SUPPLEMENTAL MATERIAL
Table S1. Adult rat cardiomyocyte isolation buffer composition.

| 10X KHB Stock Solution (Total volume= 1L) | Molarity (mM) | Amount (g) |
|-----------------------------------------|--------------|------------|
| NaCl                                    | 1180         | 68.9       |
| KCl                                     | 48           | 3.5        |
| HEPES                                   | 250          | 59.7       |
| MgSO$_4$                                | 12.5         | 1.4        |
| K$_2$HPO$_4$                            | 12.5         | 2.1        |
| Adjust pH to 7.4 with 4M NaOH (~20mL), store at 4°C |

| KHB Solution, 500 mL | Amount |
|----------------------|--------|
| 10X KHB              | 50 mL  |
| Glucose              | 0.99 g |
| Taurine              | 0.31 g |
| Add H$_2$O to bring volume to 500mL; pH should be ~7.35 |

| Solution A | Amount |
|------------|--------|
| KHB solution | 375 mL (10 mM) |
| BDM        | 0.375 g |
| Oxygenate with 100% O$_2$ and warm to 37°C |

| Solution B, 50mL | Amount |
|------------------|--------|
| Solution A       | 50 mL  |
| BSA              | 0.5 g  |
| 0.1 M CaCl$_2$ (Ca$^{++}$=0.1 mM) | 50 µL |

| Solution E, 50mL | Amount |
|------------------|--------|
| Solution A       | 50 mL  |
| BSA              | 0.05 g |
| Collagenase type II (263 units/mg) | 35 mg |
| Hyaluronidase (Type I-S) | 10 mg |
| 0.1 M CaCl$_2$ stock | 12.5 µL |
| Mix well |

| CaCl$_2$ Stock, 0.1M | Amount |
|----------------------|--------|
| CaCl$_2$             | 7.35 g |
| H$_2$O               | 500 mL |
| Then store at 4°C |
### Table S2. siRNAs Sequences.

| siRNA         | Sequence                  |
|---------------|---------------------------|
| siRb1         | CCAGUACCAAAGUUGAUATT      |
| siMeis2       | CCACGAUGAUGCAACCUCATT     |
| Cel-miR-67    | UCACAAACCUCUUAGAAAGGUAGA |
Table S3. Homology analysis of siRb1 with rat genome.

| Description                                                                 | Max score | Total score | Query cover | E value | Ident     | Accession   |
|----------------------------------------------------------------------------|-----------|-------------|-------------|---------|-----------|-------------|
| Rattus norvegicus retinoblastoma 1 (Rb1), mRNA                              | 38.2      | 38.2        | 100%        | 0.006   | 100%      | NM_017045.1 |
| Rattus norvegicus mRNA for retinoblastoma protein, partial sequence        | 38.2      | 38.2        | 100%        | 0.006   | 100%      | D25233.1    |
| Rattus norvegicus Y Chr BAC RNECO-131C03 (Amplicon Express Rat SHR-Akr (EcoR1 Digest) BAC library) complete sequence | 28.2      | 78.8        | 94%         | 5.4     | 100%      | AC246525.4  |
| Rattus norvegicus BAC CH230-6C14 () complete sequence                       | 28.2      | 80.8        | 100%        | 5.4     | 100%      | AC094946.6  |
| Rattus norvegicus BAC CH230-12M22 () complete sequence                     | 28.2      | 186         | 94%         | 5.4     | 100%      | AC141398.4  |
| Rattus norvegicus 2 BAC CH230-7P18 (Children's Hospital Oakland Research Institute) complete sequence | 28.2      | 80.8        | 100%        | 5.4     | 100%      | AC095504.7  |
| Rattus norvegicus Y Chr BAC RNECO-145B11 (Amplicon Express Rat SHR-Akr (EcoR1 Digest) BAC library) complete sequence | 28.2      | 54.5        | 73%         | 5.4     | 100%      | AC240959.5  |
| Rattus norvegicus TL0AE76YE18 mRNA sequence                                | 28.2      | 28.2        | 73%         | 5.4     | 100%      | FQ231233.1  |
| Rattus norvegicus TL0ACA17YL01 mRNA sequence                               | 28.2      | 28.2        | 73%         | 5.4     | 100%      | FQ217794.1  |
| Rattus norvegicus TL0ADA42YK07 mRNA sequence                               | 28.2      | 28.2        | 73%         | 5.4     | 100%      | FQ220503.1  |

