Sfrp5 identifies murine cardiac progenitors for all myocardial structures except for the right ventricle

Masayuki Fujii1, Akane Sakaguchi2,3, Ryo Kamata1, Masataka Nagao4, Yutaka Kikuchi5, Silvia M. Evans6, Masao Yoshizumi1, Akihiko Shimono7,†, Yumiko Saga2,3,8 & Hiroki Kokubo1

Upon acquirement of pulmonary circulation, the ancestral heart may have been remodelled coincidently with, or accompanied by, the production and rearrangement of progenitor cells. However, the progenitor populations that give rise to the left ventricle (LV) and sinus venosus (SV) are still ambiguous. Here we show that the expression of Secreted frizzled-related protein Sfrp5 in the mouse identifies common progenitors for the outflow tract (OFT), LV, atrium and SV but not the right ventricle (RV). Sfrp5 expression begins at the lateral sides of the cardiac crescent, excluding early differentiating regions, and continues in the venous pole, which gives rise to the SV. Lineage-tracing analysis revealed that descendants of Sfrp5-expressing cells at E7.5 contribute not only to the SV but also to the LV, atria and OFT and are found also in the dorsal splanchnic mesoderm accompanied by the expression of the secondary heart field marker, Islet1. These findings provide insight into the arrangement of cardiac progenitors for systemic circulation.

1Department of Cardiovascular Physiology and Medicine, Graduate School of Biomedical and Health Sciences, Hiroshima University, 1-2-3 Kasumi, Minamiku, Hiroshima 734-8551, Japan. 2Department of Mammalian Development, National Institute of Genetics, Mishima 411-8540 Japan. 3The Graduate University for Advanced Studies (SOKENDAI), Hayama, Kanagawa 240-0193, Japan. 4Department of Forensic Medicine, Graduate School of Biomedical and Health Sciences, Hiroshima University, 1-2-3 Kasumi, Minamiku, Hiroshima 734-8551, Japan. 5Department of Biological Science, Graduate School of Science, Hiroshima University, 1-3-2 Kagamiyama, Higashi-hiroshima 739-8511, Japan. 6Department of Medicine, School of Medicine UC San Diego, 9500 Gilman Drive, La Jolla, California 92039, USA. 7Center for Developmental Biology (CDB), RIKEN Kobe, 2-2-3 Minatojima-minamimachi, Chuo-ku, Kobe 650-0047, Japan. 8Department of Biological Sciences, Graduate School of Science, The University of Tokyo, Hongo 7-3-1, Bunkyo-ku, Tokyo 113-0033, Japan. †Present address: Bristol-Myers K.K. 6-5-1 Nishi-Shinjuku, Shinjuku-ku, Tokyo 163-1328, Japan. Correspondence and requests for materials should be addressed to Y.S. (email: ysaga@nig.ac.jp) or to H.K. (email: hkokubo@hiroshima-u.ac.jp).
Mammalian cardiac development begins with the cardiac crescent, which is composed of a bilateral cardiogenic splanchnic mesoderm, and the progressive differentiation of progenitors into cardiomyocytes to form the primary heart tube. During the successive development of the four-chambered heart, two independent pulmonary and systemic circulatory systems are established. In this process, the production and rearrangement of progenitor cells may account for remodelling of the ancestral heart into these two separate systems. Recent findings pertaining to another cardiac lineage called the secondary heart field (SHF) modified the classical view of cardiac development that the myocardium was derived from a single source. The SHF emerges in the splanchnic mesoderm to supply cells at both the arterial and venous poles of the heart tube, including the right ventricle (RV), most of the atria and outflow tract (OFT)\(^1\)\(^-\)\(^3\). However, the progenitor population that forms the first heart field (FHF), which gives rise to the left ventricle (LV) and the rest of the atria, and the sinus venosus (SV) are still ambiguous.

