A

		Before Tamoxifen		24 hours after tamoxifen		
			P-value*		P-value*	P-value†
FWWS	Control	1.07 +/- 0.04	NS	0.82 +/- 0.03	<0.0001	<0.0001
	YAP5SA OE	1.05 +/- 0.03		1.38 +/- 0.06		0.0002
FWWD	Control	0.75 +/- 0.02	NS	0.74 +/- 0.02	<0.0001	NS
	YAP5SA OE	0.78 +/- 0.03		1.01 +/- 0.04		0.0004
EF	Control	57.4 +/- 1.9	NS	36.0 +/- 3.6	<0.0001	<0.0001
	YAP5SA OE	59.1 +/- 3.5		72.5 +/- 4.7		0.01
FS	Control	29.9 +/- 1.2	NS	17.4 +/- 3.6	<0.0001	<0.0001
	YAP5SA OE	30.9 +/- 1.4		41.4 +/- 3.5		0.01
ESD	Control	2.74 +/- 0.10	NS	3.60 +/- 0.18	<0.0001	<0.0001
	YAP5SA OE	2.66 +/- 0.15		1.87 +/- 0.28		0.003
EDD	Control	3.92 +/- 0.09	NS	4.34 +/- 0.14	<0.0001	0.007
	YAP5SA OE	3.84 +/- 0.17		3.14 +/- 0.25		0.005

