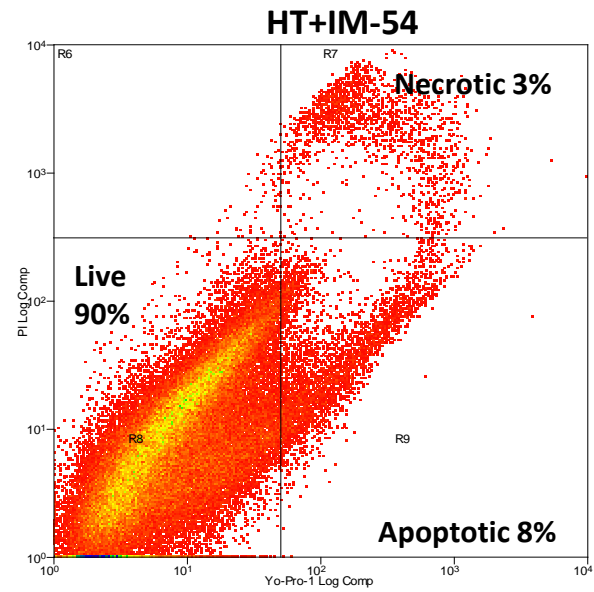
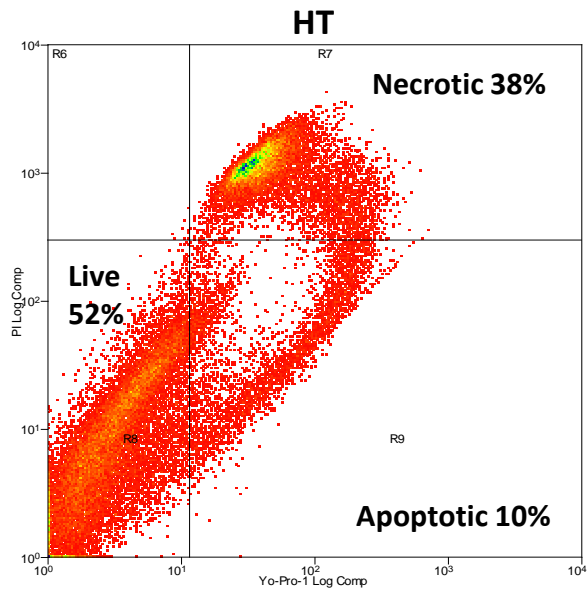
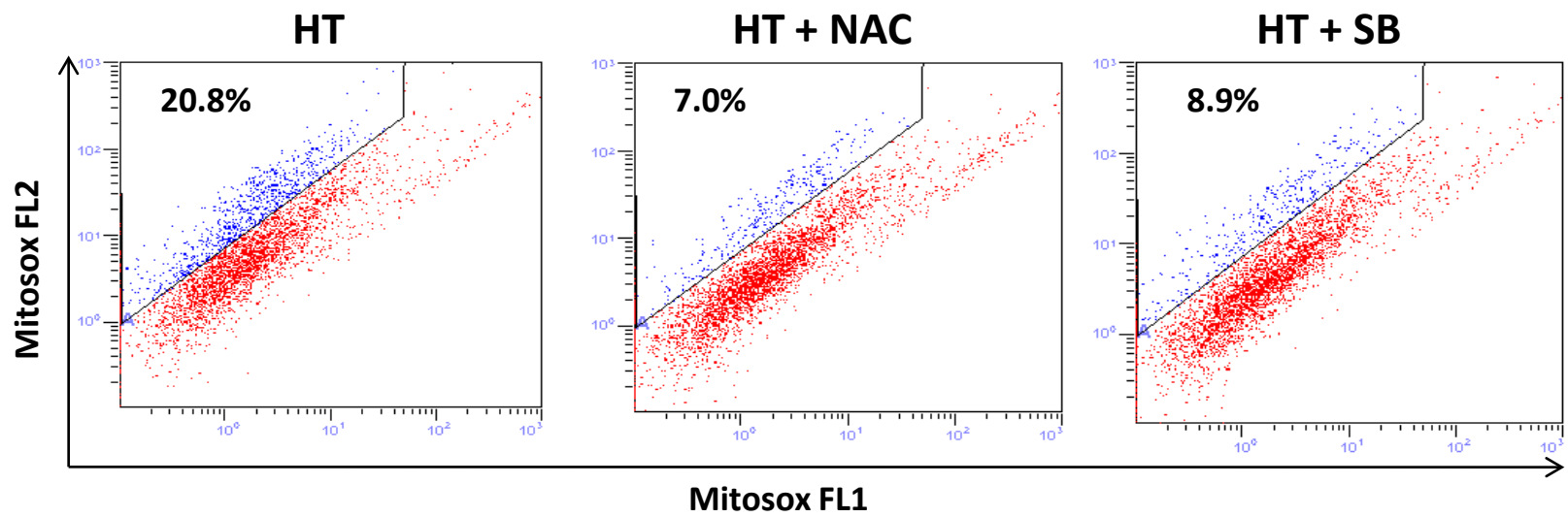


