cGATA6 (-1420/-1319, 102 bp)

GGGGGTGGCGGCGATAACAGAGTATTTGAAGGCGCTGGGATAAGGAGCGGAGGGACGCGCGGGCGGCACGTCGGCGCTCGGGGCGCTGCGTTATCGGCCCGG

cGATA6(GATA)$_2$ (-1420/-1374, 47 bp)

GGGGTGGCGGCGATAACAGAGTATTTGAAGGCGCTGGGATAAGGAGC

cGATA6muSP(GATA)$_2$ (47 bp)

GGCAAGCTTGCGATAACGCTGAAGCTTCATCGCGAGGGATAAGCGCT

TBX2 (-2700/-2300, 380 bp)

GCTGAGCGCGCGTCCCCCACCCGTCTGCGGGTCCCGGCCGATGCGAAGTGACAGGGGCCGGAGCTTTGTTGTGGAGCCTCGGCCGCCTGGCGCCAGGCGCCCTCGTCCGTGCCCTCCCCCTCCAGCCCCGGGCGGCCCGCGAGGCGCCCCCCGGGGCCTCTTAAAGAGACACGCACACTCTGCCCAGGGGACTCCCCCAGGGGCGCCTGTTCCTGGGAAGGGGAGATGAGTGGGGGTAGACCCAGGCGGGGGTAGGGCGAAGCAGGAGTTGCGAGCCGCAAGGCGCCGCTCGAGAGGCTGCTTAGGCCGCCCCAGCACCAGTTTTCGATGCTACAAGGATATTCTAAGAAAATTTTGTTGGGGATGGGTCGATAAAGAATCTTAAG

GJD3 (-2900/-2300, 660 bp)

GACCCCTGCCACTCACTTCCGGCCGAGCTGCCGTCTGCACCCTGTCCTCAGCTATGGAGGCTCAGGGTCACATCTCTTCTTTCCTAACTACCCAGGAATGGTCCCAGCCCCAGCCCCCATTGCTCCTGCTTCGTCAGGCTGGAAAAAGCCCACCAGACCCCACCCCACCTGAGCCCACCCGACCCCACCCAGTGTGACTCTGCAGGGAGTGGGATCGTGCCACAGAGATGCTAGGGTGGGCAGCAGATGGAGAGCCACTGTCACTCTGCCCTGTCCCCCACCTAGCTCCCCATGCCAGAGGAGACTCTACAATCTCTAGGACCCAGTCCTAGATAGCCGTGGGCAGCCCGGGAGGTGTTGCTGACAATACCCAGAGCTGGTGGCATTGGCCCTTCTTGGCCCTGCTTGAAGAAGTTCTGGGCTGAGAGGGTTCTTCTGTGTCCCAGACACCTCAGTCTGGCTGTGGAGCCATAGTCCCGCATCAGTCACAGGTGGACATACACAGGGGCGTGGACGTGTTCATATCTAATAAGTTCATAACAGTACCGTAATTGACACATGACAACATTTTTTGAAAATGAAAGAAATAAGCAAAAACAAAACAGAAAAACAACAAAAAAACCCAAGGCCGAATGGAATCTCTCCCAGCCTGCTCAGTA

TNNI3 (-230/+126, 356 bp)

GTCTAGACTCTTGGATTTGAGAGAAGAGGGACCTTGCTCCGGGTTTTCCTAAGTTTGAGGGAGGAGGGAGCTGGGGCGCTAGAGTCAAAGGAGGAGGGGTGTAGATCCTGGGCACCTTGGTTGACCCAACTGGAGCTTTGCACACGGCTCCCCTCACACCCTGTTATCGCTTATCCTGGGCAGGGGAGGAGACAGCAGTATATTTAGTCTTTGTCCTCGCCCCTTATCTC***AGTGTCCTCAGTGAGGCTTGAGCAGCCCAGAGGAAACCCAACCTCTAGAGACCTCCAAGGTCACCAGGGACACCCTTCCAGGACCCTCCAGGAATCTCCGATCCTGTTCTCTGCCTCTGGAGATCA***

TNNT2 (-497/+192, 711 bp)

CTGCAGAGCCATCTGCCAGAGGCCGCTTAGCCCATAAGAAGCAGCCAACAGCCCAGACAACATTTTTAACCCCCGACTTCATTTCCAACGGAGTTCTAGCCCTCTCCTCCCCAGGACCTGGCTCAGAGGCTCGTGAAGTGTCTCTGACGTTGACTACTTCTGCCCTCAGCTCTGCTCCCAGCCTGCTCTTCTGGACCTGGGCTTCTGGCGTCTGCTTTATCGGGATTCTCAAGAGGGACAGCTGGTTTATGTTACAAGCCTGTTCCCTGCATATCTGCTCTGGTTTTAAATAGCTTTATCTGAGCAGCTGGAGGACCACATGAGCTTATATGGCGTGGGGTACTTGTTCCTTTAGCCCTGTGCCGGGCACCTGTCAAAATAGTAGCCAACACCCCCCCCATTGTGTTGTCCCCCCCCCATCTCCTGCTGCACATTCCTCCCACCGTGGGGCTTGGCTCACAAGGCCCCAGCCCACATGCTTGCTTAAAGCTCTCCCC***ATGCCCTGCCCTCAGCCAGTCCCTGCTGAGGCTGAGCAGACACCTCAAGTCCTGAGTGCAGGTCCCTGTTCAGAGGTAAGACAAGGGGAGGCTCTAGGAAGGGACAGAAGTCGGCGGGAGAACGAGAGATTTGGCCCTGACACCTTTCCCTAGCCGTGTGCTTGTGTCTACCCTGAGTGGTTGGGTACCAGGAAATCCAGGGCCCTTTCTAGA***

