Supporting Information

Table S1. Theoretical and observed masses of SAMP and UbaA proteins[a].

Protein	AVERAGE MASS (Da)		
	Theoretical	Observed	
		ESI-MS	SDS-PAGE
Flag-His_6-SAMP1[b]	10,912.81	10,912.50 ± 0.35 11,088.23 ± 0.33	~14,500
Flag-His_6-SAMP1ΔGG	10,798.70	10,798.34 ± 0.11 10,986.36 ± 0.08	~12,000
Flag-His_6-SAMP2[b]	9,093.92	9,093.34 ± 0.25 9,191.43 ± 0.38	~18,000 ~16,000
Flag-His_6-SAMP3	12,153.44	12,153.07 ± 0.11	~15,500
His_6-UbaA	30,848.03	30,776.40[c]	~35,000

[a]SAMPs and UbaA were purified from recombinant *E. coli* and *Hfx. volcanii* HM1052, respectively, and analyzed by ESI-MS and reducing SDS-PAGE. [b]Masses of 11,010.31 ± 0.30 Da and 9,180.49 ± 0.20 Da were detected by ESI-MS at peak heights of >10-fold less than the major peaks observed for Flag-His-SAMP1 and Flag-His_6-SAMP2, respectively. [c]Mass corresponds to theoretical mass of His_6-UbaA with N-terminal Met cleaved and associated with 2 Na^+ adducts. Accurate mass of protein samples was determined by electrospray ionization time-of-flight mass spectrometry (ESI-TOF). Purified recombinant proteins were desalted by triple dialysis against 20 mM ammonium acetate in water. Samples were treated with acetonitrile (50% v/v) and formic acid (1% v/v) and loaded into an AB Sciex QSTAR XL quadrupole (Q) TOF MS equipped with a nanoelectrospray source (Protana XYZ manipulator). Concentrates of proteins (200 μl) were also pre-treated with 5 μl formic acid (1% v/v) and loaded into an Agilent 6210 time-of-flight mass spectrometer (Agilent Technologies, Inc., Santa Clara, CA) with a nanoelectrospray ionization source electrospray in positive mode. Mass Hunter software (Agilent Technologies, Inc., Santa Clara, CA) was used for analysis.

Table S2. List of strains and plasmids used in this study[a].

Strain, plasmid or primer	Description	Source or reference
Strains:		
E. coli		
Top10	F^- *recA1 endA1 hsdR17*(r_K^- m_K^+) *supE44 thi-1 gyrA relA1*	Invitrogen
GM2163	F^- *ara-14 leuB6 fhuA31 lacY1 tsx78 glnV44 galK2 galT22 mcrA dcm-6 hisG4 rfbD1 rpsL136 dam13*::Tn*9 xylA5 mtl-1 thi-1 mcrB1 hsdR2*	New England Biolabs
Rosetta (DE3)	*F- ompT hsdSB(rB- mB-) gal dcm (DE3) pRARE (Cmr)*	Novagen
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
H. volcanii		
DS2	Dead Sea isolate	[1]
DS70	DS2 cured of plasmid pHV2	[2]
H26	DS70 *pyrE2*	[3]
HM1052	H26 *ubaA*	[4]
HM1096	H26 *samp1 samp2 samp3*	[4]
NH02	H26 *ubaA samp1 samp2 samp3* (HM1096 *ubaA*)	This study
Plasmids:		
pET15b	Ap^r; expression vector	Novagen
pET24b	Km^r; expression vector	Novagen
pJAM809	Ap^r Nv^r; pJAM202 containing $P2_{rrnA}$-*hvo1862-strepII* (*KpnI* site upstream of StrepII coding sequence)	[5]
pJAM202c	Ap^r Nv^r; expression vector	[6]
pJAM957	Ap^r Nv^r; pJAM202 $P2_{rrnA}$-*hvo_0558-strepII* (UbaA-StrepII)	[4]
pJAM1209	Ap^r Nv^r; $P2_{rrn}$ -His_6-thrombin cleavage site-*hvo_0558* (His_6-UbaA)	This study
pJAM1214-35a	Ap^r Nv^r; pET15b P_{T7}-His_6-thrombin cleavage site-*hvo_0558* (His_6-UbaA)	This study
pJAM1131	Km^r; pET24b P_{T7}-Flag-His_6-*hvo_2619* (Flag-His_6-SAMP1)	[7]
pJAM1174	Km^r; pET24b P_{T7}-Flag-His_6-*hvo_2619* (Flag-His_6-SAMP1ΔGG)	This study
pJAM1132	Km^r; pET24b P_{T7}-Flag-His_6-*hvo_0202* (Flag-His_6-SAMP2)	This study
pJAM1175	Km^r; pET24b P_{T7}-Flag-His_6-*hvo_0202* (Flag-His_6-SAMP2ΔGG)	This study
pJAM1196	Km^r; pET24b P_{T7}-Flag-His_6-*hvo_2177* (Flag-His_6-SAMP3)	This study
pJAM 2704	Km^r; pET24b P_{T7}-Flag-His_6-*hvo_2177* (Flag-His_6-SAMP3ΔGG)	This study

