

Figure 12 | Abnormalities of the great arteries and dilation of coronary artery branch. **a-e**, Photograph of the hearts at E17.5 from vehicle control (**a**), RA-treated (**b, c**), *Ednra*-null (**d**), and *Edn1*-null (**e**) embryos. **f-j**, Hematoxylin-eosin stained sections of vehicle control (**f**), RA-treated (**g, h**), *Ednra*-null (**i**) and *Edn1*-null (**j**) hearts at E17.5 (black asterisk indicate the enlarged septal branch). Ao, aorta; LV, left ventricle; PA, pulmonary artery; RV, right ventricle. Scale bars, 1 mm (**a-e**), 500 μ m (**f-j**).

the case of NCC-ablated chicks and *Edn1/Ednra*-null mice (Fig. 12i, j)⁹. Fundamentally these coronary abnormalities were not observed in embryos of pregnant mice administered orally with the same dose of RA at 9.5 pdc (Fig. 13d, e) or intraperitoneally with 40 mg/kg bw RA at 8.5 dpc (Fig. 14), indicating that a peak elevation with a narrow time window is critical as seen in the effect of RA on craniofacial development.

The enlarged vessel was further characterized by immunoperoxidase method with anti-SM22 α (also known as transgelin), which is a calponin-related protein serving as a vascular smooth muscle marker. In vehicle control embryos, major coronary branches have the tunica media composed of smooth muscle cells surrounding an endothelial sheet (Fig. 15a). In RA-treated embryos, however, the wall of enlarged septal branches was deficient in smooth muscle layer (Fig. 15b-d). These observations suggest that a disturbance in the formation of the smooth muscle layer may cause the dilatation of coronary arteries in RA-treated embryos, and therefore RA may affect the contribution of preotic NCCs to cardiac development, although another possibility that the defects in the smooth muscle layer might be secondary to vascular enlargement cannot be discarded.

Effects of retinoic acid treatment on the distribution of neural crest cells

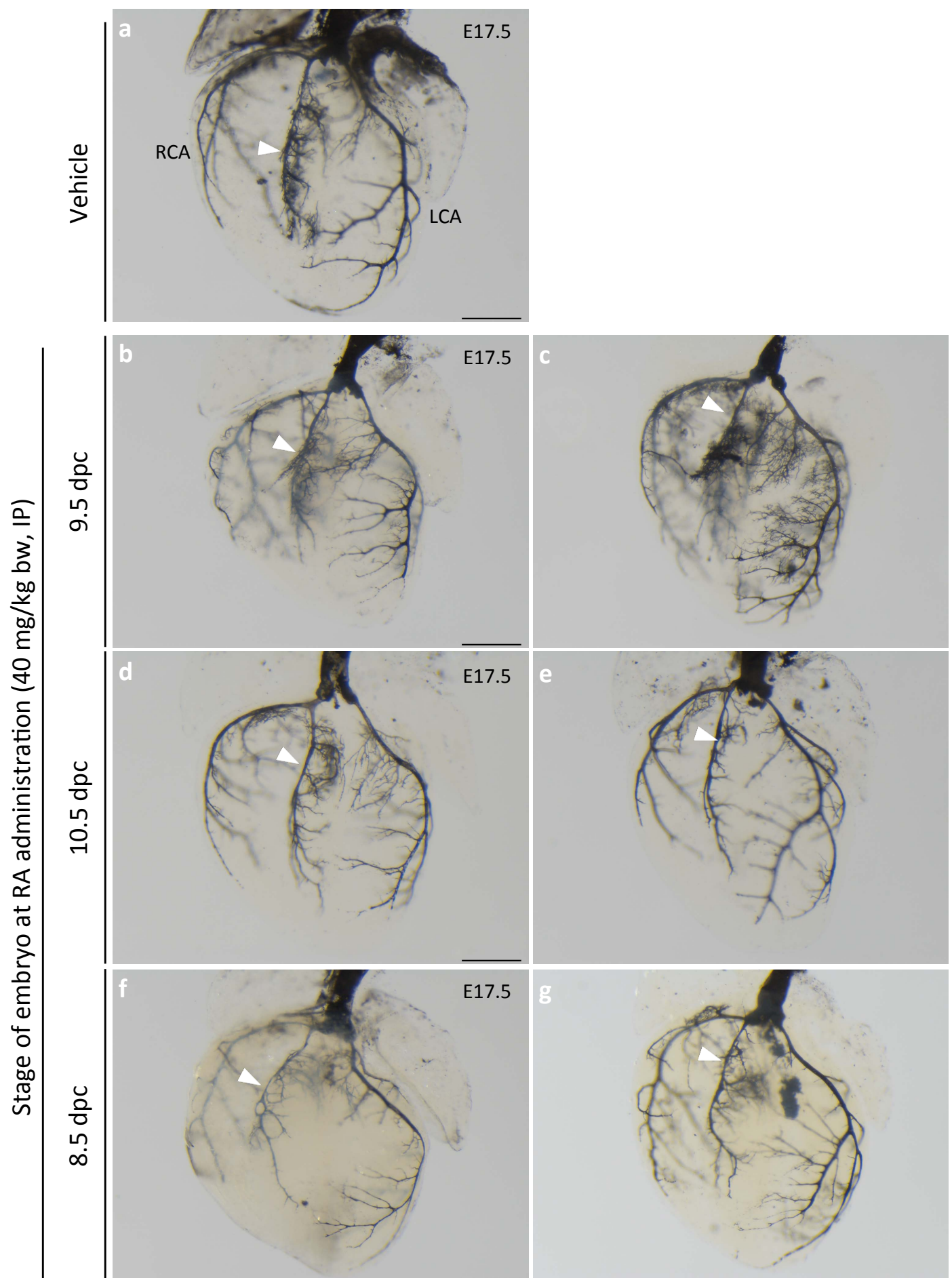


Figure 14 | Coronary orifice abnormalities were not observed by intraperitoneally administration. a-g, Coronary angiography by ink injection in vehicle (a) and RA-treated (b-g) hearts at E17.5 (white arrowheads indicate the septal branches). RA-treated performed at 9.5 (b, c), 10.5 (d, e) or 8.5 dpc (f, g). bw, body weight; dpc, day post-coitum; IP, intraperitoneal administration; LCA, left coronary artery; RCA, right coronary artery. Scale bars, 500 μ m.

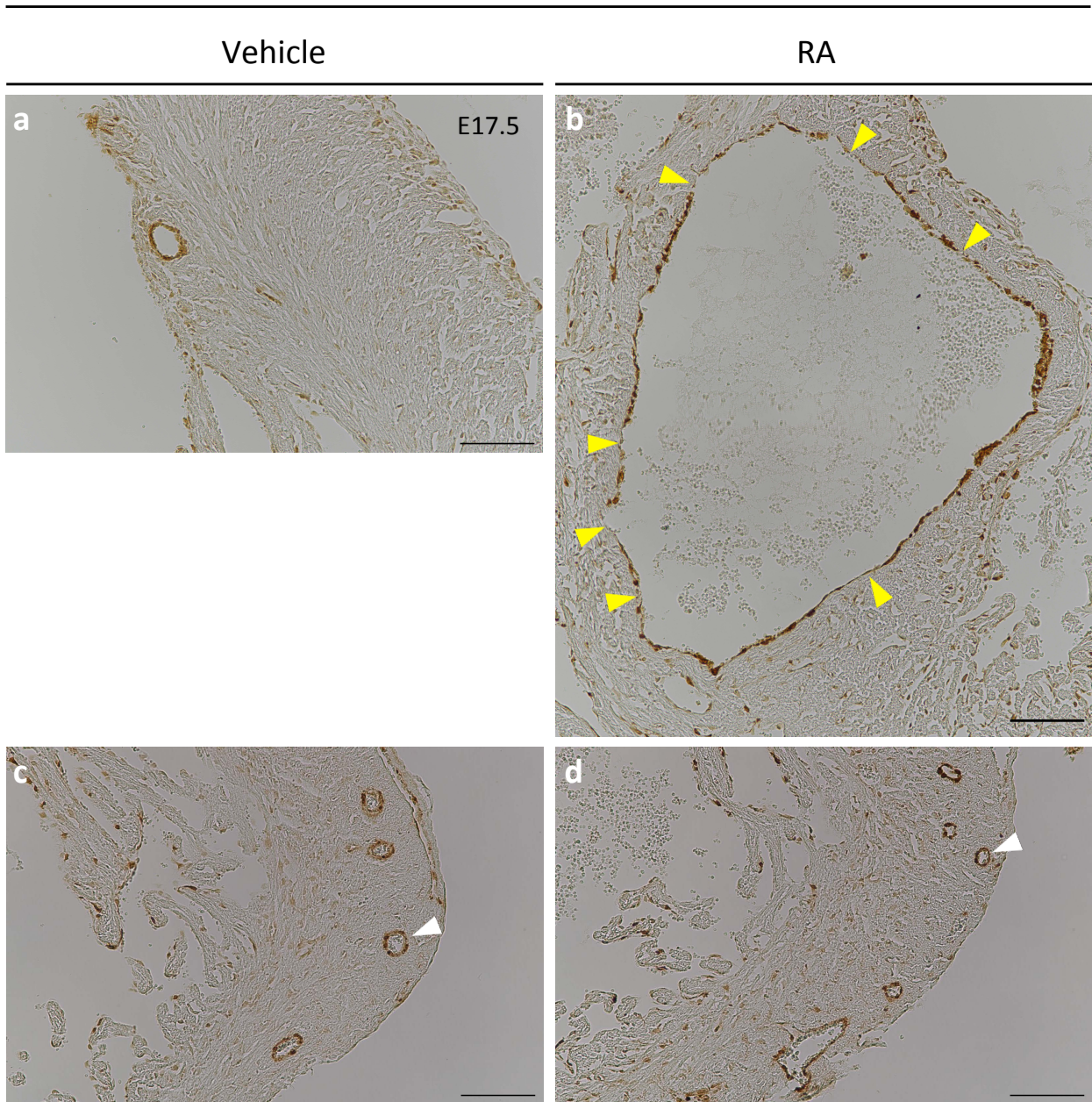


Figure 15 | Enlarged septal branches partially lack smooth muscle cells. a-d, Immunoperoxidase method was performed with anti-SM22 α to detect the smooth muscle cells in vehicle control (**a, c**), RA-treated (**b, d**) hearts at E17.5 (yellow arrowheads indicated discontinuous smooth muscle layers in septal branches). White arrowheads indicate the left coronary artery in the ventricular wall (**c, d**). Scale bars, 100 μ m (**a-d**).

Treatment with RA at 8.5 but not 9.5 dpc resulted in coronary artery malformations similar to those induced by preotic NCC ablation or *Edn1/Ednra* inactivation, indicating that the critical time window seemed to be around the stage of NCC migration much before entering the heart to contribute to coronary artery formation. To examine the effect of RA on NCC migration and resultant distribution, I used *Wnt1-Cre;R26R-lacZ* reporter mice that mark NCCs with permanent β -galactosidase expression. Pregnant mice carrying *Wnt1-Cre;R26R-lacZ* embryos re treated with 70 mg/kg bw RA or vehicle orally at 8.5 dpc and embryos were examined for β -galactosidase expression at E9.5 and E10.5 (24 and 48 hours after treatment). In vehicle-treated control embryos, β -galactosidase-positive NCCs were detected in the outflow tract and extended to the ventricular wall (Fig.16a-f, 17a, b). The longitudinal length of the β -galactosidase-positive region in the outflow tract was shortened and β -galactosidase signals in the ventricular wall were decreased in RA-treated embryos (Fig.16a, b, 17a, b).

