Supplemental Methods

Amniotic fluid collection

Human amniotic fluid was collected at the University of Heidelberg for routine amniocentesis in healthy women aged ≥ 38 years with normal pregnancies between the 15th and 18th weeks of gestation for detection of fetal chromosomal disorders. Samples were stored at -80 °C after removal of cells by centrifugation at 250 g for 5 minutes until measurements were performed in series. Detailed clinical data was not collected due to a restricted approval of the ethics committee of the University of Heidelberg.

Fetal skin and boy foreskin collection and immunohistochemical staining

Specimens of fetal periumbilical skin were taken after legal termination of pregnancy and foreskin was obtained after routine circumcision. The study was approved by the local ethics committee of the Medical University of Vienna (Nr.:1892/2020) and conducted in accordance with the Declaration of Helsinki Principles. Tissue biopsies were fixed in 10% formalin, rehydrated and embedded in paraffin. Immunohistochemical stainings for TF were performed with a monoclonal anti-TF antibody (No. 4509, American Diagostica, Inc., Greenwich, CT) at a dilution of 1:200 as described previously [1].

Amniotic fluid fractionation by size exclusive chromatography

Cryopreserved amniotic fluid was thawed in a water bath at 37 °C. Thawed samples (1 mL) were loaded to the Telos size exclusion chromatography (SEC) column (15 mL, Kinesis, Vernon Hills, IL) which packed with 10 mL of Sepharose CL-2B (GE healthcare, Pittsburgh, PA). The column was eluted with 0.9% NaCl after the loaded sample entirely entered the column. Twenty-six eluted fractions (each 500 uL) were collected in total. F8-10 and F18-20 were collected and pooled respectively for the subsequent assays. For western blot analysis,

the total protein present in each SEC fraction was precipitated using trichloroacetic acid, pelleted at 21 000 g for 10 minutes, and pellets were re-suspended in 20 µL PBS. The pH was adjusted to about 7.0 in each fraction, and proteins were dissolved in sample buffer (homemade PBS) for the subsequent western blot analysis.

Western blot

Samples were resuspended in laemmli buffer (mixed with 10% of β-mercaptoethanol if for reducing) and proteins were denatured at 100 °C for ten minutes, and then loaded on a 4-12% gradient gel (PAGEr EX gel, Lonza, Rockland, ME; Mini-PROTEAN® TGX™ Precast Protein Gels, Bio-rad Laboratories, Inc., Hercules, CA). Proteins were blotted onto a polyvinylidene difluoride (PVDF) membrane (Millipore; Amsterdam, The Netherlands). Following blocking with 5% Protifar (low fat milk powder, Nutrition), the PVDF membranes were incubated with anti-FVII (clone EP6185(2), Abcam, Cambridge, UK; 1:1000) at 4 °C overnight. After incubation with a secondary antibody, bands were visualized by Lumi-Light PLUS Western Blotting Substrate (Sigma-Aldrich, St. Louis, MO) and analyzed by Image Quant LAS 4000 (GE life science, Eindhoven, The Netherlands).

HaCaT cell culture

The HaCaT cell line was a gift from the Center for Reproductive Medicine, Amsterdam UMC, University of Amsterdam. HaCaT cells were cultured in serum free medium (Keratinocyte basal medium-2; Lonza Walkersville, Inc., Walkersville, MD) which was supplemented with a kit formulated by the manufacturer including bovine pituitary extract, human epidermal growth factor, insulin, hydrocortisone, transferrin, epinephrine and gentamicin/amphotericin-B (KGM™-2 SingleQuots™ Kit; Lonza Walkersville, Inc.). HaCaT cells were passaged at confluency of 50% - 75% as previously described [3]. For differentiation

culture, cell passage between 14 to 20 was used. Cells were seeded at the density of 80 000 per cm^2 to get full confluency after overnight incubation. Culture medium was changed every second day. Cells were well differentiated after 7-9 days being kept in a 37°C±1°C, 5% CO_2, 90% ± 2% humidity incubator as published previously [2].

