Supplementary Figure legends

Figure S1. (A) A549 cells were infected with lentivirus encoding control shRNA or 40 DUB shRNAs individually. After 2 weeks, cell viability was determined by colony formation assay. Error bars represent the SEM of three independent experiments. (B) A549 cells stably infected with the indicated shRNA were treated with different dosages of IR. After 2 weeks, cell viability was determined by colony formation assay. Error bars represents the SEM of three independent experiments. (C) Lists of USP20 associated protein identified by mass spectrometric analysis. (D) mRNAs extracted from cells stably expressing control shRNA or USP20 shRNAs were subjected to QRT-PCR. Error bar represents the SEM of three independent experiments.

Figure S2. (A-B) mRNAs were extracted from cells treated as indicated and subjected to QRT-PCR. Error bar represents the SEM of three independent experiments. (C) A549 cells stably expressing control shRNA or USP20 shRNAs were transfected with the indicated constructs. After 48 hours, cells were left untreated or treated with UV (30 J/m2). One hour later, cells were lysed and cell lysates were then blotted with indicated antibodies.

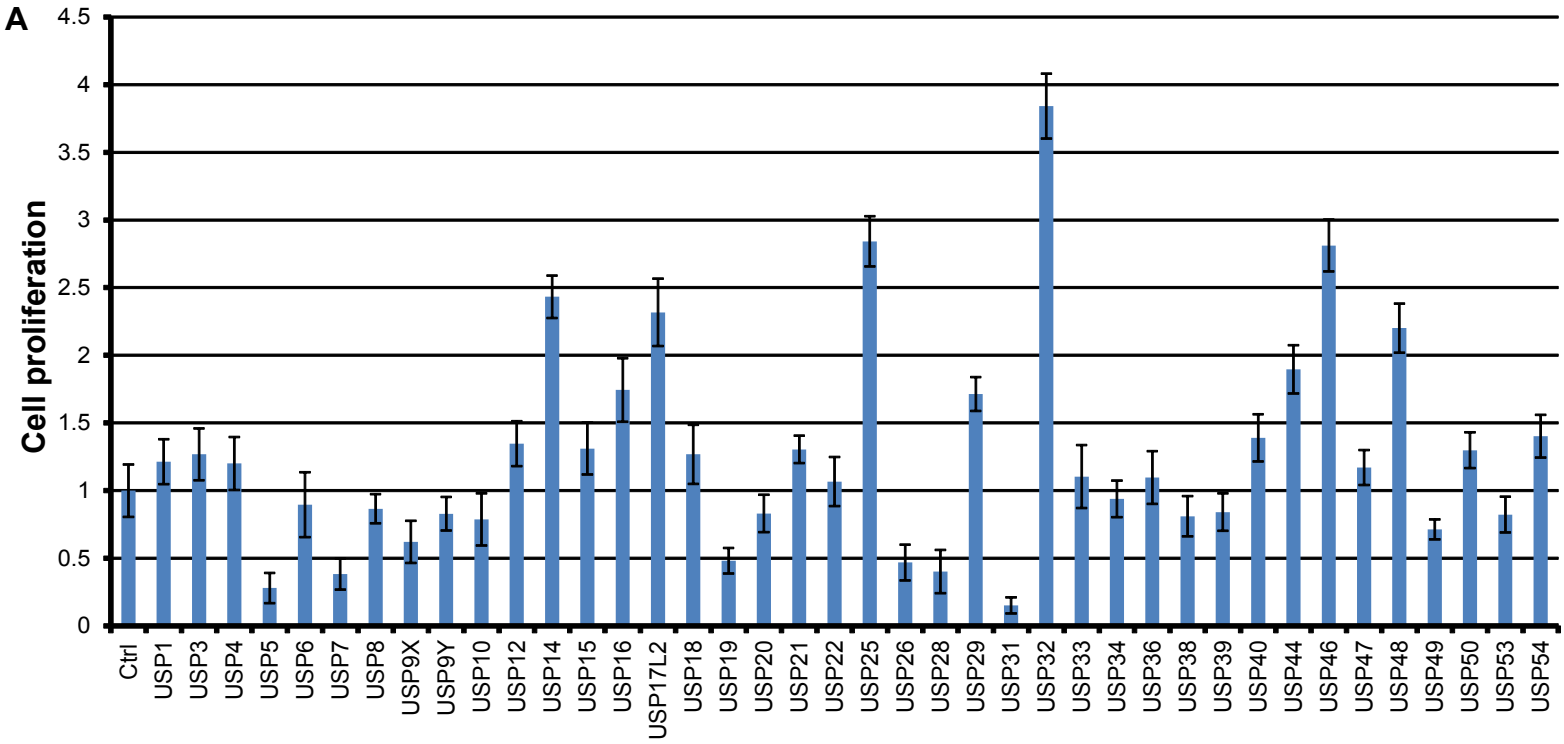
Figure S3. (A) FLAG-USP20 full length and different fragments were transfected into HEK293T cells. 48hrs later, cells were lysed and immunoprecipitated with anti-FLAG antibody. The immunoprecipitates were then blotted with the indicated antibodies. (B) Cells stably expressing Ctrl or HERC2 shRNA were lysed and immunoprecipitated with IgG or anti-USP20 antibody. The immunoprecipitates were then blotted with indicated antibodies.

