

Supplementary Material

Parapoxvirus Interleukin-10 Homologues Vary in Their Receptor Binding, Anti-Inflammatory, and Stimulatory Activities

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Table S1. Human and parapoxvirus IL-10 gene source, loci, length, and cloning details, with protein length and MW estimates after removal of signal peptides

IL-10 source (Virus strain)	GenBank accession no.	Gene locus (CDS)	Gene length (NT)	5' and 3' cloning modifications	Protein length (AA)	Protein MW (kDa)
Human	NM_000572.3	(60..596)	537	5'GGATCCCACCATGCACAGC' 5'AGATCTGATAATGAACAGTAG- CAGCAGGAACATCAGCAAA- GCATAGAA3' [33]	160	18.65
ORFV (NZ2)	DQ184476.1	ORFV127 (127856..128416)	561	5'CGCTCTAGAGCCAC- CATGTCTGAAGAACAAAATTCT3' 5'CGCTCTAGATTACTT- GTCATCGTCGTCCTTGTA- TCCATTTTAGTAG- TCATGTATG3' [32]	161	18.87
BPSV (BV-TX09c1)	KM875472.1	BVTX09c1_12 (127282..127842)	561	5'GGATCCCACCATGGCTAAT3' 5'AGATCTGATAGTGAACAGTAG- CAGCAGAACATCAGGCAG- TACATTCAGAG3'	166	19.46
RDPV (HL953)	NC_025963.1	SB87_gp125 (132854..133396)	577	5'GGATCCCACCATGGGCAAA3' 5'AGATCTGATAGTGAACAGTAG CAGCAGGAACATC AGAAAGTAC- CACCA3'	154	17.72
PCPV (F00.120R)	GQ329669.1	ORF001.3 (2186..2785)	607	5'GGATCCCACCATGGCTAAC3' 5'ATAGTGAACAGTAGCAGCAG- GAACATCAGACACAGTACAT3'	177	20.69
GSPV (AFK76s1)	NC_035188.1	CGV03_gp114 (120415..121029)	625	5'GGATCCCACCATGGCCTAC3' 5'AGATCTGATGAACAGTAGCAG- CAGGAACATCAGGAAGTAGAA3'	178	20.51

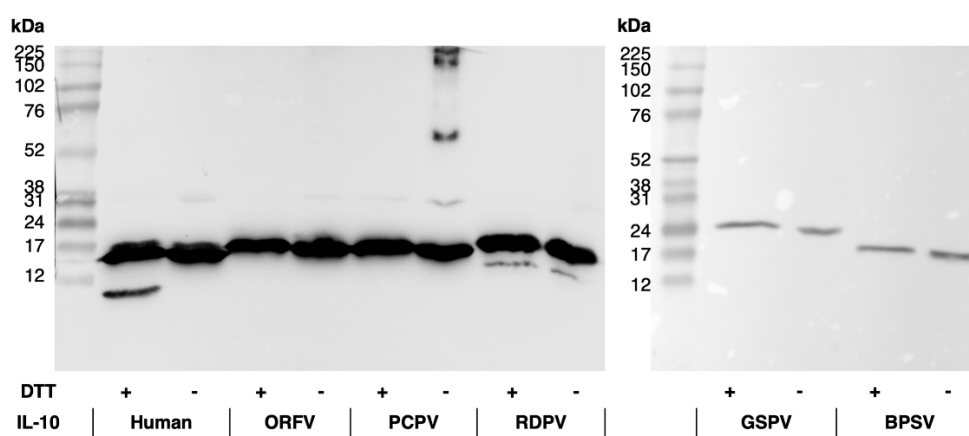


Figure S1. Anti-FLAG Western blot showing protein bands corresponding to the human and parapoxvirus IL-10 proteins. Molecular weight markers and the presence or absence of reducing agent (DTT) are indicated.

