

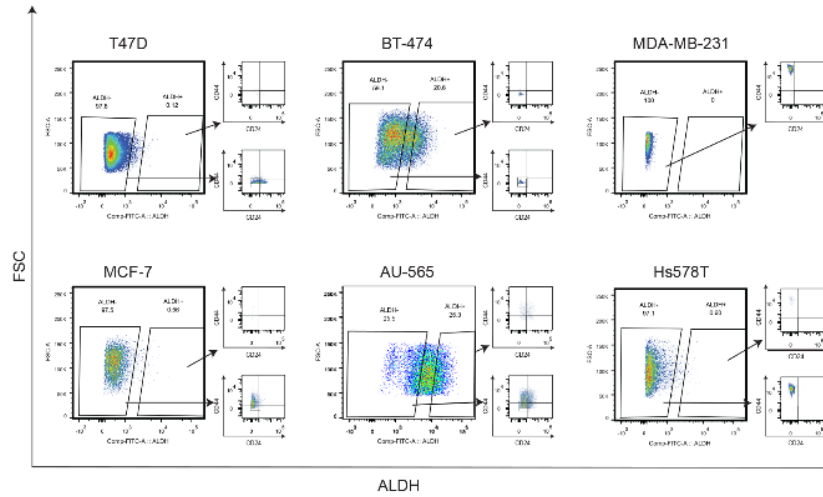
Table S1: Antibodies and reagents used

Antibody	Company	Catalog No	Host Species
Anti-CD44-APC	Thermofisher	MA1-10226	Mouse
Anti-CD24-PerCP-eFluor 710	Thermofisher	46-0247-42	Mouse
Primary anti-CD44	Abcam	ab157107	Rabbit
Primary anti-CD24	Abcam	ab202073	Rabbit
Primary Anti-ALDH1A3	Abcam	ab129815	Rabbit
Anti-ALDH1A1- Alexa Fluor 488	Abcam	ab195254	Rabbit
Primary Anti-NANOG	Abcam	ab109250	Rabbit
Anti-KI67-Alexa Fluor 647	Abcam	ab196907	Rabbit
Secondary- Goat anti-rabbit FITC	Jackson ImmunoResearch	111-095-003	Goat
Secondary- Goat anti-rabbit TRITC	Jackson ImmunoResearch	111-025-003	Goat
Reagent			
Penicillin-Streptomycin,-100X	ThermoFischer	15140-122	
B27 supplement -50x	Gibco	17504-044	
Fibroblast growth factor	Gibco	PHG0314	
Epidermal growth factor	Gibco	PHG0314	
D-MEM/F-12	Gibco	12500-039	

Table S2: Primers used for qRT-PCR

Gene	Transcript	Forward primer	Reverse primer
EMT/Metastasis			
SNAIL1	ENSG00000124216	TAATCCAGAGTTTACCTTCCAGCA	AGCCTTTCCCACTGTCCTCA
SNAIL2	ENSG00000019549	GCCAAACTACAGCGAACTGG	AGGAGGTGTCAGATGGAGGA
FOSL1	ENSG00000175592	GCAGGCGGAGACTGACAA	GGGAAAGGGAGATACAAGG
VIM	ENSG00000026025	CAGATGCGTGAAATGGAAGA	TGGAAGAGGCAGAGAAATCC
CDH2	ENSG00000170558	CATTATCAACCCCATCTCGG	ACTGTCCCATTCCAAACCTG
Pluripotency			
POU5F1	ENSG00000204531	CGAAAGAGAAAGCGAACCAG	AACCACACTCGGACCACATC
NANOG	ENSG00000111704	CCTATGCCTGTGATTTGTGG	AAGTGGGTTGTTTGCCTTG
SOX2	ENSG00000181449	ACACCAATCCCATCCACT	CCTCCCAGGTTTTCTCTGT
REX1/ZFP42	ENSG00000179059	CGCGGTAACAGGGACAAATG	TGCAGGTCTTGGCTTGACTT
KLF4	ENSG00000136826	ACCTTCTTCACCCCTAGAGC	CGGTAGTGCCTGGTCAGTTC
Proliferation			
CCNA2	ENSG00000145386	AAGACGAGACGGGTTGC	GGCTGTTTACTGTTTGCTTCC
ERBB2	ENSG00000141736	GGCACAGTCTACAAGGGCA	ATTTCTTTGTTGGCTTTGGG
MKI67	ENSG00000148773	TGGGTCTGTTATTGATGAGCC	CATCAGGGTCAGAAGAGAAGC
ALDH Isotype			
ALDH1A2	ENSG00000128918	CCATTGGAGTGTGTGGACAG	GATGAGGGCTCCCATGTAGA
ALDH1A1	ENSG00000165092	GCACGCCAGACTTACCTGTC	CCTCCTCAGTTGCAGGATTAAG
ALDH1A3	ENSG00000184254	AAAAAGAGCGAATAGCACCG	GCATAGAGGGCGTTGTAGCA
Surface markers			
CD44	ENSG00000026508	GAAGAAGGTGTGGGCAGAAGA	ACCATTTCTGAGACTTGCTG
CD24	ENSG00000272398	GCTCCTACCCACGCAGATT	GCTCCTACCCACGCAGATT

