Supplementary Materials

Materials and Methods

In Vitro Cell Proliferation Assay and Analysis of Combinational Drug Effect

The percentages of surviving cells from each group relative to the cultured cells as a control, which was defined as 100% viability, were estimated. Data obtained from the growth-inhibitory experiments were used to perform these analyses. The isobologram method is a graphical representation of the pharmacologic interaction and is formed by selecting a desired fractional cell kill (Fa) and plotting the individual drug doses required to generate that Fa on their respective x- and y-axes. A straight line is then drawn to connect the points. The observed dose combination of the two agents that achieved that particular Fa is then plotted on the isobologram. Combination data points that fall on the line represent an additive drug interaction, whereas data points that fall below or above the line represent synergism or antagonism, respectively. The combination-index (CI) method is a mathematical and quantitative representation of a two-drug pharmacologic interaction. Using data from the growth-inhibitory experiments and computerized software, CI values were generated over a range of Fa levels from AS703026 and SAR245409 treatment. CI values <0.10 indicated very strong synergism; 0.10-0.30, strong synergism; 0.31-0.70, synergism; 0.71-0.85, moderate synergism; 0.86-0.90, slight synergism; 0.91-1.10, nearly additive; 1.11-1.20, slight antagonism; 1.21-1.45, moderate antagonism; 1.46-3.30, antagonism; 3.31-10, strong antagonism; >10, very strong antagonism.[1]

Reference

1. Zhang C, Zhai S, Li X, Zhang Q, Wu L, Liu Y, et al. Synergistic action by multi-targeting compounds produces a potent compound combination for human NSCLC both in vitro and in vivo. Cell Death Dis. 2014; 5: e1138.

Figure legends

Figure S1. Ingenuity pathway analysis of 5.1% of the gene set. We used IPA to analyze the 5.1% of genes that led to no projections in the 3D MCTS system and were unviable in the 2D monolayer system. Three of the five top-ranking pathways are shown here. (A) ErbB Signaling Pathway, (B) Renin-Angiotensin Signaling, (C) Natural Killer Cell Signaling.

Figure S2. The combination of AS703026 and SAR245409 inhibited proliferation of TNBC cells. Cells were treated with AS703026 and SAR245409 for 72 h, and a Cell-Titer Blue proliferation assay was performed. For analysis, the non-linear fit curve method was used via GraphPad Prism software.

Supplementary Figure S3. The anti-tumor effect of AS703026 and SAR245409 with near-IC50 concentrations in a soft agar assay. MDA-MB-468 breast cancer cells were overlaid onto the bottom agar layer (0.8%) with the indicated drugs (1 μM) for 4 weeks. For visualization, colonies were stained with 200 μl of MTT (1 mg/ml) solution for 2 h and counted using the GelCount colony-counting system according to the manufacturer's instructions. Statistical significance was evaluated by *t* test using GraphPad Prism software. Error bars represent the standard deviation of three independent experiments.

Figure S4. Inhibitory effect of pan-RSK inhibitor (BI-D1870) on the TNBC cell migration and invasion capability. Migration and invasion assays were performed in triplicate using a 48-well microchemotaxis chamber for 6 h and 24h respectively. Cells (1×10^5) were added into the upper well with or without BI-D1807 and the bottom wells were filled with 2% fetal bovine serum and 100 ng/ml EGF as an attractant. Results were quantitated by counting migrated cells in five randomly chosen high-power fields under a light microscope in triplicate. Columns, mean; bars, standard deviation. Statistical significance was evaluated by paired *t*-test using GraphPad Prism software.

