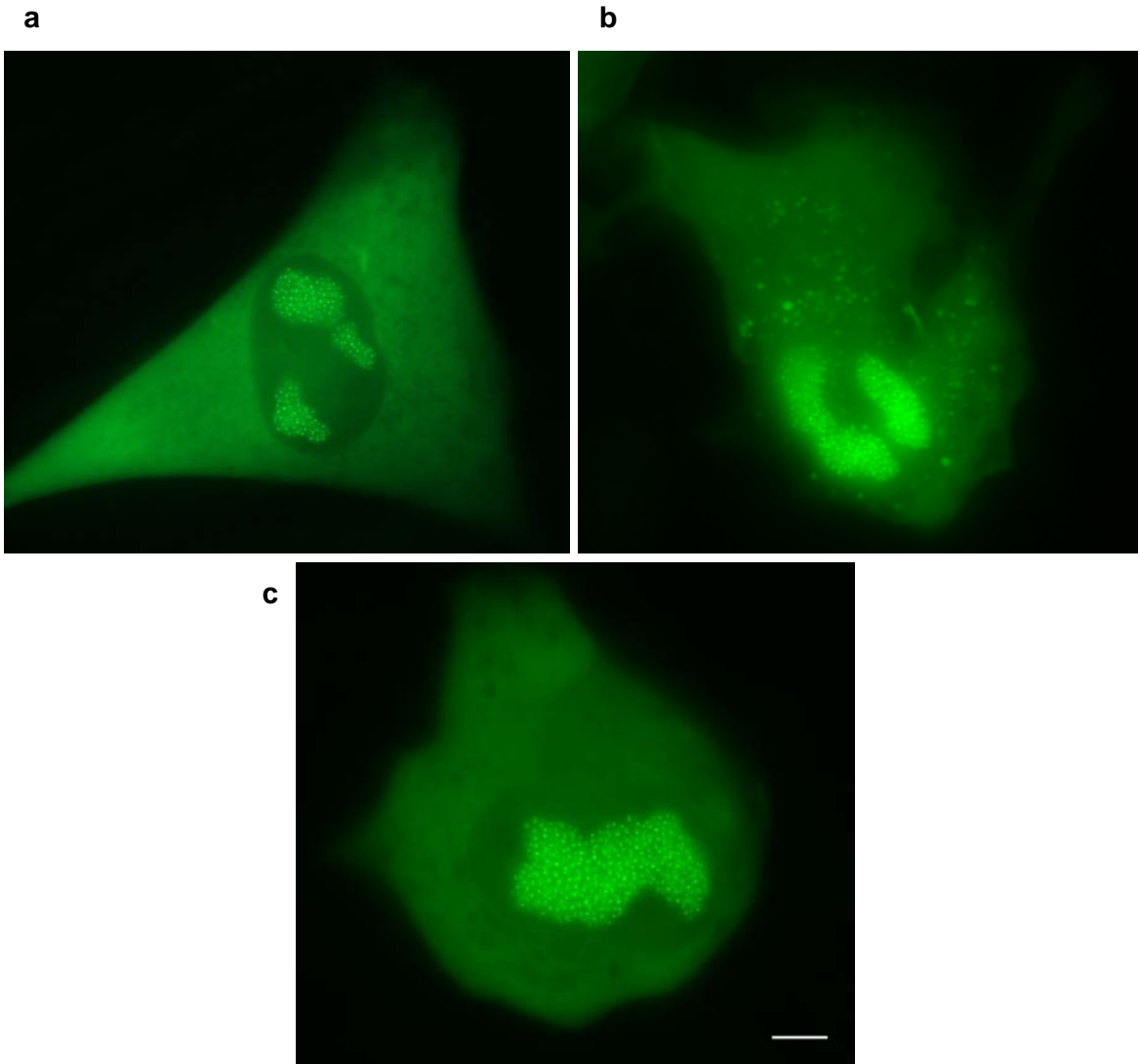
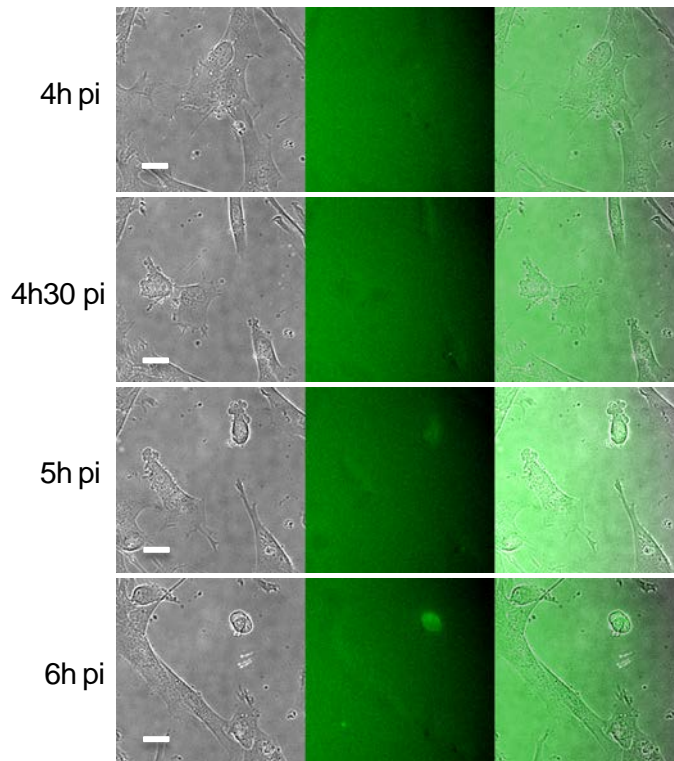


