Supporting Information

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SI Experimental Procedures

Bacterial Strains and Plasmids. All *B. subtilis* strains used in this study were derivatives of the WT strain PY79 (1) and were constructed by transformation with either plasmid, purified *B. subtilis* chromosomal DNA or a linear DNA fragment. Plasmids and linear DNA fragments used for transformation of *B. subtilis* were constructed by standard cloning methods, PCR, site-directed mutagenesis (2), PrimeSTAR mutagenesis (Takara), Gibson assembly (3), or combinations of these methods. We used the sets of oligo DNA primers in combination with template plasmid or *B. subtilis* chromosomal DNA as indicated in Tables S2, S3, and S4. PrimeSTAR mutagenesis was carried out according to the manufacturer's instructions with the following minor modifications: (*i*) PrimeSTAR GXL DNA Polymerase instead of PrimeSTAR Max DNA Polymerase was used for PCR, (*ii*) PCR products were treated with DpnI (New England Biolab) for 1 h at 37 °C to remove template DNA enzymatically, and (*iii*) a fraction of the PCR products were circularized by recombining their ends intramolecularly by Gibson assembly.

When an engineered gene was introduced into the *B. subtilis* chromosome by recombination, the recombinants were checked for their antibiotic resistance, inactivation of *amyE*, and the absence of any drug resistance markers originally present on the plasmid backbone. The nucleotide sequence of the chromosomal DNA was also analyzed if necessary. As the reporter to measure the levels of *yidC2* induction, we used the *lacZ* translational fusion gene *mifM-yidC2′-lacZ*, in which *lacZ* was fused in-frame after the sixth codon of *yidC2* (4).

NEM Reactivity Assay. For NEM reactivity assay, *B. subtilis* cells from 2-mL portions of cultures were collected by centrifugation (20,400 × *g* for 3 min at 4 °C), washed with 1 mL of 50 mM Tris·HCl (pH 6.8), and then resuspended in 50 μL of TSM buffer [20 mM Tris·HCl (pH 6.8), 0.5 M sucrose, 20 mM $MgCl_2$]. Cells were then treated with 20 mM NEM (Sigma) on ice for 90 min in TSM buffer to alkylate the engineered Cys residue of SpoIIIJ if water was available around it. The NEM modification was stopped by adding DTT (final 100 mM), followed by washing the cells twice with 1 mL of TSM to remove excess DTT. Cells were then mixed with 400 μL of 5% (wt/vol) TCA, left on ice for 20 min, and centrifuged. Pelleted cells were washed with 800 μL of 1 M Tris·HCl (pH 8.0), resuspended in 20 μL of TSE buffer [33 mM Tris·HCl (pH 8.0), 40% (wt/vol) sucrose, 1 mM EDTA] containing 1 mg/mL lysozyme and incubated at 37 °C for 10 min. Samples were solubilized by adding 20 μL of 2× SDS loading buffer [250 mM Tris·HCl (pH 6.8), 4% (wt/vol) SDS, 30% (vol/vol) glycerol, 0.2% bromophenol blue] without added DTT. They were divided into two portions, a control portion that received 64 mM DTT and an experimental portion that did not, and subjected to countermodification with 3.5 mg/mL Mal-PEG (5000; Fluka) at 37 °C for 90 min with vigorous mixing. The Mal-PEG modification reaction was then stopped by adding DTT (final concentration of 60 mM) to the experimental samples. Modified and unmodified SpoIIIJ species were separated by SDS/PAGE and detected by anti-SpoIIIJ immunoblotting to assess the NEM reactivity of a target Cys.

Growth Complementation Assay. For the growth complementation assay, *B. subtilis* cells harboring pCH1805, a rescue plasmid encoding IPTG-inducible *spoIIIJ-FLAG*, along with a spectinomycin resistance gene, were cultured at 37 °C in LB supplemented with 100 μg/mL spectinomycin and 1 mM IPTG. Four-microliter portions of serially diluted full-growth cultures were then spotted onto LB-spectinomycin agar plates with and without 1 mM IPTG and incubated at 37 °C for 17.5 h.

1. Youngman P, Perkins JB, Losick R (1984) A novel method for the rapid cloning in Escherichia coli of Bacillus subtilis chromosomal DNA adjacent to Tn917 insertions. *Mol Gen Genet* 195(3):424–433.
2. Sawano A, Miyawaki A (2000) Directed evolution of green fluorescent protein by a new versatile PCR strategy for site-directed and semi-random mutagenesis. *Nucleic Acids Res* 28(16):E78.
3. Gibson DG, et al. (2009) Enzymatic assembly of DNA molecules up to several hundred kilobases. *Nat Methods* 6(5):343–345.
4. Chiba S, Lamsa A, Pogliano K (2009) A ribosome-nascent chain sensor of membrane protein biogenesis in Bacillus subtilis. *EMBO J* 28(22):3461–3475.


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Bs_SpoIIIJ       1 -MLLKRRIGLLLSMVGVFMLLAGCSSVKEPITADSPHFWDKYVVYPLSEL  49
                    ..:|||: |||:.:.:.:.|||||:. :|||::|...|:.:.|||:|.|
Bh_YidC2         1 MNYMKRRL-LLLAGILLLVALAGCSTT-DPITSESEGIWNHFFVYPMSWL  48

Bs_SpoIIIJ      50 ITYVAKLTGDNYGLSIILVTILIRLLILPLMIKQLRSSKAMQALQPEMQK  99
                   ||.||.|...:||||||:|||||||.:|||.:||.:|.:|||.::|||:.
Bh_YidC2        49 ITTVANLLNGSYGLSIIIVTILIRLALLPLTLKQQKSMRAMQVIRPEMEA  98

Bs_SpoIIIJ     100 LKEKY---SSKDQKTQQKLQQETMALFQKHGVNPLAGCFPILIQMPILIG 146
                   :::||   :|||.|.||::|:|.:.|:|||||||:|||.|:.||:|||:.
Bh_YidC2        99 IQKKYKEKASKDPKVQQEMQKELLGLYQKHGVNPMAGCLPLFIQLPILMA 148

Bs_SpoIIIJ     147 FYHAIMRTQAISEHSFLWFDLGEKDPYYILPIVAGVATFVQQKLMMAGNA 196
                   ||.|||||:.|..|:||||||||:.|  ||||.|||:.|:.|.|:.|:...
Bh_YidC2       149 FYFAIMRTEEIRYHTFLWFDLGQPD--YILPFVAGITTYFQFKMTMSHQQ 196

Bs_SpoIIIJ     197 QQ---NP-------------QMAMMLWIMPIMIIVFAINFPAALSLYWVV 230
                   |.   ||             ||.:||::||:|||:..::.|:|||||||:
Bh_YidC2       197 QMQKTNPSDSDNPMANMMQMQMKVMLYVMPVMIIIAGLSLPSALSLYWVI 246

Bs_SpoIIIJ     231 GNLFMIAQTF--LIKGPDI------KKNPEPQKAGGKKK 261
                   ||:|||.||:  ::|.|.:      :|:.:|.||
Bh_YidC2       247 GNIFMIIQTYFIVVKAPPLEVEQTKQKSSKPNKA----- 280
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Fig. S1. Pairwise protein sequence alignment between *B. subtilis* SpoIIIJ and *B. halodurans* YidC2. The amino acid sequence alignment was generated by EMBOSS Needle. The crucial Arg73 of *B. subtilis* SpoIIIJ and its homologous Arg72 of *B. halodurans* YidC2 are shown in red.

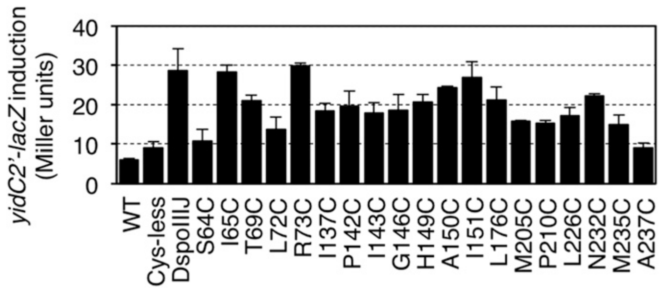

Fig. S2. Activities of the monocysteine variants of SpoIIIJ to insert MifM into the membrane. The efficiency of MifM insertion was determined by β-gal activity (mean ± SD, $n = 3$) from the *yidC2′-lacZ* reporter gene expressed in each *spoIIIJ* mutant strain. After preliminary blue-white assessment of β-gal activities of all the monocysteine derivatives of *spoIIIJ* in colonies on an LB agar plate supplemented with 60 μg/mL X-Gal (5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside), mutant strains giving blue colonies were subjected to the quantitative β-gal activity assays. All strains except WT and Δ*spoIIIJ* (DspoIIIJ) had the C134A mutation in addition to the second site mutation indicated at the bottom of each bar graph. MifM insertion efficiency is inversely correlated with the level of the *yidC2′-lacZ* induction. Eight mutants (I65C, T69C, R73C, H149C, A150C, I151C, L176C, and N232C) exhibiting β-gal activities of 20 units or higher are regarded as nonfunctional spoIIIJ mutants and are shown in striped colors in Fig. 2 *A* and *B*).

