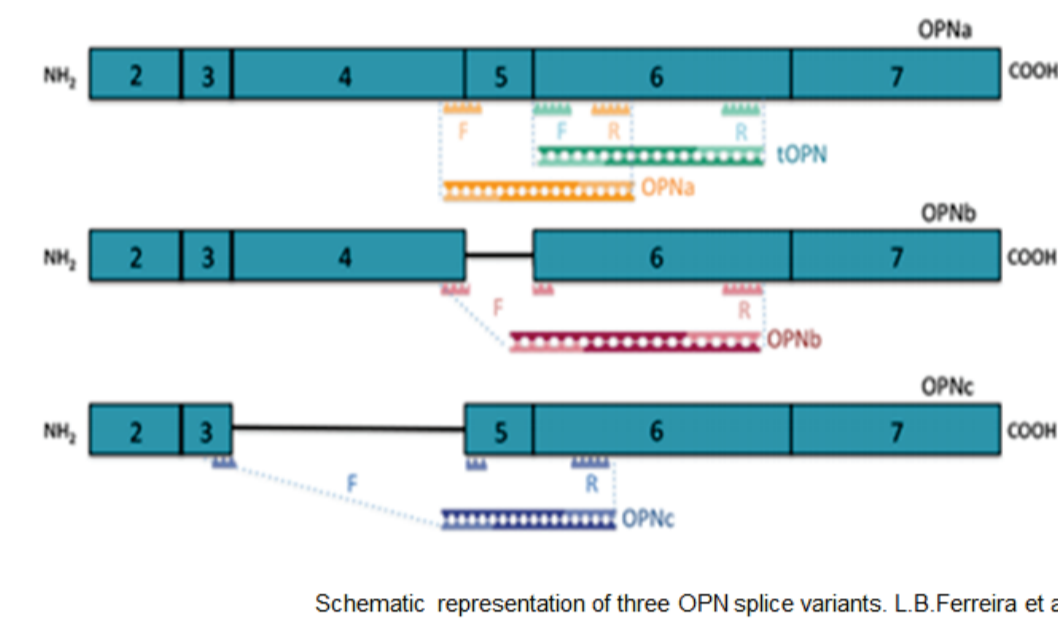


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INTRODUCTION

Osteopontin (OPN) has been described as an important gene product mediating resistance to chemotherapeutic drugs. However, the specific roles of each OPN splice variant on mediating chemoresistance should be further investigated. In this context, this work aimed to evaluate OPNc expression patterns and its correlations with resistance to doxorubicin (DOX) and cisplatin (CIS) in breast and ovarian tumor cell lines, respectively.



