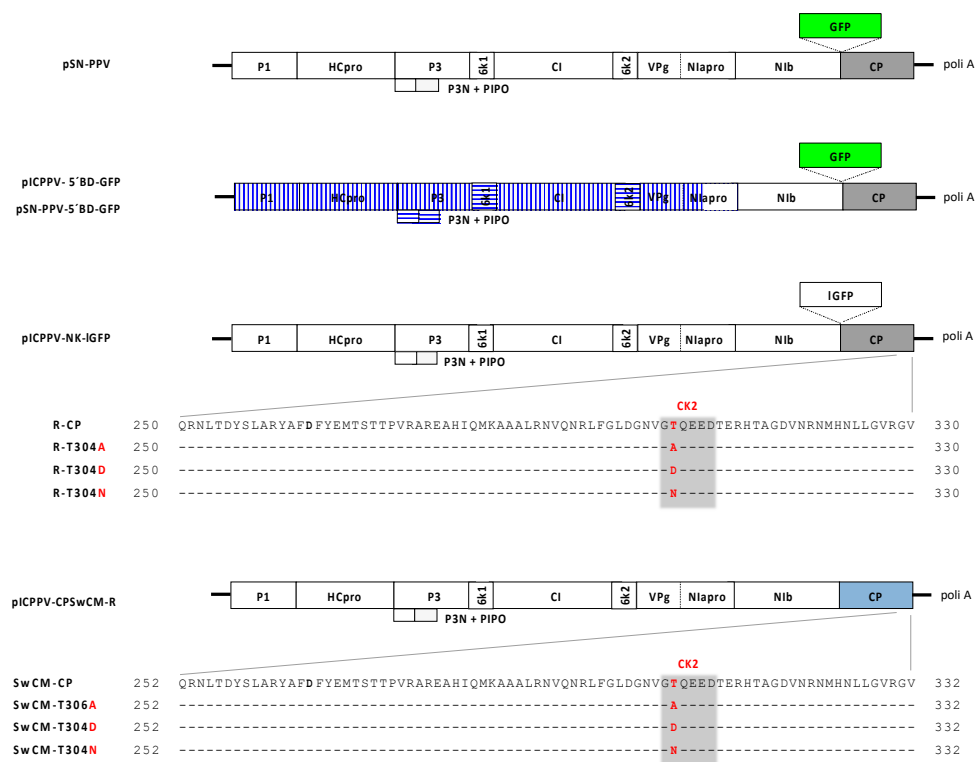


1 SUPPLEMENTARY INFORMATION

2 SUPPLEMENTARY FIGURES

3 Figure S1



4

5 Schematic representation of PPV full-length cDNA clones. Sequences of C-terminal region

6 of PPV-R and PPV-SwCM CPs are displayed below corresponding constructs. Mutations

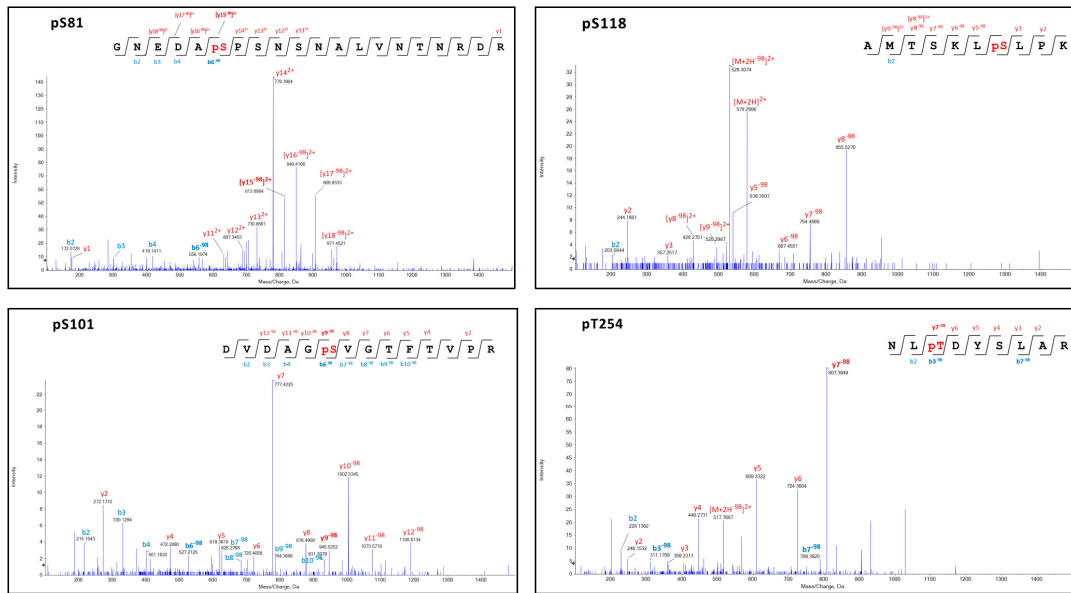
7 engineered into the casein kinase II (CK2) motif (yellow background) in constructs pICPPV-

