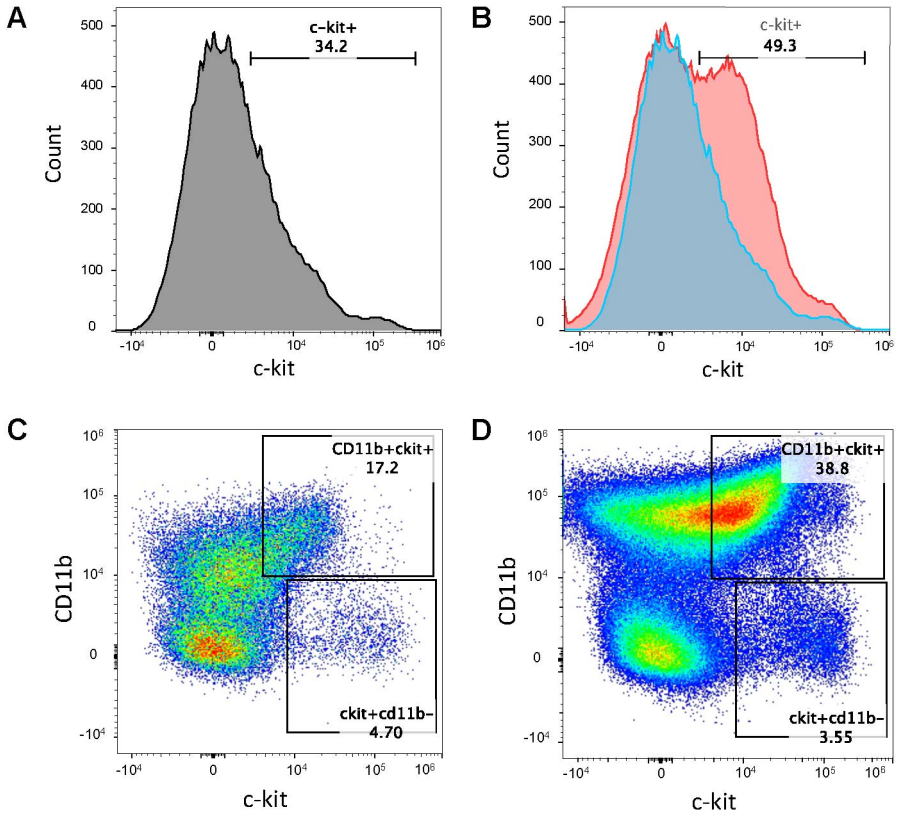


Figure S1. Murine bone marrow HSPC preparations are enriched for c-kit+/CD11b+ cells.

Mouse bone marrow cells were isolated, lineage depleted and incubated with an antibody cocktail containing murine anti-CD45, anti-c-kit and anti-CD11b antibodies. A–B) A c-kit histogram plot of CD45+ live pre-gated cells show bone marrow cells prior A) and after B) HSPCs enrichment (prior and post enrichment in blue and red, respectively). The enriched cell preparation contains ~50% of c-kit+ cells. C–D) Dot plots of CD45+ live pre-gated bone marrow cells analyzed for c-kit+/CD11b+ prior C) and after D) HSPCs enrichment. The enriched bone marrow cell preparation contains ~39% of c-kit+/CD11b+ cells. N= 2 independent experiments.

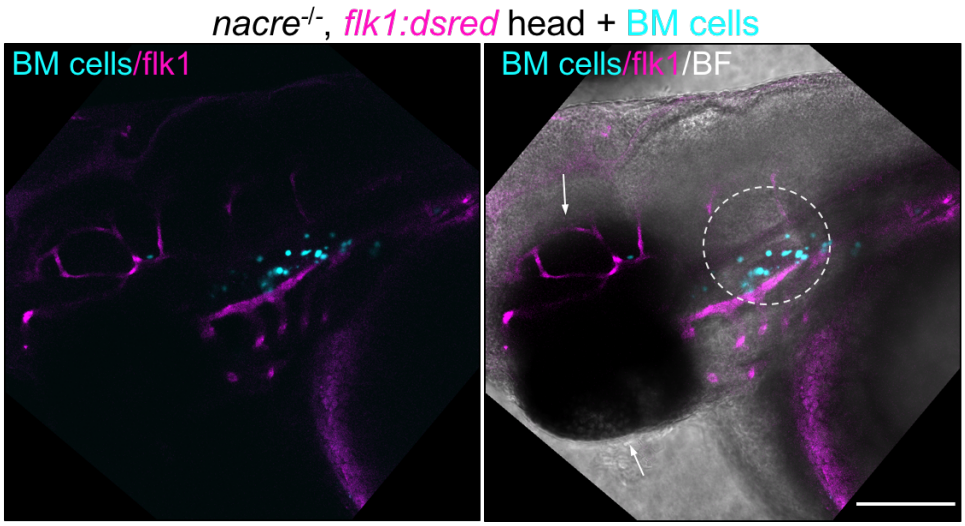


Figure S2. Xenotransplanted mouse cells HSPCs migrate to the thymic rudiment of the zebrafish host.

Mouse bone marrow cells were isolated, lineage depleted, labeled with CellTrace Violet, transplanted into *nacre*$^{-/-}$, *flk1:dsred* embryos and visualized at 3 dpf. The pseudo-colored z-stack confocal image shows mouse cells (cyan) clustered near the position of the developing thymus of the fish larva. Dashed circle depicts the otic vesicle and arrows both eyes. BF: bright field. BM cells: mouse bone marrow cells. Scale bar: 135 µm.

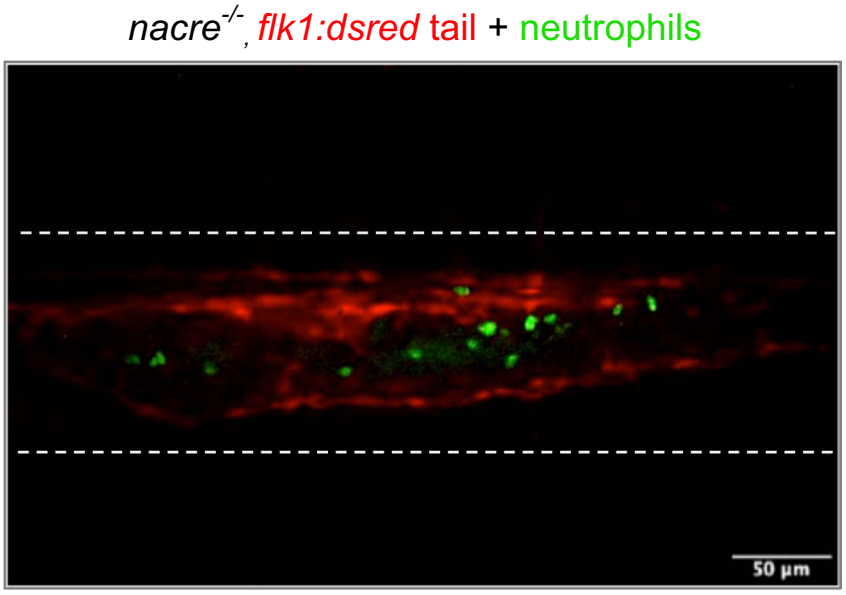


Figure S3. Mouse bone marrow neutrophils also colonize the developing hematopoietic tissues of zebrafish embryos.

Mouse bone marrow cells were isolated and neutrophils were obtained by means of negative selection. Mouse cells were labeled with CellTrace CFSE (green emission) and transplanted into nacre$^{-/-}$, *flk1:dsred* transgenic fish. Pseudo-colored z-stack confocal image of the fish PBI from a 2 dpf transplanted embryo. Area within dashed white lines indicate the PBI.

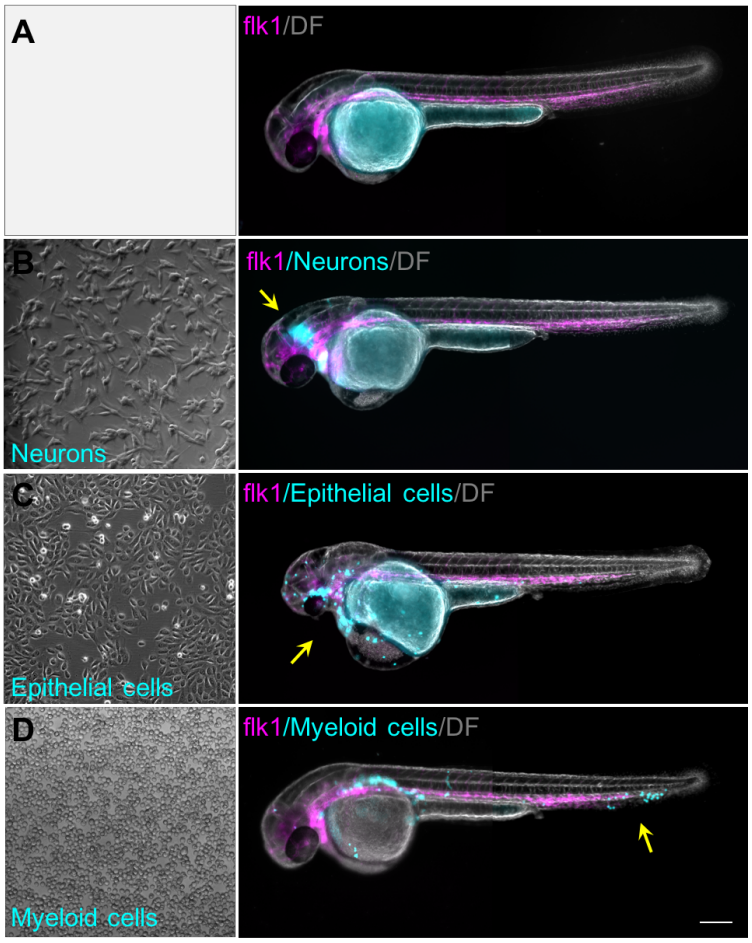


Figure S4. Transplantation of mammalian neuronal or epithelial cells does not result in colonization of the fish caudal hematopoietic tissue.

Pseudo-colored images of *nacre*$^{-/-}$, *flk1:dsred* transgenic animals of A) control and B) transplanted with CellTrace Violet stained human SH-SY5Y neuronal cells, C) human T24 epithelial bladder cells or D) murine LADMAC myeloid cells within the blastomers of 3–5 hpf zebrafish embryos. Yellow arrows indicate main localization of transplanted cells. Scale bar: 100 μm. DF: dark field.

