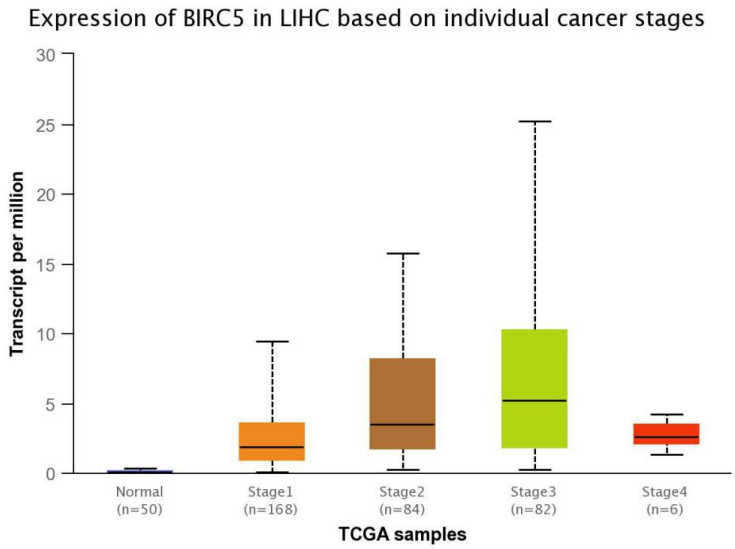
Answering Reviewers

Dear Reviewer,

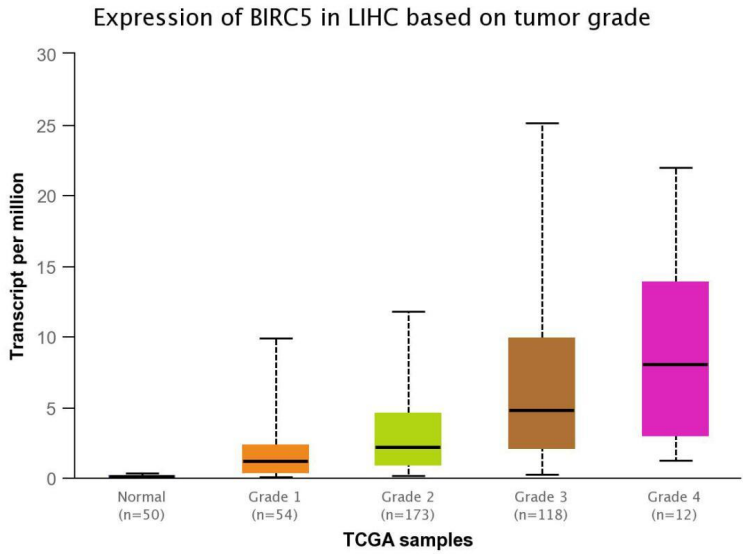
Thanks for your comments.

1. According to the TCGA analysis data, the expression of survivin in HCC tissue was significantly associated with stage and tumor grade. The transcript per million of survivin expression in patients with stage III and IV HCC was significantly higher than that in patients with stage I and II HCC($P<0.05$). And the transcript per million of survivin expression in patients with grade 3 and 4 HCC was significantly higher than that in patients with grade 1 and 2 HCC ($P<0.05$). You can see the results in supplementary figure 1 A and B.

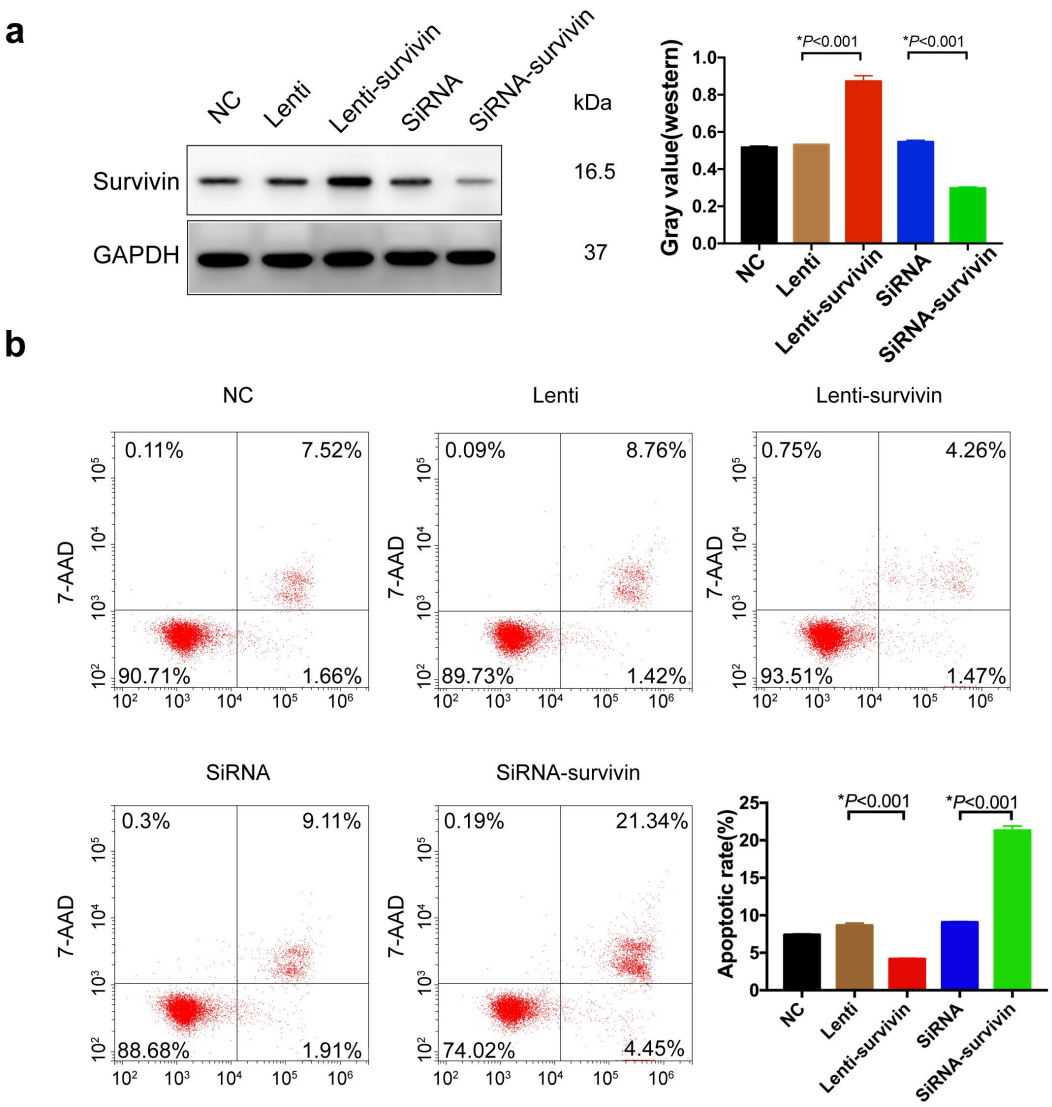
   A.



