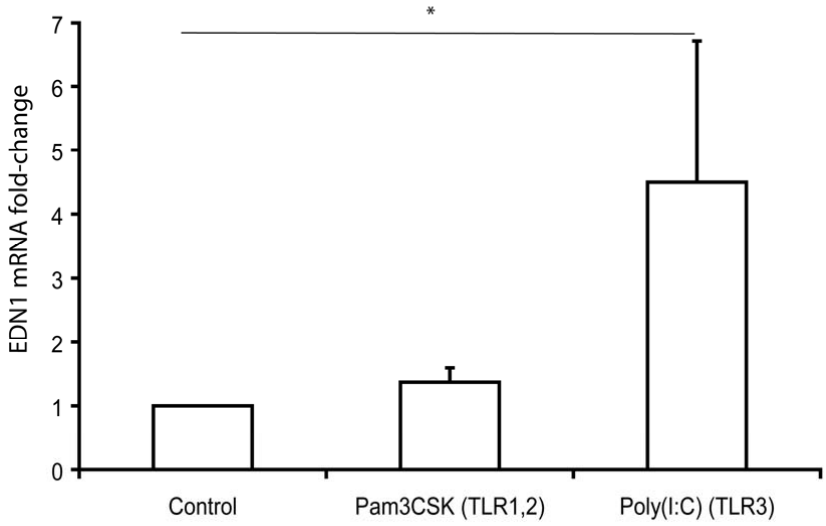
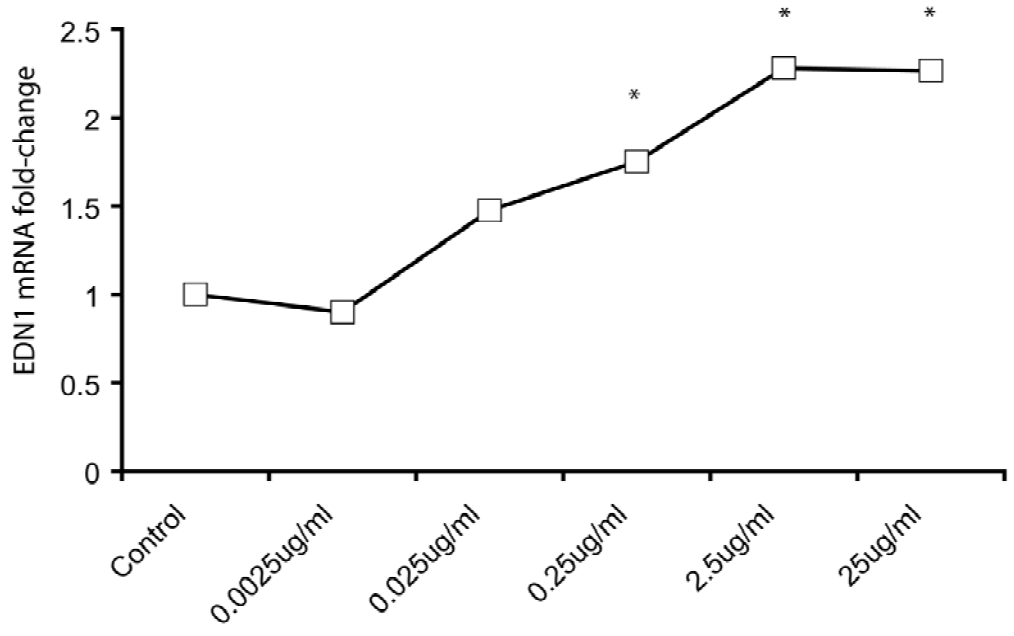
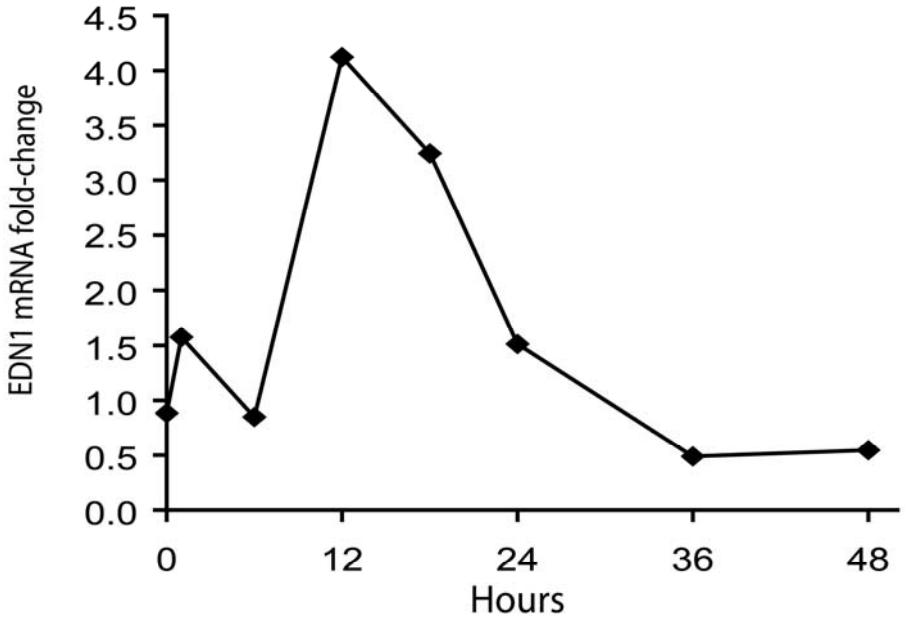


