Supplemental figure 1

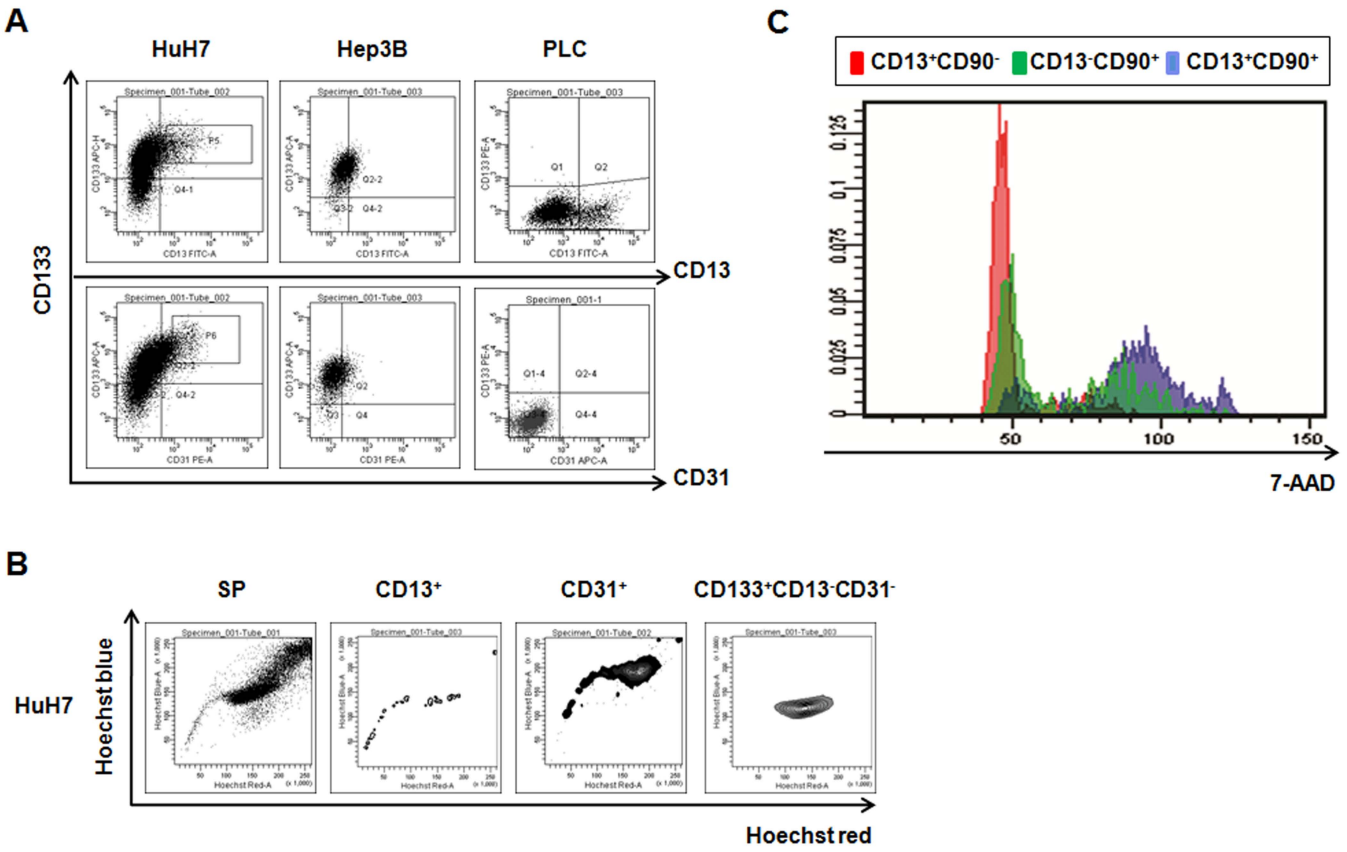

Figure S1. Evaluation of the CD31 expression and cell cycle status.

(A) The expression of the CD31 was assessed in HuH7, Hep3B, and PLC/PRF/5 cells. There was no apparent expression of the CD31 in PLC/PRF/5 cells.

(B) The relations of the CD13 and SP fraction. The CD31+ cells mainly exists in G2/M/SP fraction.

