Visualization of DNA G-quadruplexes in cells during herpes simplex virus 1 infection

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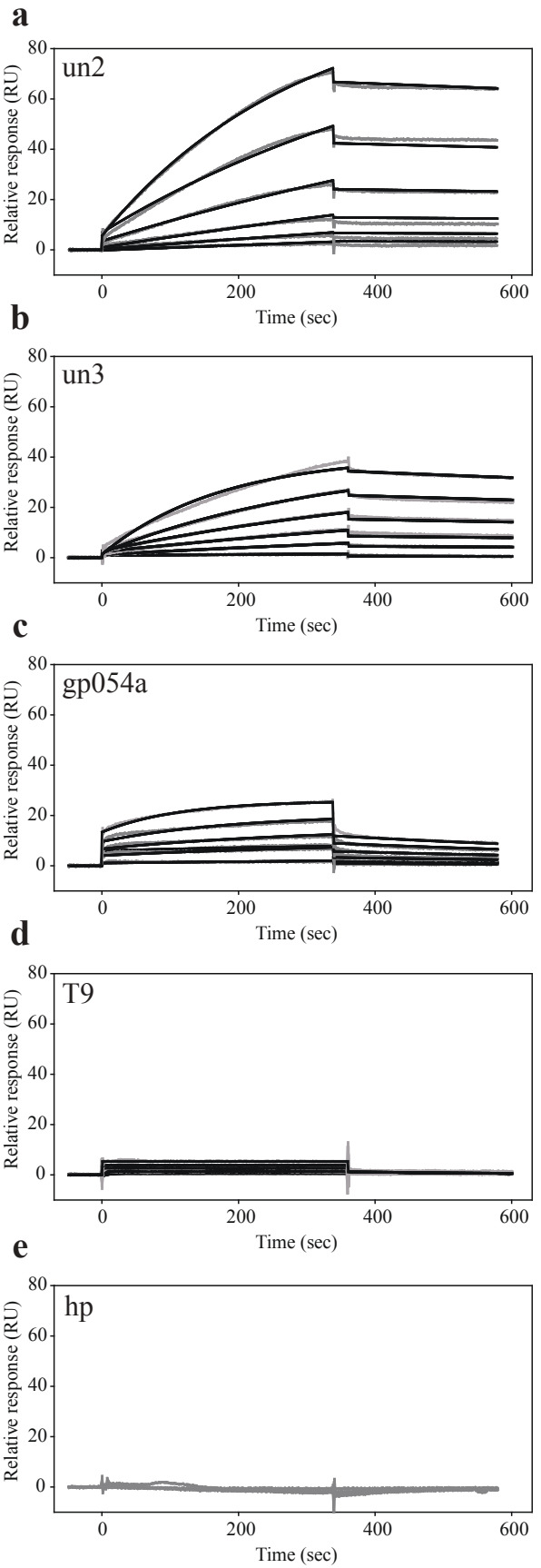
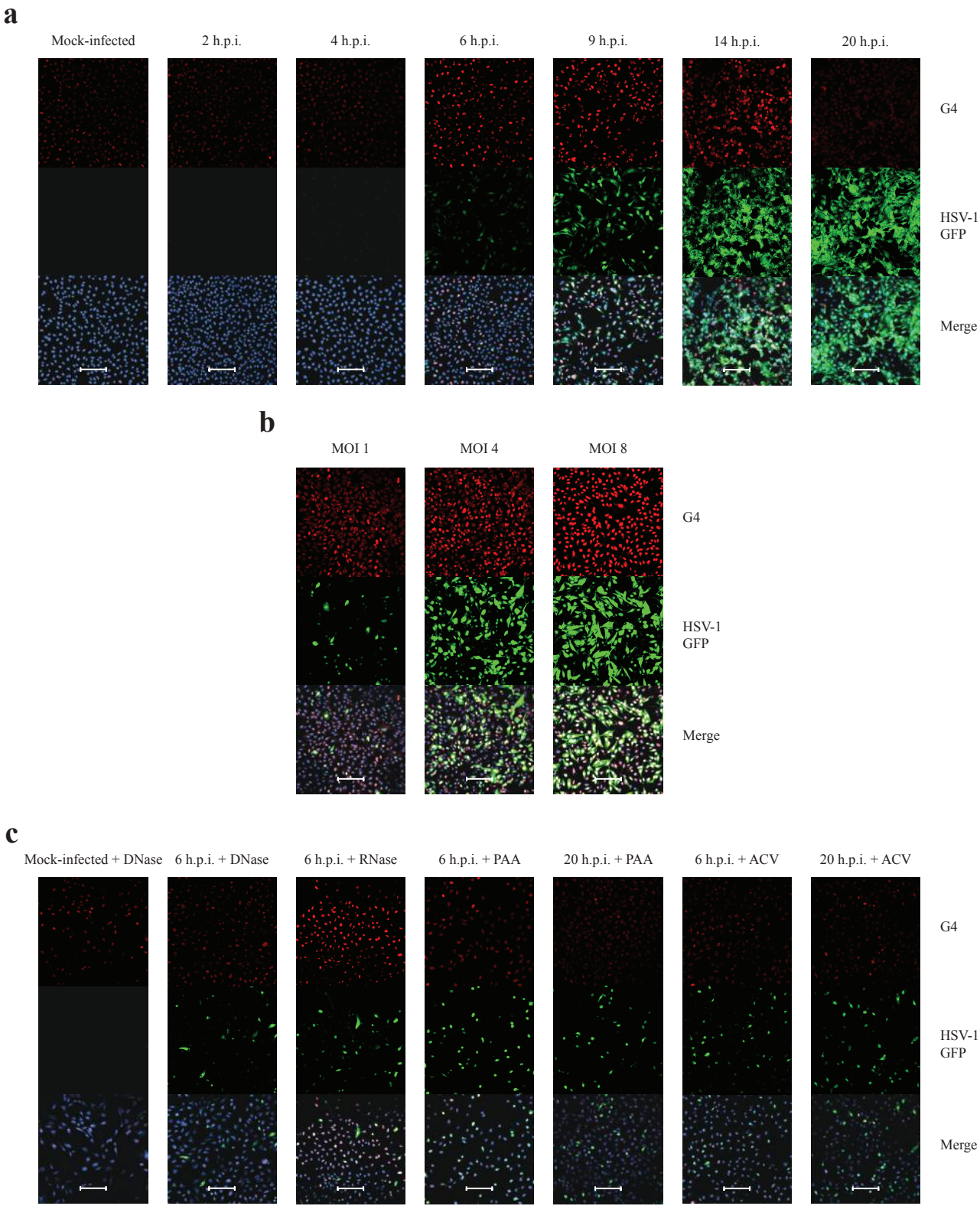
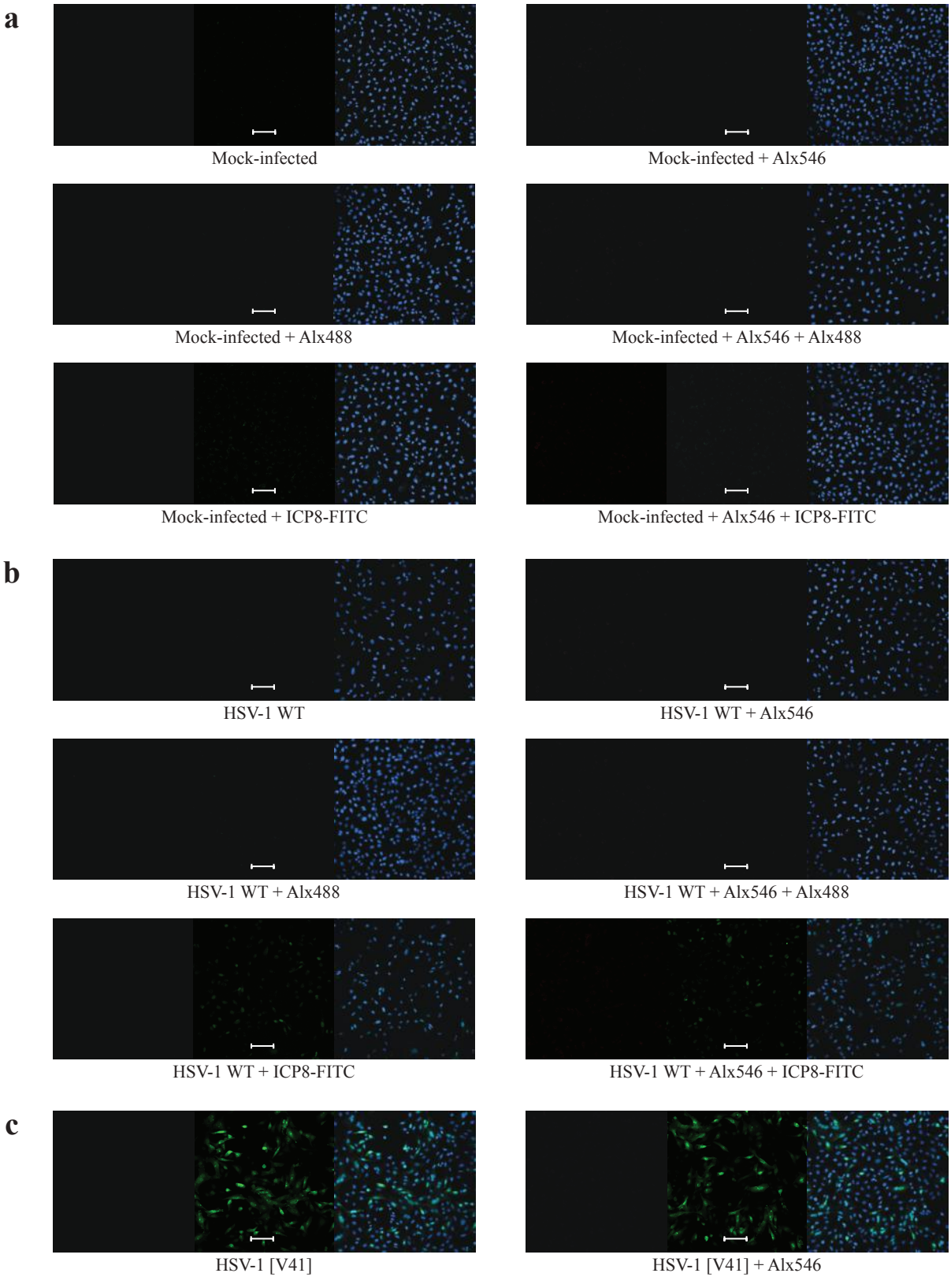


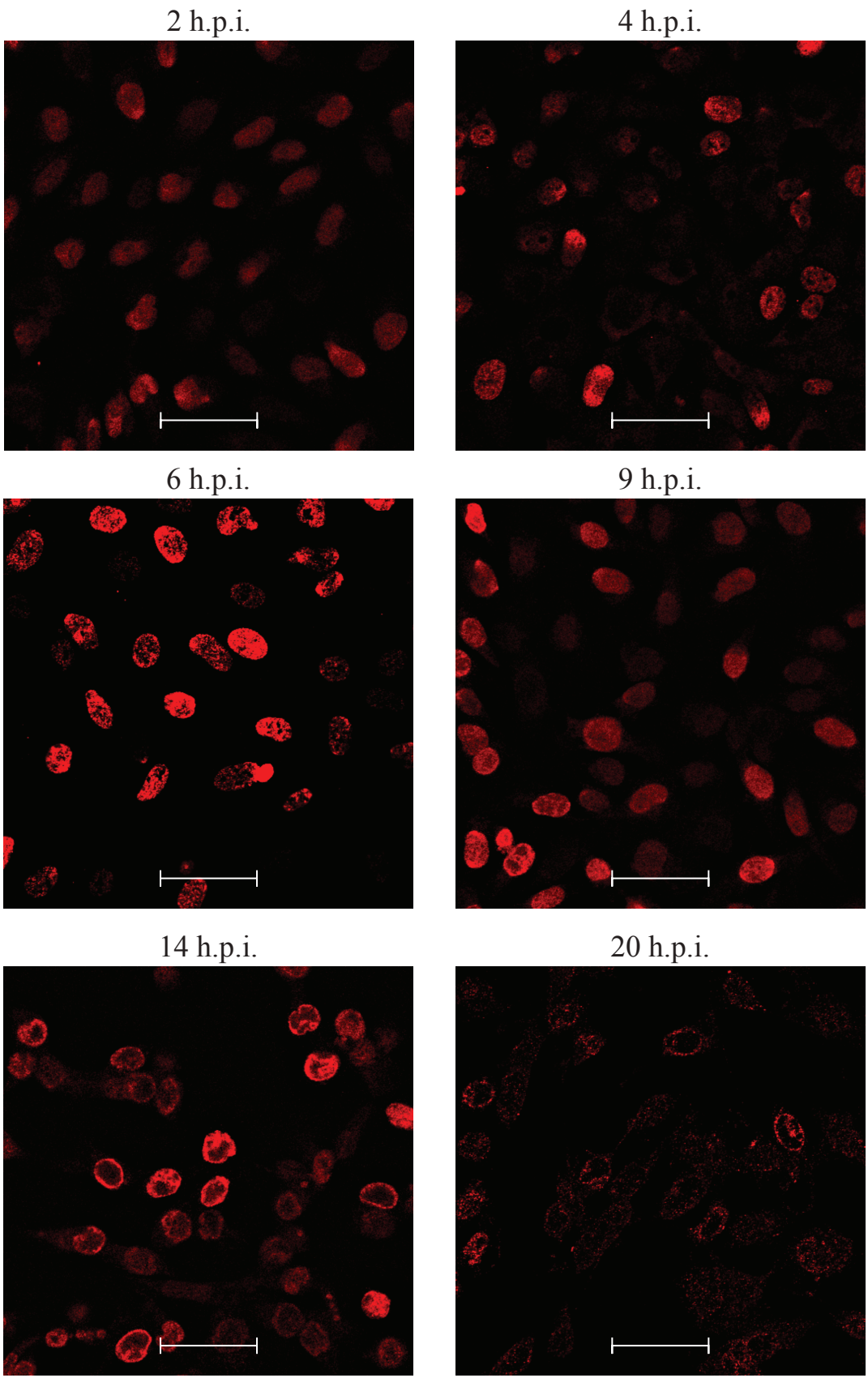
Supplementary Figure 1. Sensorgrams obtained in SPR analysis. Shown are representative sensorgrams of oligonucleotides/1H6 binding which a), b) and c) fitted the binding equation (un2, un3 and gp054a) and thus allowed calculation of KD, d) showed unspecific interaction (T9) and e) did not show any interaction (hp). RU stands for Response Units.


a
Mock-infected
2 h.p.i.
4 h.p.i.
6 h.p.i.
9 h.p.i.
14 h.p.i.
20 h.p.i.
G4
HSV-1 GFP
Merge
b
MOI 1
MOI 4
