Protection from UV light is an evolutionarily conserved feature of the haematopoietic niche

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Haematopoietic stem and progenitor cells (HSPCs) require a specific microenvironment, the haematopoietic niche, which regulates HSPC behaviour1,2. The location of this niche varies across species, but the evolutionary pressures that drive HSPCs to different microenvironments remain unknown. The niche is located in the bone marrow in adult mammals, whereas it is found in other locations in non-mammalian vertebrates, for example, in the kidney marrow in teleost fish. Here we show that a melanocyte umbrella above the kidney marrow protects HSPCs against ultraviolet light in zebrafish. Because mutants that lack melanocytes have normal steady-state haematopoiesis under standard laboratory conditions, we hypothesized that melanocytes above the stem cell niche protect HSPCs against ultraviolet-light-induced DNA damage. Indeed, after ultraviolet-light irradiation, unpigmented larvae show higher levels of DNA damage in HSPCs, as indicated by staining of cyclobutane pyrimidine dimers and have reduced numbers of HSPCs, as shown by cmyb (also known as myb) expression. The umbrella of melanocytes associated with the haematopoietic niche is highly evolutionarily conserved in aquatic animals, including the sea lamprey, a basal vertebrate. During the transition from an aquatic to a terrestrial environment, HSPCs relocated into the bone marrow, which is protected from ultraviolet light by the cortical bone around the marrow. Our studies reveal that melanocytes above the haematopoietic niche protect HSPCs from ultraviolet-light-induced DNA damage in aquatic vertebrates and suggest that during the transition to terrestrial life, ultraviolet light was an evolutionary pressure affecting the location of the haematopoietic niche.

Many aspects of the haematopoietic niche have been elucidated1,4. However, little is known about the selective pressures during evolution that influenced the location of the niche in diverse tissues such as the bones in mammals and the kidney in teleost fish. One year after the hypothesis of the existence of a specialized niche for HSPCs in 19785, it was hypothesized that HSPCs evolved to reside in the bone marrow of terrestrial animals to seek protection from ionizing irradiation, with bone then fulfilling the protective role of water6. Although this hypothesis is attractive, ionizing irradiation is mostly filtered out by Earth’s atmosphere and there is no direct evidence that HSPCs would be susceptible to DNA damage by non-ionizing irradiation such as ultraviolet B (UVB) light in vivo and that this vulnerability could determine the location and characteristics of the haematopoietic niche.

To better understand the definitive haematopoietic niche in zebrafish, we examined HSPCs in their surrounding tissues, using the Tg(runc:mCherry) line that specifically labels HSPCs7. We were intrigued to find that an umbrella of melanocytes located dorsal to the kidney marrow obscured visualization of HSPCs throughout development (Fig. 1, left, and Extended Data Fig. 1a, top left). HSPCs could more easily be observed in nacre mutants, which lack all melanocytes due to a mutation in the transcription factor mitfa (Fig. 1, right, and Extended Data Fig. 1a, bottom left; see also Extended Data Table 1). We confirmed the close spatial relationship between melanocytes and kidney HSPCs in larvae carrying Tg(mitfa:GFP) and Tg(runc:mCherry) transgenes that label melanocytes and HSPCs, respectively (Extended Data Fig. 1a, bottom right and Supplementary Video 1). To determine whether melanocytes serve as classical niche cells that support HSPC homing, expansion or maintenance, we compared HSPC numbers in mitfa−/− larvae and their pigmented siblings at 5 and 7.5 days post fertilization by whole-mount in situ hybridization analysis of the expression of cmyb—a transcription factor and master regulator of vertebrate haematopoiesis. These time points assess the homing of HSPCs into the kidney marrow and the initial expansion therein8. Equivalent numbers of HSPCs that were positive for cmyb were present in pigmented and unpigmented siblings, and all larvae had the same staining intensity in the thymus, kidney and caudal haematopoietic tissue as a representatively stained pigmented larva (Extended Data Fig. 1b). We also evaluated adult haematopoiesis in different pigment-deficient fish by analysing the kidney marrow of 2–6-month-old Tg(runc:mCherry) casper mutant fish and their pigmented siblings (Extended Data Table 1) by flow cytometry. Neither HSPC abundance nor the relative abundance of blood progenitors, myelomonocytes or lymphocytes were affected by pigment loss (Extended Data Fig. 1c–f).

In summary, we conclude that melanocytes are dispensable for steady-state haematopoiesis under laboratory lighting conditions.

