SUPPLEMENTAL MATERIAL

DETAILED METHODS

Exclusion criteria and study design

We aimed to recruit a population of obese subjects at the very early stage of the disease to dissect the independent role of obesity in endothelial function and to investigate metabolic microvascular dysfunction in its very early stage. The exclusion criteria were: age <18/> 80 years, history or clinical evidence of hypertension, clinical or biochemical evidence of diabetes or endocrine dysfunction, ethanol consumption >60 g per day, dyslipidemia, smoking habits, renal or liver impairment, menopausal status, and any documented CV disease. Patients assuming any chronic pharmacologic therapy were also excluded, as well as those taking non-steroidal anti-inflammatory drugs within the last 48 hours from surgery. Control subjects' body mass index (BMI) had to be below 30 Kg/m^2. No blinding procedures were adopted in the study design. Given the large number of hypothesis-driven experiments reported in the study and their novelty that makes preliminary data unavailable, no formal sample size calculation was performed for each assay. However, the number of subjects/samples included in each assay was defined based on our laboratory experience and previous studies. As some experiments required a substantial amount of material, for each experiment, the experimental groups' size was decided based on sample availability and quality and the experiment's feasibility. The obtained groups' sizes aligned with previous studies [8, 81-87]. In the case of exploratory or confirmatory experiments, we decided to analyze smaller groups. In particular, we adopted a small sample size (n=5 or n=6) for experiments exploring opposite phenotypes (*e.g.* Young Nonobese vs Old Obese) and a minimal sample size (n=3) for confirmatory experiments (see, e.g. **Supplementary Figure 1** and **Supplementary Figure 4**).

Clinical history and visit

We obtained complete medical history via interview or from the patient clinical records (including smoking history and current or previous use of medications). Blood pressure and heart rate were measured with an oscillometric device three times, with the participant in a seated position, after 5 minutes of rest in a quiet room. The average of the three readings was used for the analyses. High and weight were measured with the participant wearing light clothes and without shoes. The BMI was calculated as weight (kg) divided by squared height (m^2). HOMA-IR was calculated as [fasting glucose (mmol/l) x fasting insulin (pmol/l)]/135.

Biochemistry

Fasting blood samples were collected from each patient before the surgical procedure. Plasma glucose, insulin, total serum cholesterol, triglycerides and HDL-cholesterol levels were assessed by photometric analysis (Roche Modular Series, Indianapolis, IN, USA) according to standard procedures (https://wwwn.cdc.gov/nchs/data/nhanes/2019-2020/manuals/2020-MEC-Laboratory-Procedures-Manual-508.pdf); LDL-cholesterol levels were calculated according to the Friedewald formula [88].

Preparation of small arteries, structural and functional parameters

Biopsies of subcutaneous adipose tissue were recovered during the surgical procedures and processed to isolate small resistance arteries (150-300 µm lumen diameter) as previously described [81]. After isolation, arteries were mounted in a pressurised myograph (DMT 114P, Danish Myo Technology A/S, Hinnerup-Denmark) to assess their structural and functional characteristics. Vessels were then rested in a Krebs [89] solution at 37°C and perfused ad +60

mmHg. KCl (125 mM) + norepinephrine (1 μM, Sigma Aldrich SRL, Milano-Italy) were added to the solution to assess vessel viability and vitality. The obtained maximal contractility response was used to measure the minimum vessel diameter (D_m). KCl solution was then washed out with Krebs solution, and vessels were left quiescent for at least 45 minutes before starting the functional experiments.

Endothelium-dependent and -independent relaxations were assessed by cumulative concentrations of acetylcholine (ACh, 0.001-100 μM, Sigma Aldrich SRL, Milano-Italy) and sodium nitroprusside (0.01-100 μM, Sigma Aldrich SRL, Milano-Italy) in vessels precontracted with norepinephrine (1 μM, Sigma Aldrich SRL, Milano-Italy). Vasodilation response was described as % changes to the maximal vessel diameter (D_M). D_m was subtracted to both terms of the equation:

$$(d-D_m)/(D_M-D_m)*100,$$

where d is the diameter measured when exposed to a specific ACh concentration.

NO availability and the contributions of SIRT1, mtROS and NADPH oxidase to the endothelial dysfunction were investigated by repeating the dose-response curves to acetylcholine after incubation with the NO synthase inhibitor L-NAME (100 μM, 30 min-incubation, Sigma Aldrich SRL, Milano-Italy), the SIRT1 agonist SRT1720 (1 μM, 4-hour incubation; Sigma Aldrich SRL, Milano-Italy), the SIRT1 siRNA sc-40986 (overnight transfection, Santa Cruz Biotechnology, Inc., Dallas-Texas USA), the mitochondrial ROS scavenger MitoTEMPO (1 μM, 30 min-incubation, Sigma Aldrich SRL, Milano-Italy), the whole-cell ROS scavenger tempol (1 μM, 30 min-incubation, Sigma Aldrich SRL, Milano-Italy), the electron transport chain complex I inhibitor rotenone [27] (1 μM, 1-hour incubation, Sigma Aldrich SRL, Milano-Italy) and the NADPH oxidase inhibitor gp91ds-tat (1 μM; DBA, Milano-Italy). Concentration and timing were based on our previous protocols and preliminary data [8, 81, 90]. SIRT1

silencing through sc-40986 was performed by overnight transfecting human small vessels through Lipofectamine RNAiMAX according to the manufacturer's instructions (13778075, Invitrogen, Waltham, MA 02451, USA).

To confirm that a possible recovery of endothelial function after the addition of SRT1720 was due to improved function of the eNOS, SRT1720 and L-NAME were incubated simultaneously. In turn, to verify that the selective inhibition of SIRT1 impairs endothelial function through eNOS, L-NAME was incubated after sc-40986 transfection. L-NAME maximal vasoconstrictor response was calculated as the difference between the maximal vasodilation to ACh and the maximal vasodilation to L-NAME. L-NAME maximal vasoconstrictor response improvement after incubation with SRT1720 was calculated as the difference between L-NAME maximal vasoconstrictor response after and before the incubation with SRT1720.

Similarly, to explore the potential additive effect between restored SIRT1 activity and reduced mtROS production or inhibition of the NADPH oxidase, acetylcholine was repeated after incubation of the vessel with both SRT1720 and MitoTEMPO or SRT1720 and gp91ds-tat.

To eliminate the contribution of the myogenic tone, structural parameters were assessed after placing the vessels in a physiological salt solution without $CaCl_2$ plus 10 mmol/L EGTA [89]. Media thickness and lumen diameter were measured in 3 different points from each small artery to obtain the media-lumen ratio (M/L). Media cross-sectional area (MCSA) was obtained by subtracting the internal from the external cross-sectional areas using outer plus lumen diameters, as previously described [91].

qPCR assay for SIRT1 and mitochondria proteins involved in the mtROS production

Total RNA was extracted using TRIzol Reagent (Invitrogen, Carlsbad, CA) according to the manufacturer's recommendations. Before extraction, vascular samples were lysed by using a Precellys homogenizer. Conversion of total cellular RNA to cDNA was carried out with Moloney murine leukaemia virus reverse transcriptase and random hexamers (Amersham Bioscience, Piscataway, USA) in a final volume of 33 µl, using 1 µg of cDNA. Real-time PCR was performed using the SYBR Select Master Mix (Applied Biosystems, Thermo Fischer Scientific, Zug, Switzerland) on a Quant Studio 5 and 7 cyclers (Life Technologies, Thermo Fischer Scientific, Zug, Switzerland) according to the manufacturer's instructions. Primers are available in the Major resources table. TBP was used as an 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 and an annealing and elongation phase of 1 min at 60˚C. A melting curve analysis was performed after amplification to verify the accuracy of the amplicon. Differences in Ct values between test genes and endogenous controls (TBP, ΔCt) were calculated and used for statistical analysis.

Vascular mtROS and NO production assays

The *in situ* production of mtROS and NO was measured using the fluorescent dyes MitoSOX Red (ThermoFischer Scientific, Milano, Italy) and 4-Amino-5-Methylamino-2',7'-Difluorofluorescein (DAF-FM; Sigma Aldrich SRL, Milano, Italy), respectively. Isolated segment vessels from each patient were cut into 30-µm-thick sections and placed on a glass slide. Three slides per segment were analyzed simultaneously after incubation with Krebs solution at 37°C for 30 min. Krebs-HEPES buffer containing 2 µM MitoSOX Red or 5 µM DAF-FM was then applied to each section and evaluated under fluorescence microscopy. The percentage of arterial wall area stained with the red signal was evaluated using imaging software (McBiophotonics Image J, version 1.53; National Institutes of Health, Bethesda, MD).

