SUPPLEMENTAL DATA

Inhibition of TGFβ enhances chemotherapy action against triple negative breast cancer by abrogation of cancer stem cells and interleukin-8

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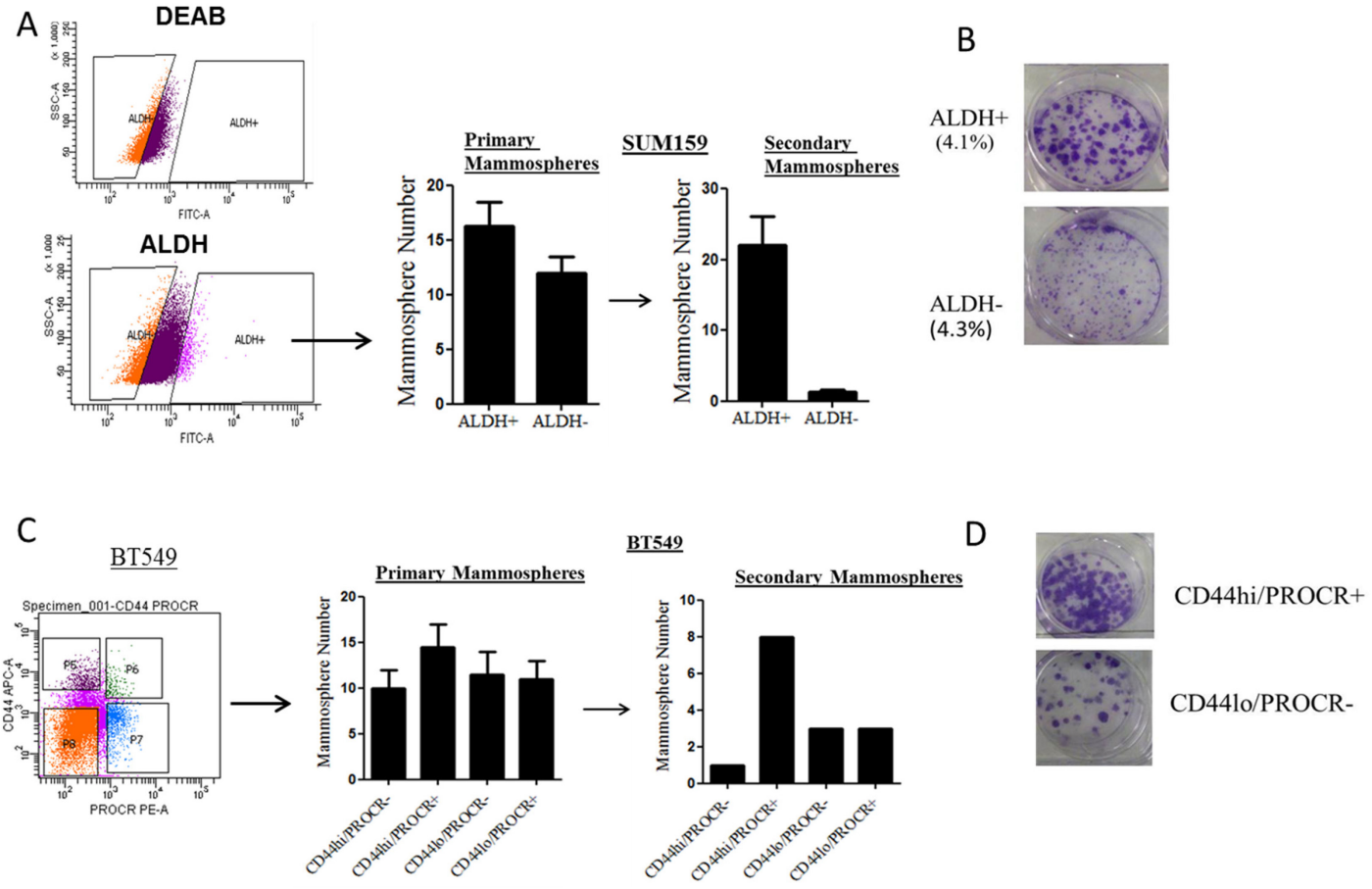
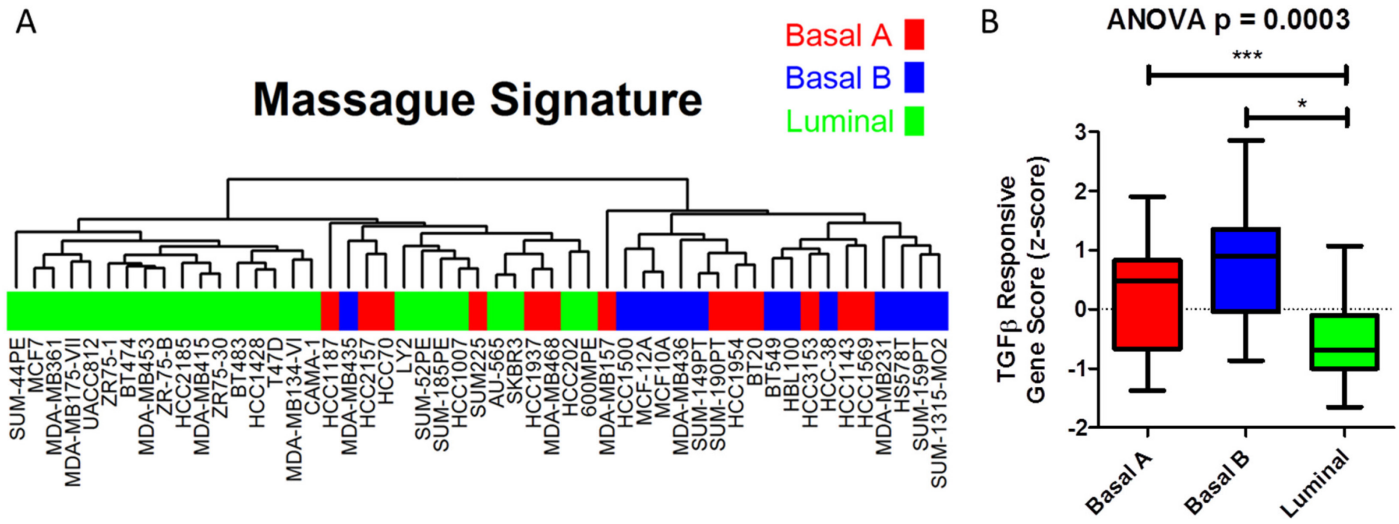
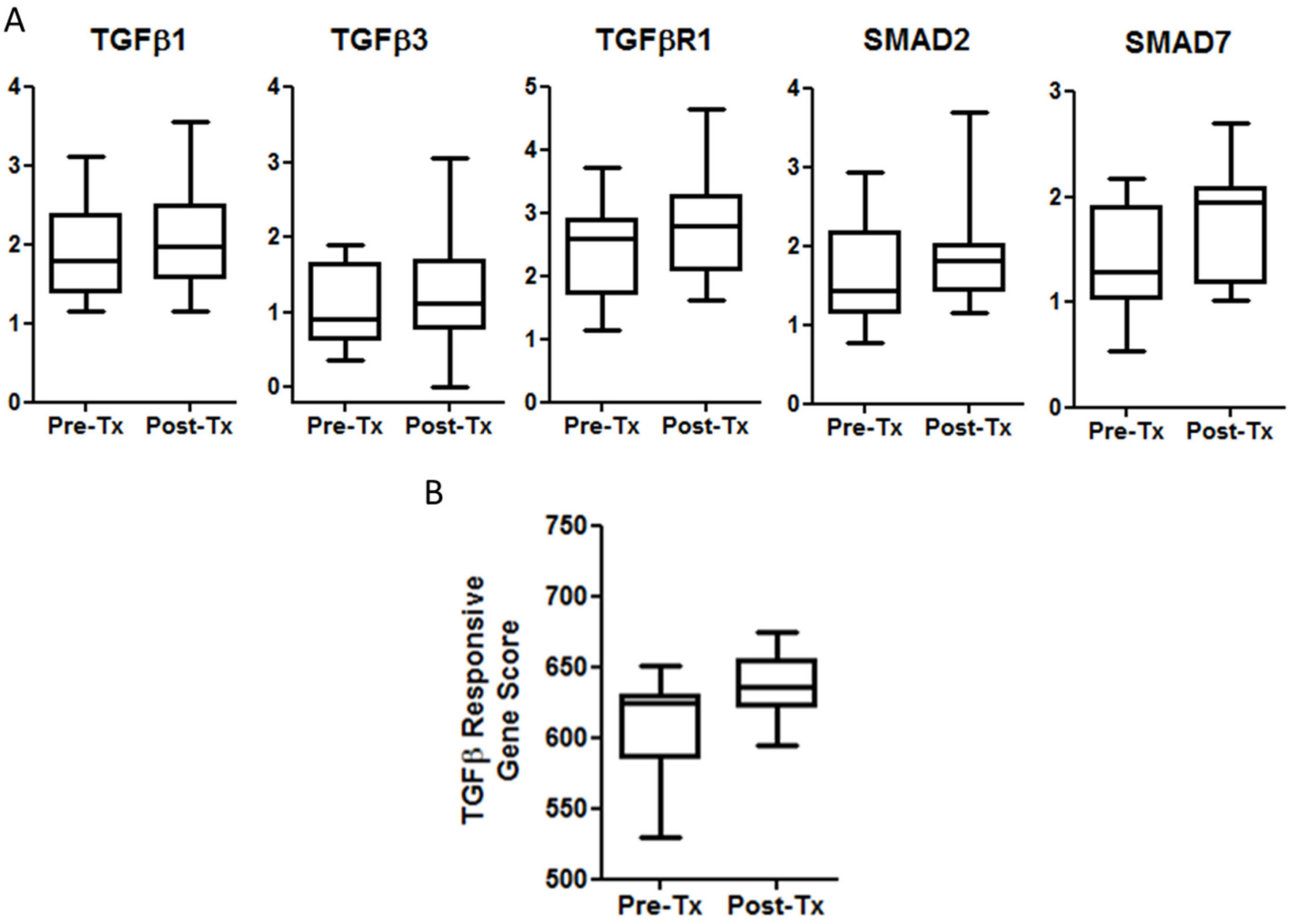
SUPPLEMENTAL FIGURES



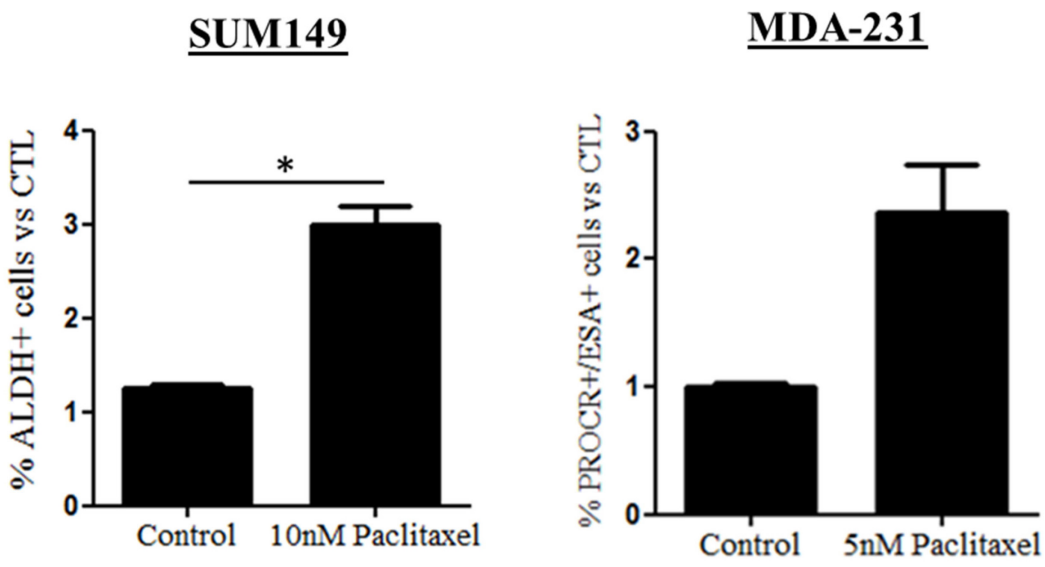
Supplemental Figure 1. Validation of CSC markers in SUM159 and BT549 cells. (A) ALDH+ and ALDH- fractions from SUM159 cells were FACS-sorted and cultured as mammospheres (primary and secondary) in 6-well ultra-low adherent plates (n=3). Diethylaminobenzaldehyde (DEAB) was used to inhibit ALDH and used to set the gate for ALDH+ and ALDH- cells. (B) SUM159 FACS-sorted ALDH+ and ALDH- cells were cultured (1,000 cells/well in triplicate) in 6-well plates. Cells were cultured for 14 days. Colonies were washed with PBS and stained with crystal violet (C) CD44+ and PROCR+ fractions of BT549 cells were FACS-sorted and cultured as primary and secondary mammospheres (n=3) (D) CD44hi/PROCR+ and CD44lo/PROCR- cells (1,000 cells/well in triplicate) were cultured in 6-well plates for 14 days. Colonies were washed with PBS and stained with crystal violet.



