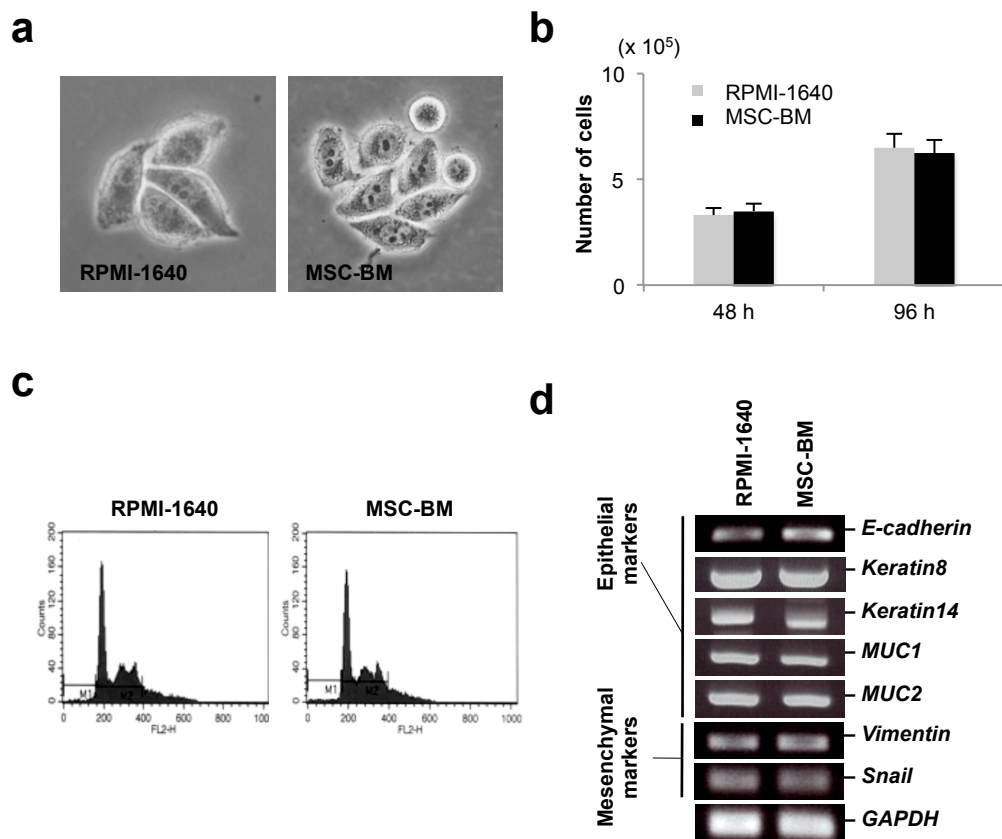


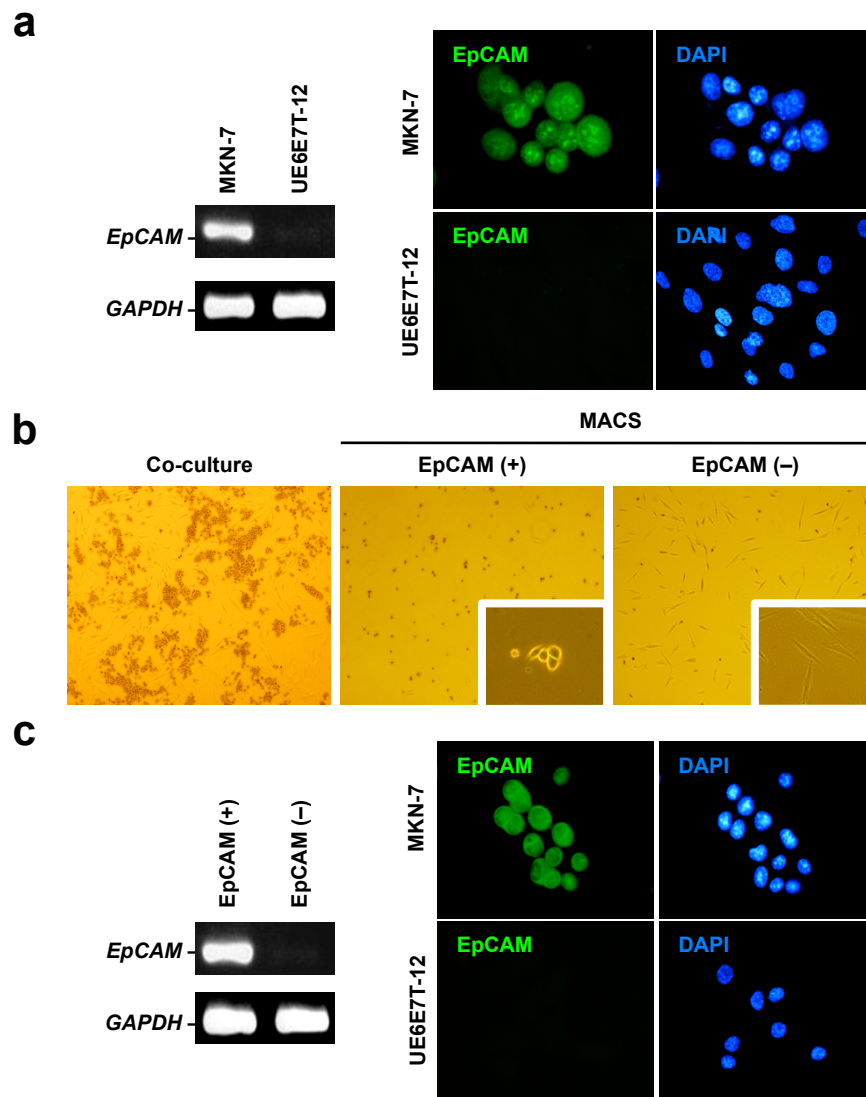
# Supplemental Figure S1



## Supplemental Fig. S1

A comparative study of culturing media RPMI-1640 and MSC-BM on the nature of MKN-7 cells. RPMI-1640 supplemented with 10% FBS is originally recommended for maintenance of MKN-7 cells. The effects of MSC-BM was tested beforehand the co-clulture with UE6E7T-12 BM-MSCs. **(a)** Morphology of the MKN-7 cells maintained in RPMI-1640 and MSC-BM media. **(b)** Results of cell growth test. MKN-7 cells ( $1 \times 10^5$ /dish) were incubated for 48 h and 96 h with each medium. **(c)** Results of flow cytometric analysis. After fixation of cells, DNA was stained with propidium iodide. **(d)** Results of RT-PCR analysis. Expressions of epithelial markers (*E-cadherin*, *Keratin 8* and *Keratin 14*), mucins (*MUC1* and *MUC2*), and mesenchymal markers (*vimentin* and *Snail*) were tested. The primer set for *GAPDH* was used as a control.

