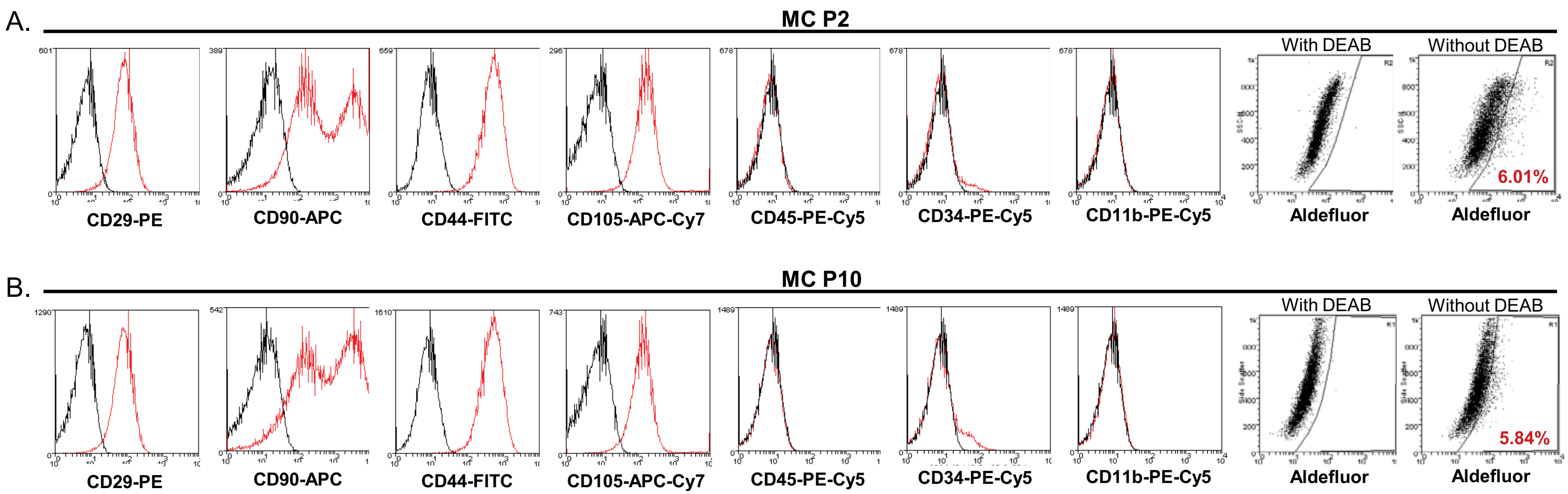

Figure S1. Characterization of MC with MSC markers and Aldefluor assay

MC from Passage 2 and Passage 10 were analyzed for different surface markers (CD29, CD90, CD44, CD105, CD45, CD34 and CD11b) which are shown in the histograms (Black: Isotype control; Red: Test) and the Aldefluor assay.

(A) Representative results of flow cytometry analysis of MC at passage 2.

(B) Representative results of flow cytometry analysis of MC at passage 10.

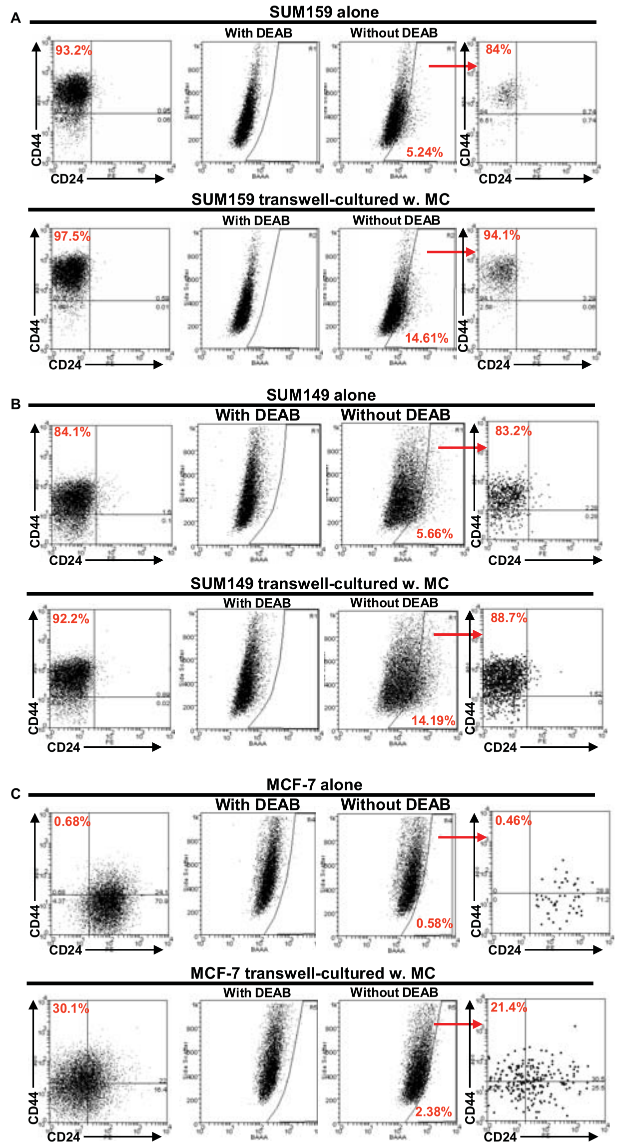

Figure S2. MC increases breast cancer stem/progenitor cell in the transwell-culture
(A) Effects of MCs on the $CD24^{-}CD44^{+}$ population (*Left*), ALDEFLUOR-positive population (*Middle*) and CD24-CD44+ population from ALDEFLUOR-positive population of SUM159 cells.
(B) Effects of MCs on the CD24-CD44+ population (*Left*), ALDEFLUOR-positive population (*Middle*) and CD24-CD44+ population from ALDEFLUOR-positive population of SUM149 cells.
(C) Effects of MCs on the CD24-CD44+ population (*Left*), ALDEFLUOR-positive population (*Middle*) and CD24-CD44+ population from ALDEFLUOR-positive population of MCF-7 cells.