SHF progenitor cells, which transiently expressIsl1 (Isl1), have been shown to express cardiac markers, including Fibroblast growth factor 10 (Fgf10), Myocyte enhancer factor 2c (Mef2c), T-box transcription factor 1 (Tbx-1), as well as other markers, and contribute to the RV, the OFT and most of the atria\(^1\)\(^-\)\(^2\)\(^,\)\(^3\)\(^-\)\(^9\). FHF progenitor cells have yet to be defined because the FHF lineage, which subsequently gives rise to the LV and a subset of the atria, is recognized only after the expression of differentiation markers such as Myosin light chain 2a (Mlc2a) and NK-2 transcription factor related, locus 5 (Nkx2-5) in the cardiac crescent\(^3\)\(^,\)\(^10\)\(^,\)\(^11\).

The SV is a cavity formed by a thin cardiac muscular wall, which drains blood from systemic veins and the coronary sinus into the right atrium, and gives rise to the sinoatrial node at the joint to the superior cardinal vein during embryogenesis. The origin of the SV has been previously addressed and mapped to the lateral rim of the heart fields in chickens by dye injection experiments\(^12\). Recently, the cell population in the lateral rim of the heart fields, which was defined by the expression of mutG5/hsp68/LacZ in mice, was found to contribute to the SV\(^13\). The venous pole was reported to be the precursor of the SV, which is marked by the expression of Tbx18 from E8.25 (refs 13,14). Lineage-tracing experiments with Tbx18-Cre mice revealed that Tbx18-expressing cells form the SV and proepicardium\(^15\)\(^,\)\(^16\). Thus the origin of the SV has been suggested to be in the caudal lateral regions of the cardiac crescent, although its relation to the FHF or SHF remains unclear.

Wnt signalling is one of the most important regulatory pathways in vertebrate cardiogenesis\(^17\)\(^-\)\(^21\). Wnt antagonism by Dkk1 or Crescent induces heart formation in vertebrates\(^22\)\(^-\)\(^24\), and Dkk1 is required for head induction in mammals\(^25\). Secreted frizzled-related protein (Sfrp) 1 and Sfrp2, which are also Wnt inhibitors, are expressed in the heart and are involved in cardiac tissue repair\(^26\)\(^,\)\(^27\), while the expression pattern and function of another subfamily member, Sfrp5, during cardiac development are unknown.

In this report, we show that Sfrp5 expression is detected in lateral and caudal regions of the cardiac crescent, is expressed in cells distinct from those positive for markers of differentiated cardiomyocytes and the SHF and continues to be expressed in the developing SV. However, descendants of Sfrp5-expressing cells notably contribute to cardiomyocytes in all chambers of the heart, except for the RV. These observations suggest that Sfrp5-expressing cells in the lateral region of the cardiac crescent are progenitor cells for the OFT, the LV, the left and right atria and the SV. Hyperplasia in the inflow tract (IFT) was observed in Sfrp1/2/5 triple knockout (TKO) mouse embryos, suggesting that Sfrp5 regulates the proliferation of cardiac progenitor cells in the venous pole.

**Results**

**Sfrp5 is expressed in progenitor cells of the SV and epicardium.** Wnt signalling is required for myocardial induction and is implicated in several steps of heart development\(^17\)\(^-\)\(^21\). The Sfrp subfamily genes (Sfrp1, 2 and 5) are known to encode secreted decoy receptors for Wnt signalling\(^28\) and to participate in the maintenance of adult cardiac function\(^26\)\(^,\)\(^29\). Among the three Sfrp genes, we found that Sfrp5 was specifically expressed in a particular part of the cardiac crescent at E7.5. Sfrp5 was detected in regions lateral and caudal to the region of Mlc2a and Nkx2-5 expression, which marks differentiating cardiomyocytes (Fig. 1a,b). The expression area of Sfrp5 was clearly distinct from those of Mlc2a and Nkx2-5 (Supplementary Fig. 1). During the heart tube looping stage (E8.0–E8.5), the expression of Sfrp5 was observed continuously in the venous pole of the heart (Fig. 1c–e), the pattern of which was distinct from Mlc2a and Nkx2-5, indicating that the Sfrp5-expressing area excludes differentiating cardiomyocytes. At E9.5, Sfrp5 expression was confined to the ventral venous pole, which is known to contain cardiac progenitors for the SV as well as the epicardium, suggesting that Sfrp5 might be expressed in such progenitors.

