

Modulation of early host innate immune response by an avipox vaccine virus' lateral body protein

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Supplementary Materials:

Supplementary Tables

Table S1: List of cloning, PCR and qPCR primers used in the study, in the order they appear in the Materials and Methods.

Table S2: Excel workbook file (.XLS) with 6 spreadsheets showing full lists of the upregulated genes, which were determined in the 6 comparisons as summarised in Fig. 1b. Each individual tab shows one of the following comparisons: **(a)** FP9-infected versus mock-infected CEF, **(b)** FWPVΔ012 -infected versus mock-infected CEF, **(c)** FWPVΔ184 -infected versus mock-infected cells, **(d)** IFN- α stimulated versus mock-stimulated CEF, **(e)** FWPVΔ012 -infected versus FP9-infected CEF, and **(f)** FWPVΔ184 -infected versus FP9-infected CEF. Headings are: Probeset ID, Gene Symbol, Gene Title, RefSeq Transcript ID, p-value (FDR) and Fold-change.

Supplementary figures

Figure S1: **(a)** Log2 expression levels of FPV184 (Chicken array probe: NC-002188.CDS185.S1_at) in mock-infected, and infected CEF with FP9 and knock-out viruses. **(b)** Log2 expression levels of FPV094 (Chicken array probe: NC-002188.CDS95.S1_at) in mock-infected, and infected CEF with FP9 and knock-out viruses. **(c)** Intermediate/late transcription of *FPV184* is consistent for a structural gene but unusual for an immunomodulatory gene, usually expressed at early stages of infection. Figure shows log2 expression values determined by microarrays of: *FPV184* and *FPV168* (late; structural genes) as well as *FPV012* and *FPV014* (early; immunomodulatory genes) during infection of CEF with FP9 (at 4, 8, 16 and 24 h p.i.). Analysis of microarray data (ArrayExpress accession: E-MTAB-5455) was conducted with Genespring GX (Agilent).

Figure S2: Correlative super-resolution light and electron microscopy of virions expressing FPV184 fused with EGFP at its N-terminus, immunolabelled with anti-GFP nanobodies. STORM images of the FPV184 protein were registered with EM micrographs of whole virions. Overview of two representative virions (i and ii), which are shown in higher magnification in Fig. 3e. Scale bar: 2 μ m.

Figure S3: Luciferase reporter gene assay. DF-1 cells were transfected for 48 h with *FPV012*, *FPV014* and/or *FPV184* expression plasmids and a luciferase reporter plasmid directed by the chicken Mx1 promoter, as well as an internal control. Cells were either treated overnight with chicken recombinant IFN- α (1000 IU/ml) or left untreated. 'Uninduced' correspond to cells transfected only with the reporter plasmid. The relative luciferase activities are shown.

Figure S4: Multiple amino acid sequence alignment of FPV184 orthologues from each genera of chordopoxviruses: genus of the chordopoxviruses: CNPV (canarypox virus), VACV (vaccinia virus), MYXV (myxoma virus), DPV (deerpox virus), SPPV (sheeppox Virus), SWPV (swinepox virus), YMTV (Yaba monkey tumor virus), MOCV (molluscum contagiosum virus), ORFV (Orf virus), CRV (crocodilepox virus). The alignment was performed by importing the corresponding amino acid sequences into CLC Workbench (CLC Bio, Qiagen, Aarhus, Denmark).

