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## Supplemental Information

# SPOP Promotes Nanog Destruction to Suppress Stem Cell Traits and Prostate Cancer Progression

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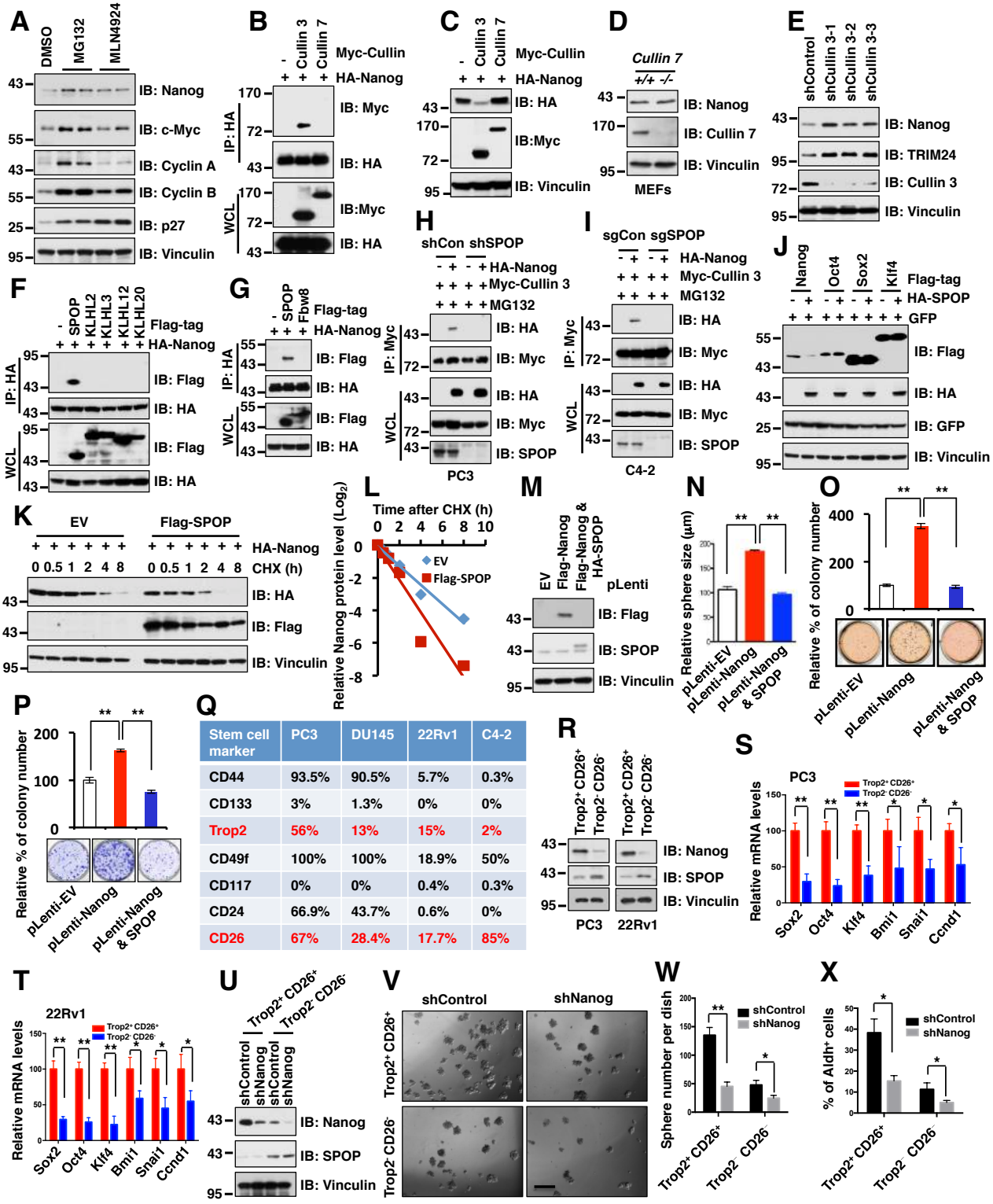
## Supplemental Information

# SPOP promotes Nanog destruction to suppress stem cell traits and prostate cancer progression

Jinfang Zhang[1,12], Ming Chen[2,9,12], Yasheng Zhu[3,12], Xiangpeng Dai[1], Fabin Dang[1], Junming Ren[1], Shancheng Ren[3], Yulia V. Shulga[2], Francisco Beca[1,11], Wenjian Gan[1], Fei Wu[1,4], Yu-Min Lin[5], Xiaobo Zhou[6,7], James A. DeCaprio[8], Andrew H. Beck[1], Kun Ping Lu[5], Jiaoti Huang[9], Cheryl Zhao[10], Yinghao Sun[3], Xu Gao[3,*], Pier Paolo Pandolfi[2,*], Wenyi Wei[1,13,*]

**SUPPLEMENTAL SECTION INVENTORY**

# Figure S1




| Stem cell marker | PC3 | DU145 | 22Rv1 | C4-2 |
|---|---|---|---|---|
| CD44 | 93.5% | 90.5% | 5.7% | 0.3% |
| CD133 | 3% | 1.3% | 0% | 0% |
| Trop2 | 56% | 13% | 15% | 2% |
| CD49f | 100% | 100% | 18.9% | 50% |
| CD117 | 0% | 0% | 0.4% | 0.3% |
| CD24 | 66.9% | 43.7% | 0.6% | 0% |
| CD26 | 67% | 28.4% | 17.7% | 85% |

**Figure S1: Cullin 3$^{SPOP}$ suppresses prostate cancer stem cell traits largely through promoting Nanog poly-ubiquitination and degradation (related to Figure 1).**

**A.** Immunoblot (IB) analysis of whole cell lysates (WCL) derived from DU145 cells with indicated antibodies. Where indicated, cells were treated with MG132 (10 μM) or MLN4924 (1 μM) for 12 hours (h) before harvesting.

**B.** IB analysis of WCL and anti-HA immunoprecipitates (IP) derived from 293T cells transfected with indicated constructs.

