SUPPLEMENTAL INFORMATION

Transcription Factor TEAD1 is Essential for Vascular Development by Promoting Vascular Smooth Muscle Differentiation

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Summary:

Online Figures I – X:

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Supplemental Figure Legends

Online Figure I. Characterization of Tagln gene promoter-directed Cre recombinase activity by using mTmG reporter mice. **A.** Schematic diagram to show smooth muscle-specific gene Tagln promoter-driven Cre mediated lineage tracing by crossing Tagln-Cre male mice with female mTmG reporter mice. The mTmG reporter mice contain a dual fluorescence reporter transgene integrated into the ROSA 26 locus. Upon Cre-mediated removal of membrane localized Tomato (mT)-stop cassette thereby inactivating mT expression, membrane localized GFP (mG) will be switched on to permanently mark the Cre-expressing cells. **B.** E13.5 Tagln-Cre^{-}/mTmG$^{+/-}$ or Tagln-Cre^{+}/mTmG$^{+/-}$ embryos were collected for photographing under a stereoscope with bright field or GFP filter. Only Tagln-Cre^{+}/mTmG$^{+/-}$ embryos show the GFP signal specifically detected in the heart and dorsal aorta (DA). RV: right ventricle. LV: left ventricle. **C.** Heart sections were prepared from E13.5 Tagln-Cre^{-}/mTmG$^{+/-}$ or Tagln-Cre^{+}/mTmG$^{+/-}$ embryos and IF was performed to examine the GFP (red) and early CMC marker ACTA2 (green) or CMC marker MYH1E (green) as indicated. Nuclei were counter stained by DAPI. **D.** Dorsa aorta sections were prepared from E13.5 Tagln-Cre^{-}/mTmG$^{+/-}$ or Tagln-Cre^{+}/mTmG$^{+/-}$ embryos and IF was performed to examine the GFP (red) and smooth muscle-specific marker ACTA2 (green). Nuclei were counter stained by DAPI. Tagln gene promoter directed Cre recombinase activity is only detected in CMCs and VSMCs during mouse embryonic development.

Online Figure II. Validation of targeted deletion of *Tead1* in CMCs and VSMCs. A. Schematic diagram to generate *Tead1* CMC/VSMC-specific KO mouse with mTmG reporter. After crossing Tagln-Cre male mice with Tead1$^{F/F}$ female mice, the resultant male heterozygous mice were crossed with mTmG$^{+/+}$/Tead1$^{F/F}$ female mice to generate CMC/VSMC-specific *Tead1* KO mouse. This breeding strategy is expected to yield 4 different genotype progenies in mTmG reporter background as indicated. **B.** Example of genotyping result of E13.5 embryos collected from breeding pair of Tagln-cre^{+}/Tead1$^{F/F}$ male mouse with Tead1$^{F/F}$/mTmG$^{+/+}$ female mouse. F: Floxed; W: WT; M: DNA marker. **C.** Total RNA was extracted from the GFP positive cells sorted from E13.5 control (Tagln-cre^{+}/Tead1$^{F/W}$/mTmG$^{+/-}$) or *Tead1* KO (Tagln-cre^{+}/Tead1$^{F/F}$/mTmG$^{+/-}$) heart and aortic tissues. Following cDNA synthesis, RT-PCR was performed to determine the deletion of *Tead1* in embryonic CMCs and VSMCs. **D.** IF was performed by using anti-TEAD1 antibody to determine TEAD1 expression (red) or anti-MYH1E to mark CMCs in the heart from E13.5 control or KO embryos. DAPI was used to stain nuclei. RV: right ventricle. Arrows point to epicardium and arrow heads indicate endocardium, respectively. **E.** IF was performed as described in "D" except using ACTA2 antibody to mark VSMCs in dorsal aorta (DA). Endothelium toward lumen or adventitial cells was indicated by arrows or arrow heads, respectively. Note that the TEAD1 immuno-signal is specifically diminished in CMCs and medial layer VSMCs in KO embryos compared to control.

Online Figure III. *Tead1* mutant embryos exhibit pericardial/peripheral perfusion, hypoplastic cardiac wall, DORV and VSD phenotype. A. Gross pictures of E13.5 or E14.5 (**B**) control and TEAD1 KO embryos show pericardial effusion (red arrows) or/and peripheral edema (green arrows). **C.** Representative HE stained images from E14.5 embryo hearts show *Tead1* KO display double outlet right ventricle (DORV) phenotype in addition to thinning myocardium wall with underdeveloped trabeculae. While pulmonary aorta derives from RV in both control and KO embryos (dark arrows, top panels), the ascending aorta from *Tead1* KO embryo rises from right ventricle (RV, bottom right panel, dark arrow) whereas the aorta in control embryo derives from left ventricle (LV, bottom left panel, dark arrow). The *Tead1* KO embryo also shows membranous ventricular septal defect (VSD, bottom right panel, green arrow) in which an abnormal opening between RV and LV can be seen in the KO embryo heart.

Online Figure IV. E12.5 *Tead1* KO mutants display reduced proliferation of CMCs. Heart sections from E12.5 control or *Tead1* mutant embryos were stained with proliferative marker MKI67 (**A**, red) or mitosis marker phospho-H3F3A (pH3F3A, **C**, red), and early cardiomyocyte marker ACTA2 (**A** and **C**, green). DAPI staining (blue) was utilized to visualize nuclei. Images were taken within right ventricle (RV), left ventricle (LV) and septum area as indicated. The percentage of MKI67 or pH3F3A positive CMCs was plotted as shown along with representative staining photos (**B** and **D**). N=3 embryos per genotype. *$p<0.05$.

