Table S1

Vaccinia gene (WR strain)	Protein topology[1]	Evidence for translation[2,46]	Full length (amino acids)	Signal peptide (Sig)/transmembrane domain [3]	Primers[4]	Restriction endonuclease sites	Final protein insert (amino acids)[5]
F12L	IEV (E)[6]; Cytosol-oriented (E)	Mut[6]	635		tagtt*aagctt*gccaccatggACAGGGTACAAATCTTGATGA tggag*gcggccgc*gTTACCATCTGACTCATGGATT	*Hind*III/*Not*I	4-633
F15L[7]	Cytosolic/viral membrane assembly (I)		147		tagttaagcttgccaccatgGAAATTTTTAATGTAGAAGAATTGA tggag*gcggccgc*gCTATTTCGAATTTAGGCTTCC	*Hind*III/*Not*I	2-146
G4L	Cytosol/IMV surface (I)[8]	MS[9]	124		tagtt*aagctt*gccaccatggcgAAGAACGTACTGATTATTTTCG ttggag*gcggccgc*gctGTAACAGGTGGCCAAACTC	*Hind*III/*Not*I	2-123
L1R	IMV surface (E)[10]	Ab[10]; MS[9]	250	tm: 182-204	tagtt*aagctt*gccaccatgGCGGCAAGCATACAGACGA tggag*gcggccgcg*TACCAGCAACTTGTTTAGGTG	*Hind*III/*Not*I	4-182
L4R	nucleoprotein core (E)[12]	Seq[11]; F[12]; MS[9]	251		tagtt*aagctt*gccaccatggTACTGCTAGAAAACCTCATCG tggag*gcggccgc*gCATCCTTTGTCGGAATATCTGT	*Hind*III/*Not*I	4-251
J5L	IMV surface (I)	Mut[13], MS[9]	133	tm: 110-132	tagtt*aagctt*gccaccatggACGAACAAATTTATGCATTCT tggag*gcggccgc*gCTCTAATTTCCTGATTCAGATAGC	*Hind*III/*Not*I	3-109
H3L	IMV surface (E)[15]	Ab[14]; Mut[15]; Seq[11]; MS[9]	324	tm: 283-305	tagtt*aagctt*gccaccatggcGGCGAAAACTCCTGTTATT tggag*gcggccgc*gctGAAATCAGTGGAGTAGTAAACG	*Hind*III/*Not*I	4-281
D6R	nucleoprotein core (E)[16]	Ab[17]; Mut[16]; MS[9]	637	tm: 41-63	tagtt*aagctt*gccaccatggcgAGATTTAAAAAGGTTTACATTCTA tggag*gcggccgc*gcTGGAGAAGATACCACGTTA	*Hind*III/*Not*I	64-637
D8L	IMV surface (E)[18]	Seq[11]; MS[9]	304	tm: 275-294	tagtt*aagctt*gccaccatggcgCCGCAACAACTATCTCCTATT tggag*gcggccgc*gctTTCTCTTCGATATATTTTTGAT	*Hind*III/*Not*I	2-274
D13L	IMV inner surface (E)[19]	F[19]; Ab[19]; MS[9]	551		tagtt*ggtacc*gccaccatggcGATCGGTGGGGATGACTC tggag*gcggccgc*gctTTATCTCCCATAATCTTGGTAA	*Kpn*I/*Not*I	10-549
A4L	nucleoprotein core (E)[20]	Ab[20]; MS[9]	281		tagtt*aagctt*gccaccatggcgTTCTTTAACAAGTTCTCACAGG tggag*gcggccgc*gctAATCGTTCAAAACCTTTGACT	*Hind*III/*Not*I	2-281
A11R	Non-structural assembly (E)[21]	Ab[21]; Mut[21]; MS[9]	318	tm: 241-259; 293-312	tagtt*aagctt*gccaccatggcgCCAGTGACGGATATACAAA tggag*gcggccgc*gTTCTACTTTCGTGATATTGTTT	*Hind*III/*Not*I	2-240
A12L	nucleoprotein core (E)[22]	Seq[11]; MS[9]	192		tagtt*aagctt*gccaccatggcgAAAAATTTAGCCGTTAGAAGC tggag*gcggccgc*gcgTACATTCCCATATCCAGACAA	*Hind*III/*Not*I	2-192
A27L	IMV surface (E)[23]	Ab[24]; Mut[23]; Seq[11]; MS[9]	110		tagtt*aagctt*gccaccatggcgCTTTTCCCCGGAGATGA tggag*gcggccgc*gCATATGGGCGCCGTCCAGTC	*Hind*III/*Not*I	5-110
A33R	EEV surface	Ab[26]; Mut[27];	185	tm: 34-56	tagtt*aagctt*gccaccatggGCCTAAATCAATGCATGTCT	*Hind*III/*Not*I	58-183

