

## **SUPPLEMENTARY MATERIAL**

### **Detailed Methods**

#### **Cell culture**

Human aortic endothelial cells (HAECs, passages 5 to 7) were purchased from Clonetics (Allschwil, Switzerland) and grown in fibronectin-coated 75cm<sup>2</sup> flasks in optimized endothelial growth medium-2 (EGM-2, Clonetics, Allschwil, Switzerland) supplemented with 10% FBS. The cells were detached by using Trypsin/EDTA for 2 minutes and reseeded in fibronectin-coated 10 cm-cell culture dishes or 6 multiwell plates. HAECs were cultured in EGM-2 containing 2% FBS and exposed for 3 days either to normal glucose (5 mmol/L) or high glucose concentration (25 mmol/L). Mannitol was purchased from Sigma Aldrich (St Louis, USA) and used at the final concentration of 25 mmol/L as osmotic control.

#### **Reagents**

Streptozotocin (STZ), citrate, norepinephrine (NE), acetylcholine (Ach), sodium nitroprusside (SNP) and polyethylene glycol-superoxide dismutase (PEG-SOD) were purchased from Sigma Aldrich (St Louis, USA).

#### **Western Blotting**

After incubation cells were washed twice with PBS and lysed for immunoblotting (150 mmol/L sodium chloride, 50 mmol/L Tris, 1 mmol/L sodium fluoride, 1 mmol/L DTT, 1 mmol/L EDTA, 10 µg/µL leupeptin, 10 µg/µL aprotinin, 0.1 mmol/L sodium vanadate, 1 mmol/L PMSF, and 0.5% NP-40). Equal amounts (20 µg) of samples were subjected to SDS-PAGE gel for electrophoresis followed by semidry transfer onto Immobilon-P filter papers (Millipore, Billerica, USA). The membranes were blocked with 5% dry milk or 5% BSA in PBS-Tween buffer (0.1% Tween 20; pH 7.5) for 60 minutes and incubated with anti-Shc and anti-eNOS (BD Transduction Laboratories™, Franklin Lakes, USA), anti-Ser-36 phospho-p66<sup>Shc</sup> (Cell Signaling Technology, Danvers, USA), anti-Pin1, anti-cytochrome *c*, anti-NF-κB p65, anti-Histone 1, anti-COX4 (Santa Cruz Biotechnology, Nunningen, Switzerland), anti-Ser-116 phospho eNOS and GAPDH (Millipore, Billerica, USA). Anti-rabbit and anti-mouse secondary antibodies were bought from GE Healthcare (Buckinghamshire, United Kingdom). The immunoreactive bands were detected by an enhanced chemiluminescence system (Millipore, Billerica, USA). Related signals were quantified using a Scion image software (Scion Corp, Frederick, USA).

#### **Real time-PCR**

Conversion of the total cellular RNA to cDNA was carried out with Moloney murine leukemia virus reverse transcriptase and random hexamers (Amersham Bioscience, Piscataway, USA) in a final

volume of 33  $\mu\text{L}$ , using 1  $\mu\text{g}$  of cDNA according to the manufacturer's recommendations. Real-time PCR amplification was performed in an MX3000P PCR cycler (Stratagene) using the SYBR Green JumpStart kit (Sigma Aldrich, St Louis, USA) in 25  $\mu\text{L}$  final reaction volume containing 2  $\mu\text{L}$  cDNA, 10 pmol of each primer, 0.25  $\mu\text{L}$  of internal reference dye, and 12.5  $\mu\text{L}$  of JumpStart Taq ReadyMix (buffer, dNTP, stabilizers, SYBR Green, Taq polymerase, and JumpStart Taq antibody). GAPDH or TBP was used as endogenous control for normalizing RNA concentration. The amplification program consisted of 1 cycle at 95 °C for 10 min, followed by 40 cycles with a denaturing phase at 95 °C for 30 s, an annealing phase at 60 °C for 30 s, and an elongation phase at 72 °C for 30 s. Differences in Ct values between test gene and endogenous controls (GAPDH and TBP,  $\Delta\text{Ct}$ ) were calculated and used for statistical analysis.

