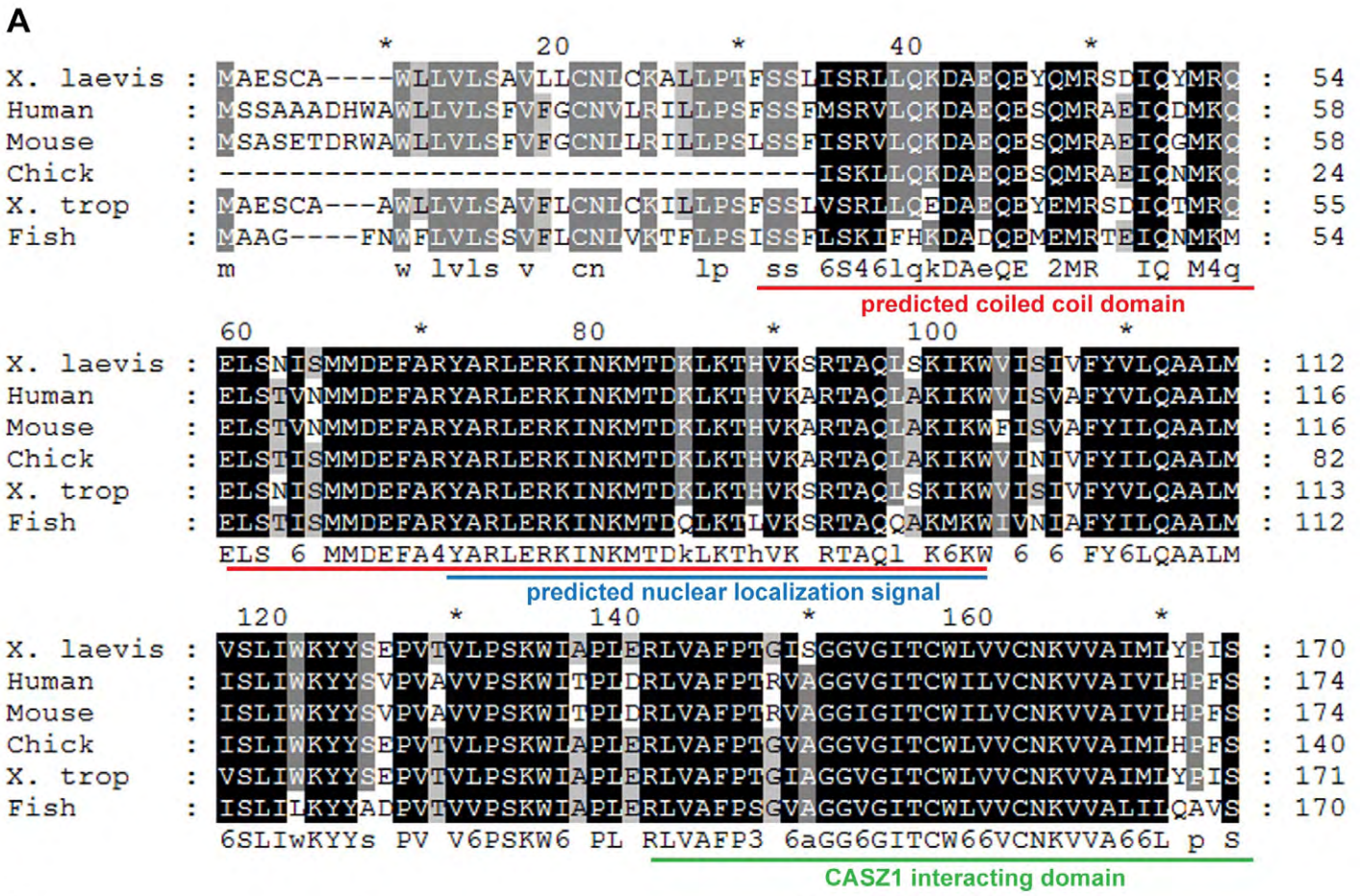
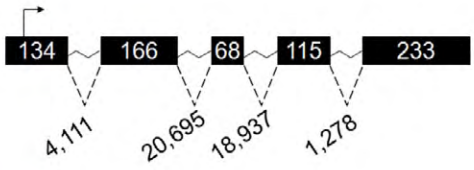
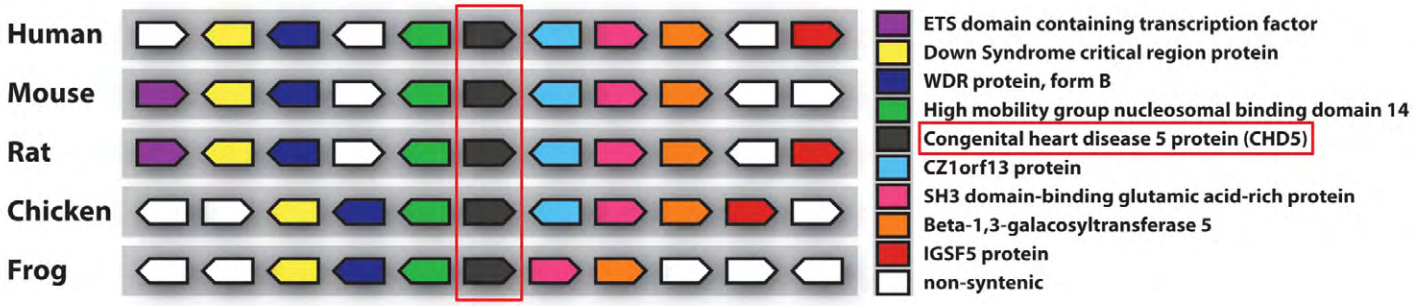


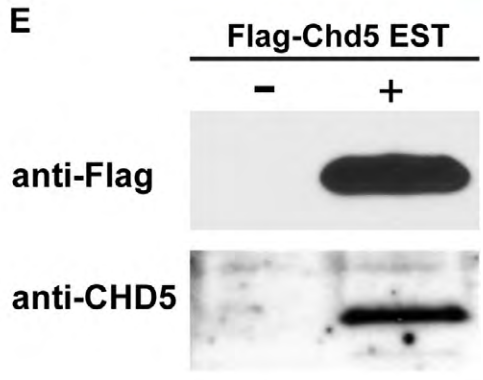

B

*X. laevis* CHD5 homologues

| | Human | Mouse | Chick | *X.tropicalis* | Fish |
|---|---|---|---|---|---|
| % identity | 74 | 72 | 72 | 94 | 69 |
| % similarity | 89 | 87 | 80 | 98 | 88 |