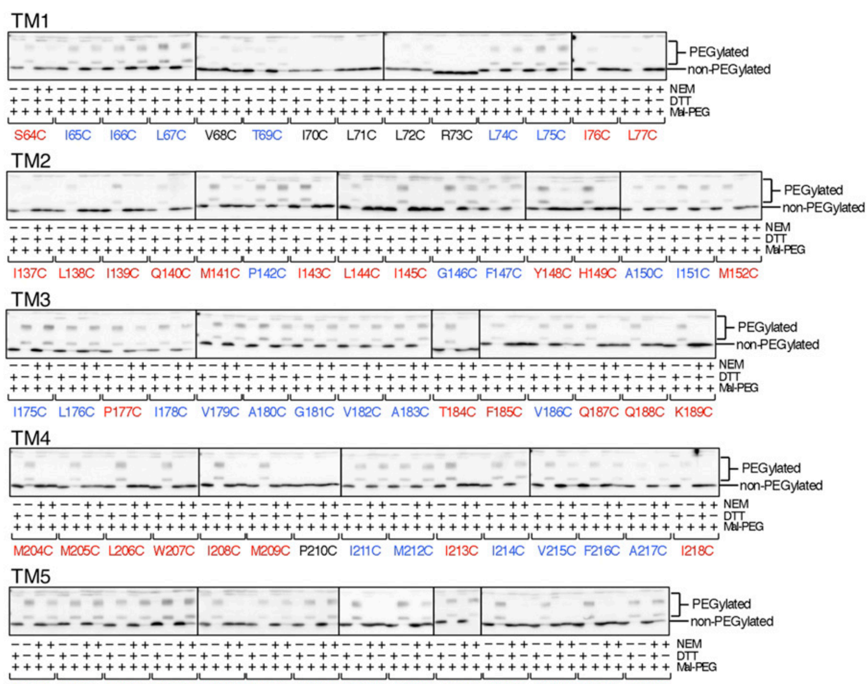

Fig. S3. NEM reactivity assay to assess water accessibility of Cys introduced into the SpoIIIJ TM regions. *B. subtilis* strains expressing a monocysteine derivative of SpoIIIJ were subjected to the NEM and Mal-PEG modification experiments as described in Fig. 2. The NEM-unmodified but PEG-modified and NEM-unmodified but PEG-modified SpoIIIJ species were separated by electrophoresis and detected by anti-SpoIIIJ immunoblotting. Positions of Cys introduced into SpoIIIJ are indicated at the bottom. Each sample received the different treatments as indicated by +. DTT in excess was included in alternate samples at the PEGylation step to give unmodified controls. PEGylated SpoIIIJ forms multiple slow-migrating bands due to heterogeneity of the Mal-PEG preparation. Mutant proteins that received high-efficiency (>50%) NEM modification are shown in red, and those mutant proteins that received lower (<50%) NEM modification are shown in blue. SpoIIIJ derivatives that could not be assessed because of the lack of PEGylation, even without NEM treatment, are shown in black.

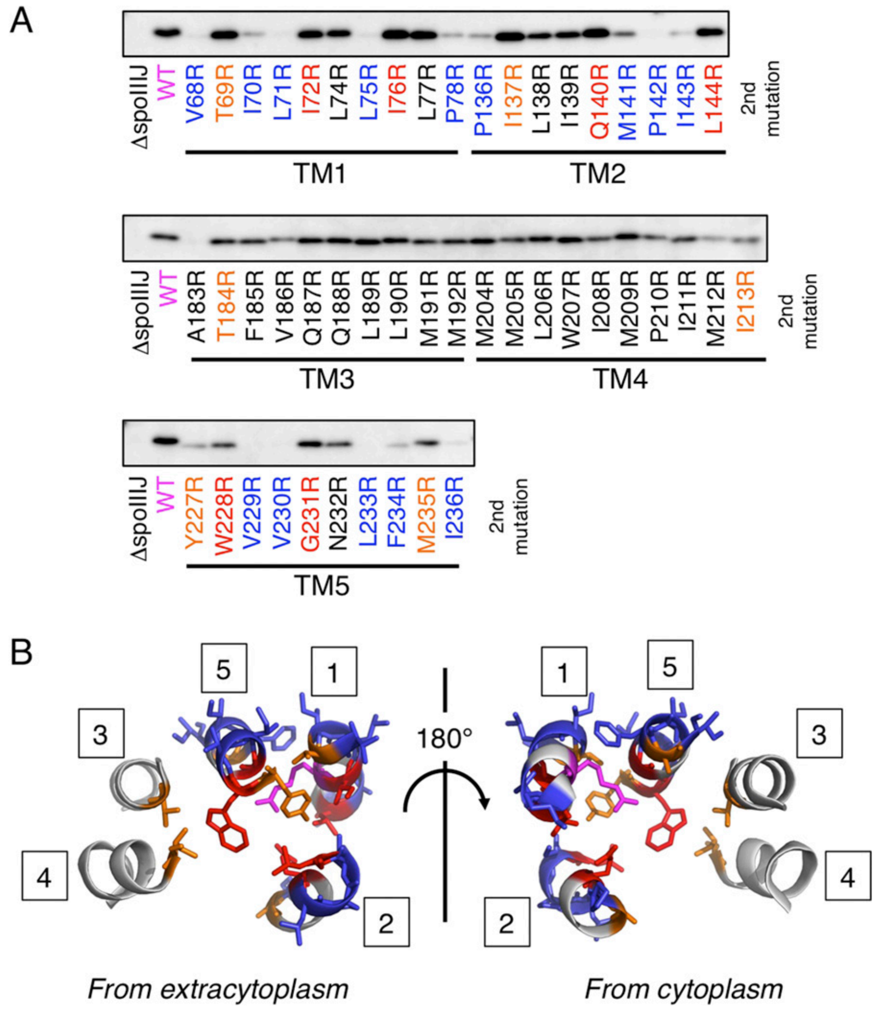

Fig. S4. Cellular accumulation of the Arg-relocated mutants of SpoIIIJ. (*A*) Cellular accumulation of SpoIIIJ was determined by anti-SpoIIIJ immunoblotting. Red and orange labels represent mutant *spoIIIJ* derivatives that exhibited low (<15 units) and intermediate (15–25 units) levels of β-gal activities expressed from the *yidC2′-lacZ* reporter gene (Fig. 4), indicating high (red) and intermediate (orange) efficiencies of MifM insertion, respectively. Blue labels represent the mutant SpoIIIJ proteins that accumulated at a very low level, whereas black labels represent the mutant SpoIIIJ proteins with a normal cellular accumulation level but a defective activity to insert MifM. (*B*) Schematic representation of the TM regions of *B. halodurans* YidC2, with the sites (in *B. subtilis* SpoIIIJ numbering) of Arg relocation highlighted. The Arg introduction into the equivalent residues in the *B. subtilis* SpoIIIJ-R73A destabilized the SpoIIIJ protein itself (blue) or restored the MifM insertion activity to a high (red) or an intermediate (orange) level as shown in Fig. 4. The original position of the crucial Arg (Arg73 in *B. subtilis* numbering) is shown in magenta. The black numbers in squares represent numbers of TM segments.

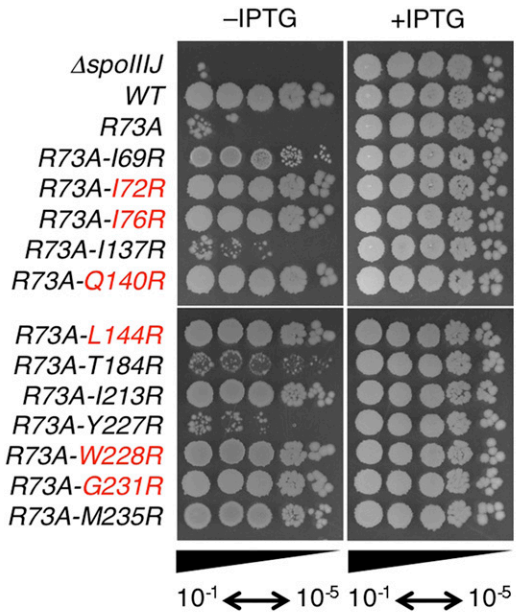

Fig. S5. Growth-supporting function of the Arg-relocated SpoIIIJ mutants. Growth complementation tests were carried out as described in the legend for Fig. 3*C*, using the indicated *spoIIIJ* alleles on the chromosome. The *spoIIIJ* mutations that efficiently supported the MifM insertion (Fig. 4) are shown in red.

