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Supplemental Information

Specifying the Anterior Primitive Streak by Modulating YAP1 Levels in Human Pluripotent Stem Cells

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Figure S1.

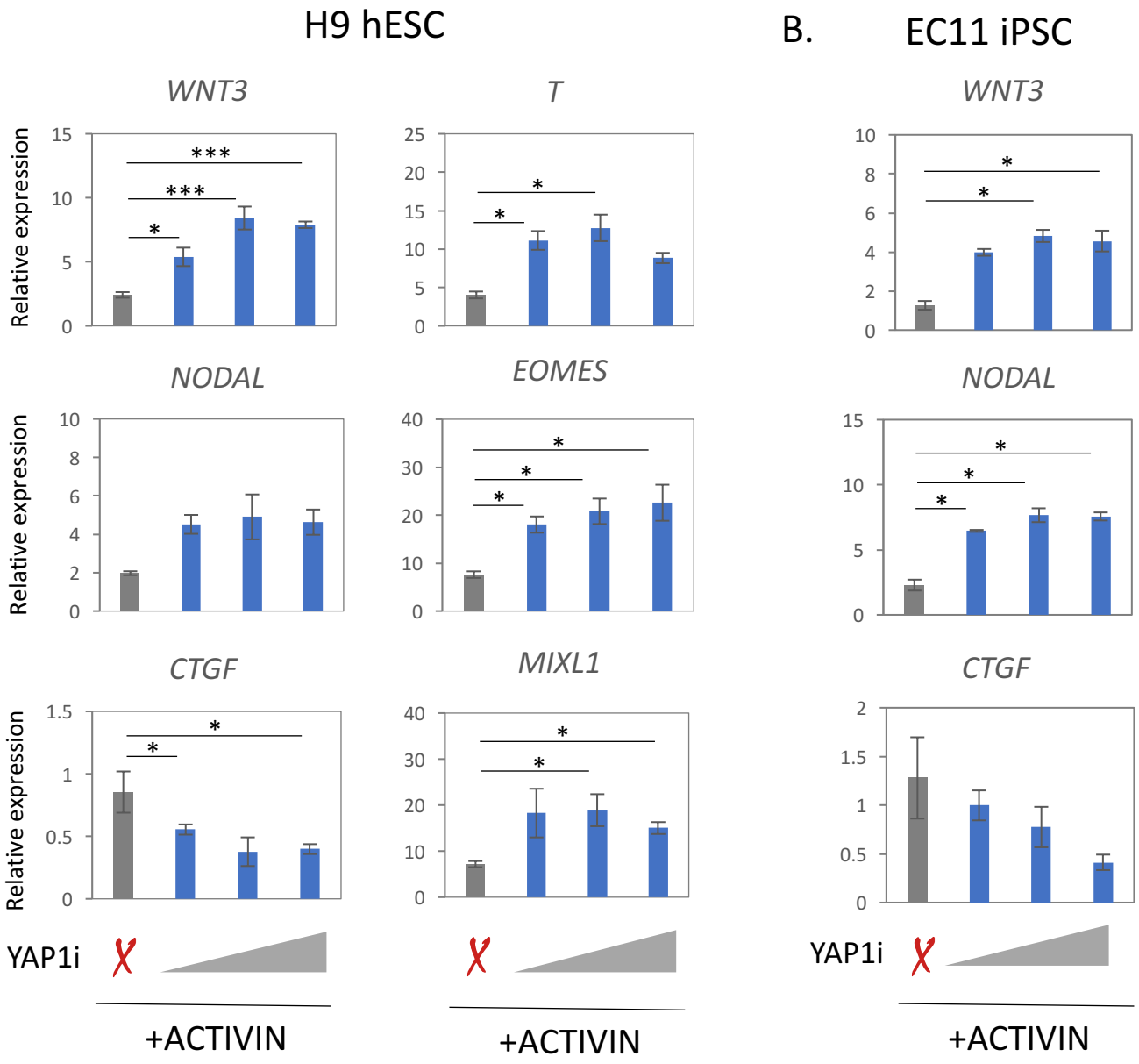

A.
H9 hESC
B.
EC11 iPSC
WNT3
T
NODAL
EOMES
CTGF
MIXL1
Relative expression
YAP1i
+ACTIVIN
+ACTIVIN
WNT3
NODAL
CTGF
YAP1i
+ACTIVIN

Figure S2.

