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# Differential Role of G Protein-Coupled Receptor Kinase 5 in Physiological Versus Pathological Cardiac Hypertrophy

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## Abstract

**Rationale—**G protein-coupled receptor (GPCR) kinases (GRKs) are dynamic regulators of cellular signaling. GRK5 is highly expressed within myocardium and is up-regulated in heart failure (HF). Although GRK5 is a critical regulator of cardiac GPCR signaling, recent data has uncovered non-canonical activity of GRK5 within nuclei that plays a key role in pathological hypertrophy. Targeted cardiac elevation of GRK5 in mice leads to exaggerated hypertrophy and early HF after transverse aortic constriction (TAC) due to GRK5 nuclear accumulation.

**Objective—**In this study we investigated the role of GRK5 in physiological, swimming induced hypertrophy (SIH).

**Methods and Results—**Cardiac-specific GRK5 transgenic mice (TgGRK5) and non-transgenic littermate control (NLC) mice were subjected to a 21-day high intensity swim protocol (or no swim sham controls). SIH and specific molecular and genetic indices of physiological hypertrophy were assessed including nuclear localization of GRK5 and compared to TAC.

Unlike after TAC, swim-trained TgGRK5 and NLC mice exhibited similar increases in cardiac growth. Mechanistically, SIH did not lead to GRK5 nuclear accumulation, which was confirmed in vitro as insulin-like growth factor-1, a known mediator of physiological hypertrophy, was unable to induce GRK5 nuclear translocation in myocytes. We found specific patterns of altered gene expression between TAC and SIH with GRK5 overexpression. Further, SIH in post-TAC TgGRK5 mice was able to preserve cardiac function.

**Conclusions—**These data suggest that while nuclear-localized GRK5 is a pathological mediator after stress, this non-canonical nuclear activity of GRK5 is not induced during physiological hypertrophy.

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**Disclosures**: None.

**Subject Terms**

Exercise; hypertrophy; heart failure; Cell Signaling/Signal Transduction; Genetically altered and Transgenic Models

## Introduction

G protein-coupled receptors (GPCRs) regulate various intracellular pathways, and are known to play an integral role in modulation of the cardiovascular system. GPCR's are regulated by GPCR kinases (GRKs) in a process termed “desensitization” leading to halting of the signal, receptor internalization and degradation or resensitization[1, 2]. GRK2 and GRK5 are the predominant GRKs expressed in the myocardium, and are known to be up-regulated in human heart failure (HF) where they can shut-off over-stimulated GPCRs such as β-adrenergic receptors[1]. The role of GRK2 in HF development after myocardial injury has been well documented[1, 3], however only recently has a critical role for GRK5 in HF pathogenesis begun to be elucidated.

Studying the role of increased myocardial GRK5, as seen in human HF, in a cardiac-specific transgenic mouse model (TgGRK5) has revealed a key role in HF pathogenesis after ventricular pressure-overload following transverse aortic constriction (TAC)[4-6]. With cardiac elevation of GRK5, it was found that following TAC there is exaggerated hypertrophic growth of the heart with accelerated maladaptation and early HF[4-6]. Interestingly, this phenotype does not depend on the canonical activity of GRK5 but rather its ability to localize in the nucleus of myocytes wherein it acts as a Class II histone deacetylase (HDAC) kinase resulting in nuclear export of HDAC5 and de-repression of cardiac hypertrophic gene transcription through myocyte enhancer factor 2 (MEF2)[4, 5]. Recently, we have discovered that in addition to the de-repression of MEF2 activity after TAC, GRK5 has the ability to bind DNA directly and in a kinase-independent manner act as a positive co-regulator of nuclear factor of activated T-cells (NFAT)-mediated hypertrophic gene transcription[6]. To confirm whether myocardial GRK5 is an endogenous HDAC5 kinase, mice with either global or cardiac myocyte specific GRK5 knockout (GRK5 KO) displayed significantly less hypertrophy and prevention of maladaptation after TAC with less HDAC5 exported from the nucleus[7]. These data confirm that GRK5 is a potent regulator of pathological hypertrophy; however, a role for this GRK in another form of hypertrophy, physiological hypertrophy, has yet to be elucidated.

Physiological hypertrophy occurs during pregnancy and after endurance training such as swimming. This form of hypertrophy is denoted by more uniform growth, with proportional increases in myocyte cell length and width resulting in favorable cardiac adaptations (i.e. anti-apoptotic, stimulation of myocyte renewal)[8, 9]. Most importantly, this form of hypertrophy does not lead to maladaptation and HF. Since TAC and other hypertrophic stimuli (such as the α-adrenergic agonist, phenylephrine (PE) and angiotensin II (AngII)) induce the nuclear localization and the above non-canonical activities of GRK5, we were interested in whether this also occurs within the context of physiological hypertrophy and if so, what mechanisms may be in play to prevent GRK5-mediated pathology. In the present

study, we used a high intensity swim training protocol to investigate whether increased myocyte GRK5 would alter the phenotype of swim induced hypertrophy (SIH) and whether any nuclear non-canonical actions of GRK5 are involved in this model of physiological hypertrophy.

## Methods

### Cell culture

Neonatal rat ventricular myocytes (NRVMs) were isolated from 1 to 2 day old rats, as previously described[10]. NRVMs were infected with recombinant, replication deficient adenovirus (GRK5, LacZ, GFP) at a MOI of 1-10 particles per cell.

### Experimental animals

All animal procedures were performed in accordance with the guidelines of the Institutional Animal Care and Use committee at Temple University School of Medicine.

### Swim protocol

A 21-day high intensity swim training protocol was utilized to induce SIH in mice, as previously described[11] (Online Figure I-A). Subsequently, a moderate exercise protocol was adapted from this protocol to train pressure-overload mice (Online Figure I-B).

### Transverse aortic constriction

Transverse aortic constriction (TAC) surgery was performed, as previously described[4-7].

### Echocardiography

Echocardiography was performed with the VisualSonics VeVo 2100 imaging system in anesthetized animals (2.0% isoflurane, vol/vol) to evaluate in vivo cardiac function, as described previously[6, 7].

### Cardiac myocytesize measurement

Cardiac myocyte cell diameter was determined from left ventricular sections from hearts of all study groups. Microphotographs of these specimens with Masson's trichrome staining were taken by a Nikon digital camera, and analyzed using ImageJ software. Similarly, NRVM cell size was evaluated in α-smooth muscle actin (SMA) stained cells.

### Nuclear fraction extraction

Nuclear extracts were prepared from NRVMs using a nuclear/cytosol fractionation kit (BioVision Inc., Milpitas, CA, USA), as previously described[6].

### RNA isolation

Total RNA was isolated from tissue samples for Real-time Polymerase Chain Reaction using the Trizol method, as previously described[7]. RT-PCR was performed for Brain Natriuretic Peptide (BNP), Regulator of Calcineurin (RCAN), Connective Tissue Growth Factor (CTGF), Collagen 3 (Col3) and Osteonectin (Sparc), as previously described[6]. Ultra-pure

RNA was isolated from tissue samples for RNA deep sequencing using the Norgen Total RNA purification kit, as previously described[12].

### RNAseq data analysis

Raw sequence reads were aligned to the mouse mm10 genome using the Tophat algorithm[13]; Cufflinks algorithm was implemented to assemble transcripts and estimate their abundance[14]. Cuffdiff was used to statistically assess expression changes in quantified genes in different conditions[15]. A false discovery rate (FDR) < 0.05 was used to define differential expression.

### Ingenuity pathway analysis (IPA)

Biological and interaction networks of candidate genes were generated using Qiagen's Ingenuity Pathway Analysis (IPA, Qiagen, Redwood City, CA, USA www.qiagen.com/ingenuity). IPA explores the set of input genes to identify networks by using Ingenuity Pathways Knowledge Base for interactions between genes. The candidate gene list was uploaded into the application for biological function enrichment analysis, and networks of Network Eligible Molecules were then algorithmically generated based on their connectivity.

### Drugs

In vitro hypertrophy was induced via either Insulin-like growth factor-1 (IGF-1, Sigma-Aldrich, St. Louis, MO, USA) or Phenylephrine (PE, Sigma-Aldrich, St. Louis, MO, USA).

### Protein-Immunoblotting

Immunoblotting was performed for GRK5, Total AKT, phospho-AKT (p-AKT), laminin A/C, fibrillarin, and GAPDH, as previously described[4, 5].

### Statistics

All the values in the text and figures are presented as mean +/- SEM. Statistical significance was determined by Student's t-test or ANOVA. P values of <0.05 were considered significant.