**Fig.S1**



**Figure S1. TB40-ANCHOR3 HCMV has retained the cellular tropism of the parental TB40 strain.**  
a) TB40-ANCHOR3 HCMV infection of MRC5 cells, 48h pi.; b) TB40-ANCHOR3 HCMV infection of ARPE-19 cells, 120h pi.; c) TB40-ANCHOR3 HCMV infection of HUVEC cells, 120h pi.. Barr scale 5 $\mu$ m.

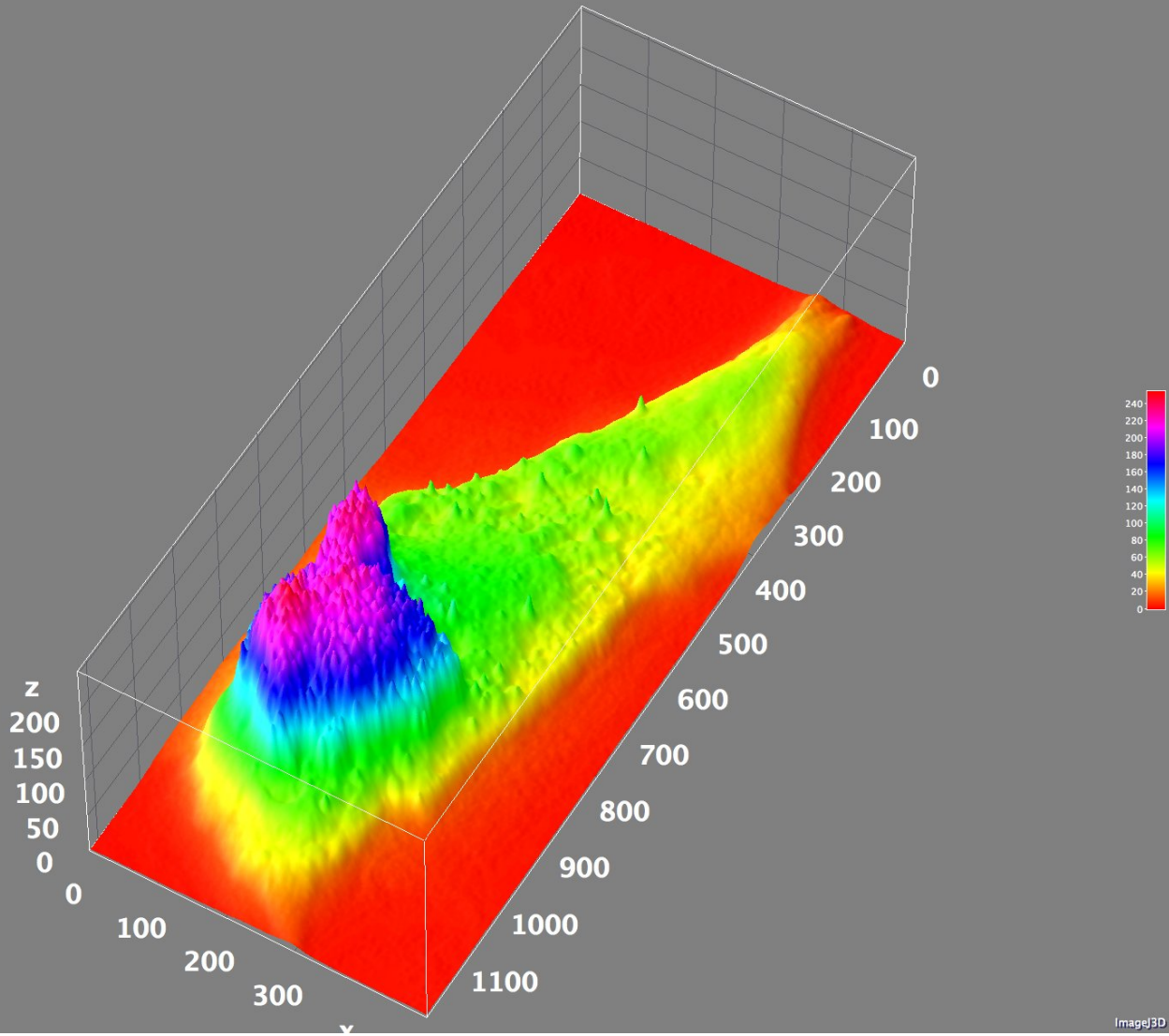
**Fig.S2**



**Figure S2. Initial demonstration of TB40-ANCHOR3 HCMV infection in MRC5 cells.**

MRC5 cells were infected with TB40-ANCHOR3 HCMV viruses at an MOI of 0.5 and the first fluorescent cells could be detected between 4 and 5 hours pi. Infection induces rounding up of the cells which later turn back to their normal spindle shape (40X, scale bar 20 $\mu$ m);

