Figure S1

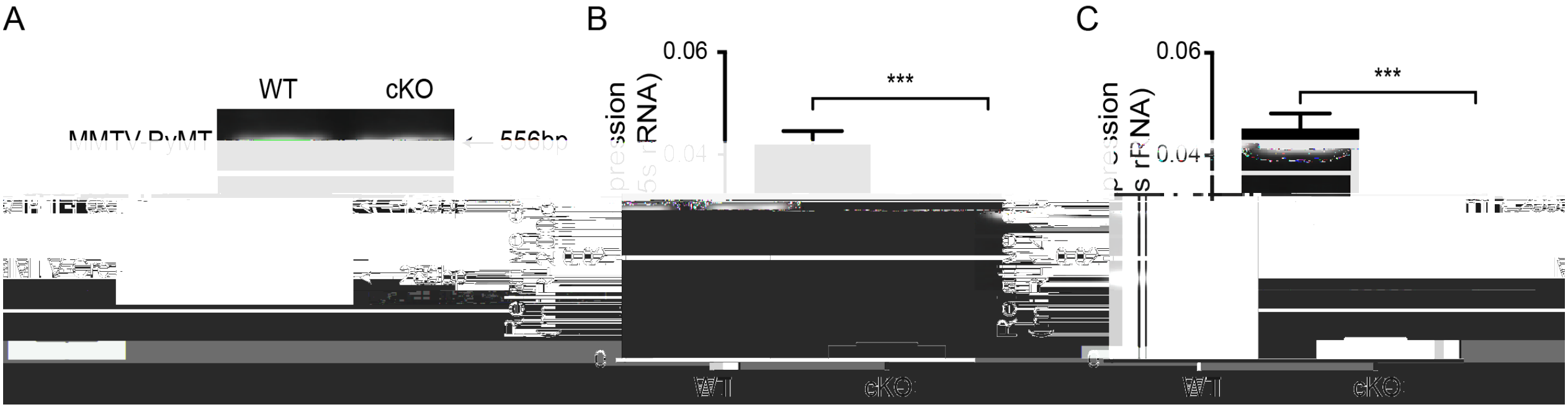

Figure S1. Verification of miR-200c/141 cluster knockout mouse model.

(A) Genotyping of indicted transgenic mice by PCR.

(B) Analysis for miR-200c expression in mammary tumors from 3-week old transgenic mice as indicated by qRT-PCR. ***p < 0.001

(C) Analysis for miR-141 expression in mammary tumors from 3-week old transgenic mice as indicated by qRT-PCR. ***p < 0.001.

