Supplemental material

Table S1. Oligonucleotides used in this study

Name	Sequence (5'-3')	Comments
Cloning		**Restriciton sites**
Pf (0772)	ATATCCATGGGTTCTGCCACATCAAATATAAG	NcoI
Pr (0772)	TTAAAAGCTTTCAGTGGTGGTGGTGGTGGGAATTTCCAATTATATCACC	HindIII
Pf (1049)	ATATCATATGTTAGATGAATTGGTTAAAAAGG	NdeI
Pr (1049)	TTAACTCGAGTCATAATTGTTCTTCAATATCTATC	XhoI
EMSA or helicase assays		
S1	CAGTGAATTCGAGCTCGGTACCCGGGGATCCTCTAGAGTCGACCTGCAGGCATGCAAGCTTG	62-nt ssDNA with a random sequence
S2	CAAGCTTGCATGCCTGCAGGTCGACTCTAGAGGATCCCCGGGTACCGAGCTCGAATTCACTG	62-nt ssDNA complementary to S1
oligo$(dT)_{62}$	TTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTT	
oligo$(dA)_{62}$	AAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA	
ologo$(dC)_{62}$	CCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCC	
ologo$(dAdG)_{62}$	AGAGGAGGGAAGAAGGAAGGGAGGGGAAGGGAGGAGAAAGGGAAGAGGGGAGAGAGAGGAGG	
oligo$(ddAC)_{62}$	ACACCACCCAACAACCAACCCACCCCAACCCACCACAAACCCAACACCCCACACACACCACC	
S3	CAGTGAATTCGAGCTCGGTACCCGGGGATCCTCTAGAGTCGA	42-nt ssDNA with a random sequence
S4	TCGACTCTAGAGGATCCCCGGGTACCGAGCTCGAATTCACTG	42-nt ssDNA complementary to S3
S5	TTTTTTTTTTTTTTTTTTTTCAGTGAATTCGAGCTCGGTACCCGGGGATCCTCTAGAGTCGA	62-nt ssDNA with 5'-$(dT)_{20}$
S6	TCGACTCTAGAGGATCCCCGGGTACCGAGCTCGAATTCACTGTTTTTTTTTTTTTTTTTTT	62-nt ssDNA with 3'-$(dT)_{20}$
S7	GTTTGGCTCCCTTGACTACG	20-nt ssDNA with a random sequence
S8	ACCTCTACCTGGACGACCGGGTTTTTTTTTTTTTTTTTTTTGGGCCAGCAGGTCCATCACCA	62-nt ssDNA with a $(dT)_{20}$ region in the middle
S9	TGGTGATGGACCTGCTGGCCCTTTTTTTTTTTTTTTTTTTTCCCGGTCGTCCAGGTAGAGGT	62-nt ssDNA with a $(dT)_{20}$ region in the middle and both terminal regions complementary to those in S8