8 NK-IGFP and pICPPV-CPSwCM-R are shown in red.

9

10 **Figure S2**

11

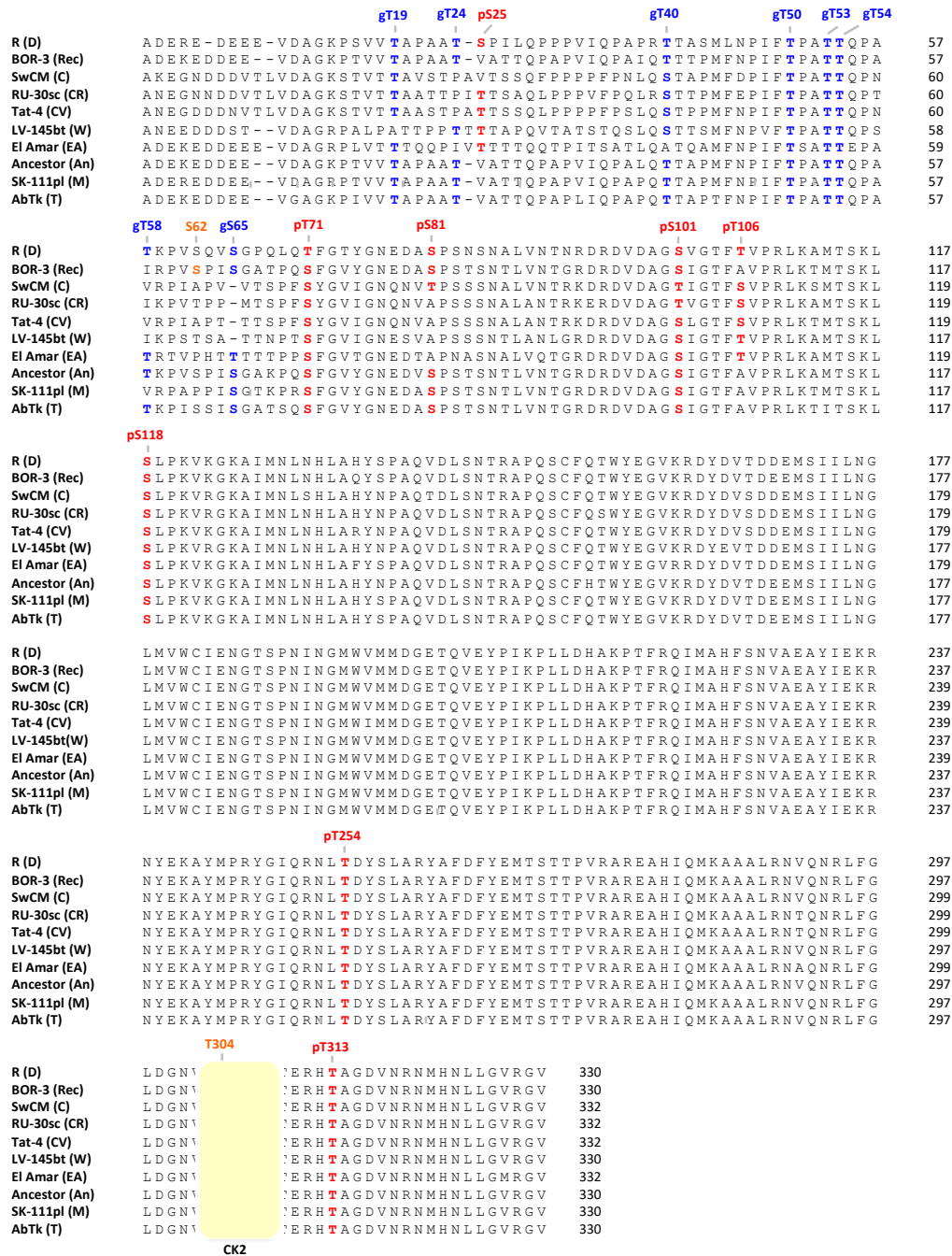


12

13 MS/MS fragmentation spectra identifying phosphorylations at S81, S101, S118 and T254
 14 in the capsid protein of PPV-R virions purified from *Prunus persica* plants. Details of
 15 identified phospho-peptides are provided in Supplementary Table S3.

