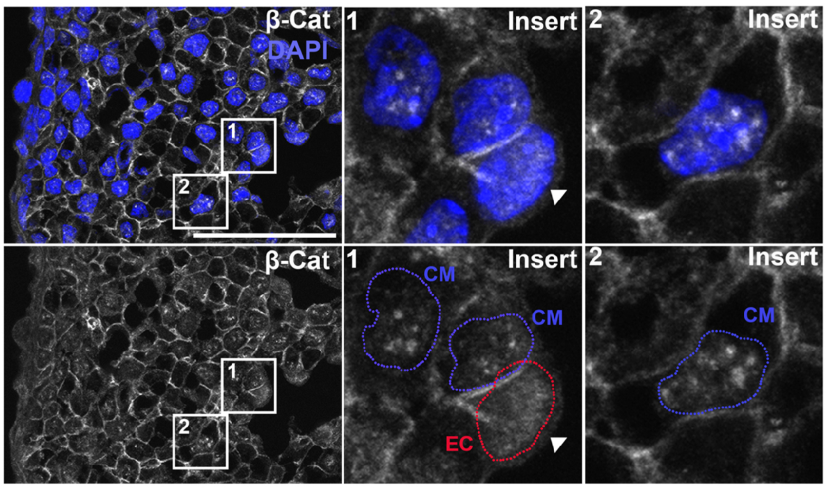
Supplemental Material

Supplementary figures and figure legends

A



B

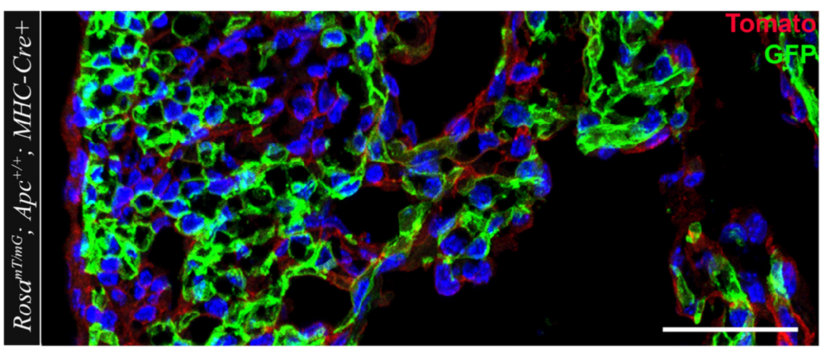


Figure S1. A, Nuclear β-catenin is clearly seen at high power magnification (100X) in CMs of WT mice at E13.5. In addition to weak diffuse nuclear staining, distinctive bright foci are present in areas lacking DAPI (euchromatin) in CMs. Endothelial cells (triangle) also contain nuclear β-catenin with pannuclear stain and no bright foci. B, Efficient *αMHC-Cre*-mediated conversion of membrane-targeted red to green fluorescent proteins is observed in both compact and trabecular layers at E13.5. Scale bar=50 µm.