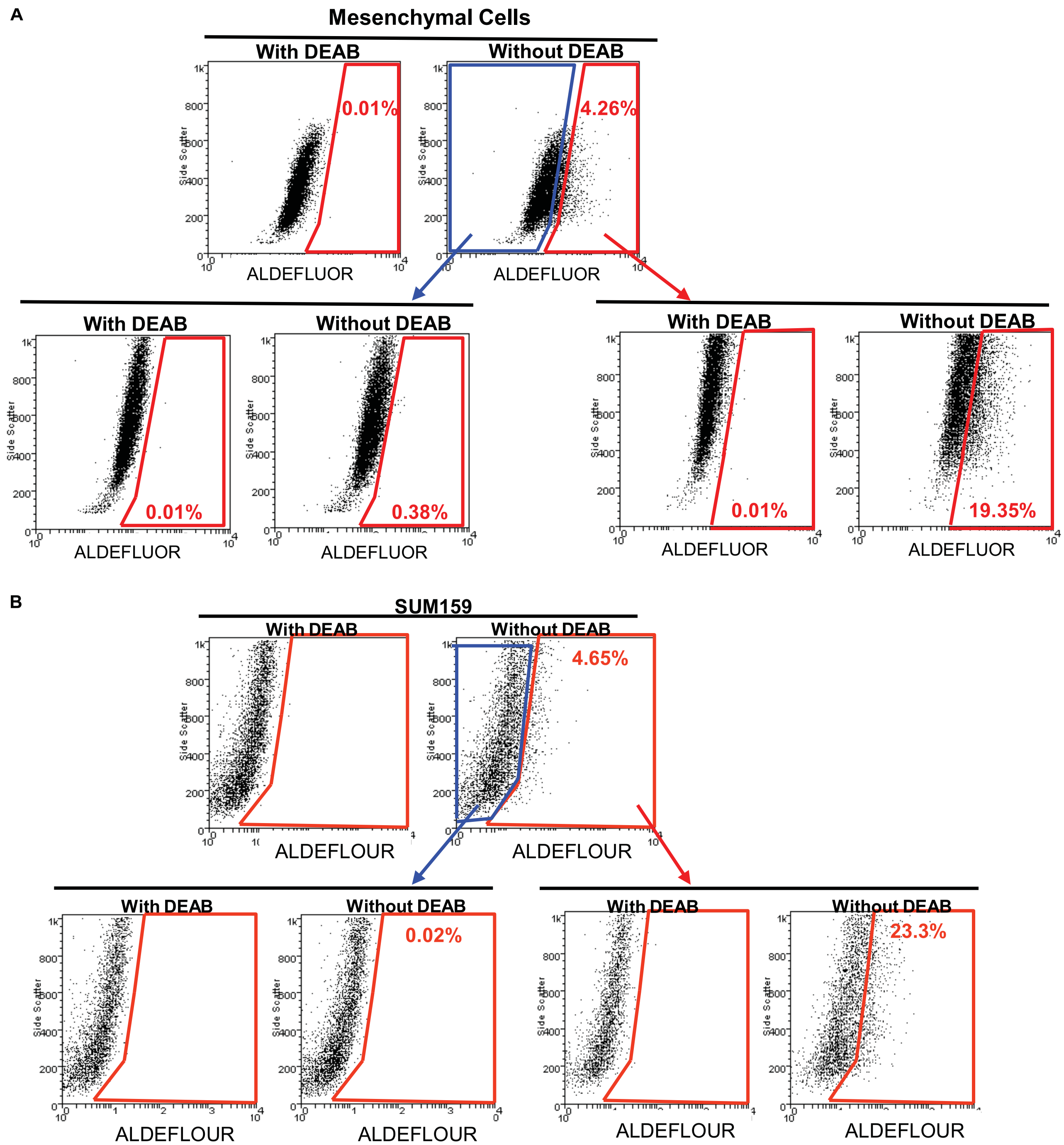

Figure S3. ALDEFLUOR-positive cells can regenerate both ALDEFLUOR-positive and ALDEFLUOR-negative cells

A. ALDEFLUOR-positive MCs can regenerate both ALDEFLUOR-positive and ALDEFLUOR negative MCs. ALDEFLUOR-positive and ALDEFLUOR-negative MCs were sorted and cultured in 10-cm plates in regular MSCM. After 3 days, the cells were collected and were analyzed by ALDEFLUOR assay. ALDEFLUOR-positive MCs can regenerate both ALDEFLUOR-positive and ALDEFLUOR-negative MCs, but ALDEFLUOR-negative MCs can not regenerate ALDEFLUOR-positive MCs.

B. ALDEFLUOR-positive SUM159 can regenerate both ALDEFLUOR-positive and ALDEFLUOR-negative SUM159. ALDEFLUOR-positive and ALDEFLUOR-negative SUM159 were sorted and cultured in 10-cm plates in regular medium. After 3 days, the cells were collected and were analyzed by ALDEFLUOR assay. ALDEFLUOR-positive SUM159 can regenerate both ALDEFLUOR-positive and ALDEFLUOR-negative SUM159, but ALDEFLUOR-negative SUM159 can not regenerate ALDEFLUOR-positive SUM159.

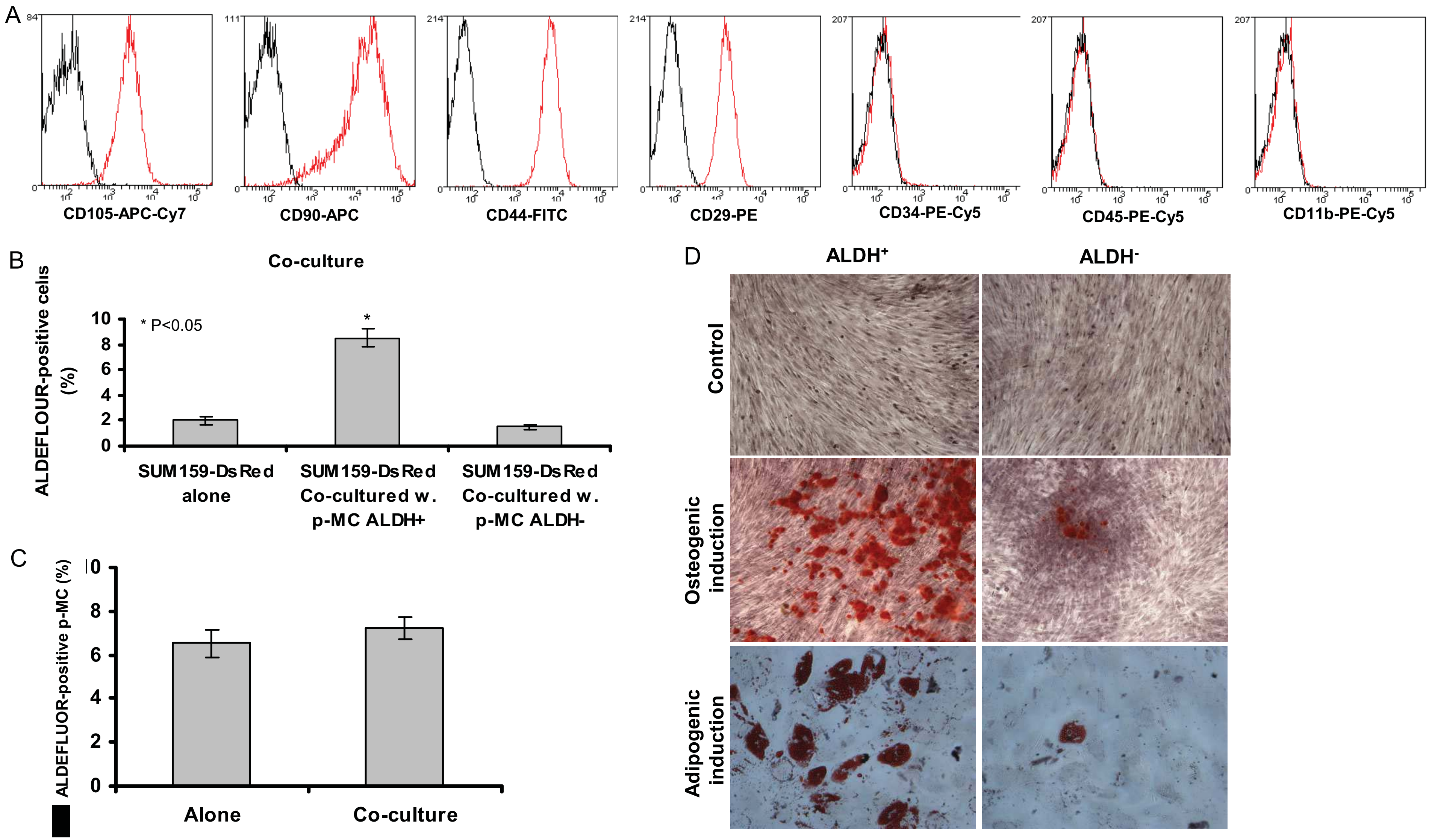

Figure S4. ALDEFLUOR-positive primary Mesenchymal cells (p-MSCs) regulate breast cancer stem cell populations and are capable of Osteogenic and Adipogenic differentiation

(A) Primary MCs were analyzed for different surface markers (CD29, CD90, CD44, CD105, CD45, CD34 and CD11b) (Black: Isotype control; Red: Test).

