

## **SUPPLEMENTAL MATERIALS**

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Disruption of myocardial Gata4 and Tbx5 results in defects in cardiomyocyte proliferation and atrioventricular septation

## SUPPLEMENTAL FIGURE LEGENDS

**Supplemental Figure S1. *Gata4* and *Tbx5* are co-expressed in developing endocardial cushions at E11.5 but not E14.5.** Immunofluorescent staining for *Gata4* (A,D) and *Tbx5* (B,E) in E11.5 (A-C) and E14.5 (D-F) endocardial cushions demonstrates co-localization at E11.5 but not E14.5.

**Supplemental Figure S2. *Gata4;Tbx5* compound heterozygote embryos display reduced endocardial cushion cell numbers at E12.5 and E13.5.** Quantification of atrioventricular cushion number in *Gata4;Tbx5* compound heterozygotes at E11.5 (A), E12.5 (B) and E13.5 (C). \*, p value <0.05 comparison to wild type; #, p value <0.05 comparison between single heterozygotes and double heterozygotes.

**Supplemental Figure S3. Dorsal mesenchymal protrusion (DMP) is absent in *Tbx5* heterozygote and *Gata4;Tbx5* compound heterozygote embryos at E11.5 as compared to wildtype and *Gata4* heterozygotes.** \*, DMP.

**Supplemental Figure S4. Expression of *Gata4* was reduced in *Gata4<sup>MyoDel/wt</sup>;Tbx5<sup>+/-</sup>* (*Gata4<sup>flox/wt</sup>;Tbx5<sup>wt/-</sup>;αMHC-Cre<sup>+</sup>*) embryos in the atria and ventricle. (A-H)** Immunohistochemistry staining reveals the loss of *Gata4* expression in the atrial and ventricular myocardium in *Gata4<sup>MyoDel/wt</sup>;Tbx5<sup>+/-</sup>* embryos. Endocardial expression is maintained (arrows). Scale bars indicate 200 μm.

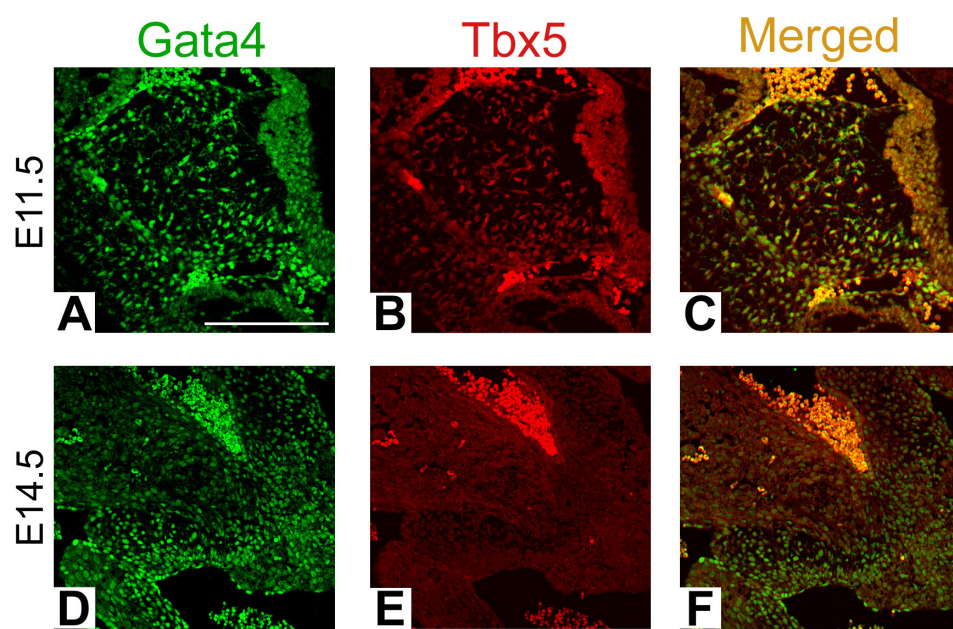
**Supplemental Figure S5. Haploinsufficiency of *Tbx5* and endocardial-*Gata4* (*Gata4*<sup>EndoDel/wt</sup>;*Tbx5*<sup>+/-</sup>) showed normal cardiac phenotype and cardiomyocyte proliferation.**

(A) Survival table for *Gata4*<sup>EndoDel/wt</sup>;*Tbx5*<sup>+/-</sup> (*Gata4*<sup>flox/wt</sup>;*Tbx5*<sup>wt/-</sup>;Tie2-Cre<sup>+</sup>) embryos at E11.5-E16.5. (B) Images and histologic sections of E14.5 embryos show no growth retardation and normal septation in *Gata4*<sup>EndoDel/wt</sup>;*Tbx5*<sup>+/-</sup> mutants. There was normal myocardial proliferation as measured by phospho-histone H3 staining (C-O). \*, p value < 0.05. Scale bars indicate 200  $\mu$ m.