Figure S2

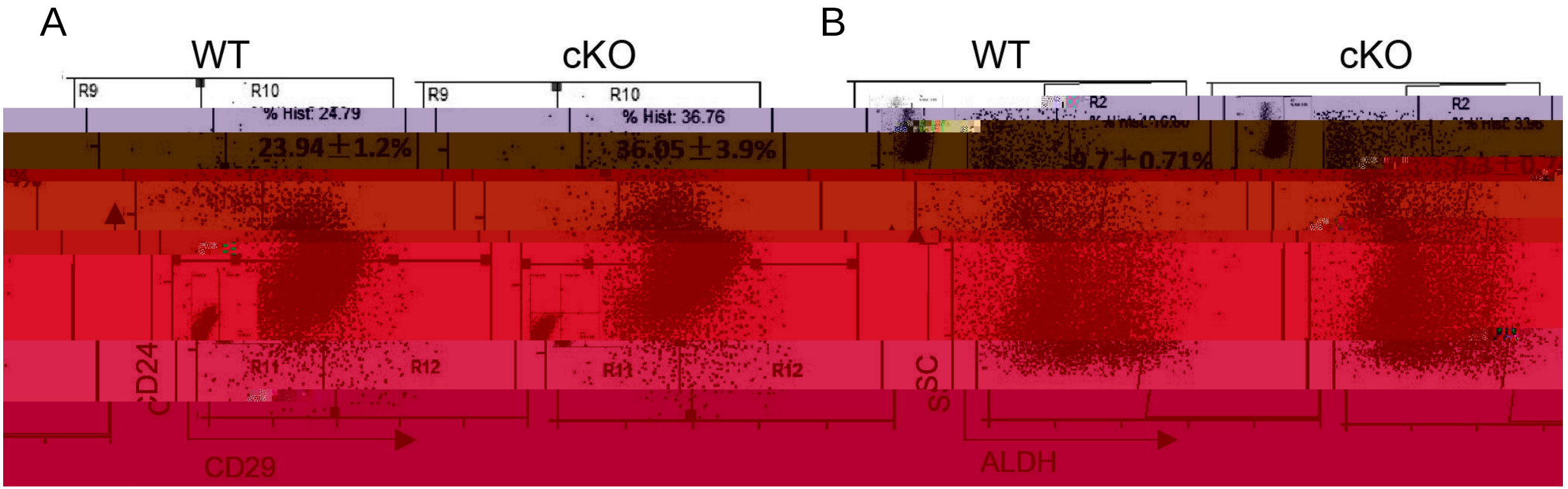

Figure S2. FACS was performed to detect the effect of miR-200c/141 cluster on breast cancer stem cells (BCSCs) with CD24/CD29 (A) and ALDEFLUOR assay (B).

