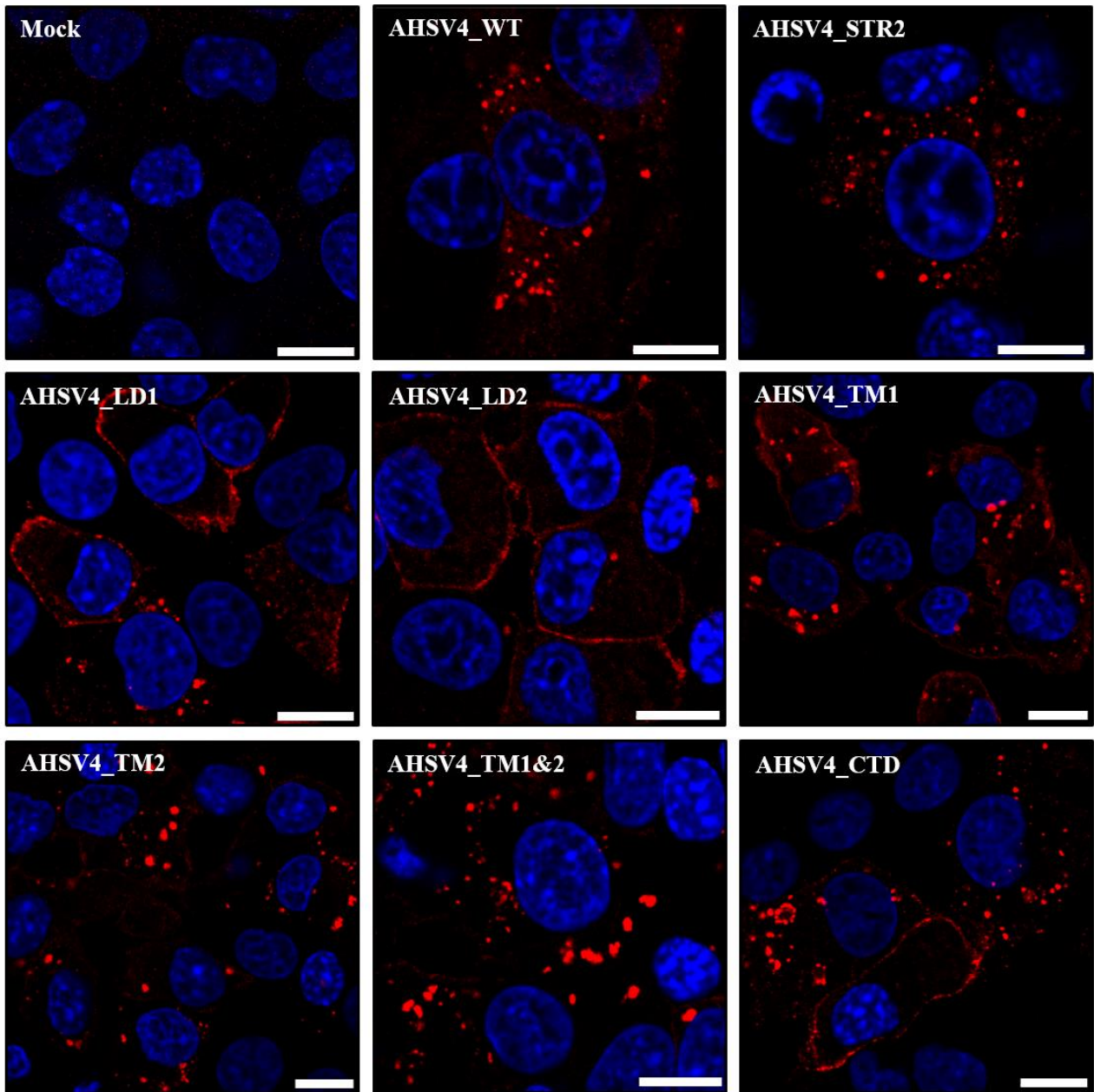


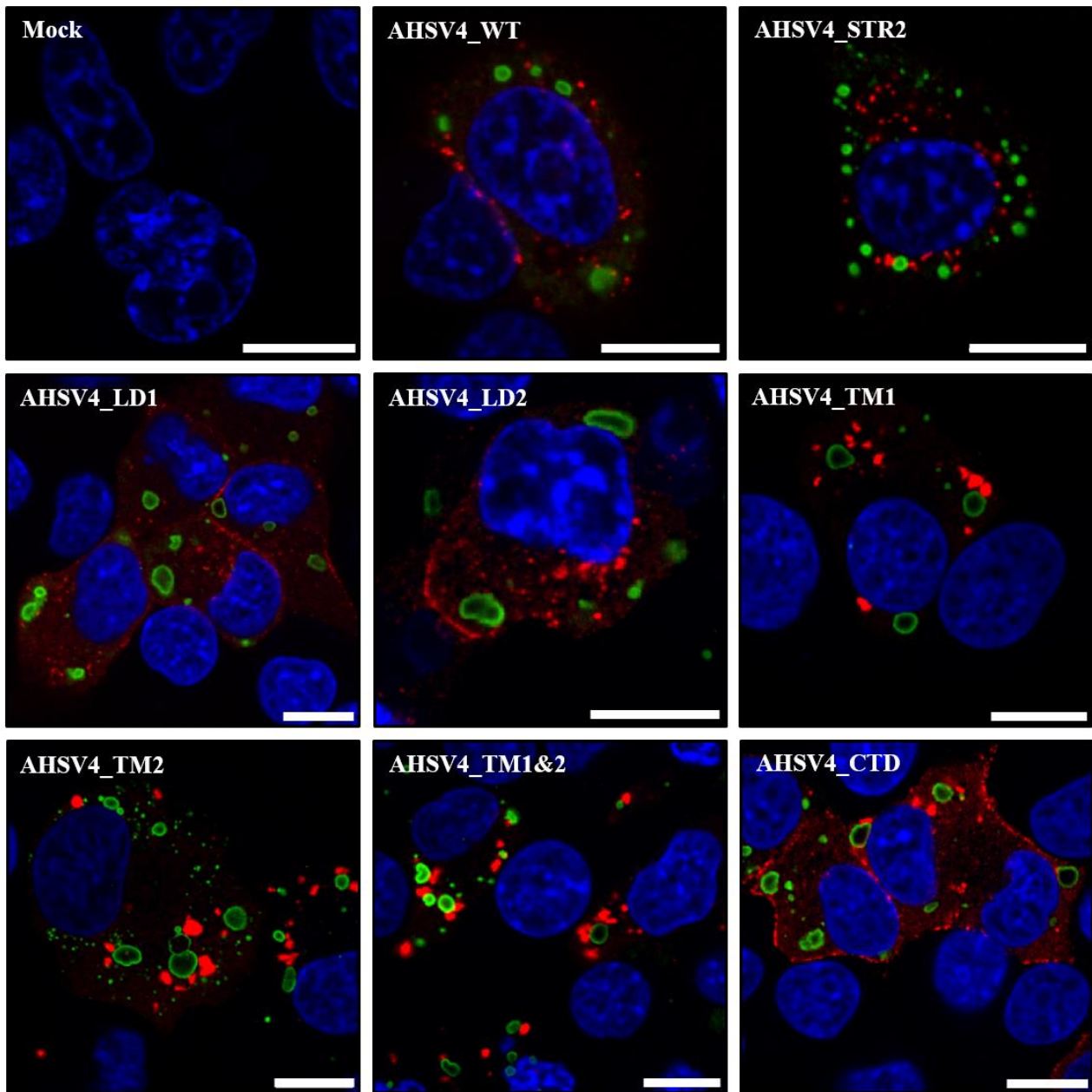
Average titre ( $\text{TCID}_{50}/\text{ml}$ )								
12 h.p.i.								
Sample	WT	STR2	LD1	LD2	TM1	TM2	TM12	CTD
Cell associated	8,14E+04	1,11E+05	1,01E+06	6,34E+06	9,08E+05	2,07E+05	2,19E+05	5,22E+06
Released	8,20E+03	1,01E+05	8,14E+03	5,16E+03	6,92E+02	0,00E+00	0,00E+00	1,32E+03
Total yield	8,96E+04	2,12E+05	1,02E+06	6,34E+06	9,09E+05	2,07E+05	2,19E+05	5,23E+06
24 h.p.i.								
Sample	WT	STR2	LD1	LD2	TM1	TM2	TM12	CTD
Cell associated	8,59E+05	8,77E+05	3,21E+07	5,17E+07	4,16E+06	6,92E+05	1,02E+06	4,69E+06
Released	7,26E+05	5,83E+05	9,36E+04	1,28E+05	4,06E+03	2,90E+03	8,55E+02	6,85E+03