Schematic representation of three OPN splice variants. L.B. Ferreira et al. 2016

RESULTS

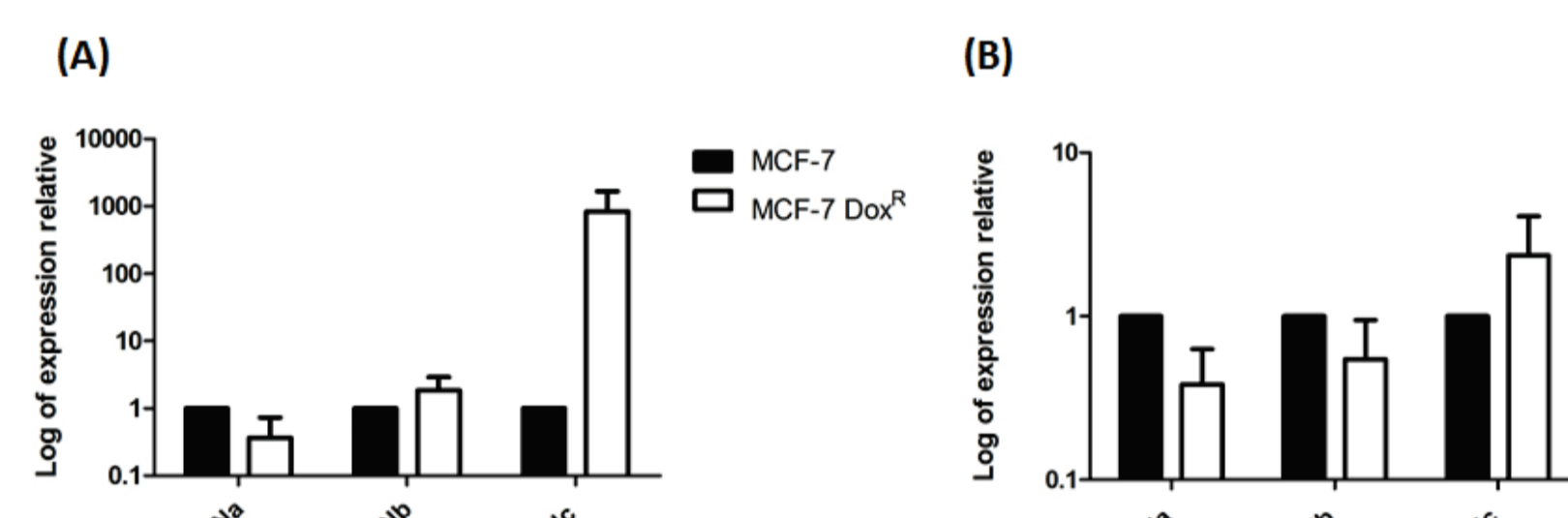


Figure 1: OPN splicing isoforms expression in MCF-7 Dox^R and ACRP resistant cell lines and their corresponding parental cell lines (A and B). OPNc isoform is expressed in higher levels in resistant cell lines in relation to parental cell lines. Besides, OPNc is overexpressed in relation to OPNa and OPNb isoforms.

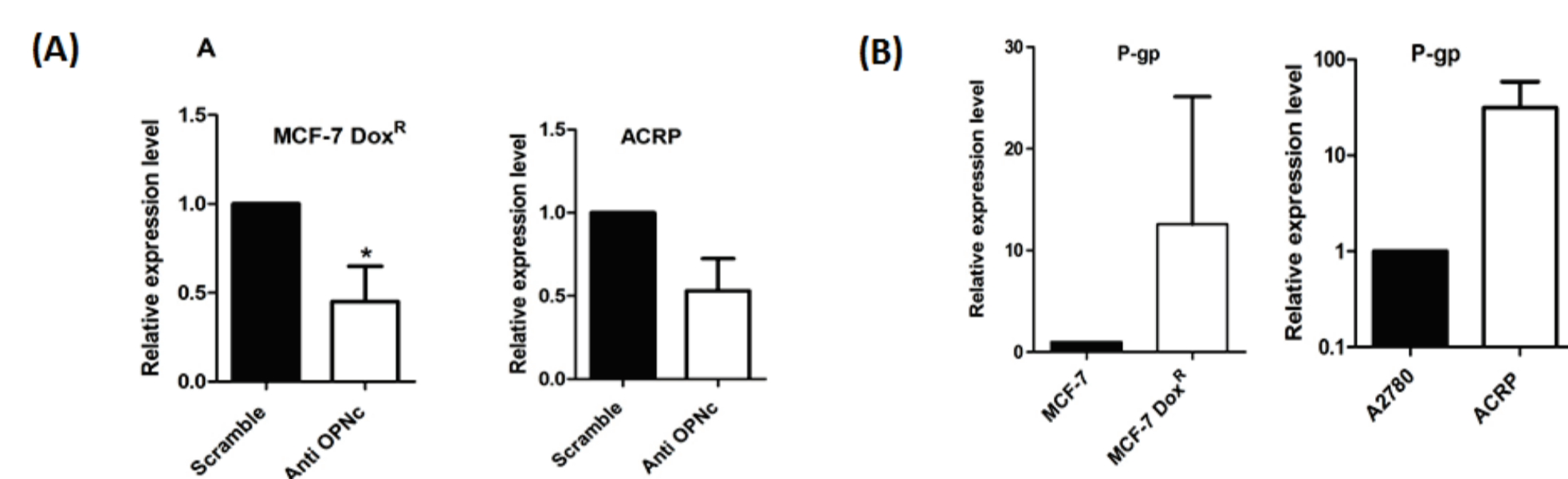


Figure 2: OPNc knockdown using anti-OPNc oligomers inhibits OPNc and P-glycoprotein (P-gp) expression in MCF-7 Dox^R and ACRP resistant cell lines. MCF-7 Dox^R and ACRP resistant cell lines were transfected with the anti-OPNc oligomers and 24 h after transfection OPNc (A) and P-gp (B) transcriptional expression levels have been measured. Transfection with the anti-OPNc oligomers reduced OPNc (A) and P-gp (B) expression in both MCF-7 Dox^R (right graphs) and ACRP (left graphs) resistant cell lines. *p<0,05.

