

SIRT7 Deficiency Suppresses Inflammation, Induces EndoMT, and Increases Vascular Permeability in Primary Pulmonary Endothelial Cells

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Supplementary Figure 1

Full-length WBs for SIRT7 (**A**), ICAM1 (**B**), α -tubulin (**C**), VCAM1 (**D**), and α -tubulin (**E**) for panel C in Figure 3. α -tubulin in panel **C** is from the same WB membrane as ICAM1; α -tubulin in panel **E** is from the same membrane as VCAM1.

Supplementary Figure 2

Full-length WBs for SIRT7 (**A**), VCAM1 (**B**), NF κ B (**C**) and α -tubulin (**D**) for panel D in Figure 3.

Supplementary Figure 3

Effects of LPS on SIRT7 and SIRT7 silencing on inflammatory mediators in airway epithelial cells. **A**: SIRT7 mRNA normalized to β -actin levels in airway epithelial cells stimulated with PBS (\square , n=5) or 1 μ g/ml LPS (\blacksquare , n=3) for 24 hours. **B**: SIRT7, IL6, and IL8 mRNA levels normalized to β -actin in airway epithelial cells transfected with CTRL (\square , n=3) or SIRT7 (\blacksquare , n=3) siRNA. Data are expressed as fold changes relative to average mRNA levels in unstimulated (LPS 0) cell cultures (**A**) or CTRL-silenced cell cultures (**B**). Bars represent averages and brackets standard deviations. P-values were calculated using the Student's *t*-test.

Supplementary Figure 4

Full-length WBs for SIRT7 (**A**), NF κ B (**B**), α -tubulin (**C**), and HDAC2 (**D**) for panel A in Figure 4.

Supplementary Figure 5

Full-length WBs for SIRT7 (**A**), phosphorylated NF κ B (**B**), and α -tubulin (**C**) for panel B in Figure 4.

Supplementary Figure 6

Phase contrast images of CTRL- and SIRT7-silenced HPAEC cultures viewed under the light microscope at 10x objective 48 hours after transfection.

Supplementary Figure 7

Time course of LPS effects on pro-inflammatory and pro-fibrotic gene expression in CTRL- and SIRT7-silenced HPAEC. mRNA levels of each indicated RT-qPCR target

at each time point in CTRL-silenced (□) or SIRT7-silenced (■) HPAEC cultures were normalized to GAPDH and expressed as fold changes relative to average mRNA levels in unstimulated (LPS 0), CTRL-silenced cell cultures. Bars represent averages and brackets standard deviations of measurements for two samples per condition. P-values were calculated using the Student's *t*-test. Significant differences ($P < 0.05$) between and CTRL- and SIRT7-silenced cultures for each LPS concentration are denoted by stars.

Supplementary Figure 8

Full-length WBs for SIRT7 (**A**), VE-Cadherin (**B**), GAPDH (**C**), PECAM1 (**D**), α -tubulin (**E**), α -SMA (**F**), SNAIL (**G**), and GAPDH (**H**) for panel A in Figure 6. Samples for SIRT7 (**A**), VE-Cadherin (**B**), and GAPDH (**C**) were run on a 15-well gel. These images were scaled up proportionally to match widths of protein bands in panels **D-H**, which represent the same samples run on a 10-well gel.

Supplementary Figure 9

Full-length WBs for SIRT7 (**A**), PECAM1 (**B**), and α -tubulin (**C**) for panel C in Figure 6.

