Supplemental materials

Supplemental figures

Fig. S1. Number of ArgD$^+$ colonies that harbored a donor marker at the given position. Two continuous donor SNPs were considered as one donor marker. For example, two donor markers at the position nt 14 and 15 were defined as one donor marker, then we used nt 14.5 to represent its nucleotide position for plotting. The 380-bp donor DNA corresponding to the position [256-261] in the recipient was defined as 'selectable donor marker', and was represented at nt 258 (dashed line).

Fig, S2. Schematic of *lacS* gene deletion via the one-step, microhomology-mediated gene inactivation system. The non-replicative plasmid pRJW10 was used as a template for PCR amplification to generate *lacS* gene deletion cassettes, in which the *StoargD* marker was flanked by different size of homology (0-40 bp) to the *lacS* gene. The resulting PCR products were purified and highly concentrated, and amount of 1.5 μg DNA was elctroporated into the genetic host RJW008, selecting agmatine prototrophic colonies on agmatine-free plates. The colonies were stained with 2 mg/ml X-gal solution to confirm inactivation of the *lacS* gene.

Fig. S3. PCR analysis of ArgD$^+$ transformants derived from TA5 knockout experiments. **(**A). Genomic context of TA5 module (*M.164_0621*/*M164_0622*) in the genetic host RJW008. *M.164_0621* is a putative antitoxin-encoding gene and *M164_0622* is a putative toxin-encoding gene.

(B-D). Ten individual ArgD$^+$ transformants were analyzed by PCR analysis using three primer sets, i.e. TA5-FlankP-F/R, UR-F/R and *StoargD*-F1/R1 to examine the TA5 loci (B), *argD* locus (C) and existence of *StoargD* marker (D) in the chromosome, respectively. The genomic DNA of genetic host RJW008 or/and wild-type *S. islandicus* M.16.4 was used as a control. L: GeneRuler Express DNA Ladder (Thermo Fisher Scientific, USA).

Fig. S4. Validation of the one-step, microhomology-mediated gene inactivation system in *S. islandicus* REY15A by deleting *upsAB* genes in the *ups* (UV-inducible pilus of Sulfolobales) operon.

(A). A graphic representation of the *ups* gene cluster in the genome of *S. islandicus* E235 (derived from the original isolate *S. islandicus* REY15A) before and after gene disruption.

(B). Transformation of *S. islandicus* E235 with the *upsAB* gene deletion cassette. Transformation with a replicative plasmid pSeSd-StoargD was used a positive control whereas transformation without DNA added was used a negative control. A total of 1.5 μg of a replicative plasmid or an *upsAB* gene deletion cassette was electroporated into competent cells (50 μl) respectively. Following electroporation, 800 μl of incubation buffer was added and then incubated for 1 h at 76-78°C without shaking. Transformed-cells were plated onto DYU plates lacking agmatine and incubated at 76-78°C for 8-12 days.

(C). PCR verification of *upsAB* deletion mutant. The approximate positions of primer sets *upsAB*-flankP-F/R used to confirm the deletion of *upsAB* were showed in (A), and the expected lengths of DNA bands amplified from the genetic host and *upsAB* mutant were also indicated. L: GeneRuler Express DNA Ladder (Thermo Fisher Scientific, USA).

Fig. S5. Phase contrast microscopy analysis of *upsAB* deletion mutant. Cells with (+UV; 75 J/m^2) or without UV treatment (-UV) were re-cultivated for 6 hours, and then observed with a phase contrast microscope. The genetic host *S. islandicus* E235 was used as positive control. Scale bars correspond to10 μm.

