pharmaceutics

Supplementary Materials: Intracellular Delivery of siRNAs Targeting AKT and ERBB2 Genes Enhances Chemosensitization of Breast Cancer Cells in a Culture and Animal Model

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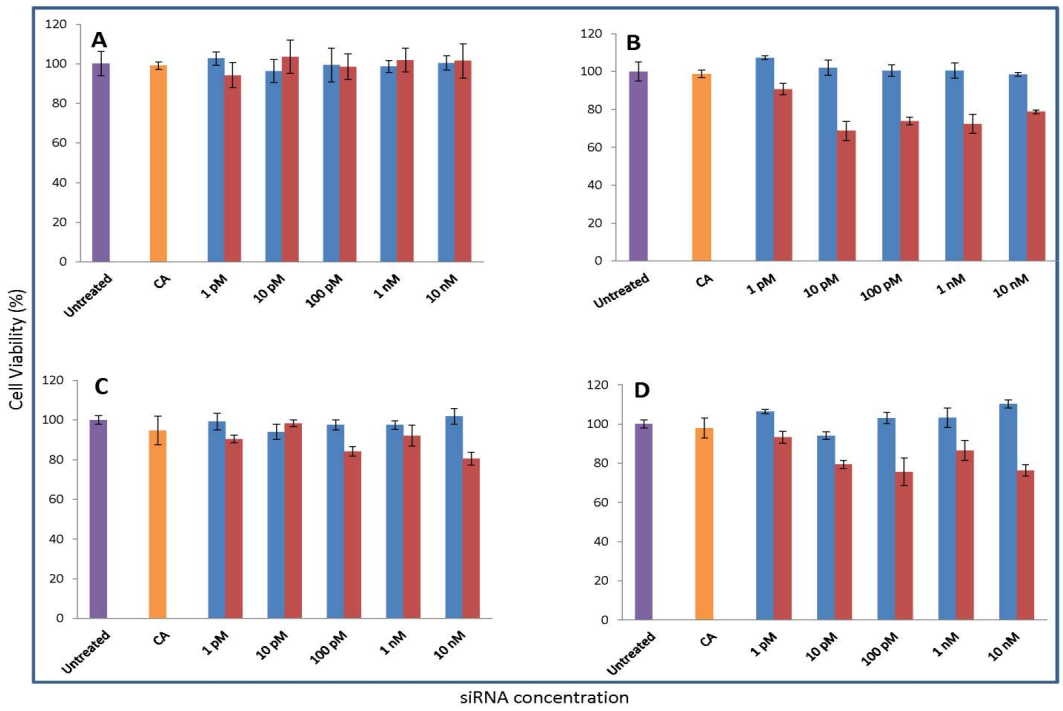

Figure S1. Cell viability assessment in 4T1 cells treated with siRNA loaded carbonate apatite. Nanoparticles were formed by using 4 mM of Calcium together with 1 pM to 10 nM of each siRNA (**A**: AKT, **B**: ERBB2, **C**:MAPK, **D**: ROS1). Two days after treatment, MTT assay was performed. Values are presented as cell viability (%) compared to untreated cells. ●Free siRNA ●CA/siRNA.

