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Supporting Information Appendix for

Epigenetic competition reveals density-dependent regulation and target site plasticity of phosphorothioate DNA modifications in bacterial genomes

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Datasets S1 to S6

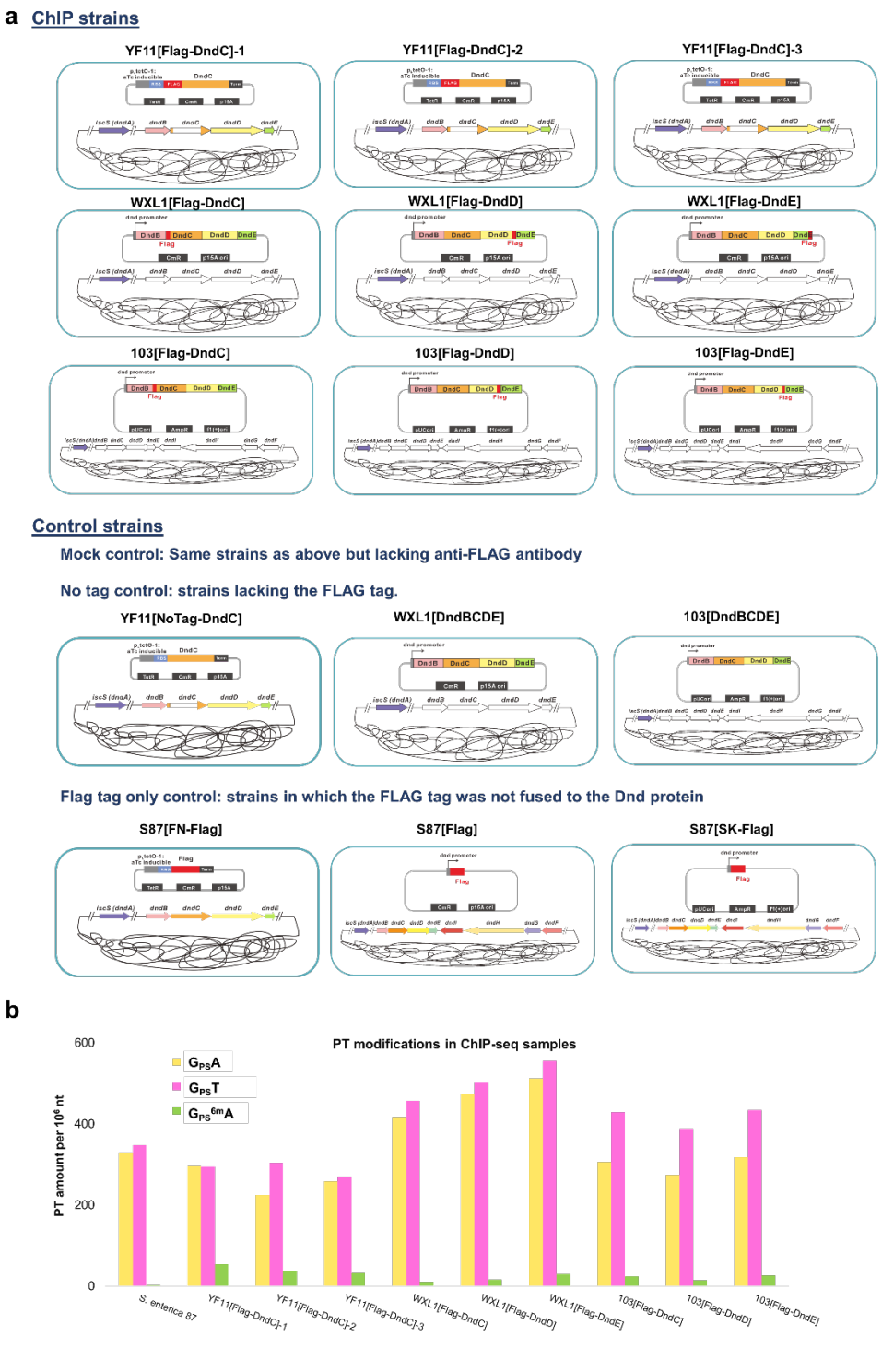

Fig. S1. Construction and validation of expression systems for FLAG-tagged Dnd proteins in *S. enterica serovar* Cerro 87 Dnd for ChIP-seq analyses. (**a**) Construction of different Dnd expression systems for ChIP-seq analysis. (**b**) The PT abundance in DNA from the same samples used for ChIP-seq from *S. enterica serovar* Cerro 87. PT dinucleotides were quantified by LC-MS/MS.