Online Figure V. E13.5 *Tead1* KO mutants exhibit reduced proliferation of CMCs. Heart sections from E13.5 control or *Tead1* mutant embryos were stained with proliferative marker MKI67 (**A**, red), mitosis marker pH3F3A (**C**, red), EdU that incorporates into active DNA synthesis in proliferating cells (**E**, red), or early CMC marker ACTA2 (**A** and **C**, green) and CMC marker MYH1E (**E**, green). Nuclei were stained by DAPI (blue). The percentage of MKI67, pH3F3A and EdU positive CMCs within right ventricle (RV), left ventricle (LV) and septum areas was plotted and shown in **B**, **D** and **E**, respectively. N=4-6 embryos per genotype. *$p<0.05$.

Online Figure VI. Deletion of *Tead1* in CMCs attenuates *Pitx2c* expression and CMC differentiation. A. Schematic workflow to identify the differentially expressed genes in E13.5 Tagln-Cre^{+}/Tead1$^{F/W}$/mTmG$^{+/-}$ heterozygous control versus Tagln-Cre^{+}/Tead1$^{F/F}$/mTmG$^{+/-}$ mutants by RNA-seq. GFP-positive control and *Tead1* KO cardiac and DA tissues were dissected under a fluorescent stereoscope (left panel) and subsequently GFP positive CMCs and VSMCs were dissociated by enzymatic digestion and sorted by FACS (middle panel). RNA was then extracted from the purified cells and subjected to whole transcriptome RNA-seq analysis. Note that KO heart and DA emit weaker GFP signal compared to control embryo (left panel). Representative FACS scatterplots (middle panel) further indicate that *Tead1* KO heart and DA have less population of GFP positive cells. N=3 embryos per genotype. LV: left ventricle; RV: right ventricle; DA: dorsal aorta. **B.** Top 10 significant GO and (**C**) KEGG terms enriched in down-regulated genes are plotted based on their P values. V: ventricle. **D.** Gene set enrichment analysis (GSEA) plot is to the significant enrichment of gene set of "muscle contraction" in *Tead1* KO CMC/VSMCs compared to control. NES, normalized enrichment score; FDR, false discovery rate. **E.** Comparison of RNA-seq read coverage on the *Pitx2* gene exons between control and *Tead1* KO samples (N=3 each genotype). The height of peaks (arrows, top panel) mapped to corresponding *Pitx2* gene exons (bottom panel, blue boxes) indicates the transcriptional activity of each exon. Only *Pitx2c* transcripts present in E13.5 CMCs/VSMCs and are down-regulated in *Tead1* KO samples.

Online Figure VII. Deletion of *Tead1* in CMCs attenuates the expression of cardiac contractile proteins. A. Representative Western blotting using protein lysates from E13.5 control or *Tead1* KO heart tissue is shown. **B.** Immunoblot signals as shown in "C" were normalized to TUBA loading control then expressed relative to signals from control heart (set to 1, red line). N=4 embryos per genotype. *$p<0.05$.

