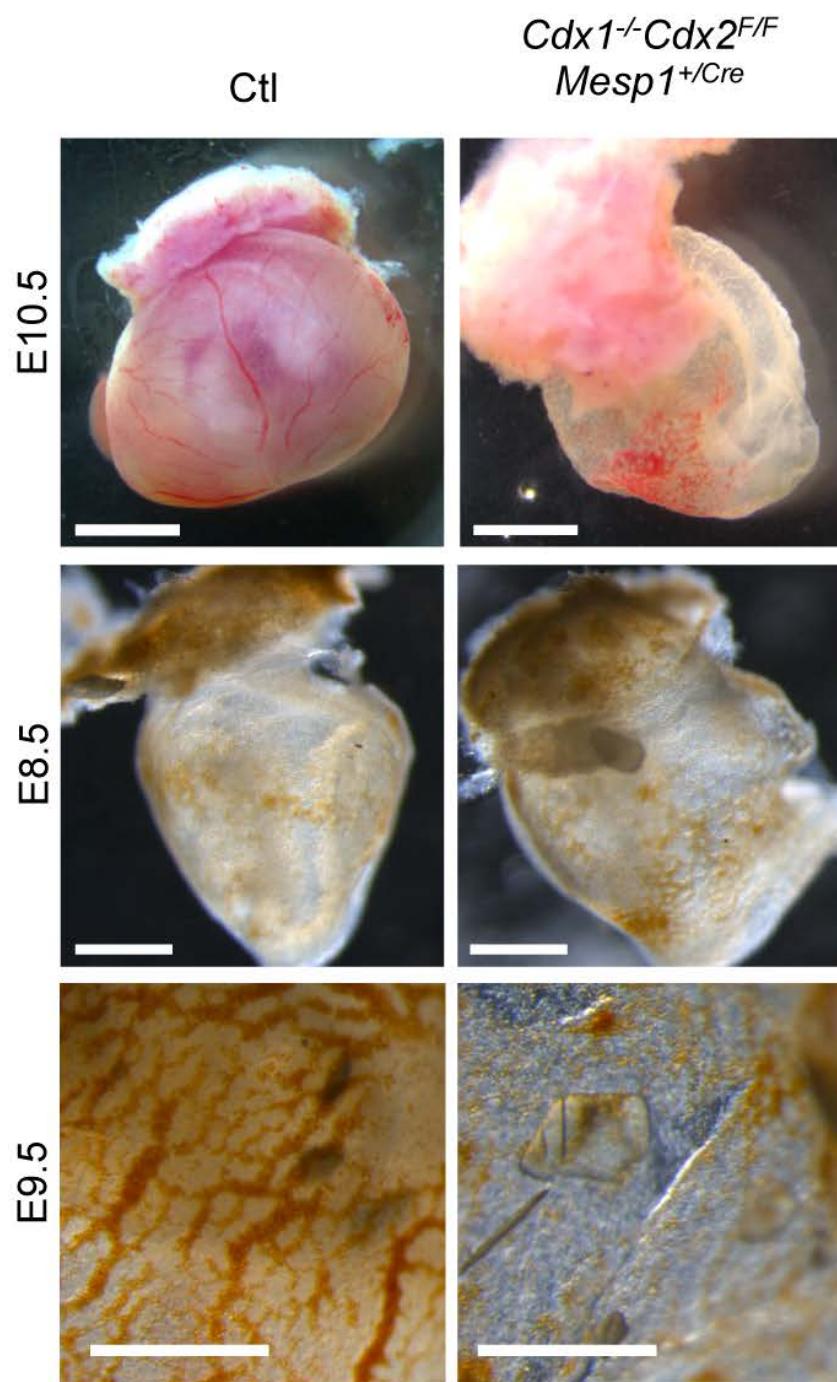
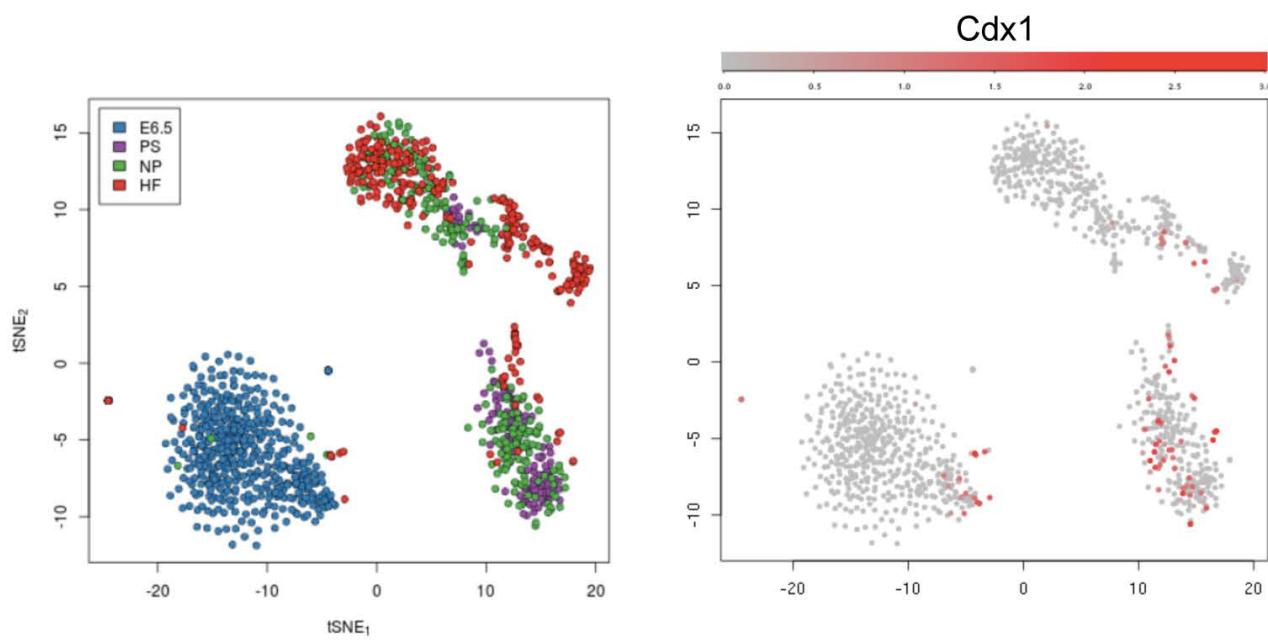
