J Blood Lymph
Volume 13:3, 2023

Journal of Blood and lymph

Research Article

Open Access

TM4SF1 is Essential for Embryonic Blood Vessel Development

Chi Iou Lin[1^], Anne Merley[1,2^], Hiromi Wada[1,3], Jianwei Zheng[1,4] and Shou Ching S. Jaminet[1,5*]

[1]*Department of Pathology, Beth Israel Deaconess Medical Center and Harvard Medical School, Boston, MA, United States of America*

[2]*Department of Center for Animal Resources and Education, Brown University, Providence, RI, United States of America*

[3]*Department of Isotope Science Center, The University of Tokyo, Tokyo, Japan*

[4]*Department of General Surgery, TianTan Hospital, Beijing, China*

[5]*Angiex Inc, Cambridge, MA, United States of America*

^ *Co- first author*

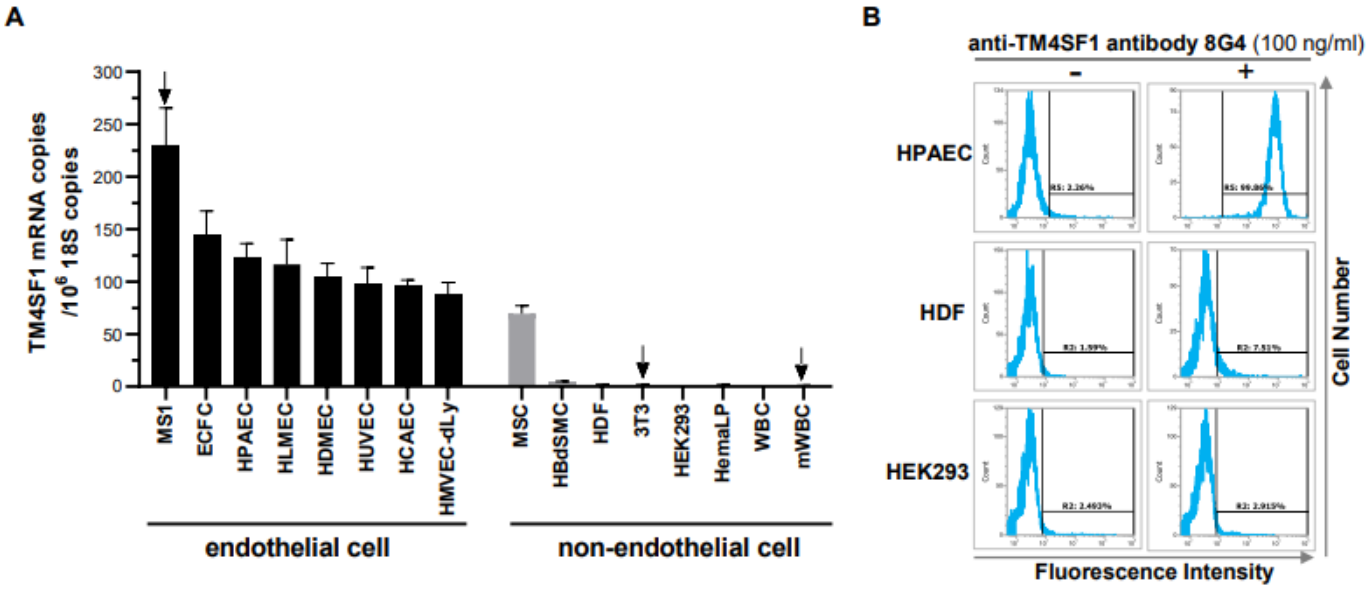

Figure S1. TM4SF1 expression in cultured cells *in vitro*. TM4SF1 expression in various cultured cells *in vitro* was examined by (A) quantitative real-time PCR and (B) Flow cytometry. (A) All cultured endothelial cells (EC), regardless of their tissue origin, expressed high levels of TM4SF1. In non- endothelial cell types, with the exception of mesenchymal stem cells (SMC, bone marrow derived), TM4SF1 expression is either very low as in smooth muscle cells (HBdSMC, bladder) and fibroblasts (HDF, dermal; 3T3, embryo), or not expressed, as in epithelial cells (HEK293, kidney), epidermal melanocytes (HemaLP, lightly pigmented adult skin), and White Blood Cells (human and murine WBC). Arrows indicate the cells that originated from mouse tissues (MS1, 3T3, and mWBC). (B) Representative TM4SF1 protein expression in HPAEC, HDF, and HEK293 cells tallies with their mRNA results presented in (A). Protein abundance was high in endothelial cells (HPAEC), very low in fibroblasts (HDF), and no expression was observed in epithelial cells (HEK293). Abbreviations: ECFC, Endothelial Colony Forming Cells; (PA) Pulmonary Artery; (LM) Lung Microvascular; (DM) Dermal Microvascular; (UV) Umbilical vein; (CA) Coronary Artery; (dLY) Adult lymphatic; (MS1) Mouse SV1 Immortalized Islet EC.

***Corresponding Author:** Dr. Shou-Ching S. Jaminet, Angiex Inc, Cambridge, MA, United States of America; Email: scj@jaminetlab.com*

Received: 19-Sep-2023, Manuscript No. JBL-23-114116; **Editor assigned:** 22-Sep-2023, PreQC No. JBL-23-114116(PQ); **Reviewed:** 06-Oct-2023, QC No. JBL-23-114116; **Revised:** 13-Oct-2023, Manuscript No. JBL-23-114116 (R); **Published:** 20-Oct-2023, DOI: 10.37421/2165-7831.2023.13.310

