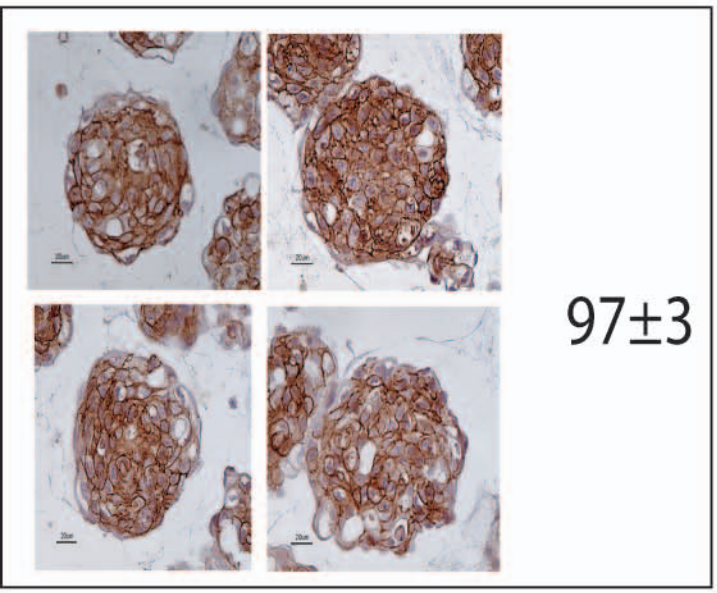
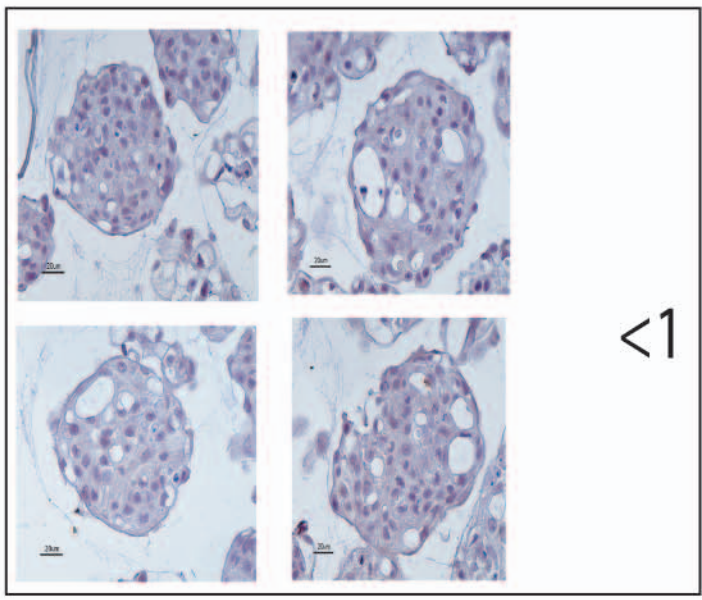
T-MS (sample 4)

CD44 (%)



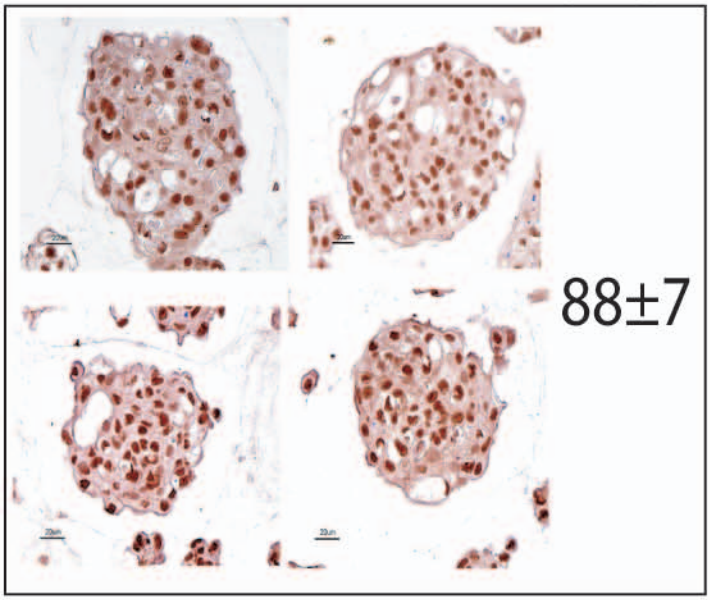
97±3

CD24 (%)