1) GATA binding motifs identified by ChIP sequencing indicated in literature[1-3]

WGATAAC NGATN

2) SMAD binding motifs identified by ChIP sequencing indicated in literature[4]

CAGAGCCa CTTCtCTG TCTGCtCT GgGTGTGG GAGGtGGG GGCGGGGc

SMAD consensus binding sites indicated in literature[5-9]

GTCTG GTCT GGCGCC GCCG GCAT CAGC CGCC

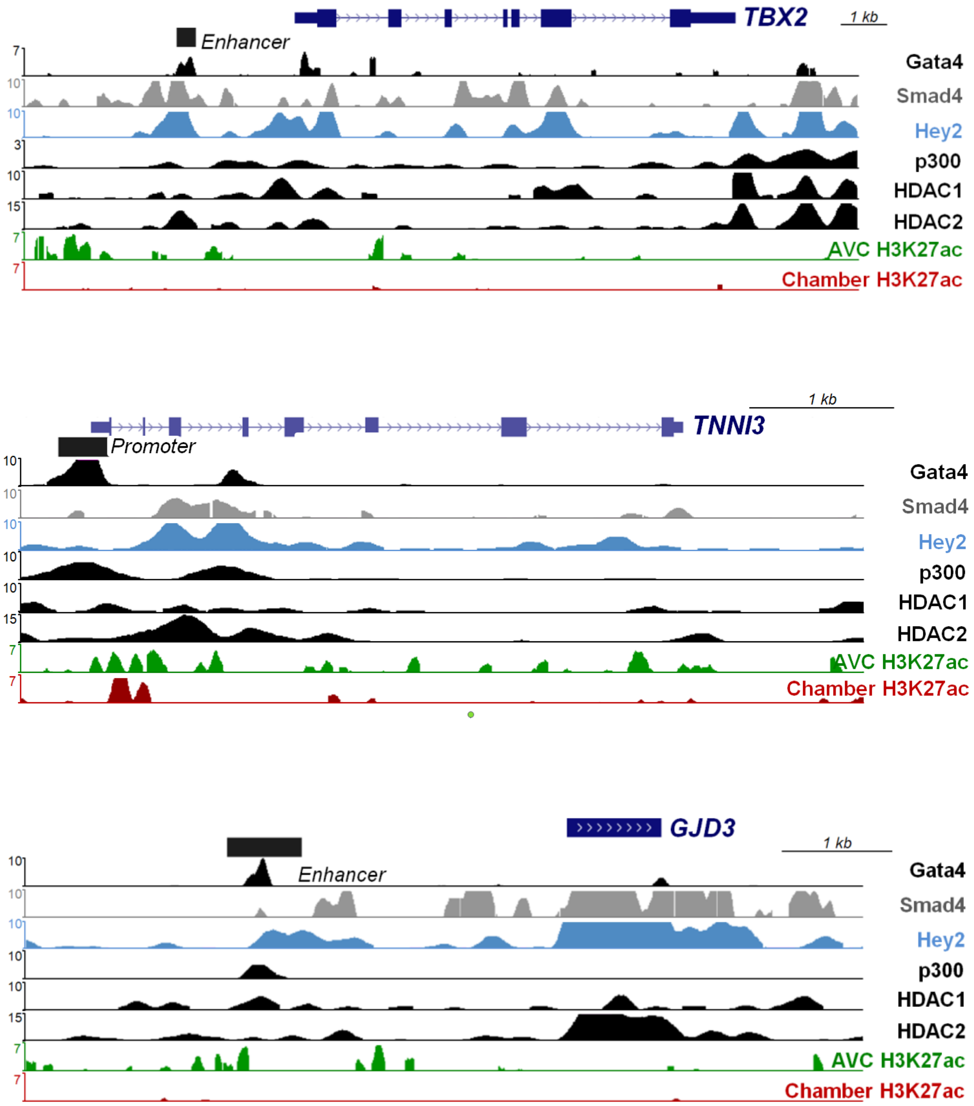
3) E-box binding motifs identified by ChIP sequencing indicated in literature[10]

