

Figure S1. PL increases ROS production in HuCCT-1 and OCUG-1 cells. (A) HuCCT-1 and (B) OCUG-1 cells were pretreated with or without NAC for 1 h. The cells were then incubated with 2',7'-dichlorodihydrofluorescein diacetate in the dark at 37°C and treated with the indicated concentrations (in  $\mu\text{M}$ ) of PL for 1 h. Next, the cells were trypsinized, washed and analyzed by flow cytometry to determine ROS levels. Data are presented as the mean  $\pm$  SD of at least three independent experiments, with representative examples included. Dye, Blank control; NAC, *N*-acetyl-L-cysteine; PL/P, piperlongumine; ROS, reactive oxygen species.

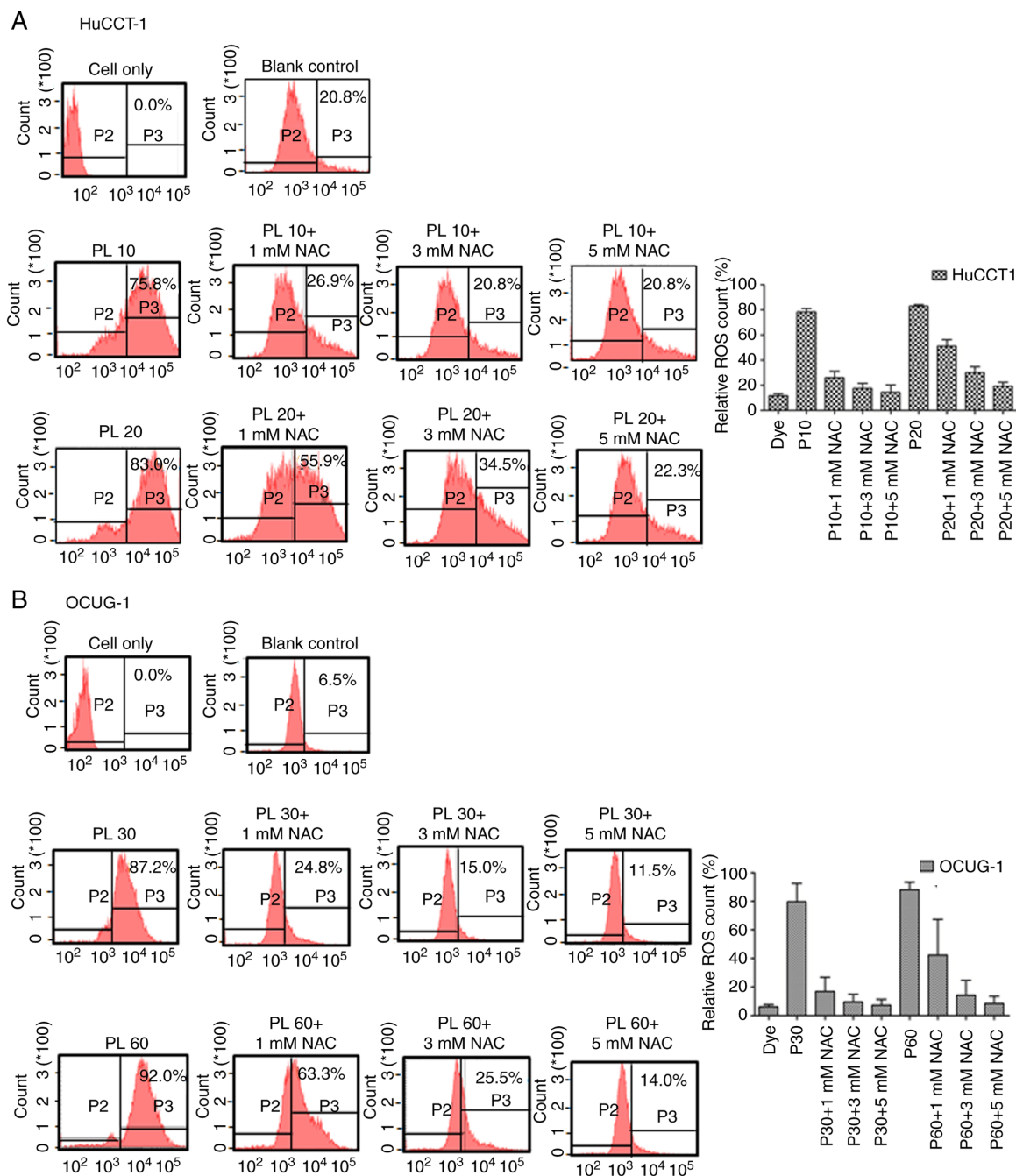


Figure S2. ROS production is upstream of Erk signaling activation in PL-treated HuCCT-1 cells. HuCCT-1 cells were pretreated for 1 h with PD98059, at the indicated concentrations. Next, the cells were treated with different concentrations (in  $\mu\text{M}$ ) of PL for 24 h. The cells were then incubated with 2',7'-dichlorodihydrofluorescein in the dark at 37°C for 1 h, trypsinized, washed and analyzed by flow cytometry to determine ROS levels. Data are presented as the mean  $\pm$  SD of at least three independent experiments, with representative examples included. PL, piperlongumine; ROS, reactive oxygen species.