**Fig.S3**



**Figure S3. Visualization of viral replication in TB40-ANCHOR3 HCMV infected ARPE-19 cells.** ARPE-19 cells were infected with TB40-ANCHOR3 HCMV at an MOI of 0.5 and analyzed 5 days pi. Quantification as in Fig.9c and d.

**Fig. S4**

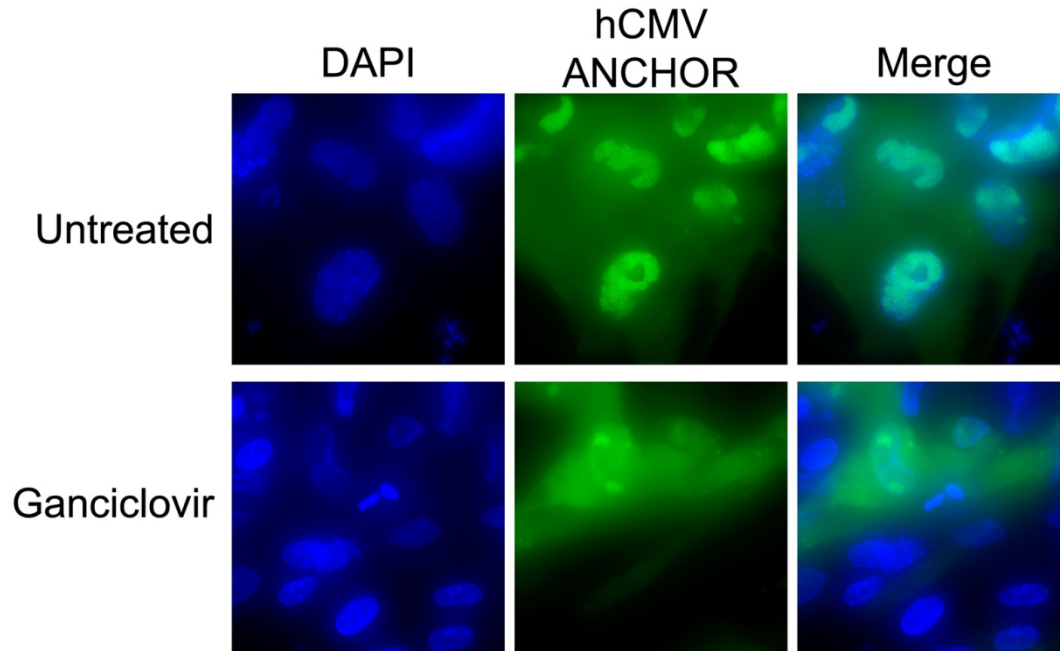


Figure S4. TB40-ANCHOR3 infected MRC5 cells, treated or not with 2.5 $\mu$ M Ganciclovir, were imaged 72h pi. In untreated cells, large RC are clearly visible at this stage of infection; on the contrary, only pre-replicative structures are observed at the same time in Ganciclovir treated cells.

1 **Supplemental Material**

2 **Video 1:** Time lapse imaging of MRC5 cells infected at a MOI of 1. Pictures were taken  
3 every 3h from 16h post infection to 97h pi on a wide-field Zeiss Axiovert, Observer Z1,  
4 1.4NA objective, 40X.

5 **Video 2:** Time lapse imaging of MRC5 cells infected at a MOI of 0.5. Pictures were taken  
6 every 5 min from 63h pi to 66h pi on a wide-field Zeiss Axiovert, Observer Z1, 1.4NA  
7 objective, 63X. Apoptosis results in lysis which induces significant liberation of fluorescent  
8 material, possibly hCMV particles either alone or in vesicle, into the surrounding medium.

9 **Video 3:** Time lapse imaging of MRC5 cells infected at a MOI of 0.5. Pictures were taken  
10 every 5min from 48h pi to 67h pi on a wide-field Zeiss Axiovert, Observer Z1, 1.4NA  
11 objective, 63X. Example of lysis events by blebbing induced by hCMV ANCHOR.

12 **Video 4:** Time lapse imaging of ARPE-19 cells infected at a MOI of 1. Pictures were taken  
13 every 30min from 72h pi to 6 days pi on a wide-field Zeiss Axiovert, Observer Z1, 1.4NA  
14 objective, 63X. Example of infection to lysis event by blebbing induced by hCMV ANCHOR  
15 (upper cell left).

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