	(E)[25]				tggag*gcggccgc*gcTGTTTTAACACAAAAATACTTTCTA		
A36R	IEV (not EEV) surface (E)[28]	F[29]; Mut[29]	221	tm: 5-24	tagtt*aagctt*gccaccatggcgAGGAAAAAGATACGTACTGTCTATAA tggag*gcggccgc*gATGATACGACCGATGATTCTA	*Hind*III/*Not*I	26-219
A38L	EEV surface (I)[30]	Ab[31]; F[31]; Mut[31]	277	Sig: 1-21 tm: 125-147; 154-176; 186-208; 215-237; 247-269	tagtt*aagctt*gccaccatggCTACAAAGACTATAGAGTATACAGCA tggag*gcggccgc*gTTTGGTAATCATTAAACCAATTA	*Hind*III/*Not*I	20-124
A39R[32]	secreted (I)[33]	Ab[33]; Mut[33]	295	tm: 4-21; 28-50	tagtt*aagctt*gccaccatggGTATCGAATGGCATAAGTTTG tggag*gcggccgc*gGAATAGGTACATAATGCGGACT	*Hind*III/*Not*I	53-294
A40R	Cell surface (E)[34, 35]	Ab[35]; Mut[35]	159	tm: 7-29	tagtt*aagctt*gccaccatggcgTTAAAAGTTGTAGAACGTAAATTAG tggag*gcggccgc*gcAGTGTAACACGAATGCAGTTT	*Hind*III/*Not*I	30-158
A56R	EEV surface (E)[36]	Ab[37]; Mut[37]	314	Sig: 1-17 tm: 279-301	tagtt*aagctt*gccaccatggcgCCTTTTCCTCAGACATCTAAA tggag*gcggccgc*gcTTCTACAAAGTCCTTGGTTTT	*Hind*III/*Not*I	18-278
B5R	EEV surface (E)[38, 39]	Ab[38,39]	317	Sig: 1-20 tm: 280-302	tagtt*aagctt*gccaccatggcgCCCACTATGAATAACGCTAAA tggag*gcggccgc*gctTGATAAGTTGCTTCTAACGATT	*Hind*III/*Not*I	24-278
B19R	Secreted (I)	Mut[40]	351	Sig: 1-24	tagtt*aagctt*gccaccatggCGATCGAAAATGAAATCACAGAA tggag*gcggccgc*GCTACTGTAGTTGTAAGGGTTTTT	*Hind*III/*Not*I	26-348

Initially tested but later excluded from the array

F13L	IEV surface (E)[41]; EEV inner membrane (E)[41, 42],	Ab[41]; Mut[41]	372		tagtt*aagctt*gccaccatggCTGCGGGAGCAAAATGTA tggag*gcggccgc*gcGTGGCTAGATACCCAATCTCT	*Hind*III/*Not*I	9-366
A32L	Non-structural assembly (E)[21]	Ab[21]; Mut[43]	270	tm: 21-43	tagtt*aagctt*gccaccatggtaTTCTTGTTTACACCCGTTT tggag*gcggccgc*gtTTTTGACGACGATGATT	*Hind*III/*Not*I	44-270
A34R	EEV[43,44]	Ab[44], Mut[44,45]	168	tm: 15-37	tagtt*aagctt*gccaccatggACAAAGAAGAACTGATGCCTA tggag*gcggccgc*gctTTTTTAACACATAGTACAGATTGA	*Hind*III/*Not*I	39-165

[1] Topology considered experimentally-demonstrated (E) based on indicated reference or indirect (I) based on bibliographic, domain and bioinformatic analysis.

