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Generation and Characterization of Blood Vessel Specific EGFP Transgenic Zebrafish *via Tol2* Transposon Mediated Enhancer Trap Screen*

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Abstract Cardiovasculature forms during early stages of embryonic development and enables other organs to develop, maintain and regenerate. Imbalanced growth of blood vessels can give rise to numerous pathological disorders. However, the genes involved in blood vessel development remain largely elusive. Zebrafish (*Danio rerio*) is an ideal vertebrate model organism for the study of developmental processes, especially for that of cardiovascular formation. 26 transgenic fish lines with blood vessel-specific EGFP expression were identified *via* a large scale enhancer trap screen mediated by *Tol2* transposon in zebrafish. The EGFP expression in some of these lines shows different and unique patterns in different part of blood vessels. The genomic sequences flanking the *Tol2* insertion sites have been successfully cloned from 22 lines *via* linker-mediated PCR, among which 17 sequences could be mapped to a unique location within current zebrafish genome assembly. Expression of 8 flanking genes from 9 transgenic lines was confirmed to recapitulate the expression of EGFP reporter gene in their corresponding lines *via* RNA whole mount *in situ* hybridization (ISH). Three of these genes, *hhex*, *ets1a* and *dusp5* are known to be important for vasculargenesis. Since *hhex* and *ets1a* are also expressed in hematopoietic precursors, these transgenic zebrafish should be very useful for the study of both hematopoiesis and vasculargenesis. The rest of these genes, namely *zvsg1*, *micall2a*, *arl8b* (*1* of *2*), *zgc*:*73355* and *hecw2* (*1* of *2*), are either novel or functionally unknown in zebrafish. Further investigation of these fish lines and corresponding genes will give important insights of blood vessel developmental mechanisms including hemangioblast formation and differentiation, as well as genes and enhancer elements important for cardiovascular system. In addition, these transgenic fish lines could also make invaluable contributions to small molecule screen for drug discovery.

Key words zebrafish, *Tol2*, enhancer trap, blood vessel, transgene
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Cardiovasculature has very important functions in both physiological and pathological processes. In embryos, the vasculature system forms and starts to function at very early stage. Besides their normal physiological functions in supporting blood circulation, blood vessels also secrete nutrient factors to guide the formation of other organs. However, due to the limitation of experimental models, the studies of embryonic blood vessel formation are relatively inadequate compared with those in adults. Zebrafish (*Danio rerio*) shows great advantages for vasculature study. Their *ex vivo* fertilization and development, as well as transparency of the embryo makes the observation of early organ formation easier than many other model organisms. Their tiny bodies and capability of laying large number of eggs make large

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scale genetic screening as feasible as in *Drosophila* and other invertebrate models. Moreover, zebrafish embryos can survive for several days without circulation, while in mice this defect will lead to immediate death of embryo [1]. This provides a good opportunity to dissect causes and mechanisms underlying the embryonic lethal phenotypes, which is impossible in mice [2]. Thus, zebrafish is an excellent model for the study of blood vessel development.

Here we report the screening for blood vessel specific transgenic zebrafish *via Tol2* transposon mediated enhancer trap, as well as novel or functionally unknown genes that are potentially important for blood vessel development. We obtained 26 different enhancer trap lines, including *Et* (*hhex*:*EGFP*) and *Et*(*ets1a*:*EGFP*) (ET represents enhancer trap) which label progenitor cells for both blood vessels and blood, and five genes whose functions have not been characterized in zebrafish. Moreover, some of our transgenic fish lines exhibit diverse and unique expression patterns in different sections of blood vessel system, which could facilitate the study of specific tissues in details as well as small molecule screen. Our results could also facilitate studies on gene function and regulation and even mutagenesis of specific genes to elucidate the mechanisms of blood vessel development.

1 Materials and methods

1.1 Generation of enhancer trap constructs

The *Tol2*-GT2MP construct used for enhancer trap was derived from T2KXIG[3] and comprises a 792 bp fragment containing a 249 bp minimal promoter, exon 1, intron 1 and partial exon 2 of the zebrafish *gata2* gene followed by the coding region of EGFP reporter gene. The mRNA encoding *Tol2* transposase was synthesized *in vitro* using pCS-TP [3] and the mMACHINE SP6 kit (Ambion, Inc) and purified with the RNeasy Mini Kit (QIAGEN).

1.2 Screening for transgenic fish lines and imaging for live embryos

Adult zebrafishes and embryos were maintained at 28.5℃ following standard protocol[3]. Embryos injected with *Tol2*-GT2MP plasmid and transposase mRNA were raised to adulthood as founders and crossed to get F_1 embryos. The embryos were observed and selected at 24 h post-fertilization(hpf) under the Axioimager A1, Z1 or Axiovert 200 fluorescence microscope (Zeiss, Germany) equipped with 5×, 10× and 20× objectives, and filter set 10 (excitation: 450～490 nm, barrier: 510 nm, emission: 515～565 nm) was used for the detection of EGFP expression. Images of live embryos with specific green fluorescent signal were acquired with AxioCam MRm or AxioCam MRc5 and AxioVision AC software.

1.3 Linker-mediated PCR

For the identification of the genomic sequences flanking the *Tol2* insertions, genomic DNA from 3 EGFP positive F_2 embryos and a pool of 6~10 negative embryos (as control) were extracted separately for each fish line. 50 μl of lysis buffer (10 mmol/L Tris pH 8.2, 10 mmol/L EDTA, 200 mmol/L NaCl, 0.5% SDS, 200 mg/L proteinase K) was added to each embryo and the DNA was precipitated by ethanol after overnight incubation at 50℃.

Linker-mediated PCR (LM-PCR) was carried out by a protocol modified from Wu *et al*.[4] Linker 1 and linker 2 (Table S1, See Supplement online, http://www.pibb.ac.cn/cn/ch/common/view_abstract.aspx?file_no=20100301&flag=1) were dissolved in 100 mmol/L STE buffer (10 mmol/L Tris pH 8.0, 50 mmol/L NaCl, 1 mmol/L EDTA) separately. A mixture of 40 μl linker 1 and 40 μl linker 2 was denatured at 98℃ and annealed to 4℃ at a rate of 1℃/min. Genomic DNA were diluted to 5～10 mg/L, digested by an appropriate restriction enzyme (*e. g.*, *Mse* Ⅰ, *Bgl* Ⅱ or *Sau*3AI, NEB, Inc) at 37℃ for 4 h and ligated with the annealed linker mixture by T4 ligase (NEB, high concentration) at 16℃ overnight. Nested PCR for the amplification of the 3′ junctions were carried out by primer pairs Afl5 and *Tol3* in the first round and Afl5nest and *Tol3*nest in the second round (Table S1). The amplification of the 5′ junctions were carried out by primer pairs Afl5 and *Tol5* in the first round and Afl5nest and *Tol5*nest in the second round (Table S1). The products from the final reaction were separated by 3% agarose gel and the band present in all three EGFP positive embryo samples and absent in the negative control was chosen for cloning into the pMD18-T vector (Takara, Japan) and subsequent sequencing. Genomic sequences flanking the *Tol2* insertion sites were aligned to Ensembl zebrafish genome through BLASTN (http://www.ensembl.org/). A scheme was provided to show the principle of LM-PCR and relative positions of all the primers in Figure S1 (See Supplement online, http://www.pibb.ac.cn/cn/ch/common/view_abstract.aspx?file_no = 20100301& flag=1).