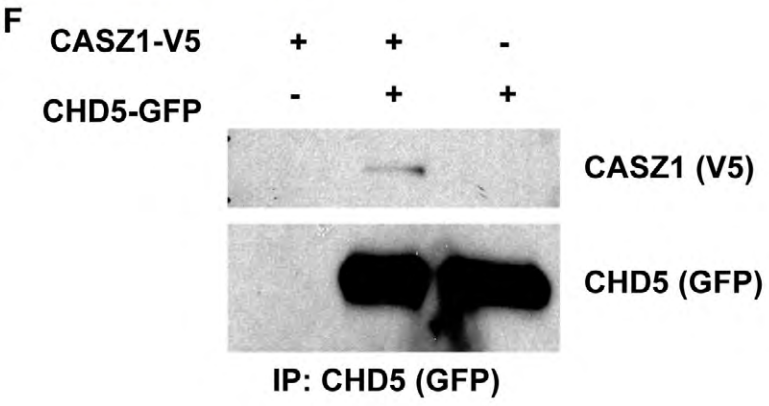



**Figure S1. Characterization of *Xenopus* CHD5. A,** Protein sequence alignment of *Xenopus* CHD5 cDNA via BLAST (GeneDoc) compared to CHD5 orthologues. Red line underlines predicted coiled coil domain, blue line predicted nuclear localization signal and green line the sequence derived from the minimum cDNA clone identified in a yeast two-hybrid screen to interact with CASZ1. **B,** Percent identity and similarity between CHD5 orthologues. **C,** Predicted genomic locus structure of *Xenopus* CHD5 (5' to 3', not to scale). Exons are shown in boxes with the corresponding size given in basepairs. **D,** A synteny comparison of *Xenopus* CHD5 and other vertebrate homologues, demonstrating strong conservation in its chromosomal position across vertebrate species (Metazome). **E,** Western analysis of a FLAG-tagged protein product of a putative *Xenopus* CHD5 homologue indicated protein runs at the predicted molecular weight, 19 kDa, as recognized by both anti-FLAG and anti-CHD5 antibodies. **F,** Western analysis demonstrating *in vivo* co-immunoprecipitaion of CASZ1-V5 and CHD5-GFP in *Xenopus* embryos.

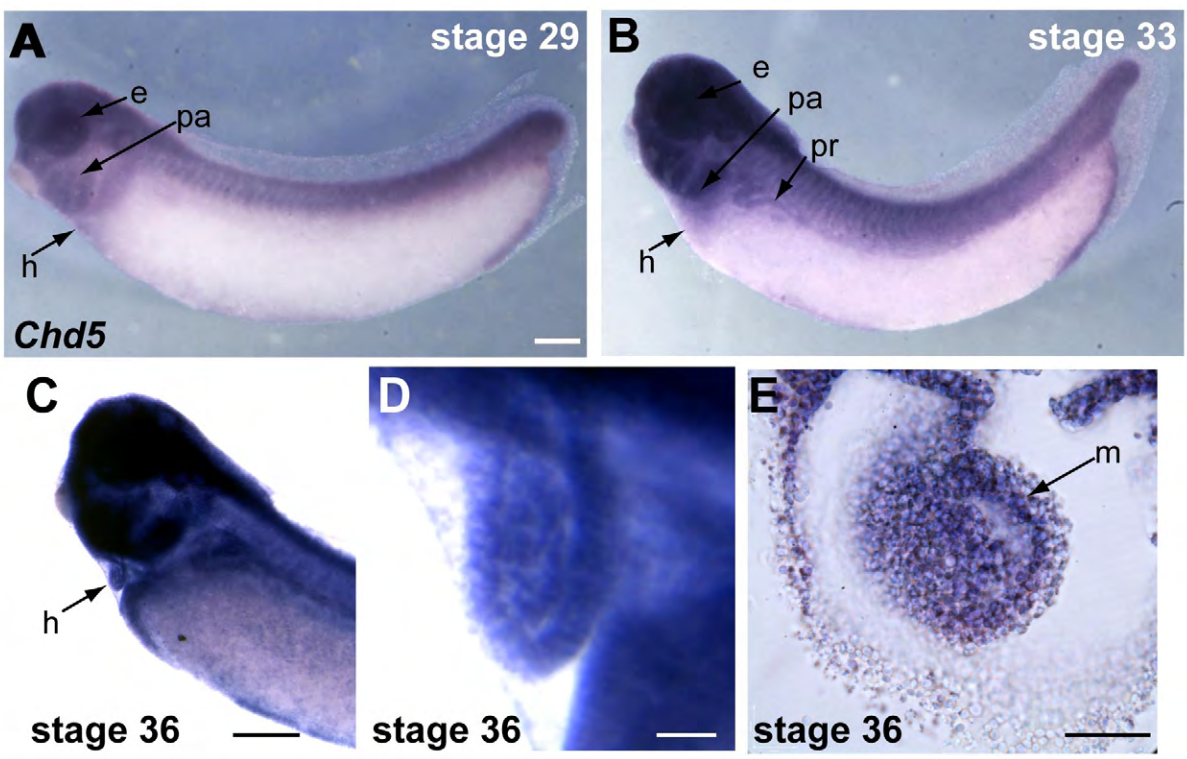



**Figure S2. *Chd5* is expressed in the heart and anterior tissue. A-B,** *in situ* images of late tailbud (stage 29) and early tadpole (stage 33) *Xenopus* embryos demonstrating *Chd5* expression in the developing heart (h) as well as in other anterior structures such as pronephros (pr), eye (e), and pharyngeal arches (pa). **C-E,** cleared *in situ* images of early tadpole (stage 36) embryo demonstrating *Chd5* expression in the developing heart (h). **D** represents 40x magnification of embryo in panel **C**. **E** represents transverse section of embryo in panel **C** and demonstrates *Chd5* expression in developing myocardial tissue (m). Scale bar: (A-C, E)=100 mm, (D)=10 μm

