# Expression of Stage Specific Embryonic Antigen-4 (SSEA-4) defines spontaneous loss of epithelial phenotype in human solid tumor cells

**Running title: SSEA-4 defines changes in epithelial phenotype of solid tumors**

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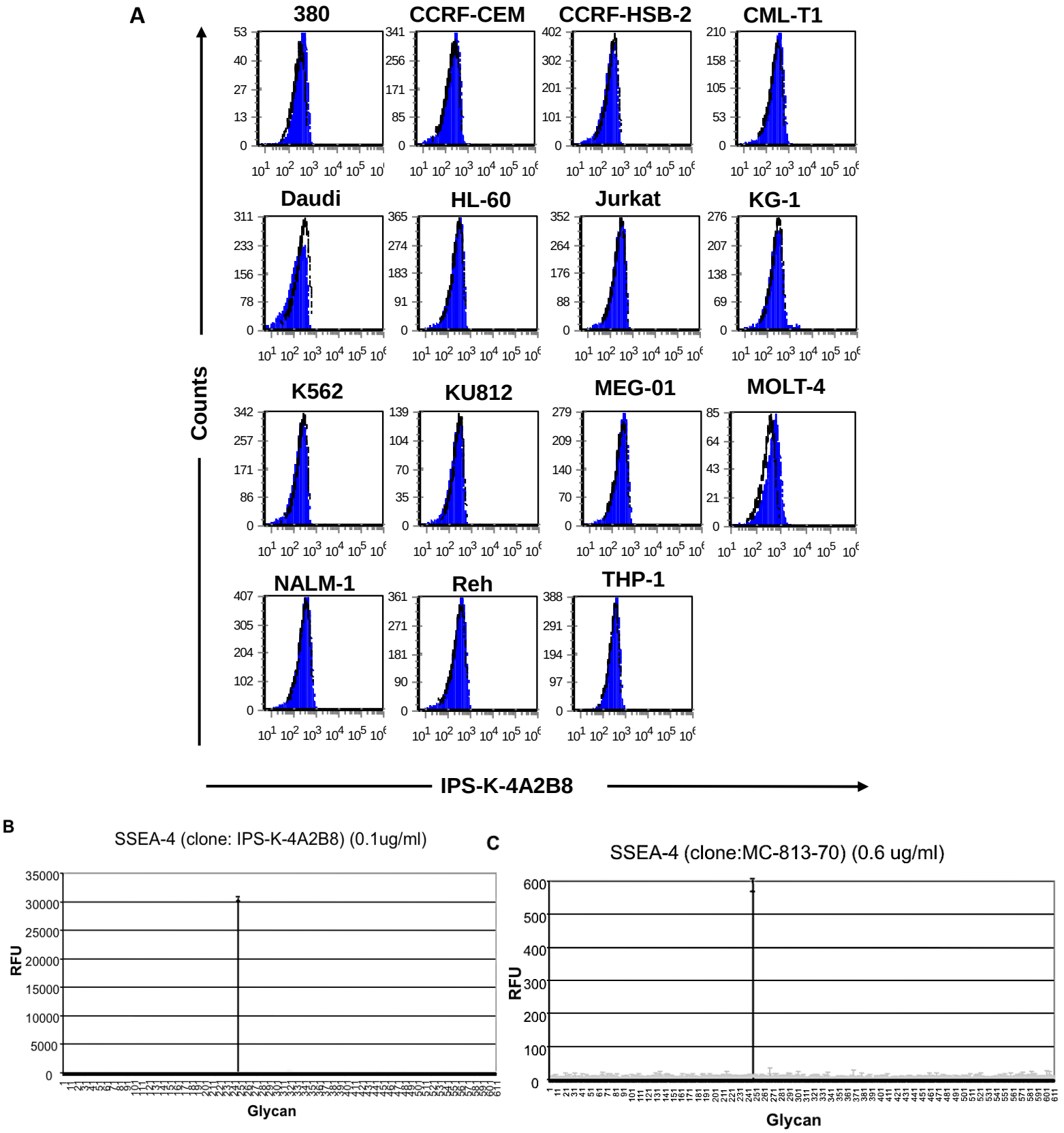
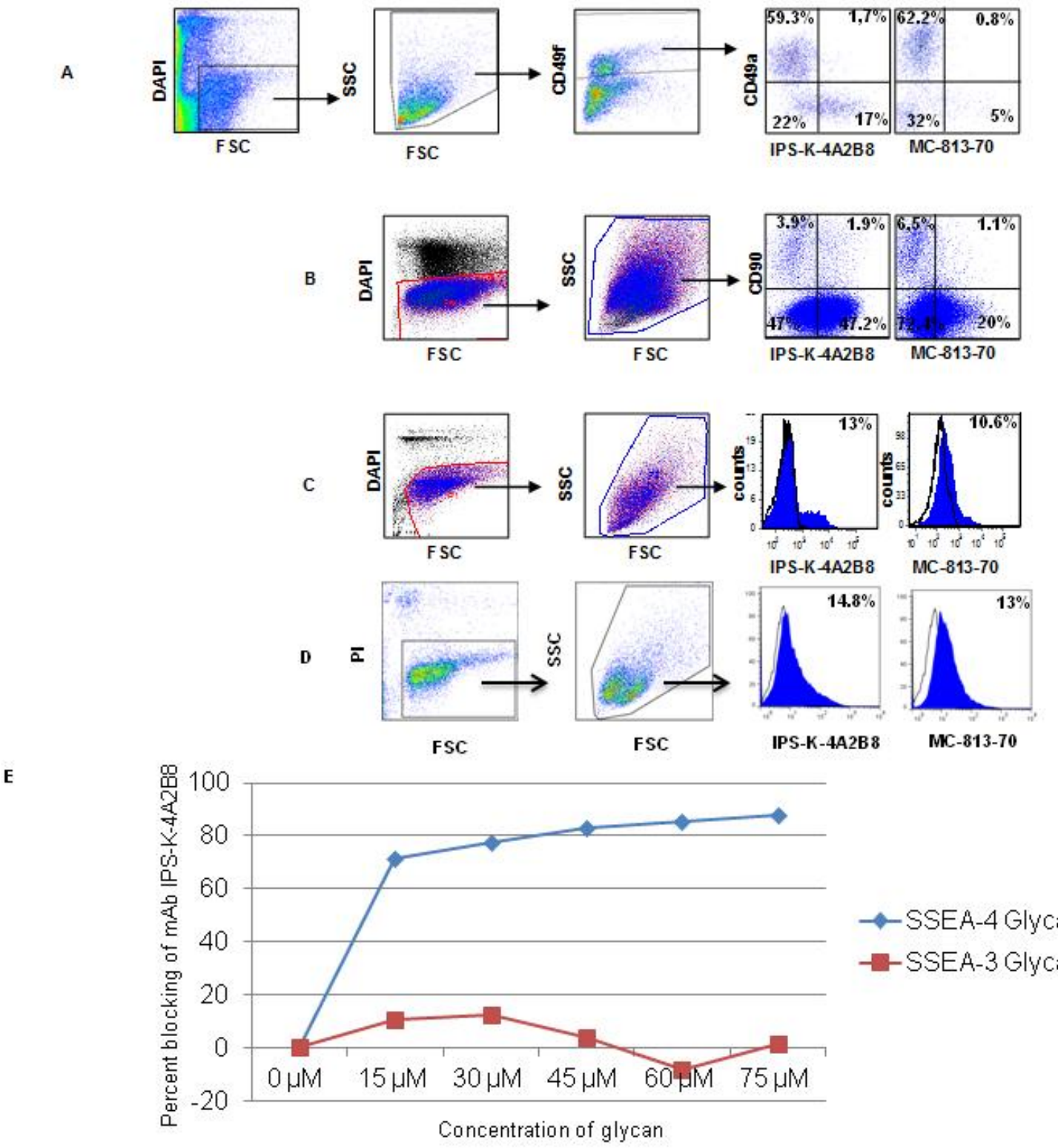
## Supplementary Figure 1