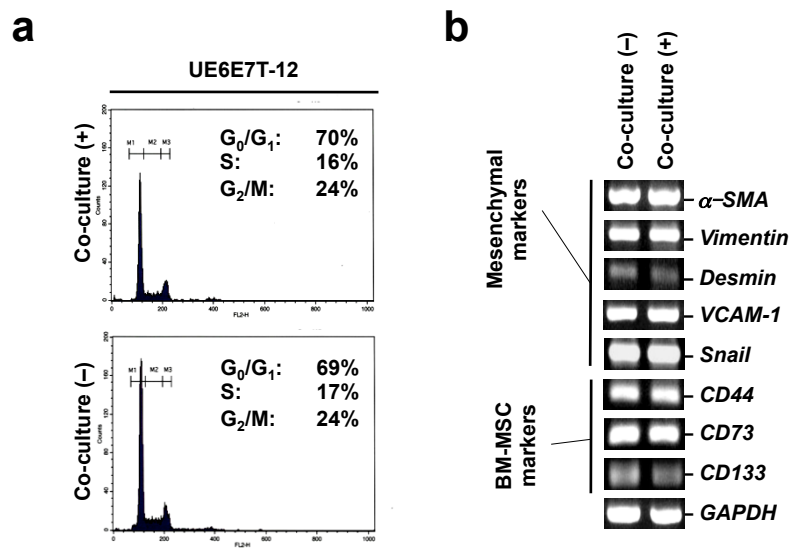
## Supplemental Figure S2



### Supplemental Fig. S2

Cell separation by MACS with anti-*EpCAM* antibody after co-culture of MKN-7 cells and UE6E7T-12 BM-MSCs. (a) Expression of *EpCAM* is detected only in MKN-7 cells but not in UE6E7T-12 BM-MSCs. Left panels, the *EpCAM* mRNA expression detected by RT-PCR; Right panels, immunofluorescence of *EpCAM*. Nuclei were stained with DAPI. (b) Representative images of after separation of MKN-7 cells from UE6E7T-12 BM-MSCs. Dissociated cells were centrifuged, rinsed with sterile MACS buffer and labeled with anti-*EpCAM* antibody directly conjugated with ferromagnetic beads. Morphologically, *EpCAM*<sup>+</sup> cells showed cobble stone-like colonies but *EpCAM*<sup>-</sup> cells exhibited spindle fibroblast-like shape. (c) Expression of *EpCAM* is detected only in *EpCAM*<sup>+</sup> cells but not *EpCAM*<sup>-</sup> cells. Left panels; the *EpCAM* mRNA expression detected by RT-PCR; Right panels, immunofluorescence of *EpCAM*. Nuclei were stained DAPI.

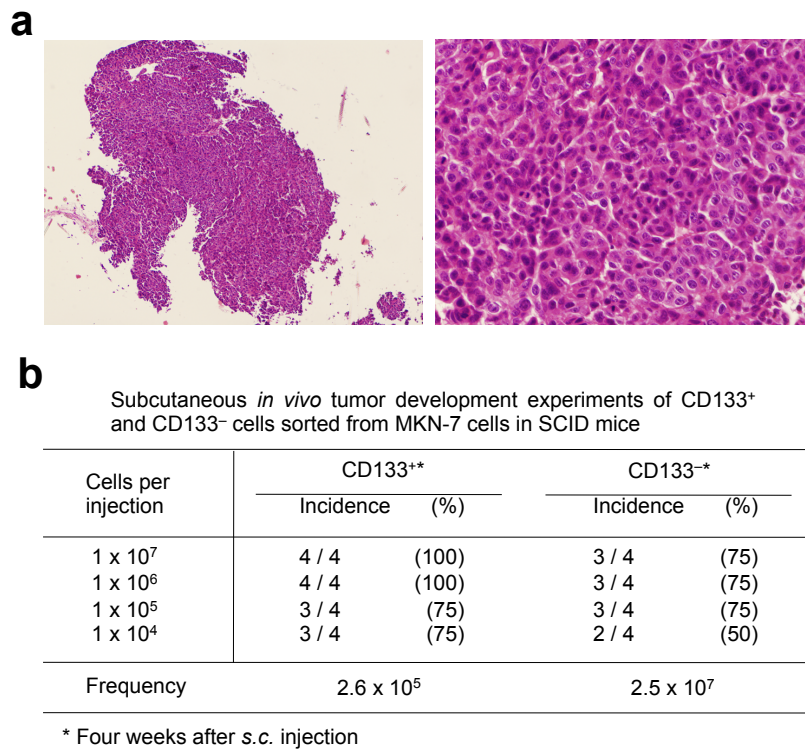
