Cerebrospinal fluid regulates skull bone marrow niches via direct access through dural channels

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It remains unclear how immune cells from skull bone marrow niches are recruited to the meninges. Here we report that cerebrospinal fluid (CSF) accesses skull bone marrow via dural-skull channels, and CSF proteins signal onto diverse cell types within the niches. After spinal cord injury, CSF-borne cues promote myelopoiesis and egress of myeloid cells into meninges. This reveals a mechanism of CNS-to-bone-marrow communication via CSF that regulates CNS immune responses.

CNS borders, including meninges and choroid plexus, harbor a rich repertoire of immune cells with important roles in brain function1–5. Importantly, myeloid populations, including monocytes and neutrophils, residing in the meninges arise predominantly from local bone marrow niches housed in the skull and vertebrae rather than from blood6. The factors influencing the cellular makeup of these compartments, and the origin of cues calling immune cells into meningeal tissue, however, remain largely unknown.

We hypothesized that local CNS cues, contained in CSF, could instruct myeloid cell recruitment to the meninges. Efflux of molecules from the brain is achieved via the blood–brain barrier7,8, and via CSF perfusion through the brain in a process termed ‘glymphatic clearance’9. Through a glymphatic mechanism, brain-derived molecules are continuously cleared via CSF, efflux to the parasagittal dura mater and subsequently drain through meningeal lymphatic vessels, enabling immune surveillance of the CNS from distant sites10–12. Recent studies demonstrated that skull bone marrow also connects directly to the underlying dura through ossified vascular channels13–15. Although these channels have been previously described to allow myeloid and lymphoid cell migration from the skull bone marrow to the dura, we speculated that these pathways might be bi-directional, allowing bone marrow direct access to the CSF. Here we show that CSF accesses skull bone marrow niches, where it regulates myelopoiesis and egress to meninges in physiology and pathology.

To test the possibility that CSF interfaces with CNS bone marrow niches, we injected fluorescent ovalbumin (OVA, ~45 kDa) into CSF via the cisterna magna (intra-cisterna magna (ICM)) and examined its efflux to skull bone marrow after 1 hour. Whole-mounted decalcified and cleared skullcaps with underlying dura revealed uptake of tracer along dural sinuses (Fig. 1a,b), as previously described4. Three-dimensional reconstructions of skull bone marrow regions also showed tracer along perivascular conduits within ossified channels and within the bone marrow niche (Fig. 1a,b and Supplementary Video 1).

Although the dorsal aspect of the skull bone marrow contains ossified channels13,15 that provide myeloid and lymphoid populations directly to the underlying dura14–16, whether similar anatomical structures are present in the skull base is unclear. Examination of the skull base revealed enriched pockets of marrow (Fig. 1c,d) with an equivalent array of stem, progenitor, myeloid and lymphoid populations (Extended Data Fig. 1a–c). Assessment of skull base bone marrow niches demonstrated the conserved presence of channels and similar perivascular conduits of CSF tracer efflux as those observed in dorsal skull (Fig. 1e). To exclude any potential contribution of postmortem artifact, we employed intravital two-photon microscopy and observed the CSF tracer in the dorsal skull bone marrow niche 30 minutes after ICM injection (Fig. 1f).

To confirm that CSF interfaces with cells in the skull bone marrow, we assessed accumulation of OVA signal in macrophages and observed uptake in both dorsal and basal skull niches after ICM injection (Fig. 1g,i). To demonstrate that CSF interacts with other cells within the bone marrow niche, we assessed labeling of hematopoietic stem cells (HSCs) (Lin–Sca1+c-Kit+ (LSK)) by flow cytometry of young-adult mice after ICM injection of an anti-c-Kit antibody. One hour after ICM delivery, 99% of LSK cells in skull bone marrow were labeled with the antibody (Fig. 1h,i,k). Intracerebral injection of OVA and anti-c-Kit antibody also labeled macrophages and HSCs, respectively, suggesting that parenchymal solutes traffic into the CSF, as previously described13,14, and subsequently access skull bone marrow (Fig. 11–n). Notably, 1 hour after either ICM or intracerebral (IC) injection, neither c-Kit-labeled nor OVA-labeled cells were found in peripheral tibial bone marrow, confirming that this CSF access reflects direct CSF-to-skel bone marrow communication rather than peripheral blood recirculation (Fig. 1l–n).

Paravascular glymphatic fluid flow and meningeal lymphatic drainage of CSF change throughout the lifespan17–19. We, therefore, wondered whether CSF access to skull bone marrow similarly changes throughout postnatal development and aging because of altered CSF dynamics. Surprisingly, no major changes in CSF–skull bone marrow accessibility were observed beyond the second postnatal week (Fig. 1o,p), suggesting that CSF-derived factors have the potential to shape skull bone marrow niches throughout the entire lifespan.

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CSF composition shapes neurogenic niches during CNS development through direct ligand–receptor signaling. Given that CSF directly accesses skull bone marrow, we asked whether exposure to CSF affords the skull marrow niche a unique phenotype. To this end, we performed single-cell RNA sequencing (scRNA-seq) of dorsal skull and tibial bone marrow from young-adult mice. Phenotypic analysis revealed expected bone marrow populations, including HSCs, monocytes, neutrophils, basophils, mast cells, erythroblasts, sensory neurons, dendritic cells, natural killer cells, T cells and B cell developmental trajectories (Fig. 2a, b and Extended Data Fig. 2a). Although there were no major differences in the cellular composition of skull versus tibia bone marrow niche, differences were observed within neutrophil, monocyte, macrophage and HSC populations, including downregulation of genes involved in proliferation in HSCs, reactive oxygen species production in monocytes and macrophages and myeloid cell differentiation in neutrophils.
Fig. 1 | CSF accesses skull bone marrow niches. a, Representative maximum intensity projection of a decalcified and cleared skull cap–dura whole mount 1 hour after an ICM injection of OVA-A594. Scale bar, 2 mm. b, Z-sections through the dura, cortical skull bone and trabecular bone marrow in a region of interest from the skull cap–dura whole mount. Arrowheads highlight perivascular OVA accumulation. Arrows denote OVA+ cells within the bone marrow cavity. Scale bars, 100 μm. c, Anatomy of skull bone marrow niches in the dorsal and basal skull. d, Sagittal sections through the dorsal and basal skull. Scale bars, 3 mm. e, High-magnification images of tracer accumulation in the dorsal (left) and basal (right) bone marrow of the skull 1 hour after an ICM injection of OVA-A594. Scale bar, 50 μm. f, Representative two-photon image of skull bone marrow in a live mouse 30 minutes after administration of 70 kDa FITC-dextran (i.v.) and OVA-594 (ICM). Scale bar, 100 μm. g, Gating strategy and representative plots of ICM OVA-A488 labeling in bone marrow macrophages. h, Gating strategy and representative plots of ICM c-Kit-PE labeling in bone marrow HSCs. i–k, Experimental design (i), percentage of macrophages positive for ICM OVA-A488 (j) and percentage of HSCs positive for ICM c-Kit-PE (k) 1 hour after injection. n = 4 or 5 mice. Data are means ± s.e.m.; P values represent a one-way ANOVA with Tukey’s post hoc test. l–n, Experimental design (l), percentage of macrophages positive for IC OVA-A594 (m) and percentage of HSCs positive for IC c-Kit-PE (n) 1 hour after injection. n = 4 mice. Data are means ± s.e.m.; P values represent a one-way ANOVA with Tukey’s post hoc test. o, p, Experimental design (o) and percentage of bone marrow HSCs positive for c-Kit-PE (p) 1 hour after ICM injection in P7, P14, P21, adult (2–3 months old) and aged (20–24 months old) mice. n = 4 mice. Data are means ± s.e.m.; P values represent a two-way ANOVA with Dunnett’s post hoc test versus 2–3 months. BM, bone marrow.