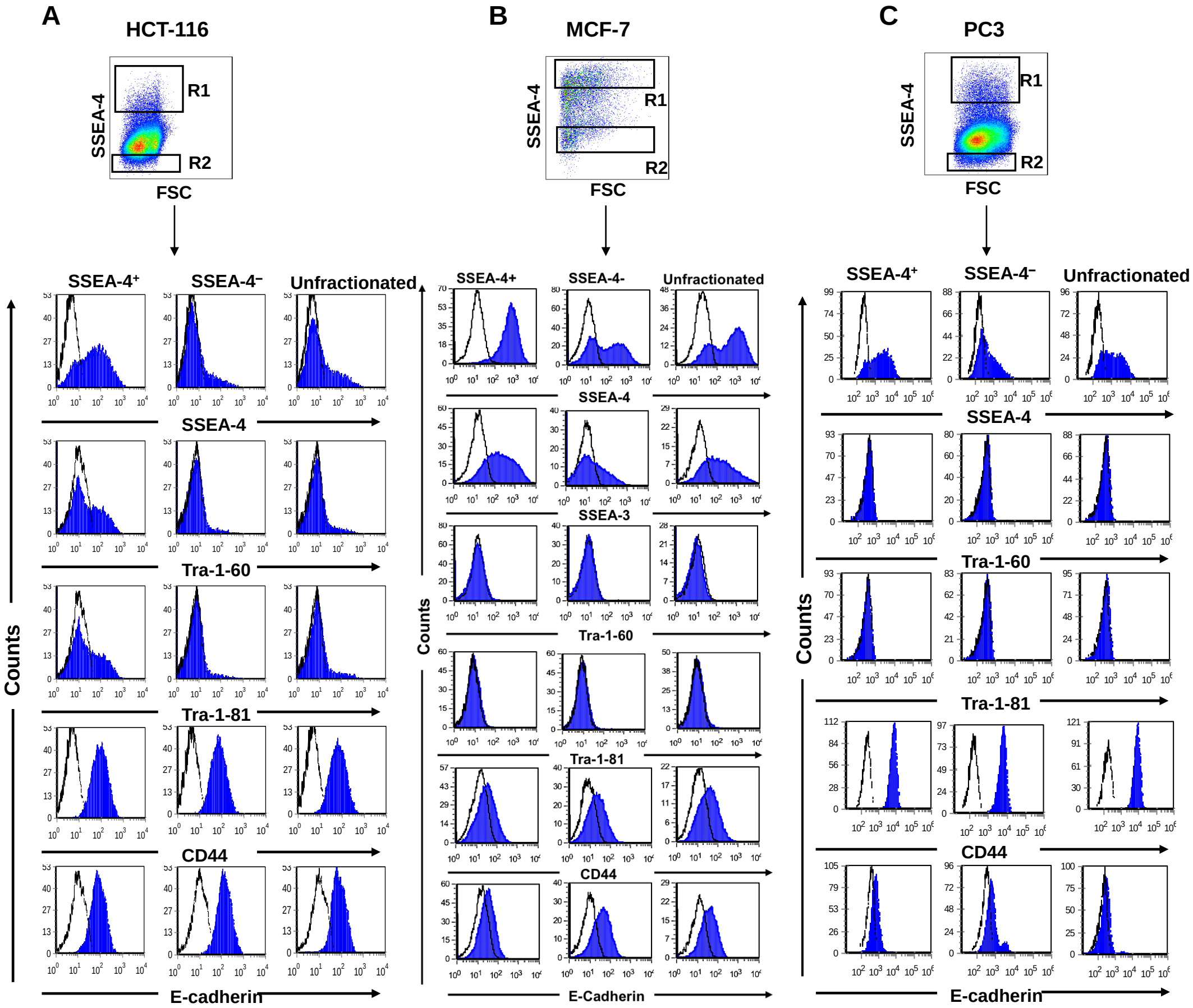

**Supplementary Figure 1. (A) Reactivity profiles of mAb IPS-K-4A2B8 on leukemic cell lines.** Cells were labeled with mAb IPS-K-4A2B8 using indirect immunofluorescence staining as described in the supplementary methods. Cells were analyzed on a FACSCanto flow cytometer. Data were processed using the FCS Express software. Note that the antibody IPS-K-4A2B8 does not react with any of the tested leukemic cell lines. **(B, C) Binding of mAb IPS-K-4A2B8 and mAb MC-813-70 to 611 different carbohydrate structures on a glycan array chip.** The value of relative fluorescence units (RFU) is related to the amount of antibody bound to each of the individual glycans. Note that mAb IPS-K-4A2B8 (B) and mAb MC813 (C) bind only to one glycan corresponding to SSEA-4 with RFU values of 30,000 and 600 respectively, indicating a 50 times stronger binding of IPS-K-4A2B8 compared to MC-813-70.

