SUPPLEMENTARY DATA

A novel host-specific restriction system associated with DNA backbone S-modification in *Salmonella*

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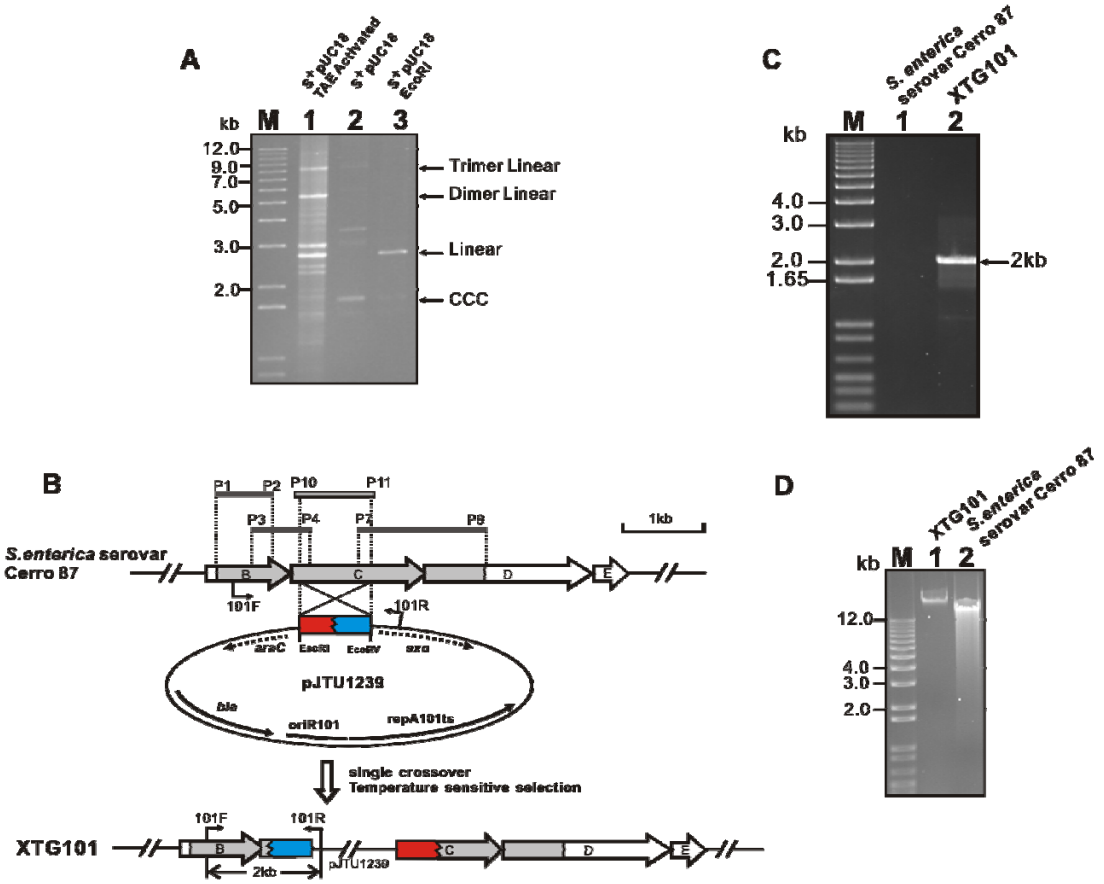

Figure S1: Dnd (DNA degradation) phenotype of *S. enterica* serovar Cerro 87, *dptC*-specific gene probe, and construction of the *dptC* mutant by single crossover gene disruption.

A: Agarose gel showing S-modified (S^+) pUC18 isolated from *S. enterica* serovar Cerro 87. The gel buffer contained thiourea to prevent oxidative cleavage of S-modified DNA. M, 1 kb ladder; lane 1, S^+ pUC18 pre-treated with activated TAE buffer creating double-strand breaks at the sites of S-modification by oxidative cleavage; lane 2, S^+ pUC18 control without pre-activation treatment; lane 3, S^+ pUC18 partially digested using EcoRI, generating the 2.7-kb linear form, and very faint bands of linear dimers and trimers in the positions indicated.

B: Construction of mutant XTG101 in which *dptC* was disrupted by the single crossover insertion of pJTU1239.

The sequenced 7.8-kb chromosomal region *S. enterica* serovar Cerro 87 spanning *dptB,C* and *D* is highlighted in grey. The DNA fragments that were amplified using the degenerate

oligonucleotide primer pairs p1/p2, p3/p4, p7/p8 and p10/p11 are indicated above the gene cluster. pJTU1239 was constructed by cloning a 0.7-kb EcoRI-EcoRV fragment obtained from pJTU1231, which carrying 0.9-kb PCR-amplified fragment P10-P11, into the thermo-sensitive plasmid pKD46. Mutant XTG101 was obtained by single crossover integration of pJTU1239 into the *S. enterica* serovar Cerro 87 genome. The oligonucleotide primers that were used to confirm the integration event are indicated with short angled arrows. XTG101 had no complete copy of *dptC* and was thus expected to have lost the ability to add sulfur to the DNA backbone.