Supplementary Figure 10

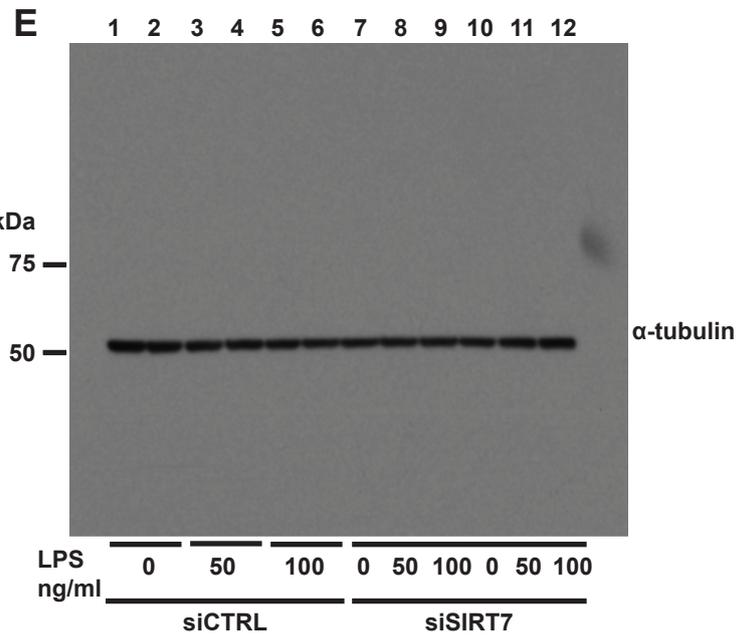
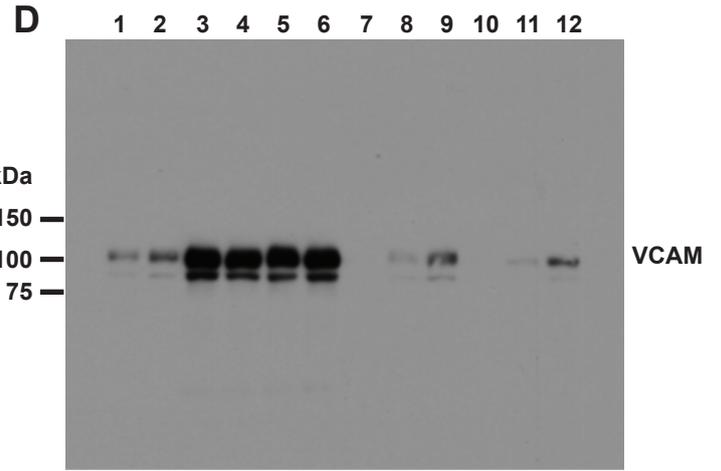
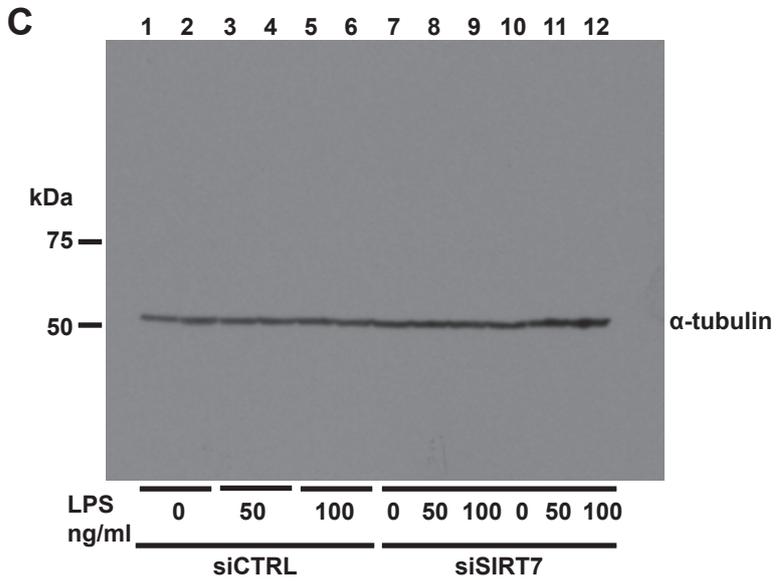
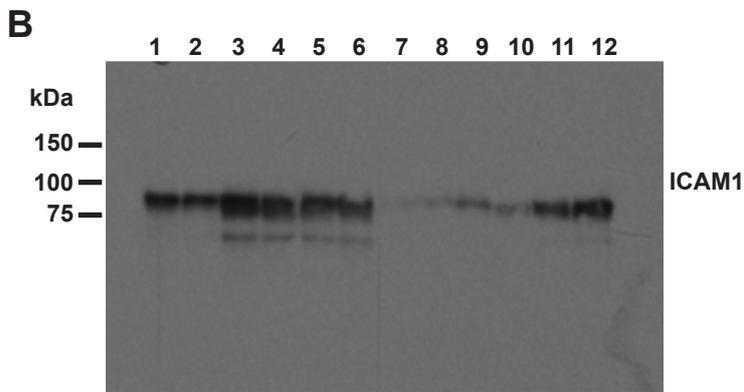
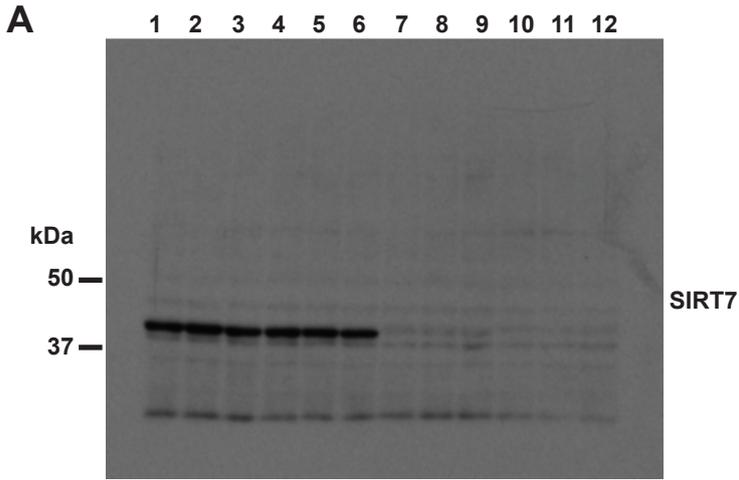
HPAEC plated on microelectrodes were transfected with CTRL or SIRT7 siRNA and transendothelial electric resistance (TER) measurements performed after stimulation with LPS (100 ng/ml) (**A**) or thrombin (0.2 U/ml) (**B**). Resistance data were normalized to the initial voltage and plotted as a normalized resistance. Data points represent averages and brackets standard deviations of three samples per condition in (**A**) and two samples per condition in (**B**). Experiments were performed on at least two separate occasions with similar results.