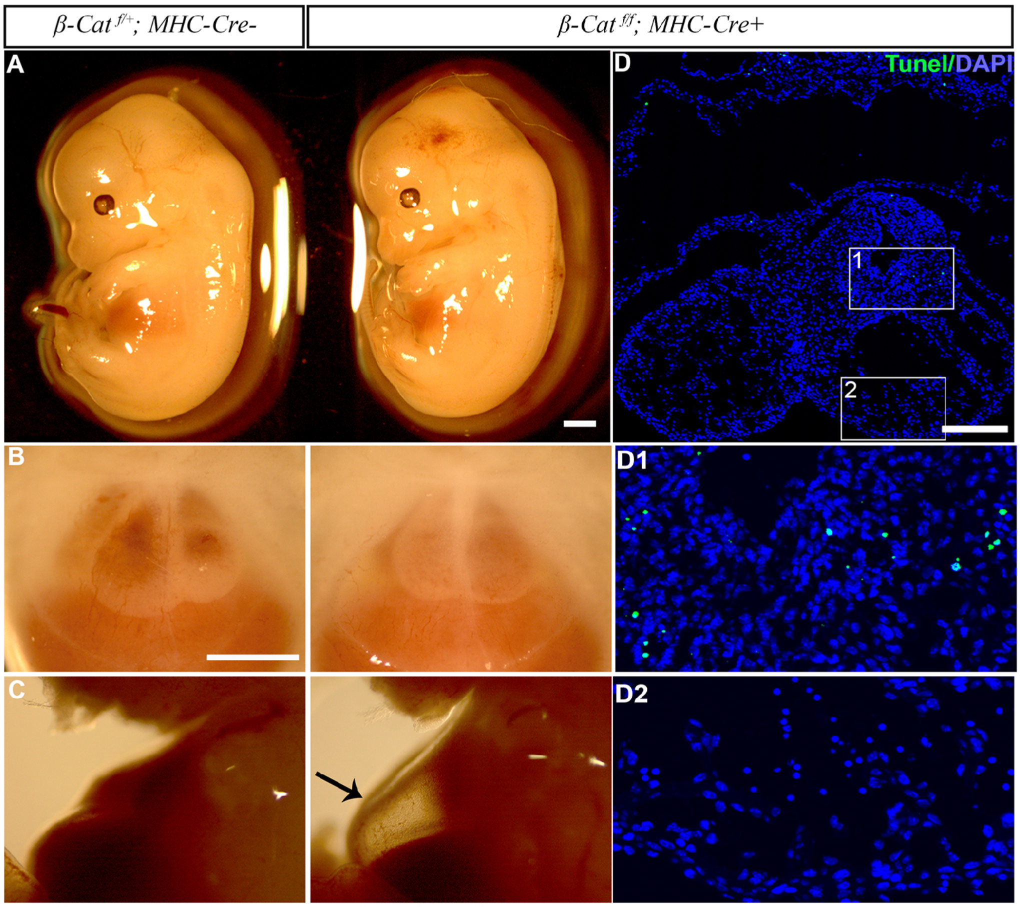


Figure S2. Gross morphology of E13.5 embryos shows that *β-Cat* cKO mice have similar body profiles to WT mice (A), but show smaller heart and bigger pericardial cavity than WT mice (B) indicating pericardial edema (C, arrow). Scale bar=1 mm. D, No apoptotic cells are observed in the ventricle wall (D2) of *β-Cat* cKO at E13.5 although both WT and KO mice have apoptotic cells in the valvular area (D1). Scale bar=200 μm.

