Supplementary Information

Insertion sequence transposition inactivates CRISPR-Cas immunity

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Supplementary Figures

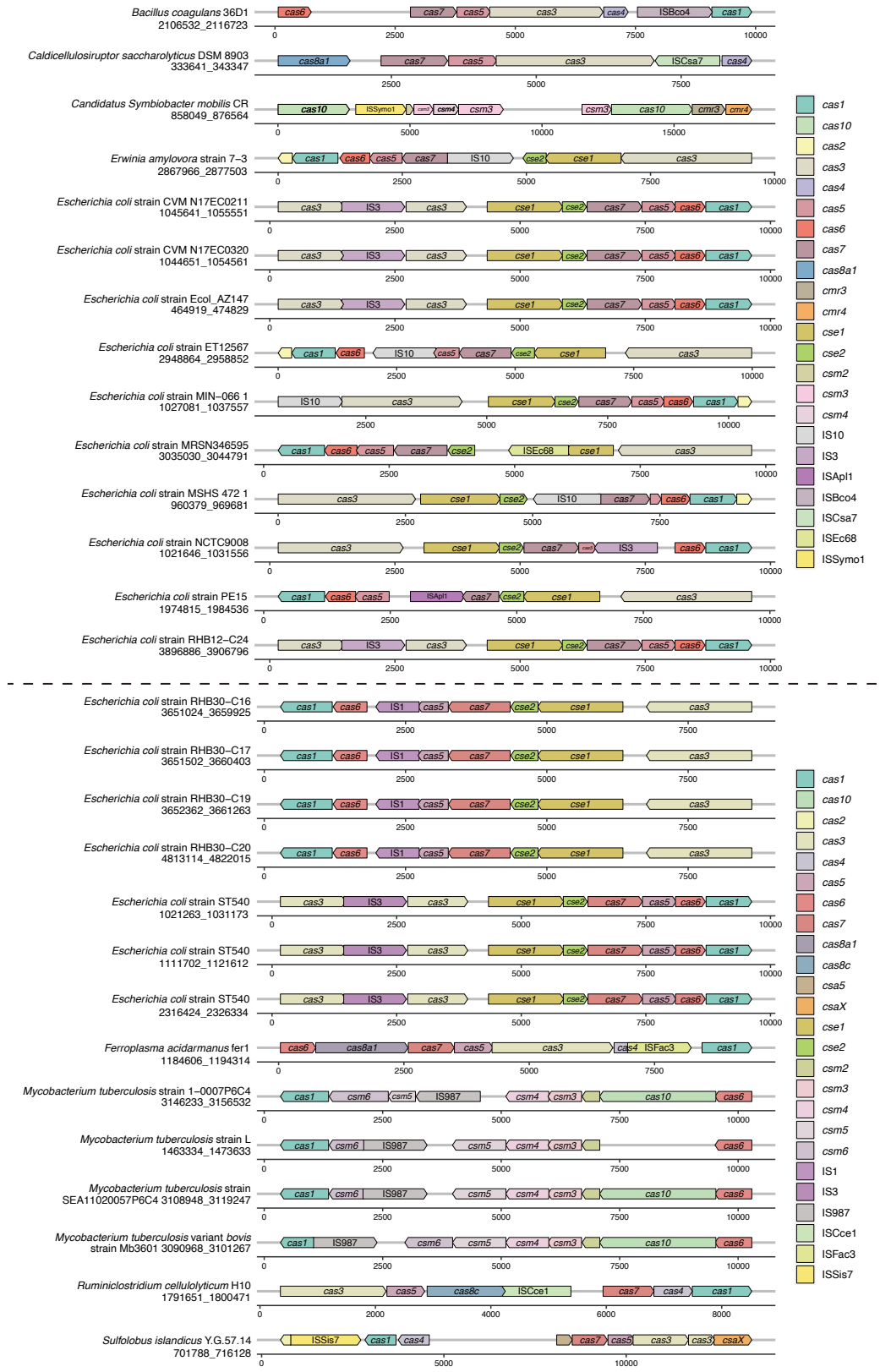
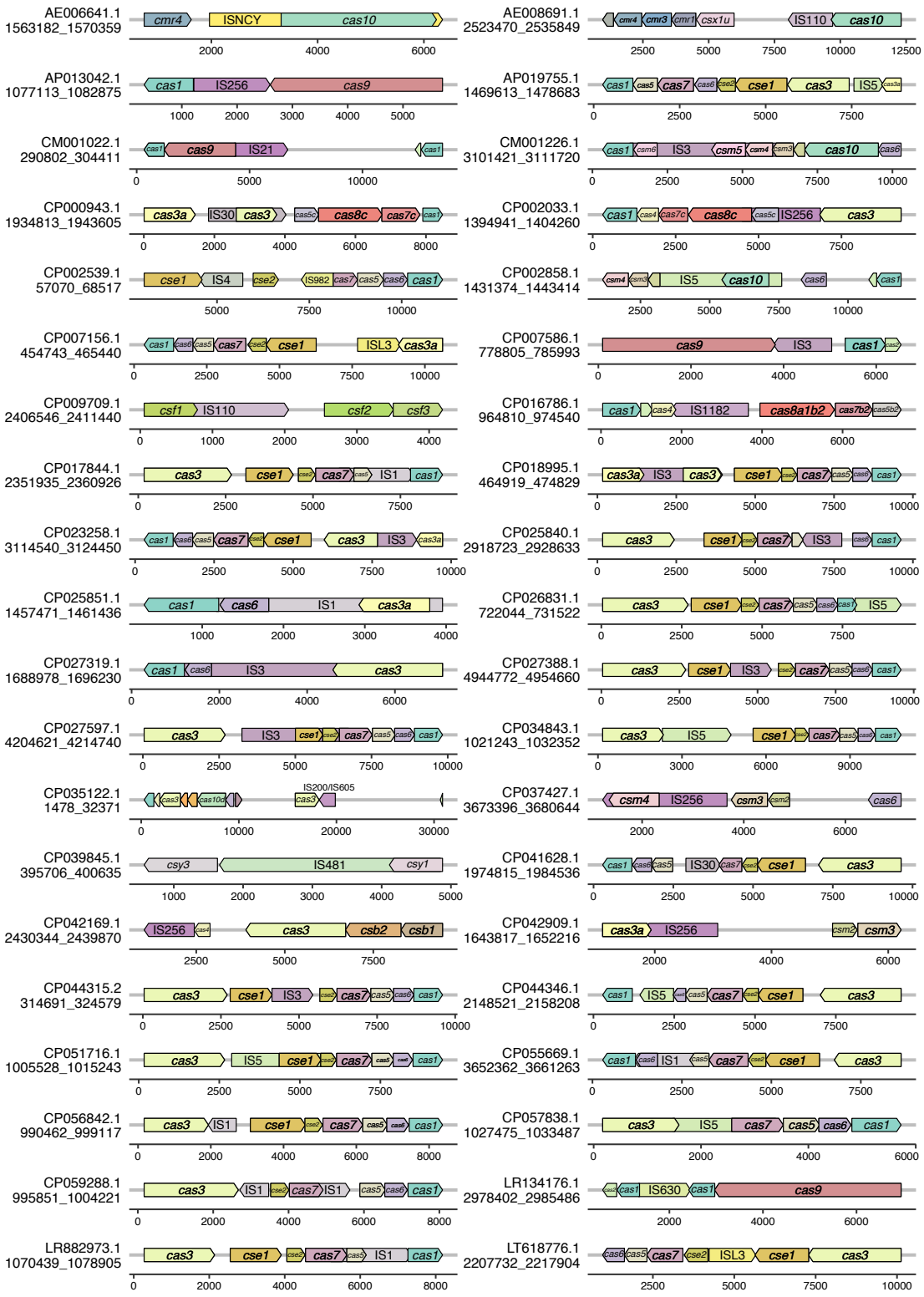


Figure S1. Identification of naturally occurring transpositions of ISs into *cas* genes based on ISfinder database. All 28 identified transposition events are shown above.

Each IS element in the figure represents a unique IS type.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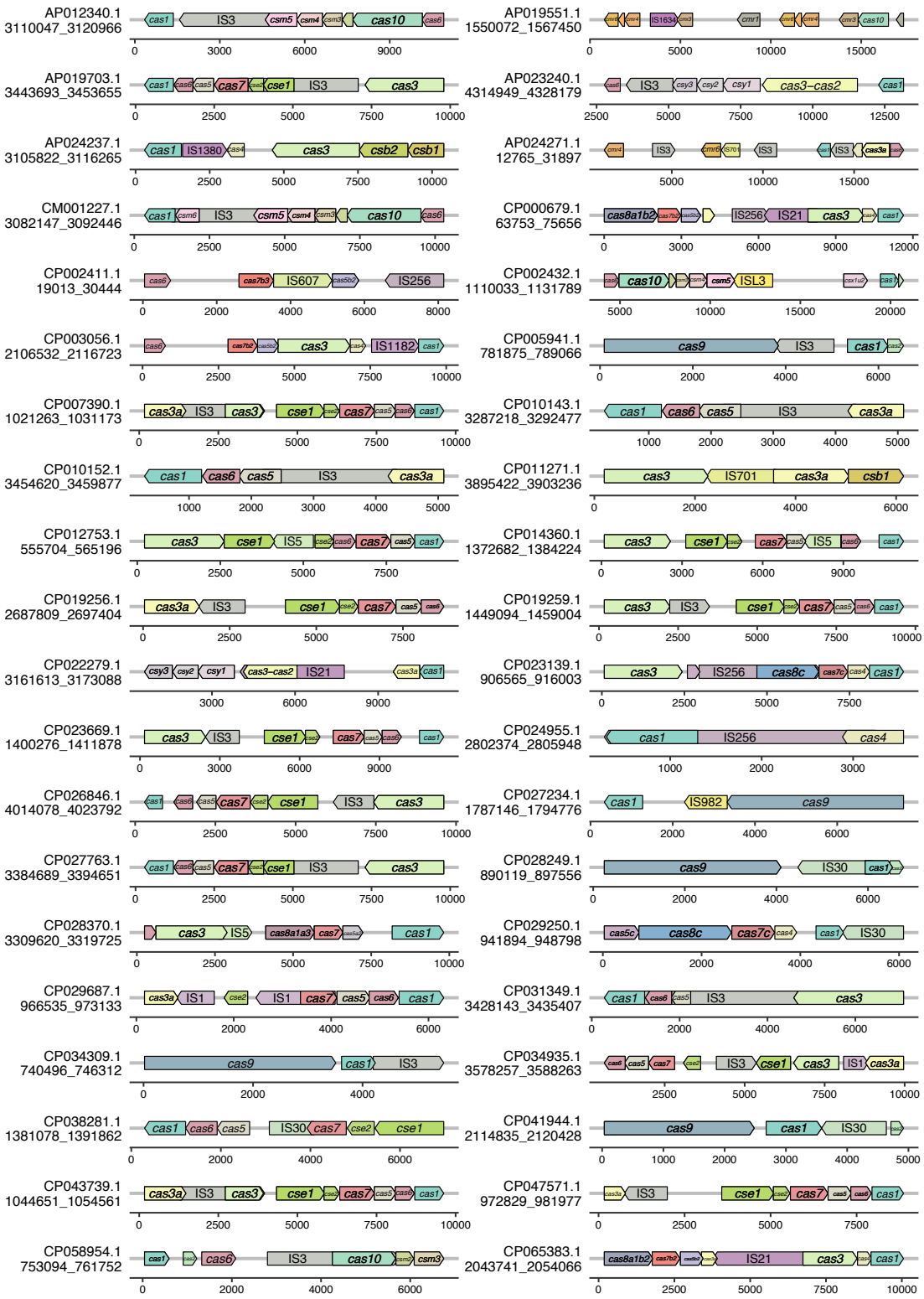
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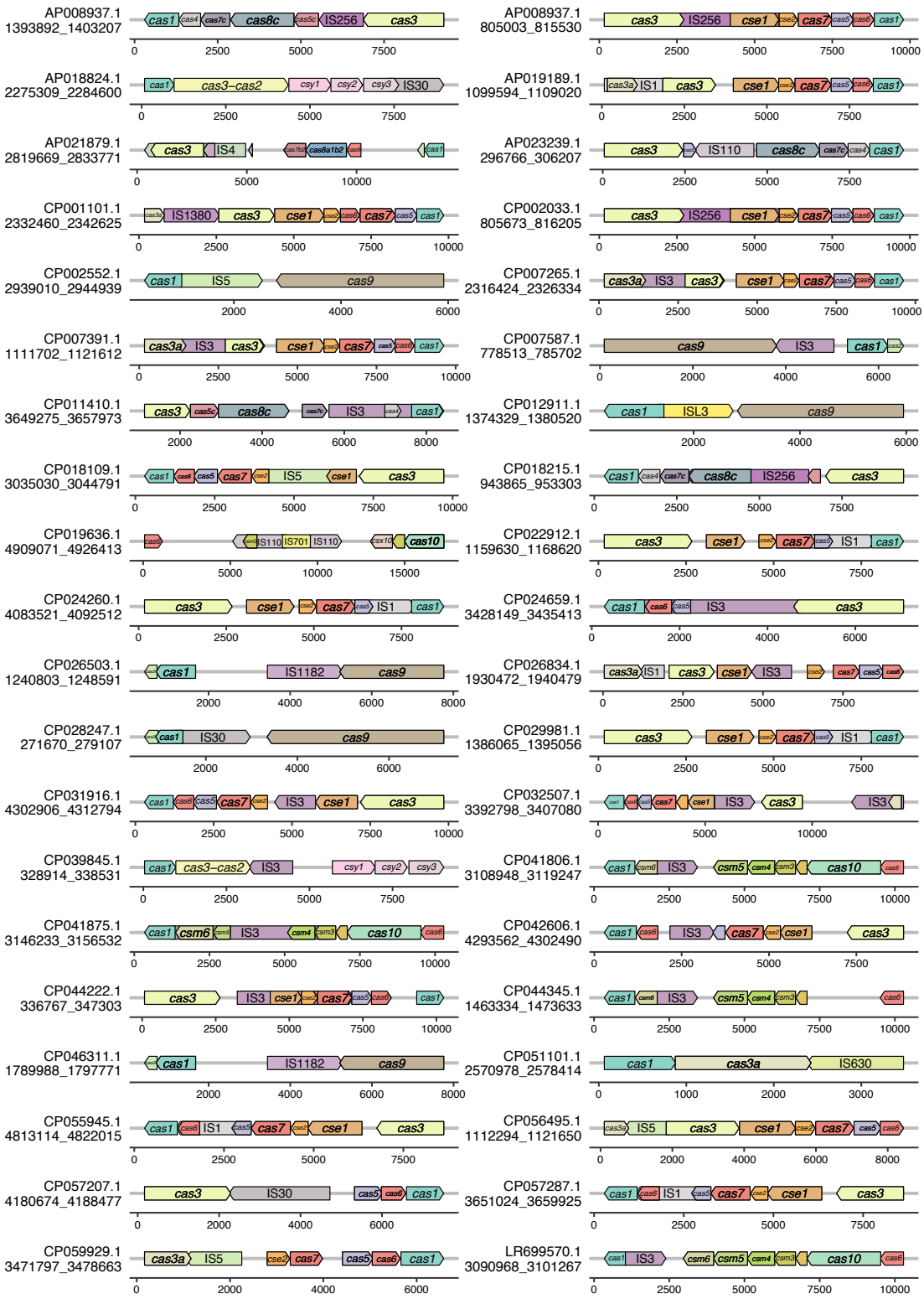
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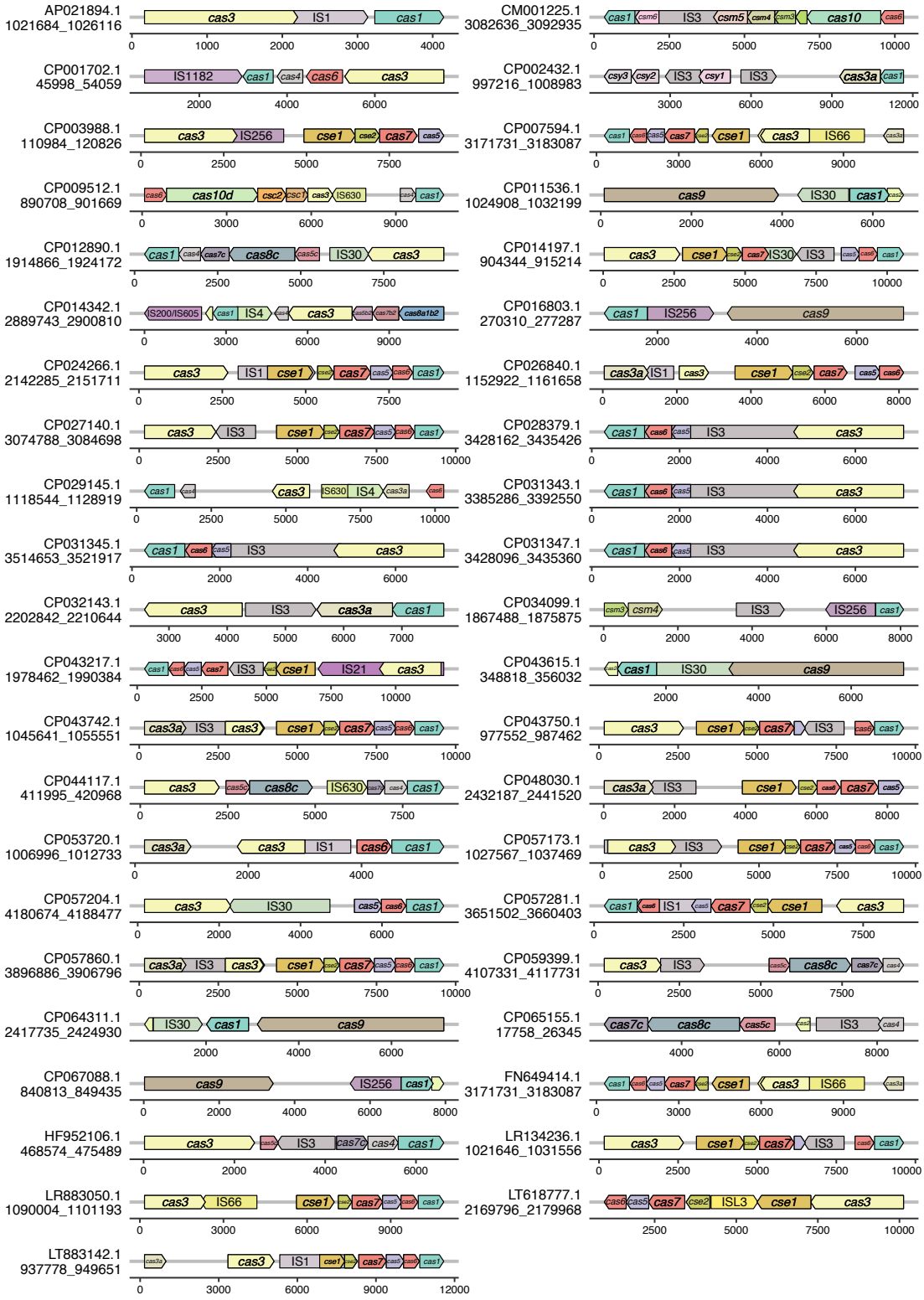

