zebraflash transgenic lines for in vivo bioluminescence imaging of stem cells and regeneration in adult zebrafish

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ABSTRACT
The zebrafish has become a standard model system for stem cell and tissue regeneration research, based on powerful genetics, high tissue regenerative capacity and low maintenance costs. Yet, these studies can be challenged by current limitations of tissue visualization techniques in adult animals. Here we describe new imaging methodology and present several ubiquitous and tissue-specific luciferase-based transgenic lines, which we have termed zebraflash, that facilitate the assessment of regeneration and engraftment in freely moving adult zebrafish. We show that luciferase-based live imaging reliably estimates muscle quantity in an internal organ, the heart, and can longitudinally follow cardiac regeneration in individual animals after major injury. Furthermore, luciferase-based detection enables visualization and quantification of engraftment in live recipients of transplanted hematopoietic stem cell progeny, with advantages in sensitivity and gross spatial resolution over fluorescence detection. Our findings present a versatile resource for monitoring and dissecting vertebrate stem cell and regeneration biology.

KEY WORDS: Zebrafish, Live imaging, Luciferase, Stem cells, Regeneration, Heart regeneration

INTRODUCTION
Zebrafish have been a valuable model system for vertebrate embryologists for nearly three decades, and the past decade has also seen their widespread use in stem cell and regeneration studies. Zebrafish are highly regenerative as adults, being equipped to regrow tissues such as amputated fins, injured retinae, transplanted optic nerves and spinal cord, lost heart muscle, brain, hair cells, pancreas, liver, skeletal muscle and kidney (Johnson and Weston, 1995; Bernhardt et al., 1996; Becker et al., 1997; Vihtelic and Hyde, 2000; Poss et al., 2002; Sadler et al., 2007; Ma et al., 2008; Jacoby et al., 2009; Moss et al., 2009; Diep et al., 2011; Andersson et al., 2012; Goldshmit et al., 2012; Kizil et al., 2012). Adult zebrafish present advantages for mechanistic studies as they are small, easy to raise in large numbers and accessible to cell transplantation and molecular genetic manipulations.

Zebrafish lose transparency during larval development. Thus, although fluorescent reporter proteins provide excellent optical tools for phenotyping and real-time imaging in zebrafish embryos, adult zebrafish only have marginal advantages over adult mice in visualizing stem cell and regenerative events by fluorescence detection. casper fish, with homozygous mutations in two pigmentation loci, were developed to assist imaging of fluorescent stem cells or melanoma cells after transplantation (White et al., 2008). Although this resource has enhanced the study of transplanted fluorescent cells in vivo, detection in these semi-transparent animals is semi-quantitative, can show a low signal-to-noise ratio, and is mainly limited to the labeled cells that are close to the body surface.

Luciferase-based imaging has been widely used in mice for monitoring gene expression, cell proliferation and migration, and bacterial and viral infections (Contag and Bachmann, 2002; Luker and Luker, 2008; Liu et al., 2011; Tinkum et al., 2011; Martinez-Corral et al., 2012; Maguire et al., 2013). Detection of bioluminescent signals generated from the luciferase-luciferin reaction is sensitive, virtually free of background noise, and can reveal biological information even from deep tissues. To date, reports on luciferase imaging in zebrafish have been few and limited to embryos (Mayerhofer et al., 1995; Liang et al., 2000; Weger et al., 2013).

Here, we describe a suitable methodology and several new transgenic lines to facilitate luciferase-based imaging in zebrafish. We find that luciferase activity in adult zebrafish can be reproducibly monitored in various settings, including deep tissues of freely swimming animals. We have applied this technique to monitor the progress of heart regeneration in individual zebrafish with cardiac injuries, removing the need to sacrifice animals for histological analysis. Moreover, stem cell transplantation experiments using transgenic donors enabled noninvasive quantitative assessment of engraftment in live animals, and unambiguously identified colonization and proliferation sites for these cells. We expect luciferase-based imaging in adult zebrafish to have multiple applications in stem cell and regeneration research.