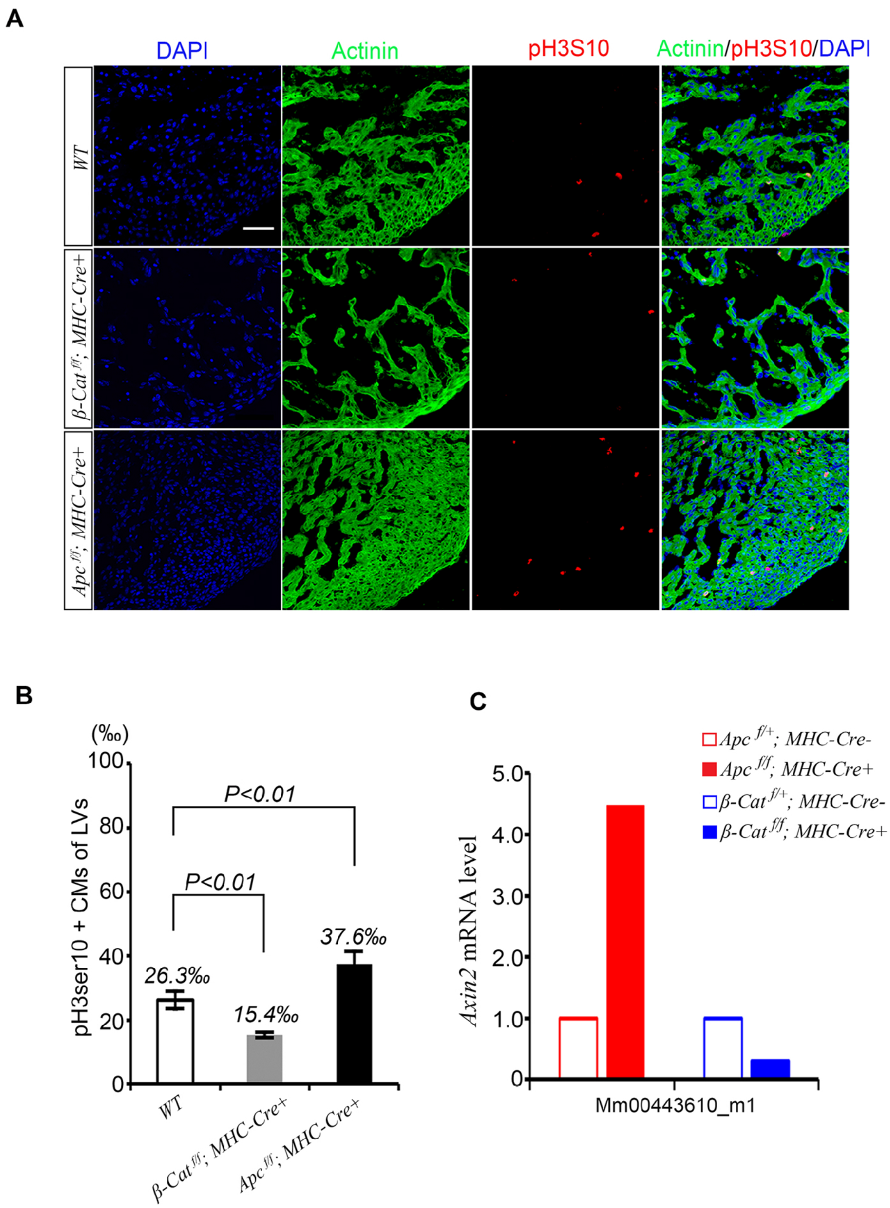
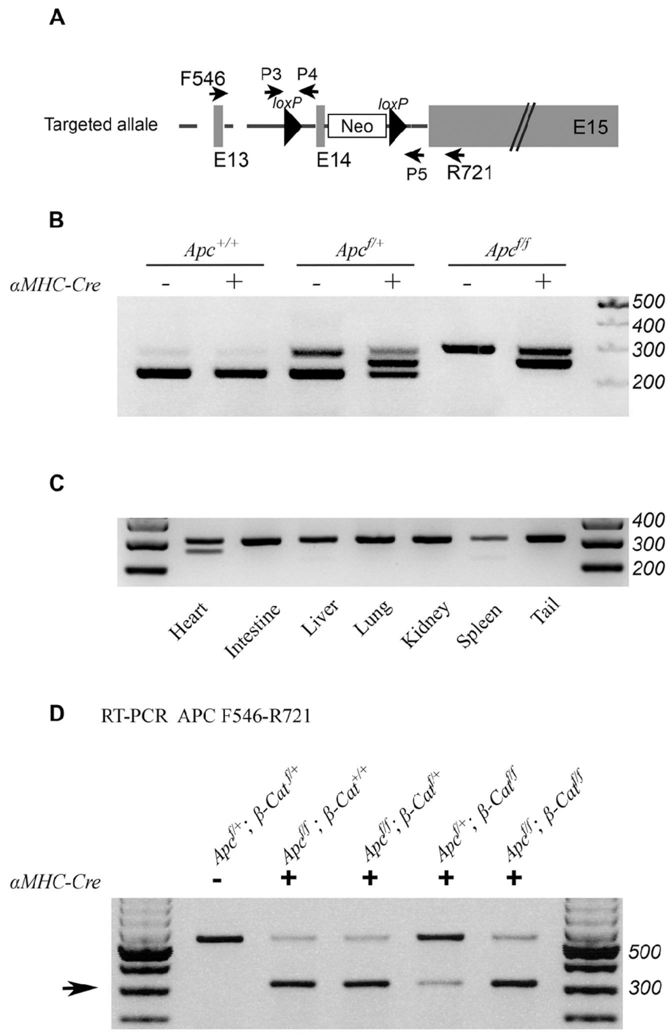


Figure S3. At E13.5, the intensity of sarcomeric α-actinin did not show significant difference between compact and trabecular layers or among different genotypes. However, pH3S10 is decreased in *β-Cat* cKO and increased in *Apc* cKO mice (A and B). Data are presented as mean ± SD. N=3-4 for each genotype. Scale bar=50 µm. C, A bar graphs of Taqman real-time PCR in E13.5 *Apc* cKO and *β-Cat* cKO shows relative fold change of *Axin2* over WT. The Taqman probe for *Axin2* is listed below the bar.