**Homology analysis of siRb1 with rat genome:** Table represents the significance base top 10 homology results, which demonstrates the specific and 100% identity of siRb1 with rat Rb1 gene with a significant $p$-value ($p=0.002$).
Table S4. Homology analysis of siMeis2 with rat genome.

| Description                                                                 | Max score | Total score | Query cover | E value | Ident | Accession         |
|----------------------------------------------------------------------------|-----------|-------------|-------------|---------|-------|-------------------|
| PREDICTED: Rattus norvegicus Meis homeobox 2 (Meis2), transcript variant X13, mRNA | 38.2      | 38.2        | 100%        | 0.007   | 100%  | XM_006234755.3    |
| PREDICTED: Rattus norvegicus Meis homeobox 2 (Meis2), transcript variant X12, mRNA | 38.2      | 38.2        | 100%        | 0.007   | 100%  | XM_006234754.3    |
| PREDICTED: Rattus norvegicus Meis homeobox 2 (Meis2), transcript variant X11, mRNA | 38.2      | 38.2        | 100%        | 0.007   | 100%  | XM_006234753.3    |
| PREDICTED: Rattus norvegicus Meis homeobox 2 (Meis2), transcript variant X10, mRNA | 38.2      | 38.2        | 100%        | 0.007   | 100%  | XM_006234752.3    |
| PREDICTED: Rattus norvegicus Meis homeobox 2 (Meis2), transcript variant X9, mRNA | 38.2      | 38.2        | 100%        | 0.007   | 100%  | XM_006234751.3    |
| PREDICTED: Rattus norvegicus Meis homeobox 2 (Meis2), transcript variant X8, mRNA | 38.2      | 38.2        | 100%        | 0.007   | 100%  | XM_006234750.3    |
| PREDICTED: Rattus norvegicus Meis homeobox 2 (Meis2), transcript variant X7, mRNA | 38.2      | 38.2        | 100%        | 0.007   | 100%  | XM_006234749.3    |
| PREDICTED: Rattus norvegicus Meis homeobox 2 (Meis2), transcript variant X6, mRNA | 38.2      | 38.2        | 100%        | 0.007   | 100%  | XM_006234748.3    |
| PREDICTED: Rattus norvegicus Meis homeobox 2 (Meis2), transcript variant X5, mRNA | 38.2      | 38.2        | 100%        | 0.007   | 100%  | XM_006234747.3    |
### Homology analysis of siMeis2 with rat genome

Table represents the significance base top 17 homology results, which demonstrates the specific and 100% query coverage of siMeis2 with rat Meis2 gene (variants) with a significant p-value ($p=0.002$).

| Predicted Gene                                      | Alignment Score | Coverage | p-value | 100% Query Coverage | Accession       |
|------------------------------------------------------|-----------------|----------|---------|----------------------|----------------|
| **PREDICTED: Rattus norvegicus Meis homeobox 2 (Meis2), transcript variant X4, mRNA** | 38.2            | 100%     | 0.007   | 100%                 | XM_006234746.3 |
| **PREDICTED: Rattus norvegicus Meis homeobox 2 (Meis2), transcript variant X3, mRNA** | 38.2            | 100%     | 0.007   | 100%                 | XM_006234745.3 |
| **PREDICTED: Rattus norvegicus Meis homeobox 2 (Meis2), transcript variant X2, mRNA** | 38.2            | 100%     | 0.007   | 100%                 | XM_006234744.3 |
| **PREDICTED: Rattus norvegicus Meis homeobox 2 (Meis2), transcript variant X1, mRNA** | 38.2            | 100%     | 0.007   | 100%                 | XM_006234743.3 |
| **Rattus norvegicus Meis homeobox 2 (Meis2), mRNA** | 38.2            | 100%     | 0.007   | 100%                 | NM_001107758.1 |
| **Rattus norvegicus serine/arginine repetitive matrix 2 (Srrm2), mRNA** | 28.2            | 73%      | 7.1     | 100%                 | NM_001277154.1 |
| **PREDICTED: Rattus norvegicus cytidine monophospho-N-acetylneuraminic acid hydroxylase (Cmah), transcript variant X7, mRNA** | 26.3            | 68%      | 28      | 100%                 | XM_017600609.1 |
| **PREDICTED: Rattus norvegicus cytidine monophospho-N-acetylneuraminic acid hydroxylase (Cmah), transcript variant X6, misc_RNA** | 26.3            | 68%      | 28      | 100%                 | XR_001841737.1 |
Table S5. List of antibodies used in the study.

