Supporting Information

LSD1/KDM1A promotes hematopoietic commitment of hemangioblast through downregulation of Etv2

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SI Figure Legends

Fig. S1. Identification of LSD1 as a responsive gene for the *it627* mutant. (*A*) A phylogenetic tree constructed by the neighbor joining method for the full-length amino acid sequences of LSD1 proteins. ce, *C. elegans*; d, *D. melanogaster*; h, human; m, mouse; sp, *S. pombe*; x, *X. laevis*; z, zebrafish. (*B*) The *gata1* expression in the LPM at 14 hpf in wild-type embryos injected or not with 1 pmole each of the indicated morpholino oligonucleotides. (*C*) The effects of tranylcypromine on the *gata1* expression. The embryos were treated with 300 μM tranylcypromine in wild-type embryos from 6 to 24 hpf. (*D*) The *gata1* expression at 14 hpf in wild-type or *lsd1*^{it627} embryos injected with or without mRNA encoding the indicated LSD1 proteins. (*E*) The expression and stability of wild-type LSD1 and LSD1ΔC609 proteins in 4- and 14-hpf embryos were examined by immunoblotting using anti-FLAG antibody.

Fig. S2. Non-hematopoietic defects in *lsd1*^{it627} embryos. (*A*) Ubiquitous expression of *lsd1* in zebrafish embryos was impaired in *lsd1*^{it627} mutants. The *lsd1* expression at the indicated developmental stages was analyzed in wild-type or *lsd1*^{it627} embryos. (*B*) The survival rates of *lsd1*^{it627} mutants calculated using the Kaplan-Meier method. (*C*) The results of a comparison of the phenotypes between *lsd1*^{it627} and *gata1*^{m651} mutants at 4 dpf. The arrows and arrowheads indicate the swim bladder and erythrocytes in the heart, respectively. The presence of pale erythrocytes due to erythropoietic defects was detected in both *lsd1*^{it627} and *gata1*^{m651} mutants, while defects in the swim bladder were only observed in *lsd1*^{it627} mutants.

Fig. S3. Defects in the expression of hematopoietic markers by the *lsd1* downregulation. (*A*) Defects in *gata1* upregulation in *lsd1*^{it627} embryos. The *gata1* expression at the indicated developmental stages was analyzed in wild-type or *lsd1*^{it627} embryos. (*B*) Effects of tranylcypromine on ectopic *pu.1* expression in *gata1*^{m651} mutants. The embryos were treated with 300 μM tranylcypromine from 6 to 24 hpf . Arrowheads indicate ectopic *pu.1* expression.

Fig. S4. Expression of *scl* and *lmo2* in *lsd1*^{it627} embryos. The expression of *scl*, *lmo2* and *gata1* at 15 hpf in wild-type or *lsd1*^{it627} embryos. The arrowheads indicate the posterior LPM.

Fig. S5. Expression of hemangioblast markers in *lsd1*^{it627} embryos. The expression of the indicated hemangioblast markers at 12 hpf (*A*) and 15 hpf (*B*) in wild-type, *lsd1*^{it627} or *gata1*^{m651} embryos. The arrowheads indicate the upregulation of *etv2* and *fli1a* in the posterior LPM of *lsd1*^{it627} embryos.

Fig. S6. Expression of *fli1a* in *lsd1*^{it627} embryos. The *fli1a* expression at the indicated developmental stages in wild-type or *lsd1*^{it627} embryos. It is noted that no difference was observed between wild-type or *lsd1*^{it627} embryos before 15 hpf.

Fig. S7. Effects of the *lsd1* mutation on angiogenesis and vascular morphogenesis. Confocal images of *Tg(kdrl:EGFP)*^{s843} transgenic wild-type or *lsd1*^{it627} embryos at 30 hpf (*A*) and 55 hpf (*B*). Fluorescent images of *Tg(kdrl:EGFP)*^{s843} transgenic wild-type or *lsd1*^{it627} embryos at 5 dpf (*C*).

Fig. S8. Expression of hematopoietic and endothelial cell markers in *etv2*-knocked-down *lsd1*^{it627} embryos. The expression of *fli1a* (*A*), *alas2* (*B*), *biklf* (*C*), *flk1* (*D*) and *tie1* (*E*) in wild-type or *lsd1*^{it627} embryos injected or not with 0.5 pmole *etv2*MO. The downregulation of *alas2* and *biklf* in *lsd1*^{it627} embryos (white arrowheads) was rescued by the *etv2*MO injection (black arrowheads). The upregulation of *flk1* and *tie1*, but not *fli1a*, in *lsd1*^{it627} embryos was rescued by the *etv2*MO injection (arrowheads).

