SUPPLEMENTAL METHODS AND FIGURES 1 -3

SUPPLEMENTAL METHODS

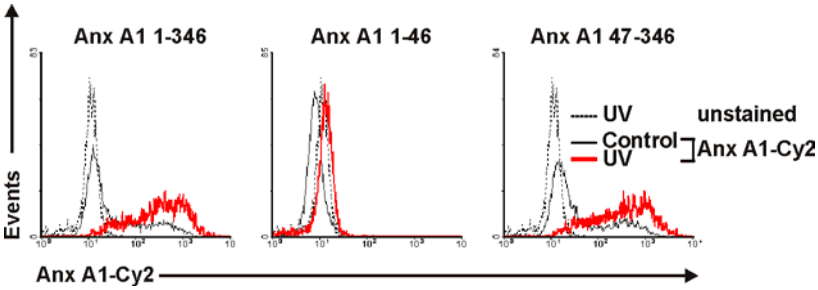
Preparation of primary human PHA-stimulated lymphoblasts, monocytes and neutrophils

Human PBMCs and neutrophils were prepared from heparinized blood by double-Ficoll gradient purification (Histopaque 1.119 g/ml, Sigma and Ficoll-Paque 1.077 g/ml, GE Healthcare). Primary human monocytes were positively selected from PBMCs with anti-CD14 magnetic beads (Miltenyi, Bergisch Gladbach, Germany) according to the manufacturer's recommendation. PHA-stimulated lymphoblasts were prepared by cultivating monocyte-depleted human PBMCs for 2 days in RPMI-1640 supplemented with 10% heat-inactivated fetal calf serum, 100 units of penicillin/ml, 0.1 mg streptomycin/ml, 10 mM HEPES and 0.5 µg/ml PHA (Biochrom, Berlin, Germany) and 5 further days in PHA-free medium.

Flow cytometric triple staining for PS exposure, membrane permeabilization and anx A1 externalization

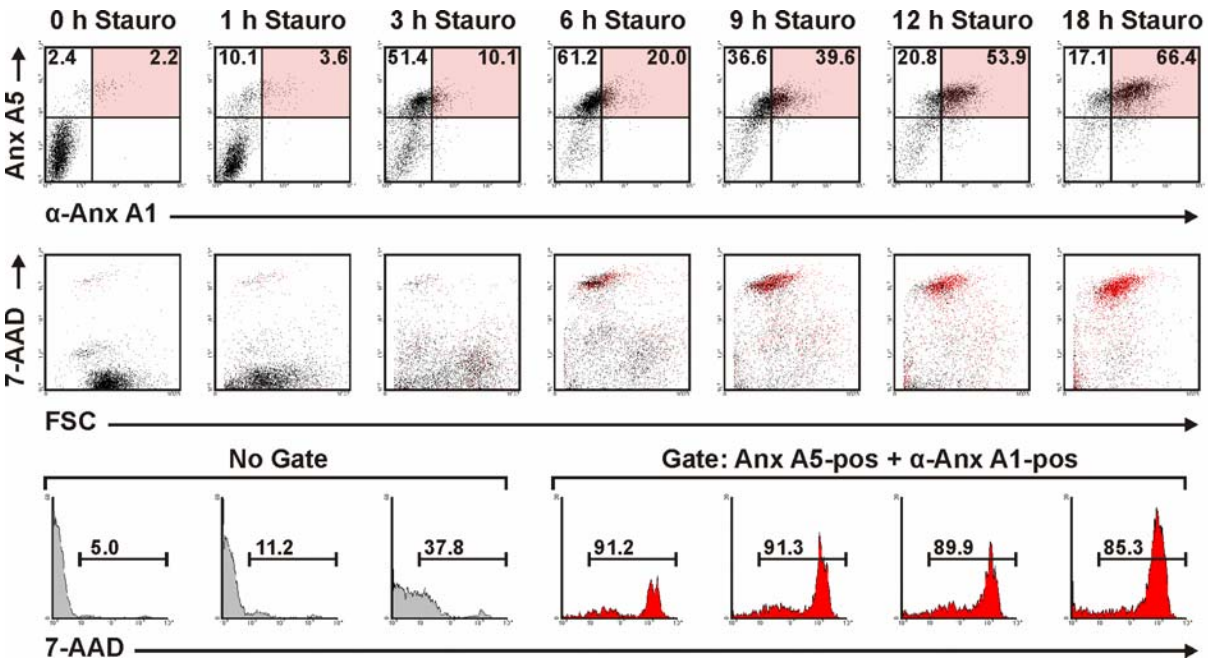
Staining with the GFP-certified apoptosis/necrosis detection kit (Enzo Life Sciences, Loerrach, Germany) was performed according to the manufacturer's instructions. Anti-anx A1 staining was combined with the procedure by pre-incubating the cells with anti-anx A1 for 30 min and subsequently adding the secondary anti-mouse-IgG-Cy2 antibody to the anx A5-Cy3/7-AAD staining mixture.