To further characterize the distribution of β -galactosidase-positive NCCs around the outflow tract region, I stained sections of RA- or vehicle-treated embryos at E9.5 (24 after treatment) for β -galactosidase activity. I also used *Isl1-Cre;R26R-lacZ* reporter mice that mark cells derived from the second heart field for comparison. In vehicle-treated control embryos, *Wnt1-Cre*-labeled cells were detected along the wall of the aortic sac and arch

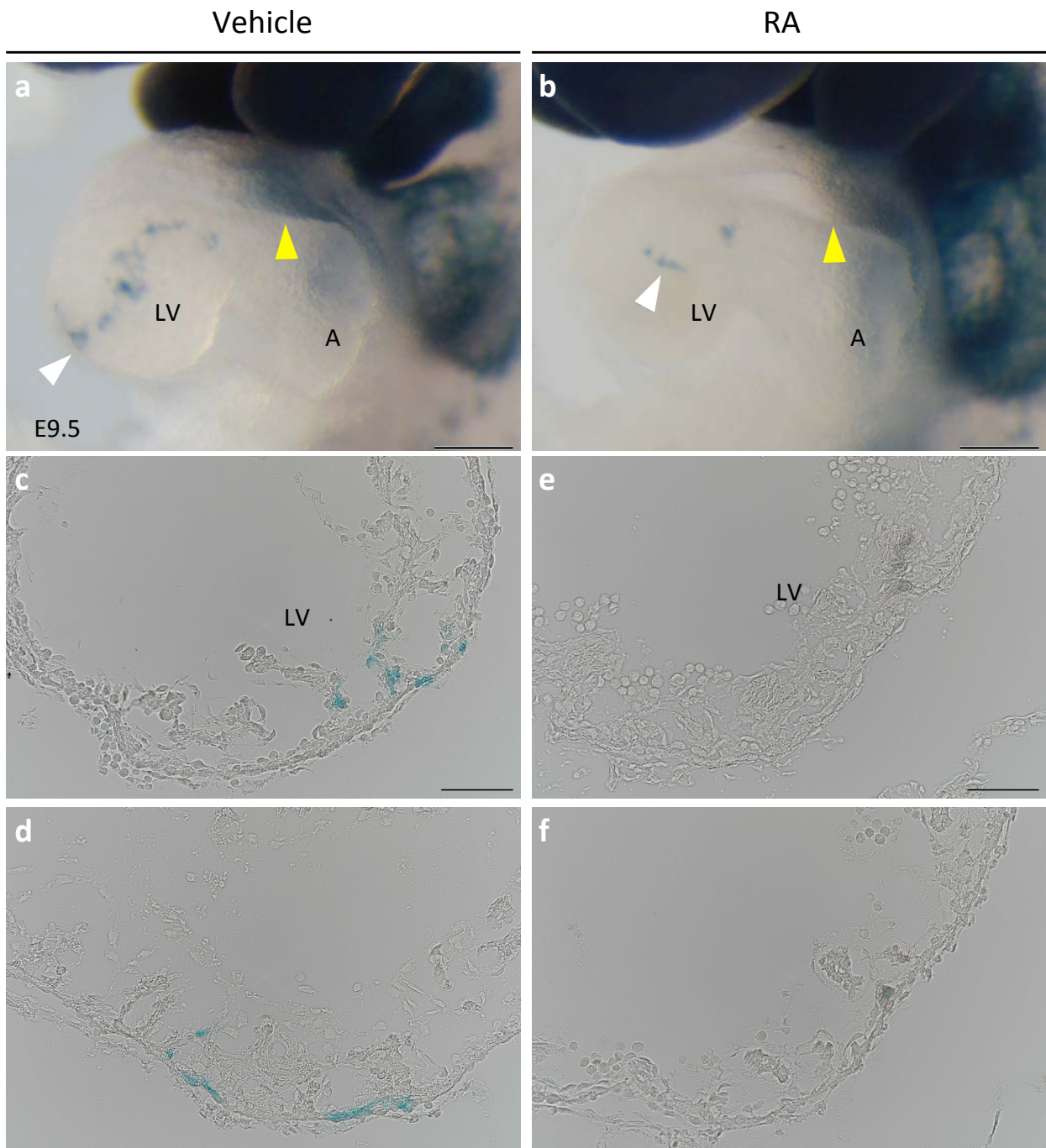


Figure 16 | β -galactosidase signals in the ventricular wall were decreased by retinoic acid administration. **a, b**, Left ventral views of the *Wnt1-Cre;R26R-lacZ* embryos stained β -galactosidase activity at E9.5 (white and yellow arrowheads indicate Cre-labeled populations). **c-f**, β -galactosidase stained sections of left ventricles in vehicle (**c, d**) and RA-treated (**e, f**) embryos. A, atrium; LV, left ventricle. Scale bars, 200 μ m (**a, b**), 50 μ m (**c, e**).

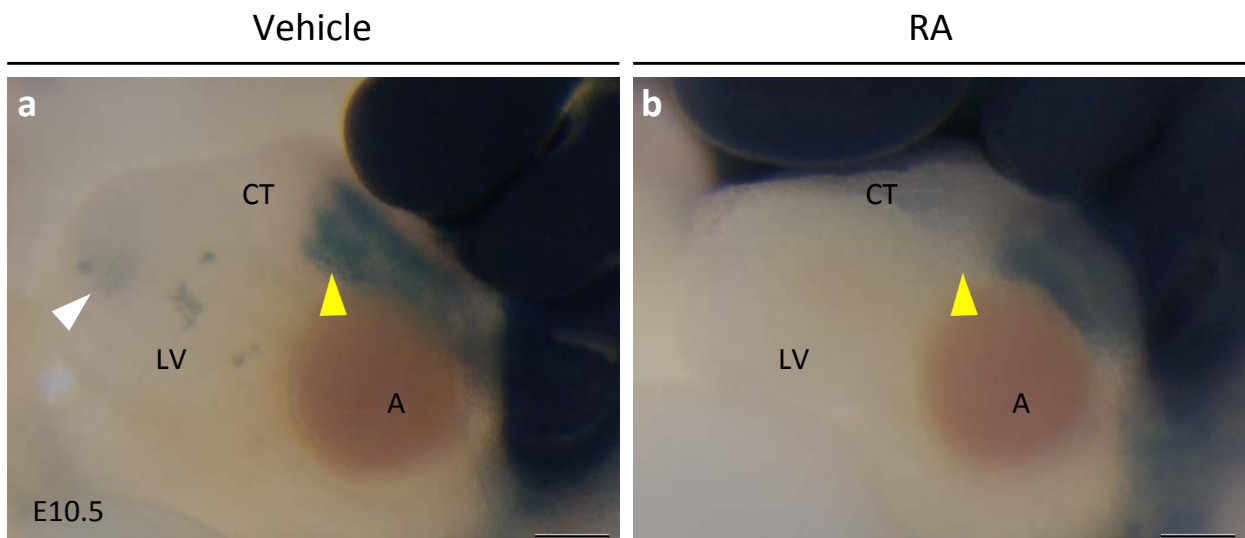


Figure 17 | β -galactosidase-positive region in the outflow tract was shortened by retinoic acid administration. a, b, Left ventral views of the *Wnt1-Cre;R26R-lacZ* embryos stained β -galactosidase activity at E10.5 (white and yellow arrowheads indicate Cre-labeled populations). A, atrium; CT, conotruncus; LV, left ventricle. Scale bars, 200 μ m.

arteries (Fig. 18a). In RA-treated embryos, *Wnt1*-Cre-labeled cells were detected abundantly in the area between the arch arteries and foregut (Fig. 18b), typically at the second pharyngeal arch level, although *Wnt1*-Cre-labeled cells were not prominent in the aortic sac. *Isl1*-Cre-dependent β -galactosidase signals were also detected in the endodermal layer and underlying mesodermal cells in this area, *Isl1*-Cre-labeled cells appeared to be not increased in this area (Fig. 18c, d). Since the second pharyngeal arch is mainly colonized by r4-derived NCCs, which serve as a main preotic NCC population contributing to coronary artery formation, the maldistribution of NCCs in the second pharyngeal arch region may reflect the possibility that RA may cause misdirection of NCC migration at their early migratory stage, leading to a decrease in NCC population migrating into the heart.

Single-cell gene expression analysis of intracardiac neural crest cells

Previous studies have shown that NCCs differentiate into a variety of cell types in the heart. To provide a basis for characterizing such cellular heterogeneity of intracardiac NCCs and understanding their developmental and (patho-)physiological roles, I performed gene expression analysis at a single-cell level. For this purpose, I used *Wnt1*-Cre;*R26R-eYFP* reporter mice, which enabled us to separate NCCs from embryonic tissues by

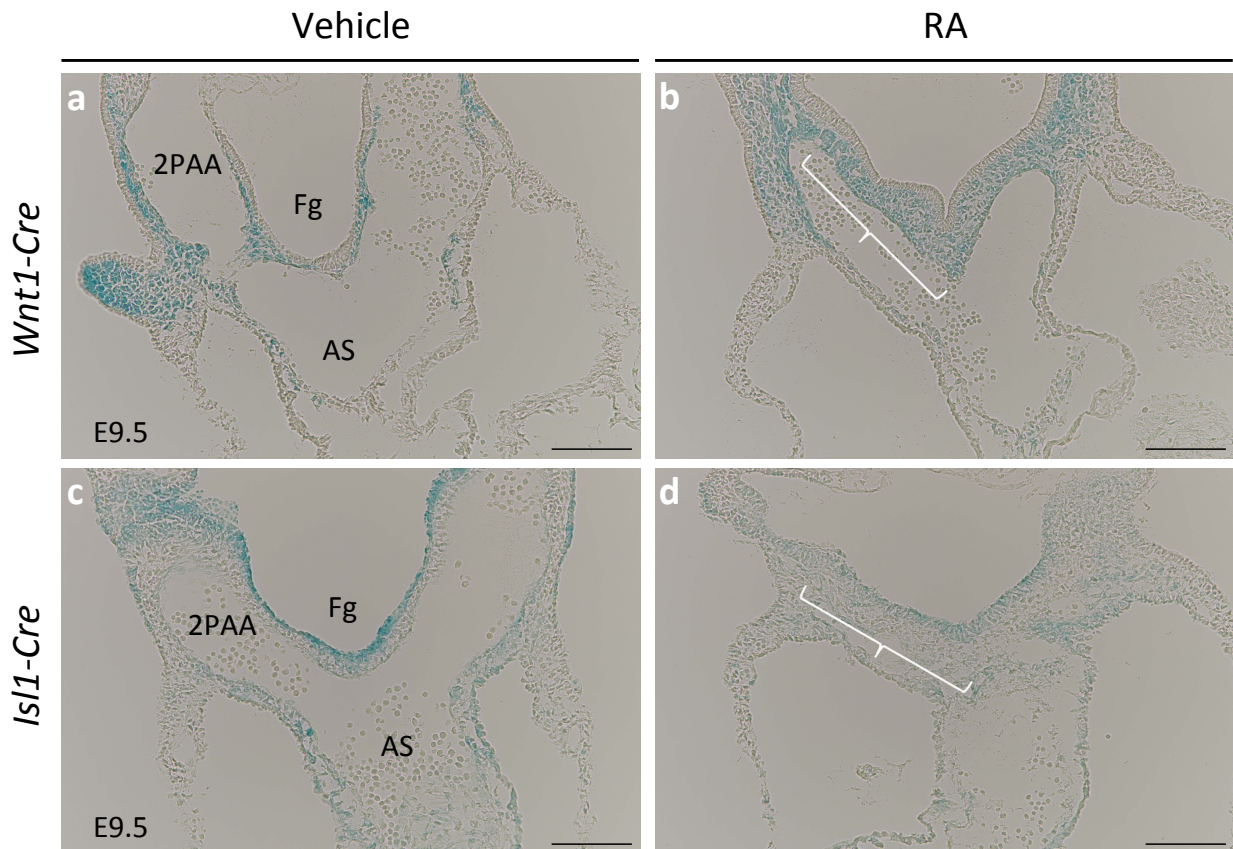


Figure 18 | Neural crest cells were detected abundantly in the area between the arch arteries and foregut. a-d, β -galactosidase stained sections of pharyngeal arch region in *Wnt1-Cre;R26R-lacZ* (**a, b**) and *Isl1-Cre;R26R-lacZ* (**c, d**) embryos at E9.5 (bracketed region indicate the clustered cells). AS, aortic sac; Fg, foregut; 2PAA, second pharyngeal arch artery. Scale bars, 100 μ m.