MOI 8
G4
HSV-1 GFP
Merge
c
Mock-infected + DNase
6 h.p.i. + DNase
6 h.p.i. + RNase
6 h.p.i. + PAA
20 h.p.i. + PAA
6 h.p.i. + ACV
20 h.p.i. + ACV
G4
HSV-1 GFP
Merge

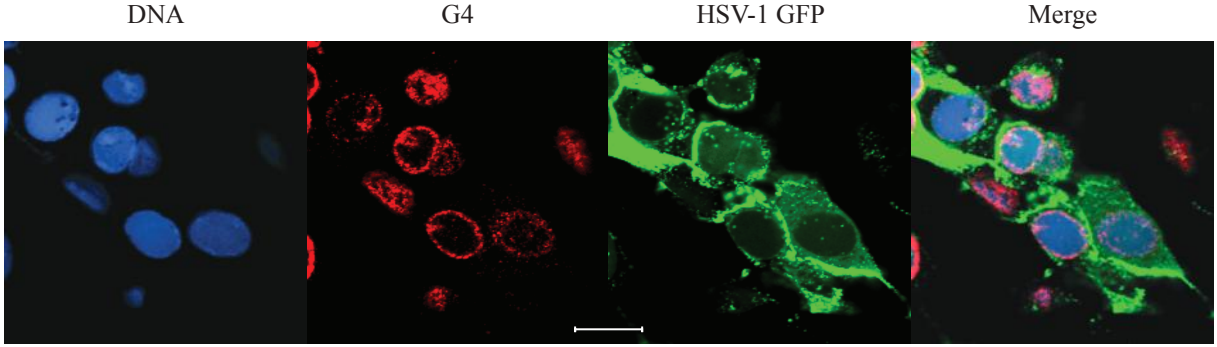
Supplementary Figure 2. Immunofluorescence confocal microscopy of HSV-1 v41-infected mammalian cells at 20X magnification. a) Infected cells visualized at different times p.i., covering a single cycle of replication. b) Cells infected with increasing MOI of HSV-1 v41 visualized at 6 h.p.i.. c) Infected cell treated with DNase, RNAse and PAA or ACV, two inhibitors of the viral DNA polymerase, visualized either at 6 h.p.i. or 20 h.p.i.. In all images: the red signal refers to G4s, detected with the 1H6 primary Ab and Alexa Fluor-546 secondary Ab; the green signal corresponds to the viral recombinant late protein VP16-GFP; the blue signal is obtained staining the DNA with DRAQ5®. In the merged images the blue signal is shown overlapped with the red and green fluorescence. Mock-infected cells were treated in the same conditions except that they lacked HSV-1 infection. Each condition was tested at least three times, always including mock-infected and infected cells as reference. Scale bar: 100 μm. For quantification, the intensity of fluorescence at 20X calculated by the instrument was normalized for the total number of G4-positive cell nuclei and then mock-infected cells. Cells were counted using ImageJ software; at least three pictures per condition (200-500 cells/picture) were considered.



