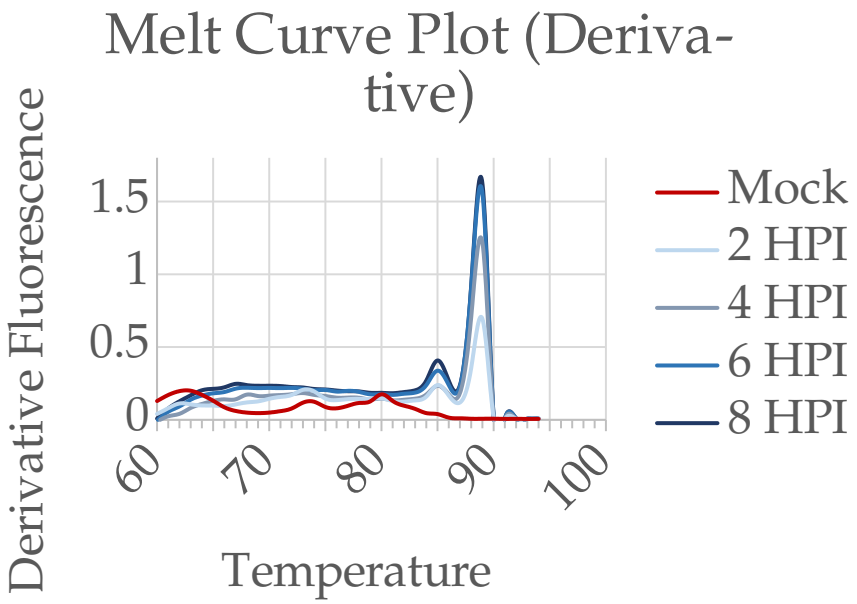
Evidence of a protein-coding gene antisense to the U_L5 gene in Bovine Herpesvirus I

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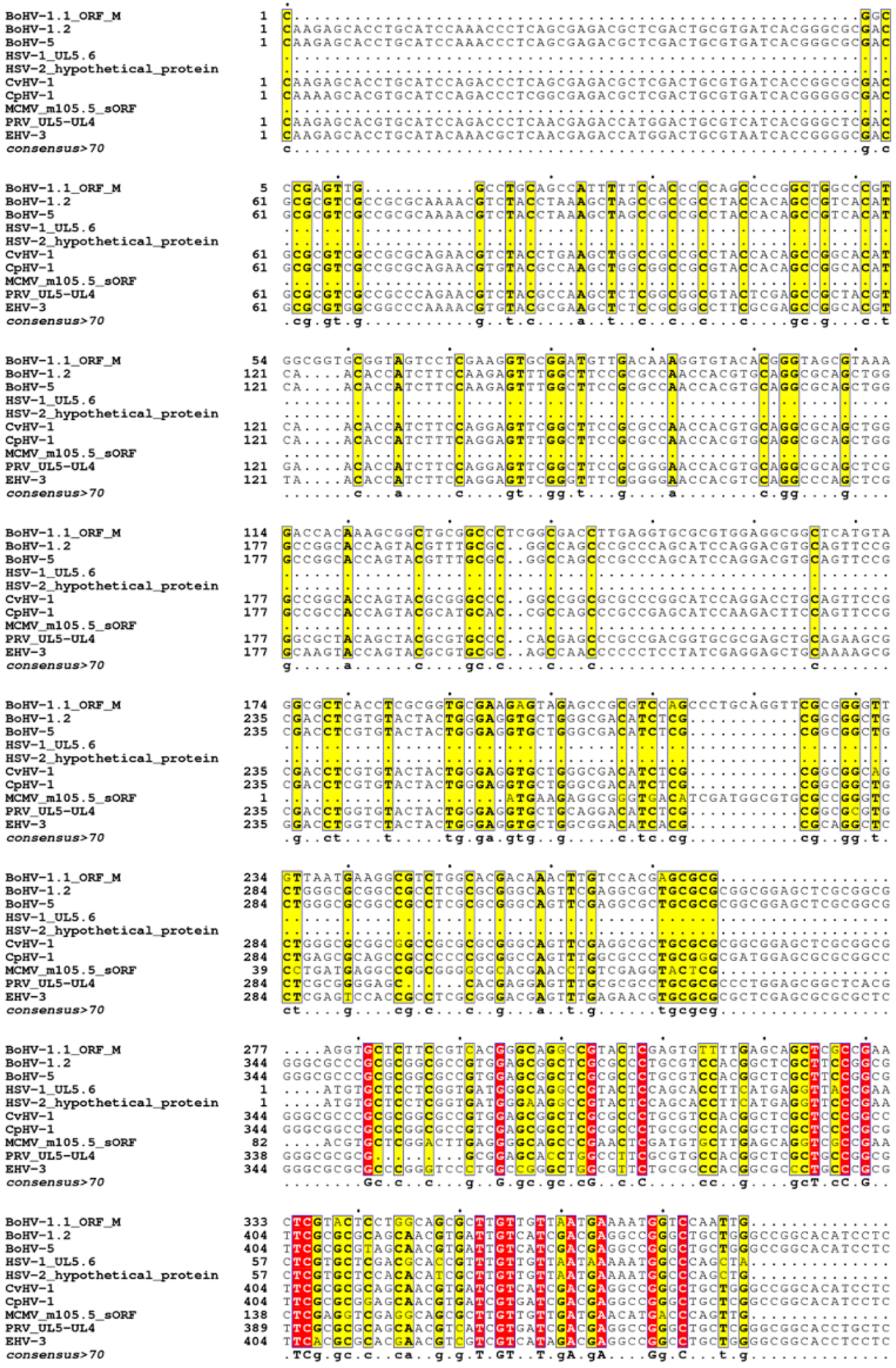
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Supplementary Figures S1-S6