A.

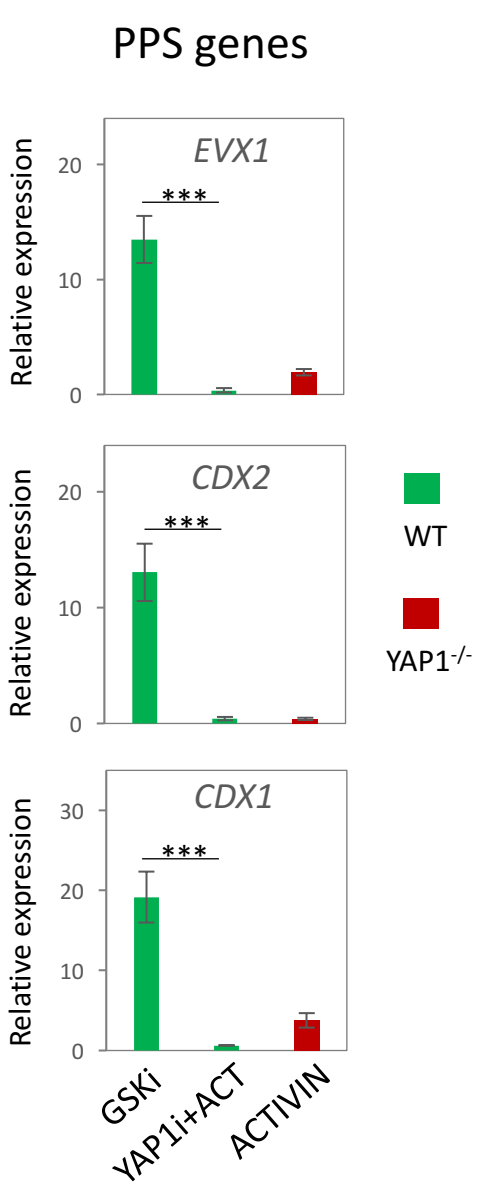
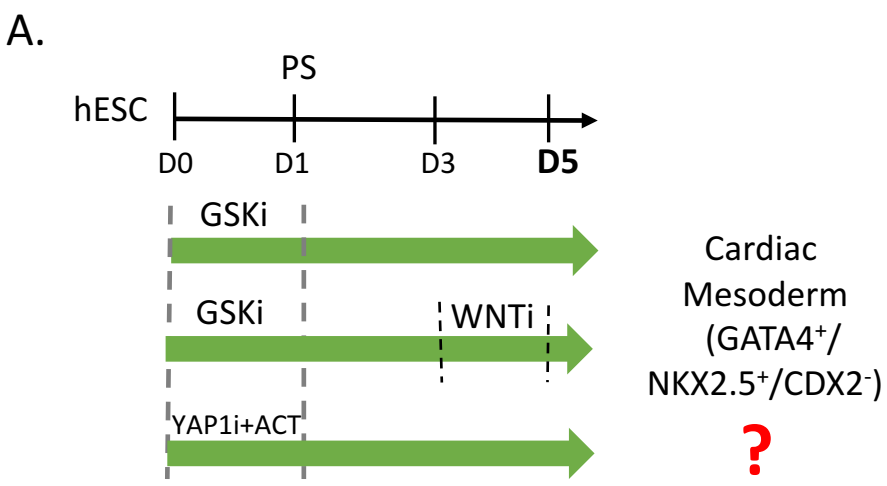
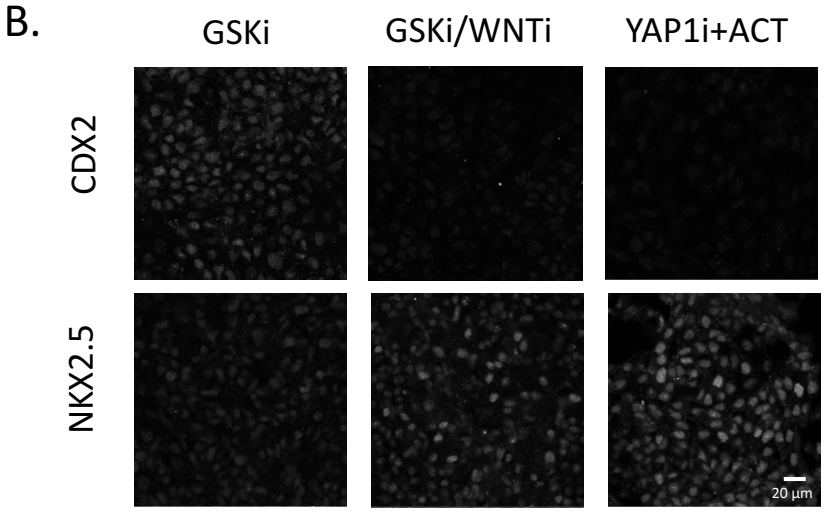
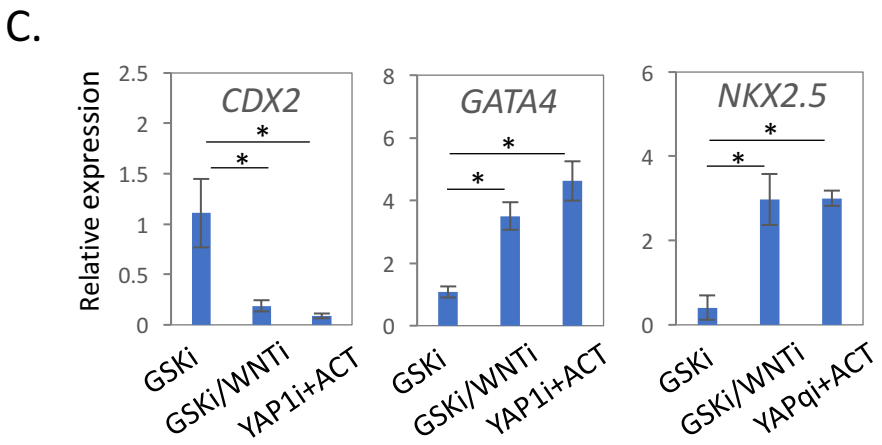