1.4 RNA whole-mount *in situ* hybridization

RNA whole-mount *in situ* hybridization for *hhex*, *ets1a*, *dusp5*, *zvsg1*, *micall2a*, *arl8b* (*1* of *2*), *zgc:73355* and *hecw2* (*1* of *2*) was performed as described previously[5]. The cDNA (partial) of these genes were amplified from total RNA of 24 hpf embryos by TRIZOL extraction followed by reverse transcription (Promega, primers shown in Table S1). Digoxigenin-UTP (Roche) labeled antisense RNA probe was generated by *in vitro* transcription (Promega) and subjected to purification (QIAGEN). The hybridization signals were developed *via* anti-digoxingenin-AP (Roche) and purple AP substrate (Promega). Photographs were taken using AxioCam MRc5 with AxioVision AC software (Zeiss, Germany).

2 Results

2.1 Generation and identification of blood vessel specific transgenic fish lines

To create a collection of blood vessel specific transgenic zebrafish as well as to identify genes with blood vessel specific expression patterns, we performed a large-scale enhancer trap screen in zebrafish *via Tol2* transposon (unpublished data). *Tol2* transponson mediates efficient insertion of exogenous DNA into zebrafish genome, and has no significant preference on sequences for its insertion[6]. In addition, the insertion sites and the flanking genomic sequences can be easily identified *via* PCR[6]. To carry out the enhancer trap, we modified *Tol2* transponson[3] by inserting an EGFP reporter gene driven by zebrafish *gata2* minimal promotor, and co-injected the modified construct together with *in vitro* synthesized *Tol2* transposase mRNA into 1-cell stage zebrafish embryos. By incrossing the founder fish, we obtained ~1 000 F_1 fish lines with specific EGFP expression (unpublished data), among which 26 lines exhibit EGFP expression in blood vessels [partially shown in Figure 1 and Figure 2, and detailed descriptions on the expression patterns were shown in Table S2 (See Supplement online, http://www.pibb.ac.cn/cn/ch/common/view_abstract.aspx?file_no=20100301 &flag=1)]. While many of the transgenic lines display EGFP expression in the whole blood vessel system, there are also some fish

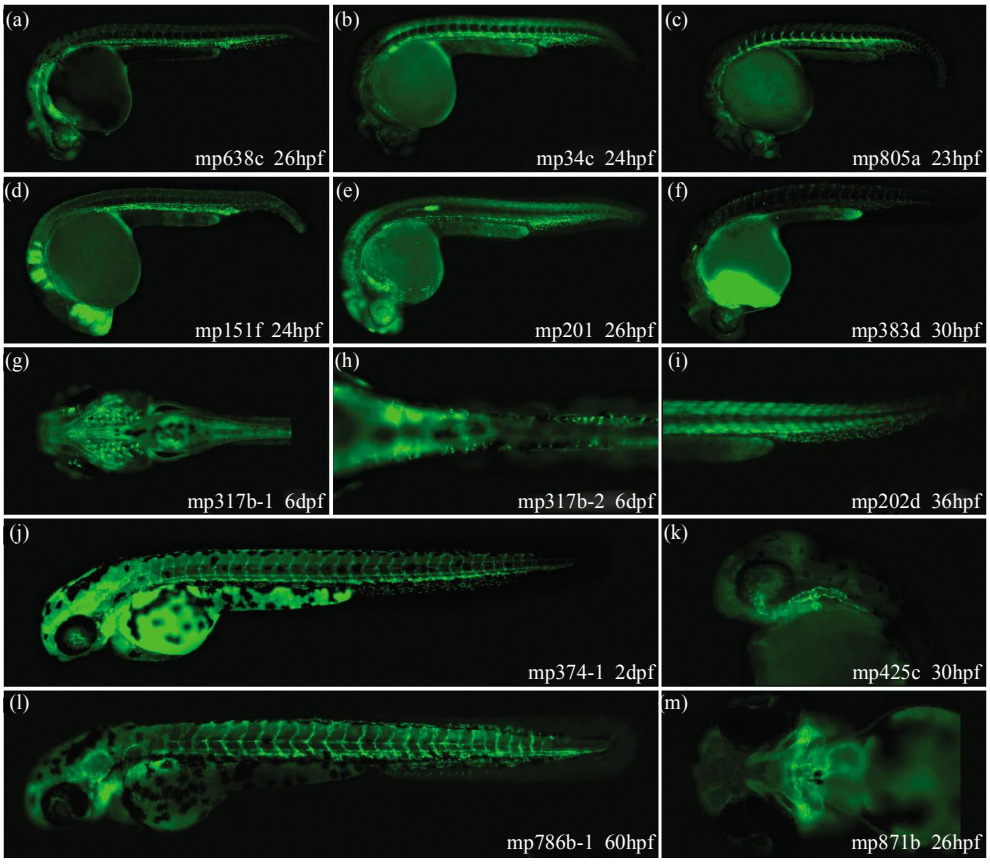

Fig. 1 Representative expression patterns of blood vessel-specific transgenic fish lines during early embryonic development stages

The names of each fish line as well as the corresponding developmental stage are shown at the lower right corner. hpf, hours post fertilization. dpf, days post fertilization.

lines showing interesting expression patterns in specific locations. Lines mp317b-1 (Figure 1g), mp425c (Figure 1k) and mp871b (Figure 1m) show distinct EGFP patterns of blood vessels in gill. Green fluorescent signals of mp317b-2 were found in blood vessels from gut (Figure 1h). mp202d show strong EGFP expression in posterior cardinal vein (Figure 1i). mp374-1 (Figure 1j) and mp786b-1 (Figure 1l) only show strong EGFP expression in blood vessels starting from 2dpf.

To identify the endogenous genes that recapitulate each EGFP expression pattern, the genomic sequences flanking the *Tol2* insertion sites were cloned by linker-mediated PCR (LM-PCR). The genomic flanking sequences from 22 lines were successfully recovered, among which 17 sequences could be mapped to a unique location within current zebrafish genome assembly (Zv8). The enzymes used for digesting the genome of each fish line are shown in Table S2 and the cloning results are shown in Table S2 and S4 (See Supplement online, http://www.pibb.ac.cn/cn/ch/common/view_abstract.aspx?file_no=20100301&flag=1). The endogenous genes corresponding to the observed reporter gene expression pattern were searched by BLASTN and verified *via* ISH.

2.2 Transgenic fish lines with known important genes

Four out of these 26 fish lines, mp378b, mp430c-2, mp380g-1 and mp638c, carry *Tol2* insertions within or close by and therefore recapitulate the expressions of well characterized genes in zebrafish (Table 1). In addition, mp378b and mp430c-2 show EGFP expression in both blood and vessels, potentially label progenitor cells for both lineages (Figure 2a, b, d and e). mp378b most likely trapped the enhancer of *hhex* (*hematopoietically expressed homeobox*, Figure 2c), a gene encoding a transcription factor which acts downstream of *cloche* to regulate the differentiation of both endothelial and hematopoietic cells together with *scl* [7]. The EGFP expression pattern of mp378b fully recapitulates that of *hhex*, including RBI (rostral blood island, Figure 2a) and liver primordium (Figure 2b). mp430c-2 trapped the enhancer of another transcription factor encoding gene *ets1a* (*erythroblastosis virus oncogene homolog E twenty-six-1a*, Figure 2f) with *Tol2* element inserted in its first intron (Table 1). Mouse Ets1a has been shown to regulate *Lmo2* together with Fli1 and Elf1 in hematopoietic progenitors and endothelial cells [8]. In particular, The EGFP expression pattern of mp430c-2 fully recapitulates the expression of *ets1a* in ICM, cardiovasculature and neural crest cells (Figure 2e, f). To our knowledge, these are the first transgenic zebrafish reported to recapitulate the expression patterns of *hhex* and *ets1a*, respectively.