(C) Cell cycle assay of the CD13+CD90−, CD13+CD90+, and CD13−CD90+ fractions of PLC/PRF/5 cells with 7-AAD.

Supplemental figure 2

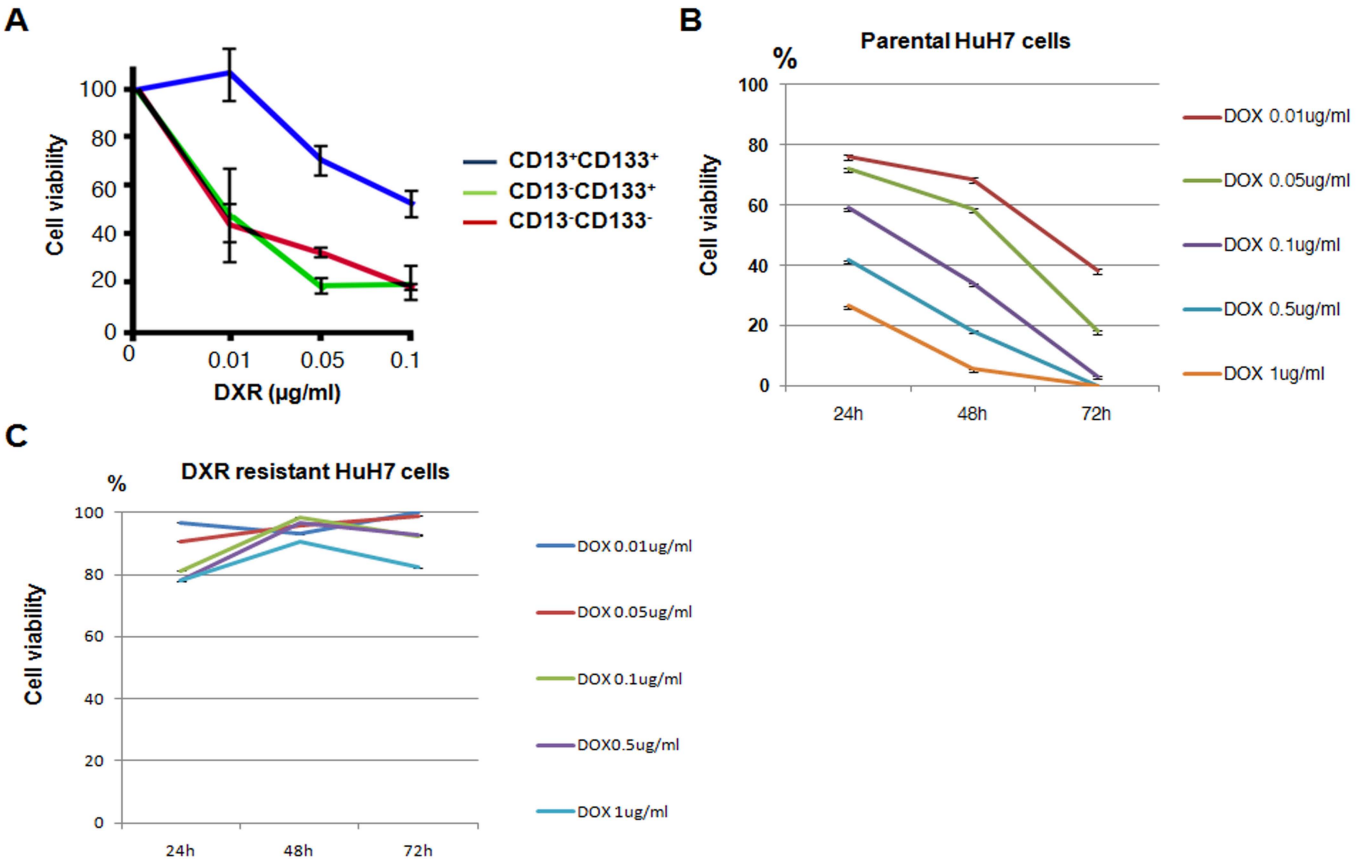

Figure S2. The CD13+ cells resist chemotherapy.

(A) Cell fractions CD13+CD133+, CD13−CD133+, and CD13−CD133− were isolated and the chemo-sensitivity was assessed in each fraction. Cells were treated with various concentrations of DXR for 72 h. Bars represent the error range.