Fig. 2 | Functional interactions between CSF and the skull bone marrow niche. a, b, t-distributed stochastic neighbor embedding (t-SNE) visualizations of scRNA-seq from dorsal skull and tibial bone marrow from 2-month-old mice colored by cell type (a) or sample (b). c, Chord plot detailing between CSF ligands identified by unlabeled LC–MS and receptors on skull bone marrow HSCs, macrophages, monocytes and neutrophils identified by scRNA-seq. d–e, Gene Ontology (GO) pathway analysis on receptor genes with at least one CSF ligand in monocytes (d) and neutrophils (e). f, Representative immunohistochemistry of CD3+ and Ly6b+ cells at the superior sagittal sinus (S.S. Sinus) and transverse sinus (T. Sinus) of the dura mater, 24 hours after an ICM injection of 10 μg AMD3100 or aCSF. Scale bar, 200 μm. g, Flow cytometry gating strategy (g) and frequency of Ly6C+ monocyte (h), neutrophil (i) and T cell proportions (j) after an ICM injection of 10 μg AMD3100 or aCSF. n = 5 mice per group. Data are means ± s.e.m.; P values represent a two-sided Student’s t-test. DCs, dendritic cells; NK, natural killer; DN, double negative.
Fig. 3 | CSF-contained cues mobilize the skull bone marrow in response to CNS injury. a, Experimental design for spinal cord injury experiments. Spinal cord injury was performed in 2-month-old mice at T7 after laminectomy (sham group included laminectomy). EdU (10 mg kg\(^{-1}\)) was injected 1 hour before collection. Three hours later, skull bone marrow was processed for flow cytometry. b, Gating strategy for MDPs, cMoPs and actively proliferating Ki67+, Gating strategy for MDPs, cMoPs and actively proliferating before collection. Three hours later, skull bone marrow was processed for flow cytometry.

(Extended Data Fig. 2b–j). We, therefore, wanted to further explore the nature of steady state signaling between CSF and skull bone marrow to determine if CSF factors might modify bone marrow physiology. We performed a proteomic analysis of CSF from young-adult mice, and, leveraging our scRNA-seq dataset, uncovered a set of potential ligand–receptor interactions between CSF proteins and diverse cells in the skull bone marrow (Fig. 2c, Extended Data Fig. 2k and Supplementary Table 1).

Examining Gene Ontology pathways of these ligand–receptor interactions in monocytes and neutrophils revealed signaling mechanisms enriched for leukocyte migration, cell adhesion and phagocytosis (Fig. 2d,e). These enriched pathways suggested that
CSF-derived factors could instruct the mobilization and recruitment of myeloid cells from the skull bone marrow. To test this, we injected AMD3100, a CXCR4 antagonist, into the CSF and assessed monocyte and neutrophil egress from skull bone marrow to the dura. By immunostaining, we found that Ly6b+ monocytes and neutrophils in the dura were significantly enriched after AMD3100 administration and preferentially clustered at nearby sinuses underlying skull bone marrow niches (Fig. 2f and Extended Data Fig. 3a–d). Using flow cytometry, we confirmed a significant increase in Ly6C+ monocytes and neutrophils in the dura, along with a concomitant decrease in overlying skull bone marrow, suggesting local bone marrow egress rather than dural proliferation (Fig. 2g–1 and Extended Data Fig. 3e,f). Notably, we did not detect a change in dural T cells (Fig. 2j) or any changes in tibial monocytes or neutrophils (Extended Data Fig. 3g), consistent with the notion of T cell trafficking through blood vasculature22,23, whereas myeloid cells migrate directly from skull bone marrow niches24. These results confirmed that myeloid cells can egress from the skull bone marrow in response to CSF-derived cues, suggesting an ability to dynamically respond to altered brain states.

We next asked whether physiological changes in CSF composition—for example, after CNS injury—could instruct skull bone marrow mobilization. To test this, we performed a spinal cord crush injury—during which a laminectomy is performed and the spinal cord meninges are left intact, thus preventing CSF leakage—in young-adult mice and asked whether a distant CNS injury could be sensed by the skull bone marrow. Notably, laminectomy did not impair CSF access to the skull bone marrow niche (Extended Data Fig. 4a–d). Three hours after spinal cord injury, vertebrae and skull bone marrow were collected, and myeloid progenitors were assessed. As expected, vertebrae bone marrow was highly activated after the injury, characterized by elevated numbers of proliferating monocytes (Extended Data Fig. 5a–e). Interestingly, remote skull bone marrow niches also showed a substantial increase in the percent of monocyte dendritic precursor (MDPs), common monocyte progenitors (cMops) and proliferating monocytes compared to sham controls (Fig. 3a–g). Additionally, assessment of CSF composition after spinal cord injury revealed elevated levels of the monocyte chemoattractant CCL2 (MCP-1) (Extended Data Fig. 5a–e). Interestingly, remote CSF access to the skull bone marrow niche (Extended Data Fig. 4a–d). Three hours after spinal cord injury, vertebrae and skull bone marrow were collected, and myeloid progenitors were assessed. As expected, vertebrae bone marrow was highly activated after the injury, characterized by elevated numbers of proliferating monocytes (Extended Data Fig. 5a–e). Interestingly, remote skull bone marrow niches also showed a substantial increase in the percent of monocyte dendritic precursor (MDPs), common monocyte progenitors (cMops) and proliferating monocytes compared to sham controls (Fig. 3a–g). Additionally, assessment of CSF composition after spinal cord injury revealed elevated levels of the monocyte chemoattractant CCL2 (MCP-1) (Extended Data Fig. 5a–e). These results suggest that CSF carries cues to neighboring bone marrow niches to induce myelopoiesis and provide myeloid cells to underlying dura or brain tissue after CNS insults.