We obtained two independent fish lines, mp380g-1 (data not shown) and mp638c (Figure 1a), that trapped the enhancer of the same gene, *dusp5* (*dual specificity phosphatase 5*, Figure 3a). This gene was found to be down-regulated in the zebrafish *cloche* mutant in two microarray studies[9–10]. It was reported in 2009 to function downstream of Snrk-1 in angioblast populations in the lateral plate mesoderm in zebrafish[11]. The *Tol2* insertion is in the 5′ UTR of *dusp5* in mp380g-1 and 15 kb upstream of *dusp5* in mp638c. The two fish lines exhibit similar EGFP expression profiles and recapitulate the expression of *dusp5* in angioblasts and all blood vessels.

Table 1 Endogenous genes that recapitulate the EGFP expression patterns in corresponding fish lines (Zv8)

Identified gene	The corresponding fish line	Chr.[1)]	Functions in zebrafish	*Tol2* Insertion site (to the identified gene)	Ensembl ID
hhex	mp378b	12	Known	Downstream 20 kb	ENSDARG00000074250
ets1a	mp430c-2	Scf. [2)]	Known	Intron 1	ENSDARG00000024431
dusp5	mp380g-1	22	Known[3)]	5′ UTR	ENSDARG00000019307
	mp638c			Upstream 15 kb	ENSDARG00000019307
zvsg1	mp34c	11	Unknown	Downstream 35 kb	ENSDARG00000045003
micall2a	mp805a	3	Unknown	Intron 1	ENSDARG00000052694
arl8b (*1* of *2*)	mp151f	11	Unknown	Downstream 70 kb	ENSDARG00000070318
zgc:73355	mp201	23	Unknown	Upstream 80 kb	ENSDARG00000022509
hecw2 (*1* of *2*)	mp383d	1	Unknown	Downstream 100 kb	ENSDARG00000063253

[1)] Chromosome. [2)] Scaffold. [3)] The function of this gene was not characterized until recently[11] after we identified it.

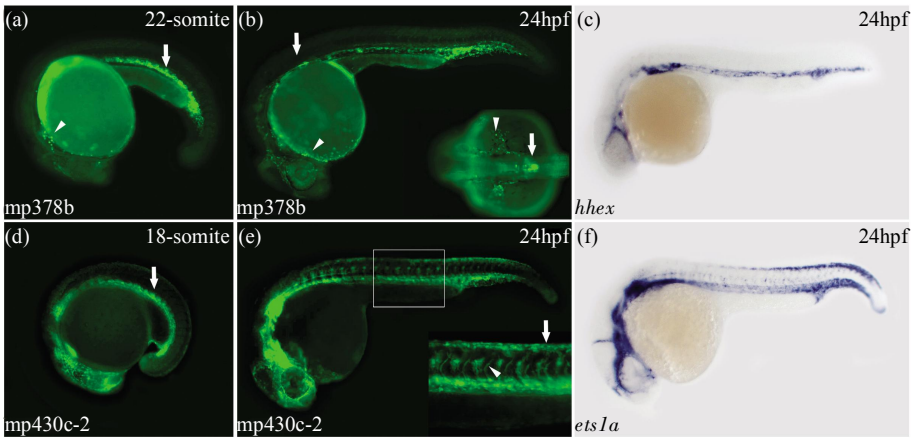

Fig. 2 The EGFP expression recapitulates that of *hhex* and *ets1a* in *Et*(*hhex*:*EGFP*) and *Et*(*ets1a*:*EGFP*) transgenic lines, respectively

(a, b) The EGFP expression pattern of mp378b (*Et*(*hhex*:*EGFP*)). (c) The expression pattern of *hhex* gene by ISH. (d, e) The EGFP expression pattern of mp430c-2 (*Et*(*ets1a*:*EGFP*)). (f) The expression pattern of *ets1a* gene by ISH. Arrowhead in (a) shows RBI (rostral blood island), in (b) shows blood cells and in (e) shows intersegmental vessels (ISV). Arrows in (a) and (d) show ICM (intermediate cell mass), in (b) shows liver primordium and in (e) shows neural crest cells. The inset in (b) shows dorsal view and in (e) shows the boxed area at higher magnification.

2.3 Transgenic fish lines with functionally unknown genes

From the rest of the blood vessel specific enhancer trap lines, we identified five more corresponding genes, most of which are new concerning blood vessel specific expression and function (Table 1). The enhancer of *zvsg1* (*zebrafish vessel-specific gene 1*, Figure 3b) was probably trapped by mp34c(Figure 1b). This gene is also down-regulated in *cloche*[9], but its function in zebrafish is still unclear. In 2009, its human ortholog was reported to function in leukocyte transendothelial migration [12]. The enhancer of *micall2a* (*molecule interacting with CasL-Like 2a*, Figure 3c) was likely trapped by mp805a (Figure 1c). Its mouse ortholog, *Micall2*, is involved in axon guidance [13], vesicle transportation and functional cell-cell junction formation[14–16]. To our knowledge, this gene as well as its orthologs has not been reported to be expressed in blood vessels before and we are interested in elucidating its potential roles in zebrafish vasculature development in the future.

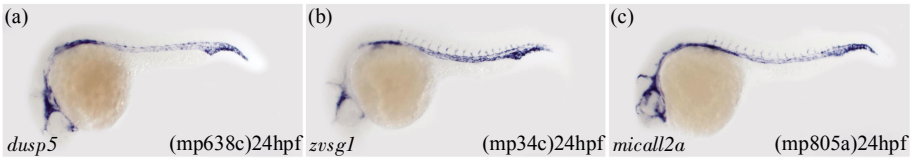

Fig. 3 The expression pattern of corresponding genes that recapitulate EGFP expression in Figure 1a~c

The names of each gene as well as the corresponding developmental stage are shown at the bottom. The corresponding fish line is shown in bracket.

arl8b (*1* of *2*) (*ADP-ribosylation factor like-8B*, Figure S2a, See Supplement online, http://www.pibb.ac.cn/cn/ch/common/view_abstract.aspx?file_no=20100301&flag=1) has not been reported in zebrafish before. Mouse ARL8B protein was reported to accumulate at the growth cone in primary neuronal cells with its mRNA widely expressed in brain[17]. It is thought to be involved in neurite formation[17], lysosome trafficking [18] and associated with microtubules [19]. It is also suggested to be a substrate of MAK3 (maintenance of killer protein 3) due to its altered localization when *MAK3* was knocked down [20]. This gene is the closest to and lies 70 kb upstream of the *Tol2* insertion site in our fish line mp151f (Figure S3c, See Supplement online, http://www.pibb.ac.cn/cn/ch/common/view_abstract.aspx?file_no=20100301&

flag=1). The EGFP expression in mp151f recapitulates the expression pattern of this gene in blood vessels and developing eyes [21]. But the enhancer driving EGFP expression in the rhombomeres in mp151f has not been identified yet (Figure 1d).

The endogenous expression of *zgc:73355* (Figure S2b) in vasculature, epiphysis and forebrain was faithfully recapitulated by the EGFP pattern in mp201 (Figure 1e). This gene is a potential ortholog of human *COX4I2* (*cytochrome c oxidase subunit* Ⅳ *isoform 2*). *COX4I2* gene was reported to be highly expressed in lung of fetal and adult human, as well as fetal muscles[22]. Its mutation was reported to cause exocrine pancreatic insufficiency, dyserythropoietic anemia, and calvarial hyperostosis in five patients [23]. The function of this gene has not been studied in zebrafish and here we report its expression in blood vessels for the first time. The EGFP signal in lateral line is perhaps due to the enhancer of gene *FAM110A* (*1* of *2*) in our study (data not shown).

