Epidermal growth factor receptor-dependent maintenance of cardiac contractility

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Aims
Epidermal growth factor receptor (EGFR) is essential to the development of multiple tissues and organs and is a target of cancer therapeutics. Due to the embryonic lethality of global EGFR deletion and conflicting reports of cardiac-overexpressed EGFR mutants, its specific impact on the adult heart, normally or in response to chronic stress, has not been established. Using complimentary genetic strategies to modulate cardiomyocyte-specific EGFR expression, we aim to define its role in the regulation of cardiac function and remodelling.
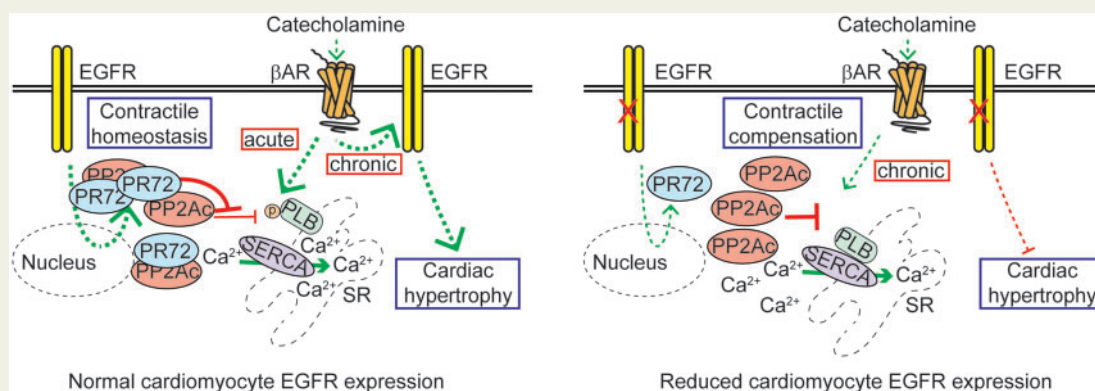
Methods and results
A floxed EGFR mouse model with α-myosin heavy chain-Cre-mediated cardiomyocyte-specific EGFR downregulation (CM-EGFR-KD mice) developed contractile dysfunction by 9 weeks of age, marked by impaired diastolic relaxation, as monitored via echocardiographic, haemodynamic, and isolated cardiomyocyte contractility analyses. This contractile defect was maintained over time without overt cardiac remodelling until 10 months of age, after which the mice ultimately developed severe heart failure and reduced lifespan. Acute downregulation of EGFR in adult floxed EGFR mice with adeno-associated virus 9 (AAV9)-encoded Cre with a cardiac troponin T promoter (AAV9-cTnT-Cre) recapitulated the CM-EGFR-KD phenotype, while AAV9-cTnT-EGFR treatment of adult CM-EGFR-KD mice rescued the phenotype. Notably, chronic administration of the β-adrenergic receptor agonist isoproterenol effectively and reversibly compensated for the contractile dysfunction in the absence of cardiomyocyte hypertrophy in CM-EGFR-KD mice. Mechanistically, EGFR downregulation reduced the expression of protein phosphatase 2A regulatory subunit Ppp2r3a/PR72, which was associated with decreased phosphorylation of phospholamban and Ca^{2+} clearance, and whose re-expression via AAV9-cTnT-PR72 rescued the CM-EGFR-KD phenotype.

Conclusions
Altogether, our study highlights a previously unrecognized role for EGFR in maintaining contractile homeostasis under physiologic conditions in the adult heart via regulation of PR72 expression.

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Graphical Abstract

1. Introduction

The erythroblastic leukaemia viral oncogene homolog (ErbB) family of homo- and hetero-dimerizing receptor tyrosine kinases, consisting of epidermal growth factor receptor (EGFR/ErbB1) and ErbB2–4, have been studied extensively for their roles in cancer biology.[1] However, ErbB receptors are also expressed in the heart where they have been recognized to regulate a number of biological processes. For instance, ErbB2 has been known for some time to relay cardiotoxicity related to cancer therapeutics,[2] engagement of ErbB2/ErbB4 signalling promotes cardiomyocyte proliferation,[3–5] and stimulation of EGFR enhances both survival and hypertrophic signalling in isolated cardiomyocytes.[6–8] Ligands for ErbB receptors, particularly heparin-binding epidermal growth factor (HB-EGF) and neuregulins, have also been shown to be essential in the regulation of cardiac development and post-injury regeneration.[9,10]

Aside from direct ligand stimulation, several cardiac-expressed G protein-coupled receptors (GPCR) indirectly transactivate ErbB receptors, most commonly EGFR via autocrine release of HB-EGF, to engage pro-survival and -growth responses including activation of extracellular-regulated kinase (ERK1/2) and Akt.[11] Due to enhanced levels of neurohormone released during heart failure, several studies have explored the impact of neurohormone GPCR-mediated EGFR transactivation on cardiac remodelling outcomes, as reviewed previously.[11,12] In particular, our lab has investigated the molecular mechanisms and impact of the β-adrenergic receptor (βAR)-mediated EGFR transactivation in the heart,[6,11,13] which have supported the notion that EGFR promotes cardiomyocyte survival signalling in the face of cardiac stress but may also promote hypertrophy.

Although EGFR has been suggested to influence several cardiac parameters, either directly or indirectly via GPCR-mediated transactivation, definitive studies into its impact on cardiac structure or function, and the mechanism(s) by which it would do so, are lacking. Genetic mouse models were previously developed to determine the role of EGFR *in vivo*, initially using global knockdown/knockin expression strategies that resulted in inconclusive cardiac-specific effects due to embryonic/postnatal lethality or other developmental abnormalities.[14–18]

Subsequent studies have employed more cardiomyocyte-selective approaches, including dominant-negative EGFR transgenic overexpression as well as EGFR deletion in combined vascular smooth muscle cell/cardiomyocyte populations, producing disparate cardiac phenotypes with mechanistically unclear causes.[19–21] A more recent study using a cardiomyocyte-specific EGFR knockdown mouse model indicated that EGFR provides protection against acute ischaemic stress,[22] but with no insight into cardiac function, long-term survival, or other remodelling parameters over time. Overall, there lacks a succinct *in vivo* analysis of the impact of cardiomyocyte-expressed EGFR on cardiac function normally or in conjunction with chronic cardiac stress in the adult heart. Here, using complimentary mouse models, we have identified a previously unrecognized role for EGFR in maintaining contractile homeostasis under physiologic conditions and shown that cardiomyocyte-expressed EGFR is a requirement for the hypertrophic response to chronic catecholamine stress.

2. Methods

2.1 Animal models and experimental procedures

All animal experiments were conducted under the National Institutes of Health (NIH) Guide for the Care and Use of Laboratory Animals and approved by the Institutional Animal Care and Use Committee (IACUC) at Temple University under Animal Care and Use Protocol #4902. The mouse strain used for this research project, B6.129S6-Egfrtm1Dwt/Mmnc, identification number 031765-UNC, was obtained from the Mutant Mouse Regional Resource Center (MMRRC), a NIH-funded strain repository, and was donated to the MMRRC by David Threadgill, Ph.D., North Carolina State University. These mice contain a conditional allele of Egfr with exon 3 flanked by two loxP sites (EGFR[f/f]), deletion of which results in early termination of translation.[23] EGFR[f/f] mice were crossed with αMHC-Cre transgenic mice[24] to produce cardiomyocyte-specific EGFR knockdown (CM-EGFR-KD). Unless specifically stated, all experiments were initiated in adult mice between 12 and 16 weeks of age. Both male and female CM-EGFR-KD and EGFR[f/f] (Cre⁻) littermates

were used throughout the study with numbers of mice used per experiment indicated in figure legends.

Recombinant AAV9 vectors encoding Cre recombinase, enhanced green fluorescent protein (eGFP), human EGFR, murine PR72, or LacZ under the control of the cardiomyocyte-specific troponin T (cTnT) promoter were generated by Vector Biolabs (Malvern, PA, USA). Anaesthetized adult EGFR[f/f] (Cre[-]) mice received either AAV9-cTnT-Cre-eGFP or AAV9-cTnT-eGFP, while adult CM-EGFR-KD mice received either AAV9-cTnT-LacZ, AAV9-cTnT-flag-EGFR, or AAV9-cTnT-PR72, via retro-orbital injection of 1×10^{12} genome copies (GC)/mouse in 150 µL.

Echocardiography, haemodynamic assessment, osmotic minipump insertion, and adult mouse cardiomyocyte isolation were performed as described previously[13,25–27] and in the Supplementary material online.

Anaesthesia was induced and maintained for the duration of each procedure (echocardiography, retro-orbital injection, osmotic minipump implantation, haemodynamic assessment) via inhalation of isoflurane (3–5%, Patterson Veterinary, Greeley, CO, USA). Immediately following osmotic minipump implantation surgery, mice were given a subcutaneous injection of buprenorphine (0.1 mg/kg). At study end, mice were euthanized via inhalation of CO_2 (100%) followed by cervical dislocation as a secondary confirmation of death, or removal of the heart.

2.2. Radioligand binding, skinned myocyte analysis, cardiomyocyte contractility, and Ca^{2+} transient analyses

Measurements of left ventricular βAR density, myocyte force generation and shortening, as well as Ca^{2+} sensitivity and transients were performed as described previously[25,26,28] and in the Supplementary material online.

2.3 Immunohistochemistry, immunoblotting, quantitative real-time PCR (RT-qPCR), and RNA-seq analyses

Parameters of cardiac remodelling (cardiomyocyte size, cardiomyocyte death, and left ventricular fibrosis), as well as immunoblotting, RT-qPCR, and RNA-seq analyses, were carried out as described previously[13,26] and in the Supplementary material online.