| Antibody                                      | Catalog # | Company                      |
|-----------------------------------------------|-----------|------------------------------|
| Rabbit anti-β-actin                          | A1978     | Sigma-Aldrich                |
| Rabbit anti-GAPDH                            | G9545     | Sigma-Aldrich                |
| Rabbit anti-aurora B                         | A5102     | Sigma-Aldrich                |
| Mouse anti- cardiac troponin-T                | MA5-12960 | Thermo Scientific            |
| Rabbit-anti-Rb1                              | 10048-2-Ig| Proteintech                  |
| Rabbit anti-cardiac troponin-I               | sc-15368  | Santa Cruz Biotechnology     |
| Goat-anti-Meis1/2                            | Sc-10599  | Santa Cruz Biotechnology     |
| Rabbit anti-VEGF                             | sc-152    | Santa Cruz Biotechnology     |
| Rabbit-anti-Bax                              | 2772S     | Cell Signaling Technology    |
| Rabbit anti-β-catenin                        | 9562S     | Cell Signaling Technology    |
| Rabbit anti-p16                              | ab51243   | Abcam                        |
| Mouse-anti-Histone H3                        | Ab6002    | Abcam                        |
| Mouse anti-Ki67                              | 550609    | BD Pharmingen                |
| ECL anti-mouse-HRP                           | NA931V    | GE Healthcare                |
| ECL anti-rabbit-HRP                          | NA9340V   | GE Healthcare                |
| Goat anti-mouse Alexa fluor 488              | A11029    | Life Technologies            |
| Goat anti-mouse Alexa fluor 594              | A11005    | Life Technologies            |
| Goat anti-rabbit Alexa fluor 488             | A11008    | Life Technologies            |
| Goat anti-rabbit Alexa fluor 594             | A11037    | Life Technologies            |
Table S6. RT PCR primer sequences.

| Gene     | 5’ to 3’               |
|----------|------------------------|
| Rb1      | Forward: GTCTGCCAACAACCCACAAACAA  |
| Rb1      | Reverse: ATCCTTCGATGTCAAAGCGC  |
| Meis2    | Forward: TGATAACTTCTGCCACCGGT |
| Meis2    | Reverse: GGTGGCATCATCGTGGTCTC |
| Aurora B | Forward: CAGGGAGAGCTGAAGATTGC |
| Aurora B | Reverse: ACTGTGGCTAGGGCTCTCAA |
| Cyclin D1| Forward: CCTGGACCGTTTCTTGTC  |
| Cyclin D1| Reverse: CCATTGAGCCTGTTTACCA |
| E2F2     | Forward: GGCAGACAGTCTACCAAGG |
| E2F2     | Reverse: CAAGGGGACAAGGGATGGTG |
| E2F3     | Forward: CGAGAGTGCCATCAGTACC |
| E2F3     | Reverse: ACTCTTGGTGAGCAGACCG |
| VEGF     | Forward: CTCTCTCCGAGTGACGGT |
| VEGF     | Reverse: CTCTCTCCGAGTGACGGT |
| IL6      | Forward: CCCTCTCCTCCTCAGTACCA |
| IL6      | Reverse: TCTGACAGTGATCATCGCT |
| β-actin  | Forward: ACCCTAAGGCCAACCGTGAAA |
| β-actin  | Reverse: GTAGCAGAGGCTACAGG |
A day wise analysis demonstrates the 59.58±2.38% ACM survival on day 7 after the Lipofectamine 2000 treatment. N= 3 rats, n= 6 experimental replicates each.
Figure S2. Transfection efficacy.