16

17 **Figure S3**



18

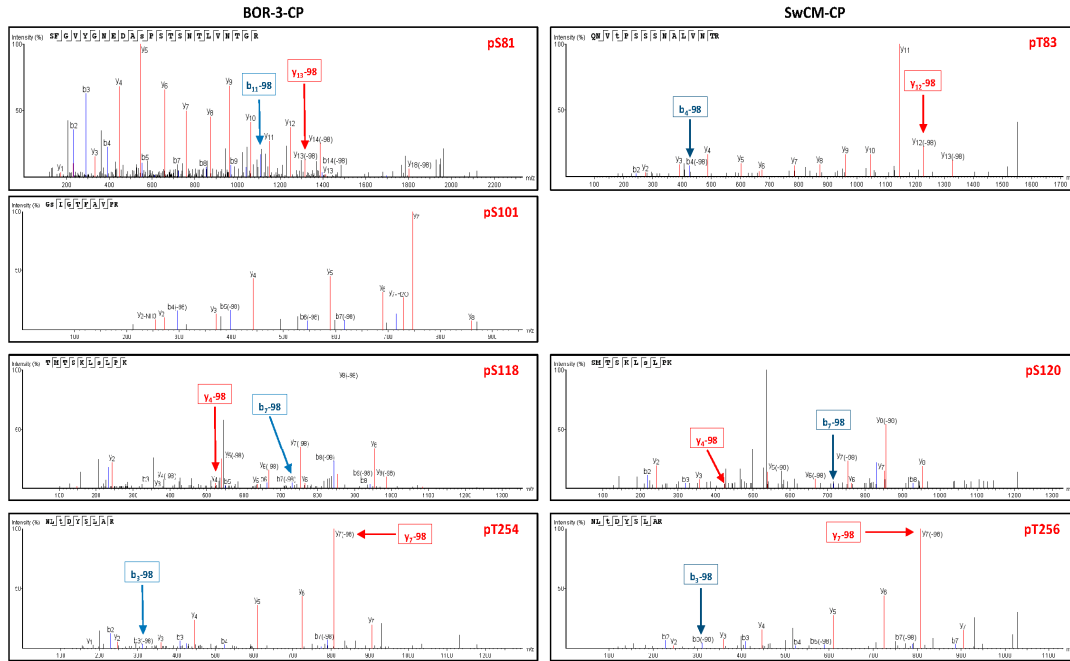
19 **Supplementary Figure S3:** Alignment of coat protein (CP) sequences from representative
20 isolates of the ten strains proposed for *Plum pox virus* (PPV). Respective strains are in
21 parentheses. Accession numbers for the aligned sequences are: R, EF569215; BOR-3,
22 AY028309; SwCM, sequence not deposited in a public repository; RU-30sc, KC020126;
23 Tat-4, MF447180; LV-145bt, HQ670748; El Amar, AM157175; Ancestor, HF674399; SK-
24 111pl, HF585099; AbTk, EU734794. Residues in which phosphorylations (red) and O-

25 GlcNAcylations (blue) had been previously identified in the CP of PPV-R virions [1-4], as
26 well as the equivalent residues, potentially modifiable in other isolates, are highlighted.
27 Amino acids found phosphorylated just in the CP of BOR-3 (pS62) and SwCM (pT306) are
28 highlighted in orange. Casein kinase II (CK2) motif, largely conserved among potyviruses,
29 is shown on a yellow background. Proteins were aligned using Clustal Omega program
30 (*European Bioinformatics Institute*).

31

32 **Figure S4**

33

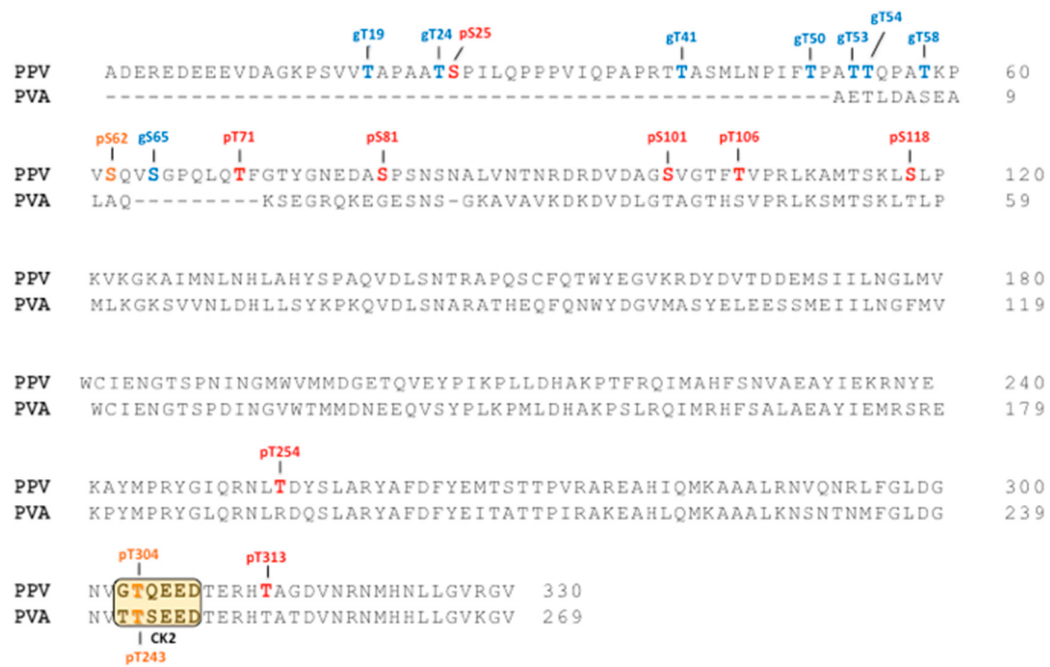


34

35 MS/MS fragmentation spectra showing phosphorylations in the coat protein (CP) of PPV-
 36 BOR-3 (BOR-3-CP) and PPV-SwCM (SwCM-CP) virions. Details of phospho-peptides
 37 corresponding with these spectra are provided in Table 1.