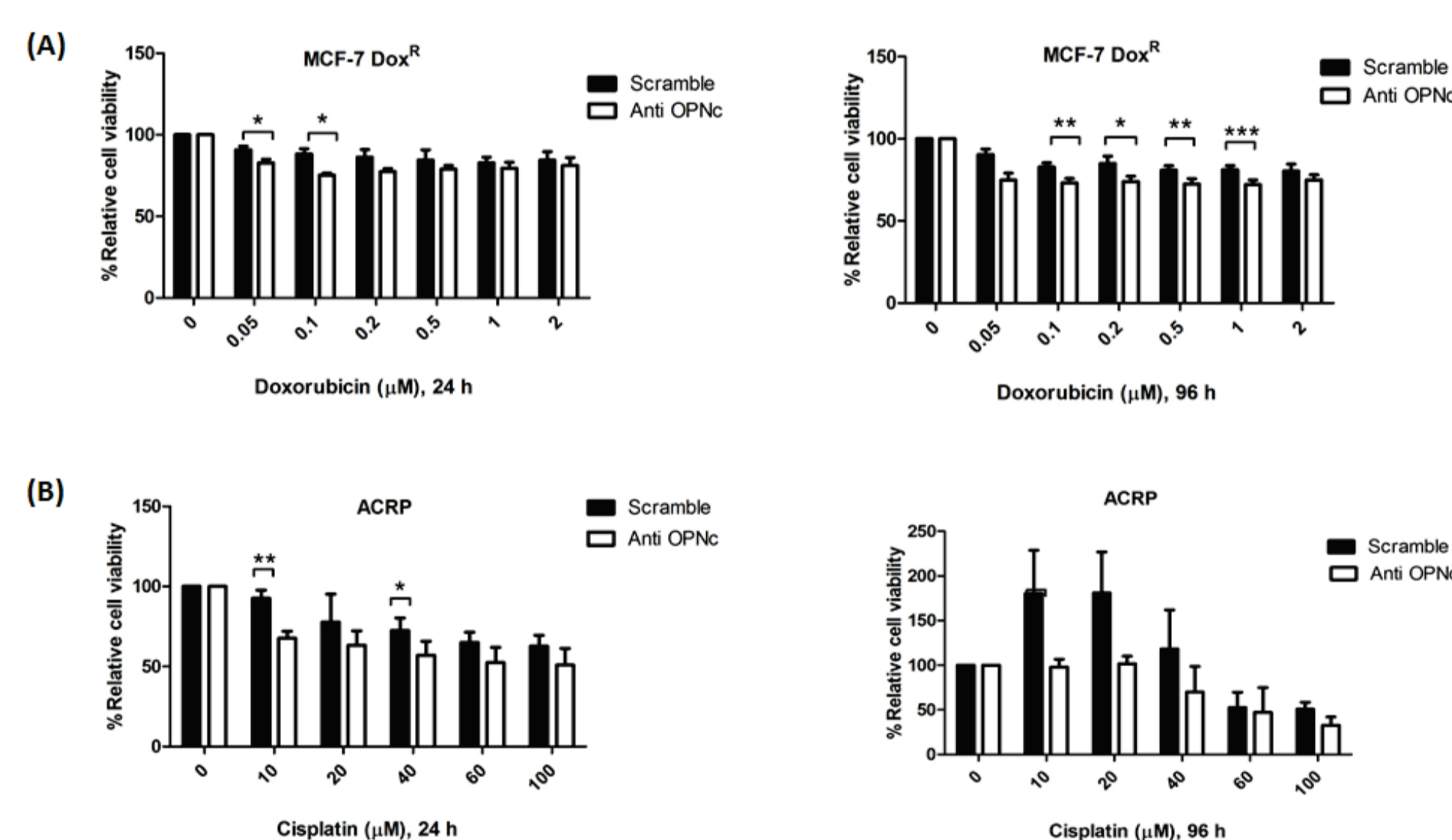


Figure 3: OPNc knockdown sensitizes drug resistant MCF-7 Dox^R and ACRP cells to cisplatin and doxorubicin treatment. MCF-7 Dox^R and ACRP cells were treated with several increasing concentrations of cisplatin (0 μM-100 μM) and doxorubicin (0 μM-2 μM) for 24 and 96 h after drug treatment cell viability has been measured using MTT assays. Bar graphs represent relative viability of MCF-7 Dox^R (A) and ACRP (B) cells in response to cisplatin and doxorubicin treatment. OPNc knockdown using anti-OPNc oligomers decrease MCF-7 Dox^R and ACRP cell viability, indicating OPNc role on mediating cell survival. *p<0,05.

