# Online Methods

## Expanded Methods and Results

### Cardiomyocyte Isolation and Culture

Neonatal rat ventricular myocytes (NRVMs) are prepared by enzymatic digestion of hearts obtained from newborn (0–2 day old) Sprague–Dawley rat pups using percoll gradient centrifugation and plated on six-well cell culture grade plates (coated with gelatin) at a density of $0.85 \times 10^6$ cells/well in F-10 medium with 10% FBS and maintained at 37°C in humid air with 5% $CO2$[1]. Adult feline left ventricular myocytes were isolated as described previously[2, 3]. Isolated myocytes were washed 3 times with serum free medium and them plated on laminin coated dishes with M199 medium (Sigma Aldrich) supplemented with penicillin, streptomycin and gentamicin.

### MicroRNA treatment and quantification

Cells are transfected with mouse miR-294-3p (mimic) or negative control mimics. NRVMs are grown in media without antibiotics and transfected with either miRNA mimics or controls (50nM, Invitrogen, CA, USA) using Lipofectamine RNAiMAX (Invitrogen, CA, USA) for 24 hrs as per manufacturer instructions.

Total RNA from the cells and heart tissue is extracted using the miRNeasy Mini Kit (Qiagen) according to the manufacturer's protocol including a DNase step. RNA concentrations are verified on the NanoDrop Spectrophotometer (NanoDrop, Thermo Scientific, DE, USA). Equal amount of RNA (5ng) is reverse transcribed using the Taqman MicroRNA Reverse Transcription Kit (Applied Biosystems, CA, USA) using a specific miRNA primer to generate cDNA for use with individual Taqman MicroRNA Assays (Applied Biosystems, CA, USA). Real time Reactions are performed in triplicate on a ABI StepOne Plus Real-Time PCR system (Applied Biosystems, CA, USA). Ct values are averaged and normalized to U6. Relative expression is determined by ΔΔCt comparative threshold method.

### In vivo administration of LNA-microRNA mimic

LNA-microRNA-294-3p mimic was purchased from Exiqon together with control LNA-mimic. For in vivo delivery, 1mg/kg of LNA microRNA-294-3p mimic or control mimic were mixed with In vivo RNA-LANCER II Maxsuppressor reagent (Bioo Scientific, Austin, Tx, USA) in a 0.5% (w/v) solution followed by single intramyocardial injection immediately after MI.

### Live cell imaging

NRVMs are plated on gelatin coated chamber slides respectively. Cells are treated with miR-294-3p mimic followed by live imaging on lecia TCS Sp8 confocal microscope. Cells are analyzed every 24hrs till 72hrs after miRNA treatment to determine effect of the miRNA on cardiomyocyte cell division. Nuclei were visualized by addition of live cell nuclear stain Nucblue.

### In Situ Hybridization

Heart sections from AAV9 administered animals as well as mouse whole embryo sections are assessed for expression miR-294 by miRCURY LNA microRNA ISH Optimization Kit (Exiqon) according to manufacturer's instructions. Briefly, sections are treated with proteinase K before incubation with custom double DIG (5' and 3') labeled miR-294-3p probe followed by detection by anti-DIG-AP antibody. LNA U6snRNA and scrambled-miR are used as controls. Nuclei are stained with nuclear fast red dye.

### Luciferase assay

NRVMs are transfected with mouse *Wee1* 3'-UTR and control luciferase reporter (GeneCopoeia, Rockville, MD, USA) plasmids together with treatment with miR-294-3p mimic. After 48hr, Firefly and Renilla luciferase reporter activity is measured using Luc-Pair Duo-Luciferase Assay Kit 2.0 (GeneCopoeia, Rockville MD, USA) per manufacturer's instructions.

**CDK1 activity assay**
Activity of CDK1 kinase is measured in NRVMs treated with miR-294 mimic or control using MESACUP Cdc2/Cdk1 Kinase assay Kit (MBL, Japan) according to the manufacturer's protocol. Briefly, 2 x $10^6$ NRVMs/group are treated with either miR-294-3p or negative control followed by addition of biotinylated MV peptides. Samples transferred to 96-well plate are treated with anti-phosphoMV to capture phosphorylated peptides and incubated with streptavidin for color development. Plates are read at OD492 in a spectrophotometer.

**Western blotting**
Western blot analysis is carried out as previously described[4-6]. Briefly, NRVMs are lysed with 0.1M Tris, 0.3 M NaCl, 0.1% SDS, 0.5% sodium deoxycholate, and 1% Triton X-100 in a cocktail of antiproteases (Sigma-Aldrich Corporation, St. Louis, MO, USA); then, the nuclei and membranes are cleared by centrifugation (15,000*g* for 10 minutes). Protein extracts are separated on an 8% SDS-PAGE gel, blotted on Nitrocellulose membrane (Millipore, Billerica, MA, USA), incubated with specific antibodies and visualized with LI-COR based detection system (LI-COR, Nebraska, USA). Images are acquired with a LI-COR Odyssey CLX Imaging system (LI-COR, Nebraska, USA).

**Real-Time Quantitative reverse transcription Polymerase chain reaction**
Total RNA is isolated from frozen heart or cultured cells using miRNeasy Kit (Qiagen, USA) and reverse-transcribed into cDNA using iScript cDNA Synthesis kit (Bio-Rad, CA, USA). Quantitative reverse transcriptase polymerase chain reaction is performed on all samples in triplicate using iQ SYBR Green (Bio-Rad, CA, USA) according to the manufacturer's instructions. Primer sequences are provided in Online Table I.

**$RT^2$ Profiler PCR Arrays**
Single-stranded cDNA is synthesized from all samples using the $RT^2$ First Strand Kit (Qiagen) as described in the Qiagen protocol for $RT^2$ profiler array sample preparation. The reverse transcribed product is used to run real time PCR reactions using RT2 SYBR Green ROX qPCR mastermix on a ABI stepOneplus system (Applied Biosystems).

**Animal Studies**
All mice (C57BL/6; 8-12 weeks old) used in this study are obtained from The Jackson Laboratories (Bar Harbor, ME). All surgical procedures and animal care protocols were approved by the Temple University Animal Care and Use Committee.

**Randomization and Blinding**
All animals were assigned animal study numbers and randomly assigned by simple randomization method to ensure equal sample size across groups for experimental procedures. Animal weights were not significantly different between groups. All researchers performing animal surgeries, AAV delivery, echocardiography and hemodynamic measurements or data analyses were completed blinded.

**Induction of Acute Myocardial Infarction and Injections**
Myocardial infarction. Mice underwent surgery to ligate the left anterior descending coronary artery as reported previously[4, 5] followed by administration of AAV9-miR-294 (n= 25)/ AAV9-Ctrl (n=25) or miRNA-LNA control (n=20) and miRNA-LNA-miR-294-3p (n=20) suspended in PBS intramyocardially into the left ventricular wall (border zone) at four different locations (AAV exp) and single location (LNA-exp) immediately after left anterior descending ligation. Tissue was harvested at 2, 7 days and 8weeks after AMI for histological analysis.

Acute myocardial infarction (AMI) was induced as described previously[4, 5]. Briefly, mice are anesthetized with isoflurane (2-4%), orally intubated and placed in a supine position. Respiration is controlled by mechanical ventilation using a rodent ventilator (Nemi Scientific, Inc., Framingham, MA) with tidal volume set to 0.4 ml at a rate of 110 strokes/min. The chest is then shaved, cleaned free of hair and sterilized. Under a dissecting microscope, a left thoracotomy is performed in the fourth intercostal space. After displacing the pericardium, an 8-0 monofilament nylon suture on a curved tapered needle is passed under the left anterior descending coronary artery (LAD) 4 mm below the left atrium and permanently tied to eliminate blood flow distal to the suture. Following verification of induced ischemia via epicardial blanching, AAV or LNA suspended in sterile PBS are injected into the infarct border zone. Pericardium is re-draped over heart, and the chest was then closed following the injection. A 22-gauge syringe is used to re-establish negative pressure within the chest cavity prior to extubation. Animals received post-surgical pain management with buprenorphine and surgical inflammation control with meloxicam. Animals are recovered until freely mobile on a heating pad at which point they are then placed into a clean cage and housed for the duration of the experiment.