(B) Chemoresistance assay. Parental HuH7 cells were cultured in the medium with DXR as indicated and cell viability was analyzed.

(C) Doxorubicin resistant (DXR-R) HuH7 cells were cultured in the medium with DXR as indicated and cell viability was analyzed.

Supplemental figure 3

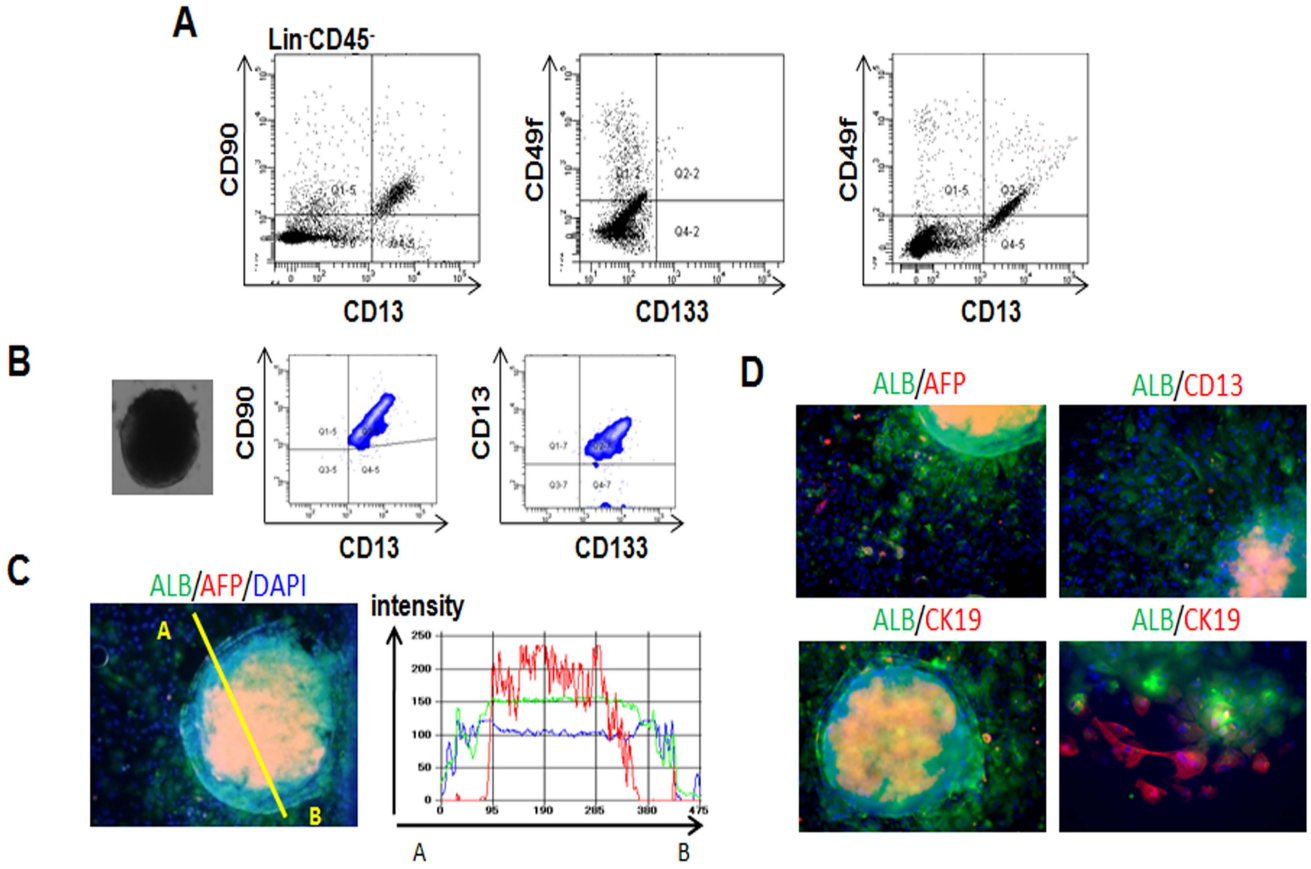

Figure S3. Enrichment of CD13+CD90+ normal hepatocytes in sphere formation, showing multi-differentiation potential

(A) Fresh normal hepatocytes, obtained in liver surgery, were analyzed for the CD13, CD90, and CD133 expression by multi-color flow cytometry.