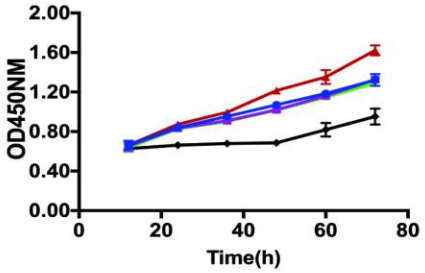
   B.

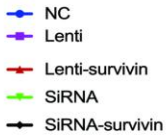


2. I revised the comparisons in figure 2 as below. Comparisons were made as Lenti. vs. Lenti-survivin, siRNA vs. siRNA-survivin.


a
NC
Lenti
Lenti-survivin
SiRNA
SiRNA-survivin
kDa
Survivin
16.5
GAPDH
37
*P<0.001
*P<0.001
Gray value(western)
1.0
0.8
0.6
0.4
0.2
0.0
NC
Lenti
Lenti-survivin
SiRNA
SiRNA-survivin
b
NC
Lenti
Lenti-survivin
0.11%
7.52%
90.71%
1.66%
0.09%
8.76%
89.73%
1.42%
0.75%
4.26%
93.51%
1.47%
7-AAD
SiRNA
SiRNA-survivin
0.3%
9.11%
88.68%
1.91%
0.19%
21.34%
74.02%
4.45%
*P<0.001
*P<0.001
Apoptotic rate(%)
25
20
15
10
5
0
NC
Lenti
Lenti-survivin
SiRNA
SiRNA-survivin

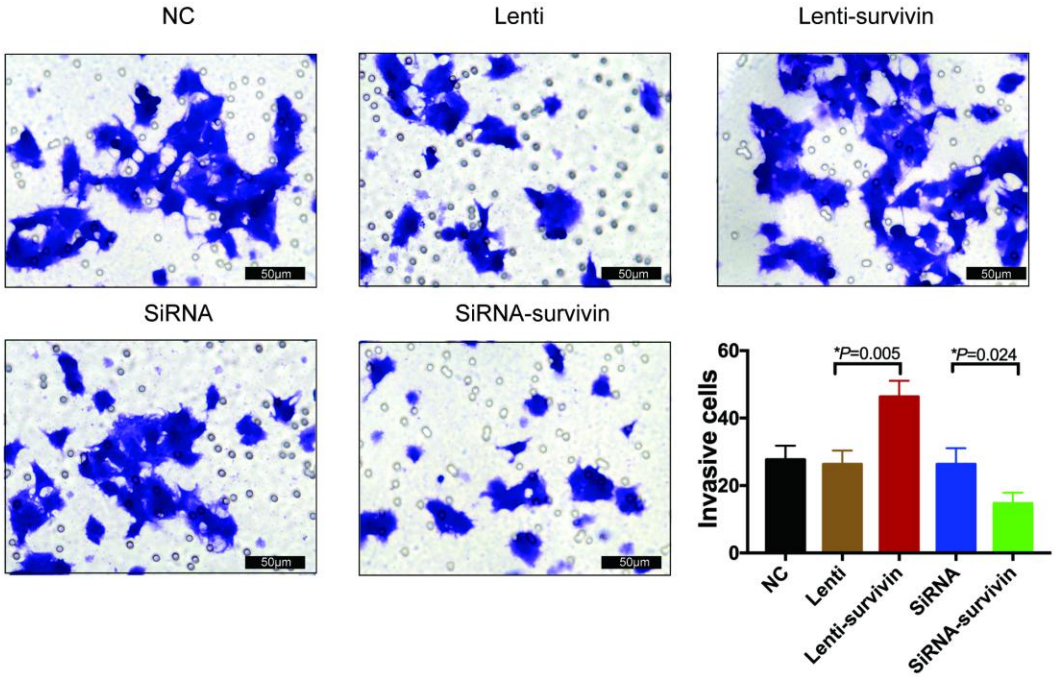
a





P-value	Lenti vs Lenti- Sur	SiRNA vs SiRNA-Sur
60hours	*P*<0.001	*P*<0.001
72hours	*P*<0.001	*P*<0.001