To determine whether the skull bone marrow response after spinal cord injury is indeed mediated by CSF-containing signals, and to confirm that this is sufficient to promote myeloid cell trafficking, we performed CSF transfer experiments. Sham (that included laminectomy) or spinal cord injury was performed on young-adult mice, and, 3 hours later, their CSF was collected and transferred into naive mice (Fig. 3h). Six hours after CSF transfer, we observed a significant increase in the number of monocytes in the dura of mice receiving CSF from injured mice compared to CSF obtained from sham donors (Fig. 3i–k). As CSF can be drained via meningeal lymphatics and into peripheral blood, we cannot entirely exclude the possibility that some of the observed response was systematically driven. However, neither a sham surgery nor a laminectomy resulted in redistribution of CSF to a distant peripheral bone marrow niche in the tibia (Extended Data Fig. 4a–d), suggesting that the phenotype observed was through direct CSF-to-skull-bone-marrow signaling.

Beyond CNS injury, we hypothesized that direct CSF access to skull bone marrow could play an important role in CNS pathogen sensing. Indeed, injection of lipopolysaccharide (LPS), an outer cell membrane component of Gram-negative bacteria, into the CSF of mice resulted in expansion of skull bone marrow HSCs and myeloid progenitors and a concomitant increase in dural monocytes and neutrophils (Extended Data Fig. 6a–e).

Understanding of the mechanisms regulating meningeal immune supply is evolving. In this study, we describe a previously unrecognized form of neuroimmune communication between the CNS and its surrounding immune reservoirs. We show that CSF accesses skull bone marrow niches and mobilizes HSCs and myeloid cells after CNS injury or infection (Extended Data Fig. 7). Additionally, we show that skull bone marrow populations have a unique transcriptional identity compared to non-CNS-associated bone marrow, suggesting that CSF-derived factors might instruct the phenotype of skull bone marrow populations. Indeed, access of brain-derived antigens along with CSF to skull bone marrow may underlie central tolerance of B cells educated in this niche.

Understanding how changes in CSF composition affect local immune supply from bone marrow niches will shed light on pathogenic mechanisms contributing to neurodevelopmental disorders, neurodegeneration, autoimmunity and CNS cancers.

**Online content**

Any methods, additional references, Nature Research reporting summaries, source data, extended data, supplementary information, acknowledgements, peer review information; details of author contributions and competing interests; and statements of data and code availability are available at https://doi.org/10.1038/s41593-022-01029-1.

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Methods

Mice. Mice were housed under pathogen-free and temperature- and humidity-controlled conditions with a 12-hour light cycle. Mice were housed no more than five animals to a cage, with rodent chow and water provided ad libitum. In all experiments, male mice were used. Adult mice (8–12 weeks old) used in this study were C57BL/6J mice purchased from Jackson Laboratory (WT; IAX000664). Mice at different developmental stages (postnatal day 7 (P7), P14 and P21) were obtained from the National Institutes of Aging. All experiments were performed under the approval of the Institutional Animal Care and Use Committee at Washington University in St. Louis (200–043).

Tracer injection and CSF collection. Mice were anesthetized via intraperitoneal injection of ketamine (100 mg kg\(^{-1}\)) and xylazine (10 mg kg\(^{-1}\)) in saline and placed on a stereotactic frame. The fur over the incision site was clipped, and the skin was disinfected with three alternating washes of alcohol and Betadine. For intracerebral injections, a midline incision was made along the scalp, exposing the dorsal skull. A burr hole was carefully made using a dental drill. A 1:1 ratio of OVA-594 and anti-κ-IgE-Fc (1 μl) was injected using a glass capillary attached to a microinjector (World Precision Instruments) over 2 minutes at the following coordinates: +1.5 A1R, −1.5 M1L, −2.5 D1V. The glass capillary was left in place for another 2 minutes to prevent backflow. For ICM injections, the posterior scalp and neck were shaved and prepared with iodine antiseptic. The head was placed in a stereotactic frame with the neck flexed. A midline incision was made and the posterior nuchal musculature divided, exposing the inferior, dorsal aspect of the occipital bone. The posterior dura overlying the cisterna magna. A glass capillary attached to a microinjector (World Precision Instruments) was used. Volumes of 1,2 and 3 μl were infused in P7, P14 and P21 pups, respectively, whereas 5 μl was infused in adult and aged mice. Injection rates were adjusted to achieve a 5-minute injection, followed by a 5-minute wait period to prevent backflow. For CSF collection, a glass capillary was inserted through the dorsal dura mater into the superficial cisterna magna, and approximately 15 μl of CSF was drawn by capillary action. For CSF transfer experiments, 10 μl of CSF was transferred. For AMD3100 experiments, mice received a 10 μg injection in 5 μl of artificial cerebrospinal fluid (aCSF). For LPS injections, mice received 1.25 μg of LPS from Escherichia coli O111:B4 (Sigma-Aldrich) dissolved in aCSF via intraperitoneal injection. For CSF collection and proteomics, Mouse CSF (4 μl) was added to 16 μl of digestion buffer (100 mM Tris–HCl, pH 8, containing 8 M urea). The samples were reduced with 5 mM DTT and incubated at 37 °C for 1 hour. The reduced protein was alkylated with 10 mM iodoacetamide for 30 minutes at room temperature. The samples were washed three times in PBS-T, and secondary antibodies (Supplementary Table 2) were added for 2 hours at room temperature. Sections were washed in PBS-T, dried, mounted with 4',6-diamidino-2-phenylindole (DAPI, 0.5 μg ml\(^{-1}\), Thermofisher Scientific) for 10 minutes and washed a final time in PBS-T. Sections were co-stained with ProLong Gold AntiFade Mountant (Thermo Fisher Scientific) and glass coverslips. Before imaging, skull–dura whole mounts were cleared using RapiClear 1.52 (SUNjin Lab) for 30 minutes.

