Supplemental Table 1. Primers used in this study.

Primer Names (paired as used)	PCR Product/Description	Primer Sequences[a]
psmC **(Hvo_2923):**		
02057-BamHI Forward 02057-HindIII Reverse	~500 bp of genomic DNA flanking 5' and 3' of *psmC* generated using *H. volcanii* DS70 genomic DNA as a template; includes BamHI and HindIII sites for cloning into pTA131 to generate pJAM2024	5'-ACTGATGGATCCGGTCGTGGGAGGTGTTCGTCG-3' 5'-TGACTGCAAGCTTGGCTTCGGTGTCGTCGG-3'
02057-Inverse Forward 02057-Inverse Reverse	*psmC*-suicide plasmid pJAM2025 generated by inverse PCR using pJAM2024 as template	5'-CGACGAGACGGACGAACGCGAGGAGTAGATGACAGACG-3' 5'-CTGCTTGTCGTTTCGGTTCATCGTGCGACCTCCTCG-3'
02057-Negative-Forward 02057-Negative-Reverse	~500 bp within *psmC* coding region; used to screen Δ*psmC* mutants	5'-CCCCGACGGACGCATCTATCAGGTC-3' 5'-GAGATTCAGTTCGTCGCTCTCG-3'
02057-Confirm-Forward 02057-Confirm-Reverse	~700 bp of genomic DNA flanking 5' and 3' of *psmC*; used to confirm Δ*psmC* mutation by PCR	5'-CCTCGGCACCTGGGAGTCGAACGGC-3' 5'-CGGTCGAACAGGTGGTCCTCCCAGAACG-3'
02057-Inverse Forward 02057-HindIII Reverse	~500 bp probe 2057, generated using pJAM2024 as template, used to confirm Δ*psmC* mutation by Southern blot	5'-CGACGAGACGGACGAACGCGAGGAGTAGATGACAGACG-3' 5'-TGACTGCAAGCTTGGCTTCGGTGTCGTCGG-3'
psmA **(Hvo_1091):**		
00857-BamHI Forward 00857-HindIII Reverse	~500 bp of genomic DNA flanking 5' and 3'of *psmA* generated using *H. volcanii* DS70 genomic DNA as a template; includes BamHI and HindIII sites for cloning into pTA131 to generate pJAM2027	5'-ACTAATGGATCCCAAACACCTCCGGCCGC-3' 5'-TGACTGCAAGCTTGGAACTCATCCGCGAGTCG-3'
00857-Inverse Forward 00857-Inverse Reverse	*psmA*-suicide plasmid pJAM2029 generated by inverse PCR using pJAM2027 as template	5'-ACCGCGTCCCGCGGTTCCCGTTCTCGCGCGTTCTTCTTCGAC-3' 5'-GGAATATCACTCGAAATCGAGTTGCGTCGCGCCCGTG-3'
00857-Negative-Forward 00857-Negative-Reverse	~500 bp within *psmA* coding region; used to screen Δ*psmA* mutants	5'-GCCGGATGGTCGACTCTATCAGG-3' 5'-GACAGCTCGACGAAGCGCTCCG-3'
00857-Confirm-Forward 00857-Confirm-Reverse	~700 bp of genomic DNA flanking 5' and 3'of *psmA*; used to confirm Δ*psmA* mutation by PCR	5'-CGCCGACGAGTGAACTGACCCGATGAAACACCTG-3' 5'-GGCGTCCGCGTCGTCGTCAACGTC-3'
00857-BamHI Forward 00857-Inverse Reverse	~500 bp probe 857, generated using pJAM2027 as template, used to confirm Δ*psmA* mutation by Southern blot	5'-ACTAATGGATCCCAAACACCTCCGGCCGC-3' 5'-GGAATATCACTCGAAATCGAGTTGCGTCGCGCCCGTG-3'
panA **(Hvo_0850) :**		
01103-BamHI Forward 01103- Inverse Reverse	~500 bp of genomic DNA flanking 5' of *panA* generated using *H. volcanii* DS70 genomic DNA as a template; includes BamHI site used for three-way cloning with the ~500 bp fragment 3' of *panA* (see below) and pTA131 to generate suicide plasmid pJAM2026	5'-ACTATGGATCCGGGAGTCGACCGCGCCCTCGTCTTCCAGTCG-3' 5'-CCGGCATAGGGTGCAGGTTCATAAGAGGCTTCGGGTGTGTG-3'
01103-Inverse Forward	~500 bp of genomic DNA flanking 3' of *panA* generated using *H.*	5'-CCGACGCGAGCCGCGGTTCGCTCCGTTTCGGACCGTGTCC-3'