The enhancer of *hecw2* (*1* of *2*, Figure S2c), a potential ortholog of human *HECW2* (*HECT, C2, AND WW DOMAINS-CONTAINING E3 UBIQUITIN-PROTEIN LIGASE 2*), was probably trapped by mp383d (Figure 1f). Although the function of this gene has not been studied in any species, the family of E3 ubiquitin ligase has been reported to have numerous functions, including in regulating epithelial Na^+ channel activity in hypertension and cardiac hypertrophy in mice [24]. Our transgenic fish line mp383d faithfully recapitulate the expression of *hecw2* (*1* of *2*) both in blood vessels and in hatching gland.

3 Discussion

Here we report 26 blood vessel specific transgenic fish lines identified from a large-scale enhancer trap screen mediated by *Tol2* transposon. For the first time, we report two transgenic fish lines recapitulating the expression pattern of two important genes, *hhex* and *ets1a*, respectively. We also identified 5 novel or functional unknown genes corresponding to some of the fish lines. Based on these results, further studies on gene function, *cis*-acting element regulation, small molecule screening and even mutation of specific genes could be carried out, which will certainly help to elucidate the mechanisms of embryonic blood vessel development.

In our enhancer trap screen, the overall frequency of EGFP-positive F1 embryos by incross is approximately 21%, and the average number of *Tol2* insertions per F_1 is four (unpublished data). Since we did not count the embryos with faint or non-specific EGFP patterns, the actual rate of germ line transmission would be higher. The F_1 fish were then outcrossed with wild type strains for at least three generations to dilute the insertions until the expression pattern exhibit the segregation ratio of a single locus Mendelian inheritance.

The controversy about the existence of hemangioblast and its regulation mechanism still needs further investigation. Thus, *Et* (*hhex:EGFP*) (mp378b) and *Et* (*ets1a:EGFP*) (mp430c-2, Figure 2) could be used to trace the dynamic formation of hemangioblast. Advanced techniques, such as combining cagged fluorescent dye or Kaede for lineage tracing with Two-Photon Microscopy to record fluorescent signals in live embryos, provide new possibilities to access to and clarify the mystery. These fish lines can also be used for stem cell research by FCM (Flow Cytometry), microarray analysis, deep sequencing and small-molecule screening for drug discovery. Besides, transgenic fish can impartially reflect the location and intensity of reporter/endogenous gene expression comparing with ISH, regardless the accessibility of probes.

Gene regulation by *cis*-acting elements is an important issue in understanding vertebrate genome organization. Our enhancer trap transgenic lines represent an invaluable resource for the identification of enhancer elements specific for blood vessels. Further analyses on the relationship between the *Tol2* insertion sites (Table S2, S4), flanking genes and the expression patterns of our fish lines should help to identify blood vessel specific enhancer elements. Along this line, it is interesting to note that more than half of our fish lines express EGFP not only in vasculature, but also in some other tissues, including blood, rhombomeres, hatching gland, skin, lateral line etc (Figure 1). While some of the co-expression with blood vessels indeed reflects the expression pattern of a single endogenous gene, other transgenic lines are certainly representing a combination of expression patterns of at least two endogenous flanking genes. In the case of mp378b and mp430c-2, where the enhancer of only one single gene was trapped in each fish line, the expression in blood cells in addition to blood vessels suggests that the corresponding endogenous genes might show expression as early as in common

progenitors of both blood vessels and blood, hypothetically the hemangioblasts. In addition, there are some fish lines seem to have trapped two different enhancers. *Tol2* element in mp34c (Figure 1b) was inserted inside the gene *gtpbp3*(*GTP binding protein 3*) (Figure S3a, See Supplement online, http://www.pibb.ac.cn) which has been shown to be expressed in spinal cord, and this fish line seems to exhibit both the spinal cord expression of *gtpbp3* (http://zfin.org/) and the blood vessel expression of the neighboring gene *zvsg1* (Figure 3b). Similar situation of combined expression patterns can be seen in mp201 (Figure 1e) which possibly exhibits the lateral line expression of the nearest gene *FAM110A* (*1* of *2*) and the blood and vessel patterns of a 80kb downstream gene *zgc*:*73355* (Figure S3b). The additional EGFP expression in rhombomeres in mp151f (Figure 1d) may also reflect this situation, although we have not identified the corresponding gene yet (Figure S3c). As the complex EGFP expression pattern did not separate after four generations in all these three lines, the recapitulation of the expression patterns of two genes at the same time might provide special opportunities for the identification of tissue specific enhancers.

Another potential application for enhancer trap transgenic lines is to generate mutations. Unfortunately, we have not found any visible phenotype in embryos homozygous for any *Tol2* insertions in our blood-vessel specific transgenic fish lines, perhaps due to the fact that most of the insertions are located in introns or intergenic regions and no insertions have been identified to be in coding regions. Nevertheless, there are still good opportunities to create deletion mutations from these fish lines by inducing imprecise mobilization of *Tol2* transposon. The imprecise mobilization of P-element in *Drosophila* has been widely adopted to create mutants [25]. Similar strategy has been reported with *Tol2* in zebrafish [26] and has also been successfully confirmed in our laboratory (unpublished data). Our transgenic fish lines are invaluable for this purpose, especially those with *Tol2* insertions inside endogenous genes.

Furthermore, the functionally unknown genes identified from this research, such as *zgc*:*73355*, *micall2a* and *hecw2* (*1* of *2*) will be the future focus for our study. In addition, our fish lines with EGFP expression in specific regions of blood vessels could also be used for certain particular screening, such as small molecules affecting specific developmental processes. Moreover, the transgenic fish lines lacking known corresponding genes are still in need of further identification.

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利用 *Tol2* 转座子介导的增强子诱捕技术获得血管相关转基因斑马鱼系 *

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摘要 心血管系统形成于胚胎发育极早期并为其他器官的发育、维持、修复所必需，血管生长异常可造成多种疾病．然而，由于研究对象所限，胚胎血管的发育机制尚未完全阐明，调控血管发育的基因也所知有限．通过 *Tol2* 转座子介导的大规模增强子诱捕筛选到 26 个血管特异表达绿色荧光蛋白(EGFP)报告基因的转基因斑马鱼系，其中有一些品系在胚胎的某些特异血管结构中表达绿色荧光．通过 linker-mediated PCR 克隆到 22 个鱼系中 *Tol2* 插入位点附近的斑马鱼基因组序列，其中有 17 个鱼系的 *Tol2* 插入可定位到现有的斑马鱼基因组中的单一位点．通过整体胚胎原位杂交对插入位点附近的基因进行表达谱分析，得到 8 个表达谱与转基因鱼系一致的基因，涵盖了 9 个鱼系，其中 *dusp5* 基因对应于 2 个不同的鱼系．这 8 个基因中包括 *hhex*、*ets1a* 和 *dusp5* 等 3 个功能已知的基因，但是大部分(5 个)基因在斑马鱼中尚无功能研究，分别为 *zvsg1*、*micall2a*、*arl8b* (*1* of *2*)、*zgc:73355* 以及 *hecw2* (*1* of *2*)．*hhex* 和 *ets1a* 基因对血管与血细胞前体的发育具有重要作用，所获得的 EGFP 报告基因受 *hhex* 或 *ets1a* 基因增强子控制的转基因斑马鱼(mp378b 和 mp430c-2)为国际首例，为深入研究这两个基因在血管与血液发育中的作用机制提供了新的机遇．筛选到的功能未知基因可以用来进一步研究其在血管发育中的功能；同时，利用所获得的转基因鱼系，可以实现实时、动态观察成血管细胞的起源、分化与基因表达调控，并可用于高通量小分子药物筛选等重要研究．

