

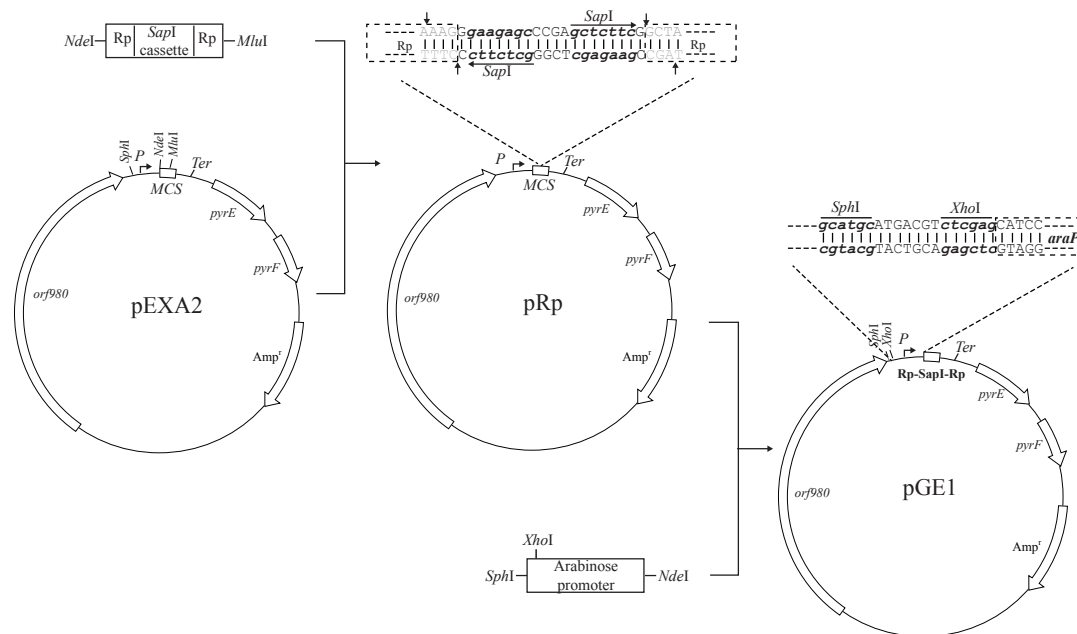
Supplementary figures and tables for

CRISPR-Cas type I-A Cascade complex couples viral infection surveillance

to host transcriptional regulation

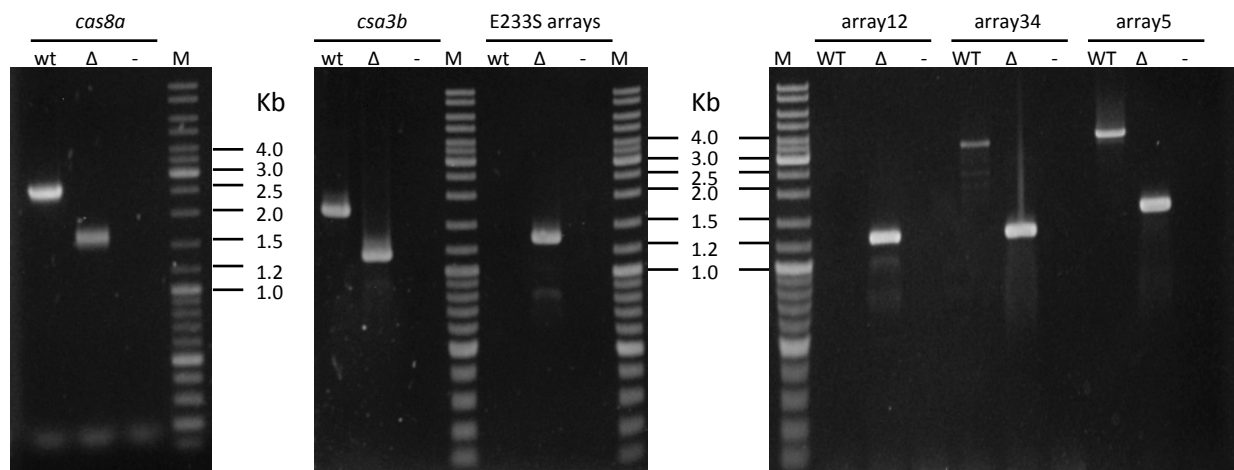
Fei He¹, Gisle Vestergaard², Wenfang Peng^{1,3}, Qunxin She¹ & Xu Peng^{1, *}

Supplementary Figure 1



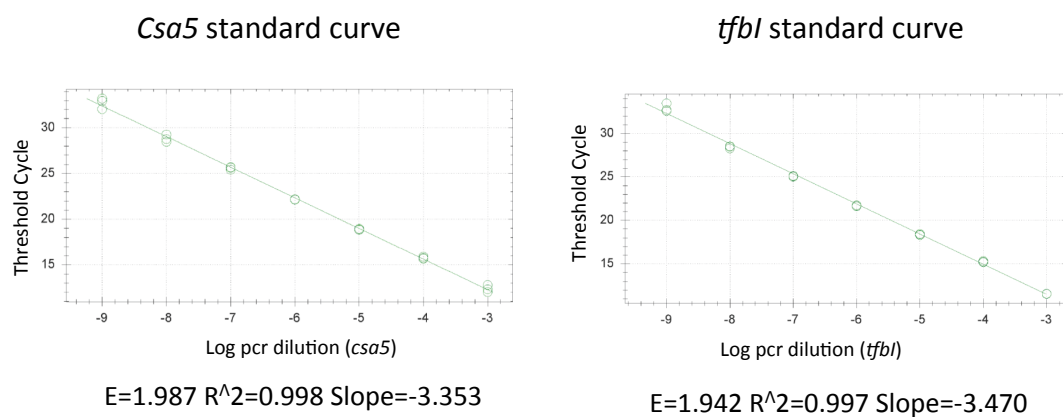
Supplementary Figure 1. Construction of pGE1 plasmid. A DNA fragment carrying two copies of type I-A CRISPR repeat separated by two oppositely oriented *SapI* recognition sequences were inserted into pEXA2 between *NdeI* and *MluI* sites adjacent to the arabinose promoter. To facilitate the insertion of recombination sequences, an *XhoI* restriction enzyme site was added adjacent to *SphI* to construct the basic genome-editing plasmid pGE1.

Supplementary Figure 2



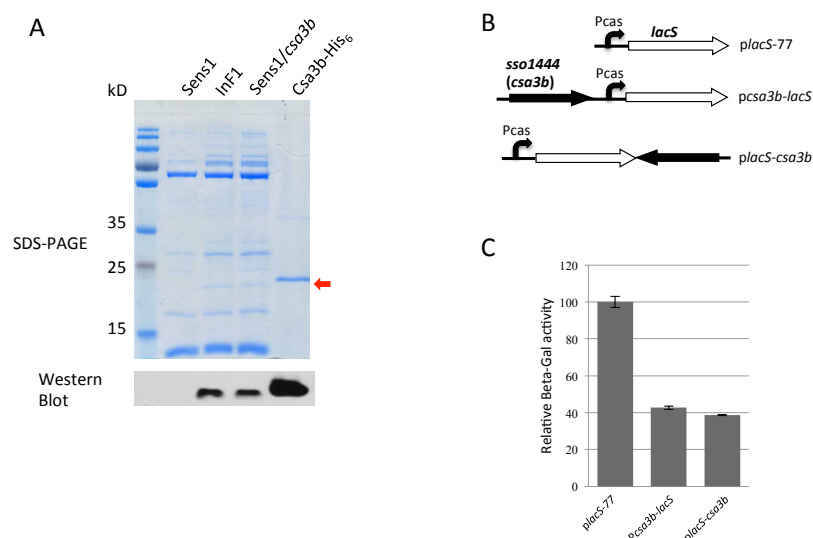
Supplementary Figure 2. PCR verification of $\Delta cas8a$ (Left), $\Delta csa3b$ and E233S Δ arrays (Middle) and LAL Δ arrays (Right). PCR was conducted with specific primers (Table S2) using genomic DNAs extracted from *S. islandicus* E233S (wt), *S. islandicus* LAL549 (WT) and corresponding deletion mutants (Δ). The expected size is 19010 bp for E233S arrays wt and 20092 bp for array12 WT, which are too big to be amplified under the condition used in the PCR. - , no template DNA was added (negative control). M, DNA size marker.

