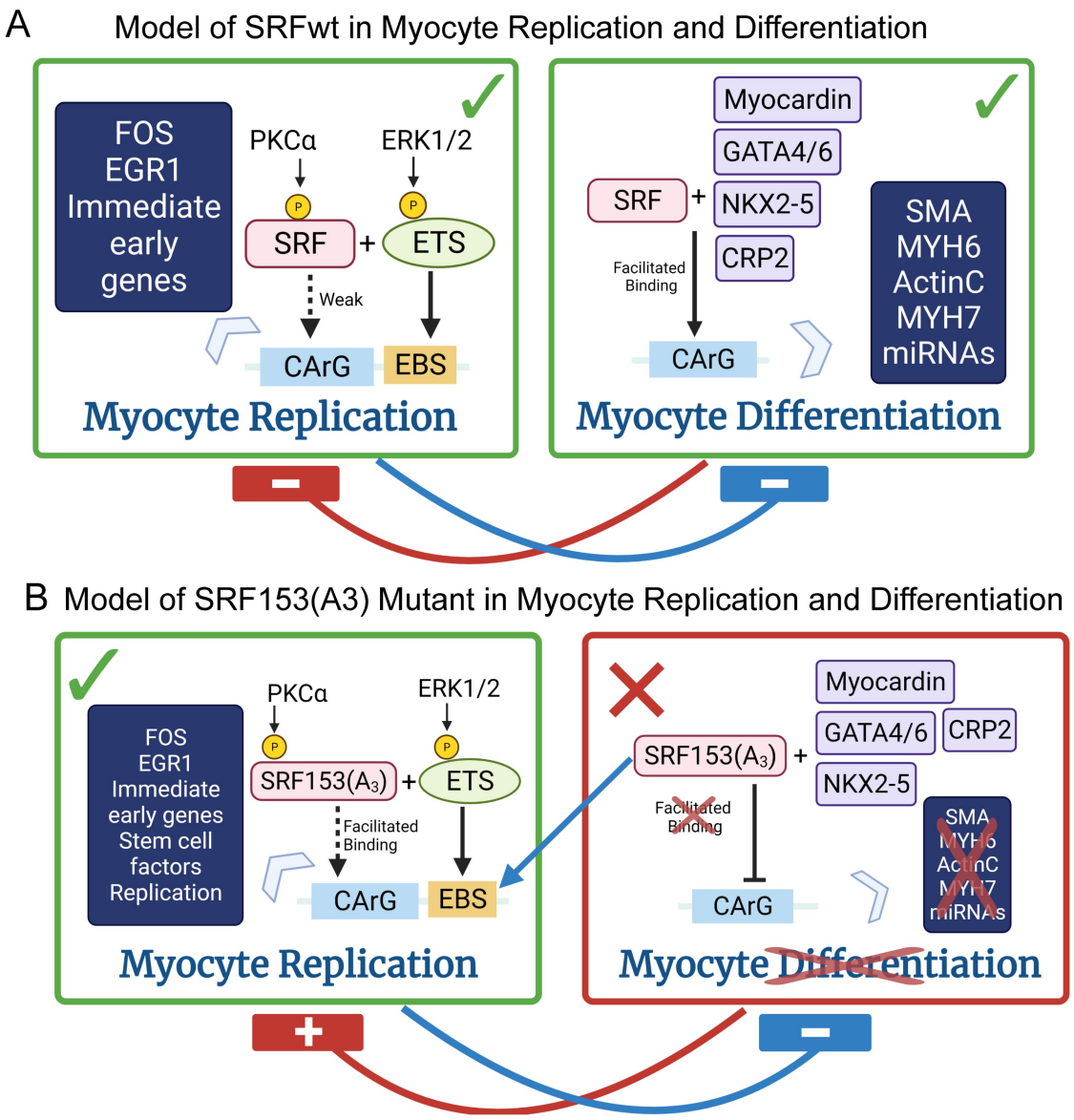
Mutant SRF and YAP synthetic modified mRNAs drive cardiomyocyte nuclear replication

Siyu Xiao, Rui Liang, Azeez B. Muili, Xuanye Cao,
Stephen Navran, Robert J. Schwartz and Dinakar Iyer

