# Supporting Information

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### SI Text

**Immunofluorescence Confocal Microscopy.** HeLa cells ($3 \times 10^4$) plated on coverslips were either mock transfected or transiently transfected with 2 $\mu$g of pcDNA-DEST40 expressing VARV-G1R, codon-optimized MPXV-003, or codon-optimized CPXV-006. At 24-h posttransfection, cells were mock treated or treated with 20 ng/mL TNF$\alpha$ for 30 min. Coverslips were then washed with PBS and fixed with 2% paraformaldehyde (Sigma) for 12 min, permeabilized with 1% Nonidet P-40 (Sigma) and blocked with 3% BSA in PBS. Coverslips were incubated with both rabbit anti-p65 (Santa Cruz) and mouse anti-V5 (Invitrogen) diluted 1:150 in PBS containing 3% BSA for 30 min at 37 °C. Coverslips were incubated in these secondary antibodies: goat anti-rabbit-Alexa Fluor 546 and goat anti-mouse-Alexa Fluor 488 (Invitrogen) diluted at 1:400 in PBS containing 3% BSA for 30 min at 37 °C in the dark. After staining, coverslips were mounted on microscope slides with 7.5 $\mu$L of 50% glycerol containing *N*-propyl-gallate and 250 $\mu$g/mL 4′,6-diamino-2-phenylindole (DAPI) (Invitrogen) to visualize the nuclei. Cells were visualized using the 40X objective of an Olympus DSU-IX81 Spinning Disc Confocal/Deconvolution fluorescent microscope.

**Yeast 2-Hybrid Screening.** PCR reagents were purchased from Sigma-Aldrich Co., Applied Biosystems, and Stratagene, and were prepared according to recipes developed at Myriad Genetics. Upon approval from the WHO, the corresponding DNA for each of the variola virus proteins, or one of its domains, was PCR amplified at the WHO Collaborating Center for Smallpox and other Poxvirus Infections at the CDC, gel purified from variola genomic material, then shipped to and cloned at Myriad Genetics into the DNA-binding domain vector, pGBT.superB, creating an ORF for each of the variola virus proteins fused to the C terminus of the GAL4 DNA-binding domain (residues 1–147). Each of the bait plasmids was introduced into Myriad's ProNet yeast strain PNY200 (*MAT*$\alpha$ *ura3-52 ade2-101 trp1-901 his3-*$\Delta$*200 leu2-3,112 gal4*$\Delta$ *gal80*$\Delta$). The bait yeast cells were allowed to mate with Myriad's ProNet MATa yeast cells, BK100 (*MAT***a** *ura3-52 trp1-901 his3-*$\Delta$*200 leu2-3,112 gal4*$\Delta$ *gal80*$\Delta$ *GAL2-ADE2 LYS2*::*GAL1-HIS3 met2*::*GAL7-lacZ*) containing 4 independent cDNA libraries (prey libraries) prepared from human tissues, cancer cell lines, or a collection of clones (human tongue/tonsil, human spleen, a combination of breast tumor and prostate tumor cell lines, and the National Institutes of Health sponsored Mammalian Gene Collection (MGC, http://mgc.nci.nih.gov) respectively), fused to the C terminus of the Gal4 activation domain (residues 768–881) of the activation domain vector, pGAD.PN2. Each library is generated from random-primed, directionally cloned cDNA and is typically composed of >5 million independent clones with an average fragment size of 600–900 bp. After mating, at least 5 million diploid yeast cells were obtained from each library and selected in the presence of 3 mM 3-amino-1,2,4-triazole for the ability to synthesize tryptophan (bait), leucine (prey), histidine (bait/prey interaction), and adenine (bait/prey interaction). The auxotrophy is suppressed if the bait and prey proteins interact. The prey plasmids were isolated from the positive colonies, and the interactions were confirmed by transforming bait and prey constructs into naive yeast cells and performing liquid culture $\beta$-galactosidase assays. cDNAs in the positive prey plasmids were sequenced.

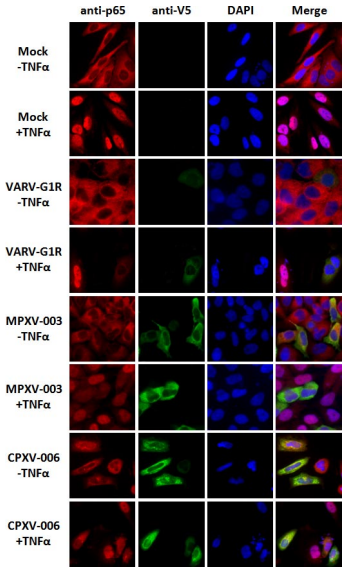


**Fig. S1.** Variola G1R family protein expression inhibits nuclear translocation of NF-κB. Indirect immunofluorescence and confocal microscopy analysis demonstrating the effect of VARV-G1R, MPXV-003, or CPXV-006 on TNFα-induced nuclear translocation of NF-κB/p65 in HeLa cells. The cells were either mock transfected or transfected with V5-VARV-G1R, V5-MPXV-003, or V5-CPXV-006 expression plasmids. The cells were labeled after treatment for 30 min in the absence or presence of TNFα.

## Other Supporting Information Files

Table S1 (PDF)
Table S2 (PDF)
Table S3 (PDF)