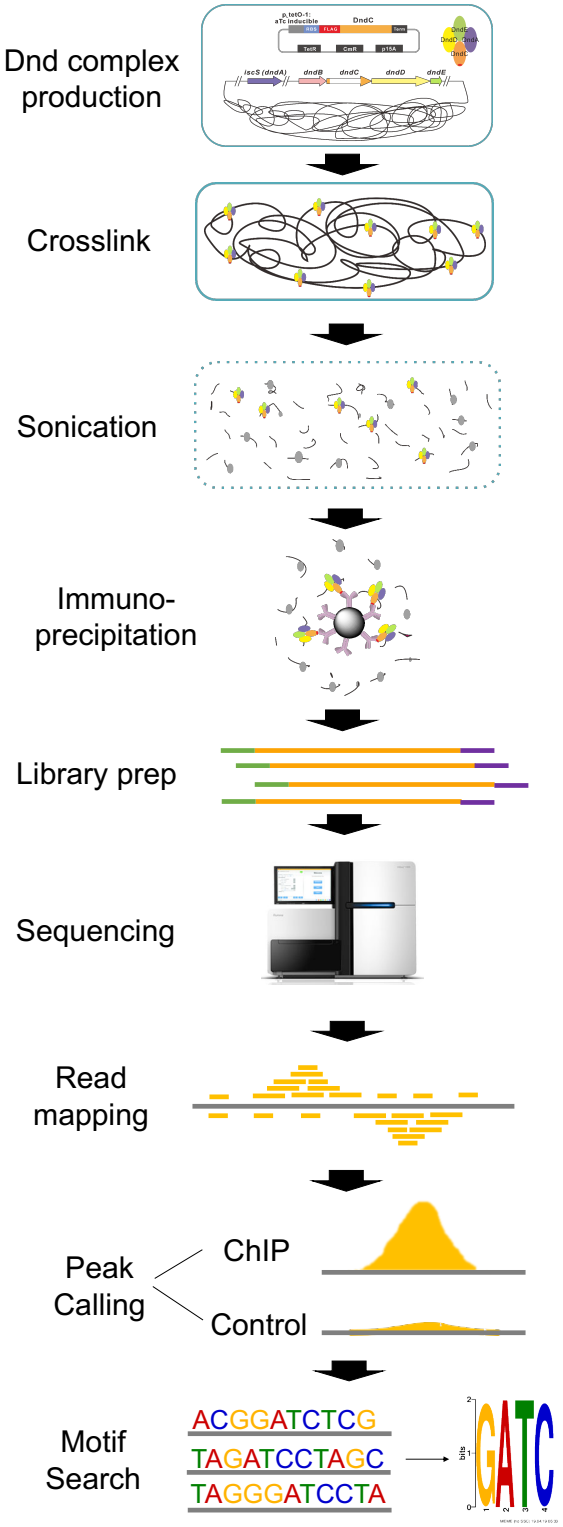

Fig. S2. Workflow for the ChIP-seq experiments.

