## Supplementary materials

# Embryonic hematopoiesis in vertebrate somites gives rise to definitive hematopoietic stem cells

Juhui Qiu[1*], Xiaoying Fan[2], Yixia Wang[1], Hongbin Jin[1], Yixiao Song[1], Yang Han[3], Shenghong Huang[1], Yaping Meng[1], Fuchou Tang[2], Anming Meng[1*]

[1]State Key Laboratory of Membrane Biology, Tsinghua-Peking Center for Life Sciences, School of Life Sciences, Tsinghua University, Beijing 100084, China

[2]Biodynamic Optical Imaging Center and Department of Obstetrics and Gynecology, College of Life Sciences, Third Hospital, Peking University, Beijing 100871, China; Ministry of Education Key Laboratory of Cell Proliferation and Differentiation, Beijing 100871, China; Peking-Tsinghua Center for Life Sciences, Peking University, Beijing 100871, China; Center for Molecular and Translational Medicine, Peking University Health Science Center, Beijing 100191, China.

[3]College of Biological Sciences, China Agricultural University, Beijing 100083, China.

**Corresponding authors:** Anming Meng, E-mail: mengam@mail.tsinghua.edu.cn; and Juhui Qiu: E-mail: jhqiu2008@126.com; Tel: 86-10-62772257.

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## Supplementary Table

**Table S1.** RNA seq data from four types of cells.

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**Movie S1.** GFP-positive cells circulate through the heart in a 36-hpf *Tg(ripply1:gfp)* transgenic embryo.

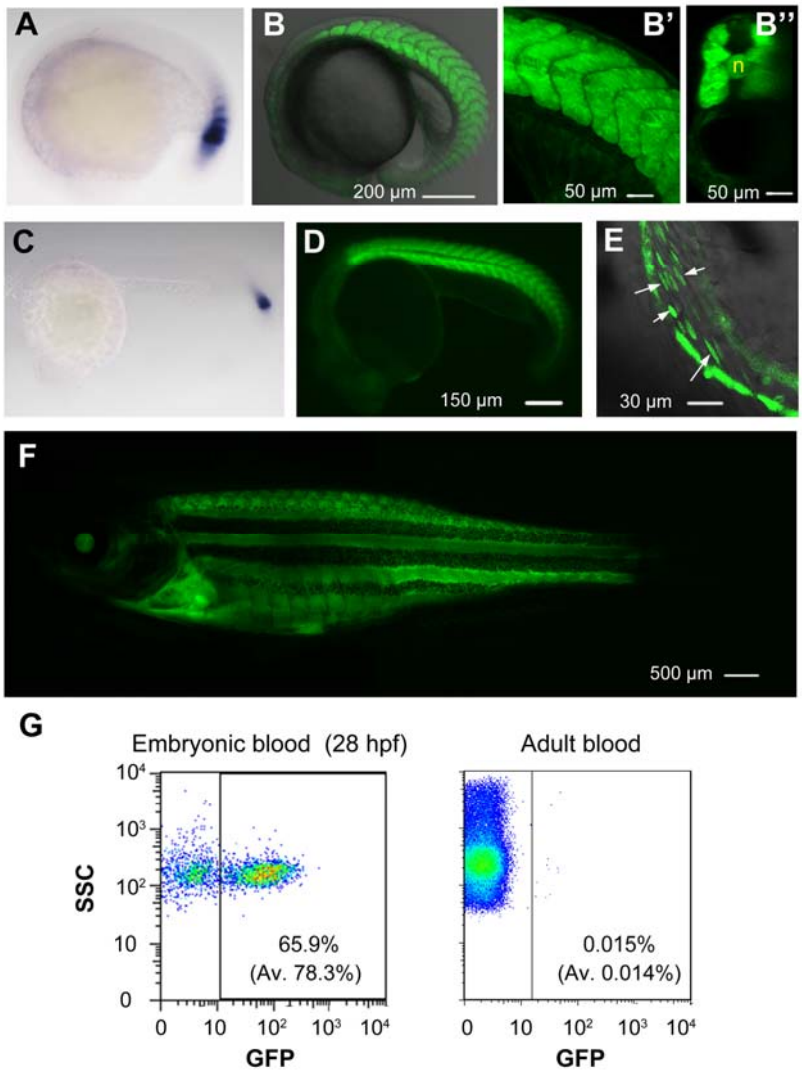
**Movie S2.** GFP-positive cells circulate through the heart in a 36-hpf *Tg(fmyhc2:gfp)* transgenic embryo.

