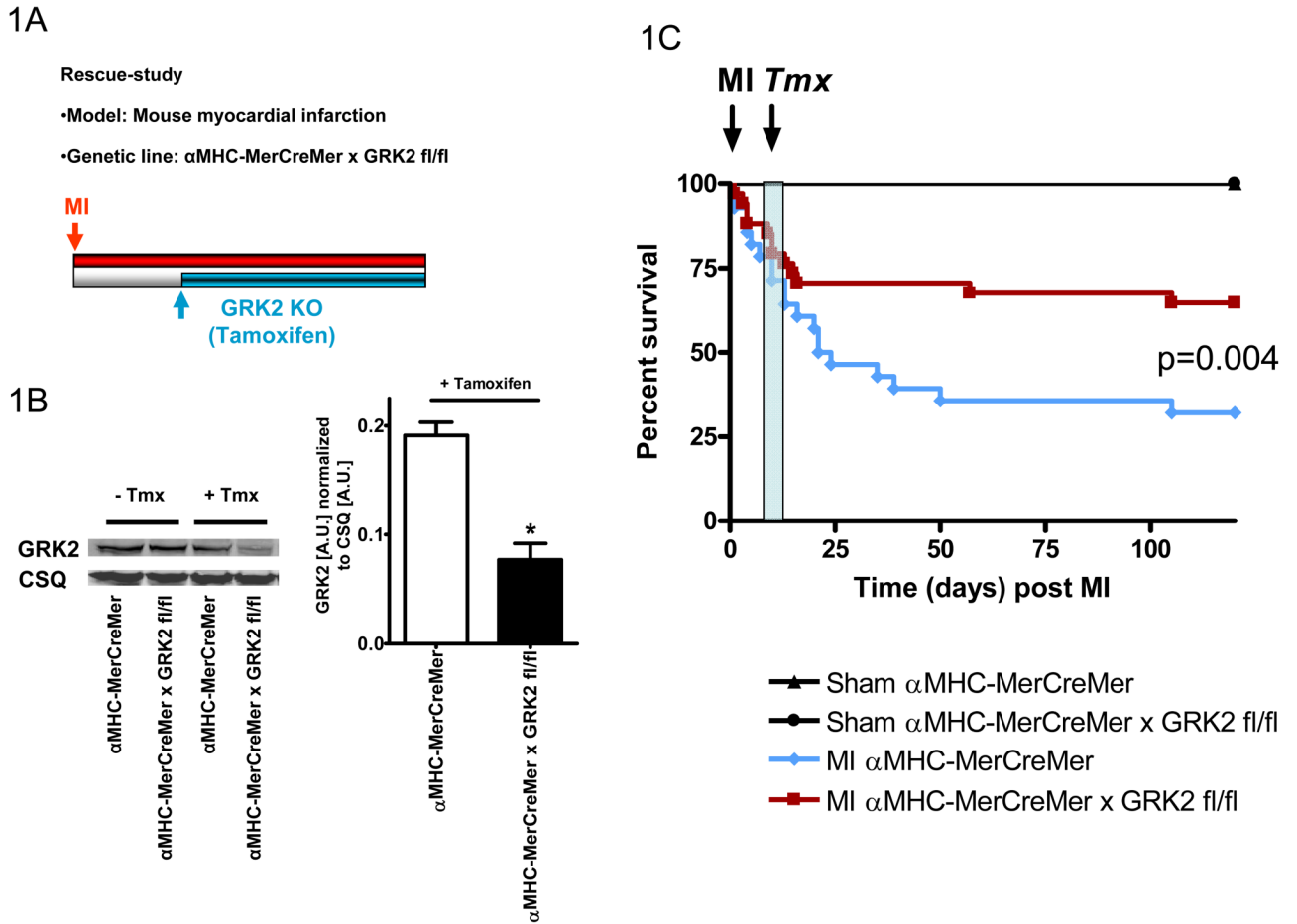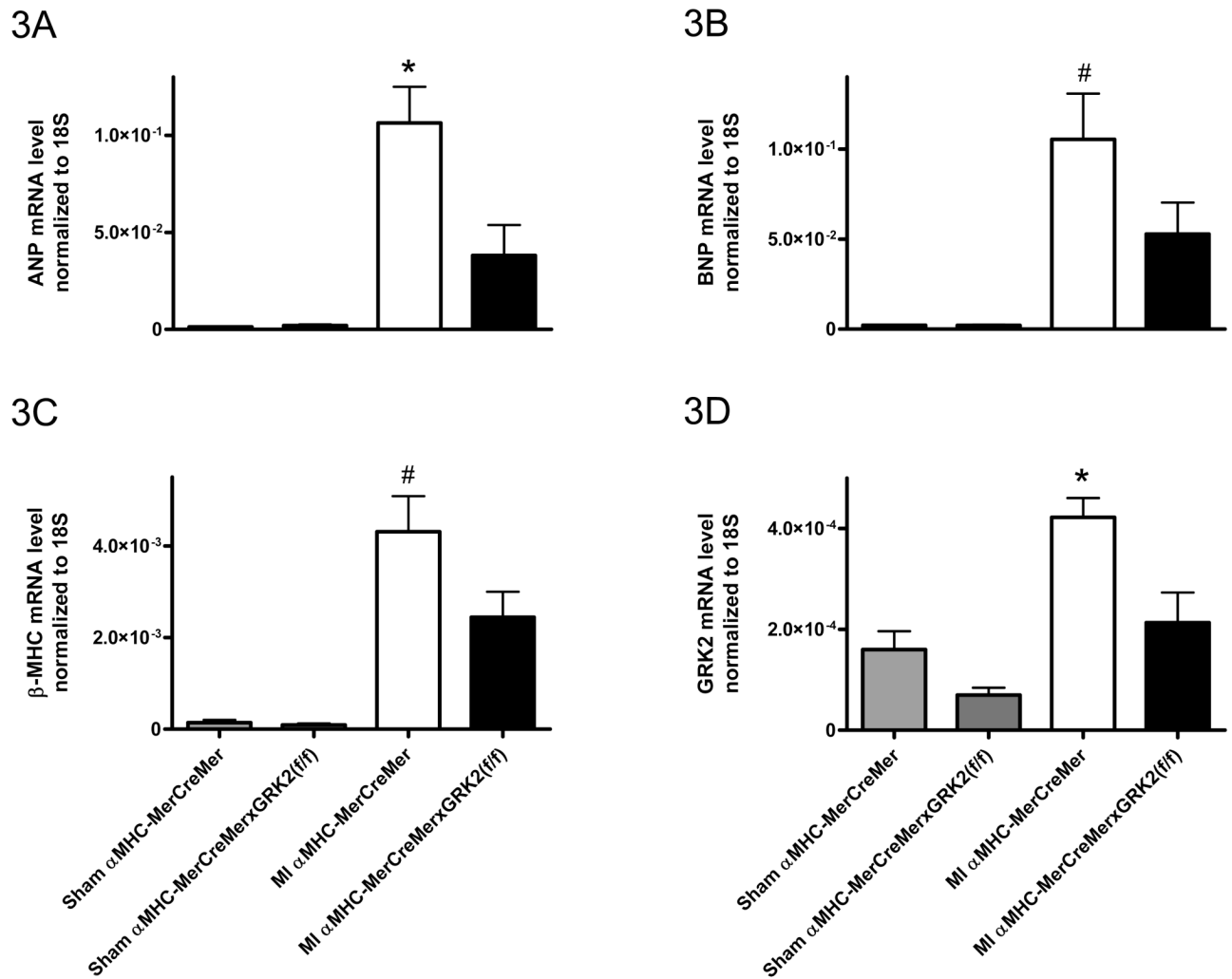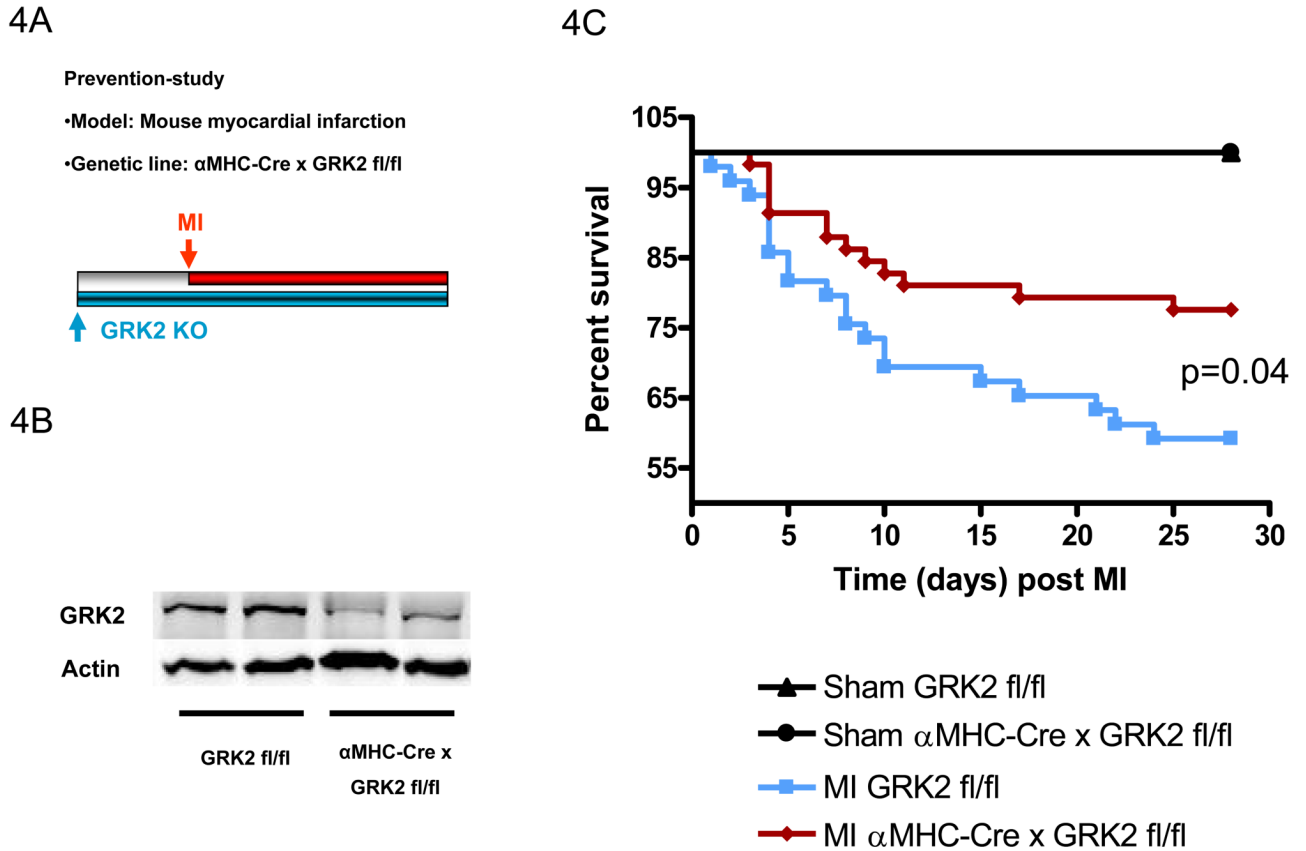
Figure 1.
Survival of HF is improved after post-MI induced loss of GRK2 in cardiac myocytes. A, Experimental protocol showing the study-design of the rescue-study using Tmx-inducible GRK2KO mice (αMHC-MerCreMer × GRK2 fl/fl, GRK2 fl/fl and αMHC-MerCreMer mice). B, Time-defined controlled loss of GRK2 in cardiac myocytes from Tmx-inducible αMHC-MerCreMer × GRK2 fl/fl and αMHC-MerCreMer mice. Impact of Tmx-treatment on cardiac myocyte GRK2 protein levels determined via Western Blotting. Representative Western Blot of GRK2 and CSQ (as a loading control) and quantification of the results, n=6 animals/group, *P<0.05 αMHC-MerCreMer × GRK2 fl/fl vs. αMHC-MerCreMer, unpaired two-tailed t-test. **C**, Survival of Tmx treated sham and infarcted αMHC-MerCreMer and