Figure 1

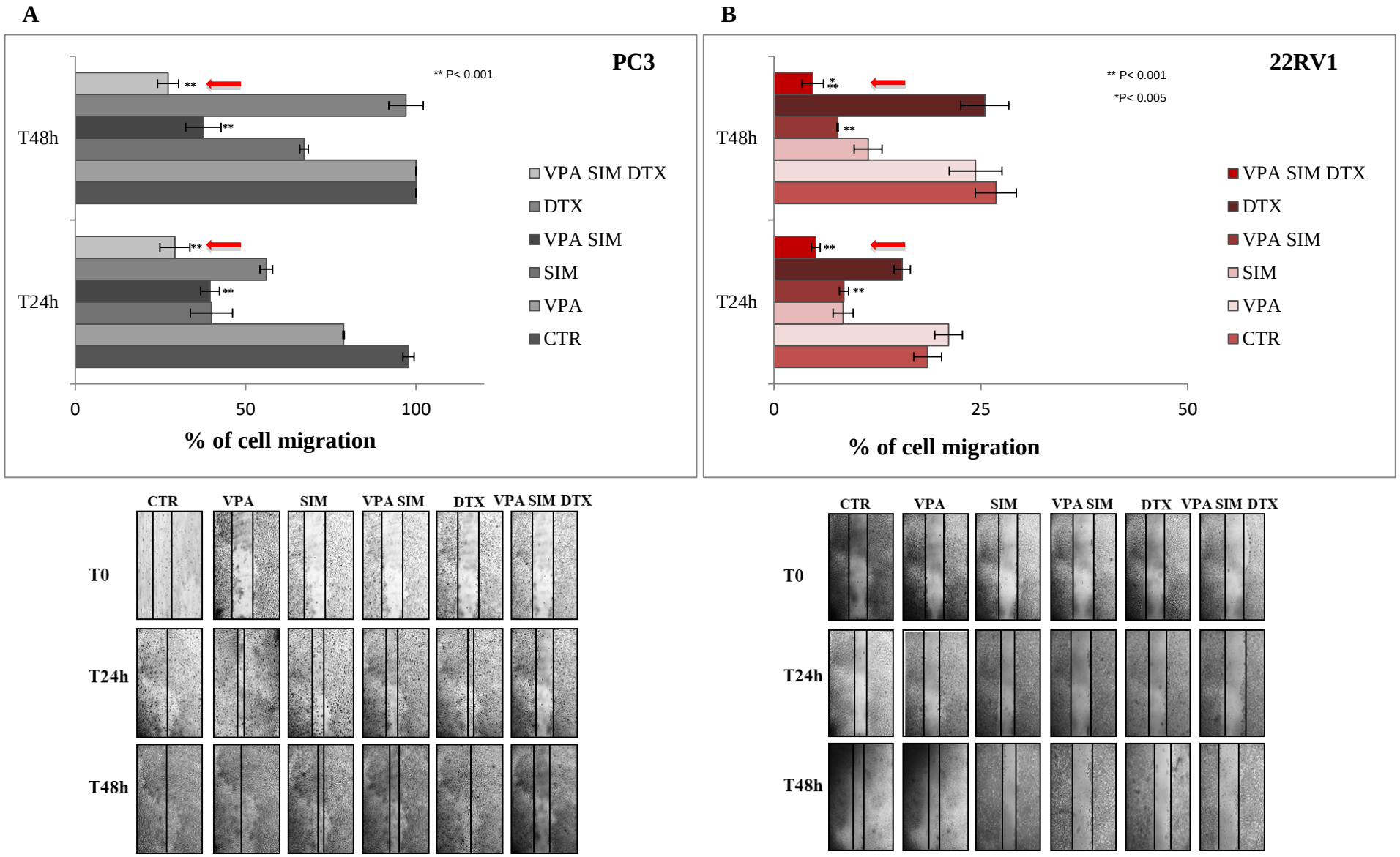

Figure 1. Migration was evaluated by a wound healing assay. PC3 (A) and 22RV1 (B) cells were seeded and cultured to 90% confluence in 24 well plates and treated withIC_{50}^{96h} doses of Valproic Acid (VPA),simvastatin (SIM) and Docetaxel (DTX) as single agents or in combination for 48h. Images were acquired 24 and 48h after wounding by phase-contrast microscopy at 10X magnification. Quantitative measurements were made by determining the distances between the wound edges. The migration values are calculated as means ±SD.

