# Supporting Information

## Deubiquitylase USP9X suppresses tumorigenesis by stabilizing large tumor suppressor kinase 2 (LATS2) in the Hippo pathway

Chu Zhu, Xinyan Ji, Haitao Zhang, Qi Zhou, Xiaolei Cao, Mei Tang, Yuan Si, Huan Yan, Li Li, Tingbo Liang, Xin-Hua Feng, Bin Zhao

Supplemental materials provided:

Supplemental Figures (6)

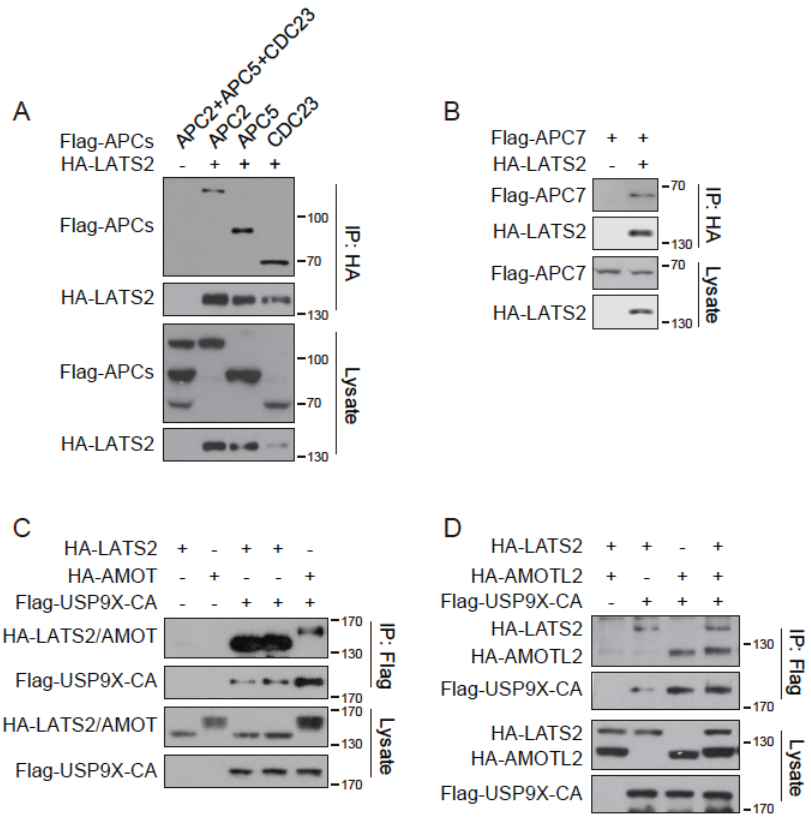


**Figure S1. Data related to Figure 1.**

A, APC2, APC5, and CDC23 of the APC/C complex also interact with LATS2. Indicated plasmids were transfected into HEK293T cells and immunoprecipitation was performed with anti-HA antibody. Samples were analyzed by western blotting.

B, APC7 interacts with LATS2. Experiments were similar to those in A.

C, The interaction between USP9X and AMOT is much weaker than that with LATS2. HEK293T cells were transfected with indicated plasmids and USP9X was immunoprecipitated with anti-Flag antibody. Samples were examined by western blotting.

D, AMOTL2 does not affect the interaction between USP9X and LATS2. Experiments were similar to those in C.

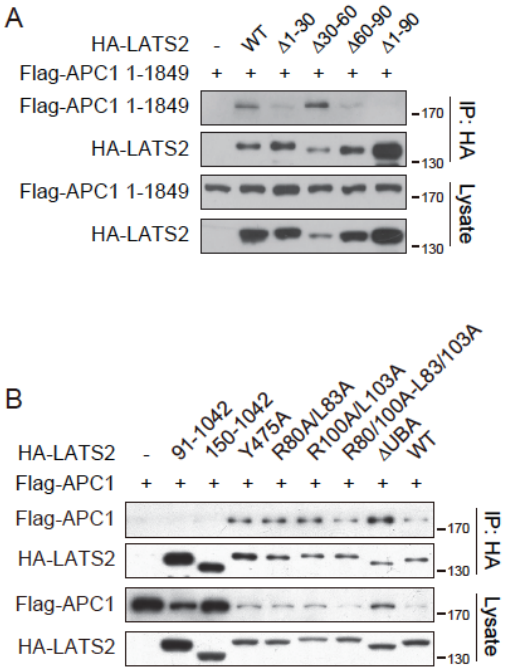


**Figure S2. Data related to Figure 2.**

A, The interaction of LATS2 deletion mutants with APC1. Experiments were similar to those in Fig. 2C.

