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Supplemental Data

Deubiquitination of FANCD2 Is Required for DNA Crosslink Repair

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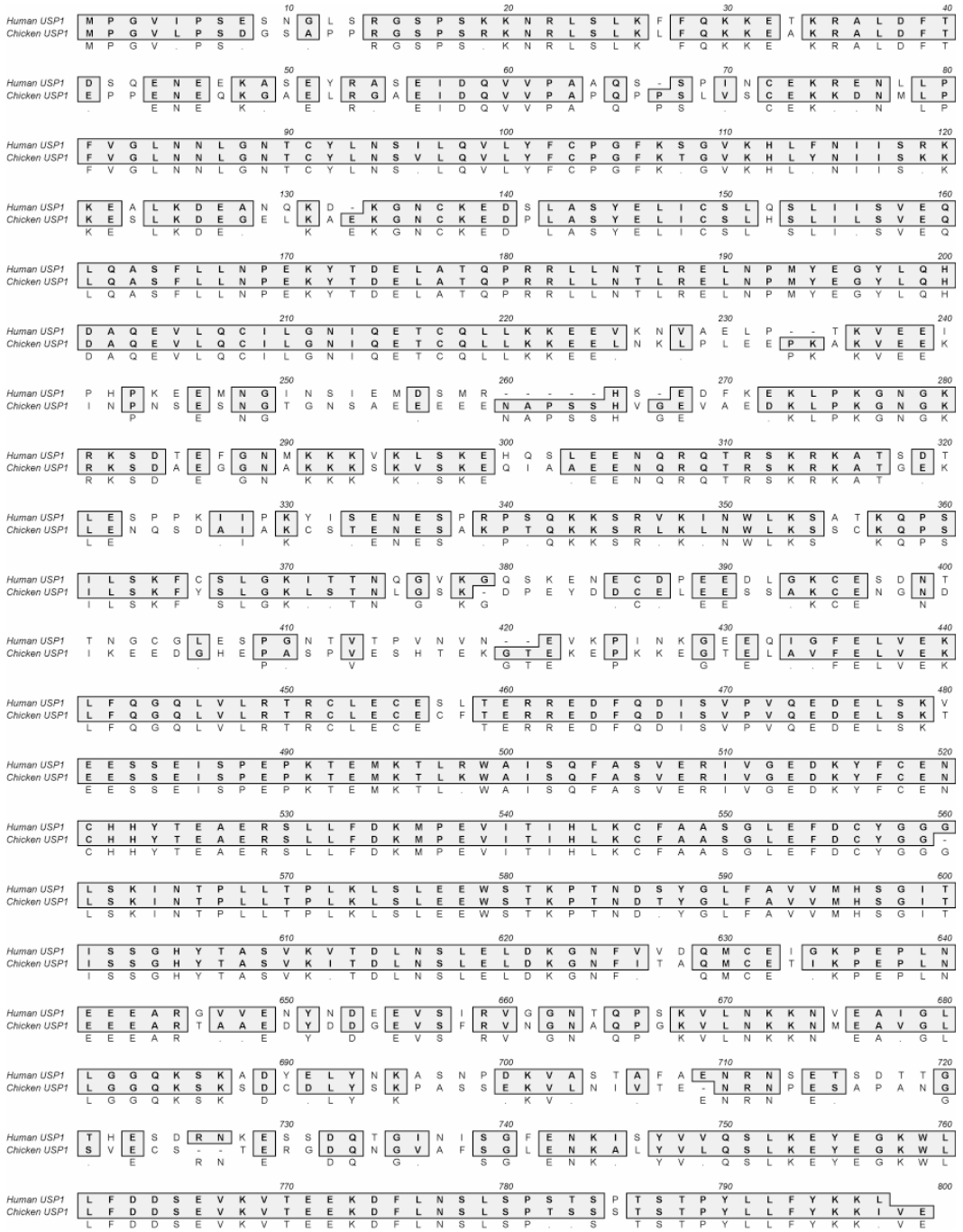

Figure S1. Alignment of the Human and Chicken USP1 Protein Sequences
The sequences share 73% identity.