Supplementary Figure 3

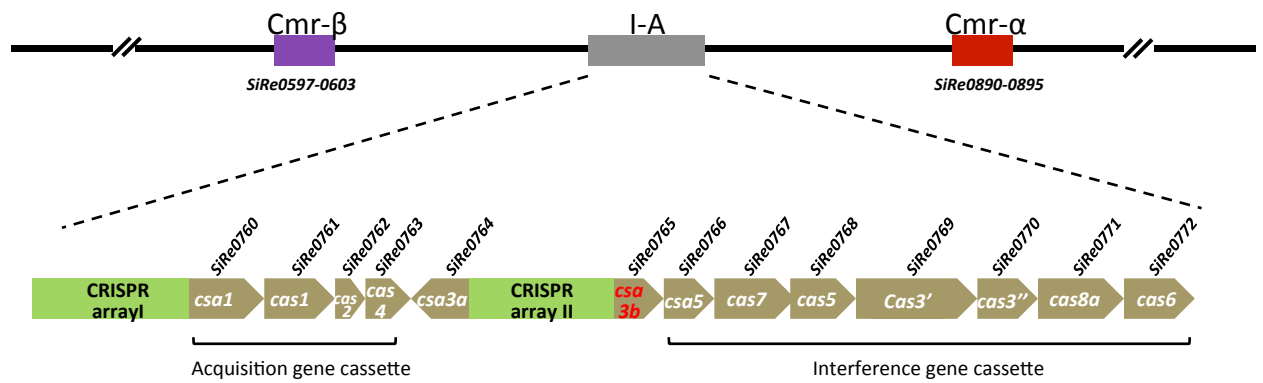


Supplementary Figure 3. Determination of qPCR efficiencies for primer sets against *csa5* and *tfbI*.

Ten-fold dilutions of PCR products (10^{-9} to 10^{-3}) were prepared. After amplification, the slope was determined for both primer sets by plotting the threshold cycle (Ct) versus the log DNA dilution. PCR efficiencies (E) were calculated using the equation $E=10^{-1}/\text{slope}$. The amplification efficiencies for *csa5* and *tfbI* are 1.987 and 1.942 (located within the range of 1.8 to 2.2) which are qualified for the comparative Ct method.



Supplementary Figure 4. Interaction of Csa3b with Pcas. (a) Pull-down assay using a biotin-labeled DNA fragment containing RI and RII. Pull-down samples from Sens1, InF1 and Sens1 carrying a plasmid-borne *csa3b* were run in SDS-PAGE together with the His-tagged Csa3b (Csa3b-His₆). Western blot using an antibody against Csa3b (SS01444) is shown at the bottom. Red arrow indicates the position of Csa3b obtained from pull-down assay. **(b)** Inserts cloned into the three constructs. **(c)** Comparison of glycosidase activity expressed from Pcas in the presence (*pcsa3b-lacS* and *placS-csa3b*) or absence (*placS-77*) of *csa3b* in Sens1 cells.



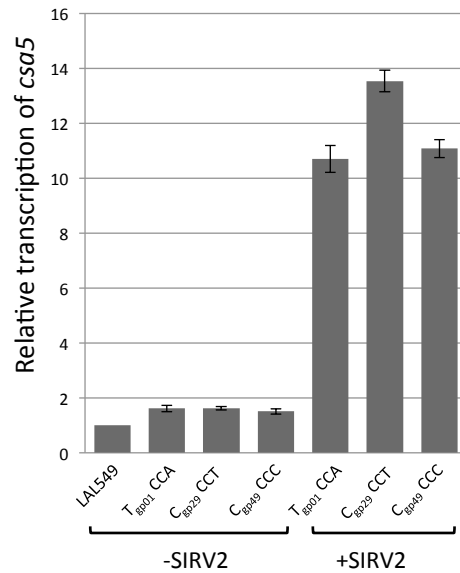
Supplementary Figure 5. Schematic presentation of CRISPR-Cas systems in *Sulfolobus islandicus* E233S (a Rey15A derivative carrying a *pyrEF* mutation).

Supplementary Figure 6

T _{gp38} CCG	CCGTCTGAATTATTGAATTTGTATACAAGTTATTATCAACATT
T _{gp38} CGT	CGTCTGAATTATTGAATTTGTATACAAGTTATTATCAACATTT
C _{gp38} CCA	CCATGTCTAGCTAATTGTAGATGATAATTAATAATTTTGGATA
C _{gp38} CAT	CATGTCTAGCTAATTGTAGATGATAATTAATAATTTTGGATAT

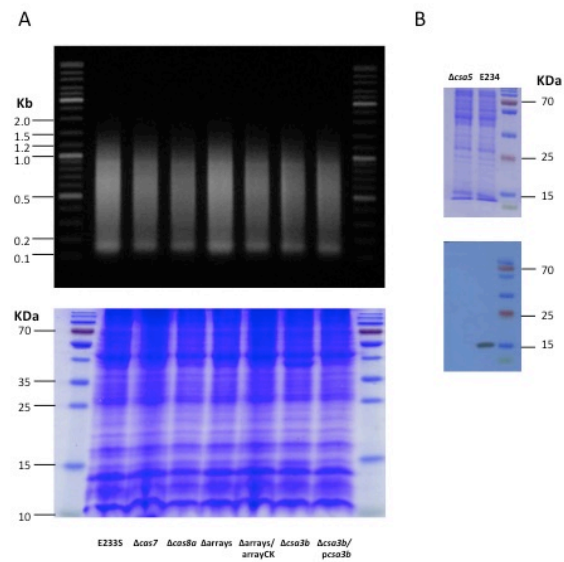
Supplementary Figure 6. Protospacer (black coloured) and PAM sequences (red coloured) corresponding to the mini-CRISPRs carried on the four constructs.

Supplementary Figure 7



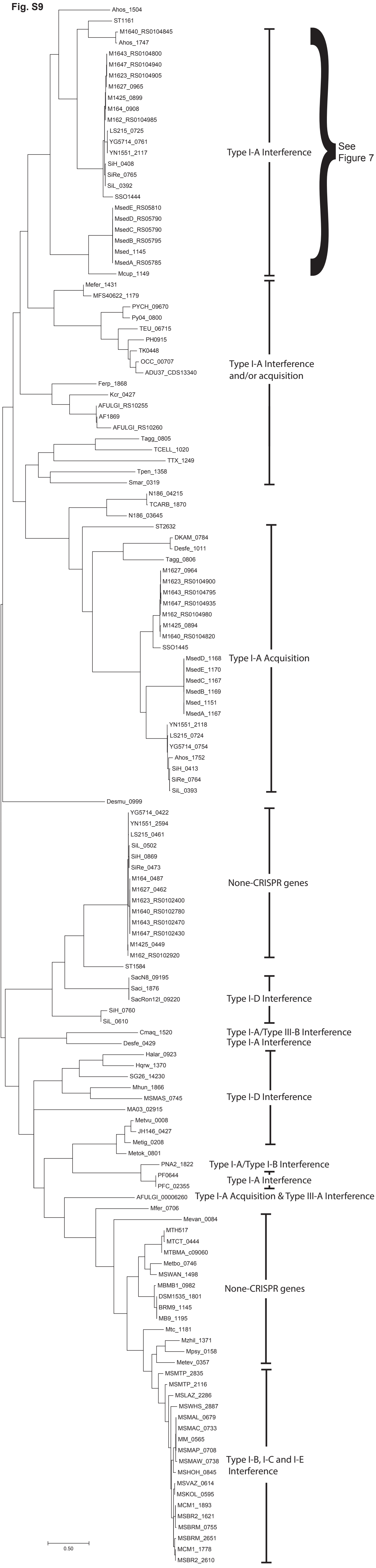
Supplementary Figure 7. Relative transcript level of *csa5* in CRISPR deletion mutant

LALΔarrays carrying plasmid-borne spacers against SIRV2 *gp01*, *gp29* or *gp49*. Values for the *tfbI* gene were used for normalization. The normalized value for *csa5* transcript level in LAL549 was set to 1. LALΔarrays transformed with construct Tgp01 CCA, Cgp29-CCT and Cgp49-CCC, as well as the wild-type strain LAL549, were incubated with (+ SIRV2) or without SIRV2 (- SIRV2) before RNA extraction and RT-qPCR. Results from three technical replicates are shown, and error bars indicate standard deviation.



Supplementary Figure 8. (A) size range of the de-crosslinked and sonicated DNA from ChIP (top gel) and loading control (SDS-PAGE) of the cell lysates used in the ChIP assay (bottom gel). Strain names are indicated at the bottom. **(B)** Western blot using Csa5-Ab to show the specificity of the antibody. Top: Coomassie blue staining of the SDS-PAGE gel; bottom: Western blot. Sizes of the DNA marker and protein markers are indicated.