E

Apc$^{f/+}$; *β-Cat*$^{f/+}$; *αMHC-Cre+* × *Apc*$^{f/f}$; *β-Cat*$^{f/f}$; *αMHC-Cre-*

	Apc$^{f/+}$; *β-Cat*$^{f/+}$		*Apc*$^{f/f}$; *β-Cat*$^{f/+}$		*Apc*$^{f/+}$; *β-Cat*$^{f/f}$		*Apc*$^{f/f}$; *β-Cat*$^{f/f}$		*Total*
αMHC-Cre	-	+	-	+	-	+	-	+	
E17.5	47	44	42	45	46	23	56	11	314
	15.0%	14.0%	13.4%	14.3%	14.7%	7.3%	17.8%	3.5%	100%

Figure S4. Cardiac-specific deletion of *Apc* at E17.5. A, Diagram shows the position of primers used for RT-PCR (F546 and R721) and genomic PCR (P3, P4 and P5). B, Targeted (314 bp) and WT (226 bp) alleles were detected in mice with or without *αMHC-Cre* while deleted allele (258 bp) was only amplified in hearts with *αMHC-Cre*. C, PCR of genomic DNA from different organs revealed that deleted allele was only seen in the heart, but not from other organs. D, Representative RT-PCR results demonstrated that hearts with *αMHC-Cre* contained exon 14 deleted transcript (313 bp, black arrow) as well as wild type transcript (528 bp) as non-cardiomyocytes should have intact exon 14. E, Genotype frequency of *β-Cat* and *Apc* compound cKO mice at E17.5.