pJAM1860	Ap^r; Nv^r; pJAM202c $P2_{rrn}$-*hvo_0558 G39,41A -StrepII* (UbaA G39A,G41A -StrepII)	This study
pJAM1849	Ap^r; Nv^r; pJAM202c $P2_{rrn}$-*hvo_0558 G42A -StrepII* (UbaA G42A -StrepII)	This study
pJAM1844	Ap^r; Nv^r; pJAM202c $P2_{rrn}$-*hvo_0558 D63N -StrepII* (UbaA D63N -StrepII)	This study
pJAM1837	Ap^r; Nv^r; pJAM202c $P2_{rrn}$-*hvo_0558 D65N -StrepII* (UbaA D65N -StrepII)	This study
pJAM1855	Ap^r; Nv^r; pJAM202c $P2_{rrn}$-*hvo_0558 S70A -StrepII* (UbaA S70A -StrepII)	This study
pJAM1861	Ap^r; Nv^r; pJAM202c $P2_{rrn}$-*hvo_0558 L72P -StrepII* (UbaA L72P -StrepII)	This study
pJAM1862	Ap^r; Nv^r; pJAM202c $P2_{rrn}$-*hvo_0558 R74Q -StrepII* (UbaA R74Q -StrepII)	This study
pJAM1124	Ap^r; Nv^r; pJAM202c $P2_{rrn}$-*hvo_0558 K87R -StrepII* (UbaA K87R -StrepII)	This study
pJAM1708	Ap^r; Nv^r; pJAM202c $P2_{rrn}$-*hvo_0558 D131N -StrepII* (UbaA D131N -StrepII)	This study
pJAM1121	Ap^r; Nv^r; pJAM202c $P2_{rrn}$-*hvo_0558 R136A -StrepII* (UbaA R136A -StrepII)	This study
pJAM1127	Ap^r; Nv^r; pJAM202c $P2_{rrn}$-*hvo_0558 C171S -StrepII* (UbaA C171S -StrepII)	This study
pJAM1123	Ap^r; Nv^r; pJAM202c $P2_{rrn}$-*hvo_0558 C174S -StrepII* (UbaA C174S -StrepII)	This study
pJAM1116	Ap^r; Nv^r; pJAM202c $P2_{rrn}$-*hvo_0558 C188S -StrepII* (UbaA C188S -StrepII)	[4]
pJAM1700	Ap^r; Nv^r; pJAM202c $P2_{rrn}$-*hvo_0558 C188A -StrepII* (UbaA C188A -StrepII)	This study
pJAM1126	Ap^r; Nv^r; pJAM202c $P2_{rrn}$-*hvo_0558 C203S -StrepII* (UbaA C203S -StrepII)	This study
pJAM1707	Ap^r; Nv^r; pJAM202c $P2_{rrn}$-*hvo_0558 C245S -StrepII* (UbaA C245S -StrepII)	This study
pJAM1706	Ap^r; Nv^r; pJAM202c $P2_{rrn}$-*hvo_0558 C248S -StrepII* (UbaA C248S -StrepII)	This study
pJAM1701	Ap^r; Nv^r; pJAM202c $P2_{rrn}$-*hvo_0558 C265S -StrepII* (UbaA C265S -StrepII)	This study
pJAM993	Ap^r; Nv^r; pJAM202c carries $P2_{rrn}$-Flag-SAMP1, UbaA-StrepII	[4]
pJAM1860	Ap^r; Nv^r; pJAM202c carries $P2_{rrn}$-Flag-SAMP1, UbaA G39,41A -StrepII	This study
pJAM1849	Ap^r; Nv^r; pJAM202c carries $P2_{rrn}$-Flag-SAMP1, UbaA G42A -StrepII	This study
pJAM1853	Ap^r; Nv^r; pJAM202c carries $P2_{rrn}$-Flag-SAMP1, UbaA D63N -StrepII	This study
pJAM1845	Ap^r; Nv^r; pJAM202c carries $P2_{rrn}$-Flag-SAMP1, UbaA D65N -StrepII	This study
pJAM1869	Ap^r; Nv^r; pJAM202c carries $P2_{rrn}$-Flag-SAMP1, UbaA S70A -StrepII	This study
pJAM1172	Ap^r; Nv^r; pJAM202c carries $P2_{rrn}$-Flag-SAMP1, UbaA N71A -StrepII	This study
pJAM2708	Ap^r; Nv^r; pJAM202c carries $P2_{rrn}$-Flag-SAMP1, UbaA L72P -StrepII	This study
pJAM1764	Ap^r; Nv^r; pJAM202c carries $P2_{rrn}$-Flag-SAMP1, UbaA R74Q -StrepII	This study
pJAM1164	Ap^r; Nv^r; pJAM202c carries $P2_{rrn}$-Flag-SAMP1, UbaA K87R -StrepII	This study
pJAM1731	Ap^r; Nv^r; pJAM202c carries $P2_{rrn}$-Flag-SAMP1, UbaA D131N -StrepII	This study
pJAM1179	Ap^r; Nv^r; pJAM202c carries $P2_{rrn}$-Flag-SAMP1, UbaA R136A -StrepII	This study
pJAM994	Ap^r; Nv^r; pJAM202c carries $P2_{rrn}$-Flag-SAMP1, UbaA C188S -StrepII	[4]
pJAM1829	Ap^r; Nv^r; pJAM202c carries $P2_{rrn}$-Flag-SAMP1, UbaA C188A -StrepII	This study

