Frizzled-7 is required for Xenopus heart development

Muhammad Abu-Elmagd\textsuperscript{1,2*}, Joanna Mulvaney\textsuperscript{2}\textdagger, Grant N. Wheeler\textsuperscript{2}$

\textsuperscript{1}Center of Excellence in Genomic Medicine Research, King Abdulaziz University, P.O. Box 80216 Jeddah 21589, Kingdom of Saudi Arabia

\textsuperscript{2}School of Biological Sciences, University of East Anglia, Norwich Research Park, Norwich, NR4 7TJ, UK

*Authors contributed equally to this work

$Author for correspondence:
Grant N Wheeler, Ph.D.
E-mail address: grant.wheeler@uea.ac.uk
Address: University of East Anglia, School of Biological Sciences, NR4 7TJ, Norwich, UK
Tel.: +44 (0) 1603 59 3988/3245
Fax: +44 (0) 1603 59 2250

E-mail addresses:
Muhammad Abu-Elmagd: mabuelmagd@kau.edu.sa
Joanna Mulvaney: jfm30@sri.utoronto.ca

Present Address: Sunnybrook Research Institute, 2075 Bayview Ave, Toronto, Canada

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Summary Statement

Wnt signalling has been shown to be important in heart development. Here, we demonstrate that the wnt receptor Fzd7 is required in mediating these Wnt signals.

Abstract

Wnt signalling regulates cardiogenesis during specification of heart tissue and the morphogenetic movements necessary to form the linear heart. Wnt11 mediated non-canonical signalling promotes early cardiac development whilst Wnt11-R, which is expressed later, also signals through the non-canonical pathway to promote heart development. It is unclear which Frizzleds mediate these interactions. Frizzled-7 (fzd7) is expressed during gastrulation in the mesodermal cells fated to become heart and then in the primary heart field. This expression is complementary to the expression of wnt11 and wnt11-R. We further show co-localisation of fzd7 with other early and late heart-specific markers using double in situ hybridisation. We have used loss of function analysis to determine the role of fzd7 during heart development. Morpholino antisense oligonucleotide-mediated knockdown of Fzd7 results in effects on heart development, similar to that caused by Wnt11 loss of function. Surprisingly, overexpression of dominant-negative Fzd7 cysteine rich domain (Fzd7 CRD) results in a cardia bifida phenotype, similar to the loss of wnt11-R phenotype. Overexpression of Fzd7 and activation of non-canonical wnt signalling can rescue the effect of Fzd7 CRD. We propose that Fzd7 has an important role during Xenopus heart development.
Introduction

During embryogenesis, the heart is one of the first organs to form. Development of the heart includes specification of cardiac progenitors and formation of the linear heart tube by cell migration and morphogenetic movements (Mohun et al., 2000). In *Xenopus*, the heart begins to form during early gastrula stages when the cardiac progenitors arise in the dorsolateral mesoderm. Cell movements during gastrulation result in the dorso-anterior translocation of these regions and subsequent ventral migration during neurulation. The heart progenitors, which comprise cells fated to become primary or secondary heart field, form a linear heart tube at the ventral midline before looping and remodelling to form the beating heart (Kriegmair et al., 2013). Understanding the processes underlying heart development and morphogenesis are important for understanding congenital heart disease.

Heart formation is controlled by many signalling pathways including wnt signalling. Wnt6, 11, and 11-R have all been implicated in *Xenopus* heart development (Garriock et al., 2005; Gessert et al., 2008; Lavery et al., 2008a; Pandur et al., 2002). Wnt antagonists such as Dickkopf-1, Crescent and Sfrp1 have also been reported to control early heart formation (David et al., 2008; Foley and Mercola, 2005; Gibb et al., 2013; Marvin et al., 2001; Schneider and Mercola, 2001). Little is known however about which frizzleds mediate these signals. Frizzled-7 (Fzd7) has been well characterised in *Xenopus laevis* and other species. It has been shown to be involved in numerous developmental processes as well as being shown to be active in several forms of cancer (Huang and Klein, 2004; Liu et al., 2016; Schiffgens et al., 2016; Xu et al., 2016). Fzd7 has been demonstrated to interact with several wnts including Wnt5a (animal cap elongation assays), Wnt6 (in somite development), Wnt8 (co-immunoprecipitation assays, Xenopus axis duplication) and Wnt11 (gastrulation movements, neural crest development)(Hsieh et al., 1999; Linker et al., 2005; Medina et al., 2000; Medina and Steinbeisser, 2000; Umbhauer et al., 2000; Witzel et al., 2006). It has also been shown to genetically interact with the co-receptors ror2 and ryk (Hikasa et al., 2002; Kim et al., 2008). *Xenopus* Fzd7 has been implicated in gastrulation movements, tissue separation, and neural crest induction (Abu-Elmagd et al., 2006; Djiane et al., 2000; Wheeler et al., 2000; Winkelbauer et al., 2001). We have previously shown *fzd7* to be expressed in the cardiac region throughout development (Wheeler and Hoppler, 1999). It has also been shown that specific depletion of *fzd7* function in Xenopus foregut lead to impaired cardiac morphogenesis but no effect on heart specification (Zhang et al., 2013). Here, we further characterise its expression
relative to known heart markers and then use whole-embryo experiments to show that fzd7 is required for heart formation during early embryonic development.

