Supplementary Materials

Supplementary Figure Legends

Figure S1. (A) Venn diagram showed the overlapped identified proteins between our A549 cells dataset and the published USP9x knock-down proteome in MCF-7 cells by Wang *et al*. **(B)** Venn diagram showed the overlapped down-regulated proteins between our A549 cells dataset and the published USP9x knock-down proteome in MCF-7 cells by Wang *et al*. using the same criterion represented by Wang *et al*. (protein ratio of [≤ 0.770, ≥ 1.3], and *P* value < 0.05).

Figure S2. (A-C) NCI-H1975, NCI-H125 and NCI-H3122 cells were transfected with either two different siRNAs targeting USP9X or a control siRNA respectively. After 48 hours, cells were harvested and proteins were analyzed by immunoblotting. **(D)** Multiple kinds of lung cancer cells were harvested for immunoblotting, the correlated pattern of the protein expression levels of USP9X and TTK in these cell lines was shown. **(E)** Flag-TTK, ubiquitin K63-only plasmids, siRNA or siUSP9X were co-transfected into 293T cells. The indicated cells were treated with MG132 overnight. Then cells were extracted for immunoprecipitation with anti-flag agarose, furthermore, analyzed by western blotting. **(F)** A549 cells were transfected with either control or USP9X siRNA. After 48 hours, DMSO (D) or proteasome inhibitor Bortezomib (B) was added as indicated and cells were harvested for immunoblotting.

Figure S3. (A) A549 cells were transfected with either three different siRNAs targeting TTK or a control siRNA. After 48 hours, cells were harvested and proteins analyzed by immunoblotting. **(B)** Stable knockdown of either USP9X or TTK decreased HeLa cells migration ability which was measured by wound-healing assay. The wound edges are indicated by black lines. Representative images are shown. The quantitative results (left) and WB result (right) are shown at the bottom. The y axis represents percentage of wound closure. Data are represented as mean ± s.d. (n = 3, *** *P* < 0.001, t-test). **(C)** A549 cells were treated with WP1130 as the indicated drug concentration for 8 hours. Then protein samples were harvested for immunoblotting. **(D)** A549 cells were treated with WP1130 as the indicated time points at 10 μM. Then protein samples were harvested for immunoblotting.

Figure S4. (A) A549 cells stably transfected with shRNAs targeting USP9X or shGFP were harvested for immunoblotting. **(B)** A549 cells stably transfected with shRNAs targeting TTK or shGFP were harvested for immunoblotting. **(C)** A549 cells stably expressing shGFP was

transiently overexpressed empty vector control (EV), shUSP9X were transiently overexpressed EV or TTK respectively. After 48 hours, cells were harvested and proteins were analyzed by immunoblotting. **(D)** TTK transient overexpression significantly increased cell proliferation in USP9X stable knockdown A549 cells. Each bar represents the mean ± s.d. (n = 4, *** $P < 0.001$, two-way ANOVA)

