Fig. S1. Comparative and phylogenetic analysis of archaeal *apt* homologues

A. Schematic overview of the *apt* gene that is conserved in some well-studied microorganisms from *Sulfolobales* and *Desulfurococcales*. Homology is indicated by the same colors.

B. Phylogenetic analysis of Apt and Gpt homologues from hyperthermophilic crenarchaea. Phylograms of Apt and Gpt proteins were constructed using the Phylogeny-fr platform (www.phylogeny.fr). For each run, proteins corresponding to hits pertaining to archaea, with BLAST e-value lower than 2E-50 (for Apt) and 3E-36 (for Gpt) and covering at least 40% of query sequence, were selected and fed to the ‘‘One Click Mode Phylogeny analysis’’ pipeline available on www.phylogeny.fr.

Fig. S1

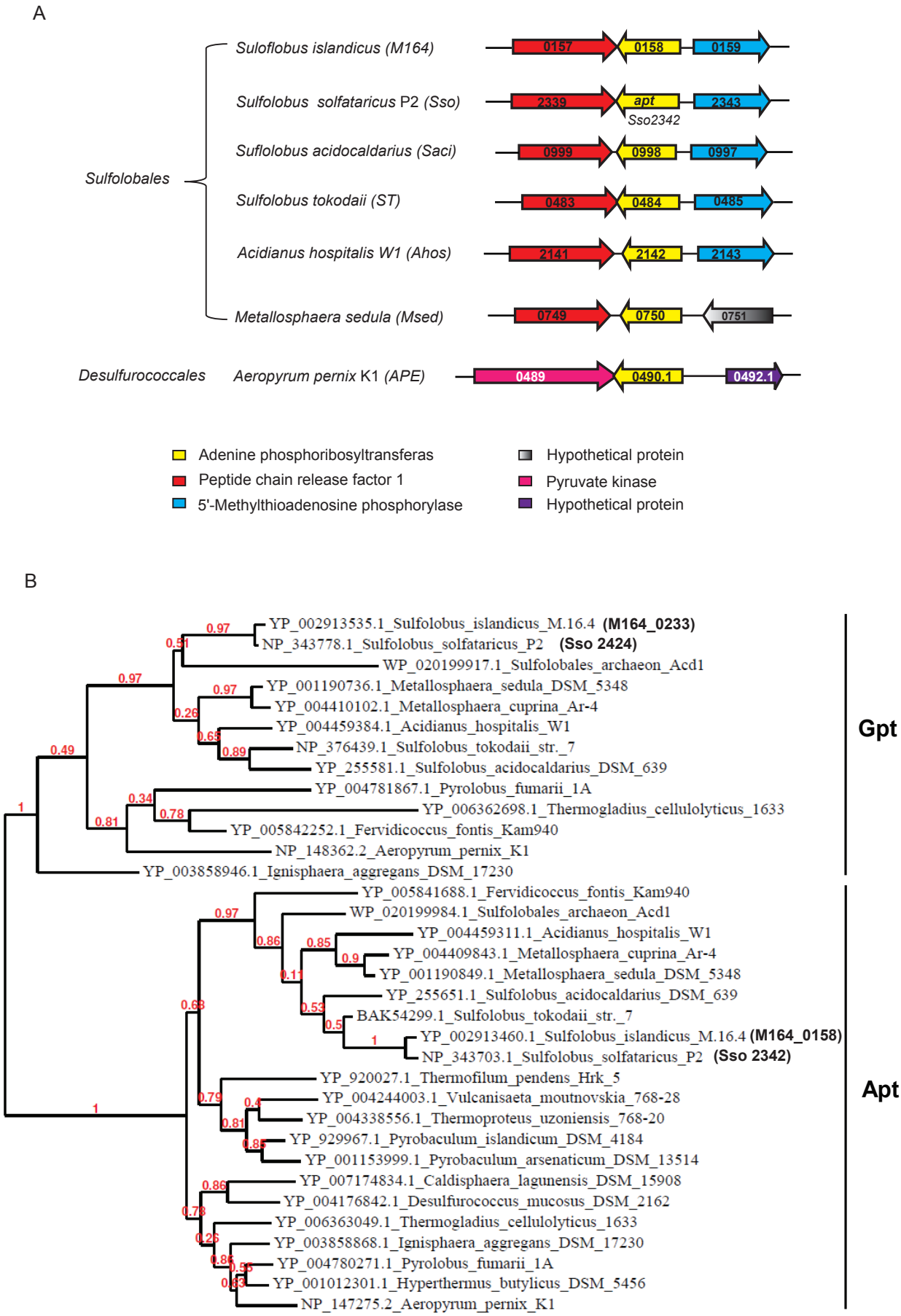

A
Suloflobus islandicus (M164)
0157
0158
0159
Sulfolobus solfataricus P2 (Sso)
2339
apt
Sso2342
2343
Suflolobus acidocaldarius (Saci)
0999
0998
0997
Sulfolobales
Sulfolobus tokodaii (ST)
0483
0484
0485
Acidianus hospitalis W1 (Ahos)
2141
2142
2143
Metallosphaera sedula (Msed)
0749
0750
0751
Desulfurococcales
Aeropyrum pernix K1 (APE)
0489
0490.1
0492.1
Adenine phosphoribosyltransferas
Peptide chain release factor 1
5'-Methylthioadenosine phosphorylase
Hypothetical protein
Pyruvate kinase
Hypothetical protein
B
YP_002913535.1_Sulfolobus_islandicus_M.16.4 (M164_0233)
NP_343778.1_Sulfolobus_solfataricus_P2 (Sso 2424)
