Supporting Materials

Figure legends

Supplemental Fig 1. Representative MS/MS spectrum of the iTRAQ-labeled peptide KVESLQEEIAFLK derived from Vimentin. Observed b-, y-ions and the iTRAQ reporter ions are annotated.

Supplemental Fig 2. Summary of Hep3B cancer /stem cell proteomic results obtained from (A) Mascot (B) ProteinPilot. The iTRAQ labeled samples were fractionated by SCX, solution-IEF and basic-RP prior LC-MS/MS analysis.

Supplemental Fig 3. Peptide fractionation summary results (A) SCX, (B) basic RP, (C) solution-IEF. (a) The protein identification results in every fraction search by Mascot. The dark bars represent the number of proteins identified only in the fractionation approach. (b) Percentage of peptide distribution for each fraction. Basic RP strategy (B-b) shows the highest percentage of the peptides identified in single fraction, which indicates this strategy has better focusing power.

Supplemental Fig 4. Orthogonality of 2D LC separation. Retention time plots for selected 2D-LC systems. (A) SCX, (B) basic RP, (C) solution-IEF. The x-axis represents fraction number for each fraction strategy and y-axis represents associated retention time of nanoLC-MS.

Supplemental Fig 5. Functional importance of vimentin in CSC-like Hep3B cells. (A) Two clones of Hep3B cells were treated with withaferin A (1 μM) for one hour. The expression levels of vimentin in these two clones of Hep3B in control group (C) or treated with withaferin A (WA) were shown. (B) Two clones of Hep3B cells were treated with withaferin A (1-2 μM) for one hour. The abilities of spheroid formation in these cells were observed daily after cells were plated. Images of spheroid formation on day 2 and day 5 were shown. (C) CSC-like Hep3B cells were pre-treated with withaferin A (2 μM) for one hour before being used in scratch assay (N=3).

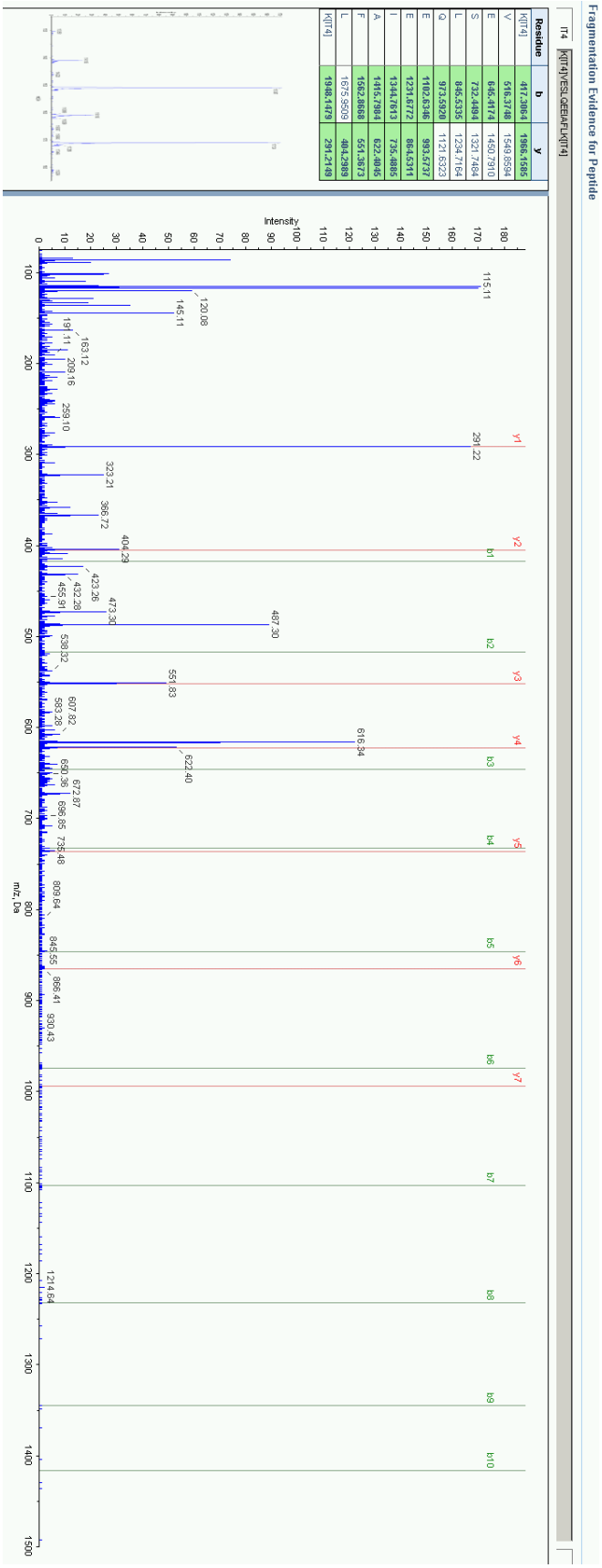
Supplemental Table 1. Expression of CSC markers in Hep3B cells.

