Supplemental Material

Supplemental Methods

Mouse breeding and genotyping. YAP flox mice were generated as previously described in details [1]. SM22α-Cre mice were purchased from the Jackson Laboratory [2]. To generate mice in which the cre transgene induces a smooth muscle cell-specific knockout of YAP, we first generated SM22α-Cre^{+}/YAP$^{F/W}$ mouse, by crossing SM22α-Cre^{+} male mice with YAP$^{F/F}$ female mice. Then SM22α-Cre^{+}/YAP$^{F/W}$ male mice were used to breed with YAP$^{F/F}$ female mice to achieve cardiac/smooth muscle-specific YAP deletion. This breeding strategy yielded 25% progeny with the homozygous smooth muscle specific deletion (SM22α-Cre^{+}/YAP$^{F/F}$) and 25% SM22α-Cre^{-}/YAP$^{F/F}$ as control littermates (Online Table I). Embryonic day (E) 0.5 was defined as noon of the day when the vaginal plug was detected. Genotyping of these mice was performed as previously described (Online Figure I A) [1, 2]. The use of experimental animals has been approved by the IACUC at Georgia Regents University and Albany Medical College in accordance with NIH guidelines.

Sections, Hematoxylin/Eosin (HE) staining, immunofluorescence (IF) and immunohistochemistry (IHC). Embryos or neonatal pups were euthanized by decapitation, fixed with 4% paraformaldehyde over-night at 4°C and embedded in paraffin. Histological assays were performed as described in our previous report [3, 4]. Briefly, sections were cut at 7-um thickness, deparaffinized and antigen retrieval was done by using microwave to heat at 98°C for 5 minutes in citric acid buffer (10mM, pH6.0). After goat serum (10%, Invitrogen) blocking for 30 minutes, sections were then incubated with anti SM α-actin (Sigma, mouse, 1:600), anti MF20 (Developmental Studies Hybridoma Bank, mouse, 1:50), anti Ki-67 (Thermo Scientific,

rabbit, 1:30), anti SM MHC (Biomedical Technologies Inc, rabbit 1:30), or anti-phospho-histone H3 (PH3) (Millipore, rabbit, 1:50) antibodies. For IF, the sections were stained with secondary antibody (488nm anti-rabbit secondary antibody or 647nm anti-mouse secondary antibody, 1:250 dilution, Invitrogen) diluted in blocking buffer for 1 hour at room temperature. Sections were then immersed with mounting medium (ProLong Gold antifade reagent with DAPI, Invitrogen) to visualize nuclei. Sections stained with IF were imaged using confocal microscopy (LS510 meta, Zeiss). The fluorescence intensity was quantified by Image J software. For IHC, sections were stained using the Vectastain Elite ABC Kit (Vector Laboratories) by using anti YAP antibody (Cell Signaling, rabbit, 1:30) as described in our recent report [4]. HE staining was performed following standard protocol as previously described [3, 4]. HE staining images were acquired using an Olympus BX51 inverted microscope. The thickness of myocardium wall, arterial wall and arterial lumen area was measured by Image J software.

Preparation of primary aortic SMCs from adult rat or mouse embryos. Rat arterial tissues were harvested and primary rat aortic SMCs were prepared and cultured as described in our previous reports [3-5]. For preparation mouse embryonic vascular SMCs, dorsal aorta from mouse E15.5 embryos was dissected and isolated. After removing periadventitial tissues under dissecting microscope, the aorta was then minced into small pieces and placed as explants under glass cover-slips in 24-well plate containing 10% FBS DMEM medium. After 5-7 days, SMCs that migrated out from the aortic explants were trypsinized and replated into 6-well plate. Staining with SM MHC antibody confirmed >99% SMC purity (Online Figure VI). SMCs were used for experiments from passage 3 to passage 6.

siRNA transfection. Scrambled control siRNA and siRNA targeting mouse and rat Gpr132 (ON-TARGETplus SMARTpool) were purchased from Thermo Scientific. Delivery siRNA into rat

primary aortic SMCs and mouse embryonic aortic SMCs was done using Neon transfection system (Invitrogen) essentially following the manufacturer's protocol and as described in our previous report [3]. After electroporation for 24 hours cells were equally plated for BrdU incorporation assays or counted at each time point as indicated in figures. After electroporation 48 hours cells were harvested to extract total RNA for qRT-PCR to validate the knock-down efficacy.

BrdU incorporation assay. Dorsal aortic SMCs were prepared from E15.5 control or mutant embryos and grown (passage 4) on coverslips. After 16 hours serum starvation cells were treated with 10uM BrdU (5-bromodeoxyuridine, Sigma) for 16 hours. Subsequently cells were then fixed, permeabilized by using Triton X-100, and incubated with monoclonal anti BrdU antibody (Sigma, mouse, 1:200), followed by incubation with 647nm anti-mouse secondary antibody (1:250) (Molecular Probes). Cells were then immersed in ProLong Gold mounting medium with DAPI (Invitrogen) to visualize nuclei. BrdU incorporation assay in rat aortic primary SMCs (passage 6) was performed as described as above except cells were heated at 98°C for 5 minutes in citric acid buffer for antigen retrieval to replace Triton X-100 permeabilization. Images were obtained using a Zeiss Axio Observer Z1 inverted microscope at 20x magnification. For SMC counting, SMCs were seeded in 12-well plate in triplicates at equal density and then cell numbers were manually counted at indicated days by a hemocytometer.

