

Supplemental material

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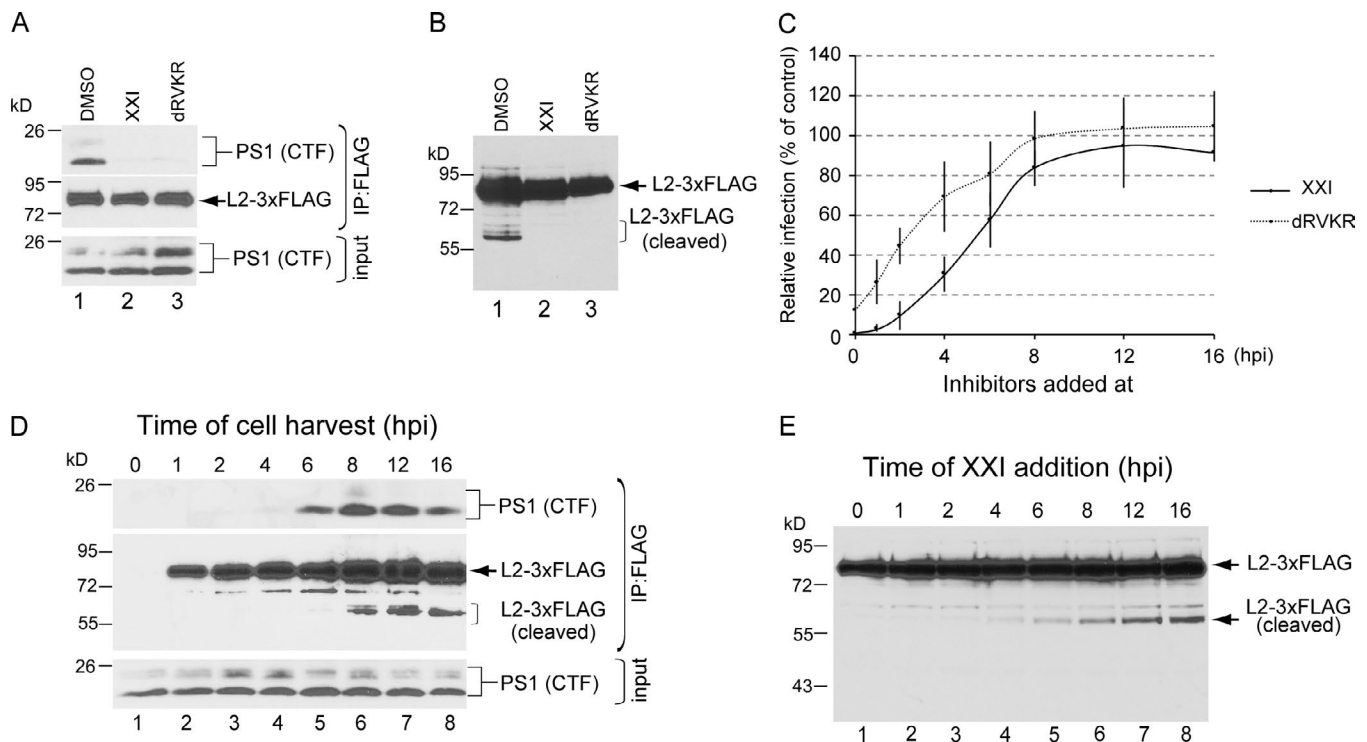


Figure S1. γ -Secretase engages and cleaves L2 in the endosome after furin-mediated cleavage. (A) HeLa cells were uninfected or infected with WT HPV16.L2F at MOI = 100 for 16 h in the presence of 0.1% DMSO, 1 μ M XXI, or 1 μ M dRVKR, a furin inhibitor. The resulting WCLs were subjected to anti-FLAG immunoprecipitation, followed by SDS-PAGE and immunoblotting with antibodies recognizing PS1 (CTF) and FLAG, as in Fig. 1D. (B) HeLa cells were infected with WT HPV16.L2F at MOI = 100 for 16 h and incubated in the presence of 1 μ M XXI or 1 μ M dRVKR. The resulting WCLs were analyzed by SDS-PAGE and immunoblotting with an antibody recognizing FLAG. (C) HeLa cells were infected at MOI = 100 with WT HPV16.L2F containing a reporter plasmid expressing secreted luciferase, followed by addition of 1 μ M XXI or 1 μ M dRVKR at the indicated time points, and further incubated in the presence of the inhibitors. At 36 hpi, luciferase activity was measured in culture supernatant. Data are normalized against the value derived from HPV16.L2F-infected cells incubated with DMSO and represent the means \pm standard deviations of data from at least three independent experiments. (D) Infection was initiated by adding WT HPV16.L2F at MOI = 100 to the medium of HeLa cells at 0 hpi. At the indicated time points, cells were harvested and lysed, and the resulting WCLs were subjected to immunoprecipitation with an antibody recognizing FLAG. Immunoprecipitated samples were analyzed by immunoblotting with antibodies recognizing PS1 (CTF) and FLAG. WCLs were also analyzed by SDS-PAGE and immunoblotting with an antibody recognizing PS1 (CTF) in the absence of immunoprecipitation (input) as a loading control. (E) HeLa cells were infected with WT HPV16.L2F at MOI = 100, followed by addition of 1 μ M XXI at the indicated time points, and then further incubated in the presence of XXI. At 16 hpi, cells were harvested and lysed, and the resulting WCLs were analyzed by SDS-PAGE and immunoblotting with an antibody recognizing FLAG.

