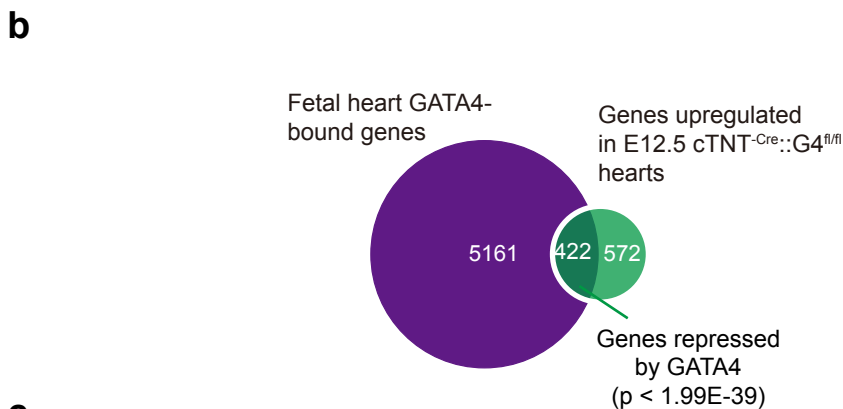


a

Downregulated in GATA4 KO	Go terms	Genes Count	p-Value	Adjusted p-Value
		heart development	19	1E-10
	cardiac muscle tissue development	8	4E-06	0.0025
	regulation of system process	12	2E-05	0.0095
	regulation of heart contraction	7	2E-05	0.0072
	muscle cell differentiation	9	7E-05	0.016
	striated muscle tissue development	9	0.0001	0.024
	muscle tissue development	9	0.0002	0.033
	heart morphogenesis	7	0.0002	0.033
	muscle organ development	10	0.0002	0.029
	muscle cell development	6	0.0007	0.082
	mitochondrion organization	7	0.001	0.099

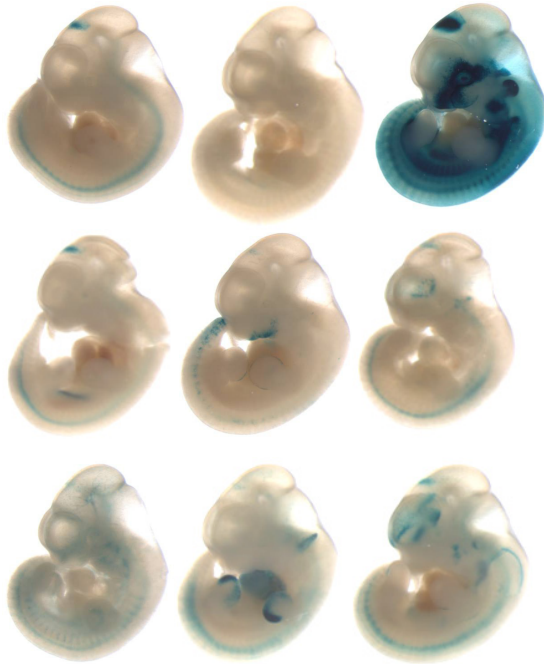


c

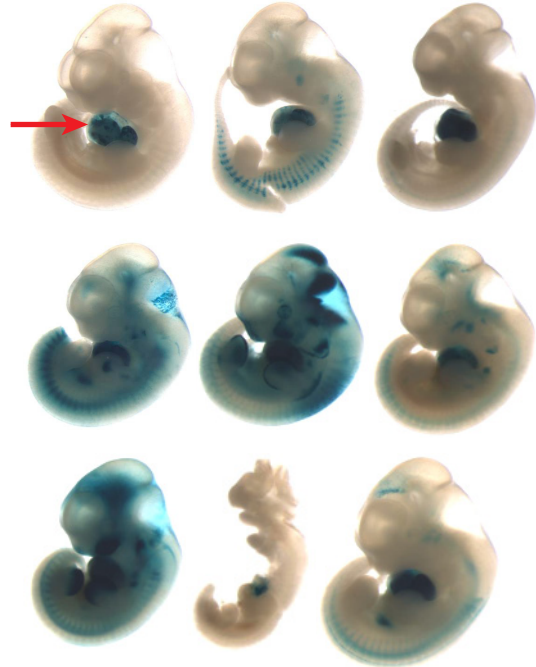
Upregulated in GATA4 KO	Go terms	Genes Count	p-Value	Adjusted p-Value
		cell adhesion	49	7.E-15
	biological adhesion	49	8.E-15	7.E-12
	tube development	26	4.E-09	2.E-06
	morphogenesis of a branching structure	17	4.E-08	2.E-05
	extracellular structure organization	18	9.E-08	3.E-05
	extracellular matrix organization	15	1.E-07	3.E-05
	vasculature development	23	1.E-07	3.E-05
	enzyme linked receptor protein signaling pathway	24	1.E-07	3.E-05
	heart development	21	4.E-07	7.E-05
	blood vessel development	22	4.E-07	7.E-05