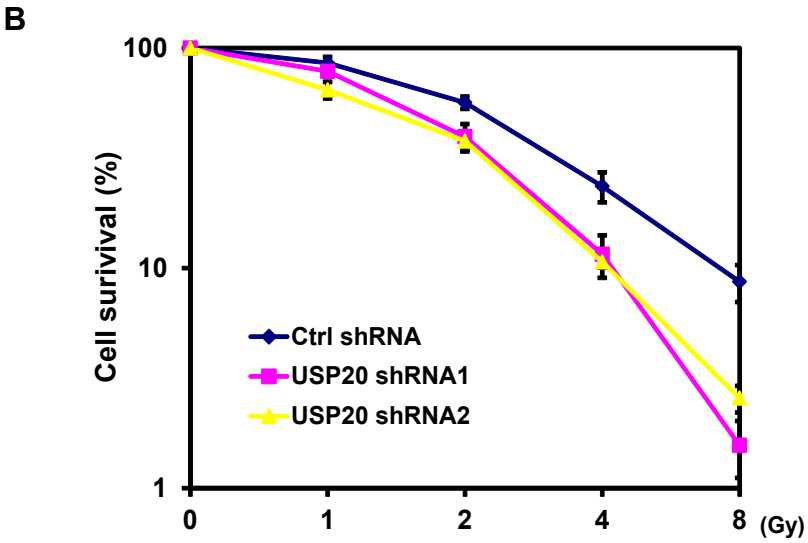
Figure S4. (A) Cells were left untreated or treated with UV. After 1hr, cells were lysed and cell lysates were incubated with GST or GST-USP20 coupled to GSH-Sepharose. Proteins retained on Sepharose were then blotted with the indicated antibodies. (B) Cells treated with UV were harvested at indicated time points, lysed and cell lysates were immunoprecipitated with anti-USP20 antibody. The immunoprecipitates were then blotted with indicated antibodies. (C) Cells stably expressing control shRNA or ATR shRNA were left untreated or treated with UV. After 1hr, cells were lysed and cell lysates were blotted with the indicated antibodies. (D) Quantification of the HA-USP20 protein levels relative to β-actin in Fig. 5I-J. (E) Cells stably expressing USP20 shRNA were transfected with shRNA resistant HA-USP20 WT or USP20-4A.

After 48hrs, cells were treated with UV and harvested at indicated time points, lysed and cell lysates were then blotted with the indicated antibodies. (F) Cells expressing the indicated constructs were treated with MG132 for 4 hrs. Cells were then lysed and immunoprecipitated with anti-Claspin antibody. The immunoprecipitates were then blotted with the indicated antibodies.