Supplemental tables

Table S1. Nucleotide sequence variations between the donor DNA (*SsoargD)* and the regions surrounding *argD* mutant allele in chromosome of recipient strain RJW008

			No. of bp[b]	
Given nt. position in *argD* deletion locus of the recipient	Recipient	Donor	Before	After
14	G	T	13	0
15	C	G	0	2
18	A	G	2	4
23	T	C	4	16
40	G	A	16	0
41	T	C	0	3
45	C	T	3	20
66	A	G	20	2
69	A	T	2	2
72	T	G	2	26
99	C	T	26	6
106	G	C	6	10
117	G	A	10	10
128	T	C	10	21
150	G	C	21	4
155	T	A	4	54
210	G	A	54	29
240	A	G	29	12
253	A	G	12	2
[256-261][a]	GTACC+Del	380	2	2
263	T	C	2	15
[278-279]	2 gaps	CT	15	4
283	T	G	4	28
312	T	G	28	11
324	A	1 gap	11	4
[328-329]	1 gap	T	4	0
329	C	A	0	31
361	T	C	31	8
370	T	A	8	5
376	A	G	5	2

a The introduced KpnI site (GGTACC) in RJW008 chromosome has a match (underlined sequence) with that from donor DNA in position nt 255.

b Number of base pairs between the indicated marker and the previous or subsequent marker.

Table S2. Predicted VapBC toxin-antitoxin (TA) loci in *S. islandicus* M.16.4

Given name	Locus tag*	Start position	Stop position

TA1	M164_0369 (T)	317841	318194
	M164_0370 (A)	318191	318418
TA2	M164_0407 (T)	345045	345440
	M164_0408 (A)	345430	345672
TA3	M164_0437 (T)	373641	374003
	M164_0438 (A)	374026	374247
TA4	M164_0609 (T)	549270	549683
	M164_0610 (A)	549655	549867
TA5	M164_0621 (A)	557633	557887
	M164_0622 (T)	557853	558227
TA6	M164_0721 (A)	667263	667499
	M164_0722 (T)	667496	667915
TA7	M164_0828 (A)	770694	770924
	M164_0829 (T)	770917	771303
TA8	M164_0872 (A)	821356	821580
	M164_0873 (T)	821570	821962
TA9	M164_0918 (T)	875867	876265
	M164_0919 (A)	876243	876479
TA10	M164_0961 (T)	909461	909871
	M164_0962 (A)	909868	910113
TA11	M164_0965 (A)	912372	912599
	M164_0966 (T)	912586	912975
TA12	M164_1023 (A)	962567	962803
	M164_1024 (T)	962790	963206
TA13	M164_1221 (A)	1159805	1160047
	M164_1222 (T)	1160044	1160442
TA14	M164_1587 (A)	1467837	1468076
	M164_1588 (T)	1468066	1468494
TA15	M164_2192 (T)	2011729	2012205
	M164_2193 (A)	2012168	2012467
TA16	M164_2194 (A)	2012842	2013099
	M164_2195 (T)	2013087	2013500
TA17	M164_2232 (A)	2044555	2044782
	M164_2233 (T)	2044742	2045176
TA18	M164_2286 (A)	2093409	2093603
	M164_2287 (T)	2093584	2093988
TA19	M164_2326 (A)	2144973	2145266
	M164_2327 (T)	2145220	2145630
TA20	M164_2407 (T)	2231329	2231766
	M164_2408 (A)	2231751	2231984
TA21	M164_2522 (T)	2340231	2340467
	M164_2523 (A)	2340630	2340815
TA22	M164_2714 (T)	2522335	2522721
	M164_2715 (A)	2522702	2522941

*** T denotes toxin and A denotes antitoxin.**

Table S3. Predicted length of PCR products amplified from the genetic host (WT) and TA knockout (KO) strains using the primers located outside of TA loci

TA loci	Length (bp)	Disruption part (bp)	Replacement part (bp)	Amplicon from WT (bp)	Amplicon from KO (bp)
TA1	578	535	740	851	1056
TA2	628	550	740	762	952
TA3	607	493	740	703	950
TA4	598	566	740	962	1136
TA5	595	514	740	714	940
TA6	653	563	740	744	921
TA7	610	538	740	835	1037
TA8	607	535	740	793	998
TA9	613	520	740	846	1066
TA10	653	590	740	684	834
TA11	604	514	740	814	1040
TA12	640	563	740	829	1006
TA13	638	596	740	769	913
TA14	658	614	740	825	951
TA15	739	622	740	746	864
TA16	659	604	740	882	1018
TA17	622	581	740	797	956
TA18	580	518	740	714	936
TA19	658	588	740	911	1063
TA20	656	591	740	818	967
TA21	585	563	740	810	987
TA22	607	542	740	792	990