pJAM1722	Ap^r; Nv^r; pJAM202c carries $P2_{rrn}$-Flag-SAMP1, UbaA C171S -StrepII	This study
pJAM1166	Ap^r; Nv^r; pJAM202c carries $P2_{rrn}$-Flag-SAMP1, UbaA C174S -StrepII	This study
pJAM1716	Ap^r; Nv^r; pJAM202c carries $P2_{rrn}$-Flag-SAMP1, UbaA C245S -StrepII	This study
pJAM1726	Ap^r; Nv^r; pJAM202c carries $P2_{rrn}$-Flag-SAMP1, UbaA C248S -StrepII	This study
pJAM1163	Ap^r; Nv^r; pJAM202c carries $P2_{rrn}$-Flag-SAMP1, UbaA C203S -StrepII	This study
pJAM1728	Ap^r; Nv^r; pJAM202c carries $P2_{rrn}$-Flag-SAMP1, UbaA C265S -StrepII	This study
pJAM995	Ap^r; Nv^r; pJAM202c carries $P2_{rrn}$-Flag-SAMP2, UbaA-StrepII	[4]
pJAM1178	Ap^r; Nv^r; pJAM202c carries $P2_{rrn}$-Flag-SAMP2, UbaA N71A -StrepII	This study
pJAM1743	Ap^r; Nv^r; pJAM202c carries $P2_{rrn}$-Flag-SAMP2, UbaA R74Q -StrepII	This study
pJAM1183	Ap^r; Nv^r; pJAM202c carries $P2_{rrn}$-Flag-SAMP2, UbaA K87R -StrepII	This study
pJAM1717	Ap^r; Nv^r; pJAM202c carries $P2_{rrn}$-Flag-SAMP2, UbaA D131N -StrepII	This study
pJAM1180	Ap^r; Nv^r; pJAM202c carries $P2_{rrn}$-Flag-SAMP2, UbaA R136A -StrepII	This study
pJAM1722	Ap^r; Nv^r; pJAM202c carries $P2_{rrn}$-Flag-SAMP2, UbaA C171S -StrepII	This study
pJAM1166	Ap^r; Nv^r; pJAM202c carries $P2_{rrn}$-Flag-SAMP2, UbaA C174S -StrepII	This study
pJAM996	Ap^r; Nv^r; pJAM202c carries $P2_{rrn}$-Flag-SAMP2, UbaA C188S -StrepII	[4]
pJAM2758	Ap^r; Nv^r; pJAM202c carries $P2_{rrn}$-Flag-SAMP2, UbaA C188A -StrepII	This study
pJAM1163	Ap^r; Nv^r; pJAM202c carries $P2_{rrn}$-Flag-SAMP2, UbaA C203S -StrepII	This study
pJAM1716	Ap^r; Nv^r; pJAM202c carries $P2_{rrn}$-Flag-SAMP2, UbaA C245S -StrepII	This study
pJAM1726	Ap^r; Nv^r; pJAM202c carries $P2_{rrn}$-Flag-SAMP2, UbaA C248S -StrepII	This study
pJAM1728	Ap^r; Nv^r; pJAM202c carries $P2_{rrn}$-Flag-SAMP2, UbaA C265S -StrepII	This study
pJAM2759	Ap^r; Nv^r; pJAM202c carries $P2_{rrn}$-Flag-SAMP2, UbaA C143S,C188S -StrepII	This study
pJAM1865	Ap^r; Nv^r; pJAM202c carries $P2_{rrn}$-Flag-SAMP2, UbaA C174S,C188A -StrepII	This study
pJAM1864	Ap^r; Nv^r; pJAM202c carries $P2_{rrn}$-Flag-SAMP2, UbaA C203S,C188A -StrepII	This study
pJAM2762	Ap^r; Nv^r; pJAM202c carries $P2_{rrn}$-Flag-SAMP2, UbaA C188S,C248S -StrepII	This study
pJAM2763	Ap^r; Nv^r; pJAM202c carries $P2_{rrn}$-Flag-SAMP2, UbaA C188S,C265S -StrepII	This study
pJAM1306	Ap^r; Nv^r; pJAM202c carries $P2_{rrn}$-Flag-SAMP3, UbaA-StrepII	[8]
pJAM1753	Ap^r; Nv^r; pJAM202c carries $P2_{rrn}$-Flag-SAMP3, UbaA N71A -StrepII	This study
pJAM1754	Ap^r; Nv^r; pJAM202c carries $P2_{rrn}$-Flag-SAMP3, UbaA K87R -StrepII	This study
pJAM1736	Ap^r; Nv^r; pJAM202c carries $P2_{rrn}$-Flag-SAMP3, UbaA D131N -StrepII	This study
pJAM1756	Ap^r; Nv^r; pJAM202c carries $P2_{rrn}$-Flag-SAMP3, UbaA C188S -StrepII	This study
pJAM1757	Ap^r; Nv^r; pJAM202c carries $P2_{rrn}$-Flag-SAMP3, UbaA C188A -StrepII	This study
pJAM1737	Ap^r; Nv^r; pJAM202c carries $P2_{rrn}$-Flag-SAMP3, UbaA C171S -StrepII	This study
pJAM1759	Ap^r; Nv^r; pJAM202c carries $P2_{rrn}$-Flag-SAMP3, UbaA C174S -StrepII	This study