WP_020199917.1_Sulfolobales_archaeon_Acd1
YP_001190736.1_Metallosphaera_sedula_DSM_5348
YP_004410102.1_Metallosphaera_cuprina_Ar-4
YP_004459384.1_Acidianus_hospitalis_W1
NP_376439.1_Sulfolobus_tokodaii_str._7
YP_255581.1_Sulfolobus_acidocaldarius_DSM_639
YP_004781867.1_Pyrolobus_fumarii_1A
YP_006362698.1_Thermogladius_cellulolyticus_1633
YP_005842252.1_Fervidicoccus_fontis_Kam940
NP_148362.2_Aeropyrum_pernix_K1
YP_003858946.1_Ignisphaera_aggregans_DSM_17230
Gpt
YP_005841688.1_Fervidicoccus_fontis_Kam940
WP_020199984.1_Sulfolobales_archaeon_Acd1
YP_004459311.1_Acidianus_hospitalis_W1
YP_004409843.1_Metallosphaera_cuprina_Ar-4
YP_001190849.1_Metallosphaera_sedula_DSM_5348
YP_255651.1_Sulfolobus_acidocaldarius_DSM_639
BAK54299.1_Sulfolobus_tokodaii_str._7
YP_002913460.1_Sulfolobus_islandicus_M.16.4 (M164_0158)
NP_343703.1_Sulfolobus_solfataricus_P2 (Sso 2342)
YP_920027.1_Thermofilum_pendens_Hrk_5
YP_004244003.1_Vulcanisaeta_moutnovskia_768-28
YP_004338556.1_Thermoproteus_uzoniensis_768-20
YP_929967.1_Pyrobaculum_islandicum_DSM_4184
YP_001153999.1_Pyrobaculum_arsenaticum_DSM_13514
YP_007174834.1_Caldisphaera_lagunensis_DSM_15908
YP_004176842.1_Desulfurococcus_mucosus_DSM_2162
YP_006363049.1_Thermogladius_cellulolyticus_1633
YP_003858868.1_Ignisphaera_aggregans_DSM_17230
YP_004780271.1_Pyrolobus_fumarii_1A
YP_001012301.1_Hyperthermus_butylicus_DSM_5456
NP_147275.2_Aeropyrum_pernix_K1
Apt
0.4