Supplementary Figure S1. PolyI:C (TLR3 ligand) but not Pam3CSK (TLR1,2 ligand) induces EDN1 mRNA in HDMECs. HDMECs were treated with PolyI:C (25µg/ml) and Pam3CSK (1µg/ml) and fold-change of EDN1 mRNA was measured after 18hour incubation. Graph depicts the mean of three separate experiments.

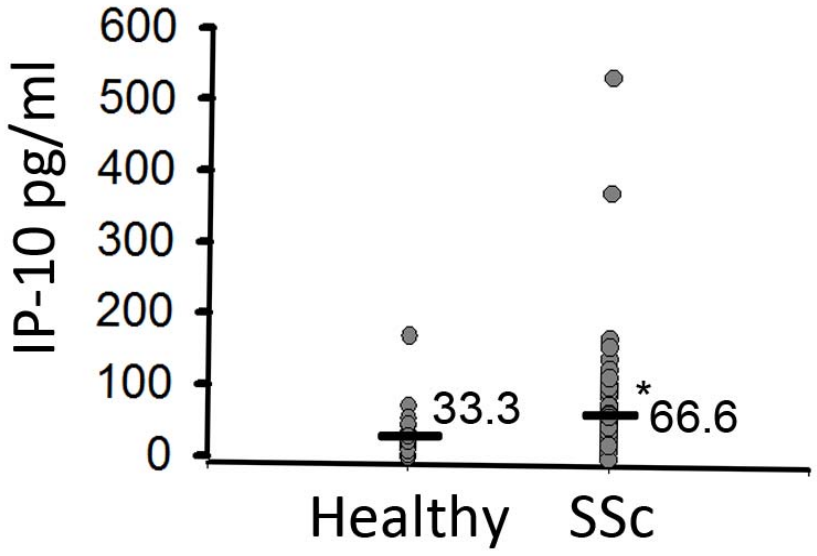


A.

