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A membrane associated mCherry fluorescent reporter line for studying vascular remodeling and cardiac function during murine embryonic development

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Abstract

The development of the cardiovascular system is a highly dynamic process dependent on multiple signaling pathways regulating proliferation, differentiation, migration, cell-cell and cell-matrix interactions. To characterize cell and tissue dynamics during the formation of the cardiovascular system in mice, we generated a novel transgenic mouse line, Tg(Flk1::myr-mCherry), in which endothelial cell membranes are brightly labeled with mCherry, a red fluorescent protein. Tg(Flk1::myr-mCherry) mice are viable, fertile and do not exhibit any developmental abnormalities. High levels of mCherry are expressed in the embryonic endothelium and endocardium, and expression is also observed in capillaries in adult animals. Targeting of the fluorescent protein to the cell membrane allows for sub-cellular imaging and cell tracking. By acquiring confocal time lapses of live embryos cultured on the microscope stage, we demonstrate that the newly generated transgenic model beautifully highlights the sprouting behaviors of endothelial cells during vascular plexus formation. We have also used embryos from this line to imaging the endocardium in the beating embryonic mouse heart, showing that Tg(Flk1::myr-mCherry) mice are suitable for the characterization of cardio dynamics. Furthermore, when combined with the previously described Tg(Flk1::H2B-EYFP) line, cell number in addition to cell architecture is revealed, making it possible to determine how individual endothelial cells contribute to the structure of the vessel.

Keywords

live imaging; mCherry; Flk1; heart dynamics; vasculogenesis; angiogenesis; embryonic development; cell tracking; yolk sac

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Introduction

Cardiovascular development is tightly regulated by many factors, including an array of signaling molecules as well as mechanical stimuli acting through a set of highly interdependant signaling pathways (Risau, 1997; Daniel and Abrahamson, 2000; Lucitti et al., 2007). The complexity in signaling events required for the normal development of the cardiovascular system is likely to account for the high probability of congenital birth defects. According to the American Heart Association, congenital abnormalities in the cardiovascular system are not only among the most common birth defects, but are also the leading cause of death in children born with congenital defects.

The mouse provides an excellent model for studying cardiovascular development and disease because of the resemblance to humans, potential for genetic manipulation, and rapid generation time. Many mouse mutants with cardiovascular defects linked to human diseases have been identified and are providing great insights into understanding cardiovascular development from a molecular genetic perspective (Conway et al., 2003). How cell-cell associations and mechanical force relate to key signaling pathways is less well understood. Much of the analysis of mutant phenotypes has relied on static images or extracted tissues that cannot provide insights about dynamic processes such as cell migration or tissue mechanics. However, the recent development of many *in vivo* imaging methods is allowing for a more thorough understanding of developmental events in real time.

Live, confocal and multiphoton imaging has been used in many developing systems as a way to study the cellular dynamics of tissue development *in vivo* (Lichtman and Fraser, 2001; Hadjantonakis et al., 2003; Kulesa, 2004). The use of tagged fluorescent proteins under control of specific regulatory elements has significantly increased the ability to track individual cells, characterize tissue dynamics, and understand the formation of complex cytoarchitectures (Hadjantonakis et al., 2003; Megason and Fraser, 2003). Even though mouse embryos develop inside the uterus, which restricts access for traditional light microscopy, protocols for growing mouse embryos on the microscopic stage to allow direct visualization of labeled tissues have recently been established (Jones et al., 2002; Megason and Fraser, 2003; Jones et al., 2004; Jones et al., 2005). By controlling temperature, pH, and supplementing growth medium with a number of factors, embryos can be grown in static culture for more than 24 hours. Using these methods, we have been successful in quantifying early circulation events, in studying the initiation of blood formation, and in characterizing mutants with circulation defects (Jones et al., 2004; Lucitti et al., 2007). The limits to what can be studied using live imaging at these early stages of development rest with the ability to mark particular cells of interest. Thus, there is a need for a greater library of available vital reporter lines for imaging live development.

