

Supplementary Materials:

Thyroid hormone derivatives reduce proliferation and induce cell death and DNA damage in ovarian cancer

Elena Shinderman-Maman^{1,2,3}, Keren Cohen^{1,2,3}, Dotan Moskovich^{1,2,3}, Aleck Hercbergs⁴, Haim Werner^{2,3}, Paul J Davis⁵, Martin Ellis^{1,3} and Osnat Ashur-Fabian^{1,2,3}

¹ Translational Hemato-Oncology Laboratory, The Hematology Institute and Blood Bank, Meir Medical Center, Kfar-Saba, Israel

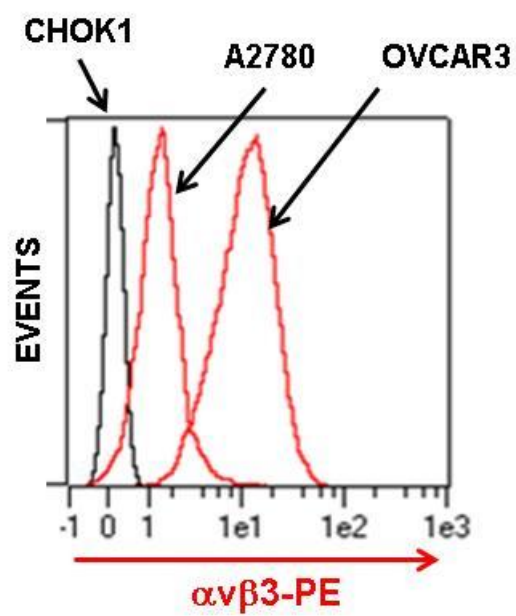
² Department of Human Molecular Genetics and Biochemistry

³ Sackler Faculty of Medicine, Tel Aviv University, Tel Aviv, Israel

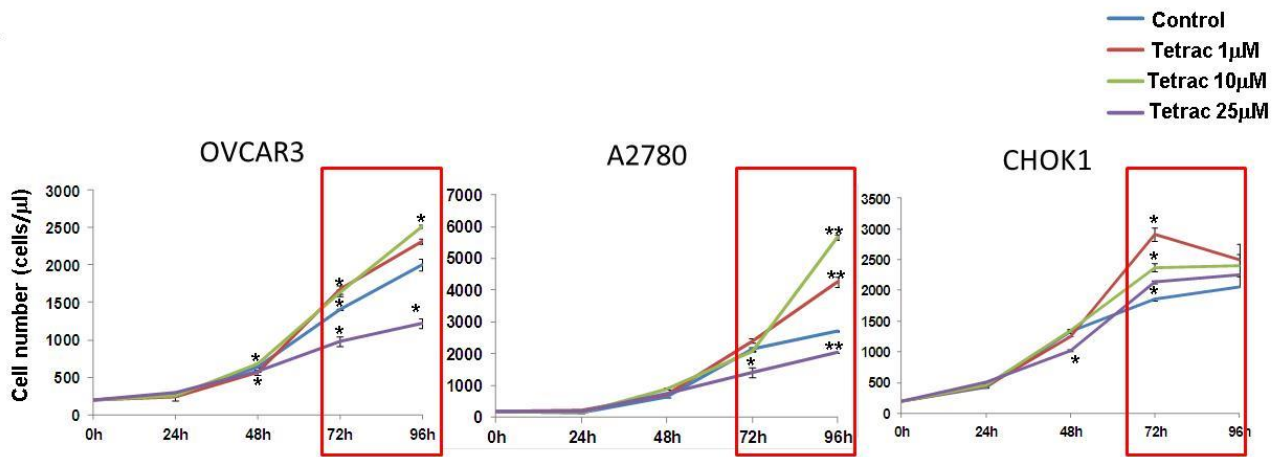
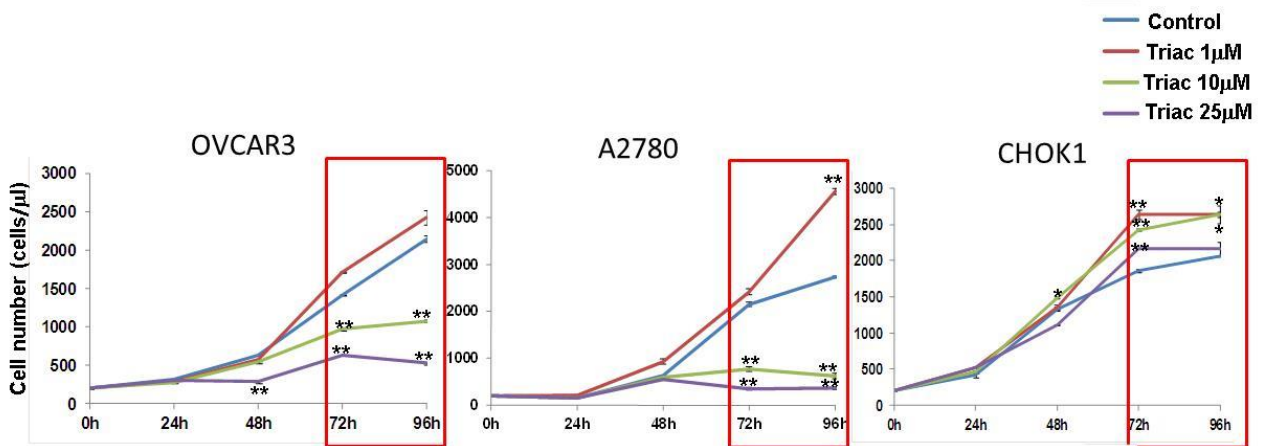
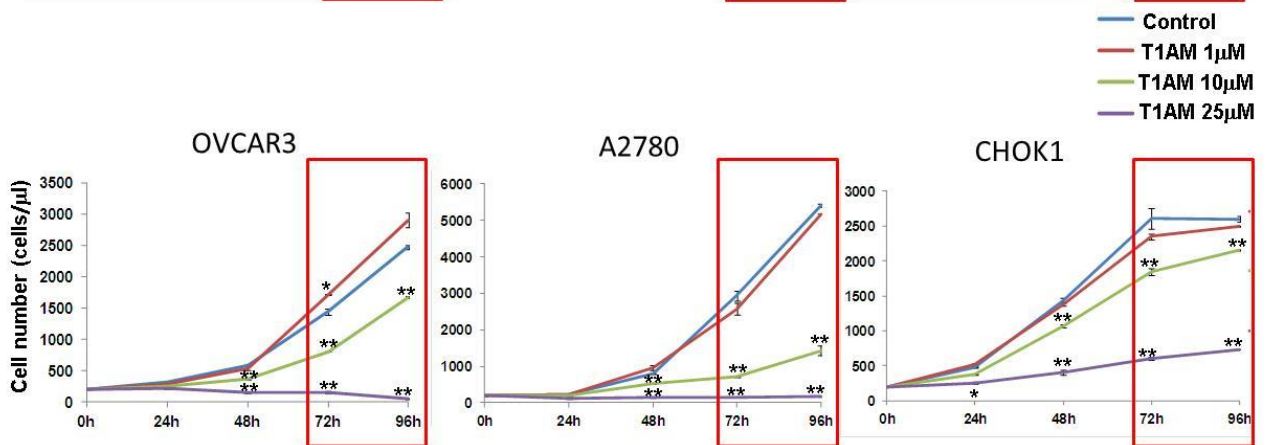
⁴ Radiation Oncology, Cleveland Clinic, Cleveland, OH, USA