S16	CGTAGTCAAGGGAGCCAAAC	20-nt ssDNA complementary to S7
S17	CAGTGAATTCGAGCTCGGTACCCGGGGATC	30-nt ssDNA with a random sequence
S18	GATCCCCGGGTACCGAGCTCGAATTCACTG	30-nt ssDNA complementary to S17
S19	"TCCAGTGGGAATCAAGGGTAGGAGATGGTTTGGAAGGCGG AAGTATAGTACGTAAGCGAAATCTCCGCGCCAGGCCATCA GAGTAAGTTC GTAGTTGCCT	100-nt ssDNA complementary to OligoA
S10	TGGTGATGGACCTGCTGGCCC	
S11	CCCGGTCGTCCAGGTAGAGGT	
D1	Annealing product of S1 and S2	62-bp blunt-ended DNA
D2	Annealing product of S4 and S5	dsDNA with a 5'-tail
D3	Annealing product of S3 and S6	dsDNA with a 3'-tail
D4	Annealing product of S5 and S6	Forked DNA
D5	Annealing product of S8 and S9	Bubbled DNA
D6	Annealing product of S8, S10 and S11	Gapped DNA
D7	Annealing product of S3 and S4	42-bp blunt-ended DNA
D8	Annealing product of S7 and S16	20-bp blunt-ended DNA
D9	Annealing product of S17 and S18	30-bp blunt-ended DNA
D10	Annealing product of S19 and OligoA	100-bp blunt-ended DNA
L1	TCGACTCTAGAGGATCCCCGGGTACCGAGCTCGAATTCACTGTTT TTTTTTTTTTTTTTTT-cy3	Cy3-labeled S6
L2	cy5-TTTTTTTTTTTTTTTTTTTTCAGTGAATTCGAGCTCGGTACCCG GGGATCCTCTAGAGTCGA	Cy5-labeled S5
LD1	Annealing product of L1 and L2	Foked DNA with a 3’-tail labeled with Cy3 and a 5’-tail labeled with Cy5
LD2	Annealing product of L1 and S5	Forked DNA with a 3’-tail labeled with Cy3
S12	TTTTTTTTTTTTTTTTTTTTCGTTTTAGCGAACCTCCCGACTTGC	45-nt primer, with 5’-$(dT)_{20}$, used in the preparation of the M13-based helicase substrate
S14	Biotin-CAGTGAATTCGAGCTCGGTACCCGGGGATCCTCT	5’labelded with biotin
S15	CAGTGAATTCGAGCTCGGTACCCGGGGATCCTCTAGAGTCGA-bioti n	3’labeled with biotin
S20	TCGACTCTAGAGGATCCCCGGGT	3’dideoxy
Preparation of a 200-nt circular substrate		
Oligo A	AGGCAACTACGAACTTACTCTGATGGCCTGGCGCGGAGATTTCGC TTACGTACTATACTTCCGCCTTCCAAACCATCTCCTACCCTTGATTC CCACTGGA	100-nt ssDNA with a random sequence
Oligo B	GCTAACCCACATTTTGACGGATACCGCTCAGATTTATTCTCGGGAC	100-nt ssDNA with a

	CGCCATTTGTTACGGTACATCTAGCGTCGCACACGAGTTGGGCCA TGGTGACCC	random sequence
Bridge AB	TTCGTAGTTGCCT GGGTCACCATGG	
Bridge BA	ATGTGGGTTAGC TCCAGTGGGAATC	
S13	TTTTTTTTTTTTTTTTTTTTTGAGCGGTATCCGTCAAAATGTGGGTT AGC TCCAGTGGGA ATCAAGGGTA GGAGATGGTT TGGAAGGCGG AAGTATAGTA CGTAAGCGAA ATCTCCGCGC	Primer, with 5'-$(dT)_{20}$, used in the preparation of a 200-nt circular substrate

