**Supplementary Materials and Methods.**

**Mammosphere Assay**

Cells were suspended in MammoCult™ medium plus (MC+) (STEMCELL Technologies Inc., Vancouver, BC, Canada) and seeded at 2,000 to 5,000 cells/well in MC+ (300 μl) in 24-well ultra-low attachment plate (Corning, Lowell, MA). Treatments of CQ (0, 0.1, 1 and 5 μM) were followed cell seeding. 100 μL of MC+ per well were added every 3 days. After 5-14 days, depending on the cells, mammospheres formed and were counted with GelCount (Oxford Optronix, Oxford, UK) and its bundled software. For the secondary MS assay, cells in each group were pulled and dispersed to single cells by treating with 0.05% trypsin for 5 to 10 minutes followed by neutralization with 10% FBS. The cells were then re-suspended in MC+ medium and counted for seeding at 3,000 cells per well. MS assays were repeated at least three times. Analysis of MSFE was repeated three times with 6 replicates for each group. FACS analysis *in vitro* was repeated two times with three replicates for each group. Two-tailed t-test was used for the statistical analysis for MSFE.

**Transmission Electron Microscope (TEM)**

Hs578T cells were grown at 2 x 105 cells in a 6-well plate and treated with PBS, CQ (1 µM), PTX, and the combination for 48 hours. Cells were then washed gently two times with ice cold DPBS. Samples were fixed with a solution containing 3% glutaraldehyde plus 2% paraformaldehyde in 0.1 M cacodylate buffer (Electron Microscopy Sciences, Hatfield, PA), pH 7.3, for 1 hour. After fixation, samples were washed and treated with 0.1% Millipore-filtered cacodylate buffered tannic acid (Electron Microscopy Sciences), postfixed with 1% buffered osmium tetroxide (Electron Microscopy Sciences) for 30 min, and stained en bloc with 1% Millipore-filtered uranyl acetate (Sigma-Aldrich, St. Louis, MO). Samples were dehydrated in increasing concentrations of ethanol, infiltrated, and embedded in LX-112 medium (Thermo Fisher Scientific, Pittsburgh, PA). The samples were polymerized in a 70°C oven for 2 days. Ultrathin sections were cut in a Leica Ultracut microtome (Leica, Deerfield, IL), stained with uranyl acetate and lead citrate in a Leica EM Stainer, and examined in a JEM 1010 transmission electron microscope (JEOL, USA, Inc., Peabody, MA) at an accelerating voltage of 80 kV. Digital images were obtained using AMT Imaging System (Advanced Microscopy Techniques Corp, Danvers, MA).

**ALDEFLUOR Assay**

ALDEFLUOR assay (STEMCELL Technologies Inc.) was performed to identify ALDH1 expressing breast cancer stem cells as suggested by the manufacturer. Briefly, after 48 hours of treatment with either vehicle (PBS) or CQ (1μM or 5 μM), cells were suspended in ALDEFLUOR assay buffer containing ALDH substrate (BAAA, 1 µM per 1 × 106 cells). In each experiment, negative controls were prepared for each sample, stained under identical conditions, with 50 mM of diethylaminobenzaldehyde (DEAB), a specific ALDH inhibitor. Cells were then incubated at 37°C in a water bath for 60 minutes and collected in ice cold ALDFLOURE assay buffer. The ALDEFLUOR positive gates were established based on viability (SYTOX-Blue negative cells) and DEAB-negative control.

