Supplementary figure 2: Nitrosylated hAAT does not cause macrophage death.

In order to investigate that S-NO-hAAT does not cause macrophage death, we assessed S-NO-hAAT toxicity towards macrophages. THP-1 cells $(0.3 \times 10^6 \text{ per well})$ were incubated for 24-hours with 27.5 μ M of hAAT or S-NO-hAAT, or 10 μ M Cytarabine (Sigma-Aldrich) as a positive control. Cell viability was assessed by supernatant relative LDH content (cytotox-96 kit, Promega), and AnnexinV-FITC/PI staining (BioLegend). Flow cytometric experiments were done using BD Canto II (BD Biosciences, Singapore) and data were analyzed by FLOWJO 7.6.3 software (Flowjo, LLC Data Analysis Software, Ashland, OR).

As our Cytarabine treatment indicates, Higher levels of supernatant LDH or double positive AnnexinV-FITC/PI cells (both are presented as mean ± SEM) are correlated with more dead cells. In contrast with the cytarabine treatment, the amount of surviving cells was high and comparable between S-NO-hAAT, hAAT and untreated cells, indicating that S-NO-hAAT does not cause macrophage death.





Annexin V FITS