In this paper, we describe a novel transgenic model, in which mCherry fluorescent protein is expressed in the embryonic endothelium and endocardium. The mCherry reporter is fused to a myristoylation motif (myr) which results in membrane localization of the fluorescent marker. We show here that myr-mCherry nicely outlines endothelium and endocardial cells, is compatible with live imaging, and can be combined with another transgenic line Tg(Flk1::H2B-EYFP) (Fraser et al., 2005) in which the reporter protein is confined to the nucleus.

Materials and Methods

Generation of transgenic mice

A construct containing the myr-mCherry clone was obtained from S. Megason, Caltech and the myr-mCherry sequence was subcloned into pFlk1::H2B-EYFP plasmid (Fraser et al.,

2005) to replace H2B-EYFP fragment. A diagram of the resulting construct pFlk1::myr-mCherry is shown in figure 1. The construct was tested by transient transfection of HeLa cells which resulted in bright staining of the cell membrane (data not shown). The Flk1::myr-mCherry fragment containing Flk1 regulatory regions (Kappel et al., 1999) and myr-mCherry fusion protein followed by an SV40 polyadenylation site was excised from the plasmid and microinjected into single cell FVB mouse zygotes by the Baylor College of Medicine Transgenic Core. PCR genotyping revealed several transgenic founders, from which earpunch biopsies were obtained and microscopically analyzed for expression of the fluorescent marker. Based on the fluorescence analysis, the four founders with the brightest signal were used for further analysis. Embryos from all the founders exhibited the same pattern of the mCherry expression. Transgenic lines were homozygosed and maintained on the FVB background. Tg(Flk1::myr-mCherry) mice were crossed to Tg(Flk1::H2B-EYFP) line (Fraser et al., 2005).

Embryo manipulations

For embryo studies, Tg(Flk1::myr-mCherry) and Tg(Flk1::myr-mCherry × Flk1::H2B-EYFP) males were mated to wild type CD-1 or Tg(Flk1::myr-mCherry) females. Females were examined for vaginal plugs daily and the presence of a plug was taken as 0.5 dpc (days post coitum). Embryos were dissected with the yolk sac intact at 7.5 to 12.5 dpc and were imaged using confocal microscopy. For time lapse microscopy, embryos were dissected and cultured on the microscope stage according to methods reported previously (Fraser et al., 2005).

Microscopic imaging

Fluorescence imaging was performed using two different confocal microscopes, the ZEISS LSM 5LIVE and ZEISS LSM 510META (Carl Zeiss Inc.). The META detector provided detailed spectral analysis of emitted light in each pixel of the acquired image and was used to confirm the presence of mCherry fluorescence (Figure 1(B–C)). Images of mCherry were obtained using 543-nm (LSM 510META) or 532-nm excitation (LSM 5LIVE) at different magnifications ranging from 10× to 63×; EYFP was excited using 488 nm laser on both microscopes. Time lapse images of 7.5 dpc embryo cultures were taken every 10 minutes for 26 hrs with LSM 5LIVE confocal microscope at 15% of total laser power, 532-nm excitation. Time lapse images of the 8.5 dpc heart were acquired at a rate of 6 frames per second (fps) with LSM 510LIVE microscope at 50% total laser power, 532-nm excitation.

Results

Generation of Tg(Flk1: :myr-mCherry) mice

Previously, we showed that Flk1 regulatory elements could be used to drive the expression of an H2B-YFP fusion in developing endothelial cells (Fraser et al., 2005). A diagram of the construct used for generation of Tg(Flk1::myr-mCherry) mice is shown in Figure 1A. The mCherry fluorescent protein fused to a myristoylation motif (myr) and followed by a SV40 polyadenylation site was cloned between Flk1 regulatory elements. We chose to use the Flk1 promoter because, as was shown previously (Fraser et al., 2005), it drives expression in the embryonic vasculature in the Tg(Flk1::H2B-EYFP) line. The myristoylation motif allows for membrane localization of the fluorescent protein, and therefore, outlines the structure of the vasculature and reveals cellular morphology and cell-cell boundaries.