## Supplementary Figure 2



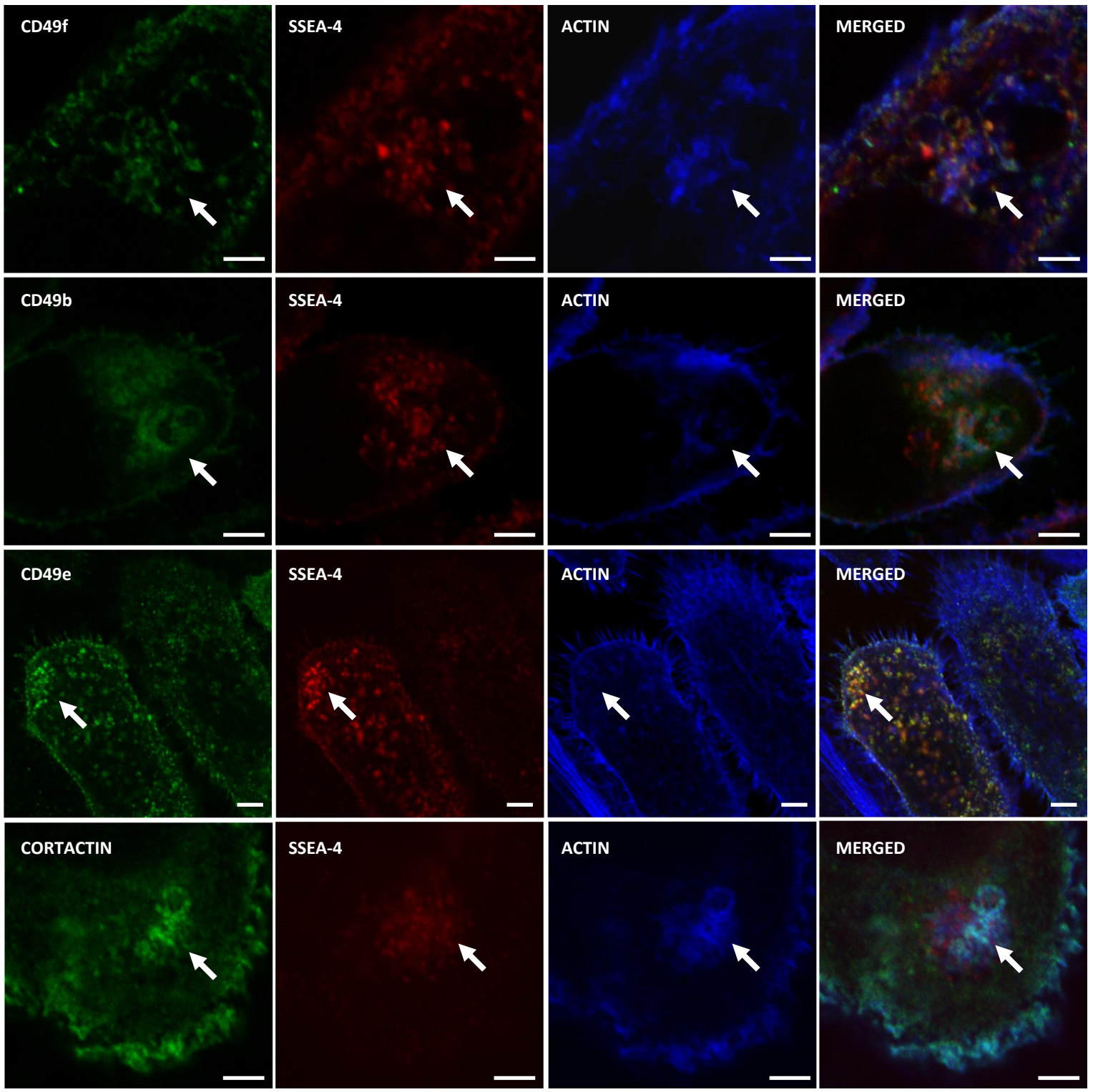

**Supplementary Figure 2. (A-D) Reactivity profiles of SSEA-4 specific antibodies IPS-K-4A2B8 and MC-813-70. (A)** Enzymatically isolated cells from human testis were stained with CD49f-FITC, CD49a-Alexa Fluor 647 and IPS-K-4A2B8-PE or MC-813-70-PE. Cells were gated on $CD49f^+$ cells and analyzed for coexpression of CD49a and SSEA-4. **(B)** Enzymatically isolated cells from amniotic membrane of human placenta were stained with CD90 and SSEA-4 specific IPS-K-4A2B8-PE or MC-813-70-PE. **(C)** Cultured cells (passage 2) derived from amniotic membrane of placenta were stained with IPS-4A2B8-PE or MC-813-70-PE. Note that the reactivity of IPS-4A2B8 is stronger than MC-813-70 in all the analyzed cell types. **(D)** Reactivity profile of IPS-K-4A2B8-PE or MC-813-70-PE on DU145 cell line **(E)** Blocking of the binding of mAb IPS-K-4A2B8 to NT-2 cells by SSEA-3 and SSEA-4 glycan.