Figure S2. Identification of naturally occurring transpositions of ISs into *cas* genes using a highly sensitive software pipeline, ISEScan. A total of 163 transposition events are shown above. Each IS element in the figures represents a unique IS family.

Horizontal axes indicate the relative position of each gene or IS within the targeted genomic region.

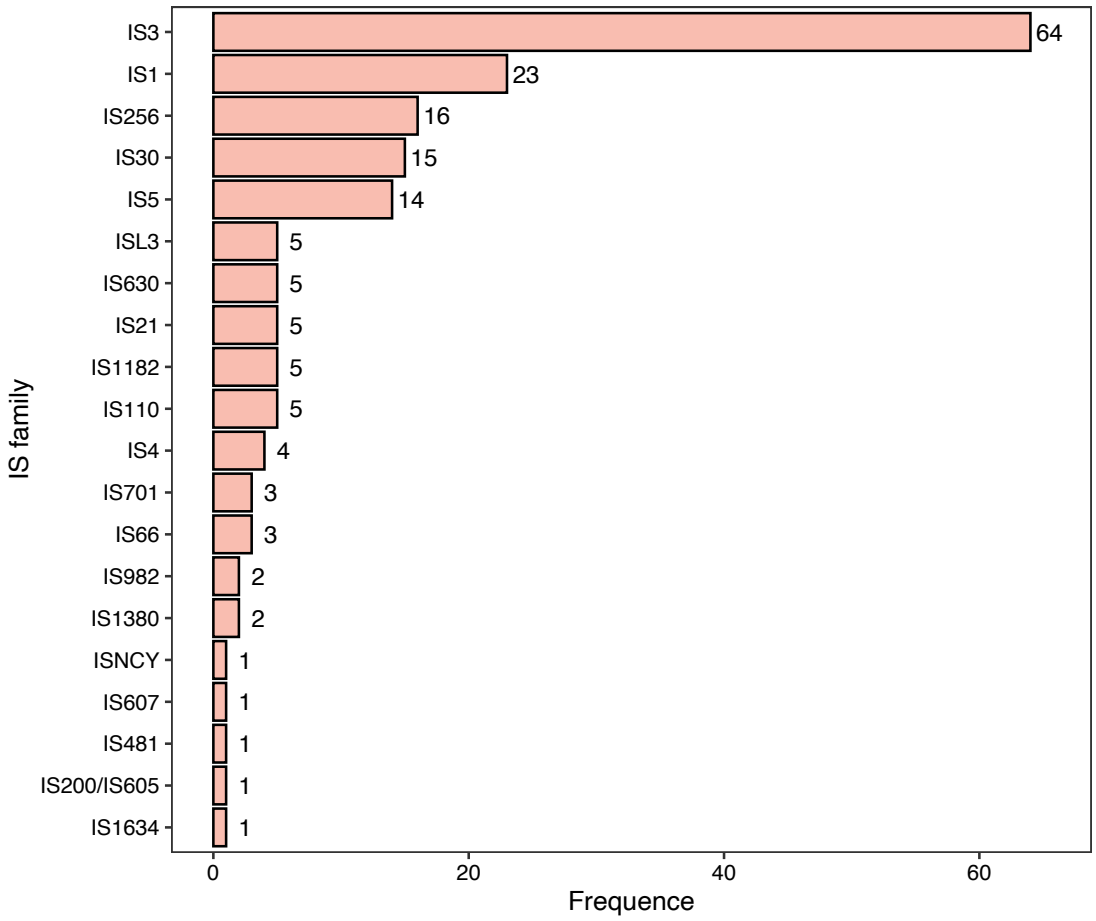

Figure S3. Analysis of ISs engagement in 163 cases of naturally occurring transpositions of ISs into *cas* genes. It is important to note that certain transposition events within the analyzed cases involve the participation of multiple IS families, rather than a single IS family.

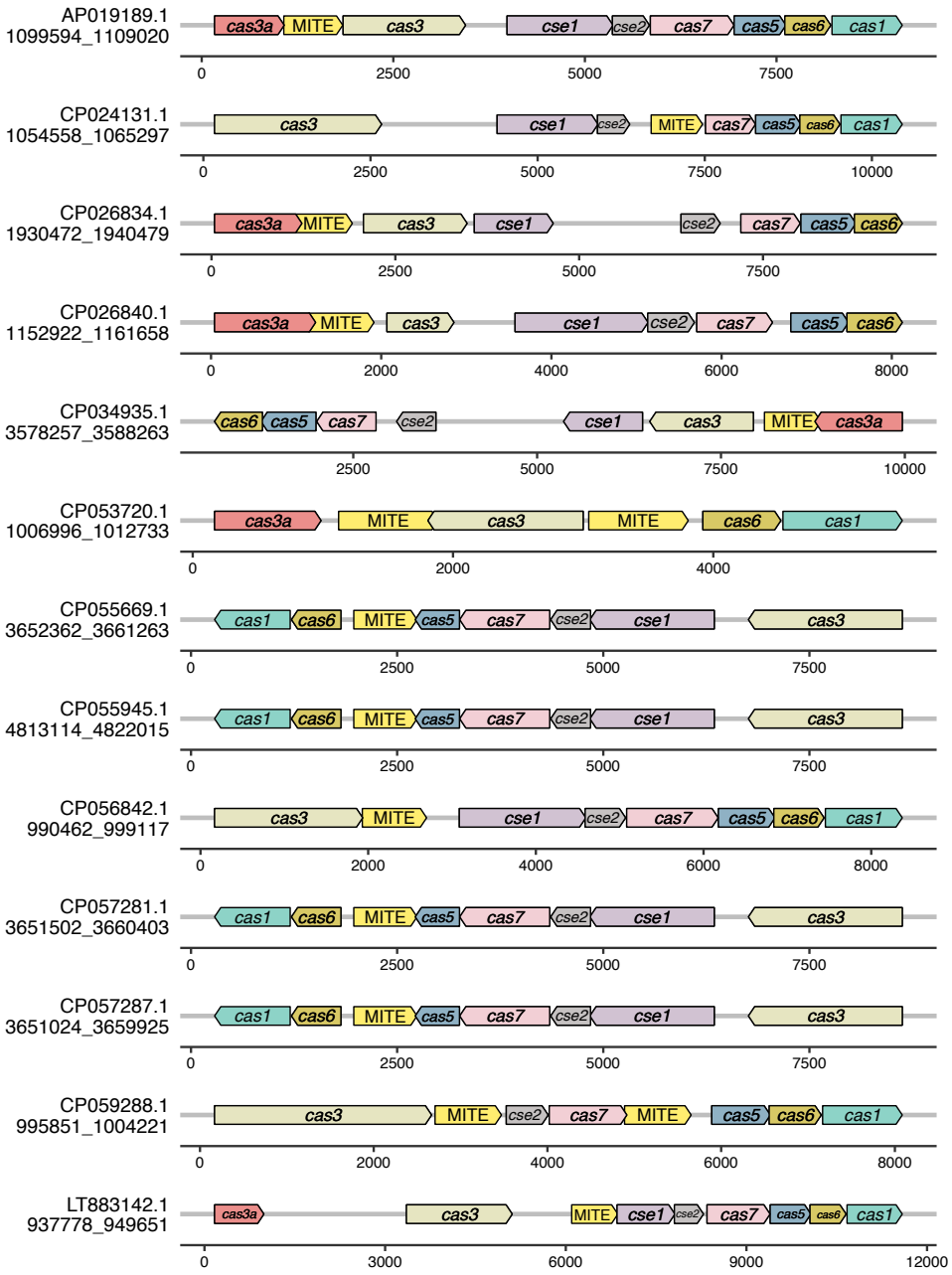

Figure S4. Naturally occurring transpositions of MITEs into *cas* genes. Horizontal axes indicate the relative position of each gene or MITE within the targeted genomic region.

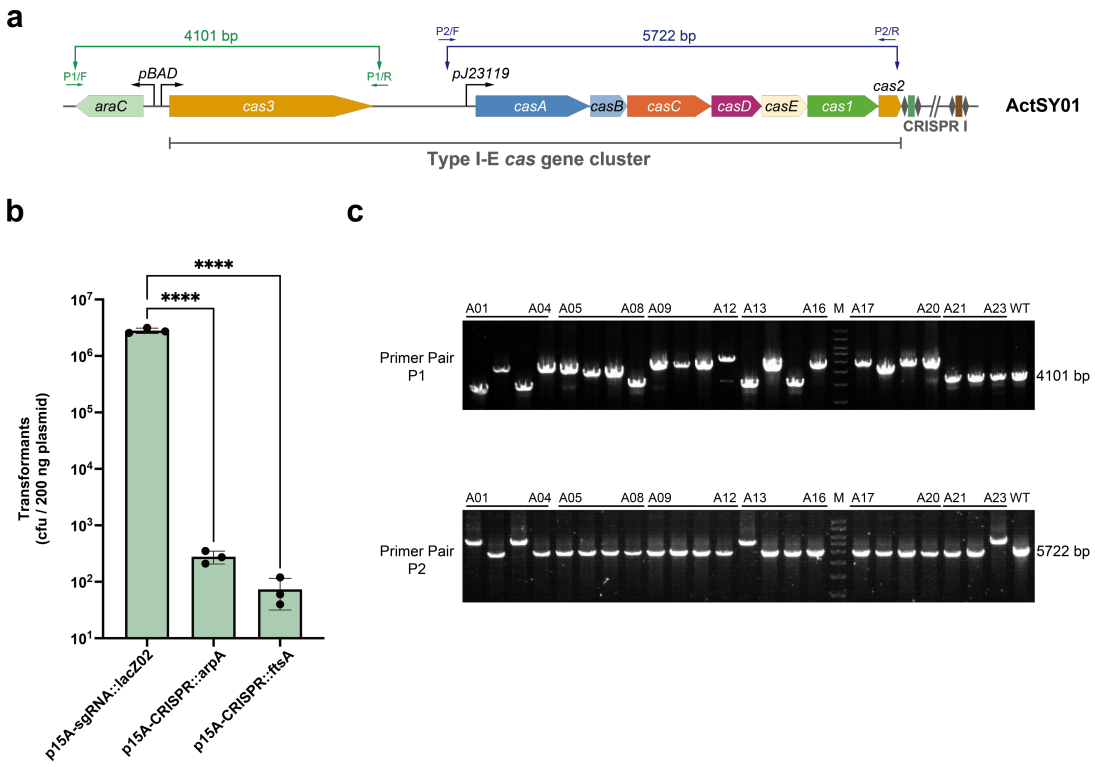

Figure S5. ISs mediate the fitness trade-off between the benefits of acquiring plasmids and the genotoxicity induced by the type I-E CRISPR/Cas system of *E. coli* DH10B under antibiotic pressure. a Schematic of the engineered type I-E CRISPR-Cas locus of *E. coli* DH10B. The strain ActSY01 was generated by replacing the native promoters of *casABCDE12* operon and *cas3* with a constitutive promoter J23119 and an arabinose-inducible promoter (pBAD), respectively. Primer pairs P1 and P2 were designed to amplify the entire *cas* cluster of the strain ActSY01; expected amplicon sizes are shown. **b** In the presence of L-arabinose induction, transformation efficiencies of p15A-sgRNA::lacZ02 (control, lacking the targeting spacer for the type I-E CRISPR/Cas system), p15A-CRISPR::arpA (targeting the non-essential gene *arpA* of the strain ActSY01) and p15A-CRISPR::ftsA (targeting the essential gene *ftsA* of the strain ActSY01) into the strain ActSY01 were determined through counting CFUs per 200 ng of plasmids. Error bars denote means ± S.D. from three biological replicates;

****, $P < 0.0001$, using two-tailed unpaired t-test; P = 9.03e-05, 9.03e-05 (from top to bottom). Source data are provided as a Source Data file. **c** Colony PCR screening with primer pairs P1 and P2 for IS insertions into the *cas* cluster of the 23 escapers (A01-A23). Lanes "WT", control amplicons with the strain ActSY01 template; larger PCR products suggest IS transposition events. Except for samples A8, A15, A21 and A22, IS insertions were detected within the *cas* genes of all other 19 samples. Detailed information regarding the IS types involved in transposition and their resulting TSD sequences can be found in Table S4. The experiment was conducted independently three times and yielded consistent results. Source data are provided as a Source Data file.