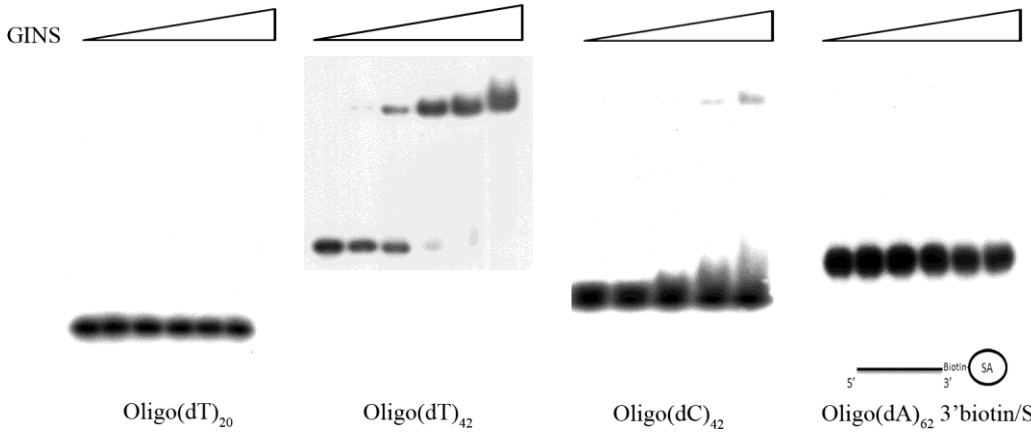


Figure S1. Binding of SsoGINS to oligo$(dT)_{20}$, oligo$(dT)_{42}$, oligo $(dC)_{42}$ or oligo $(dA)_{62}$ with 3'biotin/SA. Concentrations of SsoGINS used in the assays were 0, 0.1, 0.2, 0.4, 0.8, and 1.6 μM, as indicated by a triangle. The protein-DNA complexes were subjected to polyacrylamide gel electrophoresis. The gel was exposed to X-ray film.

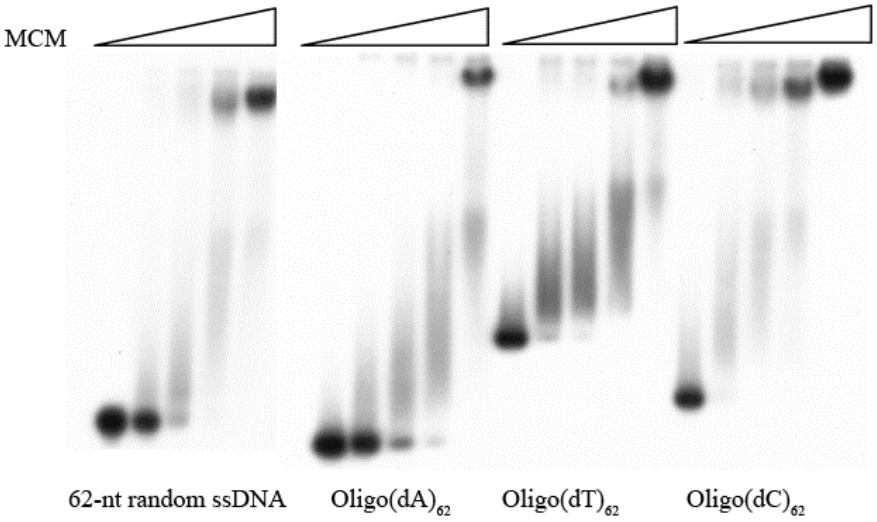


Figure S2. Binding of SsoMCM to ssDNA with different sequences. SsoMCM was mixed with a ^{32}P-labeled 62-nt ssDNA with a random sequence (S1), oligo(dA)$_{62}$, oligo(dT)$_{62}$ or oligo(dC)$_{62}$, as indicated. Concentrations of SsoMCM were 0, 0.2, 0.4, 0.8, and 1.6 μM. The protein-DNA complexes were subjected to polyacrylamide gel electrophoresis. The gel was exposed to X-ray film.