Sample preparation for permeability assay

Because amniotic fluid is not an optimal medium for HaCaT cells, we concentrated amniotic fluid or amniotic fluid SEC fractions 10-fold with a 10 kDa filtration unit (Amicon® Ultra-2 Centrifugal Filter Devices, Millipore, Darmstadt, Germany) and added 40 µl of concentrated amniotic fluid or amniotic fluid SEC fraction to 360 µl of culture medium for the subsequent TEER and permeability experiments.

Transepithelial electrical resistance measurement

Transepithelial electrical resistance (TEER) was measured by electric cell-substrate impedance sensing (ECIS). An ECIS Zθ (Theta) system (Applied BioPhysics, Troy, NY) was used to measure the permeability of the in vitro differentiated HaCaT cell layers. HaCaT cells differentiated in ECIS 8W10E+ cultureware (Applied BioPhysics) which holded 400 µL of culture medium with a growth area of 0.8 cm^2 and measurement area of 0.0196 cm^2 until a stable plateau of resistance was reached after 7-9 days. Human FVIIa (Enzyme Research Laboratories, South Bend, IN) was added to 200 µl serum free medium (Lonza) and warmed at 37°C for 5 minutes. TEER measurement was paused, half of the medium (200 µL) in the well was discarded, and 200 µL of the medium containing FVIIa was added and mixed briefly. TEER measurement was immediately re-started and resistance was recorded. To investigate the effect of amniotic fluid on HaCaT cell layers, amniotic fluid was 10-fold concentrated (detailed description see section “Sample preparation for permeability assay”).

40 µL of the 10- fold concentrated amniotic fluid were added to 160 µL serum free medium and warmed at 37°C for 5 minutes prior to the measurement. TEER measurement was paused, half of the cell culture medium (200 µL) was discarded and 200 µL medium containing amniotic fluid were added, briefly mixed, and the measurement was immediately re-started. To investigate the effect of amniotic fluid SEC fractions on HaCaT cell layers, SEC fractions 8-10 and SEC fractions 18-20 were pooled, concentrated, and 10 µL of these concentrated SEC fractions were mixed with 190 µL serum free medium and added to HaCaT cell layers as described for amniotic fluid, and measurements were performed in the absence and presence of anti-FVII antibodies clone CLB (Sanquin, Amsterdam, the Netherlands; final concentration, 30 µg/mL), clone 3G12 (30 µg/mL final concentration), and clone 12C7 (30 µg/mL final concentration; 3G12 and 12C7 were generous gifts from Wolfram Ruf). Resistance was measured at frequency 500 Hz. TEER values (ohms × cm^2) were normalized by the measurement area of 8W10E+ cultureware (0.0196 cm^2) and were calculated by subtracting the blank values from the cell-free-medium-control well.

Epithelial permeability by lucifer yellow rejection assay

Differentiated cultured HaCaT cells were kept in transwell inserts (6.5 mm diameter, 0.4 µm pore size, Corning Inc., Corning, NY). Lucifer yellow ([LY] Yellow CH dipotassium salt, Sigma Aldrich, Darmstadt, Germany), a fluorescent membrane-impermeable tracers with a molecular mass of 0.5 kDa, was dissolved in HEPES buffer saline solution (HBSS, Lonza Walkersville, Inc. Walkersville, MD) to a final concentration of 0.1 mg/mL at the apical compartment. Then the fluorescent intensity was measured for 4 hours in the apical- and basal chamber with a SpectraMax i3 microplate detector (Molecular Devices, Sunnyvale, CA) at 485 nm excitation wavelength and 535 nm emission wavelength.

