# Supplementary Materials: USP19-Mediated Deubiquitination Facilitates the Stabilization of HRD1 Ubiquitin Ligase

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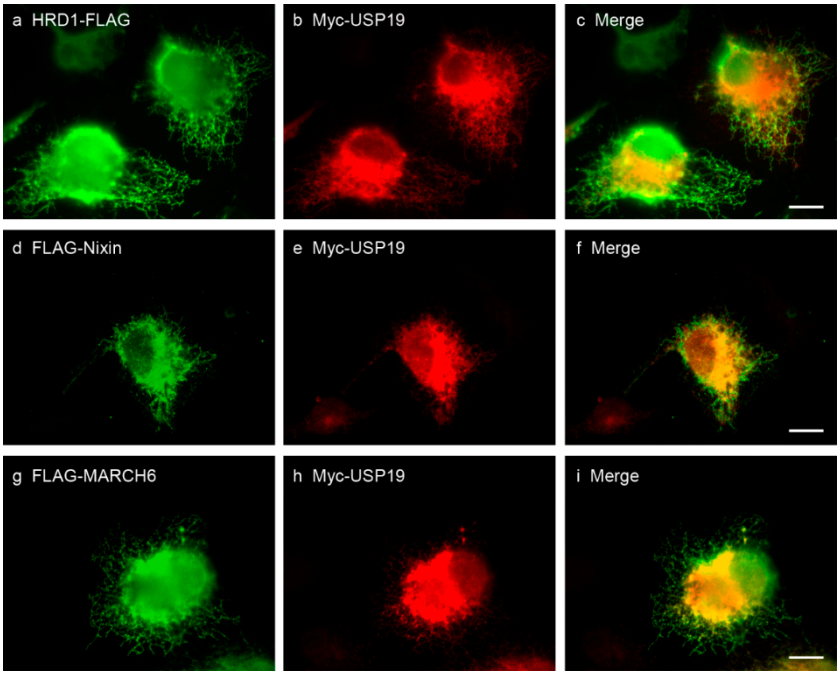


**Figure S1.** Co-localization of USP19 with ER-localized ubiquitin ligases. COS7 cells were transfected with Myc-USP19 along with either HRD1-FLAG (**a**–**c**), FLAG-Nixin (**d**–**f**) or FLAG-MARCH6 (**g**–**i**). The cells were stained with antibodies against FLAG (green) and USP19 (red). The signals were observed under fluorescence microscopy. Bars, 10 μm.

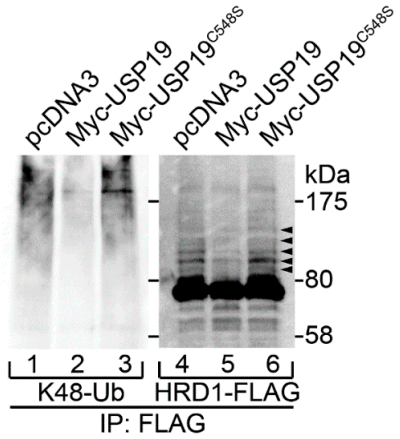


**Figure S2.** The brightness and contrast of the FLAG blot in Figure 2A were adjusted to make the bands of ubiquitinated HRD1-FLAG (arrowheads) more visible.