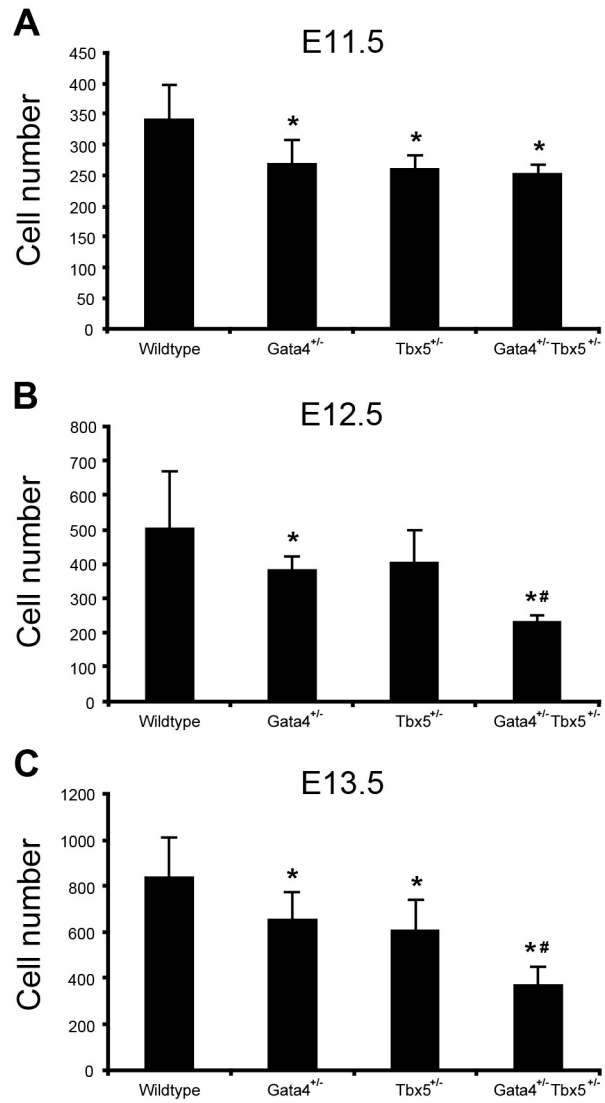
**Supplemental Figure S6. Knockdown of *Gata4* and *Tbx5* using siRNA in cultured cardiomyocytes shows decreased expression of *Cdk4* and reduced *Ki67* expression.**

(A) Decreased expression of *Cdk4* by immunoblotting in cultured E11.5 murine cardiomyocytes after knockdown of *Gata4* and *Tbx5* with *Gata4* and *Tbx5*-specific siRNA. Immunoblot for *Gata4* and *Tbx5* is shown to demonstrate efficiency of siRNA. Actin expression is shown as loading control. (B) Immunofluorescence staining demonstrates decreased *Ki67* expression in cultured cardiomyocytes, consistent with reduced cardiomyocyte proliferation. Scale bars indicate 200  $\mu$ m.