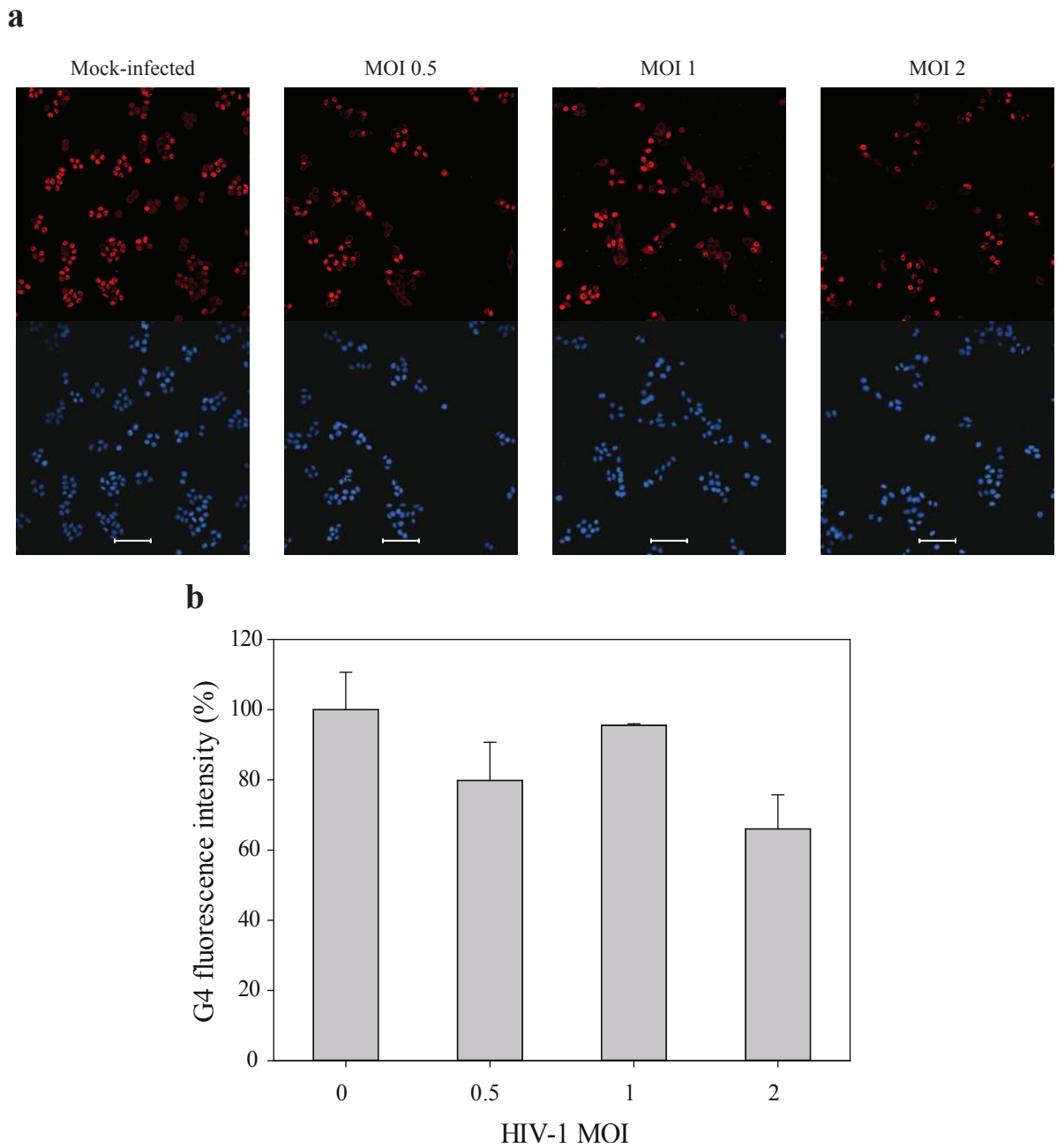
Supplementary Figure 3. Immunofluorescence confocal microscopy of negative control for mock-infected (panel a), wt HSV-1-infected (panel b) and HSV-1 v41-infected (panel c) mammalian cells at 20X magnification. All images represent negative control cells lacking treatment with primary Abs (i.e. 1H6 and anti-ICP8 serum) and treated with secondary Abs (i.e. Alexa Fluor-546 goat anti-mouse IgG or Alexa Fluor-488 goat anti-rabbit IgG). In all images, the blue signal is obtained staining the DNA with DRAQ5®. In panel b the green signal was obtained treating cells with FITC-conjugated anti-HSV-1 ICP8; in panel c the green signal represents the GFP signal from the HSV-1 v41 strain. The upper left subpanels represent DRAQ5® cell nuclear staining, the upper right subpanels show virus labelling, the lower left subpanels labelling for G4s and the lower right subpanels are merged images. Each condition was tested three times. Scale bar: 100 μm.


2 h.p.i.
4 h.p.i.
6 h.p.i.
9 h.p.i.
14 h.p.i.
20 h.p.i.