38

39 **Figure S5**



40

41 Alignment of coat protein (CP) sequences from the *Plum pox virus* (PPV) isolate PPV-R
42 (EF569215) and *Potato virus A* (CAC17411). Residues in which phosphorylations (red) and
43 O-GlcNAcylation (blue) had been identified in the CP of PPV-R virions [1-4] are
44 highlighted. Amino acids equivalents to those residues found phosphorylated in the CPs
45 of PPV-BOR3 and PPV-SwCM (see Figure S3) are highlighted in orange. Casein kinase II
46 (CK2) motif, largely conserved among potyviruses, is shown on a yellow background.
47 Proteins were aligned using Clustal Omega program (*European Bioinformatics Institute*).
48

49 **Figure S6**

Virus name	Acronym	Id	282	329
<i>Plum pox virus (isolate R)</i>	PPV	EF569215	QMKAAALRNVDNRLLFGLDGNV	QKAEEDTERHRTAGDVNRNMHLLGVRI
<i>Algerian watermelon mosaic virus</i>	AWNV	YP_001931956.1	QMKAAALIRGGNNRLLFGLDGNV	GGGDEENTERHTVDDVSRNMHSLLGMRK
<i>Banana bract mosaic virus</i>	BBMV-B	YP_001427389.1	QMKAAALIRGSNTRLFLGLDGNL	GPGEENTERHTVDDVSRNMHSLLGMRK
<i>Bean common mosaic necrosis virus</i>	BCMNV	BBH10738.1	QMKAAALANVNTRLFLGLDGNVA	TTSENTERHTARDVNRNMHLLGMTS
<i>Bean common mosaic virus</i>	BCMV	AAB28498.1	QMKAAALANVNTRLFLGLDGNVA	TTSENTERHTARDVNRNMHLLGMTS
<i>Bean yellow mosaic virus</i>	BYMV-J	NP_612218.1	QMKAAAVRGGKSTRFLGLDGNV	GTDDEENTERHTAGDVNRDMHTMLGVR
<i>Beet mosaic virus</i>	BTMV	AAF80972.1	QMKAAALASVSNKFLGLDGSAA	TTTSEEDTERHTATDVNAHMHMMGVRRQ
<i>Bidens mosaic virus</i>	BIMV	AAX63416.1	QMKAAALKSSQTRMFLGLDGGI	GTTQENTERHTTDEDVSPNMHTLLGVRN
<i>Blackberry virus Y</i>	BVY	YP_851006.1	QMKAAALLESASSKVFLGLDGGSA	RAIDTERHTTATEDATARTHNLRGAAM
<i>Canna yellow streak virus</i>	CaYSV	AIX02891.1	QMKAAASIRGGTNNLFLGLDGNV	GEDSENTERHVAATDVNKNTHSYRGAQI
<i>Celery Mosaic Virus</i>	CeMV	YP_004376199.1	QMKAAALRNKTTRLFLGLDGSVS	GKKEEDTERHTVDDVNRNMHSLLGMRQ
<i>Chilli ringspot virus</i>	ChRSV	YP_004875339.1	QMKAAALVMHAKNNMFLGLDGNVS	TKEENTERHTATDVNRDMHMLMGVRG
<i>Clover yellow mosaic virus</i>	CIYMV-1	NP_613273.1	QMKAAALIRGKSNHMFGLDGNV	GTDDEENTERHTANDVNRNMHLLAGARF
<i>Cucurbit vein banding virus</i>	CVBV	YP_009388623.1	QMKAAALSNVKTTRMFLGLDGSV	GNSEENTERHTAHADVNRQNLHTLMGVRG
<i>Daphne mosaic virus</i>	DapMV	YP_610949.1	QMKAAALRNASTHFLGLDGNV	GNAAENTERHTTDDVDVNTHTSFTGAR
<i>Daphne virus Y</i>	DVY	YP_009508399.1	QMKAAALKNASTTRMFLGLDGKV	GATENTERHTADDVNRNMHSLSGARI
<i>Dasheen mosaic virus</i>	DaMV	QB177037.1	QMKAAALSNVSTTRFLGLDGNVS	TSSSEENTERHTAKDVTNNMHTLLGVSP
<i>Dendrobium chlorotic mosaic virus</i>	DeCMV	QBS16347.1	QMKAAALLVNVSTKFLGLDGNL	GTTQEEDTERHVAIDVNRNMHLLGMRH
<i>Dioscorea mosaic virus Florida</i>	DMV-FL	AZB50213.1	QMKAGALLRGVTTKFLGLDGGI	IRNSEEDTEHPSTSGDVSRNMPHFLGERG
<i>East asian passiflora virus</i>	EAPV	BAR88501.1	QMKAAALANVSTTRFLGLDGNVS	TGTENTERHTADDVNRNMHSLLGMRN
<i>Euphorbia ringspot virus</i>	EuRSV	YP_009305422.1	QMKAAALRNKNNLFLGLDGNV	GSKKEENTERHTADDVNRNMHSLLGMRQ
<i>Freesia mosaic virus</i>	FreMV	YP_003587807.1	QMKAAALVANTTAKMFLGLDGNL	GTTQEENTERHTVTDVSRNMHSLLGVRH
