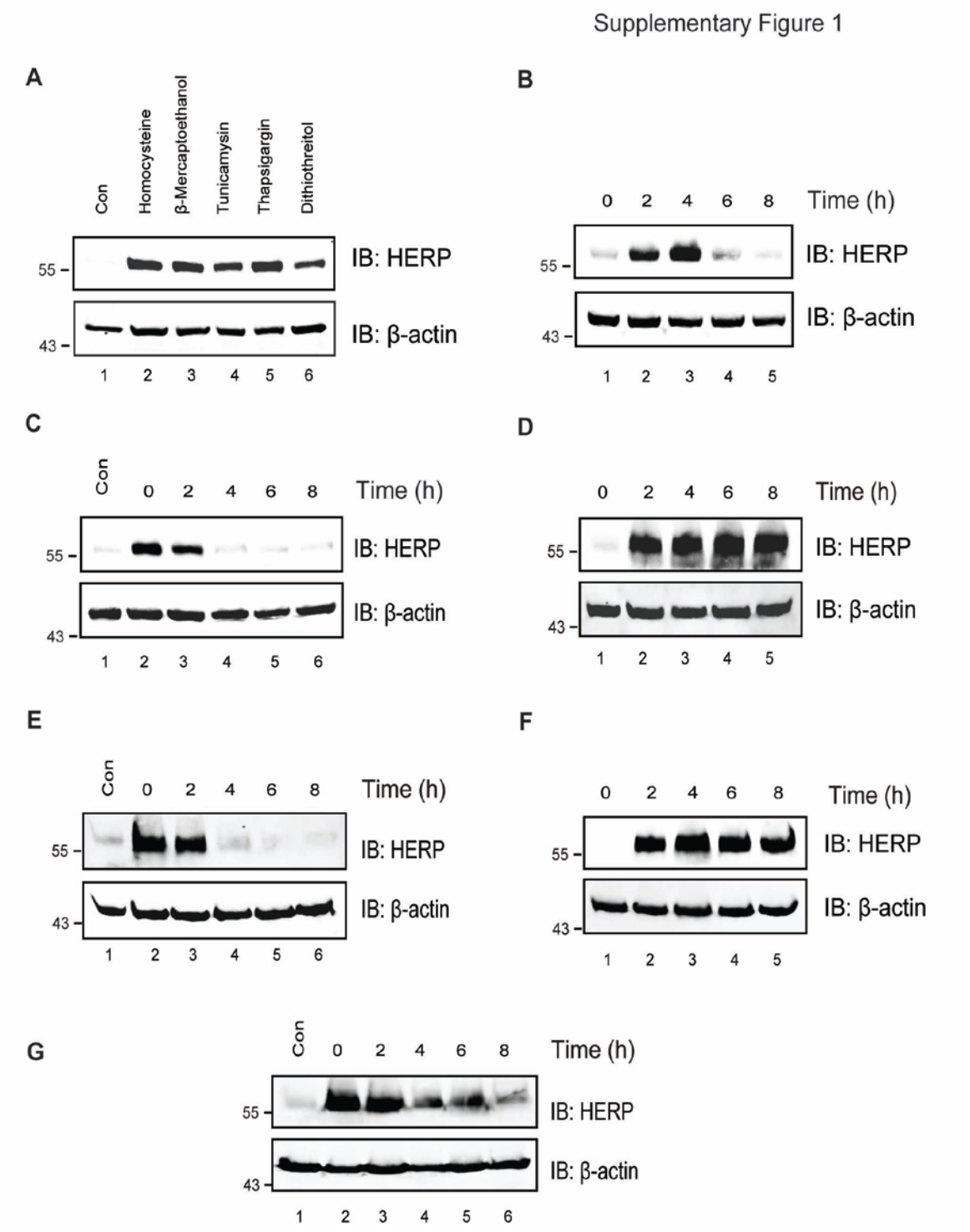

Figure S1. HERP was strongly induced by ER stress and quickly degraded after ER stress. (A) HERP was strongly induced by ER stress. HeLa cells were untreated or treated by 10mM homocysteine, 10mM β-mercaptoethanol, 12μM tunicamycin, 1μM thapsigargin, 500μM Dithiothreitol for 4h. Cell extracts were subjected to