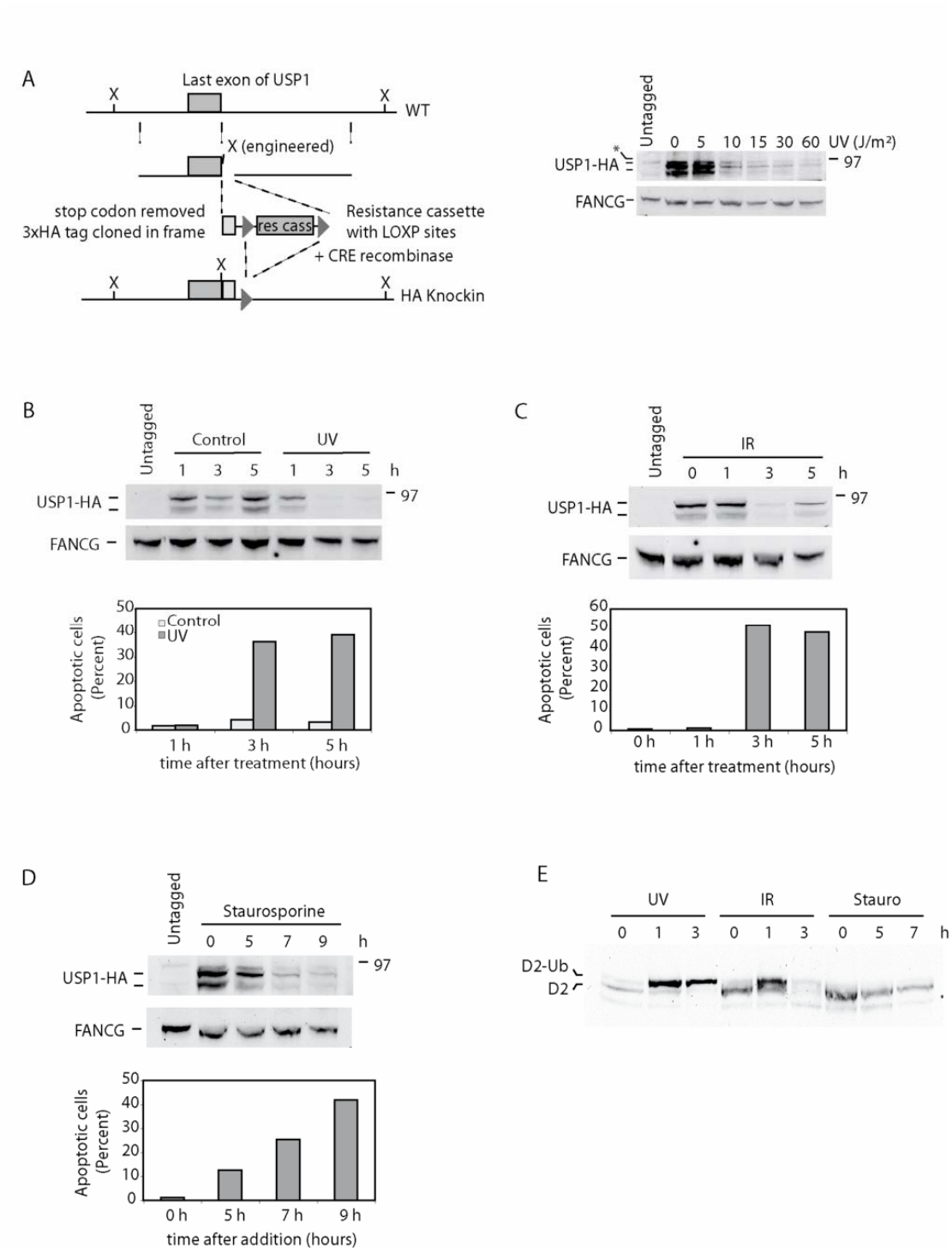

Figure S2. USP1 Degradation Monitored in USP1 *In Situ* HA-Tagged Cells
(A) Left - map of the *USP1* locus and strategy for the generation of the *in situ* HA-tag into the last exon of *USP1*. After recycling the drug resistant cassette the locus expresses USP1-3xHA tag. Right - Western blot to detect USP1-HA after exposing cells to different doses of UV light. The asterisk denotes a cross reacting band. For unknown reasons the HA antibody recognises two specific bands for the USP1-HA tagged cell line. The lower band, however, is not a USP1 self cleavage product. The C-terminal HA-tagged self cleavage product would be around 15 kDa. Western blot against FANCG was used as a loading control.

