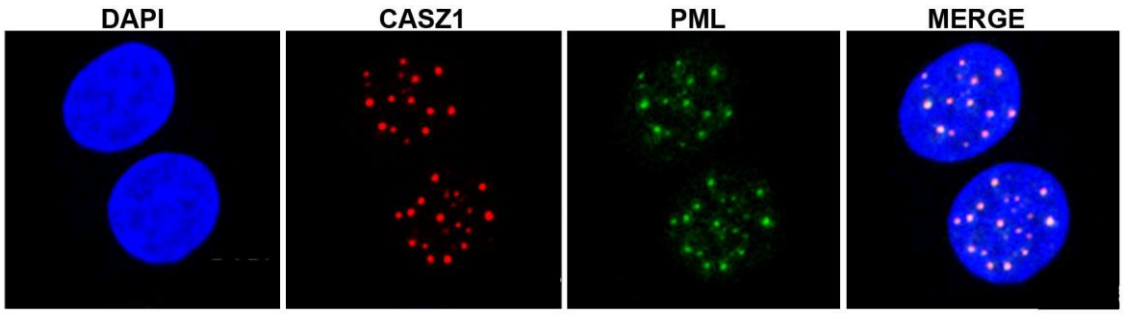
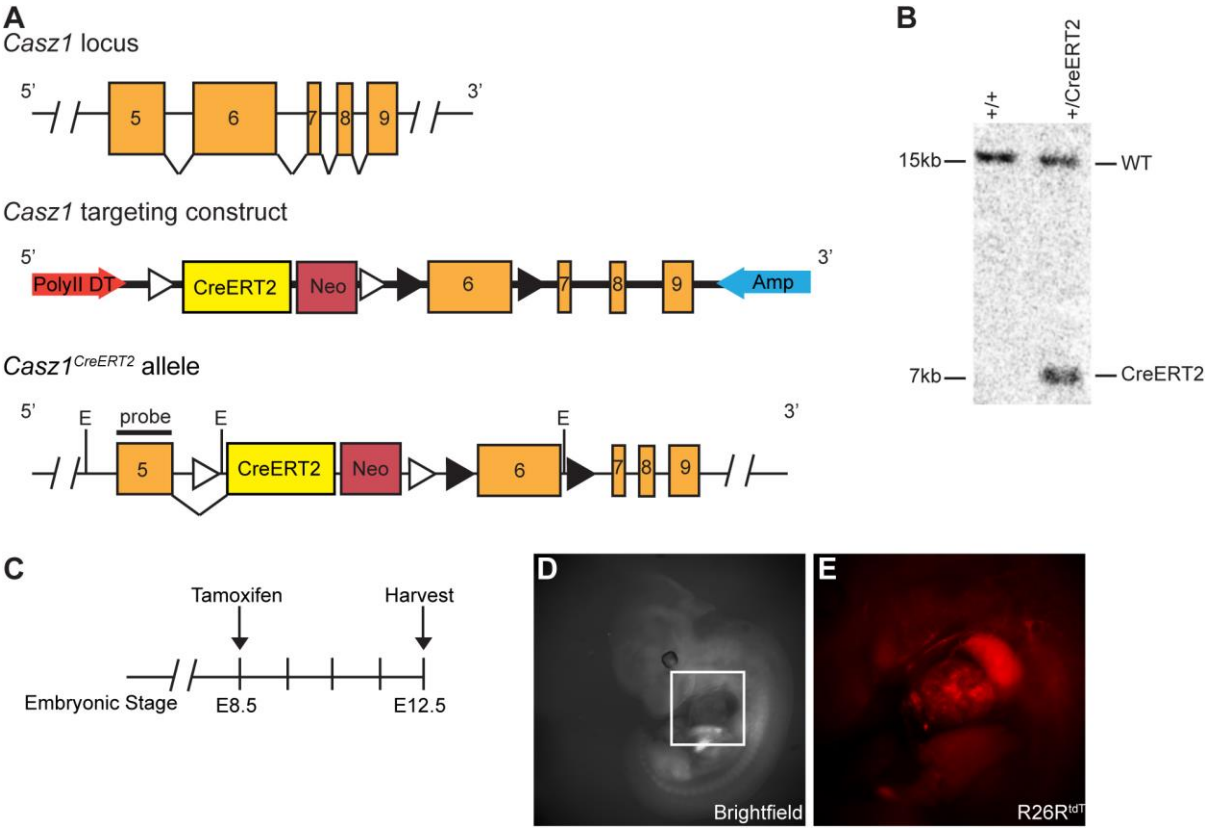


