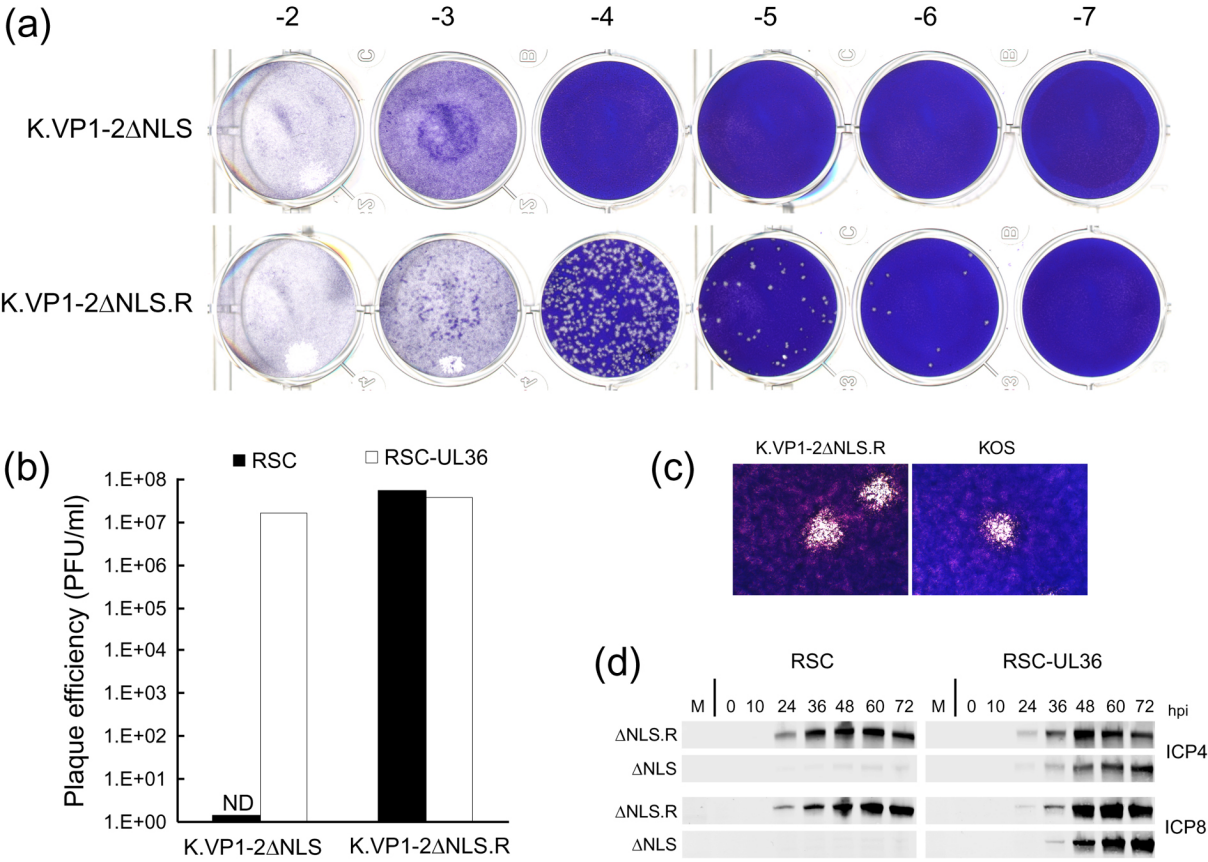
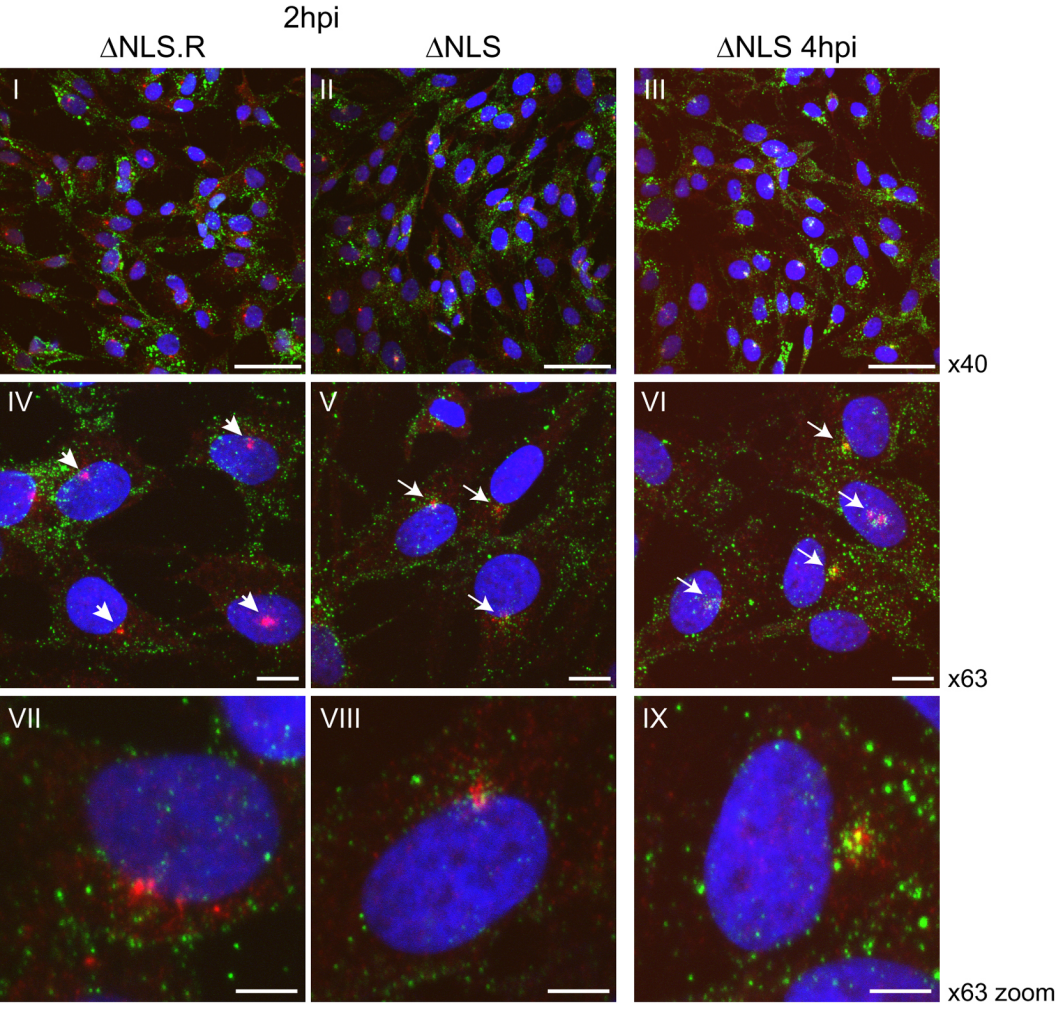
Supplementary Figures