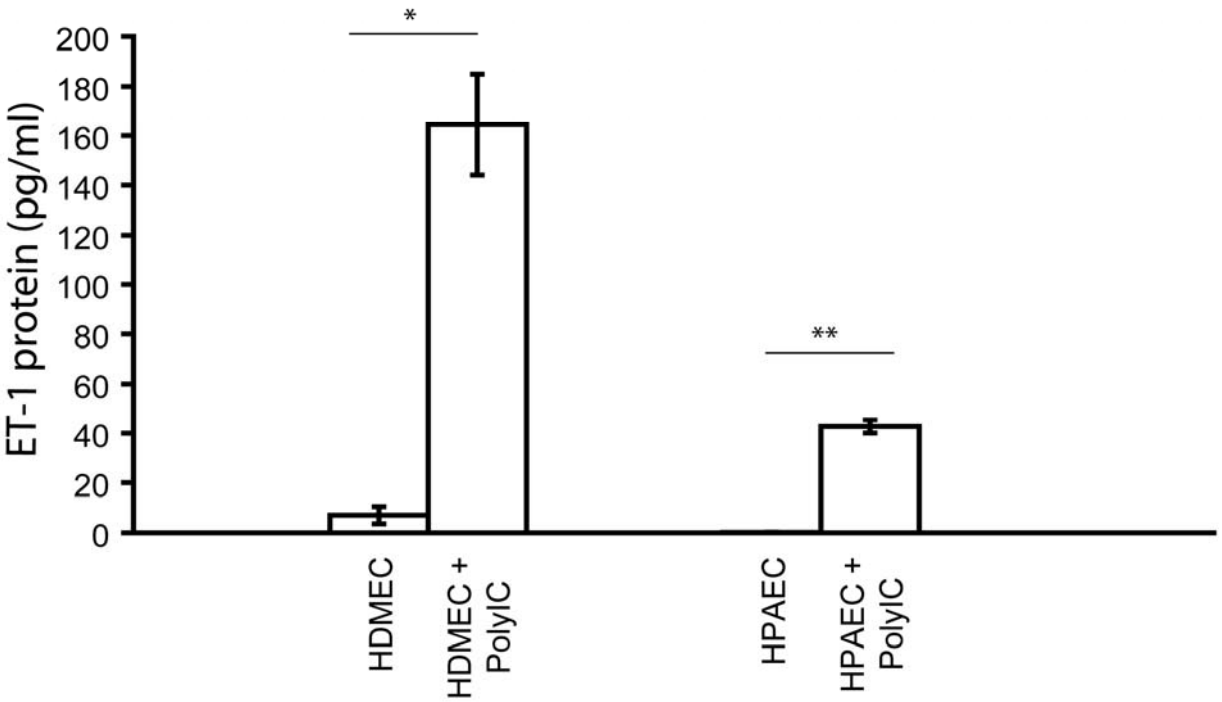


B.

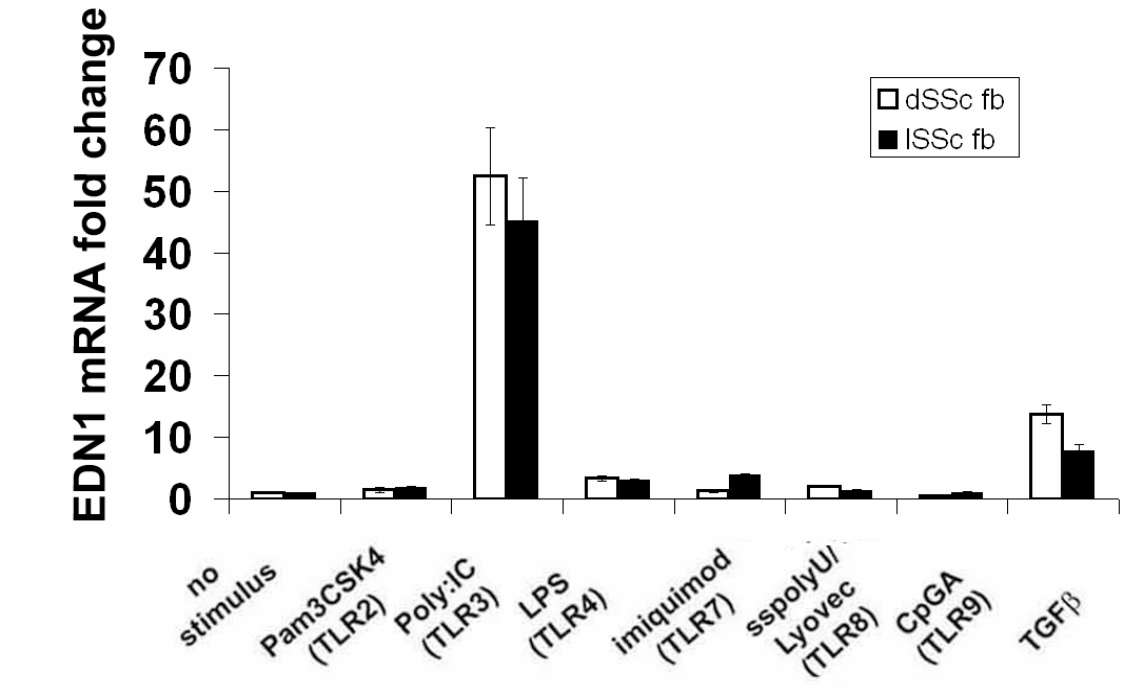
Supplementary Figure S2. Regulation of Endothelin-1 mRNA in dermal microvascular endothelial cells is dose and time dependent. Panel a**:** mRNA expression of EDN1 in HDMEC. Fold-changes shown on the graph are normalized to mRNA expression of untreated cells. Results are the mean of three experiments with $p<0.05$ for 0.025µg/ml, 2.5µg/ml and 25µg/ml doses. Panel b: HDMECs were treated with poly(I:C) 25µg/ml at 0, 1,6,12, 18, 24, 36 and 48 hours and fold-change of EDN1 mRNA expression compared with 0hr time is depicted. Maximal induction was seen at 18hrs.