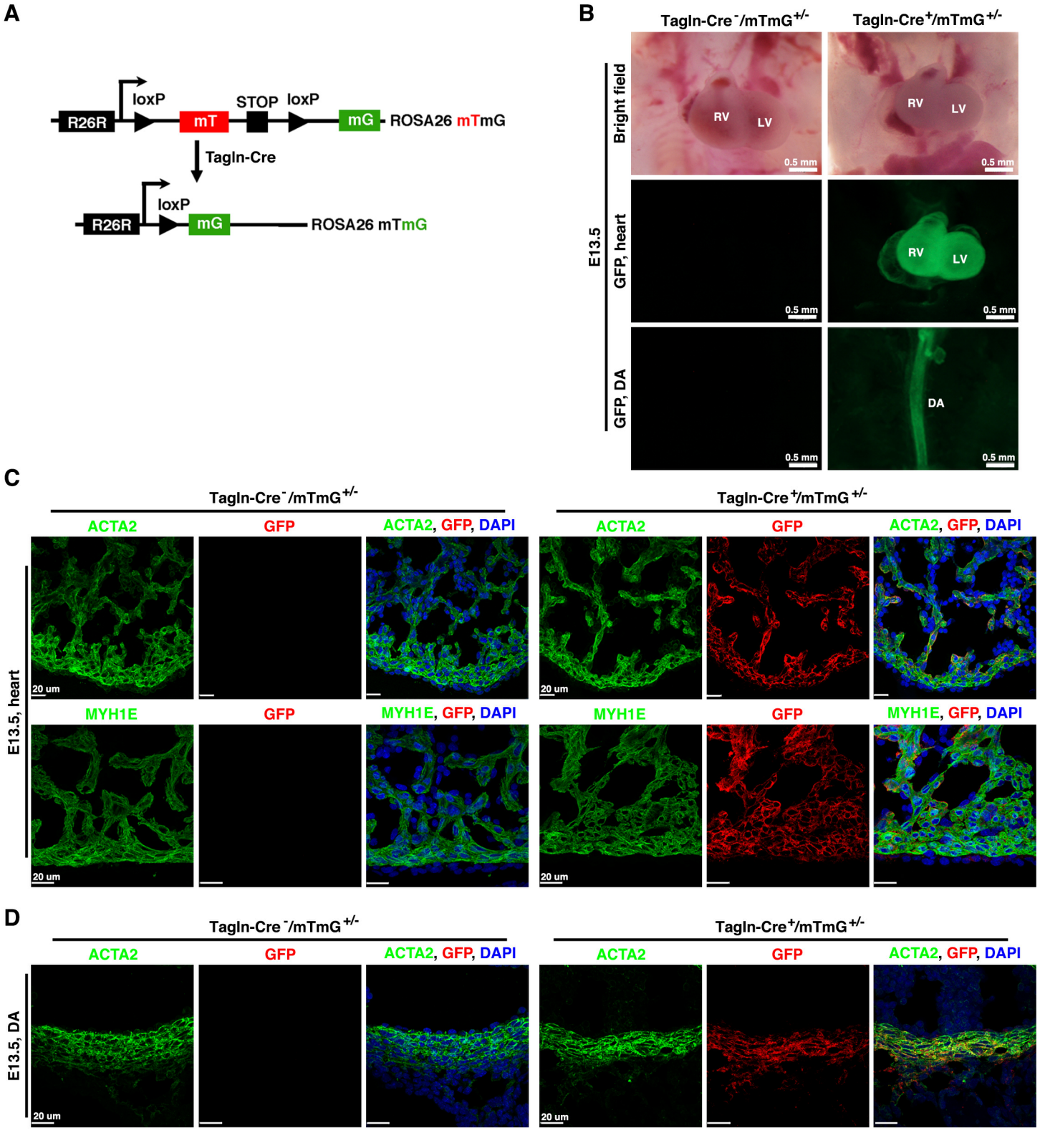
Online Figure VIII. YAP1 suppresses VSMC differentiation. A. GFP or YAP1 adenovirus was transduced into primary aortic VSMCs isolated from E13.5 control or *Tead1* mutant embryos and then expression of smooth muscle-specific genes was analyzed by Western blotting. The arrow indicates the exogenous YAP1 band. In contrast to TEAD1, over-expression of YAP1 in *Tead1*-null VSMCs exacerbates *Tead1* deficiency-induced smooth muscle differentiation defects. **B.** Immunoblot signals as shown in "A" were normalized to VCL loading control then expressed relative to signals from control cells infected with GFP (set to 1, red line). N=4. *$p<0.05$, vs control cells infected with GFP; $^{\#}p<0.05$, vs KO cells infected with GFP. **C.** Smooth muscle-specific gene

Tgfb1I1 or *Lmod1* promoter luciferase reporters were transfected into control primary VSMCs with or without co-transfection with YAP1 expression plasmid. After 36-hour transfection, the effects of YAP1 on the promoter activity were determined by the dual luciferase assay. The basal promoter activity without YAP1 co-transfection was set to 1. N=4. *P<0.05.

Online Figure IX. TEAD1 suppresses smooth muscle-specific gene expression in mature VSMCs. Primary aortic VSMCs derived from adult *Tead1* flox mouse dorsal aorta were infected with control GFP or Cre adenovirus. 48 hours post infection the cells were harvest for DNA to examine *Tead1* deleted allele by PCR (**A**), for total RNA to assess *Tead1* deletion by RT-PCR (**B**) and smooth muscle-specific gene expression by qRT-PCR (**C, N=4**), and for protein lysate to determine smooth muscle contractile protein expression by Western blotting (**D**). **E.** Immunoblot signals shown in "D" were normalized to ACTB loading control then expressed relative to signals from cells infected with GFP (set to 1, red line). N=3. *P<0.05. In contrast to TEAD1 function in embryonic VSMCs, deletion of *Tead1* in mature VSMCs promotes smooth muscle differentiation.

Online Figure X. *Myocd* is a direct target of TEAD1 and is sufficient to promote smooth muscle-specific gene expression. A. Chromatin was harvested from E13.5 heart and aortic tissues and then was immunoprecipitated with either IgG or anti-TEAD1 antibody. Immunoprecipitated DNA was subsequently analyzed by quantitative PCR using the primers flanking the enhancer region containing a MCAT element that has been shown to direct *Myocd* specifically express in VSMCs. The TEAD1 binding is expressed relative to IgG control (set to 1). N=4. *p<0.05. **B.** GFP or MYOCD adenovirus was transduced into control or *Tead1*-null embryonic VSMCs. After 48 hours infection protein lysate was harvested for Western blot using the antibodies as indicated. **C.** Immunoblot signals shown in "B" were normalized to VCL loading control then expressed relative to signals from control cells infected with GFP (set to 1, red line). N=4. *P<0.05, vs control cells infected with GFP; $^{\#}$p<0.05, vs KO cells infected with GFP.