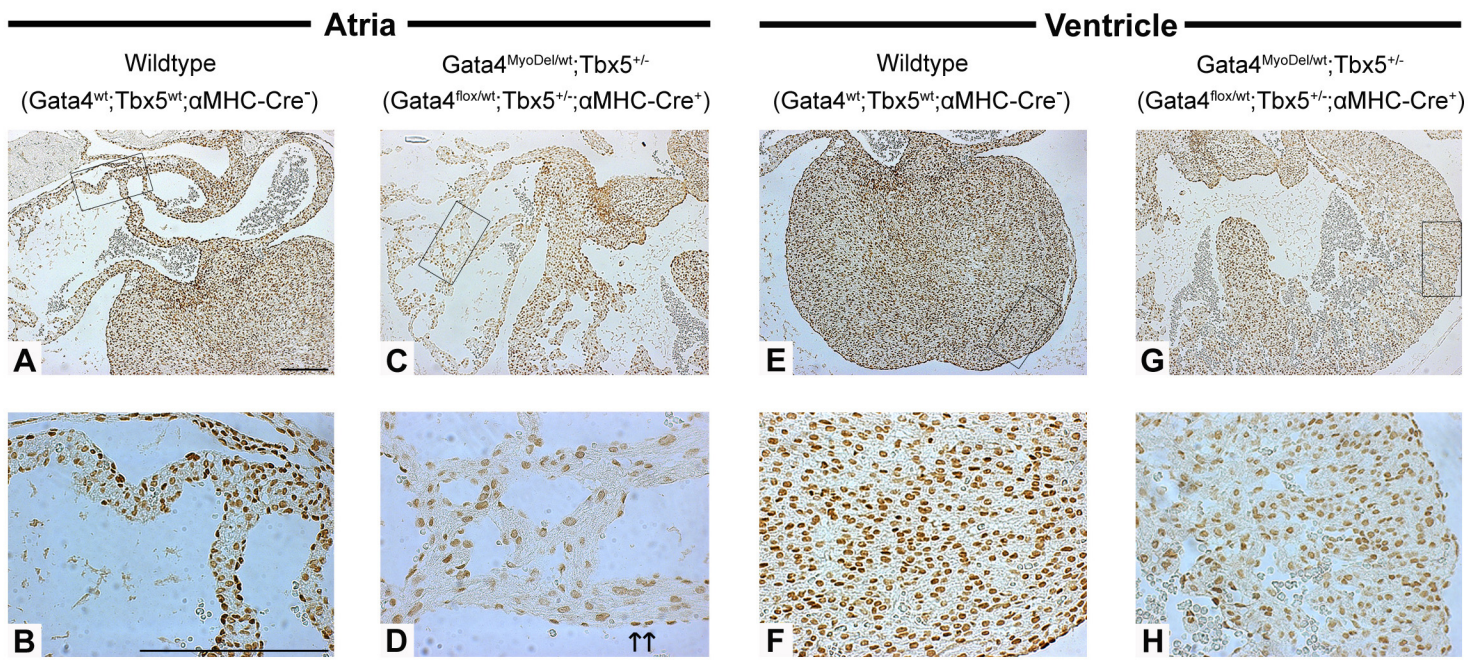
**Supplemental Figure S7. Table summarizing published ChIP-seq studies using *Gata4* and *Tbx5* in adult hearts and HL-1 cardiomyocytes.**



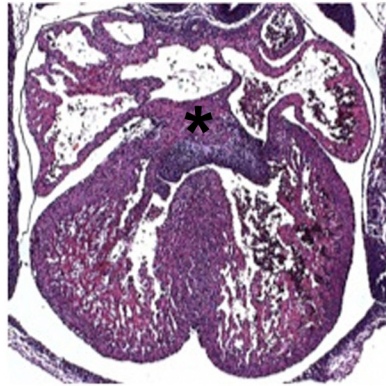
Supplemental Figure 1.



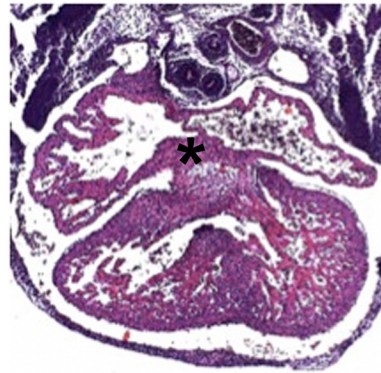
Supplemental Figure 2.



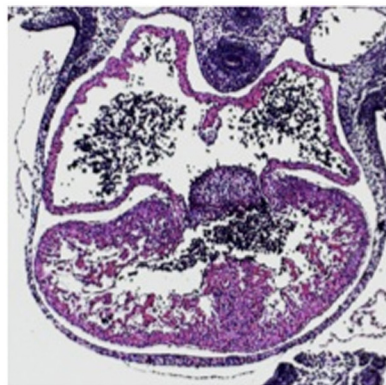
Supplemental Figure 4.



