Supplementary Figure Legends

Supplementary Figure 1. USP9X regulates YAP1 protein level and protein stability.

(A) MDA-MB-231 cells were transfected with the indicated plasmids and Western blot was performed with the indicated antibodies. (B) OVCAR8 cells stably expressing control or USP9X shRNAs were generated and Western blot was performed with the indicated antibodies. (C-D) MDA-MB-231 cells stably expressing control or USP9X shRNAs were generated and Western blot was performed with the indicated antibodies. (E) CHX pulse-chase assay was performed in cells as in (A). Right panel: quantification of the YAP1 protein levels relative to β-actin. Error bars represent the s.e.m. of three independent experiments.

Supplementary Figure 2. USP9X deubiquitinates YAP1.

(A) MDA-MB-231 cells were transfected with the indicated plasmids and treated with MG132 for 6h before cells were lysed under denaturing conditions. Immunoprecipitation was performed with HA beads. The polyubiquitylated YAP1 protein was detected by anti-K48 Ub antibody.
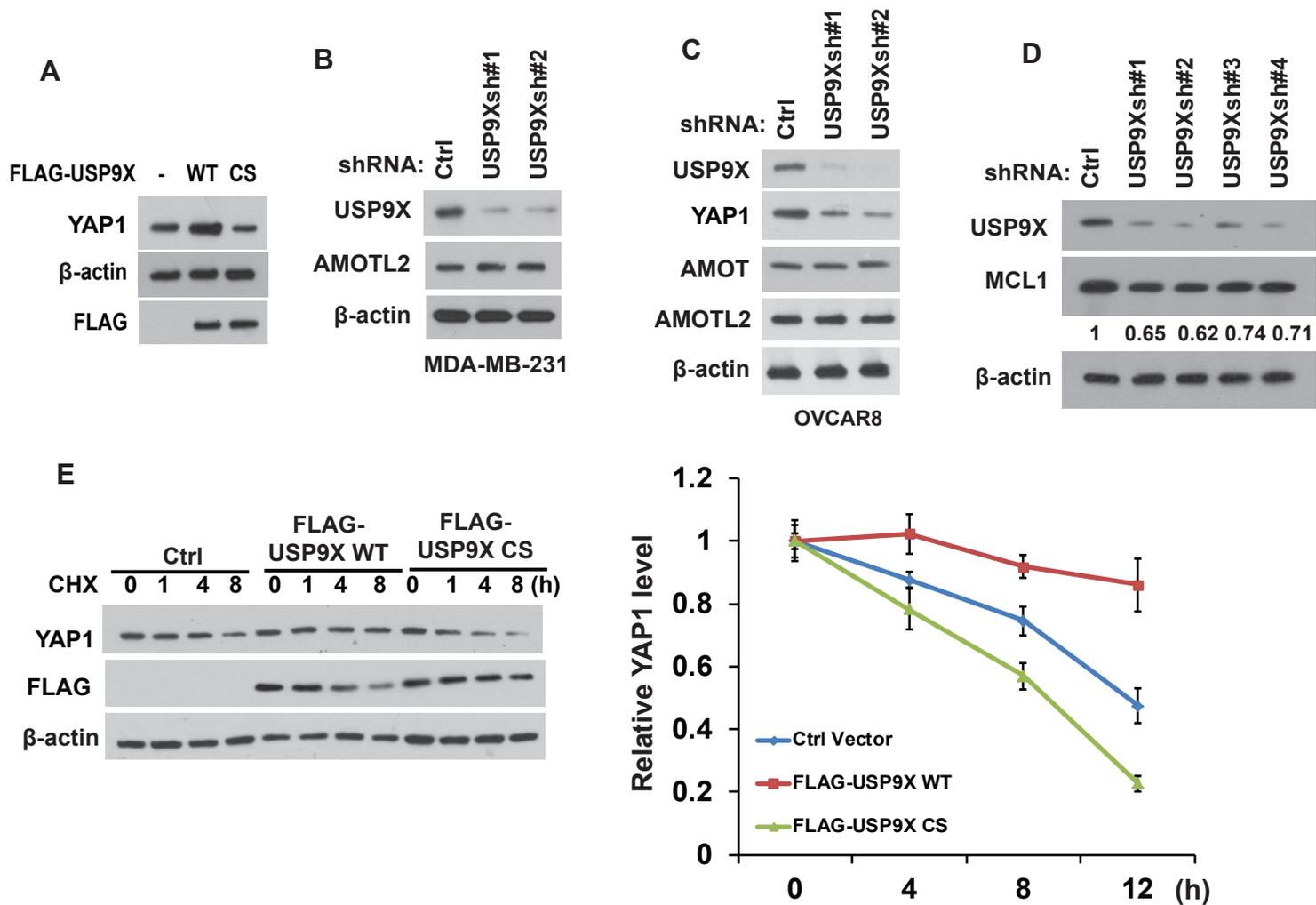
Supplementary Figure 3. USP9X regulates cell proliferation and anchorage-independent growth independent of AMOT.