**Stable cell transfection and Puncta Formation**

MDA-MB-468 cells were stably transfected with pEGFP-LC3 vector (Addgene, Cambridge, MA) using Fugene6 (Roche Applied Science, Indianapolis, IN) according to the manufacturer’s manual. Briefly, after forming DNA/Fugene6 complex with 1:3 ratio in OptiMem (Invitrogen), MDA-MB-468 cells in 100 mm dishes were transfected and selected using G418 (200ng/ml) for three months. GFP positive cells were then flow-sorted and maintained in 10% FBS DMEM plus G418 (50 ng/ml). For puncta formation assay, MDA-MB-468 cells (30,000 cells) were seeded on a cover slip bottom 35 mm dish (Cell E&G, Houston, TX) and incubated overnight in the humidified CO2 incubator at 37°C. Cells were cultured further under the 4-treatment regime for 24 hours. After changing to fresh growth medium containing the 4-treament regimen, LysoTracker® Red DND-99 \*1 mM solution in DMSO (Invitrogen) was added at a final concentration of 50 nM, and cells were further incubated in the CO2 incubator at 37°C for 30 minutes. After media were replaced with fresh media containing the 4-treatment regimen, cells were examined under a humidified CO2 chamber using a confocal microscope (Olympus FluoView FV1000 with bundled operating software, OlympusUSA, Center Velly, PA**)**. Cells were imaged with an oil-emersion 100x objective lens (Olympus) with 2.5 optical zoom. Nuclei were stained by adding Hoechst 34442 dye (0.5µg/ml) into the culture medium.

**Apoptosis Analysis**

Hs578T, MDA-MB-231, and SUM159PT cells in 6 well plates were treated with the 4-treatment regimen for 48 hours and subjected to apoptotic assay using an AnnexinV-FITC and SYTOX-Blue dead cell staining kit (Invitrogen). Cells (1 x 106 cells) in the binding buffer were mixed with 5 ul of Annexin V antibody solution for 15 minutes on ice and washed two times with fresh binding buffer. After the final wash, cells were suspended in the fresh binding buffer containing SYTOX-Blue (500nM). Cells were then analyzed immediately using BD FACS Fortessa. The experiments in triplicates per group were repeated two times with similar results.

**Western blot and Immunoprecipitation Assays**

After treatments of DMSO (Con), CQ (1µM), PTX (5nM) in DMSO (5nM), and CQ-PTX, cells were lysed in a lysis buffer (1.5% Triton X-100 and 10% glycerol in DPBS) containing a proteinase and phosphatase inhibitor cocktail (Thermo Scientific Pierce Protein Biology, Rockford, IL). For immunoprecipitation, total 500 µg of cell extracts were incubated for 24 hours with rabbit anti-Jak2 antibodies conjugated to sepharose beads (Cell Signaling Technology) according to the manufacturer’s recommendation. Normal rabbit IgG conjugated with sepharose (Cell Signaling Technology) was also used as an internal negative control that confirmed that there was no non-specific binding (Data not shown).

**In vivo experiments**

For orthotopic xenograft models, SUM159PT cells (3 x 106) in PBS (100 µl) and MDA-MB-231 cells (5 x 106) in PBS (100 µl) were injected into the left mammary fat pad. When the tumor volume reached roughly 150-200 mm3, mice were randomly separated into groups and were treated with PTX at 15 mg/kg (*i.p.* twice weekly), or CQ at 10 mg/kg (*i.p.* daily), or the combination. Tumor volumes were measured two times per week at 3- or 4-day intervals. Tumors were collected for digestion as described previously with collagenase 3 (Worthington Biochemical Corporation, Lakewood, NJ) and Dispase (Invitrogen) for the MS assay 1. For metastasis and recurrence experiments, 5 x 106 MDA-MB-231 cells expressing GFP tagged Luciferase (MDA-MB-231 G/L) were injected into the mammary fat pad as above. After forming palpable tumors in 4 days, mice were randomly separated into groups (n=10). Con (PBS) and CQ (10mg/kg) treatments were initiated for the study of metastasis, and PTX and CQ-PTX treatment were administered for recurrence study. For the recurrence study, PTX at 15 mg/kg (*i.p.* weekly) was used for the first four weeks and then increased to 30 mg/kg (*i.p.* weekly) for an additional four weeks. The dose of CQ was 10 mg/kg (*i.p.* daily) throughout the two-cycle treatment period. Tumor volumes were measured every third day. The tumor size was calculated as A × B2 / 2 where A was the largest and B the smallest diameter.

**Ex vivo imaging for metastasis study**

Ex vivo bioluminescence imaging was performed using a Xenogen IVIS image station (PerkinElmer, Waltham, MA). D-Luciferin potassium salt (Caliper, CO), was injected at a dose of 150 mg/kg (*i.p*.) per mouse. After resting for 5 minutes, animals were sacrificed according to the recommendation from the Methodist Hospital Research Institute Animal Care and Use Committee. Lungs were then extracted and kept in pre-warmed DMEM until *ex vivo* imaging. Lungs were incubated in warm DMEM with D-Luciferin (150 µg/ml) for 10 minutes before imaging. To compare the tumor burden, images were normalized, and identical regions of interest were used for each lung. The average photon counts were then measured via IVIS bundled software. The measurements were converted to Log10 values and graphed.