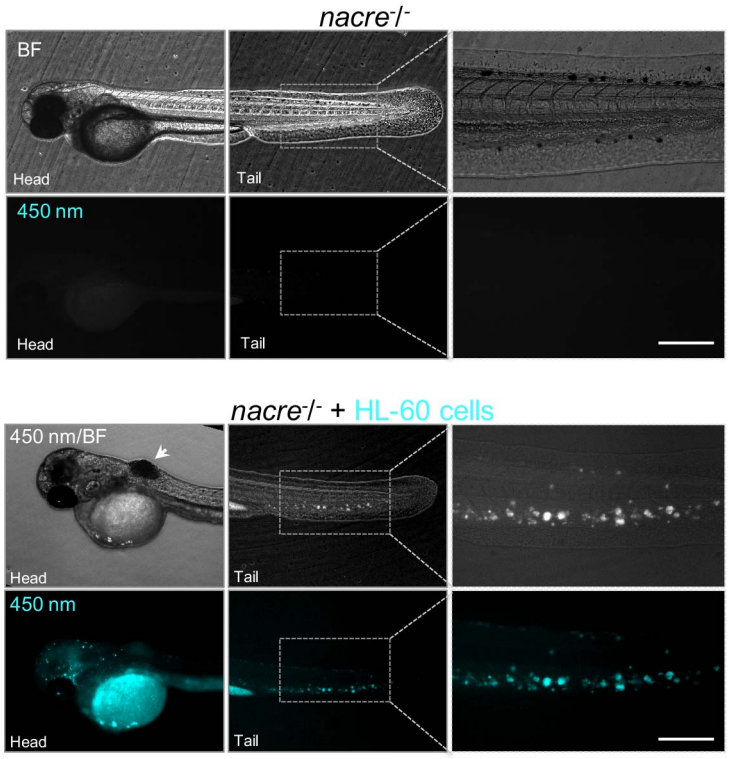


Figure S5. Human promyelocytic HL-60 cells colonize the CHT of zebrafish embryos. Human promyelocytic leukemia HL-60 cells were labeled with CellTraceViolet, transplanted into *nacre*$^{-/-}$ embryos and visualized at 3 dpf. Pseudo-colored epifluorescence images of control (upper panel) and transplanted (lower panel) zebrafish embryos showing HL-60 cells colonizing the CHT. Arrow depicts a cellular mass observed in some larvae transplanted with this cell line. BF: bright field. Scale bars: 100 µm.

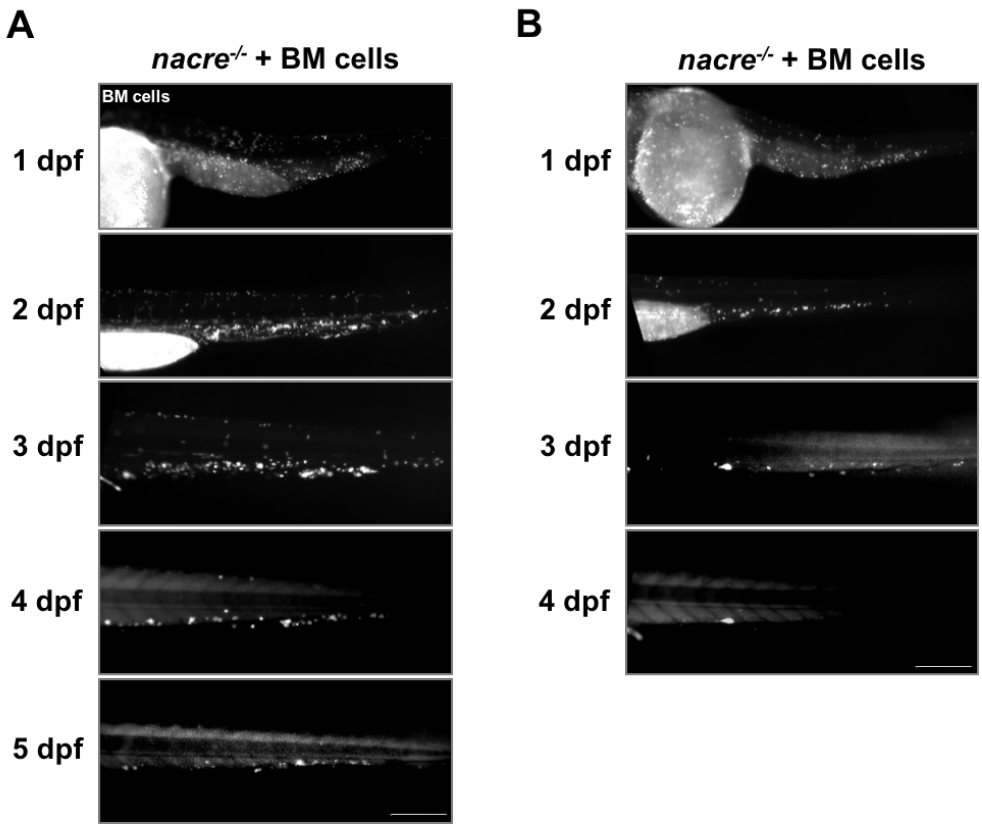


Figure S6. Representative images of the tail region of individual chimeric animals during early larval stage.

Mouse bone marrow cells were isolated, lineage depleted, labeled with CellTrace Violet and transplanted into *nacre*$^{-/-}$ zebrafish. A) Images of a chimeric animal showing a peak of mouse cell infiltration into the hematopoietic tissue at 2 dpf. B) Images of a chimeric animal showing a steady reduction of mouse cells in the hematopoietic tissue over time. BM cells: mouse bone marrow cells. Scale bar: 250 µm.

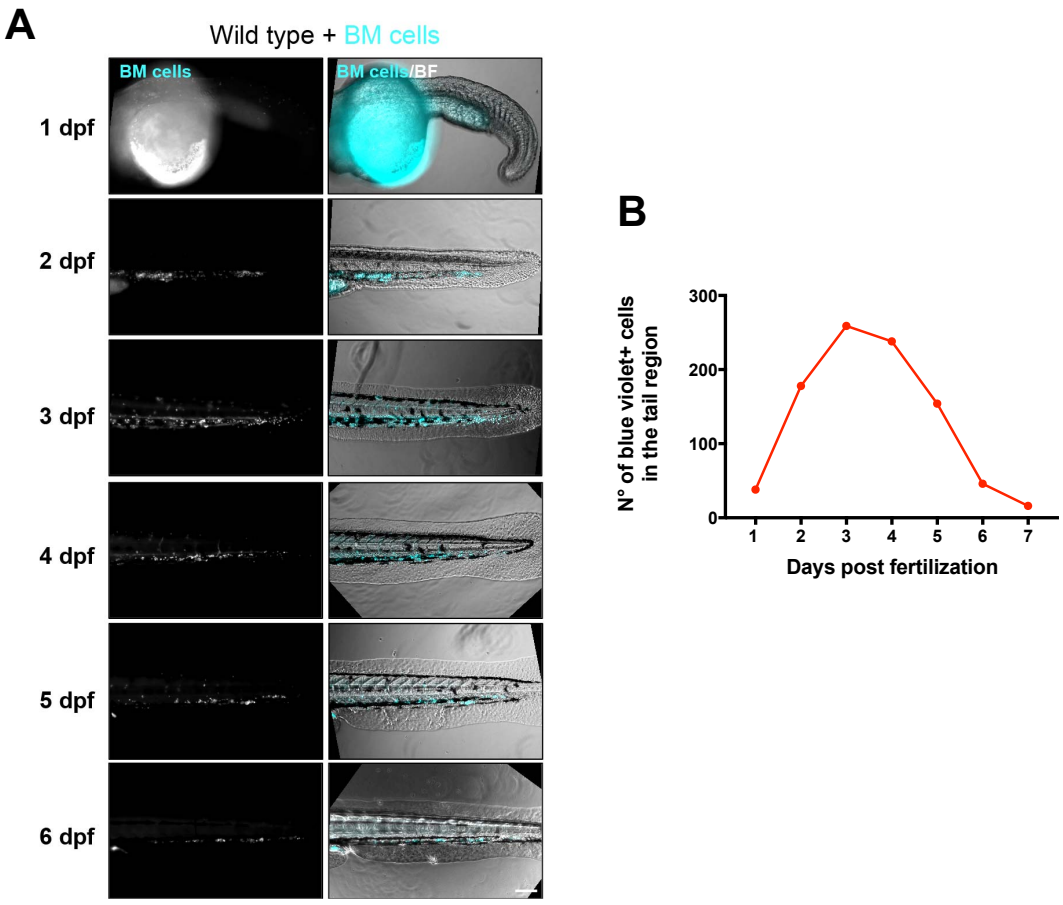


Figure S7. Mouse cells can be detected in chimeric animals up to 6 days post fertilization. Mouse bone marrow cells were isolated, lineage depleted, labeled with CellTrace Violet and transplanted into wild type zebrafish. A) Pseudo-colored epifluorescence images from a single transplanted embryo continuously imaged until 6 dpf. B) Quantification of murine cells numbers at the indicated days post fertilization. BF: bright field. BM cells: mouse bone marrow cells. Scale bar: 100 µm.

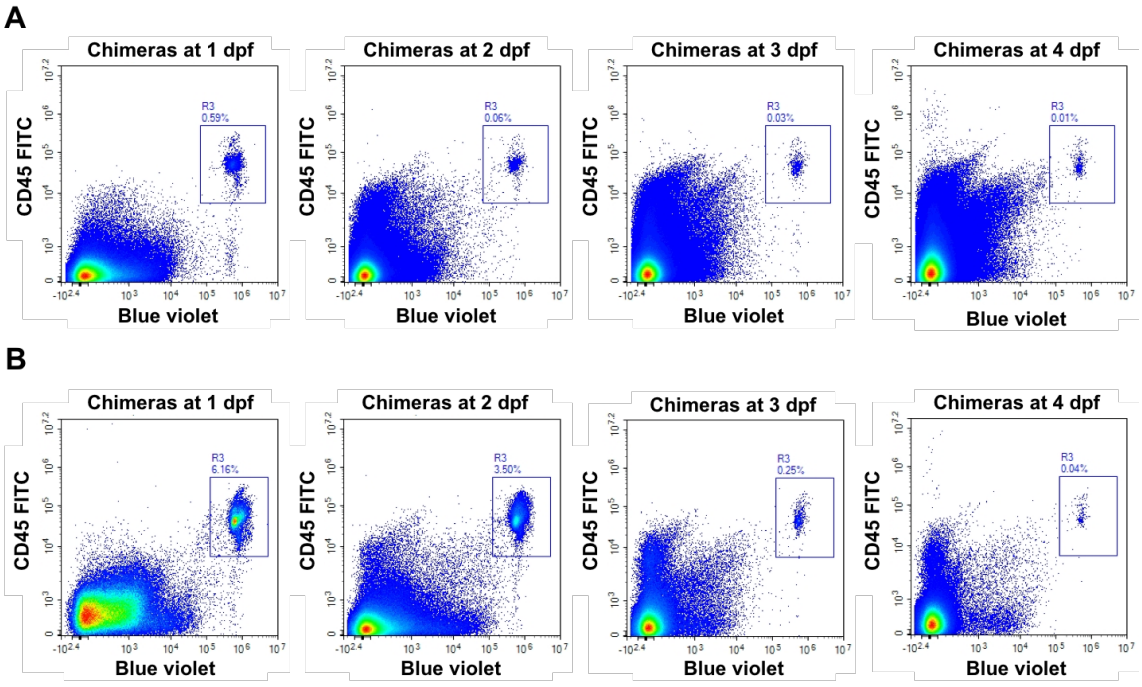


Figure S8. Analysis of total mouse cell numbers in chimeric animals during early larval stage.