CGCGTC CACGTG CACGCG

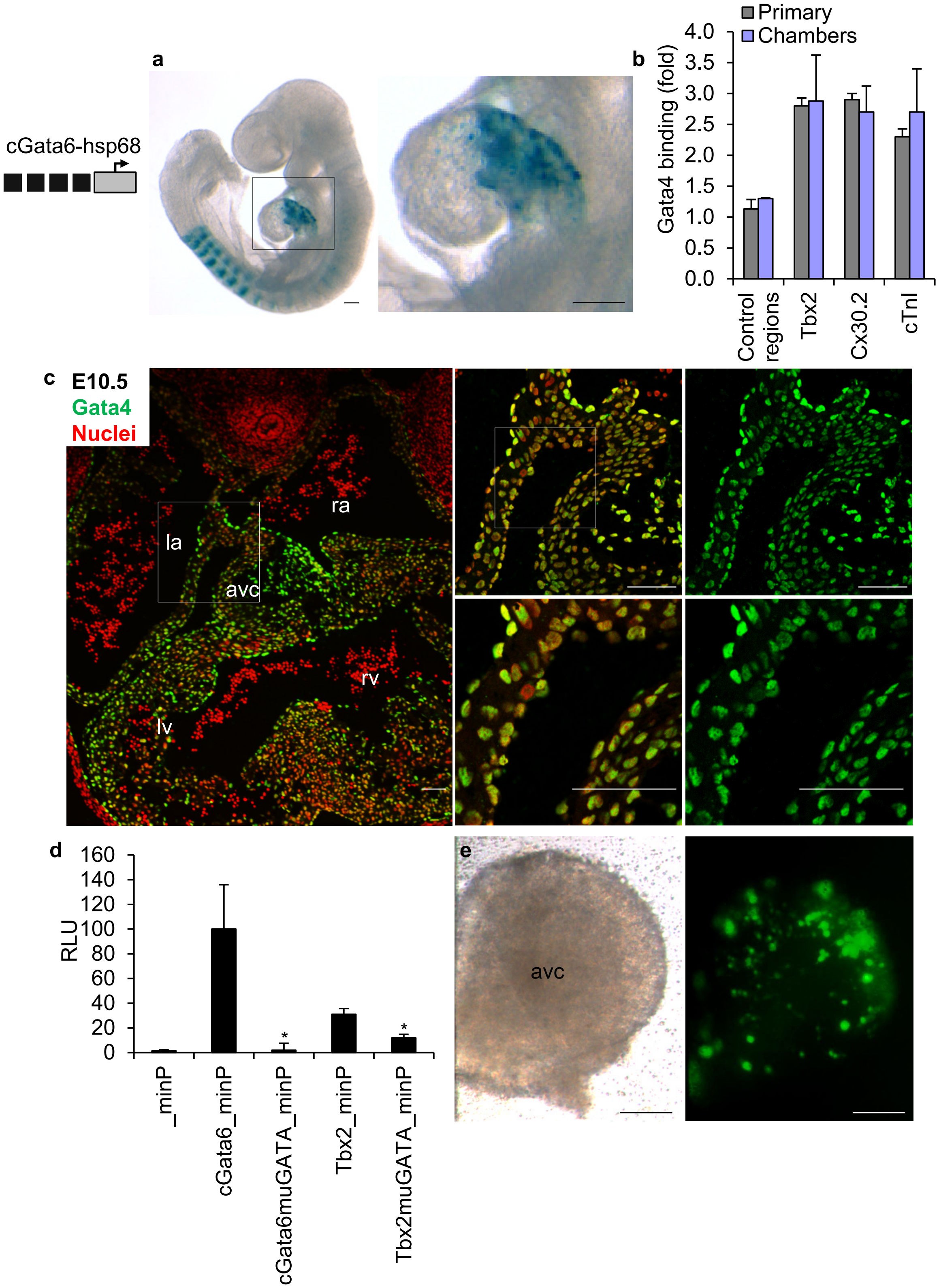
4) SP consensus binding element indicated in literature[11,12]

(G/T)GGGCGG(G/A)(G/A)(C/T)
GAGGGAGGAGGGAG
AAAGGAGGAGGGG

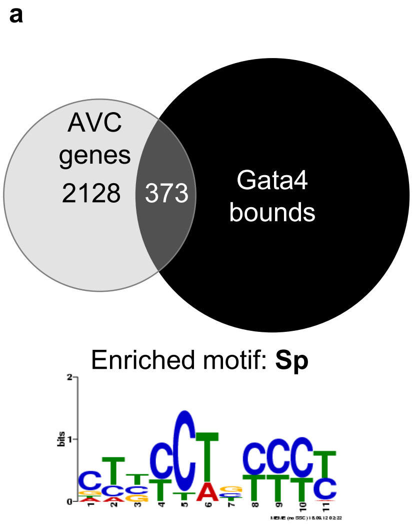
Supplementary Figure 1: Transcription binding sites in the cGata6, Tbx2, Cx30.2, cTnI and cTnT regulatory regions (GATA binding sites are depicted in green, SMAD in grey, E-box in blue and SP in red).



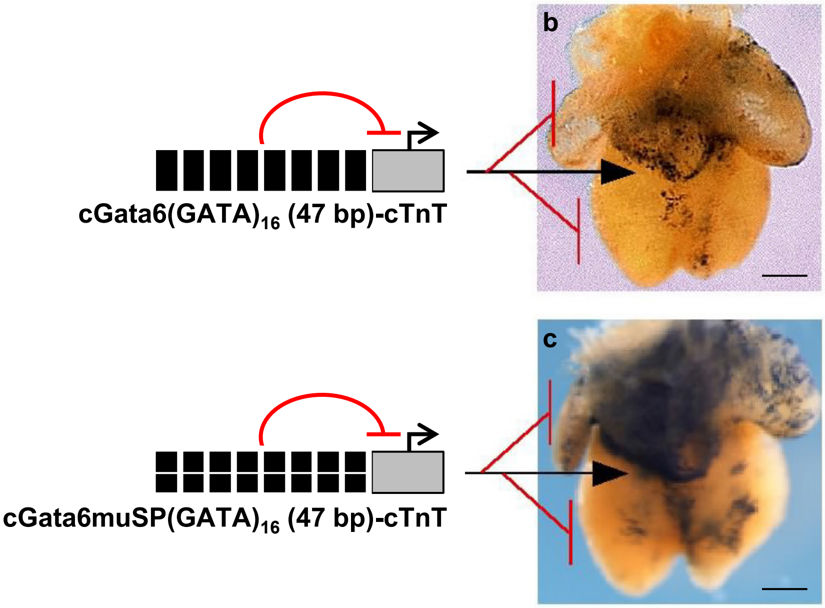
Supplementary Figure 2: UCSC genome browser views of ChIP-seq data for Tbx2, Gjd3 and cTnnI loci with ChIP-seq profiles of Gata4 (black) and p300 (black) in heart, Smad4 (grey) in human ESC, Hdac1, -2[26] (black) and Hey2 (blue) in mouse ESC. AV canal specific regions are depicted as a black box.