Mini-Osmotic pump implantation. Osmotic minipumps (Alzet; Cupertino, CA) are loaded with solution containing 39.0625 mg/mL EdU (Life Technologies, Carlsbad, CA) dissolved in 50/50% mixture of DMSO/ddH2O. Pumps are prepared as previously described[7] and implanted subcutaneously between the two scapulae at the time of MI surgery. The minipumps deliver a continuous infusion of EdU over the course of 1 week, and after 1 week all pumps are removed.

AAV generation, dose optimization and administration. Custom-made AAV9 vector carrying microRNA-294 under TRE promoter together with EGFP and α-MHC-rtTA was purchased from Vector Biolabs (Malvern, PA, USA). The virus titer is 1 x $10^{13}$ gc/ml and is administered retro-orbitally to mice in low (5.4 x $10^{11}$) and high dose (1.08 x $10^{12}$). Based on expression analysis, high dose is selected for the in vivo experiment and the AAV is administered 2 weeks before MI.

**Echocardiography**

Transthoracic two-dimensional M-mode echocardiography is performed using the Vevo770 (VisualSonics, Toronto, ON, Canada) equipped with a 30-MHz transducer. Mice are anesthetized for analysis with a mixture of 1.5% isoflurane and oxygen (1 L/min) before AMI (baseline) at 1, 2, 3, 4, 6 and 8 weeks after AMI. M-mode tracings are used to measure left ventricular wall thickness and left ventricular inner diameter in systole and diastole. The mean value of three measurements is determined for each sample. Percentage fractional shortening and percentage ejection fraction are calculated as described previously[4-6].

**Hemodyanamic Measurements**

LV pressures are measured with a 1.4-Fr Millar pressure catheter (SPR-671, Millar Instruments, Houston, TX) connected to an ADInstruments PowerLab 16/30 (ADInstruments, Colorado Springs, CO) with LabChart Pro 6.0 software. Mice are anesthetized with 2.5% isoflurane to maintain HRs in the 450–470 beats/min range, and then a midline neck incision is made to expose right carotid artery for catheter insertion. The pressure catheter is then advanced through the aortic valves into the LV. The catheter is carefully adjusted to avoid direct contact with the ventricular wall so that smooth intra-LV pressure traces are recorded. Five minutes of baseline pressure are recorded, and intra-LV blood pressure is continuously measured. Additionally, isoproterenol is infused in concentrations of 0.1ng, 0.5ng, 1ng, 5ng, 10ng and LV pressure data is measured. Pressure data are analyzed offline with the blood pressure module in the LabChart6.0 software.

**Histology and Staining**

Immunostaining of NRVMs is performed on cells grown on permanox or glass chamber slides. Cells are fixed by 4% paraformaldehyde (PFA), permeabilized in PBS supplemented by 0.2% Triton-X for 10 min and blocked in PBS supplemented with 10% horse serum for 1 hr. Primary antibodies diluted are applied overnight at 4°C after blocking in PBS with 10% horse serum. The next day, cells are washed with PBS and incubated for 1 h at room temperature with secondary antibodies (Jackson Laboratories, USA) diluted in blocking solution. DAPI (Calbiochem, USA) is diluted in Vectashield (Vector Labs, CA, USA) mounting media at 1:500 vol/vol and used as nuclear staining.

Paraffin heart sections are deparaffinized in xylene and rehydrated through graded alcohols to distilled water. Antigen retrieval is achieved by boiling the slides in 10 mmol/L citrate pH 6.0 for 12–15 min. Slides are washed several times with distilled water and once with TN buffer (100 mmol/L Tris, 150 mmol/L NaCl). Endogenous tissue peroxidase activity is quenched with TN buffer supplemented with 3% $H_2O_2$ for 20 min whenever necessary. Slides are then washed in TN buffer and blocked in TNB buffer (TSATM kit from Perkin-Elmer) at room temperature for at least 30 min. Primary antibodies are applied overnight at 4°C in TNB buffer. The next day, samples are washed in TN buffer and incubated with secondary antibodies at room temperature in the dark for 1 hr. Slides are washed in TN buffer and coverslipped using Vectashield in the presence of DNA staining. List of primary and secondary antibodies is reported in OnlineTable II. For all immunostaining experiments, secondary antibody staining is used as control for determination of background immunofluorescence. For TUNEL, Ki67, pH3 and AurB staining, 5-7 field of view/5 slides/heart were analyzed. Moreover, in total 10,000 cells/heart and 5000 cells/heart were counted for WGA staining experiment.

Terminal deoxynucleotidyl transferase-mediated deoxyuridine triphosphate nick-end labeling (TUNEL) is performed on paraffin-embedded tissues fixed at 2 days after MI and treatments. Samples are then immunostained to quantify the number of apoptotic cells.

**Infarct Size Determination**

All fixed hearts are sectioned starting from the height of the ligating suture and then sequentially at 250-μm distances below the suture as far as effective sectioning would permit. Infarct size is evaluated on Masson's trichrome-stained heart sections cut 500 μm below the ligation point with ImageJ (National Institutes of Health), and the transmural, fibrotic infarct area is then assessed as a percentage of the entire LV chamber area.

**Single cell isolation and EdU detection**

Mouse cardiac myocytes are isolated as previously described[8]. Briefly, the aorta is cannulated and perfused retrograde to enzymatically digest the heart. When softened, the atria are removed and the ventricles are mechanically dissociated to obtain single cells. The cell solution is exchanged with a BSA containing solution and allowed to settle. Next, the cells are washed in normal tyrode's solution and then fixed with 4% paraformaldehyde in PBS. Care is taken between solution exchanges to minimize myocyte loss. Following enzymatic digestion, LV myocytes are fixed in paraformaldehyde (4% in PBS, Affymetrix) and nuclei stained with Click-iT EdU Alexa Fluor 488 Imaging Kit (Life Technologies; Eugene, OR) and DAPI (Calbiochem). Total number of myocytes is determined by hemocytometer and EdU+ myocytes are counted using a fluorescent microscope (Nikon eclipse TiE). Nuclei distribution are evaluated using ImageJ software. On average ~ 150 myocytes per mouse were analyzed from 6 mice.

**Oxygen consumption rate (OCR) and ECAR measurements**

A Seahorse Bioscience XF96 Extracellular Flux Analyzer is utilized to measure oxygen consumption rates (OCR) in NRVMs using a protocol similar to that previously reported[5]. NRVMs

are plated at 50,000 cells per well in XF media supplemented with 1 mM pyruvate, 2 mM glutamine, 10 mM glucose. Three independent OCR measurements are acquired for each condition: baseline, following the addition of 3 μM oligomycin, 2 μM FCCP, and 0.5 μM Rotenone. For, ECAR measurements, NCRMs are plated at 50,000 cells per well in XF media supplemented with 1mM glutamine. Three independent ECAR measurements are acquired for each condition, baseline, following addition of Glucose 100mM, Oligomycin 100uM and 2-DG 500mM. At the conclusion of each experiment, cells are lysed in RIPA buffer and protein concentration is determined for each well using a standard Bradford assay. All calculations for assessment of OCR/ECAR were evaluated from the third reading in each condition and all values reported as mean +/- SD, (pmoles O2/min/mg).