Total yield	1,58E+06	1,46E+06	3,22E+07	5,18E+07	4,17E+06	6,95E+05	1,02E+06	4,70E+06
48 h.p.i.								
Sample	WT	STR2	LD1	LD2	TM1	TM2	TM12	CTD
Cell associated	9,77E+06	6,90E+05	6,78E+06	8,86E+06	6,77E+05	8,38E+05	7,26E+05	7,90E+05
Released	8,35E+06	6,97E+05	4,73E+05	8,18E+05	8,64E+04	1,01E+05	2,91E+05	1,09E+05
Total yield	1,81E+07	1,39E+06	7,26E+06	9,68E+06	7,64E+05	9,39E+05	1,02E+06	8,99E+05
72 h.p.i.								
Sample	WT	STR2	LD1	LD2	TM1	TM2	TM12	CTD
Cell associated	8,41E+06	6,70E+06	2,12E+07	9,92E+06	3,88E+07	2,61E+07	7,05E+06	9,24E+06
Released	5,77E+06	4,00E+06	3,88E+06	7,23E+05	8,87E+05	8,74E+05	6,38E+05	9,31E+05
Total yield	1,42E+07	1,07E+07	2,50E+07	1,06E+07	3,97E+07	2,70E+07	7,69E+06	1,02E+07