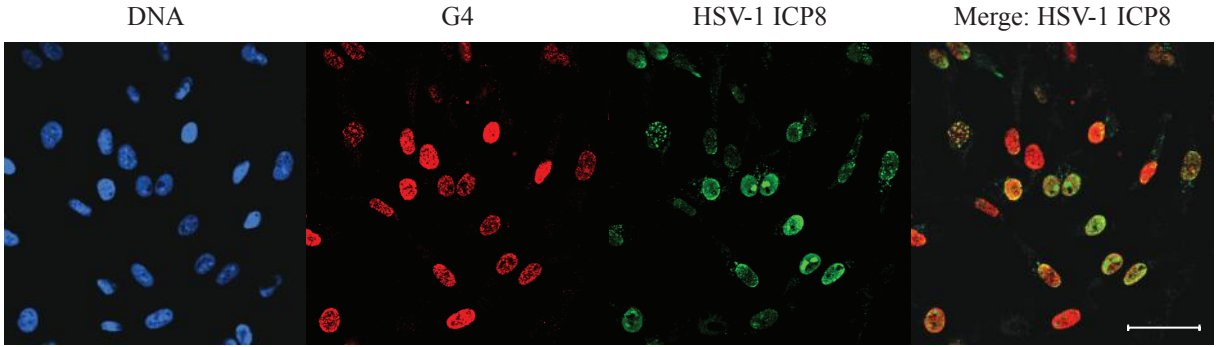
Supplementary Figure 4. Enlargement of the G4 signal at different h.p.i of Fig. 2a in the main text. Immunofluorescence confocal microscopy of HSV-1 v41-infected mammalian cells at 60X magnification. The red signal indicates G4s, detected with the 1H6 primary Ab and Alexa Fluor-546 secondary Ab. 60X magnification. Scale bars: 50 μm.



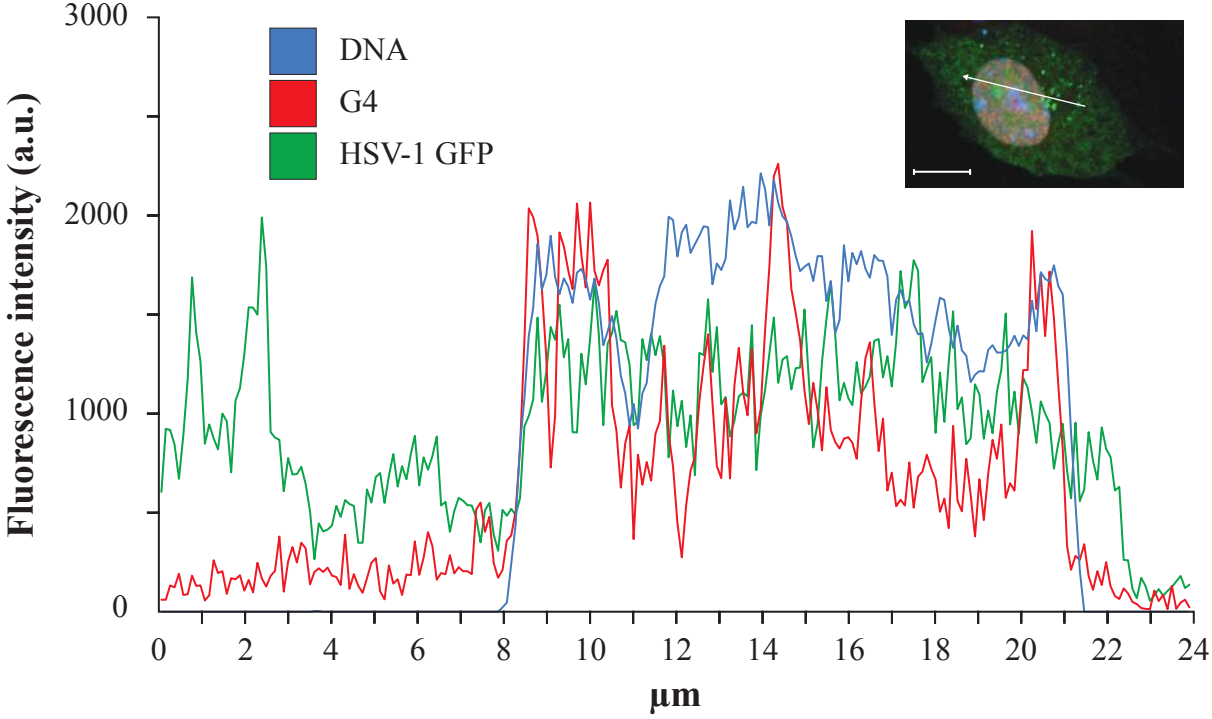
Supplementary Figure 5. Two-dimension (2D) confocal microscopy of HSV-1 v41 infected cell at 15 h.p.i.. Red fluorescence indicates G4s (1H6), which are mainly localized at the internal layer of the nuclear membrane; the green fluorescence shows the late viral protein VP16-GFP, mainly accumulated in the cytoplasm at this time p.i.; the blue fluorescence shows the DNA dispersed in the cell nucleus. Scale bar: 20 μm.