[2] Evidence from vaccinia mutant analysis (Mut) or functional activity (F), from detection of virus-induced protein-specific antibodies (Ab), or from detection in purified IMV preparations by direct protein sequencing (Seq) or by mass spectroscopy analysis of tryptic digests (MS).

[3] Signal peptides and transmembrane domains predicted by the *Simple Modular Architecture Research Tool* (SMART) sequence analysis tool (Letunic I, Copley RR, Pils B, Pinkert S, Schultz J, Bork P. (2006) SMART 5: domains in the context of genomes and networks. *Nucleic Acids Res*. **34**:D257-60).

[4] Forward and reverse primers for amplification from vaccinia WR genomic DNA. Capitalized sequence represents vaccinia-encoded region; lower case sequence is filler including restriction endonuclease site (italicized) and, for the forward primer, an optimal Kozak sequence incorporating a start codon (gccaccATGg).

[5] Representing domain inserted upstream of myc-His$_{(6)}$ tag encoded in pcDNA3.1 vector.

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[30] Ref. 31 below addresses the topology of A38L, finding that the N-terminal hydrophilic region (amino acids 20-124 used here) is probably intraluminal with respect to the endoplasmic reticulum placing it appropriately both for expression on the EEV and on the plasma membrane with a structure and topology similar to that of CD47. Using an antibody developed against an N-terminal region linear peptide, no expression is found on EEV or IMV, although the authors caution that the antibody reagent may be weak.

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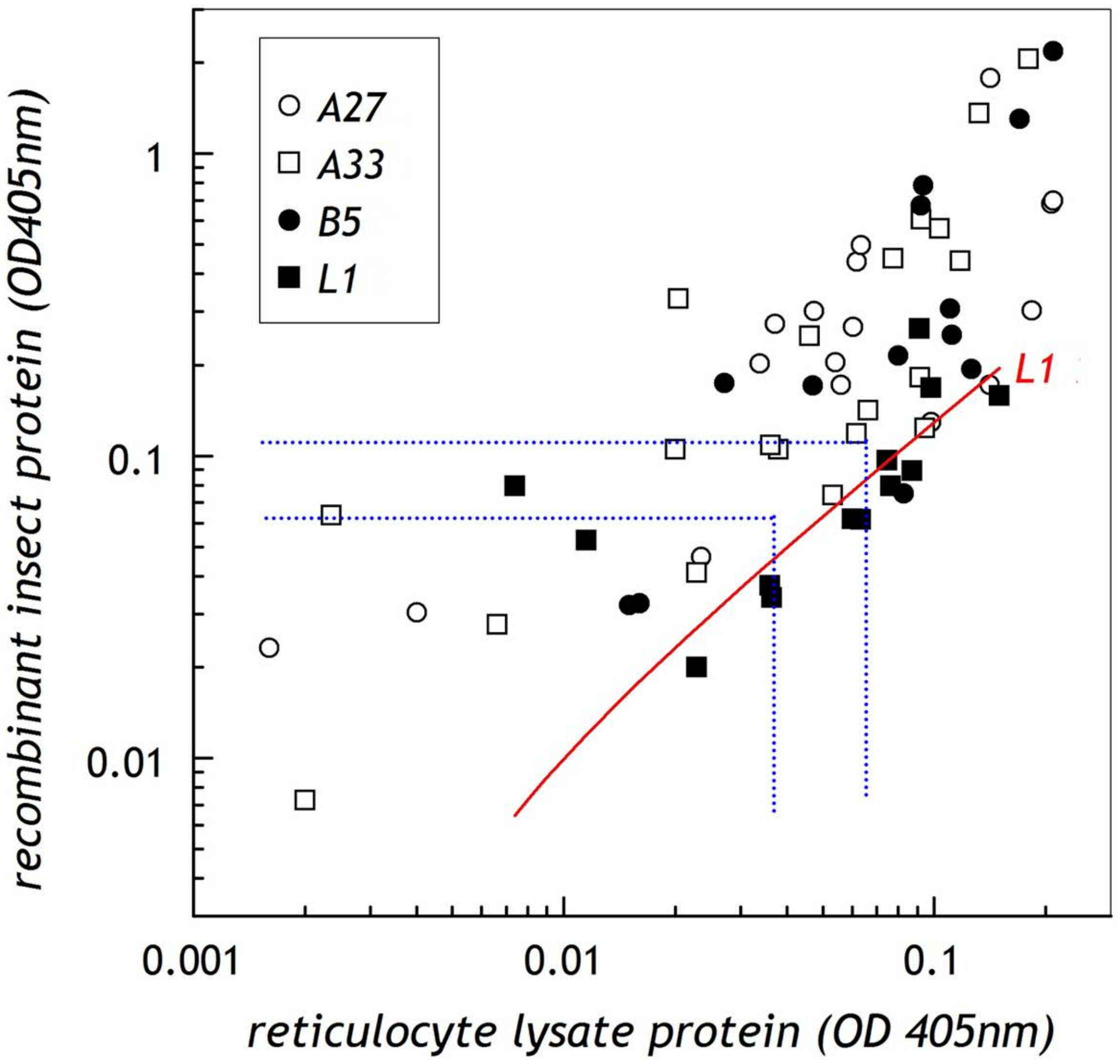
[46] A recent article using high-resolution reversed-phase nano-LC-MS/MS of purified IMV and EEV confidently identifies tryptic peptides derived from every protein used in our array except for A38L. Manes NP, Estep RD, Mottaz HM, Moore RJ, Clauss TRW, Monroe ME, Du X, Adkins JN, Wong SW, Smith RD (2008) Comparitive proteomics of human monkeypox and vaccinia intracellular mature and extracellular enveloped virions. *J. Proteome Res*. **7**: 960-8