⁵ Department of Medicine, Albany Medical College, Albany, NY, USA

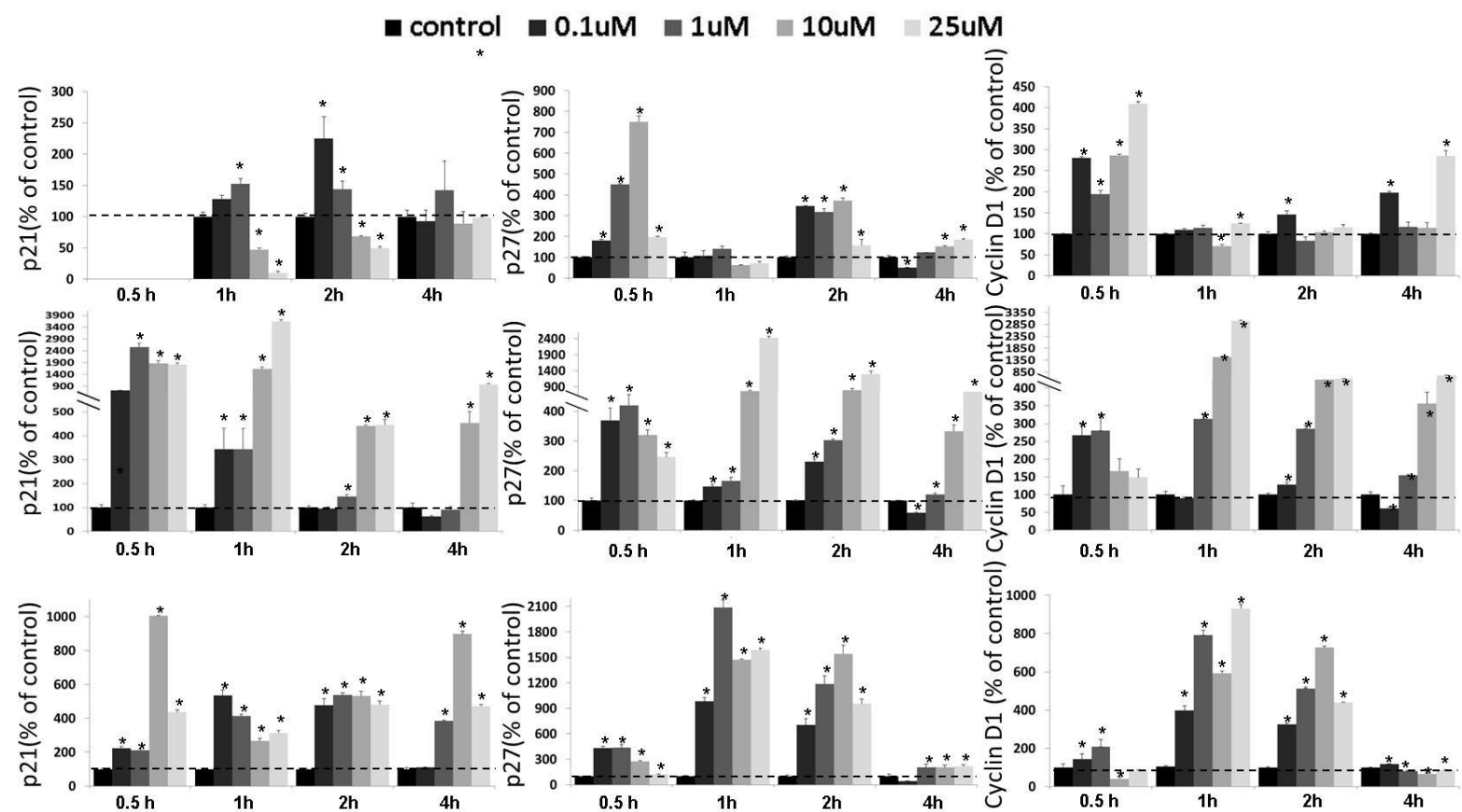
Correspondence: Dr. Osnat Ashur-Fabian, Translational Hemato-Oncology Laboratory, The Hematology Institute and Blood Bank, Meir Medical