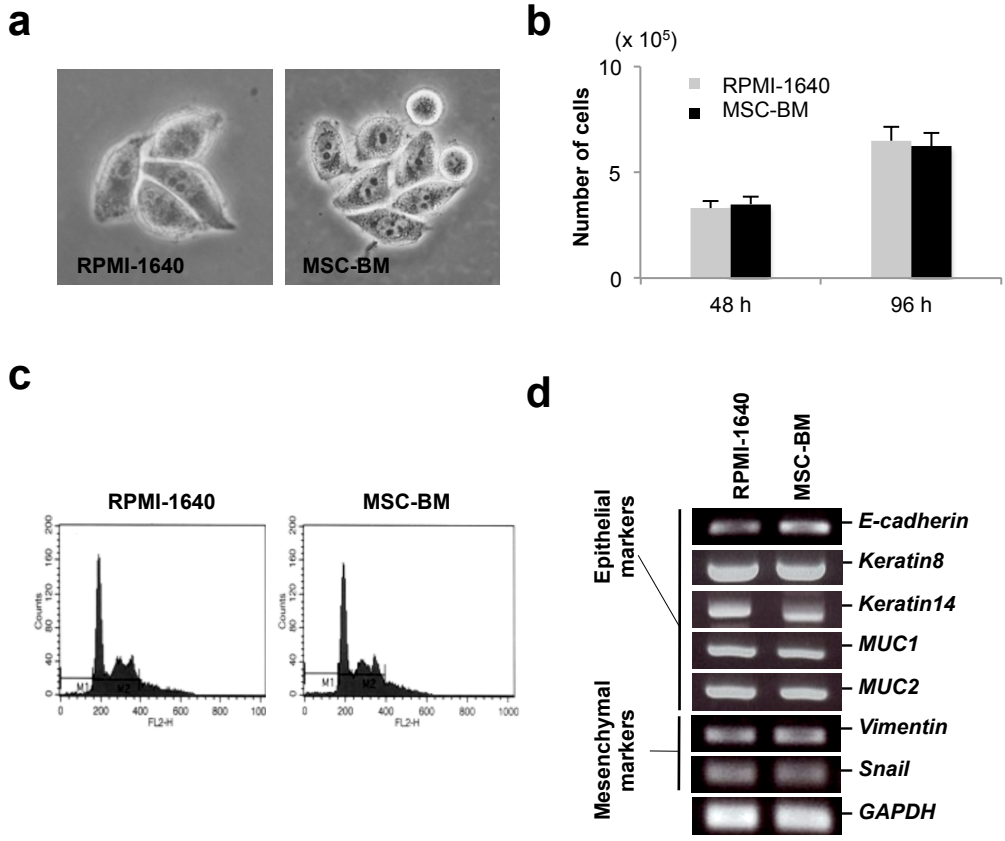
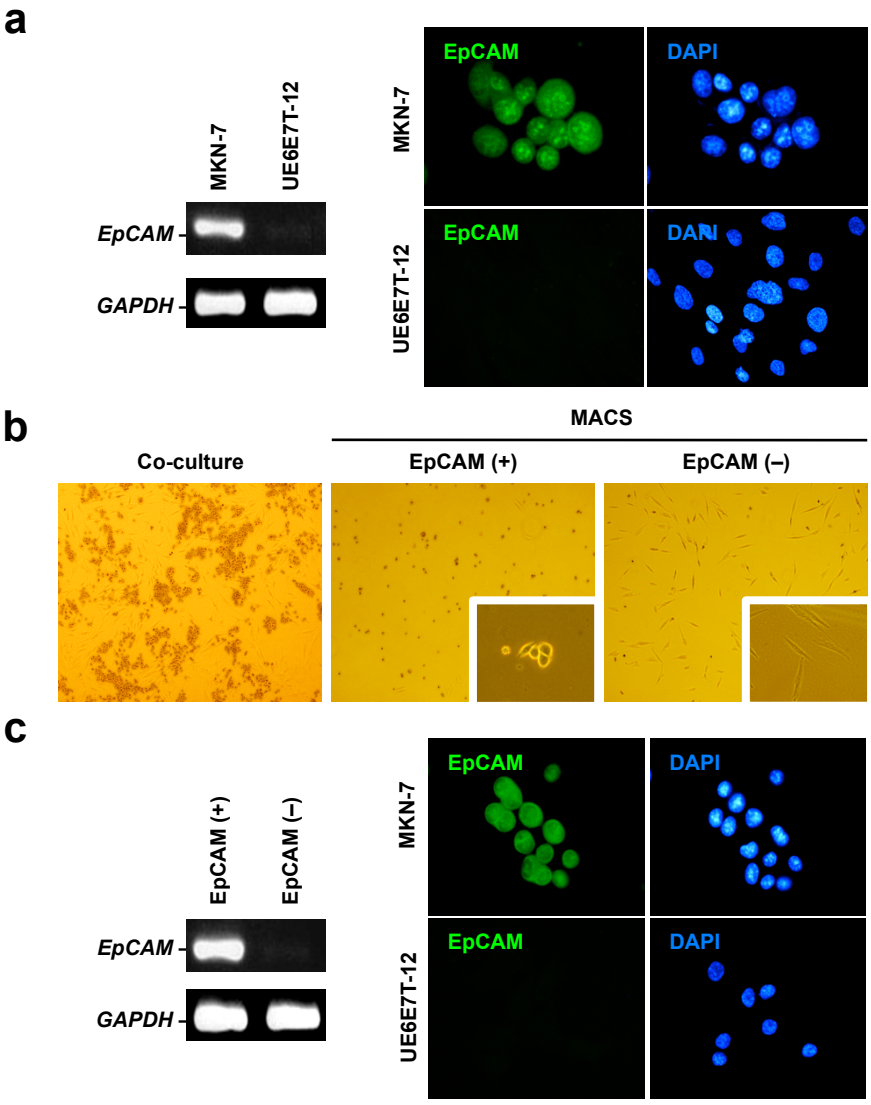
Supplemental Figure S1