Fig. S9. Model: the LSD1-Etv2 paradigm in hemangioblast. Etv2 maintains the endothelial characteristics in hemangioblasts and inhibits the hematopoietic commitment. LSD1 negatively regulates the gene expression of Etv2 and promotes the initiation of hematopoietic differentiation.

Movie S1. The blood circulation in the dorsal aorta and axial vein at 2 dpf in a wild-type embryo.

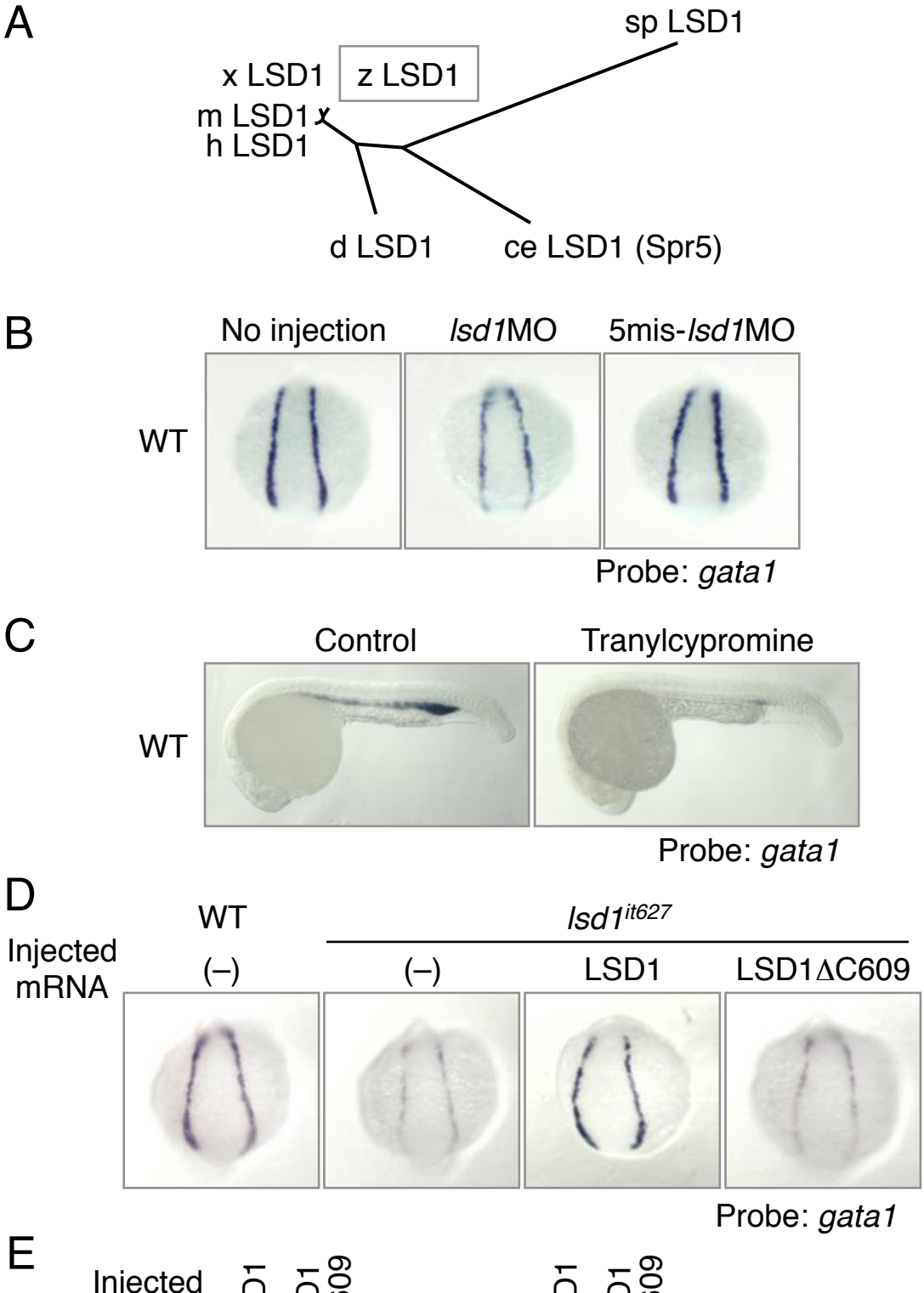
Movie S2. The blood circulation in the dorsal aorta and axial vein at 2 dpf in an *lsd1*^{it627} embryo.

Movie S3. The blood circulation in the dorsal aorta and axial vein at 4 dpf in a wild-type embryo.

Movie S4. The blood circulation in the dorsal aorta and axial vein at 4 dpf in an *lsd1*^{it627} embryo.

Movie S5. The blood circulation in the dorsal aorta and axial vein at 6 dpf in a wild-type embryo.