Center, Tchernichovsky 59, Kfar-Saba 6997801, Israel. E-mail: osnataf@gmail.com



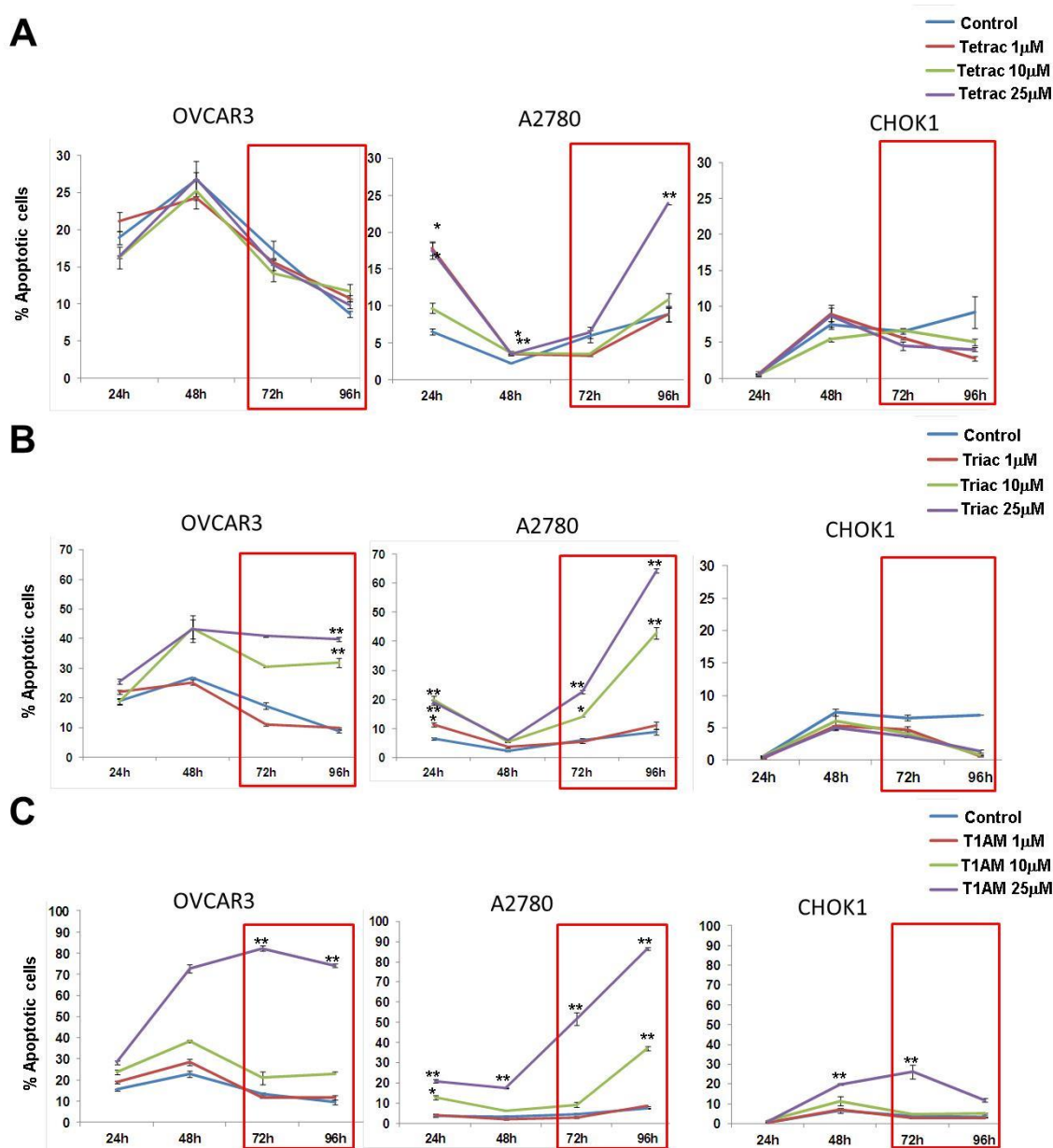
Supplementary Figure 1: OVCAR3, A2780 and CHOK1 cell lines were analyzed by flow-cytometry for $\alpha v \beta 3$ expression