To investigate this possibility, we performed double fluorescent immunohistochemistry using Tbx18 and WT1, specific markers for the SV and epicardium, respectively. To monitor the Sfrp5 expression, we used Sfrp5-venusYFP embryos. At E9.5, yellow fluorescent protein (YFP) was detected strongly in the ventral side and weakly in the dorsal side of the venous pole, similar to the pattern of Sfrp5 expression (Fig. 1f). The YFP signal highly overlapped with Tbx18 staining and moderately with WT1 staining (Fig. 1g), suggesting that Sfrp5 is expressed in progenitor cells of the SV and the epicardium.

Cardiomyocytes in the SV show a unique gene expression profile, that is, Troponin T (TnT) and Hcn4-positive but Nkx2-5-negative. This differs from the Nkx2-5- and TnT-positive chamber myocardium\(^30\). To further examine the expression profile of Sfrp5-venusYFP-expressing cells, we conducted marker expression analysis. At E9.5, Sfrp5-venusYFP-expressing cells were negative for Nkx2-5, TnT and Hcn4 (Supplementary Fig. 2a), whereas, at E10.5, these cells expressed TnT and Hcn4 but not Nkx2-5 (Supplementary Fig. 2b). These results indicate that Sfrp5-expressing cells in the venital venous pole at E9.5 represent progenitors for the SV and that they differentiate into cardiomyocytes in the SV at E10.5. Thus the Sfrp5-expressing area at E7.5 may be the precursor of the SV.

**Sfrp5 is expressed in progenitors of cardiac components except for RV.** We next determined whether progenitors of the SV correspond to Sfrp5-expressing cells in the cardiac crescent. To test this possibility, we generated a Sfrp5-Cre line (Supplementary Fig. 3) and investigated the lineages of Sfrp5-expressing cells by crossing this line with Rosa26R or CAG-floxed-CAT-eGFP reporter mice\(^31\). At E8.5, $\beta$-galactosidase (LacZ) staining was unexpectedly observed in the entire heart tube, except for the most rostral portion (Fig. 2a). At E9.5, LacZ staining was observed in the OFT, LV, atria and venous pole but not in the RV (Fig. 2b). Labelled cells overlapped with TnT in the OFT, LV and atria, suggesting that descendants of Sfrp5-expressing cells differentiate into chamber myocardium in addition to the SV (Fig. 2c). At E13.5, LacZ-stained cells mostly occupied not only the LV but were also found in the WT1-expressing pericardium (Fig. 2d,e) and the cluster of differentiation 31 (CD31)- or platelet endothelial cell adhesion molecule-expressing endocardium (Fig. 2f). Labelled cells were found in part of the interventricular septum and rarely in the RV (Fig. 2b,d) and those that were found in the RV tended to localize in the
trabecular myocardium (Fig. 2f). These observations suggest that Sfrp5 is expressed in progenitors of the SV, pericardium, epicardium and endocardium, as well as in all chamber myocardium except for the RV. Given that Isl1 lineages contribute to all myocardium except for the LV,22,23, the fate of Isl1 lineages to the RV or Sfrp5 lineages to the LV may be exclusively determined during the earliest stage of cardiogenesis.