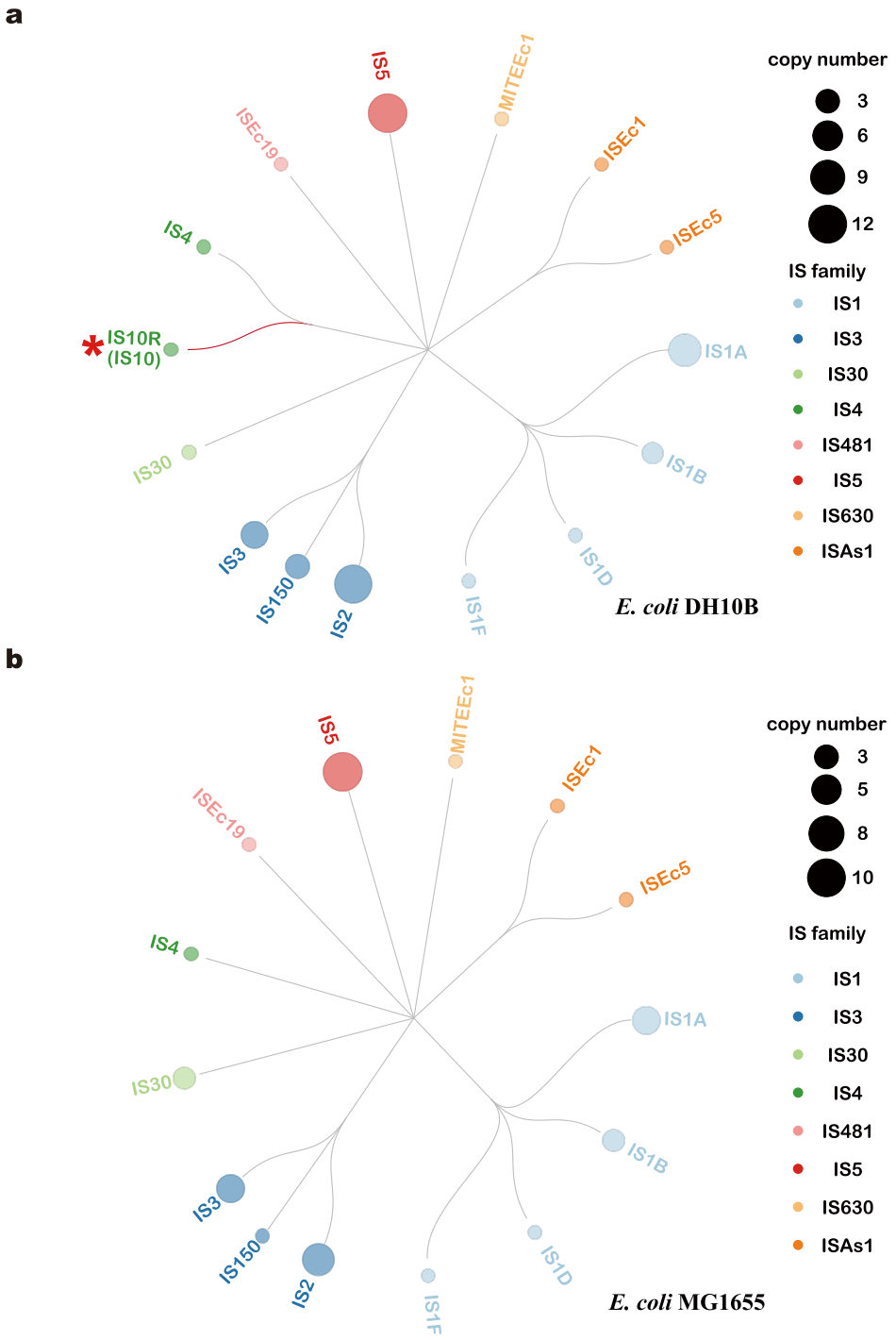

Figure S6. Analysis of the diversity and copy number of different insertion sequences within *E. coli* DH10B (a) and *E. coli* MG1655 (b). Each colored circle represents a specific IS family, and the circle size reflects the copy number of the IS element on the chromosome with a larger size indicating greater copy number. Except for the IS10 element, the same ISs were detected in both strains.

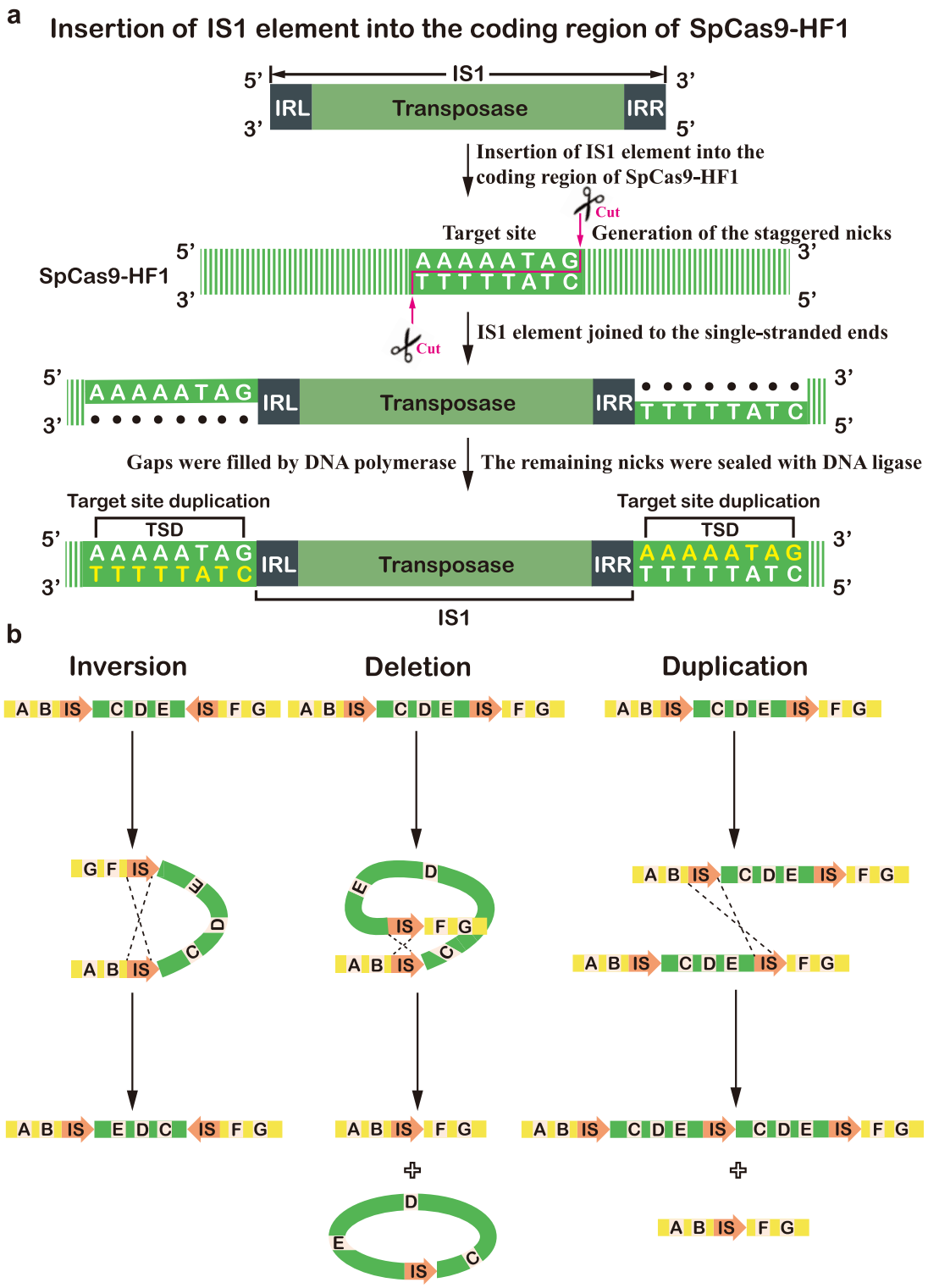

Figure S7. Schematic illustrations of the process for ISs insertion into the coding region of the Cas protein (a) and of the DNA rearrangements resulting from homologous recombination between two identical IS elements (b). a Transposition

example showing the insertion of IS1 into the coding region of SpCas9-HF1. IS1 is composed of the internal transposase flanked at both ends by two inverted repeats (IRL and IRR). The transposase cleaves at the target site within the coding region of SpCas9-HF1 and introduces the staggered nicks, followed by joining to the resulting single-stranded ends. Subsequently, the DNA gaps are filled by DNA polymerase, and the remaining nicks are sealed with DNA ligase, generating an 8 bp or 9 bp target site duplication around IS1 within the SpCas9-HF1 coding region. **b** Inversion, deletion, and duplication of the DNA segment resulting from recombination between two copies of the same IS element. The intervening sequence is likely inverted by homologous recombination when an IS element is inserted into the target sequence in two opposite orientations, whereas copies of the IS element in the same orientation may trigger either deletion or duplication of the intervening sequence.

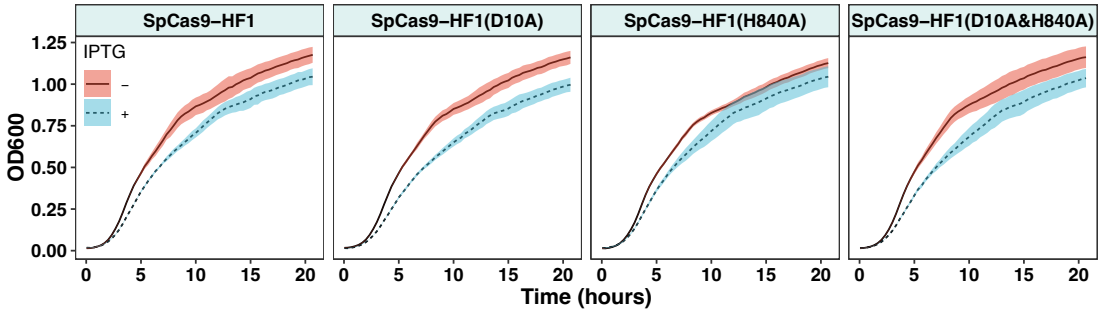

Figure S8. The mere induction of Cas protein expression does not lead to noticeable growth defects in the host strains. Growth curves of the strains encoding different Cas protein variants under both IPTG-induced and non-induced conditions. Shaded areas indicate the means ± S.D. from five biological replicates. Source data are provided as a Source Data file.

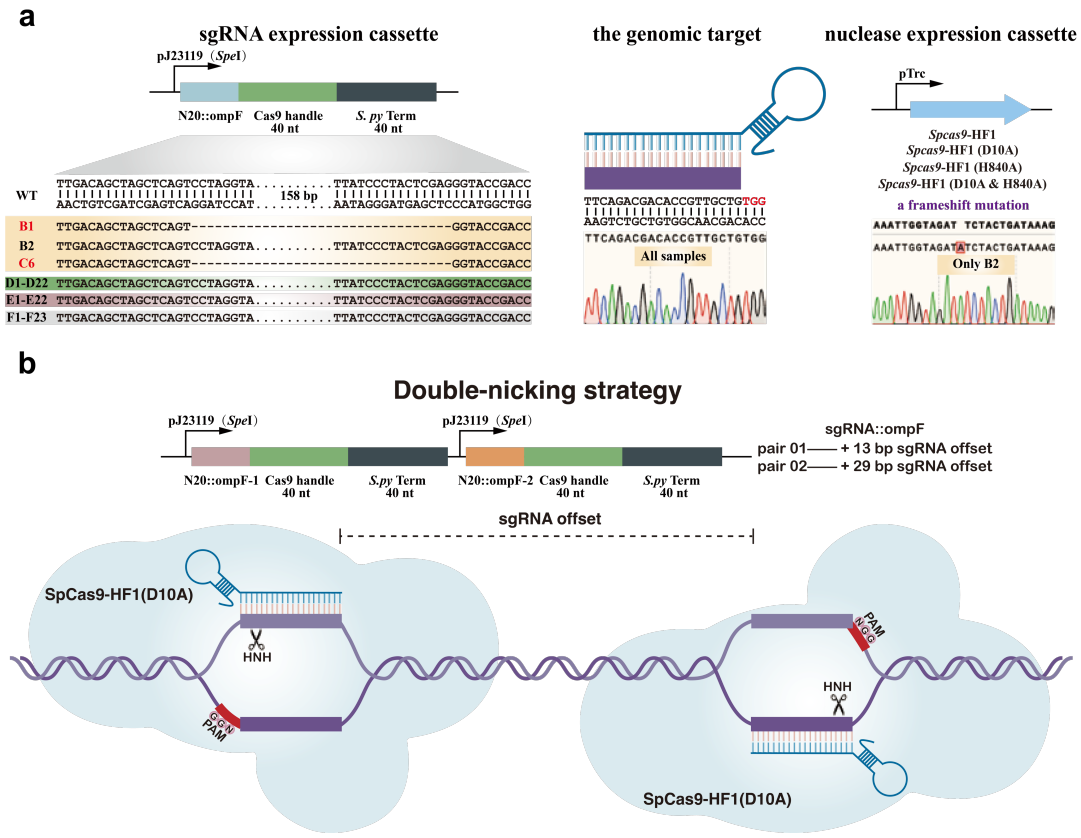

Figure S9. Identification of mutations in the CRISPR-tolerant mutants (a) and schematic illustration of the CRISPR double-nicking strategy (b). a Except for the colonies with IS insertions into the *cas* gene, all the remaining CRISPR-tolerant mutants (targeted by sgRNA::ompF) described in Figure 4c were further analyzed to determine the other factors that enabled escape from CRISPR-Cas interference. All genetic components necessary for the proper functioning of CRISPR-Cas system, including the sgRNA expression cassette, the genomic target, and the nuclease expression cassette, were sequenced by the Sanger method. Sequence analysis revealed a deletion mutation within the sgRNA expression cassettes of the mutant B1 and C6, as well as a frameshift mutation within the coding region of SpCas9-HF1 of the mutant B2, all of which led to their escape from SpCas9-HF1-mediated DSB. However, in contrast to SpCas9-HF1, the variants containing mutated nuclease domains, including

SpCas9-HF1 (D10A), SpCas9-HF1 (H840A), and SpCas9-HF1 (D10A & H840A), were unable to induce any detectable mutations which might potentially compromise CRISPR-Cas systems, indicating that they were incapable of triggering genotoxic stress. **b** The CRISPR double-nicking strategy with two pair of offset sgRNAs. The schematic depiction of the staggered DSB formation by SpCas9-HF1 (D10A) nickase in the presence of paired sgRNAs.

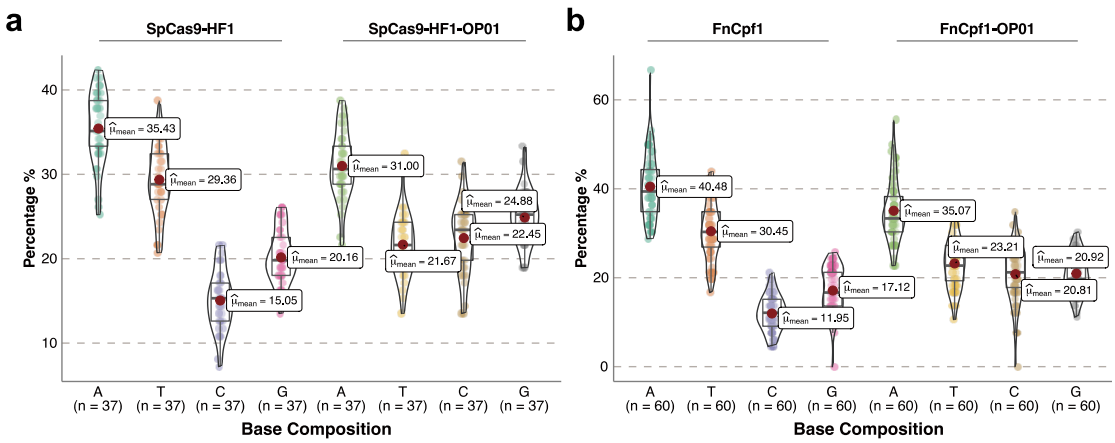

Figure S10. Comparison of base composition between the coding regions of SpCas9-HF1 and SpCas9-HF1-OP01 (a), and FnCpf1 and FnCpf1-OP01 (b). To determine the local base composition features within a nucleotide sequence, the coding regions of SpCas9-HF1, SpCas9-HF1-OP01, FnCpf1, and FnCpf1-OP01 were individually divided into 37, 37, 60, and 60 fragments, respectively, and the strand bias in nucleotide composition was computed for each segment. The total number of samples analyzed was indicated under each boxplot (n = 37 for SpCas9-HF1 and SpCas9-HF1-OP01; n = 60 for FnCpf1 and FnCpf1-OP01). The boxplots show medians (midlines), interquartile ranges (boxes) and ranges (whiskers).