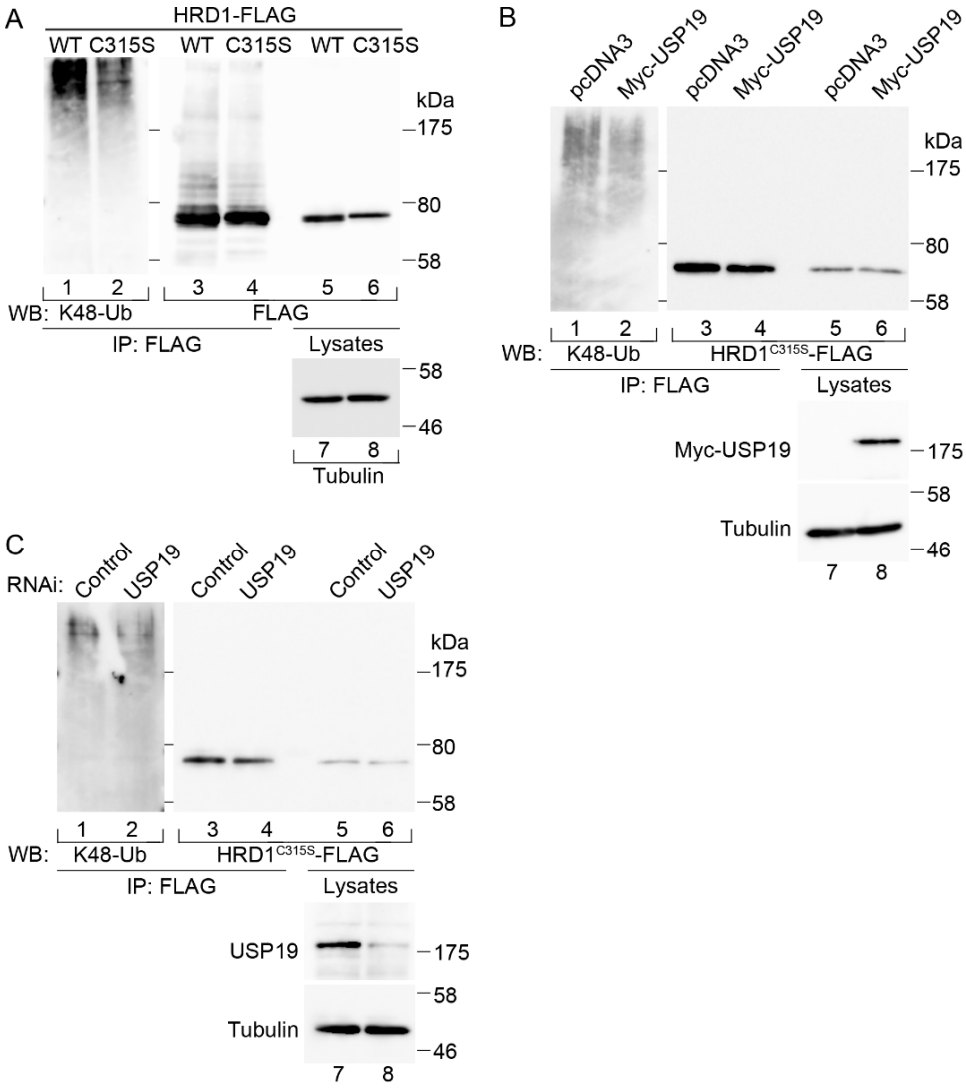


**Figure S3.** Both overexpression and knockdown of USP19 do not affect K48-linked ubiquitination of HRD1$^{C315S}$-FLAG. (**A**) Confirmation of autoubiquitination of HRD1. HRD1-FLAG (WT; lanes 1, 3, 5 and 7) and its C315S mutant (C315S; lanes 2, 4, 6 and 8) were transfected into 293T cells After epoxomicin treatment for 6 h, the cells were lysed and then subjected to immunoprecipitation (IP) with an anti-FLAG antibody under denaturing conditions. The immunoprecipitates (50% of the eluates) were analyzed by Western blotting (WB) with antibodies against K48-linked ubiquitin (lanes 1 and 2) and FLAG (lanes 3 and 4). The lysates (20 μg of protein; lanes 5–8) were analyzed by Western blotting with antibodies against FLAG (lanes 5 and 6) and $\alpha$-tubulin (lanes 7 and 8); (**B**) HRD1$^{C315S}$-FLAG was transfected into 293T cells along with either pcDNA3 (lanes 1, 3, 5 and 7) or Myc-USP19 (lanes 2, 4, 6 and 8). After epoxomicin treatment, the cells were lysed and then subjected to immunoprecipitation (IP) with an anti-FLAG antibody under denaturing conditions. The immunoprecipitates were analyzed by Western blotting (WB) with antibodies against K48-linked ubiquitin (lanes 1 and 2) and FLAG (lanes 3 and 4). The lysates (20 μg of protein; lanes 5–8) were analyzed by Western blotting with antibodies against FLAG (**top** panel), Myc (**middle** panel) and $\alpha$-tubulin (**bottom** panel); (**C**) 293T cells were transfected with control siRNA (lanes 1, 3 and 5) or USP19-specific siRNA duplexes (lanes 2, 4 and 6). Two days after transfection, the cells were further transfected with HRD1$^{C315S}$-FLAG. After epoxomicin treatment, the cells were lysed and then subjected to immunoprecipitation (IP) with an anti-FLAG antibody under denaturing conditions. The immunoprecipitates were analyzed by Western blotting (WB) with antibodies against K48-linked ubiquitin (lanes 1 and 2) and FLAG (lanes 3 and 4). The lysates (20 μg of protein; lanes 5–8) were analyzed by Western blotting with antibodies against FLAG (**top** panel), USP19 (**middle** panel) and $\alpha$-tubulin (**bottom** panel).