2.4 Statistical analyses

GraphPad Prism 9.0.2 software (GraphPad Software Inc., San Diego, CA, USA) was used for all statistical analysis. Data represent the mean ± standard error of the mean (SEM) of at least three individual samples of each group. Two-tailed unpaired *t*-tests were used for comparison between two groups, and ordinary one-way ANOVA with Tukey's multiple comparisons test was used for comparison across multiple groups at a single time point. Comparison across two or more groups during time-course studies was performed using ordinary two-way ANOVA with Bonferroni's multiple comparisons test. Intergroup differences in survival rate were tested by the log-rank (Mantel–Cox) test. Differences were considered significant at $P < 0.05$.
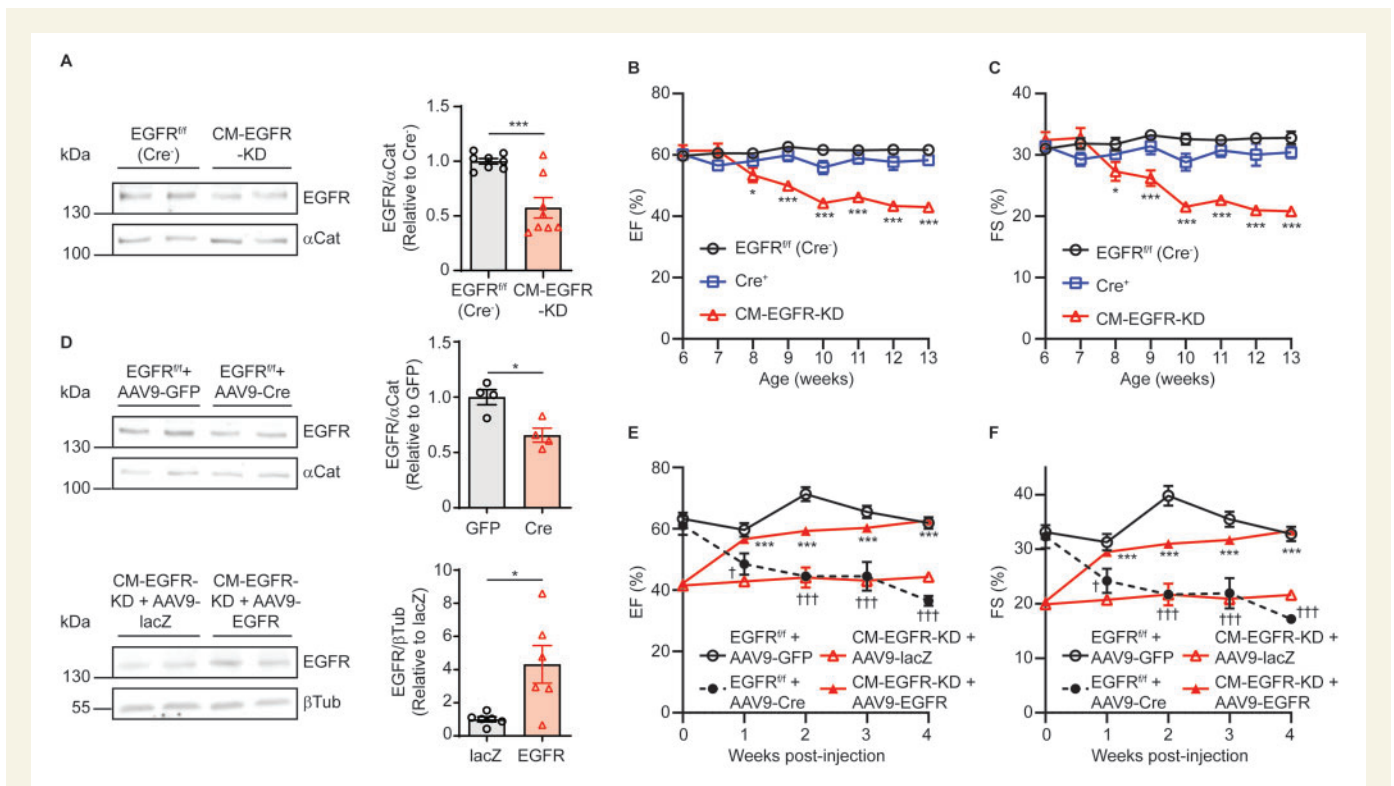
3. Results

3.1 Cardiomyocyte-specific EGFR downregulation acutely decreases cardiac function

To determine the impact of cardiomyocyte-specific EGFR expression on cardiac structure and function, we crossed mice containing a conditional allele of Egfr, with exon 3 flanked by two loxP sites (EGFR[f/f]), with αMHC-Cre transgenic mice to produce mice with cardiomyocyte-specific EGFR knockdown (CM-EGFR-KD). These mice displayed ∼50% knockdown of EGFR in adult (12–16-week-old) cardiac membranes (*Figure 1A*); however, there were no alterations in the expression of EGFR heterodimer partner ErbB2 or the phosphorylation status of known EGFR signalling kinases ERK1/2 and Akt (Supplementary material online, *Figure S1A–C*), indicating that EGFR knockdown does not lead to aberrant regulation of these canonical effectors. To monitor the impact of cardiomyocyte-specific EGFR knockdown on cardiac function over time, we performed serial echocardiography (Supplementary material online, *Table S2*). Compared to either αMHC-Cre[+] or EGFR[f/f] (Cre[-]) controls, CM-EGFR-KD mice exhibited a decrease in both % ejection fraction (EF, *Figure 1B*) and % fractional shortening (FS, *Figure 1C*) by 9 weeks of age that persisted thereafter, with no differences noted between male and female CM-EGFR-KD mice (Supplementary material online, *Figure S2A and B*).

Although we did not observe a decrease in cardiac contractility in CM-EGFR-KD mice until ∼9 weeks of age, αMHC-Cre-mediated deletion of EGFR would occur during early developmental stages[29] that could impact contractility at later timepoints. Thus, to understand whether alterations in EGFR expression in fully differentiated adult hearts alter cardiac function, we employed AAV9-mediated gene delivery in order to acutely delete or re-express cardiomyocyte-specific EGFR. To this end, we retro-orbitally injected AAV9-cTnT-Cre-eGFP to 12-week-old EGFR[f/f] (Cre[-]) mice, with AAV9-cTnT-eGFP used as a control vector in a subset of EGFR[f/f] (Cre[-]) mice (*Figure 1D*, Supplementary material online, *Figure S1D*). Indeed, AAV9-mediated induction of Cre in EGFR[f/f] (Cre[-]) mouse hearts led to cardiac dysfunction that was detectable as early as 1-week post-AAV9 injection (Supplementary material online, *Table S3*) and by 4 weeks post-injection achieved a similar decrease in %EF (*Figure 1E*) and %FS (*Figure 1F*) as had been observed in the CM-EGFR-KD mice. Conversely, to assess whether contractile function could be acutely restored with EGFR re-expression in adult CM-EGFR-KD mice, we delivered AAV9-cTnT-flag-EGFR to 12-week-old CM-EGFR-KD mice, after cardiac dysfunction had manifested, whereas AAV9-cTnT-lacZ was given as a control vector to a subset of CM-EGFR-KD mice (*Figure 1D*, Supplementary material online, *Figure S1E*). As early as 1-week post-delivery, AAV9-cTnT-EGFR improved cardiac function in CM-EGFR-KD mice (*Figure 1E and F*, Supplementary material online, *Table S3*), which was recovered to EGFR[f/f] (Cre[-]) levels by 4 weeks post-injection. These results demonstrate that cardiac contractility is acutely sensitive to the manipulation of cardiomyocyte-specific EGFR expression.

3.2 Cardiomyocyte-specific EGFR downregulation induces delayed cardiac remodelling

We next assessed whether CM-EGFR-KD-induced changes in contractile function were associated with structural alterations or the development of pathological cardiac remodelling. Despite the presence of

Figure 1 Cardiomyocyte-specific EGFR downregulation acutely decreases cardiac function. (*A*) EGFR protein expression in adult EGFR$^{f/f}$ (Cre$^-$) vs. CM-EGFR-KD left ventricular (LV) membrane samples normalized to α-catenin. ***$P < 0.001$, two-tailed unpaired t-test, $n = 8$ each. Ejection fraction (%EF, *B*) and fractional shortening (%FS, *C*) of EGFR$^{f/f}$ (Cre$^-$) ($n = 12$), αMHC-Cre$^+$ ($n = 8$) and CM-EGFR-KD ($n = 11$) mice were monitored via echocardiography from 6 to 13 weeks of age. *$P < 0.05$, ***$P < 0.001$ vs. EGFR$^{f/f}$ (Cre$^-$), ordinary two-way ANOVA with Bonferroni's multiple comparisons test. (*D*) EGFR protein expression in the hearts of adult EGFR$^{f/f}$ (Cre$^-$) mice 4 weeks after treatment with AAV9-GFP vs. AAV9-Cre (top panels, $n = 4$ each) or in the hearts of adult CM-EGFR-KD mice 4 weeks after treatment with AAV9-lacZ vs. AAV9-EGFR (bottom panels, $n = 6$ each). *$P < 0.05$, two-tailed unpaired t-test. %EF (*E*) and %FS (*F*) of adult EGFR$^{f/f}$ (Cre$^-$) mice treated with AAV9-GFP ($n = 5$) vs. AAV9-Cre ($n = 6$) or adult CM-EGFR-KD mice treated with AAV9-lacZ ($n = 10$) vs. AAV9-EGFR ($n = 10$) were monitored via echocardiography for 4 weeks following AAV administration (at time 0). ***$P < 0.001$ AAV9-EGFR vs. AAV9-LacZ in CM-EGFR-KD mice and $^\dagger P < 0.05$, $^{\dagger\dagger\dagger}P < 0.001$ AAV9-Cre vs. AAV9-GFP in EGFR$^{f/f}$ (Cre$^-$) mice at corresponding timepoints, ordinary two-way ANOVA with Bonferroni's multiple comparisons test.

cardiac dysfunction in adult CM-EGFR-KD mice (12 weeks of age), there were no changes in cardiac remodelling parameters including cardiomyocyte size, cardiomyocyte cell death, or cardiac fibrosis (Supplementary material online, *Figure S3A–C*). As detected via echocardiography, there were no consistent differences in diastolic wall thicknesses, though there were decreases in systolic wall thicknesses in CM-EGFR-KD mouse hearts over time (Supplementary material online, *Table S2*), which were also observed in the acute AAV-Cre modified mice and normalized in the acute AAV-EGFR modified mice (Supplementary material online, *Table S3*). However, these small changes in systolic wall thicknesses did not translate into differences in heart weights in any of the cohorts (Supplementary material online, *Figure S3D*) or isolated cardiomyocyte size (Supplementary material online, *Figure S3E*). Thus, cardiomyocyte-specific EGFR downregulation does not acutely induce cardiac remodelling despite diminished contractile function.