Figure 2

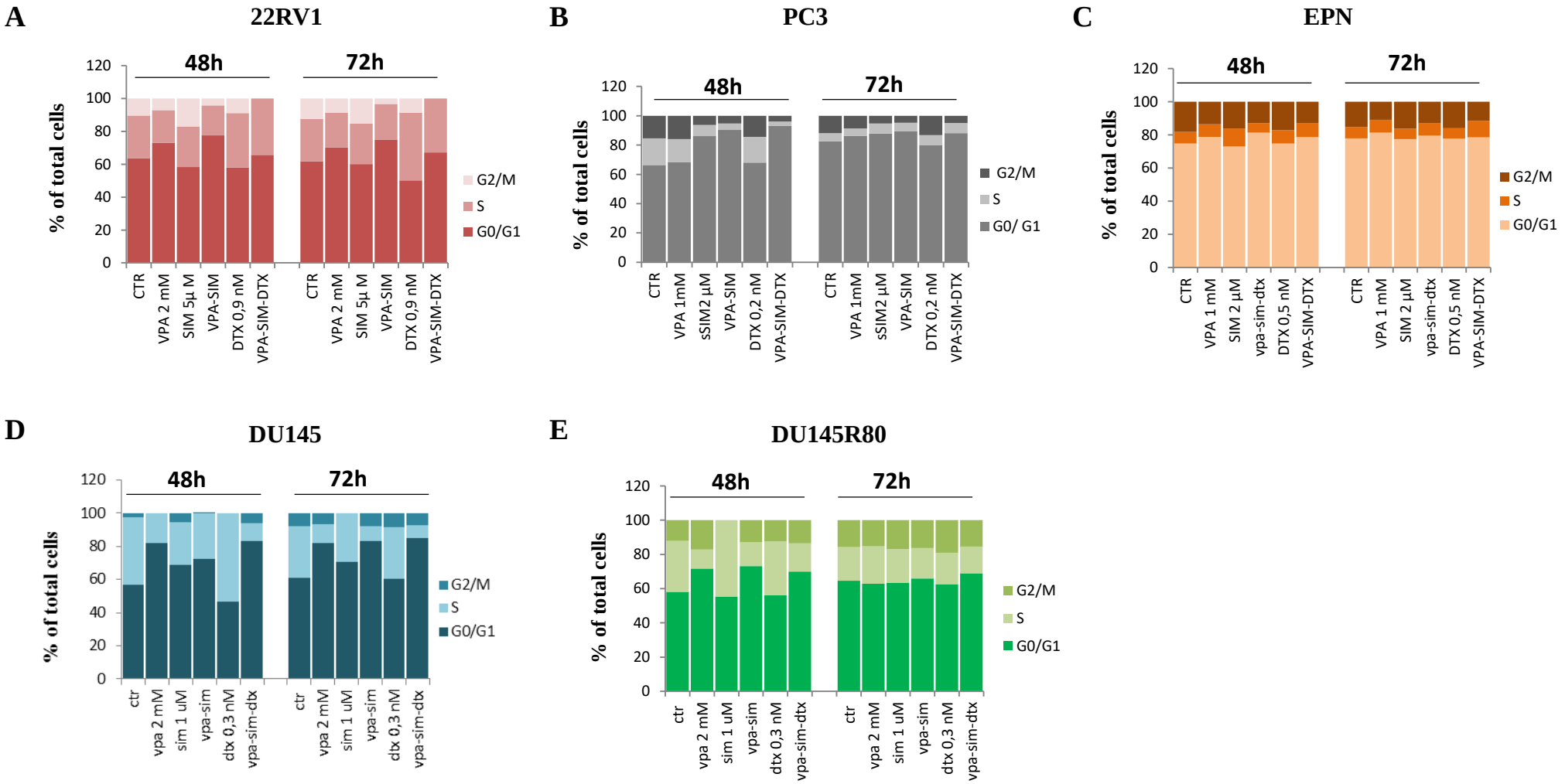

Figure 2. Nuclear DNA staining was performed by propidium iodide (PI). DNA-flow cytometry was performed by a FACScan flow cytometer (Becton Dickinson) acquiring 20000 events for each sample. We evaluated the effects of VPA, SIM and DTX, either alone or in combination on the cell cycle kinetics of 22RV1(A), PC3 (B), EPN (C), DU145 (D) and DU145R80 (E) cells. We demonstrated that concomitant treatment with VPA/SIM or VPA/SIM/DTX after 72h, induced a late-middle S-phase cell cycle arrest (about 30% of total events in 22RV1, while in PC3 DU145 and DU145R80 cells, treatment with DTX alone or in combination with VPA and SIM induces an increase of G1 phase (about 90% of total events). Notably, in normal prostate epithelial cell line EPN, no effect on cell cycle was observed.