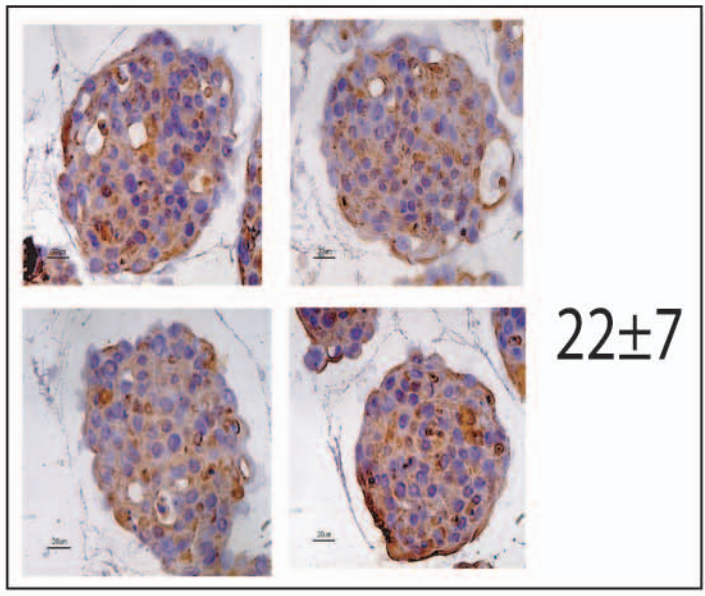
<1

Oct-4 (%)



88±7

CK-5 (%)



22±7

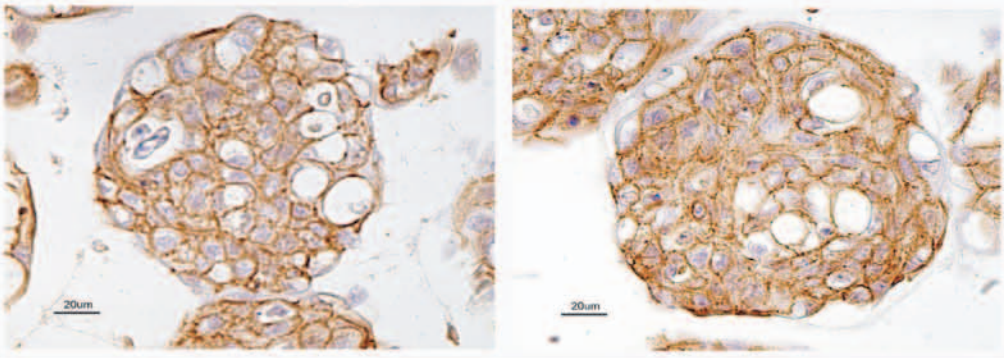
Immunohistochemistry (IHC): % of positive cells (±SD) is reported

Sansone et al, Supplementary Figure 1

T-MS (sample 4)

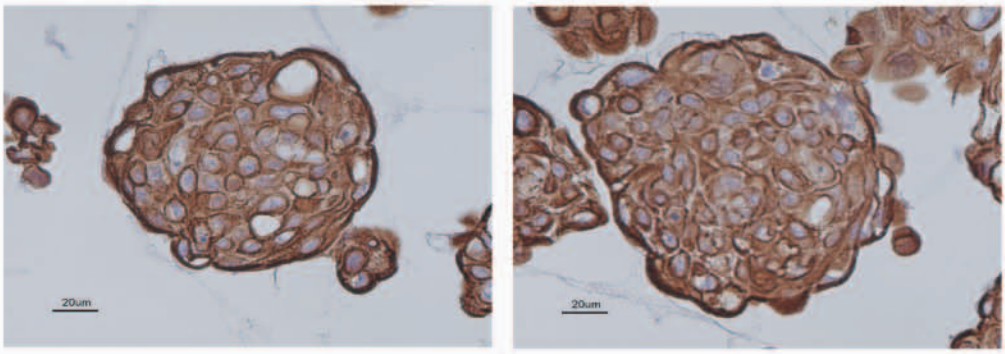
E-Cadherin

(97±2)