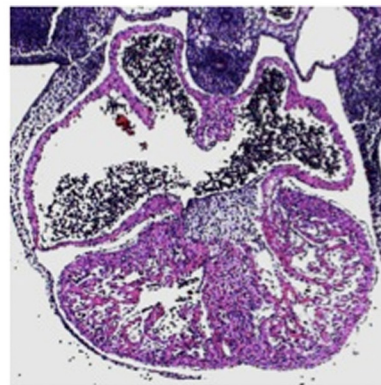
Wildtype



*Gata4*<sup>+/-</sup>



*Tbx5*<sup>+/-</sup>

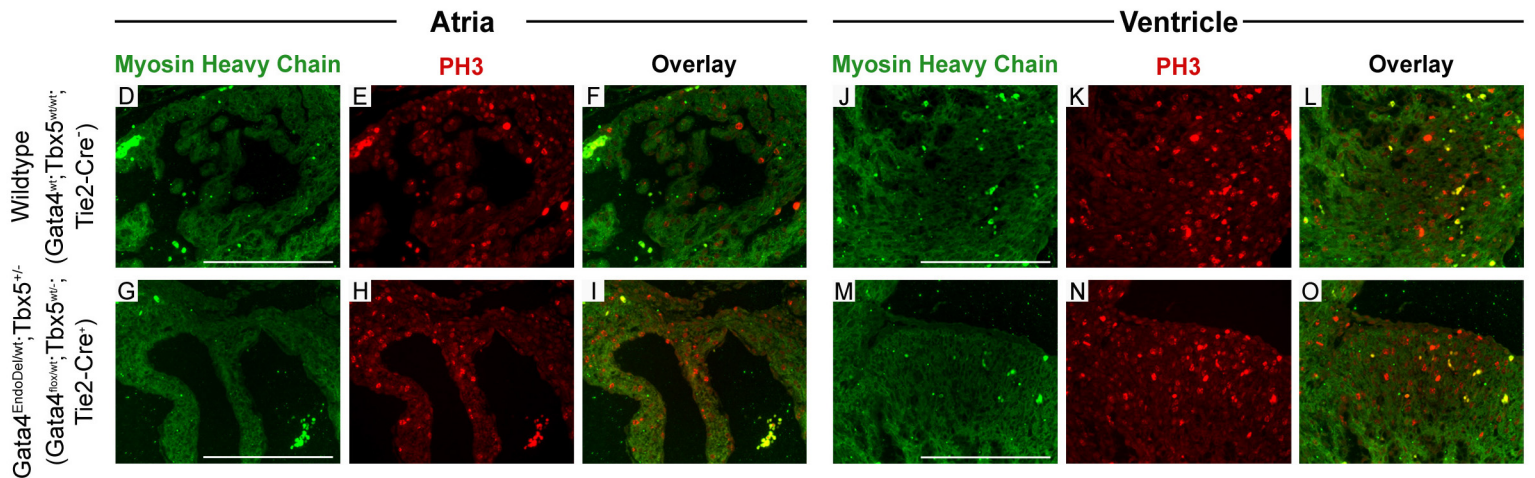
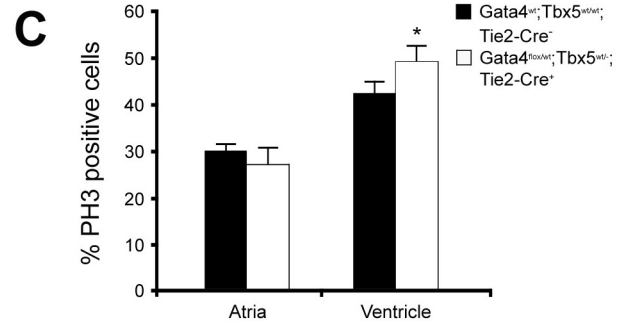
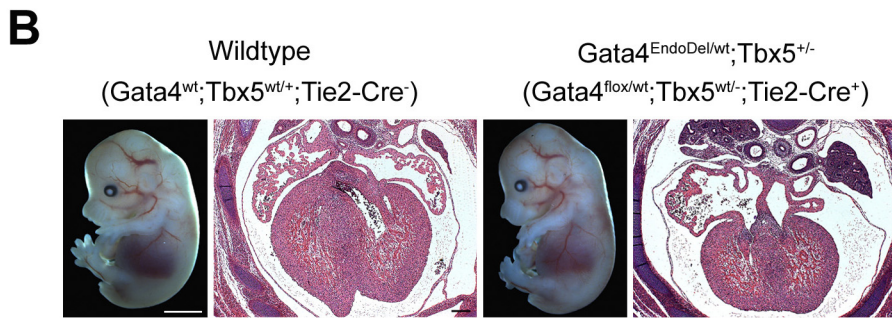


*Gata4*<sup>+/-</sup>; *Tbx5*<sup>+/-</sup>

Supplemental Figure 3.

