Appendix S1. Supplementary materials and methods

Mammosphere formation assay. For mammosphere formation, HMLE cells were suspended at a concentration of $1x10^4$ cells/ml in serum-free culture DMEM medium, supplemented with 1:50 B27 medium supplement, 20 EGF and 10 ng/ml basic FGF. Cells were seeded in a 6-well ultra-low adherent plate (2 ml/well). Mammospheres were counted 7 days after seeding. After the culture period, mammospheres with a diameter >80 μm were manually counted under a light microscope (Axiovert 25, Zeiss) with an objective magnification of 100x. Mammosphere forming efficiency (MFE) was calculated as follows: MFE (%)=(# of mammospheres/well)/(# of cells seeded/well) x100. For each cell line, three independent experiments were performed.

Irradiation. γ-irradiation was performed on a GSR D1 irradiator (Gamma Medical Service). This self-shielded device irradiates with four sources of 137Cs, with a total activity ~180.28 TBq (measured in March 2014). The samples were irradiated at 0, 2, 4, 6 and 10 Gy, with a dose rate of 2.7 Gy/min, taking the radioactive decay into account. The samples were irradiated in 25 or 75-cm^2 flasks or 6- or 12-well plates (density 50%).

Prior to irradiation, dosimetry was performed. A cylindrical ionizing chamber 31,010 (PTW Freiburg GmbH, Germany) was used as the recommendation of the AAPM'S TG-61 protocol for clinical reference dosimetry. This ionizing chamber has a cavity of 0.125 cm^3 calibrated in 137Cs kerma at the PTB [Physikalisch-Technische Bundesanstalt (Braunschweig)] (facility no. 1904442). The polarity and the ion recombination were measured for this 137Cs source. Each measurement was corrected by KTP factor to take the variation of temperature and atmospheric pressure into account.

Primers used for reverse transcription-quantitative PCR. All the primers were from Taqman® Assays from Applied Biosystems (Thermo Fisher Scientific, Inc.). Cat. nos. were as follows: CD24: Hs02379687_s1; CD44: Hs01081473_m1; epithelial cell adhesion molecule, Hs00901885_m1; E-Cadherin (Cad): Hs01013965_m1; N-Cad: Hs00983056_m1; fibronectin 1: Hs01549976_m1; Twist1: Hs00361186_m1; Twist2: Hs00382379_m1; zinc finger E-box binding homeobox (Zeb)1: Hs00232783_m1; Zeb2: Hs00207691_m1; Snai1: Hs00195591_m1; Snai2: Hs00950344_m1; Vimentin: Hs00185584_m1; Keratin 14: Hs00265033_m1; Ovo-like zinc finger 2: Hs01067398_m1; DNP63a: Hs00978339_m1; superoxide dismutase 2: Hs00167309_m1; heme oxygenase 1: Hs01110250_m1; glutathione-disulfide reductase: Hs00167317_m1; thioredoxin reductase 1: Hs00917067_m1; GAPDH: Hs99999905_m1 and RPLPO: Hs99999902_m1.

Figure S1. $CD24^{-/low}$ phenotype of epithelial HMLE cells is reversible. $CD24^{-/low}$ HMLE cells were analyzed for CD24 expression by flow cytometry and plated for long-term culture. CD24 expression was analyzed 1, 6 and 21 days after FACS sorting.

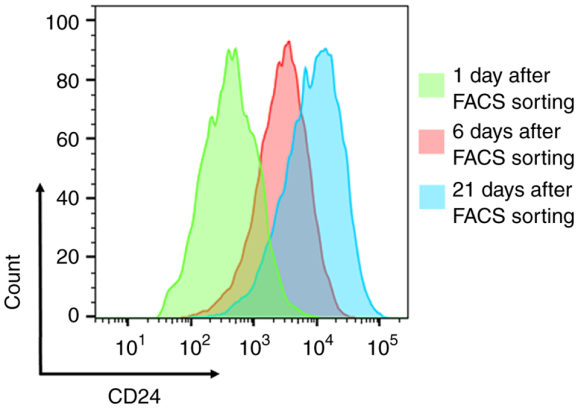

Figure S2. Analysis by reverse transcription-quantitative PCR of relative expression of the CD24 mRNA in E, E_CD24-, E_CD24-c and M cells. Expression in E cells was normalized to 1. Data are presented as the mean ± SD of three independent experiments. E, epithelial; M, mesenchymal.