fluorescence-activated cell sorting (FACS) (Fig. 19a, a'). I isolated EYFP-positive NCCs from the cardiac base region including the semilunar valves of E17.5 *Wnt1-Cre;R26R-eYFP* hearts and applied to the Fluidigm C₁ system (Fig 19b-c'). RNA samples extracted from each of captured cells were subjected to gene expression analysis by reverse transcription and subsequent multiplexed qPCR using a primer set for 96 genes selected as potential markers of differentiated cell types (Table 2).

In my first preliminary trial, two characteristic cell subpopulations emerged (Fig. 20). One exhibited a gene expression profile indicative of smooth muscle cells (5 of 49 cells analyzed). They expressed smooth muscle myosin heavy chain (*Myh11*), *SM22 α* (transgelin; *Tagln*) and angiopoietin1 (*Angpt1*). This population may represent smooth muscle cells of the aorta, pulmonary artery or coronary vessels. In these cells, there were found no other characteristic gene expression patterns such as upregulation of osteoblastic markers such as *Runx2* and *Spp1* (osteopontin). The other subpopulation showed a gene expression profile characteristic of stem/progenitor cells. Ten of 49 cells expressed *Sox10*, a sex-determining region Y-related high mobility group (HMG) transcription factor expressed in undifferentiated NCCs particularly in the lineage of melanocytes and neural cells. Among the *Sox10*-positive cells, 6 cells were positive for *c-Kit*, a stem cell marker. The 6 *Sox10*- and

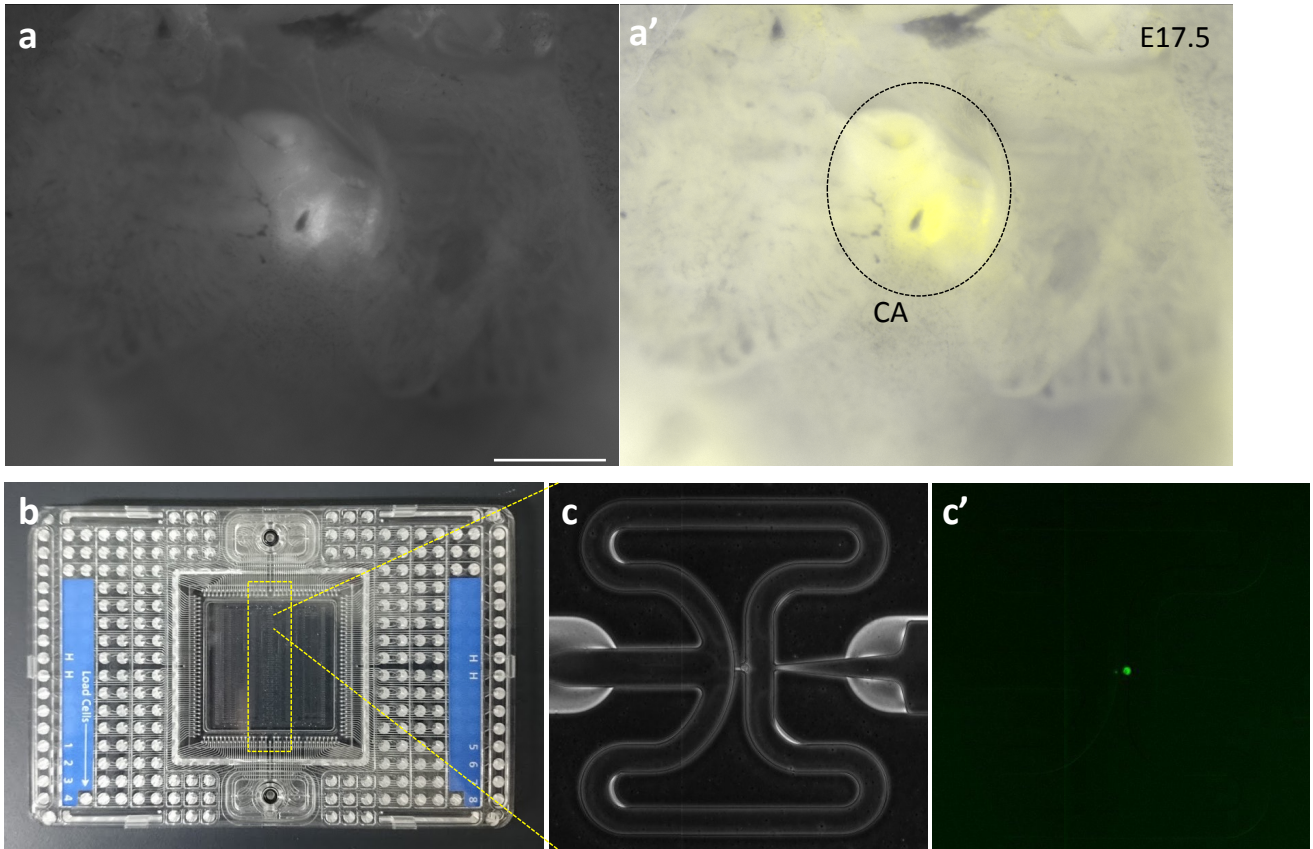


Figure 19 | Single-cell analysis usage. **a, a'**, EYFP-positive neural crest cells isolated from the *Wnt1-Cre;R26R-eYFP* mice embryos at E17.5 (dotted circles in **a'**). **b**, Fluidic circuit plates ($\phi 5-10\ \mu\text{m}$). **c, c'**, Captured EYFP-positive cell. CA, conus arteriosus. Scale bar, $500\ \mu\text{m}$.

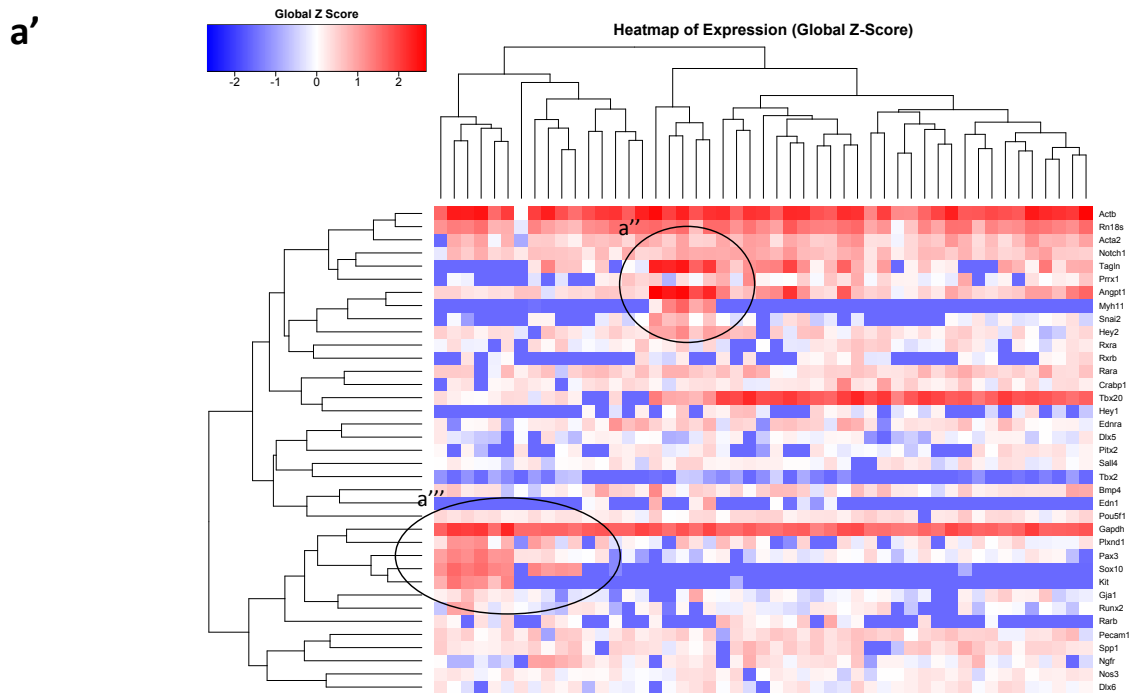
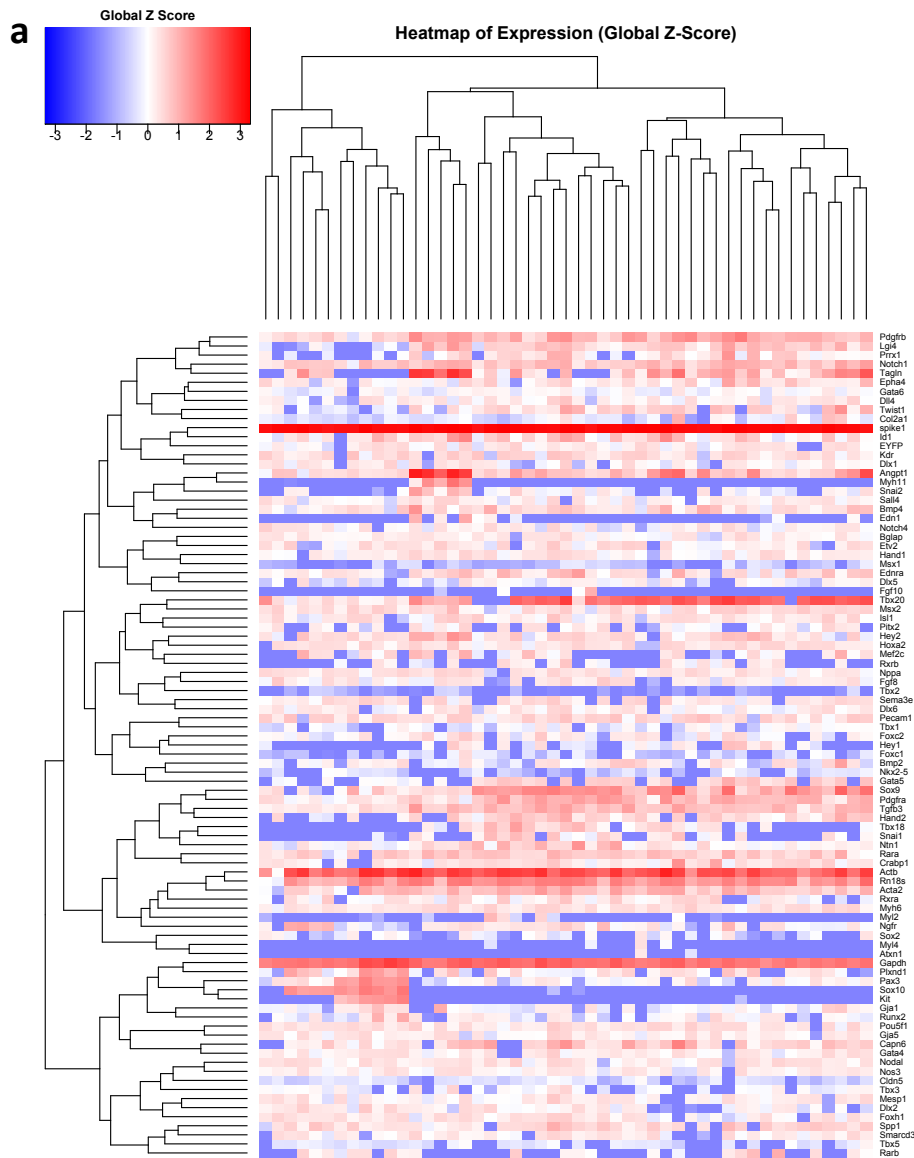