**Movie S3.** EOS-positive cells in somites migrate into intermediate cell mass in a

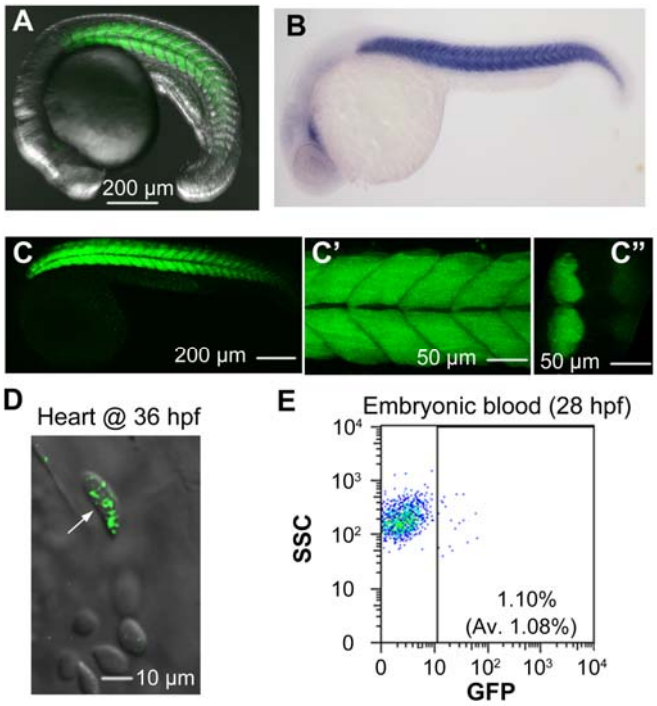
*Tg(foxc1b:EOS)* transgenic embryo.

**Movie S4.** Medial migration of photoactivated somitic cells in the middle region of somites.

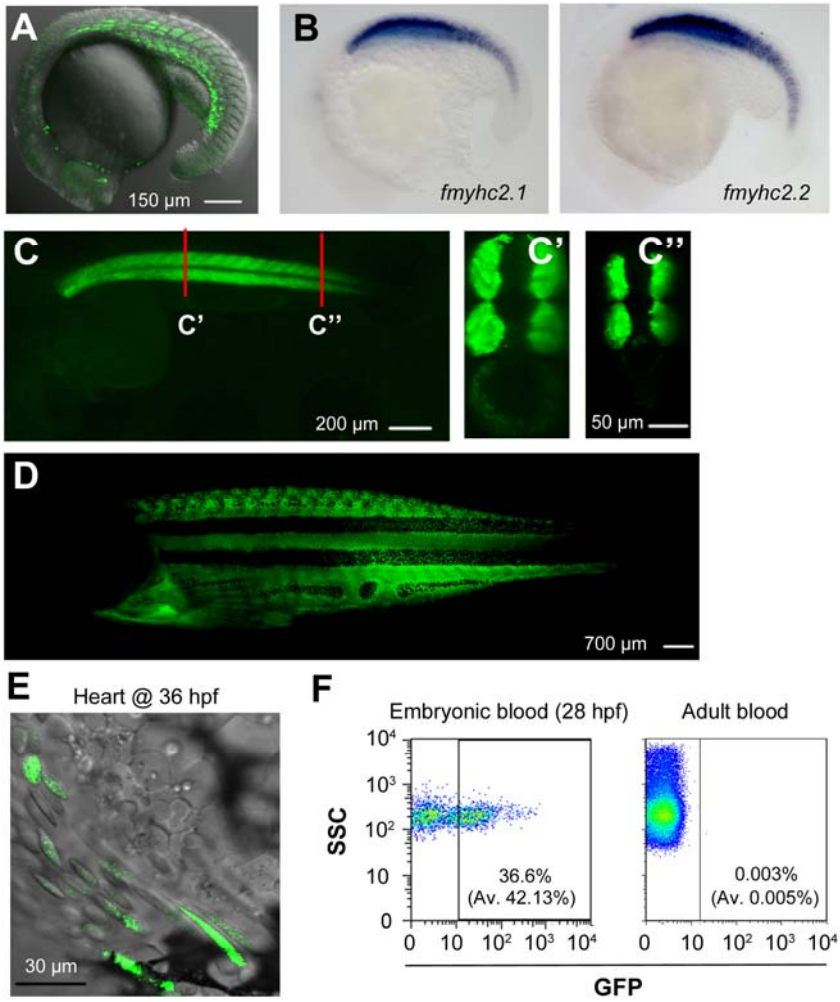
**Movie S5.** red-EOS$^{+}$ blood cells circulate through the heart in a 36-hpf *Tg(foxc1b:EOS;fli1a:gfp)* transgenic embryo.