Results

1. fzd7 expression overlaps with early heart markers

Expression pattern analysis shows *Xenopus* fzd7 is expressed in the heart forming regions throughout development (Wheeler and Hoppler, 1999). At stage 10.5 fzd7 is expressed in the dorsal mesoderm from which cardiac tissue originates (Wheeler and Hoppler, 1999) and Fig. 1A). As development progresses, fzd7 expression at stage 25 is maintained in the presumptive cardiac mesoderm as it migrates dorso-laterally to the ventral midline (Fig. 1C-Cii). By stage 29, fzd7 is expressed throughout the cardiac crescent in the cardiac mesoderm (Fig. 1E, Ei). fzd7 expression correlates with that of wnt11 (Fig. 1B, stage 10.5) where expression of both genes seem to be complementary in the presumptive heart region in the dorsal side of the embryo. fzd7 expression also correlates to that of wnt11-R (Fig. 1D-Dii, F, Fi, stages 25 and 29) where it is expressed in the anterior endoderm at stage 25 when fzd7 is expressed in the heart field. By stage 29 the expression of fzd7 and wnt11-R overlaps (Fig. 1E-Fi).

As the heart continues to form, fzd7 is strongly expressed in the lateral plates of mesoderm, cardiac mesoderm, myocardium and over time, is restricted to the pericardium (Wheeler and Hoppler, 1999)), Fig. 2A-Aii, Bii, Cii and Dii). Using double in situ hybridisation, we analysed fzd7 expression in correlation to that of early heart markers including nkx2-5, troponin-ic (tnnic) and gata6, which are all known to be required for Xenopus cardiogenesis (Afouda and Hoppler, 2011; Afouda et al., 2008; Drysdale et al., 1994; Flaherty and Dawn, 2008; Fu et al., 1998; Garriock et al., 2005; Jiang and Evans, 1996; Martin et al., 2010). fzd7 expression overlaps with that of nkx2.5 (Fig. 2B-Bii), tnnic (Fig. 2C-Cii) and gata6 (Fig. 2D-Dii) in the forming heart. Interestingly, none of these markers are seen in the pericardium except for fzd7 (Fig. 2Aii, Bii, Cii, Dii).

2. fzd7 is required for heart induction or specification

Microinjection into *Xenopus* embryo dorsal blastomeres at the 4 or 8 cell stage targets prospective mesoderm including cardiac tissue. In order to test the role of fzd7 in heart development, we inhibited its function by injecting either fzd7 morpholino (fzd7 MO) or its dominant negative form expressing only the extracellular domain (cysteine
rich domain, fzd7 CRD), which would disrupt Fzd7 mediated signalling (Abu-Elmagd et al., 2006).

Microinjection of fzd7 MO into the dorsal blastomeres of 4 or 8 cell embryos leads to a reduction of both early cardiac marker nkx2-5 (Fig. 3B-Bii) and later cardiac marker tnnic expression (Fig. 3E-Eii). Adding increasing amounts of fzd7 MO leads to a progressively more severe phenotype with a greater number of embryos affected (Fig. 3C). *In situ* hybridisation for nkx2-5 and tnnic show embryos with mild convergent extension phenotypes (Fig. 3B, E) but a severe decrease in cardiac gene expression (Fig. 3Bi and Ei while control morpholino (CMO) show normal heart (Fig. 3A, Ai and D, Di). Some embryos also showed anterior defects (not shown). Sections through the cardiac region showed not only a decrease of nkx2-5 and tnnic expression, but an absence of recognisable heart structures (Fig. 3Bii and 3Eii) compared to CMO (Fig. 3Aii and Dii). The number of embryos injected with fzd7 MO which showed heart and/or convergent extension and anterior defects are shown in Table S1.

