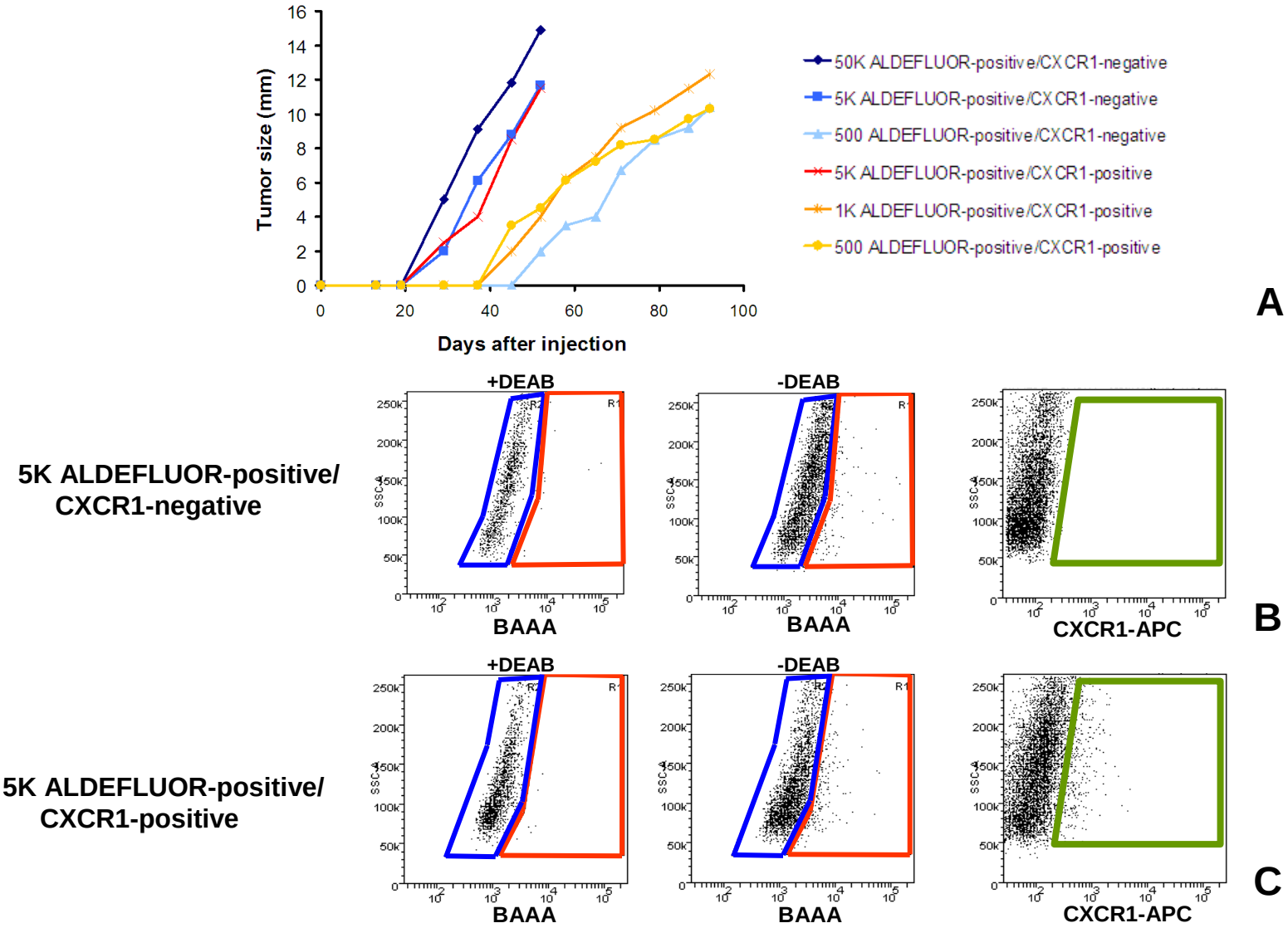
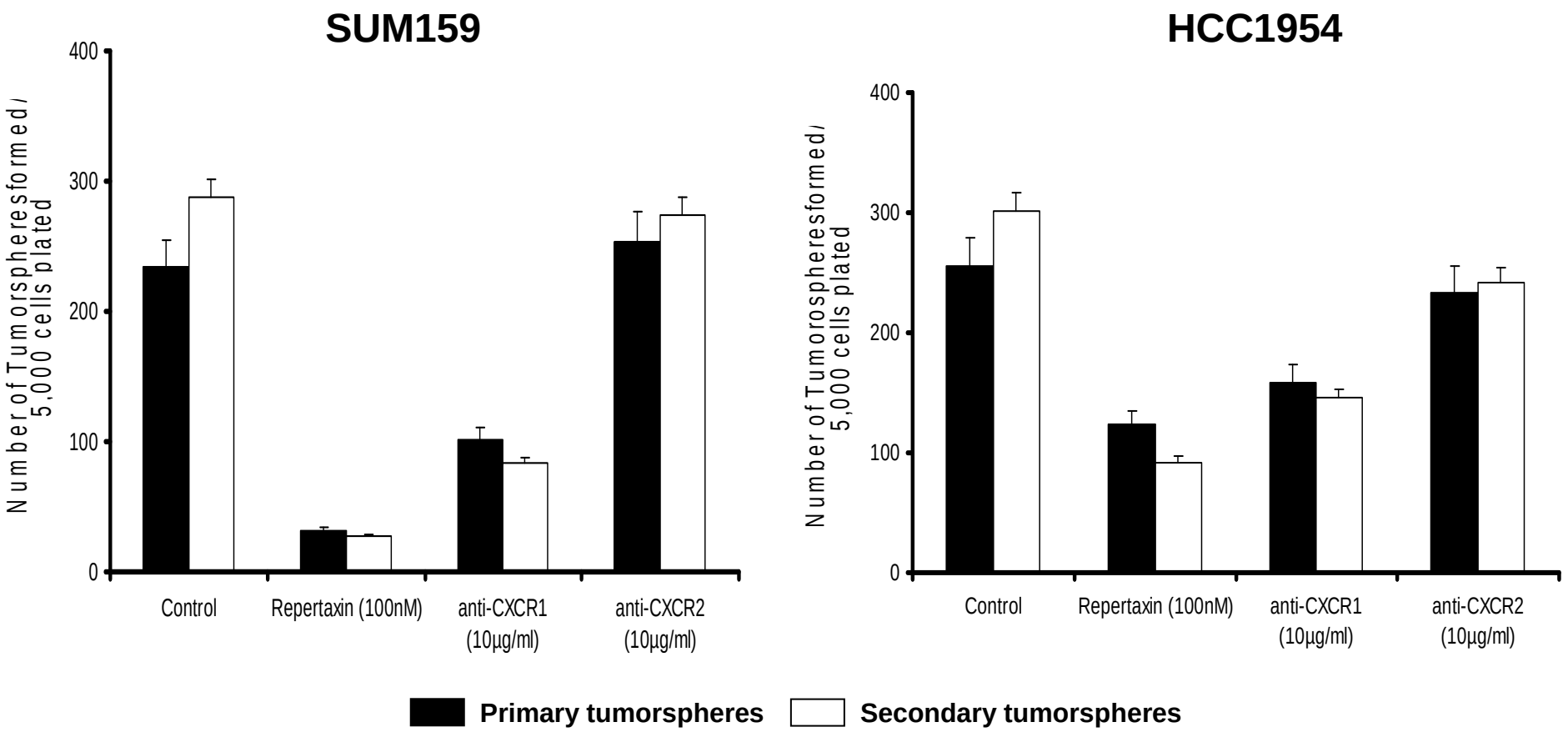
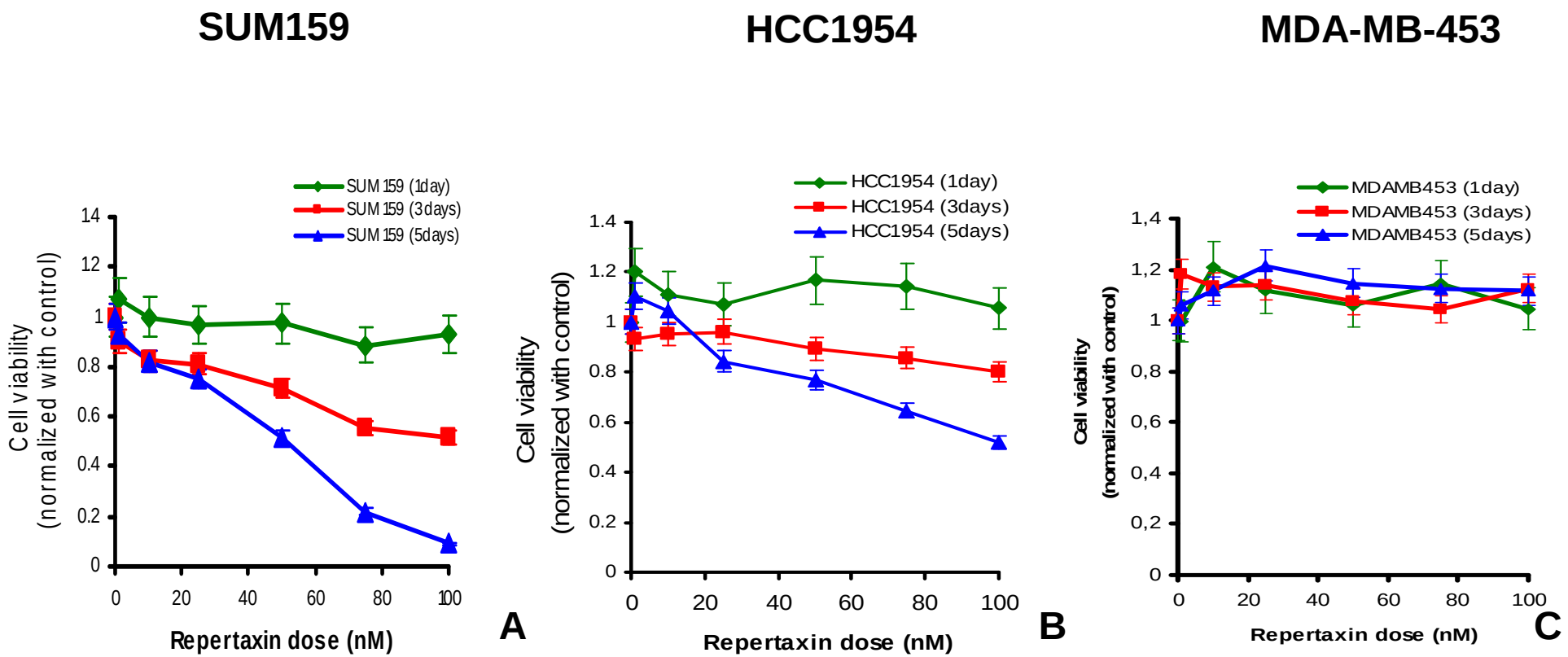