b



3. I modified all the figures as below.

Figure 1

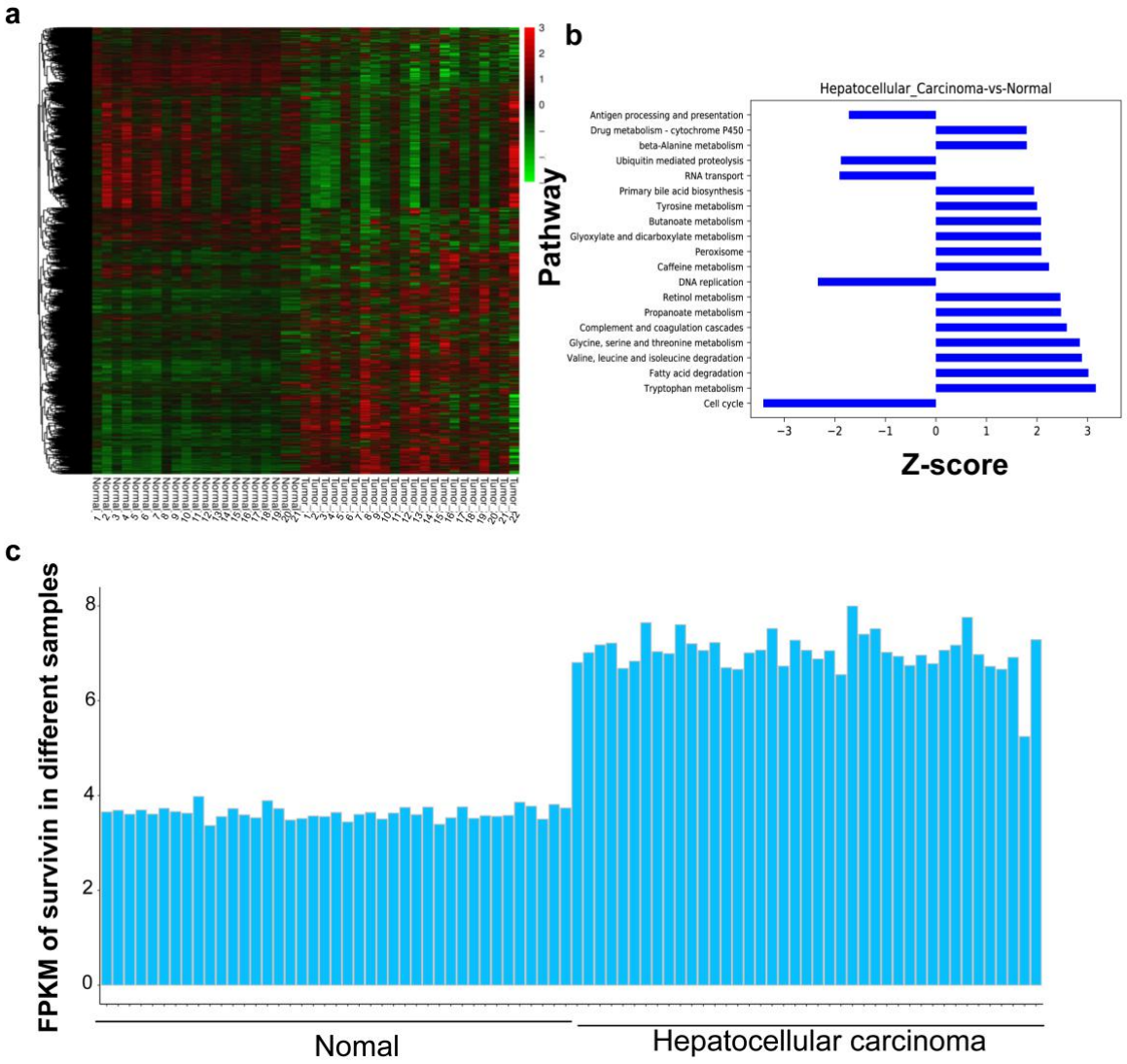

a
b
Hepatocellular_Carcinoma-vs-Normal
Antigen processing and presentation
Drug metabolism - cytochrome P450
beta-Alanine metabolism
Ubiquitin mediated proteolysis
RNA transport
Primary bile acid biosynthesis
Tyrosine metabolism
Butanoate metabolism
Glyoxylate and dicarboxylate metabolism
Peroxisome
Caffeine metabolism
DNA replication
Retinol metabolism
Propanoate metabolism
Complement and coagulation cascades
Glycine, serine and threonine metabolism
Valine, leucine and isoleucine degradation
Fatty acid degradation
Tryptophan metabolism
Cell cycle
Pathway
Z-score
c
FPKM of survivin in different samples
Nomal
Hepatocellular carcinoma