**Supplementary Figure 3**



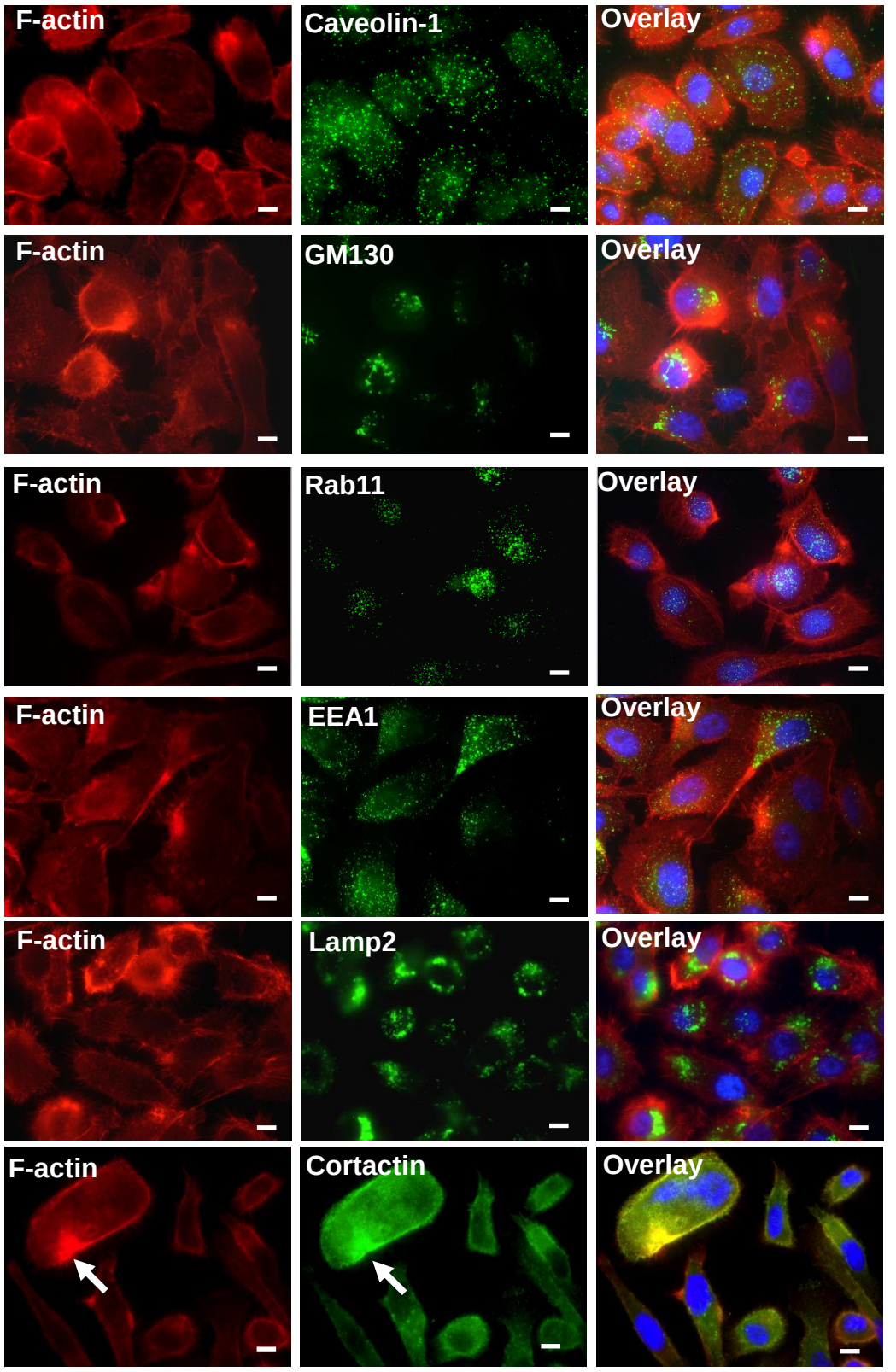

**Supplementary Figure 3. SSEA-4 defines a distinct subpopulation of HCT-116, MCF-7 and PC3 cells.** Display of SSEA-4 expression on **(A)** HCT-116, **(B)** MCF-7 and **(C)** PC3 cells  cells. Sort windows R1 and R2 were set and cells were sorted on a FACS Aria cell sorter. Sorted cells were cultured for 4- 11 days, followed by phenotypic characterization by FACS analysis.

## Supplementary Figure 4




**Supplementary figure 4. Localization of SSEA-4, F-actin, cortactin and integrins on PC3 cells.** Three color staining of adherent PC3 cells with phalloidin (F-actin), IPS-K-4A2B8 mAb and anti-integrins or anti-cortactin shows that CD49f, CD49e, CD49b and cortactin colocalize with SSEA-4 and F-actin accumulating spots. Arrows point to accumulation of integrins as well as cortactin at F-actin and SSEA-4 accumulating structures. Observed