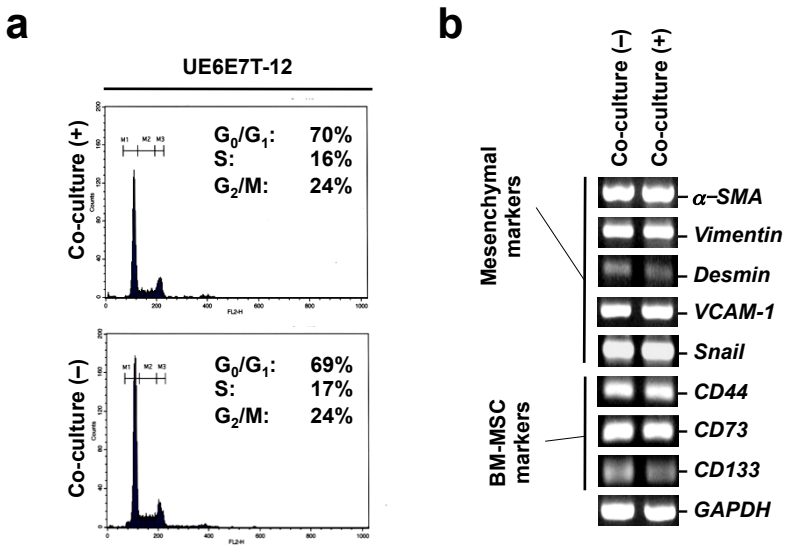
Supplemental Fig. S1
A comparative study of culturing media RPMI-1640 and MSC-BM on the nature of MKN-7 cells. RPMI-1640 supplemented with 10% FBS is originally recommended for maintenance of MKN-7 cells. The effects of MSC-BM was tested beforehand the co-cluture with UE6E7T-12 BM-MSCs. (**a**) Morphology of the MKN-7 cells maintained in RPMI-1640 and MSC-BM media. (**b**) Results of cell growth test. MKN-7 cells (1 x 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, $EpCAM^+$ cells showed cobble stone-like colonies but $EpCAM^-$ cells exhibited spindle fibroblast-like shape. (**c**) Expression of EpCAM is detected only in $EpCAM^+$ cells but not $EpCAM^-$ cells. Left panels; the *EpCAM* mRNA expression detected by RT-PCR; Right panels, immunofluorescence of EpCAM. Nuclei were stained DAPI.