**Online Table I: Quantitative Real time PCR**

| Primer Name | Forward/Reverse | Product size | Sequence |
|---|---|---|---|
| Wee1 | Forward | 363 | TCTTACCGTAGTCGGAGGCA |
| Wee1 | Reverse | | GCACATGACATTTCTGTTGCGA |
| Lin28a | Forward | 78 | TTTGCCTCCGGACTTCTCTG |
| Lin28a | Reverse | | CCCATGGTCGTCTGCTGAG |
| Cyclin B1 | Forward | 134 | GGTGGAACGACTGTTGGTCT |
| Cyclin B1 | Reverse | | TTTCGTGTTCCTGGTGACCC |
| Cyclin A2 | Forward | 77 | AGGACAAAGCTGGCCTGAATC |
| Cyclin A2 | Reverse | | GGTCCATGAGGCAAGGCTTA |
| Cyclin D1 | Forward | 145 | ATTTCCAACCCGCCTTCCAT |
| Cyclin D1 | Reverse | | GACAGTCCGCGTCACACTTG |
| Cyclin D2 | Forward | 165 | GCTCTGTGTGCTACCGACTT |
| Cyclin D2 | Reverse | | CACATCGGTGTGGGTGATCT |
| Cyclin E1 | Forward | 243 | TCCAGGAAAAGAAAGGCAAATGT |
| Cyclin E1 | Reverse | | TGCCCAGTTCAGTATAGGCAG |
| CDK1 | Forward | 78 | CCTCTAAGCTCCCGGAGTCG |
| CDK1 | Reverse | | CAACGGACCCTCTCTGTTCC |
| E2F1 | Forward | 178 | GCCTCGAATAGGCAACCTGA |
| E2F1 | Reverse | | ACCCTCCTCGAGACCAAAGT |
| c-myc | Forward | 187 | ACTCGGTGCAGCCCTATTTC |
| c-myc | Reverse | | GTAGCGACCGCAACATAGGA |
| CDK2 | Forward | 84 | CTTTGCCGAAATGGTGACCC |
| CDK2 | Reverse | | CCCAGAGTCCGAAAGATCCG |
| HK1 | Forward | 75 | GATCGTTGGAGCAGACCACA |
| HK1 | Reverse | | TGTACAAACACCCCGAGACG |
| ALDO1 | Forward | 80 | CCTTAGTCCTTTCGCCTACCC |
| ALDO1 | Reverse | | CGTTGCCATGGGTCACCTTG |
| TPI1 | Forward | 61 | GAGAGCCGTGCGTTTGTACT |
| TPI1 | Reverse | | CTGGTAGGCGCCATTGTACC |
| ENO1 | Forward | 61 | TCCTTAAGGCTCTCCTCGGT |
| ENO1 | Reverse | | AGTAGGATCGCTGCAAAGCA |
| PGAM | Forward | 56 | TTGCCAGTGGTCAGGACTTG |
| PGAM | Reverse | | CCTGTCAGACCGCCATAGTG |
| PKM2 | Forward | 135 | CGCCTGGACATTGACTCTG |
| PKM2 | Reverse | | GAAATTCAGCCGAGCCACATT |
| PDK4 | Forward | 80 | ACGTCCTTTGCTTTTCTGCG |
| PDK4 | Reverse | | CGGTCAGGCAGGATGTCAAT |
| GAPDH | Forward | 267 | GAAGCTCATTTCCTGGTATGACA |
| GAPDH | Reverse | | TATTGATGGTATTCGAGAGAAGGG |