Figure S3

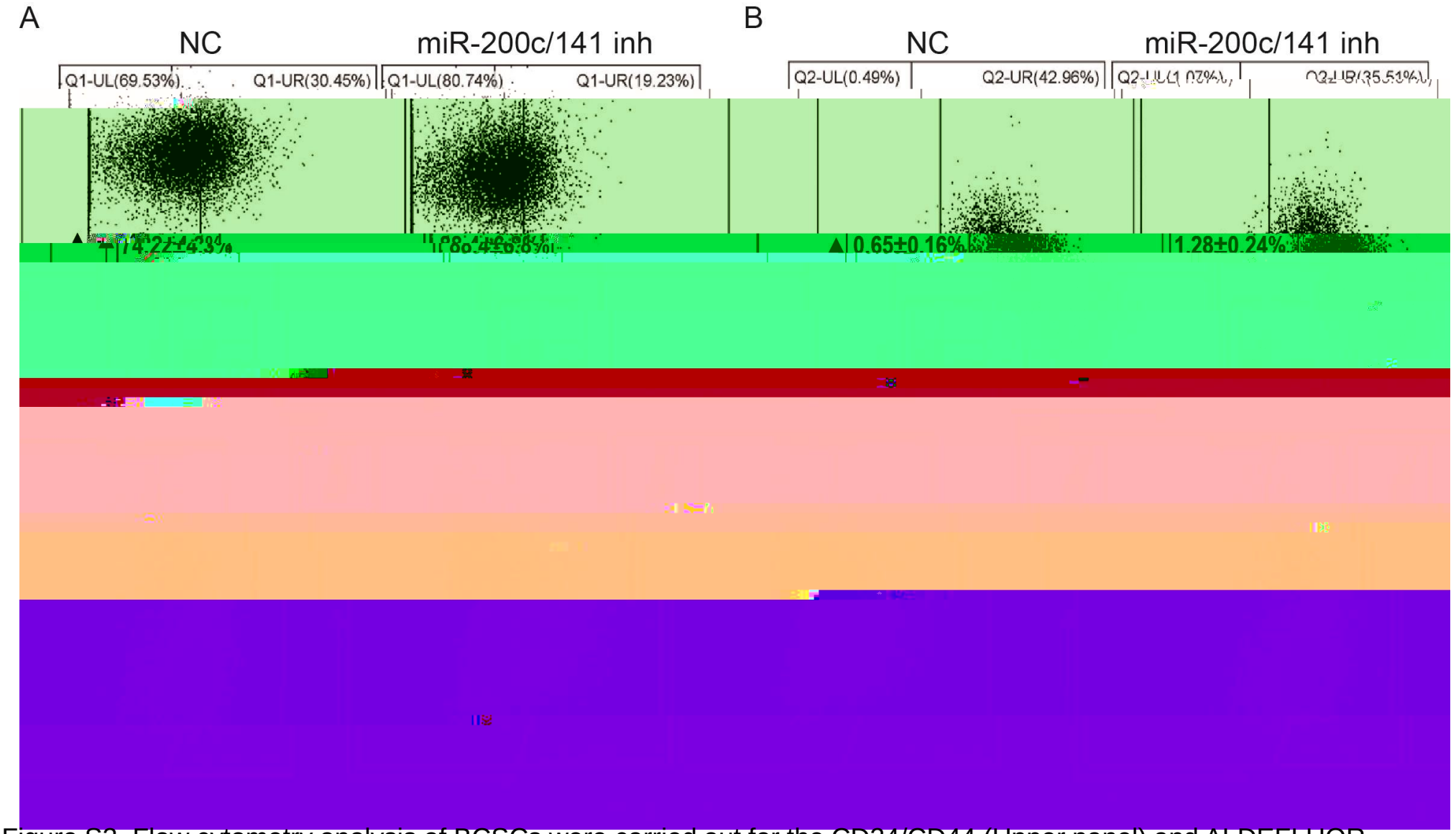

Figure S3. Flow cytometry analysis of BCSCs were carried out for the CD24/CD44 (Upper panel) and ALDEFLUOR assay (Lower panel) in treated SUM149 (A) and T47D (B) cells.

Figure S4

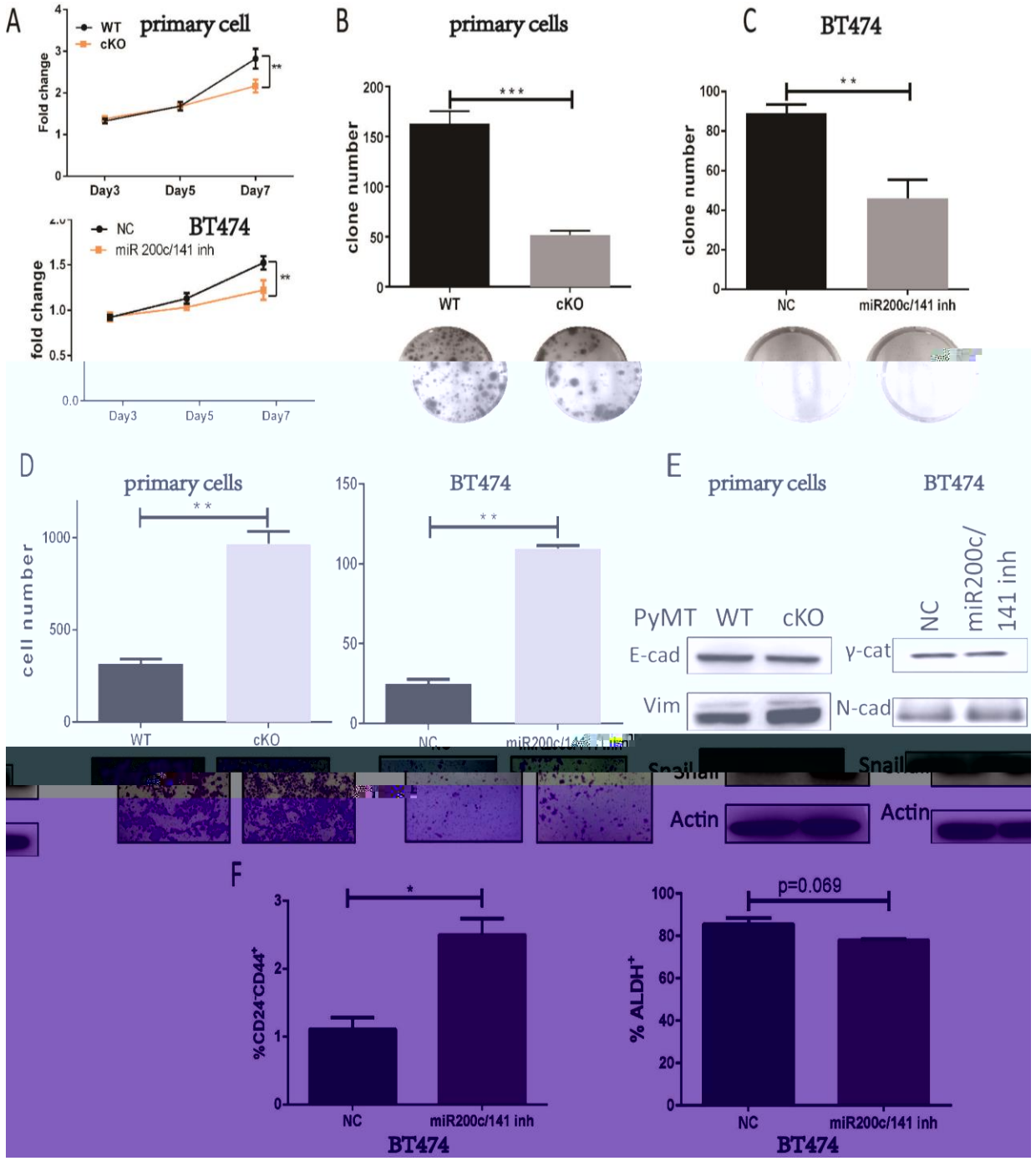
A
primary cell
WT
cKO
Fold change
Day3
Day5
Day7
BT474
NC
miR 200c/141 inh
fold change
B
primary cells
clone number
WT
cKO
C
BT474
clone number
NC
miR200c/141 inh
D
primary cells
cell number
WT
cKO
BT474
NC
E
primary cells
BT474
PyMT
WT
cKO
NC
miR200c/
141 inh
E-cad
γ-cat
Vim
N-cad
Snail
Actin
F
%CD24⁻CD44⁺
NC
miR200c/141 inh
BT474
% ALDH⁺
p=0.069
NC
miR200c/141 inh
BT474

