Supplementary material

1. Antibodies, chemicals, cell culture supplies

Anti-CD68 (cat. # IMG80071) and anti-CD34 (cat. # IMG80073) monoclonal antibodies were from Imgenex. Anti-β3 integrin (cat. # MAB197Z), anti-β1 integrin (cat. # MAB17781), anti E-selectin (cat. # AF724), and anti-VCAM-1 (cat. # AF809) monoclonal antibodies were from R&D Systems. Anti-ICAM-1 monoclonal rabbit antibody was from Epitomics (cat. # 1929-1). Calcein-AM was obtained from BD Biosciences (cat. # 354216). Cell culture inserts were from Millipore. Kinase inhibitors LY294002 (cat. # 44024), SB20380 (cat. # 559398), PD98059 (cat. # 98059), FAK inhibitor II (cat. # 324878), and Rho kinase inhibitor (cat. # 55552) were obtained from Calbiochem. NFκB inhibitor BMS-345541 (cat. # B9935) was obtained from Sigma-Aldrich. Anti-human TF monoclonal antibody 6B4 was described previously (van den Berg et al, PNAS '09 and references therein). Anti-human flTF antibody 10H10 (mouse monoclonal) was previously described (van den Berg et al, PNAS '09 and references therein); custom anti-human flTF and anti-human asTF rabbit polyclonal antibodies were previously described (Tardos et al, JTH '08). Isotype control IgG (ChromPure grade) were obtained from Jackson ImmunoResearch.

2. Immunofluorescence studies.

Paraffin embedded specimens of human cervical adenocarcinoma and lipid-rich aortic plaques were used to study the co-localization of flTF, asTF, and CD68. Tissue sections were deparaffinized, blocked, and incubated with specific anti-flTF antibody 10H10 and specific polyclonal anti-asTF antibody for 3 hrs at RT. The slides were then washed and incubated with Alexa fluor-488 labeled goat anti-rabbit antibody and Alexa fluor-633 labeled goat anti-mouse

antibody (Invitrogen) for 1hr at RT. The slides were then extensively washed and incubated with phycoerythrin-labeled anti-CD68 antibody for 1hr at RT. The slides were further washed and mounted with vectashield containing DAPI (Vector Labs). Images were captured and pseudo-colored when needed to discriminate between asTF (green), flTF (purple), and CD68 (red).

3. RT-PCR

Total RNA was isolated using RNAeasy Kit (Qiagen), reverse transcribed using Transcriptor (Roche Diagnostics), and PCR-amplified using FastStart Taq polymerase (Roche Diagnostics). Amplicons were separated on 2% agarose and visualized using ethidium bromide. Real time qRT-PCR for E-selectin, VCAM-1, and ICAM-1 was performed using TaqMan probes from the Universal Probe Library, Roche Diagnostics.

4. Western blotting

MVEC were grown to confluence in 6-well plates, treated with asTF or vehicle control for 4 hrs, and then lysed directly in Laemmli sample buffer. Lysates were loaded on 10% SDS-PAGE gels and, following electrophoresis, transferred to PVDF membranes (Roche Diagnostics). Membranes were blocked with 2% dry skim milk in Tris-buffered saline/Tween-20 (TBST) overnight at 4ºC, incubated with the primary antibodies against ICAM-1, VCAM-1, or E-selectin for 3 hrs at room temperature, washed in TBST, and then incubated with the corresponding HRP-conjugated secondary antibodies (Invitrogen) for 1 hr room temperature. Chemiluminescence was obtained using LumiLight (Roche Diagnostics). For the characterization of anti-human TF antibodies' reactivity and specificity, recombinant TF protein

variants and cervical cancer lysates, respectively, were probed with 6B4, 10H10, and custom polyclonal antibodies selectively recognizing flTF and asTF.