Overexpression of fzd7 full length (fzd7 FL) results in severe convergent extension defects, but no cardiac phenotype (Fig. S1A and B). Knockdown with fzd7 MO can also cause a mild convergent extension phenotype and anterior defects (Abu-Elmagd et al., 2006). In order to test whether this cardiac effect is specific to fzd7, we rescued the fzd7 MO cardiac phenotype with fzd7 full-length that has been mutated to not bind the fzd7 MO (fzd7SDM as described in (Abu-Elmagd et al., 2006)). Titrating increasing amounts of fzd7SDM capped RNA from 250 pg to 1 ng results in a modest rescue of the cardiac phenotype (Fig. 3F, Fi and Table S2), thus showing that fzd7 is required for normal heart development.

Interestingly, injecting fzd7 full length at 8-cell stage embryos shows detectable expression of tnnic and nkx2-5 despite some of these embryos showing severe convergent extension movements phenotype (head arrows in Fig. S1A and B). This leads to the suggestion that heart phenotypes are not necessarily due to convergent extension secondary effects.

3. Fzd7 CRD mimics wnt11R morpholino cardia bifida phenotype and is required for non-canonical signalling

To further look at the effect of inhibiting Fzd7 function we took a dominant negative approach using fzd7 CRD. Surprisingly this did not give a similar result to the MO knockdown. Instead, increasing amounts of fzd7 CRD results in a dose dependent
increase in frequency and severity of cardia bifida. This was very similar to the phenotype seen for wnt-11R knockdown (Garriock et al., 2005). Embryos with very mild convergent extension movement defects displayed a severe cardia bifida phenotype as shown by tnnic (Fig. 4B-Bii and C) and nkh2-5 (Fig. 4G, Gi) expression. Control embryos showed normal expression of tnnic (Fig. 4A-Aii) and nkh2-5 (Fig. 4F, Fi). These results suggest that the cardia bifida phenotype is not a secondary effect of the convergent extension defect. Overexpression of fzd7FL gives a severe convergent extension phenotype but no cardiac phenotype (Fig. S1A and B). Embryos with cardia bifida were unable to recover and form a normal heart when incubated up to stage 41 (n= 23, data not shown). Embryos injected with a dominant negative form of fzd3 (fzd3 CRD) into the dorsal blastomeres at 4 cell stage did not show cardia bifida (n= 27, Fig. 4E, Ei) indicating that the cardia bifida phenotype is specific to fzd7 CRD. Furthermore, this phenotypic specificity to fzd7 CRD was confirmed by rescuing the cardia bifida with full length fzd7 capped RNA (Fig. 5A-D and F).

It has been previously reported that a Jun N-terminal kinases (Jun) inhibitor phenocopies the wnt11-R cardiac phenotype of effects on cardiac morphogenesis and heart tube fusion, suggesting signalling through the non-canonical pathway (Garriock et al., 2005; Gessert et al., 2008). We therefore determined to rescue the fzd7 CRD phenotype with Dishevelled1-Delta-N (dvl1ΔN) capped RNA. Dvl1ΔN capped RNA can rescue fzd7 CRD (Fig. 5E, Ei and G, Gi and Table S3) suggesting that fzd7 is required for non-canonical wnt signalling during heart development.