Two-photon microscopy of skull bone marrow. Mice were anesthetized via intraperitoneal administration of ketamine/xylazine and placed on a stereotactic frame. After ICM OVA-594 tracer (1 μg ml\(^{-1}\)) injection, calvarial bone marrow exposure was achieved via midline skin incision. To visualize the vasculature, 100 μl of 70 kDa FITC-dextran (5 μg ml\(^{-1}\), Sigma–Aldrich) in saline was infused retro-orbitally immediately before imaging. Image acquisition was performed using a Coherent Chameleon Ultra II tuned to 820 nm. Fluorescence emission was detected using the following filters: 492 SP for second-harmonic generation, 525/50 for intravascular FITC-Dextran and 575/25 for ICM OVA-594 (Thermo Fisher Scientific).

Single-cell isolations. Mice were humanely euthanized with a lethal dose of Euthasol (10% v/v, intraperitoneal), followed by transcardiac perfusion of PBS supplemented with heparin (5 U ml\(^{-1}\)). Skulls were cut from the foramen magnum at the back along the parietal ridge to the olfactory bulbs at the front and divided into rostral, middle, and caudal portions. A single tibia was taken from each mouse. All bones were cleaned by removal of attached soft tissues, and the dura was peeled from the skulls. Bone marrow suspensions were obtained as described previously, with the skull undergoing mechanical dissociation by chopping with scissors and crushing with a pestle in PBS, and tibia bone marrow was obtained by flushing. All bone marrow cells were passed through a 70 μm cell strainer before centrifugation. Strained marrow samples were pelleted (450g for 5 minutes) and resuspended in ACK lysis buffer (Quality Biological) for 5 minutes. Samples were pelleted (450g for 5 minutes) and resuspended in fluorescence-activated cell sorting (FACS) buffer (2% BSA and 1 mM ethylenediamine tetracetic acid (EDTA)). Dural meninges were peeled from the inner aspect of the skullcap and enzymatically dissociated in pre-warmed buffer containing RPMI (Gibco) with collagenase VIII (1 mg ml\(^{-1}\), Sigma–Aldrich), DNase I (0.5 mg ml\(^{-1}\), Sigma–Aldrich) and FBS (2% v/v, Gibco). Meningeal samples were incubated at 37 °C for 20 minutes with five triturations with a P1000 pipette at 10 minutes. Samples were then resuspended and passed through a 70 μm cell strainer. The digestion process was neutralized by the addition of an equal volume of DEME with 10% FBS (v/v). Dermal meninges were pelleted (450g, 5 minutes) and resuspended in FACS buffer before staining.

Flow cytometry. Cell suspensions were prepared as described above and transferred into a 5-bottom plate. Viability staining was performed using Zombie Ned (1:5000 in PBS, 10 minutes) at 4 °C. Dead cells were stained with Calcein AM (1 μM, 30 minutes) and analyzed with FlowJo (version 10, BD Biosciences). Ki-67 staining was performed using an Aurora spectral flow cytometer (Cytek Biosciences), and data were analyzed with Flowjo (version 10, BD Biosciences).

EdU and Ki-67 staining for proliferation analysis. EdU staining was performed on selected flow cytometry samples with the Click-IT Plus EdU Alexa Fluor 647 Flow Cytometry Assay Kit (Thermo Fisher Scientific). Mice received intraperitoneal injection of 10 μg kg\(^{-1}\) EdU 4 or 7 hours before sacrifice. After single-cell isolations described above, cell surface antibody staining, fixation and permeabilization, EdU staining was performed according to the manufacturer’s instructions. Ki-67 staining was performed after EdU staining.

CSF collection and proteomics. Mouse CSF (4 μl) was added to 16 μl of digestion buffer (100 mM Tris–HCl, pH 8, containing 8 M urea). The samples were reduced with 5 mM DTT and incubation at 37 °C for 1 hour. The reduced protein was alkylated with 10 mM iodoacetamide for 30 minutes at room temperature in the dark. The urea concentration was diluted to 2 M urea by the addition of 50 mM Tris, pH 8. The proteins were digested with LysC (1 μg/μl) for 2 hours at room temperature, followed by digestion with trypsin (1 μg/μl) overnight. The filter units were then centrifuged at 14,000g for 15 minutes to collect the peptides in the flow-through. The filters were washed with 50 μl of 100 mM ammonium bicarbonate buffer, and the wash was collected with the peptides. The peptides were acidified with trifluoroacetic acid (TFA) (1% final concentration) and desalted using a two-micro-tip (porous graphite carbon, BIOMETNT3FCAR) (GlyGen) on a Beckman rotor (Biomex NX)\(^{30}\). The peptides were eluted with 60% MeCN in 0.1% TFA and dried in a Speed-Vac (Thermo Fisher Scientific, Savant DNA 120 concentrator), after adding TFA to 5%. The peptides were dissolved in 10 μl 1% MeCN in water. An aliquot (10%) was removed for quantification using the Pierce Quantitative Fluorometric Peptide Assay Kit (Thermo Fisher Scientific, 23290). The remaining peptides were transferred to autosampler vials (SUN-5RL, 200046), dried and stored at −80 °C for liquid chromatography–mass spectrometry (LC–MS) analysis. LC–MS analysis and identification and quantification of proteins were performed as described previously\(^{31}\).

Bone marrow FACS and scRNA-seq. Tibias and skulls were collected from five wild-type, 8-week-old male C57BL/6J mice, and the surrounding flesh was removed. For skulls, the dura was peeled and removed with fine forceps. Both the tibia and skull were then cut into small pieces using sterile scissors and mechanically dissociated in FACS buffer with a pestle, followed by a filtration step through a 70 μm cell strainer. Samples were centrifuged for 5 minutes at 420g, and red blood cell lysis was performed with ACK lysis buffer. Samples were washed in FACS buffer, stained with DAPI (0.2 μg ml\(^{-1}\)), and viable (DAPI−, 7AAD−) single cells were sorted on a FACSARia II (BD Biosciences) into 1% BSA-coated 1.5 ml Eppendorf tubes with 500 μl of DEME. Cells were then centrifuged at 1500g for 5 minutes.
Mice were anesthetized using ketamine (100 mg kg⁻¹) and xylazine (10 mg kg⁻¹). The skin over the upper thoracic area was shaved and cleaned with alternating Betadine solution and alcohol swabs. A midline incision was made and the paraspinal musculature divided, exposing the dorsal aspect of the spinal column. Hemostasis was achieved. A laminectomy was performed at T7 using a high-speed drill (Friedman-Pearson Rongeurs, 16221-14). At this juncture, control mice underwent closure of the muscles and skin in layers. Spinal cord injury cohort mice underwent spinal cord crush with fine forceps and then muscle and skin closure. Ketoprofen (2–5 mg kg⁻¹) was subcutaneously injected for postoperative analgesia, and mice were euthanized after 3 hours.