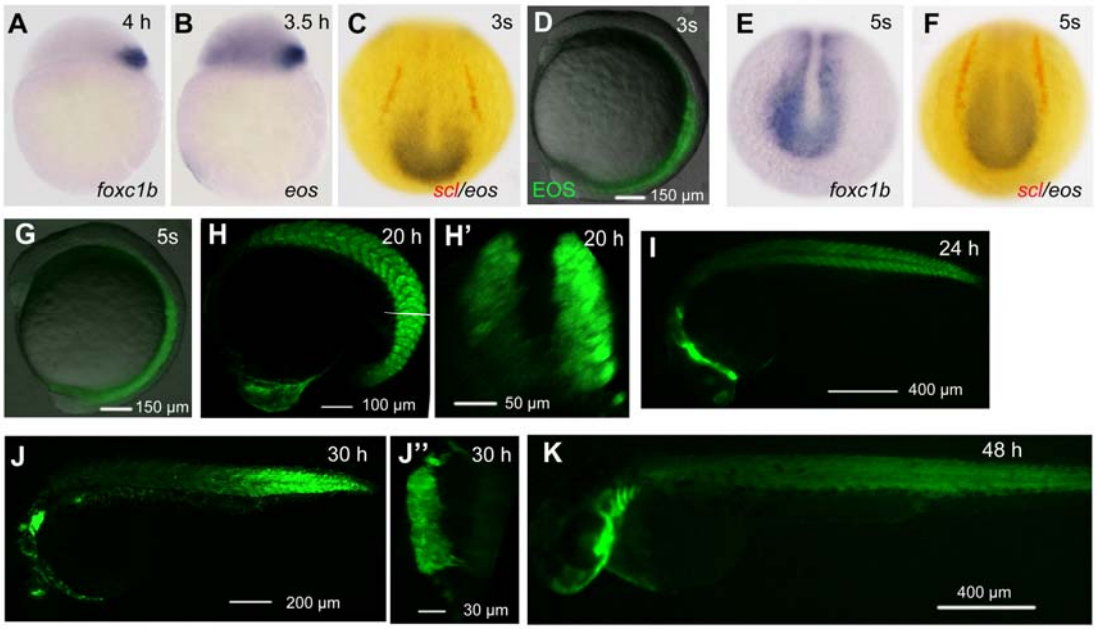

**Supplementary Figure S1. GFP expression pattern in *Tg(ripply1:gfp)* transgenic line.** (**A** and **C**) Detection of *ripply1* expression in nascent somites of wild-type embryos at 22s stage (**A**) and at 24 hpf (**C**) by in situ hybridization. (**B-B"**) Confocal image of GFP expression in somites of a *Tg(ripply1:gfp)* embryo at 22s stage (**B**). The enlarged trunk region (**B'**) and an optical cross section (**B"**) were shown. n, notochord. (**D**) GFP expression in transgenic embryos at 24 hpf. (**E**) $GFP^+$ circulating hematopoietic cells (indicated by arrows) in the heart were observed by confocal microscopy at 36 hpf. See also Movie S1. (**F**) GFP was still expressed in adult fish. (**G**) Flow cytometry results showing the existence of $GFP^+$ blood cells in the embryonic circulation at 28 hpf (left) and in the circulation of adults (right). Embryonic blood cells were sucked from the hearts of 5-10 embryos at 28 hpf. Adult blood cells were taken from the heart. The showed were representative results with average (Av) from 3 independent experiments in parenthesis.



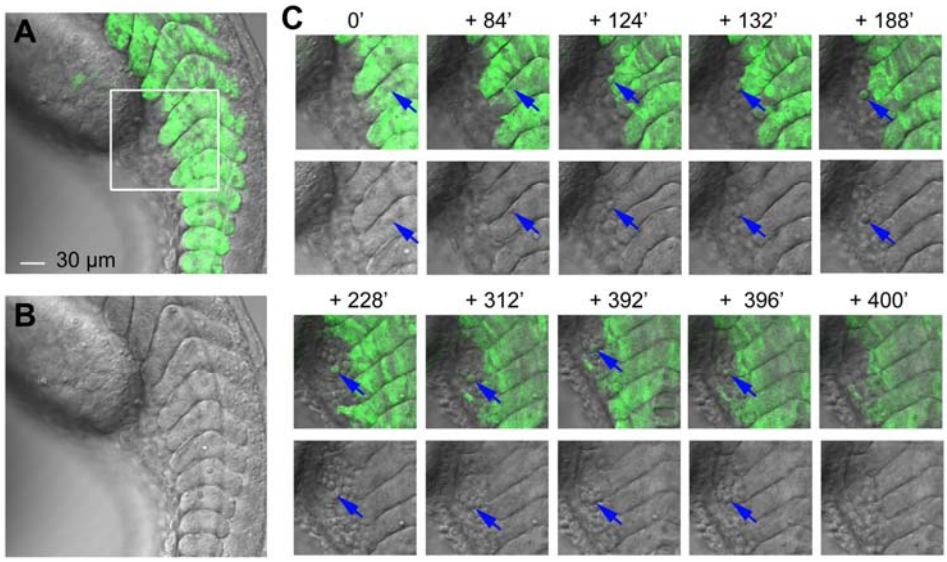

**Supplementary Figure S2. GFP expression pattern in *Tg(rbfox1l:gfp)* transgenic line.** (**A**) Confocal image showing GFP expression in somites of a *Tg(rbfox1l:gfp)* transgenic embryo at 22s stage. (**B**) The expression pattern of endogenous *rbfox1l* in wild-type embryo at 28 hpf, revealed by in situ hybridization. (**C-C"**) GFP expression in somites of a transgenic embryo at 28 hpf (**C**). The enlarged trunk region (**C'**) and an optical cross section (**C"**) were shown. (**D**) $GFP^+$ circulating hematopoietic cells (indicated by an arrow) in the heart were observed by confocal microscopy at 36 hpf. (**E**) Flow cytometry result showing the existence of $GFP^+$ blood cells in the embryonic circulation. Embryonic blood cells were sucked from the hearts of 5-10 embryos. A representative result was shown with the average (Av) from 3 independent experiments in parenthesis.



