

A

Breast milk from women with breast cancer,
at high risk, mutation carriers, or healthy donors



Culture separately under modified epithelial reprogramming assay (Molecular Cancer Research 17:1556-1570);

Typically 5 cell passages in <2 weeks with 2-5 millions of cells and cryopreserve for future use.

Comparative analysis

1. Flow cytometry for CSC markers
2. CSC assays such as mammospheres
3. RNA -Seq
4. DNA sequencing
5. Copy number variation analysis

- 1) Detection of aberrant cells with driver mutations
2) Test the effects of drugs that may target driver mutations

Time required to complete assay ~30 days

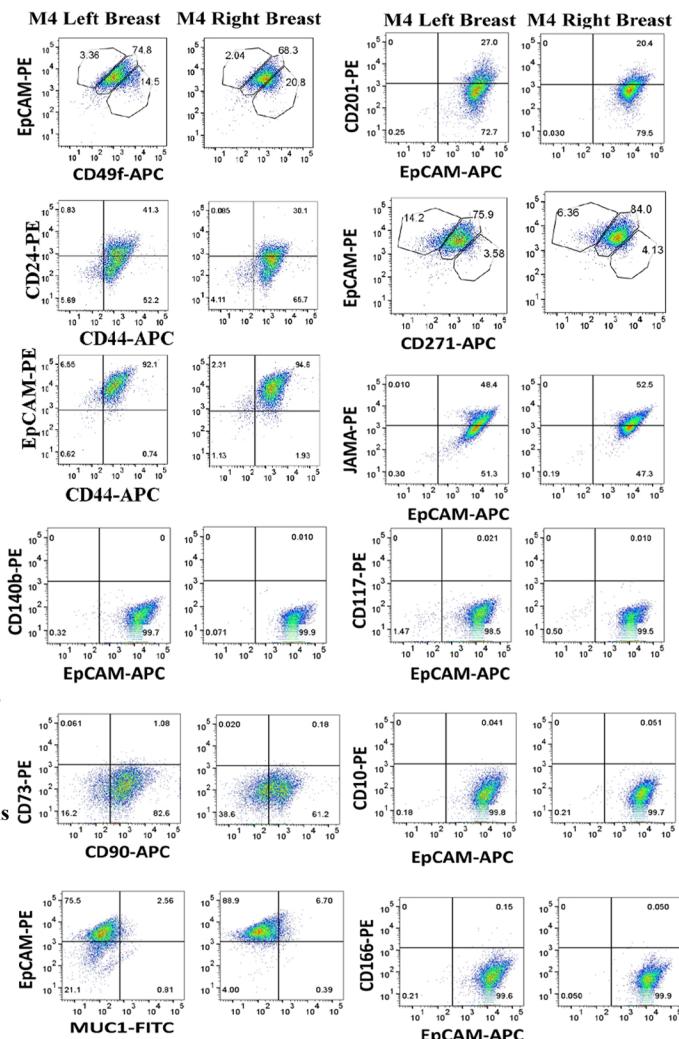
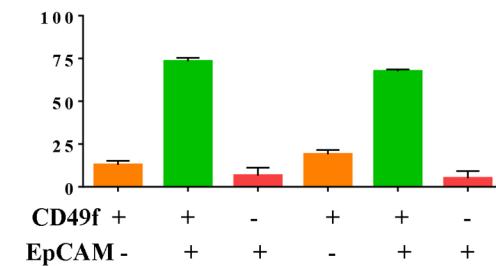
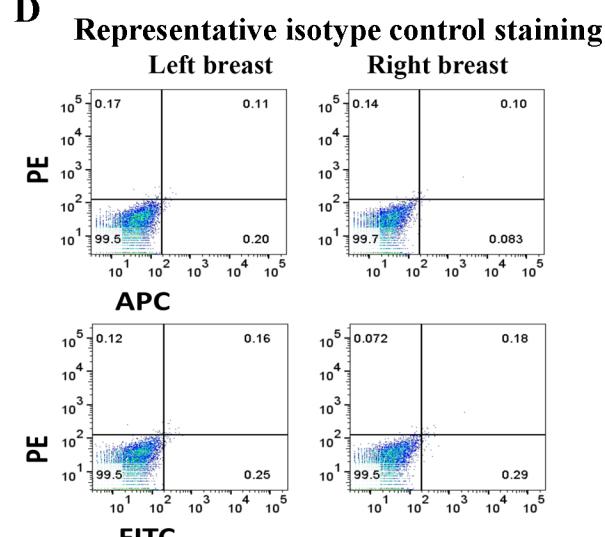
**C****D**