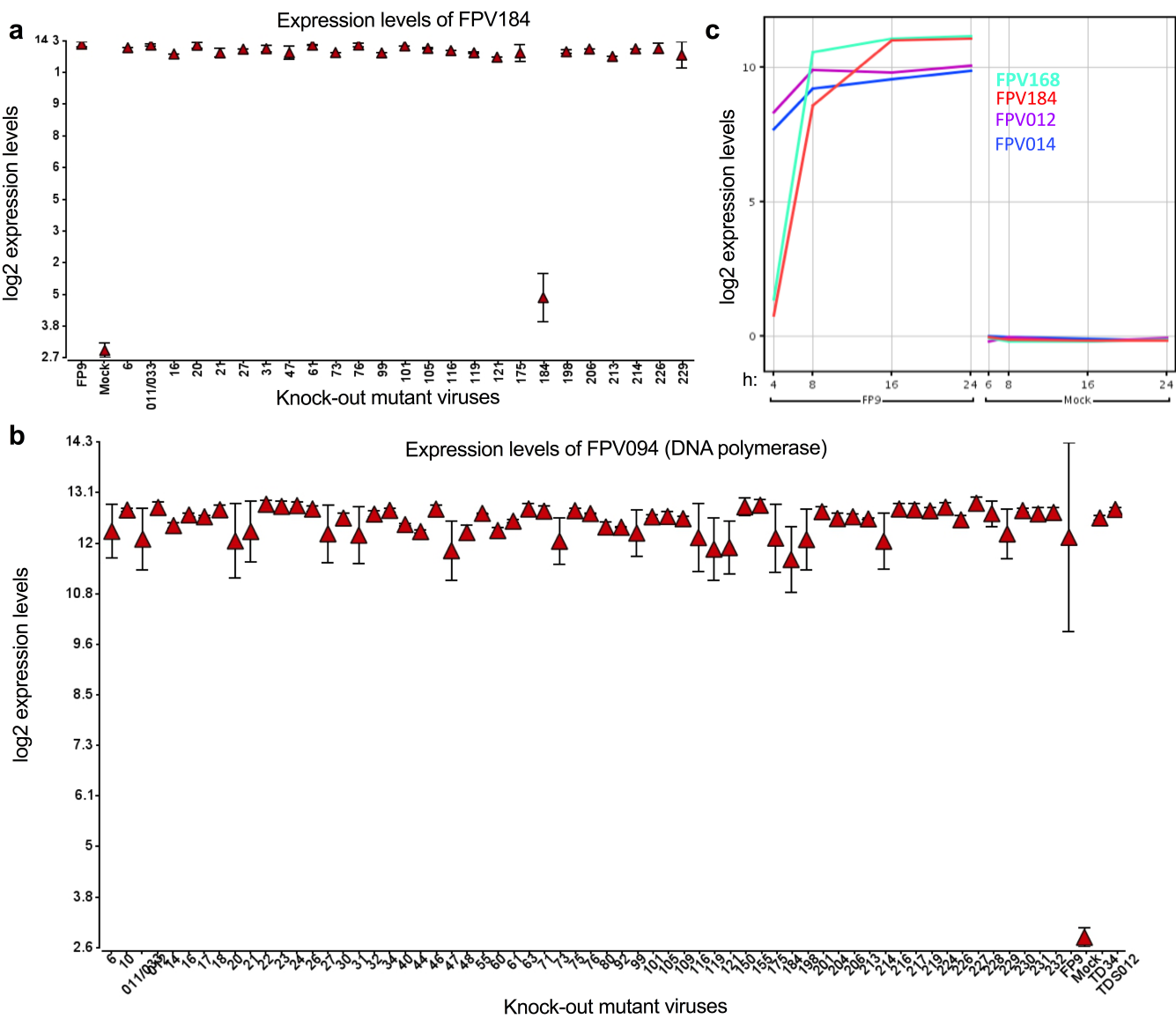
Figure S5: qPCR analysis of FPV184 or VACV A19 confirming transfections presented in Fig. 4d-e. HEK293T cells were infected with parental FP9 or FWPVΔ184 for 4 h (MOI: 5) and/or transfected with FPV184 or VACV A19 expression plasmids. Error bars indicate SEM; $n=3$. One-way ANOVA with Dunnett's *post hoc* test were used to compare induction of mRNA expression against that of cells transfected with the empty vector. **** $p<0.001$.

Figure S6: Confocal analysis of CEF infected with recombinant EGFP-expressing viruses as summarised in Fig. 5c. Confocal microscopy was performed using a Leica TCS NT confocal microscope.