# Supplemental Figure S3



## Supplemental Fig. S3

Impact of co-culture with MKN-7 cells on the nature of UE6E7T-12 BM-MSCs. Cell separation by MACS with anti-EpCAM antibody was performed 48 h after co-incubation. **(a)** Results of flow cytometric analysis. The percentage of each fraction (G<sub>0</sub>/G<sub>1</sub>, S and G<sub>2</sub>/M) were shown in right upper corner of each fluorogram. **(b)** Differential gene expression detected by RT-PCR. Mesenchymal ( $\alpha$ -smooth muscle actin [SMA], vimentin, desmin, VCAM-1 and Snail) and BM-MSC markers (CD44, CD73 and CD133) were examined. GAPDH was used as a control.

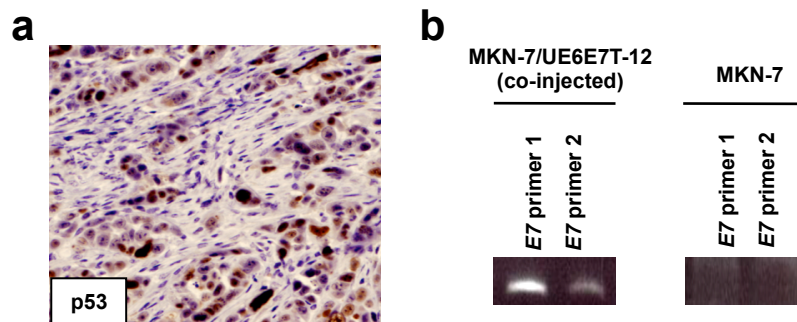
# Supplemental Figure S4



## Supplemental Fig. S4

CD133 is a CSC marker in MKN-7 cells. Subcutaneous *in vivo* tumor development experiments of CD133<sup>+</sup> and CD133<sup>-</sup> cells sorted from MKN-7 cells in SCID mice. (a) Representative images of subcutaneous tumor developed in SCID mouse injected with CD133<sup>+</sup> MKN-7 cells. Four weeks after injection, tumors were removed, fixed, stained with hematoxylin & eosin. (b) Results of limiting dilution assay. According to the incidence of subcutaneous tumors, the theoretical frequencies of cells that developed subcutaneous tumor were calculated.

