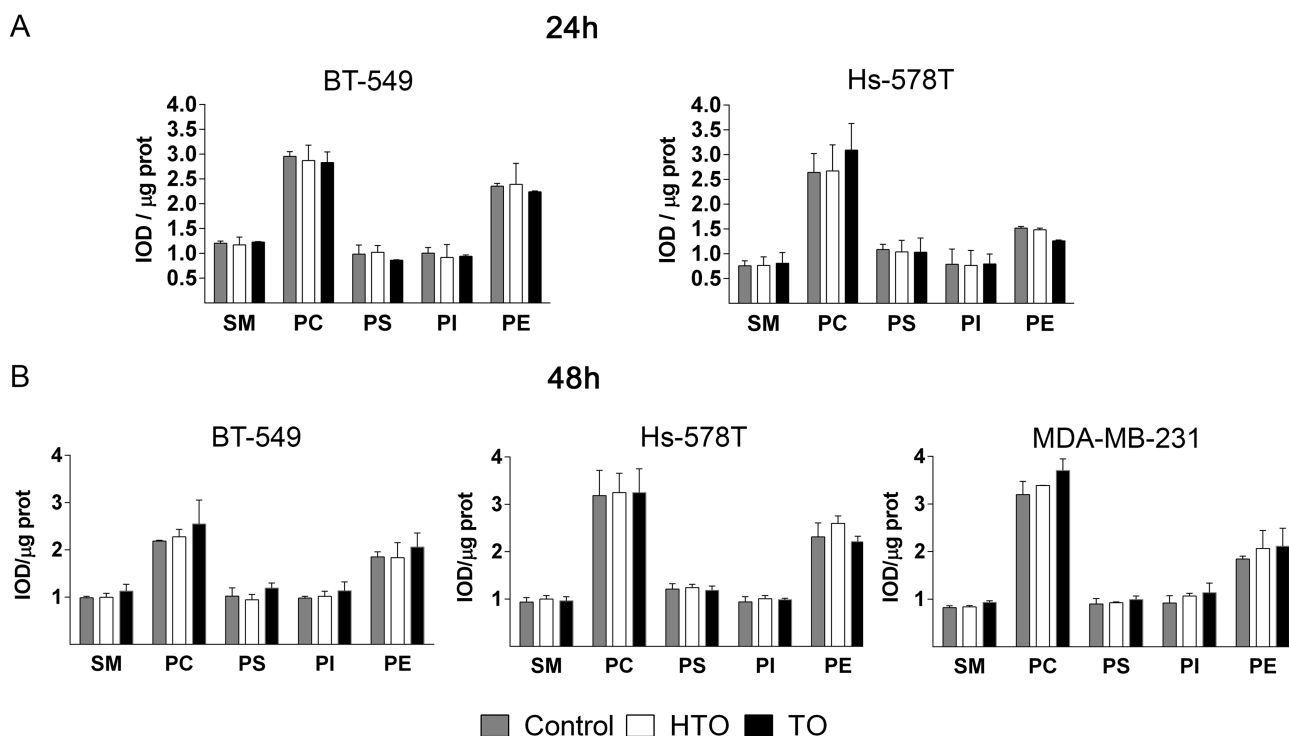