**Preparation of Tumor Tissue Lysates**

Snap-frozen tissues in PBS with 2% TritonX-100 containing the proteinase/phosphatase inhibitor cocktail were homogenized using Tissue lyser II with a Stainless Steel Bead (Valencia, CA) for each tumor. The cell lysates were then centrifuged at 4°C for 10 minutes at 12,000 RPM. Supernatants were used for western blot assays.

**Immunohistochemistry**

All tumors were fixed in 4% formalin solution and processed for paraffin embedding in the preclinical core facility in Houston Methodist Hospital, Houston TX. The tumors were then sectioned at 4 μm thickness and mounted on positively charged glass slides (Croning). After deparaffination and rehydration, antigen retrieval and blocking endogenous peroxidase were performed with EnVision™ FLEX Systems (Dako, Carpinteria, CA) according to the manufacturer’s mannual. After blocking steps in 5% BSA in PBS, primary antibody staining was performed at room temperature for 1 hour with monoclonal mouse anti-human DNMT1 (1:300 in the blocking solution, GeneTex, Irvine, CA) and rabbit anti-human Jak2 (1:100 in the blocking solution, Cell Signaling Technology). Tissues were washed three times in PBS containing 0.1% Tween-20 for 5 minutes. EnVision™ FLEX Systems was used for the colorimetric reaction according to the manufacturer’s manual. Images were taken using Nikon Eclipse Ti microscope (Melville, NY).

**Transfection of siRNA for DNMT1, Jak2, and STAT3.**

All silencing RNAs (Silencer® Select Pre-Designed & Validated siRNA) were purchased from Invitrogen. siRNA transfection was performed using siPORT™ NeoFX™ transfection agent (Invitrogen) according to the manufacturer’s protocol. Scrambled siRNAs (Scr) (Invitrogen) were used as the comparison control for all siRNA experiments. For transfection, cells were seeded in 60 mm dishes (5 x 105 cells) or in 6-well plates (1 x 105 cells/well) (Corning Inc.) with the complex of siRNAs- siPORT™ NeoFX™ transfection agent (Invitrogen) in Opti-MEM® I Reduced Serum Medium (Invitrogen) according to the manufacturer’s manual. For western blot assay, the final concentrations of siRNA-SOCS3 and siRNA-DNMT1 were 30 nM and 15 nM respectively. Cells were treated with CQ (1 µM), PTX (5 nM), or the combination, and harvested after 30 hours. In order to assess the impact of each gene (Jak2, STAT3, and DNMT1) on CSCs via FACS analysis, the final siRNA concentrations were; siRNA-DNMT1 (D) at 15 nM, siRNA-JAk2 (J) at 5 nM, siRNA-STAT3 (S) at 20 nM. siRNAs-DNMT1/Jak2 (D/J), siRNAs-DNMT1/STAT3 (D/S), or siRNAs-Jak2 /STAT3 (J/S) was the double silencing combination. For triple silencing, siRNAs against DNMT1, JAk2, and STAT3 were co-transfected (D/J/S). The final total concentration of the triple silencing siRNA was 45 nM. The scrambled siRNA was used to match the final siRNA concentration to 45 nM for the single or the dual silencing groups. After 48 hours of transfection, cells were subjected to FACS analysis for CSCs.

**Immunofluorescence staining for the co-localization of Jak2 and SOCS3.**

SUM159PT CSCs on a cover- slip bottom 35 mm dish (Cell E&G, Houston, TX) were treated with PTX alone (5 nM) or in combination with CQ (1 µM) for 36 hours. For the co-localization test for Jak2 and SOCS3, Rabbit monoclonal anti-Jak2 antibodies (Cell signaling Technology) and mouse monoclonal anti-SOCS3 (Abcam, Cambridge, MA) were diluted at 1:100 in the blocking solution. For the secondary antibody, anti-mouse antibodies-Alexa 488 and the secondary anti-rabbit antibodies-Alexa 568 (Invitrogen) were diluted at 1:1000 in blocking solution. Hoechst 33342 (100 ng/ml) was layered on top of the cells for imaging under a confocal microscope using an oil-immersion 100x objective lens with 2.5x optical zoom (Olympus FluoView FV1000 with bundled operating software, OlympusUSA, Center Velly, PA). The co-localization of SOCS3 and Jak2 was analyzed using Image J software.