PPS genes
EVX1
CDX2
CDX1
Relative expression
***
0
10
20
30
WT
YAP1-/-
GSKi
YAP1i+ACT
ACTIVIN

Figure S3.


A.
PS
hESC
D0
D1
D3
D5
GSKi
GSKi
WNTi
YAP1i+ACT
Cardiac Mesoderm (GATA4+/ NKX2.5+/CDX2-)
?


B.
GSKi
GSKi/WNTi
YAP1i+ACT
CDX2
NKX2.5
20 µm


C.
Relative expression
CDX2
GATA4
NKX2.5
GSKi
GSKi/WNTi
YAP1i+ACT
YAPqi+ACT

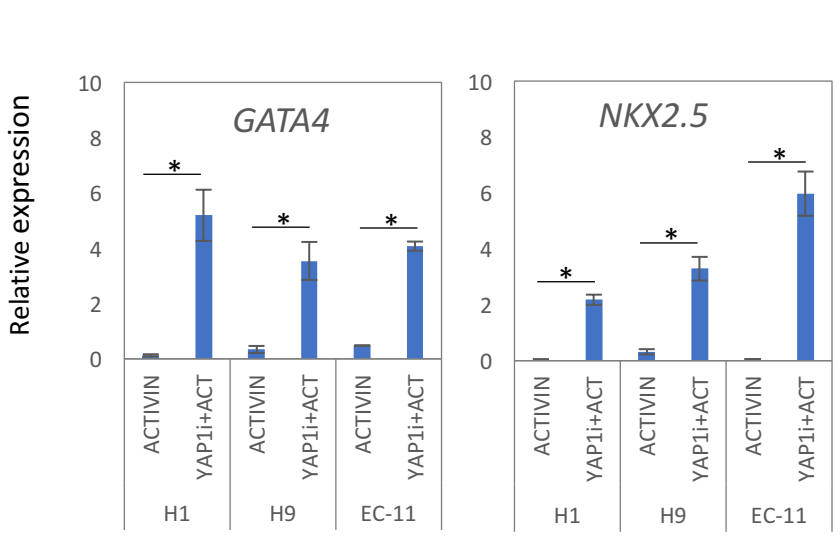
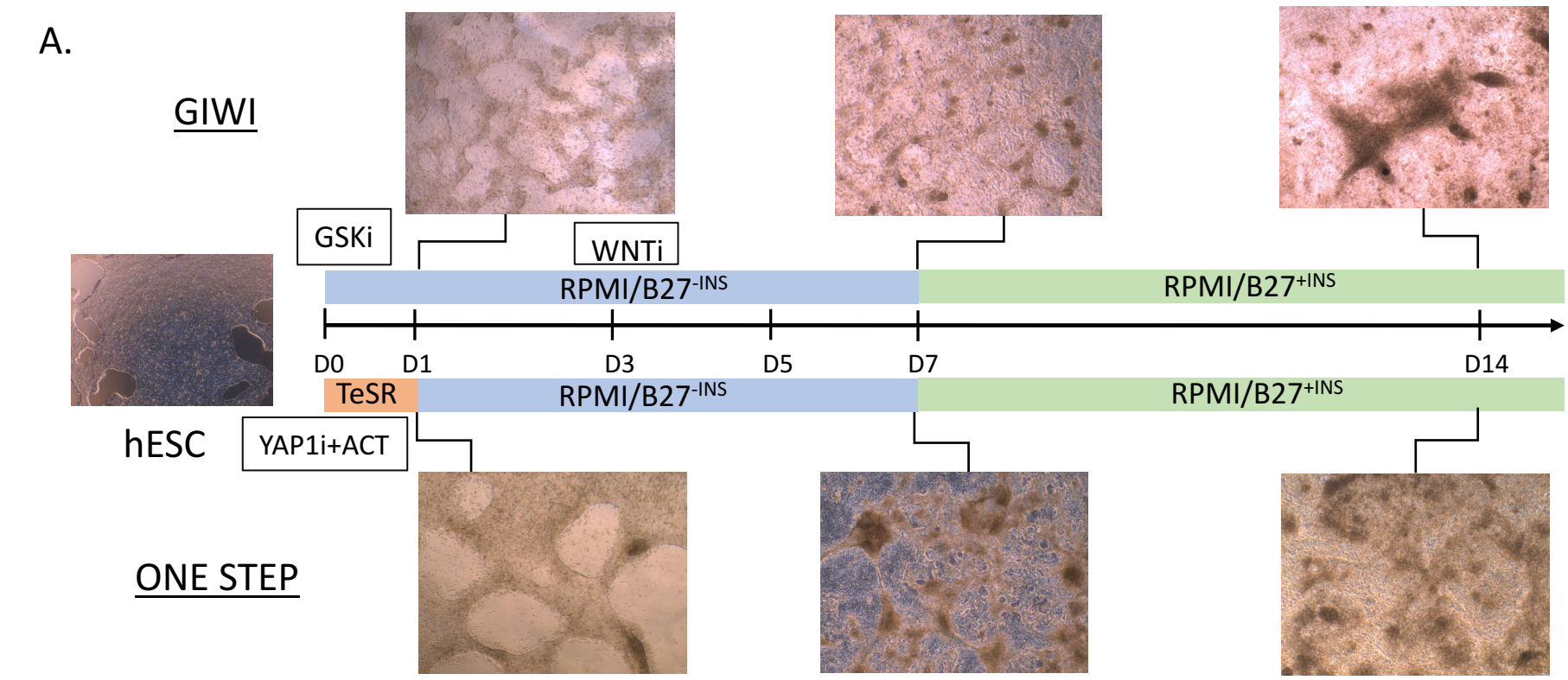
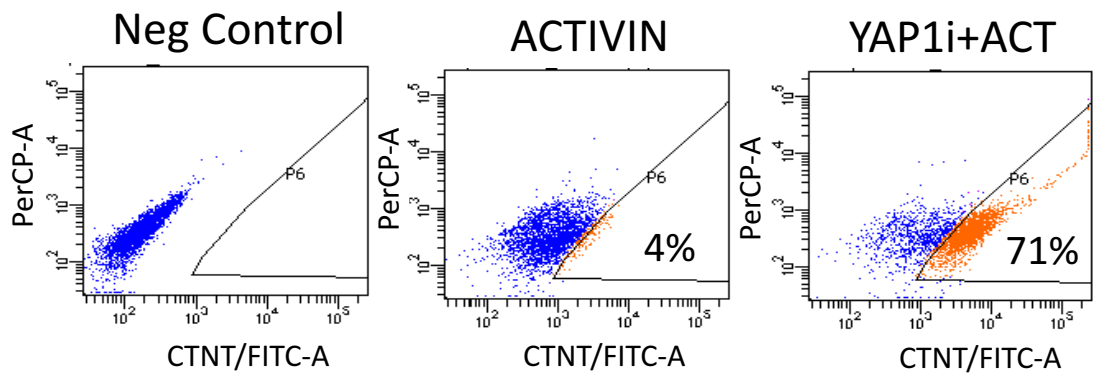