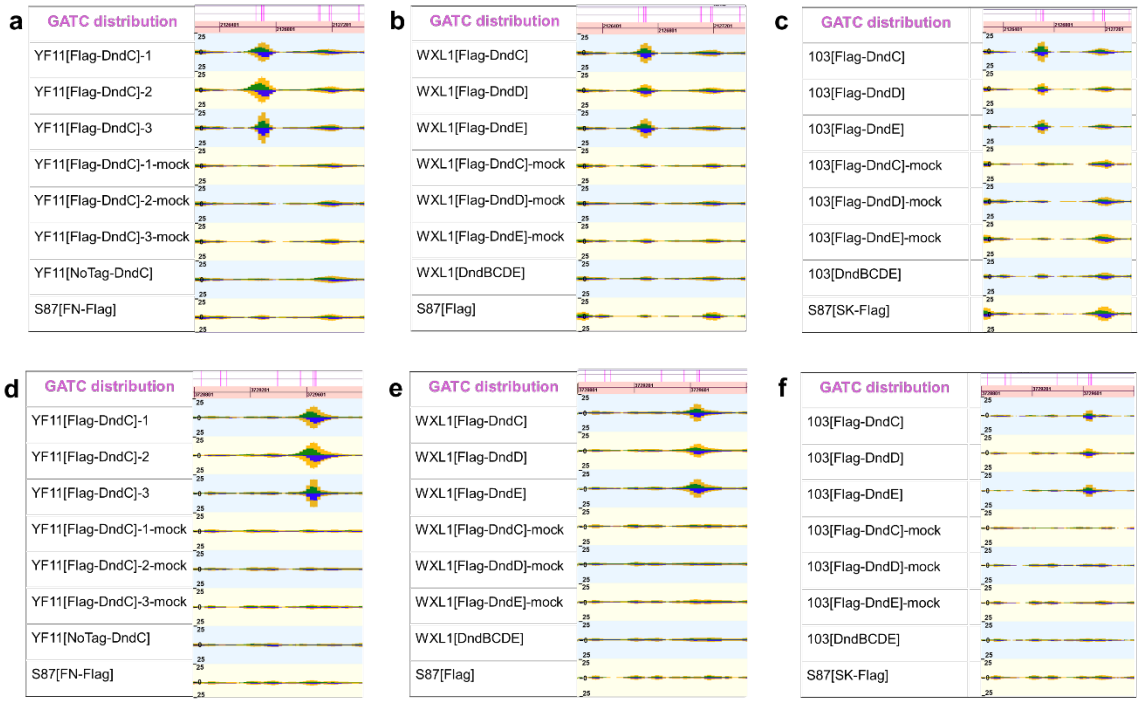

Fig. S3. By combining the ChIP-seq datasets, we were able to identify the 75 Dnd binding regions shown in **Figure 1a**. Here we show two examples of these 75 sites and the signals generated with each Dnd protein and replicate analysis. (**a,b,c**) ChIP-seq site #1, (**d,e,f**) ChIP-seq site #2. (**a,d**) ChIP-seq analyses for the three replicate analysis with FLAG-tagged DndC (YF11[FLAG-DndC]-1, 2, 3), no FLAG antibody (YF11[FLAG-DndC]-1, 2, 3-mock), no FLAG tag on DndC (YF11[NoTag-DndC]), and a FLAG-only control (S87[FN-FLAG]). (**b,e**) ChIP-seq analyses for the individual FLAG-tagged DndC, DndD, and DndE proteins (WXL1[FLAG-DndC, D, E]), no FLAG antibody control (WXL1[FLAG-DndC, D, E]-mock), a no-FLAG control (WXL1[DndBCDE]), and a FLAG-only control (S87[FLAG]). (**c,f**) ChIP-seq analyses for the individual FLAG-tagged DndC, DndD, and DndE proteins (103[FLAG-Dnd C, D, E],), no FLAG antibody control (103[FLAG-DndC, D, E]-mock), a no-FLAG control (103[DndBCDE]), and a FLAG-only control (S87[SK-FLAG]).