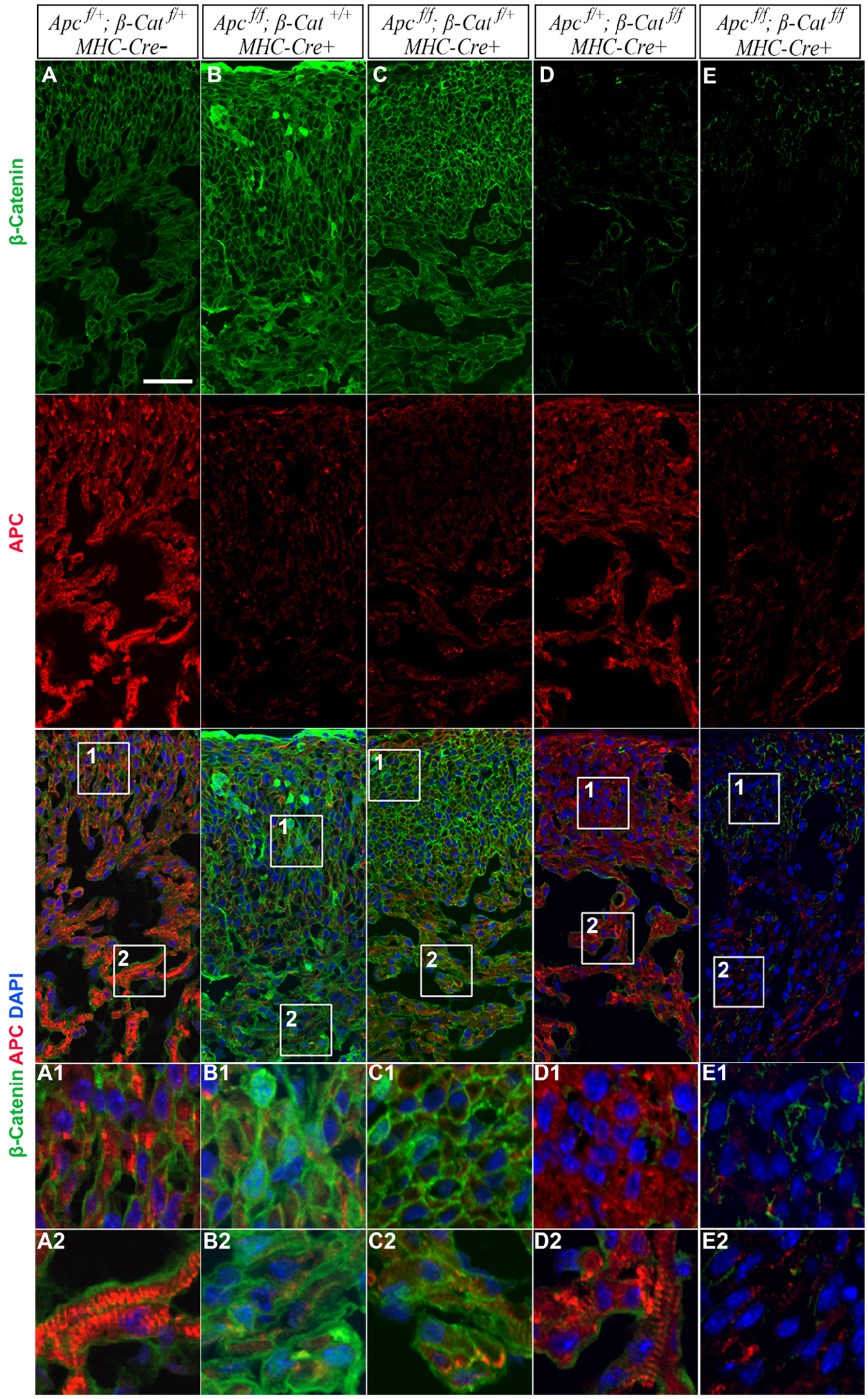


Figure S5. Representative confocal images of β-catenin and APC costained with DAPI in the left ventricle at E17.5 embryos. A-E, *Apc* deletion increases β-catenin expression in *β-Cat* WT hearts, which was partially attenuated by heterozygous *β-Cat* deletion. On the other hand, single or double *Apc* cKO could not prevent the loss of β-catenin in *β-Cat* cKO hearts at E17.5. Scale bar=50 μm.