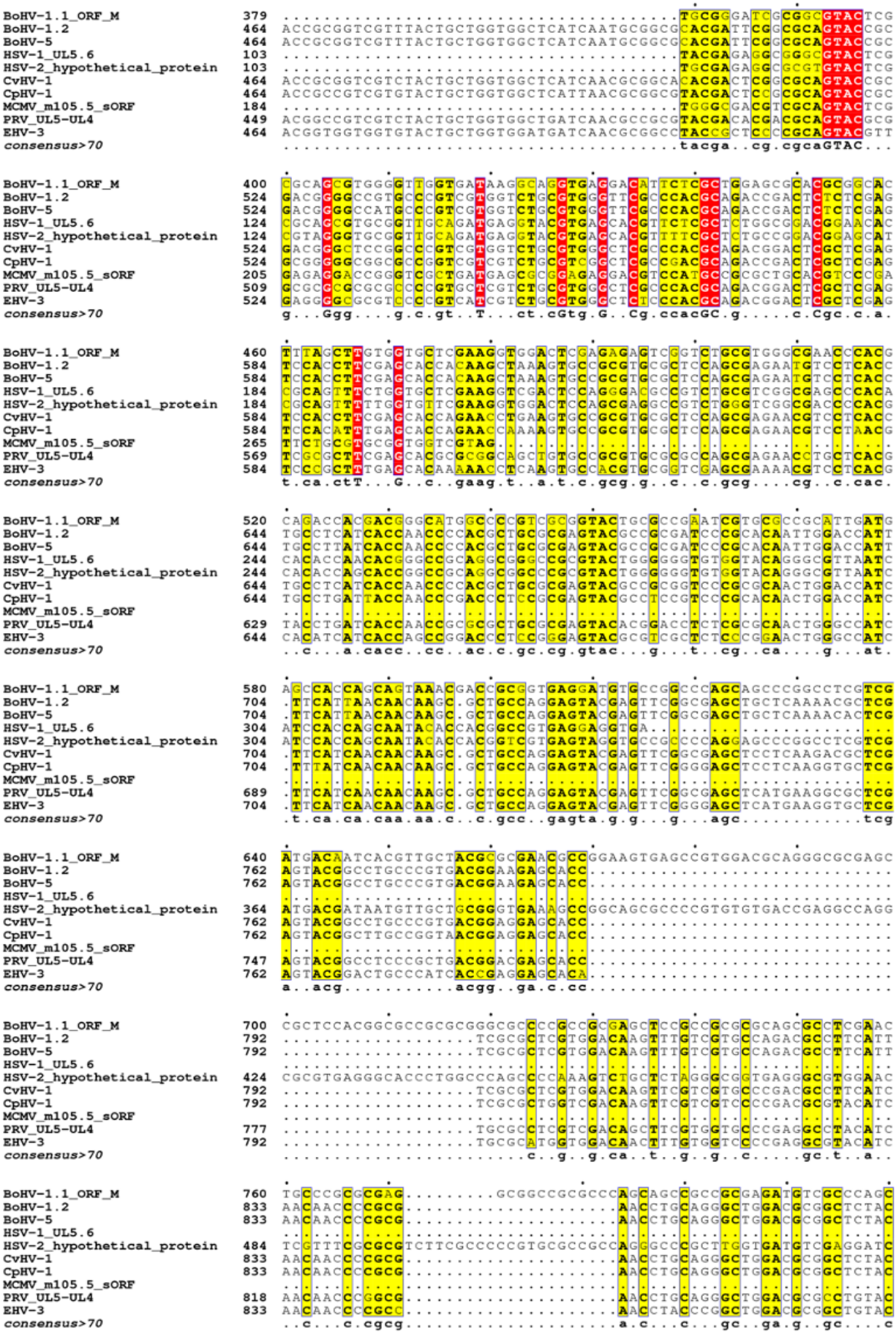


Supplementary Figure S1. Derivative melt curve plot for ORF M primers. The derivative fluorescence detected from 60 to 100C is illustrated for mock-infected and cells infected for 2, 4, 6, and 8 hours. All infected