## Results

### SIH in mice with altered myocyte GRK5 levels

TgGRK5 mice with ∼30 fold overexpression of GRK5 in cardiac myocytes and non-transgenic littermate control (NLC) mice were put through a 21-day high intensity swim training protocol, which yields hypertrophy similar to that observed with treadmill training[11] (Online Figure I-A). At the conclusion of this protocol, cardiac function was assessed by echocardiography and heart weight to body weight (HW/BW) and heart weight to tibia length (HW/TL) ratios were obtained to measure hypertrophy. Both TgGRK5 and NLC mice exhibited significant increases in the HW/BW ratio after training compared to their respective untrained sham controls (Figure 1A, Online Figure II). Importantly, HW/BW and HW/TL ratios were not statistically different between the TgGRK5 and NLC trained groups. To reaffirm this finding, mean cell diameter was quantified in both sham and trained

TgGRK5 and NLC mice, and both the NLC and TgGRK5 trained groups exhibited significant increases in myocyte cell diameter relative to sham controls that again were not different between mice with endogenous levels of GRK5 or increased myocyte levels (Figure 1B-C). These data suggest that overexpression of GRK5 does not alter the development of SIH. To address the ultimate role of GRK5 in physiological hypertrophy, global GRK5 KO animals were subjected to swim training. GRK5 KO mice exhibited significant hypertrophy after training, which was not statistically different when compared to control and TgGRK5 mice (Online Figure II).

Exercise is commonly associated with favorable cardiac adaptations[16, 17], thus cardiac function was evaluated via echocardiography pre and post training in NLC and TgGRK5 mice. Echocardiography revealed no significant changes in left ventricular (LV) ejection fraction % (EF%) in trained mice compared to their corresponding sham controls (Figure 1D). In agreement with the presence of SIH, echocardiography revealed that both TgGRK5 and NLC mice exhibited significant but identical increases in systolic posterior wall thickness (sPWT) post training relative to sham controls (Figure 1E). All other functional data has been tabulated and presented in Online Table I. To confirm effectiveness of our training protocol, we evaluated heart rate to stroke volume (HR/SV) ratio in trained NLC and TgGRK5 mice relative to sham controls (Figure 1F, Online Table I). As expected the HR/SV relationship was significantly reduced in both trained groups relatively to their respective sham controls showing the effectiveness of the training. Taken together, these data suggest that GRK5 does not actively play a role in SIH, which is in stark contrast to GRK5 being critically involved in pressure-overload induced hypertrophy[4-7].

Swim training is a standard method to study physiological hypertrophy within the cardiovascular field[11, 18]; however, a criticism of forced training protocols is that they have been reported to induce stress during training, including adrenergic stress[19, 20]. To address this question, we administered isoproterenol (ISO, 30 mg/kg/day), a chronic stress, via mini-osmotic pumps to TgGRK5 and NLC mice. Mice were pumped with phospho-buffered saline (PBS) to serve as an appropriate control. ISO administration led to significant and identical increases in HW/BW ratios in both TgGRK5 and NLC mice relative to their respective PBS pumped controls (Online Figure III). In addition, the hypertrophy observed in both TgGRK5 and NLC mice after ISO infusion was similar to that observed with training, in agreement with prior findings showing that ISO pumping does not lead to more cardiac hypertrophy than swim-training[21]. Taken together, GRK5 overexpression does not appear to significantly influence cardiac growth induced by swim training or chronic β-adrenergic stimulation.

### Swim training does not alter nuclear GRK5 levels

Our initial findings show that manipulation of GRK5 levels in the heart (i.e. ablation or overexpression) produces a characteristic hypertrophic response after swimming that appears identical to mice with endogenous levels of GRK5. Since GRK5-mediated pathological hypertrophy is dependent on its nuclear localization, we wanted to determine whether swimming alters the localization of GRK5 within the myocyte. Accordingly, cardiac nuclear fractions were generated from sham and trained TgGRK5 mice along with post-TAC

TgGRK5 mice serving as a positive control for nuclear GRK5 accumulation (Figure 2, Online Figure IV-A-E). As expected, TgGRK5 mice that underwent TAC displayed significantly increased nuclear GRK5 levels compared to sham, however, swim-training did not alter nuclear localization. Moreover, GRK5 remained at the membrane of myocytes after exercise (Online Figure IV-F). Therefore, swim training as a physiological hypertrophic stimuli does not appear to induce GRK5 nuclear targeting.

### Role of GRK5 on the IGF-1 hypertrophic signaling axis

Prior studies have demonstrated that SIH exerts its beneficial effects, in part via activation of the IGF-1/PI3K/AKT/PKB/mTOR signaling axis[16]. Thus, to reproduce our in vivo findings above in vitro, we treated NRVMs that had either endogenous or overexpressed levels of GRK5 after adenoviral (Ad)- mediated gene delivery (with Ad-LacZ or Ad-GFP as a control) with IGF-1 treatment and measured cell growth. Following IGF-1 stimulation (10 nM/L for 24 hrs) we stained for α-SMA in NRVMs and measured mean cell area (Figure 3A-B). IGF-1 stimulation produced significant increases in myocyte growth that was equal regardless of GRK5 expression levels (Figure 3A-B). In contrast, using PE to stimulate α-1 adrenergic receptors that have been linked to pathological myocyte growth, we found that myocytes with elevated GRK5 expression had an enhanced response (Figure 3A-B) consistent with previous reports [4-6]. Mechanistically, we found that consistent with elevated GRK5 levels not altering IGF-1 induced myocyte hypertrophy, this ligand did not promote nuclear accumulation of GRK5, while PE did (Figure 3C-D, Online Figure V-B-F). In addition, PE led to GRK5 nuclear localization in adult ventricular myocytes while IGF-1 did not (Online Figure V-A).

Phosphorylation leading to activation of AKT is known to occur downstream of IGF-1 signaling, and this is believed to be a key molecule involved in the beneficial aspects of SIH. Therefore, we measured p-AKT at Serine 473 as a surrogate marker of the IGF-1 dependent SIH stimulus pathway in control and GRK5 overexpressing myocytes. As expected, IGF-1 stimulation (10 nM/L for 10 minutes) led to significantly increased p-AKT levels, however an equivalent activation regardless of GRK5 expression was observed (Figure 3E-F). Importantly, pre-incubation with LY 294,002, a selective PI3K inhibitor (LY, 10 uM/L for 10 minutes), significantly blunted IGF-1 induced p-AKT levels in all cells. Taken together, IGF-1, a ligand previously known to be associated with induction of physiological hypertrophy, produces its results in myocytes without an influence by GRK5 levels, which is consistent with our observed results found with swimming.

### Swim training after TAC slows the progression towards HF in TgGRK5 mice

Swim training is known to promote favorable cardiac outcomes including protection from various cardiac insults[17, 22, 23]. We have shown that when GRK5 is elevated in cardiac myocytes an early and progressive HF phenotype arises after TAC[4-6]. Since the above data demonstrates minimal involvement of GRK5 in the induction of SIH and a lack of GRK5 nuclear targeting, which is a novel regulator of maladaptive hypertrophy and HF[4-7], we tested whether training could alter the post-TAC phenotype of TgGRK5 mice. To accomplish this, we adopted a modified exercise protocol in which one week post-TAC TgGRK5 mice, a time period where it is well established that these GRK5 overexpressing

mice have an exaggerated hypertrophic response, underwent a moderate swim training program (TAC+EX) for one week (Online Figure I-B). Sham, un-trained TgGRK5 mice served as controls. TAC+EX TgGRK5 mice exhibited significantly increased HW/BW ratios relative to sham controls (Figure 4A); however, HW/BW ratios were not statistically different between TAC+EX and TAC TgGRK5 mice. Mean myocyte diameter was also evaluated via Mason's Trichrome stained heart sections from these mice, and in agreement with our previous findings, post-TAC TgGRK5 mice exhibited significantly increased cell diameter relative to sham controls that was not further altered by swimming (Figure 4C). Interestingly, although these data suggest that swimming has no effect on the cardiac growth observed post-TAC, we found that swim-training significantly ameliorated the accelerated maladaptation and ventricular dysfunction after TAC due to GRK5 overexpression (Figure 4). As shown in Figure 4D, LV EF% as determined by echocardiography was significantly decreased 2 weeks after TAC in TgGRK5 mice, but significantly rescued by one week of swimming. The beneficial effect of swimming on GRK5-mediated cardiac dysfunction post-TAC was also maintained for up to 3 weeks (data not shown). Additional functional data have been tabulated and are presented in Online Figure II.

Mechanistically, we examined the nuclear translocation of GRK5 in myocytes after PE to stimulate TAC-induced hypertrophy followed by IGF-1 treatment to mimic molecular induction of SIH. Importantly after IGF-1 treatment, there were similar levels of GRK5 in the nucleus downstream of PE stimulation (Figure 4E-F, Online Figure VI-A). Similar data were found in vivo (Online Figure VI-B). Taken together, these findings suggest that swim training after TAC leads to hypertrophy similar to that observed with TAC alone, but can slow the deterioration of cardiac performance in TgGRK5 mice post-TAC. Further, inhibition of GRK5 nuclear accumulation does not appear to be the mechanism by which this protection occurs.

