Supplemental Information

Table S1. List of strains and plasmids used in this study.

Strain, plasmid	Description[a]	Ref./source
E. coli		
TOP10	F⁻ *mcrA* Δ(*mrr-hsdRMS-mcrBC*) Φ80*lacZ*ΔM15 Δ*lacX74 recA1 araD139* Δ(*ara leu*)*7697 galU galK rpsL* (Str^r) *endA1 nupG*	Invitrogen
XL-1 Blue	*recA1 endA1 gyrA96 thi-1 hsdR17 supE44 relA1 lac*, F´ *proAB lacI*qZΔM15 Tn10 Tet^r	Stratagene
XL10-Gold	Tet^r *Δ(mcrA)183 Δ(mcrCB-hsdSMR-mrr)173 endA1 supE44 thi-1 recA1 gyrA96 relA1 lac* Hte [F´ *proAB lacIqZΔM15 Tn10* (Tet^r) Amy Cm^r]	Stratagene
Rosetta (DE3)	F$^-$ *ompT* $hsdS_B$(r_B^- m_B^-) *gal dcm* (DE3) pRARE (Cm^r)	Novagen
GM2163	*dam-13::*Tn *9 dcm-6 hsdR2 leuB6 his-4 thi-1 ara-14 lacY1 galK2 galT22 xyl-5 mtl-1 rpsL136 tonA31 tsx-78 supE44* McrA- McrB	New England Biolabs
H. volcanii		
DS70	Wild-type isolate DS2 cured of plasmid pHV2	(Wendoloski *et al.*, 2001)
H26	DS70 *pyrE2*	(Allers *et al.*, 2004)
HM1041	H26 *samp1*	(Miranda *et al.*, 2011)
HM1052	H26 *ubaA*	(Miranda et al., 2011)
HM1096	H26 *samp1 samp2 hvo_2177**	(Miranda et al., 2011)
HM1109[b]	HM1041 *moaE*	This study
Plasmids[c]		
pET15b	Amp^r; expression vector	Novagen
pTA131	Amp^r; devoid of *H. volcanii* origin of replication, carries *pyrE2* marker used for homologous recombination	(Allers et al., 2004)
pJAM809	Amp^r Nv^r; StrepII cloning vector for *H. volcanii*	
pJAM939	Amp^r Nv^r; Flag-HVO_0383 in *H. volcanii*	(Humbard *et al.*, 2010)
pJAM947	Amp^r Nv^r; Flag-SAMP1 in *H. volcanii*	(Humbard *et al.*, 2010)
pJAM949	Amp^r Nv^r; Flag-SAMP2 in *H. volcanii*	(Humbard *et al.*, 2010)
pJAM979-1c	Amp^r Nv^r; pJAM809-derived, HvJAMM1-StrepII in *H. volcanii*	This study
pJAM991	Amp^r; pET15b-derived, His_6-HvJAMM1 in *E. coli*	This study
pJAM1114	Amp^r; ‘knockout’ plasmid for *moaE*	(Miranda *et al.*, 2011)
pJAM1119	Amp^r Nv^r; MoaE-StrepII in *H. volcanii*	(Miranda *et al.*, 2011)
pJAM1191	Amp^r; ‘pre-knockout’ plasmid, carries HvJAMM1 gene and 500 bp DNA flanking gene in pTA131	This study
pJAM1197	Amp^r Nv^r; Flag-SAMP1 S85K in *H. volcanii*	This study
pJAM1213	Amp^r; pET15b-derived, His_6-HvJAMM2 in *E. coli*	This study
pJAM1214	Amp^r; pJAM991-derived, His_6-HvJAMM1 H90Q in *E. coli*	This study
pJAM1215	Amp^r; pJAM991-derived, His_6-HvJAMM1 D101E in *E. coli*	This study
pJAM1314	Amp^r Nv^r; pJAM947- and pJAM1119-derived, Flag-SAMP1 and MoaE-StrepII in *H. volcanii*	This study

pJAM1769	Amp^r; 'knockout' plasmid for HvJAMM1; carries in-frame deletion of HvJAMM1 gene with 500 bp flanking DNA in pTA131	This study
pJAM1770	Amp^r; pJAM991-derived, His_6-HvJAMM1 H88N in *E. coli*	This study
pJAM1771	Amp^r; pJAM991-derived, His_6-HvJAMM1 D94N in *E. coli*	This study
pJAM1772	Amp^r; pJAM991-derived, His_6-HvJAMM1 S98A in *E. coli*	This study
pJAM1778	Amp^r Nv^r ; pJAM991-derived, His_6-HvJAMM1 E31D in *E. coli*	This study
pJAM1777	Amp^r Nv^r; pJAM1197- and pJAM1119-derived, Flag-SAMP1 S85K and MoaE-StrepII in *H. volcanii*	This study
pJAM1786	Amp^r Nv^r; HvJAMM1-StrepII E31D in *E. coli*	This study
pJAM1791	Amp^r; pJAM991-derived, His_6-HvJAMM1 C115S in *E. coli*	This study
pJAM1796	Amp^r Nv^r; pJAM1314-derived, Linear Flag-SAMP1-MoaE M1A-StrepII in *H. volcanii*	This study
pJAM1804	Amp^r Nv^r; pJAM1314-derived, Linear Flag-SAMP1ΔGG-MoaE M1A-StrepII in *H. volcanii*	This study
pJAM1806	Amp^r Nv^r; pJAM1314-derived, Linear Flag-SAMP1ΔGG-MoaE-StrepII in *H. volcanii*	This study
pJAM1807	Amp^r Nv^r; pJAM1314-derived, Linear Flag-SAMP1ΔVSGG-MoaE-StrepII in *H. volcanii*	This study
pJAM1810	Amp^r Nv^r; pJAM947- and pJAM979-1c-derived, Flag-SAMP1 and HvJAMM1 genes in tandem	This study
pJAM1811	Amp^r Nv^r; pJAM947 and pJAM1786-derived, Flag-SAMP1 and HvJAMM1 E31D genes in tandem	This study
pJAM1812	Amp^r Nv^r; pJAM949-and pJAM979-1c derived, Flag-SAMP2 and HvJAMM1 genes in tandem	This study
pJAM1814	Amp^r Nv^r; pJAM949 and pJAM1786-derived, Flag-SAMP2 and HvJAMM1 E31D genes in tandem	This study

[a]Str^r, Tet^r, Cm^r, Amp^r and Nv^r denote streptomycin, tetracycline, chloramphenicol, ampicillin and novobiocin resistance.

[b] HM1109 was constructed using the pop-in/pop-out method (Allers et al., 2004) with plasmid pJAM1114 and parent strain HM1041. PCR primers used to confirm integration of the plasmid on the genome and to identify strains in which resolution of this integrant generated a deletion of *moaE* on the genome were as previously described (Miranda *et al.*, 2011).

[c]Primer pairs used for generation of the plasmids in this study are numbered and listed in Table S2. Plasmids pJAM991 and pJAM1213 used for synthesis of N-terminal His_6-tagged HvJAMM1 (HVO_2505) and HvJAMM2 in recombinant *E. coli* were generated as follows. The HvJAMM1/2 genes were amplified by PCR from *H. volcanii* DS70 genomic DNA using primer pairs 1/2 and ligated into the NdeI to BlpI sites of plasmid vector pET15b. For co-expression of Flag-SAMP1 and MoaE-StrepII genes, the NotI to BamHI fragment of pJAM1119 (encoding MoaE-StrepII) was blunt-end ligated into the BlpI site of pJAM947 (encoding Flag-SAMP1) to generate plasmid pJAM1314. The plasmid with the *pyrE2* marker of pTA131 that was constructed for targeted in-frame deletion of the HvJAMM1 gene from the *H. volcanii* chromosome by the pop-in/pop-out method (Allers et al., 2004) was constructed as follows. First, a pre-knockout plasmid (pJAM1191) was generated by ligation of a PCR-derived BamHI-to-HindIII fragment of DNA from *H. volcanii* DS70 (that carries the HvJAMM1 gene and ~500 bp of flanking DNA) with primer pair 12 into plasmid pTA131. Plasmid pJAM1191 was used as a template for inverse PCR with primer pair 13 to generate knockout plasmid pJAM1769, which had an in-frame deletion of the HvJAMM1 gene and was used to transform the Δ*pyrE2 H. volcanii* strain H26 to uracil prototrophy. For site-directed mutagenesis of the HvJAMM1 encoding gene, the QuikChange site-directed mutagenesis kit (Stratagene) was used with plasmid pJAM991 as the template and primer pairs 3-9, which resulted in the generation of plasmids pJAM1214, pJAM1215, pJAM1770, pJAM1771,

pJAM1772, pJAM1778, and pJAM1791. For site-directed mutagenesis of the SAMP1 encoding gene, primer pair 10 and template pJAM947 were used for PCR. The resulting "SAMP1 S85K" PCR product was ligated into the KpnI to BlpI sites of plasmid pJAM939 to generate plasmid pJAM1197. For co-expression of genes encoding Flag-SAMP1 S85K and MoaE-StrepII, the NotI to BamHI fragment of pJAM1119 (encoding MoaE-StrepII) was ligated into the BlpI site of pJAM1197 (encoding Flag-SAMP1 S85K) to generate plasmid pJAM1777. To generate a Flag-SAMP-MoaE-StrepII linear fusion and variants, inverse PCR was used with primer pairs 11-14 and plasmid pJAM1314 as a template. The resulting linear PCR products were treated with T4 polynucleotide kinase prior to generating plasmids pJAM1796, pJAM1806, pJAM1804 and pJAM1807 by ligation. To generate plasmids for expression of HvJAMM1-StrepII in *H. volcanii*, PCR was performed using primer pair 18 and *H. volcanii* DS70 genomic DNA as template. The PCR product was ligated into the NdeI and KpnI sites of pJAM809 to generate plasmid pJAM979-1c. Plasmid pJAM979-1c served as a template for QuikChange (Stratagene) site-directed mutagenesis with primer pair 3 to generate plasmid pJAM1786. For *in trans* expression of the Flag-SAMP genes with either the HvJAMM1-StrepII wild-type or E31D gene, the NdeI to BlpI fragments of plasmids pJAM979-1c and pJAM1786 were ligated into the blunt-ended BlpI site of plasmids pJAM947 and pJAM949 to generate plasmids pJAM1810, pJAM1811, pJAM1812 and pJAM1814 (with gene orientation confirmed by restriction mapping and DNA sequencing).