A**B****C**

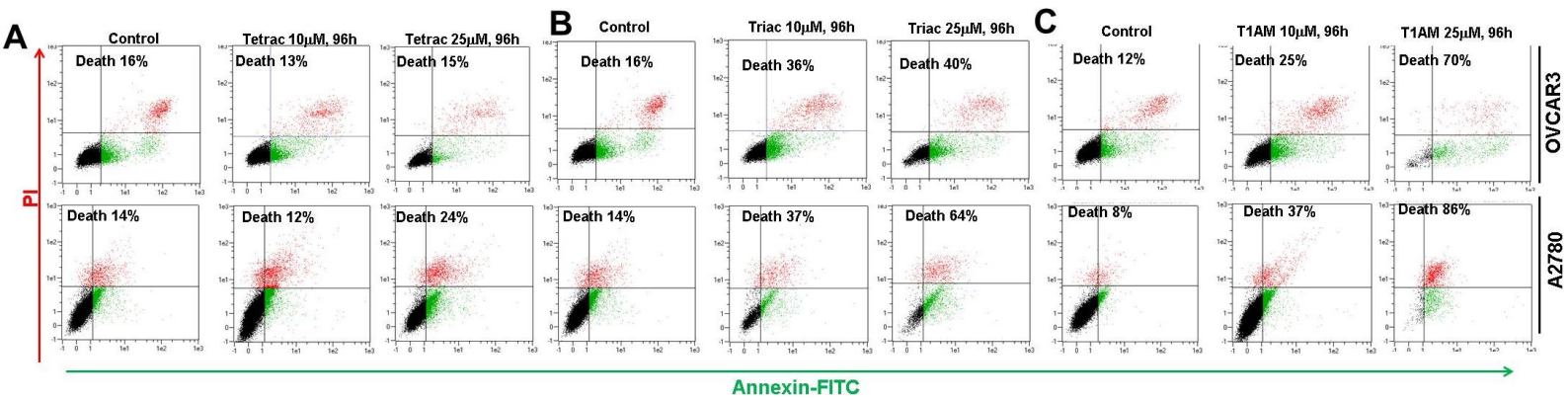
Supplementary Figure 2: Cells were treated with 1, 10 or 25 μ M of (A) Tetrac (B) Triac or (C) T1AM for 24h-96h and cell number was evaluated using flow cytometry. Experiments were repeated three times in triplicates. Significance (* $p < 0.05$, ** $p < 0.005$) from vehicle control is shown.