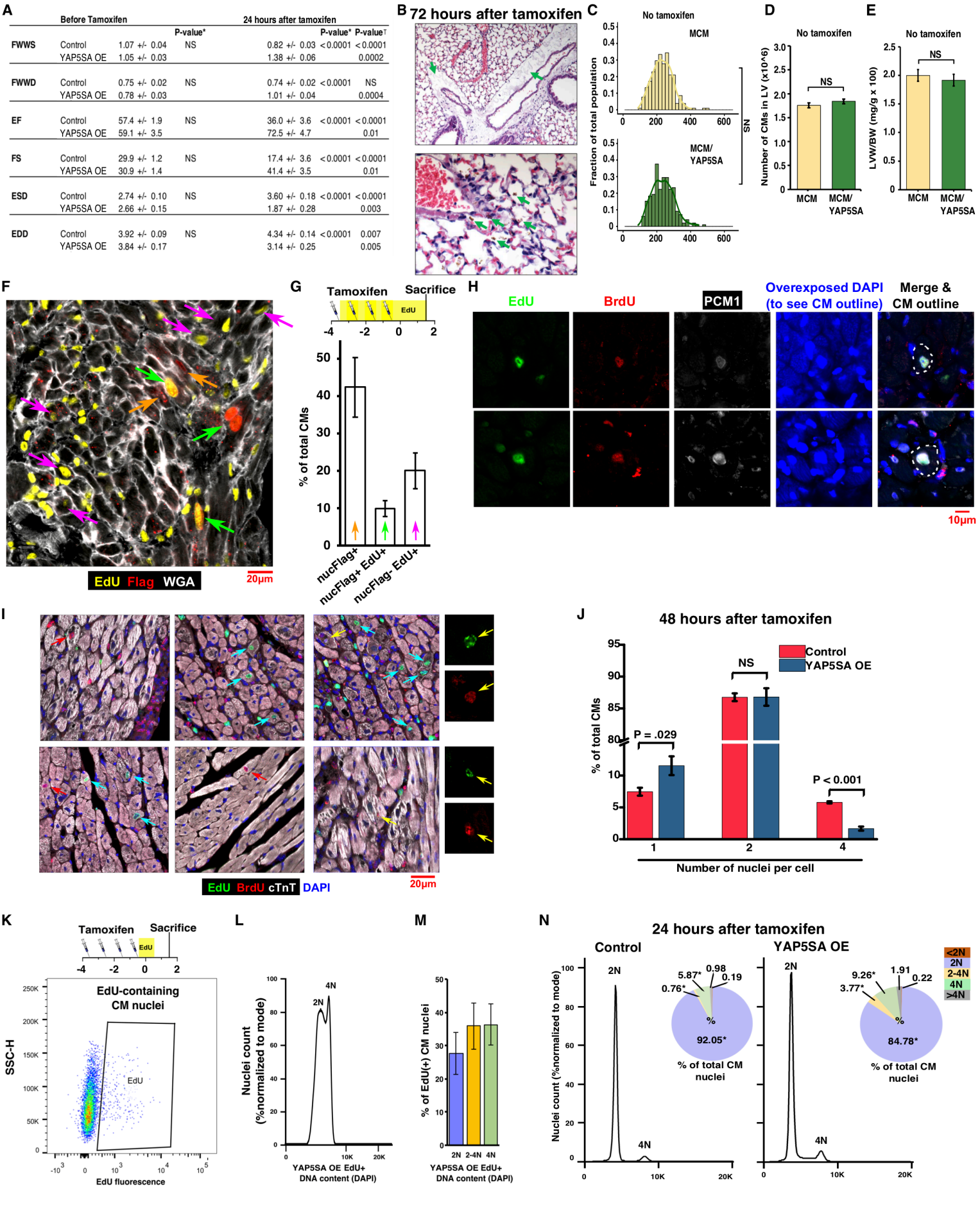

Figure S1. Echocardiography; Histology; Heart size measurements from mice without tamoxifen; Nucleotide incorporation; Flag IF; and DNA content. Related to Figures 1 and 2. (A) Echocardiography measurements. Data are before the first tamoxifen injection and one day after a fourth tamoxifen injection. Control n=11, YAP5SA OE n=8. * indicates a comparison at the same time point between genotypes. +indicates comparison within the same genotype before and after tamoxifen. Controls are MCM with tamoxifen. Data shown as mean +/- SEM. Groups compared by ANOVA with post hoc Bonferroni test. **(B)** Moribund YAP5SA OE lung histology n=3 mice. (Top, green arrows) Fluid build-up showing perivascular space expanded by edema. (Bottom, green arrows) Brown granules of hemosiderin are present in macrophages, secondary to congestive heart failure. **(C)** Histograms showing the areas of isolated CMs from MCM and YAP5SA & MCM mice. n=3 mice/genotype, ~100 cells/heart, without tamoxifen. **(D)** Myocyte number in LV from MCM and YAP5SA & MCM mice (n=3 mice/genotype). **(E)** Ratio of LV weight to body weight from MCM and YAP5SA & MCM mice (n=3 mice/genotype). C-E groups compared by ANOVA with post-hoc Bonferroni tests. C,D mean +/- SEM. **(F)** Example EdU and Flag staining (from protocol in Fig. S1G) in a YAP5SA OE heart. EdU(+) CMs with no active YAP5SA indicated with pink arrows. EdU(+) & active YAP5SA (nuclear Flag) CMs indicated with green arrows. Active YAP5SA CMs without EdU indicated with orange arrows. **(G)** Quantification of active YAP5SA (nuclear Flag) and EdU(+) CMs (mean +/- SEM. n=5, ~200 CMs/mouse). **(H)** EdU(+) & BrdU(+)example CMs, marked with PCM-1. **(I)** EdU & BrdU labeling example CMs, marked with troponin T. Singly labeled CMs are indicated with either blue [EdU(+)] or red [BrdU(+)] arrows. CMs positive for both EdU and BrdU are marked with yellow arrows and shown in separated colors on the side **(J)** CM nucleation. (n=6 hearts per group, 400-500 CMs per heart, 48 hours after the fourth tamoxifen injection). Groups compared by ANOVA with post-hoc Bonferroni tests. Controls are MCM mice injected with tamoxifen. Mean +/- SEM. **(K)** EdU-labeling and gating used for detecting EdU(+) CM nuclei for DNA content. **(L)** Representative histogram of DNA content from EdU(+) YAP5SA OE CM nuclei. **(M)** Ploidy of EdU(+) YAP5SA OE CM nuclei. K-M n=3, 48 hours after the fourth tamoxifen injection. Mean +/- SEM. **(N)** Representative histograms from flow cytometry analysis of isolated CM nuclei from control and YAP5SA overexpressing (OE) CMs, stained with DAPI (control, n=4; YAP5SA OE, n=4 control, 24 hours after the fourth tamoxifen injection). Controls are MCM mice injected with tamoxifen. Data in pie charts represent the mean values. *$P < .05$ for control vs YAP5SA OE. ANOVA with the Bonferroni post-hoc test