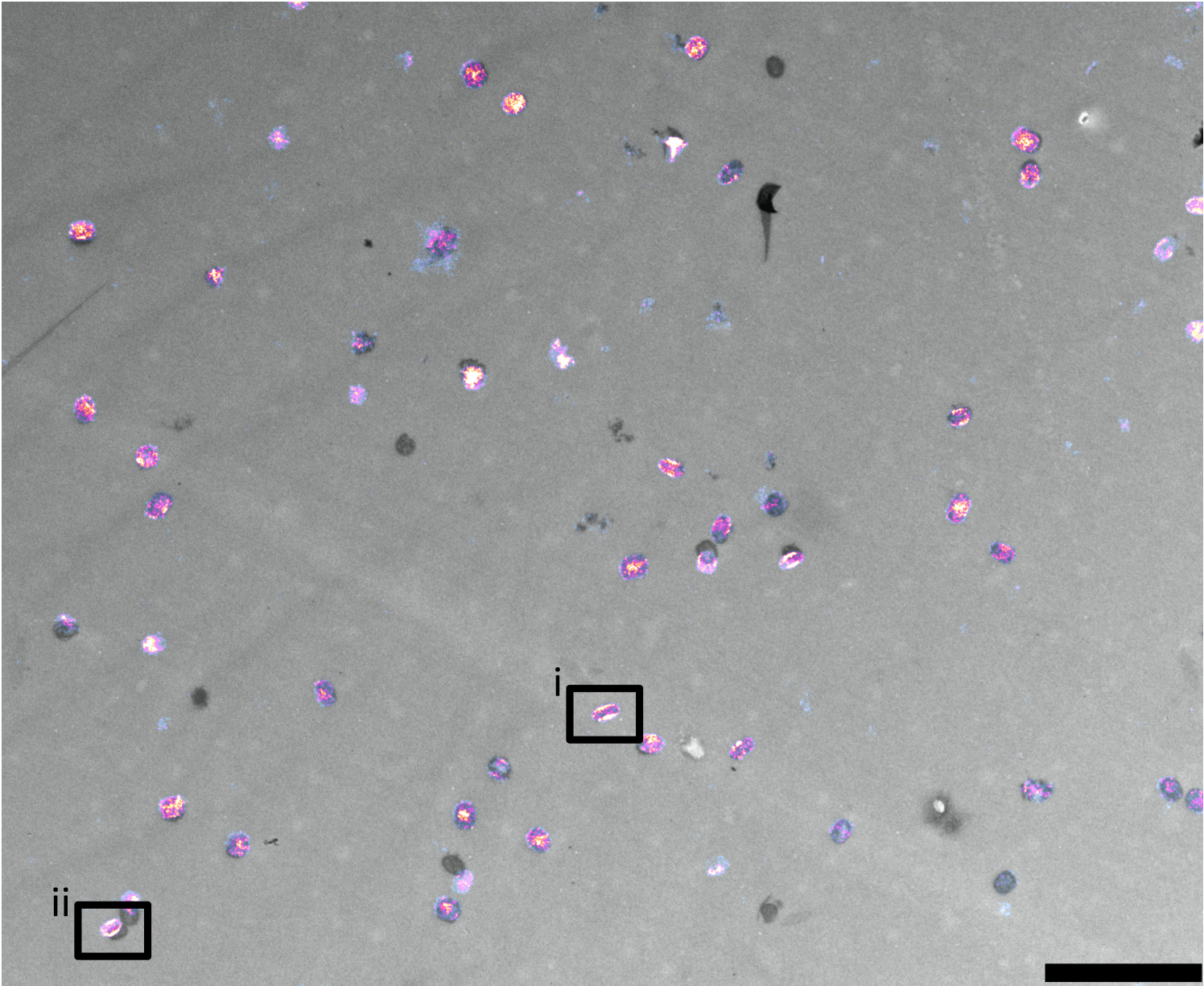
S1 Table

Name	Sequence (5'-3')	Role
M2840	GCTTCTAAATTTATCAAACATC	PCR cloning to create FPVΔ184 with insertional mutagenesis
M2841	ATCGAGAACACAACCTTTGACTGGCATATAG	PCR cloning to create FPVΔ184 with insertional mutagenesis
M2842	GTCAAAGTTGTGTCTCGATGTGAATAGC	PCR cloning to create FPVΔ184 with insertional mutagenesis
M2843	GAAACATAACGGATGCAGCCTCCAGAATAC	PCR cloning to create FPVΔ184 with insertional mutagenesis
M2844	GGCTGCATCCGTTATGTTTTCTGGGGTTAG	PCR cloning to create FPVΔ184 with insertional mutagenesis
M2845	CATCGATAAAGGAAATAAAATC	PCR cloning to create FPVΔ184 with insertional mutagenesis
M2854	CGCGGATCCCTTGATAAATTATAGCTTTACACC	PCR cloning to create transient dominant FPVΔ184
M2856	GATCCGCACGTCGTGTGGATGTAGCGGATGTAGATC	PCR cloning to create transient dominant FPVΔ184
M2857	CATCCACACGACGTGCGGATCTTCATTATGCC	PCR cloning to create transient dominant FPVΔ184
M2855	CGCGCGAAGCTTATATCCCAAACCTTAGATAGCCTGTTC	PCR cloning to create transient dominant FPVΔ184
M530	CACCGTTACAAAAATCC	PCR primer to confirm FPVΔ184 (flanking)
M1257	AGGCAAATATACGAAAC	PCR primer to confirm FPVΔ184
M2952	GCGCCCGCGGGCGCAGACTCTACAGCAGGTGCTAAG	PCR cloning of FPV184
M2951	CGCGCCCGGGTTAGGATTTAGAGAGGTCTCTCAAATGGC	PCR cloning of FPV184 and GFP184 mutant viruses
M2953	GCGCCCGCGGGCGCAGACTCTACAGCAGGTGCTTCTACATCCGCTACATCCACAG	PCR cloning of GFP184Mut1
M2954	GCGCCCGCGGGCGCAGACTCTACAGCAGGTGCTGCTGCTGCTGCTCGTAAAGAACCGCTACCGTTATACC	PCR cloning of GFP184Mut2