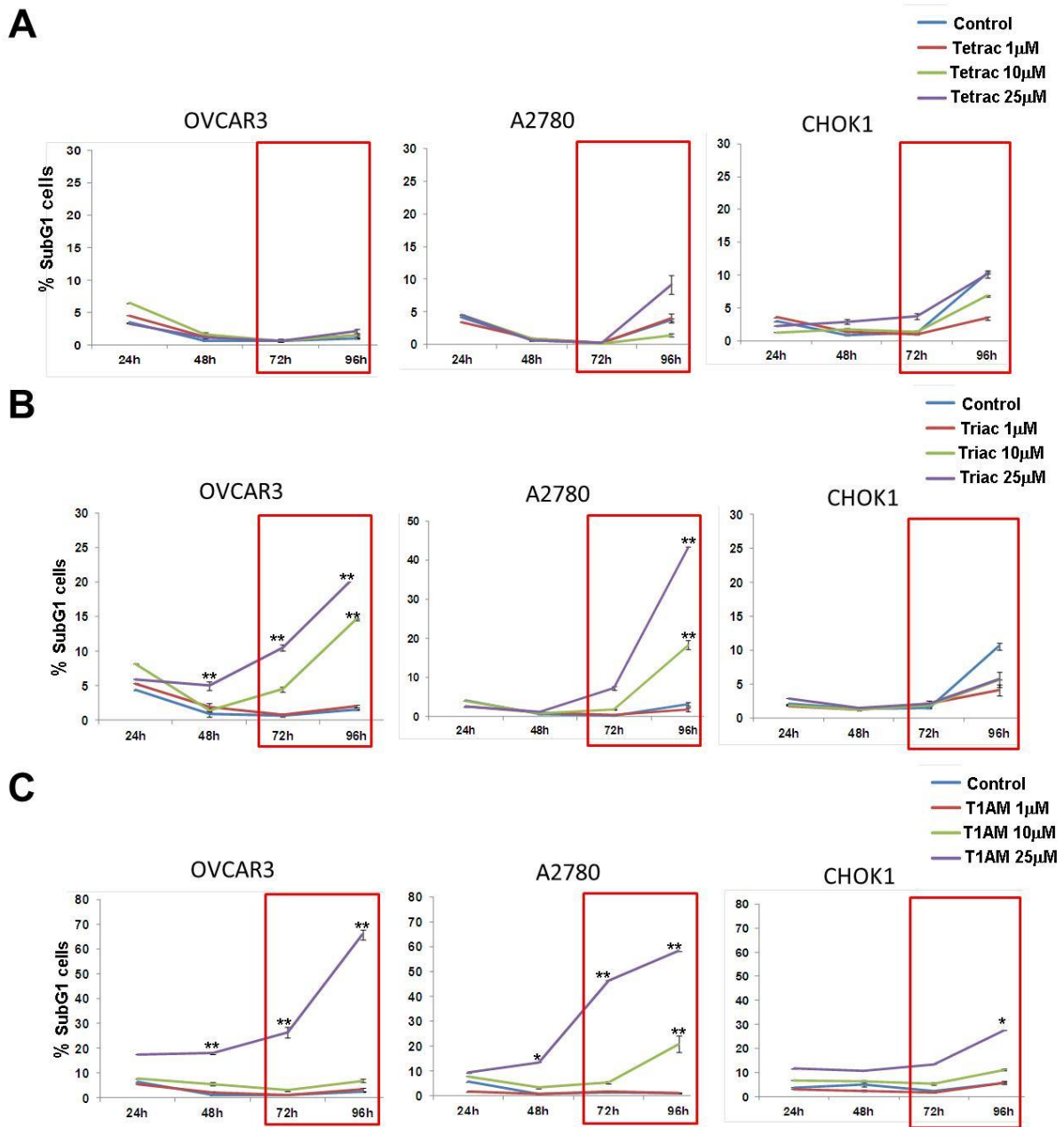
Supplementary Figure 3: Western blots quantification of p21, p27 and cyclin D1 protein levels (normalized to protein loading) following treatments with 0.1, 1, 10 or 25μM (A) tetrac (B) triac (C) T1AM for 0.5-4 hours in OVCAR3 cells.. * p<0.05.