αMHC-MerCreMer×GRK2 fl/fl mice throughout the study period. n=8–11 animals/group for Sham mice, n=30–34 animals/group for infarcted mice, survival was analyzed by the Kaplan-Meier method and between-group differences in survival were tested by the log-rank test.
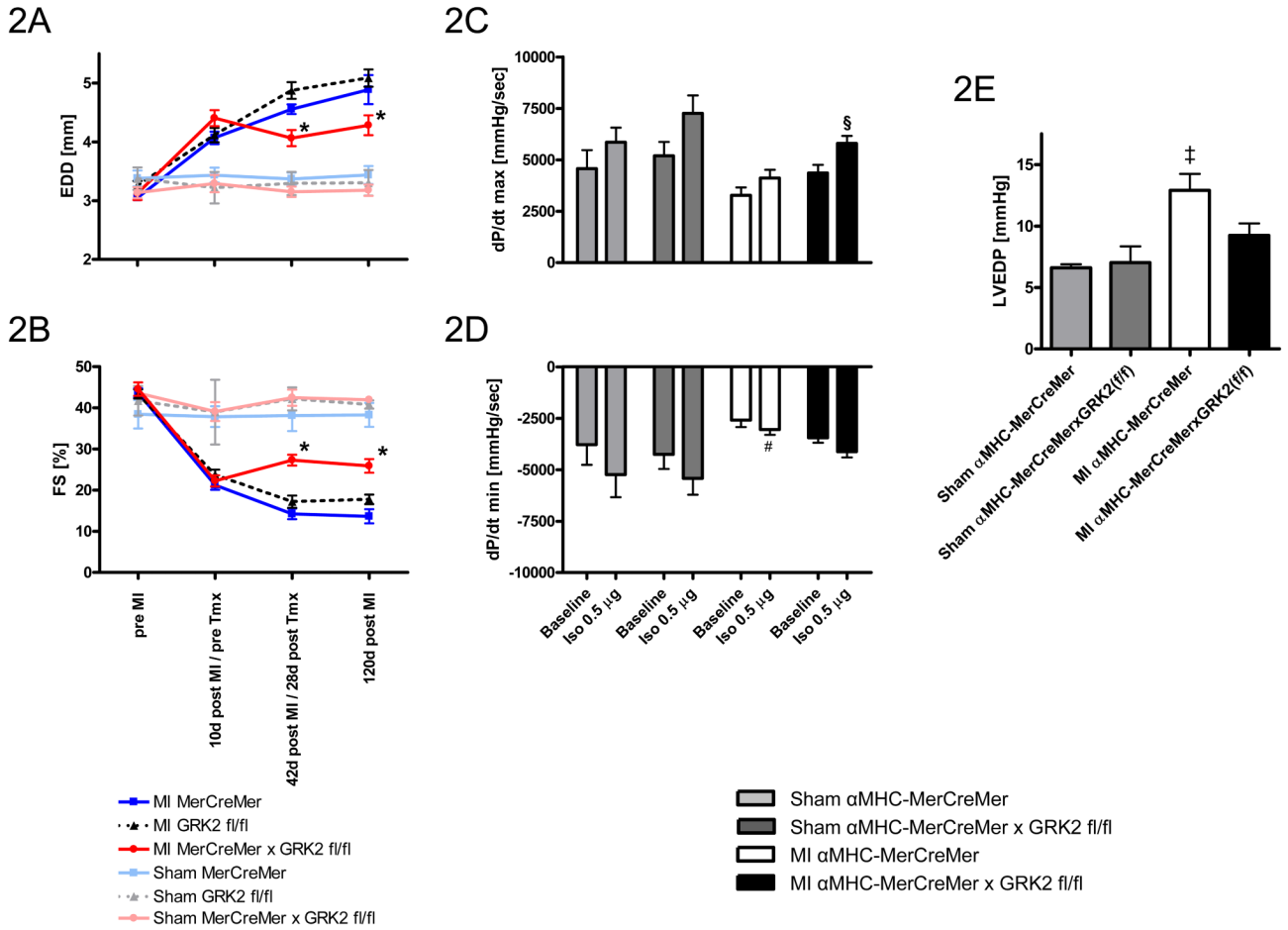
Figure 2.
Induced loss of GRK2 rescues adverse LV remodeling and cardiac function post-MI. A, Echocardiographic assessment of EDD pre-MI, 10d post-MI (prior to Tmx treatment), 42d post-MI (28d post-Tmx treatment) and 120d post-MI. B, Echocardiographic assessment of FS pre-MI, 10d post-MI (prior to Tmx treatment), 42d post-MI (28d post-Tmx treatment) and 120d post-MI. n=6–8 animals/group for sham-operated mice, n=12–16 animals/group for MI mice, *P<0.05 vs. MI αMHC-MerCreMer and MI GRK2 fl/fl. Hemodynamic measurements of LV contractility (as measured by LV dP/dt$_{max}$) (C), and