However, by 10 months of age CM-EGFR-KD mice displayed evidence of pathological cardiac remodelling including cardiomyocyte hypertrophy and death as well as interstitial fibrosis (*Figure 2A–C*). At a molecular level, there was increased expression of several genes responsive to cardiac stress and involved in remodelling and inflammatory

processes, including *Acta1, Myh7, Nppa, Il6,* and *Tnfa* (*Figure 2D*). Additionally, compared to younger CM-EGFR-KD mice (Figure *1*), contractile dysfunction was severely reduced by 10 months, as evidenced by decreases in %EF and %FS to ~20% and 10%, respectively (*Figure 2E and F*). Ultimately, CM-EGFR-KD mice began to exhibit enhanced mortality vs. their littermate controls at ~1 year of age, with median survival at 13 months of age (*Figure 2G*) with no significant differences between male and female CM-EGFR-KD mice amongst these parameters (Supplementary material online, *Figure S2C–H*). These findings demonstrate that cardiomyocyte-specific downregulation of EGFR directly mediates a rapid loss in contractile function in the absence of cardiac remodelling; however, without intervention to normalize EGFR expression, this loss in contractile function eventually promotes pathological cardiac remodelling, heart failure, and enhanced mortality.

3.3 Chronic catecholamine stimulation reversibly rescues contractile dysfunction without hypertrophy in CM-EGFR-KD mice

Adult CM-EGFR-KD mice display reduced cardiac contractility in the absence of gross morphological remodelling, suggesting that the molecular machinery for contractility remains intact, one of the major drivers of
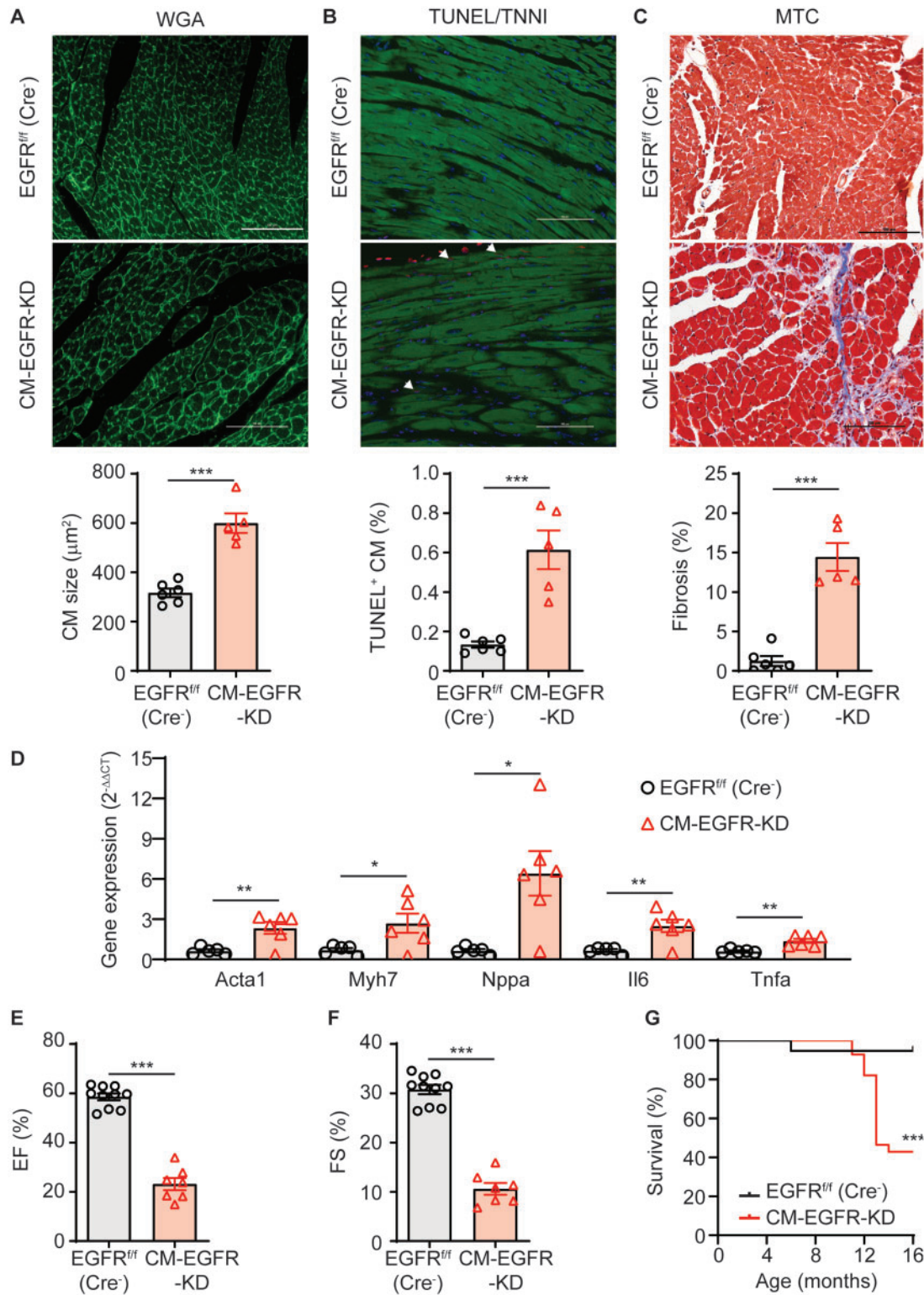
Figure 2 Cardiomyocyte-specific EGFR downregulation induces delayed cardiac remodelling. Cardiac structure was assessed in the LV of 10-month-old EGFR$^{f/f}$ (Cre$^-$) vs. CM-EGFR-KD mice via WGA (*A*), TUNEL/TNNI (*B*), and MTC (*C*) staining. Panels shown are representative images from $n = 6$ (EGFR$^{f/f}$ (Cre$^-$)) and $n = 5$ (CM-EGFR-KD) mouse hearts; scale bars denote 100 μm and arrowheads denote TUNEL-positive cardiomyocytes. Quantification of cardiomyocyte size, death, and cardiac fibrosis are shown in accompanying histograms. ***$P < 0.001$, two-tailed unpaired *t*-test. (*D*) RT-qPCR was used to measure the expression of *Acta1, Myh7, Nppa, Il6,* and *Tnfa* in the LV of 10 month-old EGFR$^{f/f}$ (Cre$^-$) ($n = 5$) vs. CM-EGFR-KD ($n = 6$) mice. $P < 0.05$, **$P < 0.01$, two-tailed unpaired *t*-test. %EF (*E*) and %FS (*F*) of 10-month-old EGFR$^{f/f}$ (Cre$^-$) ($n = 10$) and CM-EGFR-KD ($n = 7$) mice were measured via echocardiography. ***$P < 0.001$, two-tailed unpaired *t*-test. (*G*) EGFR$^{f/f}$ (Cre$^-$, $n = 19$) and CM-EGFR-KD ($n = 28$) mice were monitored for survival over time. ***$P < 0.001$ vs. EGFR$^{f/f}$ (Cre$^-$), Log-rank (Mantel–Cox) test.

which is the βAR system. Thus, we next sought to determine whether chronic βAR stimulation could rescue the contractile dysfunction already manifested in CM-EGFR-KD mice. To test this, we infused adult CM-EGFR-KD and EGFR[f/f] (Cre[-]) littermate control mice with the βAR agonist ISO[13,30] for up to 4 weeks and monitored cardiac function via weekly echocardiography. Chronic ISO infusion improved contractility in CM-EGFR-KD mice to levels not different from EGFR[f/f] (Cre[-]) littermate controls throughout the infusion period (*Figure 3A and B*, Supplementary material online, *Table S4*). Notably, the chronic catecholamine-enhanced contractility in CM-EGFR-KD mice was reversible upon cessation of ISO infusion as withdrawal of the minipumps after 2 weeks resulted in a return of contractility to baseline CM-EGFR-KD levels. Thus, the βAR system remains responsive to sympathetic stimulation in CM-EGFR-KD mice and can chronically compensate for the EGFR-dependent loss in baseline contractility.

