Supplementary Table 1.

Coordinates of putative *TBX5 cis* regulatory elements (Hg19), selection criteria.

Chrm	Start	End	Name	Conserved	Predicted	p300	TFBS	H3K4me1
chr12	114424501	114429332	CRE1	X		X		
chr12	114463712	114464080	CRE2	X			X	
chr12	114474932	114475752	CRE3	X			X	X
chr12	114483374	114483832	CRE4	X				
chr12	114589767	114590570	CRE5	X			X	X
chr12	114623787	114624349	CRE6	X				
chr12	114658356	114658783	CRE7	X			X	
chr12	114675208	114678568	CRE8	X				X
chr12	114701207	114704691	CRE9	X				
chr12	114704177	114704691	CRE9B	X				
chr12	114721588	114726517	CRE10	X				
chr12	114749686	114750464	CRE11	X				
chr12	114780115	114781227	CRE12	X				
chr12	114796322	114799754	CRE13	X		X	X	X
chr12	114828046	114828948	CRE14	X				
chr12	114832650	114834460	CRE15	X	X		X	
chr12	114853271	114858238	CRE16	X		X	X	X
chr12	114882633	114886962	CRE17	X	X			X
chr12	114892203	114896076	CRE18	X			X	
chr12	114938655	114939454	CRE19	X			X	

See main text for references to datasets. All elements tested were conserved down to opossum.

Supplementary Table 2.

Confirmed sequence variants in *TBX5* enhancers.

Enhancer	Internal ID	rsNumber	# of Patients	Alleles	Chr12 Location (hg19)
2		rs13377891	4	CG	chr12:114463917
2		rs61933018	common	CT	chr12:114463973
2	3465	NA	3	GA	chr12:114464054
9		rs1920600	common	TC	chr12:114701311
9		rs141837565	2	TC	chr12:114701338
9		rs114880578	1	GA	chr12:114701342
9		rs58562176	common	CT	chr12:114701454
9		rs12426785	common	CT	chr12:114701598
9	243692	NA	1	GA	chr12:114704281
9		rs1920596	common	TA	chr12:114704412
9		rs141875471	1	GT	chr12:114704515
9		rs143640200	1	CT	chr12:114704571
16		rs2686564	6	GT	chr12:114857694
16	2872	NA	1	CT	chr12:114857726
16		rs1895595	common	TC	chr12:114857388
16		rs2551389	common	TC	chr12:114857820
16		rs2686565	common	CT	chr12:114857824
16		rs4536277	common	AG	chr12:114857780
16		rs2551388	common	CT	chr12:114858247

Supplementary Figures 1-3

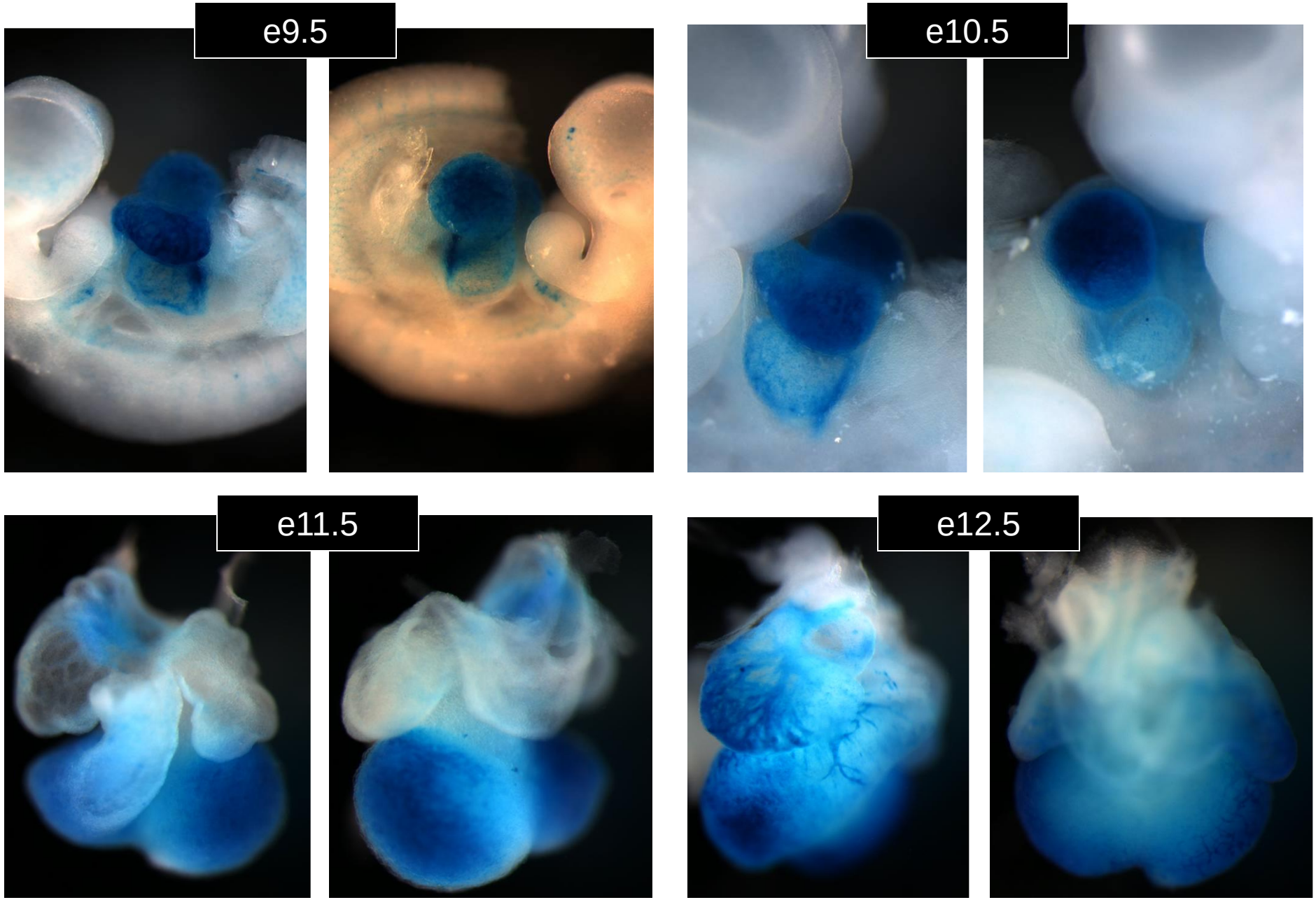
Characterization of *TBX5* enhancers at multiple time points in development

Supplementary Figure 1. **TBX5 enhancer 2 time course.**
Whole mount, ß-gal staining of embryonic mice transgenic for hsTBX5-2.
Two lines of mice with similar expression patterns were derived for enhancer 2, and their common domains of expression were as follows. At day 9.5, expression was already observed throughout the heart, though most strongly in the ventricle. At e10.5 expression was symmetric and uniform throughout the myocardium of both ventricles, and not observed in the ventricular septum. Atrial expression was observed in one line, most strongly in free wall of the left atrium (LA), but not in the atrial septum or the atrioventricular canal (AVC). By e11.5, the ventricular expression was strongest in the trabeculated myocardium of the left ventricle though substantial expression was also observed in the right ventricle. Notably, at e12.5 ventricular expression was limited to the compact myocardium.