samples display sharp peaks, and no distinct peak is produced by mock samples.

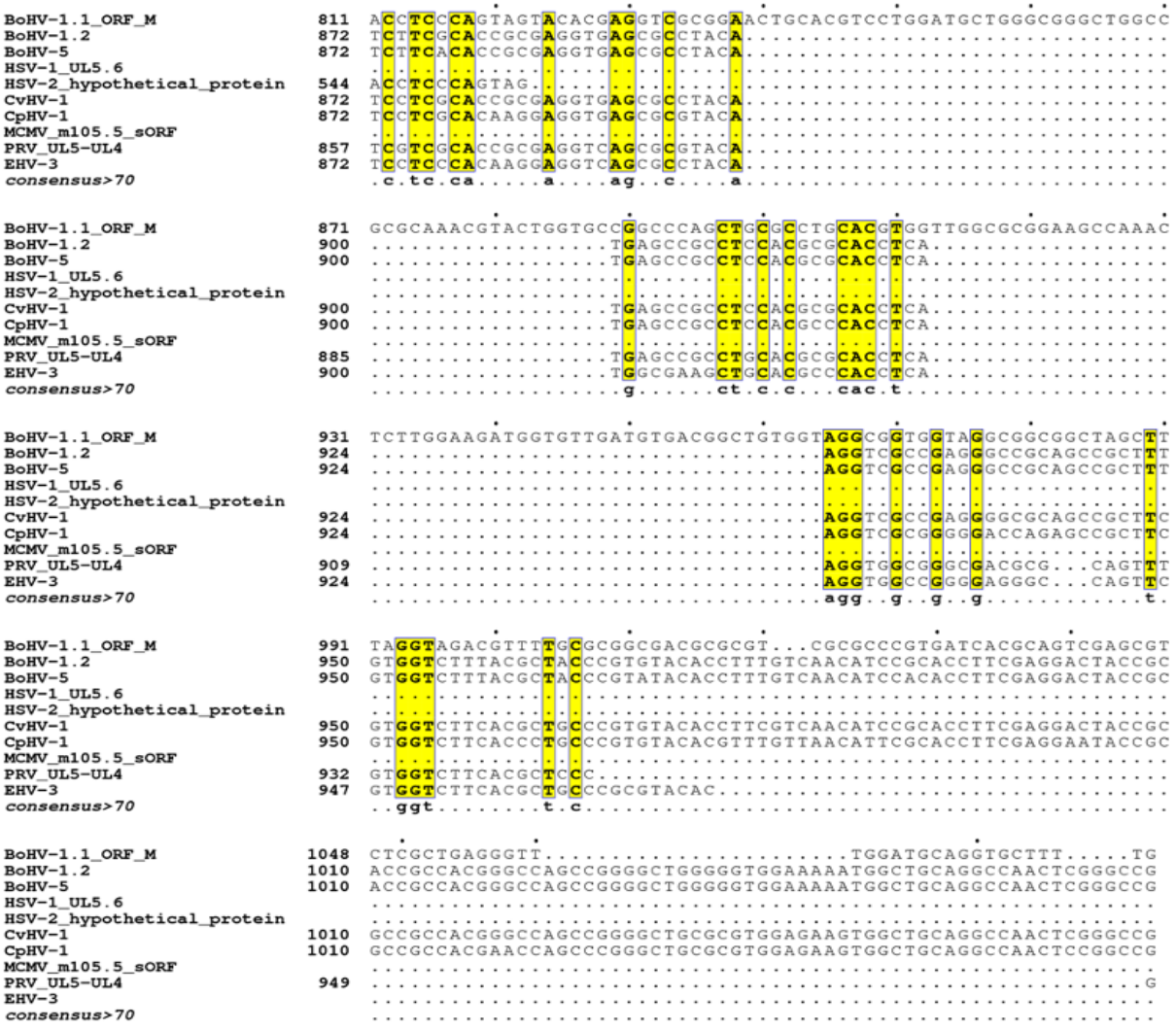
S2.1


BoHV-1.1_ORF_M
BoHV-1.2
BoHV-5
HSV-1_UL5.6
HSV-2_hypothetical_protein
CvHV-1
CpHV-1
MCMV_m105.5_sORF
PRV_UL5-UL4
EHV-3
consensus>70