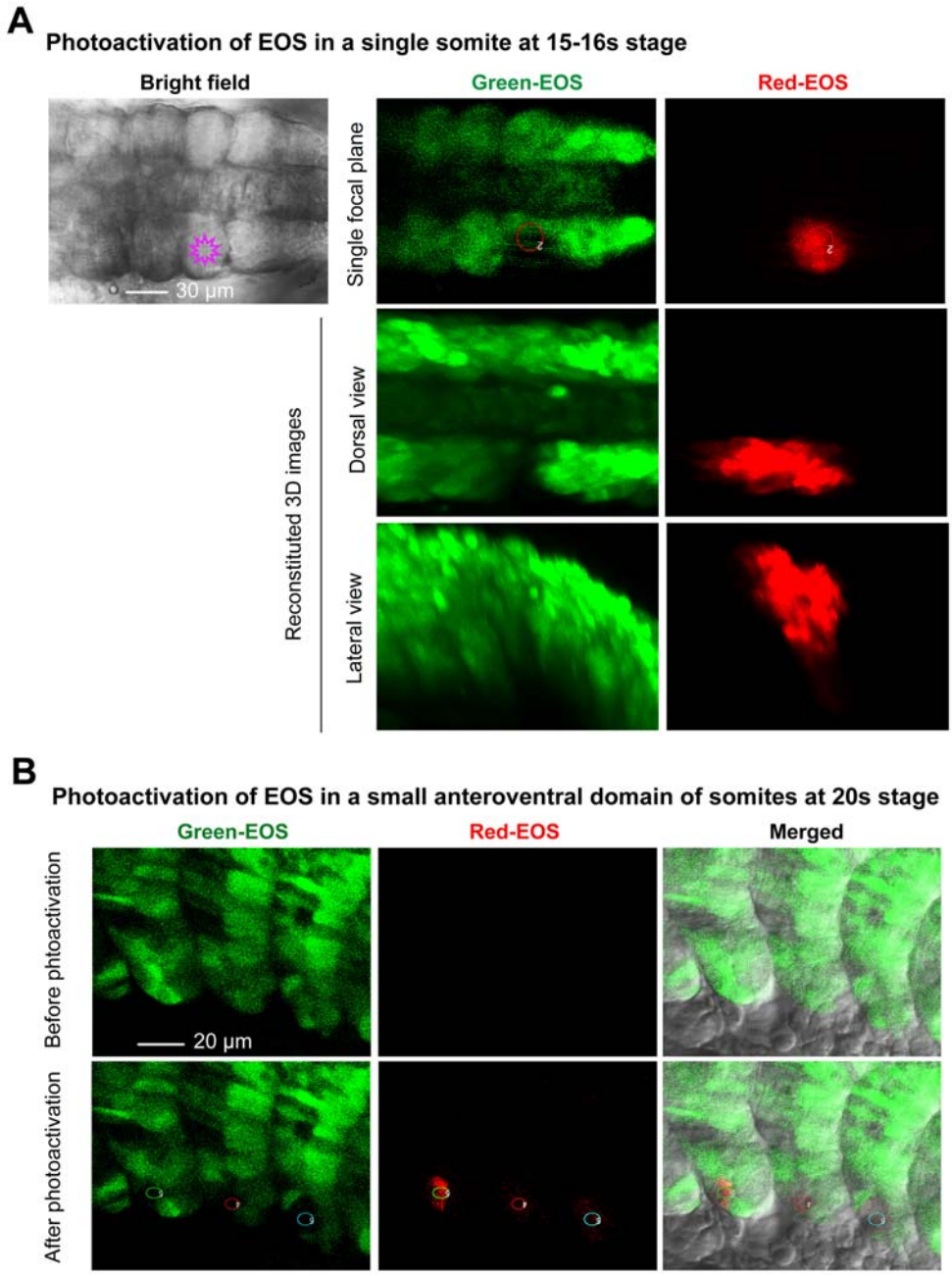

**Supplementary Figure S3. GFP expression pattern in *Tg(fmyhc2:gfp)* transgenic line.** In this line, the Tol2-based enhancer trap element was inserted in between the fast muscle heavy chain genes *fmyhc2.1*/*myhz2* and *fmyhc2.2*/*myhc4*. (**A**) GFP expression in somites of a transgenic embryo at 22s stage. (**B**) Endogenous *fmyhc2.1* and *fmhc2.2* genes share a similar expression pattern in 22s wild-type embryos. (**C-C"**) GFP expression pattern in a 28-hpf embryo with two optical cross sections (**C'**, **C"**) at indicated positions. Note that GFP expression was restricted to somites. (**D**) GFP expression in adult fish (laterally positioned). (**E**) Some $GFP^+$ hematopoietic cells were circulating through the heart in an embryo at 36 hpf. See also Movie S2. (**F**) Flow cytometry results showing the existence of $GFP^+$ blood cells in the embryonic circulation (left) and in the circulation of adults (right). Embryonic blood cells were sucked from the hearts of 5-10 embryos at 28 hpf. Adult blood cells were taken from the heart. The showed were representative results with average (Av) from 3 independent experiments in parenthesis.