ErbB Signaling

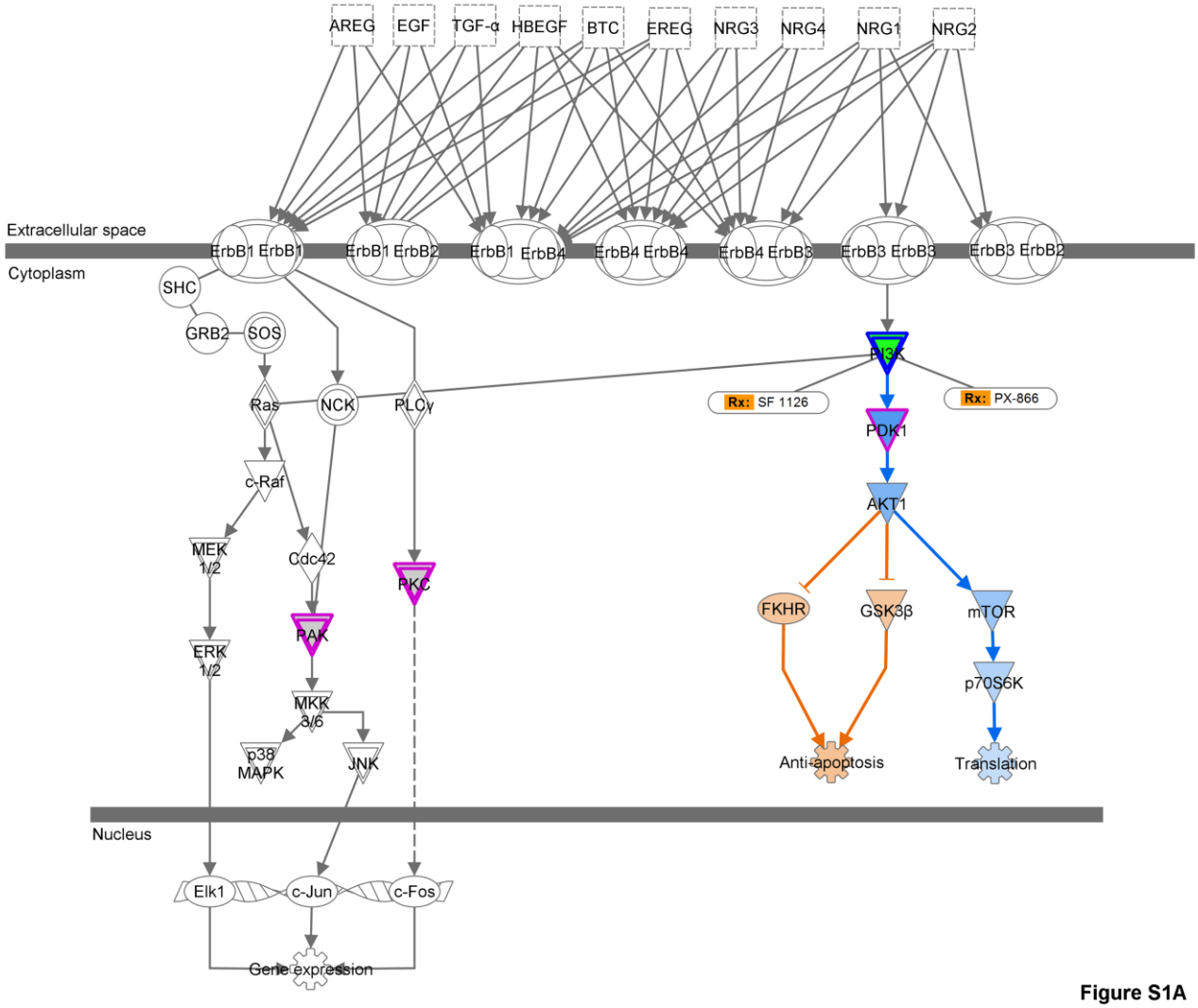

Figure S1A

Renin-Angiotensin Signaling

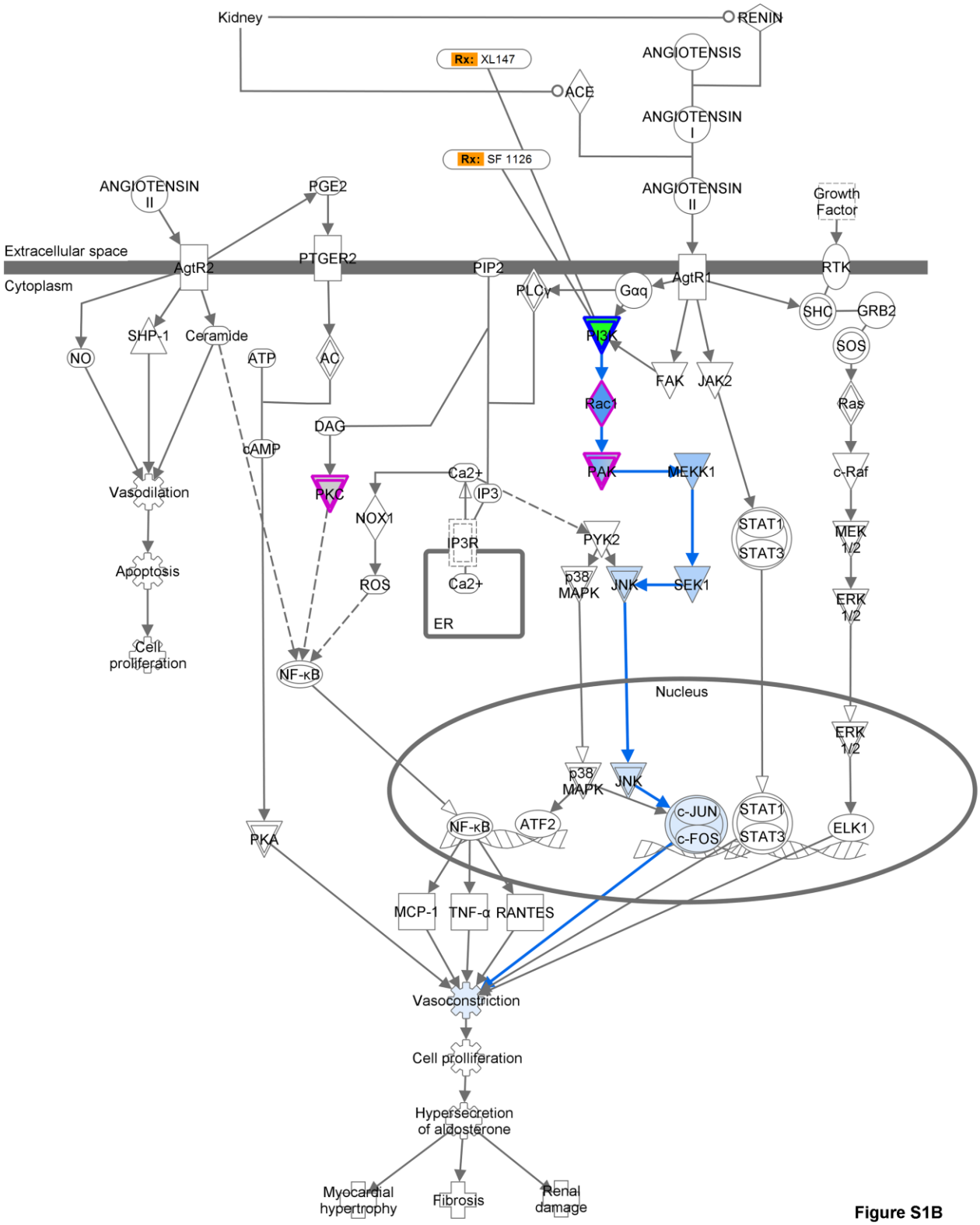

Figure S1B