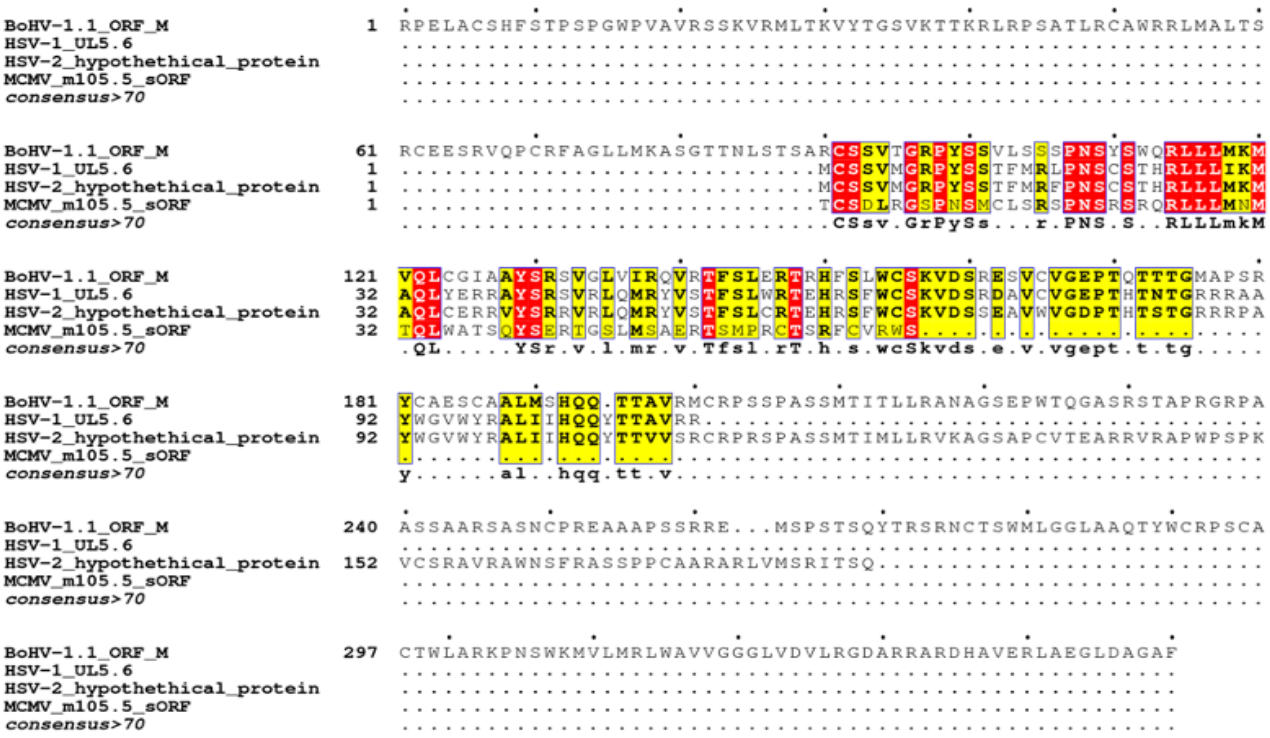
S2.2


BoHV-1.1_ORF_M
BoHV-1.2
BoHV-5
HSV-1_UL5.6
HSV-2_hypothetical_protein
CvHV-1
CpHV-1
MCMV_m105.5_sORF
PRV_UL5-UL4
EHV-3
consensus>70