Table S1. Strain list

Strains	Genotype	Source
SCB2972	*ΔspoIIIJ::tet, amyE::mifM-yidC2′-lacZΩcat*	Kumazaki et al. (1)
SCB2974	*spoIIIJΩkan, amyE::mifM-yidC2′-lacZΩcat*	Kumazaki et al. (1)
SCB3045	*ΔspoIIIJ::tet, ΔyidC2::erm/pCH1805 (Pspac spoIIIJ-FLAG)*	Kumazaki et al. (1)
SCB3055	*spoIIIJΩkan, ΔyidC2::erm/pCH1805 (Pspac spoIIIJ-FLAG)*	Kumazaki et al. (1)
SCB3142	*spoIIIJ-C134AΩkan, amyE::mifM-yidC2′-lacZΩcat*	This study
SCB3210	*spoIIIJ-L71C-C134AΩkan, amyE::mifM-yidC2′-lacZΩcat*	This study
SCB3211	*spoIIIJ-R73C-C134AΩkan, amyE::mifM-yidC2′-lacZΩcat*	This study
SCB3212	*spoIIIJ-C134A-V186CΩkan, amyE::mifM-yidC2′-lacZΩcat*	This study
SCB3213	*spoIIIJ-C134A-Q187CΩkan, amyE::mifM-yidC2′-lacZΩcat*	This study
SCB3214	*spoIIIJ-C134A-W228CΩkan, amyE::mifM-yidC2′-lacZΩcat*	This study
SCB3303	*spoIIIJ-C134A-E169CΩkan, amyE::mifM-yidC2′-lacZΩcat*	This study
SCB3304	*spoIIIJ-C134A-V179CΩkan, amyE::mifM-yidC2′-lacZΩcat*	This study
SCB3305	*spoIIIJ-C134A-A180CΩkan, amyE::mifM-yidC2′-lacZΩcat*	This study
SCB3306	*spoIIIJ-C134A-G181CΩkan, amyE::mifM-yidC2′-lacZΩcat*	This study
SCB3307	*spoIIIJ-C134A-V182CΩkan, amyE::mifM-yidC2′-lacZΩcat*	This study
SCB3308	*spoIIIJ-C134A-A183CΩkan, amyE::mifM-yidC2′-lacZΩcat*	This study
SCB3309	*spoIIIJ-C134A-F185CΩkan, amyE::mifM-yidC2′-lacZΩcat*	This study
SCB3310	*spoIIIJ-C134A-Q188CΩkan, amyE::mifM-yidC2′-lacZΩcat*	This study
SCB3311	*spoIIIJ-C134A-K189CΩkan, amyE::mifM-yidC2′-lacZΩcat*	This study
SCB3425	*spoIIIJ-C134A-I137CΩkan, amyE::mifM-yidC2′-lacZΩcat*	This study
SCB3426	*spoIIIJ-C134A-L138CΩkan, amyE::mifM-yidC2′-lacZΩcat*	This study
SCB3427	*spoIIIJ-C134A-I139CΩkan, amyE::mifM-yidC2′-lacZΩcat*	This study
SCB3428	*spoIIIJ-C134A-M141CΩkan, amyE::mifM-yidC2′-lacZΩcat*	This study
SCB3429	*spoIIIJ-C134A-I143CΩkan, amyE::mifM-yidC2′-lacZΩcat*	This study
SCB3430	*spoIIIJ-C134A-L145CΩkan, amyE::mifM-yidC2′-lacZΩcat*	This study
SCB3431	*spoIIIJ-C134A-G146CΩkan, amyE::mifM-yidC2′-lacZΩcat*	This study
SCB3432	*spoIIIJ-C134A-L224CΩkan, amyE::mifM-yidC2′-lacZΩcat*	This study
SCB3433	*spoIIIJ-C134A-S225CΩkan, amyE::mifM-yidC2′-lacZΩcat*	This study
SCB3434	*spoIIIJ-C134A-L226CΩkan, amyE::mifM-yidC2′-lacZΩcat*	This study
SCB3435	*spoIIIJ-C134A-L233CΩkan, amyE::mifM-yidC2′-lacZΩcat*	This study
SCB3439	*spoIIIJ-C134A-Y227CΩkan, amyE::mifM-yidC2′-lacZΩcat*	This study
SCB3440	*spoIIIJ-C134A-V229CΩkan, amyE::mifM-yidC2′-lacZΩcat*	This study
SCB3441	*spoIIIJ-C134A-Q140CΩkan, amyE::mifM-yidC2′-lacZΩcat*	This study
SCB3442	*spoIIIJ-C134A-P142CΩkan, amyE::mifM-yidC2′-lacZΩcat*	This study
SCB3443	*spoIIIJ-C134A-F147CΩkan, amyE::mifM-yidC2′-lacZΩcat*	This study
SCB3444	*spoIIIJ-C134A-V230CΩkan, amyE::mifM-yidC2′-lacZΩcat*	This study
SCB3445	*spoIIIJ-C134A-L144CΩkan, amyE::mifM-yidC2′-lacZΩcat*	This study
SCB3516	*spoIIIJ-C134A-T184CΩkan, amyE::mifM-yidC2′-lacZΩcat*	This study
SCB3517	*spoIIIJ-C134A-G231CΩkan, amyE::mifM-yidC2′-lacZΩcat*	This study
SCB3518	*spoIIIJ-C134A-I175CΩkan, amyE::mifM-yidC2′-lacZΩcat*	This study
SCB3519	*spoIIIJ-C134A-L176CΩkan, amyE::mifM-yidC2′-lacZΩcat*	This study
SCB3520	*spoIIIJ-C134A-P177CΩkan, amyE::mifM-yidC2′-lacZΩcat*	This study
SCB3521	*spoIIIJ-C134A-I178CΩkan, amyE::mifM-yidC2′-lacZΩcat*	This study
SCB3535	*spoIIIJ-C134A-N232CΩkan, amyE::mifM-yidC2′-lacZΩcat*	This study
SCB3536	*spoIIIJ-C134A-I65CΩkan, amyE::mifM-yidC2′-lacZΩcat*	This study
SCB3537	*spoIIIJ-C134A-I66CΩkan, amyE::mifM-yidC2′-lacZΩcat*	This study
SCB3538	*spoIIIJ-C134A-L67CΩkan, amyE::mifM-yidC2′-lacZΩcat*	This study
SCB3539	*spoIIIJ-C134A-V68CΩkan, amyE::mifM-yidC2′-lacZΩcat*	This study
SCB3540	*spoIIIJ-C134A-I70CΩkan, amyE::mifM-yidC2′-lacZΩcat*	This study
SCB3541	*spoIIIJ-C134A-L74CΩkan, amyE::mifM-yidC2′-lacZΩcat*	This study
SCB3542	*spoIIIJ-C134A-I76CΩkan, amyE::mifM-yidC2′-lacZΩcat*	This study
SCB3543	*spoIIIJ-C134A-L77CΩkan, amyE::mifM-yidC2′-lacZΩcat*	This study
SCB3544	*spoIIIJ-C134A-Y148CΩkan, amyE::mifM-yidC2′-lacZΩcat*	This study
SCB3545	*spoIIIJ-C134A-A150CΩkan, amyE::mifM-yidC2′-lacZΩcat*	This study
SCB3546	*spoIIIJ-C134A-I151CΩkan, amyE::mifM-yidC2′-lacZΩcat*	This study
SCB3547	*spoIIIJ-C134A-M152CΩkan, amyE::mifM-yidC2′-lacZΩcat*	This study
SCB3548	*spoIIIJ-C134A-W207CΩkan, amyE::mifM-yidC2′-lacZΩcat*	This study
SCB3549	*spoIIIJ-C134A-M209CΩkan, amyE::mifM-yidC2′-lacZΩcat*	This study
SCB3550	*spoIIIJ-C134A-P210CΩkan, amyE::mifM-yidC2′-lacZΩcat*	This study
SCB3551	*spoIIIJ-C134A-I211CΩkan, amyE::mifM-yidC2′-lacZΩcat*	This study
SCB3552	*spoIIIJ-C134A-M212CΩkan, amyE::mifM-yidC2′-lacZΩcat*	This study
SCB3553	*spoIIIJ-C134A-I213CΩkan, amyE::mifM-yidC2′-lacZΩcat*	This study
SCB3554	*spoIIIJ-C134A-V215CΩkan, amyE::mifM-yidC2′-lacZΩcat*	This study

Table S1. Cont.

