Supplementary material

Treponema pallidum (syphilis) antigen TpF1 induces angiogenesis through the activation of the IL-8 pathway

Running title: TpF1 stimulates angiogenesis

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Supplementary Figures

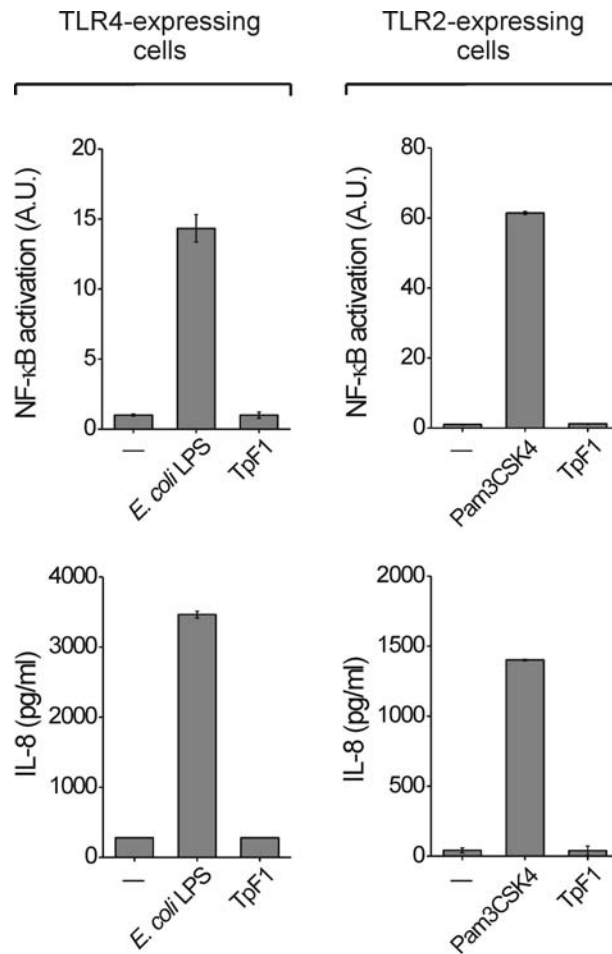

Figure S1. TpF1 preparations are free from Gram-negative and Gram-positive contaminants. HEK-293 cells expressing human Toll-Like-Receptor 4 (hTLR4)/MD2/CD14 and HEK-293 cells expressing human Toll-Like-Receptor 2 (hTLR2) were challenged with TpF1 (100 ng/ml) and, as positive controls, with *E. coli* LPS (100 ng/ml) and Pam3CSK4 (1 μg/ml). After 4 h (hTLR4) and 6 h (hTLR2), NF-κB activation was measured using the luciferase reporter assay system, and normalized to that of non-stimulated cells. Luciferase activity values from unstimulated cells were set at 1 arbitrary unit (A.U.). IL-8 production was quantified after 18 h stimulation with the specified agonists. Data are the mean values obtained from three independent experiments repeated twice.

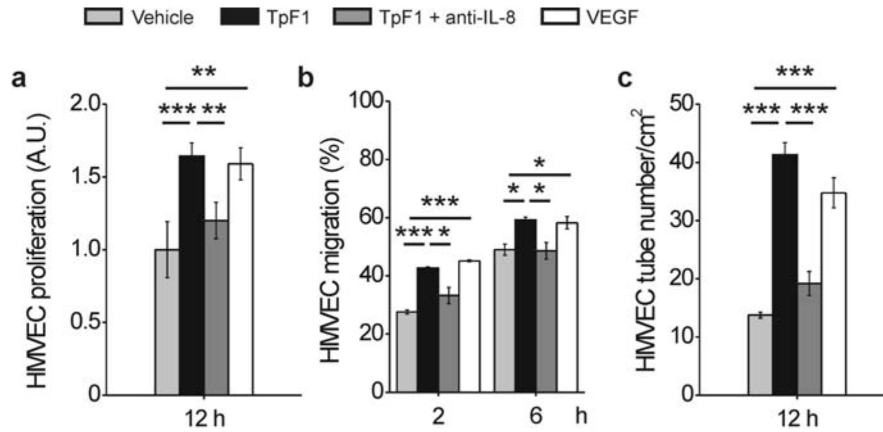

Figure S2. TpF1 induces IL-8-dependent proliferation and migration of dermal microvascular endothelial cells and their organization in microcapillary-like structures. (**a**) HMVEC-D were exposed to TpF1 (in the presence or absence of anti-IL-8 blocking antibody), VEGF or vehicle. After 12 h, cells were counted and normalized to the number of plated cells set as 1 arbitrary unit (A.U.). The graph shows cell proliferation under the different conditions. Data are represented as the mean ± S.D. of three independent experiments. (**b**) HMVEC-D, grown to confluence in 6-well plates, were exposed to TpF1 for 24 h. Supernatants were collected and incubated or not with anti-IL-8 blocking antibody for 45 min, before transferring to the lower chamber of 24 Transwell plates with Fast DiI-labelled HMVEC-D seeded on the upper chamber. VEGF and vehicle added to the lower chamber were used as positive and negative control, respectively. Migrated cells were quantified after 2 and 6 h. Data are represented as the mean ± S.D. of three independent experiments. (**c**) HMVEC-D were seeded on Matrigel-coated coverslips and exposed to TpF1 (in the presence or absence of anti-IL-8 blocking antibody), VEGF or vehicle, for 12 h. Cells were stained with Phalloidin-Alexa Fluor 546 and analyzed by confocal microscope. The number of tubes formed was counted and expressed as number of tubes/cm^2.