Table S2. List of primers used in this study.

Primer pair no.	Primer Name	Oligonucleotide Sequence[a]
1	HvJAMM1 NdeI up	5’-*TTCCGGG*catatgACTTCGAGTAGGCTCTCTCTC-3’
	HvJAMM1 BlpI stop	5’-*AAT*gctcagcTCACTCGACGGCGACGGAGAG-3’
2	HvJAMM2 NdeI up	5’- *CCCACTT*catatgCGACTCTTCCGGTCG-3’
	HvJAMM2 BlpI stop	5’- *AAT*gctcagcTCATCGGTCCAACTCCGC-3’
3	HvJAMM1 E31D fwd	5’-GGCGACCCGCCCGCtGAcGTCTGtGGaGTCCTCGC-3’
	HvJAMM1 E31D rev	5'- GCGAGGACtCCaCAGACgTCaGCGGGCGGGTCGCC -3'
4	HvJAMM1 H88N fwd	5’-GTCGGCTTCTACaACTCGCACCCCGA-3’
	HvJAMM1 H88N rev	5’-GACTCGGGGTGCGAGTtGTAGAAGC-3’
5	HvJAMM1 H90Q fwd	5’-TACCACTCGCAgCCCGA-3’
	HvJAMM1 H90Q rev	5’-TCGGGcTGCGAGTGGTA-3’
6	HvJAMM1 D94N fwd	5’-CCCCGAGTCCaACCCGGTCCCGAG-3’
	HvJAMM1 D94N rev	5’-CTCGGGACCGGGTtGGACTCGGGG-3’
7	HvJAMM1 S98A fwd	5’-CCCGGTCCCGgcCGCGACGGACC-3’
	HvJAMM1 S98A rev	5’-GGTCCGTCGCGgcCGGGACCGGG-3’
8	HvJAMM1 D101E fwd	5’-GCGACGGAaCGGGAACGGGC-3’
	HvJAMM1 D101E rev	5’-GCCCGTTCCCGtTCCGTCGC-3’
9	HvJAMM1 C115S fwd	5'- CGTCTATCTCATCTcCTCGCCCGACGGCC -3'
	HvJAMM1 C115S rev	5'- GGCCGTCGGGCGAGgAGATGAGATAGACG -3'
10	SAMP1 S85K KpnI up	5’-*AA*ggtaccGAGTGGAAGCTGTTCGCCGACCTCG-3’
	SAMP1 S85K BlpI down	5'-*TAAT*gctcagcCTAGCCGCCctTGACCGG -3'
11	SAMP1 dwn inv	5'- GCCACCACTGACCGGCGGGAACAG -3'
	MoaE fwd up M1A	5'- gctCACGTCCTCGGAATCGTCGGCG -3'
12	SAMP1 dw inv ΔGG	5'- ACTGACCGGCGGGAACAGCGCGAG -3
	MoaE FW up	5'- ATGCACGTCCTCGGAATCGTCGGCG -3'
13	SAMP1 dw inv ΔGG	5'- ACTGACCGGCGGGAACAGCGCGAG -3
	MoaE fwd up M1A	5'- gctCACGTCCTCGGAATCGTCGGCG -3'
14	SAMP1 dw inv ΔVSGG	5'- CGGCGGGAACAGCGCGAGTTCG -3'
	MoaE FW up	5'- ATGCACGTCCTCGGAATCGTCGGCG -3'
15	Hvo_2505 511 BamHI FWD	5’-*AT*ggatccGCAGCGCAGGACGTCAT-3’
	Hvo_2505 521 HindIII REV	5’-*GCG*aagcttGAGACGTTGACGACGC-3’
16	Hvo_2505 inv up REV	5’-GGCGCATGGCGATTTCGAACCACGGA-3’
	Hvo_2505 inv down FWD	5’-TCGGCTCTCGGCCAGTCGCC-3’
17	Hvo_2505 720 up FWD	5’-GCGCCGTCAACGACCTCATCACCC-3’
	Hvo_2505 687 dwn REV	5’-GGTCGGTTCGCCTCCTCGCC-3’
18	HVO2505 NdeI up	5’-*TTCCGGG*catATGACTTCGAGTAGGCTCTCTCTC-3’
	Hvo2505 KpnI StrepII down	5’-*AA*ggtaccCTCGACGGCGACGGAGAGTTCG-3’

[a]Lowercase letters indicate restriction enzyme site or site directed mutation that was introduced into the primer sequence. Underlined letters indicate modified codon. Italicized uppercase letters indicate random bases introduced into primer to enhance cleavage of PCR product by restriction enzyme.

Table S3. Mov34-MPN-PAD-1 superfamily (cl13996) protein sequences included in the dendrogram (Fig. 1).

Protein family	Protein	Species	GI number
cd08072 MPN archaeal	Hmuk_1908	*Halomicrobium mukohataei*	GI:257387955
	rrnAC1439	*Haloarcula marismortui*	GI:55378222
	HacjB3_10795	*Halalkalicoccus jeotgali*	GI:299125203
	Huta_0716	*Halorhabdus utahensis*	GI: 257051801
	Nmag_0620	*Natrialba magadii*	GI:289580302
	Htur_2117	*Haloterrigena turkmenica*	GI:284165394
	Hlac_2405	*Halorubrum lacusprofundi*	GI:222480813
	NP3222A	*Natronomonas pharaonis*	GI:76802251
	OE2140R	*Halobacterium salinarum*	GI:169235664
	HQ1580A	*Haloquadratum walsbyi*	GI:110667539
	HVO_1016 (HvJAMM2)	*Haloferax volcanii*	GI:292655176
	Hbor_21950	*Halogeometricum borinquense*	GI:313126933
	Mpal_0311	*Methanosphaerula palustris*	GI:219850984
	MCP_1751	*Methanocella paludicola SANAE*	GI:282164421
	AfJAMM	*Archaeoglobus fulgidus*	GI:11499780
	MTBMA_c13570	*Methanothermobacter marburgensis*	GI:304315110
	MTH_971	*Methanothermobacter thermautotrophicus*	GI:74548643
	Msp_1146	*Methanosphaera stadtmanae*	GI:84489938
	Mfer_0041	*Methanothermus fervidus*	GI:312136270
	MCON_1492	*Methanosaeta concilii*	GI:330507535
	Mbur_0623	*Methanococcoides burtonii*	GI:91772652
	Mmah_0889	*Methanohalophilus mahii*	GI:292666563
	MA1736	*Methanosarcina acetivorans*	GI:20090588
	MM_2631	*Methanosarcina mazei*	GI:21228733
	Mbar_A3046	*Methanosarcina barkeri*	GI:73670502
cd08070, MPN-like archaeal	Hlac_1268	*Halorubrum lacusprofundi*	GI:222479693
	HacjB3_14840	*Halalkalicoccus jeotgali*	GI:299126008
	NP0062A	*Natronomonas pharaonis*	GI:76800686
	HQ3151A	*Haloquadratum walsbyi*	GI:110669036
	Htur_3493	*Haloterrigena turkmenica*	GI:284166749
	Nmag_1532	*Natrialba magadii*	GI:289530757
	OE3561F	*Halobacterium salinarum*	GI:169236469
	HVO_2505 (HvJAMM1)	*Haloferax volcanii*	GI:292656626
	Hbor_09090	*Halogeometricum borinquense*	GI:313125679
	Mpal_1140	*Methanosphaerula palustris*	GI:219851774

