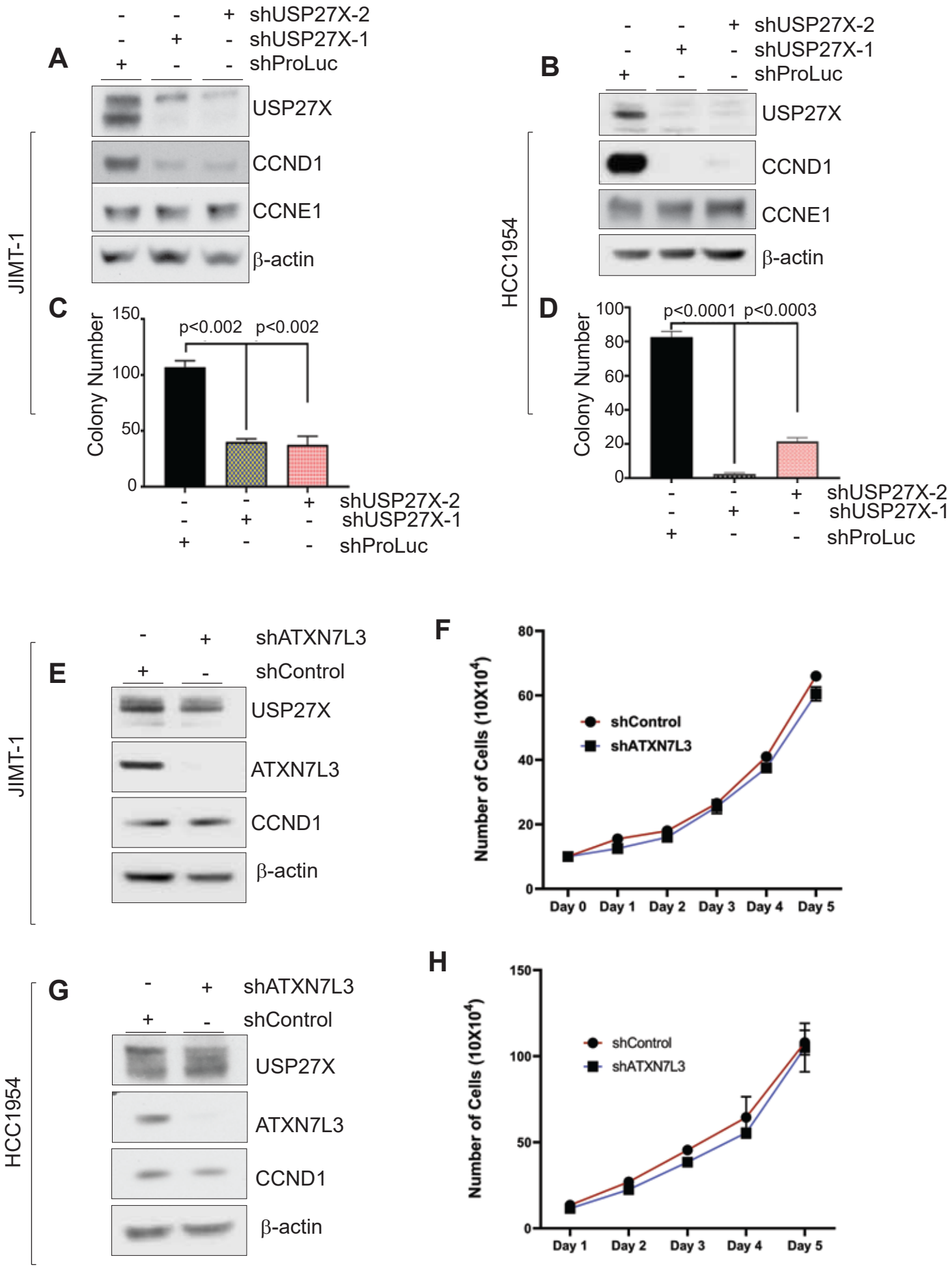