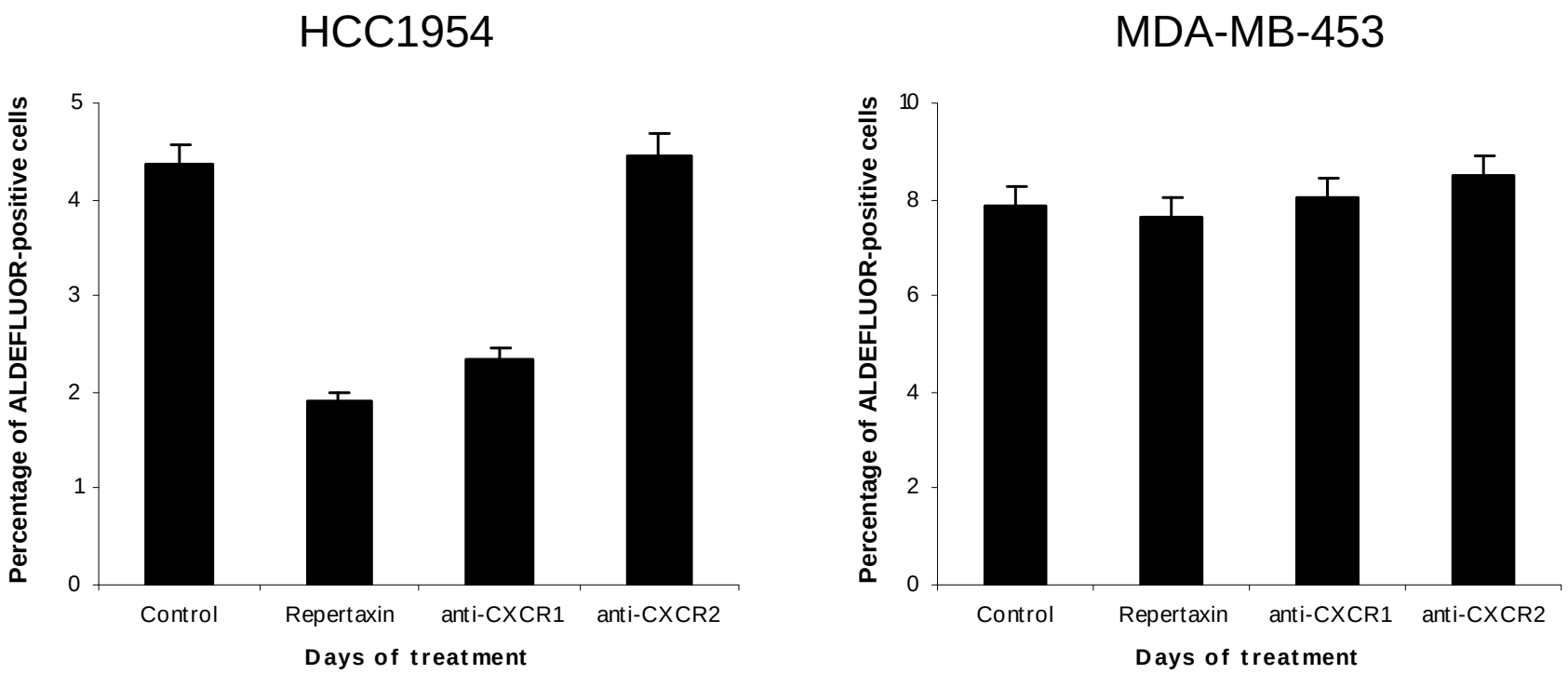
Supplemental Figure 1. Tumorigenicity of the ALDEFLUOR-positive/CXCR1-positive and ALDEFLUOR-positive/CXCR1-negative cell populations from SUM159 cell line. **A.** Tumor growth curves were plotted for different numbers of cells injected (50,000 cells, 5,000 cells, 1,000 cells, and 500 cells) and for each population (ALDEFLUOR-positive/CXCR1-positive, ALDEFLUOR-positive/CXCR1-negative). Both cell populations generated tumors. Tumor growth kinetics correlated with the latency and size of tumor formation and the number of cells injected. **B-C**. Tumors generated by the ALDEFLUOR-positive/CXCR1-positive population reconstituted the phenotypic heterogeneity of the initial tumor upon serial passages whereas the ALDEFLUOR-positive/CXCR1-negative population gave rise to tumors containing only ALDEFLUOR-positive/CXCR1-negative cells. We transplanted both cell population for three passages. Data represent mean ± SD.