Microinjection of the linearized Flk1::myr-mCherry construct into single-cell embryos produced several founders with identical pattern of expression (See Materials and Methods). The mCherry expression in the newly generated line was confirmed by the spectral analysis. Figure 1B shows an image of adult earpunch biopsy acquired in the emission fingerprinting

mode using the META detector at 543 nm excitation. The emission spectrum was measured from 560 to 700 nm. The detected spectra in three representative regions marked on the image are shown in Figure 1C. The fluorescence from the vasculature has a clear peak at 610 nm (blue and green lines in figure), which matches the published spectral emission profile for mCherry (Shaner et al., 2004). The background autofluorescence (red line) has very little contribution at the measured spectral range.

Pattern of expression of the mCherry fluorescent protein

To characterize the expression pattern of mCherry in embryonic tissues, staged embryos ranging from 7.5 dpc to 12.5 dpc were studied using confocal microscopy. In embryos homozygous for the Flk1::myr-mCherry transgene, the level of fluorescence was notably higher than in embryos heterozygous for the insertion. Fluorescence of mCherry was first detectable at the early headfold stage (7.5 dpc) in the blood islands of the yolk sac (Figure 2A). At this stage, the fluorescence was relatively dim and was only detectable in homozygous embryos. At 8.0 dpc, mCherry outlined the vascular plexus as it developed in the yolk sac. By 8.5 dpc the vascular plexus was brightly highlighted by the transgene (Figure 2B), and membrane boundaries between endothelial cells could be detected in some cells at 63× magnification (Figure 2C). At this stage, mCherry was expressed in both the embryonic vasculature as well as the endocardium of the heart (Figure 2D).

Multicolor analysis of vessel development

One benefit of the Tg(Flk1::myr-mCherry) transgenic line is the ability to cross this line with other fluorescent protein markers with minimal spectral overlap. As mCherry has an emission peak at 610nm, it is easily separated from blue, green or yellow fluorescent proteins. We have crossed Tg(Flk1::myr-mCherry) mice with mice from the Tg(Flk1::H2B-EYFP) line, which expresses H2B-EYFP in the nuclei of embryonic endothelial cells (Fraser et al., 2005). This cross produced embryos in which both the membrane (red) and the nucleus (yellow) of endothelial cells are labeled. This combination of markers allowed us to identify each endothelial cell within a vessel to reveal the distribution of individual cells within each vessel segment. In addition, because the Tg(Flk1::H2B-EYFP) line has been well characterized (Fraser et al., 2005) and the expression of fluorescent proteins in both lines is driven by the same Flk1 regulatory elements, we used this cross to verify overlap of the expression patterns. Our analysis has shown that mCherry and EYFP were expressed in the same cells in the embryonic vasculature (Figures 2E–K). Both markers were clearly co-expressed in the embryonic trunk at 9.5 dpc (Figure 2E–F) and 12.5 dpc (Figure H–I), the 12.5 dpc yolk sac, (Figure 2G) the 12.5 dpc brain (Figure 2J) and the 12.5 dpc eye (Figure 2K). The detailed subcellular localization can be appreciated in a high magnification view of embryonic vessels (Figure 2I) showing clear membrane boundaries and brightly labeled nuclei.

During late gestation, expression of the mCherry (and the H2B-EYFP) decreased in major blood vessels, but remained in some smaller vessels throughout postnatal life (data not shown). The mCherry could be detected in the adult transgenic mice in many of the micro vessels throughout the body, for example, in the skin, the brain, and the muscle. Figure 2L shows a projection of a confocal stack of images acquired from an adult earpunch biopsy. The mCherry expression in the small capillaries is evident in the image providing a quick and convenient way to genotype transgene carriers.