Figure 2

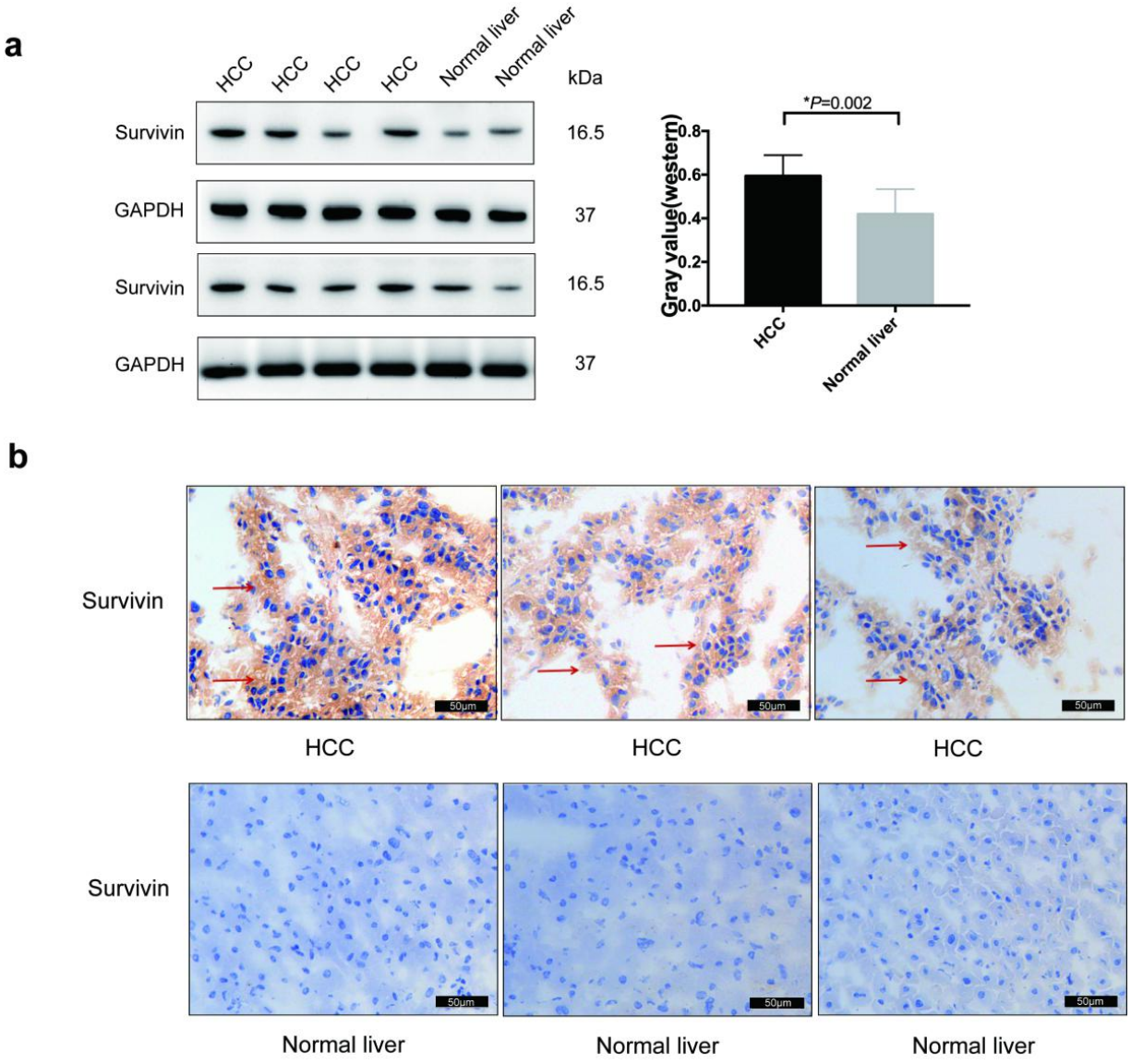

a
HCC
HCC
HCC
HCC
Normal liver
Normal liver
kDa
Survivin
16.5
GAPDH
37
Survivin
16.5
GAPDH
37
*P=0.002
Gray value(western)
0.8
0.6
0.4
0.2
0.0
HCC
Normal liver
b
Survivin
50μm
HCC
HCC
HCC
Survivin
Normal liver
Normal liver
Normal liver

Figure 3

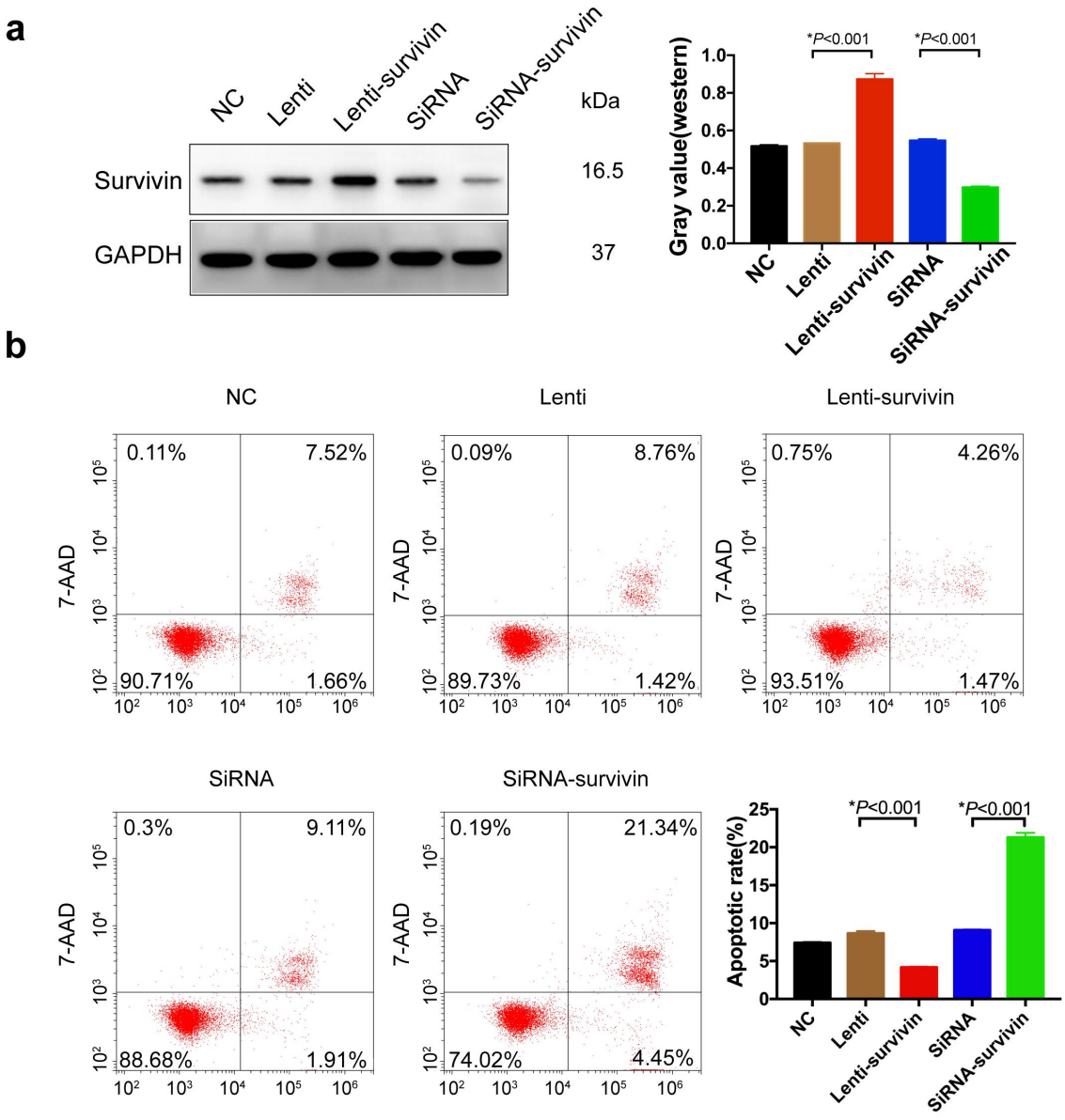
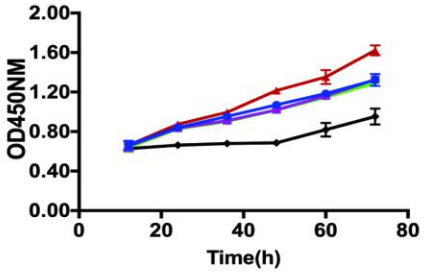

a
NC
Lenti
Lenti-survivin
SiRNA
SiRNA-survivin
kDa
Survivin
16.5
GAPDH
37
*P<0.001
*P<0.001
Gray value(western)
b
NC
Lenti
Lenti-survivin
0.11%
7.52%
90.71%
1.66%
0.09%
8.76%
89.73%
1.42%
0.75%
4.26%
93.51%
1.47%
SiRNA
SiRNA-survivin
0.3%
9.11%
88.68%
1.91%
0.19%
21.34%
74.02%
4.45%
7-AAD
*P<0.001
*P<0.001
Apoptotic rate(%)