Fig. S9



Supplementary Figure 9. Phylogenetic tree of all Csa3 sequences present in Refseq database.

Supplementary Tables

Supplementary Table 1. Sulfolobus strains used in this study.

Strains	Genotype and features	References
<i>S. solfataricus</i> Sens1	Δ pyrEF and Δ CRISPR loci A-D	Deng <i>et al.</i> (1)
<i>S. solfataricus</i> InF1	Δ pyrEF	Deng <i>et al.</i> (2)
<i>S. islandicus</i> E233S	Δ pyrEF and Δ lacS	Deng <i>et al.</i> (3)
<i>S. islandicus</i> LAL549	Δ pyrF of <i>S. islandicus</i> LAL14/1	This work
Δ csa3	In frame deletion of <i>csa3</i> in <i>S. islandicus</i> E233S	This work
Δ cas8a	In frame deletion of <i>cas8a</i> in <i>S. islandicus</i> E233S	This work
Δ csa5	In frame deletion of <i>csa5</i> in <i>S. islandicus</i> E233S	Peng <i>et al.</i> (4)
Δ cas7	In frame deletion of <i>cas7</i> in <i>S. islandicus</i> E233S	
Δ cas5	In frame deletion of <i>cas5</i> in <i>S. islandicus</i> E233S	
Δ cas3'	In frame deletion of <i>cas3'</i> in <i>S. islandicus</i> E233S	
Δ cas3''	In frame deletion of <i>cas3''</i> in <i>S. islandicus</i> E233S	
Δ cas6	Carrying deletion of the entire <i>cas6</i> gene plus 277 bp downstream flanking sequence	
Δ cmr α and <i>cmr</i> δ	Deletion of both type III-B Cmr loci	
E233S Δ arrays	Deletion of both arrays including acquisition <i>cas</i> genes in <i>S. islandicus</i> E233S	This work
LAL Δ array12	Deletion of both array1 and array2 including interspaced acquisition <i>cas</i> genes in <i>S. islandicus</i> LAL549	This work
LAL Δ array125	Deletion of array5 in LAL Δ array12	This work
LAL Δ arrays	Deletion of both array3 and array4 including interspaced <i>cmr</i> δ cassette in LAL Δ array125	This work

Supplementary Table 2. Construct names as well as primers and vectors used for their construction.

Construct	Primers	Sequences (5'-3')	Constructs based on
p <i>csa5-39</i>	<i>csa539fwd-SphI</i>	CAT GCATG CTAGAAATGTTTATATAGTGAATAATAATAGAG	pEXA2 (2)
	<i>csa5rev-NotI</i>	ATTT GCGGCCG CAACCTAACTGAACCGCTTATC	
p <i>csa5-62</i>	<i>csa562fwd-SphI</i>	CAT GCATG CAAAGAGTTTTCTGACTAGAAATAG	
	<i>csa5rev-NotI</i>	ATTT GCGGCCG CAACCTAACTGAACCGCTTATC	
p <i>csa5-77</i>	<i>csa577fwd-SphI</i>	CAT GCATG CTTTTCATATTTATGAAAAGAG	
	<i>csa5rev-NotI</i>	ATTT GCGGCCG CAACCTAACTGAACCGCTTATC	
p <i>lacS-77</i>	<i>csa577fwd-SphI</i>	CAT GCATG CTTTTCATATTTATGAAAAGAG	
	<i>csa5lacSrev</i>	CCTAAAGTTTTTGGAAAAGAATACATGTTATTCTCTATTATTCCACT	
	<i>csa5lacSfwd</i>	AGTGGAAATAATAATAGAGAATAACATGTATTCTTTCCAAAAAAGCTTTAGG	
	<i>lacsrev-NotI</i>	ATTT GCGGCCG CCTAGTGCCTTAATGGTCTTATAGG	
p <i>lacS-39</i>	<i>csa539fwd-SphI</i>	CAT GCATG CTAGAAATGTTTATATAGTGAATAATAATAGAG	
	<i>lacsrev-NotI</i>	ATTT GCGGCCG CCTAGTGCCTTAATGGTCTTATAGG	
p <i>lacS-77</i> Rleft	Rleft- <i>SphI</i>	CAT GCATG CGGGGATATTATGAAAAGAG	
	<i>lacsrev-NotI</i>	ATTT GCGGCCG CCTAGTGCCTTAATGGTCTTATAGG	
p <i>lacS-77</i> Rright	Rright- <i>SphI</i>	CAT GCATG CTTTTCATATTATCCCCGAGTTTTCGTACAC	
	<i>lacsrev-NotI</i>	ATTT GCGGCCG CCTAGTGCCTTAATGGTCTTATAGG	
p <i>lacS-77</i> Rlleft	Rlleft- <i>SphI</i>	CAT GCATG CTTTTCATATTATGAAAAGAGGGGGGGTACACTAGAAATAGAAATG	
	<i>lacsrev-NotI</i>	ATTT GCGGCCG CCTAGTGCCTTAATGGTCTTATAGG	
p <i>lacS-77</i> Rlright	Rlright- <i>SphI</i>	CAT GCATG CTTTTCATATTATGAAAAGAGTTTTCGTACACTACCCCTAGAAATGTTTATATAG	
	<i>lacsrev-NotI</i>	ATTT GCGGCCG CCTAGTGCCTTAATGGTCTTATAGG	
pCsa3bpartN-His ₆	<i>csa3bfwd-NdeI</i>	CGCCATATGATATTGATTTTGACACTTGGAT	pET-30a(+)
	<i>csa3bpartNrev-XhoI</i>	CCGCTCGAGGTCCTCAGTCTCTATCTCTATAGTAG	
pCsa3b-His ₆	<i>csa3bfwd-NdeI</i>	CGCCATATGATATTGATTTTGACACTTGGAT	pEXA3 (5)
	<i>csa3brev-XhoI</i>	CCGCTCGAGGAACCCATAGATTTTCAATAAAATCTC	
pAPCsa3b-His ₆	<i>csa3bfwd-NdeI</i>	CGCCATATGATATTGATTTTGACACTTGGAT	pEXA2
	<i>csa3brev-NotI</i>	ATTT GCGGCCG CGAACCCATAGATTTTCAATAAAATCTC	
p <i>csa3b-lacS</i>	<i>csa3bfwd-SphI</i>	CAT GCATG CTTTGGTGAATTAGGTTGACGTAATAC	pEXA2
	<i>csa5lacSrev</i>	CCTAAAGTTTTTGGAAAAGAATACATGTTATTCTCTATTATTCCACT	
	<i>csa5lacSfwd</i>	AGTGGAAATAATAATAGAGAATAACATGTATTCTTTCCAAAAAAGCTTTAGG	
	<i>lacsrev-NotI</i>	ATTT GCGGCCG CCTAGTGCCTTAATGGTCTTATAGG	
p <i>lacS-csa3b</i>	<i>csa577fwd-SphI</i>	CAT GCATG CTTTTCATATTTATGAAAAGAG	pMID
	<i>csa5lacSrev</i>	CCTAAAGTTTTTGGAAAAGAATACATGTTATTCTCTATTATTCCACT	
	<i>csa5lacSfwd</i>	AGTGGAAATAATAATAGAGAATAACATGTATTCTTTCCAAAAAAGCTTTAGG	
	<i>lacsrev-NotI</i>	ATTT GCGGCCG CCTAGTGCCTTAATGGTCTTATAGG	
	<i>lacS-csa3bfwd-NotI</i>	ATTT GCGGCCG CTTTGGTGAATTAGGTTGACGTAATAC	
	<i>lacS-csa3brev-NotI</i>	ATTT GCGGCCG CTTAGAACCATAGATTTTCAATAAAATCTC	
pMID- <i>csa3b</i>	<i>csa3bLfwd-NcoI</i>	AATTCATGGAGTTACAGCAAAATGGTCAA	pMID
	<i>csa3bLrev-XhoI</i>	ATCCTCGAGTATCATACCCTAAATCCGAAA	