Discussion

Wnt signalling through the canonical and non-canonical pathways has been implicated in many aspects of heart development (Gessert and Kuhl, 2010; Ruiz-Villalba et al., 2016). How the wnt signals that arise from both non-cardiogenic and cardiogenic tissue are integrated into heart development is less well understood. Frizzled receptors are only a part of the increasingly complicated wnt-receptor complex found at the cell membrane which can also include Lrp5/6, Ror2, Ryk and Kremen (Bryja et al., 2009; Korol et al., 2008; Mazzotta et al., 2016; van Wijk et al., 2009); however, Frizzleds are critical components of the Wnt receptor complex and so understanding their role in heart development is necessary to fully understand the signalling involved. We have previously shown that fzd7 is expressed throughout heart development and in this study, we show that it is functionally required in both early and late heart development.
Morpholino knockdown of Fzd7 leads to effects on heart development including in some cases a complete loss of heart (Fig. 3). Overexpression of Fzd7 gives rise to convergent extension defects as previously reported (Abu-Elmagd et al., 2006; Sumanas and Ekker, 2001; Winklbauer et al., 2001) but does not affect heart development. We can rescue the fzd7 MO phenotype by co-injecting site directed mutagenized full length fzd7 (Fig. 3). These results suggest fzd7 is required for initial heart development though we cannot exclude the possibility that it may also be playing a more general role in dorsoventral mesoderm patterning. Fzd7 could be interacting with Wnt11 (Kim et al., 2008; Tao et al., 2005; Witzel et al., 2006) or another wnt ligand such as Wnt3a (Mazzotta et al., 2016), Wnt6 (Gibb et al., 2013; Lavery et al., 2008a; Lavery et al., 2008b) or Wnt8c (Ruiz-Villalba et al., 2016; Schneider and Mercola, 2001) during these stages of development.

As suggested, it is possible that the fzd7 morphant cardiac phenotype is a secondary effect of failures in mesoderm specification, patterning, gastrulation, axis formation and tissue separation. We have made efforts to inject embryos at the 4 and 8 cell stages to give as small a convergent extension phenotype as possible to generate normal looking embryos but with clear heart phenotypes. The results suggest that the effect of Fzd7 during early heart development is not secondary to convergent extension defects or mesoderm development, however, this cannot be ruled out completely (Fig. 3).

An interesting feature of the loss of function analysis using fzd7 morpholino and a dominant negative fzd7 CRD, is that they give different cardiac phenotypes. fzd7 morphants have anterior defects, convergent extension defects and reduction in nkx2-5 expression, whereas fzd7 CRD capped RNA injections result in embryos with convergent extension defects and cardia bifida but no head defects or loss of cardiac markers. Interestingly it has been shown that the only way to replicate the anterior defect phenotype with a fzd7 CRD construct is to inject the capped RNA into oocytes (Medina et al., 2000). This could be because the relevant signalling event has been completed by time the product of mRNA injected at the 4 or 8 cell stage has been generated. It is possible that if we injected oocytes with fzd7 CRD then we might find embryos showing loss of the heart. Another possibility is that the Morpholino is able to disrupt all Wnt signalling through fzd7 by preventing translation of Fzd7 protein, but fzd7 CRD only disrupts non-canonical signalling in this context. The requirement for co-receptors in canonical signalling may allow the CRD to interact with endogenous Fzd7 and any Lrps present allowing the receptor complex aggregates to form. In
addition to this, it has been shown to be possible to activate canonical Wnt signalling using CRD constructs (Carron et al., 2003). Perhaps canonical Wnt signalling mediated by Fzd7 early on during development is allowed to proceed by the Fzd7 CRD, but then when Fzd7 switches to mediate non-canonical signalling, the CRD starts to behave as a dominant negative. Other possibilities are that the Morpholino may have a broader specificity than thought or that the injected RNA of the fzd7 CRD construct may not be very stable and thus only provide a short term effect compared to the Morpholino. These options remain to be tested further.

The fzd7 CRD phenotype is very similar to the wnt11-R Morpholino phenotype (Garriock et al., 2005). It has previously been shown that DM-GRASP/alcam expression lies downstream of wnt11-R signalling and that DM-GRASP/alcam can mediate non-canonical wnt signalling effects on morphogenetic movements involved in the developing heart. The DM-GRASP/alcam morpholino phenotype is also similar to the fzd7 CRD phenotype in that they both lead to a cardia bifida like phenotype and a thickening of the myocardium. This suggests Fzd7 could be mediating the Wnt11-R control of DM-GRASP/alcam expression. This needs to be investigated further.

Ruiz-Villalba and colleagues (Ruiz-Villalba et al., 2016) suggest a model where periodic switching between proliferation and differentiation within the developing heart is mediated by the periodic and reciprocal activity of the canonical and non-canonical wnt pathways. fzd7 could be playing a crucial role in this process depending upon the Wnts and other receptors expressed at specific times.

In conclusion, we have shown fzd7 to be involved in heart development. Further investigation is required to determine the specific wnt(s) it is interacting with at different stages of heart development.