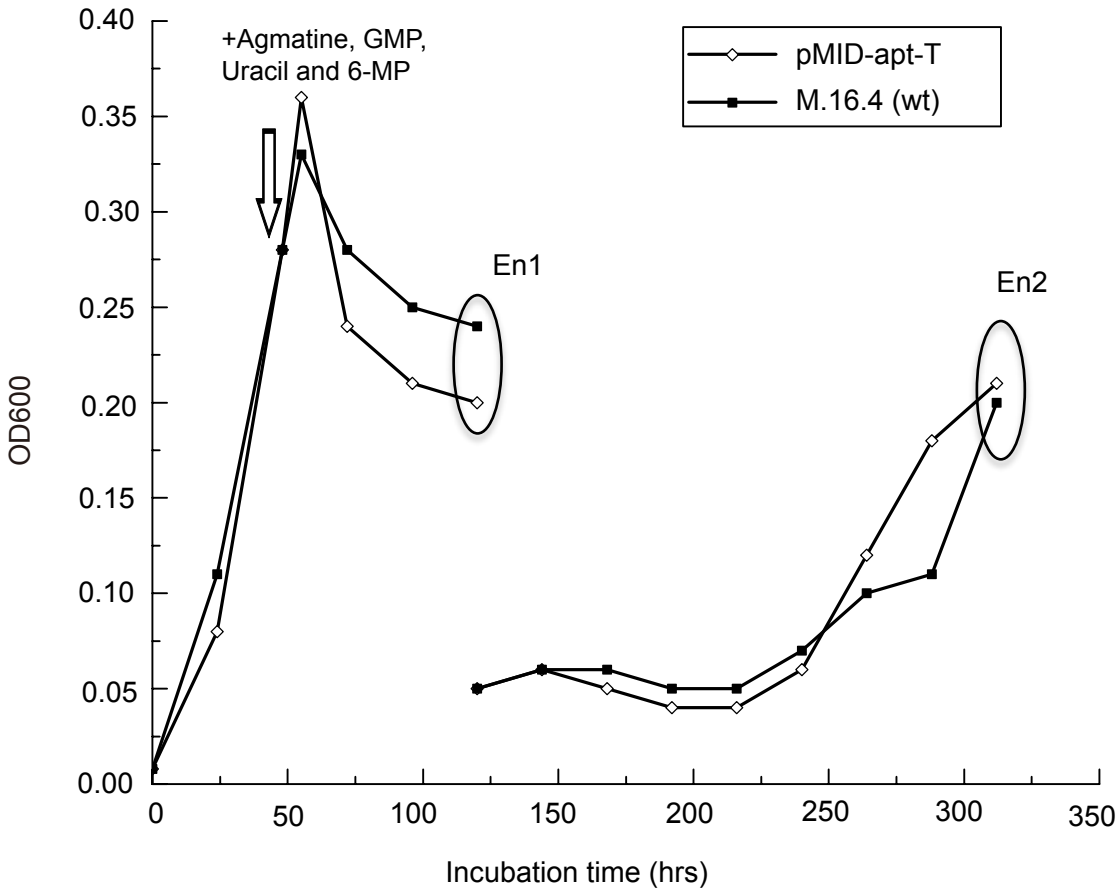
Fig. S2. Generation of △*apt* mutant cells via a mutant propagation assay

A. △*apt* mutant cells were enriched via a mutant propagation assay as described previously (1), with slight modifications. The transformant pMID-apt-T and a control strain *S. islandicus* M.16.4 were inoculated into DTU liquid medium. When the OD_{600} of cells achieved around 0.26, 6-MP (0.15 mM), agmatine (1 mg/ml) and GMP (0.5 mM) were added. After another three days' incubation, cells were collected (En1), transferred into fresh medium with initial OD_{600}=0.05 and then cultivated until obvious growth were observed (~8 days), as indicated by the En2 (A). B. X-gal staining of pMID-apt-T-En2 and M.16.4-En2 cultures. C. Isolation of single 6-MP^R colonies by directly plating pMID-apt-T-En2 on medium plates supplemented with 6-MP, GMP, agmatine and uracil. X-gal staining of colonies indicated that △*apt* mutants (White color) were successfully enriched and dominant.

1. **Zhang C, Guo L, Deng L, Wu Y, Liang Y, Huang L, She Q.** 2010. Revealing the essentiality of multiple archaeal pcna genes using a mutant propagation assay based on an improved knockout method. Microbiology **156:**3386-3397.