Natural Killer Cell Signaling

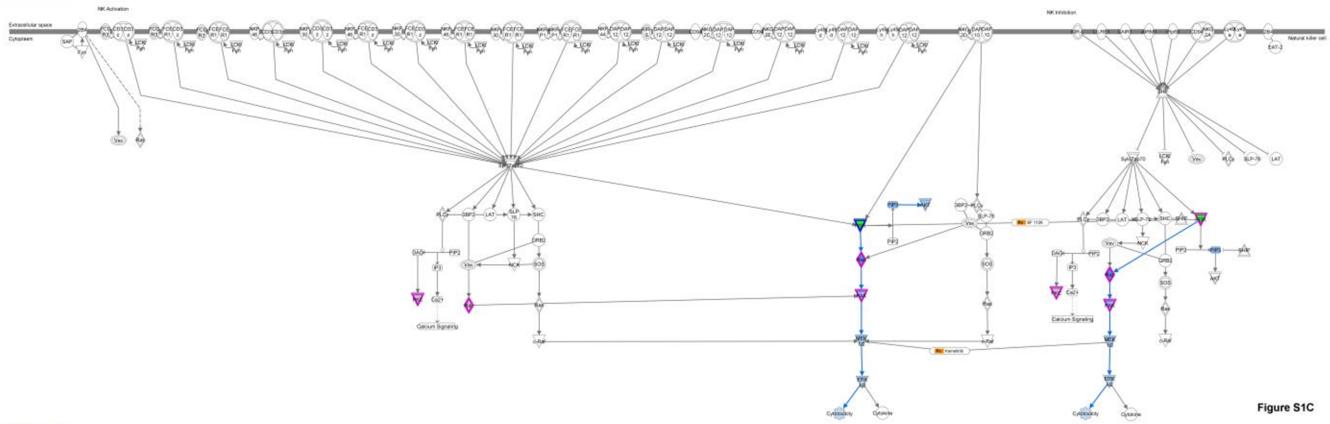

Figure S1C

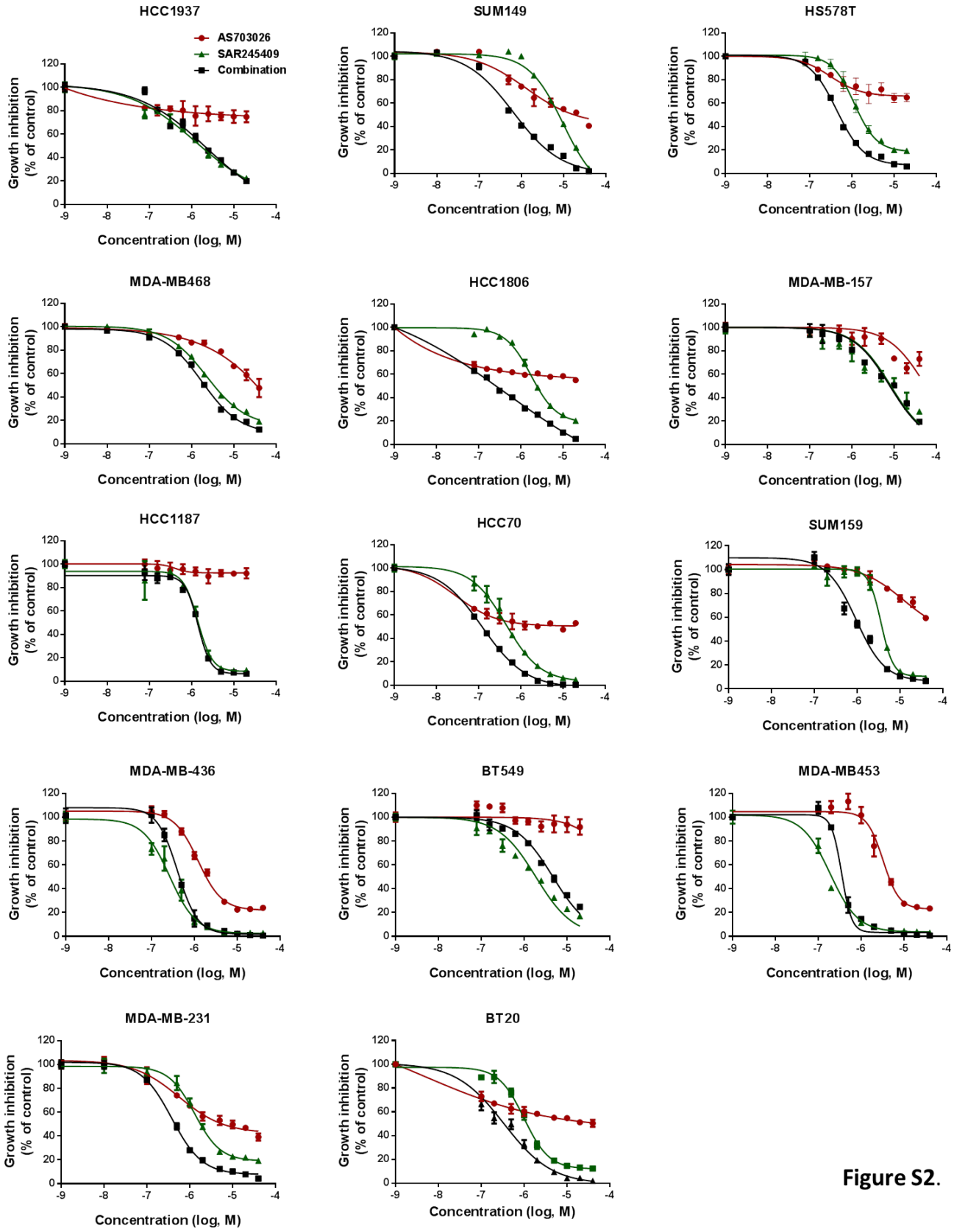
HCC1937
AS703026
SAR245409
Combination
SUM149
HS578T
MDA-MB468
HCC1806
MDA-MB-157
HCC1187
HCC70
SUM159
MDA-MB-436
BT549
MDA-MB453
MDA-MB-231
BT20
Growth inhibition (% of control)
Concentration (log, M)

Figure S2.

