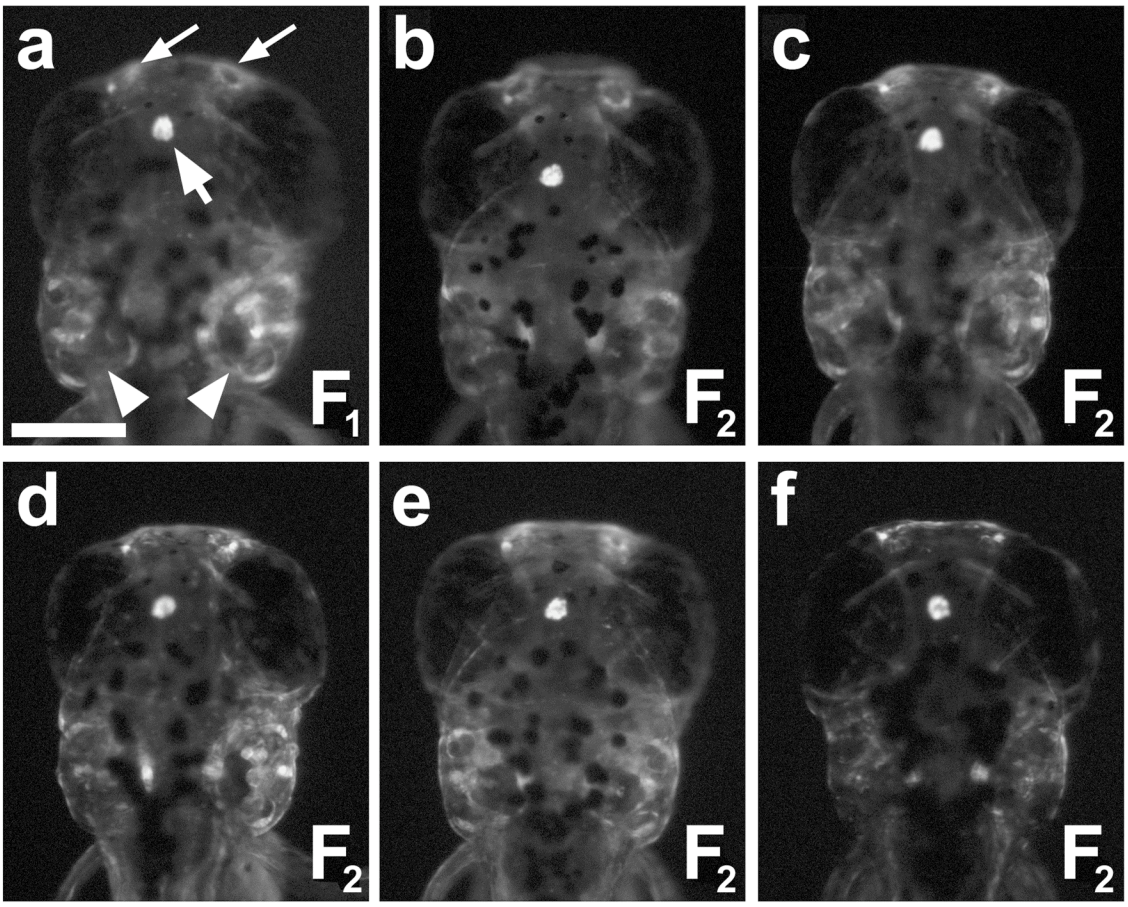
Supplementary Figure 1



Supplementary Figure 1: Uniformity and consistency of expression. Kaede expression is shown for one F_1 larva (a) and five randomly selected F_2 larvae (b-f) for expression pattern *et4.3* at 3dpf. The expression pattern and intensity of expression is roughly the same in all animals, with expression in pineal organ (arrow in a), the otic capsule (arrowheads in a), and the olfactory pits (small arrows in a). The scale bar in panel a is approximately 200 μm, and corresponds to all panels.

Supplementary Table 1: Summary of ET lines

ET insertion	Transgenic line number	Description of expression pattern
et10.1	*s1000t*	Pallium and hindbrain
et10.4	*s1001t*	Cristae and lateral line neuromasts
et11.1	*s1002t*	Cristae and circulating blood cells
et19.1	*s1003t*	Retina, spinal cord, and blood vessels
et25.2	*s1004t*	Spinal cord and habenulae
et28.2	*s1005t*	Gut and sparse cells in ear and spinal cord
et29.4	*s1006t*	Sensory cranial ganglia and lateral line ganglia
et30.1	*s1007t*	Eye, pallium, hindbrain, and spinal cord
et30.2	*s1008t*	Neurons in lower jaw
et30.3	*s1009t*	Hindbrain
et30.4	*s1010t*	Trigeminal and vagal sensory ganglia
et32	*s1011t*	Habenulae, hindbrain, and spinal cord
et33.3	*s1012t*	Hindbrain, spots on skin
et36.1	*s1013t*	Pallium, tectum, hindbrain, and spinal cord
et41.3	*s1014t*	Paired midbrain nuclei
et53.1	*s1015t*	Paired forebrain nuclei
et58.1	*s1016t*	Blood vessels, neurons in two rhombomeres
et68	*s1017t*	Retina, cristae, and hypothalamus
et204	*s1018t*	Tectum, muscle
et206	*s1019t*	Habenulae
et208.1	*s1020t*	Thalamus, spinal cord
et210.1	*s1021t*	Pallium and sensory cranial ganglia