Figure 4

a



P-value	Lenti vs Lenti- Sur	SiRNA vs SiRNA-Sur
60hours	*P*<0.001	*P*<0.001
72hours	*P*<0.001	*P*<0.001

b

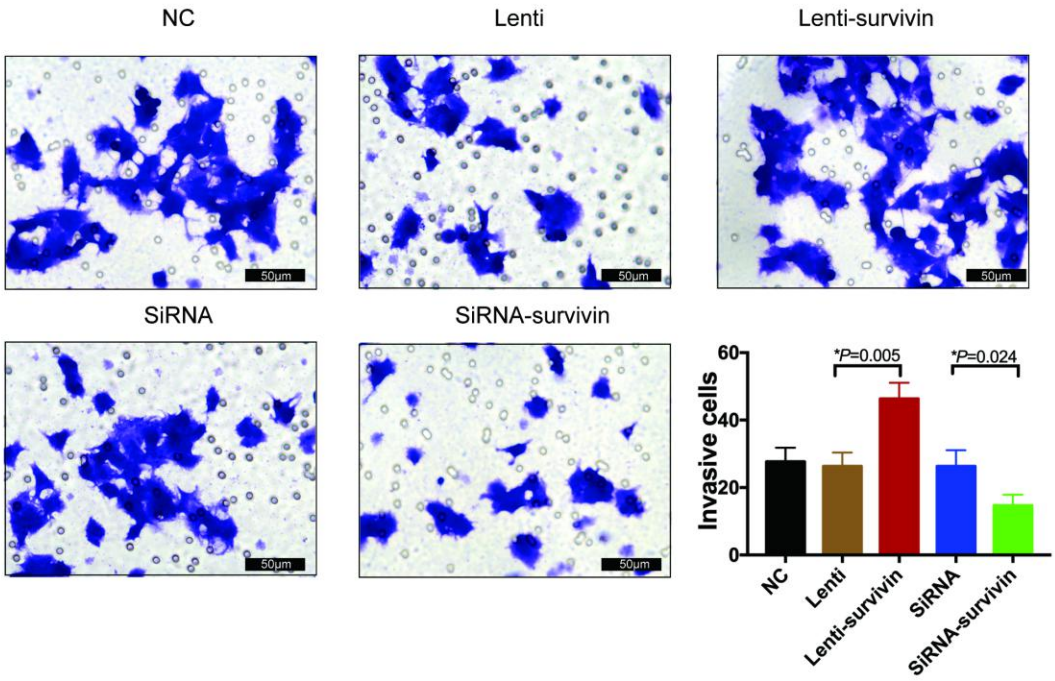


Figure 5

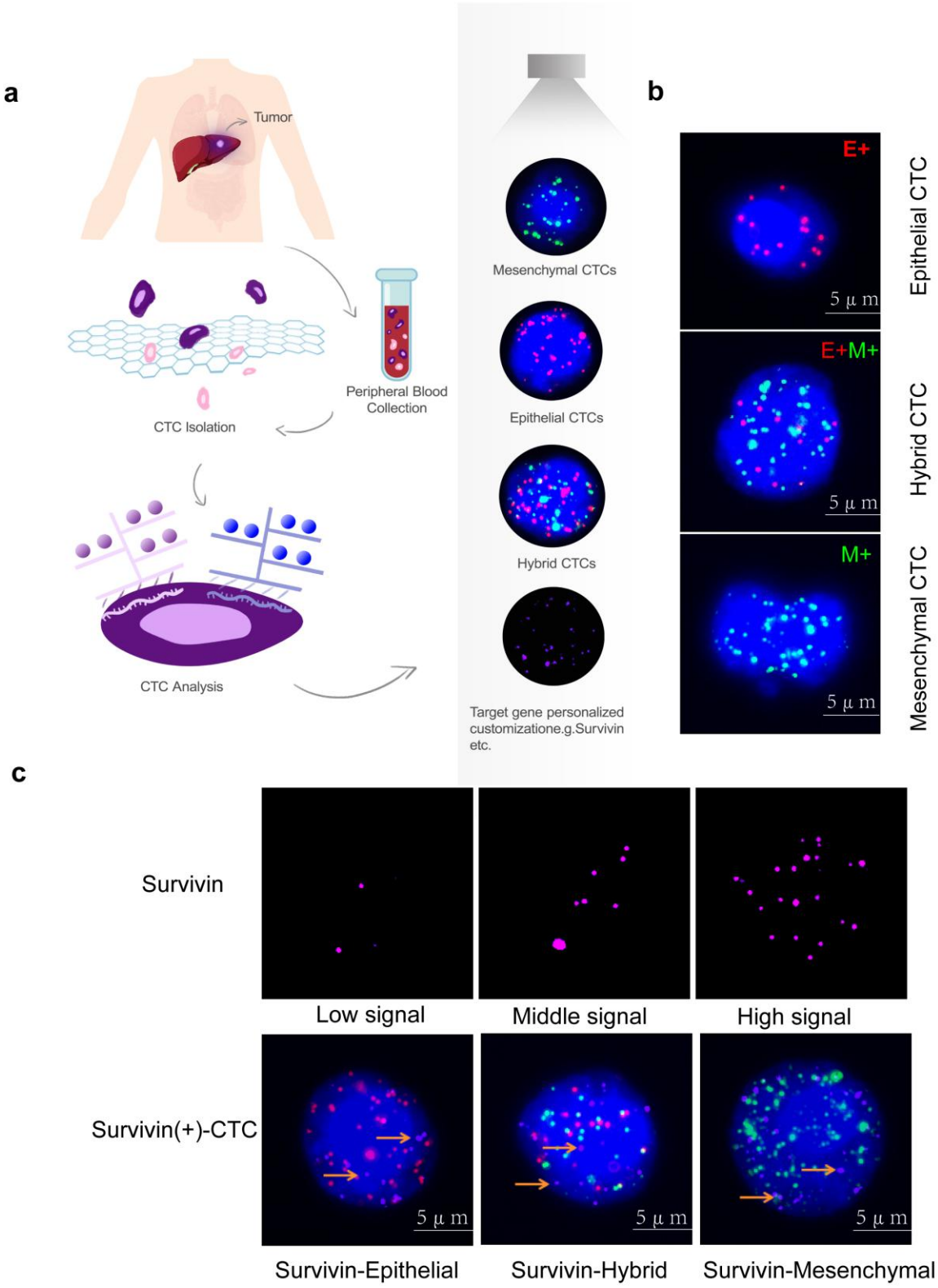

a
Tumor
Peripheral Blood Collection
CTC Isolation
CTC Analysis
Mesenchymal CTCs
Epithelial CTCs
Hybrid CTCs
Target gene personalized customizatione.g.Survivin etc.
b
E+
E+M+
M+
5 μ m
Epithelial CTC
Hybrid CTC
Mesenchymal CTC
c
Survivin
Low signal
Middle signal
High signal
Survivin(+)-CTC
Survivin-Epithelial
Survivin-Hybrid
Survivin-Mesenchymal