relaxation (as measured by LV dP/dt$_{min}$) (D), and LV end diastolic pressure (LVEDP) (E), 120 d post-MI (106 d post-Tmx). n=5 animals/group for sham-operated mice, n=9–11 animals/group for MI mice, §P<0.05 MI αMHC-MerCreMer × GRK2 fl/fl vs. MI αMHC-MerCreMer under respective condition, #P<0.05 MI αMHC-MerCreMer vs. Sham groups under respective condition, ‡P<0.05 MI αMHC-MerCreMer vs. MI αMHC-MerCreMer × GRK2 fl/fl and Sham groups, two-way ANOVA for A, B, C, and D, one-way ANOVA for E.
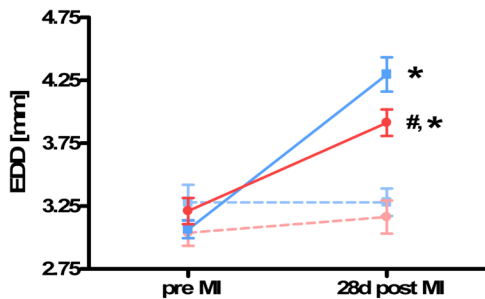
Figure 3.
GRK2 abolishment in the post-MI failing heart alters gene expression. Quantitative RT-PCR analysis on cardiac myocytes isolated from the LV 5 weeks post MI (3 weeks after the last dose of Tmx). A, ANP; B, BNP; C, β-MHC; and D, GRK2. All values normalized to 18S levels. n=5–6 animals/group for Sham mice, n=7–11 animals/group for MI mice, *P<0.05 MI αMHC-MerCreMer vs. MI αMHC-MerCreMer × GRK2 fl/fl and Sham groups, #P<0.05 MI αMHC-MerCreMer vs. Sham groups, one-way ANOVA.

Figure 4.
Infarct-related mortality is reduced with loss of GRK2 in cardiac myocytes prior to MI. A, Experimental protocol showing the study-design of the prevention-study using conditional GRK2KO mice (αMHC-Cre × GRK2 fl/fl and GRK2 fl/fl mice). B, Conventional conditional loss of GRK2 in cardiac myocytes in αMHC-Cre × GRK2 fl/fl and GRK2 fl/fl mice. Western blot analysis for GRK2 protein and actin (as a loading control) in isolated cardiac myocytes. C, Survival of male GRK2 fl/fl and αMHC-MHC × GRK2 fl/fl mice throughout the study period. n=8 animals/group for Sham mice, n=49–58 animals/group for infarcted mice, survival was analyzed by the Kaplan-Meier method and between-group differences in survival were tested by the log-rank test.