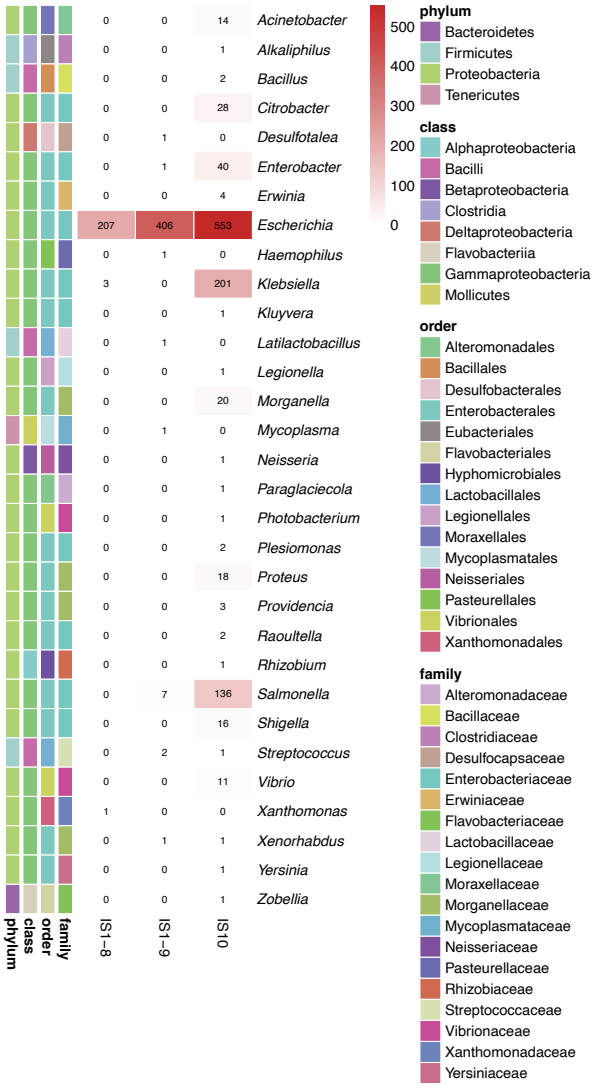

Figure S11. Enrichment analysis of the naturally occurring transposition events of IS1 and IS10 across bacterial taxonomic groups. The number of genomes per genus in which IS1 or IS10 had been transposed is indicated by a color gradient, and the actual numbers are displayed in boxes.

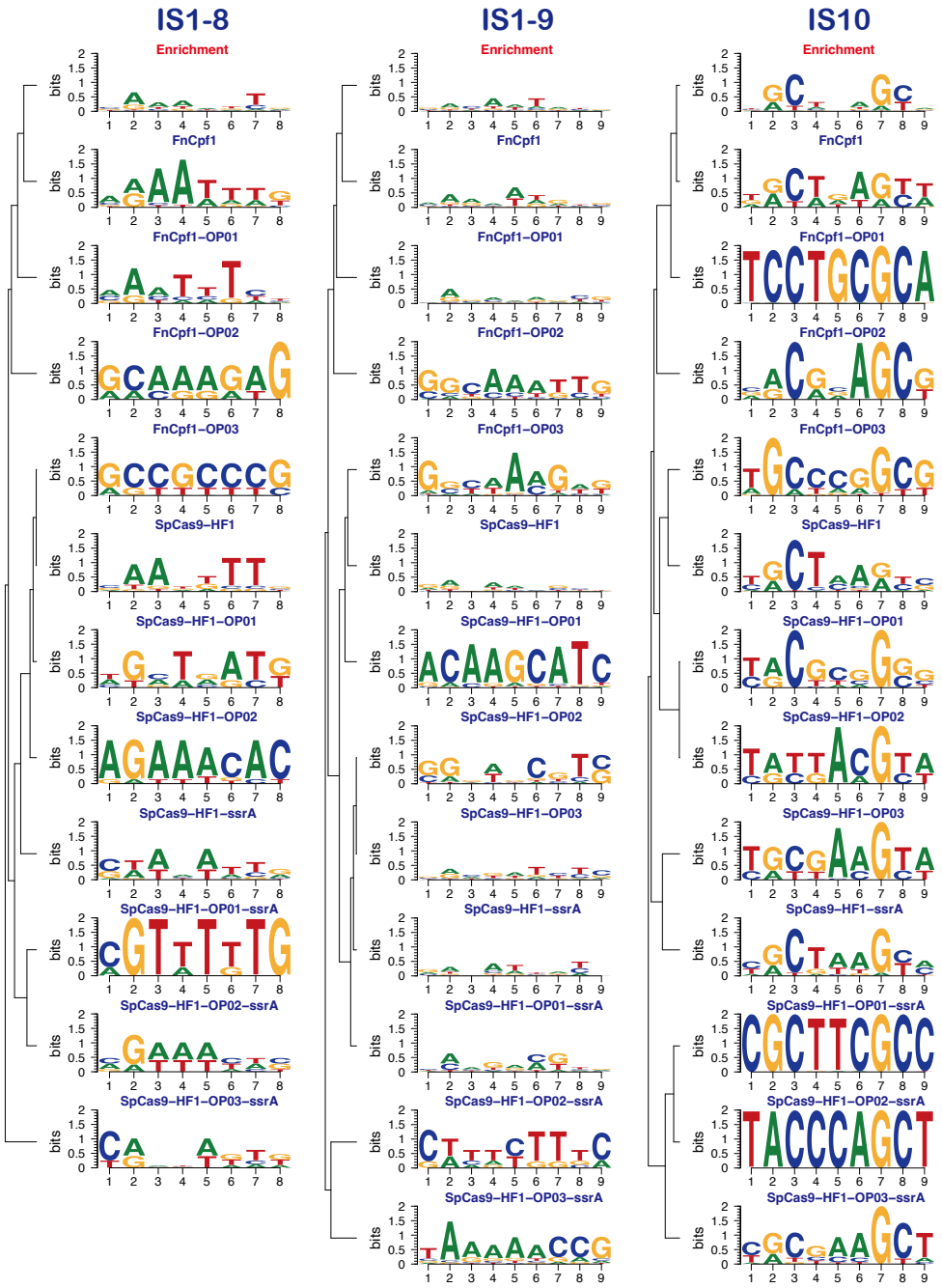

Figure S12. Comprehensive comparison of various TSD motifs generated by IS1 and IS10 within all constructed *cas* genes. Naturally occurring TSD motifs are labeled with "Enrichment" in red text (in reference to their enrichment/retrieval through bioinformatics analysis), and the name of each *cas* gene from which TSD sequences were collected is marked above the corresponding sequence logo.

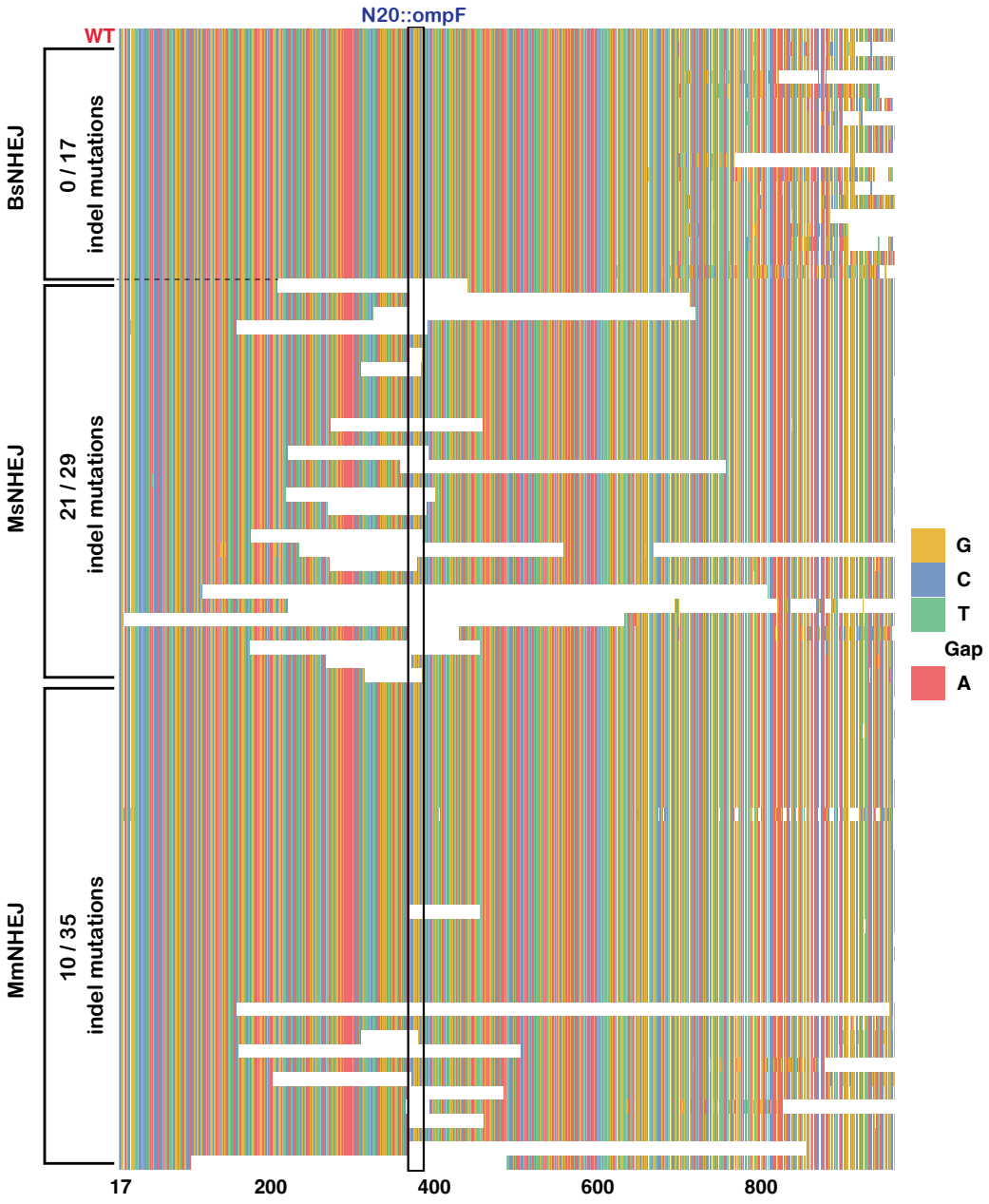

Figure S13. Detection of targeted indel mutations induced by NHEJ repair. The sgRNA targeting site (N20::ompF) is marked with a black rectangle, and the sequencing result from WT (without CRISPR/Cas-induced DSB) served as the control. A series of pixels was used to represent each nucleotide sequence, and the four distinct colors represent each base (A, T, C, G). Note that only the confidence intervals of sequencing results should be considered.

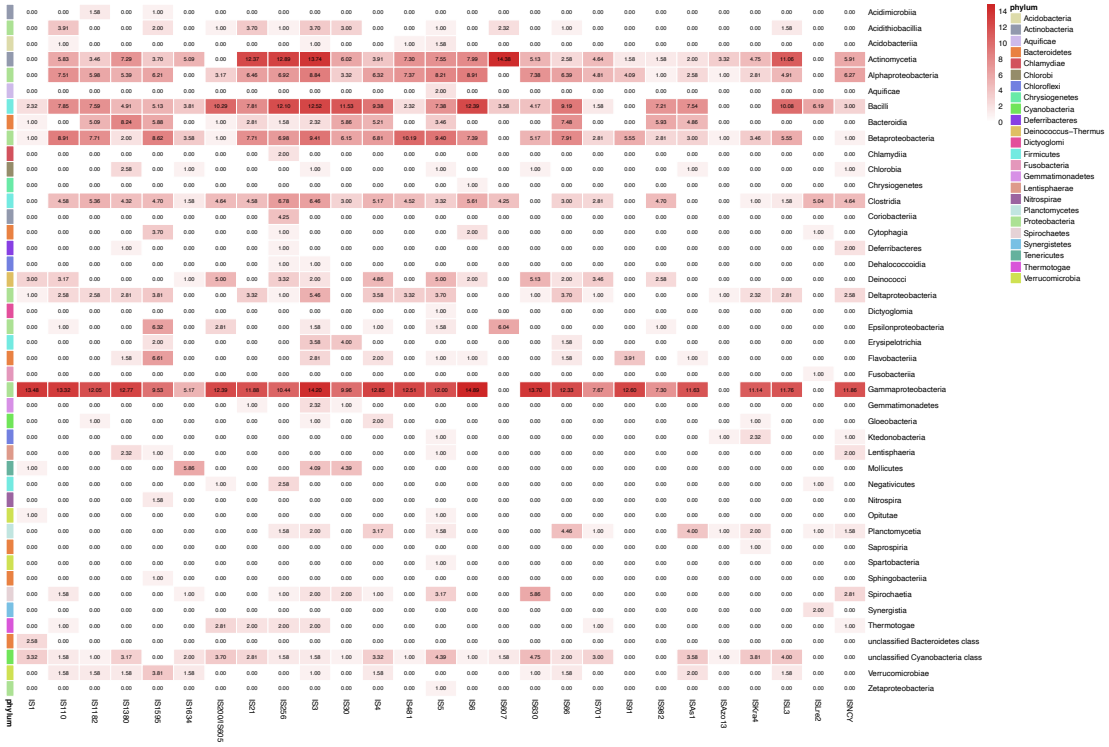

Figure S14. Diversity distribution of different IS families across bacterial taxonomic groups. The number of genomes per class containing a specific IS family is indicated by the color gradient from white (less) to firebrick red (more). Due to the different scales of the values within the heatmap analysis, the numbers in boxes were log-transformed (log2(x+1)) for better visualization.

