Supplemental Material, Lin et al.

Cardiac specific YAP activation post myocardium infarction improved cardiac function and mouse survival

A. Detailed Materials and Methods.

Animal experiments

All animal procedures were approved by the Institutional Animal Care and Use Committee. TetO-YAP[1], Rosa26$^{fs\text{-}rtTA2}$, and MHCα–Cre[3] alleles were previously described. Dox was administered at 1 mg/mL in drinking water. 5-ethynyl-2'deoxyuridine (EdU) was administered at 5 μg/g body weight IP. To induce MI, mice aged 8 weeks were subjected to LAD ligation as described previously.[4]

Echocardiography was performed on a VisualSonics Vevo 2100 with Vevostrain software. Magnetic resonance imaging (MRI) was carried out at Small Animal Imaging Core in Beth Israel Hospital. For in vivo bioluminescent imaging, mice were administered Luciferin (150 μg/gram body weight IP), sedated using isoflurane, and imaged 10 min later on a Xenogen IVIS System.

MI, echocardiography, and MRI were performed blinded to genotype and treatment group.

Immunohistochemistry.

Hearts were fixed and embedded in OCT. 8 μm cryosections were used for immunostaining, using antibodies listed in Online Table 1. EdU was detected with the Click-iT EdU imaging kit (Invitrogen). TUNEL staining was performed using the Roche in situ death detection kit. Imaging was performed on a Fluoview 1000 confocal microscope, or a Nikon TE2000 epifluorescent microscope.

AAV9 packaging

3Flag-hYAP and Luciferase were separately cloned into ITR-containing AAV plasmid (Penn Vector Core P1967) harboring the chicken cardiac TNT promoter, to get pAAV.cTnT::3Flag-hYAP and pAAV.cTnT::Luciferase, respectively. AAV9 was packaged in 293T cells with AAV9:Rep-Cap and pHelper (pAd deltaF6, Penn Vector Core) and purified and concentrated by gradient centrifugation[5]. AAV9 titer was determined by quantitative PCR.

Gene Expression

Real time PCR was performed with Syber Green or Taqman detection using an ABI 7500 thermocycler. PCR primers are listed in Online Table II. Expression profiling of total RNA from heart apex was performed using Affymetrix 2.0 ST microarrays. The microarray data is available at GSE54612 and tabulated in Online Table III. GO term analysis used DAVID.[6]

Infarct size measurement

Heart sections were collected at regular intervals. Sections were stained with Sirius Red-Fast Green. Digital images were captured and infarct sizes were calculated according to the formula: [infarct perimeter (epicardial +endocardial)/ total perimeter (epicardial + endocardial)] x 100.[7]

Statistics

Values are expressed as mean ± SEM. For two group comparisons, Student's t-test was used to test for statistical significance. To analyze data containing more than two groups, we used ANOVA with the Tukey HSD post-hoc test. Both tests were performed using JMP 10.0 (SAS).

B. Supplemental References

1. Camargo FD, Gokhale S, Johnnidis JB, Fu D, Bell GW, Jaenisch R, Brummelkamp TR. YAP1 increases organ size and expands undifferentiated progenitor cells. Curr Biol. 2007;17:2054-2060.
2. Belteki G, Haigh J, Kabacs N, Haigh K, Sison K, Costantini F, Whitsett J, Quaggin SE, Nagy A. Conditional and inducible transgene expression in mice through the combinatorial use of Cre-mediated recombination and tetracycline induction. Nucleic Acids Res. 2005;33:e51.
3. Agah R, Frenkel PA, French BA, Michael LH, Overbeek PA, Schneider MD. Gene recombination in postmitotic cells. Targeted expression of Cre recombinase provokes cardiac-restricted, site-specific rearrangement in adult ventricular muscle in vivo. J Clin Invest. 1997;100:169-179.

4. Tarnavski O, McMullen JR, Schinke M, Nie Q, Kong S, Izumo S. Mouse cardiac surgery: comprehensive techniques for the generation of mouse models of human diseases and their application for genomic studies. Physiol Genomics. 2004;16:349-360.
5. Grieger JC, Choi VW, Samulski RJ. Production and characterization of adeno-associated viral vectors. Nat Protoc. 2006;1:1412-1428.
6. Huang da W, Sherman BT, Lempicki RA. Systematic and integrative analysis of large gene lists using DAVID bioinformatics resources. Nat Protoc. 2009;4:44-57.
7. Pasumarthi KB, Nakajima H, Nakajima HO, Soonpaa MH, Field LJ. Targeted expression of cyclin D2 results in cardiomyocyte DNA synthesis and infarct regression in transgenic mice. Circ Res. 2005;96:110-118.