**Genomic DNA Isolation**

Hs578t, MDA-MB-231, and SUM159 cells (1 x 106 cells in 100 mm dish) were cultured in the presence of the four-treatment regimen (Con, CQ, PTX, and CQ-PTX) for 36 hours, and the genomic DNA was extracted using a genomic DNA extraction kit (BioVision, Mountain View, CA**)** according to the manufacturer’s manual. Briefly, after lysing cell in the provided lysis buffer (25 μl) at 50°C for 30 minutes, 100 % ethanol (100 μl) was added, and the samples were kept at -20°C for 10 minutes. The genomic DNA was then precipitated and pelleted by 10 minute centrifugation at 13,000g at room temperature, and washed with 1 ml of 70% ethanol three times. After air drying for 5 minutes on a 37°C heat block, molecular biology grade pure water (20 μl) was added, and the DNA concentration was measured using NanoDrop 2000 (Thermo Scientific). The ratio of all extracted DNA was 1.8 or above. The DNA solution was then adjusted to 0.1 mg/ml concentration with the pure water. For the genomic DNA of the CD44+/CD24-/low CSCs, SUM159PT cells were first treated with drugs and flow-sorted, and the collected cells were subjected to the genomic DNA extraction, as described above.

**Quantification of Global DNA Methylation**

For quantification of Global DNA methylation, we purchased Methylamp™ Global DNA Methylation Quantification Ultra Kit (Epigentek, *Farmingdale, NY),* a colorimetric ELISA kit, which detects 5-methylcytosine via antigen-antibody reaction. We performed the experiment as instructed by the manufacture’s protocol using total 300 ng of genomic DNA for each sample and read the plate using a microplate reader at 450 nm. Each sample was triplicated, and experiments were repeated twice. The percentage of methylation was calculated using the suggested formula in the manufacture’s protocol. The formula, shown below, has the assumption; 41% of GC contents in human genomic DNA.

*Methylation % =(Methylated DNA Amount / X\*)x 100% / (Sample DNA Amount Added) where X\* is the human GC contents (%).*

**Methylated DNA Immunoprecipitation (MeDIP)**

Intact Genomic DNA from PTX- or CQ-PTX treated SUM159PT cells was sheared to ~500-bp fragments using Bioruptor® Standard (Diagenode Inc., Denville, NJ) as suggested by the manufacturer’s protocol. The fragmented DNA was then subjected to MeDIP using Methylamp Methylated DNA Capture (MeDIP) Kit (Epigentek Group Inc., Farmingdale, NY) according to the manufacture’s protocol. First, 100 µl of immunoprecipitation (IP) buffer was added to each well of IP 96 well plate (5 wells per group) with 1 µl Anti-5-methylcytosine antibody or 1 µl of Normal Mouse IgG as the negative control. The resulting Input and IP DNA were then used for SOCS3 promoter PCR. The details of MeDIP follow. The strip wells were covered with parafilm M and incubated at room temperature for 60 min. The fragmented DNA (1 µg/well) in the provided antibody buffer was cooled down on ice immediately after the incubation at 95°C for 2 min. The rest of the sonicated DNA was used as input DNA. After the 60 minutes of incubations, the IP wells were washed three times with wash buffer then one time with antibody buffer. The sonicated DNA solution in antibody buffer (100 μl) was added to each well and covered with parafilm M to incubate at room temperature for 90 minutes on an orbital shaker (75 rpm). After removing the supernatant, the wells were washed 3 times with the wash buffer, and 60 μl of DNA release buffer containing 1 μl of proteinase K was added to each sample (including “input” vials). Each well in a strip was covered with strip caps and incubated at 65°C in a water bath for another 60 minutes. After the incubation, 180 μl of 100% ethanol was added, and the solution was transferred to a provided spin column, containing 100 μl of binding buffer. After spinning down at 12,000 rpm for 20 sec, the column was washed with 90% ethanol twice. Subsequently, the DNA was eluted with 20 μl of elution buffer by centrifugation at 12,000 rpm for 20 sec. Input and IP DNA were then used for SOCS3 promoter PCR.