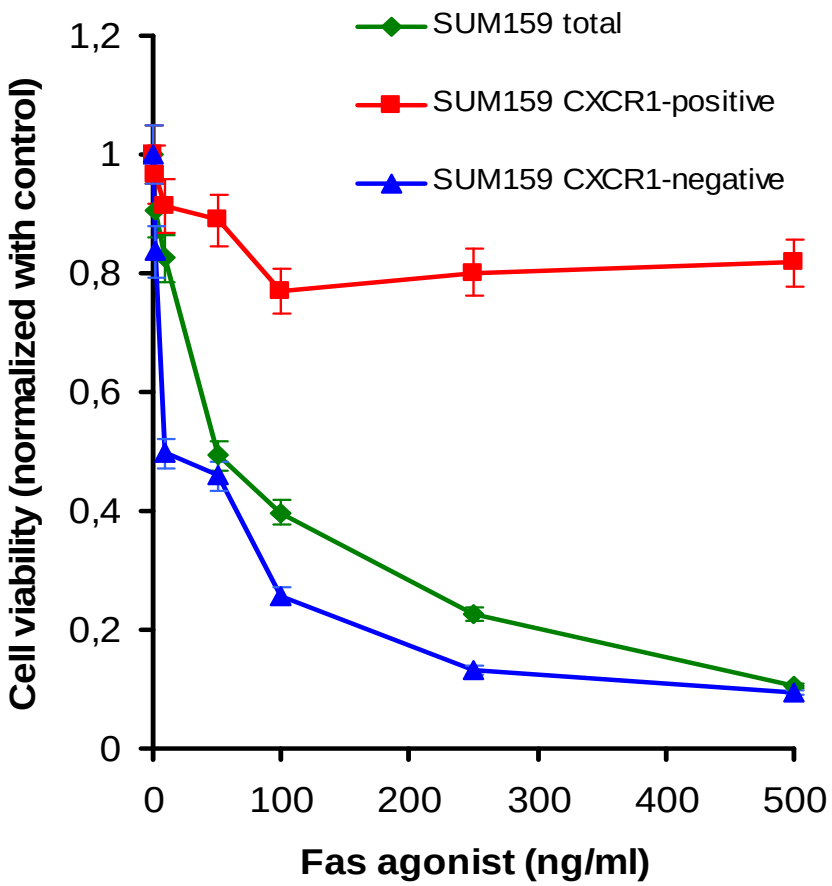
Supplemental Figure 2. Effect of CXCR1 blockade on tumorsphere formation. SUM159 and HCC1954 cells were cultured in adherent conditions and treated for three days with repertaxin (100nM), an anti-CXCR1 blocking antibody (10µg/ml), or an anti-CXCR2 blocking antibody (10µg/ml). After three days of treatment, cells were detached and cultured in suspension. The number of tumorspheres formed after 5 days of culture were evaluated. Similar results were observed for the both cell lines with a significant decrease in primary and secondary tumorosphere formation in the repertaxin and anti CXCR1-treated conditions compared to controls. In contrast, anti-CXCR2 blocking antibody had no effect on tumorosphere formation. Error bars represent mean ± SD.



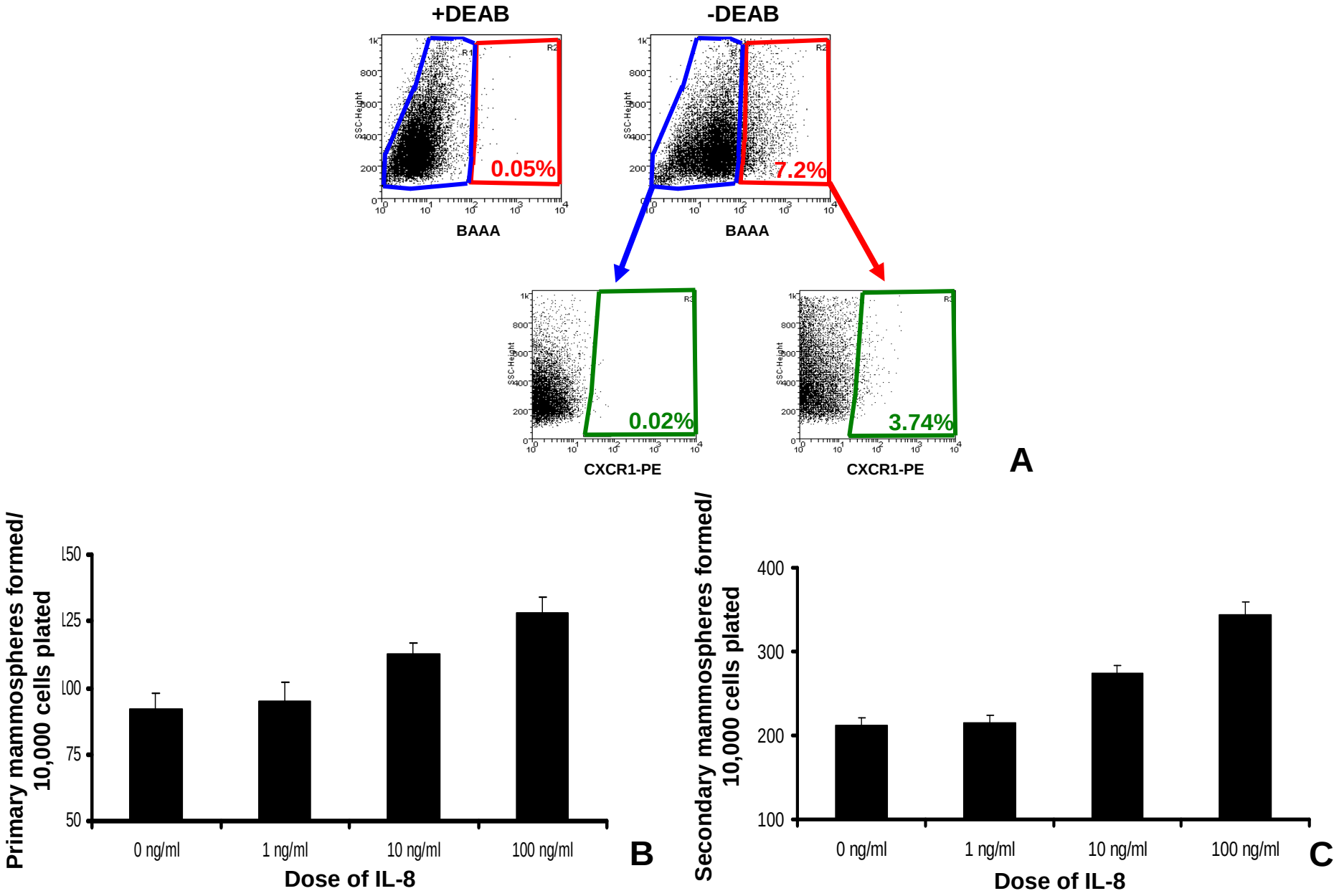
Supplemental Figure 3. Effect of repertaxin treatment on cell viability of SUM159, HCC1954, and MDA-MB-453 cell lines. Three different cell lines (SUM159, HCC1954, MDA-MB-453) were cultured in adherent conditions and treated with repertaxin (100nM). Cell viability was evaluated after one, three, and five days of treatment using the MTT assay. We observed a decrease in cell viability after 3 days of treatment for SUM159 and HCC1954 cell line. However, repertaxin did not effect the viability of MDA-MB 453 cells. Error bars represent mean ± SD.