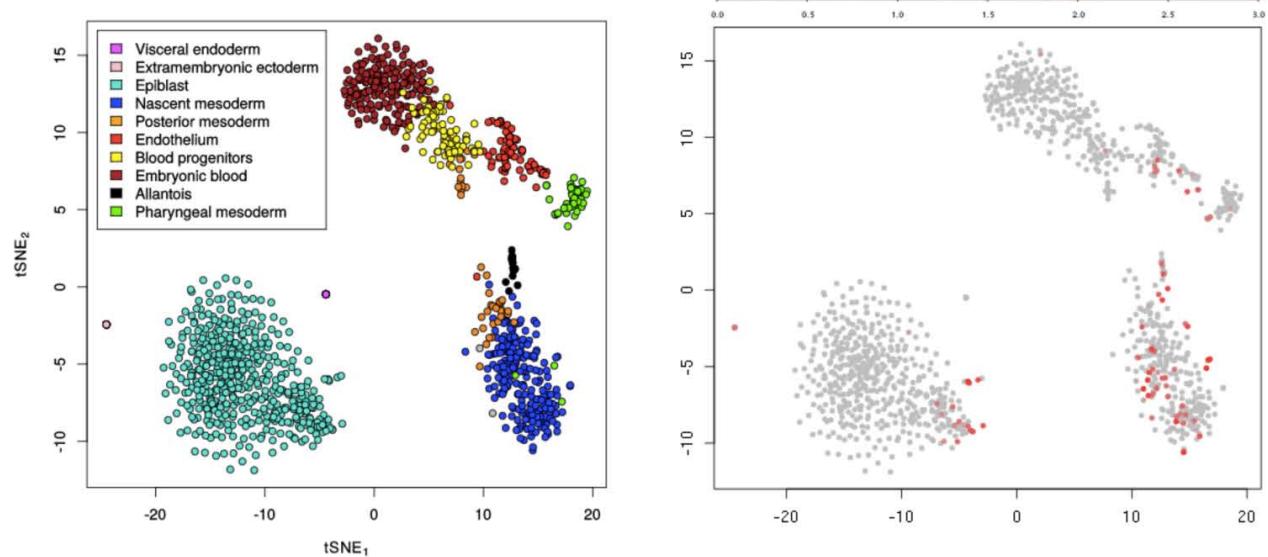


