**Supplemental Methods**

**Modulation of EMP2 expression in cell lines.** EMP2 expression levels were modified in several breast cancer cell lines including HS578t, MDA-MB-231, MDA-MB-468, SKBR3 and BT474. To increase EMP2 levels, as indicated in the figures, cells were stable transfected with a human EMP2-GFP fusion protein [9](#_ENREF_9), [10](#_ENREF_10). In addition, where indicated, EMP2 was overexpressed through retroviral transduction as previously described[5](#_ENREF_5). In order to reduce EMP2 levels, breast cancer lines were infected with either a non-targeting shRNA control or EMP2 specific shRNA (911; TRCN0000322911); (385; TRCN0000322385) in pLKO.1-puro as per manufacturer’s instructions (Sigma-Aldrich). All experiments were done with sub-confluent cells.

In some experiments, SKBR3 and MDA-MB-468 breast cancer cells were treated with small interfering control RNA (siRNA) or epithelial membrane protein-2 siRNA. Briefly, cells were transiently transfected with lipofectamine-2000 (Thermo Fisher Scientific, Waltham, MA) and EMP2 siRNA (L-016226-00; GE Lifesciences, Lafayette, CO). As a negative control, cells were transfected with 120 picomoles scrambled control siRNA (D-001206-13-05; GE Lifesciences). The EMP2 siRNA and control siRNA are derived from a pool of four siRNAs targeting EMP2 or a pool of four nontargeting siRNAs, respectively. Cells were harvested for Western blot 48-72 hours post-transfection as detailed below.

**RNA-sequencing.** Total RNA was prepared from SUM149 vector control and shRNA lentiviral knockdown cells using the RNAeasy kit (Qiagen). Two samples per group were subjected to Illumina sequencing using the UCLA Pathology and Laboratory Medicine Core. The libraries used for RNA-seq were prepared with KAPA Stranded RNA-Seq Kit. The workflow consisted of mRNA enrichment, cDNA generation, end repair to generate blunt ends, A-tailing, adaptor ligation and PCR amplification based on previously published procedures[11](#_ENREF_11). For differential expression analysis, 25,344 human genes were pair-wise analyzed with DESeq[1](#_ENREF_1" \o "Anders, 2010 #3058). The TMM normalized read counts from select genes was also used to generate a heatmap using the R package, pheatmap. Statistics were obtained for globally normalized data, and genes were classified as differentially expressed if the p-value (Student’s t test) was smaller than 0.05 (5%) and had a fold change>0.5 (log2 scale). Based on this criteria, 333 genes were classified as differentially expressed. GSEA was performed as above using the Molecular Signatures Database V5.0.

The data presented in this publication has been deposited into the NCBI Gene Expression Omnibus and is accessible through GEO Series accession number GSE134522.

**Single Staining Immunohistochemistry.** Single stain immunohistochemistry was performed on paraffin embedded, formalin fixed 5m sections. Sections were deparaffinized in xylene and rehydrated using graded alcohols. For antigen retrieval, slides were incubated at 95°C for 20 minutes in 0.01 M citrate buffer, pH 6.0. Tissues were blocked with DAKO Serum-Free Protein Block for 10 minutes, and incubated in primary antibody overnight at 4°C. For EMP2, samples were stained with rabbit anti-human EMP2 antisera at a 1:500 dilution [8](#_ENREF_8). Rabbit sera at the same concentration was utilized as an isotype control. For ALDH1 staining, samples were stained with ALDH1 (clone: 44/ALDH) at a 1:100 dilution. The following day, staining was amplified using the species appropriate DAKO Envision+ kit according to manufacturer’s instructions followed by detection using the 3,3'-diaminobenzidine (DAB) substrate. All samples were counterstained using diluted Harris Modified Method Hematoxylin (Vector Labs, Burlingame, CA).

**Double Staining Immunohistochemistry.** Double staining utilized the DAKO Envision+ HRP Mouse kit (DAB+, K400711-2), Vector Vectastain ABC-Alkaline Phosphatase Kit (AK-5001), and Vector Red Alkaline Phosphatase Substrate kit (SK-5100) as previously described[7](#_ENREF_7). Briefly, sections were deparaffinized, re-hydrated, treated via heat-induced epitope retrieval, and blocked with DAKO Peroxidase Blocking Reagent and DAKO Serum-free Protein Block. Sections were incubated with both the anti-EMP2 and anti-ALDH antibodies diluted in DAKO Antibody Diluent overnight at 4°C. The next day, sections were treated with DAKO Labeled Polymer-HRP anti-Mouse for 30 minutes, and ALDH antigenic sites were visualized using diaminobenzidine. EMP2 staining was then detected by incubating the sample with biotinylated anti-rabbit followed by the Streptavidin reagent from the Vector ABC-Alkaline Phosphatase Kit for 30 minutes. Staining was visualized with Vector Red. The slides were then dehydrated in graded alcohol and counterstained with hematoxylin. Samples were analyzed using an Olympus BX51 light microscope using a 20 X objective connected to a DP72 digital camera.