### **Pin1 promoter methylation**

Genomic DNA was digested with methylation sensitive enzyme (Ms) or with methylation dependent enzyme (Md) and also with both methylation sensitive and dependent enzymes (Msd). A predesigned primer for human Pin1 promoter was obtained from SABiosciences (Frederick, MD, USA) and Real-Time PCR reactions were performed according to the manufacturer's instructions as previously reported.<sup>1</sup>

### **Pin1 activity assay**

Pin1 activity was assessed by using a commercially available kit (R&D Systems, Inc., Minneapolis, USA).

### **Measurements of mitochondrial $\text{O}_2^-$ and NO by ESR spectroscopy**

Mitochondrial  $\text{O}_2^-$  generation and NO release were assessed by electron spin resonance (ESR) spectroscopy. Mitochondrial  $\text{O}_2^-$  generation in HAECs and mouse aortas was assessed by using the spin trap 1-hydroxy-3-methoxycarbonyl-2,2,5,5-tetramethyl-pyrrolidine (CMH). Twenty five  $\mu\text{g}$  of isolated mitochondria were resuspended in Krebs Hepes Buffer (KHB, PH 7.35) containing desferoxamine (25  $\mu\text{M}$ ) and diethyldithiocarbamate (DETC, 5  $\mu\text{M}$ ).  $\text{O}_2^-$  production was determined by following the oxidation of 1-hydroxy-3-carboxy-pyrrolidine (CP-H) to paramagnetic 3-carboxy-proxyl (CP). Endothelial NO release from HAECs was examined by ESR spectroscopy with the use of the spin-trap colloid  $\text{Fe}(\text{DETC})_2$ . Briefly, cells were washed and resuspended in 900  $\mu\text{L}$  of Krebs-HEPES buffer (37°C), 300  $\mu\text{L}$  of colloid  $\text{Fe}(\text{DETC})_2$  (final concentration 285  $\mu\text{mol/L}$ ) was added to each sample and incubated at 37°C for 60 minutes. Samples were scraped on ice, cells were collected from each sample and tubes were centrifuged at 4°C at 5000 rpm for 10 minutes. Cells were resuspended and aspirated into 1 mL syringes which were frozen immediately in liquid nitrogen. Signals were quantified by measuring the total amplitude after correction of baseline and subtraction of background signals.

### **Small interfering RNA transfection**

Transient transfection with commercially available human and mouse Pin1 siRNAs (Santa Cruz Biotechnology, Nunningen, Switzerland) was performed using Lipofectamine Reagent (Invitrogen, Basel, Switzerland). As a control, predesigned scrambled siRNA was used (Microsynth, 5' UAC ACA CUC UCG UCU CU dTdT 3'). Cytotoxicity was assessed by a colorimetric assay to detect lactate dehydrogenase (LDH; Roche, Basel, Switzerland).

### **Immunoprecipitation**

HAECs and segments of mouse aorta were lysed in ice-cold lysis buffer containing 50 mmol/L Tris-HCl, pH 7.4, 100 mmol/L NaF, 15 mmol/L  $\text{Na}_4\text{P}_2\text{O}_7$ , 1 mmol/L  $\text{Na}_3\text{VO}_4$ , 1% Triton X-100, and 1 mmol/L phenylmethylsulfonyl fluoride. Lysates were centrifuged at 10,000 *g* to remove insoluble material. For immunoprecipitation, precleared lysates were incubated with anti-Pin1 and anti-caveolin-1 antibodies (Santa Cruz Biotechnology, Nunningen, Switzerland) overnight at 4°C. Lysates were precleared by incubation with 50  $\mu\text{L}$  of protein A/G-agarose (Santa Cruz Biotechnology, Nunningen, Switzerland) for 2 h at 4°C with rocking. Agarose beads were pelleted by centrifugation at 1000 *g*. Immunoprecipitated proteins were eluted from the beads by boiling for 5 min in SDS sample buffer and immunoblotted with anti-p66<sup>Shc</sup> (1:5000), anti-eNOS antibody (1:1000), and anti-NF- $\kappa\text{B}$  p65 (1:1000). Bound antibody was visualized using an enhanced chemiluminescence system (Millipore, Billerica, USA) after incubation of the blot with peroxidase-conjugated secondary antibody for 1 h.