Online Figure I



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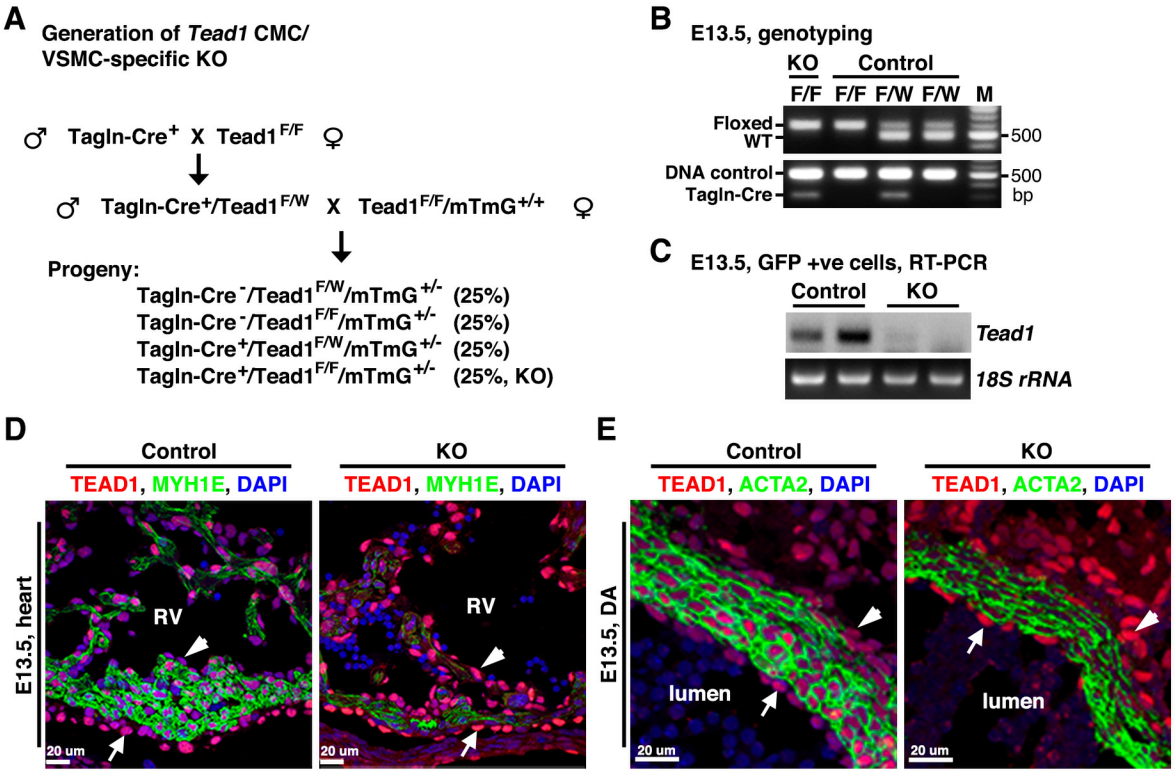

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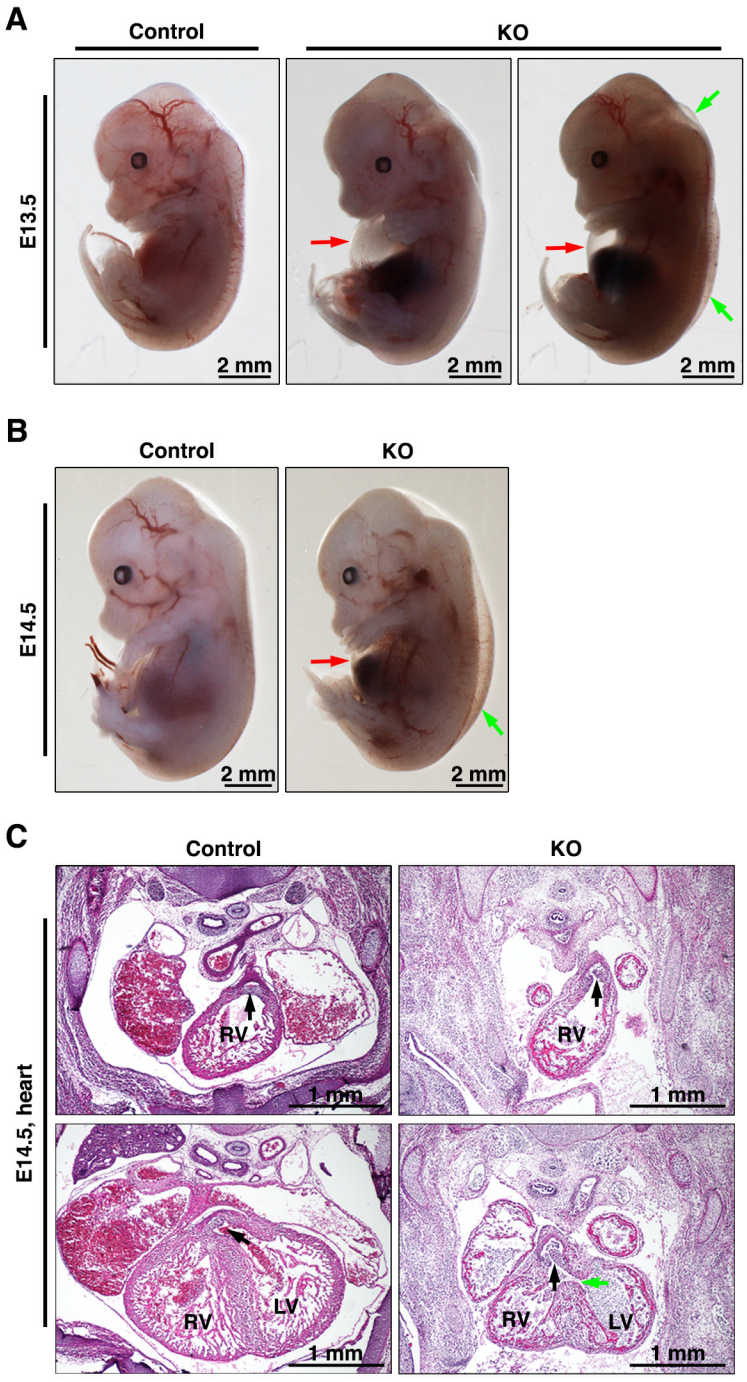