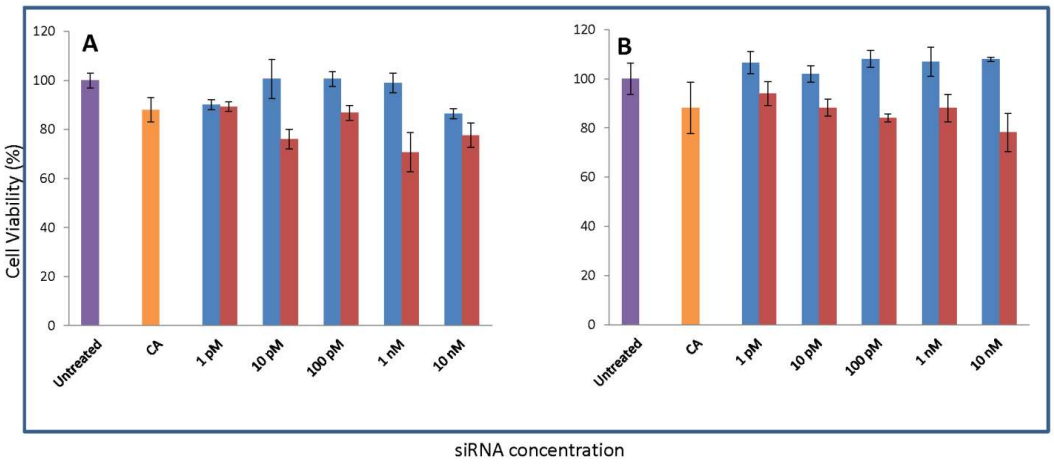

Figure S2. Carbonate apatite facilitated delivery of ERBB2 (**A**) and ROS1 (**B**) siRNA to MCF-7 cells. With 4 mM of $CaCl_2$ and various concentrations of siRNA (1 pM to 10 nM), cells were treated and MTT assay was performed two days after treatment. Values are presented as cell viability (%) compared to untreated cells. ●free siRNA ● CA/siRNA.

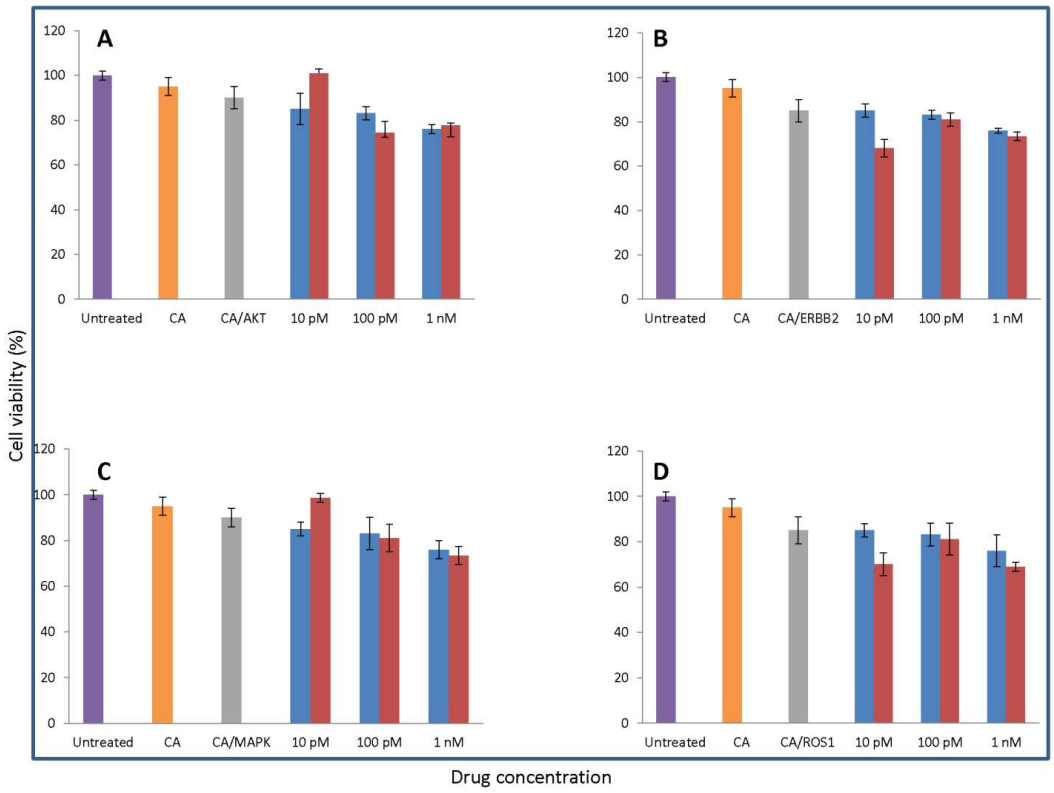

Figure S3. Effect of single pathway silencing on cytotoxicity of paclitaxel on 4T1 cells. By incorporation of 3 mM Ca, 1 pM AKT (**A**), ERBB2 (**B**), MAPK (**C**) and ROS1 (**D**) siRNA together with 10 pM, 100 pM and 1 nM paclitaxel into apatite structure, cells were treated. Two days post treatment, MTT assay was conducted to produce results of cell viability (%) compared to untreated cells. ● CA/Pac, ● CA/siRNA/Pac.