Supplemental Figure Legends

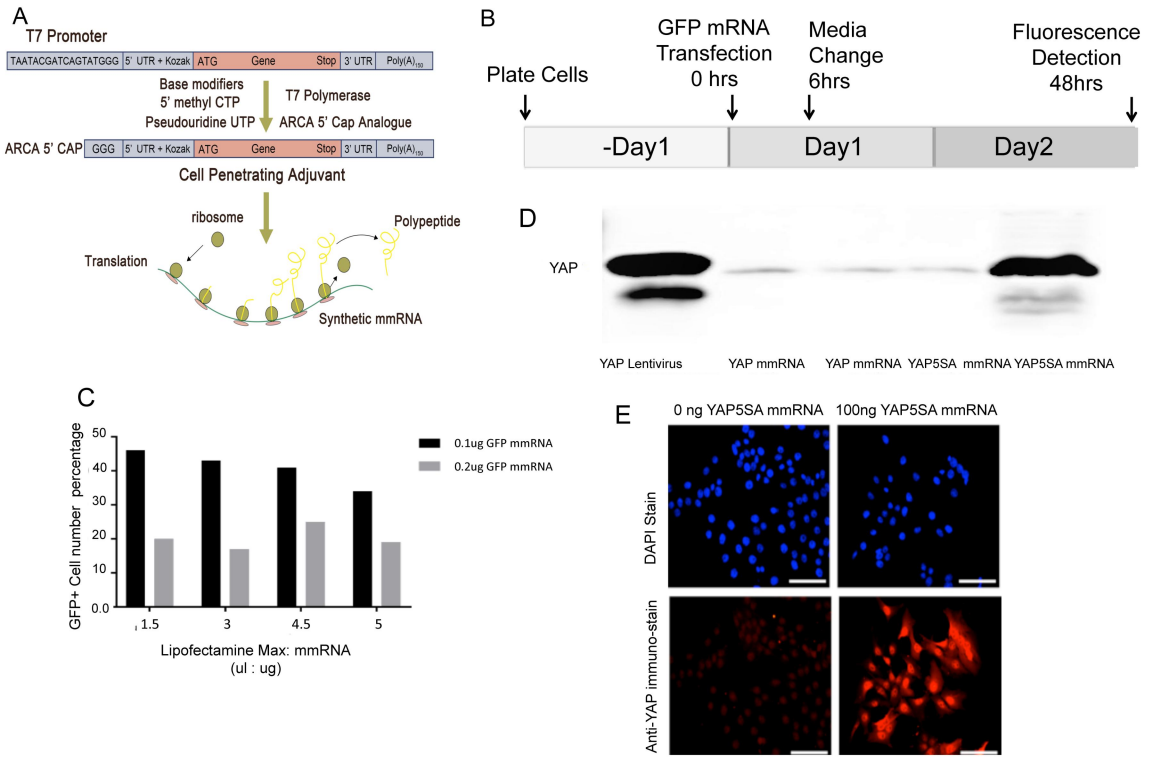


Supplemental Figure 1. Molecular models of the wildtype SRF and SRF 153(A3) mutant roles in cardiac replication and differentiation. Diagrams show the mutual inhibitory activity shared between myocyte replication and myocyte differentiation.

A. Schematic diagram shows how phosphorylated ETS factors facilitate phosphorylated SRF binding to the c-fos promoter[5,11] to propel cardiomyocyte replication, while Nkx2.5 and Gata4 facilitate SRF binding to dependent cardiac actin gene promoters[8,9] to drive cardiomyocyte differentiation.

B. Schematic model of SRF mutant SRF153(A3) supports cardiac myocyte replication, because Elk1 stabilized SRF153(A3) binding to c-fos promoter due to an adjacent ETS binding sequence (EBS). SRF153(A3) blocks cardiac differentiation, because Nkx2.5 and

Gata4 cofactors fail to facilitate SRF(153A3) DNA binding to the cardiac sarcomeric actin gene.



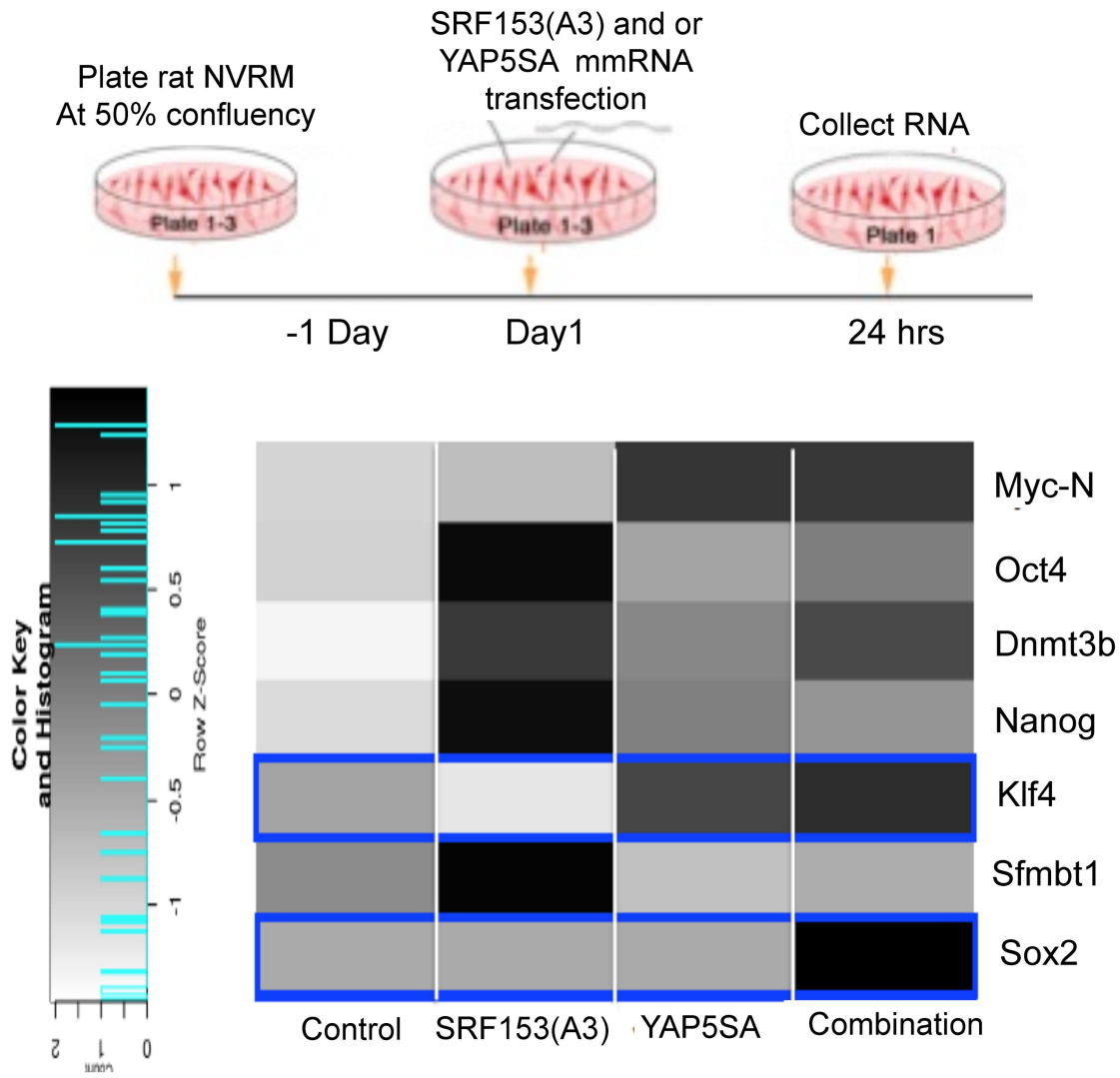
Supplemental Figure 2. Synthetic mmRNA transfection optimized in NIH 3T3 fibroblasts.

