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Supplemental Information

Tbx5-Hedgehog Molecular Networks

Are Essential in the Second Heart Field

for Atrial Septation

Linglin Xie, Andrew D. Hoffmann, Ozanna Burnicka-Turek, Joshua M. Friedland-Little, Ke Zhang, and Ivan P. Moskowitz

Inventory of Supplementary Information

Figure S1, related to Figure 4. *Tbx5* is not required for pSHF progenitor survival.

Table S1, related to Figure 4. Genes with > 2 fold expression change in *Tbx5*^{+/-} versus wildtype E9.5 pSHF.

Table S1, related to Figure 6. Genomic regions with *Tbx5* binding sites included in Luciferase reporter assay and ChIP assay.

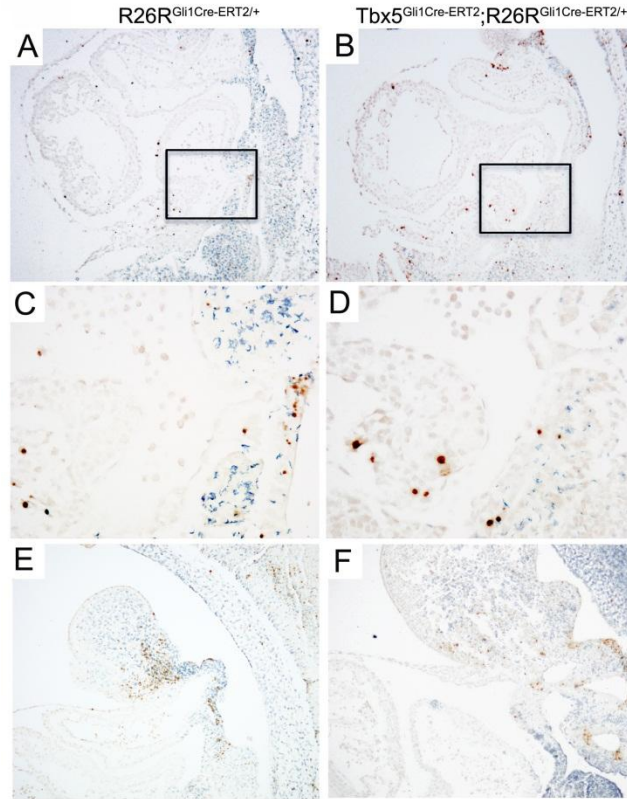


Figure S1, related to Figure 4. *Tbx5* is not required for pSHF progenitor survival.

A-D) Cell survival in the second heart field and DMP region is measured by TUNNEL staining at E9.5. Very few apoptotic cells are detected in these regions in both $Tbx5^{Gli1Cre-ERT2};R26R^{Gli1Cre-ERT2/+}$ and $R26R^{Gli1Cre-ERT2/+}$ embryos at E9.5.

E and F) Apoptotic cells in the pharyngeal ridge and the out flow tract serve as positive controls in TUNNEL staining.

Table S1. Genes with > 2 fold expression change in *Tbx5*^{+/-} versus wildtype E9.5 pSHF .

(see accompanying Excel file)

Table S2. related to Figure 6. Genomic regions with *Tbx5* binding sites included in Luciferase reporter assay and ChIP assay.

GENE NAME	LUCIFERASE ASSAY				CHIP		
	Genomic fragment	Locus	Luciferase results	<i>Tbx5</i> binding sites in subcloned fragments	Genomic fragment	Locus	ChIP results
<i>Cdk6</i>	Cdk6Fr	chr5:3341397-3344463	10.55±4.27 P=0.0009	chr5:3343142-3343151 chr5:3343285-3343292 chr5:3344080-3344089 chr5:3344441-3344452			
<i>Gas1</i>	Gas1Fr	chr13:60277613-60279287	4.29±1.43 p=0.0012	chr13:60278328-60278336			
<i>Osr1</i>	Osr1Fr1	chr12:9585330-9588700	5.82±1.42 p=0.0009	chr12:9586539-9586548 chr12:9586598-9586605 chr12:9588539-9588548 chr12:9588580-9588591	Osr1Fr1	chr12:9588536-9588611	82.54±54.06 p<0.05
	Osr1Fr2	chr12:9591000-9592000	1.4±0.59 p=0.61	Not found in mouse sequence, in human sequence chr2:19411576-19411587			
	Osr1Fr3	chr12:9595401-9596946	1.5 ±0.41 p=0.28				
	Osr1Fr4	chr12:9601880-9602629	5.37±0.37 p=0.0009	chr12:9602410-9602421	Osr1Fr4	chr12:9602333-9602473	98.14±45.72 p<0.05

Potential *Tbx5* binding sites were predicted from overlap of evolutionary conservation and ChIP-seq studies in the atrial cardiomyocyte HL-1 cell line (He et al., 2011). One *Cdk6* genomic fragment, one *Gas1* genomic fragment and four *Osr1* genomic fragments containing potential *Tbx5* binding sites were sub-cloned for luciferase reporter assay. Conserved canonical *Tbx5* binding sites were identified by rVISTA within these regions. Osr1fr1 and Osr1Fr4, demonstrating *Tbx5* responsiveness, were further tested for direct *Tbx5*-binding in the pSHF by ChIP-PCR.