Chronic catecholamine stress-induced cardiac hypertrophy has been postulated to be EGFR-sensitive, but with variable results across studies using pharmacologic (EGFR inhibitors) or genetic (overexpression of EGFR mutants) approaches.[11,13,19,21,30–33] Thus, using our CM-EGFR-KD model, we investigated whether cardiomyocyte-specific EGFR downregulation impacts chronic ISO-induced cardiac hypertrophy. Although chronic ISO infusion for either 2 or 4 weeks significantly increased cardiomyocyte size in control mice, this effect was abrogated in CM-EGFR-KD mice (*Figure 3C and D*). Notably, we found that βAR density was not different at baseline in Veh-infused CM-EGFR-KD and EGFR[f/f] (Cre[-]) control hearts and that receptor density became reduced to a similar extent in each mouse line by chronic ISO infusion (*Figure 3E*), thus EGFR downregulation does not impact βAR downregulation during chronic catecholamine stimulation. Collectively, these findings demonstrate that βAR responsiveness to catecholamine stimulation is maintained in the face of EGFR downregulation and, despite some degree of receptor desensitization, can reversibly rescue the decreased cardiac contractility in CM-EGFR-KD mice. Further, these data highlight that cardiomyocyte-specific EGFR expression is required to promote cardiac hypertrophic remodelling in response to chronic catecholamine stimulation.

3.4 Cardiomyocyte-specific EGFR downregulation leads to differential temporal effects on cardiac gene expression

To begin to attain molecular insight into the contractile dysfunction manifested in CM-EGFR-KD mice, we next investigated whether they exhibited alterations in cardiac gene expression over time. To this end, we performed RNAseq analysis of LV samples from 7-week-old (no dysfunction) and 9-week-old (earliest dysfunction manifested) CM-EGFR-KD mice vs. EGFR[f/f] (Cre[-]) littermate controls. Cardiomyocyte-specific downregulation of EGFR resulted in a distinct profile of gene expression at 7 vs. 9 weeks of age. Although 284 genes were differentially altered at 7 weeks (Supplementary material online, *Table S5*), with most upregulated, these changes were not sustained over time as only 67 were altered at 9 weeks (Supplementary material online, *Table S6*), with most downregulated (*Figure 4A*). Pathway analysis confirmed that a majority of the canonical signalling pathways identified increased at 7 weeks and decreased at 9 weeks in CM-EGFR-KD vs. EGFR[f/f] (Cre[-]) control hearts (*Figure 4B*). Several genes encoding proteins involved in regulating cardiac contractility via cAMP-dependent signalling, including *Adcy5*, *Prkaa2*, *Prkab2*, and *Pde3a*, were indicated to be altered in CM-EGFR-KD vs. EGFR[f/f] (Cre[-]) littermate control hearts at 7 weeks of age, but to be

normalized by 9 weeks of age. In conjunction with our data above, these results are consistent with the contractile defect in CM-EGFR-KD mice not being a result of aberrant βAR signalling.

After assessing the canonical signalling pathways most significantly altered in CM-EGFR-KD hearts, we focused in particular on the Ca^{2+} and cytoskeleton groups as they were downregulated at 9 weeks when cardiac dysfunction had manifested. RT-qPCR analysis of genes clustered within these groups, including several myosins and myofibril-related proteins (Supplementary material online, *Table S6*), indeed demonstrated decreased expression at the 9-week timepoint (*Figure 4C*), which could suggest an abnormality in the regulation of contractile dynamics at the level of the myofilament. However, using permeabilized myocytes from adult CM-EGFR-KD or EGFR[f/f] (Cre[-]) littermate controls, we observed no difference in maximal Ca^{2+}-activated force (F_{max}) or sensitivity (EC_{50}) (*Figure 4D–F*), indicating that, despite the reduction in contractility, myofilament sensitivity to Ca^{2+} and contractile potential remained unchanged in the absence of EGFR. Consequently, dysregulation of cardiomyocyte Ca^{2+} handling itself may play a role in manifesting the contractile dysfunction observed in CM-EGFR-KD mice.

3.5 Cardiomyocyte-specific EGFR downregulation alters Ca^{2+} handling during relaxation
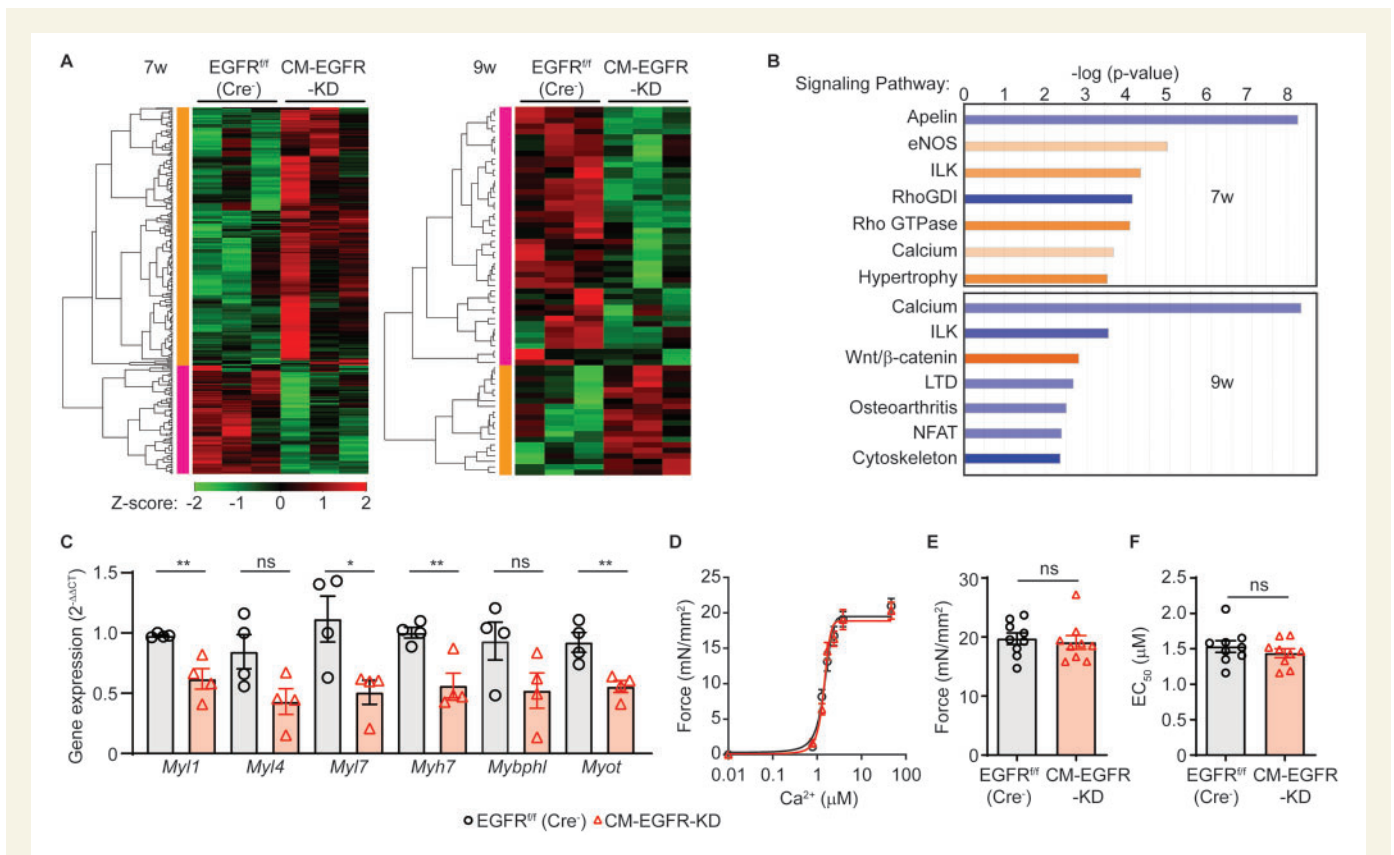
Cardiomyocyte-specific EGFR downregulation induced a rapid onset of contractile dysfunction without overt cardiac remodelling or sustained changes in broad gene expression patterns, suggesting that one or more components of the cardiac excitation–contraction cycle may be altered more directly. To more precisely assess parameters of cardiac contractility, we subjected adult CM-EGFR-KD and EGFR[f/f] (Cre[-]) littermate control mice to terminal haemodynamics at baseline and upon challenge with ISO. Although there was no difference in mean systemic pressure (*Figure 5A*) or heart rate (*Figure 5B*), we observed decreased rates of contraction (*Figure 5C*) and relaxation (*Figure 5D*) at baseline. Acute ISO infusion was sufficient to enhance heart rate and rate of contraction similarly in both CM-EGFR-KD and EGFR[f/f] (Cre[-]) littermate mice, again consistent with the βAR system remaining intact when cardiomyocyte-expressed EGFR is downregulated. We next evaluated contractile parameters at a cellular level using field-stimulated isolated AMCM (*Figure 5E*). There were no significant differences between CM-EGFR-KD and EGFR[f/f] (Cre[-]) littermate control AMCM with respect to the magnitude of contraction produced either at baseline or following ISO stimulation (*Figure 5F*). However, similar to the haemodynamics data, CM-EGFR-KD AMCM displayed prolonged relaxation at baseline (*Figure 5G*).

Changes in contraction/relaxation dynamics, as well as the high prediction of alterations in Ca^{2+} signalling from the RNASeq analysis, suggest aberrant Ca^{2+} handling or sensitivity. Conventional cardiomyocyte contraction–relaxation mechanisms depend on Ca^{2+} mobilization to and clearance from the sarcomere,[12] thus we performed comparative Ca^{2+} transient analyses between CM-EGFR-KD and littermate EGFR[f/f] (Cre[-]) control AMCM (*Figure 6A*). Consistent with our findings above, the magnitude of Ca^{2+} transients were not different between CM-EGFR-KD and EGFR[f/f] (Cre[-]) littermate control AMCM at baseline or in response to ISO (*Figure 6B*), but EGFR downregulation did produce delayed Ca^{2+} clearance, both basally and in response to ISO (*Figure 6C*).

