# Appendix for “Distinct heterochromatin-like domains promote transcriptional memory and silence parasitic genetic elements in bacteria”

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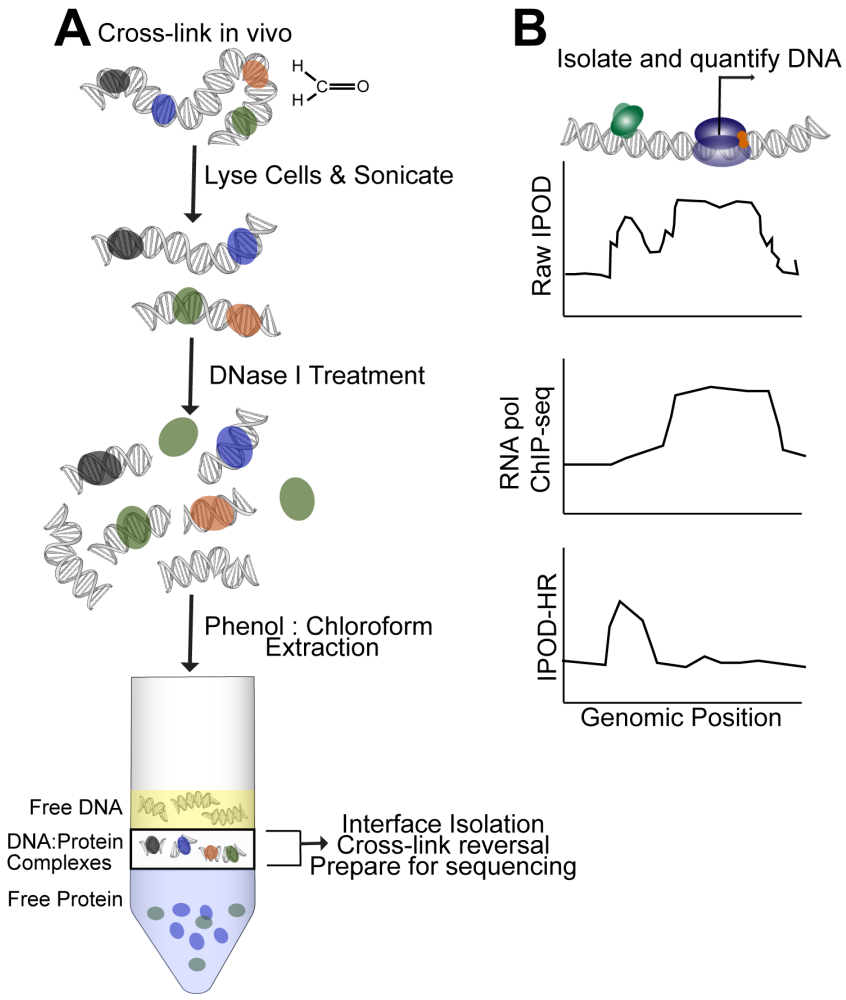
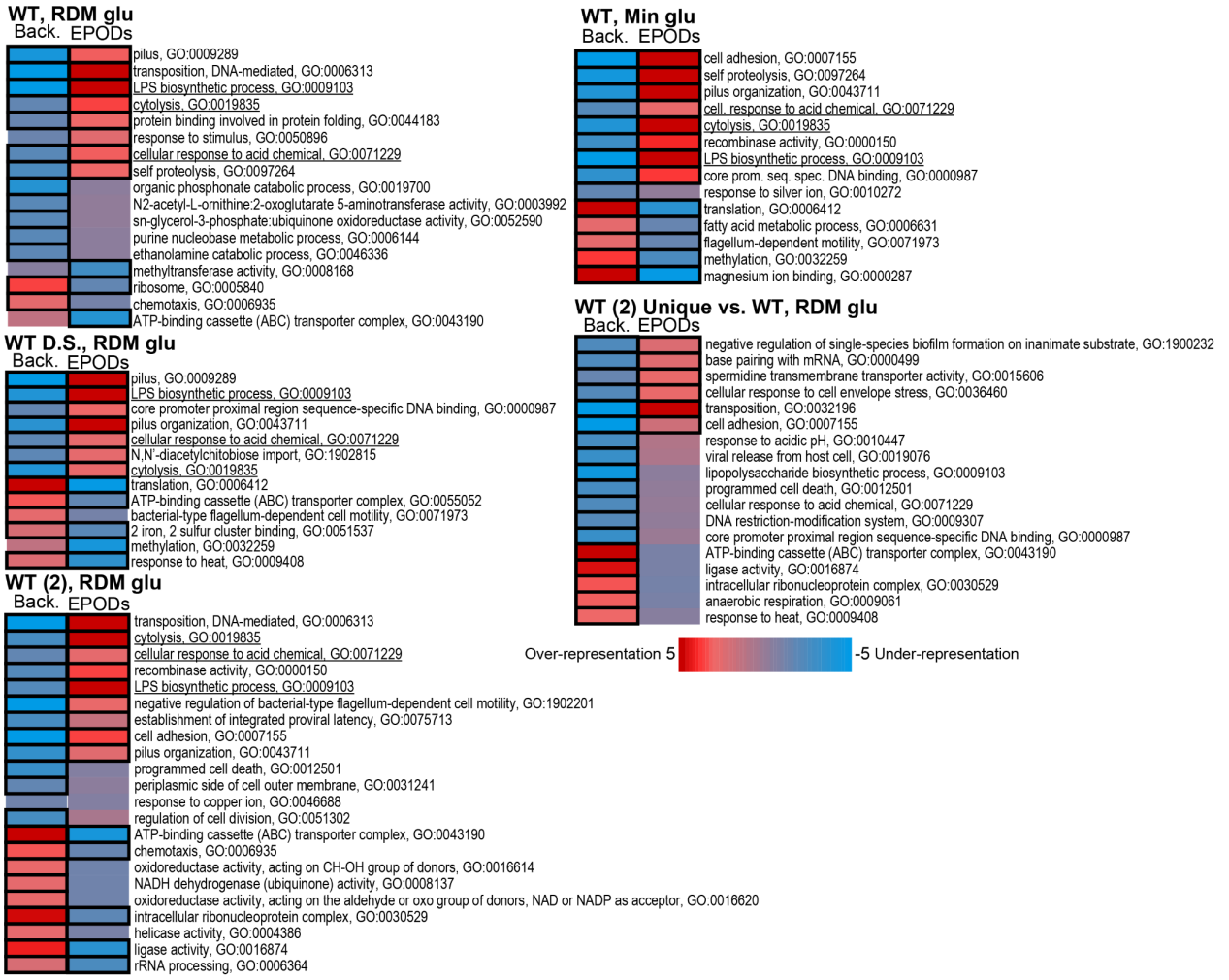
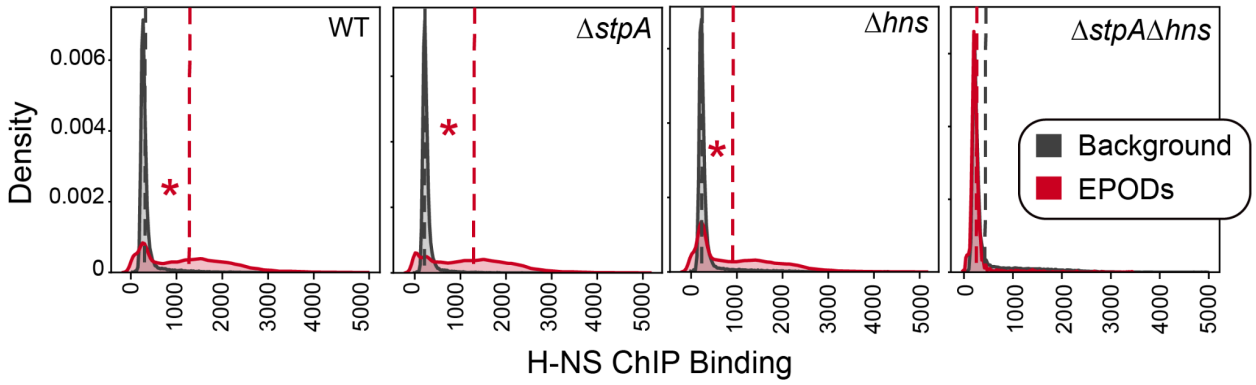
## Appendix Figures