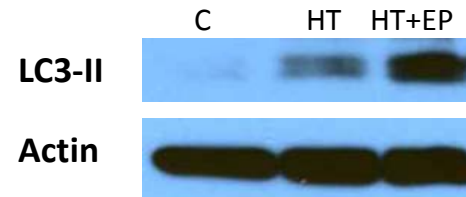
Supplementary Figure 1



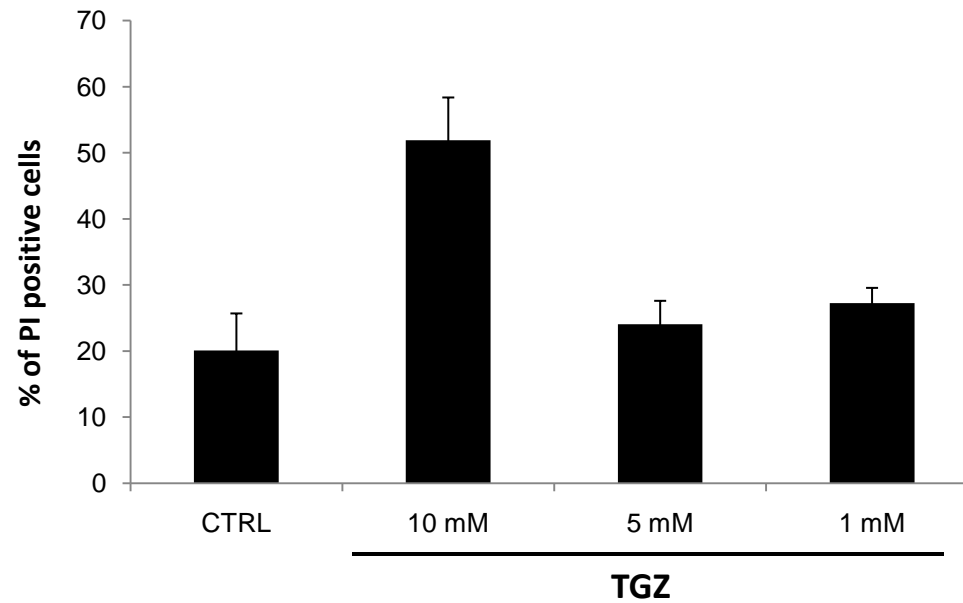
Supplementary Figure 2



Supplementary Figure 3



Supplementary Figure 4



Legends for Supplementary Figures

Figure 1. IM-54, a selective inhibitor of necrotic cell death, substantially reduced HRG/TGZ-induced cell death.

MCF-7 cells were pre-treated with 5 μ M of IM-54 followed by HRG/TGZ treatment (HT+IM-54, right). MCF-7 cells treated with HRG/TGZ in the absence of IM-54 were used as a control (HT, left). Cells were then harvested and the percentage of apoptotic and necrotic cells was determined as described in Figure 3b legend. Please note that cells were retrieved at an earlier time point (30-36 hour after HRG/TGZ treatment and thus showed a lower level of necrotic cells compared to corresponding cells treated for 48 hours. Representative data from one of three independent experiments are shown.

Figure 2. Effect of NAC and p38 MAPK inhibitor on HRG/TGZ-mediated ROS generation.

MCF-7 cells were pretreated with either 5 mM NAC (HT+NAC) or 4 μ M SB 203580 (HT+SB) followed by HRG/TGZ for 16 hours. The cells were then stained with Mitosox Red as described in Figure 4b legend. Numbers in the upper left of each dot blot indicate the percentage of cells staining positively with Mitosox Red. Representative data from one of three independent experiments are shown.

Figure 3. HRG/TGZ can induce autophagy in the presence of lysosomal inhibitors.

MCF-7 cells were treated with HRG/TGZ. At 4 hours after HRG/TGZ treatment, lysosomal inhibitors E64D (10 μ g/ml) and Pepstatin A (10 μ g/ml) were added into the culture medium and the cells were cultured for additional 44 hours. Cells were then lysed and blotted for anti-LC3II. C: control, untreated cells, HT: HRG and TGZ, HT+EP: HT+E64D/Pepstatin A

Figure 4. Dose response analysis of HRG/TGZ-induced cell death.

MCF-7 cells were treated with varying concentrations of TGZ for 36 hours in the presence of HRG (100 ng/ml). Cell death was analyzed by propidium iodide (PI) staining as described in the legend to Figure. 1. Data are the mean percentage of PI-positive cells \pm S.E. from five independent experiments.