## **Electronic supplementary material.**



**Supplementary Fig. 1. (A)** HUVEC, 48h after transduction with empty vector, stained with anti-TM4SF1 antibodies, phalloidin and DAPI. Inset boxes demonstrate that nanopodia projecting from a lamellipodium at the leading front stained intermittently with TM4SF1 to form TMED (white arrows) at intervals of 1-3 per micron. (B) Migration of GFP-transduced HUVEC (frame from Supplemental Video 1) left behind substantial, intermittently-GFP-positive debris that remained attached to substrate (white arrows). (C) GFP-transduced HUVEC project long nanopodia (white arrows), but phalloidin staining, when present, is confined proximally (pink arrows). White scale, 10 μm.

**Supplemental Figure 1** 

**Video 1. Migration and nanopodia projection of GFP-transduced HUVEC.** Cells were plated at ~60% density, cultured for 2h, and live cell imaging was performed, taking frames every 1.5 minutes for 2.5h. HUVEC consistently projected prominent, rounded lamellipodia from the leading front whose position often reverted to the trailing edge as cells changed direction. Nanopodia projections were visualized with Photoshop enhancement of images captured in Fig. 1D and 1E.

## Video 2. Live cell imaging of HUVEC transduced with both GFP- and TM4SF1.

HUVEC were transduced with 50 moi TM4SF1-adenovirus and 15 moi GFPadenovirus for 48h. Cells so transduced expressed ~400 mRNA copies of TM4SF1/cell. Cells were plated at ~60% density and were observed by live cell imaging with frames taken at 1.12 min intervals for 5.35h. TM4SF1-OE cells projected nanopodia from the entire cell perimeter and cell polarization and movement were impaired.