Figure 20 | First trial single-cell analysis reveals the characteristic gene expression. a-a''', Heat map of single-cell gene expression profile (a, a'). Analyzed cells indicated smooth muscle cells markers (a'', *Myh11*, *SM22α* and *Angpt1*) and stem/progenitor cells markers (a''', *c-Kit* and *Sox10*).

c-Kit-positive cells also highly express *Pax3*, a paired-domain-containing transcription factor expressed in NCC progenitors. In the second preliminary trial with some modification of gene primers, only 3 cells were Sox10-positive among 46 analyzable cells (Fig. 21). The 3 cells did not express c-*Kit*, but expressed other genes characteristic of NCC progenitors such as *Foxd3* (a forkhead family transcription factor), *Ngfr* (nerve growth factor receptor) and *Ednrb* (endothelin receptor type-B). Among the total of 95 cells, there were no cells with a profile of cardiomyocytes.

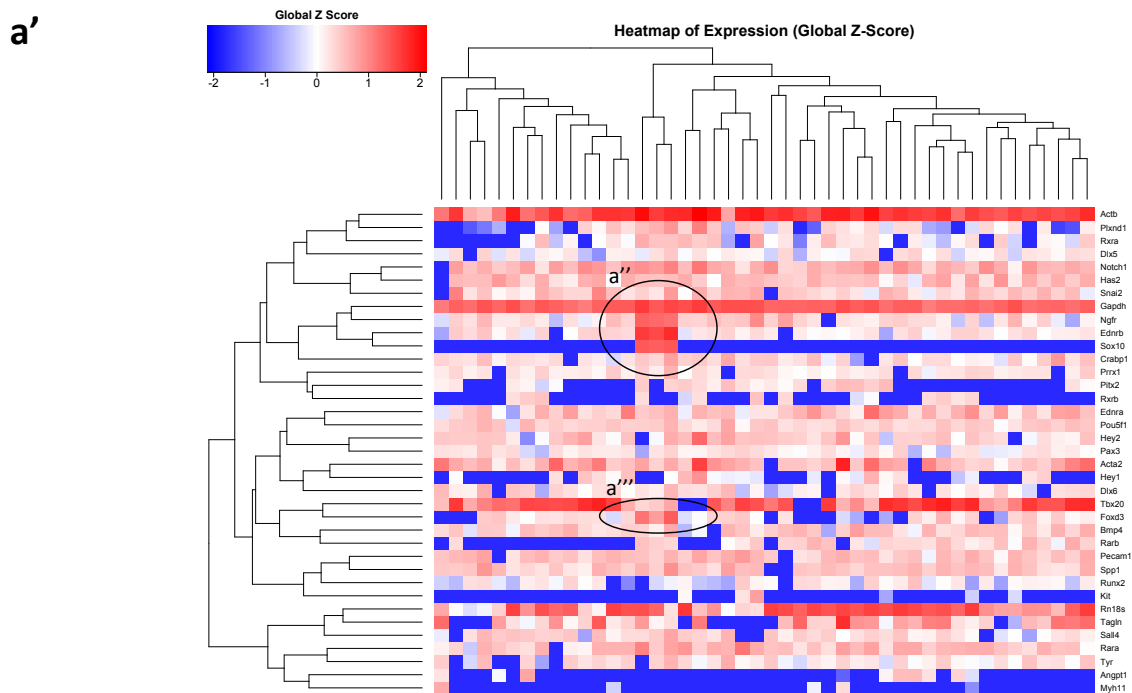
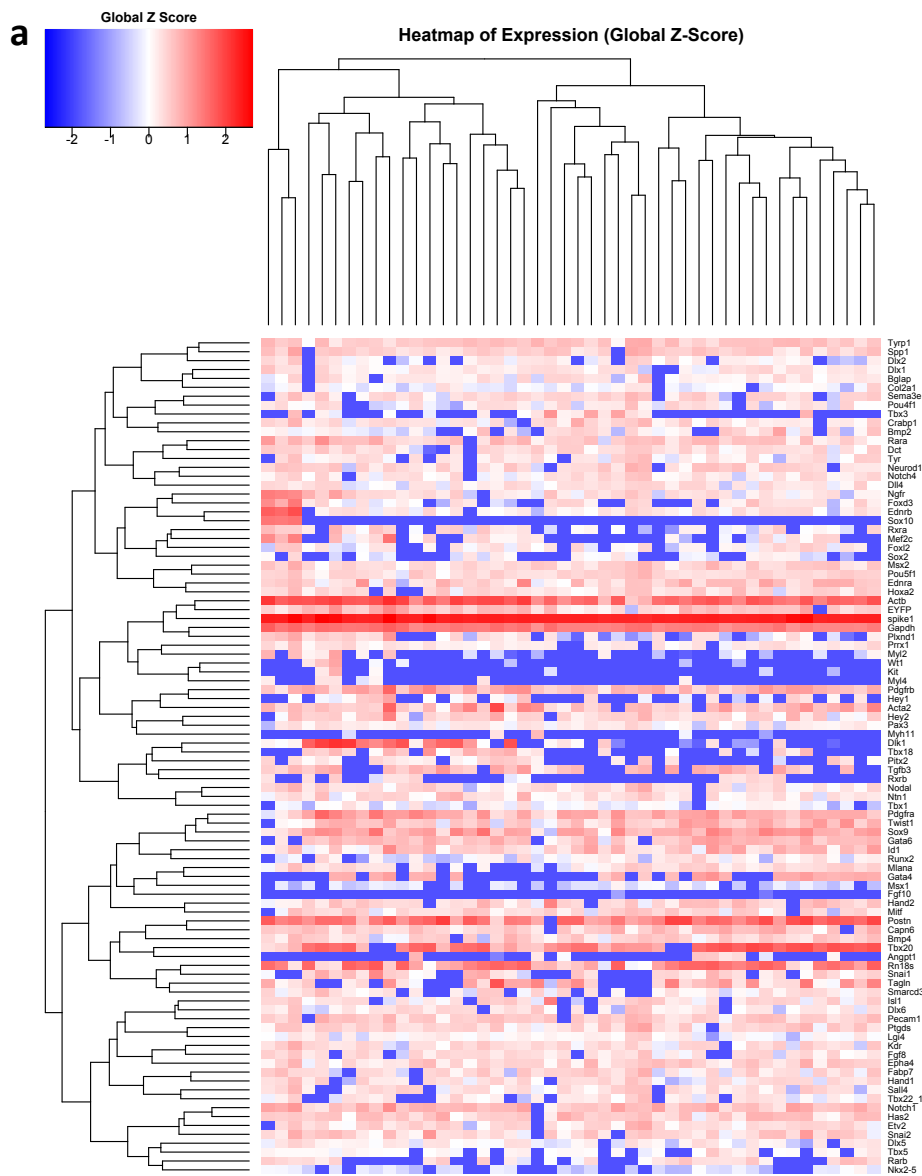


Figure 21 | Second trial single-cell analysis reveals characteristic progenitor markers expression on neural crest cells. a-a''', Heat map of single-cell gene expression profile (**a**, **a'**). Analyzed cells indicated NCC progenitor markers (**a''**, **a'''**, *Foxd3*, *Ngfr*, *Sox10* and *Ednrb*).

Discussion

Intracardiac distribution of preotic neural crest derivatives

In the present study, I first elucidated the broad distribution of preotic NCCs in the heart using a quail-chick chimera technique and compared it to the intracardiac distribution patterns of postotic NCCs. Preotic NCCs were broadly distributed in the heart, especially in the semilunar valves, right ventricular outflow region, interventricular septum and coronary arteries. This distribution pattern is in contrast to that of postotic NCCs, which are predominantly located to the outflow and aorticopulmonary septal regions. The semilunar valves contain both preotic and postotic NCCs, but their distributions were different. These differences may reflect the order of the migration of preotic and postotic NCCs, the former of which migrate in advance and are likely to locate distally in the direction of migration⁹. Previous study has reported the contribution of NCCs to the outflow cushions and their orchestrating role in the remodeling and maturation of the semilunar valves⁵⁹. Although the functional implication of the differences between preotic and postotic NCC distributions in this role remains largely unclear, it may be associated with differences in lineage direction and multipotential characteristics⁵⁸. Indeed, coronary artery smooth muscle cells are derived from preotic but not from postotic NCCs⁹, suggesting possible difference in the

predisposition to differentiation in a certain direction.

Possible involvement of preotic neural crest cells in retinoic acid-induced cardiac anomalies

It has been previously reported that coronary artery abnormalities are often associated with cardiac malformations in the outflow tract⁶⁰⁻⁶². Ratajska et al. described RA-induced outflow malformations such as DORV, TGA and PTA accompanied by coronary anomalies similar to mine present results and argued that the disrupted positioning and septation of the great vessels may affect coronary artery development⁶³. However, our previous findings indicate that NC ablation and inactivation of Edn signaling causes coronary artery abnormalities independent of other obvious cardiac defects such as ventricular septal defect (VSD)⁹. In addition, RA-induced coronary anomalies were preceded by disrupted NCC migration before entering the heart. Considering the substantial contribution of preotic NCCs to the formation of coronary artery smooth muscle layers, the effect on preotic NCC migration at the early migratory stage may be another mechanism underlying RA-induced cardiac abnormalities. Although it remains unsolved whether the effect is direct or indirect, these findings indicate that preotic NCCs destined to the heart are sensitive to RA at early embryonic stages.