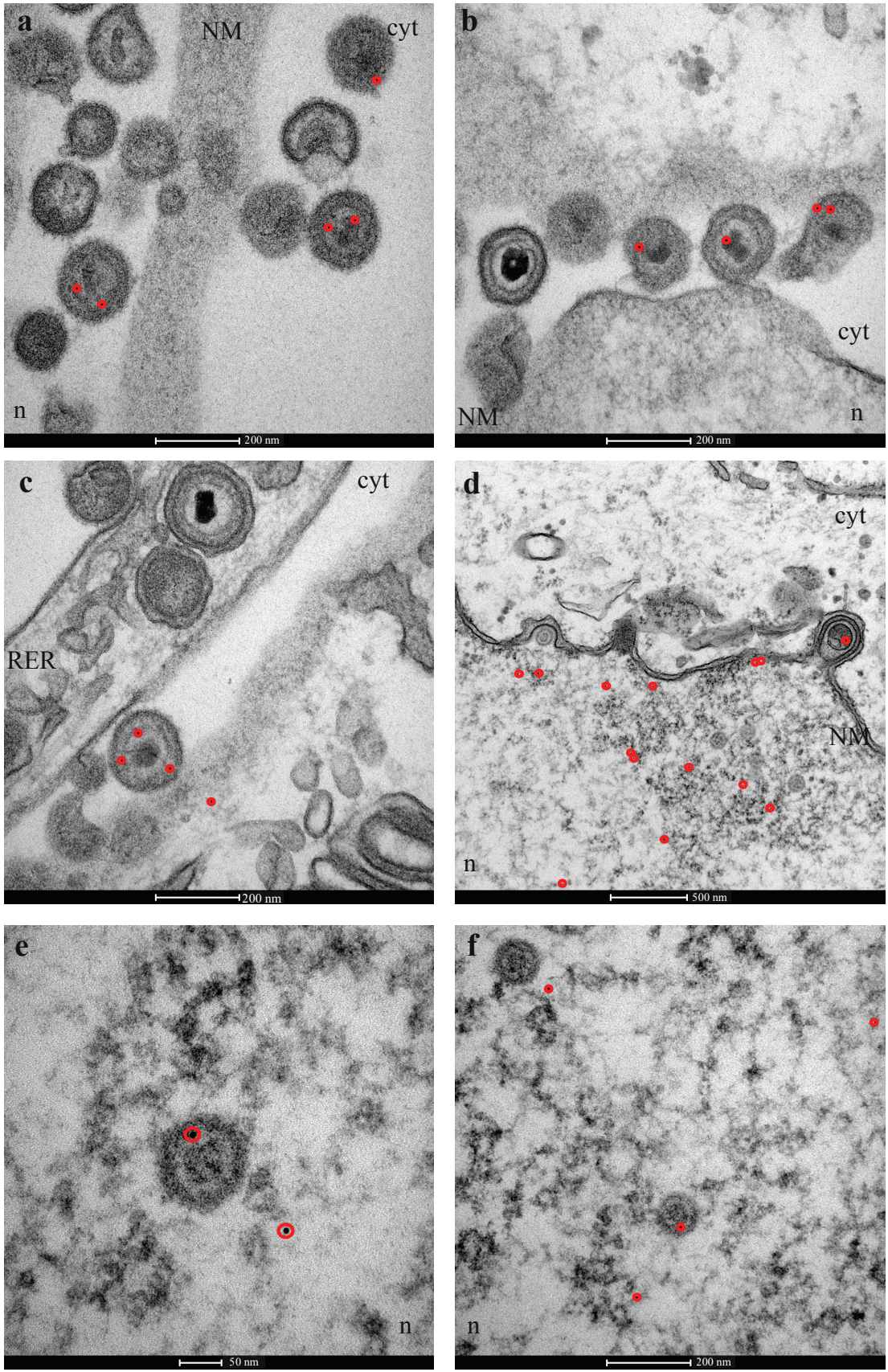
Supplementary Figure 6. Immunofluorescence confocal microscopy of HIV-1 -infected mammalian cells at 20X magnification. Cells were infected at increasing MOI (0.5-2); at 24 h.p.i. cells were fixed for imaging. In all images: the red signal refers to G4s, detected with the 1H6 primary Ab and Alexa Fluor-546 secondary Ab; the blue signal is obtained staining the DNA with DRAQ5®. Mock-infected cells (MOI = 0) were treated in the same conditions except that they lacked HIV-1 infection. Each condition was tested at least three times, always including mock-infected and infected cells as reference. Scale bar: 100 μm. For quantification, the intensity of fluorescence at 20X calculated by the instrument was normalized for the total number of G4-positive cell nuclei and then mock-infected cells. Cells were counted using ImageJ software; at least three pictures per condition (200-500 cells/picture) were considered.



Supplementary Figure 7. Immunofluorescence of HSV-1 v41-infected mammalian cells at 6 h.p.i., at 60X magnification. G4s staining (1H6) is in red, green refers to the 3-83 anti-ICP8 serum and anti-mouse Alexa Fluor-488 (Alx488), DNA nuclear staining is in blue (DRAQ5®). The blue signal is shown overlapped with the red and green fluorescence. Mock-infected 1H6-stained cells are shown in Fig. 2A. Scale bar: 50 μm