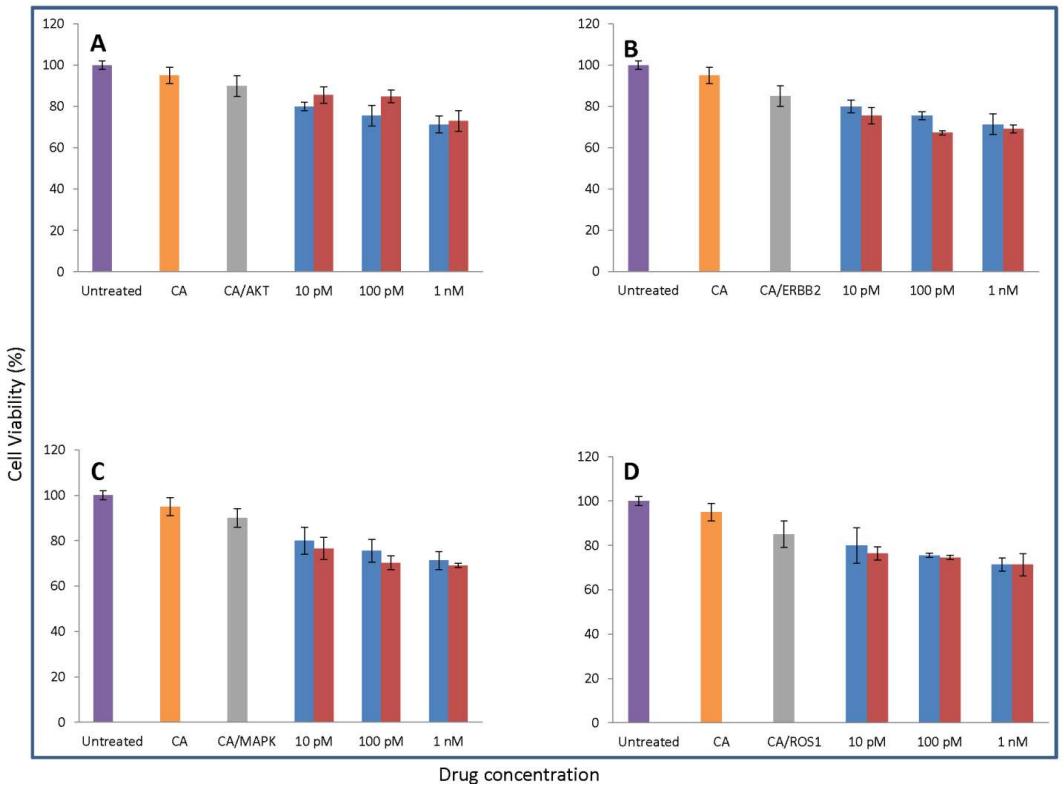

Figure S4. Effect of single pathway silencing on cytotoxicity of docetaxel on 4T1 cells. By incorporation of 3 mM Ca, 1 pM AKT (**A**), ERBB2 (**B**), MAPK (**C**) and ROS1 (**D**) siRNA together with 10 pM, 100 pM and 1 nM docetaxel into apatite structure, cells were treated. Two days post treatment, MTT assay was conducted to produce results of cell viability (%) compared to untreated cells. ● CA/Doc, ● CA/siRNA/Doc.

