

Gammaherpesviruses are important pathogens in human and veterinary medicine. During co-evolution with their hosts, they developed many strategies allowing them to shed infectious particles in presence of immune response. Understanding these strategies is likely to be important to control infection. Interestingly, we recently observed that the Murid Herpesvirus 4 (MuHV-4), a gammaherpesvirus infecting laboratory mice, could be excreted in the female genital mucosa and be sexually transmitted. This model offers therefore the opportunity to understand mechanisms underlying natural transmission. In this study, we tested the importance of a specific viral glycoprotein, gp150, in the context of MuHV-4 sexual transmission by comparing the capacity of transmission of a wild type and a gp150 deficient strains.

1. Gp150 promotes sexual transmission from female to male after intranasale infection



In order to follow lytic infection in mice, we used an *in vivo* imaging system based on the insertion of the luciferase gene into the viral genome. BALB/c females were infected intranasally with MuHV-4 (wild type or gp150 deficient strains, both of them expressing the luciferase (WT Luc+ or gp150- Luc+ strains)). Mice were followed by *in vivo* imaging at days 7, 14 and 17 to 21 p.i. (post-infection). Females were mated with naive males when genital signal appeared, and males were imaged to determine transmission rate.

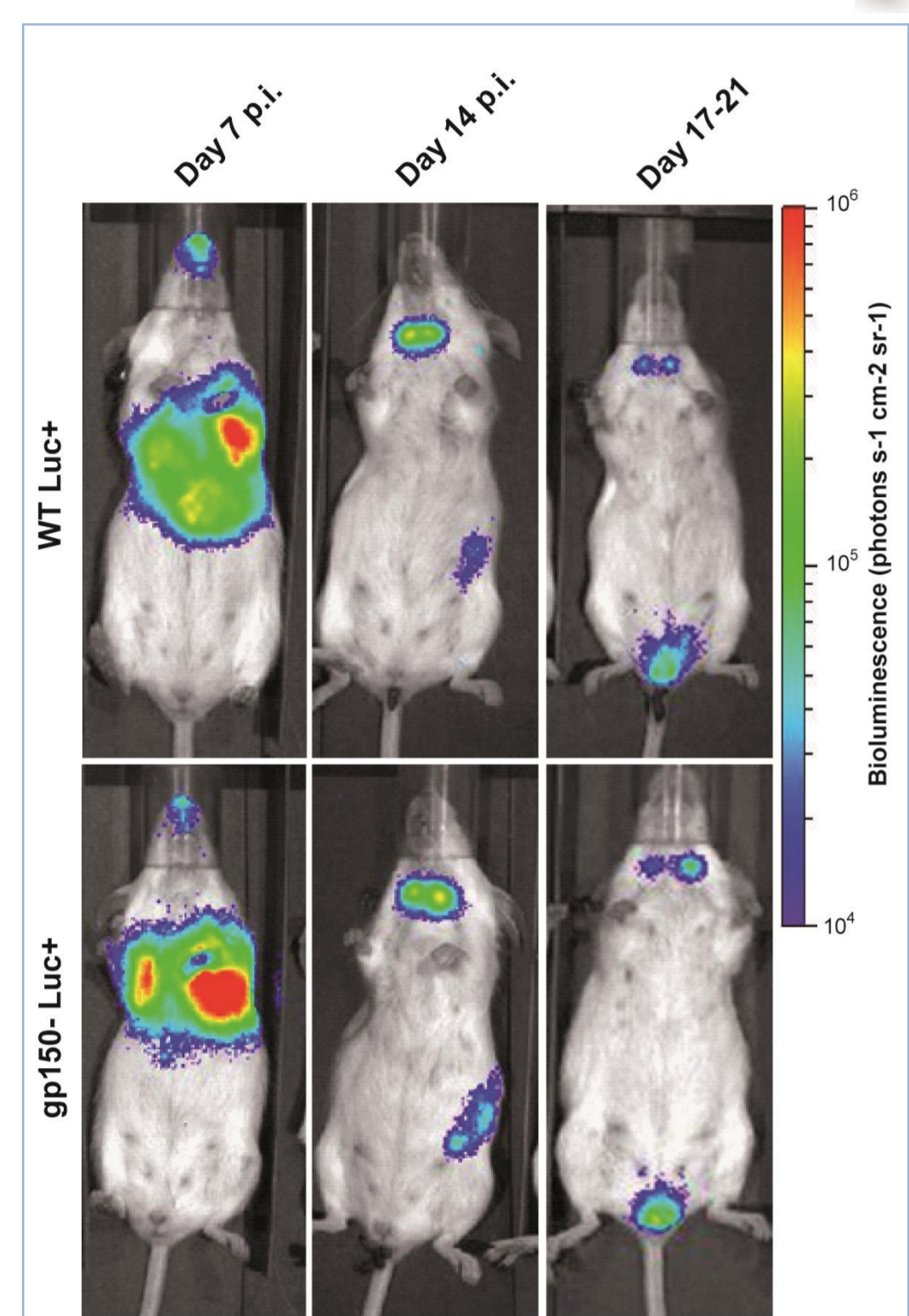


Figure 1: The gp150- Luc+ strain does not display any deficit of lytic infection.

At day 7 p.i., lytic infection was observed in regions corresponding to the nose and the lungs. At day 14 p.i., signal was observed in the SCLNs (superficial cervical lymph nodes) and in the spleen. At days 17 to 21, signal in SCLNs and spleens decreased. No significant difference in signal intensities was observed between the two strains ($p > 0.05$ by linear model) excepted for the SCLNs ($p < 0.05$), where the signal observed in the gp150- group was higher than the WT group. pi: post-infection.