Primer Names (paired as used)	PCR Product/Description	Primer Sequences[a]
01103-HindIII Reverse	*volcanii* DS70 genomic DNA as a template; includes HindIII site used for three-way cloning with the ~500 bp fragment 5' of *panA* (see above) and pTA131 to generate suicide plasmid pJAM2026	5'-ACTGCAAGCTTCTCGAACAGCTCCGCGGTGACCTTGTTGACGC-3'
01103-Negative-Forward 01103-Negative-Reverse	~500 bp within *panA* coding region; used to screen Δ*panA* mutants	5'-CGTCGCAGCAGGAGAAGATCACCG-3' 5'-GATGCCGACCTCAGCGAACATCTC-3'
01103-Confirm-Forward 01103-Confirm-Reverse	~700 bp of genomic DNA flanking 5' and 3' of *panA*; used to confirm Δ*panA* mutation by PCR	5'-GACATCGTCGGGAGCGGGCGAAGACATACGG-3' 5'-CCTCGCCGTCGTGTTTCTCGATGATGTCCGC-3'
01103-BamHI Forward 01103-Inverse Reverse	~500 bp probe 1103, generated using pJAM2026 as template, used to confirm Δ*panA* mutation by Southern blot	5'-ACTATGGATCCGGGAGTCGACCGCGCCCTCGTCTTCCAGTCG-3' 5'-CCGGCATAGGGTGCAGGTTCATAAGAGGCTTCGGGTGTGTG-3'
***panB* (Hvo_1957) :**		
03059-BamHI Forward 03059-HindIII Reverse	~500 bp of genomic DNA flanking 5' and 3' of *panB* generated using *H. volcanii* DS70 genomic DNA as a template; includes BamHI and HindIII sites for cloning into pTA131 to generate pJAM2022	5'-ACTGATGGATCCTACGACGGCAGCTATCAGG-3' 5'-TAACTTAAGCTTCGTGCGTGCCGGTGGTGAGC-3'
03059-Inverse Forward 03059- Inverse Reverse	*panB*-suicide plasmid pJAM2023 generated by inverse PCR using pJAM2022 as template	5'-GGCTTCACGGACTACCAGTACTGAGGTCGG-3' 5'-CCGGAACGTCCTCATCTCGATCTGGACCC-3'
03059-Negative-Forward 03059-Negative-Reverse	~500 bp within *panB* coding region; used to screen Δ*panB* mutants	5'-CTCCAGAACGCCGATGACCGCCACG-3' 5'-CGATGCCGACCTCAGCGAACATCTC-3'
03059-Confirm-Forward 03059-Confirm-Reverse	~700 bp of genomic DNA flanking 5' and 3' of *panB*; used to confirm Δ*panB* mutation by PCR	5'-CTCATGCGGAACTCCTGTATGCTGAACACGG-3' 5'-CAGGACGACGAACAGCCATCCCTTCACCG-3'
03059-BamHI Forward 03059-Inverse Reverse	~500 bp probe 3059, generated using pJAM2022 as template, used to confirm Δ*panB* mutation by Southern blot	5'-ACTGATGGATCCTACGACGGCAGCTATCAGG-3' 5'-CCGGAACGTCCTCATCTCGATCTGGACCC-3'
***psmB* (Hvo_1562) :**		
00375-BamHI Forward 00375-HindIII Reverse	~500 bp of genomic DNA flanking 5' and 3' of *psmB* generated using *H. volcanii* DS70 genomic DNA as a template; includes BamHI and HindIII sites for cloning into pTA131 to generate pJAM2030	5'-TGACGAGGATCCGAATTCGCGACGCTCC-3' 5'-TGACTGCAAGCTTCTCACCGCGCTCTGGATGG-3'
00375-Inverse Forward 00375- Inverse Reverse	*psmB*-suicide plasmid pJAM2031 generated by inverse PCR using pJAM2030 as template	5'-GTCGACATCCAGCGCCACCAGAACTTCGAAGGC-3' 5'-CGGAGACAATGCGTACCCCGACTCACGACG-3'
00375-Negative-Forward 00375-Negative-Reverse	~500 bp within *psmB* coding region; used to screen Δ*psmB* mutants	5'-AGTTCTCCGGCCGTCTCGACTCGCTG-3' 5'-CGACTCCCTTCGCCTCCTCGATGCTG-3'
00375-Confirm-Forward 00375-Confirm-Reverse	~700 bp of genomic DNA flanking 5' and 3' of *psmB*; used to confirm Δ*psmB* mutation by PCR	5'-CGCCGACTTCGACCTCGACGTACTCCTTGTCC-3' 5'-CTCGAAGGCGCGTTCTCGGACTGGTTCTGACG-3'