Supplementary Figures

Figure S1

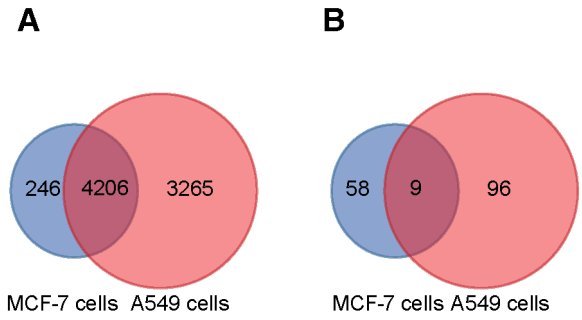


Figure S2

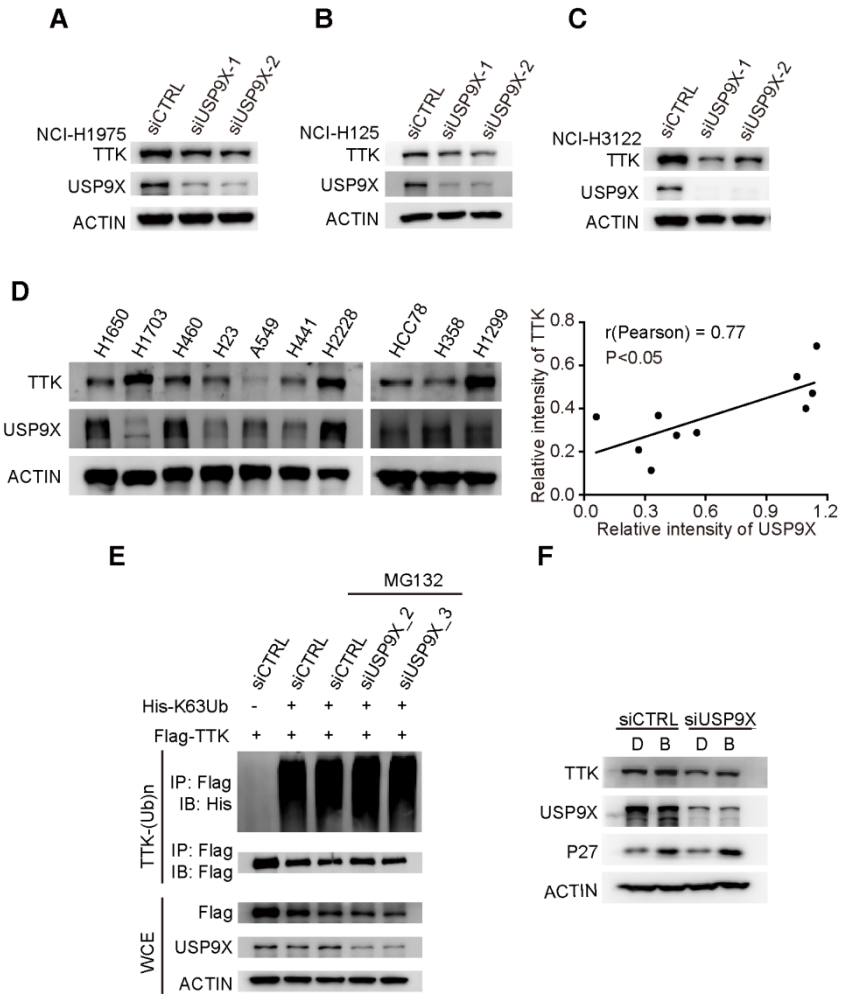

A
B
C
siCTRL
siUSP9X-1
siUSP9X-2
NCI-H1975
NCI-H125
NCI-H3122
TTK
USP9X
ACTIN
D
H1650
H1703
H460
H23
A549
H441
H2228
HCC78
H358
H1299
r(Pearson) = 0.77
P<0.05
Relative intensity of TTK
Relative intensity of USP9X
E
MG132
siUSP9X_2
siUSP9X_3
His-K63Ub
Flag-TTK
TTK-(Ub)n
IP: Flag
IB: His
IP: Flag
IB: Flag
WCE
Flag
F
siCTRL siUSP9X
D B D B
P27