D.
Relative expression
GATA4
NKX2.5
ACTIVIN
YAP1i+ACT
H1
H9
EC-11

Figure S4.


A.
GIWI
GSKi
WNTi
RPMI/B27-INS
RPMI/B27+INS
D0
D1
D3
D5
D7
D14
TeSR
RPMI/B27-INS
RPMI/B27+INS
hESC
YAP1i+ACT
ONE STEP


B.
Neg Control
ACTIVIN
YAP1i+ACT
PerCP-A
CTNT/FITC-A
P6
4%
71%

Supplemental Figure Legends

Supplemental Figure 1. ACTIVIN+YAP1i treatment in hESCs and iPSCs induces panPS gene expression.
A. mRNA abundance of panPS genes and YAP1 target (*CTGF*) in H9 cells treated with ACTIVIN or YAP1i+ACTIVIN for 24 hr. B. mRNA abundance of panPS genes and YAP1 target (*CTGF*) in EC11 cells treated with ACTIVIN or YAP1i+ACTIVIN for 24 hr. The final concentration of YAP1i in culture was from 0, 5, 10 to 20 nM. N=3 biological independent experiments, mean ± SD.

Supplemental Figure 2. PPS gene expression is YAP1 independent.
mRNA abundance of *EVX1*, *CDX2*, and *CDX1* in WT and YAP1$^{-/-}$ hESCs with indicated treatments for 24 hr. The final concentration of YAP1i was 10 nM. N=3 biological independent experiments, mean ± SD.

Supplemental Figure 3. PS progenitors can be reprogrammed.
A. A diagram to illustrate the procedures of treatments for differentiation. B. Immunostaining of CDX2 and NKX2.5 on the 5th day from the initial treatments in WT hESCs indicated in the previous diagram. C. mRNA abundance of *CDX2*, *GATA4*, and *NKX2.5* on the 5th day from the initial treatment in WT hESCs. D. mRNA abundance of *GATA4* and *NKX2.5* on the 5th day from the initial treatment in H1, H9 and EC-11 cells. N=3 biological independent experiments, mean ± SD.

Supplemental Figure 4. One-Step protocol for cardiomyocyte differentiation.
A. Bright field images of cells on day 1, 7, and 14, under treatments of GiWi or One-Step protocols for cardiomyocyte differentiation. B. A representative example of FACS analysis of CTNT positive cells in culture on day 14 of differentiation. WT hESCs served as a negative control for comparing both differentiation efficiency and staining signal.