Fig. S1: Experimental design and cell surface marker profiles of breast milk derived cells of donor M4. A) Schematic view of the experimental design. B) Representative flow cytometry patterns of breast milk-derived cells of M4. C) Bar graphs showing similar CD49f/EpCAM and CD271/EpCAM staining patterns of breast milk-derived cells of this donor. D) Representative isotype antibody control staining patterns.

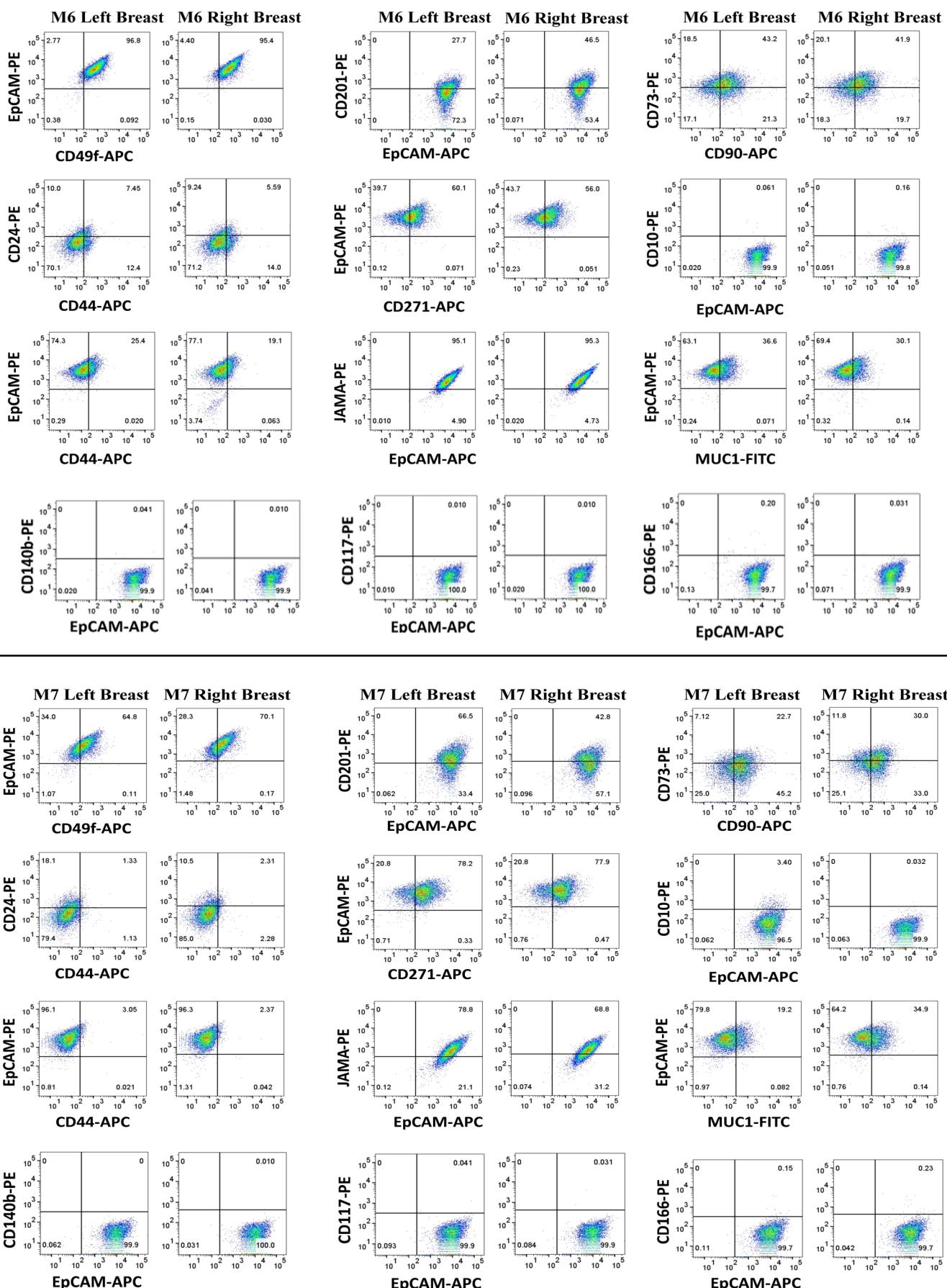


Fig. S2: Phenotypic similarities in cells derived from left and right breast milk of two other donors (M6 and M7). Assays are done as in Fig. S1.

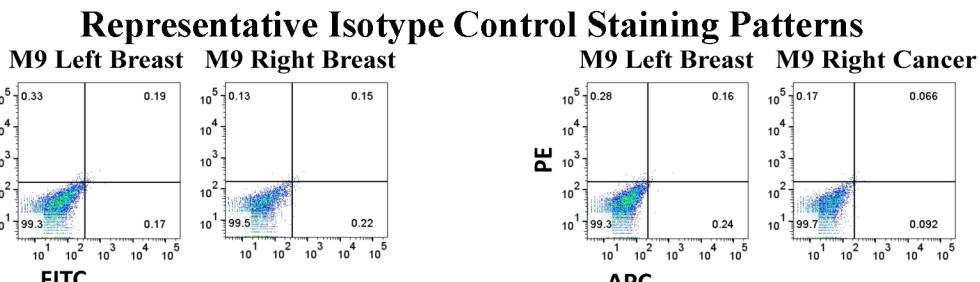
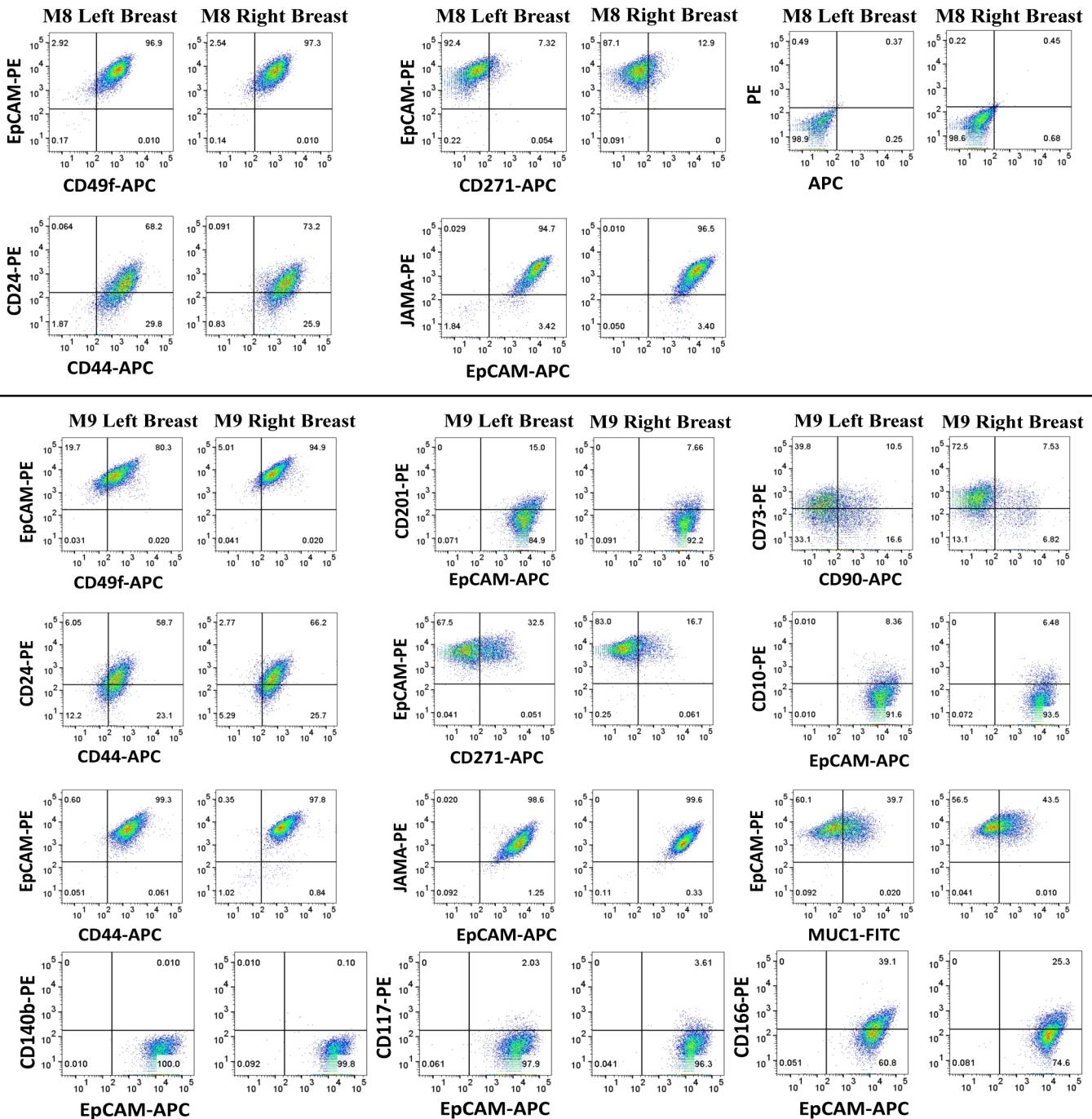