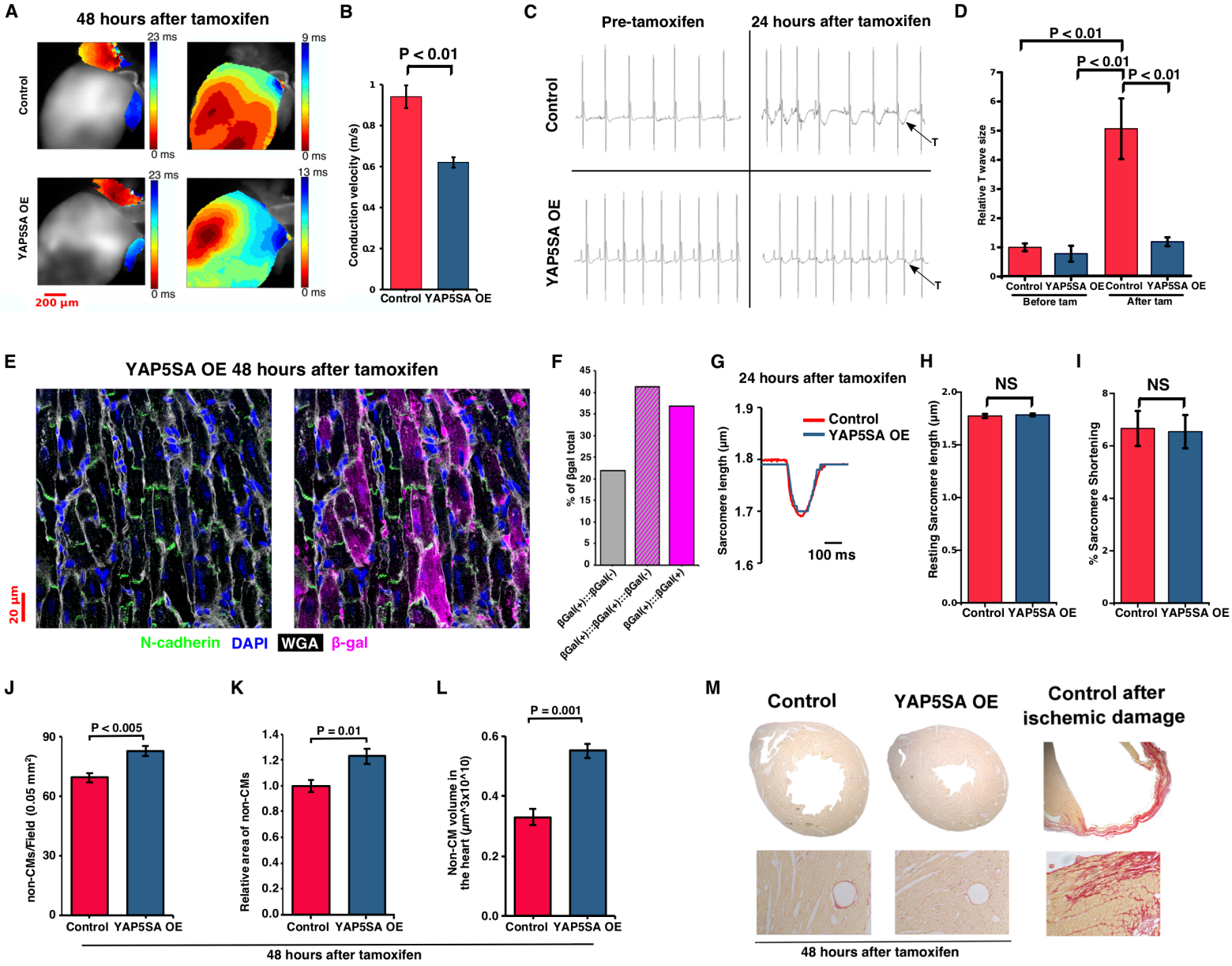
A
48 hours after tamoxifen
Control
YAP5SA OE
23 ms
0 ms
9 ms
13 ms
200 µm
B
P < 0.01
Conduction velocity (m/s)
Control YAP5SA OE
C
Pre-tamoxifen
24 hours after tamoxifen
Control
YAP5SA OE
T
D
P < 0.01
Relative T wave size
Control YAP5SA OE Control YAP5SA OE
Before tam
After tam
E
YAP5SA OE 48 hours after tamoxifen
20 µm
N-cadherin DAPI WGA β-gal
F
% of βgal total
βGal(+)::βGal(-)
βGal(+)::βGal(+)::βGal(-)
βGal(+)::βGal(+)
G
24 hours after tamoxifen
Control
YAP5SA OE
Sarcomere length (µm)
100 ms
H
NS
Resting Sarcomere length (µm)
Control YAP5SA OE
I
NS
% Sarcomere Shortening
Control YAP5SA OE
J
P < 0.005
non-CMs/Field (0.05 mm²)
Control YAP5SA OE
K
P = 0.01
Relative area of non-CMs
Control YAP5SA OE
L
P = 0.001
Non-CM volume in the heart (µm^3x10^10)
Control YAP5SA OE
48 hours after tamoxifen
M
Control
YAP5SA OE
Control after ischemic damage
48 hours after tamoxifen