Supplemental Table 1. 1424 differentially expressed genes from RNA-Seq analysis in Figure 1D and Figure 2A, 2B. PanPS genes include genes in group 0 and 3. APS genes contain genes listed in group 2 and 6. PPS genes are in group 9.

Supplemental Table 2. RNA-Seq of cardiomyocytes obtained by GiWi and One-Step protocols.

Supplemental Table 3. Lists of primers and antibodies used.

Supplemental Video 1. Beating cells on day 14 of YAP1i+ACTIVIN treatment.

Supplemental Experimental Procedures

RNA-Seq analysis

RNA-Seq analysis was carried out as previously described [ref: G&D paper]. In brief, reads were mapped to the hg19 reference by STAR [v2.5.1b, ref: 10.1093/bioinformatics/bts635. pmid:23104886] with default parameters. Only the uniquely mapped reads were used by HOMER [v4.8, ref: PMID: 20513432; http://homer.ucsd.edu/homer/] for expression quantification. Gene expression levels were calculated by summing reads that were mapped across all exons of RefSeq genes. The differential expression (DE) analysis was performed using edgeR (v3.16.1, ref: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3378882/) to compare the raw count data from differentiated cells (this study) with wild-type undifferentiated cells [ref: G&D paper]. Genes with a false discovery rate (FDR) < 0.05 and log fold-change (logFC) > 1 were identified as significantly differentially expressed genes.

Clustering

Gene expression and DE data from the previous study [ref: G&D paper] were used for this analysis. A list of 1424 genes that showed differential expression between any of the three comparisons (FDR < 0.05 and logFC > 2) was clustered into 12 groups by K-Means algorithm using Cluster 3.0 [ref: https://www.ncbi.nlm.nih.gov/pubmed/14871861?dopt=AbstractPlus]. The expression FPKM (fragment per kilobase per million mapped reads) value was log transformed and centered before clustering (normalized value available at Supplemental Table 1). Group of genes that were preferentially up-regulated in response to GSK3i (in WT and YAP1-/- cells) and in response to ACTIVIN (in YAP1-/- cells) was defined as the panPS gene group, that were preferentially up-regulated in response to ACTIVIN (in YAP1-/- cells) was defined as the APS gene group, and that were preferentially up-regulated only in response to GSK3i (in WT and YAP1-/- cells) was defined as the PPS gene group. For Figure 4E, the expression value of 50 cardiac genes (G&D paper) was log transformed and z-scale normalized before hierarchical clustering.

Statistical analysis and figure plotting

All the statistics were performed in the R environment unless mentioned specifically [ref: R Core Team (2013). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL http://www.R-project.org/]. R packages ggplot2 [ref: H. Wickham. ggplot2: Elegant Graphics for Data Analysis. Springer-Verlag New York, 2009. https://cran.r-project.org/web/packages/ggplot2/index.html], VennDiagramm [ref: https://cran.r-project.org/web/packages/VennDiagram/index.html], and gplots [ref: https://cran.r-project.org/web/packages/gplots/index.html] were used to plot the figures.

Immunofluorescence

After treatments, cells were fixed with 2% formaldehyde for 10 min followed by incubating with PBS containing 0.1% Triton for 10 min to permeabilize cells. For CTNT staining, cardiomyocytes were dissociated by incubating with Accutase and seeded on Matrigel-coated chamber slides (Millipore PEZGA0416) and maintained in RPMI/B27 media. Re-plated cardiomyocytes were recovered in culture for 3-5 days before fixation for immunostaining. Samples were then incubated in blocking solution (PBS with 0.1% Tween 20, 0.1% BSA and 10% FBS) for 30 min at room temperature before incubating with primary antibodies for overnight at 4°C. After three washes, samples were incubated with Alexa-conjugated IgG secondary antibodies for 2hr at room temperature. Lastly, samples were mounted by SlowFade Gold Antifade Mountant with DAPI (ThermoFisher #S36942). Images were captured by Zeiss LSM 780 confocal microscope and analyzed by ZEN 2011 software.