Supplementary Table 1: Expression patterns for ET lines. For each expression pattern, the corresponding transgenic line number and a brief description of the tissues with the most prominent expression are given. Most lines also have background expression in the heart and muscle. Lines *s1000t* through *s1017t* carry the 1.5kb HSP promoter while lines *s1018t* through *s1021t* carry the 600bp HSP promoter.

Supplementary Table 2: Summary of expression patterns

Enhancer trap number	Description of expression pattern
et1	weak olfactory pit, muscle, heart
et2	muscle
et3	scattered neurons throughout CNS
et4.1	hindbrain, subset of heart
et4.2	eye, pineal
et4.3	olfactory pit, jaw cartilage
et5.1	cerebellum, posterior tip of spinal cord
et5.2	pineal, cerebellum, hindbrain
et6.1	olfactory pit, ear, spinal cord
et6.2	pineal, midbrain-hindbrain boundary
et7	scattered hindbrain neurons
et8.1	eye, olfactory bulb, pineal, spinal cord
et8.2	dorsal eye
et9	sensory cranial ganglia VII and VIII
et10.1	pallium, hindbrain
et10.2	habenulae, hypothalamus
et10.3	olfactory bulb, midbrain/hindbrain boundary, spinal cord, liver
et10.4	cristae, lateral line neuromasts, eye
et10.5	pharyngeal arches
et11.1	cristae, cerebellum, blood cells, liver
et11.2	pineal, spinal cord
et12.1	single head neuromast
et12.2	midbrain/hindbrain boundary
et13	neuromasts, pineal, notochord
et14	muscle, heart
et15	pineal, thalamus
et16	muscle, heart
et17.1	cells ventral and anterior to the eye
et17.2	habenulae, midbrain/hindbrain boundary
et18.1	midbrain/hindbrain boundary
et18.2	pineal, spinal cord
et18.3	broad CNS expression
et19.1	retina, spinal cord, blood vessels
et19.2	head neuromasts, spinal cord
et20	blood vessels
et21	gut, skin
et22	broad expression
et23	broad in CNS
et24.1	pineal, habenulae, dorsal midbrain, hindbrain
et24.2	subpallium, lower jaw
et25.1	head neuromasts
et25.2	habenulae, spinal cord

et26.1	spinal cord
et26.2	midbrain/hindbrain boundary, spinal cord
et27	pineal, habenulae, midbrain, hindbrain
et28.1	eye, hindbrain, spinal cord
et28.2	sensory cranial ganglion neurons, spinal cord, gut
et28.3	olfactory bulb and pit, hindbrain, spinal cord
et29.1	lens, pineal, midbrain
et29.2	cristae, lateral line neuromasts, hypothalamus
et29.3	head neuromasts
et29.4	lateral line ganglia
et29.5	hindbrain
et30.1	eye, pallium, hindbrain, spinal cord
et30.2	possible gustatory neurons in lips
et30.3	inner ear
et30.4	trigeminal and vagal sensory ganglia
et31.1	broad in CNS
et31.2	jaw, thalamus
et32	habenulae, hindbrain, spinal cord
et33.1	retina, olfactory bulb, pineal, hindbrain
et33.2	olfactory bulb, spinal cord
et33.3	hindbrain
et33.4	olfactory bulb, dorsal midbrain
et33.5	eye, pineal
et34	pineal
et35.1	broad neural
et35.2	cerebellum
et36.1	pallium, tectum, cerebellum, hindbrain
et36.2	pineal, hindbrain, gut
et37	broad in CNS
et38	pineal, vagal sensory ganglion
et39.1	lens, hindbrain, spinal cord
et39.2	trigeminal sensory ganglion
et40.1	eye, olfactory pit, cristae, pineal, heart
et40.2	pineal
et41.1	pallium
et41.2	pharyngeal arches
et41.3	paired midbrain nuclei
et42.1	broad expression
et42.2	heart, gut
et42.3	broad CNS
et43.1	hindbrain, liver
et43.2	gut, pancreas
et44.1	eye, cristae, tectum, spinal cord
et44.2	broad CNS
et44.3	olfactory pit and bulb, pineal, tectum
et44.4	neuromasts, gut liver

