Supporting information of

"Archaeal Protein Phosphorylation: Impact of Phosphatase Deletion on Motility and Energy Metabolism in *Sulfolobus acidocaldarius*"

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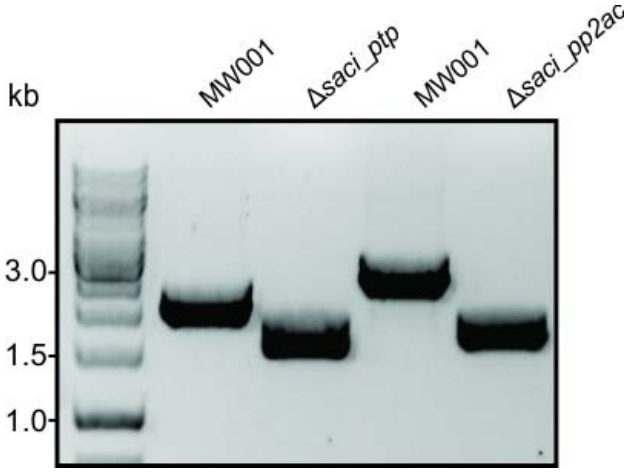

Figure S1. PCR verification of both phosphatase deletion mutants. PCR of the markerless in-frame deletion mutants Δ*saci_ptp* and Δ*saci_pp2a* resulted in smaller PCR products of the genomic region compared to the parant strain MW001 (1786 nt vs. 2238 nt and 1869 nt vs 2733 nt, respectively). The deletion mutants were additionally confirmed by sequencing.

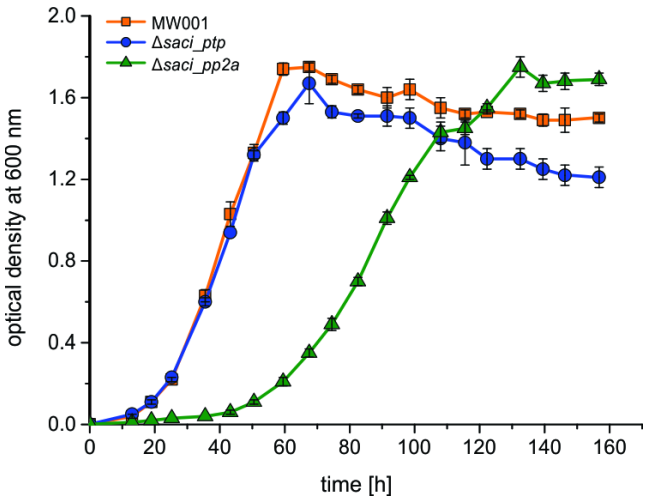

Figure S2. Slower growth rate of Δ*saci_pp2a* in comparison to MW001 and Δ*saci_ptp*. All three strains were grown for 160 hours in Brock medium supplemented with 0.1% NZ-amine, 0.2% sucrose and 10 µg/ml uracil at 76°C. Δ*saci_ptp* was comparable in growth to the background strain MW001, whereas the *saci_pp2a* deletion mutant displayed an extended lag-phase and also a slower growth rate. All growth experiments were performed in triplicate, and the standard deviation is shown.