In craniofacial development, increased RA signaling causes malformations of NC-derived structures, leading to mandibulofacial deformities and microphthalmia^{49,64,65}. The effects of RA on NCCs appear to be largely indirect through alterations of extracellular signals derived from adjacent cell populations considering the lack of retinoid responsive transgene induction upon RA treatment⁶⁶. Vieux-Rochas et al. have reported that RA treatment reduces the territories of *Fgf8* and *Edn1* expression in the pharyngeal epithelium, leading to craniofacial malformations⁴⁹. The inhibitory effect of RA on *Edn1* expression was also shown in vascular endothelial cells⁶⁷. These findings suggest that the suppression of *Edn1* expression may be involved in RA-induced disruption of NCC migration and coronary artery malformations. In the present study, however, the origin and timing of Edn signaling responsible for NC migration into the heart and the effect of RA on it were not clarified. It requires further investigation to determine whether and how the effect of RA may involve the suppression of Edn signaling.

On the other hand, several reports have demonstrated direct effects of RA on NCC migration and survival. RA and *Pitx2a* cooperatively regulate early cranial NC migration, and excess RA activity inhibits *Pitx2a* expression, causing microphthalmia in zebrafish⁶⁵. In cardiac NC development, RA is shown to inhibit migration by blocking the activation of

c-Jun N-terminal kinase (JNK) signaling⁶⁸. These molecular effects may also underlie the RA-induced abnormalities of NCC development, although further studies are required to clarify the mechanism.

Heterogeneity of intracardiac neural crest cells

The present study also provides a preliminary result of single-cell gene expression analysis showing heterogeneity in intracardiac NCC population. A subpopulation with a gene expression profile of smooth muscle cells may reflect those in the great vessel wall and/or coronary arteries. Further analysis using RNA-sequencing will reveal the characteristics of NC-derived smooth muscle cells and differences between preotic and postotic NC-derived ones. RNA-sequencing may also identify various cell types that are derived from the NC lineage, including neural cells and melanocytes.

The other subpopulation with a gene expression profile of stem/progenitor cells is consistent with previous findings. Several reports have identified melanocytes in the heart, which are likely to be of neural crest origin^{58,69-71}. The combinatory expression of *Sox10* and melanoblast-related genes may reflect this NC subpopulation. Furthermore, a part of Sox10-positive cells also expressed *c-Kit*, indicating a stem/progenitor character. Although

the present result alone cannot define the cardiac NC subpopulations, it may give a clue to future lineage analysis to clarify the mechanisms underlying developmental processes. A recent report has shown that a subpopulation of c-kit-positive cardiac progenitors emerges from the NC with the capacity to give rise to cardiomyocytes⁷². Further analysis will disclose their detailed characteristics and roles in cardiac development, function and disease processes. The present transcriptome analysis on cardiac NCs did not identify cells showing a gene expression profile of cardiomyocytes, which are expected to be in the lineage of c-kit-positive cardiac progenitors⁷². The absence of NC-derived cardiomyocytes in the analyzed population may be due to too low percentage of their existence to be detected in the limited cell number.

Clinical implications

Preotic NCCs mainly migrates into the head and pharyngeal arches, forming craniofacial skeletons through their differentiation into ectomesenchymal lineages, giving rise to chondroblasts and osteoblasts. My serial studies have revealed the contribution of preotic NCCs to various cardiac structures such as the proximal portions of the coronary arteries and semilunar valves. These structures are known to be susceptible to calcification, leading to

coronary artery sclerosis and aortic valve stenosis^{73,74}. Although evidence is still lacking, it is an important future issue to determine whether preotic NCCs with a potential for osteoblast-like differentiation may contribute to the susceptibility to calcification.

The present study also demonstrates that RA treatment can provoke coronary artery abnormalities, which resemble those in preotic NC-ablated chick embryos and *Edn1/Ednra* signal-inactivated mice. RA embryopathy is a disease entity which occurs in babies of pregnant women who took excessive vitamin A or its derivatives during the first weeks of pregnancy^{22,75}. Although this disease is hardly encountered in current practice, it is still an important subject of clinical research as a useful disease model. Moreover, coronary artery anomalies are rare but important diseases, which often require surgical repair⁶¹. Thus, elucidation of the mechanism underlying RA-induced coronary malformations may contribute to the understanding of the pathogenesis of RA embryopathies and congenital diseases involving the coronary artery.

Although the present study provides only limited information about NC-derived cell types within the heart, there are a few points that need to be addressed. First, a subpopulation of smooth muscle cells possibly of the great vessel wall and/or coronary arteries was negative for osteoblastic markers, indicating that the osteoblastic potential of preotic NCCs may not

appear to manifest at the prenatal stage. Serial changes in gene expression patterns throughout life is expected to reveal the implication of cellular origins in above-mentioned pathogenesis. Second, some cells showed a gene expression profile compatible to melanoblasts. The existence of melanocytes in the heart and their possible involvement in arrhythmogenesis has been reported⁶⁹. Analysis of their detailed gene expression profile and exact intracardiac location will give a clue to their further clinical implications. Finally, c-kit-positive cardiac progenitors may play a role in post-injury cardiac repair and regeneration. The existence of stem/progenitor cell population in the heart has been widely accepted since the discovery and extensively studied as a potential source of cardiac regeneration^{76,77}. Although NC-derived cardiomyocytes were not detected in the present experiment, gene expression analysis after myocardial injury may reveal the pathophysiological role of this NC-derived stem/progenitor population. Recently, Kramann et al. reported the existence of undifferentiated progenitors in the vascular wall, which may drive vascular calcification⁷⁸. NC-derived stem/progenitor population with a potential for osteoblast-like differentiation, if there are, may play a similar role in the calcification of the coronary artery and semilunar valves.

Conclusion

- Quail-chick chimera experiments revealed the contribution of NCCs derived from r4 and more cranial region to the coronary artery smooth muscle layer.
- Preotic NCCs contributed to semilunar valve formation with a distribution different from that of postotic NCCs. Comparison between mouse and chick embryos suggested possible species differences in distribution patterns.
- Treatment of E8.5 mouse embryos with RA, a negative regulator of Edn signaling, caused coronary artery branching abnormalities and septal branch dilatation with defective smooth muscle integrity, as observed in cranial NC ablation in chick and blockade of Edn signaling.
- RA treatment at E8.5 disturbed preotic NCC migration before entering the heart, which might be primarily responsible for coronary artery malformation.
- Single-cell transcriptome analysis of NCCs in the mouse E17.5 heart revealed heterogeneity of intracardiac NCCs with two distinct subpopulations; i) Myh11- and SM22 α -positive vascular smooth muscle-like cells and ii) undefined cells with stem/progenitor cell markers Sox10- and c-Kit.
- Overall, the present findings will serve as a basis for understanding the distinctive roles

of preotic NCCs in cardiac development, function and disease processes such as in coronary artery and semilunar valve diseases.

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Table 1 | Primers used for genotyping.

| | Forward (5'-3') | Reverse (5'-3') |
|--------------------|-----------------------------|--------------------------------|
| <i>Cre</i> | ACATGTTCAGGGATCGCCAG | TAACCAGTGAAACAGCATTGC |
| <i>Edn1</i> -null | TGCGCTGCTAGATCTGACCTG | ACTGCCTTGGGAAAAGCGCCTC |
| | GCAGATAAACTGCGCGCGATCTGT | |
| <i>Edn1</i> WT | TGCGCTGCTAGATCTGACCTG | CCAGTAGTAAGAGATGGCTAG |
| | GCAGATAAACTGCGCGCGATCTGT | |
| <i>Ednra</i> -EGFP | GACGTAAACGGCCACAAGTTCA | GAACTCCAGCAGGACCATGTGAT |
| <i>Ednra</i> WT | AGTATCTTTTGCCTTGCGGCATAC | CAGAGCACATCAGAGCAAGCGTCA |
| <i>lacZ</i> | GAAGTTCAGATGTGCGGCGAGTTGCGT | CCGCACCTCGCGGAAACCGACATCGCAGGC |
| <i>R26R</i> -eYFP | AAAGTCGCTCTGAGTTGTTAT | AAGACCGCGAAGAGTTTGTC |
| <i>R26R</i> WT | AAAGTCGCTCTGAGTTGTTAT | GGAGCGGGAGAAATGGATATG |

WT, wild-type.

Table 2 | Primers list using single-cell analysis.

| | |
|-----------------------|---------------------------|
| <i>Acta2</i> .F | CGGGCATCCACGAAACCA |
| <i>Acta2</i> .R | CCCTGACAGGACGTTGTTAG |
| <i>Actb</i> .F | GCGAGCACAGCTTCTTTGC |
| <i>Actb</i> .R | TCGTCATCCATGGCGAACT |
| <i>Angpt1</i> .F | GCAGAAGCAACAACCTGGAGC |
| <i>Angpt1</i> .R | TCCTCCCTTTAGCAAAACACCT |
| <i>Atxn1</i> .F | GCCGTGATACAGTTTGCTGTT |
| <i>Atxn1</i> .R | TGACCTGTATGACCCTGGTGAG |
| <i>Bglap</i> .F | AGCAGACACCATGAGGACCA |
| <i>Bglap</i> .R | GAGGTCAGAGAGACAGAGCG |
| <i>Bmp2</i> .F | GCATGTTTGGCCTGAAGCA |
| <i>Bmp2</i> .R | CCTGCGGTACAGATCTAGCA |
| <i>Bmp4</i> .F | GCCAAACGTAGTCCCAAGCAT |
| <i>Bmp4</i> .R | AATGGCGACGGCAGTTCTT |
| <i>Capn6</i> .F | CATCAACGGAGATCTGGTCTTCT |
| <i>Capn6</i> .R | GATGGTCAGACCATCCAAAGC |
| <i>Cdh5</i> .F | CAACTTCACCCTCATAAACAACCAT |
| <i>Cdh5</i> .R | ACTTGGCATGCTCCCGATT |
| <i>Cldn5</i> .F | CCAATGGCGATTACGACAAGA |
| <i>Cldn5</i> .R | GGCTTAGCTGCGGGAAGAG |
| <i>Col2a1</i> .F | GGACGCTACACTCAAGTCACTGA |
| <i>Col2a1</i> .R | CAAGTGCGAGCAGGGTTCTT |
| <i>Crabp1</i> .F | CGCACCACGGAGATCAACT |
| <i>Crabp1</i> .R | TTGCGTCCGTCCACTGTCT |
| <i>Dct</i> .F | TTGGTCCACAACCTCTCTCAG |
| <i>Dct</i> .R | GAAGAAAAGCCAGCAACCC |
| <i>Dkk1</i> .F | CCCCGGGAACACTACTGCAAAA |
| <i>Dkk1</i> .R | TTCCCCTCGAGGAAAATGGC |
| <i>Dlk1</i> .F | TTCGGCCACAGCACCTATG |
| <i>Dlk1</i> .R | TGGCACCTGCAGACATTGTC |
| <i>Dll4</i> .F | AGTGCCTGAACAGAGGTCCAA |
| <i>Dll4</i> .R | ATCGTGATGTGCAGTTCACA |
| <i>Dlx1</i> .F | CTGGGCTACCCCTACGTCAA |
| <i>Dlx1</i> .R | TAGGACTGCACGGAACCTGATGT |
| <i>Dlx2</i> .F | TCTGGGAAACTACCCGTGGTA |
| <i>Dlx2</i> .R | GTCTGCGAAGGATGCAGAAGT |
| <i>Dlx5</i> .F | GGCTACTGCTCTCCTACCTCTG |
| <i>Dlx5</i> .R | CTGGTAGGGGTTGAGCGCTT |
| <i>Dlx5</i> .F nested | CACGGCTACTGCTCTCCTACCT |
| <i>Dlx5</i> .R nested | CGTTCACGCCGTGGTACTG |
| <i>Dlx6</i> .F | TCCAGGCTTTAAACCATCGCT |
| <i>Dlx6</i> .R | TAAGGAAGCAGCCAGTTCGG |
| <i>Edn1</i> .F | CACAGACCAGGCAGTTAGATG |
| <i>Edn1</i> .R | GCTTGGCAGAAATTCCAGCACTT |
| <i>Edn1</i> .F nested | GGCCACAGACCAGGCAGTT |
| <i>Edn1</i> .R nested | GGTACTTTGGGCCCTGAGTTC |