(B) Effects of ALDEFLUOR-positive (ALDH+) or ALDEFLUOR-negative (ALDH-) primary MCs on the percent of ALDEFLUOR positivity of SUM159 cells. ALDEFLUOR-positive MCs increase ALDEFLUOR-positive cancer cells.

(C) Effects of SUM159 cells on ALDEFLUOR-positive population of primary MCs. The ALDEFLUOR-positive population of MCs is approximately 6% and is not changed by co-culture with SUM159 cells.

(C) Differentiation potential of ALDEFLUOR-positive (ALDH+) or ALDEFLUOR-negative (ALDH-) primary MCs. The ALDEFLUOR-positive population of MCs is the population capable of osteogenic and adipogenic differentiation.

Data are shown as means ± STDEV.

A

	CXCL1	CXCL5	CXCL6	CXCL7	IL6	IL8
MC alone (A)	154	391	450	27	23	5,083
SUM159 alone (B)	697	7,436	411	0	51	3,111
A+B (1+1)	388	2,636	638	41	45	4,301
MC + SUM159 Co-culture	115,291	937,229	176,496	840,042	39,241	104,278
MC alone (A)	543	5,501	142	0	18	2,088
SUM149 alone (B)	378,693	891	449	1,253	152	51,123
A+B (1+1)	165,797	1,133	1,524	887	100	30,191
MC + SUM149 Co-culture	687,946	1,737,385	2,002,894	955,518	106,262	296,597
MC alone (A)	148	388	345	17	12	18
MCF-7 alone (B)	796	8,219	564	10	51	3,062
A+B (1+1)	338	2,821	423	24	40	1,531
MC + MCF-7 Co-culture	221,433	1,294,931	346,585	700,353	54,097	192,277

B

	CXCL1	CXCL5	CXCL6	CXCL7	IL6	IL8
MC w. CXCL1 (+ SUM159 alone)	74,090	2,176	0	10	19	3,189
MC w. CXCL5 (+ SUM159 alone)	774	999,699	526	22	47	6,206
MC w. CXCL6 (+ SUM159 alone)	1,040	5,921	364,414	39	54	6,261
MC w. CXCL7 (+ SUM159 alone)	175,772	1,421,838	370,208	804,124	47,914	163,177
MC w. IL6 (+ SUM159 alone)	319,215	1,795,366	555,815	546,084	78,065	265,062
MC w. IL8 (+ SUM159 alone)	366	1,979	384	56	36	74,588

C

	CXCL1	CXCL5	CXCL6	CXCL7	IL6	IL8
SUM159 w. CXCL7 (+ MC alone)	139,347	877,694	226,446	690,700	43,992	151,218
MC+SUM159 w. anti-IL6 and CXCL7	162,111	990,310	265,111	697,171	41,619	100,222
MC+SUM159 w. anti-IL6	1,651	1,518	1	130	0	4,898
MC+SUM159 w. anti-CXCL7	377	1,591	0	44	21	5,112

Figure S5. Luminex bead assay is utilized to access cytokine protein expression.

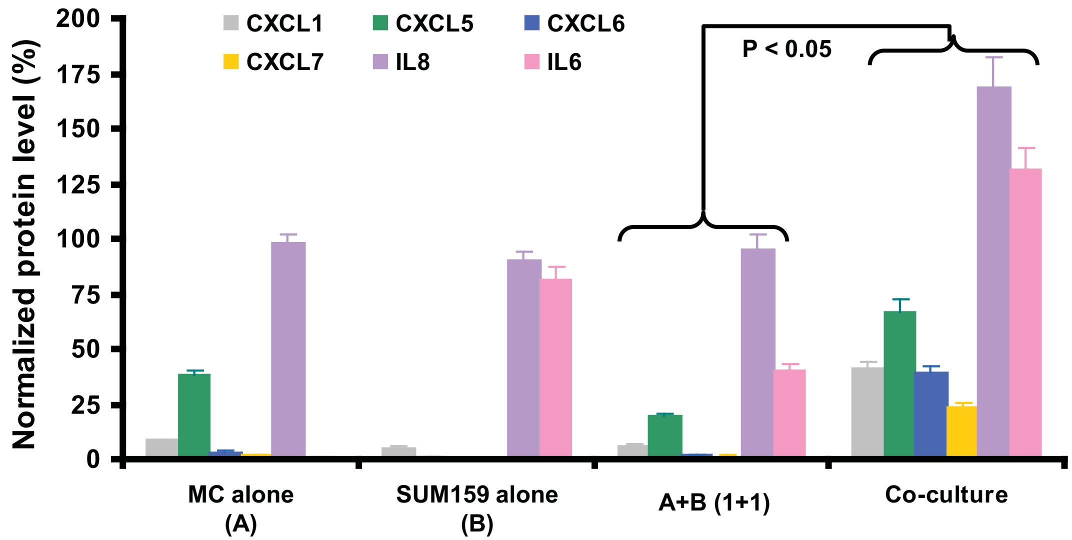

Figure S6. Cytokines are induced by co-culture of SUM149 cells and MCs

Induction of cytokine proteins by co-culture of MCs and SUM149 cells as determined by antibody array. Protein level of CXCL5 (ENA78), CXCL6 (GCP2), CXCL1 (Gro-α), CXCL7, IL-6 and IL-8 are increased in the co-culture of breast cancer cells and MCs. The $p<0.05$ refer to the significant difference of the individual cytokine from the "co-culture" group in comparison to the corresponding cytokine from "A+B" group. Error bar represent the SD.

