Supporting Information

Cohen et al. 10.1073/pnas.0915018107

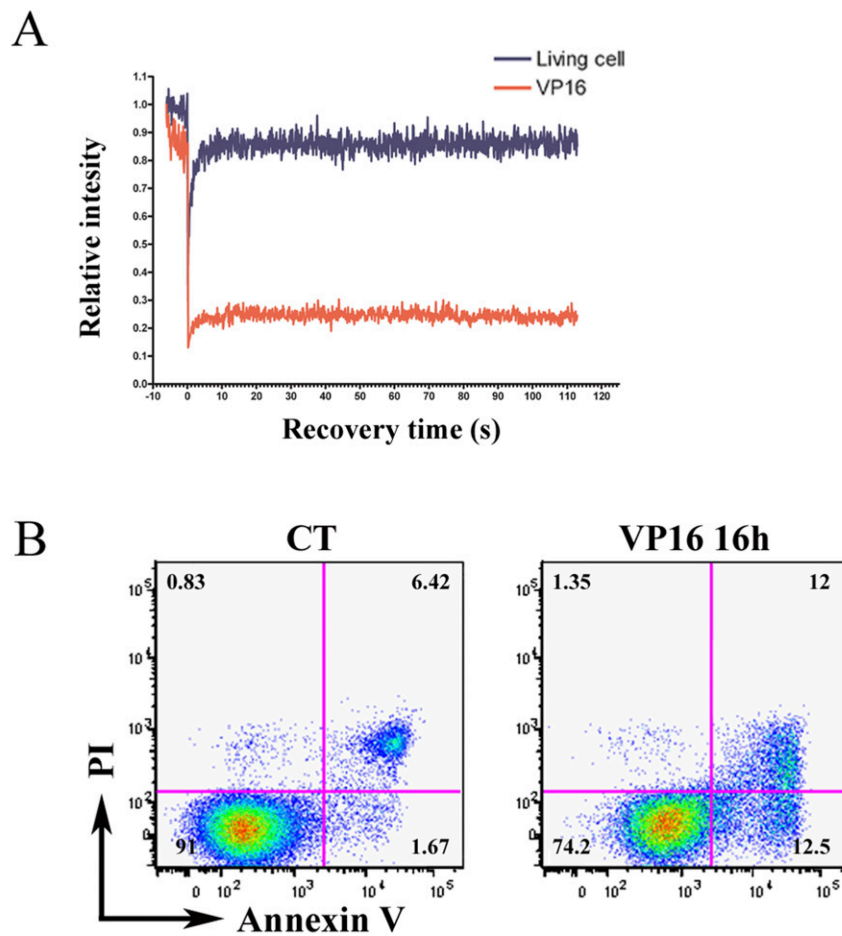

Fig. S1. Dynamics of precIL-1α in living and VP16-treated cells. (*A*) Kinetics of nucleoplasmic FRAP in living or VP16-treated B16 melanoma cells transfected with precIL-1α-GFP. Fluorescence intensity in the bleached region was measured for at least 110 s and expressed as the relative intensity. Data values in FRAP kinetics represent one of six independent experiments. (*B*) FACS analysis of annexin-V/PI–stained B16 melanoma cells treated with 20 μg/mL VP16.