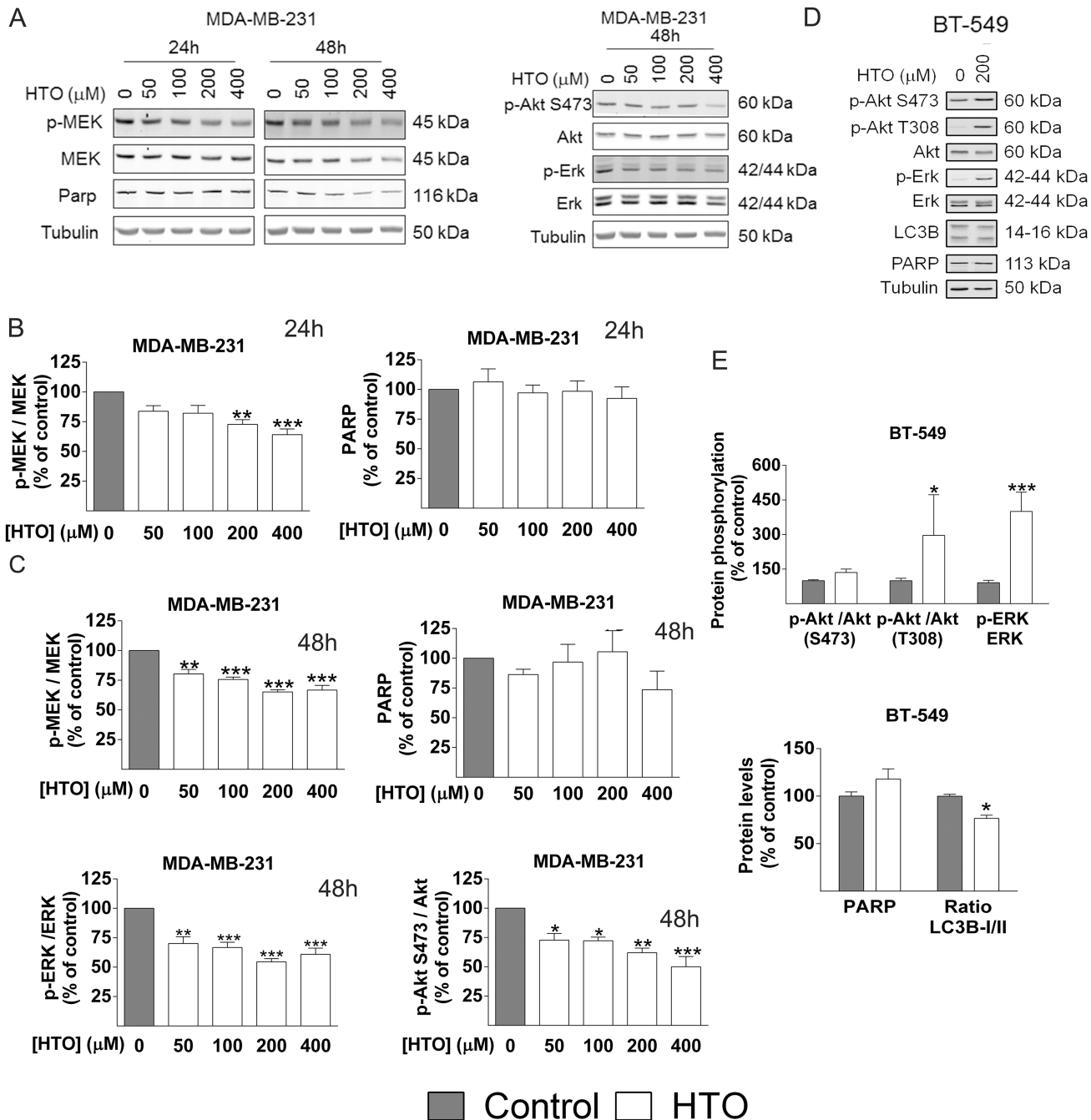