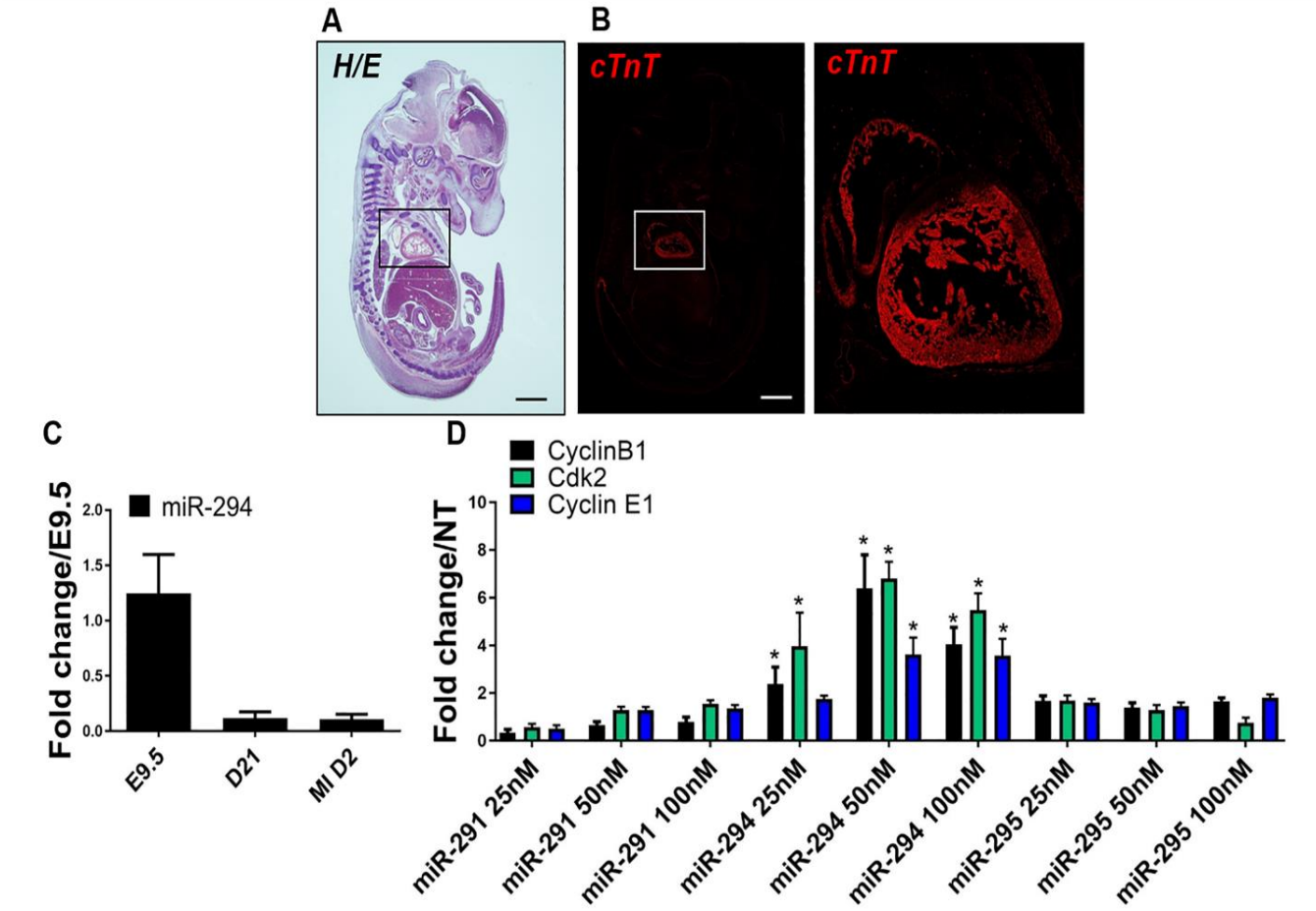
**Online Table II**

**Antibodies**

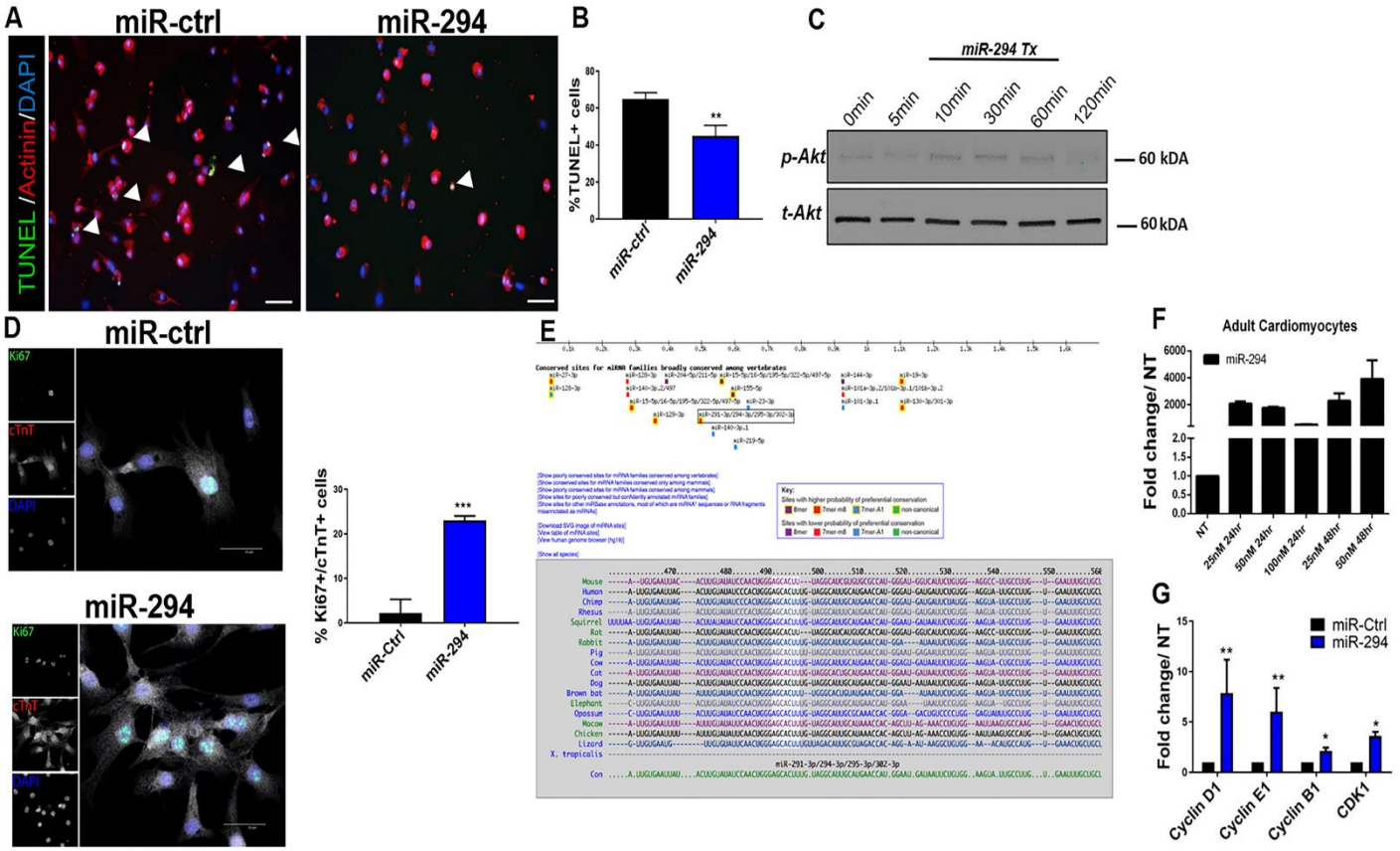
| Application | Antibody | Dilution | Cat No. | Company |
|---|---|---|---|---|
| Western Blot | Wee1 | 1:300 | Ab137377 | Abcam |
| Western Blot | Lin28a | 1:500 | SC-6216 | Santa Cruz |
| Western Blot | Cyclin B1 | 1:1000 | 4138 | Cell Signaling |
| Western Blot | Cyclin D1 | 1:3000 | 2922S | Cell Signaling |
| Western Blot | CDK1 | 1:500 | ab18 | Abcam |
| Western Blot | p-CDK tyr15 | 1:500 | 9114 | Cell signaling |
| Western Blot | βactin | 1:1000 | 9664S | Cell Signaling |
| Western Blot | p-AKT S473 | 1:500 | 4060 | Cell Signaling |
| Western Blot | AKT | 1:500 | 9272 | Cell Signaling |
| ICC | BrdU | 1:100 | Ab6326 | Abcam |
| ICC | Sarcomeric Actin | 1:100 | A2172 | Sigma Aldrich |
| ICC | Aurora B | 1:50 | Ab2254 | Abcam |
| ICC | Phospho Histone 3 | 1:100 | 441190G | Life technologies |
| ICC | Ki67 | 1:100 | Ab15580 | Abcam |
| ICC | SMA | | A2547 | Sigma Aldrich |
| IHC | WGA-488 | 1:200 | W11261 | Life technologies |

**Online Table III – Cell cycle gene array targets**

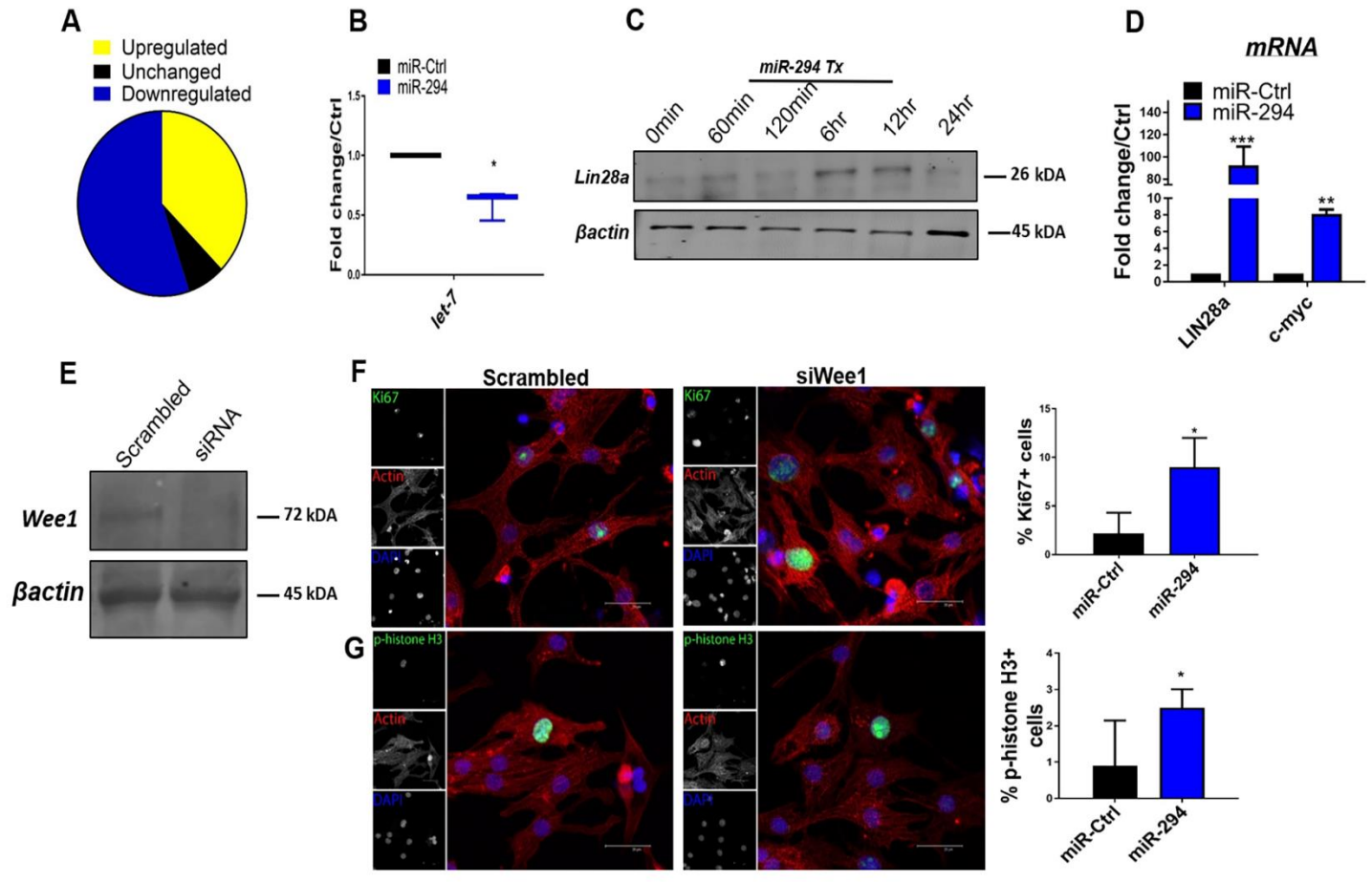
| | Gene | Cumulative Fold change (n=3) | | Gene | Cumulative Fold change (n=3) |
|---|---|---|---|---|---|
| 1. | Abl1 | 0.46 | 43. | Gad45a | 66.89 |
| 2. | Atm | 11.25 | 44. | Gpr132 | 3.231 |
| 3. | Aurka | 18.65 | 45. | Hus1 | 13.095 |
| 4. | Aurkb | 6.56 | 46. | Inha | 0.02 |
| 5. | Bcl2 | 98.10 | 47. | Itgb1 | 1.055 |
| 6. | Birc5 | 72.8 | 48. | Mcm3 | 12.04 |
| 7. | Brca1 | 0.86 | 49. | Mad2l1 | 6.25 |
| 8. | Brca2 | 1.23 | 50. | Mcm2 | 3.1066 |
| 9. | Casp3 | 2.01 | 51. | Mcm4 | 4.7743 |
| 10. | Ccna1 | 2.42 | 52. | Mdm2 | 4.6 |
| 11. | Ccna2 | 8.2233 | 53. | Mki67 | 2.4533 |
| 12. | Ccnb1 | 6.2033 | 54. | Mre11a | 0.023 |
| 13. | Ccnb2 | 2.533 | 55. | Msh2 | 0.19 |
| 14. | Ccnc | 0.966 | 56. | Nbn | 3.26 |
| 15. | Ccnd1 | 16.533 | 57. | Nek2 | 24.245 |
| 16. | Ccnd2 | 21.23 | 58. | Notch2 | 0.34 |
| 17. | Ccnd3 | 4.8766 | 59. | Pkd1 | 0.04 |
| 18. | Ccne1 | 8.343 | 60. | Pmp22 | 0.02 |
| 19. | Ccnf | 13.21 | 61. | Ppm1d | 0.15 |
| 20. | Cdc20 | 8.73667 | 62. | Rad17 | 9.61 |
| 21. | Cdc25a | 11.2933 | 63. | Rad21 | 5.96 |
| 22. | Cdc25b | 33.21 | 64. | Rad51 | 0.2 |
| 23. | Cdc25c | 2.01 | 65. | Rad9 | 0.01 |
| 24. | Cdc6 | 3.87 | 66. | Ran | 0.04 |
| 25. | Cdc7 | 10.8533 | 67. | Rb1 | 2.07 |
| 26. | Cdk1 | 7.1133 | 68. | Rbl1 | 0.1833 |
| 27. | Cdk2 | 6.1366 | 69. | Rbl2 | 0.72 |
| 28. | Cdk4 | 1.7433 | 70. | RGD1560225 | 0.01 |
| 29. | Cdk5rap1 | 11.495 | 71. | Sesn2 | 70.235 |
| 30. | Cdk6 | 2.525 | 72. | Sfn | 7.59 |
| 31. | Cdkn1a | 0.04 | 73. | Shc1 | 0.42 |
| 32. | Cdkn1b | 0.4433 | 74. | Skp2 | 0.01 |
| 33. | Cdkn2a | 1.32333 | 75. | Slfn1 | 0.01 |
| 34. | Cdkn2b | 0.22333 | 76. | Smc1a | 0.15 |
| 35. | Cdkn3 | 2.1365 | 77. | Stag1 | 0.035 |
| 36. | Chek1 | 0.12 | 78. | Stmn1 | 22.975 |
| 37. | Chek2 | 0.02 | 79. | Terf1 | 20.67 |
| 38. | Ddit3 | 0.585 | 80. | Tfdp2 | 19.75 |
| 39. | Dst | 0.075 | 81. | Tp53 | 1.09 |
| 40. | E2f1 | 11.9333 | 82. | Tp63 | 13.01 |
| 41. | E2f3 | 6.4133 | 83. | Tsg101 | 1.02 |
| 42. | E2f4 | 12.23 | 84. | Wee1 | 0.47667 |