### GRK5-dependent transcriptome analysis following exercise and pressure-overload

It is well known that physiological and pathological hypertrophy differentially activate various signaling pathways, leading to distinct gene regulation programs[8, 24]. However, since GRK5 itself can induce hypertrophic gene regulation, at least in part through NFAT activation[6], we used RNA Seq technology to assess transcripts expressed in mouse hearts after TAC, and also after swim training when GRK5 is elevated. In Figure 5A, a heat map is displayed that illustrates 43 genes up-regulated (red) and 10 mRNAs downregulated (green) in post-TAC (2 weeks) TgGRK5 mouse hearts relative to sham controls. The genes upregulated within this group are predominated by known regulators of cardiac apoptosis and fibrosis associated with HF[25]. Conversely, Figure 5B illustrates and lists the 37 gene transcripts that are elevated (green) with SIH when GRK5 is elevated in myocytes as well as 20 mRNAs down-regulated (red) with exercise relative to sham controls. Interestingly, these transcripts are from genes largely associated with metabolism. We next directly compared genes selectively regulated by TAC versus swim (Figure 5C-D). This analysis revealed that 59 gene transcripts are increased (red) in trained TgGRK5 mice compared to post-TAC TgGRK5 mice, and these are primarily genes associated with metabolism and cell maintenance (Figure 5C). Conversely, 84 genes are upregulated (green) after TAC in TgGRK5 mice compared to swim training (Figure 5D-E). These upregulated genes are

known regulators of inflammation, fibrosis, and apoptosis; all of which play prominent roles in the transition of the myocardium to a diseased state and presumably are active post-TAC when GRK5 is overexpressed. Subsequently, Ingenuity network analysis[26] illustrates genes upregulated (red) and downregulated (green) Post-TAC relative to Post-Swim in TgGRK5 mice with an emphasis on (Figure 6A) Connective Tissue Disorders, Organismal Injury and Abnormalities, Dermatological Diseases and Conditions, and (Figure 6B) Organismal Injury and Abnormalities, Cellular Development, Connective Tissue Development and Function. These genes are known to be involved in cardiac hypertrophic signaling, interferon signaling, and also regulation of cardiac hypertrophy by NFAT. These findings suggest that the maladaptive hypertrophy mediated through GRK5 overexpression after TAC is due primarily to activation of various interaction networks leading to cell death. Since GRK5 overexpression doesn't alter SIH, the gene regulation imparted by GRK5 overexpression is likely minimal. However both sets of gene expression data are unique, thereby linking GRK5 to its canonical and non-canonical actions in swimming and TAC, respectively. A complete list of these transcriptomes are included in Online Tables III-VIII.

Several transcripts from the RNA Seq data were confirmed by RT-PCR and transcripts upregulated in TgGRK5 post TAC hearts, but not swim and vice versa were indeed confirmed. Some of these are shown in Online Figure VII.

## Discussion

The present study presents evidence that GRK5, via its non-canonical localization and activity, is a critical regulator of pathological hypertrophy with no apparent role in physiological hypertrophy as a result of swim training. Training of both TgGRK5 (cardiac specific GRK5 overexpressors) and NLC mice led to similar increases in cardiac hypertrophy, which could be recapitulated in vitro via IGF-1 stimulation of cardiac myocytes. GRK5 nuclear localization, which is the triggering mechanism for pathological hypertrophy induced by GRK5 after pressure-overload, was unaltered in SIH. Overexpression of GRK5 is relevant to cardiac growth since it has been found to be up-regulated in compromised hearts including failing myocardium[27]. Interestingly, although exercise did not alter hypertrophic growth in post-TAC TgGRK5 mice, it did slow the progression to maladaptation and ventricular dysfunction, which provides evidence that GRK5-mediated pathology can be potentially deterred even when GRK5 is significantly up-regulated.

Cardiac hypertrophy is an example of a compensatory adaptation that can occur in the heart with increased demand and can be either ultimately irreversible and pathological or reversible and physiological in nature[8]. Physiological hypertrophy occurs with exercise or pregnancy and is associated with favorable cardiac adaptation[8, 24]. Conversely, pathological hypertrophy can ultimately lead to decreased cardiac function, cell death and HF[8, 24]. We have previously found that GRK5, a kinase shown to be upregulated in the diseased heart[27], is a facilitator of maladaptive or pathological hypertrophy[4-7]. Pathological hypertrophic stimuli that act through the activation of Gq-coupled receptors (e.g. TAC, α-1 adrenergic agonists, or AngII) can induce GRK5's negative effects on cardiac growth via first its Calcium ($Ca^{2+}$) -calmodulin-dependent translocation to the nucleus followed by novel, non-

canonical actions of this kinase that involve its catalytic activity as well as kinase-independent DNA binding. In the nucleus of myocytes GRK5 can act as a HDAC5 kinase, which leads to induction of MEF2-mediated hypertrophic gene transcription[4, 5, 7]. In addition, we have recently found that in a kinase-independent manner, GRK5 in the nucleus can be a robust modulator of NFAT-mediated hypertrophic gene transcription[6]. Thus, there are multiple mechanistic signaling pathways involved in GRK5's role in pathological hypertrophy. In fact, nuclear targeting of GRK5 is the sole determinant of its pathological effects as mice with cardiac-specific overexpression of a mutant GRK5 that cannot localize to the nucleus are devoid of any negative effects after pressure-overload[4]. Since GRK5 is up-regulated in diseased myocardium, it was important in this study to determine if it also has a negative role in swim-induced cardiac growth since GRK5 appears to be a potential therapeutic target in maladaptive hypertrophic conditions. Therefore, our present study is promising since we have shown that GRK5 plays a minimal role in SIH suggesting that inhibition of GRK5 in the nucleus would not have an influence on this favorable myocyte growth condition.

Physiological hypertrophy has been induced in murine models via various exercise training models ranging from forced/voluntary treadmill training to forced swim training[18]. The fact that our modest training model induced similar cardiac growth and functional changes regardless of GRK5 expression levels also supports the findings that NFAT is not involved in SIH[11] since GRK5 appears to be an important cofactor[6]. This conclusion is most supported by our data that SIH does not induce the nuclear translocation and accumulation of GRK5, which prevents it from interacting with NFAT and its DNA binding targets. That is, GRK5 nuclear levels are similar between sham and SIH TgGRK5 mice. The lack of nuclear targeting of GRK5 post-swim suggests that the signaling pathways responsible for induction of SIH do not involve the Gq-mediated signals that key the $Ca^{2+}$-calmodulin dependent mechanisms responsible for GRK5 nuclear translocation[5]. A recent study has suggested that ISO induced cardiac hypertrophy leads to similar levels of hypertrophy to that observed with swim training, due to an Inositol trisphosphate ($IP_3$) receptor augmented $Ca^{2+}$ release activated by a concomitant catecholamine release[21]. Our data reaffirms this finding, and present further evidence that the detrimental effects of GRK5 are not reliant on β-adrenergic receptor stimulation, consistent with a lack of GRK5 nuclear accumulation after chronic ISO admistration[5].

Importantly, in vitro experiments in adult mouse and neonatal rat myocytes stimulated with IGF-1, a ligand known to induce SIH[16], reaffirmed our in vivo findings such that unlike PE and Gq activation, IGF-1 does not lead to appreciable nuclear GRK5 accumulation nor did GRK5 overexpression alter AKT activation downstream of IGF-1 treatment in NRVMs. It has been demonstrated that chronic training in an ischemic rat HF model leads to reduced responsiveness to α-1 adrenergic stimuli[28]. Clinically, this has been supported by the observation that walking training in chronic human HF patients leads to a significant reduction in AngII levels[29]. Further in myocytes, IGF-1 stimulation has been shown to increase both cytosolic and nuclear levels of $[Ca^{2+}]_i$ in an $IP_3$ dependent manner[30]. Although $[Ca^{2+}]_i$ and thus, $Ca^{2+}$-calmodulin association increase with IGF-1 stimulation, it is likely that the $Ca^{2+}$ pool from this stimulation and hence exercise, does not appear to interact with GRK5. Accordingly, we speculate that pathological hypertrophic stimuli (i.e.

Gq activation, PE), activate a pool of $Ca^{2+}$ unique from that activated in physiological hypertrophy, ultimately leading to GRK5 nuclear translocation and its subsequent deleterious nuclear signaling paradigm.

Several previous studies in the literature have been performed in non-transgenic rodents to demonstrate the beneficial effects of exercise either prior to or after cardiac insult[22, 31-33]. Thus, we didn't study non-transgenic animals alone for swimming since our goal was to specifically address whether exercise in the face of cardiac-specific overexpression of GRK5 led to pathological changes and if swimming caused the nuclear localization of GRK5. Although swimming post-TAC TgGRK5 mice one week after TAC led to similar degrees of cardiac hypertrophy to those observed in untrained post-TAC TgGRK5 mice, swim trained mice had less ventricular dysfunction. A one week recovery period post-TAC prior to starting exercise was instituted due to the high mortality associated with pressure-overload surgery[34, 35], and the fact that cardiac-specific GRK5 overexpression accelerates HF development [4-7]. Our finding showing that swimming ameliorated cardiac dysfunction, but not cardiac growth was consistent with our mechanistic studies in vivo and in vitro which demonstrated that physiological stimulus did not alter GRK5 nuclear accumulation in myocytes. Thus, the beneficial effects seen in trained post-TAC GRK5 overexpressing mice must be due to a non-GRK5 event, much like the overall swimming phenotype presented in present study. Further, these findings agree with recent clinical findings which suggest moderate exercise shortly after cardiac insult can improve cardiac function and ultimately lifespan in humans[36]. The mechanism by which cardiac function is preserved in trained TAC TgGRK5 mice remains to be elucidated.