Movie S6. The blood circulation in the dorsal aorta and axial vein at 6 dpf in an *lsd1*^{it627} embryo.



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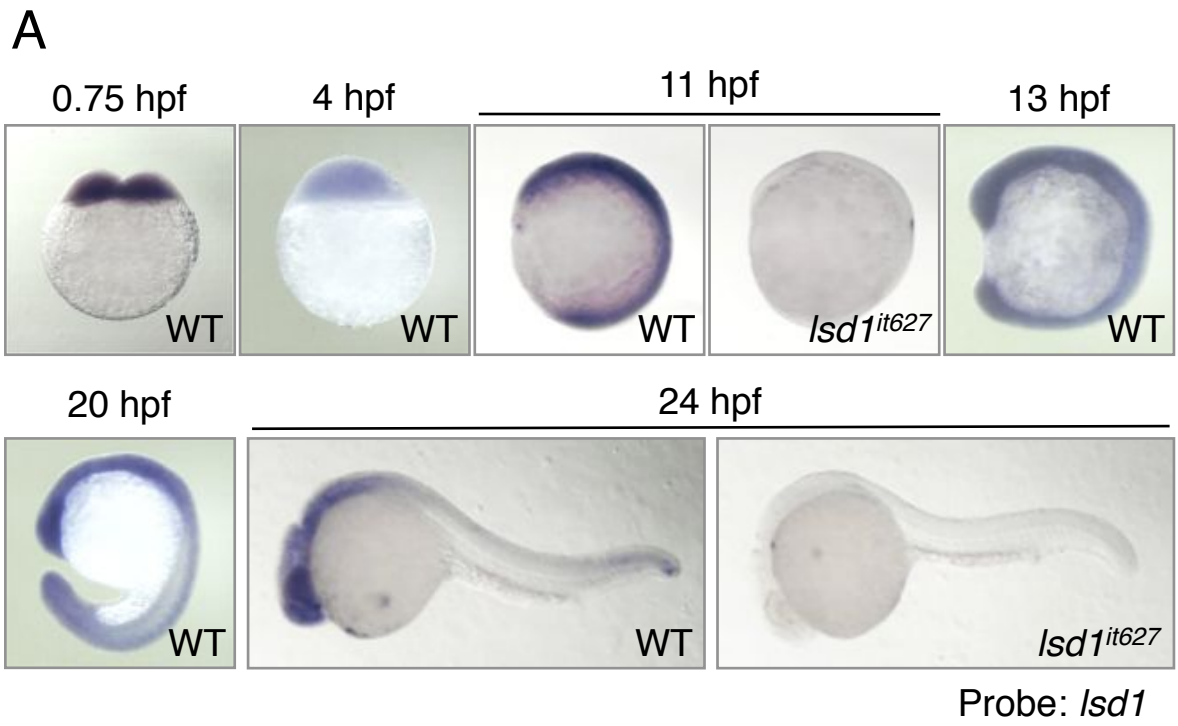