关键词 斑马鱼，*Tol2*，增强子诱捕，血管，转基因
学科分类号 Q3，Q7 **DOI:** 10.3724/SP.J.1206.2010.00301

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Supplement

Table S1　Primers used for cDNA cloning, LM-PCR and making ISH probes

Name	Primer 1	Primer 2
Linker 1	ggatttgctggtgcagtacaggccttaagagggac	–
Linker 2	PO_4-tagtccctcttaaggcct-NH_2	–
Afl5	ggatttgctggtgcagtacag	–
Afl5nest	agtacaggccttaagagga	–
Tol3	ccctaagtacttgtactttcacttg	–
*Tol3*nest	aatttttgagtacttttttacacctc	–
Tol5	tacagtaatcaagtaaaattactca	–
*Tol5*nest	gtaaaattactcaagtactttacac	–
hhex	gcacccgacgcccttcta	cattaagtcagtggtcaggtatg
ets1a	aaactcttggcgtctattc	ctttactcgtccgtgtcg
dusp5	tacgcagggtttagtaggc	aggaggtagggaaggtgaa
zgc113451	cacaacaagctccaagaatc	ccagcccatgaatacaac
micall2a	atggcggctattaaag	agctgagcttcactctcccatc
arl8b (*1* of *2*)	tttctatctccagcaggaacac	tccgagattttgaatgttgga
zgc:73355	tatgcttcgcttgacagcgg	tgtgacgctgcattccct
hecw2 (*1* of *2*)	aaaacctccgcagcctttcg	ctgcaccgggattatcagct

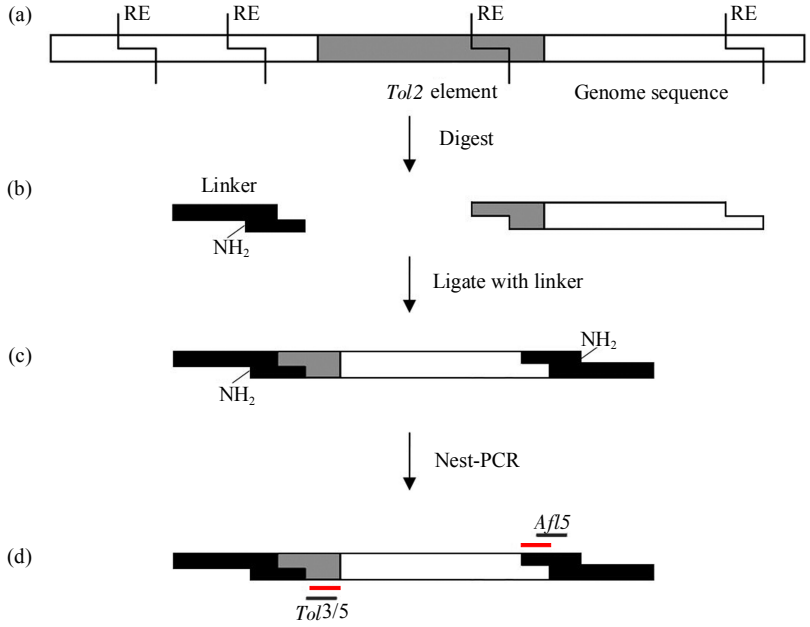

Fig. S1　The scheme of linker mediated PCR

(a) The genomic DNA of transgenic fish lines were digested by restrict enzyme of selection (*e. g.*, *Mse* Ⅰ). Bended lines show the recognition sites for the restriction enzyme. (b) The fragments resulted from (a) were ligated with 3'-amino-modified Linkers. The modification prevents non-specific amplification introduced by linkers at two sides. (c) The ligation result of the fragment with partial *Tol2* element. (d) Position of Nest-PCR primers. Black bars show primers used for the first PCR cycle of Nest-PCR. Red bars show primers used for the second PCR cycle of Nest-PCR, which contains "nest" in the names of primers.

Table S2　Detailed information of EGFP pattern and LM-PCR result for each of the 26 transgenic fish line

Fish line	EGFP pattern[1)]	3′Enzyme[1)]	3′L[1)](bp)	5′Enzyme[1)]	5′L[1)](bp)	8 bp repeat[1)]
mp34c	vs, sp.(ubi)	*Mse* Ⅰ	173	*Mse* Ⅰ	72	Y
mp65c	vs, tel, hb, sp., phar.	Bgl Ⅱ	988	Bgl Ⅱ	423	Y
mp69b	ht, ar, ls, nch	*Mse* Ⅰ	384	*Mse* Ⅰ	55	Y
mp151f	bl, vs, dien, ret, robo	*Mse* Ⅰ	181	*Mse* Ⅰ	228	Y
mp201	vein(+), ht, endo, pin, cns, ll	*Mse* Ⅰ	35	*Mse* Ⅰ	51	Y
mp202d	vein(+), ms(+), hm	–	–	–	–	–
mp214c-1	vein(-), phar, tel, mdb, ncc	*Mse* Ⅰ	166	*Mse* Ⅰ	84	Y
mp266a	vein(+), phar, tec, sp.n, pd	–	–	–	–	–
mp317b-1	vs(-), bl(-), hg(+), hpcd, gvs(+)	*Mse* Ⅰ	149	*Mse* Ⅰ	115	Y
mp317b-2	gvs, guvs	–	–	–	–	–
mp319a-1	vs, phar, ht, hg, ot, sp. n	*Mse* Ⅰ	16	*Mse* Ⅰ	80	Y
mp321b-1	vein, tel, ls, phar, endo, fb	*Sau*3AI	520	–	–	–
mp374-1	vs(+)	*Sau*3AI	372	*Sau*3AI	596	Y
mp378b	vs, ICM, RBI	*Mse* Ⅰ	109	*Mse* Ⅰ	252	Y
mp380g-1	vs(+), skin, hg	*Sau*3AI	70	*Sau*3AI	106	Y
mp383d	vs(-), hg(+)	*Sau*3AI	130	*Sau*3AI	204	Y
mp425c	PICA-AA1-LDA(+), axvs(-), hcd	*Sau*3AI	307	*Sau*3AI	143	Y
mp430c-2	vs, ICM, ncc	*Mse* Ⅰ	45	*Mse* Ⅰ	21	Y
mp589b	vein(-), ls, ot, skin, hm, mp	*Sau*3AI	95	*Sau*3AI	101	Y
mp638c	vs, hg, cns	*Mse* Ⅰ	21	*Mse* Ⅰ	72	Y
mp673b	vs(-), ncc, dien	*Mse* Ⅰ	115	*Mse* Ⅰ	89	Y
mp786b-1	ubi(24h), vs (+, 2.5d)	*Mse* Ⅰ	45	*Mse* Ⅰ	84	Y
mp805a	vs(+), sp.n	*Mse* Ⅰ	60	*Mse* Ⅰ	39	Y
mp817a	axvs, sp.n, tel, phar, hbn	*Mse* Ⅰ	157	*Mse* Ⅰ	85	Y
mp871b	axvs(-), phar	*Mse* Ⅰ	37	*Mse* Ⅰ	20	Y
mp908	axvs(-), cns	*Mse* Ⅰ	125	*Mse* Ⅰ	220	Y

[1)] Abbreviations are shown in Table S3. 3′/5′ Enzyme indicates the enzymes used in successful LM-PCR cloning. 3′/5′L (bp) enumerates the sequence length of the LM-PCR results of *Tol2* 3′/5′ flanking sequences. All the sequences are shown in Table S4. 8bp repeat in the host genome is the feature of *Tol2* transposon. Y means yes. The lines that the flanking genes have been confirmed by *in situ* hybridization (see Table 1) are marked in red.