Time lapse imaging of the embryonic yolk sac

To assess the applicability of the Tg(Flk1::myr-mCherry) mice for studying the vascular development in the embryonic yolk sac *in vivo*, we performed time lapse confocal microscopy of embryonic cultures grown on the microscope stage. Figure 3 shows images of

Tg(Flk1::myr-mCherry) embryonic yolk sac, which was cultured for 18 hours. The mCherry labeled the immature vascular plexus of the yolk sac at 8.5 dpc (Figure 3A), a time when blood flow is just beginning. Figure 3B shows the corresponding place of the yolk sac 18 hours later. A major refinement of the vascular structure has occurred during this time with some vessels regressing and those with along the major flow trajectories remaining.

To study the formation of the vasculature in the primitive plexus of the yolk sac, we acquired a time-lapse sequence of a 7.5dpc embryo carrying both transgenes, Tg(Flk1::myr-mCherry) and Tg(Flk1::H2B-EYFP). Figure 4(A–F) shows images from a time lapse sequence (images taken every 10 minutes, but shown for 4 hr intervals). At the beginning of the culture, mCherry was detected only in the region of the blood islands, but 8 hours later, mCherry expression was evident throughout the yolk sac. Defined vascular structures were difficult to visualize until 12 hours after the beginning of culture. The level of expression of the fluorescent protein increased continuously during the course of the time lapse, and by 20 hours the entire vascular plexus was brightly outlined. mCherry was expressed early enough to allow us to detect cellular movements during the formation of the vascular plexus. Figure 4 (G–I) shows multiple sprouting events of endothelial cells in the same area of the yolk sac and formation of vascular branches, which are marked by arrows. This is in contrast to data taken from embryos at later stages which shows the regression of many vessel segments.

Live imaging of embryonic cardiodynamics

Because of the high level expression of the mCherry in the embryonic endocardium, we tested whether we could acquire rapid images of the beating heart. Until embryonic turning (about 16 somites on day 8.5) the developing heart is positioned on the outside of the embryo under the yolk sac, making it accessible for confocal imaging. Figure 5 (A–E) shows images of an 8.5 dpc beating heart acquired at 6 fps. Successive panels show different phases of the cardiac cycle. mCherry fluorescence beautifully outlined the endocardium within the heart tube. Average image intensity measurements taken from the marked regions illustrate the tissue motion during the time lapse sequence (Figure 5A,F). The fluorescence intensity changed with a periodicity that could be used to estimate the heart rate. In the example shown, the heart rate was around 80 beats per minute, which is within the normal physiological range for this stage of development (Jones et al., 2004). These results show that endocardial expression in Tg(Flk1::myr-mCherry) embryos could be a very exciting tool for studying early cardiac function in mammals, using methods similar to those used to characterize heart function and biomechanics in zebrafish embryos (Liebling et al., 2005; Forouhar et al., 2006; Liebling et al., 2006).

Discussion

It this paper we describe a new transgenic mouse line Tg(Flk1::myr-mCherry) in which the mCherry fluorescent protein is expressed in the membrane of embryonic endothelial cells. mCherry is an excellent fluorescent protein to use in combination with other fluorescent proteins because the emission signal (610 peak) can be separated easily from blue, cyan, green or yellow fluorescent protein variants. By crossing the new Tg(Flk1::myr-mCherry) line with mice from the Tg(Flk1::H2B-EYFP) line, we showed that both fluorescent proteins can be easily detected in different subcellular compartments. In fact, these markers provide a powerful combination since the EYFP marker allows for the analysis of cell division and the tracking of each endothelial cell whereas the membrane-targeted mCherry marker reveals cell boundaries and highlights vessel organization.

Even though the Tg(Flk1::myr-mCherry) line was designed to allow live imaging of developing vasculature, the mCherry marker can be fixed to allow immunohistochemical analysis in combination with other fluorescent markers. According to the original designers

of the mCherry protein, this marker is quite stable to photobleaching (Shaner et al., 2004); mCherry is over tenfold more photostable than mRFP1 and only about 40% less photostable than EGFP. In consistence with this report, no decrease in fluorescence intensity of mCherry due to photobleaching was noticed during our experiments.