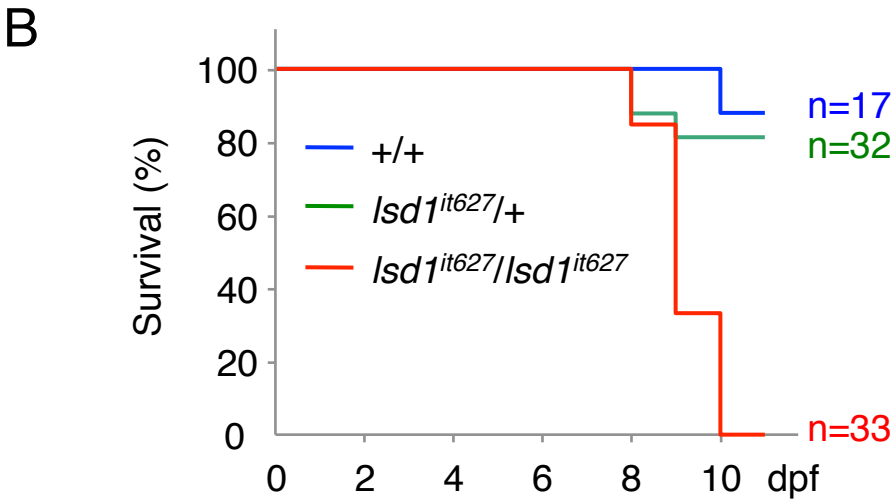

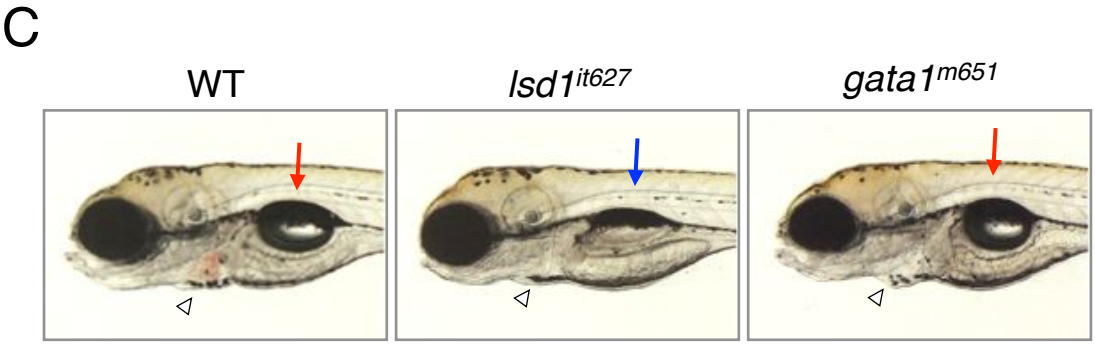

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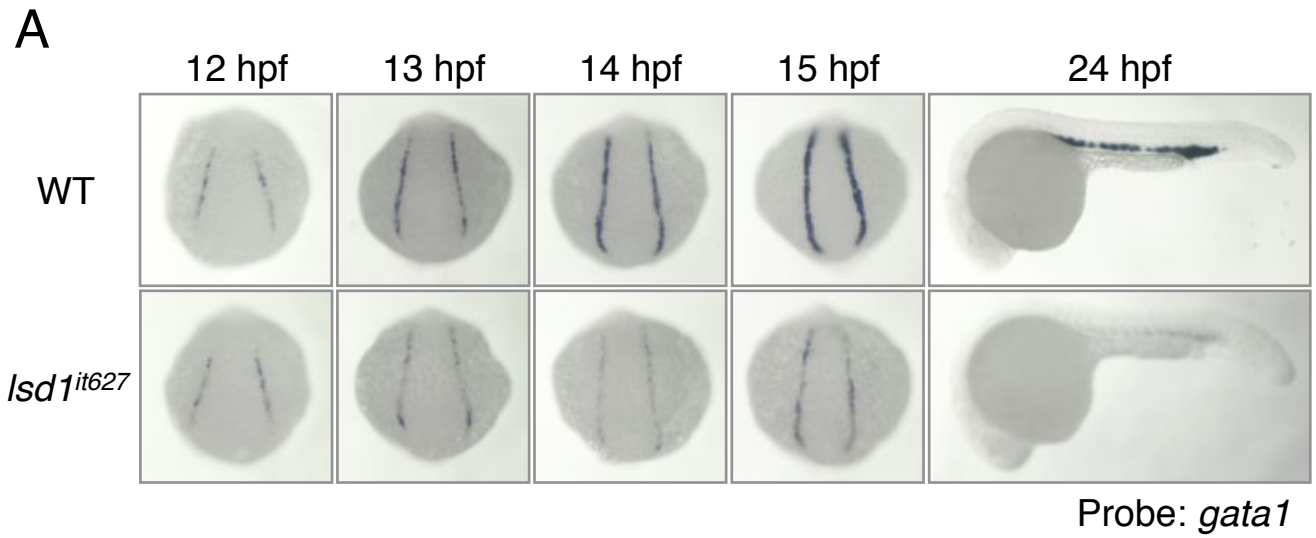