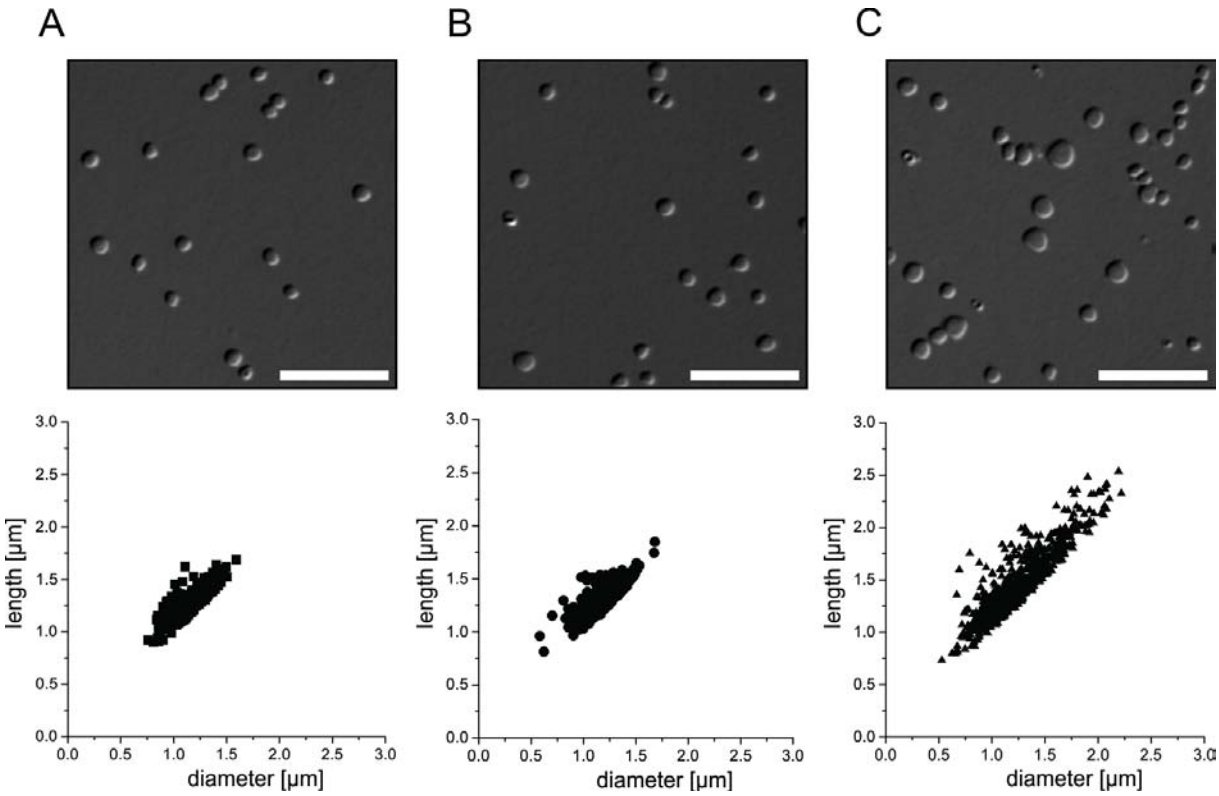

Figure S3. Cell shape and size of the phosphatase deletion mutant Δ*saci_pp2a*, Δ*saci_ptp* and MW001. MW001 and Δ *saci_ptp* show the regular spherical cell shape of *Sulfolobales* (A and B, upper panel), whereas the *saci_pp2a* deletion mutant showed a significantly increased amount of larger cells (C, upper panel). Evaluation of the cell size distribution using ObjectJ revealed a spread distribution in Δ*saci_pp2a* towards larger sizes (C, lower panel), compared to the parent strain MW001 (A, lower panel) and the *saci_ptp* deletion strain (B, lower panel). The calibration bar indicates 10 µm.

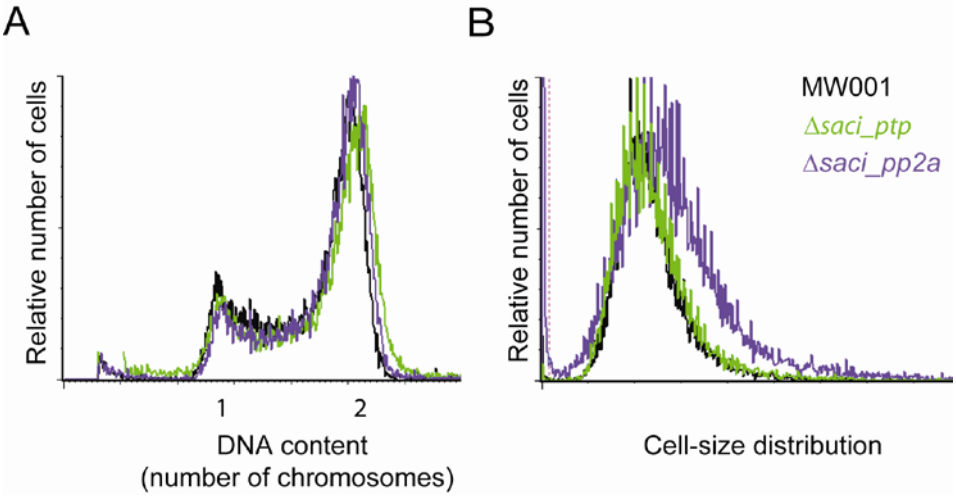

Figure S4. Flow cytometry of MW001 in comparison to Δ*saci_ptp* and Δ*saci_pp2a*. (A) All three strains show the typical DNA content distribution of exponentially growing *Sulfolobus* cultures [1]. (B) In the cell size the Ser/Thr phosphatase deletion mutant (Δ*saci_pp2a*) shows an increase in the relative number of larger cells as compared to the parent strain and the Tyr phosphatase deletion mutant *(Δsaci_ptp*).