**C.** IB analysis of WCL derived from 293T cells transfected with indicated constructs.

**D.** IB analysis of WCL derived from *Cullin 7*$^{+/+}$ and *Cullin 7*$^{-/-}$ MEFs.

**E.** IB analysis of WCL derived from DU145 cells infected with the indicated lentiviral shRNAs against *Cullin 3* and selected with puromycin (1 μg/ml) for 3 days.

**F-G.** IB analysis of WCL and anti-HA immunoprecipitations (IP) derived from 293T cells transfected with indicated constructs.

**H-I.** IB analysis of WCL and anti-Myc IP derived from PC3 or C4-2 cells stably expressing shSPOP or sgSPOP to deplete endogenous *SPOP*. Cells were treated with MG132 (30 μM) for 6 h before harvesting.

**J.** IB analysis of WCL derived from 293T cells transfected with indicated constructs.

**K-L.** IB analysis of WCL derived from 293T cells transfected with indicated constructs. 36 h post transfection, cells were treated with 100 μg/ml cycloheximide (CHX) as indicated time points. Nanog protein abundance in (**K**) was quantified by ImageJ and plotted in (**L**).

**M.** IB analysis of WCL derived from PC3 cells infected with the indicated lentiviral constructs and selected with Hygromycin B (100 μg/ml) for 3 days.

**N.** Quantification of sphere size in **Figure 1K** was showed. Data were presented as mean ± S.D. (n=3). $^{**}p<0.01$ (*t*-test).

**O.** Representative images and quantification of soft agar assays for PC3 cells stably expressing indicated constructs was showed. Data were presented as mean ± S.D. (n=3). $^{**}p<0.01$ (*t*-test).

**P.** Representative images and quantification of colony formation assays for PC3 cells stably expressing indicated constructs were shown. Data were presented as mean ± S.D. (n=3). $^{**}p<0.01$ (*t*-test).

**Q.** Summary for FACS results to show different stem cell maker positive cells in various prostate cancer cells including PC3, DU145, 22Rv1 and C4-2.

**R.** IB analysis of WCL derived from Trop2$^{+}$CD26$^{+}$ and Trop2$^{-}$CD26$^{-}$ cells in PC3 or 22Rv1 cell line, respectively.

**S-T.** Quantitative RT-PCR analysis of Nanog downstream target genes from Trop2$^{+}$CD26$^{+}$ and Trop2$^{-}$CD26$^{-}$ cells in PC3 or 22Rv1 cell line, respectively. Data were presented as mean ± S.D. (n=3). $^{*}p<0.05$, $^{**}p<0.01$ (*t*-test).

**U.** IB analysis of WCL derived from FACS-sorted PC3 cells infected with the indicated lentiviral shRNAs and selected with puromycin (1 μg/ml) for 3 days.

**V-W.** The stable cell lines generated in (**U**) were performed for *in vitro* prostate sphere forming assays. Representative images (**V**) and quantification of sphere numbers per dish (**W**) were shown. Data were presented as mean ± S.D. (n=3). $^{**}p<0.01$, $^{*}p<0.05$ (*t*-test).

**X.** The Aldh$^{+}$ cell population of stable cell lines generated in (**U**) were measured through detecting Aldh enzymatic activity and analyzed by flow cytometry. Data were presented as mean ± S.D. (n=3). $^{**}p<0.01$, $^{*}p<0.05$ (*t*-test).