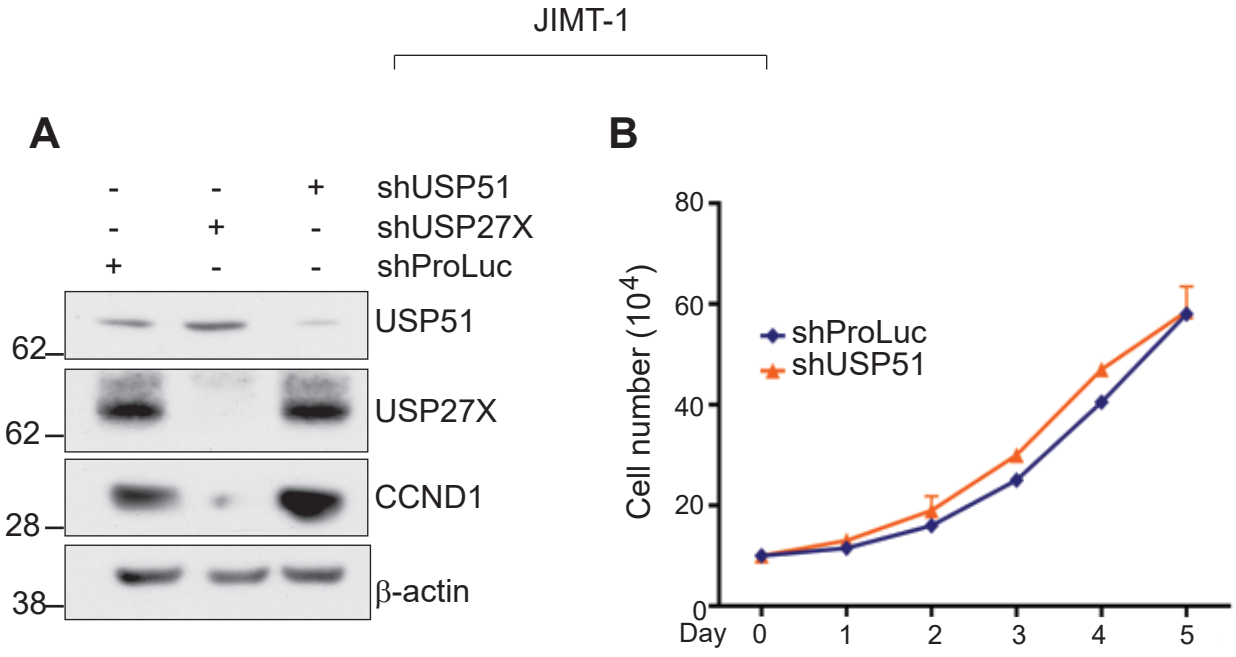
Alam et al., Supplementary Fig 1