C. Confirmation of mutant XTG101 using PCR amplification. Primers 101F/101R (101F: 5' CAACGCACGCTGAATAAA 3'; 101R: 5' GACAACTTGACGGCTACA 3') were expected to produce no bands from *S. enterica* serovar Cerro 87 DNA (lane 1) and a 2.0-kb band from mutant XTG101 (lane 2). Lane M, 1 kb ladder.

D. Agarose gel showing DNA degradation assay of XTG101. Lane M, 1 kb ladder; lane 1, XTG101 genomic DNA (no degradation); lane 2 genomic DNA from *S. enterica* serovar Cerro 87, noticeably degraded during electrophoresis.

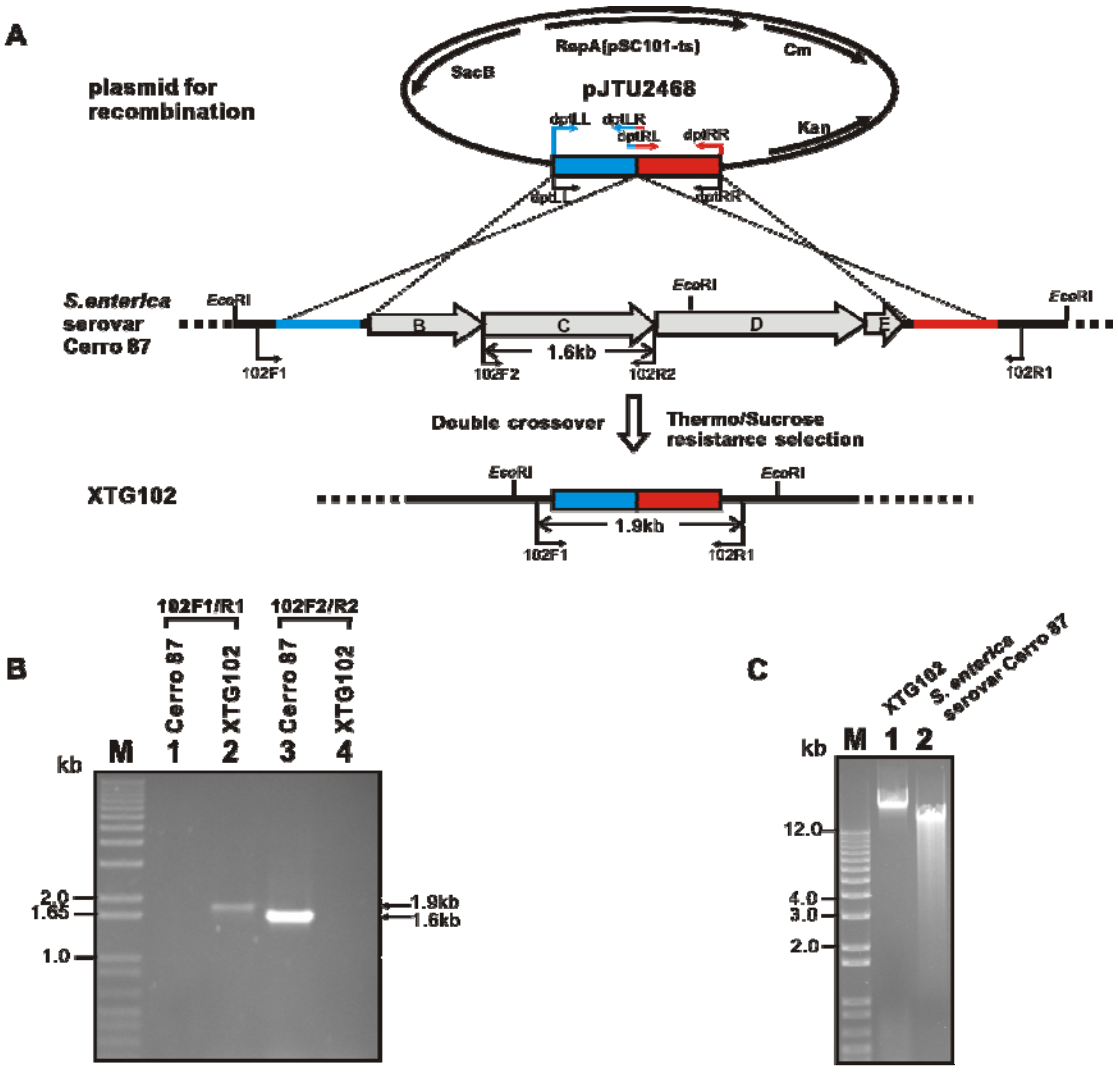

Figure S2: Construction of mutant XTG102 by double crossover replacement of *dptB-E*