Figure 6

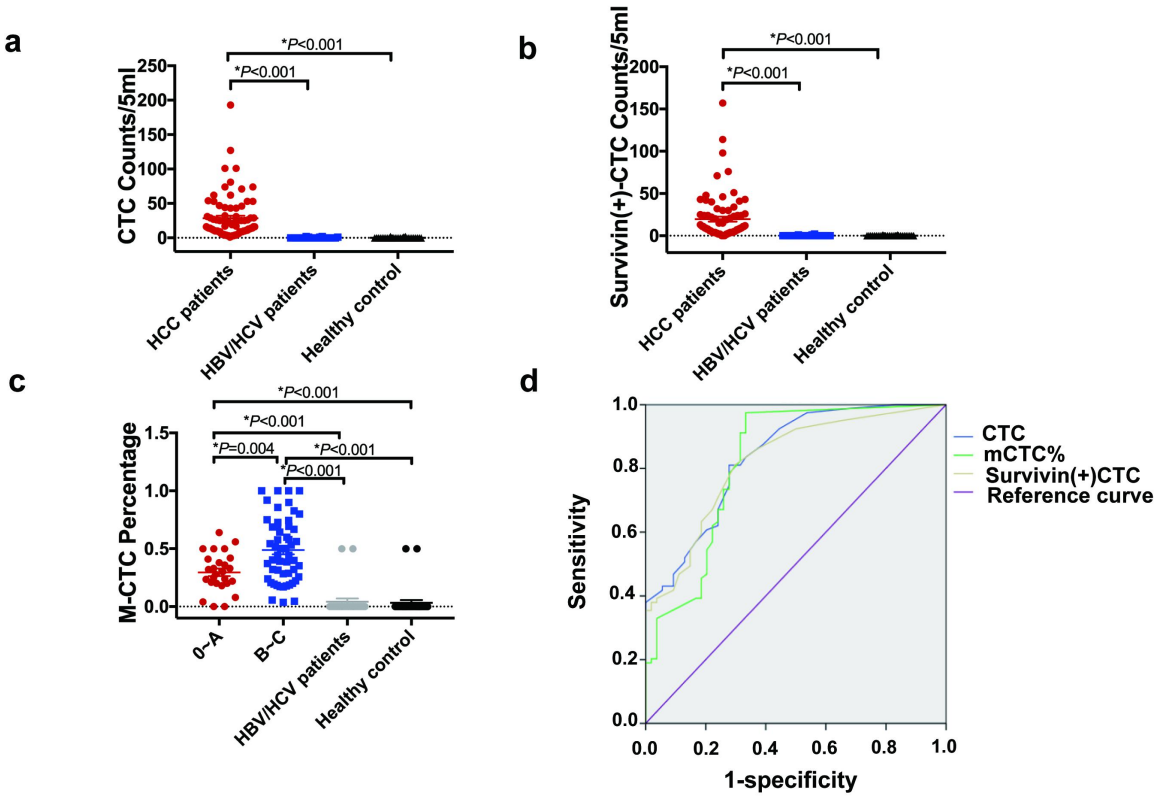


Figure 7

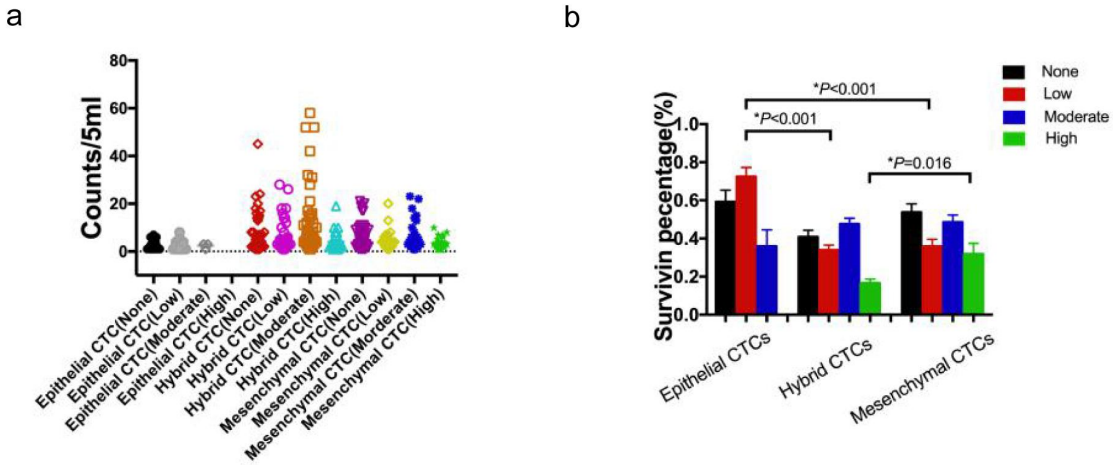


Figure 8

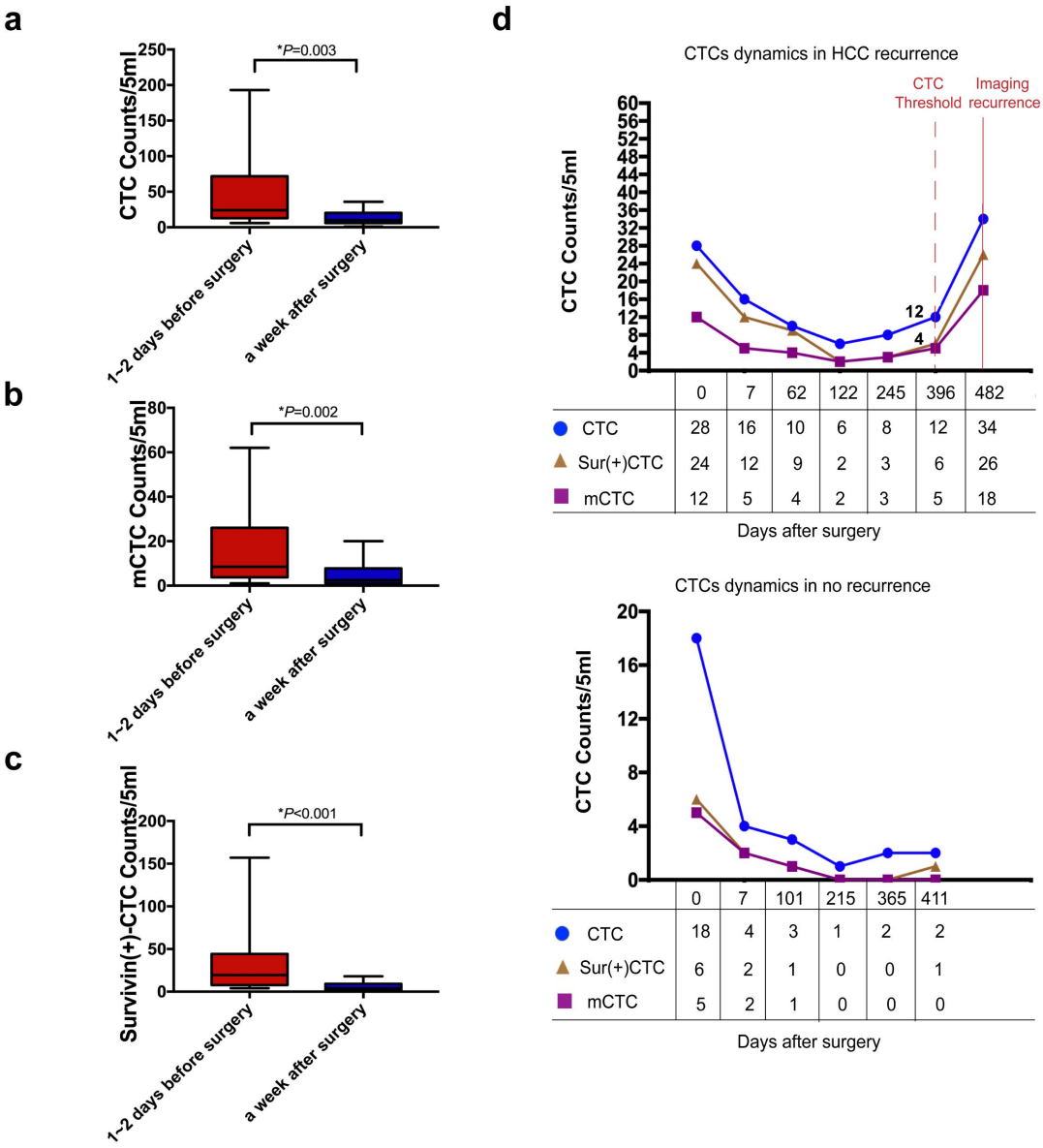

a
*P=0.003
CTC Counts/5ml
250
200
150
100
50
0
1~2 days before surgery
a week after surgery
b
*P=0.002
mCTC Counts/5ml
80
60
40
20
0
1~2 days before surgery
a week after surgery
c
*P<0.001
Survivin(+)-CTC Counts/5ml
200
150
100
50
0
1~2 days before surgery
a week after surgery
d
CTCs dynamics in HCC recurrence
CTC Threshold
Imaging recurrence
CTC Counts/5ml
60
56
52
48
44
40
36
32
28
24
20
16
12
8
4
0
12
4
0 7 62 122 245 396 482
CTC 28 16 10 6 8 12 34
Sur(+)CTC 24 12 9 2 3 6 26
mCTC 12 5 4 2 3 5 18
Days after surgery
CTCs dynamics in no recurrence
CTC Counts/5ml
20
16
12
8
4
0
0 7 101 215 365 411
CTC 18 4 3 1 2 2
Sur(+)CTC 6 2 1 0 0 1
mCTC 5 2 1 0 0 0
Days after surgery