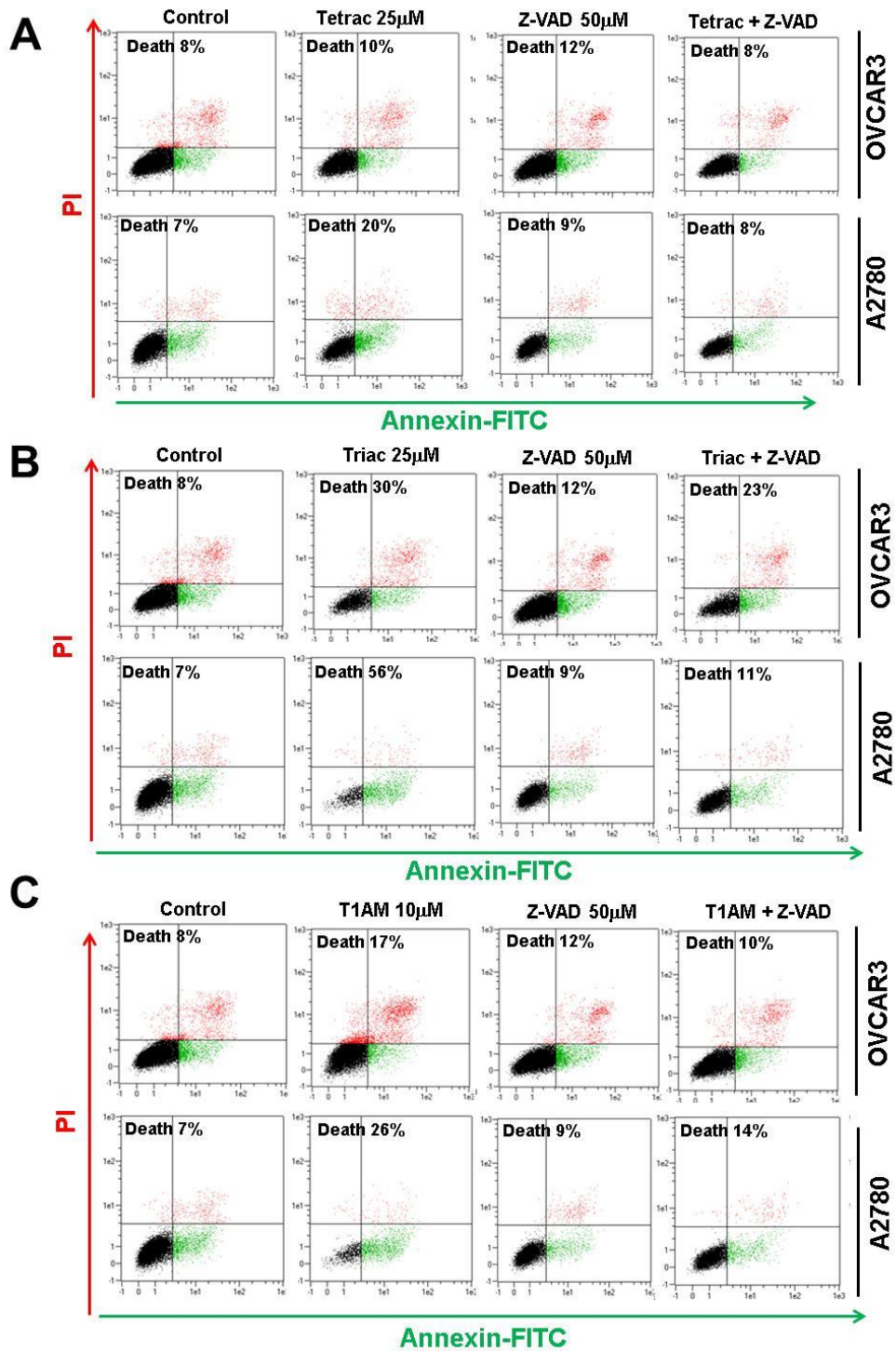
Supplementary Figure 4: Cells were treated with 1, 10 or 25 μ M of (A) Tetrac (B) Triac or (C) T1AM for 24h-96h and analyzed for annexin-FITC/PI by flow-cytometry. Experiments were repeated three times in triplicates. Significance (* $p < 0.05$, ** $p < 0.005$) from vehicle control is shown.