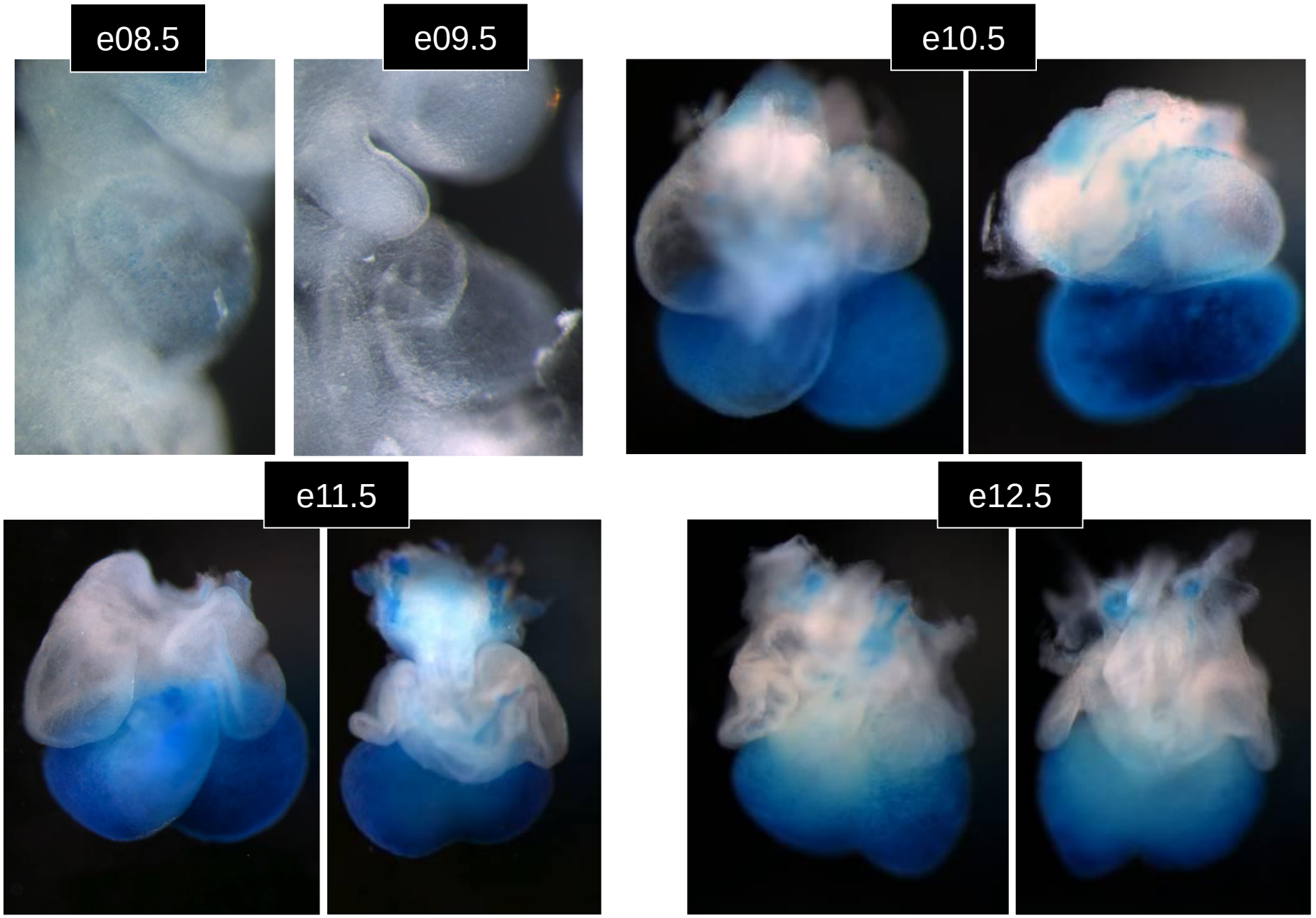
Supplementary Figure 2: ***TBX5* enhancer 9 time course.**
Whole mount, ß-gal staining of embryonic mice transgenic for hsTBX5-9.
Two lines of mice with very similar expression patterns were derived for enhancer 9. Robust expression was observed throughout the entire heart at e9.5, and at e10.5 and beyond, expression in one line was limited to the ventricles. Histological sectioning revealed strong expression throughout both compact and trabeculated myocardium, as well as the in posterior (cardiac) portion the ventricular septum, and at e11.5 moderate expression was detected in the AV canal. At e12.5 expression began to wane in an anterior to posterior fashion.

Supplementary Figure 3: ***TBX5* enhancer 16 time course.**
Whole mount, ß-gal staining of embryonic mice transgenic for hsTBX5-16.
Two lines were generated for enhancer 16 with virtually identical qualitative patterns of expression, though they differed in terms of quantitative output. In both expression is moderate by e9.5 in the AV canal and extending outward to both the ventricle and atria. At e10.5 and e11.5 expression is strongest in the left ventricle, strong in the right ventricle and more modest in the atrial free walls. Expression begins to fade at e12.5, with almost no atrial expression.

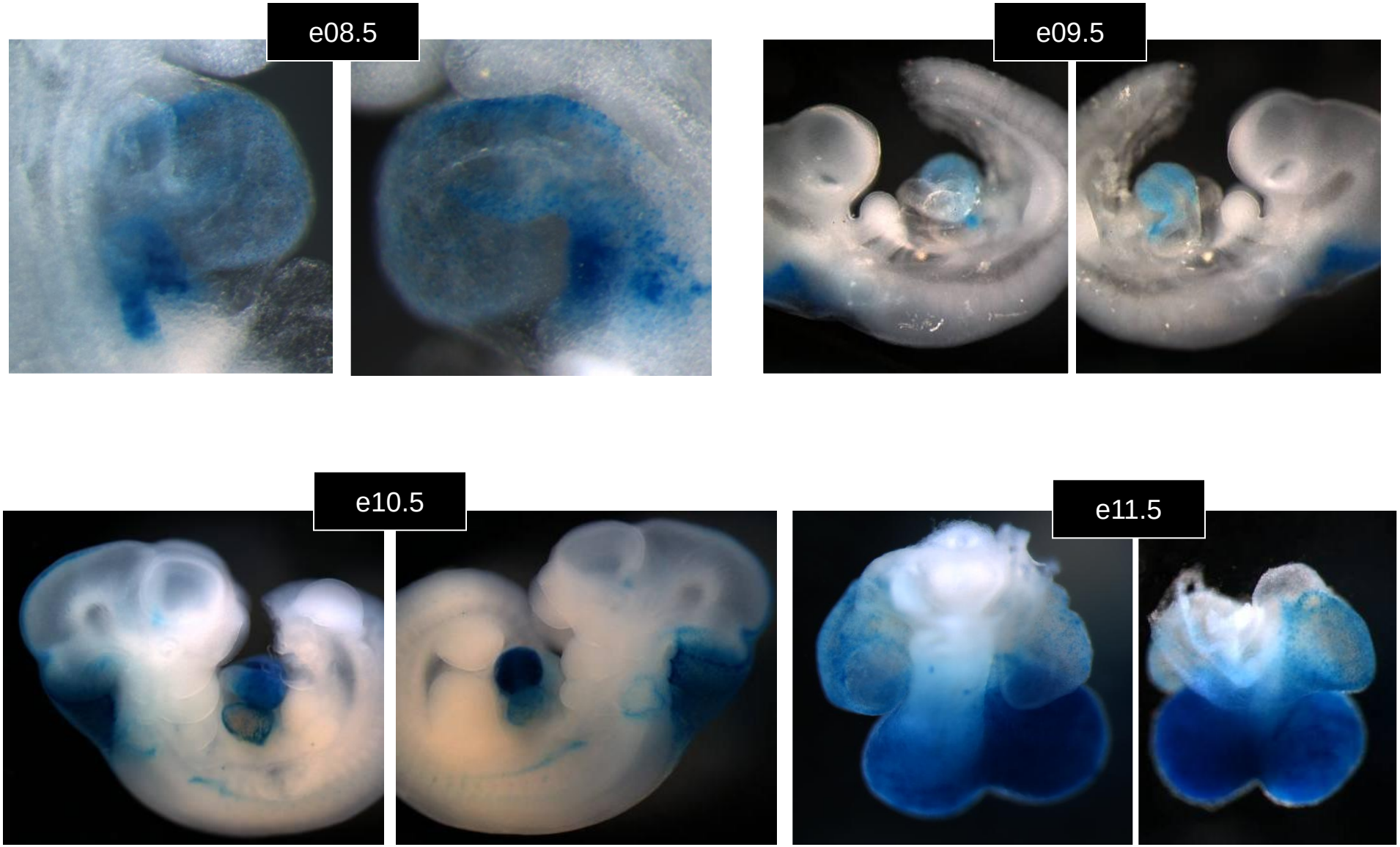
Supplementary Figure 1. ***TBX5* enhancer 2 time course**