S2.3

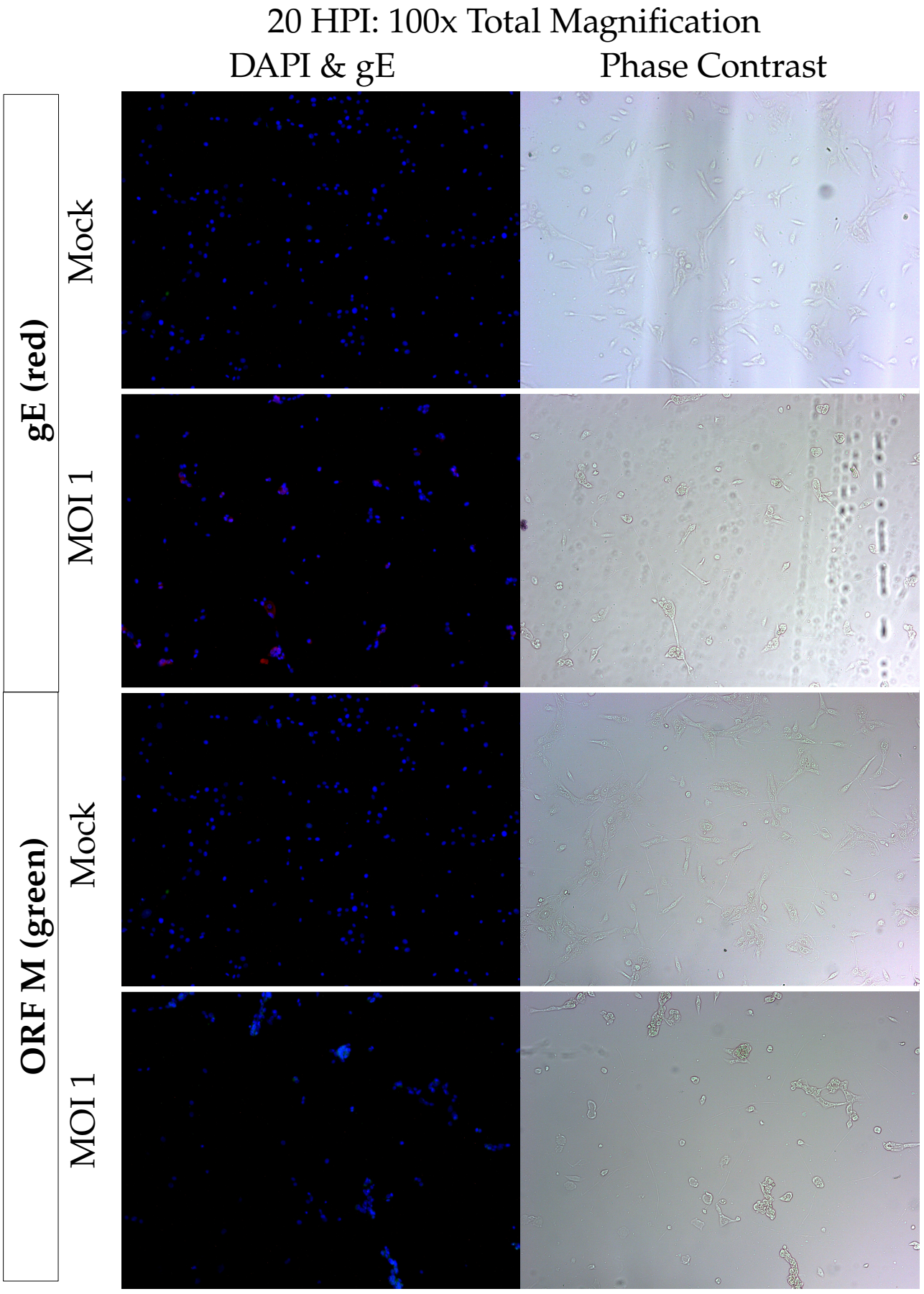


Supplementary Figure S2. Multiple sequence alignment using full ORF M nucleotide sequence (divided in three panels).