Statistics and reproducibility. Statistical methods were not used to recalculate or pre-determine study sizes but were based on similar experiments previously published[14]. Experiments were blinded, where possible, for at least one of the independent experiments. No data were excluded for analysis. For all experiments, animals from different cages were randomly assigned to different experimental groups. All experiments were replicated in at least two independent experiments of at least five mice per group, and all replication was successful. For all representative images shown, images are representative of at least three independent experiments. Statistical tests for each experiment are provided in the respective figure legends. Data distribution was assumed to be normal, but this was not formally tested. In all cases, measurements were taken from distinct samples. Statistical analysis was performed using Prism (version 8.0, GraphPad Software).

Ligand–receptor interaction network analysis. The list of proteins identified in the CSF-MW/MS (Supplementary Table 1) was converted to coding genes with the use of biomaRT using the UniProt ID and Ensembl gene name as conversion factors. This list was then filtered to include only genes contained in the list of ligands in the annotated reference provided by RNAMagnet[21] with the function getLigandsReceptors with the cellularCompartment parameter set to ‘secreted’, ‘ECM’ or ‘both’ and the version set to 3.0.0. The receptors matching those ligands were mined from the reference, and their expression was plotted for cell types of interest as average normalized mRNA transcripts per population with the circlize package in R.

Spinal cord injury. Mice were anesthetized using ketamine (100 mg kg⁻¹) and xylazine (10 mg kg⁻¹). The skin over the upper thoracic area was shaved and cleaned with alternating Betadine solution and alcohol swabs. A midline incision was made and the paraspinal musculature divided, exposing the dorsal aspect of the spinal column. Hemostasis was achieved. A laminectomy was performed at T7 using a high-speed drill (Friedman-Pearson Rongeurs, 16221-14). At this juncture, control mice underwent closure of the muscles and skin in layers. Spinal cord injury cohort mice underwent spinal cord crush with fine forceps and then muscle and skin closure. Ketoprofen (2–5 mg kg⁻¹) was subcutaneously injected for postoperative analgesia, and mice were euthanized after 3 hours.

Statistics and reproducibility. Statistical methods were not used to recalculate or pre-determine study sizes but were based on similar experiments previously published[14]. Experiments were blinded, where possible, for at least one of the independent experiments. No data were excluded for analysis. For all experiments, animals from different cages were randomly assigned to different experimental groups. All experiments were replicated in at least two independent experiments of at least five mice per group, and all replication was successful. For all representative images shown, images are representative of at least three independent experiments. Statistical tests for each experiment are provided in the respective figure legends. Data distribution was assumed to be normal, but this was not formally tested. In all cases, measurements were taken from distinct samples. Statistical analysis was performed using Prism (version 8.0, GraphPad Software).

Reporting Summary. Further information on research design is available in the Nature Research Reporting Summary linked to this article.
Extended Data Fig. 1 | Characterization of stem and immune cell populations in the basal skull marrow. **a**, Flow cytometry gating strategy for major immune populations in the skull bone marrow. **b**, Absolute numbers of CD45+ cells in the dorsal and basal skull marrow. n = 3 mice. Mean ± SEM. **c**, Relative frequencies of immune populations in the dorsal skull, basal skull, and tibial bone marrow. n = 3 mice. Mean ± SEM.
Extended Data Fig. 2 | Characterization of differences between the skull and tibial marrow populations. **a**, Dot plot demonstrating scaled gene expression and percentage of cells expressing genes for cluster phenotyping markers for bone marrow cell types from scRNA-seq analysis. **b**, Analysis of cluster proportions in skull and tibial bone marrow. **c-f**, Volcano plots of differentially expressed genes in neutrophils, monocytes, macrophages, and HSCs. Magenta dots represent upregulated transcripts, while cyan dots represent downregulated transcripts in skull populations compared to the tibia. y-axes represent adjusted log2 p value for cluster changes between skull and tibia. Dotted line represents an adjusted p value of 0.05 (general linear mixed model with Benjamini-Hochberg correction). **g-j**, Top 10 downregulated gene ontology pathways in skull vs. tibia for differentially expressed genes in neutrophils, monocytes, macrophages, and HSCs. **k**, Dot plot of receptor expression in skull bone marrow cells, scaled by gene expression and percentage of cells expressing the gene, showing expression of receptors for which there is a cognate CSF ligand.
**Extended Data Fig. 3 | Effects of AMD3100 on immune cell composition of the dura and bone marrow.**

**a,** Experimental design for injections for skull bone marrow egress experiments. AMD3100 (10 μg) or artificial cerebrospinal fluid (aCSF) was injected intra-cisterna magna (i.c.m.), and mice were left for 24 hours. The following day, tissues were processed for immunolabeling or flow cytometry. **b,** Representative images of Ly6b<sup>+</sup> cells and CD3<sup>+</sup> cells in non-sinus regions of the dura. Scale bar: 200 μm. **c, d,** Regional analysis of Ly6b<sup>+</sup> myeloid and CD3<sup>+</sup> cells in the dura following AMD3100 administration. *n* = 3 mice per group. Data are means ± SEM, *p* values represent two-way ANOVA with Sidak’s post hoc test. **e,** Flow cytometry gating strategy for neutrophils, Ly6C<sup>+</sup> monocytes, macrophages, and T cells in the bone marrow following AMD3100 administration. **f, g,** Relative numbers of neutrophils, Ly6C<sup>+</sup> monocytes, macrophages, and T cells in the skull and tibial bone marrow 24 hours following i.c.m. AMD3100 administration. *n* = 5 mice per group. Data are means ± SEM, *p* values represent a two-sided Student’s t test.
Extended Data Fig. 4 | Laminectomy does not affect CSF efflux to skull bone marrow. 