Supplementary Figure 2: ***TBX5* enhancer 9 time course**

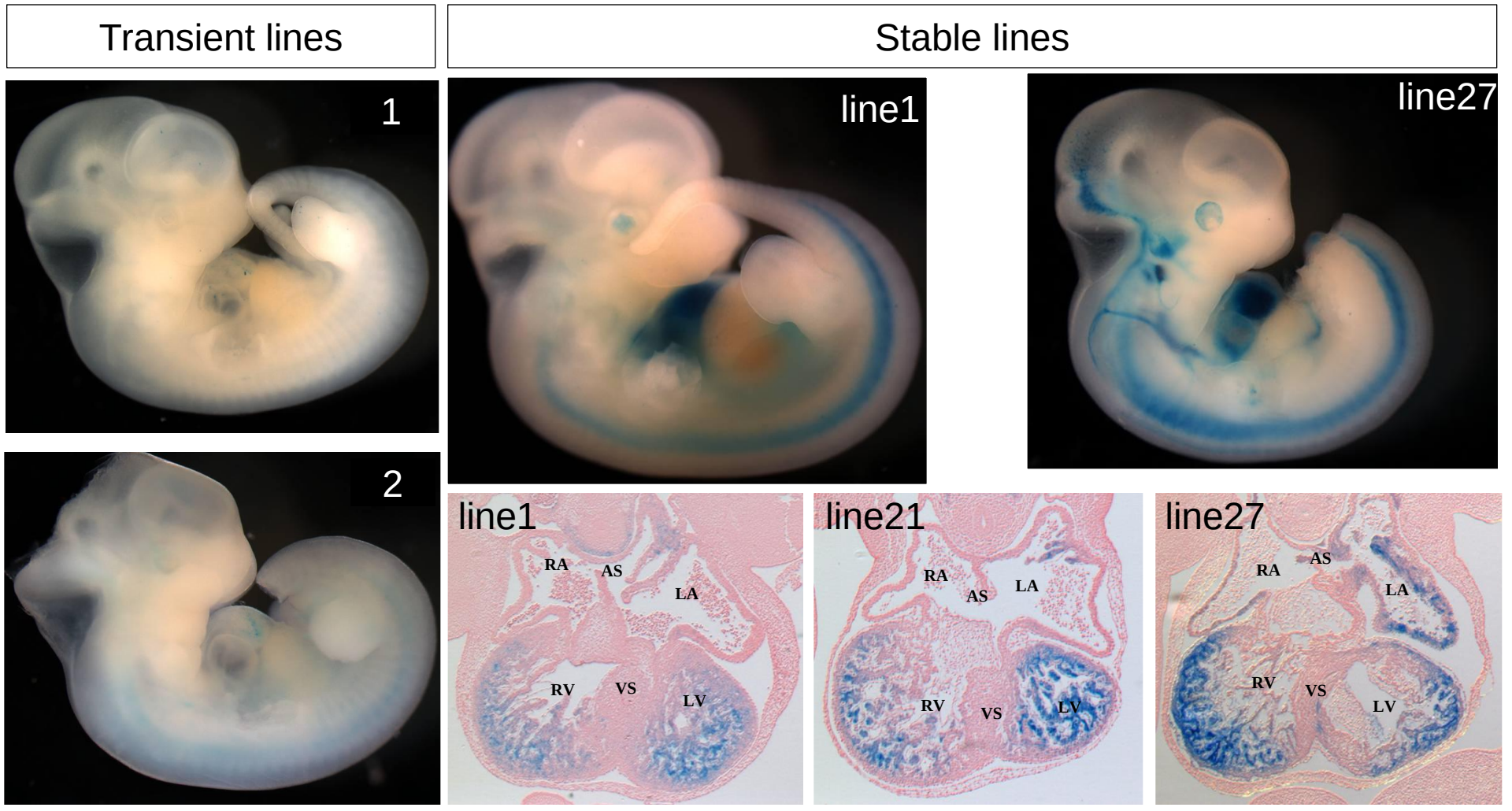


Supplementary Figure 3: ***TBX5*** **enhancer 16 time course**

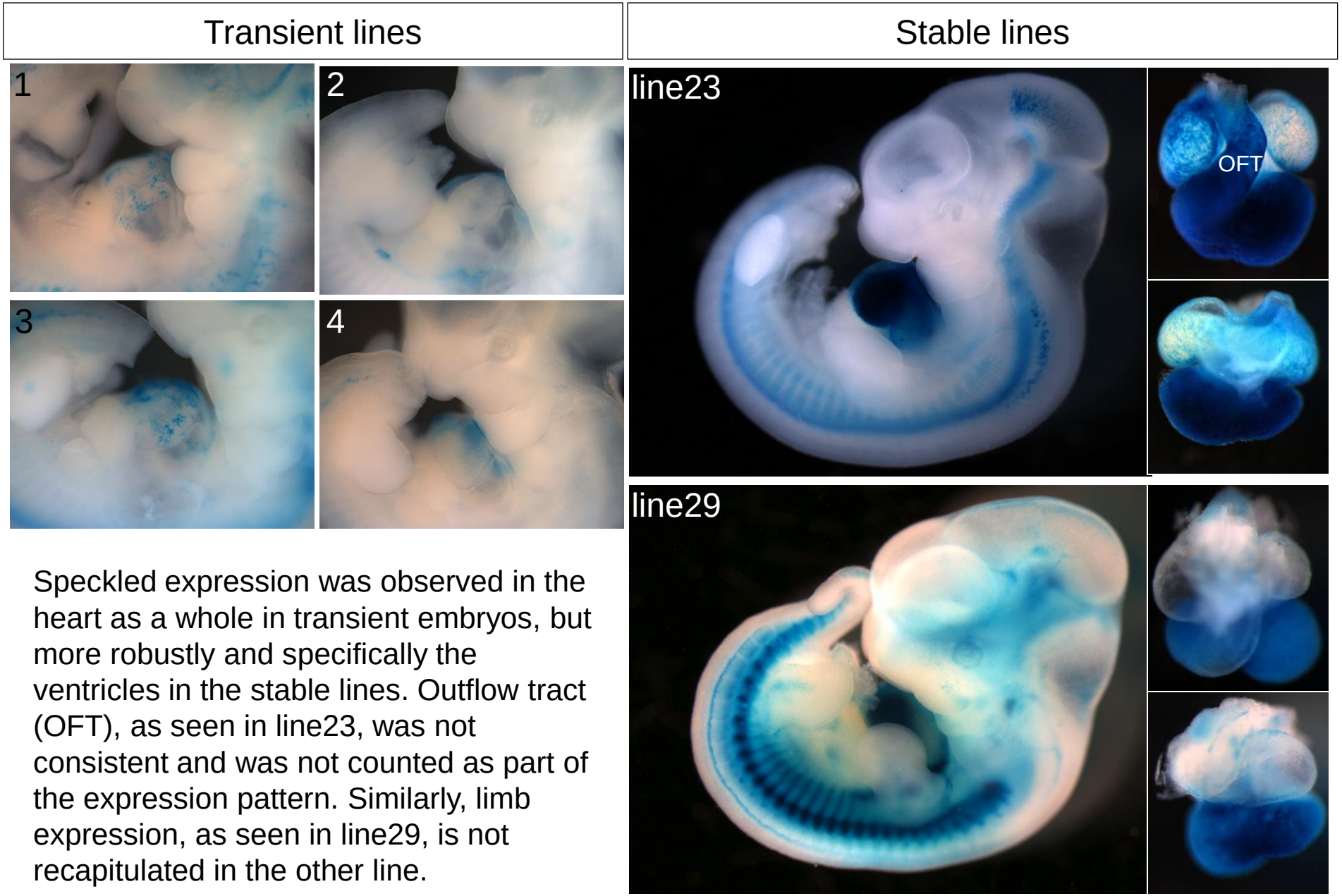


Supplementary Figure 4. ***TBX5* enhancer 2 lines.** Each embryo represents an independent insertion of the reporter construct into the genome. The precise expression pattern observed is dependent upon both positional and copy number effects. Consistent expression can nonetheless be seen in the heart and eyes. Staining within the somites is due to the minimal promoter used and not part of the enhancer-driven expression. All embryos E11.5.

RA right atrium, LA left atrium, AS atrial septum, RV right ventricle, LV left ventricle, VS ventricular septum.