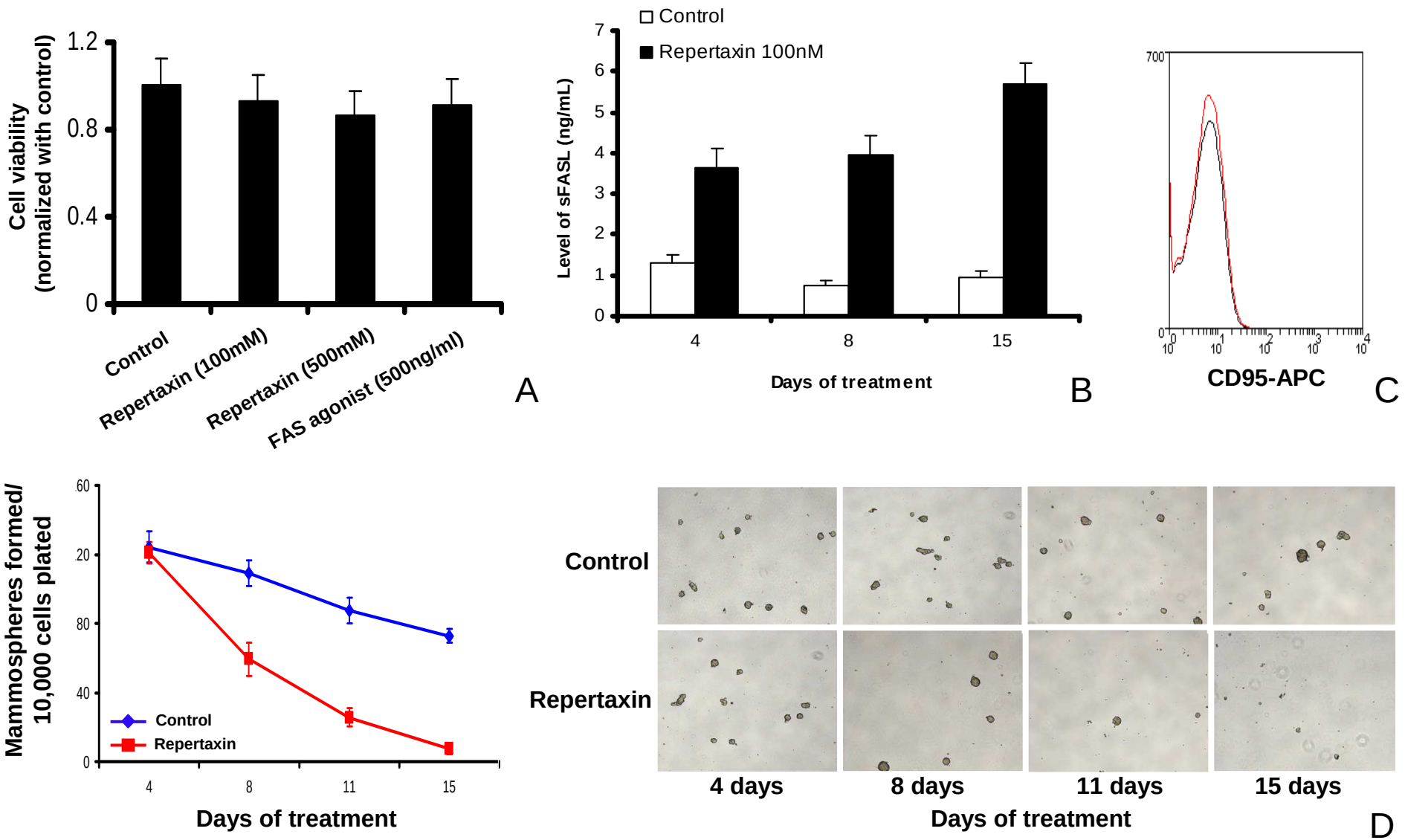
Supplemental Figure 4. Effect of CXCR1 blockade on the ALDEFLUOR-positive population *in vitro*. A-B. HCC1954 (**A**) and MDA-MB-453 (**B**) cells were cultured in adherent conditions and treated with repertaxin (100nM) or two specific blocking antibodies for CXCR1 (10µg/ml) or CXCR2 (10µg/ml). After three days, the effect on the cancer stem cell population was analyzed using the ALDEFLUOR assay. For HCC1954, a significant reduction of the ALDEFLUOR-positive population and cell viability was observed following treatment with repertaxin or anti-CXCR1 antibody. In contrast no significant effect was observed with anti-CXCR2 antibody (**A**). For MDA-MB-453, we did not observe any effect on the ALDEFLUOR-positive population (**B**). Error bars represent mean ± SD.



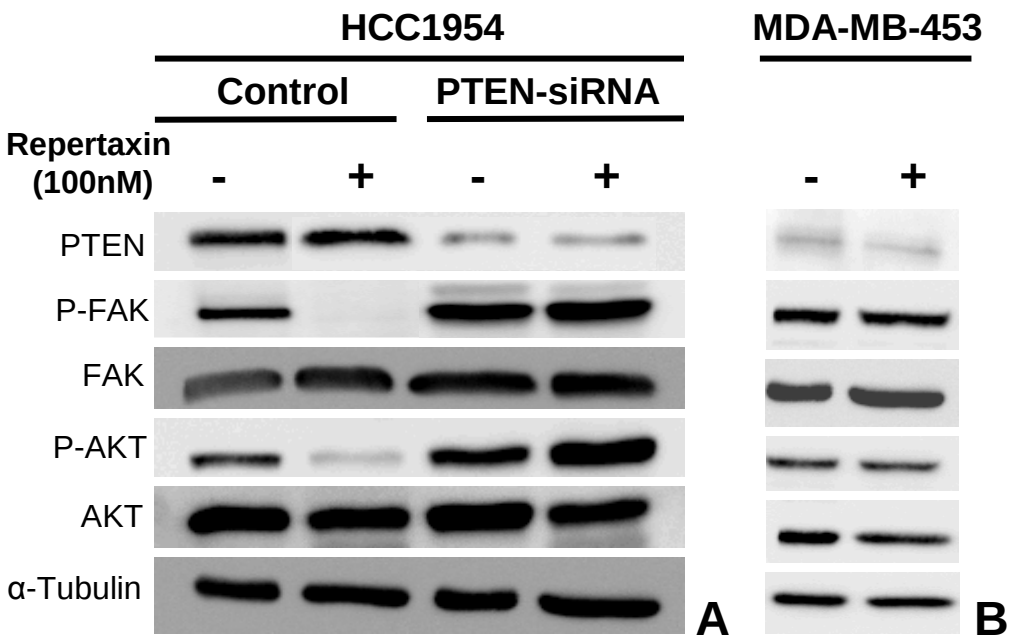
Supplemental Figure 5. Effect of FAS agonist on CXCR1-positive and CXCR1-negative cells. To determine whether cell death was mediated via a bystander effect FAS induced. CXCR1-positive and CXCR1-negative populations were flow sorted and each population treated with various concentrations of FAS agonist. A decrease in cell viability in CXCR1-negative and unsorted populations were detected whereas no effect was observed in the CXCR1-positive population**.** Error bars represent mean ± SD.