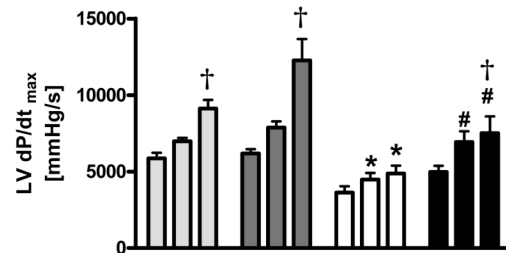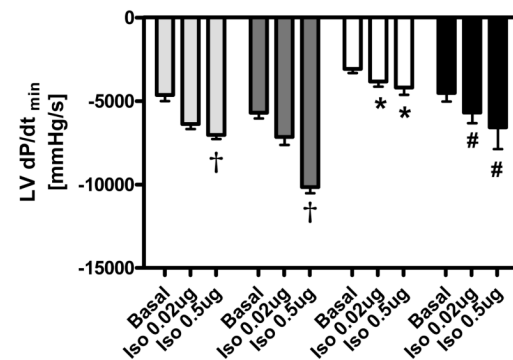
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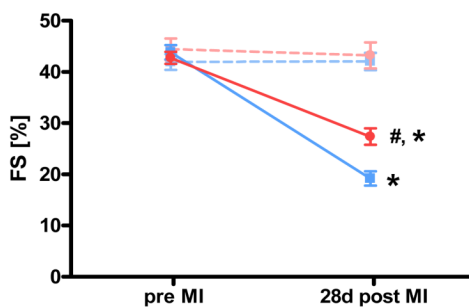
- -■- Sham GRK2fl/fl
- -●- Sham αMHC-Cre x GRK2 fl/fl
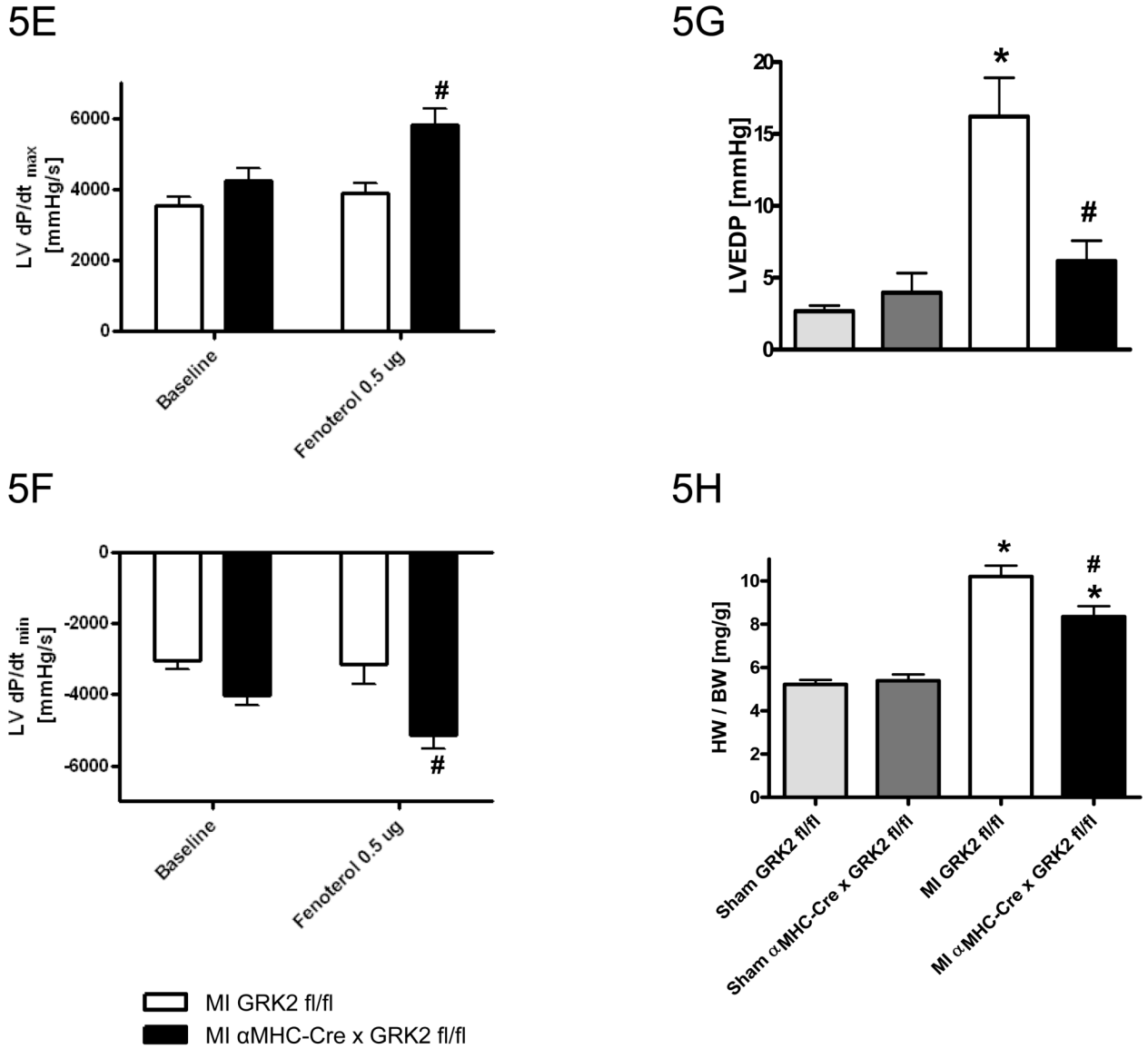- -■- MI GRK2 fl/fl
- -●- MI αMHC-Cre x GRK2 fl/fl

5C

5D

- □ Sham GRK2 fl/fl
- ■ Sham αMHC-Cre x GRK2 fl/fl
- □ MI GRK2 fl/fl
- ■ MI αMHC-Cre x GRK2 fl/fl

5E

5F

5G


5H

□ MI GRK2 fl/fl
■ MI αMHC-Cre x GRK2 fl/fl

Figure 5.