Blood plasma clotting assay

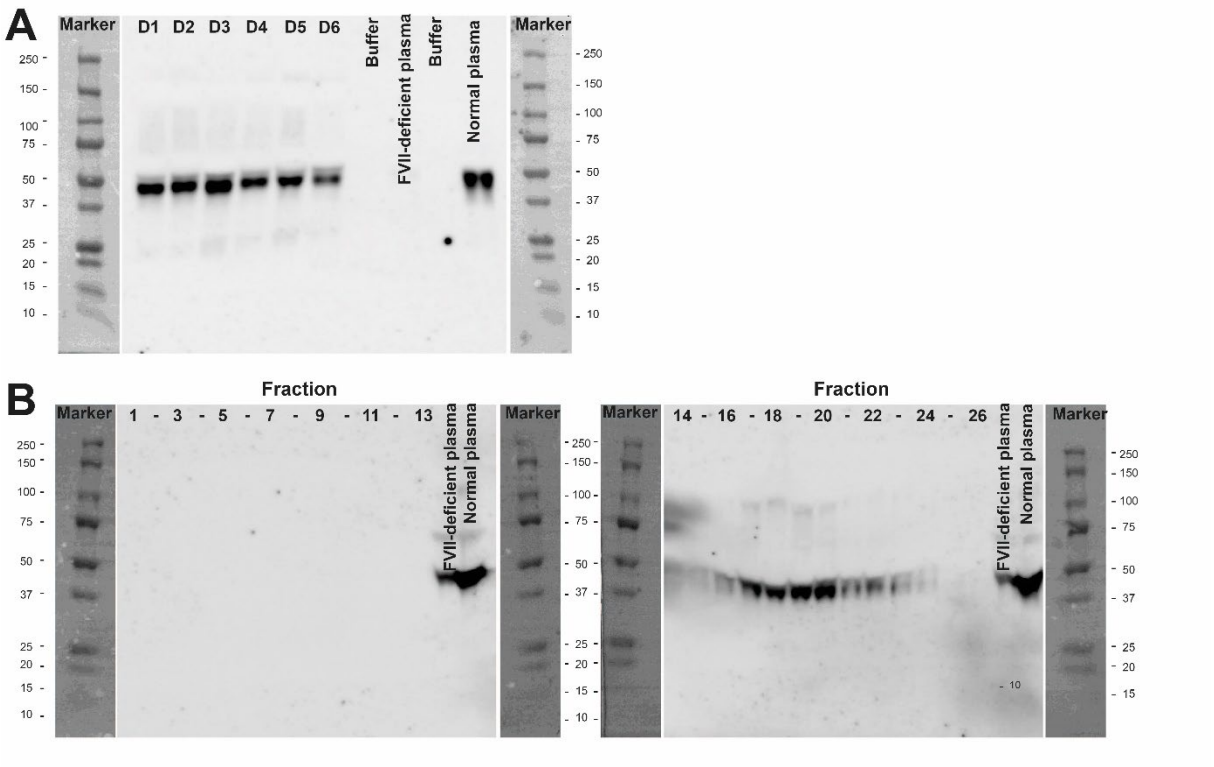
Plasma was prepared as described previously [3]. For detection of HaCaT surface TF activity, cells were collected and resuspended. 20 µL of cell suspension (containing about 1 000 cells) were added to 70 µL plasma in the presence of absence of an anti-TF antibody (clone HTF-1, eBiosciences, San Diego, CA; 30 µg/mL final concentration). After five minutes pre-warming at 37 °C, blood plasma clot formation was initiated by addition of 15 µL $CaCl_2$ (100 mM stock concentration). Blood plasma clot formation was monitored as optical density change by using a SpectraMax i3 microplate detector (Molecular Devices, Sunnyvale, CA) at the wavelength of 405 nm for one hour at 37 °C.