# Figure S2

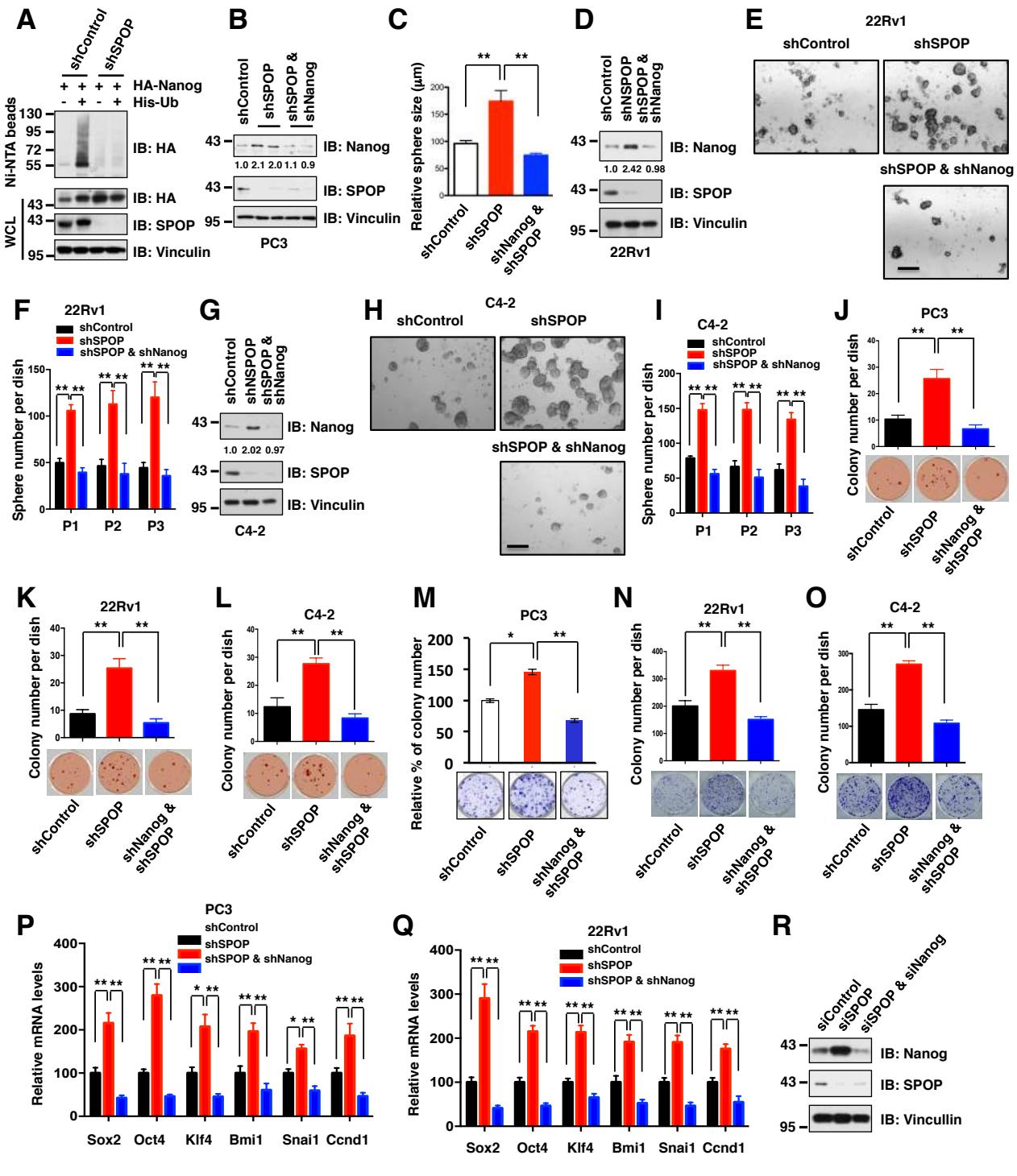

A
shControl
shSPOP
HA-Nanog
His-Ub
Ni-NTA beads
IB: HA
WCL
IB: HA
IB: SPOP
IB: Vinculin
B
shControl
shSPOP
shSPOP & shNanog
IB: Nanog
IB: SPOP
IB: Vinculin
PC3
C
Relative sphere size (μm)
shControl
shSPOP
shNanog & shSPOP
D
shControl
shNSPOP
shSPOP & shNanog
IB: Nanog
IB: SPOP
IB: Vinculin
22Rv1
E
22Rv1
shControl
shSPOP
shSPOP & shNanog
F
22Rv1
shControl
shSPOP
shSPOP & shNanog
Sphere number per dish
P1
P2
P3
G
shControl
shNSPOP
shSPOP & shNanog
IB: Nanog
IB: SPOP
IB: Vinculin
C4-2
H
C4-2
shControl
shSPOP
shSPOP & shNanog
I
C4-2
shControl
shSPOP
shSPOP & shNanog
Sphere number per dish
P1
P2
P3
J
PC3
Colony number per dish
shControl
shSPOP
shNanog & shSPOP
K
22Rv1
Colony number per dish
shControl
shSPOP
shNanog & shSPOP
L
C4-2
Colony number per dish
shControl
shSPOP
shNanog & shSPOP
M
PC3
Relative % of colony number
shControl
shSPOP
shNanog & shSPOP
N
22Rv1
Colony number per dish
shControl
shSPOP
shNanog & shSPOP
O
C4-2
Colony number per dish
shControl
shSPOP
shNanog & shSPOP
P
PC3
shControl
shSPOP
shSPOP & shNanog
Relative mRNA levels
Sox2
Oct4
Klf4
Bmi1
Snai1
Ccnd1
Q
22Rv1
shControl
shSPOP
shSPOP & shNanog
Relative mRNA levels
Sox2
Oct4
Klf4
Bmi1
Snai1
Ccnd1
R
siControl
siSPOP
siSPOP & siNanog
IB: Nanog
IB: SPOP
IB: Vincullin

**Figure S2: Depletion of *SPOP* stabilizes Nanog to promote prostate cancer stem cell properties. (related to Figure 2).**

**A.** Immunoblot (IB) analysis of whole cell lysates (WCL) and Ni-NTA pull-down products derived from the lysates of PC3 cells transfected with the indicated constructs. Cells were treated with 30 $\mu$M MG132 for 6 h before harvesting

**B.** IB analysis of WCL derived from PC3 cells infected with the indicated lentiviral shRNAs and selected with puromycin (1 μg/ml) for 3 days. Nanog band intensities were quantified using ImageJ, normalized to corresponding Vinculin band intensities, and then normalized to shControl.

**C.** Quantification of sphere size in **Figure 2G** was showed. Data were presented as mean ± S.D. (n=3). $^{**}p<0.01$ (*t*-test).

**D** and **G.** IB analysis of WCL derived from 22Rv1 or C4-2 cells infected with the indicated lentiviral shRNAs and selected with puromycin (1 μg/ml) for 3 days. Nanog band intensities were quantified using ImageJ, normalized to corresponding Vinculin band intensities, and then normalized to shControl.

**E-F.** 22Rv1 cells stably expressing indicated constructs were analyzed by *in vitro* prostate sphere forming assays. Representative images (**E**) and quantification of sphere numbers at different passages (**F**) were shown. The scale bar represents 50 $\mu$m. Data were presented as mean ± S.D. (n=3). $^{**}p<0.01$ (*t*-test).

**H-I.** C4-2 cells stably expressing indicated constructs were analyzed by *in vitro* prostate sphere forming assays. Representative images (**H**) and quantification of sphere numbers at different passages (**I**) were shown. The scale bar represents 50 $\mu$m. Data were presented as mean ± S.D. (n=3). $^{**}p<0.01$ (*t*-test).

**J-L.** Representative images and quantification of soft agar assays for PC3, 22Rv1 or C4-2 cells stably expressing indicated lentiviral shRNAs was shown. Data were presented as mean ± S.D. (n=3). $^{*}p<0.05$, $^{**}p<0.01$ (*t*-test).

**M-O.** Representative images and quantification of colony formation assays for PC3, 22Rv1 or C4-2 cells stably expressing indicated lentiviral shRNAs. Data were presented as mean ± S.D. (n=3). $^{*}p<0.05$, $^{**}p<0.01$ (*t*-test).

**P-Q.** Quantitative RT-PCR analysis of Nanog downstream target genes from PC3 or 22Rv1 cells stably expressing indicated lentiviral shRNAs. Data were presented as mean ± S.D. (n=3). $^{*}p<0.05$, $^{**}p<0.01$ (*t*-test).