	Mmah_0694	*Methanohalophilus mahii*	GI:292666370
	Ferp_0703	*Ferroglobus placidus*	GI:288893336
	TERMP_1706 (TERMP_01270)	*Thermococcus barophilus*	GI:315231033
	TK1033	*Thermococcus kodakarensis*	GI:57640968
	TSIB_0762	*Thermococcus sibiricus*	GI:242398748
	TON_1119	*Thermococcus onnurineus*	GI:212224268
	TAM4_1711	*Thermococcus* sp. AM4	GI:223478291
	PAB1919	*Pyrococcus abyssi*	GI:14520886
	PH1488	*Pyrococcus horikoshii*	GI:14591273
	PF1070	*Pyrococcus furiosus*	GI:18893137
	CSUB_C0703	Candidatus *Caldiarchaeum subterraneum*	GI:343484908
	CENSYa_0531	*Cenarchaeum symbiosum* A	GI:118194246
	Nmar_1227	*Nitrosopumilus maritimus*	GI:161528735
	APE0681 (APE0681.1)	*Aeropyrum pernix*	GI:116062476
	Igag_1456	*Ignisphaera aggregans*	GI:305663850
	Pcal_1011	*Pyrobaculum calidifontis*	GI:126459624
	Tneu_1283	*Thermoproteus neutrophilus*	GI:171185737
	Pars_2085	*Pyrobaculum arsenaticum*	GI:145592281
	Kcr_0989	Candidatus *Korarchaeum cryptofilum*	GI:170290602
cd08070, MPN-like bacterial	Mec+ (Rv1334)	*Mycobacterium tuberculosis*	GI:54042514
	TTC1133	*Thermus thermophilus*	GI:46199435
	QbsD	*Pseudomonas fluorescens*	GI:28192389
[1]**un-classified**	CSUB_C1473 (*rpn11l*)	Candidatus *Caldiarchaeum subterraneum*	GI:315426919
cd08066, AMSH-like	AMSH-LP[2]	Human (*Homo sapiens*)	GI:71153542
	AMSH	Human (*Homo sapiens*)	GI:71153538
cd08067, histone H2A DUB-type	2A-DUB	Human (*Homo sapiens*)	GI:74756898
cd08068, BRCC36-type	BRCC36	Human (*Homo sapiens*)	GI:20532383
cd08069, Rpn11 and CSN5-type	Rpn11	Yeast (*Saccharomyces cerevisiae*)	GI:1171012
	Poh1	Human (*Homo sapiens*)	GI:51701716
	CSN5	Yeast (*Saccharomyces cerevisiae*)	GI:239938615
	CSN5	Human (*Homo sapiens*)	GI:55976562

[1]GenBank GI:315426919 annotation only classified CSUB_C1473 to level of MPN superfamily (cl13996).

[2]Abbreviations: AMSH, associated molecule with the SH3 domain of STAM or endosome-associated ubiquitin isopeptidase; AMSH-LP, AMSH-like protein; 2A-DUB, histone H2A deubiquitinase MYSM1
2A-DUB, BRCC36, a Lys-63-specific deubiquitinase subunit of two different complexes through interactions with two different adaptor proteins Abraxas and ABRO1; Rpn11, 26S proteasome regulatory particle subunit 11; Poh1,26S proteasome-associated PAD1 homolog 1; CSN5, COP9 signalosome complex subunit 5.

Suppl. Figure Legends

Figure S1. Gene neighborhood and co-occurrence patterns of representative archaeal JAMM domain proteins from group I (cd08070 family) and group II (cd08072 family), as determined using STRING 9.0 (Szklarczyk et al., 2011).

Figure S2. Multiple amino acid sequence alignment of JAMM/MPN+ domain proteins. Proteins for alignment included HvJAMM1 (HVO_2505), HvJAMM2 (HVO_1016), AfJAMM (AF2198) and the central domain of human AMSH (GI: 4098124) (where gene locus tag and GI numbers are indicated in parenthesis). Identical and conserved amino acid residues are highlighted in black/red and grey, respectively. Conserved residues of the JAMM/MPN+ motif ($EX_nH[S/T]HX_7SX_2D$, where X represents any residue) including residues likely to coordinate the catalytic Zn^{2+} and water molecule are highlighted in red. A conserved cysteine residue predicted to reside near the active site of HvJAMM1 is highlighted in pink. Amino acid position number indicated on left and right.

Figure S3. Purification of HvJAMM1 and HvJAMM2. The HvJAMM1/2 proteins were purified as depicted schematically and described in methods. In general, A) the HvJAMM genes were cloned into pET15b to generate plasmids pJAM991 and 1213, B) the HvJAMM proteins were expressed in recombinant *E. coli* (Rosetta) after addition of IPTG and C) the HvJAMM proteins were purified to apparent homogeneity by Ni^{2+}-affinity chromatography. Proteins were separated by reducing 12% SDS-PAGE and stained with Coomassie Brilliant Blue. Molecular mass standards (M_r) indicated on left.

Figure S4. HvJAMM1 is not a broad spectrum protease. In contrast to proteinase K, HvJAMM1 is not a broad spectrum protease. Hemoglobin (lane 1), cytochrome *c* (cyt *c*)(lane 2), carbonic anhydrase (CA)(lane 3), creatine phosphokinase (CPK)(lane 4), β-amylase (lane 5) and bovine serum albumin (BSA)(lane 6) were incubated alone (A), with proteinase K (B), and with HvJAMM1 (C). Assay mixtures were as described in Experimental Procedures with reaction products separated by 12% SDS-PAGE and stained with Coomassie blue. Molecular mass markers are indicated on left.

Figure S5. HvJAMM1 did not cleave the amide bond linking aminomethylcoumarin (AMC) to ubiquitin (Ub-AMC). Reaction conditions were as described in Experimental Procedures. In contrast, cleavage of Ub-AMC by the eukaryotic cysteine-type peptidase isopeptidase T (ubiquitin specific peptidase 5, USP5) was readily detected by the assay. USP5 and HvJAMM1 did not hydrolyze GG-AMC using similar methods to monitor cleavage of the amide bond by fluorescence (data not shown).

Figure S6. HvJAMM1-mediated desampylation of SAMP conjugates. All reaction mixtures were in 10 µl volume using 10 µM HvJAMM1 and 'enriched' Flag-SAMP2 conjugates (5 µg protein) and were incubated at 50 °C for 2 h in 20 mM HEPES buffer at pH 7.5 with 2 M NaCl unless otherwise indicated. Where indicated, HvJAMM1 was inactivated by addition of 50 µM EDTA or boiling (•) for 10 min prior to assay. After assay, samples were separated by reducing 12 % SDS-PAGE and analyzed by α-Flag immunoblot (see methods for details). Molecular mass standards are indicated on left. A) Optimal enzyme concentration for HvJAMM1 activity. Assays were performed with 0 to 50 µM HvJAMM1 supplemented with 500 µM $ZnCl_2$. B) Time course of enzyme activity with assay

performed using 50 µM HvJAMM1 and 'enriched' Flag-SAMP2 conjugates (20 µg protein). C) Optimal temperature for HvJAMM1 activity. Assays were performed from 20 to 70 °C. D) Optimal salt concentration for HvJAMM1 activity. Assays were performed using 50 µM HvJAMM1 and 'enriched' Flag-SAMP2 conjugates (20 µg protein) with 0.15 to 2 M NaCl. E) Optimal pH and buffer for HvJAMM1 activity. Assays were performed for 2 h at 25 °C in the presence and absence of 10 µM HvJAMM1 with 2 M NaCl using 20 mM buffer and pH as indicated.

Figure S7. Mapping of sites of sampylation for the SAMP1-MoaE conjugate formed in *H. volcanii*. The conjugate was digested with trypsin and analyzed on a nanoflow HPLC-LTQ Orbitrap XL mass spectrometer. (A) MS/MS spectrum showing Gly-Gly modified Lys240 in peptide LKDEVPIFK. The precursor mass, b ion series and y8 ion are evidence for the modification identified on the peptide. (B) MS/MS spectrum showing Gly-Gly modified Lys247 in peptide LKDEVPIFKK. The precursor mass, b9 ion and the y ion series are evidence for the modification identified on the peptide.

Figure S8. HvJAMM1 gene appears essential. Plasmids and primers used for this analysis are detailed in Table S1-S2. Integration of the HvJAMM1 'knockout' plasmid pJAM1769 onto the genome of *H. volcanii* (pop-ins) was confirmed by PCR (lanes 3,4). PCR-based screening of 'pop-outs' for deletion of the HvJAMM1 gene (lanes 5-9, with 5 representative clones depicted out of 460 total) revealed only reversion to wild-type. Hi-Lo DNA markers (M, lane 1) with molecular masses are indicated on left. Strain H26 was the parental control (wt, lane 2). PCR products were generated using Taq DNA polymerase, primer pair 14 (Table S2) and genomic DNA as template according to supplier (New England Biolabs). PCR products were separated by 0.8 % (w/v) agarose

gels in TAE buffer at pH 8.0, stained with ethidium bromide, and visualized by fluorescence according to standard methods.

Figure S9. Linear-linked SAMP1-MoaE generates functional SAMP1 protein for protein modification (A, B) and MoCo biosynthesis (C) when synthesized in *H. volcanii*. Samp1ylation was monitored by expression of linear-linked SAMP1-MoaE in *H. volcanii* strains with (+) and without (-) a functional *ubaA* gene, with distinguishing features for each strain indicated on the top (A and B). Linear SAMP1-MoaE included an N-terminal Flag tag and C-terminal StrepII tag to allow for visualization of the proteins by α-FLAG and α-StrepII immunoblot, respectively. C) MoCo biosynthesis was monitored by growth of *H. volcanii* on ATCC974 medium with DMSO as a terminal electron acceptor as previously described (Humbard et al., 2010). Strains included H26 parent (■), HM1109 (□), HM1109-pJAM202c (○), HM1109-pJAM1314 (▲), HM1109-pJM1796 (●) and HM1109-pJAM1804 (Δ) with distinguishing features indicated on right (C).