C. Online Tables

Online Table I. Antibodies used in this study

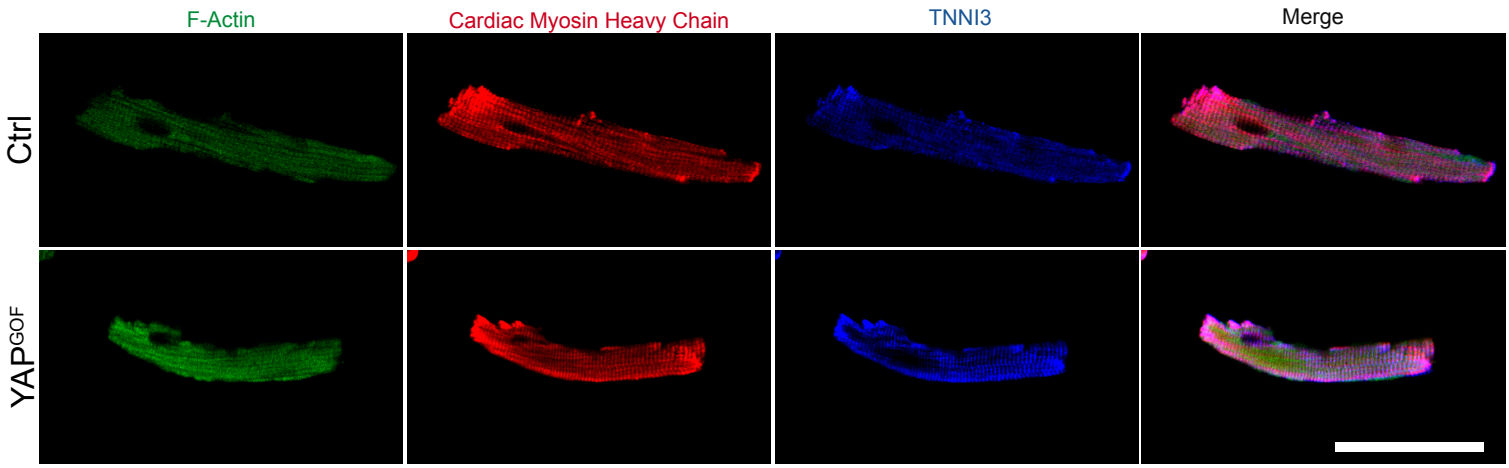
Primary antibodies			
Antigen	**Company**	**Origin**	**Working dilution**
Cardiac troponin I (TNNI3)	Abcam	Goat	1:200 for IF
Flag	Sigma	Rabbit	1:200 for IF
Luciferase	Abcam	Rabbit	1:200 for IF
488 phalloidin	IncPHDG1-A	NA	1:200 for IF
GAPDH	Sigma	Mouse	1:200,000 for WB
Cardiac Myosin Heavy chain	Abcam	Mouse	1:200 for IF
WGA-555	Invitrogen	NA	1:250 for IF
YAP	Sigma	Rabbit	1:1000 for WB
Secondary antibodies			
Anti-Goat Alexa488	Invitrogen	Donkey	1:500 for IF
Anti-Goat Alexa647	Invitrogen	Donkey	1:500 for IF
Anti-Rabbit Alexa555	Invitrogen	Donkey	1:500 for IF
Anti-Rabbit HRP	Invitrogen	Goat	1:10,000 for WB