western blot with the indicated antibodies. Con, untreated. (B) HERP was strongly induced by DTT and then quickly degraded after 4 hours. HeLa cells were treated with 500 μM DTT for different time. (C) HERP degraded during the ER stress recovery process. 500μM DTT was used to treat HeLa cells for 4h, then transferred to fresh medium and continued culturing for different time. Con, untreated. （D）HERP was strongly induced by thapsigargin and maintained the induced level until 8 hours. HEK293T cells were treated with 1μM thapsigargin for different times. (E) HERP degraded during the ER stress recovery process. 1μM thapsigargin was used to treat HEK293T cells for 4h, then transferred to fresh medium and continued culturing for different time. Con, untreated. （F）HERP was strongly induced by homocysteine and maintained the induced level until 8 hours. HEK293T cells were treated with 10mM homocysteine for different times. (G) HERP degraded during the ER stress recovery process. 10mM homocysteine was used to treat HEK293T cells for 4h, then transferred to fresh medium and continued culturing for different time. Con, untreated.

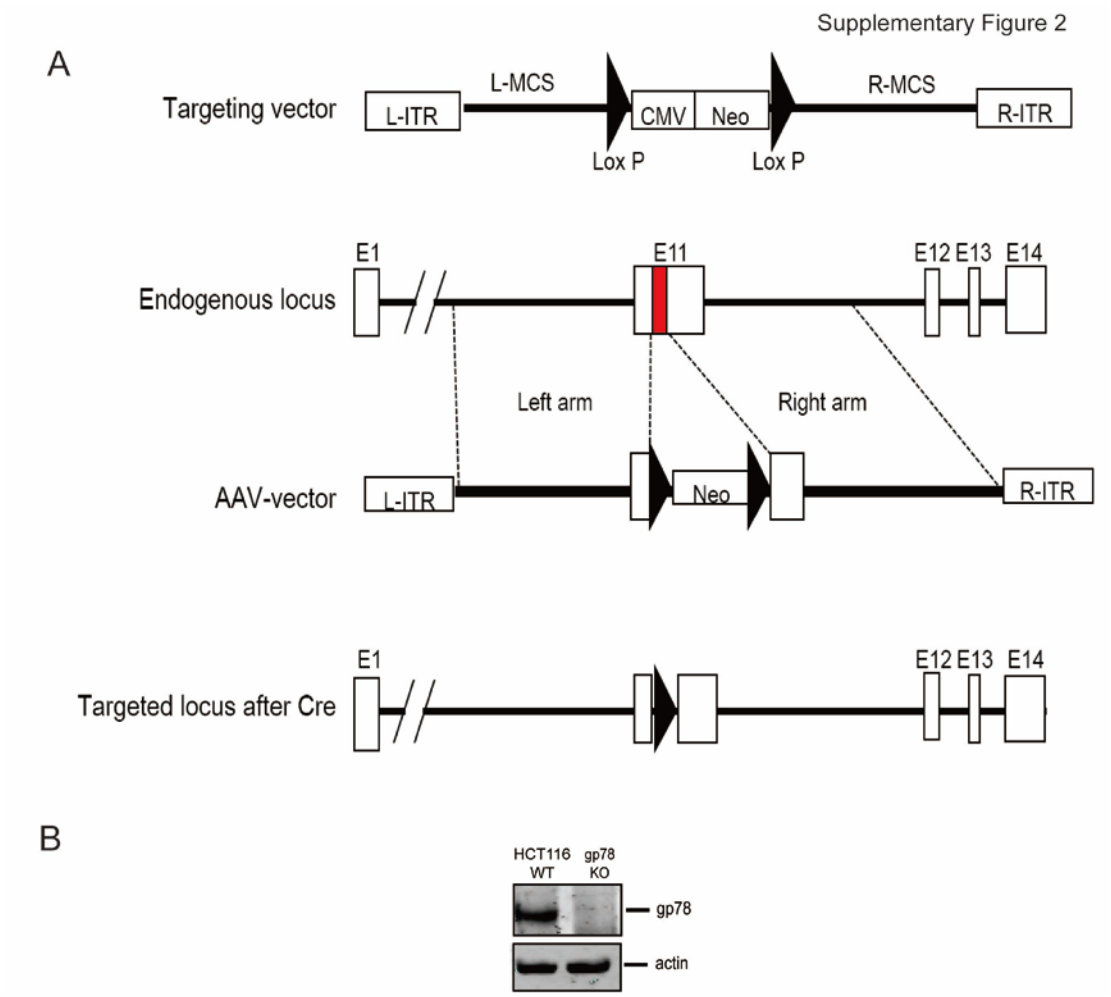

Figure S2. Establishment of gp78 knockout cell line. (A) Construction of a somatic cell gp78 KO vector. (B) Western blotting confirmation of the gp78 knockout.

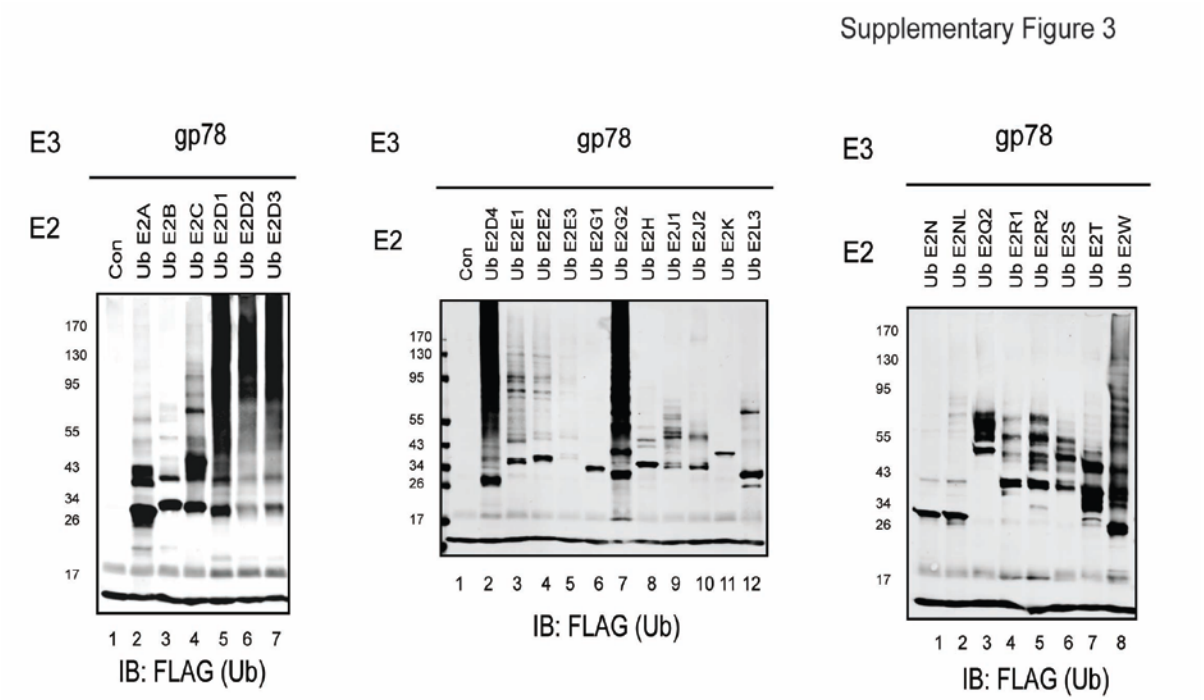

Figure S3. Screening for E2 of gp78. Polyubiquitination reactions conducted with E1, different E2s, gp78c, FLAG-Ub and ATP at 37℃ for 15min. Immunoblotting with anti-FLAG antibody in non-reducing condition. Con, without E2.