**SDS-PAGE/Western Blot Analysis**. Protein lysates were prepared through lysing cell cultures in Laemmli lysis buffer. In some experiments, cells were treated for with 50 g/ml of anti-EMP2 mAb or control IgG and lysed after 24 hrs. As indicated in the figure legends, treatments of the anti-EMP2 mAb were compared to responses observed with either anti-CD20 IgG (Rituximab; Roche) or human IgG (Sigma-Aldrich). CD20 and EMP2 are both tetraspan proteins, but in the case of the former, its expression is restricted to human B cells[2](#_ENREF_2).

Western blot analysis was performed as previously described[7](#_ENREF_7). The following anti-human antibodies were diluted in 5% milk in TBST: -actin (US Biologicals), HIF-1(BD Biosciences; clone 610959), CD44 (R&D Systems; clone 691534), ALDH1 (BD Biosciences; clone 44/ALDH), or OCT-4A (Cell Signaling; clone C30A3). For EMP2 detection, lysates were treated with N-glycosidase F (New England Biolabs, Beverly, MA) to remove N-link glycosylation, and blots were probed with rabbit, anti-human EMP2 antisera (1:2000 dilution). Horseradish peroxidase conjugated secondary antibodies were utilized for protein visualization (BD Biosciences or Southern Biotechnology Associates, Birmingham, AL) followed by Immobilon Crescendo Western substrate (EMD Millipore, Burlington, MA). Levels of β-actin were used to normalize protein expression. Relative concentrations were assessed by densitometric analysis. At least three independent experiments were performed and, where indicated, the results were evaluated for statistical significance using a Student’s t-test (unpaired, two-tailed). A level of p<0.05 was considered to be statistically significant.

**Flow cytometric staining.** Cells growing in culture were harvested using a 0.5% EDTA solution in HBSS. Immunostaining of cells for EMP2 was performed by incubating cells with concentrations of anti-EMP2 mAb ranging from 0-15g/ml on ice. Cells were washed and then incubated with a goat anti-human Fc-PE (Invitrogen) in a HBSS buffer containing 0.02% sodium azide and 0.2% BSA.

Stem cell-like CD44+/CD24- populations were identified using an anti-human CD44 antibody conjugated to PerCP (clone: G44-26; BD Biosciences) and a FITC conjugated anti-CD24 (clone SN3 A5-2H10; ThermoFisher Scientific). Appropriate fluorescently conjugated isotype control antibodies were utilized (clones: 27-35 and P3.6.2.8.1 for CD44 and CD24, respectably). ALDH activity was determined using the ALDEFLUOR assay kit (StemCell Technologies, Vancouver, BC) according to the manufacturer’s guidelines. Analysis was performed at the UCLA flow core using a FACSCalibur or LSRFortessa X-20 SORP (Becton Dickinson, Palo Alto, CA, USA) flow cytometer. Cells were first gated by FSC-A vs. SSC-A and then for singlets (SSC-H vs. SSC-A). Cellular viability was then determined using a combination of DAPI, 7AAD, or Live/Dead Aqua staining (ThermoFisher Scientific).

ALDHhigh and ALDHlow subsets were isolated from MDA-MB-468, BT474, and SKBR3 cell lines using an ARIA III flow cytometric machine (BD Biosciences) using the ALDEFLUOR assay kit (StemCell) as described above. Following FACS isolation, resulting populations were evaluated using Western Blot analysis. In some experiments, to determine the percentage of ALDHhigh and EMP2+, cells were stained with anti-EMP2 mAbs first and then using the ALDEFLUOR assay kit. Controls included staining with the secondary only and using Diethylaminobenzaldehyde (DEAB), a specific inhibitor of ALDH activity.

**Treatment and analysis using mouse xenograft model.** Animal studies were performed in strict accordance with the recommendations in the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health. The Animal Research Committee at the University of California, Los Angles, approved all procedures. All efforts were made to minimize animal suffering. The supplemental methods section describes the models created.

Tumor pieces from the HCI-002 triple negative patient derived tumorgraft was generously provided by Dr. Alana Welm (Huntsman Cancer Institute, University of Utah)[3](#_ENREF_3) and expanded as previously described[4](#_ENREF_4). Experiments were performed within the first 2 generations of receipt of tumor pieces. A 2x2mm tumor fragment was implanted into the 4th mammary fat pad of six week old NOD-SCID mice (Jackson Labs) as previously described[4](#_ENREF_4). The take rate in all cases was 100%. When tumor load approached ~100mm3, animals were divided into two treatments. Control human Abs or anti-EMP2 mAb were administered IP at 10mg/kg, twice a week. Animals were euthanized when the average tumor volume within the control group approached 1500mm3.

