Supplemental Information

Table of Contents

1) Expanded Methods and Materials

2) Supplemental Figures

3) Supplemental Tables

4) Supplemental Movies

5) Author Contributions

1. Methods and Materials

Mice. All animal procedures were performed under protocols approved by the Icahn School of Medicine at Mount Sinai Institutional Animal Care and Use Committee (IACUC). CFW or Rosa26^{mTmG} mice strains, male and female, were used. Different modRNAs (100 or 150 µg/heart) were injected directly into the myocardium during open chest surgery. 3 to 8 animals were used for each experiment. For long-term survival, (8-10-week-old) CFW mice treated with $_{CMS}$Luc or $_{CMS}$Pkm2 modRNAs (n=10) post MI induction were allowed to recover for 6 months in the animal facility. Deaths were monitored and documented. Tamoxifen-inducible CM-restricted deletion of Pkm2 ($_{CMS}$KO-Pkm2) was generated by crossing Pkm2$^{flox/flox}$ (purchased from Jackson Laboratories (B6;129S-Pkmtm1.1Mgvh/J)) to Tnnt2$^{MerCreMer/+}$ mice (made by Dr. Chen-Leng Cai, a co-author on this manuscript). Tamoxifen (Sigma-Aldrich) was dissolved in sesame oil at 10 mg/ml as stock solution. To induce Cre nuclear translocation, tamoxifen was administered to mice by intraperitoneal (IP) injection for two consecutive days (E9-E10) (24h interval between administrations) at 0.05 mg/g body weight/day for embryonic stages. The tissues were harvested on E18 for analysis. Mouse husbandry was carried out according to the protocol approved by the IACUC at Icahn School of Medicine at Mount Sinai. Oligonucleotide sequences for genotyping these mouse lines: Tnnt2-F-AGGAACATGAAATCCAGGGTGGCT, Tnnt2-R-GTTCAGCATCCAACAAGGCACTGA; Pkm2-F-CCTTCAGGAAGAC-AGCCAAG, Pkm2-R – AGTGCTGCCTGGAATCCTCT.

modRNA synthesis. ModRNAs were transcribed *in vitro* from plasmid templates (see complete list of open reading frame sequences used to make modRNA in **Table S1**). Using a customized ribonucleotide blend of anti-reverse cap analog, 3´-O-Me-m7G(5')ppp(5')G (6 mM, TriLink Biotechnologies), guanosine triphosphate (1.5 mM, Life Technology), adenosine triphosphate (7.5 mM, Life Technology), cytidine triphosphate (7.5 mM, Life Technology) and N1-Methylpseudouridine-5'-Triphosphate (7.5 mM, TriLink Biotechnologies) as described previously in our recent protocol paper [31]. mRNA was purified using the Megaclear kit (Life Technology) and treated with antarctic phosphatase (New

England Biolabs), followed by re-purification using the Megaclear kit. mRNA was quantitated by Nanodrop (Thermo Scientific), precipitated with ethanol and ammonium acetate and resuspended in 10 mM TrisHCl, 1 mM EDTA.

modRNA transfection. *In vivo* modRNA transfection was done, as described previously in our recent method paper [32], using sucrose citrate buffer containing 20µl of sucrose in nuclease-free water (0.3g/ml) and 20µl of citrate (0.1M pH=7; Sigma) mixed with 20µl of different modRNA concentrations in saline to a total volume of 60µl. The transfection mixture was directly injected (3 individual injections, 20µl each) into the myocardium. For *in vitro* transfection, we used RNAiMAX transfection reagent (Life Technologies) according to manufacturer's recommendations.

Mouse MI model and histology. All surgical and experimental procedures with mice were performed in accordance with protocols approved by IACUC and the MSSM Center for Comparative Medicine and Surgery (CCMS). CFW, Rosa26^{mTmG} mice (6-8 weeks old) were anesthetized with isoflurane. MI was induced by permanently ligating the LAD, as previously described [67]. Briefly, the left thoracic region was shaved and sterilized. After intubation, the heart was exposed through a left thoracotomy. A suture was placed to ligate the LAD. The thoracotomy and skin were sutured closed in layers. Excess air was removed from the thoracic cavity, and the mouse was removed from ventilation when normal breathing was established. In order to determine the effect of modRNA on cardiovascular outcomes after MI, modRNAs (100-150 µg/heart) were injected into the infarct zone immediately after LAD ligation. The peri-infarct zone near the apex was either snap-frozen for RNA isolation and subsequent real-time qPCR studies or fixed in 4% PFA for cryo-sectioning and immunostaining. In all experiments, the surgeon was blinded to the treatment group. To assess heart histology, hearts were collected at the end of each study. The hearts were excised, briefly washed in PBS, perfused with perfusion buffer, weighed and fixed in 4% PFA at 4°C overnight. The next day, hearts were washed with PBS and incubated overnight in 30% sucrose. Next, hearts were put in OCT, frozen and stored at -80°C. The heart blocks were transverse sectioned at 8-9µm using cryostat. The slides were further processed for evaluation using immunostaining (see below) or histological scar staining using Masson's trichrome staining kit (Sigma) and hematoxylin and eosin (H&E) staining, all performed according to standard procedures. Measuring the heart-weight to body-weight ratio was done using a scale at the end point of each experiment. This ratio was calculated as the heart tissue weight relative to the mouse total body-weight in grams (g).

Immunostaining heart sections following modRNA treatment. Mice hearts were harvested and perfused using perfusion buffer and 4% paraformaldehyde (PFA). Hearts were fixed in 4% PFA/PBS overnight on a shaker and then washed with PBS for 1 hr and incubated in 30% sucrose/PBS at 4°C overnight. The next day, hearts were fixed in OCT and frozen at -80°C. Transverse heart sections (8-10 µM) were made by cryostat. Frozen sections were rehydrated in PBS for 5 min followed by permeabilization with PBS and 0.1% triton X100

(PBST) for 7 min. Slides were then treated with 3% H_2O_2 for 5 min. After 3 washes with PBST for 5 minutes each, the samples were blocked with PBS + 5% Donkey normal serum + 0.1% Triton X100 (PBSST) for 2 hrs at room temperature and primary antibodies diluted in PBSST were added (see complete list of primary antibodies used for this study in **Table S2**). Slides were then incubated overnight at 4°C. Slides were washed with PBST (5 times for 4 min each) followed by incubation with a secondary antibody (Invitrogen, 1:200) diluted in PBSST for 2 hrs at room temperature. The samples were further washed with PBST (3 times for 5 min each) and stained with DAPI or Hoechst 33342 (1μg/ml) diluted in PBST for 7 min. After 5 washes with PBST for 4 min each and one wash with tap water (for 4 min), slides were mounted with mounting medium (VECTASHIELD) for imaging. Stained slides were stored at 4°C. All stainings were performed on 3-8 hearts/group, with 2-3 sections/heart. In the case of immunostaining with wheat germ agglutinin (WGA) for CM size quantification, images at 40X magnification were captured and the ImageJ program was used to determine the area of each cell. Quantitative analyses involved counting multiple fields from 3-6 independent hearts/group and 3 sections/heart (~50 cells per field assessed, to a total ~250 cells per sample). For BrdU immunostaining, BrdU (1mg/ml, Sigma) was added to the drinking water of adult mice (2-3 months old) for 7-10 days before hearts were harvested. Quantitative analyses involved counting BrdU-positive CMs in multiple fields from three independent samples/group and 3 sections/heart. The total number of CMs counted was ~1-2 $x10^3$ CMs per section. TUNEL immunostaining was performed according to the manufacturer's recommendations (*In Situ* Cell Death Detection Kit, Fluorescein, Cat# 11684795910, Roche). For immunostaining neonatal rat or mouse CMs following modRNA treatment, modRNA-transfected neonatal CMs were fixed on coverslips with 3.7% PFA for 15 min at room temperature, then washed 3 times with PBS. Following permeabilization with 0.5% Triton X in PBS for 10 min at room temperature, cells were blocked with 5% normal goat / donkey serum + 0.5% Tween 20 for 30 min. Coverslips were incubated with primary antibodies in a humid chamber for 1 hr at room temperature followed by incubation with corresponding secondary antibodies conjugated to Alexa Fluor 488, Alexa Fluor 647, Alexa Fluor 555 and Hoechst 33342 staining for nuclei visualization (all from Invitrogen). The fluorescent images were taken on a Zeiss fluorescent microscope at 10X, 20X and 40X magnification.

Western blot analysis. We isolated total protein from the respective cells or tissues at given time points. In brief, equal amounts of protein were resolved using a SDS-PAG Electrophoresis system in 4%-15% Mini-PROTEAN TGX stain-free gels (Bio-Rad) and blotted onto polyvinylidene fluoride (PVDF) membranes. The membranes were blocked (5% BSAin Tris-buffered saline (TBS; 50 mM Tris-HCl [pH 7.4], 150 mM NaCl) for 1 hr at room temperature and were then incubated with primary antibodies diluted in 5% BSA in TBS (overnight at 4C). We used anti-Pkm2 (1:1,000, Cell Signaling, #4053); anti-Pkm1 (1:1,000, Cell Signaling, #7067); anti-G6pd (1:1,000, Abcam, #ab993); anti-β-catenin (1:1,000, Cell Signaling, #9582); anti-GAPDH (horseradish peroxidase [HRP]

conjugate 1:3,000, Cell Signaling, #8884) and mouse monoclonal anti-β-actin (horseradish peroxidase [HRP] conjugate 1:3,000, Cell Signaling, #12262) antibodies. Anti-rabbit and anti-mouse HRP-conjugated secondary antibodies were purchased from Sigma-Aldrich. Antigen or antibody complexes were visualized with the ChemiDoc Touch imaging system (Bio-Rad).

Magnetic Resonance Imaging (MRI) and Echocardiography (Echo). In a double-blind study (i.e. neither surgeon and MRI nor Echo technician knew the type of modRNA treatment), CFW mice (8 weeks old) treated with Luc k motif, Luc k motif + miR1-208 ($_{CMS}$Luc), miR1-208, Pkm2 k motif and Pkm2 k motif + miR1-208 ($_{CMS}$Pkm2) modRNAs were subjected to MRI assessment on day 28 post LAD ligation. We obtained delayed-enhancement CINE images on a 7-T Bruker Pharmascan with cardiac and respiratory gating (SA Instruments). Mice were anesthetized with 1-2% isoflurane/air mixture. ECG, respiratory and temperature probes were placed on the mouse, which was kept warm during scans. Imaging was performed 10 to 20 min after IV injection of 0.3 mmol/kg gadolinium-diethylene triamine pentaacetic acid. A stack of 8 to 10 short-axis slices spanning from the heart apex to its base were acquired with an ECG-triggered and respiratory-gated FLASH sequence with the following parameters: echo time (TE) 2.7 msec with resolution of 200 μm x 200 μm; slice thickness of 1 mm; 16 frames per R-R interval; 4 excitations with flip angle at 60°. Ejection fraction was calculated as the difference between end-diastolic and end-systolic volumes, divided by the end-diastolic volume. MRI studies and analyses were performed blinded to treatment groups. For Echo evaluation of left ventricular systolic function, a GE Cares Insite (V7R5049) equipped with a 40 MHz mouse ultrasound probe was used. Fractional shortening was calculated based on end diastolic and end systolic dimensions obtained from M-mode ultrasound. Echocardiograms were performed on 6-8 hearts/treatment groups.

RNA interference. For siRNA knockdown, CMs were transfected 48 to 72 hrs after seeding by lipofectamine RNAiMAX kit (Invitrogen) with validated siRNAs or All Stars Negative Control siRNA (Qiagen) (100 nM) and washed after 5-6 hrs. Gene expression inhibition was verified by antibody staining. siRNA sequences: β- catenin- 5'-UAGUCGUGGGAUCGCACCCTG-3', Pkm2- PUUGGUGAGGACGAUUAUGGUU, Pkm1- PUUAUAAGAAGCCUCCACGCUU Hif1α- UGAGGUACUUGGGUAGAAGGT, G6pd- AUAAGUUAGAUCCAGCUCCGA.

RNA isolation and gene expression profiling using Real-Time PCR. Total RNA was isolated using the RNeasy mini kit (Qiagen) and reverse transcribed using Superscript III reverse transcriptase (Invitrogen) according to the manufacturer's instructions. Real-time qPCR analyses were performed on a Mastercycler Realplex 4 Sequence Detector (Eppendorf) using SYBR Green (Quantitect^{TM}SYBR Green PCR Kit, Qiagen). Data were normalized to *18s* expression; fold-changes in gene expression were determined by the ∂∂CT method and presented relative to an internal control. PCR primer sequences are shown in **Table S3.**

Lineage tracing in R26^{mTmG} mice. Rosa26^{mTmG} mice were obtained from the Jackson Laboratory (B6.129(Cg)-*Gt(ROSA)26Sor*$^{tm4(ACTB-tdTomato,-EGFP)Luo}$/J). All experiments were performed on age- and sex-matched mice with equal numbers of male and female mice. Healthy mice were chosen randomly from the expansion colony for each experiment. In this mouse line, membrane-targeted tdTomato is expressed under the control of the ubiquitous promoter on Rosa26 locus, whereas membrane-targeted eGFP becomes active after Cre-mediated excision of floxed tdTomato. $_{CMS}$Cre modRNA (Cre K-motif + miR1-miR208) was used to express Cre exclusively in transfected CMs. This allowed for lineage tracing of the transfected CMs and their progeny long after modRNA expression was diminished (>10 days). Rosa26^{mTmG} mice were genotyped by PCR with tail DNA as described in the Jackson Laboratory Genotyping Protocols. Primer sequences: Rosa26$^{mT/mG}$, wild type forward, 5' CTCTGCTGCCTCCTGGCTTCT-3', wild type reverse, 5'- CGAGGCGGATCACAAGCAATA-3' and mutant reverse, 5'- TCAATGGGCGGGGGTCGTT-3'. In this model, we measured the $_{CMS}$Cre modRNA transfection level, CM size, CM numbers and the number of nuclei in CMs post transfection with $_{CMS}$Luc or $_{CMS}$Pkm2 modRNAs.

Neonatal rat and fetal or adult mouse CMs isolation. CMs from E18 fetal mice (Pkm2 KO or wild type) or 3-4-day-old neonatal rats' hearts were isolated as previously described [68, 69]. E18 fetal mice hearts or neonatal rats' ventricular CMs were isolated from 4-day-old Sprague Dawley rats (Jackson). We used multiple rounds of digestion with 0.14-mg/mL collagenase II (Invitrogen). After each digestion, the supernatant was collected in horse serum (Invitrogen). Total cell suspension was centrifuged at 300 g for 5 min. Supernatants were discarded and cells were resuspended in DMEM (GIBCO) medium with 0.1 mM ascorbic acid (Sigma), 0.5% Insulin-Transferrin-Selenium (100X), penicillin (100 U/mL) and streptomycin (100 µg/mL). Cells were plated in plastic culture dishes for 90 min until most of the non-myocytes attached to the dish and myocytes remained in suspension. Myocytes were then seeded at 1×10^5 cells/well in a 24-well plate. Isolated CMs were incubated for 48 hrs in DMEM medium containing 5% horse serum plus Ara c. After incubation, cells were transfected with varied doses of different modRNAs as described in the main text. Adult CMs were isolated from CFW mice 28 days after MI and modRNA injection using Langendorff's method as previously described [68, 70, 71]. For CM count, we averaged 3 different counts/sample and 3 hearts/group using a hemocytometer. The total number of CMs counted was approximately 150-200 CMs/aliquot (10ul aliquot samples using a wide-bore pipette from the total volume of CMs obtained following digestion). The cultured CMs were stained with α-actinin (CMs, Red) antibody (abcam) and Hoechst 33342 for nuclei counts. For nuclei count, approximately $1x10^3$ CMs were counted per sample, using 3-4 independent samples per group. Nuclei count was plotted as percentage of counted CMs. For isolation of transfected adult CMs and RNA see **Figure S18**.