Figure S1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A33
B5
L1
L1
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0.001
0.01
0.1
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reticulocyte lysate protein (OD 405nm)

Figure S2

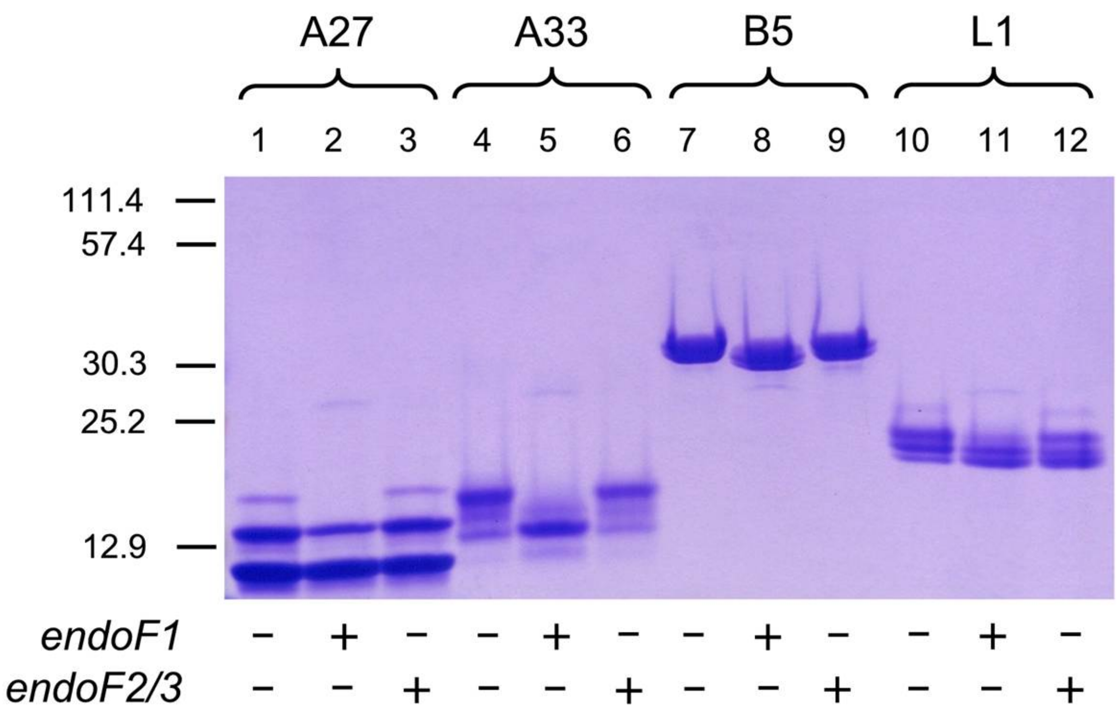