Supplementary Figure 8. Intensity profile of a representative HSV-1 v41-infected cell (MOI 0.5) at 6 h.p.i.. DNA is in blue (DRAQ5®), G4s are in red (1H6), green represents the viral recombinant VP16-protein. The profile was obtained with NIS-Elements Advanced Research software, along an ideal 24 µm-long straight line (in white), crossing the nucleus of the cell (right inset). The DNA profile defines the nuclear area where G4 is clearly enclosed. Differently, VP16-GFP fluorescence profile extends also to the cytoplasm. G4 and VP16-GFP exhibit discordant fluorescence profile. Scale bar: 20 µm.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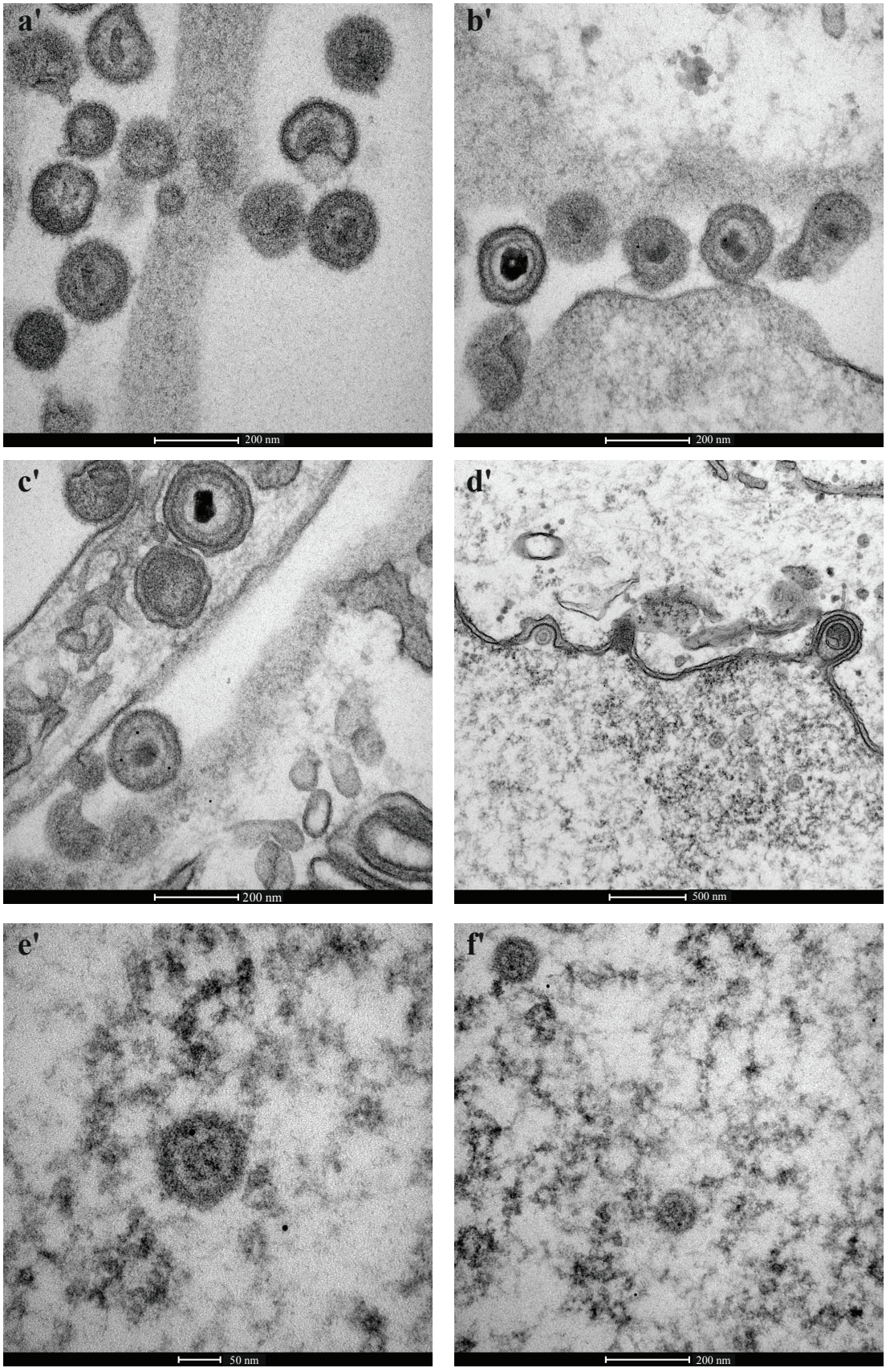
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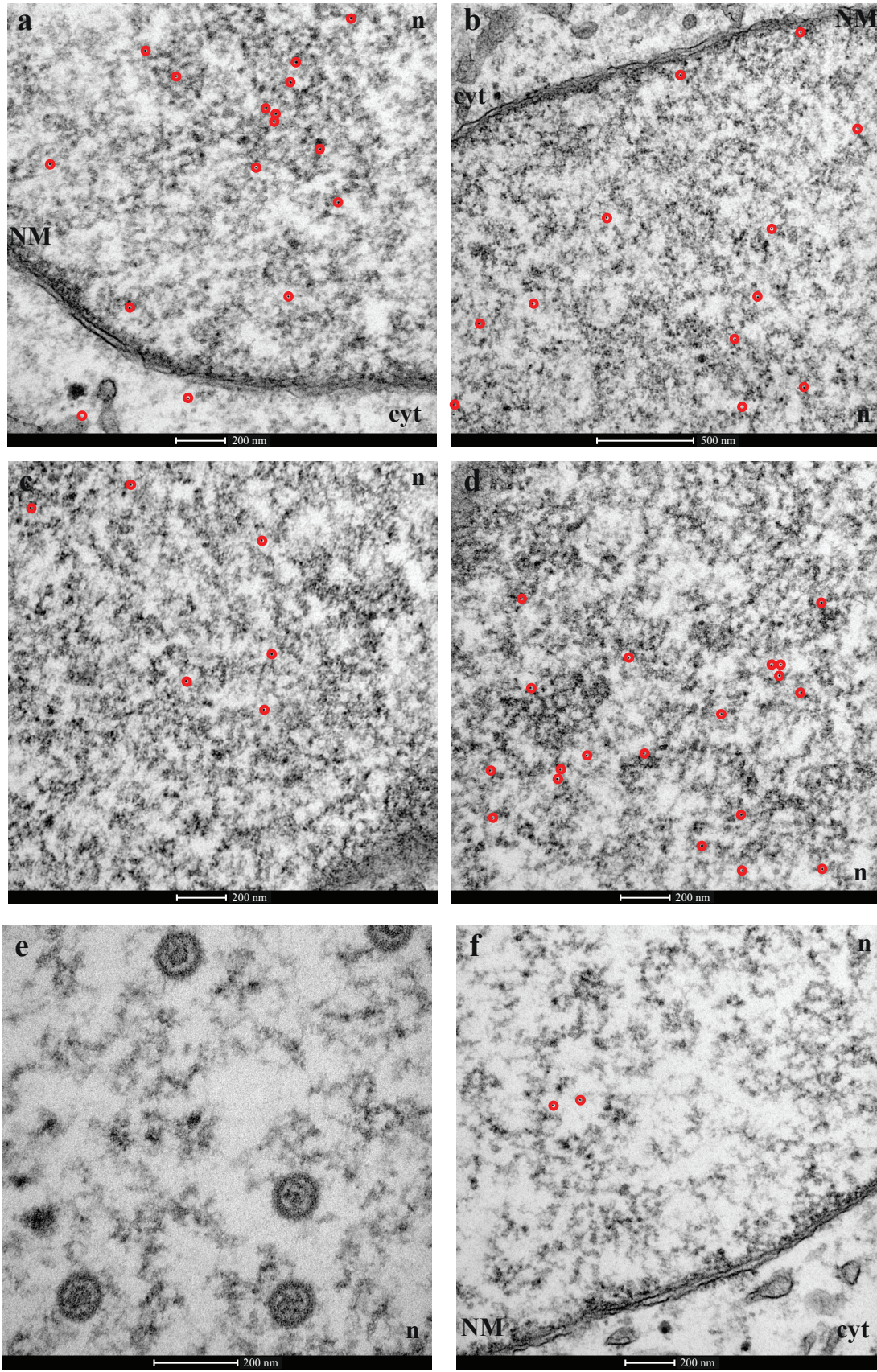
Supplementary Figure 9. Immuno-EM of HSV-1 infected cells, fixed at 15 h.p.i. and incubated with anti-G4 Ab (1H6) and/or 3-83 anti-ICP8 serum. Primary 1H6 and anti-ICP8 Abs were detected with 5-nm and 10-nm gold particles, respectively. To improve image clarity, gold particles were highlighted by red dots (indicating G4s) and green dots (indicating ICP8) in a-f. The original images are provided in the corresponding panels a'-f'. The images show the presence of G4s in HSV-1 virions both in the nucleus and right after nuclear egress. The number of G4s in virions appears higher in virions