Because the melanocytes form an opaque umbrella dorsal to the kidney marrow, we hypothesized that melanocytes shield HSPCs from UV-induced DNA damage, similar to their role in the skin. To test this, we irradiated unpigmented mitfa−/− larvae and their pigmented siblings and assayed the most common form of UV-induced DNA damage, cyclobutane pyrimidine dimers (CPDs), in HSPCs. After UVC irradiation, kidney HSPCs positive for Tg(runc:mCherry) were immediately isolated by fluorescence-activated cell sorting (FACS) (Fig. 2a) and stained with an antibody that recognizes CPDs. After irradiation HSPCs isolated from unpigmented larvae showed higher levels of DNA damage than HSPCs isolated from pigmented larvae (Extended Data Fig. 2a); this difference was significant when the fluorescence per cell was quantified (P ≤ 0.01, Fig. 2b). Since UVC light is usually filtered out by Earth’s atmosphere, we next tested biologically more relevant light (UVB), which also penetrates well into clear water. We exposed larvae to UVB and performed paraffin sections afterwards to assess...
Melanocytes protect HSPCs from UV-induced DNA damage.

a. Experimental layout. Animals were exposed to UV light at either 5 or 6 dpf and were processed immediately after UV irradiation.

b. Quantification of immunostaining intensity per cell. Data are mean ± s.d., data were analysed by ANOVA with post hoc Bonferroni’s multiple comparison test. HSPCs from 5 kidneys for each condition; n = 45, 33, 16 and 33 isolated cells from left to right. Dot plots are shown in Extended Data Fig. 2b. c. Anti-CPD immunostaining of a pigmented irradiated fish (transverse paraffin section). Black arrows indicate melanocytes. Dashed black lines indicate areas below the melanocytes with only few CD41+ nuclei. Scale bar, 50 μm. d. Magnification of the indicated area (thymus) in c. *P < 0.05; **P < 0.01; ***P < 0.0001.

The observed adverse effect of UV light on HSPCs is consistent with previous in vitro studies, in which it was shown that much lower UVB doses of 100–200 J m−2 completely abrogated colony forming potential of human HSPCs10. We chose a UVB dose that corresponds to a sunlight exposure of approximately 10–20 min at UV indices (a measurement of sunlight intensity) of 5–10; this dose corresponds to a UV exposure that would give a fair-skinned person sunburn. Wild zebrafish are found in rice paddies and small, often clear pools11, and other fish species also swim close to the water surface during the middle of the day in clear water (Extended Data Fig. 4). Since UVB penetrates well into clear water12, HSPCs in fish would be exposed to UV light in natural conditions and would thus benefit from optical protection. Fish have evolved other protective mechanisms against the accumulation of UV-induced DNA damage, such as light-dependent photoenzymatic repair13 and the expression of a sunscreen compound, gadosol14. These findings highlight the importance of coping strategies against UV-induced DNA damage even in aquatic animals.

To test whether the protective melanocyte umbrella was specific to zebrafish larvae, we performed comparative histology along the evolutionary tree of fish and other vertebrates (Fig. 4a). The adult zebrafish kidney is covered with melanocytes, which can readily be identified on histology slides stained with haematoxylin and eosin (Fig. 4f). We found that all analysed teleost fish (Ictalurus punctatus (channel catfish), Gasterosteus aculeatus (stickleback), Lepomis macrochirus (blue gill) and Lepomis microlophus (redear sunfish), Fig. 4e, g–i) had melanocytes covering the haematopoietic kidney marrow. This was also true in a member of the chondrostei, Acipenser fulvescens (lake sturgeon), and a member of the holostei, Atractosteus spatula (alligator gar) (Fig. 4c, d), that both diverged from teleost species about 250–350 million years ago15,16. Even the ancestral jawless vertebrate Petromyzon marinus, at the base of the vertebrate lineage (sea lamprey, post metamorphic stage), which diverged approximately 500 million years ago, showed melanocytes around its haematopoietic niche in the supraspinal organ17 (Fig. 4b). In more-recently diverged aquatic members of the vertebrate lineage, such as the sarcopterygian Protopterus annectens (West African lungfish), melanocytes also covered the haematopoietic kidney marrow. Scale bars, 100 μm (bright field) and 50 μm (fluorescence). WT, wild type.
kidney marrow (Fig. 4). In aquatic tetrapod larvae, from the amphibians *Xenopus laevis* (African clawed frog) and *Epipedobates anthonyi* (Anthony’s poison arrow frog), a melanocyte umbrella was present above the haematopoietic niche in the liver and kidney, respectively (Fig. 4k,l). It is known that in terrestrial amphibians, the adult haematopoietic niche is located in the bone marrow, which we confirmed in *Phyllobates terribilis* (golden poison frog; Fig. 4n). Using the anuran amphibian *Dendrobates tinctorius* (Dyeing poison frog; Fig. 4n) and analysing its haematopoietic niche at different developmental time points from tadpole to froglet (Extended Data Fig. 5a), we were able to show that the transition from a melanocyte-covered niche to the bone marrow occurred when the tadpoles first develop legs while still in an aquatic environment (Extended Data Fig. 5b–f). We then confirmed that cortical bone does indeed provide shielding against UV light by performing anti-CPD immunostaining of a hind leg of the *D. tinctorius* tadpole depicted in Extended Data Fig. 5e that was exposed to UVB light post mortem (Extended Data Fig. 6). This finding might indicate that the cortical bone around the bone marrow serves as a UV-protective layer in lieu of melanocytes, which might explain why all terrestrial animals have their haematopoietic niche in the bone marrow. Notably, some mammals have genetically programmed melanocytes in the spleen, and these melanocytes might represent an evolutionary remnant of the melanocyte umbrella that we discovered in zebrafish. Some frog species, such as certain *Rana* species, exhibit shifting sites of haematopoiesis in adulthood with the bone marrow serving as the main and the liver as a minor haematopoietic site. In addition, seasonal variation can be observed in these species, with the liver being more haematopoietic during the winter and the bone marrow during the summer, which might reflect an adaption to changing UV levels.