Supplementary Fig. 1, Supporting Fig. 1. Enriched GO terms for genes bound by GATA4 and downregulated or upregulated in embryonic heart with GATA4 loss of function. All terms with p-value < 0.001 are shown. (a) GO terms for GATA4 enriched genes downregulated in E12.5 cTNT-Cre::G4^{fl/fl} embryonic heart. (b) Overlapping of GATA4 enriched genes with genes upregulated in E12.5 cTNT-Cre::G4^{fl/fl} embryonic heart. (c) GO terms for GATA4 enriched genes upregulated in E12.5 cTNT-Cre::G4^{fl/fl} embryonic heart.

a Enhancer ID: 11
Negative for heart



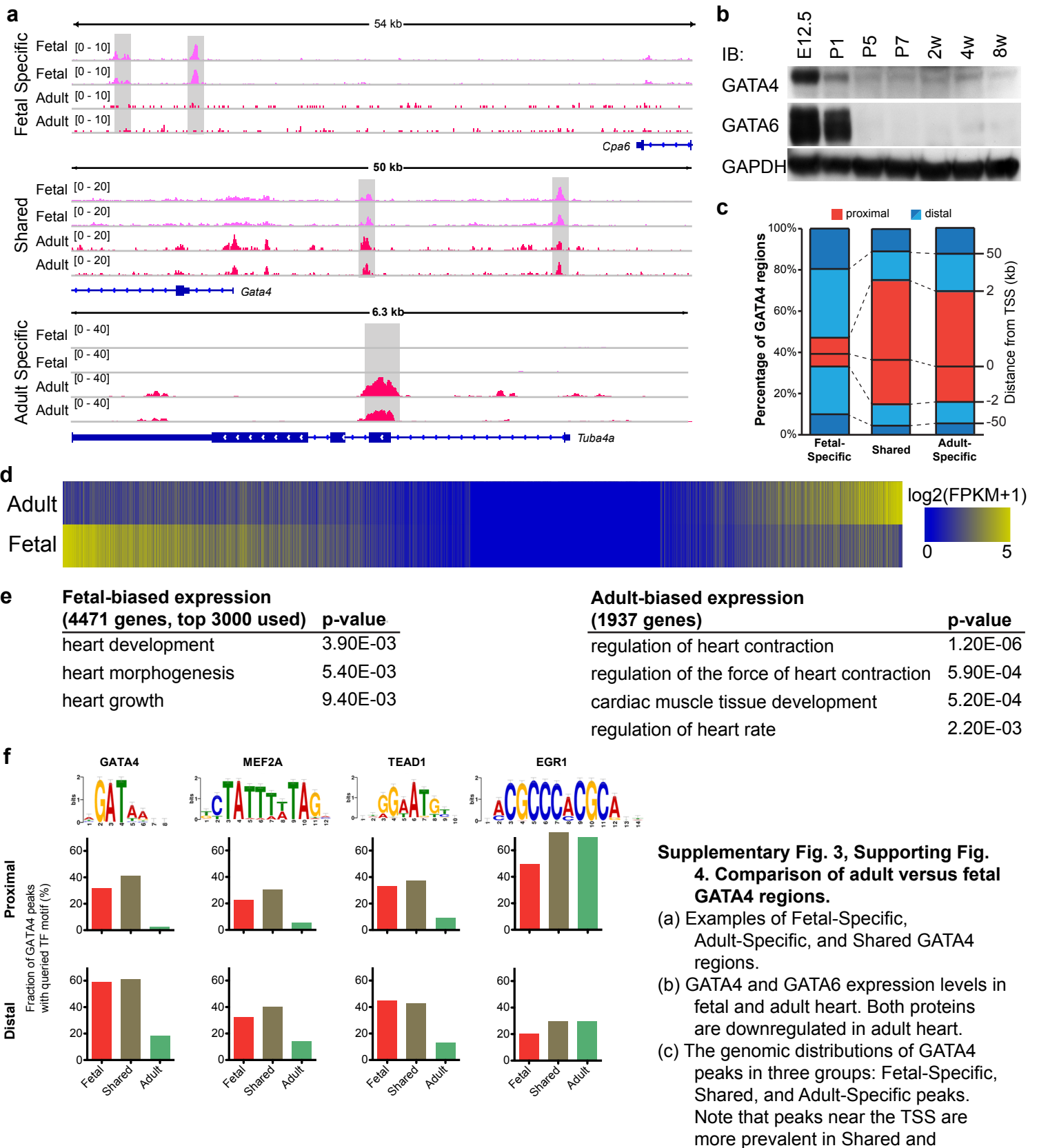
b Enhancer ID: 10
Positive for heart



Supplementary Fig. 2, Supporting Fig. 2. Examples of criteria used to score heart enhancer activity. A positive heart enhancer met three criteria: (1) at least 5 transgene positive embryos were obtained; (2) 3 or more embryos showed comparable staining patterns with positive heart staining; (3) embryos with widespread blue staining were excluded.