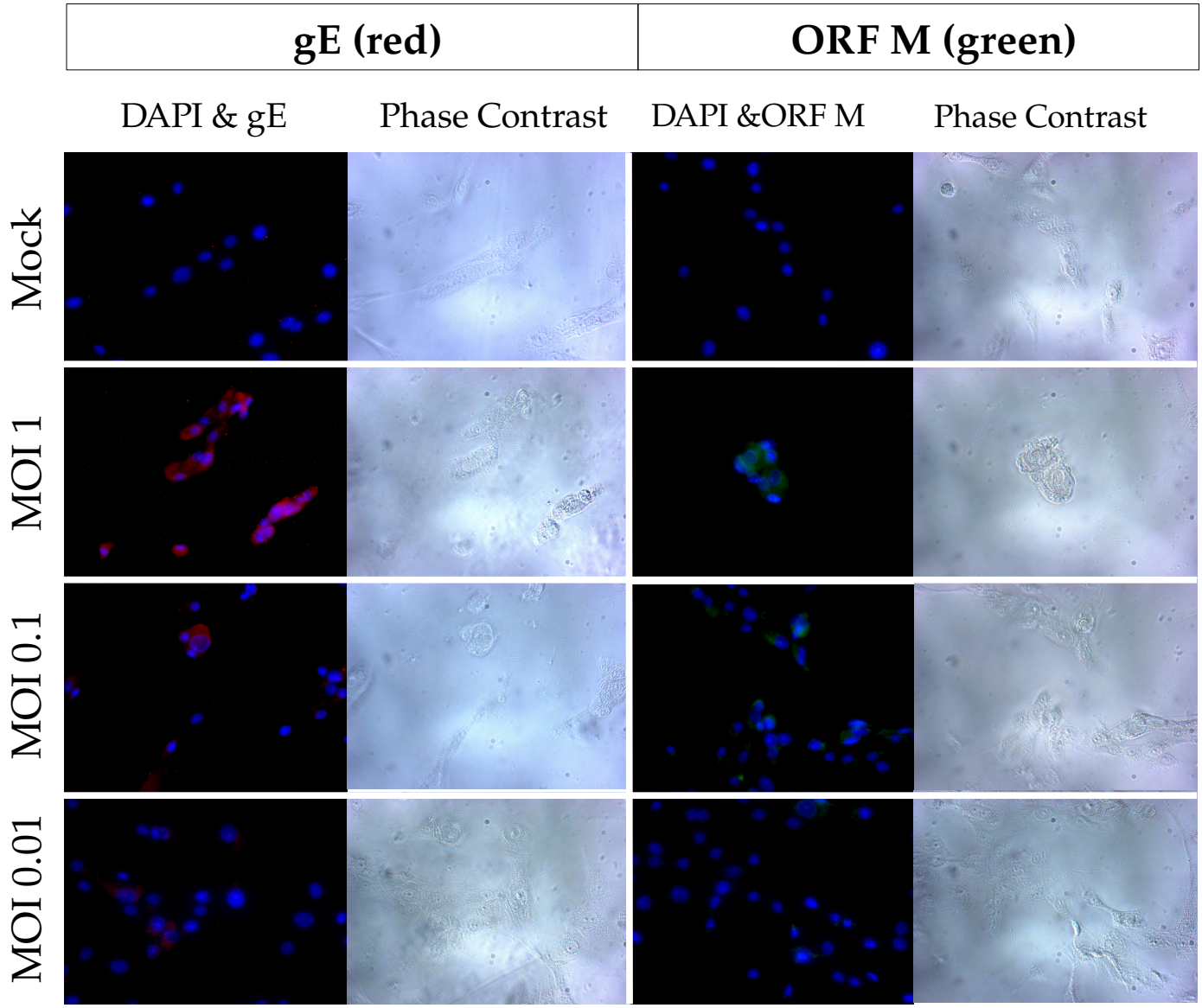
Supplementary Figure S3. Multiple sequence alignment using the full ORF M amino acid sequence.