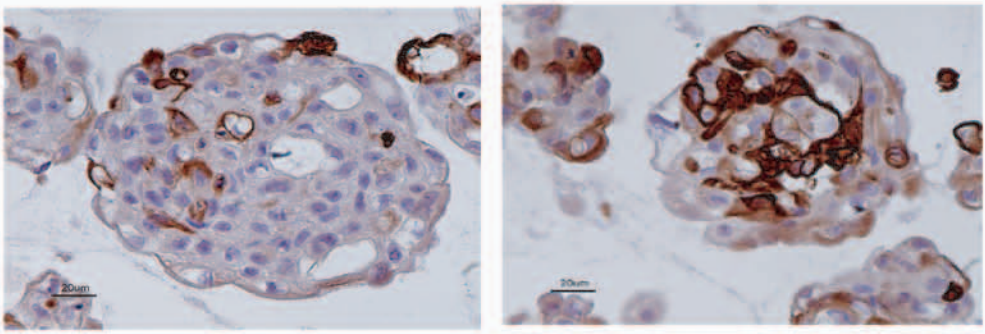
CK- 14

(99±1)



CK- 18

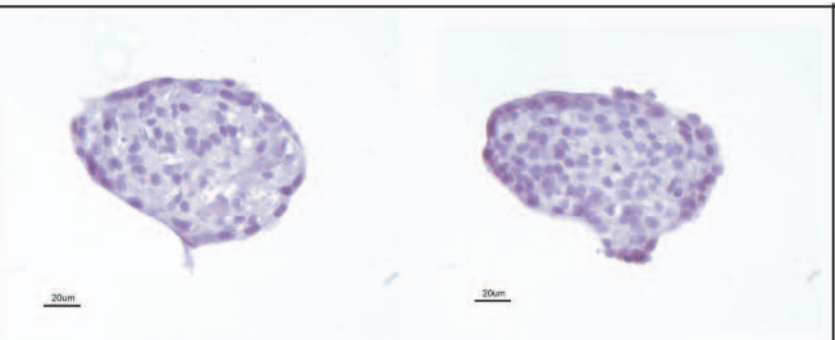
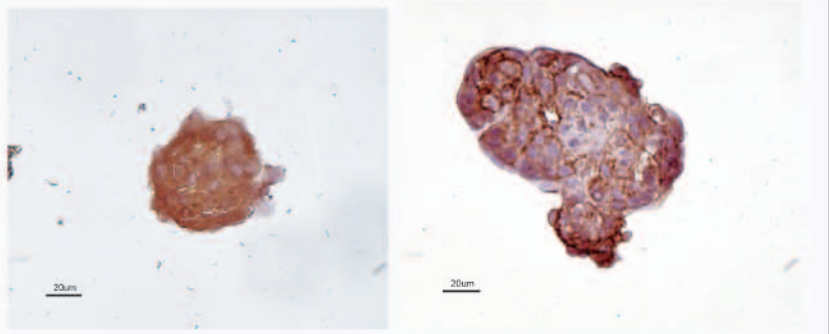
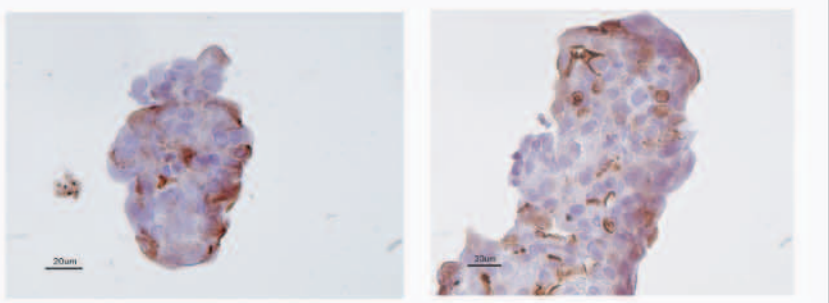
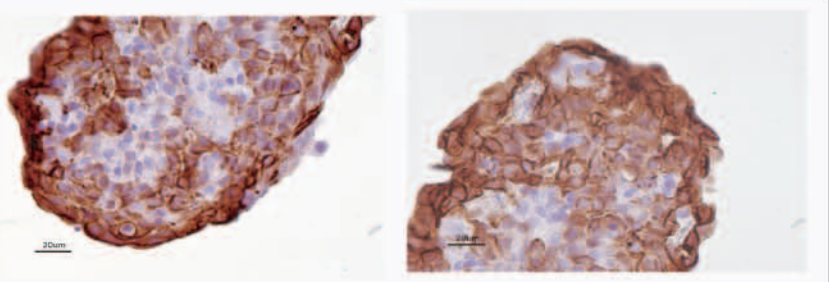
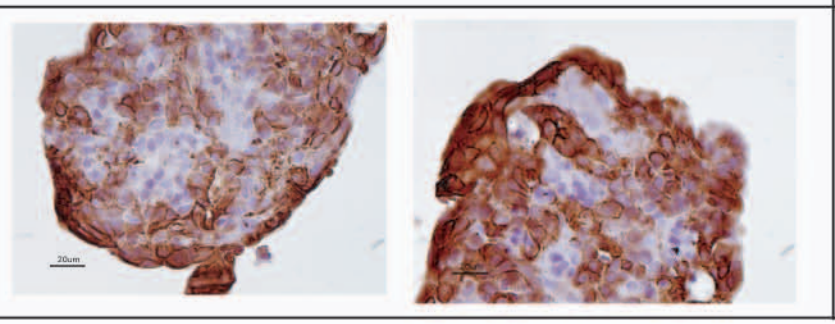
(24±7)



