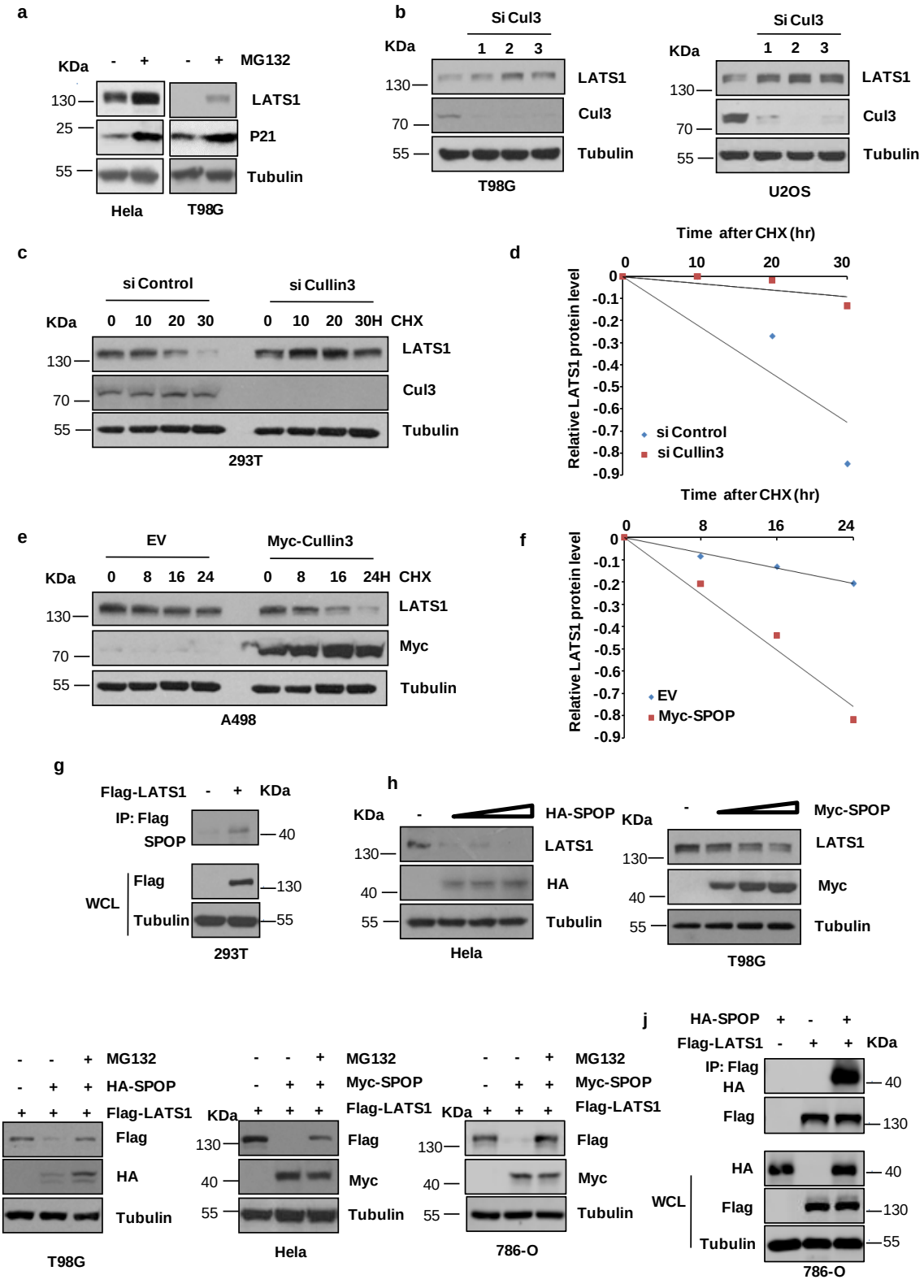
a
KDa
- + - + MG132
130
LATS1
25
P21
55
Tubulin
Hela
T98G
b
Si Cul3
KDa
1 2 3
130
LATS1
70
Cul3
55
Tubulin
T98G
Si Cul3
KDa
1 2 3
130
LATS1
70
Cul3
55
Tubulin
U2OS
c
si Control
si Cullin3
KDa
0 10 20 30 0 10 20 30H CHX
130
LATS1
70
Cul3
55
Tubulin
293T
d
Time after CHX (hr)
0 10 20 30
0
-0.1
-0.2
-0.3
-0.4
-0.5
-0.6
-0.7
-0.8
-0.9
Relative LATS1 protein level
si Control
si Cullin3
Time after CHX (hr)
e
EV
Myc-Cullin3
KDa
0 8 16 24 0 8 16 24H CHX
130
LATS1
70
Myc
55
Tubulin
A498
f
0 8 16 24
0
-0.1
-0.2
-0.3
-0.4
-0.5
-0.6
-0.7
-0.8
-0.9
Relative LATS1 protein level
EV
Myc-SPOP
g
Flag-LATS1 - + KDa
IP: Flag
SPOP
40
WCL
Flag
130
Tubulin
55
293T
h
KDa
- HA-SPOP
130
LATS1
40
HA
55
Tubulin
Hela
KDa
- Myc-SPOP
130
LATS1
40
Myc
55
Tubulin
T98G
i
- - + MG132
- + + HA-SPOP
KDa + + + Flag-LATS1
130
Flag
40
HA
55
Tubulin
T98G
- - + MG132
- + + Myc-SPOP
KDa + + + Flag-LATS1
130
Flag
40
Myc
55
Tubulin
Hela
- - + MG132
- + + Myc-SPOP
KDa + + + Flag-LATS1
130
Flag
40
Myc
55
Tubulin
786-O
j
HA-SPOP + - +
Flag-LATS1 - + + KDa
IP: Flag
HA
40
Flag
130
WCL
HA
40
Flag
130
Tubulin
55
786-O