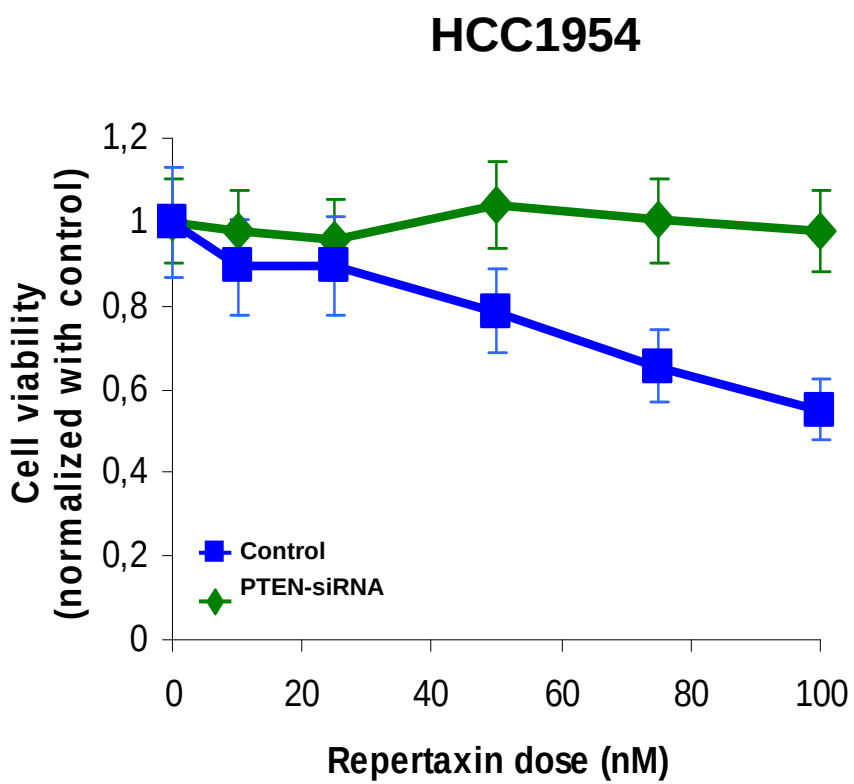
Supplemental Figure 6. Analysis of CXCR1 protein expression in the normal breast stem/progenitor population and effect of IL-8 treatment on mammosphere formation. A. The ALDEFLUOR-positive and -negative population from normal breast epithelial cells isolated form reduction mammoplasties was isolated by FACS, fixed, and analyzed for the expression of CXCR1 protein by immunostaining and FACS analysis. ALDEFLUOR-positive cells were highly enriched in CXCR1-positive cells compared to the ALDEFLUOR-negative population. **B-C.** Effect of IL8 treatment on mammosphere formation. IL8 treatment increased the formation of primary (**B**) and secondary mammospheres (**C**) in a dose-dependent manner. Error bars represent mean ± SD.



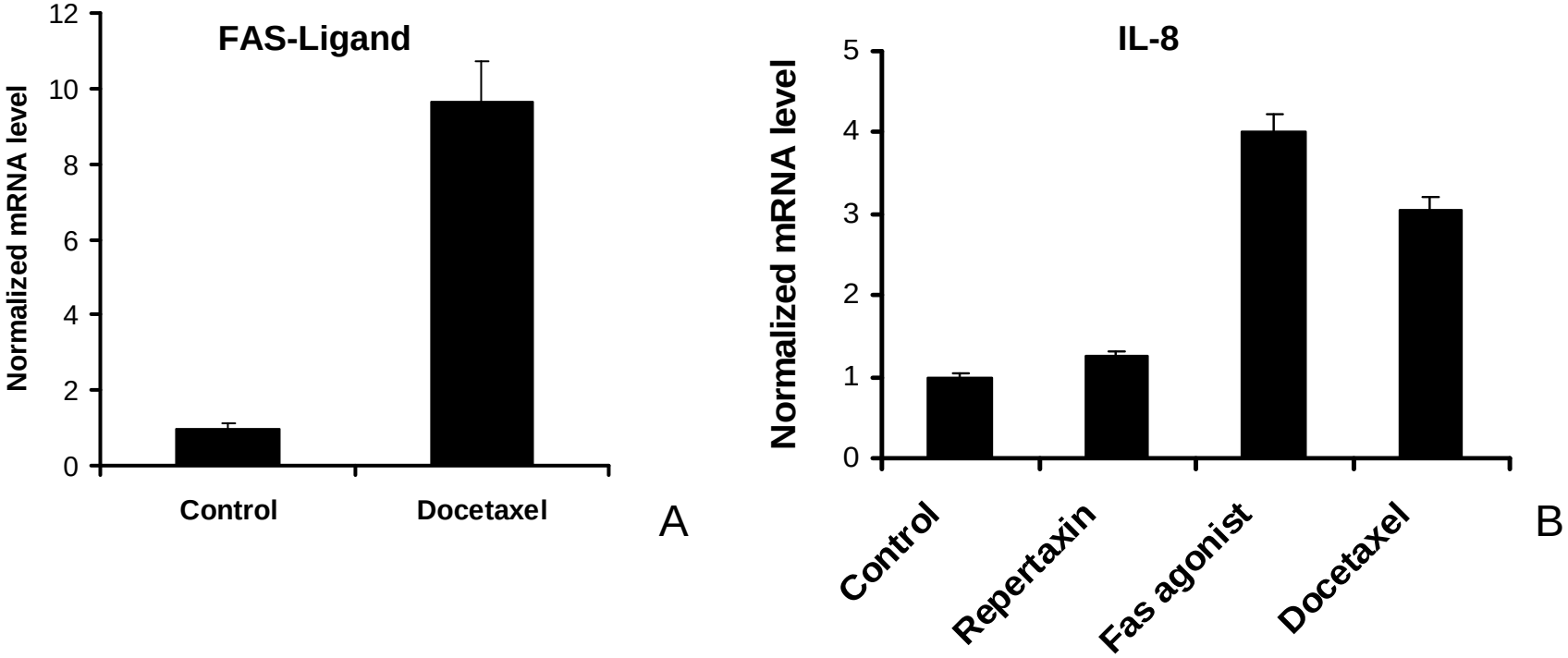
Supplemental Figure 7. Effect of repertaxin treatment on the normal mammary epithelial cells. A. Normal mammary epithelial cells isolated from reduction mammoplasties were cultured in adherent condition and treated with repertaxin (100nM or 500nM) or FAS agonist (500ng/ml). After five days of treatment cell viability was evaluated using MTT assay. Repertaxin treatment or the FAS agonist had no effect on the viability of normal mammary epithelial cells cultured in adherent conditions, even when high concentrations of repertaxin (500nM) were utilized. **B.** The level of soluble FAS-ligand was evaluated by Elisa assay in the medium of normal mammary epithelial cells treated with repertaxin. After 4 days of treatment an increase of soluble FAS-ligand was detected in the medium from treated cells. **C.** Analysis of FAS/CD95 expression in the normal mammary epithelial cells by FACS analysis. No FAS/CD95 expression was detected in the normal mammary epithelial cells cultured in adherent condition. **D.** Effect of repertaxin treatment on mammosphere formation. Normal mammary epithelial cells were cultured in adherent condition and treated during four, eight, eleven and fifteen days with repertaxin (100nM). After repertaxin treatment cells were detached and cultured in suspension. A significant decrease of mammosphere-initiating cells was observed in the repertaxin-treated condition. Error bars represent mean ± SD.