IHC (% of positive cells±SD is reported)

Sansone et al, Supplementary Figure 2

IHC N-MS (sample 4)

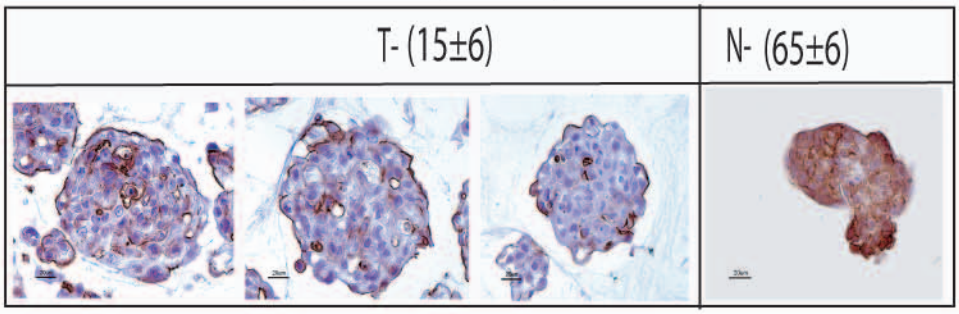
		(%±SD)
CD24		<1
CD44		95±3
CK-5		14±3
CK-14		78±7
CK-18		75±9

Sansone et al, Supplementary Figure 3

A

MS (Sample 4)

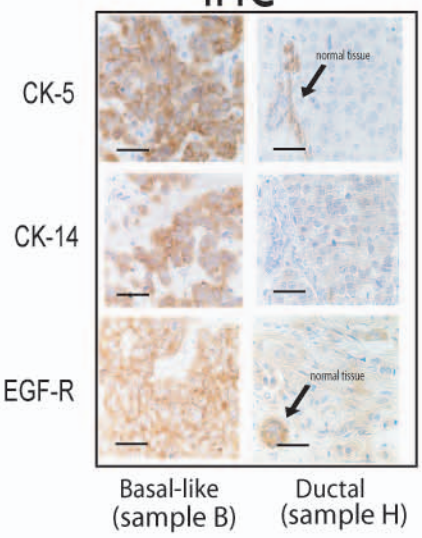
IHC (CD133, % of positive cells ±S.D.)