A. Schematic drawing for generating synthetic mmRNA.

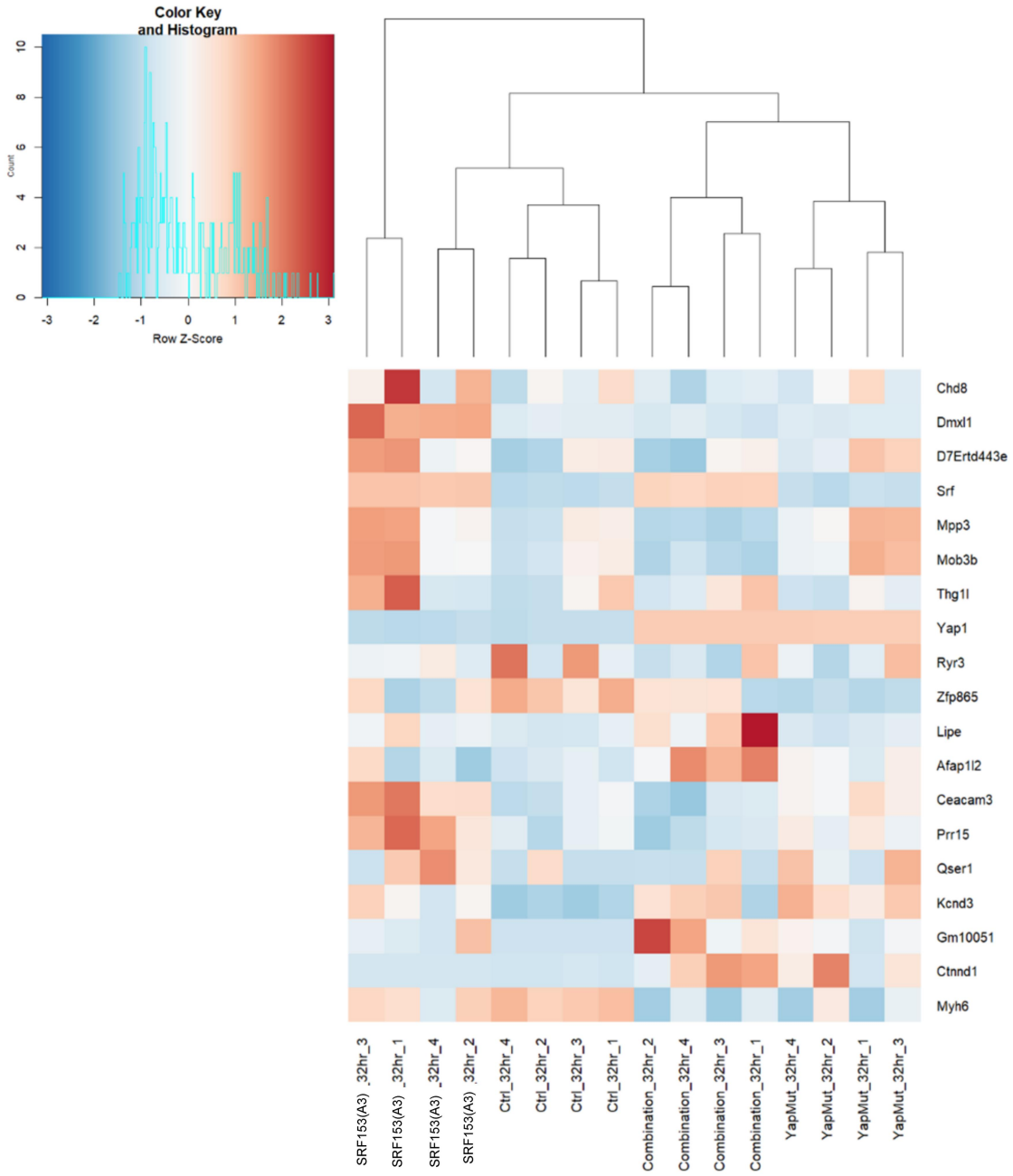
B. Schematic diagram of mRNA transfection efficiency assay protocol shows a pre-transfection period of 24 hours, a 6 hour transfection period, change of media, and analysis by YAP antibody staining after 48 hours.