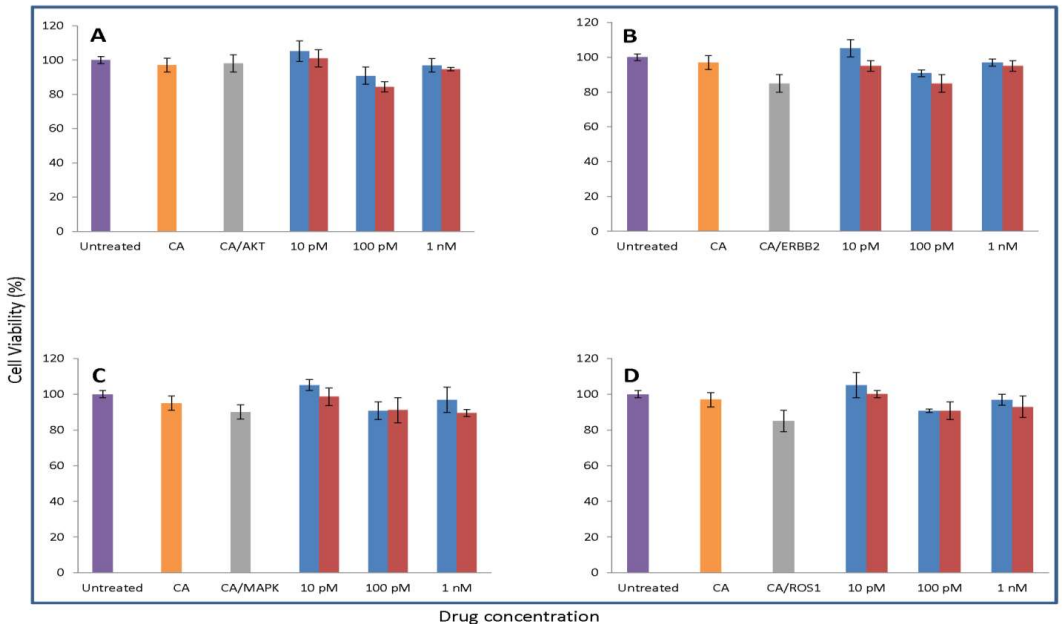

Figure S5. Effect of single pathway silencing on cytotoxicity of mitomycin C on 4T1 cells. By incorporation of 3 mM Ca, 1 pM AKT (**A**), ERBB2 (**B**), MAPK (**C**) and ROS1 (**D**) siRNA together with 10 pM, 100 pM and 1 nM Mitomycin C into apatite structure, cells were treated. Two days post treatment, MTT assay was conducted to produce results of cell viability (%) compared to untreated cells. ● CA/Mito, ● CA/siRNA/Mito.