Mouse bone marrow cells were blue labelled and transplanted into zebrafish blastulae. At the indicated days post fertilization (top of each plot), 30 chimeric animals were randomly selected and a whole body cell suspension was prepared and analyzed by flow cytometry. Dot plots of live pre-gated color based analysis show the selected region (R3 area) utilized to quantify mouse cells as shown in graph from Figure 2G, for embryos initially transplanted with A) ~2,000 cells and with B) ~3,500 cells, respectively.

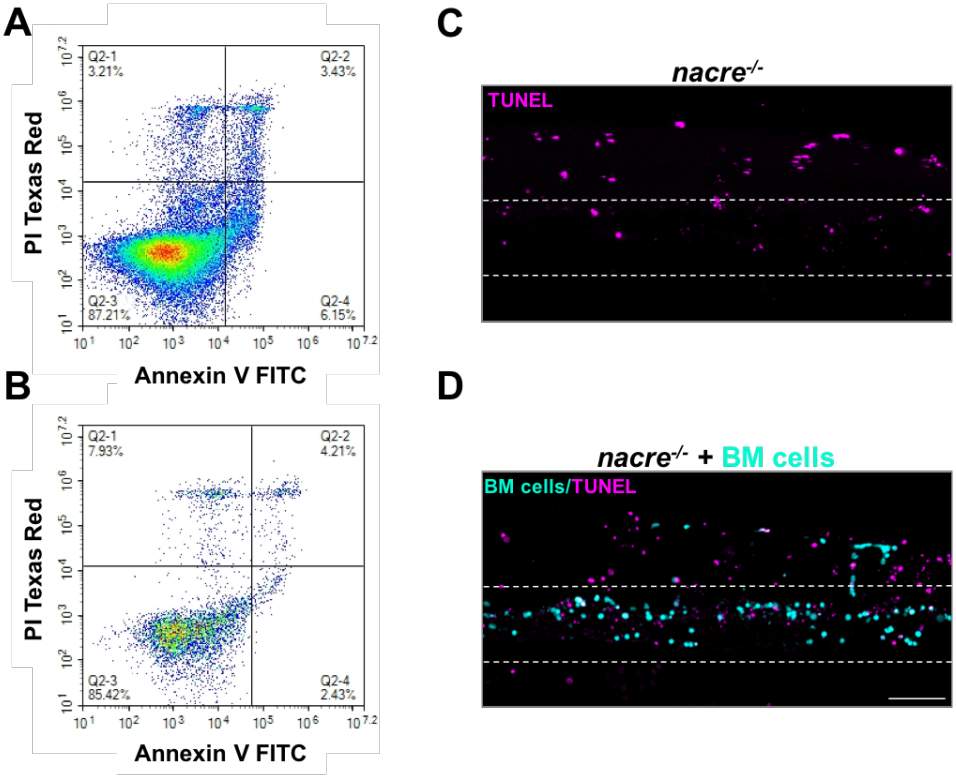


Figure S9. Analysis of cell death in xenotransplanted animals shows few dying mouse cells.

Mouse bone marrow cells were isolated, depleted of mature hematopoietic cells, labeled with Celltrace Violet and transplanted into *nacre*$^{-/-}$ embryos. Flow cytometry analysis of cell death markers before enrichment in A) freshly isolated bone marrow cells shows 6.64% of dead cells with 6.15% of early apoptotic cells (annexin V positives). B) Evaluation in HSPCs enriched and blue violet stained bone marrow cells prior transplantation show 11.36% of dead cells with 2.43% of early apoptotic cells. Graphs are representative of 2 independent experiments. C) TUNEL assays were performed at 2 dpf to evaluate mouse cell apoptosis in chimeric animals. Pseudo-colored image from a representative control animal showing few detected TUNEL positive cells, and D) a representative image of a chimeric animal showing few blue violet+/TUNEL+ labeled cells (yellow arrow heads). Area within dashed white lines indicates the CHT. Immunostaining determined that 5.4% ± 0.5 (mean, SE) of murine cells were TUNEL positives. N=10 animals. BF: bright field. BM cells: mouse bone marrow cells. Scale bar: 100 µm.

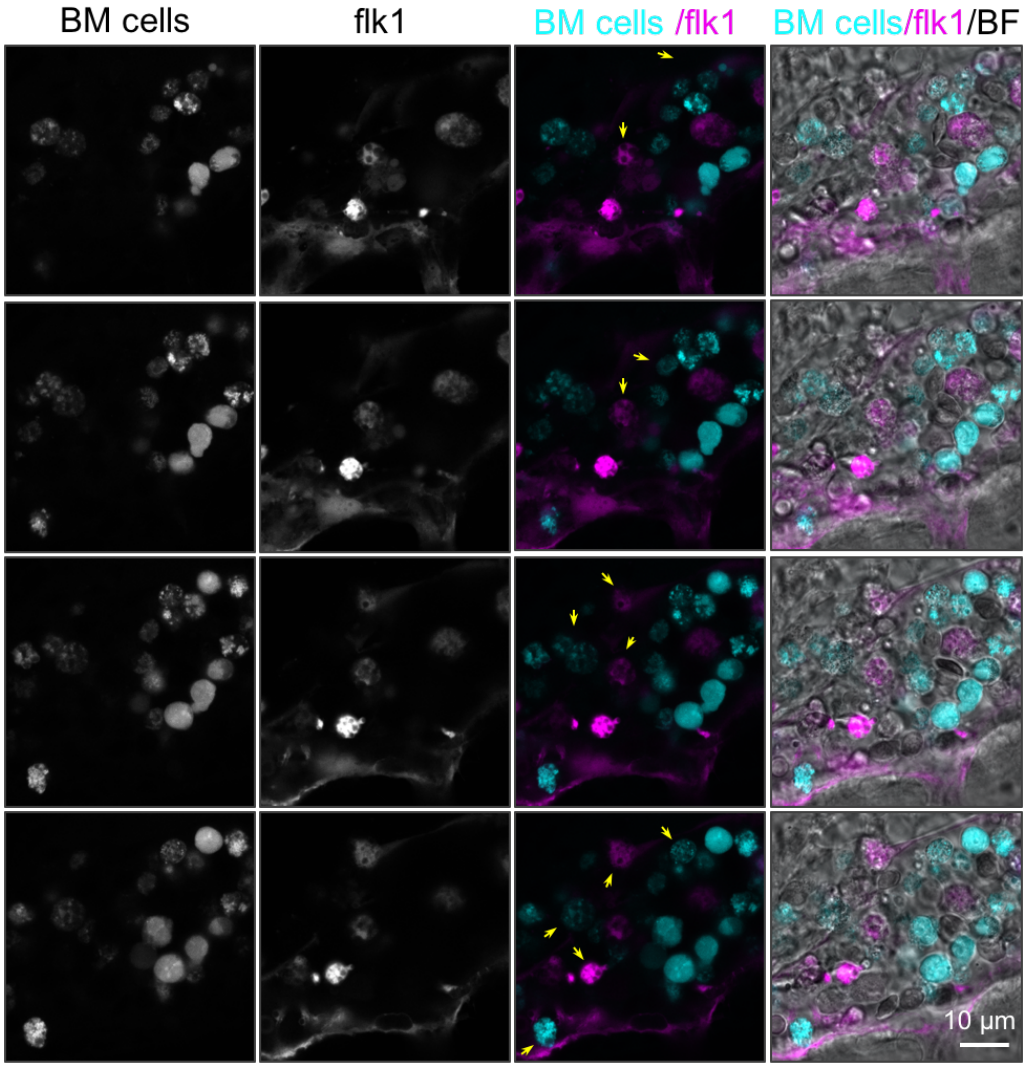


Figure S10. Visualization of transplanted mouse bone marrow cells in the fish CHT and morphological comparison with endogenous cells.

Mouse bone marrow cells were isolated, lineage depleted, labeled with CellTrace Violet, transplanted into *nacre*$^{-/-}$, *flk1:dsred* embryos and visualized *in vivo* within the fish CHT at 3 dpf. Pseudo-colored high-magnification z-stack confocal imaging reveals mouse cells with different levels of fluorescence emission and displaying similar size and morphology to endogenous fish CHT cells (yellow arrows). BF: bright field; BM cells: mouse bone marrow cells.

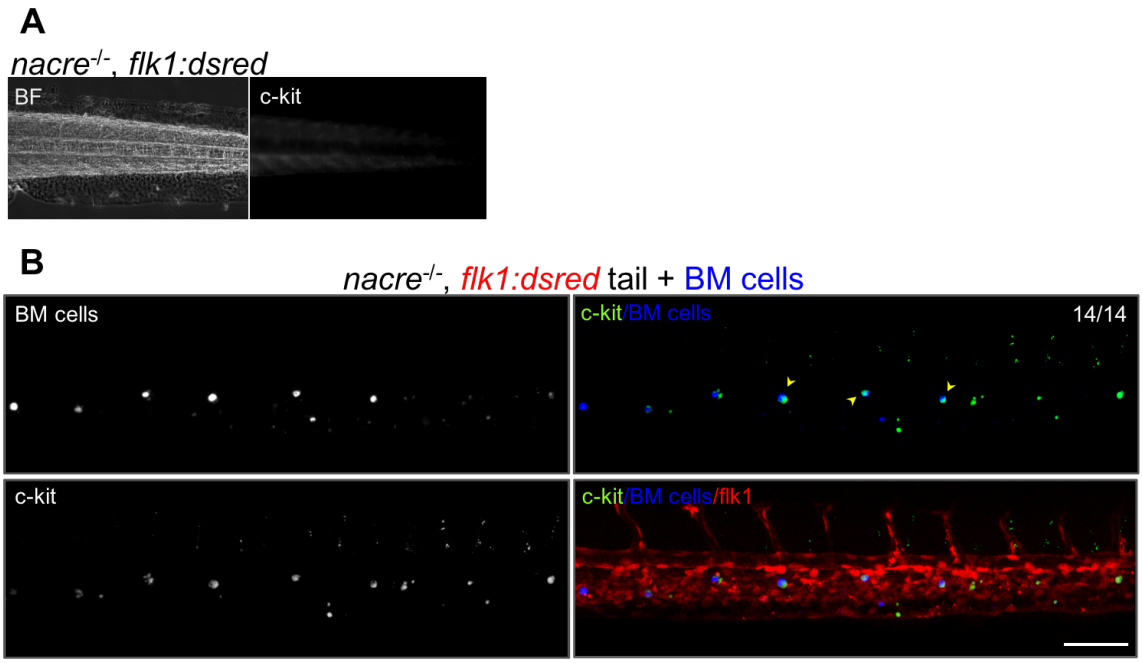


Figure S11. Mouse cells in the fish CHT display markers related to HPSCs lineages. Mouse bone marrow cells were isolated, lineage depleted, labeled with CellTrace Violet, transplanted into *nacre*$^{-/-}$, *flk1:dsred* embryos and processed for immunohistochemistry at 2–3 dpf to detect the murine c-kit protein, a marker associated with hematopoietic stem and progenitor cells. A) Immunostaining in control larvae shows no cross reactivity with fish cells. B) Raw and pseudo-colored image of the CHT of an immunostained animal at 3 dpf. Yellow arrow heads depict double stained cells. Immunostaining in 2 dpf chimeric larvae determined that 14 out of 14 tested animals (numbers in top right panel) had c-kit+ cells in the fish PBI, with 35.6% ± 6 (average, SE) of cells displaying reactivity against the antigen. BF: bright field; BM cells: mouse bone marrow cells; c-kit: tyrosine-protein kinase Kit/CD117. Scale bar: 100 µm.

