

Supplementary Table 1. Primers and restriction sites for cloning UL50 and UL53 for bacterial expression and immunofluorescence (IF) imaging

^a UL53 constructs for expression in <i>E. coli</i>	Forward and reverse primers	Restriction sites for cloning
UL53Δ321	5'- CGATATCGGCAT ATG TCTAGCGTGAGCGGCGTG 5'- CGATCGGAAT TCTCAGCCGTTGGTGGAAAC	<i>Nde I & EcoR I</i>
UL53Δ292	5'- CGATATCGGCAT ATG TCTAGCGTGAGCGGCGTG 5'- CGATCGGAAT TCTTAGCCGCTGGACTGACACAGCTC	<i>Nde I & EcoR I</i>
UL53Δ50	5'- CGATATCGGCAT ATG TCTAGCGTGAGCGGCGTG 5'- CGATCGGAAT TCTTAGCCGCTGGACTGACACAGCTC	<i>Nde I & EcoR I</i>
^b UL53 site-directed alanine substitutions		
L61A	5'- CCGCGCCTCACG GCA CACGACCTGCAC 5'- GTGCAGGTCGTG TGCC GTGAGGCCGCGG	
H62A	5'- GCGCCTCACGCTG GCC GACCTGCACGAC 5'- GTCGTGCAGGTC GCC CAGCGTGAGGCGC	
L64A	5'- CTCACGCTGCACGAC GCG CACGACATCTTCCG 5'- CGGAAGATGTCGTG GCG TCTGTCAGCGTGAG	
I67A	5'- ACGACCTGCACGAC GCC TTCGCGAGCACC 5'- GGTGCTCGCGAA GCG TCTGTCAGGTCGT	
F68A	5'- CCTGCACGACAT GCC CGCGAGCACCCC 5'- GGGGTGCTCGCG GCG GATGTCGTGCAGG	
R69A	5'- CTGCACGACATCTT GCG GAGCACCCGAACTG 5'- CAGTTCGGGGTCT CGCC GAAGATGTCGTGCAG	
H71A	5'- ACATCTCCGCGAG GCC CCGAACTGGAGC 5'- GCTCCAGTTCGGGG GCC TGCGGAAGATGT	
L74A	5'- GAGCACCCCGAA GCG GAGCTCAAGTAC 5'- GTACTTGAGCT GCG TTCGGGGTGCTC	
E75A	5'- GAGCACCCGAACTG GCG TCAAGTACCTTAAC 5'- GTTAAGGTACTTGAG GCC CAGTTCGGGGTGCTC	
L76A	5'- CGAGCACCCGAACTGGAG GCG AAGTACCTTAACATGATGAA 5'- TTCATCATGTAAAGTACTT GCC TCCAGTTCGGGGTGCTCG	
L79A	5'- GAGCTCAAGTAC GCG AACATGATGAAG 5'- CTTTCATCATGTT GCG TACTTGAGCTC	
M82A	5'- TACCTTAACATG GCG AAGATGGCCATC 5'- GATGGCCATCTT GCC CATGTTAAGGTA	
E73A/K77A	5'- GAGCACCC GCG CTGGAGCT GCG TACCTTAAC 5'- GTTAAGGTA GCG GAGCTCCAG GCG GGGGTGCTC	
D63A/D66A/E70A	5'- TGCAC GCC TGCAC GCC ATCTTCCGC GCG CACC 5'- GGTG GCG CGCGGAAGAT GCG CTGCAG GCG GTGCA	
^c UL50 constructs for expression in <i>E. coli</i>		
UL50Δ349	5'- CGATATCGGCAT ATG GAGATGAACAAGTTCTC 5'- CGATCGGAAT TCTT ACCCGAGCGCGCTCGCCACCA	<i>Nde I & EcoR I</i>
UL50Δ310	5'- GATTACGGAT CCATG GAGATGAACAAGTTCTC 5'- CGATCGGAAT TCTCA ACTCACGTGCTCCTCCAG	<i>BamH I & EcoR I</i>
UL50Δ208	5'- CGAACGGCGGGATAACGGTCTCTCGG 5'- CCGAGAGGACCG TAT CCCCCGTTCCG	
UL50Δ169	5'- GAGCTGTACCCAAGT AAG CCCGCTCGACG 5'- CGTCGACGCGG CTT ACTTGCGGTACAGCTC	
^d HA-UL50 construct for IF	5'- AT GGTACC TATCCATATGACGTCCAGACTATGCCATGGAGATGA ACAAGG 5'- GATAGAAT TCTC AGTCGCGGTGTGCGGAGCGTGTCTCG	<i>Kpn I & EcoR I</i>
^e FLAG-UL53 construct for IF	5'- AT GGTACC GATTATAAAGATGACGATGACAAA ATG TCTAGCGTGA GCGGC 5'- GATATCGGGAAT TCTCA AGGCGCACGAATGCTGTT	<i>Kpn I & EcoR I</i>

^a *Nde* I and *Eco*R I sites are underlined; start and stop codons are shown in bold.

^b Altered codons are shown in red.

^c *Nde* I and *Eco*R I sites are underlined; start and stop codons are shown in bold.

^d *Kpn* I and *Eco*R I sites are underlined; start and stop codons are shown in bold; nucleotides coding for the HA tag are italicized.

^e *Kpn* I and *Eco*R I sites are underlined; start and stop codons are shown in bold; nucleotides coding for the FLAG tag are italicized.