Adenoviral construction and cell infection. Adenovirus encoding Flag tagged YAP was generated as described previously [4, 5]. To generate mouse Gpr132 adenovirus, a cDNA clone encoding mouse Gpr132 was purchased from Thermo Scientific (Clone ID: 40129445) and subcloned into AdTrack shuttle vector by PCR with primers harboring KpnI and HindIII restriction enzyme sites (primer sequences were listed in the Online Table III). As this vector

contains an independent cytomegalovirus promoter-driven transcription cassette for green fluorescent protein (GFP) in addition of Gpr132, the efficiency of transduction can be directly monitored by visualization of GFP expression. Transferring the Gpr132 and GFP expression cassettes into AdEasy viral backbone, viral packaging and cell infection was performed as we previously reported [3, 5].

Cell cycle analysis by flow cytometry. Rat aortic SMCs were transduced with control GFP adenovirus or adenovirus co-expressing Gpr132 and GFP for 48 hours. Cells were then harvested and washed with phosphate-buffered saline and fixed overnight with 70% ice-cold ethanol at -20 °C. The fixed cells were stained with a solution containing propidium iodide (25 μg/ml) and then analyzed with a Becton Dickinson FACSAria II SORP cell sorter flow cytometer at the core facility. Only GFP positive cells in both control and over-expressing Gpr132 groups were gated for further cell cycle analysis. G_0/G_1, S and G_2/M phases were assessed by fitting the cell distribution using Cell Quest and Modfit software (Becton Dickinson).

Quantitative real time RT-PCR (qRT-PCR) analysis. Total RNA from control or mutant embryo dorsal aorta tissues was extracted by RNeasy Micro Kit (Qiagen). Total RNA from rat primary aortic SMCs was isolated with TRIzol reagent. 0.5-1 μg of RNA was utilized as a template for RT with random hexamer primers using the High Capacity RNA-to-cDNA Kit (Invitrogen). PCR array was performed to screen cell cycle related gene expression profile using Cell Cycle PCR Array kit (SABiosciences, mouse) as described in our previous report [5]. qRT-PCR was performed with respective gene-specific primers as we previously reported otherwise were listed in Online Table III [3-5]. All samples were amplified in duplicate and every experiment was repeated independently 2 times. Relative gene expression was converted using the $2^{-\triangle\triangle ct}$

method against the internal control acidic ribosomal phosphoprotein P0 (RPLP0) house-keeping gene for rat and hypoxanthine phosphor ribosyl transferase 1 (HPRT) for mouse.

Co-immunoprecipitation. Rat primary aortic SMCs were transduced with adenovirus encoding YAP. 48 hrs after transduction, nuclear protein was harvested for co-immunoprecipitation assays with anti-HDAC4 antibody (Millipore, rabbit) using Nuclear Complex Co-IP Kit (Active Motif) as described in our previous reports [6, 7]. Western bot was performed using HDAC4, YAP, TEAD1 antibody as described below.

Protein extraction and Western blotting. Dorsal aortae were dissected from E15.5 control or mutant mouse embryos and periadventitia tissues were removed under a stereoscope. Tissues were cut into small pieces and ground with a glass homogenizer in RIPA buffer (Fisher) with 1% proteinase inhibitor cocktail (Pierce) and 1% PMSF. After sonication and centrifugation of the cell lysate, proteins were quantified by BCA assay and then loaded in a 6-9% SDS-PAGE gel at 5-10 μg per lane. Protein was extracted from retrovirus infected rat primary aortic SMCs as previously described [3]. Antibodies used in this study were: YAP (Sigma, mouse, 1:2000), SM α-actin (Sigma, AC-74, 1:4000), calponin (Santa Cruz, N-15R, 1:1000), Hic-5 (BD, 1:5000), MLCK (Sigma, clone K36, 1:5000), Gpr132 (Proteintech, rabbit, 1:2000), Trp63 (Santa Cruz, 4A4, mouse, 1:1000), stratifin (Sfn, Millipore, AB9742, rabbit, 1:2000), PCNA (Santa Cruz, 1:500), cyclin D1 (Ccnd1, Cell signaling, #2926, 1:1000), α-tubulin (Cell Signaling, 1:5000), GAPDH (Santa Cruz, 1:2000), TEAD1 (BD, 1:2000), HDAC4 (Millipore, 1:2000). Images were taken by ImageQuant LAS 4000 Imaging Station (GE) and band densities were quantified using the ImageQuant TL software (GE).

Luciferase reporter assays. Cloning of GPR132 promoter luciferase reporter and dual luciferase assay were performed as described in our recent report [4]. A 1453bp fragment spanning GPR132 first intron (mouse Chr12: 112,857,974 - 112,859,426) was amplified by PCR with primers harboring KpnI and XhoI restriction enzyme sites by using mouse genomic DNA as template (Promega) (primer sequences were listed in the Online Table III). The PCR product was first cloned into pSC-B blunt vector (Stratagene) then subcloned into pGL2-Enhancer (Promega) luciferase reporter vector. A conserved MCAT element within GPR132 gene promoter was identified by a sequence alignment among mouse, rat and human (http://genome.ucsc.edu). Mutation of the MCAT element in the GPR132 promoter was carried out with QuickChange Site-Directed Mutagenesis kit (Stratagene). All plasmids were sequenced to verify the integrity of the insert. Mammalian expression plasmids for YAP and HDAC4 were generously provided by Dr. Kun-liang Guan, UCSD [8] and Dr. Stuart L. Schreiber at Harvard University [9], respectively. Transfection was carried out with X-tremeGENE 9 transfection reagent (Roche) essentially following manufactory's protocol. The promoter activity was evaluated by measurement of the firefly luciferase activity relative to the internal control TK-renilla luciferase activity using the Dual Luciferase Assay System as described by the manufacturer (Promega). A minimum of six independent transfections was performed and all assays were replicated at least twice. Results are reported as the mean ± SE.