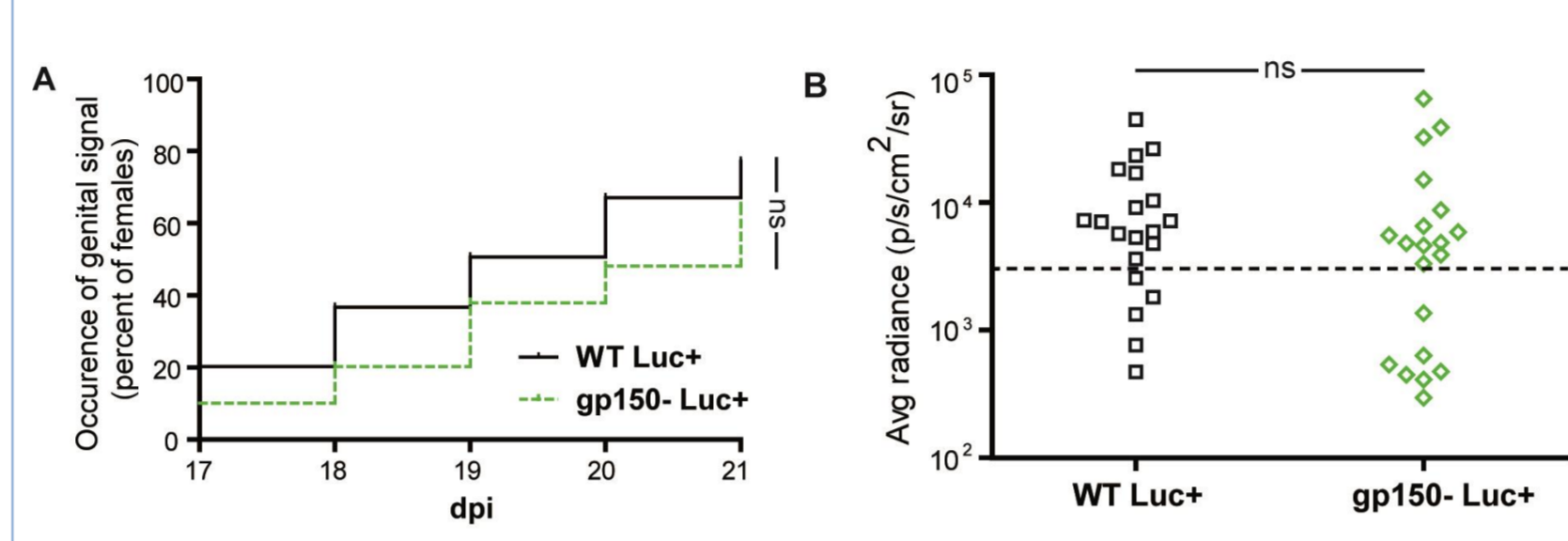


Figure 2: Replication in genital tract is similar for the gp150- Luc+ and the WT Luc+ strains.

Females were followed by *in vivo* imaging every day between 17 and 21 days p.i. to detect a genital reexcretion of the virus. **A.** Occurrence of genital signal during these five days was compared: 77% and 64% of mice showed genital signal for the WT Luc+ and the gp150- Luc+ strains respectively ($p > 0.05$ by Chi² test). **B.** Maximal genital signals observed for each mice ($p > 0.05$ by student test) also showed no significant difference in genital tract colonization for the two viruses. Altogether, the two strains reach and replicate similarly in the genital tract of females.