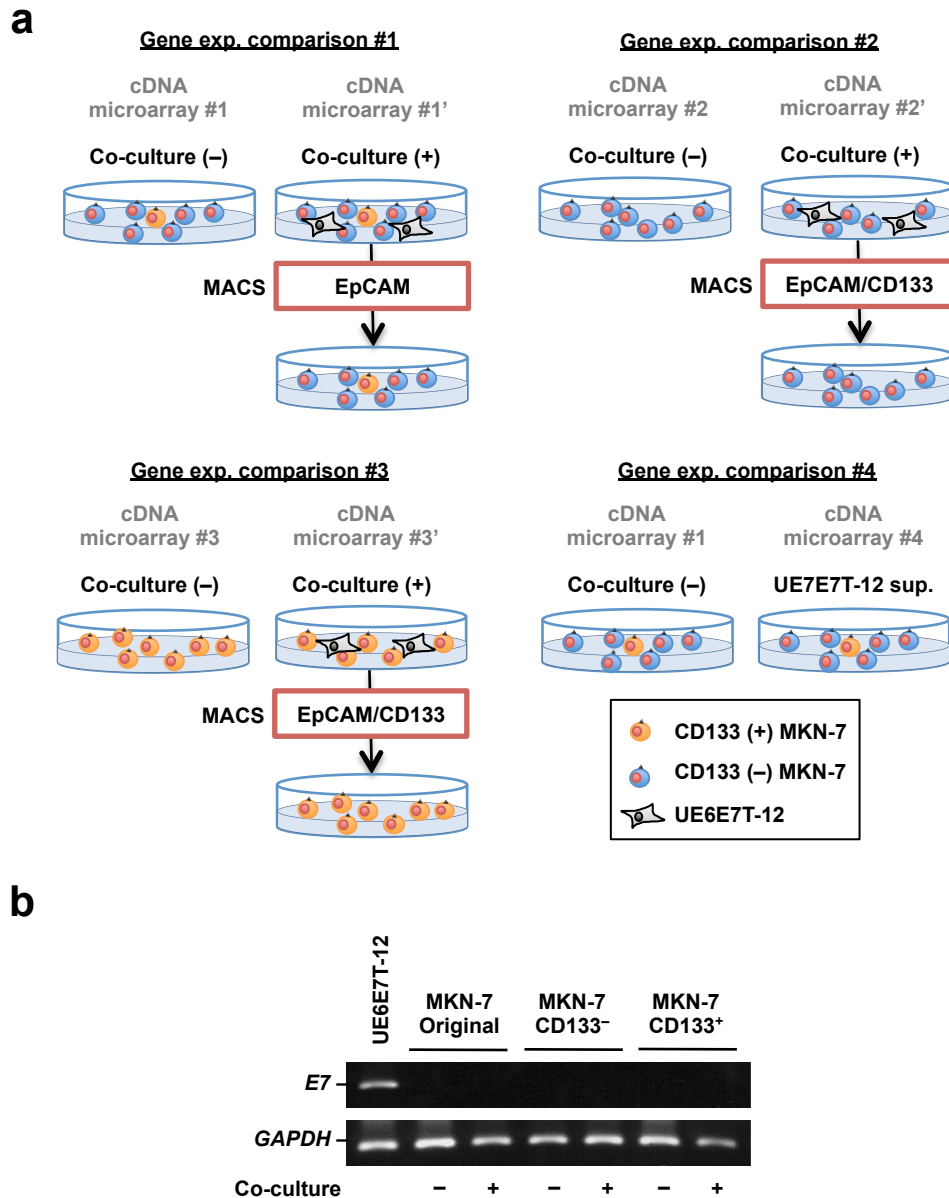
## Supplemental Figure S5



### Supplemental Fig. S5

Detection of UE6E7T-12 BM-MSCs within tumors developed in mouse xenografts co-injected with MKN-7 cells. **(a)** Expression of p53 tumor suppressor protein. In MKN-7 cells, genetic alteration of the *p53* gene, CCT (Pro) to TCT (Ser) at codon 278, has been identified, and therefore MKN-7-derived cells show abnormal nuclear accumulation of p53 protein. **(b)** Results of genomic amplification of the *E7* gene by genomic PCR. UE6E7T-12 BM-MSCs were established by transduction of the *E6* and *E7* genes into original BM-MSCs to obtain their immortalized clone. DNAs were extracted from subcutaneous tumors developed in the MKN-7/UE6E7T-12 (co-injection) and MKN-7 xenografts. The DNA sequences of the primer sets used are following: *E7* primer 1: 5'-GAT ACA CCT ACA TTG CAT GA-3'/5'-CCA TCT ATT TCA TCC TCC TC -3'; *E7* primer 2: 5'-ATT GTA ACC TTT TGT TGC AA-3'/5'-ACA ATT CCT AGT GTG CCC AT-3'.

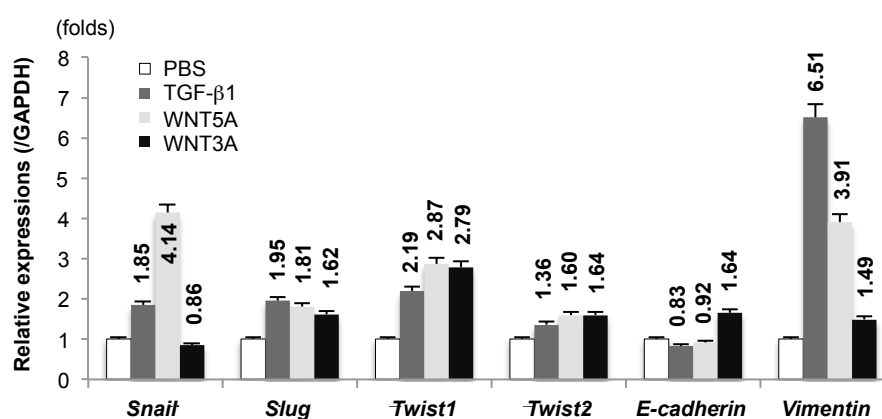
# Supplemental Figure S6



## Supplemental Fig. S6

Preparation of total RNAs for cDNA microarray analysis. (a) Illustration of the isolation, selection and culture condition for MKN-7 cells. The cell sorting experiments