Constitutive loss of GRK2 in cardiac myocytes prior to MI reduces the extent of cardiac dysfunction. Echocardiographic assessment 28d post-MI of EDD (A) and FS (B), n=8–10 animals/group for Sham mice, n=21–30 animals/group for MI mice, *P<0.01 MI vs. Sham for respective group, #P<0.05 MI αMHC-Cre × GRK2 fl/fl vs. MI GRK2 fl/fl, one-way ANOVA. Hemodynamic measurements of LV contractility (as measured by LV dP/dt$_{max}$) (C), and relaxation (as measured by LV dP/dt$_{min}$) (D) at baseline and in response to increasing intraperitoneal injected doses of isoproterenol. E, left ventricular enddiastolic pressure (LVEDP) n=5 animals/group for sham-operated mice, n=9–10 animals/group for MI mice; †P<0.05 Iso 0.5µg vs. basal for each group, *P<0.05 MI GRK2 fl/fl vs. Sham GRK2 fl/fl for each condition, #P<0.05 MI αMHC-Cre × GRK2 fl/fl vs. MI GRK2 fl/fl for each condition, two-way ANOVA for C, and D, one-way ANOVA for E. F, Heart-to-body weight ratio at day 28 post MI. *P<0.01 MI vs. Sham, #P<0.05 MI αMHC-Cre × GRK2 fl/fl vs. MI GRK2 fl/fl, one-way ANOVA.

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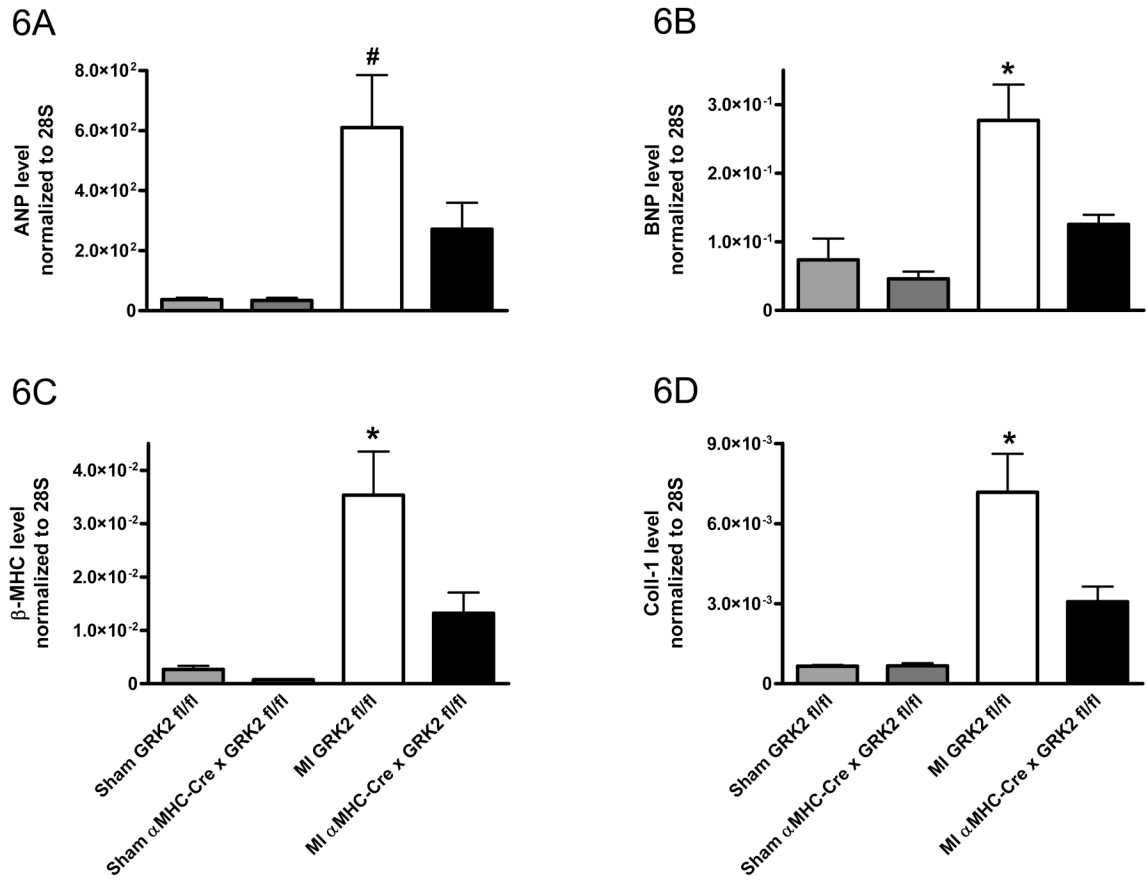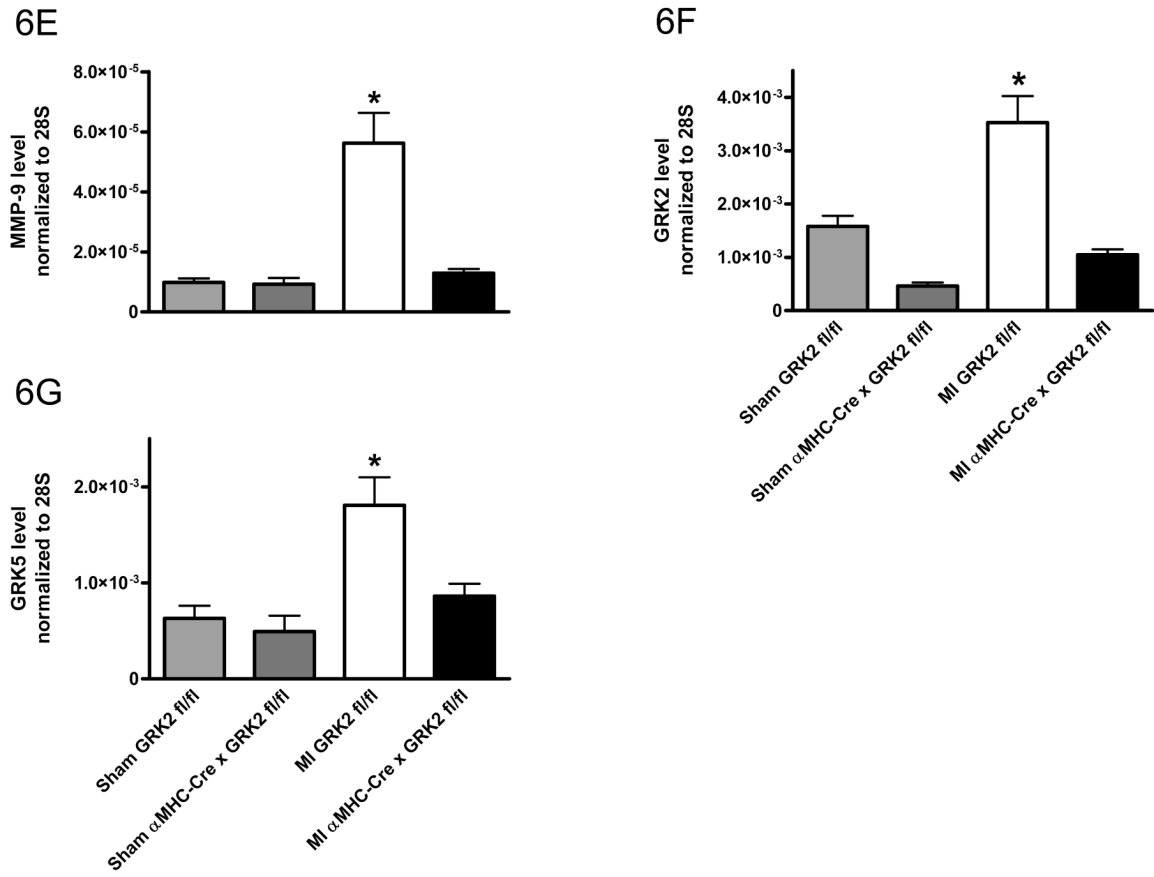
Figure 6.