A. The red 752-bp and blue 819-bp chromosomal regions were amplified using *S.enterica* serovar Cerro 87 genomic DNA as a template, and the primers indicated. Note: primers dptLR and dptRL had 40-bp complementary tails. The two fragments were then combined into a single 1.5-kb fragment by a further round of PCR using the primers dptLL and dptRR. The 1.5-kb fragment was sequenced and inserted into BamHI-SalI-digested pKOV-Kan, generating pJTU2468. pJTU2468 was firstly transferred into *E. coli* DH5α (pJTU1238, *dptB-E*, Figure 2) for S-modification (phosphorothioation), and then into *S. enterica* serovar Cerro 87 for homologous recombination. The single crossover intermediate was selected at 43°C on an LA plate added with corresponding antibiotics and confirmed by PCR. Double crossover gene replacement mutants like XTG102 were obtained by selective growth at 43°C on a 15% sucrose LA plate, followed by PCR screening using primer pairs 102F1/102R1 (102F1: 5' GCCACAAATTGAAGATTACA 3'; 102R1: 5' AATGCTAAGGGCTAGGAAAA 3') and 102F2/102R2 (102F2: 5' TTGCCATATGAGTAAATTAGTTCAGGC 3'; 102R2: 5' TACCGGATCCTTAATGAGCACGTTCAT 3').

B. Confirmation of mutant XTG102 using PCR amplification.

Primers 102F1 and 102R1 were expected to amplify no bands from *S. enterica* serovar Cerro 87 (lane 1), and a 1.9-kb fragment from mutant XTG102 (lane 2). Primers 102F2 and 102R2 were expected to amplify a 1.6-kb fragment from *S. enterica* serovar Cerro 87 (lane 3), and none from mutant XTG102 (lane 4). Lane M, 1-kb ladder and Cerro 87 is short for *S. enterica* serovar Cerro 87.

C. DNA degradation assay of XTG102 genomic DNA. Lane M, 1-kb ladder; lane 1, intact DNA from XTG102 (not degraded during electrophoresis); lane 2, S-modified genomic DNA from *S. enterica* serovar Cerro 87 (degraded).

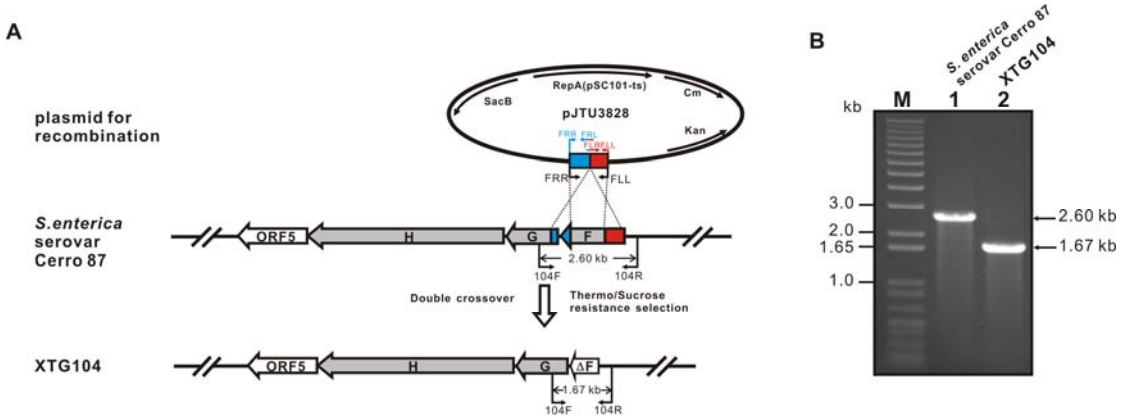

Figure S3: Construction of *dptF* deletion mutant XTG104

A. The construction of mutant XTG104 was similar to that of XTG102. Briefly, 535-bp left arm (red), and 580-bp right arm (blue) fragments were combined using PCR to produce the 1075-bp blue-red fragment flanked by BamHI and SalI sites for cloning into pKOV-Kan to give pJTU3828. Double crossover recombination produced XTG104 which has a 921-bp in-frame deletion in *dptF*. The mutant XTG104 was PCR-confirmed using primers 104F/104R (104F: 5'

CTGCCAGTTGTCCAGATTGA 3'; 104R: 5' CGGAAAGAAAGGTCGGTATT 3'). The corresponding primers used for PCR are indicated with short angled arrows and the primers introducing the overlapped region with two colours. "ΔF" means mutated *dptF* with no function.