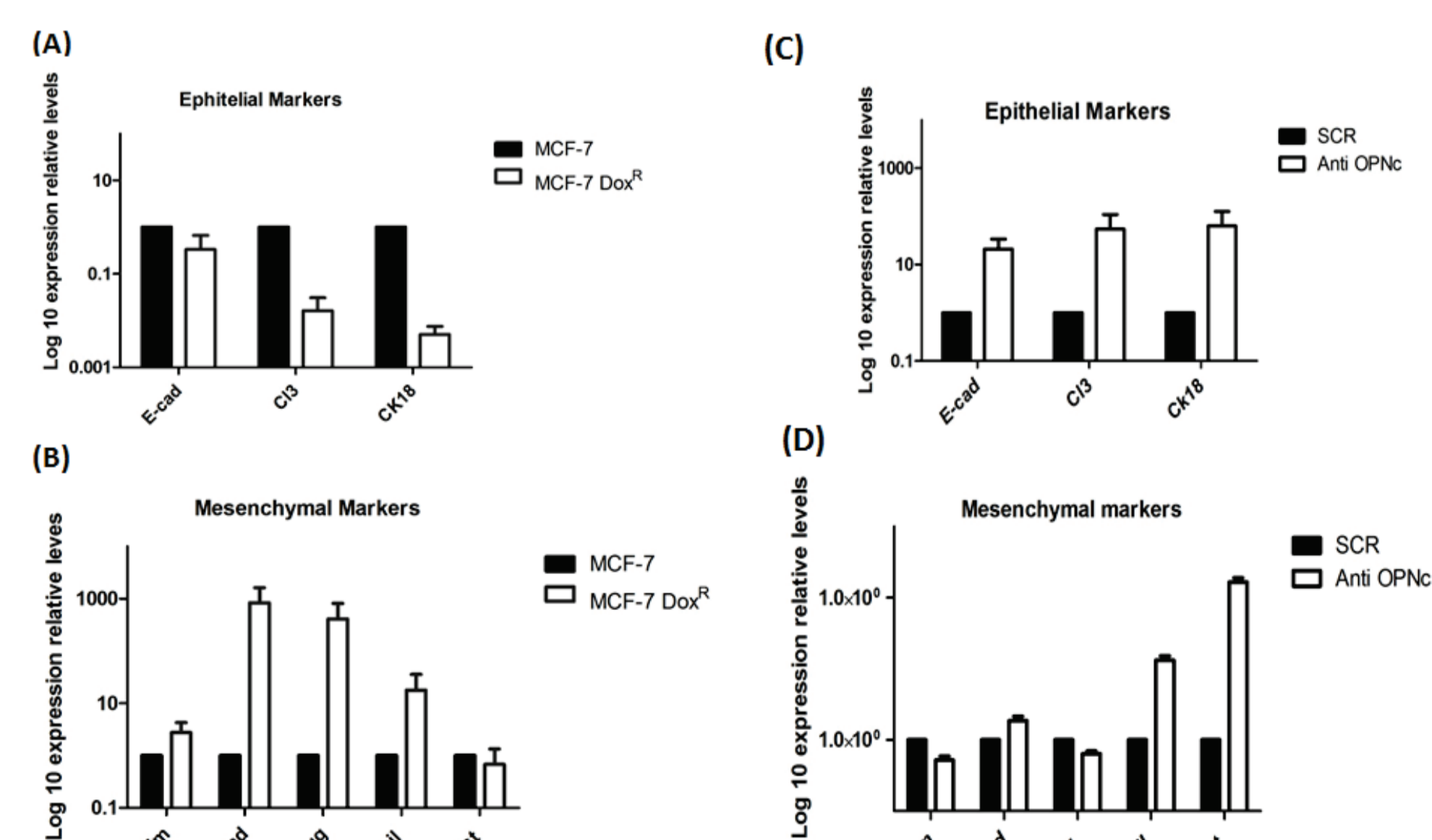


Figure 4: OPNc knockdown reverses the drug resistant MCF-7 Dox^R epithelial-mesenchymal transition (EMT) transcriptional phenotype. The transcriptional pattern of epithelial (E-cadherin (E-cad), Claudin-3 (Cl-3) and Cytokeratin-18 (CK18)) and mesenchymal (Vimentin (Vim), N-cadherin (N-cad), Slug (Slug), Snail (Snail) and Twist (Twist)) markers has been measured by quantitative real time PCR. Bar graphs on the left panels represent the relative transcriptional levels of epithelial (A) and mesenchymal markers (B) in MCF-7 Dox^R in relation to MCF-7 parental cell line. MCF-7 Dox^R exhibit a classical EMT phenotype in relation to MCF-7. Bar graphs on the right panels represent the expression levels of epithelial (C) and mesenchymal markers (D) of MCF-7 Dox^R cells when transfected with the anti-OPNc oligomers in relation to cells transfected with the scramble control oligomer. In response to OPNc knockdown, the MCF-7 Dox^R EMT phenotype has been reversed, acquiring a predominant epithelial phenotype.

CONCLUSION

In summary, our data provide early evidence that OPNc could represent a potential additional molecular target to therapeutic strategies. The specific OPNc isoform knockdown approach has the potential to surpass/overcome resistance to chemotherapeutic drugs.