Expression of CSC markers in control and CSC-like Hep3B cells were detected by flow cytometer and analyzed by comparing mean fluorescence intensity (MFI) of each marker in these cells (N=3).

Supplemental Table 2. Cell viability of CSC-like Hep3B cells in response to co-treatment of withaferin A and chemotherapeutic reagents.

CSC-like Hep3B cells were treated chemotherapeutic reagents alone or co-treated with withaferin A and chemotherapeutic reagents. Dose-dependent cell viability of Hep3B cells in each group was examined by MTT assay.

Figure S1.


Fragmentation Evidence for Peptide
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K(IT4)VESLQEEAAFLK(IT4)
Residue
b
y
K(IT4)
V
E
S
L
Q
E
E
I
A
F
L
K(IT4)
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291.22
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538.92
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583.28
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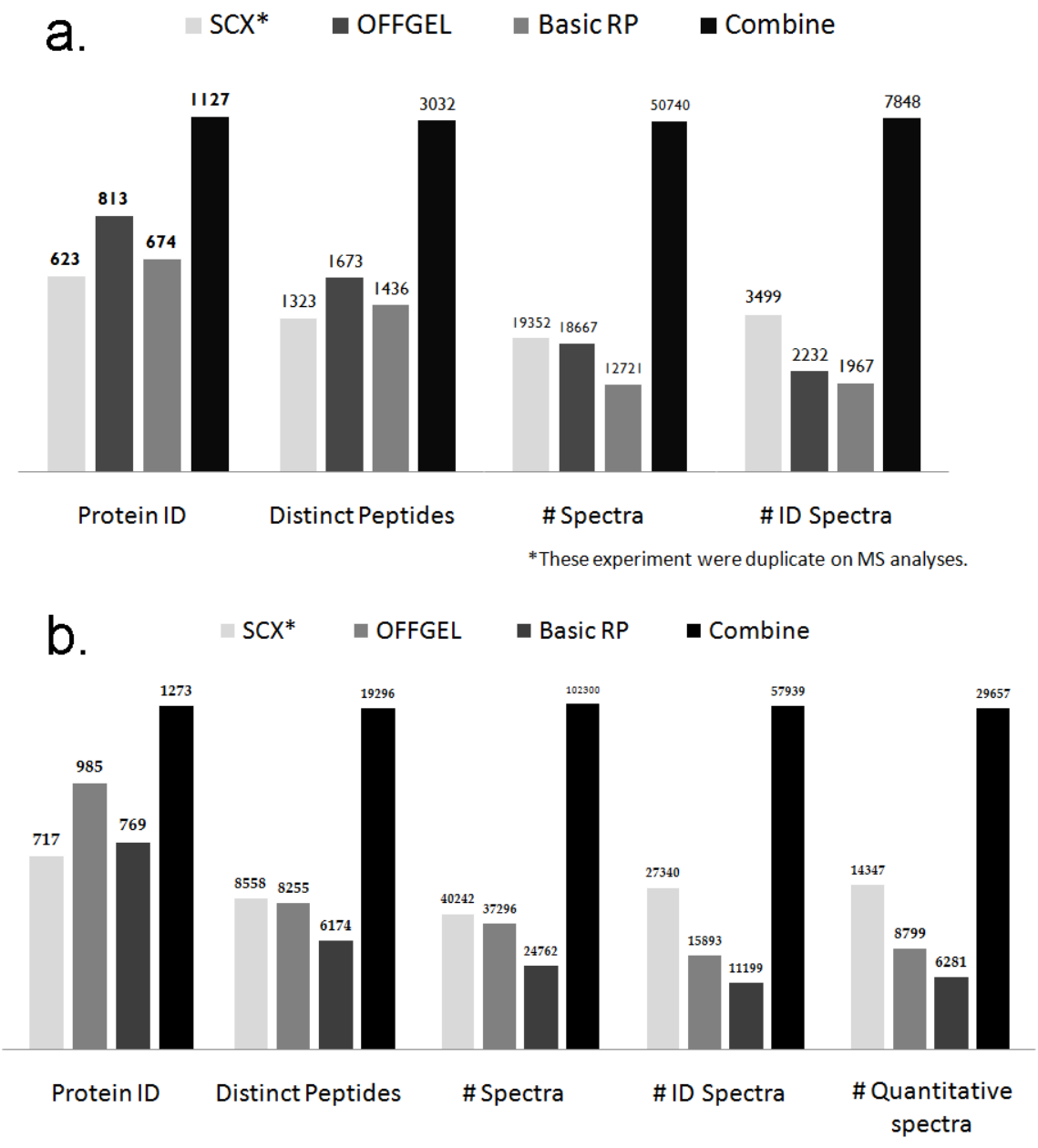
866.41
930.43
1214.64
y1
y2
b1
b2
y3
y4
b3
y5
b4
b5
y6
b6
y7
b7
b8
b9
b10