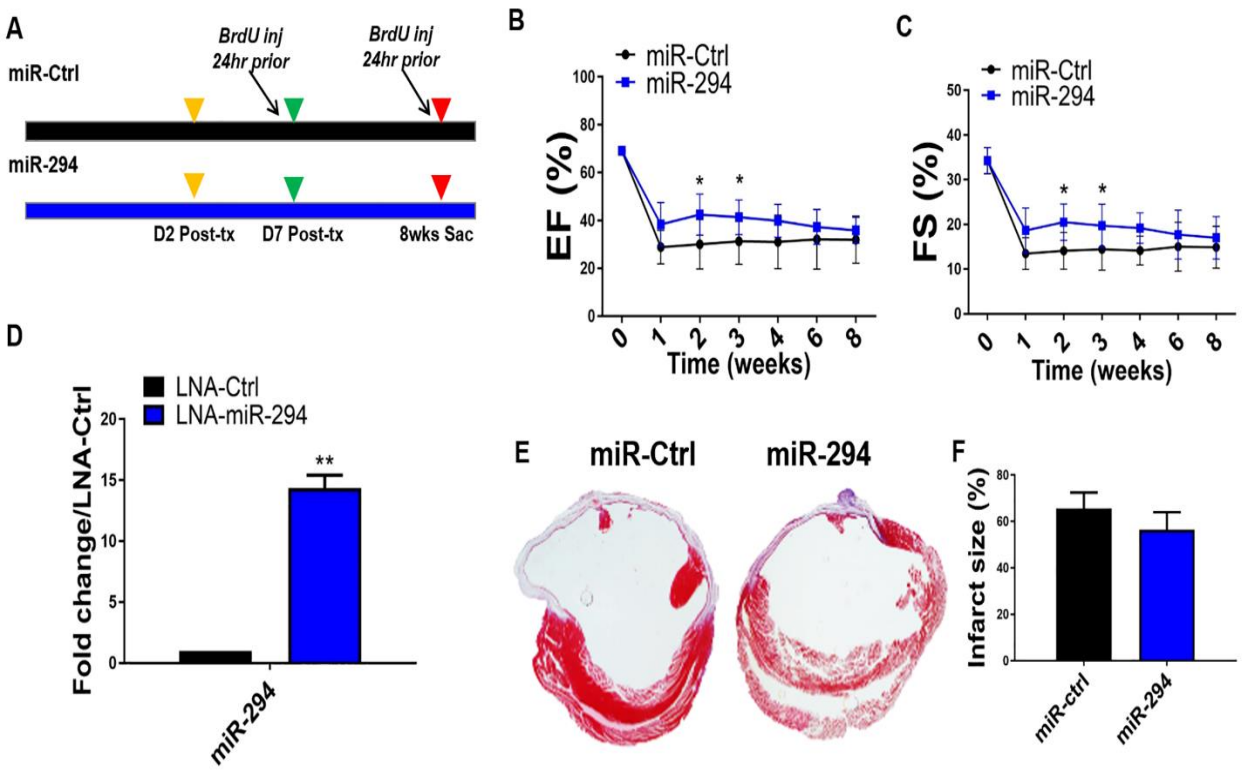

**Online Figure I:** A) Hematoxylin-eosin staining of 14.5 embryo. Inset shows the heart. B) Whole mount embryo stained with cardiac troponin T (red) labels the heart (inset) and higher magnification images of cTNT stained heart.. Scale bar = 50µm. C) Myocardial injury does not increase miR-294 levels in the heart (n=3). D) NRVMs treated with miR-291, miR-294 and miR-295 mimics to determine the potency of the miRs. Significantly high expression of cell cycle markers *Cyclin B1, CDK2,* and *cyclin E1* in the miR-294 treated groups compared to the other treatments (n=3). No treatment vs. treated cells *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, data was assessed using unpaired student's t test.



