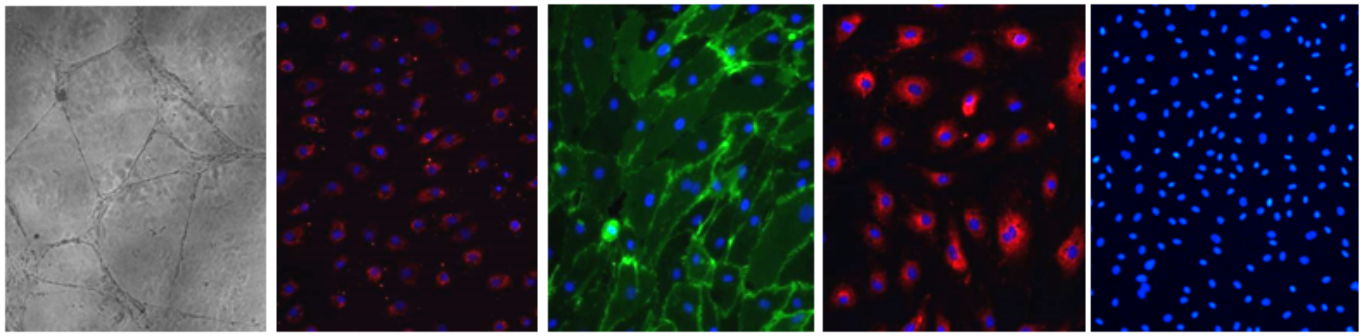
Trypsinogen 4 boosts tumor endothelial cells migration through proteolysis of tissue factor pathway inhibitor-2

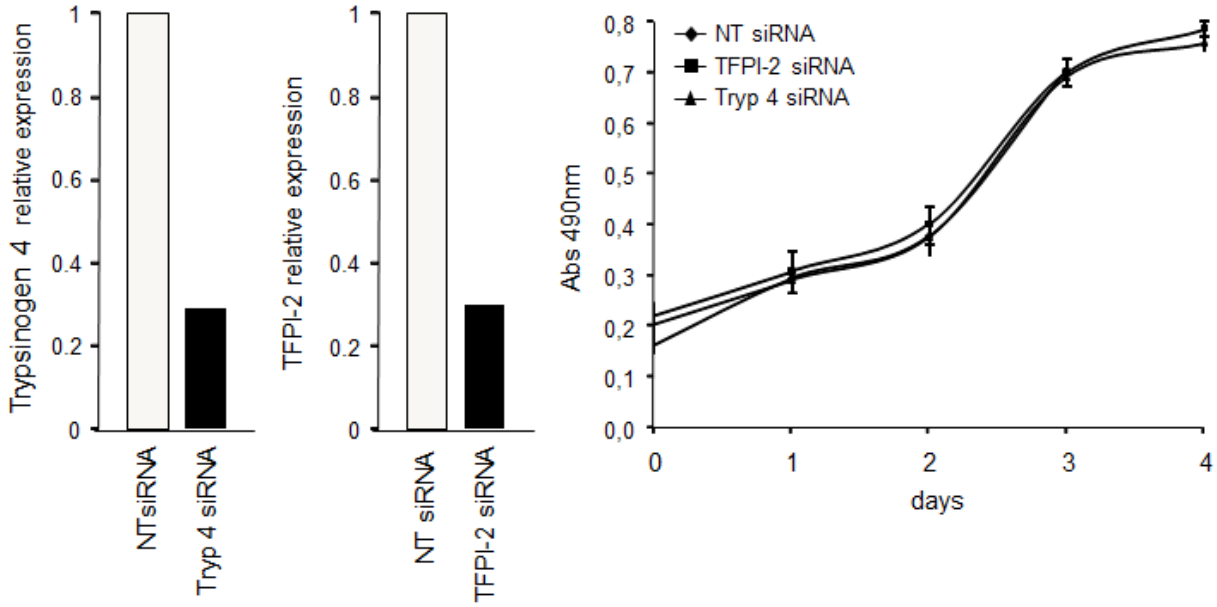
Supplementary Material

Cord formation	LDL uptake	CD31	vWF	aSMA
	% positive cells 99.2 (96.9-100)	% positive cells 99.2 (96.5-100)	% positive cells 99.9 (99.6-100)	% positive cells 0.7 (0-1.4)



Supplementary Figure 1: Characterization of endothelial cells

Endothelial cells isolated from human tumor tissues were characterized to confirm their endothelial origin. Cells formed cord-like structures on matrigel and took up LDL (red), stained positively for the typical endothelial markers CD31 (green) and von Willebrand Factor (red), but not for αSMA, typical of smooth muscle cells (blue, DAPI-counterstained nuclei). Percentages of positive cells were calculated by analyzing the immunofluorescence results for not less than 500 cells. The values [average (range)] for 17 different endothelial cells populations are shown, together with representative captions.



