Supplementary Material For

SIRT3 mediates the effects of PCSK9 inhibitors on inflammation, autophagy, and oxidative stress in endothelial cells

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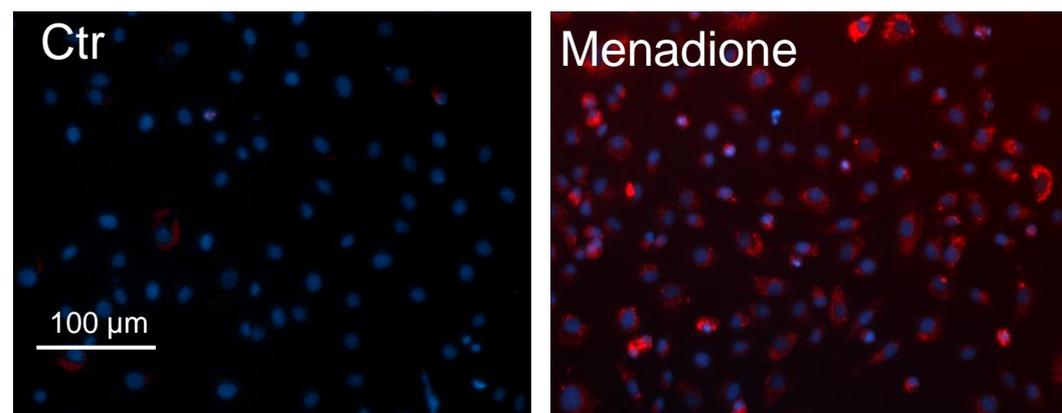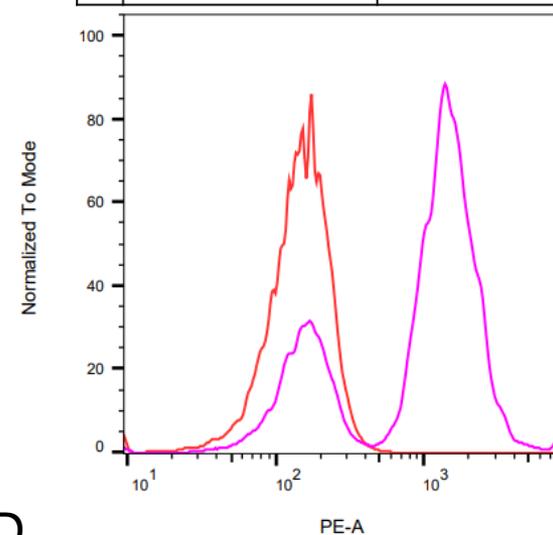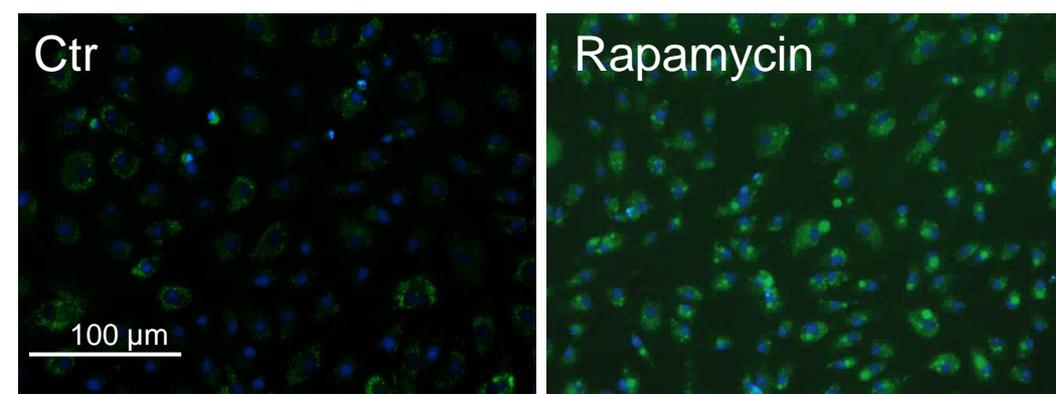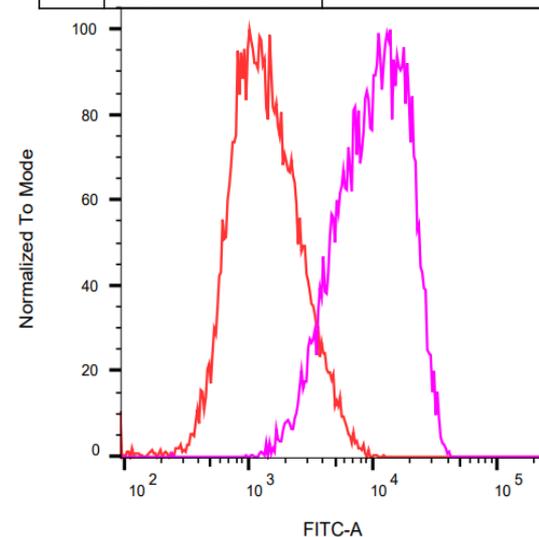
Supplementary Figure 1. Positive controls for experiments assessing induction of mitochondrial ROS and autophagy. (A) Representative images and (B) cytometer analysis, expressed as red fluorescence median, of ROS detection in EC treated for 30 min with 50 µM menadione, the ROS inducer, or with the corresponding highest volume of HBSS-10 mM Hepes (Ctr) and stained with MitoSOX Red probe. (C) Representative images and (D) cytometer analysis, expressed as