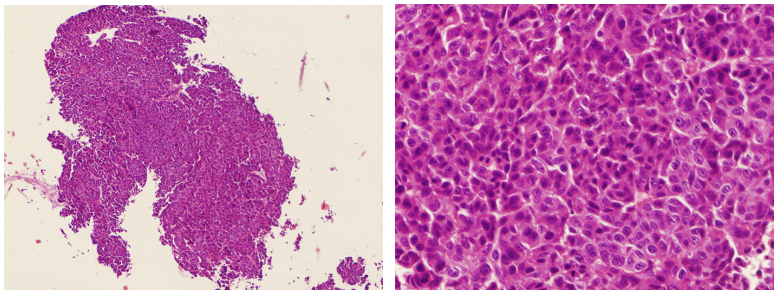
Supplemental Figure S3



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_0/G_1, S and G_2/M) were shown in right upper corner of each fluorogram. (**b**) Differential gene expression detected by RT-PCR. Mesenchymal (a-*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

a



b

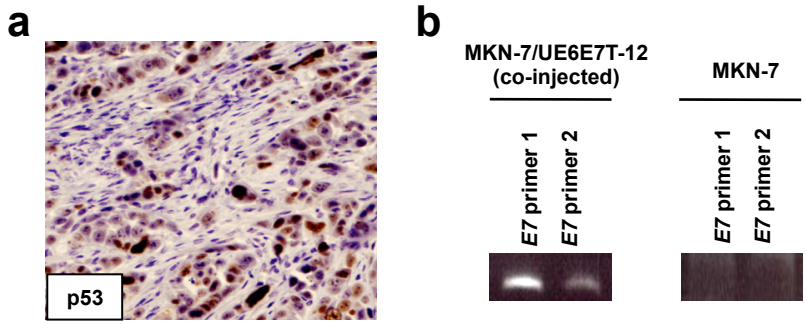
Subcutaneous *in vivo* tumor development experiments of $CD133^+$ and $CD133^-$ cells sorted from MKN-7 cells in SCID mice

Cells per injection	$CD133^{+*}$		$CD133^{-*}$	
	Incidence	(%)	Incidence	(%)
1×10^7	4 / 4	(100)	3 / 4	(75)
1×10^6	4 / 4	(100)	3 / 4	(75)
1×10^5	3 / 4	(75)	3 / 4	(75)
1×10^4	3 / 4	(75)	2 / 4	(50)
Frequency	2.6×10^5		2.5×10^7	