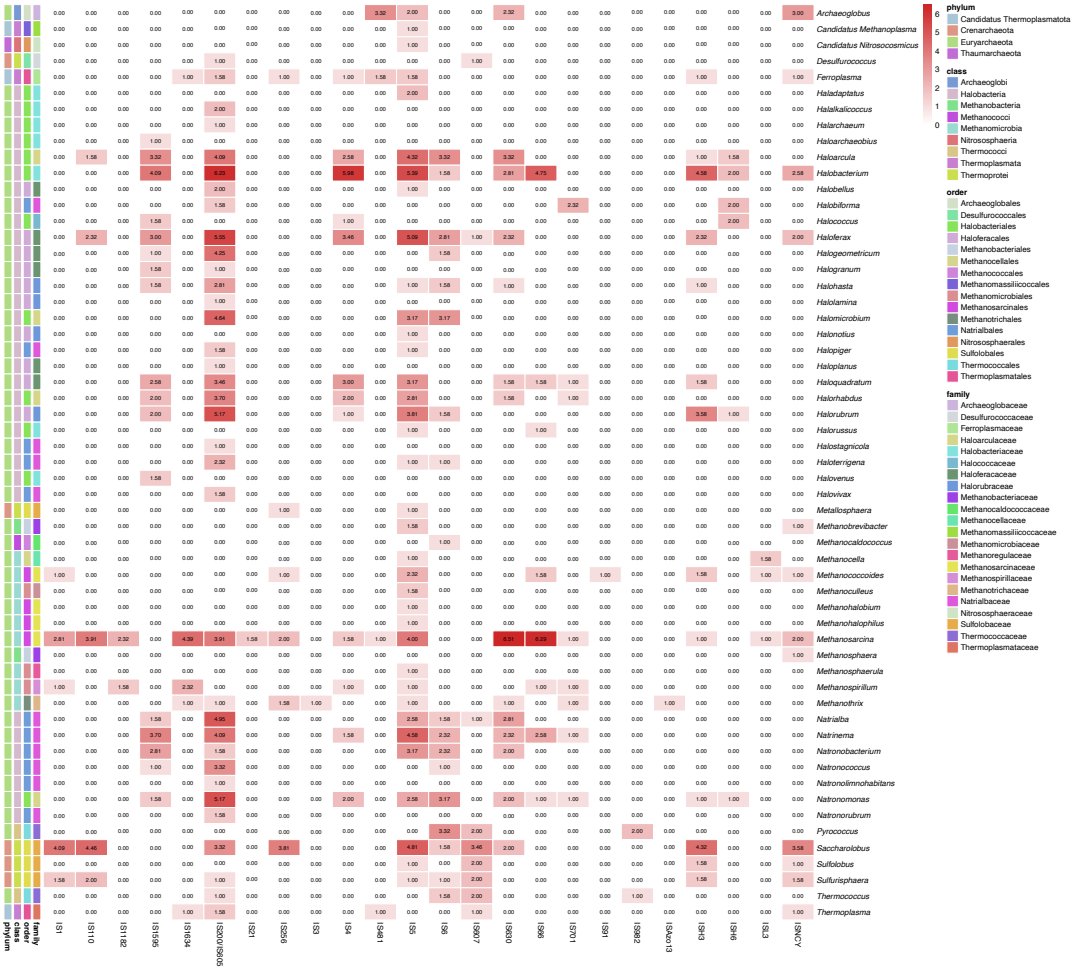

Figure S15. Diversity distribution of different IS families across archaeal taxonomic groups. The number of genomes per genus containing a specific IS family is indicated by the scaled color coding. The values in boxes were log-transformed (log2(x+1)) for better visualization.

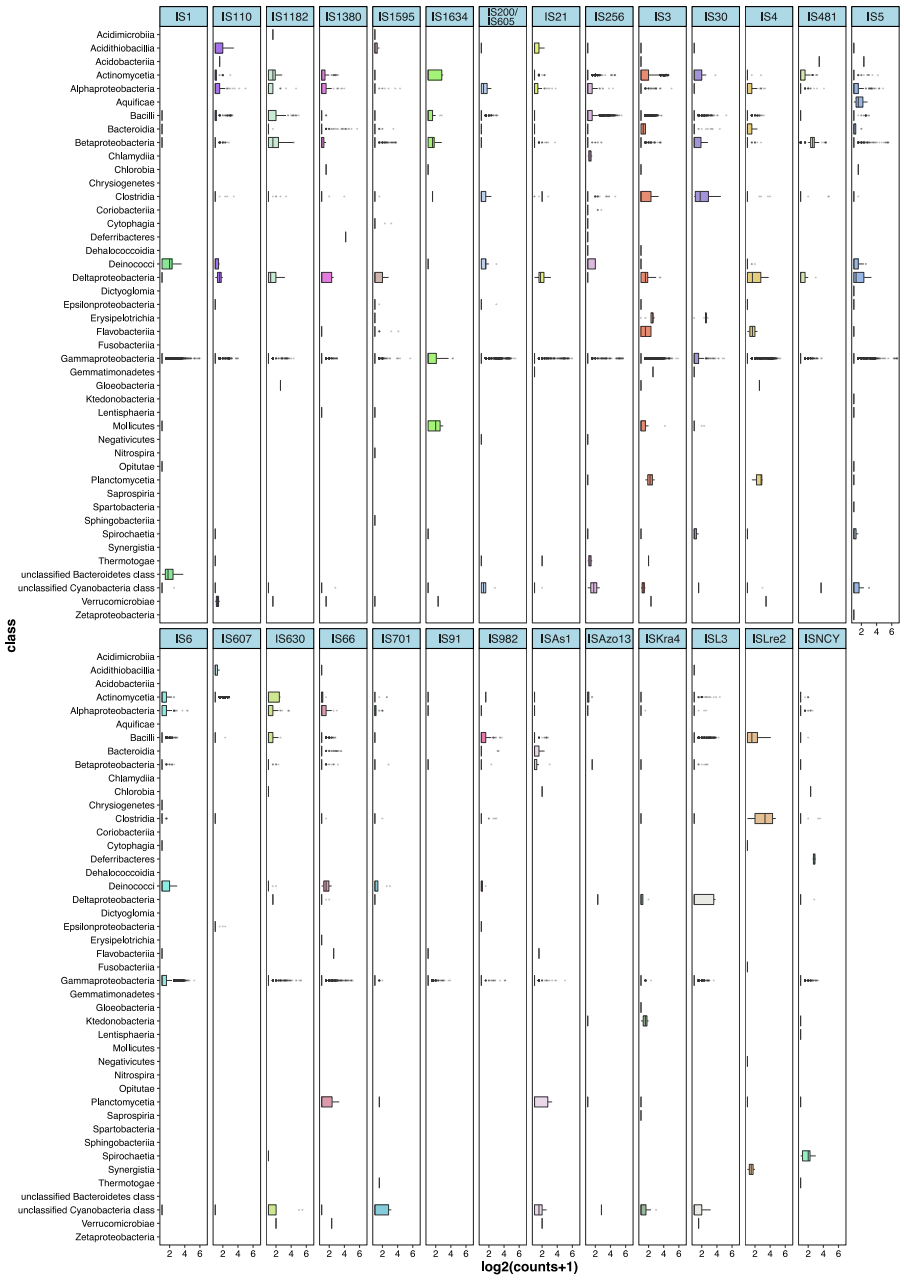

Figure S16. Distribution of different IS family counts among bacterial taxonomic groups. The boxplot diagram shows the counts per genome of each IS family across various bacterial genomes at the class level. The counts were log-transformed (log2(x+1)) for enhanced resolution, and only non-zero counts were plotted. The boxplots show medians (midlines), interquartile ranges (boxes) and ranges (whiskers). As the figure includes multiple boxplots, the number of genomes analyzed in each individual boxplot is provided in Supplementary Data 3.

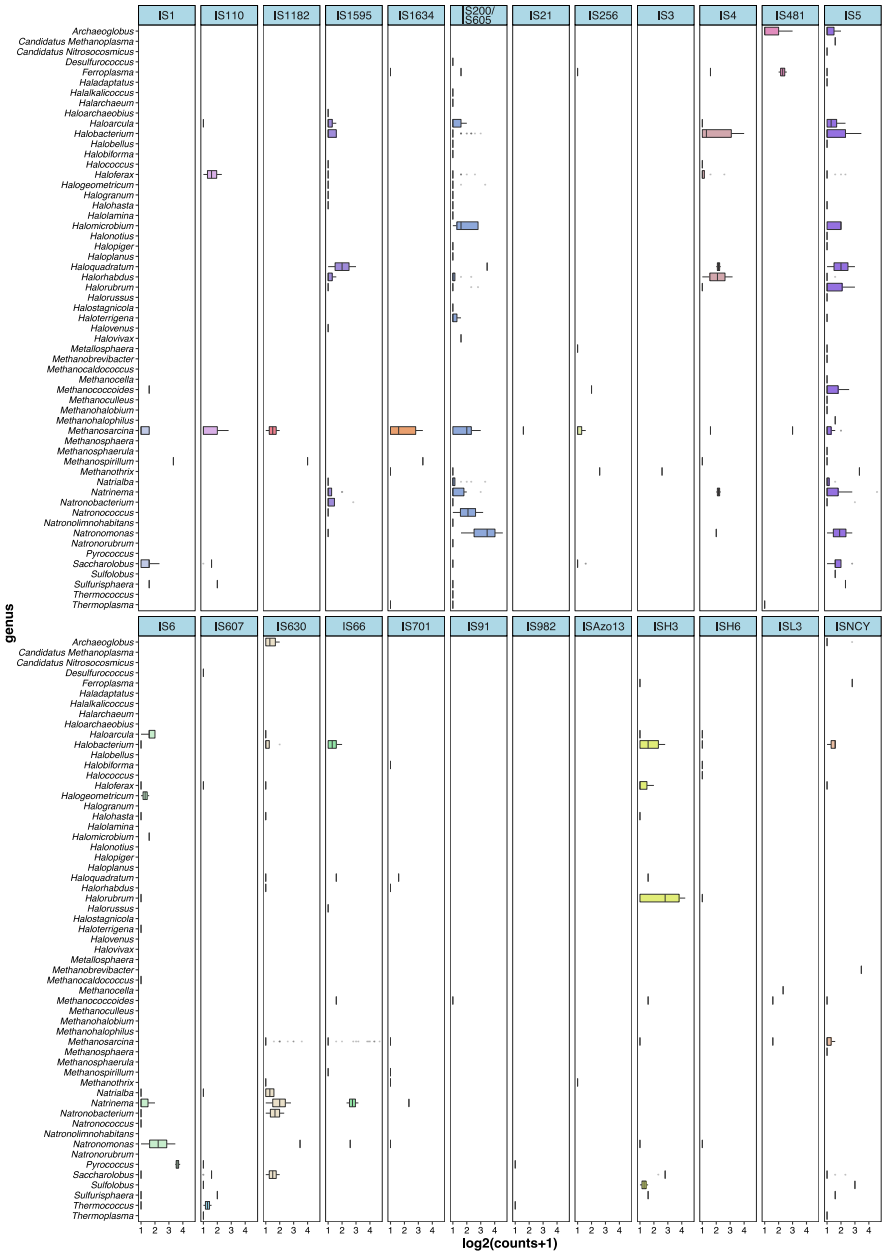

Figure S17. Distribution of different IS family counts among archaeal taxonomic groups. The boxplot diagram shows the counts per genome of each IS family across various archaeal genomes at the genus level. The count distributions were plotted on a log2(x+1) scale (x-axis) for better data visualization, and only non-zero values were plotted. The boxplots show medians (midlines), interquartile ranges (boxes) and ranges (whiskers). As the figure includes multiple boxplots, the number of genomes analyzed in each individual boxplot is provided in Supplementary Data 4.

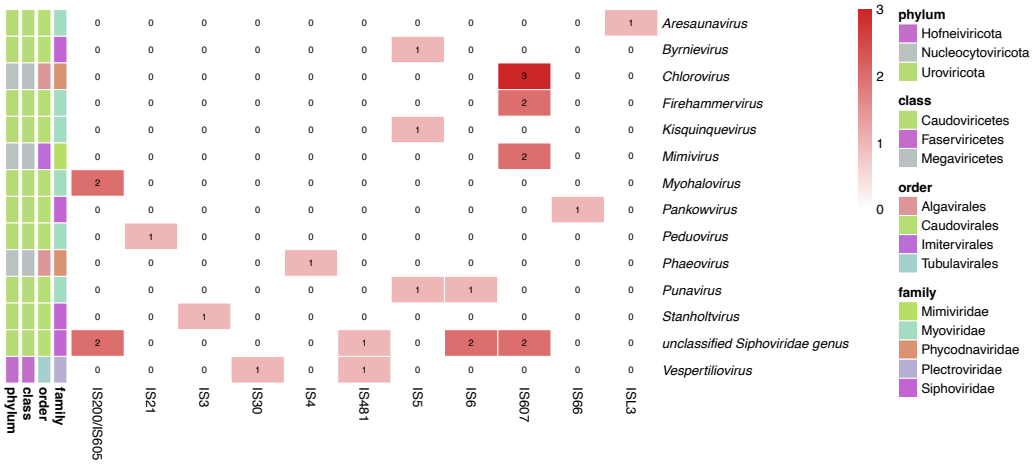

Figure S18. Diversity distribution of different IS families across viral taxonomic groups. The number of genomes per genus containing a specific IS family is indicated by a color gradient, and the numbers in boxes represent the actual values.

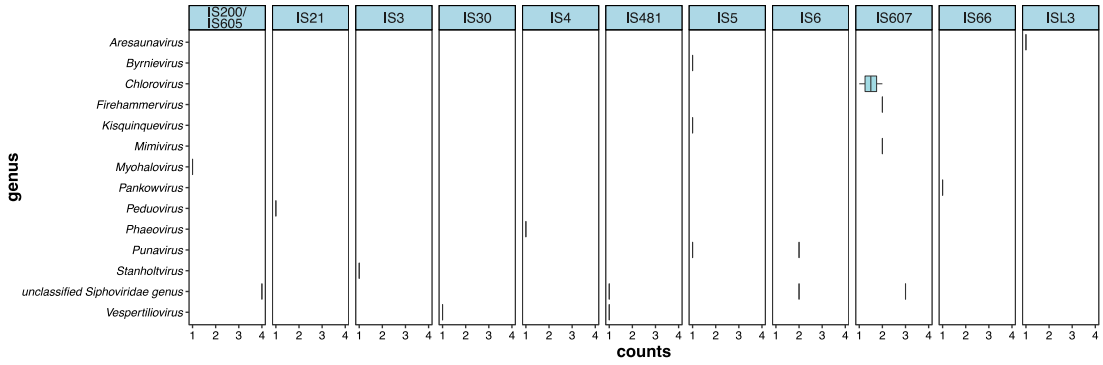

Figure S19. Distribution of different IS family counts among viral taxonomic groups. The boxplot diagram shows the counts per genome of each IS family across the various viral genomes at the genus level, and the horizontal coordinates represent the actual numbers. Only non-zero observations are shown. The boxplots show medians (midlines), interquartile ranges (boxes) and ranges (whiskers). As the figure includes multiple boxplots, the number of genomes analyzed in each individual boxplot is provided in Supplementary Data 5.