<i>Gomphocarpus mosaic virus</i>	GoMV	BB033993.1	QMKAAALVNTNTRLFLGLDGNV	TTSSEENTERHTADDVNRNMHLLGVRRQ
<i>Habenaria mosaic virus</i>	HaMV	YP_008240477.1	QMKAAALRNSTSRMFLGLDGKV	GTTQVEDTERHTAEDVNRNMHLLGVRRQ
<i>Hubei poty-like virus 1</i>	HuPLV1	APG79040.1	QMKAAALVRGKSNRLLFLGLDGNV	GTDDEENTERHTAGDVNRNMHMLGVR
<i>Japanese yam mosaic virus</i>	JYMV	AID23401.1	QMKAAALIRGVQNKFLFLGLDGNVS	TMEENTERHTAEDVNRNMHSLLGVRG
<i>Johnsongrass mosaic virus</i>	JGMV	ALIS88434.1	QMKAAALIRGSTNNHMFGLDGNV	GESSENTERHTAADVSRNVHSHYRGAQI
<i>Keunjangong mosaic virus</i>	KJMV	YP_004934107.1	QMKAAALRNNTNTRLFLGLDGNVS	TTEENTERHTATDVNRNMHLLGVRRQ
<i>Konjac mosaic virus</i>	KoMV	YP_529485.1	QMKAAALRSANTRMFLGLDGKV	TKEEDTERHTAEDVNRNLHTLMGVRG
<i>Lettuce Italian necrotic virus</i>	LINV	YP_009162372.1	QMKAAALRNASTTRFLGLDGNV	GTTKEENTERHTAEDVNRNMHMLGMRG
<i>Lettuce mosaic virus</i>	LMV	NP_619667.1	QMKAAALVGTQNNRFLGMDGGG	GTTQEENTERHTAADVNRNMHLLGVRRQ
<i>Lily yellow mosaic virus</i>	LYMV	YP_009553508.1	QMKAAALRNAANTLFLGLDGNV	GTTAEEDTERHTAADDVNRNMHLLGVRRQ
<i>Maize dwarf mosaic virus</i>	MDMV	NP_569138.1	QMKAAALVRGGSNTRMFLGLDGNV	GTTQENTERHTAGDVSRNIRHSLLGVRQ
<i>Moroccan watermelon mosaic virus</i>	MWMV	YP_001552410.1	QMKAAALIRGANTRLFLGLDGNV	GGGEENTERHTVDDVSRNMHSLLGMRK
<i>Onion yellow dwarf virus</i>	OYDV	BAE96765.1	QMKAAALIRGATNRLFLGLDGNV	TTEEDTERHTAADINKNQHTLLGIKM
<i>Panax virus Y</i>	PanVY	YP_003725718.1	QMKAAALKNSTRRLFLGLDGSVS	KKEEDTERHTVEDVNRNMHSLLGMRQ
<i>Papaya leaf distortion mosaic virus</i>	PLDMV	NP_870995.1	QMKAAALRDANNKMFGLDGKVN	GNATENTERHTADDVNRNMHSLLGVR
<i>Papaya ringspot virus</i>	PRV	AAB23789.1	QMKAAALRNSTSRMFLGMDGSV	SNKEENTERHTVEDVNRNMHSLLGMRN
<i>Paris mosaic necrosis virus</i>	PMNV	AUB51246.1	QMKAAALTSVNNKFLGLDGNVS	TTSENTERHTADDVNRNMHSLLGMRN
<i>Passionfruit Vietnam virus</i>	PMNV-DakNong	AZF86246.1	QMKAAALTGTVNKLFLGLDGNV	GTTTSEEDTERHTARDVNRNMHMLGVGP
<i>Pea enation mosaic virus</i>	PeMV	AAC42975.1	QMKAAALVRGGSNTRMFLGLDGNV	GESQENTERHTAGDVSRNMHSLLGVRQ
<i>Pea seed-borne mosaic potyvirus</i>	PSBMV	QBL54815.1	QMKAAALIRGKSNLSFLGLDGNV	GTTQEENTERHTAEDVNRNMHMLGMR
<i>Peanut mottle virus</i>	PEMVM-1	NP_068348.2	QMKAAALLNNVAIKFTFLGLDGNV	GTTQEDTERHTANDVNRNMHSLLGMRQ
<i>Peanut stripe virus</i>	PSV	AG292019.1	QMKAAALSNVNSKFLGLDGNVA	TTSENTERHTARDVNRNMHMLGMRG
<i>Pecan mosaic-associated virus</i>	PMAV	YP_009256204.1	QMKAAALLRGSNTRMFLGLDGKAN	TTEGENTERHTTDDVKDQNMHSLGVIN
<i>Pennisetum mosaic virus</i>	PeMV	YP_249455.1	QMKAAALVRGGSNTRMFLGLDGNV	GESQENTERHTAGDVSRNMHSLLGVRQ
<i>Pepper mottle virus</i>	PepMOV	NP_041276.1	QMKAAALKSQATRLFLGLDGGI	GTTQGENTERHTTDEDVSPNMHTLLGVRE
<i>Pepper severe mosaic virus</i>	PepSMV	YP_778468.1	QMKAAALKSQATRLFLGLDGGI	GTTQGENTERHTTDEDVSPNMHTLLGVRRQ
<i>Pepper yellow mosaic virus</i>	PepYMV	YP_003778216.1	QMKAAALKSQATRLFLGLDGGI	GTTQGENTERHTTDEDVSPNMHTLLGVRRQ
<i>Platyedon mild mottle virus</i>	PlaMMV	AYA60486.1	QMKAAALIRNAHNRLLFLGLDGNV	GTTTEEDTERHTAEDVNRNMHMLGMAQL