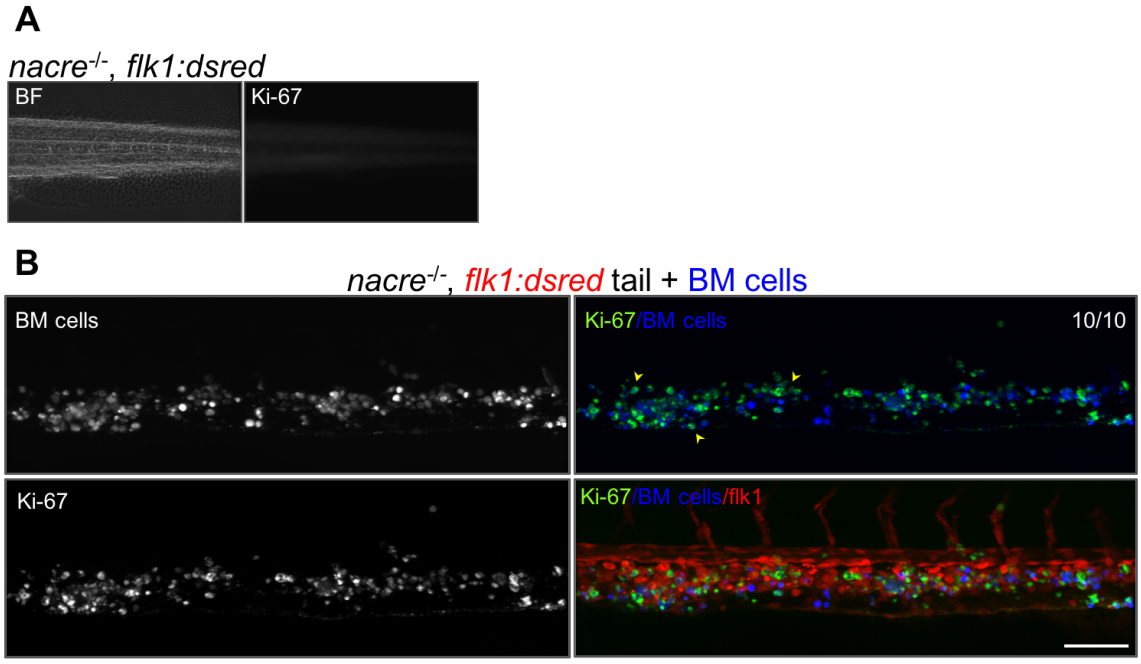


Figure S12. Mouse cells in the fish CHT display markers related to active cell proliferation.

Mouse bone marrow cells were isolated, lineage depleted, labeled with CellTrace Violet, transplanted into *nacre*$^{-/-}$, *flk1:dsred* embryos and processed for immunohistochemistry at 2–3 dpf to detect the murine Ki-67 protein, a marker associated with cell proliferation. A) Immunostaining in control larvae shows no cross reactivity with fish cells. B) Raw and pseudo-colored image of the CHT of an immunostained animal at 3 dpf. Yellow arrow heads depict double stained cells. Quantification of immunostaining in 2 dpf larvae determined that 10 out of 10 tested animals (numbers in top right panel) had Ki-67+ cells in the fish PBI, with 21.1% ± 2.8 (mean, SE) of cells displaying reactivity against the antigen. BF: bright field; BM cells: mouse bone marrow cells. Scale bar: 100 µm.

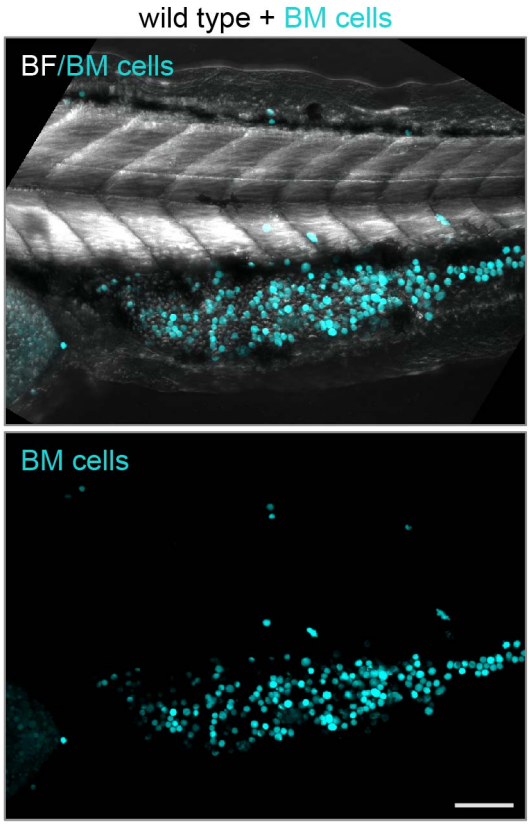


Figure S13. Xenotransplanted mouse cells display variable fluorescence emission levels in an expanded caudal hematopoietic tissue.

Mouse bone marrow cells were isolated, lineage depleted, labeled with CellTrace Violet, transplanted into wild type embryos and visualized at 3 dpf. Pseudo-colored z-stack confocal image shows mouse cells (cyan) within the caudal hematopoietic tissue. BF: bright field. BM cells: mouse bone marrow cells. Scale bar: 100 μm.

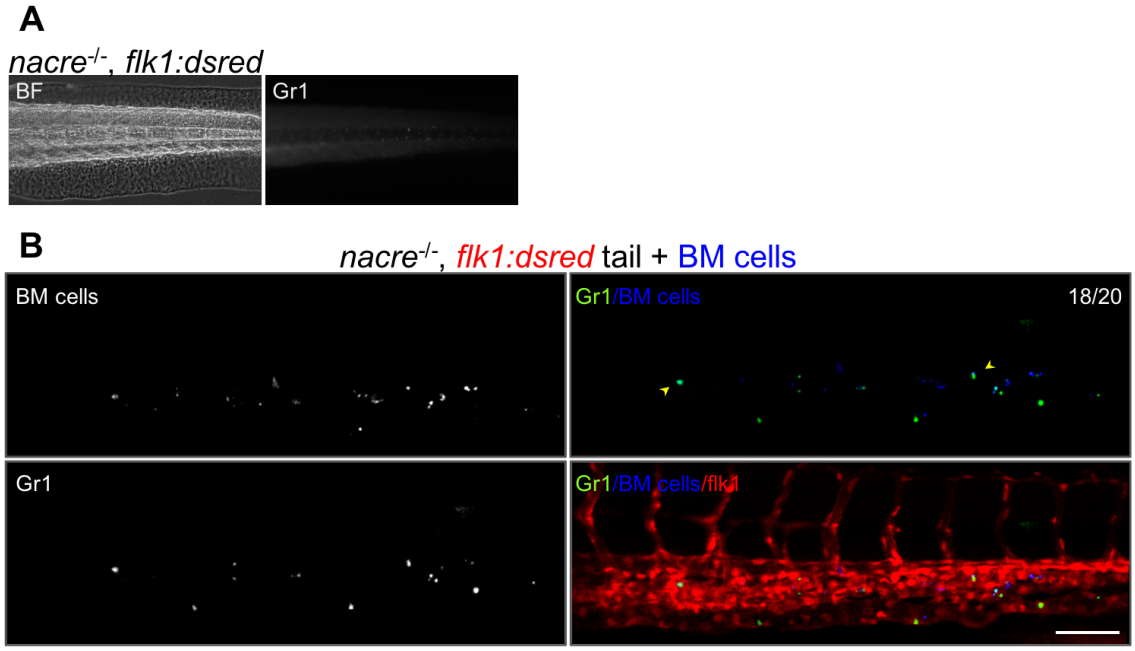


Figure S14. Mouse cells in the fish CHT express a granulocyte cell lineage marker. Mouse bone marrow cells were isolated, lineage depleted, labeled with CellTrace Violet, transplanted into *nacre*$^{-/-}$, *flk1:dsred* embryos and processed for immunohistochemistry at 2–3 dpf to detect the murine Gr1 protein which labels granulocytes. A) Immunostaining in control larvae shows no cross reactivity with fish cells. B) Raw and pseudo-colored image of the CHT of an immunostained animal at 3 dpf. Yellow arrow heads depict double stained cells. Immunostaining in 2 dpf chimeric larvae determined that 18 out of 20 tested animals (numbers in top right panel) had Gr1+ cells in the fish PBI, with 32.2% ± 7.8 (average, SE) of cells displaying reactivity against the antigen. BF: bright field; BM cells: mouse bone marrow cells; Gr1: myeloid differentiation antigen Gr-1. Scale bar: 100 µm.

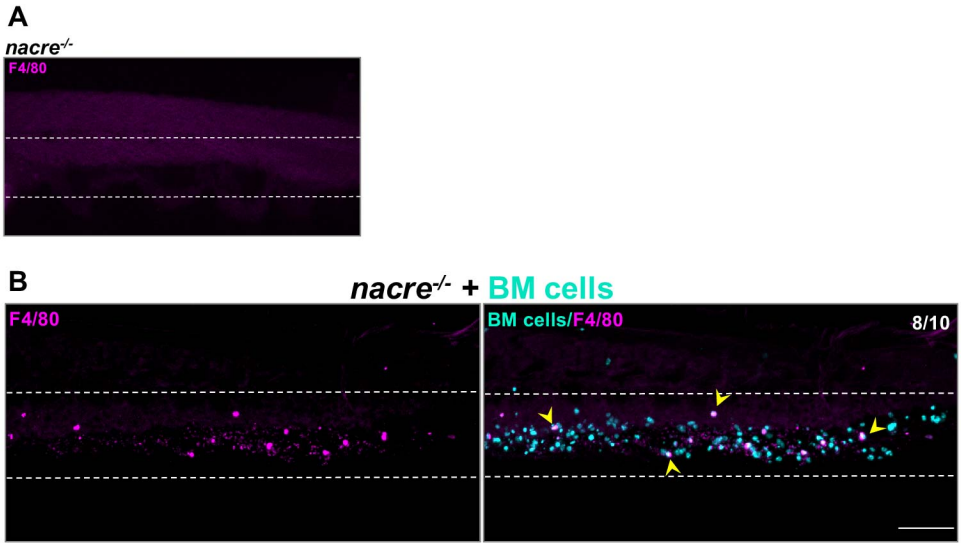


Figure S15. Mouse cells in the fish CHT express a monocyte-macrophage cell marker. Mouse bone marrow cells were isolated, lineage depleted, labeled with CellTrace Violet, transplanted into *nacre*$^{-/-}$ embryos and processed for immunohistochemistry at 2 dpf to detect the murine F4/80 protein, a marker associated with monocytes and macrophage cells. A) Immunostaining in control larvae shows no cross reactivity with fish cells. B) Pseudo-colored image of the PBI of an immunostained animal. Dashed white lines indicate the CHT. Yellow arrow heads depict double stained cells. Quantification determined that 8 out of 10 tested animals (numbers in top right panel) had F4/80+ cells in the fish PBI, with 13.4% ± 2.5 (average, SE) of cells displaying reactivity against the antigen. BF: bright field; BM cells: mouse bone marrow cells; F4/80: EGF-like module-containing mucin-like hormone receptor-like, also known as F4/80. Scale bar: 100 µm.