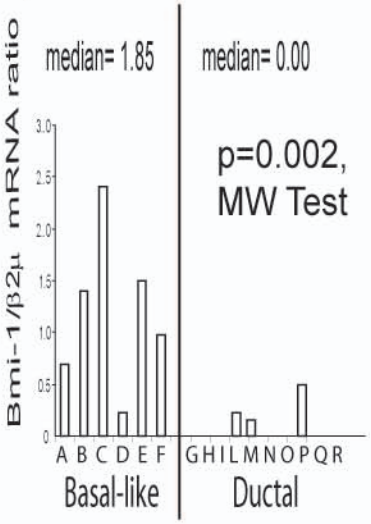
B

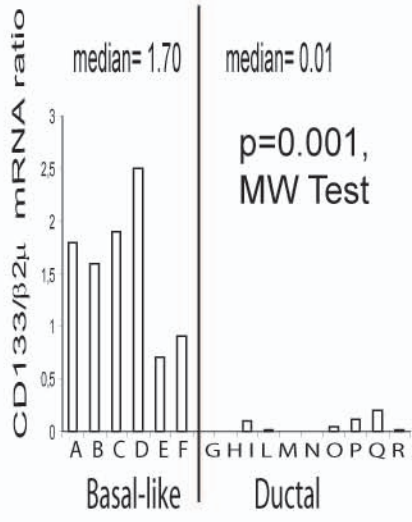
Breast carcinoma tissues

IHC



RT-PCR analysis

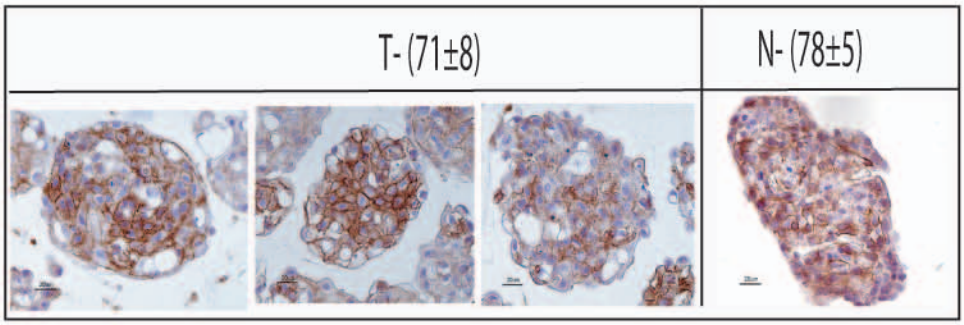




C

MS (Sample 4)

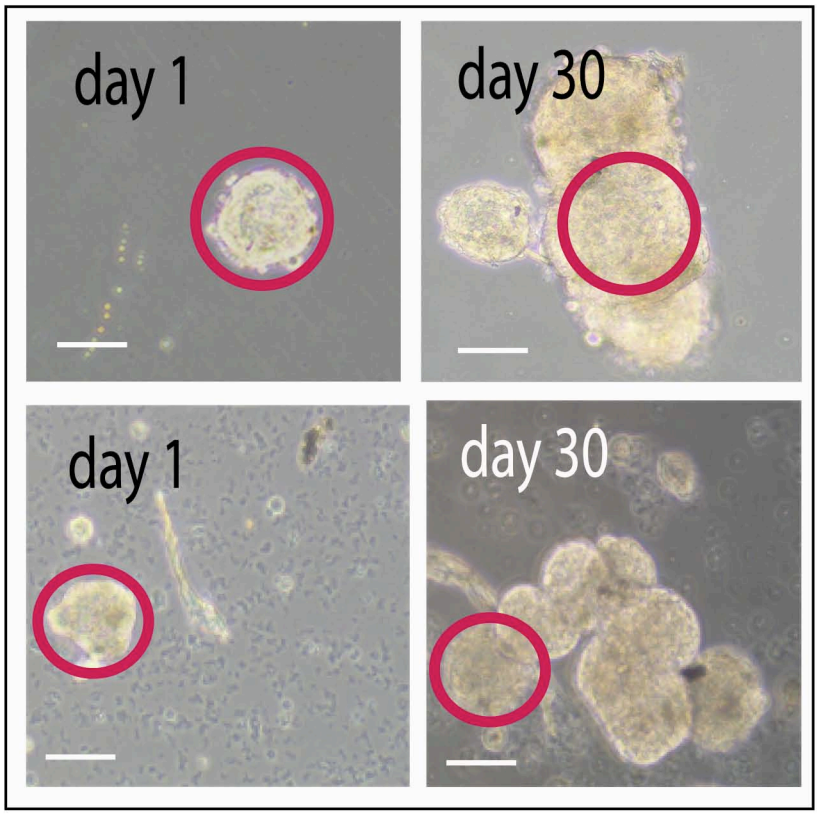
IHC (EGF-R, % of positive cells ±S.D.)