B, D box and UBA domain of LATS2 are not required for its interaction with APC1. Experiments were similar to those in Fig. 2C. R80A/L83A is mutation of D box 1, and R100A/L103A is mutation of D box 2. Y475A is mutation of the PPXY motif of LATS2.

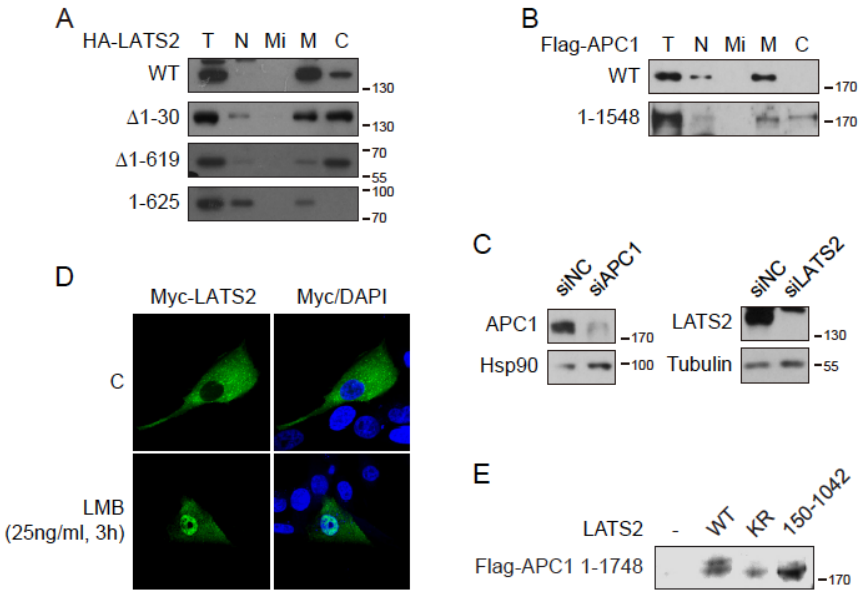


**Figure S3. Data related to Figure 3.**

A, Subcellular fractionation of LATS2 wild-type and mutants. HeLa cells transfected with indicated plasmids were fractionated by differential centrifugation speed. T, total; N, nucleus; Mi, mitochondria; M, membrane; C, cytoplasm.

B, Subcellular fractionation of APC1 wild-type and mutant. Experiments were similar to those in A.

C, Knockdown efficiency of siRNAs for APC1 and LATS2. APC1 and LATS2 were knocked down by a mix of two siRNA oligonucleotides in HeLa cells. Samples were examined by western blotting.

D, Blockade of nuclear export strongly promotes LATS2 nuclear localization. MCF10A cells transfected with Myc-LATS2 were treated with LMB before processed for immunofluorescence staining.

E, LATS2 induces an upshift of APC1 on Phos-tag gel. HEK293T cells were transfected with indicated plasmids and cell lysates were resolved on gels containing Phos-tag before western blotting.

