Figure 1

(A)

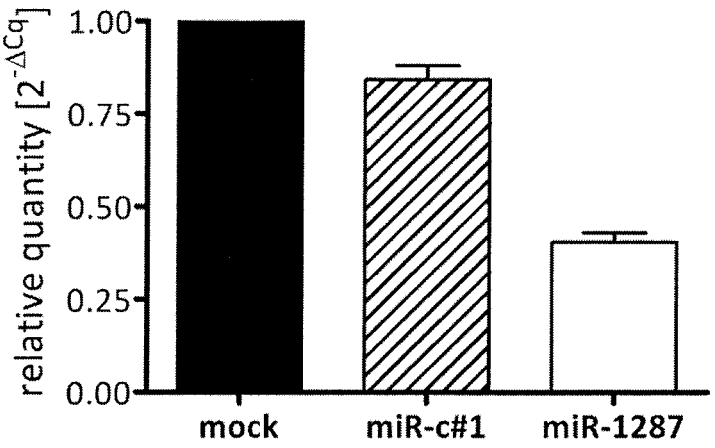
miRNA screen hit	NGS hit RefSeq	NGS hit Locus	Gene symbol	Gene description	normalized fold change gene expression (|log2|)
hsa-miR-1287 SEQ ID NO:32	XM_003508493.1	NW_003614548	Atf6b	activating transcription factor 6 beta	2.16 (1.11)
hsa-miR-1978 SEQ ID NO:33	XM_003498655.1	NW_003613699	Cers2	ceramide synthase 2	2.54 (1.34)
	XM_003511491.1	NW_006879419	Tbc1d20	TBC1 domain family, member 20	2.28 (1.19)

(B)

siRNA	Sequence	SEQ ID NO
siAtf6b#1	CCUCCUCAGGUUCAGUUCA	SEQ ID NO: 9
siAtf6b#2	GCAGCGAAUGAUCAAGAAC	SEQ ID NO: 10
siAtf6b#3	AGACACCUUCUAUGUUGUC	SEQ ID NO: 11
siTbc1D20#1	AGAACUAAUCGACAUUAUC	SEQ ID NO: 7
siCerS2#1	AGAGUCGGCUCCUAUCUAA	SEQ ID NO: 8
shRNA	Antisense target site	
shAtf6b#1	UCCAUCUUCACACUGAGGACC	SEQ ID NO: 15
shTbc1D20#1	AAUCCUUGCUCAACUGUCGAA	SEQ ID NO: 12
shCerS2#1	UUAAGUUCACAGGCAGCCAUA	SEQ ID NO: 13
shCerS2#2	UGAUGUAGAGGUCUGAGGCUU	SEQ ID NO: 14

Figure 2

(A)


relative quantity [2-ΔCq]
1.00
0.75
0.50
0.25
0.00
mock
miR-c#1
miR-1287

(B)

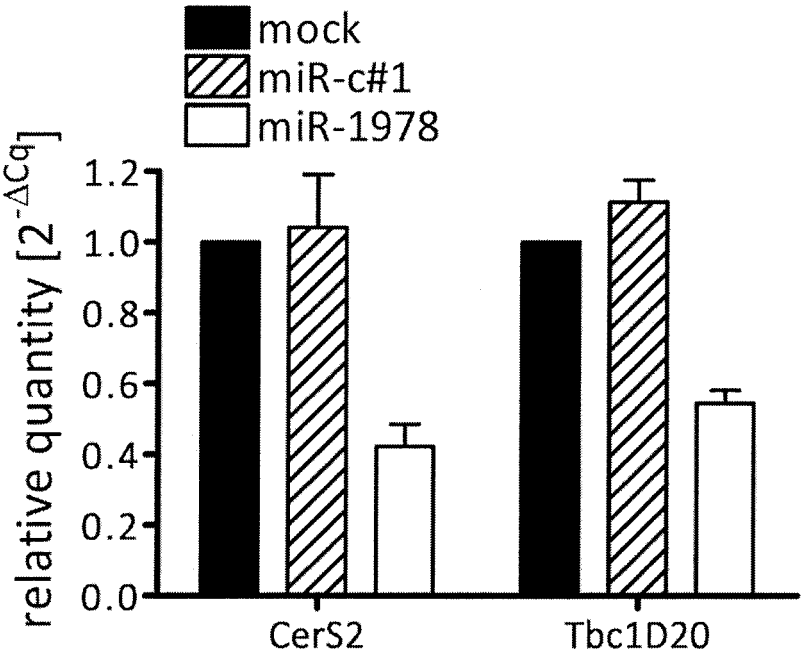
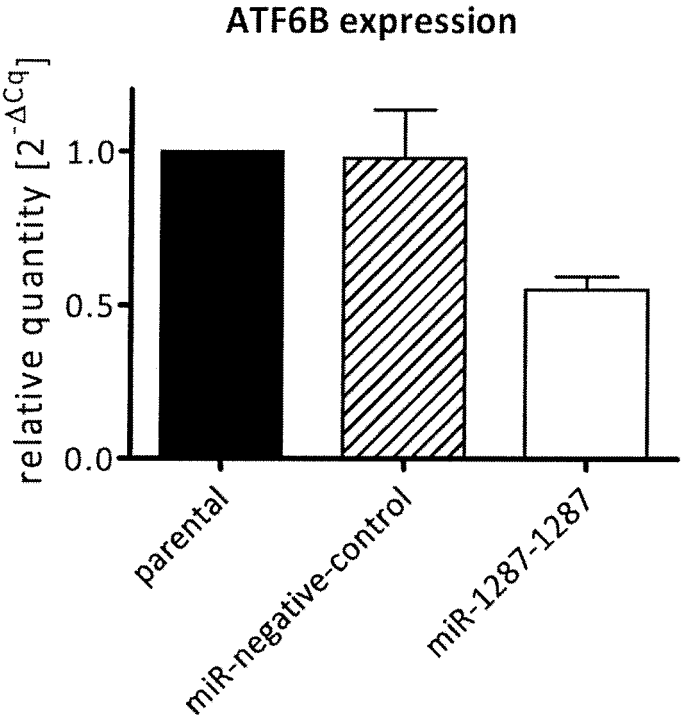

mock
miR-c#1
miR-1978
relative quantity [2-ΔCq]
1.2
1.0
0.8
0.6
0.4
0.2
0.0
CerS2
Tbc1D20

Figure 2

(C)


ATF6B expression
relative quantity [2^-ΔCq]
1.0
0.5
0.0
parental
miR-negative-control
miR-1287-1287

(D)

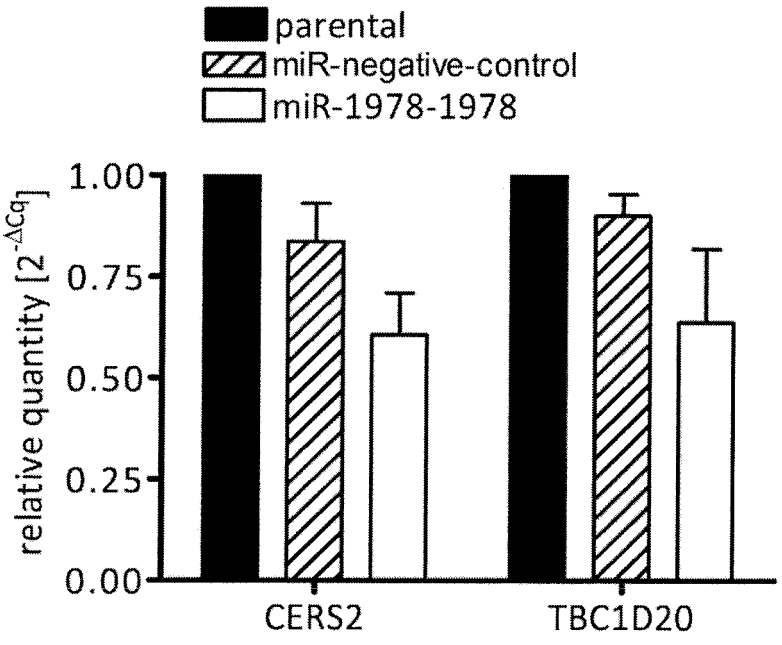
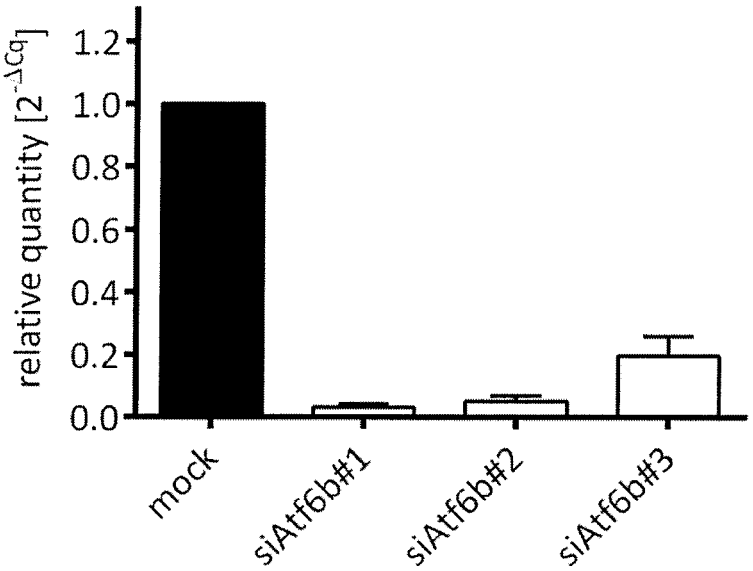

parental
miR-negative-control
miR-1978-1978
relative quantity [2^-ΔCq]
1.00
0.75
0.50
0.25
0.00
CERS2
TBC1D20