Supplementary Figure 4

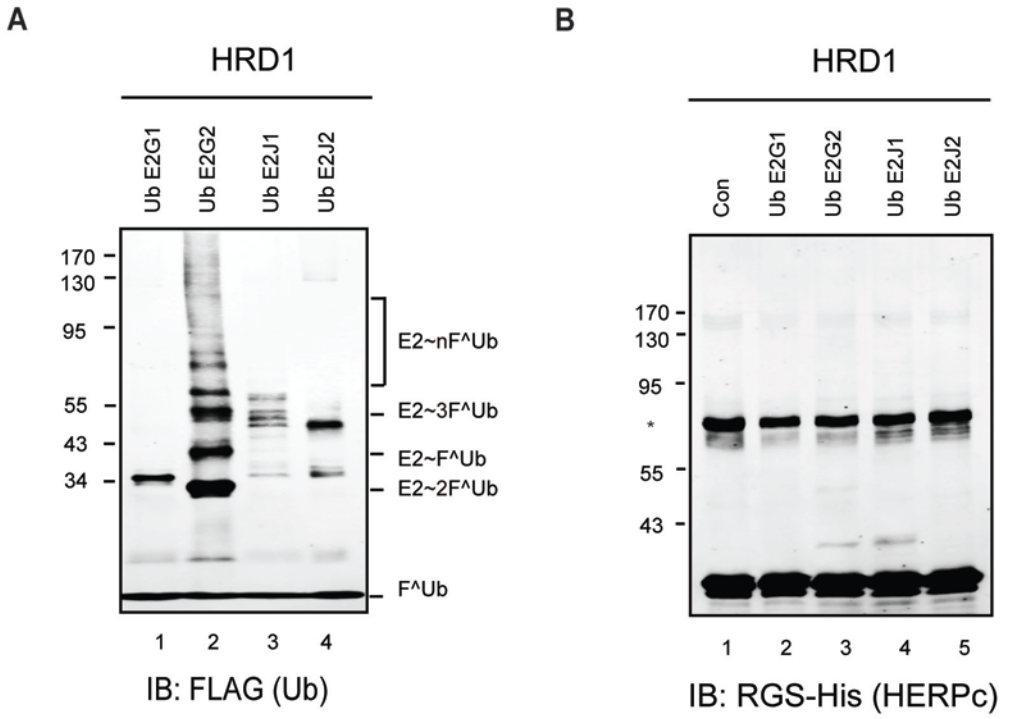

Figure S4. **HRD1 was not the E3 for HERP ubiquitination.** (A) HRD1 catalyzed polyubiquitin chain formation in cooperation with Ube2g2 (B) HRD1 failed to build polyubiquitin chain on HERPc in the *in vitro* ubiquitination assay. The polyubiquitination reactions were conducted as in Figure 2 E and F. * indicates a nonspecific band.

Supplementary Figure 5

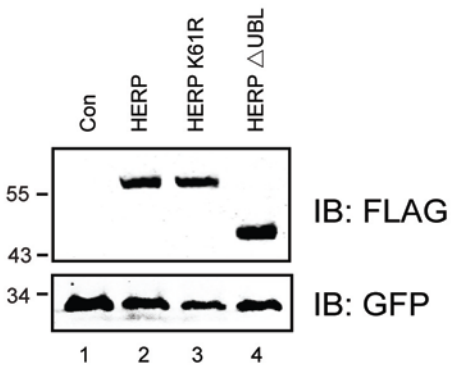

Figure S5. **The expression level of Herp mutants in HEK293T.** HEK293T cells co-transfected with GFP, pRK-*HERP*, pRK-*HERP ⊿UBL* or pRK-*HERP K61R*. GFP was transfected as control. Con, only GFP transfection.