Strains	Genotype	Source
SCB3555	*spoIIIJ-C134A-F216CΩkan, amyE::mifM-yidC2′-lacZΩcat*	This study
SCB3556	*spoIIIJ-C134A-A217CΩkan, amyE::mifM-yidC2′-lacZΩcat*	This study
SCB3557	*spoIIIJ-C134A-I218CΩkan, amyE::mifM-yidC2′-lacZΩcat*	This study
SCB3558	*spoIIIJ-C134A-M235CΩkan, amyE::mifM-yidC2′-lacZΩcat*	This study
SCB3559	*spoIIIJ-C134A-I236CΩkan, amyE::mifM-yidC2′-lacZΩcat*	This study
SCB3560	*spoIIIJ-C134A-A237CΩkan, amyE::mifM-yidC2′-lacZΩcat*	This study
SCB3562	*spoIIIJ-C134A-L75CΩkan, amyE::mifM-yidC2′-lacZΩcat*	This study
SCB3564	*spoIIIJ-C134A-M204CΩkan, amyE::mifM-yidC2′-lacZΩcat*	This study
SCB3565	*spoIIIJ-C134A-M205CΩkan, amyE::mifM-yidC2′-lacZΩcat*	This study
SCB3566	*spoIIIJ-C134A-I214CΩkan, amyE::mifM-yidC2′-lacZΩcat*	This study
SCB3567	*spoIIIJ-C134A-T69CΩkan, amyE::mifM-yidC2′-lacZΩcat*	This study
SCB3568	*spoIIIJ-C134A-I72CΩkan, amyE::mifM-yidC2′-lacZΩcat*	This study
SCB3570	*spoIIIJ-C134A-H149CΩkan, amyE::mifM-yidC2′-lacZΩcat*	This study
SCB3571	*spoIIIJ-C134A-S64CΩkan, amyE::mifM-yidC2′-lacZΩcat*	This study
SCB3587	*spoIIIJ-C134A-F234CΩkan, amyE::mifM-yidC2′-lacZΩcat*	This study
SCB3588	*spoIIIJ-C134A-L206CΩkan, amyE::mifM-yidC2′-lacZΩcat*	This study
SCB3589	*spoIIIJ-C134A-I208CΩkan, amyE::mifM-yidC2′-lacZΩcat*	This study
SCB2989	*spoIIIJ-R73AΩkan, amyE::mifM-yidC2′-lacZΩcat*	This study
SCB3056	*spoIIIJ-R73AΩkan, ΔyidC2::erm/pCH1805 (Pspac spoIIIJ-FLAG)*	This study
SCB3141	*spoIIIJ-R73A-Q140RΩkan, amyE::mifM-yidC2′-lacZΩcat*	This study
SCB3177	*spoIIIJ-R73A-G231RΩkan, ΔyidC2::erm/pCH1805 (Pspac spoIIIJ-FLAG)*	This study
SCB3178	*spoIIIJ-R73A-Q140RΩkan, ΔyidC2::erm/pCH1805 (Pspac spoIIIJ-FLAG)*	This study
SCB3412	*spoIIIJ-R73A-W228RΩkan, amyE::mifM-yidC2′-lacZΩcat*	This study
SCB3413	*spoIIIJ-R73A-L233RΩkan, amyE::mifM-yidC2′-lacZΩcat*	This study
SCB3414	*spoIIIJ-R73A-N232RΩkan, amyE::mifM-yidC2′-lacZΩcat*	This study
SCB3415	*spoIIIJ-R73A-M235RΩkan, amyE::mifM-yidC2′-lacZΩcat*	This study
SCB3416	*spoIIIJ-R73A-I236RΩkan, amyE::mifM-yidC2′-lacZΩcat*	This study
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SCB3418	*spoIIIJ-R73A-V230RΩkan, amyE::mifM-yidC2′-lacZΩcat*	This study
SCB3419	*spoIIIJ-R73A-F234RΩkan, amyE::mifM-yidC2′-lacZΩcat*	This study
SCB3612	*spoIIIJ-V68R/R73AΩkan, amyE::mifM-yidC2′-lacZΩcat*	This study
SCB3614	*spoIIIJ-T69R/R73AΩkan, amyE::mifM-yidC2′-lacZΩcat*	This study
SCB3616	*spoIIIJ-I70R/R73AΩkan, amyE::mifM-yidC2′-lacZΩcat*	This study
SCB3619	*spoIIIJ-I72R/R73AΩkan, amyE::mifM-yidC2′-lacZΩcat*	This study
SCB3620	*spoIIIJ-R73A/L74RΩkan, amyE::mifM-yidC2′-lacZΩcat*	This study
SCB3622	*spoIIIJ-R73A/L75RΩkan, amyE::mifM-yidC2′-lacZΩcat*	This study
SCB3624	*spoIIIJ-R73A/I76RΩkan, amyE::mifM-yidC2′-lacZΩcat*	This study
SCB3626	*spoIIIJ-R73A/L77RΩkan, amyE::mifM-yidC2′-lacZΩcat*	This study
SCB3628	*spoIIIJ-R73A/P78RΩkan, amyE::mifM-yidC2′-lacZΩcat*	This study
SCB3630	*spoIIIJ-R73A/P136RΩkan, amyE::mifM-yidC2′-lacZΩcat*	This study
SCB3632	*spoIIIJ-R73A/I137RΩkan, amyE::mifM-yidC2′-lacZΩcat*	This study
SCB3634	*spoIIIJ-R73A/L138RΩkan, amyE::mifM-yidC2′-lacZΩcat*	This study
SCB3636	*spoIIIJ-R73A/I139RΩkan, amyE::mifM-yidC2′-lacZΩcat*	This study
SCB3639	*spoIIIJ-R73A/M141RΩkan, amyE::mifM-yidC2′-lacZΩcat*	This study
SCB3641	*spoIIIJ-R73A/P142RΩkan, amyE::mifM-yidC2′-lacZΩcat*	This study
SCB3642	*spoIIIJ-R73A/I143RΩkan, amyE::mifM-yidC2′-lacZΩcat*	This study
SCB3644	*spoIIIJ-R73A/L144RΩkan, amyE::mifM-yidC2′-lacZΩcat*	This study
SCB3646	*spoIIIJ-R73A/A183RΩkan, amyE::mifM-yidC2′-lacZΩcat*	This study
SCB3648	*spoIIIJ-R73A/T184RΩkan, amyE::mifM-yidC2′-lacZΩcat*	This study
SCB3650	*spoIIIJ-R73A/F185RΩkan, amyE::mifM-yidC2′-lacZΩcat*	This study
SCB3652	*spoIIIJ-R73A/V186RΩkan, amyE::mifM-yidC2′-lacZΩcat*	This study
SCB3654	*spoIIIJ-R73A/Q187RΩkan, amyE::mifM-yidC2′-lacZΩcat*	This study
SCB3656	*spoIIIJ-R73A/Q188RΩkan, amyE::mifM-yidC2′-lacZΩcat*	This study
SCB3658	*spoIIIJ-R73A/K189RΩkan, amyE::mifM-yidC2′-lacZΩcat*	This study
SCB3660	*spoIIIJ-R73A/L190RΩkan, amyE::mifM-yidC2′-lacZΩcat*	This study
SCB3662	*spoIIIJ-R73A/M204RΩkan, amyE::mifM-yidC2′-lacZΩcat*	This study
SCB3664	*spoIIIJ-R73A/M205RΩkan, amyE::mifM-yidC2′-lacZΩcat*	This study
SCB3666	*spoIIIJ-R73A/L206RΩkan, amyE::mifM-yidC2′-lacZΩcat*	This study
SCB3669	*spoIIIJ-R73A/W207RΩkan, amyE::mifM-yidC2′-lacZΩcat*	This study
SCB3670	*spoIIIJ-R73A/I208RΩkan, amyE::mifM-yidC2′-lacZΩcat*	This study
SCB3673	*spoIIIJ-R73A/M209RΩkan, amyE::mifM-yidC2′-lacZΩcat*	This study
SCB3676	*spoIIIJ-R73A/I211RΩkan, amyE::mifM-yidC2′-lacZΩcat*	This study
SCB3678	*spoIIIJ-R73A/M212RΩkan, amyE::mifM-yidC2′-lacZΩcat*	This study

Table S1. Cont.

Strains	Genotype	Source
SCB3680	*spoIIIJ-R73A/I213RΩkan, amyE::mifM-yidC2′-lacZΩcat*	This study
SCB3682	*spoIIIJ-R73A/Y227RΩkan, amyE::mifM-yidC2′-lacZΩcat*	This study
SCB3686	*spoIIIJ-R73A/M191RΩkan, amyE::mifM-yidC2′-lacZΩcat*	This study
SCB3687	*spoIIIJ-R73A/M192RΩkan, amyE::mifM-yidC2′-lacZΩcat*	This study
SCB3688	*spoIIIJ-R73A/P210RΩkan, amyE::mifM-yidC2′-lacZΩcat*	This study
SCB3711	*spoIIIJ-L71R/R73AΩkan, amyE::mifM-yidC2′-lacZΩcat*	This study
SCB3732	*spoIIIJ-T69R-R73AΩkan, ΔyidC2::erm/pCH1805 (Pspac spoIIIJ-FLAG)*	This study
SCB3733	*spoIIIJ-I72R-R73AΩkan, ΔyidC2::erm/pCH1805 (Pspac spoIIIJ-FLAG)*	This study
SCB3734	*spoIIIJ-R73A-I76RΩkan, ΔyidC2::erm/pCH1805 (Pspac spoIIIJ-FLAG)*	This study
SCB3735	*spoIIIJ-R73A-I137RΩkan, ΔyidC2::erm/pCH1805 (Pspac spoIIIJ-FLAG)*	This study
SCB3736	*spoIIIJ-R73A-L144RΩkan, ΔyidC2::erm/pCH1805 (Pspac spoIIIJ-FLAG)*	This study
SCB3737	*spoIIIJ-R73A-T184RΩkan, ΔyidC2::erm/pCH1805 (Pspac spoIIIJ-FLAG)*	This study
SCB3738	*spoIIIJ-R73A-I213RΩkan, ΔyidC2::erm/pCH1805 (Pspac spoIIIJ-FLAG)*	This study
SCB3739	*spoIIIJ-R73A-Y227RΩkan, ΔyidC2::erm/pCH1805 (Pspac spoIIIJ-FLAG)*	This study
SCB3740	*spoIIIJ-R73A-W228RΩkan, ΔyidC2::erm/pCH1805 (Pspac spoIIIJ-FLAG)*	This study
SCB3741	*spoIIIJ-R73A-M235RΩkan, ΔyidC2::erm/pCH1805 (Pspac spoIIIJ-FLAG)*	This study
SCB3742	*spoIIIJ-6AΩkan, ΔyidC2::erm/pCH1805 (Pspac spoIIIJ-FLAG)*	This study
SCB3743	*spoIIIJ-6LΩkan, ΔyidC2::erm/pCH1805 (Pspac spoIIIJ-FLAG)*	This study
SCB3745	*spoIIIJ-5N1QΩkan, ΔyidC2::erm/pCH1805 (Pspac spoIIIJ-FLAG)*	This study
SCB3746	*sdm3-spoIIIJ-Ωkan, ΔyidC2::erm/pCH1805 (Pspac spoIIIJ-FLAG)*	This study
NAB316	*spoIIIJ-6A (Q140A/T184A/Q187A/Q188A/G231A/N232A)Ωkan, amyE::mifM-yidC2′-lacZΩcat*	This study
NAB323	*spoIIIJ-C134A-W228C-6A (Q140A/T184A/Q187A/Q188A/G231A/N232A)Ωkan, amyE::mifM-yidC2′-lacZΩcat*	This study
NAB327	*spoIIIJ-Q140LΩkan, amyE::mifM-yidC2′-lacZΩcat*	This study
NAB328	*spoIIIJ-C134A-I213C-6A (Q140A/T184A/Q187A/Q188A/G231A/N232A)Ωkan, amyE::mifM-yidC2′-lacZΩcat*	This study
NAB331	*spoIIIJ-6L (Q140L/T184L/Q187L/Q188L/G231L/N232L)Ωkan, amyE::mifM-yidC2′-lacZΩcat*	This study
NAB339	*spoIIIJ-Q140NΩkan, amyE::mifM-yidC2′-lacZΩcat*	This study
NAB343	*spoIIIJ-5N1Q (Q140N/T184N/Q187N/Q188N/G231N/N232Q)Ωkan, amyE::mifM-yidC2′-lacZΩcat*	This study
NAB345	*spoIIIJ-C134A-I213C-6L (Q140L/T184L/Q187L/Q188L/G231L/N232L)Ωkan, amyE::mifM-yidC2′-lacZΩcat*	This study
NAB347	*spoIIIJ-C134A-W228C-6L (Q140L/T184L/Q187L/Q188L/G231L/N232L)Ωkan, amyE::mifM-yidC2′-lacZΩcat*	This study
NAB349	*spoIIIJ-C134A-I213C-5N1Q (Q140N/T184N/Q187N/Q188N/G231N/N232Q)Ωkan, amyE::mifM-yidC2′-lacZΩcat*	This study
NAB351	*spoIIIJ-C134A-W228C-5N1Q (Q140N/T184N/Q187N/Q188N/G231N/N232Q)Ωkan, amyE::mifM-yidC2′-lacZΩcat*	This study
NAB360	*sdm3-spoIIIJΩkan, amyE::mifM-yidC2′-lacZΩcat*	This study

1. Kumazaki K, et al. (2014) Structural basis of Sec-independent membrane protein insertion by YidC. *Nature* 509(7501):516–520.