A novel data set revealed by our study using RNA Seq technology was the swim training transcriptome versus the post-TAC transcriptome with elevated myocyte GRK5 expression. It is well known that physiological and pathological hypertrophy differentially activate various signaling pathways, leading to distinct gene regulation programs[8, 24]. However to date, no study has compared the differences in gene regulation in TgGRK5 mice during both pathological and physiological hypertrophy. A recent study utilized deep RNA-sequencing to characterize gene regulation in TgGRK5 mice with specific focus on modified gene expression that was dependent on Gq-mediated signaling as it pertains to pathological hypertrophy and the post-TAC micro-enviroment[37]. Accordingly, we utilized this approach to screen differences in gene regulation between trained and TAC TgGRK5 mice. This data revealed that the genes upregulated with training in TgGRK5 mice were primarily related to cell maintenance, metabolism and growth. Conversely, TAC induced up-regulation of various detrimental genes associated with cell death, inflammation, fibrosis, and cardiac remodeling. Importantly, a role for nuclear GRK5 actions in maladaptive hypertrophy and not physiological hypertrophy were largely confirmed in this gene expression data set such that genes upregulated in TgGRK5 mice post-TAC have been identified previously with pressure-overload[37]. However, several genes were significantly more induced with GRK5 overexpression. Conversely, we checked several genes in TgGRK5 mice after swim training, and SIH leads to no observed changes relative to untrained mice suggesting a negligible role of GRK5 in this form of adaptive cardiac hypertrophy.

Due to this non-canonical action of GRK5 in the nucleus of myocytes and the fact that this kinase is up-regulated in diseased myocardium it has been suggested that it could be a target for inhibition in HF. Accordingly, screens have been conducted to evaluate potential GRK5 inhibitors, and a recent study demonstrated that a small molecule, amlexanox, was able to inhibit GRK5 thus blocking myocyte MEF2 activation after PE stimulation, which is consistent with an involvement of GRK5 in this aspect of pathological hypertrophy[38]. Further studies are needed to evaluate in vivo if compounds such as amlexanox are able to reverse the deleterious effects associated with TAC in TgGRK5 mice.

In summary, the present study presents evidence that GRK5 acts as a hypertrophic facilitator only under pathological conditions and does not affect physiological growth of the heart even when robustly overexpressed. This appears to be due to the fact that mediators of SIH, including IGF-1, do not induce the nuclear localization and accumulation of GRK5, which is needed for its pathological effects. The differences between GRK5's role in both of these hypertrophic conditions are summarized in Figure 7. Further studies will continue to assess genes specifically regulated by nuclear GRK5 levels and their association with either maladaptive or physiological cardiac hypertrophy.

## Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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## References

1. Lymperopoulos A, Rengo G, Koch WJ. Adrenergic nervous system in heart failure: Pathophysiology and therapy. Circ Res. 2013; 113:739–753. [PubMed: 23989716]
2. Kamal FA, Travers JG, Blaxall BC. G protein-coupled receptor kinases in cardiovascular disease: Why “Where” Matters. Trends Cardiovasc Med. 2012; 22:213–219. [PubMed: 23062971]
3. Woodall MC, Ciccarelli M, Woodall BP, Koch WJ. G protein-coupled receptor kinase 2: A link between myocardial contractile function and cardiac metabolism. Circ Res. 2014; 114:1661–1670. [PubMed: 24812353]
4. Martini JS, Raake P, Vinge LE, DeGeorge BR Jr, Chuprun JK, Harris DM, Gao E, Eckhart AD, Pitcher JA, Koch WJ. Uncovering g protein-coupled receptor kinase-5 as a histone deacetylase kinase in the nucleus of cardiomyocytes. Proc Natl Acad Sci U S A. 2008; 105:12457–12462. [PubMed: 18711143]
5. Gold JI, Martini JS, Hullmann J, Gao E, Chuprun JK, Lee L, Tilley DG, Rabinowitz JE, Bossuyt J, Bers DM, Koch WJ. Nuclear translocation of cardiac g protein-coupled receptor kinase 5 downstream of select gq-activating hypertrophic ligands is a calmodulin-dependent process. PLoS One. 2013; 8:e57324. [PubMed: 23472081]

6. Hullmann JE, Grisanti LA, Makarewich CA, Gao E, Gold JI, Chuprun JK, Tilley DG, Houser SR, Koch WJ. Grk5-mediated exacerbation of pathological cardiac hypertrophy involves facilitation of nuclear nfat activity. Circ Res. 2014; 20:304475.
7. Gold JI, Gao E, Shang X, Premont RT, Koch WJ. Determining the absolute requirement of g protein-coupled receptor kinase 5 for pathological cardiac hypertrophy: Short communication. Circ Res. 2012; 111:1048–1053. [PubMed: 22859683]
8. van Berlo JH, Maillet M, Molkentin JD. Signaling effectors underlying pathologic growth and remodeling of the heart. J Clin Invest. 2013; 123:37–45. [PubMed: 23281408]
9. Huang CY, Yang AL, Lin YM, Wu FN, Lin JA, Chan YS, Tsai FJ, Tsai CH, Kuo CH, Lee SD. Anti-apoptotic and pro-survival effects of exercise training on hypertensive hearts. 1985. 2012; 112:883–891.
10. Piper, H. Cell culture techniques in heart and vessel research. Springer; Berlin Heidelberg: 1990.
11. Wilkins BJ, Dai YS, Bueno OF, Parsons SA, Xu J, Plank DM, Jones F, Kimball TR, Molkentin JD. Calcineurin/nfat coupling participates in pathological, but not physiological, cardiac hypertrophy. Circ Res. 2004; 94:110–118. [PubMed: 14656927]
12. Mahler GJ, Frendl CM, Cao Q, Butcher JT. Effects of shear stress pattern and magnitude on mesenchymal transformation and invasion of aortic valve endothelial cells. Biotechnol Bioeng. 2014; 111:2326–2337. [PubMed: 24898772]
13. Trapnell C, Pachter L, Salzberg SL. Tophat: Discovering splice junctions with rna-seq. Bioinformatics. 2009; 25:1105–1111. [PubMed: 19289445]
14. Trapnell C, Williams BA, Pertea G, Mortazavi A, Kwan G, van Baren MJ, Salzberg SL, Wold BJ, Pachter L. Transcript assembly and quantification by rna-seq reveals unannotated transcripts and isoform switching during cell differentiation. Nat Biotechnol. 2010; 28:511–515. [PubMed: 20436464]
15. Trapnell C, Hendrickson DG, Sauvageau M, Goff L, Rinn JL, Pachter L. Differential analysis of gene regulation at transcript resolution with rna-seq. Nat Biotechnol. 2013; 31:46–53. [PubMed: 23222703]
16. McMullen JR, Shioi T, Huang WY, Zhang L, Tarnavski O, Bisping E, Schinke M, Kong S, Sherwood MC, Brown J, Riggi L, Kang PM, Izumo S. The insulin-like growth factor 1 receptor induces physiological heart growth via the phosphoinositide 3-kinase (p110alpha) pathway. J Biol Chem. 2004; 279:4782–4793. [PubMed: 14597618]
17. Bostrom P, Mann N, Wu J, Quintero PA, Plovie ER, Panakova D, Gupta RK, Xiao C, MacRae CA, Rosenzweig A, Spiegelman BM. C/ebpbeta controls exercise-induced cardiac growth and protects against pathological cardiac remodeling. Cell. 2010; 143:1072–1083. [PubMed: 21183071]
18. Wang Y, Wisloff U, Kemi OJ. Animal models in the study of exercise-induced cardiac hypertrophy. Physiol Res. 2010; 59:633–644. [PubMed: 20406038]
19. Zheng L, Zheng X. Integration of animal behaviors under stresses with different time courses. Neural Regen Res. 2014; 9:1464–1473. [PubMed: 25317159]
20. Droste SK, Chandramohan Y, Hill LE, Linthorst AC, Reul JM. Voluntary exercise impacts on the rat hypothalamic-pituitary-adrenocortical axis mainly at the adrenal level. Neuroendocrinology. 2007; 86:26–37. [PubMed: 17595533]
21. Nakayama H, Bodi I, Maillet M, DeSantiago J, Domeier TL, Mikoshiba K, Lorenz JN, Blatter LA, Bers DM, Molkentin JD. The ip3 receptor regulates cardiac hypertrophy in response to select stimuli. Circ Res. 2010; 107:659–666. [PubMed: 20616315]
22. Moreira-Goncalves D, Henriques-Coelho T, Fonseca H, Ferreira RM, Amado F, Leite-Moreira A, Duarte JA. Moderate exercise training provides left ventricular tolerance to acute pressure overload. Am J Physiol Heart Circ Physiol. 2011; 300:H1044–1052. [PubMed: 21186273]
23. Xu X, Wan W, Powers AS, Li J, Ji LL, Lao S, Wilson B, Erikson JM, Zhang JQ. Effects of exercise training on cardiac function and myocardial remodeling in post myocardial infarction rats. J Mol Cell Cardiol. 2008; 44:114–122. [PubMed: 17980387]
24. Dorn GW 2nd. The fuzzy logic of physiological cardiac hypertrophy. Hypertension. 2007; 49:962–970. [PubMed: 17389260]
25. Garcia M, Kassab GS. Right coronary artery becomes stiffer with increase in elastin and collagen in right ventricular hypertrophy. 1985. 2009; 106:1338–1346.