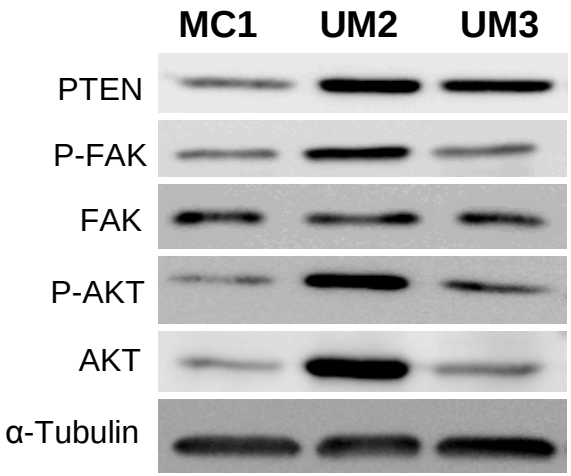
Supplemental Figure 8. Effect of repertaxin treatment on FAK/AKT activation in HCC1954 and MDA-MB-453 cell lines. To evaluate the effect of repertaxin treatment on CXCR1 downstream signaling we utilized a lentiviral construct knocking down PTEN expression via a PTEN-siRNA **A.** HCC1954 control and HCC1954 PTEN-siRNA cells were cultured in adherent conditions for two days in the absence or presence of 100nM repertaxin and the activation of the FAK/AKT pathway was accessed by western blotting. Repertaxin treatment led to a decrease in FAK Tyr397 and AKT Ser473 phosphorylation whereas PTEN deletion blocked the effect of repertaxin treatment on FAK and AKT activity. **B.** Repertaxin treatment did not have any effect on cell viability of MDA-MB-453 cell line wich harbor PTEN mutation. Utilizing western blot analysis we confirmed that FAK/AKT pathway was not perturbated by repertaxin treatment.



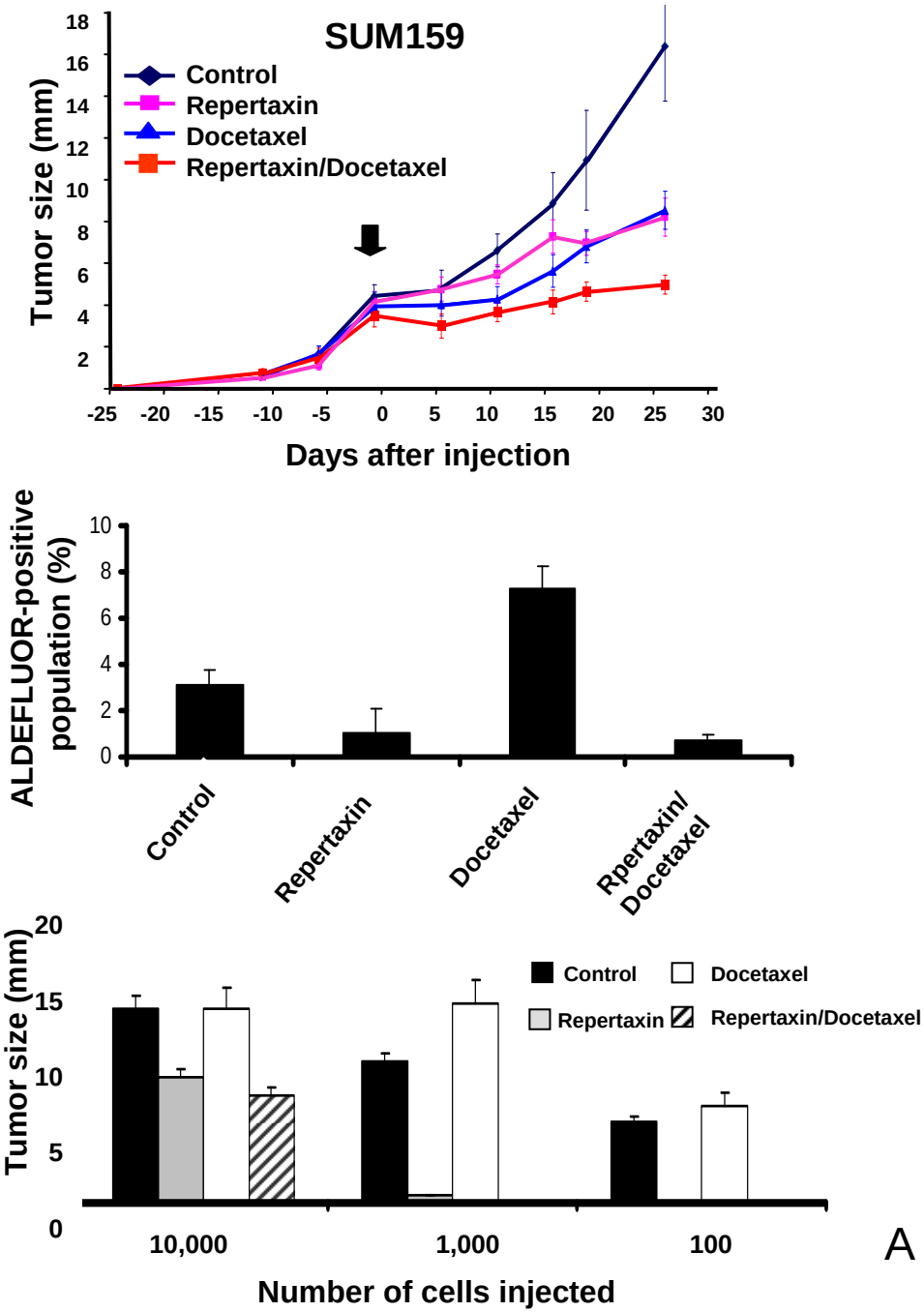
Supplemental Figure 9. The effect of repertaxin on the HCC1954 PTEN-siRNA cell viability was assessed utilizing the MTT assay. After 3 days of treatment, cells with PTEN deletion developed resistance to repertaxin. Error bars represent mean ± SD.