RESULTS
Ubiquitous and tissue-specific luciferase-based transgenic zebrafish lines
To directly compare fluorescence-based and luciferase-based detection in adult zebrafish, we generated several transgenic lines that enable expression of both mCherry and firefly luciferase (Fluc) from a single transcript. Regulatory sequences for these lines were: (1) a 3.5 kb ubiquitin (ubi) promoter that drives constitutive transgene expression during all developmental stages (Fig. 1A) (Mosimann et al., 2011); (2) a 5.8 kb β-actin2 promoter that drives widespread transgene expression (supplementary material Fig. S1A) (Higashijima et al., 1997); (3) a 600 bp cryaa promoter that drives transgene expression specifically in the lens (Fig. 1B) (Kurita et al., 2003); and (4) a 1.6 kb cmlc2 (myl7) promoter that drives transgene expression in zebrafish cardiomyocytes (Fig. 1C) (Huang et al., 2003). A cmlc2-driven luciferase line with EGFP replacing mCherry was also generated (supplementary material Fig. S1B). As expected, fluorescent protein expression was easily visualized at the surface.
of the fish (Fig. 1A,B), but was difficult to detect in an internal organ such as the heart (Fig. 1C). By contrast, we readily detected strong bioluminescence from both external and internal sources of expression upon luciferin delivery (Fig. 1D-F). Notably, although melanin absorbs light, the photons emitted from internal heart tissue do not show signs of signal attenuation by the longitudinal stripes of black melanophores, presumably owing to the short distance through which the emitted light has to travel (Fig. 1F; supplementary material Fig. S1B). Using a high-speed imaging camera, we were able to reliably localize cardiac and lens bioluminescence in adult zebrafish to specific structures at low magnification from overhead (Fig. 1G-I). Thus, bioluminescence in these transgenic zebrafish lines, which we have named zebrafish for their capacity to produce light, readily penetrates through adult tissues.

Quantitative and multiplex luciferase imaging in freely moving adult zebrafish

To optimize protocols for luciferase-based imaging in adult zebrafish, we used the cardiac zebrafish line cmilc2:mCherry-N-2A-Fluc (Fig. 1C). We tested two different routes to deliver luciferin: bathing and intraperitoneal injection (Fig. 2A). In both cases, cmilc2:mCherry-N-2A-Fluc produced a steady bioluminescent signal lasting for more than an hour (supplementary material Fig. S2A-C). Next, we determined the kinetics of bioluminescence and the optimal concentration of luciferin. To monitor the kinetic curve of the signal, we bathed adults in luciferin and immediately started imaging. Bioluminescence began to peak and reach a steady state ~8-10 minutes after initiating treatment (Fig. 2B; supplementary material Fig. S2D). 100 mM luciferin consistently produced more light than 50 mM luciferin (~2.5 fold increase; Fig. 2C) and was employed for most experiments presented here.

In bioluminescence assays of adult mice, animals are fully sedated during imaging. The plateau phase of the signal usually occurs 15 minutes after luciferin injection and endures for another 20 minutes (Zinn et al., 2008). Sequential measurements are recorded during the plateau phase and averaged for comparisons across different mice. We found that it was challenging to keep fish anesthetized for 20 minutes after luciferin delivery without affecting survival. To image conscious zebrafish, we acquired data from the entire region of interest (ROI) from animals maintained in tissue culture wells. Animal movement during the plateau phase did not affect measurements of bioluminescence (Fig. 2D). We routinely imaged six to nine adult fish at a time (Fig. 2E) or more for juvenile fish (Fig. 2F). Twenty sequential measurements were recorded from individual animals during the plateau phase (Fig. 2D). We measured bioluminescence among several fish in these experiments and found that, although the relative signal strength could vary between animals, it was consistent within each animal over 5 days of daily independent measurements (Fig. 2C). We determined the posture of the animals during imaging could affect the measured bioluminescence up to 2-fold, and thus required monitoring (Fig. 2E,F). Because of the high bioluminescent output of zebrafish tissues, we were able to capture a series of real-time movies with 30 to 50 msecond exposure per frame from freely swimming 4-week-old juveniles (supplementary material Movie 1) or 3-month-old adults.
These results indicated that it is practical to reliably capture and quantify bioluminescence from the internal organs of several fish swimming together.