Materials and Methods

1. Embryo manipulation

All experiments were performed in compliance with the relevant laws and institutional guidelines at the University of East Anglia. The research was approved by the local ethical review committee according to UK Home Office regulations. Xenopus laevis embryos were obtained as previously described (Harrison et al., 2004). Staging of the embryos was carried out according to the normal time table of Nieuwkoop and Faber (Nieuwkoop and Faber, 1994). Embryos at the required stages were fixed in MEMFA, washed in PBS, dehydrated in ascending grades of Methanol/PBS, then
stored in 100% MeOH at -20°C until processing for single or double in situ hybridisation.

2. Constructs

fzd7 full-length (fzd7-FL) and dominant negative form fzd7-cysteine rich domain (fzd7-CRD) were sub-cloned into pCS2+ at Cla1–Xho1 restriction sites as described in (Wheeler et al., 2000). Fzd7 MO titration by RNA in the rescue experiments was avoided by creating a site directed mutagenesis construct of the full coding sequence of fzd7 (fzd7 SDM) as described in (Abu-Elmagd et al., 2006). fzd3 full-length (fzd3 FL) and fzd3 CRD were kind gifts from Peter Klein. Dishevelled construct (Dvl1-Delta-N) was a gift from Roberto Mayor (De Calisto et al., 2005).

3. In vitro capped mRNA synthesis and embryo microinjections

All Capped mRNAs of all genes used for RNA injections were prepared according to the manufacturer’s instructions using the SP6 mMessage mMachine Ambion kit (Invitrogen™ AM1340). Anti-sense oligonucleotides, morpholinos (MOs), were obtained and designed by Gene Tools (www. gene-tools.com, Oregon, USA) using the reported sequence for the control morpholino (CMO) (5’-CCTCTTACCTCAgTTACAATTTATA-3’) and fzd7MO (5’-GCGGAGTGAGCAGAAATCGGCTGA-3’)(Sumanas and Ekker, 2001). MOs were diluted, prepared before use according to the manufacturer’s instructions and tested using the in vitro translation assay (TNT coupled reticulocyte lysate system, Promega-L4600). For targeting the heart, the dorsal blastomeres of the 4 and 8 cell stage embryos were injected as previously described (Lavery et al., 2008a). Capped mRNA and MOs were co-injected with lac-Z for lineage tracing. Each experiment was carried out as an internally controlled group. Each experiment was carried out 3 times and the numbers of embryos in each class were pooled.

4. RNA probe synthesis and in situ hybridisation

fzd7 in pBluescript was linearised with XbaI and transcribed by T7, nkx2-5 was linearised with BamH1 and transcribed with T7, troponin-IC (tnnic) was linearised with XhoI and transcribed with T3, gata6 was linearised with XbaI and transcribed with T7. Promega probe synthesis manufacturing instructions were followed with fzd7 probe labelled with Fluorescene-substituted nucleotide (Fl-UTP) and for other heart makers labelled with DIG-substituted nucleotide. Each RNA probe was added to 10ml hybridisation buffer and stored at -20°C for in situ hybridisation. Single (Harland, 1991)
or double (Knecht et al., 1995) in situ hybridization was carried out as previously described (Abu-Elmagd et al., 2006). Anti-Fluorescein was detected using Fast Red tablets (Kelloff et al., 2006) while anti-Digoxigenin was detected with NBT/BCIP. Frozen sectioning and wax sectioning were carried out as described (Harrison et al., 2004; Hatch et al., 2016). Images were taken using Leica microscope and Axiovision software.

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Competing Interests
No competing interests declared

Author contributions
Conception and design - GW
Investigation and data acquisition – MAE and JM
Analysis and interpretation of data – GW, MAE and JM
Material support - GW
Writing, review and/or revision of manuscript – GW, MAE and JM
Study supervision - GW

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significance of novel (Frizzled-7) and established (MGMT, IDH1) biomarkers in glioblastoma. Oncotarget 7, 55169-55180.