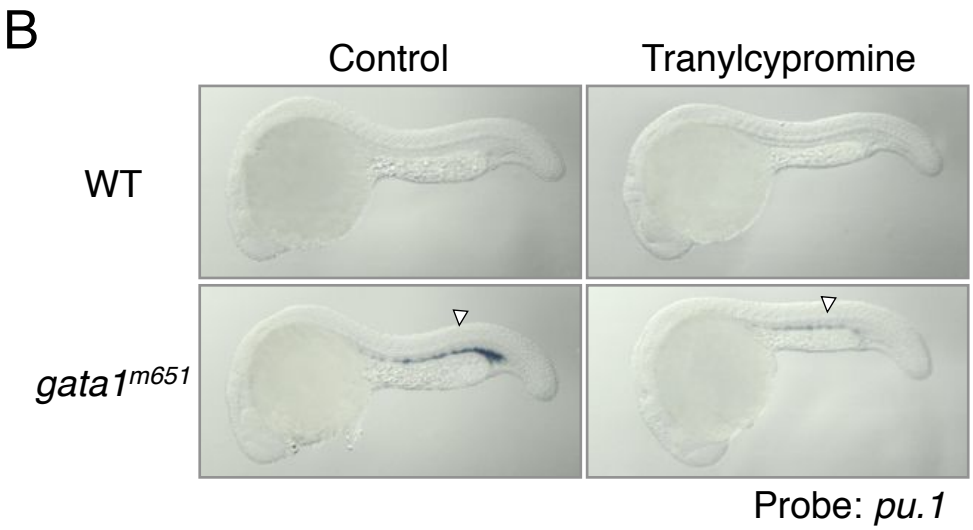

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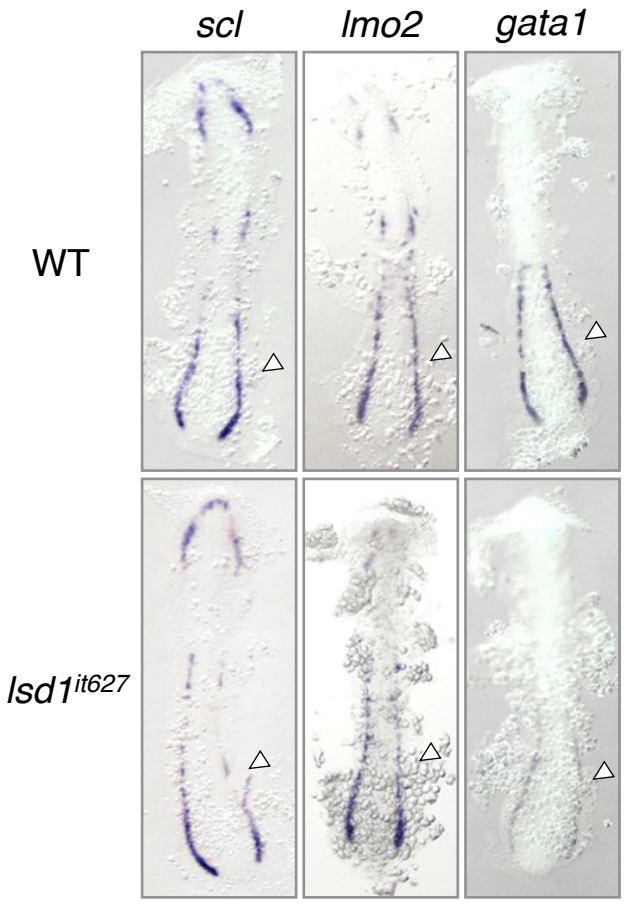

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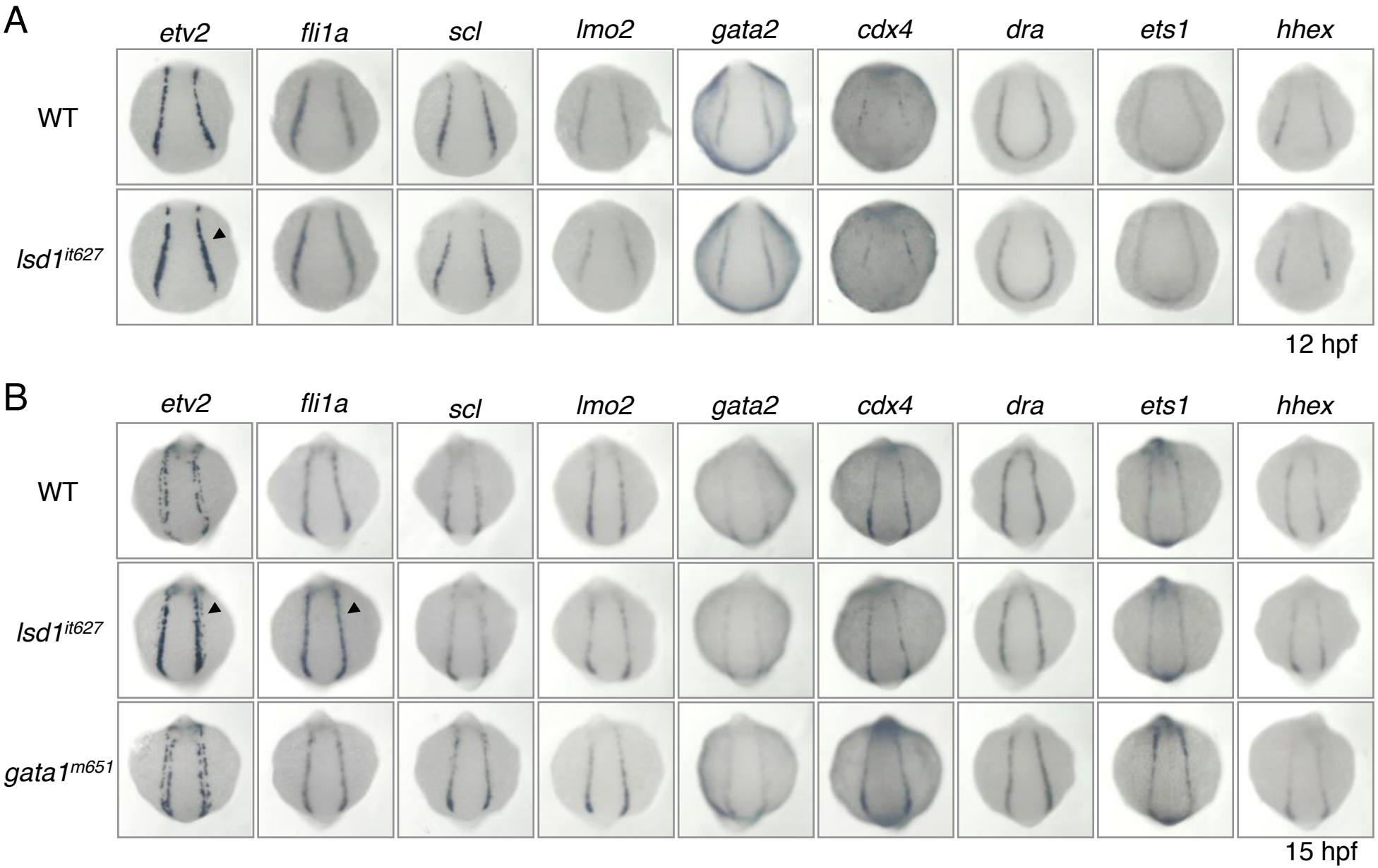

