Supplementary Methods

Drug screen

SUM159 cells were seeded into 24-well ultra-low attachment plates (Corning, cat# 3473) at a density of 5,000 cells per well in serum-free DMEM/F-12 medium supplemented with 20 ng/mL basic FGF, 20 ng/mL EGF, 4 μg/mL insulin, 4 μg/mL heparin, 0.5 μg/mL hydrocortisone, 0.4% BSA and B27 (Invitrogen). Twenty-four hours after plating, cells were dosed with a library of 165 small molecule compounds purchased from LKT laboratories (St. Paul, MN) and Selleckchem (Houston, TX) at 5 μM final concentration in triplicates. Mammospheres were counted using an inverted microscope three days after drug treatments. Inhibition was calculated using the following method: % inhibition = (1 - Number of mammospheres generated from test compound-treated cells/Number of mammospheres from vehicle (DMSO)-treated cells) x 100.

$ESA^+/CD44^+/CD24^-$ cell sorting

Tumorigenic breast CSCs have been shown to be enriched in the $ESA^+/CD44^+/CD24^-$ sub-population (1). To isolate breast CSCs, flow cytometry based cell sorting was performed using method as previously described (1). Briefly, SUM159 or SUM149 cells were re-suspended in PBS containing 2%FBS at a concentration of 10^6 cells per 100 μl and incubated with FITC-conjugated mouse anti-human epithelial specific antigen (ESA), APC-conjugated mouse anti-human CD44 and BV421-conjugated mouse anti-human CD24 (all from BD Biosciences) for 20 min on ice. Cells were washed twice with PBS and re-suspended in 2%FBS/PBS before flow cytometry analysis. $ESA^+/CD44^+/CD24^-$ cells were sorted using a FACSAria III Cell Sorter (BD Biosciences).

Real-time reverse transcriptase-PCR (RT-PCR)

Total cellular RNA was prepared using TRIzol reagent (Invitrogen). Quality and quantity of RNA samples were assessed using a Nanodrop spectrometer. First-strand cDNA was synthesized from 1 μg of total RNA using a High-Capacity cDNA Reverse Transcription Kit (ThermoFisher) according to the manufacturer's instructions. MYC mRNA expression levels were measured using TaqMan® Gene Expression Assay (ThermoFisher, Assay ID: Hs00153408_m1) on a LightCycler® 480 System (Roche). Fold change in mRNA levels were calculated using the $2^{-\Delta\Delta C}{}_{\mathrm{T}}$ method and normalized to GAPDH (ThermoFisher, Assay ID: Hs99999905_m1) levels as described previously (2).

Flow cytometric apoptosis assay

Human TNBC cells were treated with compounds for 24 h and then stained with FITC-conjugated anti-Annexin V antibody and PI using a FITC Annexin V Apoptosis Detection Kit (BD Biosciences, cat# 556547) according to the manufacturer's protocol. Apoptotic cells were measured using a BD LSRFortessa™ X-20 cell analyzer (Becton Dickinson, San Jose, CA) and FACSDiva™ software. Data analysis was performed using FlowJo software (Tree Star, Ashland, OR).

Immunohistochemical analysis (IHC)

Xenograft tumors were fixed with formalin and embedded in paraffin. Tissue sections (5 μm thick) were prepared. Endogenous peroxidase activity was blocked by incubation with 3 % hydrogen peroxide for 30 min and followed by heating in 1mM EDTA for antigen retrieval. The sections were then blocked with 5 % normal goat serum in 0.1 % Triton X-100/PBS for 1 h and

incubated overnight at 4 degree with rabbit anti-human c-Myc monoclonal antibody (1:200, cell signaling). After wash with PBS, slides were incubated with ABC reagent (Vector) for 30 min. Immunostaining was visualized by DAB and the slides were counterstained using hematoxylin.

Supplementary references

1. Al-Hajj M, Wicha MS, Benito-Hernandez A, Morrison SJ, Clarke MF. Prospective identification of tumorigenic breast cancer cells. Proc Natl Acad Sci U S A 2003; 100: 3983-8.

2. Livak KJ, Schmittgen TD. Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) Method. Methods 2001; 25:402-8.