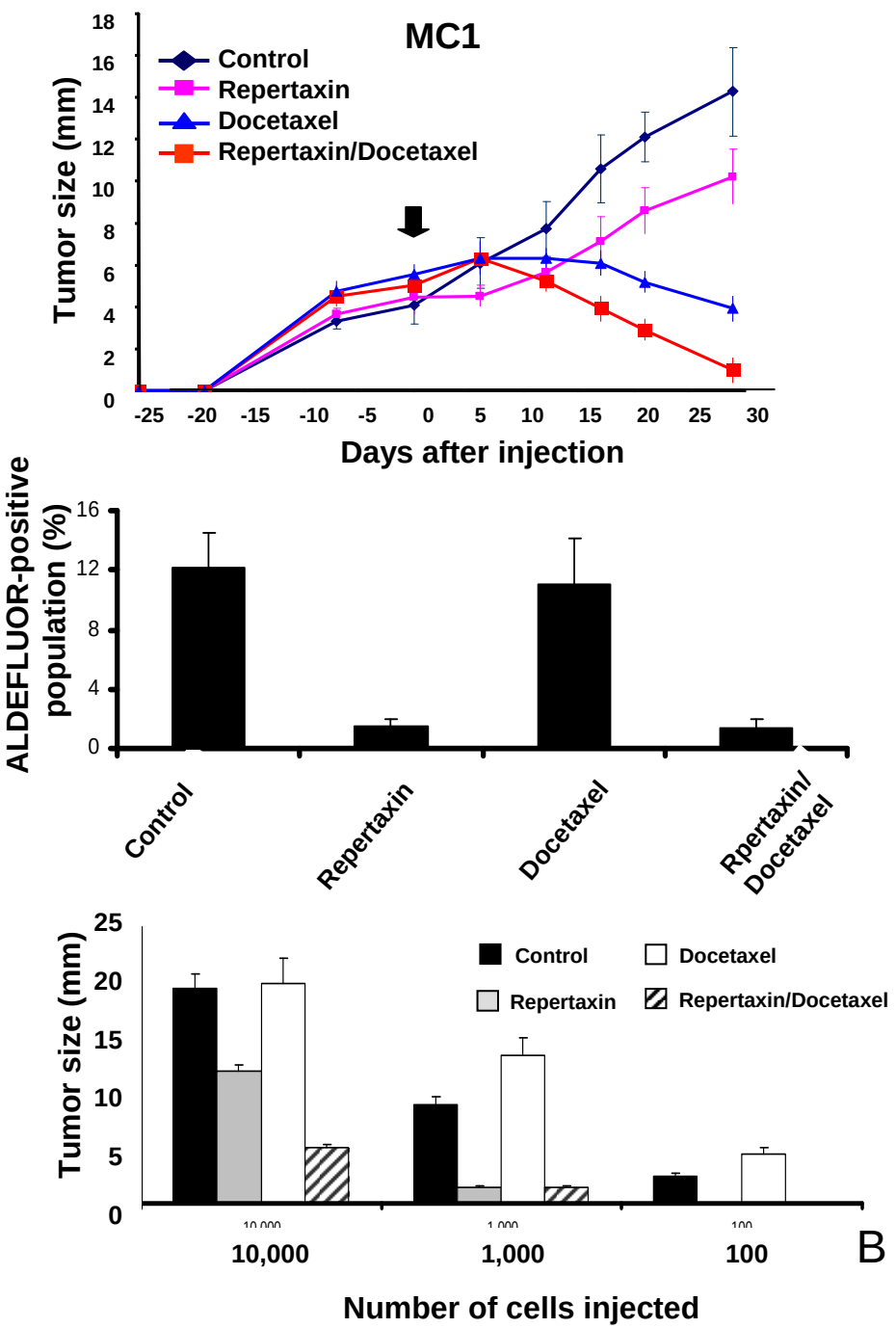


Supplemental Figure 10. Expression of FAS-ligand and IL-8 mRNA after docetaxel or repertaxin treatment measured by quantitative RT-PCR.
A-B. SUM159 cells cultured in adherent condition were treated with repertaxin (100nM), FAS agonist (500ng/ml) or docetaxel (10nM). After three days of treatment cells were collected and RNA extracted. We confirmed that the commonly utilized chemotherapeutic agent, Docetaxel, induced both FAS-ligand (**A**) and IL-8 (**B**) mRNA in SUM159 cells. We also detected a 4-fold increase of IL-8 mRNA level after FAS agonist or docetaxel treatment (**B**). Error bars represent mean ± SD.

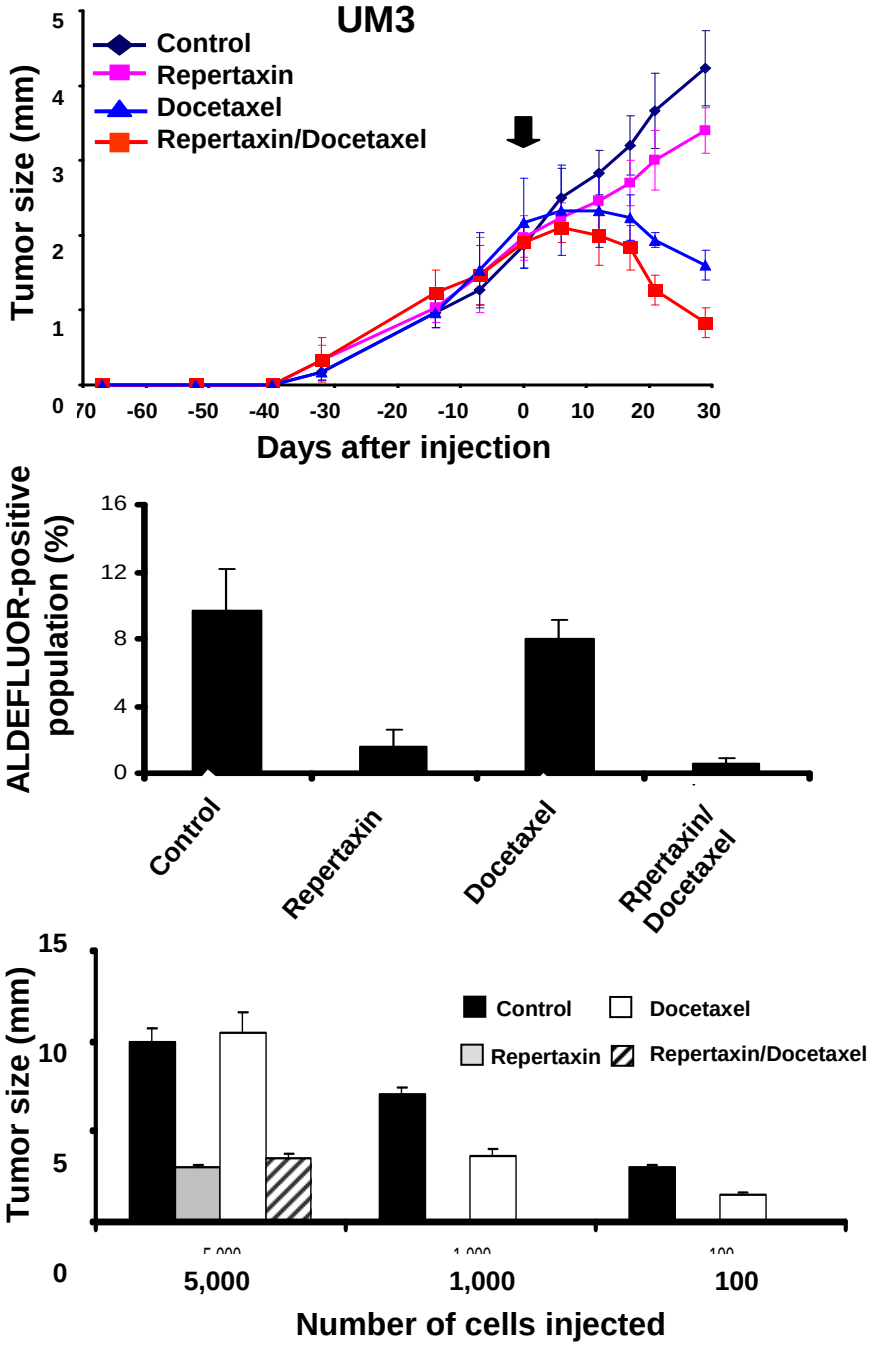


Supplemental Figure 11. Evaluation of PTEN/FAK/AKT activation in the three different breast cancer xenografts. Western blot analysis revealed that both xenografts presented an expression of PTEN and an activation of FAK/AKT pathway as shown by FAK Tyr397 and AKT Ser473 phosphorylation.