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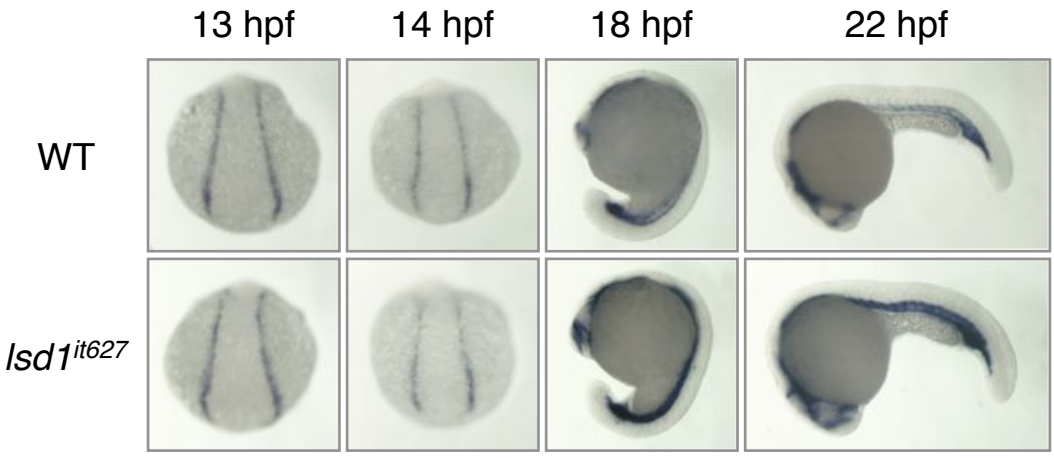