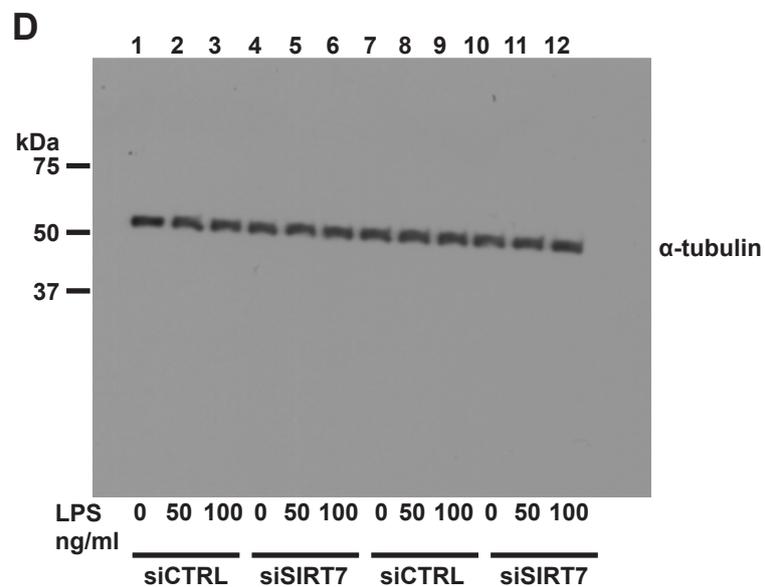
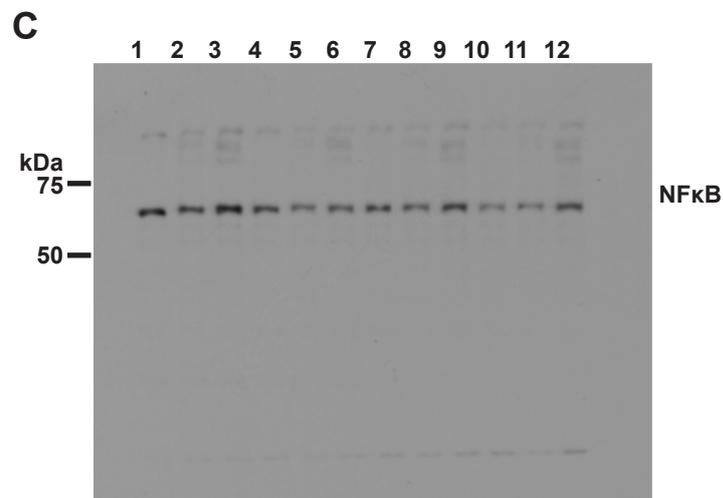
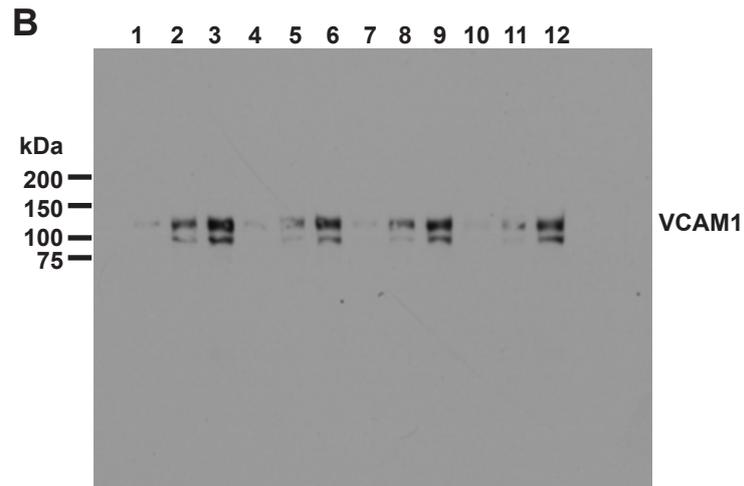
Supplementary Figure 11

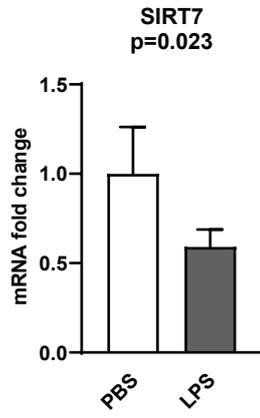
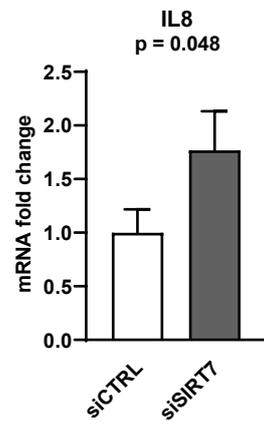
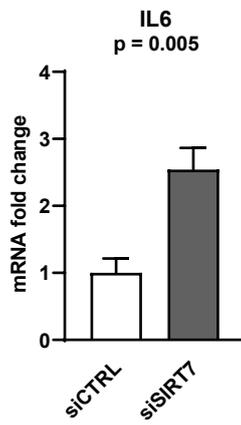
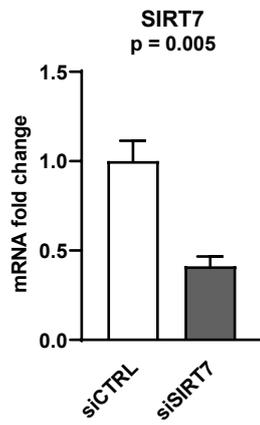
Full-length WBs for SIRT7 (**A**), total β -Catenin (**B**), α -tubulin (**C**), phosphorylated β -Catenin (**D**), and α -tubulin (**E**) corresponding to panel C in Figure 7.