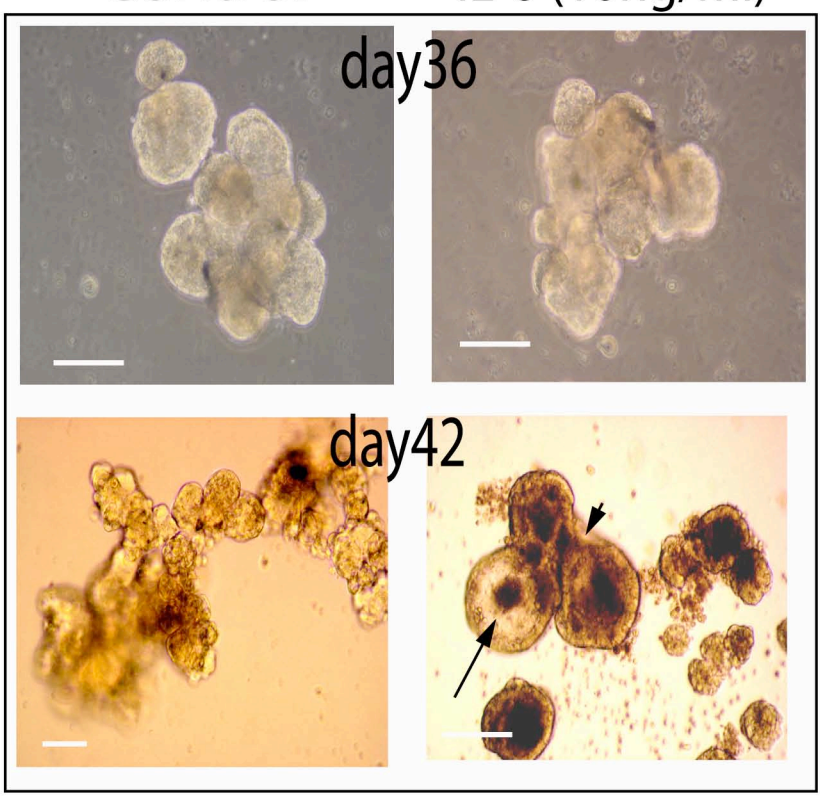
Sansone et al, Supplementary Figure 4

Normal sample 1: starting from entire MS.