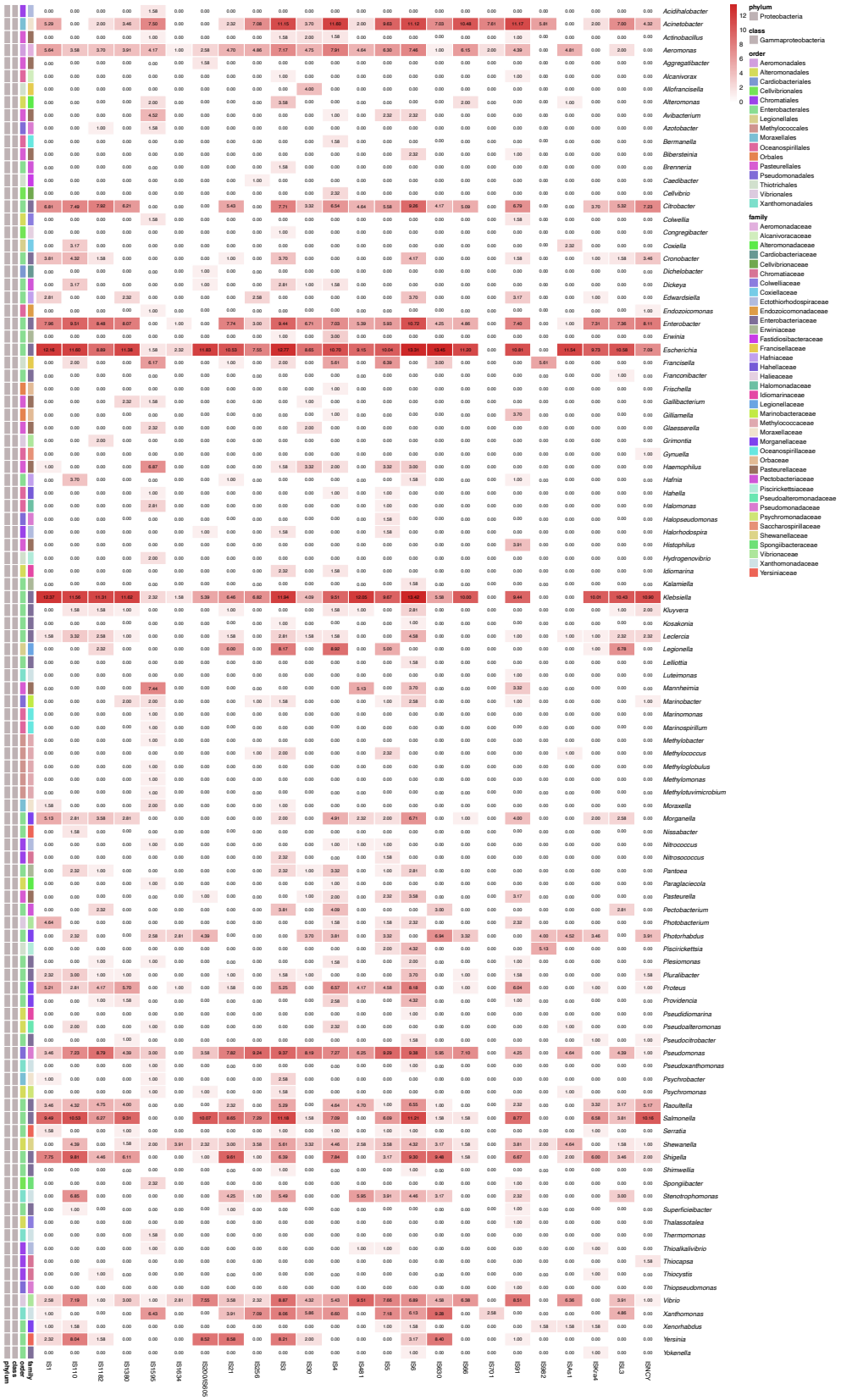

Figure S20. Diversity distribution of different IS families within the class Gammaproteobacteria. Heatmap analysis of the diversity distribution of different IS

families within the class Gammaproteobacteria at the genus level. The color gradient indicates the number of genomes per genus that contained the given IS family. The values in boxes were log-transformed (log2(x+1)) for greater clarity.

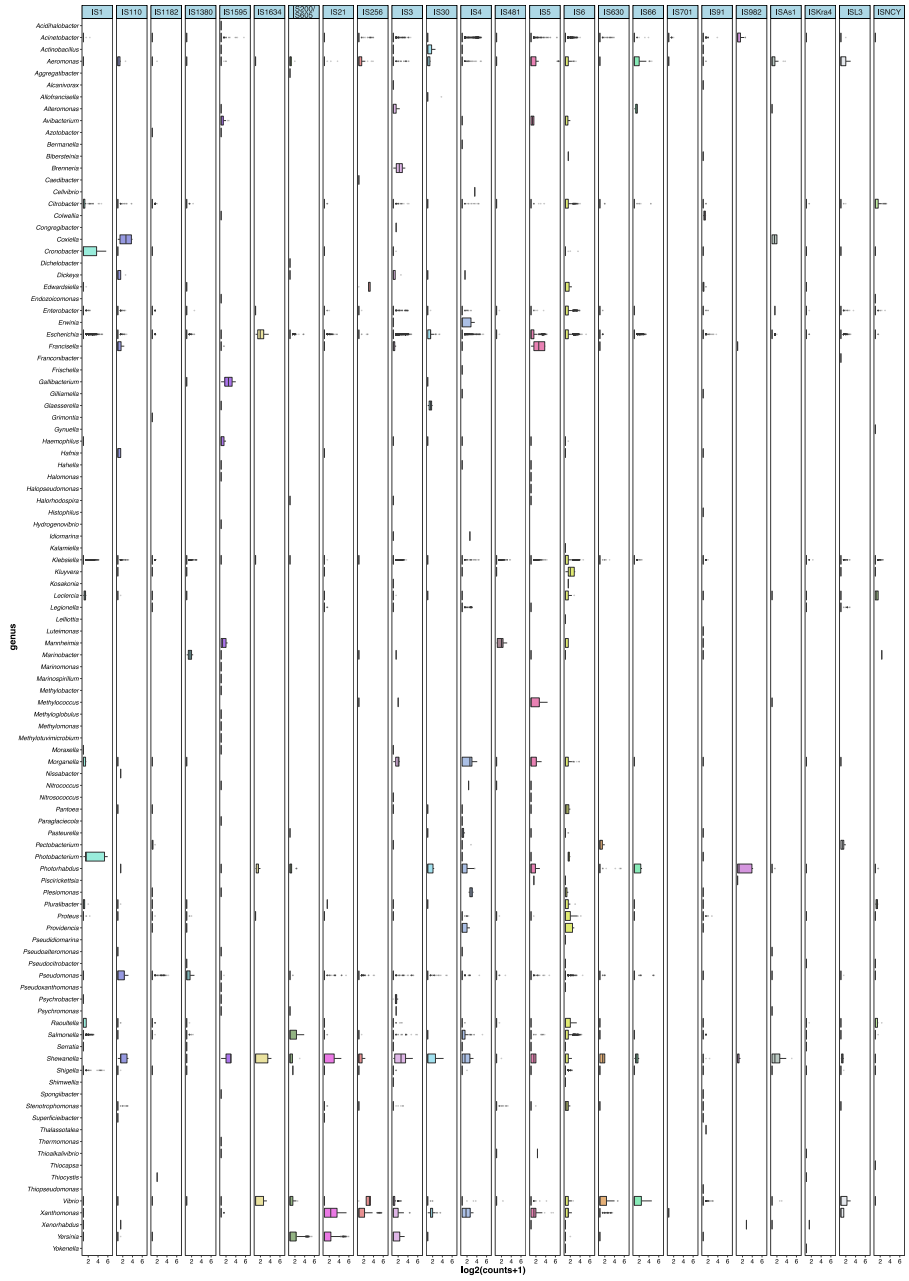

Figure S21. Distribution of different IS family counts among the class Gammaproteobacteria. Boxplot diagram of counts per genome for each IS family within Gammaproteobacteria genomes is shown at the genus level. On the x-axis, the counts were log-transformed (log2(x+1)), and only non-zero values were plotted. The boxplots show medians (midlines), interquartile ranges (boxes) and ranges (whiskers). As the figure includes multiple boxplots, the number of genomes analyzed in each individual boxplot is provided in Supplementary Data 6.

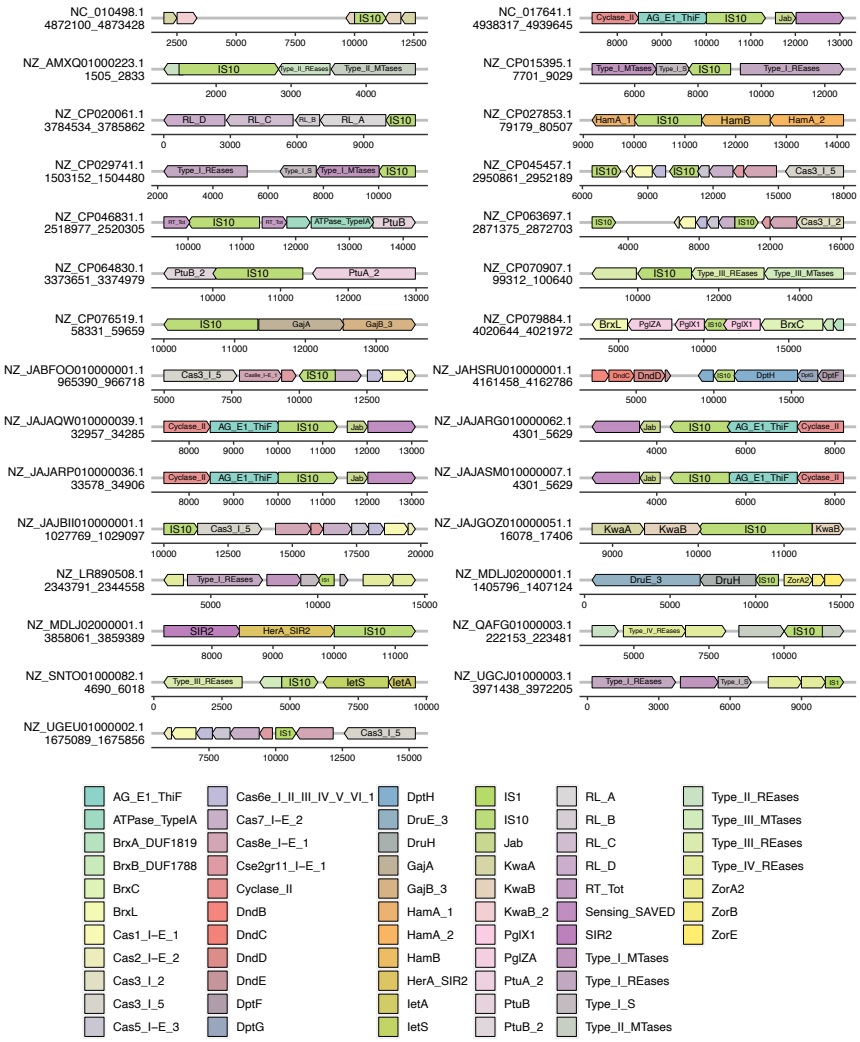

Figure S22. Schematic diagram of compromised defense systems resulting from naturally occurring transpositions of IS1 and IS10. All identified transposition events are shown.

Supplementary Tables

Table S1. Primers used in this study.

Assembly cloning of the type I-E CRISPR-Cas system of *Erwinia amylovora* 7-3	
EraCas-locus234-2-F	AGATCTCAGGCAGACTGCCG
EraCas-locus234-2-R	CTGCGCGTCAGACGGCATCC
EraCas-locus234-3-F	CTGCCTGTTACAAACGCTGT
EraCas-locus234-3-R	AGGTGGGCGGCAATAGTGAG
EraCas-locus234-4-F	GTGCAAGCCGTGAGCAGGCG
EraCas-locus234-4-R	AAGCTTGCCCAGTATTCTTC
T-Apr-IS10-Era-1-F	ATTATCAGCAGCACCTTGGGGAATCAAGCATGCGCACCACTGA GAGATCCCCTCATAAT
T-Apr-IS10-Era-1-R	TTATCATCCCCTTTTGCTGATCATGACCAATTTCTAGCCG
T-Apr-IS10-Era-2-F	CGGCTAGAAATTGGTCATGATCAGCAAAAGGGGATGATAA
T-Apr-IS10-Era-2-R	GAGCAATGTTCAAAGAAAGCTCATGAGCTCAGCCAATCGA
T-Apr-IS10-Era-3-F	TCGATTGGCTGAGCTCATGAGCTTTCTTTGAACATTGCTC
T-Apr-IS10-Era-3-R	GACGGGCATGAACCACATTACGCAGATCCGCCAGATGGATGG TGCGCACTGATGAATCCCCTAATG
T-Apr-pBAD-1-F	CCGTCTGATAATGGAATATAACGGTCGTAAAGGGAGCTTCTCG TTCTCCGCTCATGAGC
T-Apr-pBAD-1-R	CGCTCATGTCTAGACGATCGTCAGCAAAAGGGGATGATAA
T-Apr-pBAD-2-F	TTATCATCCCCTTTTGCTGACGATCGTCTAGACATGAGCG
T-Apr-pBAD-2-R	CTTACCCCAATAGCGGTAATACTCAGGCTGCGAATTCATTTTTT ATAACCTCCTTAGAG
T-EraP-pro-Ichl-F	AGGGCCGCAGATGCGACCCTTGTGTATCAAACAAGACGAACG CACCGGTGCAGCCTTTT
T-EraP-pro-Ichl-R	ATTGACTATCCGGTATTACCCGGCATGACAGGAGTAAAACGCG CGCGCAATTGAAAAAT
RT-qPCR analysis	
qPCR-Eracas3-1-F	GCGGCTATATCTACGGCAACAC
qPCR-Eracas3-1-R	GGTTCTGCATCATCGGCTTCG
qPCR-Eracse1-2-F	AGCTGCTGATCTGCCTGTTACA
qPCR-Eracse1-2-R	GCTGCTGCGGATGGTTGAGA
qPCR-Eracse2-3-F	GCCTAGCGAAGATAAGCAGCAA
qPCR-Eracse2-3-R	CACCTCGGGTTCGGCATGAATA
qPCR-Eracas7-4-F	CTGATGAGCGAGGTGGGTAACG
qPCR-Eracas7-4-R	AGAACTCCTGGGTGCCAAGATG
qPCR-Eracas5-5-F	GCAGCATCAGGCGGAACATG
qPCR-Eracas5-5-R	AACTGGCGTGGTAGGTGGATT
qPCR-Eracas6-6-F	AAGCAGGCGGTGGCAGGAAA
qPCR-Eracas6-6-R	AGGCACGGGCGATGGATAACA
qPCR-Eracas1-7-F	AATGGCGTGGGCGTCGTTAC
qPCR-Eracas1-7-R	GCGGCTTGCCACTGTGAACA

qPCR-Eracas2-8-F	ATGGCTGCTGGAAGTGCGTG
qPCR-Eracas2-8-R	AAACCCGACTCGCTGTTAGTGG
16sRNA-F	ACCCTTATCCTTTGTTGCC
16sRNA-R	TATGAGGTCCGCTTGCTCT
Construction of the strain ActSY01	
T-Pcas3-aprpBAD-F	TTTGGCTTAAAAAGGGAATGTGGGTTACACGAAGGGTAACTCG TTCTCCGCTCATGAGC
T-Pcas3-aprpBAD-R	TTTTCCCCAGTAATGGCATATATATTTAAAAGGTTCCATTTTTT ATAACCTCCTTAGAG
T-Pcas-kan23119-1-F	GAAGGGATGACCAGAGTCATCCCTGCAAATCCCAAATAAGGC GTCCCGGAAAACGATTC
T-Pcas-kan23119-1-R	TAGTAGCTAGCATTATACCTAGGACTGAGCTAGCTGTCAACAC GCTGCCGCAAGCACTCA
T-Pcas-kan23119-2-F	AGGTATAATGCTAGCTACTAGAGAAAGAGGAGAAATACTAGA TGAATTTGCTTATTGATA
T-Pcas-kan23119-2-R	CCAACAGTTTTTCCATTGGA
T-Pcas-kan23119-2-II-R	CGGGCGTACAGGGATCCAGT
Constructions of the *recA*-deficient knock-out mutants	
ΔrecA-FRT-MG1655-F	GACTATCCGGTATTACCCGGCATGACAGGAGTAAAAATGATTC CGGGGATCCGTCGACC
ΔrecA-FRT-MG1655-R	GCCGCAGATGCGACCCTTGTGTATCAAACAAGACGATTATGTA GGCTGGAGCTGCTTC
ΔrecA-FRT-MDS42-F	ATGGCTATCGACGAAAACAAACAGAAAGCGTTGGCGGCAATT CCGGGGATCCGTCGACC
ΔrecA-FRT-MDS42-R	TTAAAAATCTTCGTTAGTTTCTGCTACGCCTTCGCTATCTGTAG GCTGGAGCTGCTTC
Constructions of CRISPR interference plasmids in the ISs trapping system	
Tar-tet-F	ATCAGGTTTGTGCCAATACCAGTAGAAACAGACGAAGAATTG GCCAGAAAAAAAAGAATATATAAG
Tar-tet-R	GCGCAACGCAATTAATGTAAGTTAGCGCGAATTGATCTGGTGG CCACGCCGGCTTCCATTCAGGTC
Tar-SmR-pTrc-1-F	GCACCCTCGCAAGCTCGGTTGCGGCCGCAATCGGGCAAATCTC GAGTTCATGTGCAGCTCCATAA
Tar-SmR-pTrc-1-R	TAATTGTCAACAGCTCATTTAGATCTCTCGAGACTGGCGAGCG GCATCTTAT
Tar-SmR-pTrc-2-F	ATAAGATGCCGCTCGCCAGTCTCGAGAGATCTAAATGAGCTGT TGACAATTA
Tar-SmR-pTrc-2-R	TTGTGCCGATATCTAAGCCTATTGAGTATTTCTTATCCATAGAT CCTTTCTCCTCTTTGC
SpCas9-HF1-1-F	TTCGAATTCCTTATTATGTT
SpCas9-HF1-1-R	GAAGATTTTTATCAAACGCTGTCATGCGTTCAATA
SpCas9-HF1-2-F	TATTGAACGCATGACAGCGTTTGATAAAAATCTTC
SpCas9-HF1-2-R	TCAATTTTCGAGACAACGCTCCCCAACCAGTATAA
SpCas9-HF1-3-F	TTATACTGGTTGGGGAGCGTTGTCTCGAAAATTGA