(B) Multi-color flow cytometry indicated that normal hepatocyte sphere cells were predominantly CD13+CD90+CD133+.

(C) Multi-differentiation potential of normal hepatocyte sphere cells. Formed spheres were fixed and subjected to immunostaining with specific antibodies. The computed dissection analysis for differentiation markers detected the expression of albumin (blue) and alfa-fetoprotein (red) inside of spheres. Nuclei were stained by DAPI.

(D) The red staining indicates immature liver marker alfa-fetoprotein (the top left panel), cholangio-marker CD13 (the top right), cholangio-marker CD19 (the bottom pannels). Albumin, green.

Supplemental figure 4

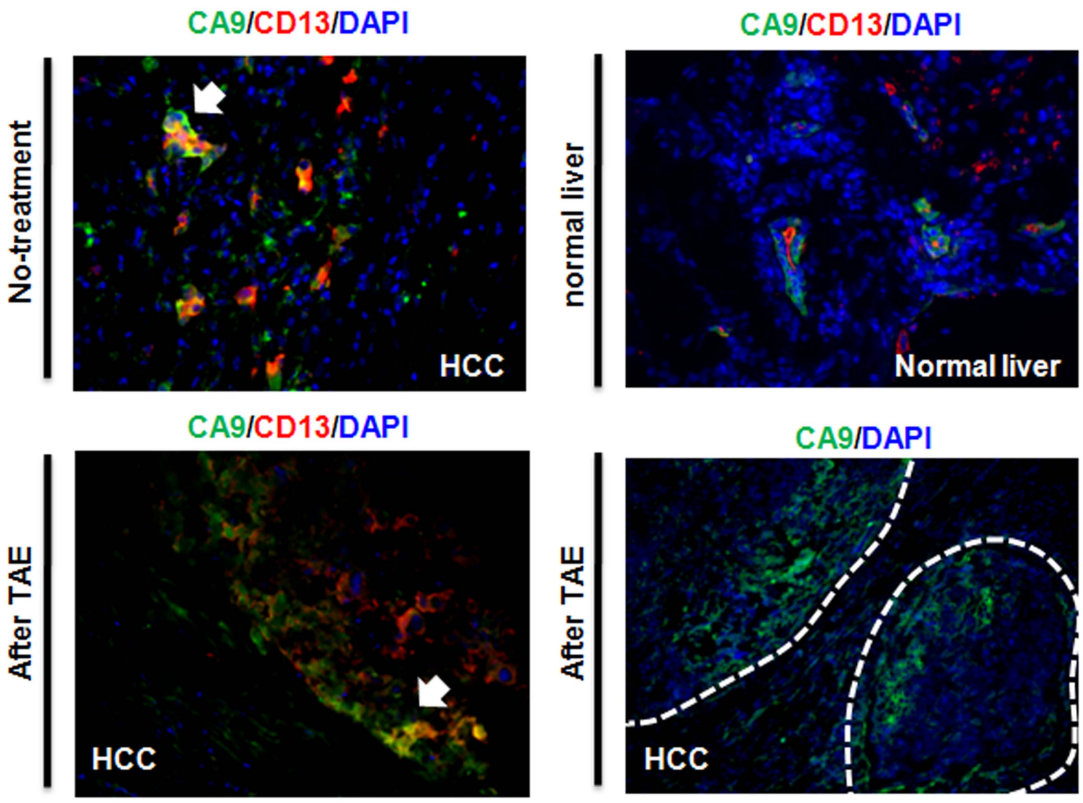

Figure S4. Evaluation of the expression of CA9 and CD13.

The expression of CD31 and hypoxia marker CA9 were assessed in no-treatment, after TAE and normal liver clinical samples. CD13 (red), CA9 (green) and nuclei (blue). The white dot lines indicate fibrous capsule and tumor cells exists inside of white dot circle. White arrow shows double positive cells of CD13 and CA9.