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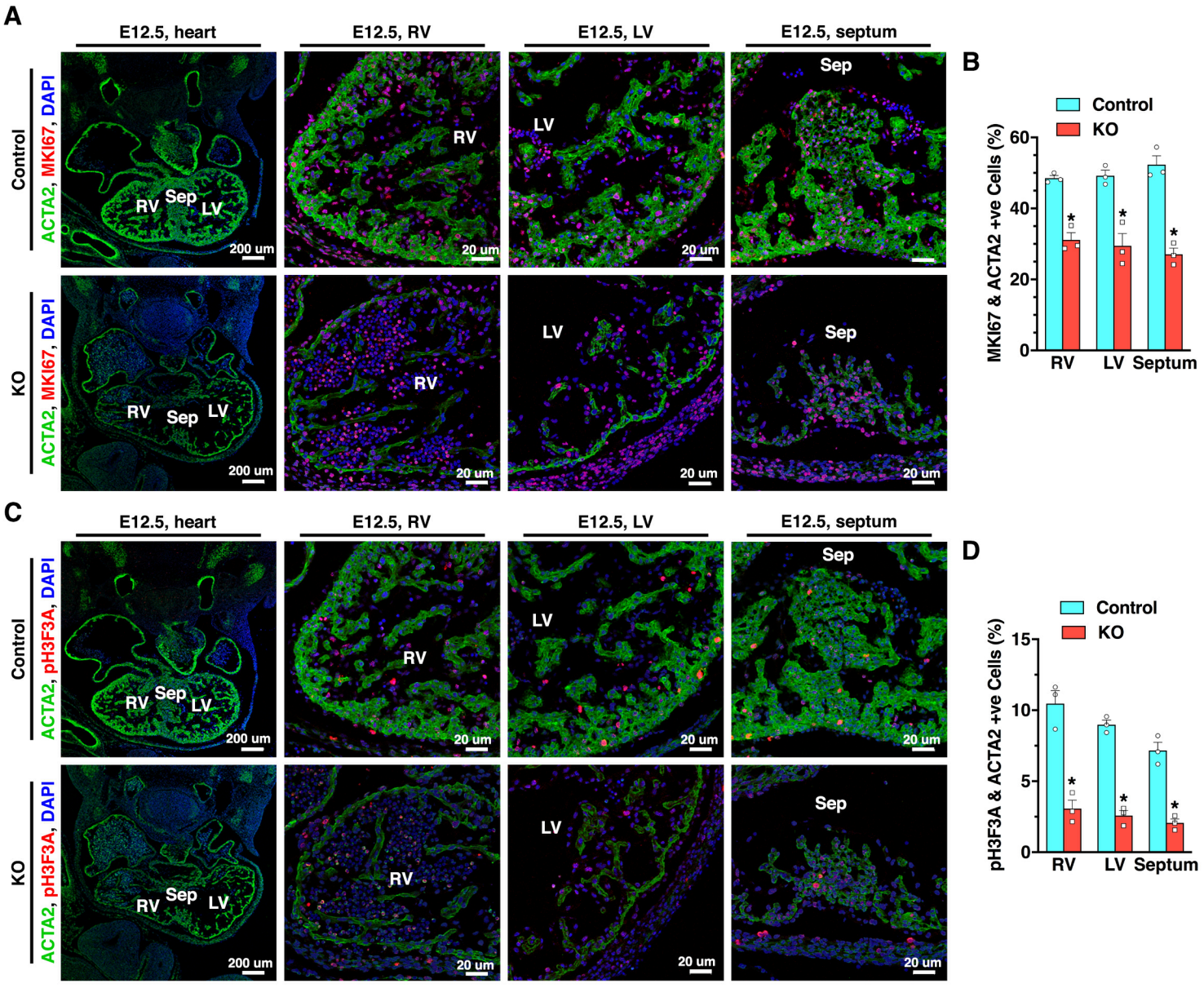