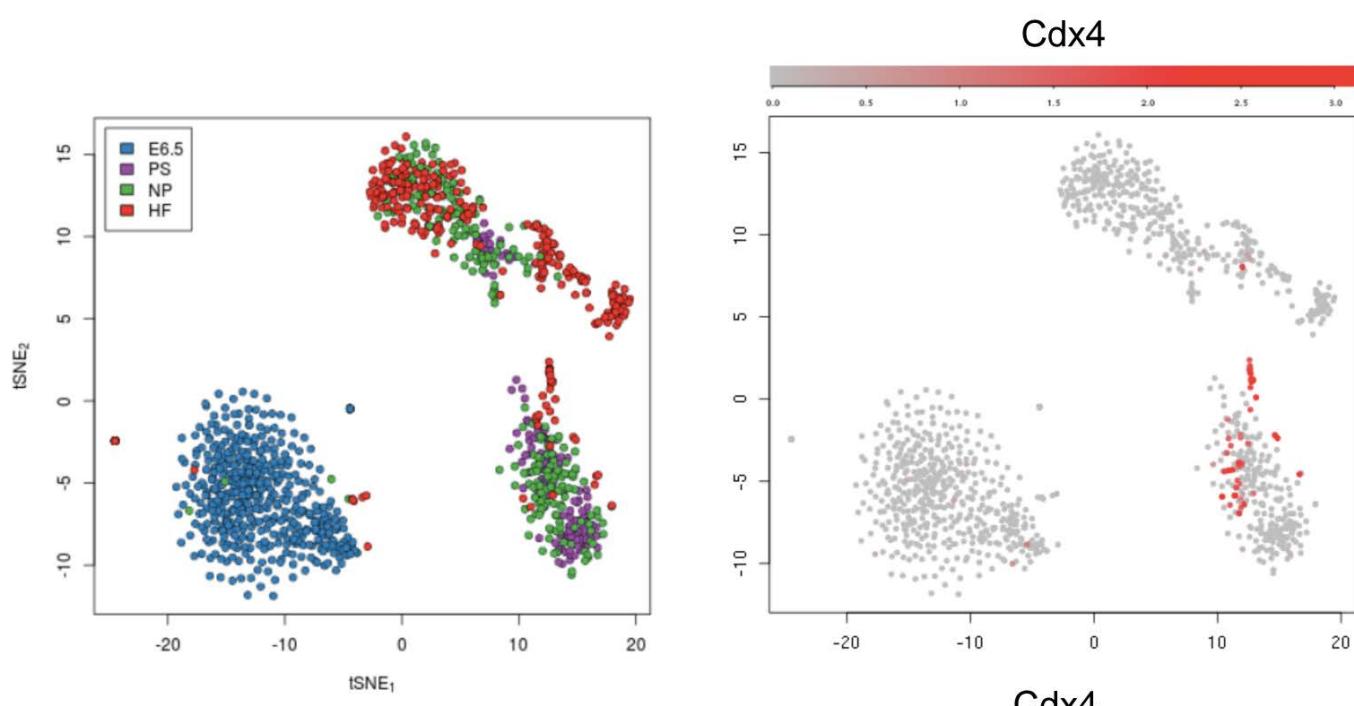
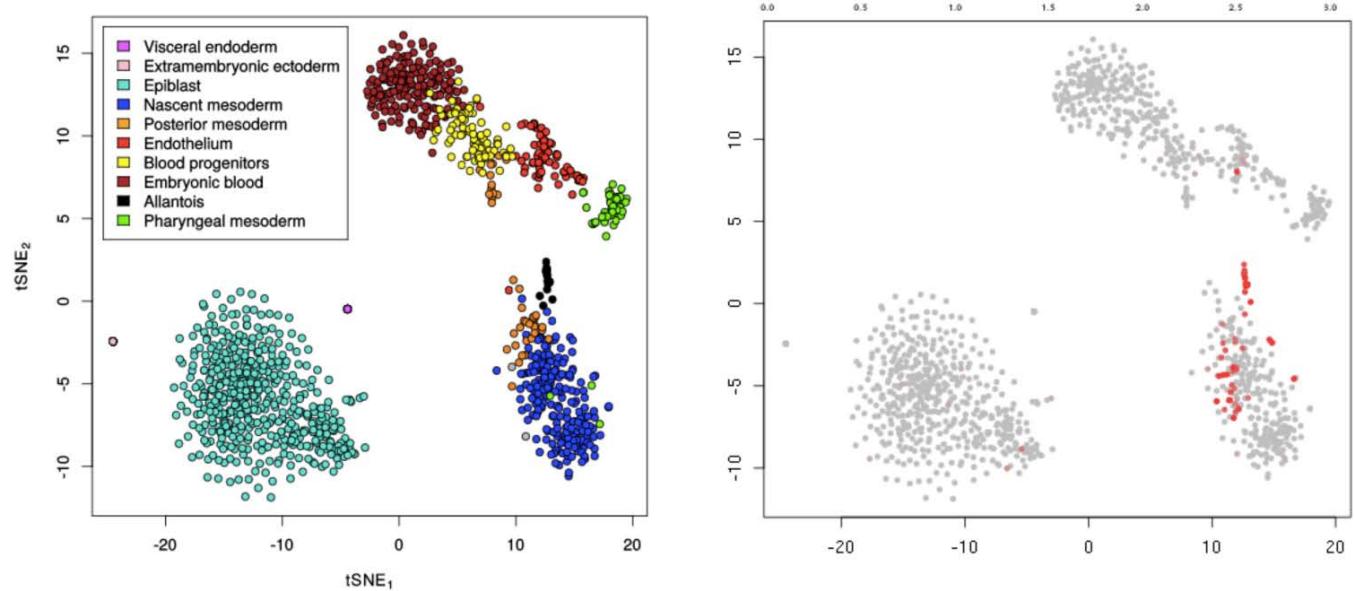
**Figure S1. Tamoxifen-mediated Cdx deletion at E6.5 induces a cardiac phenotype.** Cardiac defects in E9.5 *DKO* mutants treated with tamoxifen at E6.5 compared to stage-matched control. Scale bars, 0.5mm (top) and 0.25mm (bottom). Images are representative of 8/9 mutants and 4/4 wild type controls.