Figure .S1

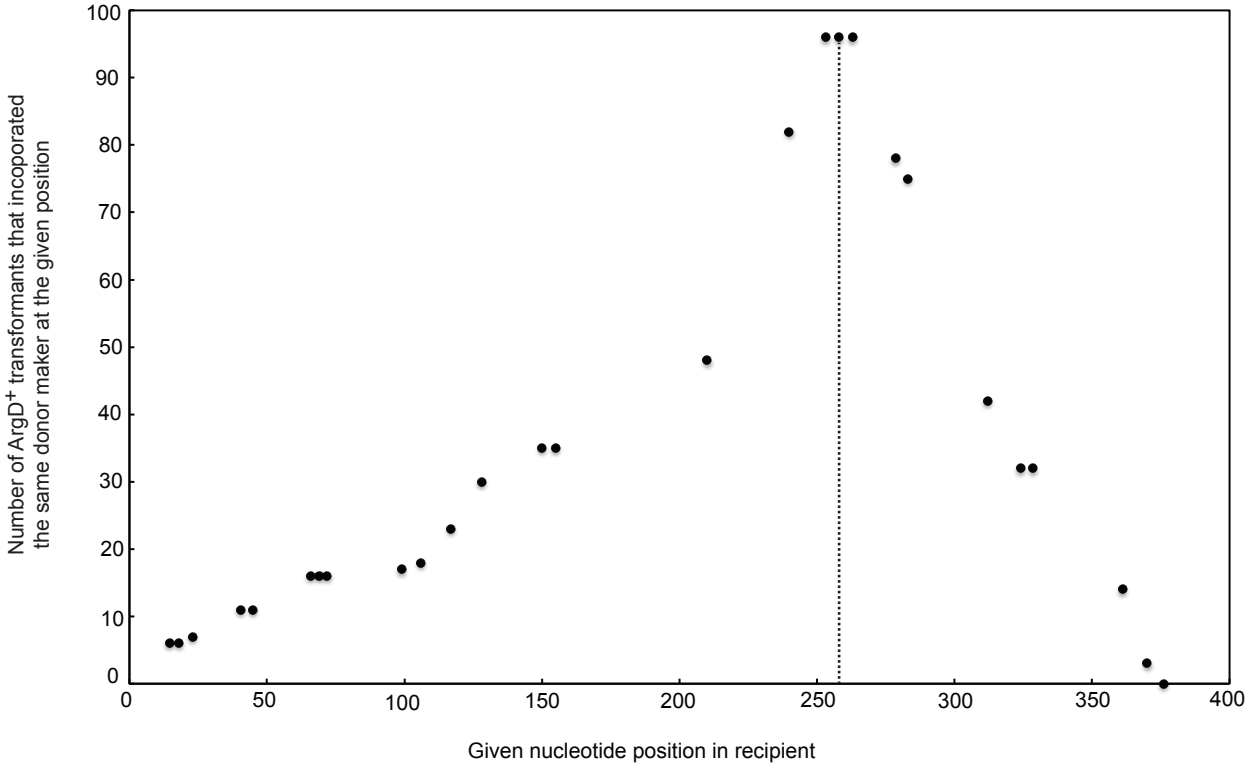

Number of ArgD+ transformants that incoporated the same donor maker at the given position
Given nucleotide position in recipient
0
10
20
30
40
50
60
70
80
90
100
0
50
100
150
200
250
300
350
400