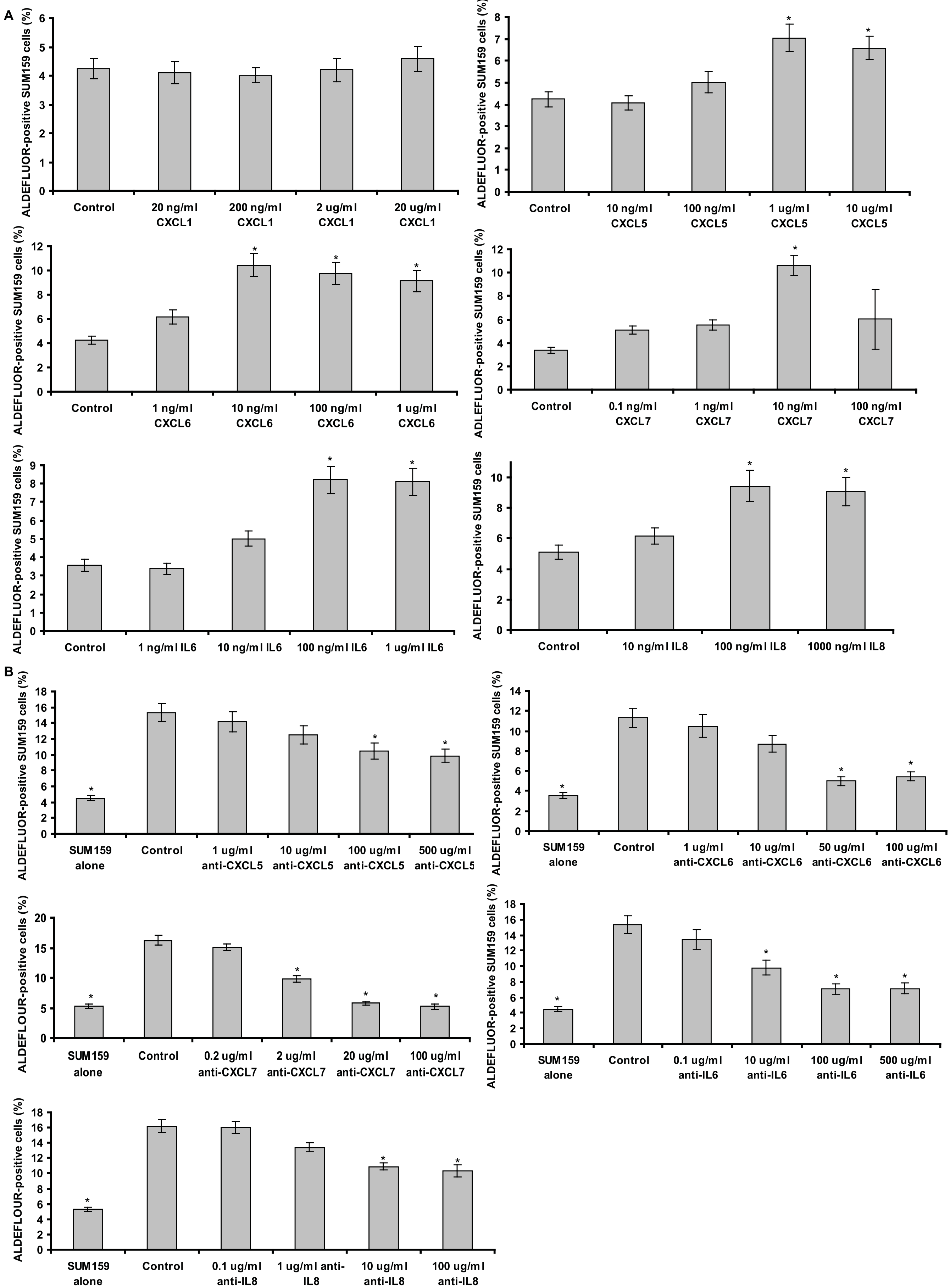

Figure S7. Optimization of the concentrations of cytokines and cytokine blocking antibodies

(A) Effects of different concentrations of each cytokine on the percentage of ALDEFLUOR-positive SUM159.

(B) Effects of different concentrations of each cytokine blocking antibody on the percentage of ALDEFLUOR-positive SUM159 from the co-culture of MCs and SUM159 cells.

The * $p<0.05$ refers to the significant difference of sample groups in comparison to the "Control" group. Error bar represent the SD.

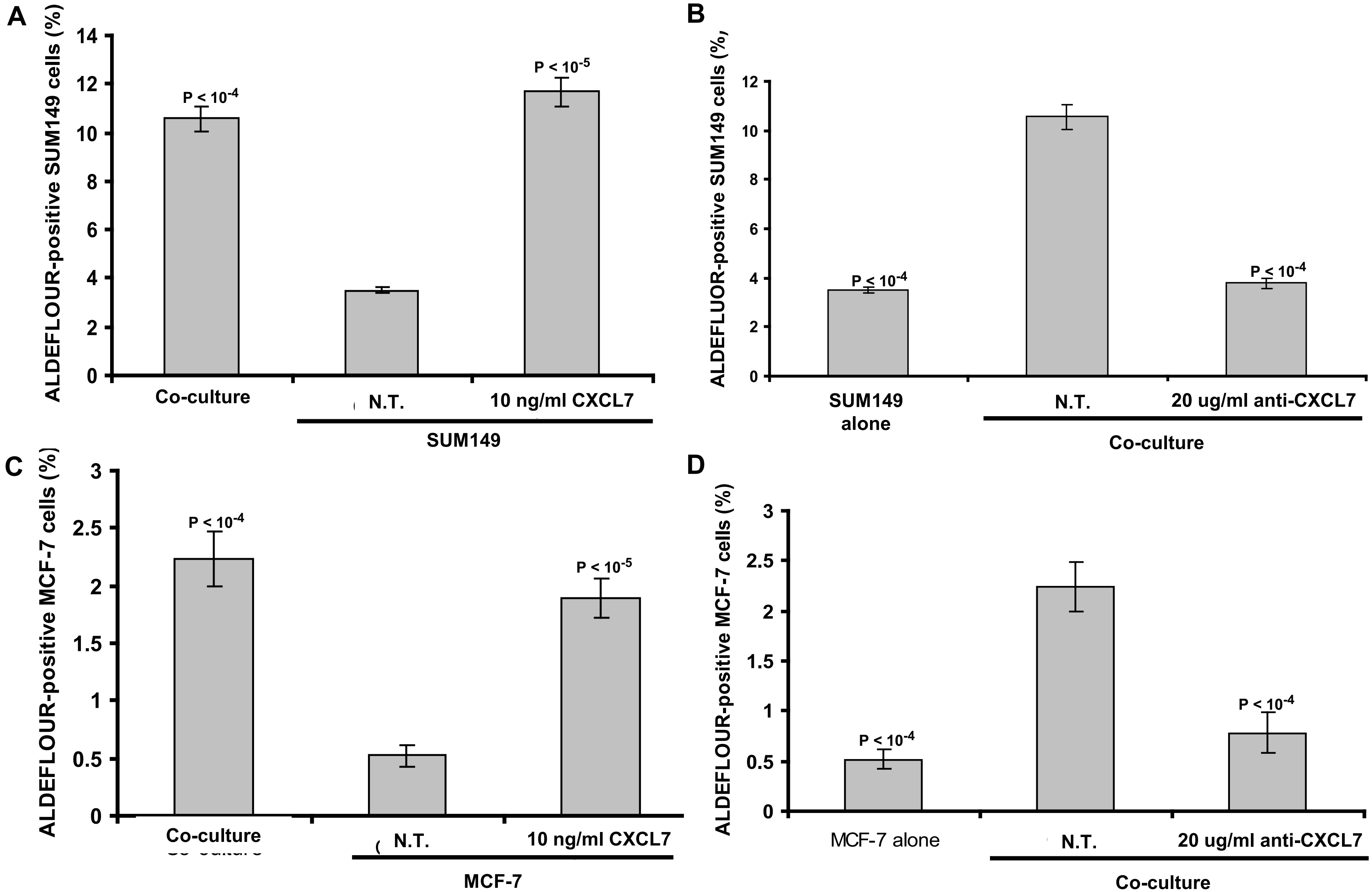

Figure S8. CXCL7 plays key role in the interaction of MCs and breast cancer cells

(A) Effects of optimized concentration of CXCL7 on the percent of ALDEFLUOR-positive population of SUM149 cells. CXCL7 significantly increases the percent of ALDEFLUOR-positive population of SUM149 cells.