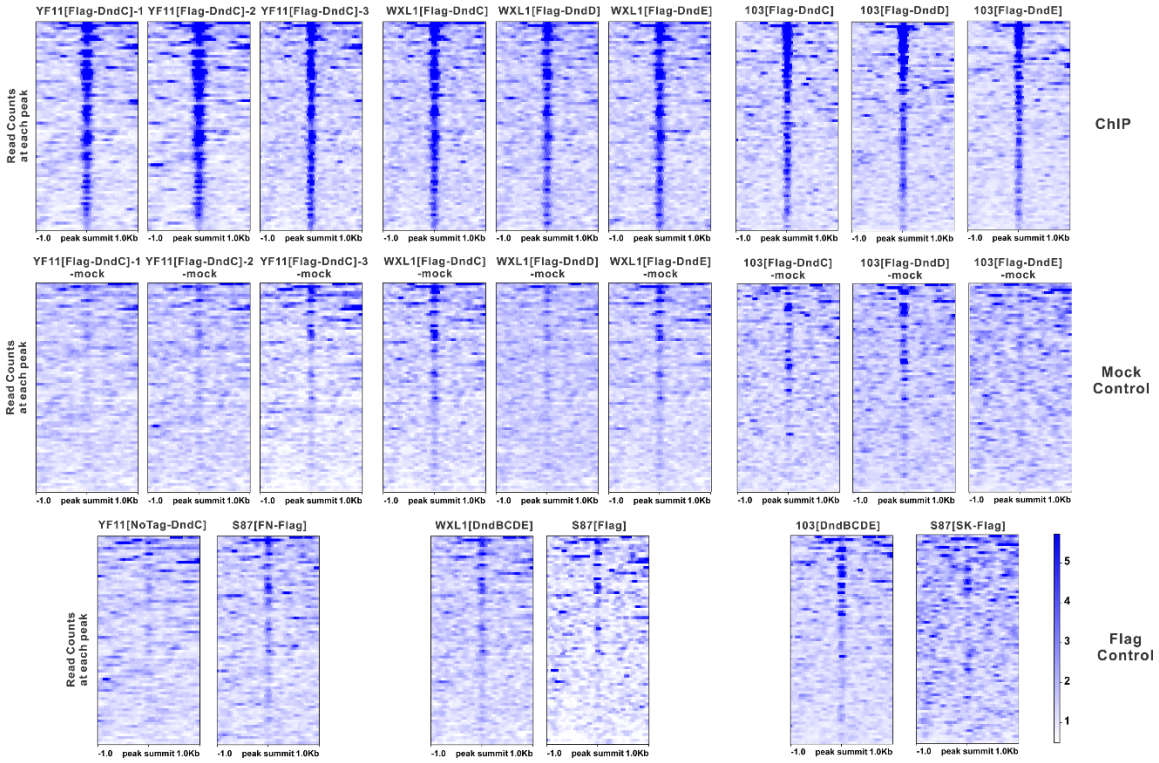

Fig. S4. **Read counts around centers of Dnd-bound regions.** The analysis was performed separately for 75 Dnd ChIP-seq regions detected in 9 FLAG-tagged Dnd strains (top), 9 control stains without anti-FLAG antibody (middle) and 6 controls strains either without FLAG tag or FLAG tag not fused to Dnd proteins (bottom). Read counts for each nucleotide were normalized to 1x coverage and colored according to scale. Each row shows the read pile ups (normalized to 1x coverage) for 1000 bp on either side of the ChIP-seq site, with colors indicating the number of reads in each pile up. The rows are sorted in descending order of mean coverage value.

