

1 **Supplementary materials**

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3 **Induction of a toxin-antitoxin gene cassette under high hydrostatic pressure**
4 **enables markerless gene disruption in the hyperthermophilic archaeon**

5 *Pyrococcus yayanosii*

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Table S1 Type II TA systems in *P. yayanosii* were identified using TAFinder^a

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Sequence Name: *Pyrococcus yayanosii* CH1 chromosome, complete genome

#	T/A ^b	Locus tag	Location	Len (a.a.)	Strand	Family	Domain	Score_TA
1	T	PYCH_04900	408569..409093	174	+	-	pfam13420	9.46
	A	PYCH_04890	408024..408449	141	+	-	cd00090	
2	T	PYCH_05070	419237..419434	65	-	MNT-like	-	60.21
	A	PYCH_05080	419440..419589	49	-	HEPN-like	-	
3	T	PYCH_05650	472157..472528	123	-	vapC	cd09981	86.63
	A	PYCH_05660	472498..472728	76	-	vapB	COG1753	
4	T	PYCH_13120	1152428..1153837	469	+	-	pfam00583	8.39
	A	PYCH_13110	1151801..1152361	186	+	-	cd00090	
5	T	PYCH_13440	1178632..1179141	169	+	-	COG1246	4.70
	A	PYCH_13430	1177992..1178504	170	+	-	cd00090	
6	T	PYCH_17720	1583761..1584165	134	-	PIN-like	cd09886	57.48
	A	PYCH_17710	1583428..1583589	53	-	vapB	COG3077	
7	T	PYCH_17720	1583761..1584165	134	-	PIN-like	cd09886	81.40
	A	PYCH_17730	1584152..1584373	73	-	RHH-like	PHA00617	
8	T	PYCH_18030	1611534..1611872	112	-	vapC	cd09981	39.85
	A	PYCH_18040	1611833..1612117	94	-	RHH-like	COG1753	
9	T	PYCH_18760	1674438..1674839	133	+	PIN-like	cd09886	75.91
	A	PYCH_18750	1674185..1674454	89	+	AbrB-like	COG2002	
10	T	PYCH_18760	1674438..1674839	133	+	PIN-like	-	47.19
	A	PYCH_18770	1674851..1675417	188	+	Xre-like	-	
11	T	PYCH_18840	1678921..1679298	125	+	PIN-like	cd09886	74.56
	A	PYCH_18830	1678712..1678948	78	+	RHH-like	COG1753	

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^a<http://202.120.12.133/TAFinder/index.php>

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^bT/A: This shows the type of the protein. T for Toxin and A for Antitoxin.

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Table S2. Type II TA systems in *P. furiosus* were identified using TAffinder^a

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Sequence Name: *Pyrococcus furiosus* DSM 3638 chromosome, complete genome

#	T/A ^b	Locus tag	Location	Len (a.a.)	Strand	Family	Domain	Score_TA
1	T	PF0233	244161..245558	465	+	-	pfam00583	8.88
	A	PF0232	243538..244101	187	+	-	cd00090	
2	T	PF0355	367848..368303	151	-	vapC	cd09981	99.99
	A	PF0355.1n	368300..368509	69	-	vapB	COG1753	
3	T	PF0574	594811..595212	133	+	vapC	COG5611	99.99
	A	PF0573	594596..594823	75	+	vapB	COG2002	
4	T	PF0575	595671..596009	112	-	vapC	-	99.99
	A	PF0576	596048..596188	46	-	vapB	-	
5	T	PF0773	765121..765216	31	-	vapC	-	99.99
	A	PF0774	765189..765497	102	-	vapB	-	
6	T	PF0776	765933..766259	108	+	PIN-like	-	99.99
	A	PF0775	765537..765860	107	+	RHH-like	-	
7	T	PF0778	767181..767360	59	-	PIN-like	-	76.11
	A	PF0776.1n	766956..767174	72	-	vapB	-	
8	T	PF0778	767181..767360	59	-	PIN-like	-	99.99
	A	PF0779	767357..767491	44	-	AbrB-like	-	
9	T	PF0812	794106..794522	138	-	vapC	cd09886	99.99
	A	PF0813	794519..794749	76	-	vapB	COG2002	
10	T	PF0839	813549..813929	126	+	vapC	-	99.99
	A	PF0838	813298..813549	83	+	vapB	-	
11	T	PF0944	909057..909425	122	+	MNT-like	-	99.99
	A	PF0943	908759..909094	111	+	HEPN-like	-	
12	T	PF1206	1147304..1147774	156	-	vapC	cd09981	99.99
	A	PF1207	1147771..1148001	76	-	vapB	COG1753	
13	T	PF1223	1159977..1160348	123	+	PIN-like	cd09981	99.99
	A	PF1222	1159693..1160004	103	+	RHH-like	COG1753	
14	T	PF1225	1161130..1161417	95	-	MNT-like	-	73.11
	A	PF1224	1160968..1161111	47	-	vapB	-	
15	T	PF1322	1244575..1244805	76	+	-	-	99.99
	A	PF1323	1244802..1245029	75	+	hicB	-	
16	T	PF1352	1270974..1271378	134	-	vapC	cd09886	99.99
	A	PF1353	1271365..1271586	73	-	vapB	PHA00617	
17	T	PF1600	1492512..1493033	173	-	-	pfam13420	10.21
	A	PF1601	1493159..1493584	141	-	-	cd00090	
18	T	PF2057	1897201..1897371	56	-	PIN-like	-	99.99
	A	PF2058	1897530..1897727	65	-	AbrB-like	-	