(B) Effects of optimized concentration of CXCL7 blocking antibody on the interaction of MCs and SUM149 cells. CXCL7 blocking antibody significantly decreases the ALDEFLUOR-positive population of SUM149 cells in the co-culture.

(C) Effects of optimized concentration of CXCL7 on the percent of ALDEFLUOR-positive population of MCF-7 cells. CXCL7 significantly increases the percent of ALDEFLUOR-positive population of MCF-7 cells.

(D) Effects of optimized concentration of CXCL7 blocking antibody on the interaction of MCs and MCF-7 cells. CXCL7 blocking antibody significantly decreases the ALDEFLUOR-positive population of MCF-7 cells in the co-culture.

The p values refer to the significant difference of sample groups in comparison to the "N.T." group. Data are shown as means $\pm$ STDEV.

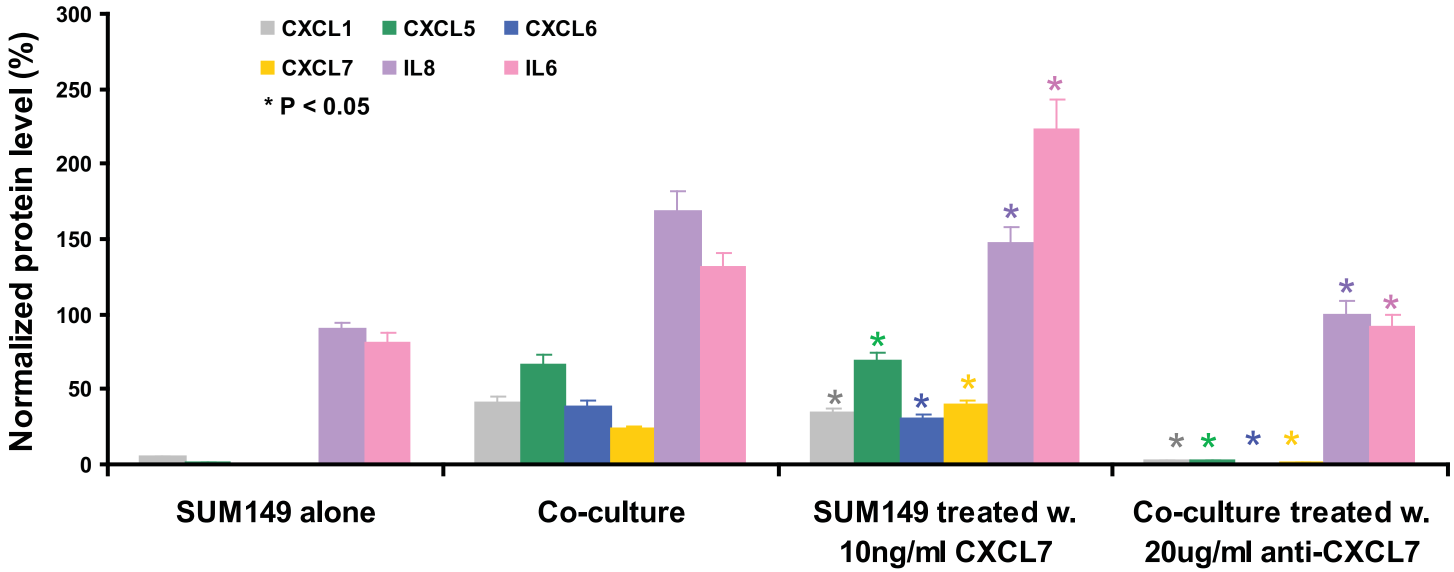

Figure S9. Effects of CXCL7 on cytokine production by SUM149 cells

Addition of CXCL7 to SUM149 cells induces a cytokine expression pattern which recapitulated that of co-culture. Alternatively, the addition of blocking antibody to CXCL7 to the co-culture completely blocked expression of induced cytokines.

N.T. represents No Treatments. Data are shown as means ± STDEV. The * p<0.05 refer to the significant difference of the individual cytokine from the "co-culture treated with 20ug/ml anti-CXCL7" group in comparison to the corresponding cytokine from "co-culture" group, or the significant difference of the individual cytokine from the "SUM149 treated with 10ng/ml CXCL7" group in comparison to the corresponding cytokine from "SUM149 alone" group.

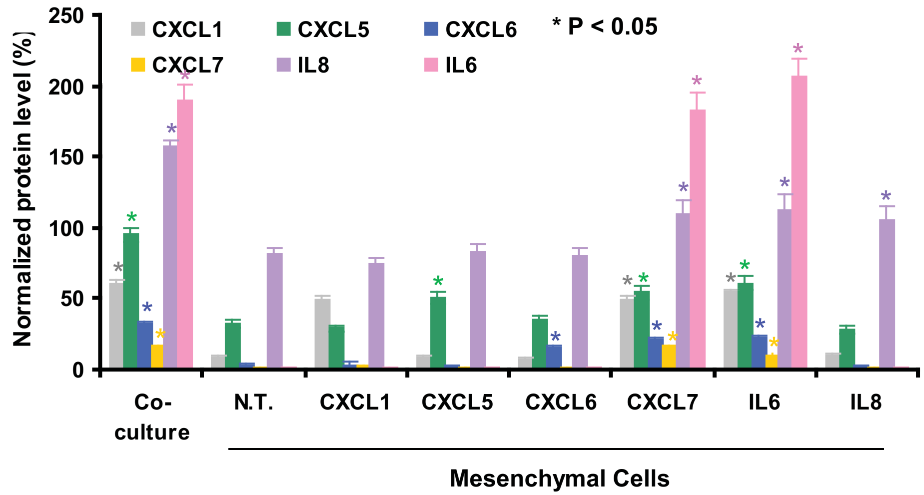

Figure S10. Effects of addition of recombinant cytokines on cytokine production by MCs
The addition of IL6 or CXCL7 to MCs induces cytokines in a pattern that resembles the induced-cytokine pattern produced by co-culture of MCs and SUM159 cells. N.T. represents No Treatments. The * $p<0.05$ refer to the significant difference of the individual cytokine from the sample groups in comparison to the corresponding cytokine from "N.T." group Data are shown as means ± STDEV.