Figure S4. Inhibition of miR-200c and miR-141 results in reduced cell proliferation and increased cell invasion in vitro by regulating BCSC plasticity.

Primary cells derived from PyMT mouse tumors (WT, cKO) were cultured in vitro. BT474 cells were treated with 200nM miR-200c and miR-141 inhibitors (miR-200c/141 inh) or negative control inhibitor (NC) for 48h.

(A) The proliferation of primary cells and BT474 cells was measured by MTT assay **p<0.01

(B-C) The plate colony formation assay was carried out with primary cells (B) or treated BT474 cells (C) in 6-well culture plates for two weeks and colonies were counted in the whole field for statistics. **p<0.01

(D-E) The invasive ability of primary (D) or treated BT474 (E) cells was measured with the transwell assay . Quantitative analysis of the total invasive cells from three independent experiments. *p<0.05, **p<0.01

(F). Flow cytometry analysis of BCSCs was carried out for the CD24/CD44 and ALDEFLUOR assay in treated BT474 cells.*p<0.05, **p<0.01

(G) Primary cells and treated BT474 cells were harvested to detect the protein expression levels of E-cadherin (E-cad), Snail ,γ-catenin (γ-cat), N-cadherin (N-cad) and Vimentin (Vim) with Western Blotting.

Figure S5

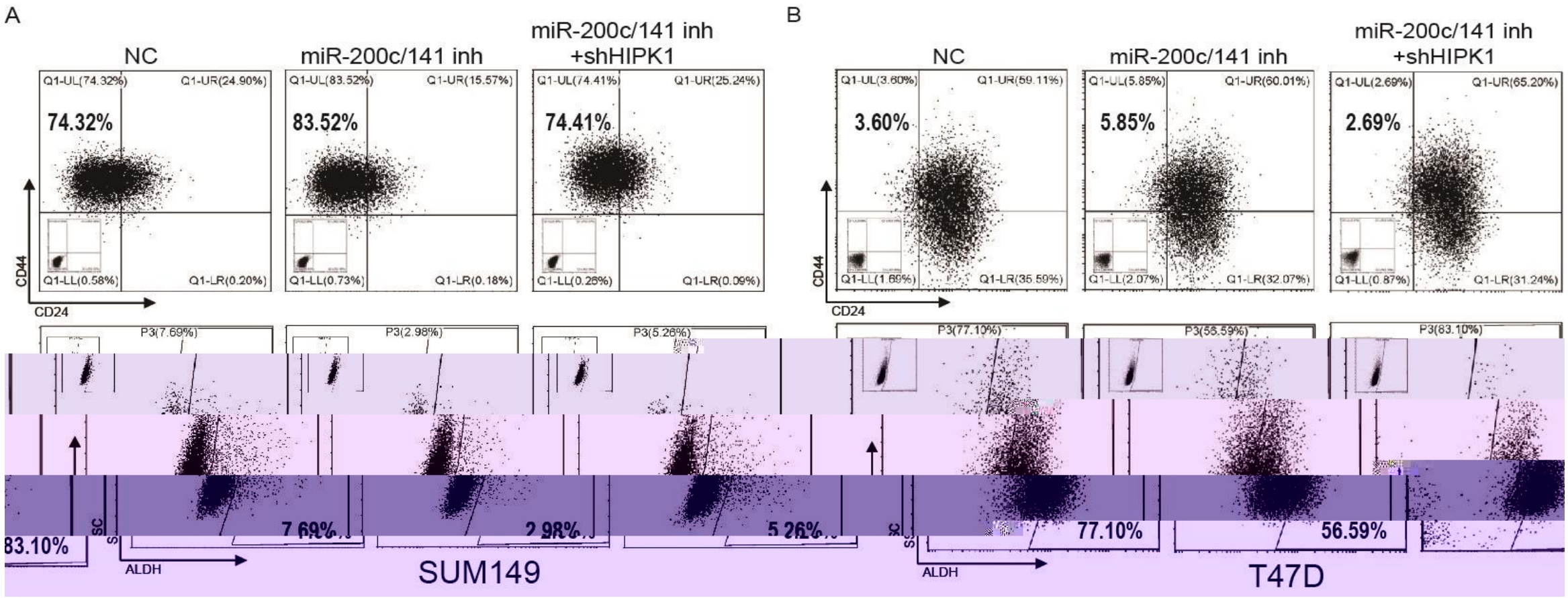

Figure S5. The heterogeneity of BCSCs was analyzed by Flow cytometry with two sets of markers (CD24-CD44+ and ALDH+) in treated SUM149 (A) and T47D (B) cells.