**Longitudinal monitoring of heart regeneration by luciferase-based imaging**

Currently, it is possible to estimate readouts of regeneration of internal tissues in individual zebrafish by assessing organ function. For example, glucose levels provide a surrogate readout for islet cell number or function (although this assay requires a cardiac stab injury) (Moss et al., 2009), and swimming endurance recovers concomitant with regeneration of heart muscle (Wang et al., 2011). However, it is not possible to directly monitor the recovery of new internal tissue after injury. Heart regeneration occurs robustly in adult zebrafish after surgical resection, cryoinjury or induced cell death by activating the proliferation of spared cardiomyocytes (Poss et al., 2002; Jopling et al., 2010; Kikuchi et al., 2010; González-Rosa et al., 2011; Wang et al., 2011). Studies of heart regeneration typically employ a large number of animals for each experiment, all of which are sacrificed for tissue collection at various time points to approximate the progression of regeneration. To test whether bioluminescence is capable of interpreting regenerative progress, we first examined its correlation with cardiac size in adult cm02: mCherry-N-2A-Fluc animals inside an imaging chamber. (F) Snapshot of a movie of ten swimming 4-week-old juvenile cm02: mCherry-N-2A-Fluc animals, indicating cardiac bioluminescence. Luminescence signals are reported as radiance (p/sec/cm²/sr) with a color bar.
For these experiments, we crossed cmlc2:mCherry-N-2A-Fluc into a strain that enables inducible diphtheria toxin A-mediated ablation of cardiomyocytes (Wang et al., 2011) (Fig. 4B). We found that the addition of tamoxifen, to induce cardiomyocyte ablation throughout both chambers, significantly reduced cardiac bioluminescence by 10 days post-treatment in most animals (Fig. 4C). Loss of cardiac bioluminescence was visually apparent in movies of swimming vehicle- and tamoxifen-treated zebrafish (Fig. 4D; supplementary material Movie 4). The quantified bioluminescence intensity at this time point was predictive of the extent of cardiomyocyte ablation, as determined by histology and muscle quantification (supplementary material Fig. S3B-D). Next, we examined whether luciferase-based imaging could monitor the progress of heart regeneration within a single animal. To increase the throughput of the imaging, we first tagged individual animals with commercially available p-Chips (supplementary material Fig. S4; see Materials and methods for details). We then induced cardiomyocyte ablation and chose, for longitudinal monitoring, those animals with at least a 25% reduction in bioluminescence at 12 days post-injury. Notably, bioluminescence gradually recovered and reached the approximate pre-ablation level by ~35 days post-injury (n=17; Fig. 4E), consistent with the published kinetics of heart regeneration in this injury model (Wang et al., 2011). Both males and females recovered similarly (Fig. 4F). We also performed a second ablation injury in a portion of these animals, and found that they recovered similar bioluminescence levels as prior to the initial injury, although after a longer period of 100 days (n=6; supplementary material Fig. S3E,F).