Figure S2.


a.
SCX*
OFFGEL
Basic RP
Combine
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674
1127
1323
1673
1436
3032
19352
18667
12721
50740
3499
2232
1967
7848
Protein ID
Distinct Peptides
Spectra
ID Spectra
*These experiment were duplicate on MS analyses.
b.
SCX*
OFFGEL
Basic RP
Combine
717
985
769
1273
8558
8255
6174
19296
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37296
24762
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15893
11199
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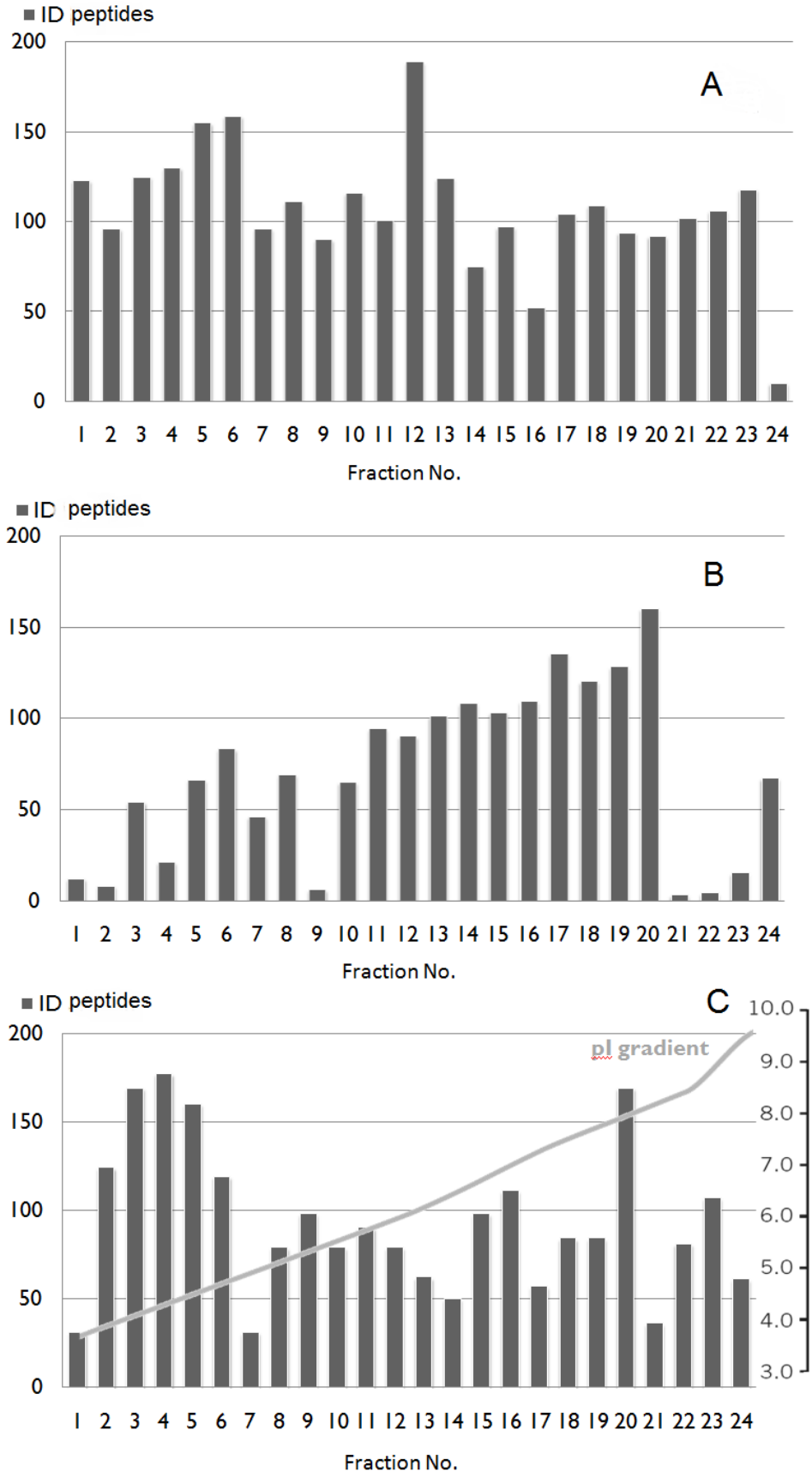
Protein ID
Distinct Peptides
Spectra
ID Spectra
Quantitative spectra
*These experiment were duplicate on MS analyses.

Figure S3.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100
50
0
A
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Fraction No.
ID peptides
200
150
100
50
0
B
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24
Fraction No.
ID peptides
C
200
150
100
50
0
pI gradient
10.0
9.0
8.0
7.0
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3.0
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24
Fraction No.

Figure S4.