MADM mice. All animal procedures were performed under protocols approved by the Icahn School of Medicine at Mount Sinai Institutional Care and Use Committee. The 8-10-week-old MADM-ML-11$^{GT/TG}$ heterozygous mice were

purchased from Jackson Laboratory (*Igs2*$^{tm1(ACTB-EGFP,-tdTomato)Zng}$/Igs2$^{tm2(ACTB-tdTomato,-EGFP)Zng}$/J (Cat no. 030578)). Acute and chronic MI models were used to evaluate CM cell division in MADM mice. In the acute MI models, 8-12-week-old MADM mice were injected immediately after MI, and in chronic MI models mice were injected 14 days post MI with $_{CMS}$Cre + $_{CMS}$Luc or $_{CMS}$Pkm2 modRNA. 14 days after modRNA injection, adult CMs were isolated from MADM mice using Langendorff's method or harvested and immunostained to evaluate single-color % of total labeled (Cre-activated cells) CMs.

^{13}C isotopic tracers for metabolic flux analysis. 2 X10^{6} P3 neonatal rat CMs were cultured in DMEM medium and transfected with Luc or Pkm2 modRNA. After 6 hrs, medium was changed with ^{13}C Glucose containing DMEM media. The samples were collected at 10 min, 2 hrs and 18 hrs by flash freezing the cell culture plates using liquid nitrogen and then freezing the plates at -80^{0}C. The metabolites were extracted from cells using 80% methanol [72, 73]. Targeted LC/MS analyses were performed on a Q Exactive Orbitrap mass spectrometer (Thermo Scientific) coupled to a Vanquish UPLC system (Thermo Scientific). The Q Exactive was operated in polarity-switching mode. A Sequant ZIC-HILIC column (2.1 mm i.d. × 150 mm, Merck) was used to separate metabolites. Flow rate was 150 μL/min. Buffers consisted of 100% acetonitrile for A and 0.1% NH4OH/20 mM CH3COONH4 in water for B. Gradient ran from 85 to 30% A in 20 min followed by a wash with 30% A and re-equilibration at 85% A. Metabolites were identified on the basis of exact mass within 5 ppm and standard retention times. Relative metabolite quantitation was performed based on peak area for each metabolite. All data analyses were done using scripts written in house (Metabolomics Facility, Biotechnology Resource Center, Cornell University).

HPLC measurements of ROS. ROS production in heart tissue was measured using HPLC for dihydroethidium (DHE) oxidation products [74, 75]. Immediately after harvesting hearts, a 20 mg segment was cut into small pieces and incubated with DHE (100 μM) in PBS containing diethylenetriaminepentaacetic acid DTPA (100 μM) at 37°C for 30 min. The sample was washed with PBS/DTPA, extracted with acetonitrile (500μl) and briefly sonicated (3x30 sec, 8W). After spinning (13,000Xg, 10 min at 4°C), the supernatant was collected and dried under vacuum, and pellets were stored at -20°C in the dark until analysis. Samples were resuspended in 120 μl PBS-DTPA and injected into the HPLC system. The superoxide-specific DHE oxidation product, 2-hydroxyethidine (EOH), was quantified by comparing the peak signal between samples and standard solutions under identical chromatographic conditions and then expressed as EOH/mg tissue.

HPLC measurements of glutathione. The glutathione (GSH) level in heart tissue was measured by an HPLC method with electrochemical detection, similar to a process previously described [9]. Heart samples were homogenized in a 100 mM acetate buffer (pH 5.4, containing 10 μM DTPA). A small aliquot was used to quantify total protein. The remaining sample was deproteinized with 10% TCA

and centrifuged at 10,000 g for 5 min at 4^0C; the supernatant was filtered (0.45 μm) and stored at -80 ^{0}C. Samples were applied to a C18 column (Phenomenex C18, 3 μm, 150 × 4.6 mm) at 0.5 $ml.min^{-1}$ using isocratic mobile phase solution (25 mM NaH_2PO_4, 1 mM 1-octane sulfonic acid, 6% acetonitrile, pH 2.6,with phosphoric acid). GSH levels were quantified by comparing to standards subjected to the same HPLC conditions. Glutathione disulphide (GSSG) levels were indirectly determined by enzymatic reduction with glutathione reductase (0.6 U/ml plus 0.2 mg/ml NADPH) for 30 min. The reaction was stopped by adding 5% TCA on ice. The GSH levels were normalized by the amount of protein. GSSG quantification was achieved by the difference between total reduced GSSG and total GSH in each sample.

NADP/NADPH quantitation kit. Samples were isolated, according to manufacturer instructions, from cultured 1-day neonatal CMs 24 hours after being transfected with Luc or Pkm2 modRNA. Metabolic calorimetric of NAD/NADH Quantitation Kit (Cat no. MAK037) and NADP/NADPH Quantitation Kit (Cat no. MAK038) were used to evaluate the different ratios. Assays were performed according to manufacturers' instructions.

Co-immunoprecipitation and western blotting. 2 X 10^6 P3 neonatal rat CMs were rinsed with PBS and harvested in ice-cold RIPA lysis buffer (50 mM Tris-HCl, 150 mM NaCl, 1.0% NP-40, 0.5% sodium deoxycholate and 0.1% SDS, Boston Bioproducts) containing the protease and phosphatase inhibitor cocktail (Pierce). The sonicated cell lysates were clarified by centrifugation at 13,000 X g for 20 min at 4^oC. Protein concentration was determined using a BCA protein assay kit (Pierce). The supernatants containing cell lysates were incubated with either the normal IgG or the indicated antibodies overnight on a rotator at 4^oC. The immunocomplexes were incubated with protein A/G Plus-Agarose beads (Millipore) for 1 hr at 4^oC. The immunoprecipitates were washed three times with ice-cold lysis buffer and eluted from the beads by boiling in 2X SDS sample buffer (Bio-Rad). The immunoprecipitates (800 μg) and total cell lysates (30~50 μg) were analyzed by western blot. The boiled samples were separated on 4-12% SDS-PAGE gels (NuPAGE Novex 4-12% Bis-Tris Gels, Thermo Fisher Scientific), and transferred to nitrocellulose membrane (Bio-Rad). The blots were stained with 0.2%(w/v) Ponceau S (Sigma-Aldrich) to ensure successful transfer. After blocking with 1% BSA (Sigma-Aldrich) in TBS, the membranes were incubated with the corresponding antibodies overnight at 4^oC. The antibodies were detected with HRP-conjugated immunoglobulin and visualized by chemiluminescent detection (ECL, Pierce) with a ChemiDoc imager (Bio-Rad). The following primary antibodies were used: anti-PKM2 (Rabbit mAb #4053, Cell Signaling Technology); anti-PKM2 (Mouse mAb #MAB7244, Novous Biologicals); anti-HIF1α (Rabbit ChIP Grade #ab2185, Abcam); anti-β catenin (Rabbit mAb #9582, Cell Signaling Technology); anti-β catenin (Mouse mAb #610154, BD Biosciences); GAPDH (Mouse mAb #G8795, Sigma-Aldrich) and anti-IgG (Abcam). Anti-rabbit and anti-mouse HRP-conjugated secondary antibodies were purchased from Sigma-Aldrich.

β-catenin/TCF activity. 1 X 10^5 P3 neonatal rat CMs were cultured in a 24-well plate with DMEM medium and co-transfected with TOPflash or FOPflash reporter plasmids (0.5 µg, Addgene) and 0.5 µg pGL4.75 (hRluc/CMV) vector (Promega) using Viromer Yellow for 48 hrs (efficiency of transfection: 30-50%). Subsequently, cells were transfected with Luc or Pkm2 modRNA. Reporter activity was measured using the Dual Luciferase Assay System (Promega) after 24 hrs. TOPflash or FOPflash activity was normalized to Renilla luciferase activity of pGL4.75, an internal standard for transfection efficiency according to the manufacturer.

Live cell imaging of isolated rat neonatal CMs. The time-lapse images of isolated rat neonatal CMs after either transfection with nGFP CM-specific modRNA or co-transfection with $_{CMS}$nGFP + $_{CMS}$Luc or $_{CMS}$Pkm2 modRNAs were acquired with a 10x objective lens every 10 sec with a confocal spinning disk microscope (Zeiss) following 24 hrs of time-lapse acquisition.

Supplemental Figures

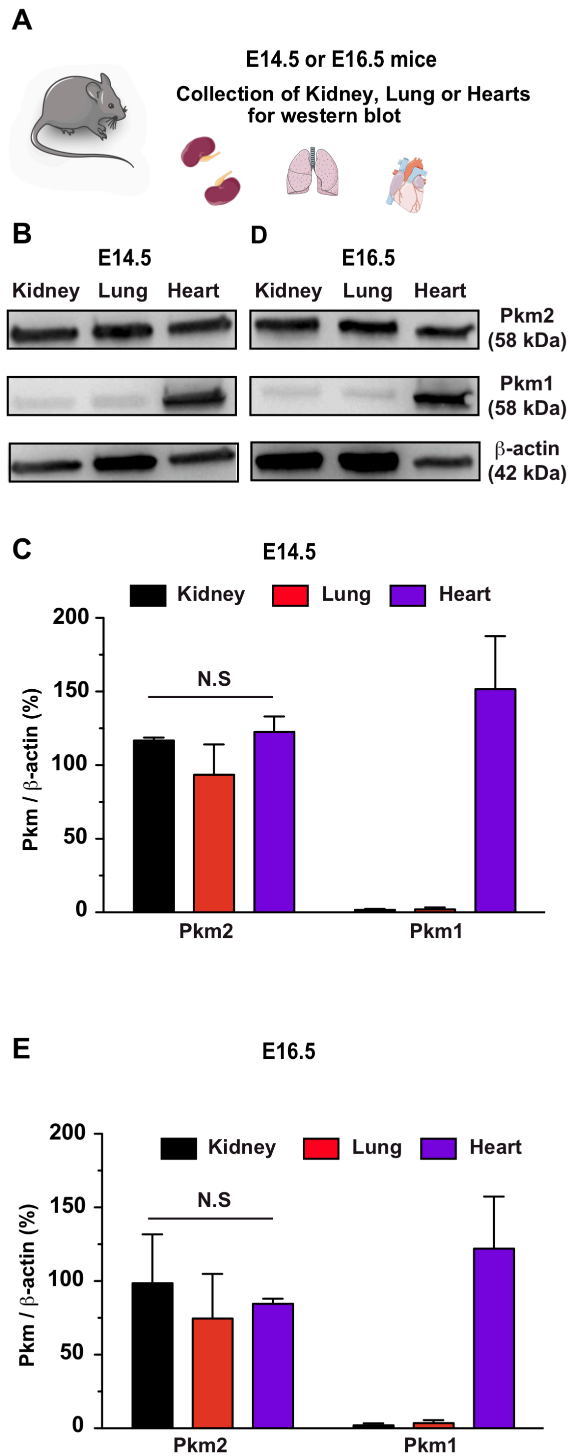


Figure S1. Pkm2 expression in different organs at E14.5 and E16.5.

A. Organs collected from E14.5 or E16.5 mice for western blot analysis. **B.**

Western blot of Pkm2 or Pkm1 expression in different organs (Kidney, Lung and Heart) at E14.5. **C.** Quantitative analysis of B. **D.** Western blot of Pkm2 or Pkm1 expression in different organs (Kidney, Lung and Heart) at E16.5. **E.** Quantitative analysis of D. One-way ANOVA, Tukey's Multiple Comparison, N.S, Not Significant.

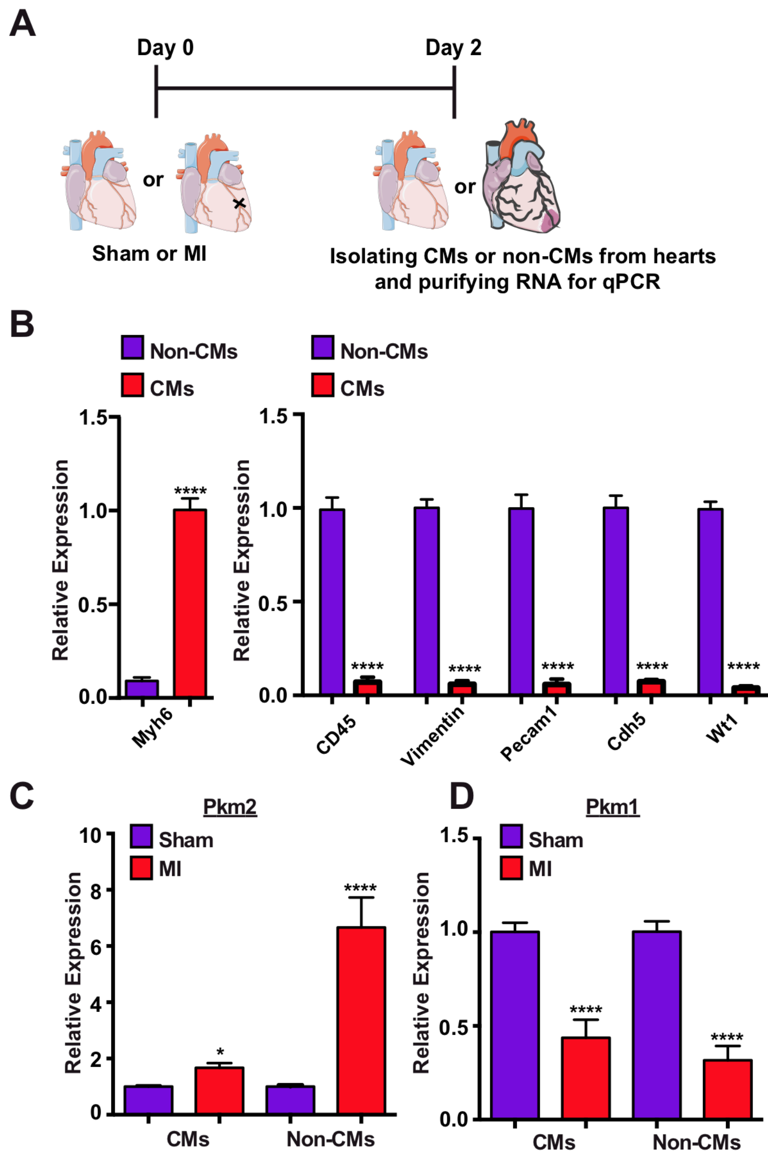


Figure S2. Pkm1 or Pkm2 gene expression in CMs or non-CMs in sham-operated hearts or post MI.

A. Experimental timeline for evaluating Pkm1 and Pkm2 expression in isolated cells from sham-operated hearts or 2 days post MI. **B-D**. Cells were isolated, and CMs and non-CMs were purified by sequential centrifugation and filtration. **B.** qRT-PCR analysis to validate purity of CM and non-CM populations. **C&D.** Pkm2 (**C**) or Pkm1 (**D**) expression in CM and non-CM populations from sham-operated

hearts or 2 days post MI (n=3). Unpaired two-tailed t-test for B. Two-way ANOVA for C&D. ****, $P<0.0001$, *, $P<0.05$.

