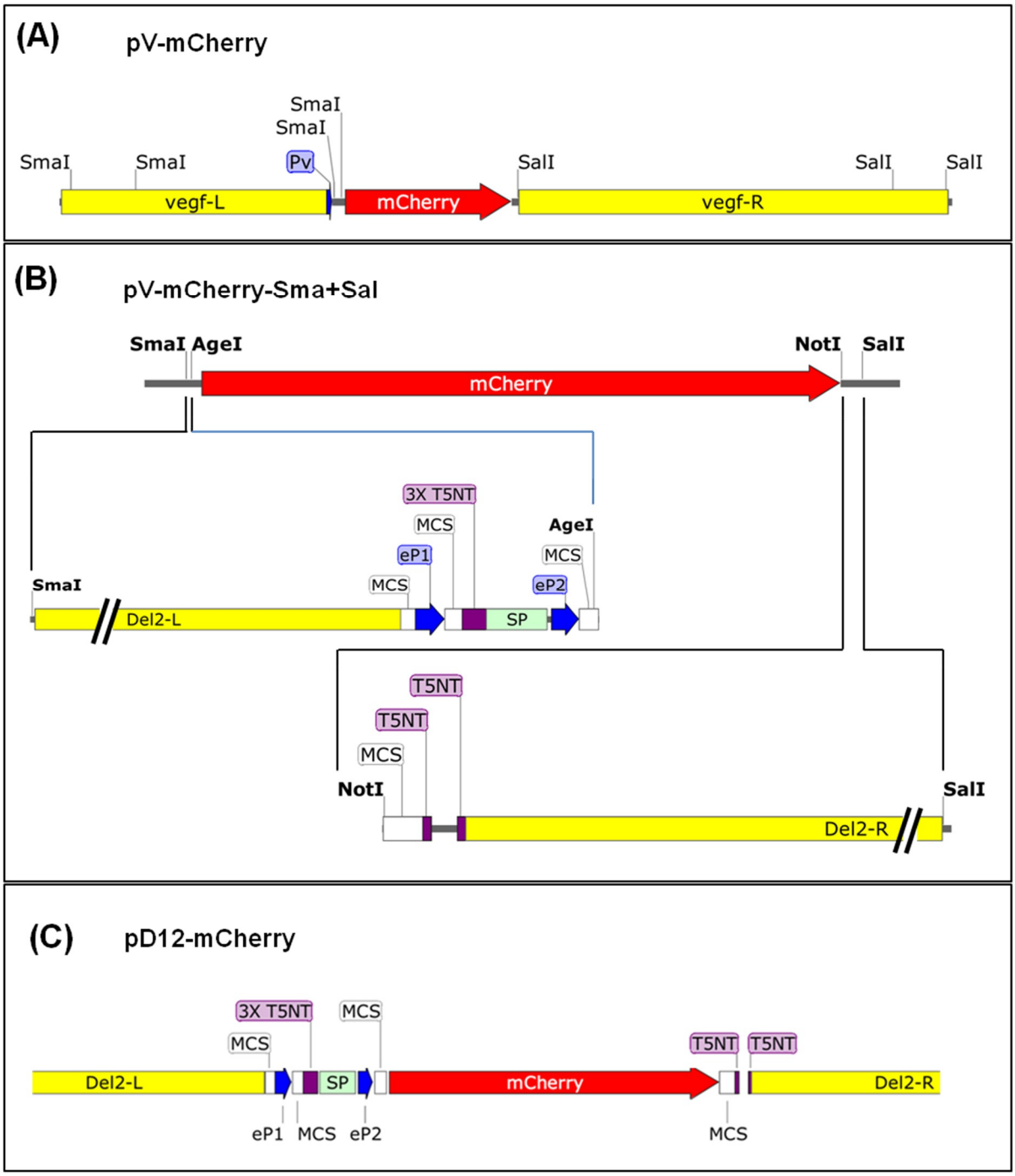
Supplementary Figure S1. Construction of plasmid pD12-mCherry



Legend to Figure S1: Construction of plasmid pD12-mCherry

(A) The *Sma*I and *Sal*I restriction sites in plasmid pV-mCherry are shown, which are necessary for the following plasmid construction. Left and right vegf-e gene homology

arms vegf-L and vegf-R and the promoter Pv are indicated.

