Survival analysis of patients with colon cancer, divided into two groups (high and low expression profiles of the indicated cytokines), based on the TCGA expression data.
Representative images of cell death detected by IncuCyte imaging analysis of melanoma (A), lung cancer (C), and leukemia (E) cells treated with TNF-α and IFN-γ at 48 h post-treatment. Scale bar, 50 μM. Time-course analysis of cell death of melanoma (B), lung cancer (D), and leukemia (F) cells treated with TNF-α alone, IFN-γ alone, or TNF-α plus IFN-γ, assessed over the course of 48 h post-stimulation. Data are representative of three independent experiments (A-F). Data are presented as the mean ± SEM (B,D,F). *P < 0.05; **P < 0.01; ****P < 0.0001. Analyses were performed using one-way ANOVA (B,D,F).
Supplementary Figure 3: TNF-α and IFN-γ treatment triggers PANoptotic cell death in human melanoma, lung and leukemia cancer cell lines

(A-C) Western blot analysis of PANoptosis components in melanoma cancer cells treated with cytokines as indicated and assessed in culture at 48 h post-stimulation. (A) Western blot analysis of the pyroptosis markers: pro- (P45) and activated (P20) caspase-1 (CASP1), pro- (P53) and activated (P30) gasdermin D (GSDMD), and pro- (P53) and activated (P34) gasdermin E (GSDME). (B) Western blot analysis of the apoptosis markers: pro- (P55) and cleaved caspase-8 (P18), pro- (P35) and cleaved caspase-3 (P19 and P17), and pro- (P35) and cleaved caspase-7 (P20). (C) Western blot analysis of necroptosis components: total MLKL (T-MLKL) and total RIPK3 (T-RIPK3).

(D-F) Western blot analysis of PANoptosis components in lung cancer and leukemia cells treated with cytokines as indicated and assessed in culture at 48 h post-stimulation. (D) Western blot analysis of the pyroptosis markers: pro- (P45) and activated (P20) CASP1, pro- (P53) and activated (P30) GSDMD, and pro- (P53) and activated (P34) GSDME. (E) Western blot analysis of the apoptosis markers: pro- (P55) and cleaved CASP8 (P18), pro- (P35) and cleaved CASP3 (P19 and P17), and pro- (P35) and cleaved CASP7 (P20). (F) Western blot analysis of necroptosis components: T-MLKL and T-RIPK3. Western blot of β-Actin was used as loading control. Asterisks indicate non-specific bands. Data are representative of at least three independent experiments (A-F).
Supplementary Figure 4: Nitric oxide inhibition does not prevent the TNF-α and IFN-γ-dependent cancer cell death in human melanoma cells

Representative images (A) or time course analyses (B) of cell death detected by IncuCyte imaging of indicated human melanoma cells treated for 48 h with TNF-α and IFN-γ, in the presence or absence of the nitric oxide inhibitors L-NAME (nitric oxide inhibitor) or 1400W (inducible nitric oxide inhibitor). Scale bar, 50 μM. Data are representative of two independent experiments (A-B). Data are presented as the mean ± SEM (B). ns, not significant; ****P < 0.0001. Analyses were performed using two-way ANOVA (B).