Table S3　Abbreviation illustration for Table S2

Abb.[1)]	Full name	Abb.[1)]	Full name	Abb.[1)]	Full name
+/–	Strong/weak expression	hg	Hatching gland	ot	Otic vesicle
AA1	Mandibular arch	hm	Head mesenchyme	pd	Pronephric duct
ar	Aortic arch	hpcd	Hypochord	phar	Pharyngeal arch
axvs	Axial vessel	ht	Heart	PICA	Primitive internal carotid artery
bl	Blood	ICM	Intermediate cell mass	pin	Pineal gland
cns	Central neuron system	LDA	Lateral dorsal aorta	RBI	Rostral blood island
dien	Diencephalon	ll	Lateral line	ret	Retina
endo	Endoderm	ls	Lens	robo	Rhombomere
fb	Fin bud	mdb	Midbrain	sm	Somite
gvs	Vessel in gill	mp	Muscle pioneer	sp.	Spinal cord
guvs	Vessel in gut	ms	Mucus cell	sp.n	Spinal cord neuron
hb	Hind brain	ncc	Neural crest cell	tec	Tectum
hbn	Hind brain neuron	nch	Notochord	tel	Telencephalon
hch	Hypochord	olf	Olfactory pit	vs	Vessel

[1)] Abb. means abbreviation.

Table S4 Zebrafish genomic sequences flanking *Tol2* insertion sites recovered by LM-PCR in Table S2

Fish line	Flanking side[1)]	Sequence (*Tol*3/5 to Afl5)[2)]
mp34c	3'	**CTATTTGT**ACATATCAGTGGGAGGAAAGCCCCGCCCATTAGTGATGATCTCTTCC
		TCATTAGCATAGGATGTTACTCTTGTTTTTGAATCTGCCACTATGCTGAAACATAT
		GCATTTGTAGCTCCGCCCTCTTTTGAAAAAAAGGGCTGGGAGCACAGTCTCATTT
		GAATTTA
	5'	**ACAAATAG**AGAATGTCAATCTAAATGTTTCTGCAGACATAATTAGATATAATCAT
		CTAATGAATACATTTTA
mp65c	3'	**CTAGTGAC**CATCTCTCCCTCATTAATATCGGATGTTAGACTTGTTTTTGAATCCGC
		CTCTATCTGCTTGTTAAGTGGTGACGTGTTTGTGACGTTTGTAATACTGTTTCCGG
		GTCCAAGCCGCCGTTCATTTGAGTGGAGAAATCATTCGTTTATGATCACGTTTTAT
		TCCTATCACACATTAAAGTTGTTTTTTATATAAAATCATAGTGTACACAACAATCT
		CTGGGCTTGCTTCATAACAAATACCAAAAATTTAACAATTTCTAAAAAAAATGAA
		TCCCTTTCTGGCCATCGGATATTAGGCATGGACATATATACTGCCATCGTGATTTT
		CGAAAGCATTAAATCGCTTCGTAAACGTTTATTATTACCATTTCATAAGTGTCCTC
		ATTCAGTTGAATAAAGCGCTTGGACCTGGAAGCCGCTTCGCGTGACGTTACACTT
		AACAGCAAAATCTTAGAAGAGACTGTGCAAGTTATGAAAACATCAACCTGCAGT
		CTTGACACGCTCCCCACATCATTCTTCAAAACGGTGTTTACCTGTTTAGAAATGGA
		TCTTCTAAAAGTGGTAAATGCTTCACTTCTATCAGGGATTTTTCCAACCTCACTTA
		GAACTGCAGTTGTTAAACCCCTCTTGAAGAAGAGCAACCTGGATAACACCCTATT
		GAGCAATTACAGGCCAATCTCAAATCTCCCTTTCATTGGCAAAATCATTGAAAAA
		GTTTTTTTTAATCAGGTTAACAAGTTCTTAAACTTCAAGGGGTGTTTAGACAATTT
		TCAATCGGGGTTCAGAGCACATCACAGTACAGAGAGCGCTCTTATAAAGATAATC
		AATGATATACGCCTAAATACAGATTCAGGTAAAATAACAGTGCTGGTATTGTTGG
		ATCTCAGTGCTGCATTTGACACTGTCGATCACAGCATACTTCTGGATAGGCTGGA
		AAACTGGGTTGGGCTGTCTGGGACGGTCCTCAAATGGTTTAGATC
	5'	**GTCACTAG**TGGCGGGGCTTTGCCCCACTGATGACACGTATAAAGGGAGAATGTC
		AATCAAAGTGTTTCTGCAGACTGATTTAATCAAGTCAGATTATAACCAATAGGAT
		TAATTAACTTTTATCATTAGAGGCTGACTATGTTCACAAGATGTTGCTACACAATT
		ATGTTTAAACCCCTAATAAAAATGATTTTTGCATAATAGGTCCCCTTTAAAAAAA
		GGCACCATCGAAATAAATTGATACCATTTTTATGTGTTTCCTACATGAATGATTGT
		GAACTGGATGTCTTCAAAGTTTCCCAGCCTGTGGCCTCAAACACTGGGATGAGCT
		CCAGCATCCCTTTGACTCTACATACTGTGTGACTCTACATATTTTCTGTAGTATTTG
		TGCTGAGGAGTTAGCAGGTCACGGGCCTTGAGATC

Continued

Fish line	Flanking side[1)]	Sequence (*Tol*3/5 to Afl5)[2)]
mp69b	3'	**CCAAAGGA**TCAGTCTTAGGATTTGATATTCATAATGACACTACAGTGTTTCCAAA
		GGCTTCACTCACGGTCAGCTGATCGACTGCTGAGCTGCGCATGCGCATTCACACT
		CGACAGCGACACATGAAGGTGTGGAGTTTGAATGGAAAGCTGTGTTTGATGTAAC
		CACTGTAGAAAACTAAAAAAAAAAACTATGTAAAATAGGACGCAAACACACAACA
		AATGTAGGTAAGTGAAAGTAAACTCAATTTGGCGCTGATAATAACAGCCTTTACT
		TTCACTGATGACTGCACACATCACAAACTCACCCACATGAACAAATACTACAGTG
		GTTTATAGTAAACACTAGTGTTATTGAACCATACTTTAGTAAAGTACTTCACTTA
	5'	**TCCTTTGG**ACATTATTTTGTTGAAATTACCAAATAATCGCCACGTCGTCTCATTA
mp151f	3'	**GGTCTCAC**CCATTCATCGAGTCTCACAGCTTGGCAGGTCTGCCTTGCTTTCAGAA
		AGTAAGTGTATGAATAATCTAGAAGCAGGGTCTTCTCATAGGATAAGAGCACTCT
		GCTATGAATAATAATGAGAAACCGACGCATCATCACTCCACTAGCAGATTCACAC
		TACGTCAAAGATTTTA
	5'	**GTGAGACC**ATGCACAGCATGTGTCGTATGTGTTTGTTTCCATGATCCACGGGGTT
		TGTTCTGGCCGCAGCCGTATGTAACCTTCAGCTCAATAACCATGTGGATGGATGA
		TAACAGCCTTCTAAACCTACTAGTAGGCGCAGAAGTCCCCTACTGGCCCATATCT
		ATCAGCTGATAACCACTGATAACACCCATTCAATCGAGTGATAACATTCGAACTG
		ACTGGTTA
mp201	3'	**TGTATAAC**GATGGTTTGTCCTGTAGTAGACTATTA
	5'	**GTTATACA**ATAACTTGCCTAATTACCCTAACCTAACTAGTAAACCTAATTA
mp214c-1	3'	**AGTGTGAG**TGCGTTGAATCGGGCTCAAGCACGGTTCACTTGGCCGGCCCTGGCCC
		GGTTGGAAGAGGTGTGCCTGAGCGCGGTACACTTGGGCTTTGGCGCGGTACGCTT
		GTGTGTGAGCGCGAAACGCGCCAAAGCCCGAAACCGAAAGCGAGACGTGACTTT
		TA
	5'	**CTCACACT**TCTCAAACGATCCGGGAAACGGGCCTGGGCACGGTACGGATGGCAT
		AGTGTGAGTAGGCCCATATGACAGAGGTTA
mp317b-1	3'	**TGTGTGTG**TGTTTCATACTATTGCATTTAGCTGTTACGTGTCCTGCTCACTCATTA
		GGTTTTCTAGCAACAATTTAGTGGACATTGCAATCCTTTCCAAAATCTTGTTTTTT
		GTTTTATAGTCAAATACGCTTTATTTCATCTAGCTTA
	5'	**CACACACA**CACACACACACGCACGCACGCACACACACACACACACACACACA
		CACACACAGTCTTCTTATATCTATTCTTGATATAATATTTAGATAAAAATCTAAAA
		TATTA
mp319a-1	3'	**ATAAGAGC**TGTTATTA
	5'	**GCTCTTAT**AAATATCAATGTCAAAACATTTTGCATCATATATTTAGTGGATTTTTC
		TTTGTGCAACAAAAATGACTCTTA

