Supplementary Information

for

**Inhibition of USP14 influences alphaherpesvirus proliferation by degrading viral VP16 protein via ER stress-triggered selective autophagy**

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**Figure S1.** Examination of anti-PRV activity of USP inhibitors.(**A**) A list of thirteen USPs inhibitors. (**B-N**) PK-15 cells were infected with PRV-GFP (MOI = 0.01) and simultaneously treated with indicated concentrations of inhibitors for 36 h. The percentage of GFP-positive cells that represented PRV replication was measured by flow cytometry. Data were shown as mean ± SD based on three independent experiments. \* *P* < 0.05, \*\* *P* < 0.01, \*\*\* *P* < 0.001 determined by two-tailed Student’s *t*-test.

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**Figure S2.** b-AP15 inhibits the transcription of PRV *IE180* and *EP0* at early stage of infection. (**A and B**) PK-15 cells were infected with PRV-QXX (MOI = 0.1) and treated with b-AP15 (1 μM) for 0-120 min. The mRNA levels of PRV *IE180* (**A**) and *EP0* (**B**) were assessed by qRT-PCR analysis. mpi, minutes post infection. Data were shown as mean ± SD based on three independent experiments. \* *P* < 0.05, \*\* *P* < 0.01, \*\*\* *P* < 0.001 determined by two-tailed Student’s *t*-test.

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**Figure S3.** USP14 directly interacts with PRV VP16. (**A**) Schematic of generation of rPRV Δ*UL48* using CRISPR-Cas9 through homology-directed recombination. (**B**)HEK293 cells were infected with PRV WT (MOI = 0.1), or HEK293 cells expressing FLAG-VP16, FLAG-VP16 (K168R) or FLAG-VP16 (K305R) were infected with rPRV Δ*UL48* (MOI = 0.1) for 24 h. Viral titers were assessed by the plaque assay. (**C**) Quantification of colocalization of USP14 and VP16 from **Figure 5A**. (**D**)Quantification of colocalization of USP14 and VP16 from **Figure 5E**. (**E**)Purification of GST-USP14 and GST-USP14-ΔUBL from *E. coli*. (**F**)Purification of FLAG-VP16, FLAG-VP16 (K168R) and FLAG-VP16 (K305R) from HEK293T cells. Data were shown as mean ± SD based on three independent experiments. \*\* *P* < 0.01, \*\*\* *P* < 0.001 determined by two-tailed Student’s *t*-test. ns, no significance.

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**Figure S4.** Quantification of EIF2AK3/PERK and ERN1/IRE1 target genes with qRT-PCR analysis. (**A**) PK-15 cells were treated with b-AP15 (1 μM) for 0-36 h. The mRNA levels of *ATF4*, *PPP1R15A* and *DDIT3* were assessed by qRT-PCR analysis. hpt, hour post treatment. (**B**) PK-15 cells were treated with b-AP15 (1 μM) for 0-36 h. The mRNA levels of *XBP1(s):XBP1(t)* and *DNAJB9* were assessed by qRT-PCR analysis. (**C**) PK-15 cells were treated with b-AP15 (1 μM) for 0-36 h. The mRNA level of *FOXO1* was assessed by qRT-PCR analysis. Data were shown as mean ± SD based on three independent experiments. \* *P* < 0.05, \*\* *P* < 0.01, \*\*\* *P* < 0.001 determined by two-tailed Student’s *t*-test.

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**Figure S5.** b-AP15 induces the interaction of SQSTM1/p62 with VP16. (**A**)Quantification of colocalization of UB, SQSTM1 and VP16 from **Figure 8A**. (**B**) sgControl and sg*ATG5* PK-15 cells were transfected with plasmids encoding SQSTM1-EGFP and FLAG-VP16 and untreated or treated with b-AP15 (1 μM) as indicated for 24 h. The interaction of SQSTM1 with VP16 was assessed by co-IP assay. (**C**)Purification of His6-SQSTM1 and His6-SQSTM1-ΔUBA from *E. coli*. Data were shown as mean ± SD based on three independent experiments. \*\*\* *P* < 0.001 determined by two-tailed Student’s *t*-test. ns, no significance.

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**Figure S6.** b-AP15 activates ER stress and macroautophagy *in vivo*. (**A­**)Schematic representation of b-AP15 treatment in mice. (**B**) Micewere intraperitoneally injected with DMSO or b-AP15 (8 mg/kg) on day -4 and day -2. On day 0, HSPA5, ATF6, p- EIF2AK3, EIF2AK3, p-EIF2A, EIF2A, ATF4, XBP1 and FOXO1 levels in the lung were assessed by immunoblot analysis (n = 3). (**C**) Mice were treated as in **B**. The mRNA levels of *Atf4*, *Ppp1r15a*, *Ddit3*, *Xbp1(s):Xbp1(t)* and *Dnajb9* in the lung were assessed by qRT-PCR analysis (n = 5). (**D**) Mice were treated as in **B**. LC3-I, LC3-II, SQSTM1, ATG5 and ATG12 levels in the lung were assessed by immunoblot analysis (n = 3). Data were shown as mean ± SD based on three independent experiments. \*\* *P* < 0.01, \*\*\* *P* < 0.001 determined by two-tailed Student’s *t*-test.