Figure S10. Effect of various chemical compounds on HvJAMM1 activity. All reactions (10 µl) were in 20 mM HEPES buffer at pH 7.5 with 2 M NaCl and included 10 µM HvJAMM1, Flag-SAMP2 conjugate (in 5 µg cell lysate), and chemical compound in molar ratio with the enzyme as indicated above each lane. Chemicals included: A) phenylmethanesulfonylfluoride (PMSF), B) *N*-ethylmaleimide (NEM), C) 1,10-phenalthroline (phen), and D) N,N,N′,N′-tetrakis (2-pyridyl-methyl) ethylenediamine or TPEN. After incubation for 2 h at 50 °C, samples were separated by reducing 12 % SDS-PAGE and analyzed by α-Flag immunoblot (see methods for details). Molecular mass standards are indicated on left.

Figure S11. Comparison of HvJAMM1 wild-type (WT) and site-directed variants by SDS-PAGE and circular dichroism. A) Purified HvJAMM1 proteins (1 ug per lane) were separated by reducing 12 % SDS-PAGE and stained by Coomassie Blue. Molecular mass standards (M_r Std) are indicated on left. B and C) CD spectra for purified HvJAMM1 WT and site-directed variants in the absence (B) and presence of supplementation with 500 µM $ZnCl_2$ (C). Average absorbance of triplicate measurements for each sample (+/- 0.001) are presented and were used as basis for converting CD signal in mdeg to molar ellipticity.

Suppl. References

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Fig. S1

cysteine synthase

MoeB/ThiF/UBA family

e.g., Archaeal JAMM – group I

OE2456F tuf yrhA moeB hom cysK lipA OE3561F hemC idiA trh3

Input:

OE3561F hypothetical protein (136 aa)
(Halobacterium salinarum)

Predicted Functional Partners:

Partner	Description	Neighborhood	Gene Fusion	Cooccurrence	Coexpression	Experiments	Databases	Textmining	[Homology]	Score
moeB	molybdenum cofactor biosynthesis protein MoeB (278 aa)	•		•						0.839
yrhA	cysteine synthase (326 aa)	•								0.722
cysK	cysteine synthase (327 aa)	•								0.717
idiA	isopentenyl-diphosphate delta-isomerase, type I; Catalyzes the 1,3-allylic rearrangement of the [...] (209 aa)	•								0.691
tuf	elongation factor 1-alpha; This protein promotes the GTP-dependent binding of aminoacyl-tRNA to [...] (421 aa)	•								0.665
OE2456F	hypothetical protein (69 aa)	•								0.659
hemC	hydroxymethylbilane synthase (porphobilinogen deaminase); Tetrapolymerization of the monopyrrol [...] (396 aa)	•								0.608
trh3	putative transcription regulator (137 aa)	•								0.577
hom	homoserine dehydrogenase (317 aa)	•								0.574
lipA	lipoyl synthase; Catalyzes the radical-mediated insertion of two sulfur atoms into the C-6 and [...] (311 aa)	•								0.551

SAMP1 homolog

e.g., Archaeal JAMM – group II

RecJ-like

Lon B-type protease

moaD argC HQ1580A HQ2953A taqD HQ1027A HQ3426. HQ1358A HQ1581A HQ1519A lon

Input:

HQ1580A hypothetical protein (223 aa)
(Haloquadratum walsbyi)

Predicted Functional Partners:

Partner	Description	Neighborhood	Gene Fusion	Cooccurrence	Coexpression	Experiments	Databases	Textmining	[Homology]	Score
taqD	glycerol-3-phosphate cytidyltransferase-like protein (179 aa)	•								0.964
lon	ATP-dependent protease Lon (749 aa)	•		•						0.822
HQ1581A	RecJ like exonuclease (644 aa)	•		•						0.785
HQ1027A	hypothetical protein (167 aa)			•						0.738
moaD	molybdopterin converting factor, small subunit (118 aa)			•						0.733
HQ1519A	hypothetical protein (81 aa)			•						0.732
HQ3426A	hypothetical protein (154 aa)			•						0.699
HQ2953A	cell division control protein cdc6-like protein (129 aa)			•						0.699
argC	N-acetyl-gamma-glutamyl-phosphate reductase (346 aa)	•								0.697
HQ1358A	hypothetical protein (217 aa)			•						0.696

Fig. S2

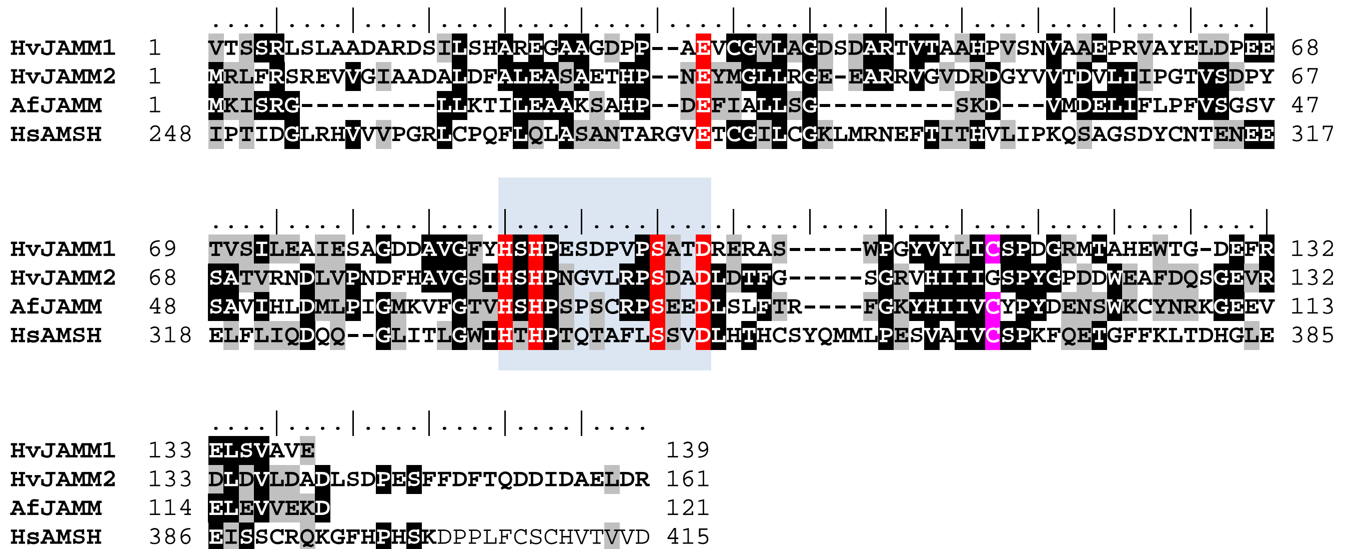
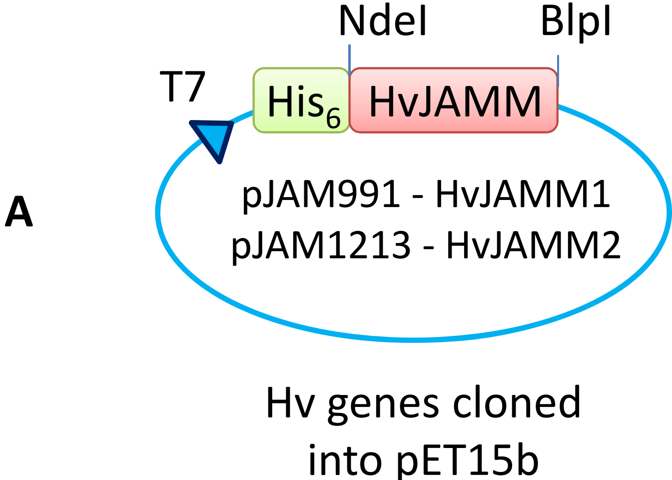
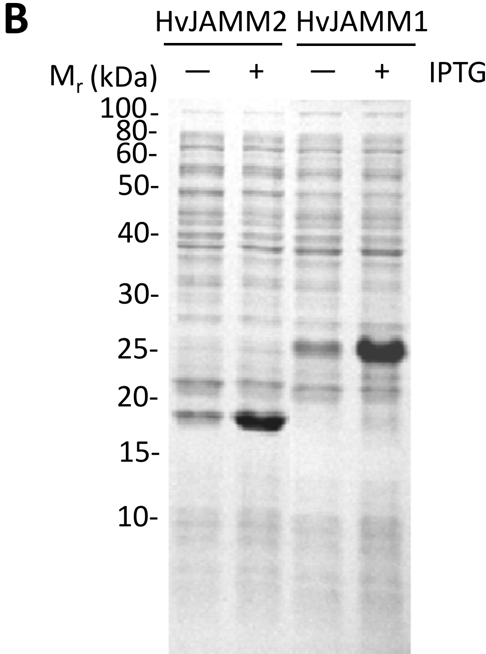
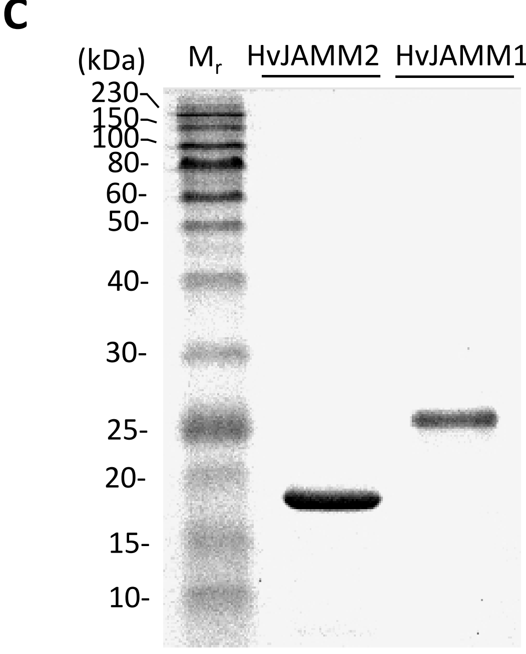


Fig. S3





HvJAMM
produced in *E. coli*



HvJAMM
protein purified

Figure S4.