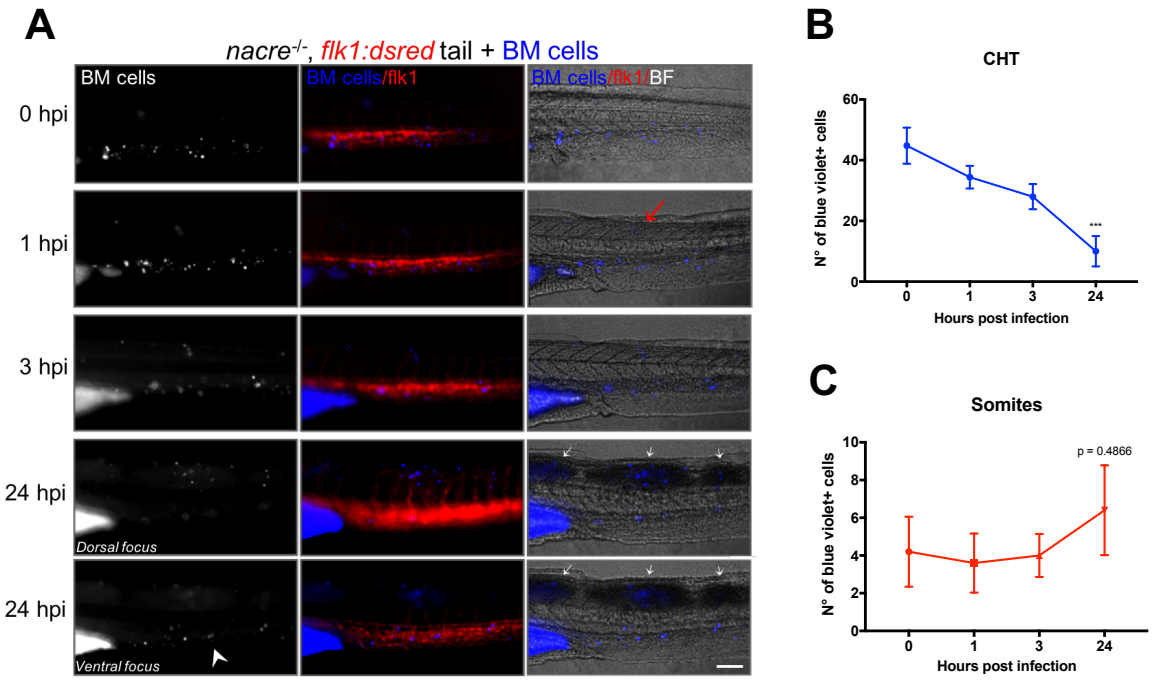


Figure S16. Mouse bone marrow cells in zebrafish chimeras respond to a localized infection of *Klebsiella pneumoniae*.

Mouse bone marrow cells were isolated, lineage depleted, labeled with CellTrace Violet and transplanted into nacre-/-, *flk1:dsred* embryos. At 2 dpf, embryos were intramuscularly infected with ~400 cfu of live *K. pneumoniae* within the dorsal tail muscles (red arrow) and incubated at 30°C. A) *In vivo* visualization over time showed that 4 out of 5 infected animals evidenced mouse cells infiltrated the necrotized muscle tissue starting at 1 hpi (white arrows), while at 24 hpi the CHT become depleted of these cells (white arrowhead). hpi: hours post infection. B-C) Quantification of murine cells in the caudal hematopoietic tissue B) and C) dorsal somites at the indicated time points post infection. Statistical significance was analyzed by a two-way ANOVA with Bonferroni posttest ($p \leq 0.001$) and with a two-tailed unpaired student t-test, respectively. cfu: colony-forming units. Scale bar: 100 µm.

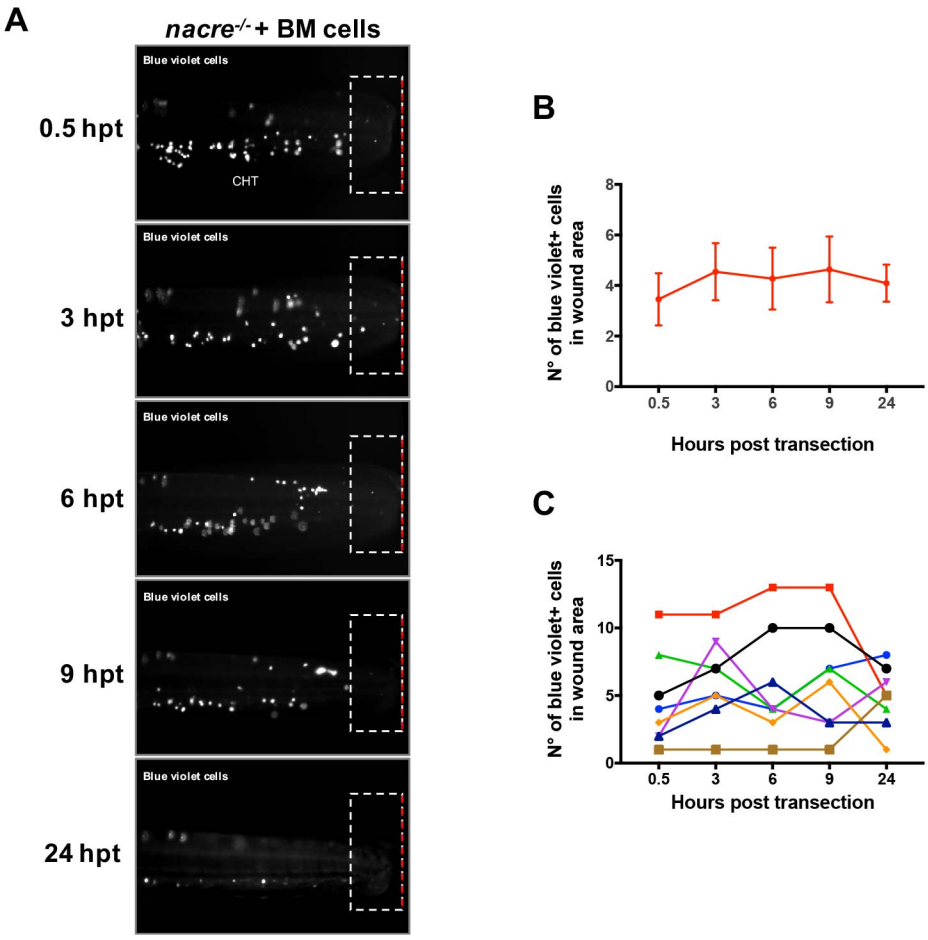


Figure S17. Mouse bone marrow cells in zebrafish chimeras do not infiltrate the wounded tissue in a tail cut assay. Mouse bone marrow cells were isolated, lineage depleted, labeled with CellTrace Violet and transplanted into *nacre*$^{-/-}$ embryos. At 2 dpf, 11 embryos were tail transected (red dashed line) and animals were imaged by epifluorescence microscopy. A) Images of a representative chimeric animal imaged at the indicated hours post transection. B) Quantification of murine cell numbers in the wounded area (white dashed lines rectangle) at the indicated hours post transection. C) Quantification of infiltrated murine cells in 8 individual animals at the indicated hours post transection. N= 11 animals. Results are representative of 2 independent experiments.

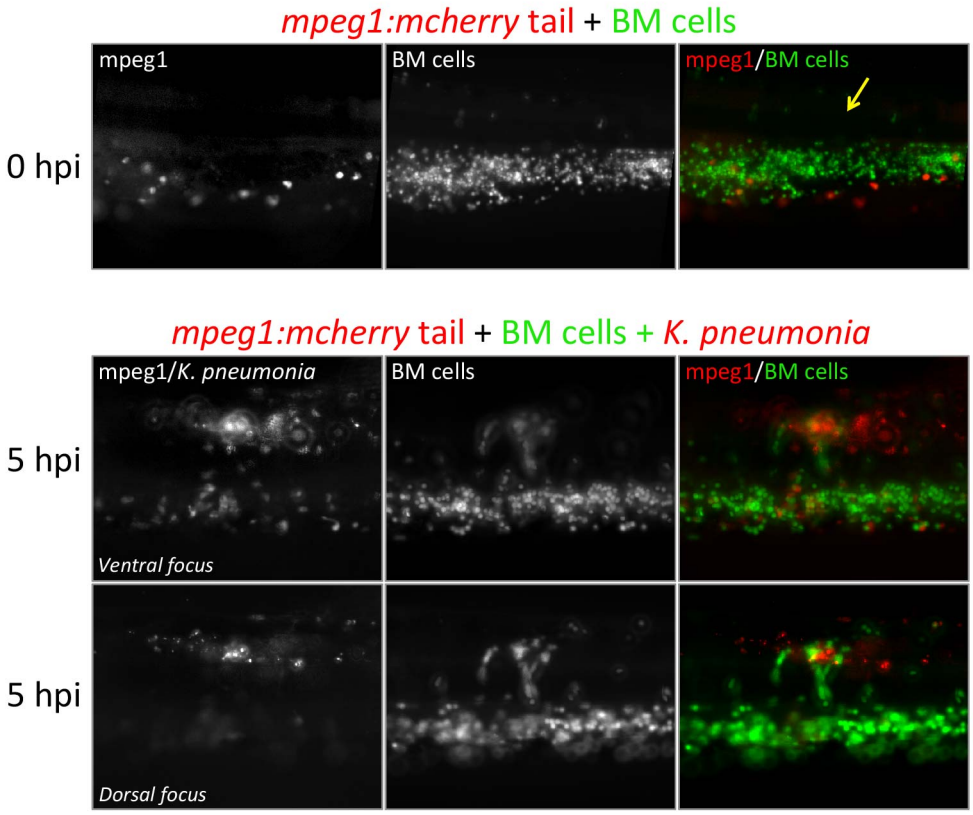


Figure S18. Murine cells and endogenous macrophages in infected chimeras respond and infiltrate to the infection site. Mouse bone marrow cells were isolated, lineage depleted, labeled with CellTrace Violet and transplanted into *mpeg1:mcherry* embryos. At 3 dpf, embryos were intramuscularly infected with ~400 cfu of *K. pneumoniae* within the dorsal tail muscles (yellow arrow) and incubated at 30°C. Visualization at the injection site at 0 and later 5 hours post infection evidence murine cell (pseudo-colored green) and macrophage cells infiltrated in the infected area. N= 20 animals.