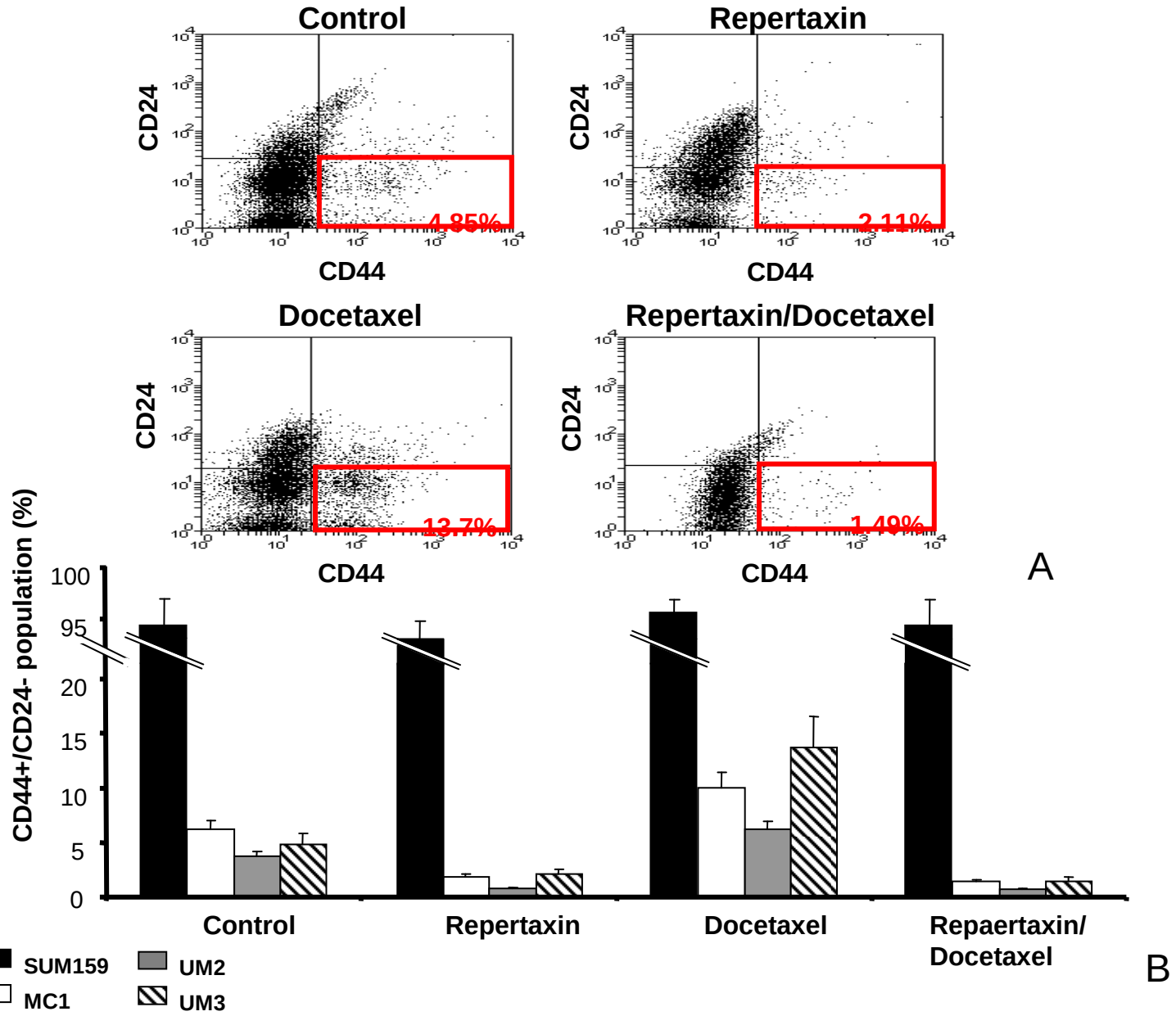


Supplementary Figure 12 (1)



C

Supplemental Figure 12. Effect of Repertaxin treatment on the breast cancer stem cell population *in vivo*. A-C. To evaluate the effect of repertaxin treatment on tumor growth and the cancer stem cell population *in vivo* we utilized a breast cancer cell line (SUM159, **A**) and three human breast cancer xenografts generated from different patients (MC1,**B** ; UM2,**Figure 4** ; UM3,**C**). For each sample, we injected 50,000 cells into the humanized mammary fat pad of NOD/SCID mice and monitored tumor size. When the tumors were about 4 mm, we initiated s.c. injection of repertaxin (15mg/Kg) twice/day for 28 days or once/week I.P. injection of docetaxel (10mg/Kg) or the combination (repertaxin/docetaxel). The graph shows the tumor size before and during the course of each indicated treatment (arrow, beginning of the treatment). Similar results were observed for each sample (**A.** SUM159; **B**. MC1, and **C.** UM3) with a statistically significant reduction of the tumor size in docetaxel alone or the combination repertaxin/docetaxel treated groups compared to the control ($p<0.01$) whereas no difference was observed between the growth of the control tumors and the tumors treated with repertaxin alone. Evaluation of repertaxin, docetaxel, or the combined treatment on the cancer stem cell population was assessed by the ALDEFLUOR assay and by reimplantation into secondary mice. Docetaxel-treated tumor xenografts showed similar or increased percentage of ALDEFLUOR-positive cells compared to the control, whereas repertaxin treatment alone or in combination with docetaxel produced a statistically significant decrease in ALDEFLUOR-positive cells with a 65% to 85% decrease in cancer stem cells compared to the control ($p<0.01$). Serial dilutions of cells obtained from primary tumors, non-treated (control), and treated mice were implanted in the mammary fat pad of secondary NOD/SCID mice which received no further treatment. Control and docetaxel treated primary tumors formed secondary tumors at all dilutions whereas, only higher numbers of cells obtained from primary tumors treated with repertaxin or in combination with docetaxel were able to form tumors. Furthermore, tumor growth was significantly delayed and resulting tumors were significantly smaller in size than the control or docetaxel treated tumors ($p<0.01$). Error bars represent mean ± SD.



Supplemental Figure 13. Effect of repertaxin treatment on the breast cancer stem cell population as assessed by the CD44+/CD24- phenotype. A-B. Evaluation of repertaxin, docetaxel, or the combined treatment on the cancer stem cell population were assessed by the presence of CD44+/CD24- cells. In residual tumors treated with docetaxel alone, we consistently observed either an unchanged or increased percent of CD44+/CD24- cells whereas repertaxin treatment alone or in combination with docetaxel resulted in a reduction of the CD44+/CD24- cell population. **A.** Flow chart analysis for UM3 xenograft is presented. **B.** Similar results were observed for MC1, UM2, and UM3. Almost all of SUM159 cells are CD44+/CD24- under all treatment conditions. Error bars represent mean ± SD.

Supplemental Table 1.

	ALDEFLUOR (%)	CXCR1 (%)	Overlap CXCR1/ALDEFLUOR (%)
Breast cancer cell lines			
HCC1954	3.42	1.72	0.94
MDA-MB-453	4.22	0.8	0.5
SUM159	5.24	0.52	0.48
Human breast cancer xenografts			
MC1	12.3	1.81	1.32
UM2	8.4	1.23	0.88
UM3	9.7	0.84	0.76

Supplemental Table 2. Overlap between CD24-/CD44+ cells and CXCR1-positive cells in human breast cancer xenografts.

	$CD24^-/CD44^+$ (%)	CXCR1 (%)	Overlap $CD24^-/CD44^+/CXCR1^+$ (%)
Human breast cancer xenografts			
MC1	6.8	1.8	0.5
UM2	3.7	1.2	0.3
UM3	4.8	0.8	0.2

Supplemental Table 3.

	Tumors/Injections						
	number of cells injected						
	10,000	5,000	2,500	1,000	500	250	100
Control	6/6	2/2	---	8/8	---	---	6/8
Repertaxin	4/4	2/2	2/2	4/8	1/3	0/2	0/9
Docetaxel	2/2	4/4	2/2	6/6	3/4	2/3	8/9
Repertaxin/Docetaxel	2/2	3/4	2/2	1/6	1/4	0/4	0/9