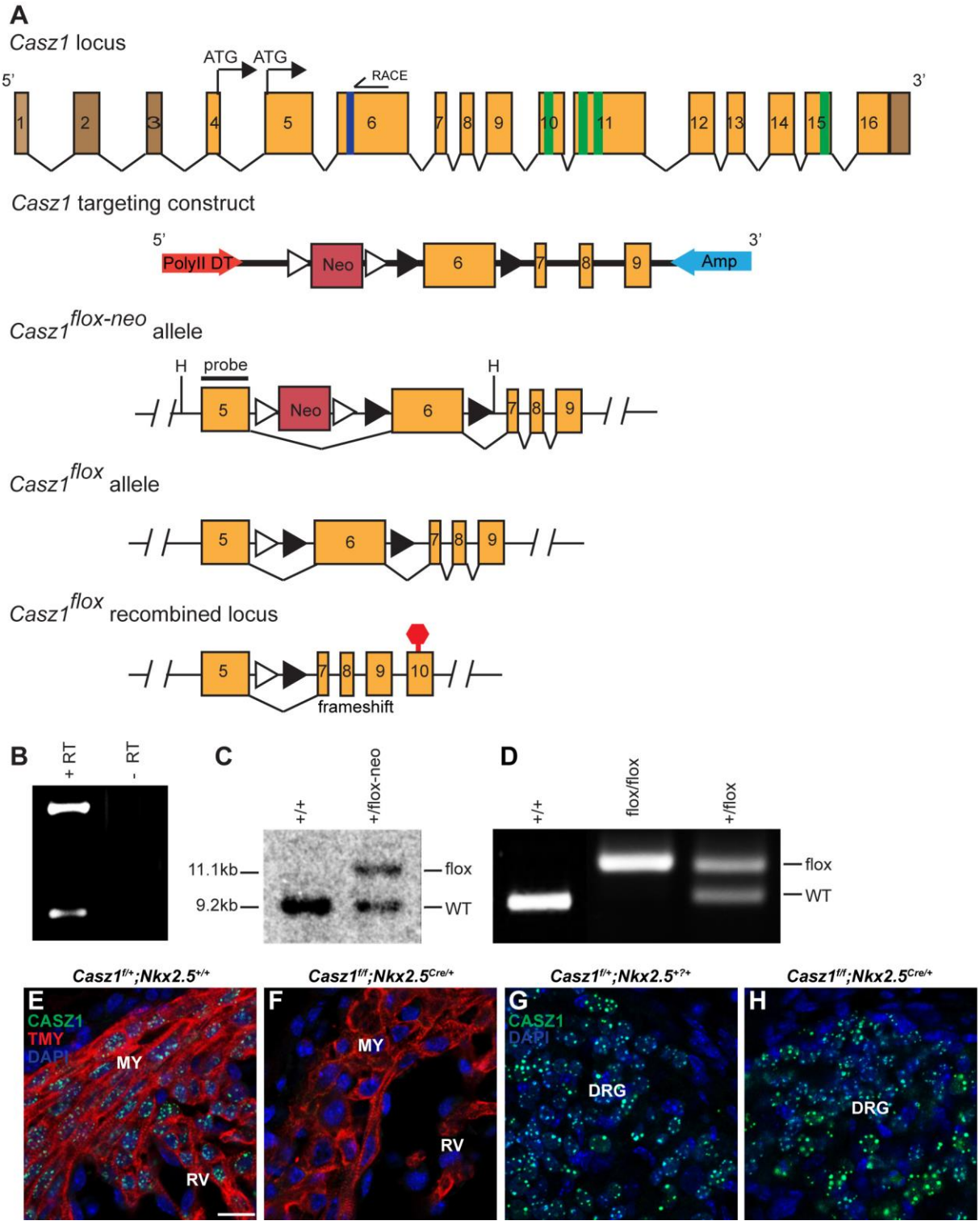
Supplementary Figure 1. CASZ1 is expressed in PML bodies

Immunofluorescent staining reveals that CASZ1 is co-expressed with PML in the nucleus of HUVECS.