Figure .S2

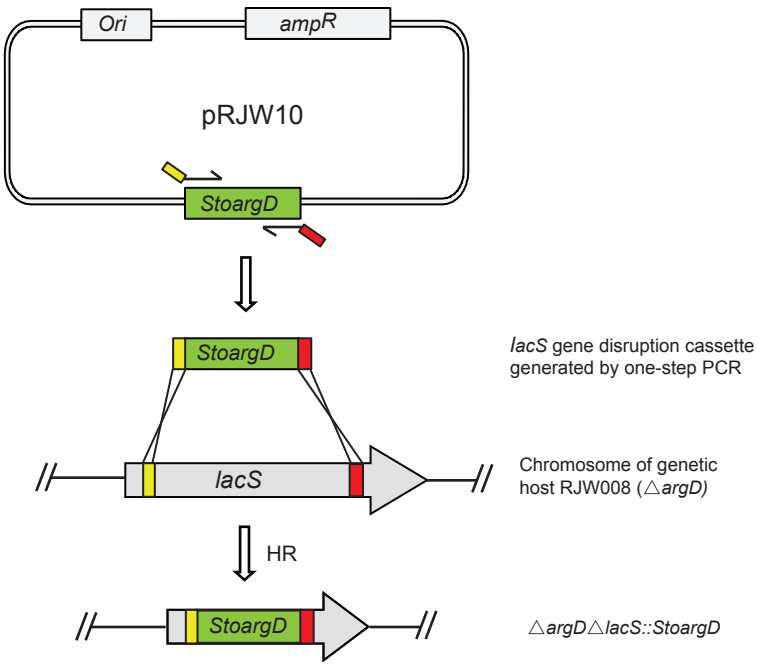

Ori
ampR
pRJW10
StoargD
StoargD
lacS gene disruption cassette
generated by one-step PCR
lacS
Chromosome of genetic
host RJW008 (△argD)
HR
StoargD
△argD△lacS::StoargD