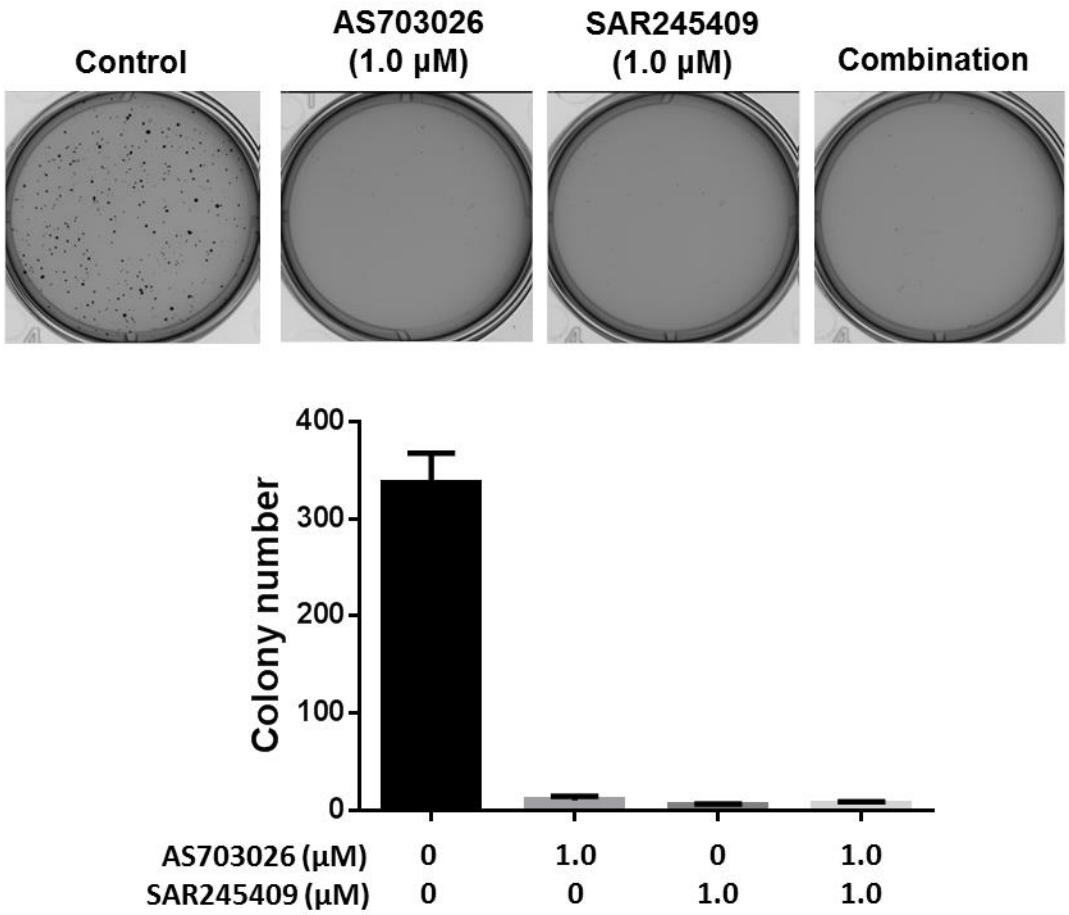
Control
AS703026
(1.0 μM)
SAR245409
(1.0 μM)
Combination
Colony number
400
300
200
100
0
AS703026 (μM) 0 1.0 0 1.0
SAR245409 (μM) 0 0 1.0 1.0

Figure S3.