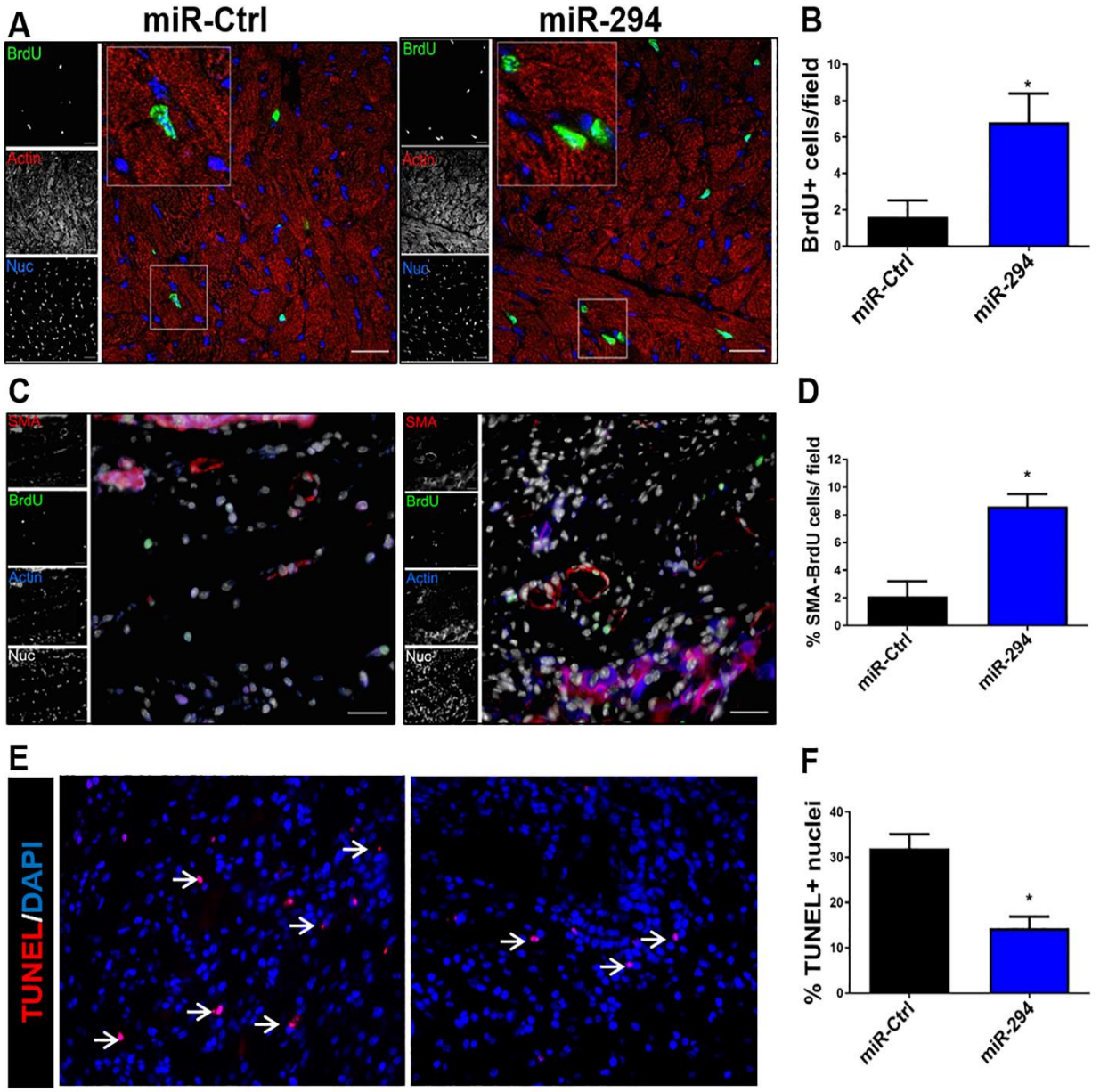

**Online Figure II:** A-B) Decreased number of TUNEL+ NRVM nuclei in response to miR-294 treatment compared to control under stress from H2O2 along with quantification (n=3). TUNEL (green), Actinin (red) and nuclei (Blue). Scale bar = 40µm. C) Increase AKT serine 473 phosphorylation in NRVMs treated with miR-294 mimic compared to control mimic(n=3). D) Validation of miR-294 proliferative effect on NRVMs cultures by immunostainig with Ki67 and cardiac troponin T (cTnT) in cells treated with miR-294 mimic or control mimic. Ki67 (green), cTnT (white), nuclei (blue). Scale bar = 20µm. E) Target scan analysis of conserved sites for miR-294 for cell cycle genes across species. F) Expression of miR-294 in adult cardiomyocyte transfected with miR-294-3p mimic (n=3) G) Elevated mRNA expression of cell cycle markers (*cyclins D1, E1, B1, CDK1*) in adult myocyte after miR-294 treatment (n=3). miR-Ctrl vs. miR-294 **p* < 0.05, ***p* < 0.01, ****p* < 0.001, data was assessed using unpaired student's t test.



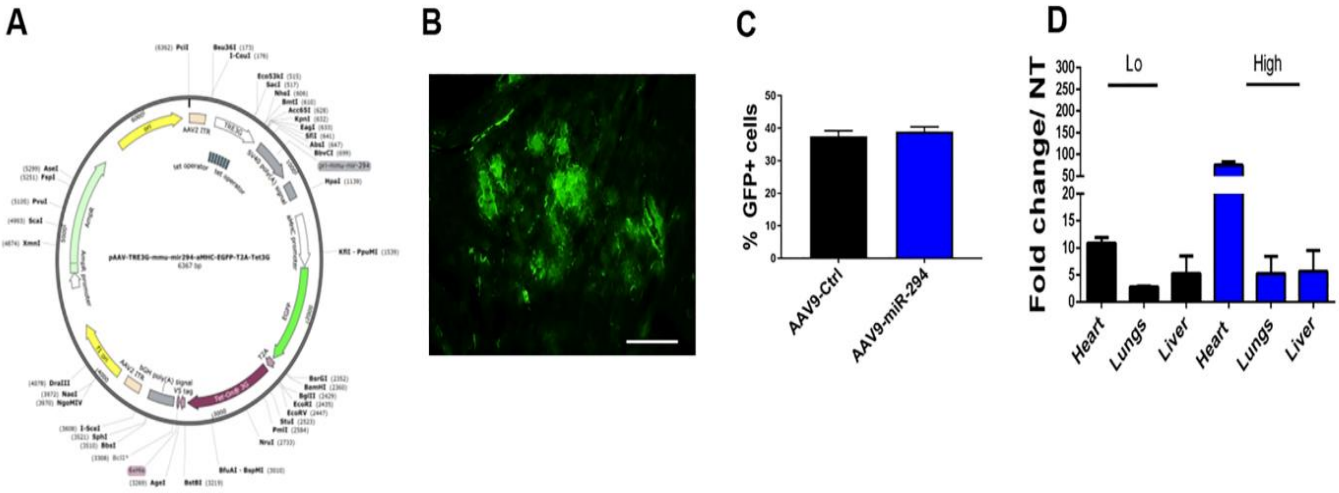

**Online Figure III:** A) Distribution of upregulated, downregulated and unchanged genes after miR-294 treatment in NRVMs (n=3). B) miR-294 treatment reduces let-7 expression in NRVMs compared to control treated cells (n=5). C-D) Increased expression of miR-294 signaling targets *LIN28a* and *c-myc* as confirmed by immunoblot and mRNA respectively. E) Wee1 expression analysis after siRNA Wee1 treatment of NRVMs. F-G) Immunostaining with Ki67 and p-histone H3 of NRVMs treated with siWee1 and scrambled along with the corresponding quantification. Ki67/p-histone H3 (green), Actinin (red), nuclei (blue). Scale bar =20 μm. miR-Ctrl vs. miR-294 $*p < 0.05$, $**p < 0.01$, $***p < 0.001$, data was assessed using unpaired student's t test.



