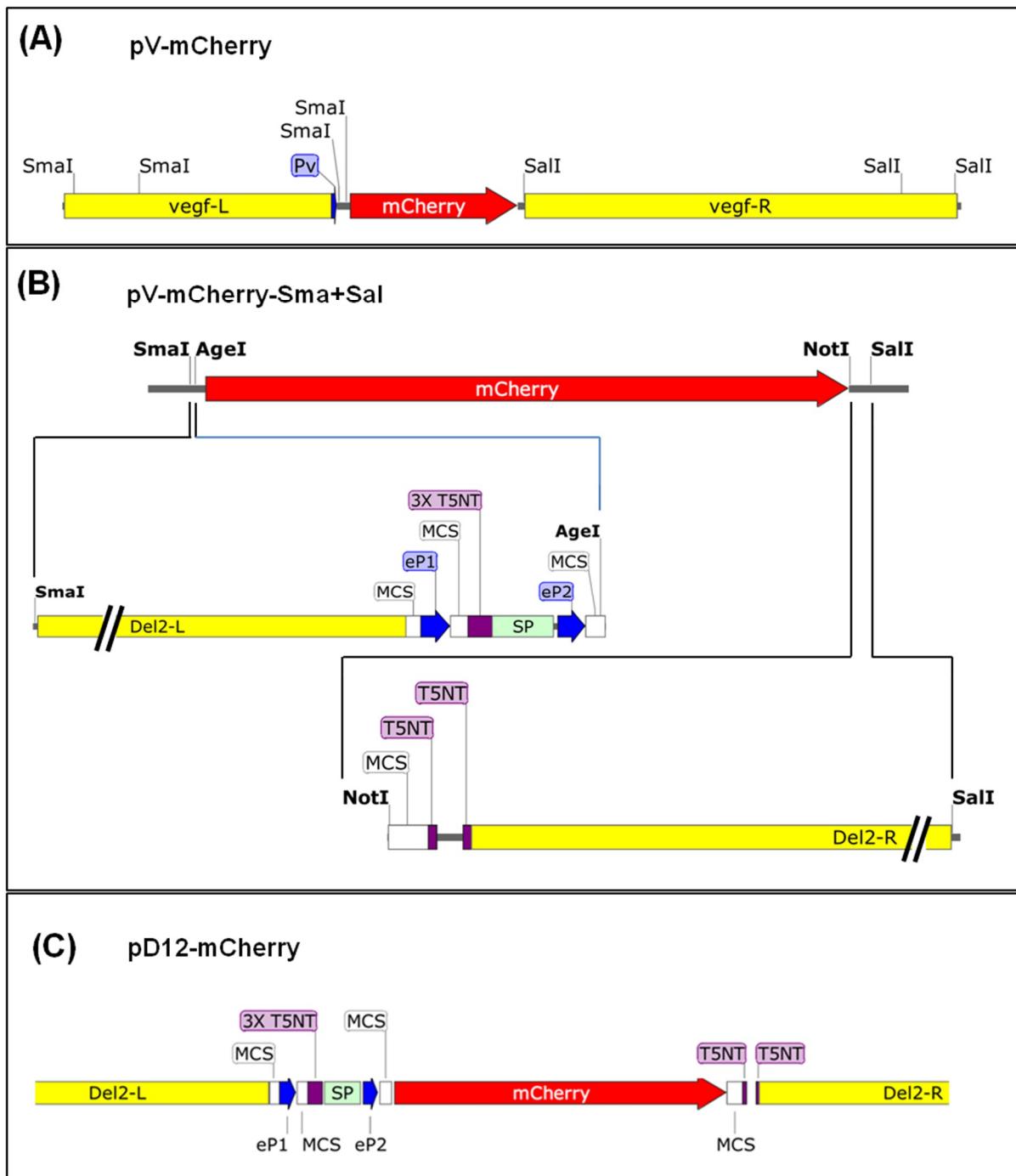


Supplementary Figure S1. Construction of plasmid pD12-mCherry



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-VTATTTTTT		
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