We hypothesized that during the evolution of tetrapods, UV light was a selective pressure in for the location of the HSPC niche. Larvae in which HSPCs colonized the bone marrow before the transition from aquatic to terrestrial life were at an advantage due to the selective pressures from higher UV levels in terrestrial conditions, although other factors, such as a more hypoxic microenvironment, might also have contributed to this process. Our hypothesis is also consistent with the development of the bone marrow in early sarcopterygians (lobe-finned fish) and with the observation that traits of terrestrial animals were often acquired before the transition out of the water. As to why HSPCs are located in the bone marrow of terrestrial animals, where they find shelter from harmful irradiation.

### Online content

Any Methods, including any statements of data availability and Nature Research reporting summaries, along with any additional references and Source Data files, are available in the online version of the paper at [https://doi.org/10.1038/s41586-018-0213-0](https://doi.org/10.1038/s41586-018-0213-0).

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Competing interests L.I.Z. is a founder and stockholder of Fate Therapeutics, Inc., Scholar Rock and Camp4 Therapeutics. D.E.F. has a financial interest in Soltego, Inc., a company developing SIK inhibitors for topical skin darkening treatments that might be used for a broad set of human applications. The interests of D.E.F. were reviewed and managed by Massachusetts General Hospital and Partners Healthcare in accordance with their conflict of interest policies.

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METHODS

Zebrafish husbandry. Zebrafish maintenance and breeding were performed at 28.5 °C with a 14 h:10 h light:dark cycle.[26] These standard laboratory conditions do not comprise exposure to UV light. All experiments were performed according to protocols approved by the Institutional Animal Care and Use Committees (IACUCs) of Harvard University and Boston Children’s Hospital, or by the Regierungspräsidium Freiburg, and were in accordance with the German laws for animal experiments.[26]

Frog methods. E. anthonyi, P. terribilis and D. tinctorius were reared in a captive colony. Animals were anaesthetized with 20% benzocaine followed by euthanasia by cervical transection. Specimens were then placed in 4% paraformaldehyde. All poison frog protocols were approved by the IACUC at Harvard University.

Statistics and reproducibility. Owing to animal welfare regulations in Germany, complete experiments involving zebrafish older than 5 dpf could only be performed once, since repeat experiments are not permissible once a statistically significant result has been obtained. Small-scale pre-experiments have been performed to estimate the effect strength in the assays performed, and the animal experiments were statistically planned and approved by the Regierungspräsidium Freiburg with sufficient numbers of animals to obtain statistically significant results. Confidence in the observed results and their reproducibility is strengthened by an experimental strategy, in which successive experiments not only investigate new biological questions, but are also based on and thus add supportive information to the preceding experiment (for example, irradiation of different pigment mutants in Fig. 3c, anaesthesia experiment in Fig. 3e, or thrombocyte count in Extended Data Fig. 3e). The sex of the animals was not or could not be determined before the conduction of the experiments.