SpCas9-HF1-3-R	TATCATCATGGATCAGCGCCATAAAATTGCGATTG
SpCas9-HF1-4-F	CAATCGCAATTTTATGGCGCTGATCCATGATGATA
SpCas9-HF1-4-R	CCACATGCTTAGTGATCGCGCGAGTTTCAACCAAT
SpCas9-HF1-5-F	ATTGGTTGAAACTCGCGCGATCACTAAGCATGTGG
SpCas9-HF1-5-R	TCTAGACGATCGTTAAGCAGCCAGAGCGTAGTTTTCGTCGTTA GCAGCGTCACCTCCTAGCTGACT
Tar-Δλ-red-kan-F	TATGAAACACGCATTGATTTGAGTCAGCTAGGAGGTGACTAAG GCGTCCCGGAAAACGATTC
Tar-Δλ-red-kan-R	TTGCGCCTACCCGGATATTATCGTGAGGATGCGTACGTACACG CTGCCGCAAGCACTCA
Tar-Δλ-red-kan-ssrA-F	GCGCACATTTCCCCGAAAAGTGCCACCTGCATCGATTTAGGCG TCCCGGAAAACGATTC
Tar-Δλ-red-kan-ssrA-R	TTGCGCCTACCCGGATATTATCGTGAGGATGCGTACGTACACG CTGCCGCAAGCACTCA
Tar-spe-FnCpf1-1-F	AGCGCAATGACATTCTTGCAGGTATCTTCGAGCCAGCCACGAT CGACATTGATCTGGCT
Tar-spe-FnCpf1-1-R	AATTCTTGATAAATTGACATAGATCCTTTCTCCTCTTTGC
Tar-spe-FnCpf1-2-F	GCAAAGAGGAGAAAGGATCTATGTCAATTTATCAAGAATT
Tar-spe-FnCpf1-2-R	TTCTAAATACATTCAAATATGTATCCGCTCATGTCTAGACGATC GTTAGTTATTCCTAT
Tar-spe-SpCas9-HF1(D10A)-1-F	CAGCGCAATGACATTCTTGC
Tar-spe-SpCas9-HF1(D10A)-1-R	CTATTTGTGCCGATAGCTAAGCCTATTGAGT
Tar-spe-SpCas9-HF1(D10A)-2-F	ACTCAATAGGCTTAGCTATCGGCACAAATAG
Tar-spe-SpCas9-HF1(D10A)-2-R	CTGACCGCTTCCTCGTGCTT
Tar-spe-SpCas9-HF1(H840A)-1-F	CAGCGCAATGACATTCTTGC
Tar-spe-SpCas9-HF1(H840A)-1-R	CTTTGTGGAACAATGGCATCGACATCATAATC
Tar-spe-SpCas9-HF1(H840A)-2-F	GATTATGATGTCGATGCCATTGTTCCACAAAG
Tar-spe-SpCas9-HF1(H840A)-2-R	CTGACCGCTTCCTCGTGCTT
Tar-spe-F	CAGCGCAATGACATTCTTGC
Tar-spe-CasOP01-R	ATGCTATACTTTTTATCCATAGATCCTTTCTCCTCTTTGC
Tar-spe-CasOP01-1-F	GCAAAGAGGAGAAAGGATCTATGGATAAAAAGTATAGCAT
Tar-spe-CasOP01-1-R	CGGGTCATCCAGGCGAAGCGACTGTTCCCGCGAGCCAGAG
Tar-spe-CasOP01-2-F	CTCTGGCTCGCGGGAACAGTCGCTTCGCCTGGATGACCCG
Tar-spe-CasOP01-2-R	ATTGCACGGGTCTCTACCAGCTGACGTTTGATAAAACCCG
Tar-spe-CasOP01-3-F	CGGGTTTTATCAAACGTCAGCTGGTAGAGACCCGTGCAAT
Tar-spe-CasOP01-3-R	TTTCTAAATACATTCAAATATGTATCCGCTCATGTCTAGACGAT CGTCAGTCTCCCCCCAGCTGACTCA
Tar-spe-CasOP01-ssrA-3-R	TTTCTAAATACATTCAAATATGTATCCGCTCATGTCTAGACGAT CGTCAGGCGGCCAGGGCGTAGT
Tar-spe-Cpf1OP01-R	AATTCCTGGTAAATCGACATAGATCCTTTCTCCTCTTTGC
Tar-spe-FnCpf1OP01-1-F	GCAAAGAGGAGAAAGGATCTATGTCGATTTACCAGGAATT
Tar-spe-FnCpf1OP01-1-R	TCAGTTCCTGTTCTTTTTTCGAAGGATTATCGAGATTTTT
Tar-spe-FnCpf1OP01-2-F	AAAAATCTCGATAATCCTTCGAAAAAAGAACAGGAACTGA
Tar-spe-FnCpf1OP01-2-R	CGTAAAGCGCTTATCCTTGATCAGATCATATTCAAACACG

Tar-spe-FnCpf1OP01-3-F	CGTGTTTGAATATGATCTGATCAAGGATAAGCGCTTTACG
Tar-spe-FnCpf1OP01-3-R	TTTCTAAATACATTCAAATATGTATCCGCTCATGTCTAGACGAT CGTCAATTATTACGGTTCTGGA
Tar-spe-CasOP02-1-R	GCCACCATCGATGTAGCCCGCATATCCGTTTTTTGACTG
Tar-spe-CasOP02-2-F	CAGTCAAAAAACGGATATGCGGGCTACATCGATGGTGGC
Tar-spe-CasOP02-2-R	TTTACGGGTCATCCAGGCAAACCTACTGTTCCCGCGAGC
Tar-spe-CasOP02-3-F	GCTCGCGGGAACAGTAGGTTTGCCTGGATGACCCGTAAA
Tar-spe-CasOP02-3-R	TCGTATCGGTTTGTCCCGGTGTTTATTATACGCTGAAAGCAC
Tar-spe-CasOP02-4-F	GTGCTTTCAGCGTATAATAAACACCGGGACAAACCGATACGA
Tar-spe-III-R	GTGGCACTTTTCGGGGAAAT
Tar-spe-FnCpf1OP02-1-R	ATTACGGATGCGCAGAATGTCTTCAGACGGA
Tar-spe-FnCpf1OP02-2-F	TCCGTCTGAAGACATTCTGCGCATCCGTAAT
Tar-spe-CasOP03-1-R	CGACTCTTCCAGGCGGTGAAAAAAGCTATCATCTACCTTCGC
Tar-spe-CasOP03-2-F	TTCACCGCCTGGAAGAGTCGTTTCTGGTTGAAGAGGATAAAAA ACACGAACGCCAT
Tar-spe-CasOP03-2-R	GTATTTCTCATGGTACGCCACCTCGTCCACTATATTA
Tar-spe-CasOP03-3-F	TAATATAGTGGACGAGGTGGCGTACCATGAGAAATAC
Tar-spe-CasOP03-3-R	GATTGATCCATTATCGAATGTTCGCTGTTTACGCAGC
Tar-spe-CasOP03-4-F	GCTGCGTAAACAGCGAACATTCGATAATGGATCAATC
Tar-spe-CasOP03-4-R	GTGTTCTTTCAGAATTTGGCTCCCCAGTTCT
Tar-spe-CasOP03-5-F	AGAACTGGGGAGCCAAATTCTGAAAGAACAC
Tar-spe-FnCpf1OP03-1-R	AAAGAGATACAATTTACCTTGGTTCACCACC
Tar-spe-FnCpf1OP03-2-F	GGTGGTGAACCAAGGTAAATTGTATCTCTTT
Tar-spe-FnCpf1OP03-2-R	TGCCTTTTCTTTCAGTAAGAGGTTGATTTCATC
Tar-spe-FnCpf1OP03-3-F	GATGAAATCAACCTCTTACTGAAAGAAAAGGCA
Constructions of NHEJ expression plasmids	
pBAD-Mm-ku-1-F	GCGGCCGCCGATCGTCTAGACATGAGCG
pBAD-Mm-ku-1-R	CCCTTCCAGATGGAGCGCATTTTTTATAACCTCCTTAGAG
pBAD-Mm-ku-2-F	CTCTAAGGAGGTTATAAAAAATGCGCTCCATCTGGAAGGG
pBAD-Mm-ku-2-R	ACTAGTTGCTTGGATTCTCACCAATA
pBAD-Ms-ku-1-F	ACATGTCGATCGTCTAGACATGAGCG
pBAD-Ms-ku-1-R	CCCTTCCAGATGGATCGCATTTTTTATAACCTCCTTAGAG
pBAD-Ms-ku-2-F	CTCTAAGGAGGTTATAAAAAATGCGATCCATCTGGAAGGG
pBAD-Ms-ku-2-R	ACTAGTTGCTTGGATTCTCACCAATA
pBAD-Bs-ku-1-F	GCGGCCGCCGATCGTCTAGACATGAGCG
pBAD-Bs-ku-1-R	AGAGAAGGAGTACGATTCATTTTTTATAACCTCCTTAGAG
pBAD-Bs-ku-2-F	CTCTAAGGAGGTTATAAAAAATGAATCGTACTCCTTCTCT
pBAD-Bs-ku-2-R	ACTAGTTGCTTGGATTCTCACCAATA
Determination of the indel mutations in the gene *ompF*	
V-ompF-F	GTGCGAGCACGTTTGTCATT
V-ompF-R	CTTCGTTGGTCGTGTTGGCG
Detection of the target site duplications	
S-IS1-1-F	CGCCATTCATGGCCATATCA

S-IS1-2-F	CCTGAAGGGAAAGCTGCACG
S-IS2-1-F	CCTGTATGTGCCCGTTTCGA
S-IS2-2-F	CCGGAGAGTAACGGAATAGC
S-IS3-1-F	CCGATGCGTTCAGCAAGCTT
S-IS3-2-F	GAACACTTTATCAGCCGGGA
S-IS5-1-F	GTACCAATGCTGCATGCAGT
S-IS5-2-F	ATACGTCAGTGGGAGAGATC
S-IS10-1-F	TTGGTTGGCAGGTTACGGCC
S-IS10-2-F	GCTCAGAAACAAGGTTGGGA
S-IS150-1-F	GAGCGCGCGTAAACCAGCTT
S-IS150-2-F	CTGTTCTGCACTCTGACCAG
PCR amplifications of the complete coding regions of SpCas9-HF1, FnCpf1 and their derivatives	
S-univer-c&9-F	TAAGATGCCGCTCGCCAGTC
S-univer-c&9-R	CTGACCGCTTCCTCGTGCTT
Sanger sequencing for the complete coding regions of SpCas9-HF1, FnCpf1 and their derivatives	
S-SpCas9-HF1-1M-F	GCTGGTTTTCGCATTCCTTC
S-SpCas9-HF1-2-F	CAAATTCACTTGGGTGAGCT
S-SpCas9-HF1-3-F	CGCCAAGTTAATCACTCAAC
S-SpCas9-HF1-4-F	GGTAATCACTTTAACCTCTC
S-FnCpf1-1-F	CCCACAGGGTAAAACACTTG
S-FnCpf1-2-F	GGCAGAAGAGCTAACCTTTG
S-FnCpf1-3-F	GCAAACTTTGCGGCTATTCC
S-FnCpf1-4-F	TAAGCATCCGGAGTGGAAAG
S-FnCpf1-5-F	CTCAAAATAGCCCTTATCAAGG
S-SpCas9-HF1-OP01-1-F	CTTTTCATCCAGCTGGTGCA
S-SpCas9-HF1-OP01-2-F	CCGCACCAGATTCACTTGGG
S-SpCas9-HF1-OP01-3-F	GTACGCCCATCTGTTTGATG
S-SpCas9-HF1-OP01-4-F	CCCGCAGTGACAAAAACCGT
S-SpCas9-HF1-OP01-5-F	TGTGGGATAAAGGTCGGGAC
S-FnCpf1-OP01-1-F	GCAGCAATGATATTCCGACC
S-FnCpf1-OP01-2-F	CCGCGGTGTTGGAATACATT
S-FnCpf1-OP01-3-F	CGGCGTTATGAACAAGAAAA
S-FnCpf1-OP01-4-F	CCCGAAAAAGGAAAGCGTGT
Sanger sequencing for the sgRNAs and crRNAs expression cassettes	
V-p15A-cm-circle-F	CAAGGCGACAAGGTGCTGAT
V-p15A-cm-circle-R	GGTAGCTCAGAGAACCTTCGA

Table S2. Plasmids used in this study.

Plasmids	Descriptions	Source
General plasmids		
pBluescript SK(+)	the vector backbone for cloning PCR products	Stratagene
p15A-cm	the verctor backbone for cloning both sgRNAs and crRNAs expression cassettes	[1]
pUC57-1.8K	the vector backbone for cloning NHEJ expression cassettes	Qingke[a], General[b]
pClone007	the vector backbone for cloning the type I-E CRISPR Cas system of *Erwinia amylovora* 7-3	Qingke[a]
pSET152	the template plasmid for amplifying the apramycin selective marker	[2]
the plasmids associated with the type I-E CRISPR-Cas system of *Erwinia amylovora* 7-3		
pClone007-EraCas01	the plasmid pClone007 containing the fragment EraCas01	This study
pClone007-EraCas02	the plasmid pClone007 containing the fragment EraCas02	This study
pClone007-EraCas03	the plasmid pClone007 containing the fragment EraCas03	This study
pClone007-EraCas04	the plasmid pClone007 containing the fragment EraCas04	This study
pClone007-EraCas05	the plasmid pClone007 containing the fragment EraCas05	This study
pEraCas234	the plasmid pClone007 containing the fragment EraCas02, EraCas03 and EraCas04	This study
pEraCas1234	the plasmid pClone007 containing the fragment EraCas01, EraCas02, EraCas03 and EraCas04	This study
pEraCas	the plasmid pClone007 containing the engineered type I-E CRISPR-Cas locus of *E. amylovora* 7-3	This study
pEraCas-IS10-Apr	derived from the plasmid pEraCas, in which the IS10 element together with the apramycin resistance marker were inserted into the coding region of *cas7*	This study
pEraCas-IS10	derived from the plasmid pEraCas-IS10-Apr, in which the apramycin resistance marker was removed	This study
p15A-Eraprotos	the plasmid p15A-cm containing the engineered protospacers that were designed from the plasmid p7-3	This study
SK(+)-spe	the plasmid pBluescript SK(+) containing the spectinomycin resistance cassette	This study
p15A-Eraprotos-spe	the plasmid p15A-Eraprotos with the insertion of the spectinomycin resistance cassette	This study
p15A-spe	derived from the plasmid p15A-Eraprotos-spe, in which all protospacers were deleted	This study
pEraCas-pBAD	derived from the plasmid pEraCas, in which the *araC* gene and pBAD promoter were inserted in front of *cas3* to dirve the expression of *cas* operon in the presence of L-arabinose	This study