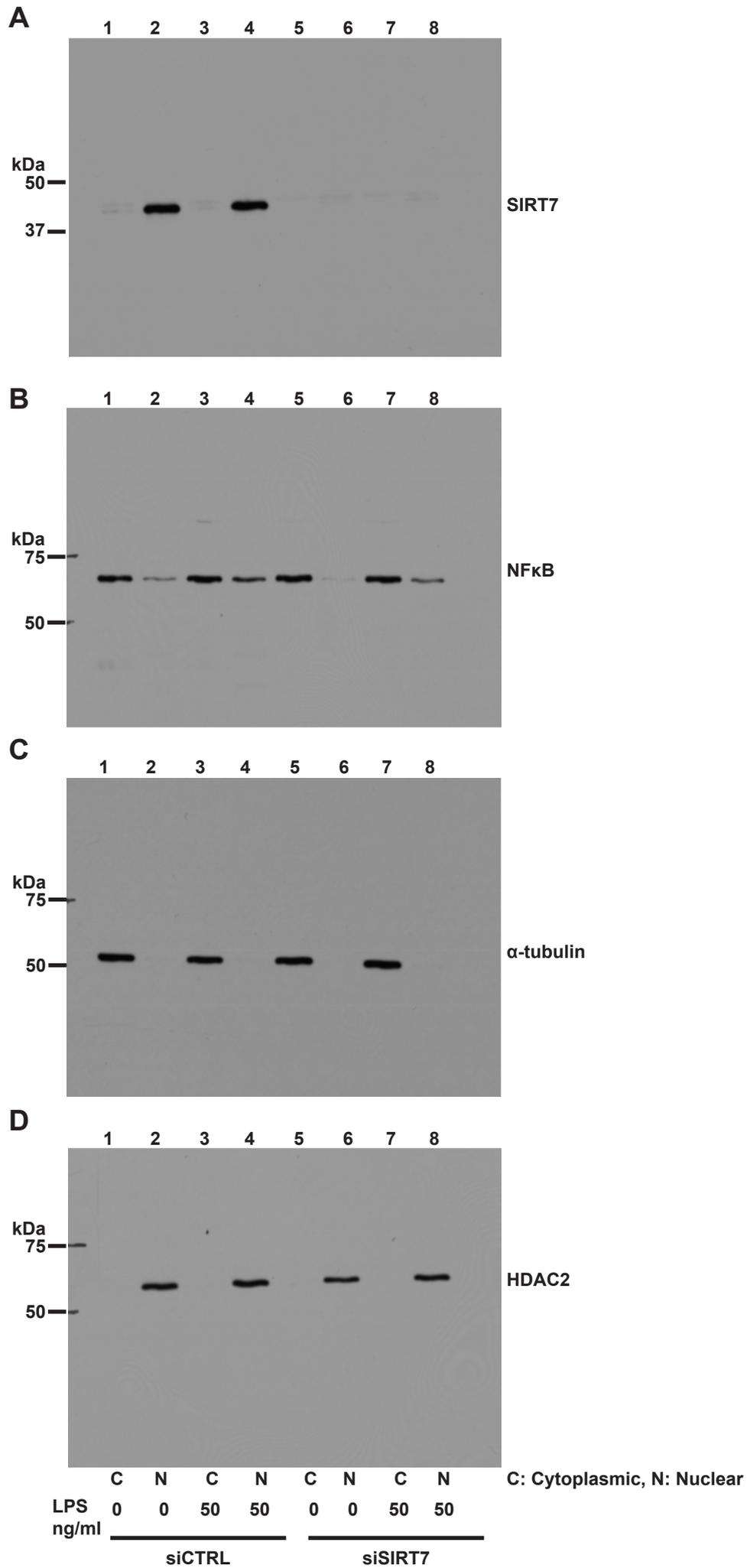
Supplementary Figure 12

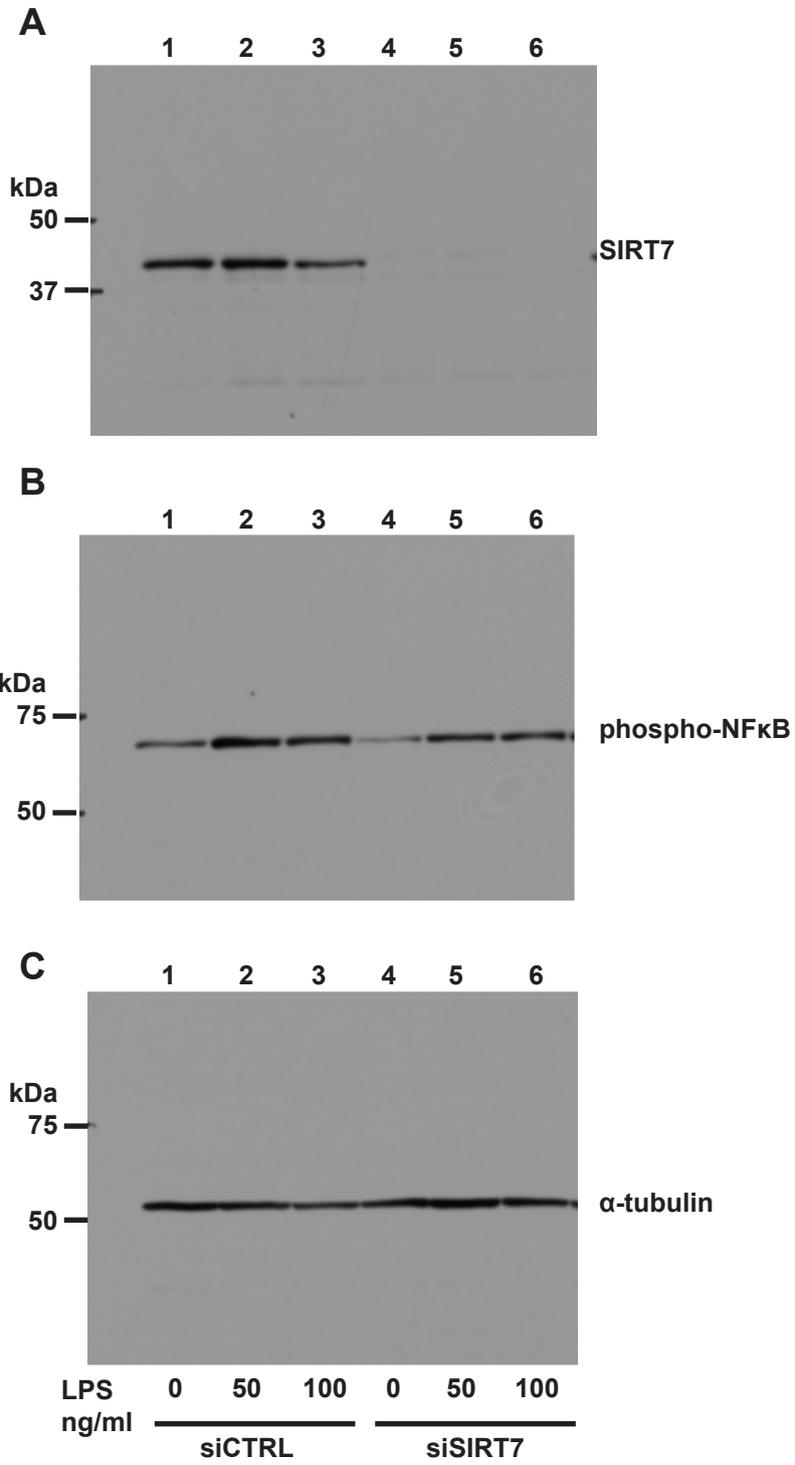
Effects of SIRT7 silencing on Rho signaling in HPAEC. **A:** Effect of SIRT7 silencing in HPAEC on thrombin-induced protein levels of phosphorylated and total myosin light chain 2 after thrombin stimulation for 15 minutes. Densitometry measurements relative to α -tubulin (RDU) are shown. Full-length WBs for SIRT7 (**B**), phosphorylated MLC2 (**C**), total MLC2 (**D**), and α -tubulin (**E**) in panel A.