(a) Enhancer 11 (associated with *Kcne1*) was negative in heart.

(b) Enhancer 10 (also associated with *Kcne1*) was determined positive in heart as indicated by the red arrow.



Supplementary Fig. 3, Supporting Fig. 4. Comparison of adult versus fetal GATA4 regions.

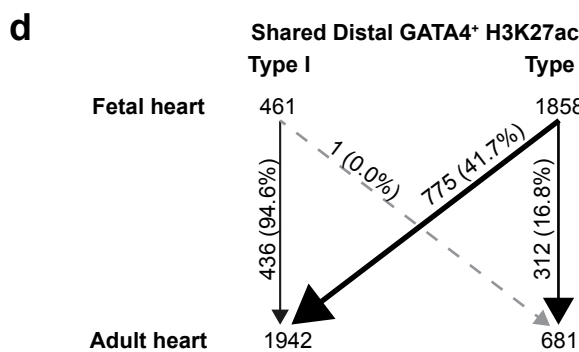
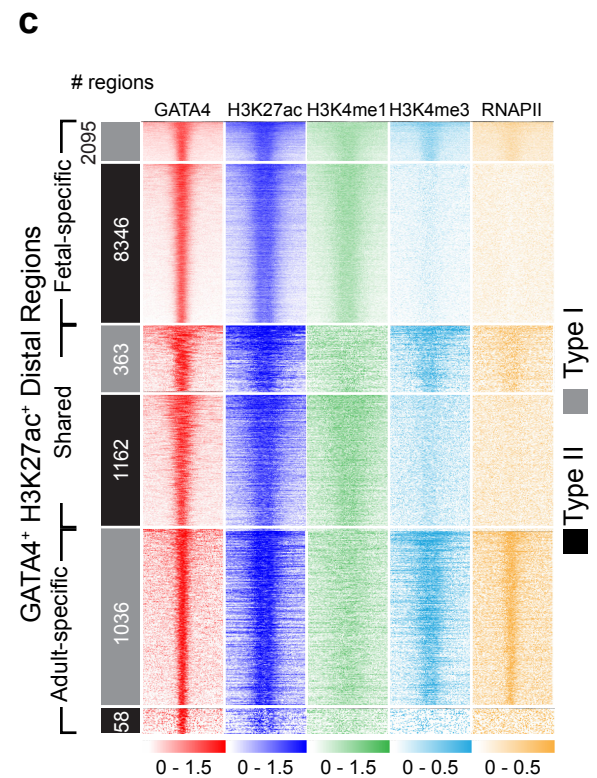
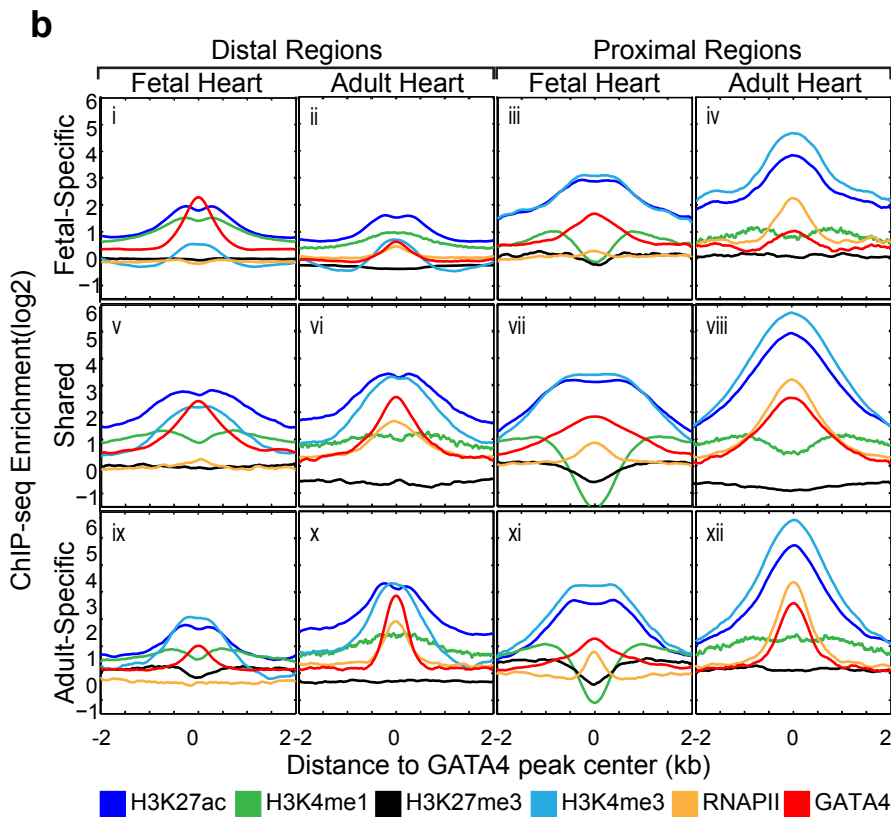