pJAM1739	Ap^r; Nv^r; pJAM202c carries $P2_{rrn}$-Flag-SAMP3, UbaA C245S -StrepII	This study
pJAM1738	Ap^r; Nv^r; pJAM202c carries $P2_{rrn}$-Flag-SAMP3, UbaA C248S -StrepII	This study
pJAM1304	Ap^r; Nv^r; pJAM202c carries $P2_{rrn}$-Flag-SAMP1ΔGG, UbaA-StrepII	This study
pJAM1305	Ap^r; Nv^r; pJAM202c carries $P2_{rrn}$-Flag-SAMP2ΔGG, UbaA-StrepII	This study
pJAM1308	Ap^r; Nv^r; pJAM202c carries $P2_{rrn}$-Flag-SAMP3ΔGG, UbaA-StrepII	This study

[α] Ap^r, ampicillin resistance; Nv^r, novobiocin resistance; Km^r, kanamycin resistance; Tet^r, tetracycline resistance; Cm^r, chloramphenicol resistance. *Hfx. volcanii* strain NH02 was generated by deletion of *ubaA* from HM1096 by uracil selection and 5-fluoroorotic acid (FOA) counter selection as previously described [3]. *E. coli* TOP10 was used for routine recombinant DNA experiments, and *E. coli* GM2163 was used to prepare plasmid DNA for transformation into *Hfx. volcanii* as previously described [8, 9]. *E. coli* strains were grown at 37 °C in Luria-Bertani medium. PCR-generated DNA fragments encoding the full-length *ubaA* (HVO_0558) were amplified using Phusion DNA polymerase and cloned into the NdeI-to-BlpI sites of pJAM503 (with an N-terminal His_6-tag and thrombin-cleavage site, His_6-) and pJAM809 (with a C-terminal StrepII-tag, -StrepII) to generate pJAM1209 and pJAM957, respectively. These plasmids were used to produce epitope-tagged UbaA in *Hfx. volcanii.* UbaA variants with single-point and combinatorial amino acid exchanges were generated by site-directed mutations using plasmid pJAM957 purified from *E. coli* TOP10 as a template and Pfu Turbo DNA polymerase as described in QuikChange Lightning mutagenesis protocol (Stratagene, La Jolla, CA). PCR products were treated with DpnI (37 °C for 2-3 h), purified using MinElute purification kit (Qiagen) and transformed into *E. coli* XL10-Gold. Other UbaA variants were generated by inverse PCR-based mutagenesis using Phusion DNA polymerase. DpnI-treated PCR amplicons were phosphorylated by T4 polynucleotide kinase and circularized by T4 DNA ligase prior to transformation into *E. coli* TOP10. All pJAM957-derived plasmid constructs were verified by DNA sequencing. For co-expression of UbaA-StrepII proteins with SAMPs that had N-terminal Flag tags (Flag-), the BamHI to BlpI fragment of pJAM957 and pJAM957-derived plasmids (encoding UbaA-StrepII and its variants) was blunt-end ligated into the BlpI site of plasmid pJAM947 (SAMP1), pJAM949 (SAMP2), and pJAM977 (SAMP3). Genes encoding N-terminal Flag-His_6-SAMPs were cloned in pET24b vector (Km^r) to generate pJAM1131 (SAMP1), pJAM1132 (SAMP2), pJAM1160 (SAMP3), pJAM1174 (SAMP1ΔGG), and pJAM1175 (SAMP2ΔGG). Expression plasmid pJAM2704 (Flag-His_6-SAMP3ΔGG) was derived from pJAM1160 truncation using a 3' PCR primer with the appropriate bases deleted. For other details see Materials and Methods.

Table S3. List of primers used in this study.