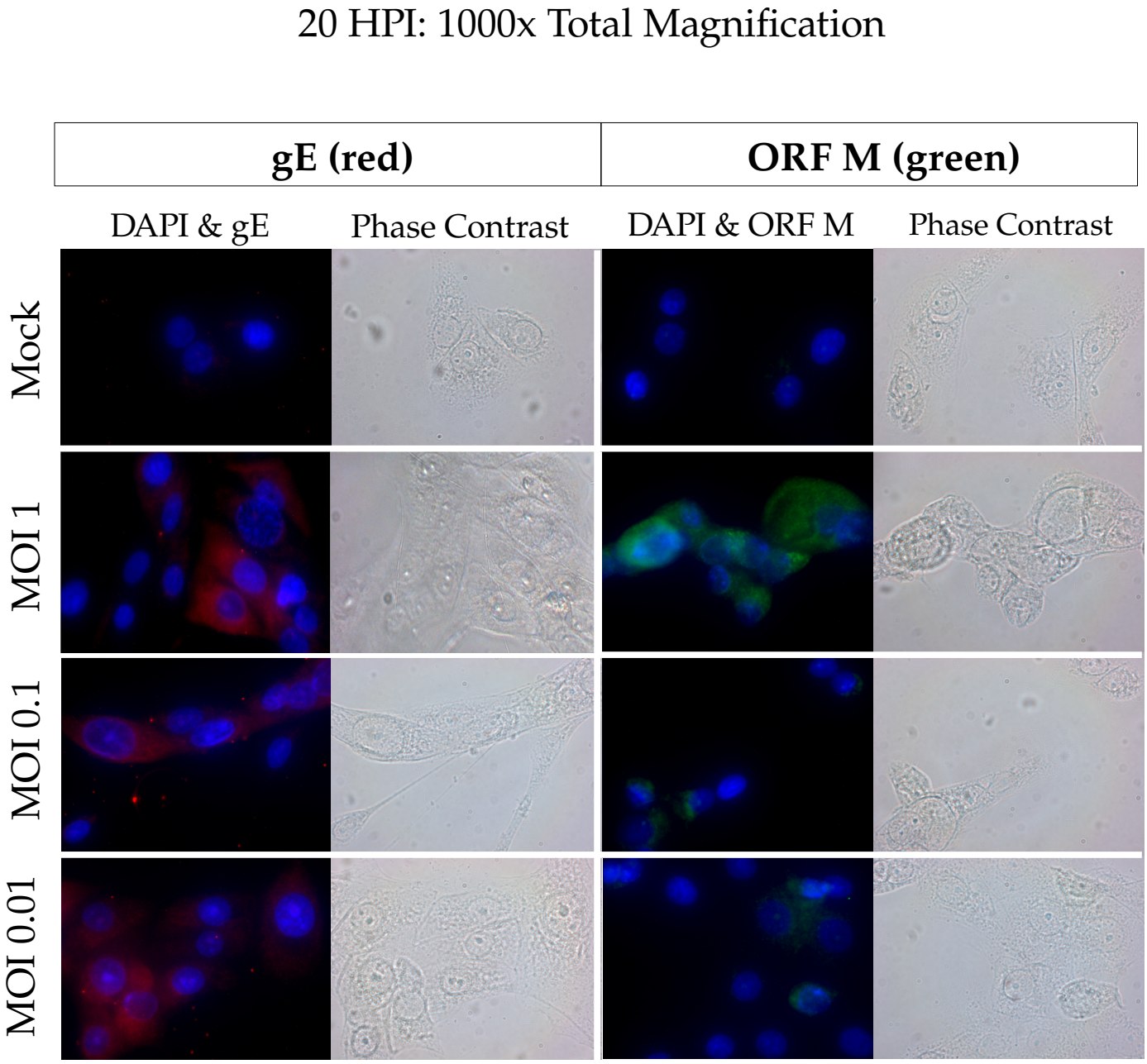
S4.1


20 HPI: 100x Total Magnification
DAPI & gE
Phase Contrast
gE (red)
ORF M (green)
Mock
MOI 1
Mock
MOI 1

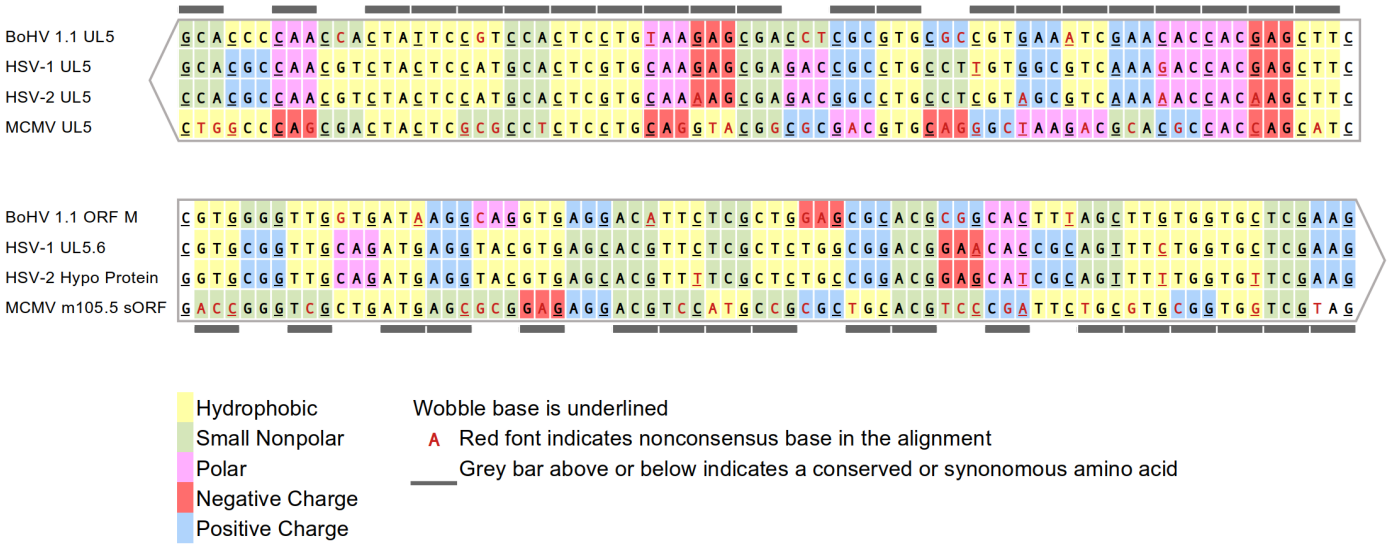
S4.2

20 HPI: 400x Total Magnification

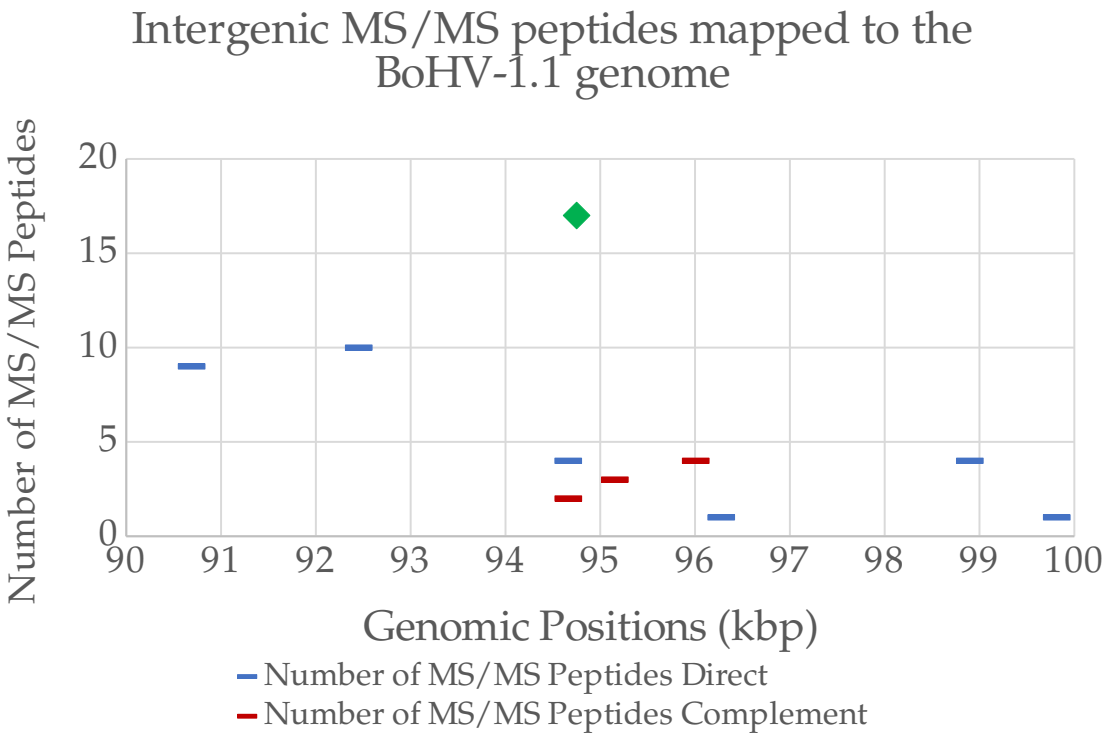




Supplementary Figure S4. Images of MDBK cells infected at various MOIs for 20 hours displayed at 100x, 400x, and 1000x (total) magnification. Images were captured with DAPI (blue), Texas Red (red) or FITC (green) filters. The phase contrast image to the right shows the appearance of cultured cells. Viral glycoprotein E was detected using an AlexaFluor594 secondary antibody (red), while ORF M was detected using an AlexaFluor488 conjugate secondary antibody (green). Nuclei were stained with DAPI stain (blue).



Supplementary Figure S5. Amino Acid Conservation of U_L5 Homologs and Antisense Sequences. Using the nucleotide sequence of the p-loop (phosphate binding region) of the helicase subunit coded by UL5 that overlaps ORF M and its homologs, this diagram illustrates how conservation of UL5 amino acid sequence does not necessarily transfer to the same conservation in the antisense amino acid sequence. Direction of each amino acid sequence is indicated with the gray arrow-shaped outline.



Supplementary Figure S6. Visual representation of intergenic peptides detected by MS/MS that mapped to genomic coordinates 90,000-100,000 Peptides mapping to both direct and complement strands are shown. ORF M is differentiated from other complement peptides by the green diamond.