To assess the impact of a restored SIRT1 activity on the vascular mtROS and NO production in vessels obtained from Old Obese patients, the MitoSOX Red staining and the DAF-FM staining were repeated after pre-incubation with the SRT1720. To assess the NO impairment and mtROS production induced by selective inhibition of SIRT1, the colourations were repeated on small resistance arteries obtained from the Young Nonobese group after an overnight transfection with sc-40986. The staining was also performed on vessels from the same Young Nonobese at baseline and after an overnight transfection with scrambled siRNA (Santa Cruz Biotechnology, Switzerland), respectively, as control groups.

To provide consistency in our results, in a small sample of patients (n=3 for each group), we adopted highly-specific fluorescence probes [67-69]: ENZ-51013 ROS-ID® NO Detection kit (Enzo Life Sciences Inc, Milano, Italy; Detection Reagent diluted 1:400 as per manufacturer instruction) and 10 μM Mito peroxy yellow-1 (MITOPY1; Bio-Techne srl, Milano, Italy). Young Nonobese vessels stimulated with L-Arginine (as NO inducer) and Old Obese vessels stimulated with high-dose (20 μM) rotenone were used as a positive control. Old Obese vessels stimulated with c-PTIO (as NO scavenger) and Young Nonobese vessels stimulated with mitoTEMPO (as mtROS scavenger) were used as a negative control. After incubation, prepared vessels were immediately mounted on coverslips and observed under a fluorescent/confocal microscope. For each figure, representative images were chosen as a balance between the high quality of the image and the most accurate representation of the mean value for each experimental group.

Western blot

Specimens of small visceral arteries were homogenized in radioimmunoprecipitation assay buffer (RIPA buffer, R0278, Sigma-Aldrich, St. Louis, MO 63103, USA) supplemented with

protease inhibitor cocktails (P8340, Sigma-Aldrich, St. Louis, MO 63103, USA) with a polytron homogenizer and centrifuged at 13.000× g at 4°C for 20 min. The resulting supernatants were separated from pellets and stored at -20°C. Proteins' concentration was measured using Bradford method (B6916, Sigma-Aldrich, St. Louis, MO 63103, USA).

An equal amount of proteins (30 µg) were diluted in 4x Laemmin Sample Buffer and heated at 70°C for 10 min. Proteins were separated on 8% SDS-PAGE gel and then transferred to Amersham Protan Nitrocellulose 0.45 µm (GE10600002, GE Healthcare, Marlborough, MA, USA). After blocking the membrane using BSA 5% in TTBS (TBS-Tween-20 0.1%) for 1 h at room temperature, blots were washed three times in TTBS and incubated overnight at 4°C with anti-SIRT1 diluted 1:1000 (SIRT1(H-300): sc-15404, Santa Cruz Biotechnology, Dallas, TX 75220 USA) and anti-β-Actin diluted 1:1000 (A2066 Sigma-Aldrich, St. Louis, MO 63103, USA). Following three washes with TTBS, blots were incubated with secondary antibody Anti-Rabbit IgG (whole molecule–Peroxidase antibody produced in goat; 1:80.000, A0545, Sigma-Aldrich Louis, MO 63103, USA) for 1 h at room temperature. The bands were detected by incubating the nitrocellulose membranes with Clarity MAX Western ECL Substrate for 5 minutes and acquired using ChemiDoc Imaging System (Bio-Rad Laboratories).

Mitochondria isolation and swelling assay

Mitochondria swelling assay was performed to characterize further the protection induced by SIRT1 function on the mitochondria functional and structural integrity. Indeed, mitochondria resistance to swelling might be considered a summary measure of mitochondria health. Thus, its results are important for accurately observing how the SIRT1 pathway modulates the mitochondria homeostasis.

Vessels were suspended in the mitochondrial buffer containing 250 mmol/L sucrose, 10 mmol/L MOPS, 5 µmol/L EGTA, 2 mmol/L MgCl2, 5 mmol/L KH2PO4, 5 mmol/L pyruvate, 5 mmol/L malate, 10 µg/mL leupeptin, and 10 µg/mL aprotinin, 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 mitochondrial buffer.

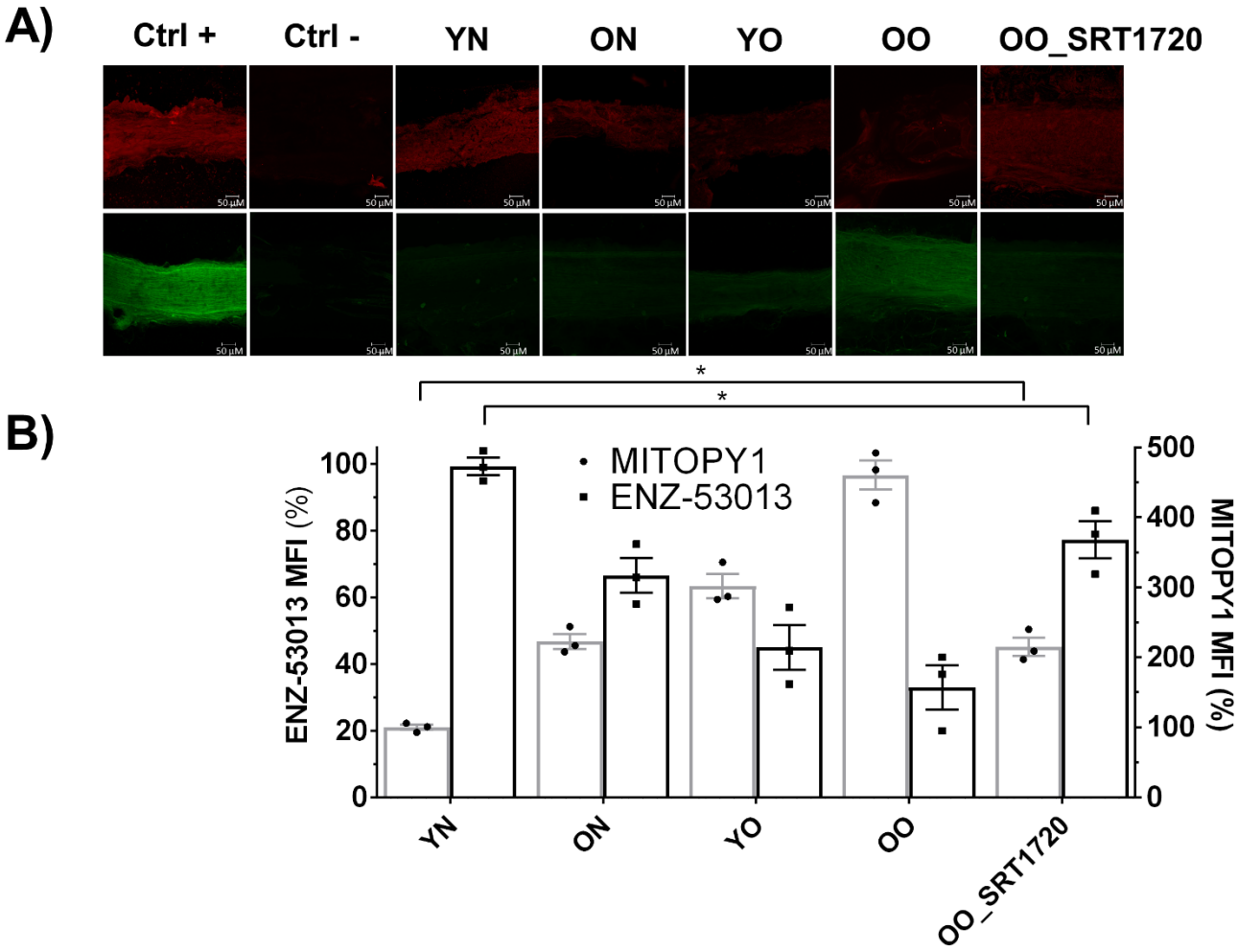
Forty µg of isolated mitochondria in mitochondrial buffer were incubated with 150 µmol/L of calcium chloride ($CaCl_2$) in a final volume of 200 µL in a 96-well plate for 20 minutes. Light scattering measured through absorbance at 520 mm was read every 30 seconds at 520 nm. Calcium stimulates the permeability of the inner mitochondrial membrane, namely by opening the mitochondrial permeability transition pore. This translates into a decreased light absorbance. In healthy mitochondria, there is no substantial difference in the light absorbance because Calcium is not able to induce a significant swelling. When the mitochondrion is damaged, a relevant difference is observed [92, 93]. Absolute values of mitochondrial swelling assay do not allow for discrimination between healthy and disease, as they are generally reported compared to a healthy group [92]. Also, their absolute value in terms of absorbance decrease depends on the solution's concentration [94].

