Legends Supplemental Tables

Supplemental Table 1. List of embryos in the Carnegie collection that were used to reconstruct the heart and prepare 3D-PDF files. The list includes the correlation between the Carnegie stage of development of an embryo and its estimated post-fertilization (pf) age in days (range; [1]), its crown-rump length (CRL), the average CRL of same-stage embryos in the collection that had been graded "good" or "excellent" [2], with standard deviation (SD) and number of embryos (N).

Supplemental Table 2. List of structures identified in each of the reconstructed embryos. In total 76 items representing 70 different structures were reconstructed. Bilateral structures are usually shown separately so as not to hinder views. Eight structures not belonging to the cardiovascular system served as topographical landmarks. Almost all structures were identified in 2 or more successive Carnegie stages of development. Reconstructed cardiovascular structures are arranged in the upstream-to-downstream order that is also used in the description of heart morphology according to the "sequential segmental analysis" protocol [3]. Because of limited space that is available to describe the structures in the model trees accompanying the reconstructions, the following abbreviations were used: AV: atrioventricular; card: cardinal; CCS: (ventricular) cardiac conduction system; DMP: dorsal mesenchymal protrusion; HCC: hepatocardiac vein; L: left; LA: left atrium; LV: left ventricle; musc: muscular; NCCs: neural crest cells; non-adj: non-adjacent; OFT: outflow tract; PAAs: pharyngeal arch arteries; pulm: pulmonary; R: right; RA: right atrium; RV: right ventricle; SAN: sinuatrial node; subpulm: subpulmonary; Umb; umbilical; Vit: vitelline.

Legends Supplemental Figures

Supplemental Figure 1. Relation between developmental stages in men and mice, or chickens. Developmental stages in man (horizontal axis) are expressed in Carnegie Stages [1]; those in mice (left Y-axis) in days of embryonic development corresponding with Streeter's horizons [4]; and those in chickens (right Y-axis) in Hamburger-Hamilton stages [5]. Black

symbols relate development of men and mice, while blue symbols relate men and chickens. Although established staging systems for mouse embryos exist (e.g. [4]), they are rarely used in mouse developmental cardiology.

Supplemental Figures 2-13. Interactive 3D-PDFs of human hearts between 3.5 and 8 weeks of development. Click on a hyperlink below to enable one of these Supplements and click on 'trust this document only once'. *(Note for Mac users: click on a hyperlink, download the 3D-PDF and open with adobe PDF reader to enable the interactive options).* Subsequently, the 3D-PDF becomes activated by "clicking" with the mouse on the reconstruction. A toolbar appears at the top of the screen that includes the option "model tree". The model tree displays a material list of structures in the upper box, and preset viewing options (cameras) in the lower box. The sequence of items corresponds to that in Supplemental Table 2. The list of visible structures can be modified by marking or unmarking a structure. To manipulate the reconstruction, press the left mouse button to rotate it, the scroll button to zoom in or out, and the left and right mouse buttons simultaneously to move the embryo across the screen. A structure can be rendered transparent by selecting that option from the drop-down menu after selecting the structure with the right mouse button. To inspect a combination of structures, one is advised to build up the composition, beginning with a familiar component, such as a lumen, rather than deleting non-relevant structures one-by-one. The slicer button in the toolbar allows making cross sections. The plane of section can be adjusted with the offset and tilt options. The "loop wires" in Supplemental Figures 3-6, which are drawn through the center of the endocardial heart tube, emphasize the changing shape of the heart loop during CS10-13. The side length of the scale cubes is 200 μm. The preset views correspond to the images shown in Figures 1-12. Note that items that are visible in these views can be altered by marking or unmarking a structure in the model tree.

Supplemental Figure 2. CS9 embryo at ~26 days after fertilization. The cardiac jelly, which is produced by endoderm and future myocardial cells in the visceral layer of the pericardium [6], probably represents the boundary of the developing primary myocardium within in this visceral layer (*cf.* [7]). We have, therefore, marked its X-shaped distribution with a black contour line on the visceral wall.