Primer Names (paired as used)	PCR Product/Description	Primer Sequences[a]
00375-BamHI Forward 00375-Inverse Reverse	~500 bp probe 375, generated using pJAM2030 as template, used to confirm Δ*psmB* mutation by Southern blot	5'-TGACGAGGATCCGAATTCGCGACGCTCC-3' 5'-CGGAGACAATGCGTACCCCGACTCACGACG-3'
P_{tnaA} promoter fusions:		
XbaI_P_{tnaA} up NdeI_P_{tnaA} down	337-bp P_{tnaA} promoter generated using *H. volcanii* DS70 genomic DNA as a template; includes XbaI and NdeI sites for cloning into pJAM202 and pJAM204	5'-GCTCTAGAACGACGCCATCACCTCC-3' 5'-ACGATTTCATATGGCCCGCAATAGGTCCG-3'
XbaI_P_{tnaA}_*psmA* 00857-Confirm-Forward	531-bp flanking 5' of *psmA* generated using *H. volcanii* DS70 genomic DNA as a template; includes XbaI site for cloning to generate pTA131-derived P_{tnaA}-*psmA* suicide plasmid	5'-TGTTGTTGCGCTTGTCGTCTAGAGGAATATCACTCGAAATC-3' 5'-CGCCGACGAGTGAACTGACCCGATGAAACACCTG-3'
SpeI_P_{tnaA}_*psmB* 00375-Confirm-Forward	834-bp flanking 5'of *psmB* generated using *H. volcanii* DS70 genomic DNA as a template; includes SpeI site for cloning to generate pTA131-derived P_{tnaA}-*psmB* suicide plasmid	5'-GGCCACTAGTTGTCTCCGATTGCACTTCG-3' 5'-CCTCGGCACCTGGGAGTCGAACGGC-3'
00857-Confirm-Forward 00857-Negative-Reverse	~1.1 kb of genomic DNA flanking 5' and within *psmA*; used to confirm insertion of P_{tnaA} upstream of *psmA* by PCR	5'-CGCCGACGAGTGAACTGACCCGATGAAACACCTG-3' 5'-GACAGCTCGACGAAGCGCTCCG-3'
00375-Confirm-Forward 00375-Negative-Reverse	~1.4 kb of genomic DNA flanking 5' and within *psmB*; used to confirm insertion of P_{tnaA} upstream of *psmB* by PCR	5'-CGCCGACTTCGACCTCGACGTACTCCTTGTCC-3' 5'-CGACTCCCTTCGCCTCCTCGATGCTG-3'

[a]Restriction sites are underlined.

Supplemental Table 2. Summary of chromosomal knockout and P_{tnaA} promoter fusion mutant strains of 20S proteasome and proteasome-activating nucleotidase genes as confirmed by PCR and Southern blot analyses.