Chromatin immunoprecipitation (ChIP) assay

Chromatin immunoprecipitation was performed in human vascular samples using the Magna ChIP Assay Kit (Millipore, Billerica, USA), according to the manufacturer's instructions. Briefly, human vessels were fixed for 10 minutes with 37% paraformaldehyde. After stopping cross-linking by adding 0.1 M glycine, the tissues were sonicated and centrifuged. ChIP was performed using 10 µg of anti-SIRT1 (Millipore, Billerica, USA) and equivalent amounts of

mouse IgG (Millipore, Billerica, USA) as a negative control. Washes and elution of the IP DNA were performed according to the Magna ChIP protocol. Quantifications of Sirt1 binding on $p66^{Shc}$ promoter, ArgII promoter and Sirt3 promoter were performed by Real time PCR (primers are available in the major resource table). Quantifications were performed using the comparative cycle threshold method and are reported as the n fold difference in antibody-bound chromatin against the input DNA.

198 **SUPPLEMENTAL FIGURES AND FIGURES LEGENDS**

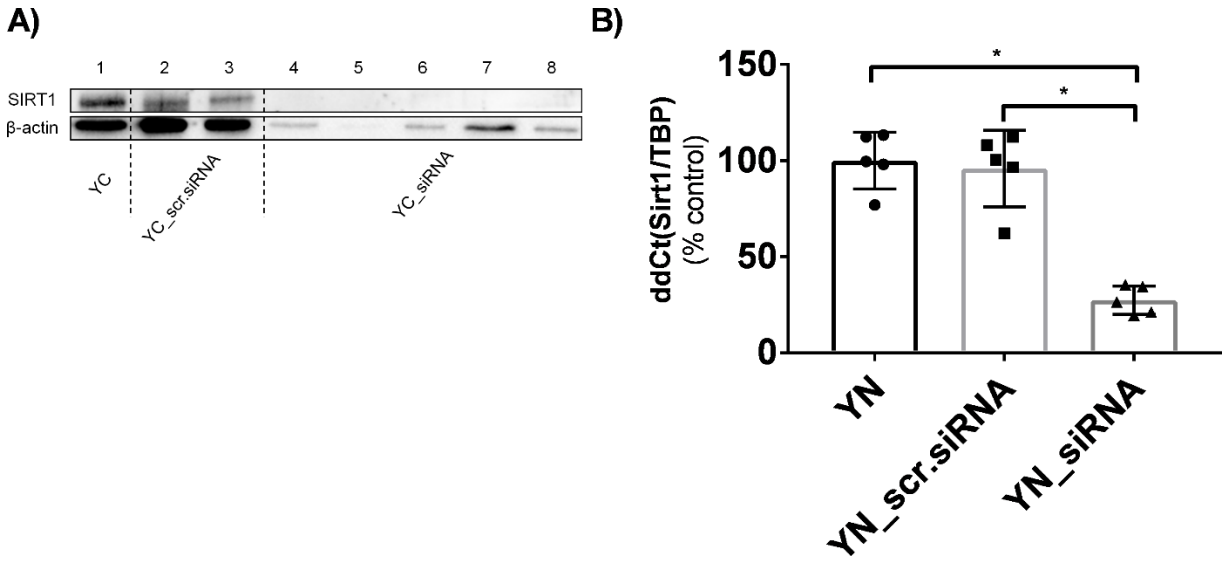


199

200 **Supplementary Figure 1. High-specific fluorescent probes assay**

201 A) Differences in NO levels (red staining) and mtROS (green staining) assessed by ENZ-53013
202 and MITOPY1 fluorescence in the Young Nonobese, Old Nonobese, Young Obese, Old Obese
203 and Old Obese after incubation with SRT1720 (n=3 for each group). Young Nonobese vessels
204 stimulated with L-Arginine (as NO inducer) and Old Obese vessels stimulated with high-dose
205 rotenone were used as a positive control. Old Obese vessels stimulated with c-PTIO (as NO
206 scavenger) and Young Nonobese vessels stimulated with mitoTEMPO (as mtROS scavenger)
207 were used as a negative control. B) Data are presented as mean±SEM and were compared by
208 the Kruskal-Wallis test. Fluorescence is calculated as mean fluorescence intensity (MFI) and

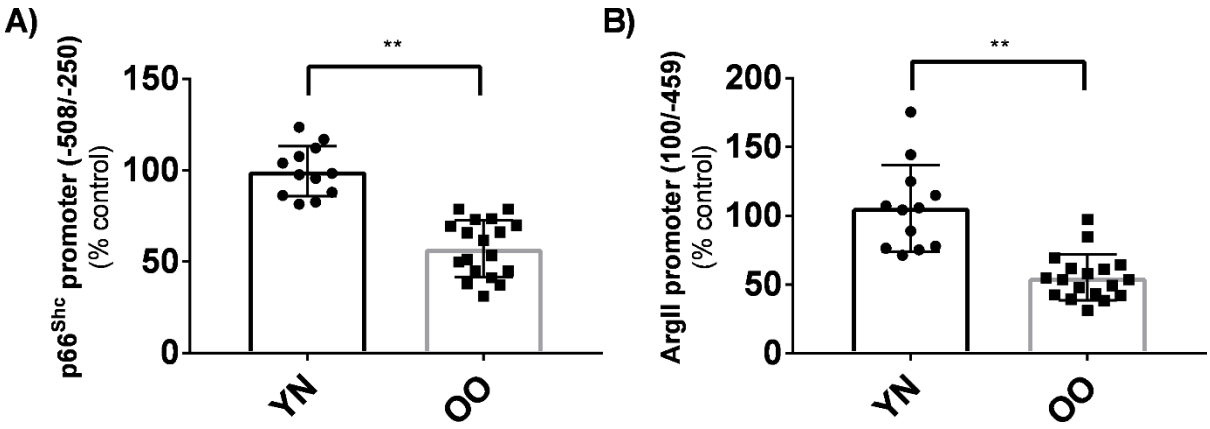
209 expressed as % of the Young Nonobese group. Original magnification is 20 x. *ON: Old*
210 *Nonobese; OO: Old Obese; YN: Young Nonobese; YO: Young Obese.*



211

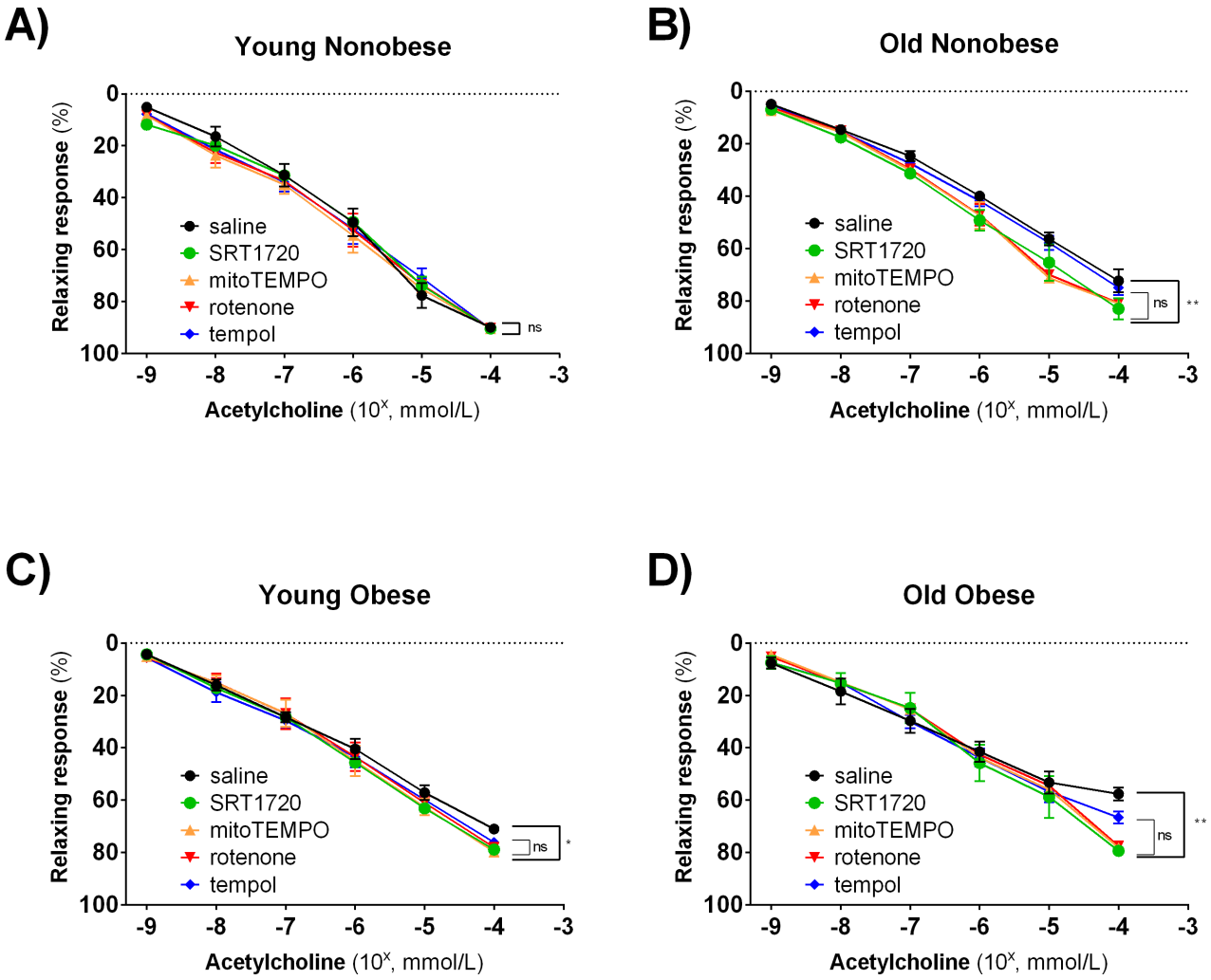
212 **Supplemental Figure 2. Confirmatory Western Blot and qPCR of Young Nonobese**
213 **vessels treated with sc-40986.**