Flk1 (also known as VEGF receptor-2) is the earliest known marker of endothelial cells and their precursors and is known to be expressed in nascent mesoderm. It marks all early endothelial cells during angiogenesis and vasculogenesis (Yamaguchi et al., 1993). Expression of mCherry in Tg(Flk1::myr-mCherry) embryos is first detected at low levels at 7.5 dpc within the blood islands of the yolk sac and correlates well with endogenous Flk1 expression. From 7.5 to 8.5 dpc, the expression in the yolk sac increases dramatically, outlining the detailed structure of the vascular plexus. Transgene expression continues in the yolk sac during remodeling, as the vascular plexus is transformed from a polygonal primitive structure to a branched hierarchy of large and small caliber vessels. In the embryo proper, mCherry is expressed throughout the vasculature from 8.5 dpc until late gestation, when expression disappears in large vessels and detected in many small capillaries throughout adulthood.

The bright expression in Tg(Flk1::myr-mCherry) embryos facilitates confocal time lapse analysis. One of the limitations of time lapse imaging of live embryos is that repetitive imaging with high intensity lasers can be harmful to embryo growth. Here we show that normal time lapse sequences can be acquired for at least 24 hours, similar to other fluorescent protein transgenic lines that we have used (Fraser et al., 2005).

The expression of this marker from early stages, when the vascular plexus is forming, throughout the remodeling process has provided useful insights into the dynamics of vessel formation and maturation. At early stages, confocal time lapse analysis shows many small sprouts forming to increase the number of vessel branches, whereas at later stages, after blood flow begins, many vessel branches regress. We have recently shown that vessel remodeling in the yolk sac is triggered by hemodynamic force (Jones et al., 2004; Lucitti et al., 2007). Further studies are underway to determine if sprouting persists and vessel regression is absent if normal blood flow is disrupted.

Expression of the mCherry reporter in the embryonic endocardium provided sufficient contrast to acquire images of the mouse embryonic heart tube as it was beating. Recently, great strides have been made in understanding how the zebrafish embryonic heart functions. Rapid confocal imaging of transgenic zebrafish embryos expressing fluorescent proteins in cardiac cells has been used to define how the embryonic heart acts as a pump and how blood flow changes as the morphology of the heart becomes more complex (Liebling et al., 2005; Forouhar et al., 2006; Liebling et al., 2006). Similar studies have not been undertaken in mouse embryos because reporter lines with robust expression in the early endocardium have not been available for similar analysis. Here we have show that Tg(Flk1::myr-mCherry) embryos are suitable for repeated imaging at 6 frames per second, opening the door for further analysis into the mechanisms of heart function and provide a vital marker for studying aberrant heart function in mutant models.

Virtually nothing is known about human embryonic cardiac function because the heart begins to beat in the first few weeks of pregnancy and imaging tools are insufficient to characterize early function. Thus, mouse models are an invaluable tool for understanding defects in critical aspects of development that can result in miscarriage or later cardiovascular abnormalities at birth. Imaging tools combined with high-contrast fluorescent protein reporter lines enable the advanced phenotypic analysis of mutant animals and can

provide significant insights into understanding how genetic signaling pathways and physiological inputs converge to regulate normal development.

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References

Conway SJ, Kruzynska-Frejtag A, Kneer PL, Machnicki M, Koushik SV. What cardiovascular defect does my prenatal mouse mutant have, and why? Genesis. 2003; 35:1–21. [PubMed: 12481294]

Daniel TO, Abrahamson D. Endothelial Signal Integration in Vascular Assembly. Annual Review of Physiology. 2000; 62:649–671.