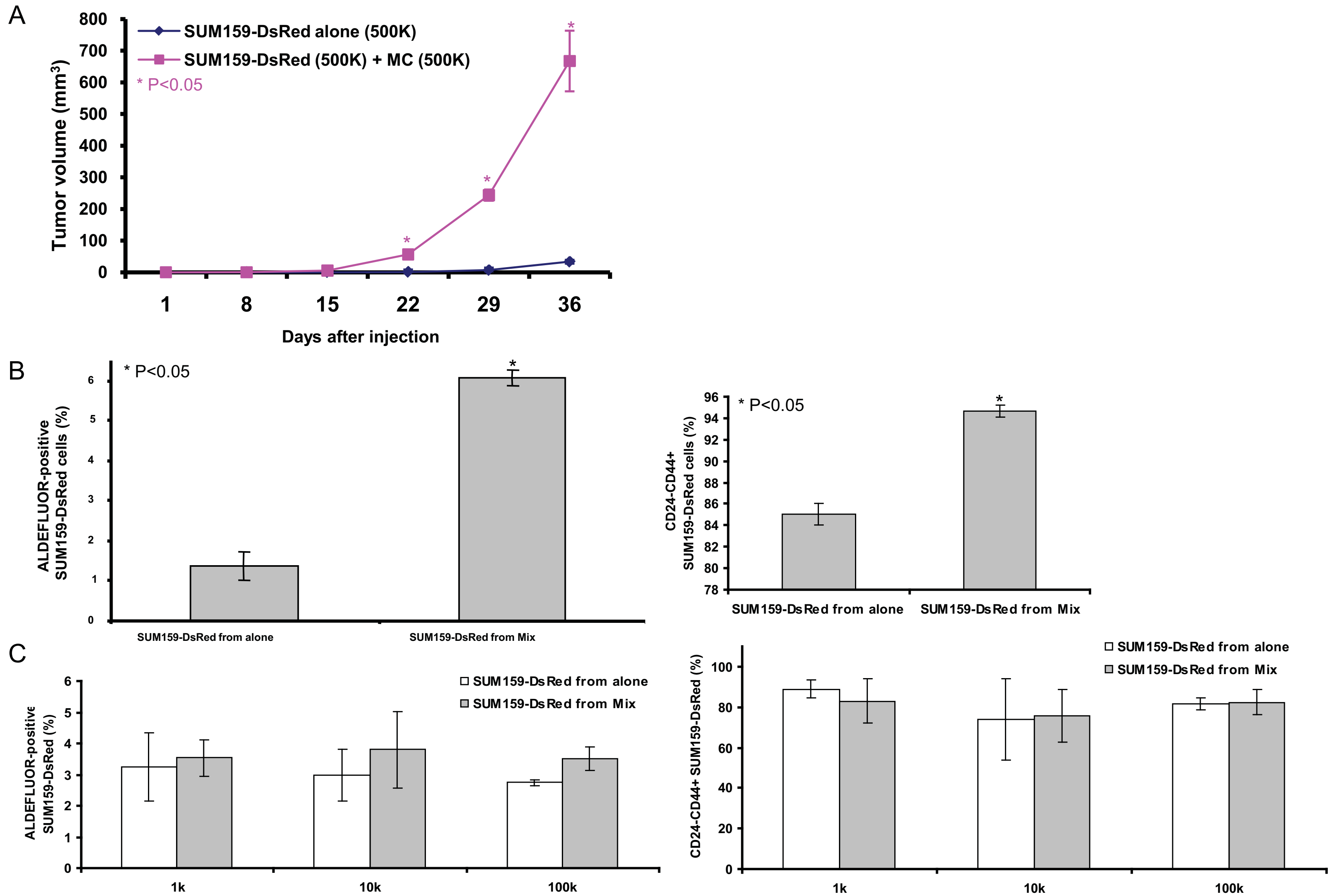

Figure S11. MCs induce cancer stem cells stimulating breast tumor growth in NOD/SCID mice

(A) Effect of admixture (1:1) of MCs on the growth of SUM159 (Total) tumor xenograft.
MCs stimulate primary tumor growth of SUM159 cells in NOD/SCID mice, which is consistent with that shown in Figure 6.

(B) Effects of admixture (1:1) of MCs on the percent of ALDEFLUOR-positive (*Left*) or $CD24^{-}CD44^{+}$ (*Right*) SUM159 cells isolated from SUM159 (Total) primary tumor xenograft. MCs increase the stem/progenitor cell population of SUM159 cells in NOD/SCID mice.

(C) The percentage of ALDEFLUOR-positive (*Left*) or $CD24^{-}CD44^{+}$ (*Right*) SUM159 cells isolated from SUM159 secondary tumor xenografts derived from primary tumors (A) at serial dilutions (1k, 10k, 100k).

Data are shown as means ± STDEV.

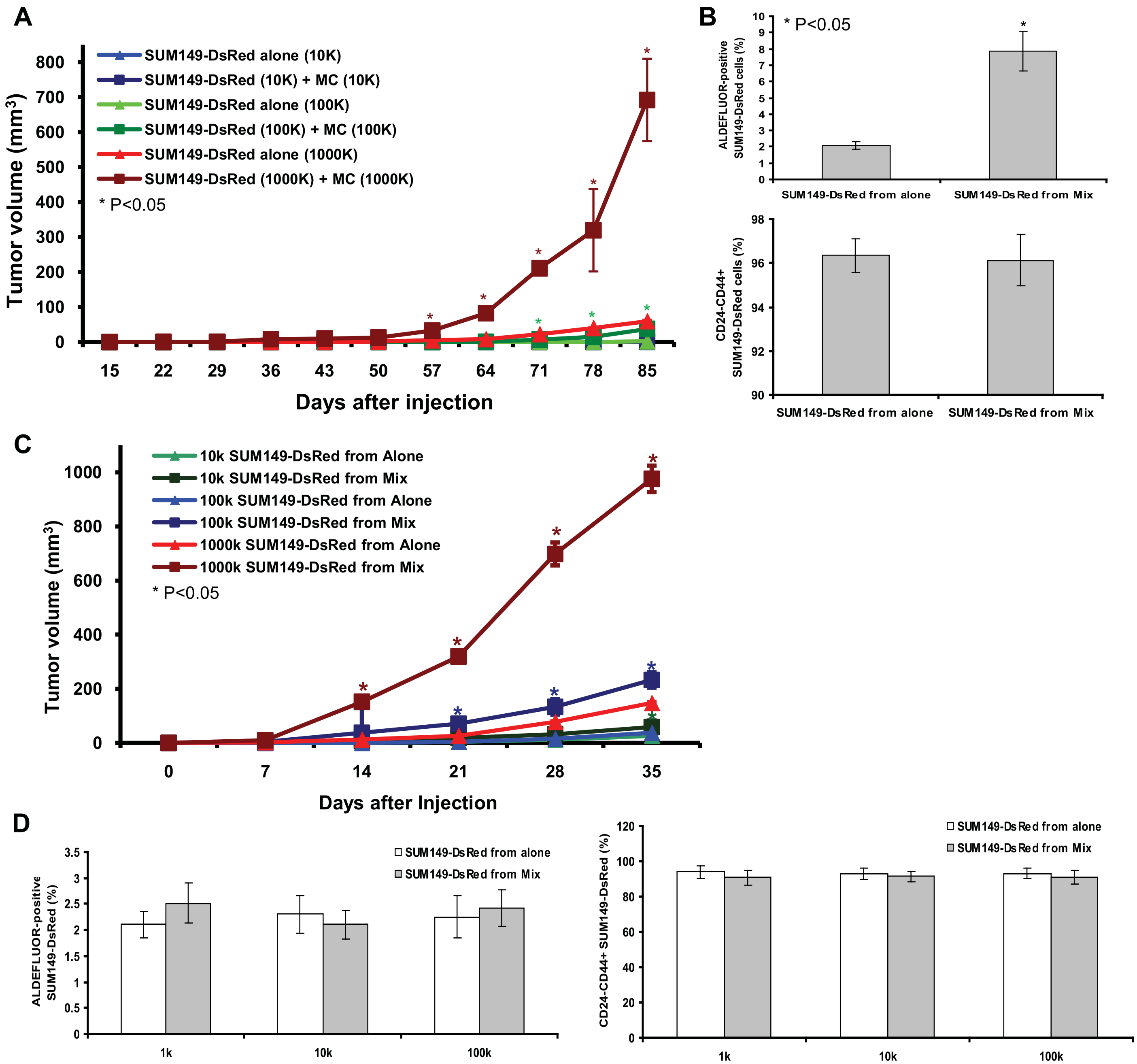

Figure S12. MCs induce cancer stem cells stimulating breast tumor growth in NOD/SCID mice

(A) Effect of admixture (1:1) of MCs on the growth of SUM149 (Total) tumor xenograft. MCs stimulate tumor growth of SUM149 cells in NOD/SCID mice. The * p<0.05 refer to the significant difference of the "SUM149-DsRed + MC" in comparison to the "SUM149-DsRed alone" injected with the same number of cells.