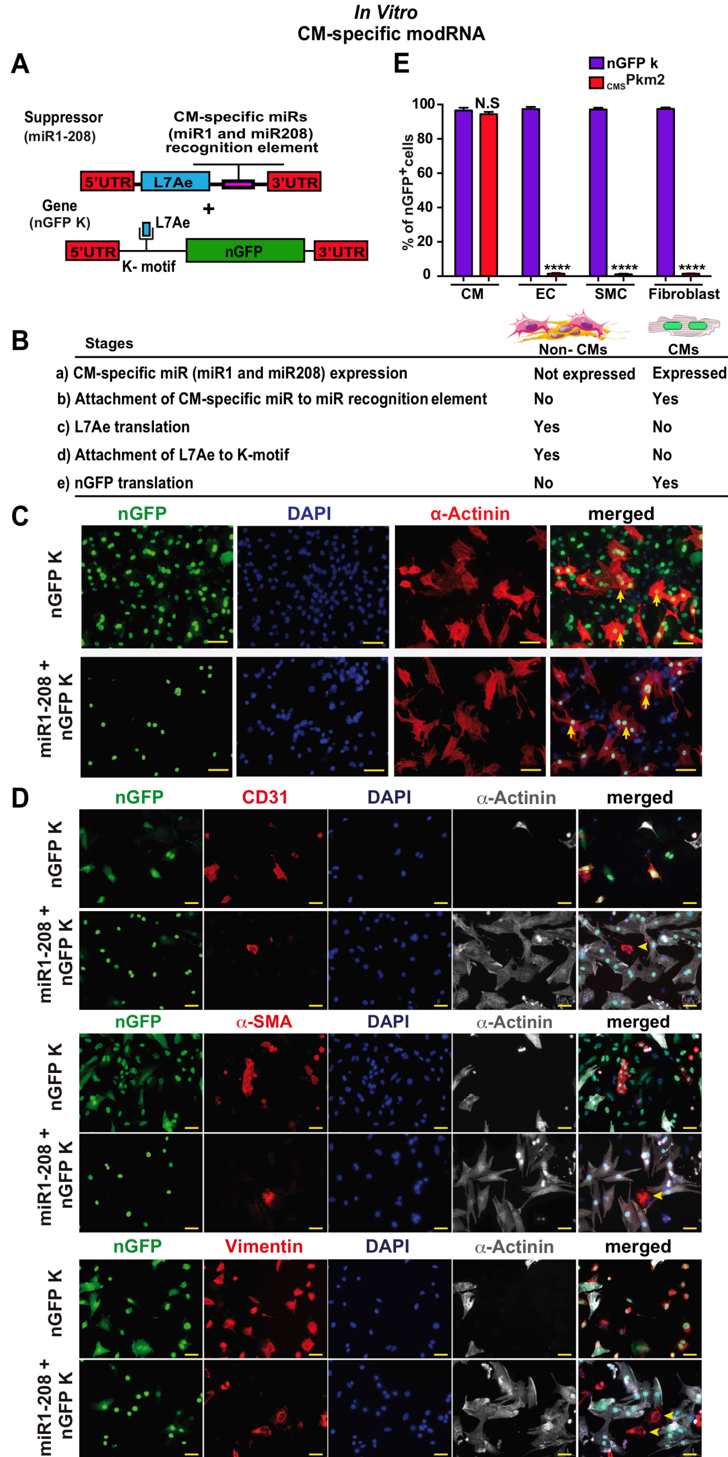
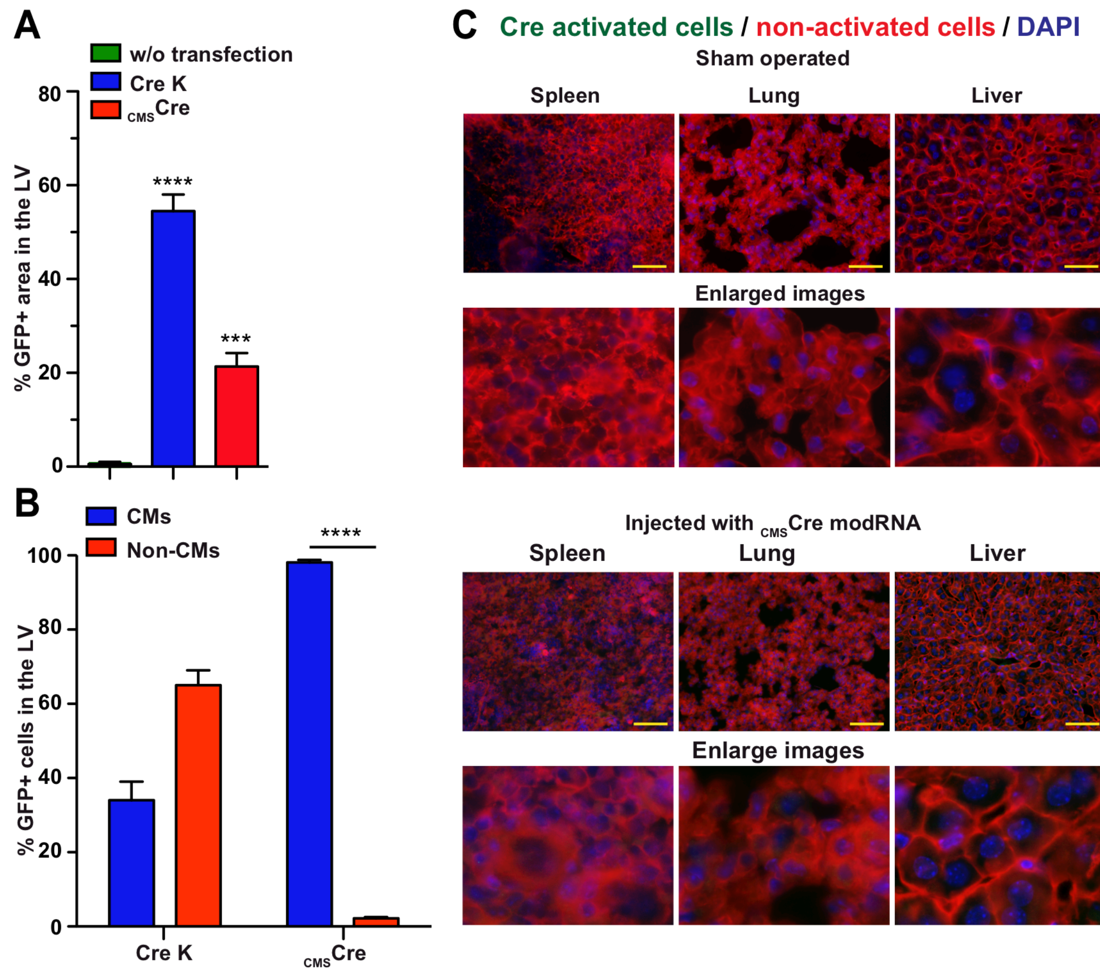


Figure S3. Design of $_{CMS}$modRNA constructs and cell type specificity *in vitro*. A. Two modRNAs were co-transfected into cardiac cells. One modRNA is a suppressor gene (L7Ae) that contains a $_{CMS}$miR recognition element for miR1

and miR208 upstream of its 3'UTR. The second modRNA is the gene of interest (nGFP) regulated by a K-motif downstream of its 5'UTR. **B.** When translated, L7Ae binds to the K-motif, which prevents translation of nGFP modRNA. Upon transfection into CMs, L7Ae is suppressed by endogenous ${}_{CMS}$miRs (miR1-208). Lack of L7Ae translation into protein results in nGFP translation (nGFP+ cells) only in CMs. In non-CMs, expressed L7Ae suppresses nGFP expression. This system allows transient gene expression exclusively in CMs. **C.** Validation of system specificity: representative images of neonatal rat CMs transfected with either nGFP K modRNA alone or CM-specific nGFP modRNA, stained for nGFP or CM-marker (α-actinin). **D.** Representative images validate the specificity of the ${}_{CMS}$modRNA expression tool. Neonatal rat cardiac cells were transfected with either nGFP K modRNA alone or CM-specific nGFP modRNA and stained for nGFP with CD31 (marker for endothelial cells), α-SMA (marker for smooth muscle cells) or vimentin (marker for non-CM cells such as cardiac fibroblasts) and nucleus (DAPI). Scale bar = 50μm. **E.** Quantitative analysis of C. & D. (n=3). Unpaired two-tailed t-test. ****, $P<0.0001$, N.S, Not Significant. Scale bar = 25μm.



<u>Figure S4. Design of $_{CMS}$modRNA constructs and cell type specificity *in vivo*.</u> **A&B.** Quantitative analysis of GFP^+ area (**A**) and percentage of GFP^+ cell types (**B**) in the LV, 7 days post Cre K or $_{CMS}$Cre modRNA injections (n=3). **C.** Representative images of spleen, lung and liver of $Rosa26^{mTmG}$ adult mice stained for GFP, DsRed and DAPI, 7 days post Sham operation or MI and delivery of $_{CMS}$Cre modRNA. One-way ANOVA, Tukey's Multiple Comparison Test for A and Two-way ANOVA for B. ****, P<0.0001, ***, P<0.001, Scale bar = 25µm.

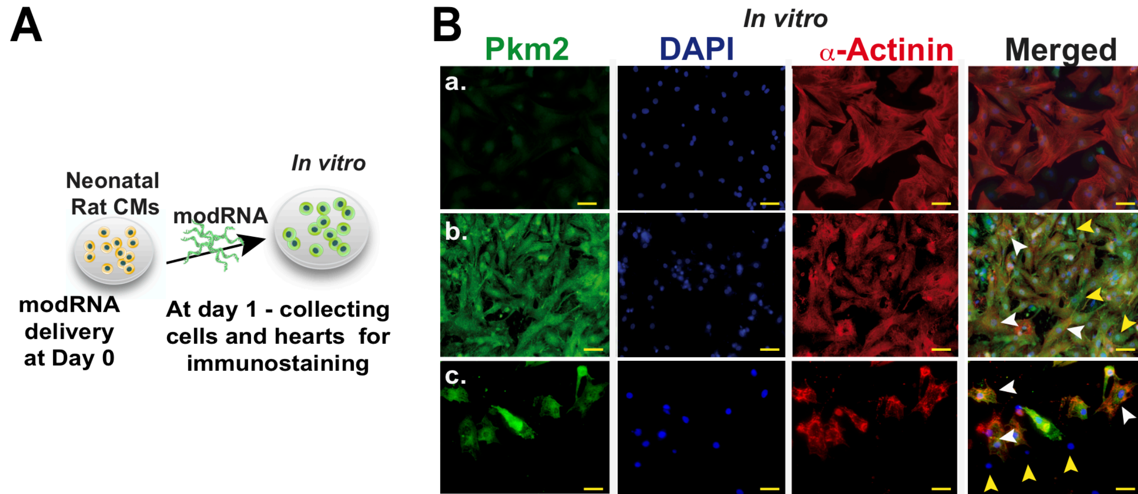


Figure S5. Design of $_{CMS}$Pkm2 modRNA for *in vitro* study. A. Experimental timeline for evaluating Pkm2 expression and transfection of Luc, Pkm2 and $_{cms}$Pkm2 modRNAs in neonatal rat CMs. **B.** Representative *in vitro* immunostaining images of Pkm2 and α-Actinin 1 day post modRNA delivery.

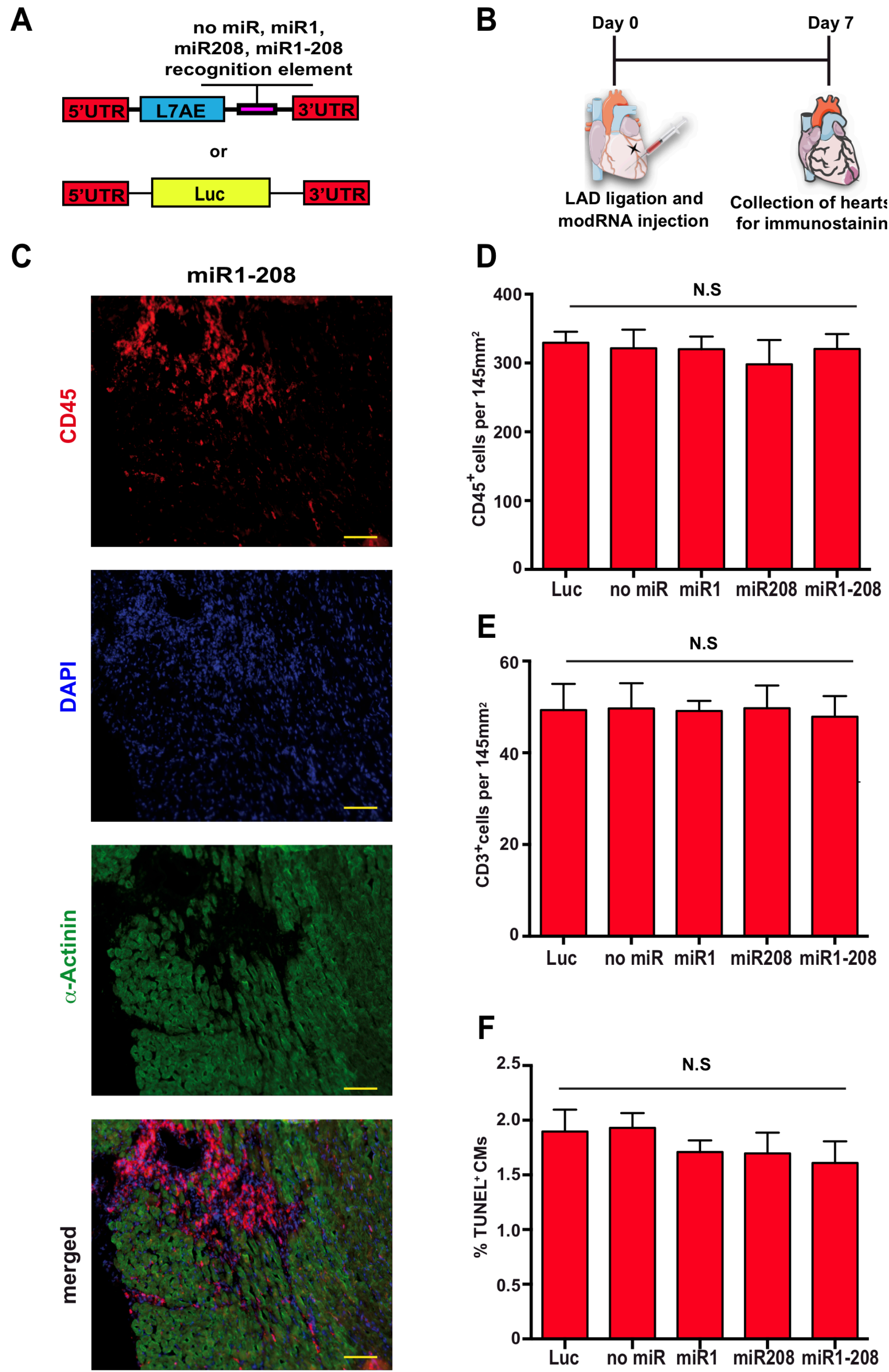


Figure S6. L7Ae modRNA does not elevate immune response in a mouse MI model. **A.** Diagram of the modRNA construct used in the experiments. **B.**

Experimental timeline. **C.** Representative pictures of $CD45^+$ cells infiltrating the left ventricle 7 days post MI and treatment with L7Ae carrying recognition elements for miR1 and miR208. Quantification of $CD45^+$ (**D.** n=6) and $CD3^+$ cells (**E.** n=6) or $TUNEL^+$ CMs (**F.** n=6) in the left ventricle 7 days post MI and delivery of different modRNAs. Results depict 2 independent experiments. One-way ANOVA, Tukey's Multiple Comparison Test for b. N.S, Not significant. Scale bar = 50μm.