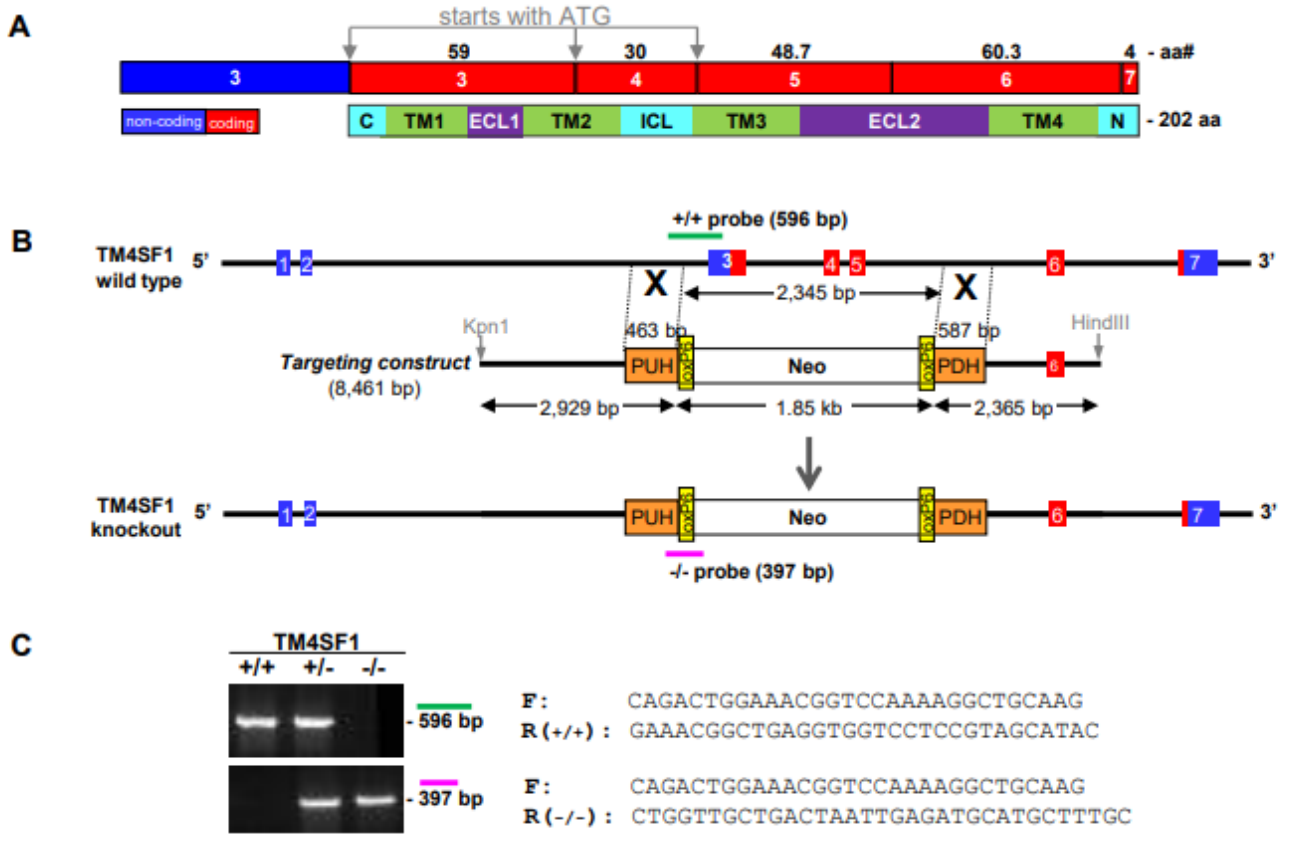

Figure S2. Genomic structure of *Tm4sf1* and targeting strategy to generate *Tm4sf1*-knockout mice. The mouse TM4SF1 gene constitutes seven exons (Ex) with the translation initiation site lies within Ex3 and encodes 202 amino acids of mature TM4SF1 protein from Ex3 to Ex7 (red boxes). (A) *Tm4sf1* coding exons 3-7, with the count of amino acids (aa) coded by each exon are indicated above the exons, and the respective translated TM4SF1 protein domains below [C, cytosolic C-terminal; TM1 to 4, transmembrane domain 1 to 4; (ECL1 and 2) Extra Cellular Loop 1 & 2; (ICL) Intracellular Loop; N, cytosolic N-terminal]. The initial codon for the first three coding exons, Ex3, Ex4, and Ex5, is an ATG initiation codon thus offering two extra potential independent translation start sites through Ex4 and Ex5. (B) The wild-type murine *Tm4sf1* locus, the targeting construct, and targeted *Tm4sf1* knockout locus with Ex3 to 5 (2,345 bp) replaced by the neomycin resistant loxP-Neo-loxP reporter cassette (3,167 bp). Orange boxes (PUH and PDH) indicate the homology arms of TM4SF1 genomic region that were used for homologous recombination. The probe positions of wild type (596 bp) and knock out (397 bp) are indicated in green and pink lines respectively. (C) PCR analysis was used to distinguish the *Tm4sf1* wild-type (+/+) and targeted alleles (-/-). The PCR primer sequences used for genotyping are shown. We generated stabilized *Tm4sf1* +/- C57BL/6 mice after eight generations of intercross mating of *Tm4sf1* +/- males with wild type females before they were used in this study.