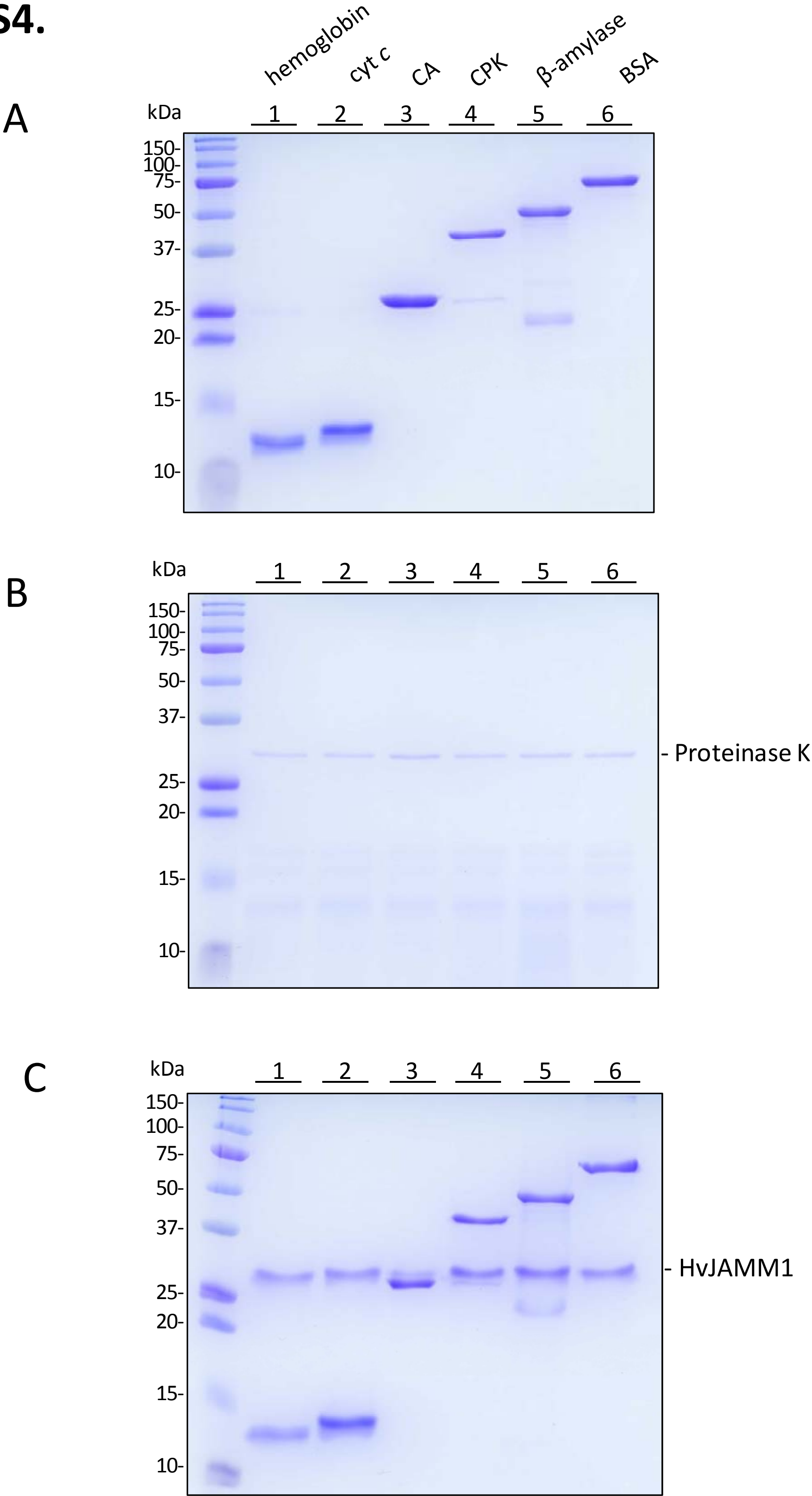


Figure S5.

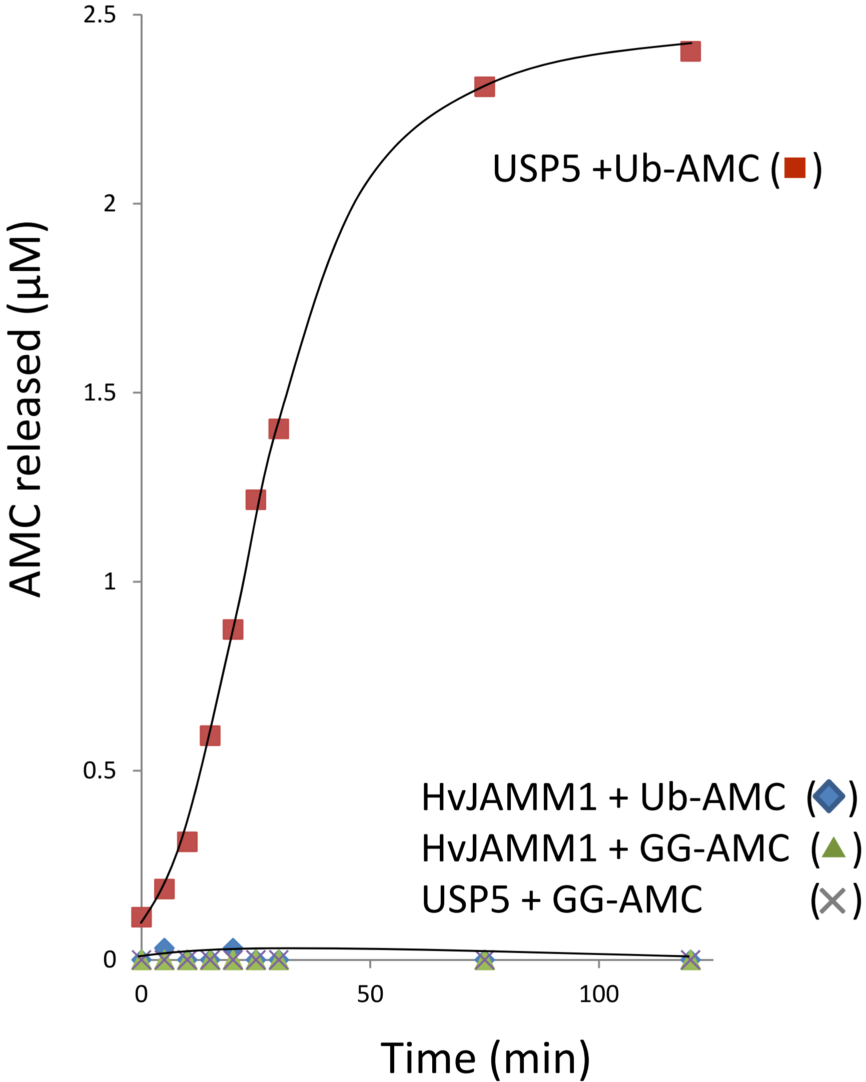
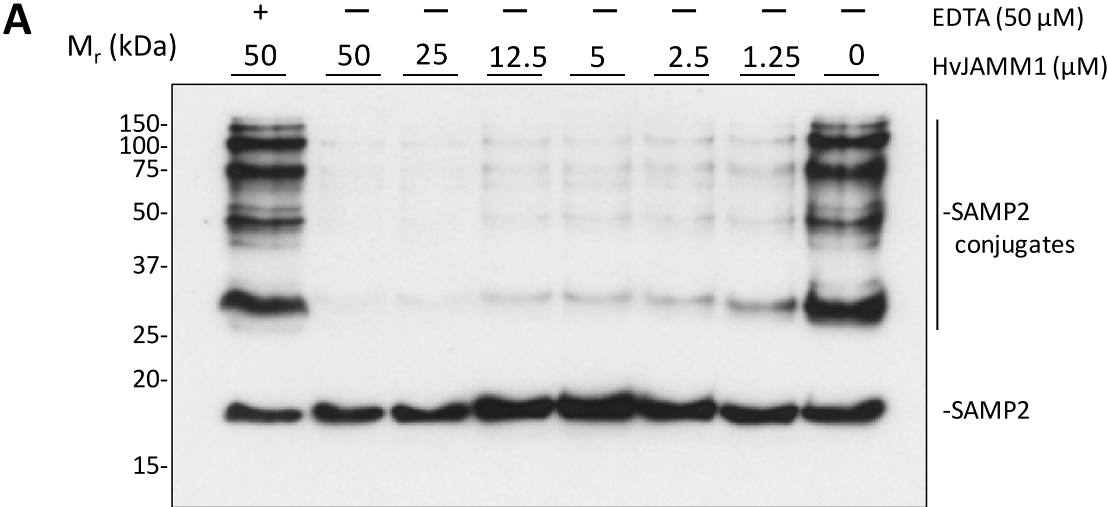
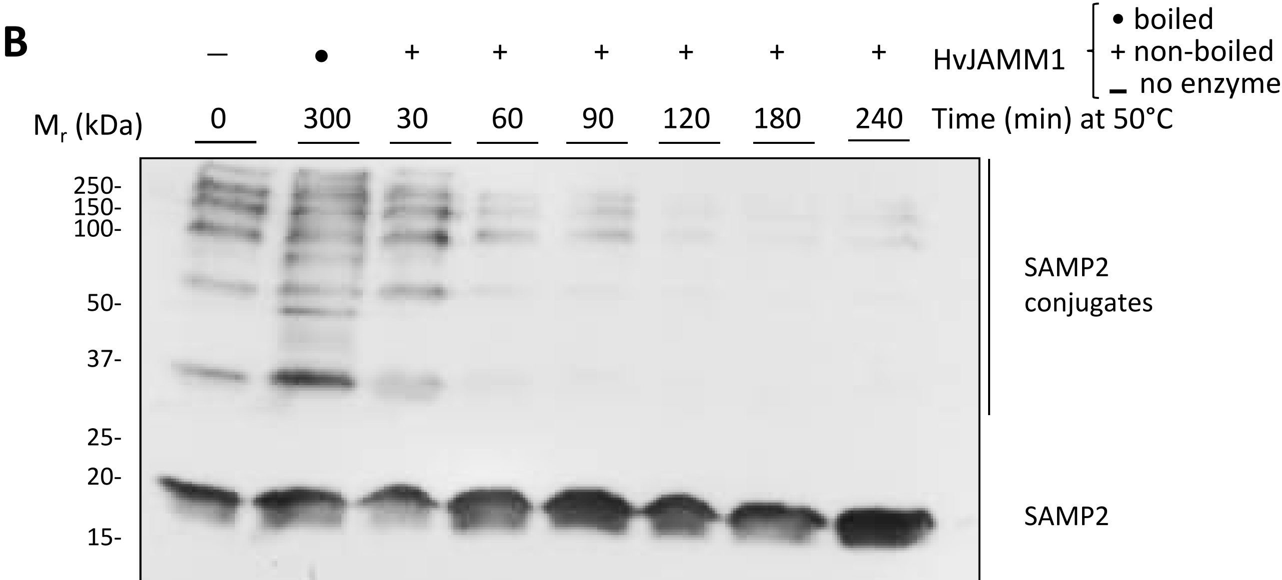