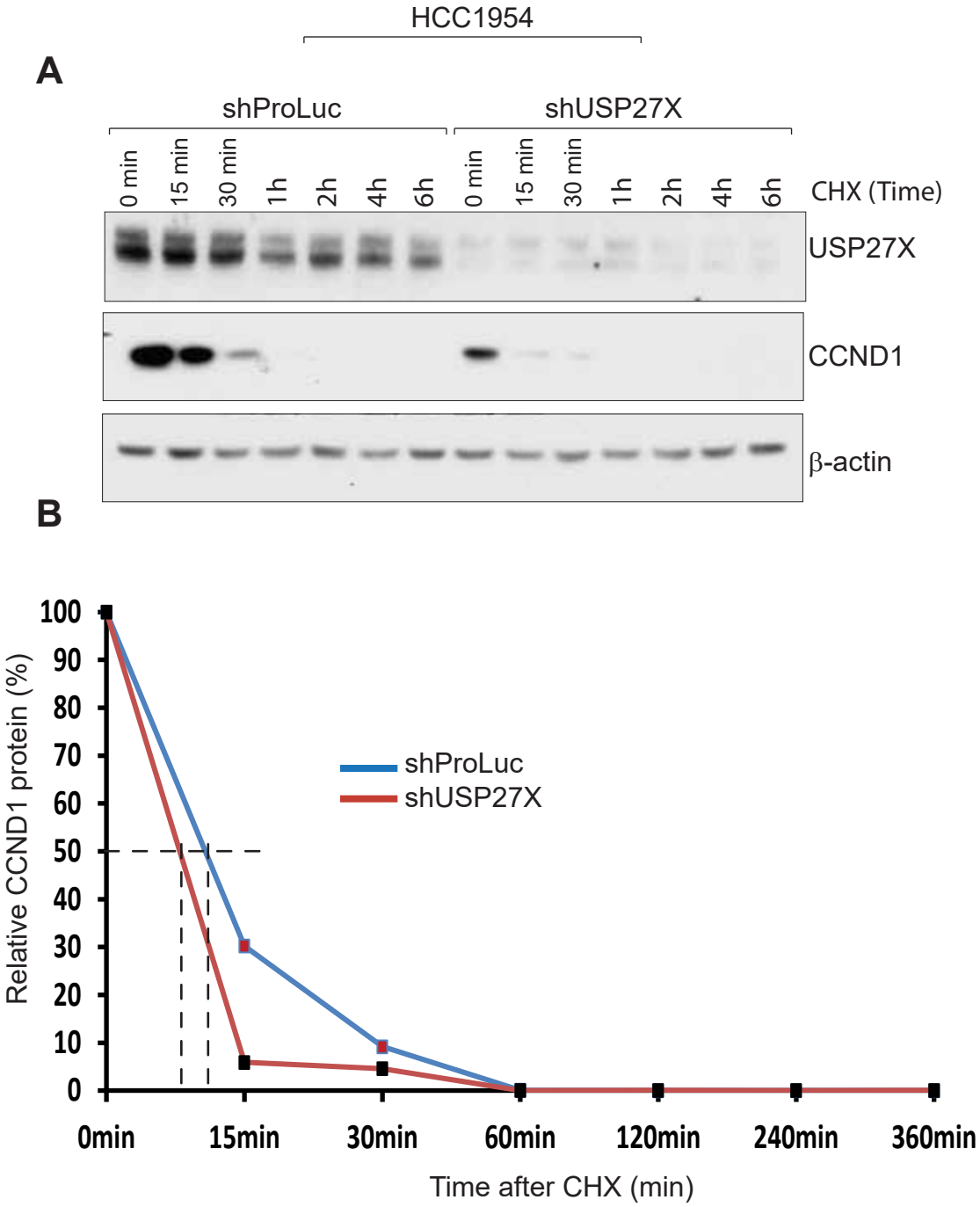
Supplementary Figure 1. Ablation of USP27X, but not ATXN7L3, impairs CCND1 protein levels and cell proliferation. (A and B) Immunoblots showing efficient silencing of USP27X and reduced levels of CCND1 in JIMT-1 and HCC1954 cells, respectively. USP27X depletion does not impact the steady levels of CCNE1 in HCC1954 cells. (C and D) Quantification of colony formation assays as performed in Figure 1. Ablation of USP27X significantly impacts the colony formation ability of JIMT-1 and HCC1954 cells, respectively. (E and G) Immunoblots showing efficient depletion of ATXN7L3 in JIMT-1 and HCC1954, respectively. (F and H) ATXN7L3 depletion has no effect on the proliferation rates of JIMT-1 and HCC1954 cells, respectively, compared to controls. Cells were counted over the course of 5 days.