close to or just outside the nuclear membrane. Panels d-f were treated with anti-G4 Ab only. n indicates the nucleoplasm, cyt the cytoplasm, RER the rough endoplasmic reticulum and NM the nuclear membrane. Bars length is indicated in each panel.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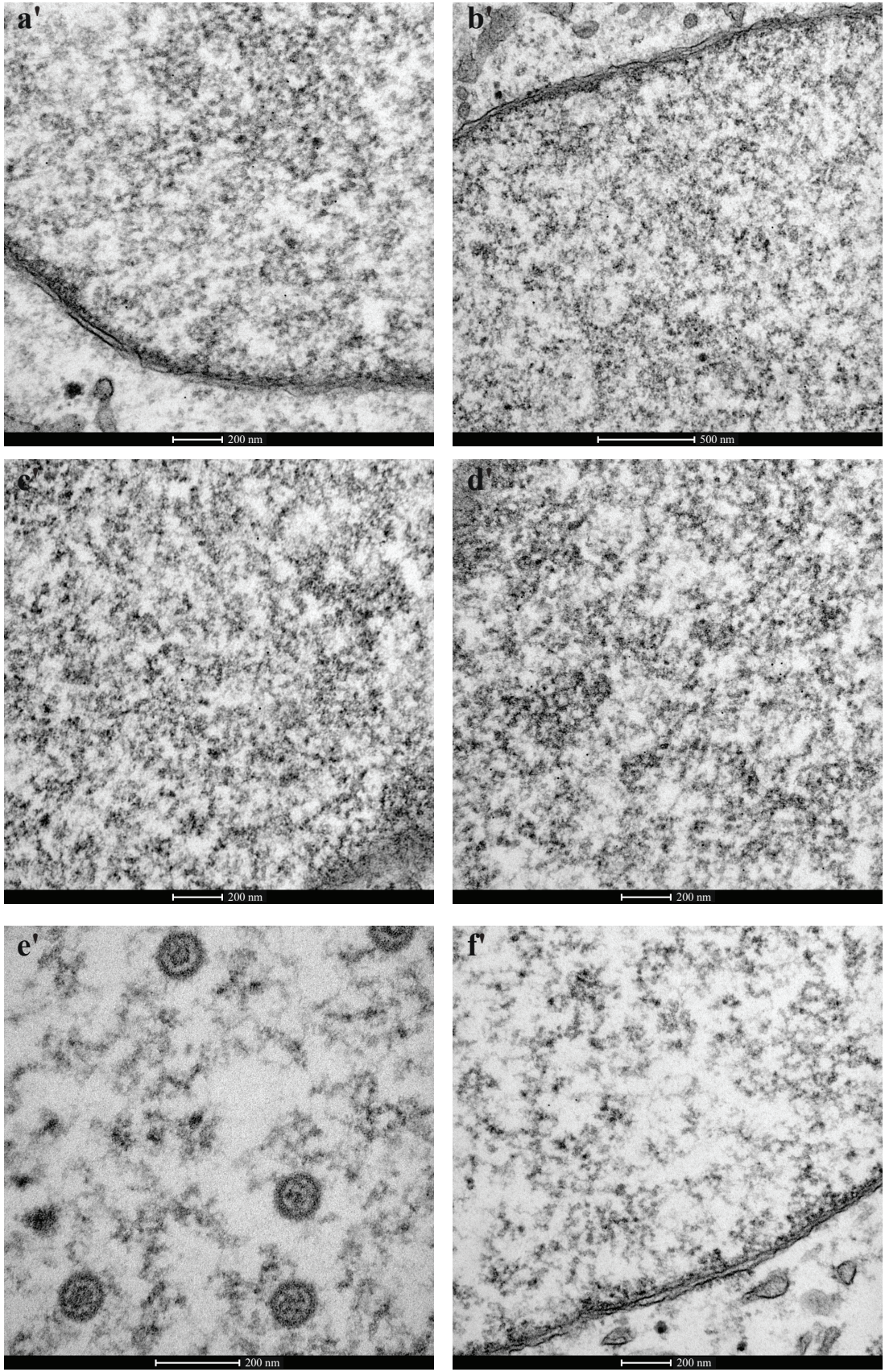
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Supplementary Figure 10. Immuno-EM of mock-infected or HSV-1 infected cells, fixed at 15 h.p.i. Primary 1H6 and anti-ICP8 Abs were detected with 5-nm and 10-nm gold particles, respectively. To improve image clarity, gold particles were highlighted by red dots (indicating G4s) and green dots (indicating ICP8). The original images are provided in the corresponding panels a'-f'. Panels a-d). Mock-infected cells were incubated with anti-G4 Ab (1H6) and 3-83 anti-ICP8 serum. The images show that no ICP8 is unspecifically detected, whereas cellular G4s are detected in a much lower amount and less close to the nuclear membrane than G4s in HSV-1 infected cells (Fig. 5). Panels e- f). HSV-1 infected cells lacking treatment with anti-G4 Ab were treated with gold-particle-conjugated secondary Ab. A very low rate of unspecific labelling is detected (see also Fig. 5g). n indicates the nucleoplasm, cyt the cytoplasm and NM the nuclear membrane. Bars length is indicated in each panel.