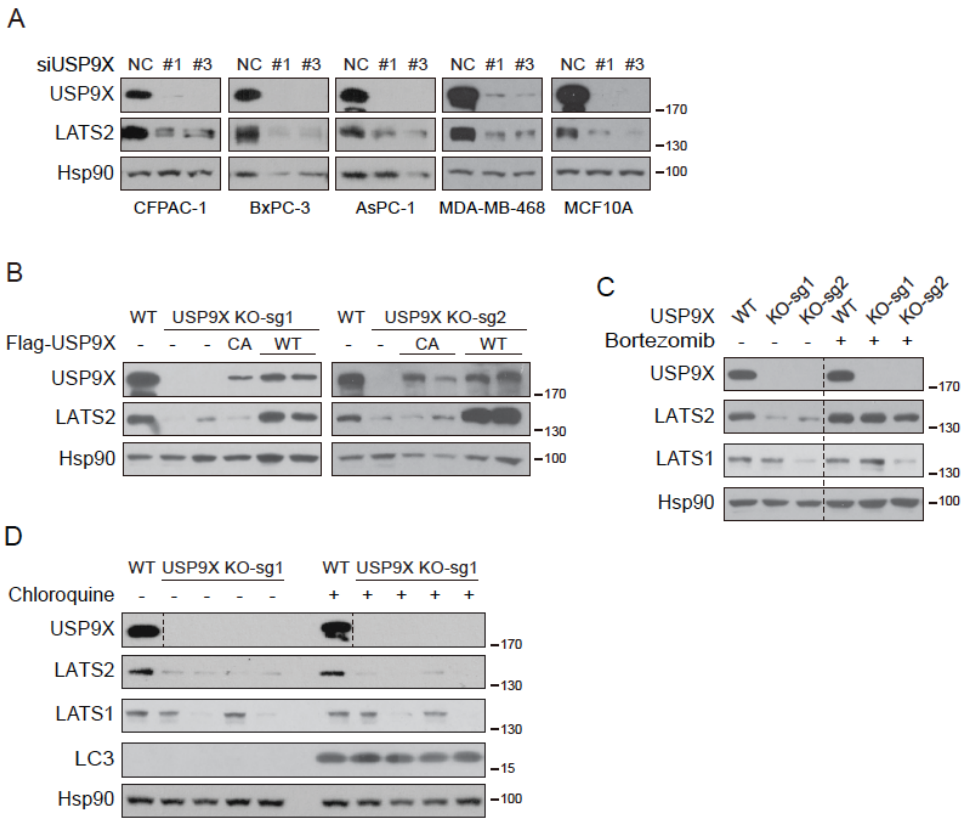


**Figure S4. Data related to Figure 4.**

A, Knockdown of USP9X diminishes LATS2 protein levels in various cell lines. Experiments were similar to those in Fig. 4A.

B, Enzymatically inactive USP9X fails to rescue LATS2 protein level in USP9X knockout cells. Experiments were similar to those in Fig. 4D except that HeLa cells were used.

C, Proteasome inhibitor rescues LATS2 protein level in USP9X knockout cells. Experiments were similar to those in Fig. 4G except that control and USP9X knockout HeLa cells were used.

D, Inhibition of autophagy could not rescue LATS2 protein level in USP9X knockout cells. Wide-type and four clones of USP9X knockout HeLa cells were treated with 50 μM Chloroquine for 24 hours before collection.