<i>Pleione flower breaking virus</i>	PFBV	YP_009552772.1	QMKAAALLNNSVSTRFLGLDGNV	TTEENTERHTAADVNRNMHSLLGVRH
<i>Pokeweed mosaic virus</i>	PKMV	YP_008719787.1	QMKAAALRNASTKMFGLDGNV	GTEKENTERHTAEDVSRNMHMLGVR
<i>Potato virus A</i>	PVA	CAC17411.1	QMKAAALKNSTNNMFLGLDGNV	TTSSEEDTERHTATDVNRNMHMLGVG
<i>Potato virus Y</i>	PVY	BAH16607.1	QMKAAALKSAQSRLFLGLDGGI	GTTQEENTERHTTDEDVSPNMHTLLGVKN
<i>Potato yellow blotch virus</i>	PYBV	AFF52882.1	QMKAAALKNANTSMFLGLDGNV	TTSSEEDTERHTAEDVNRNMHMLGVGK
<i>Saffron latent virus</i>	SalV	YP_009455737.1	QMKAAALTNVDSTRFLGLDGNAS	TNLENTERHTAKDVNRNMHMLGMRG
<i>Shallot yellow stripe virus</i>	SYSV	YP_331412.1	QMKAAALVRGVANRMFLGLDGNIS	TDDENTERHTAADVNRNMHMLFGLMR
<i>Soybean mosaic virus</i>	SMV	AAB22819.2	QMKAAALSGVNNKFLGLDGNIS	TNSSEENTERHTARDVNRNMHMLLGMRP
<i>Sudan watermelon mosaic virus</i>	SuWMV	YP_009407951.1	QMKAAALIRNTNRRMFLGLDGS	IGGGEENTERHTVDDVSRNMHSLLGMRK
<i>Sugarcane mosaic virus</i>	SCMV-Sp	CAI33884.1	QMKAAALVRGGSNTRFLGLDGNV	GTTQENTERHTAGDVNRNMHSLLGVRQ
<i>Sunflower chlorotic mottle virus</i>	SuCMoV	ADF31932.1	QMKAAALKSQATRLMFLGLDGGI	GTTKEENTERHTTDEDVSPNMHTLLGVRR
<i>Sunflower ring blotch virus</i>	SuRBV	YP_009351870.1	QMKAAALKSQATRLMFLGLDGGI	GTTQENTERHTTDEDVSPNMHTLLGVRR
<i>Sweet potato feathery mottle virus</i>	SPFMV	BAJ04334.1	QMKAAALKNARNRLLFLGLDGNVS	TQEEDTERHTTTDVTNRNIHNLGMRG
<i>Sweet potato latent virus</i>	SPLV	YP_007697620.1	QMKAAALTNTHHRLFLGLDGNVS	TTEENTERHTATDVTNRNIHNLGMRG
<i>Sweet potato virus 2</i>	SPV2	YP_006382460.1	QMKAAALKNAQNRLLFLGLDGNIS	TQEEDTERHTTTDVTNRNIHNLGMRG
<i>Sweet potato virus G</i>	SPVG	AFJ68039.1	QMKAAALKNAQNRLLFLGLDGNIS	TQEEDTERHTTTDVTNRNIHNLGMRG
<i>Telasma mosaic virus</i>	TeMV	YP_001427386.1	QMKAAALVGTTRNMFGSDGSVS	TACEDTERHTARDVNRNMHMLGVGS
<i>Thunberg fritillary mosaic virus</i>	TFMV	YP_25413.1	QMKAAALKNARTRMFLGLDGSV	GTTTEEDTERHTTDDVNRNMHSMGLGIRM
<i>Tobacco etch virus</i>	TEV	ABJ16044.1	QMKAAALVRNSGTRFLGLDGNV	GTTAEEDTERHTAHDVNRNMHMLGVRRQ
<i>Tobacco vein mottling virus</i>	TVMV	NP_056867.1	QMKAGRTPQFAAAMFLCLDGSVS	GQGEENTERHTVDDVNAQNMHMLGVGK
<i>Turnip mosaic virus</i>	TuMV	QBQ58063.1	QMKAAALRGANNLFLGLDGNV	GTTVENTERHTTDEDVNRNMHMLGVQ
<i>Turnip mosaic virus</i>	WMV	AXU24938.1	QMKAAALLAGINSLFLGLDGNIS	TNSSEENTERHTADDVNRNMHMLLGMRP
<i>Wild melon vein banding virus</i>	WMVBV	YP_009407950.1	QMKAAALRNANRLLFLGLDGSVS	GSGEENTERHTVEDVDRNMHMLLGMRK
<i>Wild tomato mosaic virus</i>	WTMV	QB878857.1	QMKAAALRNANRMLFLGLDGKV	GTTQEEDTERHTAEDVNRNMHMLGVRRQ
<i>Yambean mosaic virus</i>	YBMV	YP_004936165.1	QMKAAALSNVNNKFLGLDGNL	ATTSSEENTERHTARDVNRNMHMLLGMRP
<i>Yellow mosaic virus</i>	YMV	NP_612218.1	QMKAAALIRGNVNMKMFGLDGKV	GLPEEDTERHAAAGDVTQDMHSLLGMRG
<i>Zucchini shoestring virus</i>	ZSSV	YP_009665156.1	QMKAAALIRGANRLLFLGLDGSVS	SEGGEENTERHTVEDVTRDMHSLLGMRN
<i>Zucchini tiger mosaic virus</i>	ZTMV	AGY36215.1	QMKAAALRNANRLLFLGLDGSVS	NRREENTERHTVEDVDRDMHSLLGMRK
<i>Zucchini yellow mosaic virus</i>	ZYMV	AWX33674.1	QMKAAALSNVSSRLFLGLDGNV	ATTSSEEDTERHTARDVNRNMHMLGVNT