### **Isolation of mitochondrial and cytosolic fraction**

HAECs and thoracic aortas were suspended in the mitochondrial buffer containing 10mM MOPS (pH 7.2), 10 mM KCl, 1.5 mM  $\text{MgCl}_2$ , 1 mM EDTA, 10  $\mu\text{g}/\text{mL}$  leupeptin, 10  $\mu\text{g}/\text{mL}$  aprotinin, and 0.25 M sucrose, and gently homogenized with a Dounce homogenizer (30 strokes). The homogenate was centrifuged at 750 *g* for 10 minutes at 4°C to remove nuclei and unbroken cells, and the supernatant was centrifuged at 10,000 *g* for 15 minutes. The resultant mitochondrial pellet was resuspended in lysis buffer (see Western blot for details) while the supernatant was collected as the cytosolic fraction.

### **Immunocytochemistry**

HAECs were washed with PBS, fixed in 4% PFA for 20 minutes and blocked with 1% BSA for 1 h. Cells were incubated for 1 h at room temperature with anti-cytochrome *c* antibodies (1:400, Santa Cruz Biotechnology, Nunningen, Switzerland). Fluorescence-labeled secondary antibody (mouse IgG 488, Molecular Probes, USA) was applied for 1 h. Successively, slides were incubated with 4,6 diamidino-2-phenylindole hydrochloride (DAPI) solution (Vector Laboratories, CA, USA) for 10 minutes and images were captured at 60X magnification with a confocal microscope (Leica).

### **Mitochondrial network extent**

A semiquantitative scale was used to rate the extent of mitochondrial networks in HAECs under different experimental conditions, as previously reported. Images were captured at 60X magnification with a confocal microscope (Leica).

### **Mitochondrial DNA damage detection**

Briefly, each 30 ng of total DNA was used in two PCR reactions, one amplifying a short 117-bp product of mtDNA and the other amplifying a long fragment of 10-kb product of mtDNA. The amount of PCR products from the 10-kb amplification reflects the quality of the template mtDNA, whereas the 117-bp amplification serves as an internal control for template concentration. PCR reaction was run with the following conditions: 10-kb PCR, denaturation at 94°C for 15 seconds, annealing, and extension at 68°C for 12 minutes for 20 amplifying cycles, followed by a final 10 minutes extension; 117-bp PCR, 94°C for 30 seconds, 60°C for 45 seconds, 72°C for 45 seconds for 20 cycles, and followed by final extension at 72°C for 10 minutes. mtDNA integrity was determined by calculating the ratios of amount of 10 kb products to that of 117-bp products.

### **p66<sup>Shc</sup> mitochondrial translocation and cytochrome *c* release**

Mitochondrial and cytosolic proteins (30 µg) were separated on 10% and 15% polyacrylamide gel and then transferred to nitrocellulose membranes. The membranes were blocked in 5% milk, followed by incubation with antibodies against p66<sup>Shc</sup> (BD Transduction Laboratories™, Franklin Lakes, USA) and cytochrome *c* (Santa Cruz Biotechnology, Nunningen, Switzerland). After washing, the membranes were incubated with anti-rabbit and anti-mouse IgG horseradish peroxidase-conjugated antibodies in blocking buffer for 1 hour, washed, and developed using a Western blot chemiluminescence detection kit (Millipore, Billerica, USA). Cytochrome *c* release and mitochondrial translocation of p66<sup>Shc</sup> were shown as percentage change of protein content in the cytosol vs. mitochondria and vice versa. GAPDH and COX4 antibodies were used as loading controls for cytosolic and mitochondrial fraction, respectively.

### **Mitochondrial swelling assay**

Forty µg of isolated mitochondria from either HAECs or mouse aortas in swelling buffer (250 mmol/L sucrose, 10 mmol/L MOPS, 5 µmol/L EGTA, 2 mmol/L MgCl<sub>2</sub>, 5 mmol/L KH<sub>2</sub>PO<sub>4</sub>, 5 mmol/L pyruvate, 5 mmol/L malate) were incubated with 150 µmol/L of calcium chloride (CaCl<sub>2</sub>) in a final volume of 200 µL in a 96-well plate for 20 minutes. Absorbance was read every 30 seconds at 520 nm.