Fig. S1. Cullin3^{SPOP} is the Physiological E3 Ubiquitin Ligase for LATS1.

(a) IB analysis of WCLs derived from HeLa cells and T98G cells treated with 10μM MG132 for 10 hr.

(b) IB analysis of WCLs derived from T98G and U2OS cells transfected with Cullin3 siRNA.

(c) B analysis of WCLs derived from 293T cells after the specified duration of 100μg/ml cycloheximide (CHX) transfected with Cullin3 siRNA.

(d) The abundance of LATS1 protein in (c) was quantified and plotted.

(e) IB analysis of WCLs derived from A498 cells after the specified duration of 100μg/ml cycloheximide (CHX) transfected with Cullin3 plasmids.

(f) The abundance of LATS1 protein in (E) was quantified and plotted.

(g) IB analysis of WCLs and immunoprecipitates (IPs) derived from 293T cells transfected with indicated constructs. Cells were treated with MG132 (10μM) before harvesting.

(h) IB analysis of WCLs derived from HeLa or T98G cells transfected with increasing doses of plasmid encoding SPOP.

(i) IB analysis of WCLs derived from T98G cells or HeLa cells transfected with indicated constructs. Where indicated, cells were treated with 10μM MG132 before harvesting.

(j) IB analysis of WCLs and immunoprecipitates (IPs) derived from 786-O cells transfected with indicated constructs. Cells were treated with MG132 (10μM) before harvesting.