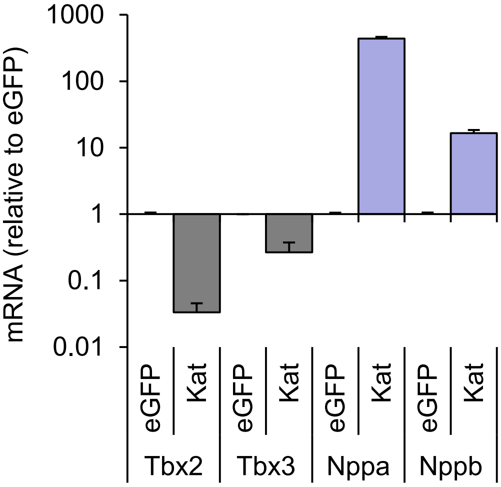
Supplementary Figure 3: (a) Tandemly repeated copies of a 102 bp cGata6 enhancer fragment drive expression in the developing AV canal at E9.5 (scale bars represent 100 µm). (b) Gata4 ChIP-qPCR on E10.5 AV canal/outflow tract and chamber extracts shows enrichment for AV enhancers. (c) Immunohistochemistry for Gata4 protein on sections of E10.5 hearts (scale bars represent 25 µm). (d) Activity of wt and GATA mutated cGata6 and Tbx2 enhancers in AV canal explants (Bars represent mean sem; n=4; *p < 0.05 by Student's t-test) (e) AV canal explant transfected with a CMV-EGFP reporter (scale bars represent 25 µm).



	motif name	p-value	corrected p-value
1	Gata1	1.18E-61	5.63E-59
2	GAT1	8.09E-34	3.85E-31
3	Tal1::Gata1	1.80E-31	8.57E-29
4	GZF3	8.63E-17	4.11E-14
5	Klf4	2.63E-14	1.25E-11
6	MEF2A	2.38E-12	1.13E-09
7	Evi1	4.48E-12	2.13E-09
8	SP1	1.04E-10	4.96E-08
9	btd	4.24E-10	2.02E-07
10	hkb	2.86E-09	1.36E-06



Supplementary Figure 4: (a) Sp binding motif is significantly enriched in Gata4- bound sites. To identify Gata4-cofactor networks operating proximally and distally through cis- and transacting elements with respect to the Gata4 occupancy, we crossed our Gata4 ChIP-seq**[18]** with the gene expression profiling of the AV canal[19] using Galaxy. We found that 373 peaks were present in or near AV canal genes (flanking regions of 50 kb on either side of the AV canal loci). We then searched for enrichments in co-occurring transcription factor binding site motifs within the 373 Gata4 ChIP-seq peaks. In line with this we now provide the top 10 motifs recovered by MEME in the set of 373 peaks, together with a screenshot of the STAMP match for SP1 from Jaspar. The scoring was conducted using the Fisher-exact test. (b) cTnT transgenic harbouring tandemly repeated copies of a 47 bp cGata6 show predominant expression in the AV canal myocardium, similar to the pattern of the cTnT transgenic in which only the GATA sites were preserved (c). Scale bars represent 100 µm.



Supplementary Figure 5: mRNA isolated from AV canal-EGFP and chambers-Katushka sorted cells and expression of AV canal (*Tbx2*, *Tbx3*) and chamber markers (*Nppa*, *Nppb*) were analyzed with RT-qPCR. Data were normalized to *Hprt* and expressed as folds of increase over eGFP (mean sd; n=3).

Established AV canal gene loci
Bmp2[13-15]
Cacna1g[16]
Cacna2d2[17]
Gjd3[18]
Id1[19]
Id2[20,21]
Kcna6[22]
Msx2[23]
Robo1[24]
Tbx2[25]
Tbx3[25]

Established myocardial gene loci
Actc1
Atp2a2
Des
Gata4
Gata6
Mef2c
Mybpc3
Myh6
Myh7
Myl2
Myl4
Myl7
Nkx2-5
Pln
Tbx20
Tbx5
Tnnc1
Tnnt2
Tpm1
Zfpm2

Top-50 enriched AV canal gene loci
5730559C18Rik

5930412G12Rik
9030425E11Rik
9030425E11Rik
Abi3
Afp
Crlf1
Crmp1
Cthrc1
Cyp26b1
Dhh
Dnm3os
Ebf3
Emid2
Foxc2
Gata3
Gngt2
Hapln1
Hmgcs2
Hoxa5
Hs3st1
Icam2
Igf1
Itih2
Klk8
Matn4
Mfsd2
Mmp2
Msx1
Olfml1
Papss2
Papss2
Prdx2
Ptprb
Ramp2
Sall3
Serpina1b
Serpina1d
Serpinf1
Serpinf2

Sox10
Sox17
Sox9
Sp5
Tgfbi
Tgfbi
Trpv4
Tspan8
Twist1
Twist2

Supplementary Table 1: List of genes expressed specifically in AV canal or in all myocardium, and of the top-50 AV canal-enriched gene loci.