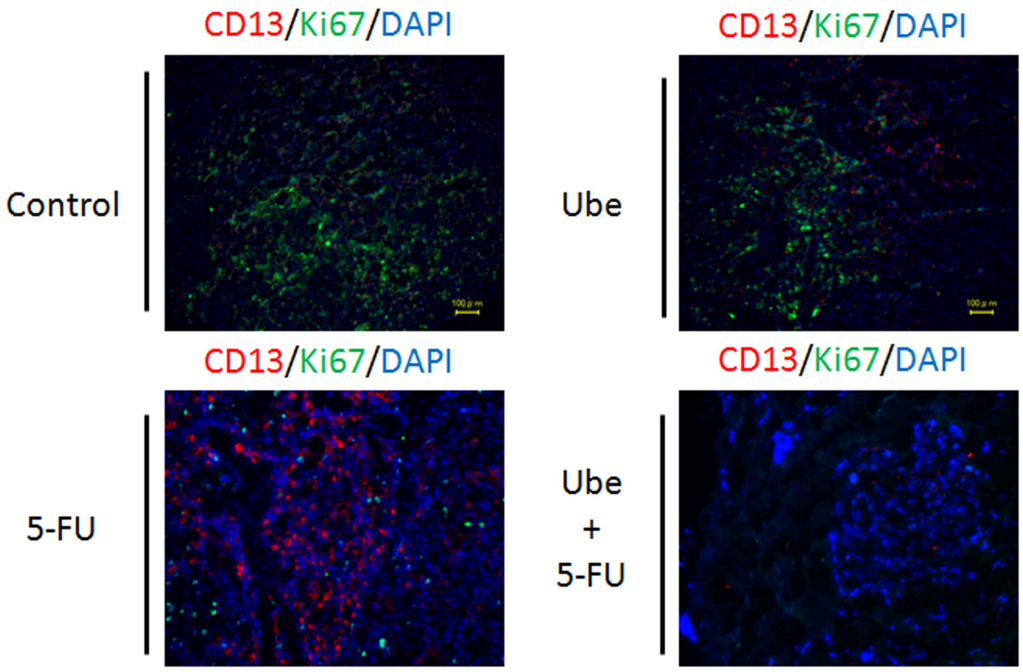

Figure S5. Evaluation of the CD13 and Ki67 expression in tumors of PLC/RLF/5 xenografted mice.
The PLC/PRF/5 cells xenografted mice were treated with 5-FU, Ubenimex and Ubenimex plus 5-FU for 14 days. Enucleated tumors were stained with CD13 (red), Ki67 (green) and nuclei for DAPI (blue).