under 100x magnification in Zeiss LSM 510 META confocal laser scanning microscope . Scale bars: 5 μm

## Supplementary Figure 5



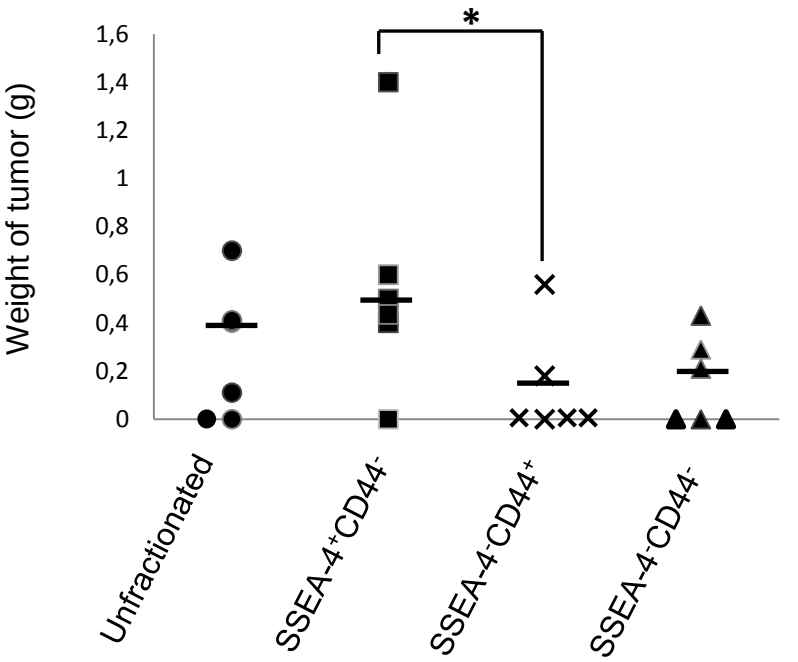

**Supplementary Figure 5. Localization of F-actin and organelle specific markers on PC3 cells.** Adherent PC3 cells were stained for organelle specific markers like caveolin-1 (caveolae), GM130 (Golgi apparatus), Rab11 (recycling endosomes), EEA1 (early endosomes), Lamp2 (lysosomes) or cortactin (invadopodia) and F-actin. F-actin accumulating puncta showed no colocalization with caveolin-1, GM130, Rab11, EEA1 and Lamp2 suggesting that F-actin accumulating structures do not correspond to caveolae, Golgi apparatus, recycling endosomes, early endosomes and lysosomes. In contrast, F-actin strongly colocalized with cortactin (marked by ↑) indicating that F-actin accumulating puncta correspond to invadopodia. Scale bars: 10 µm.