### **Nuclear translocation of NF-κB p65**

Nuclear protein was obtained by using a nuclear extraction kit (Active Motif, Rixensart, Belgium) and NF-κB p65 nuclear translocation was assessed by Western blotting and analyzed as the percentage

change of protein content in the nucleus vs. cytosol. GAPDH and Histone 1 antibodies were used as loading controls for cytosolic and nuclear fraction, respectively.

### **NF- $\kappa$ B p65 binding activity**

The DNA binding reaction was carried out with 10  $\mu$ g of nuclear protein in a 96-well plate coated with consensus sequences for NF- $\kappa$ B (GGGACTTTCC) for 1 h at room temperature. After washing, NF- $\kappa$ B p65 antibody (Active Motif, Rixensart, Belgium) was added and incubated for 1 h, followed by incubation with a horseradish peroxidase-conjugated secondary antibody.

### **Animals**

Four to six months male C57BL/6 mice, Pin1 WT mice, and *Pin1*<sup>-/-</sup> mice were used in all the experiments. Homozygous Pin1-mutant mice on a pure C57BL/6 background and Pin WT littermates were provided from Dr. Giannino Del Sal (University of Trieste). Genotyping was performed by PCR on tail DNA, as previously described.<sup>2</sup> Diabetes was induced by a single high dose of streptozotocin (STZ, 180 mg/Kg, via intraperitoneal injection) dissolved in sterile 0.025 M citrate buffer (PH 4.5) and injected within 10 minutes. Control animals received an equal volume of citrate buffer. Mice were housed in temperature-controlled cages (20–22°C), fed ad libitum, and maintained on a 12:12-h light/dark cycle. Tail-cuff systolic blood pressures were measured in all the experimental groups. After 4 weeks all mice were euthanized. Animal experiments were conducted in accordance with the guidelines approved by the Institutional Animal Care Committee of the University of Zürich, Switzerland, University of Rome “Sapienza” and University of Trieste, Italy.

### **In vivo knockdown of Pin1**

In vivo knockdown of Pin1 was performed in male C57BL/6 mice by injecting a predesigned siRNAs targeting mouse Pin1 (Santa Cruz Biotechnology, Nunningen, Switzerland). A scrambled-siRNA was used as a control (Microsynth, 5'-UAC ACA CUC UCG UCU CU dTdT-3'). Amount of Pin1 siRNA was selected based on dose optimization studies (data not shown). The siRNA mix at the final dose of 20  $\mu$ M was incubated with the in vivo-jetPEI delivery reagent (Polyplus Transfection) for 15 min at room temperature, in a final volume of 100  $\mu$ L, as previously reported.<sup>1</sup> Based on time-course studies, Pin1 siRNA was injected intravenously every 5 days for 4 weeks.

### **Tissue harvesting and organ chamber experiments**

Mice were euthanized by intraperitoneal administration of 50 mg/Kg sodium pentobarbital. The entire aorta from the heart to the iliac bifurcation was excised and placed immediately in cold modified Krebs-Ringer bicarbonate solution (pH 7.4, 37°C, 95% O<sub>2</sub>; 5% CO<sub>2</sub>) of the following composition (mmol/L): NaCl (118.6), KCl (4.7), CaCl<sub>2</sub> (2.5), KH<sub>2</sub>PO<sub>4</sub> (1.2), MgSO<sub>4</sub> (1.2), NaHCO<sub>3</sub> (25.1), glucose (11.1), and calcium EDTA (0.026). The aorta was cleaned from adhering connective tissue under a

