

Supplementary Material

A synergistic combination of niclosamide and doxorubicin as an efficacious therapy for all clinical subtypes of breast cancer

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Supplementary figures

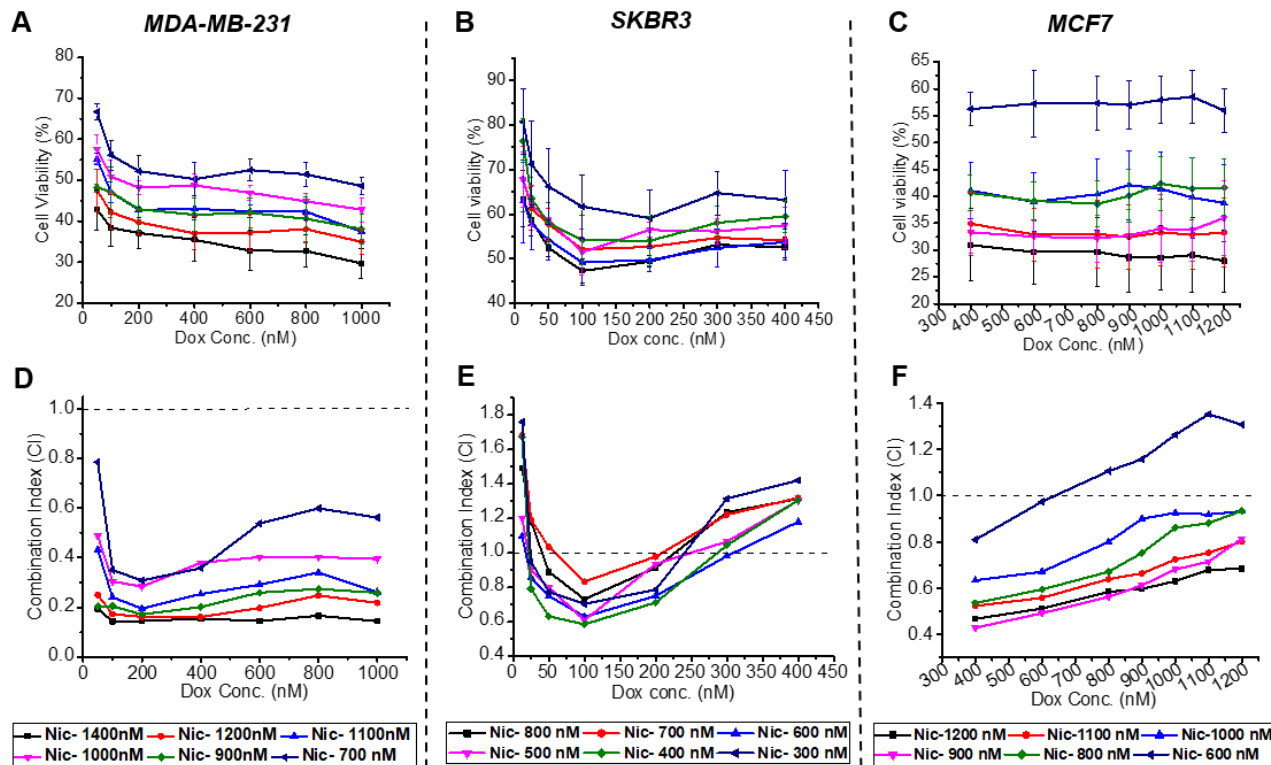


Figure S1. Effect of sequential treatment of Nic and Dox on breast cancer cells.

Cell viability line plots of different sequential combinatorial concentrations of Nic and Dox (24 h of Nic treatment followed by 24 h of Dox treatment) treated against A) MDA-MB-231, B) SKBR3 and C) MCF7 cells. Combination index line plots for D) MDA-MB-231, E) SKBR3 and F) MCF7 cells.

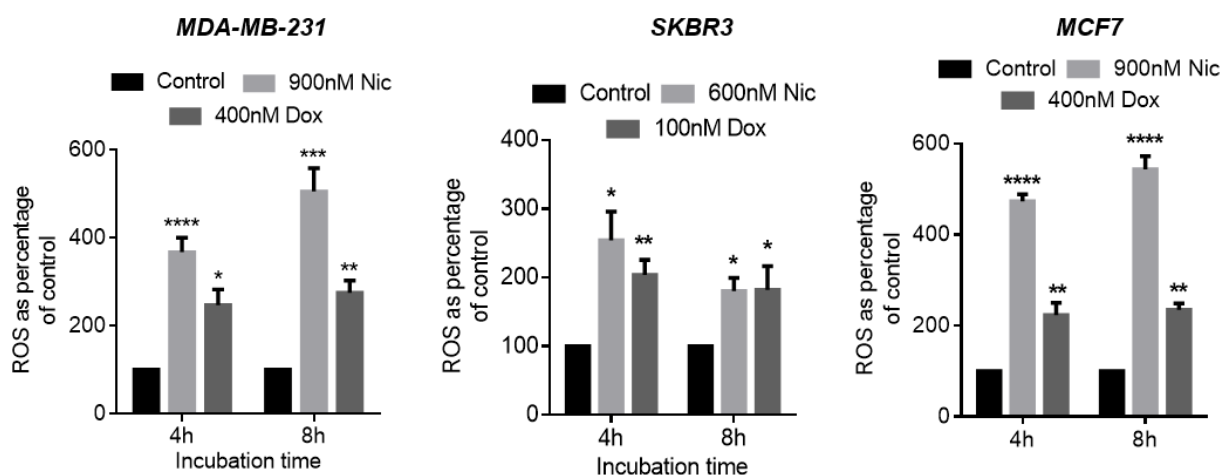


Figure S2. ROS analysis in breast cancer cells.