Average titre ( $10\log \text{TCID}_{50}/\text{ml}$ )								
12 h.p.i.								
Sample	WT	STR2	LD1	LD2	TM1	TM2	TM12	CTD
Cell associated	4,91	5,04	6,00	6,80	5,96	5,32	5,34	6,72
Released	3,91	5,00	3,91	3,71	2,84	0,00	0,00	3,12
Total yield	4,95	5,33	6,01	6,80	5,96	5,32	5,34	6,72
24 h.p.i.								
Sample	WT	STR2	LD1	LD2	TM1	TM2	TM12	CTD
Cell associated	5,93	5,94	7,51	7,71	6,62	5,84	6,01	6,67
Released	5,86	5,77	4,97	5,11	3,61	3,46	2,93	3,84
Total yield	6,20	6,16	7,51	7,71	6,62	5,84	6,01	6,67
48 h.p.i.								
Sample	WT	STR2	LD1	LD2	TM1	TM2	TM12	CTD
Cell associated	6,99	5,84	6,83	6,95	5,83	5,92	5,86	5,90
Released	6,92	5,84	5,67	5,91	4,94	5,00	5,46	5,04
Total yield	7,26	6,14	6,86	6,99	5,88	5,97	6,01	5,95
72 h.p.i.								
Sample	WT	STR2	LD1	LD2	TM1	TM2	TM12	CTD
Cell associated	6,92	6,83	7,33	7,00	7,59	7,42	6,85	6,97
Released	6,76	6,60	6,59	5,86	5,95	5,94	5,81	5,97
Total yield	7,15	7,03	7,40	7,03	7,60	7,43	6,89	7,01

**Fig. S1.** Growth kinetics of all virus strains in BSR cells over 72 h.p.i.. Titres of virus released, cell associated virus and total virus yield indicated. Tables representing the  $\text{TCID}_{50}$  titre values and log conversions of the titres included. Titrations were done in quadruplicate for all biological repeats at all three time points.