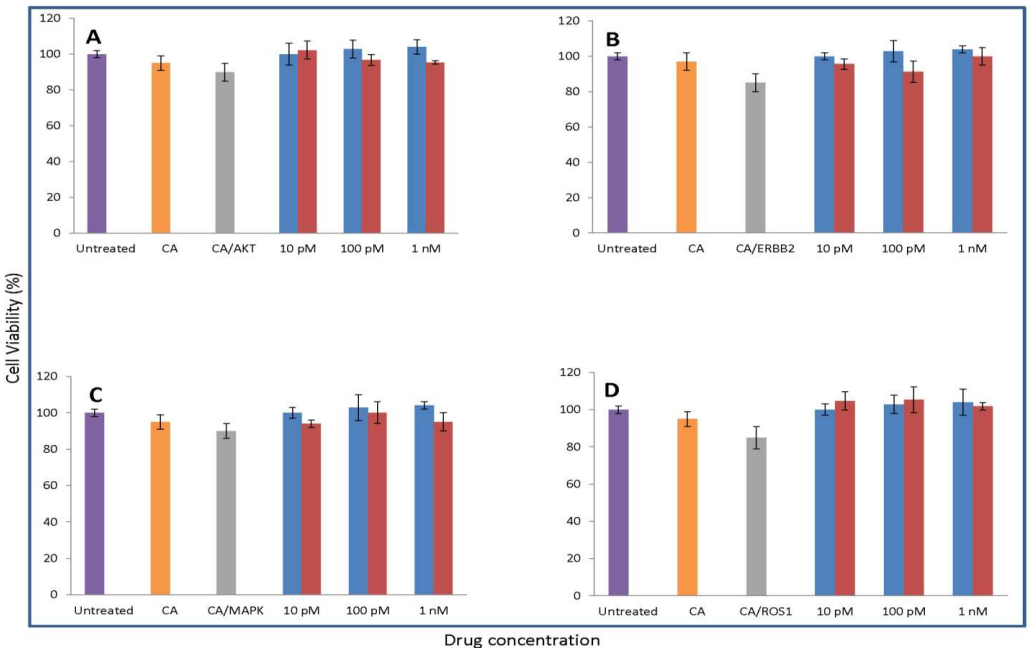

Figure S6. Effect of single pathway silencing on cytotoxicity of topotecan on 4T1 cells. By incorporation of 3 mM Ca, 1 pM AKT (**A**), ERBB2 (**B**), MAPK (**C**) and ROS1 (**D**) siRNA together with 10 pM, 100 pM and 1 nM topotecan into apatite structure, cells were treated. Two days post treatment, MTT assay was conducted to produce results of cell viability (%) compared to untreated cells. ● CA/Topo, ● CA/siRNA/Topo.