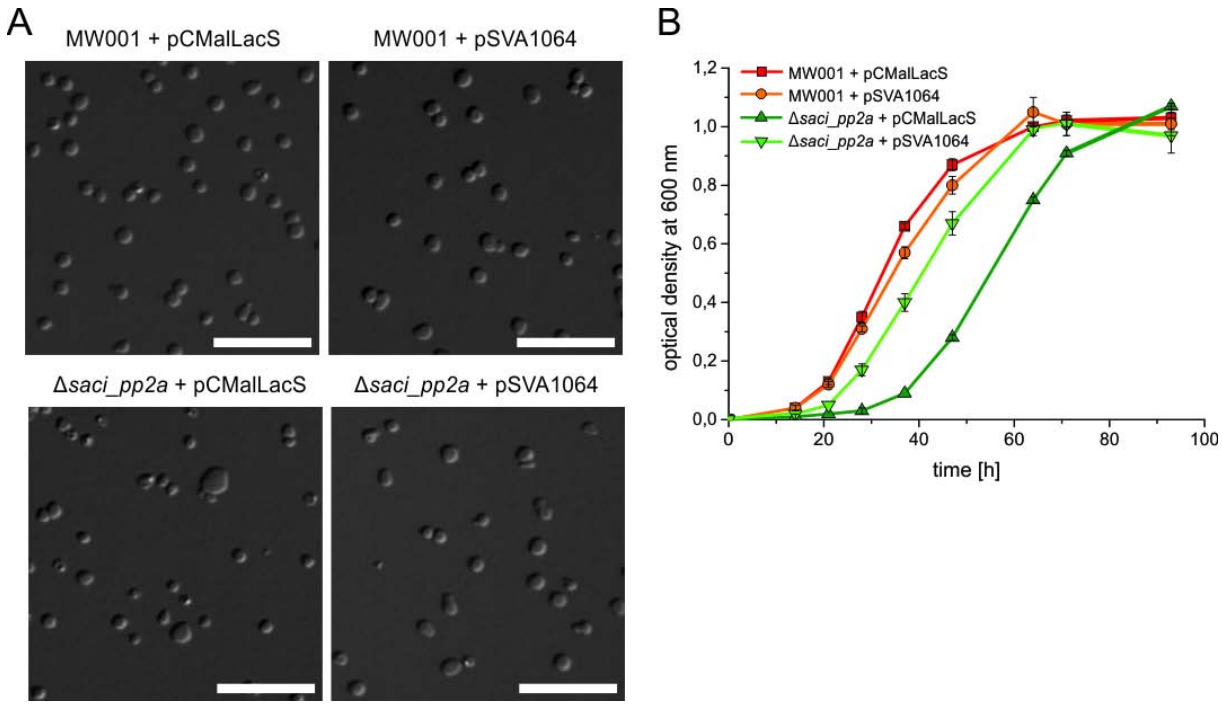

Figure S5. Complementation of the Δ*saci_pp2a* deletion. Δ*saci_pp2a* and MW001 were transformed with the control plasmid pCMalLacS and the complementation plasmid pSVA1064. (A) DIC light microscopy images of Δ*saci_pp2a* in comparison to MW001. In the complemented strainΔ *saci_pp2a* + pSVA1064, the cells became smaller and more homogeneous as compared to the parent strain. (B) Growth curve at 76°C in Brock medium supplemented with 0.1% NZ-amine and 0.2% sucrose. The complemented strain Δ*saci_pp2a* + pSVA1064 grew similar to the wild type strain. The scalebar indicates 10 µm in all images. All growth curves were performed in triplicate, and the standard deviations are shown.