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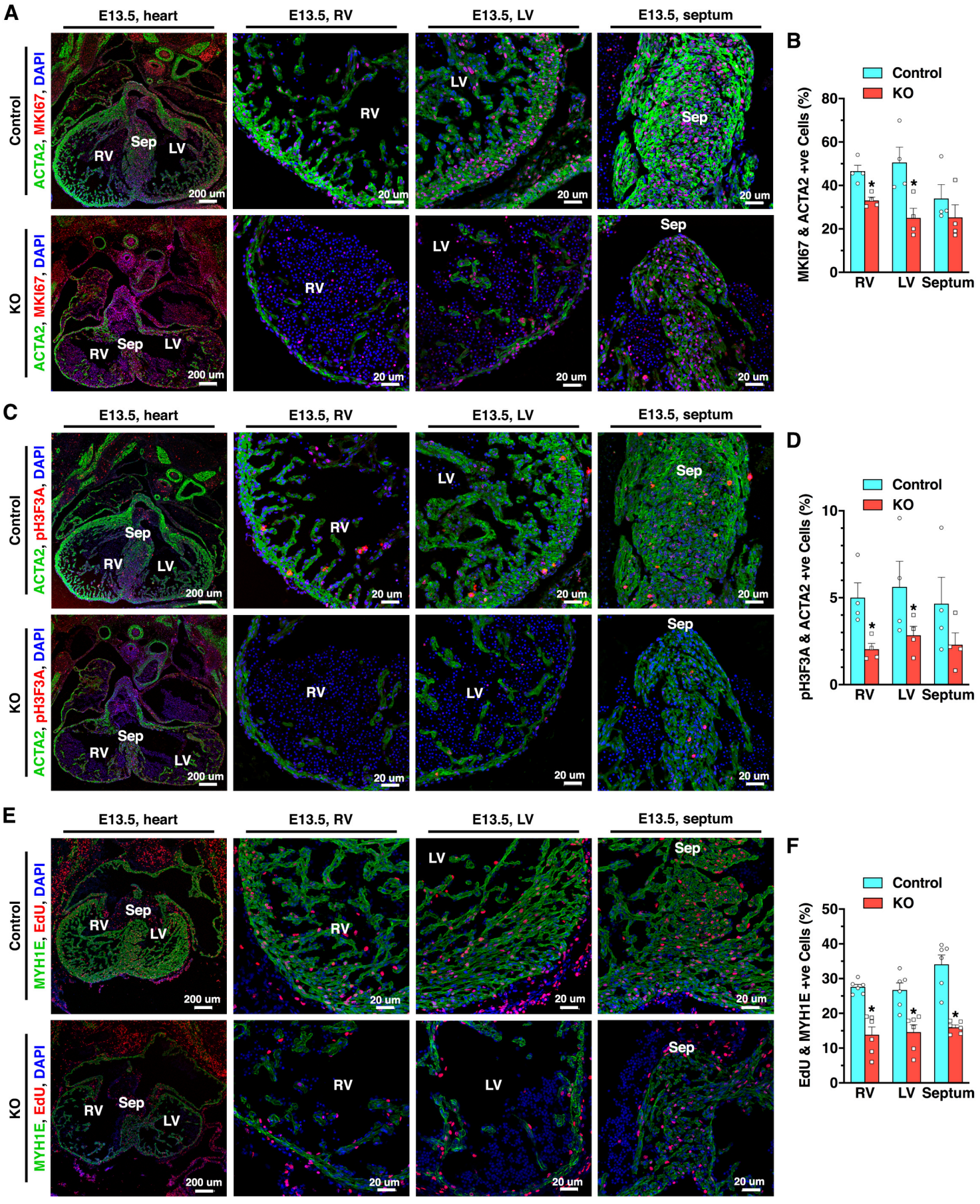

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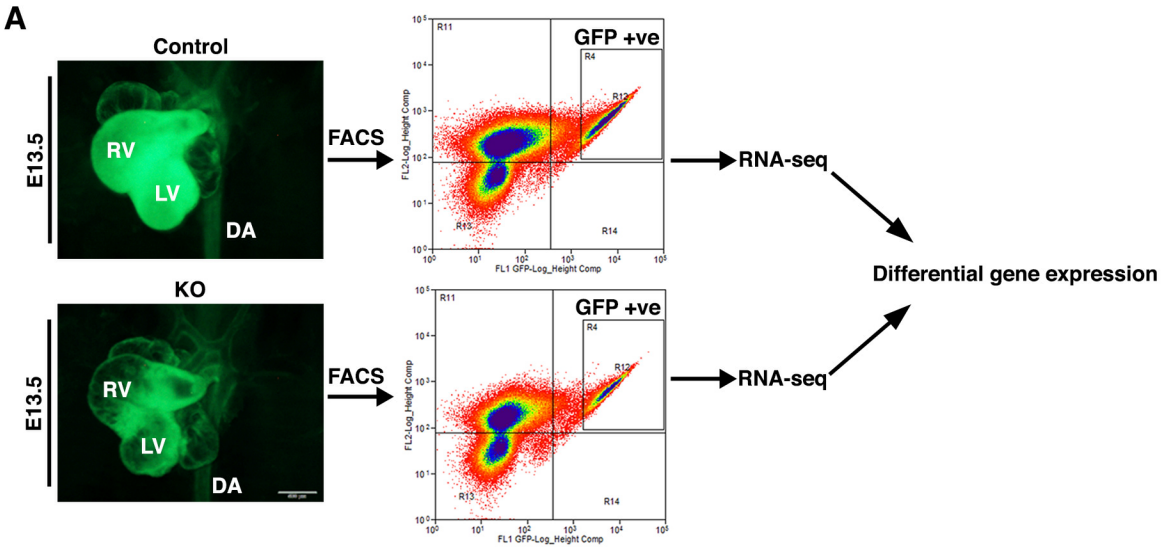

