Premature senescence of endothelial cells upon chronic exposure to TNFα can be prevented by N-acetyl cysteine and plumericin

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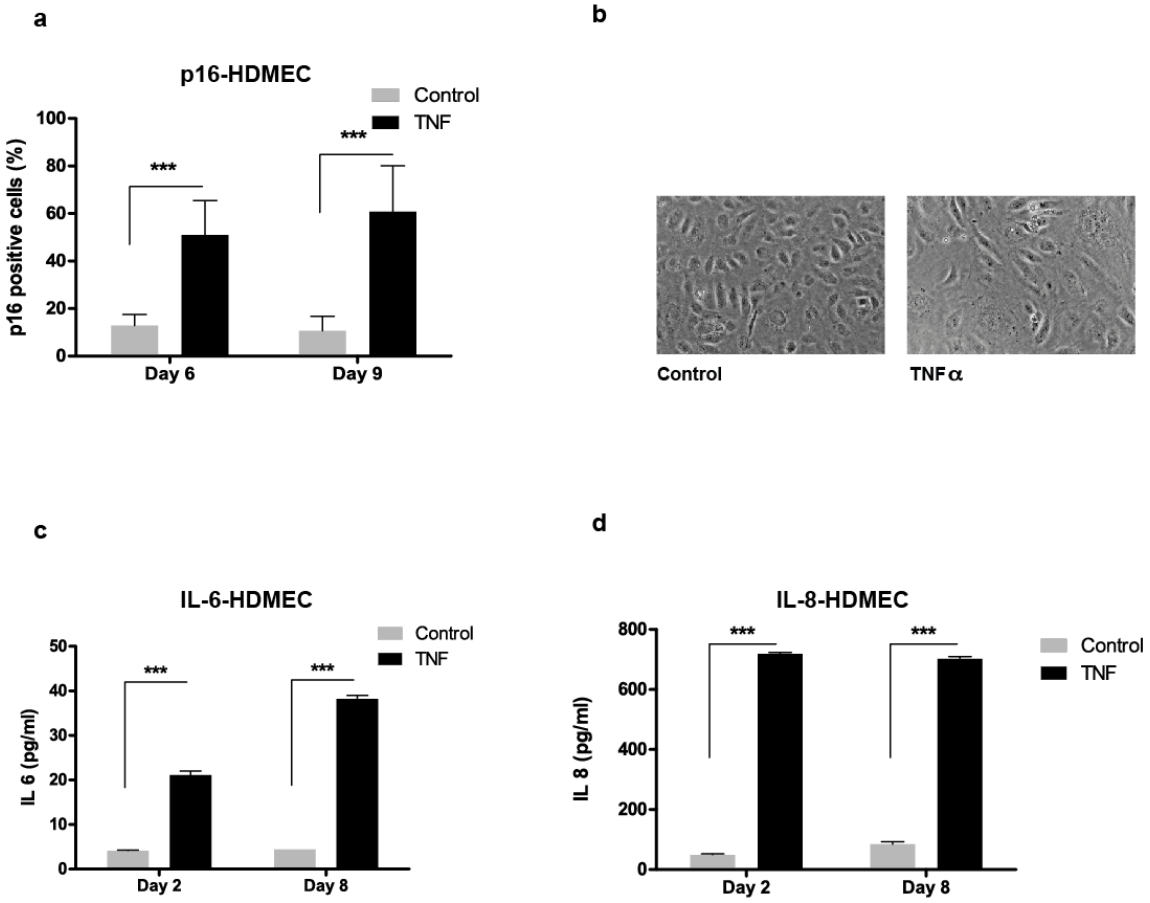
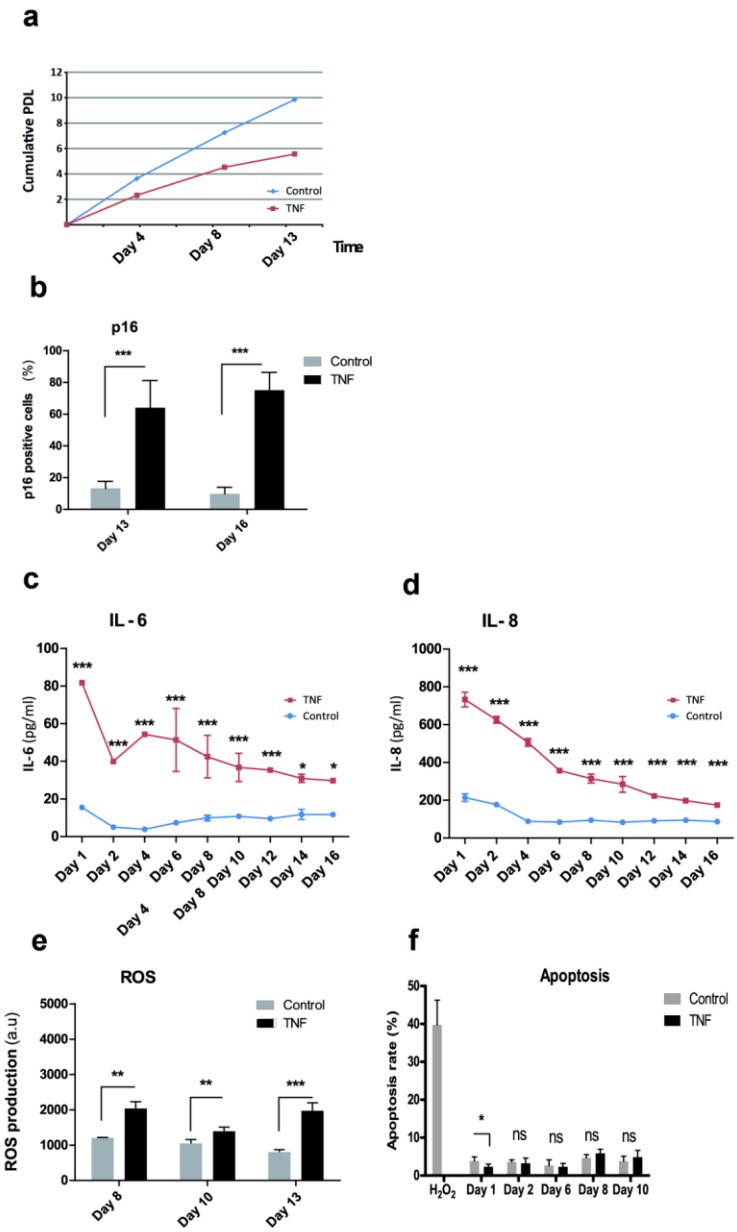
Supplemental Information