	csa3bGfwd- <i>Sall</i>	AATT GTCGAC GTTTTGAGGAAGAGAAGGCTA	
	csa3bGrev- <i>MluI</i>	CC ACGCGT CATAAACCTGGAAAAATTAGG	
	csa3bRfwd- <i>XhoI</i>	AT CCTCGAGT CCTAATTTTCCAGGTTTAT	
	csa3bRrev- <i>SphI</i>	AATT GCATG CCACCACCTTGTTCACTCTTAC	
pUC19- <i>cas8a</i>	cas8aLfwd- <i>Sall</i>	GTAC GTCGAC CTCGGTCGGCTCTATTTTC	pUC19
	cas8aLrev	ATCTTGAAAAATTAATGGCACTTATTATAAAGTGGAACCTCCATT	
	cas8aRfwd	AAATGGAGGTTCCACTTTATAATAAGTGCCATTAATTTTCAAGAT	
	cas8aRrev- <i>PstI</i>	CCG ACTGCA GACCGGTAACCTGGATGTAAATC	
	cas8aGfwd- <i>EcoRI</i>	AAGG GAA TTCCGAGGGGTATTCTCTACAGTT	
	cas8aGrev- <i>BamHI</i>	CAT GGGATC CTTAATGGCACTATCCTTCTCC	
	lacS-pyrEFfwd- <i>BamHI</i>	CCAC GGA TCCAATCCTATCACATCAGCACC	
	lacS-pyrEFrev- <i>Sall</i>	CTGC GTCGAC TTAGTGCCTTAATGGCTTTAC	
<i>pcsA3b</i>	Ecsa3bfwd- <i>SphI</i>	CAT GCATG CTTTAGTGAATTTACTGCACGT	pEXA2
	Ecsa3brev- <i>NotI</i>	TT GCGGCCG CATAAACCTGGAAAAATTAGG	
pGE1-Array12-E233S	Earray12-cas1fwd	aagTAGTTGAAATCAGAGAGGAAGATAAGACTAATATTTTCGTC	pGE1
	Earray12-cas1rev	agcGACGAAATATTAGTCTTATCTCCTCTCTGATTCAACTA	
	Earray12fwd- <i>SphI</i>	AATT GCATG CGGAAAAGAATAGAAGATAATATTGC	
	Earray12rev	GAAAGGAAAATTGAGGATAATATACCTCCTACAACCTCC	
	Earray12fwd	AGGAGGTATATTATCCTCAATTTTCCTTCATAATCAAATT	
	Earray12rev- <i>XhoI</i>	AT CCTCGAG AGGATAGGGAAGGTAAAGTAAC	
pGE1-Array12-LAL	Earray12-cas1fwd	aagTAGTTGAAATCAGAGAGGAAGATAAGACTAATATTTTCGTC	pGE1
	Earray12-cas1rev	agcGACGAAATATTAGTCTTATCTCCTCTCTGATTCAACTA	
	Larray12fwd- <i>SphI</i>	AATT GCATG CTTAGTGCCTGTGGAGCT	
	Larray12rev	GAAAGGAAAATTGAGGTAATTTATTATTAGTTAAACGGG	
	Larray12fwd	CTAATAATAAATTACCTCAATTTTCCTTCATAATCAAATT	
	Earray12rev- <i>XhoI</i>	AT CCTCGAG AGGATAGGGAAGGTAAAGTAAC	
pGE2-Array34-LAL	Larray34	aacATAAAATTTTACTAACTTAAACTCATTAAATTTCCCTAA	pGE2
	Larray34	tacTTAGGGGAAATTAATGAGTTTAAAGTTAGTAAATTTTAT	
	Larray34fwd- <i>SphI</i>	AATT GCATG CATATGATAATTTCCAAGATTCCTC	
	Larray34rev	TTTCTATTACTTACGTTAGATTACGTTTGGTTGATTG	
	Larray34fwd	CAAACGTAAATCTAACGTAAGTAATAGAAAAATTAAGAAGC	
	Larray34rev- <i>XhoI</i>	AT CCTCGAG CAATCATCTTTCTGCTCTACC	
pGE1-Array5-LAL	Larray5-spacerfwd	aagATAGAGTACAAGCGCGTTGAAGTAGAATAAACTCAGAAAA	pGE1
	Larray5-spacerrev	agcTTTTCTGAGTTTATTCTAGTTCAACGCGCTTGACTCTAT	
	Larray5fwd- <i>SphI</i>	AATT GCATG CATAGGTTGATCTGGATATAAACG	
	Larray5rev	TCTGGAACTACCTCGTATTGGCAACGAACCTTTG	
	Larray5fwd	GGTTCGTTGCCAATACGAGGTAGTCCAGAGTTAC	
	Larray5rev- <i>XhoI</i>	AT CCTCGAG CATAATGTCTATTTCATCCAGT	
primers used for confirming gene or array deletion mutants	csa3bcheck-fwd	GGCGTCTAAAAAATTGTTGTC	
	csa3bcheck-rev	TGAACCACTTATCATTTCTTCTC	
	cas8acheck-fwd	ATAAACAGTAAGGGAAGTTCTG	

	Cas8acheck-rev	CAACCTCTTATCTGGAATGTC	
	Earray12check-fwd	GGAAAAGAATAGAAGATAATATTGC	
	Larray12check-fwd	TTAGTGCCTGTGGAGCT	
	E or L array12check-rev	CATAAACCTGGAAAAATTAGG	
	Larray34check-fwd	AACAATTAGTTGAACTGTTGAAG	
	Larray34check-rev	GAAGGATTGCGTTAACTGG	
	Larray5check-fwd	GAGACGATAAGAGTTCTTGAG	
	Larray5check-rev	GTCATTGATATTACTTAACATTCC	
pGE1-arrayCK	Earray12-cas1fwd	aagTAGTTGAAATCAGAGAGGAAGATAAGACTAATATTCGTC	
	Earray12-cas1rev	agcGACGAAATATTAGTCTTATCTCCTCTCTGATTCAACTA	
pGE1-T _{gp38} CCG	T _{gp38} spacerCCG-fwd	aagTCTGAATTATTGAATTTGTATACAAGTTATTATCAACATT	
	T _{gp38} spacerCCG-rev	agcAATGTTGATAATAACTTGTATACAAATCAATAATTCAGA	
pGE1-T _{gp38} CGT	T _{gp38} spacerCGT-fwd	aagCTGAATTATTGAATTTGTATACAAGTTATTATCAACATTT	
	T _{gp38} spacerCGT-rev	agcAAATGTTGATAATAACTTGTATACAAATCAATAATTCAG	
pGE1-C _{gp38} CCA	C _{gp38} spacerCCA-fwd	aagTGTCTAGCTAATTGTAGATGATAATTAATAATTTTGGATA	
	C _{gp38} spacerCCA-rev	agcTATCCAAAAATTATTAATTATCATCTACAATTAGCTAGACA	
pGE1-C _{gp38} CAT	C _{gp38} spacerCAT-fwd	aagGTCTAGCTAATTGTAGATGATAATTAATAATTTTGGATAT	
	C _{gp38} spacerCAT-rev	agcATATCCAAAAATTATTAATTATCATCTACAATTAGCTAGAC	
pGE1-T _{gp01} CCA	T _{gp01} spacerCCA-fwd	aagGGAAGGGTTTCACAGTTGAAAAGATAGCAAGTTTACTAGG	
	T _{gp01} spacerCCA-rev	agcCCTAGTAACTTGCTATCTTTTCAACTGTGAAACCCCTCC	
pGE1-C _{gp29} CCT	C _{gp29} spacerCCT-fwd	aagACAAACATATCTTTTATCATATCCTTTATCATGTCTATAA	
	C _{gp29} spacerCCT-rev	agcTTATAGACATGATAAAGGATATGATAAAAGATATGTTTGT	
pGE1-C _{gp49} CCC	C _{gp49} spacerCCC-fwd	aagACTAATATTCTGTTACTGCAAAAAATCCATATAGTGCTC	
	C _{gp49} spacerCCC-rev	agcGAGCACTATATGGATTTTTCAGTAACAGGAATATTAGT	

pGE1

Restriction sites are indicated in boldface. Sequences shared between primers in fusion PCR are underlined. Mutated nucleotides are in boldface and underlined.

Supplementary Table 3. Oligonucleotides used in pull-down, footprinting and qPCR assays.

