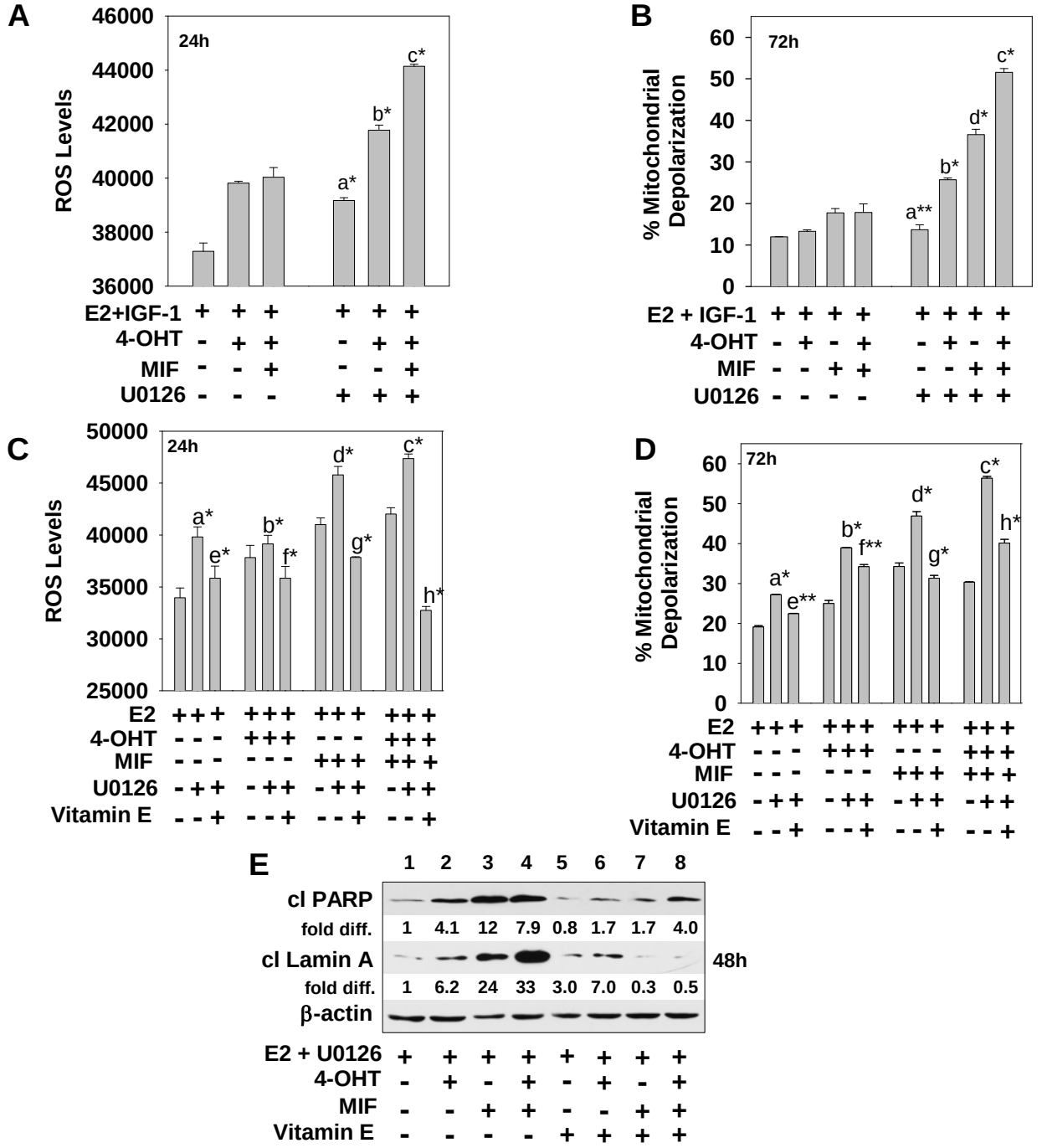

Additional File 2. U0126, a selective inhibitor of MEK1, induces ROS-dependent apoptosis in MCF-7 cells undergoing hormonal treatments in the presence or absence of IGF-1. (A-B) U0126 (5μM; 30 min pretreatment) significantly enhanced ROS levels **(A)** and the percent of mitochondrial depolarization **(B)** in cells treated with hormones in the presence of IGF-1 (20ng/ml). **(C-E)** Vitamin E (500 uM, 30 min pretreatment) blocked 4-OHT- and/or MIF- induced ROS **(C)**, and reduced the percent of mitochondrial membrane depolarization **(D)** and cleavage of PARP and lamin A **(E)** in cells treated in medium supplemented with E2 +/- U0126 (5μM; with 30 min pretreatment). *fold diff.*, the increase in signal intensity of cleaved PARP or lamin A relative to the signal intensity in E2 + U0126-treated cells (lane 1), arbitrarily set to a value of 1.0; corrections for loading were based on β-actin signal intensity per sample. Values are expressed as mean ± S.D. Significant differences between treatments are designated as follows: [a] E2 versus E2 + U0126 (+/-IGF-1); [b] E2 + 4-OHT versus E2 + 4-OHT + U0126 (+/-IGF-1); [c] E2 + 4-OHT+ MIF versus E2 + 4-OHT + MIF + U0126 (+/-IGF-1); [d] E2 + MIF versus E2 + MIF + U0126 (+/-IGF-1); [e] E2 + U0126 (+/- vitamin E); [f] E2 + 4-OHT + U0126 (+/- vitamin E); [g]E2 + MIF + U0126 (+/-vitamin E); [h]E2 + 4-OHT + MIF + U0126 (+/- vitamin E) The symbol * and ** represent statistical significance at $P<0.001$ and $P<0.05$, respectively.