(A) The MDA-MB-231 cells stably expressing Ctrl shRNA or USP9X shRNA along with HA-AMOT or vector control were utilized to perform colony formation assays. Error bars represent the s.e.m. of three independent experiments. Statistical analyses were performed with the ANOVA. *, $p<0.05$; **, $p<0.01$.

Supplementary Figure 4. USP9X expression positively correlates with YAP1 expression in clinical breast cancer samples
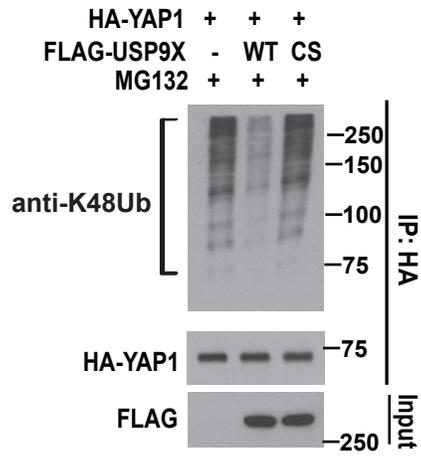
(A) Representative images of immunohistochemical staining of USP9X and MCL1 in breast carcinoma. Right panel: Correlation study of USP9X and MCL1 in breast carcinoma. Statistical analyses were performed with the χ2 test. R: The Pearson correlation coefficient. (B) Representative images of immunohistochemical staining of USP9X and AMOT in breast carcinoma. Right panel: Correlation study of USP9X and AMOT in breast carcinoma. Statistical analyses were performed with the χ2 test. R: The Pearson correlation coefficient.
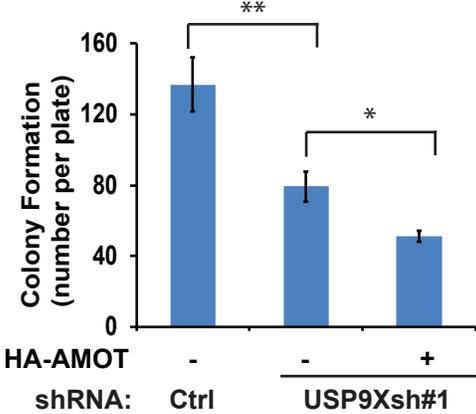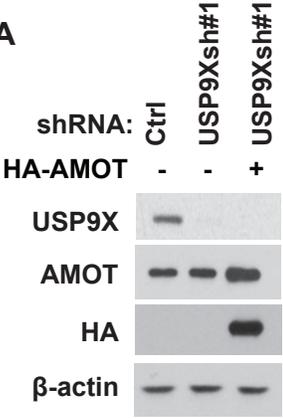
Supplementary Figure 1

A

FLAG-USP9X   -   WT   CS

YAP1

β-actin

FLAG

B

shRNA: Ctrl   USP9Xsh#1   USP9Xsh#2

USP9X

AMOTL2

β-actin

MDA-MB-231

C

shRNA: Ctrl   USP9Xsh#1   USP9Xsh#2

USP9X

YAP1

AMOT

AMOTL2

β-actin

OVCAR8

D

shRNA: Ctrl   USP9Xsh#1   USP9Xsh#2   USP9Xsh#3   USP9Xsh#4

USP9X

MCL1

  1   0.65   0.62   0.74   0.71

β-actin

E

     Ctrl   FLAG-USP9X WT   FLAG-USP9X CS

CHX   0   1   4   8   0   1   4   8   0   1   4   8 (h)