Figure 3

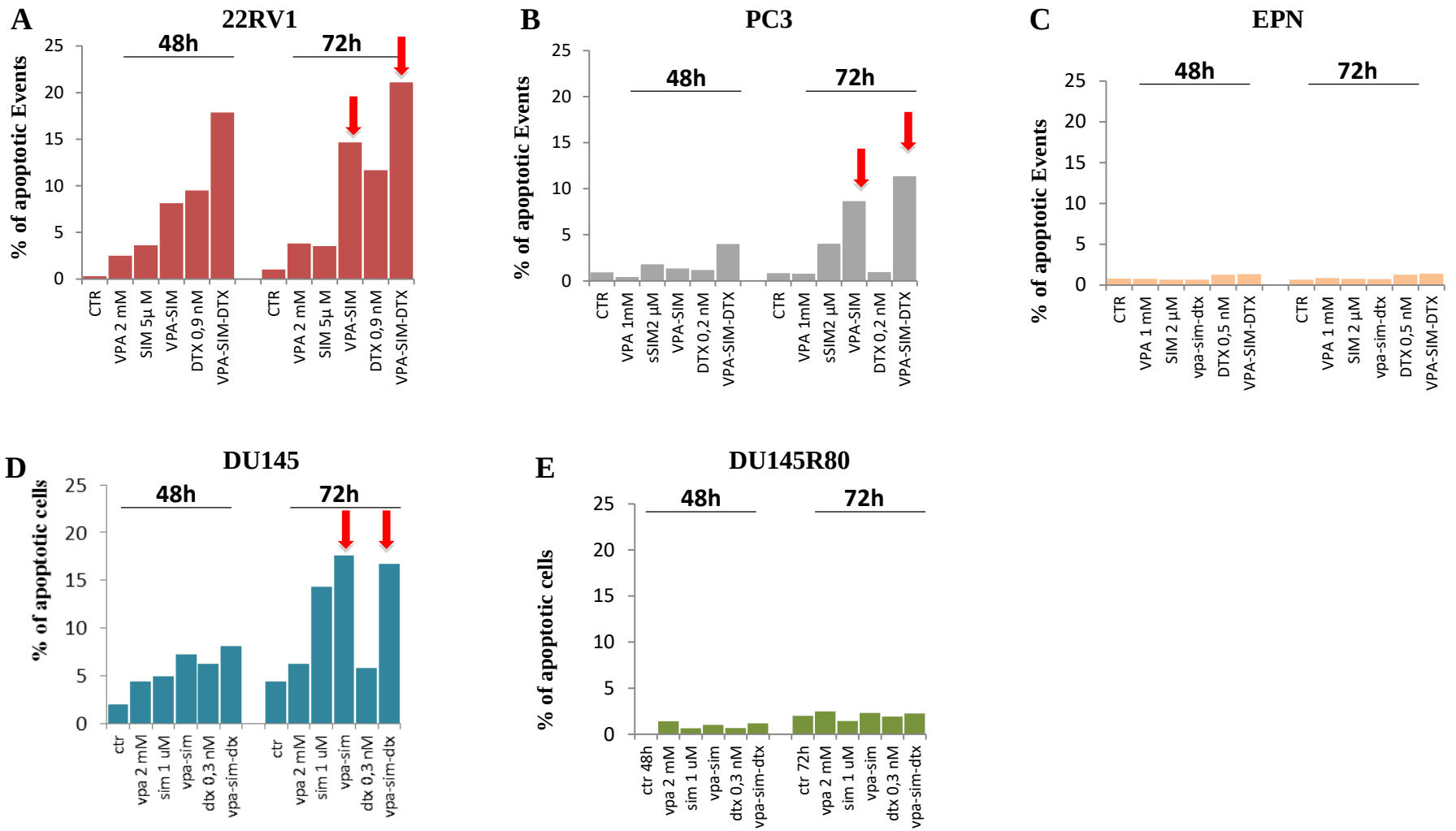

Figure 3. Nuclear DNA staining was performed by propidium iodide (PI). DNA-flow cytometry was performed by a FACScan flow cytometer (Becton Dickinson) acquiring 20000 events for each sample. The percentage of apoptotic cells was calculated in the sub-diploid region of the DNA content, registered as FL2 signals in linear scale.
We evaluated the effects of VPA, SIM and DTX, either alone or in combination on apoptosis, measured by the appearance of a hypo-diploid population (sub G0-G1). We observed in presence of double and triple combination after 72h of treatment an induction of apoptosis in 22RV1(A), PC3 (B), DU145 (D) and DU145R80 (E) cells. Notably, in normal prostate epithelial cell line EPN (C), no effect on apoptosis was observed.