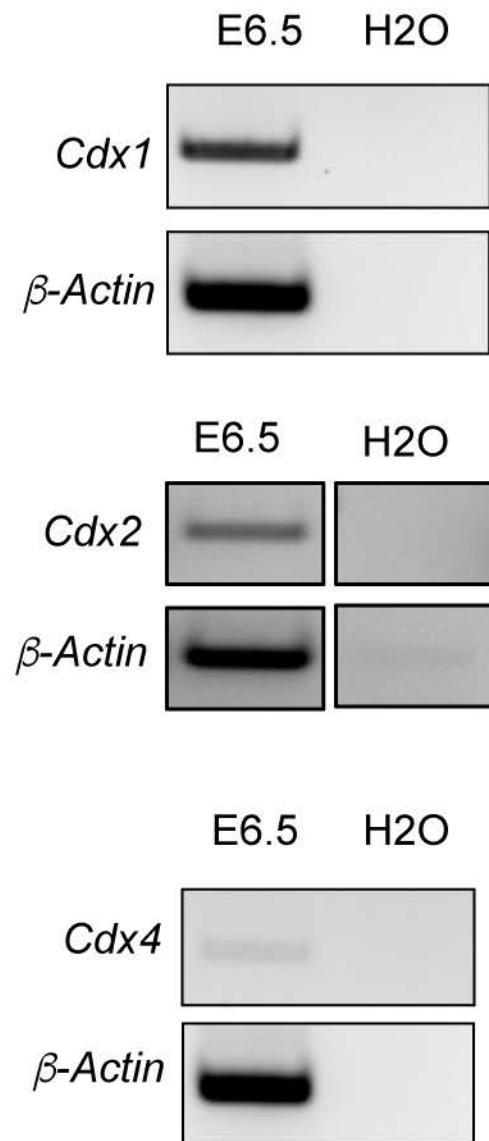
**Figure S2. Disrupted yolk sac vascularization in *Cdx1<sup>-/-</sup>Cdx2<sup>-/-</sup>Mesp1<sup>+/-Cre</sup>* and *Cdx1<sup>-/-</sup>Cdx2<sup>-/-</sup>Tie2<sup>+/-Cre</sup>* mutants.** *Cdx1<sup>-/-</sup>Cdx2<sup>F/F</sup>Mesp1<sup>+/-Cre</sup>* and littermate control embryos with affixed yolk sacs collected at E10.5, E8.5, and E9.5 and DAB stained. Scale bars, 2mm (top panel) and 0.5mm (middle and bottom panel). Images at E10.5 are representative of 3/4 mutants and 7/7 controls; E9.5, 2/3 mutants and 3/3 controls; at E8.5, 1/1 mutant and 4/4 controls.