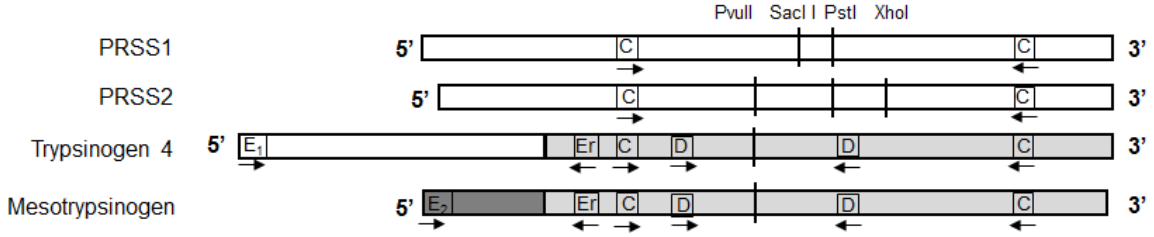
Supplementary Figure 2: Effect of trypsinogen 4 and TFPI-2 silencing on tumor EC proliferation

The proliferative activity of endothelial cells transfected with trypsinogen 4, TFPI-2, or not targeting siRNA was the same. Proliferation was measured using the CellTiter96® AQueous Non-Radioactive Cell Proliferation Assay (Promega). Briefly, $3x10^3$ HOC-EC were plated into 96-well plates (six replicates) and placed at 37°C in 5% CO_2. After 3h (time=0), 1, 2, 3 and 4 days 20 µl MTS solution were added to each well and incubated for 3h at 37°C in 5% CO_2. The plates were thoroughly shaken, and the absorbance at 490nm was recorded with a microplate reader (Infinite®200, Tecan).

Supplementary Table 1. Primers for RT-PCR analysis

The primer sequences used for RT-PCR with the amplicon lengths and target transcripts.

Primer pair	Target transcript	Primer	Primer Sequences	Amplicon length
C	PRSS1 (NM_002769) PRSS2 (NM_002770) PRSS3 (NM_007343, NM_002771)	Forward Reverse	5' CAGGTGAGACTGGGAGAGCACAA 3' 5' ATAGTTGTAGACCTTGGTGTAGACTCCAGG 3'	498 bp
D	PRSS3 (NM_007343, NM_002771)	Forward Reverse	5' CCTAAATACAACAGGGACACTCTGGAC 3' 5' GTCAGCACCAAAGCTCAGAGTGTTG 3'	170 bp
E_1+Er	TRYPSINOGEN 4 (NM_007343)	Forward Reverse	5' ATGTGCGGACCTGACGAC 3' 5' GCAGTGAGCTGCTGATACCAC 3'	345 bp
E_2+Er	MESOTRYPSINOGEN (NM_002771)	Forward Reverse	5' ATGAATCCATTCCTGATCCTTGC 3' 5' GCAGTGAGCTGCTGATACCAC 3'	191 bp



Supplementary Table 2. Gene expression assays for quantitative RealTime PCR

Shown are the Specific TaqMan® Gene Expression Assays used for RT-qPCR: the last column indicates whether the amplification was successful (+) or not (-) when RNA from tumor-EC was assayed (N= 7)

Target transcript	Assay ID	Product length	Tumor EC
PRSS1 (NM_002769)	Hs00605631_g1	108 bp	-
PRSS2 (NM_002770)	Hs00828418_gH	150 bp	-
PRSS3 (NM_007343, NM_002771)	Hs00605637_m1	157 bp	+
TFPI-2 (NM_006528)	Hs00197918_m1	84 bp	+
18s rRNA	Hs99999901_s1	187 bp	+