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METHODOLOGY

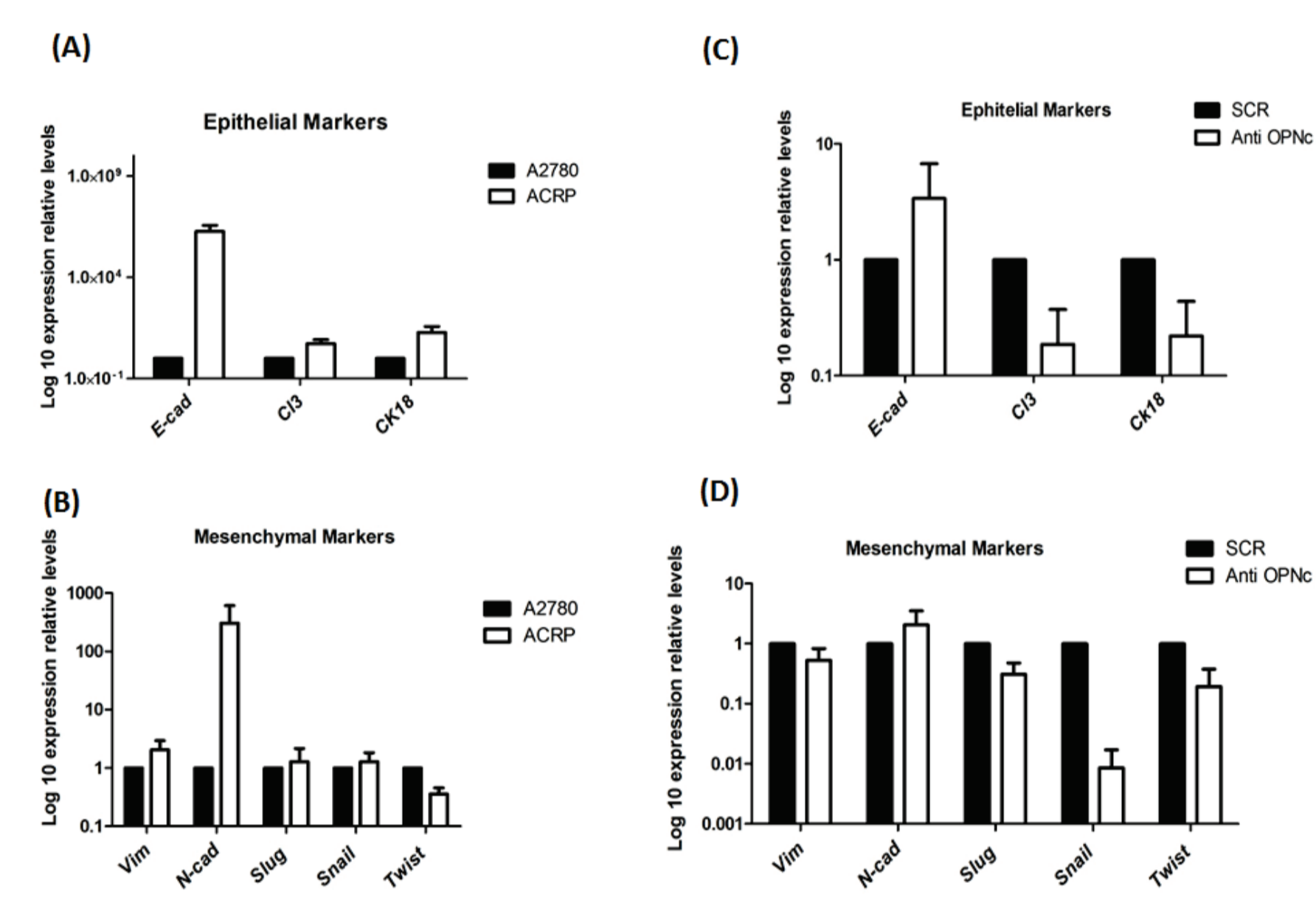
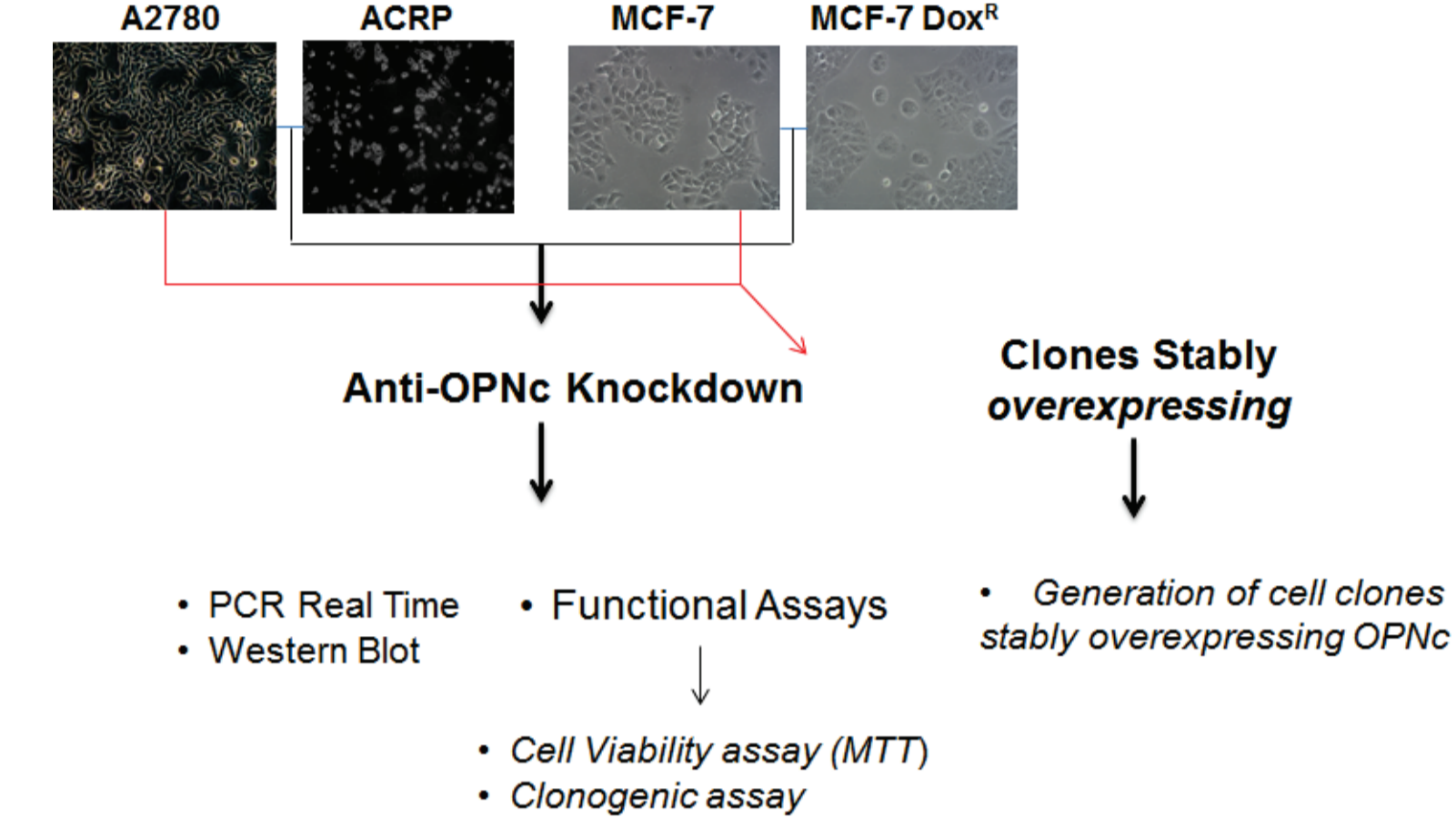