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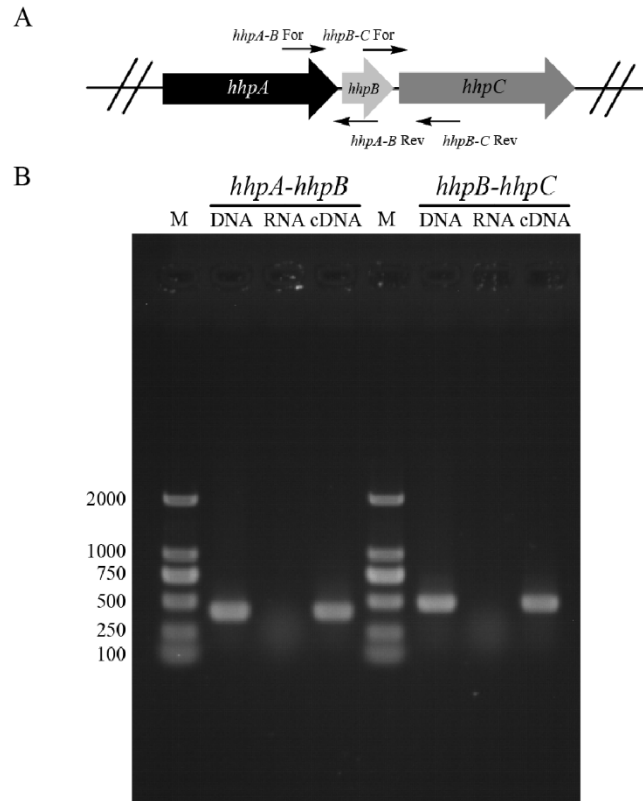
^a<http://202.120.12.133/TAfinder/index.php>

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^bT/A: This shows the type of the protein. T for Toxin and A for Antitoxin.

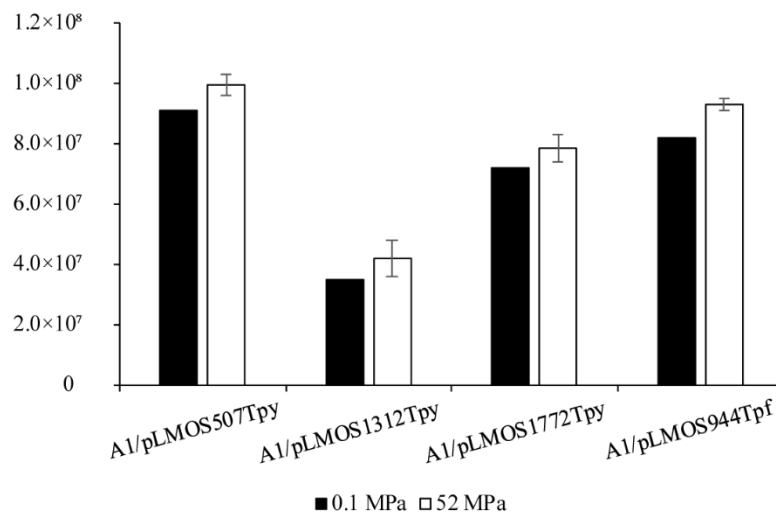
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27 Fig S1. A. Sketch map of primers designed for detection of co-transcription. B. The PCR analysis
 28 of co-transcription of the three hypothetical pressure related genes. Lane DNA is positive control;
 29 Lane RNA is negative control; Lane cDNA is the result of co-transcription.