214 **A)** Protein expressions (Young Nonobese: n=1, Young Nonobese_scr.siRNA: n=2; Young
215 Nonobese_siRNA: n=5) and **B)** qPCR expression of SIRT1 in small vessels' pools (n=5 each
216 group) from the same Young Nonobese subjects of the functional and fluorescence staining
217 experiments (**Figure 5A** and **5B-C**, respectively). Results are expressed as % to control. Data
218 are presented as mean±SD and were compared by the Kruskal-Wallis test with Dwass-Steel-
219 Critchlow-Flinger post-hoc test. A p-value <0.05 was considered significant. *: p<0.05.**:
220 p<0.01. *YN: Young Nonobese.*



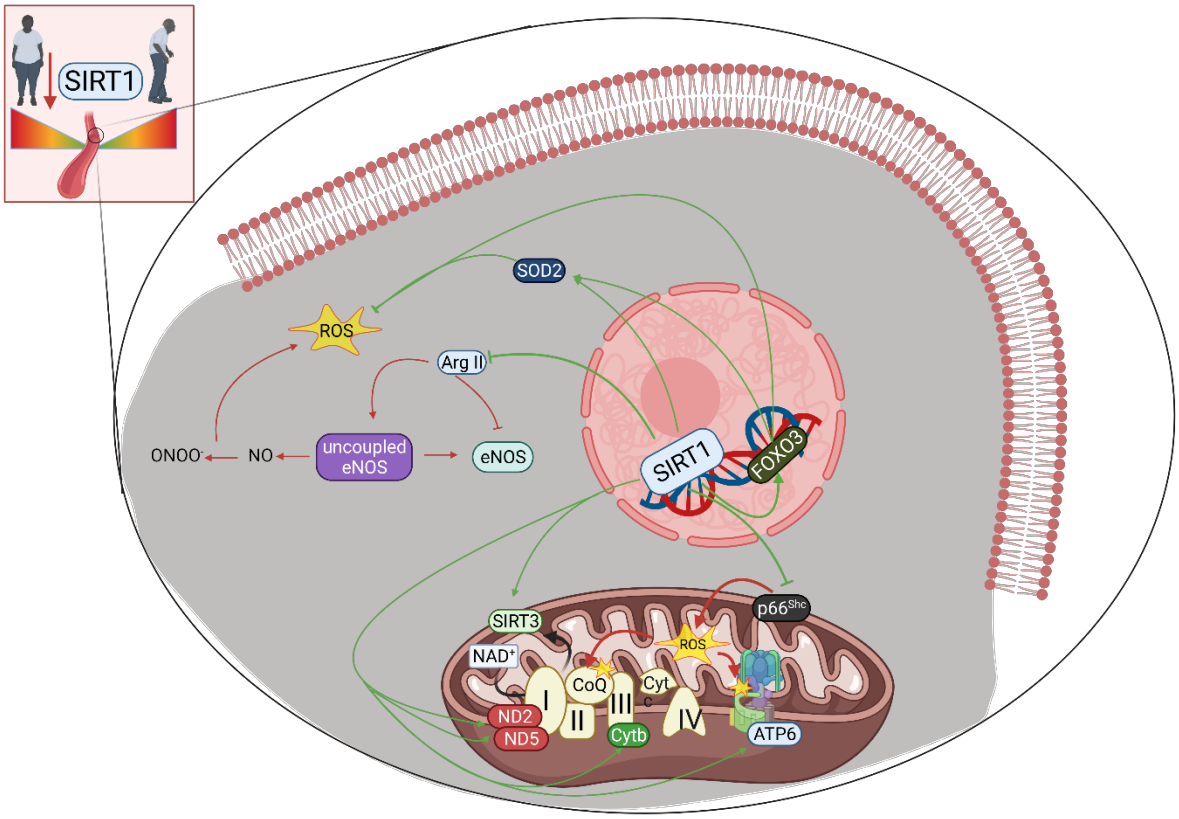
Supplementary Figure 3. SIRT1 binding to promoter region p66^{Shc} (A) and Arginase II (B).

qPCR after ChIP assay showing binding of SIRT1 on the promoter region of p66^{Shc} (A) and Arginase II (B) genes in Young Nonobese (n=12) and Old Obese (n=18) patients. Shapiro-Wilk test was adopted to assess normality. Binding to the promoter of Arginase II was natural log-transformed for the means of the analyses. Data are presented as mean±SD and were compared by independent samples Student's t-test. A p-value <0.05 was considered significant. *: p<0.05.**: p<0.01. *ArgII: Arginase II; OO: Old Obese; YN: Young Nonobese.*



Supplementary Figure 4. Comparison of SRT1720, mitoTEMPO, rotenone and tempol rescuing microcirculatory dysfunction in the four groups.

A-D) Relaxing response to cumulative concentration to Ach in vessels precontracted with norepinephrine in the four groups (n=3 for each group). Vasodilatory response is expressed as % of the maximal diameter. The experiment was repeated five times for each patient by incubating the vessel with saline (black circle), SRT1720 (green circle), mitoTEMPO (orange triangle), rotenone (red reverse triangle), tempol (blue rhombus). Data are presented as mean±SD and were compared by the Friedman test followed by Durbin-Conover as a post-hoc test. A p-value <0.05 was considered significant. *: p<0.05.**: p<0.01.



Supplemental Figure 5. SIRT1 regulates a plethora of genes implicated in mitochondrial and endothelial function.

SIRT1 is a novel central modulator of obesity and ageing vascular impairment, protecting microcirculation from endothelial dysfunction. Indeed, SIRT1 gene expression is lower during ageing and obesity in the microvessel. Due to its pleiotropic involvement in regulating NO availability and cellular and mitochondrial ROS production, its downregulation impairs endothelial function mainly by damaging mitochondrial function. These activities are primarily exerted through epigenetic mechanisms and make SIRT1 the centre of the cross-talk between substrates availability, cellular metabolism and vascular phenotype. *ArgII: Arginase II; ATP synthase 6; Cytb: Cytochrome b; FOXO3: Forkhead box protein O3; ND2: NADH dehydrogenase 2; ND5: and NADH dehydrogenase 5; SOD-2: superoxide dismutase-2.*