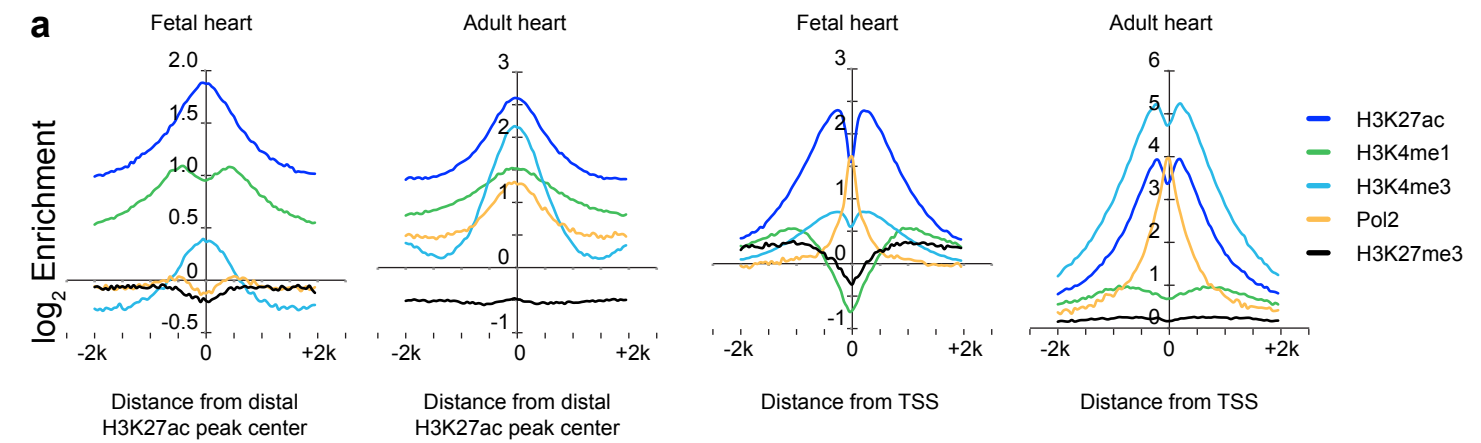
- (a) Examples of Fetal-Specific, Adult-Specific, and Shared GATA4 regions.
- (b) GATA4 and GATA6 expression levels in fetal and adult heart. Both proteins are downregulated in adult heart.
- (c) The genomic distributions of GATA4 peaks in three groups: Fetal-Specific, Shared, and Adult-Specific peaks. Note that peaks near the TSS are more prevalent in Shared and