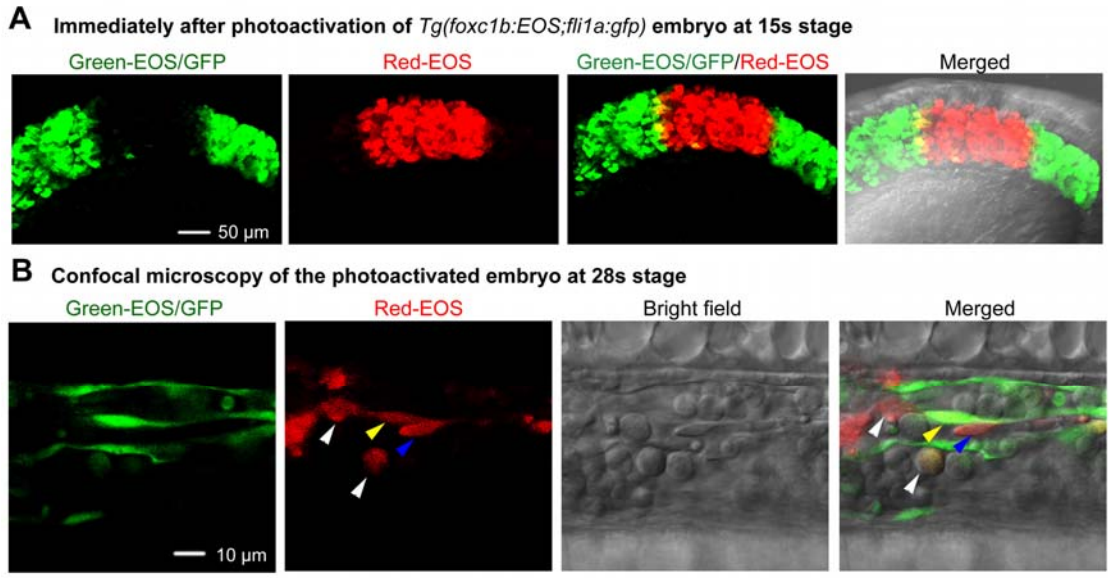

**Supplementary Figure S4. EOS expression pattern in *Tg(foxc1b:EOS)* transgenic line.** This line was identified from the founder fish that was injected with the *Tol2(foxc1b:EOS)* construct at the one-cell stage. The expression of EOS was driven by a 3.6-kb promoter of the *foxc1b* gene. (**A**, **B**, **E**) Expression patterns of *foxc1b* mRNA in wild-type embryos (**A** and **E**) and *eos* mRNA in *Tg(foxc1b:EOS*) embryo (**C** and **F**). The expression of *scl* (red) and *eos* mRNAs (black/blue) was detected by double in situ hybridization. The posterior part of the embryos in (**C**, **E** and **F**) was dorsally viewed. The stages were indicated. (**D** and **G-K**) Confocal images showing EOS expression in transgenic embryos at indicated stages. Embryos were laterally viewed. (**H'**) An optical cross section at the position indicated in (**H**). (**J'**) An optical cross section was reconstituted from the posterior trunk region of the embryo shown in (**J**).