Quantitative Chromatin immunoprecipitation (ChIP) assays. After infection with adenovirus expressing GFP or Flag-tagged YAP, rat primary aortic SMCs were fixed with formaldehyde, and ChIP was performed by using an anti-Flag (Sigma) or anti-HDAC4 (Millipore) antibody as described by the manufacturer (Active Motif) and in our previous report [4, 6]. Sequential ChIP with anti-HDAC4 antibody or IgG control was performed with anti-YAP antibody immunoprecipitated chromatin using Re-ChIP-IT kit (Active Motif). ChIP assay was repeated

twice and each assay was duplicated. Primers for quantitative evaluation of enrichment of the Gpr132 promoter MCAT region and exon 3 are listed in Online Table III.

Statistical analysis. Data are expressed as means ± SE, and statistical analysis using unpaired t test was done with Prism software (Graphpad). Differences with p values < 0.05 were considered significant.

References

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Online Figure Legends

Online Figure I. Validation of targeted deletion of YAP. A. Genomic DNA was extracted from yolk sacs of mouse E13.5 embryos and PCR was performed to detect YAP floxed allele and SM22α-Cre transgene. F: Floxed; W: WT. **B.** IHC was performed by using anti-YAP antibody to determine YAP expression (brown) in the heart and descending aorta (De.A) from E15.5 control or KO embryo sections. Species-matched IgG served as a negative control. Nuclei were visualized by hematoxylin counter staining (blue). Note that thinning myocardium wall and descending aorta wall, and enlargement of arterial lumen in mutant embryos. RV: Right Ventricle; ES: esophagus. Scale bar: 100um. **C.** Western blotting was carried out to determine YAP expression in the heart from E13.5 control, heterozygote, or KO embryos. An arrow pointed to a non-specific band of YAP.

Online Figure II. YAP mutant pups develop cyanosis. Photograph of P0 control and cardiac/smooth muscle-specific YAP knock out (KO) pups as indicated (N=8). Shortly after birth mutant pups displayed blue coloration, an indicative of lack of oxygenated blood circulation.

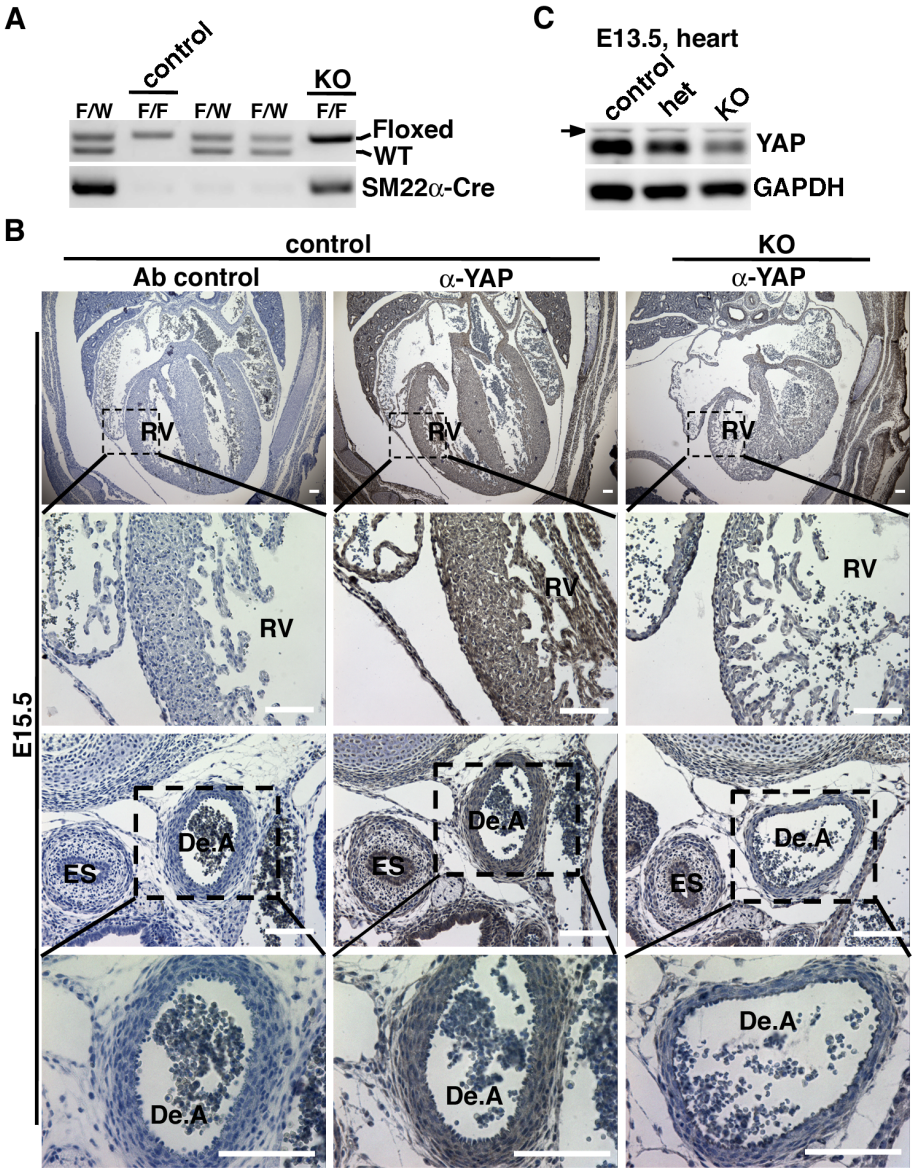
Online Figure III. YAP mutants exhibit severe hypoplastic myocardium. A-G. HE staining of heart sections from control or YAP mutants of E11.5, E12.5, E13.5, E14.5, E15.5, E18.5 embryos and P0 neonates as indicated. Magnifications of right ventricle (RV) or left ventricle (LV) shown below each whole heart sections indicate drastic hypoplasia of compact myocardium in the mutants. Scale bar: 100um. **H.** The thickness of RV and LV myocardium wall at each time point as shown in "A-G" was quantified and presented relative to control (set to 1). N=5-6 embryos per genotype. *$p<0.05$.