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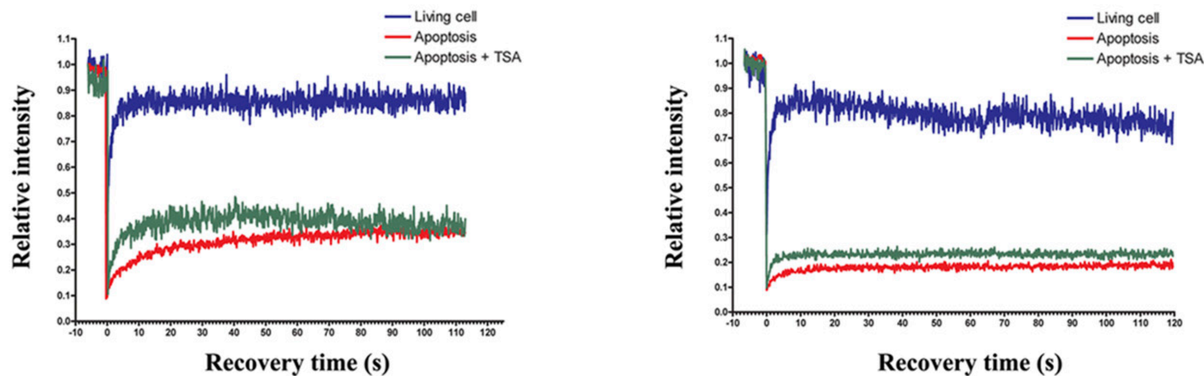

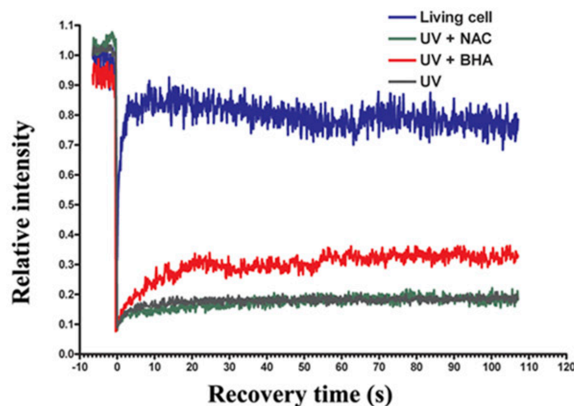

Fig. S2. ROS scavengers and a histone deacetylase inhibitor do not alter the nonreversible nature of IL-1α proteins in the nucleus of apoptotic cells. (*A*) Kinetics of nucleoplasmic FRAP in living cells, UV-irradiated cells and UV-irradiated cells treated with 200 ng ml^{-1} TSA. B16 melanoma cells transfected with ppIL-1α-GFP (*Left*) and precIL-1α-GFP (*Right*). (*B*) Kinetics of nucleoplasmic FRAP in living cells and UV-irradiated cells treated with the ROS scavengers NAC or BHA. Fluorescence intensity in the bleached region was measured for at least 110 s and expressed as the relative intensity. Data values in FRAP kinetics represent one of six independent experiments.

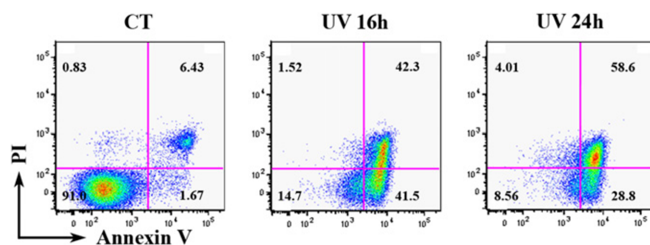

Fig. S3. Progression of the apoptotic process in UV-irradiated murine B16 melanoma cells. Apoptosis was induced in B16 melanoma cells by 3 min of UV irradiation. The rate of apoptosis was evaluated by annexin-V/PI staining and analyzed by FACS 16 h or 24 h after UV irradiation.