Alam et al., Supplementary Fig 2



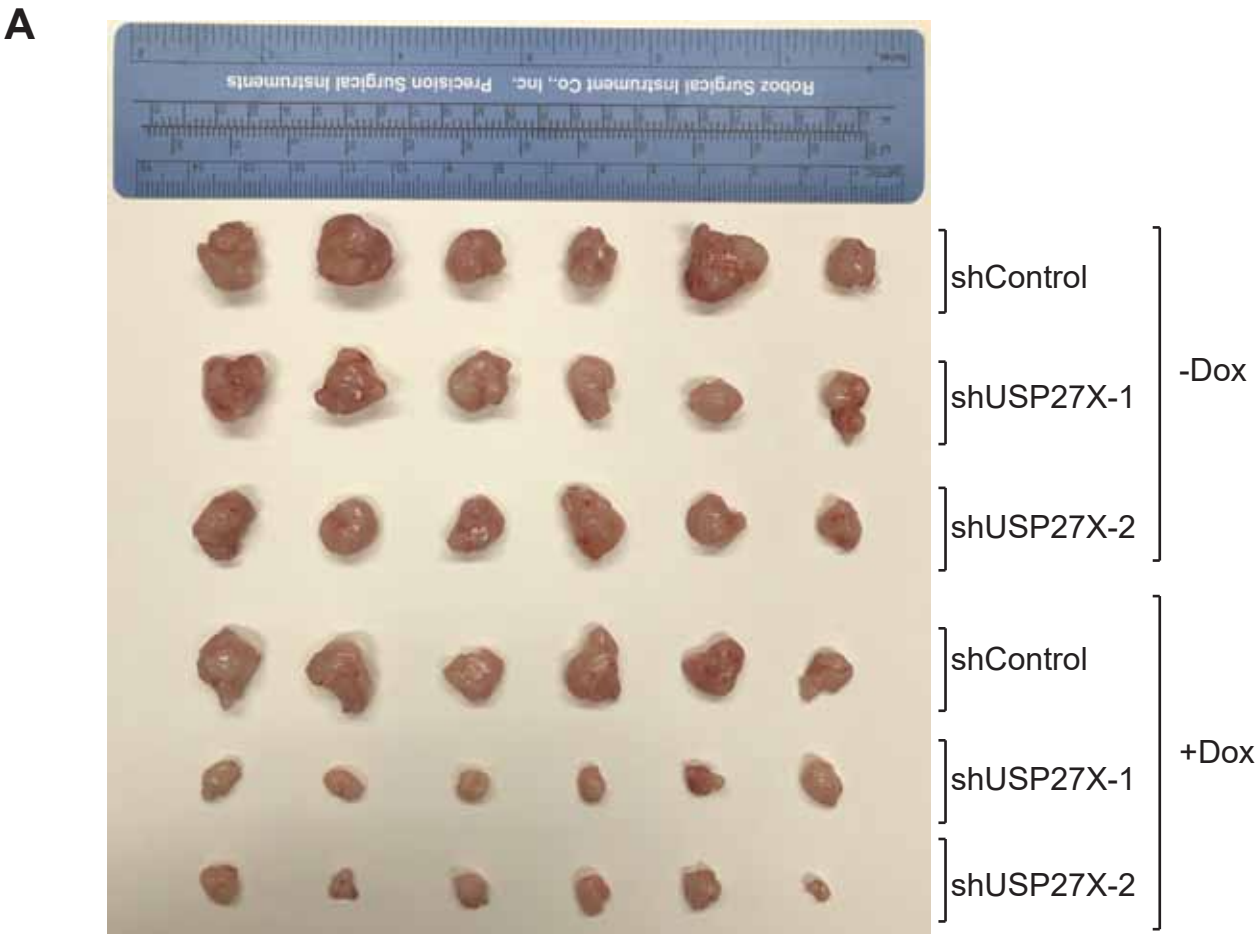
Supplementary Figure 2. USP51, a DUB highly similar to USP27X, does not regulate CCND1 levels. (A) USP51 depletion has no impact on CCND1 protein levels, despite its similarity to USP27X. (B) USP51 depletion has no effect on cell proliferation. Equal number of control or USP51-depleted JIMT-1 cells were seeded and counted over the course of 5 days.