Online Figure IV. YAP KO mutants display reduced proliferation of cardiomyocytes. Heart sections from E11.5 control or mutant embryos were stained with proliferative marker Ki67 or mitosis marker phospho-histon H3 (PH3) (green), and cardiomyocyte marker MF20 (red). DAPI staining (blue) was utilized to visualize nuclei. Images were taken within right ventricle (RV) (**A**), left ventricle (LV) (**C**) and septum (**E**) area as indicated. The percentage of Ki67 or PH3 positive cardiomyocytes was plotted as shown along with representative staining photos (**B, D, F**). N=4 embryos per genotype. Scale bar: 50um *$p<0.05$.

Online Figure V. YAP mutants exhibit reduced SMC investment in arterial wall without affecting SM MHC expression. A. Sections from E15.5 control or mutant left carotid artery were stained with smooth muscle-specific marker SM MHC (green) and counter-stained with DAPI (blue) to visualize nuclei. SM MHC positive cells were then counted and plotted (**B**). The average of SM MHC immuno-fluorescence intensity of each SMC was quantified by Image J software and presented in **C**. N=4 embryos per genotype. *$p<0.05$.

Online Figure VI. High purity SMCs cultured from embryonic dorsal aorta. Primary aortic SMCs derived from E15.5 control or YAP mutant dorsal aorta were stained with smooth muscle-specific marker SM MHC (green) and counter-stained with DAPI (blue). More than 99% are SM MHC staining positive. Scale bar: 50um.

Online Figure I



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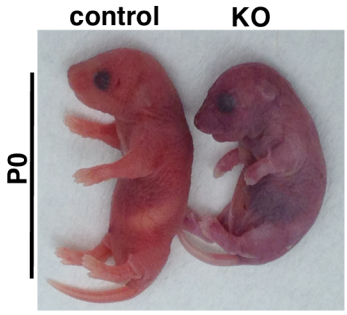

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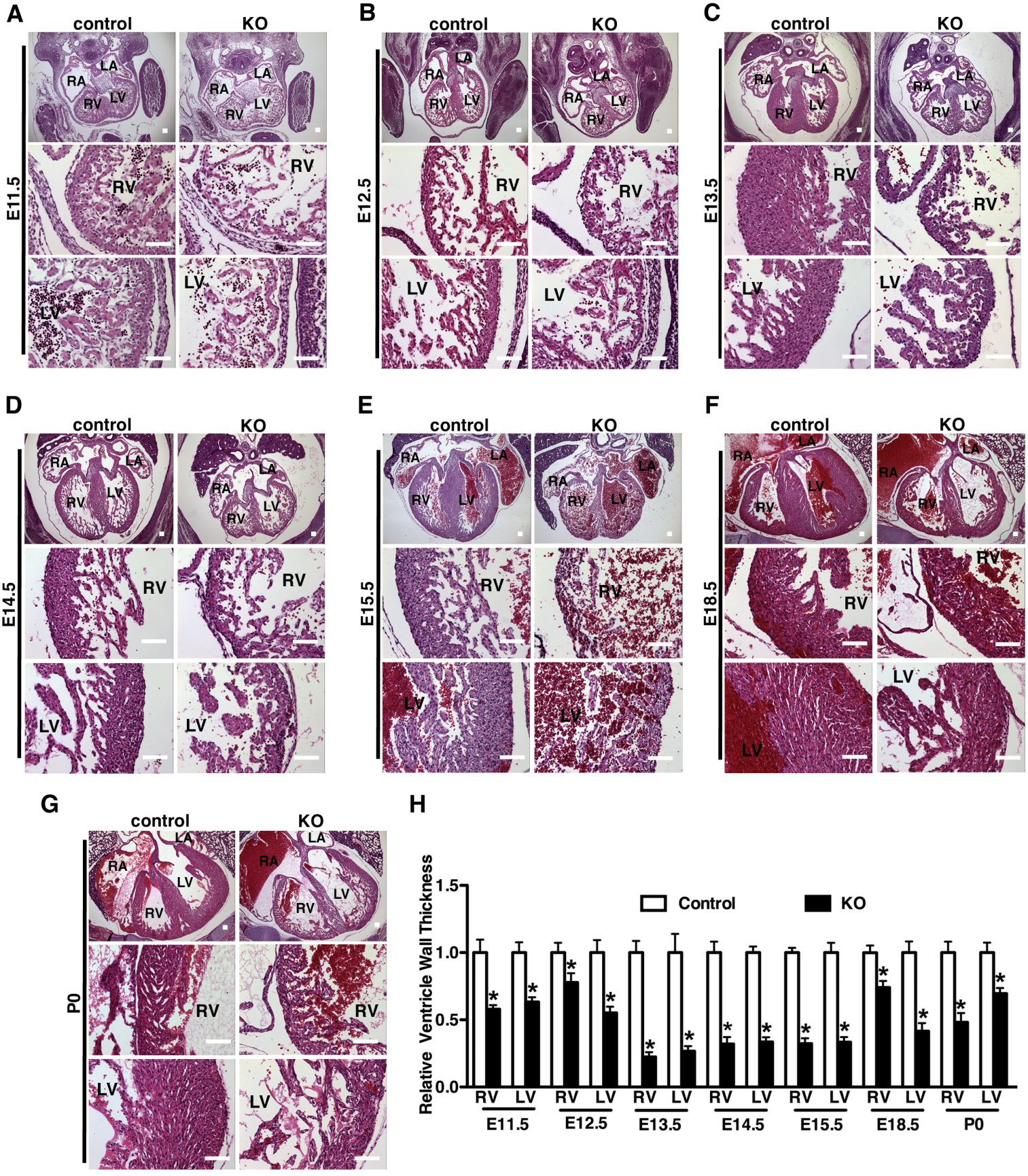