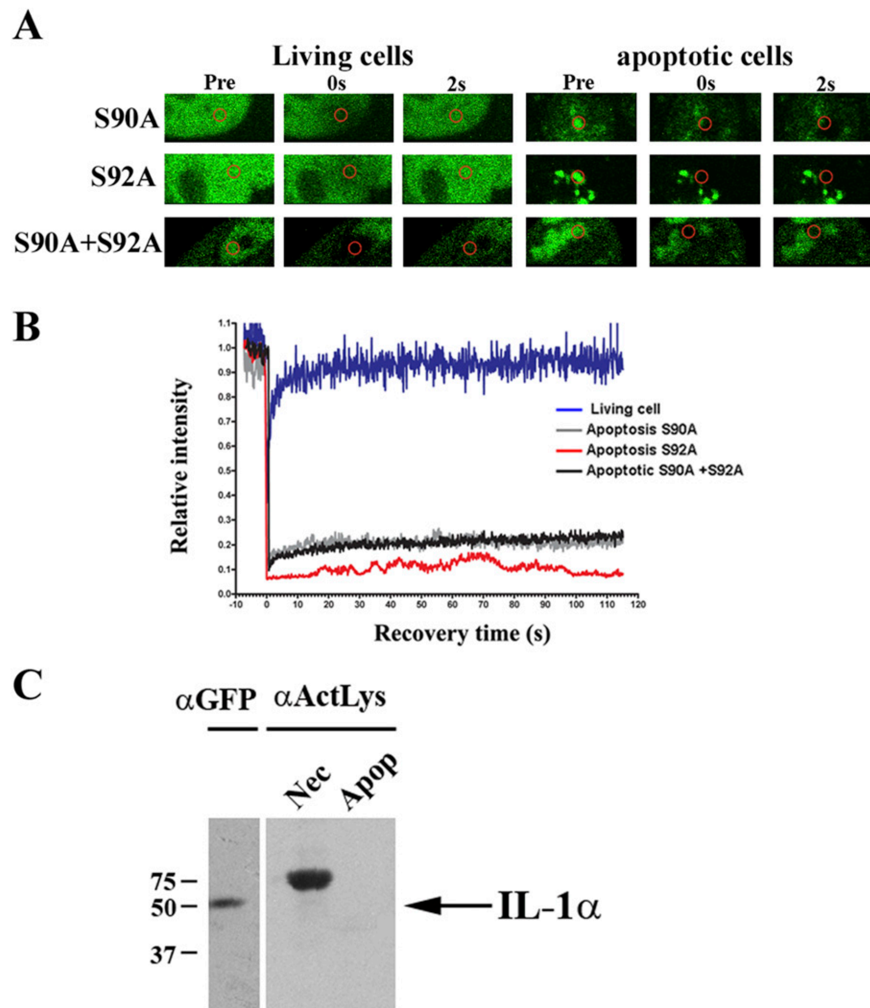

Fig. S4. Posttranslational modifications do not affect IL-1α precursor mobility during apoptosis. (*A*) FRAP experiments in living and apoptotic cells expressing precIL-1α-GFP and mutants of precIL-1α-GFP within serine 90, 92, and 90+92 (substitution of serine residues in positions 90, 92, and 90+92 to alanine). The area of the bleach spot is indicated with a red circle. (*B*) Kinetics of nucleoplasmic FRAP in living or apoptotic transfectants of precIL-1α-GFP and mutants of precIL-1α-GFP within serine 90, 92, and 90+92. Fluorescence intensity in the bleached region was measured for at least 110 s and expressed as the relative recovery. Data values in FRAP kinetics represent one of six independent experiments. (*C*) Western blot analysis using antiacetylated lysine antibody was used to detect acetylated lysine residues within apoptotic and necrotic cell lysates. IL-1α position was detected with anti-GFP antibody.

Fig. S5. IL-1α affects the recruitment of both macrophages and neutrophils to the site of injury. Number of infiltrating cells into Matrigel plugs 20 h after injection into mice with supernatants of necrotic and apoptotic WT or IL-1$\alpha^{-/-}$ fibroblasts without or with pretreatment with neutralizing anti-IL-1α antibodies. *Top:* Total infiltrate number. *Middle:* Numbers of macrophages out of the total of infiltrating cells per plug were calculated from the percentage of Ly-6C^{high}/CD115^{+}/F4-80^{+} cells. *Bottom:* Numbers of neutrophils out of the total infiltrating cells per plug were calculated from the percentage of Ly-6G ^{high}/Ly-6C^{dull}/CD115neg cells. Recombinant mIL-1α and PBS were used as positive and negative controls. Data represent an average (*n* = 3) of one of three independent experiments.