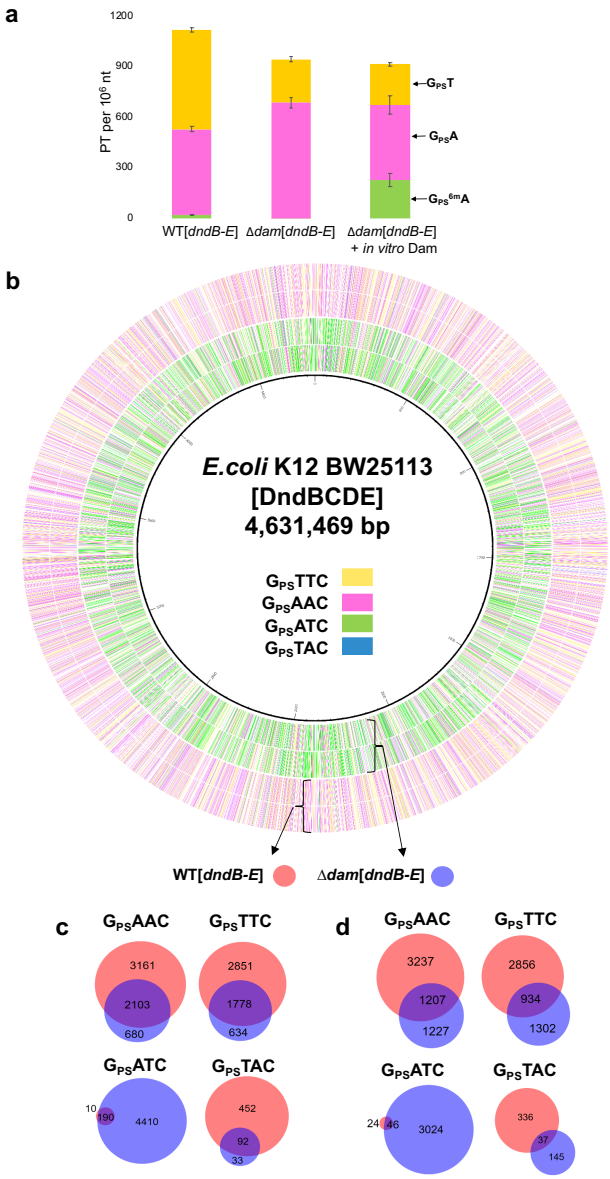

Fig. S5. Effect of the number of copies of the Dnd-expressing plasmid on PT modification sites and levels in *E. coli* possessing or lacking Dam. Studies presented in **Figure 2** used a high copy-number plasmid for expressing *S. enterica dnd* genes in *E. coli* BW25113. Here we repeated the studies with a low copy-number plasmid to compare the effect of plasmid copy number on the Dam-dependent changes in PT locations and levels. (**a**) Dam-dependent changes in the levels of PT dinucleotides measured by LC-MS. (**b**) TdT-seq map of PT sites across the *genomes of E. coli* BW25113 [*dndB-E*] and Δ*dam*[*dndB-E*]. From inner to outer circles: Circles 1 and 2, (forward, reverse strands) PT sites in Δ*dam*[*dndB-E*]. Circles 3 and 4, (forward, reverse strands) PT sites in WT[*dndB-E*]. GPSAAC: purple, GPSTTC: yellow, GPSATC: green, and GPSTAC: blue. (**c,d**) Quantification of Dam-dependent changes in PT modification sites in the *E.coli* BW25113 [*dndB-E*] and Δ*dam*[*dndB-E*] genomes. (**c**) Low copy-number and (**d**) high copy-number *dnd* expression plasmids.