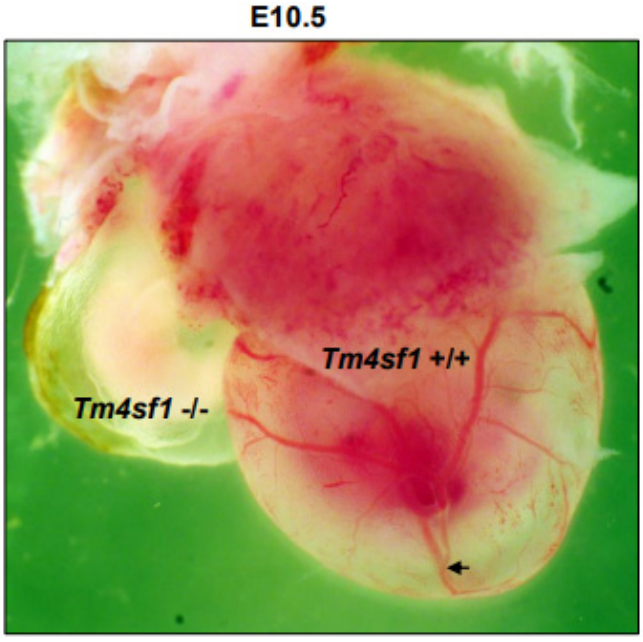

Figure S3. Defective in yolk sac vascular development in *Tm4sf1* knockout embryo. Bright field E10.5 yolk sac image show a side-by-side *Tm4sf1* +/+ and *Tm4sf1* -/- embryos. Note presence of dense capillary plexus and large vitelline artery (black arrow) in *Tm4sf1* +/+ embryo. Unlike *Tm4sf1* +/+ embryo, *Tm4sf1* -/- lack developing vasculature in the yolk sac and embryo.

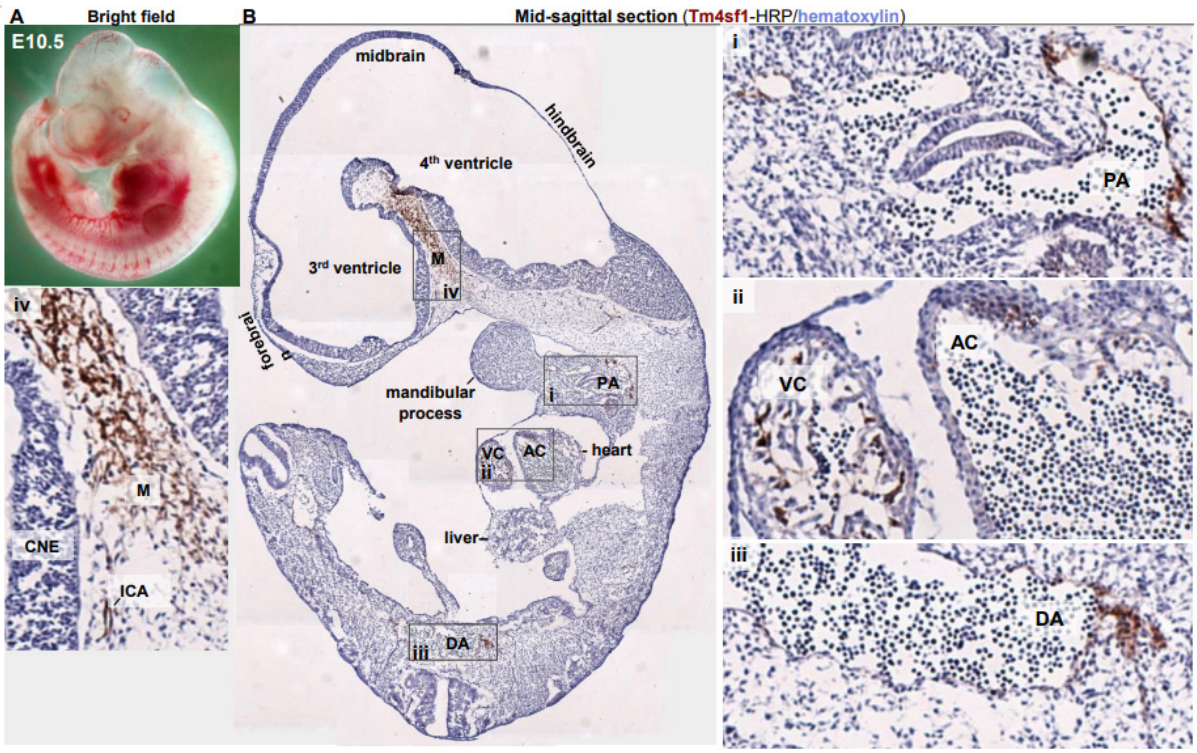

Figure S4. TM4SF1 protein expression in E10.5 wild type embryos. (A) Bright field image shows a *Tm4sf1* +/+ embryo at E10.5. The E10.5 embryo was processed for (B) TM4SF1 IHC staining with anti-mouse TM4SF1 antibody 2A7A followed by HRP-conjugated 2nd antibody with hematoxylin counterstain. Staining of a representative mid-sagittal section revealed TM4SF1 protein localization to all major vessels such as (i) pharyngeal arches (PA), (ii) Ventricular Chamber (VC) and Arterial Chamber (AC), (iii) Dorsal Aorta (DA), and (iv) Internal Carotid Artery (ICA), and to cephalic Mesenchyme (M). There was no noticeable TM4SF1 expression in Cortical Neuro Epithelium (CNE).