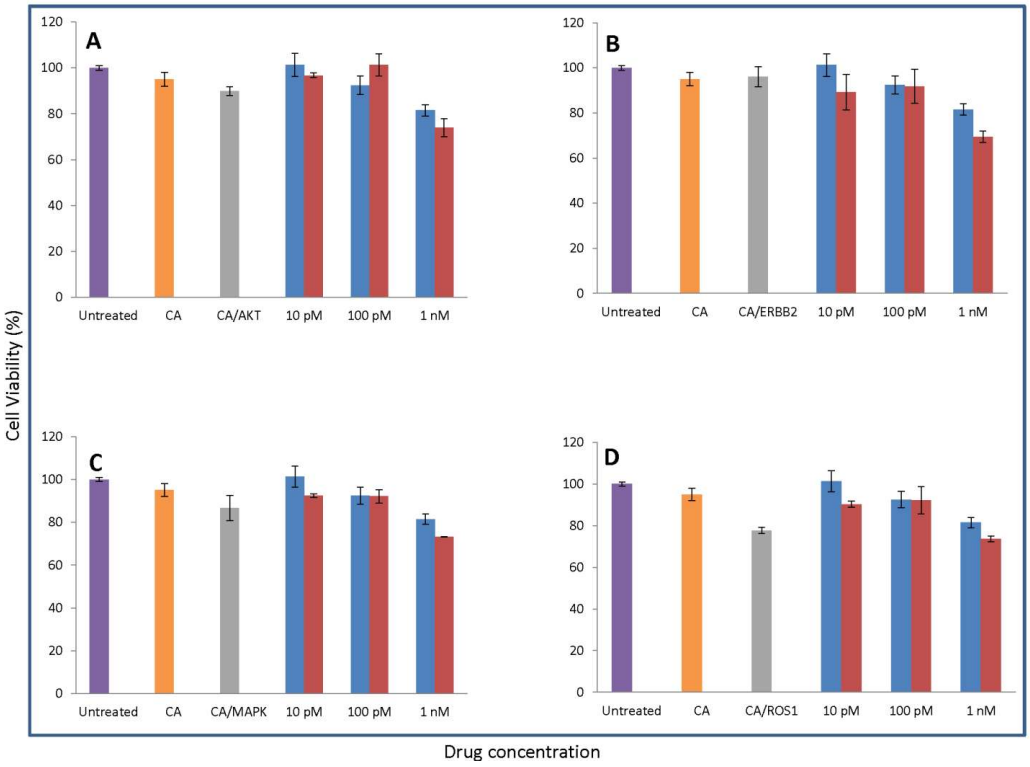

Figure S7. Effects of single pathway silencing on paclitaxel cytotoxicity in MDA-MB-231. Cells were treated with complexes of apatite formed with 3 mM of Ca together with 1 nM of AKT (**A**), ERBB2 (**B**), MAPK (**C**) and ROS1 (**D**) siRNA and 10 pM, 100 pM and 1 nM of Pac. MTT assay was performed two days after treatment and results were calculated as cell viability (%). ● CA/Pac, ● CA/siRNA/Pac.

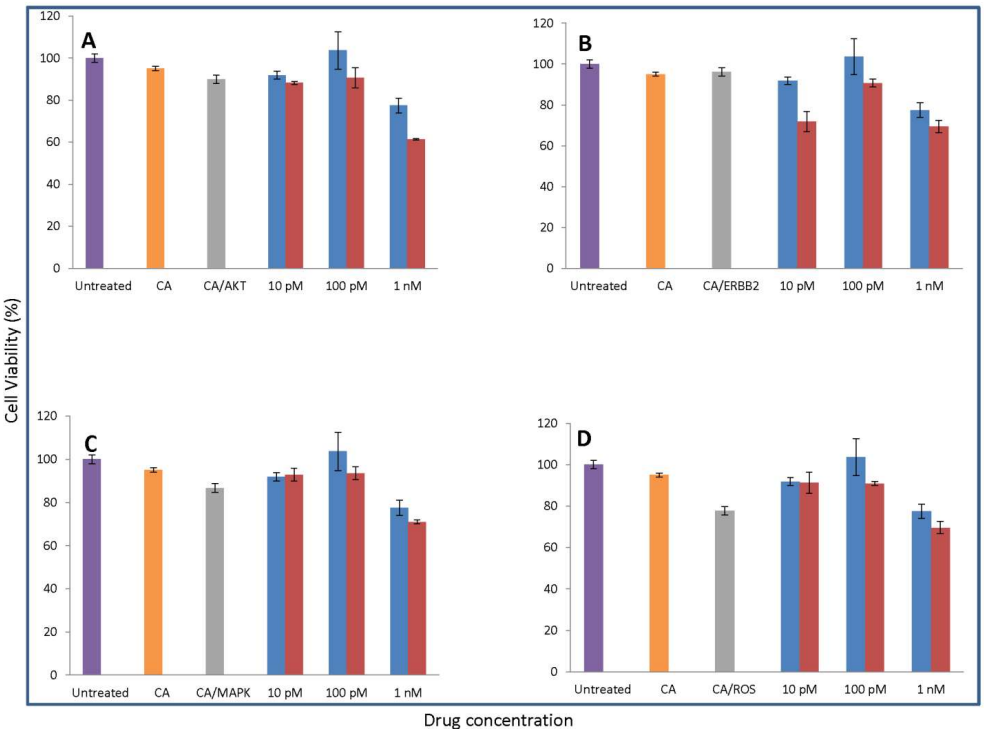

Figure S8. Effects of single pathway silencing on docetaxel cytotoxicity. MDA-MB-231 cells were treated with complexes of apatite formed with 3 mM of Ca together with 1 nM of AKT (**A**), ERBB2 (**B**), MAPK (**C**) and ROS1 (**D**) siRNA and 10 pM, 100 pM and 1 nM of Doc. MTT assay was performed two days after treatment and results were calculated as cell viability (%). ●CA/Doc, ● CA/siRNA/Doc.