Figure S5. (A) Cells used in Figure 6A and 6C were lysed and cell lysates were blotted with the indicated antibodies.

Supplementary Figure 1

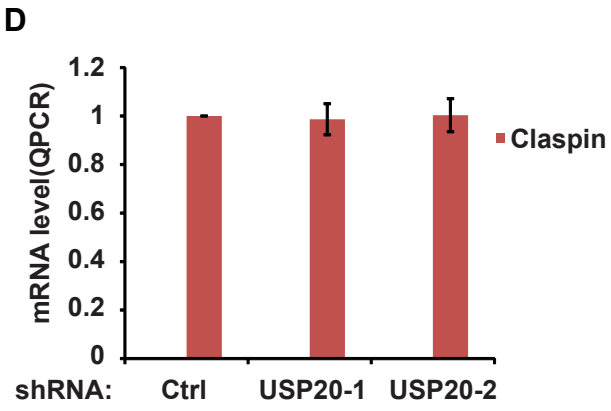




C

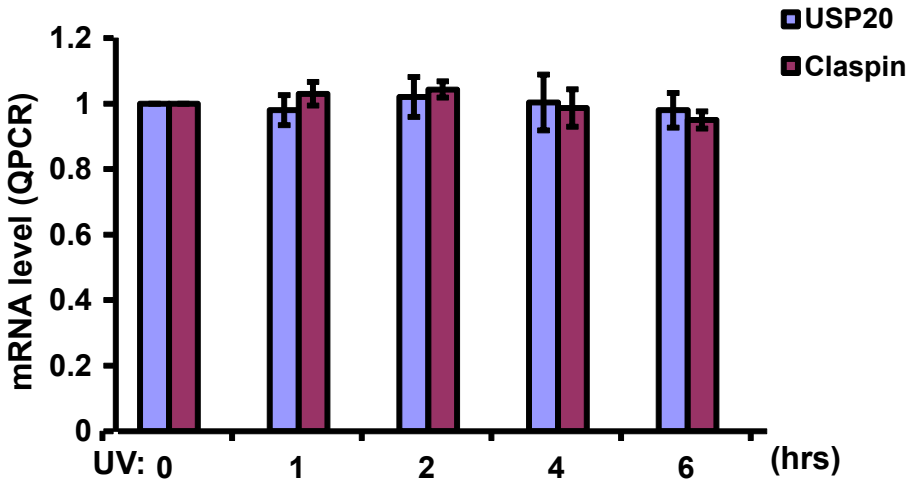
USP20 Mass specturm data

Protein	Number of Peptides
USP20	32
Claspin	14
SAMHD1	10
SSBP1	9
HNRPUL1	8
HERC2	8
ZNF770	4
EIF3S2	3
ZFP106	3
CCDC82	2



Supplementary Figure 2

A



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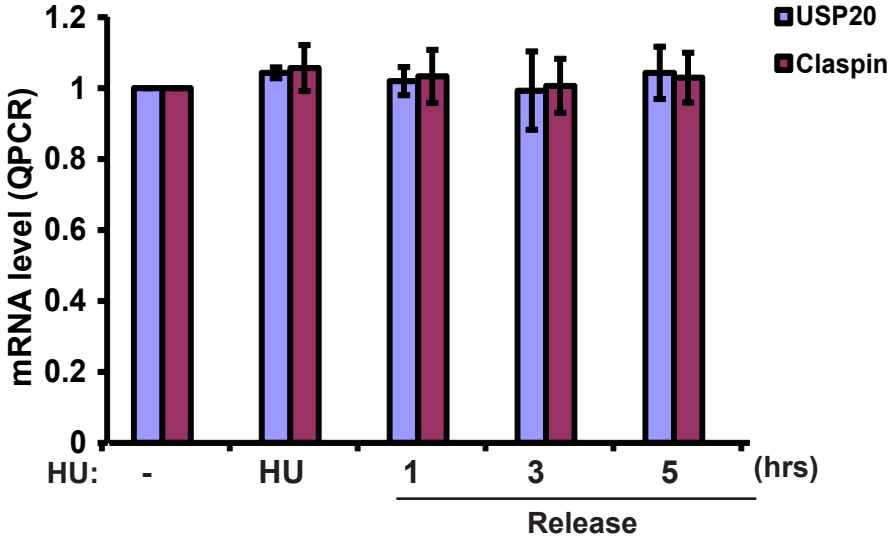

