MiR-15b-5p and PCSK9 inhibition reduces lipopolysaccharide-induced endothelial dysfunction by targeting SIRT4

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Additional file 1

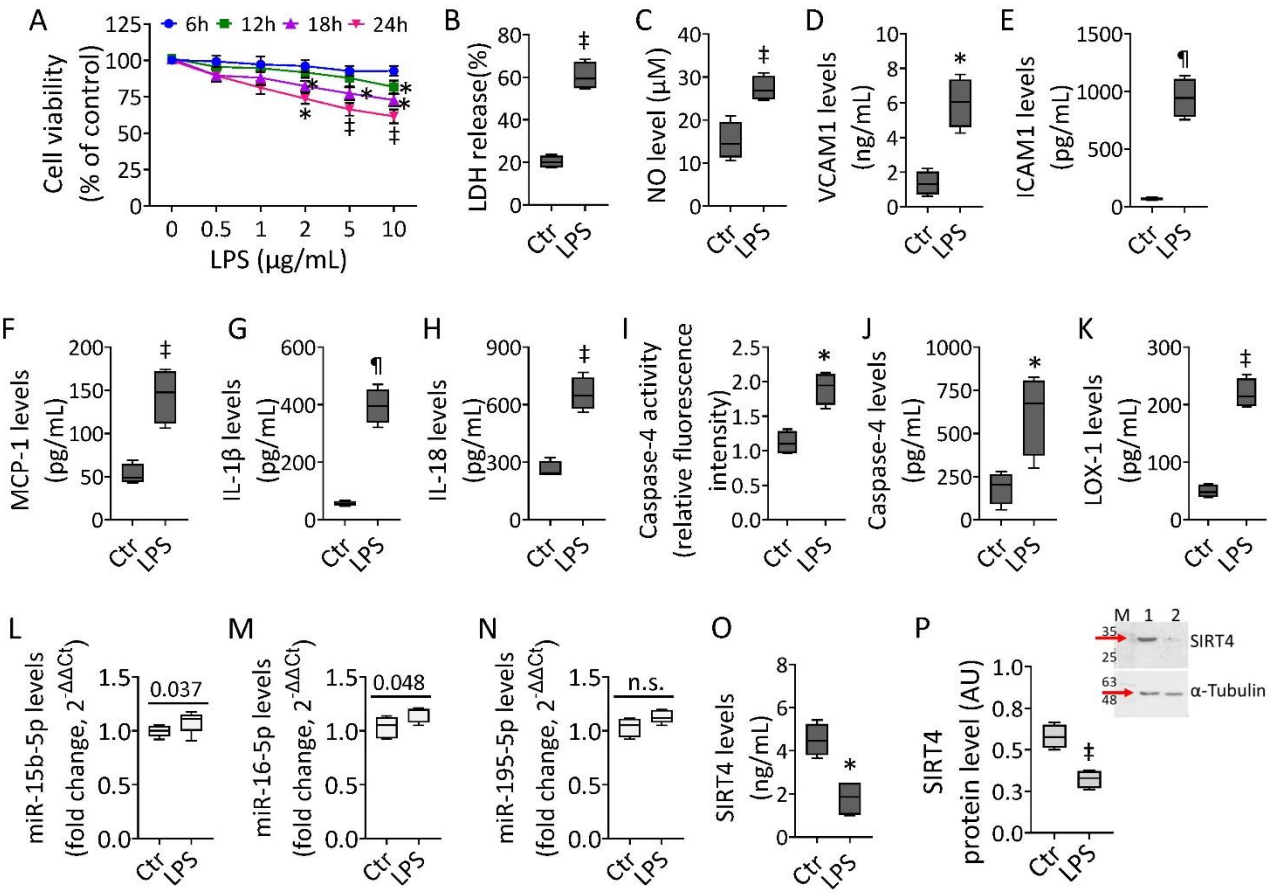

Fig. S1. *LPS-mediated inflammation on HUVEC cells*. (A) Cell viability on HUVEC exposed to LPS. Detection of (B) LDH, (C) NO, (D) VCAM1 (E) ICAM1, (F) MCP-1, (G) IL-1β and (H) IL-18 levels. Caspase-4 (I) activity and (J) levels and (K) LOX-1 evaluation by ELISA. Representation of (L) hsa-miR-15b-5p, (M) hsa-miR-16-5p and (N) hsa-miR-195-5p levels measured by qRT-PCR. SIRT4 levels assessed by (O) ELISA and (P) immunoblotting. Mean ± SD, n = 3. M = molecular