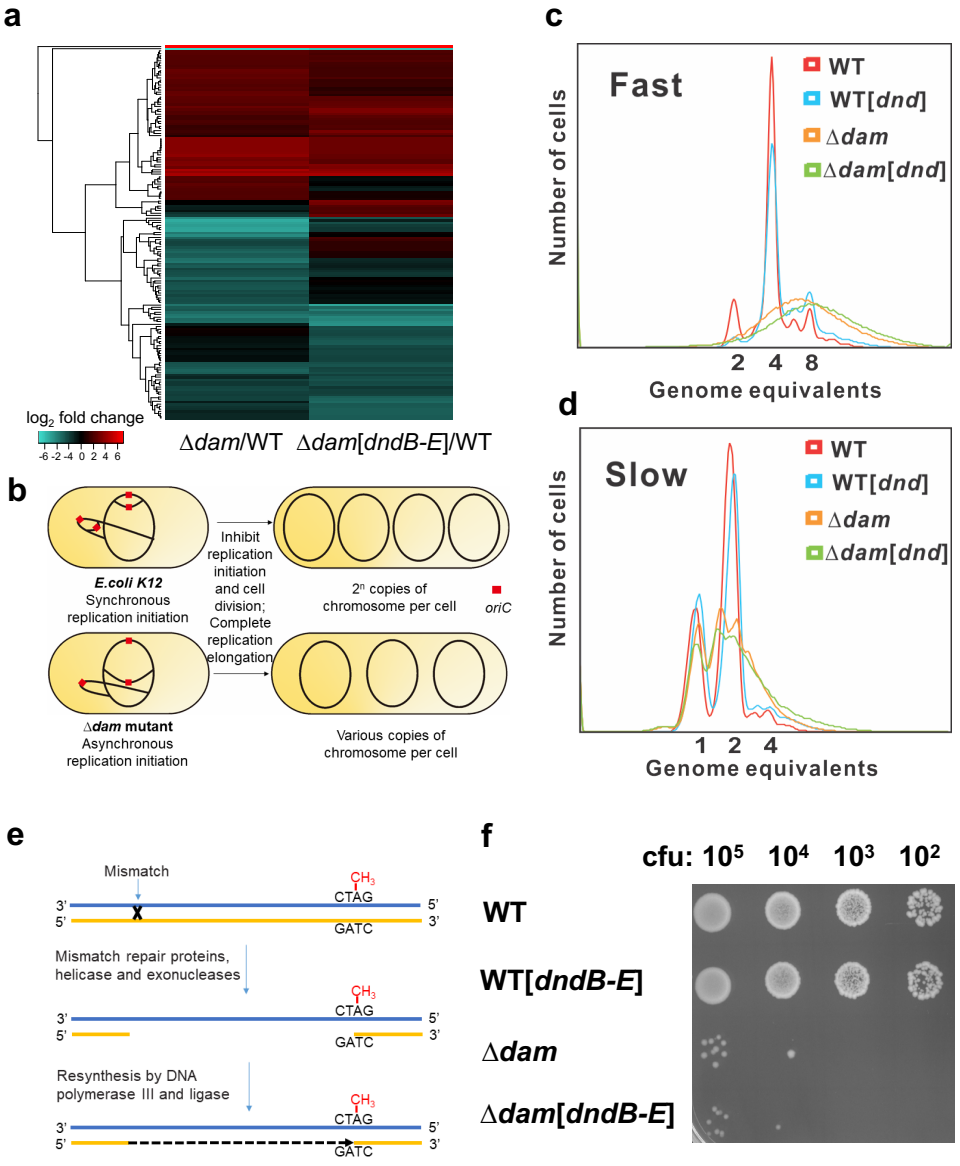

Fig. S6. Tests of PT function in the Δ*dam* mutant. (a) Gene expression changes in *E. coli* Δ*dam* mutant harboring *dnd*BCDE or empty plasmid relative to wild-type *E. coli* BW25113 (red represents up-regulation; blue represents down-regulation). (**b**) Dam regulates chromosome replication. (**c, d**) Flow cytometric analysis of DNA content in *E. coli*. Log-phase *E. coli* BW25113 and its *dam* and *dnd* derivates were analyzed after replication-run out in the presence of rifampicin and cephalexin which will block replication initiation and cell division. Above 100,000 cells were detected in each sample. (**e**) Dam's function in DNA mismatch repair. (f) 2AP sensitivity test on *E.coli dam* and *dnd* derivates. Each log-phase culture was 10-fold serial diluted and spotted on LB agar plates containing 2AP (350 mg/ml) followed by growing in 37 ℃ for 24 h. Without drug, plating efficiency and colony size were similar for all strains tested.