Figure S6

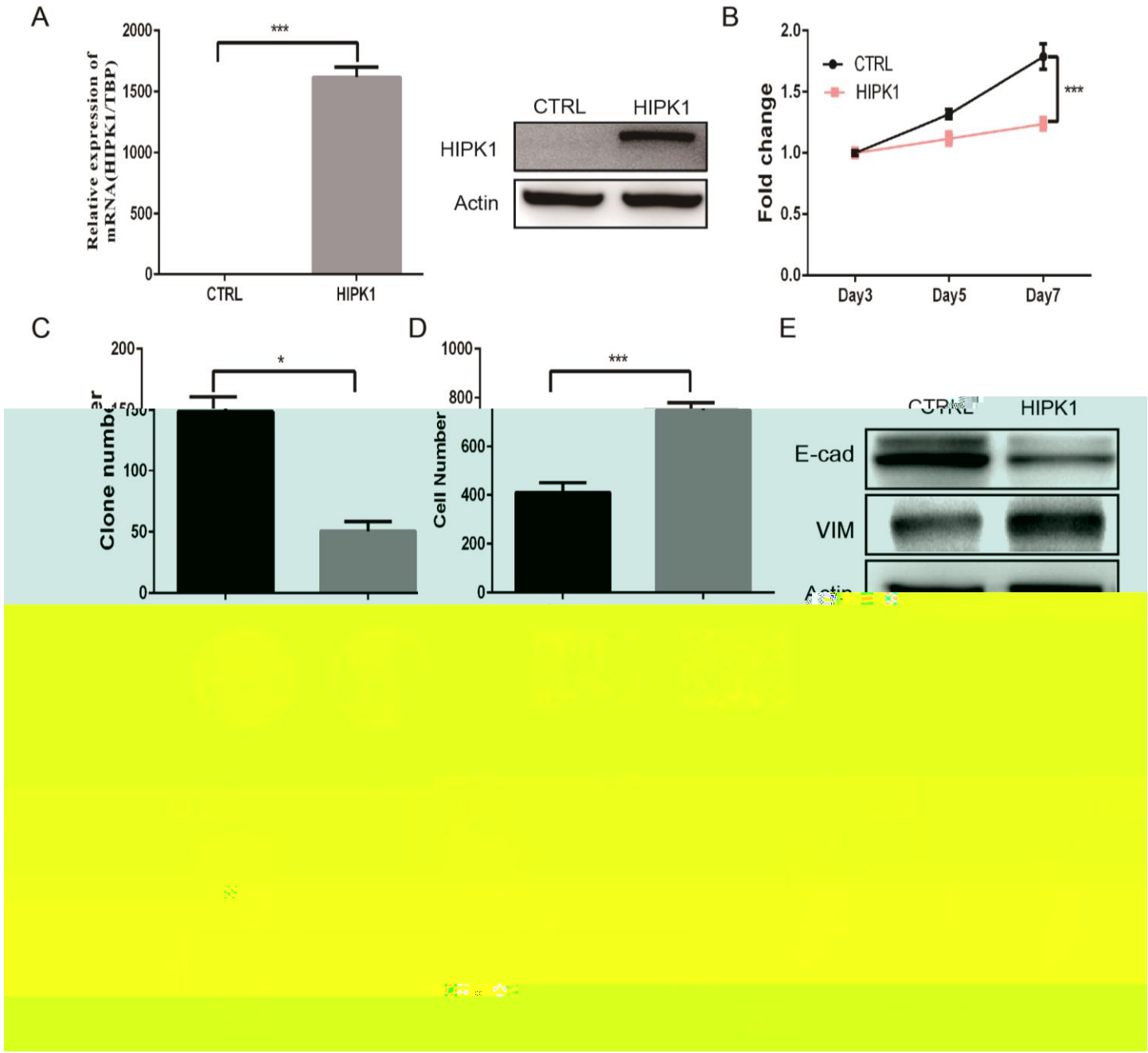
A
Relative expression of mRNA(HIPK1/TBP)
2000
1500
1000
500
0
***
CTRL
HIPK1
CTRL
HIPK1
HIPK1
Actin
B
Fold change
2.0
1.5
1.0
0.5
0.0
CTRL
HIPK1
***
Day3
Day5
Day7
C
200
100
50
0
*
D
Cell Number
1000
800
600
400
200
0
***
E
CTRL
HIPK1
E-cad
VIM
Actin

Figure S6. overexpression of HIPK1 reduced cell proliferation and increased cell invasion in vitro by regulating BCSC plasticity.

(A)Ectopic expression of HIPK1 in SUM149 cell line.

(B-C) The proliferation of SUM149 cells was measured by MTT assay(B) and plate colony formation assay(C). Colonies were counted in the whole field for statistics. **p<0.01

(D) The cell invasive ability was measured by the transwell assay. Quantitative analysis of the total invasive cells was done from three independent experiments. *p<0.05, **p<0.01

(E) The protein expression levels of E-cadherin (E-cad) and Vimentin (Vim) in two group cells were measured by Western Blotting.

(F) Flow cytometry analysis of BCSCs was carried out for the CD24/CD44 analysis and ALDEFLUOR assay in SUM149 cells.