The experiments in the figures were performed as follows: Figure 1: imaging experiments were repeated >3 times independently with similar results (biological replicates). Figure 2b: the experiment was performed once. Figure 2c: the experiment was performed twice with similar results (biological replicate). Figure 3c, e: the experiments were performed once. Figure 4b–m: experimental results were confirmed in at least a second animal of the same species (except lungfish, owing to scarcity of material). Extended Data Fig. 1a (and Supplementary Video 1): the experiment was performed at least twice with similar results (biological replicate). Extended Data Fig. 1b: the experiment was performed three times with similar results (biological replicates). Extended Data Fig. 1c–f: the experiment was performed twice with similar results (biological replicates). Extended Data Fig. 2a, b: the experiment was performed once (same experiment as Fig. 2b). Extended Data Fig. 2c: the experiment was performed twice with similar results (biological replicate). Extended Data Fig. 3b: the experiment was performed twice with similar results (biological replicate). Extended Data Fig. 3c: the experiment was performed once. Extended Data Fig. 3d–f: the experiments were each performed once (the same larvae were used for the thrombocyte count as in the histology experiments to reduce variability, and the animals were anaesthetized). Extended Data Fig. 5: the experiment was performed once owing to scarcity of material, but the analyses at different developmental time points support each other. Extended Data Fig. 6: the experiment was performed once owing to scarcity of material. Extended Data Fig. 7: N/A.

Experiments were conducted in a blinded fashion, whenever possible. Animals were randomly assigned to treatment and control groups.

Imaging. Using the recently developed transgenic reporter line Tg(Mntu.runx1.1LS-mCherry)[27] (here called Tg(runx1.mCherry)) that labels HSPCs,[28] we imaged the location of HSPCs relative to the kidney tubule in a cross with the Tg(cdhl17:GFP)[29] line[30] in zebrafish larvae at different developmental stages and also assessed the spatial relationship with melanocytes, labelled by the transgenic reporter line Tg(mitfa:GFP)[31,32] Fish were anaesthetized with 0.168 mg tricaine per ml egg water for the duration of the procedure and were imaged on a Zeiss CellObserver, Zeiss Examiner or a Zeiss LSM700 system. For the thrombocyte count, Tg(CD41:GFP)[33] larvae were analysed two days after irradiation by imaging on a Zeiss Examiner with a 20× objective and time lapse videos of 10 s were recorded. Afterwards, a 2 projection was performed and the circulating thrombocytes were counted and normalized to the area of the vessel (Extended Data Fig. 3f). Statistical analysis was performed using ANOVA with post hoc Bonferroni tests.

Flow cytometry. Kidney marrow was isolated from adult wild-type, mitfa−/−, cmyb−/− and casper−/− fish and analysed as previously described.[36] For gating strategy see Supplementary Fig. 1.

UVC and UVB irradiation. Pigmented and unpigmented larvae were placed in 6-cm Petri dishes and egg water was added to a volume of 10 ml. For each irradiation protocol, pigmented and unpigmented larvae were placed in the same dish to achieve identical UV exposure in pigmented and unpigmented fish. For UVC irradiation a Stratalinker 1800 (Stratagene) was used, for UVB irradiation an UV 801 BL unit (Waldmann GmbH, Germany) was used. The Petri dish was placed in a cardboard container to reduce the amount of UV light reaching the larvae from the side (see experimental setup displayed in Extended Data Fig. 7). The administered dose of UVB corresponded to approximately 10 min at a UV index of 10; both a UV index of 90 for approximately 50 s (for example, Fig. 3c, e) and a UV index of 20 for approximately 5 min (Extended Data Fig. 3c) led to the same results; of note, even a UV index of 20 is slightly higher than usually encountered in natural conditions. The UV index was measured using a Solarmeter 6.5 (Solar Light Company, Inc.). The device was also calibrated to the UV Sensor Variocolor (Waldmann, Germany) and corresponded to 500 μJ m−2. Before and after the irradiation, larvae were kept in the dark at 28.5 °C until the end of the experiment. After irradiation, larvae were transferred to a 10-cm dish containing 30–40 ml of egg water to maintain good water quality.

PTU treatment. To prevent pigmentation in wild-type TU (Tubingen) embryos, embryos were treated with 150 μl 1-phenyl 2-thiourea (PTU; Sigma-Aldrich) at 24 hours post-fertilization (hpf), a slightly reduced dose of PTU that consistently gave very good results.[31] To avoid interaction of PTU with the UV light, PTU was washed off and the embryo medium was replaced 12 h before irradiation.