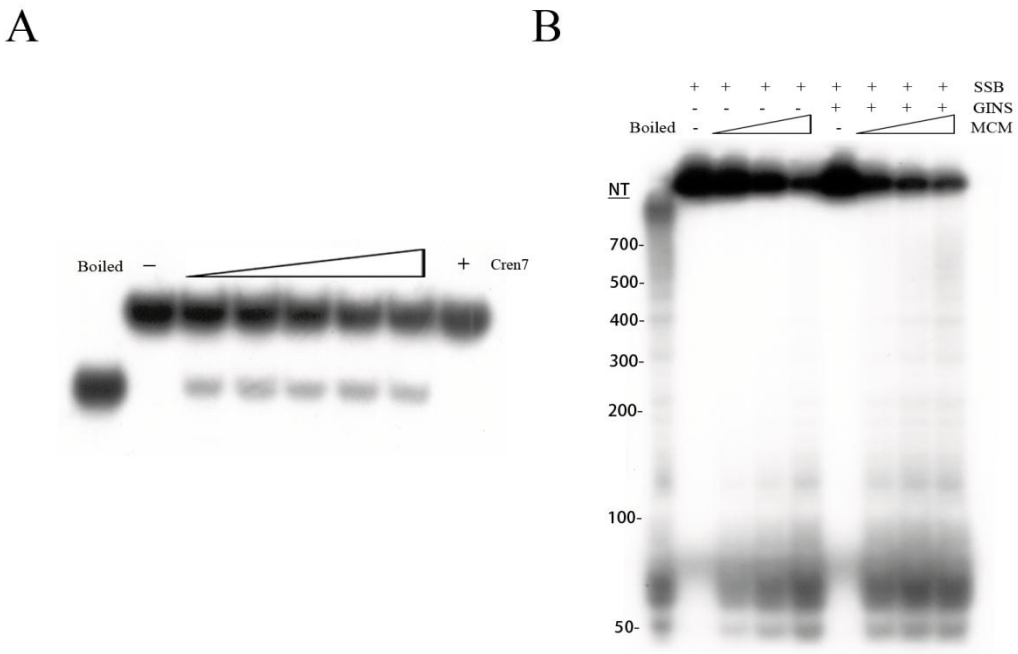


Figure S3. Effect of Cren7 on the helicase activity and processivity of SsoMCM. (A) Helicase activity assays were performed with 0.2 μM SsoMCM on a forked DNA substrate (D4) in the presence of increasing amounts of Cren7 (0, 0.5, 1, 2, and 4 μM). (B) Helicase processivity assays were performed with various amounts of SsoMCM (0, 0.1, 0.2, and 0.4 μM) on a radiolabeled M13-based substrate in the presence of 1 μM Cren7. SsoGINS (1 μM) was added as indicated. The gel was exposed to X-ray film.

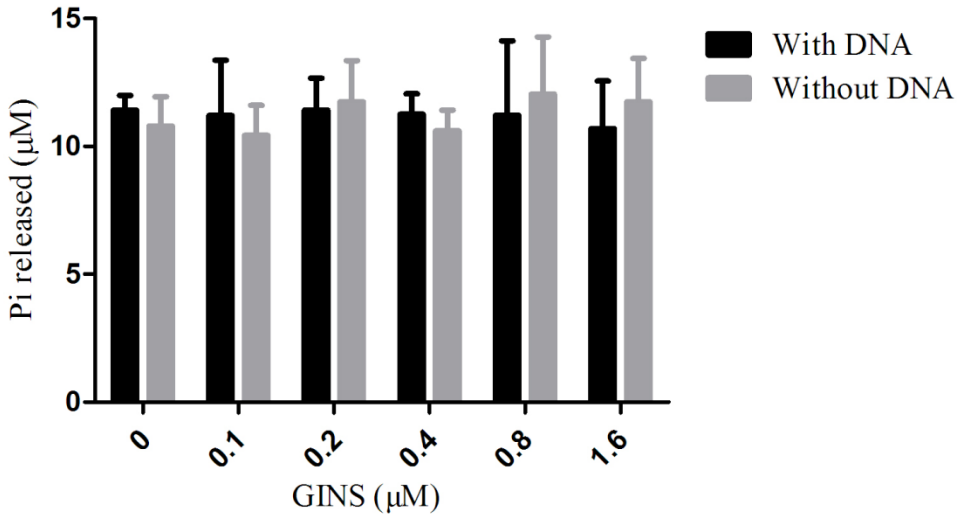


Figure S4. Effect of SsoGINS on the ATPase activity of SsoMCM. The standard reaction mixture (20 μl) contained 25 mM HEPES-NaOH, pH 7.0, 50 mM sodium acetate, 5 mM $MgCl_2$, 1 mM DTT, 0.1 mg/ml BSA, 100 μM [γ-^{32}P]ATP (1 μCi), and 0.2 μM SsoMCM. Increasing amounts of SsoGINS (0, 0.1, 0.2, 0.4, 0.8, and 1.6 μM) were added in the presence or absence of DNA (D4, 0.5 μM). Reaction mixtures were incubated for 30 min at 55°C, and quenched with 5 μl of 50 mM EDTA. Aliquots (1 μl) of the reaction mixtures were subjected to thin-layer chromatography on a polyethyleneimine-cellulose plate (Macherey-Nagel) in 1 M formic acid–0.5 M LiCl. ATP hydrolysis was quantitated by Phosphorimaging.

