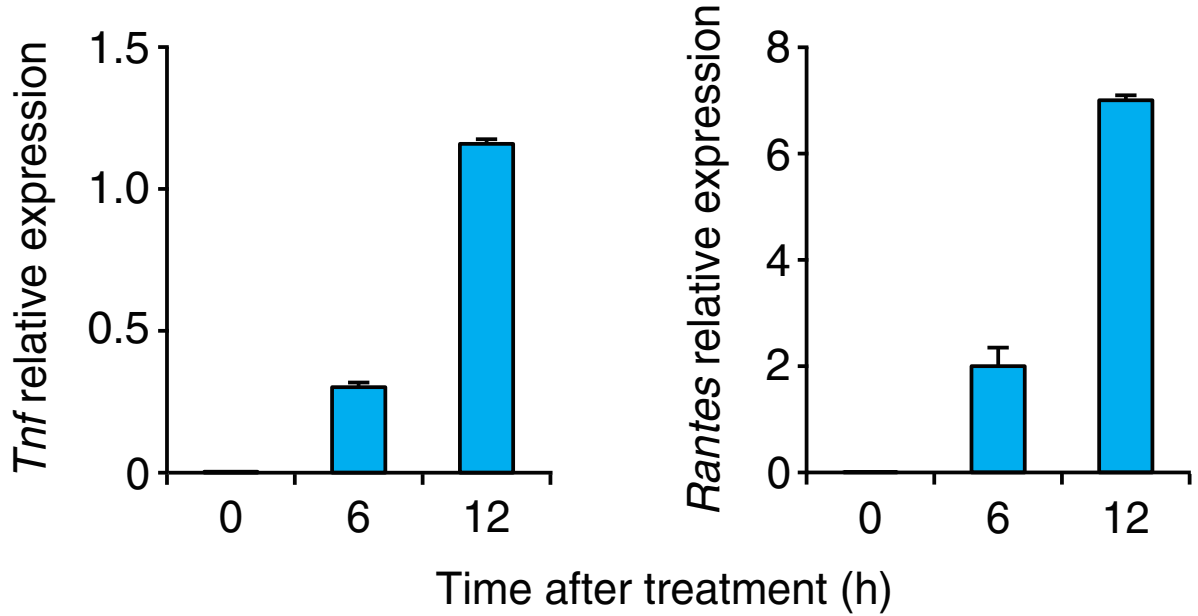

Fig. S1. SINCRO induces TNF and RANTES mRNAs expression. B16F1 cells (8×10^4 cells) were treated with SINCRO (10 μg/ml) for the indicated time. TNF and RANTES mRNAs expression was quantified by qRT-PCR analysis. Data are shown as mean ± SEM.