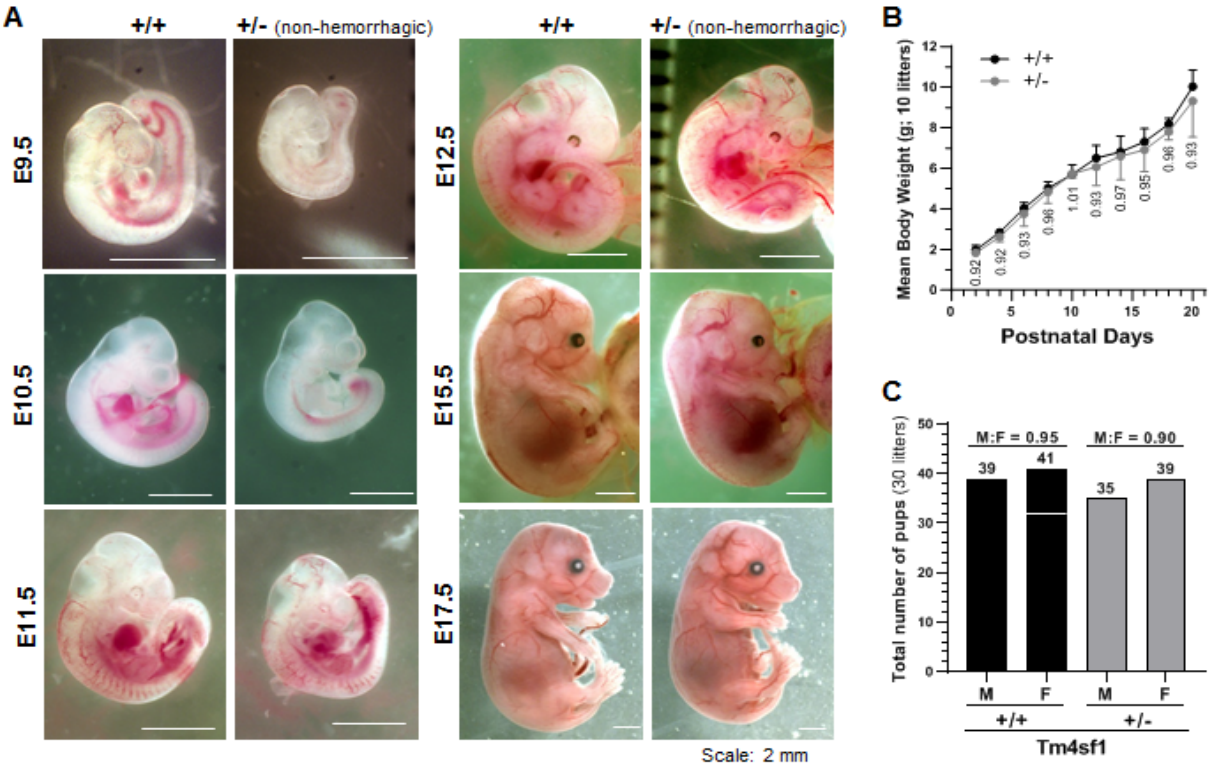

Figure S5. Growth of *Tm4sf1* +/+ and non-hemorrhagic +/- mice during prenatal and postnatal stages. (A) Representative prenatal images of *Tm4sf1* +/+ and non hemorrhagic *Tm4sf1* +/- embryos at different embryonic developmental stages showed that the earlier the developmental stage, the larger the difference in body size between the +/+ and +/- embryos. Body size became similar at birth. (B) Postnatal body weight of 10 litters of mice was tracked, and revealed a similar growth rate between the *Tm4sf1* +/+ and *Tm4sf1* +/- mice. (C) Ratio of males and females born to *Tm4sf1* +/+ and *Tm4sf1* +/- were similar (0.95 for *Tm4sf1* +/+ and 0.90 for *Tm4sf1* +/-).