26. Calvano SE, Xiao W, Richards DR, Felciano RM, Baker HV, Cho RJ, Chen RO, Brownstein BH, Cobb JP, Tschoeke SK, Miller-Graziano C, Moldawer LL, Mindrinos MN, Davis RW, Tompkins RG, Lowry SF. A network-based analysis of systemic inflammation in humans. Nature. 2005; 437:1032–1037. [PubMed: 16136080]
27. Aguero J, Almenar L, Monto F, Oliver E, Sanchez-Lazaro I, Vicente D, Martinez-Dolz L, D'Ocon P, Rueda J, Salvador A. Myocardial g protein receptor-coupled kinase expression correlates with functional parameters and clinical severity in advanced heart failure. J Card Fail. 2012; 18:53–61. [PubMed: 22196842]
28. Palmer BM, Olsson MC, Lynch JM, Mace LC, Snyder SM, Valent S, Moore RL. Chronic run training suppresses alpha-adrenergic response of rat cardiomyocytes and isovolumic left ventricle. Am J Physiol. 1999; 277:H2136–2144. [PubMed: 10600831]
29. Braith RW, Welsch MA, Feigenbaum MS, Kluess HA, Pepine CJ. Neuroendocrine activation in heart failure is modified by endurance exercise training. J Am Coll Cardiol. 1999; 34:1170–1175. [PubMed: 10520808]
30. Ibarra C, Estrada M, Carrasco L, Chiong M, Liberona JL, Cardenas C, Diaz-Araya G, Jaimovich E, Lavandero S. Insulin-like growth factor-1 induces an inositol 1, 4, 5-trisphosphate-dependent increase in nuclear and cytosolic calcium in cultured rat cardiac myocytes. J Biol Chem. 2004; 279:7554–7565. [PubMed: 14660553]
31. McMullen JR, Amirahmadi F, Woodcock EA, Schinke-Braun M, Bouwman RD, Hewitt KA, Mollica JP, Zhang L, Zhang Y, Shioi T, Buerger A, Izumo S, Jay PY, Jennings GL. Protective effects of exercise and phosphoinositide 3-kinase (p110alpha) signaling in dilated and hypertrophic cardiomyopathy. Proc Natl Acad Sci U S A. 2007; 104:612–617. [PubMed: 17202264]
32. Lachance D, Plante E, Bouchard-Thomassin AA, Champetier S, Roussel E, Drolet MC, Arsenault M, Couet J. Moderate exercise training improves survival and ventricular remodeling in an animal model of left ventricular volume overload. Circ Heart Fail. 2009; 2:437–445. [PubMed: 19808374]
33. Konhilas JP, Watson PA, Maass A, Boucek DM, Horn T, Stauffer BL, Luckey SW, Rosenberg P, Leinwand LA. Exercise can prevent and reverse the severity of hypertrophic cardiomyopathy. Circ Res. 2006; 98:540–548. [PubMed: 16439687]
34. Mohammed SF, Storlie JR, Oehler EA, Bowen LA, Korinek J, Lam CS, Simari RD, Burnett JC Jr, Redfield MM. Variable phenotype in murine transverse aortic constriction. Cardiovasc Pathol. 2012; 21:188–198. [PubMed: 21764606]
35. deAlmeida AC, van Oort RJ, Wehrens XH. Transverse aortic constriction in mice. J Vis Exp. 2010; (38):1729. [PubMed: 20410870]
36. Giallauria F, Acampa W, Ricci F, Vitelli A, Torella G, Lucci R, Del Prete G, Zampella E, Assante R, Rengo G, Leosco D, Cuocolo A, Vigorito C. Exercise training early after acute myocardial infarction reduces stress-induced hypoperfusion and improves left ventricular function. Eur J Nucl Med Mol Imaging. 2013; 40:315–324. [PubMed: 23224706]
37. Zhang Y, Matkovich SJ, Duan X, Gold JI, Koch WJ, Dorn GW 2nd. Nuclear effects of g-protein receptor kinase 5 on histone deacetylase 5-regulated gene transcription in heart failure. Circ Heart Fail. 2011; 4:659–668. [PubMed: 21768220]
38. Homan KT, Wu E, Cannavo A, Koch WJ, Tesmer JJ. Identification and characterization of amlexanox as a g protein-coupled receptor kinase 5 inhibitor. Molecules. 2014; 19:16937–16949. doi:16910.13390/molecules191016937. [PubMed: 25340299]


## Nonstandard Abbreviations and Acronyms

| | |
|---|---|
| **Ad** | Adenoviral |
| **AngII** | Angiotensin II |
| **BNP** | Brain Natriuretic Peptide |
| **$Ca^{2+}$** | Calcium |

| | |
|---|---|
| **Col3** | Collagen 3 |
| **CTGF** | Connective Tissue Growth Factor |
| **EF%** | Ejection Fraction % |
| **FDR** | False Discovery Rate |
| **GPCR** | G protein-coupled receptor |
| **GRK** | G protein-coupled receptor kinase |
| **GRK5 KO** | GRK5 knockout |
| **HDAC** | Histone deacetylase |
| **HF** | Heart failure |
| **HR/SV** | Heart rate/Stroke volume Ratio |
| **HW/BW** | Heart weight/Body weight Ratio |
| **HW/TL** | Heart weight/Tibia length Ratio |
| **IGF-1** | Insulin-like growth factor 1 |
| **IPA** | Ingenuity Pathway Analysis |
| **$IP_3$** | Inositol trisphosphate |
| **ISO** | Isoproterenol |
| **LV** | Left ventricular |
| **LY** | LY 294,002 |
| **MEF2** | Myocyte enhancer factor 2 |
| **NFAT** | Nuclear factor of activated T cells |
| **NLC** | Non transgenic littermate control |
| **NS** | Non-significant |
| **NRVMs** | Neonatal rat ventricular myocytes |
| **p-AKT** | Phosphorylated AKT (Serine 473) |
| **PBS** | Phospho buffered saline |
| **PE** | Phenylephrine |
| **RCAN** | Regulator of calcineurin |
| **SIH** | Swim induced hypertrophy |
| **SMA** | α-Smooth muscle actin |
| **Sparc** | Osteonectin |
| **sPWT** | Systolic posterior wall thickness |
| **TAC** | Transverse aortic constriction |

| | |
|---|---|
| **TAC+EX** | Trans aortic constriction followed by exercise |
| **TgGRK5** | Transgenic GRK5 |
| **Unt** | Untreated |

## Novelty and Significance

**What Is Known?**

- G protein-coupled receptor kinase 5 (GRK5) is upregulated in models of heart failure (HF), as well as in the failing human heart.
- GRK5 translocates from the membrane to the nucleus via a functional nuclear localization sequence, where it has non-canonical GRK functions involved in pathological hypertrophy including being a histone deacteylase 5 (HDAC5) kinase and acting as a co-factor of nuclear factor of activated T-cells (NFAT) at the level of DNA.
- Increased nuclear GRK5 in the setting of pressure-overload and specific hypertrophic agonists is pathological.

**What New Information Does This Article Present?**

- Physiological hypertrophy, induced by exercise (swim training) leads to similar levels of cardiac growth regardless of the level of GRK5 expression (i.e. ablation, overexpression)
- Nuclear accumulation of GRK5, a hallmark of its role in pathological hypertrophy, is not induced during physiological hypertrophy.
- Swim training before pressure-overload can preserve cardiac function in mice with cardiac-specific overexpression of GRK5

During maladaptive hypertrophy, GRK5 in cardiac myocytes becomes localized to the nucleus, leading to increased transcription of various hypertrophic genes via its ability to act as a HDAC5 kinase and due to its association with DNA in concert with NFAT. Previous studies have shown that GRK5 ablation reduces pathological hypertrophy. However, a role for this GRK in physiological hypertrophy, has yet to be elucidated. To study this, we used a high intensity swim training protocol to induce physiological hypertrophy. Unlike pressure-overload, swim training in cardiac-specific GRK5 overexpressing mice led to similar increases in cardiac growth relative to control mice, and training did not lead to nuclear accumulation of GRK5. Swim training in GRK5 overexpressing mice under conditions of pressure-overload delayed the onset of early HF. Moreover, during pathological and physiological hypertrophy GRK5 overexpressing mice show different patterns of changes in gene expression. These findings suggest that GRK5 acts as a hypertrophic facilitator only under pathological conditions and does not affect physiological cardiac growth even when robustly overexpressed.