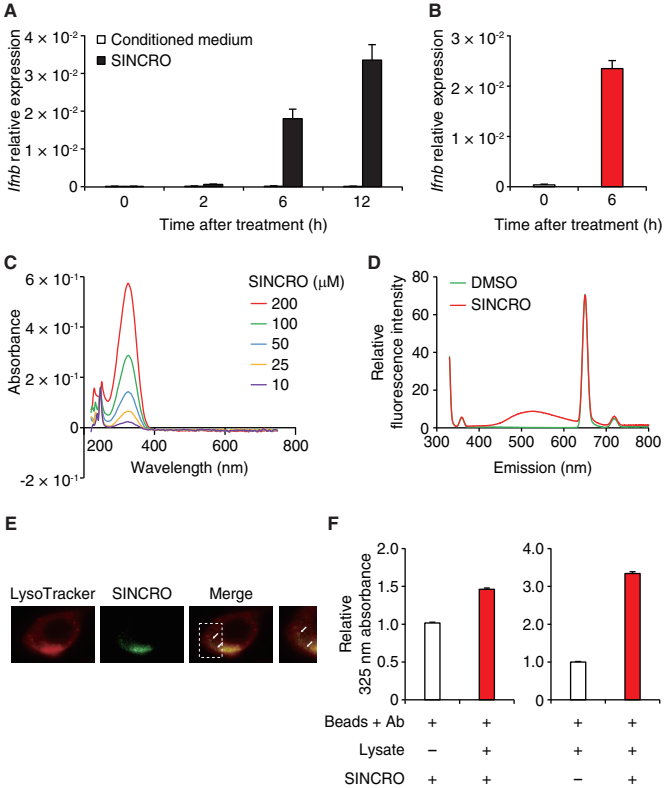

Fig. S2. SINCRO is internalized into cells and induces IFN-β mRNA expression through STING. (A) B16F1 cells (8×10^4 cells) were stimulated with SINCRO (10 μg/ml) for 6 hours, extensively washed with medium, and incubated for another 6 hours. Supernatants were collected as conditioned medium from the culture and B16F1 cells (8×10^4 cells) were treated with the medium for the indicated time. The cells were also treated with SINCRO (10 μg/ml). IFN-β mRNA induction was measured by qRT-PCR analysis. (B) B16F1 cells (8×10^4 cells) were treated with SINCRO (10 μg/ml) for 0 or 6 hours. The cells were extensively washed and incubated for another 6 hours. IFN-β mRNA induction was determined by qRT-PCR analysis. (C) Absorbance spectrum of SINCRO (10, 25, 50, 100 or 200 μM) was measured and depicted. Indicated absorbance value shows the light absorbance of SINCRO solution subtracting that of DMSO. (D) Emission spectrums of SINCRO (2 μM) or DMSO excited with a 325 nm light are shown. (E) B16F1 cells (1.5×10^6 cells) were treated with SINCRO (10 μg/ml) for 3 hours. After the treatment, the cells were incubated with LysoTracker for 30 minutes. Fluorescence images of SINCRO (Green) and LysoTracker (Red) were visualized by confocal fluorescence microscope analysis. Right panel shows enlarged image of the area surrounded by white dot line. Arrows indicate dot-like SINCRO location. (F) HA-STING was transiently expressed in HEK293T cells. Cell lysate (500 μg) was prepared from those cells and HA-STING was immunoprecipitated with anti-HA antibody (2 μg/ml) and protein G-conjugated Dynabeads. The precipitate obtained with or without the cell lysate was incubated with SINCRO (100 μg/ml) for 2 hours, and SINCRO was then released by boiling, followed by the absorbance measurement at 325 nm (Left panel). Similar assay was performed with or without incubation with SINCRO (Right panel). Data are shown as mean ± SEM.