Supplementary Figure 5. ***TBX5* enhancer 9 lines**



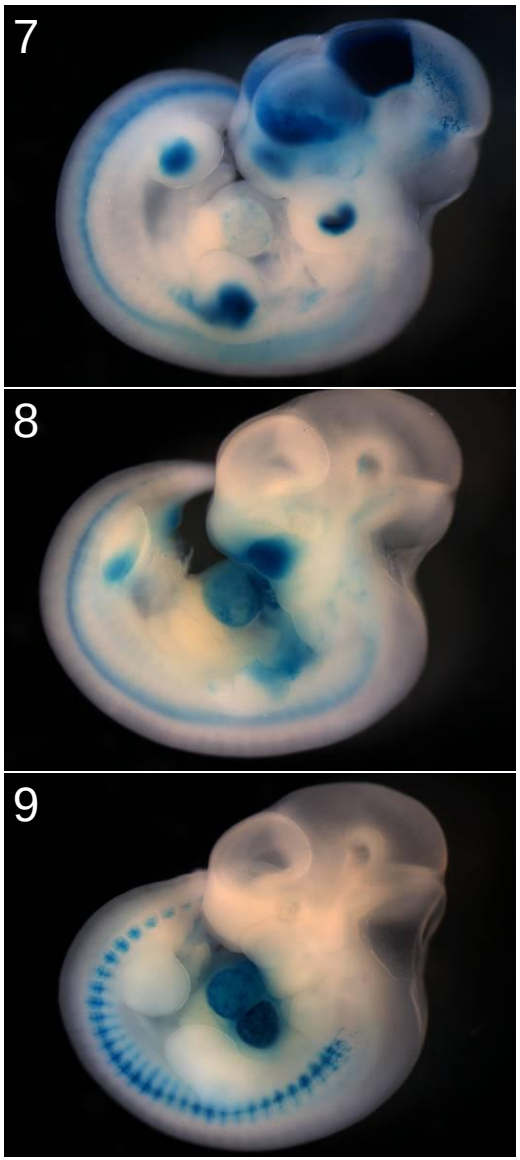
Speckled expression was observed in the heart as a whole in transient embryos, but more robustly and specifically the ventricles in the stable lines. Outflow tract (OFT), as seen in line23, was not consistent and was not counted as part of the expression pattern. Similarly, limb expression, as seen in line29, is not recapitulated in the other line.

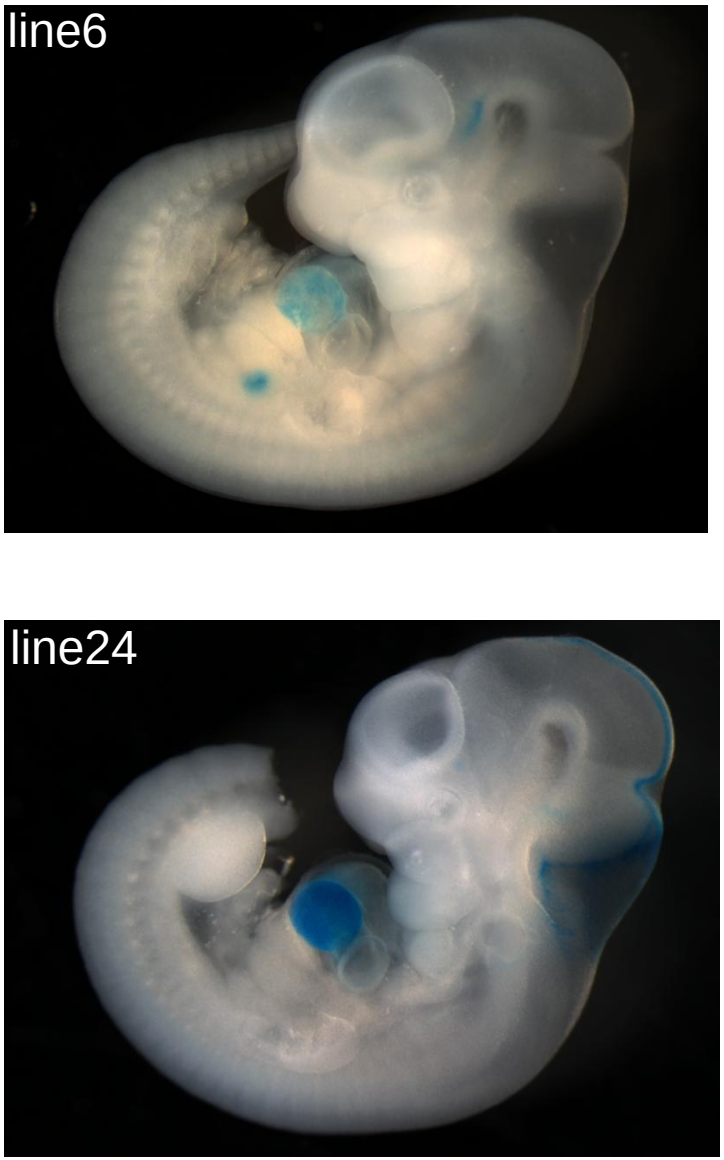
Supplementary Figure 6. ***TBX5* enhancer 16 lines**

Although atrial expression was seen in transient line 9, it was not recapitulated in the stable lines, which are otherwise very similar and do not have limb expression (not shown, limbs removed here). Robust expression was common in all left ventricles.