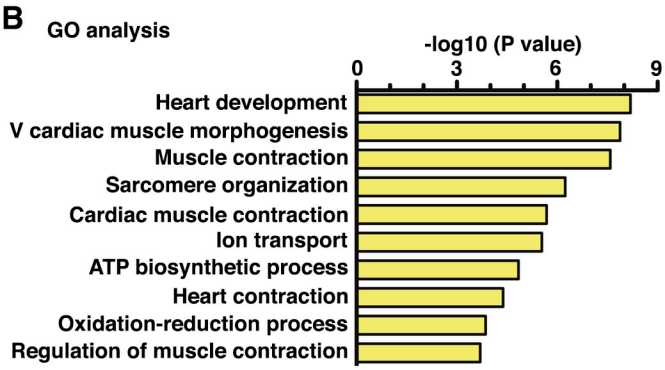

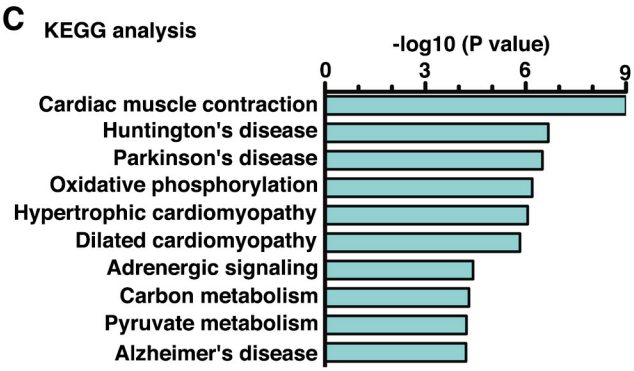

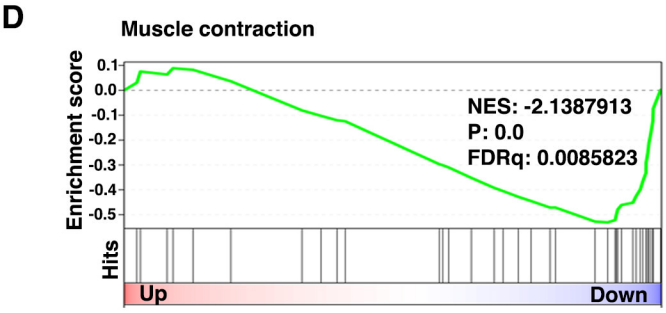

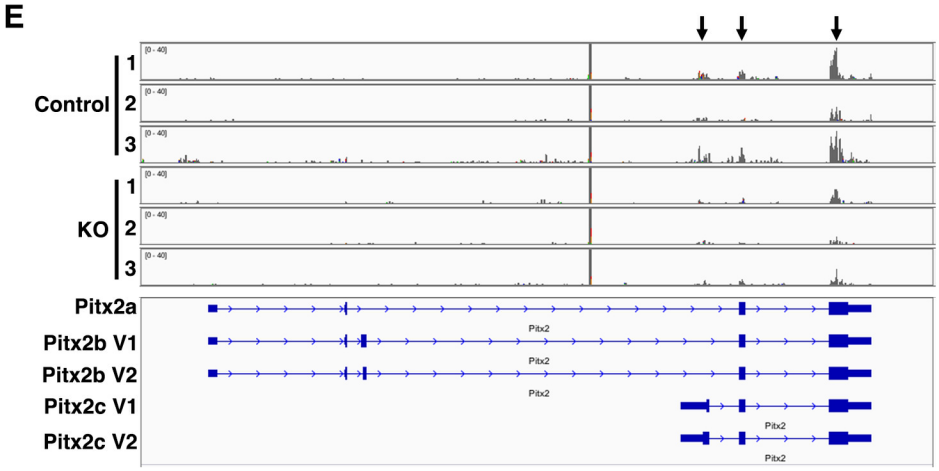

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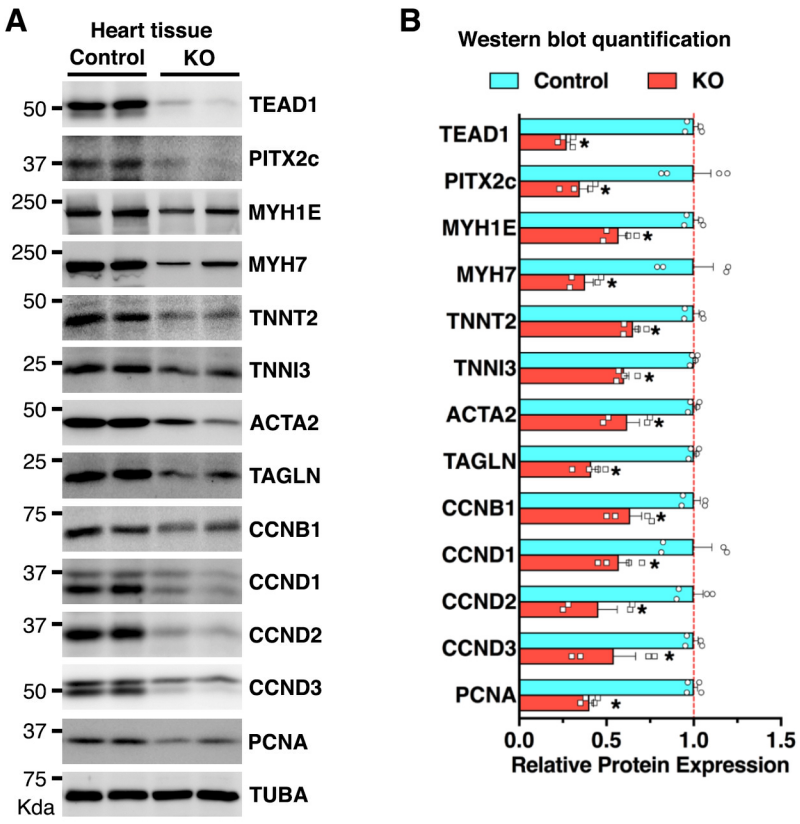