M2955	GCGCCCGCGGGCGCAGACTCTACAGCAGGTGCTAAGAAGAGGAAGAAAAGAGCTGCTGCTGCTACATCCACACGTAAAGAACCGCCTACCG	PCR cloning of GFP184Mut3
M2956	GCGCCCGCGGGCGCAGACTCTACAGCAGGTGCTAAGAGCCGCAAAAAGAAGCCCAAGACTACCGTTATACCAGAAGATGAA	PCR cloning of GFP184Mut4
M2892	CGCGAAGCTTATTATGGCAGACTCTACAGCAGGTGC	PCR cloning of FPV184V5
M2893	CGCGCTCGAGGGATTTAGAGAGGTCTCTCAAATG	PCR cloning of FPV184V5
M4279	GATCCTCGAGGCAGACTCTACAGCAGGTGCTAAG	FPV184 forward primer to clone into pCI-FLAG/Xho/Sal
M4280	GATCGTCGACTTAGGATTTAGAGAGGTCTCTCAAATG	FPV184 reverse primer to clone into pCI-FLAG/Xho/Sal
M4344	GATCCTCGAGGATAGCACCAATGTGCGTTCCGGAATG	Forward primer for cloning of VV A19 in pCI+FLAG-Xho site
M4345	GATCGTCGACTTAAATAGAATATTTAACTCGCAAAATC	Reverse primer for cloning of VV A19 in pCI+FLAG-SAL site
chGAPDH	GGCACTGTCAAGGCTGAGAA	qRT-PCR forward primer
chGAPDH	TGCATCTGCCCATTTGATGT	qRT-PCR reverse primer
chMx1	CACACCCAAGTGTGACGAT	qRT-PCR forward primer
chMx1	ATGTCCGAAACTCTCTGCGG	qRT-PCR reverse primer
chIFI6	TGACCAGAACGTCCACAAAGCCG	qRT-PCR forward primer
chIFI6	ACCTGCTCCTGGACCGATGCTT	qRT-PCR reverse primer
chIFIT5	TGCTTCACCAGCTAGGACTCTGC	qRT-PCR forward primer
chIFIT5	TGGCTTTTGCTCTGTCAACACTTTG	qRT-PCR reverse primer
FPV168	ACCTCAAACAACCTCATC	qRT-PCR forward primer
FPV168	GTTAATACTTGTGACTGCTG	qRT-PCR reverse primer
M2919	CCTGTATTTAGATAATCGC	PCR primer to confirm FPVΔ184 (internal)
M192	AGGTGACGATGTATTTTTTCGCTCAT	PCR primer to confirm FPVΔ184 (GPT)
M2854	CGCGGATCCCTTGATAAATTATAGCTTTACACC	PCR primer to confirm FPVΔ184 (GPT)