Figure S2. YAP5SA OE electrophysiology & non-CM content. Related to Figures 1 and 2. (A) Representative action potential conduction velocity maps from Control and YAP5SA OE hearts 48 hours after the fourth tamoxifen injection. Controls are MCM mice injected with tamoxifen. **(B)** Quantification of right atrium to Left ventricle conduction velocity. n=3 per condition. **(C)** Representative electrocardiogram traces from Control and YAP5SA OE mice before and one day after a fourth tamoxifen injection. Post-tamoxifen T-waves indicated with arrows. Controls are MCM mice injected with tamoxifen. **(D)** Relative area under the curve of T wave. Control n=3; YAP5SA OE n=4. Controls are MCM mice injected with tamoxifen. **(E)** Representative YAP5SA CMs connected to other CMs through intercalated disks, indicated by n-cadherin and β-galactosidase staining (left image without β-galactosidase channel for clarity). **(F)** Quantification of CMs that β-gal(+) CMs are connected to with n-cad. ::: represents CM-CM coupling. n=3 mice, >500 CMs imaged. **(G)** Representative plot of sarcomere shortening. **(H)** Resting sarcomere length. **(I)** Percent sarcomere shortening. G-I control n=5 mice, 27 cells. YAP5SA OE n=5 mice, 31 cells, 24 hours after the fourth tamoxifen injection. Controls are MCM mice injected with tamoxifen. **(J)** Density of non-CMs in YAP5SA OE and Control hearts 48 hours after the fourth tamoxifen injection. **(K)** Relative area of non-CMs in imaging frames. **(L)** Volume of non-CMs (area extrapolated to the volume of the heart). H-J n= 5 mice/group. Control are MCM with tamoxifen. 10 images (> 1000 cells/mouse). **(M)** Picrosirius Red stain of YAP5SA OE and control hearts at the 48 hour post-tamoxifen time point. Control is MCM with tamoxifen. A representative heart with damage and fibrosis also shown for comparison. B, D, H-L shown as means +/- SEM. B, D, H-L ANOVA with post hoc Bonferroni test.