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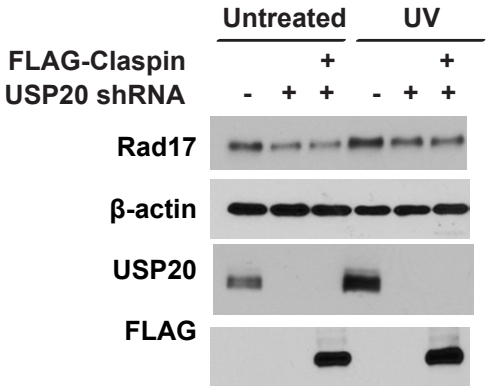
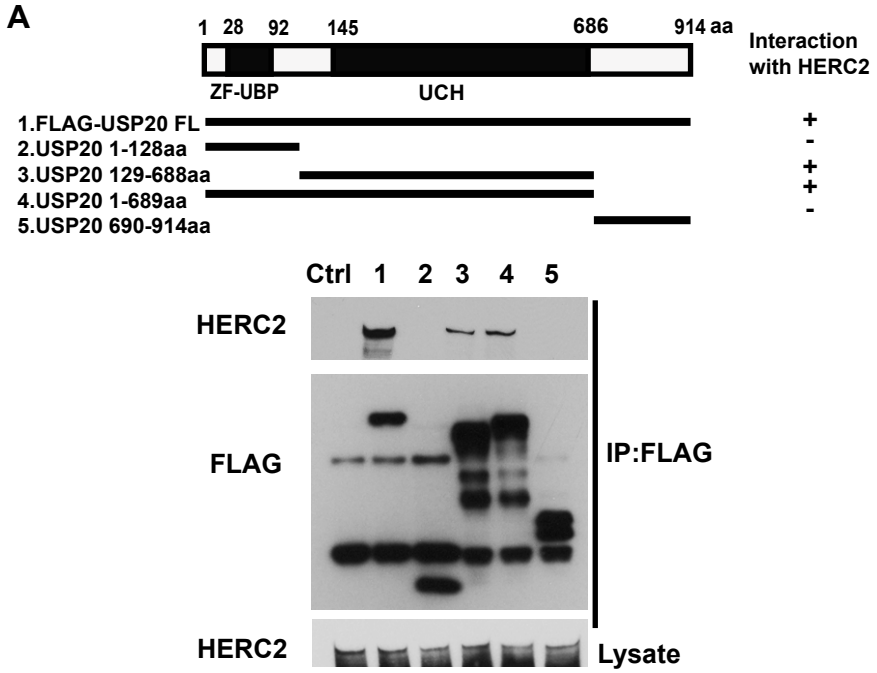