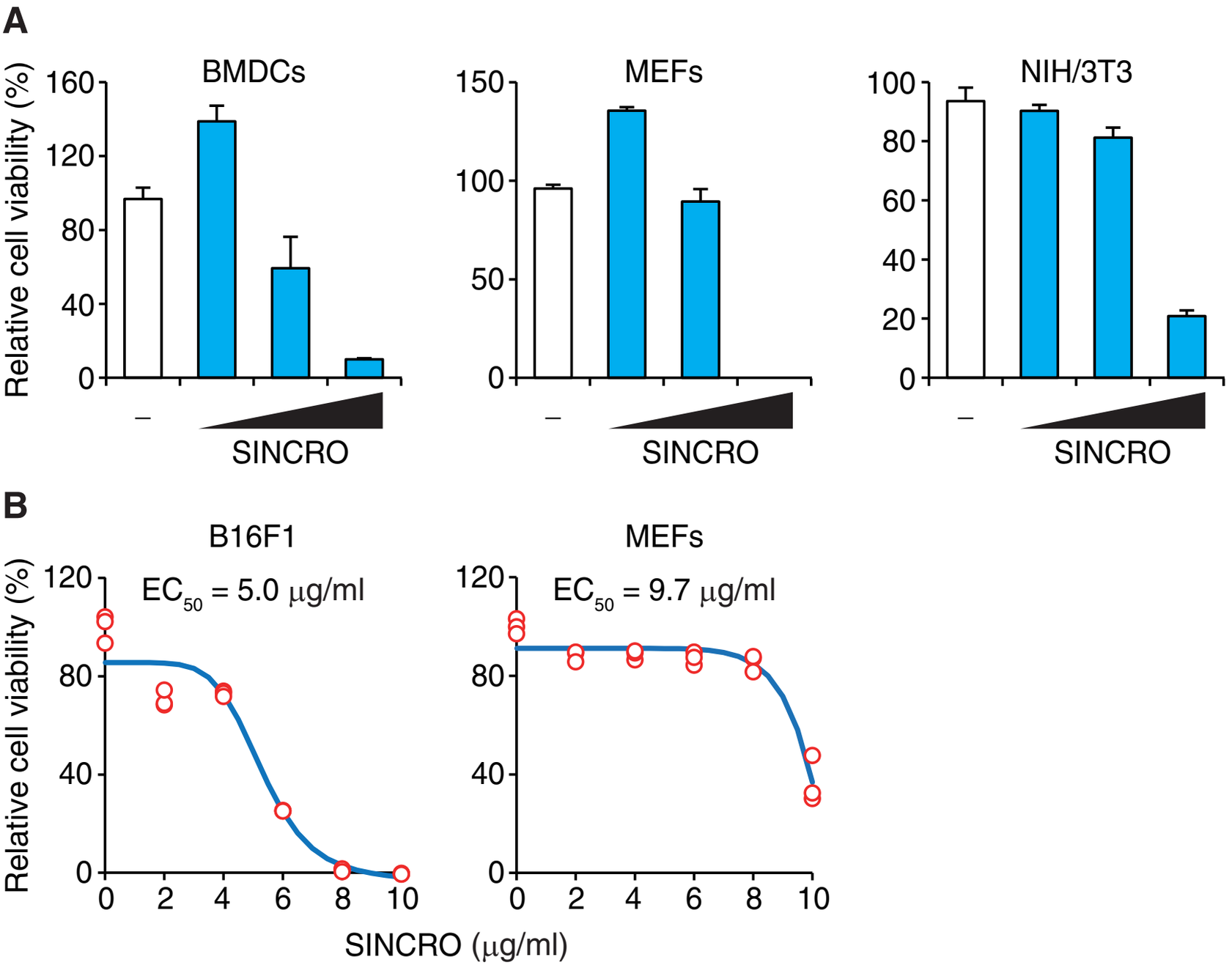

Fig. S3. SINCRO possesses cytocidal activity against non-transformed cells. (A) BMDCs (7×10^4 cells), MEFs (1×10^4 cells) or NIH/3T3 (1×10^4 cells) cells were treated with SINCRO (2.5, 5 or 10 μg/ml) or DMSO for 40 hours. Cell viability was evaluated by MTT assay. (B) B16F1 cells or MEFs (1×10^4 cells) were incubated with SINCRO (2, 4, 6, 8 or 10 μg/ml) or DMSO for 40 hours. Measurement of cell viability was performed with MTT assay and EC_{50} of SINCRO is calculated. Data are shown as mean ± SEM.

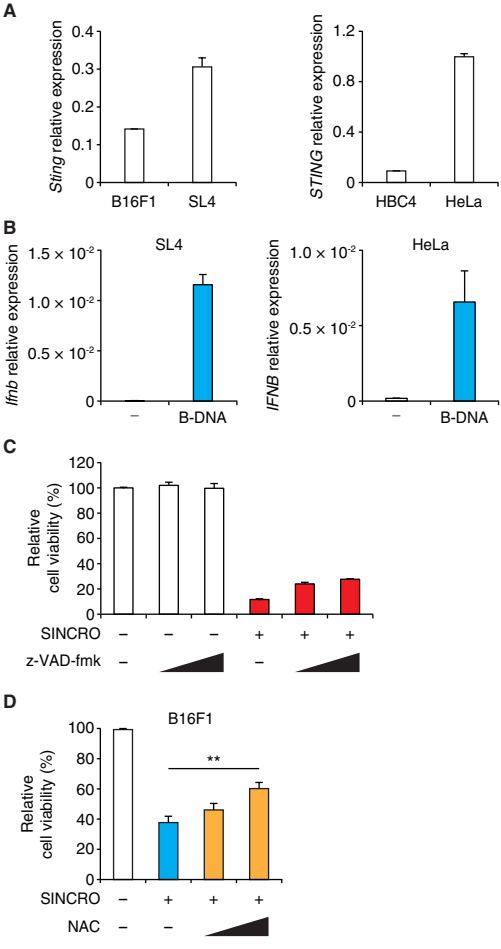

Fig. S4. SINCRO induces cell death independently of caspases. (A) The expression level of STING mRNA in B16F1, SL4, HBC4 and HeLa cells were measured by qRT-PCR. (B) SL4 cells (8×10^4 cells) or HeLa cells (8×10^4 cells) were treated with or without B-DNA (10 μg/ml) for 6 hours. IFN-β mRNA expression was quantified by qRT-PCR analysis. (C) B16F1 cells (1×10^4 cells) were pre-treated with z-VAD-fmk (20 or 40 μM) or DMSO for 1 hour. Then the cells were incubated with SINCRO (10 μg/ml) or DMSO for 40 hours. Cell viabilities were measured by MTT assay. (D) B16F1 cells (1×10^4 cells) were treated with NAC (1 or 3 mM) or water under the stimulation with SINCRO (10 μg/ml) or DMSO. Cell viability was evaluated by MTT assay. Data are shown as mean ± SEM.