	1	▼	▼		50
D1701-B	MACLRVFLAV	LALCGSVHSA	RWIGERDFCM	AHAQDVFARL	QVWMRIDRNV
D1701-McG	MACLRVFLAV	LALCGSVHSA	RWIGERDFCM	AHAQDVFARL	QVWMRIDRNV
NZ2	MACLRVFLAV	LALCGSVHSA	QWIGERDFCT	AHAQDVFARL	QVWMRIDRNV
OV-IA82	MACLRVFLAV	LALCGSVHSA	QWIGERDFCT	AHAQDVFARL	QVWMRIDRNV
B029	MACLRVFLAV	LALCGSVHSA	QWIGERDFCT	AHAQDVFARL	QVWMRIDRNV
	:	○			
	51	▼			100
D1701-B	TAADNSSACA	LAIETPPSNF	DVDVYVAAAG	INVSVAINC	GFFNMRQVET
D1701-McG	TAADNSSACA	LAIETPPSNF	DVDVYVAAAG	INVSVAINC	GFFNMRQVET
NZ2	TAADNSSACA	LAIETPPSNF	DADVYVAAAG	INVSVAINC	GFFNMRQVET
OV-IA82	TAADNSSACA	LAIETPPSNF	DADVYVAAAG	INVSVAINC	GFFNMRQVET
B029	TAADNSSACA	LAIETPPSNF	DADVYVAAAG	INVSVAINC	GFFNMRQVET
	:				
	101	▼	▼		150
D1701-B	TYNTARRQMY	VYMDTWPDPWV	LNDPQPLFSQ	EHENETLPYL	LEVLELARLY
D1701-McG	TYNTARRQMY	VYMDTWPDPWV	LNDPQPLFSQ	EHENETLPYL	LEVLELARLY
NZ2	TYNTARRQMY	VYMDSWDPWV	IDDPQPLFSQ	EYENETLPYL	LEVLELARLY
OV-IA82	TYNTARRQMY	VYMDSWDPWM	LDDPQPLFSQ	EYENETLPYL	LEVLELARLY
B029	TYNTARRQMY	VYMDSWDPWV	IDDPQPLFSQ	EYENETLPYL	LEVLELARLY
	:	::	:		
	151	▼			200
D1701-B	IRVGCTVPGE	QPFEVIPGTD	YPHTGMFQL	HVLRPNRRFA	PAKLHMDLEV
D1701-McG	IRVGCTVPGE	QPFEVIPGTD	YPHTGMFQL	HVLRPNRRFA	PAKLHMDLEV
NZ2	IRVGCTVPGE	QPFEVIPGID	YPHTGMFQL	HVLRPNRRFA	PAKLHMDLEV
OV-IA82	IRVGCTVPGE	QPFEVIPGID	YPHTGMFQL	HVLRPNRRFA	PAKLHMDLEV
B029	IRVGCTVPGE	QPFEVIPGID	YPHTGMFQL	HVLRPNRRFA	PAKLHMDLEV
	○				
	201	▼	▼	▼	250
D1701-B	DYRCVSAHV	KAFLQDAGA	RKARTPLYFA	GHGSNHPDRR	PKNPVPRPQH
D1701-McG	DYRCVSAHV	KAFLQDACS	RKARTPLYFA	GHGSNHPDRR	PKKPSTAPSA
NZ2	DHRCVSAHV	KAFLQDACS	RKARTPLYFA	GHGCNHPDRR	PKNPVPRPQH
OV-IA82	DHRCVSAHV	KAFLQDACS	RKARTPLYFA	GHGCNHPDRR	PKNPVPRPQH
B029	DHRCVSAHV	KAFLQDACS	RKARTPLYFA	GHGCNHPDRR	PKNPVPRPQH
	:				
	251	265			300
D1701-B	VSSPMRKCC	MQTAR~~~~~	~~~~~	~~~~~	~~~~~
D1701-McG	CVVADVQEVL	HADSALRALT	ALTAVVVCAI	AIALEREAEA	DAVLILIKE
NZ2	VSSPISRKCS	MQTAR~~~~~	~~~~~	~~~~~	~~~~~
OV-IA82	VSSPISRKCS	MQTAR~~~~~	~~~~~	~~~~~	~~~~~
B029	VSSPISRKCS	MQTAR~~~~~	~~~~~	~~~~~	~~~~~
	301				
D1701-B	~~~~~				
D1701-McG	SMIC				
NZ2	~~~~~				
OV-IA82	~~~~~				
B029	~~~~~				

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	.	AAAAAAACCCAGTA			
D1701-V	CGGCC	.	AAAAAAACCCAGTA			
D1701-McG	CGGCC	A AAAAAAACCCAGTA				
	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.

pV	AAAATGTAAATACTA
eP1	AAAATTGAAA A TTA
eP2	AAAATTGAAATTCTA
PrS	AAAATTGAAATTTA
7.5K	AAAgTaGAAAataTA
	* * * * * * * * *
E1.1	AAAANTGAAAANNNA

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.