**A.****B.**

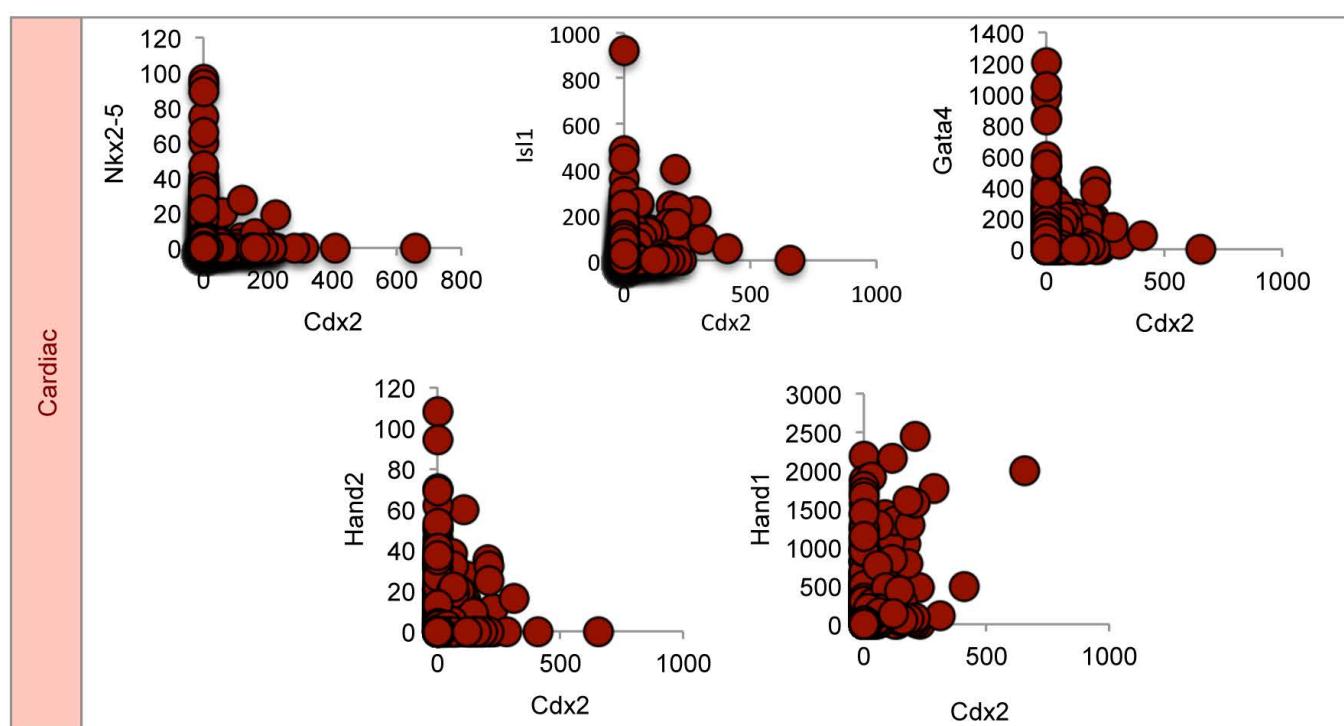
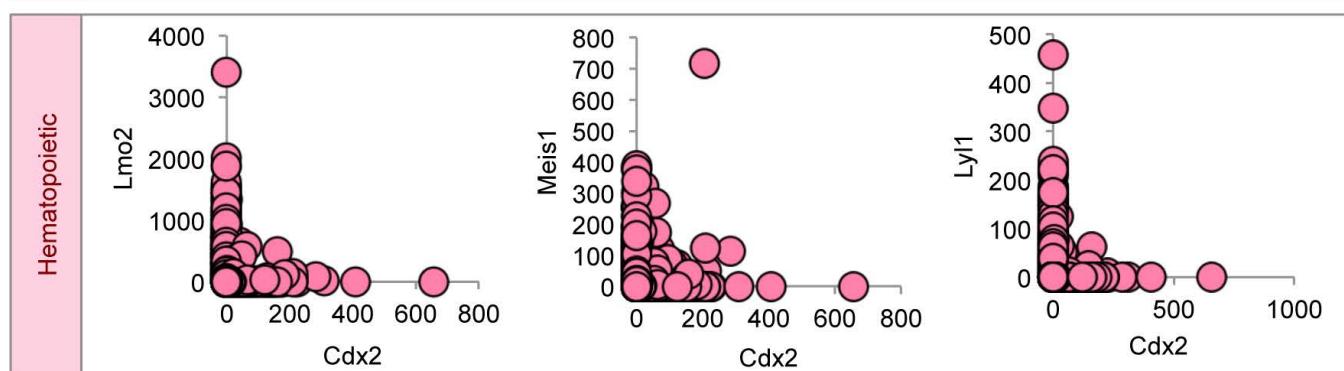
**Figure S3. Cdx1 expression in E6.5-E7.75 embryos by single-cell RNA-seq.** **A.** Cdx1 transcripts were detected in cells isolated from embryos at E6.5 (epiblast, blue), primitive streak stage (E7.0, purple), neural plate stage (E7.5, green), and head fold stage (E7.75, red) embryos (Scialdone et al., 2016). **B.** Cdx1 transcripts were detected in cells associated with a number of progenitor lineages, including epiblast cells (turquoise), nascent mesoderm (blue), posterior mesoderm (orange), pharyngeal mesoderm (green), endothelium (red), blood progenitors (yellow), and extraembryonic ectoderm (pink).

**A.****B.**

**Figure S4. Cdx4 expression in E6.5-E7.75 embryos by single-cell RNA-seq.** **A.** Cdx4 transcripts were detected in cells isolated from embryos at E6.5 (epiblast, blue), primitive streak stage (E7.0, purple), neural plate stage (E7.5, green), and head fold stage (E7.75, red) embryos (Scialdone et al., 2016). **B.** Cdx4 transcripts were detected in cells associated with a number of progenitor lineages, including epiblast cells (turquoise), endothelium (red), allantois (black), nascent mesoderm (blue), and posterior mesoderm (orange).