Figure 3

(A)


relative quantity [2-ΔCq]
1.2
1.0
0.8
0.6
0.4
0.2
0.0
mock
siAtf6b#1
siAtf6b#2
siAtf6b#3

(B)

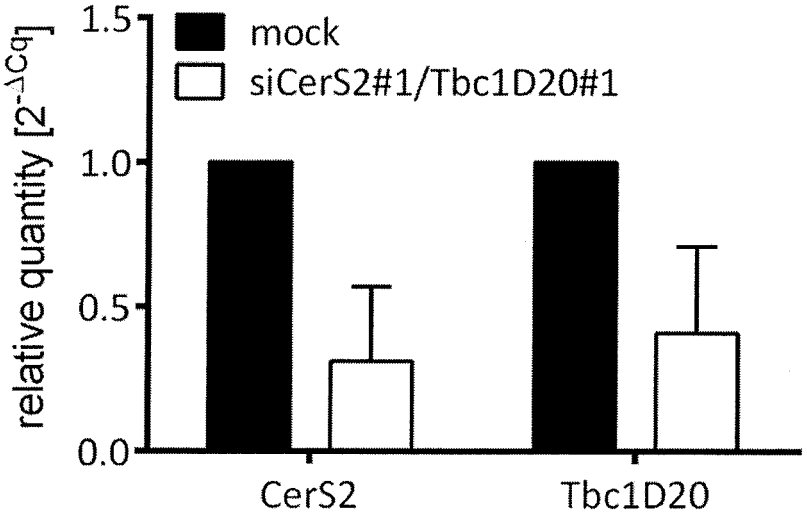

relative quantity [2-ΔCq]
1.5
1.0
0.5
0.0
mock
siCerS2#1/Tbc1D20#1
CerS2
Tbc1D20

Figure 4

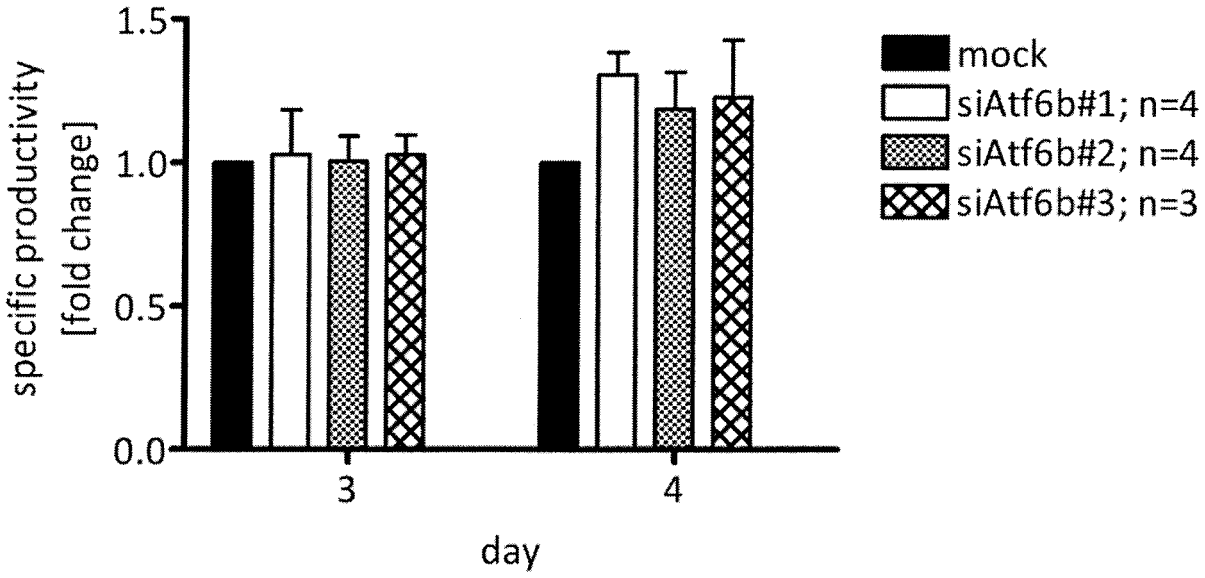
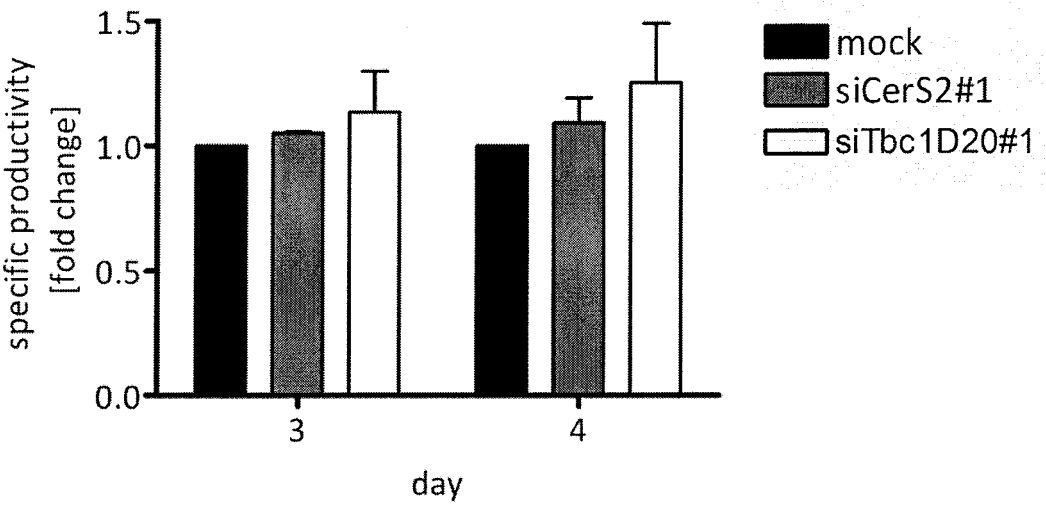

specific productivity
[fold change]
1.5
1.0
0.5
0.0
3
4
day
mock
siAtf6b#1; n=4
siAtf6b#2; n=4
siAtf6b#3; n=3

Figure 5

(A)


specific productivity
[fold change]
1.5
1.0
0.5
0.0
3
4
day
mock
siCerS2#1
siTbc1D20#1

(B)

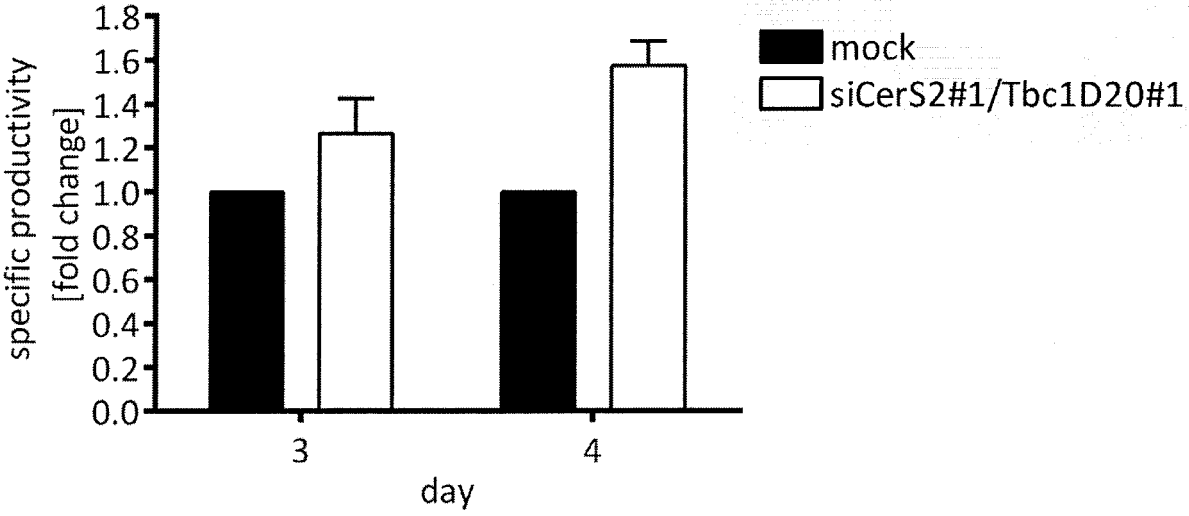

specific productivity
[fold change]
1.8
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
3
4
day
mock
siCerS2#1/Tbc1D20#1