Continued

Fish line	Flanking side[1)]	Sequence (*Tol*3/5 to Afl5)[2)]
mp321b-1	3'	TCCGCTCCACCTCCCGCCTGAACAGTATTAGGAGTGTCTGGAAGCCACTCCTTAG
		AGGGGGGGCTCTGTCATGTTCACCAGCGAACAACCACCAGATGTCTCTGGTAAAC
		ACTCACTCATAAAACTACATATCTGCCTTCTTCTGTGCCTTCTTCTGGACTACATA
		TGCATACATGCACTTCTCCTAGACACTCACACCTGCTCACTCATCCAGCTTGATTA
		CATTTGCACACCTGAGGACTTTCAGAAACTGATTAACACACACTATTTAAGCCAC
		ACATTCACCCCTTCAGTTTGCCGAGTCTTGTTTATCTGTAAGGTGACATTACAACG
		CGTTATCCTGGTCTTGTTTCTCTGTGTTTTGAACCTAGCCTTGTTTATTTAGTTTCC
		TTGTCTGCCGCCTGCCTTTCGACCTCTTGATTGTTTAACTGACTACGATTCTGGATT
		GTTCTCGCATACCTGTTTGCTCCCGTATTGACCACTGCTTGCCTGACTGTGAATAA
		ACCTGCATATTTGATC
mp374-1	3'	**GGAATATT**TGCATAATTGACCCATAACAATGTACAGTACCTTTTACTCTCAGTAT
		GTTTGTGAGCCCTTTATATTGTTTCTATTCTAATTTGCAGTTGAGATGAAAAAATA
		AATACATTGAAATCAAAGTATAAATAGCAGAAGTGAACAAATAGATTTTCCCTAC
		CAGCAAATATTAGTATATATAATAAGTTTAGGCGACGCAATGGTGCAGTAGGTAA
		TGCTTTCGCCTCACAGCAAGAAGGTCGCTGGTTTGAGCCTCGACTAGGCCAGTTG
		GCGTTTCTGTGAGGAGTTTGCATGTTCTCCCTGCGTTCGCGTGGGTTTCCTCCGGG
		TGCTCTGGTTTCCCCCACAGTCCAAAGACATGCGGTACAGGGGAATTGGGTAGGC
		TAAATTGTCCATAGTGTATGTGTGAATGAGTGTGTATGGATGTTTTCCATAGTAAG
		GTTGCAGCTGGAAGAGCATCTGCTGCATAAAACATATGCTGGATATGTTGGCAGT
		TTGTTCCGCTGTGGCAACCCCAGATCAATAAAGGGACTAAGCCAAAAAGAGAAT
		AATGAATGATAAGTGTATTGACTGCAAGTGCTCAGTTTGGGATC
	5'	**AATATTCC**CCACAAATCCATCAATTTCTGGCTGTTCTTTCTATAACTGAATAATGT
		TAAAGCACTGTCCATGCGCGTTTCCTTGTCAGAATGTAACACAATACACTATTGC
		ACAACAATTACTCTATCAGGCTTTTTGGCTAAAAAAAAAATCACGGTTTCATTTGTT
		ATTATTGGATTATTTTTAAATCTCAAAAATTACTAATTATATTCCTGGGAATGTCT
		GACACCGGCGCATACACACTGTAATAGATGAGCCAGGCACGAAAGCTTGACAGG
		CGTAGCAACAGTAACTAAGGAGGGCGGAACTTAGCAAAGGGTCAATTTTAAATG
		CACGGCTACACGCATTGTATGCACCATGGGTCTAGATGATC
mp378b	3'	**GTGTGTAC**TTTACAAACACCAAATACAGTTTCACCACGGACACAAAGACCTAAT
		CATAAGAACGAATCAAAAAGGGACACTTCACTGTCAAAATTATGTCAGCGCATTA
	5'	**GTACACAC**ATCATAAATTTATCAGTCGTATACAAACAGACAAAGAAAATGTCGT
		ATCTGTGTCTGTTCTAATCGCAGACTTTGGAATTGTACCCTCGTAGAATTACGAGT
		ACGCTCCCATTTTCTTTTACGGTTTTATAATTACAGATGTGCGAATTTTATTTGCGC
		ATGAAATATTTATGACAGGGATTTTATTCCATAATAATAATAAAAAATAAAACAT
		CCATCATGGAGCAGTCTAGCAGAAAAATTA

Continued

Fish line	Flanking side[1)]	Sequence (*Tol*3/5 to Afl5)[2)]
mp380g-1	3'	**GTAGGTTG**CCTACTAAACCCTGCGTACTGAGCCTGAGTCTACATTTCCTCCAAGG
		GGATTCCGTCTGATC
	5'	**CAACCTAC**ACCCCTGAAACATCCACATCCCTATTTAAAACACATTTCACTTTGTC
		ATTTCAAACATGAAGGTCTCCAGCATAGATTGCCGACGCCTGAGGAAGATC
mp383d	3'	**TGCTAAA**CGCAAATATTAAACATTAGATTTACATTTAATTCAAGTAGAGATTAAA
		TAGCACTGATTATTGATGGTATTAACTCTGTTAGTAATGGCCAAATCATTTCACTT
		CAAGTCTCAATGTATGATC
	5'	**TTTAGCA**TTATGAATCGTTAATCTTTTGGGATAGCTATGTGGCGATTTTACTACCA
		TCTTATTAACATCAAGGACATGCCACATTGGCAATCAATATTTCTGAAATAAATC
		CCAATTAAACTCACACTGAGGAAAAACGTGCACGAATAACCAAGCAGCAGGTCT
		TTAGCCTTGAAAACATGTGCATTTACGCAACATTAGATC
mp425c	3'	**GTCACATT**GTCCGCTGTGGTTGTGTTTTGACTCTGAAATTCAGCGCGCCCAAATA
		GACACTCCCACACCAAGCCTCTTTTCTTCCTCCGACACTCCCCCCTAAACAGAGCT
		GGACACACCCACTTTTCTGACTTTTTCCAAAGTAGAGGTGTGAAAACACCCTGCT
		GAAACGAGGGGGTTTCATGGCCCTTTAACAAGCTGTCTGTATGAACACACACACT
		CACACACACAACACCAAGCAAGTGACACAGACCGCATCACGCTCTCTCTCATTCA
		CACACATACACACACACACAGCATTAAGATC
	5'	**AATGTGAC**TGTGTTTACATGGACATCTGTTGTCGAATTATTTGCCAAATTATTAAA
		TGGTGGACTTTAACTGCAGTTTGGCTCTTTCATTCAGGGAATTCATTCATGTCCCT
		CCCGACAAACGAGATATTTGATTCAAGGATC
mp430c-2	3'	**CTCAACAT**GCAATCACTTATAACTGGGCCAAATAATCTGGGATTA
	5'	**ATGTTGAG**ATTAGTCCTATTA
mp589b	3'	**GGCATAAG**TCATCCAAAGATGTTAAATGGGCTACCTGGATGAAGTCCTCCACGT
		ATTCCTCTATAGACCGGGCTTCCTGACGCAGGCACACGATC
	5'	**CTTATGCC**TAATGATTTTCTTTCGTGGAGGACTTTCTGAGCCCCTATATTCCACAA
		TGCCTTTGCATGACCCCCACGGGACTCTAGAAGGTTATATTGATC
mp638c	3'	**CCAATGAA**AGCATTTGTTTTA
	5'	**TTCATTGG**CCTCTGGCATGTTGTCTTTTAGCATGTCGAAAAACAAAGGAATGCAA
		GGTTGCAGAAATGCTTA
mp673b	3'	**ATTATATG**GTATTTTTATGGTGAGTTTCTGGCAACCATAGCAGCCAGTTTTTTACC
		ATAAATTTTACAGGATTGTTTTTACTGTGTGGATGTTTTGGCGGTATGTGATTGAG
		TTA
	5'	**CATATAAT**GTTTTTCAATTTTACGGTAGCAATAACCATAAAATTTTCAGGGAAAA
		ACTGTAAGCTGTGGTTGACAAAAATATACCGTTA