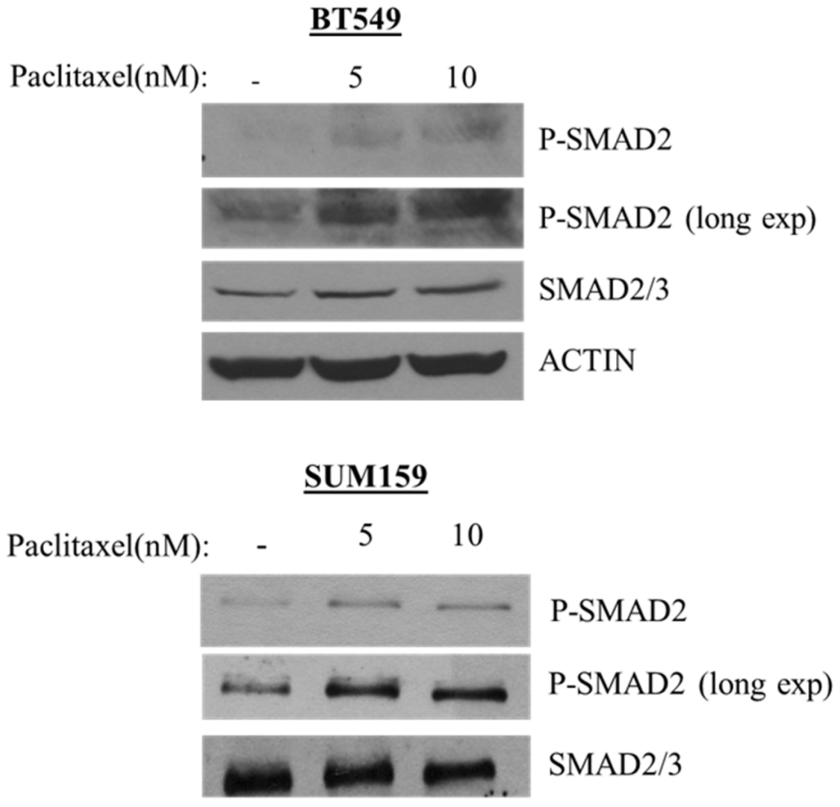
Supplemental Figure 2. TGFβ activation signature marks human breast cancer cell lines with basal-like gene expression. A) A TGFβ gene signature generated in TGFβ-stimulated-breast cancer cells was used to cluster breast cancer cell lines as described in Methods. The signature separated breast cancer cell lines of basal-like vs. luminal subtype. B) Plot of the Z-normalized signature scores according to breast cancer subtype. The TGFβ gene signature was enriched in basal-like breast cancer cell lines, particularly in Basal B cell lines. The difference among all three groups were statistically significant ($p = 0.0003$; ANOVA).