Figure 6

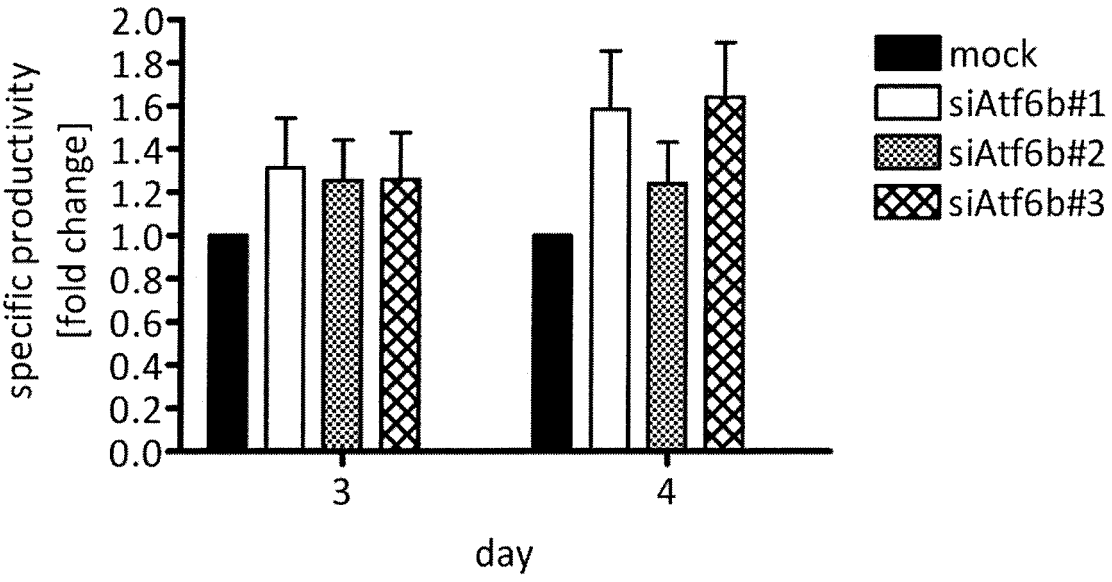

specific productivity
[fold change]
2.0
1.8
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
3
4
day
mock
siAtf6b#1
siAtf6b#2
siAtf6b#3

Figure 7

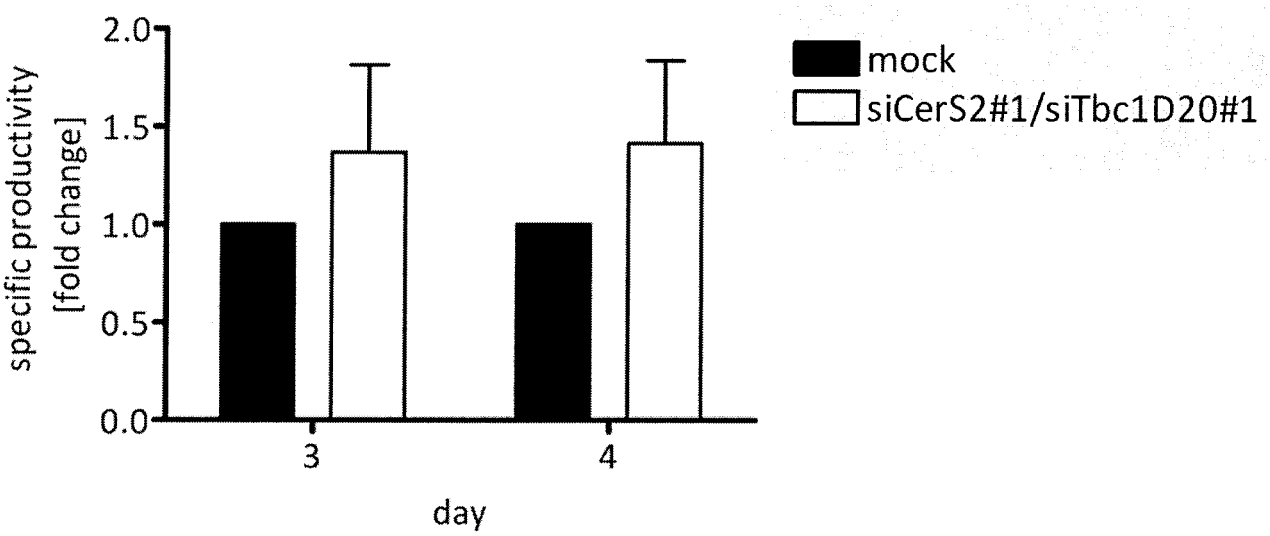
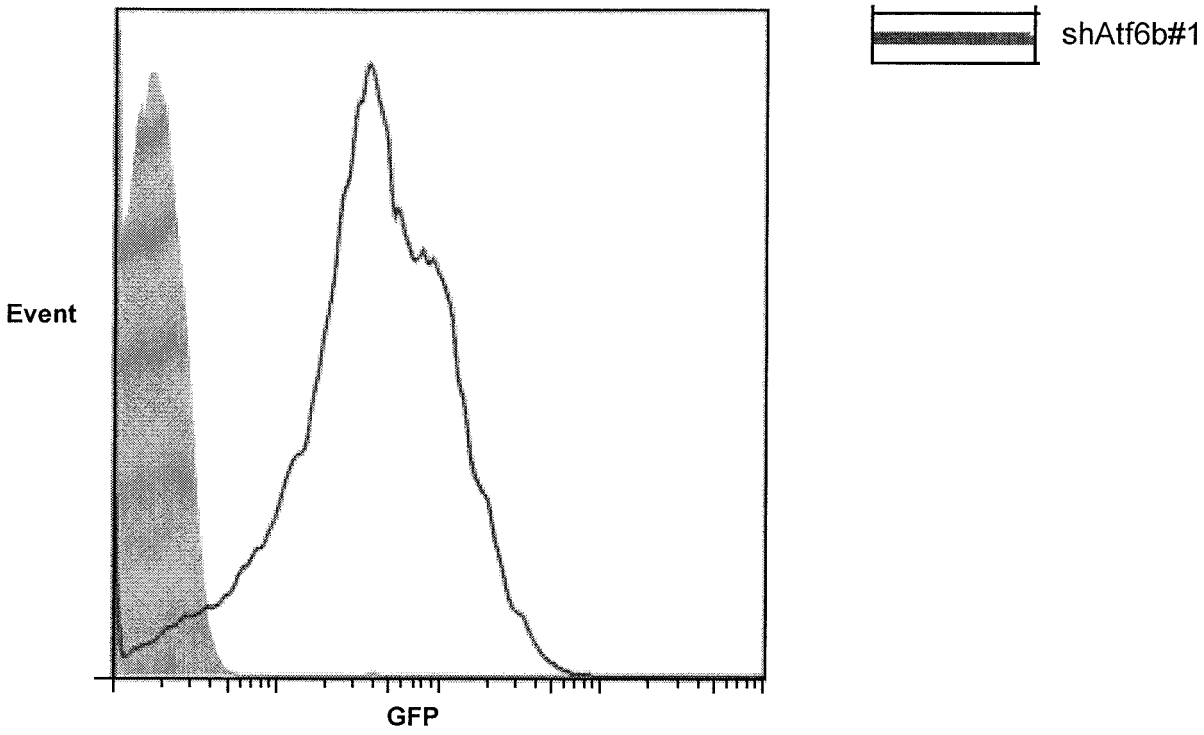

specific productivity
[fold change]
2.0
1.5
1.0
0.5
0.0
3
4
day
mock
siCerS2#1/siTbc1D20#1

Figure 8

(A)


shAtf6b#1
Event
GFP

(B)