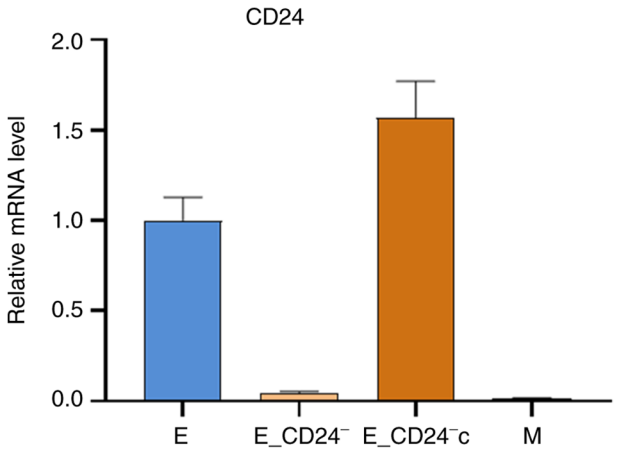

Figure S3. Analysis by reverse transcription-quantitative PCR of relative expression of the mRNAs encoding CD24, Vim and ΔNp63α in T47D and T47D_CD24$^-$ cells. Expression in E cells was normalized to 1. Data are presented as the mean ± SD of three independent experiments. Significant differences were analyzed by Mann-Whitney tests. *P<0.05 and **P<0.01. Vim, vimentin.

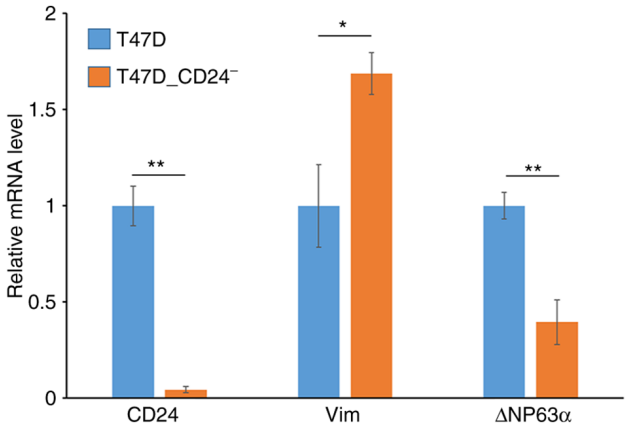

Figure S4. Representative FACS analysis of the ALDH$^+$ subpopulation using Aldefluor assay. Cells incubated with the specific inhibitor of ALDH, diethylaminobenzaldehyde, were used to establish the baseline fluorescence and define the ALDH positive population. ALDH, aldehyde dehydrogenase.