Since Sfrp5 expression is confined to the venous pole of the heart at E8.5, which does not include chamber myocardium, we speculated that cells expressing Sfrp5 prior to E8.5 might include progenitors of the FHF. To evaluate the fate of Sfrp5-expressing cells at specific developmental stages, we generated a tamoxifen-inducible (Estrogen Receptor T2 (ERT2)) Cre mouse line (Supplementary Fig. 3). After crossing Sfrp5-ERT2Cre mice with reporter mice, Sfrp5-expressing cells were labelled at E7.5 via tamoxifen injection and analysed at E10.5. LacZ staining revealed that cells labelled at E7.5 were found in the OFT, LV, atria, SV and venous pole but not in the RV (Fig. 3a,b). Interestingly, labelled cells were still found in the atria at E8.5 and E9.5 (Supplementary Fig. 4), indicating that Sfrp5-expressing cells at the venous pole may supply cardiomyocytes for the IFT. These data suggest that Sfrp5-expressing cells at E7.5 include common progenitors for the SV, OFT and all chamber myocardium except for the RV. There is therefore a strong possibility that Sfrp5 expression marks progenitors of the FHF.

Sfrp5 derivatives contribute to the FHF and part of the SHF lineages. To test this possibility, the expression of Sfrp5 was compared with that of the FHF marker genes, Hcn4 and Tbx5. Hcn4 is expressed in the prospective LV at E7.5, with expression shifting towards the SV at E8.5. Tbx5 is expressed in the FHF lineage at E6.5–E7.5 (refs 35–37). The expression of Hcn4 was observed in the anteromedial region compared with Sfrp5, while region marked by Tbx5 overlapped with and extended anteriorly from that marked by Sfrp5 (Fig. 3c,d). Since the expression of Hcn4 was restricted to the anterior portion of the Tbx5 expression area, we concluded that Hcn4 might mark only the early differentiated FHF lineage. Conversely, Tbx5 marks both undifferentiated and differentiating FHF progenitors, among which Sfrp5 expression is restricted to undifferentiated FHF progenitors existing in the lateral sides of the cardiac crescent. Moreover, cells weakly expressing YFP in the primitive ventricle and IFT were co-distributed with HCN4 in the Sfrp5-venusYFP mouse embryo at E8.5 (Fig. 3e), suggesting that descendants of Sfrp5-expressing cells contribute to the FHF lineage.

Lineage analysis also revealed that Sfrp5-expressing cells at E7.5 contributed to the OFT and atria, indicating that these cells also include progenitors of the SHF. However, at E7.5, the expression pattern of Sfrp5 was distinct from that of Isl1, an SHF marker, although some overlap was found in the expression in the caudal region (Fig. 3c,d). At E9.5, Isl1 transcripts and protein were expressed in the splanchnic mesoderm, including the dorsal venous pole, but were rarely expressed in the ventral venous pole, which contains Sfrp5-expressing cells (Supplementary Figs 5 and 6). To clarify whether the expression of Sfrp5 precedes that of Isl1 in the splanchnic mesoderm, we conducted lineage-tracing analysis using Isl1-Cre and CAG-floxed-CAT-mRFP reporter/Sfrp5-venusYFP mice. We found that the YFP signal in the ventral venous pole rarely overlapped with derivatives of Isl1-expressing cells (Fig. 3f). In Sfrp5-Cre/CAG-floxed-CAT-eGFP-reporter mice, however, GFP-labelled cells expressed Isl1 and Foxf1 in the dorsal splanchnic mesoderm and part of the OFT (Fig. 3g and Supplementary Fig. 7). These findings suggest that derivatives of Sfrp5-expressing cells also contributed to part of the SHF lineage, which may give rise to the OFT and atria. Notably, these GFP-labelled cells were rarely found in the anterior splanchnic mesoderm, where Tbx1, which is known to also be expressed in the SHF,38,39 was distributed (Fig. 3g).