5. Removal of N-His tag from recombinant asTF

Recombinant N-His tagged asTF was treated with 1U of rEnterokinase (rEK, Novagen) for 16 hrs at room temperature, following which rEK was removed using enterokinase cleavage-capture kit (Novagen). The asTF purity and MW were confirmed on 10% PAGE stained with Coomasie brilliant blue. Prior to experiments, asTF concentration was measured using Bradford microassay.

Supplementary Figures

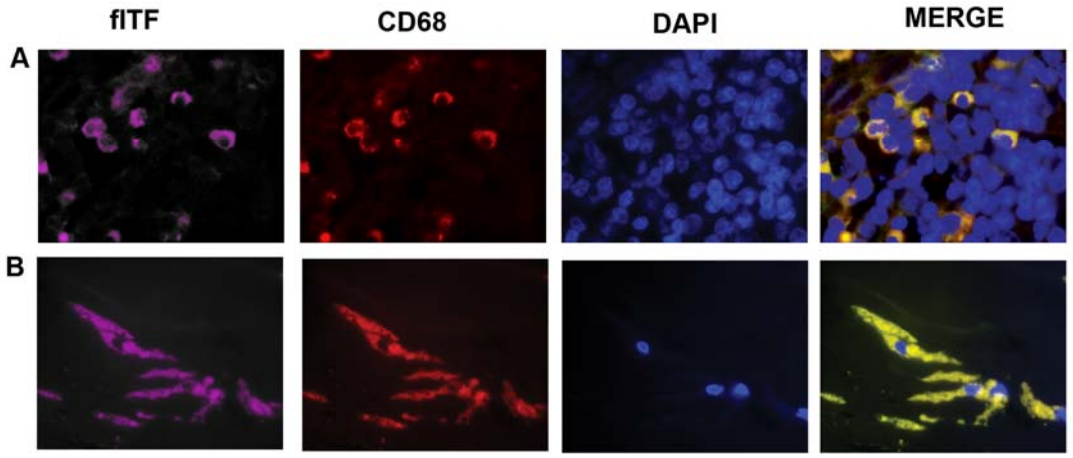

Fig. S1. Representative images, co-localization of flTF and CD68 in paraffin-embedded specimens of cervical cancers (A) and aortic plaques (B) assessed using flTF-specific rabbit polyclonal antibody and anti-CD68 monoclonal antibody. Following incubation with the primary antibodies, slides were washed, incubated with fluorophore-labeled corresponding secondary antibodies, and mounted with vectashield containing DAPI (Vector Labs). Visualization was performed in a Nikon-Photostat fluorescent microscope; images were captured using a CCD camera.

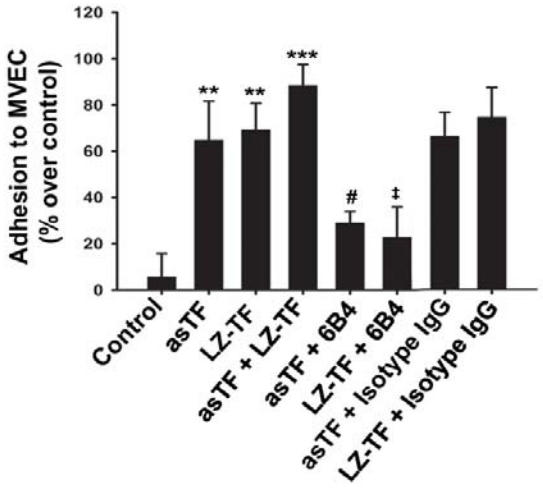

Fig. S2. LZ-TF and asTF promote MVEC-monocyte interactions via integrin ligation. Blockade of integrin binding by anti-TF antibody 6B4 inhibits MVEC-THP-1 interactions. n=3 for each assay, the values are mean ± SD. *, **, *** $p<0.05$, $p<0.01$ and $p<0.001$, respectively, compared to control. # $p<0.05$ compared to asTF. ‡ and ‡‡ $p<0.05$ and $p<0.01$, respectively, compared to LZ-TF.