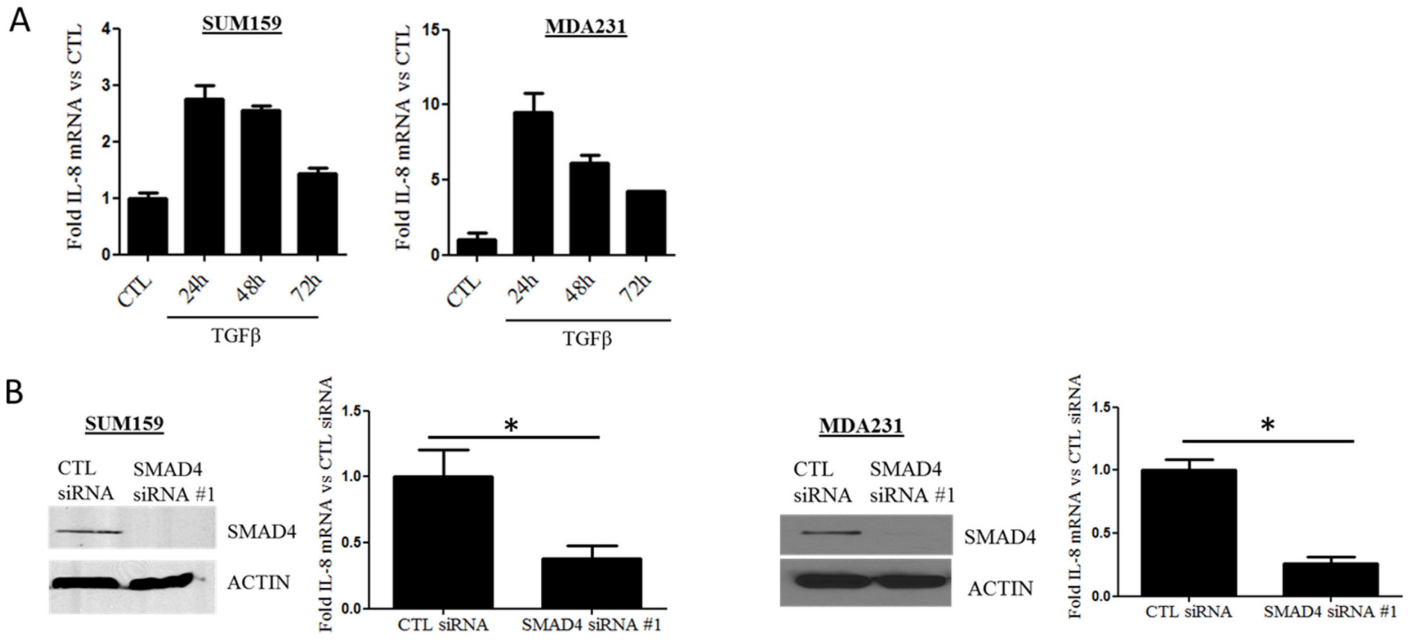
Supplemental Figure 3. TGFβ pathway genes are upregulated following neoadjuvant chemotherapy. (A) NanoString analysis of TGFβ pathway genes in RNA extracted from matched pre- and post-chemotherapy breast cancer biopsies (n=17). Transcript levels of TGFβ1, TGFβ3, TGFβR1, SMAD2 and SMAD7 were compared and are shown as box plots. (B) Gene expression data from pre and post-treatment breast cancer biopsies acquired from a cohort of 21 patients treated with neoadjuvant systemic therapy for 4-6 months was used to generate a TGFβ responsive gene score as indicated in Methods ($p < 0.0001$).