Sfrp5-YFP-expressing ES cells differentiate into cardiac components. We next tested whether Sfrp5-venusYFP-expressing embryonic stem (ES) cells could differentiate into cardiac...
components, such as endothelial, smooth muscle and myocardial cells. On day 2, the peak of YFP expression was found to be ahead of those of other differentiation markers (Supplementary Fig. 8). On day 8, CD31-, β-smooth muscle actin- and TnT-positive cells were found in the cell mass (Supplementary Fig. 8), suggesting that Sfrp5 could also serve as a marker gene of precursor cells for cardiac components in an in vitro differentiation system.

Sfrps may not be involved in cardiac differentiation. Our results imply that Sfrp5 is expressed and retained in cardiac progenitors but lost upon their differentiation, indicating that the loss of Sfrp5 might enhance the differentiation of these cardiac progenitors. To test this possibility, we generated Sfrp1/2/5 TKO mice. As reported previously, only TKO mice show severe growth retardation due to the compensatory functions of Sfrp1 and Sfrp2 (ref. 40). We focussed our analysis on the cardiac phenotype and analysed several lineage markers via in situ hybridization (ISH) at E9.5, the latest stage at which TKO embryos can be collected. Similar to controls, Nkx2-5 was present in the heart tube but not in the venous pole in TKO embryos (Fig. 4a). This indicates that the differentiation of cardiomyocytes occurred normally until this stage. The expression of Tbx5, Isl1 and Tbx18 was normal (Fig. 4a), suggesting that formation of the FHF, SHF and SV was not affected. These observations suggest that Sfrps may not affect differentiation.

Sfrps prevent proliferation by inhibiting canonical Wnt signalling. TKO embryos appeared to have an enlarged IFT, as compared with the OFT. To determine whether Sfrps might regulate the
proliferation of cardiac progenitors, we next assessed whether Wnt/β-catenin signalling and progenitor cell proliferation in the venous pole were affected in TKO embryos. In control embryos, β-catenin accumulated in the ventricles and atria, but less β-catenin was observed in YFP-positive cardiac progenitor cells in the ventral venous pole (Fig. 4b). In TKO embryos, however, β-catenin accumulated even in YFP-positive cardiac progenitor cells (Fig. 4b). In control mice, Ki67 was more highly expressed in YFP-negative cells than in YFP-positive cells, suggesting that proliferation may be inhibited in Sfrp5-expressing cells (Fig. 4c,d). In contrast, the percentage of Ki67-positive cells in TKO embryos increased in YFP-positive cells, as compared with the percentage in control samples (Fig. 4c,d). These results indicate that Sfrps prevent the proliferation of cardiac progenitors by inhibiting canonical Wnt signalling.

Discussion

Based on our findings, we propose a model for the contribution of Sfrp5-expressing cardiac precursors to heart development, except for the RV (Supplementary Fig. 9). We found that Sfrp5-expressing cells at E7.5 contributed not only to the OFT, LV and atria and also to the SV by fate mapping experiments using Sfrp5-Cre and -ERT2Cre mice. This observation gives rise to the possibility that the FHF lineage and the SV might share a common origin. This idea has been implied previously by the expression and lineage-tracing experiments of Hcn4, because Hcn4-expressing cells ultimately yield most of the LV, portions of the atria and the cardiac conduction system30,41–44. We found that the expression of Sfrp5 rarely overlapped with that of Hcn4, while cells weakly expressing YFP in the PV and IFT at E8.5 largely overlapped with Hcn4. The expression of Sfrp5 also...
Ki67 positive (located in the right bottom window, show that TKO embryos with probes for Sfrp5 suggesting that
6
Figure 4 | Sfrp1/2/5 TKO embryos exhibit hyperplasia of the IFT accompanied by activated canonical Wnt signalling. (a) ISH of control and TKO embryos with probes for Nkx2-5, Tbx5, Tbx18 and Isl1 genes. PV: primitive ventricle. (b, c) Double immunohistochemistry using anti-β-catenin (b) or anti-Ki67 (c) antibodies in control and TKO embryos. Rectangles from the left end panels are magnified in the right panels. Further magnified photos, located in the right bottom window, show that β-catenin accumulated in YFP-expressing cells in the ventral venous pole in TKO embryos. (d) Percentage of Ki67 positive (+) cells in Sfrp5-YFP-expressing (+) or negative (−) cells in control and TKO embryos (n = 3). Data are presented as mean ± s.d.; **P<0.01, as compared with control samples. Scale bars = 50 μm (5 μm in dotted rectangles).