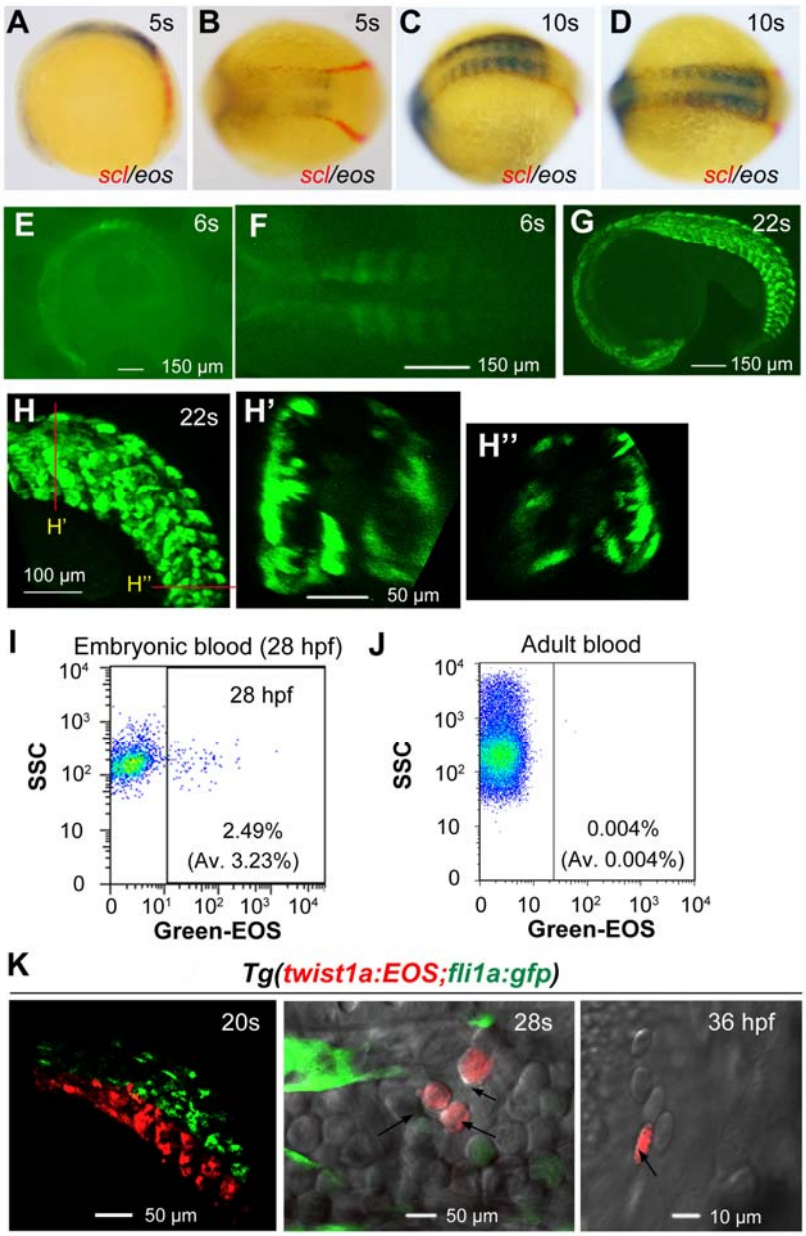

**Supplementary Figure S5. Emigration of an EOS-positive cell from the ventral domain of the somite in *Tg(foxc1b:EOS)* transgenic embryo.** The embryo was laterally orientated and observed continuously from 22 to 30 hpf with focus on posterior somites by confocal microscopy. The imaging time points were indicated. An emigrating cell was indicated by arrows. See also Movie S3. Note that the embryo looked younger because its posterior trunk could not extend well when it was embedded in agarose gel. Beside, the tracked cell was vanished in the last frame most likely because it had flowed away with the circulation.




**Supplementary Figure S6. Photoactivation of the reporter EOS in defined embryonic locations.** *Tg(foxc1b:EOS)* transgenic embryos were used throughout. (**A**) Photoactivation of a single somite at 15-16s stages. The irradiated somite on one side was marked in the left bright-field image (dorsal view). The green EOS in the exposed area started to emit red fluorescence immediately after irradiation (right panel). The embryo was orientated with anterior to the left. Note that fluorescent cells in between the paraxial mesoderm (in the middle panel) should be emigrating somitic cells, either sclerotomal or transdifferentiating hematopoietic cells. (**B**) A small anteroventral region of three consecutive somites of an embryo at 20s stage was irradiated and red fluorescence was detected by confocal microscopy immediately.