Adult-Specific groups compared to Fetal-Specific.

(d) Heatmap of fetal and adult gene expression. Gene expression was measured by RNA-seq. Genes are sorted by the adult/fetal expression ratio.

(e) GO term analysis of genes with fetal or adult biased expression. Biased expression was defined as fold-change of greater than 2.

(f) Motif scanning with the indicated motifs showed their frequency in Fetal-Specific, Adult-Specific, and Shared GATA4 peak sets. The GATA, MEF2, and TEAD1 motifs were most frequent in Fetal-Specific and Shared regions. Proximal adult regions were notably depleted for the GATA motif but frequently contained the EGR1 motif, suggesting that proximal adult GATA occupancy may be driven by "piggy-back" binding of GATA4 to EGR family transcription factors.



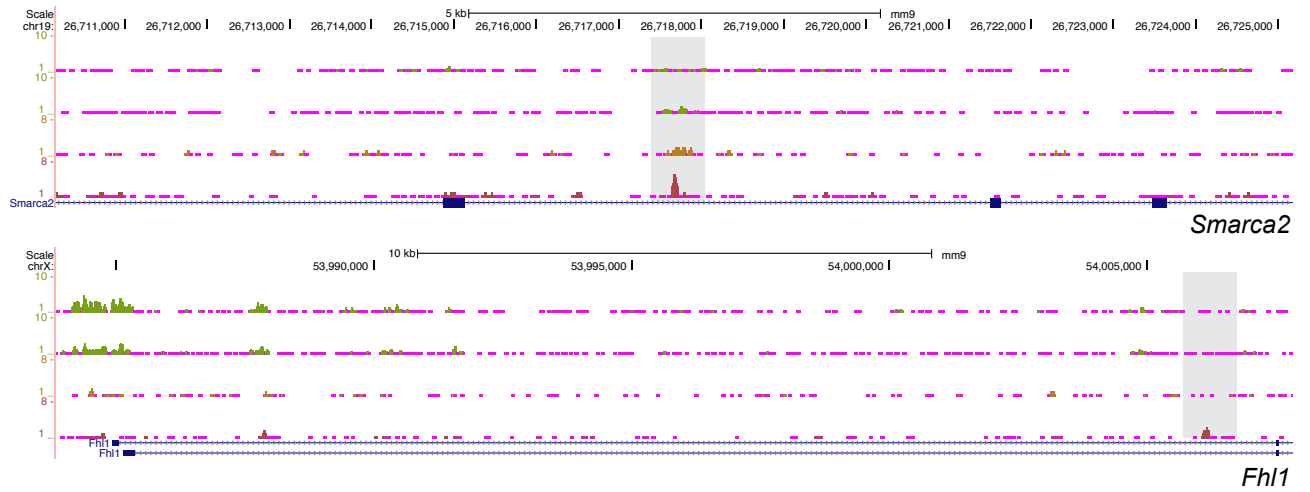
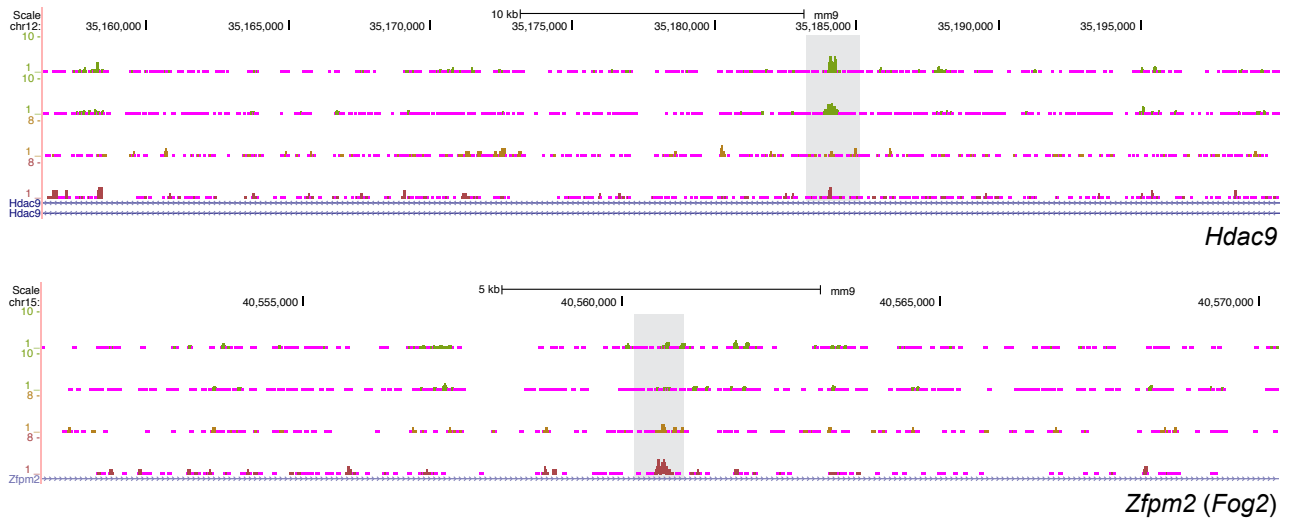
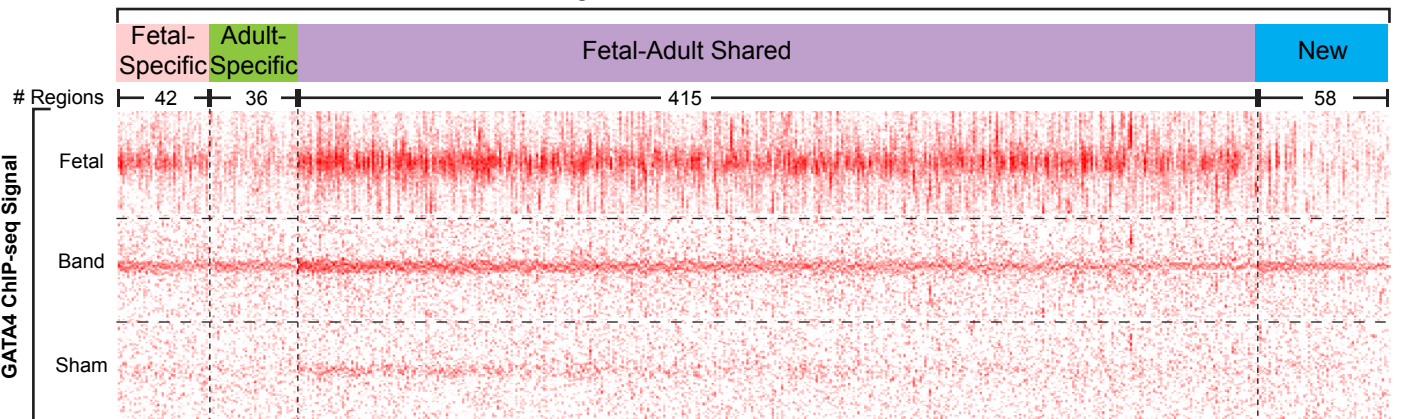
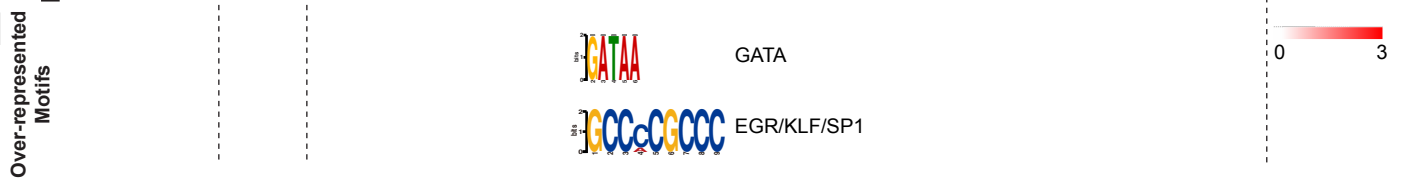
Supplementary Fig. 4, supporting Fig. 5.