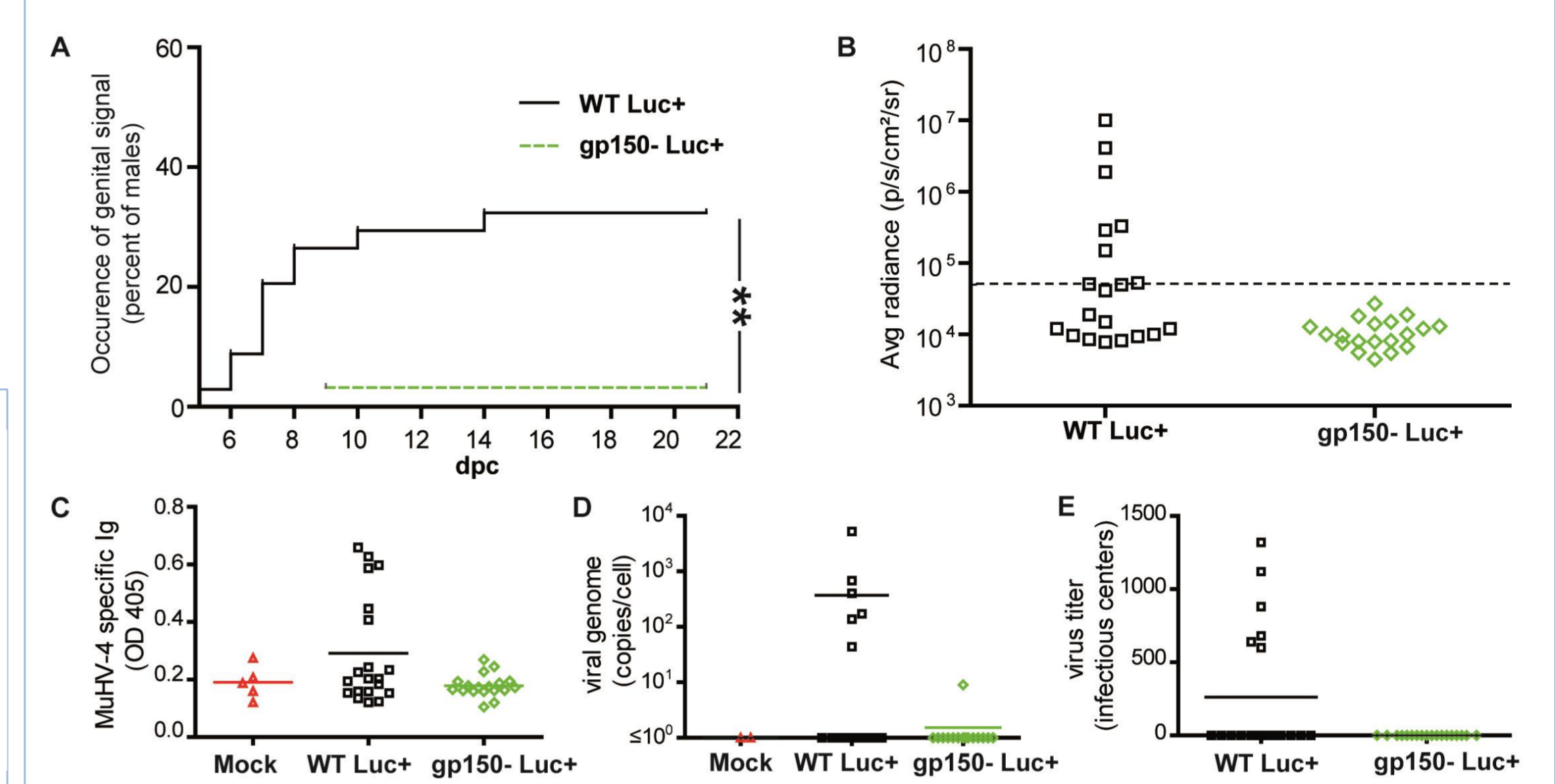


Figure 3: Infection of males after transmission from infected females.

WT Luc+ and gp150- Luc+ infected females were mated with uninfected males at the moment of genital reexcretion of the virus. Males were imaged between 3 and 21 days post-contact with females. **A.** Occurrence of positive genital signal in males was compared: while 32% of males (11 males) were infected in the WT Luc+ group, only 3% (1 male) became infected in the gp150- Luc+ group ($p < 0.01$ by Chi² test). **B.** Maximal genital signals observed in each male. **C-E.** ELISA on sera (**C**) and measure of latency in spleens (**D** and **E**) confirmed the reduced infection in the gp150- group. These results therefore show that the absence of gp150 leads to a strong deficit of genital transmission of the MuHV-4. dpc: days post-contact.

2. Gp150 promotes sexual transmission by increasing the release of infectious particles from vaginal cells

In order to explain how gp150 promotes sexual transmission, several hypotheses were proposed, based on *in vitro* studies that focussed on gp150.

Hypothesis 1:
Reduced tropism for genital cells in males?