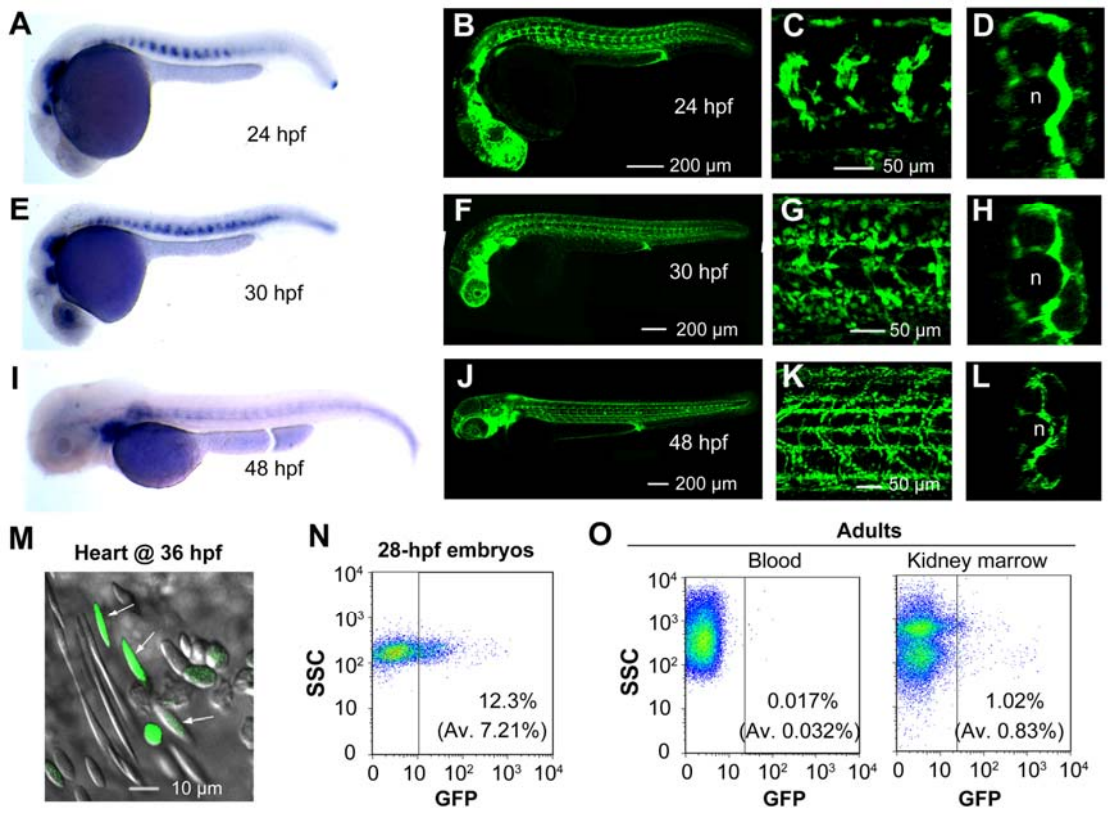



**Supplementary Figure S7. Cells of somite origin transform into hematopoietic cells, endothelial cells or pericytes.** *Tg(foxc1b:EOS;fli1a:gfp)* double transgenic embryos were used. (**A**) Four pairs of somites in the middle trunk of an embryo at 15s stage were irradiated by laser and imaged immediately by confocal microscopy (lateral view). (**B**) The trunk ventral region of the photoactivated embryo was observed by confocal microscopy at 28s stage. The embryo was orientated with anterior to the left. The red-EOS$^{+}$;GFP$^{+}$ blood cells and endothelial cells were indicated by white and yellow arrowheads respectively, and a red-EOS$^{+}$ cell of unknown identity was indicated by a blue arrowhead.

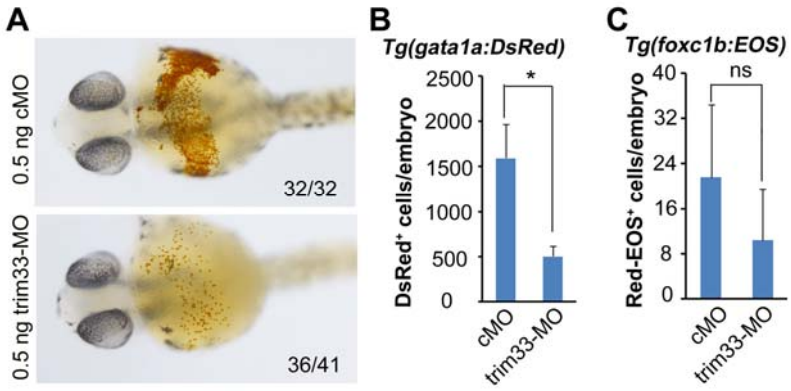



**Supplementary Figure S8. EOS expression pattern in *Tg(twist1a:gfp)* transgenic line.** This line was identified from the founder fish that was injected with the *Tol2(twist1a:EOS)* construct at the one-cell stage. The expression of EOS was driven by a 4.9-kb promoter of the *twist1a* gene. (**A**-**D**) The expression of *scl* (red) and *eos* (black/blue) mRNAs in the transgenic embryos at indicated stages, as detected by double in situ hybridization. The embryos were orientated with anterior to the right and viewed either laterally (**A**) or dorsally (**B**-**D**). (**E**-**H”**) The fluorescence of EOS protein in the transgenic embryo at indicated stages. (**E** and **F**) Weak EOS expression was observed by confocal microscopy in a transgenic embryo at 6s stage. The embryo was laterally (**E**) or dorsally (**F**) viewed with anterior to the left. (**G**) EOS was expressed in the migrating sclerotomal cells in a 22s embryo. (**H**-**H”**) A trunk region of the embryo shown in (**G**) was enlarged (**H**) and two optical cross sections at indicated positions were shown in (**H’** and **H”**). (**I**) The proportion of $EOS^{+}$-blood cells in the circulation of *Tg(twist1a:EOS)* embryonic hearts at 28 hpf was analyzed

by flow cytometry. The average (Av) was the mean of three independent experiments, each including 5-10 embryos. The showed was the representative result of one experiment. (**J**) Green-EOS$^{+}$ cells in the circulation of *Tg(twist1a:EOS)* transgenic adult was analyzed by flow cytometry. The showed was the representative result from a single fish with the average from three fish in parenthesis. (**K**) Photoconverted red-EOS$^{+}$ sclerotomal cells gave rise to hematopoietic cells. The ventral region of posterior somites of *Tg(twist1a:EOS;fli1a:gfp)* embryos at 20s stage was photoconverted (left) and red-EOS$^{+}$ (indicated by arrows) was found in the ICM at 28s stage (middle) and in the heart at 36 hpf.