Six to eight week old Balb/c nude mice (Nu/J; Jackson) were used to create the human cell line xenograft models. To determine the effects of EMP2 on tumor growth, animals were inoculated subcutaneously (s.c.) or within the mammary fat pad as indicated in the figure legends. 2x106 MDA-MD-231, 4x106 BT474, or 1x106 SUM149 cells with up- or down-regulated EMP2 levels mixed 1:1 with Matrigel (BD Biosciences) or Basement Membrane Extract (Cultrex, type 3; Trevigen, Gaithersburg, MD). Every 3-4 days, tumors were measured with digital calipers, and tumor volumes were calculated using the formula (length x width2)/2. In antibody efficacy studies, cells were injected into the mammary fat pad using a 5% matrigel suspension as above. Tumors were treated IP with 10mg/kg anti-EMP2 mAb or control human IgG (Sigma Aldrich, I4506) twice a week. The number of animals used per experiment is indicated in the figure legends. In all cases, mice were euthanized once the tumors approached 1.5cm3. Following euthanization, tumors were isolated, fixed overnight in 10% formalin, and embedded for immunohistochemical staining.

To determine the ability of anti-EMP2 therapy to reduce the BCSC population *in vivo*, tumors were dissociated and analyzed by flow cytometry or secondary tumor implantation. Tumors were disassociated through incubation with DMEM/F12K (Corning) media supplemented with ultra-pure collagenase III and hyaluronidase for 60min at 37ºC. Following incubation, cells were washed in saline, filtered through a 40 micron nylon mesh, and incubated in ACK Lysing buffer for 3 min at RT to remove red blood cells. ALDH activity was measured in some cells using flow cytometry. To determine if treatment reduced tumor initiation in some experiments, single cell suspensions of dissociated primary tumors treated with control human IgG or anti-EMP2 mAb were reinjected into the left mammary fat pad at concentrations of 50,000, 5,000, or 500 cells in a 1:1 saline and matrigel suspension. Tumor size was monitored as previously stated, and mice were euthanized once tumors reached 1.5 cm in diameter. Tumors were isolated, fixed and processed for hematoxylin and eosin staining. These experiments were independently repeated twice.

***In vivo* Imaging System (IVIS).** In order to monitor tumor load in metastatic models, 5x104 MDA-MB-231-Luciferase or 4T1-Luciferase positive cells were injected either intracardiac or orthotopically into the mammary fat pad, respectively of 6 week old Balb/c female mice. The substrate luciferin was injected into the intraperitoneal cavity at a dose of 150 mg/kg body weight (30 mg/ml luciferin), approximately 5 minutes before imaging. Mice were anesthetized with isoflurane/oxygen and placed on the imaging stage. Ventral and dorsal images were collected for 30 seconds to 2 minutes using the IVIS (Xenogen Corp., Alameda, CA). Photons emitted from the primary tumor and lung region were quantified using Living Image software (Xenogen Corp., Alameda, CA).

**Tumorspheres**. EMP2 modified or wildtype breast cells were plated in 2% polyHEMA coated 48-well plates at low density (5,000-50,000 cells/well). Cells were grown in Mammocult media supplemented with L-glut, P/S, sodium pyruvate, heparin, hydrocortisone, and proliferation supplement for one to three weeks. In some instances, tumorspheres were dissociated with Accutase (Stem Cell Technologies, Seattle, WA) and replated for secondary tumorsphere formation. Treatment of tumorspheres with control or anti-EMP2 mAb was done at time of plating with varying doses of the antibodies. Tumorsphere forming capacity was calculated using the formula: (number or tumorspheres formed ÷ number of cells seeded) × 100.

In some experiments, 1, 10 or 50 live cells were FACS sorted into 50l of Enhanced serum-free media (+/- 10ug/mL anti-EMP2 mAb or a control Ab) per well in non-treated 384-well plates. Spheres were counted 2 weeks later. The frequency of tumorsphere-initiating cells (TIC) was calculated using the online Extreme Limiting Dilution Analysis (ELDA) calculator [http://bioinf.wehi.edu.au/software/elda/index.html][6](#_ENREF_6). P-values were calculated by testing for inequality in frequency between multiple groups.

**Statistical analysis.** Western blot band intensities were quantitated using Image J, and differences between cell lines were compared using Student’s unpaired, two tailed t-test at a 95% confidence level (GraphPad Prism version 5.0; GraphPad Software, La Jolla, CA). Differences in the rate of growth over time between cell lines were determined using a two way ANOVA (GraphPad Prism version 8.0; GraphPad Software, La Jolla, CA) with post hoc Bonferroni test. P values <0.05 were considered significant.

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