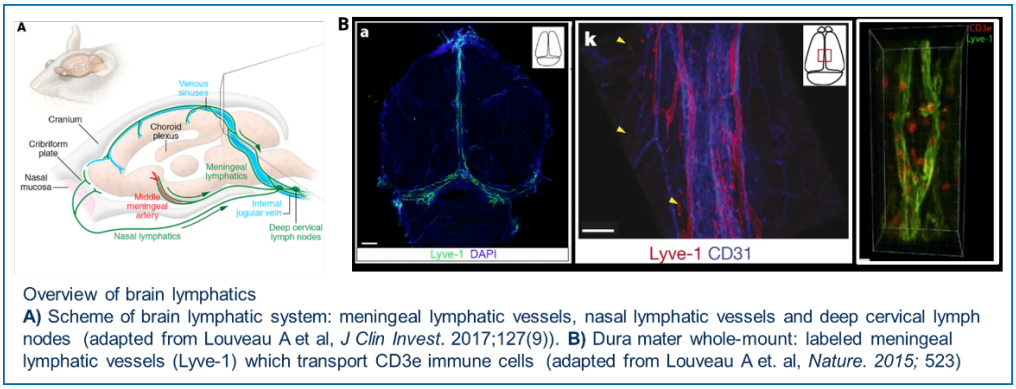
Role of EGFL7 in brain lymphatics and malignant brain tumors

The (re)discovery of the lymphatic system within the CNS is considered to be one of the most astonishing discoveries of the last decade, prompting scientists to challenge the long-held assumption that the brain was an 'immune-privileged' site (Louveau et al., 2015). Indeed, the CNS possesses distinctive anatomical characteristics, including the blood–brain barrier and the absence of conventional lymphatic vessels (LVs) within the parenchyma. However, there is evidence that CNS-derived soluble molecules and lymphocytes circulate throughout the healthy brain. The meningeal lymphatic vessels (MLVs) and nasal lymphatic vessels provide a route for draining macromolecules and trafficking immune cells from the CNS into the deep cervical lymph nodes (CLNs). Consequently, the cerebrospinal fluid (CSF) is collected from the subarachnoid space, while the interstitial fluid (ISF) is gathered from the brain parenchyma via the perivascular space. Nevertheless, further research is required to fully examine and define the location and organization of the brain lymphatic system in the CNS. The brain lymphatics vessels are impaired under pathological conditions: Dysfunction of MLVs has been shown to be an aggravating factor in the pathology of Alzheimer's disease and aging (Ma, Ineichen, Detmar, & Proulx, 2017). Further, it has been reported that MLVs play a role in brain tumors by means of immunomodulation (Hu et al., 2020). They are critical for the drainage of intratumor fluid, macromolecules (antigens), metastasis, and immunity. They provide a route for brain tumor immune cells to enter to the CLNs to generate an immune response against the tumor through the trafficking of dendritic cells from the tumor to the CLNs. Additionally, in context of brain tumors, these lymphatic vessels undergo remodeling compared to their physiological state, characterized by increased lymphangiogenesis. However, little is currently known regarding the modulation of the maintenance and growth of brain lymphatic vessels and the therapeutic potential of the lymphatic drainage system for brain tumors.



Overview of brain lymphatics
A) Scheme of brain lymphatic system: meningeal lymphatic vessels, nasal lymphatic vessels and deep cervical lymph nodes (adapted from Louveau A et al, *J Clin Invest*. 2017;127(9)). **B)** Dura mater whole-mount: labeled meningeal lymphatic vessels (Lyve-1) which transport CD3e immune cells (adapted from Louveau A et. al, *Nature. 2015;* 523)

One promising modulating factor is the protein EGFL7, which is a highly conserved secreted pro-angiogenic factor. Primarily, it is expressed in endothelial cells while blood vessels form under both physiological and pathological conditions, which includes tumor angiogenesis in malignant brain tumors (Dudvarski Stankovic et al., 2018; Nikolic et al., 2013). Despite its critical role in angiogenesis, any involvement of EGFL7 in lymphangiogenesis remains largely uncertain. However, a recent study suggests that EGFL7 is expressed by brain lymphatic endothelial cells in zebrafish which are essential for meningeal angiogenesis (Chen et al., 2024). Furthermore, data suggest that EGFL7 plays a role in modulating the composition of glioma-infiltrating immune cells which points towards a role of EGFL7 in modulating the immune response of the host versus malignant brain tumors. Nevertheless, the mechanisms underlying these effects remain elusive and might very well involve the immune system.

<u>Project outline:</u>

- Unravel the role of EGFL7 in regulating meningeal lymphatic vessels under physiological conditions
  - Localization of EGFL7 in MLVs and nasal LVs
  - Impairment of EGFL7 on lymphangiogenesis: characterize vessel morphology of MLVs and nasal LVs at different time points
  - Impact of EGFL7 on physiological immune cell composition in the CSF and dCLNs (altered immune cell status through EGFL7-/- endothelial cells and EGFL7-/- lymphatic endothelial cells due to modulatory molecules)
- Influence of EGFL7 on pathological lymphangiogenesis in glioblastoma (characterize vessel morphology of MLVs and nasal LVs)
- Impact of EGFL7 on lymphatic drainage in gliomblastoma: immune cell composition and immune cell status in dCLNs, tumor and CSF

Course of Study/Expertise of Candidat: Biology, molecular biology, cell biology, biochemistry, microscopy, neuroscience

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Louveau, A., Smirnov, I., Keyes, T. J., Eccles, J. D., Rouhani, S. J., Peske, J. D., . . . Kipnis, J. (2015). Structural and functional features of central nervous system lymphatic vessels. *Nature, 523*(7560), 337-341. doi:10.1038/nature14432

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Nikolic, I., Stankovic, N. D., Bicker, F., Meister, J., Braun, H., Awwad, K., . . . **Schmidt, M. H. H.** (2013). EGFL7 ligates alphavbeta3 integrin to enhance vessel formation. *Blood, 121*(15), 3041-3050. doi:10.1182/blood-2011-11-394882