weight markers; lane 1 = Ctr; lane 2 = LPS. *p<0.05 vs. 0 µg/mL or Ctr; ‡p<0.01 vs. 0 µg/mL or Ctr; ¶p<0.001 vs. Ctr; n.s., non-significant. Statistical analysis of data was performed using Student's t-test.

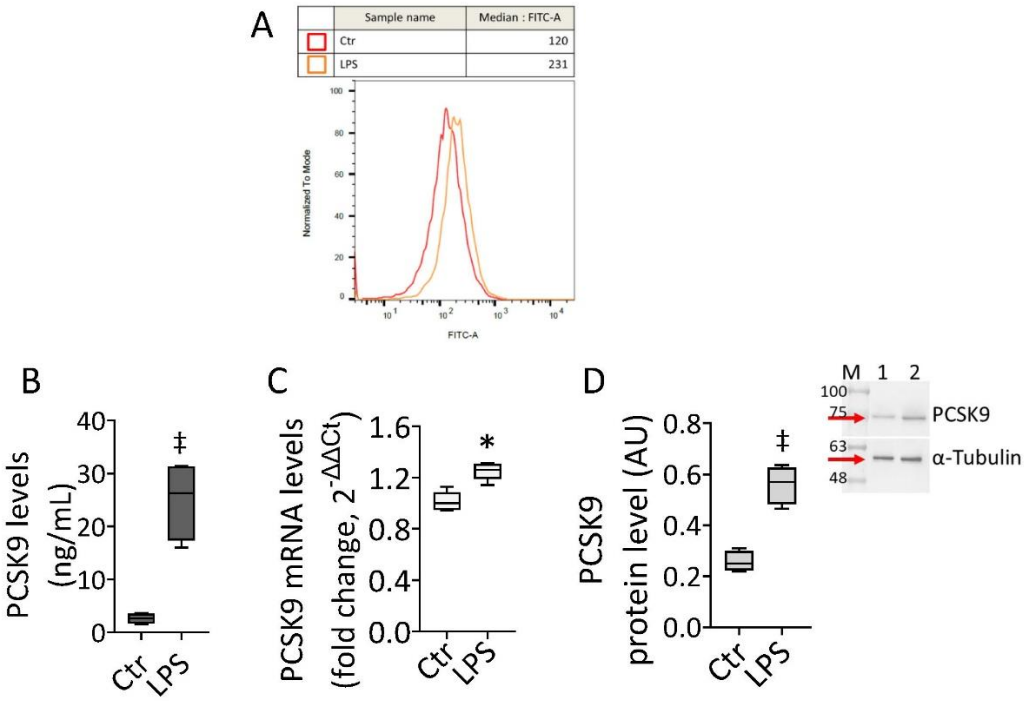

Fig. S2. *LPS modulation of PCSK9 protein on HUVEC cells*. (A) Representative intracellular PCSK9 protein content on TeloHAEC detected by FACS analysis. Detection of PCSK9 by (B) ELISA, (C) mRNA levels by qRT-PCR and (D) immunoblotting analysis on HUVEC. Mean ± SD, n = 3. M = molecular weight markers; lane 1 = Ctr; lane 2 = LPS. *$p<0.05$ vs. Ctr; ‡$p<0.01$ vs. Ctr. Statistical analysis of data was performed using Student's t-test.