**Figure S5. *Cdx1*, *Cdx2*, and *Cdx4* are expressed in E6.5 whole embryos.** *Cdx1*, *Cdx2*, and *Cdx4* transcripts were detected in pooled litters of E6.5 embryos by RT-PCR.  $\beta$ -Actin was used as a loading control. RT-PCR images are representative of four independent experiments in which similar results were obtained.

**A.****B.**

**Figure S6. Cdx2 expression relative to cardiac and hematopoietic marker genes in E6.25-E7.5 embryos.** Correlation plots were generated using a single-cell RNA-seq dataset generated by Scialdone and colleagues (Scialdone et al., 2016) using E6.25-E7.5 whole embryos to demonstrate co-expression of Cdx2 and select cardiac (A) and hematopoietic (B) transcripts.

**Table S1 – Select transcription and signaling factors with peak scores below 800**

Gene Name	Peak Score	Binding Region
<i>Bmpr1a</i>	346.6	promoter-TSS (NM_009758)
<i>Wnt7b</i>	351.89	promoter-TSS (NM_009528)
<i>Sox6</i>	351.38	promoter-TSS (NM_001025560)
<i>Smad5</i>	509.91	Intergenic
<i>Lmo4</i>	488.81	promoter-TSS (NM_001161770)
<i>Ptch1</i>	478.92	Intergenic
<i>Sox3</i>	468.75	Intergenic
<i>Isl1</i>	423.56	Intergenic
<i>Id2</i>	422.99	Intergenic
<i>Hes1</i>	411.9	Intergenic
<i>Notch2</i>	400.99	promoter-TSS (NM_010928)
<i>Pax2</i>	396.03	Intergenic
<i>Neurog2</i>	390.89	Intergenic
<i>Foxa1</i>	388.42	Intergenic
<i>Dll1</i>	386.31	Intergenic
<i>Gsx2</i>	377.44	Intergenic
<i>Wnt2b</i>	373.98	promoter-TSS (NM_009520)
<i>Wnt7b</i>	351.89	promoter-TSS (NM_009528)
<i>Sox6</i>	351.38	promoter-TSS (NM_001025560)
<i>Bmpr1a</i>	346.6	promoter-TSS (NM_009758)
<i>Neurod2</i>	339.47	Intergenic
<i>Neurog1</i>	336.61	Intergenic
<i>Sox3</i>	317.7	Intergenic
<i>Lbx2</i>	314.86	promoter-TSS (NM_010692)
<i>Hoxb1</i>	312.16	Intergenic
<i>Fgf8</i>	308.96	Intergenic
<i>Foxa2</i>	308.16	Intergenic
<i>Esx1</i>	306.91	Intergenic
<i>Nkx3_2</i>	305.43	Intergenic
<i>Bmpr1b</i>	301.93	Intergenic
<i>Smad3</i>	297.39	promoter-TSS (NM_016769)
<i>Tbx3</i>	293.08	Intergenic
<i>Tbx20</i>	292.35	Intergenic