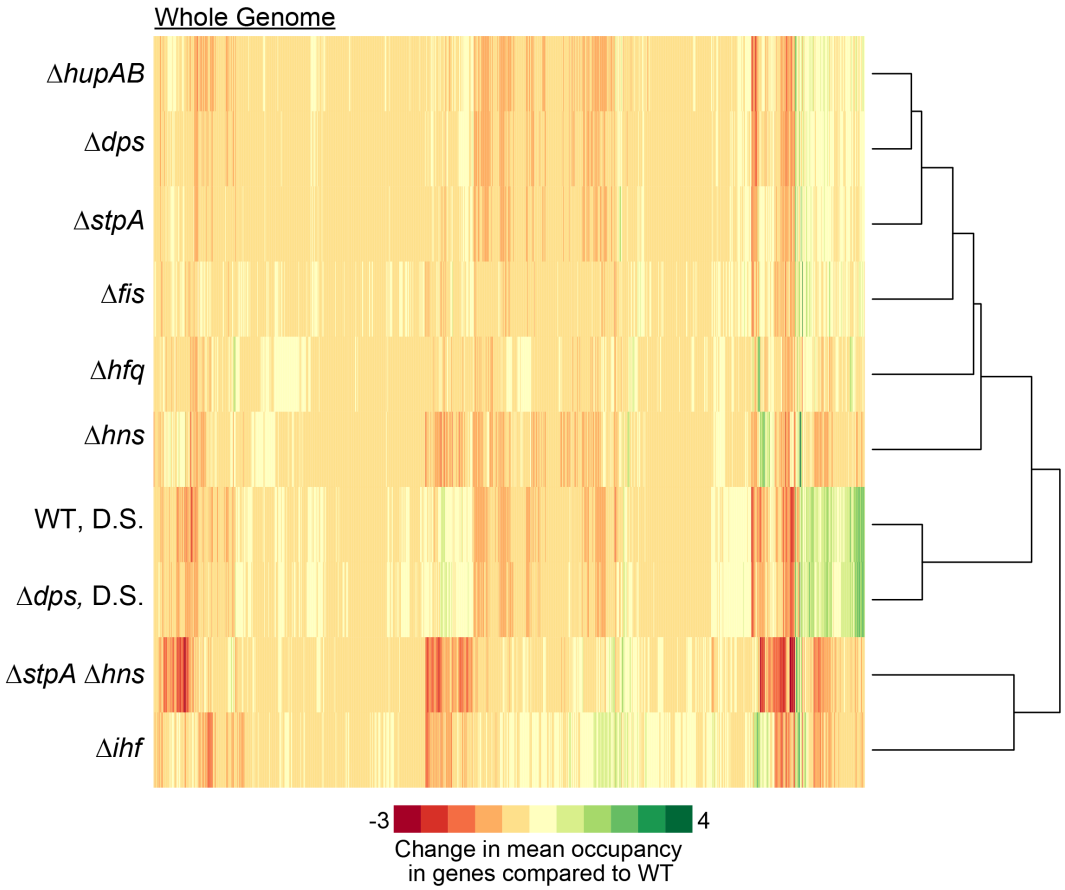

**Appendix Figure S1: Schematic of the IPOD-HR method.**
(A) Summary of the key steps in IPOD-HR: proteins are crosslinked to bound DNA with formaldehyde, cells are lysed and enzymatically treated to minimize footprints, and then subjected to a phenol-chloroform extraction. The interphase layer between the aqueous and organic phases is isolated, and DNA recovered and prepared for sequencing. Further details are in [12,13]. (B) Postprocessing of IPOD signal (log ratio of the DNA abundance in the interphase sample to a corresponding input control); values from an RNA polymerase ChIP experiment (again in the form of log ratio relative to an input control) are subtracted to yield the final IPOD-HR signal (bottom). Further details are given in [13].