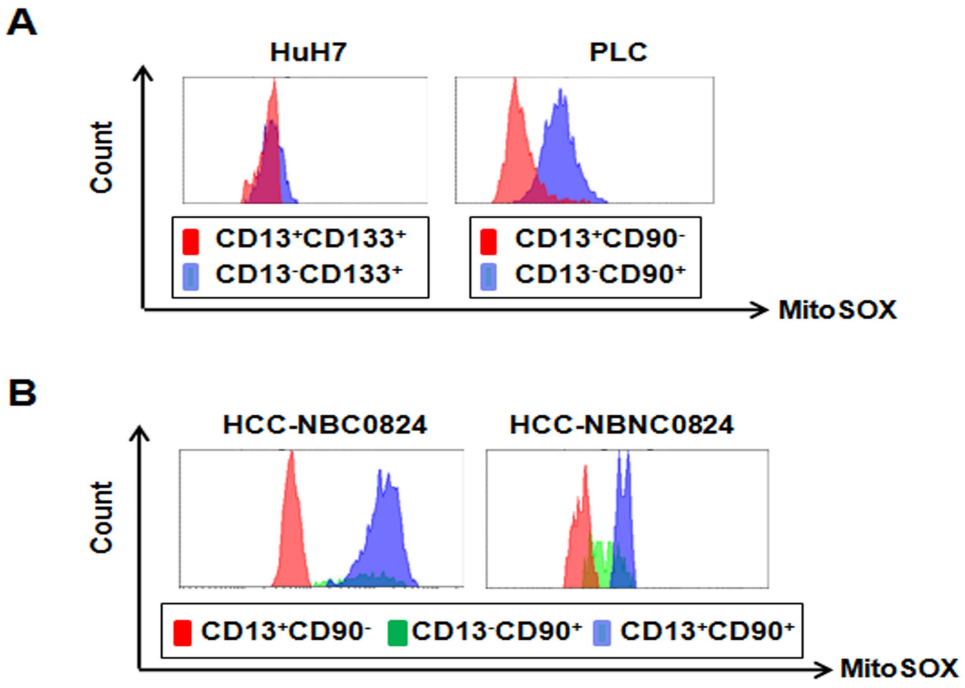

Figure S6. Evaluation of the MitoSOX expression

(A) The expression of mitochondrial ROS (MitoSOX) in HuH7 and PLC/PRF/5

(B) The expression of mitochondrial ROS (MitoSOX) in 2 clinical HCC samples.

Supplementary Table 1. Lists of 56 up-regulated membrane associated genes in the SP cells

ANKRD3	NM_020639	Homo sapiens ankyrin repeat domain 3 (ANKRD3), mRNA
SLC15A2	NM_021082	Homo sapiens solute carrier family 15 (H+/peptide transporter), member 2 (SLC15A2), mRNA
LCT	NM_002299	Homo sapiens lactase (LCT), mRNA
FER1L3	NM_133337	Homo sapiens fer-1-like 3, myoferlin (C. elegans) (FER1L3), transcript variant 2, mRNA
GBP3	NM_018284	Homo sapiens guanylate binding protein 3 (GBP3), mRNA
PRO0659	NM_014138	Homo sapiens PRO0659 protein (PRO0659), mRNA
PECAM1	NM_000442	Homo sapiens platelet/endothelial cell adhesion molecule (CD31 antigen) (PECAM1), mRNA
CDKL5	NM_003159	Homo sapiens cyclin-dependent kinase-like 5 (CDKL5), mRNA
CDH1	NM_004360	Homo sapiens cadherin 1, type 1, E-cadherin (epithelial) (CDH1), mRNA
ANPEP	NM_001150	Homo sapiens alanyl (membrane) aminopeptidase (aminopeptidase N, aminopeptidase M, microsomal aminopeptidase, CD13, p150) (ANPEP), mRNA
SLC37A1	NM_018964	Homo sapiens solute carrier family 37 (glycerol-3-phosphate transporter), member 1 (SLC37A1), mRNA
ABCC2	NM_000392	Homo sapiens ATP-binding cassette, sub-family C (CFTR/MRP), member 2 (ABCC2), mRNA
LRRC19	NM_022901	Homo sapiens hypothetical protein FLJ21302 (FLJ21302), mRNA
OSTalpha	NM_152672	Homo sapiens organic solute transporter alpha (OSTalpha), mRNA
UNC93A	NM_018974	Homo sapiens unc-93 homolog A (C. elegans) (UNC93A), mRNA
JAG1	NM_000214	Homo sapiens jagged 1 (Alagille syndrome) (JAG1), mRNA
OSTbeta	NM_178859	Homo sapiens organic solute transporter beta (OSTbeta), mRNA
FMOD	NM_002023	Homo sapiens fibromodulin (FMOD), mRNA
ZDHHC14	NM_024630	Homo sapiens zinc finger, DHHC domain containing 14 (ZDHHC14), mRNA
PDZGEF1	NM_014247	Homo sapiens PDZ domain containing guanine nucleotide exchange factor (GEF) 1 (PDZGEF1), mRNA
I_1002307	I_1002307	Phosphoinositide-3-kinase regulatory subunit 3, binds insulin receptor (INSR) and insulin-like growth factor receptor (IGF1R), may be involved in regulating signaling
UNC5CL	NM_173561	Homo sapiens unc-5 homolog C (C. elegans)-like (UNC5CL), mRNA
TM4SF8	NM_005724	Homo sapiens tetraspan 3 (TSPAN-3), mRNA
ABCB1	NM_000927	Homo sapiens ATP-binding cassette, sub-family B (MDR/TAP), member 1 (ABCB1), mRNA
GPM6A	NM_005277	Homo sapiens glycoprotein M6A (GPM6A), mRNA
AK026443	AK026443	Homo sapiens cDNA: FLJ22790 fis, clone KAIA2176, highly similar to HUMPMCA Human plasma membrane calcium-pumping ATPase (PMCA4) mRNA
SLC2A9	NM_020041	Homo sapiens solute carrier family 2 (facilitated glucose transporter), member 9 (SLC2A9), mRNA
CEACAM6	NM_002483	Homo sapiens carcinoembryonic antigen-related cell adhesion molecule 6 (non-specific cross reacting antigen) (CEACAM6), mRNA
ATP1B1	NM_001677	Homo sapiens ATPase, Na+/K+ transporting, beta 1 polypeptide (ATP1B1), mRNA
SLC38A2	NM_018976	Homo sapiens solute carrier family 38, member 2 (SLC38A2), mRNA
RNF19	NM_015435	Homo sapiens ring finger protein 19 (RNF19), mRNA
AB007935	AB007935	Homo sapiens mRNA for KIAA0466 protein, partial cds
C11orf11	NM_006133	Homo sapiens chromosome 11 open reading frame 11 (C11orf11), mRNA
AK025431	AK025431	Homo sapiens cDNA: FLJ21778 fis, clone HEP00201
ABHD6	NM_020676	Homo sapiens abhydrolase domain containing 6 (ABHD6), mRNA
SLCO2B1	NM_007256	Homo sapiens solute carrier family 21 (organic anion transporter), member 9 (SLC21A9), mRNA
SAT2	NM_133491	Homo sapiens polyamine N-acetyltransferase (SSAT2), mRNA
TNFSF10	NM_003810	Homo sapiens tumor necrosis factor (ligand) superfamily, member 10 (TNFSF10), mRNA
COL13A1	NM_005203	Homo sapiens collagen, type XIII, alpha 1 (COL13A1), transcript variant 1, mRNA
BC035618	BC035618	Homo sapiens somatostatin receptor 1, mRNA (cDNA clone MGC:45280 IMAGE:5175220), complete cds
BC031313	BC031313	Homo sapiens solute carrier family 1 (glial high affinity glutamate transporter), member 2, mRNA (cDNA clone IMAGE:5276194), partial cds
ATP6V0A4	NM_020632	Homo sapiens ATPase, H+ transporting, lysosomal V0 subunit a isoform 4 (ATP6V0A4), transcript variant 1, mRNA
CA4	NM_000717	Homo sapiens carbonic anhydrase IV (CA4), mRNA
ABCG2	NM_004827	Homo sapiens ATP-binding cassette, sub-family G (WHITE), member 2 (ABCG2), mRNA