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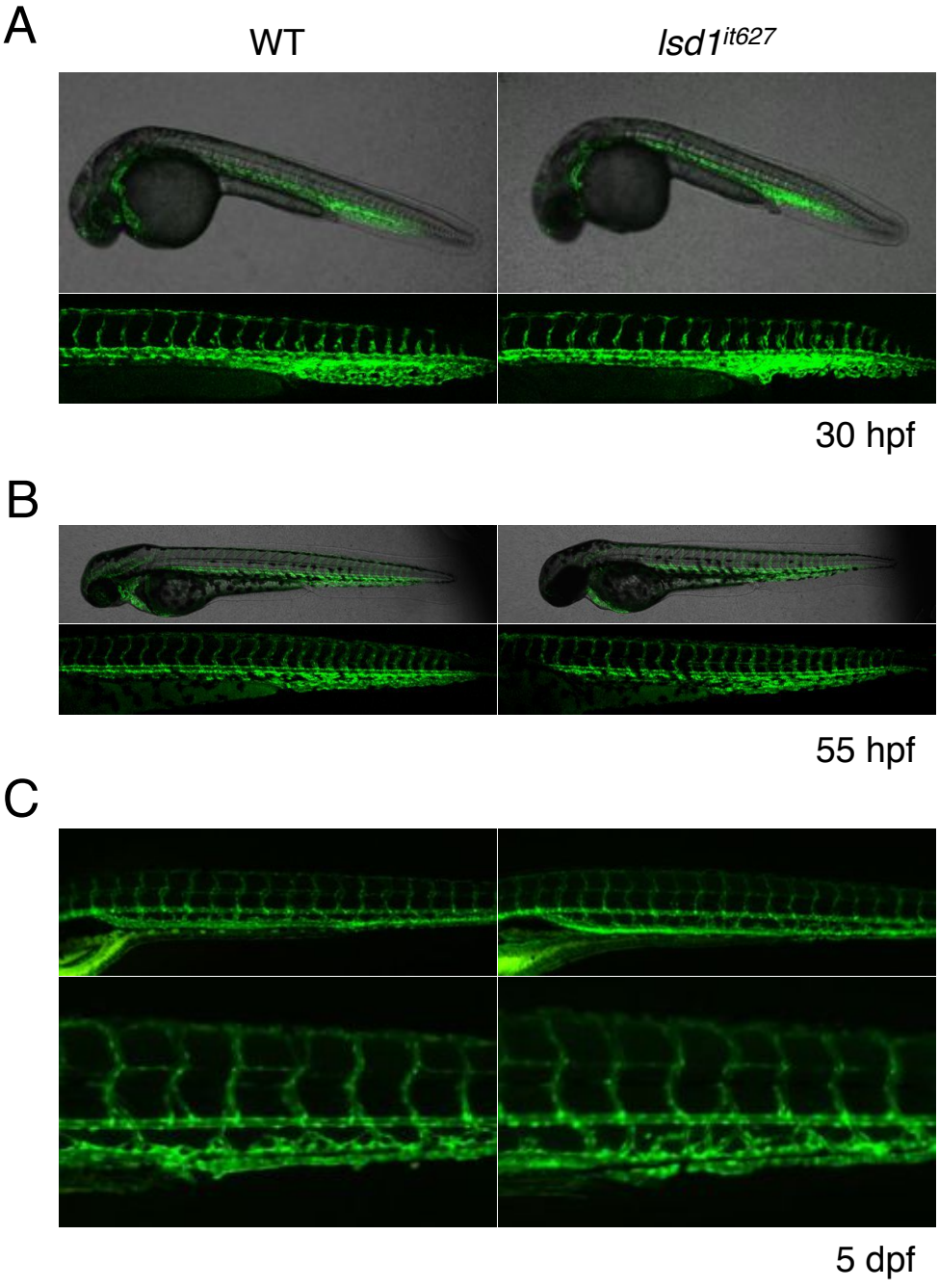

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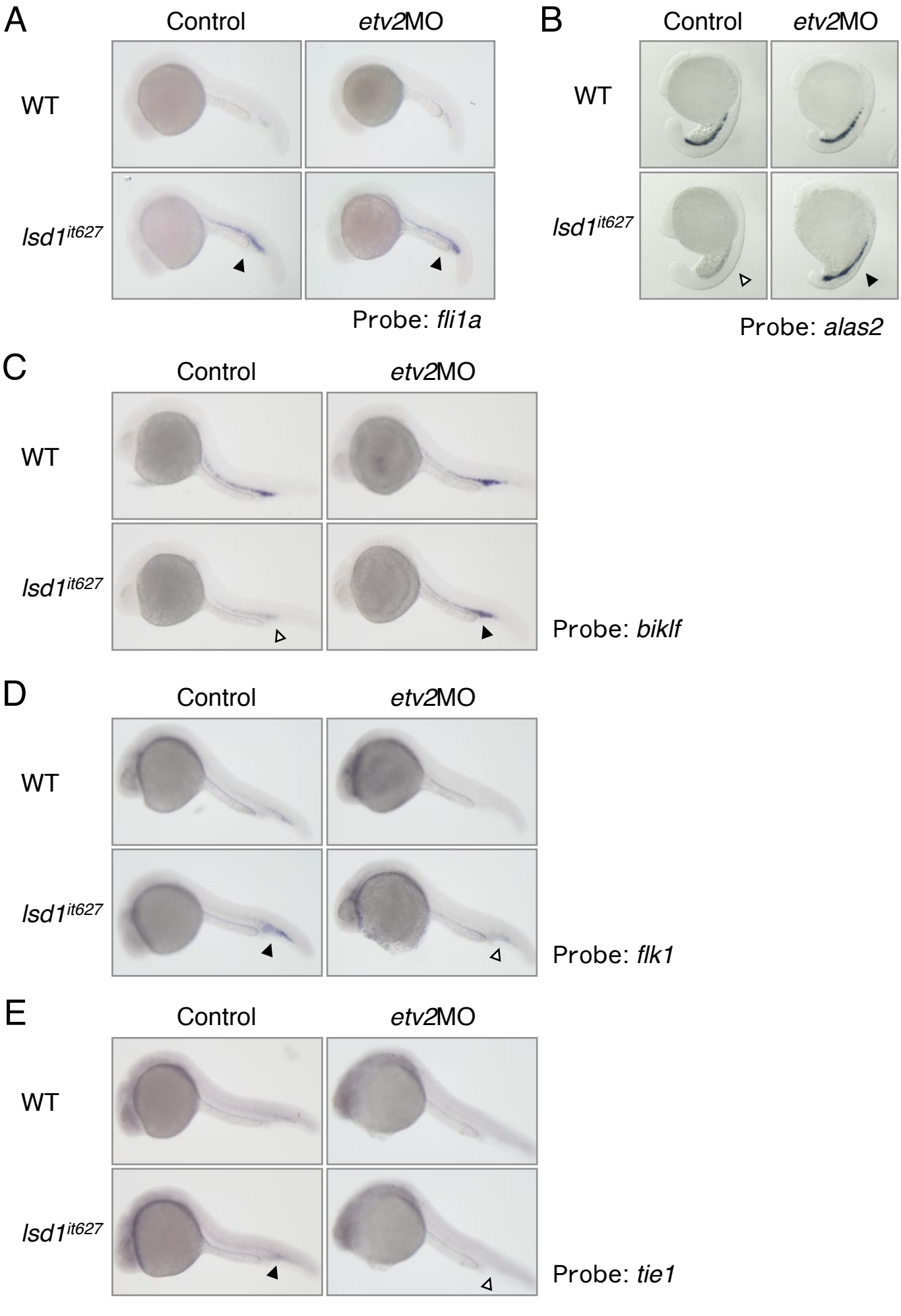