**R.** IB analysis of WCL derived from CJ7 embryonic stem (ES) cells after 72 h transfection with the indicated siRNAs.

# Figure S3

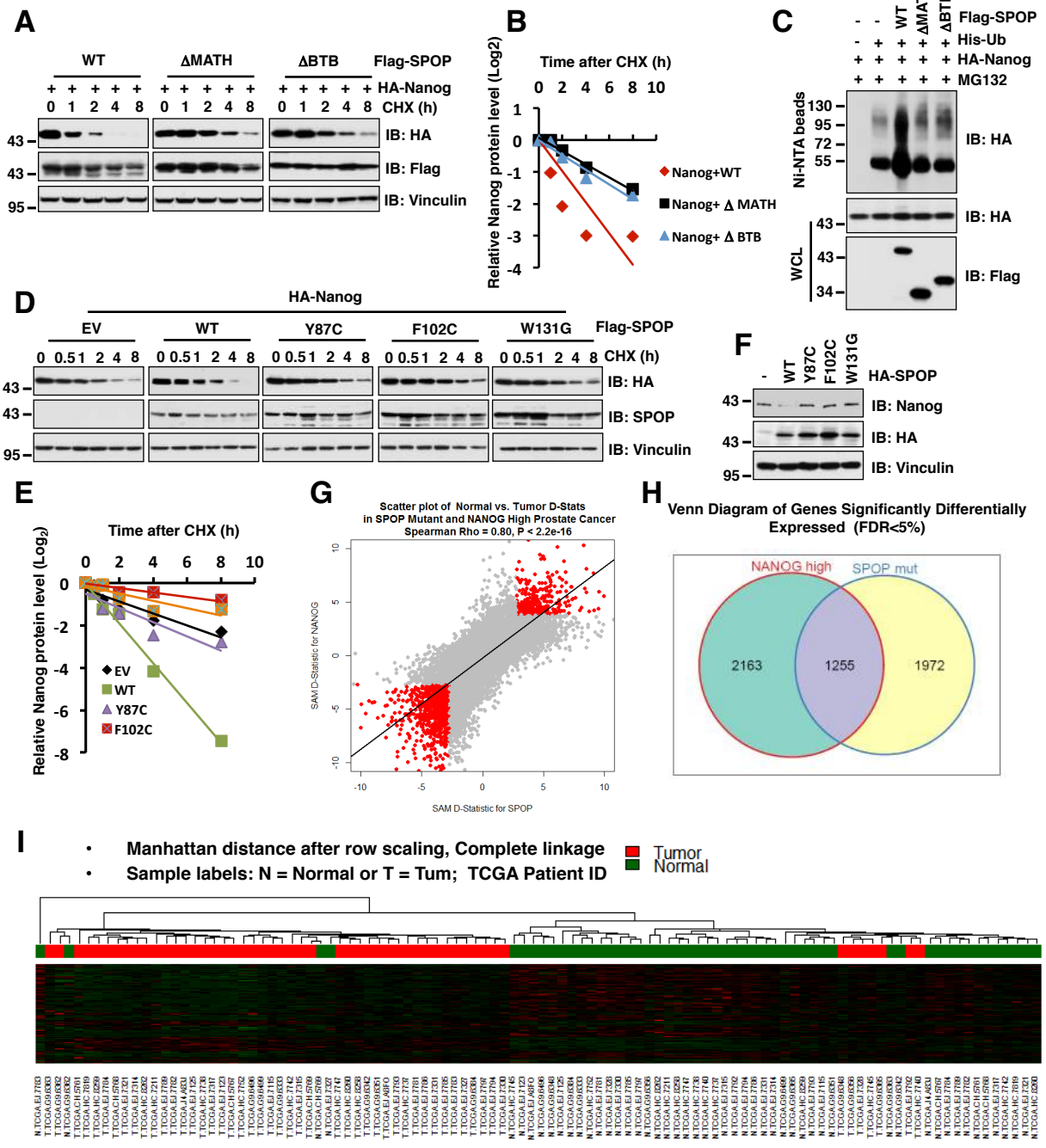

A
WT
ΔMATH
ΔBTB
Flag-SPOP
HA-Nanog
CHX (h)
IB: HA
IB: Flag
IB: Vinculin
B
Time after CHX (h)
Relative Nanog protein level (Log2)
Nanog+WT
Nanog+ Δ MATH
Nanog+ Δ BTB
C
Flag-SPOP
His-Ub
HA-Nanog
MG132
Ni-NTA beads
WCL
IB: HA
IB: Flag
D
HA-Nanog
EV
WT
Y87C
F102C
W131G
Flag-SPOP
CHX (h)
IB: HA
IB: SPOP
IB: Vinculin
F
HA-SPOP
IB: Nanog
IB: HA
IB: Vinculin
E
Time after CHX (h)
Relative Nanog protein level (Log2)
EV
WT
Y87C
F102C
G
Scatter plot of Normal vs. Tumor D-Stats
in SPOP Mutant and NANOG High Prostate Cancer
Spearman Rho = 0.80, P < 2.2e-16
SAM D-Statistic for NANOG
SAM D-Statistic for SPOP
H
Venn Diagram of Genes Significantly Differentially
Expressed (FDR<5%)
NANOG high
SPOP mut
2163
1255
1972
I
Manhattan distance after row scaling, Complete linkage
Sample labels: N = Normal or T = Tum; TCGA Patient ID
Tumor
Normal

**Figure S3: Prostate cancer-associated SPOP mutants fail to bind and promote Nanog poly-ubiquitination and degradation (related to Figure 3).**

**A**-**B.** Immunoblot (IB) analysis of whole cell lysates (WCL) derived from 293T cells transfected with indicated constructs. 36 h post transfection, cells were treated with 100 $\mu$g/ml cycloheximide (CHX) as indicated time points. Nanog protein abundance in (**A**) was quantified by ImageJ and plotted in (**B**).

**C.** IB of WCL and Ni-NTA pull-down products derived from the lysates of PC3 cells transfected with the indicated constructs. Cells were treated with 30 $\mu$M MG132 for 6 h before harvesting.

**D.** IB of WCL derived from the lysates of PC3 cells infected with the indicated lentiviral constructs and selected with Hygromycin B (100 μg/ml) for 3 days.

**E**-**F.** IB analysis of WCL derived from 293T cells transfected with indicated constructs. 36 h post transfection, cells were treated with 100 $\mu$g/ml cycloheximide (CHX) as indicated time points. Nanog protein abundance in (**E**) was quantified with ImageJ and plotted as indicated (**F**).