Table S2. Plasmids used in this study

Plasmid	Genotype	Method or reference	Primers	Template
pCH1777	*spoIIIJ-R73AΩkanΩjag*	Kumazaki et al. (1)		
pCH1805	*Pspac spoIIIJ-FLAG*	Kumazaki et al. (1)		
pCH1822	*spoIIIJ-R73A-G231RΩkanΩjag*	Site-directed mutagenesis	SP175	pCH1777
pCH1852	*spoIIIJ-R73A-Q140RΩkanΩjag*	Site-directed mutagenesis	SP176	pCH1777
pCH1853	*spoIIIJ-C134AΩkanΩjag*	Site-directed mutagenesis	SP177	pCH1763
pCH1878	*spoIIIJ-L71C-C134AΩkanΩjag*	Site-directed mutagenesis	SP178	pCH1853
pCH1879	*spoIIIJ-R73C-C134AΩkanΩjag*	Site-directed mutagenesis	SP179	pCH1853
pCH1880	*spoIIIJ-C134A-V186CΩkanΩjag*	Site-directed mutagenesis	SP180	pCH1853
pCH1881	*spoIIIJ-C134A-Q187CΩkanΩjag*	Site-directed mutagenesis	SP181	pCH1853
pCH1882	*spoIIIJ-C134A-W228CΩkanΩjag*	Site-directed mutagenesis	SP182	pCH1853
pCH1909	*spoIIIJ-C134A-E169CΩkanΩjag*	Site-directed mutagenesis	SP183	pCH1853
pCH1910	*spoIIIJ-C134A-V179CΩkanΩjag*	Site-directed mutagenesis	SP184	pCH1853
pCH1911	*spoIIIJ-C134A-A180CΩkanΩjag*	Site-directed mutagenesis	SP185	pCH1853
pCH1912	*spoIIIJ-C134A-G181CΩkanΩjag*	Site-directed mutagenesis	SP186	pCH1853
pCH1913	*spoIIIJ-C134A-V182CΩkanΩjag*	Site-directed mutagenesis	SP187	pCH1853
pCH1914	*spoIIIJ-C134A-A183CΩkanΩjag*	Site-directed mutagenesis	SP188	pCH1853
pCH1915	*spoIIIJ-C134A-F185CΩkanΩjag*	Site-directed mutagenesis	SP189	pCH1853
pCH1916	*spoIIIJ-C134A-Q188CΩkanΩjag*	Site-directed mutagenesis	SP190	pCH1853
pCH1917	*spoIIIJ-C134A-K189CΩkanΩjag*	Site-directed mutagenesis	SP191	pCH1853
pCH1926	*spoIIIJ-R73A-N232RΩkanΩjag*	Site-directed mutagenesis	SP192	pCH1777
pCH1927	*spoIIIJ-R73A-M235RΩkanΩjag*	Site-directed mutagenesis	SP193	pCH1777
pCH1928	*spoIIIJ-R73A-I236RΩkanΩjag*	Site-directed mutagenesis	SP194	pCH1777
pCH1929	*spoIIIJ-C134A-I137CΩkanΩjag*	Site-directed mutagenesis	SP195	pCH1853
pCH1930	*spoIIIJ-C134A-L138CΩkanΩjag*	Site-directed mutagenesis	SP196	pCH1853
pCH1931	*spoIIIJ-C134A-I139CΩkanΩjag*	Site-directed mutagenesis	SP197	pCH1853
pCH1932	*spoIIIJ-C134A-M141CΩkanΩjag*	Site-directed mutagenesis	SP198	pCH1853
pCH1933	*spoIIIJ-C134A-I143CΩkanΩjag*	Site-directed mutagenesis	SP199	pCH1853
pCH1934	*spoIIIJ-C134A-L145CΩkanΩjag*	Site-directed mutagenesis	SP200	pCH1853
pCH1935	*spoIIIJ-C134A-G146CΩkanΩjag*	Site-directed mutagenesis	SP201	pCH1853
pCH1936	*spoIIIJ-C134A-L224CΩkanΩjag*	Site-directed mutagenesis	SP202	pCH1853
pCH1937	*spoIIIJ-C134A-S225CΩkanΩjag*	Site-directed mutagenesis	SP203	pCH1853
pCH1938	*spoIIIJ-C134A-L226CΩkanΩjag*	Site-directed mutagenesis	SP204	pCH1853
pCH1939	*spoIIIJ-C134A-L233CΩkanΩjag*	Site-directed mutagenesis	SP205	pCH1853
pCH1941	*spoIIIJ-R73A-V229RΩkanΩjag*	Site-directed mutagenesis	SP206	pCH1777
pCH1942	*spoIIIJ-R73A-V230RΩkanΩjag*	Site-directed mutagenesis	SP207	pCH1777
pCH1943	*spoIIIJ-R73A-F234RΩkanΩjag*	Site-directed mutagenesis	SP208	pCH1777
pCH1944	*spoIIIJ-C134A-Y227CΩkanΩjag*	Site-directed mutagenesis	SP209	pCH1853
pCH1945	*spoIIIJ-C134A-V229CΩkanΩjag*	Site-directed mutagenesis	SP210	pCH1853
pCH1948	*spoIIIJ-C134A-Q140CΩkanΩjag*	Site-directed mutagenesis	SP211	pCH1853
pCH1949	*spoIIIJ-C134A-P142CΩkanΩjag*	Site-directed mutagenesis	SP212	pCH1853
pCH1950	*spoIIIJ-C134A-F147CΩkanΩjag*	Site-directed mutagenesis	SP213	pCH1853
pCH1951	*spoIIIJ-C134A-V230CΩkanΩjag*	Site-directed mutagenesis	SP214	pCH1853
pCH1952	*spoIIIJ-C134A-G231CΩkanΩjag*	Site-directed mutagenesis	SP215	pCH1853
pCH1953	*spoIIIJ-C134A-I175CΩkanΩjag*	Site-directed mutagenesis	SP216	pCH1853
pCH1954	*spoIIIJ-C134A-L176CΩkanΩjag*	Site-directed mutagenesis	SP217	pCH1853
pCH1955	*spoIIIJ-C134A-P177CΩkanΩjag*	Site-directed mutagenesis	SP218	pCH1853
pCH1956	*spoIIIJ-C134A-I178CΩkanΩjag*	Site-directed mutagenesis	SP219	pCH1853
pCH1973	*spoIIIJ-C134A-L206CΩkanΩjag*	PrimeSTAR mutagenesis	SP220/SP221	pCH1853
pCH1974	*spoIIIJ-C134A-I208CΩkanΩjag*	PrimeSTAR mutagenesis	SP222/SP223	pCH1853

1. Kumazaki K, et al. (2014) Structural basis of Sec-independent membrane protein insertion by YidC. *Nature* 509(7501):516–520.

Table S3. Strain construction procedures

Strains	Methods	Plasmid	Primers	Template DNA
SCB3142	Transformation	pCH1853		
SCB3210	Transformation	pCH1878		
SCB3211	Transformation	pCH1879		
SCB3212	Transformation	pCH1880		
SCB3213	Transformation	pCH1881		
SCB3214	Transformation	pCH1882		
SCB3303	Transformation	pCH1909		
SCB3304	Transformation	pCH1910		
SCB3305	Transformation	pCH1911		
SCB3306	Transformation	pCH1912		
SCB3307	Transformation	pCH1913		
SCB3308	Transformation	pCH1914		
SCB3309	Transformation	pCH1915		
SCB3310	Transformation	pCH1916		
SCB3311	Transformation	pCH1917		
SCB3425	Transformation	pCH1929		
SCB3426	Transformation	pCH1930		
SCB3427	Transformation	pCH1931		
SCB3428	Transformation	pCH1932		
SCB3429	Transformation	pCH1933		
SCB3430	Transformation	pCH1934		
SCB3431	Transformation	pCH1935		
SCB3432	Transformation	pCH1936		
SCB3433	Transformation	pCH1937		
SCB3434	Transformation	pCH1938		
SCB3435	Transformation	pCH1939		
SCB3439	Transformation	pCH1944		
SCB3440	Transformation	pCH1945		
SCB3441	Transformation	pCH1948		
SCB3442	Transformation	pCH1949		
SCB3443	Transformation	pCH1950		
SCB3444	Transformation	pCH1951		
SCB3445	Site-directed mutagenesis		SP1	pCH1853
SCB3516	PrimeSTAR mutagenesis		SP2/SP3	pCH1853
SCB3517	Transformation	pCH1952		
SCB3518	Transformation	pCH1953		
SCB3519	Transformation	pCH1954		
SCB3520	Transformation	pCH1955		
SCB3521	Transformation	pCH1956		
SCB3535	PrimeSTAR mutagenesis		SP4/SP5	pCH1853
SCB3536	PrimeSTAR mutagenesis		SP6/SP7	pCH1853
SCB3537	PrimeSTAR mutagenesis		SP8/SP9	pCH1853
SCB3538	PrimeSTAR mutagenesis		SP10/SP11	pCH1853
SCB3539	PrimeSTAR mutagenesis		SP12/SP13	pCH1853
SCB3540	PrimeSTAR mutagenesis		SP14/SP15	pCH1853
SCB3541	PrimeSTAR mutagenesis		SP16/SP17	pCH1853
SCB3542	PrimeSTAR mutagenesis		SP18/SP19	pCH1853
SCB3543	PrimeSTAR mutagenesis		SP20/SP21	pCH1853
SCB3544	PrimeSTAR mutagenesis		SP22/SP23	pCH1853
SCB3545	PrimeSTAR mutagenesis		SP24/SP25	pCH1853
SCB3546	PrimeSTAR mutagenesis		SP26/SP27	pCH1853
SCB3547	PrimeSTAR mutagenesis		SP28/SP29	pCH1853
SCB3548	PrimeSTAR mutagenesis		SP30/SP31	pCH1853
SCB3549	PrimeSTAR mutagenesis		SP32/SP33	pCH1853
SCB3550	PrimeSTAR mutagenesis		SP34/SP35	pCH1853
SCB3551	PrimeSTAR mutagenesis		SP36/SP37	pCH1853
SCB3552	PrimeSTAR mutagenesis		SP38/SP39	pCH1853
SCB3553	PrimeSTAR mutagenesis		SP40/SP41	pCH1853
SCB3554	PrimeSTAR mutagenesis		SP42/SP43	pCH1853
SCB3555	PrimeSTAR mutagenesis		SP44/SP45	pCH1853
SCB3556	PrimeSTAR mutagenesis		SP46/SP47	pCH1853
SCB3557	PrimeSTAR mutagenesis		SP48/SP49	pCH1853
SCB3558	PrimeSTAR mutagenesis		SP50/SP51	pCH1853

Table S3. Cont.

