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Supplemental Data

Structure of a Herpesvirus-Encoded Cysteine Protease Reveals a Unique Class of Deubiquitinating Enzymes

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Table S1: Structural homologs of the M48^{USP}. Z score, sequence identity of aligned region, rms deviations and alignment lengths were determined by using DALI structural alignment server. Total No. of residues gives the full length of the respective enzymes.

	Protein	Origin	PDB code	Z score	Sequence identity of aligned region (%)	Rms deviation [Å]	Alignment length	Total No. of residues
1	Cysteine protease fragment (staphopain B)	*S. aureus*	1x9y	7.1	10	4.3	134	346
2	mite fecal allergen der p 1 fragment	*D. pteronyssinus*	1xkg	6.0	9	3.9	124	298
3	human procathepsin l	*H. sapiens*	1cs8	6.0	9	4.0	121	316
4	cysteine protease apg4b	*H. sapiens*	2cy7	5.3	12	4.4	142	333
5	yeast bleomycin hydrolase	*S.cerevisiae*	3gcb	4.6	10	4.0	132	458
6	protease core of mu calpain	*R. norvegicus*	1kxr	4.3	9	3.9	118	320
7	ubiquitin C-terminal hydrolase (USP 6)	*S. cerevisiae*	1vjv	4.2	14	3.6	125	359
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12	ubiquitin C-terminal hydrolase (UCH-L 3)	*H. sapiens*	1uch	2.2	12	3.9	86	206

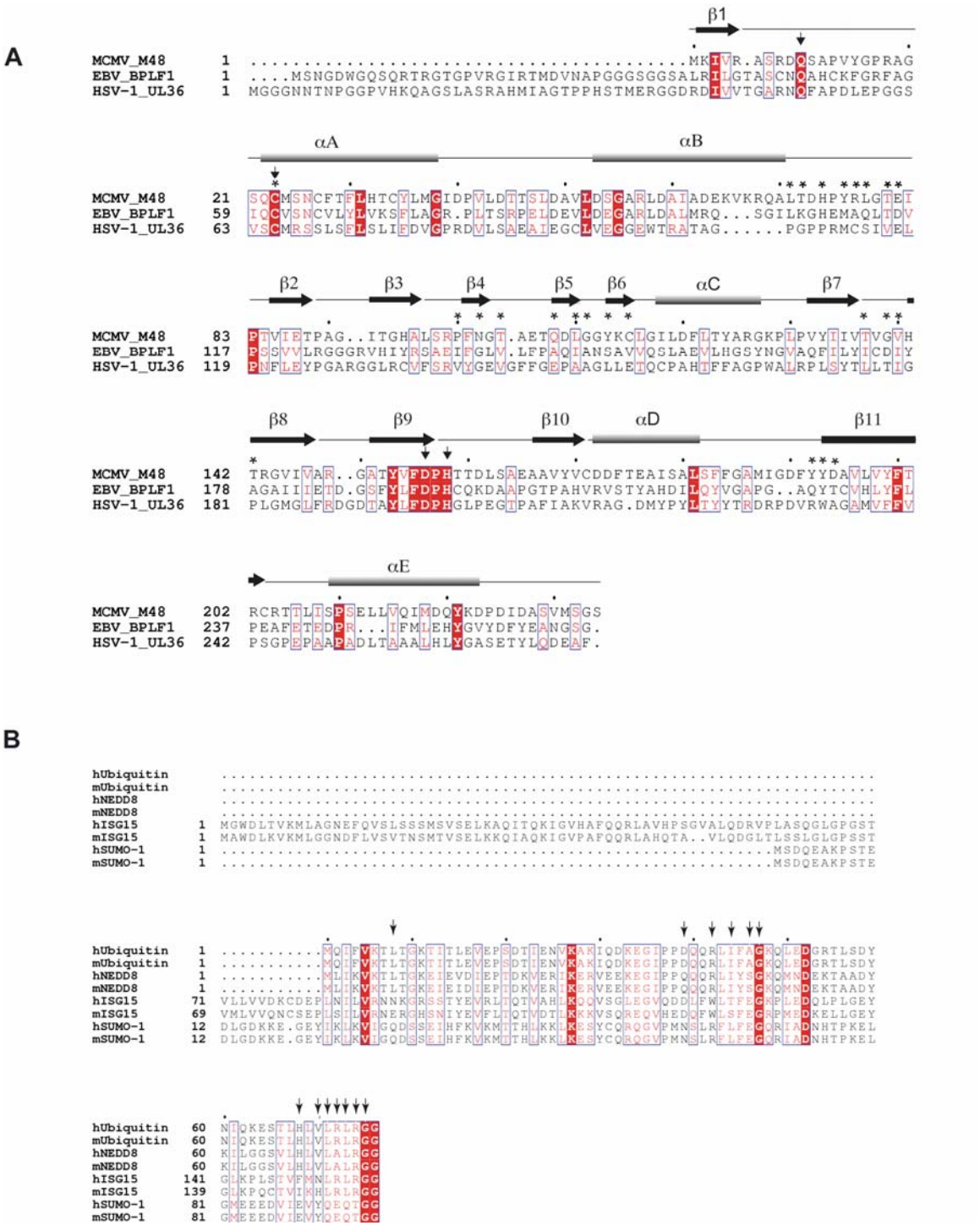

Figure S1: Sequence alignments. (A) Sequence alignment of the USP domains of M48^{USP} (MCMV), BPLF1 (EBV) and UL36 (HSV-1). Active site residues are marked by vertical arrows and residues of M48^{USP} contacting Ub are marked by stars. The secondary

structure, as observed in the M48^{USP}-UbVME structure, is depicted above the sequences, with cylinders representing helices; arrows for β-strands and lines for coils. (B) Sequence alignment of Ub and Ub-like modifiers NEDD8, ISG15 and SUMO-1. h: *Homo sapiens*, m: *Mus musculus*. Ub residues that are contacted by M48^{USP} are marked by arrows. Conserved residues are red on a white background; identical ones are white on red background.

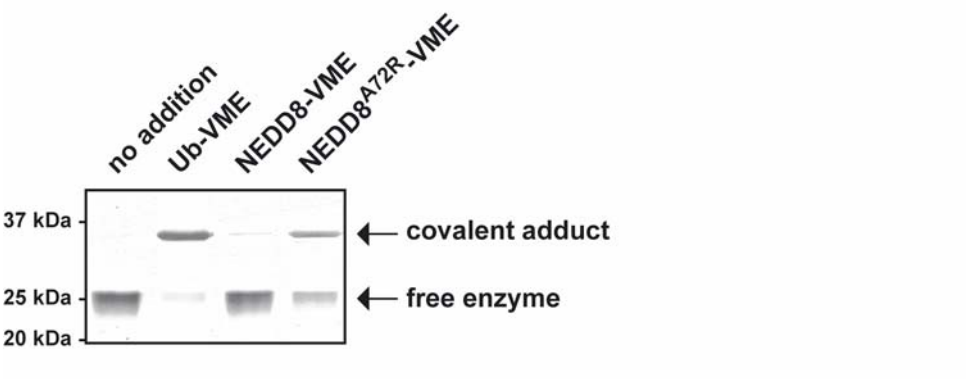

Figure S2: A single Ala to Arg substitution is sufficient to convert NEDD8 into a M48USP substrate. M48USP (2 µM) was incubated for 90 min in absence or presence of a five-fold excess of the indicated suicide substrate or the corresponding mutant derivative, and applied to SDS-PAGE and silver staining. A shift in molecular mass is indicative of a productive interaction that leads to the formation of a covalent adduct.