(B) Top –HA and FANCG Western blots from cells exposed to UV light (30 J/m^2) recovered for 1, 3 and 5 hours. Below – column chart showing the percentage of apoptotic cells detected by uptake of Annexin V and FACS analysis at the same time points.
(C) Top - HA and FANCG Western blots from cells exposed to X-rays (20 GY). Below – graph plotting the percentage of apoptotic cells detected by uptake of Annexin V and FACS analysis at the same time points.
(D) Top – HA and FANCG Western blots from cells exposed to single dose of the apoptosis-inducing drug staurosporine. Samples removed at 5, 7 and 9 hours after exposure to drug. Graph showing percentage of apoptotic cells detected by uptake of Annexin V and FACS analysis at the same time points.
(E) FANCD2 Western blot performed on cells treated with UV, X-rays or staurosporine.

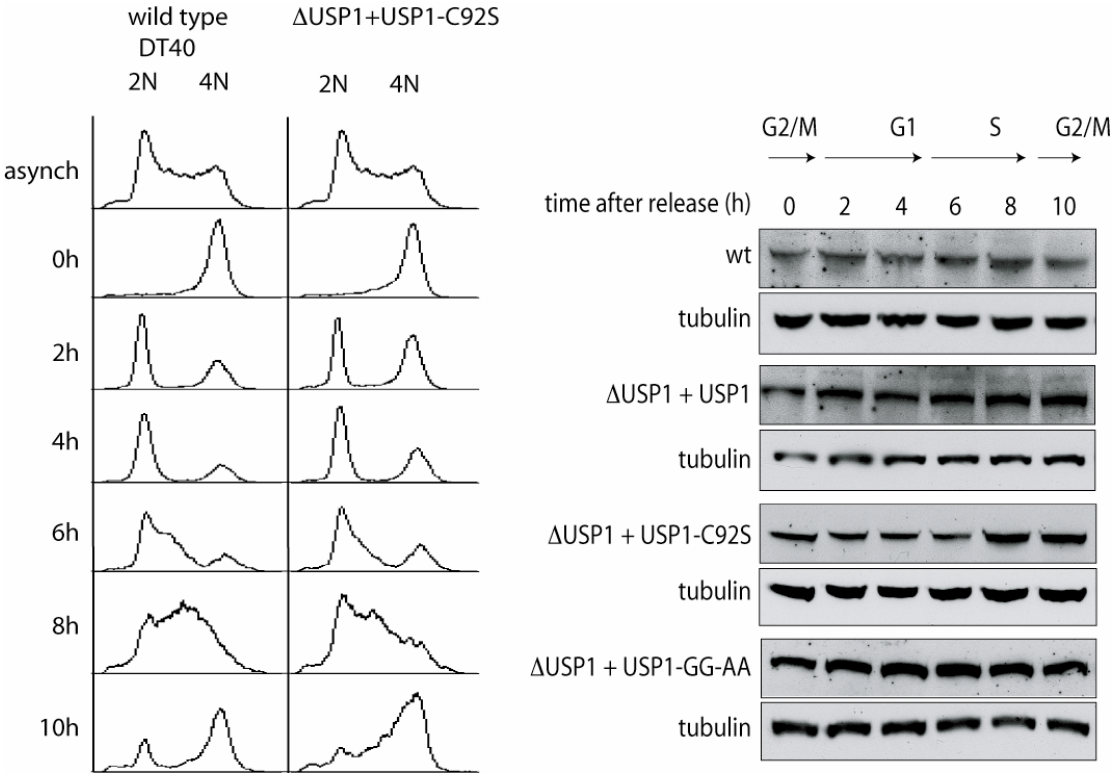

Figure S3. Following the Levels of USP1 Wild Type and Mutants throughout the Cell Cycle
Wild type DT40 and Δ*USP1* stably transfected with mutant or wild type USP1 cDNA were synchronised (in G2/M). Two hourly samples were analysed by FACS and immunoblotted with anti-USP1 and anti-tubulin (as a loading control) antibodies. USP1 and USP1-GG-AA transfected cells display identical synchrony to wild type cells as above (data not shown).