**a**, Laminectomy, or sham surgery, was performed on mice and 3 hours later OVA-488 was injected into the cisterna magna. Tissues were collected 1 hour later for flow cytometry. Representative flow plots of macrophages in skull and tibia bone marrow with either sham surgery or laminectomy. **b**, Quantification of i.c.m. injected OVA uptake in macrophages following sham surgery or laminectomy. *n* = 5 mice per group. Data are means ± SEM, *p* values represent a two-way ANOVA. **c**, Representative flow plots of i.c.m. anti-c-Kit-PE staining in LSKs in skull and tibia bone marrow with either sham surgery or laminectomy. **d**, Quantification of i.c.m. injected cKit-PE uptake in LSKs following sham surgery or laminectomy. *n* = 5 mice per group. Data are means ± SEM, *p* values represent a two-way ANOVA with Sidak’s post hoc test.
Extended Data Fig. 5 | Effects of spinal cord injury on vertebral bone marrow. a, Experimental paradigm for spinal cord injury experiments. Spinal cord injury (SCI) or laminectomy (sham) was performed, and at 3 hours post-injury vertebra adjacent to the site of injury were processed for flow cytometry. b-e, Relative numbers of monocyte dendritic precursors (MDPs), common monocyte progenitors (cMoPs), Ly6Chi monocytes, and actively proliferating (Ki-67+, EdU+) monocytes in vertebral bone marrow. n = 5 mice per group. p values represent a two-sided Student’s t test. f, Multiplexed measurement of cytokines and chemokines in the CSF of sham and SCI mice using Luminex. n = 5. p values represent two-sided t tests with Holm-Sidak’s multiplicity adjustment. Data are means ± SEM.
Extended Data Fig. 6 | Intracisternal injection of LPS enhances hematopoiesis in skull bone marrow and triggers myeloid egress to the dura. a, LPS (1.25 µg, 4 µL) was injected into the skull bone marrow. After 24 hours, skullcaps and dura were processed for flow cytometry. Representative flow plots of neutrophils in the dura in aCSF and LPS-treated mice. b, Representative flow plots of Ly6C<sup>hi</sup> monocytes in the dura in aCSF and LPS-treated mice. c, Representative flow plots of LSKs in the skull BM of aCSF and LPS-treated mice. d, Quantification of the proportion of CD45<sup>+</sup> immune cells and absolute number of neutrophils and Ly6C<sup>hi</sup> monocytes in the dura of aCSF and LPS-treated mice. n = 5 mice per group. Mean ± SEM. p values represent a two-sided Student’s t test. e, Quantification of the proportion of live cells and the proportion of actively proliferating Ki67<sup>+</sup> stem/progenitor (LSK, MDP, cMoP, GMP, GP) and myeloid (neutrophils, Ly6C<sup>hi</sup> monocytes) cells in the skull bone marrow of aCSF and LPS-treated mice. n = 5 mice per group. Mean ± SEM. p values represent a two-sided Student’s t test.
**Extended Data Fig. 7 | Summary schematic for proposed mechanism.** Brain interstitial fluid and cerebrospinal fluid can efflux to skull bone marrow during healthy conditions. During CNS insults—for example pathogenic infections or spinal cord injury—CSF-derived cues can promote skull bone marrow hematopoiesis and egress of myeloid cells to underlying dura. HSC; hematopoietic stem cell, CSF; cerebrospinal fluid, ISF; interstitial fluid, SAS; subarachnoid space, BM; bone marrow, CNS, central nervous system.
Reporting Summary

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Statistics

For all statistical analyses, confirm that the following items are present in the figure legend, table legend, main text, or Methods section.

- The exact sample size (n) for each experimental group/condition, given as a discrete number and unit of measurement
- A statement on whether measurements were taken from distinct samples or whether the same sample was measured repeatedly
- The statistical test(s) used AND whether they are one- or two-sided
  - Only common tests should be described solely by name; describe more complex techniques in the Methods section.
- A description of all covariates tested
- A description of any assumptions or corrections, such as tests of normality and adjustment for multiple comparisons
- A full description of the statistical parameters including central tendency (e.g. means) or other basic estimates (e.g. regression coefficient) AND variation (e.g. standard deviation) or associated estimates of uncertainty (e.g. confidence intervals)
- 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

Our web collection on statistics for biologists contains articles on many of the points above.

Software and code

Policy information about availability of computer code

Data collection

The following softwares were used to collect the data in this study:
- SpectroFlo v 2.2.0.3 (Cytek)
- Olympus VS200 AWS
- Leica Application Suite v 4.2.1.23810

Data analysis

The following softwares were used to analyze the data in this study:
- FIJI image processing software (NIH) - v2.0.0-rc-59/1.51n
- Prism v8.3.0 (GraphPad Software, Inc)
- FastQC v0.11.5
- R v3.5.0
- Bioconductor DESeq2 v3.5
- FlowJo software v10 (BD Biosciences)
- Custom code used for single-cell RNA-seq analysis is available from the corresponding authors upon request.

For manuscripts utilizing custom algorithms or software that are central to the research but not yet described in published literature, software must be made available to editors and reviewers. We strongly encourage code deposition in a community repository (e.g. GitHub). See the Nature Portfolio guidelines for submitting code & software for further information.
Data

Policy information about availability of data
All manuscripts must include a data availability statement. This statement should provide the following information, where applicable:

- Accession codes, unique identifiers, or web links for publicly available datasets
- A description of any restrictions on data availability
- For clinical datasets or third party data, please ensure that the statement adheres to our policy

scRNA-seq data are available from Gene Expression Omnibus under accession number GSE184766. All data are available in the main text or the supplementary information files.

Field-specific reporting

Please select the one below that is the best fit for your research. If you are not sure, read the appropriate sections before making your selection.

☒ Life sciences  ☐ Behavioural & social sciences  ☐ Ecological, evolutionary & environmental sciences

For a reference copy of the document with all sections, see nature.com/documents/nr-reporting-summary-flat.pdf

Life sciences study design

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

Sample size

Statistical methods were not used to recalculate or predetermine study sizes but were based on similar experiments previously published (Cugurra et al., Science (2021); Rustenhoven et al., Cell (2021); Da Mesquita et al., Nature (2018)).

Data exclusions

No data were excluded for analysis.

Replication

All representative images, are representative of the same experiment performed in at least 3 animals. All experiments were replicated in at least two independent experiments of at least 5 mice per group, and all replication was successful.

Randomization

For all experiments, animals from different cages were randomly assigned to different experimental groups. Because all variables were controlled for, no covariates were present.