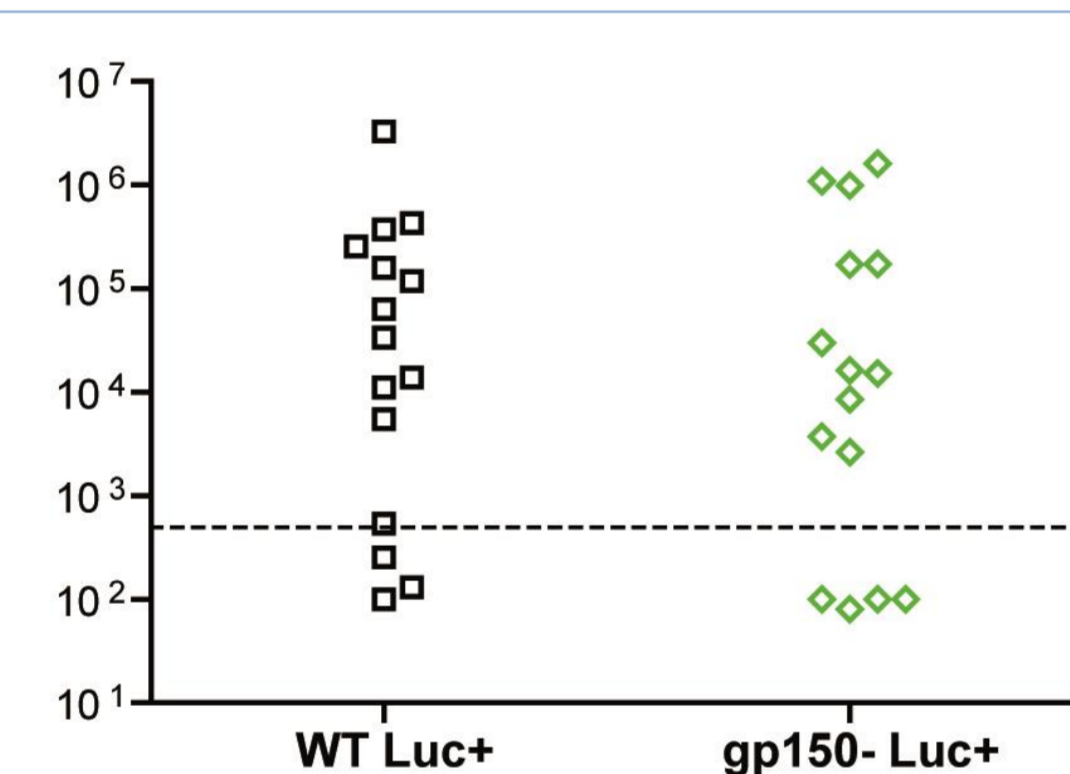


Figure 4: Intra-preputial infection of males.

In order to determine if the difference of transmission in absence of gp150 could be explained by a difference of tropism for genital cells in males, we infected directly males by the prepuce (10^5 PFU of MuHV-4 WT Luc+ or gp150- Luc+). 73% of males showed a genital signal after an intra-preputial infection. No difference of infection was observed between the two groups, in terms of occurrence or intensity of lytic infection, supporting that the deficit of transmission could not be explained by a difference of cellular tropism.

Hypothesis 2:
Enhanced neutralization of gp150- virus?