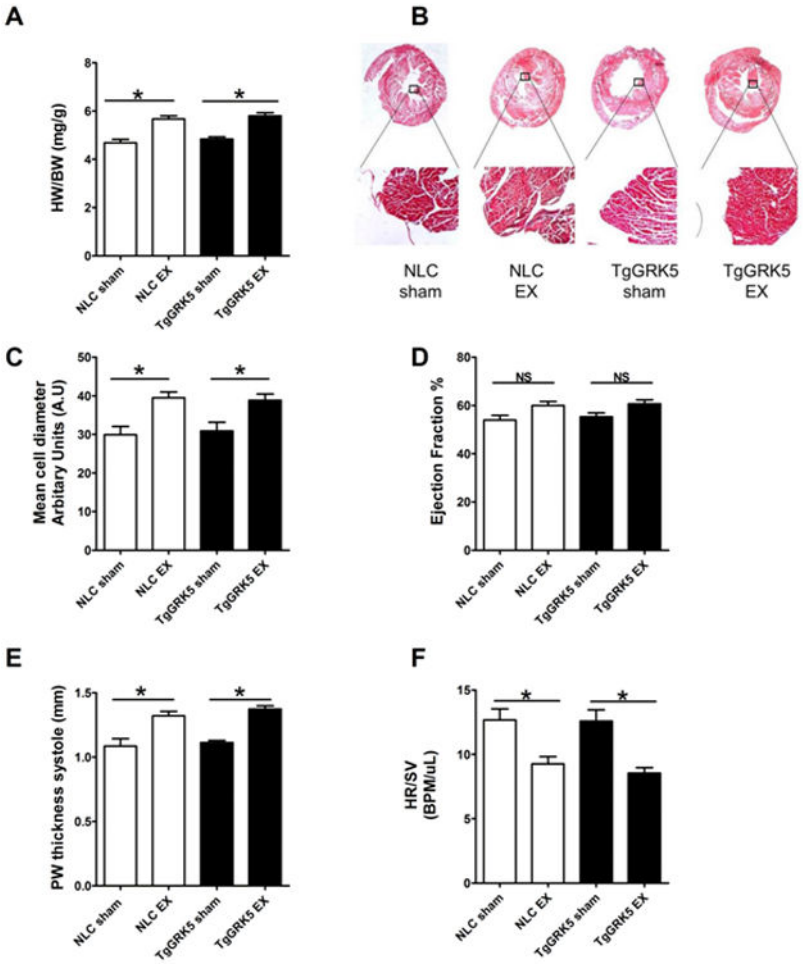



**Figure 1. Swim-induced hypertrophy (SIH) is similar in Non-Transgenic Littermate Control (NLC) mice, and cardiac-specific transgenic GRK5 (TgGRK5) mice after swimming**

(**A**) Heart weight to body weight (HW/BW) ratios in NLC and TgGRK5 mice after swim training*, $p<0.05$ vs. sham, (ANOVA, n = 11-26 mice/group). (**B**) Representative images of H&E stained cardiac sections from NLC and TgGRK5 mice after swim training. Shown in the inset are 400× Magnification images showing myocyte diameter. (**C**) Quantification of Mean Cell Diameter from H&E stained cardiac sections from NLC and TgGRK5 after swim training *, $p<0.05$ vs. sham, (ANOVA, n = 6-15 mice/group). (**D**) Cardiac function evaluated by LV EF% measured by echocardiography in the mice shown in A, Data statistically non-significant (NS), (ANOVA, n=6-26 mice/group). (**E**) Systolic posterior wall thickness (sPWT) as measured by echocardiography in the mice shown in A, *, $p<0.05$ vs. sham, (ANOVA, n=6-26 mice/group). (**F**) Stroke volume (SV)/Heart Rate (HR) as measured by echocardiography in the mice shown in A, *, $p<0.05$ vs. sham, (ANOVA, n=6-26 mice/group).

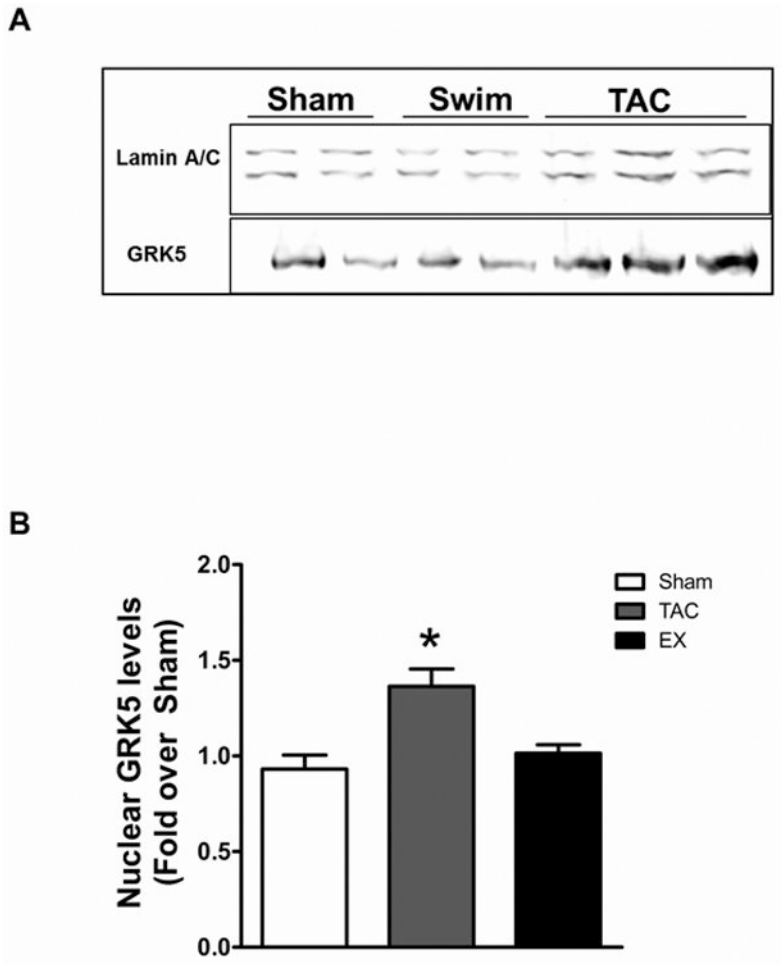



**Figure 2. Swim training does not alter nuclear levels of GRK5**

(**A**) Representative Western blot of nuclear GRK5 accumulation after swim training or TAC (bottom panel) normalized to the nuclear marker, Laminin A/C (top panel). (**B**) Quantification of nuclear accumulation in TgGRK5 mice after swim training or trans-aortic constriction (TAC) surgery *, $p<0.05$ TAC relative to swim or sham, (ANOVA, n = 6-8 mice/group).

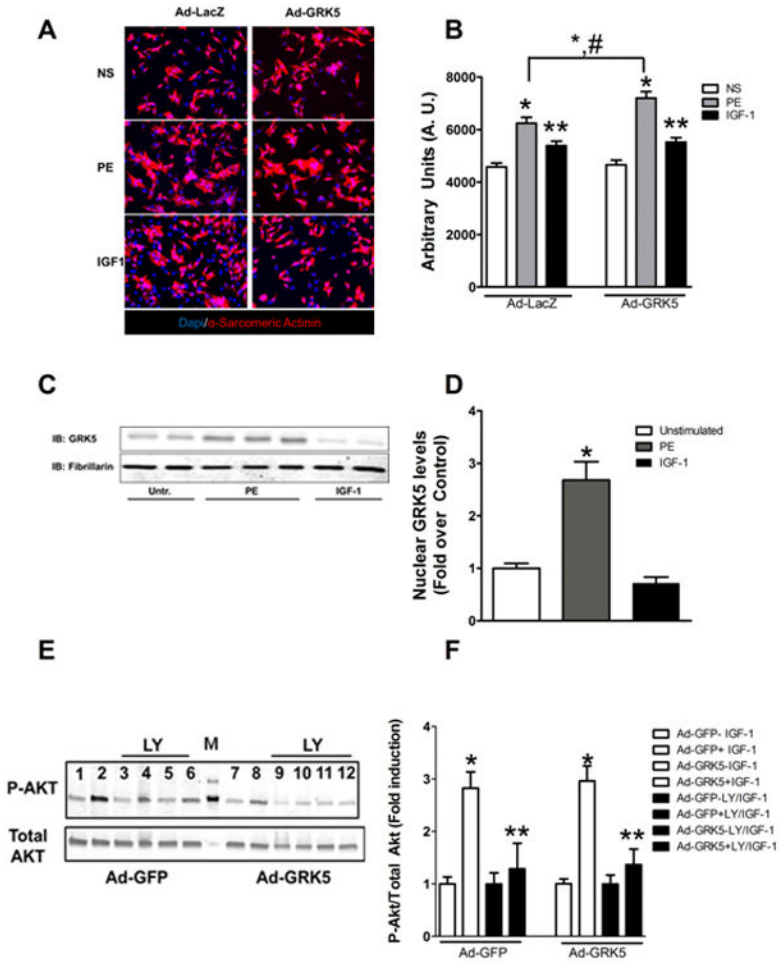



**Figure 3. In vitro Swim-induced myocyte hypertrophy when GRK5 is overexpressed**

(**A**) Representative images of SMA after treatment with insulin-like growth factor-1 (IGF-1, 10 nM/L) or phenylephrine (PE, 50 μM/L) compared to untreated (Unt). (**B**) Quantification of Mean Cell Size of SMA stained Ad-LacZ or Ad-GRK5 treated NRVMs under three conditions: Unt, IGF-1 treatment or PE stimulation *, $p<0.05$ PE vs. Unt, **, $p<0.05$ IGF-1 vs. Unt, *,#, $p<0.05$ Ad-GRK5 PE vs. Ad-LacZ PE, (ANOVA, n = 101-150 cells/group). (**C**) Nuclear GRK5 levels (top panel) visualized via Western blots of NRVM nuclear fractions following IGF-1 or PE treatment relative to Unt with Fibrillarin (bottom panel) serving as the nuclear marker. (**D**) Fold Change of nuclear accumulation in GRK5 infected NRVMs after IGF-1 or PE stimulation *, $p<0.05$ PE vs. Unt or IGF-1 treated, (ANOVA, n = 9-16 cell lysates/group). (**E**) IGF-1 stimulation increases p-AKT to similar levels in Ad-GFP and Ad-GRK5 treated NRVMs. Shown is a representative Western blot of Ad-GFP (Lanes 1-6) and Ad-GRK5 (Lanes 7-12) treated NRVMs stimulated with IGF-1 or IGF1 in combination with PI3K inhibitor (LY, 10 μM/L). Odd numbered lanes were Unt. Lanes 2 and 8 were stimulated with IGF-1, while Lanes 4, 6, 10, and 12 were treated with IGF-1 ± LY. (**F**) Quantification of p-AKT in Ad-GFP and Ad-GRK5 treated NRVMS after IGF-1 stimulation or IGF-1 + LY *, $p<0.05$ IGF-1 vs. Unt, **, $p<0.05$ IGF-1 vs. IGF-1 + LY, (ANOVA, n = 3-10 per group). M=molecular weight marker