Supplementary Figure 1. Restoration of growth and plaque formation in a revertant virus.

(a) Comparison of plaque formation by K.VP1-2ΔNLS and the revertant K.VP1-2ΔNLS.R in non-complementing RSC cells. Quantitative analysis of plaquing efficiency is shown in panel (b) indicating restoration of plaque formation in non-complementing cells and no increase in complementing cells, unlike the at least 7 logs increase for the mutant. (c) Typical and similar plaque size for the revertant and parental KOS w/t virus. (d) Comparison of protein expression by the mutant and revertant strains in multi-step growth curve after low moi infection in RSC cells or in RSC-HAUL36 cells. In RSC cells expression for the mutant was virtually undetectable, and restored by infection in complementing cells, while expression was restored for the revertant and not significantly different in the complementing cells.

Supplementary Figure 2. Higher magnification images of MTOC and tegument association of K.VP1-2ΔNLS^{NC} capsids. As described in Figure 7, RSC cells were infected with extracellular virus of either the revertant ΔNLS.R or mutant virus ΔNLS produced from non-complementing cells. Infection was at a moi of 100 for the revertant or an equivalent amount of the mutant based on standardisation of VP5 in the extracellular purified virus. Capsid localization (VP5 staining, green) is shown in relation to the MTOC (PCM1 staining red). Images at lower magnification (x 40 objective, panels I-III, scale bar 50 μm) show typical entire fields or higher magnification (x63, panels IV-VI, scale bar 10 μm) showing individual cells. Arrowheads in panels IV-VI point to the MTOC and indicate no distinct clustering of capsids for the w/t versus distinct clustering for the ΔNLS mutant. The lower panels VII-IX show higher magnification images (scale bar 5 μm) showing more detail. These data were quantitated for capsid localisation around the MTOC as described in the text for 130 cells for each virus with the results illustrated in Figure 7.


(a)
-2
-3
-4
-5
-6
-7
K.VP1-2ΔNLS
K.VP1-2ΔNLS.R
(b)
RSC
RSC-UL36
Plaque efficiency (PFU/ml)
1.E+08
1.E+07
1.E+06
1.E+05
1.E+04
1.E+03
1.E+02
1.E+01
1.E+00
ND
K.VP1-2ΔNLS
K.VP1-2ΔNLS.R
(c)
K.VP1-2ΔNLS.R
KOS
(d)
RSC
RSC-UL36
M
0 10 24 36 48 60 72
hpi
ΔNLS.R
ΔNLS
ICP4
ICP8


2hpi
ΔNLS.R
ΔNLS
ΔNLS 4hpi
I
II
III
x40
IV
V
VI
x63
VII
VIII
IX
x63 zoom