largely overlapped with that of Tbx5 at E7.5, and early labelling of Tbx5-expressing cells at E6.5–E7.5 are reported to mark cells that are predominantly restricted to the LV and atria36,37, suggesting that Sfrp5-expressing cells include progenitors for the FHF. Moreover, Sfrp5 was continuously expressed in cells giving rise to the SV, suggesting that cells that transiently express Sfrp5 contribute to the FHF. Sfrp5-expressing cells start to express Tbx18 at around E8.0, and forced expression of Tbx18 reportedly promotes differentiation of cells into the SV, including the conduction system45, and represses a subset of myocardial genes46. Thus Sfrp5-expressing cells could maintain the potential of cells to differentiate into both the SV and FHF until Tbx18 expression initiates.

Alternatively, the early Sfrp5-expressing progenitor pool might already be heterogeneous in the commitment to each differentiation program. Since Tbx5-expressing cells at E6.5–E7.5 would not contribute to the OFT (personal communication from Dr Yashiro), a precise comparison of expression domains and lineages of Sfrp5 and Tbx5 may discriminate Sfrp5-expressing progenitors that contribute to the OFT from those that contribute to other regions in the cardiac crescent. However, the expression domain of Sfrp5 determined by ISH could be more restricted than it actually is at early stages, given the possibility of Cre recombination occurring in Sfrp5-expressing progenitors at levels undetectable by ISH. Thus the timing for cell fate determination and the potential for Sfrp5-expressing progenitor cells to differentiate should be addressed in future.

Another observation was that a portion of Sfrp5-expressing cells later expressed Isl1 and contributed to the OFT and atria (Fig. 5). This suggests that the Isl1-expressing SHF progenitor population has at least two distinct origins, one of which is derived from Sfrp5-expressing cells. We also found that Tbx1 is expressed in the anterior splanchnic mesoderm in which descendants of Sfrp5-expressing cells did not appear, indicating that Tbx1 could mark the subpopulation of Isl1-expressing cells in the SHF. Moreover, lineages of Sfrp5-expressing cells did not contribute to the RV. It was previously reported that mesodermal cells of the first branchial arch are involved in RV formation and that Tbx1 is expressed in the first branchial arch and descendants of Tbx1-expressing cells contribute to the RV38,39,47–49. These findings suggest a distinct origin of the RV relative to that of the other chambers. Given that septated ventricles have been evolutionally acquired to separate systemic and pulmonary circulation50, our results provide new insight into the arrangement of cardiac progenitors before the evolutionary emergence of the RV.

The role of Wnt signalling in cardiac development has been extensively reported51. The hyperactivation of Wnt/β-catenin signalling in cardiac precursors leads to enhanced proliferation and inhibition of further differentiation51,52. In our study,
increased proliferation was observed in TKO embryos, but differentiation into primitive cardiomyocytes was not impaired. We speculate that artificial activation and lack of inhibition of Wnt/β-catenin signalling may result in different outcomes during myocardial differentiation, and a primary role of Wnt/β-catenin signalling in this context may be the regulation of proliferation rather than differentiation. Since Sfrp1/2/5 has been reported to regulate planar cell polarity pathways through the Sfrp1/2/5 signalling in this context may be the regulation of proliferation.