Supplementary Table 2. Lists of 47 Antibodies Examined in the SP Screening

Antibody	Clone	Source	Antibody	Clone	Source
CD10	eBioCB-CALLA	eBioscience	CD164	N6B6	BD
CD13	WM-15	BD	CD166	3A6	BD
CD18	CLB-LFA-1/1	eBioscience	CD167a	51D6	BioLegend
CD24	ML5	BD	CD184	12G5	eBioscience
CD26	L272	BD	CD202b	83715	R&D
CD29	4B4	BECKMANCOULTER	CD227	HMPV	BD
CD31	WM59	eBioscience	CD266	ITEM-4	eBioscience
CD34	8G12	BD	CD321	WK9	eBioscience
CD44	G44-26	BD	CD324	67A4	BioLegend
CD45	HI30	BD	CD325	401408	R&D
CD47	HCD47	BioLegend	CD326	1B7	eBioscience
CD49f	GoH3	BD	CD334	4FR6D3	BioLegend
CD51/CD61	23C6	BD	CD339		ACR
CD55	IA10	BD	ABCG2	5D3	eBioscience
CD71	M-A712	BD	Lin1(CD3,14,16,19,20,56)		BD
CD90	5E10	BioLegend	c-Met	eBioclone 97	eBioscience
CD104	439-9B	BD	aVb5 integrin	P1F6	CHEMICON
CD106	STA	eBioscience	MSC and NPC	W4A5	BioLegend
CD115	12-3A3-1B10	eBioscience	NPC	57D2	BioLegend
CD130	AM64	BD	SSEA-4	MC-813-70	BioLegend
CD133/1	13A4	eBioscience	TRA-1-81	TRA-1-81	BioLegend
CD135	BV10A4H2	BioLegend	hBMPR/ALK-3		R&D
CD144	TEA1/31	BECKMANCOULTER	ACE2	171608	R&D
CD162	KPL-1	BD			