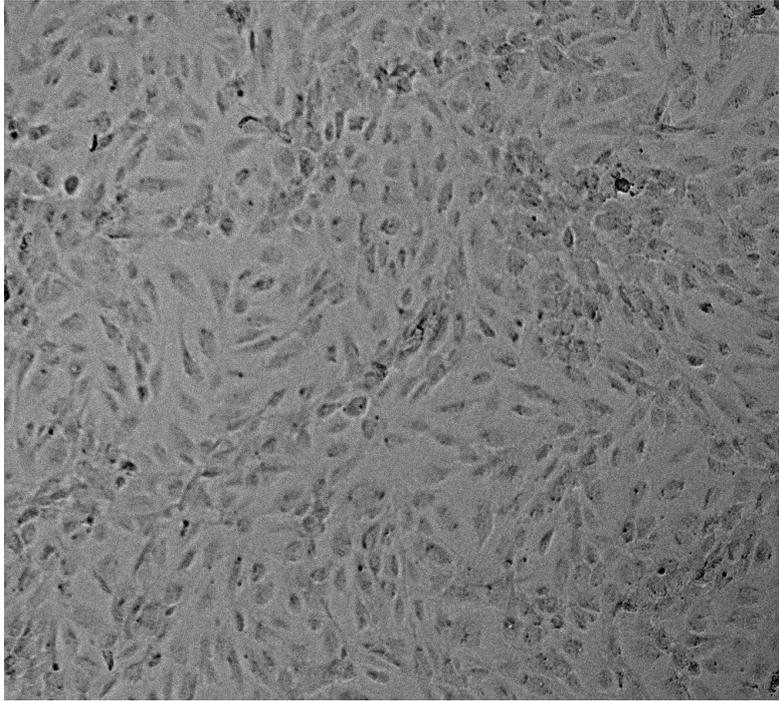


A**B**

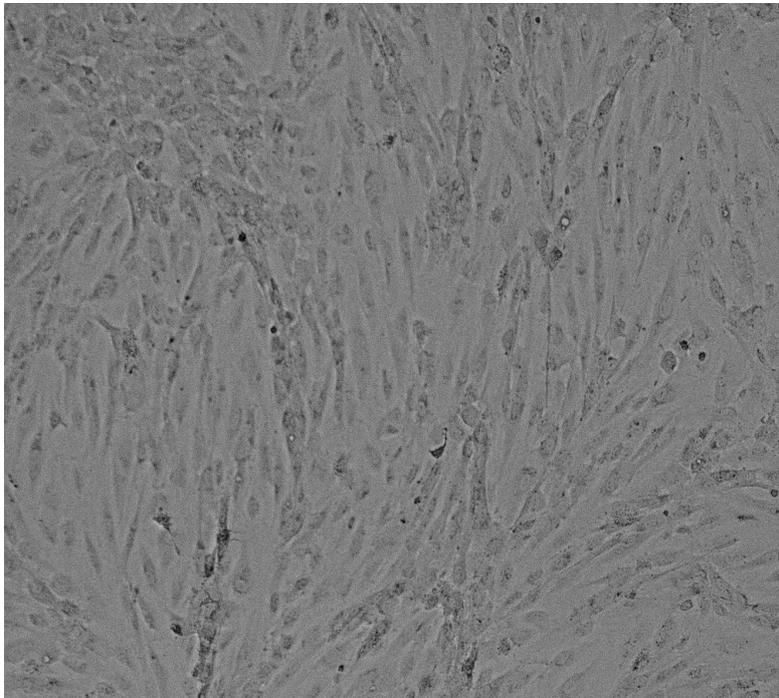




siCTRL

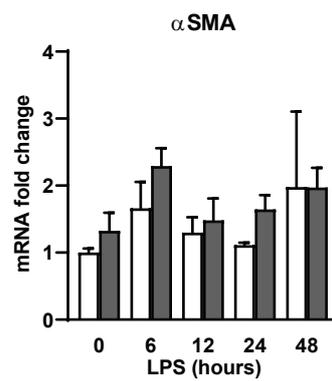
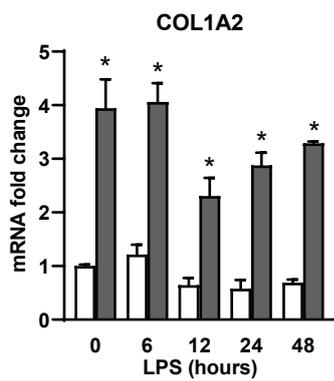
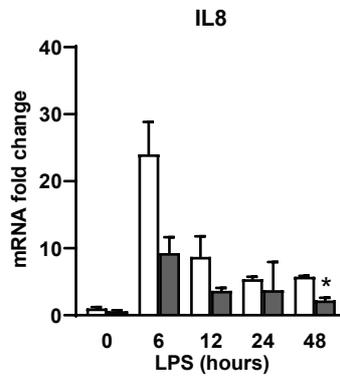
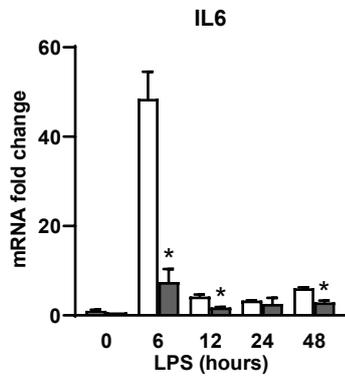
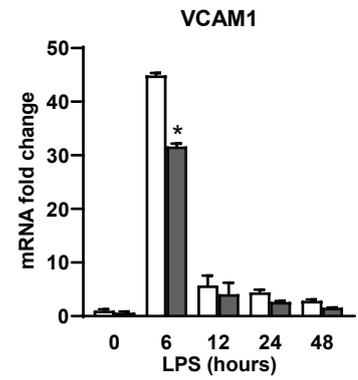
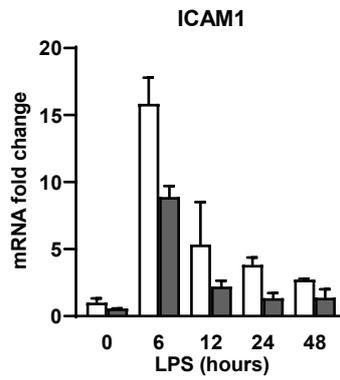
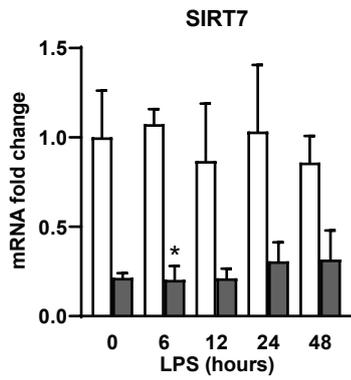