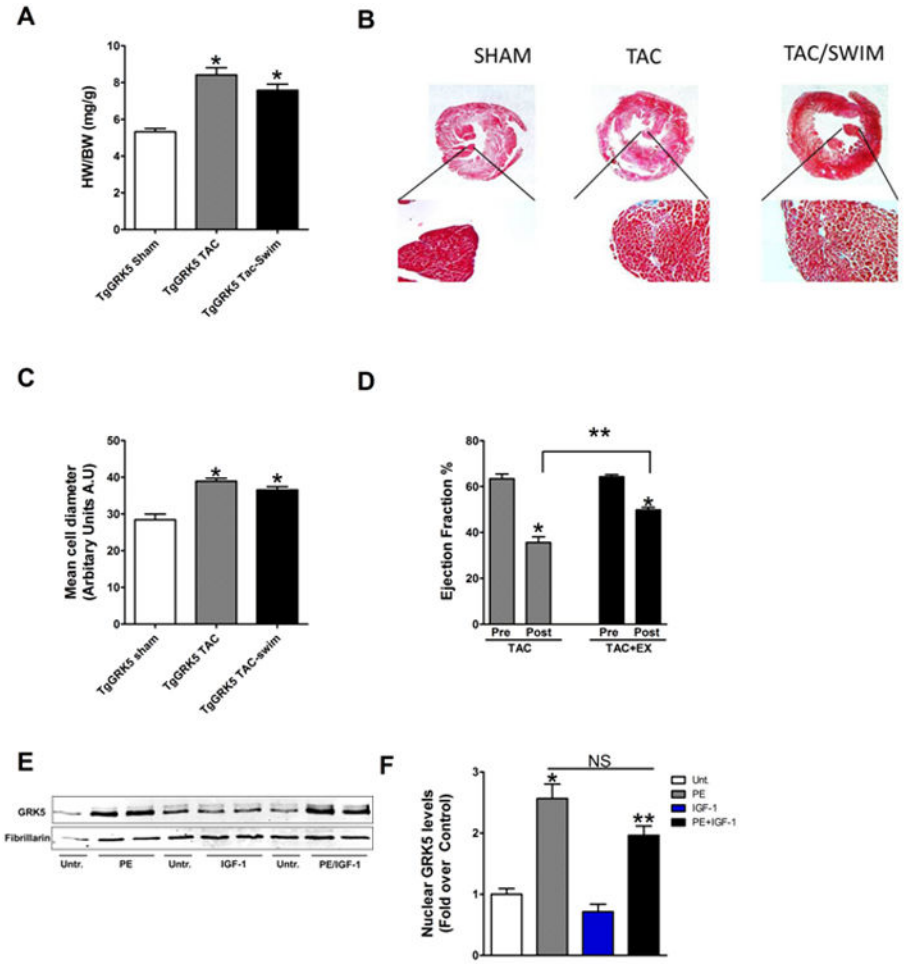



**Figure 4. Swim training delays the onset of post-TAC heart failure in TgGRK5 mice**

(**A**) HW/BW ratios in sham TgGRK5, TAC TgGRK5 and TAC/swim TgGRK5 mice*, $p<0.05$ vs. sham, (ANOVA, n = 5-15 mice/group). (**B**) Representative images of H&E stained cardiac sections from mice in A. Shown in the inset are 400× Magnification images showing myocyte diameter. (**C**) Quantification of Mean Cell Diameter from H&E stained cardiac sections from mice in A *, $p<0.05$ vs. sham, (ANOVA, n = 4-5 hearts/group). (**D**) Cardiac function (LV EF%) measured by echocardiography in the mice shown in A*, $p<0.05$ Pre-TAC vs. Post-TAC, (ANOVA, n = 4-13 mice/group). (**E**) IGF-1 stimulation after PE administration does not prevent GRK5 nuclear accumulation in myocytes. Shown is a Representative Western blot of nuclear GRK5 accumulation after IGF-1, PE, or IGF-1+ PE treatment (top panel) normalized to the nuclear marker, Fibrillarin (bottom panel). (**F**) Fold Change of nuclear accumulation in Ad-GRK5 treated NRVMs after IGF-1, PE, or IGF-1+ PE treatment *, $p<0.05$ PE vs. Unt, **, $p<0.05$ PE + IGF-1 vs. IGF-1, NS, PE vs. PE + IGF-1, (ANOVA, n = 9-16 cell lysates/group).

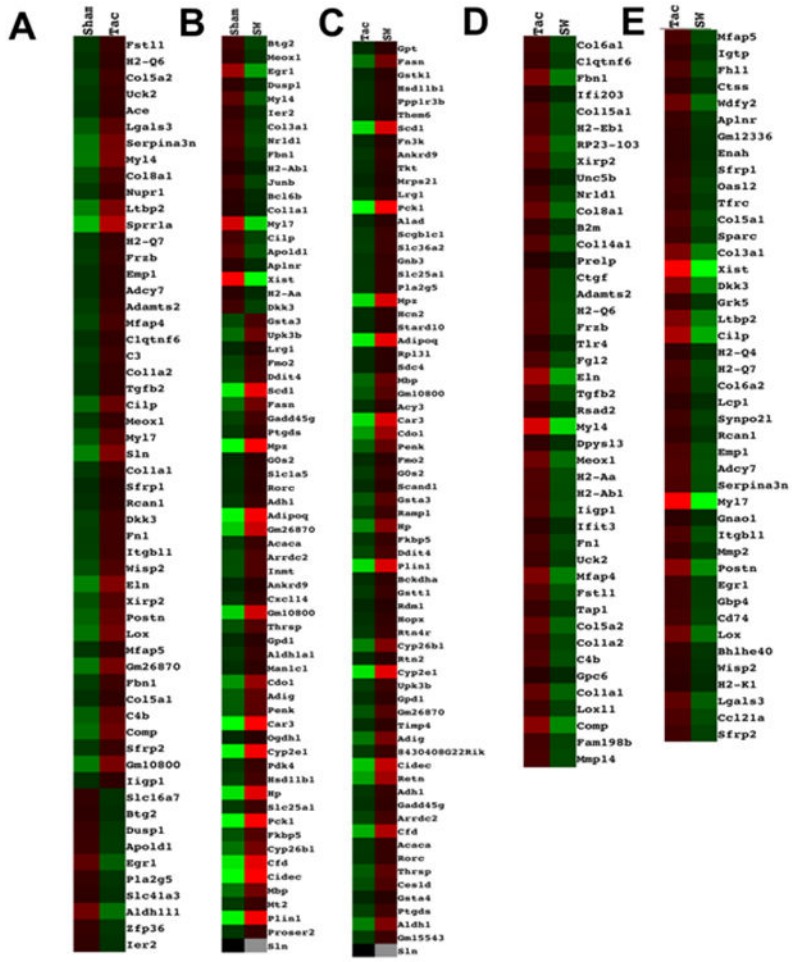
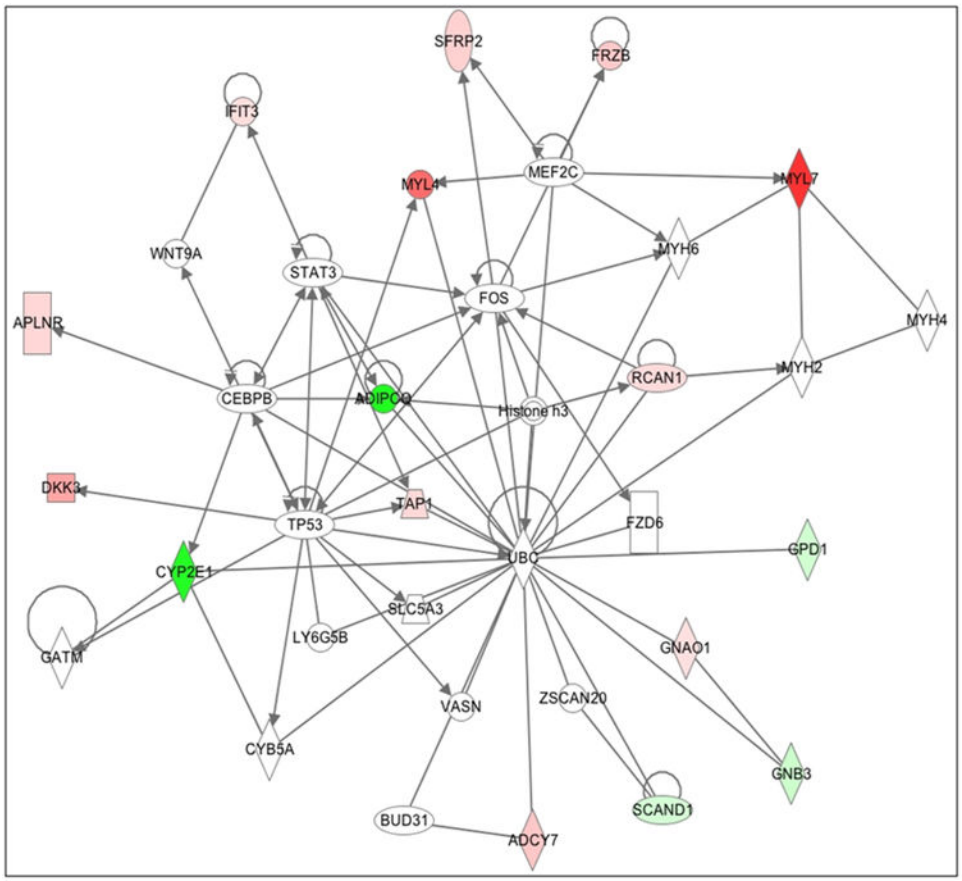