Efficient Ca^{2+} clearance during cardiomyocyte relaxation can be enhanced via several mechanisms, including Ca^{2+} reuptake into the SR by SERCA, which is enhanced by phosphorylation of the inhibitory protein phospholamban (PLB) at either Ser16 by PKA or Thr17 by CAMKII.[34]
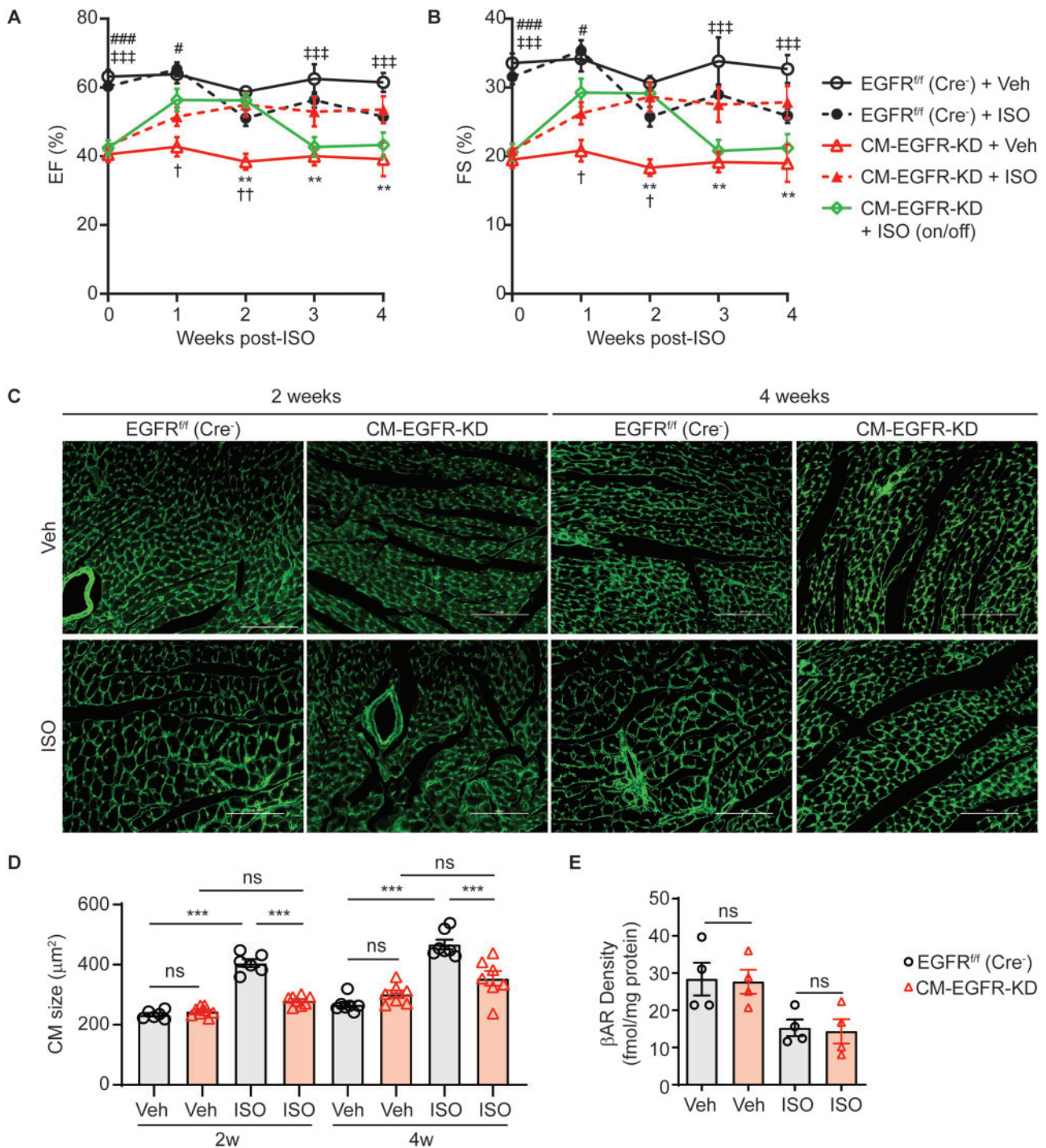
Figure 3 Chronic catecholamine stimulation reversibly rescues contractile dysfunction without hypertrophy in CM-EGFR-KD mice. %EF (*A*) and %FS (*B*) of adult EGFR^f/f (Cre^-) or CM-EGFR-KD mice infused with vehicle or ISO (3 mg/kg/day) were monitored via echocardiography for up to 4 weeks. $n = 7$ EGFR^f/f (Cre^-) + Veh, EGFR^f/f (Cre^-) + ISO, and CM-EGFR-KD + Veh, $n = 11$ CM-EGFR-KD + ISO and $n = 6$ CM-EGFR-KD + ISO (2 weeks on/2 weeks off). **$P < 0.01$ CM-EGFR-KD + Veh vs. CM-EGFR-KD + ISO, †$P < 0.05$, ††$P < 0.01$ CM-EGFR-KD + Veh vs. CM-EGFR-KD + ISO (2 weeks on/2 weeks off), #$P < 0.05$, ###$P < 0.001$ EGFR^f/f (Cre^-) + Veh vs. CM-EGFR-KD + ISO, ‡‡‡$P < 0.001$ EGFR^f/f (Cre^-) + Veh vs. CM-EGFR-KD + ISO (2 weeks on/2 weeks off) at corresponding timepoints, ordinary two-way ANOVA with Bonferonni's multiple comparisons test. (*C*) WGA staining of the LV of adult EGFR^f/f (Cre^-) vs. CM-EGFR-KD mice infused with vehicle or ISO for 2 (left) or 4 weeks (right). Panels shown are representative images from $n = 6$ [EGFR^f/f (Cre^-) Veh and ISO 2 weeks], $n = 8$ (CM-EGFR-KD Veh and ISO 2 weeks), $n = 7$ [EGFR^f/f (Cre^-) Veh and ISO 4 weeks] and $n = 7$ (CM-EGFR-KD Veh and ISO 4 weeks) mouse hearts; scale bars denote 100 μm. (*D*) Quantification of cardiomyocyte size from (*C*). ***$P < 0.001$, ordinary one-way ANOVA with Tukey's multiple comparisons test. (*E*) βAR LV membrane density was measured in adult EGFR^f/f (Cre^-) vs. CM-EGFR-KD mice ($n = 4$ each) infused with vehicle or ISO for 4 weeks. ns, not significant, ordinary one-way ANOVA with Tukey's multiple comparisons test.

Figure 4 Cardiomyocyte-specific EGFR downregulation leads to differential temporal effects on cardiac gene expression. (A) Heat maps depicting significant changes in cardiac gene expression in CM-EGFR-KD vs. EGFR$^{f/f}$ (Cre$^-$) hearts ($n = 3$ each) at 7 (left) or 9 (right) weeks of age. Red and green colours represent upregulated and downregulated genes, respectively. (B) Top signalling pathways altered at 7 (upper panel) and 9 (lower panel) weeks of age in the LV of CM-EGFR-KD vs. EGFR$^{f/f}$ (Cre$^-$) mice as determined by Ingenuity Pathway Analysis (IPA). Blue bars depict downregulation and orange bars upregulation. (C) RT-qPCR was used to measure the expression of *Myl1, Myl4, Myl7, Myh7, Mybphl,* and *Myot* in the LV of EGFR$^{f/f}$ (Cre$^-$) vs. CM-EGFR-KD mice ($n = 4$ each). ns, not significant, *$P < 0.05$, **$P < 0.01$, two-tailed unpaired *t*-test. (D) Mean force as a function of calcium concentration and fitted curves for skinned myocytes isolated from adult EGFR$^{f/f}$ (Cre$^-$) vs. CM-EGFR-KD mice, maximal Ca^{2+}-activated force (F$_{max}$, E) and sensitivity (EC$_{50}$, F) shown in histograms. Measurements were attained from 3 skinned myocytes per LV isolated from three independent LV samples per genotype.