Figure 4

Apoptotic effect induced by double nd triple combination in Pca Cells

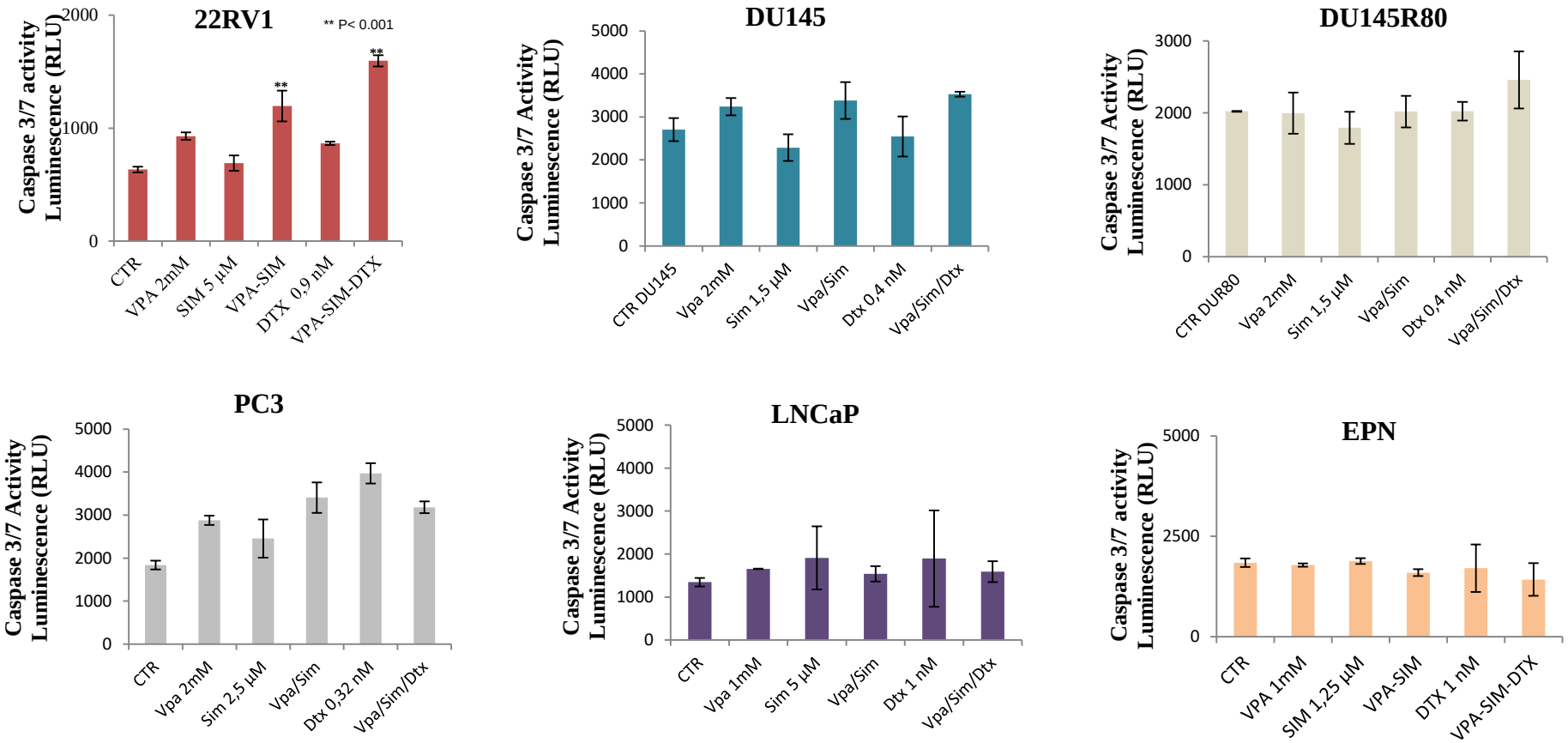

Figure 4.. The combined caspase 3/7 activity was analyzed in triplicates using the Caspase-Glo® 3/7 Assay (Promega) according to the manufacturer's protocol after 24h of treatment with VPA, SIM and DTX alone or in combination in PCa cells (22RV1, DU145, DU145R80, PC3 LNCaP and EPN). The caspase activities were assessed by measuring the luminescence in a Multilabel Reader VICTOR X4 2030 (PerkinElmer). We demonstrated in all cell lines, out of LNCaP and EPN, a clear caspase 3/7 activity induced by both combination settings of VPA/SIM and VPA/SIM/DTX, compared with control and the single agents.