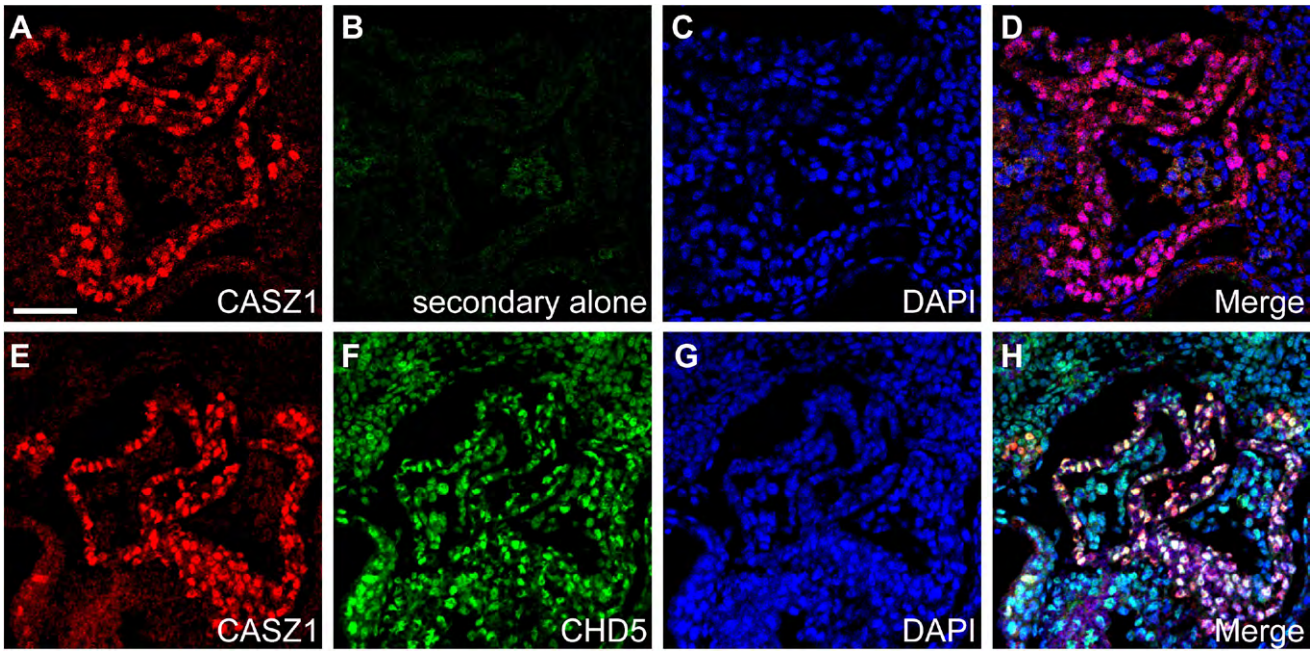



**Figure S3. CASZ1 and CHD5 co-localize in the nuclei of cardiomyocytes. A-H,** CASZ1 and CHD5 co-localize in nuclei of developing cardiomyocytes of *Xenopus* tadpoles (stage 40) as demonstrated by CASZ1 and CHD5 specific antibody staining; staining with secondary antibody alone shown to control for the specificity of the CHD5 antibody across multiple tissues (**B versus F**). Scale bar: (A-H)=50 mm

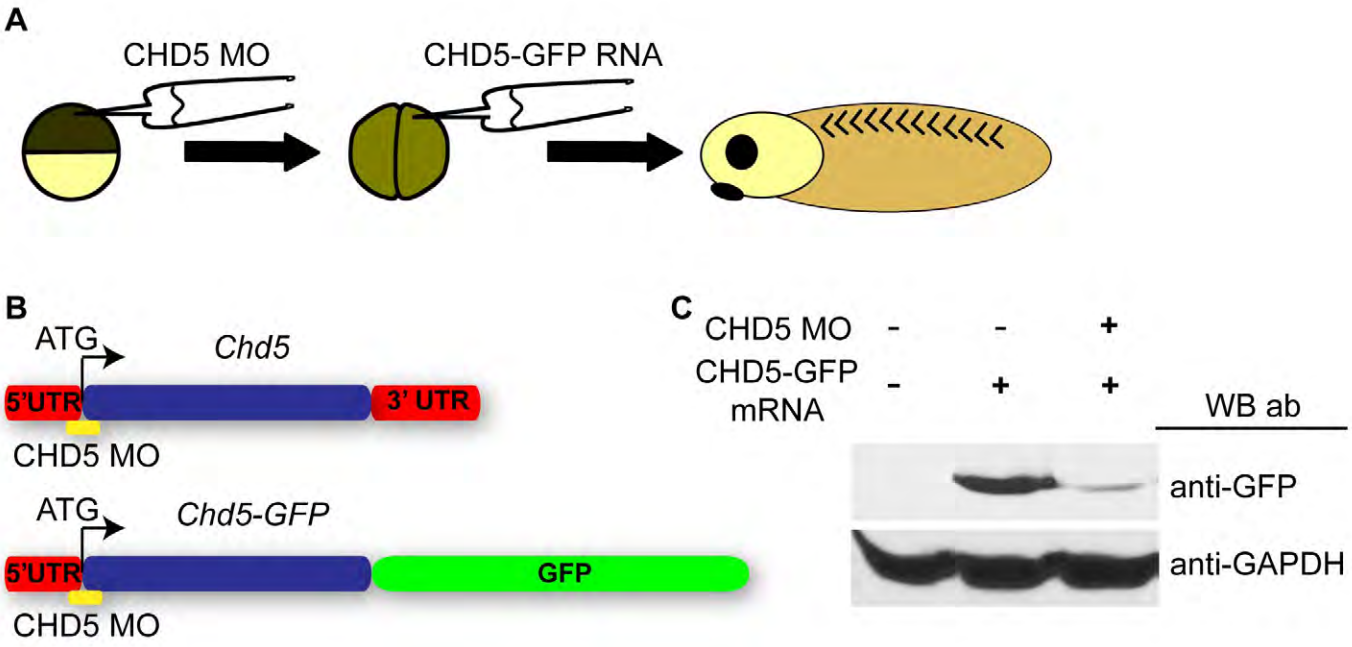



**Figure S4. Efficacy of translation blocking CHD5 morpholinos. A**, Schematic depicting sequential injections of CHD5 MO targeting 5' UTR and CHD5-GFP. **B**, Schematic of *Chd5* and the *Chd5-GFP* fusion protein generated. **C,** CHD5 MO efficiently depletes CHD5-GFP by blocking translation of injected mRNA in *Xenopus* embryos (stage 27) as assessed by western blot analysis (WB) with anti-GFP antibody. GAPDH was used as a loading control.