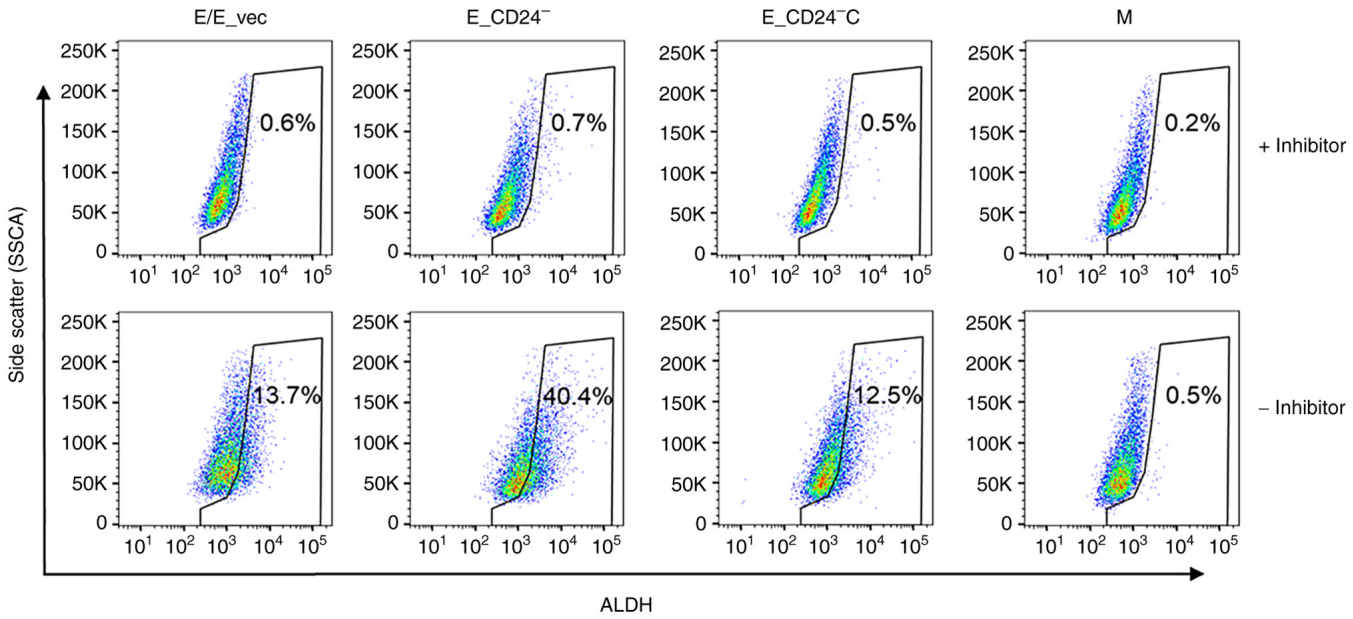

Figure S5. Forced extinction of CD24 expression alone promotes chemo-resistance of epithelial breast cell lines MCF7 and T47D. The cell lines were transfected with the p-EBV-plasmid expressing CD24 small interfering RNA to obtain MCF7_CD24^{-} and T47D_CD24^{-} cells. The parental and transfected cell lines were exposed for three days to 400 μM 5FU and 15 μM cisplatin and the percentage of dead cells was analyzed. Significant differences were analyzed by Mann-Whitney test. Data are presented as the mean ± SD of 3 independent experiments. *P<0.05. 5FU, 5-fluorouracil.

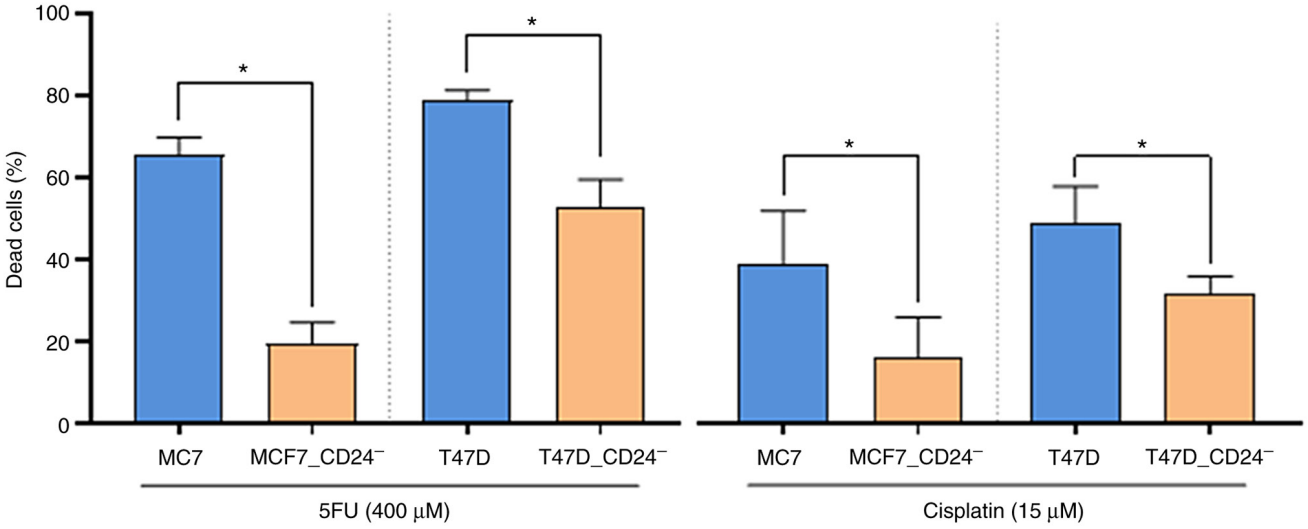

Figure S6. Ratio of fluorescence between TMRE and MTG analysis for E, E_CD24-, E_CD24-c and M cells. MTG, Mitotracker Green; TMRE, tetramethylrhodamine, ethyl ester; E, epithelial; M, mesenchymal.