| | |
|----------------|----------------------------|
| <i>Ednra.F</i> | CTGTTCCCCTTCCTGCAGAA |
| <i>Ednra.R</i> | CACACTGAGAGCACAGAGGTTCA |
| <i>Ednrb.F</i> | CTCCACGCTGCTAAGAATCATCT |
| <i>Ednrb.R</i> | AGAGCCAGACTGGCGATCA |
| <i>Epha4.F</i> | ACACCGATCCGAACCTACCA |
| <i>Epha4.R</i> | AGTCAGTTCGCAGCCAGTTGT |
| <i>Etv2.F</i> | CAAGAGGAAGCCGGGAATGA |
| <i>Etv2.R</i> | CCACCACTCTTGAGCACGAT |
| <i>EYFP.F</i> | CTGCTGCCCCGACAACCAC |
| <i>EYFP.R</i> | TCACGAACTCCAGCAGGAC |
| <i>Fabp7.F</i> | GTTGGCACCTGTAGAGTTTTA |
| <i>Fabp7.R</i> | GACATTCACAGCAATCCCAT |
| <i>Fgf10.F</i> | AGAAGAACGGCAAGGTCAGC |
| <i>Fgf10.R</i> | CGGCAACAACCTCCGATTTCC |
| <i>Fgf8.F</i> | GCTCATTGTGGAGACCGATACTT |
| <i>Fgf8.R</i> | CCTTCTTGTTTCATGCAGATGTAGAG |
| <i>Foxa2.F</i> | GTGAAGATGGAAGGGCACGA |
| <i>Foxa2.R</i> | TTGCTCACGGAAGAGTAGCC |
| <i>Foxc1.F</i> | CAACATCATGACGTCGCTGC |
| <i>Foxc1.R</i> | AGGAGGCCCGGAACCGAG |
| <i>Foxc2.F</i> | GGGACCCCGGTAGCTGA |
| <i>Foxc2.R</i> | CCTCGCTCTTAACCACGACTT |
| <i>Foxd3.F</i> | CGCTGGGAATAACTTTCCGT |
| <i>Foxd3.R</i> | CCCACAGTACTGAGAACACA |
| <i>Foxh1.F</i> | TATCCGTCAGGTCCAGGCAG |
| <i>Foxh1.R</i> | AAAGGTTGTGGCGGATGGAG |
| <i>Foxl2.F</i> | GCTCACTCTGTCCGGCATCT |
| <i>Foxl2.R</i> | ACCTTGATGAAGCACTCGTTGA |
| <i>Gapdh.F</i> | GCCTACATGGCCTCCAAGG |
| <i>Gapdh.R</i> | GAGTTGGGATAGGGCCTCTCTT |
| <i>Gata4.F</i> | GTCCTCATCACCCACAGAGC |
| <i>Gata4.R</i> | GTAAGGTTTAGGGGTGGGGC |
| <i>Gata5.F</i> | GCTCTGTGGAGACCCAGTCTA |
| <i>Gata5.R</i> | GCAGAGATCGCCAGCCAAAG |
| <i>Gata6.F</i> | GCAAGATGAATGGCCTCAGC |
| <i>Gata6.R</i> | TGACAGTTGGCACAGGACAG |
| <i>Gja1.F</i> | GATCGCGTGAAGGGAAGAAG |
| <i>Gja1.R</i> | GGAGATCCGCAGTCTTTGGA |
| <i>Gja5.F</i> | CAGCCTGGCTGAACTCTACCA |
| <i>Gja5.R</i> | CACACCCTGCCGTGACTTG |
| <i>Hand1.F</i> | TGAACCTCGTGGGCAGCTA |
| <i>Hand1.R</i> | GGAAGGGTTCGTGGAGCAT |
| <i>Hand2.F</i> | GCCGACACCAAACTCTCCAA |
| <i>Hand2.R</i> | TCTCCGTTCTGGTCGTCTT |
| <i>Has2.F</i> | ACAAAGAGGTTTCGTTCAAGTTC |
| <i>Has2.R</i> | GTGTGTTTGTTCCTTAGC |
| <i>Hey1.F</i> | CCCCAACGACATCGTCCCA |
| <i>Hey1.R</i> | CGCTTCTCGATGATGCCTCT |

| | |
|----------------------|--------------------------|
| <i>Hey1.F nested</i> | TGTTCCATGTCCCCAACGA |
| <i>Hey1.R nested</i> | GTCGGCGCTTCTCGATGAT |
| <i>Hey2.F</i> | ATGTCGCCTATCCACATCTTCA |
| <i>Hey2.R</i> | TCCGAAGAGCAGAATCTGCAT |
| <i>Hoxa2.F</i> | TCACCCACTGTTCTAACTGCTT |
| <i>Hoxa2.R</i> | GCCTCGGGACTGTCATTGTT |
| <i>Id1.F</i> | CTGCTACTCACGCCTCAAGGA |
| <i>Id1.R</i> | CAGGATCTCCACCTTGCTCACT |
| <i>Isl1.F</i> | CTGGCAGTGAAGTAGCATCGAT |
| <i>Isl1.R</i> | GCCTCAATAGGACTGGCTACCAT |
| <i>Kdr.F</i> | GCCCTGCTGTGGTCTCACTAC |
| <i>Kdr.R</i> | CAAAGCATTGCCATTTCGAT |
| <i>Kit.F</i> | GTGACGGTACATGGCTGCAT |
| <i>Kit.R</i> | GGCACAGACACAACAGGGATAG |
| <i>Lgi4.F</i> | TGGCTTCTCCTCTTAGCCCT |
| <i>Lgi4.R</i> | TTGGGGAGAGTGGGAAGGAT |
| <i>Mef2c.F</i> | GAGCCGGACAAACTCAGACA |
| <i>Mef2c.R</i> | CTGAATCGTCTGCATCGGGA |
| <i>Mesp1.F</i> | TGGCCATAGGTGCCTGACTT |
| <i>Mesp1.R</i> | GACCCCTTAGTCCAGTCTGC |
| <i>Mitf.F</i> | CCTGGGTGCAGAATTGTATC |
| <i>Mitf.R</i> | CGTTACCTTACCCAGAGGC |
| <i>Mlana.F</i> | CCCTGAGAGCCGGGA |
| <i>Mlana.R</i> | CTAAAGCGAAACACCGGC |
| <i>Msx1.F</i> | ATGACTTCTTTGCCACTCGG |
| <i>Msx1.R</i> | ATCTGTGCCCATGGCGGT |
| <i>Msx2.F</i> | CGCACACCCTTCACCACAT |
| <i>Msx2.R</i> | CGCTCTGCTATGGACAGGTACTG |
| <i>Myh11.F</i> | CACAAGGGCAAGAAAGACAGC |
| <i>Myh11.R</i> | GAAAGCCTCCAGGATTGGGT |
| <i>Myh6.F</i> | CAGAAGCCTCGCAATGTCAA |
| <i>Myh6.R</i> | TAGTCCACGGTGCCAGCAT |
| <i>Myl2.F</i> | CAGACACCATGGCACCAAAG |
| <i>Myl2.R</i> | TGGGTCTGCTCAACATGGA |
| <i>Myl4.F</i> | CAGTGCTGACCAGATCGAAGAA |
| <i>Myl4.R</i> | GCCCGTAGGTGATCTTCATCTC |
| <i>Myocd.F</i> | CATCAGCCTGCCTCCATCA |
| <i>Myocd.R</i> | TGAAGAGACCCCATGGTTCTCT |
| <i>Neurod1.F</i> | GCTGTTTGAGATGTGATGCT |
| <i>Neurod1.R</i> | GCATTACACCATAATACACAGGA |
| <i>Ngfr.F</i> | CGTGACCATCTCAGGCCTTT |
| <i>Ngfr.R</i> | GGTGCCCCTGTTACCTTCTC |
| <i>Nkx2-5.F</i> | CCGATCCATCCCACTTTATTGA |
| <i>Nkx2-5.R</i> | CCTAGTGTGGAATCCGTCGAA |
| <i>Nodal.F</i> | GCCGACATCATTTGCCAGAC |
| <i>Nodal.R</i> | CCCCAGCCAATCAGGTTGAA |
| <i>Nos3.F</i> | GCTCTCACCTACTTCCTGGACATC |
| <i>Nos3.R</i> | GCTGTTCGCTGGACTCTTCTG |