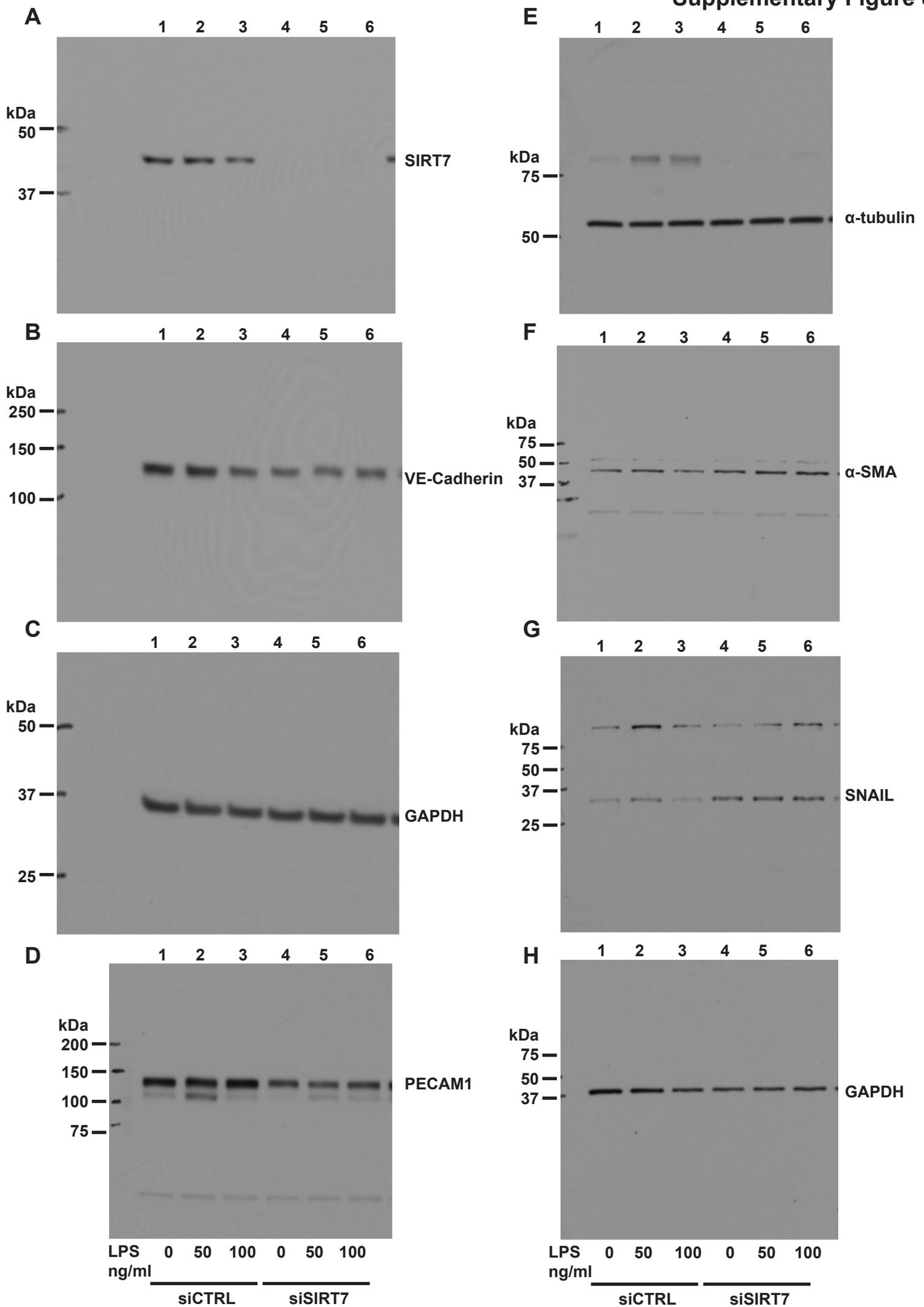
siSIRT7

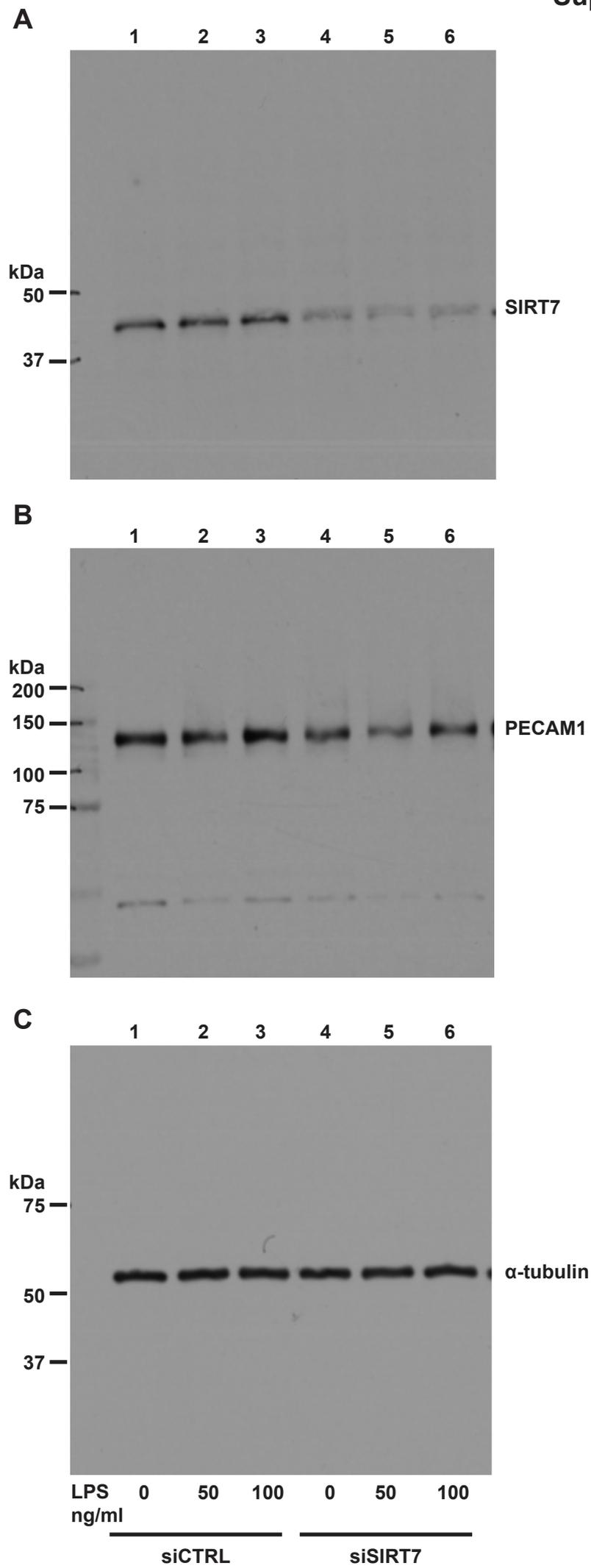