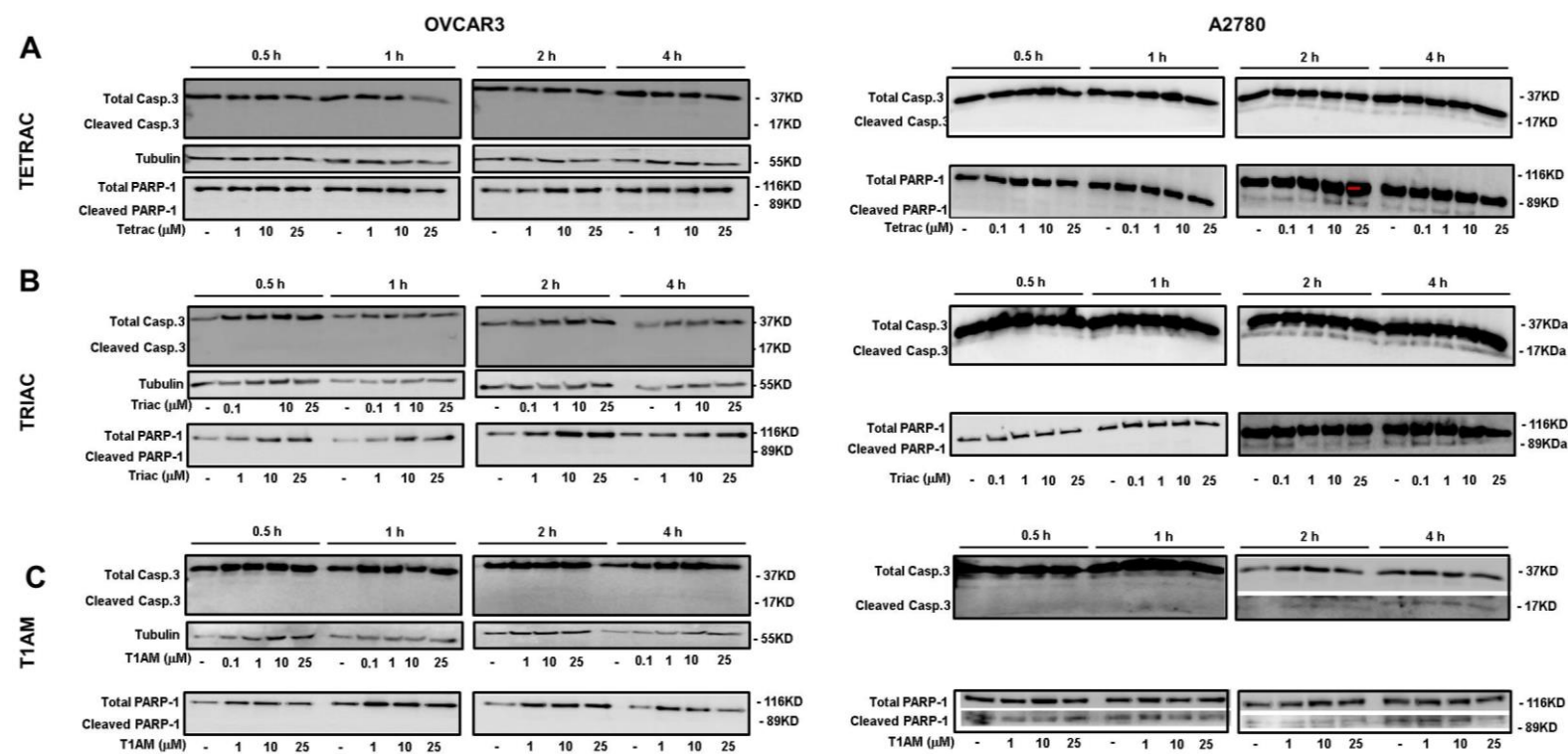
Supplementary Figure 5: Cells were treated with 10 or 25 μM of (A) Tetrac (B) Triac or (C) T1AM for 96h and analyzed for annexin-FITC/PI. Flow cytometry plots from representative experiments are shown.



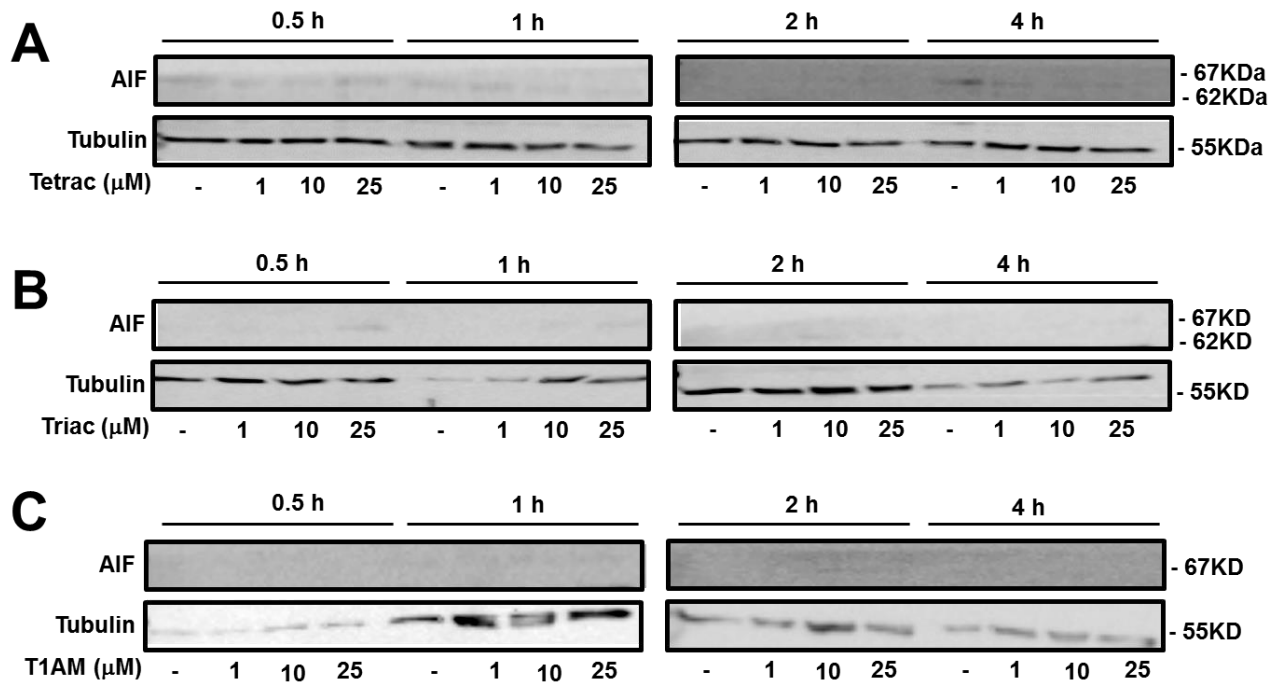
Supplementary Figure 6: Cells were treated with 1, 10 or 25 μM of (A) Tetrac (B) Triac or (C) T1AM for 24h-96h, cell cycle analysis was performed and the changes in subG1 cell fraction over time were documented. Experiments were repeated three times in triplicates. Significance (* $p < 0.05$, ** $p < 0.005$) from vehicle control is shown.