Supplemental Figure 3. CS10 embryo at ~28 days after fertilization. Note that the cardiac lumen resembles an hourglass with its neck at the junction between the embryonic left ventricle and outflow tract. When viewed from dorsal the continuity between the left- and right-sided myocardium indicates that the dorsal mesocardium has disappeared at this location so that one can pass from left to right on the dorsal side of the heart tube. Further note that the junction of embryonic ventricle and outflow tract bends slightly leftward and ventrally (see "loop wire"), which shows that cardiac symmetry has broken and cardiac looping starts.

Supplemental Figure 4. CS11 embryo at ~29 days after fertilization. Note that the common cardinal veins are still absent. Further note the topographic relation between the inflow tract and the contour of the cranial intestinal portal. The twisted loop of the heart lumen is visualized by the "loop wire".

Supplemental Figure 5. CS12 embryo at ~30 days after fertilization. Note that the "spikes" on the lumen of the embryonic left and right ventricles represent the endothelial ingressions that mark the beginning formation of the ventricular trabeculae. Also note that the loop-wire model of the heart tube now resembles two helices that connect in the right ventricle.

Supplemental Figure 6. CS13 embryo at ~32 days after fertilization. Note that the epicardium has spread over a large part of the surface of the heart, but that we have reconstructed only the areas with a thick layer of epicardium in the grooves of the atrioventricular and interventricular junctions.

Supplemental Figure 7. CS14 embryo at ~34 days after fertilization. Note that from this stage onwards we use the spinal ganglia as reference for segmental levels and that the first 4 somites do not form ganglia [8]. Further note that the parietal and septal outflow-tract ridges form within the cuff of endocardial jelly, which then becomes reduced to a very thin layer that was no longer reconstructed.

Supplemental Figure 8. CS15 embryo at ~36 days after fertilization. Note that the veins, systemic venous sinus, and both atria were not reconstructed because they were

pathologically distended. Further note that the aortic trunk consists of the intrapericardial ascending aorta and the extrapericardial brachiocephalic trunk and aortic arch.

Supplemental Figure 9. CS16 embryo at ~38 days after fertilization. Note that the right atrioventricular junction derives from the left-sided atrioventricular canal, but is from now on depicted as a right-sided connection. Also note that the interventricular foramen and its surrounding ring bundle of developing ventricular conduction system still resemble the configuration seen at CS14, but the part surrounding the connection between the left ventricle and subaortic outflow tract has disappeared (hatched section). Note further that the parietal and septal outflow-tract ridges both have a darker proximal part and a lighter distal part that contributes to semilunar valve formation. In addition, the tissue forming the intercalated spurs produces not only the future ventral and dorsal semilunar leaflets of the arterial valves (lighter shade), but also the walls of the ascending aorta and pulmonary trunk (darker shade).

Supplemental Figure 10. CS17 embryo at ~40 days after fertilization. This stage shows very pronounced changes in the shape of the outflow tract and its constituent structures.

Supplemental Figure 11. CS18 embryo at ~43 days after fertilization. Note that there is only a single (left) coronary artery that passes through the myocardium of the distal outflow tract.

Supplemental Figure 12. CS20 embryo at ~49 days after fertilization. Note that the superior and inferior atrioventricular cushions are no longer distinguishable as separate entities and are, therefore, depicted by hatching both code colors.

Supplemental Figure 13. CS23 embryo at ~56 days after fertilization. Note that the periaortic section of the GlN ring is no longer reconstructed.

Supplemental Figure 14. Measurements to assess the degree of spiraling of the walls of the heart and arterial trunks. The upper panel shows caudal views of the lumens of the outflow tract and arterial trunks, and illustrates the protocol of the measurements shown in Figure 10. The black arrows show the rotation of the arterial trunks relative to each other between CS14 and CS18, while the green arrows reveal the asymmetric growth of the

extrapericardial “horns” of the aortic trunk. The lower panel shows a ventral view of the outflow tract of a CS16 heart, with the myocardium rendered translucent. The dashed black lines indicate the position of the measurements. The degree of rotation of the subaortic and subpulmonary channels, the ridges, and the distally fused prongs of neural crest cells was determined perpendicular to the luminal axis of the outflow tract. The graph shown in Figure 10 is included for convenience.

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