Representative immunostaining images following Dy546 labeled siRNA-cel-67 transfection of ACM. 69.12% of ACMs were positive for Dy546-siR-cel-67 at 24hrs following transfection, whereas, 34.10% ACM were positive for Dy546-siR-cel-67 on day 7. Panels in white rectangles represent respective enlarged sections. Bar graph represents the percent of ACM, transfected with Dy546-siR-cel-67. N= 3 rats, n= 4 experimental replicates each, and n= 3 images each. DAPI= 4′,6-diamidino-2-phenylindole. Scale bar=200 µm.
Figure S3. Transfection efficacy following siUbc transfection.

Representative bright field images at different time points for siUbc transfection of ACMs. Bar graph represents the percent of live ACM, after siUbc transfection at different time points. Only 20% ACMs survive at 72hrs post siUbc transfection. *= p-value ≤0.05. P value ≤0.05 was considered statistically significant. N= 6 rats, n= 8 experimental replicates each, and n= 10 images each. Scale bar=200 µm.
Figure S4. Simultaneous inhibition of Rb1 and Meis2 is necessary for ACM proliferation.

(a) Quantification of DNA synthesis marker, EdU incorporated ACMs on Day 6 after siRNA transfection as depicted. siRNA-cocktail transfection shows superior induction of ACM proliferation compared to the individual Rb1 or Meis2 knockdown approach. (b) Quantification of EdU incorporated ACMs transfected with all combinations of three independent sets of siRNAs against each Rb1 and Meis2. Rb1(V1)+Meis2(V1) is referred to as “siRNA-cocktail” in this manuscript gave the highest amount of induced ACM proliferation. All the experiments were performed using ACM, isolated from rats (~12 weeks old). N= 3 rats, n= 6 experimental replicates each. EdU= 5-ethynyl-2'-deoxyuridine, V= version. *=p value ≤0.05; #= p value=0.054; $=p value >0.05.
Figure S5. Nucleation analysis of ACM.

Time course nucleation analysis reveals the state of nuclei in ACM during the course of experiments. It further demonstrates (a) significant increase in number of mono-nuclear ACM, and (b) significant decrease in number of bi-nucleated ACM in siRNA-cocktail transfected group on and after day 6 when compared to control, whereas we did not find any difference in number of multi-nucleated ACM between siRb1+siMeis2 transfected group and control (c). N= 3 rats and n= 8 experimental replicates. *= p-value ≤0.05. P value ≤0.05 was considered statistically significant.
Figure S6. Time course of ACM proliferation upon simultaneous inhibition of *Rb1* and *Meis2*.

(a,b) Immunostaining shows induction of ACM proliferation on Day 6 after siRNA-cocktail transfection. ACMs were marked by Troponin (green), DNA synthesis was marked by EdU (red), whereas, nuclei were marked by DAPI (blue). All the experiments were performed using ACM, isolated from rats (~12 weeks old). N= 3 rats, n= 8 experimental replicates each, and n= 10 images each. Scale bar=100 µm. EdU= 5-ethynyl-2’-deoxyuridine. * = p-value ≤0.05. *P* value ≤0.05 was considered statistically significant.
Figure S7. Simultaneous inhibition of Rb1 and Meis2 leads to ACM cell cycle progression.

Immunostaining shows co-localization of cardiac-specific nuclear marker Nkx2.5 and EdU in ACM in the siRNA-cocktail treated group. 26.05±1.28% of ACM were positive for both, EdU as well as Nkx2.5 in siRNA-cocktail transfected groups. White arrows indicate the co-localization of EdU with Nkx2.5 in mono-nucleated ACM. Yellow arrows indicate the ACM with Nkx2.5 without EdU. Arrowheads are indicating the EdU positive noncardiomyocytes, which are not showing the Nkx2.5. N= 3 rats and n= 5 experimental replicates each. TnI= Troponin I, EdU= 5-ethynyl-2’-deoxyuridine, DAPI= 4’,6-diamidino-2-phenylindole, NKx2.5= NK2 Homeobox 5. Scale bar=100 µm.
Figure S8. Simultaneous inhibition of *Rb1* and *Meis2* leads to ACM mitosis.