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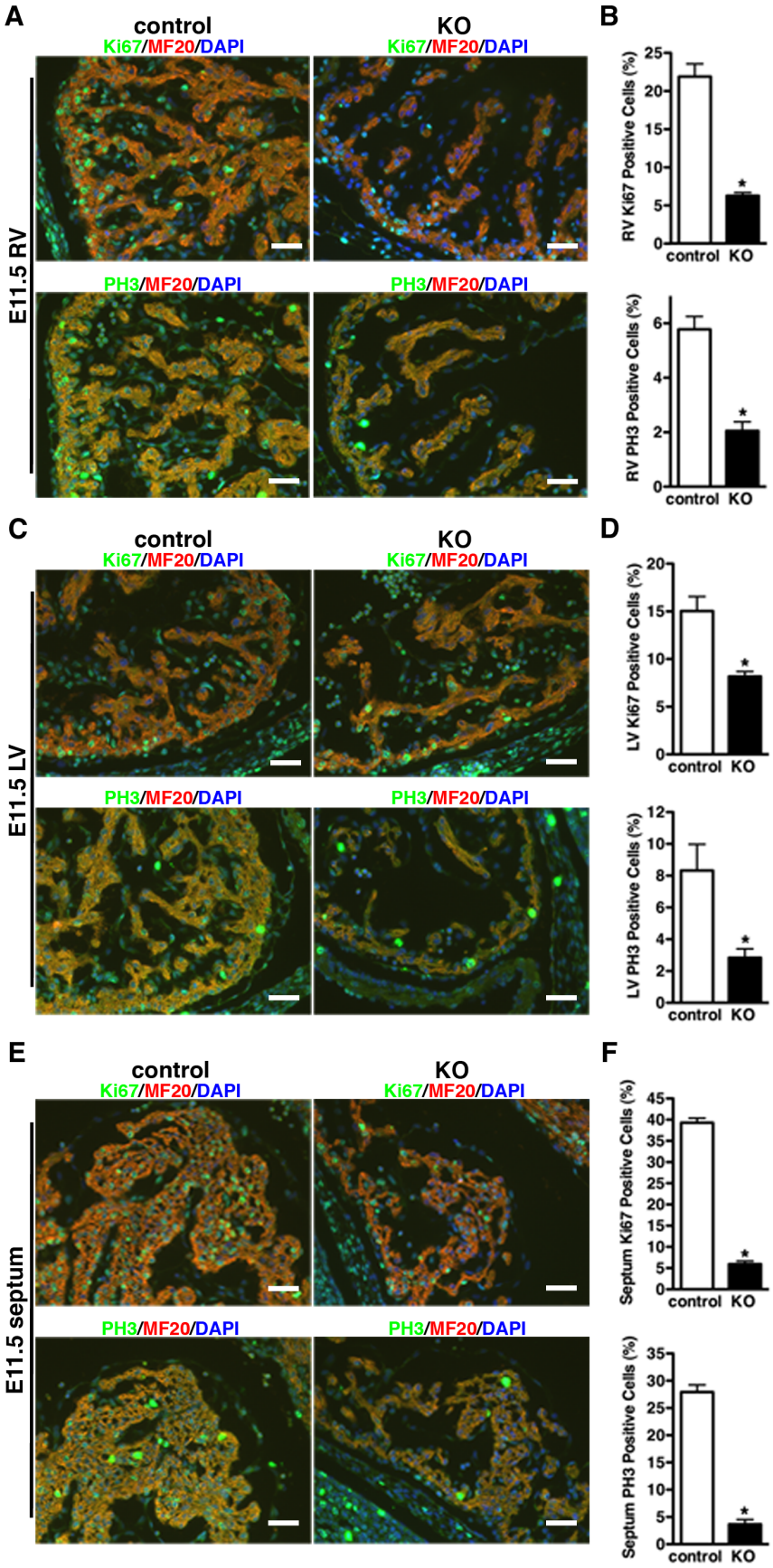

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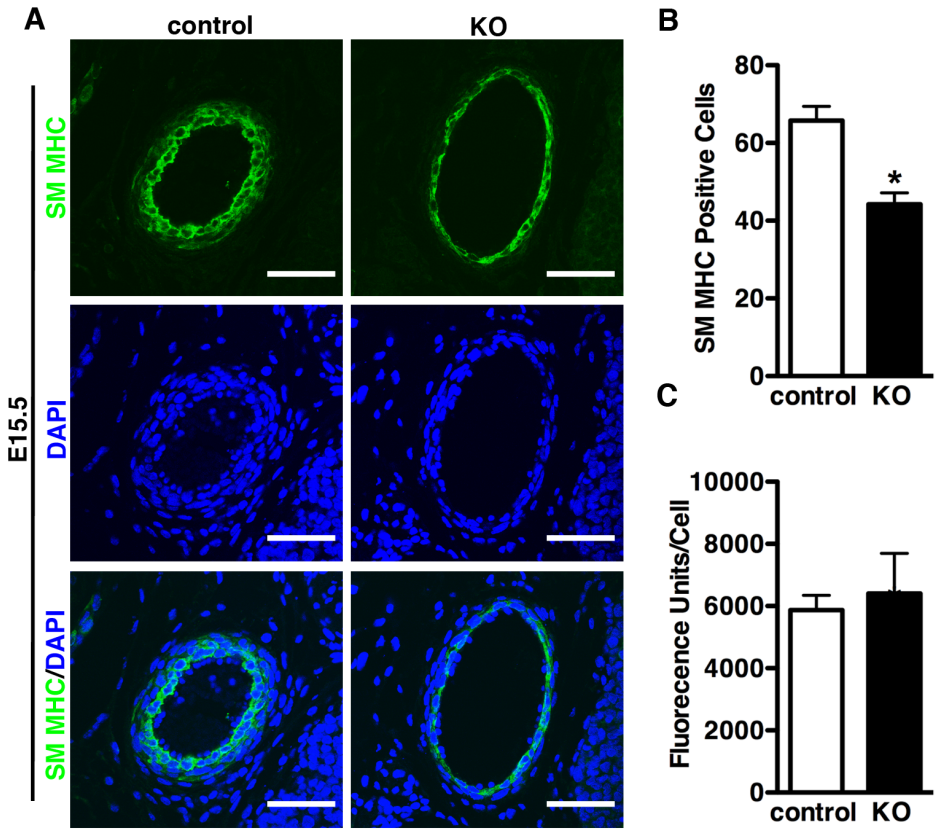