ROS analysis of Nic and Dox treated cells at indicated doses for 4 h and 8 h through DHE assay in MDA-MB-231, SKBR3 and MCF7 cells. *($p<0.05$); **($p<0.01$), ***($p<0.001$) and ****($p<0.0001$) indicate statistically significant difference with respect to untreated control group ($n=3$).

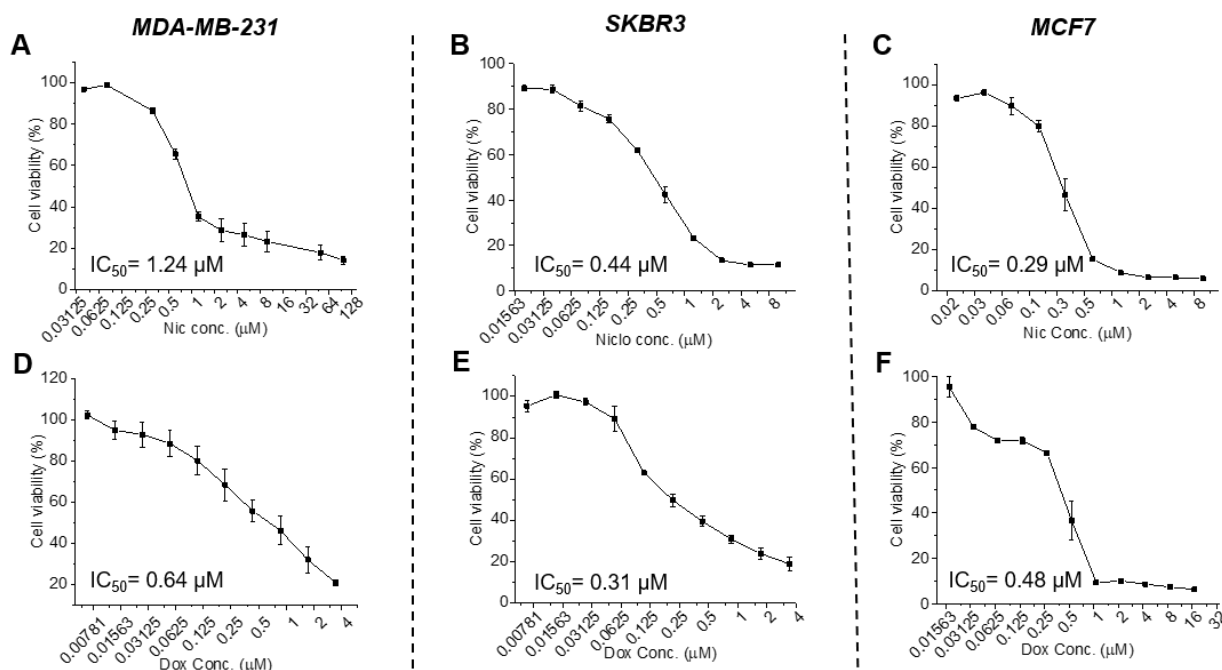


Figure S3. Effect of individual treatment of Nic and Dox on breast cancer cells for 48 h.

In vitro cytotoxicity of individual A-C) Nic and D-F) Dox on MDA-MB-231, SKBR3 and MCF7 cells at different doses for 48 h ($n=3$).