Data analysis

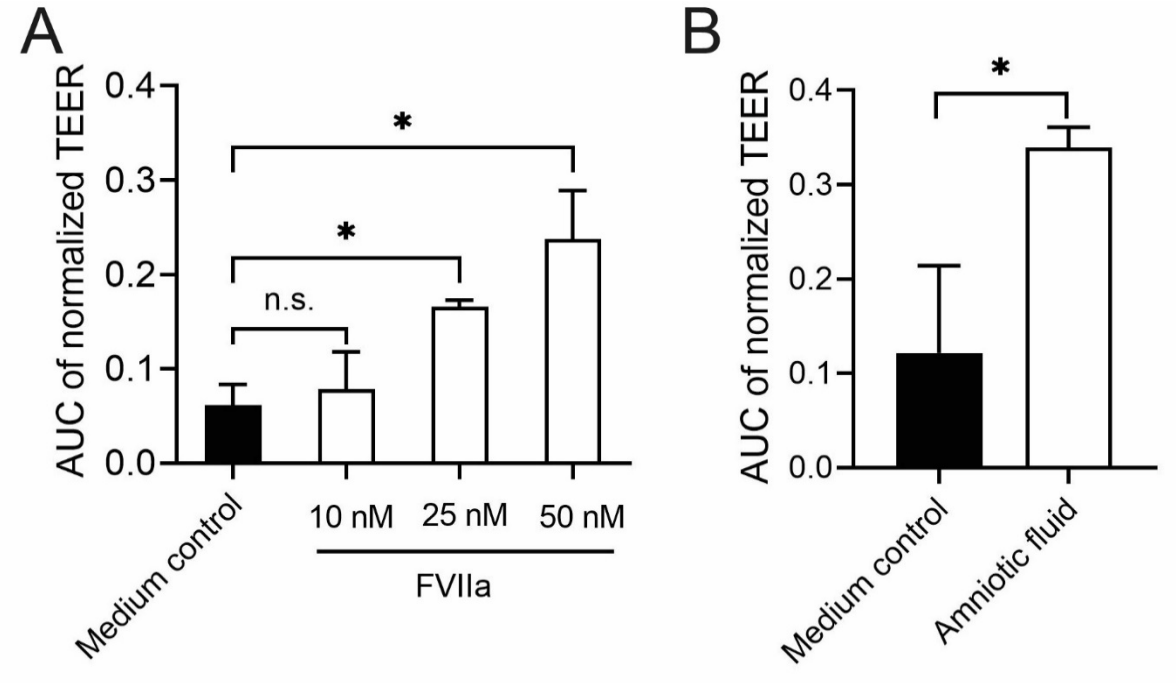
Data were analyzed by Student's *t* test (SPSS version 25.0 software, SPSS Inc., Chicago, IL). A probability value of less than 0.05 was considered to be statistically significant. Continuous variables are shown as mean ± standard deviation, unless stated otherwise.