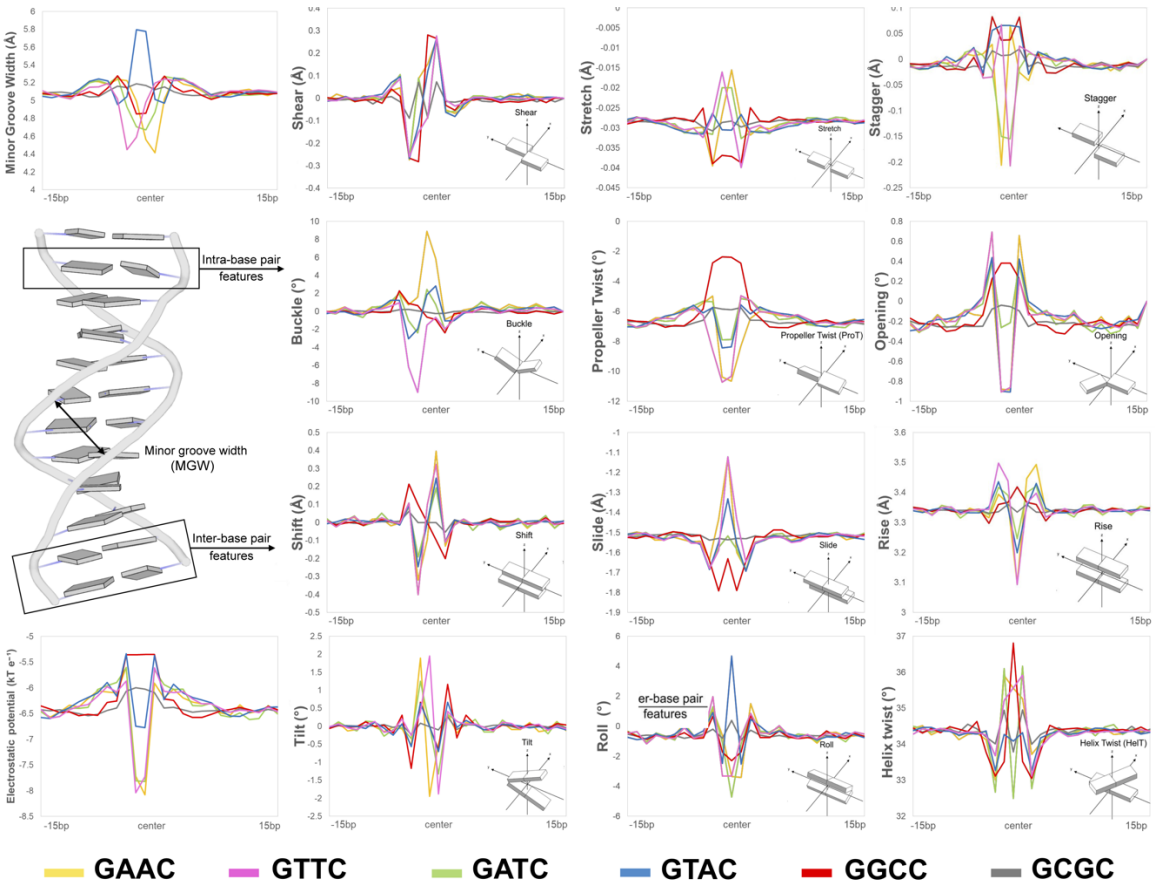

Fig. S7. Analysis of DNA shapes for GXXC motifs associated with PT modification. Here we analyzed the 6 possible GXXC motifs for 12 different DNA helical features, electrostatic potential and minor groove width, using the DNAshapeR algorithm (1). ~1,500 DNA sequences centered on the GXXC PT modification motifs were selected from the *E. coli* genome and DNAshapeR was used to calculate the DNA features for each sequence. The plots show the average value for the ~1,500 sequence contexts of each GXXC motif.

Supporting Table S1. Strains and plasmids used in this study

Strains or plasmid	Source	Characteristics
S. enterica 87	Ref (2)	*S. enterica serovar* Cerro 87, strain naturally contains *dndBCDE* gene cluster and PT modified $G_{PS}A$ and $G_{PS}T$
YF11	Ref (2)	*S. enterica serovar* Cerro 87 Δ*dndC* mutant
WXL1	Ref (2)	*S. enterica serovar* Cerro 87 Δ*dndB-E* mutant
XTG103	Ref (2)	*S. enterica serovar* Cerro 87 Δ*dndB-H* mutant
E. coli BW25113	Keio collection (3)	*E. coli* K12 derivative, F^-, Δ*(araD-araB)567*, Δ*lacZ4787*(::rrnB-3), λ^-, *rph-1*, Δ*(rhaD-rhaB)568*, *hsdR514*
E. coli JW3350	Keio collection (3)	*E. coli* BW25113 Δ*dam* mutant
pBluescript SK(+)	Ref (4)	Cloning vector, Amp^R, f1(+) and pUC origin
pACYC184	Ref (4)	Cloning vector, Cm^R, p15A origin
pJTU1238	Ref (4)	*S. enterica* 87 *dndBCDE* cloned in pBluescript SK(+)
pJTU1980	Ref (4)	*S. enterica* 87 *dndBCDE* cloned in pACYC184
pFLAG-DndC	This work	FLAG-tag fused to *S. enterica* 87 *dndC*, cloned in p15A origin plasmid
pNoTag-DndC	This work	*S. enterica* 87 *dndC*, cloned in p15A origin plasmid
pFLAG	This work	pFlag-DndC derivative, Δ*dndC*
p1980-FLAG-DndC	This work	pJTU1980 derivative, FLAG tag fused to N terminal of DndC
p1980-FLAG-DndD	This work	pJTU1980 derivative, FLAG tag fused to C terminal of DndD
p1980-FLAG-DndE	This work	pJTU1980 derivative, FLAG tag fused to C terminal of DndE
pACYC184-FLAG	This work	FLAG tag cloned in pACYC184
p1238-FLAG-DndC	This work	pJTU1238 derivative, FLAG tag fused to N terminal of DndC
p1238-FLAG-DndD	This work	pJTU1238 derivative, FLAG tag fused to C terminal of DndD
p1238-FLAG-DndE	This work	pJTU1238 derivative, FLAG tag fused to N terminal of DndE
pSK-FLAG	This work	FLAG tag cloned in pBluescript SK (+)