**Figure 5. RNA sequencing data illustrating the effects of pathological hypertrophy and swim-induced physiological hypertrophy on transcript expression in TgGRK5 mice**

(**A**) Heat map illustrating 43 genes upregulated (red) and 10 genes downregulated (green) in Post-TAC TgGRK5 mice relative to sham. (**B**) Heat map illustrating 37 genes upregulated (red) and 20 genes downregulated (green) in Swim TgGRK5 mice relative to sham. (**C**) Heat map illustrating 59 genes upregulated (red) in swim TgGRK5 mice relative to Post-TAC TgGRK5 mice. (**D-E**) Heat map illustrating 84 genes upregulated (green) in Post-TAC TgGRK5 mice relative to Swim TgGRK5 mice. Data are plotted using a $\log_2$ scale. n=2 hearts per group.



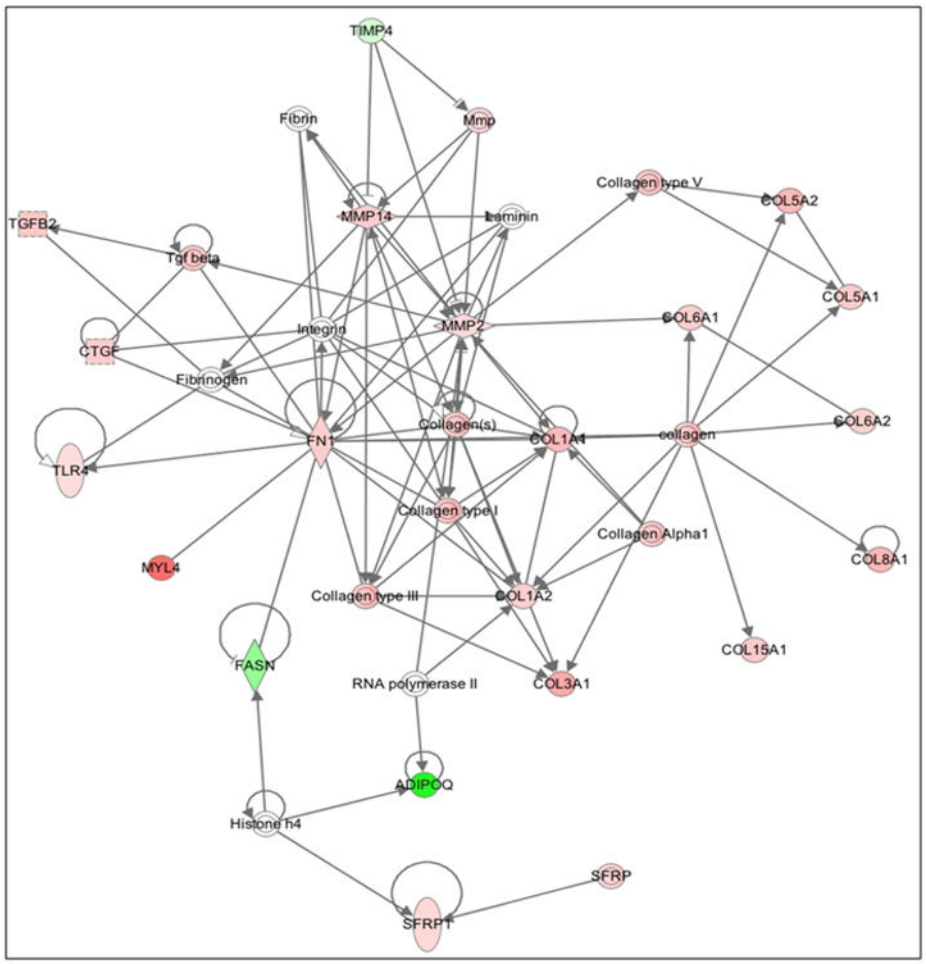



**Figure 6. Swim-induced physiological hypertrophy and pathological hypertrophy post-TAC differentially regulate various pathways in TgGRK5 mice**

(**A**) Ingenuity Pathway Analysis (IPA) illustrating genes upregulated (red) and downregulated (green) Post-TAC relative to Post-Swim in TgGRK5 mice with an emphasis on Connective Tissue Disorders, Organismal Injury and Abnormalities, Dermatological Diseases and Conditions. (**B**) IPA illustrating genes upregulated (red) and downregulated (green) Post-TAC relative to Post-Swim in TgGRK5 mice with an emphasis on Organismal Injury and Abnormalities, Cellular Development, Connective Tissue Development and Function. Lines with solid arrowheads represent direct interaction of molecules in signaling cascade. Lines with clear arrowheads represent auto-regulation in signaling cascade.

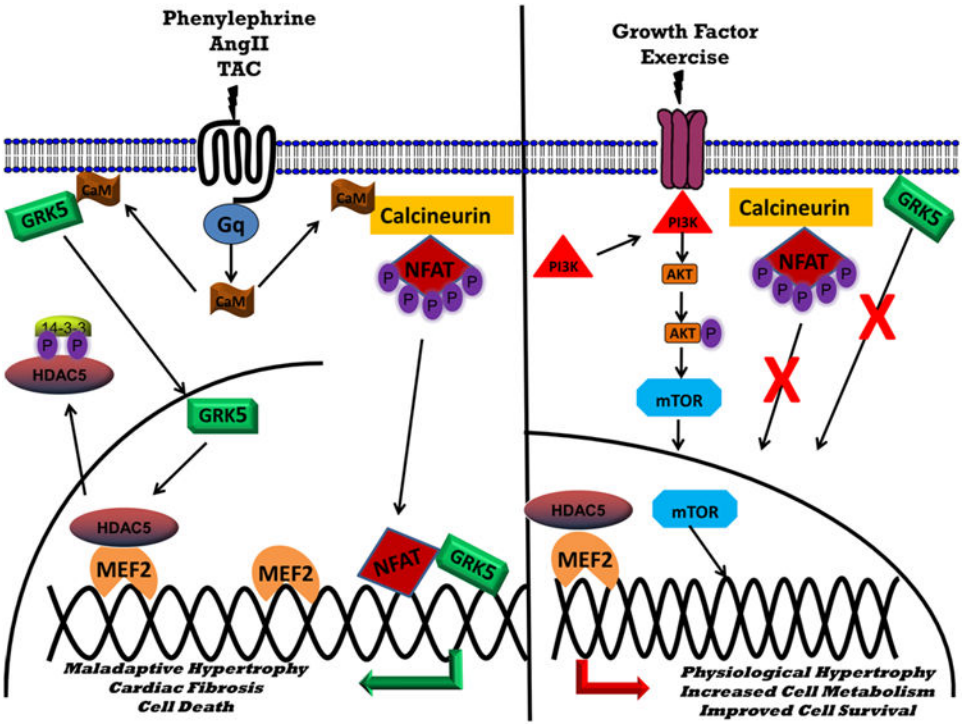



**Figure 7. Summary schematic illustrating the differences between GRK5's role in physiological and pathological cardiac hypertrophy**

(**A**) With pathological stimuli (PE, TAC), GRK5 associates with $Ca^{2+}$-calmodulin leading to translocation to the nucleus, wherein it promotes maladaptive cardiac hypertrophy via association with HDAC5 and NFAT. (**B**) Conversely, the physiological hypertrophic stimulus of exercise does not lead to translocation of GRK5 or NFAT to the nucleus, resulting in a normal adaptive hypertrophic response with GRK5 overexpression.