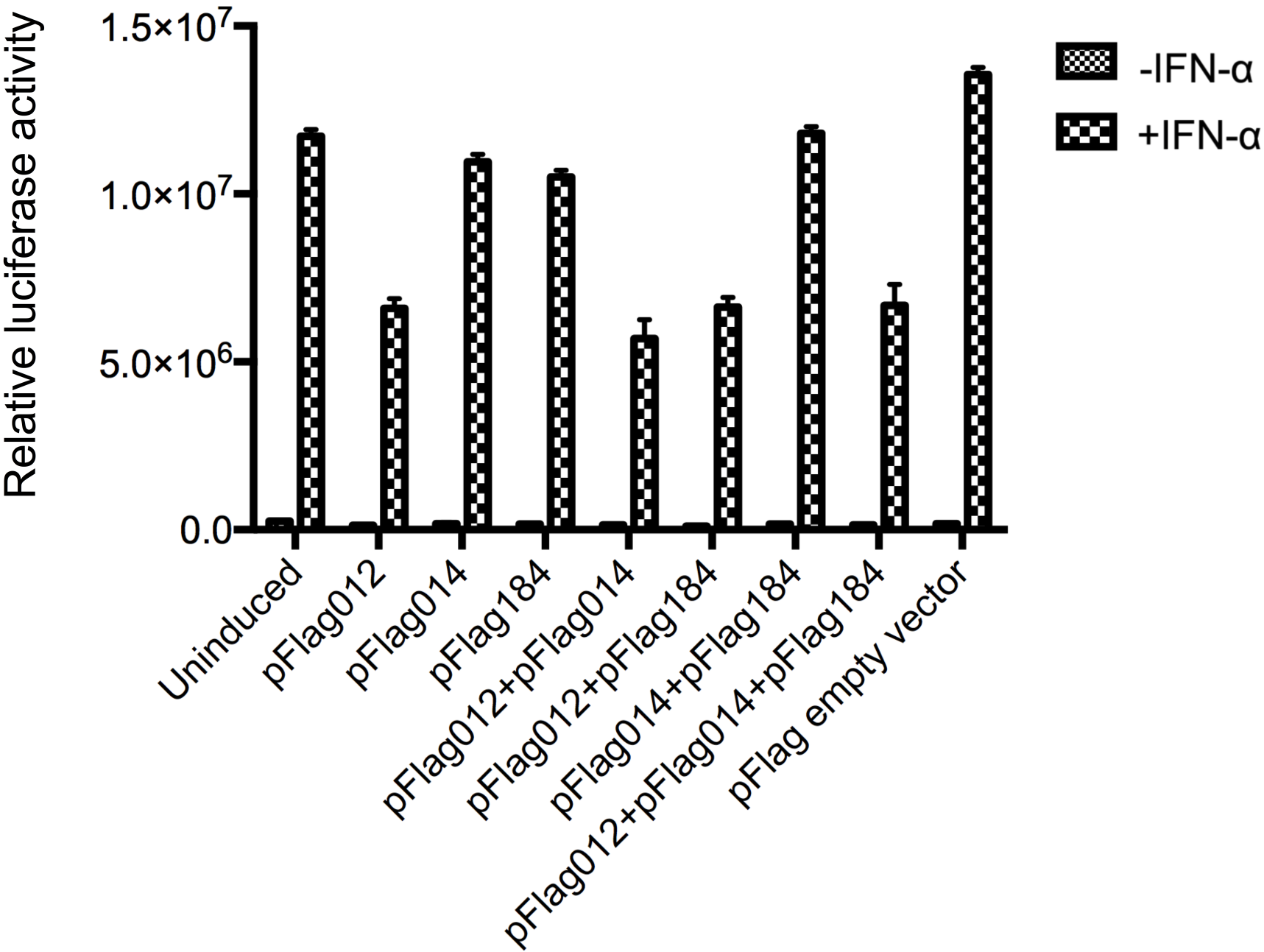
S1 Figure



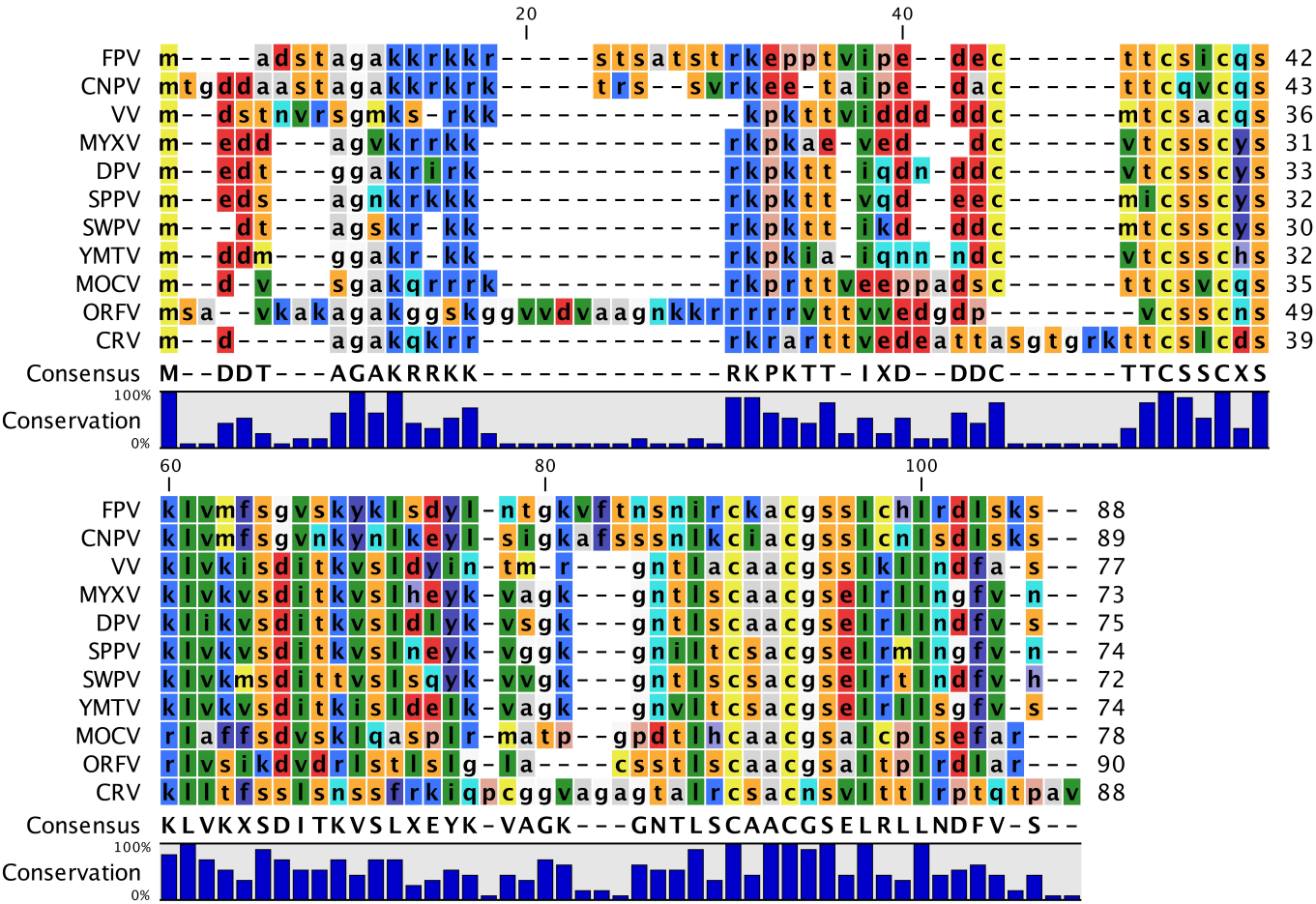