Figure 1:
Endogenous expression of *fzd7* in *Xenopus* heart and relative to *wnt11* and *wnt11-R* expression. (A, B): stage 10.5 (mid-gastrula) *fzd7* and *wnt11* expression detected at the dorsal side of the embryo and appear complementary in the presumptive heart region. (C-Cii and D-Di): *fzd7* and *wnt11-R* expression at stage 25. *fzd7* is seen in the heart field and *wnt11-R* in the anterior endoderm. *fzd7* and *wnt11-R* expression are complementary in the heart region (Cii, Dii). (E, Ei and F, Fi): stage 29 embryos with *fzd7* and *wnt11-R* expression in the heart field. hf: heart field, ae: anterior endoderm. Magnification 20x.
Figure 2:

$\textit{fzd7}$ expression coincides with expression of the early heart markers $\textit{nngx2-5}$, $\textit{tnnic}$ and $\textit{gata6}$. Lateral view of $\textit{Xenopus laevis}$ embryos at stage 31 showing $\textit{fzd7}$ expression detected in red (A-Aii) and co-localised by double $\textit{in situ}$ hybridisation with other heart markers in dark blue including $\textit{nngx2-5}$ (B-Bii), $\textit{tnnic}$ (C-Cii) and $\textit{gata6}$ (D-Dii). (Ai, Bi, Ci, Di). Magnified lateral view of the same embryos in (A, B, C and D) respectively. (Aii, Bii, Cii, Dii). Cross section through the heart region of the embryos in (A, B, C, D) respectively. $\textit{fzd7}$ is expressed in the myocardium and pericardium (Aii) and in other structures including neural crest, eye, pronephric duct and tail bud. $\textit{fzd7}$ expression shows a high degree of overlapping with the heart markers in the myocardium but not in the pericardium (Bii, Cii, Dii). h: heart, c: cement gland, e: eye, nc: neural crest, pnd: pronephric duct, tb: tail bud, mc: myodcardium, lpm: lateral plate.
of mesoderm. Magnification: 20x in (A, B, C, D), 30x in (Ai, Bi, Ci, Di), 200x in (Aii, Bii, Cii, Dii).
Figure 3:  
**Fzd7 is required for Xenopus heart development**

(A, Ai and D, Di) Lateral and ventral views of embryos injected in the dorsal blastomeres (DB) at 4 cell stage with control morpholino (CMO) showing normal *nkx2-5* (A-Ai) and *tnnic* (D, Di) expression. (Aii, Dii). Cross sections in the heart region of the embryos in (A) and (D) respectively showing normal *nkx2-5* and *tnnic* expression in the myocardium. (B, Bi and E, Ei). Lateral and ventral views of embryos injected in the DB at 4 cell stage with *fzd7* MO showing loss of *nkx2-5* (B-Bi) and *tnnic* (E, Ei) expression. (Bii, Eii). Cross sections in the heart region of the embryos in (B) and (E) respectively showing loss of the heart. (C) Graph showing that *fzd7* MO phenotype leads to reduction/loss of *nkx2-5* expression in a dose-dependent manner. (F, Fi). *Fzd7* MO phenotype can be rescued by *fzd7* SDM full length, (Fi) is the key for the phenotype scoring. Red staining in B, Bi and Bii is due to *lac-Z* lineage tracing using Red-Gal.
Figure 4:
**A dominant negative Fzd7 induces cardia bifida phenotype.**

(A-Ai and F, Fi) Lateral and ventral views of wild type embryos at stage 29 showing normal *tnnic* (A, Ai) and *nkx2-5* (F-Fi) expression in the heart. (B, Bi and G, Gi) Lateral and ventral views of embryos injected in the dorsal blastomeres at 4 cell stage with dominant negative *fzd7* (*fzd7* CRD). The cardia bifida phenotype is shown by *tnnic* (B, Bi) and *nkx2-5* (G-Gi) expression. These embryos were fixed at the same stage as the control embryos in (A and F). (C) Graph showing *fzd7* CRD cardia bifida phenotype percentages indicated by *tnnic* expression. (D, Di). Lateral and ventral views of embryos injected in the dorsal blastomeres (DB) at 4 cell stage with full length of *fzd7* showing normal heart tube. Note that embryos in (D) and (G) are showing severe convergent extension defects but cardia bifida phenotype is only induced by *fzd7* CRD. (E, Ei). Lateral and ventral views of injected embryo in the DB at 4 cell stage with *fzd3* dominant negative form (*fzd3* CRD) showing normal heart looping (at stage 38) indicating that *fzd7* CRD cardia bifida phenotype is specific to Fzd7. Magnification 20x.
(H and I). Lateral (H) and ventral (I) views of embryos injected in the DB at 4 cell stage with full length of \textit{fzd7} showing normal heart tube indicated by \textit{nkkx2-5} expression.
Figure 5:
Activation of non-canonical wnt signalling rescues fzd7 CRD induced cardia bifida.