## Supplementary Figure 6

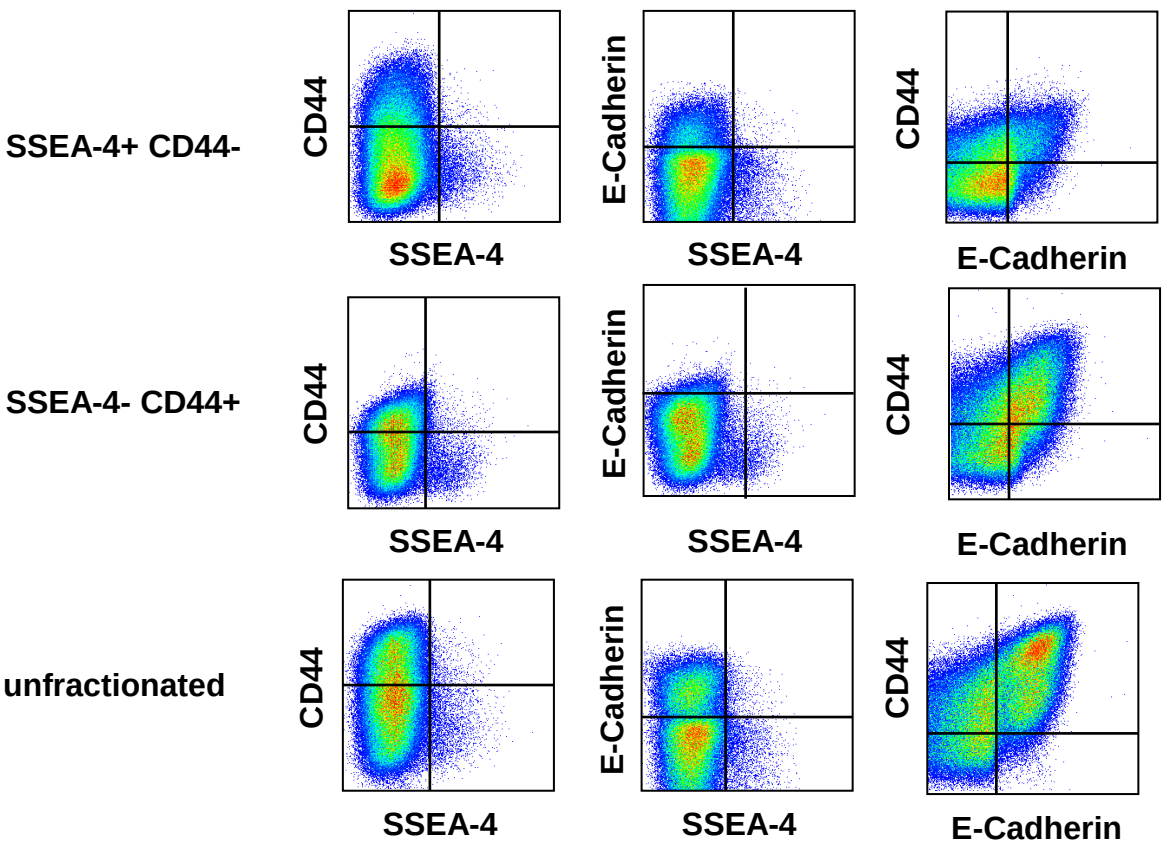
**A**

| Cell type | SSEA-4$^{+}$ CD44$^{-}$ (3000 Cells) | SSEA-4$^{-}$ CD44$^{+}$ (3000 Cells) | SSEA-4- CD44- (3000 Cells) | Unfractionated (3000 cells) |
|---|---|---|---|---|
| No.of sites of tumor formation | 5/6 | 2/6 | 3/6 | 4/6 |

**B**




**C**




**Supplementary Figure 6. Tumorigenicity of SSEA-4$^{+/-}$ cells (A)** Serial subcutaneous transplantation of SSEA-4$^{+}$CD44$^{-}$, SSEA-4$^{-}$CD44$^{+}$, SSEA-4$^{-}$CD44$^{-}$ and unfractionated DU145 cells isolated from xenografts derived from unfractionated DU145 cells show higher efficiency of tumor induction by SSEA-4$^{+}$CD44$^{-}$ cells. **(B)** Graph of statistical dot plot showing the frequency and the size of tumors generated by the marker-positive or negative cells at 80 days following transplantation. **(C) SSEA4$^{+}$ cells spontaneously give rise to SSEA4$^{-}$ cells in vivo or vice versa .** Xenografts derived from SSEA-4$^{+}$CD44$^{-}$, SSEA-4$^{-}$CD44$^{+}$ and unfractionated DU145 cells transplanted subcutaneously into NSG mice were harvested 80 days after transplantation. Tumors were dissected and digested into single cell suspension. The cells were stained for SSEA-4 and E-Cadherin or CD44 and analyzed by flow cytometry.