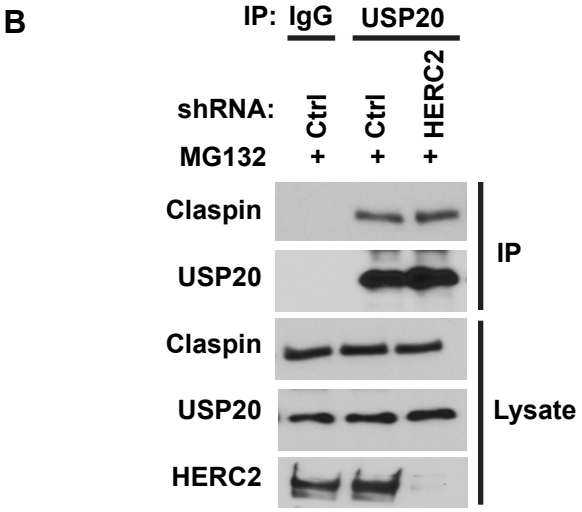
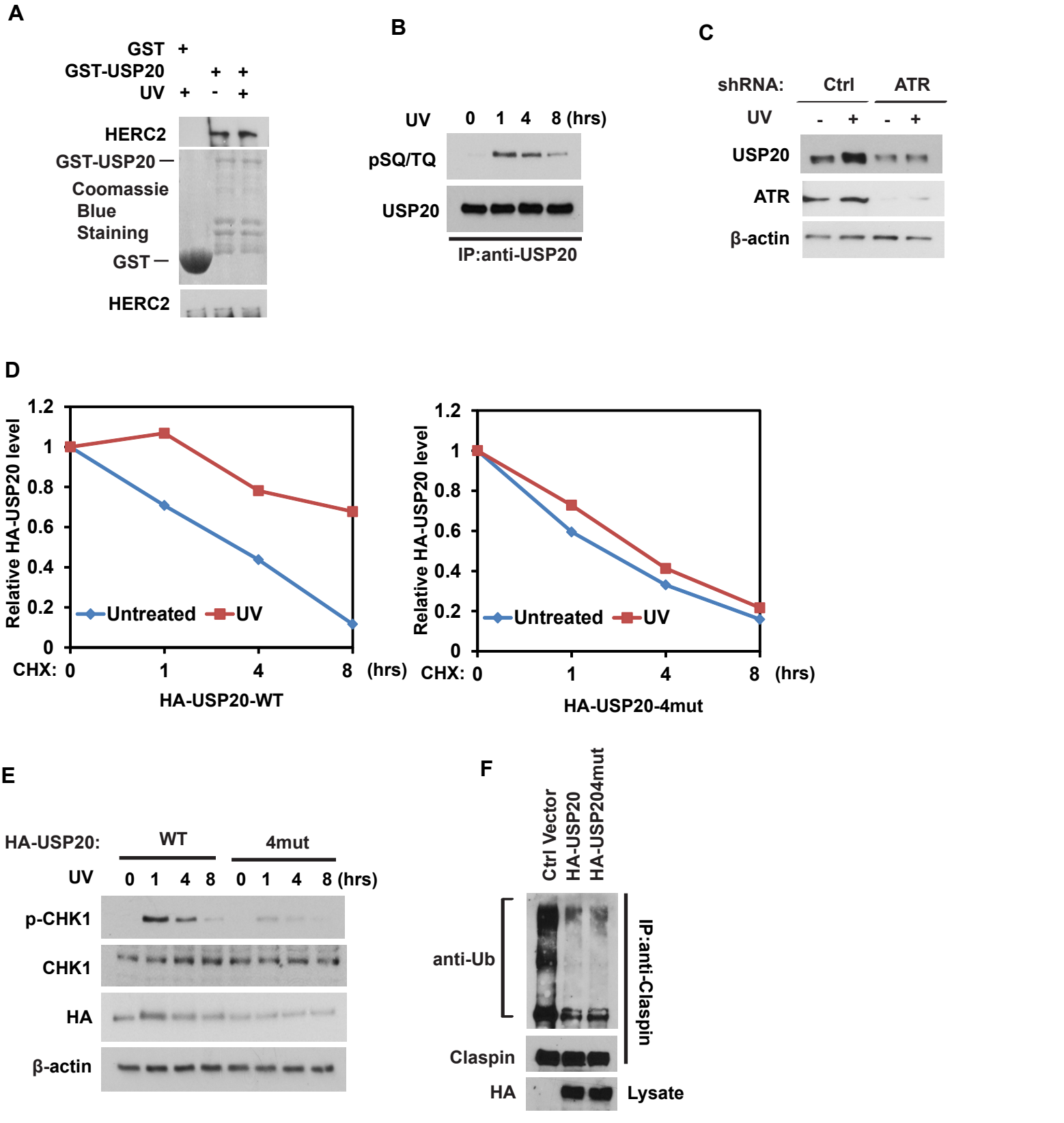


Supplementary Figure 3





Supplementary Figure 4



Supplementary Figure 5