Figure 9

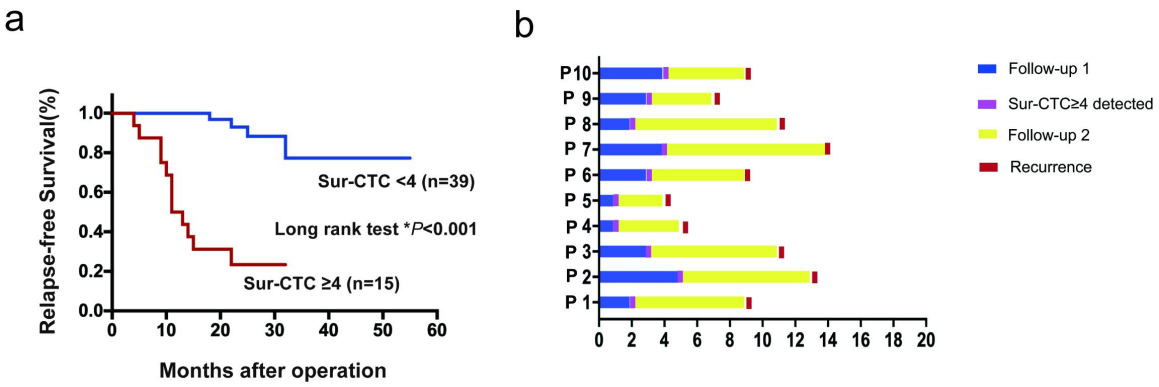
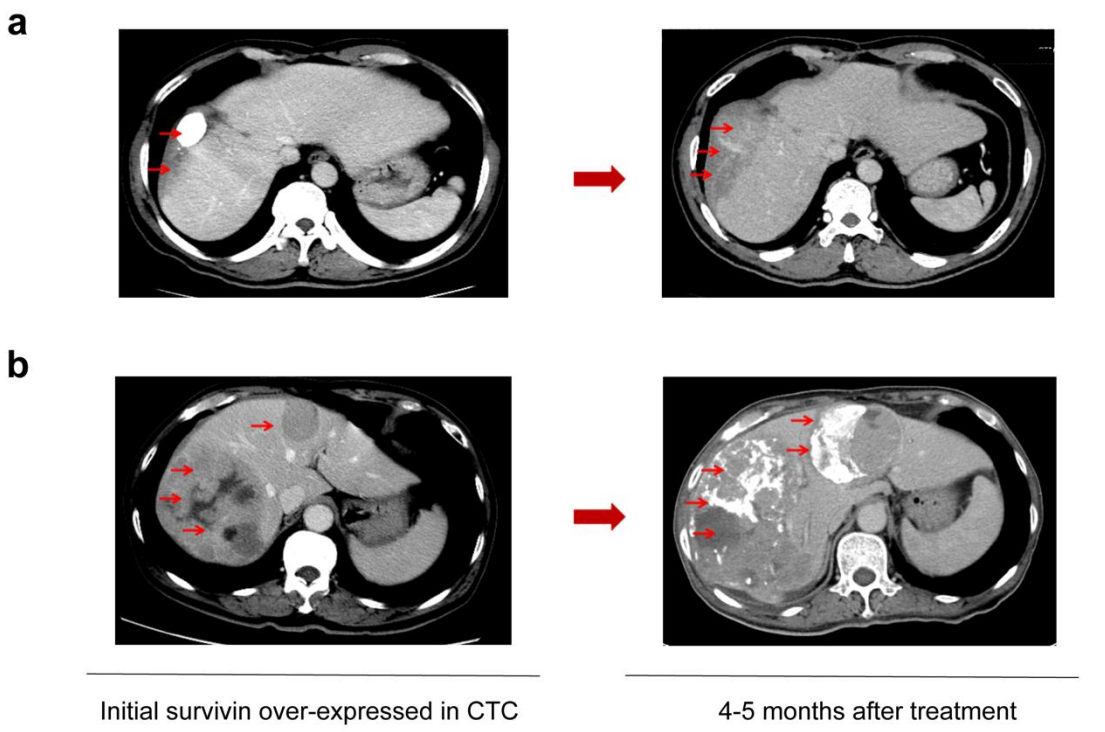
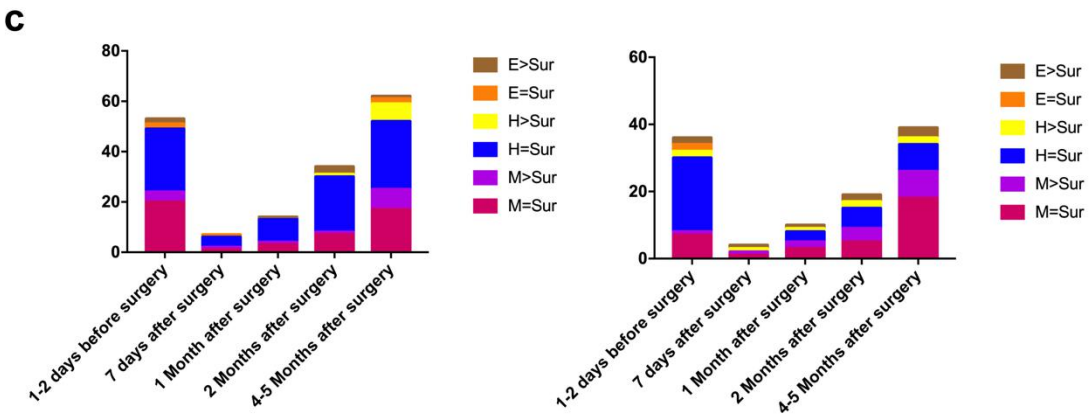

a
Relapse-free Survival(%)
1.0
0.8
0.6
0.4
0.2
0.0
0
10
20
30
40
50
60
Months after operation
Sur-CTC <4 (n=39)
Long rank test *P<0.001
Sur-CTC ≥4 (n=15)
b
P10
P 9
P 8
P 7
P 6
P 5
P 4
P 3
P 2
P 1
0
2
4
6
8
10
12
14
16
18
20
Follow-up 1
Sur-CTC≥4 detected
Follow-up 2
Recurrence

Figure10


a
b
Initial survivin over-expressed in CTC
4-5 months after treatment


c
80
60
40
20
0
1-2 days before surgery
7 days after surgery
1 Month after surgery
2 Months after surgery
4-5 Months after surgery
E>Sur
E=Sur
H>Sur
H=Sur
M>Sur
M=Sur
60
40
20
0
1-2 days before surgery
7 days after surgery
1 Month after surgery
2 Months after surgery
4-5 Months after surgery
E>Sur
E=Sur
H>Sur
H=Sur
M>Sur
M=Sur