Nevertheless, to further address the Reviewer’s concern, we have now performed a **quantitative analysis of fluorescence intensity** in the in vitro BBB spheroid model infected with **THP-1–mediated oHSV-1**. Image quantification was carried out using ImageJ software on multiple spheroids (n = 3 per condition). The resulting data were analyzed using a mixed-effects model (REML) followed by **Dunnett’s post-hoc test**, confirming a **highly significant increase in mCherry signal intensity** in infected spheroids compared to uninfected controls (\*\*\*\*p < 0.0001). The quantitative results are in full agreement with the fluorescence images already shown in the manuscript. Specifically, the mean fluorescence intensity (MFI) plot revealed a progressive increase in mCherry signal over time, with infection mediated by THP-1 cells showing an **earlier onset at 24 h** compared to the direct viral infection (Figure 1). The signal in THP-1-mediated infection then reached a plateau, while the free virus showed a slower but sustained increase at later time points (48-72 h). In addition, the quantitative analysis of mCherry fluorescence in the endothelial layer of the BBB-on-chip model (Figure 2) showed that the signal intensity was **lower in the monocytes-mediated oHSV-1 infection** compared with the direct infection with free oHSV-1. This result is in line with the fluorescence images already presented in the manuscript, indicating that **using monocytes as carriers reduces the direct infection of endothelial cells and helps preserve the integrity of the BBB**. These quantitative data therefore **further support our previous conclusion** that monocyte-mediated viral delivery allows the virus to cross the BBB **while minimizing endothelial damage**. These new quantitative data have been provided to the Reviewer for verification, but are not included in the revised manuscript to maintain focus on the feasibility objectives of this study.

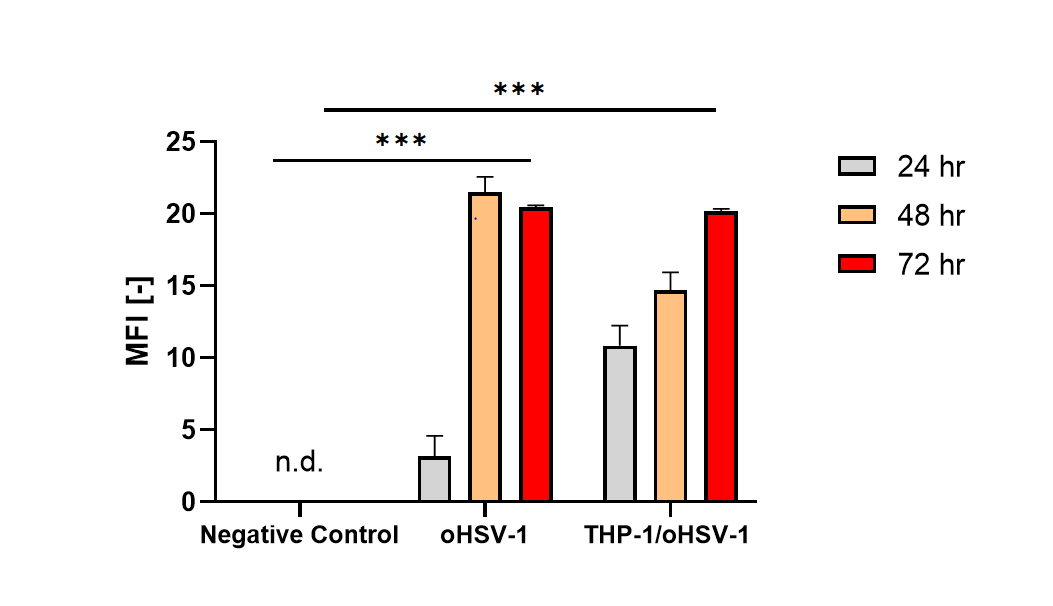


Figure 1. Quantification of mCherry fluorescence intensity in U87-MG spheroids infected in the microfluidic BBB-on-chip model with free or THP-1-mediated oHSV-1. Mean fluorescence intensity (MFI) was measured at 24, 48 and 72 hours post-infection. Both infection modes induced a significant increase in mCherry signal compared with uninfected controls (\*\*\* p < 0.001, mixed-effects model with Dunnett’s post-hoc test). Data represent mean ± SEM (n = 3).

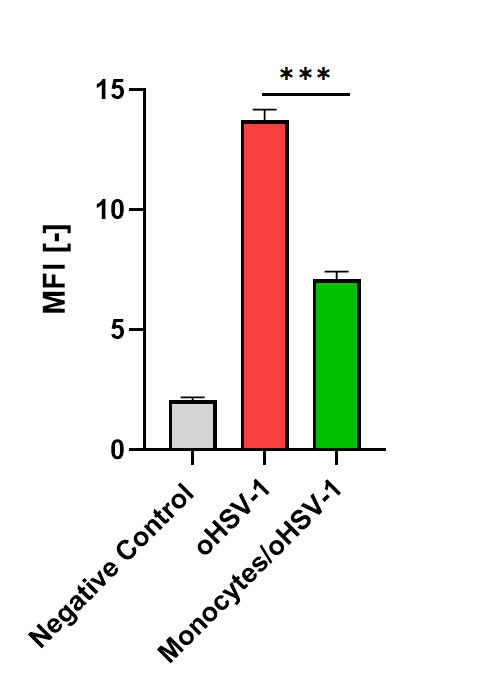


Figure 2. Quantification of oHSV-1 infection in the endothelial layer of the *in vitro* blood–brain barrier (BBB) model. Mean fluorescence intensity (MFI) was measured at 24 hours post-infection with free or primary monocytes mediated oHSV-1. Data represent mean ± SEM (n = 3, \*\*\*p < 0.001, unpaired two-tailed *t*-test).