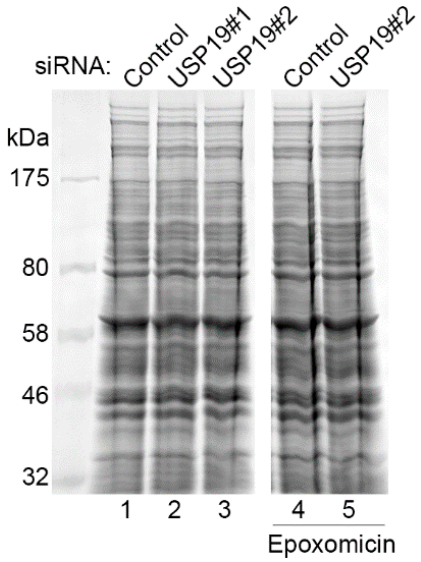


**Figure S4.** No effect or a lesser effect of USP19 knockdown and proteasomal inhibition on cell viability. The 293T cells were transfected with control siRNA (lanes 1 and 4) or USP19-specific siRNA duplexes (lanes 2, 3 and 5). The cells (lanes 4 and 5) were further transfected with HRD1-FLAG and then treated with epoxomicin for 6 h. Equal volume of cell lysates were separated by SDS–PAGE followed by Coomassie Brilliant Blue staining.

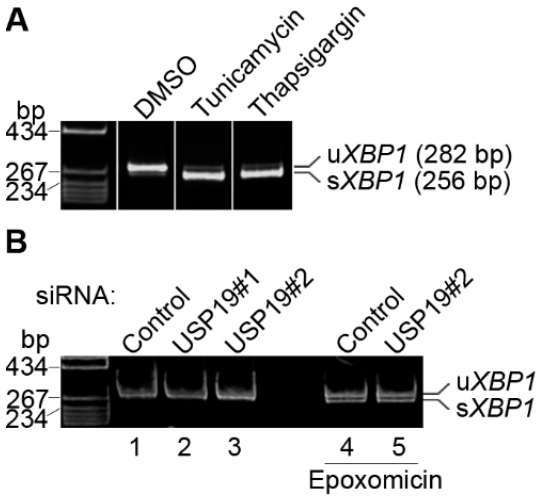


**Figure S5.** The status of ER stress in USP19-knockeddown cells. (**A**) The 293T cells were treated with vehicle (DMSO), 2 μg/mL tunicamycin or 1 μM thapsigargin for 4 h at 37 °C. RT-PCR was then performed to detect unspliced *XBP1* (u*XBP1*) and spliced *XBP1* (s*XBP1*) transcripts; (**B**) The 293T cells were transfected with control siRNA (lanes 1 and 4) or USP19-specific siRNA duplexes (lanes 2, 3 and 5). The cells (lanes 4 and 5) were further transfected with HRD1-FLAG and then treated with epoxomicin for 6 h. XBP1 transcripts were detected by RT-PCR.