Anaesthesia. To move the melanocytes out of the path of light, larvae were anaesthetized immediately before the irradiation. Tricaine was added to the 6-cm Petri dish at a final concentration of 0.168 mg tricaine per ml egg water. After irradiation, tricaine was immediately washed off and replaced with egg water.

FACS and anti-CDP antibody staining. Staining of single cells positive for Tg(runx1.mCherry) for UV-induced DNA damage was performed according to a protocol adapted from previously published methods.[33] Immediately after UV irradiation, larvae were euthanized in tricaine and transferred to ice water. The larvae were then reared behind the swim bladder and discarded, since only HSPCs in the kidney were of interest. Five larval heads were pooled according to their pigment status, incubated with liberase at 37 °C for 20 min in the dark. PBS was added to reach 10% to inhibit further digestion and larvae were dissociated by pipetting up and down. Debris was removed by pipetting through a 40-μm mesh filter. Then, 10% formaldehyde was added to reach a 4% final concentration and cells were fixed for 10 min at room temperature. Afterwards, cells were spun down at 500g for 5 min at 4 °C and washed with PBS twice. Cells were sorted onto SuperFrost slides with attached 8-well silicone insulators containing 70–100 μl ultrapure water using a FACS Aria (HSCRB FACS Core) with the 355-nm laser turned off. Slides were dried at room temperature, then put in an oven at approximately 70 °C for 10 min. Slides were kept in a humidified chamber for the following steps. Cells were washed with PBS, incubated with PBS with 0.5% Triton X-100 for 20 min, washed with PBS, DNA was denatured with 2 M HCl for 30 min at room temperature, washed with PBS, blocked with 10% NGS for 60 min, and incubated with the anti-CDP antibody TDM-2 (Cosmo Bio Co) at 1:500 in 2% NHS overnight at 4 °C. The next day, cells were washed with PBS and kept in PBS for the dark for the subsequent steps. The slides were incubated with a goat anti-mouse antibody conjugated with AlexaFluor 488 (Invitrogen) at 1:500 in 2% NHS at room temperature. Cells were washed with PBS, followed by incubation with 0.05 μg ml−1 DAPI in PBS for 5 min and washed again with PBS. Slides were analysed on a CellObserver (Zeiss, Germany) and the mean fluorescence of each cell was measured. Statistical analysis was performed using GraphPad Prism 5 software using a one-way ANOVA with a post hoc Bonferroni’s multiple comparison test.

Paraffin sections. Directly after irradiation, larvae were euthanized in Tricaine followed by fixation in 4% formaldehyde. Larvae were embedded in paraffin wax, sectioned at 5–7 μm and immunohistochemistry was performed as described above with an additional proteinase K digestion step. The secondary biotinylated anti-mouse (Vector Laboratory) (1:200) was used followed by signal detection with VECTASTAIN ABC HRP kit (Vector Laboratory).

Whole-mount in situ hybridization. Whole-mount in situ hybridization was performed as previously described[38] with the addition of 0.2% glutaraldehyde to the 4% fixation step after permeabilization with proteinase K. The cmyb probe was used at 400 ng ml−1. Staining with BCIP and NBT took approximately 4–6 h. Statistical analysis was performed using GraphPad Prism 5 software using χ² tests.

Histology. Fish specimens were euthanized and fixed in 4% formaldehyde for at least 24 hpf at 4 °C. After fixation, samples were transferred to 70% ethanol and stored at 4 °C until further processing. Samples were decalcified and afterwards embedded in paraffin wax. After hardening, samples were cut at a thickness of 4–10 μm on a microtome, transferred to charged glass slides and stained with haematoxylin and eosin and covered with a coverslip afterwards. The zebrafish histology slide (Fig. 4f) was acquired from the Zebrafish Histology Atlas (http://bio-atlas.psu.edu/zf/view.php?page=250&atlas=18&z=18c=9774.7525).