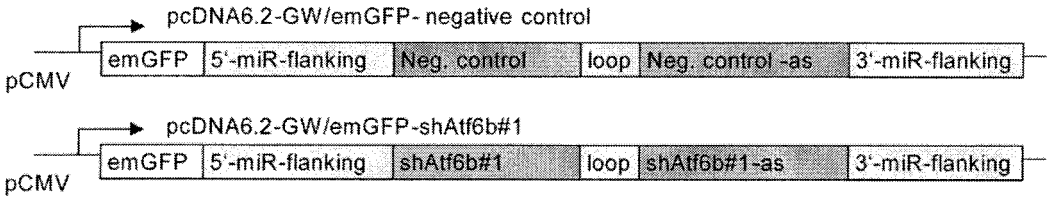
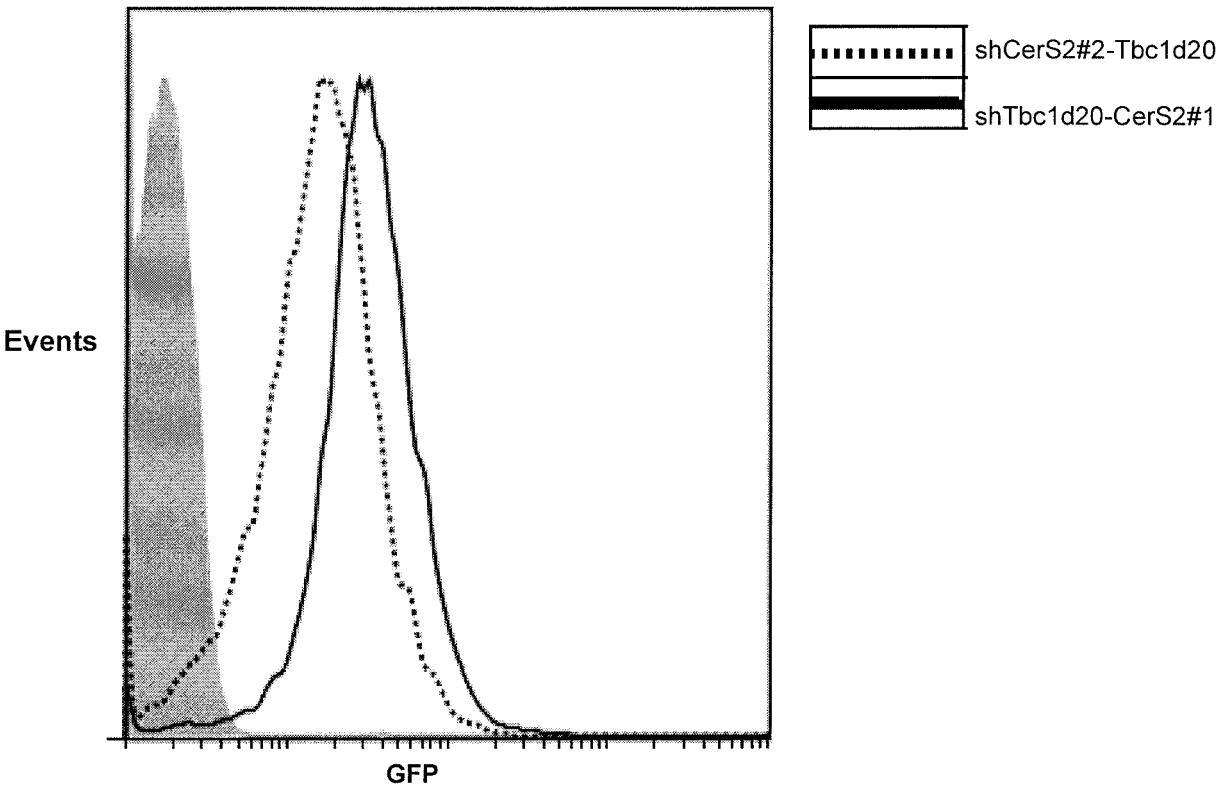

pcDNA6.2-GW/emGFP- negative control
pCMV
emGFP
5'-miR-flanking
Neg. control
loop
Neg. control -as
3'-miR-flanking
pcDNA6.2-GW/emGFP-shAtf6b#1
pCMV
emGFP
5'-miR-flanking
shAtf6b#1
loop
shAtf6b#1-as
3'-miR-flanking

Figure 8

(C)


shCerS2#2-Tbc1d20
shTbc1d20-CerS2#1
Events
GFP

(D)

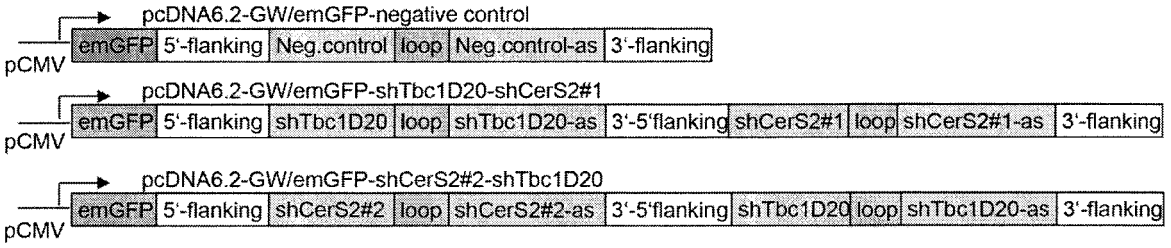
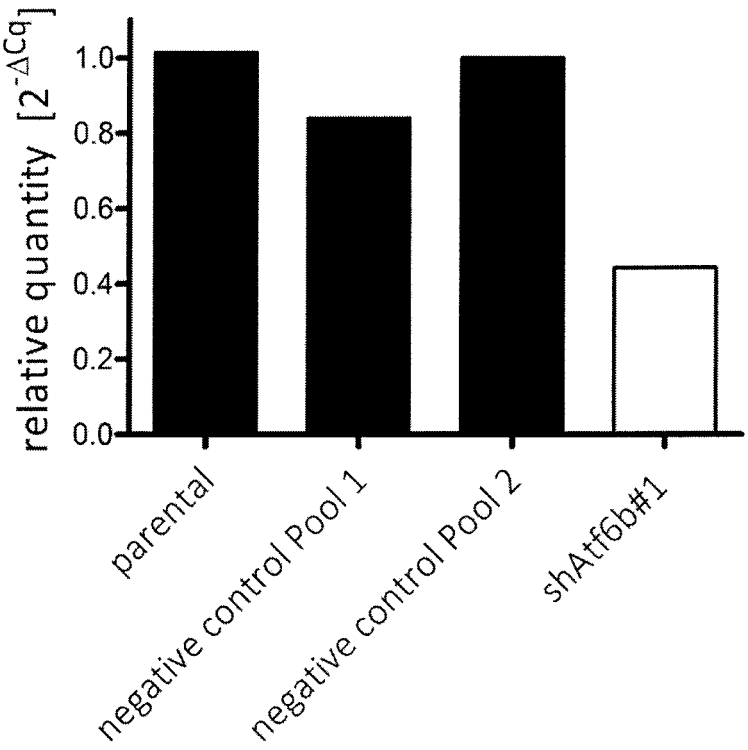

pcDNA6.2-GW/emGFP-negative control
pCMV
emGFP
5'-flanking
Neg.control
loop
Neg.control-as
3'-flanking
pcDNA6.2-GW/emGFP-shTbc1D20-shCerS2#1
pCMV
emGFP
5'-flanking
shTbc1D20
loop
shTbc1D20-as
3'-5'flanking
shCerS2#1
loop
shCerS2#1-as
3'-flanking
pcDNA6.2-GW/emGFP-shCerS2#2-shTbc1D20
pCMV
emGFP
5'-flanking
shCerS2#2
loop
shCerS2#2-as
3'-5'flanking
shTbc1D20
loop
shTbc1D20-as
3'-flanking

Figure 9

(A)


relative quantity [2-ΔCq]
1.0
0.8
0.6
0.4
0.2
0.0
parental
negative control Pool 1
negative control Pool 2
shAtf6b#1

(B)