Figure .S3

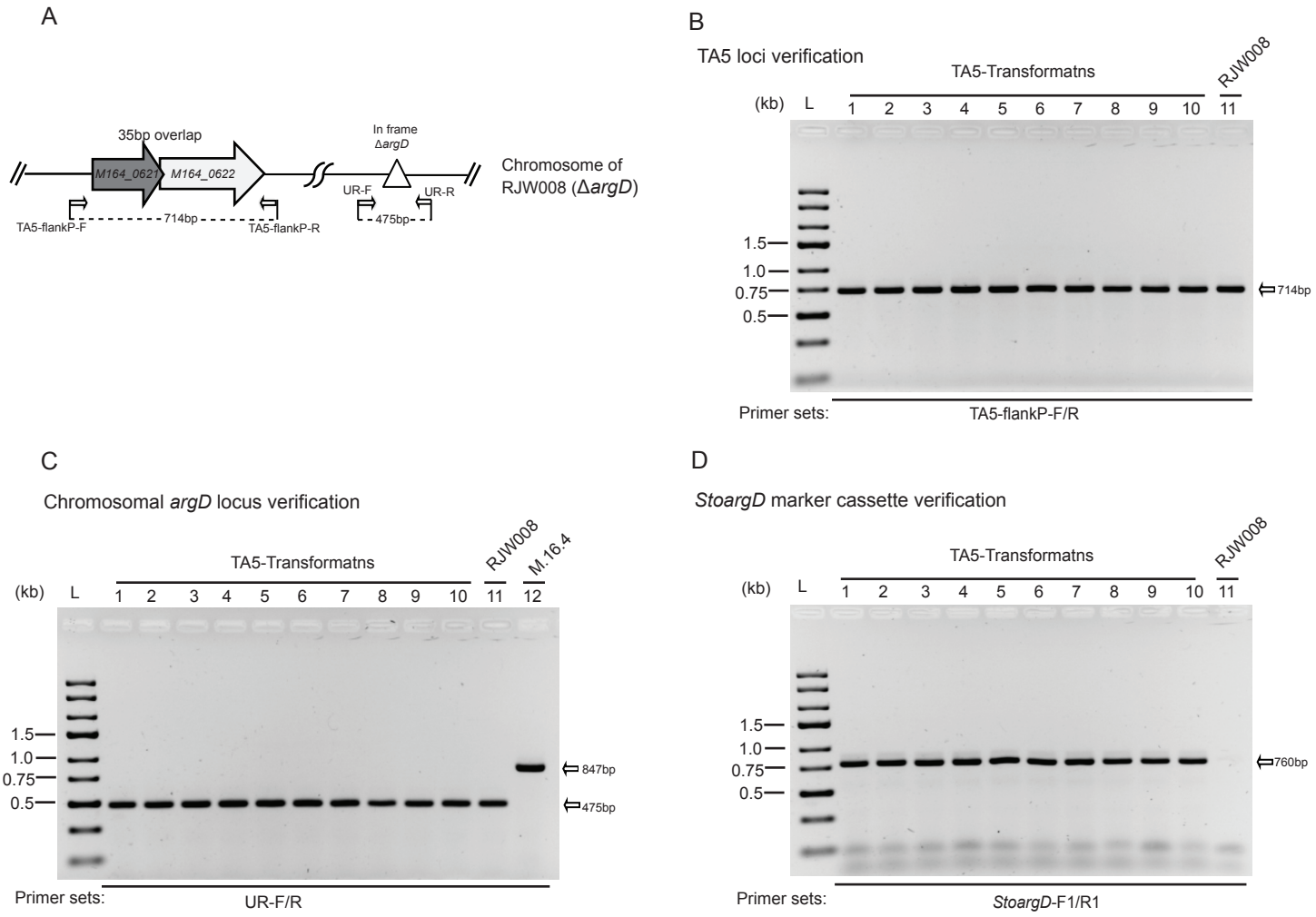