	Number of AV canal H3K27ac peaks	Number of chamber H3K27ac peaks	Ratio of H3K27ac peaks AV canal / chamber	Significance (Z-test on difference)
Random UCSC gene loci (50kb flanks)	12221	11273	1.08	0-hypothesis population
Established myocardial gene loci (2kb flanks)	132	183	0.72	1.46×10^{-3}
Established myocardial gene loci (50kb flanks)	268	375	0.71	2.15×10^{-7}
Established AV canal gene loci (50kb flanks)	96	62	1.55	2.58×10^{-2}
Top-50 AV canal-enriched gene loci (2kb flanks)	103	55	1.87	8.26×10^{-4}
Top-50 AV canal-enriched gene loci (50kb flanks)	288	187	1.54	1.39×10^{-4}
Putative enhancers of established myocardial genes	245	332	0.74	5.70×10^{-6}
Putative enhancers of established AV canal genes	92	57	1.61	1.61×10^{-2}
Promoters of established myocardial genes	13	27	0.48	1.41×10^{-2}
Promoters of top-50 AV canal-enriched genes	100	51	1.96	4.26×10^{-4}

Supplementary Table 2: Comparison of H3K27ac enrichment on AV canal gene loci in AV canal-cells or chamber cells. Values are expressed as number of peaks called from AV canal-derived and chamber-derived H3K27ac ChIP-Seq datasets, respectively. Random UCSC gene loci were selected by randomly picking 5541 genes from the total UCSC Gene list. Established myocardial gene loci were defined as 20 selected genes with a confirmed broad cardiac expression pattern. Established AV canal gene loci were 11 selected genes the expression of which has been experimentally confirmed to be limited to AV canal myocardium (see Supplemental Table S2 for the selected gene lists). Top-50 AV canal-enriched gene loci were selected by picking the 50 genes most significantly differentially expressed between AV canal and chambers as determined by micro-array data generated by Horsthuis et al. 2009[22]. All genes were extended upstream and downstream with 2kb or 50kb flanking regions to incorporate potential regulatory regions. To analyze promoter exclusive interactions, TSS coordinates were taken from all gene sets and extended 3kb upstream and 1 kb downstream. To assess H3K27ac distribution at putative enhancer regions, the genes were extended 50 kb upstream and 50 kb downstream, whereas the region spanning from −3 kb to + 1kb relative to the TSS was excluded. Peaks were considered overlapping with a gene locus or promoter if they have 1 bp or more in common.

H3K27ac enriched regions occupied by Gata4 in AV canal

Term	Count	%	P-value
GO:0006793~phosphorus metabolic process	82	9	2.76E-09
GO:0006796~phosphate metabolic process	82	9	2.76E-09
GO:0010604~positive regulation of macromolecule metabolic process	60	7	5.25E-07
GO:0006468~protein amino acid phosphorylation	60	7	7.58E-07
GO:0010628~positive regulation of gene expression	49	5	1.41E-06
GO:0045941~positive regulation of transcription	48	5	1.55E-06
GO:0016310~phosphorylation	64	7	1.71E-06
GO:0031328~positive regulation of cellular biosynthetic process	53	6	1.87E-06
GO:0045935~positive regulation of nucleobase, nucleoside, nucleotide and nucleic acid metabolic process	50	6	2.15E-06
GO:0007242~intracellular signaling cascade	76	9	2.23E-06
GO:0051173~positive regulation of nitrogen compound metabolic process	51	6	2.30E-06
GO:0007167~enzyme linked receptor protein signaling pathway	33	4	2.31E-06
GO:0009891~positive regulation of biosynthetic process	53	6	2.46E-06
GO:0045893~positive regulation of transcription, DNA-dependent	43	5	3.38E-06
GO:0051056~regulation of small GTPase mediated signal transduction	29	3	4.01E-06
GO:0051254~positive regulation of RNA metabolic process	43	5	4.06E-06
GO:0006357~regulation of transcription from RNA polymerase II promoter	56	6	4.87E-06
GO:0010557~positive regulation of macromolecule biosynthetic process	50	6	6.30E-06
GO:0007507~heart development	28	3	7.76E-06
GO:0030029~actin filament-based process	24	3	1.04E-05
GO:0045944~positive regulation of transcription from RNA polymerase II promoter	37	4	1.86E-05
GO:0030036~actin cytoskeleton organization	22	2	3.73E-05
GO:0007169~transmembrane receptor protein tyrosine kinase signaling pathway	24	3	4.28E-05
GO:0007155~cell adhesion	48	5	1.15E-04
GO:0022610~biological adhesion	48	5	1.20E-04
GO:0046578~regulation of Ras protein signal transduction	21	2	3.94E-04
GO:0007010~cytoskeleton organization	31	3	4.31E-04
GO:0035295~tube development	26	3	8.37E-04
GO:0060416~response to growth hormone stimulus	4	0	1.00E-03
GO:0060396~growth hormone receptor signaling pathway	4	0	1.00E-03
GO:0044057~regulation of system process	21	2	1.48E-03