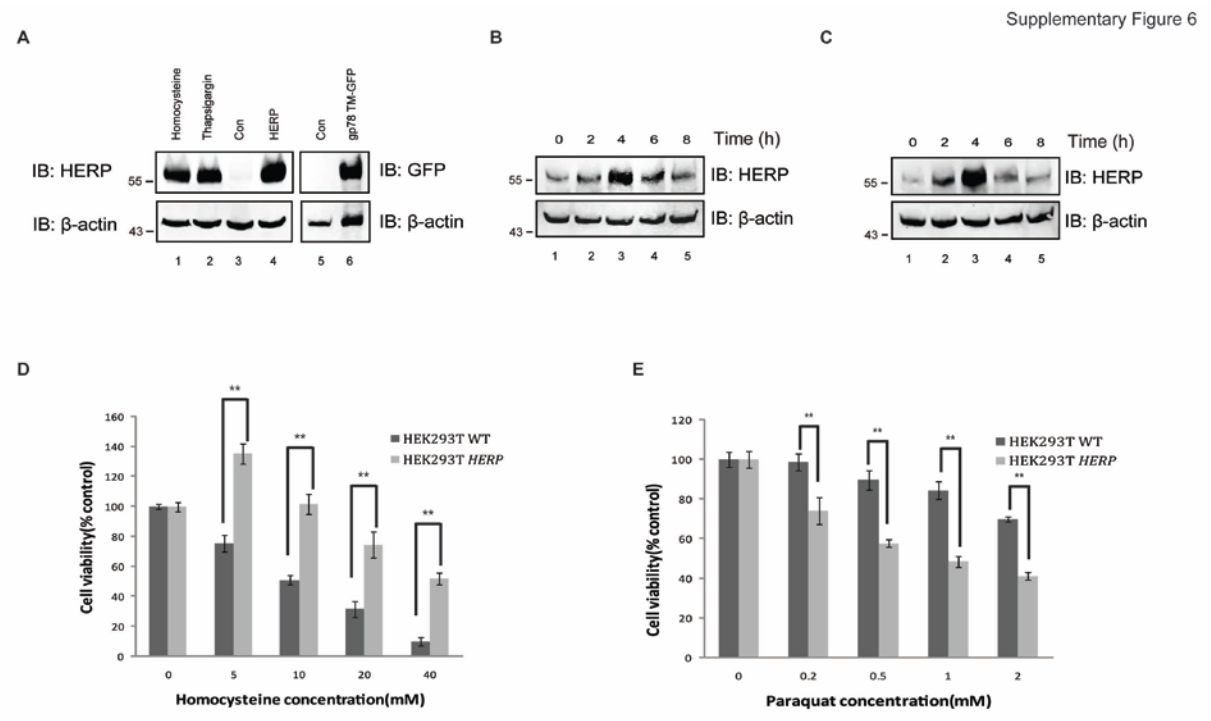

Figure S6. HERP overexpression in HEK293T cells improved their tolerance to homocysteine but impaired their tolerance to Paraquat. (A) HEK293T cells used in this experiment were transfected with *HERP*, *HERP* △*UBL* or another irrelevant membrane protein-gp78 TM domain fused with GFP, Cell extracts were subjected to western blot with the indicated antibodies. Con, without transfection. (B, C) gp78 TM-GFP overexpression didn't changing HERP degradation during ER stress recovery process. HERP was induced by DTT and then quickly degraded after 4 hours in HEK293T cells either transfected with the control plasmids or gp78 TM-GFP were treated with 500 μM DTT for different time. (D) HERP overexpression made cells grew better in the reductive stress condition. (E) HERP overexpression in HEK293T cells impaired their tolerance to oxidative stress such as different concentration of Paraquat. Values shown are means (± SEM) of six experiments. * $p<0.05$, ** $p<0.01$.