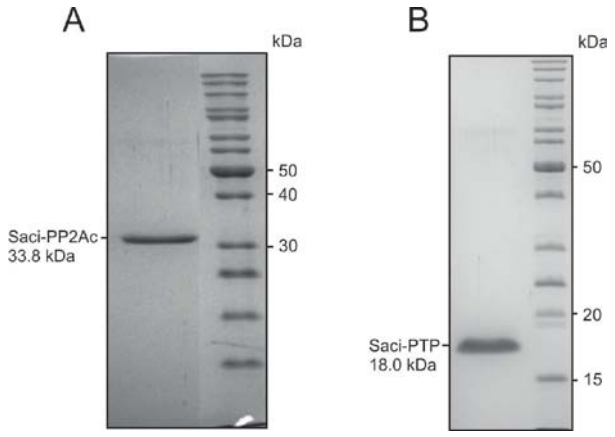

Figure S6. Purification of Saci-PP2A and Saci-PTP. 2 µg of Saci-PP2A (A) after size exclusion chromatography and Saci-PTP (B) after immobilized metal affinity chromatography were applied to SDS-PAGE. The SDS-gel was stained with Coomassie brilliant blue and PageRuler™ Unstained Protein Ladder (Fermentas) was used as size ladder.

Figure S7.

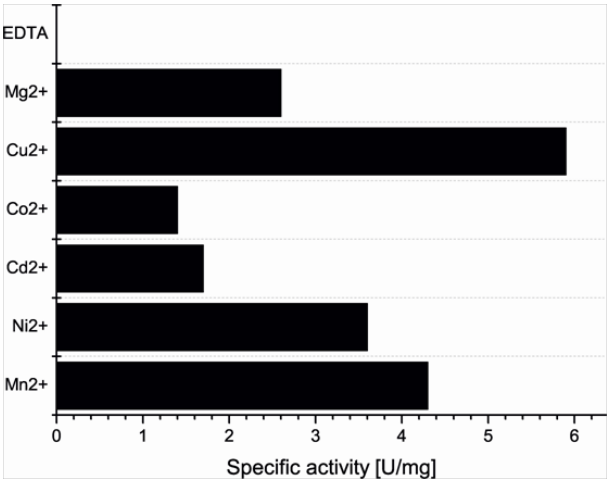

Figure S8. Metal-ion dependency of Saci-PP2A in the presence of EGTA. Enzyme activity was measured in the presence of 10 mM p-NPP, 5 mM Me^{2+} and 1 mM EGTA. The highest activity was observed with Cu^{2+} and Mn^{2+}.