GO:0032870~cellular response to hormone stimulus	10	1	1.56E-03
GO:0051270~regulation of cell motion	14	2	1.69E-03
GO:0000902~cell morphogenesis	28	3	1.76E-03
GO:0035023~regulation of Rho protein signal transduction	12	1	2.48E-03
GO:0032989~cellular component morphogenesis	30	3	2.80E-03
GO:0048514~blood vessel morphogenesis	20	2	2.90E-03
GO:0016311~dephosphorylation	16	2	2.93E-03
GO:0001568~blood vessel development	23	3	3.10E-03
GO:0001944~vasculature development	23	3	4.16E-03
GO:0003012~muscle system process	10	1	4.30E-03
GO:0032318~regulation of Ras GTPase activity	11	1	4.54E-03
GO:0006816~calcium ion transport	14	2	4.99E-03
GO:0007264~small GTPase mediated signal transduction	23	3	6.06E-03
GO:0001763~morphogenesis of a branching structure	14	2	6.55E-03
GO:0009719~response to endogenous stimulus	18	2	6.84E-03
GO:0006936~muscle contraction	9	1	7.26E-03
GO:0006470~protein amino acid dephosphorylation	13	1	8.02E-03
GO:0008016~regulation of heart contraction	8	1	9.03E-03

H3K27ac enriched regions occupied by Gata4 in chambers

Term	**Count**	**%**	**P-value**
GO:0007517~muscle organ development	43	3	2.99E-12
GO:0060537~muscle tissue development	35	2	9.21E-11
GO:0048738~cardiac muscle tissue development	22	2	1.54E-10
GO:0007507~heart development	46	3	2.15E-10
GO:0014706~striated muscle tissue development	33	2	2.65E-10
GO:0042692~muscle cell differentiation	31	2	6.06E-10
GO:0051146~striated muscle cell differentiation	24	2	5.18E-08
GO:0030029~actin filament-based process	34	2	3.39E-07
GO:0003012~muscle system process	19	1	7.81E-07
GO:0010604~positive regulation of macromolecule metabolic process	80	6	1.05E-06
GO:0007010~cytoskeleton organization	49	3	1.74E-06
GO:0030036~actin cytoskeleton organization	31	2	2.24E-06

GO:0006936~muscle contraction	17	1	3.55E-06
GO:0055002~striated muscle cell development	16	1	4.10E-06
GO:0055001~muscle cell development	17	1	4.49E-06
GO:0006793~phosphorus metabolic process	98	7	7.91E-06
GO:0006796~phosphate metabolic process	98	7	7.91E-06
GO:0007155~cell adhesion	70	5	8.62E-06
GO:0022610~biological adhesion	70	5	9.17E-06
GO:0051254~positive regulation of RNA metabolic process	56	4	1.11E-05
GO:0055007~cardiac muscle cell differentiation	11	1	1.46E-05
GO:0045893~positive regulation of transcription, DNA-dependent	55	4	1.83E-05
GO:0051270~regulation of cell motion	22	2	2.21E-05
GO:0007242~intracellular signaling cascade	100	7	2.67E-05
GO:0001944~vasculature development	37	3	5.37E-05
GO:0003007~heart morphogenesis	17	1	6.11E-05
GO:0045935~positive regulation of nucleobase, nucleoside, nucleotide and nucleic acid metabolic process	62	4	6.71E-05
GO:0001568~blood vessel development	36	3	7.36E-05
GO:0035051~cardiac cell differentiation	11	1	7.41E-05
GO:0051056~regulation of small GTPase mediated signal transduction	34	2	9.81E-05
GO:0006357~regulation of transcription from RNA polymerase II promoter	71	5	9.93E-05
GO:0045941~positive regulation of transcription	58	4	1.02E-04
GO:0016310~phosphorylation	80	6	1.04E-04
GO:0007015~actin filament organization	14	1	1.38E-04
GO:0051173~positive regulation of nitrogen compound metabolic process	62	4	1.59E-04
GO:0010927~cellular component assembly involved in morphogenesis	11	1	1.67E-04
GO:0010557~positive regulation of macromolecule biosynthetic process	62	4	1.94E-04
GO:0010628~positive regulation of gene expression	58	4	2.10E-04
GO:0030239~myofibril assembly	8	1	2.35E-04
GO:0009891~positive regulation of biosynthetic process	64	4	2.52E-04
GO:0031032~actomyosin structure organization	9	1	2.58E-04
GO:0007167~enzyme linked receptor protein signaling pathway	37	3	3.28E-04
GO:0031328~positive regulation of cellular biosynthetic process	63	4	3.40E-04
GO:0040029~regulation of gene expression, epigenetic	15	1	4.11E-04
GO:0048514~blood vessel morphogenesis	29	2	4.73E-04

GO:0006468~protein amino acid phosphorylation	70	5	5.29E-04
GO:0051051~negative regulation of transport	15	1	5.55E-04
GO:0035295~tube development	35	2	7.17E-04
GO:0008016~regulation of heart contraction	12	1	7.26E-04

Supplementary Table 3: Biological process terms selected from the 50 most significantly enriched for dynamic H3K27 regions co-occupied by Gata4.