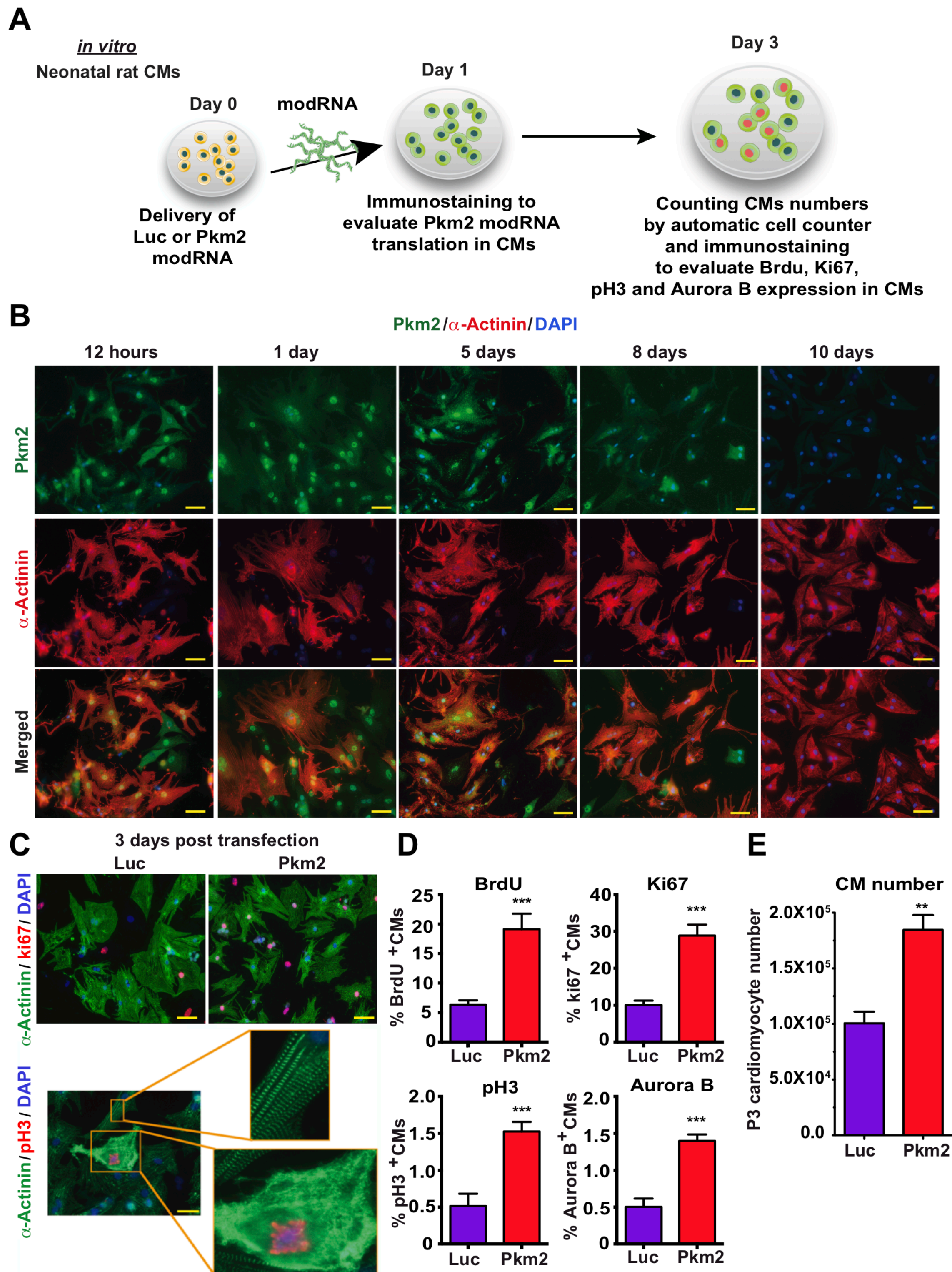


Figure S7. Pkm2 overexpression via modRNA in rat neonatal CMs *in vitro* increases their cell cycle markers and suppresses postnatal CM cell-cycle

arrest. **A.** Experimental plan for Pkm2 overexpression kinetics and its effect on P3 neonatal rat CMs' cell marker expression. **B.** Pkm2 expression kinetics at 12 hrs and 1, 5, 8 and 10 days post Pkm2 modRNA transfection *in vitro*. **C.** Representative images of Pkm2 modRNA expression in neonatal rat CMs. Note that Pkm2 expression increases cell cycle markers ($Ki67^+$) in CMs (α-$Actinin^+$) 3 days post transfection. $pH3^+$ CMs (M phase marker) with disrupted sarcomere structure compared to $pH3^-$ CMs. **D.** Quantification of cell cycle markers (Ki67, BrdU, pH3 and Aurora B) expression in neonatal rat CMs 3 days after transfection with Luc or Pkm2 modRNAs (n=3). **E.** Quantification of CM numbers by Eve Automatic cell counter 7 days after transfection with Luc or Pkm2 modRNAs (n=3). Results represent 3 independent experiments (D). Unpaired two-tailed t-test for E&D. ***, P<0.001, **, P<0.01. Scale bar = 25μm.

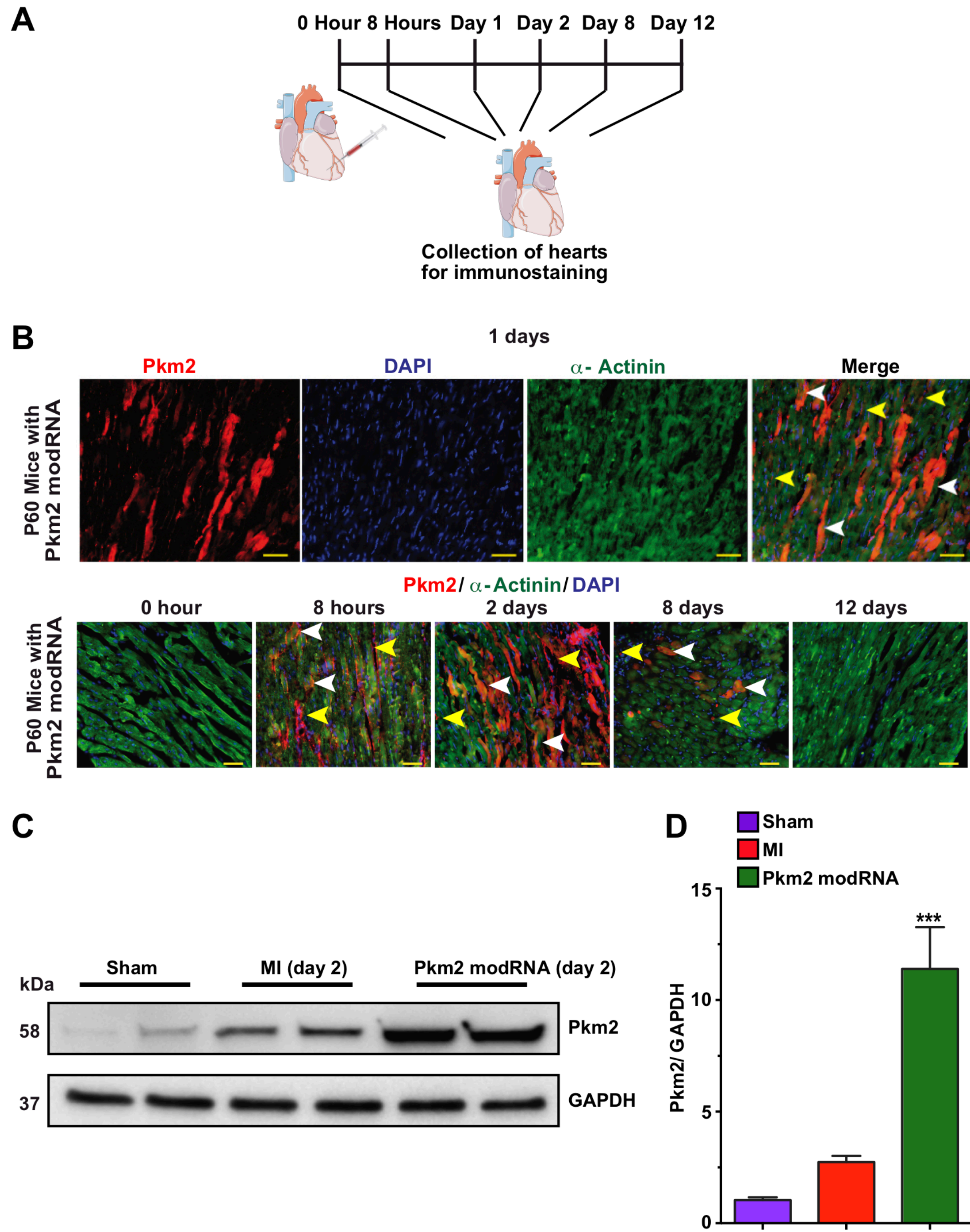


Figure S8. Pkm2 overexpression via modRNA in adult CMs after MI last for at least 8 days but not more than 12 days, *in vivo*.

A. Experimental plan for evaluating Pkm2 modRNA pharmacokinetics *in vivo*. **B.** Pharmacokinetics of Pkm2 expression post modRNA delivery *in vivo*. **C.** Western

blot analysis for LV 2 days post MI and Pkm2 modRNA delivery in comparison to MI or sham operation without injections of modRNA (n=2). **D.** Quantification of the experiment described in C (n=2). One-way ANOVA, Tukey's Multiple Comparison Test. ***, P<0.001.

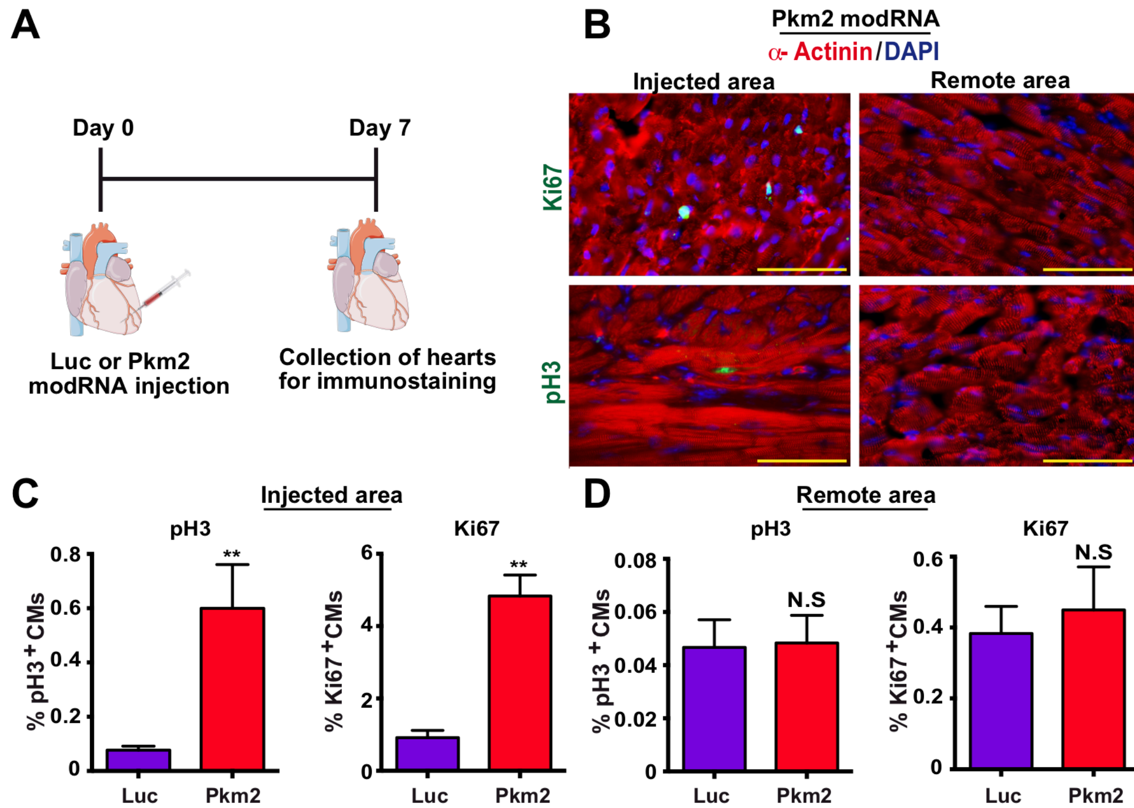


Figure S9. Pkm2 overexpression via modRNA delivery in the remote area of the uninjured heart (sham surgery) does not increase cell cycle marker expression in postnatal CMs, *in vivo*. **A.** Experimental plan for measuring the effect of Pkm2 on cell marker expression by CM from sham-operated hearts *in vivo*. **B.** Representative images of Ki67$^+$ or pH3$^+$ in CMs (α-Actinin$^+$) and non-CMs (α-Actinin$^-$) 7 days after Luc or Pkm2 modRNA delivery to injected or remote areas. **C&D.** Quantification of B. (n=4). Results depict 2 independent experiments. White arrowheads point to CMs. Yellow arrowheads point to non-CMs. Unpaired two-tailed t-test for C&D. **, P<0.01, N.S, Not Significant. Scale bar = 50µm.

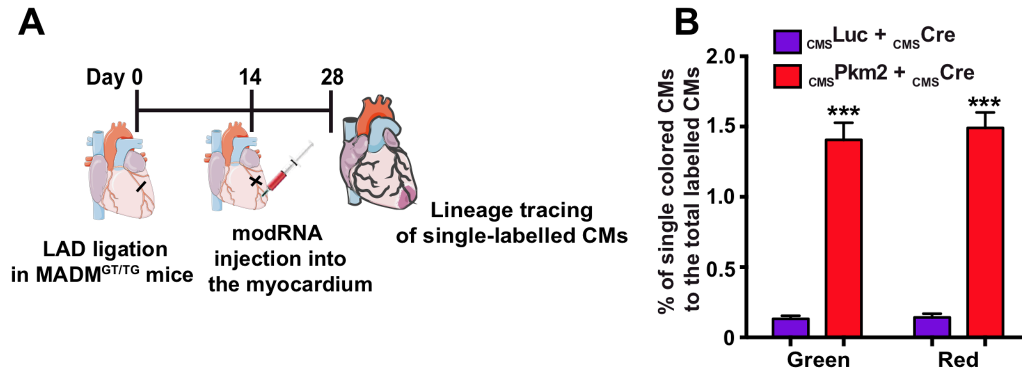


Figure S10. MADM lineage tracing in a chronic MI mouse model shows increased CM cell division after expressing $_{cms}$Pkm2 28 days post MI and 14 days post modRNA delivery.

A. Experimental timeline to trace CM cell division using a MADM lineage tracing chronic MI mouse model. **B.** Quantification of single-colored CMs (Green ($eGFP^+$/$DsRed^-$) or Red ($DsRed^+$/ $eGFP^-$) CMs) in comparison to total labeled CMs 28 days post MI and 14 days post modRNA delivery (n=5). Unpaired two-tailed t-test for B. ***, P<0.001.