	Primers	Sequences (5'-3')
Primers for pull-down assay	Biotin- <i>pcs5</i> -fwd	Biotin-GTTATTCTCTATTATTATTCCTACTATATAAACATTC
	NoBiotin- <i>pcs5</i> -rev	CTTCCGCTAATATGATAAGTAAGAC
Primers for DNaseI foot printing assay (based on pJET1.2/blunt)	Fprintingfwd	TCACTTCACCTTCACTATTCTTAAC
	Fprintingrev	CATATAGAACCTTAGTTACAGCCTC
	Fprintingfwd	TCACTTCACCTTCACTATTCTTAAC
	Rlrightrev	<u>ACTCGGGG</u> GATAAATGAAAAATTAGAACC
	Rlrightfwd	<u>TTTT</u> CATATTAT CCCC GAGTTTCGTACAC
	Fprintingrev	CATATAGAACCTTAGTTACAGCCTC
	Fprintingfwd	TCACTTCACCTTCACTATTCTTAAC
	Rllrightrev	<u>CGAAAACTCTTT</u> CATAAATATG
	Rllrightfwd	<u>TTTT</u> CATATTATGAAAAGAGTTTTCGTACACTA CCCC TAGAAATGTTTATATAG
	Fprintingrev	CATATAGAACCTTAGTTACAGCCTC
Primers for FAM labelled DNA	Fprintingfwd	TCACTTCACCTTCACTATTCTTAAC
	FAM-pJET1.2rev	FAM-CTGAGAAATATTGTAGGAGATCTTCTAG
qPCR primers	qPCR- <i>csa5</i> -fwd	AAGGAGGGTATCATAGGGAG
	qPCR- <i>csa5</i> -rev	TGAATCATAGTTGTTGTGCTC
	qPCR- <i>tfb1</i> -fwd	TTAGACCCTAAGAGAAGACTTG
	qPCR- <i>tfb1</i> -rev	ACGCTTTCTATACTCCTTCC

Sequences shared between primers in fusion PCR are underlined and mutated nucleotides are in boldface and underlined.

Supplementary Table 4. Quality control data for RNA samples (A-C) and threshold cycle (Ct) values of qPCR (A-D). (A) Data for Figure 4A. (B) Data for Figure 5. (C) Data for Supplementary Figure 7. (D) Ct values for Figure 6.

A

	RNA (No DNase treatment)		RNA (DNase treatment)		1.75 ng RNA was used for qPCR to test the contamination of DNA (<i>tfb1</i> gene)						cDNA (100X dilution)					
	C (ng/μl)	A260/A280	C (ng/μl)	A260/A280	Ct values for samples without Dnase treatment			Ct values for samples with DNase treatment			Ct values (<i>tfb1</i>)			Ct values (<i>csa5</i>)		
E233S	3625	1.94	386	2.02	16.60	16.60	16.58	N/A	N/A	N/A	22.65	22.65	22.61	21.27	21.12	21.1
Δ <i>csa3b</i>	3013	2.02	412	2.02	17.10	17.04	17.04	N/A	N/A	N/A	22.34	22.23	22.23	16.08	16.13	16.22
Δ <i>csa3b/pcs3b</i>	3123	2.01	394	2.03	17.94	18.05	18.03	N/A	N/A	N/A	21.92	21.86	21.83	19.23	19.48	19.54
Δ <i>csa5</i>	2971	2.02	396	2.01	17.23	17.24	17.42	N/A	N/A	N/A	22.33	22.21	22.22	37.4	N/A	N/A
Δ <i>cas7</i>	3540	1.96	339	2.02	16.71	16.95	17.14	N/A	N/A	N/A	23.06	23.17	23.09	16.06	16.1	16.27
Δ <i>cas5</i>	3416	1.98	397	2.02	17.17	17.15	17.38	N/A	N/A	N/A	23.19	23.14	23.1	18.48	18.45	18.72
Δ <i>cas3'</i>	3303	1.99	400	2.03	16.64	16.89	16.99	N/A	N/A	N/A	22.89	23.18	22.94	17.82	17.77	18.09
Δ <i>cas3''</i>	3143	2.01	394	2.02	17.13	17.28	17.44	N/A	N/A	N/A	22.28	22.35	22.24	16.59	16.62	16.85
Δ <i>cas8</i>	2494	2.06	378	2.03	21.01	21.08	21.06	N/A	N/A	N/A	20.01	20.00	19.77	13.82	13.83	13.63
Δ <i>cas6</i>	3325	1.99	389	2.04	16.59	16.74	16.91	N/A	N/A	N/A	22.35	22.32	22.4	16.13	16.18	16.27
Δarrays	3570	1.97	392	2.02	17.81	17.87	17.78	N/A	N/A	N/A	23.55	23.4	23.49	17.35	17.4	17.33
Δarrays/arrayCK	3400	2.00	404	2.05	17.73	17.71	17.62	N/A	N/A	N/A	22.06	21.99	21.94	18.68	18.74	18.85

B

		RNA (No DNase treatment)		RNA (DNase treatment)		1.75 ng RNA was used for qPCR to test the contamination of DNA (<i>tfb1</i> gene)						cDNA (100X dilution)					
		C (ng/μl)	A260/A280	C (ng/μl)	A260/A280	Ct values for samples without Dnase treatment			Ct values for samples with DNase treatment			Ct values (<i>tfb1</i>)			Ct values (<i>csa5</i>)		
-SIRV2	T _{gp38} CCG	2784	2.05	374	2.05	21.48	21.54	21.28	N/A	N/A	N/A	20.58	20.58	20.57	19.03	19.05	18.98
	T _{gp38} CGT	2812	2.04	372	2.04	19.98	19.98	19.97	N/A	N/A	N/A	20.12	20.10	19.87	18.35	18.31	18.13
	C _{gp38} CCA	2890	2.04	372	2.05	20.93	20.99	21.12	N/A	N/A	N/A	20.88	20.84	20.67	19.17	19.09	18.92
	C _{gp38} CAT	2876	2.05	377	2.06	20.60	20.86	21.08	N/A	35.27	N/A	20.29	20.32	20.26	18.32	18.56	18.26
	arrayCK	3196	2.03	399	2.03	25.21	25.28	25.41	N/A	N/A	N/A	19.29	19.33	19.27	17.52	17.58	17.56
	LAL549	2744	2.05	365	2.05	19.81	19.99	20.18	N/A	N/A	N/A	20.34	20.32	20.24	19.72	19.83	19.72
+SIRV2	T _{gp38} CCG	2725	2.05	370	2.05	20.33	20.36	20.29	N/A	N/A	N/A	19.69	19.77	19.63	16.26	16.16	16.09
	T _{gp38} CGT	2914	2.04	379	2.05	20.57	20.53	20.63	N/A	N/A	N/A	19.60	19.69	19.51	17.74	17.83	17.60
	C _{gp38} CCA	2783	2.06	379	2.04	22.03	22.08	22.22	N/A	N/A	N/A	19.42	19.42	19.32	15.30	15.23	15.10
	C _{gp38} CAT	2835	2.04	374	2.05	20.76	20.86	21.07	N/A	N/A	N/A	19.09	19.03	18.93	16.31	16.29	16.22
	arrayCK	3027	2.04	388	2.05	22.14	22.11	22.28	N/A	N/A	N/A	18.77	18.94	18.77	16.77	16.93	16.88
	LAL549	2574	2.06	368	2.05	21.33	21.50	21.68	N/A	N/A	N/A	19.30	19.41	19.33	15.10	15.16	15.09

C

		RNA (No DNase treatment)		RNA (DNase treatment)		1.75 ng RNA was used for qPCR to test the contamination of DNA (<i>tfb1</i> gene)						cDNA (100X dilution)					
		C (ng/μl)	A260/A280	C (ng/μl)	A260/A280	Ct values for samples without Dnase treatment			Ct values for samples with DNase treatment			Ct values (<i>tfb1</i>)			Ct values (<i>csa5</i>)		
-SIRV2	LAL549	3274	1.99	377	2.04	16.62	16.67	16.89	N/A	N/A	N/A	20.08	20.18	20.23	19.11	19.20	19.25
	T _{gp01} CCA	3477	1.99	387	2.04	23.97	23.98	24.06	N/A	N/A	N/A	20.49	20.57	20.38	18.76	18.87	18.84
	C _{gp29} CCT	3662	1.95	416	2.02	18.36	18.55	18.67	N/A	N/A	N/A	19.51	19.59	19.69	17.81	17.88	18.08
	C _{gp49} CCC	3524	1.98	392	2.06	17.76	17.88	18.17	N/A	N/A	N/A	19.62	19.83	19.73	17.98	18.23	18.28
	T _{gp01} CCA	3134	2.00	373	2.01	17.64	17.82	18.00	N/A	N/A	N/A	19.68	19.84	19.87	15.29	15.51	15.40
+SIRV2	C _{gp29} CCT	3429	1.98	389	2.03	17.42	17.56	17.78	N/A	N/A	N/A	19.34	19.50	19.63	14.63	14.79	14.85
	C _{gp49} CCC	2934	2.02	377	2.04	17.75	17.91	18.09	N/A	N/A	N/A	19.15	19.24	19.35	14.67	14.77	14.95

D

ChIP	Ct values (<i>tfb1</i>)			Ct values (<i>csa5</i>)		
E233S+Csa5 Ab	25.28	24.73	24.65	22.30	22.22	22.30
E233S-Csa5 Ab	28.14	28.53	28.07	28.34	28.39	28.37

