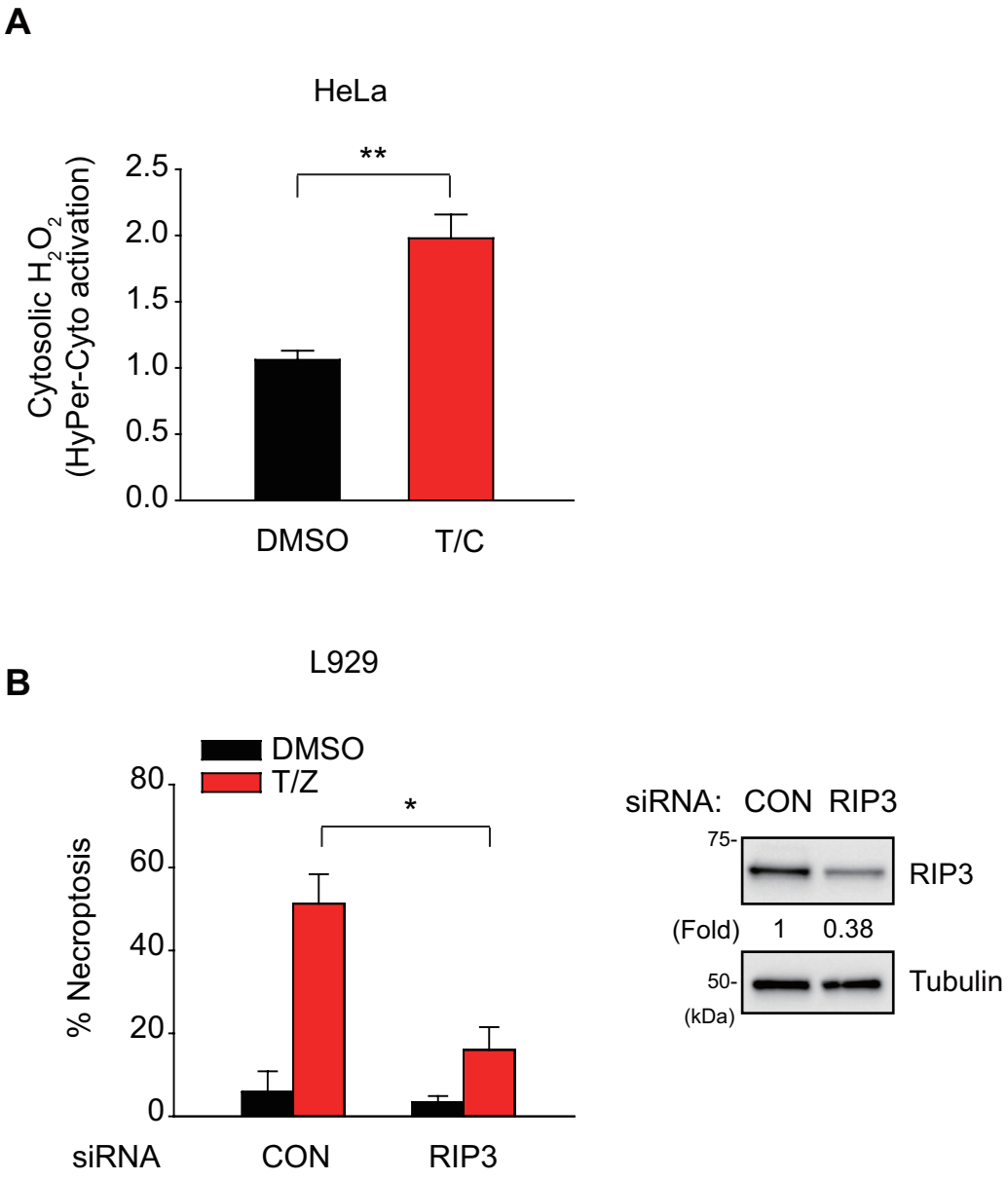
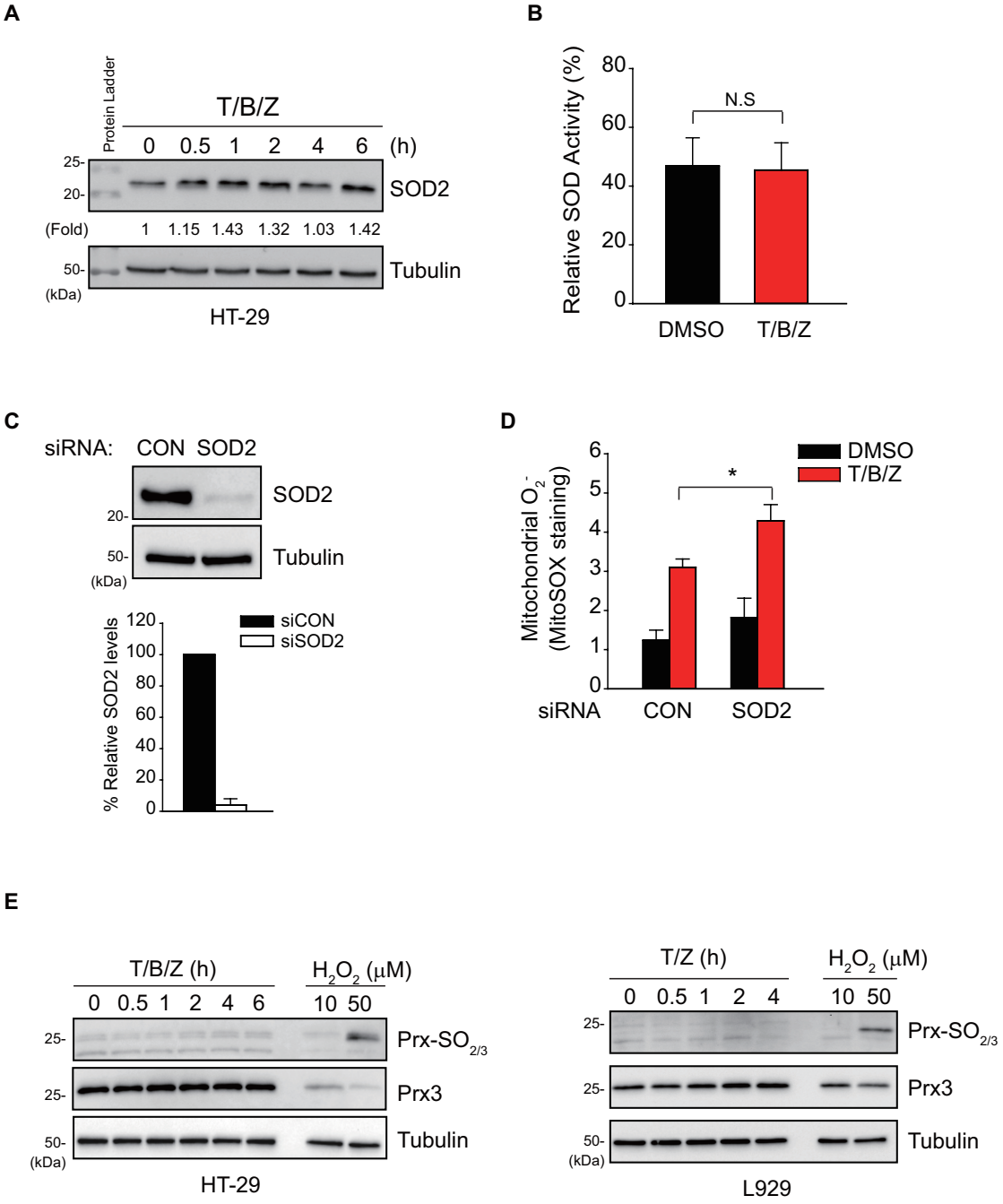


Supplementary Fig. S1. TNF-α-induced necroptosis is RIP3-dependent in L929 cells. (A) Cytosolic H_2O_2 level was monitored in the HyPer-expressing HeLa cells. Fluorescence images were taken from the HyPer-expressing HeLa cells treated with T/C. Data in the graph are means ± SD of fold change of ratio of relative fluorescence intensities at 488 nm and 405 nm of 100-120 cells from three independent experiments (n = 3, $**P < 0.005$). (B) The siRNA-transfected L929 cells were treated with vehicle control (DMSO) or T/Z combinaiton for 6 h. The cells were labeled with propidium iodide (PI) and annexin V followed by FACS analysis. The knockdown level of mouse RIP3 was shown by immunoblotting. Relative band intensity was quantified and shown as mean of fold change versus that of control siRNA (CON)-transfected cells (n = 2). Data in the graph are means ± SD of the percentage of necroptotic cell death from three independent experiments (n = 3, $*P < 0.001$).



Supplementary Fig. S2. The expression and activity of mitochondrial ROS-metabolizing enzymes during necroptosis. (A) Protein levels of SOD2 were analyzed in HT-29 cells with T/B/Z treatment for indicated time periods. Immunoblots against α-tubulin as a loading control. Relative band intensity was quantified and shown