Figure 5: OPNc knockdown partially reverses the drug resistant ACRP epithelial-mesenchymal transition (EMT)-like transcriptional phenotype. The transcriptional pattern of epithelial (E-cadherin (E-cad), Claudin-3 (Clau-3) and Cytokeratin 18 (CK18)) and mesenchymal (Vimentin (Vim), N-cadherin (N-cad), Slug (Slug), Snail (Snail) and Twist (Twist)) markers has been measured by quantitative real time PCR. Bar graphs on the left panel (A) represent the relative transcriptional levels of epithelial and mesenchymal markers in ACRP in relation to A2780 parental cell line. ACRP exhibit a EMT-like phenotype in relation to A2780; Bar graphs on the right panel (represent the expression of epithelial and mesenchymal markers when transfecting ACRP cells with the anti-OPNc oligomers in relation to cells transfected with the scramble control oligomers. In response to OPNc knockdown, the ACRP EMT-like phenotype has been partially reversed.

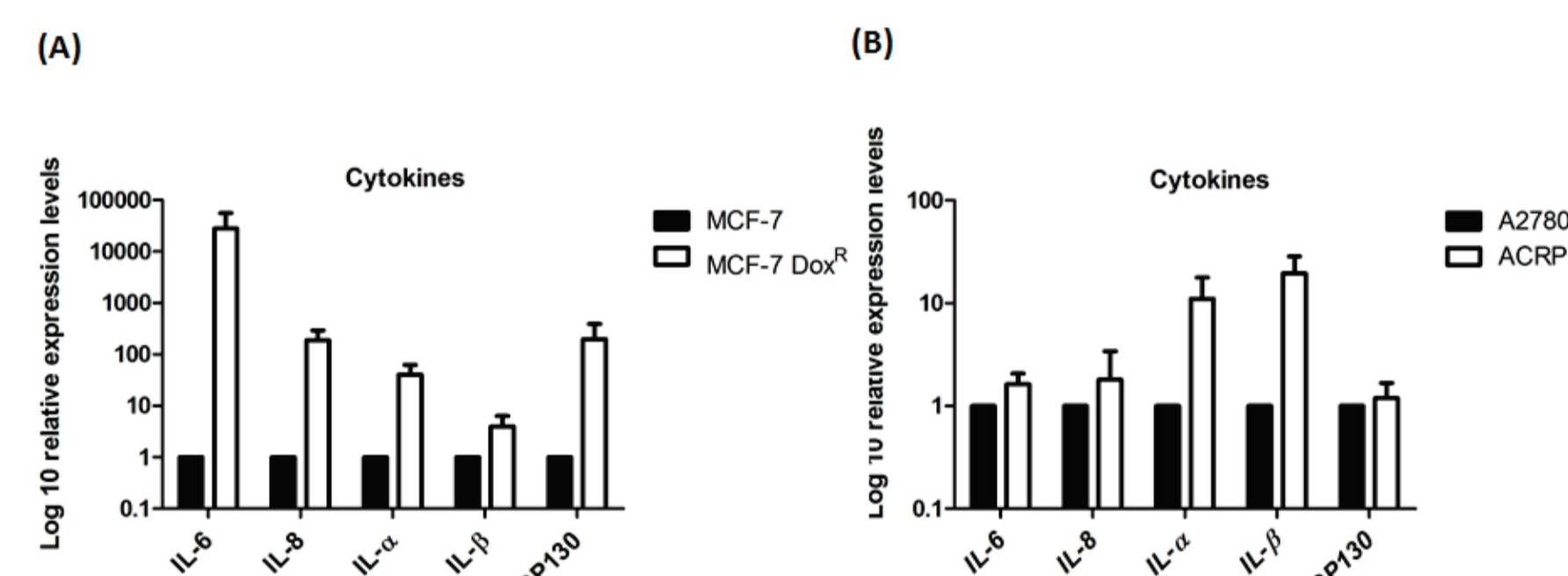


Figure 6: MCF-7 Dox^R and ACRP drug resistant cell lines display upregulated expression of epithelial-mesenchymal transition (EMT)-related cytokines. The transcriptional expression of EMT-related cytokines (Interleukin-6 (IL-6), Interleukin-8 (IL-8), Interleukin-α (IL-α), Interleukin-β (IL-β) and GP130 (GP130)) has been measured by real time PCR. The expression of EMT-related cytokines is overexpressed in MCF-7 Dox^R (A) and ACRP (B) cells in relation to MCF7 and A2780 parental cell lines, respectively.