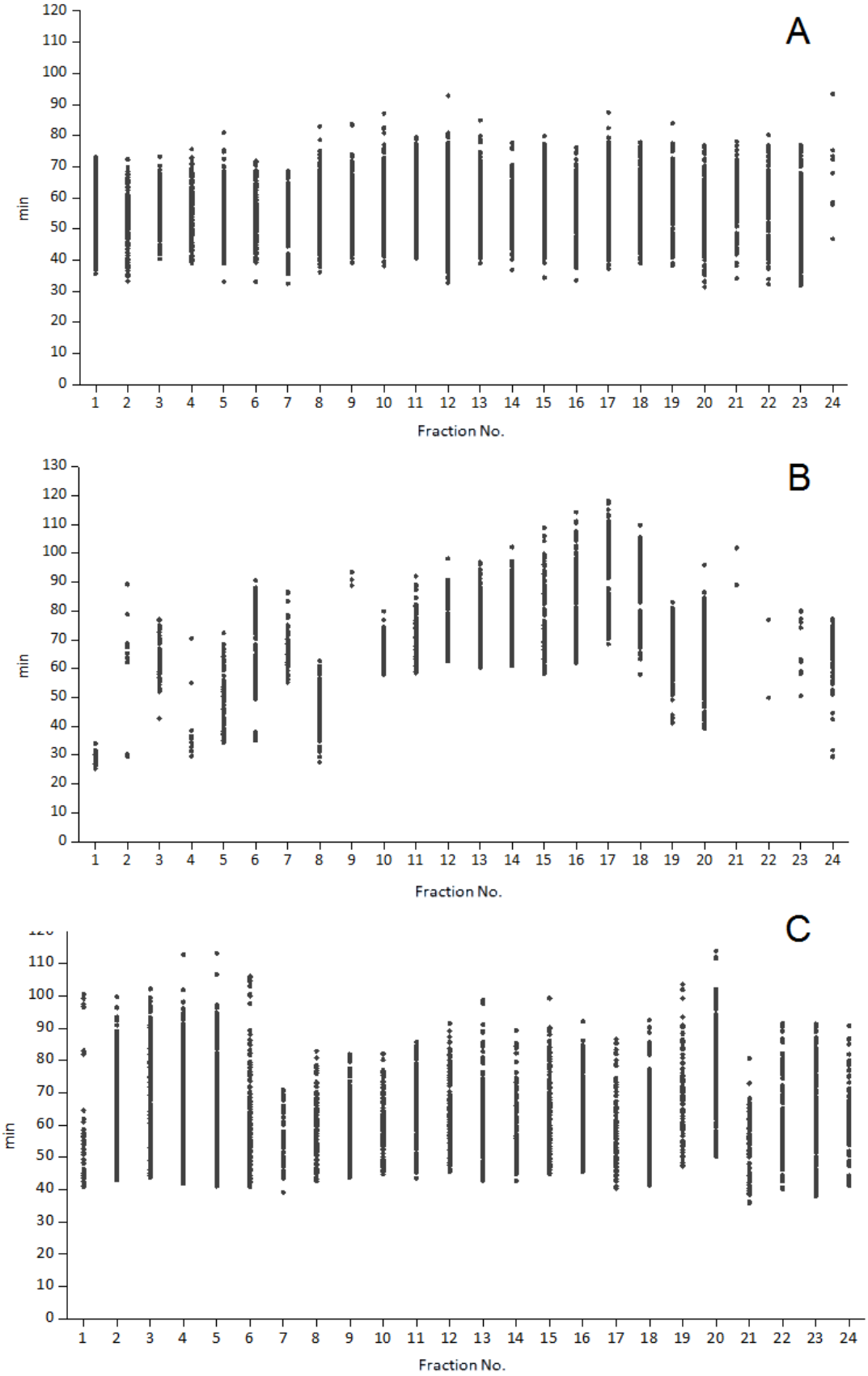
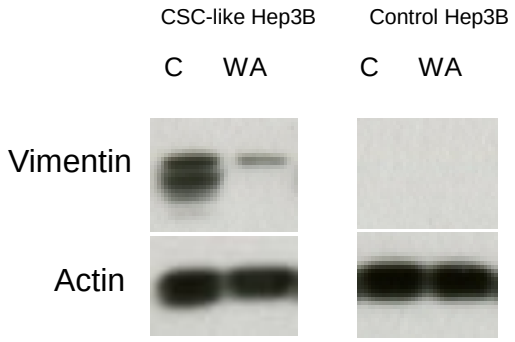
