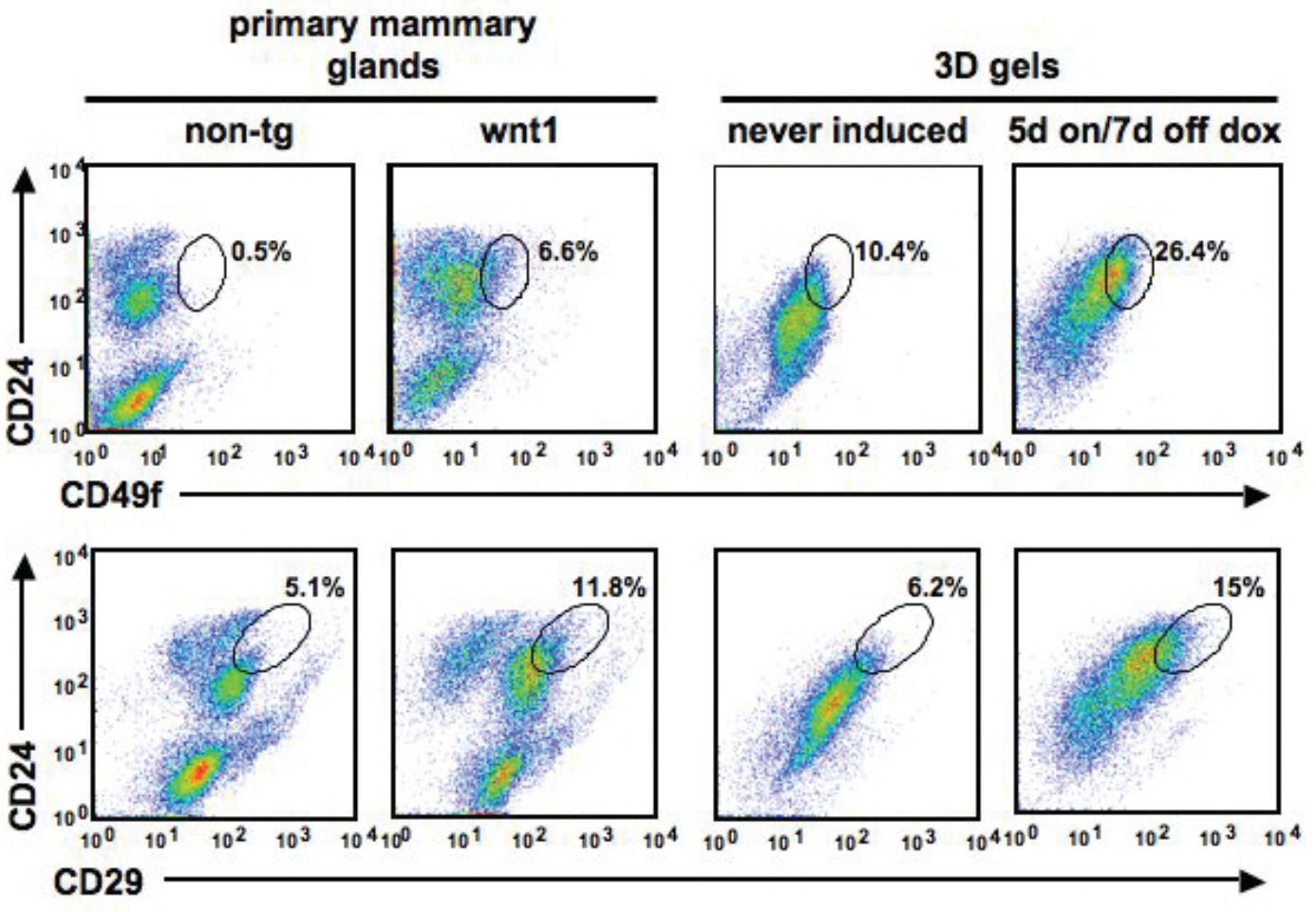

GENESDEV/2009/121111; FigureS4; Jechlinger et al.

Supplemental Figure 4 **Cells derived from the surviving rim are enriched for Lin^- $CD29^{hi}$ $CD24^+$ and Lin^-CD24^{med} $CD49f^{high}$ cells compared to cells derived from acini that had never been exposed to doxycycline**

Measurement by FACS of CD24, CD49f and CD29 expression on the surface of mammary epithelial cells after disaggregation of glands or after isolation of cells from the 3D culture system. Left panels: Non-transgenic mammary cells (1st column) and Wnt-1-expressing mammary cells from MMTV-Wnt-1 transgenic mice (2nd column) were used to establish the FACS gates---after exclusion of dead cells by 7AAD stain--- for $CD29^{hi}$ $CD24^+$ and $CD24^{med}$ $CD49f^{high}$ cells, respectively. Right panels: Distribution of 7AAD-negative cells derived from the 3D gels and percentage of $CD24^{med}$ $CD49f^{high}$ positive cells. Cells that were never exposed to doxycycline (3rd column) were kept in gels for 19 days before analyzed, cells surviving doxycycline withdrawal (4th column; cells grown for 7days without doxycycline, then for 5 days exposed to doxycycline, and subsequently for 7 days after doxycycline withdrawal) showed 2.5-fold enrichment for $CD24^{med}$ $CD49f^{high}$ positive cells and a 2.4-fold enrichment for $CD24^{med}$ $CD49f^{high}$ positive cells in the gates that had been established by analyzing freshly harvested mammary cells (Left panels).

Text to Supplemental Figure 4 We have detected the expected populations of progenitor cells in freshly prepared mammary cells from non transgenic animals and found that those fractions (Lin^-CD29^{hi} $CD24^+$ or Lin^-CD24^{med} $CD49f^{high}$) compose a larger portion of cells derived from hyperplastic mammary gland of MMTV-Wnt-1 transgenic mice than from wild-type mice, implying an enrichment in mammary stem/progenitor cells consistent with earlier reports using these stem cell markers (Stingl et al., 2006; *Nature* **439**(7079): 993-997 ; Shackleton et al., 2006; *Nature* **439**(7072): 84-88).

These findings with non-transgenic mammary cells and Wnt1-expressing mammary cells were then used to establish FACS gates to analyze cells derived from 3D cultures. Cells derived from 3D gels that were never exposed to doxycycline as well as from gels with spheres of cells surviving deinduction did not split into distinct subpopulations when stained for CD24/CD29 or CD24/CD49f. This may be due to suboptimal performance of these stem cell markers in long-term cultured cells. For example, work from the Lodish laboratory shows that the surface phenotype of ex-vivo-expanded hematopoetic stem sells (HSCs) is different from that of freshly isolated HSCs, but this plasticity of surface phenotype does not significantly alter their repopulation capability (Zhang and Lodish 2005). Despite this caveat, we observed a 2.5-fold enrichment of Lin^-CD29^{hi} $CD24^+$ and Lin^-CD24^{med} $CD49f^{high}$ cells within the established gates for the cells derived from the surviving rim, compared to the number of cells derived from acini that had never been exposed to doxycycline. These results are in line with the enhanced capacity of the surviving cells to reform acini and to repopulate the cleared mammary fat pad, as reported in this manuscript.

LITERATURE

Zhang, C.C. and Lodish, H.F. 2005. Murine hematopoietic stem cells change their surface phenotype during ex vivo expansion. *Blood* **105**(11): 4314-4320.