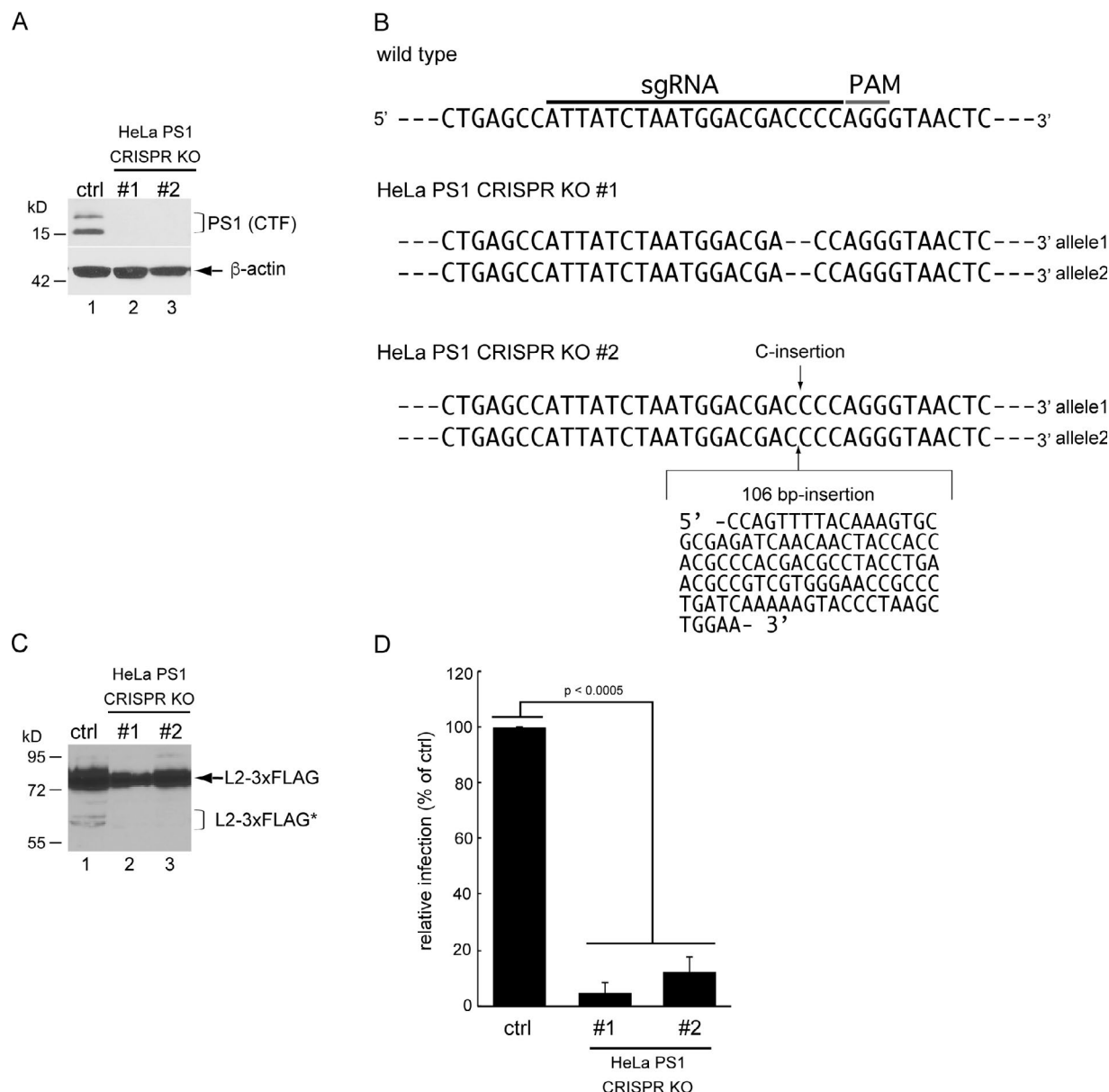


Figure S2. **Characterization of HPV16 infection in two distinct HeLa PS1 CRISPR KO cells (related to Fig. 4).** (A) Control (ctrl) and HeLa PS1 CRISPR KO #1 and #2 cells were lysed, and the resulting whole-cell extracts were analyzed by immunoblotting with the indicated antibodies. (B) Mutations in HeLa PS1 CRISPR KO #1 and #2 cells are indicated. (C) Control and HeLa PS1 CRISPR KO #1 and #2 cells were infected with WT HPV16.L2F at MOI = 100 for 16 h. The resulting WCLs were analyzed by immunoblotting with antibodies recognizing FLAG, PS1 (CTF), and β-actin as a loading control. (D) Control and HeLa PS1 CRISPR KO #1 and #2 cells were infected at MOI = 100 with WT HPV16.L2F containing a reporter plasmid expressing secreted luciferase for 36 h. Luciferase activity was measured using the culture supernatant. Data are shown relative to a value derived from control cells, which is set at 100%, and represent the means ± standard deviations of data from at least three independent experiments.