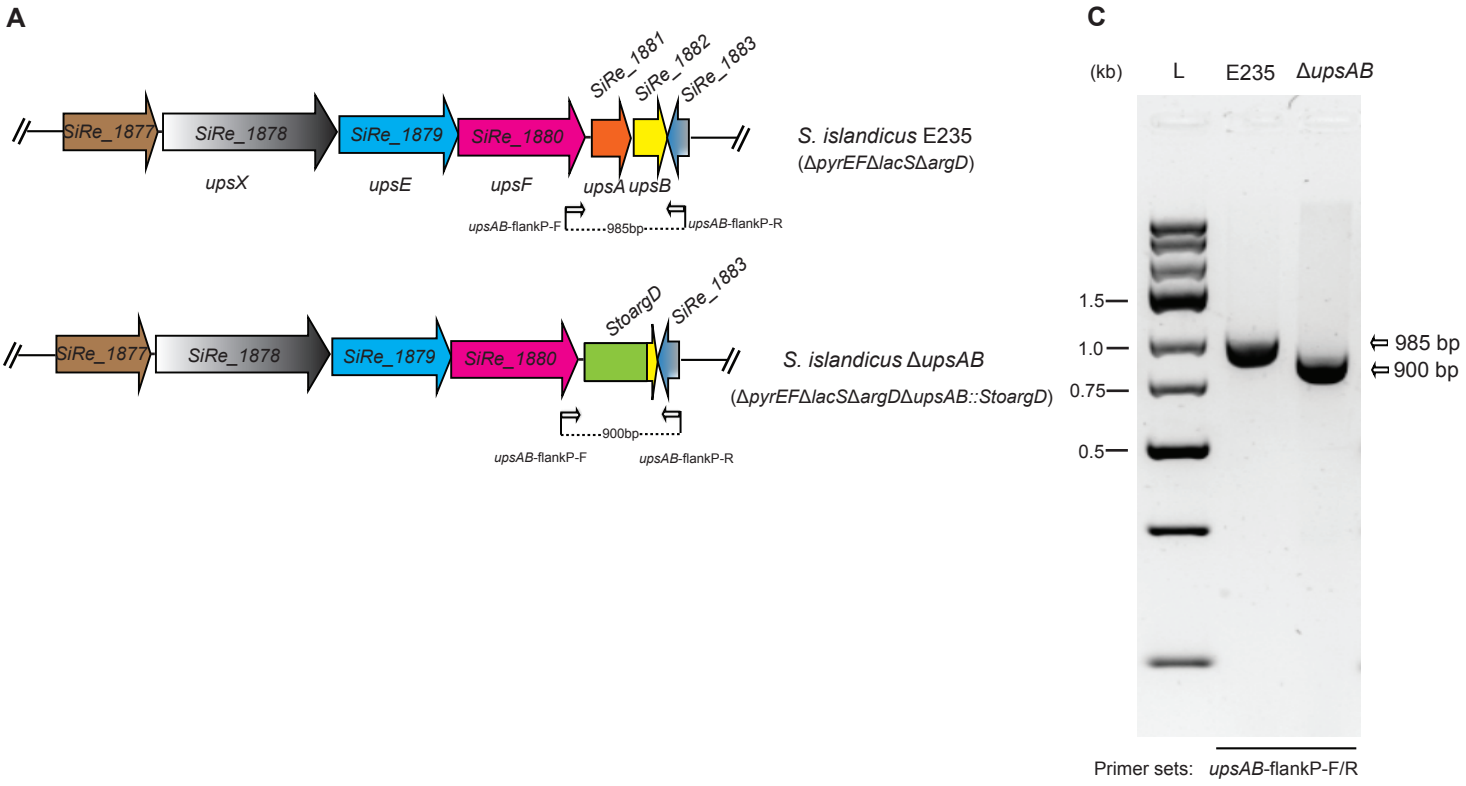
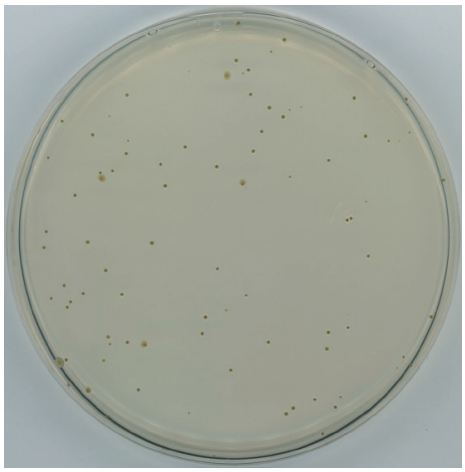