Fig. S6





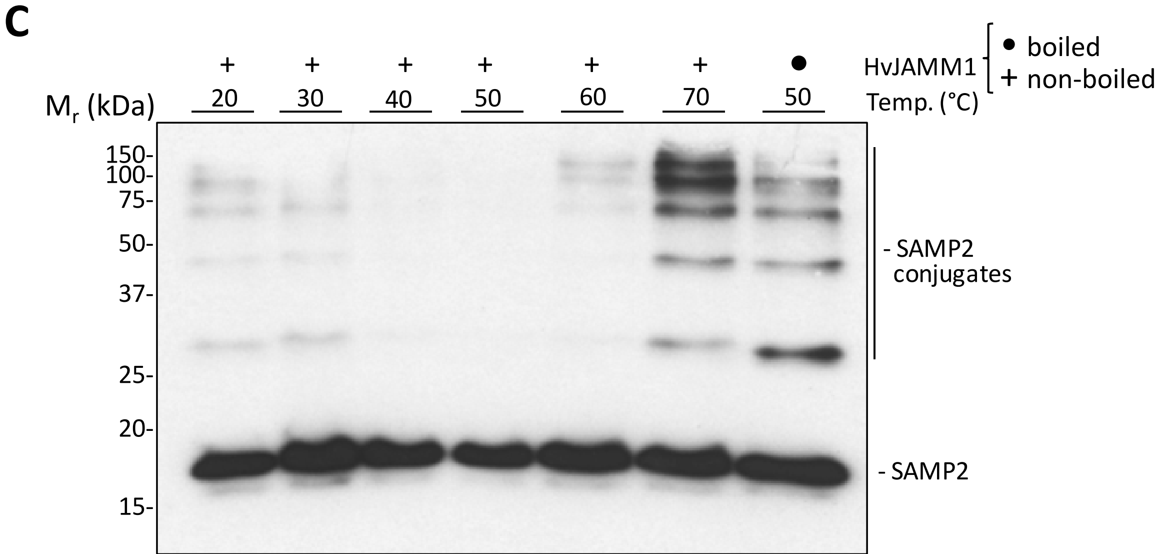
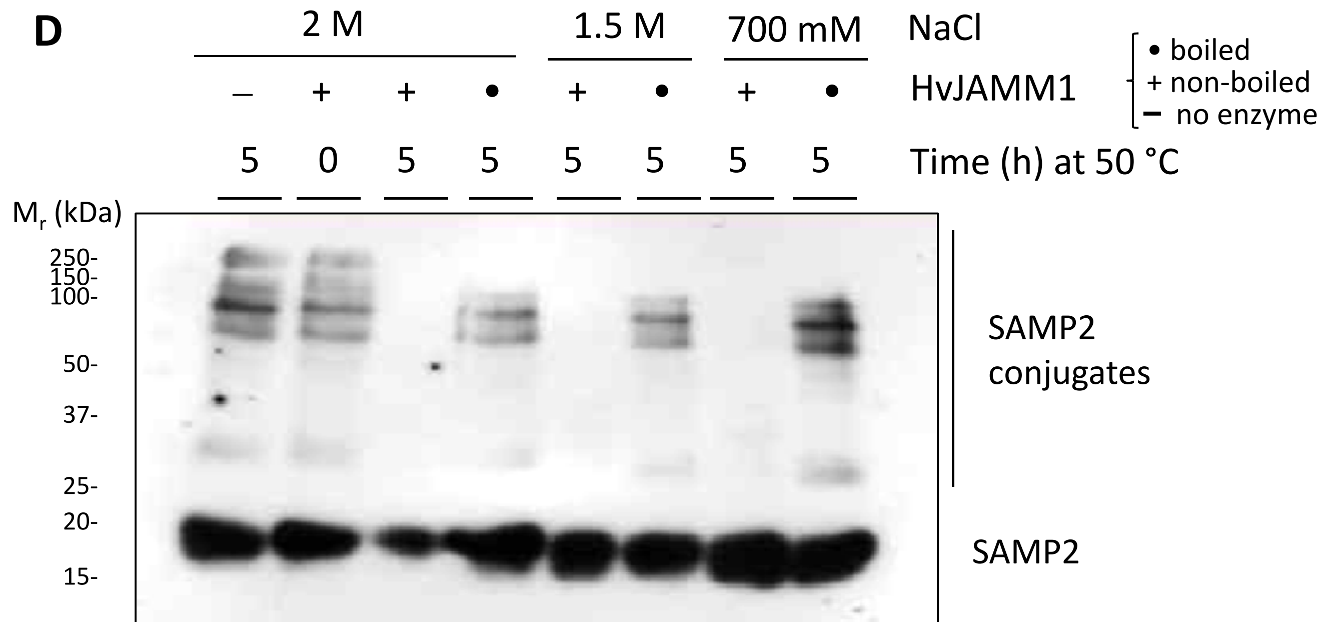