Fig. S3: Phenotypic similarities in cells derived from the left and the right breast milk of two other donors (M8 and M9). Note inter-individual differences in cell surface marker profiles, particularly for CD201/EpCAM and CD271/EpCAM.

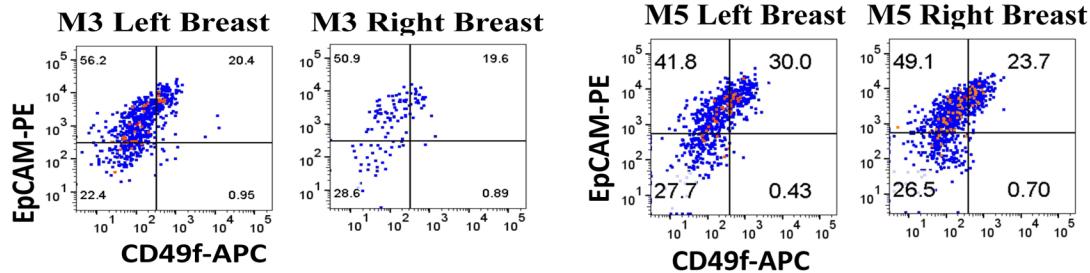
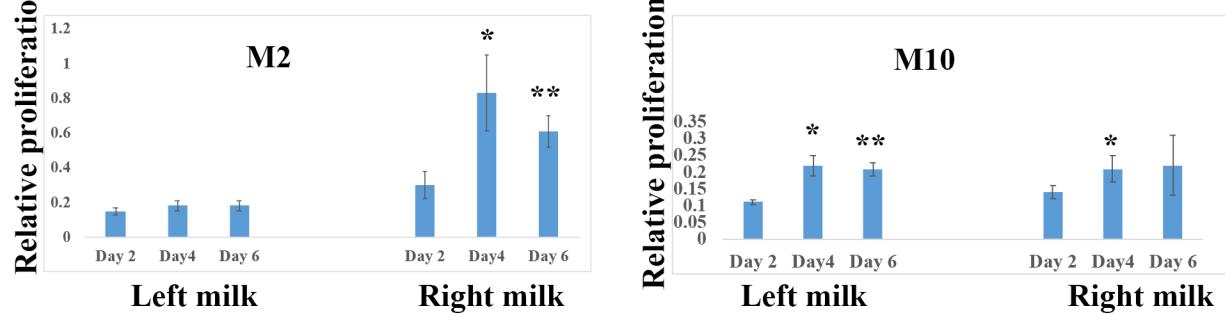
A**B**

Fig. S4: Breast milk-derived cells generate mammospheres. A) CD49f/EpCAM staining patterns of mammosphere-derived cells from M3 and M5. B) Growth rate of breast milk-derived cells of M2 and M10. *p<0.003; **p<0.0001 compared to proliferation rate at day 2.