C. To determine the optimal dose for GFP mRNA transfections in NIH 3T3 fibroblasts transfection efficiency was assayed in 96 well plates. The ratio of RNA (0.1 µg or 0.2 µg) was titrated with increasing doses of Lipofectamine Max (µl), as follows: GFP mRNA was transfected into fibroblasts with Lipofectamine MessengerMAX reagent; GFP was detected with fluorescent microscopy two days after transfection; GFP marked cells were quantified with Image J software; and the transfection efficiency was calculated by GFP+ cell number/DAPI cell number. The optimal condition for mRNA transfection was 0.1µg mmRNA in 200µL culture media with a Lipo:mRNA ratio of 1.5 resulted in a transfection efficiency of 45%.

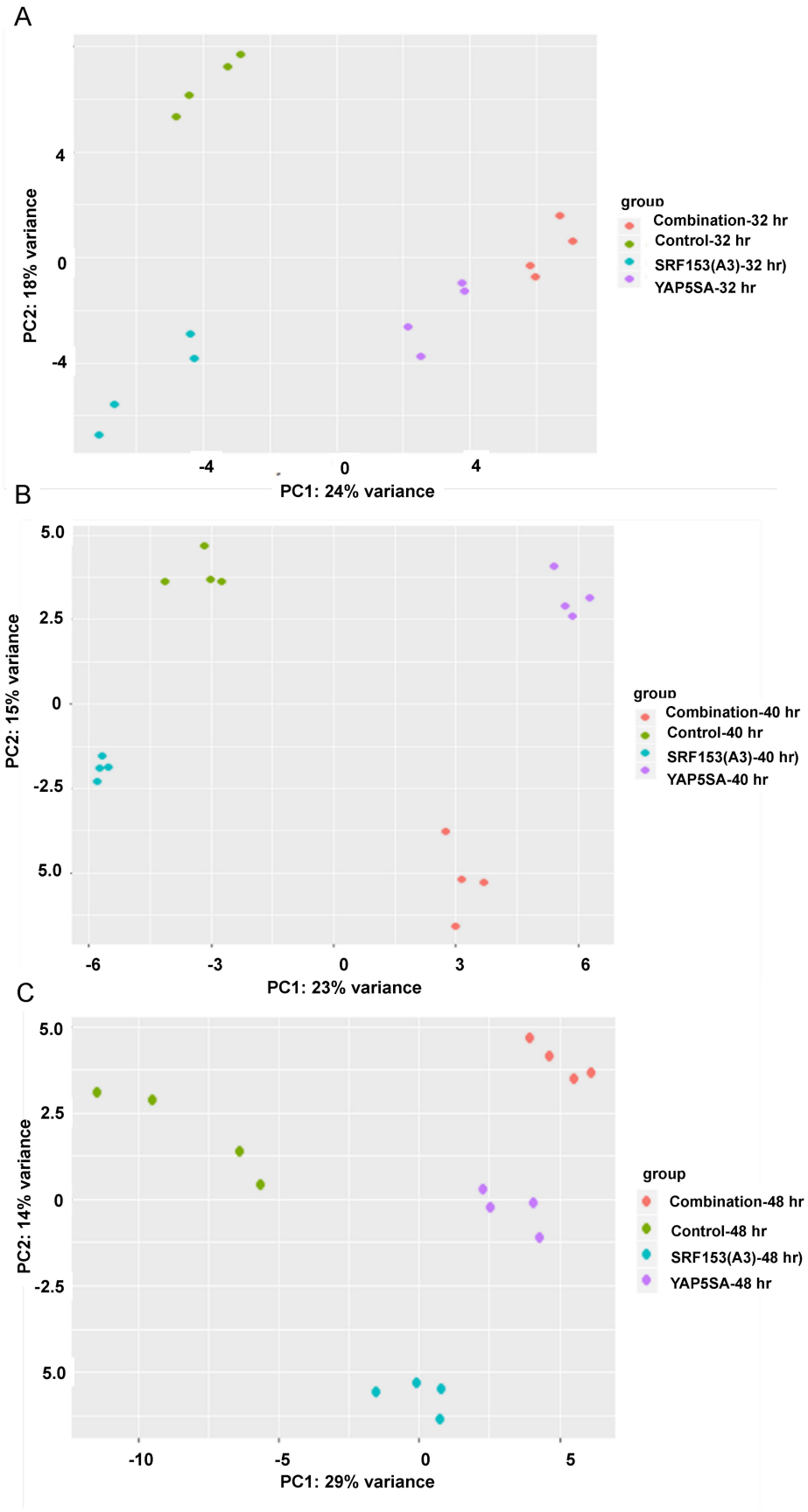
D. Synthetic wildtype YAP and mutant YAP 5SA mmRNA were tested for cellular translation by transfection into NIH3T3 cells and cellular lysates were electrophoresed in SDS-PAGE, blotted onto nitrocellulose membrane and then probed with YAP antibodies. Expression of YAP5SA mmRNA was observed by immunofluorescent staining in NIH3T3 cells, note overlap with DAPI stained nuclei. Control received only transfection reagents.