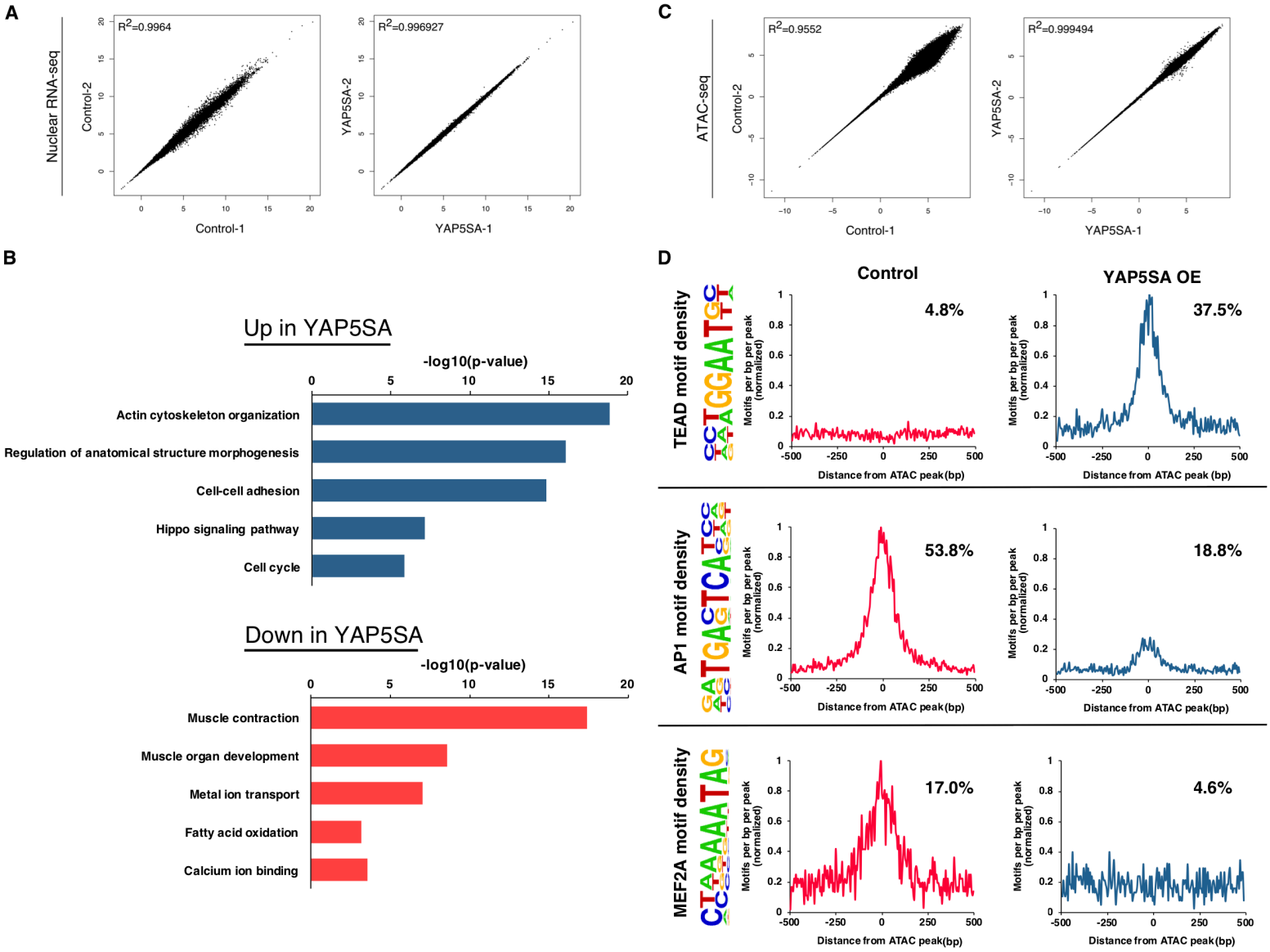
A
Nuclear RNA-seq
Control-2
Control-1
R2=0.9964
YAP5SA-2
YAP5SA-1
R2=0.996927
C
ATAC-seq
Control-2
Control-1
R2=0.9552
YAP5SA-2
YAP5SA-1
R2=0.999494
B
Up in YAP5SA
-log10(p-value)
Actin cytoskeleton organization
Regulation of anatomical structure morphogenesis
Cell-cell adhesion
Hippo signaling pathway
Cell cycle
Down in YAP5SA
-log10(p-value)
Muscle contraction
Muscle organ development
Metal ion transport
Fatty acid oxidation
Calcium ion binding
D
Control
YAP5SA OE
TEAD motif density
4.8%
37.5%
AP1 motif density
53.8%
18.8%
MEF2A motif density
17.0%
4.6%
Motifs per bp per peak (normalized)
Distance from ATAC peak(bp)

Figure S3. RNA-seq and ATAC-seq supplement. Related to Figures 3 and 4 (A) Reproducibility in both sets of RNA-seq. Reads from each indicated experiment were transformed with a regularized-logarithm transformation and displayed as scatter plots to show the relationship of replicate data sets within each group. **(B)** RNA-seq Gene Ontology analysis of up-regulated and down-regulated genes in YAP5SA OE CMs, relative to control ($P < .01$). **(C)** Reproducibility between the ATAC-seq sets. Reads from each indicated experiment were transformed with a regularized-logarithm transformation and displayed as scatter plots to show the relationship of replicate data sets within each group samples/genotype. **(D)** TEAD, AP-1, and MEF2A normalized motif densities of centered around unique ($P < 0.01$) ATAC-seq peaks from control or YAP5SA OE CMs, percentages of motif presence in those peaks is shown.