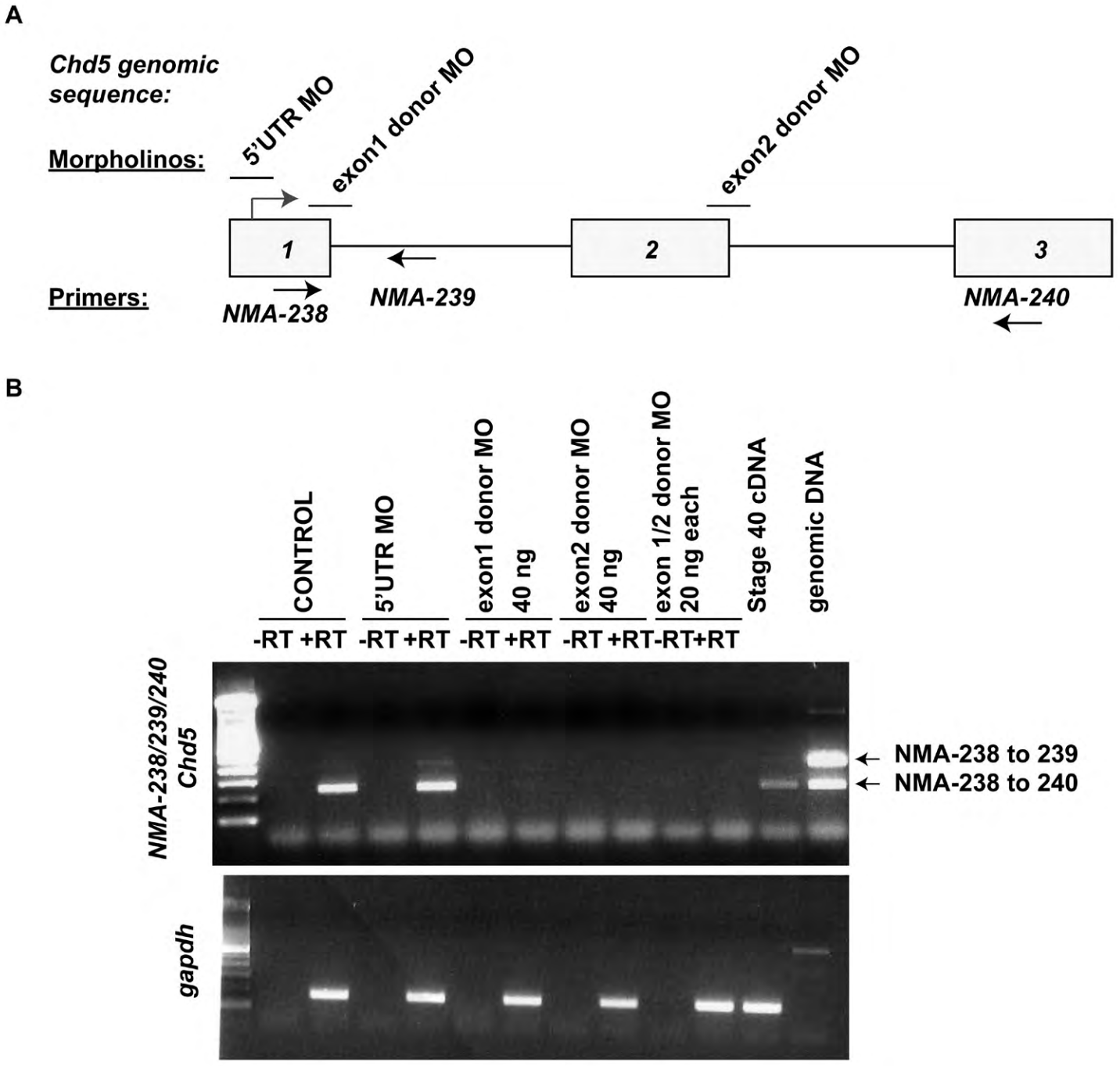
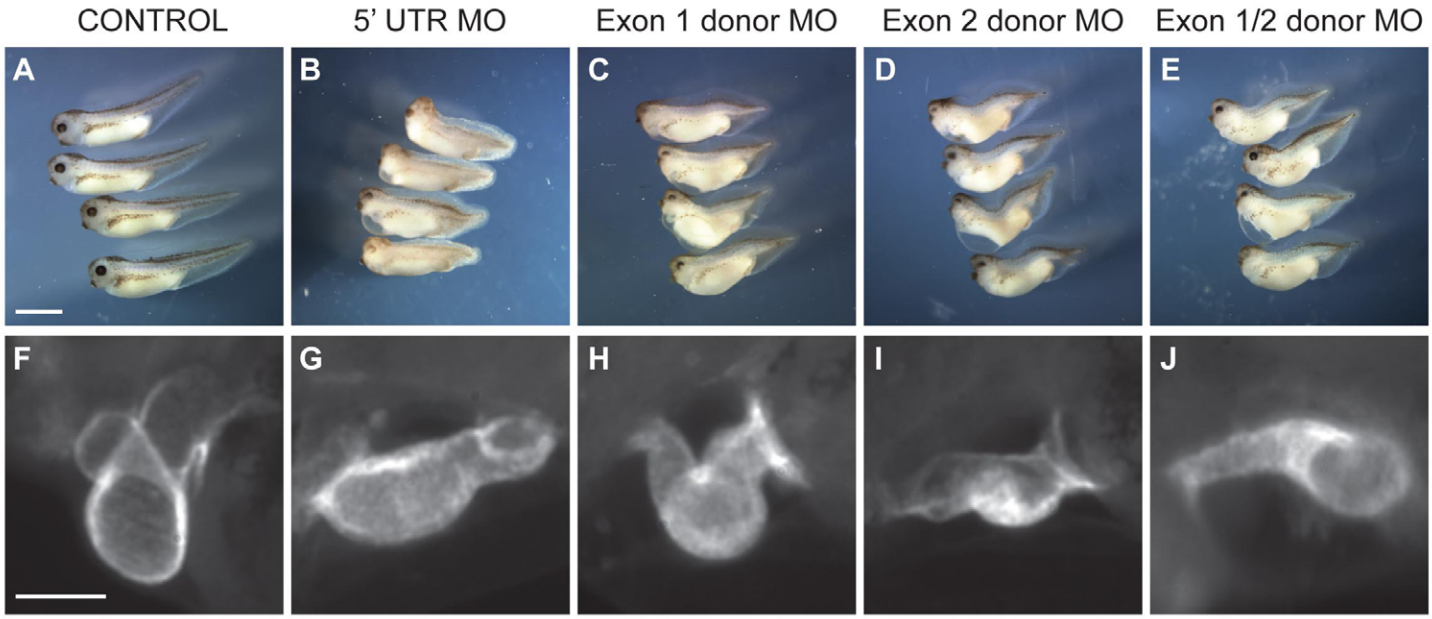