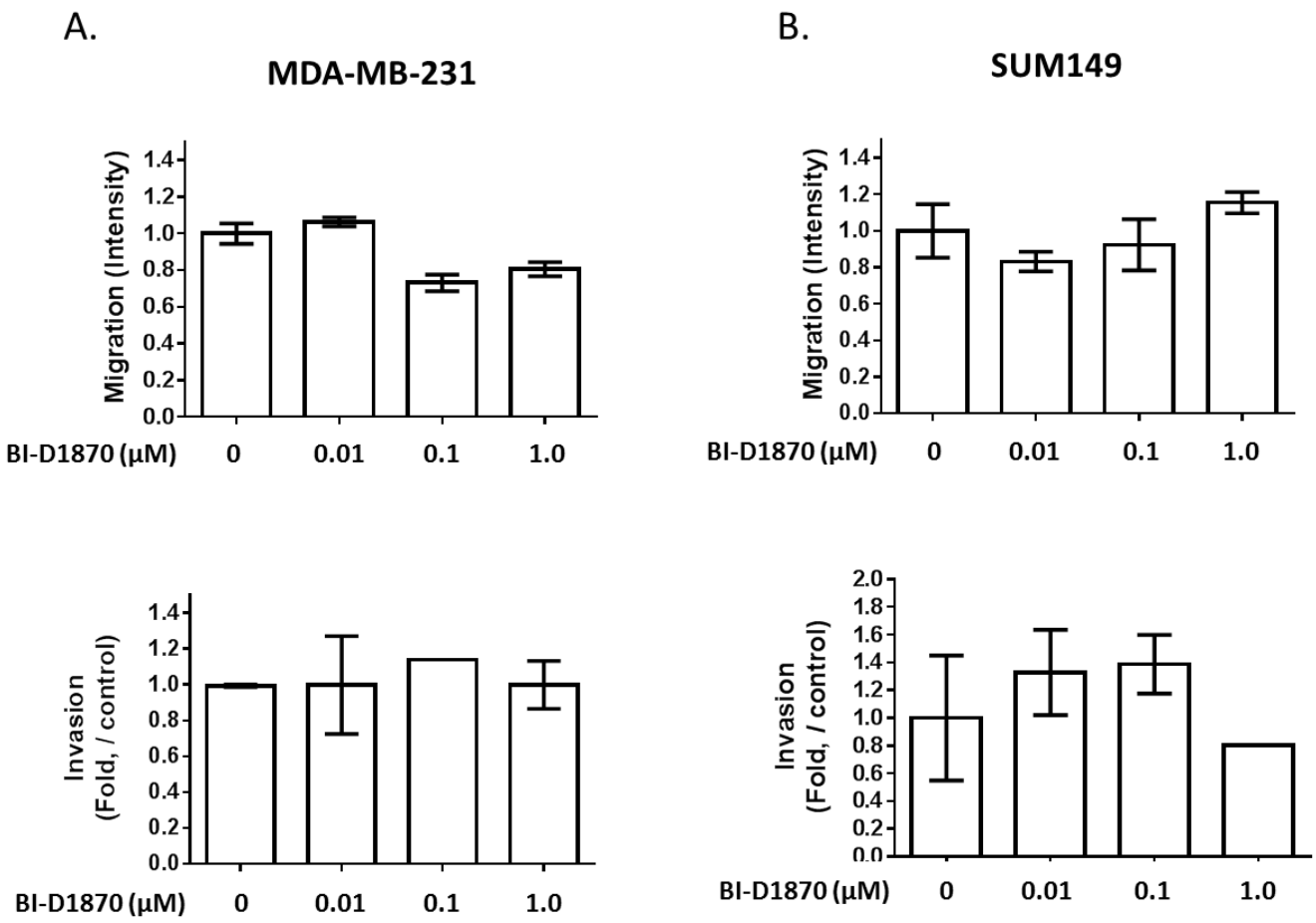
A.
MDA-MB-231
Migration (Intensity)
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
BI-D1870 (μM) 0 0.01 0.1 1.0
Invasion (Fold, / control)
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
BI-D1870 (μM) 0 0.01 0.1 1.0
B.
SUM149
Migration (Intensity)
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
BI-D1870 (μM) 0 0.01 0.1 1.0
Invasion (Fold, / control)
2.0
1.8
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
BI-D1870 (μM) 0 0.01 0.1 1.0

Figure S4.