Fig. S2

A


+Agmatine, GMP,
Uracil and 6-MP
pMID-apt-T
M.16.4 (wt)
En1
En2
OD600
0.40
0.35
0.30
0.25
0.20
0.15
0.10
0.05
0.00
0
50
100
150
200
250
300
350
Incubation time (hrs)

B


M.16.4-En2
pMID-apt-T-En2

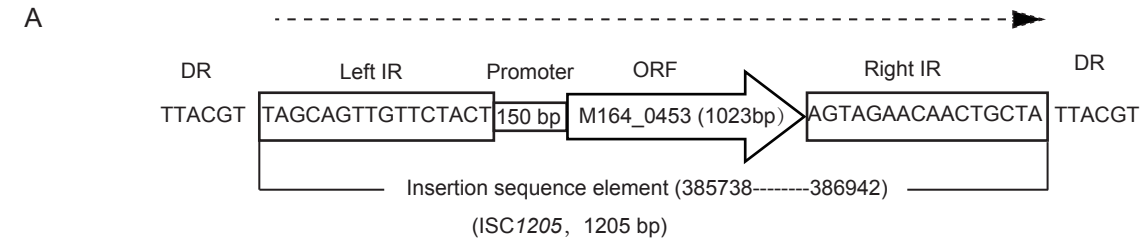
C

Fig. S3. Sequences and structural features of the insertion sequence element ISC*1205*

A. Schematic representation of ISC*1205* in the wild-type *S. islandicus* M.16.4 chromosome. The entire insertion sequence element contained a large open reading frame (M164_0453) of 1023 bp, a putative promoter region of 150 bp and terminal inverted repeats (IR, 16 bp). DR means the direct repeat with 100% match.

B. Properties of ISC*1205* insertions in chromosome of 6-MP^{R} isolates. The orientation of the ISC*1205* in the chromosome was indicated by dotted arrows.

Fig. S3



B

		DR	Insertion position	Isolates
TTAGGGA ----------►	TTAGGGA	7bp	-8 ~ -7	4
TTAGGGAG ◄----------	TTAGGGAG	8bp	-15 ~ -14	1
TGATAAA ----------►	TGATAAA	7bp	-32 ~ -31	1
AAATTGA ◄----------	AAATTGA	7bp	-36 ~ -35	1
CTACTAA ◄----------	CTACTAA	7bp	+59 ~ +60	1
AAAGAT ----------►	AAAGAT	6bp	+81 ~ +82	1
TAAAGAT ◄----------	TAAAGAT	7bp	+81 ~ +82	1
TGATAT ◄----------	TGATAT	6bp	+173 ~ +174	1
CAGATAA ◄----------	CAGATAA	7bp	+266 ~ +267	2
TATAGTA ----------►	TATAGTA	7bp	+282 ~ +283	2
TGCCAG ◄----------	TGCCAG	6bp	+409 ~ +410	1
CTACGCA ----------►	CTACGCA	7bp	+420 ~ +421	1
CTTAGTG ----------►	CTTAGTG	7bp	+489 ~ +490	1
TGAGCTTA ----------►	TGAGCTTA	8bp	+541 ~ +542	1

Table S1. MICs of 6-methylpurine (6-MP) on *Sulfolobus* species

Sulfolobus species	MIC of 6-MP (mM)	6-MP-resistant determinant
S. islandicus M.16.4	0.04	*M164_0158*
S. islandicus REY15A	0.1	*SiRe_0139*
S. solfataricus P1	0.04	--
S. solfataricus P2	0.04	*Sso2342*
S. tokodaii	0.02	*St0484*
S. acidocaldarius	> 0.4	*Saci_0998*

Sulfolobus cells were inoculated into DT medium (initial OD_{600} = 0.008) supplemented with various concentrations of 6-methylpurine. Cell growth was checked after 10 days' incubation at 75℃.

--: The sequence of 6-MP-resistant determinant in *S. solfataricus* P1 shares 100% identity as *Sso2342*.