Additionally, Ca^{2+} dissociation from TnC can be enhanced via phosphorylation of cTnI, potentially contributing to accelerated relaxation.[31] Thus, we measured the phosphorylation status of PLB and cTnI in LV lysates from adult CM-EGFR-KD or EGFR$^{f/f}$ (Cre$^-$) littermate control mice. Consistent with decreased cardiac contractility and Ca^{2+} clearance during cardiomyocyte relaxation, PLB phosphorylation at both Ser16 and Thr17 was reduced with EGFR downregulation (*Figure 6D and E*), as was phosphorylation of cTnI at Ser23/24 (Supplementary material online, *Figure S4A*). Collectively, these results demonstrate that impaired cardiac contractility caused by EGFR downregulation is associated with dysfunctional Ca^{2+} clearance.
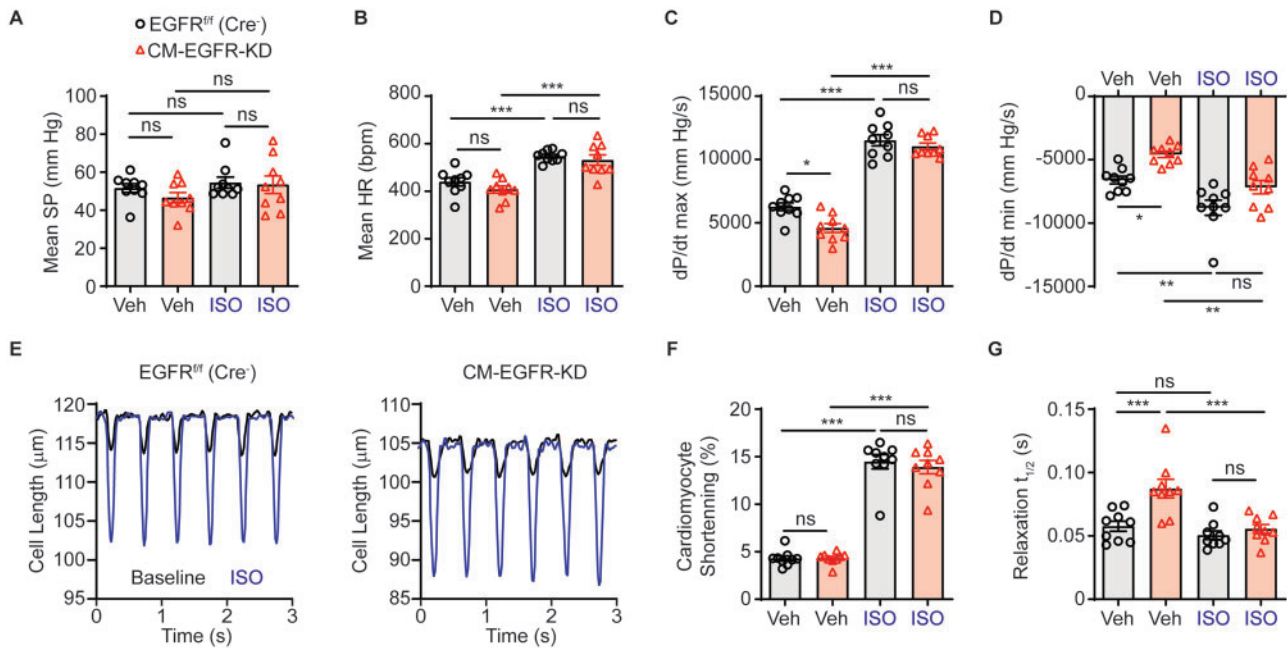
3.6 Cardiomyocyte-specific EGFR downregulation decreases expression of the PP2A B″ regulatory subunit PR72

Since PLB and cTnI phosphorylation are regulated by multiple kinases downstream of βAR, including PKA and CAMKII, but βAR signalling remains intact, we hypothesized that their reduced phosphorylation levels may be due to a change in dephosphorylation dynamics. Thus, we next tested whether EGFR downregulation in cardiomyocytes impacts the expression of phosphatase subunits belonging to the protein phosphatase 1 (PP1) and protein phosphatase 2A (PP2A) families, which have been shown to regulate the phosphorylation status of PLB.[35,36] Our RNASeq data indicated a significant difference in transcript expression between CM-EGFR-KD and EGFR$^{f/f}$ (Cre$^-$) littermate control hearts for two PP1 regulatory subunits (*Ppp1r12b, Ppp1r3a*) and one PP2A B″ alpha regulatory subunit (*Ppp2r3a*) at either the 7- or 9-week timepoints. Via RT-qPCR-mediated validation experiments with an expanded sample size across early and later age groups, we found no change in expression levels of the PP1 regulatory subunits at any time point (Supplementary material online, *Figure S4B and C*); however, the transcript level of *Ppp2r3a* was consistently reduced in CM-EGFR-KD hearts at all ages tested (*Figure 7A*). Further, we confirmed that adult CM-EGFR-KD hearts also displayed significantly decreased Ppp2r3a protein expression, specifically the PR72 isoform (*Figure 7B*). While a very small decrease in the expression of the catalytic subunit PP2Ac was detected (Supplementary material online, *Figure S4D*), its global activity was not decreased in adult CM-EGFR-KD hearts (Supplementary material online, *Figure S4E*), suggesting that an alteration in its targeting to intracellular protein substrates may be a consequence of decreased PR72 expression in CM-EGFR-KD mice.

Since a decrease in PR72 expression was associated with prolonged relaxation and Ca^{2+} clearance in CM-EGFR-KD mice, we sought to

Figure 5 Cardiomyocyte-specific EGFR downregulation impairs relaxation dynamics. Haemodynamic evaluation of mean systolic pressure (SP, *A*), mean heart rate (HR, *B*), +dP/dt max (*C*) and -dP/dt max (*D*) in adult EGFR[f/f] (Cre[-]) vs. CM-EGFR-KD mice at baseline and in response to infusion with 10 ng ISO, *n* = 9 each. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$, ordinary one-way ANOVA with Tukey's multiple comparisons test. (*E*) LV cardiomyocytes were freshly isolated from adult EGFR[f/f] (Cre[-]) vs. CM-EGFR-KD hearts and cardiomyocyte length measured at baseline (black line) and after stimulation with ISO (0.1 μM, red line), as shown in tracings representative of *n* = 9 individual cardiomyocytes isolated from *n* = 3 mice per genotype, with quantification of cardiomyocyte shortening (*F*) and time to 50% relaxation (*G*) summarized in histograms. ns, not significant, ***$P < 0.001$, ordinary one-way ANOVA with Tukey's multiple comparisons test.

determine whether rescue of PR72 expression could restore these parameters. To test this, we infected primary AMCM isolated from CM-EGFR-KD mice with adenoviruses encoding PR72 or lacZ as a control (*Figure 7C*) and measured cardiomyocyte relaxation and Ca^{2+} clearance rates. Indeed, upregulation of PR72 expression in CM-EGFR-KD AMCM improved both parameters, with decreased time to 50% relaxation (*Figure 7D*) and Ca^{2+} clearance (*Figure 7E*). To next determine if the contractile dysfunction phenotype of CM-EGFR-KD mice could be rescued by re-expression of PR72, we injected adult CM-EGFR-KD mice with AAV9-cTnT-PR72 vs. AAV9-cTnT-LacZ control (*Figure 7F*, Supplementary material online, *Figure S4F*). Similar to re-expression of EGFR in these mice, treatment with AAV9-cTnT-PR72 resulted in a restoration of contractile function over time (*Figure 7G and H*, Supplementary material online, *Table S3*).
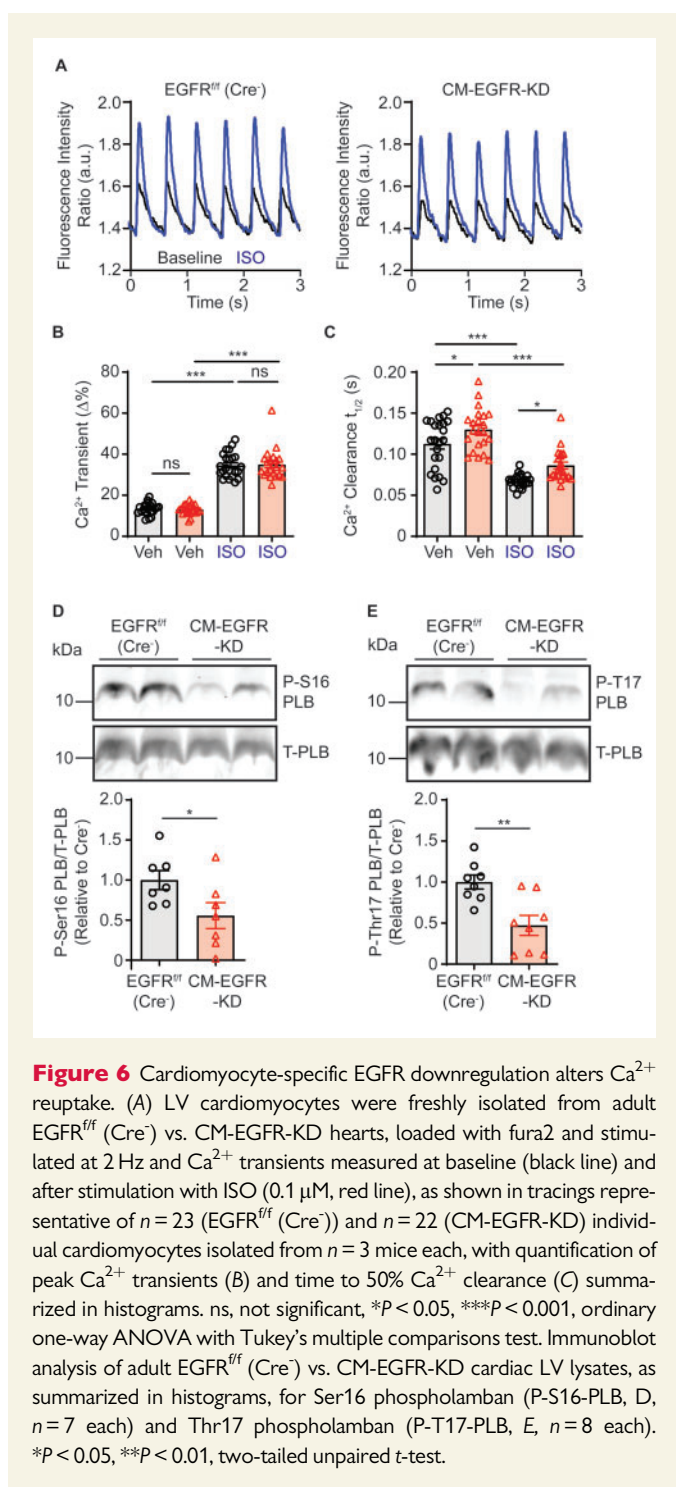
Altogether, these data demonstrate that EGFR normally exerts homeostatic control of cardiac contractility via regulation of PR72 expression, whereas EGFR downregulation in cardiomyocytes leads to the acute repression of PR72 expression and dysregulation of Ca^{2+} dynamics and cardiac contractility. Further, although chronic catecholamine stimulation normally acts via EGFR transactivation to induce cardiac hypertrophy, this response is absent when EGFR is downregulated and instead acts to compensate for the loss in contractility.