**G.** There is an observed positive correlation between gene expression changes in *SPOP* mutation and Nanog higher expression clinical specimens (See experimental procedures for details).

**H.** The *SPOP* mutation and Nanog higher expression clinical samples share common gene signatures. The Venn diagram shows the overlap of genes significantly differentially expressed in *SPOP* mutation and Naong higher expression samples from TCGA.

**I.** A heatmap of the common genes associated with *SPOP* mutation and Nanog higher expression clinical samples. N represents matched normal samples from Nanog higher expression or *SPOP* mutation patients. T represents matched tumor samples from Nanog higher expression or *SPOP* mutation patients. The numbers represents TCGA patient IDs.

## Figure S4

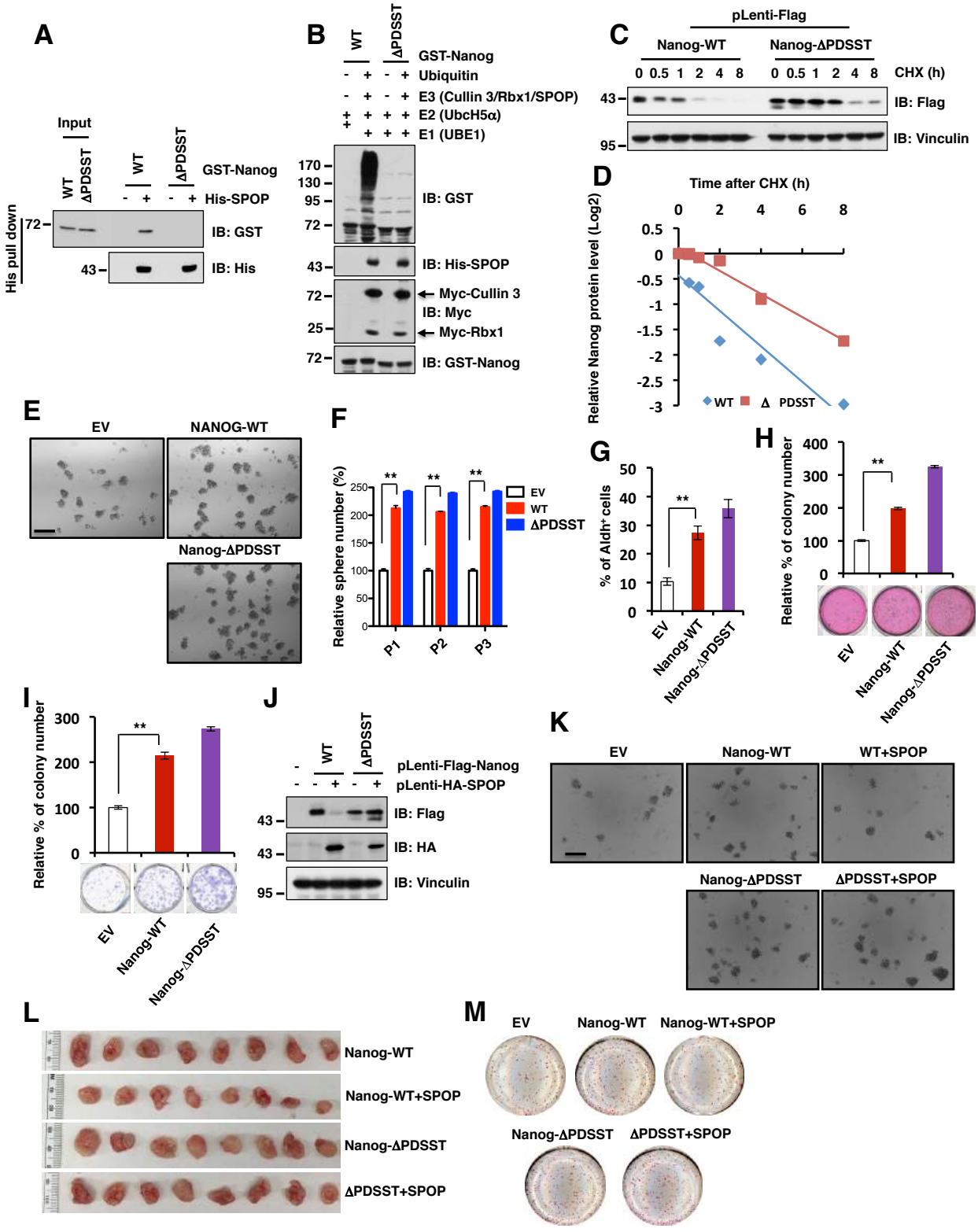

A
Input
WT
ΔPDSST
WT
ΔPDSST
GST-Nanog
His-SPOP
His pull down
72
IB: GST
43
IB: His
B
WT
ΔPDSST
GST-Nanog
Ubiquitin
E3 (Cullin 3/Rbx1/SPOP)
E2 (UbcH5α)
E1 (UBE1)
170
130
95
72
IB: GST
43
IB: His-SPOP
72
Myc-Cullin 3
IB: Myc
25
Myc-Rbx1
72
IB: GST-Nanog
C
pLenti-Flag
Nanog-WT
Nanog-ΔPDSST
0 0.5 1 2 4 8
0 0.5 1 2 4 8
CHX (h)
43
IB: Flag
95
IB: Vinculin
D
Time after CHX (h)
0 2 4 6 8
Relative Nanog protein level (Log2)
0.5
0
-0.5
-1
-1.5
-2
-2.5
-3
WT
Δ PDSST
E
EV
NANOG-WT
Nanog-ΔPDSST
F
Relative sphere number (%)
250
200
150
100
50
0
**
**
**
EV
WT
ΔPDSST
P1
P2
P3
G
% of Aldh+ cells
50
40
30
20
10
0
**
EV
Nanog-WT
Nanog-ΔPDSST
H
Relative % of colony number
400
300
200
100
0
**
EV
Nanog-WT
Nanog-ΔPDSST
I
Relative % of colony number
300
200
100
0
**
EV
Nanog-WT
Nanog-ΔPDSST
J
WT
ΔPDSST
pLenti-Flag-Nanog
pLenti-HA-SPOP
43
IB: Flag
43
IB: HA
95
IB: Vinculin
K
EV
Nanog-WT
WT+SPOP
Nanog-ΔPDSST
ΔPDSST+SPOP
L
Nanog-WT
Nanog-WT+SPOP
Nanog-ΔPDSST
ΔPDSST+SPOP
M
EV
Nanog-WT
Nanog-WT+SPOP
Nanog-ΔPDSST
ΔPDSST+SPOP