A



B

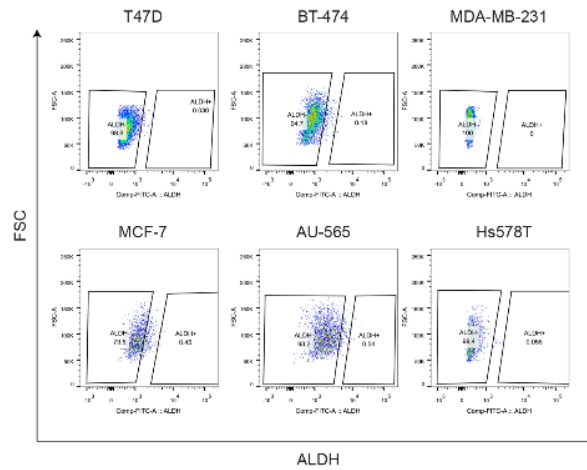


Figure S1: Gating strategy to isolate ALDH^{br}, CD44^{high}/CD24^{-low} and CD44^{-low}/CD24^{-low} populations (A) ALDH^{br} cells were the highest in HER2-OE cell lines BT-474 (~20%) and AU-565 (~6%); CD44^{-low}/CD24^{-low} in luminal T47D (~64%), MCF-7 (57%), BT-474 (~59%) and CD44^{high}/CD24^{-low} in triple negative cell lines Mda-MB-231 and Hs578T (~99%). Gating were set to DEAB inhibitor control (B).

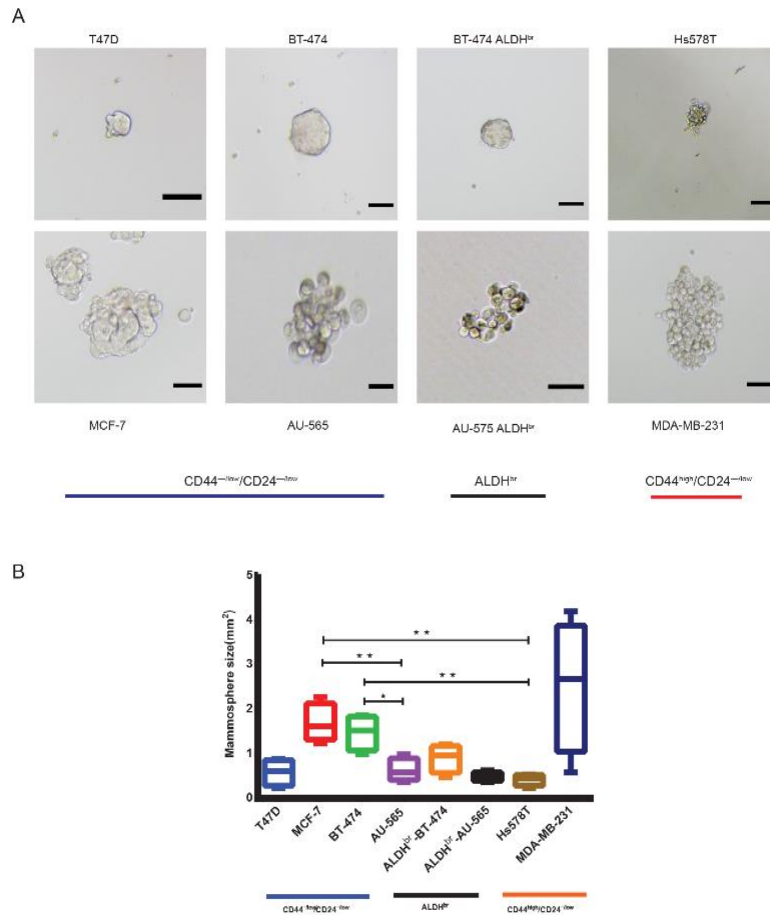
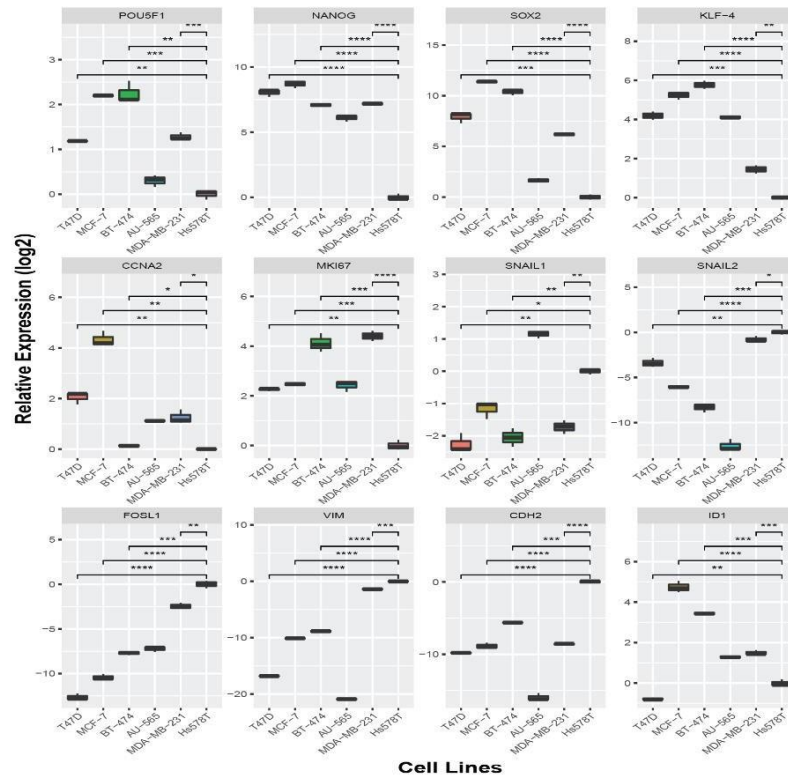