Figure 5

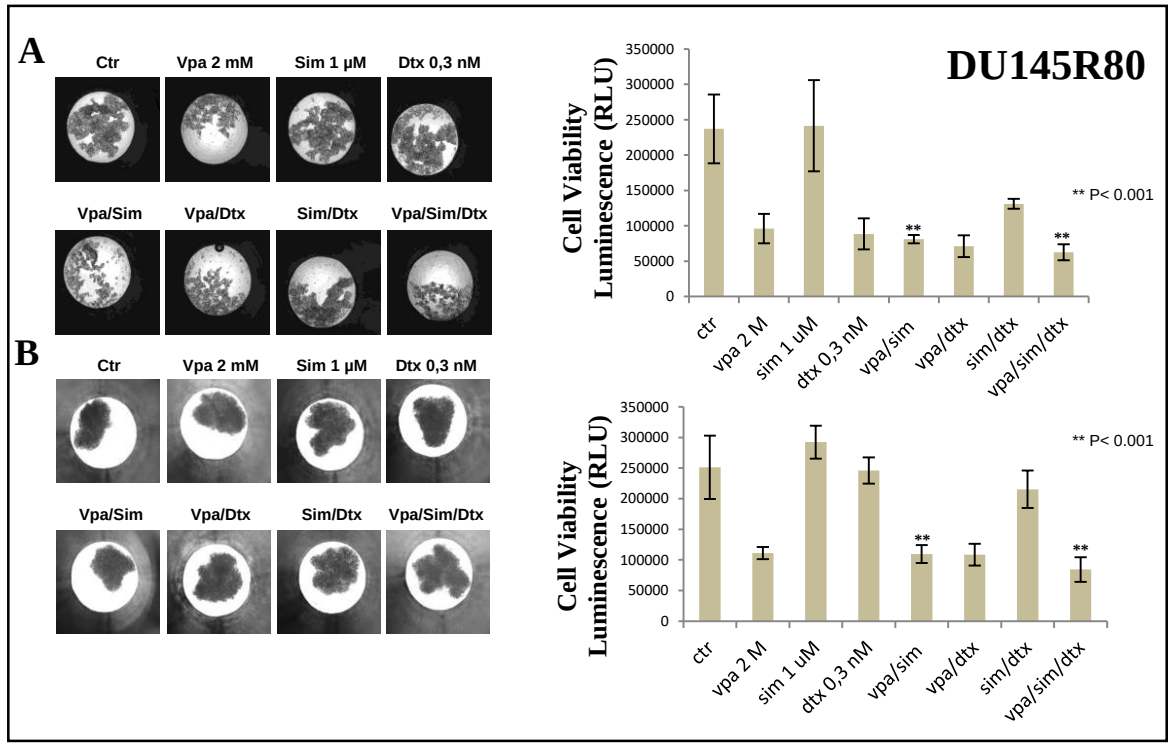

Figure 5. DU145R80 microtissues generated in 72h by GravityPlus hanging drop system in the absence or the presence of VPA, SIM, DTX, dual VPA/SIM or triple combinations, then transferred into GravityTrap plates where cell viability (bars) was evaluated by luminescence assay. (A). DU145R80 microtissues generated in 72h as above, in the absence of drugs, were the transferred into GravityTrap and treated with VPA, SIM, DTX, dual VPA/SIM or triple combinations