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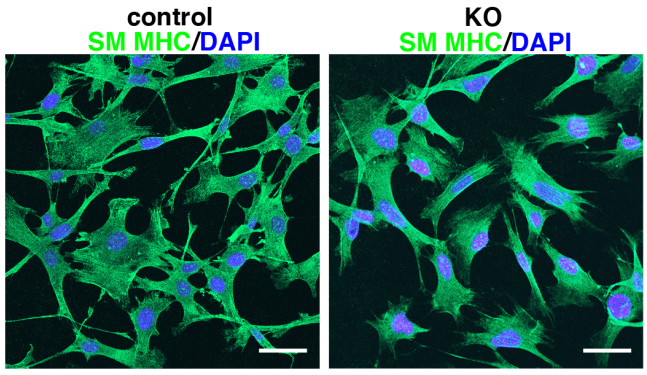

Online Table I

Genotype distribution in cardiac/smooth muscle-specific YAP knock-out offspring from mating pair of SM22α-Cre^{+}/YAP$^{F/W}$ male X YAP$^{F/F}$ female mice.

Genotype	E10.5	E11.5	E12.5	E13.5	E14.5	E15.5	E16.5	E17.5-18.5	P0	P1-P14
SM22α-Cre^{-}/YAP$^{F/W}$	6	7	8	11	9	10	24	21	30	35
SM22α-Cre^{-}/YAP$^{F/F}$ (control)	3	9	7	12	14	10	17	19	24	37
SM22α-Cre^{+}/YAP$^{F/W}$	3	9	10	13	20	18	15	21	30	34
SM22α-Cre^{+}/YAP$^{F/F}$ (KO)	4	7	8	12	13	13	11	11	8	1*
Total	16	32	33	48	56	51	67	72	92	107
KO ratio (%)	25%	22%	24%	25%	23%	25%	16%	15%	9%	1%

* died on P21

Online Table II

PCR array results using cDNA from E15.5 control and YAP KO dorsal aorta tissues.