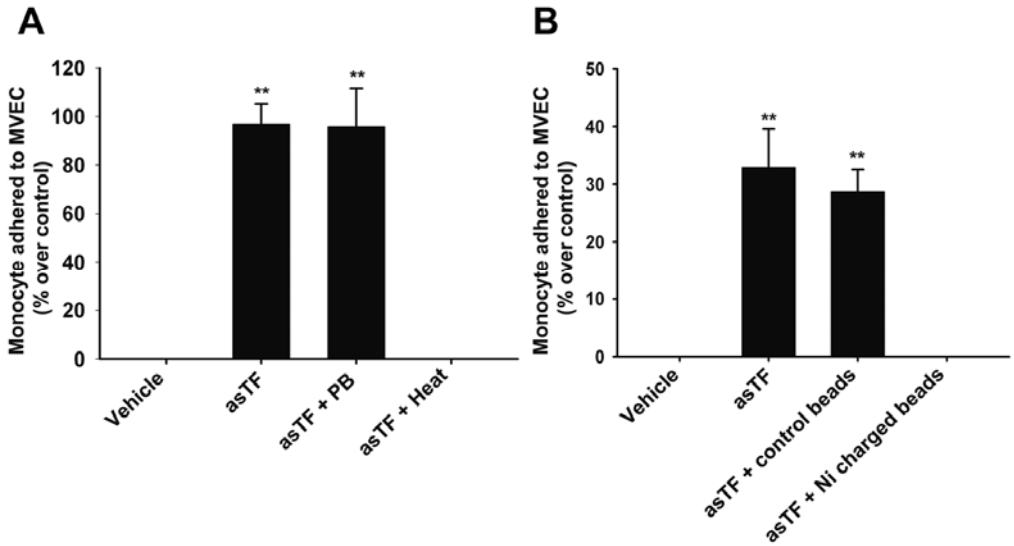

Fig. S3. (**A**) MVEC were treated with recombinant asTF, or pre-treated with Polymyxin B ("PB," 50 μg/ml) prior to the addition of recombinant asTF; in a separate sample, the medium containing recombinant asTF was heated and added to MVEC; monocyte-MVEC interactions were assessed using orbital shear assay. (B) Ni-charged and uncharged (control) beads were added to the medium containing recombinant asTF and placed on a rocker platform for 1 hr at room temperature; beads were removed by centrifugation and the medium was then added to MVEC. Monocyte-MVEC interactions were assessed using orbital shear assay. **$p< 0.001$.

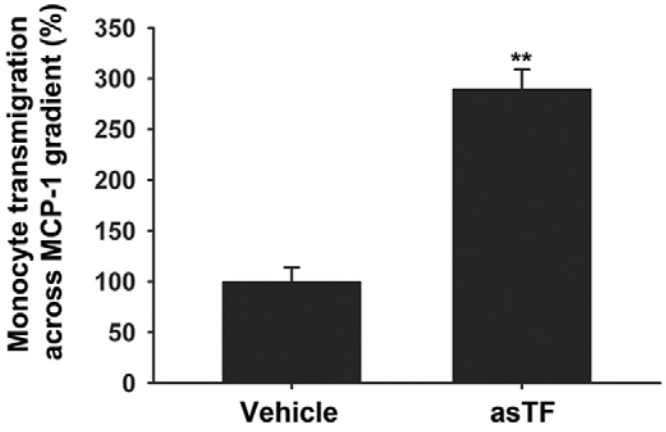

Fig. S4. Transwell assay under MCP-1 gradient (50 μg/L): THP-1 cells on the abluminal surface of the inserts chamber were stained with DAPI and counted using Image J (n=3, mean ± SD, **p<0.001).