Loss of GRK2 in cardiac myocytes alters gene expression in the failing heart. Quantitative RT-PCR analysis 28 days post MI in the remote non-infarcted region of the LV. A, ANP; B, BNP; C, β-MHC; D, Coll-1; E, MMP-9; F, GRK2; and G, GRK5. All values normalized to 28S levels. n=6–8 animals/group, *P<0.05 MI GRK2 fl/fl vs. MI αMHC-Cre × GRK2 fl/fl and Sham GRK2 fl/fl and Sham αMHC-Cre × GRK2 fl/fl, #P<0.05 MI GRK2 fl/fl vs. Sham GRK2 fl/fl, one-way ANOVA.
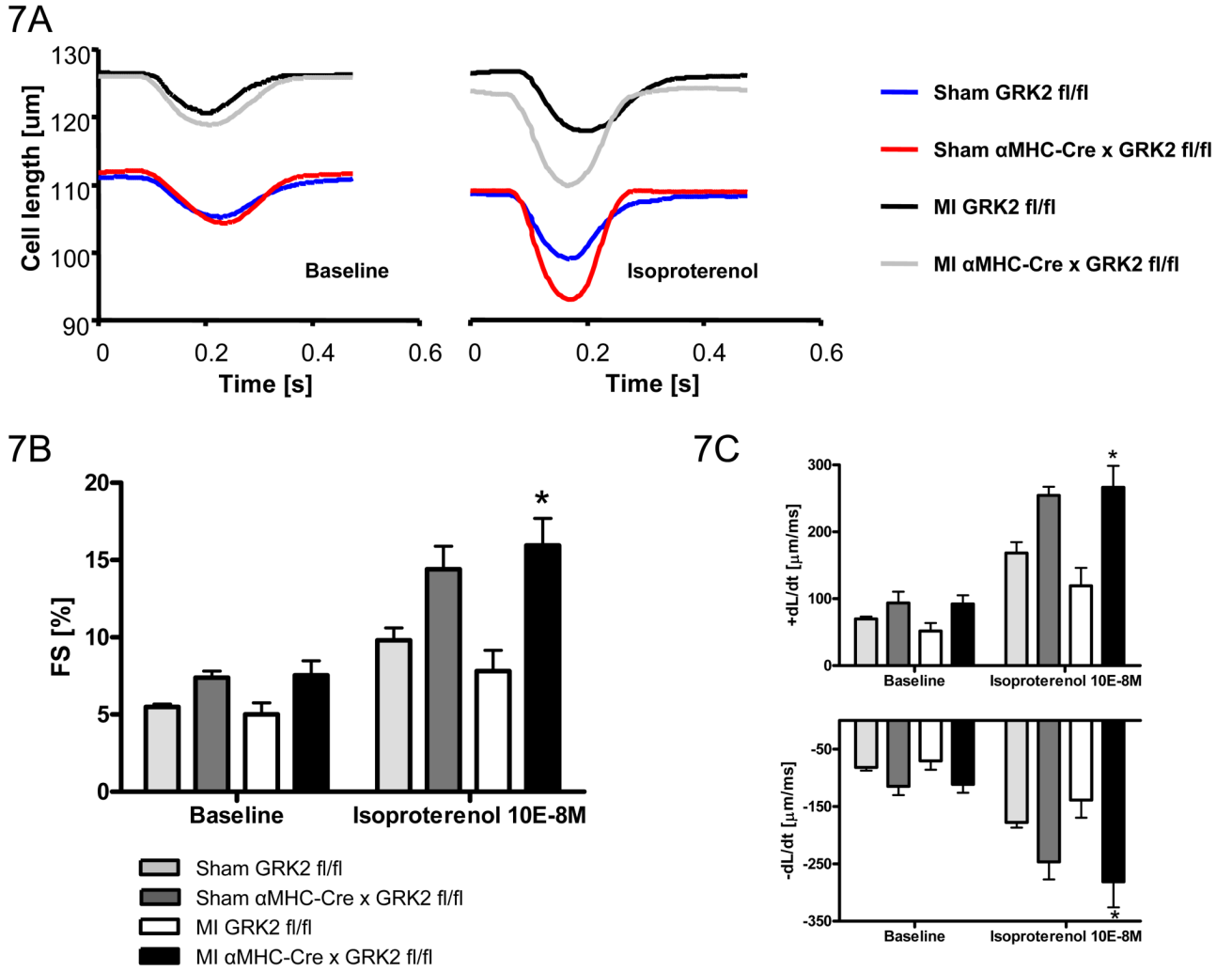
Figure 7.
Loss of GRK2 improves post-MI βAR responsiveness of single cardiac myocytes. A, Representative tracings of single myocyte contractility under baseline conditions and stimulation with isoproterenol. B, Quantitative data of FS. C, Maximum rate of cardiac myocyte contractility and relengthening. Cardiac myocytes were measured at baseline conditions and after stimulation with isoproterenol. n=3 animals/group for Sham mice, n= 6 animals/group for MI mice, *P<0.05 MI αMHC-Cre/GRK2(fl/fl) vs. MI GRK2(fl/fl) for respective condition, two-way ANOVA.