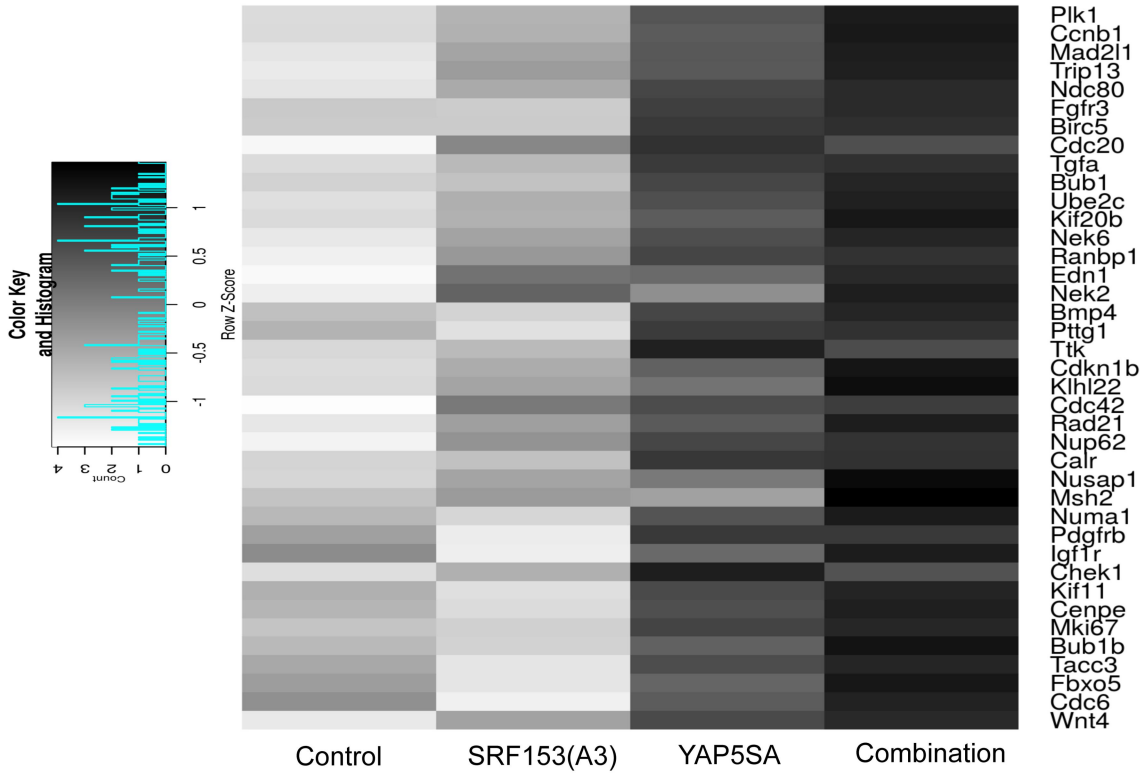
Supplemental Figure 3 SRF153(A3) mutant and YAP5SA mutant mmRNA induce stem cell gene activity NRVM. Schematic protocol of transfected synthetic SRF153 (A3) and YAP5SA mmRNA and or in combination into NRVM to test for stem cell gene expression assayed by RNA-seq. Heat maps show expression measured on a gray scale. Blue line demarcates evidence of synergy especially for Sox2 lesser so for KLf4.



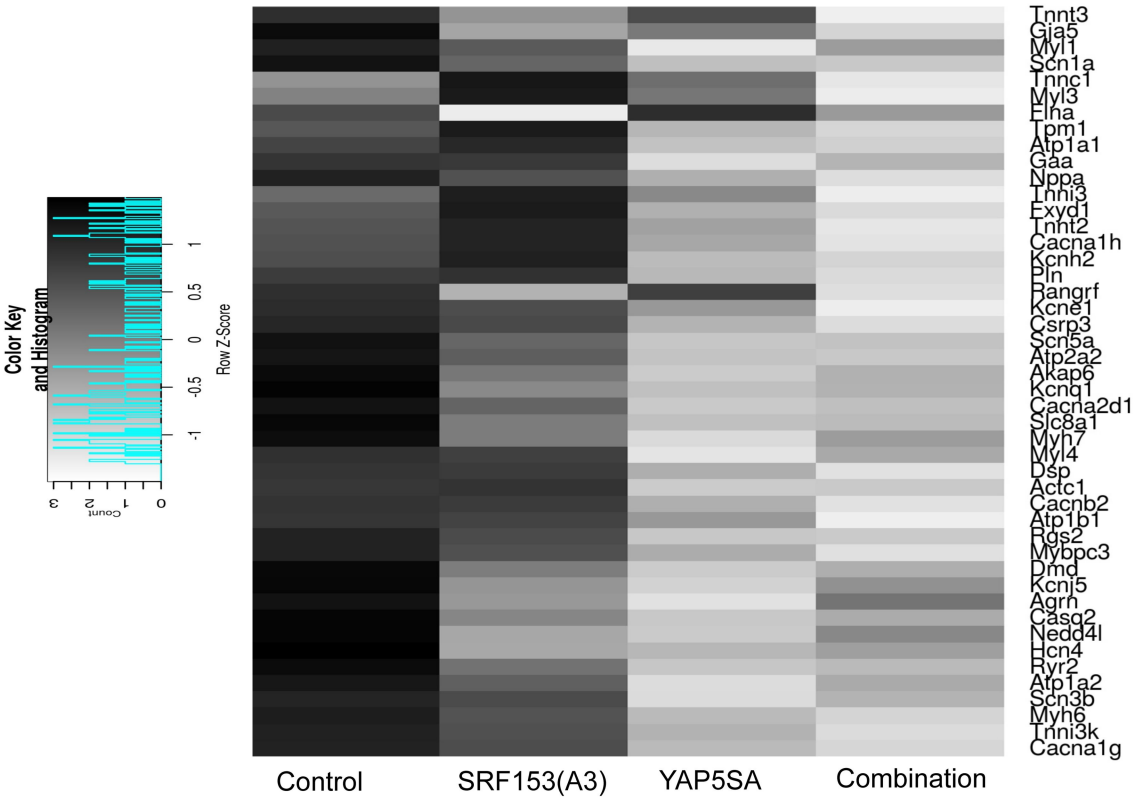
Supplemental Figure 4. Heat map of the top 20 regulated genes in 32 hours post-transfection groups of NRVM. Among the top 20 regulated genes, *Srf* and *Yap1* were increased 11-fold and 64-fold respectively in the combination treated group, with the top 2 lowest p-value respectively; thus, indicating that our mmRNA-based transfection system expressed well in NRVMs.