Alam et al., Supplementary Fig 3



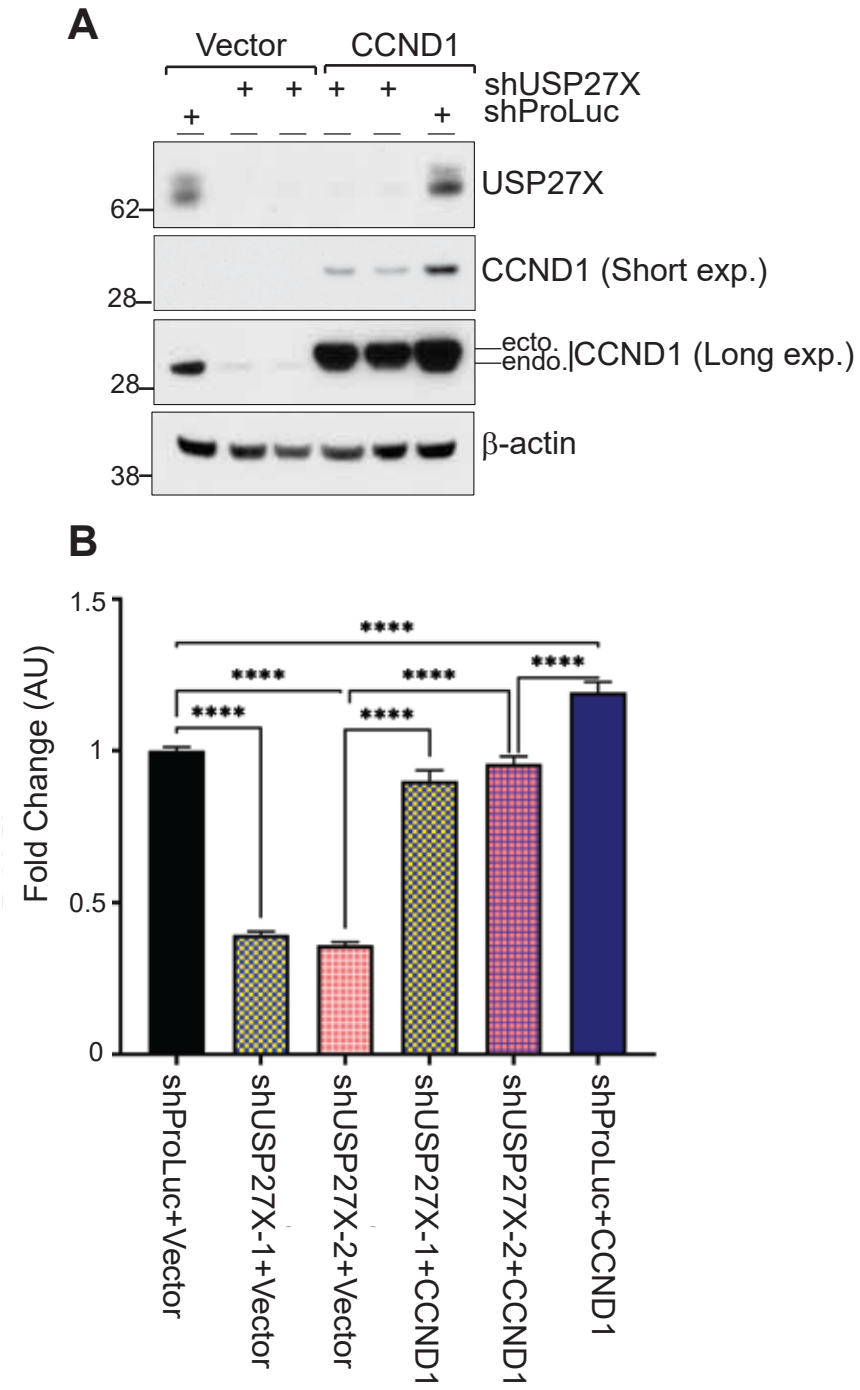
Supplementary Figure 3. USP27X ablation reduces the stability of CCND1 in HCC1954 cells (A) Immunoblots showing accelerated degradation of CCND1 in USP27X-depleted cells upon cycloheximide treatment. (B) Quantification of the data presented in A. X-ray films in A were scanned, and Image J software was used to measure the intensity of the CCND1 signal in each lane.