(B) Re-ligation after restriction digest led to plasmid pV-mCherry-Sma+Sal, and using its indicated singular restriction sites, the left Del2-L as well as the right Del2-R arms were inserted as described in the text.

(C) Shows the obtained plasmid pD12-mCherry. The multiple cloning sites MCS, the early stop motif T5NT and the early promoter eP1 separated by spacer SP (see also Table 1) from early promoter eP2 are displayed.

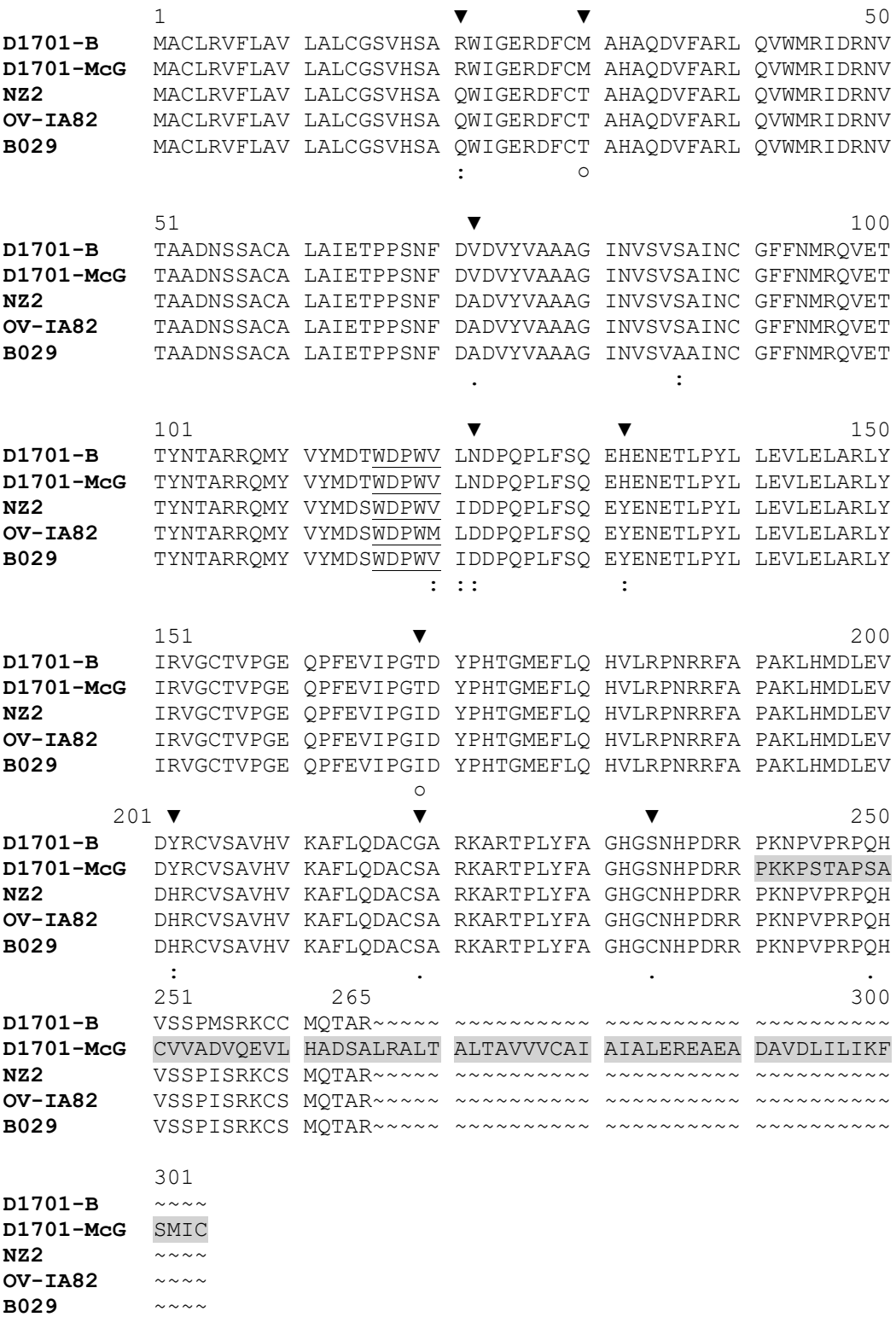
Supplementary Figure S2. DNA sequence comparison of loci AT and D of the ORFV D1701 variants

```
           3601                                                 3650
D1701-B    GACTTCTAGC TTCTTAGACC GATGCTACCA TATCGCGGCG TGCCGGCCCC
D1701-V    GACTTCTAGC TTCTTA.... ... Deletion AT ..... ..........
D1701-McG  GACTTCTAGC TTCTTAGACC GATGCTACCA TATCGCGGCG TGCCGGCCCC

           6101                                                 6150
D1701-B    TTGAACTTAT CAAACGAAAT GTTTACTCGC GGTTGTGCTT CGTATTTTTT
D1701-V    .......... .......... .......... .......... ..TATTTTTT
D1701-McG  TTGAACTTAT CAAACGAAAT GTTTACTCGC GGTTGTGCTT CGTATTTTTT



           14051                                                14100
D1701-B    CAAGACGGAG CCGGCGCGGC GCAGCACGTC CTCGCCGGGC CCTAGACGCC
D1701-V    CAAGACGGAG CCGGCG.... ..... Deletion D .... ..........
D1701-McG  CAAAACGGAG CCGGCGCGGC GCAACACGTC CTCGCCGGGC CCTAGACGCC

           16251                                                16300
D1701-B    CCAGCACGTT CTACGGCCAA ACCGCCGGTT CGCTCCAGCG AAGCTGCACA
D1701-V    .......... CTACGGCCAA ACCGCCGGTT CGCTCCAGCG AAGCTGCACA
D1701-McG  CCAGCACGTT CTACGGCCAA ACCGCCGGTT CGCTCCAGCG AAGCTGCACA
```