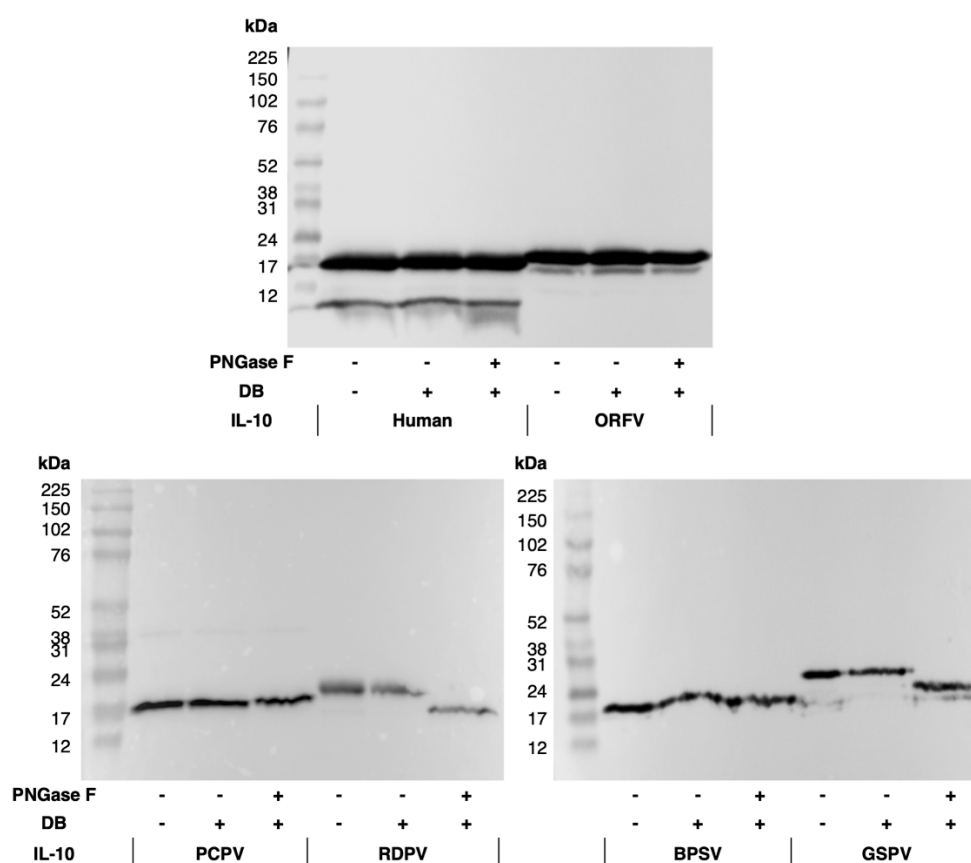


Figure S2. Anti-FLAG Western blot showing bands for human and PPV IL-10 proteins following *N*-glycosidase digestion. Molecular weight markers and the presence and absence of denaturing buffer (DB) and PNGase F are indicated.

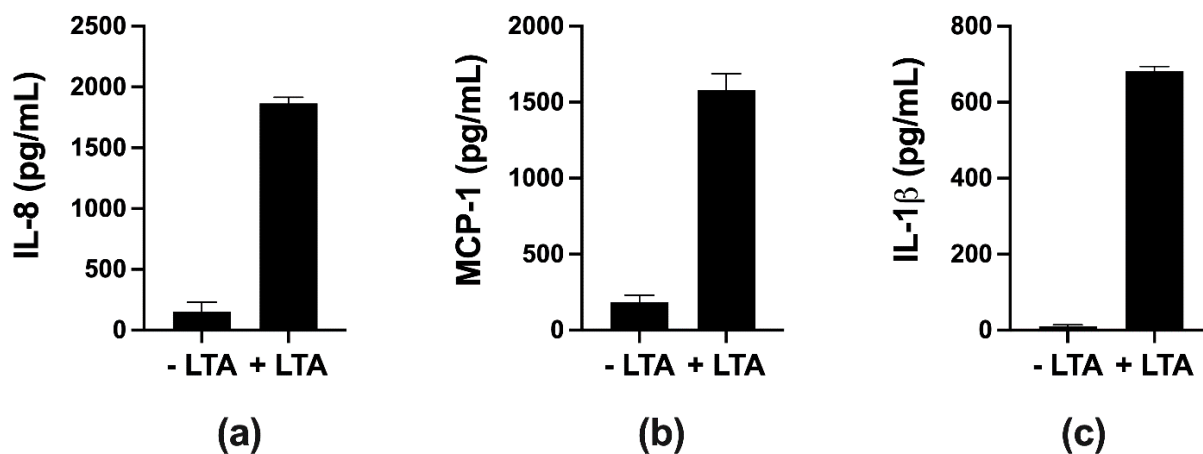


Figure S3. Pro-inflammatory chemokine and cytokine production in stimulated human monocytes. THP-1 cells were stimulated with LTA (+LTA, 1 µg.mL⁻¹) or left unstimulated (-LTA). After 24, 24, or 48 h incubation, ELISA was used to assess the levels of IL-8 (a), MCP-1 (b), IL-1β (c), respectively. Values represent the mean ± SEM of three independent experiments.

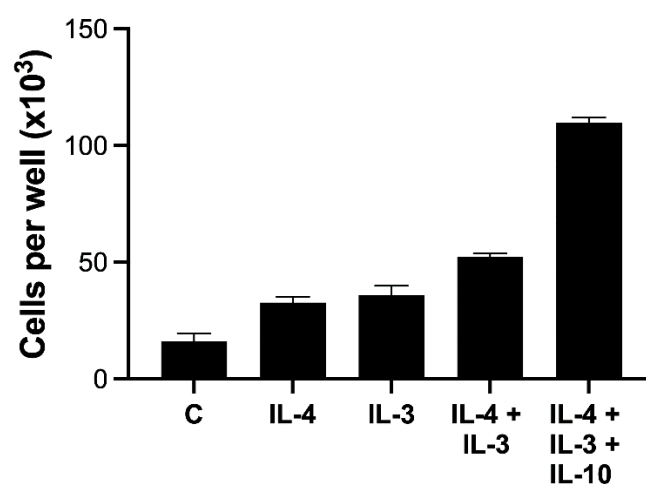


Figure S4. Stimulation of murine mast cell proliferation. MC/9 cells were co-stimulated with IL-4-conditioned medium from concanavalin A-activated murine splenocytes (IL-4), IL-3-containing conditioned medium from murine WEHI-3 cells (IL-3) and/or human IL-10 (IL-10). After 48 h incubation, the cell number was assessed through tetrazolium dye reduction and a standard curve. Values representing the mean \pm SEM of three independent experiments.