$\Delta csa3b$ +Csa5 Ab	23.10	22.72	22.84	22.78	22.75	22.76
$\Delta csa3b$ -Csa5 Ab	28.60	28.74	28.63	28.16	28.16	28.08
$\Delta csa3b/pcsa3b$ +Csa5 Ab	24.54	24.55	24.45	21.20	21.18	21.19
$\Delta csa3b/pcsa3b$ -Csa5 Ab	28.18	28.25	28.44	28.52	28.21	28.99
$\Delta arrays$ +Csa5 Ab	24.22	24.15	24.09	23.42	23.38	23.70
$\Delta arrays$ -Csa5 Ab	27.89	28.36	28.06	29.01	29.25	28.92
$\Delta arrays/arrayCK$ +Csa5 Ab	24.07	24.04	24.11	22.02	22.23	22.45
$\Delta arrays/arrayCK$ -Csa5 Ab	28.64	28.24	28.42	28.93	28.97	29.36
$\Delta cas7$ +Csa5 Ab	23.15	23.03	23.05	22.87	23.07	23.30
$\Delta cas7$ -Csa5 Ab	27.58	28.02	27.65	27.24	27.74	28.03
$\Delta cas8$ +Csa5 Ab	25.13	25.09	25.20	24.05	24.33	24.55
$\Delta cas8$ -Csa5 Ab	28.93	28.88	29.38	29.57	29.08	29.31
NTC	N/A	N/A	-	N/A	N/A	-

Supplementary Table 5A. Ratio of *cas* transcripts between individual knockout mutant and the mother strain E233S. '-' indicates low or undetectable transcription.

<i>cas</i> gene	Ratio of transcript between <i>cas</i> gene knockout mutants and E233S								
	$\Delta csa3b$	$\Delta csa3b/pcs a3b$	$\Delta csa5$	$\Delta cas7$	$\Delta cas5$	$\Delta cas3'$	$\Delta cas3''$	$\Delta cas6$	$\Delta cmrA cmrB$
<i>csa3a</i>	0.84	0.71	2.28	3.36	1.3	3.32	2.32	1.28	2.68
<i>cas4</i>	1.37	1.63	0.67	2.33	1.13	0.93	0.93	0.84	1.73
<i>cas2</i>	8	3	0.5	1.5	-	0.5	1	5	-
<i>cas1</i>	2.13	2	0.83	0.95	0.46	0.48	0.56	0.9	1.22
<i>csa1</i>	2.96	1.41	1.08	1.67	0.94	1.56	1.14	2.07	1.06
<i>csa3b</i>	-	1.33	0.66	1.59	0.24	1.36	2.28	0.75	1.42
<i>csa5</i>	40.17	2.44	-	44.78	16.5	6.38	13.59	22.37	1.08
<i>cas7</i>	38.33	2.38	9.01	-	3.94	6	9.85	21.58	0.79
<i>cas5</i>	23.95	2.35	1.68	23.81	-	2.03	6.31	14.27	0.66
<i>cas3'</i>	24.51	2.7	2.78	14.54	2.02	-	8.22	13.75	1.5
<i>cas3''</i>	14.26	1.93	1.02	7.68	0.25	0.7	-	8.59	0.83
<i>cas8a1</i>	14.89	1.66	1.99	12.62	0.26	0.15	3.12	8.92	1.08
<i>cas6</i>	15.9	2.06	2.38	12.63	0.26	0.2	1.3	1.68	0.98

Supplementary Table 6. *csa3* identified from Refseq database and the adjacent genes in corresponding genomes. Int., CRISPR-Cas interference gene cassette; acq., CRISPR-Cas acquisition gene cassette; both, both interference and acquisition gene cassettes. -, non-CRISPR genes.

Organism	Csa3 gene ID	adjacent gene IDs	Associated CRISPR-cas
<i>Sulfolobus islandicus</i> M.16.43	M1643_RS0104800	M1643_RS0104805	type I-A Int
<i>Sulfolobus islandicus</i> M.16.47	M1647_RS0104940	M1647_RS0104945	type I-A Int
<i>Sulfolobus islandicus</i> M.16.23	M1623_RS0104905	M1623_RS0104910	type I-A Int
<i>Sulfolobus islandicus</i> M.16.27	M1627_0965	M1627_0966	type I-A Int
<i>Sulfolobus islandicus</i> M.14.25	M1425_0899	M1425_0900	type I-A Int
<i>Sulfolobus islandicus</i> M.16.4	M164_0908	M164_0909	type I-A Int
<i>Sulfolobus islandicus</i> M.16.2	M162_RS0104985	M162_RS0104990	type I-A Int
<i>Sulfolobus islandicus</i> L.S.2.15	LS215_0725	LS215_0726	type I-A Int
<i>Sulfolobus islandicus</i> Y.G.57.14	YG5714_0761	YG5714_0762	type I-A Int
<i>Sulfolobus islandicus</i> Y.N.15.51	YN1551_2117	YN1551_2116	type I-A Int
<i>Sulfolobus islandicus</i> HVE10/4	SiH_0408	SiH_0407	type I-A Int
<i>Sulfolobus islandicus</i> Rey15A	SiRe_0765	SiRe_0766	type I-A Int
<i>Sulfolobus islandicus</i> LAL14/1	SiL_0392	SiL_0391	type I-A Int
<i>Sulfolobus solfataricus</i> P2	SSO1444	SSO1443	type I-A Int
<i>Sulfolobus tokodaii</i> str. 7	ST1161	transposon	-
<i>Acidianus hospitalis</i> W1	Ahos_1747	Ahos_1746	type I-A Int
<i>Sulfolobus islandicus</i> M.16.40	M1640_RS0104845	M1640_RS0104850	type I-A Int
<i>Acidianus hospitalis</i> W1	Ahos_1504	-	-
<i>Metallosphaera cuprina</i> Ar-4	Mcup_1149	Mcup_1150	type I-A Int
<i>Metallosphaera sedula</i> strain SARC-M1	MsedE_RS05810	MsedE_RS05805	type I-A Int
<i>Metallosphaera sedula</i> strain ARS120-2	MsedD_RS05790	MsedD_RS05785	type I-A Int
<i>Metallosphaera sedula</i> strain ARS120-1	MsedC_RS05790	MsedC_RS05785	type I-A Int
<i>Metallosphaera sedula</i> strain ARS50-2	MsedB_RS05795	MsedB_RS05790	type I-A Int
<i>Metallosphaera sedula</i> DSM 5348	Msed_1145	MSED_RS05800	type I-A Int
<i>Metallosphaera sedula</i> strain ARS50-1	MsedA_RS05785	MsedA_1160	type I-A Int
<i>Methanocaldococcus fervens</i> AG86	Mefer_1431	Mefer_1430 (cas2) and Mefer_1432 (Cas3")	type I-A both
<i>Methanocaldococcus</i> sp. FS406-22	MFS40622_1179	MFS40622_1180 (cas2) and MFS40622_1178 (Cas3")	type I-A both
<i>Pyrococcus yayanosii</i> CH1	PYCH_09670	PYCH_09660 (Cas3") and PYCH_09680 (cas6)	type I-A int
<i>Pyrococcus</i> sp. ST04	Py04_0800	Py04_0801 (Cas3")	type I-A Int
<i>Thermococcus eurythermalis</i> strain A501	TEU_06715	TEU_06710 (Cas3") and TEU_06720 (Cas4)	type I-A both
<i>Pyrococcus horikoshii</i> OT3	PH0915	PH0916 (Cas3")	type I-A Int
<i>Thermococcus kodakarensis</i> KOD1	TK0448	TK0447 (Cas6) and TK0449 (Cas3")	type I-A Int
<i>Thermococcus litoralis</i> DSM 5473	OCC_00707	OCC_00712 (Cas6) and OCC_00702 (Cas3")	type I-A Int
<i>Thermococcus</i> sp. 2319x1	ADU37_CDS13340	ADU37_CDS13320 (Cas3) and ADU37_CDS13350 (Cas2)	type I-A both
<i>Ferroglobus placidus</i> DSM 10642	Ferp_1868	Ferp_1867 (Cas8") and Ferp_1869 (Cas6)	type I-A Int
<i>Candidatus Korarchaeum cryptofilum</i> OPF8	Kcr_0427	Kcr_0426 (Cas4) and Kcr_0428 (Cas8")	type I-A both
<i>Archaeoglobus fulgidus</i> DSM 8774	AFULGI_RS10255	AFULGI_RS10260 (Csa3)	type I-A Int
<i>Archaeoglobus fulgidus</i> DSM 4304	AF1869	AF_1868 (Cmr1) and AF_1870 (Cas8")	Type III-B and type I-A Int and continuous I-A adaptation
<i>Archaeoglobus fulgidus</i> DSM 8774	AFULGI_RS10260	AFULGI_RS10255 (Csa3) and AFULGI_RS10265 (Csa5)	type I-A Int
<i>Thermosphaera aggregans</i> DSM 11486	Tagg_0805	Tagg_0804 (Cas8") and Tagg_0806 (Csa3)	type I-A Int
<i>Thermogladius cellulolyticus</i> 1633	TCELL_1020	TCELL_1019 (Cas8") and TCELL_1039 (Csx1p)	type I-A Int
<i>Thermoproteus tenax</i> Kra 1	TTX_1249	TTX_1248 (Cas4') and TTX_1250 (Cas8")	type I-A both