Fig. S6



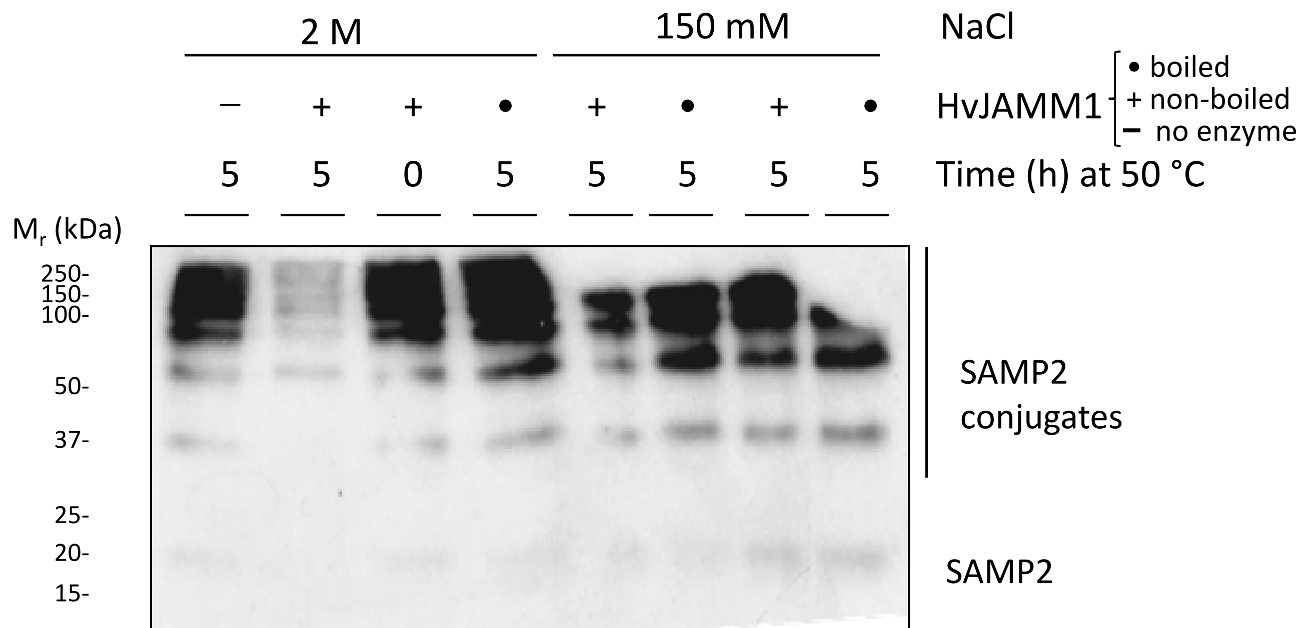


Fig. S6

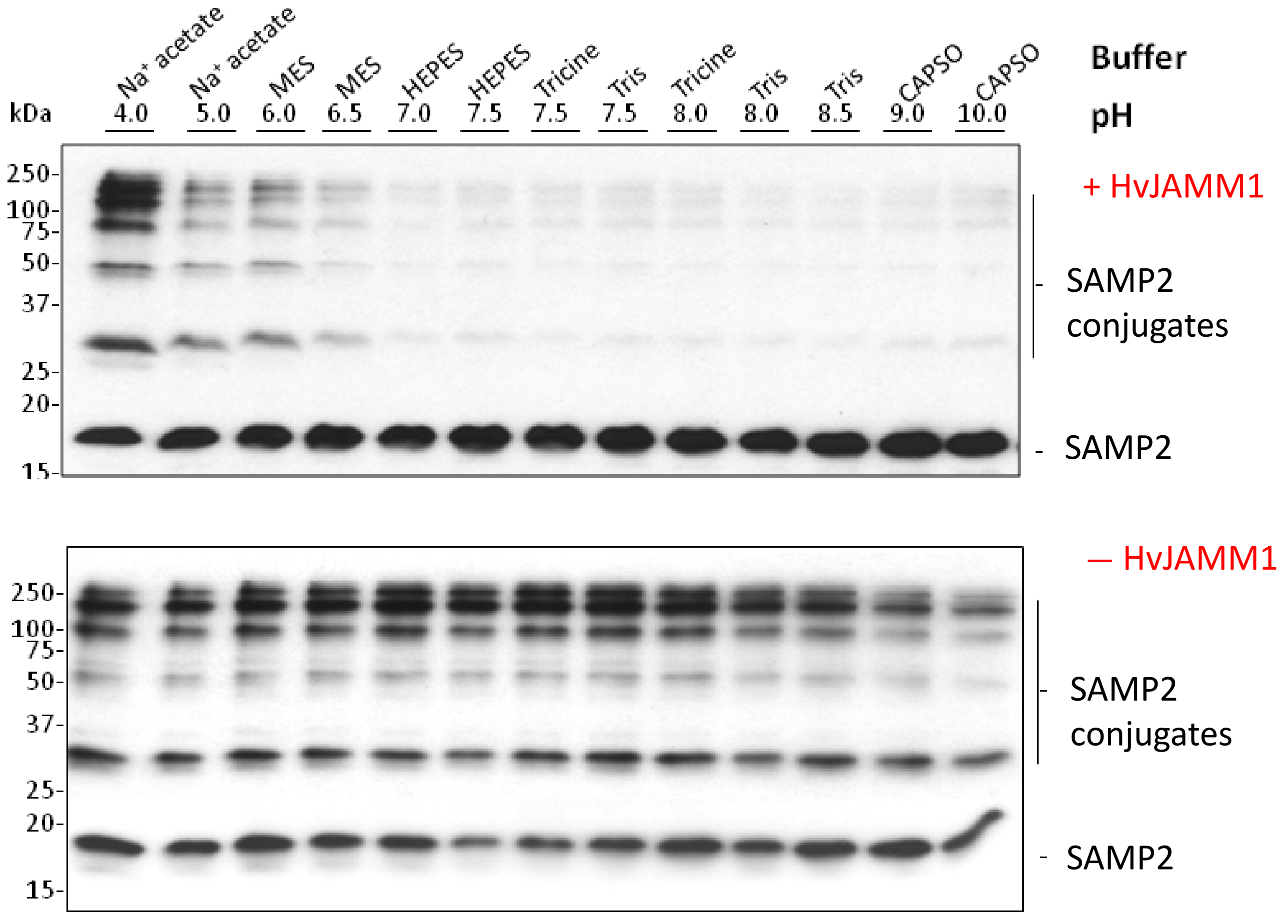
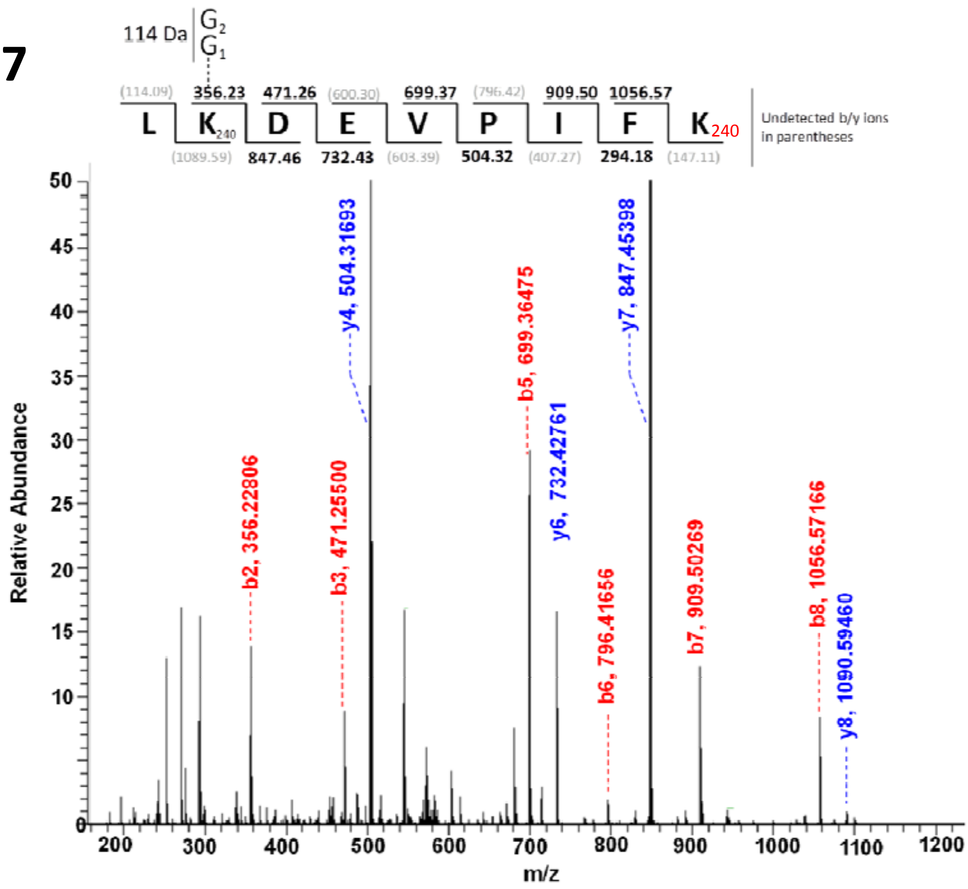
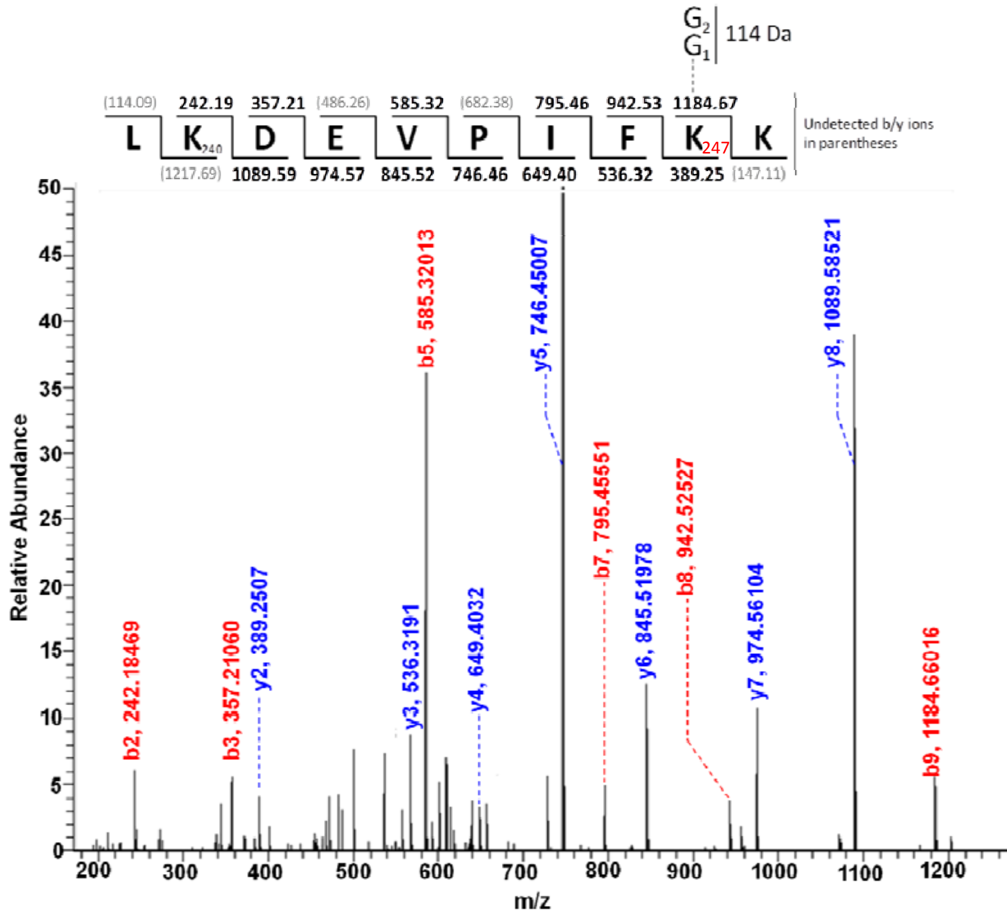