MACS with anti-EpCAM antibody was performed to exclude co-cultured UE6E7T-12 BM-MSCs. MACS with anti-CD133 antibody was conducted to divide MKN-7 cells based on expression of CD133<sup>+</sup> or CD133<sup>-</sup>. For identification of the molecules that were stimulated by soluble factors expressed from UE6E7T-12 BM-MSCs, original MKN-7 cells were maintained with the UE6E7T-12 BM-MSCs conditioned medium (UE6E7T-12 sup.). (b) Result of RT-PCR. To avoid the contamination of UE6E7T-12 BM-MSCs within MKN-7 mRNA, E7 primer 1 set was used.

# Supplemental Figure S7



## Supplemental Fig. S7

Effects of recombinant WNT5A and TGF- $\beta$ 1 on the expressions of EMT-related genes in MKN-7 cells. Primer sets used in this analysis were as follows: *Snail*, 5'-ACC ACT ATG CCG CGC TCT T-3'/5'-GGT CGT AGG GCT GCT GGA A-3'; *Slug*, 5'-ATG CCG CGC TCC TTC CT-3'/5'-TGT GTC CAG TTC GCT-3'; *Twist1*, 5'-GGA CAA GCT GAG CAA GAT TCA GA-3'/5'-TCT GGA GGA CCT GGT AGA GGA A-3'; *Twist2*, 5'-GCC GCC AGG TAC ATA GAC TT-3'/5'-CCC CAA ACA TAA GAC CCA GA-3'; *E-cadherin*, 5'-TGC CCA GAA AAT GAA AAA GG-3'/5'-GTG TAT GTG GCA ATG CGT TC-3'; and *vimentin*, 5'-GAG AAC TTT GCC GTT GAA GC-3'/5'-TCC AGC AGC TTC CTG TAG GT-3'. Recombinant WNT3A was used as a control.