4. Discussion

While EGFR signalling in cardiomyocytes may provide a potentially important target to regulate cardiac remodelling during heart failure (HF),

especially in relation to its role as a signalling node for neurohormone GPCRs in general, a major hurdle in defining its impact during HF is the interpretability of *in vivo* studies. Exploration of the impact of EGFR signalling on cardiac function and remodelling *in vivo* either via GPCR-mediated transactivation or otherwise have relied primarily upon pharmacologic EGFR inhibition. *In vivo* use of EGFR inhibitors is limited by agent selectivity profiles[37] and has produced variable and conflicting reports regarding the impact of EGFR inhibition on cardiac function and remodelling either alone or in conjunction with different models of cardiac stress.[13,30–33] Alternatively, genetic models were developed, initially to define the impact of EGFR on developmental processes, including global EGFR knockout (KO) mice, mice containing a point mutation in the kinase domain of EGFR rendering it almost inactive, as well as a humanized EGFR knock-in mouse line.[14–18] However, while these global models demonstrated varying levels of cardiac remodelling processes over time, they either experienced embryonic/post-natal lethality or displayed a number of other developmental abnormalities throughout the body that precluded a clear understanding of the impact of EGFR specifically expressed in the heart.

Subsequently, genetic models were developed in which EGFR expression or activity was manipulated more selectively in the heart. One group crossed EGFR[f/f] and smooth muscle-specific protein 22-Cre mice, which resulted in downregulation of EGFR in both vascular smooth muscle cells (VSMC) and cardiomyocytes and the development of systemic hypotension with cardiac hypertrophy.[20] Initially, interpretation of the cardiac phenotype distinct from the vascular phenotype in this model was unclear; however, a follow-up study using inducible VSMC-specific
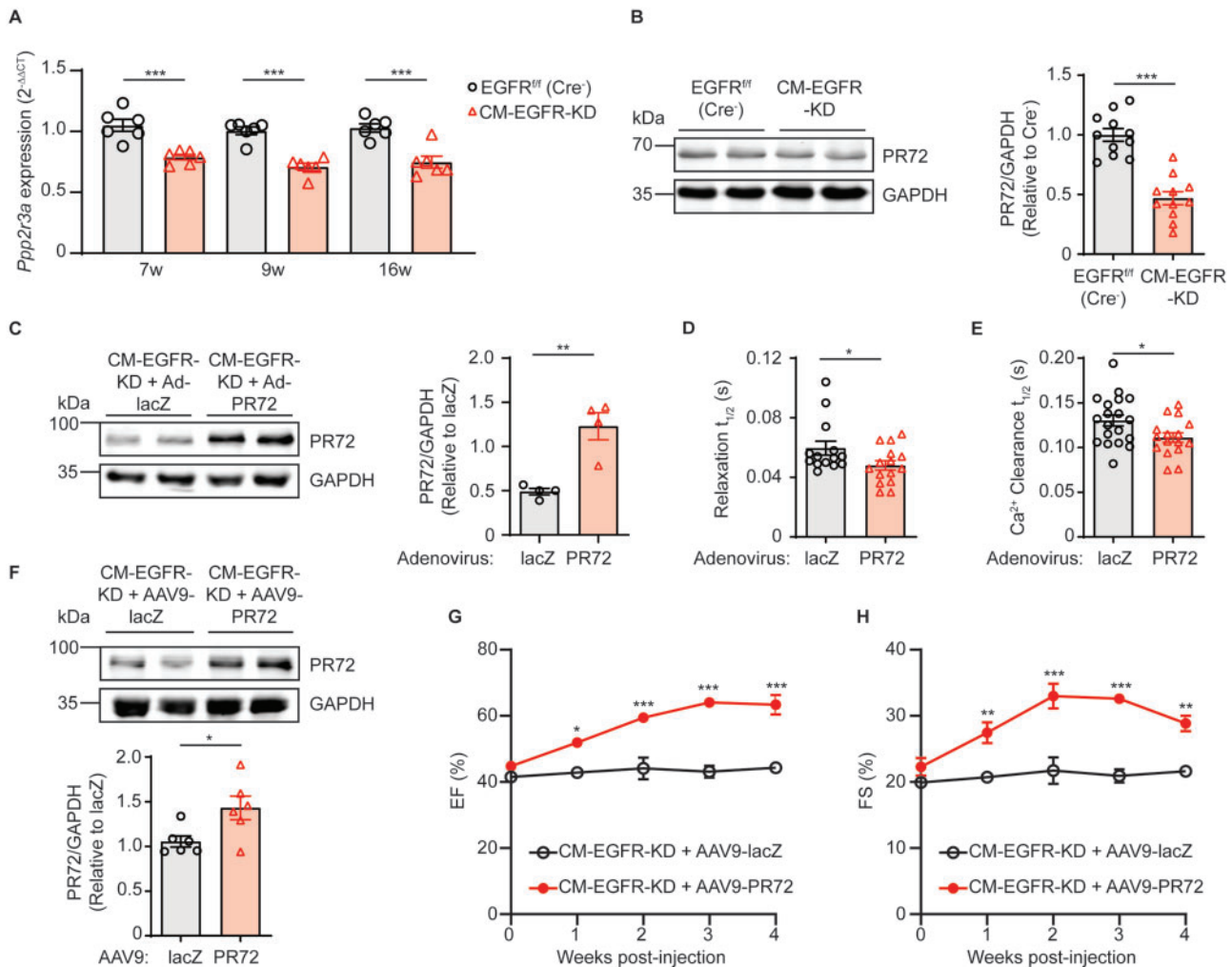
Figure 6 Cardiomyocyte-specific EGFR downregulation alters Ca^{2+} reuptake. (*A*) LV cardiomyocytes were freshly isolated from adult EGFR$^{f/f}$ (Cre$^-$) vs. CM-EGFR-KD hearts, loaded with fura2 and stimulated at 2 Hz and Ca^{2+} transients measured at baseline (black line) and after stimulation with ISO (0.1 μM, red line), as shown in tracings representative of $n = 23$ (EGFR$^{f/f}$ (Cre$^-$)) and $n = 22$ (CM-EGFR-KD) individual cardiomyocytes isolated from $n = 3$ mice each, with quantification of peak Ca^{2+} transients (*B*) and time to 50% Ca^{2+} clearance (*C*) summarized in histograms. ns, not significant, *$P < 0.05$, ***$P < 0.001$, ordinary one-way ANOVA with Tukey's multiple comparisons test. Immunoblot analysis of adult EGFR$^{f/f}$ (Cre$^-$) vs. CM-EGFR-KD cardiac LV lysates, as summarized in histograms, for Ser16 phospholamban (P-S16-PLB, D, $n = 7$ each) and Thr17 phospholamban (P-T17-PLB, *E*, $n = 8$ each). *$P < 0.05$, **$P < 0.01$, two-tailed unpaired t-test.

(smooth muscle myosin heavy chain) Cre mice showed normal cardiac EGFR expression with no hypertrophy.[38] While this indirectly suggested that loss of cardiomyocyte-expressed EGFR may promote cardiac hypertrophy, our results now clearly show that cardiomyocyte-specific downregulation of EGFR does not promote hypertrophy until a later age when cardiac function has undergone severe deterioration.

Other groups have employed cardiomyocyte-specific transgenic overexpression of an intracellular domain-deficient EGFR that acted as a dominant negative (DN-EGFR) with endogenous ErbB receptors.[19,21] However, these studies produced disparate effects on cardiac function and remodelling, with one group observing a lack of cardiac dysfunction

at any age tested in constitutive DN-EGFR-expressing mice[21] and the other group observing an immediate decline in contractility concurrent with increased cardiac dilation and hypertrophy following induction of the DN-EGFR in adult mice.[19] While the differences in outcomes in these studies could be attributable to distinct methods, the extent of induction of DN-EGFR or the construct differentially altering ErbB2 activity, ultimately using two independent methods we show via echocardiography, invasive haemodynamics, and isolated cardiomyocyte contractility assays that downregulation of EGFR expression, rather than overexpression of a mutant EGFR, leads to decreased contractile function without concurrent cardiac remodelling. Instead, pathologic cardiac remodelling appears to occur in a delayed manner in these mice that only occurs following long-term contractile dysfunction. We also demonstrate that EGFR downregulation in cardiomyocytes does not alter ErbB2 expression or downstream activation status of its common effectors ERK1/2 and Akt, although these were measured in total left ventricular lysates that contain fibroblasts as well as vascular smooth muscle, immune and endothelial cells and would contribute to the signal. Alterations in cardiomyocyte-specific ErbB2 expression have been shown to directly alter cardiomyocyte growth and proliferation at various developmental stages and in response to injury,[4,5,39] highlighting the essential role for ErbB2 in maintaining cardiac structure. Conversely, since αMHC turns on early,[29] our data indicate that cardiomyocyte-specific EGFR is not required for cardiac post-natal development, but becomes essential in maintaining contractile function as cardiac development progresses toward adulthood. Further, acute AAV9-mediated EGFR downregulation in the adult heart demonstrated that EGFR expression is required for the continued maintenance of contractility in fully matured hearts without an overt impact on the structure. Thus, while ErbB2 is essential for cardiomyocyte proliferation and maintenance of cardiac structure, EGFR plays a more predominant role in the homeostasis of contractile function.