Blinding

Experiments were blinded, where possible, for at least one of the independent experiments. Blinded experiments included tracer studies, injections of compounds (AMD3100 and LPS) into the CSF. Because spinal cord injuries cause paralysis, and because age influences the size of the skull, it was not possible for researchers to be blinded for these studies.

Reporting for specific materials, systems and methods

We require information from authors about some types of materials, experimental systems and methods used in many studies. Here, indicate whether each material, system or method listed is relevant to your study. If you are not sure if a list item applies to your research, read the appropriate section before selecting a response.

Materials & experimental systems

n/a
☒ Involved in the study
☐ Antibodies
☒ Eukaryotic cell lines
☒ Palaeontology and archaeology
☒ Animals and other organisms
☒ Human research participants
☒ Clinical data
☒ Dual use research of concern

Methods

n/a
☐ Involved in the study
☒ ChIP-seq
☒ Flow cytometry
☒ MRI-based neuroimaging

Antibodies

Antibodies used
Primary antibodies used for IHC:
- Anti-Pecam-1 A hamster - clone 2H8 MAB13982 Millipore Sigma (1:200)
- Anti-Ly-6B.2 Rat - 7-Apr ab53457 Abcam (1:200)
- Anti-CD3 Rat - 17A2 58-0032-82 Invitrogen (1:200)

Secondary antibodies used for IHC:
- Anti-A.Hamster IgG Goat Alexa Fluor 594 Polyclonal 127-585-160 Jackson ImmunoResearch (1:200)
Antibodies used for flow cytometry:
- Anti-Rat IgG Chicken Alexa Fluor 647 Polyclonal A21472 Thermo Fisher Scientific (1:200)
- Anti-Ly6C Rat Alexa Fluor 700 HK1.4 128024 Biolegend (1:200)
- Anti-Ly6C Rat BV510 HK1.4 128033 Biolegend (1:200)
- Anti-Ly6G Rat PerCP Cy5.5 HK1.4 128011 Biolegend (1:200)
- Anti-Sca1 Rat Alexa Fluor 647 E13-161.7 108118 Biolegend (1:200)
- Anti-CD150 Rat BV650 TC15-12F12.2 115931 Biolegend (1:200)
- Anti-CD48 A Hamster PerCP Cy5.5 10356107 BD Bioscience (1:200)
- Anti-CD45 Rat BV750 30-F11 746947 BD Bioscience (1:200)
- Anti-TCR-b A Hamster BUV805 H57-597 748405 BD Bioscience (1:200)
- Anti-TCR-b A Hamster PerCP Cy5.5 H57-597 109228 Biolegend (1:200)
- Anti-CD135 Rat PE CY5 A2F10 135312 Biolegend (1:200)
- Anti-CD64 Mouse APC X54-5/7.1 139306 Biolegend (1:200)
- Anti-F4/80 Rat AF700 BM8 123130 Biolegend (1:200)
- Anti-Lin - Pac blue - 133310 Biolegend (1:40)
- Anti-CD11b Rat PE-Cy7 M1/70 552850 Biolegend (1:200)
- Anti-CD4 Rat Alexa Fluor 647 RM4-5 100533 Biolegend (1:200)
- Anti-B220 Rat PE RA3-6B2 553090 BD Bioscience (1:200)
- Anti-CD8a Rat FITC 53-6.7 11-0081-82 Fisher Scientific (1:200)
- Anti-CD19 Rat BV480 1D3 566107 BD Bioscience (1:200)
- Anti-CD3e Rat AF532 17A2 58-0032-82 eBioscience (1:200)
- Anti-NK1.1 Mouse PE CY7 PK136 108724 Biolegend (1:200)
- Anti-CD11c A Hamster BUV737 N418 749039 BD Bioscience (1:200)
- Anti-CD115 Rat BUV737 AFS98 750948 BD Bioscience (1:200)
- Anti-CD11b Rat PerCP-Cy5.5 M1/70 101228 Biolegend (1:200)
- Anti-Ki67 Rat BV421 16A8 652411 Biolegend (1:200)
- Anti-CD115 Rat BUV737 AFS98 750948 BD Bioscience (1:200)
- Anti-B220 Rat PE RA3-6B2 553090 BD Bioscience (1:200)
- Anti-CD11b Rat BV750 30-F11 746947 BD Bioscience (1:200)
- Anti-Sca1 Rat BV510 1A8 108129 Biolegend (1:200)
- Anti-CD45 Rat BV750 30-F11 746947 BD Bioscience (1:200)
- Anti-F4/80 Rat AF700 BM8 123130 Biolegend (1:200)
- Anti-CD11b Rat BV480 103 566107 BD Bioscience (1:200)
- Anti-CD19 Rat BV480 103 566107 BD Bioscience (1:200)
- Anti-CD48 A Hamster BV805 H57-597 748405 BD Bioscience (1:200)
- Anti-CD135 Rat PE CY5 A2F10 135312 Biolegend (1:200)
- Anti-CD64 Mouse APC X54-5/7.1 139306 Biolegend (1:200)
- Anti-CD11b Rat PerCP-Cy5.5 M1/70 101228 Biolegend (1:200)
- Anti-CD164 Mouse BV711 X54-5/7.1 139306 Biolegend (1:200)
- Anti-CD135 Rat PE CY5 A2F10 135312 Biolegend (1:200)
- Anti-CD11c A Hamster BUV737 N418 749039 BD Bioscience (1:200)
- Anti-CD48 A Hamster BV805 H57-597 748405 BD Bioscience (1:200)
- Anti-CD11b Rat PerCP-Cy5.5 M1/70 101228 Biolegend (1:200)
- Anti-CD64 Mouse BV711 X54-5/7.1 139306 Biolegend (1:200)
- Anti-CD8a Rat FITC 53-6.7 11-0081-82 Fisher Scientific (1:200)
- Anti-CD4 Rat Alexa Fluor 647 RM4-5 100533 Biolegend (1:200)
- Anti-CD3e Rat AF532 17A2 58-0032-82 eBioscience (1:200)
- Anti-NK1.1 Mouse PE CY7 PK136 108724 Biolegend (1:200)
- Anti-CD11c A Hamster BUV737 N418 749039 BD Bioscience (1:200)
- Anti-CD115 Rat BUV737 AFS98 750948 BD Bioscience (1:200)
- Anti-CD11b Rat BV510 1A8 108129 Biolegend (1:200)
- Anti-CD45 Rat BV750 30-F11 746947 BD Bioscience (1:200)
- Anti-CD19 Rat BV480 103 566107 BD Bioscience (1:200)
- Anti-TCR-b A Hamster BV805 H57-597 748405 BD Bioscience (1:200)
- Anti-CD11b Rat BV510 1A8 108129 Biolegend (1:200)
- Anti-CD48 A Hamster BV805 H57-597 748405 BD Bioscience (1:200)
- Anti-CD135 Rat PE CY5 A2F10 135312 Biolegend (1:200)
- Anti-CD64 Mouse APC X54-5/7.1 139306 Biolegend (1:200)
- Anti-CD11b Rat PerCP-Cy5.5 M1/70 101228 Biolegend (1:200)
- Anti-CD64 Mouse BV711 X54-5/7.1 139306 Biolegend (1:200)
- Anti-CD8a Rat FITC 53-6.7 11-0081-82 Fisher Scientific (1:200)
- Anti-CD4 Rat Alexa Fluor 647 RM4-5 100533 Biolegend (1:200)
- Anti-CD3e Rat AF532 17A2 58-0032-82 eBioscience (1:200)
- Anti-NK1.1 Mouse PE CY7 PK136 108724 Biolegend (1:200)