Bmp2_F	CGGACTGCGGTCTCCTAA	RT-qPCR
Bmp2_R	GGGGAAGCAGCAACACTAGA	RT-qPCR
Gata4_F	GGAAGACACCCCAATCTCG	RT-qPCR
Gata4_R	CATGGCCCCACAATTGAC	RT-qPCR
Gata6_F	GGTCTCTACAGCAAGATGAATGG	RT-qPCR
Gata6_R	TGGCACAGGACAGTCCAAG	RT-qPCR
Hdac1_F	TGGTCTCTACCGAAAAATGGAG	RT-qPCR
Hdac1_R	TCATCACTGTGGTACTTGGTCA	RT-qPCR
Hdac2_F	GCTGCTGGACTTACGAAACa	RT-qPCR
Hdac2_R	CTTGAAATCCGGTCCAAAGT	RT-qPCR
Hdac3_F	TTCAACGTGGGTGATGACTG	RT-qPCR
Hdac3_R	TTAGCTGTGTTGCTCCTTGC	RT-qPCR
Hdac4_F	GCAAGATCCTCATTGTAGACTGG	RT-qPCR
Hdac4_R	GAACATTGGGGTCATTGTAGAAG	RT-qPCR
Hdac5_F	CAGCACCGAGGTAAAGCTG	RT-qPCR
Hdac5_R	GGAACTCTGGTCCAAAGAAGC	RT-qPCR
Hdac6_F	GCCTAGATGTGTCCCAACCT	RT-qPCR
Hdac6_R	CCATATGGGCATTGACATAGC	RT-qPCR
Hdac7_F	GCAGCCCTTGAGAGAACAGT	RT-qPCR
Hdac7_R	TGTCCAAGGGCTCAAGAGTT	RT-qPCR
Hdac8_F	GCAGCTGGCAACTCTGATT	RT-qPCR
Hdac8_R	GTCAAGTATGTCCAGCAACGAG	RT-qPCR
Hdac9_F	TTGCACACAGATGGAGTGG	RT-qPCR
Hdac9_R	GGCCCATAGGAACCTCTGAT	RT-qPCR
Hey1_F	CATGAAGAGAGCTCACCCAGA	RT-qPCR
Hey1_R	CGCCGAACTCAAGTTTCC	RT-qPCR
Hey2_F	ATTGCAAATGACAGTGGATCAT	RT-qPCR
Hey2_R	AGCATGGGCATCAAAGTAGC	RT-qPCR
Hprt_F	TGTTGGATATGCCCTTGACT	RT-qPCR
Hprt_R	GATTCAACTTGCGCTCATCT	RT-qPCR
Nppa_F	CACAGATCTGATGGATTTCAAG	RT-qPCR

Nppa_R	CCTCATCTTCTACCGGCATC	RT-qPCR
Nppb_F	GTCCAGCAGAGACCTCAAAA	RT-qPCR
Nppb_R	AGGCAGAGTCAGAAACTGGA	RT-qPCR
Tbx2_F	TCACTGCCTACCAGAATGACAA	RT-qPCR
Tbx2_R	GCGTTAGCTGCTTCCTTTTC	RT-qPCR
Tbx3_F	GTCGTCACTTTCCACAAACTGA	RT-qPCR
Tbx3_R	GGACCCAGACTATACTAAACTC	RT-qPCR
Tbx2 (-2700/-2300)_F	ACTCTGCCCAGGGGACTC	ChIP-qPCR
Tbx2 (-2700/-2300)_R	GGGTCTACCCCCACTCATCT	ChIP-qPCR
cTnI (-230/+126)_F	TGAGAGAAGAGGGACCTTGC	ChIP-qPCR
cTnI (-230/+126)_R	AGCGATAACAGGGTGTGAGG	ChIP-qPCR
Cx30.2 (-2900/-2300)_F	GAGGACAGGGTGCAGACG	ChIP-qPCR
Cx30.2 (-2900/-2300)_R	CCCCACATTCTGAAAACAGG	ChIP-qPCR
ID2 BRE (promoter)_F	ACGCGCGGTAATCAGAAG	ChIP-qPCR
ID2 BRE (promoter)_R	ATCACGCGGGGATAGTAGC	ChIP-qPCR
Nppa (promoter)_F	AACCAGAGTGGGCAGAGACAG	ChIP-qPCR
Nppa (promoter)_R	TGATGGAGAAGGAGCCCATGC	ChIP-qPCR
cTnT (promoter)_F	TCTATCTGCCTGAGGCCATT	ChIP-qPCR
cTnT (promoter)_R	ATGAGGCTGGGGGTAATGA	ChIP-qPCR
Negative control region 1 (Chr4: 147397477- 147397580)	TCCAGACACACTGAACAGCAC	ChIP-qPCR
Negative control region 1 (Chr4: 147397477- 147397580)	TCCCCGAGATCAAGTGCTAC	ChIP-qPCR
Negative control region 2 (Chr4: 147397478 - 147397598)	CCAGACACACTGAACAGCAC	ChIP-qPCR
Negative control region 2 (Chr4: 147397478 - 147397598)	TGGCAGCAGGTTCTGGAATC	ChIP-qPCR

Supplementary Table 4: Real time PCR primer sequences.

Supplementary References

1 He, A., Kong, S. W., Ma, Q. & Pu, W. T. Co-occupancy by multiple cardiac transcription factors identifies transcriptional enhancers active in heart. *Proc Natl Acad Sci U S A* 108, 5632-5637, doi:10.1073/pnas.1016959108 (2011).

2 Schlesinger, J. *et al.* The cardiac transcription network modulated by Gata4, Mef2a, Nkx2.5, Srf, histone modifications, and microRNAs. *PLoS genetics* 7, e1001313, doi:10.1371/journal.pgen.1001313 (2011).

3 van den Boogaard, M. *et al.* Genetic variation in T-box binding element functionally affects SCN5A/SCN10A enhancer. *J Clin Invest* 122, 2519-2530, doi:10.1172/jci62613 (2012).