Reporting summary. Further information on experimental design is available in the Experimental design section. This research was also linked to the Vertebrate Sensory Systems Database. Data availability. The data that support the findings of this study are available from the corresponding author upon reasonable request. Source data for Figs. 2, 3 and Extended Data Figs. 1–3, 6 are provided in the online version of the paper.
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Extended Data Fig. 1 | Melanocytes are spatially associated with the zebrafish kidney but dispensable for steady-state haematopoiesis.

a, Top right panel, schematic of an embryo. The black boxed area contains the kidney and is enlarged in the other panels. Left panel containing 4 images, zebrafish positive for Tg(cdh17:GFP) (green, labelling the kidney tubule) and Tg(runx:mcherry) (red, labelling HSPCs) are depicted at 7 dpf. The boxed areas in the left images are enlarged in the corresponding fluorescence panels in the middle; these panels show the head kidney containing the haematopoietic marrow (indicated by the dashed outline). The white arrow highlights the melanocyte umbrella. Scale bars, 100 μm (bright field) and 50 μm (fluorescence). Bottom right panels, the kidney marrow of a 6-dpf larva positive for Tg(mita:GFP) (green, labelling melanocytes) and Tg(runx:mcherry) (red, labelling HSPCs) is shown from a lateral view (bottom right, large panel) and from an orthogonal view (transverse section; bottom right, small panel). The scale bar represents 20 μm.

b, Whole-mount in situ hybridization of cmyb at different time points, a representative larva at 5 dpf is shown. Arrows indicate (from cranial to caudal) the thymus, the kidney and the caudal haematopoietic tissue; the enlarged portion of the image (dashed box) shows the thymus and the kidney. The experiment was performed with n = 10 wild-type and 10 mita−/− larvae at 5 dpf and 10 wild-type and 7 mita−/− larvae at 7.5 dpf. c, Flow cytometric analysis of the percentage of HSPCs that were positive for Tg(runx:mCherry) (red, labelling HSPCs) as a proportion of live cells in the kidney marrow of adult fish. Data are mean ± s.d.; n.s., not significant.

d–f, Relative abundance of progenitors, myelomonocytes and lymphocytes in adult wild-type fish and mita−/−, roy−/− and casper−/− pigment mutants as assessed by flow cytometry as previously described. c–f, n = 6 wild-type, 6 mita−/−, 3 roy−/− and 4 casper−/− fish. Data are mean ± s.d.; analysis by ANOVA.
Extended Data Fig. 2 | Unprotected haematopoietic cells accumulate DNA damage after UV irradiation. a, Sorted cells that were positive for Tg(runx:mCherry) after anti-CPD immunostaining. Scale bars, 10 μm. b, Dot plot representation of data in Fig. 2b (quantification of immunostaining intensity per cell). Data are mean ± s.d.; for statistics and P values please refer to Fig. 2b. c, Magnification of the thymus (dashed red outline) after anti-CPD immunostaining (counterstaining with haemalum) after UVB irradiation at 5 dpf in pigmented and non-pigmented larvae. Arrows from above indicate melanocytes; arrows from below indicate examples of nuclei with DNA damage.
Extended Data Fig. 3 | UV light is detrimental to exposed haematopoiesis. a, Experimental layout. b, Reduction in HSPCs in larvae with and without PTU treatment as assessed by cmyb in situ hybridization after UVC irradiation. $n = 26, 26, 24$ and 19 larvae in from left to right. c, Reduction in HSPCs in tyr−/− larvae and their pigmented siblings as assessed by cmyb in situ hybridization after UVB irradiation at a UV index of 20. $n = 38, 33, 29$ and 38 larvae from left to right. Results were not significant but note that the pigmented irradiated group seemed to retain more HSPCs in this experiment than in the preceding experiments with a higher UV index. d, Histology of the kidney marrow two days after irradiation. The yellow outline represents the kidney tubules, the red outline shows the aorta and the green outline indicates the haematopoietic marrow. Note the reduced area of the haematopoietic marrow in the non-pigmented, irradiated larvae (bottom right). Scale bars, 20 μm. e, Abundance of thrombocytes positive for Tg(CD41:GFP) two days after irradiation. Each data point represents the number of thrombocytes per vessel area in individual larvae. $n = 8, 7, 7$ and 7 larvae for treatment groups from left to right. Statistical significance was calculated using ANOVA with post hoc Bonferroni’s multiple comparison test, data are mean ± s.d. f, Schematic of the analysis of the number of thrombocytes that were positive for Tg(CD41:GFP). The boxed area represents the analysed area, in which the circulating cells were counted. The yellow outline represents the area of the vessel, which the number of circulating thrombocytes was normalized to.
Extended Data Fig. 4 | Sunlight exposure in fish living in the wild. Example of small fish swimming in clear and shallow water on a sunny day (photo taken at Titisee, Germany, July 2016). Field of view approximately 25–30 cm wide.
Extended Data Fig. 5 | HSPCs relocated into the bone marrow before the transition to a terrestrial environment. a, Top, a developmental time line of *D. tinctorius*. Tadpoles were staged according to Gosner. Animals (from left to right) represent Gosner stage 25, 30, 41 and 42, as well as a froglet five days after losing its tail. Stage-specific histology analyses are shown in panels b–f as indicated. Bottom, the habitat of *D. tinctorius* at the different developmental stages. b–f, Haematopoietic niches analysed by haematoxylin and eosin staining. Scale bars, 100 μm (b–f) and 1 cm (a).
Extended Data Fig. 6 | Cortical bone protects from UV-induced DNA damage. a, Paraffin section of a *D. tinctorius* hind leg (from Extended Data Fig., specimen e) after irradiation with UVB post mortem; the leg was severed from the body and irradiated with UVB. The dashed outline represents the cortical bone. Note the higher staining intensity of the anti-CPD antibody in nuclei within the muscle tissue compared to nuclei within the bone marrow. This part of the leg is not yet haematopoietic (compare to Extended Data Fig. 5e, which shows the haematopoietic marrow in the other leg), but contains chondrocytes. Note that even the chondrocyte nuclei closest to the cortical bone are stained much less than the cells outside the cortical bone (arrows from below and from above, respectively). The triangle represents the direction of the UV source; white tip towards UV source. Scale bar, 50 μm. b, Quantification of grey scale values of nuclei inside (*n* = 17) and outside (*n* = 41) the cortical bone. Each data point represents the mean grey value of a 16 × 16 pixel circle inside the nucleus, the difference is highly significant (unpaired Student’s two-tailed *t*-test, *P* < 0.0001); data are mean ± s.d.
Extended Data Fig. 7 | Experimental setup during irradiation. Fish were placed in a Petri dish inside the upper cardboard box to focus the light from above, because the Waldmann UV 801 BL unit has a curved lamp carrier. The lower cardboard box was used to place the larvae at the recommended distance from the lamps.
Extended Data Table 1 | List of causes for lack of pigmentation