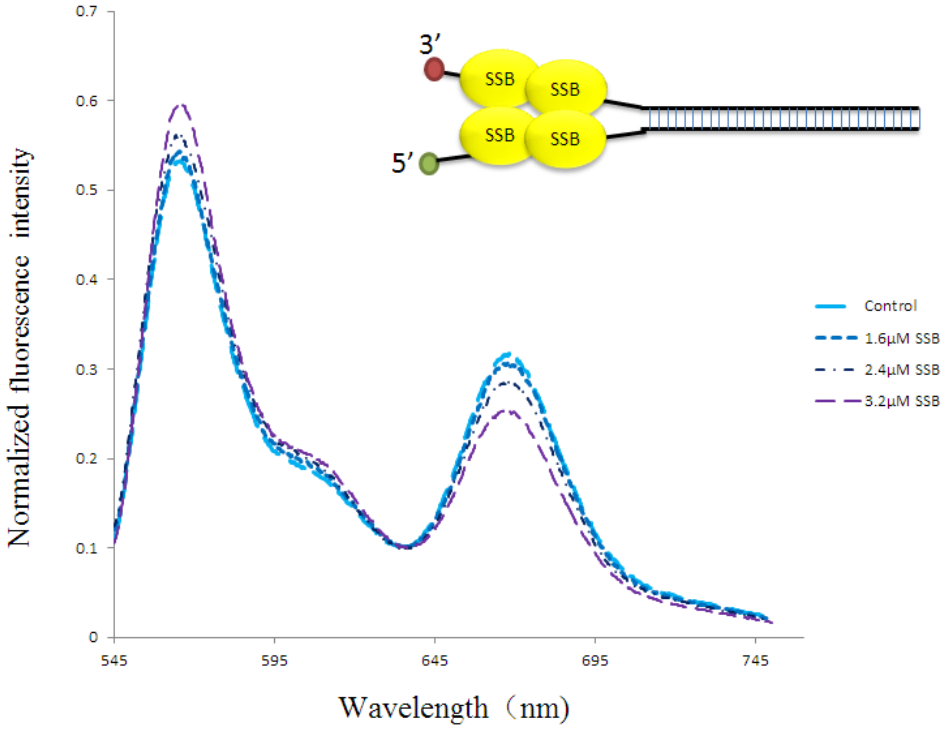