A
PC2: 18% variance
PC1: 24% variance
group
Combination-32 hr
Control-32 hr
SRF153(A3)-32 hr)
YAP5SA-32 hr
B
PC2: 15% variance
PC1: 23% variance
group
Combination-40 hr
Control-40 hr
SRF153(A3)-40 hr)
YAP5SA-40 hr
C
PC2: 14% variance
PC1: 29% variance
group
Combination-48 hr
Control-48 hr
SRF153(A3)-48 hr)
YAP5SA-48 hr

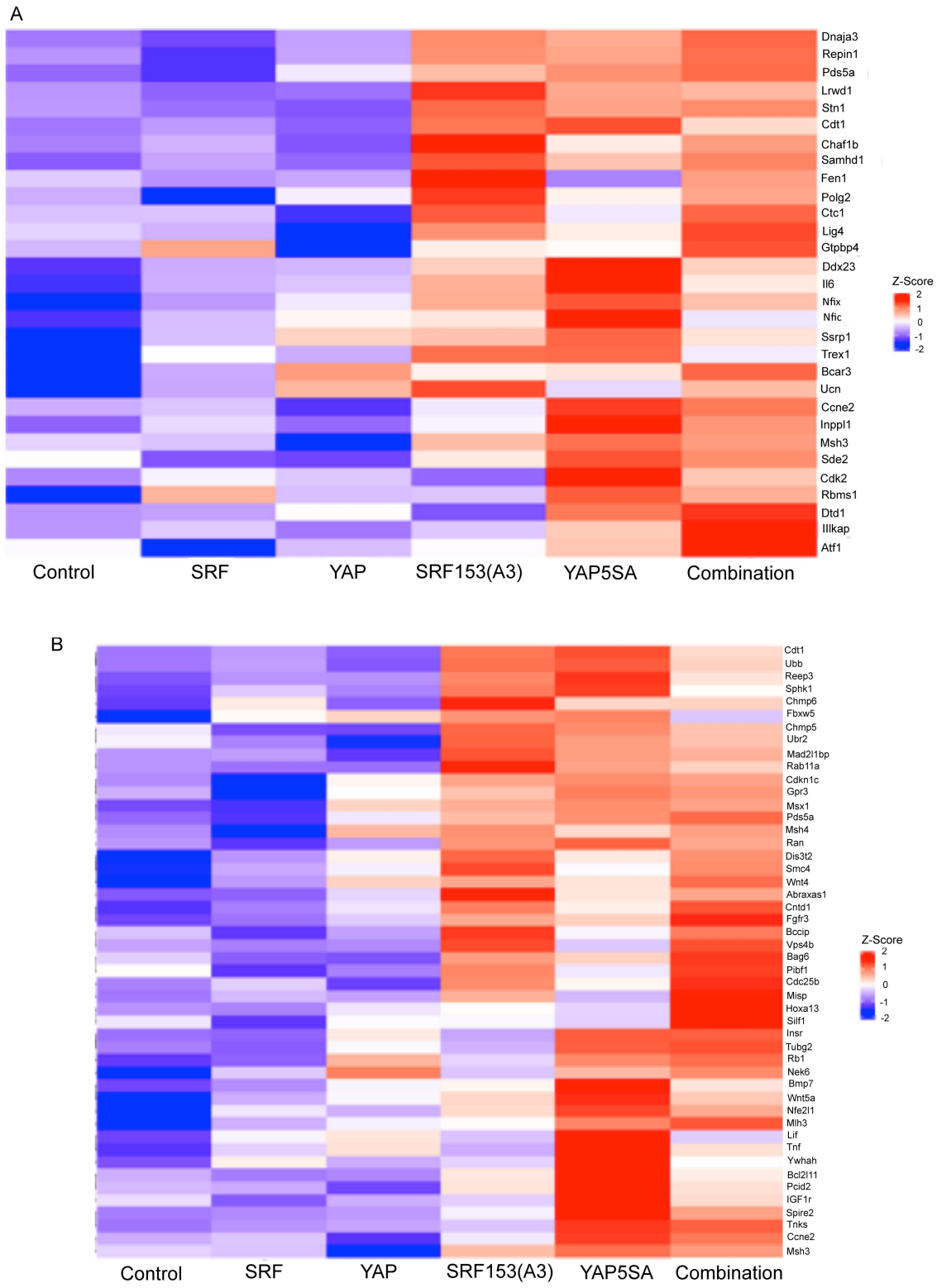
Supplemental Figure 5. Graphical presentations of the principal component analysis (PCA) plot the distance of samples under Ctrl, SRF153(A3), YAP5SA and combination treatment based on the normalized expression values using DESeq2. These PCA plots demonstrate well-separated clusters at all three time points based on the level of gene expression A. PCA plot of 32 hours post-transfection groups. B PCA plot of 40 hours post-transfection groups. C. PCA plot of 48 hours post-transfection groups.