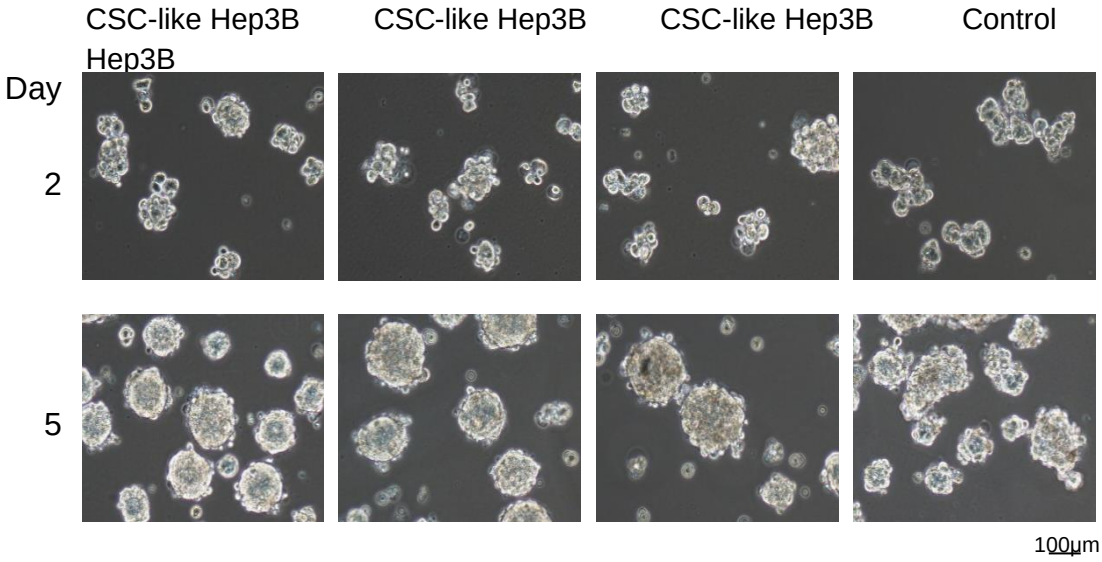