50

51 Alignment of partial sequences of the coat protein (CP) from 79 potyvirus species. Aligned
52 fragment spans from amino acids 282 to 329 of the CP of *Plum pox virus*. Casein kinase II
53 (CK2) motif is highlighted with yellow background. Residues aligning with amino acid T243
54 of PVA CP are in red font. Amino acids numbering refers to the sequence of PPV-R.
55 Proteins were aligned using the Clustal Omega software (*European Bioinformatics*
56 *Institute*).

57

58 Supplementary Tables

59 Table S1

60 **Table S1.** Cloning of plasmids bearing mutations at casein kinase II motif of the coat protein
61 of *Plum pox virus*

Construct ^a	PCR step ^b	Primers	Template
R-T304A	PCR1	SM35-F-mut-Ala	pICPPV-NK-IGFP
		SM30-R-ext	
	PCR2	SM30-F-ext	
		SM35-R-mut-Ala	
	PCR3	SM30-F-ext	PCR1 + PCR2
		SM30-R-ex	
R-T304D	PCR1	SM36-F-mut-Asp	pICPPV-NK-IGFP
		SM30-R-ext	
	PCR2	SM30-F-ext	
		SM36-R-mut-Asp	
	PCR3	SM30-F-ext	PCR1 + PCR2
		SM30-R-ex	
R-T304N	PCR1	SM37-F-mut-Asn	pICPPV-NK-IGFP
		SM30-R-ext	
	PCR2	SM30-F-ext	
		SM37-R-mut-Asn	
	PCR3	SM30-F-ext	PCR1 + PCR2
		SM30-R-ex	
SwCM-T306A	PCR1	SM56-F-mut-T306A	pICPPV-SwCMCP-R
		SM30-R-ext	
	PCR2	SM59-F-ext	
		SM56-R-mut-T306A	
	PCR3	SM30-R-ext	PCR1 + PCR2
		SM59-F-ext	
SwCM-T306D	PCR1	SM57-F-mut-T306D	pICPPV-SwCMCP-R
		SM30-R-ext	
	PCR2	SM59-F-ext	
		SM57-R-mut-T306D	
	PCR3	SM30-R-ext	PCR1 + PCR2
		SM59-F-ext	
SwCM-T306N	PCR1	SM58-F-mut-T306N	pICPPV-SwCMCP-R
		SM30-R-ext	
	PCR2	SM59-F-ext	
		SM58-R-mut-T306N	
	PCR3	SM30-R-ext	PCR1 + PCR2
		SM59-F-ext	

78 ^a Mutated residues are in red.

79 ^b Mutations at T304 and T306 of PPV CP CK2 motif were introduced by site-directed
80 mutagenesis using three-step PCR approach.

81

82 **Table S2**83 **Table S2. Primer list**

Primer name	Sequence
<i>Mutator primers for site-directed mutagenesis ^a</i>	
SM35-F-mut-Ala	5' - TGGATGGAAACGTCGGAG <u>GCA</u> CAAGAAGAGGACACAGAG-3'
SM35-R-mut-Ala	5' - CTCTGTGTCCTCTTCTTG <u>TGC</u> TCCGACGTTTCCATCCA-3'
SM36-F-mut-Asp	5' - TGGATGGAAACGTCGGAG <u>GAT</u> CAAGAAGAGGACACAGAG-3'
SM36-R-mut-Asp	5' - CTCTGTGTCCTCTTCTTG <u>ATC</u> TCCGACGTTTCCATCCA-3'
SM37-F-mut-Asn	5' - TGGATGGAAACGTCGGAA <u>AAT</u> CAAGAAGAGGACACAGAG-3'
SM37-R-mut-Asn	5' - CTCTGTGTCCTCTTCTTG <u>ATT</u> TCCGACGTTTCCATCCA-3'
SM56-F-mut-T306A	5' - TGGATGGAAACGTCGGAG <u>GCA</u> CAAGAAGAGGACACAGAG-3'
SM56-R-mut-T306A	5' - CTCTGTGTCCTCTTCTTG <u>TGC</u> TCCGACGTTTCCATCCA-3'
SM57-F-mut-T306D	5' - TGGATGGAAACGTCGGAG <u>GAT</u> CAAGAAGAGGACACAGAG-3'
SM57-R-mut-T306D	5' - CTCTGTGTCCTCTTCTTG <u>ATC</u> TCCGACGTTTCCATCCA-3'
SM58-F-mut-T306N	5' - TGGATGGAAACGTCGGAA <u>AAT</u> CAAGAAGAGGACACAGAG-3'
SM58-R-mut-T306N	5' - CTCTGTGTCCTCTTCTTG <u>ATT</u> TCCGACGTTTCCATCCA-3'
<i>Flanking primers for site-directed mutagenesis</i>	
SM30-F-ext	5' - AGTCCTGCACAGGTTGACTTGTCAAACAC-3'
SM30-R-ext	5' - CGATTTAGGTGACACTATAGAATACAAGCTTCTAG-3'
SM59-F-ext	5' - GTTTGATCCCATATTCACCTCCAGCAACAAC-3'
<i>Primers for analysis of viral progenies</i>	
2429	5' - GTCTCTTGCAAGAAGAACTAT-3'
SM13-IGFP	5' - TTACCTGTCCACAC-3'
S80	5' - TTGGGTTCTTGAACAAGC-3'