Symbol	Well	AVG ΔC$_t$ (Ct(GOI) - Ave Ct (HKG))		2^-ΔC$_t$		Fold Change	Fold Up- or Down-Regulation
		YAP KO	WT Control	YAP KO	WT Control	YAP KO /WT Control	YAP KO /WT Control
Abl1	A01	3.88	3.74	6.8E-02	7.5E-02	0.91	-1.10
Atm	A02	6.42	6.53	1.2E-02	1.1E-02	1.08	1.08
Atr	A03	5.10	5.18	2.9E-02	2.8E-02	1.06	1.06
Aurka	A04	4.48	4.57	4.5E-02	4.2E-02	1.07	1.07
Aurkb	A05	4.17	4.15	5.6E-02	5.6E-02	0.99	-1.01
Bcl2	A06	4.26	4.42	5.2E-02	4.7E-02	1.12	1.12
Birc5	A07	3.42	3.51	9.4E-02	8.8E-02	1.07	1.07
Brca1	A08	4.56	4.76	4.2E-02	3.7E-02	1.15	1.15
Brca2	A09	5.27	5.39	2.6E-02	2.4E-02	1.09	1.09
Casp3	A10	4.19	3.99	5.5E-02	6.3E-02	0.87	-1.15
Ccna1	A11	9.22	9.89	1.7E-03	1.1E-03	1.60	1.60
Ccna2	A12	2.32	2.20	2.0E-01	2.2E-01	0.92	-1.09
Ccnb1	B01	3.28	3.26	1.0E-01	1.0E-01	0.99	-1.01
Ccnb2	B02	3.41	3.57	9.4E-02	8.4E-02	1.11	1.11
Ccnc	B03	6.68	6.89	9.7E-03	8.4E-03	1.15	1.15
Ccnd1	B04	2.85	2.25	1.4E-01	2.1E-01	0.66	-1.51
Ccnd2	B05	1.27	1.17	4.2E-01	4.5E-01	0.93	-1.07
Ccnd3	B06	1.38	1.48	3.8E-01	3.6E-01	1.07	1.07
Ccne1	B07	4.70	4.82	3.9E-02	3.5E-02	1.09	1.09
Ccnf	B08	5.16	5.35	2.8E-02	2.4E-02	1.14	1.14
Cdc20	B09	4.64	4.84	4.0E-02	3.5E-02	1.15	1.15
Cdc25a	B10	5.06	5.20	3.0E-02	2.7E-02	1.10	1.10
Cdc25c	B11	5.98	6.05	1.6E-02	1.5E-02	1.05	1.05
Cdc6	B12	5.45	5.53	2.3E-02	2.2E-02	1.06	1.06
Cdc7	C01	6.49	6.95	1.1E-02	8.1E-03	1.38	1.38
Cdk1	C02	2.64	2.71	1.6E-01	1.5E-01	1.04	1.04
Cdk2	C03	3.10	3.16	1.2E-01	1.1E-01	1.05	1.05
Cdk4	C04	1.18	1.22	4.4E-01	4.3E-01	1.03	1.03
Cdk5rap1	C05	6.51	6.81	1.1E-02	8.9E-03	1.23	1.23
Cdk6	C06	3.25	3.35	1.0E-01	9.8E-02	1.07	1.07
Cdkn1a	C07	4.92	5.32	3.3E-02	2.5E-02	1.31	1.31
Cdkn1b	C08	2.39	2.60	1.9E-01	1.7E-01	1.16	1.16
Cdkn2a	C09	11.94	12.58	2.5E-04	1.6E-04	1.56	1.56
Cdkn2b	C10	9.04	9.21	1.9E-03	1.7E-03	1.13	1.13
Cdkn3	C11	5.31	5.13	2.5E-02	2.8E-02	0.88	-1.13
Chek1	C12	4.57	4.75	4.2E-02	3.7E-02	1.14	1.14
Chek2	D01	5.57	5.80	2.1E-02	1.8E-02	1.17	1.17
Cks1b	D02	3.69	3.94	7.8E-02	6.5E-02	1.19	1.19
Ddit3	D03	4.57	4.81	4.2E-02	3.6E-02	1.19	1.19
Dst	D04	2.42	2.46	1.9E-01	1.8E-01	1.03	1.03
E2f1	D05	5.85	6.05	1.7E-02	1.5E-02	1.14	1.14
E2f2	D06	7.38	7.75	6.0E-03	4.6E-03	1.29	1.29
E2f3	D07	5.12	5.09	2.9E-02	2.9E-02	0.98	-1.02
E2f4	D08	4.07	4.24	6.0E-02	5.3E-02	1.13	1.13
Gadd45a	D09	6.89	7.38	8.4E-03	6.0E-03	1.40	1.40
Gpr132	D10	11.40	11.99	3.7E-04	2.5E-04	1.51	1.51
Hus1	D11	7.66	7.74	5.0E-03	4.7E-03	1.06	1.06
Itgb1	D12	0.01	-0.14	9.9E-01	1.1E+00	0.90	-1.11
Mad2l1	E01	3.31	3.24	1.0E-01	1.1E-01	0.96	-1.05
Mcm2	E02	3.51	3.76	8.8E-02	7.4E-02	1.19	1.19
Mcm3	E03	3.03	3.04	1.2E-01	1.2E-01	1.01	1.01
Mcm4	E04	3.22	3.21	1.1E-01	1.1E-01	0.99	-1.01
Mdm2	E05	3.51	3.75	8.8E-02	7.4E-02	1.18	1.18
Mki67	E06	1.53	1.67	3.5E-01	3.1E-01	1.10	1.10
Mre11a	E07	4.99	5.08	3.2E-02	2.9E-02	1.07	1.07
Msh2	E08	5.17	5.29	2.8E-02	2.6E-02	1.08	1.08
Myb	E09	8.55	9.07	2.7E-03	1.9E-03	1.44	1.44
Nbn	E10	4.36	4.32	4.9E-02	5.0E-02	0.97	-1.03
Nek2	E11	5.26	5.44	2.6E-02	2.3E-02	1.13	1.13
Notch2	E12	3.25	3.21	1.1E-01	1.1E-01	0.98	-1.03
Pkd1	F01	2.99	2.88	1.3E-01	1.4E-01	0.92	-1.08
Pmp22	F02	0.89	1.10	5.4E-01	4.6E-01	1.16	1.16
Ppm1d	F03	4.27	4.40	5.2E-02	4.7E-02	1.10	1.10
Rad17	F04	6.02	6.06	1.5E-02	1.5E-02	1.03	1.03
Rad21	F05	2.33	2.37	2.0E-01	1.9E-01	1.03	1.03
Rad51	F06	6.59	6.90	1.0E-02	8.4E-03	1.23	1.23
Rad9	F07	5.57	5.58	2.1E-02	2.1E-02	1.01	1.01
Ran	F08	6.32	6.45	1.3E-02	1.1E-02	1.09	1.09
Rb1	F09	4.55	4.79	4.3E-02	3.6E-02	1.19	1.19
Rbl1	F10	4.45	4.53	4.6E-02	4.3E-02	1.06	1.06
Rbl2	F11	4.90	4.96	3.4E-02	3.2E-02	1.05	1.05
Sfn	F12	8.17	8.19	3.5E-03	3.4E-03	1.01	1.01
Shc1	G01	2.55	2.72	1.7E-01	1.5E-01	1.12	1.12
Skp2	G02	4.08	4.12	5.9E-02	5.7E-02	1.03	1.03
Slfn1	G03	9.10	10.21	1.8E-03	8.5E-04	2.16	2.16
Smc1a	G04	2.38	2.38	1.9E-01	1.9E-01	1.00	1.00
Stag1	G05	3.64	3.69	8.0E-02	7.8E-02	1.03	1.03
Stmn1	G06	0.64	0.52	6.4E-01	7.0E-01	0.92	-1.09
Terf1	G07	4.09	4.31	5.9E-02	5.0E-02	1.17	1.17
Tfdp1	G08	1.91	1.93	2.7E-01	2.6E-01	1.02	1.02
Trp53	G09	3.64	3.87	8.0E-02	6.8E-02	1.18	1.18
Trp63	G10	9.93	10.86	1.0E-03	5.4E-04	1.90	1.90
Tsg101	G11	3.11	3.11	1.2E-01	1.2E-01	1.00	-1.00
Wee1	G12	4.53	4.51	4.3E-02	4.4E-02	0.99	-1.01
Actb	H01	-2.76	-2.93	6.8E+00	7.6E+00	0.89	-1.13
B2m	H02	-0.04	0.26	1.0E+00	8.3E-01	1.23	1.23
Gapdh	H03	-0.80	-1.04	1.7E+00	2.1E+00	0.85	-1.18
Gusb	H04	4.22	4.35	5.4E-02	4.9E-02	1.09	1.09
Hsp90ab1	H05	-0.62	-0.63	1.5E+00	1.5E+00	0.99	-1.01
MGDC							
RTC							
RTC							
RTC							
PPC							
PPC							
PPC							

Online Table III. List of primer sequences used in the study.