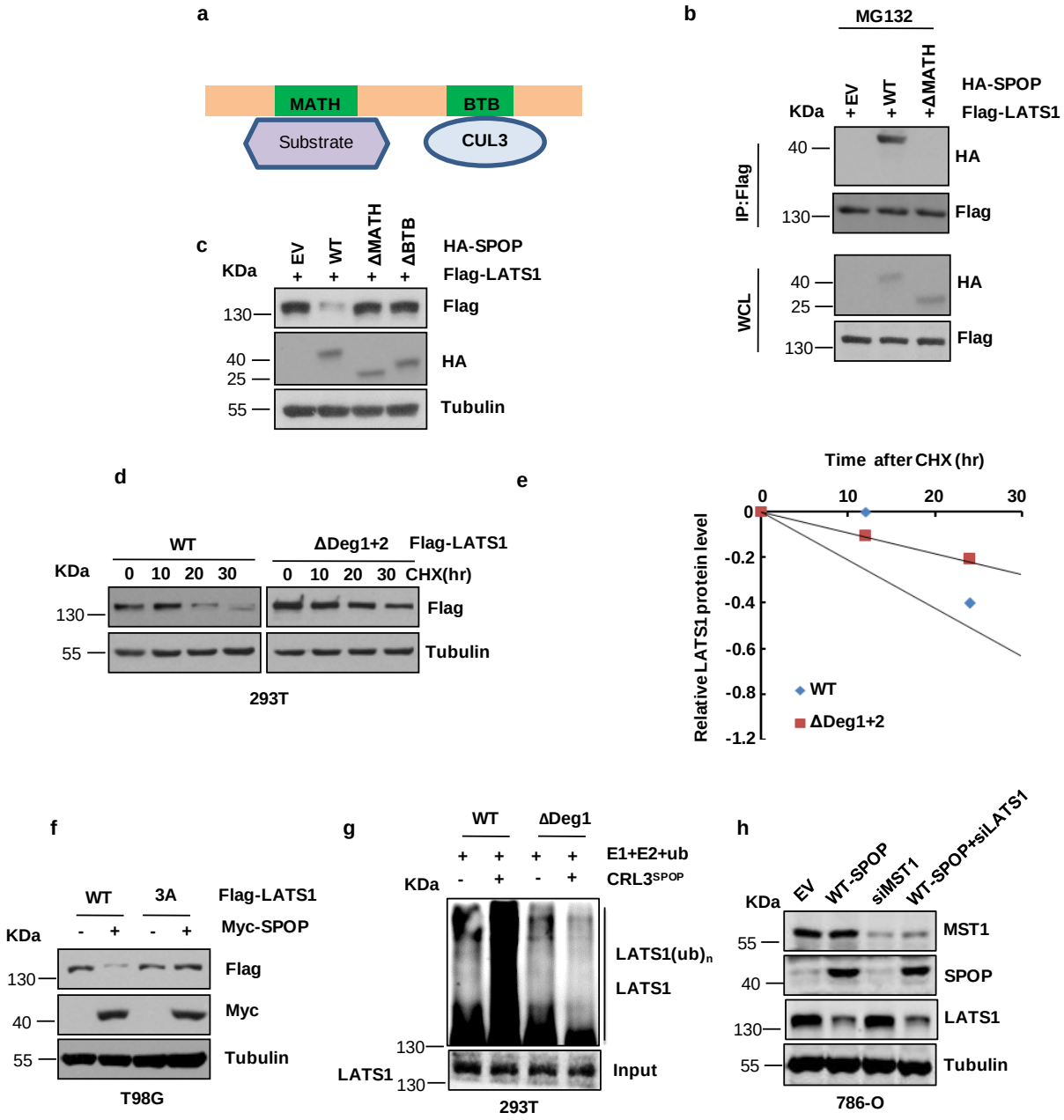
a
MATH
BTB
Substrate
CUL3
b
MG132
KDa
+ EV
+ WT
+ ΔMATH
HA-SPOP
Flag-LATS1
IP:Flag
40
130
HA
Flag
WCL
40
25
130
HA
Flag
c
KDa
+ EV
+ WT
+ ΔMATH
+ ΔBTB
HA-SPOP
Flag-LATS1
130
Flag
40
25
HA
55
Tubulin
d
WT
ΔDeg1+2
Flag-LATS1
KDa
0 10 20 30
0 10 20 30 CHX(hr)
130
Flag
55
Tubulin
293T
e
Time after CHX (hr)
0 10 20 30
0
-0.2
-0.4
-0.6
-0.8
-1.2
Relative LATS1 protein level
WT
ΔDeg1+2
f
WT
3A
Flag-LATS1
KDa
- + - +
Myc-SPOP
130
Flag
40
Myc
55
Tubulin
T98G
g
WT
ΔDeg1
+ + + +
E1+E2+ub
KDa
- + - +
CRL3SPOP
LATS1(ub)n
LATS1
130
LATS1
130
Input
293T
h
KDa
EV
WT-SPOP
siMST1
WT-SPOP+siLATS1
55
MST1
40
SPOP
130
LATS1
55
Tubulin
786-O

Fig. S2. SPOP Ubiquitinated and Degraded of LATS1 Depending on the Degron Motif.

(a) Schematic of SPOP domains.

(b) IB analysis of WCLs and immunoprecipitates (IPs) derived from 293T cells transfected with indicated plasmids.

(c) IB analysis of WCLs derived from 293T cells transfected with indicated plasmids.

(d) IB analysis of WCLs derived from 293T cells after the specified duration of 100μg/ml cycloheximide (CHX) transfected with indicated Flag-tagged LATS1 plasmids.

(e) The abundance of LATS1 protein in (D) was quantified and plotted.

(f) IB analysis of WCLs derived from 293T cells transfected with indicated plasmids.

(g) In vitro ubiquitination assay was performed to detect LATS1 ubiquitination by SPOP.