S2 Figure

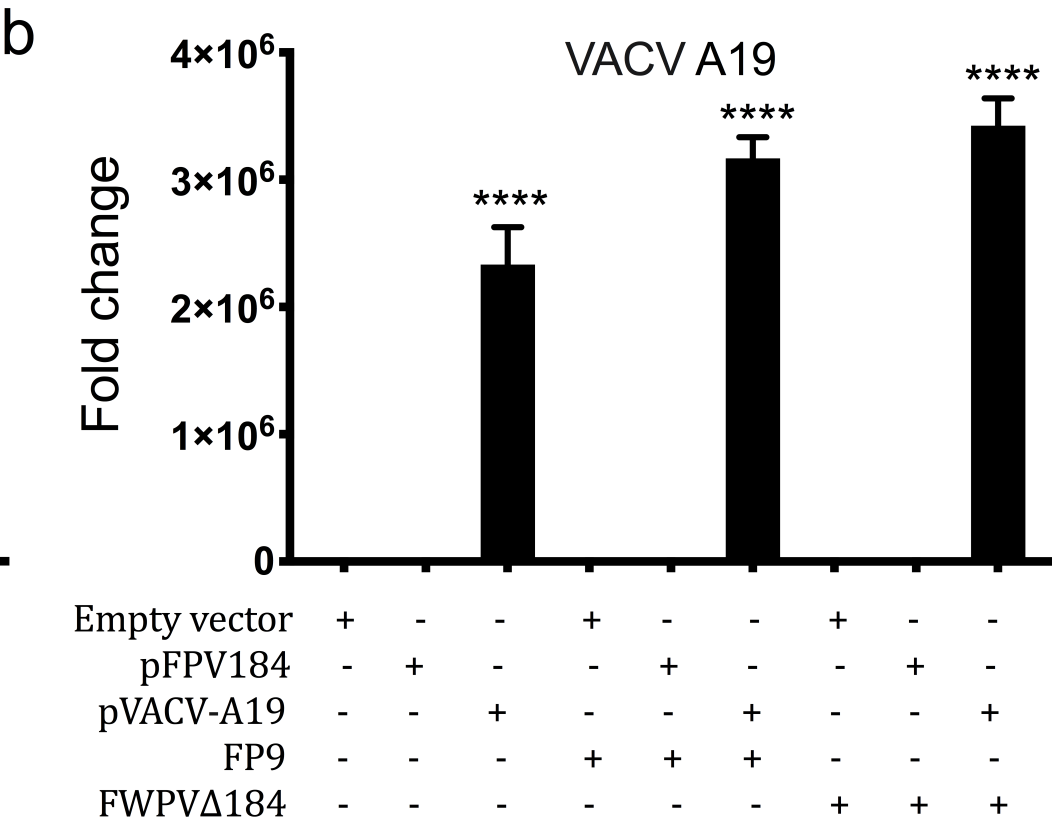