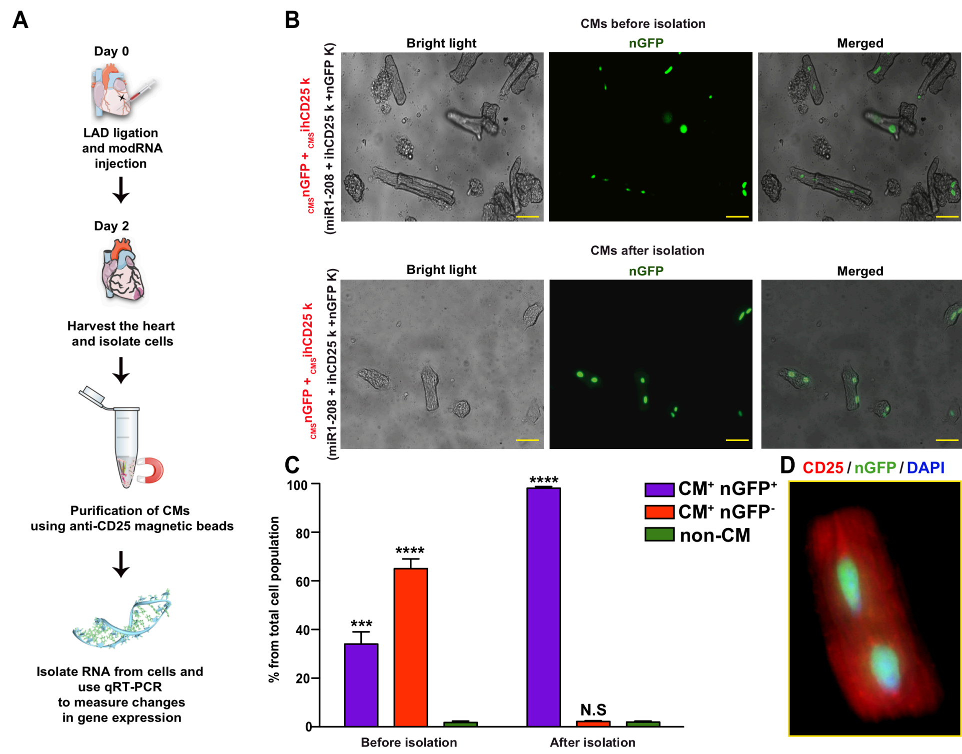


Figure S11. Isolating transfected adult CMs from mouse hearts post MI using $_{\text{CMS}}$modRNA and magnetic beads strategy. **A.** Adult CMs were isolated 2 days post MI and modRNA administration. Anti-hCD25 magnetic beads were used to isolate hCD25-positive cells. **B.** Hearts of CFW mice were co-transfected with inactive human CD25 (ihCD25) and nGFP carrying a K motif and L7Ae containing recognition elements for miR1 and miR208 ($_{\text{CMS}}$modRNA). Isolated CMs were a mixture of transfected CMs (nGFP^{+} and ihCD25^{+}) and non-transfected CMs (nGFP^{-} and ihCD25^{-}). Transfected CMs were isolated using commercially available anti-hCD25 magnetic beads. **C.** Proportion of the different CM populations before and after anti-hCD25 magnetic bead isolation (n=6). **D.** Representative image of purified CM stained for CD25, nGFP and DAPI. One-way ANOVA, Tukey's Multiple Comparison Test. ****, P<0.0001 ***, P<0.001, N.S, Not Significant. Scale bar = 50µm.

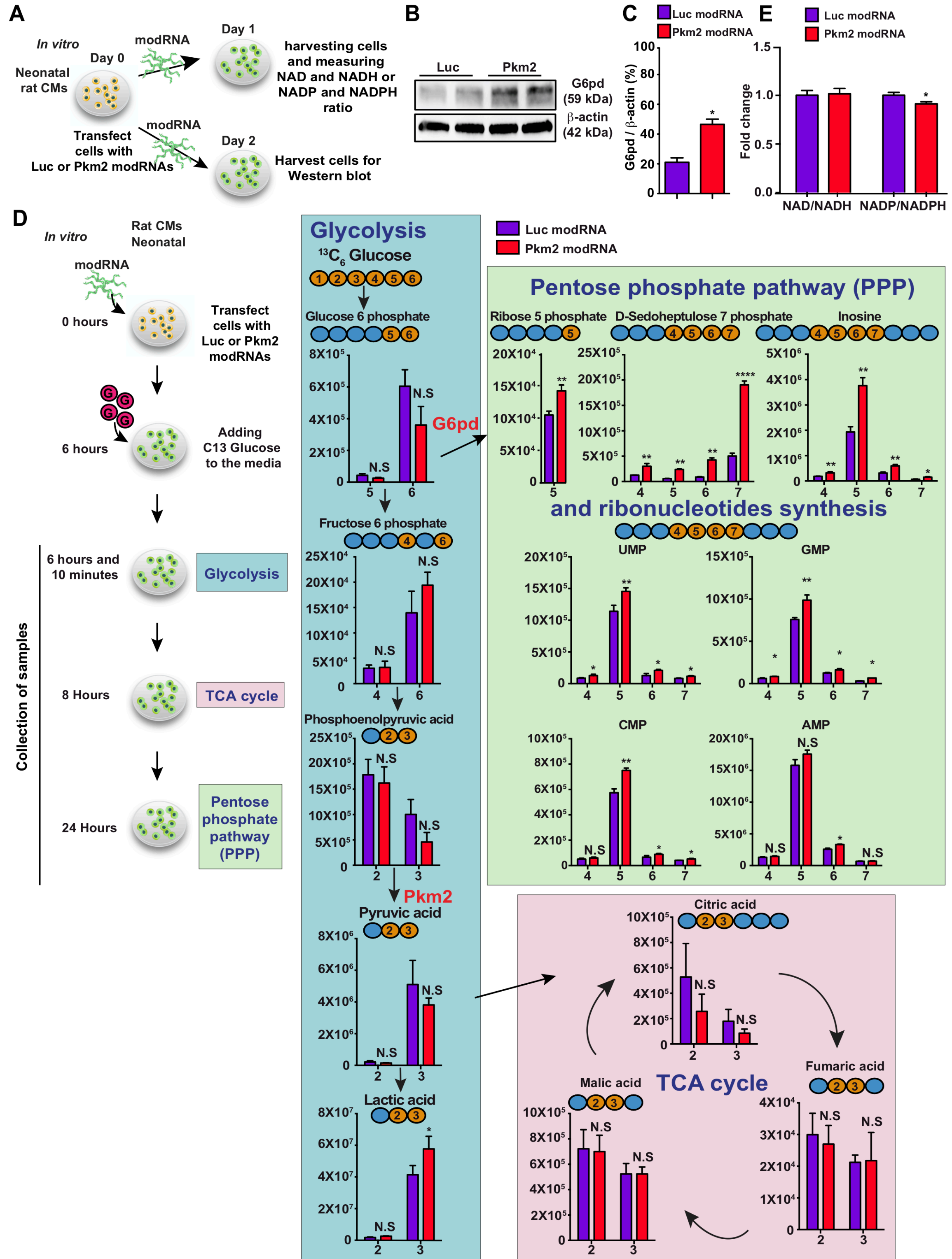


Figure S12. Pkm2 modRNA delivery into CMs upregulates G6pd and redirects glucose carbon flow into PPP, resulting in reduced oxidative

stress, *in vitro*. **A.** Experimental plan for G6PD expression analysis. **B.** Western blot of G6pd 24 hours post $_{cms}$Luc or $_{cms}$Pkm2 modRNA transfection. **C.** Quantification of B (n=2). **D.** Experimental timeline to analyze absolute intracellular labeled metabolites with [U-^{13}C] glucose flux using mass spectrometry in P3 neonatal rat CMs transfected with Luc or Pkm2 modRNA. The levels of [U-^{13}C]-labeled glycolysis metabolites were evaluated 10 min after [U-^{13}C] glucose addition, TCA metabolites were evaluated 2 hrs after [U-^{13}C] glucose addition and PPP metabolites and nucleotides synthesis was evaluated 18 hours after [U-^{13}C] glucose addition (n=3). Results include 2 independent experiments. In all panels, the X-axis represents the carbon number in the given structure of a specific molecule and the Y-axis represents absolute intensity. **E.** Quantitative analysis of NAD/NADH and NADP/NADPH ratios by enzymatic kit (n=3) in P3 neonatal CMs transfected with Luc or Pkm2 modRNA. Unpaired two-tailed t-test for C-E. ****, $P<0.0001$, **, $P<0.01$, *, $P<0.05$, N.S, Not Significant.

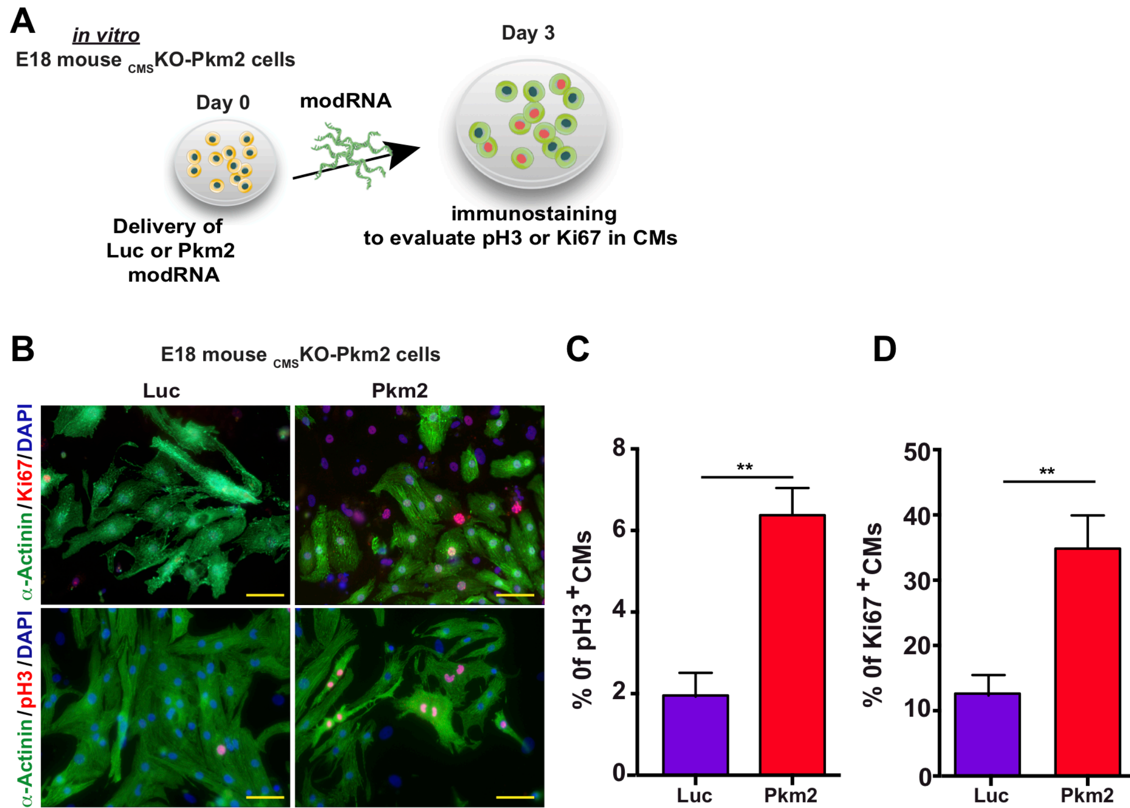


Figure S13. Rescue of $_{cms}$KO-Pkm2 CMs (E18) by overexpression of Pkm2, from modRNA, resulting in increased expression of cell cycle markers in fetal CMs, *in vitro*.

A. Experimental plan for using Pkm2 modRNA to rescue $_{cms}$KO-Pkm2 CMs (E18) by overexpressing Pkm2. **B.** Representative images of pH3$^+$ and ki67$^+$ CMs isolated from hearts of E18 $_{cms}$KO-Pkm2 mice 3 days post transfection with Luc or Pkm2 modRNA *in vitro*. **C&D.** Quantification of pH3$^+$ (**C**) or ki67$^+$ (**D**) CMs post transfection with Luc or Pkm2 modRNA (n=3). Unpaired two-tailed t-test for C&D. **, P<0.01. Scale bar = 25μm.

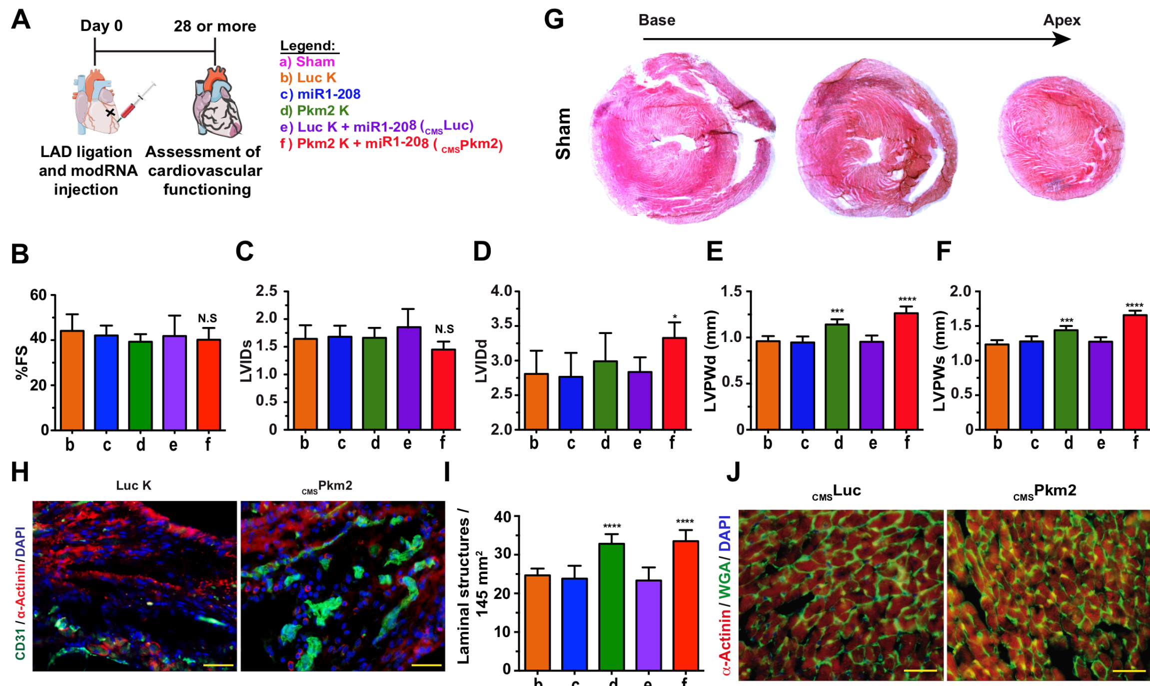


Figure S14. $_{cms}$Pkm2 modRNA improves outcome post MI. A. Experimental timeline used for evaluating the effect of $_{cms}$Pkm2 modRNA delivery on cardiac function and outcome in a mouse MI model. **B.** Echo was used to evaluate %FS 2 days post MI in the $_{cms}$Pkm2 and control groups (n=7). **C-F.** Echo was used to evaluate left ventricular internal diameter end systole (**B.** LVIDs), end diastole (**C.** LVIDd), end-diastolic left ventricular posterior wall thickness (LVPWd) or end-systolic left ventricular posterior wall thickness (LVPWs) in the $_{cms}$Pkm2 and control groups (n=7). **G.** Representative images of masson tricrome staining to evaluate cardiac scars in sham-operated hearts 28 days after surgery. **H.** Representative images of CD31 (PECAM1) to evaluate capillary density in the LV 28 days post MI and delivery of $_{cms}$Luc or $_{cms}$Pkm2 modRNA**. I.** Quantitative analysis of H (n=7). **J.** Representative images of wheat germ agglutinin (WGA) co-stained with cardiac marker (α-Actinin$^+$) and DAPI to evaluate CM size (cross-sectional area) 28 days post MI and delivery of either $_{cms}$Luc or $_{cms}$Pkm2

modRNA. One-way ANOVA Bonferroni post-hoc test. ****, P<0.0001 ***, P<0.001, *, P<0.05, N.S, Not Significant. Scale bar = 25μm.