Figure S5.

A.



B. Sphere formation



C.

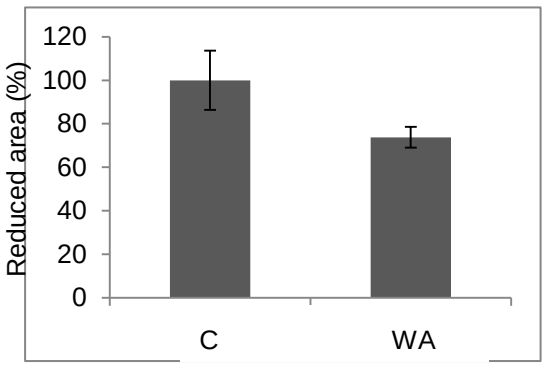


Table S1.

MFI

	CSC-like Hep3B	Control Hep3B	P value
CD13	517.70±192.94	1578.29±712.13	p=0.067
CD90	20.73±10.89	-5.47±29.93	p=0.230
CD133	640.57±70.01	73.07±79.68	P<0.001*
cKIT	-2.84±2.52	-13.74±40.43	p=0.670
EpCAM	2842.07±445.86	1498.81±824.47	p=0.068

MFI

	CSC-like Hep3B	Control Hep3B	P value
Vimentin	74.95±36.88	6.73±3.32	p=0.033*

Table S2.

Cell Drug (uM)		CSC-like Hep3B	CSC-like Hep3B + WA	P Value
Cisplatin	50	41.08±6.31%	38.15±15.81%	0.780
	25	50.93±8.63%	34.64±10.12%	0.100
	10	74.94±4.26%	41.11±9.80%	0.005*
	5	86.66±6.58%	53.73±1.76%	0.001*
Doxorubicin	5	52.33±7.02%	32.64±17.23%	0.140
	2.5	49.46±9.44%	34.79±18.01%	0.280
	1	82.23±5.53%	54.71±14.00%	0.110
	0.5	81.29±1.81%	62.63±11.75%	0.053
Idarubicin	5	45.15±6.19%	21.02±17.93%	0.092
	2.5	45.43±6.01%	22.25±18.93%	0.110
	1	35.26±10.74%	12.73±14.75%	0.099
	0.5	49.97±9.59%	42.03±16.56%	0.510