as mean of fold change versus that of untreated cells (n = 3). (B) HT-29 cells were treated with vehicle control (DMSO) or T/B/Z combination for 2 h. Cells were lysed and subjected to the total SOD activity assay as described in the Methods. Data in the graph are means ± SD of absorbance averaged from triplicate wells (n = 3, N.S, not significant). (C and D) The siRNA-transfected HT-29 cells were treated with vehicle control (DMSO) or T/B/Z for 2 h. The knockdown level of SOD2 was shown by immunoblotting. Data in the graph are means of the percentage of ralative intensities of SOD2 bands after being normailized by corresponding α-tubulin bands (n = 3) (C). Cells were stained with MitoSOX and analyzed by a confocal microscopy. Data in the graph are means ± SD of relative fluorescence intensities of 45-50 cells (n = 3, $*P < 0.05$) (D). (E) HT-29 and L929 cells were treated with combination of T/B/Z or T/Z for indicated times and lysed for immunoblotting. The hyper-oxidation of 2-Cys Prxs was immunoblotted with anti-Prx-$SO_{2/3}$ antibody. The Prx3 were re-probed with anti-Prx3 antibody. Immunoblots for α-tubulin as a loading control. For positive control, HT-29 and L929 cells were treated with increasing concentrations of H_2O_2 for 30 min and analyzed in the parallel immunoblots. Representative blots from two independent experiments are shown.