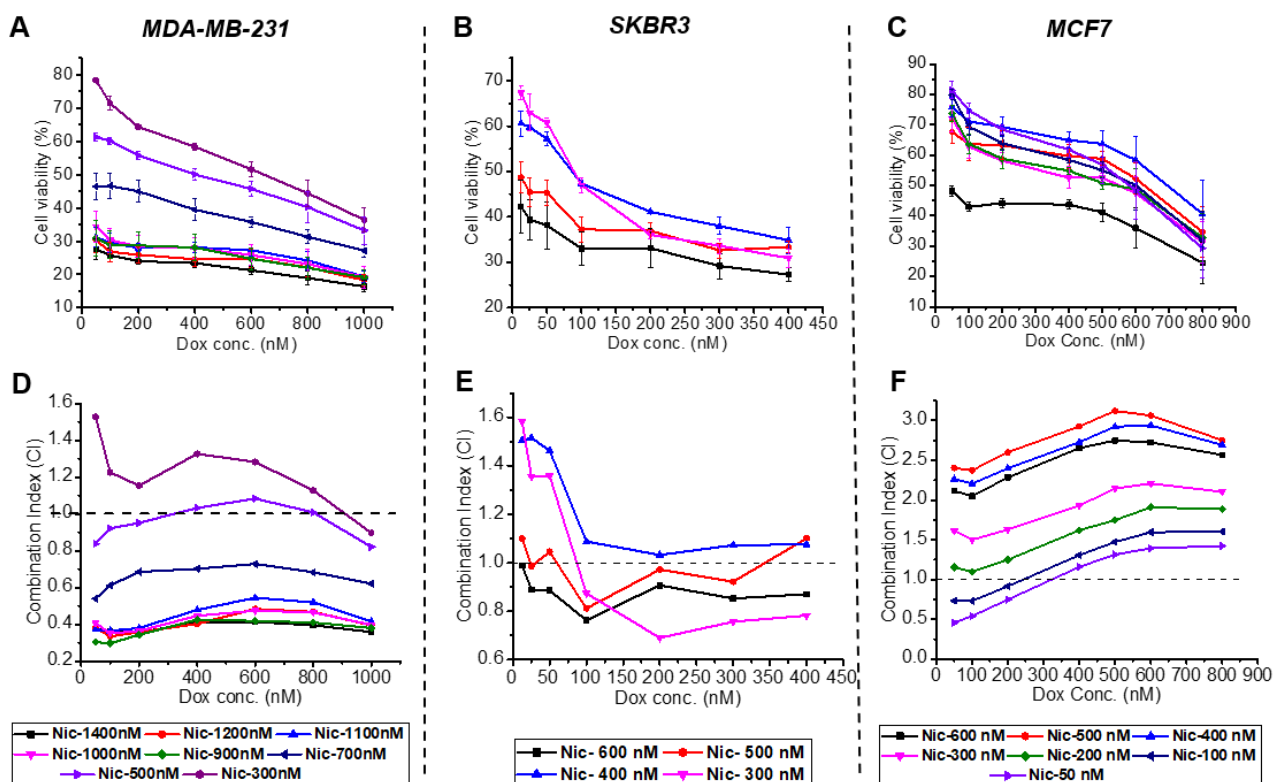


Figure S4. Effect of concurrent treatment of Nic and Dox on breast cancer cells.

Cell viability line plots of different concurrent combinatorial concentrations of Nic and Dox for 48 h treated against A) MDA-MB-231, B) SKBR3 and C) MCF7 cells. Combination index line plots of combination therapy for D) MDA-MB-231, E) SKBR3 and F) MCF7 cells.

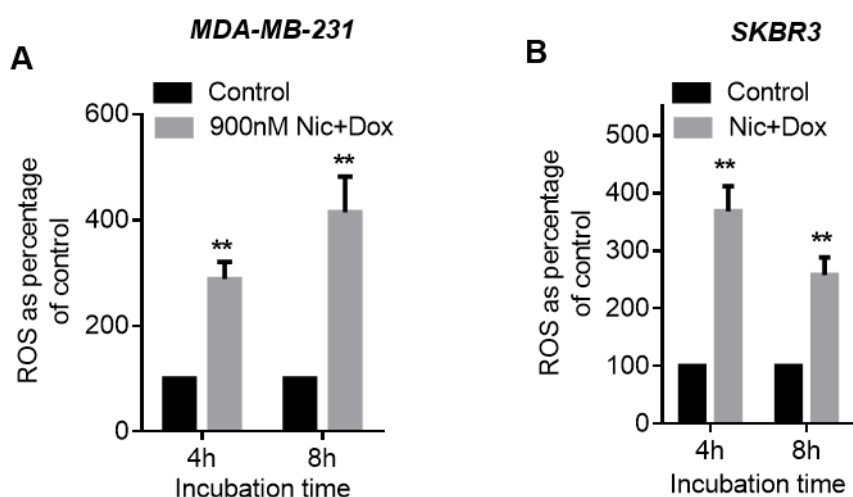


Figure S5. ROS analysis in breast cancer cells when concurrently treated with Nic and Dox.

Reactive oxygen species (ROS) analysis of Nic+Dox treated cells at indicated doses for 4 h and 8 h through DHE assay in A) MDA-MB-231, and B) SKBR3 cells. ** indicates statistically significant difference ($p < 0.01$) with respect to untreated control group ($n=3$).

Table S1. List of primer sequences.

Name	Sequence
Lymphoid enhancer factor (LEF)	F: 5' AATGAGAGCGAATGTCGTTGC 3' R: 5' GCTGTCTTTCTTTCCGTGCTA 3'
T-cell factor (TCF)	F: 5' CTGCCTTAGGGACGGACAAAG 3' R: 5' TGCCAAAGAAGTTGGTCCATTTT 3'
cellular Myelocytomatosis (c-Myc)	F: 5' CATACATCCTGTCCGTCCAAG 3' R: 5' GAGTCCGTAGCTGTTCAAGT 3'
Axix inhibition protein2 (Axin2)	F: 5' GAGTGGACTTGTGCCGACTTCA 3' R: 5' GGTGGCTGGTGCAAAGACATAG 3'
CyclinD1 (CCND1)	F: 5' GCTGCGAAGTGGAACCATC 3' R: 5' CCTCCTTCTGCACACATTTGAA 3'