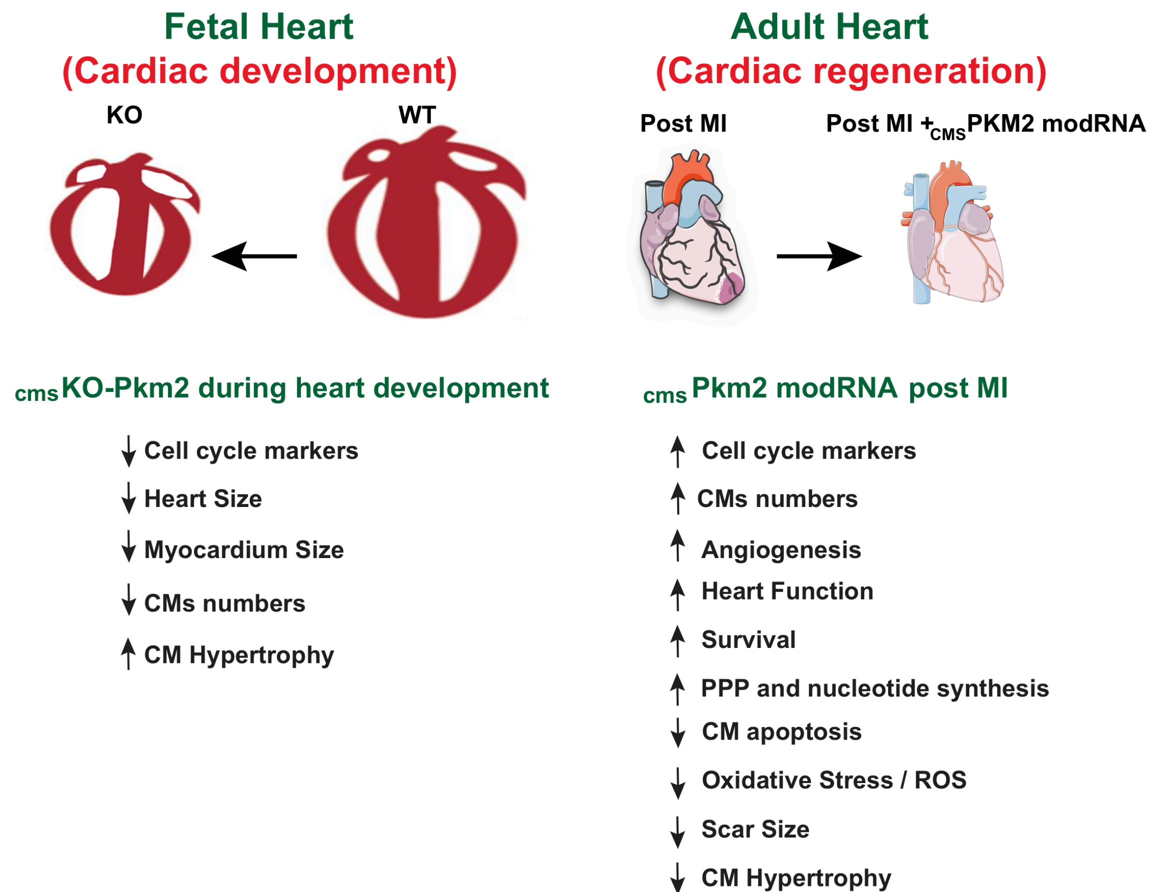


Figure S15. The effect of Pkm2 on CMs during cardiac development and regeneration. Pkm2 has key functions in inducing cell cycle marker expression in CMs, CM cell division and prevention of CM hypertrophy that allow normal heart development during embryogenesis. Gain of Pkm2 expression in the adult heart after acute or chronic MI increases cell cycle marker expression and cell division in CMs and suppresses the postnatal CM cell cycle arrest, reduced oxidative stress and CM apoptosis that lead to improve cardiac function and overall induction of cardiac regeneration post ischemic cardiac injury.

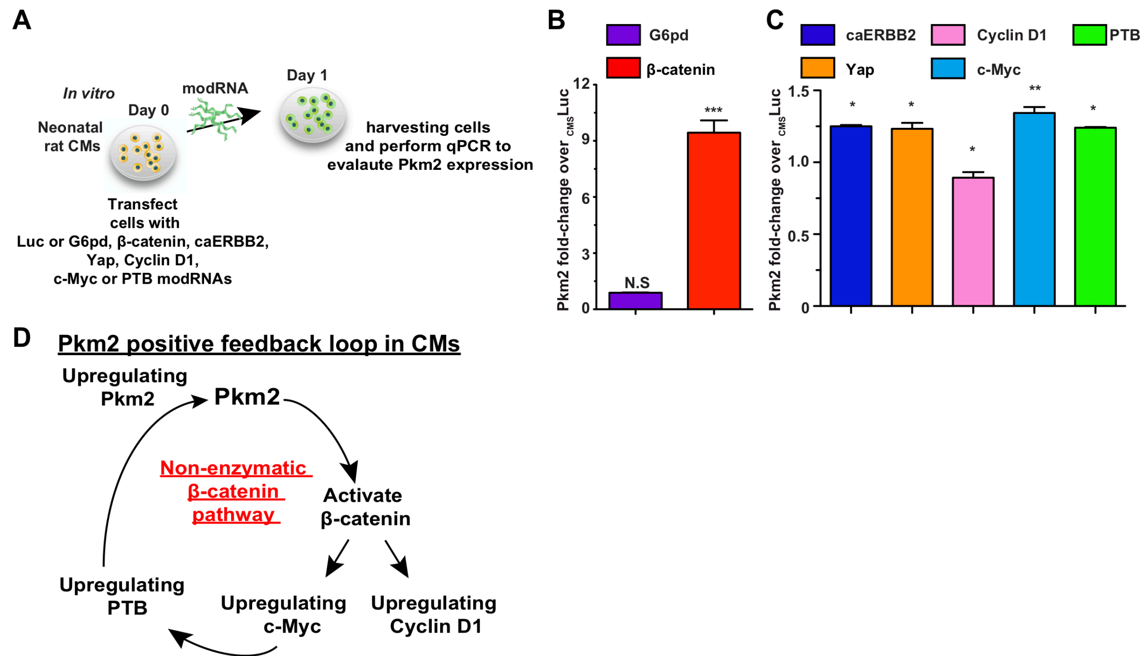


Figure S16. Activation of β-catenin- but not G6pd-elevated Pkm2 expression in CMs, *in vitro*.

A. Experimental timeline to analyze Pkm2 expression post delivery of control modRNA (Luc) or CM division-inducer modRNA (β-catenin, caERBB2, YAP, Cyclin D1, c-Myc or polypyrimidine tract-binding protein (PTB)) into P3 neonatal rat CMs. **B.** qPCR comparison of Pkm2 expression in CMs 1 day post delivery of G6pd (enzymatic pathway) or β-catenin (non-enzymatic pathway) modRNAs (n=3). **C.** qPCR comparison of Pkm2 expression in CMs 1 day post delivery of β-catenin-related modRNAs, such as caERBB2 or Yap, or direct targets of β-catenin (cyclyin D1 or c-Myc) or a direct target of c-Myc (PTB, n=3). **D.** Suggested Pkm2 positive feedback loop in CMs. One-way ANOVA, Tukey's Multiple Comparison Test. ***, P<0.001, **, P<0.01, *, P<0.05, N.S, Not Significant.

3. Supplemental Tables

Table S1: Open reading frame sequences used for modRNA production in this study

Gene	Open Reading Frame
Pkm2	atgccgaagccacacagtgaagcagggactgccttcattcagacccagcagctccatgcagccatggctg acaccttcctggaacacatgtgccgcctggacattgactctgcccccatcacggcccgcaacactggcatc atttgtaccattgggcctgcttcccgatctgtggagatgctgaaggagatgattaagtctggaatgaatgtggc tcggctgaatttctctcatggaacccatgagtaccatgcagagaccatcaagaatgtccgtgaagccacag aaagctttgcatctgatcccattctctaccgtcctgttgcggtggctctggatacaaagggacctgagatccgg actggactcatcaagggcagcggcaccgctgaggtggagctgaagaagggagccactctgaagatcac cctggacaacgcttacatggagaagtgtgacgagaacatcctgtggctggactacaagaacatctgcaag gtggtggaggtgggcagcaagatctacgtggacgatgggctcatctcactgcaggtgaaggagaaaggc gctgacttcctggtgacggaggtggagaatggtggctccttgggcagcaagaagggcgtgaacctgccgg gcgctgctgtggatctccccgctgtgtcggaaaaggacatccaggacctgaagtttggggtggagcaggat gtggacatggtgtttgcatctttcatccgcaaggcagccgacgtgcatgaagtcaggaaggtgctgggaga gaagggcaagaacatcaagatcatcagcaaaatcgagaaccatgaaggcgtccgcaggtttgatgagat cttggaggccagtgatgggatcatggtggctcgtggtgacctgggcattgagattcctgcagagaaggtcttc ctggctcagaagatgatgatcgggcgatgcaaccgagctgggaagcctgtcatctgtgccacacagatgct ggagagcatgatcaagaagccacgccccacccgtgctgaaggcagtgatgtggccaatgcagtcctgga tggagcagactgcatcatgctgtctggagaaacagccaaggggactaccctctggaggctgttcgcatgc agcacctgattgcccgagaggcagaggctgccatctaccacttgcagctattcgaggaactccgccgcctg gcgcccattaccagcgaccccacagaagctgccgccgtgggtgccgtggaggcctccttcaagtgctgca gtggggccattatcgtgctcaccaagtctggcaggagtgctcaccaagtggccaggtaccgccctcgggct cctatcattgccgtgactcgaaatccccagactgctcgccaggcccatctgtaccgtggcatcttccctgtgct gtgtaaggatgccgtgctgaatgcctgggctgaggatgtcgaccttcgtgtaaacttggccatggatgttggc aaggcccgaggcttcttcaagaagggagatgtggtcattgtgctgaccgggtggcgccctggctctggattc accaacaccatgcgtgtagtgcctgtaccttga
Luc	atggccgatgctaagaacattaagaagggccctgctcccttctaccctctggaggatggcaccgctggcga gcagctgcacaaggccatgaagaggtatgccctggtgcctggcaccattgccttcaccgatgcccacattg aggtggacatcacctatgccgagtacttcgagatgtctgtgcgcctggccgaggccatgaagaggtacggc ctgaacaccaaccaccgcatcgtggtgtgctctgagaactctctgcagttcttcatgccagtgctgggcgccc tgttcatcggagtggccgtggcccctgctaacgacatttacaacgagcgcgagctgctgaacagcatgggc atttctcagcctaccgtggtgttcgtgtctaagaagggcctgcagaagatcctgaacgtgcagaagaagctg cctatcatccagaagatcatcatcatggactctaagaccgactaccagggcttccagagcatgtacacattc gtgacatctcatctgcctcctggcttcaacgagtacgacttcgtgccagagtctttcgacagggacaaaacc attgccctgatcatgaacagctctgggtctaccggcctgcctaagggcgtggccctgcctcatcgcaccgcc tgtgtgcgcttctctcacgcccgcgaccctattttcggcaaccagatcatccccgacaccgctattctgagcgt ggtgccattccaccacggcttcggcatgttcaccaccctgggctacctgatttgcggctttcgggtggtgctgat gtaccgcttcgaggaggagctgttcctgcgcagcctgcaagactacaaaattcagtctgccctgctggtgcc aaccctgttcagcttcttcgctaagagcaccctgatcgacaagtacgacctgtctaacctgcacgagattgcc tctggcggcgccccactgtctaaggaggtgggcgaagccgtggccaagcgctttcatctgccaggcatccg

	ccagggctacggcctgaccgagacaaccagcgccattctgattaccccagagggcgacgacaagcctg gcgccgtgggcaaggtggtgccattcttcgaggccaaggtggtggacctggacaccggcaagaccctgg gagtgaaccagcgcggcgagctgtgtgtgcgcggccctatgattatgtccggctacgtgaataaccctgag gccacaaacgccctgatcgacaaggacggctggctgcactctggcgacattgcctactgggacgaggac gagcacttcttcatcgtggaccgcctgaagtctctgatcaagtacaagggctaccaggtggccccagccga gctggagtctatcctgctgcagcaccctaacattttcgacgccggagtggccggcctgcccgacgacgatg ccggcgagctgcctgccgccgtcgtcgtgctggaacacggcaagaccatgaccgagaaggagatcgtg gactatgtggccagccaggtgacaaccgccaagaagctgcgcggcggagtggtgttcgtggacgaggtg cccaagggcctgaccggcaagctggacgcccgcaagatccgcgagatcctgatcaaggctaagaaag gcggcaagatcgccgtgtaa
ihCD25	atggattcatacctgctgatgtggggactgctcacgttcatcatggtgcctggctgccaggcagagctctgtga cgatgacccgccagagatcccacacgccacattcaaagccatggcctacaaggaaggaaccatgttga actgtgaatgcaagagaggtttccgcagaataaaaagcgggtcactctatatgctctgtacaggaaactcta gccactcgtcctgggacaaccaatgtcaatgcacaagctctgccactcggaacacaacgaaacaagtga cacctcaacctgaagaacagaaagaaaggaaaaccacagaaatgcaaagtccaatgcagccagtgg accaagcgagccttccaggtcactgcagggaacctccaccatgggaaaatgaagccacagagagaattt atcatttcgtggtggggcagatggtttattatcagtgcgtccagggatacagggctctacacagaggtcctgct gagagcgtctgcaaaatgacccacgggaagacaaggtggacccagccccagctcatatgcacaggtga aatggagaccagtcagtttccaggtgaagagaagcctcaggcaagccccgaaggccgtcctgagagtg agacttcctgcctcgtcacaacaacagattttcaaatacagacagaaatggctgcaaccatggagacgtcc atatttacaacagatctccaggtagcagtggccggctgtgttttcctgctgatcagcgtcctcctcctgagtggg ctcacctggcagcggagacagaggaagagtagaagaacaatctag
L7Ae	atgtacgtgagatttgaggttcctgaggacatgcagaacgaagctctgagtctgctggagaaggttaggga gagcggtaaggtaaagaaaggtaccaacgagacgacaaaggctgtggagaggggactggcaaagct cgtttacatcgcagaggatgttgacccgcctgagatcgttgctcatctgcccctcctctgcgaggagaagaat gtgccgtacatttacgttaaaagcaagaacgaccttggaagggctgtgggcattgaggtgccatgcgcttcg gcagcgataatcaacgagggagagctgagaaaggagcttggaagccttgtggagaagattaaaggcctt cagaagtaa
Cre	Atgtccaatttactgaccgtacaccaaaatttgcctgcattaccggtcgatgcaacgagtgatgaggttcgca agaacctgatggacatgttcagggatcgccaggcgttttctgagcatacctggaaaatgcttctgtccgtttgc cggtcgtgggcggcatggtgcaagttgaataaccggaaatggtttcccgcagaacctgaagatgttcgcga ttatcttctatatcttcaggcgcgcggtctggcagtaaaaactatccagcaacatttgggccagctaaacatgc ttcatcgtcggtccgggctgccacgaccaagtgacagcaatgctgtttcactggttatgcggcggatccgaa aagaaaacgttgatgccggtgaacgtgcaaaacaggctctagcgttcgaacgcactgatttcgaccaggtt cgttcactcatggaaaatagcgatcgctgccaggatatacgtaatctggcatttctggggattgcttataacac cctgttacgtatagccgaaattgccaggatcagggttaaagatatctcacgtactgacggtgggagaatgtta atccatattggcagaacgaaaacgctggttagcaccgcaggtgtagagaaggcacttagcctgggggtaa ctaaactggtcgagcgatggatttccgtctctggtgtagctgatgatccgaataactacctgttttgccgggtca gaaaaaatggtgttgccgcgccatctgccaccagccagctatcaactcgcgccctggaagggatttttgaa gcaactcatcgattgatttacggcgctaaggatgactctggtcagagatacctggcctggtctggacacagt gcccgtgtcggagccgcgcgagatatggcccgcgctggagtttcaataccggagatcatgcaagctggtg gctggaccaatgtaaatattgtcatgaactatatccgtaacctggatagtgaaacaggggcaatggtgcgcc tgctagaagatggcgattag