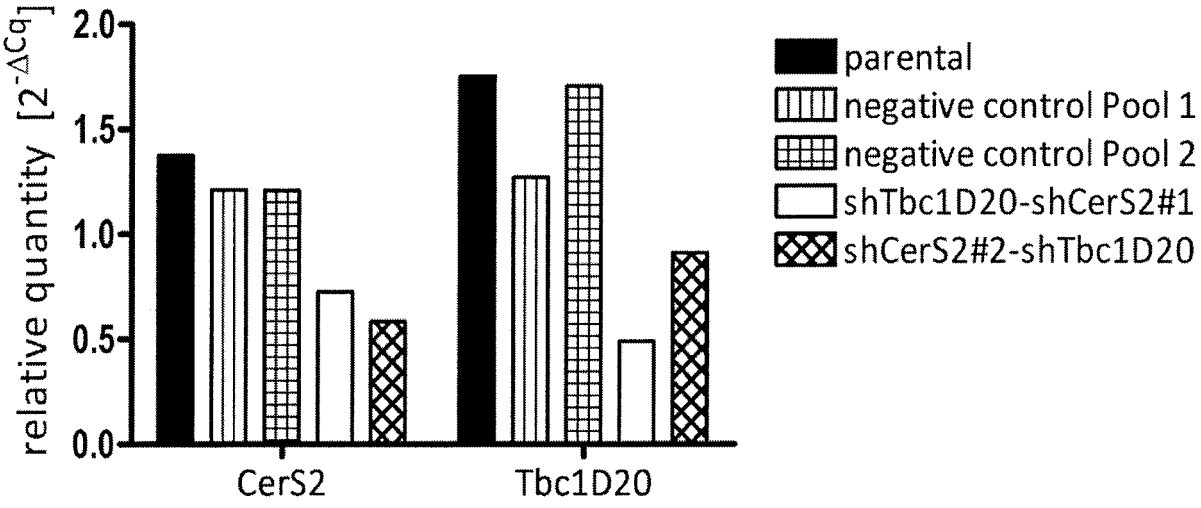
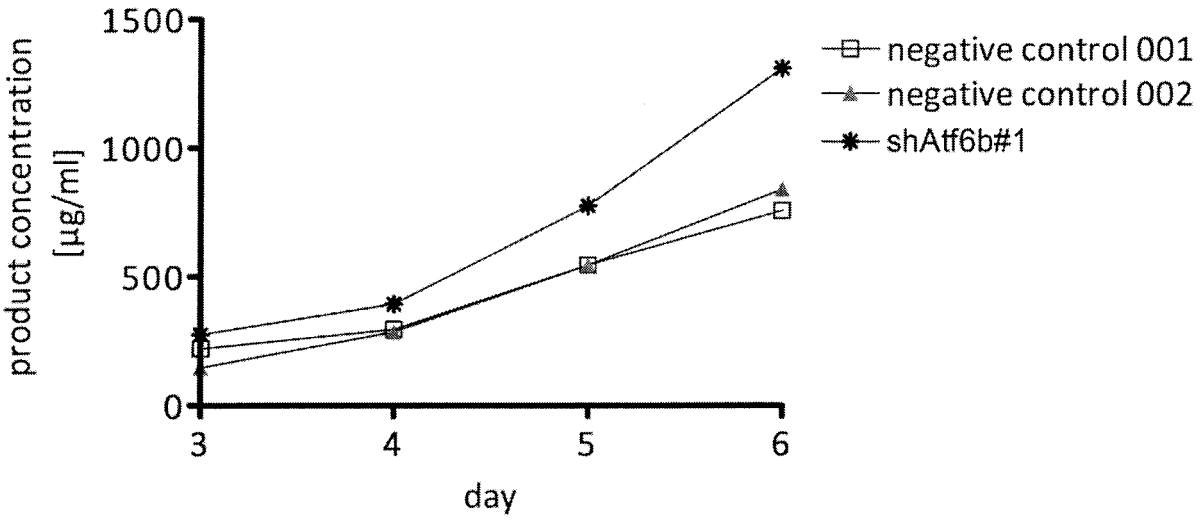

relative quantity [2-ΔCq]
2.0
1.5
1.0
0.5
0.0
CerS2
Tbc1D20
parental
negative control Pool 1
negative control Pool 2
shTbc1D20-shCerS2#1
shCerS2#2-shTbc1D20

Figure 10

(A)


product concentration [µg/ml]
1500
1000
500
0
3
4
5
6
day
negative control 001
negative control 002
shAtf6b#1

(B)

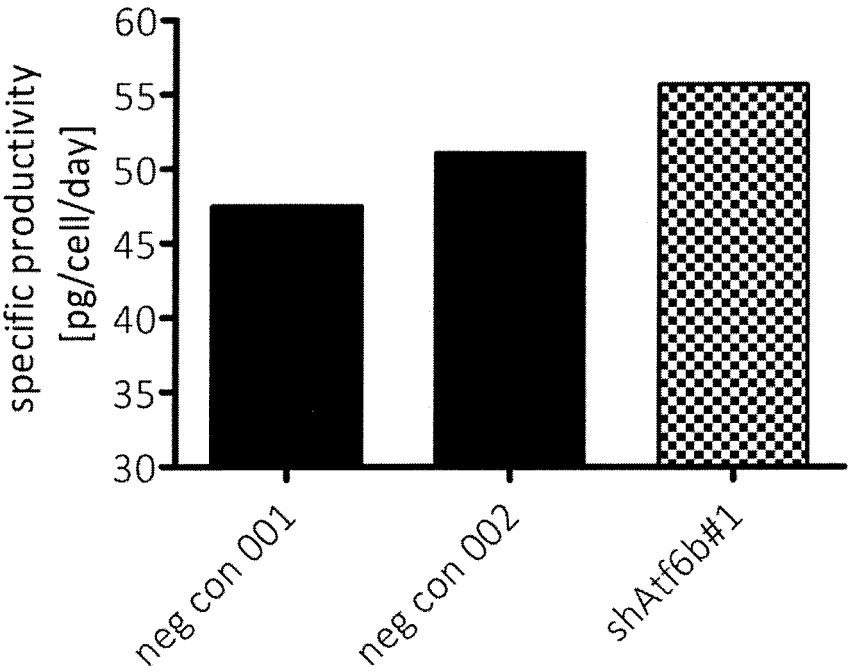

specific productivity [pg/cell/day]
60
55
50
45
40
35
30
neg con 001
neg con 002
shAtf6b#1

Figure 10

(C)

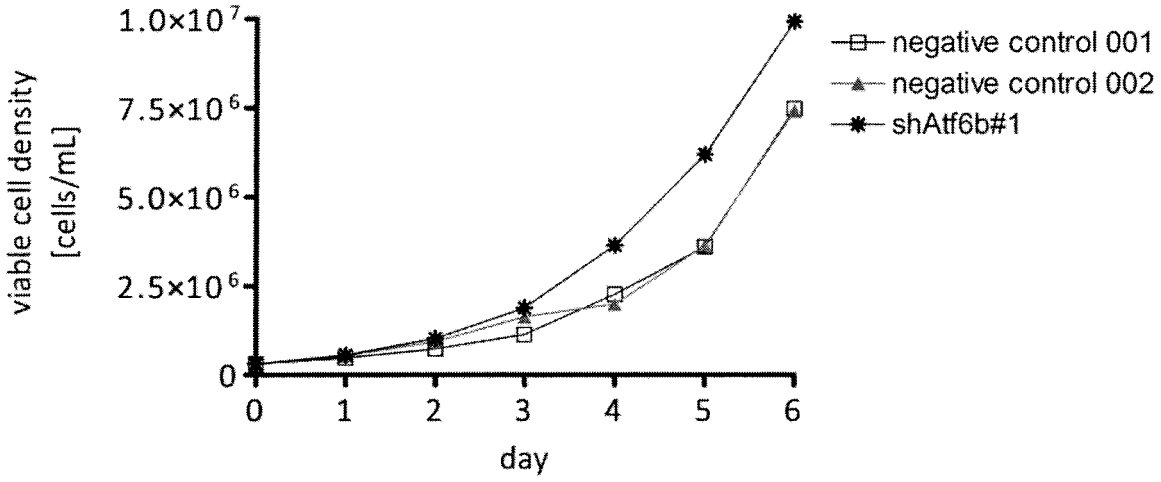
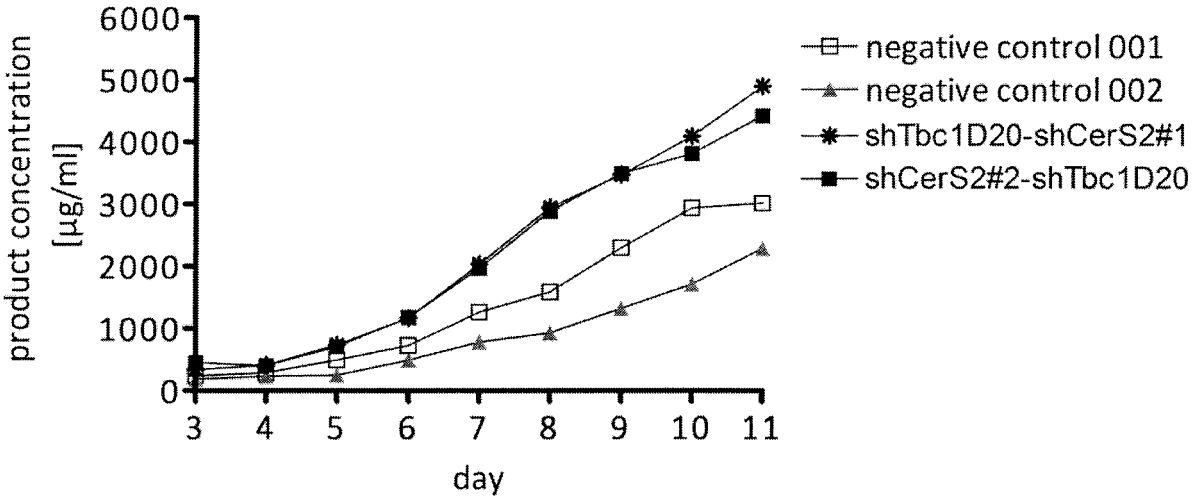

viable cell density [cells/mL]
1.0×10^7
7.5×10^6
5.0×10^6
2.5×10^6
0
0
1
2
3
4
5
6
day
negative control 001
negative control 002
shAtf6b#1