Luciferase-based live imaging after hematopoietic stem cell transplantation

Current methodology to assess stem cell engraftment involves the transplantation of cells from a transgenic donor strain expressing a fluorescent reporter protein in a tissue-specific or ubiquitous manner, often into the semi-transparent host strain casper (Traver et al., 2003; White et al., 2008; Stachura et al., 2011). To examine whether zebrafish lines offer broad-spectrum labeling for various blood lineages, we first examined mCherry expression in whole kidney
marrow (WKM) cells isolated from *ubi:mCherry-2A-Fluc* lines and subjected to flow cytometric analysis (Mosimann et al., 2011). One line \[Tg(ubi:mCherry-2A-Fluc) pd75\] displayed particularly robust and ubiquitous labeling (~87.8%) of most WKM cells (Fig. 5A,B), including erythroid (~71.1%), myeloid (~98.6%) and lymphoid (~96%) lineages and precursor cell populations (~97.1%) (Fig. 5C). Then, WKM cells isolated from this line were mixed with wild-type WKM cells in different ratios and injected into irradiated casper recipients (Fig. 6A) (Traver et al., 2003; Pugach et al., 2009). After 1 month, we quantified bioluminescence in freely swimming cell recipients. We found a high positive correlation between the intensity of whole animal bioluminescence and the input number of *ubi:mCherry-2A-Fluc* WKM cells \((n=6-9\) per group; Fig. 6B,C). The variation seen in Fig. 6C most likely reflects the variable number of successfully engrafted WKM cells after transplantation in adult zebrafish, which has been documented by FACS analysis (de Jong et al., 2011). Although we estimated the detection limit in this experiment to be ~500 cells (0.5%), we could not detect bioluminescence above background from most animals with the lowest (5-10%) dilution of transgenic cells (Fig. 6D). This sharp decline in the engraftment ratio at 5000-10,000 transgenic WKM cells closely resembles that reported previously for limiting dilution transplantation experiments (de Jong et al., 2011; Hess et al., 2013). Thus, luciferase imaging offers high sensitivity and a new opportunity for detecting the progeny of engrafted stem cells in freely moving recipient animals.

We next performed higher magnification imaging of anesthetized recipients to visualize sites of stem cell colonization/proliferation. Bioluminescence was strong throughout the entire animal (Fig. 6E,F), ostensibly owing to the proliferation and circulation of multiple WKM-derived blood lineages. By increasing the detection cut-off, we could unambiguously identify signal hotspots in the trunk kidney, the head kidney and the thymus, which are three recognized sites for stem cell colonization in adult zebrafish (Fig. 6G).
Fig. 5. FACS analysis of labeled WKM cells. Flow cytometry analysis of adult whole kidney marrow (WKM) cells from the transgenic line Tg(ubi:mCherry-2A-Fluc)pd75. mCherry-positive cells were resolved by forward scatter and side scatter (A). The majority of isolated WKM cells express mCherry fluorescent protein (B), including the erythroid, myeloid and lymphoid lineages and precursor cell populations (C).

6G) (Murayama et al., 2006). Comparisons of bioluminescence with fluorescence imaging within the same fish indicated that bioluminescence was not only qualitatively better for the detection of transplanted cells in intact animals but also improved the spatial resolution of donor-derived tissues (Fig. 6H). For instance, fluorescent signals from the thymus were completely obstructed (Fig. 6I), whereas thymic bioluminescence was strong and clear (Fig. 6G).

**DISCUSSION**

By applying the properties of luciferase-based imaging to adult zebrafish, we have established a methodology and new transgenic resources for the study of stem cells and organ regeneration. Bioluminescence is quantitative and highly sensitive, and marries well with small, thin adult zebrafish, which have no body hair to attenuate light (Sadikot and Blackwell, 2008; Curtis et al., 2011). Such a combination is ideal for whole animal imaging to monitor cell loss, migration, expansion and regeneration in internal tissues.

Although luciferase-based imaging requires administration of substrate and relies on the availability of a more sensitive camera and a light-sealed imaging chamber, there are multiple advantages over fluorescence detection for tissue regeneration research that we can identify from this study. First, bioluminescence is more sensitive for deep tissues, enabling clear visualization of signals in freely moving animals. Second, luciferase-based imaging is quantitative, allowing assessment of the amount of tissue that is removed by injury and the amount that is replaced during regenerative events in an individual animal. This allows immediate confirmation of the extent of the injury, and removes the requirement for qualitative histological analysis of fixed tissues to determine the extent of regeneration. Consequently, the ability to follow individual animals throughout the regeneration process without sacrificing them for tissue collection should considerably reduce the number of animals required for experiments. This will also help to detect subtle changes despite variation among individuals. When combined with an inducible genetic injury system, as we have shown here for the heart, the tedium of resection surgery plus that of histological detection is circumvented. These improvements in efficiency and sensitivity should lay the groundwork for discovery approaches such as chemical or genetic screens for effectors of heart regeneration.