Validation
Each antibody was validated for the species (mouse or human) and application (immunohistochemistry, flow cytometry) by the correspondent manufacturer. The usage was described in full detail the methods section of the manuscript.

Animals and other organisms

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

Laboratory animals
Mice were housed under pathogen-free, temperature (22 ± 1 °C) and humidity-controlled conditions (47 ± 5 %) with a 12-hour light cycle. Mice were housed no more than 5 animals to a cage with rodent chow and water provided ad libitum. In all experiments male mice were used. Adult mice (8-12 weeks old) used in this study were C57BL/6J purchased from Jackson Laboratory (WT; JAX000664). Mice at different developmental stages (postnatal day 7 (P7), P14, and P21) were obtained from colonies established in-house. Aged mice (20-24 months) were obtained from the National Institutes of Aging.

Wild animals
This study did not involve wild animals.

Field-collected samples
This study did not involve field-collected samples.

Ethics oversight
All experiments were performed under the approval of the Institutional Animal Care and Use Committee at Washington University in St. Louis (#200-043). Note that full information on the approval of the study protocol must also be provided in the manuscript.

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
Mice were humanely euthanized with a lethal dose of Euthasol (10 % v/v; i.p.), followed by transcardiac perfusion of phosphate-buffered saline (PBS) supplemented with heparin (5 U/mL). Skulls were cut from the foramen magnum at the back along the parietal ridge to the olfactory bulbs at the front, and divided into dorsal and basal portions. A single tibia was taken from each mouse. All bones were cleaned by removal of attached soft tissues and the dura was peeled from the skulls. Bone
marrow suspensions were obtained as described previously (Cugurra), with the skull undergoing mechanical dissociation by chopping with scissors and crushing with a pestle in PBS and tibial bone marrow was obtained by flushing. All bone marrow cells were passed through a 70 μM cell strainer prior to centrifugation. Strained marrow samples were pelleted (450 x g for 5 min) and resuspended in ACK lysis buffer (Quality Biological) for 5 min. Samples were pelleted (450 x g for 5 min) and resuspended in fluorescence activated cell sorting (FACS) buffer (2 % bovine serum albumin (BSA); company, 1 mM ethylenediamine tetraacetic acid (EDTA). Dural meninges were peeled from the inner aspect of the skullcap and enzymatically dissociated in pre-warmed buffer containing RPMI (Gibco) with collagenase VIII (1 mg/ml; Sigma-Aldrich, MO, USA), of DNase I (0.5 mg/ml; Sigma-Aldrich), and FBS (2 % v/v; Gibco). Meningeal samples were incubated at 37 °C for 20 min, with five triturations with a P1000 pipette at 10 min. Samples were then resuspended and passed through a 70 μM cell strainer. The digestion process was neutralized by addition of an equal volume of DMEM with 10 % FBS (v/v). Dural suspensions were pelleted (450 x g, 5 min) and resuspended in FACS buffer before staining. Cell suspensions were prepared as described above and transferred into a V-bottom plate. Viability staining was performed using Zombie NIR (1:500 in PBS, 10 min, room temperature; Biolegend, CA, USA). Suspensions were then pelleted (450 x g for 5 min), and resuspended in anti-CD16/32 antibody (1:100; Biolegend) diluted in FACS buffer to block Fc-receptor binding. Antibodies against cell surface epitopes were then added for 10 min at room temperature.

Instrument
Flow cytometry was performed using an Aurora spectral flow cytometer (Cytek Biosciences, CA, USA).

Software
Data were collected on SpectroFlo (v2.2.0.3; Cytek) and analyzed with FlowJo (v10; BD Biosciences, NJ, USA).

Cell population abundance
For each individual experiment, single-cell suspensions were incubated with viability dyes. Positive populations were gated based on negative control staining. In general, populations are given as a percentage of live, CD45+ cells.

Gating strategy
Gating strategies are described in figures.

Briefly:
- Dural Ly6Chi monocytes: Live/CD45+/CD11b+/Ly6G-/F4-80-/CD64-/Ly6C+
- Dural neutrophils: Live/CD45+/CD11b+/Ly6G+
- Dural B cells: Live/CD45+/CD11b-/Ly6G-/CD19+/TCR-b-
- Dural T cells: Live/CD45+/CD11b-/Ly6G-/CD19-/TCR-b-
- Dural macrophages: Live/CD45+/CD11b+/Ly6G-/F4-80+/CD64+
- Bone marrow Ly6Chi monocytes: Live/CD45+/CD11b+/Ly6G-/F4-80-/CD64-/CD115+/Ly6C+
- Bone marrow neutrophils: Live/CD45+/CD11b+/Ly6G+
- Bone marrow B cells: Live/CD45+/CD11b-/Ly6G-/CD19+/TCR-b-
- Bone marrow T cells: Live/CD45+/CD11b-/Ly6G-/CD19-/TCR-b+
- Bone marrow hematopoietic stem cells: Live/Lin-/Sca1+/cKit+
- Bone marrow macrophages: Live/CD45+/CD11b+/Ly6G-/F4-80+/CD64+
- Bone marrow MDPs: Live/CD19-/TCR-b-/Ly6G-/CD117+/CD135+/CD11b-/Ly6C-
- Bone marrow cMoPs: Live/CD19-/TCR-b-/Ly6G-/CD117+/CD135-/CD11b-/Ly6C+

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