<i>Thermofilum pendens</i> Hrk 5	Tpen_1358	Tpen_1357 (Cas8'') and Tpen_1359 (Cas6)	type I-A Int
<i>Staphylothermus marinus</i> F1	Smar_0319	Smar_0318 (Cas6) and Smar_0320 (Cas8'')	type I-A Int
<i>Thermofilum</i> sp. 1910b	N186_04215	N186_04210 (tRNA) and N186_04220 (hypothetical protein)	-
<i>Thermofilum carboxyditrophus</i> 1505	TCARB_1870	TCARB_2006 (tRNA-Ser) and TCARB_1871 (hypothetical protein)	-
<i>Thermofilum</i> sp. 1910b	N186_03645	N186_03640 (Cas4) and N186_03650 (Csa5)	type I-A both
<i>Sulfolobus tokodaii</i> str. 7	ST2632	ST2633 (Cas4')	type I-A Acq
<i>Desulfurococcus kamchatkensis</i> 1221n	DKAM_0784	DKAM_0783 (Cas4')	type I-A Acq
<i>Desulfurococcus fermentans</i> DSM 16532	Desfe_1011	Desfe_1012 (Cas4')	type I-A both
<i>Thermosphaera aggregans</i> DSM 11486	Tagg_0806	Tagg_0805 (Csa3) and Tagg_0807 (Cas4)	type I-A Acq
<i>Sulfolobus islandicus</i> M.16.27	M1627_0964	M1627_0963 (Cas4)	type I-A Acq
<i>Sulfolobus islandicus</i> M.16.23	M1623_RS0104900	M1623_RS0104895 (Cas4)	type I-A Acq
<i>Sulfolobus islandicus</i> M.16.43	M1643_RS0104795	M1643_RS0104790 (Cas4)	type I-A Acq
<i>Sulfolobus islandicus</i> M.16.47	M1647_RS0104935	M1647_RS0104930 (Cas4)	type I-A Acq
<i>Sulfolobus islandicus</i> M.16.2	M162_RS0104980	M162_RS0104975 (Cas4)	type I-A Acq
<i>Sulfolobus islandicus</i> M.14.25	M1425_0894	M1425_0895 (Cas4)	type I-A Acq
<i>Sulfolobus islandicus</i> M.16.40	M1640_RS0104820	M1640_RS0104825 (Cas4)	type I-A Acq
<i>Sulfolobus solfataricus</i> P2	SSO1445	SSO1449 (Cas4)	type I-A Acq
<i>Metalllosphaera sedula</i> strain ARS120-2	MsedD_1168	MsedD_1167 (Cas4)	type I-A Acq
<i>Metalllosphaera sedula</i> strain SARC-M1	MsedE_1170	MsedE_1169 Cas4	type I-A Acq
<i>Metalllosphaera sedula</i> strain ARS120-1	MsedC_1167	MsedC_1166 (Cas4)	type I-A Acq
<i>Metalllosphaera sedula</i> strain ARS50-2	MsedB_1169	MsedB_1168 (Cas4)	type I-A Acq
<i>Metalllosphaera sedula</i> DSM 5348	Msed_1151	Msed_1150 (Cas4)	type I-A Acq
<i>Metalllosphaera sedula</i> strain ARS50-1	MsedA_1167	MsedA_1166 (Cas4)	type I-A Acq
<i>Sulfolobus islandicus</i> Y.N.15.51	YN1551_2118	YN1551_2119 (Cas4)	type I-A Acq
<i>Sulfolobus islandicus</i> L.S.2.15	LS215_0724	LS215_0723 (Cas4)	type I-A Acq
<i>Sulfolobus islandicus</i> Y.G.57.14	YG5714_0754	YG5714_0755 (Cas4)	type I-A Acq
<i>Acidianus hospitalis</i> W1	Ahos_1752	Ahos_1751 (Cas4)	type I-A Acq
<i>Sulfolobus islandicus</i> HVE10/4	SiH_0413	SiH_0412 (Cas4)	type I-A Acq
<i>Sulfolobus islandicus</i> Rey15A	SiRe_0764	SiRe_0763 (Cas4)	type I-A Acq
<i>Sulfolobus islandicus</i> LAL14/1	SiL_0393	SiL_0394 (Cas4)	type I-A Acq
<i>Desulfurococcus mucosus</i> DSM 2162	Desmu_0999	Desmu_0998 (Cas3'') and Desmu_1000 (Cas6)	type I-A Int
<i>Sulfolobus islandicus</i> Y.G.57.14	YG5714_0422	YG5714_0421 (ATPase) and YG5714_0423 (6-phosphogluconate dehydrogenase)	-
<i>Sulfolobus islandicus</i> Y.N.15.51	YN1551_2594	YN1551_2593 (6-phosphogluconate dehydrogenase) and YN1551_2597 (ATPase)	-
<i>Sulfolobus islandicus</i> L.S.2.15	LS215_0461	LS215_0459 (ATPase) and LS215_0462 (6-phosphogluconate dehydrogenase NAD-binding)	-
<i>Sulfolobus islandicus</i> LAL14/1	SiL_0502	SiL_0501 (6-phosphogluconate dehydrogenase) and SiL_0503 (hypothetical protein)	-
<i>Sulfolobus islandicus</i> HVE10/4	SiH_0869	SiH_0868 (hypothetical protein) and SiH_0870 (transposase)	-
<i>Sulfolobus islandicus</i> Rey15A	SiRe_0473	SiRe_0471 (6-phosphogluconate dehydrogenase NAD-binding) and SiRe_0474 (hypothetical protein)	-
<i>Sulfolobus islandicus</i> M.16.4	M164_0487	M164_0486 (6-phosphogluconate dehydrogenase) and M164_0488 (ATPase)	-
<i>Sulfolobus islandicus</i> M.16.27	M1627_0462	M1627_0461 (6-phosphogluconate dehydrogenase) and M1627_0463 (hypothetical protein)	-
<i>Sulfolobus islandicus</i> M.16.23	M1623_RS0102400	M1623_RS0102395 (6-phosphogluconate dehydrogenase) and M1623_RS14705 (hypothetical protein)	-
<i>Sulfolobus islandicus</i> M.16.40	M1640_RS0102780	M1640_RS0102775 (6-phosphogluconate dehydrogenase) and M1640_RS15285 (hypothetical protein)	-