G6pd	atggcagagcaggtggccctgagccggacccaggtgtgtgggatcctgagggaagagttgtaccagggt gatgccttccaccaagctgatacacacatatttatcatcatgggtgcatcgggtgacctggccaagaagaag atctatcctaccatctggtggctgttccgggatggccttctacctgaagataccttcattgtgggctatgcccgct cacgactcacagtggacgacatccgaaagcagagtgagcccttctttaaagccactccagaagaaagac ctaagctggaggagttctttgcccgtaattcctatgtagctggccagtatgatgatgcagcctcctataagcac ctcaacagccacatgaatgccctgcaccaggggatgcaggccaaccgtctattctacctggccttgcccccc cacagtctatgaagcagtcaccaagaacattcaagagacctgcatgagtcagacaggctggaaccgcat catcgtggagaagcccttcgggagagacctacagagctccaatcaactgtcgaaccacatctcctctcttttc cgtgaggaccagatctaccgcattgaccactacctgggcaaagagatggtccagaatctcatggtgctgag gtttgccaacaggatctttggccccatctggaatcgagacaacattgcttgtgtgatcctcacatttaaagagc cctttggtactgagggtcgtgggggctattttgatgaatttgggatcatcagggatgttatgcagaaccacctcc tgcagatgttgtgtctggtggccatggaaaagcctgccactacaggttcagatgatgtccgtgatgagaaggt caaagtgttgaagtgtatctcagaggtggaaactgacaacgtggtccttggccaatatgtggggaacccca atggagaaggagaagctgccaatggatacttagatgaccccacagtaccccatgggtccaccactgcca cttttgcagcagctgtcctctatgtggagaatgaacggtgggacggggtacccttcatcctgcgctgtggcaa agccctgaatgaacgcaaagctgaagtgagactgcagttccgagacgtggcaggtgacatcttccaccaa cagtgcaagcgtaatgagctggtcatccgtgtgcagcccaatgaggcagtatacaccaagatgatgacca agaagcctggcatgttctttaaccctgaggagtcggagctggatctaacttatggcaacagatacaagaatg tgaagctccctgatgcctatgaacgcctcatcctggatgtcttctgtgggagccagatgcactttgtccggagt gatgaactcagggaagcctggcgtatcttcacaccactgctgcacaagattgatcgagaaaagccccagc ccatcccctatgtatatggcagccgcggccccacagaggcagatgagctgatgaagagagtgggcttcca gtacgagggtacctacaagtgggtgaaccctcacaagctctga
Hif1α	tggagggcgccggcggcgagaacgagaagaaaaataggatgagttctgaacgtcgaaaagaaaagtc tagagatgcagcaagatctcggcgaagcaaagagtctgaagtttttatgagcttgctcatcagttgccacttc cccacaatgtgagctcacatcttgataaagcttctgttatgaggctcaccatcagttatttacgtgtgagaaaa cttctggatgccggtggtctagacagtgaagatgagatgaaggcacagatggactgtttttatctgaaagccc tagatggctttgtgatggtgctaacagatgacggcgacatggtttacatttctgataacgtgaacaaatacatg gggttaactcagtttgaactaactggacacagtgtgtttgattttactcatccatgtgaccatgaggaaatgag agaaatgcttacacacagaaatggcccagtgagaaaagggaaagaactaaacacacagcggagcttttt tctcagaatgaagtgcaccctaacaagccgggggaggacgatgaacatcaagtcagcaacgtggaagg tgcttcactgcacgggccatattcatgtctatgataccaacagtaaccaacctcagtgtgggtacaagaaac cacccatgacgtgcttggtgctgatttgtgaacccattcctcatccgtcaaatattgaaattcctttagatagcaa gacatttctcagtcgacacagcctcgatatgaaattttcttactgtgatgaaagaattactgagttgatgggttat gagccggaagaacttttgggccgctcaatttatgaatattatcatgctttggattctgatcatctgaccaaaact caccatgatatgtttactaaaggacaagtcaccacaggacagtacaggatgcttgccaaaagaggtggat atgtctgggttgaaactcaagcaactgtcatatataatacgaagaactcccagccacagtgcattgtgtgtgt gaattatgttgtaagtggtattattcagcacgacttgattttctcccttcaacaaacagaatctgtgctcaaacca gttgaatcttcagatatgaagatgactcagctgttcaccaaagttgaatcagaggatacaagctgcctttttgat aagcttaagaaggagcctgatgctctcactctgctggctccagctgccggcgacaccatcatctctctggattt tggcagcgatgacacagaaactgaagatcaacaacttgaagatgttccattatataatgatgtaatgtttccct cttctaatgaaaaattaaatataaacctggcaatgtctcctttaccttcatcggaaactccaaagccacttcga agtagtgctgatcctgcactgaatcaagaggttgcattaaaattagaatcaagtccagagtcactgggacttt cttttaccatgccccagattcaagatcagccagcaagtccttctgatggaagcactagacaaagttcacctg agagacttcttcaggaaaacgtaaacactcctaacttttcccagcctaacagtcccagtgaatattgctttgat gtggatagcgatatggtcaatgtattcaagttggaactggtggaaaaactgtttgctgaagacacagaggca aagaatccattttcaactcaggacactgatttagatttggagatgctggctccctatatcccaatggatgatgat

	ttccagttacgttcctttgatcagttgtcaccattagagagcaattctccaagccctccaagtatgagcacagtt actgggttccagcagacccagttacagaaacctaccatcactgccactgccaccacaactgccaccactg atgaatcaaaaacagagacgaaggacaataaagaagatattaaaatactgattgcatctccatcttctacc caagtacctcaagaaacgaccactgctaaggcatcagcatacagtggcactcacagtcggacagcctca ccagacagagcaggaaagagagtcatagaacagacagacaaagctcatccaaggagccttaacctgt ctgccactttgaatcaaagaaatactgttcctgaggaagaattaaacccaaagacaatagcttcgcagaat gctcagaggaagcgaaaaatggaacatgatggctccctttttcaagcagcaggaattggaacattattgca gcaaccaggtgactgtgcacctactatgtcactttcctggaaacgagtgaaaggattcatatctagtgaaca gaatggaacggagcaaaagactattattttaataccctccgatttagcatgcagactgctggggcagtcaat ggatgagagtggattaccacagctgaccagttacgattgtgaagttaatgctcccatacaaggcagcagaa acctactgcagggtgaagaattactcagagctttggatcaagttaactga
β-catenin	atggctactcaagctgacctgatggagttggacatggccatggagccggacagaaaagctgctgtcagcc actggcagcagcagtcttacttggattctggaatccattctggtgccaccaccacagctccttccctgagtggc aagggcaaccctgaggaagaagatgttgacacctcccaagtcctttatgaatgggagcaaggcttttccca gtccttcacgcaagagcaagtagctgatattgacgggcagtatgcaatgactagggctcagagggtccga gctgccatgttccctgagacgctagatgagggcatgcagatcccatccacgcagtttgacgctgctcatccc actaatgtccagcgcttggctgaaccatcacagatgttgaaacatgcagttgtcaatttgattaactatcagga tgacgcggaacttgccacacgtgcaattcctgagctgacaaaactgctaaacgatgaggaccaggtggta gttaataaagctgctgttatggtccatcagctttccaaaaaggaagcttccagacatgccatcatgcgctccc ctcagatggtgtctgccattgtacgcaccatgcagaatacaaatgatgtagagacagctcgttgtactgctgg gactctgcacaacctttctcaccaccgcgagggcttgctggccatctttaagtctggtggcatcccagcgctg gtgaaaatgcttgggtcaccagtggattctgtactgttctacgccatcacgacactgcataatctcctgctccat caggaaggagctaaaatggcagtgcgcctagctggtggactgcagaaaatggttgctttgctcaacaaaa caaacgtgaaattcttggctattacaacagactgccttcagatcttagcttatggcaatcaagagagcaagct catcattctggccagtggtggaccccaagccttagtaaacataatgaggacctacacttatgagaagcttctg tggaccacaagcagagtgctgaaggtgctgtctgtctgctctagcaacaagccggccattgtagaagctgg tgggatgcaggcactggggcttcatctgacagacccaagtcagcgacttgttcaaaactgtctttggactctc agaaacctttcagatgcagcgactaagcaggaagggatggaaggcctccttgggactctagtgcagcttct gggttccgatgatataaatgtggtcacctgtgcagctggaattctctctaacctcacttgcaataattacaaaa acaagatgatggtgtgccaagtgggtggcatagaggctcttgtacgcaccgtccttcgtgctggtgacaggg aagacatcactgagcctgccatctgtgctcttcgtcatctgaccagccggcatcaggaagccgagatggcc cagaatgccgttcgccttcattatggactgcctgttgtggttaaactcctgcacccaccatcccactggcctctg ataaaggcaactgttggattgattcgaaaccttgccctttgcccagcaaatcatgcgcctttgcgggaacagg gtgctattccacgactagttcagctgcttgtacgagcacatcaggacacccaacggcgcacctccatgggtg gaacgcagcagcagtttgtggagggcgtgcgcatggaggagatagtagaagggtgtactggagctctcc acatccttgctcgggacgttcacaaccggattgtaatccgaggactcaataccattccattgtttgtgcagttgc tttattctcccattgaaaatatccaaagagtagctgcaggggtcctctgtgaacttgctcaggacaaggaggc tgcagaggccattgaagctgagggagccacagctcccctgacagagttactccactccaggaatgaagg cgtggcaacatacgcagctgctgtcctattccgaatgtctgaggacaagccacaggattacaagaagcgg ctttcagtcgagctgaccagttccctcttcaggacagagccaatggcttggaatgagactgcagatcttggac tggacattggtgcccagggagaagcccttggatatcgccaggatgatcccagctaccgttcttttcactctggt ggatacggccaggatgccttggggatggaccctatgatggagcatgagatgggtggccaccaccctggtg ctgactatccagttgatgggctgcctgatctgggacacgcccaggacctcatggatgggctgcccccaggt gatagcaatcagctggcctggtttgatactgacctgtaa

nGFP	Atggtgagcaagggcgaggagctgttcaccggggtggtgcccatcctggtcgagctggacggcgacgta aacggccacaagttcagcgtgtccggcgagggcgagggcgatgccacctacggcaagctgaccctgaa gttcatctgcaccaccggcaagctgcccgtgccctggcccaccctcgtgaccaccctgacctacggcgtgc agtgcttcagccgctaccccgaccacatgaagcagcacgacttcttcaagtccgccatgcccgaaggcta cgtccaggagcgcaccatcttcttcaaggacgacggcaactacaagacccgcgccgaggtgaagttcga gggcgacaccctggtgaaccgcatcgagctgaagggcatcgacttcaaggaggacggcaacatcctgg ggcacaagctggagtacaactacaacagccacaacgtctatatcatggccgacaagcagaagaacggc atcaaggtgaacttcaagatccgccacaacatcgaggacggcagcgtgcagctcgccgaccactaccag cagaacacccccatcggcgacggccccgtgctgctgcccgacaaccactacctgagcacccagtccgcc ctgagcaaagaccccaacgagaagcgcgatcacatggtcctgctggagttcgtgaccgccgccgggatc actctcggcatggacgagctgtacaagggagatccaaaaaagaagagaaaggtaggcgatccaaaaa agaagagaaaggtaggtgatccaaaaaagaagagaaaggtataa
caERBB2	Atgaagctgcggctgcccgcctctcctgagacacacctggacatgctgcggcacctgtaccagggctgtca ggtggtgcagggcaacctggaactgacctacctgcccaccaacgccagcctgagctttctgcaggacatc caggaagtgcagggctacgtcctgatcgcccacaaccaggtccgacaggtgcccctgcagagactgag aatcgtgcggggcacccagctgttcgaggacaattatgccctggccgtgctggacaacggcgacccccctg aacaataccacccctgtgacaggcgccagccctggcggactgagagaactgcagctgcggagcctgac cgagatcctgaagggcggcgtgctgatccagagaaacccccagctgtgctaccaggacaccatcctgtg gaaggacatcttccacaagaacaaccagctggccctgaccctgatcgacaccaacagaagcagagcct gccacccctgcagccccatgtgcaagggctctagatgttggggcgagagcagcgaggactgccagtccct gaccagaacagtgtgtgccggcggatgcgccagatgcaagggccctctgcctaccgattgctgccacga gcagtgtgccgctggctgtacaggccccaagcacagcgattgcctggcctgcctgcactttaaccacagcg gcatctgcgagctgcactgccctgccctggtcacctacaacaccgacaccttcgagagcatgcccaaccc cgagggcagatacaccttcggcgccagctgtgtgaccgcctgcccctacaactacctgagcaccgatgtg ggcagctgcaccctcgtgtgcccccctgcacaatcaggaagtgaccgccgaggacggcacccagagatg cgagaagtgcagcaagccctgcgccagagtgtgctacggcctgggcatggaacacctgagagaagtgc gggccgtgaccagcgccaatatccaggaattcgccggctgcaagaagatctttggctccctggcctttctgc ccgagagcttcgatggcgaccctgcctctaataccgcccctctgcagccagagcagctccaggtgttcgag acactggaagagatcaccggctacctgtacatcagcgcctggcccgacagcctgcccgatctgagcgtgtt ccagaatctgcaggtcatcagaggccggatcctgcacaacggcgcctacagcctgacactgcagggcct gggaatcagctggctgggcctgagatctctgagagagctgggcagcggcctggctctgatccaccacaac acccacctgtgcttcgtgcacaccgtgccctgggaccagctgtttagaaaccctcaccaggcactgctgcac accgccaacagacccgaggatgagtgtgtgggcgaaggcctggcttgccatcagctgtgcgctagaggc cactgttggggccctggacctacccagtgcgtgaactgctcccagttcctgcggggccaggaatgcgtgga agagtgcagagtgctgcagggactgccccgcgagtacgtgaacgccagacactgcctgccttgccaccct gagtgccagcctcagaatggcagcgtgacctgcttcggccctgaggccgatcagtgtgtggcctgcgccca ctacaaggacccccccattctgcgtggccagatgccctagcggcgtgaagcccgacctgagctacatgccc atctggaagttccccgacgaggaaggcgcctgccagccttgtcccatcaactgcacccacagctgcgtgg acctggacgacaagggctgtcctgccgagcagagagccagcccccctgacctctatcatctccgccgtgga aggcatcctgctggtggtggtgctgggcgtggtgttcggcatcctgatcaagcggcggcagcagaagatcc ggaagtacaccatgcggcggctgctgcaggaaaccgagctggtcgagcctctgacaccaagcggcgcc atgcctaaccaggcccagatgcggatcctgaaagagacagagctgcggaaagtgaaggtgctgggatc cggcgccttcggcacagtgtacaagggaatctggatccccgacggcgagaacgtgaagatccccgtggc catcaaggtgctgagagagaacaccagccccaaggccaacaaagagatcctggacgaggcctacgtg atggccggcgtgggcagcccttatgtgtccagactgctgggcatctgcctgaccagcaccgtgcagctggtc