252 **Supplementary Table 1 – Figures and Table statistics**

				p
Figure 1				
Figure 1A				
Age				$3.34*10^{-6}$
BMI group				$2.32*10^{-2}$
Age*BMI group				$9.48*10^{-3}$
Nonobese group		r=0.555	x=0.0148	$1.35*10^{-4}$
Obese group		r=0.487	x=0.0533	$5.23*10^{-4}$
Test: linear regression				
Sample size: Nonobese=42, Obese=47				
Figure 1B				
Age				$3.43*10^{-31}$
BMI				$8.82*10^{-35}$
Age*BMI				$5.25*10^{-9}$
Young Nonobese	Young Obese			$4.94*10^{-27}$
Young Nonobese	Old Nonobese			$3.97*10^{-28}$
Young Nonobese	Old Obese			$3.47*10^{-42}$
Young Obese	Old Nonobese			ns
Young Obese	Old Obese			$5.72*10^{-13}$
Old Nonobese	Old Obese			$3.88*10^{-21}$
Test: two-way ANOVA followed by Holm-Sidak post hoc correction				
Sample size: Young Nonobese=12, Old Nonobese=31, Young Obese=15, Old Obese=32				
Figure 1C				
Age				$6.48*10^{-18}$
BMI				$1.11*10^{-8}$
Age*BMI				$4.25*10^{-7}$
Young Nonobese	Young Obese			$2.19*10^{-9}$
Young Nonobese	Old Nonobese			$8.49*10^{-17}$
Young Nonobese	Old Obese			$3.53*10^{-18}$
Young Obese	Old Nonobese			$1.03*10^{-3}$
Young Obese	Old Obese			$1.16*10^{-4}$
Old Nonobese	Old Obese			ns
Test: two-way ANOVA followed by Holm-Sidak post hoc correction				
Sample size: Young Nonobese=12, Old Nonobese=31, Young Obese=15, Old Obese=32				
Figure 1D				
Age				ns
BMI				ns
Age*BMI				ns
Young Nonobese	Young Obese			ns
Young Nonobese	Old Nonobese			ns
Young Nonobese	Old Obese			ns
Young Obese	Old Nonobese			ns
Young Obese	Old Obese			ns
Old Nonobese	Old Obese			ns
Test: two-way ANOVA followed by Holm-Sidak post hoc correction				
Sample size: Young Nonobese=12, Old Nonobese=31, Young Obese=15, Old Obese=32				
Figure 1E				
Age				$1.91*10^{-2}$
BMI				$8.81*10^{-4}$
Age*BMI				ns

Young Nonobese	Young Obese	$4.47*10^{-2}$
Young Nonobese	Old Nonobese	$4.47*10^{-2}$
Young Nonobese	Old Obese	$4.47*10^{-2}$
Young Obese	Old Nonobese	ns
Young Obese	Old Obese	$4.47*10^{-2}$
Old Nonobese	Old Obese	$4.47*10^{-2}$
Test: Scheirer-Hare-Ray test followed by Dunn post hoc correction		
Sample size: Young Nonobese=5, Old Nonobese=5, Young Obese=5, Old Obese=5		
Figure 1F		
Age		$1.31*10^{-7}$
BMI		$8.73*10^{-4}$
Age*BMI		ns
Young Nonobese	Young Obese	ns
Young Nonobese	Old Nonobese	ns
Young Nonobese	Old Obese	$4.47*10^{-2}$
Young Obese	Old Nonobese	ns
Young Obese	Old Obese	$4.47*10^{-2}$
Old Nonobese	Old Obese	$4.47*10^{-2}$
Test: Scheirer-Hare-Ray test followed by Dunn post hoc correction		
Sample size: Young Nonobese=5, Old Nonobese=5, Young Obese=5, Old Obese=5		
Figure 2		
Figure 2A		
Age		$6.13*10^{-6}$
BMI		$3.88*10^{-12}$
Age*BMI		$3.12*10^{-2}$
Young Nonobese	Young Obese	$8.30*10^{-9}$
Young Nonobese	Old Nonobese	$1.07*10^{-4}$
Young Nonobese	Old Obese	$1.23*10^{-11}$
Young Obese	Old Nonobese	$1.68*10^{-3}$
Young Obese	Old Obese	$6.77*10^{-3}$
Old Nonobese	Old Obese	$8.02*10^{-7}$
Test: two-way ANOVA followed by Holm-Sidak post hoc correction		
Sample size: Young Nonobese=10, Old Nonobese=12, Young Obese=10, Old Obese=8		
Figure 2B		
Age		$4.81*10^{-7}$
BMI		$2.97*10^{-13}$
Age*BMI		ns
Young Nonobese	Young Obese	$2.74*10^{-9}$
Young Nonobese	Old Nonobese	$4.66*10^{-5}$
Young Nonobese	Old Obese	$5.45*10^{-13}$
Young Obese	Old Nonobese	$1.17*10^{-3}$
Young Obese	Old Obese	$1.17*10^{-3}$
Old Nonobese	Old Obese	$3.68*10^{-8}$
Test: two-way ANOVA followed by Holm-Sidak post hoc correction		
Sample size: Young Nonobese=10, Old Nonobese=12, Young Obese=10, Old Obese=8		
Figure 2C		
Age		$1.27*10^{-4}$
BMI		$2.22*10^{-8}$
Age*BMI		ns
Young Nonobese	Young Obese	$1.17*10^{-5}$
Young Nonobese	Old Nonobese	$1.93*10^{-3}$

Young Nonobese	Old Obese	$2.48*10^{-8}$
Young Obese	Old Nonobese	$4.26*10^{-2}$
Young Obese	Old Obese	$4.26*10^{-2}$
Old Nonobese	Old Obese	$2.83*10^{-4}$
Test: two-way ANOVA followed by Holm-Sidak post hoc correction		
Sample size: Young Nonobese=10, Old Nonobese=12, Young Obese=10, Old Obese=8		
Figure 2D		
Age		$3.00*10^{-8}$
BMI		$7.75*10^{-8}$
Age*BMI		ns
Young Nonobese	Young Obese	$1.02*10^{-4}$
Young Nonobese	Old Nonobese	$6.70*10^{-5}$
Young Nonobese	Old Obese	$2.79*10^{-10}$
Young Obese	Old Nonobese	ns
Young Obese	Old Obese	$7.58*10^{-5}$
Old Nonobese	Old Obese	$7.58*10^{-5}$
Test: two-way ANOVA followed by Holm-Sidak post hoc correction		
Sample size: Young Nonobese=10, Old Nonobese=12, Young Obese=10, Old Obese=8		
Figure 2E		
Age		$2.43*10^{-6}$
BMI		$3.00*10^{-13}$
Age*BMI		ns
Young Nonobese	Young Obese	$7.55*10^{-9}$
Young Nonobese	Old Nonobese	$4.54*10^{-4}$
Young Nonobese	Old Obese	$1.33*10^{-12}$
Young Obese	Old Nonobese	$4.54*10^{-4}$
Young Obese	Old Obese	$6.91*10^{-4}$
Old Nonobese	Old Obese	$1.36*10^{-8}$
Test: two-way ANOVA followed by Holm-Sidak post hoc correction		
Sample size: Young Nonobese=10, Old Nonobese=12, Young Obese=10, Old Obese=8		
Figure 3		
Figure 3A		
Within-subject effect		$9.23*10^{-21}$
saline	L-NAME	$2.96*10^{-7}$
saline	SRT1720	ns
saline	SRT1720+L-NAME	$1.33*10^{-7}$
L-NAME	SRT1720	$3.34*10^{-8}$
L-NAME	SRT1720+L-NAME	ns
SRT1720	SRT1720+L-NAME	$5.78*10^{-10}$
Test: ANOVA for repeated measures followed by Holm-Sidak post hoc correction		
Sample size: Young Nonobese=10, Old Nonobese=27, Young Obese=8, Old Obese=20		
Figure 3B		
Within-subject effect		$2.32*10^{-37}$
saline	L-NAME	$5.16*10^{-8}$
saline	SRT1720	$6.89*10^{-10}$
saline	SRT1720+L-NAME	$1.80*10^{-11}$
L-NAME	SRT1720	$2.14*10^{-15}$
L-NAME	SRT1720+L-NAME	$7.16*10^{-5}$
SRT1720	SRT1720+L-NAME	$2.39*10^{-18}$
Test: ANOVA for repeated measures followed by Holm-Sidak post hoc correction		
Sample size: Young Nonobese=10, Old Nonobese=27, Young Obese=8, Old Obese=20		