Supplementary Materials and Methods

Step by step protocol

Animals

6–8 week old mice.

3–5 hpf zebrafish embryos.

Equipment

Dissecting microscope.

Microinjector (Eppendorf femtojet, optional).

Micromanipulator.

Neubauer chamber (optional).

Incubators at 28°C for fish and 37°C for mouse cells.

Materials and methods

Mortar and pestle.

Surgical scissors and tweezers.

40–70 nylon cell strainer (Stemcell Technologies, 27305, optional).

100 mm × 25 mm plastic petri dishes for egg collection and microinjection.

3 x 50 mL tubes.

9 x 15 mL tubes.

1 x 1 mL plastic tube.

QuadroMACS Starting Kit (LD) (Miltenyi Biotec, MACS, 130-092-857).

Microinjection borosilicate needles.

Plastic Pasteur pipettes.

Microscope glass slides.

Microloader tips (Eppendorf, optional).

Solutions and reagents

MACS buffer.

E3 zebrafish media.

FBS.

PBS.

Pronase (1mg/mL diluted in embryo medium).

Lineage cell depletion kit, mouse (Miltenyi Biotec, MACS, 130-090-858).

Fluorescent vital dye. Celltrace violet, 5 mM stock (Thermo Fisher Scientific Inc., C34557, optional).

Ter-119 antibody (eBioscience, 14-5921, optional).

Reagent and needles setup

MACS buffer: 1x PBS supplemented with 0.5% BSA/BFS and 2 mM EDTA. Store and work at 4°C.

Puller settings for microinjection needles:

(Sutter instruments, model P-2000) Heat: 350, Fil: 4, Vel: 5, Del: 225, Pul: 150. Needles: Borosilicate glass with filament. OD: 1 mm, ID: 0.5 mm, 10 cm (Sutter instruments, BF100-50-10).

Procedure

Day 1

a) Bone marrow cell suspension preparation:

1. Euthanize the mouse, cut off both hind legs, extract femurs and tibias, cut off muscle tissue and leave them on PBS on ice.
2. Crush bones with mortar and pestle in MACS buffer, collect the buffer and filter it with 40–70 µm mesh filters in a 50 mL tube on ice. Crush and collect buffer 3 times for each pair of

femurs and tibias.

3. Bring the cell suspension to a volume of 50 mL with MACS buffer and determine cell concentration (Neubauer chamber, optional).
4. Centrifuge at 300g x 10' at 4°C, discard the supernatant and bring cells to a volume with MACS buffer in order to achieve a cell concentration of 10^7 cells for each 40 µL of MACS buffer.

b) Magnetic labeling:

1. Prepare 8 tubes with 40 µL each of bone marrow cell suspension.
2. Add 10 µL of **biotin antibody cocktail** and 10 µL of **Ter-119** to each tube.
3. Incubate at 4°C for 15'.
4. Add 30 µL of MACS buffer and 20 µL of **anti-biotin microbeads** to each tube and homogenize.
5. Incubate at 4°C for 20'.
6. Collect all 8 single reactions into a single 50 mL tube and add MACS buffer to reach a final volume of 25 mL.
7. Centrifuge at 300g x 10' at 4°C. Discard the supernatant and gently resuspend cells with 4 mL of MACS buffer and leave on ice.

c) Magnetic separation:

1. Mount both magnetic fields with 4 LD columns each, and flow through 3 mL of MACS buffer to each single column.
2. Gently homogenize the 4 mL of cell suspension and add 500 µL to each single column carefully avoiding bubble formation. Collect flow through on ice*.
3. Wash columns 4 times with 3 mL of MACS buffer and collect flow through on ice.
4. Centrifuge all 8 tubes at 300g x 10' at 4°C. Discard the supernatant and collect all cell pellets together. Bring to 1 mL of PBS in a 1.5 mL tube.

* Make sure to homogenize well and avoid bubble formation every time before loading the columns—cell overloading will clog the columns, and bubbles won't allow flow through.

d) Fluorescent labeling:

1. Centrifuge cells at 300g x 10' at 4°C and discard supernatant.
2. Resuspend cells with 1 mL of PBS containing 4–5 µL of **CellTrace Violet** (optional). Transfer to a 15 mL tube and incubate 20–30' at 37°C.
2. Add 1 mL of FBS and incubate 10' at 37°C.
3. Bring to 10 mL with PBS and centrifuge at 300g x 10' at 4°C. Discard the supernatant and resuspend to 50–100 µL with PBS (~200,000 – 300,000 cells/µL), or to achieve the desired concentration.

d) Cell transplantation into zebrafish embryos:

1. Collect zebrafish embryos in the morning and leave them at room temperature to delay development and extend the transplantation time window.
2. Load injection needles with up to 5 µL of cell suspension.
3. Open the tip with tweezers and set up the microinjector to inject between 2-10 nL of cell suspension.
4. Put a glass slide into the inner face of the top of a 100 mm plastic petri dish.
5. Collect eggs into the dish, remove E3 medium and align eggs against the side of the glass slide.
6. Go across the chorion and inject the cell suspension directly into the blastoderm of 3–5 hpf zebrafish embryos*.
7. Inject into PBS to quantify cell transplantation.
8. After transplantation, add E3 medium into the petri dish and collect the embryos with a plastic Pasteur pipette. Place the embryos into a petri dish containing E3 medium and incubate at 28°C.

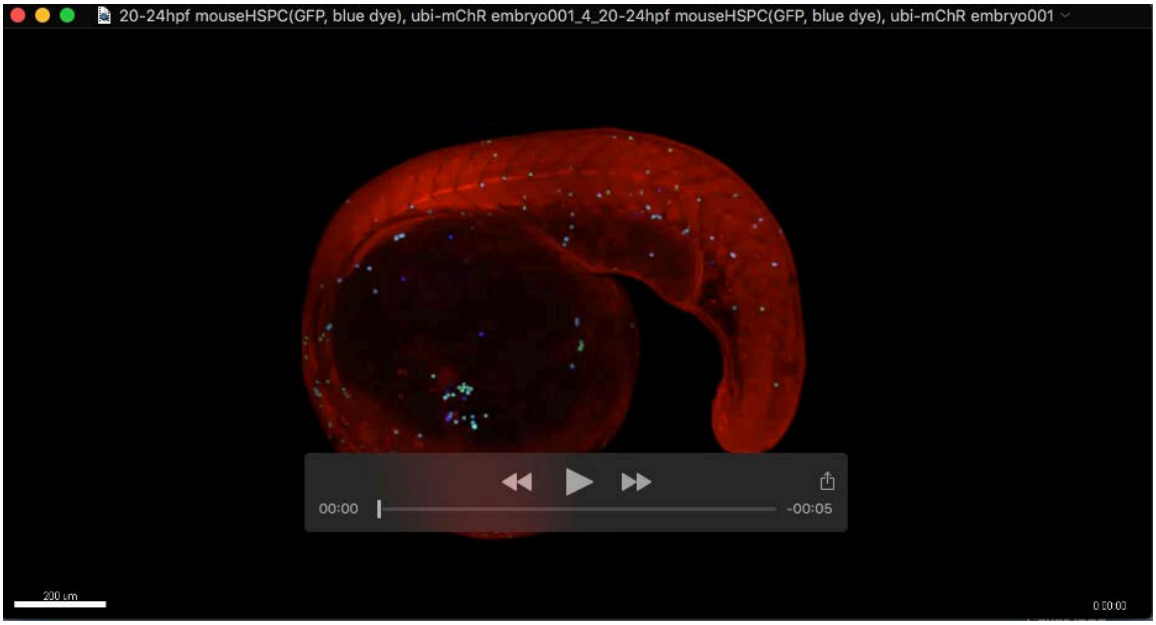
9. Repeat procedure to transplant as desired.

* Be careful not to leave embryos too much time without media, or otherwise they will dry out and die.

Day 2

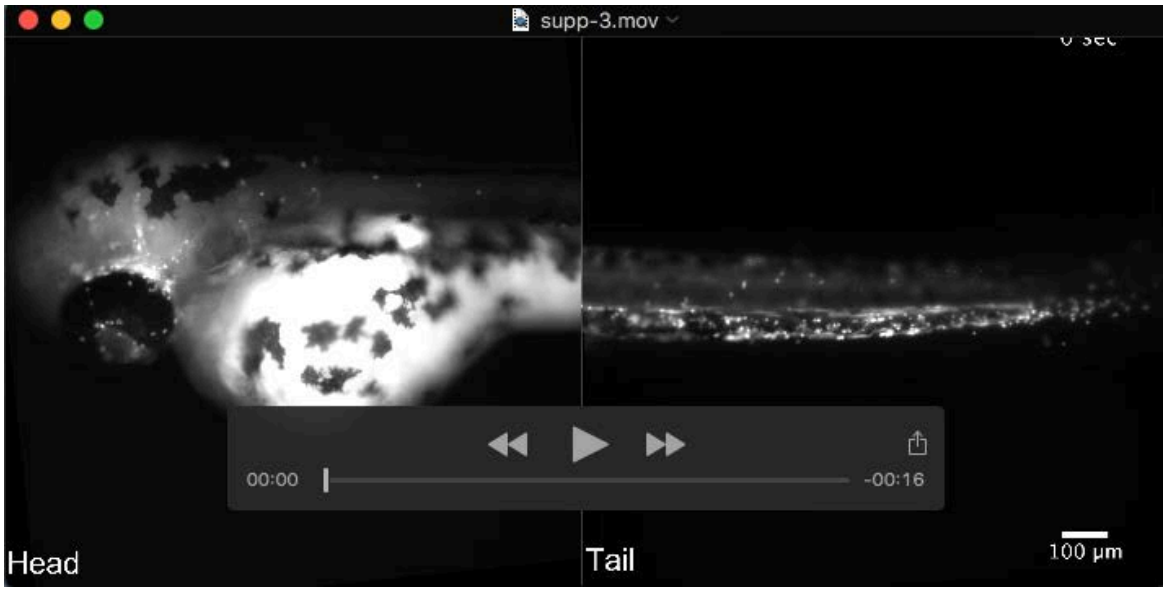
a) Selection of viable embryos:

1. The following morning visualize and select viable embryos.
2. To identify efficiently transplanted embryos, dechorionate them manually with tweezers or with a pronase solution to clean them from mouse cells that might remain outside the embryos but inside the chorion.



Movie S1: Mouse bone marrow cells follow the migratory route of endogenous primitive macrophages (439 KB).