**Supplementary Figure S9. GFP expression pattern in *Tg(pax1a:gfp)* transgenic line.** This line was identified from the founder fish that was injected with the *Tol2(pax1a:gfp)* construct at the one-cell stage. The expression of GFP was driven by a 2.4-kb promoter of the *pax1a* gene. (**A, E** and **I**) in situ hybridization indicated the expression of endogenous *pax1a* in the sclerotome of wild-type embryos at 24 hpf (**A**), 30 hpf (**E**) and 48 hpf (**I**). (**B-D**) GFP expression in transgenic embryos at 24 hpf. A trunk region (**C**) and an optical cross section (**D**) of the same embryo (**B**) were shown. n, notochord. (**F-H**) GFP expression in transgenic embryos at 30 hpf. A trunk region (**G**) and an optical cross section (**H**) of the same embryo (**F**) were shown. (**J-L**) GFP expression in transgenic embryos at 48 hpf. A trunk region (**K**) and an optical cross section (**L**) of the same embryo (**J**) were shown. (**M**) A $GFP^+$ circulating blood cells (indicated by arrows) was observed by confocal microscopy in the heart at 36 hpf. (**N**) Flow cytometry result showing the proportion of $GFP^+$ blood cells in embryos at 28 hpf. Blood cells were taken from the heart of 5-10 embryos at 28 hpf. The average in parenthesis was the mean of three experiments. (**O**) Flow cytometry results showing the proportion of $GFP^+$ cells in adult blood (left) and kidney marrow (right). The showed were representative results from single fish. The average from three fish was shown in parenthesis.




**Supplementary Figure S10. Blockage of primitive erythropoiesis by *trim33* knockdown has little effect on somite hematopoiesis.** (**A**) Effect of *trim33* knockdown on primitive erythropoiesis. Wild-type embryos at the one-cell stage were injected with the control MO (cMO) or trim33-MO and collected at 30 hpf for detection of hemoglobin by *O*-dianisidine staining. The embryos were ventrally viewed with anterior to the left. The ratio of embryos with the representative pattern was indicated. Note that the number of red-blood cells was drastically reduced in *trim33* morphants, indicating an effective knockdown. (**B** and **C**) Effect of *trim33* knockdown on LPM (**B**) and somite hematopoiesis (**C**). *Tg(gata1a:DsRed)* (**B**) or *Tg(foxc1b:EOS)* (**C**) embryos at the one-cell stage were injected with corresponding MOs. For injected *Tg(foxc1b:EOS)* embryos, five pairs of new born somites were photoactivated at 20s stage. The circulating blood cells taken from a group of 5 embryos at 30 hpf were sorted by flow cytometry and the number of DsRed$^+$ (**B**) or red-EOS$^+$ cells (**C**) per embryo was averaged from three independent experiments. *, statistically significant with $p < 0.05$); ns, non-significance, $p > 0.05$.

**Table S1. RNA seq data from four types of cells.** S1 and S2, green-EOS$^+$ sHPSCs; S3 and S4, red-EOS$^+$ sHPSCs; S5 and S6, red-EOS$^+$ somitic cells; S7 and S8, gata1a$^+$ erythroblasts. The detail of each type was described in Figure 5.

**Movie S1. GFP-positive cells circulate through the heart in a 36-hpf *Tg(ripply1:gfp)* transgenic embryo.** The embryo was orientated with anterior to the left and the heart chamber region was focused.

**Movie S2. GFP-positive cells circulate through the heart in a 36-hpf *Tg(fmyhc2:gfp)* transgenic embryo.** The embryo was orientated with anterior to the left and the heart chamber region was shown.

**Movie S3. EOS-positive cells in somites migrate into intermediate cell mass in a *Tg(foxc1b:EOS)* transgenic embryo.** The embryo was embedded in agarose gel with anterior to the left. The tail region was observed continuously from 22 to 30 hpf by confocal microscopy. A ventrally migrating cell was indicated by an arrow. Note that the embryo looked younger because its posterior trunk could not extend well when it was embedded in agarose gel.

**Movie S4. Medial migration of photoactivated somitic cells in the middle region of somites.** The middle region of about 10 somites in a *Tg(foxc1b:EOS)* embryo at 25s stage was irradiated and observed by confocal microscopy.

**Movie S5. red-EOS$^{+}$ blood cells circulate through the heart in a 36-hpf *Tg(foxc1b:EOS;fli1a:gfp)* transgenic embryo.** Five nascent somites were photoactivated by exposure to laser and the heart chamber region was observed by confocal microscopy at 36 hpf. The embryo was orientated with anterior to the left. Note that red-EOS$^{+}$ blood cells were derived from photoactivated somites and green fluorescent blood cells could be either green-EOS$^{+}$ (non-photoconverted) or GFP$^{+}$.