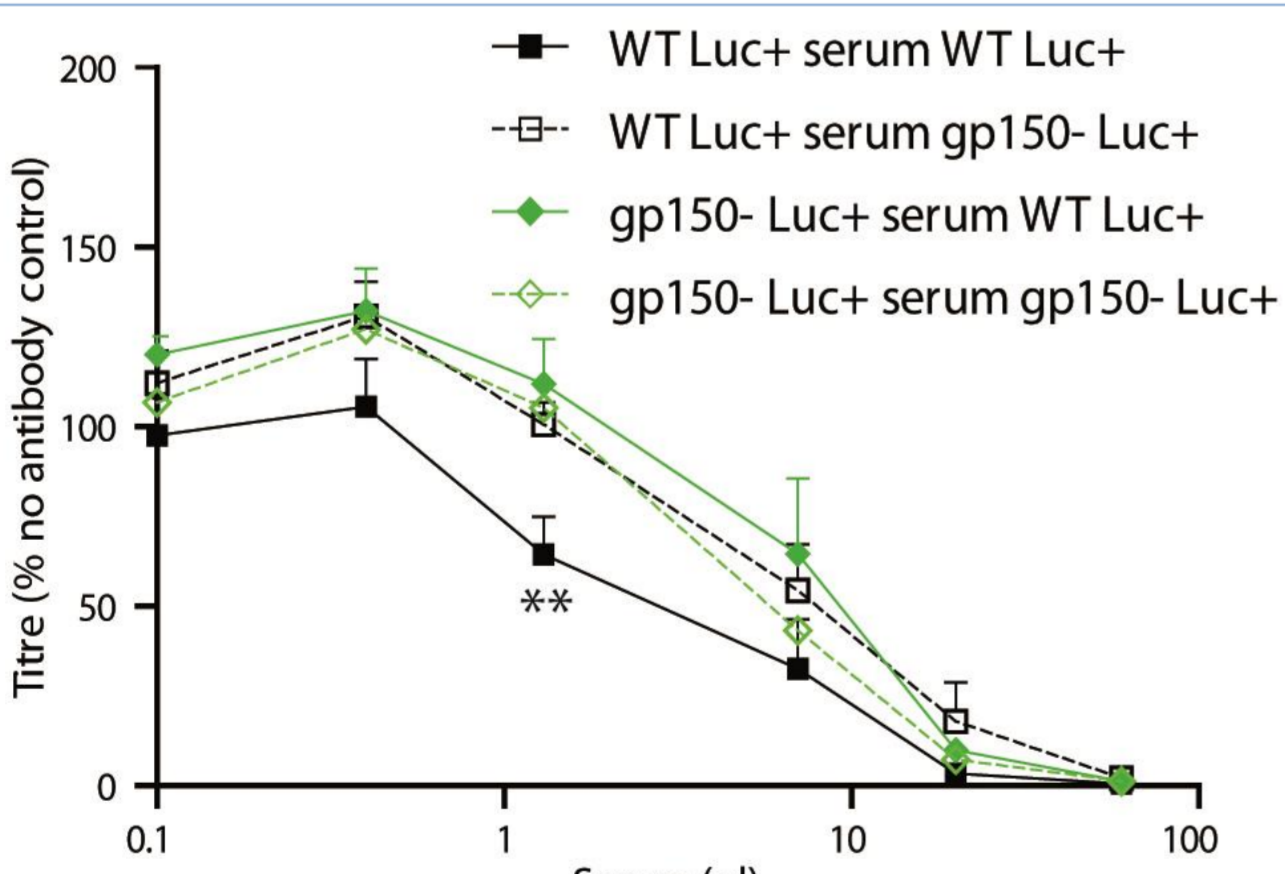


Figure 5: Importance of gp150 for protection against neutralization.

To test if gp150 improves the transmission by protecting the virus against neutralization, an *in vitro* sero-neutralization assay was performed, using WT Luc+ or gp150- Luc+ virions, neutralized with sera from mice infected with WT Luc+ or gp150- Luc+ strains. We did not highlight any increase of neutralization in absence of gp150, it is therefore unlikely that gp150 promotes sexual transmission by protecting the virus against neutralization.

Hypothesis 3: Deficit of virions release?