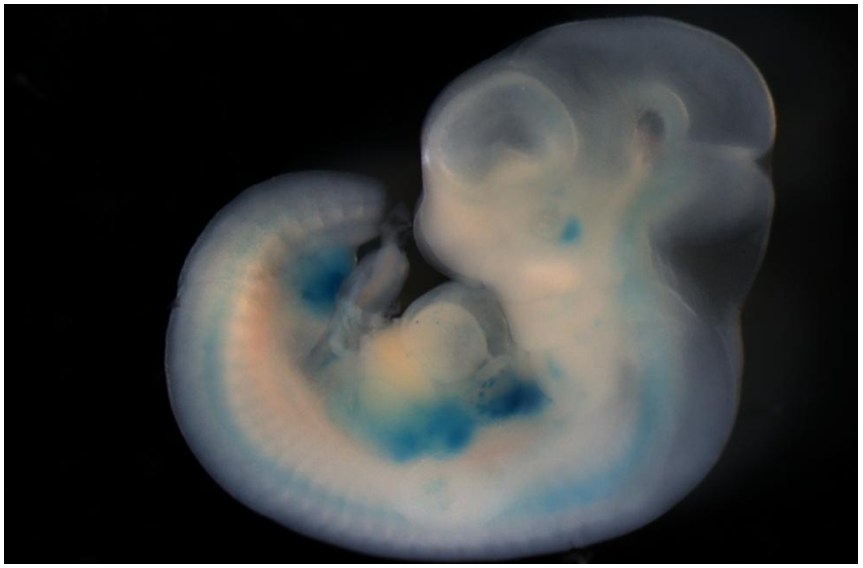
Transient lines

Stable lines

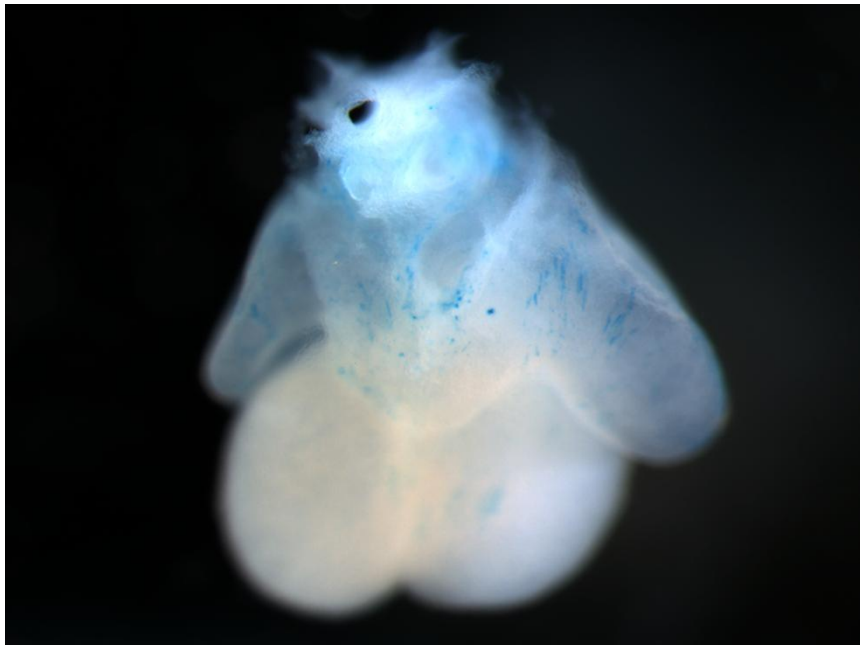


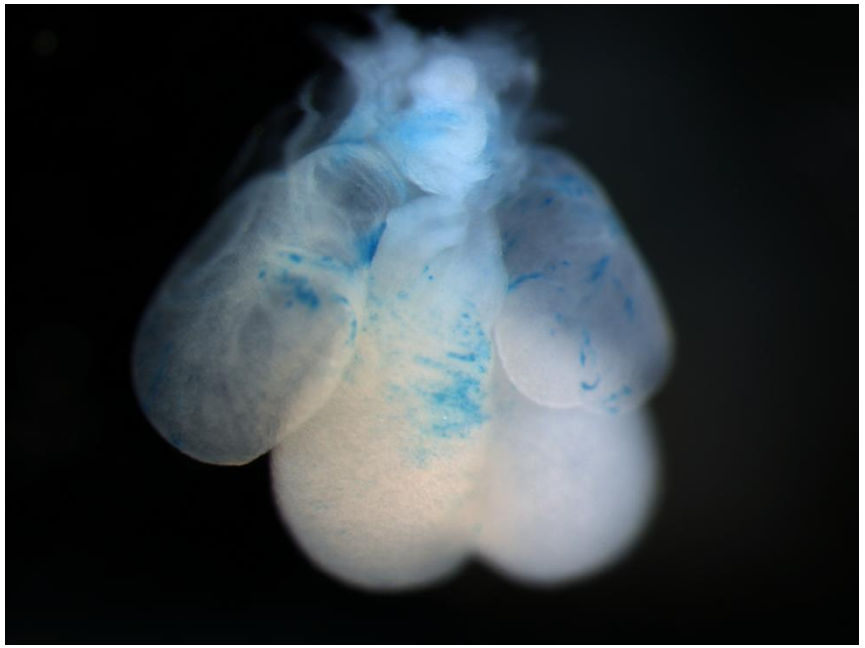


ECR8

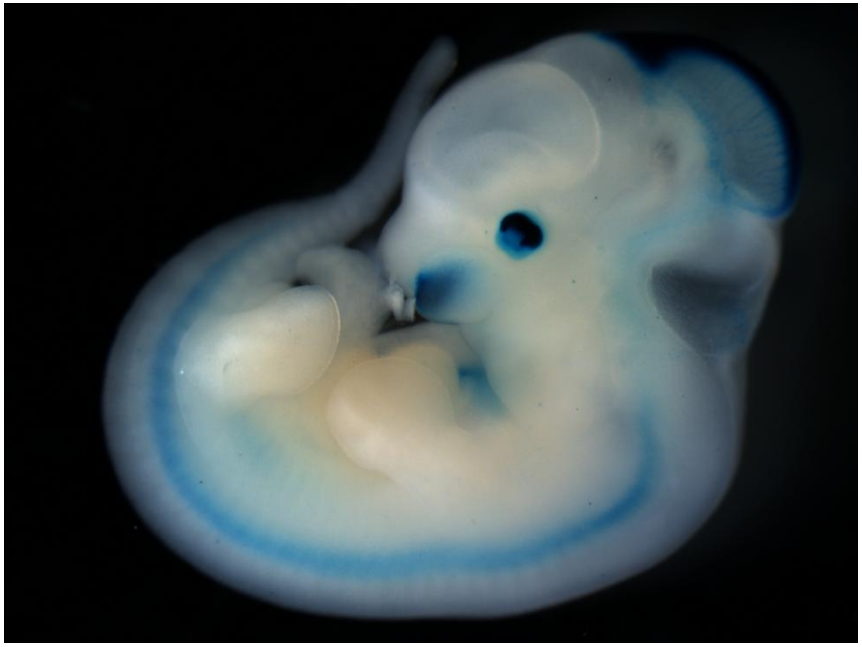


Supplementary Figure 7.
Beta-galactosidase staining driven by ECR8 at E11.5.