By swapping regulatory sequences, the imaging system described here can be readily applied to study the regeneration of other organs in adult zebrafish. Luciferase activity formally reflects gene expression and not tissue amounts. Thus, for quantitative in vivo bioluminescence assays of tissue regeneration, it is necessary to identify a tissue-specific promoter that remains largely stable during regeneration. In alternative strategies, injury-responsive or regeneration-responsive regulatory sequences can be used to drive luciferase in transgenic lines. Such tools are predicted to detect sharp changes in expression in response to injury and should be optimal for screening approaches.

The resources that we report here provide similar advantages over fluorescence detection for stem cell transplantation studies. Engraftment can be assessed rapidly, quantitatively and with higher sensitivity in individual animals, and does not require sacrificing animals for end-point flow cytometric analysis. These advantages should enable more efficient experiments with wild-type and mutant donors or recipients, which can include: (1) single-cell transplants,
as performed to identify self-renewing stem cells that contribute to murine skeletal muscle regeneration or hematopoietic stem cell reconstitution in mice (Wagers et al., 2002; Sacco et al., 2008); (2) stem cell competition assays (Harrison et al., 1993); and (3) whole animal metastasis assays (Ceol et al., 2011).

Although luciferase-based imaging provides certain advantages over fluorescence imaging, it also has limitations. In vivo bioluminescence images of swimming recipient fish transplanted with different ratios of ubi:mCherry-2A-Fluc WK6 cells. (C) Bioluminescence reading from recipient fish at 30 days post-transplant (n=6-9 per group). (D) Percentage of recipients with any above-background bioluminescence at 30 days post-transplant.

(E,F) Representative bioluminescence images of a freely moving recipient fish, viewed overhead (E) or laterally (F) under anesthesia. (G) Top Depiction of the internal anatomy of an adult zebrafish. (Bottom) Representative bioluminescence image of a recipient fish, at high magnification and a higher threshold setting, indicating signals from key hematopoietic stem cell colonization sites. Red arrows indicate the location of trunk kidney (T) and head kidney (H); the green arrowhead points to the thymus. (H,I) Fluorescence images (H) were acquired from the same recipient fish shown in G, along with a non-recipient animal (Ctrl.). Background fluorescence signal was subtracted by ImageJ with a higher threshold setting to reveal signals from key hematopoietic stem cell colonization sites (I). Luminescence signals are reported as radiance (p/sec/cm²/sr) with a color bar. Scale bars: 5 mm.

Fig. 6. Luciferase-based live imaging after hematopoietic stem cell transplantation. (A) Scheme of marrow transplantation experiments. (B) Representative bioluminescence images of swimming recipient fish transplanted with different ratios of ubi:mCherry-2A-Fluc WK6 cells. (C) Bioluminescence reading from recipient fish at 30 days post-transplant (n=6-9 per group). (D) Percentage of recipients with any above-background bioluminescence at 30 days post-transplant.

(E,F) Representative bioluminescence images of a freely moving recipient fish, viewed overhead (E) or laterally (F) under anesthesia. (G) Top Depiction of the internal anatomy of an adult zebrafish. (Bottom) Representative bioluminescence image of a recipient fish, at high magnification and a higher threshold setting, indicating signals from key hematopoietic stem cell colonization sites. Red arrows indicate the location of trunk kidney (T) and head kidney (H); the green arrowhead points to the thymus. (H,I) Fluorescence images (H) were acquired from the same recipient fish shown in G, along with a non-recipient animal (Ctrl.). Background fluorescence signal was subtracted by ImageJ with a higher threshold setting to reveal signals from key hematopoietic stem cell colonization sites (I). Luminescence signals are reported as radiance (p/sec/cm²/sr) with a color bar. Scale bars: 5 mm.