Supplementary Figure 7

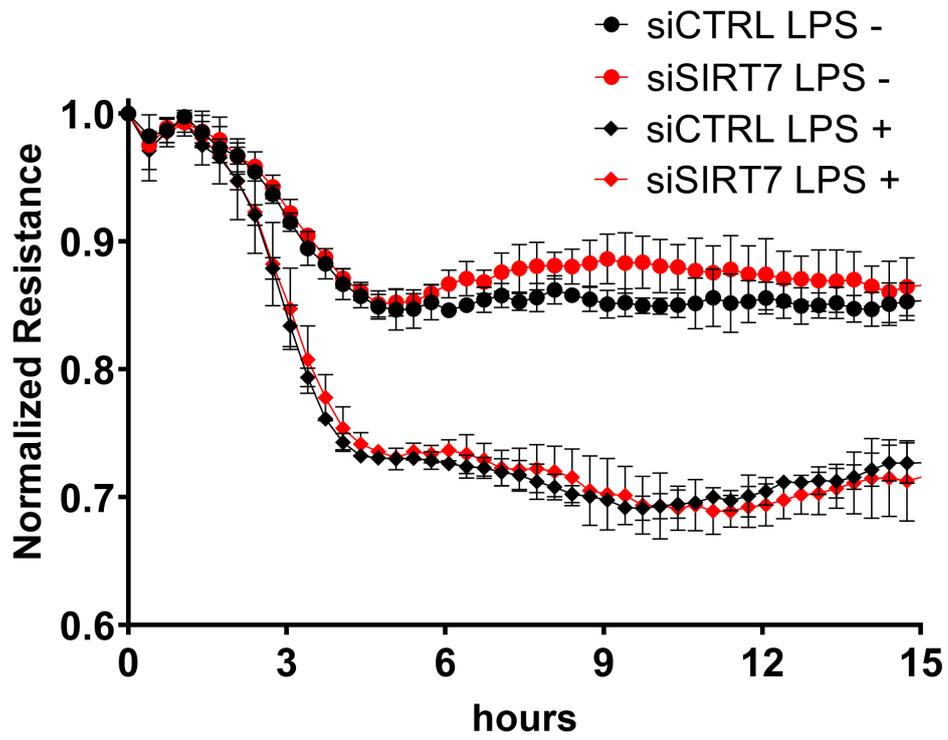
□ CTRL siRNA
 ■ SIRT7 siRNA







A



B