Figure 11

(A)


product concentration [µg/ml]
6000
5000
4000
3000
2000
1000
0
3
4
5
6
7
8
9
10
11
day
negative control 001
negative control 002
shTbc1D20-shCerS2#1
shCerS2#2-shTbc1D20

(B)

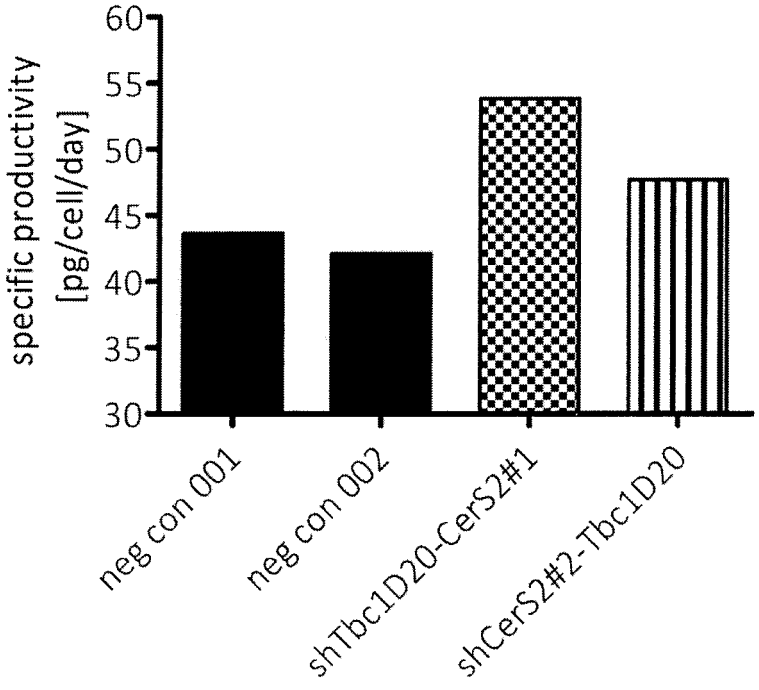

specific productivity [pg/cell/day]
60
55
50
45
40
35
30
neg con 001
neg con 002
shTbc1D20-CerS2#1
shCerS2#2-Tbc1D20

Figure 11

(C)

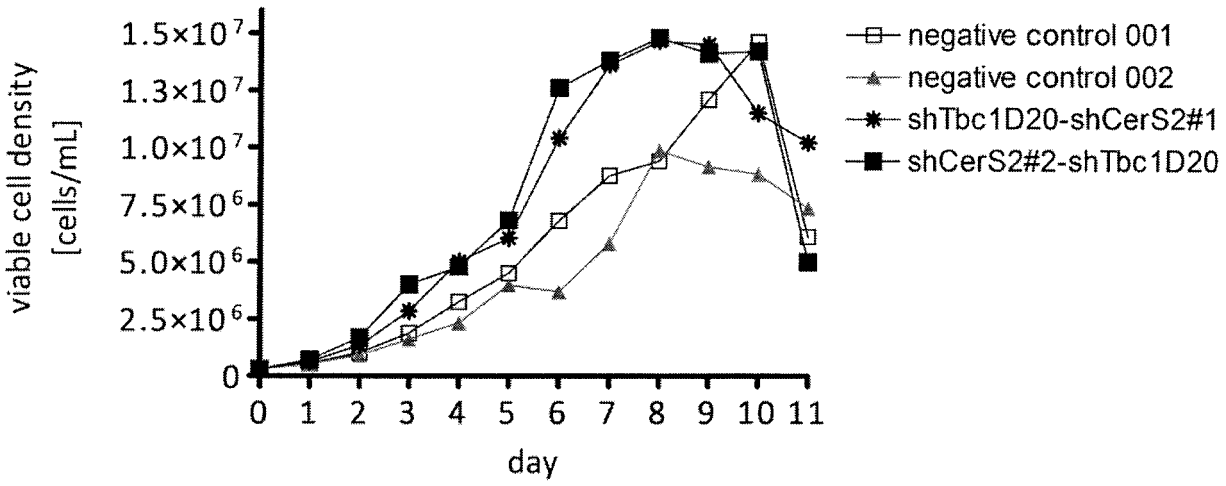
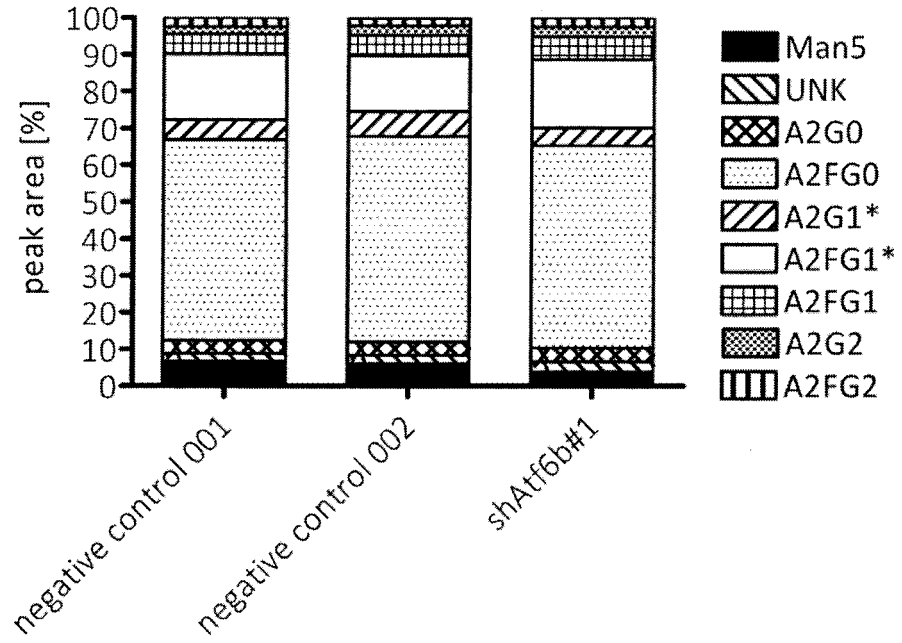
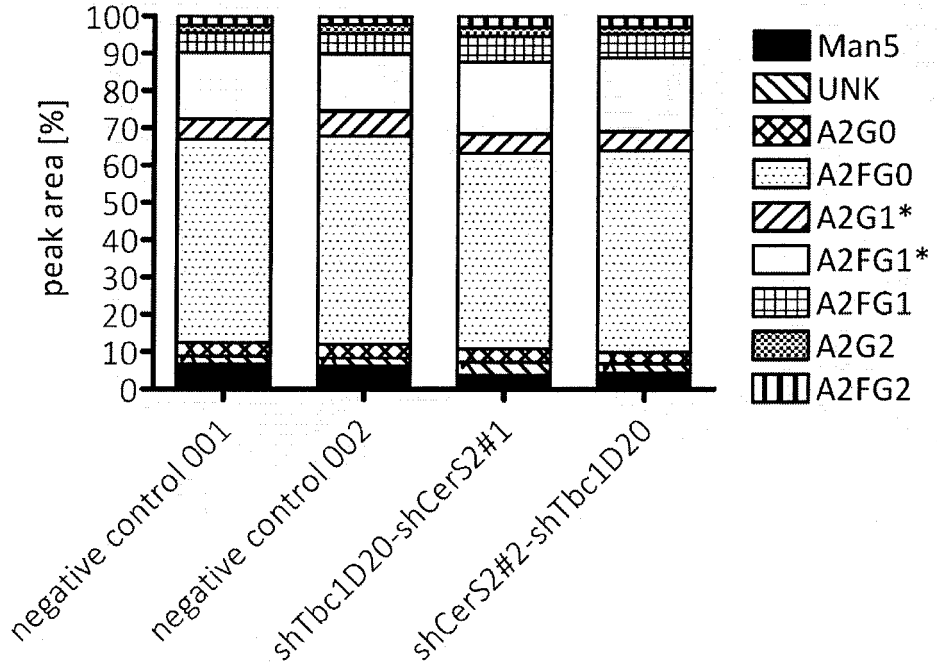

viable cell density [cells/mL]
1.5×10^7
1.3×10^7
1.0×10^7
7.5×10^6
5.0×10^6
2.5×10^6
0
0 1 2 3 4 5 6 7 8 9 10 11
day
negative control 001
negative control 002
shTbc1D20-shCerS2#1
shCerS2#2-shTbc1D20