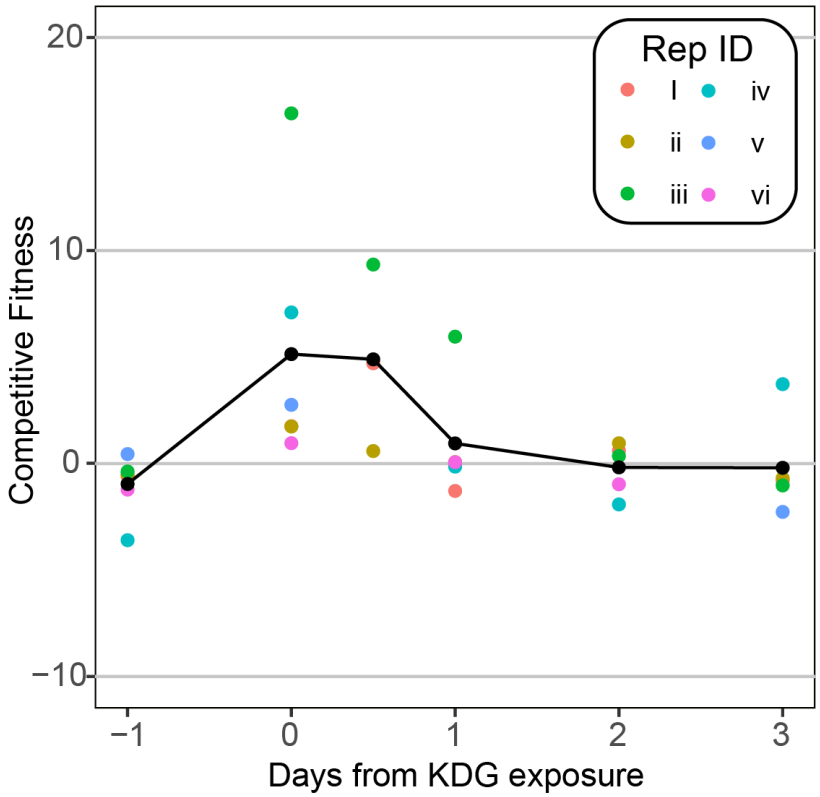

**Appendix Figure S2: Pathway analysis of EPODs across WT conditions.**
iPAGE analysis revealed key pathways overrepresented in EPODs compared to background that remain across different growth media, harvest growth phase, and parental background (underlined). Color scale represents over- or under-representation of genes with particular GO term annotations in the EPODs vs. non-EPOD regions (background). To compare the differences among two MG1655 parental strains, we performed iPAGE specifically on EPODs that were unique in WT (2) compared to WT, shown in the bottom right.




**Appendix Figure S3: Deletion of *hns* and *stpA* impacts EPODs across the genome.** Density plots exhibit enrichment of H-NS binding within EPODs that is reduced upon deletion of *hns* and the double deletion of *stpA* and *hns*. Dashed lines are the median for background (grey) and EPODs (red) for each condition. (*) indicates FDR-corrected p<0.005 via permutation test (against a null hypothesis of no difference in medians).




**Appendix Figure S4: Changes in protein occupancy across the genome.**
The average occupancy was calculated across EPODs and background regions. The change in protein occupancy was calculated by subtracting the WT average at each region for every mutant. A gain in occupancy in the mutant is represented by a positive change in occupancy, while a loss is represented by a negative change in occupancy. Hierarchical clustering distinguished NAPs that have similar impacts on protein occupancy across the genome.




**Appendix Figure S5: Memory effect of KDG exposure dissipates over time.**
Shown are the replicate-level data from the competition experiments presented in FIg. 4 of the main text, demonstrating that the competitive advantage of cells pre-exposed to KDG persists for 12-24 hours of growth in glucose minimal media, and then dissipates. Colored points show the replicate-level data contributing to the analysis in Fig. 4B, with the solid black line and associated points showing the mean value across experimental replicates at each timepoint. Statistical significance is assessed in Fig. 4B.

# Appendix Tables

| Replicate | End of KDG Growth | | | After 12 hr GLU outgrowth | | | | After 24 hr GLU outgrowth | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | *Colony count* | *Dilution* | *Cells/mL* | *Colony count* | *Dilution* | *Cells/mL* | *# Doublings from KDG* | *Colony count* | *Dilution* | *Cells/mL* | *# Doublings from KDG* |
| I | 4 | 1.0E-05 | 8.0E+07 | 5 | 1.0E-05 | 1.0E+08 | 8.0 | 24 | 1.0E-05 | 4.8E+08 | 10.2 |
| II | 8 | 1.0E-05 | 1.6E+08 | 17 | 1.0E-05 | 3.4E+08 | 8.7 | 30 | 1.0E-05 | 6.0E+08 | 9.6 |
| III | 31 | 1.0E-01 | 6.2E+04 | 15 | 1.0E-02 | 3.0E+05 | 9.9 | 16 | 1.0E-05 | 3.2E+08 | 20.0 |

**Appendix Table S1: Outgrowth of KDG-exposed cells in GLU during competition experiments.**

Colony counts for cells in KDG growth experiments at the end of KDG growth and at the early stages of outgrowth in GLU media (for the competition data shown in Fig. 4B of the main text). The three stages shown here correspond to the KDG-exposed cells at the 0 day, 0.5 day, and 1.0 day timepoints. “Colony count” refers to the actually observed counts from spottings of 5 microliters of media, and are converted to inferred cells/mL for the original culture. “# Doublings from KDG” indicates the number of doublings that the cells in GLU media have undergone after the end of their exposure to KDG, and account for the changes in cell numbers, plus the 200-fold dilution of cells from the KDG condition into the GLU condition (see Methods).