MATERIALS AND METHODS

Zebrafish

Adult zebrafish of 3-5 months of age were used for most experiments. Animal density was maintained at 3-4 per liter in all experiments. All transgenic lines were analyzed as hemizygotes. To induce cardiomyocyte
abilion, triple-transgenic cmlc2:mCherry-N-2A-Fluc; Z-CAT zebrafish were bathed in 0.5 μM tamoxifen in fish water for 16 hours as described previously (Wang et al., 2011). All animal procedures were performed in accordance with Duke University guidelines.

**Transgenic zebraflash lines**

**ubi:mCherry-2A-Fluc**
The sequences of mCherry, 2A (Kim et al., 2011) and firefly luciferase (Promega) were cloned downstream of the *ubi* promoter (3.5 kb) (Mosimann et al., 2011) by yeast recombinational cloning as described (Oldenburg et al., 1997). The construct was flanked with I-Sce1 sites to facilitate transgenesis. Purified DNA (30 ng/μl) was injected into one-cell zebrafish embryos after linearization by I-Sce1 digestion. The full name of this transgenic line is Tg(ubi:mCherry-2A-Fluc)^pd73.

**cryaa:mCherry-2A-Fluc**
The sequences of mCherry, 2A and firefly luciferase were cloned downstream of the *cryaa* promoter (0.6 kb) (Kurita et al., 2003) by yeast recombinational cloning. The entire construct was flanked with I-Sce1 sites to facilitate transgenesis. The full name of this transgenic line is Tg(cryaa:mCherry-2A-Fluc)^pd74.

**cmlc2:mCherry-N-2A-Fluc**
The sequences of mCherry-N (mCherry-bacterial nitroreductase fusion) (Grohmann et al., 2009), 2A and firefly luciferase were cloned downstream of the *cmlc2* promoter (1.6 kb) (Huang et al., 2003) by yeast recombinational cloning. The entire construct was flanked with I-Sce1 sites to facilitate transgenesis. The full name of this transgenic line is Tg(cmlc2:mCherry-N-2A-Fluc)^pd71. A second line, Tg(cmlc2:mCherry-N-2A-Fluc)^pd72, was independently selected after transgenesis.

**β-actin2:mCherry-2A-Fluc**
The sequences of mCherry, 2A and firefly luciferase were cloned downstream of the *β-actin2* promoter (5.8 kb) (Higashijima et al., 1997) by yeast recombinational cloning. The entire construct was flanked with I-Sce1 sites to facilitate transgenesis. The full name of this transgenic line is Tg(β-actin2:mCherry-2A-Fluc)^pd75.

**cmlc2:EGFP-2A-Fluc transgenic lines**
The sequences of EGFP, 2A and firefly luciferase were cloned downstream of the *cmlc2* promoter (1.6 kb) by yeast recombinational cloning. The entire construct was flanked with I-Sce1 sites to facilitate transgenesis. The full name of this transgenic line is Tg(cmlc2:EGFP-2A-Fluc)^pd73.

**Tagging adult zebrafish for long-term monitoring**
To increase the throughput of the imaging and monitor many individual adult zebrafish in the same tank over time, commercially available p-Chips (Gruda et al., 2010) were tested and implanted into the left dorsal trunk muscle (supplementary material Fig. S4A,B). We could follow tagged adult zebrafish over a 4-month period with a 96% success rate (supplementary material Fig. S4C) and with no obvious impact on animal health (supplementary material Fig. S4D).

**Transplantation of WKM cells**
WKM cells from the Tg(ubi:mCherry-2A-Fluc)^pd71 line were transplanted into irradiated casper recipient adult fish by retro-orbital injection as described previously (Traver et al., 2003; Pugach et al., 2009). Recipients were subjected to y-irradiation using a cesium-137 irradiator at a split dose of 30 gy on day −2 and day −1 prior to transplantation. On the day of transplantation, WKM cells from *ubi:mCherry-2A-Fluc* and non-irradiated casper fish were harvested and injected into recipients at doses ranging from 5000 to 100,000 cells per recipient fish. Transplanted fish were kept off-flow for 7 days post-injection in supplemented fish water, as described previously (Pugach et al., 2009).