dissection microscope, and either snap-frozen in liquid nitrogen and stored at  $-80^{\circ}\text{C}$  or used immediately for organ chamber experiments. For isometric tension studies, aorta was cut into 2 mm rings. Each ring was then connected to an isometric force transducer (Multi-Myograph 610M, Danish Myo Technology), suspended in an organ chamber filled with 6 mL Krebs-Ringer bicarbonate solution ( $37^{\circ}\text{C}$ , pH 7.4), and bubbled with 95%  $\text{O}_2$ , 5%  $\text{CO}_2$ . After a 30 minute equilibration period, rings were gradually stretched to the optimal point of their length–tension curve, as determined by the contraction in response to potassium chloride (80 mmol/L). Several rings from the same aorta were studied in parallel. Responses to Ach ( $10^{-9}$  to  $10^{-6}$  mol/L) in the presence or absence of PEG-SOD (150 U/mL) were recorded during submaximal contractions to NE ( $10^{-7}$  mol/L). The cyclooxygenase inhibitor indomethacin was used at the concentration of  $10^{-5}$  mol/L. The NO donor SNP ( $10^{-10}$  to  $10^{-5}$  mol/L) was added to test endothelium-independent responses. Relaxations were expressed as a percentage of the precontracted tension.

### **Immunofluorescence**

Thoracic aorta segments were embedded in optimum cutting temperature and stored at  $-80^{\circ}\text{C}$ . Five-micrometer-thick slices were then cut, fixed with 4% PFA, blocked with 10% goat serum for 30 minutes and incubated overnight at  $4^{\circ}\text{C}$  with anti-VCAM-1 (1:400, AbD Serotec, Oxford, UK) and anti-ICAM-1 antibodies (1:1000, Southern Biotech, Birmingham, Alabama, USA). Fluorescence-labeled secondary antibodies (goat anti-rat IgG 488, Molecular Probes, USA) were applied for 1 h. Successively, slides were incubated with 4,6 diamidino-2-phenylindole hydrochloride (DAPI) solution (Vector laboratories, CA, USA) for 10 minutes and analyzed by fluorescence microscopy (Olympus).

### **Study population**

Thirty seven patients with type 2 diabetes mellitus and twenty age-matched healthy subjects were consecutively recruited at the Cardiology Units of Sant'Andrea Hospital, University "Sapienza" and Catholic University (Rome, Italy). All patients with diabetes were free from overt atherosclerotic cardiovascular disease. Diagnosis of diabetes was made according to current recommendations.<sup>3</sup> Healthy subjects were not taking any medications, blood pressure was  $<140/90$  mm Hg, LDL cholesterol  $<160$  mg/dL, and fasting plasma glucose  $<100$  mg/dL. Furthermore, control subjects were not smoking or had stop smoking for  $> 1$  year before enrolment. On the day of the study, subjects were on a fasting state, refrained from caffeine containing beverages, and avoided exercise for at least 12h prior to the experiment. All participants underwent a full medical history and physical examination. The study protocol was approved by Local Ethics Committee and, in accordance with institutional guidelines, all participants were aware of the investigational nature of the study and gave written consent for their participation.

### **Isolation of peripheral blood monocytes**

Blood was collected in Ficoll tubes (Vacutainer CPT, BD Diagnostics, Franklin Lakes, USA) and centrifuged for 20 min at 1800 g and room temperature. The turbid white layer above the Ficoll containing the mononuclear blood cells was transferred to a clean tube and washed twice with PBS. Subsequently, monocytes were isolated using magnetic CD14-coated beads and magnetic activated cell sorting (MACS, Miltenyi Biotec).

### **Noninvasive Vascular Function Testing**

Brachial artery FMD was measured as previously described.<sup>4</sup> Vascular function was assessed in the morning at rest with subjects in the supine position from at least 15 min, in a quiet air-conditioned room (22–24°C), to minimize the possible negative effect of environmental and physiological influences, including stress. Endothelial-dependent vasodilation was assessed as dilation of the brachial artery in response to increased blood flow in accordance with current guidelines, as previously described. The exams were carried out by two sonographers (L.C and G.S) by using an echo-Doppler machine (Esaote, Philips, General Electric, Siemens). The right brachial artery was located and scanned longitudinally between 5 and 10 cm above the elbow using a linear array transducer with a frequency ranging from 7.5 to 10 MHz. The transducer was held in the same position throughout the scan by an adjustable stereotactic clamp to ensure greater image stability. A sphygmomanometer blood pressure (BP) cuff was positioned on the right forearm 2 cm below the elbow. Right brachial artery was scanned longitudinally between 5 and 10 cm above the elbow, capturing images starting 1 min prior to cuff inflation. The cuff was inflated for 5 min at 250 mmHg and then deflated to induce reactive hyperemia. Endothelium-independent vasodilation was elicited by the administration of low dose (25 µg) of sublingual glyceril-trinitrate (GTN). Recording time frames were 10 min for FMD studies (1 min for baseline, 5 min of ischemic period, 4 min for assessing changes in diameter following reactive hyperemia) and 6 min for GTN-mediated dilation (1 min for baseline, 5 min for assessing changes in diameter following GTN administration).