Immunostaining shows co-localization of mitosis marker (PH3; red) and DNA synthesis marker (EdU; far red) in TnI labeled ACMs from the siRNA- cocktail transfected groups. Arrows indicate the co-localization of Edu and PH3 in mono-nucleated ACM, whereas, the arrowhead indicates the bi-nucleated ACM, which shows EdU but not the PH3. N= 3 rats and n= 6 experimental replicates each. TnI= Troponin I, EdU= 5-ethyl-2'-deoxyuridine, DAPI= 4',6-diamidino-2-phenylindole, PH3= phosphor histone 3. Scale bar=100 µm.
Figure S9. Simultaneous inhibition of Rb1 and Meis2 improves ACM survivability.

(a,b) Immunostaining shows a significantly lower TUNEL positive ACMs after day 3 in the siRNA-cocktail transfected group when compared to control. ACMs were marked by Troponin (green), cell survivability was analyzed through TUNEL assay (red), whereas, nuclei were marked by DAPI (blue). Arrows indicate the TUNEL $^+$ ACMs. Panels in white rectangles represent respective enlarged sections. (c) Immunoblot for apoptotic marker Bax from the cell lysate of ACMs, per depicted groups. (d) The densitometric analysis shows a significant downregulation in Bax expression in the siRNA-cocktail transfected group compared to control. (e) TUNEL assay for Individual Rb1 or Meis2 knockdowns compared to siRNA-cocktail. All the experiments were performed in triplicate using ACMs, isolated from rat (~12 weeks old). N= 3 rats, n= 3 experimental replicates each, and n= 10 images each. TnI= Troponin I, TUNEL= Terminal deoxynucleotidyl transferase dUTP nick end labeling, DAPI= 4',6-diamidino-2-phenylindole. Scale bar=100 µm *= p-value ≤0.05. $P$ value ≤0.05 was considered statistically significant.
Figure S10. Expression analysis of cell cycle associated genes in ACMs after simultaneous inhibition of \textit{Rb1} and \textit{Meis2}.

(a) RT-PCR expression analysis is showing a significant increase in the expression of E2F2, E2F3, Cyclin D1, and Aurora B; whereas, \textit{Rb1}, \textit{Meis2}, and IL6 are down-regulated in the siRNA-cocktail transfected group when compared to control. (b) Representative immunoblots for β-catenin and p16 expression. Immunoblotting was performed with cell lysate from ACMs, transfected with siRNA-cocktail and control. (c,d) Densitometric analysis showed regulation in the expression of cell cycle regulators in the siRNA-cocktail transfected group in comparison to control. All the experiments were performed in triplicate using ACMs, isolated from adult rats (~12 weeks old). N= 3 rats, n= 3 experimental replicates each, and n= 10 images each. Results are presented as mean±SEM; * = \(p\)-value ≤0.05. \(P\) value ≤0.05 was considered statistically significant.
Figure S11. Improved ACMs survivability after hydrogel mediated delivery of \textit{siRb1} and \textit{siMeis2} in adult animals post-MI.

(a,b) Immunostaining images are showing a significant decrease in TUNEL positive ACMs in the siRNA-cocktail treated group versus controls. Panels in yellow rectangles represent respective enlarged sections. N= 4 rats per group, n= 5 non-serial sections were imaged each, and n= 5 separate regions quantified each. Scale bar=50 µm. WGA= Wheat germ agglutinin, TUNEL= Terminal deoxynucleotidyl transferase dUTP nick end labeling, DAPI= 4',6-diamidino-2-phenylindole. Results are presented as mean±SEM; * = \textit{p}-value ≤0.05. \textit{P} value ≤0.05 was considered statistically significant.
Figure S12. Expression of cell cycle associated genes after hydrogel mediated delivery of siRb1 and siMeis2 in adult animals post-MI.