4 Fei, T. *et al.* Genome-wide mapping of SMAD target genes reveals the role of BMP signaling in embryonic stem cell fate determination. *Genome research* 20, 36-44, doi:10.1101/gr.092114.109 (2010).

5 Jonk, L. J., Itoh, S., Heldin, C. H., ten Dijke, P. & Kruijer, W. Identification and functional characterization of a Smad binding element (SBE) in the JunB promoter that acts as a transforming growth factor-beta, activin, and bone morphogenetic protein-inducible enhancer. *J Biol Chem* 273, 21145-21152 (1998).

6 Shi, Y. *et al.* Crystal structure of a Smad MH1 domain bound to DNA: insights on DNA binding in TGF-beta signaling. *Cell* 94, 585-594 (1998).

7 Zawel, L. *et al.* Human Smad3 and Smad4 are sequence-specific transcription activators. *Mol Cell* 1, 611-617 (1998).

8 Massague, J. & Wotton, D. Transcriptional control by the TGF-beta/Smad signaling system. *EMBO J* 19, 1745-1754, doi:10.1093/emboj/19.8.1745 (2000).

9 Korchynskyi, O. & ten Dijke, P. Identification and functional characterization of distinct critically important bone morphogenetic protein-specific response elements in the Id1 promoter. *J Biol Chem* 277, 4883-4891, doi:10.1074/jbc.M111023200 (2002).

10 Heisig, J. *et al.* Target gene analysis by microarrays and chromatin immunoprecipitation identifies HEY proteins as highly redundant bHLH repressors. *PLoS genetics* 8, e1002728, doi:10.1371/journal.pgen.1002728 (2012).

11 Kadonaga, J. T., Courey, A. J., Ladika, J. & Tjian, R. Distinct regions of Sp1 modulate DNA binding and transcriptional activation. *Science (New York, N.Y.)* 242, 1566-1570 (1988).

12 Di Lisi, R. *et al.* Combinatorial cis-acting elements control tissue-specific activation of the cardiac troponin I gene in vitro and in vivo. *J Biol Chem* 273, 25371-25380 (1998).

13 Ma, L., Lu, M. F., Schwartz, R. J. & Martin, J. F. Bmp2 is essential for cardiac cushion epithelial-mesenchymal transition and myocardial patterning. *Development* 132, 5601-5611, doi:10.1242/dev.02156 (2005).

14 Rutenberg, J. B. *et al.* Developmental patterning of the cardiac atrioventricular canal by Notch and Hairy-related transcription factors. *Development* 133, 4381-4390, doi:10.1242/dev.02607 (2006).

15 Singh, R. & Kispert, A. Tbx20, Smads, and the atrioventricular canal. *Trends in cardiovascular medicine* 20, 109-114, doi:10.1016/j.tcm.2010.09.004 (2010).

16 Mizuta, E. *et al.* Different distribution of Cav3.2 and Cav3.1 transcripts encoding T-type Ca(2+) channels in the embryonic heart of mice. *Biomedical research (Tokyo, Japan)* 31, 301-305 (2010).

17 Marionneau, C. *et al.* Specific pattern of ionic channel gene expression associated with pacemaker activity in the mouse heart. *The Journal of physiology* 562, 223-234, doi:10.1113/jphysiol.2004.074047 (2005).

18 Munshi, N. V. *et al.* Cx30.2 enhancer analysis identifies Gata4 as a novel regulator of atrioventricular delay. *Development* 136, 2665-2674, doi:10.1242/dev.038562 (2009).

19 Monteiro, R. M. *et al.* Real time monitoring of BMP Smads transcriptional activity during mouse development. *Genesis* 46, 335-346, doi:10.1002/dvg.20402 (2008).

20 Jongbloed, M. R. *et al.* Expression of Id2 in the second heart field and cardiac defects in Id2 knock-out mice. *Developmental dynamics : an official publication of the American Association of Anatomists* 240, 2561-2577, doi:10.1002/dvdy.22762 (2011).

21 Moskowitz, I. P. *et al.* A molecular pathway including Id2, Tbx5, and Nkx2-5 required for cardiac conduction system development. *Cell* 129, 1365-1376, doi:10.1016/j.cell.2007.04.036 (2007).

22 Horsthuis, T. *et al.* Gene expression profiling of the forming atrioventricular node using a novel tbx3-based node-specific transgenic reporter. *Circ Res* 105, 61-69, doi:10.1161/circresaha.108.192443 (2009).

23 Boogerd, K. J. *et al.* Msx1 and Msx2 are functional interacting partners of T-box factors in the regulation of Connexin43. *Cardiovasc Res* 78, 485-493, doi:10.1093/cvr/cvn049 (2008).

24 Mommersteeg, M. T. *et al.* Slit-roundabout signaling regulates the development of the cardiac systemic venous return and pericardium. *Circ Res* 112, 465-475, doi:10.1161/circresaha.112.277426 (2013).

25 Aanhaanen, W. T., Moorman, A. F. & Christoffels, V. M. Origin and development of the atrioventricular myocardial lineage: insight into the development of accessory pathways. *Birth defects research. Part A, Clinical and molecular teratology* 91, 565-577, doi:10.1002/bdra.20826 (2011).

26 Whyte, W. A. *et al.* Enhancer decommissioning by LSD1 during embryonic stem cell differentiation. *Nature* 482, 221-225, doi:10.1038/nature10805 (2012).