<i>Sulfolobus islandicus</i> M.16.43	M1643_RS0102470	M1643_RS0102465 (6-phosphogluconate dehydrogenase) and M1643_RS0102485 (ATPase)	-
<i>Sulfolobus islandicus</i> M.16.47	M1647_RS0102430	M1647_RS0102425 (6-phosphogluconate dehydrogenase) and M1647_RS15095 (hypothetical protein)	-
<i>Sulfolobus islandicus</i> M.14.25	M1425_0449	M1425_0448 (6-phosphogluconate dehydrogenase) and M1425_0450 (hypothetical protein)	-
<i>Sulfolobus islandicus</i> M.16.2	M162_RS0102920	M162_RS14985 (hypothetical protein) and M162_RS0102925 (6-phosphogluconate dehydrogenase)	-
<i>Sulfolobus tokodaii</i> str. 7	ST1584	ST1583 (multidrug ABC transporter ATP-binding protein) and ST1585 (MBL fold metallo-hydrolase)	-
<i>Sulfolobus acidocaldarius</i> N8	SacN8_09195	SacN8_09190 (Cas8) and SacN8_09200 (Cas6) and continuous adaptation gene	Type I-D int
<i>Sulfolobus acidocaldarius</i> DSM 639	Saci_1876	Saci_1875 (Cas8) and Saci_1877 (Cas6) and continuous adaptation gene	Type I-D int
<i>Sulfolobus acidocaldarius</i> Ron12/I	SacRon12I_09220	SacRon12I_09215 (Cas8) and SacRon12I_09225 (Cas6) and continuous adaptation gene	Type I-D int
<i>Sulfolobus islandicus</i> HVE10/4	SiH_0760	SiH_0759 (Cas8) and SiH_0761 (Cas6) and continuous adaptation gene	Type I-D int
<i>Sulfolobus islandicus</i> LAL14/1	SiL_0610	SiL_0609 (Cas8) and SiL_0611 (Cas6) and continuous adaptation gene	Type I-D int
<i>Caldivirga maquilingensis</i> IC-167	Cmaq_1520	Cmaq_1519 (cmr3) and Cmaq_1521 (Cas8'')	Type III-B int and I-A int
<i>Desulfurococcus fermentans</i> DSM 16532	Desfe_0429	Desfe_0430 (Cas8'')	Type I-A int
<i>Halophilic archaeon</i> DL31	Halar_0923	Halar_0922 (Cas6) continuous adaptation gene	Type I-D int
<i>Haloquadratum walsbyi</i> C23	Hqrw_1370	Hqrw_1371 (Cas6) continuous adaptation gene	Type I-D int
<i>Haloarcula</i> sp. CBA1115	SG26_14230	SG26_14235 (Cas6) continuous adaptation gene	Type I-D int
<i>Methanospirillum hungatei</i> JF-1	Mhun_1866	Mhun_1865 (Cas6) continuous adaptation gene	Type I-D int
<i>Methanosarcina mazei</i> S-6	MSMAS_0745	MSMAS_0746 (Cas6)	Type I-D int
<i>Thermofilum</i> sp. 1807-2	MA03_02915	MA03_02905 (Cas7)	Type I-C int
<i>Methanocaldococcus vulcanius</i> M7	Metvu_0008	Metvu_0007 (Cas8) continuous adaptation gene and Metvu_0009 (Cas6)	Type I-D int
<i>Methanocaldococcus bathoardescens</i> strain JH146	JH146_0427	JH146_0426 (Cas6) and JH146_0428 (Cas10d) continuous adaptation gene	Type I-D int
<i>Methanoterris igneus</i> Kol 5	Metig_0208	Metig_0209 (Cas10d) continuous adaptation gene	Type I-D int
<i>Methanothermococcus okinawensis</i> IH1	Metok_0801	Metok_0800 (purS) and METOK_RS04090 (hypothetical protein)	-
<i>Pyrococcus</i> sp. NA2	PNA2_1822	PNA2_1821 (Csa5) continuous adaptation gene and PNA2_1824 (Cas3)	I-A int and I-B int
<i>Pyrococcus furiosus</i> DSM 3638	PF0644	PF0643 (Csa5)	Type I-A int
<i>Pyrococcus furiosus</i> COM1	PFC_02355	PFC_02350 (Csa5)	Type I-A int
<i>Archaeoglobus fulgidus</i> DSM 8774	AFULGI_00006260	AFULGI_00006250 (Cas4) AFULGI_00006270 (Csm1)	Type I-A Acq and Type III-A int
<i>Methanothermus fervidus</i> DSM 2088	Mfer_0706	Mfer_0705 (N-ethylmeline chlorohydrolase) and Mfer_0707 (glycosidase ph1107-related protein)	-
<i>Methanococcus vannielii</i> SB	Mevan_0084	Mevan_0083 (hypothetical protein) and Mevan_0085 (ATP-dependent DNA helicase)	-
<i>Methanothermobacter thermautotrophicus</i> str. Delta H	MTH517	MTH516 (hypothetical protein) and MTH518 (hypothetical protein)	-
<i>Methanothermobacter</i> sp. CaT2	MTCT_0444	MTCT_0443 (hypothetical protein) and MTCT_0445 (hypothetical protein)	-
<i>Methanothermobacter marburgensis</i> str. Marburg	MTBMA_c09060	MTBMA_c09050 (hypothetical protein) and MTBMA_c09070 (hypothetical protein)	-
<i>Methanobacterium lacus</i> strain AL-21	Metbo_0746	Metbo_0745 (hypothetical protein) and Metbo_0747 (response regulator receiver protein)	-
<i>Methanobacterium paludis</i> strain SWAN1	MSWAN_1498	MSWAN_1497 (response regulator receiver protein) and MSWAN_1499 (hypothetical protein)	-
<i>Methanobacterium</i> sp. MB1	MBMB1_0982	MBMB1_0981 (hypothetical protein) and MBMB1_0983 (hypothetical protein)	-
<i>Methanobacterium formicicum</i> genome assembly DSM1535	DSM1535_1801	DSM1535_1800 (hypothetical protein) and DSM1535_1802 (hypothetical protein)	-
<i>Methanobacterium formicicum</i> strain BRM9	BRM9_1145	BRM9_1144 (histidine kinase/GAF/PAS domain-containing protein) and BRM9_1146 (hypothetical protein)	-

<i>Methanobacterium formicicum</i> genome assembly isolate Mb9	MB9_1195	MB9_1194 (hypothetical protein) and MB9_1196 (hypothetical protein)	-
<i>Methanocella conradii</i> HZ254	Mtc_1181	Mtc_1180 (Cas2)	Acquisition
<i>Methanosalsum zhilinae</i> DSM 4017	Mzhil_1371	Mzhil_1370 (amidophosphoribosyltransferase) and Mzhil_1372 (thiazole biosynthesis enzyme)	-
<i>Methanobacterium psychrophilum</i> R15	Mpsy_0158	Mpsy_0157 (DNA helicase UvrD) and MPSY_RS00755 (hypothetical protein)	-
<i>Methanohalobium evestigatum</i> Z-7303	Metev_0357	Metev_0356 (Nuclease) and Metev_0359 (hypothetical protein)	-
<i>Methanosarcina</i> sp. MTP4	MSMTP_2835	MSMTP_2834 (Cas8) continuous adaptation gene	Type I-B int
<i>Methanosarcina</i> sp. MTP4	MSMTP_2116	MSMTP_2115 (Ribonuclease III) and MSMTP_2117 (Cas8) continuous adaptation gene	Type I-B int
<i>Methanosarcina lacustris</i> Z-7289	MSLAZ_2286	MSLAZ_2287 (Cas8b) continuous adaptation gene	Type I-B int
<i>Methanosarcina</i> sp. WWM596	MSWHS_2887	MSWHS_2888 (Cas3'') continuous adaptation gene	Type I-E int
<i>Methanosarcina mazei</i> LYC	MSMAL_0679	MSMAL_0677 (Cas7)	Type I-C int
<i>Methanosarcina mazei</i> C16	MSMAC_0733	MSMAC_0734 (Cas8b) continuous adaptation gene	Type I-B int
<i>Methanosarcina mazei</i> Go1	MM_0565	MM_0564 (Cas8b) continuous adaptation gene	Type I-B int
<i>Methanosarcina mazei</i> SarPi	MSMAP_0708	MSMAP_0709 (Cas8b) continuous adaptation gene	Type I-B int
<i>Methanosarcina mazei</i> WWM610	MSMAW_0738	MSMAW_0741 (Cas7) and continuous adaptation gene	Type I-C int
<i>Methanosarcina horonobensis</i> HB-1	MSHOH_0845	MSHOH_0846 (Cas8b) continuous adaptation gene	Type I-B int
<i>Methanosarcina vacuolata</i> Z-761	MSVAZ_0614	MSVAZ_0613 (Cas8b) continuous adaptation gene	Type I-B int
<i>Methanosarcina</i> sp. Kolksee	MSKOL_0595	MSKOL_0594 (Cas8b) continuous adaptation gene	Type I-B int
<i>Methanosarcina barkeri</i> CM1	MCM1_1893	MCM1_1894 (Cas3'') continuous adaptation gene	Type I-E int
<i>Methanosarcina barkeri</i> 227	MSBR2_1621	MSBR2_1622 (Cas3'') continuous adaptation gene	Type I-E int
<i>Methanosarcina barkeri</i> MS	MSBRM_0755	MSBRM_0754 (Cas3'') continuous adaptation gene	Type I-E int
<i>Methanosarcina barkeri</i> MS	MSBRM_2651	MSBRM_2650 (Cas8b) continuous adaptation gene	Type I-B int
<i>Methanosarcina barkeri</i> CM1	MCM1_1778	MCM1_1777 (Cas8b)	Type I-B int
<i>Methanosarcina barkeri</i> 227	MSBR2_2610	MSBR2_2609 continuous adaptation gene	Type I-B int

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