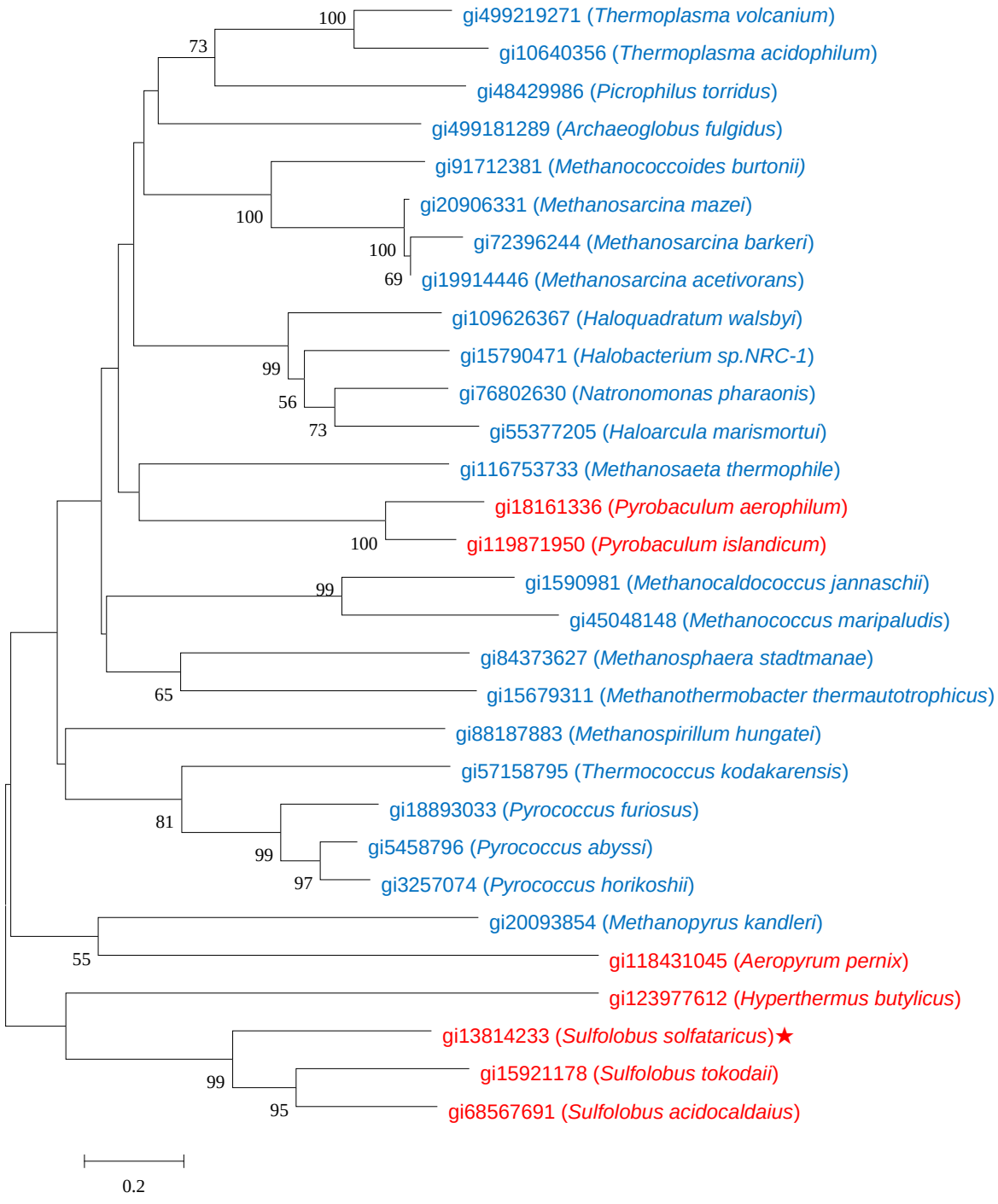