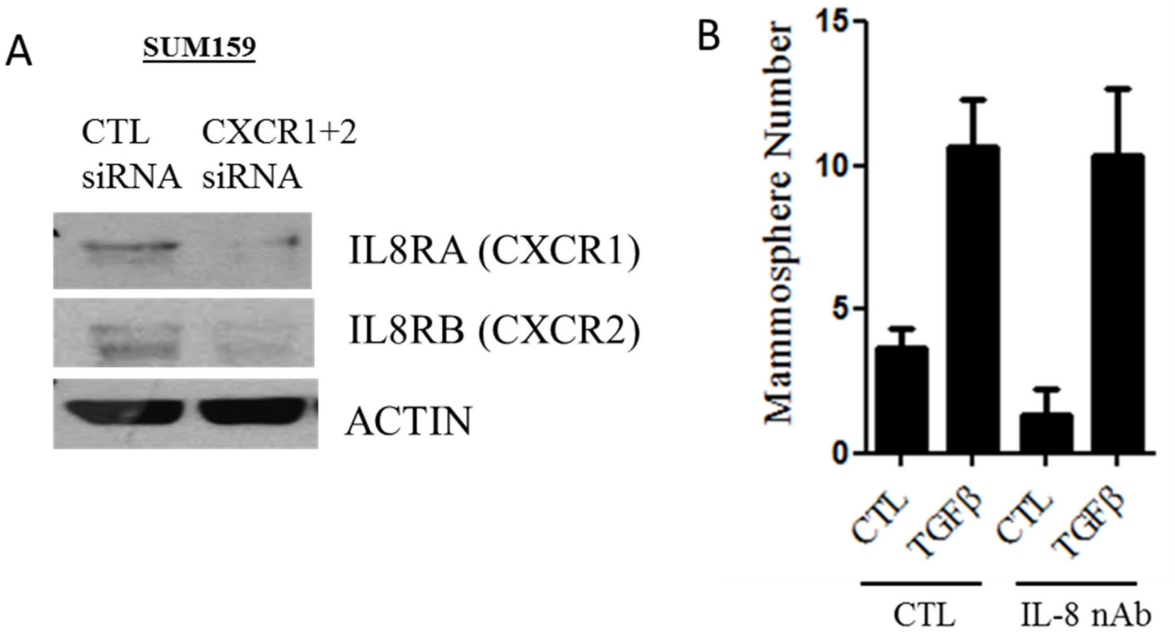
Supplemental Figure 4. Paclitaxel enriches for a CSC population in human basal-like breast cancer cell lines. SUM149 and MDA231 cells were treated with 5 nM paclitaxel for 4 days and allowed to recover in fresh media for another 3 days. Cells were trypsinized and analyzed by FACS for ALDH activity and the PROCR+/ESA+ fraction.



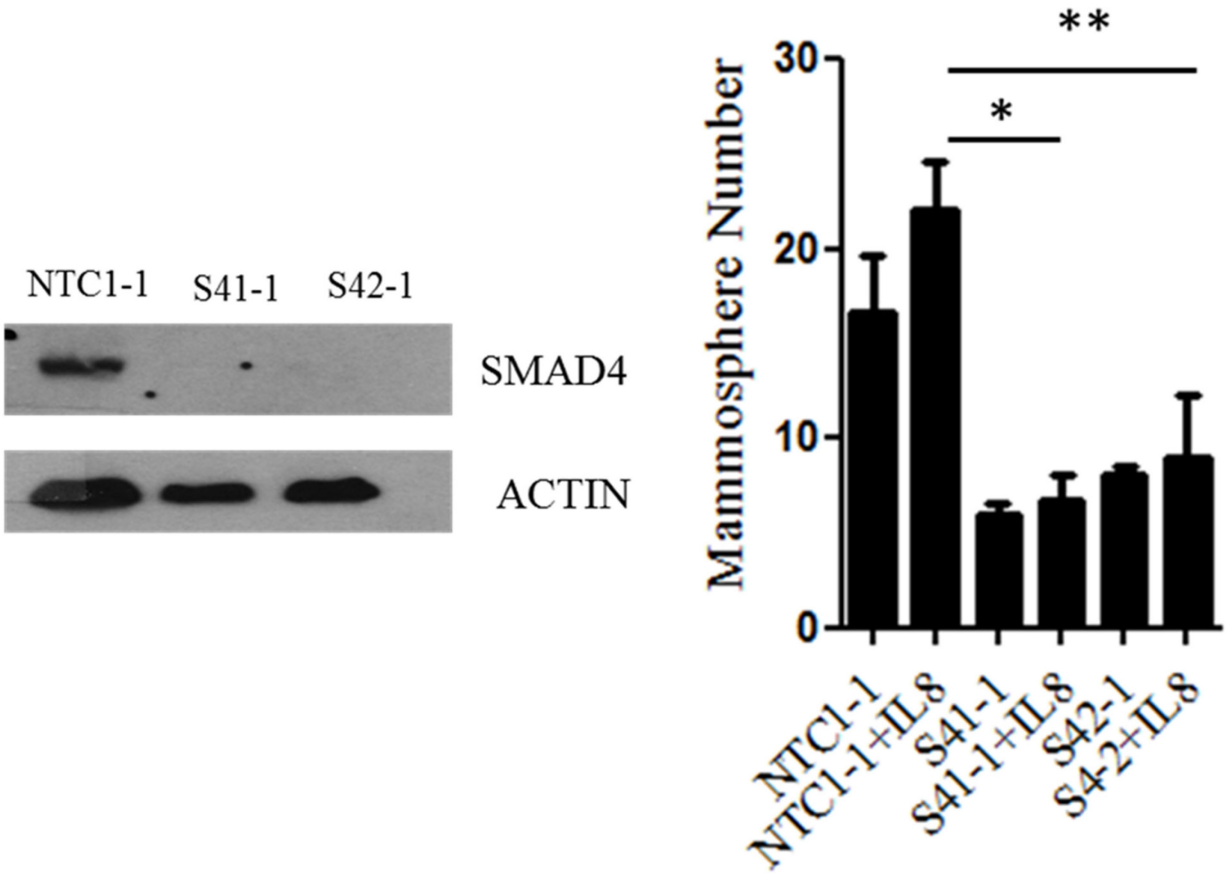
Supplemental Figure 5. SMAD2 phosphorylation is sustained following paclitaxel treatment. SUM159 and BT549 cells were treated with 5-10 nM paclitaxel for 6 days. Cell lysates were prepared and subjected to immunoblot analysis with the indicated antibodies.



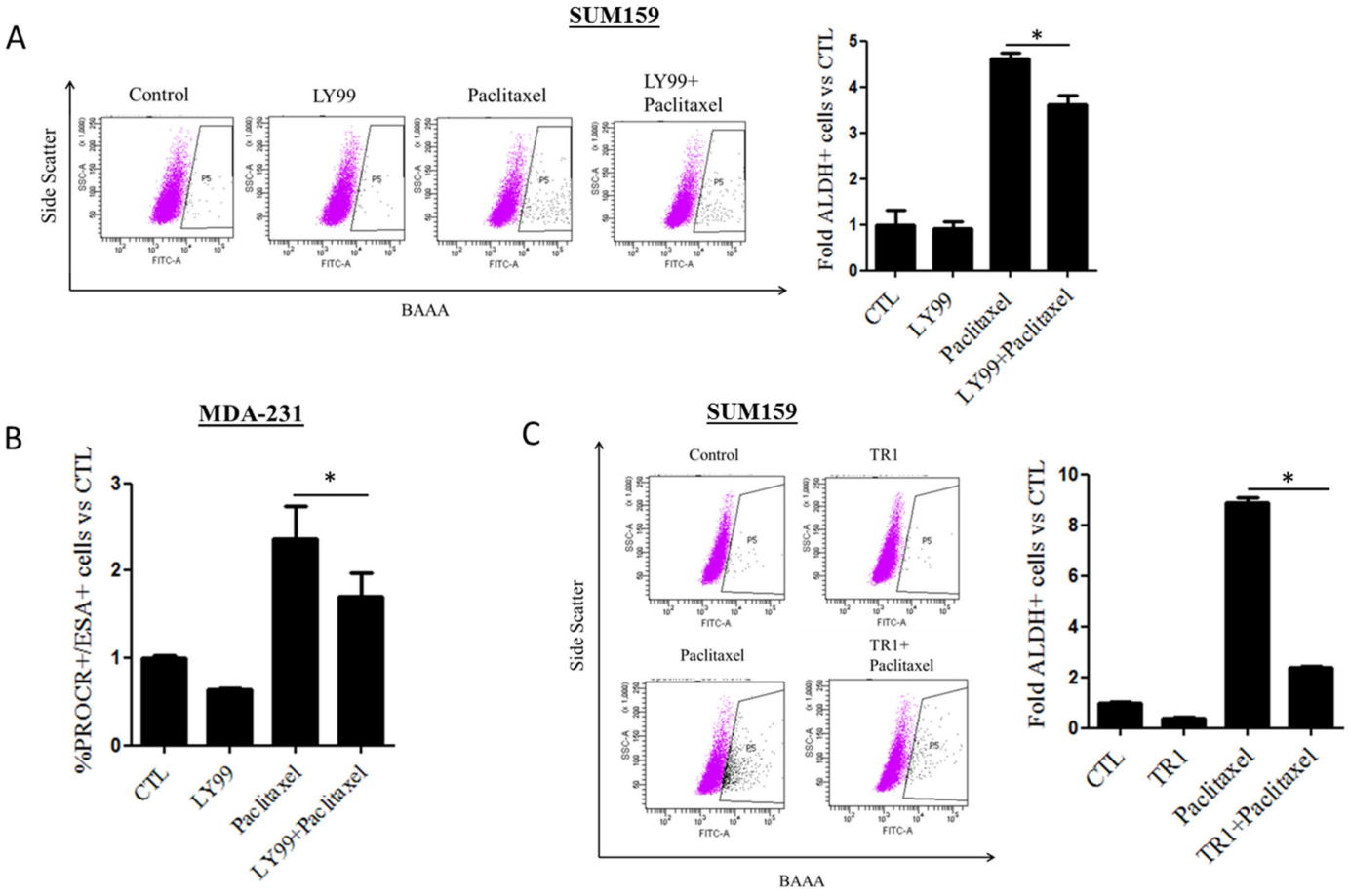
Supplemental Figure 6. Treatment with TGFβ and paclitaxel upregulate IL-8 mRNA levels. (A) SUM159 and MDA231 cells were serum-starved and treated with 2.5 ng/ml TGFβ1 for 24, 48 and 72 h. mRNA was extracted and IL-8 and GAPDH levels measured by RT-qPCR. (B) SUM159 (left) and MDA231 (right) cells were transfected with non-targeting controls (NTC) or SMAD4 siRNA for 72 h. After 72 hours, cells were harvested and their