(h) IB analysis of WCLs derived from 786-O cells transfected with indicated plasmids.

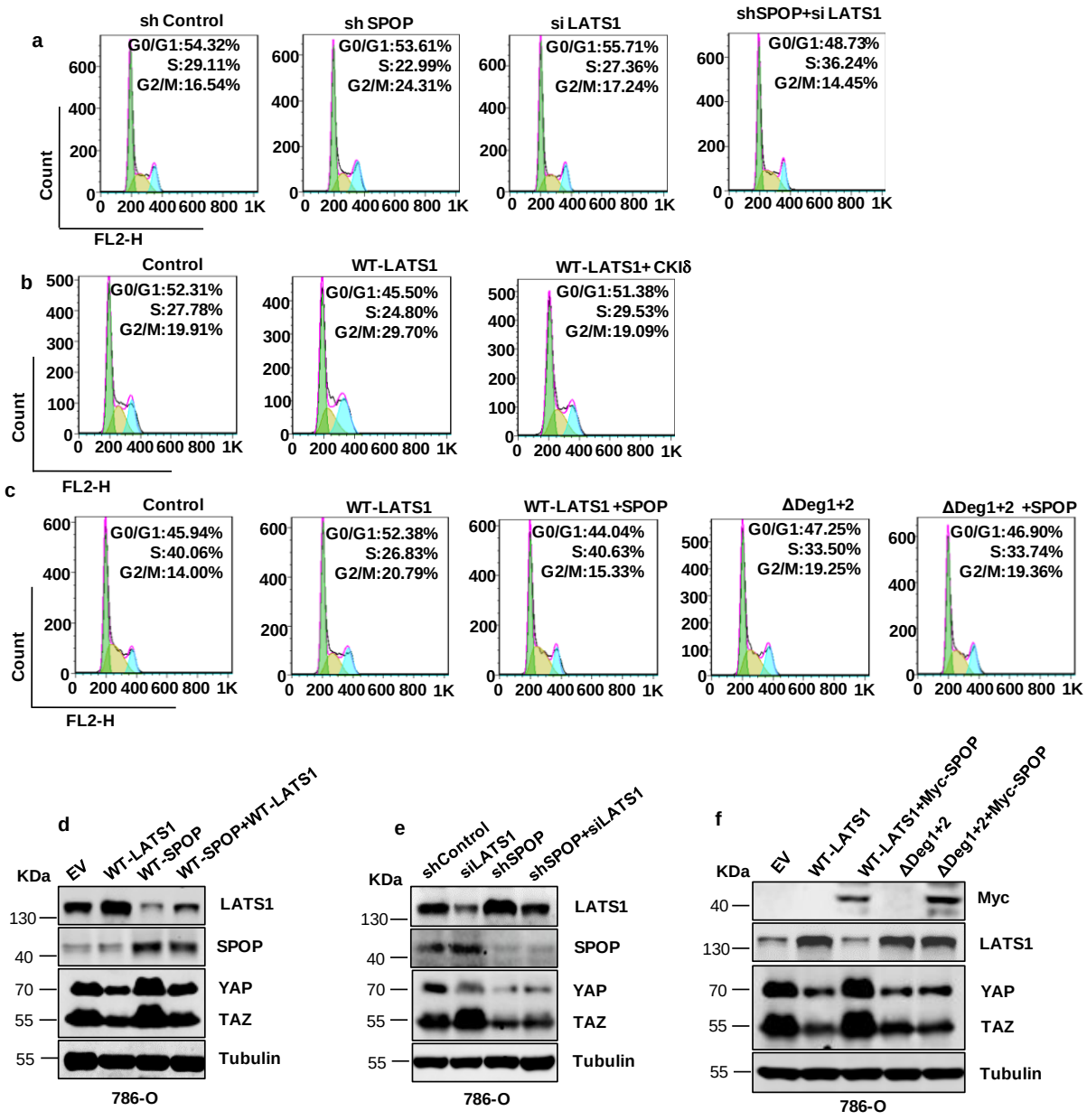

Fig. S3. SPOP Regulates the Cell Cycle Distribution of Kidney Cancer Cells.

(a) The cell cycle distribution of 786-O cells treated with downregulation of SPOP or LATS1.

(b) The cell cycle distribution of 786-O cells transfected with indicated plasmids.

(c) The cell cycle distribution of 786-O cells transfected with indicated plasmids.