A. Primers used for quantitative RT-PCR

Gene Name	*Species*	*Sequence*
Aurka[1]	Mouse	F: 5'-GGGTGGTCTGTGCATGCTCCG-3'
	Mouse	R: 5'-GCCTCAAAAGGAGGCATCCCCACTA-3'
Aurkb[1]	Mouse	F: 5'-ATGAGCAGCGGACTGCCACG-3'
	Mouse	R: 5'-GTCCAGGGTGCCGCACATGG-3'
Ccna1	Mouse	F: 5'-ATCGCCCAGACAGAGAAGAA-3'
	Mouse	R: 5'-GCATTTGACAAGCATCAGGA-3'
Ccnd1	Mouse/rat	F: 5'-GAACACTTCCTCTCCAAAATGCCAGA-3'
	Mouse/rat	R: 5'-GAAATGAACTTCACATCTGTGGCAC-3'
Foxm1	Mouse	F: 5'-GCCATGATACAGTTTGCCATC-3'
	Mouse	R: 5'-AGAGAAAGGTTGTGACGAATAGAG-3'
Cdc25b[1]	Mouse	F: 5'-TGGTGGCCCTGTTGACAGGC-3'
	Mouse	R: 5'-GCGGCACATCCGTGGTCCAC-3'
Cdc2[1]	Mouse	F: 5'-TTTCGGCCTTGCCAGAGCGTT-3'
	Mouse	R: 5'-GTGGAGTAGCGAGCCGAGCC-3'
Cdc20[2]	Mouse	F: 5'-GGCACATTCGCATTTGGAACG-3'
	Mouse	R: 5'-TAGTGGGGAGACCAGAGGATGGAG-3'
Cdkn2a	Mouse	F: 5'-CTCACCTCGCTTGTCACAGT-3'
	Mouse	R: 5'-GCACGAACTTCACCAAGAAA-3'
HMGB2	Mouse	F: 5'-AAGAGCGACAAAGCTCGTTA-3'
	Mouse	R: 5'-ATCTTTGGCAGATTGCTCAG-3'
Gadd45a	Mouse	F: 5'-CGTAGACCCCGATAACGTGGTA-3'
	Mouse	R: 5'-CGGATGAGGGTGAAATGGAT-3'
Slfn1	Mouse	F: 5'-GCCAGACCAGCACCTGCAC-3'
	Mouse	R: 5'-AAGAGGTTGGAGGGGGCTCAT-3'
Trp63	Mouse	F: 5'-CCACCATCTATCAGATTGAGCA-3'
	Mouse	R: 5'-GAGATGAGGAGGTGAGGAGAAG-3'
Sfn	Mouse	F: 5'-ATGAAGACATGGCAGCTTTC-3'
	Mouse	R: 5'-GCTCGGTCTCTACCTTCTCC-3'
Gpr132	Rat	F: 5'-TGAGGTCAGAACCCACCAATGCAAC-3'
	Rat	R: 5'-TGCTCTCCTCGTAGGACAACGTGTG-3'
	Mouse	F: 5'-TCGGCAAGAAGTGTCCAGAATCCA-3'
	Mouse	R: 5'-TAAACCTAGCTTCGCTGGCTGTGA-3'

B. Primers used for cloning of mouse Gpr132 promoter luciferase reporter and adenovirus

Primer Name	*Sequence*
Gpr132 luci	F (KpnI): 5'-AGGTACCGTCCTTATACTCGTGTGACAAGCACT-3'
	R (XhoI): 5'-ACTCGAGGCTCTTGAATCCCTACACTATAATGTG-3'
Gpr132 MCAT mutation	F: 5'-AGTGACGTGCTAAGGTCATGGTACCAGATGTGTTGCCCTTTGTCC-3'
	R: 5'-GGACAAAGGGCAACACATCTGGTACCATGACCTTAGCACGTCACT-3'
Gpr132 adenovirus	F (KpnI): 5'-AGGTACCATGAGATCAGAACCTACCAATGCAGCA-3'
	R (HindIII): 5'- GAAGCTTTTAGCAGAGCTCCTCAGGCAGTCTCT-3'

C. Primers for quantitative ChIP assay

Primer Name	*Sequence*
Gpr132 MCAT Region	F: 5'-CAGGTAGACGAGGACATCCAGGGAT-3'
	R: 5'-CCTTTAGCACGCTGAACGCCATCAG-3'
Gpr132 E3	F: 5'-ACAGGCTGTACACGGTCTCTACAGTGTT-3'
	R: 5'-TTCCACCCCGTGTGGATCCTGCACA-3'

Note: the primer sequences are from the following studies:

1. von Gise A, Lin Z, Schlegelmilch K, Honor LB, Pan GM, Buck JN, Ma Q, Ishiwata T, Zhou B, Camargo FD, Pu WT. Yap1, the nuclear target of hippo signaling, stimulates heart growth through cardiomyocyte proliferation but not hypertrophy. *Proc Natl Acad Sci U S A*. 2012;109:2394-2399

2. Heallen T, Zhang M, Wang J, Bonilla-Claudio M, Klysik E, Johnson RL, Martin JF. Hippo pathway inhibits wnt signaling to restrain cardiomyocyte proliferation and heart size. *Science*. 2011;332:458-461