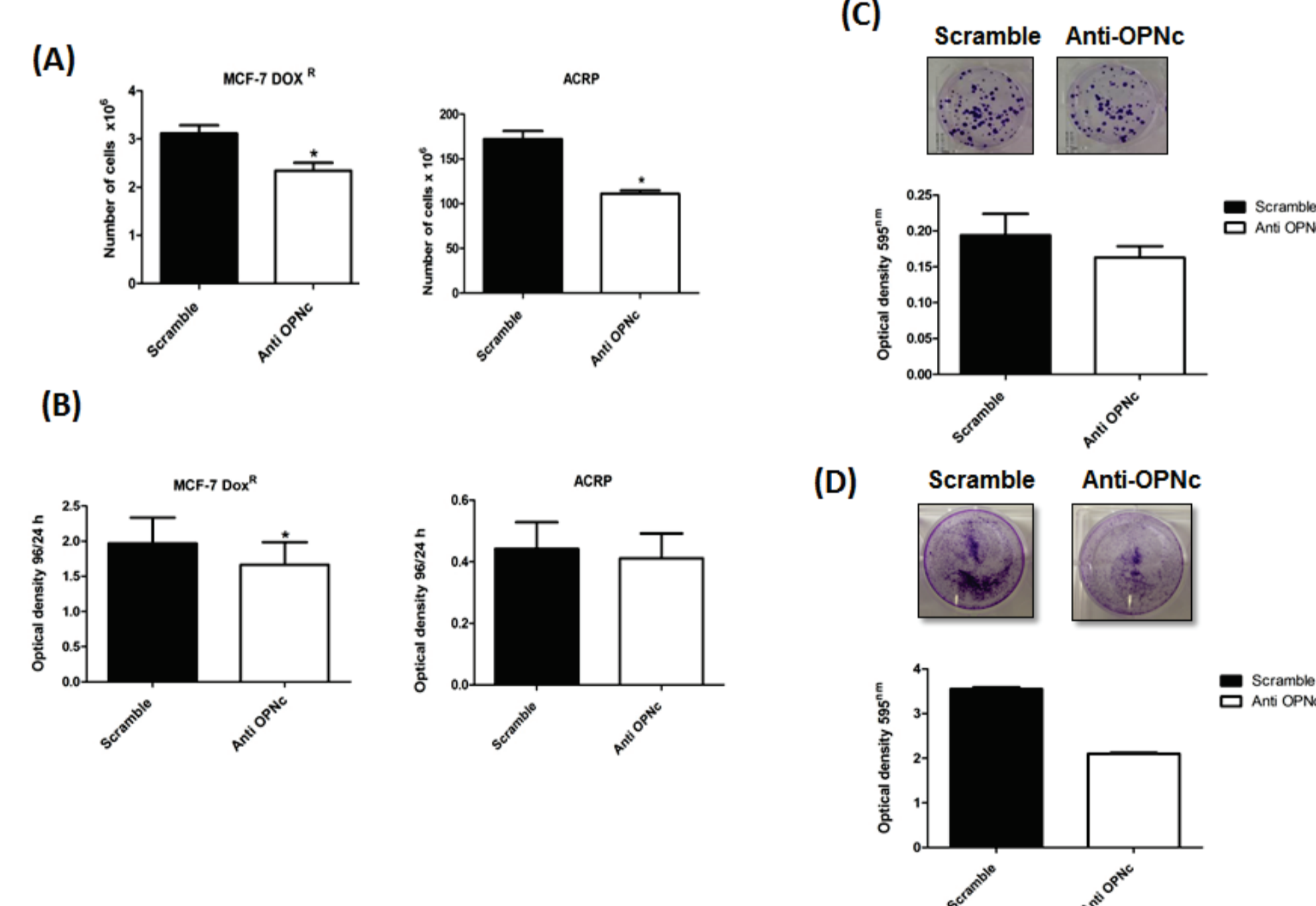


Figure 7: OPNc knockdown reduces cell growth in drug resistant MCF-7 Dox^R and ACRP cells. MCF-7 Dox^R and ACRP cells exhibit a decreased cell number in response to OPNc knockdown (A and B) and a reduced cell growth, as evidenced by clonogenic assay using crystal violet staining (C and D). *p<0,05.

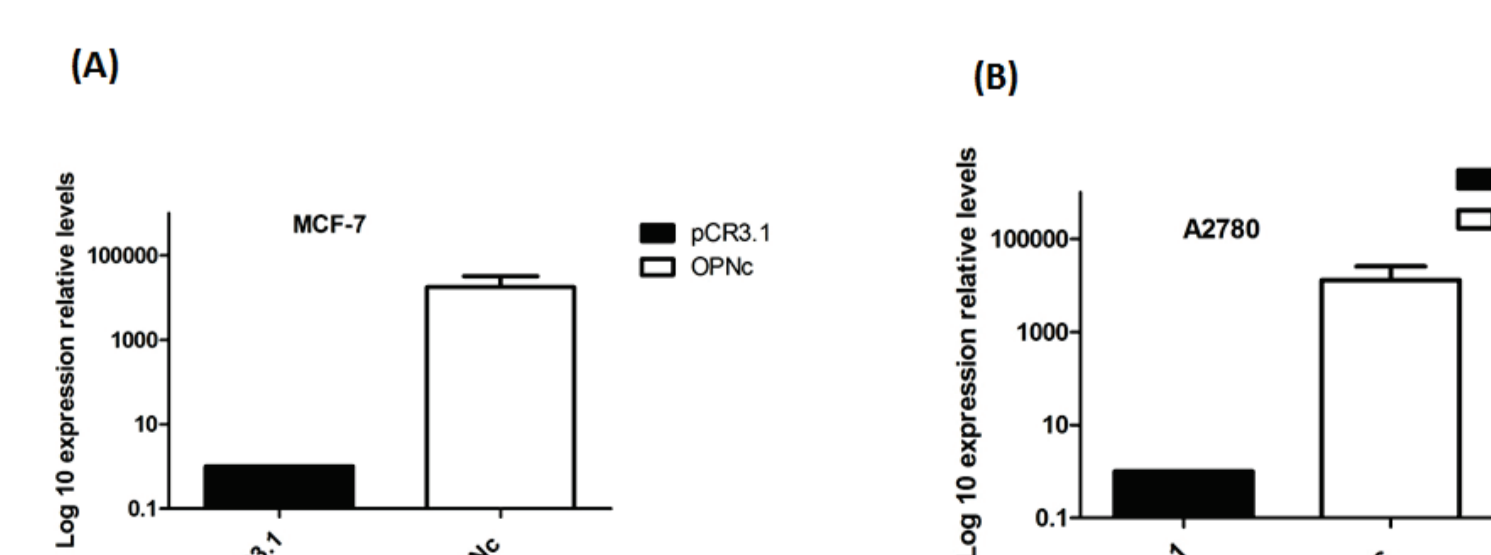


Figure 8: A2780 and MCF-7 cells ectopically overexpressing OPNc. A2780 and MCF7 cells were transfected with the plasmid pCR3.1, in which OPNc complete cDNA has been cloned. Cell clones stably overexpressing OPNc have been selected using geneticin in the culture media. These results have been analyzed using a pool of stably overexpressing cell clones. The transcriptional expression of OPNc has been evaluated in MCF-7 (A) and A2780 (B) cell clones stably expressing OPNc and in those cells transfected with the empty vector control clone.