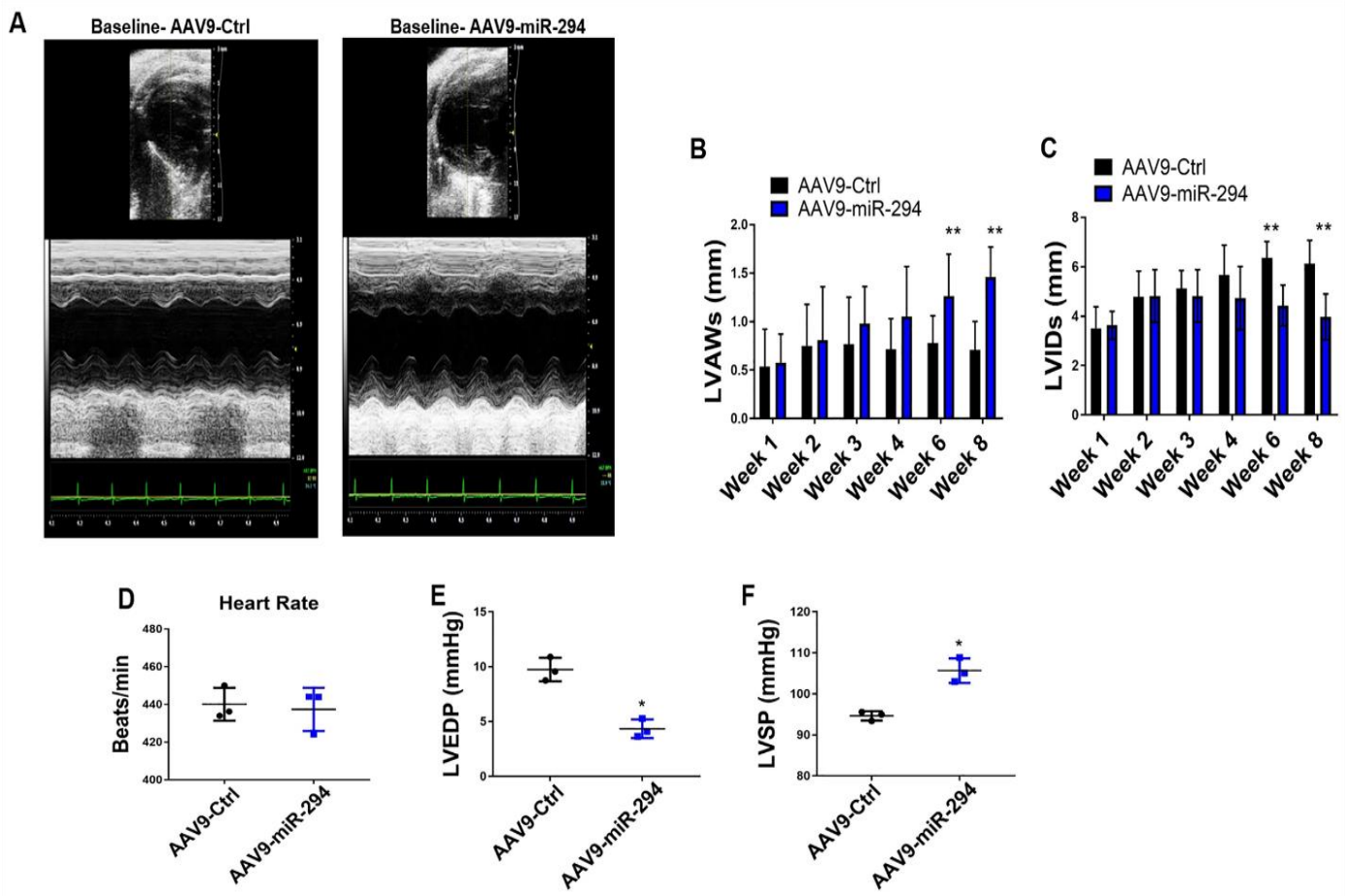

**Online Figure IV: LNA-miR-294 treatment of animals after myocardial infarction.** A) Experimental design for LNA-miR-294 (n=20) and LNA-ctrl (n=20) administration at the time of MI. Animals were followed for 8 weeks with samples being taken at day2, day7 and 8 weeks. BrdU was given intraperitoneally to each animal prior to harvesting the heart. Increased ejection fraction (B) and fractional shortening (C) in animals administered LNA-miR-294 compared to control. D) Analysis of hearts at day2 show increased expression of transfected miR-294 in the heart (n=6). E-F) No change in infarct size in animals administered LNA-miR-294 compared to controls (n=8). LNA-Ctrl vs. LNA-miR-294 *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, data in panel B, C was assessed using one-way ANOVA with bonferroni post hoc test while panel D was assessed with unpaired student's t test



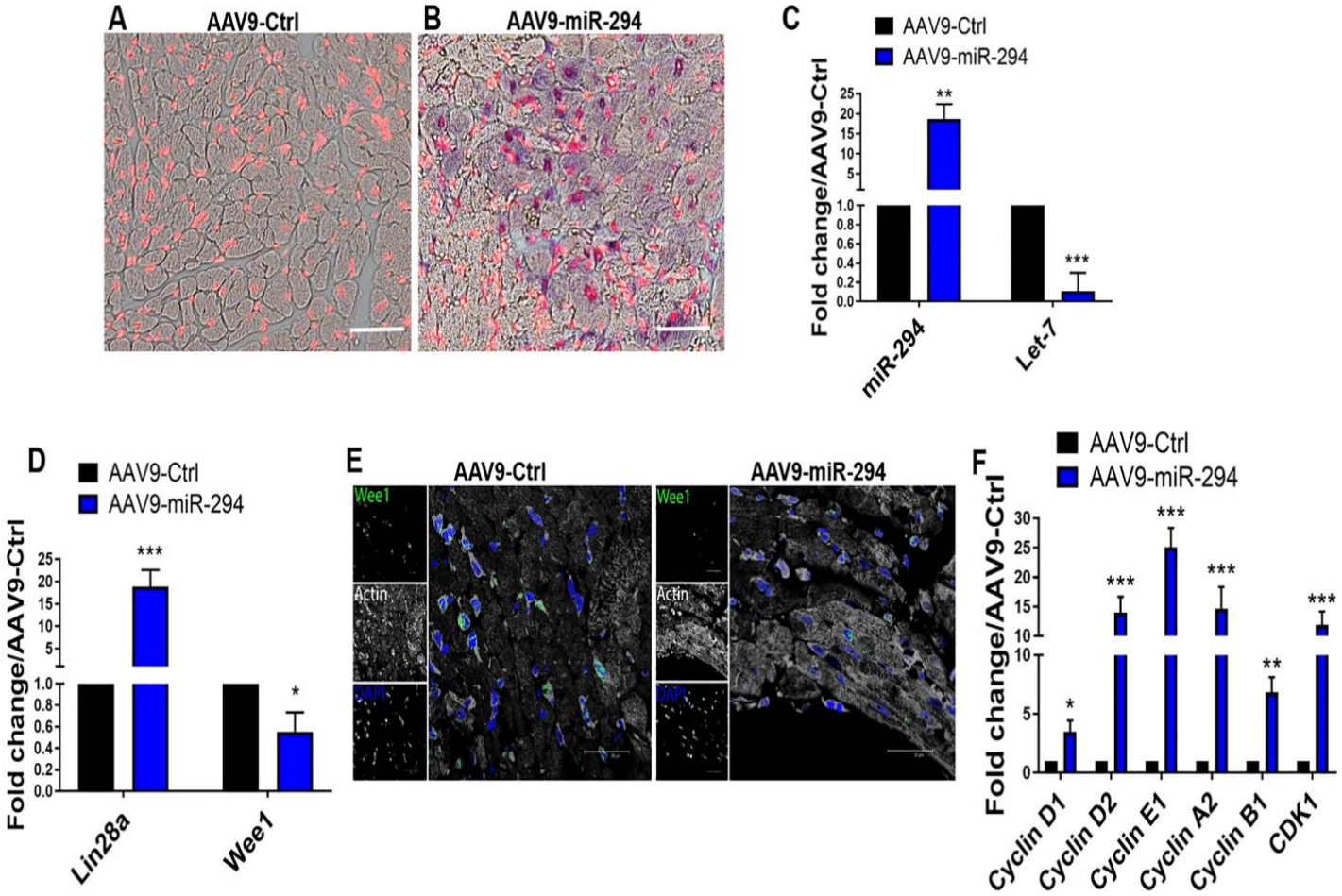

**Online Figure V: Increased proliferation and survival in the heart at 7 days after miR-294 treatment.** A-B) Increased BrdU+ cells in the hearts treated LNA-miR-294 7 days after MI and miR administration compared to control treated animals (n=5). BrdU (green), sarcomeric actin (red) and nuclei (Blue). Scale bar = 40μm. C-D) Increased number of SMA+/BrdU+ cells in the hearts treated with LNA-miR-294 compared to controls. SMA (red), BrdU (green), Actin (blue) and nuclei (white) (n=5). Scale bar = 40μm. E-F) LNA-miR-294 reduces the number of TUNEL+ nuclei in the heart 2 days after MI compared to LNA-control miR (n=5). TUNEL (red), nuclei (blue), scale bar = 40μm. LNA-Ctrl vs. LNA-miR-294 *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, data was assessed using unpaired student's t test