(a) Global view of chromatin marks in TSS and distal regions. Aggregation plots of ChIP-seq data from fetal and adult heart show the overall pattern of profiled chromatin features at H3K27ac distal peak centers and at TSSs.

(b) Biological replicate ChIP-seq data showing the relationship of chromatin marks to GATA4 bound regions. Figures are based on biological replicates of the data shown in Fig. 5a.

(c) Tag heat map of GATA4⁺ H3K27ac⁺ regions using biological replicates of the data shown in Fig. 5b.

(d) Change of chromatin marks at Distal Shared GATA4⁺ H3K27ac⁺ regions between fetal and adult. Type II regions (H3K4me3⁻ and RNAPII⁻) frequently acquire these marks during heart development, while regions that already possess these marks rarely lose them.

a**New GATA4 Binding Induced by Pressure Overload****b****Restored Fetal GATA4 Binding Induced by Pressure Overload****c****Proximal Regions with Band-Induced GATA4 Peak****d**

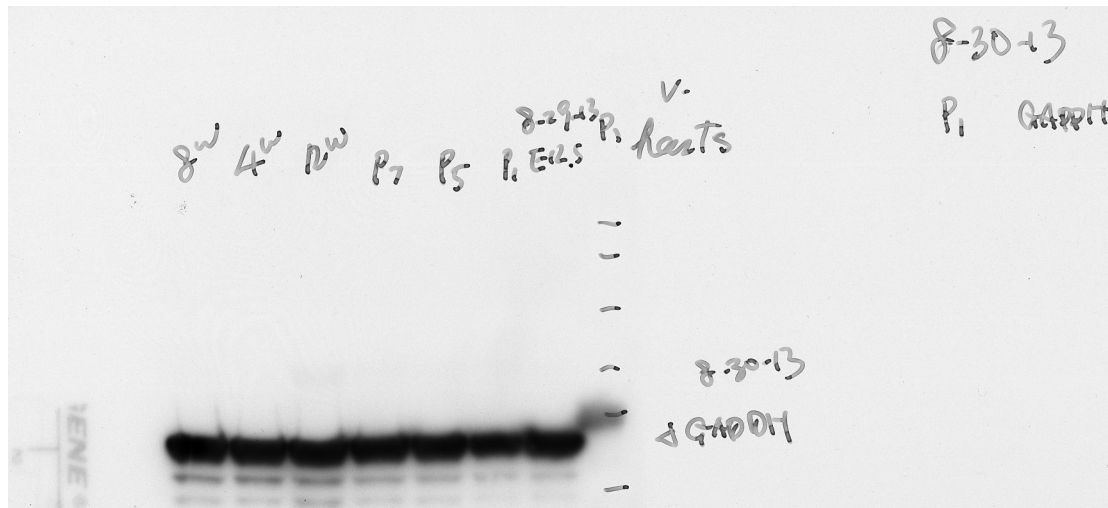
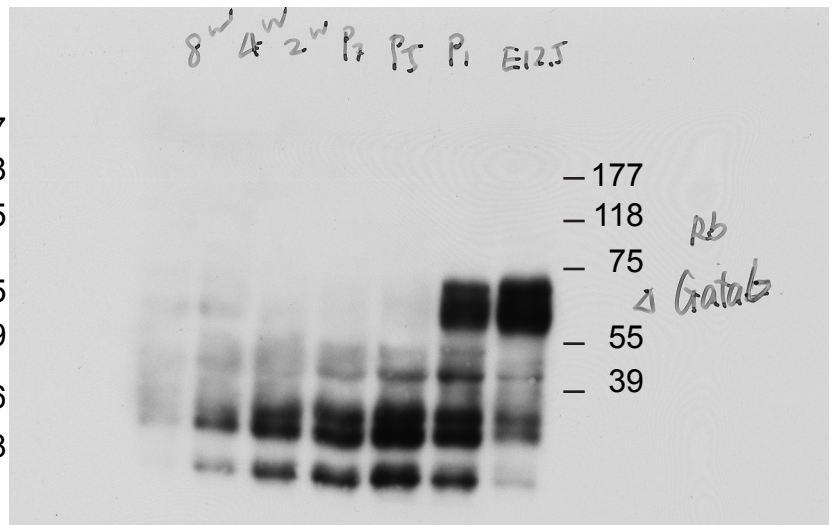
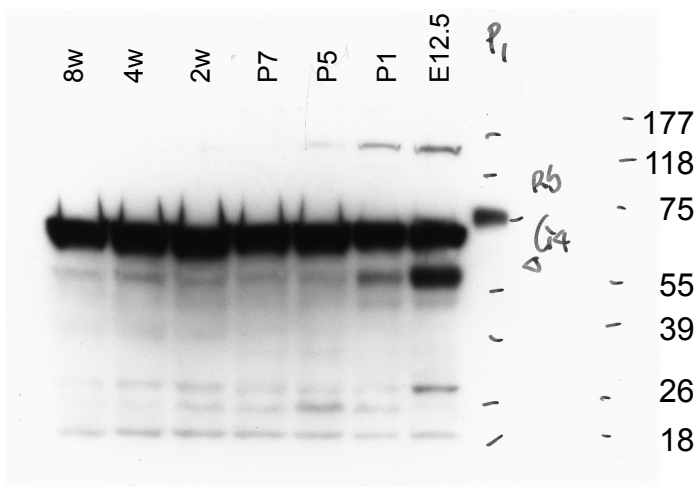
e