A
35bp overlap
M164_0621
M164_0622
In frame
ΔargD
UR-F
UR-R
TA5-flankP-F
714bp
TA5-flankP-R
475bp
Chromosome of
RJW008 (ΔargD)
B
TA5 loci verification
TA5-Transformatns
RJW008
(kb)
L
1 2 3 4 5 6 7 8 9 10 11
1.5
1.0
0.75
0.5
714bp
Primer sets:
TA5-flankP-F/R
C
Chromosomal argD locus verification
TA5-Transformatns
RJW008
M.16.4
(kb)
L
1 2 3 4 5 6 7 8 9 10 11 12
1.5
1.0
0.75
0.5
847bp
475bp
Primer sets:
UR-F/R
D
StoargD marker cassette verification
TA5-Transformatns
RJW008
(kb)
L
1 2 3 4 5 6 7 8 9 10 11
1.5
1.0
0.75
0.5
760bp
Primer sets:
StoargD-F1/R1

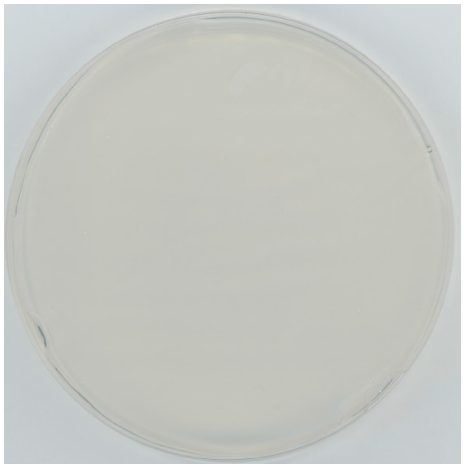
Figure .S4



B



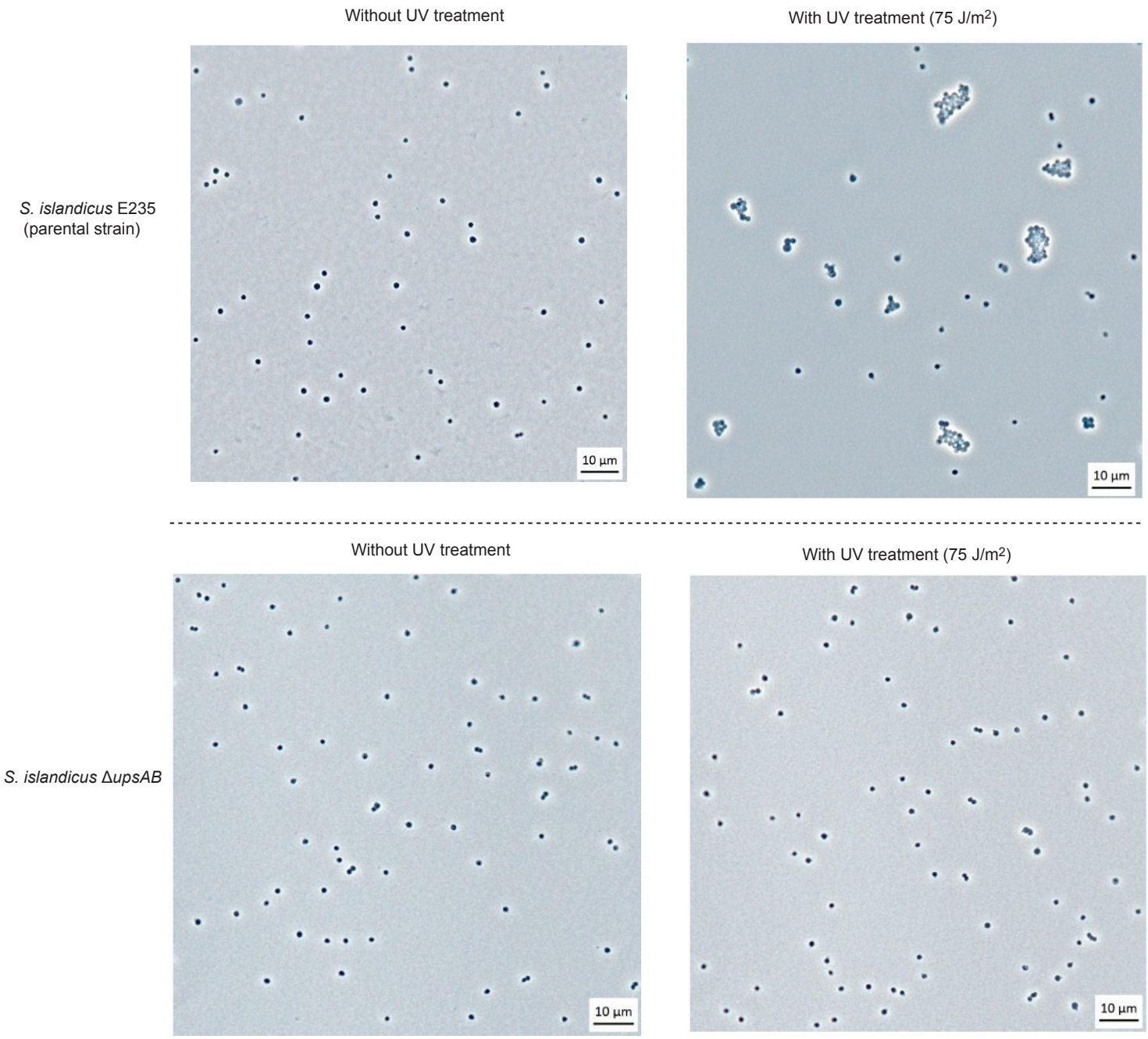
upsAB KO
(850 µl transformed-cells were plated)



Negative
(850 µl transformed-cells were plated)

Positive
(5 µl transformed-cells were plated)

Figure .S5


Without UV treatment
With UV treatment (75 J/m2)
S. islandicus E235
(parental strain)
10 µm
10 µm
Without UV treatment
With UV treatment (75 J/m2)
S. islandicus ΔupsAB
10 µm
10 µm