84

85 ^a Mutated codons in bold and underlined

86

87 **Table S3**

88 **Table S3.** Phospho-peptides identified in the coat protein of *Plum pox virus* virions
89 purified from *Prunus persica* plants

Peptide sequence	Phosphorylation site	start-end	z	m/z	Search engine	Score
ADEREDEEEVDAGKPSVVTAPAA TSPILQPPFVIQPA ^a	S25 [?]	1-39	4	1090.78	Mascot	>30
GNEDA ^a SPSNSNALVNTNRDR ^b	S81	76-95	3	737.65	Mascot	>40
DVDAG ^a SVGTFTVPR ^b	S101	96-109	2	750.84	Mascot	>50
					Peaks	>50
AMTSKL ^a SLPK ^b	S118	112-121	2	578.29	Mascot	>25
NL ^a TDYSLAR ^b	T254	252-260	2	566.75	Mascot	>40
					Peaks	>50

90

91 Summary of confident peptides and their phosphorylated sites identified by LC-MS/MS,
92 from two biological replicates. MS/MS spectra identifications were carried out by using
93 Mascot and Peaks search engines, considering specified confident threshold scores,
94 although manual validation was also performed. Data are available via ProteomeXchange
95 with identifier PXD017780.

96 ^a Both post-translational modifications, phosphorylation and O-GlcNAcylation, are
97 unambiguously detected in the identified peptide 1-39. However, y/b fragment ions
98 assigning these modifications to specific residues could not be found. Phosphorylation at
99 S25 (in green) is guessed on basis to previous results with virions purified from *Nicotiana*
100 *benthamiana* plants, but phosphorylation in S16 or T19 cannot be ruled out. O-
101 GlcNAcylation targeted one of these residues: S16, T24 or S25.

102 ^b Residues definitively found modified by phosphorylation are shown in red (see MS/MS
103 fragmentation spectra in Supplementary Figure S2).

104

105 **Table S4**

106 **Table S4.** Summary of phosphorylations affecting the coat protein (CP) of different *Plum*
 107 *pox virus* isolates, and in different hosts

Plant host	Strain Isolate	Specific residues in PPV-R CP									
		pS25	pS62	pT71	pS81	pS101	pT106	pS118	pT254	pT304	pT313
<i>Nicotiana spp.</i>	D R	M	N.D.	M	M	M	M	M	M	N.D.	M
	Rec BOR-3	N.A.	M	N.D.	M	M	N.D.	M	M	N.D.	N.D.
	Cherry SwCM	-	N.A.	N.D. (S73)	M (pT83)	N.D. (T103)	N.D. (S108)	M (pS120)	M (pT256)	M (pT306)	N.D. (T315)
<i>Prunus persica</i>	D R	?	-	N.D.	M	M	N.D.	M	M	N.D.	-

108

109 (M) phosphorylation mapped; (?) phosphorylation detected but not unambiguously
 110 assigned to that specific position; (N.D.) not detected phosphorylation; (N.A.) not
 111 applicable; (-) corresponding peptide not generated. Residues in the CP of the SwCM
 112 isolate in listed positions of R isolate are shown in brackets.

113

114 **Table S5**

115 **Table S5.** Infectivity of *Plum pox virus* mutants affected in putative phospho-target at the
116 casein kinase II motif of the capsid protein (CP)

Inoculum ^a	Clone ^a	Infected / inoculated plants ^b
R-T304A	1	2/3
	2	2/3
R-T304D	1-1	3/3
	1-2	2/3
R-T304N	1	2/3
	2	2/3
R	1	3/3
	2	2/3
SwCM-T306A	1-1	4/4
	1-2	4/4
SwCM-T306D	1	2/4
	2	3/4
SwCM-T306N	1	4/4
	2	2/4
CPSwCM-R	1	4/4

117

118 ^a *Nicotiana benthamiana* plants were manually inoculated with the DNA of indicated
119 plasmids. Two independent clones per construct were used, except in the case of mutants
120 R-T304D and SwCM-T306A for which two DNA preparations from a single clone were
121 used.

122 ^b Infectivity was estimated at 21 days post infection, based on CP detection by Western
123 blot analysis and IC-RT-PCR amplification of the viral RNA.

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142