Supplemental Figure 6. Heat map showed nuclear division (GO:0000280) genes generated from RNA-seq data at 48 hours after transfection of NRVM. Upregulation of crucial genes such as CCNB1 and CDC20 suggests that SRF153(A3) and YAP5SA promote nuclear division to foster cardiomyocyte division.



Supplemental Figure 7. Heat map showed heart contraction (GO:0060047) genes generated from RNA-seq data at 48 hours after transfection of NRVM. Down regulation of crucial genes in heart contraction such as TNNT2 and TNNI3 suggests that cardiomyocytes are brought back to a less differentiated state.

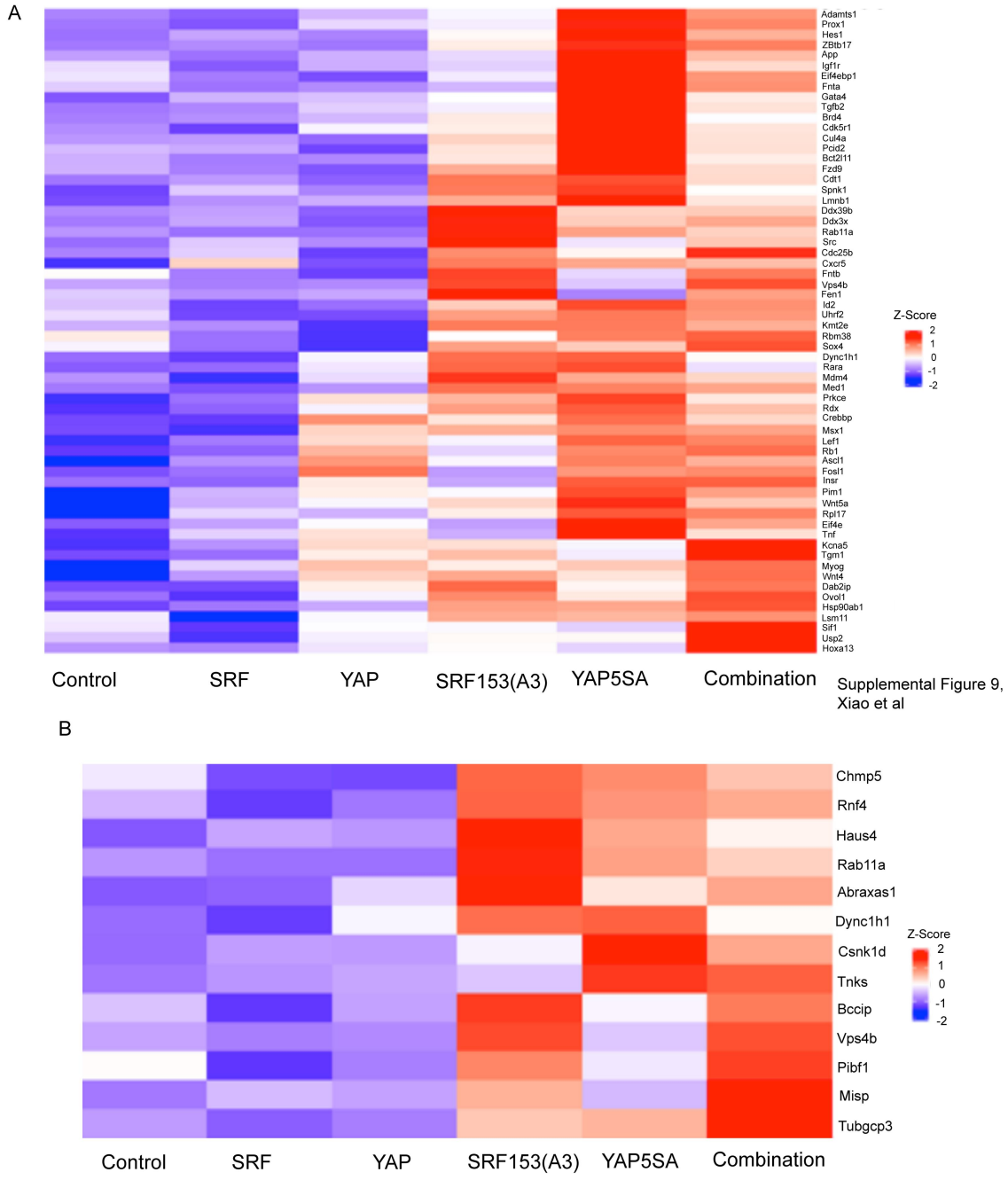


Supplemental Figure 8. Heat maps of DNA replication (GO:0006260) genes and nuclear division (GO: 0000280) genes. Heat maps were plotted using ATAC-seq signal intensity score of ATAC-seq signal intensity for each gene using the peak signal within the 2 kb

regions surrounding the TSS in each sample ATAC-seq signal intensity score.