| Unpigmented because of | Name                     | Mechanism                                                                 |
|------------------------|--------------------------|----------------------------------------------------------------------------|
| Chemical treatment     | 1-phenyl 2-thiourea (PTU)| Blocking of the enzyme tyrosinase\(^{29}\)                               |
|                        |                          | → melanocytes do not contain melanin                                      |
| Genetic cause          | nacre (mitfa\(^{22}\))  | Mutation in the transcription factor mitfa\(^{32}\)                      |
|                        |                          | → melanocytes are absent                                                  |
|                        | sandy (tyr\(^{33}\))    | Mutation in the enzyme Tyrosinase\(^{33}\)                              |
|                        |                          | → melanocytes do not contain melanin                                      |
|                        | roy (mpv1\(^{33}\))     | Mutation in the mpv17 mitochondrial inner membrane protein\(^{34}\)    |
|                        |                          | → iridophores are absent                                                  |
|                        | casper (mitfa\(^{20c2}\); mpv1\(^{30a0}\)) | double homozygous for nacre and roy\(^{34}\)                        |
|                        |                          | → melanocytes and iridophores are absent                                  |
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- The **exact sample size** (n) for each experimental group/condition, given as a discrete number and unit of measurement
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- The statistical test(s) used AND whether they are one- or two-sided
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- A description of all covariates tested
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  - *For null hypothesis testing, the test statistic (e.g. F, t, r) with confidence intervals, effect sizes, degrees of freedom and P value noted* Give P values as exact values whenever suitable.
- For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings
- For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes
- Estimates of effect sizes (e.g. Cohen's d, Pearson's r), indicating how they were calculated
- Clearly defined error bars
  - *State explicitly what error bars represent (e.g. SD, SE, CI)*

Our web collection on **statistics for biologists** may be useful.

Software and code

Policy information about availability of computer code

- **Data collection**
  - Zeiss ZEN (blue and black versions)
  - Camera (iPhone 6)
- **Data analysis**
  - FlowJo
  - ImageJ
  - Microsoft Excel
  - Microsoft Powerpoint
  - GraphPad PRISM

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Life sciences

Study design

All studies must disclose on these points even when the disclosure is negative.

- **Sample size**
  
  In our experience, approx. 60 embryos are needed per treatment group to achieve a reliable result in case of cmyb in situ. In case of low embryo availability the experiment was conducted with lower numbers and statistical significance was calculated in order to determine, whether an additional experiment to achieve the planned animal numbers is necessary. If statistical significance was reached, the conduction of an additional animal experiment was not conducted.