Supplemental Figure 1. Characterization of HDMECs. (**a**) Increased portion of p16 positive cells and (**b**) an increase in size and pronounced cell flattening after six days of TNFα (10ng) treatment. (**c**) Increased IL-6 and (**d**) IL-8 levels after six days of TNFα treatment. Values are presented as mean ± SD . (***p<0.001). The results shown are derived from a single experiment in technical triplicates (**a**) or in duplicates (**c,d**).



Supplemental Figure 2. Effects of cultivation of HUVECs in the presence of TNFα for six days, followed by an additional 7-10 days period without TNFα. (**a**) Retardation of growth in TNFα (10ng) treated HUVECs. (**b**) Increased portion of p16 positive cells after TNFα treatment, as assessed at days 13 and 16. Increased levels of (**c**) IL-6 and (**d**) IL-8 throughout the entire analyzed period. (**e**) Elevated ROS production in TNFα treated cells at days 8, 10 and 13 as compared to controls cells. (**f**) Quantification of apoptosis as determined by annexin V and propidium iodide staining and subsequent evaluating of at least 300 cells. H_2O_2 was used as positive control for the induction of apoptosis. Values are presented as mean ± SD of a single experiment in technical triplicates (**a,b,e,f**) or duplicates (**c,d**). (***p<0.001).



Supplemental Figure 3. Effects of plumericin, PHA-408, and NAC on growth rates and cytokine secretion in HUVECs. (**a**) Growth curves of HUVECs treated with inhibitors only. The results shown are representative of three independent experiments accomplished in technical triplicates. Levels of (**b**) IL-6 and (**c**) IL-8 in HUVECs treated with inhibitors only. The results shown are derived from a single experiment in technical duplicates. PL: plumericin, PHA: PHA-408, NAC: N-acetyl cysteine.