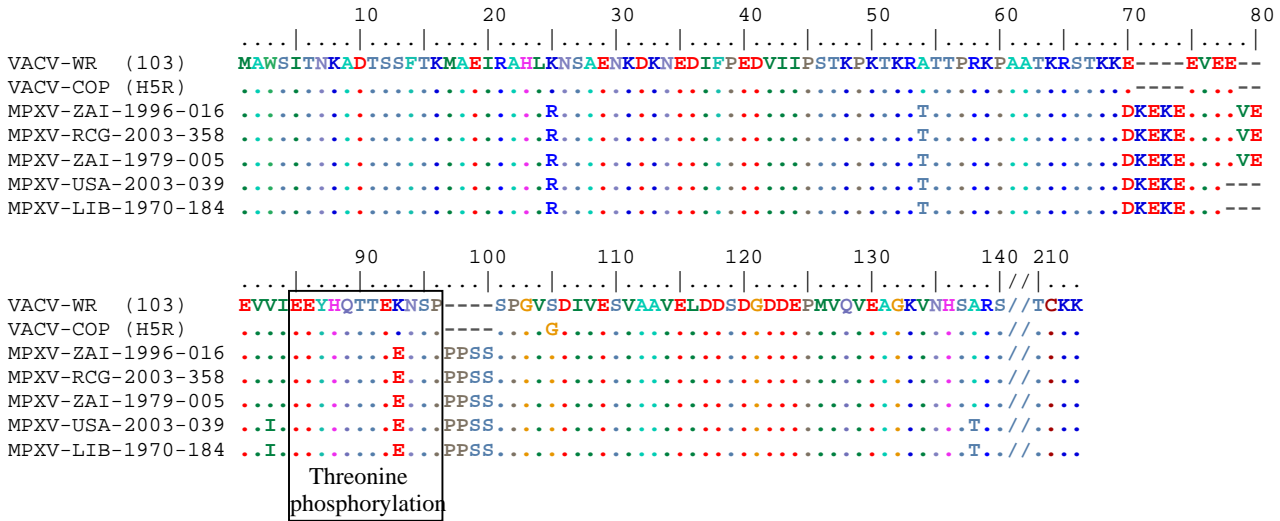
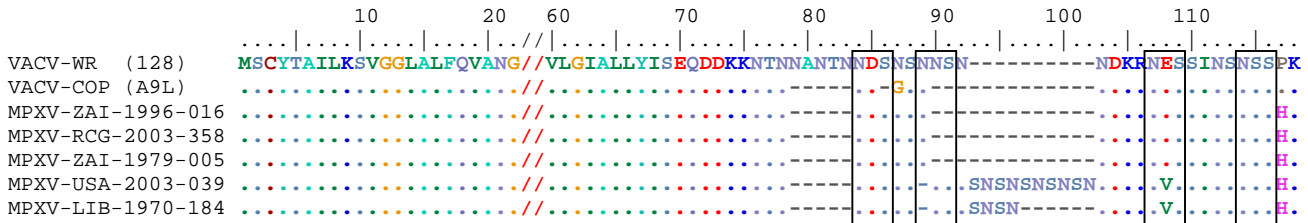


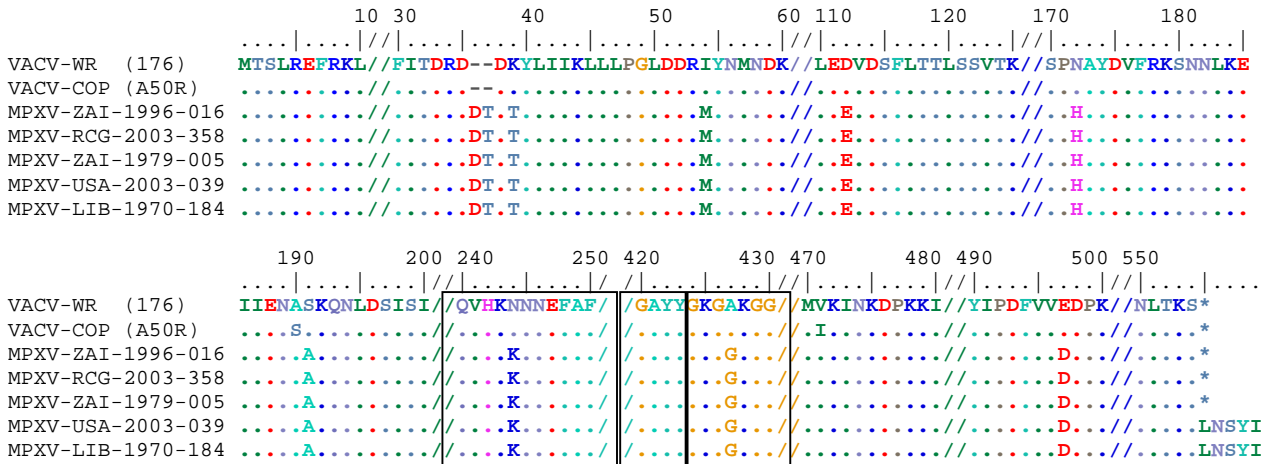
(a) H5R of VACV-COP homologues [viral late transcription factor (VLTF-4)]