A. Heat map of DNA replication (GO:0006260) genes.

B. Heat map of nuclear division (GO: 0000280) genes.

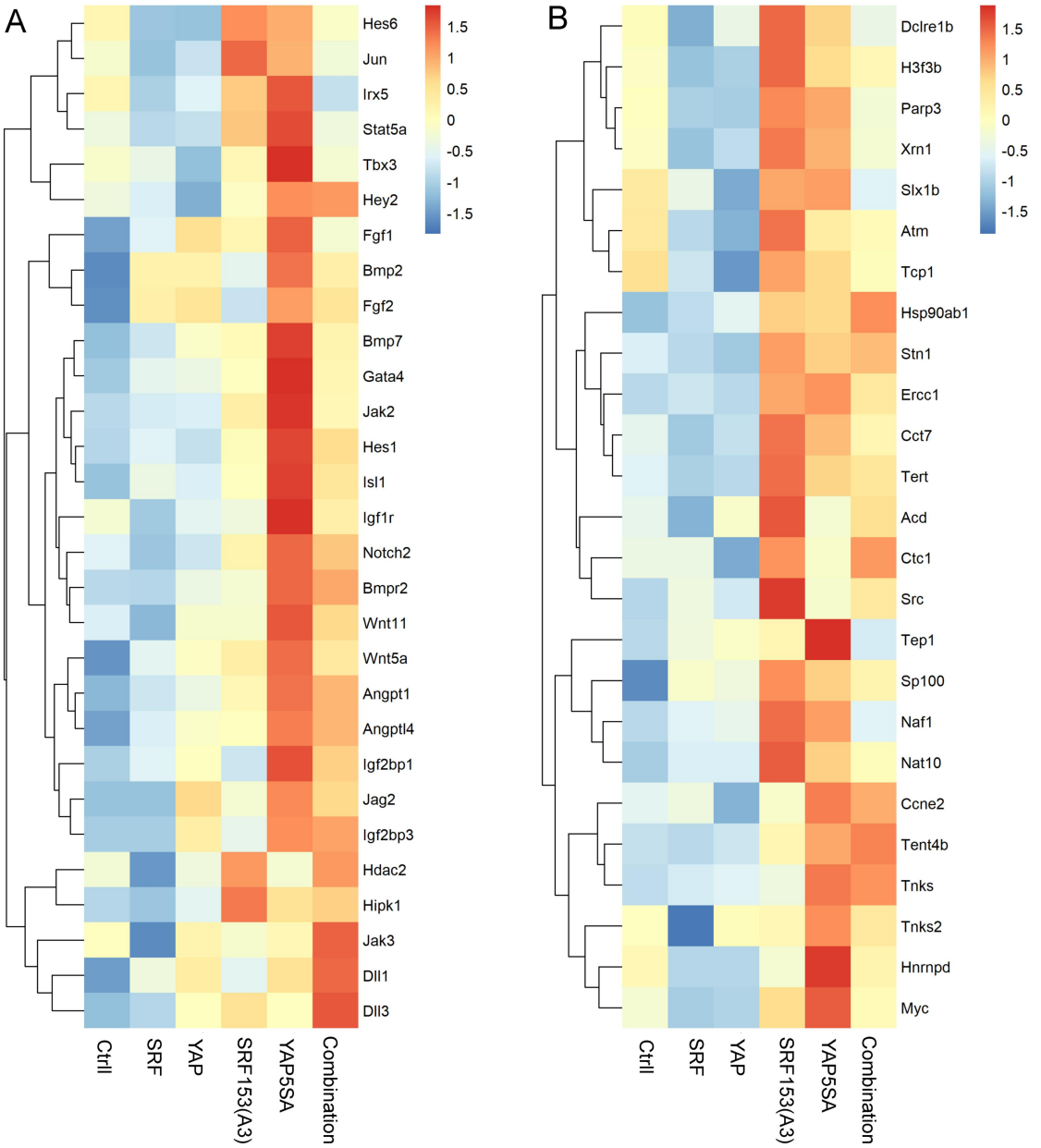


Supplemental Figure 9. Heat maps of ATAC-seq signal intensity of the genes involved in Positive regulation of cell cycle (GO:0045787) and Spindle Assembly

(GO:0051225). Some of the genes in both groups were already accessible under SRF153(A3) or YAP5SA treatment. Combination treatment of SRF153(A3) and YAP5SA further increased accessibility at cell-cycle genes and spindle assembly genes.

A. Heat map of genes involved in Positive regulation of cell cycle (GO:0045787).

B. Heat map of Spindle Assembly (GO:0051225) genes.



Supplemental Figure 10. Heat maps of ATAC-seq signal intensity of the genes involved with growth factors and telomerase maintenance genes. Some of the genes in both groups were already accessible under SRF153(A3) or YAP5SA treatment.

A. YAP5SA further increased signal intensity and accessibility at growth factor genes.

B. SRF153(A3) further increased signal intensity and accessibility of telomerase maintenance genes.