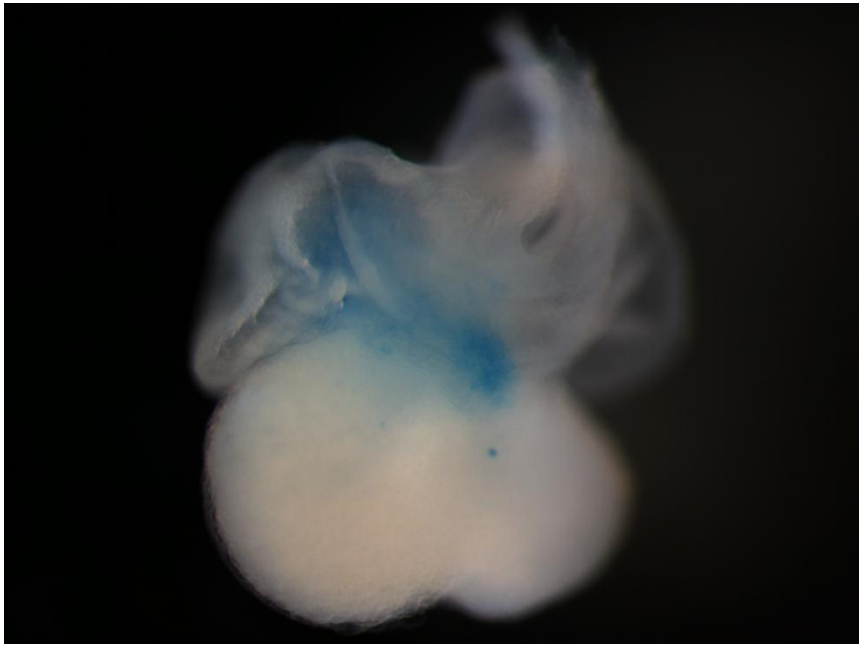


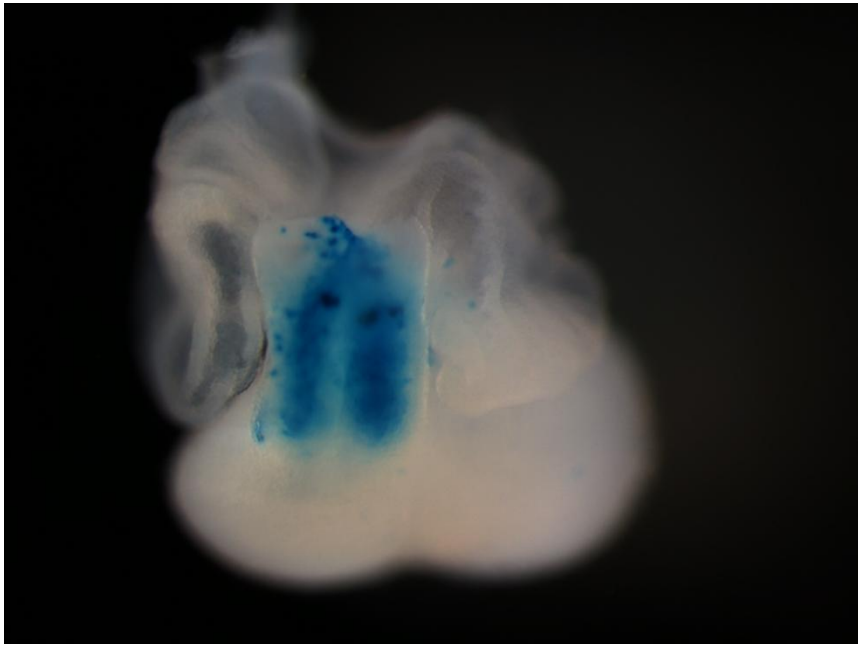


ECR15

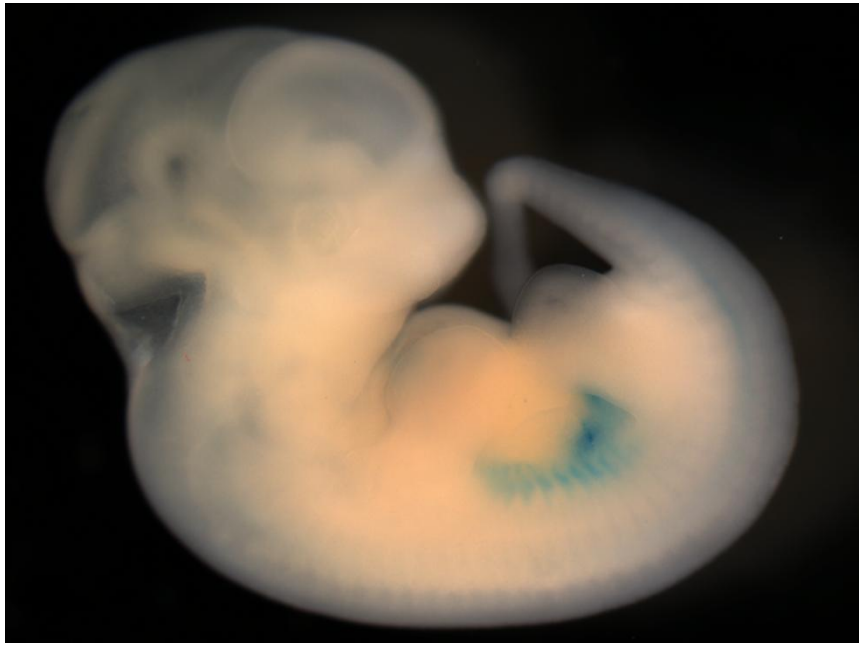


Supplementary Figure 8.
Beta-galactosidase staining driven by ECR15 at E11.5.

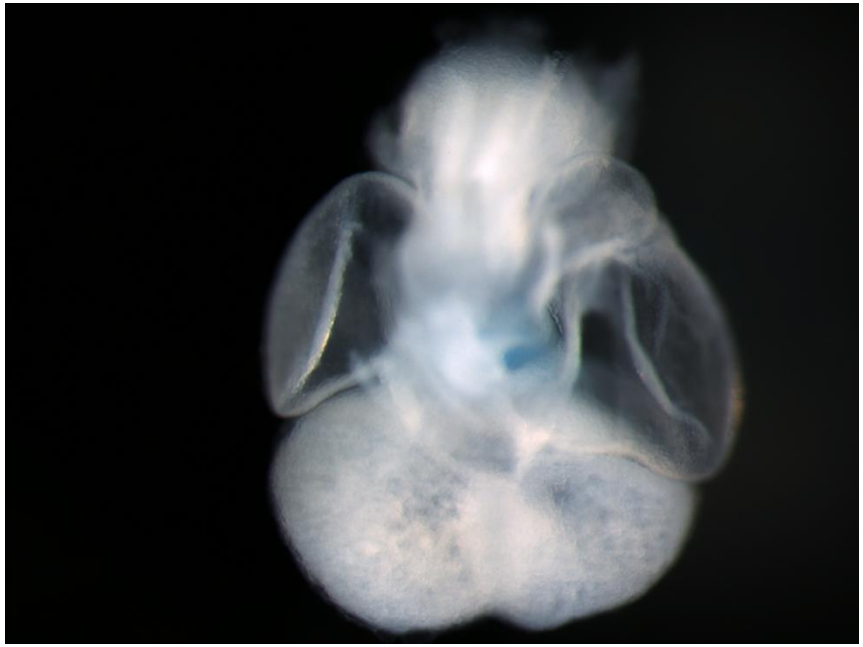


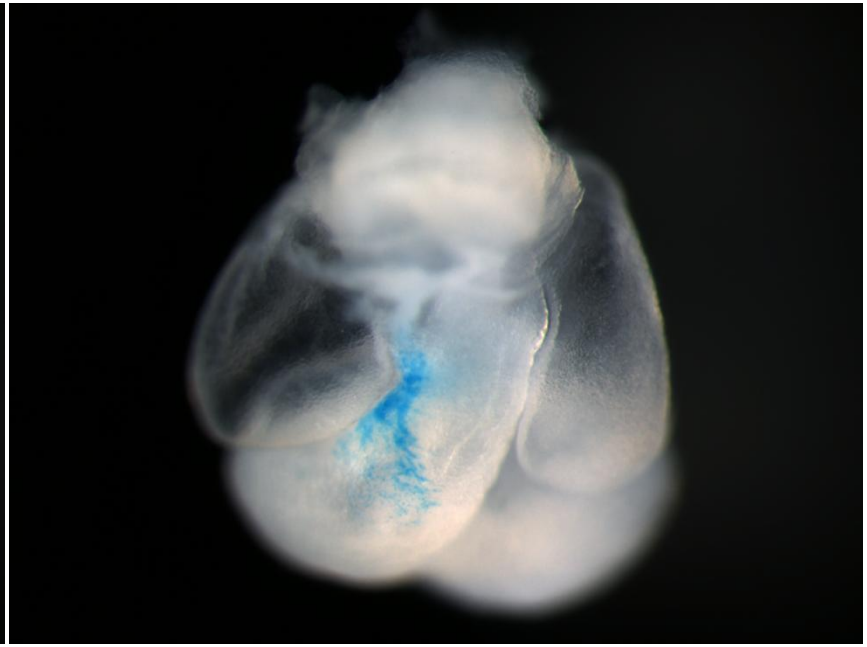


ECR19



Supplementary Figure 9.
Beta-galactosidase staining driven by ECR19 at E11.5.





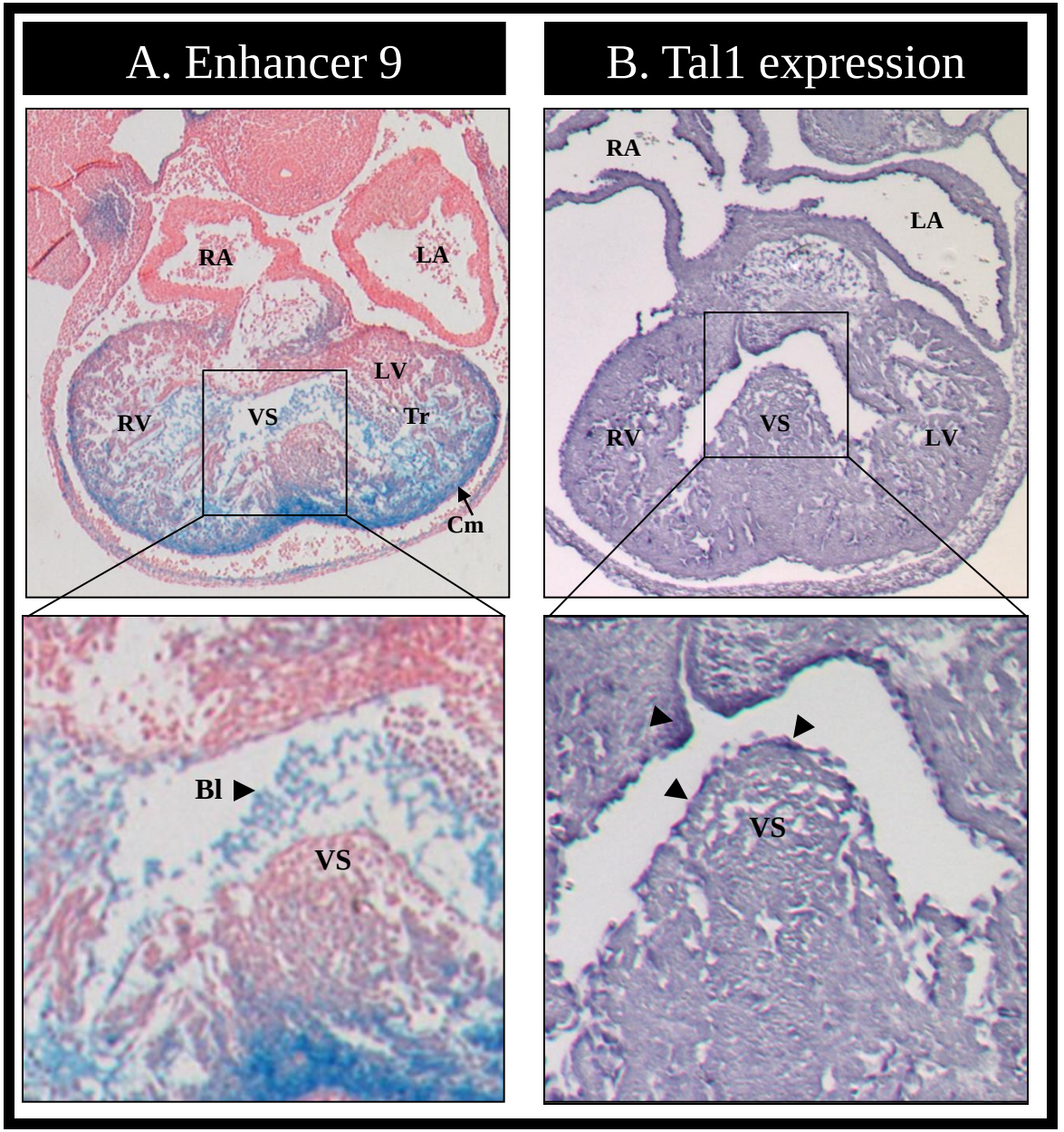
Supplementary Figure 10.