| | |
|------------------|----------------------------|
| <i>Notch1.F</i> | ACGTGCTGCACACCAACGT |
| <i>Notch1.R</i> | AGCTCTTCCTCGTGGCCATAG |
| <i>Notch4.F</i> | GCATCCCAGCCTATGACCAGTA |
| <i>Notch4.R</i> | GCGTTATTGCAGCCTTTCTCA |
| <i>Nppa.F</i> | GCTAGACCACCTGGAGGAGAAGA |
| <i>Nppa.R</i> | GCTTCCTCAGTCTGCTCACTCA |
| <i>Ntn1.F</i> | GGATCAGCCAGCTTTGGTCT |
| <i>Ntn1.R</i> | TCCCTCCCTACAGATGGTG |
| <i>Pax3.F</i> | AACCCAAGCAGGTGACAACG |
| <i>Pax3.R</i> | CATGCCCCGGTTCTCTCTTT |
| <i>Pdgfra.F</i> | TGTCACAGTGCTGGAAGTGTT |
| <i>Pdgfra.R</i> | CATCCGTCTGAGTGTGGTTGTAG |
| <i>Pdgfrb.F</i> | CTGTGAATGCCGTGCAGACT |
| <i>Pdgfrb.R</i> | TGGAAGTTCACCACATCATTGC |
| <i>Pecam1.F</i> | GAGCCCAATCACGTTTCAGTTT |
| <i>Pecam1.R</i> | TCCTTCCTGCTTCTGCTAGCT |
| <i>Pitx2.F</i> | GGGTGTCTGGAAGTCTGAGCTG |
| <i>Pitx2.R</i> | CCTCCGAGCACACCCTCC |
| <i>Plxnd1.F</i> | ACCTCACCTTCTGGTACATGCA |
| <i>Plxnd1.R</i> | GTTCCGCCAGCCACAGTGAT |
| <i>Postn.F</i> | TCATTGAAGGTGGCGATGGT |
| <i>Postn.R</i> | TCTTTGCAGGTGTGTCTCCC |
| <i>Pou4f1.F</i> | CTGGGCTCTGATTCTGTGAT |
| <i>Pou4f1.R</i> | AGTGCACTCAACGCATATTT |
| <i>Pou5f1.F</i> | GATGTGGTTCGAGTATGGTTCTGT |
| <i>Pou5f1.R</i> | TTCTCGTTGGGAATACTCAATACTTG |
| <i>Prrx1.F</i> | TCTGATGTTGGCAAAGGGGTT |
| <i>Prrx1.R</i> | TCCTACCTCCTTTTCCTCGG |
| <i>Ptgds.F</i> | GGTGAGAGAAAGTCAGTCAGAG |
| <i>Ptgds.R</i> | TCTTGAGAGTGACAGAGCAAA |
| <i>Rara.F</i> | CGCCTGAGCAAGACACAATG |
| <i>Rara.R</i> | AGCCAGCGTTGTGCATCTG |
| <i>Rarb.F</i> | AAATCACAGATCTCCGCAGCAT |
| <i>Rarb.R</i> | GGTGGCATTGATCCAGGAAT |
| <i>Rn18s.F</i> | closed |
| <i>Rn18s.R</i> | closed |
| <i>Runx2.F</i> | GTAGCCCTCGGAGAGGTACCA |
| <i>Runx2.R</i> | TCGGCGGAGTAGTTCTCATCA |
| <i>Rxra.F</i> | CTAAGATGCGTGACATGCAGATG |
| <i>Rxra.R</i> | GCCCCCTAGAGTCAGGGTTGA |
| <i>Rxrb.F</i> | CGCCTCACTGGAGACCTATTG |
| <i>Rxrb.R</i> | CGTAACAGCAGCTTGCCAAA |
| <i>Sall4.F</i> | ATTACTGGGACATGCGCGTT |
| <i>Sall4.R</i> | GGAAGAGCCCTGGTGACTTG |
| <i>Sema3e.F</i> | GAGGCTGGCTTATGGCATAGAG |
| <i>Sema3e.R</i> | ACCAGATGACTTTTGCTTGTAGTGA |
| <i>Smarcd3.F</i> | AACCACCTTGTTGAGTGGCA |
| <i>Smarcd3.R</i> | CACACTCAAGTCCCCTGGTC |

| | |
|----------------------|--------------------------|
| <i>Snai1.F</i> | CGCCGGAAGCCCAACTATA |
| <i>Snai1.R</i> | TAGGGCTGCTGGAAGGTGAA |
| <i>Snai2.F</i> | GGCTGCTTCAAGGACACATTAG |
| <i>Snai2.R</i> | TATTGCAGTGAGGGCAAGAGA |
| <i>Sox10.F</i> | CACTAGGCAAGCTCTGGAGGTT |
| <i>Sox10.R</i> | GCCTCTCAGCCTCCTCAATG |
| <i>Sox2.F</i> | GGACCGCGTCAAGAGGC |
| <i>Sox2.R</i> | GTGCATCTTGGGGTTCTCCTG |
| <i>Sox2.F nested</i> | GCGGCAACCAGAAGAACAG |
| <i>Sox2.R nested</i> | CTGATCTCCGAGTTGTGCATCT |
| <i>Sox9.F</i> | CGGCTCCAGCAAGAACAAG |
| <i>Sox9.R</i> | TTGTGCAGATGCGGGTACTG |
| <i>spike1</i> | closed |
| <i>Spp1.F</i> | TGCTTTTGCCTGTTTGGCAT |
| <i>Spp1.R</i> | TCCTCTGAGCTGCCAGAATC |
| <i>Tagln.F</i> | TCCAGTCCACAAACGACCAA |
| <i>Tagln.R</i> | ATGCCGTAGGATGGACCCTT |
| <i>Tbx1.F</i> | GAATGTTCCCCACGTTCCAA |
| <i>Tbx1.R</i> | ACAAAGTCCATGAGCAGCATGT |
| <i>Tbx2.F</i> | GTCACCATCCACCTAGCAC |
| <i>Tbx2.R</i> | ACTGGGCTCACGGCTATTTT |
| <i>Tbx3.F</i> | CGTGCCCGGACGCTG |
| <i>Tbx3.R</i> | GAGCCGGCTGTCTTCCTTG |
| <i>Tbx5.F</i> | TCCTGCTAAGAGGAAGAGGGG |
| <i>Tbx5.R</i> | CGAGGTTCTATTCTCGCTCTGT |
| <i>Tbx18.F</i> | TTGCCAAAGGTTTCCGAGACT |
| <i>Tbx18.R</i> | TGGCCTCCAGAATGCGTATG |
| <i>Tbx20.F</i> | CCACGGGTGCACATCATAAA |
| <i>Tbx20.R</i> | TGAACGTCCTGAATTCTTCTGACT |
| <i>Tbx22.F</i> | TCATCAATCCAAACAGTGGCTTAC |
| <i>Tbx22.R</i> | TGATTGGCAGAATGAGATGCTT |
| <i>Tgfb3.F</i> | GGGATATCGTTAGAGGCGGC |
| <i>Tgfb3.R</i> | TGATTCCAGGGGACTTTGGC |
| <i>Twist1.F</i> | ACTGGCGGCCAGGTACATC |
| <i>Twist1.R</i> | GCTCGTGGGCCACATAGCT |
| <i>Tyr.F</i> | ACAGCTGACAAGGGTCTTTA |
| <i>Tyr.R</i> | TGACATGAAGTGGCAAAGGA |
| <i>Tyrp1.F</i> | TGTGAATGCATTGTTATTGTTGG |
| <i>Tyrp1.R</i> | AATGTCATGTGATTTGTTGTAAC |
| <i>Wt1.F</i> | ATCCTCTGTGGTGCCCAGTA |
| <i>Wt1.R</i> | GTTGGGGCCACTCCAGATAC |

F, forward; R, reverse.

Table 3 | Preotic neural crest cells migrating into the heart in quail-chick chimera.

| Sample # | Grafted NF | Donor (quail) stage (ss) | Recipient (chick) stage (ss) | Sampling (day) | Distribution | PV | APS-OFC | RV | LV | IVS | TV | MV | CA | Others |
|----------|------------|-----------------------------|---------------------------------|----------------|--------------|----|---------|----|----|-----|----|----|----|-------------|
| 1 | R4/5/6 | 6 | 6 | 12 | AV | | | | | | | | | Epicardium |
| 2 | R4/5/6 | 7 | 8 | 12 | | | | | | | | | | |
| 3 | R4/5/6 | 8 | 9 | 12 | | | | | | | | | | |
| 4 | R4/5/6 | 10 | 8 | 12 | | | | | | | | | | |
| 12 | R4/5/6 | 8 | 9 | 10 | | | | | | | | | | |
| 13 | R4/5/6 | 9 | 9 | 10 | | | | | | | | | | |
| 14 | R1/2/3/4/5 | 6 | 6 | 8 | | | | | | | | | | |
| 16 | R1/2/3/4/5 | 6 | 9 | 10 | | | | | | | | | | |
| 18 | R1/2/3/4/5 | 6 | 7 | 10 | | | | | | | | | | |
| 19 | R1/2/3/4/5 | 5 | 8 | 10 | | | | | | | | | | |
| 20 | R1/2/3/4/5 | 6 | 7 | 10 | | | | | | | | | | |
| 21 | R1/2/3/4/5 | 6 | 7 | 10 | | | | | | | | | | |
| 22 | R1/2/3/4/5 | 7 | 7 | 10 | | | | | | | | | | |
| 23 | R1/2/3/4/5 | 5 | 6 | 10 | | | | | | | | | | |
| 24 | R1/2/3/4/5 | 7 | 6 | 10 | | | | | | | | | | |
| 25 | R1/2/3/4/5 | 6 | 6 | 11 | | | | | | | | | | |
| 26 | FB-R5 | 3 | 5 | 12 | | | | | | | | | | |
| 27 | FB-R5 | 5 | 5 | 12 | | | | | | | | | | |
| 28 | FB-R5 | 5 | 4 | 12 | | | | | | | | | | |
| 30 | FB-R5 | 4 | 6 | 12 | | | | | | | | | | |
| 31 | FB-R5 | 4 | 6 | 12 | | | | | | | | | | |
| 33 | FB-R5 | 4 | 5 | 12 | | | | | | | | | | |
| 34 | FB-R5 | 5 | 5 | 12 | | | | | | | | | | |
| 35 | FB-R5 | 4 | 4 | 12 | | | | | | | | | | |
| 36 | FB-R5 | 5 | 5 | 10 | | | | | | | | | | |
| 37 | FB-R5 | 5 | 6 | 10 | | | | | | | | | | |
| 41 | FB-R2 | 5 | 7 | 12 | | | | | | | | | | |
| 42 | FB-R2 | 5 | 5 | 12 | | | | | | | | | | |
| 43 | FB-R2 | 5 | 5 | 12 | | | | | | | | | | |
| 49 | MB-R2 | 4 | 6 | 12 | | | | | | | | | | |
| 51 | MB-R2 | 6 | 7 | 12 | | | | | | | | | | |
| 52 | MB-R2 | 5 | 7 | 8 | | | | | | | | | | |
| 55 | MB-R2 | 5 | 6 | 8 | | | | | | | | | | |
| 56 | MB-R2 | 5 | 6 | 8 | | | | | | | | | | Epicardium |
| 57 | MB-R2 | 4 | 6 | 8 | | | | | | | | | | |
| 59 | MB-R2 | 4 | 5 | 10 | | | | | | | | | | |
| 60 | MB-R2 | 5 | 6 | 4 | | | | | | | | | | OFT cushion |
| 62 | MB-R2 | 4 | 6 | 4 | | | | | | | | | | OFT cushion |

Highlighted parts in gray indicate the distribution of QCPN-positive cells. APS, aorticopulmonary septum; AV, aortic valve; CA, coronary artery; FB, forebrain; IVS, interventricular septum; LV, left ventricle; MB, midbrain; MV, Mitral valve; NF, neural fold; OFC, outflow tract cushion; OFT, outflow tract; PV, pulmonary valve; R1-6, rhombomere 1 to 6; RV, right ventricle; ss, somite stage; TV, tricuspid valve.