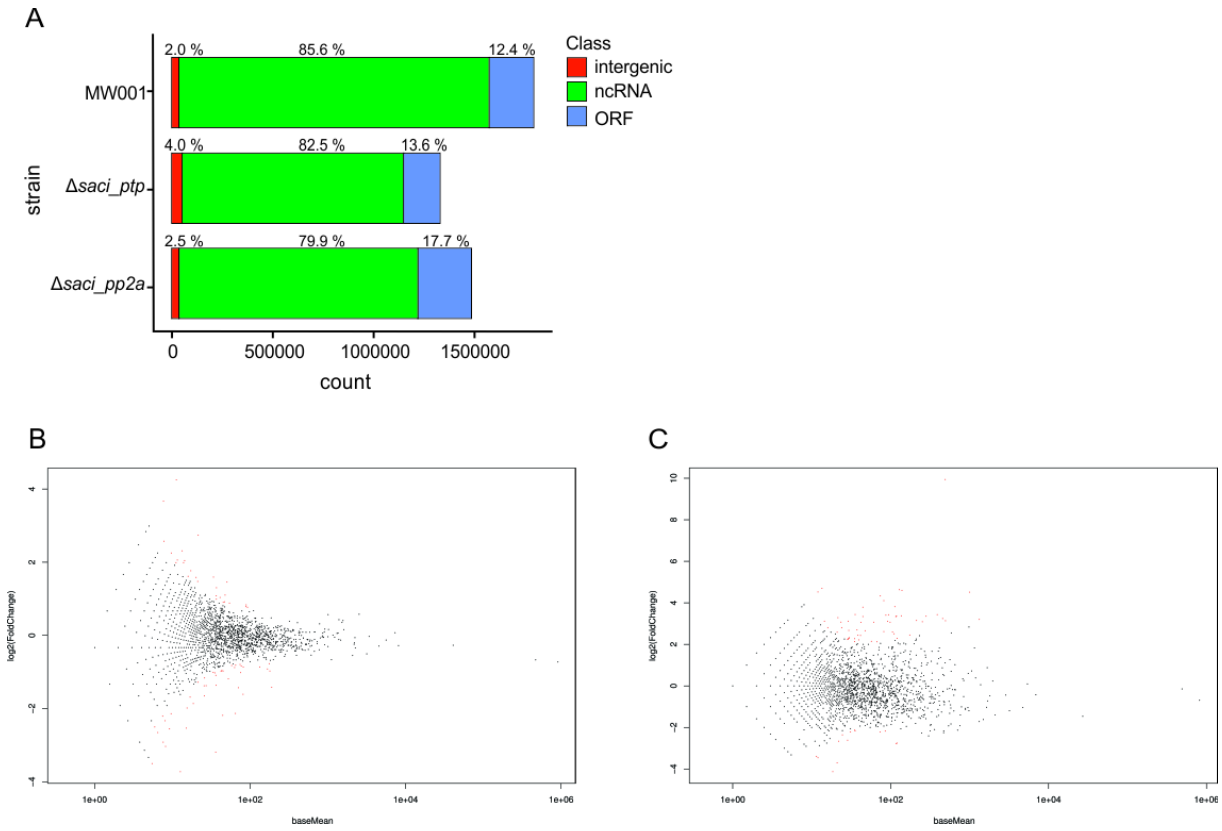

Figure S8. (A) Number of RNA-seq reads and genomic regions to which they were mapped, for samples from the parent strain MW001, Δ*saci_ptp* and Δ*saci_pp2a. (B+C)* Plots of normalized mean expression intensities versus $\log_2$ fold change for parent strain against Δ*saci_pp2a (B)* and parent strain against Δ*saci_ptp (C).* Significantly altered genes (p-value < 0.055) are indicated red.

Table S1. Phosphoproteome composition in *S. acidocaldarius* phosphatase deletion mutants *Δsaci_ptp* (S1b) and *Δsaci_pp2a (S1c)* and the parent strain MW001 (S1a). (extra files)

Table S2. Comparison between the number of phosphorylated proteins in MW001, Δ*saci_pp2a*, Δ*saci_ptp* with the number of proteins assigned to different arCOG categories in the wild type strain *S. acidocaldarius* DSM 639. Cellular metabolism: arCOGs C, E, F, G, H, I, P and Q; information storage/processes: D, M, N, T, O, U and V; information storage/processing (J, K and L); poorly characterized proteins (R, S and X).

Table S3. Differentially regulated genes in the *S. acidocaldarius* phosphatase deletion mutants Δ*saci_ptp* (Table S3b) and Δ*saci_pp2a* (Table S3a). (extra files)

Table S4. Distribution of detected mRNAs in arCOG categories.

arCOG category		Total		Δ*saci_pp2a*		Δ*saci_ptp*	
		No.	%	No.	%	No.	%
arCOG C	Energy production and conversion	15	5.7	8	5.7	7	5.7
arCOG E	Amino acid transport and metabolism	6	2.3	3	2.1	3	2.5
arCOG F	Nucleotide transport and metabolism	4	1.5	2	1.4	2	1.6
arCOG G	Carbohydrate transport and metabolism	5	1.9	4	2.9	1	0.8
arCOG H	Coenzyme transport and metabolism	5	1.9	1	0.7	4	3.3
arCOG I	Lipid transport and metabolism	6	2.3	2	1.4	4	3.3
arCOG J	Translation, ribosomal structure and biogenesis	4	1.5	1	0.7	3	2.5
arCOG K	Transcription	12	4.6	5	3.6	7	5.7
arCOG L	Replication, recombination and repair	4	1.5	2	1.4	2	1.6
arCOG N	Cell motility	4	1.5	2	1.4	2	1.6
arCOG O	Posttranslational modification, protein turnover, chaperones	3	1.1	3	2.1	0	0.0
arCOG P	Inorganic ion transport and metabolism	4	1.5	3	2.1	1	0.8
arCOG Q	Secondary metabolites biosynthesis, transport and catabolism	3	1.1	2	1.4	1	0.8
arCOG R	General function predicted only	10	3.8	3	2.1	7	5.7
arCOG S	Function unknown	34	13.0	20	14.3	14	11.5
arCOG T	Signal transduction mechanism	1	0.4	0	0.0	1	0.8
arCOG V	Defense mechanism	2	0.8	2	1.4	0	0.0
arCOG X	Not predicted	4	1.5	2	1.4	2	1.6
No arCOG	No arCOG	8	3.1	3	2.1	5	4.1
Total		262	100	140	100	122	100

Table S5. Primers used in this study.