Supplementary Figure 2. Generation of a *Casz1*$^{CreERT2\text{-}neo}$ lineage tracing allele

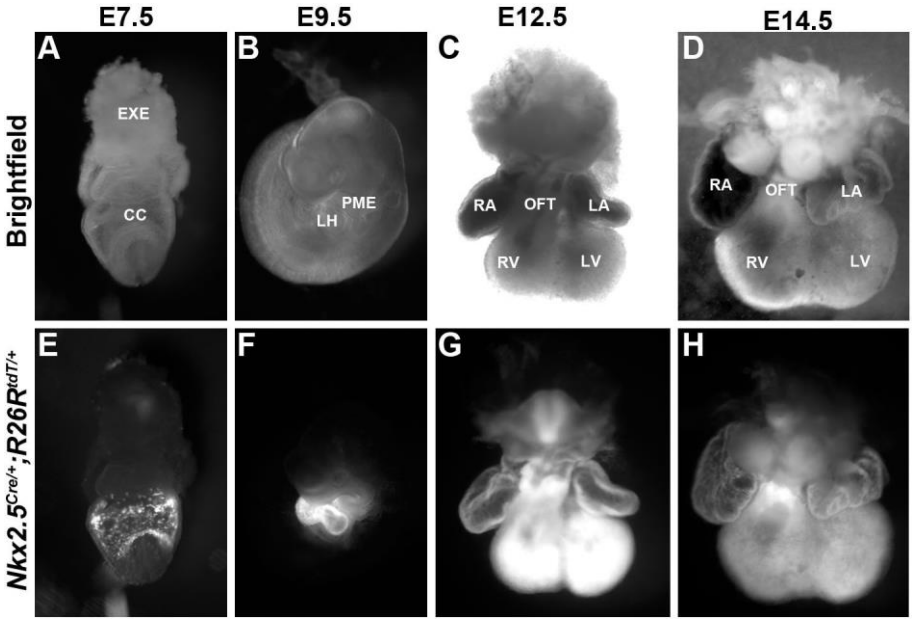
(A) Gene targeting strategy for *Casz1*^{CreERT2} allele. (B) Germline transmission was validated by Southern blot using a 5' external probe. Heterozygous mice containing the *Casz1*^{CreERT2} allele are viable, fertile, and display no obvious phenotypic abnormalities. (C) Fate mapping experimental design – pregnant females were injected with a single dose of tamoxifen at E8.5 and embryos were harvested at E12.5. (D) Image of an E12.5 *Casz1*$^{CreERT2/+}$;*R26R*$^{tdT/+}$ embryo. (E) Enlarged image of the heart in (D) shows *Casz1*-expressing cells in the embryonic heart. E – EcoRI cut sites; FRT sites – white triangles; loxP sites – black triangles.



Supplementary Figure 3. Generation of a conditional *Casz1* allele

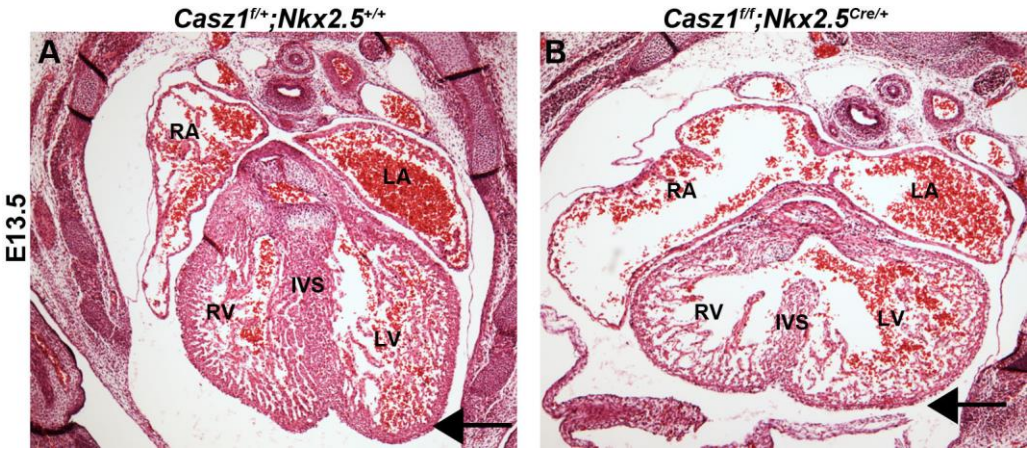
(A) Conditional gene targeting strategy for *Casz1*^{flox} allele. *loxP* sites were introduced into the *Casz1* locus flanking exon 6. (B) 5' RLM-RACE identified 2 transcription start sites in the