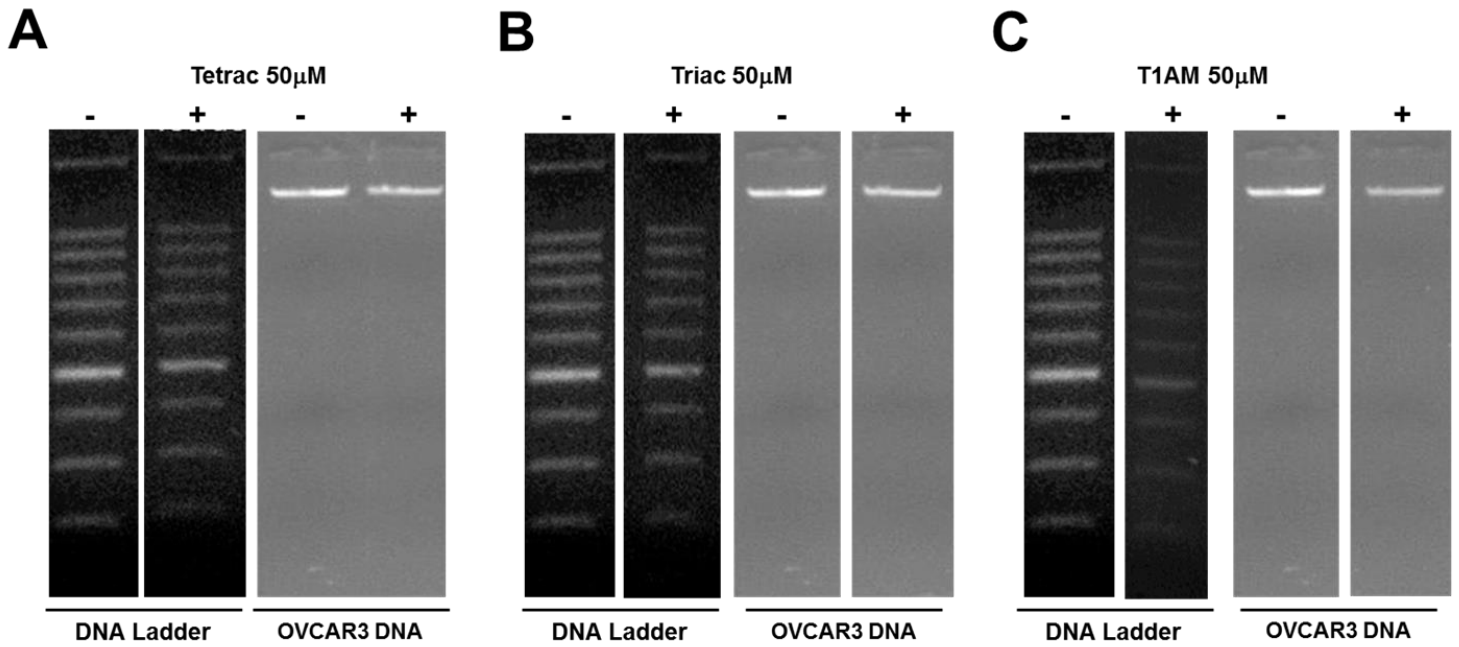
Supplementary Figure 7: Cells were treated with 25 μ M (A) tetrac (B) triac (C) 10 μ M T1AM, for 96 hours \pm Z-VAD-FMK (50 μ M) and analyzed by annexin-V/PI. Experiments were repeated twice in triplicates. Representative Flow cytometry plots are presented.



Supplementary Figure 8: Cells were treated with 0.1, 1, 10 or 25 μM (A) tetrac (B) triac or (C) T1AM, for 0.5-4 hours and caspase-3 and PARP-1 cleavage were quantified by Western blots. Representative blot is presented. Experiments were repeated twice.



Supplementary Figure 9: OVCAR3 cells were treated with 1, 10 or 25 μM (A) tetrac (B) triac (C) T1AM for 0.5-4 hours and AIF levels were evaluated by Western blots. Representative blot of duplicates is presented.



Supplementary Figure 10: Linear DNA ladder (left panels) and DNA extracted from OVCAR3 cells (right panels) were treated with 50 µM of (A) tetrac (B) triac or (C) T1AM for an overnight and separated by agarose gel. Experiments were repeated twice.