**Figure S5. Efficacy of splice blocking CHD5 morpholinos. A,** Schematic demonstrating location of splice junction morpholinos generated to block splicing between *Chd5* exon donor sites 1 and 2 and relative location of primers (NMA-238, 239, 240) used. **B,** RT-PCR demonstrating efficiency of splice junction morpholinos. Lack of expected PCR product in cDNA samples derived from morpholino injected samples (likely due to nonsense-mediated decay of aberrantly spliced mRNA). *gapdh* specific primers used as control.




**K**

| | CONTROL | 5' UTR MO | Exon 1 donor MO | Exon 2 donor MO | Exon 1/2 donor MO |
|---|---|---|---|---|---|
| cardia bifida | 0/45<br>0% | 21/43<br>49% | 16/46<br>35% | 15/39<br>38% | 2/44<br>5% |
| abnormal looping | 1/45<br>2% | 18/43<br>42% | 17/46<br>37% | 16/39<br>41% | 14/44<br>32% |

embryos totaled from two independent experiments

**Figure S6. CHD5 morpholinos induce cardiac defects. A-E,** Brightfield images of early tadpole control, 5' UTR, and splice junction MO injected embryos reveal similar morphological defects with either set of CHD5 MOs. **F-J,** Whole-mount IHC images of representative looping defects seen in embryos under all MO conditions visualized with anti-Tropomyosin staining. **K,** Quantification of cardiac defects in control and all MO conditions, total of two independent experiments (n>20 embryos per condition). Scale bars: (A-E)=2.5 mm, (F-J)=100 mm