Primer Pair	Primer Sequence (5'-3')	PCR product/ description	Source or Reference
HVO_0558 NdeI FW HVO_0558 KpnI Strep RV	5'-TTCCTTACATATGACGCTCTCACTCGACGCCACCC-3' 5'-CCGGTACCGTCGAGGCTGATTGCGCAG-3'	N-terminal His_6-tagged UbaA	[4]
Hvo_0558 BamHI 500 bp up FW Hvo_0558 HindIII 500 bp dwn RV	5'-TTATGGATCCCAGAAGTGACTCAGAACGGCGACG-3' 5'-CTAAGCTTACGTGGTTCAGGACGGGTGCGGTG-3'	used to confirm Δ*ubaA* in generating NH02	[4]
Hvo_0558 G39,41A fwd Hvo_0558 G39,41A rev	5'-CGTCGTCGTCGCCGCGGCCGGGTTGG-3' 5'-CCAACCCGGCCGCGGCGACGACGACG-3'	G39,G41A	This study
Hvo_0558 G42A fwd Hvo_0558 G42A rev	5'-GCGTTGGGCGCGCCGGCCATC-3' 5'-GCCCGCGCCGACGACGACGAC-3'	G42A	This study
Hvo_0558 D63N fwd Hvo_0558 D63N rev	5'-AACGACGACGTGGTCGAGCGG-3' 5'-GACCACGACGAGTTCGCCGACG-3'	D63N	This study
Hvo_0558 D65N fwd Hvo_0558 D65N rev	5'-AACGTGGTCGAGCGGAGCAACC-3' 5'-GTCATCGACCACGACGAGTTCGCC-3'	D65N	This study
Hvo_0558 S70A fwd Hvo_0558 S70A rev	5'-GCCAACCTCCAGCGGCAGGTC-3' 5'-CCGCTCGACCACGTCGTCGTC-3'	S70A	This study
Hvo_0558 N71A fwd Hvo_0558 N71A rev	5'-GAGCGGAGCGCCCTCCAGCGGCAG-3' 5'-CTGCCGCTGGAGGGCGCTCCGCTC-3'	N71A	This study
Hvo_0558 L72P fwd Hvo_0558 L72P rev	5'-CGGAGCAACCCCCAGCGGCAGGTC-3' 5'-GACCTGCCGCTGGGGGTTGCTCCG-3'	L72P	This study
Hvo_0558 R74Q fwd Hvo_0558 R74Q rev	5'-CAACCTCCAGCAGCAGGTCGTCC-3' 5'-GGACGACCTGCTGCTGGAGGTTG-3'	R74Q	This study
Hvo_0558 C174S fwd Hvo_0558 C174S rev	5'-CCCTGCTATCGAAGTCTGTTCCCC-3' 5'-GGGGAACAGACTTCGATAGCAGGG-3'	C174S	This study
Hvo_0558 R136A fwd Hvo_0558 R136A rev	5'-TTCCCGACGGCCTATCTCCTCAAC-3' 5'-GTTGAGGAGATAGGCCGTCGGGAA-3'	R136A	This study
Hvo_0558 K87R fwd Hvo_0558 K87R rev	5'-TGGGGACGCCGAGAGCCGAGAG-3' 5'-CTCTCGGCTCTCGGCGTCCCCA-3'	K87R	This study
Hvo_0558 D131N fwd Hvo_0558 D131N rev	5'-CGACGCCTCGAACAACTTCCCG-3' 5'-CGGGAAGTTGTTCGAGGCGTCG-3'	D131N	This study

