

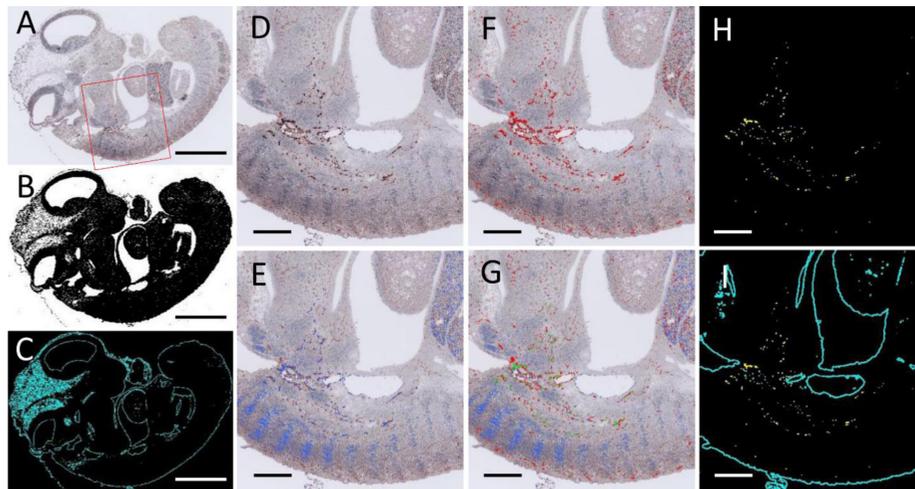
SUPPLEMENTARY MATERIAL

corresponding to:

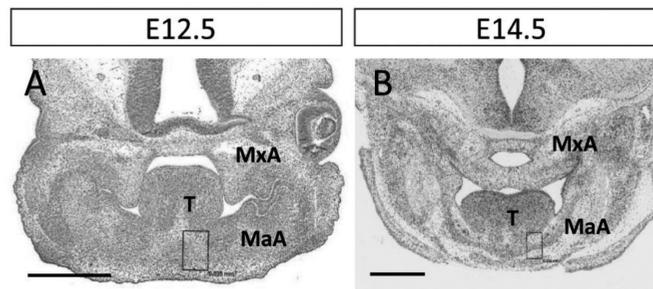
**Migration of lymphatic endothelial cells and lymphatic
vascular development in the craniofacial region
of embryonic mice**

YUJI TAYA*, KAORI SATO, YOUICHI SHIRAKO and YUUICHI SOENO

***Address correspondence to:** Yuji Taya. Department of Pathology, The Nippon Dental University School of Life Dentistry at Tokyo, 1-9-20 Fujimi, Chiyoda-ku, Tokyo 102-8159, Japan. Tel: +81-3-3261-8911. Fax: +81-3-3261-8969. E-mail: taya-yu@tky.ndu.ac.jp - web: <http://www.ndu.ac.jp/~pathhome/index.html>
-  <https://orcid.org/0000-0001-5663-0835>



Supplementary Fig. S1. Procedure for reconstruction of 3D images from serial immunostained sections of mouse embryos. (A) All sagittal sections of whole mouse embryos at E11.5 were dual immunolabeled with Prox1 (red) and Vegfr3 (blue). All tissues of the embryo were painted (B). The outline of embryonic tissues (tissue septum and body surface) is depicted (dark green pseudo color) (C). (D) Higher magnification of the box region in (A). Segmentation figures of Prox1-positive (blue pseudo color) (E) or Vegfr3-positive (red pseudo color) cells (F). (G) Merging of (E) and (F). (H) Segmentation of Vegfr3/Prox1 double-positive cells from (G) (yellow pseudo color). (I) Merging of (H) and the outline of embryonic tissues (dark green pseudo color) for 3D images. Three-dimensional images were reconstructed according to the alignment and stacking of the merged serial images. Scale bars, 1 mm (A–C) and 250 μm (D–I).



Supplementary Fig. S2. Measurement areas for morphometric analysis of lymphatic endothelial cell density. The boxes (0.035 mm^2) in hematoxylin and eosin-stained micrographs in substitution for fluorescent images show the measurement areas under lateral lingual swelling or the tongue for morphometric analysis of LEC density at E12.5 (A) and E14.5 (B). MaA, mandibular arches; MxA, maxillary arches; PS, palatal shelves; T, tongue. Scale bars, 500 μm .

Supplementary video 1. Three-dimensional movie showing a stream of lymphatic endothelial cell (LECs) in a slice of an E10.5 mouse embryo. A 3D movie was reconstructed from the 3D image of the LEC stream based on serial sagittal sections with Prox1 and Vegfr3 dual immunolabeling. Yellow areas indicate Vegfr3/Prox1 double-positive cells. Blue areas indicate the outline of embryonic tissues (including the tissue septum and body surface).

Supplementary video 2. Three-dimensional movie showing a magnified view of a lymphatic endothelial cell stream in Supplementary video 1.

Supplementary video 3. Three-dimensional movie showing a stream of lymphatic endothelial cells (LECs) in a slice of an E11.5 mouse embryo. A 3D movie was reconstructed from the 3D image of a LEC stream based on serial sagittal sections with Prox1 and Vegfr3 dual immunolabeling. Yellow areas indicate Vegfr3/Prox1 double-positive cells. Blue areas indicate the outline of embryonic tissues (including the tissue septum and body surface).

Supplementary video 4. Three-dimensional movie showing a magnified view of an LEC stream in Supplementary video 3.