(d-f) IB analysis of WCLs derived from 786-O cells transfected with indicated plasmids.

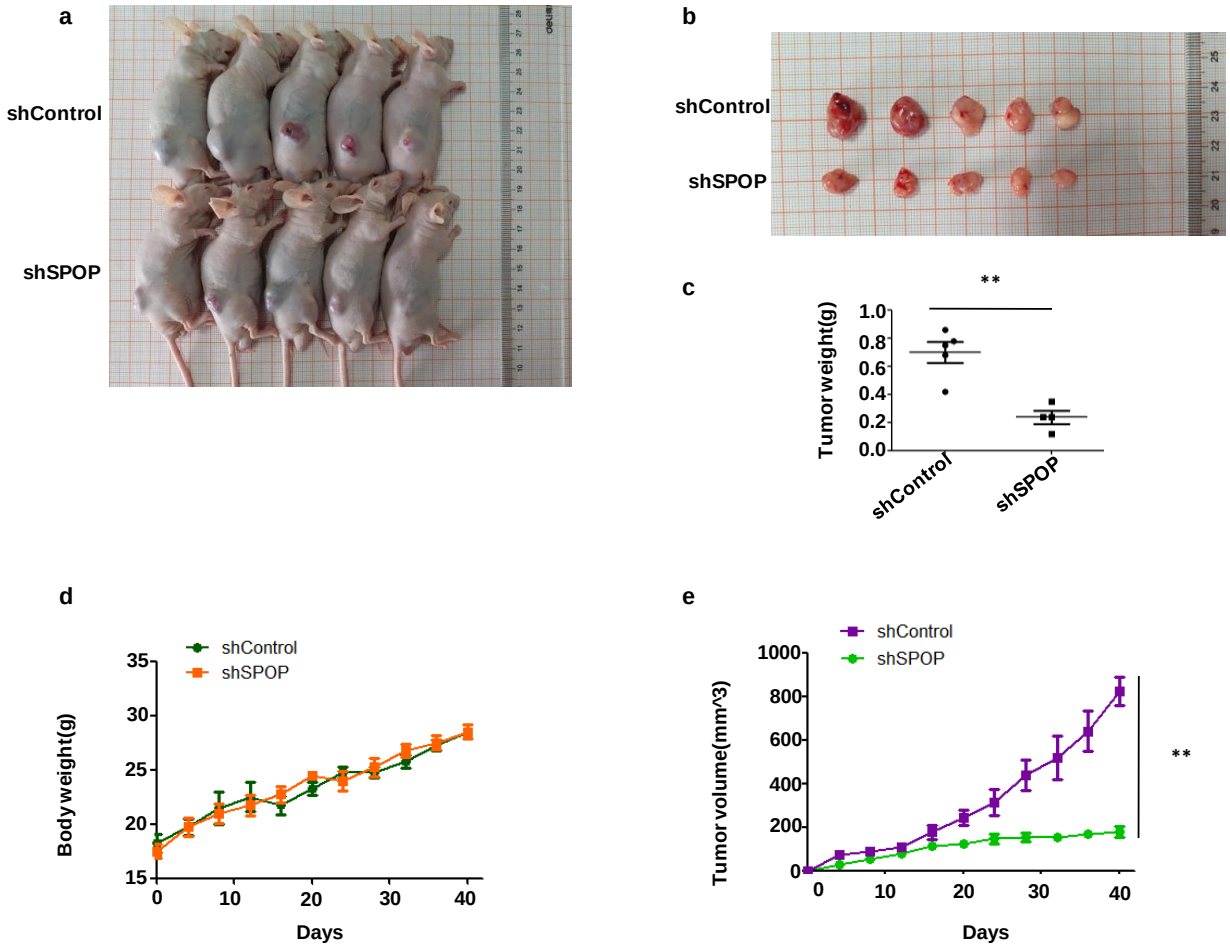

Fig. S4. SPOP Promotes Tumorigenesis.

(a) 786-O-shControl, 786-O-shSPOP polyclonal stable cell lines were injected subcutaneously into the BALB/c-nu/nu mice. 40 days later, mice were anesthetic and taken a picture.

(b) The tumor were dissected and taken a picture.

(c) The weights of the dissected tumors in B.

(d) *In vivo* tumor growth was measured over the indicated time period.

(e) The body weights of the BALB/c-nu/nu mice are measured over the indicated time period.