Strains	Methods	Plasmid	Primers	Template DNA
SCB3559	PrimeSTAR mutagenesis		SP52/SP53	pCH1853
SCB3560	PrimeSTAR mutagenesis		SP54/SP55	pCH1853
SCB3562	PrimeSTAR mutagenesis		SP56/SP57	pCH1853
SCB3564	PrimeSTAR mutagenesis		SP58/SP59	pCH1853
SCB3565	PrimeSTAR mutagenesis		SP60/SP61	pCH1853
SCB3566	PrimeSTAR mutagenesis		SP62/SP63	pCH1853
SCB3567	PrimeSTAR mutagenesis		SP64/SP65	pCH1853
SCB3568	PrimeSTAR mutagenesis		SP66/SP67	pCH1853
SCB3570	PrimeSTAR mutagenesis		SP68/SP69	pCH1853
SCB3571	PrimeSTAR mutagenesis		SP70/SP71	pCH1853
SCB3587	PrimeSTAR mutagenesis		SP72/SP73	pCH1853
SCB3588	Transformation	pCH1973		
SCB3589	Transformation	pCH1974		
SCB2989	Transformation	pCH1777		
SCB3056	Transformation	pCH1777		
SCB3141	Transformation	pCH1852		
SCB3177	Transformation	pCH1822		
SCB3178	Transformation	pCH1852		
SCB3412	Site-directed mutagenesis		SP74	pCH1777
SCB3413	Site-directed mutagenesis		SP75	pCH1777
SCB3414	Transformation	pCH1926		
SCB3415	Transformation	pCH1927		
SCB3416	Transformation	pCH1928		
SCB3417	Transformation	pCH1941		
SCB3418	Transformation	pCH1942		
SCB3419	Transformation	pCH1943		
SCB3612	PrimeSTAR mutagenesis		SP76/SP77	pCH1777
SCB3614	PrimeSTAR mutagenesis		SP78/SP79	pCH1777
SCB3616	PrimeSTAR mutagenesis		SP80/SP81	pCH1777
SCB3619	PrimeSTAR mutagenesis		SP82/SP83	pCH1777
SCB3620	PrimeSTAR mutagenesis		SP84/SP85	pCH1777
SCB3622	PrimeSTAR mutagenesis		SP86/SP87	pCH1777
SCB3624	PrimeSTAR mutagenesis		SP88/SP89	pCH1777
SCB3626	PrimeSTAR mutagenesis		SP90/SP91	pCH1777
SCB3628	PrimeSTAR mutagenesis		SP92/SP93	pCH1777
SCB3630	PrimeSTAR mutagenesis		SP94/SP95	pCH1777
SCB3632	PrimeSTAR mutagenesis		SP96/SP97	pCH1777
SCB3634	PrimeSTAR mutagenesis		SP98/SP99	pCH1777
SCB3636	PrimeSTAR mutagenesis		SP100/SP101	pCH1777
SCB3639	PrimeSTAR mutagenesis		SP102/SP103	pCH1777
SCB3641	PrimeSTAR mutagenesis		SP104/SP105	pCH1777
SCB3642	PrimeSTAR mutagenesis		SP106/SP107	pCH1777
SCB3644	PrimeSTAR mutagenesis		SP108/SP109	pCH1777
SCB3646	PrimeSTAR mutagenesis		SP110/SP111	pCH1777
SCB3648	PrimeSTAR mutagenesis		SP112/SP113	pCH1777
SCB3650	PrimeSTAR mutagenesis		SP114/SP115	pCH1777
SCB3652	PrimeSTAR mutagenesis		SP116/SP117	pCH1777
SCB3654	PrimeSTAR mutagenesis		SP118/SP119	pCH1777
SCB3656	PrimeSTAR mutagenesis		SP120/SP121	pCH1777
SCB3658	PrimeSTAR mutagenesis		SP122/SP123	pCH1777
SCB3660	PrimeSTAR mutagenesis		SP124/SP125	pCH1777
SCB3662	PrimeSTAR mutagenesis		SP126/SP127	pCH1777
SCB3664	PrimeSTAR mutagenesis		SP128/SP129	pCH1777
SCB3666	PrimeSTAR mutagenesis		SP130/SP131	pCH1777
SCB3669	PrimeSTAR mutagenesis		SP132/SP133	pCH1777
SCB3670	PrimeSTAR mutagenesis		SP134/SP135	pCH1777
SCB3673	PrimeSTAR mutagenesis		SP136/SP137	pCH1777
SCB3676	PrimeSTAR mutagenesis		SP138/SP139	pCH1777
SCB3678	PrimeSTAR mutagenesis		SP140/SP141	pCH1777
SCB3680	PrimeSTAR mutagenesis		SP142/SP143	pCH1777
SCB3682	PrimeSTAR mutagenesis		SP144/SP145	pCH1777
SCB3686	PrimeSTAR mutagenesis		SP146/SP147	pCH1777
SCB3687	PrimeSTAR mutagenesis		SP148/SP149	pCH1777

Table S3. Cont.

Strains	Methods	Plasmid	Primers	Template DNA
SCB3688	PrimeSTAR mutagenesis		SP150/SP151	pCH1777
SCB3711	PrimeSTAR mutagenesis		SP152/SP153	pCH1777
SCB3732	Transformation	SCB3614		
SCB3733	Transformation	SCB3619		
SCB3734	Transformation	SCB3624		
SCB3735	Transformation	SCB3632		
SCB3736	Transformation	SCB3644		
SCB3737	Transformation	SCB3648		
SCB3738	Transformation	SCB3680		
SCB3739	Transformation	SCB3682		
SCB3740	Transformation	SCB3412		
SCB3741	Transformation	SCB3415		
SCB3742	Transformation	NAB316		
SCB3743	Transformation	NAB331		
SCB3745	Transformation	NAB343		
SCB3746	Transformation	NAB360		
NAB316	PCR/Gibson assembly		SP154/SP155	SCB2994
			SP156/SP157	SCB2994
			SP158/SP159	SCB2994
NAB323	PCR/Gibson assembly		SP154/SP160	NAB316
			SP161/SP162	NAB316
			SP163/SP159	NAB316
NAB327	PCR/Gibson assembly		SP154/SP164	SCB3065
			SP165/SP159	SCB3065
NAB328	PCR/Gibson assembly		SP154/SP160	NAB316
			SP161/SP172	NAB316
			SP166/SP159	NAB316
NAB331	PCR/Gibson assembly		SP154/SP155	NAB327
			SP167/SP157	NAB327
			SP168/SP159	NAB327
NAB339	PCR/Gibson assembly		SP154/SP164	SCB3065
			SP169/SP159	SCB3065
NAB343	PCR/Gibson assembly		SP154/SP155	NAB339
			SP170/SP157	NAB339
			SP171/SP159	NAB339
NAB345	PCR/Gibson assembly		SP154/SP160	NAB331
			SP161/SP172	NAB331
			SP166/SP159	NAB331
NAB347	PCR/Gibson assembly		SP154/SP160	NAB331
			SP161/SP162	NAB331
			SP163/SP159	NAB331
NAB349	PCR/Gibson assembly		SP154/SP160	NAB343
			SP161/SP172	NAB343
			SP166/SP159	NAB343
NAB351	PCR/Gibson assembly		SP154/SP160	NAB343
			SP161/SP162	NAB343
			SP163/SP159	NAB343
NAB360	PCR/Gibson assembly		SP154/SP173	SCB3117
			SP174/SP159	SCB3065