**Real Time PCR for SOCS1 and SOCS3.**

Total RNA were extracted using RNeasy Plus Mini Kit (Qiagen, Valencia, CA) and cDNA was synthesized using SuperScript® Double-Stranded cDNA Synthesis Kit (Life Technologies, Grand Island, NY) according to the manufacturer’s protocols. Real-time PCR was performed on an Applied Biosystem 7900HT Fast Instrument (Applied Biosystems, Foster City, CA, USA) with TaqMan® Gene Expression Assay kits (Life Technologies) for SOCS1 and SOCS3 and 2X TaqMan® Fast Universal PCR Master Mix according to the manufacturer’s protocols. Data were evaluated as described previously1. Briefly, data were analyzed using ABI Prism RQ Manager 1.2 (Applied Biosystems, Foster City, CA, USA). The following adjustable analysis settings were used: automatic threshold (CT), automatic outlier removal, and relative quantification (RQ) min/max confidence 99%. All data were calibrated to pooled cDNA and each sample was normalized to 18S rRNA endogenous control. The experiments were repeated three times independently with similar results.

**Polymerase Chain Reaction for SOCS3 primers**

The 13 pairs of custom designed primers were purchased from Sigma. The PCR reaction was performed using GoTaq® Long PCR Master Mix with molecular biology grade water (Promega, Madison, Wisconsin) with varying % of DMSO (Sigma) on Eppendorf Mastercycler® gradient (Eppendorf, Hauppauge, NY). The primer pairs of number 2, 3, 4, 5, 9, 10, 11, and 13 were run with a final concentration of 5% DMSO and with the following cycle protocol; 1 cycle at 94°C for 3 minutes, 35 cycles of 94°C for 15 seconds, 55°C for 15 seconds, and 72°C for 30 seconds, and 1 cycle of 72°C for 3 minutes. For primers of 1 and 7, the final concentration of DMSO was 0% with the following cycle protocol; 1 cycle of 94°C for 3 minutes, 35 cycles of 94°C for 15 seconds, 50°C for 15 seconds, and 72°C for 30 seconds, and 1 cycle of 72°C for 3 minutes. For primer 8 and 12, 5% DMSO was used for the following cycle protocol; 1 cycle of 94°C for 3 minutes, 35 cycles of 94°C for 15 seconds, 50°C for 15 seconds, and 72°C for 30 seconds, and 1 cycle of 72°C for 3 minutes. For the 6th primer, 0% DMSO was used for the following cycle protocol; 1 cycle of 94°C for 3 minutes, 35 cycles of 94°C for 15 seconds, 55°C for 15 seconds, and 72°C for 30 seconds, and 1 cycle of 72°C for 3 minutes. All PCR reactions were cooled down to 4°C. The resulting PCR products were run on 2% agarose gels in TAE buffer using Mupid Kit (Clontech Laboratory Inc., Mountain View, CA) and the images were taken using the Gel Doc™ XR+ imaging system with Image Lab™ (Bio-Rad, Hercules, CA).

**Bioinformatic analysis of MBDCap-seq data**

Enriched binding sites were determined through the use of MACS-1.4.2 2 with default parameters as outlined in a reported protocol3 and a p-value ≤ 1 x 10-10. Binding site differences between control and chloroquine-treated samples were determined with MAnorm4, and differential binding events between the control and chloroquine-treated samples accepted with an absolute M\_value\_rescaled value of ≥ 1 (note: log2 value) and a Bonferroni-corrected p-value ≤ 0.05. The differential binding events were associated with promoters of protein coding genes (Ensembl GRCh37.p13) through use of a custom Python script that determines overlap between the MAnorm defined binding sites and 5000 base pairs (bp) upstream to 200 bp past the Transcriptional Start Site (TSS). Functional enrichment of discovered genes were determined with GeneCodis35.

**References**

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