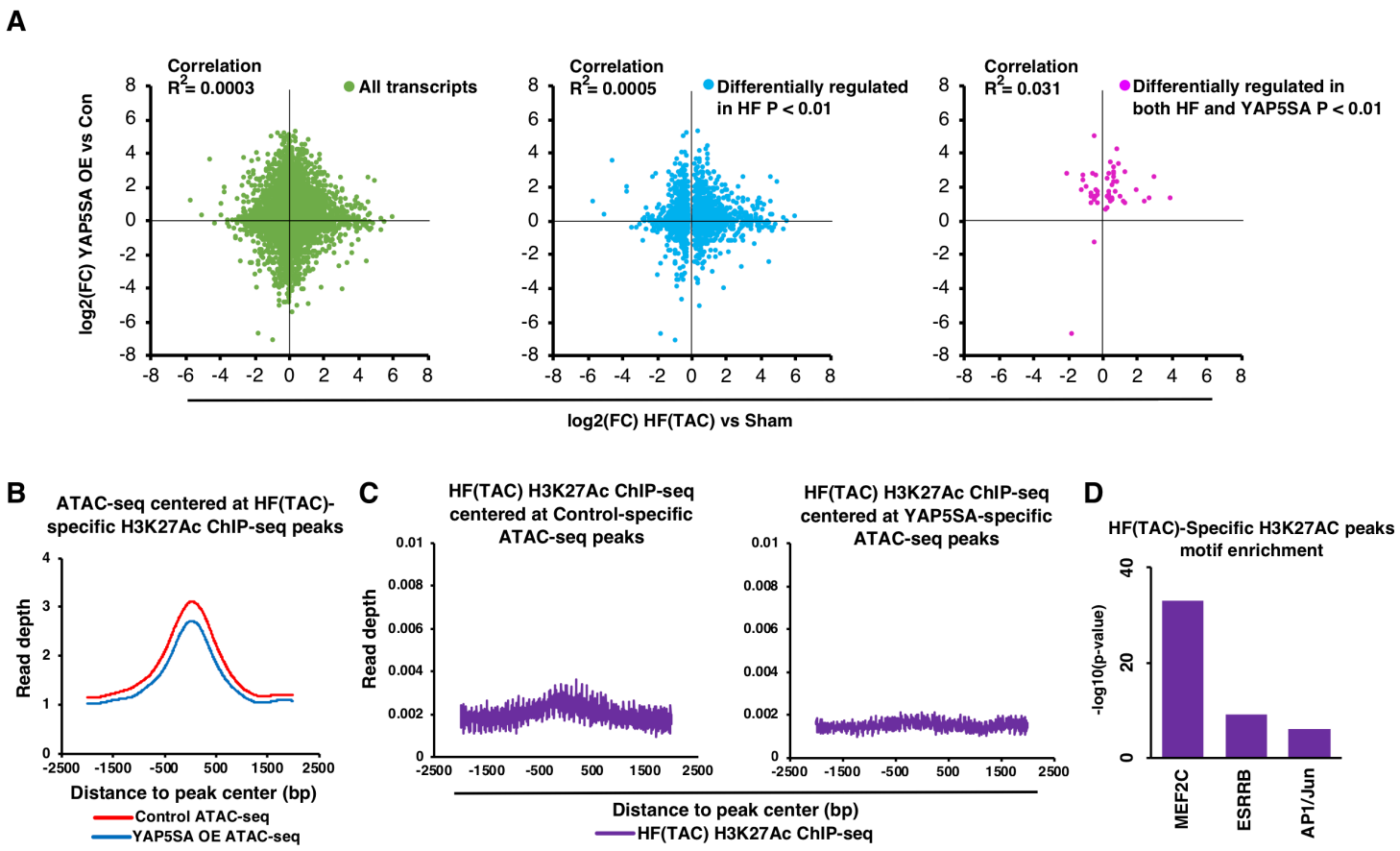

Figure S4. YAP5SA OE CMs do not genetically resemble failing CMs. Related to figures 1, 3 and 4. (A) RNA-seq comparison of mice in heart failure (HF), 8 weeks after transaortic constriction (TAC) vs sham and YAP5SA vs control. HF data from GSE112055. No significant correlation overall from the data, or from significantly differentially regulated genes from mice in heart failure compared to the YAP5SA OE vs Con data. **(B)** Global ATAC-seq depth from Control and YAP5SA OE CMs at active enhancers unique to failing hearts (8 weeks after TAC, see methods). HF data from GSM2497652 & GSM2687477 in GSE95143. **(C)** H3K27Ac reads centered at Control or YAP5SA OE-specific ATAC-seq peaks. **(D)** Motifs enriched in H3K27Ac ChIP-seq heart failure peaks 8 weeks after TAC.

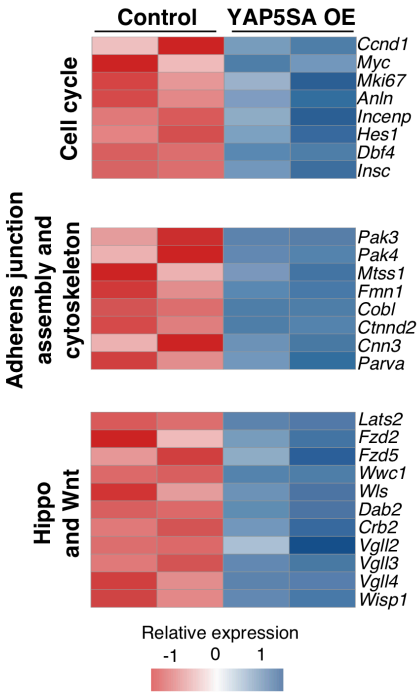

Figure S5. Heat maps of select YAP5SA direct targets. Related to Figure 5 and Table S1. Relative expression levels of select genes from the indicated categories

YAP5SA Genotyping Forward Primer	AAGCCTTGACTTGAGGTTAG
YAP5SA Genotyping Reverse Primer	CGTCATCGTCTTTGTAGTCC