Figure 12

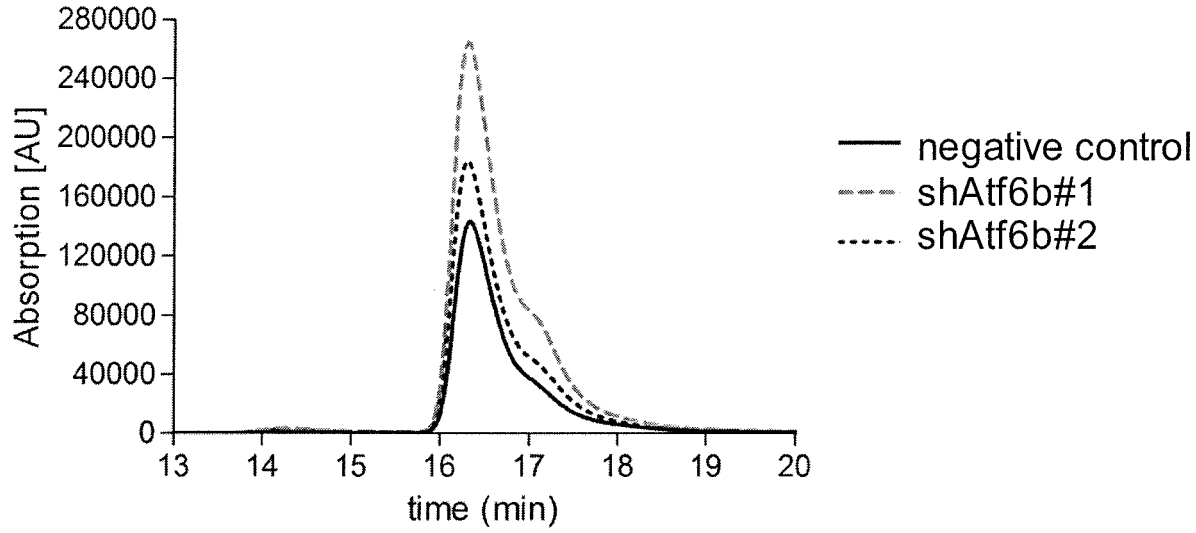
(A)


peak area [%]
100
90
80
70
60
50
40
30
20
10
0
Man5
UNK
A2G0
A2FG0
A2G1*
A2FG1*
A2FG1
A2G2
A2FG2
negative control 001
negative control 002
shAtf6b#1

(B)


peak area [%]
100
90
80
70
60
50
40
30
20
10
0
Man5
UNK
A2G0
A2FG0
A2G1*
A2FG1*
A2FG1
A2G2
A2FG2
negative control 001
negative control 002
shTbc1D20-shCerS2#1
shCerS2#2-shTbc1D20

(C)


280000
240000
200000
160000
120000
80000
40000
0
Absorption [AU]
13
14
15
16
17
18
19
20
time (min)
negative control
shAtf6b#1
shAtf6b#2

(D)

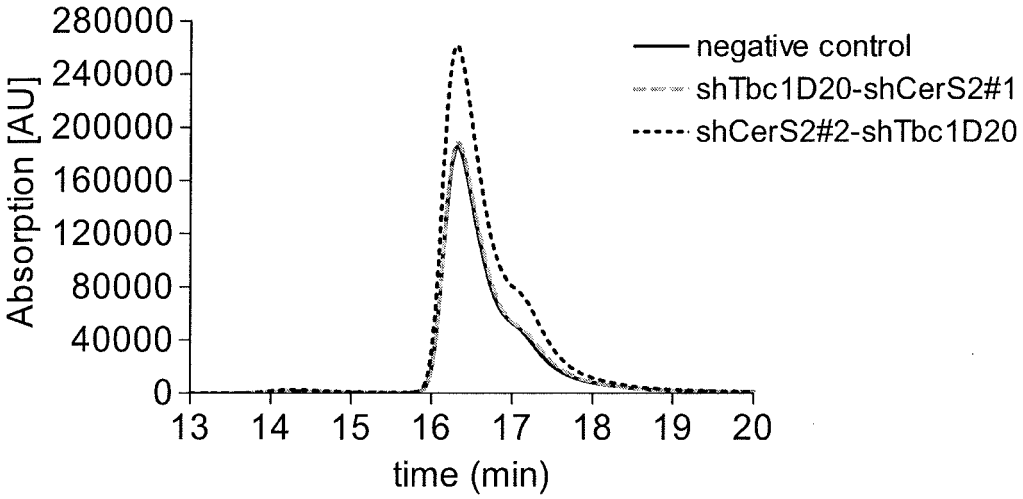
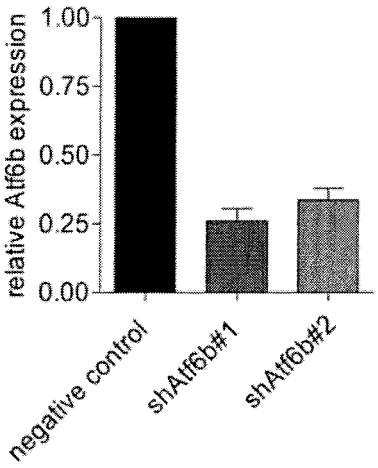
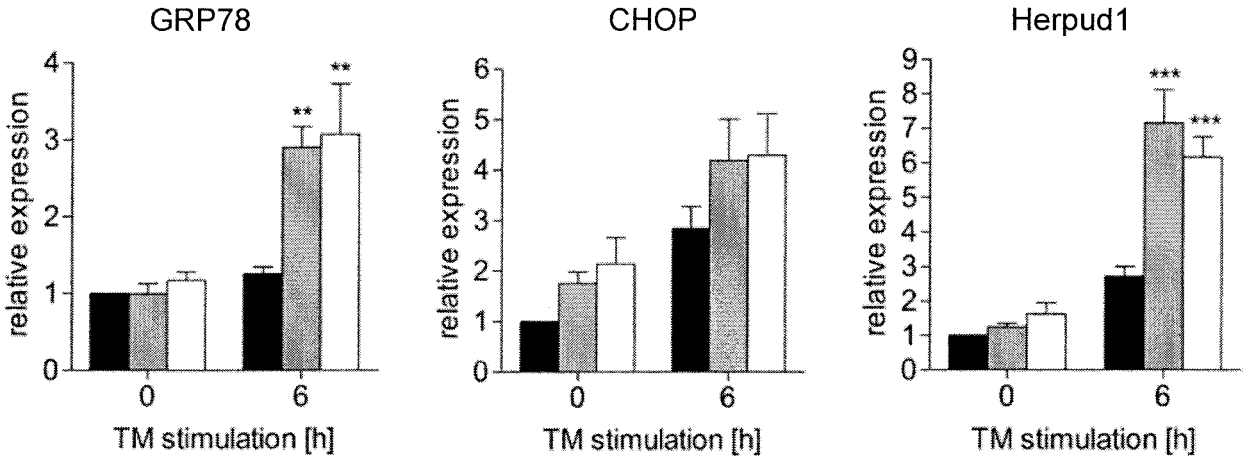

280000
240000
200000
160000
120000
80000
40000
0
Absorption [AU]
13
14
15
16
17
18
19
20
time (min)
negative control
shTbc1D20-shCerS2#1
shCerS2#2-shTbc1D20

Figure 13

(A)



(B)



(C)