Online Table II. Primers Used in This Study

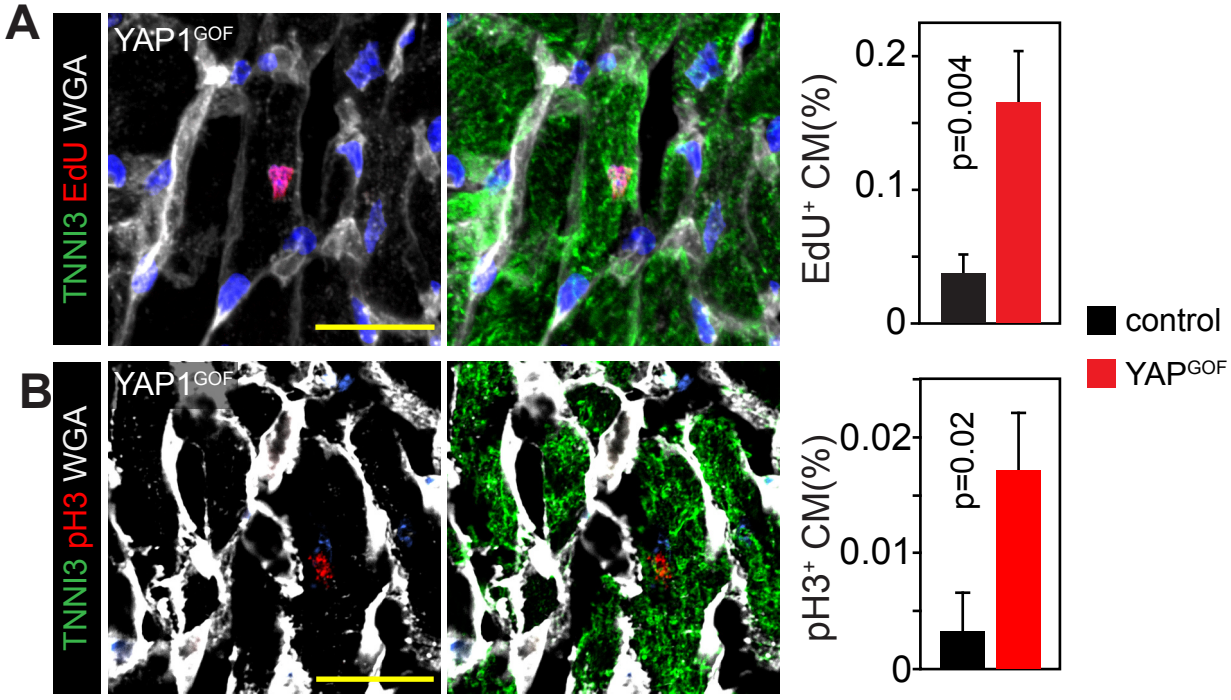
Primers		
Gene*	**Forward**	**Reverse**
m/hYAP	*GACCCTCGTTTTGCCATGA*	*ATTGTTCTCAATTCCTGAGAC*
mCCNA2	GCCTTCACCATTCATGTGGAT	TTGCTCCGGGTAAAGAGACAG
mCDK1	TTTCGGCCTTGCCAGAGCGTT	GTGGAGTAGCGAGCCGAGCC
mCCNB1	AAGGTGCCTGTGTGTGAACC	GTCAGCCCCATCATCTGCG
mCDC20	TTCGTGTTCGAGAGCGATTTG	ACCTTGGAACTAGATTTGCCAG
mAurka	GGGTGGTCGGTGCATGCTCCA	GCCTCGAAAGGAGGCATCCCCACTA
mCCL2	GTTGGCTCAGCCAGATGCA	AGCCTACTCATTGGGATCATCTTG
mCCL7	AGAAGCAAGGCCAGCACAGAGT	GAGCAGCAGGCACAGAAGCGT
mMMP8	AATCCTTGCCCATGCCTTTCAACC	CCAAATTCATGAGCAGCCACGAGA
mIl6	CACTTCACAAGTCGGAGGCT	CTGCAAGTGCATCATCGTTGT
mICAM	CAGTCCGCTGTGCTTTGAGA	AGGGTGAGGTCCTTGCCTAC
mIl10	GGTGAGAAGCTGAAGACCCTC	GCCTTGTAGACACCTTGGTCTT
mTgfb1	ACTGGAGTTGTACGGCAGTG	TCATGTCATGGATGGTGCCC
mIL1B	TGTGCAAGTGTCTGAAGCAGCTA	TCAAAGGTTTGGAAGCAGCCCT
mMyh6	CTCTGGATTGGTCTCCCAGC	GTCATTCTGTCACTCAAACTCTGG
mMybpc3	CTGTCCATGAGGCCATTGGT	GCTTTGAGTCCCTCCGGAAA
mMyom1	TGGGTACTACATCGAGGCCA	GAGACACTCCTCCCCCGATA
mMyl3	CCAAAAAGCCAGAGCCCAAG	GGCCTCCTTGAACTCTTCAATC