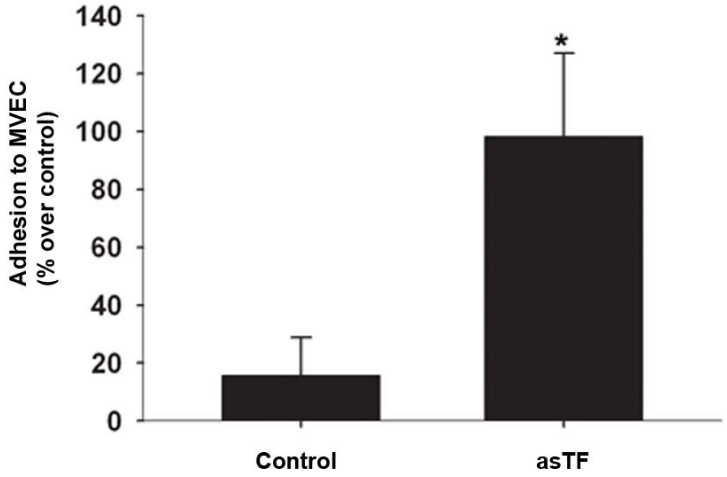

Fig. S5. Increased MVEC-monocyte adhesion elicited by recombinant asTF lacking the N-terminal His-tag, orbital shear assay. * p< 0.01