(a,b) RT-PCR analysis shows an increased expression of E2F2, E2F3, Aurora B, and VEGF; whereas, Rb1, Meis2, and IL6 are down-regulated in the siRNA-cocktail group compared to controls. RT-PCR was performed with total RNA isolated from heart tissues of adult rats from different groups (~12 weeks old). N= 4 rats per group and n= 6 experimental replicates each. Expression analysis was performed on day 21 after MI. Results are presented as mean±SEM; * = p-value ≤0.05. P value ≤0.05 was considered statistically significant.
Figure S13. Western blot analysis of cell cycle associated genes after hydrogel mediated delivery of siRb1 and siMeis2 in adult animals post-MI.

(a) Representative immunoblots for Rb1, Meis2, p16, and VEGF. Immunoblotting was performed using tissue lysates, from heart tissues of adult rats from different groups (~12 weeks old, N= 4 rats per group, and n= 3 experimental replicates each). (b,c) Densitometry analysis showed differential expression of proteins among the three groups. N= 3 rats per group and n= 3 experimental replicates each. Expression analysis was performed on day 21 after MI. Results are presented as mean±SEM; * = p-value ≤0.05. P value ≤0.05 was considered statistically significant.
Figure S14. Effects of *siRb1* and *siMeis2* knockdown on Endothelial and Fibroblast cells.

(a) Quantification of antibody labeling against a cellular marker for proliferation, Ki67, within cardiac fibroblasts showing no difference of increased proliferation on Day 6 after siRNA transfection as depicted. (b,c) Similar results were obtained when using either isolated cardiac endothelial cells (b) or HUVEC cells (c). (d) siRNA-cocktail transfection of NRCMs results in an enhancement of endogenous proliferation rate, showing a selective effect of Rb1 and Meis2 on the control of ACM senescence. N= 6 rats, n= 8 experimental replicates each. HUVEC= Human Umbilical Vein Endothelial Cells. *= p-value ≤0.05.
Figure S15. Cardiac function analysis at baseline with hydrogel injection.

Bar graph shows no significant difference in ejection fraction, fractional shortening, and cardiac output between the hydrogel alone intramyocardial injected group and the PBS injected control group at day 21. N= 6 rats per group. EF= ejection fraction, FS= fractional shortening, CO= cardiac output. #= p-value ≥0.05. P value ≤0.05 was considered statistically significant. EF; ejection fraction: FS; fractional shortening: CO; and cardiac output.
Figure S16. Cardiac function analysis with strain echocardiography after injury.

(a) A schematics overview of six segments of the myocardial wall, highlighting the segments, anticipated to be at the infarcted area. (b) Representative echocardiographic image for radial and longitudinal strain after MI in hydrogel-siRNA-cocktail treated groups. (c) The bar graph shows a significantly improved Radial velocity in hydrogel-siRNA-cocktail treated groups versus controls at day 21 post-MI. N= 6 rats per group. NT= no treatment. *= p-value ≤0.05. P value ≤0.05 was considered statistically significant.
Figure S17. Real-time imaging of proliferating ACMs after \textit{Rb1} and \textit{Meis2} knockdown.

Bright field images captured with live cell imaging with Incucyte® Live Cell Analysis System. Each row shows a cytokinesis event captured between days 3 and 6 post siRNA-cocktail transfection. Red arrows are showing the appearance of dividing ACMs. Yellow arrowheads are pointing at a non-dividing cell. \(N=3\) rats, \(n=8\) experimental replicates each (~12 weeks old). Scale bars=200 µm.
Figure S18. Homology analysis of siRb1 with rat genome.

(a) Homology alignment for siRb1 with rat genome (b) demonstrate the specific binding of siRNA with rat Rb1 gene.
Figure S19. Homology analysis of siMeis2 with rat genome.

(a) Homology alignment for siMeis2 with rat genome (b) demonstrate the specific binding of siRNA with rat Meis2 gene.
Supplemental Video Legend:

**Video S1.** Live cell video of a beating ACM on day 7. ACMs retain their physiological characteristics up until day 7, as shown by beating ACM. N= 3 rats. Best viewed with Windows Media Player.