et45	olfactory bulb, pineal, hindbrain
et46	broad CNS
et47	broad CNS
et48	retina, olfactory pit, pineal, hindbrain
et49.1	lens, notochord
et49.2	olfactory pit and bulb, spinal cord
et49.3	pineal
et49.4	pineal, hindbrain, spinal cord
et50	broad CNS
et51.1	pineal, hindbrain, base of pectoral fins
et51.2	lens, hindbrain
et52	scattered in CNS
et53.1	pallium
et53.2	olfactory pit, pineal
et54	pineal
et55.1	midbrain, hindbrain, spinal cord
et55.2	olfactory pit, pineal
et56.1	olfactory bulb, cristae, pineal, spinal cord, gut
et56.2	sensory cranial ganglia, spotty throughout CNS
et56.3	olfactory pit and bulb, subpallium
et57.1	cristae, neuromasts, hindbrain
et57.2	neuromasts, hindbrain, spinal cord
et57.3	tectum, spotty throughout CNS
et58.1	hindbrain, blood vessels
et58.2	olfactory bulb, hypothalamus
et67	Muscle
et68	eye, cristae, hypothalamus
et69	eye, gut
et70	broad CNS
et201.1	hindbrain, spinal cord
et201.2	olfactory pit
et202	olfactory pit, forebrain
et203	midbrain/hindbrain boundary, spinal cord
et204	tectum
et205.1	olfactory pit
et205.2	muscle and heart
et206	olfactory pit and bulb, habenulae
et207.1	olfactory pit and bulb, spinal cord
et207.2	hindbrain
et207.3	inner ear
et207.4	global expression
et208.1	thalamus, spinal cord
et208.2	olfactory pit
et208.3	early endoderm
et209	hindbrain, blood cells
et210.1	pallium, sensory cranial ganglia

et210.2	habenulae
et211	surface of brain
et212.1	heart, muscle
et212.2	thalamus
et213	olfactory pit, sensory cranial ganglia
et214	muscle
et215	eye, cristae

Supplementary Table 2: Location of expression in all insertion lines. For each ET insertion, a brief description is given of where expression is strongest. In addition to the listed tissues, most patterns included background expression in the heart and muscles. Insertions *et1* through *et70* contain the 1.5kb HSP promoter. Insertions *et201* through *et215* contain the 600bp HSP promoter. Numbers *et59* through *et66* were not used.

Supplementary Table 3: Integration Sites

ET insertion	Chromosome & Position	Nearest Gene and descriiption	Insertion position vs nearest gene
et10.1	Ch10: bp21,730,662	Novel predicted gene, Similar to mannose receptor, C type 2	4th intron
et10.4	Ch2: bp43,825,248	zgc:110183 unknown function	1.5kb upstream
et30.4	Ch22: bp3,944,788	zgc:110764 Mitochondrial import inner membrane translocase	12.2kb downstream
et58.1	Ch20: bp25,029,937	rtn1b reticulon 1-like	1st intron
et204	Ch11: bp44,796,716	pax7 paired box gene 7	4th intron
et206[*]	Ch10: bp11,016,855	Mrrf mitochondrial ribosome recycling factor	5.5kb downstream
et208[**]	Ch9: bp22,786 on CT030044.6	unknown	unknown

Supplementary Table 3: Insertion sites resulting in enhancer trap patterns of interest. For each enhancer trap insertion shown, the chromosome and location on the chromosome are indicated. The nearest gene or predicted gene is identified, along with the insertion's proximity to that gene.

*The genomic region flanking insertion *et206* is most strongly aligned with the indicated location on chromosome 10, but is also highly similar to the region flanking chromosome 4, bp42,770,860

***et208* was localized to a sequenced genomic clone, but could not be linked to an assembled genomic contig, and is therefore not linked to a nearest gene.

Supplementary Methods

Construction and injection of plasmid constructs

To make the trapping constructs, the 1.5kb HSP70/4 heat shock promoter[1] was subcloned into the pT2KXIGΔin vector[2] at XhoI and BamHI sites. GAL4-SV40polyA[3] was then inserted downstream of HSP using KpnI and NotI. The 600bp HSP70 contains the 3' portion of the HSP70/4 and was PCR amplified using the following primers: forward primer (5') gaattcactggaggcttccagaacag and reverse primer (3') caggaaaaaaaaaaacaattagaattaatt. This 600bp HSP70 promoter was subcloned into the pT2KXIGΔ vector at ApaI and BamHI sites.

DNA for enhancer trapping was prepared by Qiagen midiprep, and was phenol/chloroform extracted prior to injection. Transposase mRNA was prepared using the Ambion mMESSAGE mMACHINE T7 kit. Embryos were injected at single cell stage with a solution containing 25ng/μl plasmid DNA, 50ng/μl transposase mRNA, and 0.04% Phenol Red.