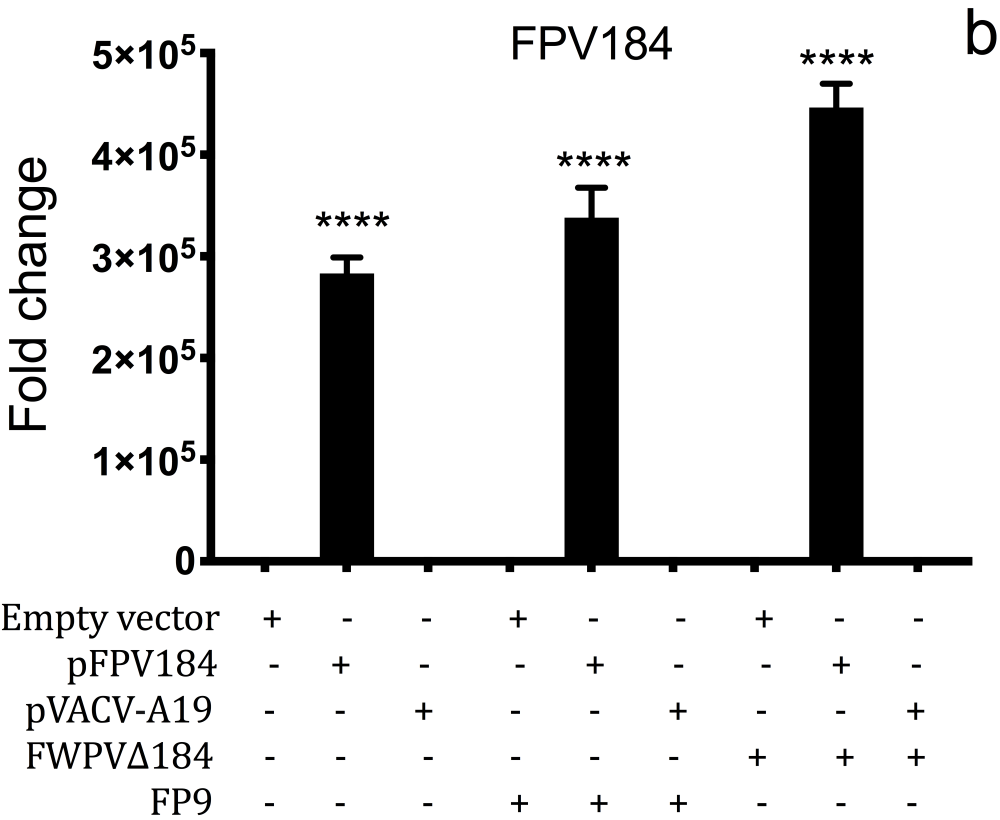


S3 Figure



S4 Figure





S6 Figure