Hvo_0558 C143S fwd Hvo_0558 C143S rev	5'-CTCGCTTCGAGGGAATCCCGC-3' 5'-AGACGTCGTTGAGGAGATAGCGC-3'	C143S	This study
Hvo_0558 C171S fwd Hvo_0558 C171S rev	5’-CGACGGACCCAGCTATCGATGTC-3’ 5’-GACATCGATAGCTGGGTCCGTCG-3’	C171S	This study
Hvo_0558 C174S fwd Hvo_0558 C174S rev	5’-CCCTGCTATCGAAGTCTGTTCCCC-3’ 5’-GGGGAACAGACTTCGATAGCAGGG-3’	C174S	This study
Hvo_0558 C188S fwd Hvo_0558 C188S rev	5’-GTCCCCGACAGCGCGACGACCGGC-3’ 5’-GCCGGTCGTCGCGCTGTCGGGGAC-3’	C188S	[4]
Hvo_0558 C188A fwd Hvo_0558 C188A rev	5’-GTCCCCGACGCCGCGACGACCGG-3’ 5’-CCGGTCGTCGCGGCGTCGGGGAC-3’	C188A	This study
Hvo_0558 C203S fwd Hvo_0558 C203S rev	5’-CACGGTCGGCAGTATTCAGGC-5’ 5’-GCCTGAATACTGCCGACCGTG-3’	C203S	This study
Hvo_0558 C245S fwd Hvo_0558 C245S rev	5’-CCCGGACAGCCCCGTCTGCG-3’ 5’-CGCAGACGGGGCTGTCCGGG-3’	C245S	This study
Hvo_0558 C248S fwd Hvo_0558 C248S rev	5’-GGACTGCCCCGTCAGCGGCGAGGG-3’ 5’-CCCTCGCCGCTGACGGGGCAGTCC-3’	C248S	This study
Hvo_0558 C265S fwd Hvo_0558 C265S rev	5'-GAGAGCTCCGCAATCAGCCTCGAC-3' 5'-GACGTAGTCGATGTCCTCGATGGAG-3'	C265S	This study
NdeI up Hvo_2177 fwd Hvo_2177 ΔGG BlpI rev	5’-ATCATATGGAGCTCGAATTACGCTTCTTCG-3’ 5’-TTGCTCAGCATCACGCGACCGGCGGGAAGACGC-3’	SAMP3 ΔGG	This study

[a]SDM, site-directed mutagenesis. Amino acid exchanges were PCR-based using plasmid pJAM957 as template with either Pfu DNA polymerase as described in QuikChange Lightning mutagenesis protocol (Stratagene, La Jolla, CA) or by inverse PCR with Phusion DNA polymerase (New England Biolabs, Ipswich, MA). See methods for details.