**Figure S4: SPOP promotes Nanog poly-ubiquitination and degradation in a degron-dependent manner (related to Figure 4).**

**A.** Immunoblot (IB) analysis of His pull-down precipitates from bacterially purified His-tagged recombinant SPOP protein incubated with bacterially purified GST-Nanog WT and mutant recombinant proteins.

**B.** IB analysis of *in vitro* ubiquitin assay for bacterially purified GST-Nanog WT and mutant recombinant proteins incubated with E1, E2, E3 and ubiquitin as indicated.

**C-D.** IB analysis of whole cell lysates (WCL) derived from PC3 cells stably expressing indicated lentiviral constructs. Cells were treated with 100 $\mu$g/ml cycloheximide (CHX) as indicated time points. Nanog WT and deletion mutant (ΔPDSST) protein abundance in (**C**) was quantified by ImageJ and plotted in (**D**).

**E-F.** *In vitro* tumor sphere forming assays were performed for PC3 cells stably expressing Nanog-WT, Nanog-ΔPDSST as well as empty vector (EV) as control. Representative images (**E**) and quantification of sphere numbers at different passages (**F**) are shown. Data were presented as mean ± S.D. (n=3). $^{**}p<0.01$ (*t*-test).

**G.** The aldehyde dehydrogenase activity positive ($Aldh^+$) cell population of PC3 cells stably expressing indicated consructs were measured through detecting Aldh enzymatic activity and analyzed by flow cytometry. Data were presented as mean ± S.D. (n=3). $^{**}p<0.01$ (*t*-test).

**H.** Representative images and quantification of soft agar assays for PC3 cells stably expressing indicated lentiviral constructs were shown. Data were presented as mean ± S.D. (n=3). $^{**}p<0.01$ (*t*-test).

**I.** Representative images and quantification of colony formation assays for PC3 cells stably expressing indicated lentiviral constructs were shown. Data were presented as mean ± S.D. from three independent experiments. $^{**}p<0.01$ (*t*-test).

**J.** IB analysis of WCL derived from PC3 cells infected with the indicated constructs and selected with hygromycin (250 μg/ml) for 3 days before harvesting.

**K.** Representative images of *In vitro* tumor sphere forming assays for PC3 cells stably cell lines generated in (**J**) were shown. The scale bar represents 50 $\mu$m.

**L.** Tumor Xenograft mouse assays were performed and after 18 days post-injection, tumors were dissected after euthanizing the mice.

**M.** Alkaline Phosphatase (AP) staining was used to quantify the percentage of pluripotent CJ7 ES cells after 72 h transfection with indicated constructs. Representative images are shown.