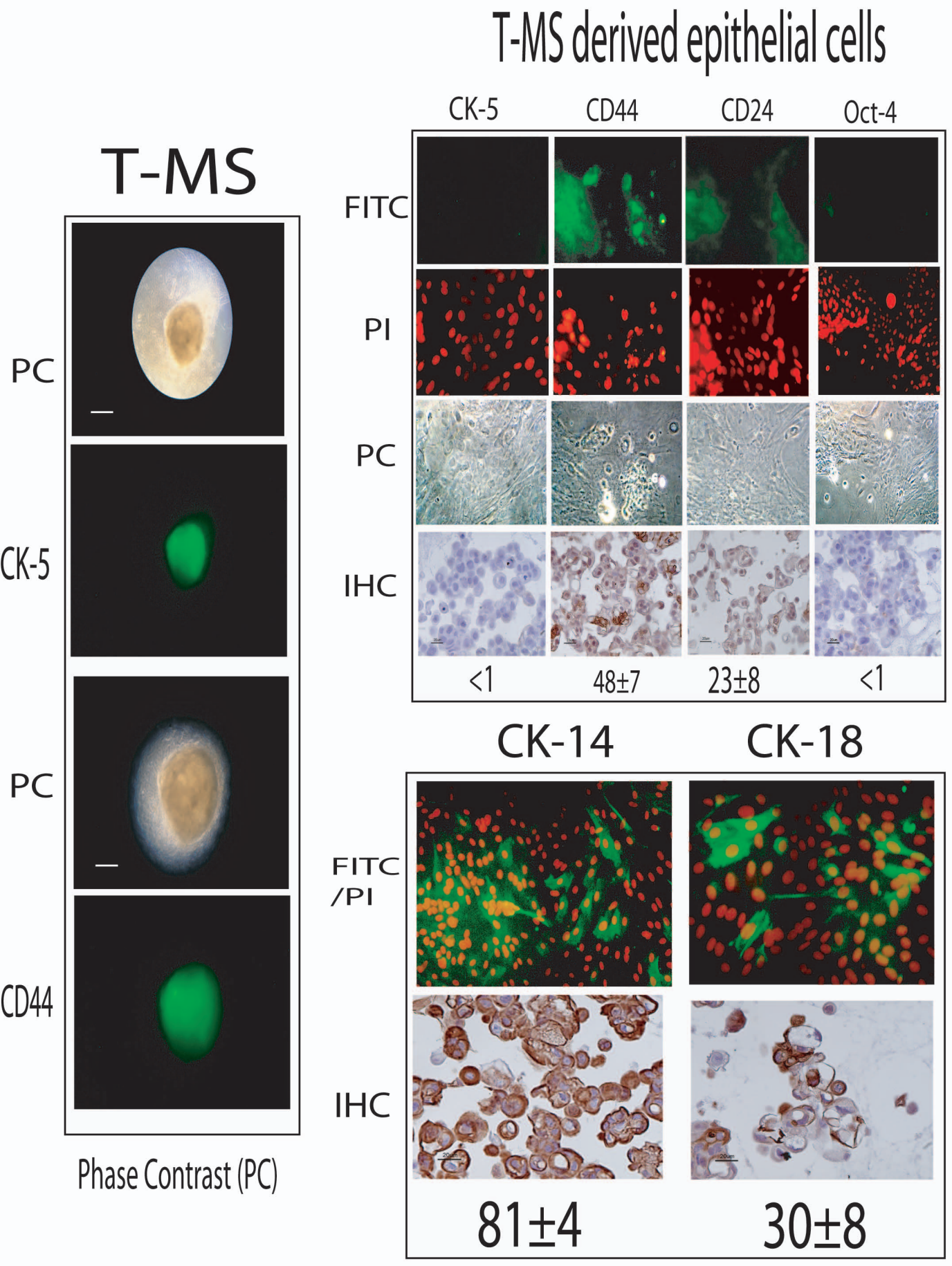
red circle represents the location of the seeded MS



Normal sample 2: starting from MS-derived cells



Sansone et al, Supplementary Figure 5



T-MS-derived cells plated on plastic: immunofluorescence (FITC), nuclear contrast (PI)
T-MS-derived cells in 3D culture: Immunohistochemistry (IHC): % of positive cells (±SD) is reported,

Sansone et al, Supplementary Figure 6

Supplementary Materials and Methods.

MS were generated from 2 breast cancer patients: Sample n.1 gave raise only to MS from the normal tissue, Sample n.2 generated MS from both normal and tumor tissues. 3D-assay was performed using Growth Factor Reduced Matrigel (BD Biosciences, Frankling Lakes, NJ), which was thawed overnight and kept at 4°C until use.

Day 14 normal MS from Sample 1 were re-suspended in 150µl complete MEGM containing 2% of Matrigel, and layered on the top of 300µl pre-solidified Matrigel, in 24 wells plates. IL-6 was added in one culture well, to a final concentration of 10ng/ml. Cultures were re-fed every 5 days with 150µl of MEGM 2% Matrigel.

Day 14 normal and tumor MS were tryspin dissociated and 2000 cells derived from each MS cultures were embedded in 600µl of cold Matrigel. 300µl of each cell suspension were seeded in 24 wells plates. IL-6 was added in one of each culture wells, to a final concentration of 10ng/ml. Cultures were re-fed every 5 days with 150µl of MEGM 2% Matrigel.

Similar protocols for MS and MS-derived cells 3D assay ,have been described (21, 24, 25). Cells from tumor MS were also allowed to grow on plastic at clonogenic density (10^2 x cm^2) and were then fixed in cold Methanol for 5 min for Immunofluoresence (IF) analysis. IHC analysis was performed on Matrigel cultures, re-suspended in 2% Agarose Fixed in Paraformaldeide and embedded in Paraffin, as described in Materials and Methods. For IF, FITC-conjugated antibodies were purchased from Santa Cruz. Propidium Iodide (Sigma) was used as nuclear counterstaining. Primary antibodies used for IHC and IF are described in Materials and Methods.