**Table S2 – Oligonucleotide sequences for ChIP-PCR**

Gene Name	Primer Sequences	
<i>Gata4 CDREs1</i>	Forward	CACGTGCGCTCCAAAAGTC
	Reverse	GCCCAGAGGGGTTAACGTG
<i>Gata4 CDREs2</i>	Forward	GAAGTATCCCCCAGCTGTT
	Reverse	AGATAGCAAAGTCTCAAAGTAGCC
<i>Hand1 CDREs1</i>	Forward	GTTGTCTGAGCTCGTCCA
	Reverse	CCCGAGAGCAACTACCAGAA
<i>Hand1 CDREs2</i>	Forward	AACTTGCCCAGTCTTGACGA
	Reverse	GAGGTCTTGCTCCTATGG
<i>Hand2 CDREs1</i>	Forward	TTAGTGTGGCCCTCTCTCCA
	Reverse	AAGTGTGTTGTGTATGGGCT
<i>Hand2 CDREs2</i>	Forward	CCCCCGTCATTCTCTGTGTG
	Reverse	GGATGAGAACCACGTACTCTT
<i>Mef2c</i>	Forward	TCCCCAAAACCCCTATAACCT
	Reverse	GTAAGTATGAAAATTGCCCT
<i>Tbx5 CDREs1</i>	Forward	ACCTCTCTGTTCTATCCAGTG
	Reverse	CGCTTAGTCGACCTCAAGTCTAA
<i>Tbx5 CDREs2</i>	Forward	GCTCGCAGCTCTCACCAAAT
	Reverse	AAACTAGAGGGGATGGGTGC
<i>Wnt3a Exon4</i>	Forward	GCCATGCCAGTCACATGCACCTCAAGTGC
	Reverse	CTACTGCGAGGTGTGCACGTATAGACACG
<i>Tbx5 Control Region</i>	Forward	ACACCAAAGCTATGACTTGTCTGGC
	Reverse	ATCCTTAGGTTCCCTGAGCTGACAT
<i>Gata4 Control Region</i>	Forward	AAGTCACTCCGCTCTTAGACAA
	Reverse	ATTAGGTCACTGTAGTAGCTT
<i>Nkx2-5 Control Region</i>	Forward	AAGCTGCTCATTTCACCCCTCTA
	Reverse	ACTTAAGAGTGACTTGTAGCTAA

**Table S3 – Oligonucleotide sequences for qRT-PCR**

Transcript	Primer Sequences	
<i>Tbx5</i>	Forward	ATGGTCCGTAACTGGCAAAG
	Reverse	ACAAGTTGTCGCATCCAGTG
<i>GAPDH</i>	Forward	GGCCGGTGCTGAGTATGTCG
	Reverse	TTCAAGTGGGCCCGGCCTT

**Table S4 – Oligonucleotide sequences for semi-quantitative RT-PCR**

Transcript	Primer Sequences	
<i>cTnT</i>	Forward	CCAGCAGGCTTTCATGC
	Reverse	TTCATTCAAGGTGGTCGATGG
<i>Nkx2-5</i>	Forward	CAGCAACTTCGTGAACCTTGCG
	Reverse	AATCTGAGGGACAGGGCATAGTGG
<i>Cdx1</i>	Forward	ACAGAGCGGCAGGTAAAGATCTGG
	Reverse	GGAGACGGTGAAGCCTCAATCTTGT
<i>Cdx2</i>	Forward	AGCCAAGTAAAACCAGGACAA
	Reverse	TGCAAGGAGGTACAGGACT
<i>Cdx4</i>	Forward	GCATGGATGCGCAAACGT
	Reverse	CCAGAGTCACTTGCACGGA
$\beta$ -Actin	Forward	GCGGGAAATCGTGCCTGACATT
	Reverse	GATGGAGTTGAAGGTAGTTCTGT

**Table S5 – Oligonucleotide sequences for FAIRE-PCR**

Gene Name	Primer Sequences	
<i>Gata4 CDRE1</i>	Forward	CACGTGCGCTCCAAAAGTC
	Reverse	GCCCCACGTCTCTGAGATG
<i>Gata4 CDRE2</i>	Forward	GAAGTATCCCCGCAGCTGTT
	Reverse	GGGTCTGCCTCCCCAAGATC
<i>Tbx5 CDREs1</i>	Forward	ACCTCTCTGTTCCCTATCCAGTG
	Reverse	GCCTGGACCGATCTCCAGCT
<i>Tbx5 CDRE2</i>	Forward	GCTCGCAGCTCTCACCAAAT
	Reverse	CCTGGGGTGTGGTGAGG
<i>Nkx2-5</i>	Forward	CTTCCTCTGTTCCCCTATTAGC
	Reverse	GGAAACTGTTTATGTGTG
<i>β-Actin</i>	Forward	CCCTAAGGCCAACCGTGAAA
	Reverse	GTCCTATGGGAGAACGGCAG