(B) Effects of admixture (1:1) of MCs on the percent of ALDEFLUOR-positive (*Left*) or $CD24^{-}CD44^{+}$ (*Right*) SUM149 cells isolated from SUM149 primary tumor xenograft. MCs increase the stem/progenitor cell population of SUM149 cells in NOD/SCID mice.

(C) The growth of secondary SUM149 tumors derived from the SUM149 primary tumors in the absence or presence of the admixture (1:1) MCs and SUM149, which were implanted at a serial dilutions. SUM149 cells from primary tumors with MC form tumors much faster and bigger than that from primary tumors without MC at the same dilution. The * p<0.05 refer to the significant difference of the "SUM149-DsRed from Mix" in comparison to the "SUM149-DsRed from Alone" group injected with the same number of cells.

(D) The percentage of ALDEFLUOR-positive (*Left*) or $CD24^{-}CD44^{+}$ (*Right*) SUM149 cells isolated from SUM149 secondary tumor xenografts derived from primary tumors (A) at a serial dilutions.

Data are shown as means ± STDEV.

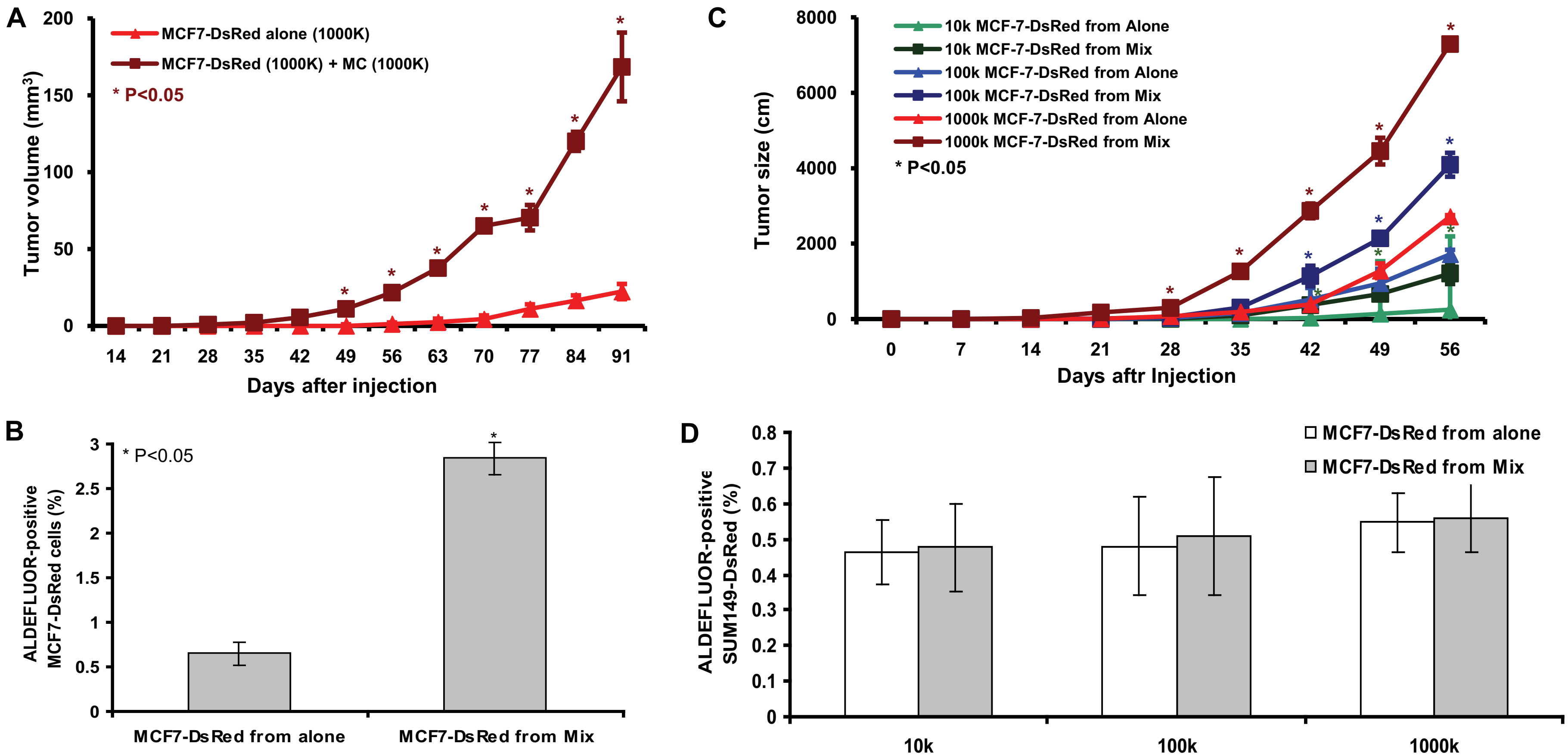

Figure S13. MCs induce cancer stem cells stimulating breast tumor growth in NOD/SCID mice

(A) Effect of admixture (1:1) of MCs on the growth of MCF-7 (Total) tumor xenograft. MCs stimulate tumor growth of MCF-7 cells in NOD/SCID mice. The * $p<0.05$ refer to the significant difference of the "MCF7-DsRed + MC" in comparison to the "MCF7-DsRed alone".

(B) Effects of admixture (1:1) of MCs on the percent of ALDEFLUOR-positive MCF-7 cells isolated from MCF-7 primary tumor xenograft. MCs increase the Aldefluor-positive population of MCF-7cells in NOD/SCID mice.

(C) The growth of secondary MCF-7 tumors derived from the MCF-7 primary tumors in the absence or presence of the admixture (1:1) MCs and MCF-7, which were implanted at a serial dilutions. MCF-7 cells from primary tumors with MC form tumors much faster and bigger than that from primary tumors without MC at the same dilution. The * $p<0.05$ refer to the significant difference of the "MCF7-DsRed from Mix" in comparison to the "MCF7-DsRed from Alone" group injected with the same number of cells.

(D) The percentage of ALDEFLUOR-positive MCF-7 cells isolated from MCF-7 secondary tumor xenografts derived from primary tumors (A) at a serial dilutions.

Data are shown as means ± STDEV.