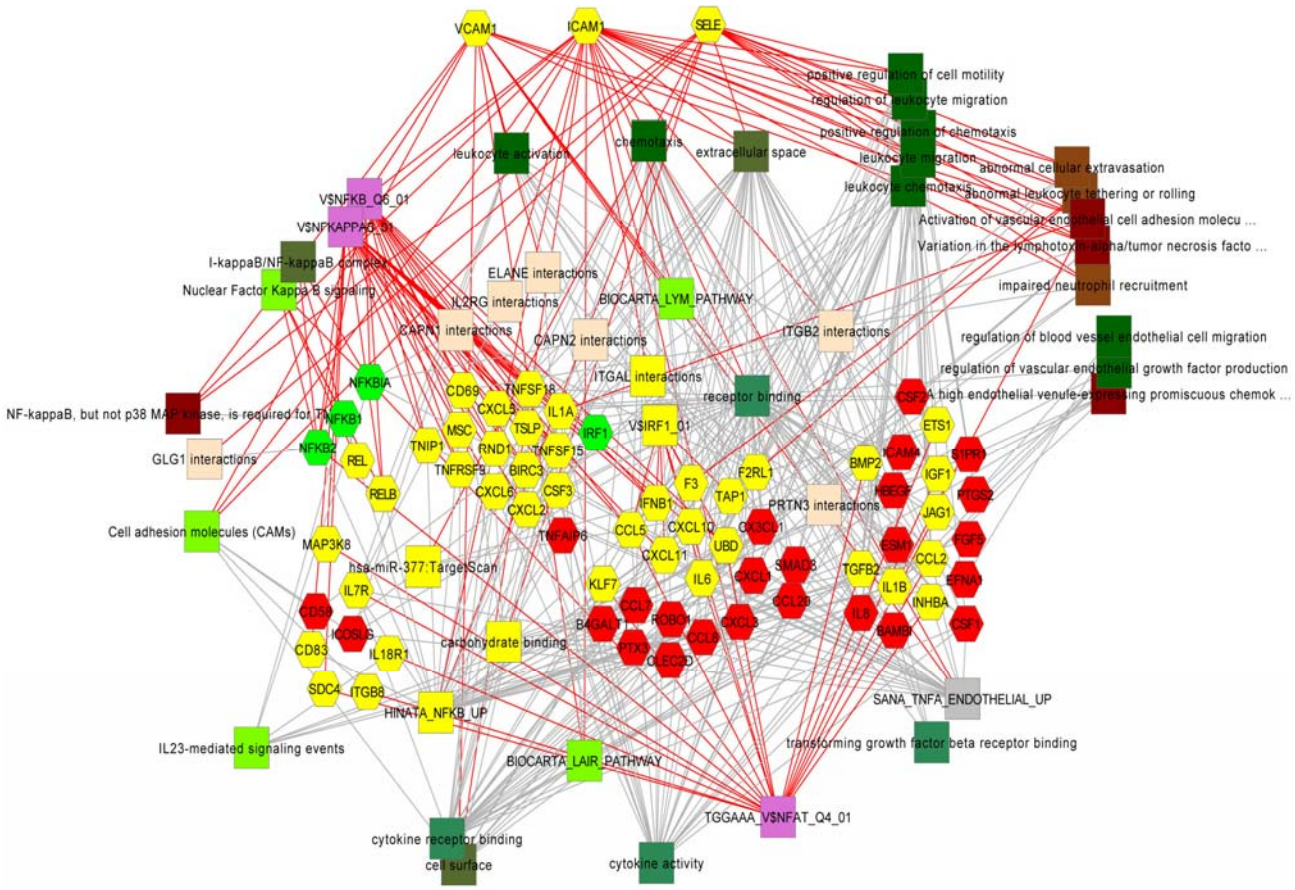

Fig. S6. Schematic representation of the interactions between the major pathways upregulated in MVEC stimulated with asTF. Yellow hexagons: primary NFkB targets; red hexagons: targets of the NFkB-upregulated genes. Grouped color-coded squares: the affected physiological and pathophysiological pathways (green and brown, respectively).

Supplementary Table

Top 30% of the genes upregulated ≥1.5 fold in cardiac and retinal MVEC in response to asTF stimulation

S.no	Gene name (HUGO)	Gene Description	Cardiac MVEC	Retinal MVEC
			Fold over control	Fold over control
1	**CCL20**	chemokine (C-C motif) ligand 20	30.4	14.8
2	**TNFAIP6**	tumor necrosis factor, alpha-induced protein 6	17.8	5.6
3	**TNIP3**	TNFAIP3 interacting protein 3	17.8	14.0
4	**CXCL3**	chemokine (C-X-C motif) ligand 3	16.3	7.8
5	**TNFAIP3**	tumor necrosis factor, alpha-induced protein 3	11.7	5.3
6	**IL18R1**	interleukin 18 receptor 1	10.3	4.6
7	**BIRC3**	baculoviral IAP repeat-containing 3	9.9	13.2
8	**IL1B**	interleukin 1, beta	9.8	1.5
9	**HIVEP2**	human immunodeficiency virus type I enhancer binding protein 2	9.5	3.9
10	**IL6**	interleukin 6 (interferon, beta 2)	9.3	7.3
11	**CXCL2**	chemokine (C-X-C motif) ligand 2	9.0	17.5
12	**IL8**	interleukin 8	8.9	12.2
13	**SELE**	selectin E (endothelial adhesion molecule 1)	8.1	108.4
14	**SLC7A2**	solute carrier family 7 (cationic amino acid transporter, y+ system), member 2	7.2	3.4
15	**RND1**	Rho family GTPase 1	6.1	3.6
16	**MSC**	musculin (activated B-cell factor-1)	6.1	1.7
17	**CXCL1**	chemokine (C-X-C motif) ligand 1 (melanoma growth stimulating activity, alpha)	5.9	7.4
18	**VCAM1**	vascular cell adhesion molecule 1	5.4	40.5
19	**CCL5**	chemokine (C-C motif) ligand 5	5.3	2.2
20	**IL1A**	interleukin 1, alpha	5.1	3.4
21	**CLEC2D**	C-type lectin domain family 2, member D	4.8	3.6
22	**NFKBIZ**	nuclear factor of kappa light polypeptide gene enhancer in B-cells inhibitor, zeta	4.7	4.0
23	**ICAM1**	intercellular adhesion molecule 1 (CD54), human rhinovirus receptor	4.6	8.7
24	**TNFAIP2**	tumor necrosis factor, alpha-induced protein 2	4.5	2.4
25	**CSF2**	colony stimulating factor 2 (granulocyte-macrophage)	4.4	2.8
26	**ELOVL7**	ELOVL family member 7, elongation of long chain fatty acids (yeast)	4.3	1.8
27	**SOD2**	superoxide dismutase 2, mitochondrial	4.2	4.4
28	**IFIH1**	interferon induced with helicase C domain 1	4.1	4.7
29	**EFNA1**	ephrin-A1	4.1	2.4
30	**RIPK2**	receptor-interacting serine-threonine kinase 2	4.1	3.4
31	**TNFRSF9**	tumor necrosis factor receptor superfamily, member 9	4.0	2.4
32	**IRF1**	interferon regulatory factor 1	3.9	1.5
33	**SLC41A2**	solute carrier family 41, member 2	3.9	2.0