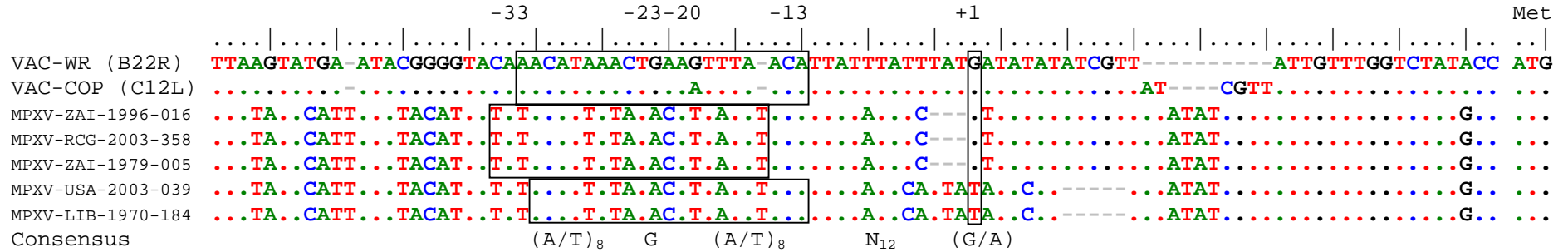
(b) A9L of VACV-COP homologues (Essential morphogenesis factor – membrane associated)



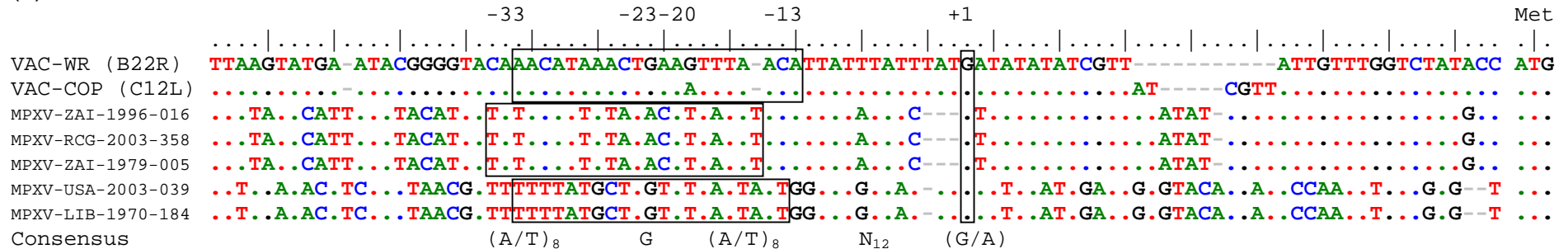
(c) A50R of VACV-COP homologues (DNA ligase)



(a)



(b)



Supplementary Fig. S2. Predicted promoter regions for SPI-1 homologues. Within vaccinia virus strain Western Reserve (VAC-WR), the transcription start site (+1, G boxed above) has been characterized for SPI-1 (B22R) (Kettle *et al.*, 1995). An upstream region fitting the consensus for early orthopoxviral promoters is found within vaccinia and all of the monkeypox virus strains (data not shown). However, a variable length insertion within the monkeypox virus strains introduces hundreds of nucleotides (~200–300 nt) between the transcription start site and the putative translational start site. This repeated insertion may disrupt efficient expression from the conserved predicted promoter within the monkeypox virus strains. Furthermore, the West African/USA MPXVs do not contain a purine at the putative transcription start site. When the sequences adjacent to the predicted translational start site for the Congo Basin MPXVs were examined, a putative promoter sequence was identified (a). The consensus sequence for early orthopoxviral promoters requires a purine (A/G) at the transcription start site (+1), with a core sequence (boxed area, –13 to –33) located 12 nt upstream (Broyles, 2003; Davison & Moss, 1989). This core sequence is variable, but always very adenosine- (A) and thymidine (T)-rich and usually contains a guanosine (G) (position –20 or –21) involved in contacting the early transcription factors (Broyles, 2003). As shown above, the promoter sequence upstream of the predicted start site of the Congo Basin SPI-1 homologues aligns well with the consensus sequence (a). This region possesses both the purine (G or A) at the transcription start site (+1) as well as a very A/T-rich core region. Although the West African/USA MPXVs also contain an A/T-rich core region, they lack a purine at the transcription start site, which may decrease transcription of the West African/USA strains from this position. Within the West African/USA MPXVs, a sequence with higher homology to early orthopoxvirus promoters is found upstream of the variable length insertions (b). The A/T-rich core contains a G, as does the putative transcriptional start site. If this promoter is used, a transcript encoding the IY repeats would be made only within the West African/USA strains. The exact location of transcriptional initiation for all monkeypox virus strains awaits experimental investigation. However, based upon nucleotide alignments, the West African/USA strains are predicted to initiate transcription prior to the repetitive IY sequence. (a) Alignment of the previously mapped vaccinia virus strain Western Reserve SPI-1 promoter nucleotide sequence compared with nucleotide sequences a similar distance from the vaccinia translational start site within monkeypox virus homologues. (b) Alignment of nucleotide sequences preceding the predicted SPI-1 translational start sites, with high similarity to consensus early orthopoxvirus