4C *Wls* primer chr3_159502003_159502235_Read1_1	TTTATGTTCCAAGGGTCGAT
4C *Wls* primer chr3_159502003_159502235_Read2_1	GACGTTTGATTTGTCTGGAT
4C *Nog* primer chr11_89163154_89164463_Read1_5	TGACCCTAAGGAGAAGGATC
4C *Nog* primer chr11_89163154_89164463_Read2_5	GGGGACTCTCACTAGCTGAG
3C Cobl promoter	GAGAACCGACTTGAAGGAAGAG
3C Cobl enhancer	TTCCGACAGAGGGCATAAAC
3C Cobl control region	CTTGAGGGTTAGGTGCATCAT
3C Crim1 promoter	CTGCTTCCCTGCATCTTGT
3C Crim1 enhancer	TCAAGGCCATTTCAGCTCTC
Crim1 3C control region	GGCCTTCATGTAATCCAAGGTA
Lats2 3C promoter	CAGTCTTCAGAGGAACCATGTTA
Lats2 3C enhancer	ATGTAGGTGGTGGGACAGA
Lats2 3C control region	CAGGCATACAGGTGATCTACAG
Nog 3C promoter	TTTCCTTTGGAGGAAGAGCTG
Nog 3C enhancer	CCTAAACAAAGAGAGGACGCTTA
Nog 3C control region	TTGCTCCCTAACAAGGTTTGA
Vgll4 3C promoter	CCAGCCCGAGTGTTTACAT
Vgll4 3C enhancer	GGCCACAAAGCAAGACAAC
Vgll4 3C control region	GTGTGTGTGTCTGCCTATGA
Wls 3C promoter	TTCTAAGCCTCTGTCCTCCATA
Wls 3C enhancer	GAGGAACTGTATTAAAGGGTTGGA
Wls 3C control region	CTGGCTAAGGGTTTATCCATCTT
Actb Forward for 3C	CTTTGCAGCTCCTTCGTTGC
Actb Reverse for 3C	CCTTCTGACCCATTCCCACC

Table S2. Primers used in this paper (Related to Figure 1, 5, 6, STAR Methods).