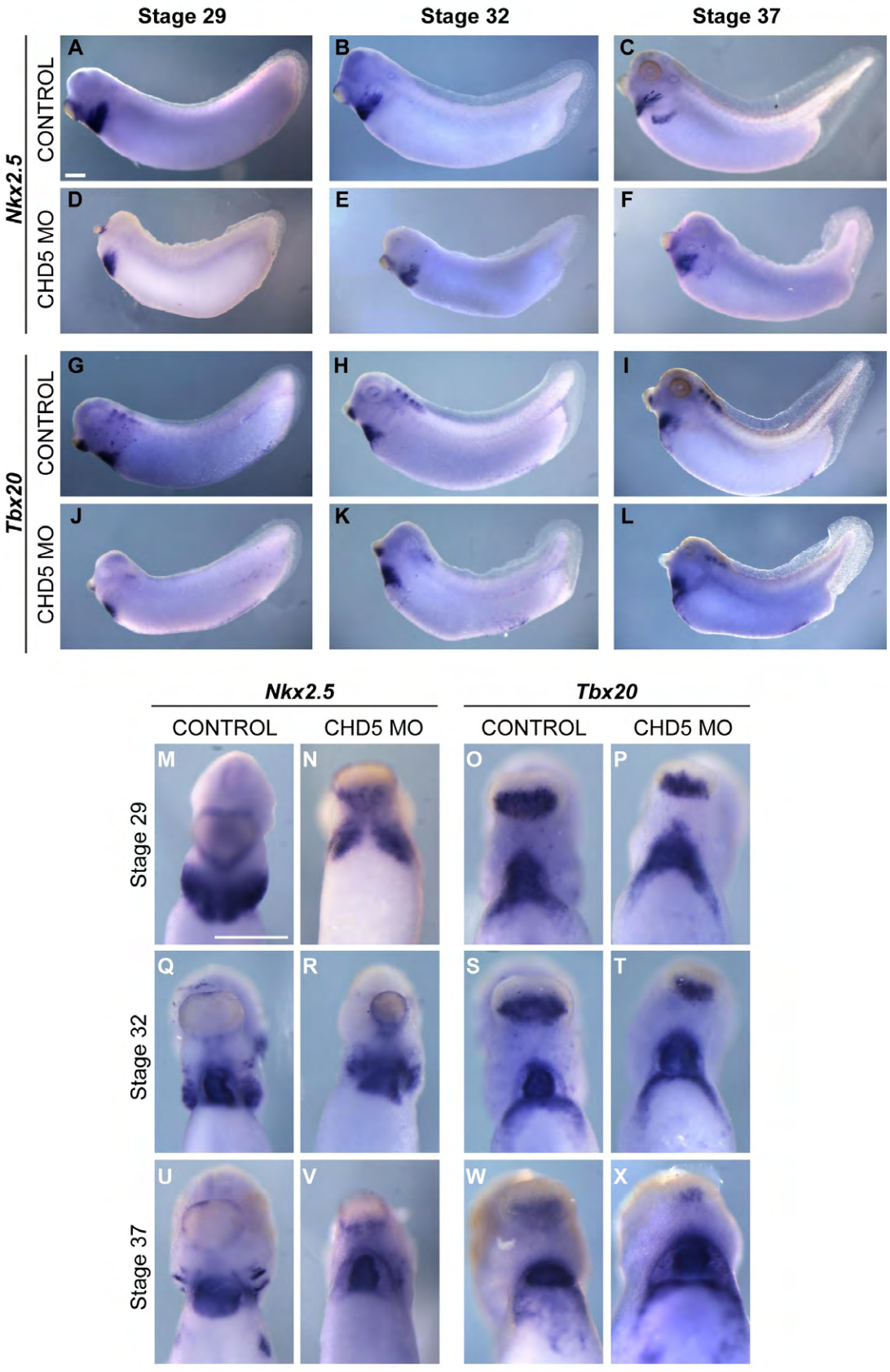



**Figure S7. CHD5 is not required for early cardiac specification or movement of cardiac cells to the ventral midline.. A-L,** CHD5-depleted embryos undergo proper early cardiac patterning as demonstrated by lateral views of *in situ* analysis with the early cardiac markers *Nkx2.5* (A-F) and *Tbx20* (G-L). Ventral views of CHD5-depleted embryos demonstrate proper movement of cardiac cells to the ventral side of the embryo as visualized by *in situ* hybridization of early cardiac markers *Nkx2.5* (M,N,Q,R,U,V) and *Tbx20* (O,P,S,T,W,X). Scale bars: (A-L)=500 mm, (M-X)=500 mm

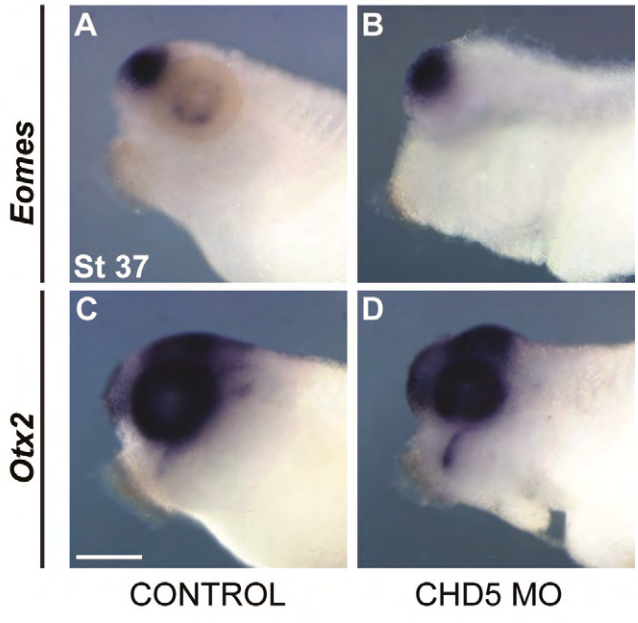



**Figure S8. CHD5 is not required for anterior patterning. A-D,** CHD5-depleted embryos (stage 37) properly express the anterior markers *Otx2* and *Eomes* as examined by *in situ* analysis. Scale bars: (A-D)=500 mm

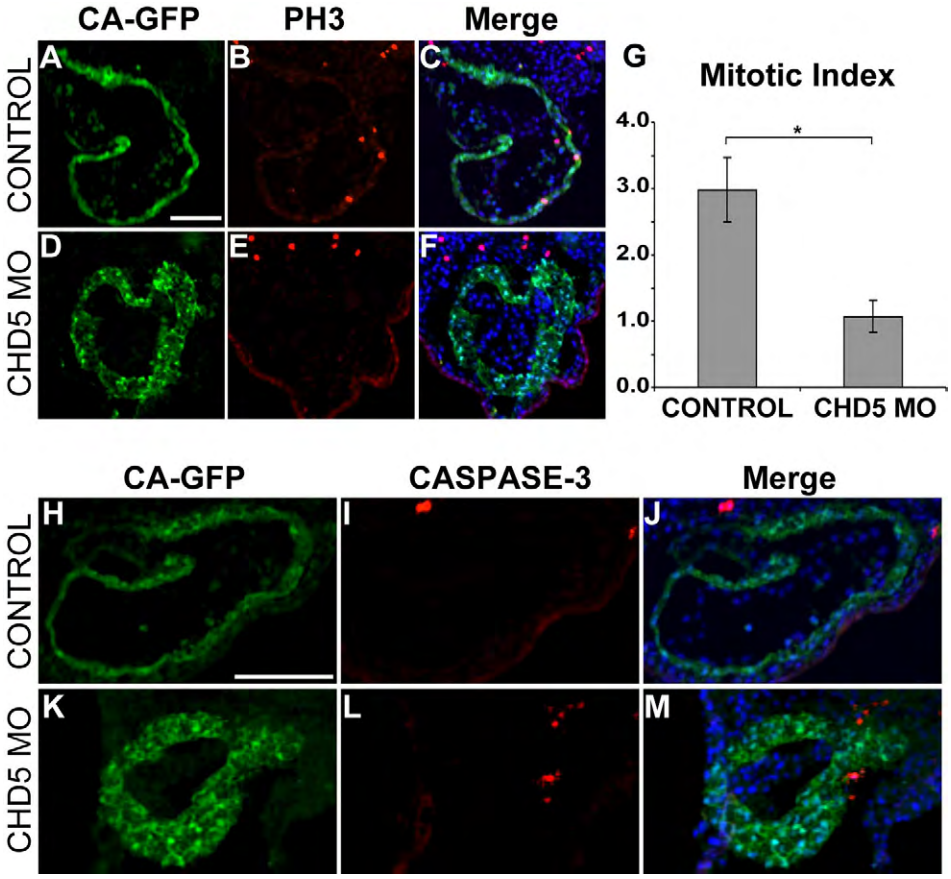



**Figure S9. CHD5-depletion reduces cardiomyocyte number but is not associated with programmed cell death. A-G,** CHD5-depleted embryos (stage 37) display a reduced number of cardiomyocytes compared and a reduced mitotic index as revealed by phospho-histone H3 (red) expression (*= $p < 0.05$). Mitotic index was calculated by dividing number of cells positive for phospho-histone H3 and cardiac actin-GFP by total number of cardiac actin-GFP positive cells **H-M,** Neither control nor CHD5-depleted embryos exhibit programmed cell death as marked by cleaved caspase-3 (red; transverse sections of CA-GFP embryos show cell death in surrounding mesenchyme but not in cardiomyocytes), n=2 per condition, 1 biological replicate). Scale bar: (A-F, H-M)=100 mm