Expression patterns of beta-galactosidase driven by enhancer 9 compared to endogenous TAL1 expression.

A. Enhancer 9 drives expression (blue) in the trabeculated and compact myocardium of both ventricles and the ventricular septum.

B. TAL1 expression (purple) is limited to the endocardial layer in all four chambers, and is not detected in the myocardium of the ventricular septum.

RA, right atrium; LA, left atrium; AS, atrial septum; RV, right ventricle; LV, left ventricle; VS, ventricular septum; Bl, blood; Tr, trabeculated myocardium; Cm, compact myocardium.

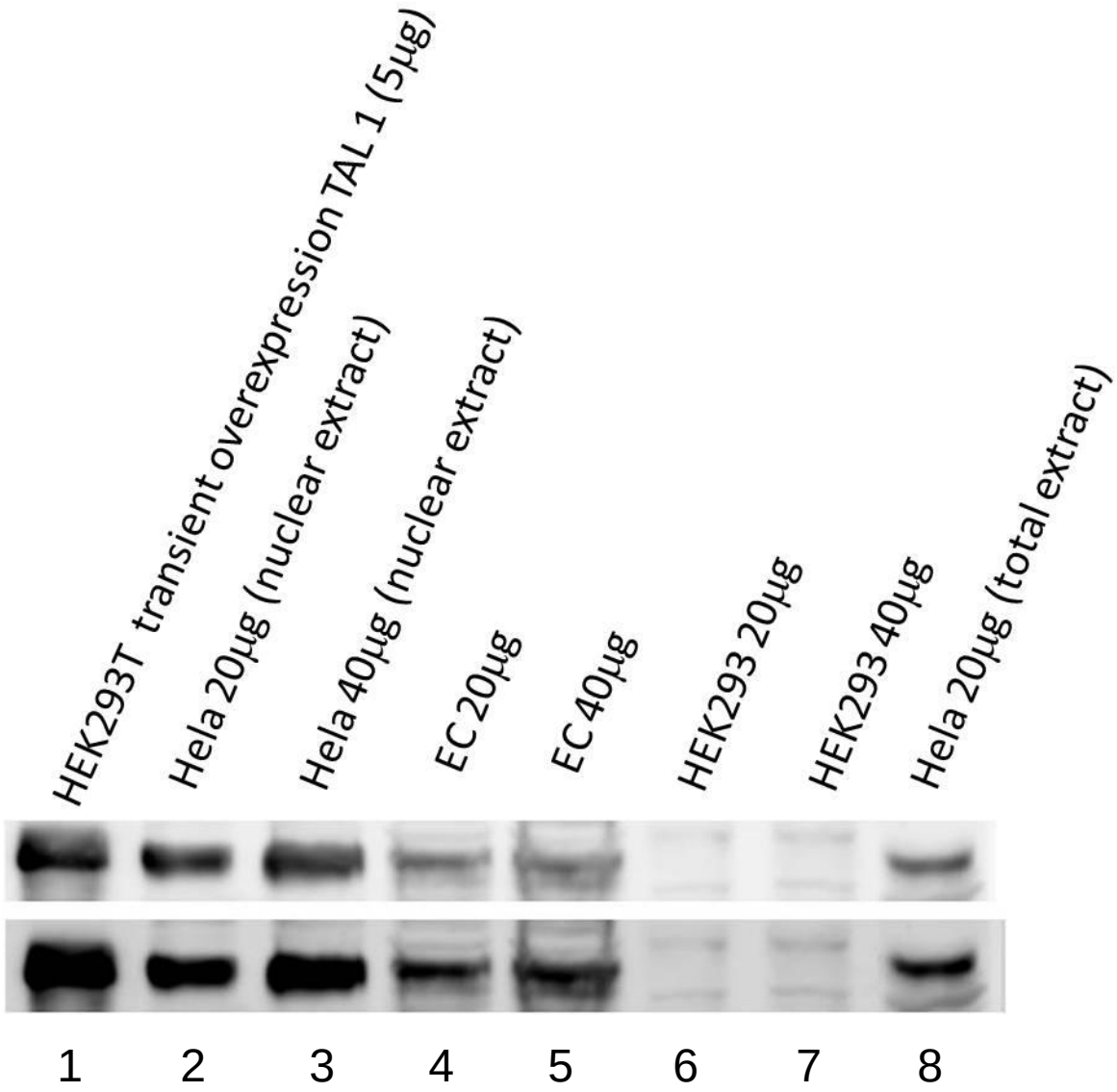


Supplementary Figure 11.

HeLa cells express TAL1

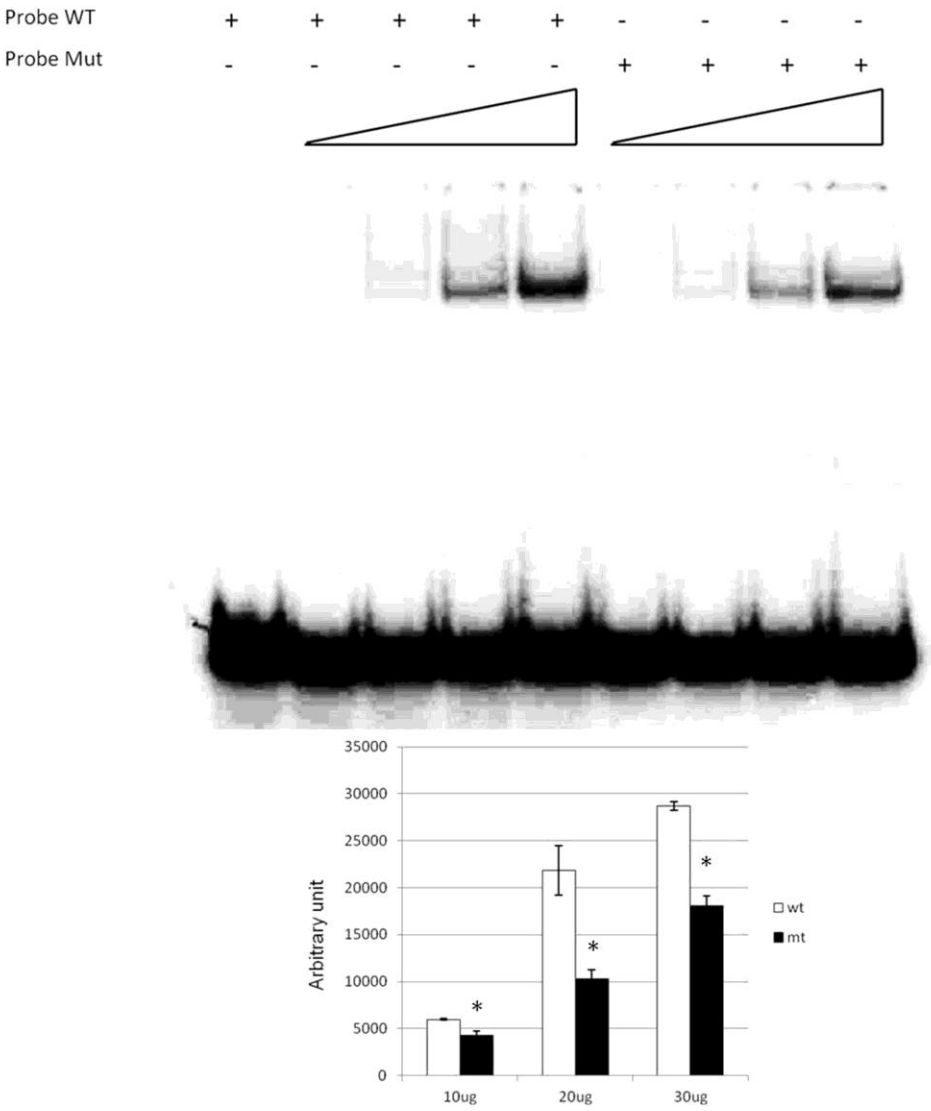
Western blot for TAL1 on various cell and nuclear extracts (as indicated).