## Figure S5

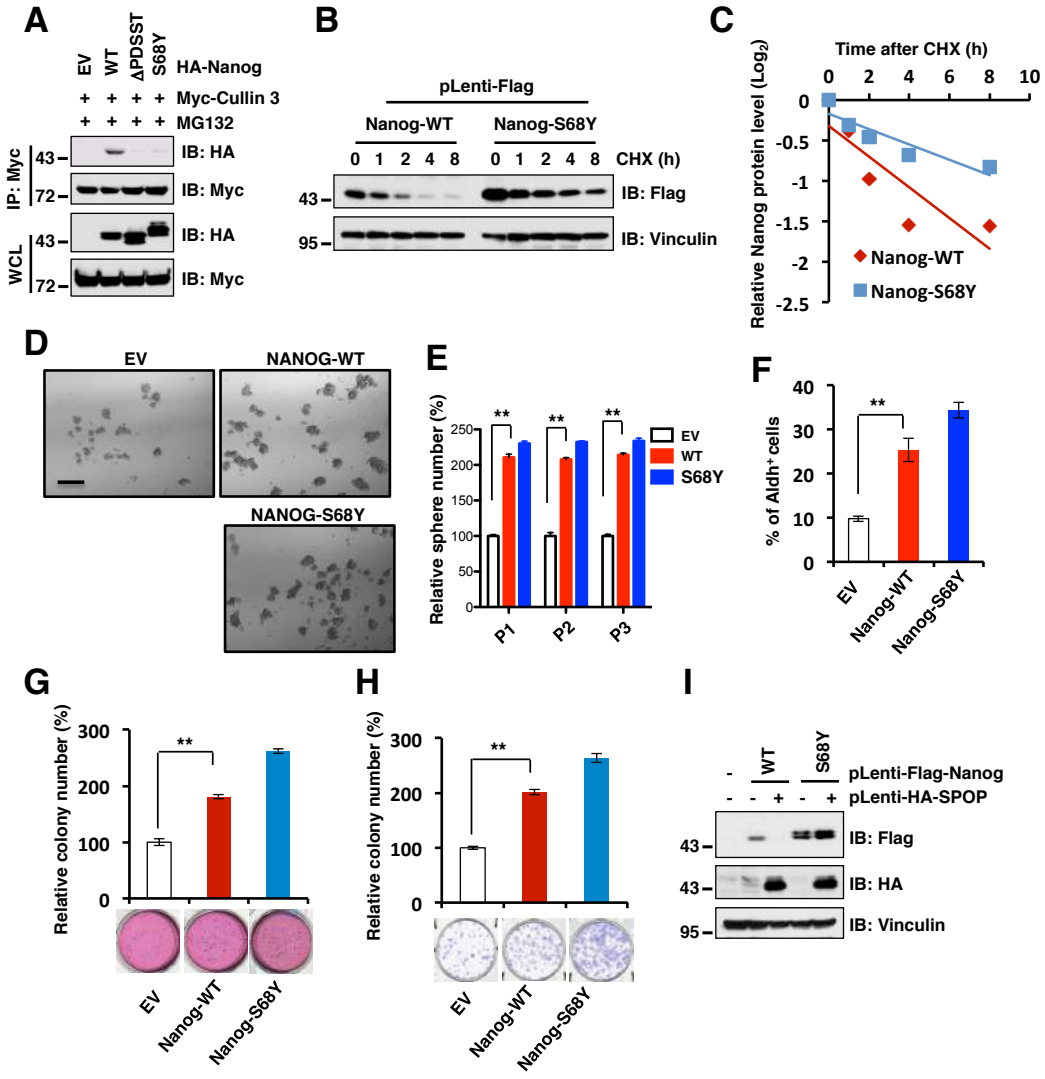

A
EV
WT
ΔPDSST
S68Y
HA-Nanog
Myc-Cullin 3
MG132
IP: Myc
IB: HA
IB: Myc
WCL
IB: HA
IB: Myc
B
pLenti-Flag
Nanog-WT
Nanog-S68Y
0 1 2 4 8 0 1 2 4 8 CHX (h)
IB: Flag
IB: Vinculin
C
Time after CHX (h)
Relative Nanog protein level (Log2)
Nanog-WT
Nanog-S68Y
D
EV
NANOG-WT
NANOG-S68Y
E
Relative sphere number (%)
EV
WT
S68Y
P1
P2
P3
F
% of Aldh+ cells
EV
Nanog-WT
Nanog-S68Y
G
Relative colony number (%)
EV
Nanog-WT
Nanog-S68Y
H
Relative colony number (%)
EV
Nanog-WT
Nanog-S68Y
I
WT
S68Y
pLenti-Flag-Nanog
pLenti-HA-SPOP
IB: Flag
IB: HA
IB: Vinculin

**Figure S5: Cancer patient-derived Nanog mutation (S68Y) in the degron motif confers resistance to SPOP-mediated destruction (related to Figure 5).**

**A.** Immunoblot (IB) analysis of whole cell lysates (WCL) and anti-Myc immunoprecipitations (IP) derived from 293 cells transfected with indicated constructs. Cells were treated with 10 $\mu$M MG132 for 12 hours before harvesting.

**B**-**C.** Immunoblot (IB) analysis of whole cell lysates (WCL) and anti-Myc immunoprecipitations (IP) derived from 293 cells stably expressing indicated lentiviral consturct. Cells were treated with 100 $\mu$g/ml cycloheximide (CHX) as indicated time points. Nanog WT and S68Y mutant protein abundance in (**B**) was quantified by ImageJ and plotted in (**C**).

**D**-**E.** *In vitro* tumor sphere forming assays were performed for PC3 cells stably expressing Nanog-WT, Nanog-S68Y as well as empty vector (EV) as control. Representative images (**D**) and quantification of sphere numbers at different passages (**E**) are shown. The scale bar represents 50 $\mu$m. Data were presented as mean ± S.D. (n=3). $^{**}p<0.01$ (*t*-test).

**F.** The aldehyde dehydrogenase activity positive ($Aldh^+$) cell population of PC3 cells stably expressing indicated constructs were measured through detecting Aldh enzymatic activity and analyzed by flow cytometry. Data were presented as mean ± S.D. (n=3). $^{**}p<0.01$ (*t*-test).

**G.** Representative images and quantification of soft agar assays for cell lines stably expression of Nanog-WT, S68Y as well as EV were shown. Data were presented as mean ± S.D. (n=3). $^{**}p<0.01$ (*t*-test).

**H.** Representative images and quantification of colony formation assays for cell lines stably expression of Nanog-WT, S68Y as well as EV were shown. Data were presented as mean ± S.D. (n=3). $^{**}p<0.01$ (*t*-test).

**I.** IB analysis of WCL derived from PC3 cells infected with the indicated constructs and selected with hygromycin (250 μg/ml) for 3 days before harvesting.