**FACS analysis of WKM cells**
WKM cells from *ubi:mCherry-2A-Fluc* fish were harvested and prepared in 0.9% phosphate-buffered saline (PBS) with 5% heat-inactivated fetal bovine serum (FBS). WKM cells were dissociated and filtered prior to FACS analysis. WKM populations were determined using an LSR-II analyzer (BD Biosciences) based on SSC and FSC profiles as previously described (Traver et al., 2003). Flow cytometry data were analyzed using FlowJo software (TreeStar).

**Histological analysis**
Zebrafish hearts were collected and fixed in 4% paraformaldehyde (PFA) at room temperature for 1 hour or at 4°C overnight. The tissue was then incubated in 30% sucrose overnight at 4°C. All histological analyses were performed using 10 μm cryosections. Immunofluorescence staining was performed as previously described (Lepilina et al., 2006). Antibodies used were: anti-Myosin heavy chain (1:100, F59, mouse monoclonal; Developmental Studies Hybridoma Bank), anti-cardiac troponin T (1:100, mouse monoclonal; Thermo Scientific, MS-295-P1-ABX) and Alexa Fluor 588 goat anti-mouse IgG (H+L; Invitrogen, A-11001). Ventricular muscle surface area was quantified as described using the three largest longitudinal sections from each ventricle (Wang et al., 2011).

**In vitro luciferase assays**
Zebrafish hearts were collected and snap-frozen in liquid nitrogen. The frozen tissue was ground in 500 μl 1× CCLR (Promega, E1500) in a homogenizer (TissueLyzer, Qiagen). After a brief centrifugation, the supernatant was assayed for protein concentration (Thermo Scientific Pierce, #23227) and in vitro luciferase activity (Promega, E1500).

**Imaging**
Fluorescence images were acquired using a Leica M205 FA dissecting microscope. For bioluminescence, adult zebrafish harboring a luciferase transgene or transplanted cells expressing luciferase were given 100 mM D-luciferin (Biosynth, L-8240) by bathing in aquarium water or in 10 μl PBS by intraperitoneal (I.P.) injection. I.P. injection is more cost effective, whereas bathing is recommended for small juvenile animals. For bath treatment, animals were allowed to swim in luciferin solution for 15 minutes to reach the plateau phase of bioluminescence. After rinsing in aquarium water, the fish were ready for imaging. For I.P. injection, fish were first anesthetized by tricaine immersion before injection, and then given 3-5 minutes recovery time in aquarium water. We found that bioluminescence immediately reached the plateau phase if luciferin was delivered by I.P. injection. For imaging, adult zebrafish were placed in a low dose of tricaine (0.01 mg/ml) to reduce stress, and placed in an enclosure with a charge-coupled device camera (IVIS Kinetic system, PerkinElmer). Individual adult zebrafish were housed in tissue culture plates with black walls (Molecular Devices, W1155) to avoid light reflection. Exposure times for imaging ranged from 10 seconds to 1 minute per frame, depending upon transgenic lines and the IVIS model used for imaging. Typically, 6-9 fish are imaged at the same time for 20 minutes during the plateau phase. Total photon output was quantified as total photon counts per second (p/s) using Living Image software (PerkinElmer). Ten median values from a total of 20 frames of recorded data were averaged and used for comparison across different fish (Fig. 2A,B). Individually tagged fish were imaged before and after heart injury to monitor their injury levels and regeneration over time. For longitudinal monitoring of the same animal over days or months, a set of control fish (5-8 fish) was repeatedly measured and the mean value of the readout was used to correct day-to-day bias from the imaging system.

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**Competing interests**
The authors declare no competing financial interests.

**Author contributions**
C.-H.C. generated zebraflash lines and performed imaging. E.D. and Li.Z. designed and performed transplantation experiments and flow cytometry. J.W. generated Z-CAT lines. C.-H.C. and K.D.P. designed the experiments, analyzed the data, and wrote the manuscript. All authors commented on the manuscript.
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Supplementary material
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