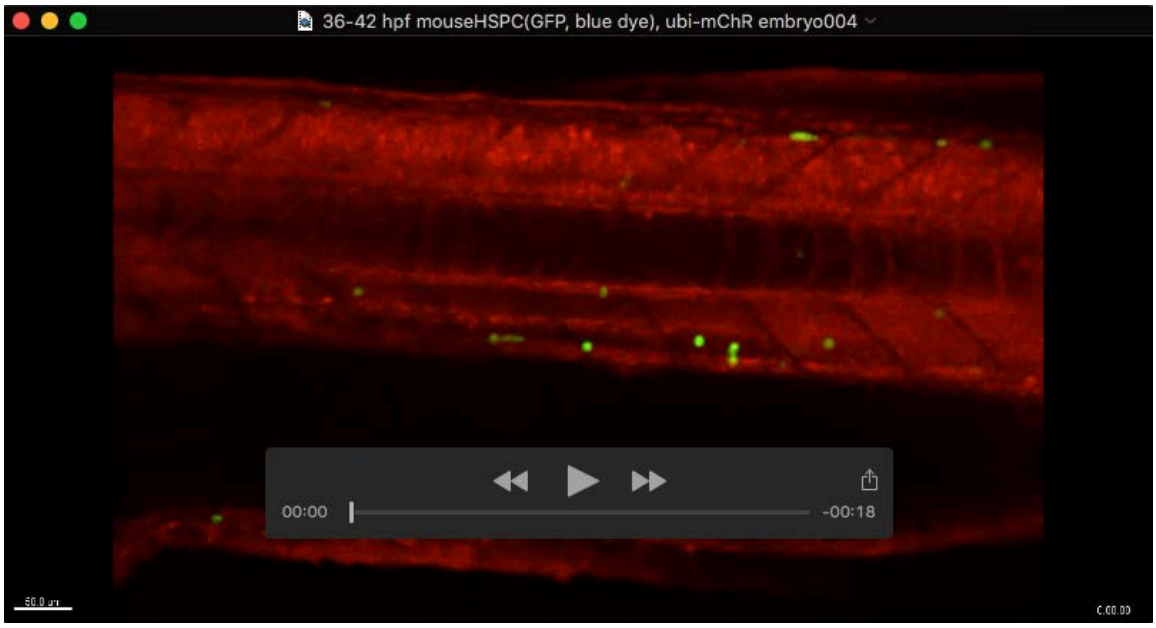
Mouse bone marrow cells from a UBI-GFP transgenic mouse (ubiquitous GFP expression) were isolated, lineage depleted, labeled with CellTrace Violet and transplanted into a *ubi:mcherry* transgenic fish (ubiquitous mCherry expression). The video was made by compiling images taken every 16 minutes for 13 hours, starting at ~20 hpf. Mouse cells (labeled in green and blue) can be seen migrating over the yolk towards the posterior trunk, a route also followed by endogenous primitive macrophages. The timing of the video is shown in the lower right corner.



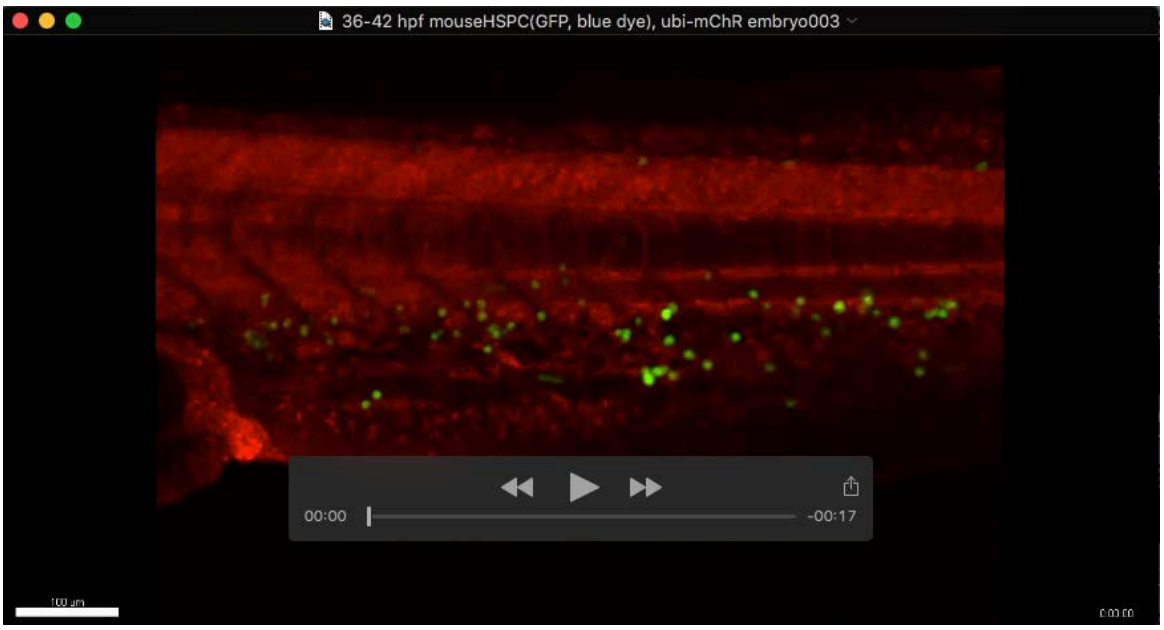
Movie S2: Live mouse cells circulate within the fish vasculature in xenografted animals (5.5 MB).

Mouse bone marrow cells were isolated, lineage depleted, labeled with CellTrace Violet and transplanted into wild type embryos. The video was made by compiling images under epifluorescence illumination at 450 nm taken approx. every 2.3 seconds for 1.8 minutes, starting at ~48 hpf. Live visualization shows mouse cells in circulation throughout the fish body. The timing of the video is shown in the upper right corner.

Movie S3

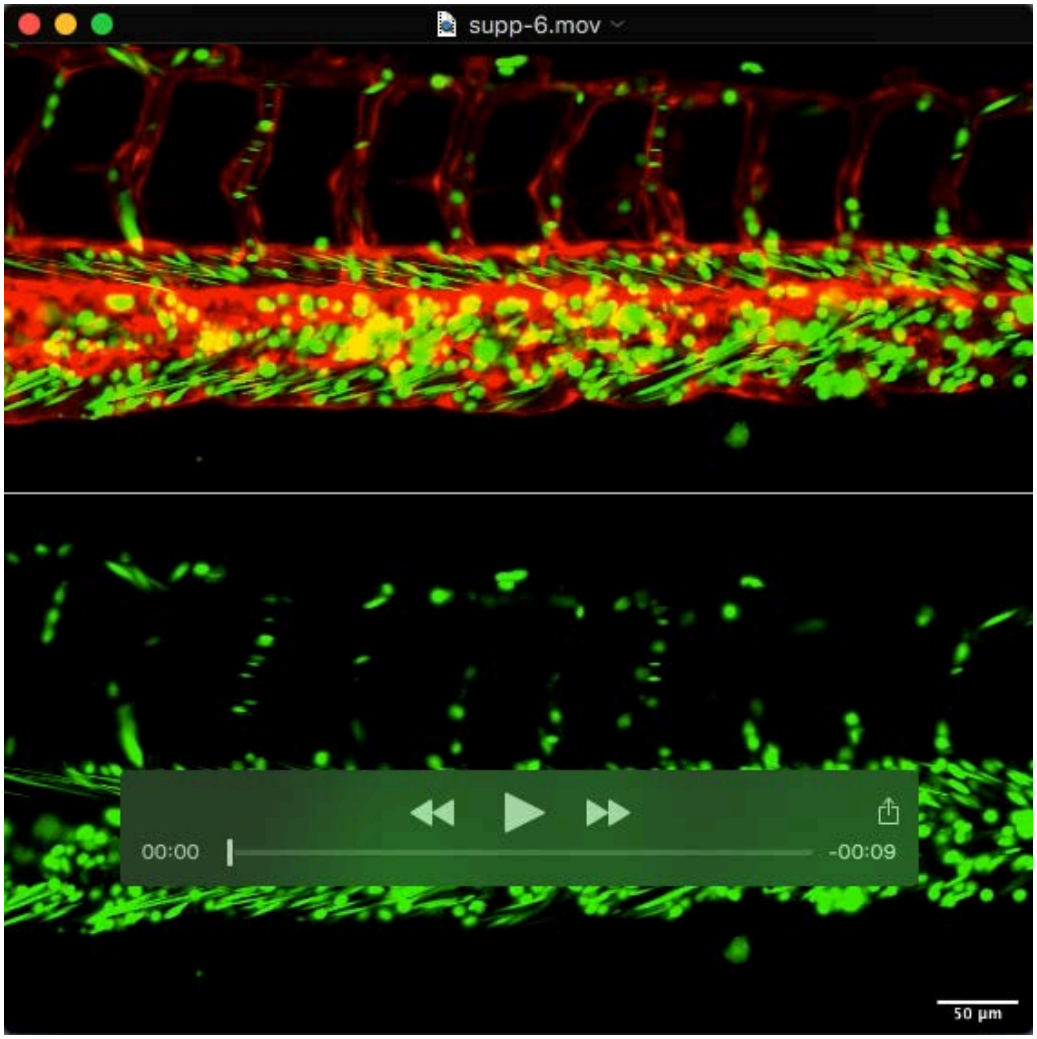


Movie S4



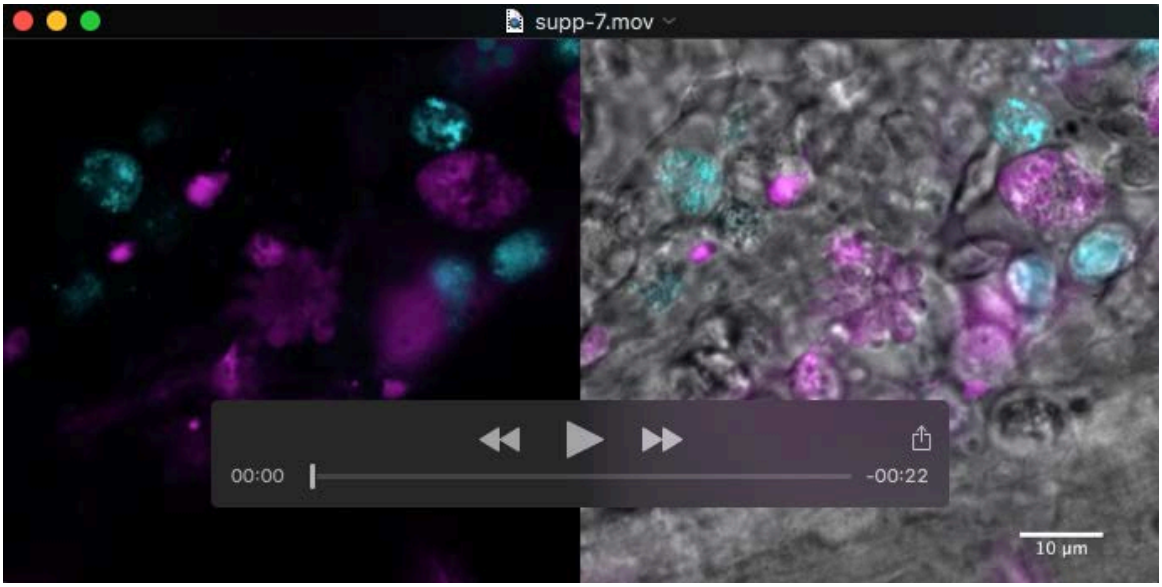
Movies S3 and S4: Circulating mouse cells show differential adherence to fish vascular endothelium (3.6 MB and 2.9 MB).