murine heart. +RT indicates cDNA sample treated with reverse transcriptase (RT); -RT indicates control cDNA sample not treated with RT. (C) Germline transmission was validated by Southern blot using a 5' external probe. (D) Mice were genotyped by PCR for the presence of the flox allele. Immunofluorescent staining for CASZ1, TMY and DAPI confirms depletion of CASZ1 in the myocardium in *Casz1*$^{f/f}$*;Nkx2.5*$^{Cre/+}$ E13.5 hearts (E,F), but is maintained in the dorsal root ganglia (DRG) (G,H). H – HindIII cut site; FRT sites – white triangles; loxP sites – black triangles; blue bar represents location of a nuclear localization signal; green bars indicate location of C_2H_2 zinc-fingers; red stop sign indicates the location of a premature stop codon subsequent to the frameshift following exon6 removal. Scale bars indicate 20 µm (Bergmann et al.).



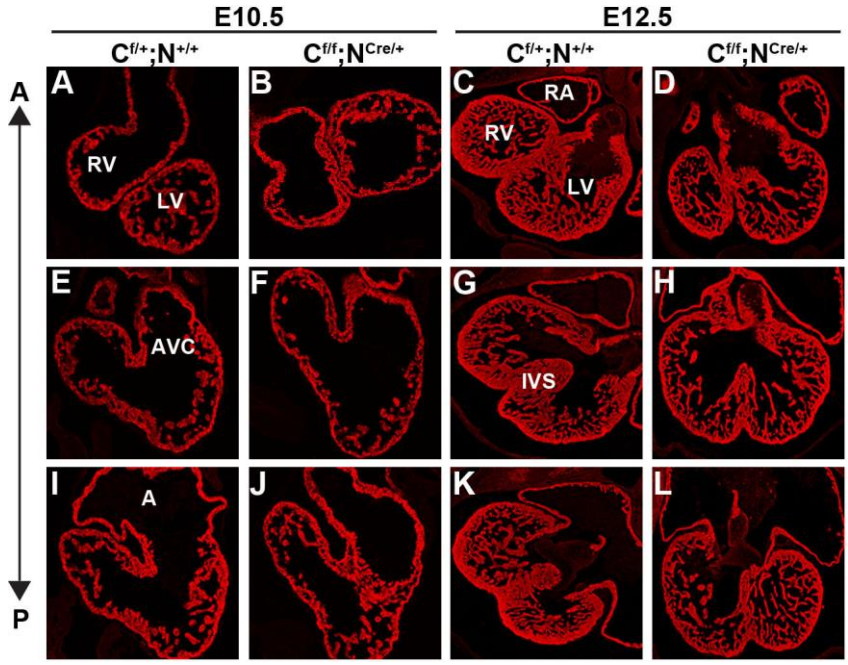
Supplementary Figure 4. *Nkx2.5*^{Cre} lineage analysis

Gross morphology of *Nkx2.5*$^{Cre/+}$*;R26R*$^{tdT/+}$ embryos at E7.5 (A,E) and E9.5 (B,F), and hearts at E12.5 (C,G) and E14.5 (D,H). Cre recombination is observed in the cardiac crescent at E7.5 (E), the looped heart at E9.5 (F), the ventricles, the OFT and the atria of the four-chambered heart at E12.5 (G) and similarly in the fetal heart at E14.5 (H). EXE, extraembryonic tissue; CC, cardiac crescent; LH, looped heart; PME, pharyngeal mesoderm; LV, left ventricle; LA, left atria; RV, right ventricle; RA, right atria; OFT, outflow tract.