Figure S7

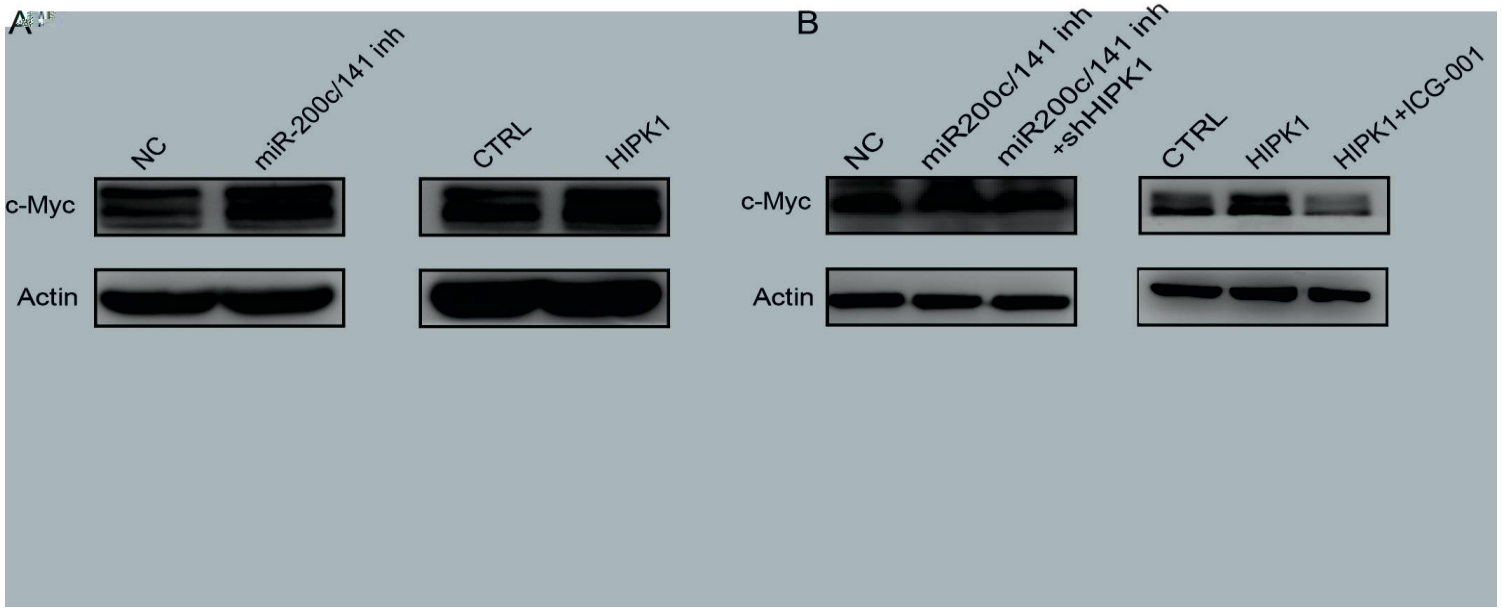

Figure S7: miR-200c/141 activates β-catenin downstream gene c-Myc through HIPK1.

The protein expression of c-Myc in miR-200c/141 inhibition, HIPK1 overexpression, and miR-200c/141 and HIPK1 double knockdown cell lines.