Fig. S7

A



B



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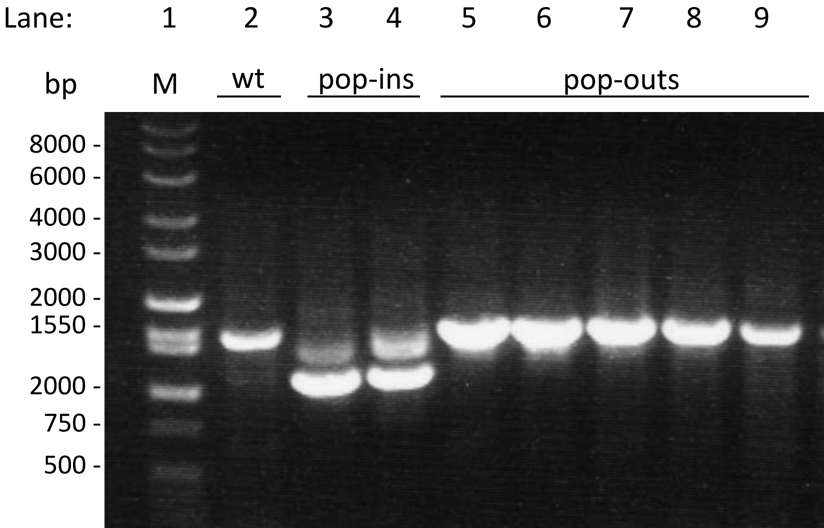
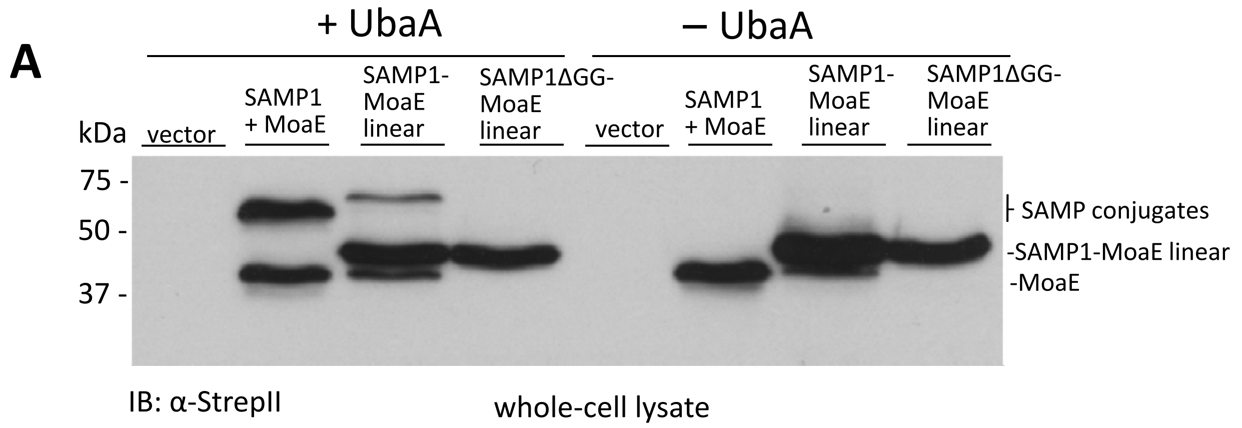


Fig. S9

A



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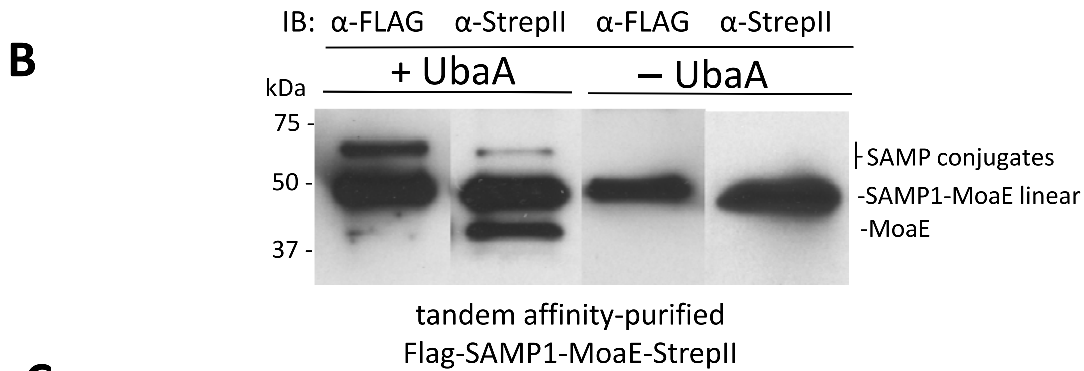

C

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0.05
0.01
OD 600 nm
parent
samp1 moaE + SAMP1 + MoaE
samp1 moaE + SAMP1-MoaE linear-linked
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samp1 moaE + SAMP1ΔGG-MoaE linear linked
10
20
30
40
50
Time (hours)

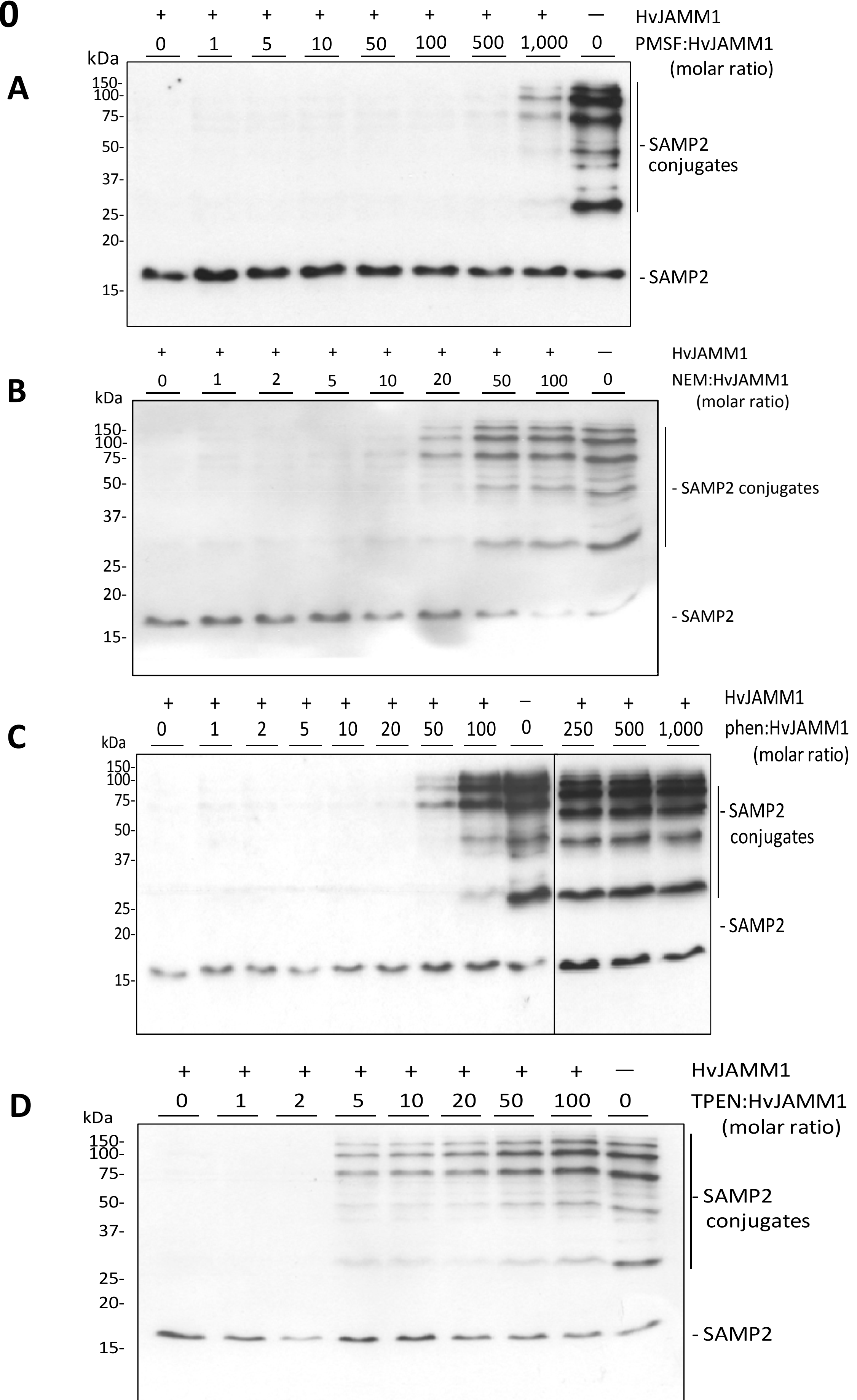
Fig. S10
A
HvJAMM1
PMSF:HvJAMM1
(molar ratio)
kDa
SAMP2 conjugates
SAMP2
B
NEM:HvJAMM1
(molar ratio)
C
phen:HvJAMM1
(molar ratio)
D
TPEN:HvJAMM1
(molar ratio)

Fig. S11