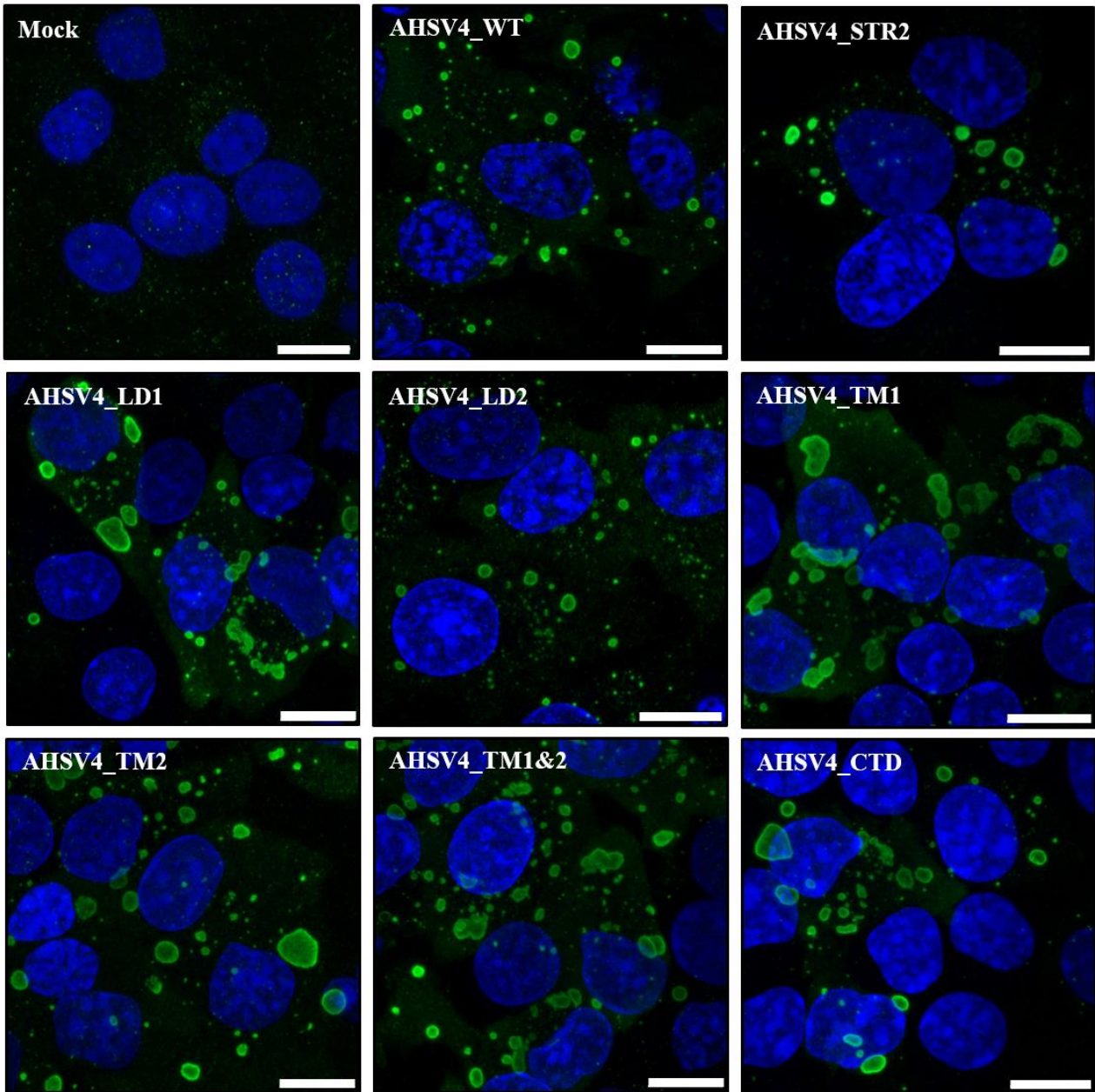


**Fig. S2.** Intracellular distribution of VP5 within AHSV-4 infected mammalian cells. BSR monolayers were infected with wild-type or mutant AHSV-4 virus strains and fixed at 24 h p.i. Mock infected cells were included as control. VP5 was detected with an anti-VP5 antibody (mouse) and Alexa Fluor™ 594 conjugated goat anti-mouse secondary antibody (red). Nuclei were stained with DAPI (blue). Scale bars represent 10 μm.



**Fig. S3.** Intracellular localisation of VP2 and NS2 within AHSV-4 infected mammalian cells. BSR monolayers were infected with wild-type or mutant AHSV-4 virus strains and fixed at 24 h p.i. Mock infected cells were included as a control. NS2 was detected with an anti-NS2 antibody (rabbit) and Alexa Fluor™ 488 conjugated goat anti-rabbit secondary antibody (green). VP2 was detected with an anti-VP2 antibody (mouse) and Alexa Fluor™ 594 conjugated goat anti-mouse secondary antibody (red). Nuclei were stained with DAPI (blue). Scale bars represent 10 μm.





**Fig. S4.** Representative MIPs of the intracellular distribution of VIBs within AHSV-4 infected mammalian cells. BSR monolayers were infected with wild-type or mutant AHSV-4 virus strains and fixed at 24 h p.i. Mock infected cells were included as control. NS2 was detected with an anti-NS2 antibody (rabbit) and Alexa Fluor™ 488 conjugated goat anti-rabbit secondary antibody (green). Nuclei were stained with DAPI (blue). Scale bars represent 10  $\mu$ m.