primer	sequence (5´ - 3´)	purpose
	primers for pSVA1016	
1535	CCCCGGATCCGTTTTCCGATTAGAACTATT	Δ*saci0545* upstr fw *Bam*HI
1536	CATAAAATCTTCCATGTCTTCATCACTCTGAAGAT	Δ*saci0545* upstr rev ol
1537	CAGAGTGATGAAGACATGGAAGATTTTATGATAGA	Δ*saci0545* downstr fw ol
1538	CCAAACTGCAGAGCCTTATGAATTAAGCTC	Δ*saci0545* downstr rev *Pst*I
1553	AACTCATAGCGTGAGATCC	Δ*saci0545* check primer fw
1554	ATCCAGCTAATGCATGTTCC	Δ*saci0545* check primer rev
	primers for pSVA1017	
1539	CCCCGGATCCGTATTTTCTTAAACCTTC	Δ*saci0884* upstr fw *Bam*HI
1540	TGTCTGCTATACTATAATGTTCACAATATTGTGGT	Δ*saci0884* upstr rev ol
1541	AATATTGTGAACATTATAGTATAGCAGACAAAAAA	Δ*saci0884* downstr fw ol
1542	CCCCCTGCAGCCATAACTTATCCTTAAT	Δ*saci0884* downstr rev *Pst*I
1601	TTCCTGCCCACTGATATTCC	Δ*saci0884* check primer fw
1602.1	CGGTTGGTTAAATCAATTAG	Δ*saci0884* check primer rev
	primers for pET15b_Saci_0545	
1602.2	AAACATATGATGTATTGGGTAAAAAAGCATGTC	Saci0545 expr. fw *Nde*I
1603.1	AAAGGATCCTCATAAAATCTTCCATTTATCTTTCAT	Saci0545 expr. rev *Bam*HI
	primers for pSVA1037	
1599	GGGCCATGGCTAACATTGAAGAAACGTATGAG	Saci0884 expr. fw *Nco*I
1600	GGGGGATCCTTAGTGGTGATGATGGTGATGTACTATCTCTTCTATTAGTTGATCGTTCAC	Saci0884 expr. rev *Bam*HI with His-tag
	Primers for pSVA1064	
1604	**GGCCGCGGCGTCAATTGAATTAAAGTATGG**	*saci0884* compl fw *Sac*II
1603.2	**GGGCGGCCGCTATACTATCTCTTCTATTAGTTGATCG**	*saci0884* compl rev *Eag*I
	Quantitative RT-PCR primers	
1480	CCTGCAACATCTATCCATAACATACCGA	*secY*-housekeep-qRT-PCR-fw
1481	CCTCATAGTGTATATGCTTTAGTAGTAG	*secY*-housekeep-qRT-PCR-rev
1424	ACTGCGTCTACTGCGTTATCTTTATC	*flaB*-qRT-PCR-fw
1425	GGAGATAAGTCTACACTAGATACACCAGAA	*flaB*-qRT-PCR-rev
1426	GCAGTTGAAGAGTTAGCCTTATCTGTG	*flaX*-qRT-PCR-fw
1427	CCTACTAACTGACTTACGGTACTAATCT	*flaX*-qRT-PCR-rev
1428	CCTGGCTGTAGTGAATTAGATGTAACTG	*flaG*-qRT-PCR-fw
1429	GTGTAGTGTATTTCGGTCCAAATGGTCA	*flaG*-qRT-PCR-rev
2308	CTCTAACCCTAGCCCTTATTATTGGAC	*flaF*-qRT-PCR-fw
2309	GGATACGGAGGATATGGCAGAATGAT	*flaF*-qRT-PCR-rev
1432	AGTTGATGTGTATCTTAAGCTCTCGG	*flaH*-qRT-PCR-fw