Figure 3C		
Within-subject effect		$4.82*10^{-14}$
saline	L-NAME	$8.08*10^{-4}$
saline	SRT1720	$7.76*10^{-4}$
saline	SRT1720+L-NAME	$5.86*10^{-4}$
L-NAME	SRT1720	$2.72*10^{-9}$
L-NAME	SRT1720+L-NAME	ns
SRT1720	SRT1720+L-NAME	$1.98*10^{-7}$
Test: ANOVA for repeated measures followed by Holm-Sidak post hoc correction		
Sample size: Young Nonobese=10, Old Nonobese=27, Young Obese=8, Old Obese=20		
Figure 3D		
Within-subject effect		$4.11*10^{-31}$
saline	L-NAME	$7.73*10^{-8}$
saline	SRT1720	$3.57*10^{-10}$
saline	SRT1720+L-NAME	$9.66*10^{-12}$
L-NAME	SRT1720	$3.12*10^{-12}$
L-NAME	SRT1720+L-NAME	ns
SRT1720	SRT1720+L-NAME	$4.15*10^{-12}$
Test: ANOVA for repeated measures followed by Holm-Sidak post hoc correction		
Sample size: Young Nonobese=10, Old Nonobese=27, Young Obese=8, Old Obese=20		
Figure 3E		
Baseline AUC comparison between groups		
Age		$4.26*10^{-3}$
BMI		$9.89*10^{-8}$
Age*BMI		$8.48*10^{-4}$
Young Nonobese group		
Within-subject effect		$8.35*10^{-13}$
saline	L-NAME	$9.19*10^{-5}$
saline	SRT1720	ns
saline	SRT1720+L-NAME	$1.10*10^{-3}$
L-NAME	SRT1720	$1.02*10^{-7}$
L-NAME	SRT1720+L-NAME	$1.61*10^{-2}$
SRT1720	SRT1720+L-NAME	$1.75*10^{-6}$
Old Nonobese group		
Within-subject effect		$9.87*10^{-32}$
saline	L-NAME	$9.81*10^{-15}$
saline	SRT1720	$7.42*10^{-9}$
saline	SRT1720+L-NAME	$6.45*10^{-9}$
L-NAME	SRT1720	$1.71*10^{-13}$
L-NAME	SRT1720+L-NAME	ns
SRT1720	SRT1720+L-NAME	$4.16*10^{-15}$
Young Obese group		
Within-subject effect		$1.95*10^{-8}$
saline	L-NAME	$3.50*10^{-2}$
saline	SRT1720	$1.31*10^{-2}$
saline	SRT1720+L-NAME	ns
L-NAME	SRT1720	$3.96*10^{-7}$
L-NAME	SRT1720+L-NAME	ns
SRT1720	SRT1720+L-NAME	$2.02*10^{-5}$
Old Obese group		
Within-subject effect		$3.12*10^{-15}$
saline	L-NAME	$4.94*10^{-4}$
saline	SRT1720	$2.39*10^{-4}$

saline	SRT1720+L-NAME		$3.15*10^{-4}$
L-NAME	SRT1720		$5.44*10^{-7}$
L-NAME	SRT1720+L-NAME		ns
SRT1720	SRT1720+L-NAME		$1.17*10^{-8}$
Figure 3F			
Baseline maximal vasodilation to ACh comparison between groups			
Age			$2.14*10^{-18}$
BMI			$1.74*10^{-25}$
Age*BMI			$3.27*10^{-8}$
Test: two-way ANOVA followed by Holm-Sidak post hoc correction			
Sample size: Young Nonobese=10, Old Nonobese=27, Young Obese=8, Old Obese=20			
Other statistics relative to Figure 3F are the same reported in Figures 3A-D			
Figure 3G			
ΔACh			
Age			$3.43*10^{-6}$
BMI			$2.04*10^{-8}$
Age*BMI			ns
Young Nonobese	Young Obese		$2.94*10^{-5}$
Young Nonobese	Old Nonobese		$9.37*10^{-6}$
Young Nonobese	Old Obese		$7.03*10^{-11}$
Young Obese	Old Nonobese		ns
Young Obese	Old Obese		ns
Old Nonobese	Old Obese		$1.72*10^{-4}$
ΔL-NAME			
Age			$2.36*10^{-5}$
BMI			$6.05*10^{-5}$
Age*BMI			$1.6*10^{-2}$
Young Nonobese	Young Obese		$7.02*10^{-4}$
Young Nonobese	Old Nonobese		$7.70*10^{-6}$
Young Nonobese	Old Obese		$1.42*10^{-7}$
Young Obese	Old Nonobese		ns
Young Obese	Old Obese		ns
Old Nonobese	Old Obese		ns
Figure 4			
Figure 4A			
Unadjusted	r=0.373	x=0.226	$2.22*10^{-3}$
Adjusted	r=0.729	x=0.247	$3.31*10^{-4}$
Test: linear regression unadjusted and adjusted for BMI, sex, mean blood pressure, creatinine and HOMA-IR			
Sample size: Young Nonobese=10, Old Nonobese=27, Young Obese=8, Old Obese=20			
Figure 4B			
Unadjusted	r=0.488	x=0.393	$3.73*10^{-5}$
Adjusted	r=0.729	x=0.322	$2.82*10^{-2}$
Test: linear regression unadjusted and adjusted for age, sex, mean blood pressure, creatinine and HOMA-IR			
Sample size: Young Nonobese=10, Old Nonobese=27, Young Obese=8, Old Obese=20			
Figure 4C			
Unadjusted	r=0.576	x=2.460	$5.18*10^{-7}$
Adjusted	r=0.800	x=3.180	$2.30*10^{-4}$
Test: linear regression unadjusted and adjusted for age, BMI, sex, mean blood pressure, creatinine and HOMA-IR			

Sample size: Young Nonobese=10, Old Nonobese=27, Young Obese=8, Old Obese=20			
Figure 4D			
Unadjusted	r=0.445	x=0.282	$2.05*10^{-4}$
Adjusted	r=0.642	x=0.305	$1.85*10^{-4}$
Test: linear regression unadjusted and adjusted for BMI, sex, mean blood pressure, creatinine and HOMA-IR			
Sample size: Young Nonobese=10, Old Nonobese=27, Young Obese=8, Old Obese=20			
Figure 4E			
Unadjusted	r=0.315	x=0.265	$1.07*10^{-2}$
Adjusted			ns
Test: linear regression unadjusted and adjusted for age, sex, mean blood pressure, creatinine and HOMA-IR			
Sample size: Young Nonobese=10, Old Nonobese=27, Young Obese=8, Old Obese=20			
Figure 4F			
Unadjusted	r=0.435	x=1.940	$2.95*10^{-4}$
Adjusted	r=0.724	x=3.397	$9.60*10^{-4}$
Test: linear regression unadjusted and adjusted for age, BMI, sex, mean blood pressure, creatinine and HOMA-IR			
Sample size: Young Nonobese=10, Old Nonobese=27, Young Obese=8, Old Obese=20			

Figure 5

Figure 5A		
saline		$3.06*10^{-3}$
Young Nonobese	Young Nonobese_scr.siRNA	ns
Young Nonobese	Young Nonobese _siRNA	$2.45*10^{-2}$
Young Nonobese _scr.siRNA	Young Nonobese _siRNA	$2.45*10^{-2}$
L-NAME		ns
Young Nonobese	Young Nonobese_scr.siRNA	ns
Young Nonobese	Young Nonobese _siRNA	ns
Young Nonobese _scr.siRNA	Young Nonobese _siRNA	ns
saline vs L-NAME		
Young Nonobese		$2.53*10^{-2}$
Young Nonobese _scr.siRNA		$2.53*10^{-2}$
Young Nonobese _siRNA		$2.53*10^{-2}$
Test: Kruskal-Wallis test with Dwass-Steel-Critchlow-Flinger post-hoc test		
Sample size: 5		
Figure 5B-C		
sc-40986 mitoSOX		$8.08*10^{-3}$
Young Nonobese	Young Nonobese_scr.siRNA	ns
Young Nonobese	Young Nonobese _siRNA	$2.45*10^{-2}$
Young Nonobese _scr.siRNA	Young Nonobese _siRNA	$2.40*10^{-2}$
Test: Kruskal-Wallis test with Dwass-Steel-Critchlow-Flinger post-hoc test		
Sample size: 5		
sc-40986 DAF-FM		$8.93*10^{-3}$
Young Nonobese	Young Nonobese_scr.siRNA	$2.40*10^{-2}$
Young Nonobese	Young Nonobese _siRNA	$2.40*10^{-2}$
Young Nonobese _scr.siRNA	Young Nonobese _siRNA	$2.40*10^{-2}$
Test: Kruskal-Wallis test with Dwass-Steel-Critchlow-Flinger post-hoc test		
Sample size: 5		
SRT1720 mitoSOX		$1.93*10^{-3}$
Young Nonobese	Old Obese	$2.45*10^{-2}$
Young Nonobese	Old Obese_SRT1720	$2.45*10^{-2}$