ABI Taqman assays	
Gene*	
mGAPDH	4352339E
Myh7	Mm00600555_m1

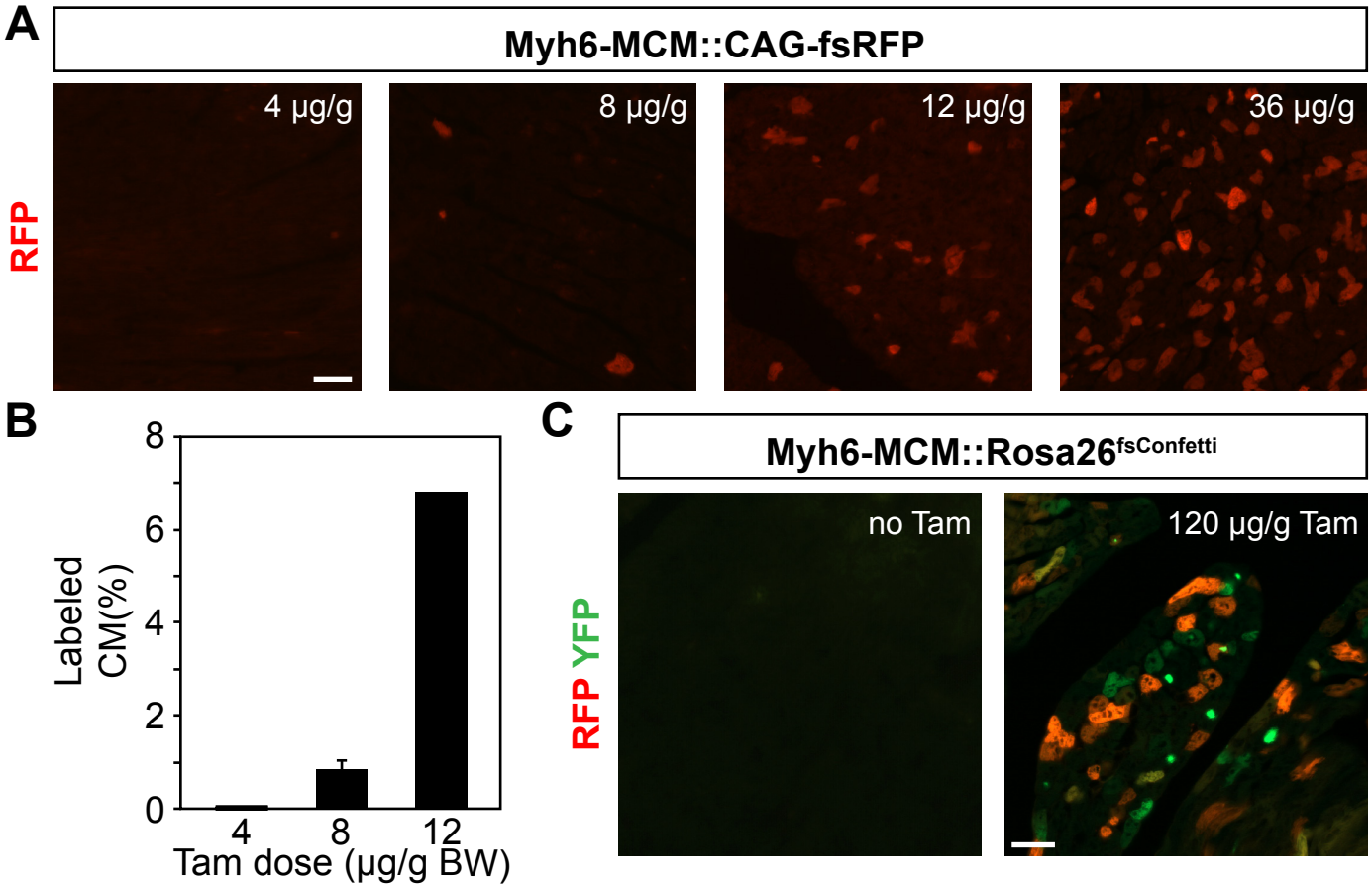
*m=mouse; h=human



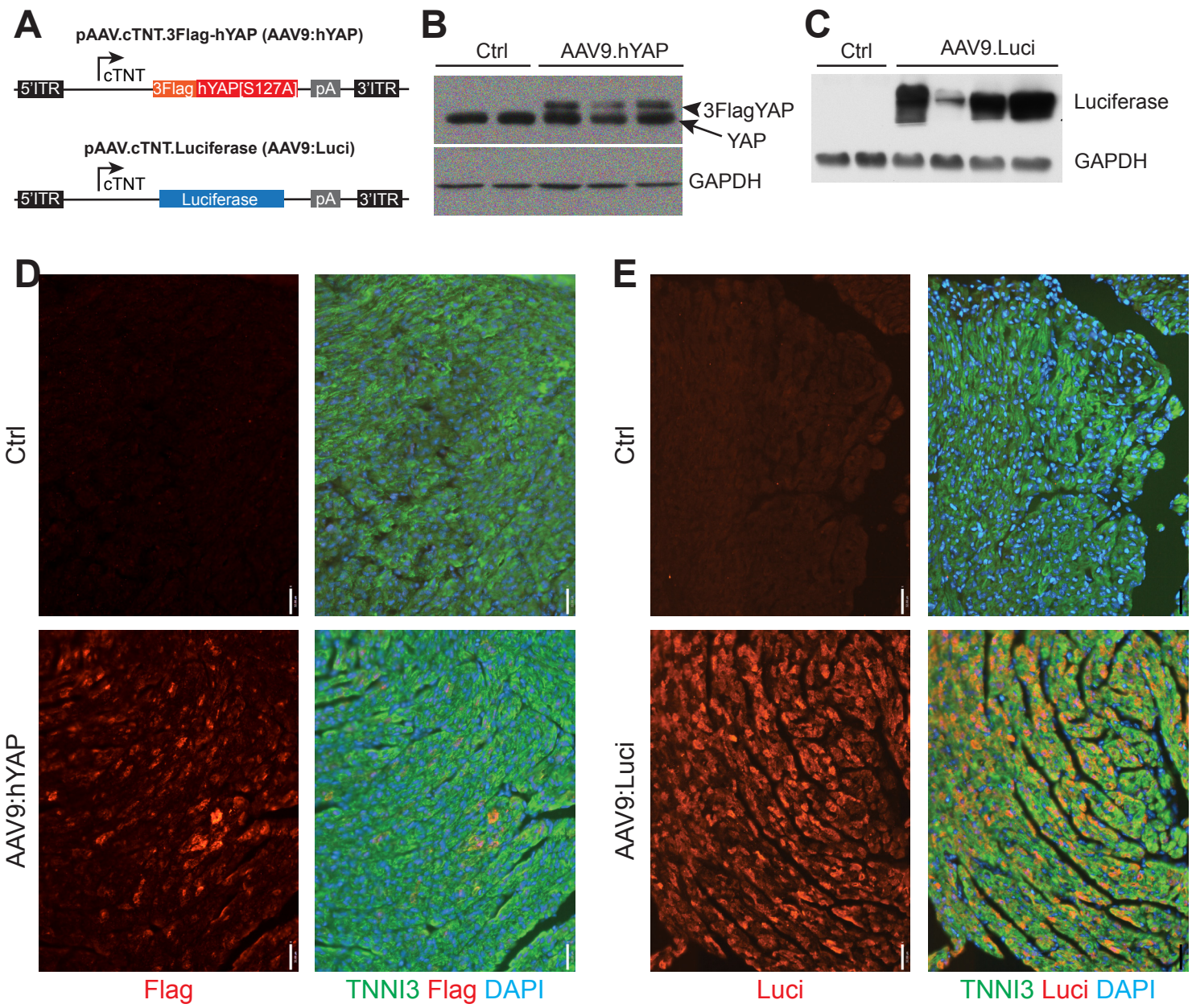
Online Figure I. Lin et al.
Representative images of sarcomere morphology of cardiomyocytes isolated from adult control and YAP^{GOF} hearts. Filamentous actin was stained with phalloidin. Cardiac myosin heavy chain antibody recognized both α and β isoforms. No difference in sarcomere organization was observed between treatment groups. Bar=50 μm