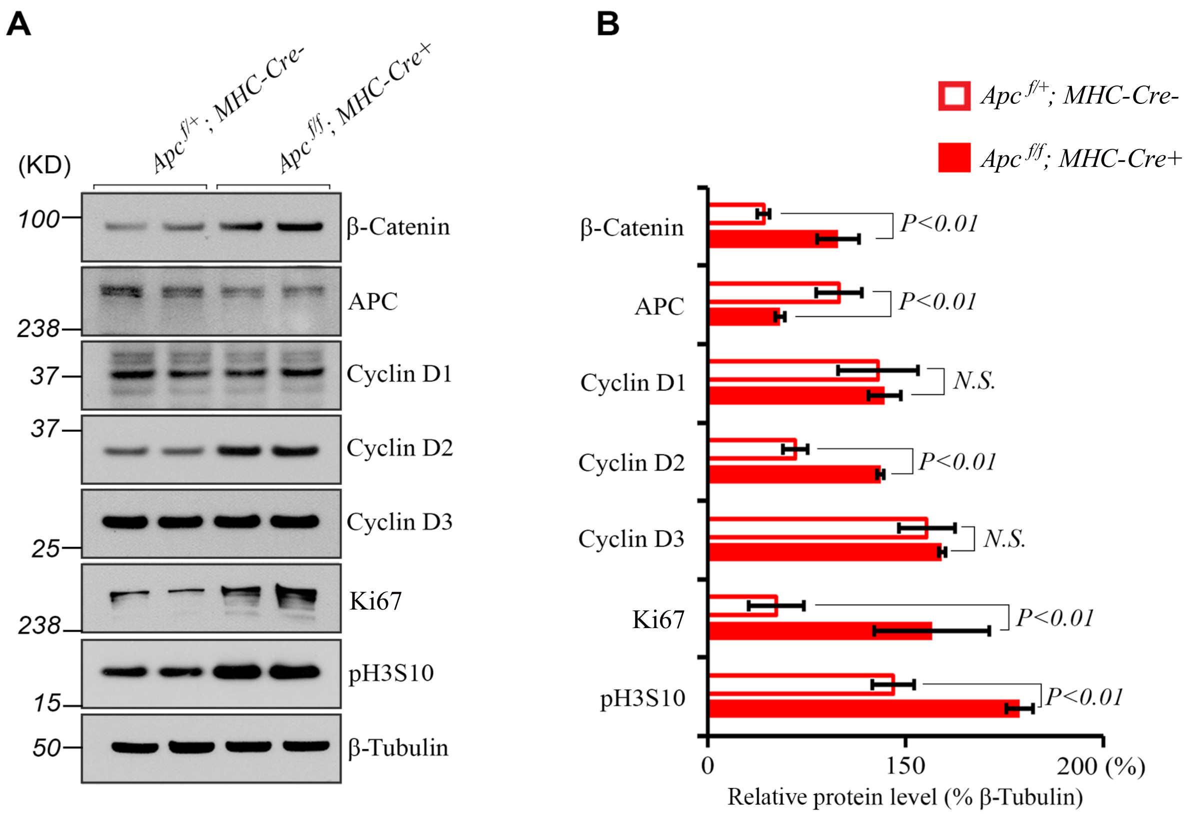


Figure S6. Representative Western blots (A) and Bar graphs (B) of relative densitomeric values show protein levels of APC, β-Catenin, cyclin Ds, Ki67, and pH3S10 in *Apc* KO and WT hearts at E17.5. Cyclin D2, β-catenin, Ki67, and pH3S10 are increased while APC is downregulated upon cardiac Apc deletion at E17.5. Band intensities are quantified by ImageJ and normalized to β-tubulin. Data are presented as mean ± SD. N=3-4 for each genotype.

Supplementary tables

Table S1. Genotyping results of embryos from timed pregnancy between *β-Cat*$^{f/f}$; *αMHC-Cre*$^{-}$ and *β-Cat*$^{f/+}$; *αMHC-Cre*$^{+}$ mice.

Age	*β-Cat*$^{f/+}$		*β-Cat*$^{f/f}$		Total
	αMHC-Cre^{-}	*αMHC-Cre*$^{+}$	*αMHC-Cre*$^{-}$	*αMHC-Cre*$^{+}$	
E13.5	16(24.2%)	18(27.3%)	14(21.2%)	18(27.3)*	66
E14.5	13(25.5%)	13(25.5%)	15(25.5%)	10(19.6%)*	51
E15.5	21(45.7%)	11(23.9%)	12(26.1%)	2(4.3%)*	46

*2 of 18 were dead at E13.5, 7 of 10 were dead at E14.5, 1 of 2 was dead at E15.5.

Table S2. Genotyping results of embryos from timed pregnancy between *Apc* $^{f/f}$; *αMHC-Cre*$^{-}$ and *Apc* $^{f/+}$; *αMHC-Cre*$^{+}$ mice.

Age	*Apc* $^{f/+}$		*Apc* $^{f/f}$		Total
	αMHC-Cre^{-}	*αMHC-Cre*$^{+}$	*αMHC-Cre*$^{-}$	*αMHC-Cre*$^{+}$	
E13.5	19(27.5%)	23(33.3%)	12(17.4%)	15(21.7)	51
E15.5	22(25.6%)	21(24.4%)	25(29.1%)	18(21.0%)	86
E17.5	38(26.6%)	36(25.2%)	33(23.1%)	36(25.2%)	143

Table S3. Primer pairs for real time RT-PCR

Targets	Primer pairs	
	Forward Primer	Reverse Primer
β-catenin	TCAAGAGAGCAAGCTCATCATTCT	CACCTTCAGCACTCTGCTTGTG
Apc	AACCTGTCTGCACACTGCAC	CAAGCTGGACACATTCCGTA
CyclinD1	GCGTACCCTGACACCAATCTC	CTCCTCTTCGCACTTCTGCTC
CyclinD2	GAGTGGGAACTGGTAGTGTTG	CGCACAGAGCGATGAAGG T
CyclinD3	TGCGTGCAAAAGGAGATCAAG	GGACAGGTAGCGATCCAGGT
GSK3β	ATGGCAGCAAGGTAACCACAG	TCTCGGTTCTTAAATCGCTTGTC
Myc	TAACTCGAGGAGGAGCTGGA	GCCAAGGTTGTGAGGTTAGG
Gata4	CCACGGGCCCTCCATCCAT	GGCCCCCACGTCCCAAGTC
Hand1	GGTCGGCAGGTCCTTCGTGTC	GTGCGGCGGGTGTGAGTGG
Hand2	CCCGCCGACACCAAACTCTC	CCCCCGGCTCACTGCTCTC
Myh6	ACGGTGACCATAAAGGAGGA	TGTCCTCGATCTTGTCGAAC
Myh7	GCCCTTTGACCTCAAGAAAG	CTTCACAGTCACCGTCTTGC
Myl2	ACTTCACCGTGTTCCTCACGATGT	TCCGTGGGTAATGATGTGGACCAA
Myl7	AAGGGAAGGGTCCCATCAACTTCA	AACAGTTGCTCTACCTCAGCAGGA
Nppa	ACCCTGGGCTTCTTCCTCGTCTT	GCGGCCCCTGCTTCCTCA
Nkx2.5	CTCCGATCCATCCCACTTTA	AGTGTGGAATCCGTCGAAAG
Pdgfra	CTGGTGCCTGCCTCCTATGAC	CACGATCGTTTCTCCTGCCTTAT
SMA	ATCAGCAAACAGGAATACGACGAA	AGGAATGATTTGGAAAGGAACTGG
Tbx2	GAACGGCCGTCGGGAGAAAAG	TGGGGGAGGGCGGTGGTT
Tbx3	ACCGGCATCCCTTTCTCATCC	CCTTACCGGCCACCATCCAC
Tbx5	CTACCCCGCGCCCACTCTCAT	TGCGGTCGGGGTCCAACACT
Tbx18	GGCGGCCGCGTTCCTGCTTCC	TGCCTCCCGAGATCTGTCCCCTTCC
Tbx20	ATCGCCGCGCTTATGTCCAG	CCCCGCCGCCAAACTCC