Identification of genomic insertion locations

Genomic sequences flanking insertions were identified as described[2] using the MboI restriction endonuclease and linker-mediated PCR. Output sequences were aligned with the zebrafish genome using NCBI BLASTn or Ensembl

BlastView. Chromosomal locations and neighboring genes were identified with Ensembl ContigView.

Generation of the Tg (UAS:Kaede)^{s1999t} and BGUG transgenic lines

The *Kaede* open reading frame[4] (kindly provided by Atsushi Miyawaki) was amplified by PCR and inserted downstream of the E1b promoter in a 14xUAS:eGFP construct[3] using the Xi Clone PCR Cloning System (Gene Therapy Systems, San Diego, CA). I-SceI meganuclease recognition sites were inserted flanking the *UAS:Kaede* construct to increase rate of transgenesis and ensure single insertions[5]. Wild-type TL embryos were injected at the 1-2 cell stage with a solution of 50 ng/µl *UAS:Kaede* DNA and 5 U/µl I-SceI endonuclease in 0.04% Phenol Red. Injected F0 animals were raised and interbred. F_1 embryos were pooled and screened by PCR for the *UAS:Kaede* transgene. F_0 founder animals giving rise to *UAS:Kaede*-positive offspring were then mated to existing GAL4 driver lines to identify expressors. All transgenic F_1 gave the identical expression pattern, regardless of their founder parent. The stable *Tg*(*UAS:Kaede*)^{s1999t} line was propagated by outcrossing a single F_0 founder to wild-type TL fish.

For *BGUG*, an EcoRI fragment containing 14xUAS-E1B (from pBUASE1B, ref [3]) was inserted into *pBrn3c:mGFP* vector[6]. A PCR product of GAL4-SV40[3] was inserted into a StuI site behind the *brn3c* promoter to generate the

Brn3c:GAL4;UAS:mGFP construct.

DNA plasmids (50 ng/µl in ddH2O with 0.05% phenol red) were injected into 1-4 cell stage embryos. Injected fish were raised to sexual maturity and crossed to identify carriers as described previously[6]. Two *Brn3c:GAL4;UAS:mGFP* (*BGUG*) transgenic lines, *TG (Brn3c:GAL4;UAS:mGFP)*^{s314t} and *TG (Brn3c:GAL4;UAS:mGFP)*^{s318t} were produced.

Screening and imaging of expression patterns

Potential founders of enhancer trap lines were raised to adulthood and crossed to carriers of *UAS:Kaede.* F_1 clutches containing at least 50 larvae were observed at three and five days postfertilization (dpf) for Kaede expression on a Leica MZFLIII fluorescence stereoscope. Expression patterns were characterized, and larvae bearing interesting patterns were raised to establish stable stocks.

Larvae were imaged live using a Spot camera (Diagnostic Instruments Inc.) on a Leica MZFLIII fluorescence stereoscope, or were imbedded live in 1% low melting temperature agarose and imaged on a Zeiss Pascal confocal microscope. Kaede photoconversions were conducted with DAPI fluorescence illumination on a Zeiss Axioplan 2 compound microscope.

Adult Imaging

Adult fish three to six months of age were anesthetized with Tricane (Western Chemicals Inc. WA, USA) and fixed in 4% PFA for 4 hours. Their brains were removed and embedded in 4% agarose in PBS. Horizontal slices (150 μm) were cut with a vibratome (VT1000S, Leica Microsystems Nussloch GmbH, Germany). Slices were then mounted for imaging in 2% agarose between two cover slips. Sections from adults were imaged on one of the two instruments above, and in some cases, the images from multiple sections were aligned and combined using ImageJ software. All confocal Z-series were produced in ImageJ, and all images were arranged with Adobe Photoshop software.

Generation and naming of transgenic lines

The expression patterns presented here carry an Enhancer Trap (et) number, such as *et25.2.* In this example, this is the second expression pattern found among the offspring of founder 25. From our 141 ETs, we established 22 as stable transgenic lines (see Supplementary Tables 1 and 2). These lines have been assigned an allele number following zebrafish nomenclature guidelines (http://zfin.org).

References:

1. Halloran, M.C. et al. *Development* **127**, 1953-60 (2000).
2. Kotani, T., Nagayoshi, S., Urasaki, A. & Kawakami, K. *Methods* **39**, 199-206 (2006).
3. Koster, R.W. & Fraser, S.E. *Dev Biol* **233**, 329-46 (2001).
4. Ando, R., Hama, H., Yamamoto-Hino, M., Mizuno, H. & Miyawaki, A. *Proc Natl Acad Sci U S A* **99**, 12651-6 (2002).
5. Thermes, V. et al. *Mech Dev* **118**, 91-8 (2002).
6. Xiao, T., Roeser, T., Staub, W. & Baier, H. *Development* **132**, 2955-67 (2005).