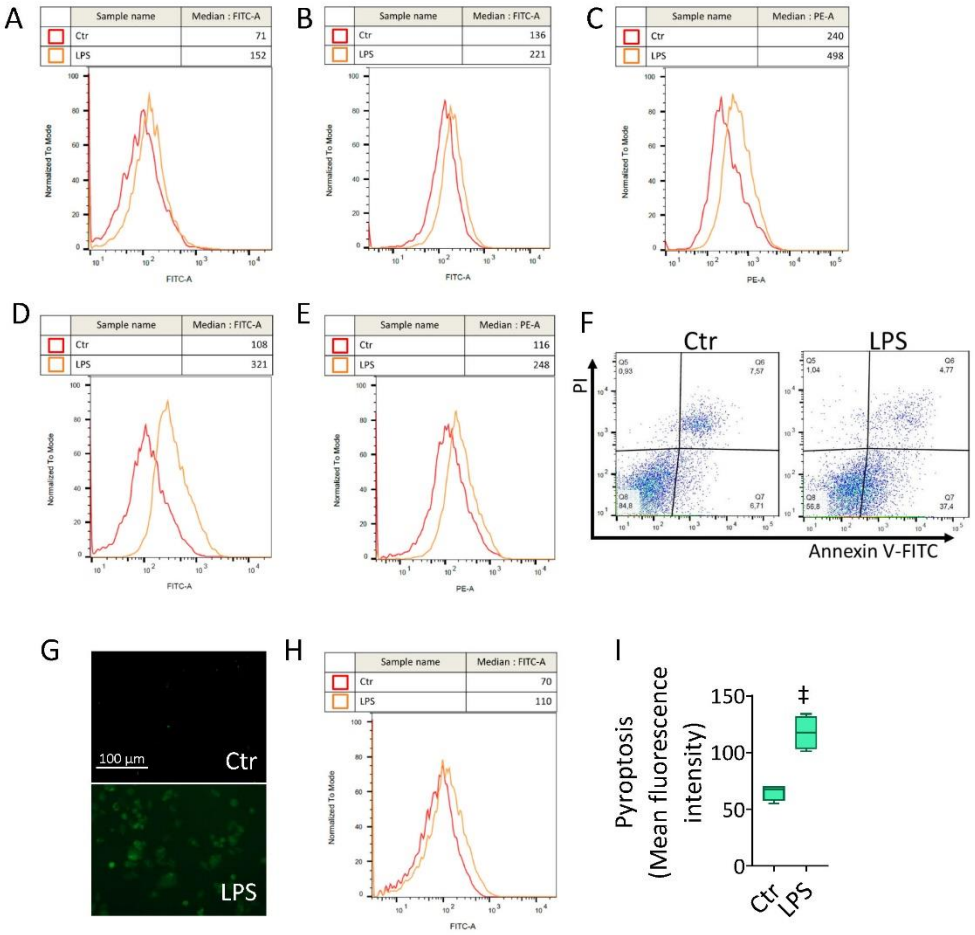

Fig. S3. *LPS-induced pyroptosis on HUVEC cells*. Representative FACS analysis of (A) pyroptosis, (B) intracellular NLRP3 levels, (C) lysosomes, (D) autophagy, (E) mitochondrial ROS levels and (F) annexin V-FITC and PI-staining on TeloHAEC. Q1: necrotic cells; Q2: late apoptotic cells; Q3: early apoptotic cells; Q4: viable cells. (G) Images and (H,I) cytometer analysis of pyroptosis on HUVEC. Mean ± SD, n = 3. Scale bars = 100 µm. ‡$p<0.01$ vs. Ctr. Statistical analysis of data was performed using Student's t-test.