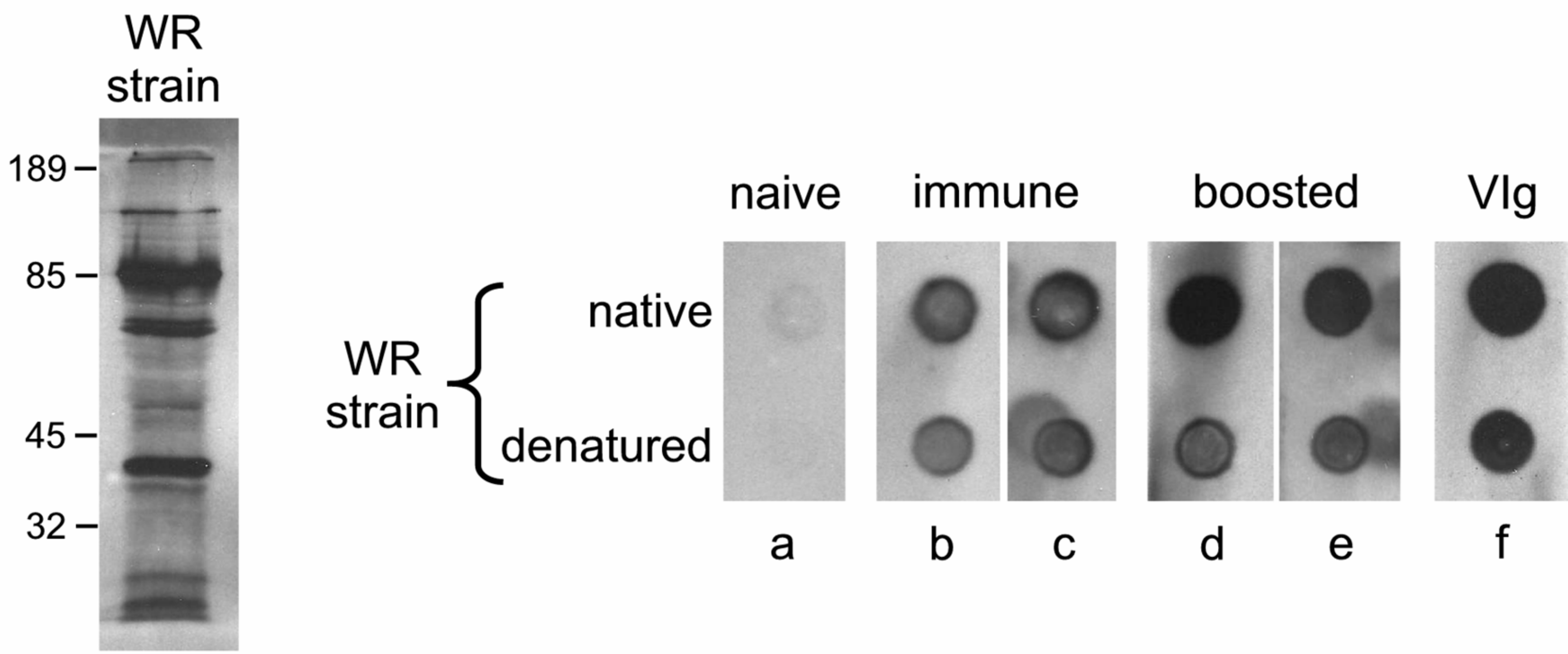

A27
A33
B5
L1
1 2 3 4 5 6 7 8 9 10 11 12
111.4
57.4
30.3
25.2
12.9
endoF1 − + − − + − − + − − + −
endoF2/3 − − + − − + − − + − − +

Figure S3


WR
strain
189
85
45
32
naive
immune
boosted
VIg
WR
strain
native
denatured
a
b
c
d
e
f