Table S4. Mean relative value of genes in *Apc* KO and *β-catenin* KO hearts over WT controls by real time RT-PCR at E13.5.

Target	Mean relative value of *Apc* KO/WT	Std. Deviation	*P* Sig. (2-tailed)	Mean relative value of *β-Cat* KO/WT	Std. Deviation	*P* Sig. (2-tailed)
β-catenin	1.4961	0.1729	0.0382	0.6848	0.0821	0.0027
Apc	0.7646	0.0909	0.0109	1.0639	0.0878	0.3349
CyclinD1	1.1438	0.3086	0.5043	0.9781	0.0405	0.4475
CyclinD2	2.1947	0.1602	0.0007	0.9084	0.0926	0.1421
CyclinD3	1.1860	0.0959	0.0784	0.9506	0.0239	0.0699
GSK3β	1.1930	0.0492	0.0210	1.0441	0.0825	0.4074
Myc	1.9395	0.0968	0.0001	0.9232	0.0780	0.2300
Gata4	1.1335	0.0901	0.0622	1.1907	0.0824	0.0160
Hand1	1.3252	0.0924	0.0259	1.2110	0.0845	0.0124
Hand2	1.3918	0.0891	0.0168	1.0639	0.0501	0.1575
Myh6	0.8885	0.0106	0.0030	1.0330	0.0419	0.2442
Myh7	1.3104	0.1177	0.0103	0.6903	0.0541	0.0006
Myl2	1.3894	0.2383	0.1054	1.9331	0.1912	0.0011
Myl7	1.6653	0.2285	0.0073	0.8287	0.1389	0.1662
Nppa	1.4189	0.0373	0.0026	1.5322	0.0761	0.0003
Nkx2.5	1.0523	0.0748	0.3494	1.1057	0.0375	0.0082
Pdgfra	1.3087	0.3594	0.2111	0.6182	0.0483	0.0053
SMA	1.2859	0.1165	0.0131	1.1161	0.1091	0.1392
Tbx2	0.7964	0.0520	0.0025	1.0988	0.0476	0.0228
Tbx3	1.1396	0.0460	0.0343	1.0721	0.0311	0.0569
Tbx5	1.2459	0.0402	0.0088	1.2594	0.0051	0.0000
Tbx18	0.7301	0.0430	0.0084	0.9608	0.0582	0.3082
Tbx20	1.2199	0.1036	0.0667	0.8450	0.0210	0.0061

Table S5. List of primary antibodies

Antibodies	Company	Catalog No.	Dilution for IHC and IF
Sarcomeric α-Actinin	Sigma	A7811	1:400
β-Catenin	Cell signaling	9582	1:200
β-Catenin	BD	610156	1:300
β-Catenin	Santa Cruz	SC-7963	1:100
APC	Santa Cruz	SC-895	1:100
Cyclin D1	Santa Cruz	SC-718	1:200
Cyclin D2	Cell Signaling	3741	1:100
Cyclin D3	Cell signaling	2936	1:300
Ki67	Abcam	ab16667	1:500
pH3S10	Millipore	06-570	1:600