Supplementary Figure 5. *Casz1* cardiac null embryos exhibit severe cardiac defects

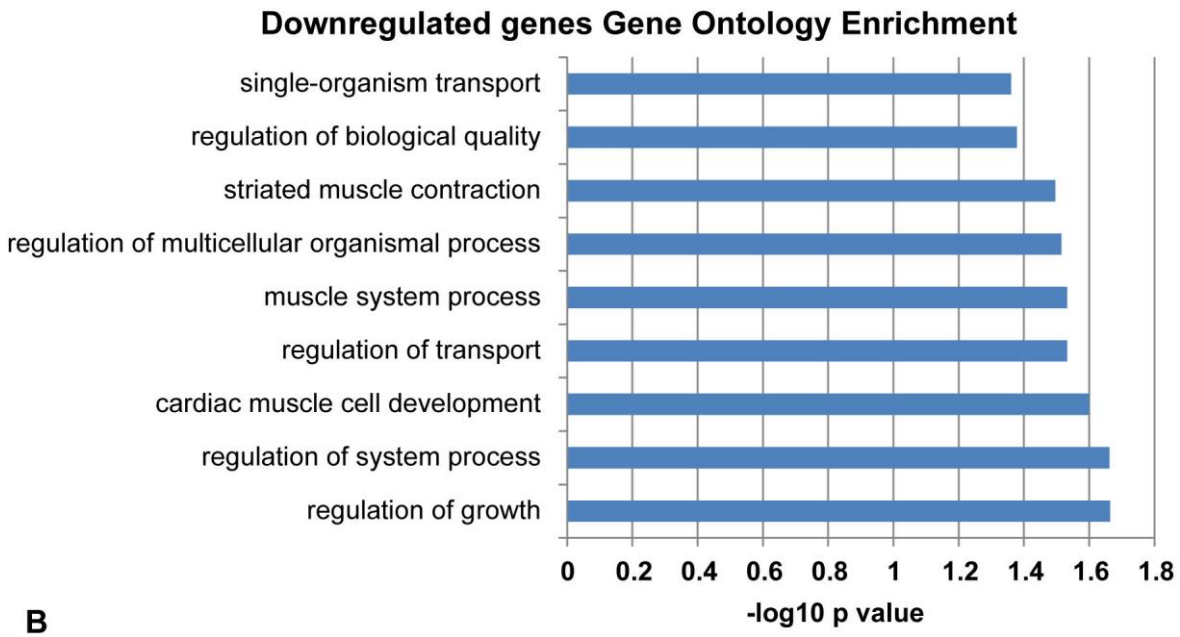
Histological analysis of WT (A) and *Casz1*$^{f/f}$*;Nkx2.5*$^{Cre/+}$ (B) hearts at E13.5 highlights the enlarged right atria observed in the SEM data. At E13.5, the cardiac hypoplasia and ventricular septal defect is more pronounced and further shows that the *Casz1*$^{f/f}$*;Nkx2.5*$^{Cre/+}$ heart is misshapen and does not form an apex for either the left or the right ventricle as seen in the wild-type (highlighted by the arrows in each panel).



Supplementary Figure 6. *Casz1* is required for myocardial development

(A-L) Immunofluorescent staining for TMY of anterior, mid and posterior sections highlights a decrease in differentiated cardiomyocytes in *Casz1*$^{f/f}$*;Nkx2.5*$^{Cre/+}$ hearts at E10.5 (A,B,E,F,I,J) and E12.5 (C,D,G,H,K,L). A, anterior; P, posterior.