Figure S3

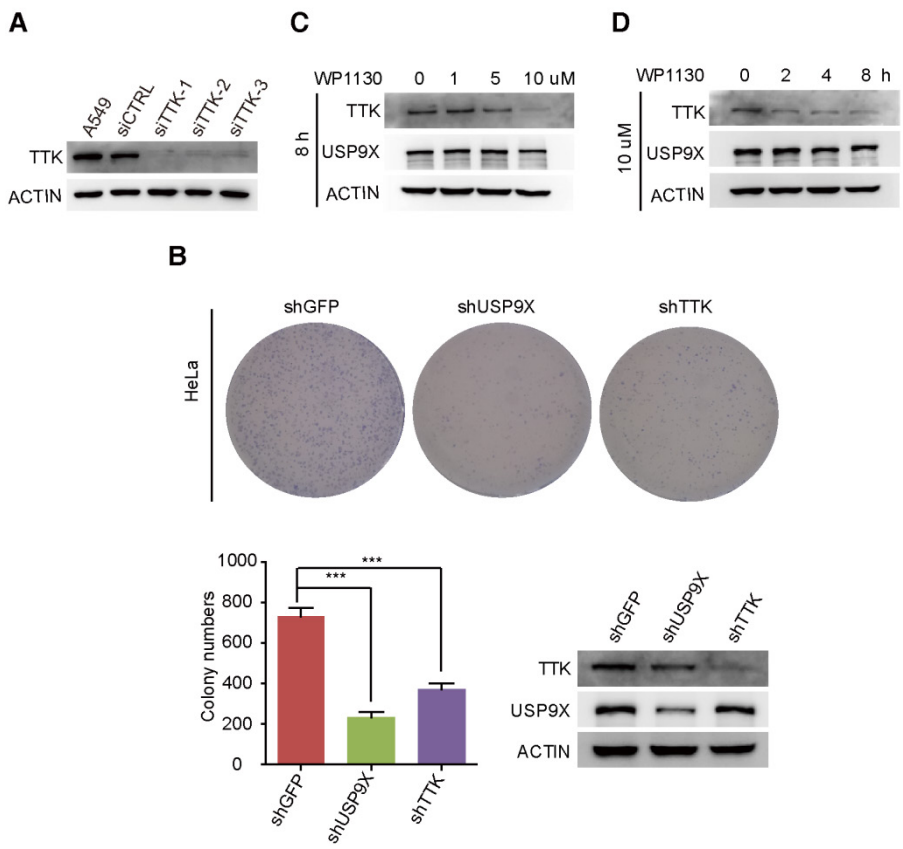
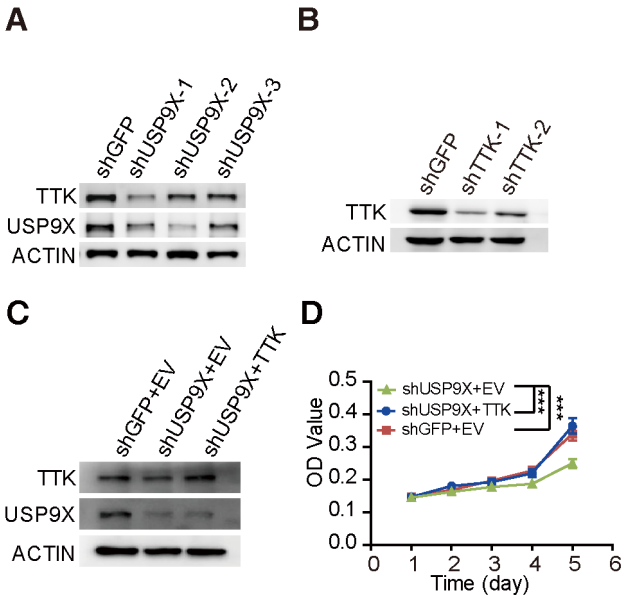

A
A549
siCTRL
siTTK-1
siTTK-2
siTTK-3
TTK
ACTIN
C
WP1130 0 1 5 10 uM
8 h
TTK
USP9X
ACTIN
D
WP1130 0 2 4 8 h
10 uM
TTK
USP9X
ACTIN
B
HeLa
shGFP
shUSP9X
shTTK
Colony numbers
1000
800
600
400
200
0
***
***
shGFP
shUSP9X
shTTK
TTK
USP9X
ACTIN

Figure S4


A
shGFP
shUSP9X-1
shUSP9X-2
shUSP9X-3
TTK
USP9X
ACTIN
B
shGFP
shTTK-1
shTTK-2
TTK
ACTIN
C
shGFP+EV
shUSP9X+EV
shUSP9X+TTK
TTK
USP9X
ACTIN
D
shUSP9X+EV
shUSP9X+TTK
shGFP+EV
***
OD Value
0.5
0.4
0.3
0.2
0.1
0.0
0
1
2
3
4
5
6
Time (day)

Supplementary Table

Table S1

The list of 22 down-regulated proteins in USP9X-knockdown cells

Gene names	Ratio(KD/Con)	T-test p-value
UBXN8	0.648	2.198E-02
PIBF1	0.658	1.505E-02
MYO15B	0.643	4.493E-02
NCOA4	0.493	3.863E-02
TAX1BP1	0.607	1.971E-02
FOPNL	0.628	3.434E-02
CPEB2	0.452	2.008E-02
MPRIP	0.610	4.695E-03
SYNM	0.585	2.702E-02
HSP90AA1	0.665	3.265E-02
TTK	0.624	2.149E-02
MLLT11	0.482	5.967E-04
MLK4	0.461	3.425E-03
CPLX2	0.434	1.724E-02
KIAA0907	0.384	4.868E-02
USP9X	0.563	4.891E-03
ITFG2	0.625	6.232E-03
FAM49B	0.666	1.747E-04
RNF19A	0.596	4.226E-02
HECTD1	0.665	1.188E-02
RNF115	0.516	3.371E-02
FAM73A	0.635	1.839E-02