Legend S2:

The DNA parts covering deletion site AT and D, respectively, were aligned by CLUSTAL Omega. Dashes indicate the deleted parts, stars mark identical nt.

Supplementary Figure S3A. Amino acid comparison of ORF117 (GIF) of different ORFV strains



Supplementary Figure S3B. Comparison of the section of the GIF gene (ORF117) sequence differing between D1701-B and D1701-McG

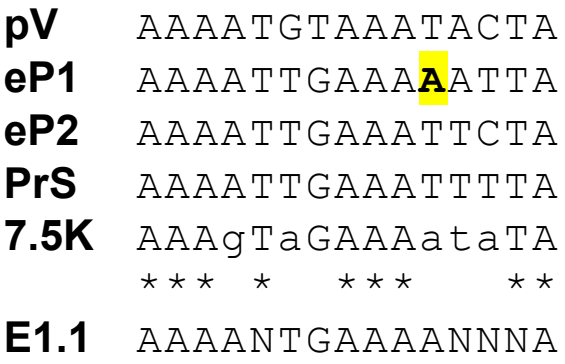
```
             R   P    K   N   P   V
D1701-B      CGGCC.AAAAAACCCAGTA
D1701-V      CGGCC.AAAAAACCCAGTA
D1701-McG    CGGCCAAAAAAACCCAGTA
             R   P   K   K   P   S
```

Legend S3:

(A) The GIF protein sequences of the D1701 variants were aligned with the indicated ORFV strains (see also Table 2) using program CLUSTAL Omega/MSF. The ▼ marks those aa differing between D1701 and other ORFV. The WSXWS motif is underlined. Completely diverging aa of D1701-McG are shaded grey.

(B) The additional A in D1701-McG results in a frame-shift of the aa sequence of the D1701 sequence published by McGuire et al. [63], and results in complete divergence to the GIF aa of other ORFV strains (see also Fig. S3A).

Supplementary Figure S4. Nucleotide sequence comparison of the core motifs of different poxviral early promoters.



Legend S4:

The critical core sequences of the ORFV early promoters used in this study were compared by CLUSTAL Omega with the VACV early promoter PrS [53], the optimized well-known early VACV promoter 7,5K; the bases not important for the promoter strength are written in small letters [54]. Stars indicate identical nt. At the bottom the recently published critical promoter core of VACV immediate early gene class E1.1 [46] is given.