Supporting Table S2. Bacterial strains designed for the ChIP-seq studies

Sample name	Role	Bacteria	Anti-FLAG Antibody	FLAG tag	FLAG fused to Dnd
YF11[FLAG-DndC]-1	ChIP	YF11[pFLAG-DndC]	+	+	+
YF11[FLAG-DndC]-1-mock	No antibody control	YF11[pFLAG-DndC]	-	+	+
YF11[FLAG-DndC]-2	ChIP	YF11[pFLAG-DndC]	+	+	+
YF11[FLAG-DndC]-2-mock	No antibody control	YF11[pFLAG-DndC]	-	+	+
YF11[FLAG-DndC]-3	ChIP	YF11[pFLAG-DndC]	+	+	+
YF11[FLAG-DndC]-3-mock	No antibody control	YF11[pFLAG-DndC]	-	+	+
YF11[NoTag-DndC]	No tag control	YF11[pNoTag-DndC]	+	-	-
S87[FN-FLAG]	Unfused tag control	*S. enterica* 87 [pFLAG]	+	+	-
WXL1[FLAG-DndC]	ChIP	WXL1[p1980-FLAG-DndC]	+	+	+
WXL1[FLAG-DndC]-mock	No antibody control	WXL1[p1980-FLAG-DndC]	-	+	+
WXL1[FLAG-DndD]	ChIP	WXL1[p1980-FLAG-DndD]	+	+	+
WXL1[FLAG-DndD]-mock	No antibody control	WXL1[p1980-FLAG-DndD]	-	+	+
WXL1[FLAG-DndE]	ChIP	WXL1[p1980-FLAG-DndE]	+	+	+
WXL1[FLAG-DndE]-mock	No antibody control	WXL1[p1980-FLAG-DndE]	-	+	+
WXL1[DndBCDE]	No tag control	WXL1[pJTU1980]	+	-	-
S87[FLAG]	Unfused tag control	*S. enterica* 87 [pACYC184 +FLAG]	+	+	-
103[FLAG-DndC]	ChIP	XTG103[p1238-FLAG-DndC]	+	+	+
103[FLAG-DndC]-mock	No antibody control	XTG103[p1238-FLAG-DndC]	-	+	+
103[FLAG-DndD]	ChIP	XTG103[p1238-FLAG-DndD]	+	+	+
103[FLAG-DndD]-mock	No antibody control	XTG103[p1238-FLAG-DndD]	-	+	+
103[FLAG-DndE]	ChIP	XTG103[p1238-FLAG-DndE]	+	+	+
103[FLAG-DndE]-mock	No antibody control	XTG103[p1238-FLAG-DndE]	-	+	+
103[DndBCDE]	No tag control	XTG103[pJTU1238]	+	-	-
S87[SK-FLAG]	Unfused tag control	*S. enterica* 87 [pBluescript SK (+) +FLAG]	+	+	-

Datasets in separate Excel spreadsheets:
Dataset S1: Dnd protein binding sites in *S. enterica* determined by ChIP-seq
Dataset S2. PT modification sites in *S. enterica* ChIP-seq samples
Dataset S3. PT modification sites in *E. coli* expressing DndB-E from *S. enterica* (high copy number)
Dataset S4. PT modification sites in *E. coli* expressing DndB-E from *S. enterica* (low copy number)
Dataset S5. RNA-seq results in *E. coli* expressing DndB-E from *S. enterica*
Dataset S6. Pearson correlation coefficients (PCC) for the different DNA shape motifs for 4-nucleotide GXXC DNA sequences

SI Appendix References

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