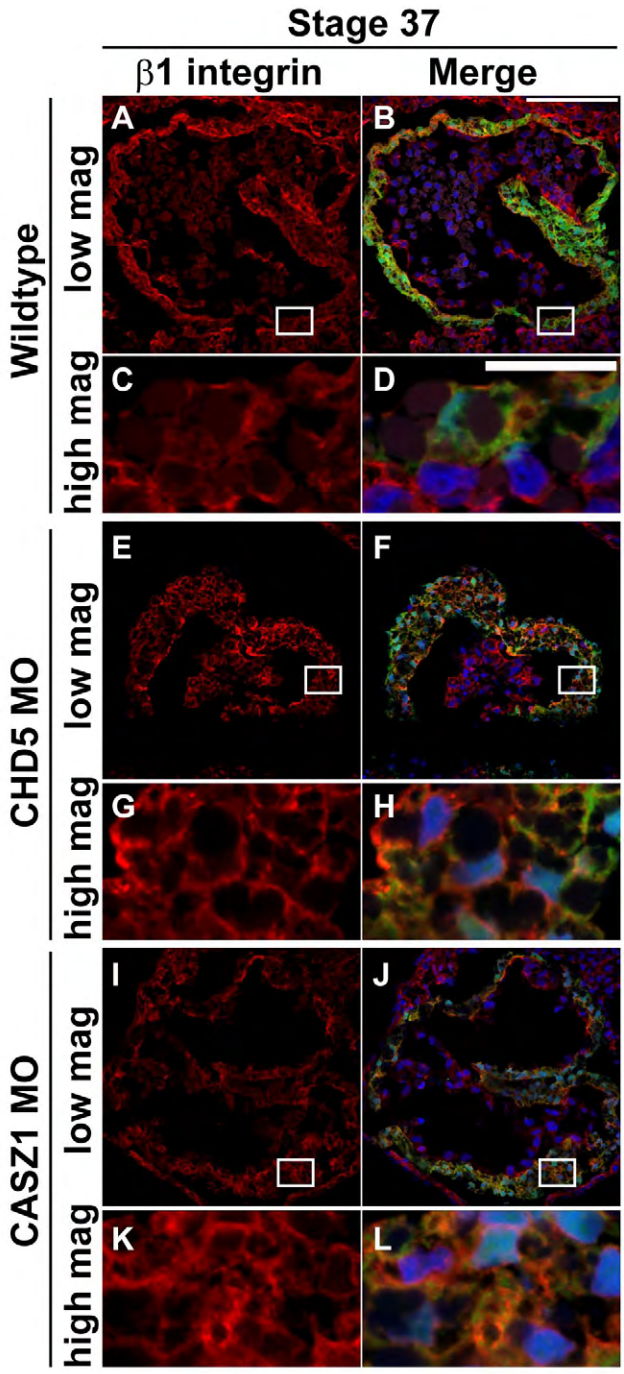



**Figure S10. β1-integrin expression is not altered in CHD5 and CASZ1-depleted embryos. A-L,**Control, CHD5-depleted, and CASZ1-depleted CA-GFP embryos were stained for β1-integrin to mark extracellular matrix-cytoskeletal junctions. The overall pattern of integrin expression within myocardial tissue remained unchanged among all three conditions. White boxes in A,B,E,F,I,J correspond to magnified images in C,D,G,H,K,L. Merge represents β1-intgerin (red), DAPI (blue), and CA-GFP (green). Scale bar: (A,B,E,F,I,J)=100μm, (C,D,G,H,K,L)=20μm

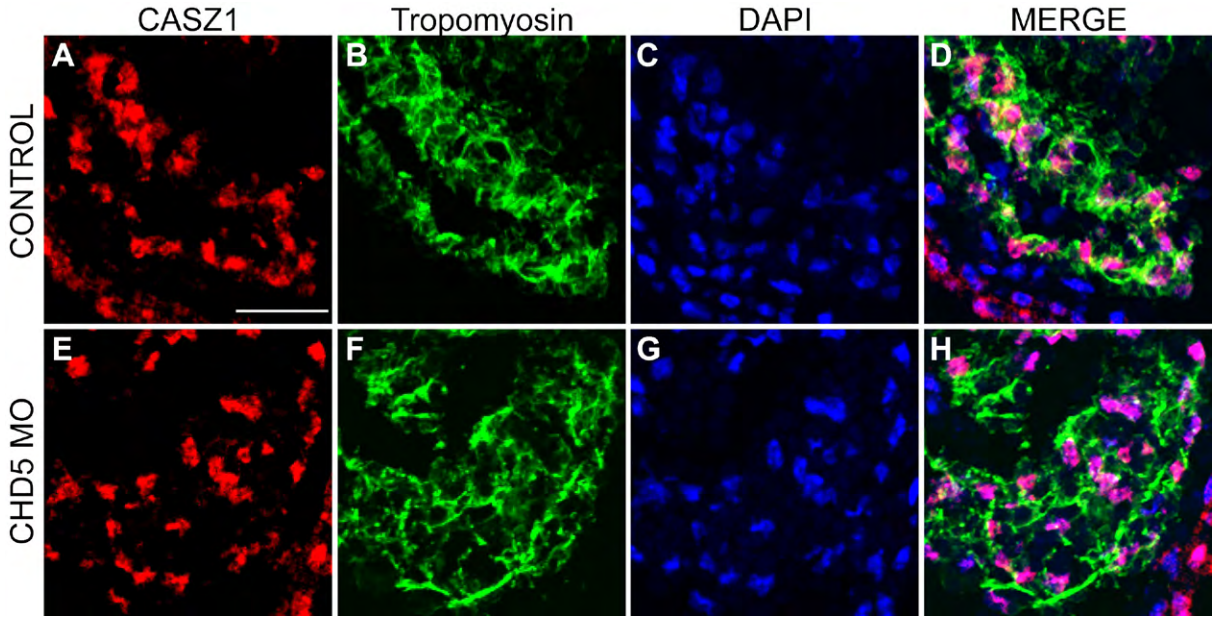



**Figure S11. CHD5 is not required for CASZ1 nuclear localization. A-H,** CASZ1 remains localized to the nucleus of cardiomyocytes in CHD5-depleted embryos as visualized by immunostaining for CASZ1, Tropomyosin and DAPI on transverse sections of stage 37 embryos. Scale bar: (A-H)=50 mm