	actcagctgatgccttacggctgcctgctggaccacgtgcgcgagaatagaggcagactgggcagccag gacctgctgaactggtgcatgcagatcgccaagggcatgagctacctcgaggacgtgcggctggtgcaca gagatctggccgccagaaacgtgctcgtgaagtcccccaaccacgtgaaaatcaccgacttcggactggc ccggctgctggacatcgacgagacagagtatcacgccgacggcggcaaggtgcccatcaagtggatgg ccctggaatccatcctgcggcggaggttcacccaccagagcgacgtgtggtcttacggcgtgaccgtgtgg gagctgatgacattcggagccaagccctacgacggcatccccgccagagagatccccgatctgctggaa aagggcgagagactgccccagcccccatctgcaccatcgacgtgtacatgattatggtcaagtgctggat gatcgacagcgagtgccggcccagattccgcgagctggtgtccgagttctccagaatggcccgggacccc cagagattcgtggtcatccagaacgaggacctgggccctgcctccccctggactccaccttttaccggtcc ctgctggaagatgacgacatgggcgacctggtggacgccgaggaatacctggtgccccagcagggcttct tctgccctgatcctgctcctggcgctggcggcatggtgcatcacagacacagaagctccagcaccagaag cggaggcggcgatctgaccctgggactggaaccttctgaggaagaggcccctagaagcccctggcccc tagtgaaggggcaggatctgatgtgttcgacggggacctgggaatgggcgctgccaaaggactgcagag tctgcccacccacgaccccagcccactgcagaggtacagcgaggatcctaccgtgcctctgcccagcga gacagatggctacgtggcccctctgacctgtagcccccagcccgagtatgtgaaccagcccgatgtgcgg cctcagcctcctagccctagagaaggacctctgcctgccgctagacctgccggcgctaccctggaaagac ccaagacactgagccccggcaagaacggcgtggtcaaggacgtgttcgcctttggcggagccgtggaaa accccgagtacctgacacctcagggcggagcagcacctcagccacaccctccaccagccttcagccccg ccttcgacaacctgtactactgggatcaggaccctcccgagagaggcgccccacctagcacctttaaggg cacccctaccgccgagaatcctgagtacctggggctggacgtgcccgtctaa
YAP	atggactacaaagacgatgacgacaagcttgcggccgcgaattcaagcttagccaccatggactacaaa gacgatgacgataaagcaaggctcgaatcggtacctaaggatcccgggcagcagccgccgcctcaacc ggcccccagggccaagggcagccgccttcgcagcccccgcaggggcagggcccgccgtccggacc cgggcaaccggcacccgcggcgacccaggcggcgccgcaggcacccccgccgggcatcagatcgt gcacgtccgcggggactcggagaccgacctggaggcgctcttcaacgccgtcatgaaccccaagacgg ccaacgtgccccagaccgtgcccatgaggctccggaagctgcccgactccttcttcaagccgccggagcc caaatcccactcccgacaggccagtactgatgcaggcactgcaggagccctgactccacagcatgttcga gctcatgcctctccagcttctctgcagttgggagctgtttctcctgggacactgacccccactggagtagtctct ggcccagcagctacacccacagctcagcatcttcgacagtcttcttttgagatacctgatgatgtacctctgcc agcaggttgggagatggcaaagacatcttctggtcagagatacttcttaaatcacatcgatcagacaacaa catggcaggaccccaggaaggccatgctgtcccagatgaacgtcacagcccccaccagtccaccagtg cagcagaatatgatgaactcggcttcaggtcctcttcctgatggatgggaacaagccatgactcaggatgg agaaatttactatataaaccataagaacaagaccacctcttggctagacccaaggcttgaccctcgttttgcc atgaaccagagaatcagtcagagtgctccagtgaaacagccaccacccctggctccccagagcccaca gggaggcgtcatgggtggcagcaactccaaccagcagcaacagatgcgactgcagcaactgcagatgg agaaggagaggctgcggctgaaacagcaagaactgcttcggcaggagttagccctgcgtagccagttac caacactggagcaggatggtgggactcaaaatccagtgtcttctcccgggatgtctcaggaattgagaaca atgacgaccaatagctcagatcctttccttaacagtggcacctatcactctcgagatgagagtacagacagt ggactaagcatgagcagctacagtgtccctcgaaccccagatgacttcctgaacagtgtggatgagatgg atacaggtgatactatcaaccaaagcaccctgccctcacagcagaaccgtttcccagactaccttgaagcc attcctgggacaaatgtggaccttggaacactggaaggagatggaatgaacatagaaggagaggagctg atgccaagtctgcaggaagctttgagttctgacatccttaatgacatggagtctgttttggctgccaccaagct agataaagaaagctttcttacatggttatag

Cyclin D1	Atggaacaccagctcctgtgctgcgaagtggagaccatccgccgcgcgtaccctgacaccaatctcctca acgaccgggtgctgcgagccatgctcaagacggaggagacctgtgcgccctccgtatcttacttcaagtgc gtgcagaaggagattgtgccatccatgcggaaaatcgtggccacctggatgctggaggtctgtgaggagc agaagtgcgaagaggaggtcttcccgctggccatgaactacctggaccgcttcctgtccctggagcccttg aagaagagccgcctgcagctgctgggggccacctgcatgttcgtggcctctaagatgaaggagaccattc ccttgactgccgagaagttgtgcatctacactgacaactctatccggcccgaggagctgctgcaaatggaa ctgcttctggtgaacaagctcaagtggaacctggccgccatgactccccacgatttcatcgaacacttcctct ccaaaatgccagaggcggatgagaacaagcagaccatccgcaagcatgcacagacctttgtggccctct gtgccacagatgtgaagttcatttccaacccaccctccatggtagctgctgggagcgtggtggctgcgatgc aaggcctgaacctgggcagccccaacaacttcctctcctgctaccgcacaacgcactttctttccagagtcat caagtgtgacccggactgcctccgtgcctgccaggaacagattgaagcccttctggagtcaagcctgcgcc aggcccagcagaacgtcgaccccaaggccactgaggaggaggggaagtggaggaagaggctggtc tggcctgcacgcccaccgacgtgcgagatgtggacatctga
C-Myc	Atgccgctgaacgtgagctttaccaaccgcaactatgatctggattatgatagcgtgcagccgtatttttattgc gatgaagaagaaaacttttatcagcagcagcagcagagcgaactgcagccgccggcgccgagcgaag atatttggaaaaaatttgaactgctgccgaccccgccgctgagcccgagccgccgcagcggcctgtgcag cccgagctatgtggcggtgaccccgtttagcctgcgcggcgataacgatggcggcggcggcagctttagc accgcggatcagctggaaatggtgaccgaactgctgggcggcgatatggtgaaccagagctttatttgcga tccggatgatgaaacctttattaaaaacattattattcaggattgcatgtggagcggctttagcgcggcggcga aactggtgagcgaaaaactggcgagctatcaggcggcgcgcaaagatagcggcagcccgaacccggc gcgcggccatagcgtgtgcagcaccagcagcctgtatctgcaggatctgagcgcggcggcgagcgaatg cattgatccgagcgtggtgtttccgtatccgctgaacgatagcagcagcccgaaaagctgcgcgagccag gatagcagcgcgtttagcccgagcagcgatagcctgctgagcagcaccgaaagcagcccgcagggca gcccggaaccgctggtgctgcatgaagaaaccccgccgaccaccagcagcgatagcgaagaagaac aggaagatgaagaagaaattgatgtggtgagcgtggaaaaacgccaggcgccgggcaaacgcagcg aaagcggcagcccgagcgcgggcggccatagcaaaccgccgcatagcccgctggtgctgaaacgctg ccatgtgagcacccatcagcataactatgcggcgccgccgagcacccgcaaagattatccggcggcgaa acgcgtgaaactggatagcgtgcgcgtgctgcgccagattagcaacaaccgcaaatgcaccagcccgc gcagcagcgataccgaagaaaacgtgaaacgccgcacccataacgtgctggaacgccagcgccgca acgaactgaaacgcagctttttgcgctgcgcgatcagattccggaactggaaaacaacgaaaaagcgcc gaaagtggtgattctgaaaaaagcgaccgcgtatattctgagcgtgcaggcggaagaacagaaactgatt agcgaagaagatctgctgcgcaaacgccgcgaacagctgaaacataaactggaacagctgcgcaaca gctgcgcgtaa
polypyrimidine tract-binding protein (PTB)	atggacggcatcgtcccagacatagcagtcggtacaaagcggggatccgacgagctcttctccacgtgtgt cagcaacggccccttcatcatgagcagctctgcctcagcagccaatggaaacgatagcaagaagttcaa aggtgacaacaggagcgcaggagtcccttccagagtcatccatgtcagaaagctgcccagcgatgtcact gagggcgaggtcatctccctagggctgccctttggaaaggttaccaaccttctcatgctgaaggggaagaa ccaggccttcattgagatgaacacagaggaggctgccaacactatggttaactactatacatcggtggcgc cagtgcttcgtggacagcccatctacatccagttctccaaccacaaagagctcaagaccgacagctcgcc caaccaggcacgtgcccaggcagccctgcaggctgtaaactccgtccagtctggaaacctggccttggca gcgtccgctgctgccgtggatgcaggaatggcaatggcagggcagagcccagtgctcaggatcattgtgg aaaaccttttctacccagtgaccctggacgtgctgcaccagatcttctctaagtttggcaccgtcctgaagatc atcacgttcaccaagaacaaccagttccaggcgctgctgcagtatgctgaccctgtgagcgcccagcatgc caagctgtccctggatggccagaacatctacaacgcctgctgcacgctgcgcatcgacttctccaagctca ccagtctcaatgtcaagtacaacaatgataagagcagagactacactcgacctgacctgccctctggaga

	cagccagccttcactagaccagaccatggcagcagcctttggtgcgcccggcataatgtcagcctctccgt atgcaggagccgggttccctcccacctttgccatccctcaggccgcaggcctctctgtccctaatgtccatgg agccttggccccccctggccatcccgtctgctgctgctgctgctgcggccagccgcattgccatcccagggttg gcaggtgctgggaattctgtccttttggtcagcaatctgaaccctgagagagtcacaccccaaagcctctttat tctcttcggcgtctacggtgatgtgcagcgggtgaagatcctgttcaataagaaggagaacgcacttgtgca gatggcagacggcagccaggcccagctggccatgagccacctgaacgggcacaagctgcacgggaa gtcagtgcgcattacactgtccaagcatcagagtgtgcagctgcctcgggagggtcaggaggaccagggc ctgaccaaggactatggcagctccccgctgcaccgcttcaagaaaccaggctccaagaacttccagaac atctttccaccctcagctaccctgcacctctccaacatcccgccctctgtgtcagaggacgacctcaagagc ctcttctccagcaacggtggtgtggtcaaaggcttcaagttcttccagaaggaccgcaagatggcactgatc cagatgggctctgtggaggaggctgtgcaggcgctgattgaactgcacaaccatgacctgggcgagaac caccacctgcgagtgtccttttccaagtccaccatctag

Table S2: Antibodies used in this study

Antigen	Dilution	Company	Catalog number
Pkm2	1:100	Cell Signaling	4053
BrdU	1:200	Abcam	ab6326
Aurora B	1:200	BD Transduction	611082
α-Actinin	1:100	Abcam	ab9465
Ki67	1:100	Abcam	ab16667
pH3	1:100	Millipore	06-570
Troponin I	1:50	Santa Cruz Biotechnology	SC-15368
CD45	1:100	BD Pharmigen	550539
CD3	1:100	Abcam	ab5690
GFP	1:500	Abcam	ab13970
CD25	1:100	Abcam	ab187683
CD31	1:100	R&D Biosystems	AF3628
G6pd	1:100	Abcam	ab993
Pkm1	1:100	Cell Signaling	7067
OHG	1:100	Abcam	ab62623
WGA	1:50	Life Technology	W11261
β-catenin	1:200	Cell Signaling	9582
Pkm2	1:100	Proteintech	15822-1-AP
Vimentin	1:200	Abcam	Ab24525
DsRed	1:200	Living Colors	632496
Troponin I	1:200	Abcam	Ab47003
GAPDH	1:1000	Cell Signaling	8884

Suppl. Table S3: Primer Sequences for qPCR in this study

Gene	Forward	Reverse
Pkm2	gtctggagaaacagccaagg	cggagttcctcgaatagctg
Tnnt2	ctgagacagaggaggccaac	ttccgctctgtcttctggat
Mhy6	cagaacaccagcctcatcaa	cccagtacctccgaaagtca
Pecam1	ctgccagtccgaaaatggaac	cttcatccaccggggctatc
Cdh5	attgagacagaccccaaacg	ttctggttttctggcagctt
αSMA	aagctgcggctagaggtca	ccctccctttgatggctgag
WT1	agacacacaggtgtgaaacca	atgagtcctggtgtgggtct
Myc	aggcagctctggagtgagag	cctggctcgcagattgtaag
Hif1α	gggtacaagaaaccacccat	gaggctgtgtcgactgagaa
Pdk1	accaggacagccaatacaag	cctcggtcactcatcttcac
Cdc20	ttcgtgttcgagagcgatttg	accttggaactagatttgccag
Cdk1	tttcggccttgccagagcgtt	gtggagtagcgagccgagcc
Ccnd2	gtcacccctcacgacttcat	ttccagttgcaatcatcgac
Ccnb1	aaggtgcctgtgtgtgaacc	gtcagccccatcatctgcg
18s	agtccctgccctttgtacaca	cgatccgagggcctcacta
HDac4	aaccttagtggggtgctgtg	aaggcacaaactcgcatctt
Hand2	ccagctacatcgcctacctc	tggttttcttgtcgttgctg
Meox2	cacagtgcctgaaatcacca	ctggctgtgtttgtcaatgg
Gata4	tccagcctgaacatctaccc	ccatagtcaccaaggctgct
Mstn	tggctcctactggacctctc	tgccttttaagatgcagcag
MYHC	cagaacaccagcctcatcaa	gctccttcttcagctcctca
Pkm1	gtctggagaaacagccaagg	tcttcaaacagcagacggtg
Bcl2	ggaaggtagtgtgtgtgg	actccactctctgggttcttgg
ANF	cacagatctgatggatttcaaga	cctcatcttctaccggcatc
BNF	aagggtctggctgctttg	cagccaggacttcctcttaatg
P27	aggagagccaggatgtcagc	cagagtttgcctgagacccaa
Cxn43	atcgtggatcagcgaccttc	gttgagtaccacctccacgg
Cxn40	cacagtcatcggcaaggtct	ctgaatggtatcgcaccgga
Serca2a	tgtgtaatgccctcaacagc	agcggtgtgatctggaaaat
PLN	ctcactcgctcagctataagaag	agagaagcatcacgatgatacag
Vegfa	ctgtgcaggctgctgtaacg	gttcccgaaaccctgaggag
G6pd	ccaggtcacccgatgcaccc	ccggaaactaactgtgcgct
P21	gacaagaggcccagtacttc	gcttggagtgatagaaatctgtc

4. Supplemental Movies

Supplemental Movie 1. $_{cms}$Pkm2 modRNA induces neonatal rat CM cell division *in vitro*. 24 hours of live cell imaging, with time-lapse acquisition, was used to evaluate the cell division rate of isolated neonatal rat CMs post transfection with $_{CMS}$nGFP modRNA (nGFP carrying a K motif and L7Ae containing recognition elements for miR1 and miR208) or co-transfection with $_{CMS}$nGFP and Pkm2 modRNA (nGFP and Pkm2 containing a K motif and L7Ae containing recognition elements for miR1 and miR208). Note that CMs transfected with $_{CMS}$nGFP and $_{CMS}$Pkm2 modRNA proliferate more and are in telophase phase post mitosis. 40 seconds long.

Supplemental Movie 2. Cine MRI of infarcted heart transfected with $_{cms}$Luc modRNA, 1 month post MI. 1 second long.

Supplemental Movie 3. Cine MRI of infarcted heart transfected with $_{cms}$Pkm2 modRNA, 1 month post MI. 1 second long.

5. Author Contributions

A.M. designed and carried out most of the experiments, analyzed most of the data and wrote the manuscript. N.S. performed experiments and analyzed qRT-PCR and immunostaining data. A.A.K., I.M., T.M. and K.B. performed histology staining, immunostaining and western blot. M.T.K.S. prepared modRNAs. E.C. performed all mice surgery and Echo analyses. S.Y. analyzed CM-specific miR. J.O. isolated adult mouse CMs. P.L. performed Co-IP experiments. S.C.X. performed and analyzed HPLC oxidative stress and ROS production evaluation. A.G.S. revised the manuscript. G.Z. performed mass spectrometer experiments and quantified ^{13}C metabolite flux data. C.C. designed and provided the Tnnt2^{merCremer} mice. S.A. designed and analyzed MADM mouse acute and chronic MI models. C.W.K. designed and analyzed Co-IP data. M.M. analyzed ^{13}C metabolite flux data. A.S. analyzed HPLC oxidative stress and ROS production evaluation. R.J.H. and H.S. designed experiments, analyzed data and revised the manuscript. L.Z. designed experiments, analyzed data and wrote the manuscript.