**Methods**

**Mice.** To generate Sfrp5-Cre recombinase (Cre) and Sfrp5-modified ERT2 conjugated with Cre knockout in mouse lines, a strategy similar to that used to generate the Sfrp5-venusYFP mouse line was adopted. Targeting vectors were constructed by using a cassette containing Cre or ERT2Cre recombinase genes with 

frt-PGK-neomycin (neo)-bpA-frt, which was ligated with KpnI-Smal and SpeI-XbaI fragments for long and short homologous arms, respectively. A MC1-DTA-negative selection marker cassette was also added to the outside of the targeting vector. Homologous recombination was then co-transfected with the targeting vector. Homologous recombination was verified by Southern blotting. The authors declared that all data supporting the findings of this study are available within the paper and its Supplementary Information files or available on reasonable request from the corresponding authors.

**References**

1. Moorman, A. F., Christofides, V. M., Anderson, R. H. & van den Hoff, M. J. The heart-forming fields: one or multiple? *Philos. Trans. R. Soc. Lond. B Biol. Sci.* 362, 1257–1265 (2007).

2. Buckingham, M., Meilicke, S. & Zaffran, S. Building the mammalian heart from two sources of myocardial cells. *Nature Rev. Genet.* 6, 826–835 (2005).
3. Abu-Issa, R. & Kirby, M. L. Heart field: from mesoderm to heart tube. *Annu. Rev. Cell Dev. Biol.* 23, 45–68 (2007).

4. Viragh, S. & Challice, C. E. Origin and differentiation of cardiac muscle cells in the mouse. *J. Ultrastr. Res.* 42, 1–24 (1973).

5. Kelly, R. G., Brown, N. A. & Buckingham, M. E. The arterial pole of the mouse heart forms from Fgf10-expressing cells in pharyngeal mesoderm. *Dev. Cell* 1, 435–440 (2001).

6. Mjaatvedt, C. H. et al. The outflow tract of the heart is recruited from a novel heart-forming field. *Dev. Biol.* 238, 97–109 (2001).

7. Cai, C. L. et al. Lsl1 identifies a cardiac progenitor population that proliferates prior to differentiation and contributes a majority of cells to the heart. *Dev. Cell* 5, 877–889 (2003).

8. Sun, Y. et al. Isl1 is expressed in distinct cardiovascular lineages, including pacemaker and coronary vascular cells. *Dev. Biol.* 304, 286–296 (2007).

9. Prall, O. W. et al. An Nkx2.5-8mp2/Smad1 negative feedback loop controls heart progenitor specification and proliferation. *Cell 128*, 947–959 (2007).

10. Snarr, B. S., Kern, C. B. & Wessels, A. Origin and fate of cardiac mesenchyme. *Dev. Dynam.* 237, 2804–2819 (2008).

11. Evans, S. M., Yelon, D., Conlon, F. L. & Kirby, M. L. Myocardial lineage development. *Circ. Res. 107*, 1428–1444 (2010).

12. Stalsberg, H. & DeHaan, R. L. The precardiac areas and formation of the tubular heart in the chick embryo. *Dev. Biol.* 19, 128–159 (1969).

13. Mommersteeg, M. T. et al. The sinus venosus progenitors separate and diversify from the first and second heart fields early in development. *Cardiovasc. Res. 87*, 92–101 (2010).

14. Christoffersen, V. M. et al. Formation of the venous pole of the heart from an Nkx2-5-negative precursor population requires Tbx18. *Circ. Res. 98*, 1555–1563 (2006).

15. Cai, C. L. et al. A myocardial lineage derives from Tbx18 epicardial cells. *Nature 454*, 104–108 (2008).

16. Christoffersen, V. M. et al. Tbx18 and the fate of epicardial progenitors. *Nature 458*, E8–E9 discussion E9–E10 (2009).

17. Brade, T., Manner, J. & Kühhl, M. The role of Wnt signalling in cardiac development and tissue remodelling in the mature heart. *Cardiovasc. Res. 72*, 198–209 (2006).