Forouhar AS, Liebling M, Hickerson A, Nasiraei-Moghaddam A, Tsai H-J, Hove JR, Fraser SE, Dickinson ME, Gharib M. The Embryonic Vertebrate Heart Tube Is a Dynamic Suction Pump. Science. 2006; 312:751–753. [PubMed: 16675702]

Fraser ST, Hadjantonakis A-K, Sahr KE, Willey S, Kelly OG, Jones EAV, Dickinson ME, Baron MH. Using a histone yellow fluorescent protein fusion for tagging and tracking endothelial cells in ES cells and mice. genesis. 2005; 42:162–171. [PubMed: 15986455]

Hadjantonakis AK, Dickinson ME, Fraser SE, Papaioannou VE. Technicolour transgenics: imaging tools for functional genomics in the mouse. Nat Rev Genet. 2003; 4:613–625. [PubMed: 12897773]

Jones EA, Baron MH, Fraser SE, Dickinson ME. Dynamic in vivo imaging of mammalian hematovascular development using whole embryo culture. Methods Mol Med. 2005; 105:381–394. [PubMed: 15492409]

Jones EA, Crotty D, Kulesa PM, Waters CW, Baron MH, Fraser SE, Dickinson ME. Dynamic in vivo imaging of postimplantation mammalian embryos using whole embryo culture. Genesis. 2002; 34:228–235. [PubMed: 12434332]

Jones EAV, Baron MH, Fraser SE, Dickinson ME. Measuring hemodynamic changes during mammalian development. Am J Physiol Heart Circ Physiol. 2004; 287:H1561–1569. [PubMed: 15155254]

Kappel A, Ronicke V, Damert A, Flamme I, Risau W, Breier G. Identification of Vascular Endothelial Growth Factor (VEGF) Receptor-2 (Flk-1) Promoter/Enhancer Sequences Sufficient for Angioblast and Endothelial Cell-Specific Transcription in Transgenic Mice. Blood. 1999; 93:4284–4292. [PubMed: 10361126]

Kulesa PM. Developmental imaging: Insights into the avian embryo. Birth Defects Res C Embryo Today. 2004; 72:260–266. [PubMed: 15495184]

Lichtman JW, Fraser SE. The neuronal naturalist: watching neurons in their native habitat. Nat Neurosci. 2001; 4(Suppl):1215–1220. [PubMed: 11687832]

Liebling M, Forouhar AS, Gharib M, Fraser SE, Dickinson ME. Four-dimensional cardiac imaging in living embryos via postacquisition synchronization of nongated slice sequences. Journal of Biomedical Optics. 2005; 10:054001. [PubMed: 16292961]

Liebling M, Forouhar AS, Wolleschensky R, Zimmermann B, Ankerhold R, Fraser SE, Gharib M, Dickinson ME. Rapid three-dimensional imaging and analysis of the beating embryonic heart reveals functional changes during development. Developmental Dynamics. 2006; 235:2940–2948. [PubMed: 16921497]

Lucitti JL, Jones EAV, Huang C, Chen J, Fraser SE, Dickinson ME. Vascular remodeling of the mouse yolk sac requires hemodynamic force. Development. 2007; 134:3317–3326. [PubMed: 17720695]

Megason SG, Fraser SE. Digitizing life at the level of the cell: high-performance laser-scanning microscopy and image analysis for in toto imaging of development. Mech Dev. 2003; 120:1407–1420. [PubMed: 14623446]

Risau W. Mechanisms of angiogenesis. Nature. 1997; 386:671. [PubMed: 9109485]

Shaner NC, Campbell RE, Steinbach PA, Giepmans BNG, Palmer AE, Tsien RY. Improved monomeric red, orange and yellow fluorescent proteins derived from Discosoma sp red fluorescent protein. Nature Biotechnology. 2004; 22:1567–1572.

Yamaguchi TP, Dumont DJ, Conlon RA, Breitman ML, Rossant J. flk-1, an flt-related receptor tyrosine kinase is an early marker for endothelial cell precursors. Development. 1993; 118:489–498. [PubMed: 8223275]

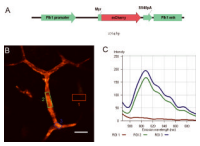

Figure 1. Flk1::myr-mCherry transgene

(A) Restriction map of the Flk1::myrm-Cherry construct that was used for injection and establishment of transgenic mouse line. (B) fluorescent image of an earpunch biopsy obtained from Tg(Flk1::myr-mCherry) mouse acquired in the emission fingerprinting mode at 543 nm excitation, and (C) corresponding emission spectra of the marked regions. Region 1 (red line) was selected outside of the vessel and corresponded to background autofluorescence; regions 2 (green) and 3 (blue) were selected on the vessel and correlating with emission spectrum of mCherry fluorescent protein. The scale bar corresponds to 20 μm.