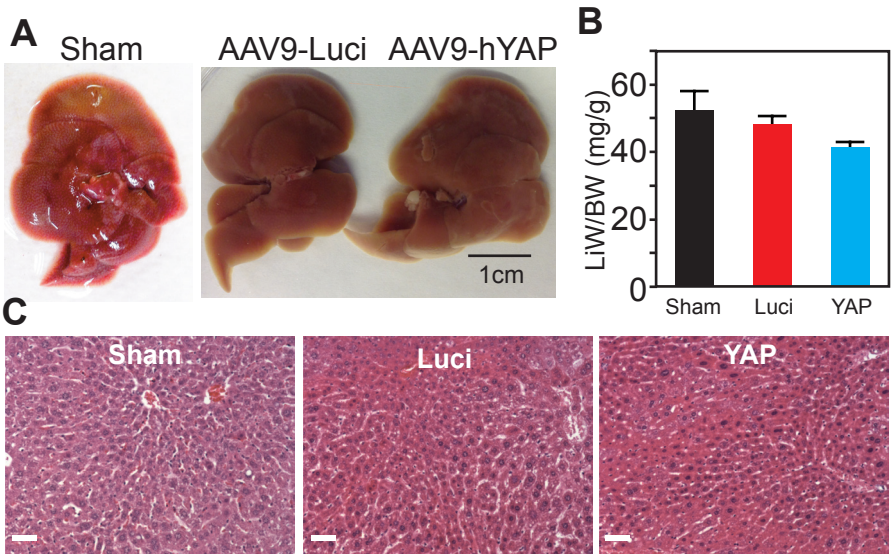
Online Figure II. Lin et al.
Cardiomyocyte cell cycle activity induced by expression of activated YAP. After Dox-induced expression of activated YAP from weeks of life 4 to 8, hearts were analyzed for cell cycle markers.
A. The fraction of cardiomyocytes in S-phase was determined by measuring EdU uptake. At least 5000 CMs were sampled per heart. n=6. Bar = 25 μm.
B. The fraction of cardiomyocytes in M-phase was determined by immunostaining for phosphohistone H3 (pH3). YAP increased the fraction of CMs in M-phase. At least 8000 CMs were sampled per heart. n=6. Bar = 25 μm.