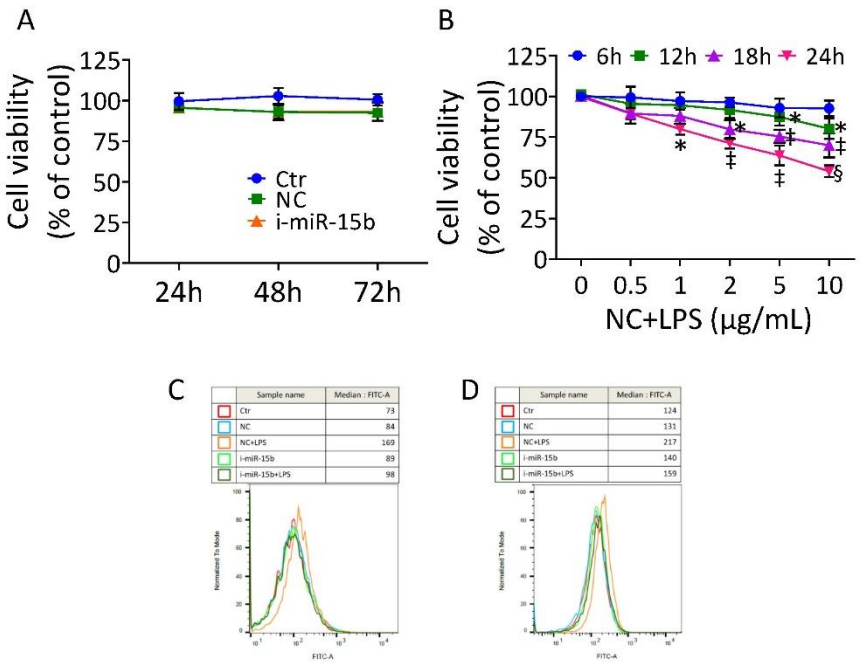

Fig. S4. *Transfection with i-miR-15b.* TeloHAEC viability evaluated (A) after antagomir Negative Control (NC) and antagomiR hsa-miR-15b-5p (i-miR-15b) transfection and (B) after exposure to LPS on NC-transfected cells. Representative FACS analysis of (C) pyroptosis and (D) intracellular NLRP3 levels detected on TeloHAEC. Mean ± SD, n = 3. *p<0.05 vs. NC; ‡p<0.01 vs. NC; §p<0.001 vs. NC. Statistical analysis of data was performed using Student's t-test.

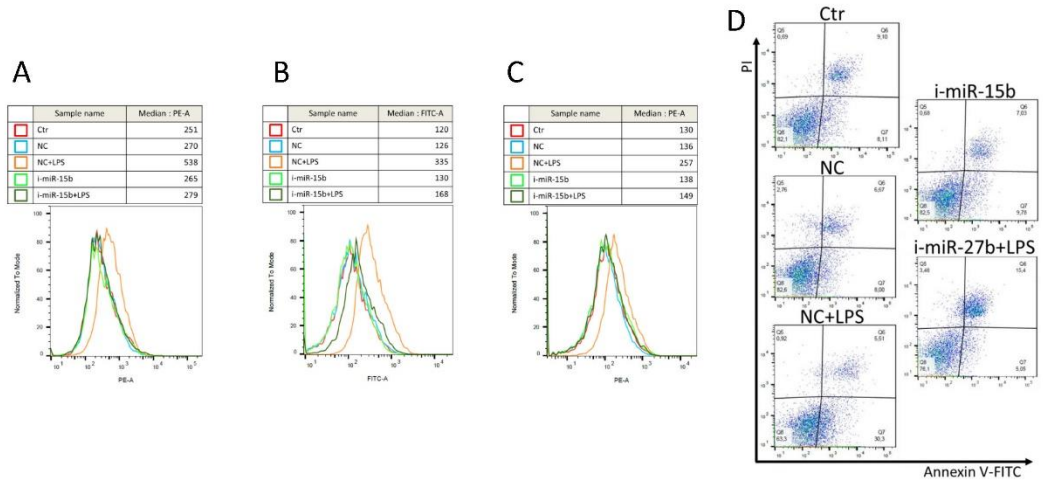

Fig. S5. *FACS analyses.* Representative FACS analysis of (A) lysosomes, (B) autophagy, (C) mitochondrial ROS levels and (D) annexin V-FITC and PI-staining performed on TeloHAEC. Q1: necrotic cells; Q2: late apoptotic cells; Q3: early apoptotic cells; Q4: viable cells.