green fluorescence median, of autophagy detection in EC treated for 16h with 1 µM rapamycin, the autophagy inducer, or with the corresponding highest volume of HBSS-10 mM Hepes (Ctr) and stained with Green detection reagent. Scale bars = 100 µm.
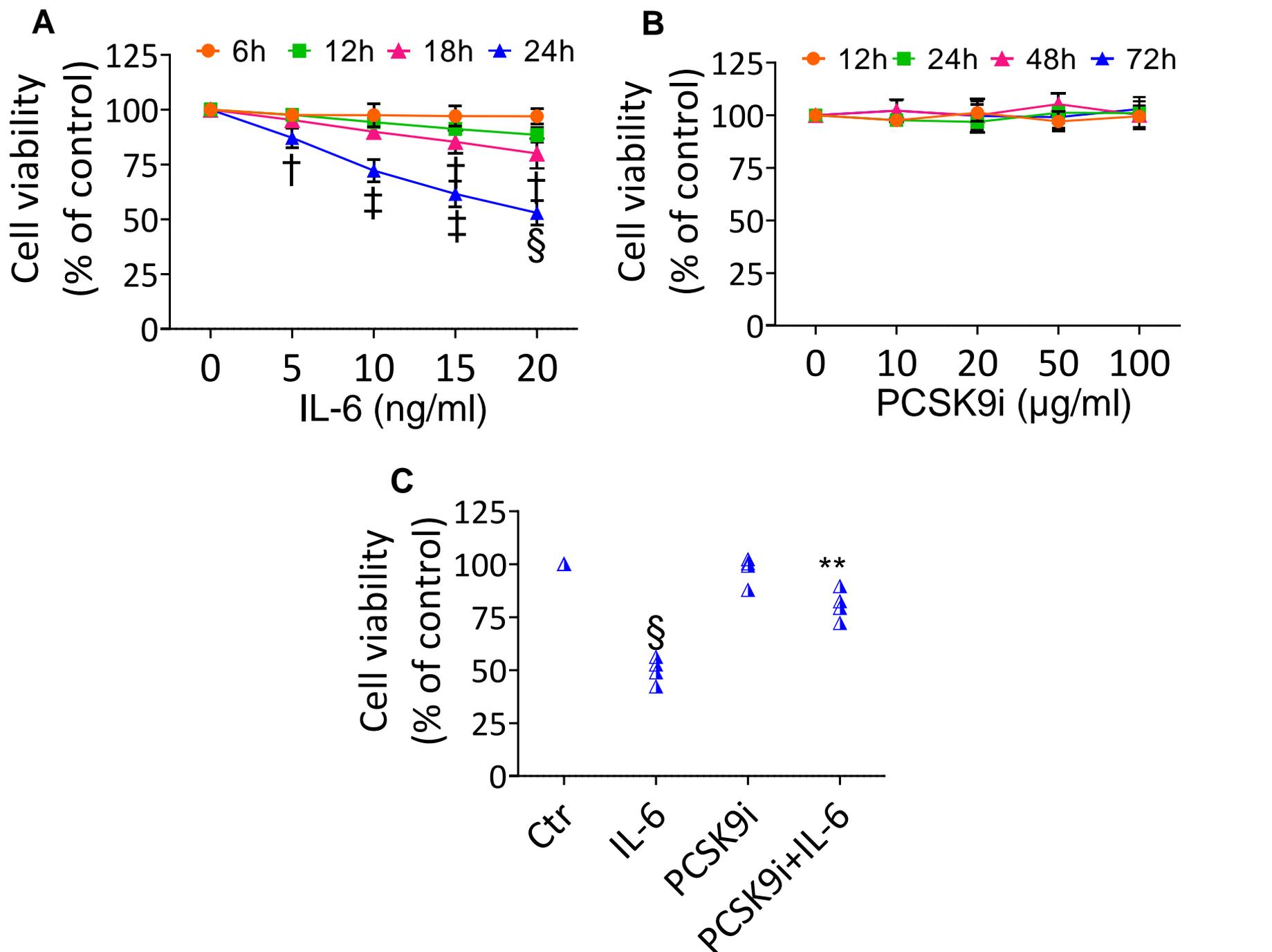
Supplementary Figure 2. IL-6 and PCSK9i dose-response experiments. EC viability after exposure to different concentrations of (A) IL-6 (0-20 ng/mL) up to 24, to PCSK9i (0-100 μg/mL) up to 72h and (C) treated for 24h with 20 ng/mL IL-6 (IL-6), with 100 μg/mL PCSK9i (PCSK9i) or pre-treated for 8h with 100 μg/mL PCSK9i before being exposed to 20 ng/mL IL-6 for 24h (PCSK9i+IL-6). Control cells (Ctr or 0 mM) were treated with the corresponding highest volume of Hanks' balanced salt solution (HBSS)-10 mM Hepes. Cell viability was assessed by Cell Counting Kit-8 assay and reported as % of control. †$p<0.05$ vs 0 ng/ml; ‡$p<0.01$ vs 0 ng/ml; §$p<0.001$ vs 0 ng/ml or Ctr; **$p<0.01$ vs IL-6.

Supplementary Table 1. Characteristics of study patients.

Variable	
N	277
Sex (male), n (%)	136 (49.1)
Age, years	65.3±5.9
BMI, kg/m^2	28±1.8
Glucose, mg/dl	162.1±53.4
HbA1c, %	7.6±4.1
Cholesterol, mg/dl	160±17.2
LDL-cholesterol, mg/dl	98.4±24.1
HDL-cholesterol, mg/dl	40.2±3.8
Triglycerides, mg/dl	177±23.8
Hypertension, n (%)	177 (63.9)
Previous cardiovascular event, n (%)	129 (46.6)
Diabetes, n (%)	56 (20.2)
Smoking, n (%)	52 (18.8)
Statins, n (%)	248 (89.5)
Ezetimibe, n (%)	74 (26.7)

Data are presented as mean ± SD or as number (%).BMI = body mass index; HbA1c= Haemoglobin A1c; HDL= high-density lipoprotein; LDL = low-density lipoprotein.