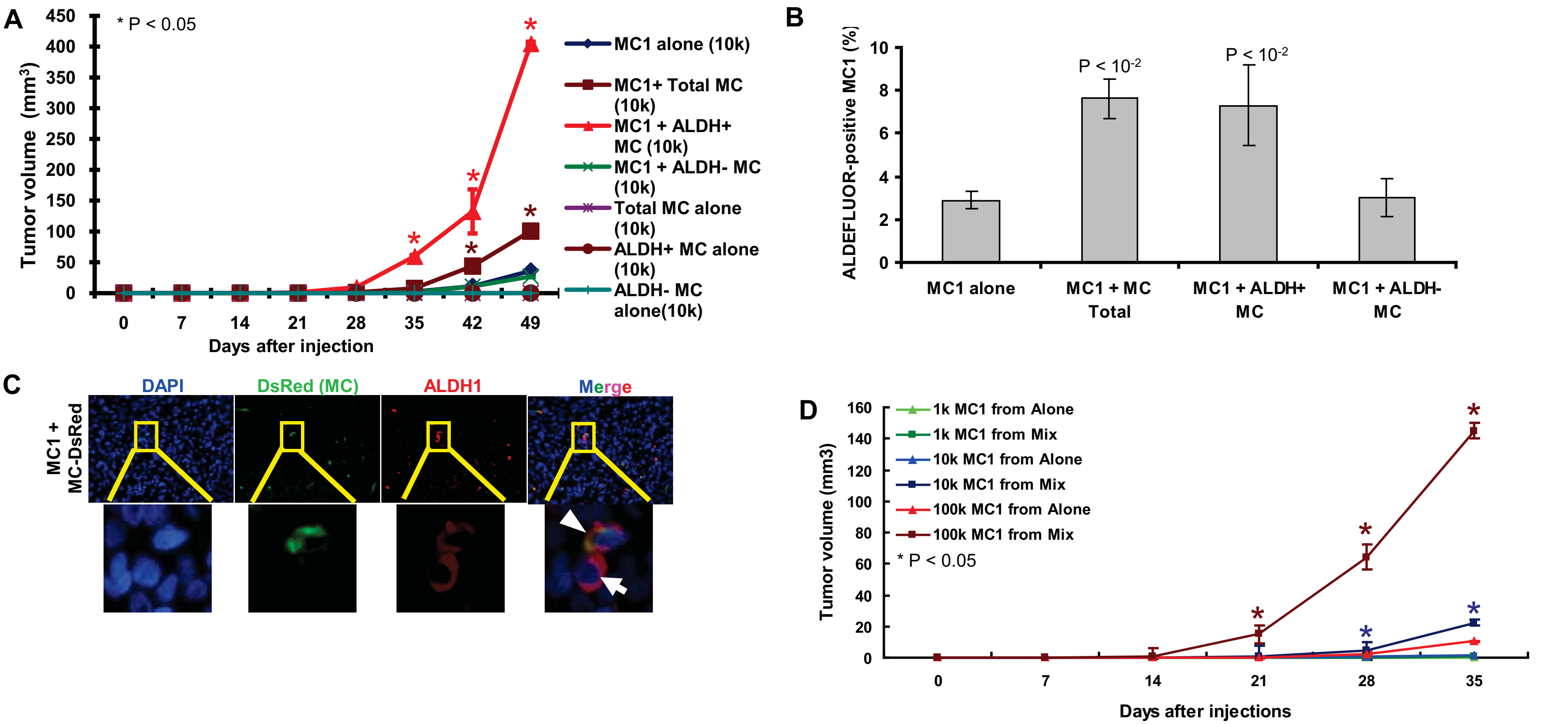

Figure S14. MCs induce cancer stem cells stimulating breast tumor growth in NOD/SCID mice

(A) Effect of admixture (1:1) of MCs on the growth of MC1 (Total) tumor xenograft. MCs stimulate tumor growth of MC1 in NOD/SCID mice. The * $p<0.05$ refer to the significant difference of the sample groups in comparison to the "MC1 alone (10k)" group.

(B) Effects of admixture (1:1) of MCs on the percentage of ALDEFLUOR-positive MC1 cells isolated from MC1 primary tumor xenograft. MCs increase the Aldefluor-positive population of MC1 in NOD/SCID mice. The p values refer to the significant difference of sample groups in comparison to the "MC1 alone" group.

(C) In situ localization of ALDH1-positive MCs and ALDH1-positive MC1 cells identifies a "cancer stem cell niche" in the primary tumors derived from admixture (1:1) MCs and MC1. In order to quantitate this association, we counted 9 fields to determine the frequency of association of ALDH-positive CSCs and MSCs. MC-DsRed: DsRed-labeled MCs; Blue: DAPI staining; Green: DsRed staining; Red: ALDH1 staining. White arrow: ALDH1+ MC1 cells; White triangle: ALDH1+ MC.

(D) The growth of secondary MC1 tumors derived from the MC1 primary tumors in the absence or presence of the admixture (1:1) MCs and MC1, which were implanted at a serial dilution. MC1 cells from primary tumors with MC form tumors much faster and bigger than that from primary tumors without MC at the same dilution. The * $p<0.05$ refer to the significant difference of the "MC1 from Mix" in comparison to the "MC1 from Alone" group injected with the same number of cells.

Error bar represent the SD.

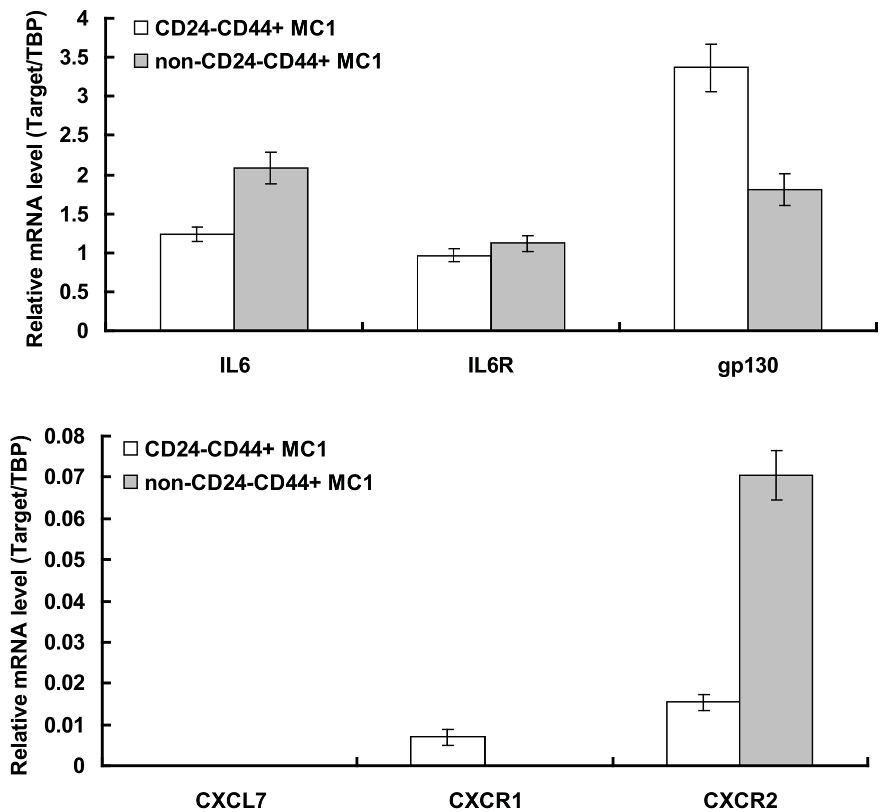

Figure S15, **The expression of Cytokines and the receptors in MC1 primary xenograft tumor cells.**
CD44^{+}/CD24- cells and the remaining cells were sorted from MC1 primary xenograft tumors, and total RNA was isolated. Real-time RT-PCR showed that IL6 and its receptors were present in both populations , whereas CXCL7 mRNA level was undetectable in both populations. The IL8 receptors CXCR1 was selectively expressed in the CD44^{+}/CD24- cells whereas CXCR2 which binds both CXCL7 and IL8 demonstrated higher expression in the bulk tumor Population.