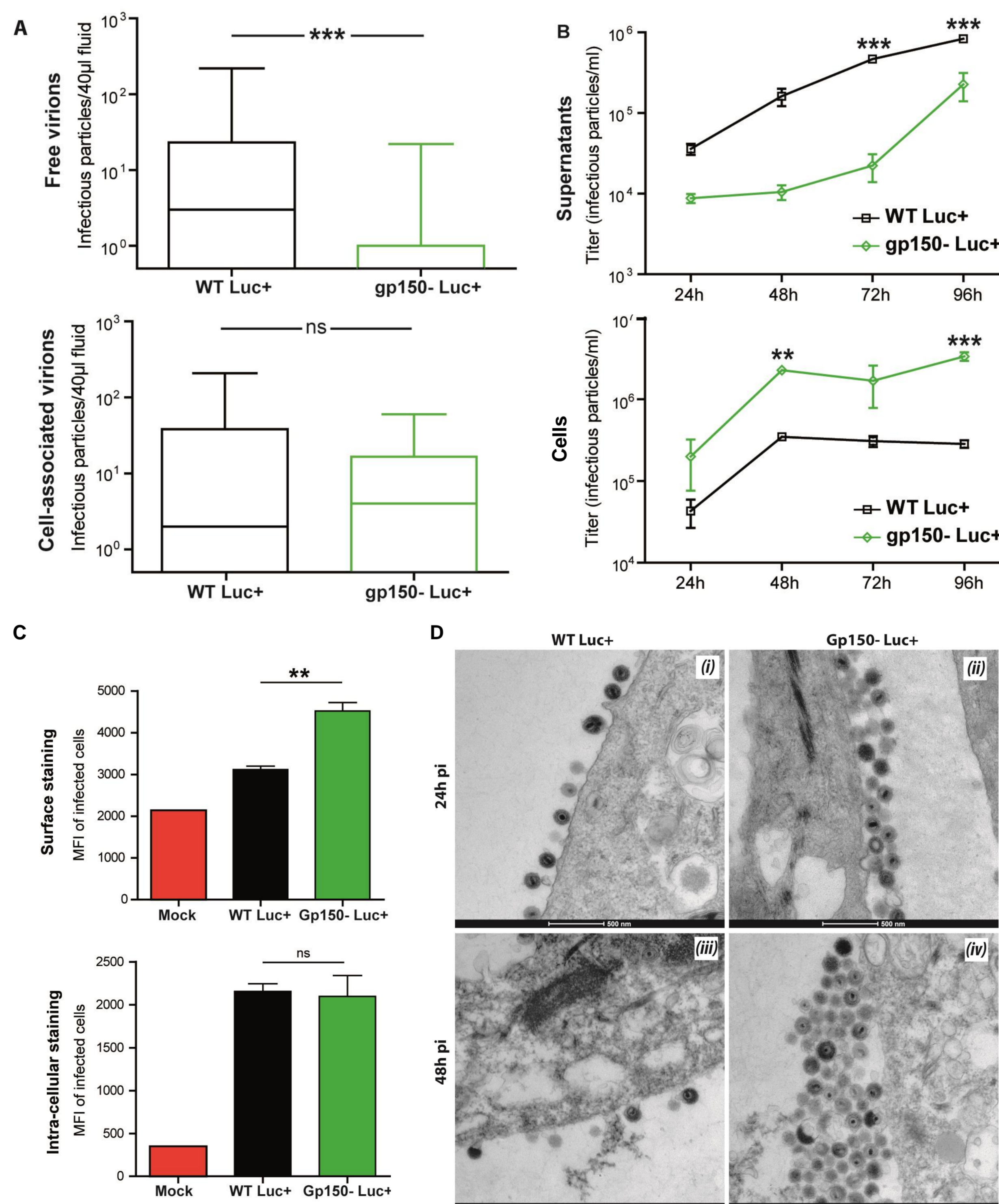


Figure 6: Efficiency of virus release from infected vaginal cells.

A. To determine if the absence of gp150 leads to a deficit of virus release from infected cells, vaginal fluids were collected from intranasally infected females during genital signal detection, and mucosal cell-associated virions were separated from free virions by successive centrifugations. Samples were plaque-assayed on BHK-21 cells. Significant difference in infectious particles amount was highlighted for free virions ($p < 0.05$ by Mann Withney test) whereas no significant difference was observed for the cell-associated virions ($p > 0.05$). Gp150- strain seems therefore to be less efficiently released in the vaginal secretions than the WT strain. **B.** To further confirm this result, we infected primary culture of epithelial vaginal cells with the two strains and compared the amount of infectious particles collected at different times post-infection in the cells and in the supernatants of cell culture. Results show that the amount of virions in supernatants is significantly higher for the WT group, whereas it is the opposite for the cell associated particles, confirming that the gp150- virions stay attached to the cells instead of being released in the culture medium. **C.** Infected vaginal cells from primary culture were also analysed by flow cytometry, to compare the amount of virions bound to the surface of cells. The surface staining showed a significantly increased level of fluorescence for the gp150- strain compared to the WT strain. An intra-cellular staining was used as control to check the level of cell infection. **D.** Infected vaginal cells (MOI = 1) were finally analysed by Transmission Electronic Microscopy. Images taken at 24h and 48h p.i. showed that gp150- virions were abundant at the surface of infected cells, unlike WT particles, which were less abundant, and became even rare at 48h p.i.. This experiment confirms therefore that WT virions, once produced, are directly released, whereas gp150- virions remain bound to the cell surface. Altogether, these experiments suggest that gp150 promotes transmission by allowing the release of virions from infected vaginal cells.

In conclusion, our results show that, while gp150 is not required for efficient dissemination and maintenance of MuHV-4 within its host, it is essential for efficient transmission, at least by the sexual route. Interestingly, the deficit of transmission observed in absence of gp150 does not result from an increased sensitivity to antibody neutralisation but reflects a release deficit of virions from vaginal epithelial cells. In the future, knowledge of the implication of viral glycoproteins during transmission is likely to be decisive to develop strategies to block infection and transmission of gammaherpesviruses.