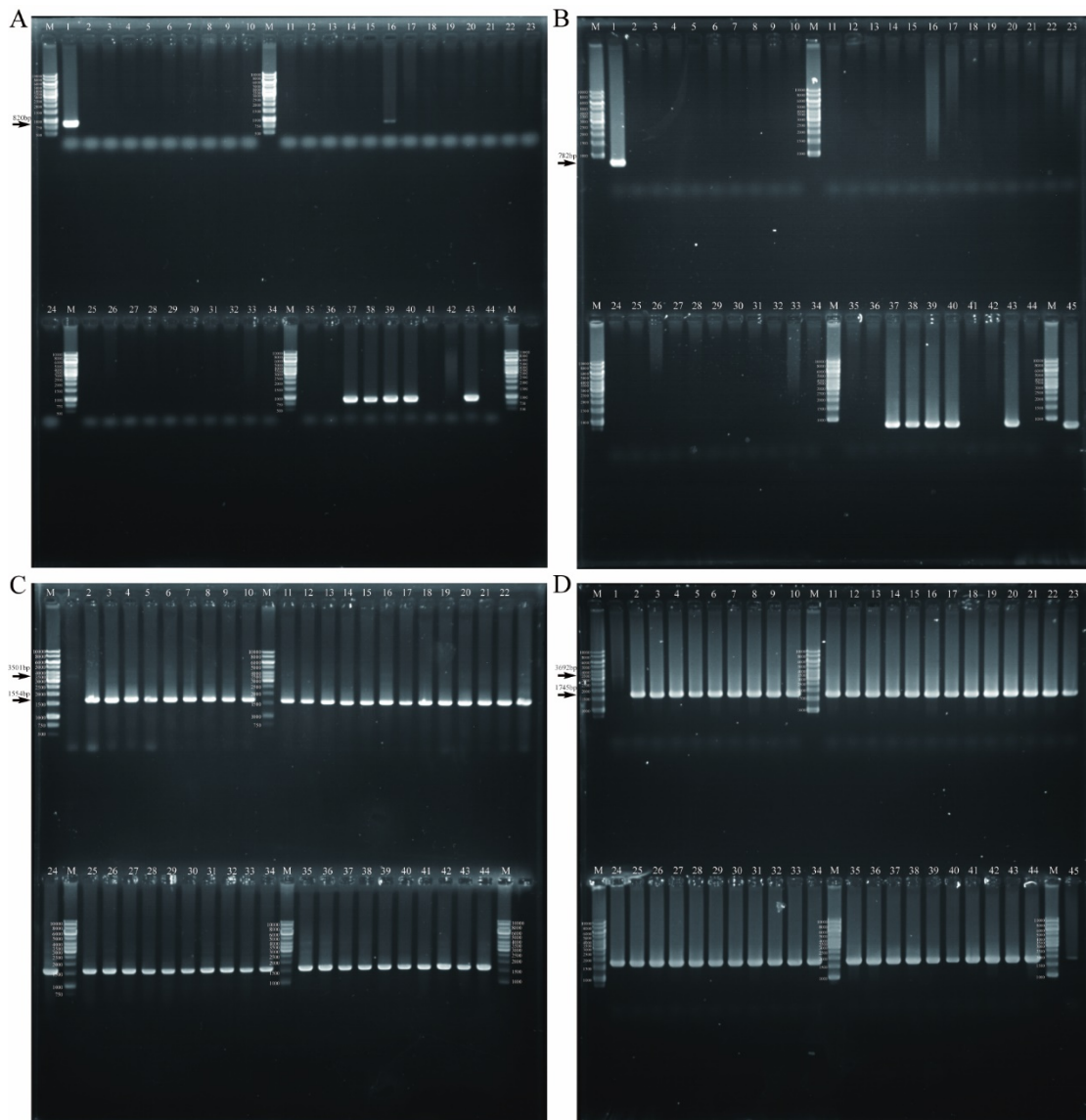
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33 Fig S2. The biomass of recombinant strains with different toxins at different pressures.
34 A1/pLMOS507Tpy, A1/pLMOS1312Tpy, A1/pLMOS1772Tpy, and A1/pLMOS944Tpf are strain
35 A1 containing plasmids pLMOS507Tpy, pLMOS1312Tpy, pLMOS1772Tpy and pLMOS944Tpf,
36 respectively. Toxins PYCH_05070, PYCH_13120 and PYCH_17720 are from *P. yayanosii*. Toxin
37 PF0944 of *P. furiosus* has 64% amino acid sequence identity with its homologue of *P. yayanosii*.
38 Black represents the biomass of strains at atmospheric pressure. Blank represent the biomass of
39 strains at 52 MPa. The results are from three independent experiments, and the error bars represent
40 the standard deviations. *p < 0.05; **p < 0.01.