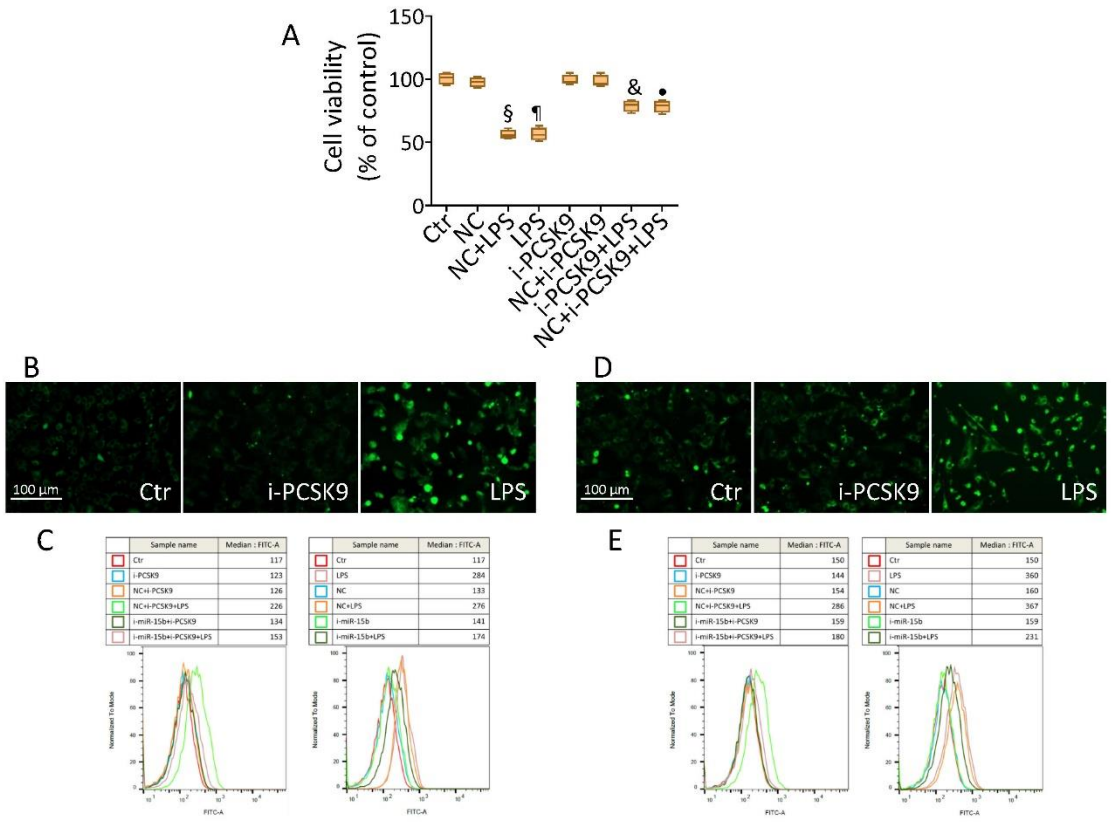

Fig. S6. *i-PCSK9 effects on LPS-induced pyroptosis and autophagy*. (A) TeloHAEC viability after treatment with LPS, i-PCSK9 or transfection with NC before i-PCSK9 and/or LPS stimulation. Representative images and FACS analysis of (B,C) pyroptosis and (D,E) autophagy performed on TeloHAEC. Mean ± SD, n = 3. Scale bars = 100 µm. ¶p<0.001 vs. Ctr; §p<0.001 vs. NC; •p<0.05 vs. NC+LPS; &p<0.05 vs. LPS. Statistical analysis of data was performed using one-way ANOVA.