## Juvenile blood vessels in the skull: implications for hematopoiesis in the cranial bone marrow

**This project aims to unravel why blood vessels in the cranium do not signs of aging like their counterparts in the periphery of the body do and what this means for hematopoiesis in the cranial bone marrow (CBM). In particular, the role of the *Egfl7* gene will be assessed in this context, which we have previously shown to govern blood vessels, stem cells and the immune system.**

The CBM forms a specialized hematopoietic niche close to the brain and harbors a unique vasculature within the body (Cugurra et al., 2021). Blood vessels in the CBM show a remarkable capacity for lifelong growth in the absence of age-dependent decline and support lifelong hematopoiesis in the CBM without typical signs of aging. Unlike other bone marrow sites, the aged CBM displays sustained hematopoiesis, reduced fat accumulation and does not exhibit a myeloid bias upon differentiation of hematopoietic stem cells (HSCs) into immune cells (Koh et al., 2024). These features highlight the CBM as a crucial, distinct hub coupling vascular development, hematopoiesis, and CNS homeostasis.

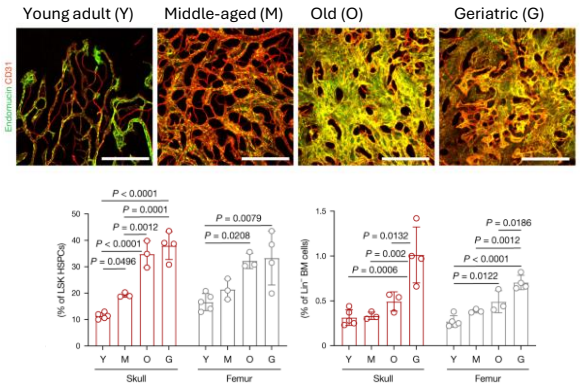


**Figure 1: Age-related expansion of vessels & HSCs/HPCs in CBM** (Upper panel) Immunofluorescence staining of blood vessels in the skull showing vascular expansion and changes in vessel morphology. (Lower panel) Comparison of HSCs/HPCs in young adult, middle-aged, old and geriatric skulls and femurs by FACS (Koh et al., 2024)

Previously, it has been shown that HSCs and hematopoietic progenitor cells (HPCs) in the CBM are governed by factors derived of endothelial cells and other local tissues and oxygen gradients (Itkin et al., 2016). Further, the CBM is connected to meningeal lymphatic vessels in the dura by specialized vascular transosseus skull channels, which allow for the trafficking of molecules and immune cells via the cerebrospinal fluid (CSF), allowing meninges to govern HSCs and HPCs (Herisson et al., 2018; Mazzitelli et al., 2022). Even pathological conditions, such as stroke, affect hematopoiesis in the CBM (Koh et al., 2024). However, the molecular mechanisms behind these observations remain barely understood.

Most likely, *Egfl7* is a central player in these biological processes (Fabian et al., 2025). This gene is transcribed by endothelial cells and encodes for the pro-angiogenic molecules EGFL7 and miR-126 (Parker et al., 2004; Wang et al., 2008). Previously, we have shown that EGFL7 is deposited in the extracellular matrix of endothelial cells, where it promotes blood vessel formation by virtue of binding to integrin $\alpha_V\beta_3$ (Nikolic et al., 2013). Likewize, EGFL7 is expressed in brain lymphatic endothelial cells in zebrafish where it binds to very same integrin (Chen et al., 2024). In malignant brain tumors, EGFL7 drives pathophysiological angiogenesis via integrin $\alpha_5\beta_1$ (Dudvarski Stankovic et al., 2018) and promotes immune evasion via the integrins $\alpha_D\beta_2$ and $\alpha_L\beta_2$ (Mahajan et al., 2026). Moreover, EGFL7 governs adult neural stem cells (Barth et al., 2023; Bicker et al., 2017; Schmidt et al., 2009), offering the possibility that EGFL7 directly regulates HSCs.

By using different tissue-specific *Egfl7* knock-out and transgenic mouse models we will investigate the role of the *Egfl7* gene for blood vessel formation in the calvaria, for lymph vessel formation in the dura and for the amount of blood vessels in the transosseus skull channels, which both compartments connect, in an age dependent manner. In parallel, we will assess how hematopoiesis gets altered in the CBM of these mice.

**Project outline**

1) Age-dependent influence *Egfl7* exerts on angiogenesis in the CBM and lymphangiogeneis in the dura
   - Quantification of sinusoidal vessels by immunohistology studies on cleared calvaria whole mounts (vascular area, vessel density and vessel morphology) in comparison to blood vessels in femur and tibia of young and old *Egfl7* knock-out and transgenic mice
   - Analysis of blood vessels in transosseus skull channels by immunohistology studies on cleared calvaria whole mounts in young and old *Egfl7* knock-out and transgenic mice
   - Characterization of lymph vessels by immunofluorescence studies in dura whole mounts (vessel morphology, vessel density) in young and old *Egfl7* knock-out and transgenic mice

2) Age-dependent influence of *Egfl7* exerts on hematopoiesis in the CBM
   - Characterization of HSCs, HPCs and mature immune cells in comparison to peripheral bone marrow derived of femur and tibia in young and old *Egfl7* knock-out and transgenic mice by single cell RNA-sequencing and FACS
   - HSC proliferation capacity (self-renewal) *in vivo* and *in vitro* using young and old *Egfl7* knock-out and transgenic mice

3) Omics-studies to identify the molecular pathways causing the phenotypes observed above
   - Bulk, single cell and spatial RNA-sequencing
   - Protein mass spectrometry
   - Immunoblotting, immunofluorescence, cell culture, FACS, molecular biological assays

**Course of Study/Expertise of Candidate**

**Biology, molecular biology, cell biology, biochemistry, microscopy, angiogenesis, neuroscience**

**References**

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