REFERENCES

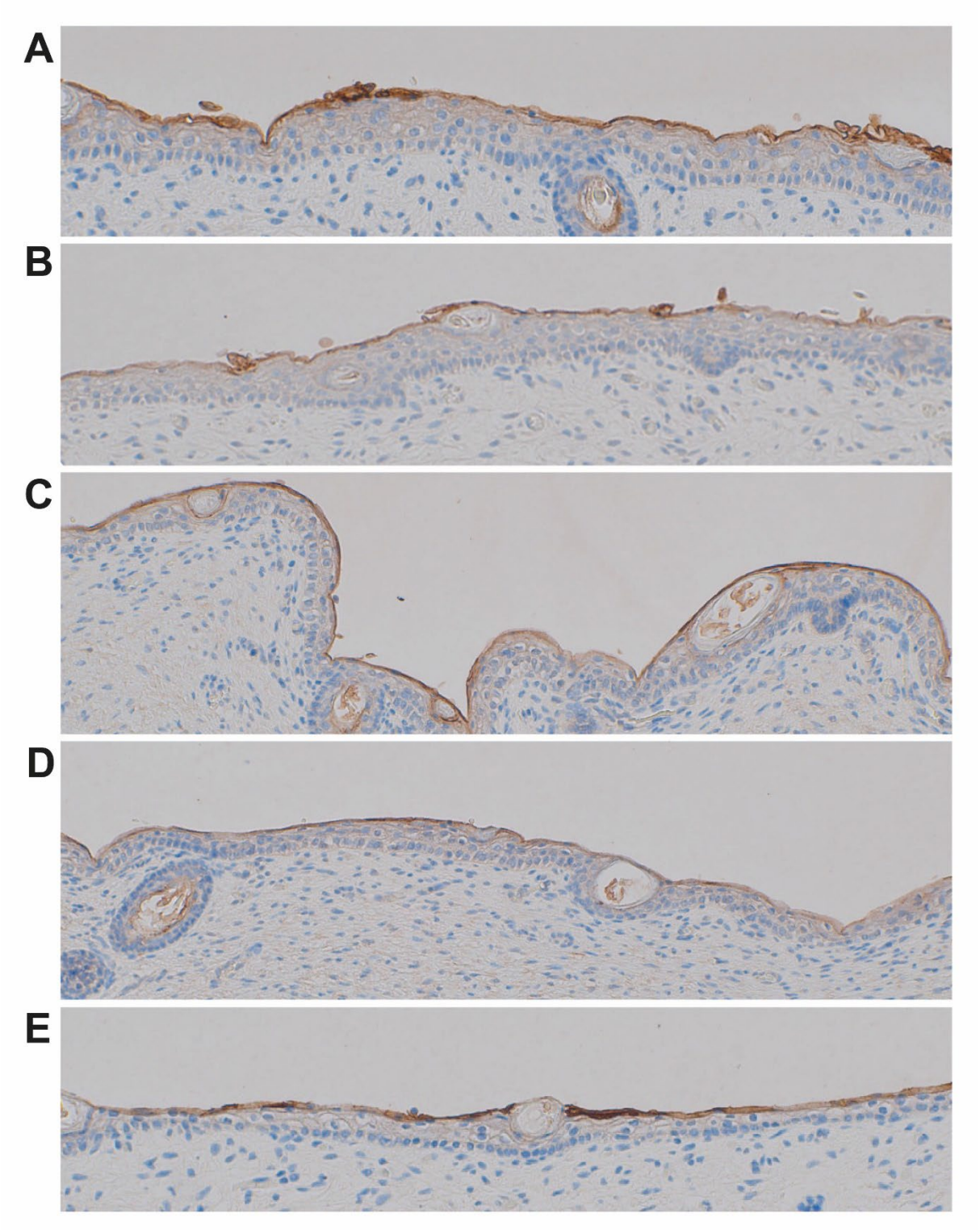
1. Thaler J, Preusser M, Ay C, et al. Intratumoral tissue factor expression and risk of venous thromboembolism in brain tumor patients. *Thromb Res*. 2013;131(2):162-165.
2. Schossleitner K, O'Mahony C, Brandstätter S, et al. Differences in biocompatibility of microneedles from cyclic olefin polymers with human endothelial and epithelial skin cells. *J Biomed Mater Res A*. 2019;107(3):505-512.
3. Berckmans RJ, Sturk A, van Tienen LM, Schaap MC, Nieuwland R. Cell-derived vesicles exposing coagulant tissue factor in saliva. *Blood*. 2011;117(11):3172-3180.



Supplemental Figure 1. Coagulation factor VII in amniotic fluid. (A) Human amniotic fluid (AF) was obtained from healthy women who underwent routine amniocentesis (n = 6). AF was blotted to detect coagulation factor VII (FVII). The FVII(a)-related enzymatic activity in amniotic fluid (quantified by its ability to generate fibrin in FVII-deficient plasma) was 53% in mean (range: 31%-72%), referring to normal plasma. (B) Human AF was fractionated by Sepharose 2B-size exclusion chromatography. All fractions were blotted for FVII. FVII deficient plasma was used as negative control; human normal plasma was used as positive control. Buffer (phosphate buffered saline) was used in blank lane.



Supplemental Figure 2. Transepithelial electrical resistance (TEER) of HaCaT cells was measured after addition of (A) human factor VIIa (FVIIa) to HaCaT cells (n=3) and (B) amniotic fluid (AF, n=3, CV=4.6%). *P < 0.05; n.s., non-significant.



Supplemental Figure 3, Tissue factor expression in skin epithelium.

Immunohistochemical staining for tissue factor (TF) in skin samples from 5 different fetuses (A-E) All pictures were taken at original 20× magnification.