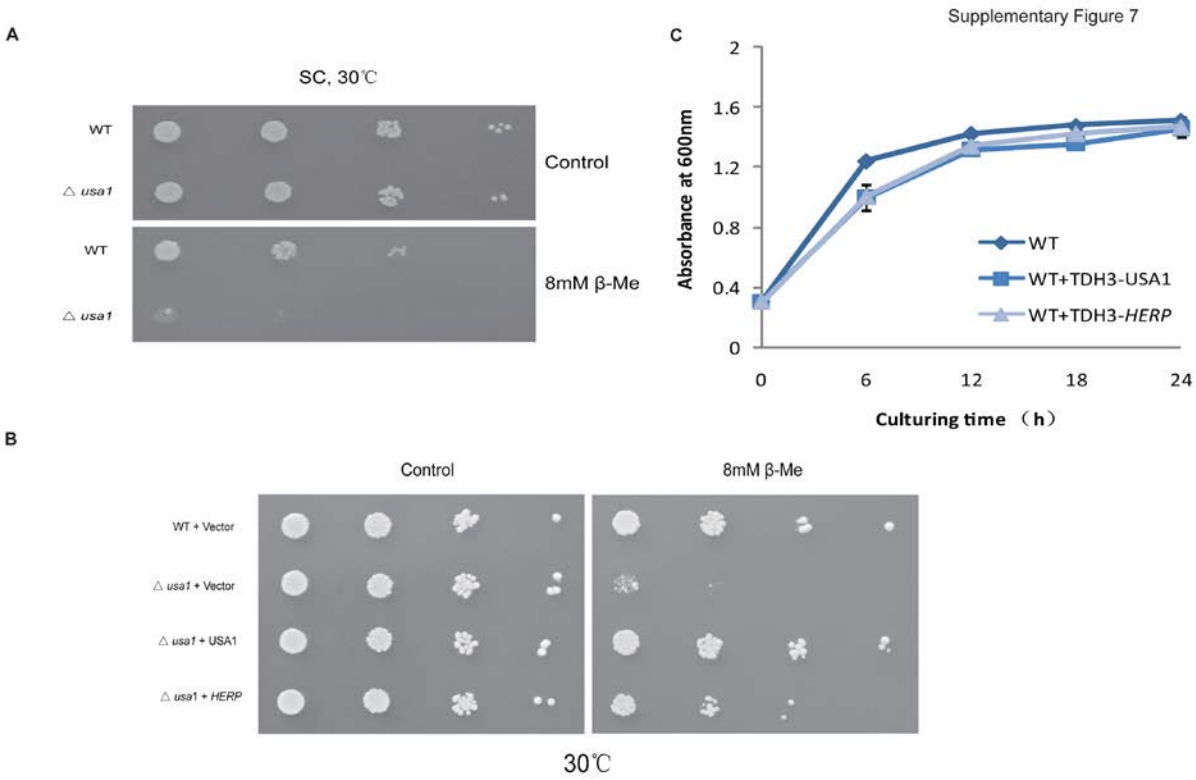

Figure S7. HERP is functionally conserved in yeast cells. (A) *⊿usa1* yeast strain was sensitive to reductive stress. WT yeast strain grew much better than *⊿usa1* strain on SC plates containing 8mM β-mercaptoethanol. (*B*) Usa1 or Herp rescued the β-mercaptoethanol sensitive phenotype of *⊿usa1* strain. （*C*）Overexpression of Usa1p slightly retarded the growth of yeast cells. The yeast strains were cultured in the selected medium (SC-Leu) and grew for 24h at 30℃, growth curves were measured by recording optical density of the cultures at 600nm. Error bars represent standard error of three independent experiments.