Cardiac contractility was decreased secondary to reduced EGFR expression, despite βAR density, responsiveness to stimulation, and downstream impact on contractility remaining intact. Further, although chronic βAR stimulation in control mice led to increased cardiomyocyte hypertrophy with a slight reduction in contractility over time, CM-EGFR-KD mice regained normal contractile function and were protected from hypertrophic remodelling. These outcomes are notable for a number of reasons. First, even though βAR and EGFR are known to associate and bi-directional regulation of their downstream signalling pathways has been reported by us and others,[6,13,30,40–42] we demonstrate here that EGFR downregulation does not alter βAR density or amplitude of catecholamine-mediated contractility. Thus, β1AR and EGFR regulate cardiac contractility via distinct mechanisms and EGFR transactivation does not directly modulate β1AR-mediated cardiomyocyte contraction. Second, although EGFR transactivation by various GPCRs has been implicated in the induction of cardiac remodelling in response to chronic neurohormone stimulation, various pharmacologic and genetic models have led to conflicting interpretation of the role of EGFR in remodelling outcomes.[13,30–33] However, we definitively showed that with reduced EGFR expression specifically in cardiomyocytes, chronic catecholamine stimulation is no longer able to induce a hypertrophic response in cardiomyocytes. Lastly, our study highlights that chronic βAR stimulation is able to stably and reversibly rescue the contractile dysfunction in CM-EGFR-KD mice in the absence of hypertrophy. Consistent with earlier studies showing that hypertrophy is not required for maintaining contractile function in the heart under conditions of stress,[43,44] our study therefore suggests that βAR-mediated promotion of contractility in the absence of EGFR-dependent hypertrophic remodelling could provide

Figure 7 Cardiomyocyte-specific EGFR downregulation decreases expression of the PP2A B'' regulatory subunit PR72. (*A*) RT-qPCR was used to measure the expression of *Ppp2r3a* in the LV of EGFR$^{f/f}$ (Cre$^-$) vs. CM-EGFR-KD mice at 7, 9, and 16 weeks of age (*n* = 6 each). ****P* < 0.001, two-tailed unpaired *t*-test. (*B*) Immunoblot analysis of PR72 expression in adult EGFR$^{f/f}$ (Cre$^-$) vs. CM-EGFR-KD cardiac lysates, as summarized in histogram. Blots shown are representative of *n* = 11 cardiac lysates per genotype. ****P* < 0.001, two-tailed unpaired *t*-test. (*C*) Immunoblot analysis of PR72 expression in adult CM-EGFR-KD mouse cardiomyocytes infected for 24 h with adenoviruses encoding lacZ or PR72, as summarized in histogram. Blots shown are representative of *n* = 4 independent cardiomyocyte preparations. ***P* < 0.01, two-tailed unpaired *t*-test. (*D*) Time to 50% relaxation measured in lacZ vs. PR72-infected AMCM from CM-EGFR-KD mice. **P* < 0.05, two-tailed unpaired *t*-test. *n* = 14–15 individual cardiomyocytes isolated from *n* = 5 mice per adenovirus infection. (*E*) Time to 50% Ca^{2+} clearance in lacZ vs. PR72-infected AMCM from CM-EGFR-KD mice. **P* < 0.05, two-tailed unpaired *t*-test. *n* = 16–19 individual cardiomyocytes isolated from *n* = 3 mice per condition. (*F*) Immunoblot analysis of PR72 expression in adult CM-EGFR-KD cardiac lysates, 2 weeks after infection with AAV9-lacZ or AAV9-PR72, as summarized in histogram. Blots shown are representative of *n* = 6 cardiac lysates per condition. **P* < 0.05, two-tailed unpaired *t*-test. %EF (*G*) and %FS (*H*) of adult CM-EGFR-KD mice treated with AAV9-lacZ (*n* = 10, reference group from *Figure 1E and F*, respectively) vs. AAV9-PR72 (*n* = 5) were monitored via echocardiography for 4 weeks following AAV administration (at time 0). **P* < 0.05, ***P* < 0.01, ****P* < 0.001 vs. reference group CM-EGFR-KD+AAV9-lacZ at corresponding timepoints, ordinary two-way ANOVA with Bonferonni's multiple comparisons test.

beneficial outcomes under stress conditions in which elevated catecholamine levels would be expected to contribute to hypertrophic cardiomyopathy. However, whether the decreased hypertrophic response to chronic catecholamine stimulation in CM-EGFR-KD mice is directly due to loss of βAR-mediated transactivation-dependent hypertrophic signalling or rather to the preservation of cardiac contractility via βAR stimulation remains to be determined.

Previous studies investigating the role of cardiomyocyte-expressed EGFR on cardiac function and remodelling have been primarily phenotype-based,[19–21] with little insight into mechanistic underpinnings that

could explain the effects of altered cardiomyocyte-specific EGFR expression/activity on cardiac parameters. Here, we show that downregulation of EGFR in cardiomyocytes results in the altered expression of cardiac genes in a temporally dependent manner. A majority of the cardiac gene expression changes were detected at 7 weeks of age, with normalization of three quarters of these by 9 weeks of age. These data suggest that many of the gene changes observed early, before cardiac dysfunction manifested, may be elevated in the CM-EGFR-KD mice as a compensatory process that is not sustained as the mice mature, wherein relatively fewer gene changes account for the actual cardiac dysfunction. Notably, of the

myofibril-related genes that were shown to be downregulated at 9 weeks of age when cardiac dysfunction was present, several encode atrial or skeletal isoforms known to be involved in development, arrhythmias or myofibrillar myopathies,[45–48] suggesting that their LV downregulation could reflect an attempt to normalize cardiac function at the level of the myofilament. While we did not detect abnormalities in either cardiac structure or myofilament Ca^{2+} sensitivity and thus did not further investigate these changes in gene expression, future work focused on how EGFR modulates the expression and function of myofilament proteins may be warranted.

Beyond the myofilament, our data clearly pointed to dysregulated Ca^{2+} handling as a possible mechanism involved in the contractile dysfunction phenotype observed in CM-EGFR-KD mice. Notably, we detected a reduction in phosphorylation of PLB and cTnI, which would be expected to decrease Ca^{2+} reuptake into the sarcoplasmic reticulum[12,34] and reduce the Ca^{2+} dissociation rate from the sarcomere,[49] respectively, leading to slower relaxation kinetics. Indeed, we observed a decrease in cardiac relaxation at baseline and following ISO stimulation in vivo that was associated with reduced Ca^{2+} reuptake as measured in isolated cardiomyocytes. While reduced phosphorylation of PLB or cTnI could be secondary to decreased kinase activity, no evidence of this arose since βAR signalling pathways and functional responsiveness to βAR stimulation were not altered. Thus, we focused instead on dephosphorylation dynamics, observing that the regulatory phosphatase subunit Ppp2r3a/PR72 in particular was downregulated at all timepoints measured in CM-EGFR-KD mice.

Despite its identification as a cardiac-expressed B″ regulatory subunit of the PP2A holoenzyme over 2 decades ago,[50] and its recently reported involvement in cardiac development in zebrafish,[51] very little is known about the role of PR72 in the regulation of cardiac function in the adult heart, representing a limitation of our study. However, increased expression of cardiac PR72 was detected in both non-ischaemic and ischaemic human HF as well as a canine model of HF,[52] which coincides with a reported increase in EGFR expression in ischaemic human heart tissue,[22] though the consequences of this upregulation are unknown. Inferences can be made based on the reported localization of PR72 in adult cardiomyocytes at Z and M lines[52] and PP2A at the myofilaments,[53,54] which would suggest a role in the regulation of PP2A activity at the level of the sarcomere and disruption of which upon reduced PR72 expression could lead to more diffuse PP2Ac localization toward other targets such as the sarcoplasmic reticulum. However, in addition to decreased PLB phosphorylation, we also observed decreased cTnI phosphorylation which may suggest enhanced accumulation of PP2Ac at the myofilaments with reduced PR72 expression, thus the impact of PR72-dependent regulation on PP2Ac localization within cardiomyocytes requires additional study. Notably, both increased and decreased cardiomyocyte-specific PP2Ac expression has been shown to reduce contractile function and promote hypertrophy and fibrosis,[53,55,56] but in our study, we observed decreased contractility in the absence of cardiac remodeling events. This would be consistent with the idea that the various B regulatory subunits of the PP2A holoenzyme specifically regulate certain pools of PP2A activity.[35,57] Therefore, since the rescue of PR72 expression restored cardiac contractility both in vitro and in vivo, PR72 may normally act to restrict excessive PP2A access to targets including PLB and cTnI, which will be the focus of ongoing investigation.

In summary, using complimentary mouse models, we have for the first time identified the impact of cardiomyocyte-expressed EGFR on the homeostatic regulation of contractility via a novel regulation of the PP2A B″ regulatory subunit PR72. Further, we have conclusively demonstrated that cardiomyocyte-expressed EGFR is required for the promotion of hypertrophy under conditions of chronic catecholamine stress. Thus, strategies to uncouple β1AR-mediated transactivation of EGFR may provide a means by which to preserve both catecholamine- and EGFR-dependent regulation of cardiac contractility in the absence of hypertrophic remodelling during the development and progression of HF.

Supplementary material

Supplementary material is available at *Cardiovascular Research* online.

Authors' contributions

The authors approve of this work and contributed as follows: experimental design (SG, DGT), data acquisition and analysis (SG, ADO, EM, JLS, RLC, VCP, TPT, ML, JS, AML, TGM, EG, SR), interpretation of data (SG, JAK, WJK, JYC, DGT), and drafting the work (SG, DGT).

Data availability

The data underlying this article will be shared upon reasonable request to the corresponding authors. RNA-Seq data underlying this article are available in GEO at https://www.ncbi.nlm.nih.gov/geo/ and can be accessed with accession number GSE140407.

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Translational perspective

Our study highlights a previously unrecognized role for epidermal growth factor receptor (EGFR) in maintaining contractile homeostasis under physiologic conditions in the adult heart via regulation of PR72, a PP2A regulatory subunit with an unknown impact on cardiac function. Further, we have shown that cardiomyocyte-expressed EGFR is required for the promotion of cardiac hypertrophy under conditions of chronic catecholamine stress. Altogether, our study provides new insight into the dynamic nature of cardiomyocyte-specific EGFR.