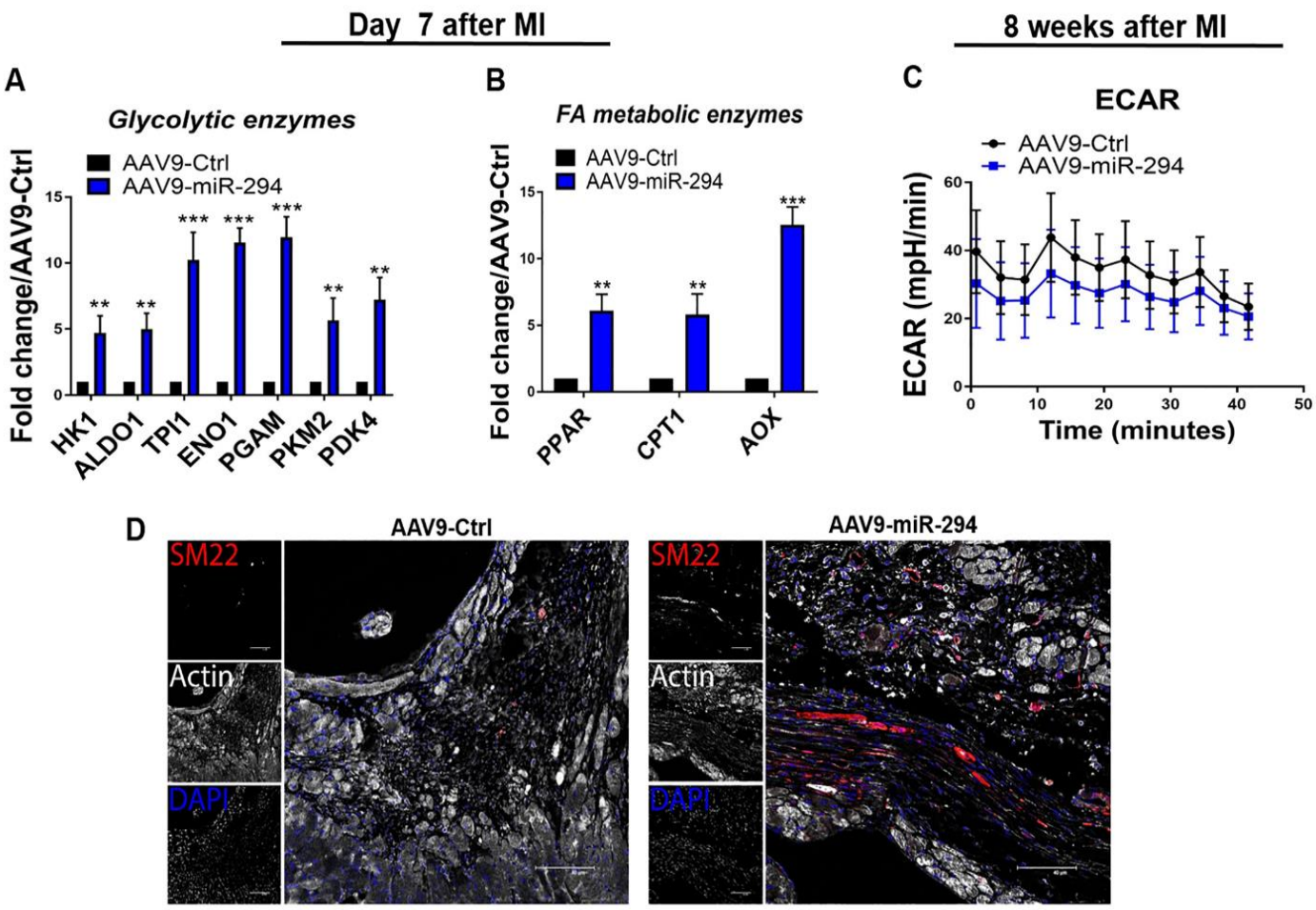

**Online Figure VI: Generation, validation and dose optimization of AAV-9 delivery to the heart.** A) Vector design for AAV9-TRE-miR-294-MHC- EGFP-T2A-Tet3G. B) AAV9-miR-294 was delivered to the heart 2 weeks before administration of doxycycline chow to mice for activation of miR-294 expression. Representative image shows GFP expression in AAV-9-miR-294 hearts. C) Transduction efficiency in both groups of animals administered by AAV9. D) Heart were harvested 7days after dox induction to assess miRNA levels. Increased expression of miR-294 was observed in the heart under low and high AAV9 dose indicating expression of the vector.




**Online Figure VII: Echocardiographic assessment and hemodynamic measurements.** A) M-mode long axis representative images of baseline echocardiography in both the groups. B) Analysis of Left ventricular anterior wall thickness (LVAW) and C) Left ventricular internal dimensions end-systolic (LVIDs) was done in mice administered AAV9-Ctrl (n=8) and AAV9-miR-294 (n=10) 8 weeks after myocardial infarction. D) Heart rate was similar between both groups during hemodynamic measurements. E-F) LV end-diastolic pressure (LVEDP) and LV systolic pressure (LVSP) hemodynamic measurements between two groups. AAV9-Ctrl vs. AAV9-miR-294 *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, data was assessed using unpaired student's t test.




**Online Figure VIII: Analysis of molecular signaling in the hearts after AAV9 administration.** A-B) miR-294 expression in the hearts of AAV9-miR-294 (n=4) treated animals was significantly elevated 7 days after mice were administered dox chow as confirmed by in situ hybridization compared to no expression in the AAV9-Ctrl hearts (n=4). Nuclei are stained with nuclear fast red stain. Scale bar = 40µm. C) Increased miR-294 levels and reduced let-7 expression 7 days after MI as confirmed by qRT-PCR analysis (n=3). D) miR-294 targets Lin28a was upregulated while Wee1 was downregulated in AAV9-miR-294 animals at day7 after MI compared to AAV9-Ctrl hearts (n=3). E) Decreased expression of Wee1 in the miR-294 treated hearts compared to control 2 days after MI. Wee1 (green), sarcomeric actin (red), DAPI (blue). Scale bar = 20µm. F). Markers of proliferation were significantly upregulated in AAV9-miR-294 administered hearts at day7 after MI compared to control animals (n=3). AAV9-Ctrl vs. AAV9-miR-294 *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, data was assessed using unpaired student's t test.




**Online Figure IX: Metabolic assessment of the heart at day 7 and 8 weeks after MI.** A-B) Increased mRNA expression glycolytic enzymes together with increased OXPHOS enzyme in AAV9-miR-294 hearts compared to AAV9-Ctrl at day7 after MI (n=3). C) Analysis of ECAR in isolated adult cardiomyocytes from hearts of both AAV9-Ctrl and AAV9-miR-294 8 weeks after MI shows decreased ECAR and glycolytic parameters (AAV9-Ctrl n=6 and AAV-miR-294 n=6/3 mice), data was assessed using unpaired student's t test. D) Increased new vessel formation was Increased SM22 staining in AAV9-miR-294 administered hearts compared to AAV9-Ctrl animals 8 weeks after myocardial infarction. SM22 (red), sarcomeric actin (white), DAPI (blue). Scale bar = 40µm.

**Online Videos**

**Online Video I:** Live cell imaging showing cell division in NRVMs treated with miR-294 for 24hours post treatment.

**Online Video II:** Z-stack image of p-histone H3+ myocyte in AAV9-miR-294 heart 8 weeks after MI. p-histon (green), Actin (red), DAPI (blue). Scale bar= 20µm

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