Table 4 | Cardiac malformation induced by retinoic acid treatment.

| Sample # | Dose (mg/kg bw) | Dosage | Administration Stage (dpc) | Dilatation | Septal branche origin | Outflow mal-alignment | VSD | RCA orifice | LCA orifice |
|----------|-----------------|--------|----------------------------|------------|-----------------------|-----------------------|---------|-------------|-------------|
| 1 | 40 | IP | 10.5 | | RCA | | unknown | | |
| 2 | 40 | IP | 10.5 | | RCA | | unknown | | |
| 3 | 40 | IP | 10.5 | | RCA | | unknown | | |
| 4 | 40 | IP | 10.5 | | RCA | | unknown | | |
| 5 | 40 | IP | 10.5 | | RCA | | unknown | | |
| 6 | 40 | IP | 10.5 | | RCA | | unknown | | |
| 7 | 40 | IP | 10.5 | | RCA | | unknown | | |
| 8 | 40 | IP | 10.5 | | RCA | | unknown | | |
| 9 | 40 | IP | 10.5 | | RCA | | unknown | | |
| 10 | 40 | IP | 10.5 | | RCA | | unknown | | |
| 11 | 40 | IP | 10.5 | | RCA | | unknown | | |
| 12 | 40 | IP | 10.5 | | RCA | | unknown | | |
| 13 | 40 | IP | 10.5 | | RCA | | unknown | | |
| 14 | 40 | IP | 10.5 | | RCA | | unknown | | |
| 15 | 40 | IP | 10.5 | | RCA | | unknown | | |
| 16 | 40 | IP | 10.5 | | RCA | | unknown | | |
| 17 | 40 | IP | 10.5 | | RCA | | unknown | | |
| 18 | 40 | IP | 10.5 | | RCA | | unknown | | |
| 19 | 40 | IP | 10.5 | | RCA | | unknown | | |
| 20 | 40 | IP | 10.5 | | RCA | | unknown | | |
| 21 | 40 | IP | 10.5 | | RCA | | unknown | | |
| 22 | 40 | IP | 10.5 | | RCA | | unknown | | |
| 23 | 40 | IP | 9.5 | | RCA | | unknown | | |
| 24 | 40 | IP | 9.5 | | RCA | | unknown | | |
| 25 | 40 | IP | 9.5 | | RCA | | unknown | | |
| 26 | 40 | IP | 8.5 | | RCA | | unknown | | |
| 27 | 40 | IP | 8.5 | | RCA | | unknown | | |
| 28 | 40 | IP | 8.5 | | RCA | | unknown | | |
| 29 | 40 | IP | 8.5 | | RCA | | unknown | | |
| 30 | 40 | IP | 8.5 | | RCA | | unknown | | |
| 31 | 40 | IP | 8.5 | | RCA | | unknown | | |
| 32 | 40 | IP | 8.5 | | RCA | | unknown | | |
| 33 | 40 | IP | 8.5 | | RCA | | unknown | | |
| 34 | 40 | IP | 8.5 | | RCA | | unknown | | |
| 35 | 40 | IP | 8.5 | | RCA | | unknown | | |
| 36 | Vehicle | IP | 9.5 | | RCA | | unknown | | |
| 37 | Vehicle | IP | 9.5 | | RCA | | unknown | | |
| 38 | Vehicle | IP | 9.5 | | RCA | | unknown | | |
| 39 | Vehicle | IP | 9.5 | | RCA | | unknown | | |
| 40 | Vehicle | IP | 9.5 | | RCA | | unknown | | |
| 41 | Vehicle | IP | 9.5 | | RCA | | unknown | | |
| 42 | Vehicle | IP | 9.5 | | RCA | | unknown | | |
| 43 | Vehicle | IP | 9.5 | | RCA | | unknown | | |
| 44 | Vehicle | IP | 9.5 | | RCA | | unknown | | |
| 45 | Vehicle | IP | 9.5 | | RCA | | unknown | | |
| 46 | Vehicle | IP | 9.5 | | RCA | | unknown | | |
| 47 | Vehicle | IP | 9.5 | | RCA | | unknown | | |

| Sample # | Dose (mg/kg bw) | Dosage | Administration Stage (dpc) | Dilatation | Septal branch origin | Outflow mal-alignment | VSD | RCA orifice | LCA orifice |
|----------|-----------------|--------|----------------------------|------------|----------------------|-----------------------|---------|-------------|-------------|
| 48 | 70 | PO | 8.5 | | LCA | TGA | | | |
| 49 | 70 | PO | 8.5 | | LCA | TGA | | | |
| 50 | 70 | PO | 8.5 | | RCA | | | | |
| 51 | 70 | PO | 8.5 | | RCA | | | | |
| 52 | 70 | PO | 8.5 | | LCA | TGA | | | |
| 53 | 70 | PO | 8.5 | | LCA | TGA | | | |
| 54 | 70 | PO | 8.5 | | LCA | TGA | | RCC | RCC |
| 55 | 70 | PO | 8.5 | | LCA | TGA | | | |
| 56 | 70 | PO | 8.5 | | LCA | TGA | | | |
| 57 | 70 | PO | 8.5 | | RCA | | | | |
| 58 | 70 | PO | 8.5 | | LCA | | | | |
| 59 | 70 | PO | 8.5 | | RCA | | | | |
| 60 | 70 | PO | 8.5 | | LCA | DORV | | RCC | RCC |
| 61 | 70 | PO | 8.5 | | LCA | DORV | | | |
| 62 | 70 | PO | 8.5 | | RCA | | | | |
| 63 | 70 | PO | 8.5 | | LCA | | | | |
| 64 | 70 | PO | 8.5 | | LCA | | | | |
| 65 | 70 | PO | 8.5 | | LCA | TGA | | | |
| 66 | 70 | PO | 8.5 | | LCA | TGA | | | |
| 67 | 70 | PO | 8.5 | | LCA | | | | |
| 68 | 70 | PO | 8.5 | | LCA | | | | |
| 69 | 70 | PO | 8.5 | | LCA | | | | |
| 70 | 70 | PO | 8.5 | | RCA | TGA | | | |
| 71 | 70 | PO | 8.5 | | RCA | TGA | | | |
| 72 | 70 | PO | 8.5 | | LCA | TGA | | | |
| 73 | 70 | PO | 8.5 | | RCA | | | | |
| 74 | 70 | PO | 8.5 | | LCA | TGA | | | |
| 75 | 70 | PO | 8.5 | | LCA | | | | |
| 76 | 70 | PO | 8.5 | | LCA | TGA | | | |
| 77 | 70 | PO | 8.5 | | RCA | | | | |
| 78 | 70 | PO | 8.5 | | LCA | | | | |
| 79 | 70 | PO | 8.5 | | RCA | | | | |
| 80 | 70 | PO | 8.5 | (+) | LCA | | | | |
| 81 | 70 | PO | 8.5 | | LCA | | | | |
| 82 | 70 | PO | 8.5 | | RCA | | | | |
| 83 | 70 | PO | 8.5 | | LCA | TGA | | | |
| 84 | 70 | PO | 8.5 | | RCA | TGA | | | |
| 85 | 70 | PO | 8.5 | | LCA | TGA | | | |
| 86 | 70 | PO | 8.5 | | LCA | TGA | | | |
| 87 | 70 | PO | 8.5 | | LCA | TGA | | | |
| 88 | 70 | PO | 8.5 | | LCA | | | | |
| 89 | 70 | PO | 8.5 | | RCA | | | | |
| 90 | 70 | PO | 8.5 | (+) | LCA | | | | |
| 91 | 70 | PO | 8.5 | | LCA | TGA | | | |
| 92 | 70 | PO | 8.5 | | RCA | TGA | | | |
| 93 | 70 | PO | 8.5 | | LCA | TGA | | | |
| 94 | 70 | PO | 8.5 | | LCA | | | | |
| 95 | 70 | PO | 8.5 | | LCA | | | | |
| 96 | 70 | PO | 8.5 | | LCA | TGA | | | |
| 97 | 70 | PO | 8.5 | | RCA | | | | |
| 98 | 70 | PO | 8.5 | | LCA | DORV | | | |
| 99 | 70 | PO | 8.5 | | RCA | | | | |
| 100 | 70 | PO | 8.5 | | LCA | | | | |
| 101 | 70 | PO | 8.5 | | LCA | | unknown | | |
| 102 | 70 | PO | 8.5 | | LCA | | unknown | RCC | RCC |
| 103 | 70 | PO | 8.5 | | RCA | | unknown | | |
| 104 | 70 | PO | 8.5 | | RCA | | unknown | | |
| 105 | 70 | PO | 8.5 | | RCA | | unknown | | |
| 106 | 70 | PO | 8.5 | | LCA | TGA | unknown | LCC | LCC |

| Sample # | Dose (mg/kg bw) | Dosage | Administration Stage (dpc) | Dilatation | Septal branch origin | Outflow mal-alignment | VSD | RCA orifice | LCA orifice |
|----------|-----------------|--------|----------------------------|------------|----------------------|-----------------------|---------|-------------|-------------|
| 107 | 70 | PO | 9.5 | | RCA | | | | |
| 108 | 70 | PO | 9.5 | | RCA | | | | |
| 109 | 70 | PO | 9.5 | | RCA | | | | |
| 110 | 70 | PO | 9.5 | | LCA | | | | |
| 111 | 70 | PO | 9.5 | | RCA | | | | |
| 112 | 70 | PO | 9.5 | | RCA | | | | |
| 113 | 70 | PO | 9.5 | | RCA | | | | |
| 114 | 70 | PO | 9.5 | | RCA | | | | |
| 115 | 70 | PO | 9.5 | | LCA | | | | |
| 116 | 70 | PO | 9.5 | | RCA | | | | |
| 117 | 70 | PO | 9.5 | | RCA | | | | |
| 118 | 70 | PO | 9.5 | | RCA | | unknown | | |
| 119 | 70 | PO | 9.5 | | RCA | | unknown | | |
| 120 | 70 | PO | 9.5 | | RCA | | unknown | | |
| 121 | 70 | PO | 9.5 | | RCA | | unknown | | |
| 122 | 70 | PO | 9.5 | | RCA | | unknown | | |
| 123 | 70 | PO | 9.5 | | RCA | | unknown | | |
| 124 | 70 | PO | 9.5 | | RCA | | unknown | | |
| 125 | 70 | PO | 9.5 | | RCA | | unknown | | |
| 126 | 70 | PO | 9.5 | | RCA | | unknown | | |
| 127 | 70 | PO | 9.5 | | RCA | | unknown | | |
| 128 | 70 | PO | 9.5 | | RCA | | unknown | | |
| 129 | 70 | PO | 9.5 | | RCA | | unknown | | |
| 130 | Vehicle | PO | 8.5 | | RCA | | | | |
| 131 | Vehicle | PO | 8.5 | | RCA | | | | |
| 132 | Vehicle | PO | 8.5 | | RCA | | | | |
| 133 | Vehicle | PO | 8.5 | | RCA | | | | |
| 134 | Vehicle | PO | 8.5 | | RCA | | | | |
| 135 | Vehicle | PO | 8.5 | | RCA | | | | |
| 136 | Vehicle | PO | 8.5 | | RCA | | | | |
| 137 | Vehicle | PO | 8.5 | | RCA | | | | |
| 138 | Vehicle | PO | 8.5 | | RCA | | | | |
| 139 | Vehicle | PO | 8.5 | | RCA | | | | |
| 140 | Vehicle | PO | 8.5 | | RCA | | unknown | | |
| 141 | Vehicle | PO | 8.5 | | RCA | | unknown | | |
| 142 | Vehicle | PO | 8.5 | | RCA | | unknown | | |
| 143 | Vehicle | PO | 8.5 | | RCA | | unknown | | |
| 144 | Vehicle | PO | 8.5 | | RCA | | unknown | | |
| 145 | Vehicle | PO | 8.5 | | RCA | | unknown | | |

Highlighted parts in gray indicate the abnormalities. bw, body weight; DORV, double outlet right ventricle; dpc, day post-coitum; IP, intraperitoneal administration; LCA, left coronary artery; LCC left coronary cusp; PO, oral administration (*per os*); RCS, right coronary artery; RCC, right coronary cusp; TGA, transposition of the great arteries; VSD, ventricular septal defect.