## The triacylglycerol, hydroxytriolein, inhibits triple negative mammary breast cancer cell proliferation through a mechanism dependent on dihydroceramide and Akt

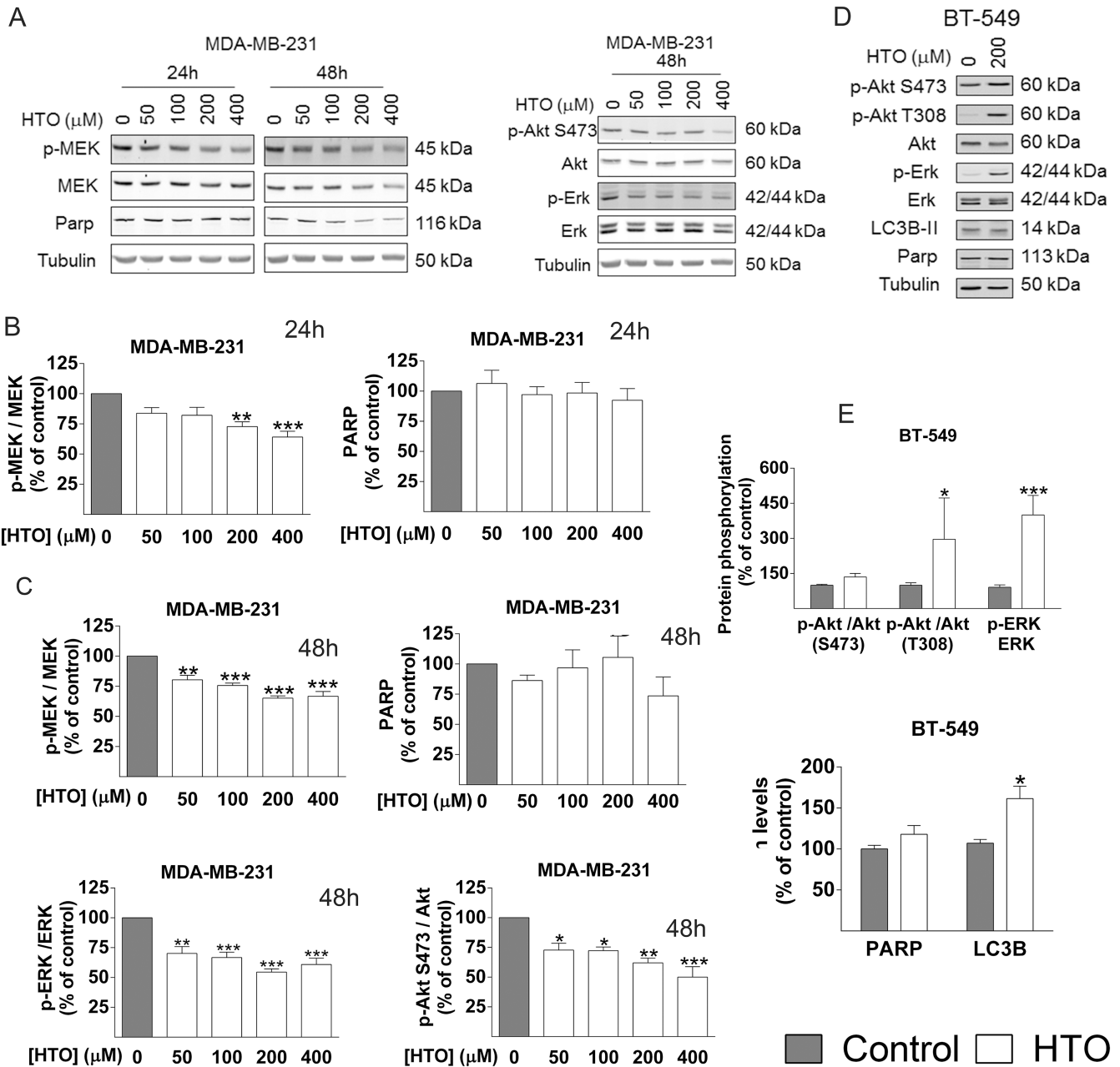
### SUPPLEMENTARY MATERIALS



**Supplementary Figure 1: HTO and TO affects the phospholipid composition of TNBC cell lines.** TNBC cell lines (MDA-MB-231, BT-549 and Hs-578T) were cultured for 24 h or 48 h in the presence or absence (grey bars) of HTO (300  $\mu$ M, open bars) or triolein (black bars), before the lipids were extracted and fractionated by TLC to measure the phospholipids. The bars correspond to the mean  $\pm$  SEM values of 2 independent experiments: \*\* $p < 0.01$ , \*\*\* $p < 0.001$  vs control (ANOVA followed by Bonferroni's test). Abbreviations: PC, phosphatidylcholine; PE, phosphatidylethanolamine; PI, phosphatidylinositol; PS, phosphatidylserine; SM, sphingomyelin.