Alam et al., Supplementary Fig 4



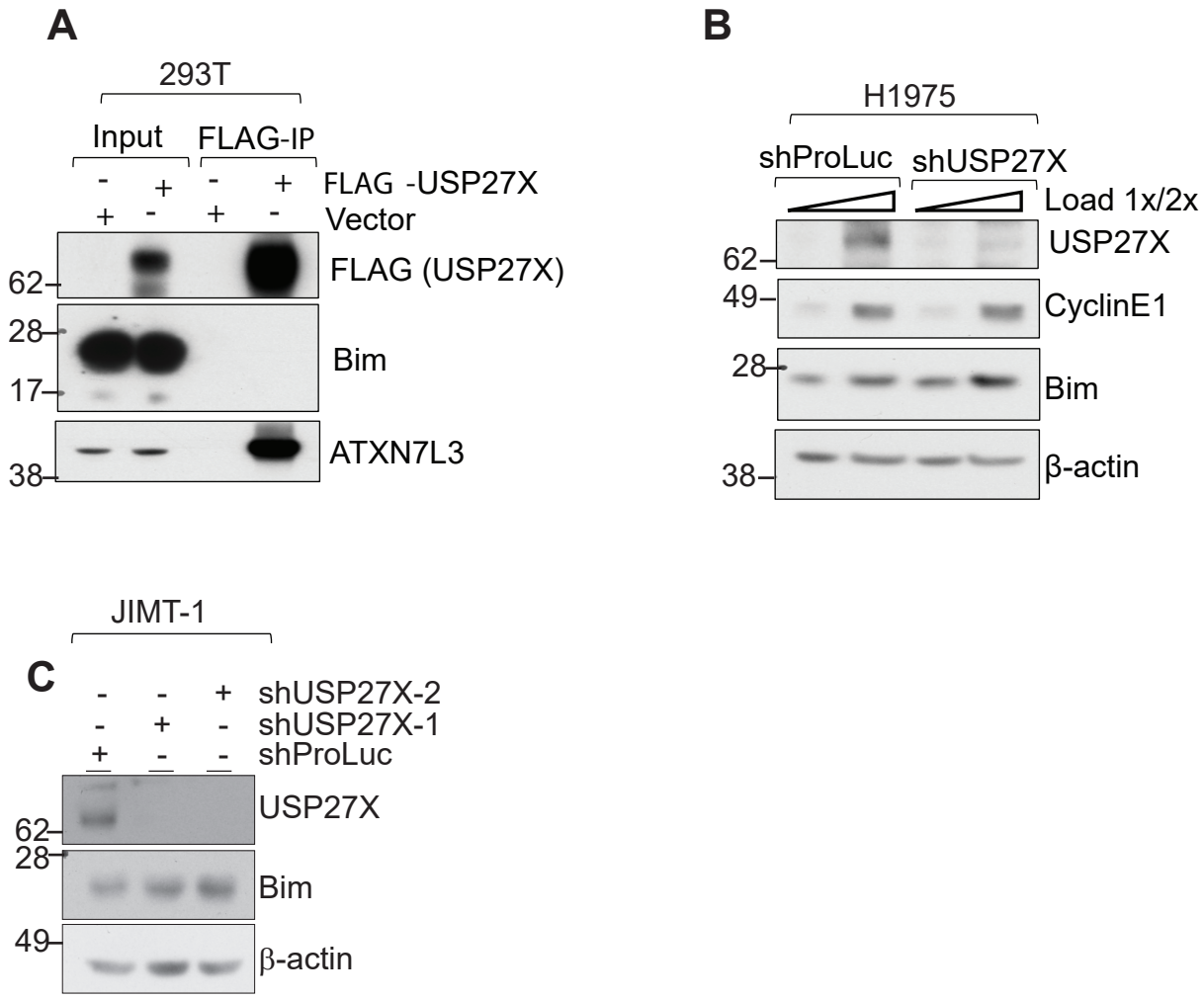
Supplementary Figure 4. Ablation of USP27X inhibits xenograft tumor growth. (A) Tumors harvested from JIMT-1 xenografts at Day 40. USP27X depletion was induced with doxycycline at Day 15, when tumors in all groups were ~200mm^3. (B) Quantification of the harvested tumor sizes presented in A. USP27X-depleted tumors weighed ~3 fold less than control tumors.

Alam et al., Supplementary Fig 5



Supplementary Figure 5. Expression of ectopic CCND1 rescues the growth defects in USP27X-depleted cells. (A) Immunoblots showing efficient depletion of USP27X and reduction of endogenous CCND1 as well as expression of ectopic CCND1 in JIMT-1 cells. USP27X was depleted in JIMT-1 cells stably expressing ectopic CCND1. (B) Cell counts for JIMT-1 cells expressing the indicated shRNAs and expression vectors. $1X10^5$ cells were seeded in 60mm culture dishes and grown for 5 days. The bar graph represents the fold change in cell numbers at day 5 of this assay. ****p<0.0001.

Alam et al., Supplementary Fig 6



Supplementary Figure 6. Depletion of USP27X has no impact on CCNE1 and Bim1 levels in H1975 and JIMT-1 cells. (A) Immunoblots demonstrating that ectopically expressed USP27X does not interact with endogenous Bim in 293T cells. ATXN7L3, an interacting partner of USP27X, is used as a positive control for this immunoprecipitation. (B) Immunoblots show no difference in the steady state levels of Cyclin E1 or Bim in USP27X-depleted H1975 cells. (C) USP27X ablation does not impact Bim steady-state levels in JIMT-1 cells.