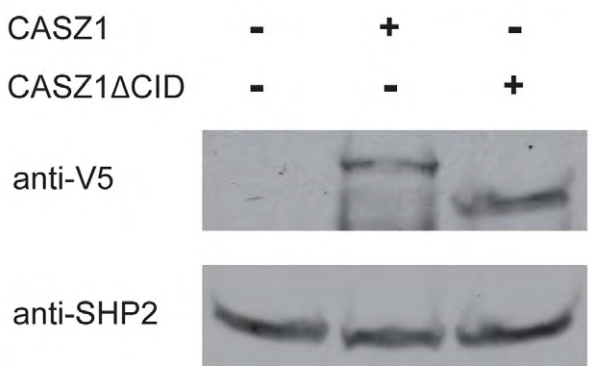



**Figure S12. CASZ1-V5 and CASZ1ΔCID-V5 are efficiently translated in *Xenopus*.** mRNA encoding CASZ1-V5 and CASZ1ΔCID-V5 was injected into *Xenopus* embryos at the one-cell stage and embryos were collected at stage 37. Western analysis with a V5-specific antibody confirmed the presence of the correct size molecular weight proteins. Anti-SHP2 was used as a loading control.


**Movie 1. 3D reconstruction of a control early tadpole stage *Xenopus* heart.** At the cardiac looping stage (stage 37) control embryos have formed a heart tube which displays a prominent tubular structure comprised of an inflow tract, ventricle and outflow tract. Scale bar=50 mm


**Movie 2. 3D reconstruction of a CHD5-depleted early tadpole stage *Xenopus* heart.** Reconstruction at the cardiac looping stage (stage 37) shows that cardiac looping and chamber formation has been aborted upon CHD5 depletion. In a dorsal view, it is also shown that the inflow tract has failed to completely close. Scale bar=50 mm

**Supplemental Table 1: Embryo statistics for CASZ1 morpholino rescue**

| | expression of markers of cardiac differentiation | cardia bifida | proper looping | | proper chamber formation |
|---|---|---|---|---|---|
| | | | partial | complete | |
| **CONTROL** | 100/100<br>100% | 0/100<br>0% | 1/100<br>1% | 99/100<br>99% | 99/100<br>99% |
| **CASZ1 MO** | 94/94<br>100% | 67/94<br>71% | 44/94<br>47% | 18/94<br>19% | 15/94<br>16% |
| **CASZ1 MO + CASZ1** | 108/108<br>100% | 13/108<br>12% | 34/108<br>31% | 63/108<br>58% | 62/108<br>57% |
| **CASZ1 MO + CASZ1ΔCID** | 82/82<br>100% | 35/82<br>59% | 35/82<br>43% | 19/82<br>23% | 18/82<br>22% |

embryos totaled from three independent experiments

Cumulative results from three independent experiments of *Xenopus* embryos scored for presence of cardia bifida defects, cardiac looping defects and chamber formation defects.

**Supplementary Table 2. Cloning strategy and PCR conditions for generation of CASZ1 deletion series**

| Construct | PRIMERS or cloning strategy | PCR conditions |
|---|---|---|
| CASZ1in pGBKT7 | Cloned 3' portion of CASZ1 into pGBKT7 using NdeI (986bp of CASZ1 ORF) and SalI (3332bp of CASZ1 ORF). Cloned 5' end into resulting construct using two NdeI sites (pcDNA3.1 MCS 5' of CASZ1 ORF and 986 bp CASZ1 ORF) | N/A |
| CASZ1 (1-998) in pGBKT7 | Removed fragment at restriction sites EcoNI (2994bp of CASZ1 ORF) and SalI (pGBKT7 MCS), Klenow treated and ligated blunt ends of remaining construct | N/A |
| CASZ1 (1-828) in pGBKT7 | Removed fragment at restriction sites ApaI (2483bp of CASZ1 ORF) and SalI (pGBKT7 MCS), Klenow treated and ligated blunt ends of remaining construct | N/A |
| CASZ1 (561-1108) in pGBKT7 | Isolated fragment at restriction sites PstI (1683bp of CASZ1 ORF) and PstI (pGBKT7 MCS), Klenow treated excised fragment and ligated into empty pGBKT7 vector | N/A |
| CASZ1 (561-998) in pGBKT7 | Isolated fragment at restriction sites Pst1 (1683 of CASZ1 ORF) and EcoNI (2994bp of CASZ ORF), Klenow treated excised fragment and ligated into empty pGBKT7 vector | N/A |
| CASZ1 (785-998) in pGBKT7 | 5'CGA**CATATG**CCTCAGACAAACAAAGTCAC<br>3'GAC**CTGCAG**AAGGTAATGCGTTGAATCCA<br><br>Cloned into pGBKT7 vector via NdeI and PstI MCS sites | 30s 98ºC<br>30x<br>(10s 98 ºC<br>30s 55 ºC<br>30s 72 ºC)<br>7min 72 ºC |
| CASZ1 (828-998) in pGBKT7 | Excised fragment at restriction sites ApaI (2483bp of CASZ1 ORF) and EcoNI (2994bp of CASZ1 ORF) | N/A |
| CASZ1ΔCID in pGBKT7 | STEP 1<br>Fragment 1(5')<br>5'AGCAACCAATCACTGGCAGACTACCTGGACTTGATTCAGG<br>3'AGAGCCTTCATCACCTTGCCAGAAGGAACCTCAGAACCAG<br>Fragment 2 (3')<br>5'CTGGTTCTGAGGTTCCTTCTGGCAAGGTGATGAAGGCTCT<br>3'TCAATGGTGATGGTGATGATGACCGGTACGCGTAGAATCG<br><br>STEP 2<br>5'TTGCCCCTTTCAACTGCTAC<br>3'AGGGTTAGGGATAGGCTTAC<br><br>Cloned CASZ1ΔCID product into CASZ1-pGBKT7 using internal CASZ1 ORF EcoRI and EcoNI sites | STEP 1<br>30s 98ºC<br>25x<br>(10s 98 ºC<br>30s 58 ºC<br>40s 72 ºC)<br>7min 72 ºC<br><br>STEP 2<br>30s 98ºC<br>30x<br>(10s 98 ºC<br>30s 58 ºC<br>1min 72 ºC)<br>7min 72 ºC |