Continued

Fish line	Flanking side[1)]	Sequence (*Tol*3/5 to Afl5)[2)]
mp786b-1	3'	**GAATAATG**TTCGGTGTCTTGCACAAACTGATCTTTCTGCTTCTTA
	5'	**CATTATTC**ACAGCATTATTACAAGCACAAGCATTTTATGAATCACAAAGTTGTTC
		ATTCAAAATAAGTCAAAATATGTTCTTTA
mp805a	3'	**AACACACT**GTCTGAAGCTATAAACAAACGCCAAAGCTGAGAACTAACATTCAGT
		TTTTTA
	5'	**AGTGTGTT**TGATTGTGTTGAGTGGAAATTTGTCAATTTA
mp817a	3'	**GCTATAGG**TGAATTGGGCAAGCTAAATTGGCCATAGTGTATGTGTGTGAATGAGT
		GTGTATTGACATTTTCCAGTGATGGGTTGCAGCTGGCATCCACTGTGTAAAACATT
		TGCTGCATAAGTTAGCAGTTCATTCCGCTGTGGCAACCCCAGATTA
	5'	**CCTATAGC**ACATTTGTTTGGACTTGTGGGGGAAACCTTGAAATTACAGTATATTC
		CTATCACAACATATATTGCAGACAAACTTA
mp871b	3'	**GAAAAGGG**TGACCTGACTTGACATGCAAAAGTACTTA
	5'	**CCCTTTTC**ATACATGATTTA
mp908	3'	**AAAATAAC**CCATCGTGCTAATTTGAGAATTAGTTTAGAGCACTATTCACAGAAAA
		CCACAGGATGTGTACATCAAATAGTTCAGGTAAGCTTTAGAATGGTTCACATCTT
		ACAAAATGTAATTTA
	5'	**GTTATTTT**ACATCTTTATACTCACTCCACCCAGCACATAGTACGTCAACCAATCA
		GAACAAGGTTTTGTCTCCGTGGTTACACTGAAGTCTGGTGTGGGCAAAATTTCCC
		CTGCCGCCTACACATGACATCATATACGAGACGACAAAAGGAAAAAAAAATCCG
		ACCGCTCGCCAAGTTTAACGGAGTGAACTTAATTTTGATGTACTATCAACGATTTA

[1)] 3' means the sequence was cloned from the 3' flanking sequences of the *Tol2* element. 5' means the sequence was cloned from the 5' flanking sequences of the *Tol2* element.

[2)] The orientation of each sequence is shown from *Tol3* or *Tol5* nest primer to Afl5 nest primer. The 8 bp genomic DNA repeat due to *Tol2* insertion is marked in Bold.

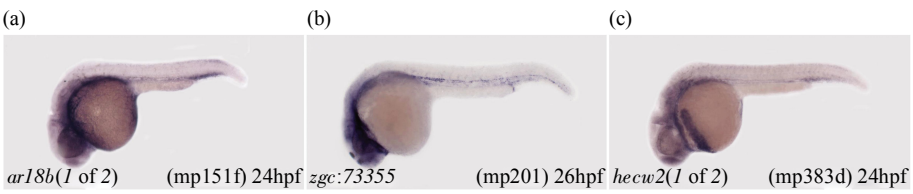

Fig. S2　The expression pattern of corresponding genes that recapitulate EGFP expression in Figure 1d～f

The names of each gene as well as the corresponding developmental stage are shown at the bottom. The corresponding fish line is shown in bracket.

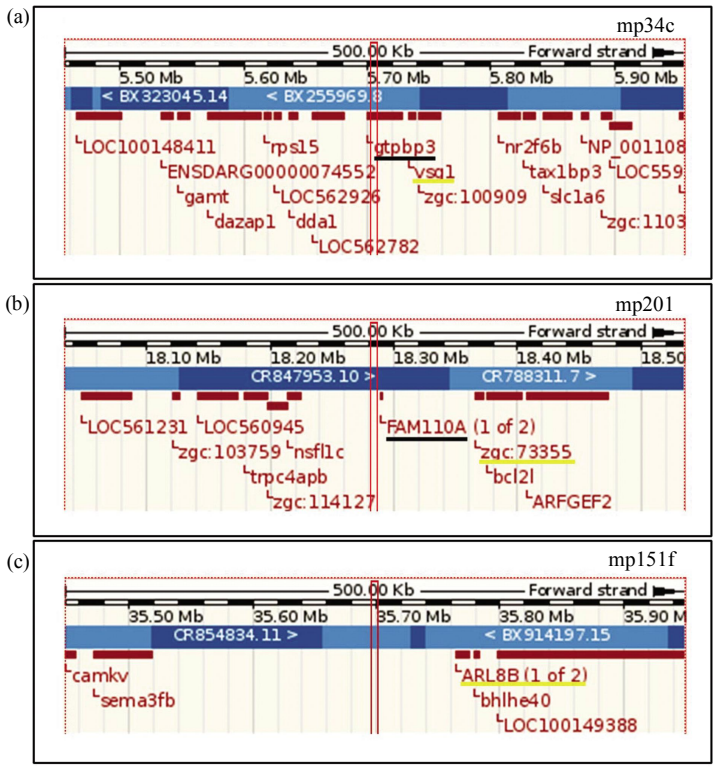

Fig. S3　The insertion sites of *Tol2* element in the corresponding chromosomes of transgenic fish lines mp34c, mp201 and mp151f shown by Ensembl database alignment (Zv8)

This figure represents the direct results from Ensembl BLASTN using the sequences obtained from LM-PCR. The corresponding fish line is shown in the upper right corner. The blue box in the middle indicates zebrafish genome sequences and the dark red color indicates Ensembl known genes. The fresh red columns indicate input sequences used for BLASTN search. The bright yellow bars underline the genes we identified as functionally unknown blood vessel specific genes. The black bars underline the other genes which correspond to other expression patterns in addition to blood vessels in the relevant transgenic fish lines. The length of the genomic region as well as its position relative to one end of the chromosome is marked in the upper ruler.