Old Obese	Old Obese_SRT1720	$2.45*10^{-2}$
Test: Kruskal-Wallis test with Dwass-Steel-Critchlow-Flinger post-hoc test		
Sample size: 5		
SRT1720 DAF-FM		$1.93*10^{-3}$
Young Nonobese	Old Obese	$2.45*10^{-2}$
Young Nonobese	Old Obese_SRT1720	$2.45*10^{-2}$
Old Obese	Old Obese_SRT1720	$2.45*10^{-2}$
Test: Kruskal-Wallis test with Dwass-Steel-Critchlow-Flinger post-hoc test		
Sample size: 5		
Figure 5D-E		
Within-subject effect		$1.27*10^{-17}$
saline	SRT1720	$5.63*10^{-5}$
saline	mitoTEMPO	$4.77*10^{-6}$
saline	gp91dstat	$2.68*10^{-5}$
saline	SRT1720+mitoTEMPO	$1.95*10^{-3}$
saline	SRT1720+gp91dstat	$6.25*10^{-6}$
saline	mitoTEMPO+gp91dstat	$4.77*10^{-6}$
SRT1720	mitoTEMPO	ns
SRT1720	gp91dstat	$3.5*10^{-3}$
SRT1720	SRT1720+mitoTEMPO	ns
SRT1720	SRT1720+gp91dstat	ns
SRT1720	mitoTEMPO+gp91dstat	ns
mitoTEMPO	gp91dstat	$5.81*10^{-4}$
mitoTEMPO	SRT1720+mitoTEMPO	ns
mitoTEMPO	SRT1720+gp91dstat	$3.97*10^{-3}$
mitoTEMPO	mitoTEMPO+gp91dstat	$2.09*10^{-2}$
gp91dstat	SRT1720+mitoTEMPO	$3.07*10^{-2}$
gp91dstat	SRT1720+gp91dstat	$2.09*10^{-4}$
gp91dstat	mitoTEMPO+gp91dstat	$5.96*10^{-4}$
SRT1720+mitoTEMPO	SRT1720+gp91dstat	ns
SRT1720+mitoTEMPO	mitoTEMPO+gp91dstat	ns
SRT1720+gp91dstat	mitoTEMPO+gp91dstat	ns
Test: ANOVA for repeated measures followed by a post-hoc test with Holm-Sidak correction		
Sample size: 6		
Figure 5F-I		
Swelling_10 min		$1.78*10^{-2}$
Young Nonobese	Old Obese	$4.31*10^{-2}$
Young Nonobese	Old Obese_SRT1720	ns
Old Obese	Old Obese_SRT1720	ns
Swelling_20 min		$3.06*10^{-3}$
Young Nonobese	Old Obese	$2.45*10^{-2}$
Young Nonobese	Old Obese_SRT1720	ns
Old Obese	Old Obese_SRT1720	$2.45*10^{-2}$
Test: Kruskal-Wallis test with Dwass-Steel-Critchlow-Flinger post-hoc test		
Sample size: 5		
Figure 6		
Figure 6A		
$p66^{Shc}$		$9.19*10^{-3}$
Young Nonobese	Old Obese	$2.45*10^{-2}$
Young Nonobese	Old Obese_SRT1720	ns
Old Obese	Old Obese_SRT1720	$2.45*10^{-2}$

Test: Kruskal-Wallis test with Dwass-Steel-Critchlow-Flinger post-hoc test		
Sample size: 5		
Figure 6B		
ArgII		$8.15*10^{-3}$
Young Nonobese	Old Obese	$2.45*10^{-2}$
Young Nonobese	Old Obese_SRT1720	ns
Old Obese	Old Obese_SRT1720	$2.45*10^{-2}$
Test: Kruskal-Wallis test with Dwass-Steel-Critchlow-Flinger post-hoc test		
Sample size: 5		
Figure 6C		
SIRT3		$3.06*10^{-3}$
Young Nonobese	Old Obese	$2.45*10^{-2}$
Young Nonobese	Old Obese_SRT1720	ns
Old Obese	Old Obese_SRT1720	$2.45*10^{-2}$
Test: Kruskal-Wallis test with Dwass-Steel-Critchlow-Flinger post-hoc test		
Sample size: 5		
Figure 6D		
SIRT1 on $p66^{Shc}$ promoter		$1.93*10^{-3}$
Young Nonobese	Old Obese	$2.45*10^{-2}$
Young Nonobese	Old Obese_SRT1720	$2.45*10^{-2}$
Old Obese	Old Obese_SRT1720	$2.45*10^{-2}$
Test: Kruskal-Wallis test with Dwass-Steel-Critchlow-Flinger post-hoc test		
Sample size: 5		
Figure 6E		
SIRT1 on ArgII promoter		$3.74*10^{-3}$
Young Nonobese	Old Obese	$2.45*10^{-2}$
Young Nonobese	Old Obese_SRT1720	ns
Old Obese	Old Obese_SRT1720	$2.45*10^{-2}$
Test: Kruskal-Wallis test with Dwass-Steel-Critchlow-Flinger post-hoc test		
Sample size: 5		
Figure 6F		
ATP6		$1.93*10^{-3}$
Young Nonobese	Old Obese	$2.45*10^{-2}$
Young Nonobese	Old Obese_SRT1720	$2.45*10^{-2}$
Old Obese	Old Obese_SRT1720	$2.45*10^{-2}$
Test: Kruskal-Wallis test with Dwass-Steel-Critchlow-Flinger post-hoc test		
Sample size: 5		
Figure 6H		
CytB		$1.93*10^{-3}$
Young Nonobese	Old Obese	$2.45*10^{-2}$
Young Nonobese	Old Obese_SRT1720	$2.45*10^{-2}$
Old Obese	Old Obese_SRT1720	$2.45*10^{-2}$
Test: Kruskal-Wallis test with Dwass-Steel-Critchlow-Flinger post-hoc test		
Sample size: 5		
Figure 6H		
ND2		$3.74*10^{-3}$
Young Nonobese	Old Obese	$2.45*10^{-2}$
Young Nonobese	Old Obese_SRT1720	ns
Old Obese	Old Obese_SRT1720	$2.45*10^{-2}$
Test: Kruskal-Wallis test with Dwass-Steel-Critchlow-Flinger post-hoc test		
Sample size: 5		
Figure 6I		
ND5		$3.06*10^{-3}$

Young Nonobese	Old Obese	$2.45*10^{-2}$
Young Nonobese	Old Obese_SRT1720	ns
Old Obese	Old Obese_SRT1720	$2.45*10^{-2}$
Test: Kruskal-Wallis test with Dwass-Steel-Critchlow-Flinger post-hoc test		
Sample size: 5		
Figure 7		
Figure 7A		
Within-subject effect		ns
saline	SRT1720	ns
saline	mitoTEMPO	ns
SRT1720	mitoTEMPO	ns
Test: Friedman test followed by Durbin-Conover as a post-hoc test		
Sample size: 5		
Figure 7B		
Within-subject effect		$7.76*10^{-12}$
saline	SRT1720	$2.94*10^{-7}$
saline	mitoTEMPO	$1.59*10^{-7}$
SRT1720	mitoTEMPO	ns
Test: ANOVA for repeated measures followed by a post-hoc test with Holm-Sidak correction		
Sample size: 16		
Figure 7C		
Within-subject effect		$5.81*10^{-6}$
saline	SRT1720	$3.46*10^{-5}$
saline	mitoTEMPO	$1.67*10^{-6}$
SRT1720	mitoTEMPO	ns
Test: ANOVA for repeated measures followed by a post-hoc test with Holm-Sidak correction		
Sample size: 8		
Figure 7D		
Within-subject effect		$4.78*10^{-23}$
saline	SRT1720	$7.33*10^{-13}$
saline	mitoTEMPO	$4.00*10^{-26}$
SRT1720	mitoTEMPO	ns
Test: ANOVA for repeated measures followed by a post-hoc test with Holm-Sidak correction		
Sample size: 22		
Supplementary Figure 1B		
mitoPY1		$1.18*10^{-2}$
ENZ-53013		$1.25*10^{-2}$
Test: Kruskal-Wallis test		
Sample size: 3 for each group		
Supplementary Figure 2B		
SIRT1		$9.00*10^{-3}$
Young Nonobese	Young Nonobese_scr.siRNA	ns
Young Nonobese	Young Nonobese_siRNA	$2.45*10^{-2}$
Young Nonobese_scr.siRNA	Young Nonobese_siRNA	$2.45*10^{-2}$
Test: Kruskal-Wallis test with Dwass-Steel-Critchlow-Flinger post-hoc test		
Sample size: 5 for each group		
Supplementary Figure 3		