GO Analysis of Distal GATA4 Band-Induced Regions

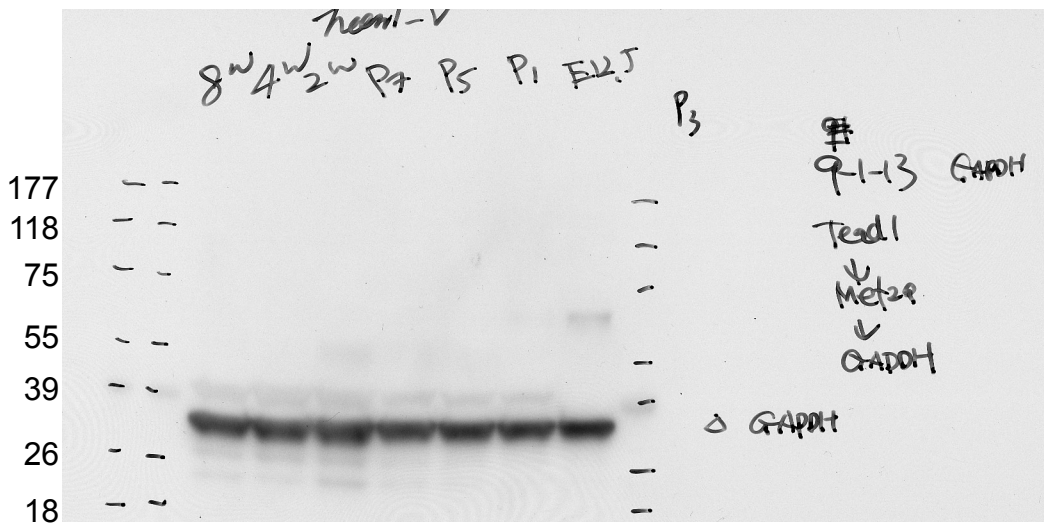
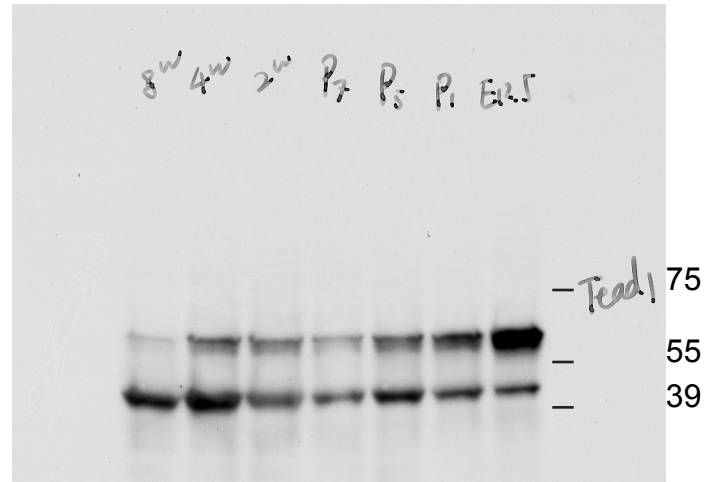
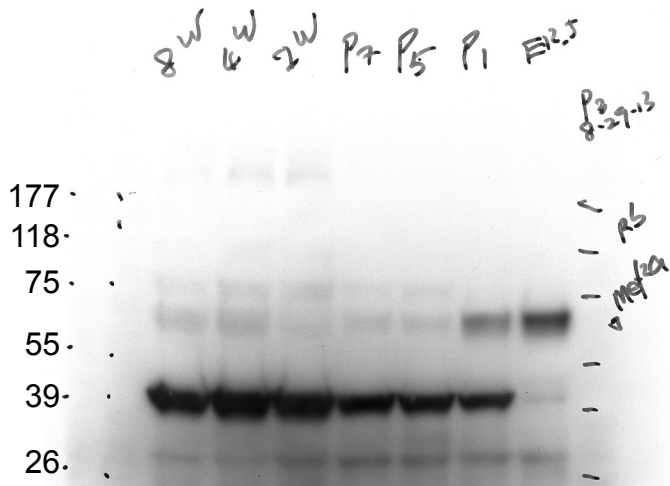
		Term Name	P-Value	Term Name	P-Value	
GO terms	Fetal-Specific	positive regulation of signal transduction	1.50E-10	cellular response to chemical stimulus	1.52E-05	
		positive regulation of response to stimulus	1.96E-09	hemopoiesis	1.60E-05	
		positive regulation of signaling	3.89E-09	cell proliferation	5.33E-05	
		positive regulation of cell communication	6.42E-09	hemopoietic or lymphoid organ development	1.00E-04	
		positive regulation of cellular protein metabolic process	1.34E-08	immune system development	1.53E-04	
		artery morphogenesis	1.50E-08	leg morphogenesis	1.82E-04	
		positive regulation of cell death	1.66E-08	muscle structure development	3.86E-07	
		regulation of protein modification process	1.89E-08	regulation of heart contraction	3.97E-06	
		induction of apoptosis	5.14E-08	mitochondrion organization	8.68E-06	
		induction of programmed cell death	5.54E-08	muscle organ development	9.57E-06	
		positive regulation of apoptosis	6.98E-08	negative regulation of catalytic activity	1.03E-05	
		positive regulation of programmed cell death	8.02E-08	striated muscle tissue development	2.24E-05	
		positive regulation of protein metabolic process	1.60E-07	response to amine stimulus	2.32E-05	
		artery development	2.25E-07	regulation of heart rate	3.56E-05	
	positive regulation of protein modification process	2.29E-07	New	regulation of smooth muscle cell migration	5.40E-05	
	muscle cell differentiation	4.81E-07				
			Adult-Specific			
			Shared			

Supplementary Fig. 5, Supporting Fig. 7. Banding-Induced GATA4 regions.

- Genome browser views of two examples of new GATA4 binding induced by pressure overload. Peak regions are highlighted in grey.
- Genome browser views of two examples of restored GATA4 binding induced by pressure overload. Peak regions are highlighted in grey.
- Tag heatmap of proximal regions containing proximal Band-induced GATA4 peaks. These regions were classified by their overlap with Fetal-Specific, Adult-Specific, or Fetal-Adult Shared peaks. Those without overlap were labeled "New". Within each group, regions were sorted by the enrichment score in Band.
- Over-represented motifs found in proximal GATA4 Band-Induced regions, based on their overlap with developmental GATA4 regions. Over-represented motifs were discovered de novo using DREME and identified by aligning known TF binding motifs using TOMTOM.
- GO term analysis of the distal GATA4 regions shown in panel (Fig.7a). Terms of interest are highlighted in red. Muscle or heart GO terms were only found in the Fetal-Specific and Shared groups.



Supplementary Fig. 6. Full scans of uncropped Western blots in Supplementary Fig. 3b.



Supplementary Fig. 7.. Full scans of uncropped Western blots in Fig.4f.