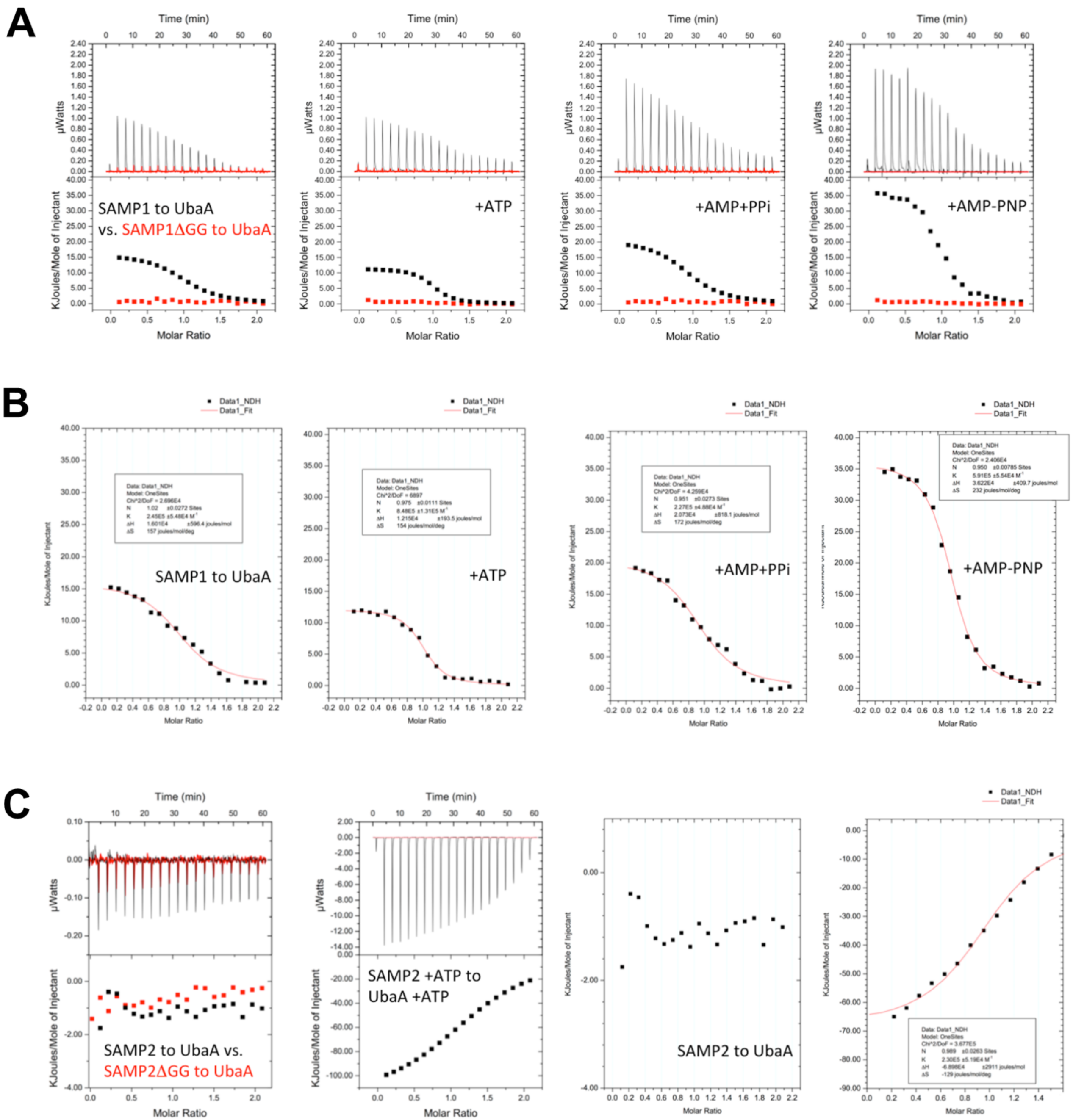

Figure S1. Raw isothermal titration calorimetry (ITC) data and corresponding enthalpy plots of UbaA:SAMP1/2 binding at 25 °C. The SAMPs (in syringe) were titrated to UbaA (in cell), with and without ATP, AMP+PPi or AMP-PNP as indicated. (A) From left to right: SAMP1 to UbaA (black) overlaid with SAMP1ΔGG to UbaA (red); SAMP1+ATP to UbaA+ATP (black) overlaid with SAMP1ΔGG+ATP to UbaA+ATP (red); SAMP1+AMP+PPi to UbaA+AMP+PPi (black) overlaid with SAMP1ΔGG to UbaA (red); and SAMP1+AMP-PNP to UbaA+AMP-PNP (black) overlaid with SAMP1ΔGG+ATP to UbaA+ATP (red). (B) Background-subtracted enthalpy plots for SAMP1 titrated to UbaA without and with nucleotide ligands, ATP, AMP+PPi and AMP-PNP as indicated from left to right. Here, heat contributions determined from control titrations, namely SAMP1 to buffer, UbaA to buffer and buffer to buffer, were subtracted from the total enthalpy. Data were subsequently fitted to a law of mass action equation, assuming a one-site binding model, and (C) From left to right: raw isothermal titration calorimetry (ITC) data for: i) SAMP2 (black) titrated to UbaA overlaid with SAMP2ΔGG (red) titrated to UbaA and ii) SAMP2 to UbaA in the presence of ATP and the corresponding background-subtracted enthalpy plots for iii) SAMP2 to UbaA without ATP and iv) SAMP2 to UbaA with ATP.

Supplemental References

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