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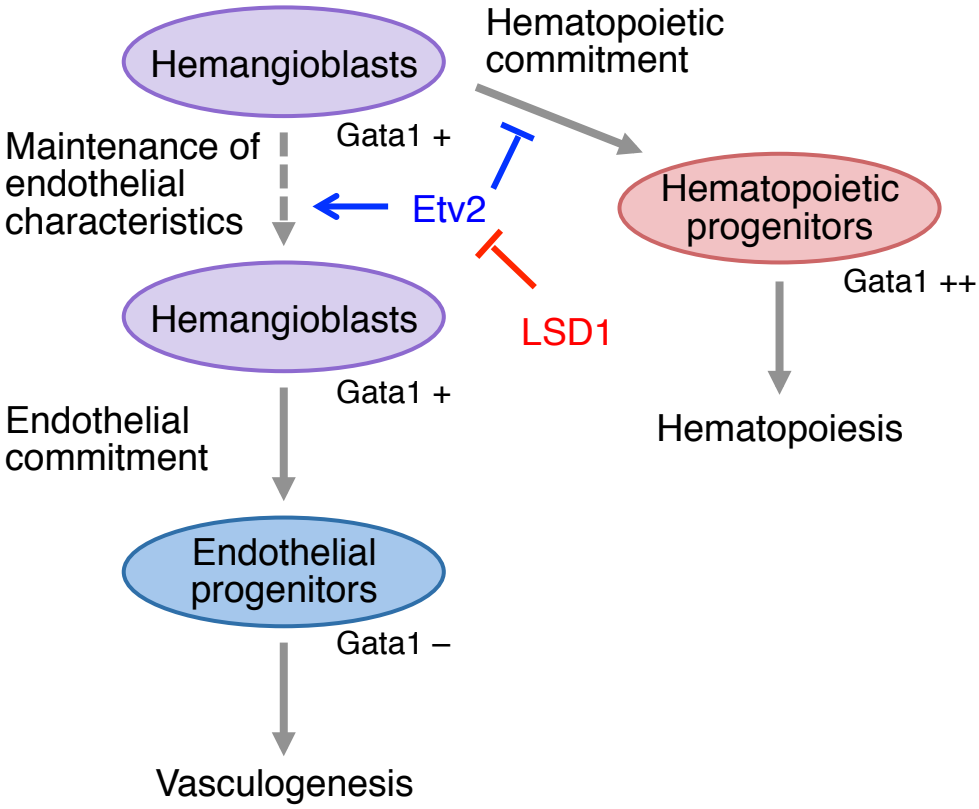

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Table S1 Oligonucleotide primers for ChIP analysis

Target regions	Names	Sequences
up1	etv2.up1.f1	5’-TCGCACAGTTTGGCAACTTAC
	etv2.up1.r1	5’-AGTGCTTTGGACAGATAAGACC
35 bp enhancer	etv2.35bp.f1	5’-AATCTGTGGTCGTGACTCCTG
	etv2.35bp.r1	5’-GTCTGTGCTGGACTGAGGAC
int2	etv2.int2.f2	5’-AACATTTCCGCTGTCCTTGAG
	etv2.int2.r2	5’-GACATCCCATTCCTAAATCTCAG
exon8	etv2.exon8.f1	5’-ACGTCTACCGCTTTGTCTGTG
	etv2.exon8.r1	5’-GCTTGTGTTTGCTACAGACTG