18. Cohen, E. D., Tian, Y. & Morrissey, E. E. Wnt signalling: an essential regulator of cardiovascular differentiation, morphogenesis and progenitor self-renewal. *Development 135*, 789–798 (2008).

19. Gessert, S. & Kühhl, M. The multiple phases and faces of Wnt signalling during cardiac differentiation and development. *Circ. Res. 107*, 186–199 (2010).

20. Martin, L. K. et al. Canonical Wnt signalling enhances stem cell expression in the developing heart without a corresponding inhibition of cardiogenic differentiation. *Stem Cells Dev. 20*, 1973–1983 (2012).

21. Ueno, S. et al. Biphasic role for Wnt/beta-catenin signalling in cardiac specification in zebrafish and embryonic stem cells. *Proc. Natl Acad. Sci. USA 104*, 9685–9690 (2007).

22. Marvin, M. J., Di Rocco, G., Gardiner, A., Bush, S. M. & Lassar, A. B. Inhibition of Wnt activity induces heart formation from posterior mesoderm. *Genes Dev. 23*, 316–327 (2009).

23. Schneider, V. A. & Mercola, M. Wnt antagonism initiates cardiogenesis in *Xenopus laevis*. *Genes Dev. 15*, 304–315 (2001).

24. Tzahor, E. & Lassar, A. B. Wnt signals from the neural tube block ectopic heart progenitor population that proliferates prior to differentiation and contributes a majority of cells to the heart. *Dev. Cell 5*, 877–889 (2003).

25. Kobayashi, K. et al. Secreted Fizzled-related protein 2 is a procollagen C proteinase enhancer with a role in fibrosis associated with myocardial infarction. *Nature Cell Biol. 11*, 46–55 (2009).

26. Hay, W. et al. Exogenously administered secreted fizzled related protein 2 (Sfrp2) reduces fibrosis and improves cardiac function in a rat model of myocardial infarction. *Proc. Natl Acad. Sci. USA 107*, 21110–21115 (2010).

27. Gekko, K. & Kiyota, R. Secreted antagonists of the Wnt signalling pathway. *J. Cell. Sci. 116*, 2627–2634 (2003).

28. Bergmann, M. W. WNT signalling in adult cardiac hypertrophy and remodeling: lessons learned from cardiac development. *Circ. Res. 107*, 1198–1208 (2010).

29. Liang, X. et al. HCN4 dynamically marks the first heart field and conduction system precursors. *Circ. Res. 113*, 399–407 (2013).

30. Soriano, P. Generalized lacZ expression with the ROSA26 Cre reporter strain. *Nature Genet. 21*, 70–71 (1999).

31. Langguth, K. L., Moretti, A., Caron, L., Nakano, A. & Chien, K. R. Islet1 cardiac progenitors: a single source for heart lineages? *Development 135*, 193–205 (2008).

32. Moretti, A. et al. Multipotent embryonic Isl1+ progenitor cells lead to cardiac, smooth muscle, and endothelial cell diversification. *Cell 127*, 1151–1165 (2006).
Additional information
Supplementary Information accompanies this paper at http://www.nature.com/naturecommunications

Competing financial interests: The authors declare no competing financial interests.

Reprints and permission information is available online at http://npg.nature.com/reprintsandpermissions/

How to cite this article: Fujii, M. et al. Sfrp5 identifies murine cardiac progenitors for all myocardial structures except for the right ventricle. Nat. Commun. 8, 14664 doi: 10.1038/ncomms14664 (2017).

Publisher’s note: Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

This work is licensed under a Creative Commons Attribution 4.0 International License. The images or other third party material in this article are included in the article’s Creative Commons license, unless indicated otherwise in the credit line; if the material is not included under the Creative Commons license, users will need to obtain permission from the license holder to reproduce the material. To view a copy of this license, visit http://creativecommons.org/licenses/by/4.0/

© The Author(s) 2017