### **Assessment of urinary 8-isoprostaglandin PGF<sub>2α</sub> levels**

Urinary levels of 8-isoprostaglandin F<sub>2α</sub> were assessed by using a commercially available kit (Cell Biolabs, San Diego, CA, USA).

### **Plasma levels of VCAM-1, ICAM-1 and MCP-1**

Plasma levels of adhesion molecules VCAM-1, ICAM-1 and MCP-1 were determined by using commercially available kits (Abcam, Cambridge, MA).

## Statistical analysis

All data are presented as means  $\pm$  SEM. Statistical comparison were made by using the Student t test for unpaired data and 1-way ANOVA, followed by Bonferroni's post-hoc test, when appropriate. The between-variable correlations were measured by Spearman's analysis. Linear regression models were generated to test the independent association of Pin1 expression/activity with endothelial dysfunction (FMD), oxidative stress (8-isoPGF<sub>2 $\alpha$</sub> ) and vascular inflammation (VCAM-1, ICAM-1 and MCP-1), respectively. Probability values less than 0.05 were considered statistically significant. All analyses were performed with GraphPad Prism (version 5.0) and SPSS (version 20) softwares.

## References

1. Paneni F, Mocharla P, Akhmedov A, Costantino S, Osto E, Volpe M, Lüscher TF, Cosentino F. Gene silencing of the mitochondrial adaptor p66(Shc) suppresses vascular hyperglycemic memory in diabetes. *Circ Res.* 2012;111:278-89.
2. Atchison FW, Capel B, Means AR. Pin1 regulates the timing of mammalian primordial germ cell proliferation. *Development* 2003; 130:3579-3586.
3. ESC Guidelines on diabetes, pre-diabetes, and cardiovascular diseases developed in collaboration with the EASD: the Task Force on diabetes, pre-diabetes, and cardiovascular diseases of the European Society of Cardiology (ESC) and developed in collaboration with the European Association for the Study of Diabetes (EASD). *Eur Heart J.* 2013;34:3035-87.
4. Ghiadoni L, Faita F, Salvetti M, Cordiano C, Biggi A, Puato M, Di Monaco A, De Siati L, Volpe M, Ambrosio G, Gemignani V, Muiesan ML, Taddei S, Lanza GA, Cosentino F. *J Hypertens.* 2012;30:1399-405.



**Table S1.** Body weight, glucose levels, blood pressure and lipids in the different experimental groups. Results are presented as mean  $\pm$  SEM. \*,  $p < 0.05$  vs. WT control.

	<b>WT Control</b> n=10	<b>WT Diabetic</b> n=12	<b><i>Pin1</i><sup>-/-</sup> Control</b> n=8	<b><i>Pin1</i><sup>-/-</sup> Diabetic</b> n=7
Body weight (g)	27.1 $\pm$ 4.6	24.4 $\pm$ 3.6	26.7 $\pm$ 3.9	24.6 $\pm$ 3.3
Blood glucose (mmol/L)	6.7 $\pm$ 1.5	28.0 $\pm$ 3.8*	6.3 $\pm$ 1.1	27.8 $\pm$ 2.0*
Blood pressure (mmHg)	101 $\pm$ 11	98 $\pm$ 9	103 $\pm$ 10	100 $\pm$ 12
Cholesterol (mmol/L)	2.1 $\pm$ 0.4	2.7 $\pm$ 0.4*	1.9 $\pm$ 0.2	2.5 $\pm$ 1.3*
Triglycerides (mmol/L)	1.2 $\pm$ 0.7	1.8 $\pm$ 1.5	1.0 $\pm$ 0.8	1.6 $\pm$ 1.0

**Table S2.** Demographics, laboratory parameters and medications in the study population. Values are expressed as mean±SD. P-value refers to Student *t* and Chi<sup>2</sup> tests. BMI, body mass index; FPG, fasting plasma glucose; ACE-I, angiotensin converting enzyme inhibitors, ARB, angiotensin receptor blockers. NS, not significant; N/A, not applicable.