Mouse bone marrow cells isolated from a ubi-GFP transgenic mouse were lineage depleted and transplanted into a *ubi:mcherry* transgenic fish. *In vivo* visualization of circulating mouse cells in the fish trunk, within the dorsal aorta and axial vein, show active mouse cell vascular endothelial interactions (Movie 3, top), while cells circulating through the fish CHT show different velocities within the caudal aorta and vein (Movie 4, bottom). For each video, images were acquired every 0.15 seconds for ~20 seconds. The timing of each video is shown in the lower right corner.



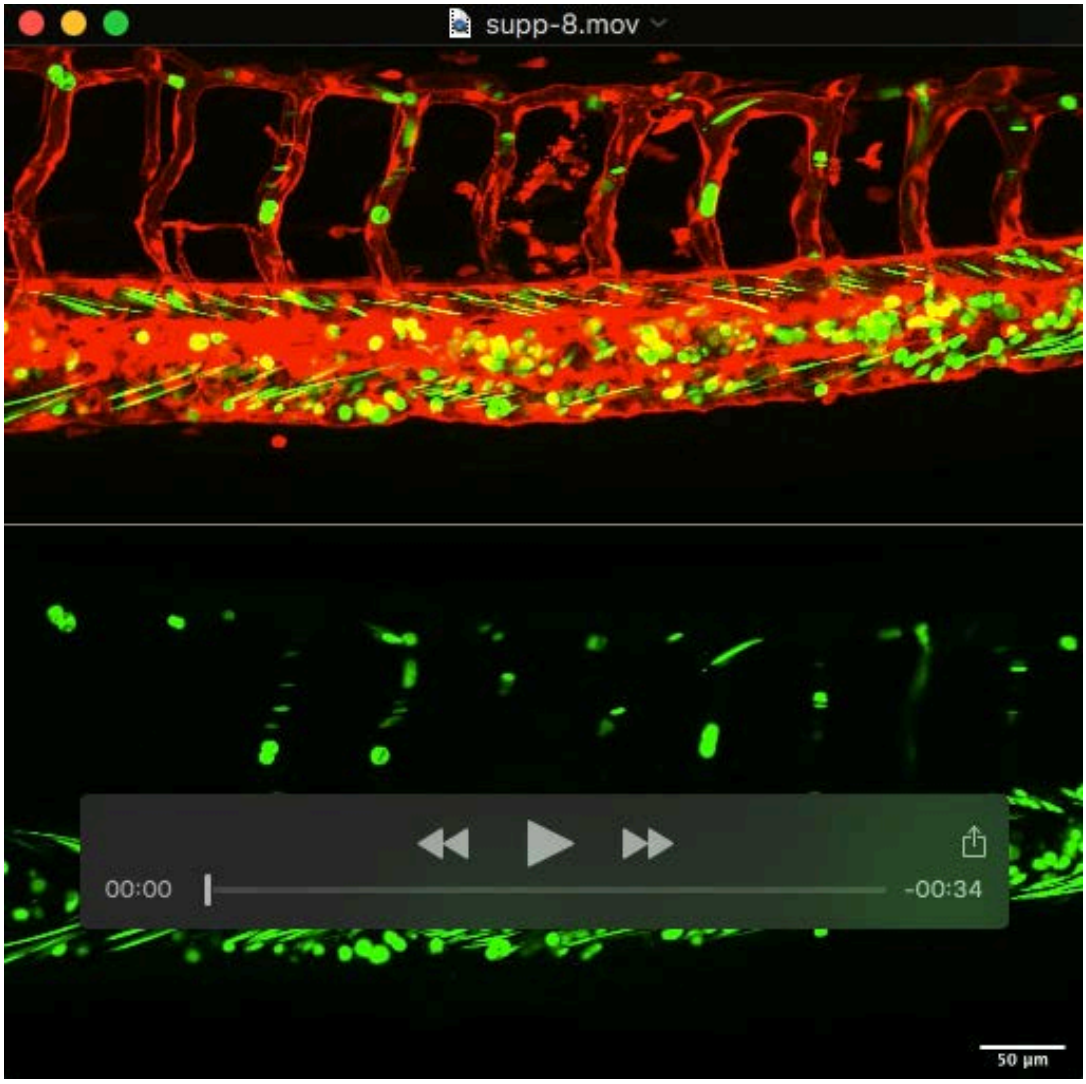
Movie S5: Long-term dynamics of mouse cells in the fish caudal hematopoietic tissue (3.4 MB).

Mouse bone marrow cells were isolated, lineage depleted, labeled with CellTrace Violet and transplanted into *nacre*$^{-/-}$, *flk1:dsred* embryos. The video was made by compiling images taken every 5 minutes for 1 hour and 25 minutes. Pseudo-colored confocal imaging shows mouse cell (green) dynamic displacements and interactions within the fish CHT (red) in a 2 dpf embryo. The lower panel shows only the mouse cells labeled. The timing of the video is shown in the upper right corner.



Movie S6: Mouse bone marrow derived cells display dynamic behaviors within the fish caudal hematopoietic tissue (19.7 MB).

Mouse bone marrow cells were isolated, lineage depleted, labeled with CellTrace Violet and transplanted into *nacre*$^{-/-}$, *flk1:dsred* embryos. The video was made by compiling images taken approx. every 2,6 seconds for 2,8 minutes. Pseudo-colored confocal high magnification imaging throughout the fish CHT of a xenotransplanted 3 dpf embryo shows mouse cells dynamic behavior. Time is indicated in seconds in the upper right corner of the video.

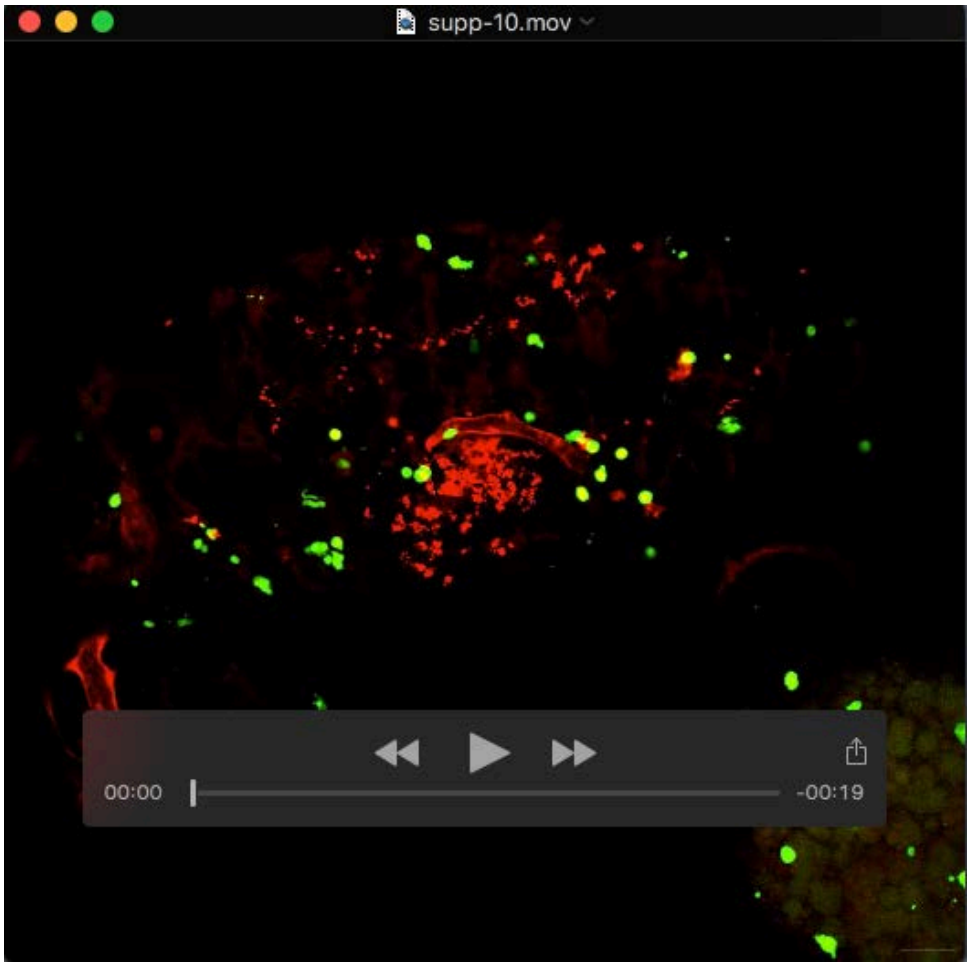


Movie S7: Xenotransplanted mouse bone marrow cells respond to bacterial infection *in vivo* (11.5 MB).

Mouse bone marrow cells were isolated, depleted of mature hematopoietic cells, labeled with CellTrace Violet and transplanted into *nacre*$^{-/-}$, *flk1:dsred* embryos. To visualize mouse cell dynamics after a bacterial infection, a 3 dpf xenotransplanted embryo was injected with ~100 cfu of *K. pneumoniae* (pRSET-tdTomato) into the tail dorsal musculature. The video was made by compiling images taken every 5 minutes for 5 hours and 35 minutes. Live imaging right after the infection shows endogenous fish (red) cells interacting with bacteria at the injection site, while mouse cells display dramatic changes in their distribution within the CHT. The lower panel shows only mouse cells. The timing of the video is shown in the upper right corner. cfu: colony forming units.

Movie S8: Mouse bone marrow derived cells respond to infection in the fish otic vesicle (1.2 MB).

Mouse bone marrow cells were isolated, lineage depleted, labeled with CellTrace Violet and transplanted into *nacre*$^{-/-}$ embryos. A 2 dpf transplanted embryo was infected with ~500 cfu of *K. pneumoniae* (pRSET-tdTomato) within the otic vesicle and visualized starting at 1 hpi. The video was made by compiling images taken every 5 minutes for 12 hours. Pseudo-colored images show mouse cells (green) migrating to and interacting with bacterial cells (red). The timing of the video is shown in the upper right corner. Scale bar: 10 µm.



Movie S9: Mouse bone marrow derived cells respond to infection in the fish otic vesicle (6.4 MB).

Mouse bone marrow cells were isolated, lineage depleted, labeled with CellTrace Violet and transplanted into *nacre*$^{-/-}$, *flk1:dsred* embryos. A 2 dpf transplanted embryo was infected with ~500 cfu of *K. pneumoniae* (pRSET-tdTomato) within the otic vesicle and visualized starting at 9 hpi. The video was made by compiling images taken every 5 minutes for 8 hours. Pseudo-colored images show mouse cells (green) migrating to and interacting with bacterial cells (red). The timing of the video is shown in the upper right corner. Scale bar: 20 µm