A



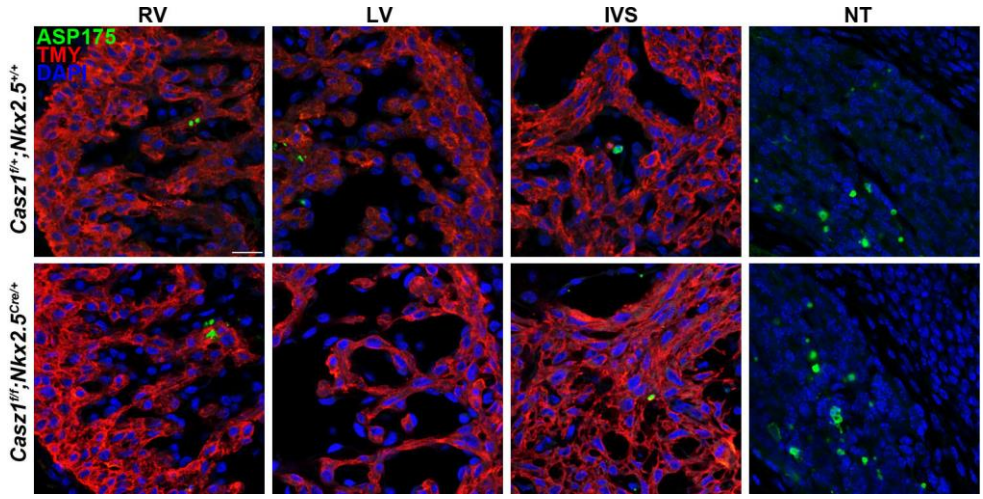
B

Cell Growth Genes Downregulated in *Casz1*^{CKO} Hearts

Gene	log2 Fold Change	Adjusted p-value	Best Blastx hit
Alox12	-0.966	0.017	arachidonate 12-lipoxygenase
Lpar3	-0.85711	0.003	lysophosphatidic acid receptor 3
Fgf9	-1.46448	0.009	fibroblast growth factor 9
Mndal	-1.95735	0.016	myeloid nuclear differentiation antigen like
Alox8	-1.12568	0.028	arachidonate 8-lipoxygenase
P2rx5	-1.23956	0.036	purinergic receptor ligand-gated ion channel, 5
Zc3h12d	-1.58106	0.039	zinc finger ccch type containing 12d
Pou3f2	-1.76676	0.005	pou domain, class 3, transcription factor 2
Hey2	-0.67981	0.0165	hairy/enhancer-of-split related with yrpw motif 2
Ntn1	-1.41721	7.19E-07	netrin 1
Nkx2-5	-0.78714	0.007	nk2 transcription factor related, locus 5 (drosophila)
Nppa	-0.98232	0.012	natriuretic peptide type a
Cav3	-0.7795	0.014	caveolin
Igfbpl1	-1.45848	7.17E-07	insulin-like growth factor binding protein-like 1

Supplementary Figure 7. RNA-seq analysis

(A) Gene Ontology (GO) Analysis – significant cellular processes downregulated in *Casz1*$^{f/f}$*;Nkx2.5*$^{Cre/+}$ E10.5 hearts. (B) Cell growth genes downregulated in *Casz1*$^{f/f}$*;Nkx2.5*$^{Cre/+}$ hearts.



Supplementary Figure 8. Loss of *Casz1* does not lead to programmed cell death

Immunofluorescent staining for cleaved caspase-3, TMY and DAPI (blue) to identify apoptotic cells shows that *Casz1*$^{f/f}$*;Nkx2.5*$^{Cre/+}$ hearts do not have an increase in programmed cell death compared to controls. As a positive control, sections of the neural tube (NT) were also analyzed. Data represents 2 independent experiements. LV, left ventricle; RV, right ventricle; IVS, interventricular septum.

Supplemental Table 1

Gene	Primer Sequence	Size
Casz1 Genotyping	Forward: 5' – CACAGGAACTGTCTCCACTGTC – 3' Reverse: 5' – GTTTCTGTTGCTGGGTTAGGTT – 3'	Flox: 571 bp WT: 471 bp
Cre Genotyping	Forward: 5' – TGGGCCAGCTAAACATGCTT – 3' Reverse: 5' – GGTGTTATAAGCAATCCCCAGA – 3'	236 bp
R26R^{tdT} Genotyping	Forward: 5' – GGCATTAAAGCAGCGTATCC – 3' Reverse: 5' – CTGTTCCTGTACGGCATGG – 3'	126 bp
Casz1 5' RACE Outer Primer	Reverse: 5' – GGATGTATTCCTCGTATTTGGAGA – 3'	N/A
Casz1 5' RACE Inner Primer	Reverse: 5' – AGCTGATCTTCTTGGCCAGCTCAT – 3'	N/A
Casz1 5' Southern Probe	Forward: 5' – CTGAAAGCACCCGGTGCACCGACC – 3' Reverse: 5' – CTGAAGCCTGCCTGGTGCTGGGGC – 3'	486 bp
Casz1 In Situ Probe	Forward: 5' – GAGAAACCTCCTCTCTGAGGGACT – 3' Reverse: 5' – CTGGCAATATATTTCTGGTAAATG – 3'	904 bp