1433	CTGAACCAGATATTCCTCCTGTAGTTTTTA	*flaH*-qRT-PCR-rev
1434	GGAGAAACCGCATCTGGAAAGACAAC	*flaI*-qRT-PCR-fw
1435	GGAACCGTCAATTCTGGAGTGTCCT	*flaI*-qRT-PCR-rev
1436	CCAGAAAGGAGCAGAACGGTAGG	*flaJ*-qRT-PCR-fw
1437	GCTATTACCGAAGCCAATTCACCAATC	*flaJ*-qRT-PCR-rev
4309	TAGTGTCGCTGCTGCTAGAG	*soxB*-qRT-PCR-fw
4310	ATTGCAGACTGCAGCGTTTG	*soxB*-qRT-PCR-rev
4311	TGGGTATCTGGTGCTACTTG	*cbsA*-qRT-PCR-fw
4312	AGCCGTGATTTCCACTATCC	*cbsA*-qRT-PCR-rev
4313	CCCTAAACGTGCCAAATGAGGG	*cbsB*-qRT-PCR-fw
4314	CAGAGATGCGGTAATCAATGTC	*cbsB*-qRT-PCR-rev
4315	AGGCGATTCGAGTGGGAGAC	*soxL*-qRT-PCR-fw
4316	GGCGGTGTACAACCCAGATG	*soxL*-qRT-PCR-rev
4317	AGCAGTGTACCTGGGCTATC	*soxN*-qRT-PCR-fw
4318	CGTAGTGCTCAGCCATGAAG	*soxN*-qRT-PCR-rev

Table S6. Strains and plasmids used in this study.

Strains	Genotype	Source/ Reference
Strains		
DH5α	*Escherichia coli* K-12 cloning strain l2 f80d/*lacZ*D*M15* D(*lacZYA-argF*)*U169 recA1 endA1 hsdR17* (rK2 mK1) *supE44 thi-1 gyrA relA1*	Gibco
Rosetta (DE3)	*Escherichia coli* expression strain	Novagen
ER1821	*Escherichia coli* propagation strain F^- *glnV44 e14*$^-$*(McrA*$^-$*) rfbD1? relA1? endA1 spoT1? thi-1* Δ*(mcrC-mrr)114::IS10*	New England Biolabs
MW001	*Sulfolobus acidocaldarius* DSM639 Δ*pyrE*	[1]
MW010	MW001 Δ*saci_ptp* (Δ*saci0545*)	This work
MW025	MW001 Δ*saci_pp2a* (Δ*saci0884*)	This work
MW156	MW001 Δ*saci2318* (Δ*aapF*)	[2]
MW455	MW001 Δ*saci2318*Δ*saci1174* (Δ*aapF*Δ*flaH*)	[3]
Plasmids		
pSVA406	Gene targeting plasmid, pGEM-T Easy backbone, $pyrEF_{SSO}$ cassette; single crossover method	[1]
pSVA407	Gene targeting plasmid, pGEM-T Easy backbone, $pyrEF_{SSO}$ and $lacS_{SSO}$ cassette; single crossover method	[1]
pΔ2*pyrEF*	Gene targeting plasmid, pBluescript backbone, $pyrEF_{SSO}$; single crossover method	[4]
pSVA1016	In-frame deletion of *saci0545*(*saci_ptp*) cloned into pΔ2*pyrEF* with *Bam*H, *Pst*I	This work
pSVA1017	In-frame deletion of *saci0884* (*saci_pp2ac*) cloned into pΔ2*pyrEF* with *Bam*H, *Pst*I	This work
pCMalLacS	pRN-1 based shuttle vector with $lacS_{SSO}$ reporter gene	[5]
pSVA1064	Complementation of *saci0884* (*saci_pp2ac*) deletion with own promoter, cloned into pCMal instead of *lacS*	This work
pETDuet-1	Amp^r, Car^r, expression plasmid containing replicon ColE1 (pBR322) and two MCS (MCS1 and MCS2)	Novagen
pET-15b	Amp^r, expression plasmid containing N-terminal His-Tag sequence	Novagen
pSVA1037	*saci0884* with C-termial His-tag cloned into pETDuet-1 with *Nco*I, BamHI in MCSI	[6]
pET15b_Saci_0545	*Saci0545* with N-terminal His-tag cloned into pET15b with NdeI, BamHI	This work

Table S7 Supplementary table showing the scores of each phosphorylation site in proteins identified by our method. DISPHOS 1.3 (http://www.dabi.temple.edu/disphos/pred.html) was used to calculate the scores. For unknown reasons DISPHOS 1.3 fails to process the sequences of: Saci_1499, Saci_2190, Saci_2201, Saci_2215, Saci_2331

References

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4. Wagner M, Berkner S, Ajon M, Driessen AJM, Lipps G, et al. (2009) Expanding and understanding the genetic toolbox of the hyperthermophilic genus *Sulfolobus*. Biochem Soc Trans 37: 97–101.
5. Berkner S, Wlodkowski A, Albers S-V, Lipps G (2010) Inducible and constitutive promoters for genetic systems in *Sulfolobus acidocaldarius*. Extremophiles 14: 249–259.
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