34	**UBD**	ubiquitin D	3.9	2.2
35	**CD69**	CD69 molecule	3.8	6.0
36	**F3**	coagulation factor III (thromboplastin, tissue factor)	3.8	2.0
37	**CXCR7**	chemokine (C-X-C motif) receptor 7	3.6	1.9
38	**PTGS2**	prostaglandin-endoperoxide synthase 2 (prostaglandin G/H synthase and cyclooxygenase)	3.6	2.9
39	**TIFA**	TRAF-interacting protein with a forkhead-associated domain	3.6	3.6
40	**UBD**	ubiquitin D	3.5	2.1
41	**ZC3H12C**	zinc finger CCCH-type containing 12C	3.4	2.6
42	**IL7R**	interleukin 7 receptor	3.4	2.4
43	**TRAF1**	TNF receptor-associated factor 1	3.3	1.8
44	**FGF5**	fibroblast growth factor 5	3.1	2.5
45	**TNFAIP8**	tumor necrosis factor, alpha-induced protein 8	3.1	1.8
46	**PDE5A**	phosphodiesterase 5A, cGMP-specific	3.0	1.9
47	**NFKB1**	nuclear factor of kappa light polypeptide gene enhancer in B-cells 1 (p105)	3.0	2.6
48	**BCL2A1**	BCL2-related protein A1	3.0	2.9
49	**IRAK2**	interleukin-1 receptor-associated kinase 2	2.9	2.0
50	**NFKBIA**	nuclear factor of kappa light polypeptide gene enhancer in B-cells inhibitor, alpha	2.9	3.6
51	**CXCL6**	chemokine (C-X-C motif) ligand 6 (granulocyte chemotactic protein 2)	2.8	2.5
52	**MAP3K8**	mitogen-activated protein kinase kinase kinase 8	2.8	2.1
53	**IFIT3**	interferon-induced protein with tetratricopeptide repeats 3	2.6	2.2
54	**CNKSR3**	CNKSR family member 3	2.5	2.2
55	**ICOSLG**	inducible T-cell co-stimulator ligand	2.5	1.6
56	**SDC4**	syndecan 4	2.5	2.7
57	**CYLD**	cylindromatosis (turban tumor syndrome)	2.4	2.1
58	**WTAP**	Wilms tumor 1 associated protein	2.4	1.5
59	**CD83**	CD83 molecule	2.3	1.6
60	**GBP4**	guanylate binding protein 4	2.3	2.3
61	**SERPINB2**	serpin peptidase inhibitor, clade B (ovalbumin), member 2	2.3	1.7
62	**CSF1**	colony stimulating factor 1 (macrophage)	2.3	1.6
63	**OSGIN2**	oxidative stress induced growth inhibitor family member 2	2.3	1.5
64	**RELB**	v-rel reticuloendotheliosis viral oncogene homolog B, nuclear factor of kappa light polypeptide gene enhancer in B-cells 3 (avian)	2.2	2.0
65	**CLDN1**	claudin 1	2.2	1.9
66	**CSF3**	colony stimulating factor 3 (granulocyte)	2.2	1.7
67	**MMP10**	matrix metallopeptidase 10 (stromelysin 2)	2.2	2.2
68	**IFIT2**	interferon-induced protein with tetratricopeptide repeats 2	2.1	2.6
69	**DRAM**	damage-regulated autophagy modulator	2.0	1.5
70	**CCL2**	chemokine (C-C motif) ligand 2	2.0	6.7
71	**CTSS**	cathepsin S	2.0	1.8