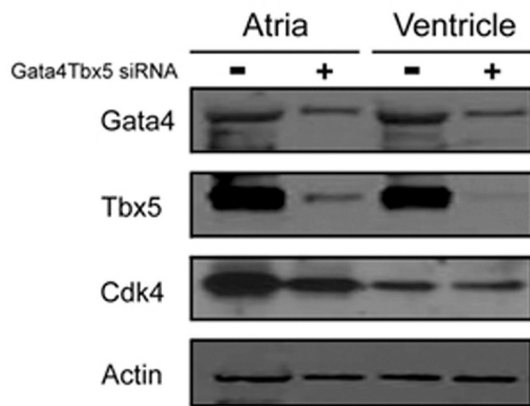
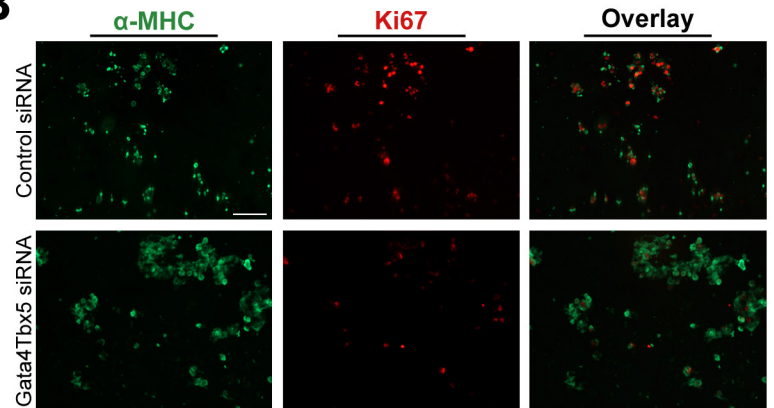
**A**  $Gata4^{flox/wt} \times Tbx5^{wt/-}; Tie2-Cre^+$

	wt	wt	wt	wt	flox	flox	flox	flox
<i>Gata4</i>	wt	wt	wt	wt	flox	flox	flox	flox
<i>Tbx5</i>	wt	wt	-	-	wt	wt	-	-
<i>Tie2-Cre</i>	-	+	-	+	-	+	-	+
E11.5-E16.5	10 (15%)	8 (13%)	9 (14%)	8 (13%)	8 (13%)	9 (14%)	5 (8%)	6 (10%)
Expected Mendelian percentage	12.5%	12.5%	12.5%	12.5%	12.5%	12.5%	12.5%	12.5%



Supplemental Figure 5.



**A****B**

Supplemental Figure 6.

### Gata4

Dataset (GEO#)	Tissue	Cell line	CDK4	CDK2	Reference
GSE 35151	Adult mouse whole heart		—	—	van den Boogaard <i>et al.</i> , 2012
GSM 862697	Adult mouse whole heart		—	—	
GSE 21529		HL-1 cells	—	—	He <i>et al.</i> , 2011

### Tbx5

Dataset (GEO#)	Tissue	Cell line	CDK4	CDK2	Reference
GSE 21529		HL-1 cells	+	—	He <i>et al.</i> , 2011
GSM 558908		HL-1 cells	—	—	

Supplemental Figure 7.

Supp Table 1. Primer sequences for gene expression

Gene name	Primer sequence
q-PCR	
Mouse CDK2 F	CTGCCATTCTCACCGTGTCC
Mouse CDK2 R	AGCTTGATGGACCCCTCTGC
Mouse CDK4 F	CGAGCGTAAGATCCCCTGCT
Mouse CDK4 R	GCACCGACACCAATTCAGC
ChIP	
Mouse CDK2 F	TCCAAAAGCAGGCGAGAGT
Mouse CDK2 R	TCCACCTGGACAAGAGAGTCT
Mouse CDK4 F	GTTGGCCCGGTTGCCATGACACCG
Mouse CDK4 R	CTGGACACGTGATCTTCACCCTTG