Figure S8

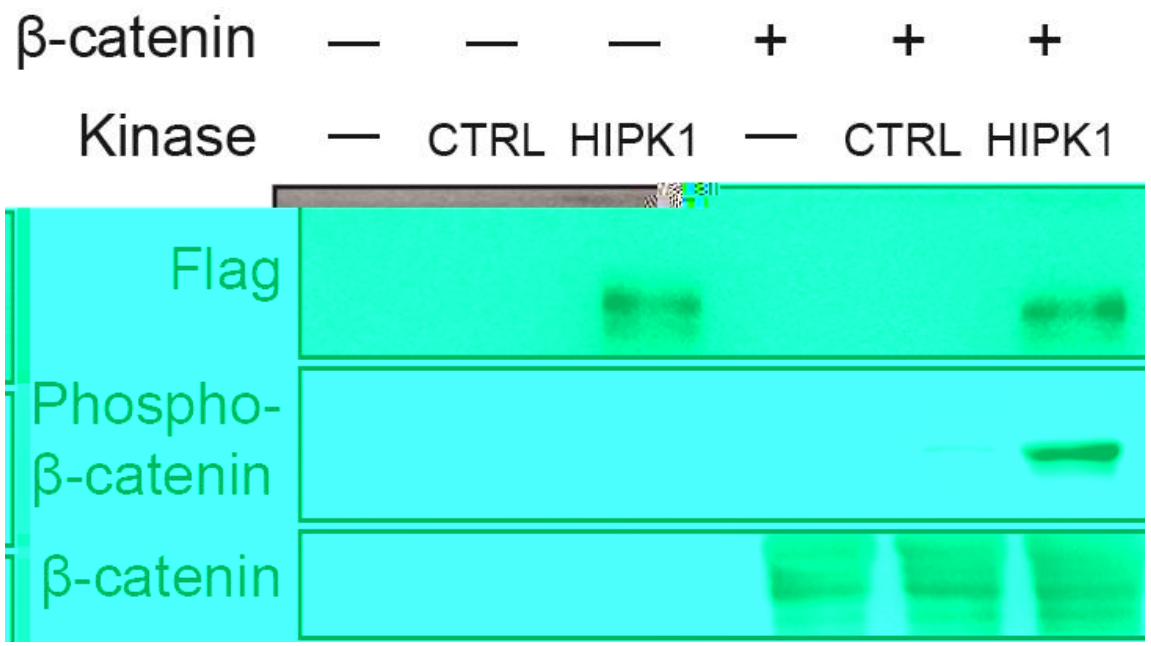

Figure S8. HIPK1 phosphorylates the human β-catenin protein at Ser 552 in vitro.

Flag-HIPK1 was transfected into HEK293T cells, recovered by anti-Flag immunoprecipitation. The beta-catenin protein is purified by prokaryotic expression system. Kinase (HIPK1) and β-catenin incubated in the presence of ATP. The in vitro reaction products were resolved by SDS-PAGE.

Table S1. The primer sequence used in qRT-PCR

Gene	*Forword primer*	*Reverse primer*
5s RNA	TACGGCCATACCACCCTGAA	TAACCAGGCCCGACCCTGCT
miR-200c	GCCCGCTAATACTGCCGGGTAAT	GTGCAGGGTCCGAGGT
miR-141	GCCCGCTAACACTGTCTGGT	GTGCAGGGTCCGAGGT
hCELF1	ACATCCGAGTCATGTTCTCTTCG	CATTGCCTTGATAGCCGTCTG
hCERS6	GGACCACAAATTGCTCCGC	GGCTTCTCCTGATTGCGTCT
hCSNK1	TGGAGATACAAAACGGGCTACA	GCAACTGTTTACCAATCCAGTCA
hDEK	AACTGCTTTACAACAGGCCAG	ATGGTTTGCCAGAAGGCTTTG
hHIPK1	TCTCAGTGCCGGAACAAAAAC	CCCTCCAGGTCTGTAGACATATT
hHIPK3	TCACAAGTCTTGGTCTACCCA	CACATAGGTCCGTGGATAGTTTC
hIHC2	CCCACAAGAACGTCCTAGCC	GCAGCTTGCCTGTGTAGATGA
hRAP2C	TCTACCGCAAAGAGATCGAAGT	ACCTTGGCCGTTTTTGATGTA
hSPAG9	CAAGCACTCCCACCAAAGG	CCCGACCCATTCCTAGTAAATCT
hYWHAG	AGCCACTGTCGAATGAGGAAC	CTGCTCAATGCTACTGATGACC
mCelf1	TGCTCTCCATAACATGAAGGTCC	CAGGTCCCCGCAATATCCG
mCers6	GATTCATAGCCAAACCATGTGCC	AATGCTCCGAACATCCCAGTC
mCsnk1	AAACTGGAGCCCATGAAATCC	TGTATTTACCACAAGGGCCAAAA
mDek	GGGCACAGTGTCCTCGTTG	CGCCTGACCTCTCTAAATCAAGA
mHipk1	TCCCGCCTAAGCAGTGAAAAT	GGCAGGTATGATTCTTGTGCTG
mHipk3	ATGGCCTCACAAGTCTTGGTC	GCACTACCTTTCGTGGAAGGAT
mIhc2	CCCAGCCATTCAAAACAGCTC	GATGAGGTTATCATGCAGGACC
mRap2c	GAACGGCCAAGGTTTCATCCT	GCCCCATTCTTGAGCCAGA
mSpag9	TGGGTCGTGAGGTGGAGAAT	TTTTTGCCTTGCATCTTCAGC
mYwhag	GTGACCGAGCTGAACGAAC	GATGCTGCTGATGACCCTCC

Table S1