YAP1

FLAG

β-actin

- Ctrl Vector
- FLAG-USP9X WT
- FLAG-USP9X CS

Supplementary Figure 2

A

HA-YAP1   +   +   +
FLAG-USP9X   -   WT   CS
MG132   +   +   +

anti-K48Ub

—250
—150
—100
—75

IP: HA

HA-YAP1   —75

FLAG

Input

—250

Supplementary Figure 3

A

Supplementary Figure 4

A
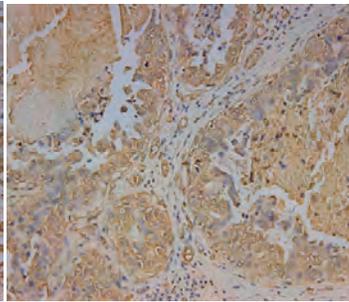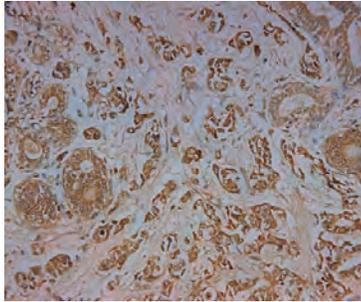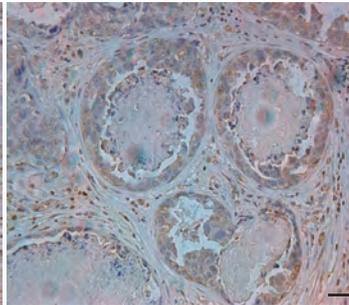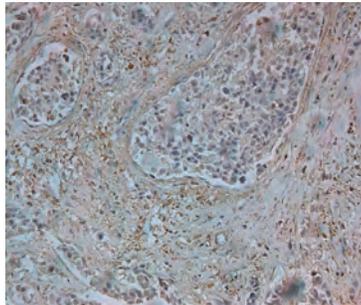
	USP9X Low	USP9X High	Total
MCL1 Low	12	43	55
MCL1 High	17	80	97

P＞0.05   R=0.052

B
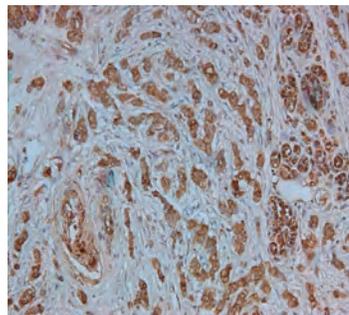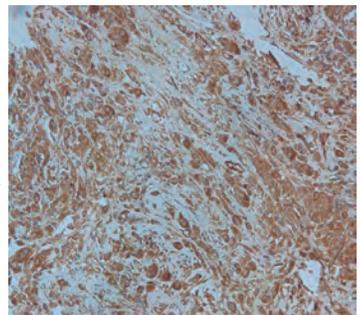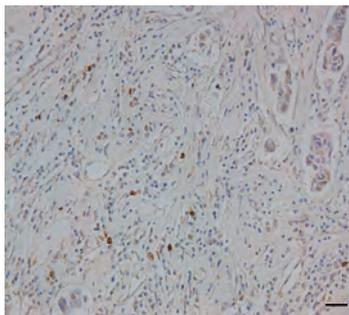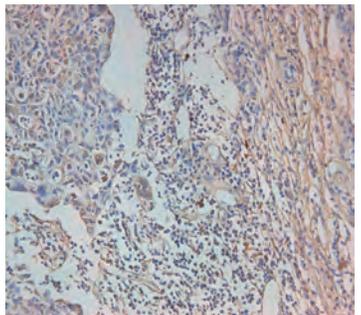
	USP9X Low	USP9X High	Total
AMOT Low	16	49	65
AMOT High	15	72	87

P＞0.05   R=0.091