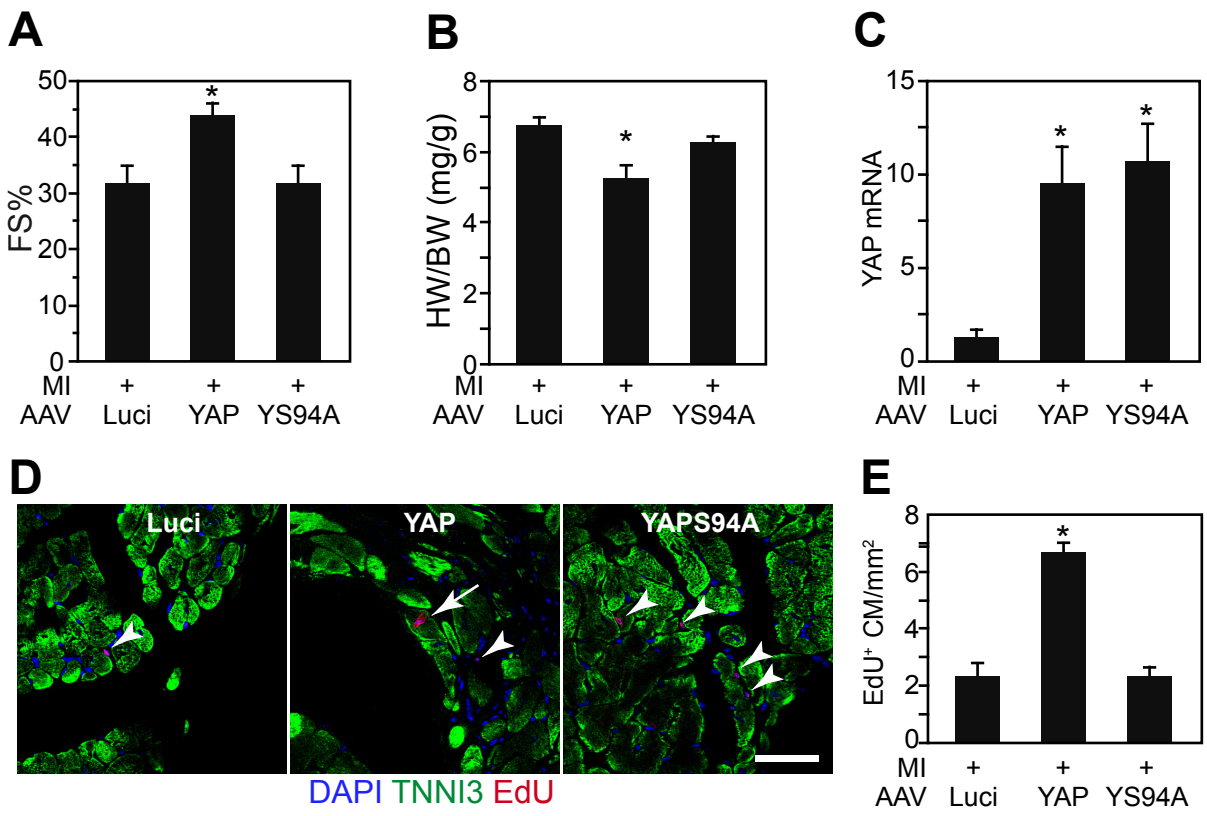
Online Figure III. Lin et al.
Tamoxifen titration to achieve infrequent cardiomyocyte labeling by Myh6-MerCreMer.
A-B. The indicated dose of tamoxifen was administered to Myh6-MCM; CAG-fs-RFP mice. We measured the frequency of labeled cardiomyocytes (B).
C. Tamoxifen-induced activation of RFP or YFP from the Rosa26^{fsConfetti} reporter. Bar, 40 µm.



Online Figure IV. Lin et al.
AAV construction and validation.
A. AAV vectors used to generate AAV9:hYAP and AAV9:Luci contained the AAV inverted terminal repeats (ITRs), the chicken TNNT2 (cTNT) promoter, and a polyadenylation (pA) signal. 3Flag-hYAP[S127A] or luciferase were cloned into this AAV backbone to generate pAAV.cTNT.3Flag-hYAP (AAV9:hYAP) or pAAV.cTNT.Luciferase (AAV9:Luci), respectively.
B-C. AAV9:hYAP or AAV9:Luci viruses were injected subcutaneously into 3 day old mice . 5 days later, hearts were analyzed by western blotting. Mice that received no AAV9 injection served as control (Ctrl). Expression of 3Flag-hYAP (B, indicated by arrowhead) and Luciferase (C) was confirmed with western blot.
D. AAV9:hYAP expressed Flag-tagged YAP in the heart. Bar = 50 μm.
E. AAV9:Luci expressed luciferase in the heart. Bar = 50 μm.



Online Figure V. Lin et al.
Direct intramyocardial injection of AAV9-hYAP did not cause liver tumors.
A. Gross morphology of livers from sham control, AAV9-Luci and AAV9-hYAP.
B. AAV9-hYAP did not alter liver weight to body weight ratio (LiW/BW). No significant difference was found between groups. Sham, n=3; AAV9-Luci, n= 3; AAV9-hYAP, n=6.
C. HE staining of liver sections did not reveal any hepatic tumors. Bar = 50 μm.



Online Figure VI, Lin et al.

YAP-TEAD interaction is required for beneficial activities of YAP after MI. Interaction of overexpressed YAP was abolished by mutation of serine 94 to alanine. Mice were treated with AAV9:YAP, AAV9:YAPS94A, or AAV9:Luci at the time of coronary artery ligation. Hearts were examined 1 month after MI.

A. Fraction shortening measured by echocardiography.

B. Heart weight to body weight ratio.

C. Total YAP mRNA level measured by qRT-PCR.

D-E. Cardiomyocyte proliferation assessed by EdU incorporation rate. EdU was administered in the fourth week after MI. White arrow heads indicate EdU positive non-cardiomyocytes. White arrow indicates EdU positive cardiomyocyte. Bar=50 μm.

*P<0.05. n=4