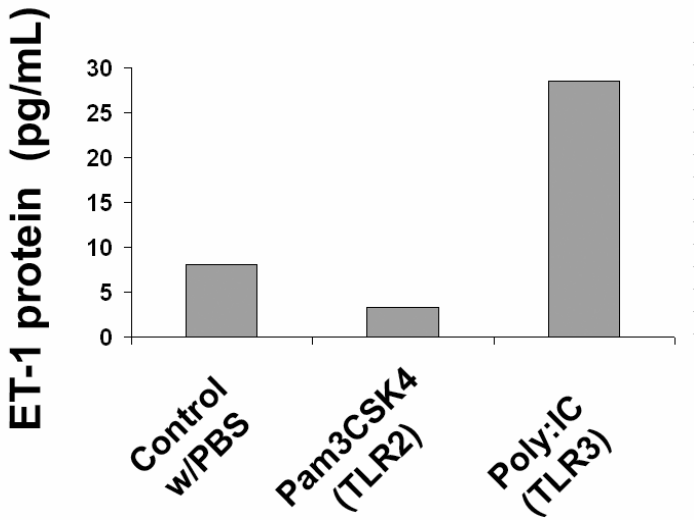
Supplementary Figure S3. Increased CXCL10/IP-10 levels are present in the serum of SSc patients. Serum from SSc patients (n=61, average 66.6) is increased compared to healthy controls (n=25, average 33.3, $p<0.005$).



Supplementary Figure S4. PolyI:C stimulates production of 21 aa bioactive ET-1 peptide in HDMECs and HPAECs. Primary HDMECs and HPAECs were stimulated with poly(I:C) 25ug/ml for 24 hours and ET-1 21 aa peptide levels were quantified by ELISA. Both HDMECs and HPAECs had increased ET-1 levels compared to untreated cells. Experiments were done in triplicate and results depict the averages of 4 experiments with HDMEC and 2 experiments with HPAECs. Compared to untreated cells, poly(I:C) stimulated a 23-fold increase in ET-1 secretion in HDMECs (mean 164, * $p<0.0001$)and over 20-fold increase in HPAEC (mean 42 , **$p=.01$) .



Supplementary Figure S5. Regulation of Endothelin-1 mRNA expression by TLR ligands in scleroderma dermal fibroblasts. Dermal fibroblasts from SSc patients with dcSSc (□) or lSSc (■) were treated with TLR ligands and TGFβ as positive control as described in the methods and END1 mRNA analyzed by qRT-PCR. Fold-change shown is normalized to mRNA expression by the corresponding unstimulated cells.



Supplementary Figure S6. In vivo effect of poly(I:C) on ET-1 21 aa serum concentration. Circulating levels of bioactive ET-1 peptide were increased following 7-day infusion of poly(I:C) (TLR3 ligand, 0.5mg/ml) but not in mice treated with PBS vehicle alone (Control w/ PBS), or Pam3CSK4 (TLR1,2 ligand, 1mg/ml). Graph depicts a representative experiment in which osmotic pumps were placed subcutaneously with TLR ligands or PBS control to infuse over 7 days. Mice were sacrificed on day 7 and serum was collected and ET-1 21 aa concentrations determined by ELISA.