Mutant Strain no.	Probe no.[a]	Mutant Strain Genotype	PCR (bp)[b] Parent vs. Mutant		Southern Blot (bp) Parent vs. Mutant		Difference (bp)
GZ108	3059	H26 Δ*panB*	2628	1463	2702	1537	1165
GZ109	1103	H26 Δ*panA*	2366	1145	2362	1141	1221
GZ114	2057	H26 Δ*psmC*	2064	1363	2307	1606	701
GZ120	3059	GZ114 Δ*psmC* Δ*panB*	2628	1463	2702	1537	1165
GZ130	857	H26 Δ*psmA*	2209	1450	1873	1114	759
GZ131	1103	GZ114 Δ*psmC* Δ*panA*	2366	1145	2362	1141	1221
GZ132	1103	GZ108 Δ*panB* Δ*panA*	2366	1145	2362	1141	1221
GZ133	857	GZ108 Δ*panB* Δ*psmA*	2209	1450	1873	1114	759
GZ134	1103	GZ130 Δ*psmA* Δ*panA*	2366	1145	2362	1141	1221
GZ136	857	H26 P_{tnaA}-*psmA*	1190	1513	1873	2196	-323
GZ137	857	H26 Δ*psmC* -P_{tnaA}-*psmA*	1190	1513	1873	2196	-323
GZ138	375	H26 P_{tnaA}-*psmB*	1431	1790	2160	2519	-359
GZ112	375	H26 Δ*psmB* (pJAM202)	2215	1547	2160	1492	668

[a]Probe number used in Southern blot.
[b]Molecular size (bp) of DNA fragments generated by PCR and Southern blot for parent and mutant strains as calculated *in silico* from the *H. volcanii* genome sequence (http://archaea.ucsc.edu/; April 2007 version). Difference in the anticipated size of DNA fragments for parent and mutant strains is also presented. Calculations for PCR were based on amplification using "Confirm-Forward" and "Confirm-Reverse" primer pairs (Suppl. Table 1) which anneal upstream and downstream (outside) of the DNA sequences cloned in the suicide plasmid used for homologous recombination for each respective gene mutation.

Supplemental Table 3. Frequency of isolating *Hfx. volcanii* mutant strains with a marker-less deletion in the proteasomal gene of interest using the 'pop-in/pop-out' method.

Strain no.	Genotype	Proteasome Genes Deleted or Modified[a]	Proteasome Protein Deficiency[b]	Knockout Frequency[c]
GZ130	H26 *psmA*	Δ*psmA*	α1	5.6% (6/108)
GZ114	H26 *psmC*	Δ*psmC*	α2	2.4% (1/41)
GZ109	H26 *panA*	Δ*panA*	PanA	14.0% (8/57)
GZ108	H26 *panB*	Δ*panB*	PanB	1.6% (1/60)
N.A.[d]	H26 *psmB*	Δ*psmB*	β	0% (0/205)
GZ112	H26 *psmB-pJAM202*	Δ*psmB* + *psmB in trans*	β	7.1% (3/42)
GZ120	GZ114 *panB*	Δ*psmC* Δ*panB*	α2, PanB	11.7% (7/60)
GZ131	GZ114 *panA*	Δ*psmC* Δ*panA*	α2, PanA	15.8% (9/57)
GZ132	GZ108 *panA*	Δ*panB* Δ*panA*	PanA, PanB	1.9% (1/52)
GZ133	GZ108 *psmA*	Δ*panB* Δ*psmA*	α1, PanB	2.9% (3/103)
GZ134	GZ130 *panA*	Δ*psmA* Δ*panA*	α1, PanA	1.9% (1/53)
N.A.	GZ114 *psmA*	Δ*psmC* Δ*psmA*	α1, α2	0% (0/252)
N.A.	GZ130 *psmC*	Δ*psmA* Δ*psmC*	α1, α2	0% (0/100)
GZ136	H26 P_{tnaA}-*psmA*	P_{tnaA}-*psmA*	α1	4.3% (2/46)
GZ137	GZ114 P_{tnaA}-*psmA*	Δ*psmC* P_{tnaA}-*psmA*	α1, α2	2.6% (4/156)
GZ138	H26 P_{tnaA}-*psmB*	P_{tnaA}-*psmB*	β	7.2% (11/152)

[a]Gene deletions (Δ), P_{tnaA} promoter fusions (P_{tnaA}-) and/or replicating plasmid pJAM202 provided in trans as indicated.
[b]α1, α2 and β, 20S proteasome core particle subunits; PanA and PanB, proteasome-activating nucleotidase proteins.
[c]Frequency of clones isolated with targeted gene deleted on the *Hfx. volcanii* genome represented as percent of total with number of clones isolated per total screened in parenthesis.
[d]N.A., not applicable. Target gene was not deleted from parent strain.