**Table 1: List of primers used for QuickChange® II modifications of Seg-10**

Primer	Primer sequence with mismatches underlined (5'-3')	Amino acid change	Length (nucleotides [nt])
<b>STR 1 Forward</b>	GTAAAAATTATCCCTTGTC <u>CG</u> GAATCTAGCTACAATCGCC	M to A	40
<b>STR 1 Reverse</b>	GGCGATTGTAGCTAGATT <u>CGCG</u> GACAAGGGATAATTTAAC		40
<b>STR 2 Forward</b>	CAATCGCCAAGAATTATAG <u>CGCG</u> CATAATGGAGAGTCGGG	M to A	40
<b>STR 2 Reverse</b>	CCCGACTCTCCATTATGC <u>CG</u> CTATAATTCTTGGCGATTG		40
<b>LD1 Forward</b>	CACCACCATAACAATTT <u>CGGAG</u> CTGCTCCGACGTTTTCTC	AS to GA	39
<b>LD1 Reverse</b>	GAGAAAACGTCGGAGC <u>AGCT</u> CGAAATTGTATGGTGGTG		39
<b>LD2 Forward</b>	GATCGTCCCTTATGT <u>GGCAG</u> CACCAGGCAATTTCCGAAAGTGCTCC	PPPY to AAPG	45
<b>LD2 Reverse</b>	GGAGCACTGCGAAATT <u>GCT</u> GGT <u>GCT</u> GCCACATAAGGGACGATC		45

**Table 2: gBlock® gene fragments for introduction of specific mutations to Seg-10**

	gBlock® gene fragment sequence with nucleotide modifications underlined (5'-3')	Amino acid change	Length (nt)
<b>TM 1</b>	GCAACAATGCGTCGAAAGAAATCGGCATTA <del>AAAA</del> AATGATCTTTATTAGT GGATG <u>CCGAACGCGAGATAAA</u> TCGATGGTTGGGGGATTGAGTATCGTTGA CGACGAAATATTAAGAGATTATAAGAACAACG	VTLAT to RTRDK	133
<b>TM 2</b>	GTATCGTTGACGACGAAATATTAAGAGATTATAAGAACAACGATTGGTTAA TGAAGACTATACATGGGCTGAATTTGTTATGTACTACA <u>AGCGCGACGTGG</u> CGGGTAAGATTTCCGATAAAATGCAAGAGGAGATTTACGGACTAAACGT GACATTGCGAAAAGAGAGTCTTACGTGTCAGCGGCGAGTATGTCGTGGAG TGGAG	VLLA to KRDV	207
<b>TM 1&amp;2</b>	GCAACAATGCGTCGAAAGAAATCGGCATTA <del>AAAA</del> AATGATCTTTATTAGT GGATG <u>CCGAACGCGAGATAAA</u> TCGATGGTTGGGGGATTGAGTATCGTTGA CGACGAAATATTAAGAGATTATAAGAACAACGATTGGTTAATGAAGACTA TACATGGGCTGAATTTGTTATGTACTACA <u>AGCGCGACGTGGCGGGTAAGA</u> TTTCCGATAAAATGCAAGAGGAGATTTACGGACTAAACGTGACATTGCGA AAAGAGAGTCTTACGTGTCAGCGGCGAGTATGTCGTGGAGTGGAG	VTLAT to RTRDK VLLA to KRDV	298
<b>CTD</b>	TGTTATGTACTACAGTTTTGTTAGCGGCGGGTAAGATTTCCGATAAAATGC AAGAGGAGATTTACGGACTAAACGTGACATTGCGAAAAGAGAGTCTTAC GTGTCAGCGGCGAGT <u>AGT</u> ATGACCTCCACGAGCGGAAAATCCATCGTGTT GGATG	Deletion of 19 aa at C-terminal end	157

**Table 3: List of primers used for pJAD.S10 linearisation**

Primer	Primer sequence (5'-3')	Length (nt)
<b>TM 1 Forward</b>	ATTATAAGAACAACGATTGGTTAATGAAGACTATAC	36
<b>TM 1 Reverse</b>	TCGACGCATTGTTGCTAACTCCATCTTTAGG	31
<b>TM 2 Forward</b>	TGTCGTGGAGTGGAGATACTGAGATG	26
<b>TM 2 Reverse</b>	CGTCGTCAACGATACTCAATCC	22
<b>CTD Forward</b>	CCATCGTGTTGGATGGATGG	20
<b>CTD Reverse</b>	CTGTAGTACATAACAAATTCAGCCCATGTATAGTC	35