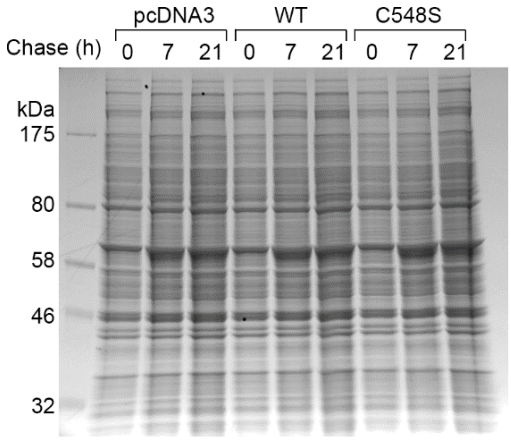


**Figure S6.** Equal volume of the cell lysates described in Figure 4A were separated by SDS-PAGE followed by Coomassie Brilliant Blue staining.

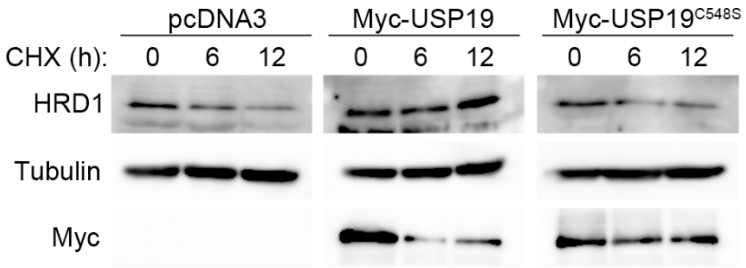


**Figure S7.** Effect of USP19 activity on the stability of endogenous HRD1. The 293T cells transfected with a pcDNA vector, Myc-USP19 or Myc-USP19$^{C548S}$ were incubated with 0.1 mg/mL cycloheximide (CHX) at 37 °C for indicated time. Whole cell lysates (25 μg of proteins) were analyzed by Western blotting with antibodies against HRD1 (**top** panels), $\alpha$-tubulin (**middle** panels) and Myc (**bottom** panels).

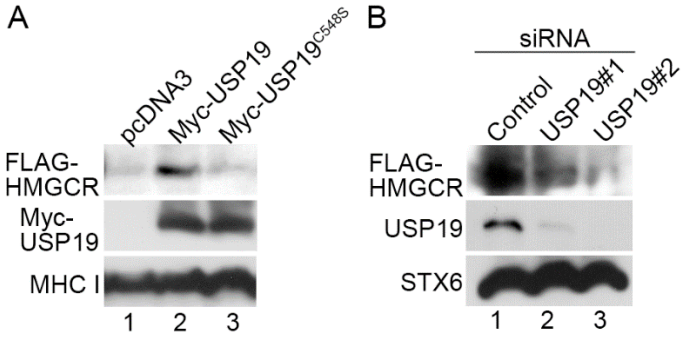


**Figure S8.** Effects of overexpression and knockdown of USP19 on FLAG-HMGCR expression. (**A**) FLAG-HMGCR was transfected into 293T cells along with either a pcDNA3 vector (lane 1), Myc-USP19 (lane 2) or Myc-USP19$^{C548S}$ (lane 3). Membrane fractions of the cells (60 μg of protein) were analyzed by Western blotting with antibodies against FLAG (**top** panel), Myc (**middle** panel) and MHC class I (as a loading control; **bottom** panel); (**B**) The 293T cells were transfected with control siRNA (lane 1) or USP19-specific siRNA duplexes (lanes 2 and 3). Three days after transfection, the cells were further transfected with FLAG-HMGCR. One day after the second transfection, membrane fractions of the cells (100 μg (**top** panel) and 10 μg of protein (**middle** and **bottom** panels)) were analyzed by Western blotting with antibodies against FLAG (**top** panel), USP19 (**middle** panel) and syntaxin 6 (STX6, as a loading control; **bottom** panel).