Figure S5. Effect of binding by SsoSSB to a forked DNA molecule on the distance between the two single-stranded tails of the DNA. SsoSSB were mixed with a forked DNA molecule with a 3'-oligo$(dT)_{20}$ tail labeled at the 3' end with Cy3 and a 5'- oligo$(dT)_{20}$ tail labeled at the 5' end with Cy5 (LD1). Fluorescence intensity was recorded over a range of wavelength from 545 to 750 nm. Cartoons show the proposed effect of the binding of SsoSSB on the distance between the two single-stranded tails of the forked DNA.


gi499219271 (Thermoplasma volcanium)
gi10640356 (Thermoplasma acidophilum)
gi48429986 (Picrophilus torridus)
gi499181289 (Archaeoglobus fulgidus)
gi91712381 (Methanococcoides burtonii)
gi20906331 (Methanosarcina mazei)
gi72396244 (Methanosarcina barkeri)
gi19914446 (Methanosarcina acetivorans)
gi109626367 (Haloquadratum walsbyi)
gi15790471 (Halobacterium sp.NRC-1)
gi76802630 (Natronomonas pharaonis)
gi55377205 (Haloarcula marismortui)
gi116753733 (Methanosaeta thermophile)
gi18161336 (Pyrobaculum aerophilum)
gi119871950 (Pyrobaculum islandicum)
gi1590981 (Methanocaldococcus jannaschii)
gi45048148 (Methanococcus maripaludis)
gi84373627 (Methanosphaera stadtmanae)
gi15679311 (Methanothermobacter thermautotrophicus)
gi88187883 (Methanospirillum hungatei)
gi57158795 (Thermococcus kodakarensis)
gi18893033 (Pyrococcus furiosus)
gi5458796 (Pyrococcus abyssi)
gi3257074 (Pyrococcus horikoshii)
gi20093854 (Methanopyrus kandleri)
gi118431045 (Aeropyrum pernix)
gi123977612 (Hyperthermus butylicus)
gi13814233 (Sulfolobus solfataricus)★
gi15921178 (Sulfolobus tokodaii)
gi68567691 (Sulfolobus acidocaldaius)
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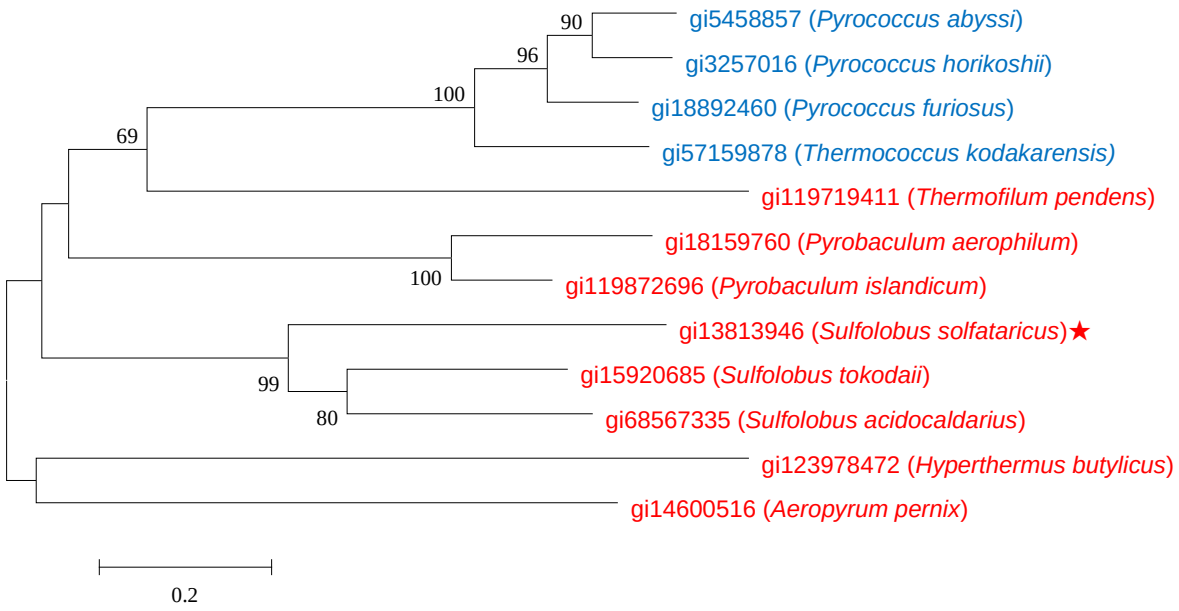

B

Figure S6. Phylogenetic dendrograms of Gins51 (A) and Gins23 (B) from archaeal GINS family members. Multiple sequence alignments were obtained by using MUSCLE (http://www.ebi.ac.uk/Tools/msa/muscle/). The tree was reconstructed using the neighbour-joining method. Bootstrap values were obtained with 1,000 replicates and only values >50 % are shown. The source organisms and GI numbers are given. The names of euryarchaea and crenarchaea are shown in blue and red, respectively. The GINS from *S. solfataricus* are denoted by a star.