Table S4. Oligo nucleotides sequence

Primer	DNA sequence
SP1	5′-TTGATCCAGATGCCGATTTGTATTGGATTCTATCATGCG-3′
SP2	5′-GTCGCTTGTTTTGTTCAGCAAAAACTGATG-3′
SP3	5′-AACAAAACAAGCGACACCGGCAACGATAGG-3′
SP4	5′-GTAGTTGGTTGTTTGTTTATGATTGCGCAAACT-3′
SP5	5′-CATAAACAAACAACCAACTACCCAATAAAGAGA-3′
SP6	5′-GATAACTACGGGCTTTCATGTATTCTAGTTACCATTTTA-3′
SP7	5′-ACATGAAAGCCCGTAGTTATCTCCCGTCAATTT-3′
SP8	5′-AACTACGGGCTTTCAATTTGTCTAGTTACCATTTTAATT-3′
SP9	5′-ACAAATTGAAAGCCCGTAGTTATCTCCCGTCAA-3′
SP10	5′-TACGGGCTTTCAATTATTTGTGTTACCATTTTAATTCGT-3′
SP11	5′-ACAAATAATTGAAAGCCCGTAGTTATCTCCCGT-3′
SP12	5′-GGGCTTTCAATTATTCTATGTACCATTTTAATTCGTTTA-3′
SP13	5′-ACATAGAATAATTGAAAGCCCGTAGTTATCTCC-3′
SP14	5′-TCAATTATTCTAGTTACCTGTTTAATTCGTTTATTAATT-3′
SP15	5′-ACAGGTAACTAGAATAATTGAAAGCCCGTAGTT-3′
SP16	5′-GTTACCATTTTAATTCGTTGTTTAATTTTACCGCTGATG-3′
SP17	5′-ACAACGAATTAAAATGGTAACTAGAATAATTGA-3′
SP18	5′-ATTTTAATTCGTTTATTATGTTTACCGCTGATGATTAAG-3′
SP19	5′-ACATAATAAACGAATTAAAATGGTAACTAGAAT-3′
SP20	5′-TTAATTCGTTTATTAATTTGTCCGCTGATGATTAAGCAG-3′
SP21	5′-ACAAATTAATAAACGAATTAAAATGGTAACTAG-3′
SP22	5′-CCGATTTTAATTGGATTCTGTCATGCGATCATGAGAACC-3′
SP23	5′-ACAGAATCCAATTAAAATCGGCATCTGGATCAA-3′
SP24	5′-TTAATTGGATTCTATCATTGTATCATGAGAACCCAGGCG-3′
SP25	5′-ACAATGATAGAATCCAATTAAAATCGGCATCTG-3′
SP26	5′-ATTGGATTCTATCATGCGTGTATGAGAACCCAGGCGATT-3′
SP27	5′-ACACGCATGATAGAATCCAATTAAAATCGGCAT-3′
SP28	5′-GGATTCTATCATGCGATCTGTAGAACCCAGGCGATTTCA-3′
SP29	5′-ACAGATCGCATGATAGAATCCAATTAAAATCGG-3′
SP30	5′-CAAATGGCGATGATGCTTTGTATTATGCCAATTATGATT-3′
SP31	5′-ACAAAGCATCATCGCCATTTGCGGATTTTGCTG-3′
SP32	5′-GCGATGATGCTTTGGATTTGTCCAATTATGATTATCGTA-3′
SP33	5′-ACAAATCCAAAGCATCATCGCCATTTGCGGATT-3′
SP34	5′-ATGATGCTTTGGATTATGTGTATTATGATTATCGTATTT-3′
SP35	5′-ACACATAATCCAAAGCATCATCGCCATTTGCGG-3′
SP36	5′-ATGCTTTGGATTATGCCATGTATGATTATCGTATTTGCG-3′
SP37	5′-ACATGGCATAATCCAAAGCATCATCGCCATTTG-3′
SP38	5′-CTTTGGATTATGCCAATTTGTATTATCGTATTTGCGATC-3′
SP39	5′-ACAAATTGGCATAATCCAAAGCATCATCGCCAT-3′
SP40	5′-TGGATTATGCCAATTATGTGTATCGTATTTGCGATCAAC-3′
SP41	5′-ACACATAATTGGCATAATCCAAAGCATCATCGC-3′
SP42	5′-ATGCCAATTATGATTATCTGTTTTGCGATCAACTTCCCG-3′
SP43	5′-ACAGATAATCATAATTGGCATAATCCAAAGCAT-3′
SP44	5′-CCAATTATGATTATCGTATGTGCGATCAACTTCCCGGCG-3′
SP45	5′-ACATACGATAATCATAATTGGCATAATCCAAAG-3′
SP46	5′-ATTATGATTATCGTATTTTGTATCAACTTCCCGGCGGCT-3′
SP47	5′-ACAAAATACGATAATCATAATTGGCATAATCCA-3′
SP48	5′-ATGATTATCGTATTTGCGTGTAACTTCCCGGCGGCTCTT-3′
SP49	5′-ACACGCAAATACGATAATCATAATTGGCATAAT-3′
SP50	5′-GTAGTTGGTAACTTGTTTTGTATTGCGCAAACTTTCCTC-3′
SP51	5′-ACAAAACAAGTTACCAACTACCCAATAAAGAGA-3′
SP52	5′-GTTGGTAACTTGTTTATGTGTGCGCAAACTTTCCTCATT-3′
SP53	5′-ACACATAAACAAGTTACCAACTACCCAATAAAG-3′
SP54	5′-GGTAACTTGTTTATGATTTGTCAAACTTTCCTCATTAAA-3′
SP55	5′-ACAAATCATAAACAAGTTACCAACTACCCAATA-3′
SP56	5′-ACCATTTTAATTCGTTTATGTATTTTACCGCTGATGATT-3′
SP57	5′-ACATAAACGAATTAAAATGGTAACTAGAATAAT-3′
SP58	5′-CAAAATCCGCAAATGGCGTGTATGCTTTGGATTATGCCA-3′
SP59	5′-ACACGCCATTTGCGGATTTTGCTGCGCATTGCC-3′
SP60	5′-AATCCGCAAATGGCGATGTGTCTTTGGATTATGCCAATT-3′
SP61	5′-ACACATCGCCATTTGCGGATTTTGCTGCGCATT-3′
SP62	5′-ATTATGCCAATTATGATTTGTGTATTTGCGATCAACTTC-3′
SP63	5′-ACAAATCATAATTGGCATAATCCAAAGCATCAT-3′

Table S4. Cont.

Primer	DNA sequence
SP64	5′-CTTTCAATTATTCTAGTTTGTATTTTAATTCGTTTATTA-3′
SP65	5′-ACAAACTAGAATAATTGAAAGCCCGTAGTTATC-3′
SP66	5′-ATTCTAGTTACCATTTTATGTCGTTTATTAATTTTACCG-3′
SP67	5′-ACATAAAATGGTAACTAGAATAATTGAAAGCCC-3′
SP68	5′-ATTTTAATTGGATTCTATTGTGCGATCATGAGAACCCAG-3′
SP69	5′-ACAATAGAATCCAATTAAAATCGGCATCTGGAT-3′
SP70	5′-TACGGGCTTTCAATTATTTGTGTTACCATTTTAATTCGT-3′
SP71	5′-ACAAATAATTGAAAGCCCGTAGTTATCTCCCGT-3′
SP72	5′-TGGGTAGTTGGTAACTTGTGTATGATTGCGCAAACTTTC-3′
SP73	5′-ACACAAGTTACCAACTACCCAATAAAGAGAAAG-3′
SP74	5′-GCGGCTCTTTCTCTTTATCGCGTAGTTGGTAACTTGTTT-3′
SP75	5′-TATTGGGTAGTTGGTAACCGCTTTATGATTGCGCAAACT-3′
SP76	5′-ATTCTACGCACCATTTTAATTGCCTTA-3′
SP77	5′-AATGGTGCGTAGAATAATTGAAAGCCC-3′
SP78	5′-CTAGTTCGCATTTTAATTGCCTTATTA-3′
SP79	5′-TAAAATGCGAACTAGAATAATTGAAAG-3′
SP80	5′-GTTACCCGCTTAATTGCCTTATTAATT-3′
SP81	5′-AATTAAGCGGGTAACTAGAATAATTGA-3′
SP82	5′-ATTTTACGCGCCTTATTAATTTTACCG-3′
SP83	5′-TAAGGCGCGTAAAATGGTAACTAGAAT-3′
SP84	5′-ATTGCCCGCTTAATTTTACCGCTGATG-3′
SP85	5′-AATTAAGCGGGCAATTAAAATGGTAAC-3′
SP86	5′-GCCTTACGCATTTTACCGCTGATGATT-3′
SP87	5′-TAAAATGCGTAAGGCAATTAAAATGGT-3′
SP88	5′-TTATTACGCTTACCGCTGATGATTAAG-3′
SP89	5′-CGGTAAGCGTAATAAGGCAATTAAAAT-3′
SP90	5′-TTAATTCGCCCGCTGATGATTAAGCAG-3′
SP91	5′-CAGCGGGCGAATTAATAAGGCAATTAA-3′
SP92	5′-ATTTTACGCCTGATGATTAAGCAGCTG-3′
SP93	5′-CATCAGGCGTAAAATTAATAAGGCAAT-3′
SP94	5′-TGTTTCCGCATTTTGATCCAGATGCCG-3′
SP95	5′-CAAAATGCGGAAACATCCCGCCAATGG-3′
SP96	5′-TTCCCGCGCTTGATCCAGATGCCGATT-3′
SP97	5′-GATCAAGCGCGGGAAACATCCCGCCAA-3′
SP98	5′-CCGATTCGCATCCAGATGCCGATTTTA-3′
SP99	5′-CTGGATGCGAATCGGGAAACATCCCGC-3′
SP100	5′-ATTTTGCGCCAGATGCCGATTTTAATT-3′
SP101	5′-CATCTGGCGCAAAATCGGGAAACATCC-3′
SP102	5′-ATCCAGCGCCCGATTTTAATTGGATTC-3′
SP103	5′-AATCGGGCGCTGGATCAAAATCGGGAA-3′
SP104	5′-CAGATGCGCATTTTAATTGGATTCTAT-3′
SP105	5′-TAAAATGCGCATCTGGATCAAAATCGG-3′
SP106	5′-ATGCCGCGCTTAATTGGATTCTATCAT-3′
SP107	5′-AATTAAGCGCGGCATCTGGATCAAAAT-3′
SP108	5′-CCGATTCGCATTGGATTCTATCATGCG-3′
SP109	5′-TCCAATGCGAATCGGCATCTGGATCAA-3′
SP110	5′-GGTGTCCGCACATTTGTTCAGCAAAAA-3′
SP111	5′-AAATGTGCGGACACCGGCAACGATAGG-3′
SP112	5′-GTCGCTCGCTTTGTTCAGCAAAAACTG-3′
SP113	5′-AACAAAGCGAGCGACACCGGCAACGAT-3′
SP114	5′-GCTACACGCGTTCAGCAAAAACTGATG-3′
SP115	5′-CTGAACGCGTGTAGCGACACCGGCAAC-3′
SP116	5′-ACATTTCGCCAGCAAAAACTGATGATG-3′
SP117	5′-TTGCTGGCGAAATGTAGCGACACCGGC-3′
SP118	5′-TTTGTTCGCCAAAAACTGATGATGGCT-3′
SP119	5′-TTTTTGGCGAACAAATGTAGCGACACC-3′
SP120	5′-GTTCAGCGCAAACTGATGATGGCTGGC-3′
SP121	5′-CAGTTTGCGCTGAACAAATGTAGCGAC-3′
SP122	5′-CAGCAACGCCTGATGATGGCTGGCAAT-3′
SP123	5′-CATCAGGCGTTGCTGAACAAATGTAGC-3′
SP124	5′-CAAAAACGCATGATGGCTGGCAATGCG-3′
SP125	5′-CATCATGCGTTTTTGCTGAACAAATGT-3′

Table S4. Cont.