# Figure S6

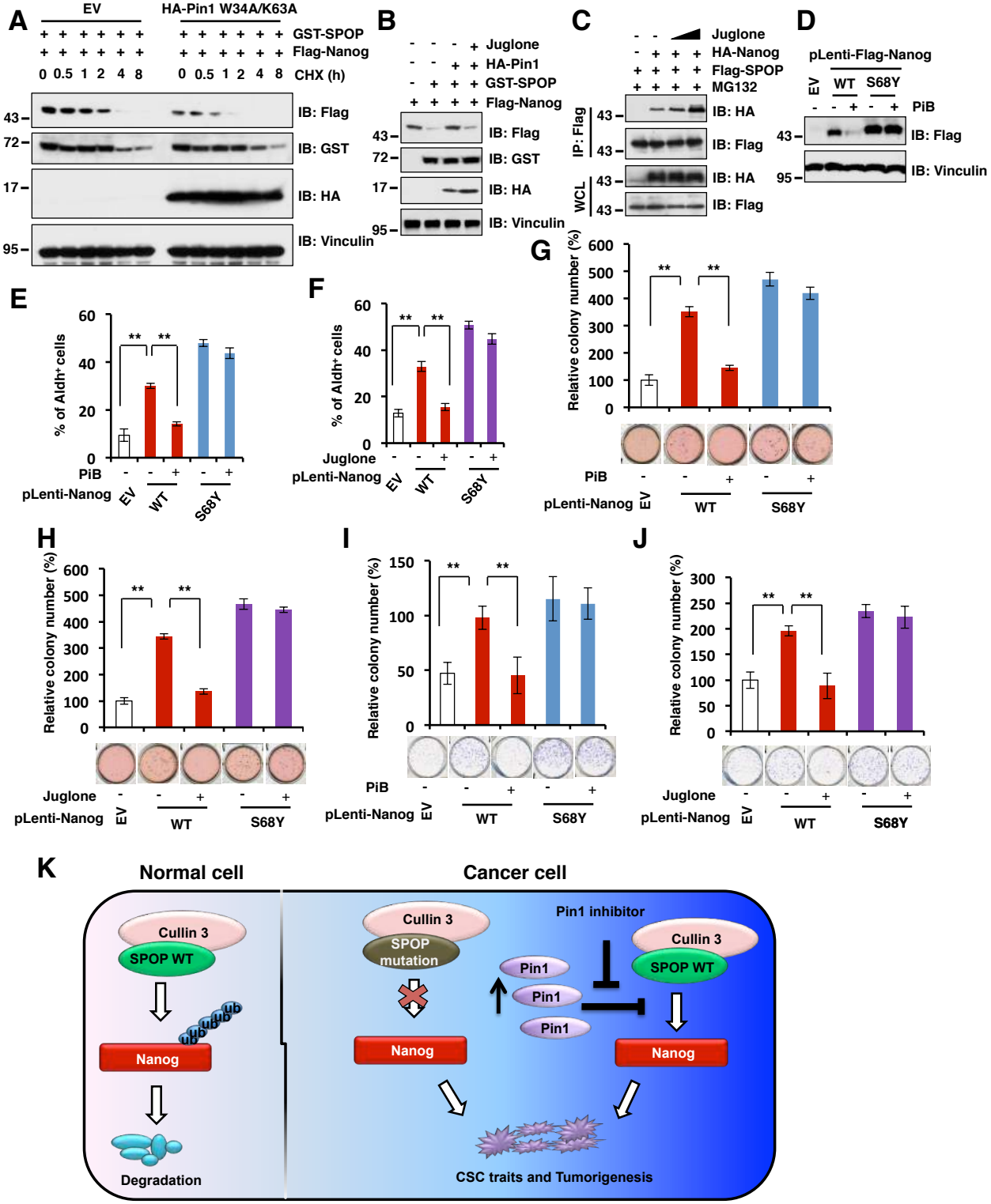

A
EV
HA-Pin1 W34A/K63A
GST-SPOP
Flag-Nanog
0 0.5 1 2 4 8 0 0.5 1 2 4 8 CHX (h)
43
72
17
95
IB: Flag
IB: GST
IB: HA
IB: Vinculin
B
Juglone
HA-Pin1
GST-SPOP
Flag-Nanog
IB: Flag
IB: GST
IB: HA
IB: Vinculin
C
Juglone
HA-Nanog
Flag-SPOP
MG132
IP: Flag
WCL
IB: HA
IB: Flag
D
pLenti-Flag-Nanog
EV
WT
S68Y
PiB
IB: Flag
IB: Vinculin
E
% of Aldh+ cells
PiB
pLenti-Nanog
EV
WT
S68Y
F
% of Aldh+ cells
Juglone
pLenti-Nanog
G
Relative colony number (%)
PiB
pLenti-Nanog
H
Relative colony number (%)
Juglone
pLenti-Nanog
I
Relative colony number (%)
PiB
pLenti-Nanog
J
Relative colony number (%)
Juglone
pLenti-Nanog
K
Normal cell
Cancer cell
Cullin 3
SPOP WT
Nanog
Degradation
SPOP mutation
Pin1 inhibitor
Pin1
CSC traits and Tumorigenesis

**Figure S6: Pin1 inhibitors promote SPOP-mediated destruction of Nanog to suppress prostate CSC traits (related to Figure 6).**

**A.** Immunoblot (IB) analysis of whole cell lysates (WCL) derived from 293T cells transfected with indicated constructs. 36 h post transfection, cells were treated with 100 $\mu$g/ml cycloheximide (CHX) as indicated time points.

**B.** IB analyses of WCL from 293T cells transfected with indicated constructs and treated with Pin1 inhibitor (Juglone, 10 μM) for 8 h before harvesting.

**C.** IB analyses of WCL and anti-Flag immunoprecipitations from 293T cells transfected with indicated constructs and were treated with MG132 (10 $\mu$M) for 12 h and Juglone (10 μM) for 8 h before harvesting.

**D.** IB analysis of WCL derived from PC3 cells stably expressing indicated constructs treated with the Pin1 inhibitor (PiB, 20 μM) for 8 h.

**E-F.** PC3 cells stably expressing indicated constructs were treated with Pin1 inhibitors (2 μM PiB or 1 μM Juglone) for 3 days. Subsequently, cells were measured through detecting aldehyde dehydrogenase (Aldh) enzymatic activity and analyzed by flow cytometry. Data were presented as mean ± S.D. (n=3). $^{**}p<0.01$ (*t*-test).

**G**-**H.** Representative images and quantification of soft agar assays for cell lines generated in (**D**) was showed. The cells were treated with Pin1 inhibitors (2 μM PiB or 1 μM Juglone) for 3 days before seeding for soft agar assays. Data were presented as mean ± S.D. (n=3). $^{**}p<0.01$ (*t*-test).

**I**-**J.** Representative images and quantification of colony formation assays for cell lines generated in (**D**) was showed. The cells were treated with Pin1 inhibitors (2 μM PiB or 1 μM Juglone) for 3 days before seeding for colony formation assays. Data were presented as mean ± S.D. (n=3). $^{**}p<0.01$ (*t*-test).

**K.** A proposed working model to illustrate how SPOP regulates Nanog in normal and cancer cell setting.

**Table S1. Quantitative RT-PCR primer sequences (relates to STAR★METHODS).**

| *Gene* | *Sequence* |
|---|---|
| Human *Sox2* | F: TGGACAGTTACGCGCACAT |
| | R: CGAGTAGGACATGCTGTAGGT |
| Human *Oct4* | F: CTGGGTTGATCCTCGGACCT |
| | R: CCATCGGAGTTGCTCTCCA |
| Human *Klf4* | F: CCCACATGAAGCGACTTCCC |
| | R: CAGGTCCAGGAGATCGTTGAA |
| Human *Bmi1* | F: GCTGCCAATGGCTCTAATGAA |
| | R: TGCTGGGCATCGTAAGTATCTT |
| Human *Snai1* | F: ACTGCAACAAGGAATACCTCAG |
| | R: GCACTGGTACTTCTTGACATCTG |
| Human *Ccnd1* | F: GCTGCGAAGTGGAAACCATC |
| | R: CCTCCTTCTGCACACATTTGAA |