the plasmids associated with the type I-E CRISPR/Cas system of *E. coli* DH10B and ActSY01		
p15A-sgRNA::lacZ02	the sgRNA::lacZ02 expression cassette was cloned into plasmid p15A-cm	This study
p15A-CRISPR::arpA	the CRISPR array expression cassette (CRISPR::arpA) was cloned into plasmid p15A-cm	This study
p15A-CRISPR::ftsA	the CRISPR array expression cassette (CRISPR::ftsA) was cloned into plasmid p15A-cm	This study
CRISPR interference plasmids in the ISs trapping system		
pCas	the original vector backbone for the constructions of the CRISPR interference plasmids	Addgene # 62225
pY001	the template plasmid containing the complete coding region of FnCpf1	Addgene # 69973
pCas-tet	pCas with the insertion of tetracycline resistance marker	This study
pCasY-1	derived from the plasmid pCas-tet, in which kanamycin resistance marker together with its neighboring sequences were substituted with both spectinomycin resistance marker and pTrc promoter	This study
pCasY-2	derived from the plasmid pCasY-1, in which the complete coding region of SpCas9 was replaced with the high-fidelity SpCas9-HF1 nuclease fused with the ssrA degratation tag to its C-terminus	This study
pCasY-3-ΔSIM-kan	derived from the plasmid pCasY-2, in which the λ-red expression cassette together with the ssrA degratation tag were substituted with the kanamycin resistance marker	This study
pCasY-3-ΔSIM-kan (D10A)	pCasY-3-ΔSIM-kan with the mutation D10A in the coding region of the SpCas9-HF1	This study
pCasY-3-ΔSIM-kan (H840A)	pCasY-3-ΔSIM-kan with the mutation H840A in the coding region of the SpCas9-HF1	This study
pCasY-3-ΔSIM-kan (D10A&H840A)	pCasY-3-ΔSIM-kan with the mutations D10A and H840A in the coding region of the SpCas9-HF1	This study
pCasY-3-ΔSIM-kan-ssrA-PvuI	the vector backbone derived from the plasmid pCasY-3-ΔSIM-kan-ssrA, in which both the partial C-terminus of spectinomycin resistance protein coding region and the complete SpCas9-HF1-ssrA expression cassette were deleted	This study
pCasY-3-ΔSIM-kan-OP01	the vector backbone pCasY-3-ΔSIM-kan-ssrA-PvuI with the insertions of both C-terminus of spectinomycin resistance protein coding region and the SpCas9-HF1-OP01 expression cassette	This study
pCasY-3-ΔSIM-kan-OP02	the vector backbone pCasY-3-ΔSIM-kan-ssrA-PvuI with the insertions of both C-terminus of spectinomycin resistance protein coding region and the SpCas9-HF1-OP02 expression cassette	This study

pCasY-3-ΔSIM-kan-OP03	the vector backbone pCasY-3-ΔSIM-kan-ssrA-PvuI with the insertions of both C-terminus of spectinomycin resistance protein coding region and the SpCas9-HF1-OP03 expression cassette	This study
pCasY-3-ΔSIM-kan-ssrA	derived from the plasmid pCasY-2, in which the λ-red expression cassette was substituted with the kanamycin resistance marker	This study
pCasY-3-ΔSIM-kan-OP01-ssrA	the vector backbone pCasY-3-ΔSIM-kan-ssrA-PvuI with the insertions of both C-terminus of spectinomycin resistance protein coding region and the SpCas9-HF1-OP01-ssrA expression cassette	This study
pCasY-3-ΔSIM-kan-OP02-ssrA	the vector backbone pCasY-3-ΔSIM-kan-ssrA-PvuI with the insertions of both C-terminus of spectinomycin resistance protein coding region and the SpCas9-HF1-OP02-ssrA expression cassette	This study
pCasY-3-ΔSIM-kan-OP03-ssrA	the vector backbone pCasY-3-ΔSIM-kan-ssrA-PvuI with the insertions of both C-terminus of spectinomycin resistance protein coding region and the SpCas9-HF1-OP03-ssrA expression cassette	This study
pCpf1Y-3-ΔSIM-kan	the vector backbone pCasY-3-ΔSIM-kan-ssrA-PvuI with the insertions of both C-terminus of spectinomycin resistance protein coding region and the FnCpf1 expression cassette	This study
pCpf1Y-3-ΔSIM-kan-OP01	the vector backbone pCasY-3-ΔSIM-kan-ssrA-PvuI with the insertions of both C-terminus of spectinomycin resistance protein coding region and the FnCpf1-OP01 expression cassette	This study
pCpf1Y-3-ΔSIM-kan-OP02	the vector backbone pCasY-3-ΔSIM-kan-ssrA-PvuI with the insertions of both C-terminus of spectinomycin resistance protein coding region and the FnCpf1-OP02 expression cassette	This study
pCpf1Y-3-ΔSIM-kan-OP03	the vector backbone pCasY-3-ΔSIM-kan-ssrA-PvuI with the insertions of both C-terminus of spectinomycin resistance protein coding region and the FnCpf1-OP03 expression cassette	This study
sgRNAs/crRNAs expression plasmids		
p15A-cm-sgRNA-nonespacer	the sgRNA cassette without any spacer sequence cloned into plasmid p15A-cm	This study
p15A-cm-sgRNA::ompF	the sgRNA::ompF expression cassette was cloned into plasmid p15A-cm	This study
p15A-cm-sgRNA::pyrF	the sgRNA::pyrF expression cassette was cloned into plasmid p15A-cm	This study
p15A-cm-sgRNA::lpp	the sgRNA::lpp expression cassette was cloned into plasmid p15A-cm	This study

p15A-cm-sgRNA::lacZ	the sgRNA::lacZ expression cassette was cloned into plasmid p15A-cm	This study
p15A-cm-sgRNA::ompF pair 01	the sgRNA::ompF pair 01 expression cassette was cloned into plasmid p15A-cm	This study
p15A-cm-sgRNA::ompF pair 02	the sgRNA::ompF pair 02 expression cassette was cloned into plasmid p15A-cm	This study
p15A-cm-crRNA-nonespacer	the crRNA cassette without any spacer sequence cloned into plasmid p15A-cm	This study
p15A-cm-crRNA::ompF	the crRNA::ompF expression cassette was cloned into plasmid p15A-cm	This study
p15A-cm-crRNA::pyrF	the crRNA::pyrF expression cassette was cloned into plasmid p15A-cm	This study
p15A-cm-crRNA::lpp	the crRNA::lpp expression cassette was cloned into plasmid p15A-cm	This study
p15A-cm-crRNA::lacZ	the crRNA::lacZ expression cassette was cloned into plasmid p15A-cm	This study
NHEJ expression plasmids		
pUC57-1.8K-Bs-ku	the complete coding region of the Ku from the *Bacillus subtilis* 168 was cloned into the plasmid pUC57-1.8K	This study
SK(+)-pBAD-Bs-ku	the gene *ku* was amplified from the plasmid pUC57-1.8K-Bs-ku and fused together with the promoter pBAD by overlap PCR, subsequently, the overlap PCR product was cloned into the plasmid pBluescript SK(+)	This study
pUC57-1.8K-Bs-ligD	the gene *ligD* from the *Bacillus subtilis* 168, was assembled with the promoter J23119, and then cloned into the plasmid pUC57-1.8K	This study
pUC57-1.8K-Ms-ku	the complete coding region of the Ku from the *Mycobacterium smegmatis* mc^2155 was cloned into the plasmid pUC57-1.8K	This study
SK(+)-pBAD-Ms-ku	the gene *ku* was amplified from the plasmid pUC57-1.8K-Ms-ku and fused together with the promoter pBAD by overlap PCR, subsequently, the overlap PCR product was cloned into the plasmid pBluescript SK(+)	This study
pUC57-1.8K-Ms-ligD	the gene *ligD* from the *Mycobacterium smegmatis* mc^2155, was assembled with the promoter J23119, and then cloned into the plasmid pUC57-1.8K	This study
pUC57-1.8K-Mm-ku	the complete coding region of the Ku from the *Mycobacterium marinum* M was cloned into the plasmid pUC57-1.8K	This study

SK(+)-pBAD-Mm-ku	the gene *ku* was amplified from the plasmid pUC57-1.8K-Mm-ku and fused together with the promoter pBAD by overlap PCR, subsequently, the overlap PCR product was cloned into the plasmid pBluescript SK(+)	This study
pUC57-1.8K-Mm-ligD	the gene *ligD* from the *Mycobacterium marinum* M, was assembled with the promoter J23119, and then cloned into the plasmid pUC57-1.8K	This study
pBs-NHEJ	the DNA cassette harboring the gene *ku* and pBAD promoter was digested from the plasmid SK(+)-pBAD-Bs-ku, and then cloned into the plamid pUC57-1.8K-Bs-ligD	This study
pMs-NHEJ	the DNA cassette harboring the gene *ku* and pBAD promoter was digested from the plasmid SK(+)-pBAD-Ms-ku, and then cloned into the plamid pUC57-1.8K-Ms-ligD	This study
pMm-NHEJ	the DNA cassette harboring the gene *ku* and pBAD promoter was digested from the plasmid SK(+)-pBAD-Mm-ku, and then cloned into the plamid pUC57-1.8K-Mm-ligD	This study

[a] denoted the Beijing Qingke Biotechnology Co., Ltd.

[b] indicated the General Biological System (Anhui) Co., Ltd (Anhui, China).

Table S3. Strains used in this study.

Strains	Descriptions	Source
Escherichia coli		
DH10B	str. K-12 F^-	Invitrogen
MG1655	K-12 F^- λ^- *ilvG*$^-$ *rfb-50 rph-1*	[3]
MG1655-Δ*recA*	MG1655 strain with *recA* gene disrupted	This study
MDS42	a derivative of *E. coli* K-12 without IS elements	[4]
MDS42-Δ*recA*	MDS42 strain with *recA* gene disrupted	This study
GB08-red	for expression of λ-red recombination system	[1]
SYHY01	DH10B equipped with an exogenous *E. amylovora* type I-E CRISPR-Cas system in a single plasmid pEraCas	This study
SYHY02	DH10B harboring an exogenous *E. amylovora* type I-E IS10-aborted CRISPR-Cas system in a single plasmid pEraCas-IS10	This study
SYHY01::p15A-Eraprotos-spe	SYHY01 bearing plasmid p15A-Eraprotos-spe	This study
SYHY02::p15A-Eraprotos-spe	SYHY02 bearing plasmid p15A-Eraprotos-spe	This study
SYHY01::p15A-spe	SYHY01 bearing plasmid p15A-spe	This study
SYHY02::p15A-spe	SYHY02 bearing plasmid p15A-spe	This study
SYHY03	DH10B containing all protospacers that were designed from plasmid p7-3	This study
DH10B::pEraCas	DH10B containing plasmid pEraCas	This study
DH10B::pEraCas-pBAD	DH10B containing plasmid pEraCas-pBAD	This study
ActSY01	DH10B harboring an engineered type I-E CRISPR/Cas system	This study

Table S4. Analysis of a total of 19 larger amplicons containing an IS element insertion into *cas* genes within the type I-E CRISPR/Cas system of the strain ActSY01.

Samples	IS type	TSD sequences	TSD length
A01	IS10	CGCAGAACA	9
A02	IS10	TGCTATGCT	9
A03	IS10	TGTCAGGCA	9
A04	IS10	TGCTATGCT	9
A05	IS5	TTAG	4
A06	IS1	ACGCCAGCG	9
A07	IS1	GGCAATGGC	9
A09	IS2	GGCAC	5
A10	IS10	TGCTATGCT	9
A11	IS10	GGCCTGGCT	9
A12	IS10	CACTCGACG	9
A13	IS5	TTAA	4
A14	IS10	CACTCGACG	9
A16	IS10	TGCTGCGCA	9
A17	IS10	CACTCGACG	9
A18	IS1	CTGGTTTCC	9
A19	IS10	GGCCTGGCT	9
A20	IS10	AGCTTCGCC	9
A23	IS10	CGCAGAACA	9

Table S5. Analysis of a total of 44 larger amplicons containing an IS element insertion into the coding region of SpCas9-HF1.

Samples	IS type	TSD sequences	TSD length
1108-02A1	IS1	GAGATGGCG	9
1108-02A2	IS1	GATTTGCGC	9
1108-02A3	IS1	GAGATGGCG	9
1108-02A5	IS5	CAAG	4
1108-02B4	IS1	GTAACCGTT	9
1110-03A1	IS5	CTAA	4
1110-03A2	IS10	TGCTAAGTC	9
1110-03A3	IS10	TGCTAAGTC	9
1110-03A4	IS10	CACTCAACG	9
1110-03A5	IS1	GTGATTATG	9
1110-03A6	IS10	TGCTAAGTC	9
1110-03A7	IS10	CACTCAACG	9
1110-03A8	IS3	GGT	3
1110-03B1	IS10	CACTCAACG	9
1110-03B2	IS10	TGCTAAGTC	9
1110-03B3	IS10	TGCTAAGTC	9
1110-03B4	IS10	TGCCCCAAG	9
1110-03B5	IS1	AGTAACCGT	9
1110-03B6	IS10	TGCTAAGTC	9
1110-03B7	IS10	CACTCAACG	9
1110-03B8	IS10	TGCTAAGTC	9
1110-03C1	IS1	CAAGGTTC	8
1110-03C2	IS5	TTAG	4
1110-03C3	IS10	TGCTAAGTC	9
1110-03C5	IS10	CACTCAACG	9
1110-03C6	IS1	CAAGGTTC	8
1110-03C7	IS10	TGCTAAGTC	9
1110-03C8	IS10	CGCATGACA	9
1110-03D1	IS10	TGCTAAGTC	9
1110-03D3	IS3	GGT	3
1110-03D4	IS3	GGT	3
1110-03D5	IS10	TGCTAAGTC	9
1110-03D6	IS10	TGCTAAGTC	9
1110-03D7	IS1	CAAGGTTC	8
1110-03D8	IS10	CACTCAACG	9
1110-03E4	IS10	CACTCAACG	9
1110-03E5	IS3	GGT	3
1110-03E6	IS10	CACTCAACG	9
1110-03E8	IS1	GTAACCGTT	9

1110-03F1	IS5	CTAA	4
1110-03F2	IS10	AGCTCTATC	9
1110-03F3	IS10	TGCTAAGTC	9
1110-03F4	IS10	CACTCAACG	9
1110-03F5	IS10	CACTCAACG	9

Table S6. Summary of whole-genome sequencing of 11 CRISPR-tolerant mutants and the control strain.

Samples	Genome accession	IS type[a]
SY221	CP106998	/
SY22A1	CP107005	IS10
SY22A3	CP107004	IS1
SY22A4	CP106999	IS5
SY22A6	CP106994	IS1
SY22B1	CP107003	IS5
SY22B2	CP107000	/
SY22B3	CP106995	IS10
SY22B5	CP107001	IS2
SY22B6	CP106997	/
SY22C3	CP107002	IS2
SY22D8	CP106996	/

[a] identified by PCR and Sanger sequencing.

"/" indicated that no IS element was inserted into the coding region of SpCas9-HF1 in the given sample.

Table S7. The non-canonical length of TSD sequences generated by IS1 in our CRISPR/Cas-mediated ISs trapping system.

Samples	TSD sequences	TSD length
0201-2F3	ATCAAGACGAT	11
0310-3A3	CAAATATACAACAAGGACTTTTC	23
0310-3D1	CAAATATACAACAAGGACTTTTC	23
0310-3E6	CAAATATACAACAAGGACTTTTC	23
0526-1B5	GTTTTAT	7

Supplementary References

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3. Blattner, F.R. *et al.* The complete genome sequence of *Escherichia coli* K-12. *Science* **277**, 1453-1462 (1997).
4. Pósfai, G. *et al.* Emergent properties of reduced-genome *Escherichia coli*. *Science* **312**, 1044-1046 (2006).