* Four weeks after *s.c.* injection

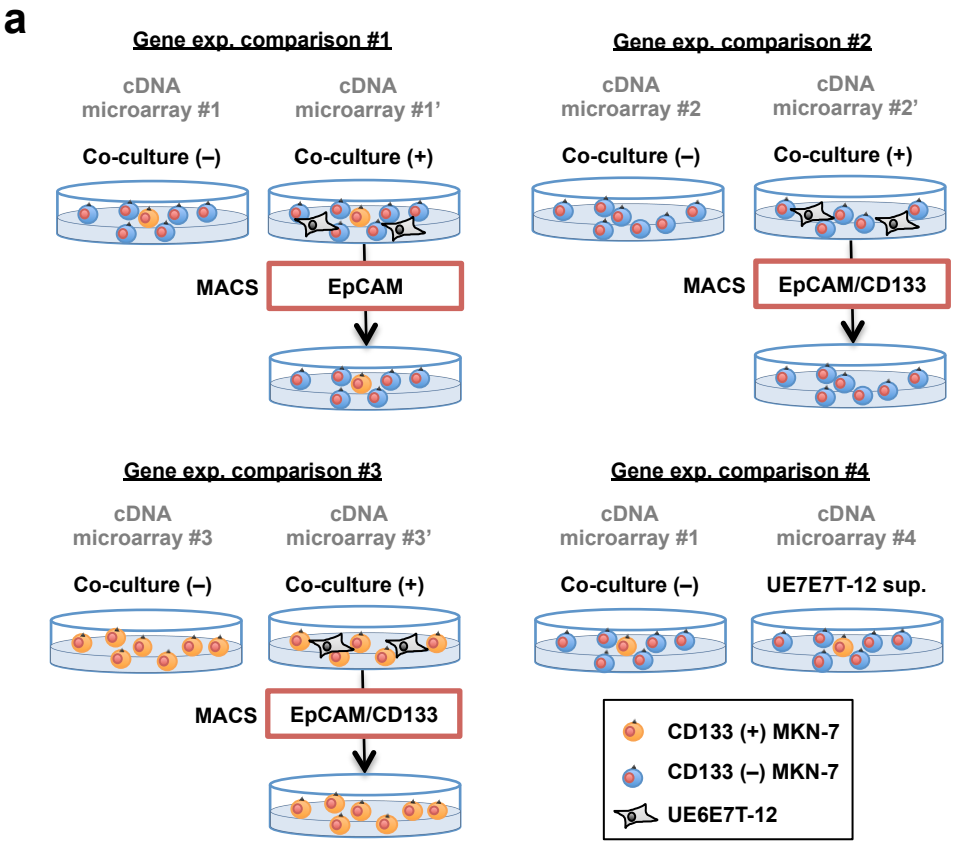
Supplemental Fig. S4
CD133 is a CSC marker in MKN-7 cells. Subcutaneous *in vivo* tumor development experiments of $CD133^+$ and $CD133^-$ cells sorted from MKN-7 cells in SCID mice. (**a**) Representative images of subcutaneous tumor developed in SCID mouse injected with $CD133^+$ MKN-7 cells. Four 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.

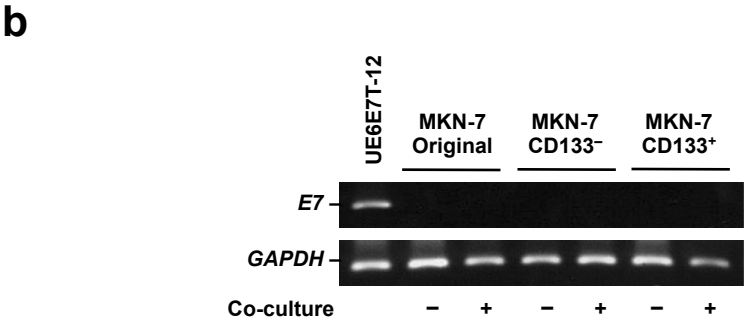
Supplemental Figure S5



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'.

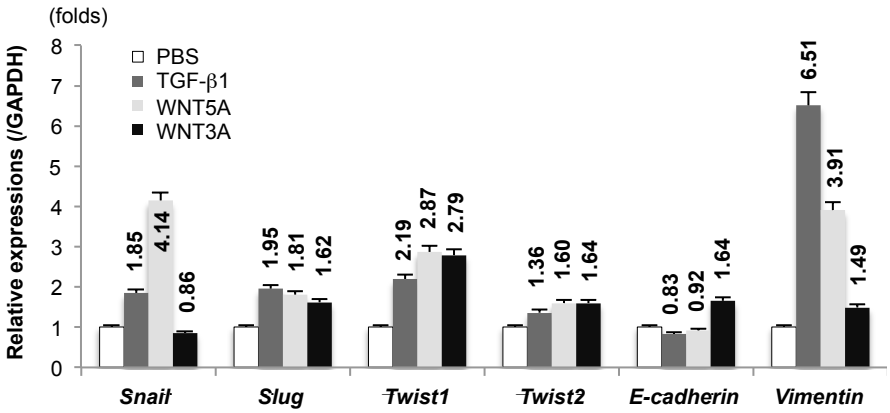
Supplemental Figure S6





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 $CD133^{+}$ or $CD133^{-}$. 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-b1 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.