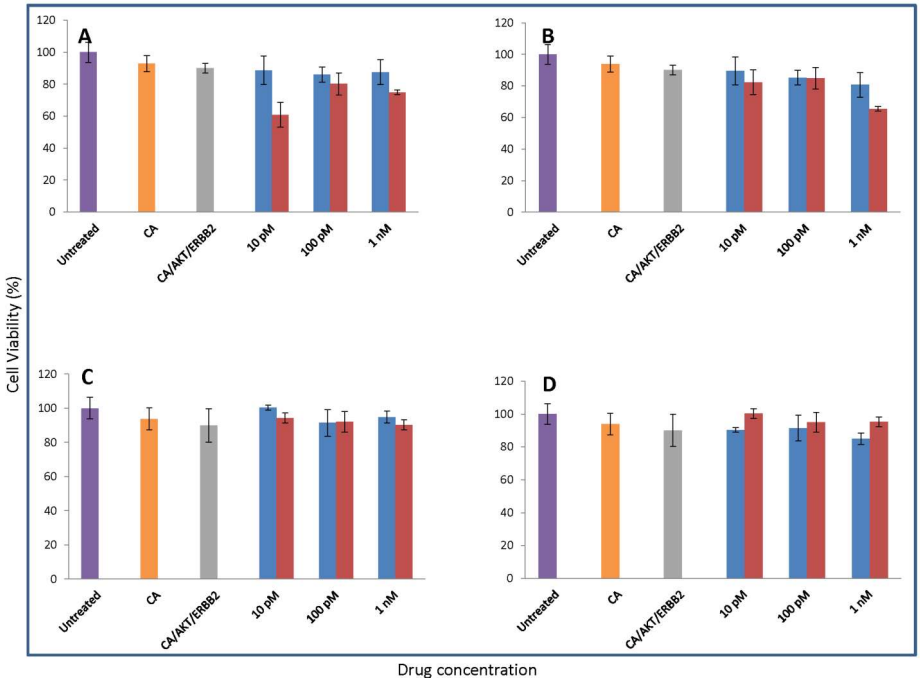

Figure S9. Effects of silencing AKT and ERBB2 oncogenes on drugs cytotoxicity in 4T1. Cells were treated with complexes of apatite formed with 3 mM of Ca together with 1 pM of AKT and ERBB2 siRNA and 10 pM, 100 pM and 1 nM of Pac (**A**), Doc (**B**), Mito (**C**), Topo (**D**). MTT assay was performed two days after treatment and results were calculated as cell viability (%). ● CA/drug, ● CA/AKT/ERBB2/drug.

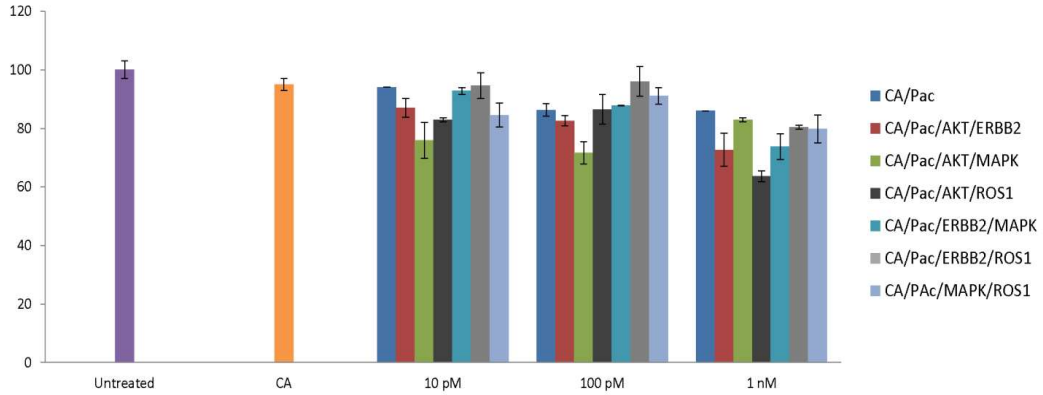

Figure S10. Cell viability assay on MDA-MB-231 cells treated with carbonate apatite complexed with paclitaxel and two siRNAs. By addition of 3 mM Ca, 1 nM siRNA and 10 pM, 100 pM and 1 nM of Pac, cells were treated and then subject to MTT assay after two days. Results are presented as cell viability (%).