- **Data exclusions**
  
  No data were excluded

- **Replication**
  
  Due to animal welfare regulations in Germany, complete experiments involving zebrafish older than 5 dpf could only be performed once, since repeat experiments are not permissible once a statistical significant result has been obtained. Small-scale pre-experiments have been performed to estimate the effect strength in the assays performed, and the animal experiments were statistically planned and approved by the Regierungspräsidium Freiburg with sufficient numbers of animals to obtain statistically significant results. Confidence in the observed results and their reproducibility is strengthened by an experimental strategy, in which successive experiments not only investigate new biological questions, but are also based on and thus add supportive information to the preceding experiment (e.g. irradiation of different pigment mutants in Fig. 3c, anaesthesia experiment in Fig. 3e, or thrombocyte count in Extended Data Fig. 3e).

- **Randomization**
  
  Sample allocation to treatment groups was random. Each treatment group contained the same amount of embryos from the same clutch.

- **Blinding**
  
  Data collection and analysis was conducted in a blinded fashion. To confirm the robustness of the in situ read-out, a total of four researchers scored the initial in situ and achieved very results.

Materials & experimental systems

Policy information about availability of materials

- n/a Involved in the study
- X Unique materials
- X Antibodies
- X Eukaryotic cell lines
- X Research animals
- X Human research participants

Antibodies

- Antibodies used
  
  Antibody recognizing Cyclobutane Pyrimidine Dimers (clone TDM2) from Cosmo Bio, Catalogue number: CAC-NM-DND-001

- Validation
  
  The TDM-2 antibody is a well established antibody. We followed the manufacturer’s protocol and tested out the protocol on unirradiated and irradiated samples first. After this validation step, we proceeded with the experiments detailed in the paper.

Research animals

Policy information about studies involving animals; ARRIVE guidelines recommended for reporting animal research

- Animals/animal-derived materials
  
  The following animals were used in the study.
  Zebrafish strains:
Most experiments were conducted up to an age of 8 dpf; sex could thus not be determined. In the experiment involving adults (age 2-6 months), sex was neglected for the analysis.

The following other species were examined in the study:
- Ictalurus punctatus (adult)
- Gasterosteus aculeatus (adult)
- Lepomis macrochirus (juvenile)
- Lepomis microlophus (juvenile)
- Acipenser fulvescens (juvenile)
- Atractosteus spatula (juvenile)
- Petromyzon marinus (post metamorphic stage)
- Protopus annectans (adult)
- Xenopus laevis (larva)
- Epipedobates anthonyi (larva)
- Phyllobates terribilis (adult)
- Dendrobates tinctorius (larva to young adult)

Method-specific reporting

| Method          | Included in the study |
|-----------------|-----------------------|
| ChIP-seq        | Yes                   |
| Flow cytometry  | Yes                   |
| Magnetic resonance imaging | Yes |

Flow Cytometry

Plots

Confirm that:
- The axis labels state the marker and fluorochrome used (e.g. CD4-FITC).
- The axis scales are clearly visible. Include numbers along axes only for bottom left plot of group (a ‘group’ is an analysis of identical markers).
- All plots are contour plots with outliers or pseudocolor plots.
- A numerical value for number of cells or percentage (with statistics) is provided.

Methodology

Sample preparation

Zebrafish kidney marrows were dissected from euthanized fish and placed in FACS buffer on ice. They were dissociated by repetitive pipetting and filtered into FACS tubes (BD Falcon). FACS buffer consisted of 0.9X Dulbecco’s phosphate buffered saline, 2% heat inactivated fetal bovine serum, 1% penicillin/streptomycin, and 1 USP unit/μL heparin.

Instrument

BD LSR II (analysis), BD FACS Aria II (sorting, only Figure 2b)

Software

BD FACS Diva (collection), FlowJo (data analysis), Prism (statistical analysis)

Cell population abundance

Sorting was only performed in the experiment displayed in Figure 2b. Since cells were sorted directly onto slides, no post-sort control could be performed.

Gating strategy

Both for analysis and sorting: SSC-A vs. FSC-A to remove debris, FSC-H vs. FSC-A to isolate single cells, SSC-H vs. SSC-W to isolate single cells, Pacific Blue-A vs. FSC-A for live-dead staining (Sytox Blue-based), PE-A vs. mCherry-A for mCherry positive. For the abundance of cell populations (lymphocytes, progenitors, myelomonocytes), a plot of FSC vs. SSC was performed after the selection of live cells.

Tick this box to confirm that a figure exemplifying the gating strategy is provided in the Supplementary Information.