	<b>Controls (n=20)</b>	<b>T2DM (n=37)</b>	<b>P value</b>
<b>Demographics</b>			
Age (years)	46±12	50±14	0.27
Gender (F:M)	12:8	17:20	0.43
BMI (kg/m <sup>2</sup> )	23±3	29±4	<0.001
Waist circumference (cm)	83±12	93±13	0.04
Systolic blood pressure (mmHg)	112±10	130±15	<0.001
Diastolic blood pressure (mmHg)	73.2±10	78.8±6	0.02
<b>Laboratory parameters</b>			
FPG (mg/dL)	80±6	187±65	<0.001
HbA <sub>1c</sub> (%)	5.1±0.4	8.2±2.2	<0.001
Triglycerides (mg/dL)	87±26	160±136	0.07
HDL cholesterol (mg/dL)	59±12	49±12	0.02
LDL cholesterol (mg/dL)	100±23	115±36	0.22
Total cholesterol (mg/dL)	183±23	182±45	0.95
<b>Medications (%)</b>			
Aspirin (%)	-/-	8 (21.6)	N/A
Statins (%)	-/-	11 (29.7)	N/A
ACE-I/ARBs (%)	-/-	18 (48.6)	N/A
Beta-blockers (%)	-/-	4 (10.8)	N/A
Diuretics (%)	-/-	8 (21.6)	N/A
Insulin (%)	-/-	10 (27.0)	N/A
Hypoglycemic agents (%)	-/-	32 (86.4)	N/A

**Table S3.** Values are expressed as mean±SD. P-value refers to Student *t* tests. FMD, flow-mediated dilation; 8-isoPGF<sub>2α</sub>, 8-iso-prostaglandin F<sub>2α</sub>; VCAM-1, vascular cell adhesion molecule-1; ICAM-1, intercellular cell adhesion molecule-1; MCP-1, monocyte chemoattractant protein-1.\* n=12 for controls.

	<b>Controls (n=20)</b>	<b>T2DM (n=37)</b>	<b>P value</b>
Flow-mediated dilation* (%)	8.8±1	5.8±3	0.002
Nitroglycerin-mediated dilation* (%)	14±4	13±3	0.77
8-isoPGF <sub>2α</sub> (pg/mg creatinine)	175±14	465±103	<0.001
VCAM-1 (ng/mL)	239±64	440±27	<0.001
ICAM-1 (ng/mL)	151±49	271±40	<0.001
MCP-1 (ng/mL)	147±35	239±47	<0.001

**Table S4.** Linear regression analysis adjusted for age, gender, BMI, blood pressure and HDL cholesterol showing the independent association of Pin1 expression and activity with endothelial dysfunction (FMD), oxidative stress (8-isoPGF<sub>2α</sub>) and soluble adhesion molecules VCAM-1, ICAM-1 and MCP-1. \*p<0.05; \*\*p<0.01. FMD, flow-mediated dilation; 8-isoPGF<sub>2α</sub>, 8-iso-prostaglandin F<sub>2α</sub>; VCAM-1, vascular cell adhesion molecule-1; ICAM-1, intercellular cell adhesion molecule-1; MCP-1, monocyte chemoattractant protein-1. β indicates regression coefficient.

	<b>Pin1 mRNA</b>	<b>Pin1 activity</b>
Flow-mediated dilation	β= -0.32*	β= -0.25*
8-isoPGF <sub>2α</sub>	β= 0.24*	β= 0.27*
VCAM-1	β= 0.42**	β= 0.35*
ICAM-1	β= 0.31*	β= 0.29*
MCP-1	β= 0.22*	β= 0.25*