Primer	DNA sequence
SP126	5′-ATGGCGCGCATGCTTTGGATTATGCCA-3′
SP127	5′-AAGCATGCGCGCCATTTGCGGATTTTG-3′
SP128	5′-GCGATGCGCCTTTGGATTATGCCAATT-3′
SP129	5′-CCAAAGGCGCATCGCCATTTGCGGATT-3′
SP130	5′-ATGATGCGCTGGATTATGCCAATTATG-3′
SP131	5′-AATCCAGCGCATCATCGCCATTTGCGG-3′
SP132	5′-ATGCTTCGCATTATGCCAATTATGATT-3′
SP133	5′-CATAATGCGAAGCATCATCGCCATTTG-3′
SP134	5′-CTTTGGCGCATGCCAATTATGATTATC-3′
SP135	5′-TGGCATGCGCCAAAGCATCATCGCCAT-3′
SP136	5′-TGGATTCGCCCAATTATGATTATCGTA-3′
SP137	5′-AATTGGGCGAATCCAAAGCATCATCGC-3′
SP138	5′-ATGCCACGCATGATTATCGTATTTGCG-3′
SP139	5′-AATCATGCGTGGCATAATCCAAAGCAT-3′
SP140	5′-CCAATTCGCATTATCGTATTTGCGATC-3′
SP141	5′-GATAATGCGAATTGGCATAATCCAAAG-3′
SP142	5′-ATTATGCGCATCGTATTTGCGATCAAC-3′
SP143	5′-TACGATGCGCATAATTGGCATAATCCA-3′
SP144	5′-TCTCTTCGCTGGGTAGTTGGTAACTTG-3′
SP145	5′-TACCCAGCGAAGAGAAAGAGCCGCCGG-3′
SP146	5′-AAACTGCGCATGGCTGGCAATGCGCAG-3′
SP147	5′-AGCCATGCGCAGTTTTTGCTGAACAAA-3′
SP148	5′-CTGATGCGCGCTGGCAATGCGCAGCAA-3′
SP149	5′-GCCAGCGCGCATCAGTTTTTGCTGAAC-3′
SP150	5′-ATTATGCGCATTATGATTATCGTATTT-3′
SP151	5′-CATAATGCGCATAATCCAAAGCATCAT-3′
SP152	5′-ACCATTCGCATTGCCTTATTAATTTTA-3′
SP153	5′-GGCAATGCGAATGGTAACTAGAATAAT-3′
SP154	5′-GGTTGACTTCATCCAAGTCTC-3′
SP155	5′-AGCGACACCGGCAACGATAGGAAGAATATAGTA-3′
SP156	5′-ATCGTTGCCGGTGTCGCTGCGTTTGTTGCGGCGAAACTGATGATGGCT-3′
SP157	5′-AACTACCCAATAAAGAGAAAGAGCCGCCGGGAA-3′
SP158	5′-TCTCTTTATTGGGTAGTTGCGGCGTTGTTTATGATTGCG-3′
SP159	5′-CATTTCCTCTACATCATCATAC-3′
SP160	5′-TCCCGCCAATGGATTGACACC-3′
SP161	5′-GGTGTCAATCCATTGGCGGGAGCATTCCCGATTTTGATC-3′
SP162	5′-ATAAAGAGAAAGAGCCGCCGG-3′
SP163	5′-CCGGCGGCTCTTTCTCTTTATTGCGTAGTTGGTAAC-3′
SP164	5′-GATCAAAATCGGGAAACATCC-3′
SP165	5′-GGATGTTTCCCGATTTTGATCCTTATGCCGATTTTAATT-3′
SP166	5′-CTTTGGATTATGCCAATTATGTGCATCGTATTTGCGATC-3′
SP167	5′-CCTATCGTTGCCGGTGTCGCTCTTTTTGTTCTTCTTAAACTGATGATGGCT-3′
SP168	5′-CTTTCTCTTTATTGGGTAGTTCTTCTTTTGTTTATGATTGCG-3′
SP169	5′-GGATGTTTCCCGATTTTGATCAATATGCCGATTTTAATT-3′
SP170	5′-ATCGTTGCCGGTGTCGCTAATTTTGTTAATAATAAACTGATGATGGCT-3′
SP171	5′-CTTTCTCTTTATTGGGTAGTTAATCAGTTGTTTATGATTGCG-3′
SP172	5′-CATAATTGGCATAATCCAAAG-3′
SP173	5′-AATTAATAAACGAATTAAAAT-3′
SP174	5′-ATTTTAATTCGTTTATTAATT-3′
SP175	5′-TCTCTTTATTGGGTAGTTCGCAACTTGTTTATGATTGCG-3′
SP176	5′-TGTTTCCCGATTTTGATCCGCATGCCGATTTTAATTGGA-3′
SP177	5′-GTCAATCCATTGGCGGGAGCCTTCCCGATTTTGATCCAG-3′
SP178	5′-ATTATTCTAGTTACCATTTGTATTCGTTTATTAATTTTA-3′
SP179	5′-CTAGTTACCATTTTAATTTGTTTATTAATTTTACCGCTG-3′
SP180	5′-GCCGGTGTCGCTACATTTTGTCAGCAAAAACTGATGATG-3′
SP181	5′-GGTGTCGCTACATTTGTTTGTCAAAAACTGATGATGGCT-3′
SP182	5′-GCGGCTCTTTCTCTTTATTGTGTAGTTGGTAACTTGTTT-3′
SP183	5′-TTATGGTTTGACTTAGGATGTAAAGATCCTTACTATATT-3′
SP184	5′-TACTATATTCTTCCTATCTGTGCCGGTGTCGCTACATTT-3′
SP185	5′-TATATTCTTCCTATCGTTTGTGGTGTCGCTACATTTGTT-3′
SP186	5′-ATTCTTCCTATCGTTGCCTGTGTCGCTACATTTGTTCAG-3′
SP187	5′-CTTCCTATCGTTGCCGGTTGTGCTACATTTGTTCAGCAA-3′

Table S4. Cont.

Primer	DNA sequence
SP188	5′-CCTATCGTTGCCGGTGTCTGTACATTTGTTCAGCAAAAA-3′
SP189	5′-GTTGCCGGTGTCGCTACATGTGTTCAGCAAAAACTGATG-3′
SP190	5′-GTCGCTACATTTGTTCAGTGTAAACTGATGATGGCTGGC-3′
SP191	5′-GCTACATTTGTTCAGCAATGTCTGATGATGGCTGGCAAT-3′
SP192	5′-CTTTATTGGGTAGTTGGTCGCTTGTTTATGATTGCGCAA-3′
SP193	5′-GTAGTTGGTAACTTGTTTCGCATTGCGCAAACTTTCCTC-3′
SP194	5′-GTTGGTAACTTGTTTATGCGCGCGCAAACTTTCCTCATT-3′
SP195	5′-TTGGCGGGAGCCTTCCCGTGTTTGATCCAGATGCCGATT-3′
SP196	5′-GCGGGAGCCTTCCCGATTTGTATCCAGATGCCGATTTTA-3′
SP197	5′-GGAGCCTTCCCGATTTTGTGTCAGATGCCGATTTTAATT-3′
SP198	5′-TTCCCGATTTTGATCCAGTGTCCGATTTTAATTGGATTC-3′
SP199	5′-ATTTTGATCCAGATGCCGTGTTTAATTGGATTCTATCAT-3′
SP200	5′-ATCCAGATGCCGATTTTATGTGGATTCTATCATGCGATC-3′
SP201	5′-CAGATGCCGATTTTAATTTGTTTCTATCATGCGATCATG-3′
SP202	5′-ATCAACTTCCCGGCGGCTTGTTCTCTTTATTGGGTAGTT-3′
SP203	5′-AACTTCCCGGCGGCTCTTTGTCTTTATTGGGTAGTTGGT-3′
SP204	5′-TTCCCGGCGGCTCTTTCTTGTTATTGGGTAGTTGGTAAC-3′
SP205	5′-TATTGGGTAGTTGGTAACTGTTTTATGATTGCGCAAACT-3′
SP206	5′-GCTCTTTCTCTTTATTGGCGCGTTGGTAACTTGTTTATG-3′
SP207	5′-CTTTCTCTTTATTGGGTACGCGGTAACTTGTTTATGATT-3′
SP208	5′-TGGGTAGTTGGTAACTTGCGCATGATTGCGCAAACTTTC-3′
SP209	5′-CCGGCGGCTCTTTCTCTTTGTTGGGTAGTTGGTAACTTG-3′
SP210	5′-GCTCTTTCTCTTTATTGGTGTGTTGGTAACTTGTTTATG-3′
SP211	5′-GCCTTCCCGATTTTGATCTGTATGCCGATTTTAATTGGA-3′
SP212	5′-CCGATTTTGATCCAGATGTGTATTTTAATTGGATTCTAT-3′
SP213	5′-ATGCCGATTTTAATTGGATGTTATCATGCGATCATGAGA-3′
SP214	5′-CTTTCTCTTTATTGGGTATGTGGTAACTTGTTTATGATT-3′
SP215	5′-TCTCTTTATTGGGTAGTTTGTAACTTGTTTATGATTGCG-3′
SP216	5′-GAAAAAGATCCTTACTATTGTCTTCCTATCGTTGCCGGT-3′
SP217	5′-AAAGATCCTTACTATATTTGTCCTATCGTTGCCGGTGTC-3′
SP218	5′-GATCCTTACTATATTCTTTGTATCGTTGCCGGTGTCGCT-3′
SP219	5′-CCTTACTATATTCTTCCTTGTGTTGCCGGTGTCGCTACA-3′
SP220	5′-CCGCAAATGGCGATGATGTGTTGGATTATGCCAATTATG-3′
SP221	5′-ACACATCATCGCCATTTGCGGATTTTGCTGCGC-3′
SP222	5′-ATGGCGATGATGCTTTGGTGTATGCCAATTATGATTATC-3′
SP223	5′-ACACCAAAGCATCATCGCCATTTGCGGATTTTG-3′