B. Confirmation of mutant XTG104 using PCR amplification.

Primers 104F/104R produced the expected 2.60-kb band from *S. enterica* serovar Cerro 87 (lane 1), and a 1.67-kb band from XTG104 (lane 2). Lane M, 1-kb ladder.

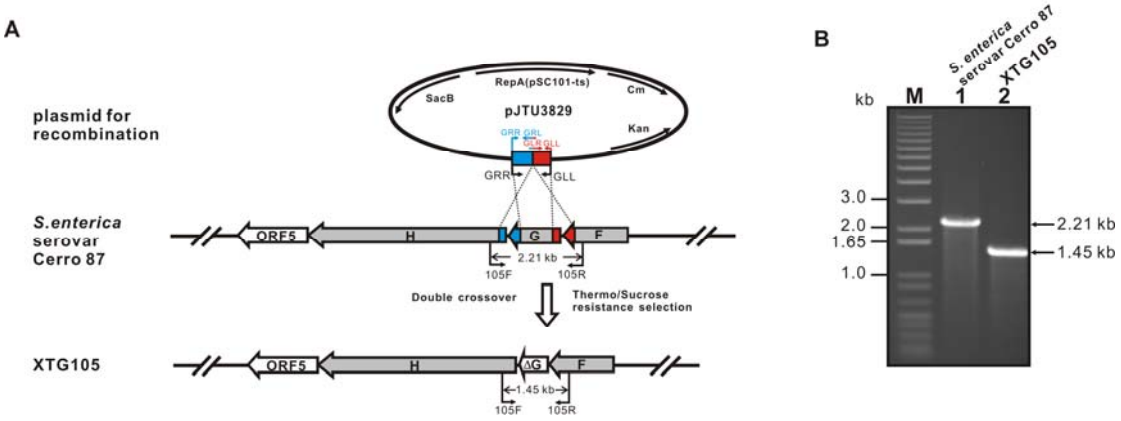

Figure S4: Construction of *dptG* deletion mutant XTG105

A. The construction of mutant XTG105 was similar with that of XTG102. Briefly, the 537-bp left arm (red), and 604-bp right arm (blue) fragments were combined using PCR to produce the 1101-bp blue-red fragment flanked by BamHI and SalI restriction sites for cloning into pKOV-Kan to give pJTU3829. Double crossover recombination produced XTG105 which has a 762-bp in-frame deletion in *dptG*. The mutant XTG105 was PCR-confirmed using primers 105F/105R (105F: 5' AAGCGATACAGCGACGAGAA 3'; 105R : 5' TTGGGTGAGTTTGGTGGAGT 3'). "ΔG" means mutated *dptG* with no function.

B. Confirmation of mutant XTG105 using PCR amplification.

Primers 105F/105R produced the expected 2.21-kb band from S. enterica serovar Cerro 87 (lane 1), and a 1.45-kb band from XTG105 (lane 2). Lane M, 1-kb ladder.

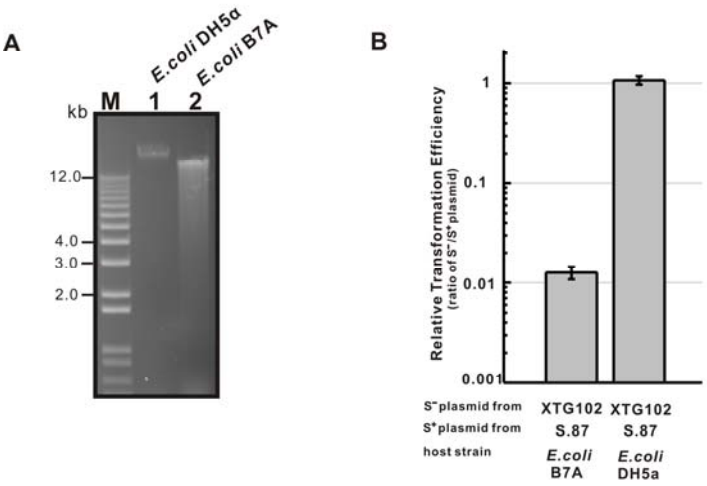

Figure S5: Oxidative DNA degradation and restriction in *E. coli* B7A**.**

A. Tris-acetate agarose gel. Lane M, 1-kb ladder; lane 1 genomic DNA from *E.coli* DH5α (no degradation during electrophoresis); lane 2, genomic DNA from *E.coli* B7A (degraded, indicating S-modification).

B. Comparative transformation frequencies of *E. coli* B7A and *E. coli* DH5α as acceptor hosts, and S-free (S^-) pKOV-Kan isolated from XTG102, and S-modified (S^+) pKOV-Kan isolated from *S. enterica* serovar Cerro 87 (*abbr.* S.87). The results are presented as relative transformation efficiencies (ratios of S^-/S^+ plasmid) obtained by parallel transformation of S^- and S^+ plasmid DNA. Error bars represent the standard deviation from three repeat experiments.