(A, Ai) Wild type control embryos showing normal tnnic expression in the heart. (B, Bi) fzd7 full length overexpression (500pg) injected into the dorsal blastomeres (DB) at the 4 cell stage show normal heart expression of tnnic despite suffering a severe extension movement defect. (C). Embryos injected with 500pg fzd7 CRD show cardia bifida phenotype, note that embryos have normal to mild convergent extension defects. (D). Rescue of the fzd7 CRD (250pg) cardia bifida phenotype with 250pg full length fzd7, embryos show normal morphology as well as tnnic expression. (F). Graph of fzd7 CRD cardia bifida phenotype rescue with fzd7 Full length. (E, Ei). Rescue of fzd7 CRD (500pg) cardia bifida phenotype with 1.25ng dishevelled1-Delta-N (Dvl1ΔN) indicating that fzd7 is required for the non-canonical signalling in the heart. (G). Graph of fzd7 CRD cardia bifida phenotype rescue with dvl1ΔN, (Gi) is the key for the cardia bifida phenotype scoring in (G). Magnification 20x.
Supplemental Tables and Figures

Table S1:

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fzd7 Morpholino dose response. Increasing amounts of fzd7 MO were injected at the 4 cell stage into both blastomeres of the dorsal side (DB) of the embryo and ventral side (VB) as a control. Observed phenotypes included a range of convergent extension phenotypes from severe to mild, varying degrees of anterior defects and a reduction of nkh2-5 or tnnic expression.
Table S2:

<table>
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<th>Embryo injections</th>
<th>Total No. of Embryos</th>
<th>No Heart</th>
<th>Reduced Heart</th>
<th>Normal Heart</th>
<th>% No Heart</th>
<th>% Reduced Heart</th>
<th>% Normal Heart</th>
</tr>
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<tr>
<td>Non-injected control</td>
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<tr>
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**fzd7 MO phenotype is rescued by fzd7 SDM.** Injecting 1ng of fzd7SDM capped RNA does not give a cardiac phenotype. Coinjecting 60 ng fzd7 MO with from 250pg –1ng of lacZ capped RNA gives between 51% and 30% embryos with no heart and between 22% and 29% embryos with normal hearts. Coinjecting with fzd7 SDM capped RNA from 250pg- 1ng gives a dose responsive decrease of embryos with no heart 33% at 250pg to 15% at 1 ng and an increase in embryos with a normal heart from 18% at 250pg to 43% at 1ng. DB: dorsal blastomeres, VB: ventral blastomeres.
### Table S3:

<table>
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<th>Embryo injections</th>
<th>Total No. of Embryos</th>
<th>Severe Cardia Bifida (%)</th>
<th>Mid. Cardia Bifida (%)</th>
<th>Partial Cardia Bifida (%)</th>
<th>Normal Heart (%)</th>
<th>% Severe Cardia Bifida</th>
<th>% Mid. Cardia Bifida</th>
<th>% Partial Cardia Bifida</th>
<th>% Normal Heart</th>
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<td>90</td>
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</table>

**fzd7 CRD is rescued by dvl1 Δ N.** Injecting 1.5 ng of dvl1 Δ N capped RNA does not give a cardiac phenotype. Coinjecting 160 ng fzd7 CRD with from 750pg –1.5ng of lacZ capped RNA gives between 33% and 46% embryos with severe cardia bifida and between 17% and 10% embryos with normal hearts. Coinjecting with dvl1 Δ N capped RNA from 750pg- 1.5ng gives a dose responsive decrease of embryos with severe cardia bifida 25% at 750pg to 7% at 1.25ng and an increase in embryos with a normal heart from 18% at 750pg to 44% at 1.25ng. DB: dorsal blastomeres, VB: ventral blastomeres.
Figure S1:

Cardiac development is independent on the convergent extension movement defects caused by overexpression of \textit{fzd7}. (A, B). \textit{fzd7} full length (250pg) injected into the dorsal blastomeres at 8 cell stage and incubated till stage-32 showing detectable \textit{tnnic} (A) and \textit{nkx2-5} (B) expression in both normal embryos and those with convergent extension movement defects (arrow heads in A and B).