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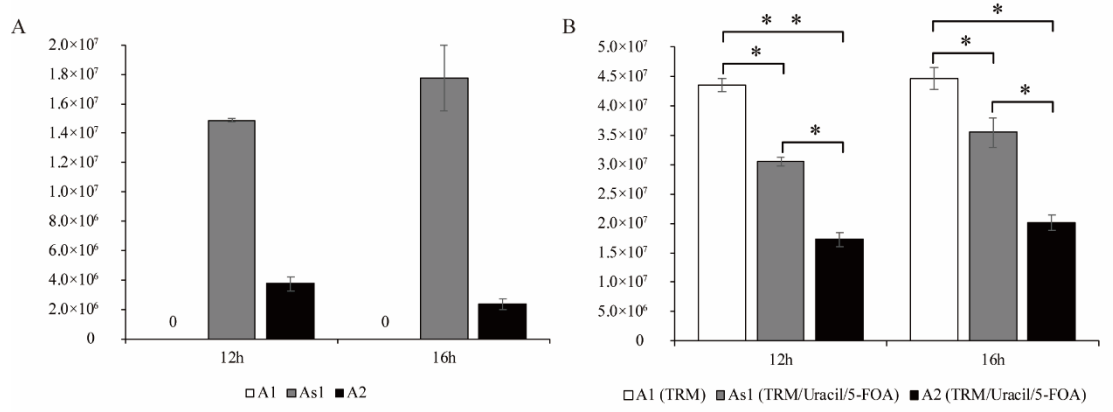
43 Fig S3. PCR analyses of the PYCH₁₃₆₉₀ loci confirming gene disruption. A. Primers PYCH₁₃₆₉-
44 F/R were designed in the internal regions of gene(s) to be disrupted. B. Primers 1369-F/R were
45 designed in the internal regions of gene(s) to be disrupted. C. Primers PYCH₁₃₆₉-up-F/
46 PYCH₁₃₆₉-dw-R were designed in the 5' and 3' flanking regions of the upstream and downstream
47 fragments of gene(s) to be disrupted. D. Primers 1369ud-F/R were designed in the 5' and 3' flanking
48 regions of the upstream and downstream fragments of gene(s) to be disrupted. M, 1-kb marker; 1,
49 wild type strain A1; 2-34, single colonies cultured in normal TRM liquid medium after second
50 recombination at 52 MPa; 35-44, single colonies cultured in normal TRM liquid medium after
51 second recombination at 45 MPa; 45, the first recombinant strain cultured in normal TRM liquid
52 medium.



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55 Fig S4. Biomass of the strain A1 and the auxotrophic strains As1 and A2. A. Biomass of the strains
 56 cultured in TRM with 1 mg/ml 5-FOA. B. Biomass of the strains cultured in TRM; the auxotrophic
 57 strains As1 and A2 were supplemented with 10 μ g/ml uracil and 1 mg/ml 5-FOA. *p < 0.05; **p <
 58 0.01.



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