SUPPLEMENTAL FIGURE LEGENDS



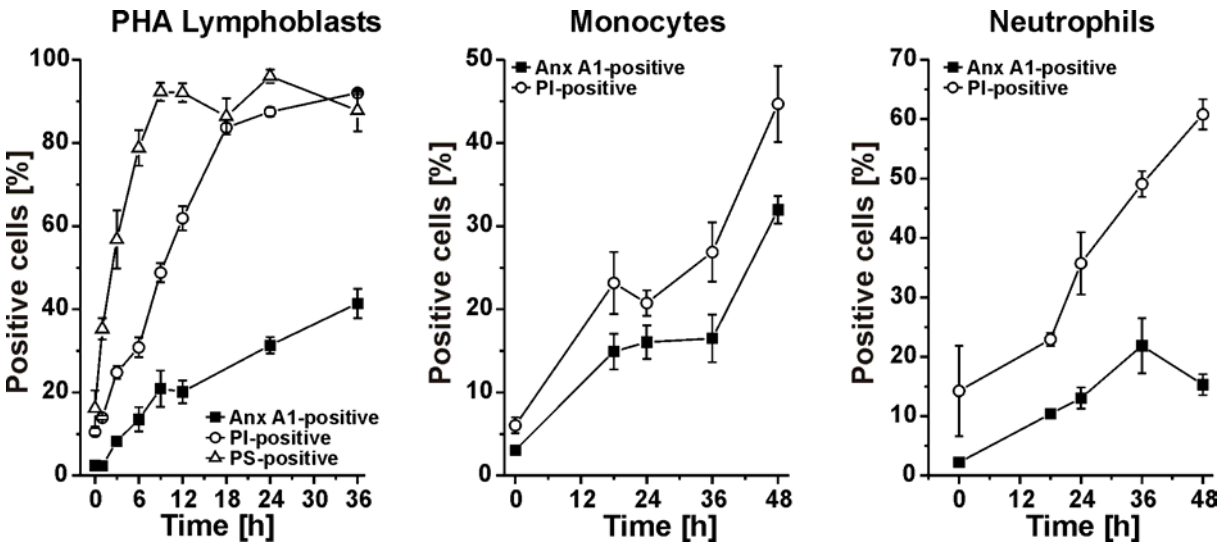
Supplemental Figure 1: Apoptotic cells bind Cy2-labeled anx A1 and an anx A1 core fragment.

THP-1 cells were left untreated or UV-irradiated with 10 mJ/cm^2. After 12 h of incubation cells were stained with purified Cy2-labeled anx A1 mutants (aa 1-346, aa 1-46 and aa 47-346) and analyzed by flow cytometry. Representative histograms of triplicates are shown (analysis gate was set on PI-negative cells).



Supplemental Figure 2: Time course of phosphatidylserine exposition, membrane permeabilization and anx A1 externalization during apoptosis and secondary necrosis.

Jurkat cells were stimulated with 2.5 µM staurosporine (Stauro) for the indicated times in order to undergo apoptosis followed by secondary necrosis. Subsequently, cells were stained with anti-anx A1/anti-mouse-IgG-Cy2, anx A5-Cy3 and 7-AAD and analyzed by flow cytometry. *Upper panel:* Dotplots showing anti-anx A1 vs. anx A5 staining. *Middle panel:* Dotplots showing FSC vs. 7-AAD. Cells positive for anti-Anx A1 and anx A5 staining (upper right quadrant in upper panel) are displayed in red. *Lower panel:* Histograms of 7-AAD fluorescence. The first 3 histograms display the whole population analyzed, whereas the last 4 histograms are gated on the population positive for anti-Anx A1 and anx A5 (upper right quadrant in upper panel). Representative plots of triplicates are shown.



Supplemental Figure 3: Exposure of phosphatidylserine and loss of plasma membrane integrity precede anx A1 externalization in primary human PHA-stimulated lymphoblasts, monocytes and neutrophils.

Primary human PHA-stimulated lymphoblasts, monocytes and neutrophils were stimulated with 2.5 µM staurosporine to undergo apoptosis and secondary necrosis. Cell surface exposure of phosphatidylserine (PS) was detected by anx A5-FITC staining. Anx A1 externalization and PI exclusion were measured as in Fig. 2A. The percentages of PS-positive, anx A1-positive and PI-positive cells (mean values ± standard deviations of triplicates) are shown.