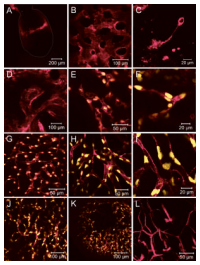

Figure 2. Expression of the mCherry fluorescent protein in the embryonic tissues

(A) blood islands of 7.5 dpc yolk sac; (B) 8.5 dpc yolk sac; (C) migrating cell in the 9.5 dpc yolk sac; (D) endocardium of 8.5 dpc embryo; (E–F) 9.5 dpc embryo trunk; (G) 12.5 dpc yolk sac; (H–I) 12.5 dpc embryo trunk; (J) 12.5 dpc embryonic brain; (K) 12.5 dpc embryonic eye; (L) adult ear. Images (E–K) correspond to embryos from a cross of Tg(Flk1::myr-mCherry) mice to earlier characterized Tg(Flk1::H2B-EYFP) line; yellow staining corresponds to nuclear-localized EYFP driven by the same Flk1 regulatory elements in the endothelial cells.

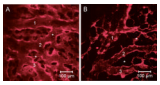

Figure 3. Vascular remodeling in the embryonic yolk sac

Images of an 8.5 dpc homozygous Tg(Flk1::myr-mCherry) embryo cultured on the microscope stage (A) at the beginning of culture and (B) 18 hours after the beginning of culture. The numbers indicate individual vessels aligned with the flow and asterisks mark the vessel branches that underwent regression during the 18 h period.

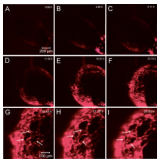

Figure 4. Time lapse imaging of embryonic yolk sac during 7.5 dpc embryo culture on the microscopic stage

(A–F) Formation of vascular plexus in the embryonic yolk sac; images are presented for 4 hour intervals. (G–I) Vascular sprouting in the embryonic yolk sac. Sprouting events and forming vascular branches are indicated with arrows.

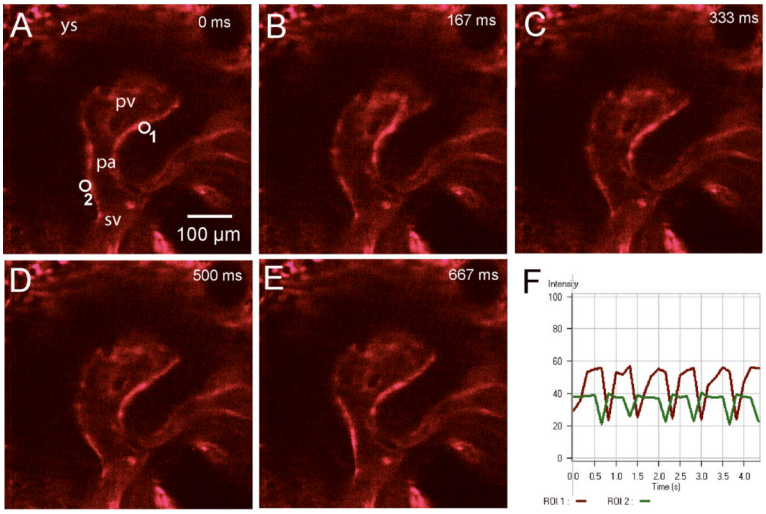

Figure 5. Time lapse imaging of embryonic cardio dynamics

(A–E) Images of an 8.5 dpc beating heart showing different phases of cardiac cycle. (F) An average fluorescence intensity of regions marked with circles (1 and 2) in (A) is plotted vs. time showing that the fluctuation in intensity, as the tissue layer moves, can be used to reveal the periodicity of the cardiac cycle. In (A), *ys* – yolk sac, *sv* – sinus venosus, *pa* – primitive atrium, *pv* – primitive ventricle.