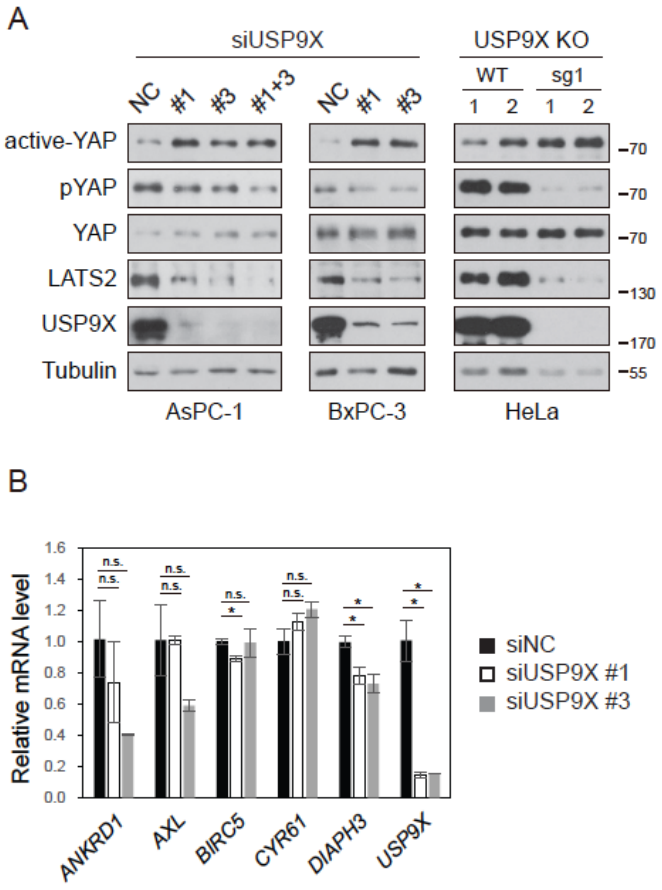


**Figure S5. Data related to Figure 5.**

A, Knockdown or knockout of USP9X increase dephosphorylated active YAP in various cancer cell lines. Lysates of siRNA-transfected cells or stable knockout cells were examined by western blotting.

B, Knockdown of USP9X does not affect the expression of YAP target genes in BT-474 cells. mRNA levels of indicated genes in siRNA-transfected BT-474 cells were determined by quantitative RT-PCR. Values represent means ± SD from three technical repeats. *P* value was calculated by Student *t* test. (n.s.) not significant; (*) $P < 0.05$.

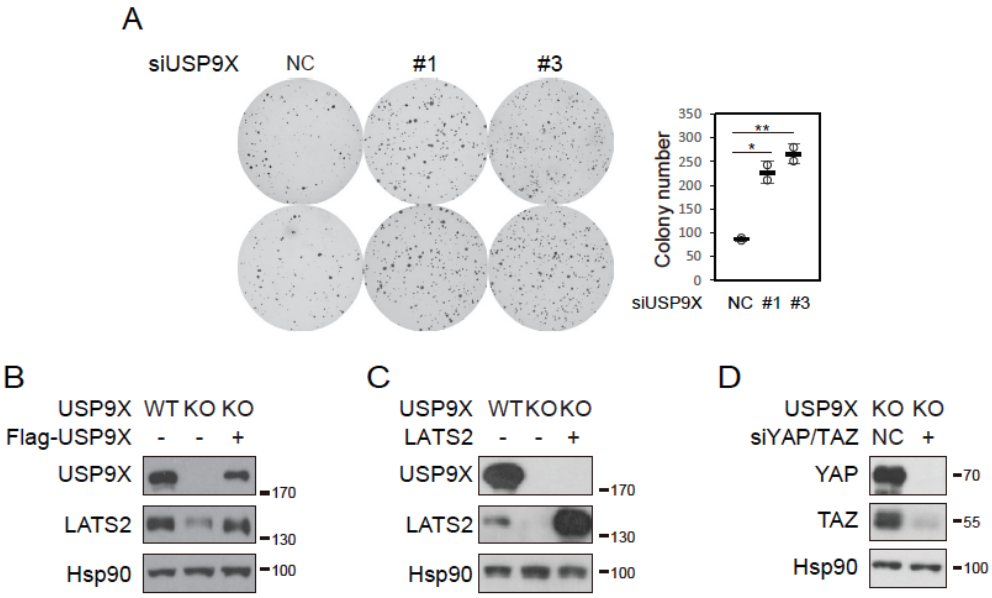


**Figure S6. Data related to Figure 6.**

*A*, USP9X knockdown promotes anchorage-independent growth. MIA PaCa-2 cells were transfected with siRNAs and were then subjected to soft agar colony formation assay. Colonies were stained by crystal violet and quantified (right panel). Values represent means ± SD. Individual data points were also shown. *P* value was calculated by Student *t* test. (*) $P < 0.05$; (**) $P < 0.01$.

*B*, Knockout and rescue of USP9X expression. Cells the same as those in Fig. 6B were examined by western blotting.

*C*, Rescue of LATS2 expression in USP9X knockout cells. Cells the same as those in Fig. 6C were examined by western blotting.

*D*, Knockdown efficiency of YAP and TAZ. Cells the same as those in Fig. 6D were examined by western blotting.