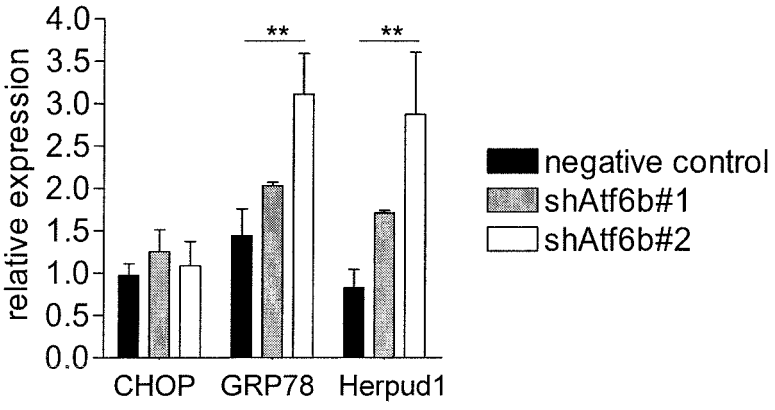


Figure 14

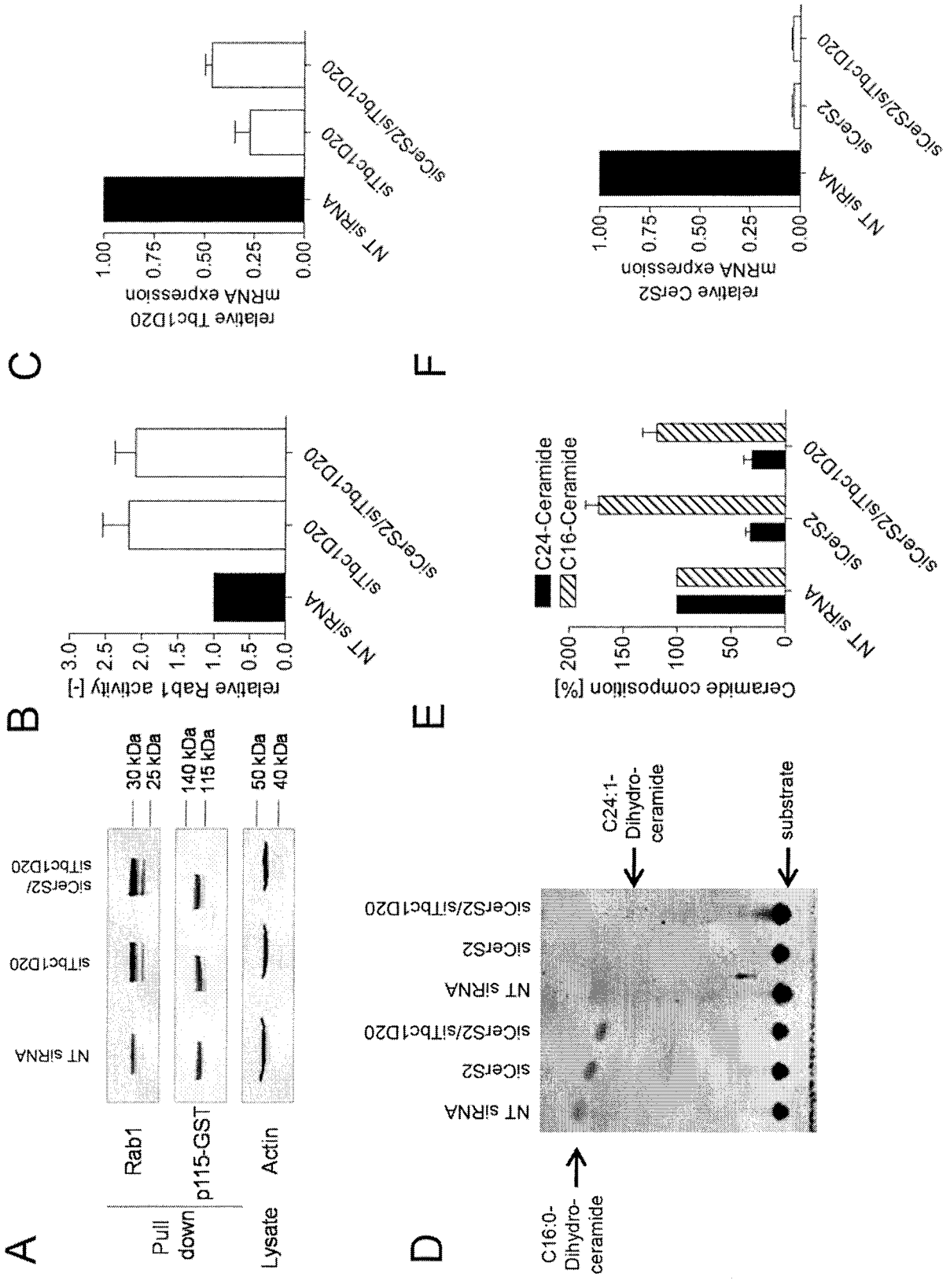
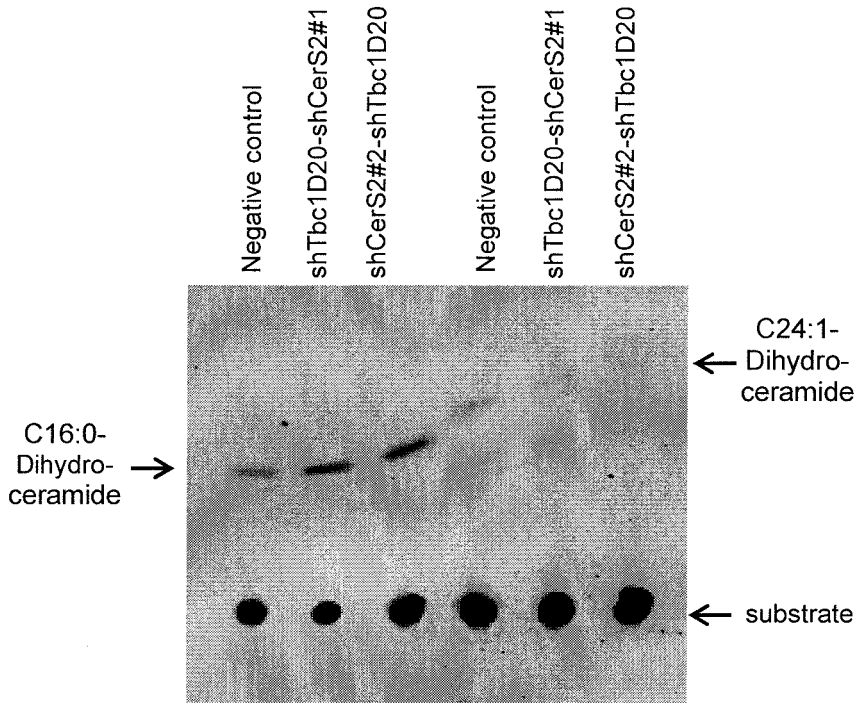
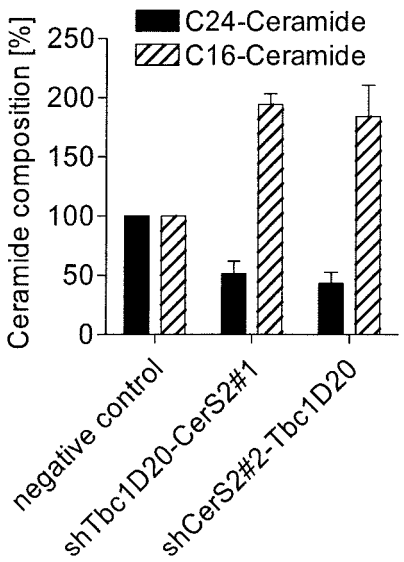

A
Pull down
Lysate
Rab1
p115-GST
Actin
30 kDa
25 kDa
140 kDa
115 kDa
50 kDa
40 kDa
NT siRNA
siTbc1D20
siCerS2/ siTbc1D20
B
relative Rab1 activity [-]
3.0
2.5
2.0
1.5
1.0
0.5
0.0
NT siRNA
siTbc1D20
siCerS2/siTbc1D20
C
relative Tbc1D20 mRNA expression
1.00
0.75
0.50
0.25
0.00
NT siRNA
siTbc1D20
siCerS2/siTbc1D20
D
C16:0-Dihydro-ceramide
C24:1-Dihydro-ceramide
substrate
NT siRNA
siCerS2
siCerS2/siTbc1D20
NT siRNA
siCerS2
siCerS2/siTbc1D20
E
Ceramide composition [%]
200
150
100
50
0
C24-Ceramide
C16-Ceramide
NT siRNA
siCerS2
siCerS2/siTbc1D20
F
relative CerS2 mRNA expression
1.00
0.75
0.50
0.25
0.00
NT siRNA
siCerS2
siCerS2/siTbc1D20

Figure 15

(A)


Negative control
shTbc1D20-shCerS2#1
shCerS2#2-shTbc1D20
Negative control
shTbc1D20-shCerS2#1
shCerS2#2-shTbc1D20
C24:1-
Dihydro-
ceramide
C16:0-
Dihydro-
ceramide
substrate

(B)


C24-Ceramide
C16-Ceramide
Ceramide composition [%]
250
200
150
100
50
0
negative control
shTbc1D20-CerS2#1
shCerS2#2-Tbc1D20