RNA extracted and subjected to RT-qPCR analysis using IL-8 and GAPDH levels (*$p<0.05$). Simultaneously, protein lysates were prepared and subjected to SMAD4 and actin immunoblot analysis to confirm knockdown of SMAD4.



Supplemental Figure 7. Knockdown of IL-8 receptors decreases basal and TGFβ-induced mammosphere formation . (A) SUM159 cells were transfected with control (CTL) or both CXCR1 and CXCR2 siRNA and Knockdown of CXCR1 and CXCR2 by siRNA were verified by immunoblot analysis of cell lysates. (B) SUM159 cells were plated as mammospheres ± 2.5 ng/ml TGFβ1 and 200 ng/ml IL-8 neutralizing antibody for 6 days. Mammosphere number was then quantitated as described in Methods.



Supplemental Figure 8. Exogenous IL-8 modestly rescues decreased mammosphere growth in SMAD4 shRNA-expressing cells. MDA231 cells stably expressing two different SMAD4 shRNA oligonucleotides (S41 and S42) or control shRNA (NTC) was verified for SMAD4 and actin (control) expression by immunoblot analysis (left). NTC, S41-1 and S42-1 cells were cultured as mammospheres in the presence or absence of 100 ng/ml IL-8 for 9 days. Mammosphere number was quantified as described in Methods (*p=0.033).



Supplemental Figure 9. Treatment with TGFβ inhibitors decreases paclitaxel-mediated expansion of CSCs. (A) SUM159 cells were treated with 5 nM Paclitaxel ± 1 μM LY2157299 and for 6 days and assessed for ALDH+ (P5) by FACS ($p < 0.02$). (B) MDA-231 cells were treated with paclitaxel and 5 μM LY2157299 for 6 days; PROCR+/ESA+ cells were assessed by FACS ($p < 0.01$) (C) SUM159 cells were treated with 10 nM paclitaxel ± 200 nM TR1 for 6 days followed by FACS analysis of ALDH+ cells ($p < 0.001$).

Supplemental Table 1. Paclitaxel IC_{50} in SUM159 and BT549 cells

Cell Line	IC_{50} (nM)
SUM159	3.16
MDA231	4.25
BT549	2
SUM149	3.7

SUM159 and BT549 cells were treated with a range of 6 concentrations of paclitaxel (1 nM to 1 μM) for 72 h in serum-containing media. MTT reagent (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) was added after 72 h and incubated for 30 minutes at 37ºC. DMSO was added and following the formation of the formazan product, plates were read at 595 nM using an ELISA plate reader. IC_{50} values were determined using GraphPad Prism Software.