1. HEK293T cells transiently transfected to overexpress TAL1, 5ug cell lysate
2. HeLa nuclear extract, 20ug
3. HeLa nuclear extract, 40ug
4. Endothelial cell lysate, 20ug
5. Endothelial cell lysate, 40ug
6. Untransfected HEK293T cell lysate, 20ug
7. Untransfected HEK293T cell lysate, 40ug
8. Hela cell lysate, 20ug



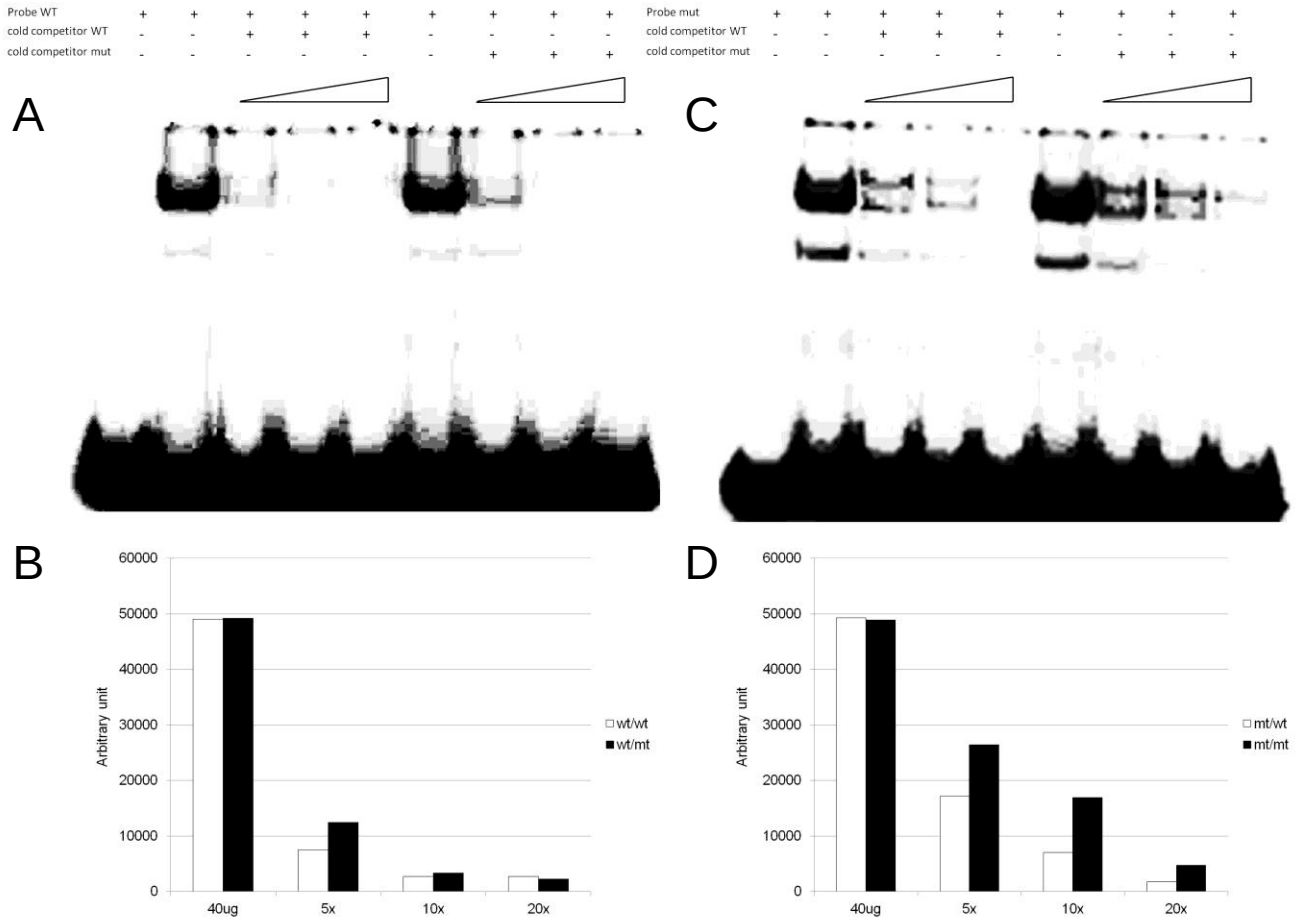
Supplementary Figure 12.
Reduced binding to mutant DNA.
(Top) EMSA shows radioactive DNA probe containing wild-type G allele (WT; lanes 1-5) is bound to a greater extent by nuclear extract than radioactive probe containing mutant T allele (Mut; lanes 6-9).

(Bottom) Quantification of same data. Statistical analyses were performed with student *t*-test. Each bar represents media ± standard error (n = 3). *P < 0.05 vs. wild type.



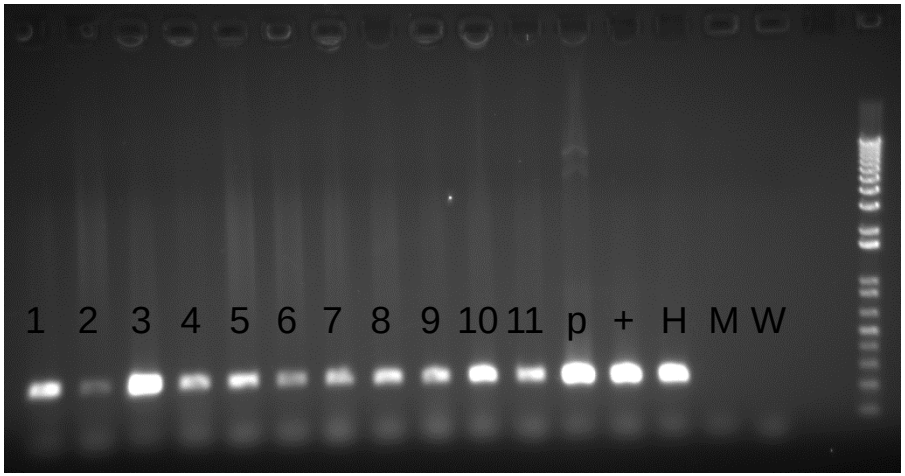
Supplementary Figure 13. **Reduced binding to mutant DNA.**
A (quantified in B). Starting with a saturated system of 40ug of lysate (lanes 2 & 6), an excess of cold mutant competitor (lanes 7-9) cannot displace hot WT probe as fast as a cold WT competitor (lanes 3-5).
C (quantified in D), the same result is seen when starting with hot mutant probe.

A and C represent individual experiments and are provided without statistical analysis.



Supplementary Figure 14.
A. PCR genotyping for presence of hsTBX5-9 enhancer.
B. Only 1 of 11 transgenic animals carrying the mutant hsTBX5-9 enhancer expressed beta-galactosidase (blue), which was only in the heart, and only in this diffuse pattern including atrial free wall (arrows) largely absent the ventricles. See Main text Figs. 2 and 5 for comparison with wild-type enhancer-driven expression.

A



Templates as follows:
1-11: Gentra-purified DNA from hsTBX5-09mut mice
p: hsTBX5-9mut plasmid
+: DNA from a hsTBX5-9wt animal
H: Human genomic DNA (Invitrogen)
M: Mouse genomic DNA (Invitrogen)
W: water (no template)

B