Figure S2: Luminal CD44^{-low}/CD24^{-low} cells form tightly packed spheres

(A). Representative images of primary mammospheres grown in serum deficient DMEM medium after 7 days. All subpopulations formed discernable spheres by day 4 of culture. Luminal (T47D, MCF-7 and BT-474) spheres consist cells more tightly packed together. Triple negative (MDA-MB-231, Hs578T) and AU-565 cell lines formed aggregates of loosely packed cells. Scale bar = 100 μ m. Sphere size comparison of CD44^{-low}/CD24^{-low}, CD44^{high}/CD24^{-low} and ALDH^{br} cell after 7 days in culture (B). CD44^{high}/CD24^{-low} cells from MDA-MB-231 formed the biggest aggregate spheres. CD44^{-low}/CD24^{-low} cells from MCF-7 and BT-474 formed significantly larger spheres than CD44^{high}/CD24^{-low} and ALDH^{br} cells from AU-565 and hs578T cell lines.). The data represent the mean \pm S.D. from three independent experiments performed in triplicate. * $P < 0.05$, ** $P < 0.01$

A



B

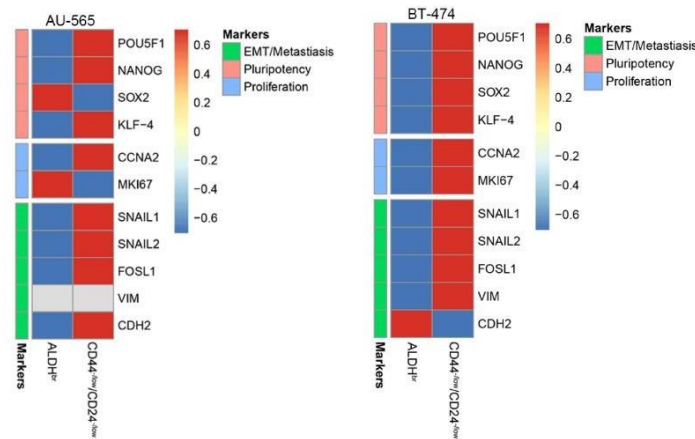


Figure S3: Relative expression of markers for pluripotency, proliferation and EMT/metastasis

(A) CD44^{-low}/CD24^{-low} luminal cells show overexpression of metastasis and proliferation marker but low expression of EMT markers compared to CD44^{high}/CD24^{-low} cells from triple negative cell line Hs578T. Both the triple negative cell lines show comparable expression of EMT genes. Hs578T has significantly lower expression of NANOG and MKI-67. (B) CD44^{-low}/CD24^{-low} cells from both HER2-OE cell lines BT-474 and AU-565 show marginal overexpression of metastasis, proliferation and EMT markers comparative to ALDH^{br} cells from their respective cell lines. All comparisons relative to bulk gene expression of respective cell lines. The data represents relative fold change (log₂) from three independent experiments. Fold change relative to Hs578T expression. **P*<0.05, ***P*<0.01, ****P*<0.001, *****P*<0.0001