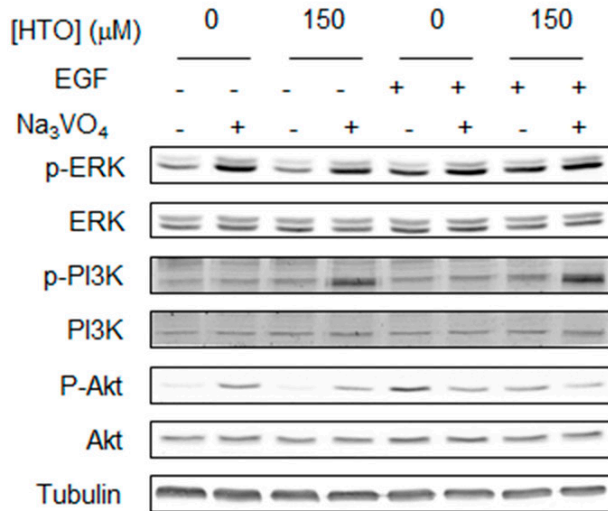


**Supplementary Figure 2: Effect of HTO on signaling proteins after a 48 h treatment.** (A–C) MDA-MB-231 cells were treated for 48 h with the concentrations of HTO indicated and different signaling proteins were quantified in immunoblots. Representative immunoblots are shown and the bars correspond to the mean ± SEM values of 3 independent experiments with duplicate samples. (D–E) BT-549 cells were cultured in the presence or absence of HTO (200 μM) for 48 h and signaling proteins were assessed in immunoblots. Representative immunoblots are shown and the bars correspond to the mean ± SEM values of 2 independent experiments with duplicate samples: \**p* < 0.05, \*\*\**p* < 0.001 (ANOVA followed by Bonferroni’s test).

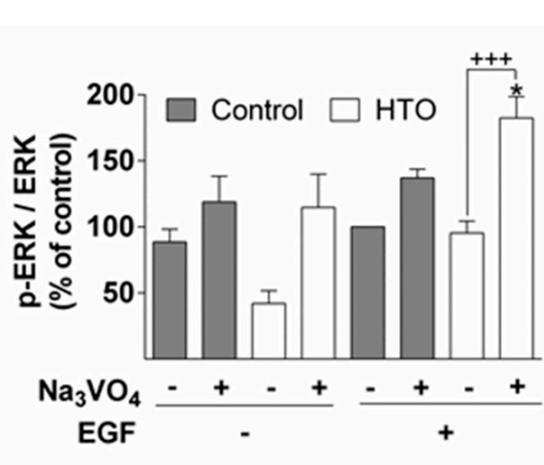


**Supplementary Figure 3: Effect of HTO on phosphatases.** MDA-MB-231 cells were serum starved, cultured for 24 h in the presence or absence of HTO (150 μM), for 2 h in the presence or absence of orthovanadate (100 nM) and stimulated for 15 min with EGF (100 ng/ml) or left untreated before the cell lysates were prepared for immunoblotting. Representative immunoblots are shown, the bars corresponding to the mean ± SEM values of 3 independent experiments: \* $p < 0.05$ , \*\* $p < 0.01$ , \*\*\*/ $p < 0.001$  (ANOVA followed by Bonferroni's test).

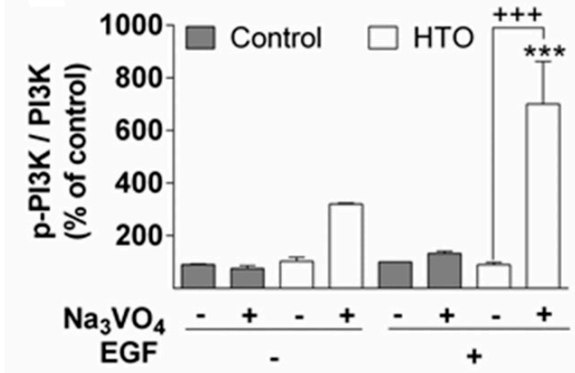
**A**



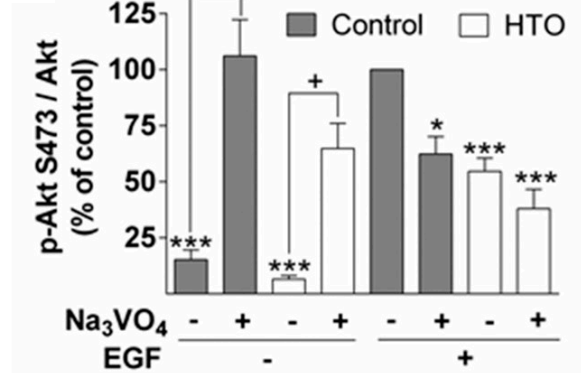
**B**



**C**



**D**



**Supplementary Figure 4: Linear correlation for the 2OHOA mass and GC peak area.** The concentrations of 2OHOA indicated were submitted transmethylation and half of the sample was then derivatized. The fatty acid levels were measured by GC, with 1  $\mu\text{l}$  of each solution applied to the GC device.