Table S1. PCR primer sequences used in generating TM4SF1 knockout mice.

Constructs/genotyping	PCR fragment ID	PCR fragment (bp)	Forward (5' to 3')	Reverse (5' to 3')
pM253/UH-DH plasmid (5.7kb)	UH: 5' arm of homology for TM4SF1 Ex3 targeting construct	657	AGGAAGGACCAGCACCAAGGGTTATGAAC	CAAGATCTGATGCCCTCTTCTG GAGTGTC
	DH: 3' arm of homology for TM4SF1 Ex5 targeting construct	634	GAGGTCTAGCCCTGTAGTGGCA ACAAG	TGTGAAAGAAAGCCAGGAAGCT CTCA
pM253/UH-HR-DH plasmid (13.5 kb)	UP: the area lies in between UH and PUH	692	AGCAACACAGTCACAGACTTGG TAAATC	CTCATCTGGAGGGTGGTCTGTT CTATC
	DP: same as DH	634	GAGGTCTAGCCCTGTAGTGGCA ACAAG	TGTGAAAGAAAGCCAGGAAGCT CTCA
PUH-loxP-Neo-loxP-PDH cassette (2.85 kb)	PUH: 5' TM4SF1 homology arm of loxP-Neo-loxP casette	463	GTGCCGTGGGCCTCCTTCACCT TTTC	GACTGTGCCAGACTTCCCCTTC GAATAGGAGACCGTGC
	PDH: 3' TM4SF1 homology arm of loxP-Neo-loxP casette	587	CTCGCGTCTCAATACCTAGTAA CACATTCTTAACCACAATGCT	TCAGTGTCGTTAAGAGAGGCCC ATTGTC
pM253/PUH-loxP-Neo-loxP-PDH targeting plasmid (12.9 kb)	TM-UPH-Neo: adjacent to 5' end of PUH and 5' end of Neo	717	GTCCAGTTGGGAATGTGACCTCTTCAC	GAAGAGTCCTGAGGCGGAAAGAACCAGCT
Genotyping	Wild type	596	CAGACTGGAAACGGTCCAAAAG GCTGCAAG	GAAACGGCTGAGGTGGTCCTCC GTAGCATAC
	Knockout	397	CAGACTGGAAACGGTCCAAAAG GCTGCAAG	CTGGTTGCTGACTAATTGAGAT GCATGCTTTGC

Underline: part of TM4SF1 genomic sequence

Table S2. Real-time PCR primer sequences used in MGTP approach to quantitative real-time PCR.

Gene	Species	Forward (5' to 3')	Reverse (5' to 3')
TM4SF1	Human	CGTGTGGTTCTTTTCTGGCA	CCAGCCCAATGAAGACAAATG
TM4SF1	Mouse	CATCACTGGGTTTGGCAGAA	TCAGTGCTGGCAAAGGTGTAGT
CD31	Mouse	GAGCCCAATCACGTTTCAGTTT	TCCTTCCTGCTTCTTGCTAGCT
CD144	Mouse	CAACTTCACCCTCATAAACAACCAT	ACTTGGCATGCTCCCGATT
Tie1	Mouse	CAAGGTCACACACACGGTGAA	GCCAGTCTAGGGTATTGAAGTAGG
Tie2	Mouse	ACTCTTCATGTACAACGGCCATT	AGTGGGTGGCTTGCTTGGTA
VEGFR1	Mouse	GAGGAGGATGAGGGTGTCTATAGGT	GTGATCAGCTCCAGGTTTGACTT
VEGFR2	Mouse	GCCCTGCTGTGGTCTCACTAC	CAAAGCATTGCCCATTCGAT
VEGFA	Mouse	GGAGATCCTTCGAGGAGCACTT	GGCGATTTAGCAGCAGATATAAGAA