at IC_{50}^{96h} doses, after additional 72h cell viability (bars) was evaluated by luminescence assay (B); the values represent means ± S.D. of technical triplicates**.**

Figure 6

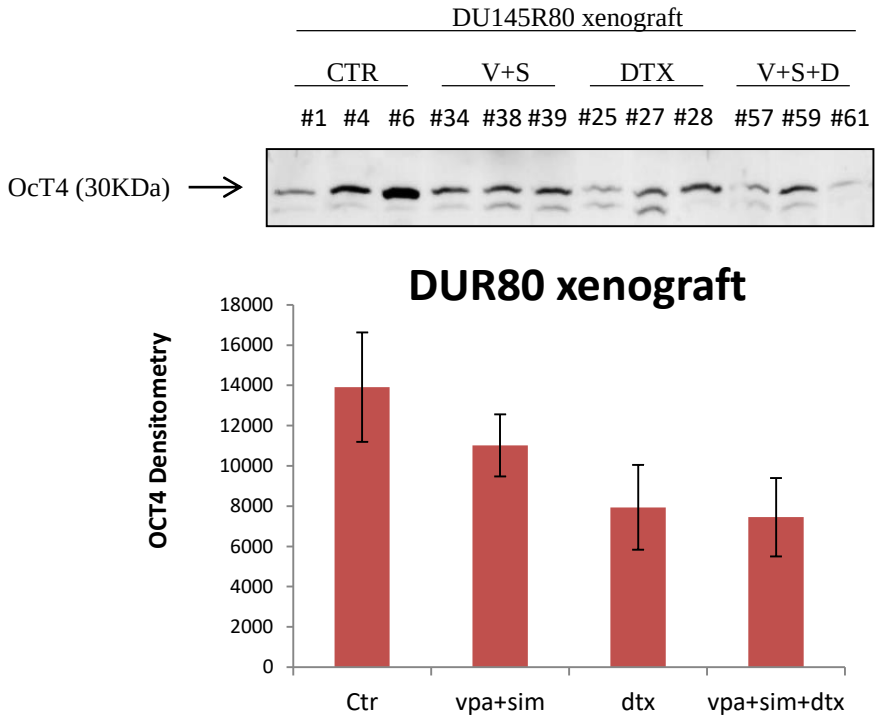

Figure 6 . Expression of OCT4 in lysates from three xenograft DU145R80 tumor samples from each treatment group evaluated by western blot (abbreviation= V+S : VPA+SIM; V+S+D: VPA+SIM+DTX). Ponceau Red was used as loading control. The reduction was scored by densitometry (bars).