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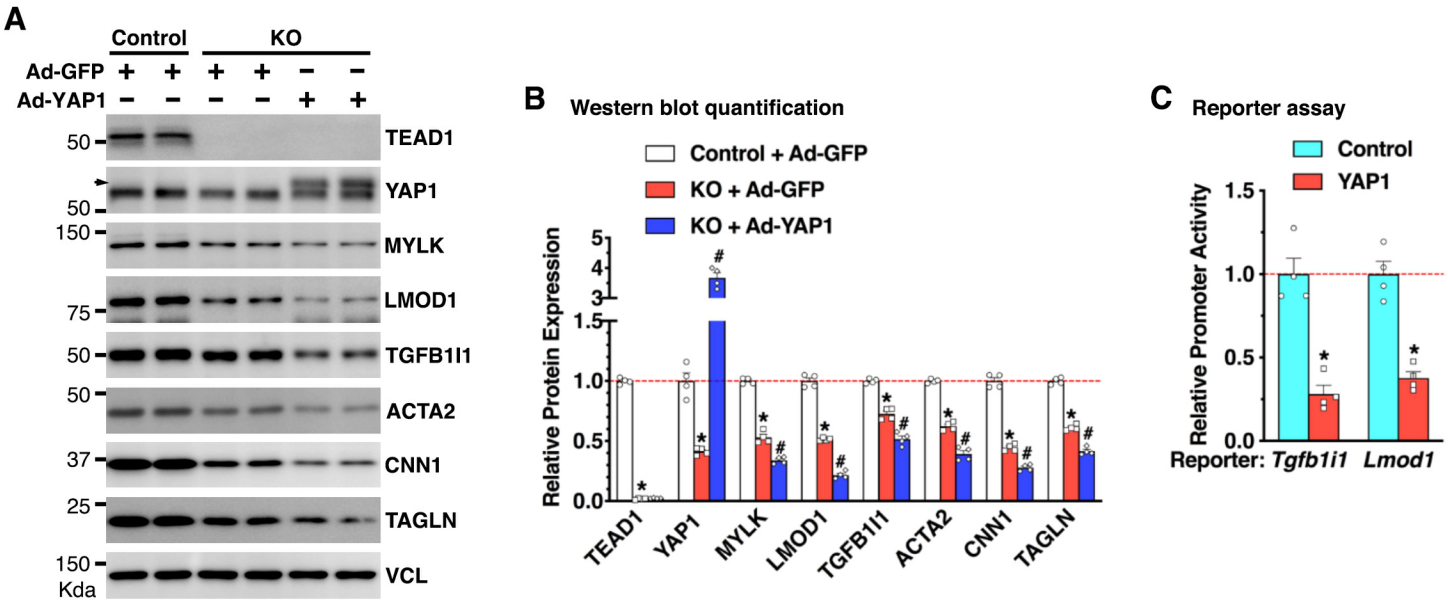

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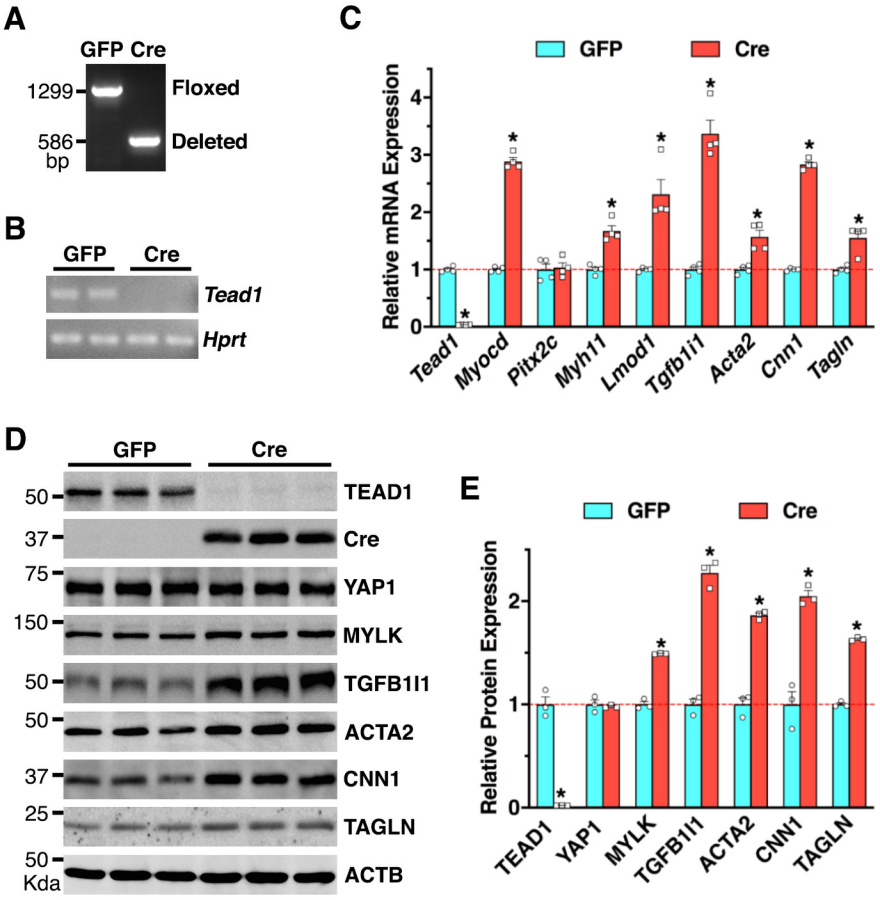

Online Figure X