SIRT1 on $p66^{Shc}$ promoter		$9.98*10^{-8}$
SIRT1 on ArgII promoter		$1.27*10^{-5}$
Test: independent samples Student's t test		
Sample size: Young Nonobese=12, Old Obese=18		
Supplementary Figure 4		
Supplementary Figure 4A		
Within-subject effect		ns
saline	SRT1720	ns
saline	mitoTEMPO	ns
saline	rotenone	ns
saline	tempol	ns
SRT1720	mitoTEMPO	ns
SRT1720	rotenone	ns
SRT1720	tempol	ns
mitoTEMPO	rotenone	ns
mitoTEMPO	tempol	ns
rotenone	tempol	ns
Test: Friedman test followed by Durbin-Conover as a post-hoc test		
Sample size: Young Nonobese=3, Young Obese=3, Old Nonobese=3, Old Obese=3		
Supplementary Figure 4B		
Within-subject effect		$1.80*10^{-3}$
saline	SRT1720	ns
saline	mitoTEMPO	ns
saline	rotenone	ns
saline	tempol	ns
SRT1720	mitoTEMPO	ns
SRT1720	rotenone	ns
SRT1720	tempol	ns
mitoTEMPO	rotenone	ns
mitoTEMPO	tempol	ns
rotenone	tempol	ns
Test: Friedman test followed by Durbin-Conover as a post-hoc test		
Sample size: Young Nonobese=3, Young Obese=3, Old Nonobese=3, Old Obese=3		
Supplementary Figure 4C		
Within-subject effect		$1.51*10^{-2}$
saline	SRT1720	ns
saline	mitoTEMPO	ns
saline	rotenone	ns
saline	tempol	ns
SRT1720	mitoTEMPO	ns
SRT1720	rotenone	ns
SRT1720	tempol	ns
mitoTEMPO	rotenone	ns
mitoTEMPO	tempol	ns
rotenone	tempol	ns
Test: Friedman test followed by Durbin-Conover as a post-hoc test		
Sample size: Young Nonobese=3, Young Obese=3, Old Nonobese=3, Old Obese=3		
Supplementary Figure 4D		
Within-subject effect		$9.00*10^{-4}$
saline	SRT1720	ns
saline	mitoTEMPO	ns

saline	rotenone	ns
saline	tempol	ns
SRT1720	mitoTEMPO	ns
SRT1720	rotenone	ns
SRT1720	tempol	ns
mitoTEMPO	rotenone	ns
mitoTEMPO	tempol	ns
rotenone	tempol	ns
Test: Friedman test followed by Durbin-Conover as a post-hoc test		
Sample size: Young Nonobese=3, Young Obese=3, Old Nonobese=3, Old Obese=3		

Table 1

Male sex		
χ^2		$5.92*10^{-4}$
Test: chi-Squared test		
Sample size: Young Nonobese=12, Young Obese=15, Old Nonobese=36, Old Obese=32		
Age		
Age		$6.12*10^{-21}$
BMI		$9.62*10^{-3}$
Age*BMI		ns
Young Nonobese	Young Obese	ns
Young Nonobese	Old Nonobese	$1.15*10^{-13}$
Young Nonobese	Old Obese	$1.38*10^{-8}$
Young Obese	Old Nonobese	$8.98*10^{-18}$
Young Obese	Old Obese	$5.14*10^{-12}$
Old Nonobese	Old Obese	$1.95*10^{-3}$
Test: two-way ANOVA followed by Holm-Sidak post hoc correction		
Sample size: Young Nonobese=12, Young Obese=15, Old Nonobese=36, Old Obese=32		
BMI		
Age		ns
BMI		$4.22*10^{-38}$
Age*BMI		ns
Young Nonobese	Young Obese	$2.75*10^{-21}$
Young Nonobese	Old Nonobese	ns
Young Nonobese	Old Obese	$3.24*10^{-27}$
Young Obese	Old Nonobese	$2.28*10^{-26}$
Young Obese	Old Obese	ns
Old Nonobese	Old Obese	$1.51*10^{-36}$
Test: two-way ANOVA followed by Holm-Sidak post hoc correction		
Sample size: Young Nonobese=12, Young Obese=15, Old Nonobese=36, Old Obese=32		
Systolic blood pressure		
Age		ns
BMI		ns
Age*BMI		ns
Young Nonobese	Young Obese	ns
Young Nonobese	Old Nonobese	ns
Young Nonobese	Old Obese	ns
Young Obese	Old Nonobese	ns
Young Obese	Old Obese	ns
Old Nonobese	Old Obese	ns
Test: two-way ANOVA followed by Holm-Sidak post hoc correction		
Sample size: Young Nonobese=12, Young Obese=15, Old Nonobese=36, Old Obese=32		

Diastolic blood pressure		
Age		$2.25*10^{-3}$
BMI		ns
Age*BMI		ns
Young Nonobese	Young Obese	ns
Young Nonobese	Old Nonobese	ns
Young Nonobese	Old Obese	ns
Young Obese	Old Nonobese	ns
Young Obese	Old Obese	$4.86*10^{-2}$
Old Nonobese	Old Obese	ns
Test: two-way ANOVA followed by Holm-Sidak post hoc correction		
Sample size: Young Nonobese=12, Young Obese=15, Old Nonobese=36, Old Obese=32		
Fasting plasma glucose		
Age		$1.42*10^{-2}$
BMI		ns
Age*BMI		ns
Young Nonobese	Young Obese	ns
Young Nonobese	Old Nonobese	ns
Young Nonobese	Old Obese	ns
Young Obese	Old Nonobese	ns
Young Obese	Old Obese	ns
Old Nonobese	Old Obese	ns
Test: two-way ANOVA followed by Holm-Sidak post hoc correction		
Sample size: Young Nonobese=12, Young Obese=15, Old Nonobese=36, Old Obese=32		
Fasting plasma insulin		
Age		$1.57*10^{-2}$
BMI		$2.87*10^{-53}$
Age*BMI		ns
Young Nonobese	Young Obese	$6.32*10^{-36}$
Young Nonobese	Old Nonobese	ns
Young Nonobese	Old Obese	$2.74*10^{-41}$
Young Obese	Old Nonobese	$8.05*10^{-41}$
Young Obese	Old Obese	ns
Old Nonobese	Old Obese	$7.69*10^{-50}$
Test: two-way ANOVA followed by Holm-Sidak post hoc correction		
Sample size: Young Nonobese=12, Young Obese=15, Old Nonobese=36, Old Obese=32		
HOMA-IR		
Age		$4.75*10^{-3}$
BMI		$1.64*10^{-25}$
Age*BMI		ns
Young Nonobese	Young Obese	$2.23*10^{-12}$
Young Nonobese	Old Nonobese	ns
Young Nonobese	Old Obese	$3.52*10^{-19}$
Young Obese	Old Nonobese	$1.35*10^{-13}$
Young Obese	Old Obese	$2.07*10^{-2}$
Old Nonobese	Old Obese	$2.81*10^{-24}$
Test: two-way ANOVA followed by Holm-Sidak post hoc correction		
Sample size: Young Nonobese=12, Young Obese=15, Old Nonobese=36, Old Obese=32		
eGFR		
Age		$9.35*10^{-4}$
BMI		$9.48*10^{-7}$
Age*BMI		ns
Young Nonobese	Young Obese	$4.00*10^{-3}$

Young Nonobese	Old Nonobese	$3.12*10^{-4}$
Young Nonobese	Old Obese	$1.14*10^{-3}$
Young Obese	Old Nonobese	$2.43*10^{-8}$
Young Obese	Old Obese	$1.68*10^{-2}$
Old Nonobese	Old Obese	$1.01*10^{-4}$
Test: two-way ANOVA followed by Holm-Sidak post hoc correction		
Sample size: Young Nonobese=12, Young Obese=15, Old Nonobese=36, Old Obese=32		
Total cholesterol		
Age		ns
BMI		ns
Age*BMI		ns
Young Nonobese	Young Obese	ns
Young Nonobese	Old Nonobese	ns
Young Nonobese	Old Obese	ns
Young Obese	Old Nonobese	ns
Young Obese	Old Obese	ns
Old Nonobese	Old Obese	ns
Test: two-way ANOVA followed by Holm-Sidak post hoc correction		
Sample size: Young Nonobese=12, Young Obese=15, Old Nonobese=36, Old Obese=32		
Media-to-lumen ratio		
Age		$8.97*10^{-5}$
BMI		$7.41*10^{-26}$
Age*BMI		$2.62*10^{-2}$
Young Nonobese	Young Obese	$8.45*10^{-11}$
Young Nonobese	Old Nonobese	ns
Young Nonobese	Old Obese	$1.76*10^{-21}$
Young Obese	Old Nonobese	$1.68*10^{-11}$
Young Obese	Old Obese	$1.91*10^{-5}$
Old Nonobese	Old Obese	$1.53*10^{-26}$
Test: two-way ANOVA followed by Holm-Sidak post hoc correction		
Sample size: Young Nonobese=12, Young Obese=15, Old Nonobese=36, Old Obese=32		
Media cross-sectional area		
Age		ns
BMI		$2.08*10^{-7}$
Age*BMI		ns
Young Nonobese	Young Obese	$6.25*10^{-3}$
Young Nonobese	Old Nonobese	ns
Young Nonobese	Old Obese	$8.65*10^{-6}$
Young Obese	Old Nonobese	$1.88*10^{-2}$
Young Obese	Old Obese	ns
Old Nonobese	Old Obese	$6.36*10^{-6}$
Test: two-way ANOVA followed by Holm-Sidak post hoc correction		
Sample size: Young Nonobese=12, Young Obese=15, Old Nonobese=36, Old Obese=32		

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