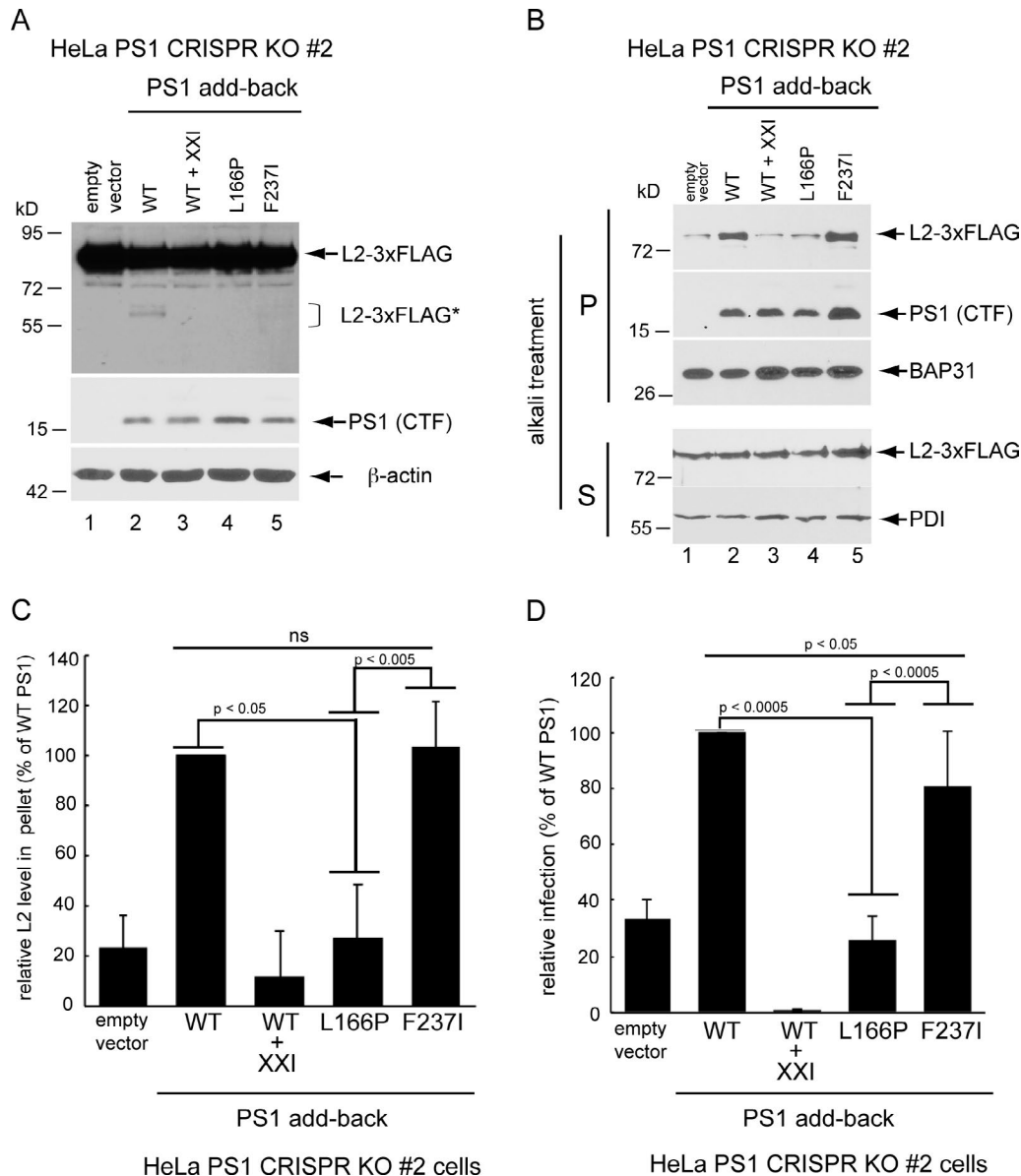


Figure S3. **Supporting data confirming that γ -secretase-dependent membrane insertion but not cleavage of L2 promotes HPV16 infection (related to Fig. 4).** (A) As in Fig. 4 A, except that HeLa PS1 CRISPR KO #2 cells were used. (B) As in Fig. 4 B, except that HeLa PS1 CRISPR KO #2 cells were used. (C) As in Fig. 4 C, except that HeLa PS1 CRISPR KO #2 cells were used. Data are normalized to values for PS1 KO cells expressing WT PS1 and represent the means \pm standard deviations of data from at least three independent experiments. Student's two-tailed *t* test was used to assess statistical significance. (D) As in Fig. 4 D, except that HeLa PS1 CRISPR KO #2 cells were used. All infection values represent the means \pm standard deviations of data from at least three independent experiments. Student's two-tailed *t* test was used to assess statistical significance.