Quantitative reverse transcription PCR

Total RNA was extracted using Quick RNA Zymo kit following manufacturer indications. Then, 0.5 µg of total RNA was reverse transcribed using Transcriptor First Strand Synthesis kit (Roche). The cDNA was amplified using SYBR green master mix (Life Technologies) on an ABI7300 (Applied Biosystems) thermo-cycler. All results were normalized to a *RPS23* gene control. The ΔΔCt method was used to calculate relative transcript abundance against an indicated reference. Unless otherwise stated, error bars denote standard deviation among three independent experiments.

FACS analysis of cardiomyocytes

Monolayers cardiomyocytes were dissociated by Accutase digestion. After washing in PBS, cells were fixed with 1% formaldehyde for 20 min followed by 15 min incubation in 90% cold methanol at 4°C. Cells were washed 3 times in FlowBuffer 1 (0.5% BSA in PBS) and incubated with a primary antibody against CTNT (Lab Vision ms-295-p1, 1:200) in FlowBuffer 2 (0.5% BSA and 0.1% Triton in PBS) overnight at 4°C. Cells were then washed twice in FlowBuffer2 and incubated with a 2^{nd} antibody (Thermo A11001, 1:1000) at room temperature for two hours. Finally, cells were washed and resuspended in FlowByffer 1 for analysis using The Becton-Dickinson LSR II flow cytometer. Percentages of CTNT-positive cells were determined by pre-gating of intact single cells based on forward and side scatter using FACSDiva version 6.1.2 software.

Supplemental Table 3. Lists of primers and antibodies.

RT-qPCR primers

Gene	Fw Primer	Rev Primer
WNT3	GACCACATGCACCTCAAATG	CAGCAGGTCTTCACCTCACA
T	ACGCCATGTACTCCTTCCTG	TGAGCTTGTTGGTGAGCTTG
EOMES	ACTGGTTCCCACTGGATGAG	ATTTGCGCCTTTGTTATTGG
MIXL1	AGCTGCTGGAGCTCGTCTT	GCAAGTGGATGTCGGGGTA
CTGF	GGCTTACCGACTGGAAGACA	CCAGGCAGTTGGCTCTAATC
NODAL	GAGATTTTCCACCAGCCAAA	AGGTGACCTGGGACAAAGTG
RPS23	TGTCGTGGACTTCGTACTGC	ATGCCACTTCTGGTCTCGTC
LHX1	GAAGGCAAACTCTACTGCAAGAA	AGTTCAGGTGAAACACTTTGCTC
EVX1	GCTGTCTCTCTGAACAAAATGCT	CATCTCTCACTCTCTCCTCCAAA
SOX17	TATTTTGTCTGCCACTTGAACAGT	TTGGGACACATTCAAAGCTAGTTA
MEOX1	GCAGGGGGTTCCAAGGAAAT	GTCAGGTAGTTATGATGGGCAAA
CDX1	AAGGAGTTTCATTACAGCCGTTAC	TGCTGTTTCTTCTTGTTCACTTTG
MSX1	CGCCAAGGCAAAGAGACTAC	GCCATCTTCAGCTTCTCCAG
BAF60C	CACTTTTAACCCTGCGAAGC	GAACTTCCGCTTCTGTTTGC
MSGN1	GCTGGAATCCTATTCTTCTTCTCC	TGGAAAGCTAACATATTGTAGTCCAC

Antibodies

ANTIBODY	COMPANY	CATALOG	APPLICATION
YAP1/TAZ	Santa Cruz	sc-101199	IF/WB
SOX2	R&D System	245610	IF
CDX2	Santa Cruz	sc-134468	IF
BAF60C	Cell Signaling	62265S	IF
NKX2.5	Santa Cruz	sc-376565	IF/WB
CTNT	Lab Vision	MS295P1	IF/WB/FACS
MIC2.A	Synaptic Systems	311011	WB
ACTININ	Santa Cruz	sc-17829	WB